CRANFIELD UNIVERSITY

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ENGINEERING DESIGN OF INSTRUMENTATION FOR LIFE DETECTION PLANETARY EXPLORATION MISSIONS

CRANFIELD HEALTH
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PhD Thesis
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Supervisor: Professor David C. Cullen
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To my childhood friend María Baltanás García (26/09/1983-27/04/2008).

Her memory will forever inspire me to pursue my dreams.

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Abstract

The aim of the research documented in this thesis was to explore issues associated with the development of instrumentation for life detection and characterisation in a planetary exploration context.

Within this aim, the following objectives had to be achieved:

1. To consider current and near-future single molecule detection (ultra-low lower limit of detection) analytical techniques that would be compatible with development into a Space qualifiable in situ analytical instrument for the detection of biomarkers in a planetary exploration context.
2. To practically consider the consequences of Planetary Protection and Contamination Control on the development of a sample return instrumentation in a planetary exploration context.
3. To consider the implications of flying an in situ instrument on-board a stratospheric balloon platform in order to apply them into a specific planetary exploration mission:

In order to achieve the objectives described above, the following work was pursued:

- A desk-based European Space Agency (ESA) study was carried out which entailed producing a literature review on single molecule detection technologies that had to be validated by the expert community. This was done by organising an International Workshop on Single Molecule Detection Technologies for Space Applications in March 2009 at Cranfield University, UK. The approved technologies then had to be analysed with standard analytical techniques (*i.e.*, trade-offs) in order to propose a specific technology for development and present its breadboard implementation and test plans at the end of the study.
- A sample return experiment implementing PP&CC constraints and protocols was designed, built, tested and flown on-board the ESA, Swedish Space Corporation (SSC), Swedish National Space Board (SNSB) and German Space Agency (DLR) BEXUS stratospheric balloon platform. The biological and engineering results obtained from the sample return flight were then analysed and lessons learnt obtained for future flights.
- Another desk-based study was performed to research future stratospheric balloon platforms for the exploration of Venus' cloud layer. The *in situ* instrument previously proposed for the detection of biomarkers for planetary exploration missions was then put forward as a possible payload for a Venusian stratospheric balloon platform and approved by experts during the Venus Exploration Analysis Group (VEXAG) conference held in August 2011 in Washington D.C, USA.

The first part of the research involved studying ultra-low lower limit of detection technologies as these have the potential to impact significantly on the technological and scientific requirements of future Space missions. Two systems were proposed: one based on Tandem Mass Spectrometry (with Cylindrical Ion Trap analysers) followed by Surface Enhanced Raman Scattering spectroscopy to create an MS/MS-SERS instrument for the detection of astrobiology biomarkers in Martian regolith, Europan ice and samples from Titan's hydrocarbon lakes; and a second one as a Stand-Alone SERS system for the detection of biomarkers in Enceladean plumes, Venusian clouds and cometary coma.

The second part of the research practically explored the design of instrumentation for stratospheric balloon platforms. CASS•E, the Cranfield Astrobiological Stratospheric Sampling Experiment, was a life detection experiment that aimed to be capable of detecting stratospheric microorganisms. The experiment consisted of a pump which drew air from the Stratosphere through a 0.2 µm collection filter which retained any microorganisms and >0.2 µm particulates present in the pumped air. Due to the expected rarity of microbes in the Stratosphere compared to the known levels of contamination at ground level, Planetary Protection and Contamination Control (PP&CC)

constraints were introduced. Therefore PP&CC protocols were followed to implement Space qualified cleaning and sterilisation techniques; biobarrier technology was implemented to prevent re-contamination of the instrument after sterilisation; and cleanliness and contamination was monitored throughout assembly, integration and testing.

The third part of the research demonstrated how an instrument from the first part of the study could be proposed as a payload on-board a stratospheric balloon platform with a focused mission context, *i.e.*, a life detection mission for Venus. Therefore, the research concluded with the proposal of a payload for a Venus mission based on SERS technology on-board a stratospheric balloon platform to search for life above or in the mid Venusian cloud cover.

Keywords:

Astrobiology, Tandem Mass Spectroscopy, SERS, Stratospheric balloons, PP&CC, Venus.

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Abbreviations

A/D: Analogue to Digital.
AFFM: The Atomic Force Fluorescence Microscope.
AFM: The Atomic Force Microscope.
AIT: Assembly, Integration and Test.
AIV: Assembly, Integration and Verification.
APCI: Atmospheric Pressure Chemical Ionisation.
ATP: Adenosine Tri-phosphate.
AR: Assessment Requirement.
BEXUS: Balloon EXperiments for University Students.
BEXUSR: BEXUS Requirement.
CARS: Coherent Anti-Stokes Raman Scattering.
CASS•E: Cranfield Stratospheric Sampling Experiment.
CDR: Critical Design Review.
CE: Capillary Electrophoresis.
CI: Chemical Ionisation.
CIT: Cylindrical Ion Trap.
COG: Centre of gravity.
COSPAR: Committee on Space Research.
COTS: Commercial Off-The-Shelf.
DART: Direct Analysis in Real Time.
DR: Design Requirement.
DI: Deionised.
DLR: Deutsches Zentrum für Luft und Raumfahrt.
DR: Design Requirement.
EAR: Experiment Acceptance Review.
EI: Electron Ionisation.
EIT: Electrical Interface Test.
ESA: European Space Agency.
ESI: Electrospray Ionisation.
Esrange: European Sounding Rocket Launching Range.
ESTEC: European Space Research and Technology Centre, ESA (NL).
EVE: European Venus Explorer.
FAB: Fast Atom Bombardment.
FCS: Fluorescence Correlation Spectroscopy.
FD: Field Desorption.
FI: Field Ionisation.
FRET: Förster Resonance Energy Transfer.
FRP: Flight Requirement Plan.
FTICR: Fourier Transform Ion Cyclotron Resonance.
FR: Functional Requirement.
GC: Gas Chromatography.
HPLC: High Pressure Liquid Chromatography.
I-BATE: ISU-Balloon ATC Technology Experiment.
IM: Ion Mobility.
IPA: Isopropyl Alcohol.
IPR: Interim Progress Review.
LC: Liquid Chromatography.
LED: Light Emitting Diode.
LOS: Line of Sight.
LQIT: Linear Quadrupole Ion Trap.
MALDI: Matrix-Assisted Laser Desorption.
Mbps: Mega Bits per second.

MORABA: Mobile Raketen Basis (DLR, EuroLaunch).
MS: Mass Spectroscopy.
MS/MS: Mass Spectrometry.
MSMSSERSR: Tandem MS-SERS Requirement.
OR: Operation Requirement.
PCB: Printed Circuit Board.
PDR: Preliminary Design Review.
PERDAIX: Proton Electron Radiation Detector.
PLED: Polymer Light Emitting Diode.
PI: Photoionisation.
PP&CC: Planetary Protection and Contamination Control.
PPT: Parts per Trillion.
PR: Performance Requirement.
QCM: Quartz Crystal Microbalance.
QIT: Quadrupole Ion Trap.
RETA: Radiation Exposure in The Atmosphere.
REXUS: Rocket Experiments for University Students.
SCRAT: Spherical Compact Rechargeable Air Thruster.
SAW: Surface Acoustic Wave.
SCOPE 2.0: Stabilized Camera Observation Platform Experiment.
SERRS: Surface Enhanced Resonant Raman Spectroscopy.
SERS: Surface Enhanced Raman Scattering.
SERSR: SERS Requirement.
SED: Student Experiment Documentation.
SNSB: Swedish National Space Board.
SPR: Surface Plasmon Resonance.
SSC: Swedish Space Corporation (EuroLaunch).
T: Time before and after launch noted with + or -.
TBC: To be confirmed.
TBD: To be determined.
TI: Thermal Ionisation.
TIRFM: Total Internal Reflection Fluorescence Microscopy.
TLR: Top Level Requirement.
TOF: Time-of-Flight.
UCZ: Ultra Clean Zone.
VCM: Venus Climate Mission.
VEXAG: Venus Exploration Analysis Group.
VFDRM: Venus Flagship Design Reference Mission.

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1. Introduction to Life Detection Missions

Humans have one fundamental question they have strived to address since their use of rational thinking: *Who are we?*

This question, calling for both scientific and philosophical arguments, addresses the unanswered questions of: *Why are we here? Where did we come from?* And, *where are we headed?* Understanding these questions developed the new science of Astrobiology which over the past two decades has been aided by the use of empirical results to painstakingly start forming the answers.

Scientifically, the questions become: *What is Life? What is the course of Life?* And, *are we alone?* We now understand the mechanics of Life due to our knowledge of molecular genetics and can differentiate between a chemical reaction and a living entity; but how different can other types of Life be from our own? (Sullivan and Baross, 2007)

If we understand when and where Life first came from and how it evolved; can we understand how future Life could be formed? Is Life then a ubiquitous characteristic of the Cosmos or a chanceful event?

And if Life can occur elsewhere in the Universe, are we then to believe that we were never alone?

The research described in this document describes the quest for detecting Life in our Solar System and how it can be achieved.

1.1 The Search for Life in Extreme Environments

According to the ESA Exobiology Team Study (1999), Life is considered to be any living chemical system able to transfer its molecular information via self-replication and to evolve by making few random errors during that transfer that can lead to higher complexity and better adaptation to its environment. Due to this, the ESA Exobiology Team Study (1999) also states that liquid water is a necessary requirement for Life as it provides the medium where molecules can dissolve in and chemical reactions can take place in order to enable Life to self-replicate and evolve. This is due to the fact that water is the only liquid that exists in a temperature range that is not too cold to sustain biochemical reactions and yet not too hot to impede organic bonds from forming.

1.1.1 The Limits of Life in Extreme Conditions

Microbes can be found in the most extreme environments on Earth: bacteria have been reported in permanently cold caves in the Arctic, at high pressures at 10,500m deep in the ocean and at hyper-saline waters all over the world, as well as at hot springs, submarine hydrothermal vents, geothermally heated oil reserves and oil wells (Satyanarayana and Raghukumar, 2005).

The following extreme environment scenarios host Life on Earth.

1.1.1.1 Extreme temperature ranges

On Earth, life is based on the chemistry of carbon in water, which makes it intrinsically linked with temperature. However, life has been found to thrive in the extreme temperature ranges of liquid water, where the maximum temperature limit of life known to be living in is above its boiling point and up to 113°C. Prokaryotes (small unicellular microorganisms without a cellular membrane, as opposed to eukaryotes, which do have a membrane) are found to be living in large populations within hydrothermal vents using its reduced chemicals for energy and growth. This is due to proteins and DNA being stable at high temperatures to at least above 107°C.

On the other hand, the lower temperature limit of life on Earth is not as clearly understood as it is difficult to understand growth and/or metabolic activity at sub-zero temperatures. There are two types of organisms: those with optimal growth temperatures below 15°C and minimal growth below 0°C (called psychrophiles) and those capable of growth at 0°C but with optimal growth temperatures above 15°C (psychrotrophs). Whereas hyperthermophily is restricted to specific types of prokaryotes, psychrophily exists in all prokaryotic and eukaryotic groups. Therefore, for life on Earth, adapting to low temperatures seems to be easier than to high temperatures. This is believed to be due to the effect of high temperatures on the rupture of covalent bonds (ESA Exobiology Team Study, 1999).

1.1.1.2 High-salt environments

Although salts are essential for Earth-based organisms due to their function as co-catalysts during enzymatic processes, they are only tolerated in very low concentrations (<0.5%) as they trigger water loss from cells. However, halophilic microorganisms that include both procaryotes and eucaryotes living in habitats such as the Dead Sea tolerate concentrations of up to 20% and even die at concentrations below 10% by producing large amounts of internal solutes or by retaining external ones (Gilmour and Sephton, 2004).

1.1.1.3 Acidic and alkaline environments

The majority of life on Earth thrives in neutral pH environments due to the DNA molecule being susceptible to destruction with high or low pHs. However, acidophiles can grow optimally in deep-sea hydrothermal vents at pH 0.7 while alkaliphiles grow in carbonate-rich environments with pHs from 9 to 12 by maintaining their intracellular pH near to neutrality by pumping or excreting protons (ESA Exobiology Team Study, 1999).

1.1.1.4 High-pressure environments

Pressure forces cell membranes to have decreased membrane fluidity; therefore biochemical reactions which result in an increase in volume are inhibited. However, barophiles have been found in the deepest parts of the ocean at 1100 bar, and still the extreme pressure limit that Life can thrive in is unknown.

1.1.1.5 Subterranean life

Although it was believed that deep subterranean environments were sterile, bacteria flourish in the terrestrial crust. These are often found in subterranean oil fields and during drilling experiments that yielded microbial populations at 750m below the ocean floor (ESA Exobiology Team Study, 1999). Heterotrophic organisms (*i.e.,* organisms that need organic compounds of carbon and nitrogen for nutrition) survive in subterranean communities using either remnant organic carbon within sediments or dissolved oxygen as a metabolite and hence become extinct once their nutrients are exhausted; while procaryotes thrive in geothermal methane and brine incursions.

1.1.1.6 Survival in Space

Space is a very extreme environment with vacuum, high radiation and extreme temperature conditions. Although microorganisms have been found to survive exposure in Space vacuum for extended periods of time, they can only do so if their genetic material is protected against solar UV radiation (Horneck and Möller, 2011). Gravity also has an effect on the forces an organism experiences and hence low gravity affects cell membranes and the reproduction of microorganisms.

1.2 Planetary Bodies Targeted in the Search for Life

Life is understood to depend on liquid water, the availability of elements to form complex organic molecules (C, H, N, O, P, S, Fe, etc.) and a source of free energy to sustain metabolic processes. Therefore, planetary bodies that seem to conform to these criteria are targeted in the search for Life. Due to the importance of liquid water to harbour life, the concept of "Habitable Zone" arose. A Habitable Zone is the distance from a Star where an Earth-like planet can have liquid water on its surface. This zone is the intersection of both the Habitable Zone in a planetary system and within a galaxy, and yields the planets and moons that could harbour life. However, in the current study, only planetary bodies within our Solar System are considered.

Targets are classified into Icy bodies (comets, Europa, Ganymede and Enceladus) and Non-Icy bodies (Titan, Venus and Mars) (Sullivan, 2007).

1.2.1 The Icy Bodies

These include comets, Europa, Ganymede and Enceladus.

1.2.1.1 Comets

Comets have a substantial amount of organic content (C, H, O, and N) and even host complex molecules such as hydrogen cyanide and formaldehyde. These findings were recently corroborated with physical evidence due to the Hayabusa sample return mission launched in 2003 which returned in 2010 after studying a small near Earth asteroid named 25143 Itokawa. Other missions to asteroids and cometary coma fly-throughs are envisaged on the search for life, such as the Rosetta mission launched in 2004 to land on comet 67P/Churyumov-Gerasimenko in 2014.

Comets are a target due to the following characteristics:

- Comets are known to contain significant amounts of water ice.
- Comet Hyakutake harboured ammonia, methane, acetylene, acetonitrile and hydrogen isocyanide. Comet Hale-Bopp also was shown to contain methane, acetylene, formic acid, acetonitrile, hydrogen isocyanide, isocyanic acid, formic acid, cyanoacetylene and thioformaldehyde (Crovisier, 1998).
- Free energy can be obtained from sun light; however it is more likely that life harboured by comets is being transported rather than flourishing.

1.2.1.2 Europa

Europa is one the four large satellites of Jupiter and is nearly the size of Earth's moon. Although covered by a thick layer of ice constantly bombarded by Jupiter's radiation and exhibiting a midday temperature of around -140°C at its equator and -190°C at its poles, Europa is considered to comply with the criteria of harbouring life due to the following (Sullivan, 2007):

- Models as well as observational evidence indicate that the tidal heating created by Jupiter's gravity, sustained by Europa's orbital resonance with Io and Ganymede, maintains liquid water below its layer of ice.
- Spectroscopy has revealed Europa's surface composition to be predominantly water ice with SO_2, H_2O_2 concentrations and a tenuous O_2 atmosphere. Lower bounds of the abundance of the biogenic elements (H, N, O, P, S, Fe, etc) can be assumed due to the evidence of cometary impacts on Europa's surface.

- Hydrothermal activity in Europa's subsurface ocean and/or organics and oxidants provided by the radiation chemistry on Europa's surface could supply the free energy needed to drive biological processes.

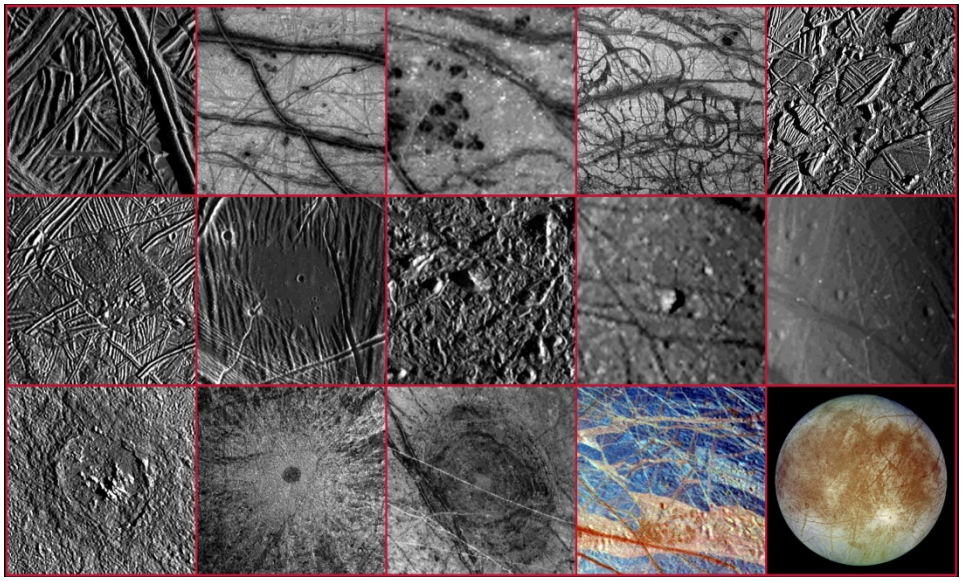


Figure 1-1: *Europa Surface Photos Obtained by the Galileo Spacecraft (NASA/JPL/DLR, 1998).*

1.2.1.3 Ganymede

Ganymede, one of Jupiter's satellites, is the largest moon in the Solar System. It exhibits a strong internal magnetic field which blocks most of the plasma from Jupiter bombarding its equatorial regions by funnelling it to its polar regions (Khurana *et al.,* 2007), has an iron-rich core, and its surface (exhibiting temperatures down to -203°C) is composed of silicate rock and water ice.

- An underground saltwater ocean is believed to exist 200km sandwiched between layers of ice (NASA, 2000).
- A tenuous oxygen atmosphere similar to Europa's exists, and although the exact chemical composition of its rock is unknown, ammonia is thought to exist as volatile ice.
- Ganymede, like Europa, is also subjected to tidal heating.

1.2.1.4 Enceladus

Enceladus, the sixth largest moon of Saturn, has a mean midday surface temperature of -198°C due to it high albedo of 0.99. It is emerging to be a very desirable target for astrobiology exploration due to the following:

- Enceladus was discovered to be venting water rich plumes into Space by the Cassini spacecraft in 2005. These Enceladean plumes are believed to be outgassing from an underground source of liquid water.
- CO_2, NH_3, deuterium, argon and potassium and sodium salts were also detected in the plume as well as complex organics such as benzene (Waite, 2009).
- Enceladus is currently geologically active (possibly from tidal heating) and so exhibits a free energy source.

Figure 1-2: *Enceladean Plumes (NASA-Cassini Mission, 2011).*

1.2.2 The Non-Icy Bodies

The Non-Icy bodies that are targeted for the search of life outside Earth are Titan, Venus and Mars.

1.2.2.1 Titan

Titan is the largest moon of Saturn primarily composed of water ice and rocky material. It receives 1% of sunlight compared to Earth and exhibits a surface temperature of -179°C.

It is considered a target for the following reasons:

- Although Titan's surface is too cold to support liquid water or water/ammonia mixtures, it has a dense atmosphere that is in an oxidation-reduction state resembling that of early Earth's prior to having oxygen.
- The compounds found in Titan's dense atmosphere include methane and nitrogen, which would create amino acids and carboxylic acids with the presence of oxygen. Reactions with liquid water (a liquid ammonia-water layer is speculated to exist in Titan's interior that could be exposed after impacts) could provide this oxygen (Grasset et al., 2000).
- Impacts and possible volcanism on the surface provide energy. Also, energy stored in the bonds of unsaturated hydrocarbons formed in its Stratosphere (such as acetylene) could be released and become available for other chemistry.

1.2.2.2 Venus

Like Mars, Venus has also had a wealth of missions directed to study its geological origin as well as potential for life. However, due to its extreme surface temperatures (up to 464°C), permanent thick clouds of H_2SO_4 and elevated surface pressure (92 times that of Earth), life is considered to only be able to survive in the higher layers of its atmosphere, *i.e.,* between 50km to 60km.

Venus is considered a target as:

- Although water concentrations are currently very low in the atmosphere and crust, researchers speculate that Life could have emerged on liquid water on the surface before it was lost and hence migrated to the global cloud layer (Grinspoon and Bullock, 2007).
- C, H, O, N, S, P, Cl, F and Fe have found to exist in the cloud region of Venus and I, Br, Al, Se, Te, Hg, Pb, Al, Sb and As are suspected to be present. Therefore, an array of elements exist that would aid Life.
- Venus currently hosts active geological and meteorological processes otherwise only found on Earth. Its active volcanism would provide the free energy needed for biological processes.

1.2.2.3 Mars

Mars is the fourth planet from the Sun. It has no magnetosphere, two permanent polar water ice caps and experiences -87°C during its polar winters and -5°C in its summers. Also, due to its thin CO_2 atmosphere, its surface is exposed to UV light and believed to be sterile. However, it is believed life could exist below the surface, shielded from the radiation and thriving on sub-surface water even if the Viking spacecraft experiments in the 1970s apparently found no organics in the Martian soil -note that this is believed to have been a false negative due to a misinterpretation of results from the control *vs* the sample response (Biancardi *et al*, 2012). The following conditions make Mars a target:

- NASA's Mars Phoenix Lander confirmed the existence of water ice in a soil sample the 31st of July 2008, (The University of Arizona, 2008) and, in August 2011, NASA's Mars Reconnaissance Orbiter (NASA, 2011) revealed possible water flowing during the summer months.
- H and O are present in water and C and O in the atmospheric CO_2 making up 95% of the atmosphere. Nitrogen is also present in the atmosphere at 3%; while 1.6% is argon and 0.4% is water and oxygen (Williams, 2004). Sulphur, phosphorus, calcium, iron, etc can also be found in its crust.
- Sunlight is abundant everywhere on the surface (although half as strong as on Earth); also the energy available from hydrothermal systems and chemical weathering reactions could have allowed for microbial life (Sullivan, 2007).

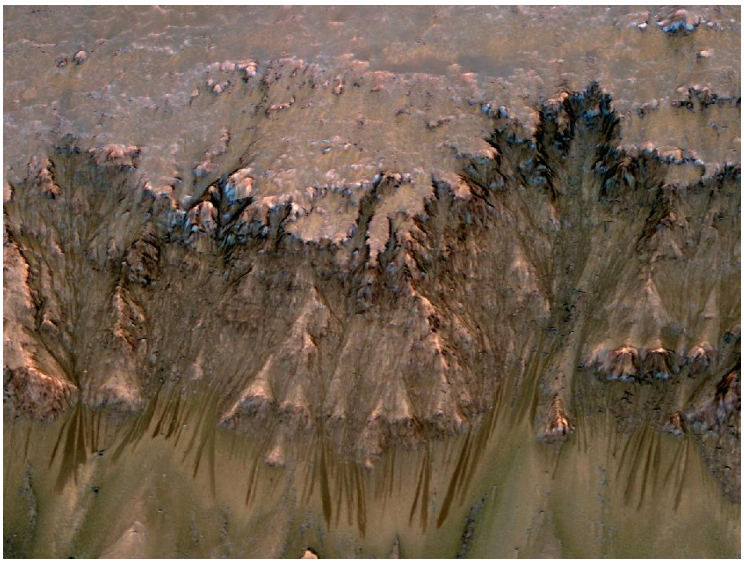


Figure 1-3: *Oblique View of Warm Seasonal Flows in Newton Crater (NASA, 2011).*

1.3 Life Detection in Planetary Exploration Missions

The Viking landers carried out the first attempt at searching for life outside Earth, and although unsuccessful, they spurred on the scientific community to push the limits of technological advances for more reliable, more robust and more efficient methods to conclusively ascertain the presence of microorganisms in other planetary bodies.

A number of missions have been suggested over the next decades to the most promising astrobiological targets, *i.e.,* Mars, Titan and Europa, which will simultaneously investigate parallel questions regarding the origin and chemical evolution of life on Earth. Future missions to planetary bodies require a low-mass, low-power instrument to conduct chemical/biological analysis on a moving platform in real-time.

Thus far, no sample return has been achieved, except from the Moon. Only Mars and the Moon has had rover missions while landers have been more common (achieved in the Moon, Mars, Venus, Titan and comets). Mars has been the most explored due to its relative proximity to Earth and more benign conditions than Venus.

Table 1–1: *Exploration Milestones.*

Planetary Body	Fly-by	Orbiter	Lander	Rover	Sample Return
MARS	X	X	X	X	-
VENUS	X	X	X	-	-
TITAN	X	X	X	-	-
ENCELADUS	X	-	-	-	-
COMETS	X	X	X	-	-
EUROPA	X	-	-	-	-
MOON	X	X	X	X	X

1.3.1 Life Detection Platforms in Planetary Exploration Platforms

Human exploration has thus far only reached the Moon; therefore we rely on rovers, minibots and aerobots for the exploration of other planetary bodies.

The Space Industry classifies rovers as macro rovers (around 1 tonne), mini rovers (around 200kg), micro rovers (less than 10kg) and nano rovers (10-100s of grams). Even though mini rovers are the current type of rover exploring Mars, distributed miniaturised systems would consider the micro and the nano rovers as candidates for the system. This is due to the possibility engineers would have of forming clusters of them to efficiently explore wider areas.

Airplanes and balloons can achieve a higher resolution than the orbiters while also covering larger areas than the rovers. There are several types: lighter-than-air concepts such as different balloon models (Montgolfier, zero pressure, super pressure and reverse fluid) as well as airships (blimps) and heavier-than-air concepts including airplanes, gliders and rotorcrafts in addition to vertical take-off (VTOL) landing concepts. Other novel concepts being researched include those based on a tumbleweed design. These are essentially an inflatable ball of several meters in diameter with a mass no larger than tens of kilograms in order to operate in Mars' low density atmosphere. This autonomous rover would move by using Mars' natural winds and it would deflate temporarily to be able to remain stationary to allow its instruments to take readings (Antol et al., 2006).

1.3.2 Defining Biomarkers

Biomarkers are molecular indicators of a diverse range of biological processes and their detection represents a key approach to understanding and diagnosing disease as well as providing a means to understand basic biological processes. They are particularly useful for Astrobiology, as biomarkers help to answer questions concerning Life in the Universe by indicating the presence of current or past Life as well as detection of molecular markers associated with pre-biotic processes.

Parnell *et al.* (2007), state that in 1996 the ESA's Microgravity Directorate listed the following criteria to test for extant or fossil life:

- Presence of water.
- Derived inorganic minerals (*e.g.*, carbonate).
- Carbonaceous debris.
- Organic matter with a complex structure.
- Chirality.
- Isotopic fractionation between reservoirs.

Therefore, biomarkers that indicate the presence of the above are needed to understand the presence of extant or fossil life.

For extant life, the following specific evidence is needed (ESA Exobiology Team Study, 1999):

- Structural: by observation of cells and subcellular structures.
- Culture: by detection of biomarkers indicating successful culturing (*e.g.,* nucleic acid sequencing and protein, lipid and sugar content).
- Metabolic: by detection of the products of metabolism (*e.g.,* gases).
- Isotopic: by discriminating against ^{13}C relative to ^{12}C.
- Chirality: by optical detection of homochirality (needed for polymerisation and replication).
- Spectral: by observing organic compounds.

For extinct life, the following specific evidence is needed (ESA Exobiology Team Study, 1999):

- Structural: by observation of microfossil structures.
- Biogeochemical: by quantifying carbon remnants.
- Isotopic.
- Molecular: by detecting biochemical compounds that withstand degradation (*e.g.,* lipid type and pigments).
- Chirality.
- Spectral.

1.3.3 *In situ vs* Sample Return Missions

Detecting key biomarkers in environmental samples from astrobiology targets such as Martian regolith and ice, possible water from Europa, Enceladean plumes and Titan's hydrocarbon lakes at levels of less than 1 part per trillion, presents significant technological challenges. These challenges are dealt with routinely using high sensitivity lab-based methods such as mass spectrometry, yet this would involve bringing the sample back to Earth to be analysed, *i.e.*, a sample return mission. These type of missions, although highly desired for the flexibility and sensitivity they would allow by placing the sample in a fully equipped laboratory, have a considerable amount of cost and risk associated to them compared to *in situ* missions, where the equipment needs to function autonomously under the extreme conditions of another planet but without compromising the sample in any of the complete failure scenarios presented with sample return (as for example during re-entry into the Earth's atmosphere).

However, a key technology challenge for *in situ* instruments is to implement the required levels of analytical performance with spaceflight compatible technologies that have the following typical mission requirements: low mass, low volume, low power requirements, low communication requirements, high automation ability and high radiation, vibration and shock tolerance.

1.3.3.1 Difficulty in bringing lab reliability to field portable instrumentation

By looking at different aspects of an extraterrestrial sample, complementary techniques will lead to an understanding of the history (geological, chemical, biological, etc.) as complete as possible.

The automated detection and unique identification of organic molecules with *in situ* platforms is very difficult. Traditionally, material has to be brought into a chamber that is sealed and heated to elevated temperatures to volatilise organic material. In Gas Chromatography/Mass Spectroscopy (GC-MS), the volatile molecules are then fed into a GC column where molecular separation occurs. From there they are introduced into a high vacuum environment where they are separated and detected based upon their mass to charge ratios. If a calibrated GC column is used, some structural information may be inferred by the retention time inside the column, otherwise just mass to charge information is generated. This is a very complicated process, which can take a majority of the available resources of an *in situ* mission.

For example, organic analyses of near surface material will be of great importance in the astrobiological exploration of Mars; therefore strategies are being developed to pursue exobiological objectives for Mars exploration such as determining the abundance and distribution of biogenic elements and organic compounds, detecting evidence of ancient biota and determining whether indigenous organisms exist (or existed) anywhere on Mars. On future Mars missions, samples will be identified, acquired and analysed by instrumentation to determine if they contain signs of biological activity (*i.e.* biosignatures).

1.3.4 Top Level Life Detection Requirements

In order to be able to detect life in planetary exploration missions, the instrumentation must comply with the requirements set in the table below.

Table 1–2: *Top Level Life Detection Requirements.*

Reference	Requirement
TLR1	Be able to detect astrobiology biomarkers.
TLR2	Be robust enough to withstand the extreme pressures, temperatures and radiation environments encountered on other planetary bodies.
TLR3	Must implement Planetary Protection and Contamination Control (PP&CC) requirements as per COSPAR (2005) regulations in order to avoid contamination of other planetary bodies (as well as to increase confidence in the detection of extra-terrestrial life by minimising the contamination of terrestrial life on the instrumentation).

Moreover, COSPAR (2005) regulations categorise missions according to their potential contamination threat to other planetary bodies and the Earth itself.

- **Category I:**
  - Description: any mission to a target body of no interest for understanding the process of chemical evolution or the origin of life.
  - Requirements: none.
- **Category II:**
  - Description: all types of missions to target bodies where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote chance that contamination carried by a spacecraft could jeopardise future exploration.
  - Requirements: simple documentation only. Preparation of a short planetary protection plan to outline intended or potential impact targets, brief Pre- and Post-launch analyses detailing impact strategies, and a Post-encounter and End-of-Mission Report which provides the location of impact if such an event occurs.
- **Category III:**
  - Description: missions (mostly fly-by and orbiter) to a target body of chemical evolution and/or origin of life interest or for which scientific opinion provides a significant chance of contamination which could jeopardise a future biological experiment.
  - Requirements: documentation (more involved than Category II) and some implementing procedures, including trajectory biasing, the use of cleanrooms during spacecraft assembly and testing, and possibly bioburden reduction. Although no impact is intended for Category III missions, an inventory of bulk constituent organics is required if the probability of impact is significant.

- **Category IV:**
  - Description: missions (mostly probe and lander) to a target body of chemical evolution and/or origin of life interest or for which scientific opinion provides a significant chance of contamination which could jeopardise future biological experiments.
  - Requirements: detailed documentation (more involved than Category III), including a bioassay to enumerate the bioburden, a probability of contamination analysis, an inventory of the bulk constituent organics and an increased number of implementing procedures. The implementing procedures required may include trajectory biasing, cleanrooms, bioload reduction, possible partial sterilisation of the direct contact hardware and a bioshield for that hardware. Generally, the requirements and compliance are similar to Viking, with the exception of complete lander/probe sterilisation.
- **Category V:**
  - Description: all Earth-return missions.
  - Requirements: As the concern for these missions is the protection of the terrestrial system, the Earth and the Moon. (The Moon must be protected from back contamination to retain freedom from planetary protection requirements on Earth-Moon travel.)
    - For solar system bodies deemed by scientific opinion to have no indigenous life forms, a subcategory “unrestricted Earth return” is defined. Missions in this subcategory have planetary protection requirements on the outbound phase only, corresponding to the category of that phase (typically Category I or II).
    - For all other Category V missions, in a subcategory defined as “restricted Earth return,” the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any un-sterilised sample collected and returned to Earth. Post-mission, there is a need to conduct timely analyses of any un-sterilised sample collected and returned to Earth, under strict containment, and using the most sensitive techniques. If any sign of the existence of a non-terrestrial replicating entity is found, the returned sample must remain contained unless treated by an effective sterilising procedure.

Therefore, the following COSPAR categories are matched to example missions:

Table 1–3: *Category-Specific Listing of Target Body/Mission Types (COSPAR, 2005).*

Category	Target Body/Mission Types
Category I	Flyby, Orbiter, Lander: Venus; Moon; Undifferentiated, metamorphosed asteroids; others TBD.
Category II	Flyby, Orbiter, Lander: Comets; Carbonaceous Chondrite Asteroids; Jupiter; Saturn; Uranus; Neptune; Pluto/Charon; Kuiper-Belt Objects; others TBD.
Category III	Flyby, Orbiters: Mars; Europa; others TBD.
Category IV	Lander Missions: Mars; Europa; others TBD.
Category V	Any Earth-return mission. “Restricted Earth return”: Mars; Europa; others TBD; “Unrestricted Earth return”: Moon; others TBD.

1.4 Aims and Objectives of the Research

The aim of the research documented in this thesis was to explore issues associated with the development of instrumentation for life detection and characterisation in a planetary exploration context.

Within this aim, the following objectives had to be achieved:

1. To consider current and near-future single molecule detection (ultra-low lower limit of detection) analytical techniques that would be compatible with development into a Space qualifiable *in situ* analytical instrument for the detection of biomarkers in a planetary exploration context.
2. To practically consider the consequences of Planetary Protection and Contamination Control (PP&CC) on the development of a sample return instrumentation in a planetary exploration context.
3. To consider the implications of flying an *in situ* instrument on-board a stratospheric balloon platform in order to apply them into a specific planetary exploration mission:

In order to achieve the objectives described above, the following work was pursued:

- A desk-based European Space Agency (ESA) study was carried out which entailed producing a literature review on single molecule detection technologies that had to be validated by the expert community. This was done by organising an International Workshop on Single Molecule Detection Technologies for Space Applications in March 2009 at Cranfield University, UK. The approved technologies then had to be analysed with standard analytical techniques (*i.e.*, trade-offs) in order to propose a specific technology for development and present its breadboard implementation and test plans at the end of the study.
- A sample return experiment implementing PP&CC constraints and protocols was designed, built, tested and flown on-board the ESA, Swedish Space Corporation (SSC), Swedish National Space Board (SNSB) and German Space Agency (DLR) BEXUS stratospheric balloon platform. The biological and engineering results obtained from the sample return flight were then analysed and lessons learnt obtained for future flights.
- Another desk-based study was performed to research future stratospheric balloon platforms for the exploration of Venus' cloud layer. The *in situ* instrument previously proposed for the detection of biomarkers for planetary exploration missions was then put forward as a possible payload for a Venusian stratospheric balloon platform and approved by experts during the Venus Exploration Analysis Group (VEXAG) conference held in August 2011 in Washington D.C, USA.

1.5 Thesis Structure

The thesis is divided into five main chapters. They are described as follows:

1.5.1 Chapter 1

Chapter 1: “Introduction to Life Detection Missions” introduces the overall research theme of the thesis by giving an overview on the search for life in extreme environments and the history on exploring other planetary bodies. It also defines the challenges and constraints imposed by planetary exploration missions and how scientists and engineers have strived to attain meaningful science while working under extremely hostile conditions.

This chapter also describes the research’s aims and objectives as well as the thesis structure of the thesis.

1.5.2 Chapter 2

Chapter 2: “Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions” describes a desk-based European Space Agency (ESA) study to review emerging single molecule detection (ultra-low lower limit of detection) technologies. As this research was conducted as part of an industrial study a lot of information was collected, hence calling for a longer chapter than usually expected in a PhD thesis.

In this chapter, the technologies are analysed as part of a trade-off study which was peer-reviewed by the scientific community and a breadboard implementation of the proposed technology as well as a test plan is presented.

1.5.3 Chapter 3

Chapter 3: “Design, Implementation, Testing and Validation of a Sample Return Instrument for a Stratospheric Balloon Mission Instrument for Life Detection” describes the design process, implementation, test and build of a microbiological sample return experiment built by a student team of engineers and biologists from Cranfield University, UK which the author of this thesis lead in order to implement PP&CC constraints and protocols into a sample return experiment.

The experiment was then validated and flown on-board the European Space Agency (ESA), Swedish Space Corporation (SSC), Swedish National Space Board (SNSB) and the German Space Agency (DLR) Balloon Experiment for University Students (BEXUS) stratospheric balloon platform and its samples and performance analysed in order to put forward lessons learnt for future missions.

1.5.4 Chapter 4

Chapter 4 “An *In Situ* Ultra-Low Lower Limit of Detection Instrument for a Stratospheric Balloon Mission Searching for Life in Venus” is considerably shorter than Chapter 2 and Chapter 3 as it extracts the outputs of these chapters in order to explore the possibility of integrating the instrument proposed in Chapter 2 with the type of platform studied in Chapter 3 and propose an instrument for a future life detection mission to Venus’ cloud layer.

1.5.5 Chapter 5

Chapter 5 “Final Discussion, Conclusions and Future Work” explores the conclusions derived from the results obtained from the work described in chapters 2, 3 and 4.

1.5.6 Appendices

Appendix A describes the methodology of the review conducted for the study described in Chapter 2 as well as complementary results. Appendix B details the Risk Register of the experiment described in Chapter 3 and Appendix C its Planetary Protection and Contamination Control protocols.

Appendix D also includes the checklists required for the launch of the experiment detailed in Chapter 3 and Appendix E includes the designs of the circuits designed and built by the author of the thesis as part of her role as the Electronic Engineer in the team she lead to design, build and fly the experiment on-board a stratospheric balloon.

2. Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions

The aim of this chapter is to consider current and near-future single molecule detection (ultra-low lower limit of detection) analytical techniques that would be compatible with development into a Space qualifiable *in situ* analytical instrument for the detection of biomarkers in a planetary exploration context.

2.1 Introduction

Single molecule (ultra-low lower limit of detection) research emerged through a variety of drivers including the desire to study biological processes and reactions which occur via the action of single or low numbers of molecules such as single enzymes, nucleic acid molecules or cell-signalling molecules; and the ability to achieve ultra-low level detection of molecular analytes. The ability to observe, and where appropriate count, these molecules one at a time provides a means to address a wide variety of scientific questions at a new level of detail and within a different regime compared to previous analytical techniques. Designing experiments and techniques to analyse the properties of isolated or distinguished single molecules has occurred since the 1970's and with an increased level of interest and activity over recent years.

The breadth of interest in single molecule detection studies is highlighted by the variety of public funding sources for such research. These include: health agencies such as the US National Institutes of Health that pursue the quick diagnosis of illnesses, governmental agencies such as the US National Science Foundation that are concerned with bioterrorism, and research and development agencies that wish to further the field such as the current European Commission Framework programme (Framework 7). Also, annual conferences around the world such as the EuroNanoForum (2011) and workshops such as those hosted by the National Institute of Medical General Sciences (2011) demonstrate the ongoing effort to develop and enhance single molecule detection techniques.

Ultra-low lower limit of detection technologies have the potential to impact significantly on the technological and scientific requirements of future Space missions as ultra-low levels of detection are necessary for astrobiology missions. Moreover, searching for life in extreme environments is especially challenging due to the harsh environmental conditions of temperature, radiation and pressure. Therefore, this chapter describes how a trade-off study was performed to propose the most suitable technology to be used in the demanding conditions of Space exploration in search for life.

2.1.1 Science and Technology Background

Biomarkers are molecular indicators of a diverse range of biological processes and questions; therefore their detection is particularly useful in the rapidly expanding areas of biosciences, medical and environmental sectors. For the biosciences and medical area, the detection of biomarkers represents a key approach to understanding and diagnosing disease as well as providing a means to understand basic biological processes. Meanwhile, used in an environmental context, the detection of biomarkers provides understanding of the presence and interaction of biology with various environments -including studies concerning pollution, microbial ecology and climate change.

Within the Space sector, both contexts are to be found; within the biosciences field, understanding of Earth Life in Space environments is vital and it requires experimental procedures to understand its interaction with Space environments (including microgravity and radiation) as well as to understand and monitor the well-being of astronauts during long duration missions. These studies

can be reduced to the technical challenge of detecting trace levels of key biomarkers in biological cells and cell cultures.

In order to better understand questions concerning Life in the Solar System and beyond, the technical challenge of detecting trace levels of biomarkers in environmental samples from cometary coma, Martian regolith, rocks and ice, possible water from Europa, Enceladean plumes, Venusian clouds and Titan's hydrocarbon lakes is key to further our understanding of how Life can evolve in the Universe. These samples could be analysed to indicate the presence of current or past Life as well as detection of molecular markers associated with pre-biotic processes.

The detection of trace levels of key biomarkers in biological cells, cell cultures and astrobiology samples is a complex technical challenge as, for many of the above examples, the concentration of the target molecules is at levels of less than 1 part per trillion, *i.e.*, approximately 10^6 molecules of a 500 $g.mol^{-1}$ molecular weight biomarker per milligram of sample. For cell biology studies, this concentration can equate to only one to two molecules per typical mammalian cell (volume assumed 10^{-12} litres).

Therefore, a key technology challenge is to implement the required levels of analytical performance in instruments and experiments compatible with spaceflight and hence compatible with key constraints that include: low mass, low volume, low power requirements, low communication requirements, high automation ability and high radiation, vibration and shock tolerance. Moreover, the *in situ* detection of extraterrestrial biomarkers is particularly challenging due to the typical mission requirements compared to typical cell biology questions that often comprise low Earth orbit manned or un-manned missions.

2.2 Aims, Objectives, Requirements and Targets for a Single Molecule Life Detection Instrument

This research was conducted as part of an ESA funded desk based study.

2.2.1 Aims and Objectives

As stated in the European Space Agency (14/05/2008) Statement of Work, the aim of the research was: "to produce an extensive review and assessment of the current scientific and technological developments in probes, optics and image informatics used for biomarker research (sensing, identifying and quantifying) on a single molecular level".

The objectives derived from the Statement of Work (ESA, 2008) were as follows:

- Produce a literature review of single molecule detection and counting technologies.
- Validate the literature review with the scientific community by organising an International Workshop on Single Molecule Detection Technologies for Space Applications and with approximately 20 international experts.
- Analyse technologies and propose a technology that conforms to ESA's set requirements for Astrobiology missions using a standard analytical procedure such as the trade-off technique.
- Work up identified technology into an implementation plan.
- Devise a test plan.
- Design a breadboard system.
- Consider implication of findings on systems aspects of instrument builds.

2.2.2 Requirements and Targets

2.2.2.1 Requirements

The Statement of Work (ESA, 2008) specifies the following technology assessment requirements for single molecule detection technologies for Space applications:

Table 2–1: Assessment *Requirements.*

Reference	Requirement
AR1	The measurement techniques or principles shall be able to identify and count non-stochastic biomarkers.
AR2	Astrobiology biomarkers to be detected according to EXO-ESA-MO-11001 (Vago, 2005) for specific biomarkers on Mars.
AR3	Cell biology biomarkers to be detected as mediators in cell biology for cell signalling mechanisms and/or transmission of neurosignals.
AR4	The selected technologies shall be able to detect, identify and count at least one representative type of biomarkers in the area of cell biology and at least one in the area of exobiology.

It also states the formal design requirements that the proposed technology has to comprise with have to be as follows:

Table 2–2: *Design Requirements.*

Reference	Requirement
DR1	Be able to detect and recognise biomarkers.
DR2	Include selected technologies that shall be compact and integrate as much as possible in order to become part of a system.
DR3	Be able to detect concentrations in a deterministic (module counting) manner.
DR4	Sample preparation and use of reagents shall be minimal.
DR5	Be able to reach at least part per trillion (design aim) level sensitivity.
DR6	Have a mass of less than 5kg.

And the following operation requirement:

Table 2–3: *Operation Requirement.*

Reference	Requirement
OR1	To be operational in human, autonomous and tele-operated modes.

2.2.2.2 Astrobiology and cell biology targets

From the technology assessment requirement AR4: “Astrobiology biomarkers to be detected according to EXO-ESA-MO-11001 (Vago, 2005) for specific biomarkers on Mars”, the targets relevant to the study were derived. These included a very broad range of molecule types encompassing inorganic ions such as calcium, amino acids, small apolar molecules, peptides through to macromolecules and proteins. Therefore, the selected instrument had to be able to detect a wide range of molecular weights and other physical and chemical characteristics.

Examples of astrobiology targets include:

- Life building blocks or traces of life (past or present), such as amino-acids and their chirality, nucleobases and sugars.
- Molecules from metabolic pathways.
- Presence of functioning metabolic pathways.
- Chemical fossils, building blocks such as, but not only, hopanes, sterane, porphyrins, chlorophyll, and similar pigments.
- Molecules that could be building blocks of life forms not recognisable by the classical biochemistry, immunochemistry or nucleic acid methods currently used on Earth -for example deviations from the expected patterns of decreasing occurrence with increased complexity in homologous series of organic molecules.

Examples of cell biology targets relevant to cell signalling mechanisms and transmission of neurosignals include:

- For neurotransmitters molecules such as glutamate, GABA, and other amino acid based signal molecules.
- G-protein coupled signal molecules such as dopamine, serotonin, inositol triphosphate (IP3) and inositol tetrakisphosphate (IP4).
- Calcium ions and related signalling molecules -cAMP.
- Neuropeptides such as neurotensin, β-endorphin, somatostatin.
- Receptor proteins such as acetylcholine receptors, glutamate receptors.

An important context for the study was the consideration of the sample matrix in which the astrobiology and cell biology biomarkers would be detected. Therefore, scenarios considered for astrobiology biomarkers included sampling of Martian regolith (or ground rock and possible ice), cometary coma, European ice (and possible water), Enceladean plumes, Venusian clouds and samples from Titan’s hydrocarbon lakes. Also, the material under study would normally be time invariant during a mission scenario and therefore high temporal resolution would not be required of the analytical technique.

However, for cell biology targets, it was assumed that their final applications would be in relatively benign environments comprising manned spaceflight or un-manned micro-gravity platforms. Therefore, the sample matrix was assumed to be cell material (including single cells) and cell culture media. Also, cell signalling biomarker concentrations can vary on various timescales during the course of a mission (from seconds to hours) and therefore high temporal resolution had to be required of the analytical technique.

2.3 Literature Review of Single Molecule Detection and Counting Technologies

Single molecule research is needed in order to be able to study the individual behaviour of distinct molecules hidden in ensembles and when concentrations are so low that single molecule detection level is required. At low concentrations detection efficiency is a challenge and there are other issues to consider such as background noise and spurious signals.

The various types of single molecule detection and counting techniques can be divided into direct and indirect detection types: the direct measurement of a biomarker can be achieved by detecting an inherent physical property such as its electrical characteristics, an spectroscopic feature, the mass or mobility properties; whereas the indirect measurement of a biomarker is achieved by detecting molecular recognition events and using added reagents (labelled) or not (labeless). All these techniques allow us to count, image, identify, manipulate, modify, move, switch, sort, and trace single atoms, molecules and nanoparticles (Basché, 2001).

As the review of technologies had to be presented to ESA and be peer-reviewed by experts during the International Workshop required by ESA, each identified technique was presented with a brief description as well as a preliminary indication of its use in order to suitably understand its development and heritage.

2.3.1 Direct Measurement of Inherent Physical Properties

There are various techniques that directly measure inherent physical properties: optical, electrical and mechanical. These techniques do not introduce a labelled component.

2.3.1.1 Optical techniques

These methods include optical traps, optical microcavities and optical nanofibres.

Raman techniques

Raman spectroscopy is a spectroscopic technique used in condensed matter physics and chemistry to study vibrational, rotational, and other low-frequency modes in a system. It relies on inelastic scattering (Raman scattering) of monochromatic light, usually from a laser in the visible, near infrared or near ultraviolet range illuminating a sample to obtain Raman signals (the nuclear vibrations of chemical bonds). The laser light interacts with phonons (vibrational motions) or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. This shift in energy gives information about the phonon modes in the system therefore helping to identify the sample.

Raman scattering is an inherently weak process with 1 in 10^6 photons being Raman scattered. However, the benefit of Raman scattering is that some molecules scatter more strongly than others and as such can be seen in the presence of weak scatterers such as water. Also, the intensity of the Raman scattered light can be magnified by attaching the target molecules onto a roughened metallic surface typically made of silver or gold. Hence, enhanced versions of this technique are used.

Surface Enhanced Raman Scattering (SERS)

Surface Enhanced Raman Scattering is a surface sensitive technique that results in the enhancement of Raman scattering by molecules absorbed on rough metal surfaces such as silver or gold. Surface plasmons of silver and gold are excited by the laser, which results in an increase in the electric field surrounding the metal (Camden *et al.,* 2008) (Costa *et al*., 2006) (Dieringer *et al*., 2008) (Zhang *et al.,* 2009).

The enhancement factor of the measured signal can be as much as 10^{14} to 10^{15}, which allows the technique to be sensitive enough to detect single molecules, (Schmidt *et al.,* 2004) (Peron *et al.,* 2009) (Etchegoin *et al.,* 2009).

Surface Enhanced Resonance Raman Spectroscopy (SERRS)

In Resonant Raman Spectroscopy, the excitation wavelength is matched to an electronic transition of the molecule or crystal so that vibrational modes associated with the excited electronic state are greatly enhanced . This is useful for studying large molecules which might show hundreds of bands in typical Raman spectra. Surface Enhanced Resonance Raman Spectroscopy (SERRS) is a combination of Surface Enhanced Raman Spectroscopy and Resonant Raman Spectroscopy. It uses proximity to a surface to increase Raman intensity, as well as an excitation wavelength which is matched to the maximum absorbance of the molecule being analysed in order to enhance the signal (Pieczonka *et al.,* 2009).

Therefore, SERRS is an advancement on the basic Raman Effect that just provides a vibrational spectrum of a molecule by measuring the difference in energy of scattered light from that of the incident light. This gives SERRS significant advantage over SERS for practical applications and in particular for biological studies.

Coherent AntiStokes Raman Scattering (CARS)

Coherent AntiStokes Raman Scattering allows identification of molecules down to nanomolar concentrations. Unlike Raman Spectroscopy, CARS employs multiple photons to address the molecular vibrations. The resulting signal is formed by the emitted waves which are coherent with one another. Therefore, CARS is orders of magnitude stronger than spontaneous Raman emission.

CARS uses three laser beams (a Pump beam, a Stokes beam and a Probe beam), which interact with the sample and generate a coherent optical signal at the Anti-Stoke frequency (Lütgens *et al,* 2012). For more complex molecules, bespoke light pulses allow detection of specific molecules while suppressing background signals. Broadband CARS is setup with shaped light pulses that probe multiple vibrational transitions simultaneously to enhance sensitivity.

Even though CARS can generate a stronger signal than ordinary Raman scattering, its sensitivity is lower than that of SERS and has not yet achieved single molecule level. However, by combining Surface enhancement (SE) techniques using metal nanoparticle structures coupled with CARS (SE-CARS) single molecule sensitivity can be achieved. This is because coupling emitters to metallic nanostructures confines the light, which in turn induces large absorption and diffusion cross sections and enhances local electromagnetic fields. Therefore, SERS and CARS are being combined into Surface Enhanced Coherent Raman Scattering (SECARS).

Optical Traps

Optical traps, also known as optical tweezers, can be used to apply force and small movements using a tightly focused laser light. This was demonstrated for the first time by Ashkin *et al.* in 1986.

There are two techniques: the single-beam optical trap or the dual-beam optical trap with a few nanometres resolution, as well as the subnanometre resolution dual-trap that allows manipulation of molecules. An optical trap consists of a laser that is strongly focused through a lens with a very short focal length (*i.e.,* a microscope objective with a high numerical aperture). Laser light entering an object is refracted and, due to the momentum of light, it exerts forces on the object and traps it. Optical traps used for the study of molecular motors have a spring constant or trap stiffness of 0.02-0.06pN/nm. Stronger traps, of up to 1pN/nm have also been designed by Smith *et al.* (1996).

Optical traps are frequently used with imaging techniques such as Single Molecule Fluorescence in order to investigate molecular motors and nucleic acids.

Optical traps can also detect forces by attaching the macromolecule to a bead and a substrate. The light force acting on the bead is then used to translate it against a force generated by the molecule.

Optical Nanofibres

Optical nanofibres create sensors that give a fast response, are highly sensitive and are immune to electromagnetic interference. The measurement is taken by quantifying the guiding loss of light through the nanofibres due to nanoscattering (induced by the particles). As the altered power is strongly dependent on the size and the refractive index of the nanoparticle as well as the diameter and the refractive index of the nanofibres, a high sensitivity can be achieved by measuring the transmission loss.

Nanofibres were first used for the detection of benzoapyrene tetrol and gave zeptomolar (10^{-21} molar) range sensitivity, yielding information about the nature and structure of the molecule (Wang *et al*., 2007).

2.3.1.2 Mechanical & electrical techniques

These methods include those that measure or manipulate the mass or electrical or mobility properties such as magnetic traps and force probes as well as the Atomic Force Microscope.

Force Probes/Atomic Force Microscope (AFM)

The Scanning Tunnelling Microscope invented by G. Binnig and H. Rohrer (1986) uses sharp and chemically treated tips in a sample proximity of 10^{-9} metres to measure tunnelling currents or weak mechanical forces to generate a real space image of a single atom or molecule. This technology has been widely used and a well known descendant of it is the Atomic Force Microscope.

The Atomic Force Microscope has two working modes: a scanning mode, where structural features of the molecule of interest are probed as well as monitoring of changes as a function of time or reaction; and a pulling mode, which allows measurement of protein folding and unfolding or unbinding forces and dynamics. This latter method provides detailed information about the folding or binding energy landscapes of biomolecules and complexes by clamping macromolecules between the AFM's tip and a substrate in order to determine the forces needed to stretch a single polymer chain.

Therefore, the AFM works as a force spectrometer which can probe the mechanical properties (*i.e.*, length and tension) of individual molecules. As well as being able to measure a molecule's length at subnanometre resolution and its tension at picoNewton resolution. Therefore it can be used both as an imaging tool and as a force transducer to manipulate and exert mechanical force on individual molecules. Also, even though the AFM is not as force sensitive as optical or magnetic traps, it can apply a much higher force.

Magnetic Traps

Magnetic traps, also known as magnetic tweezers, work on the principle that a magnetised bead experiences a force when it is in a magnetic field gradient. Therefore, a bead is trapped in the magnetic field gradient generated by two magnets and molecules are attached to the surface of the magnetic bead and to a glass surface. Forces range from 10^{-3} pN to more than 100pN (although the maximum value of force depends on the size of the bead). These traps do not currently have

as good a spatial and temporal resolution as achieved by optical traps; however they are able to apply torque and control the supercooling state of DNA as used by Strick et al. (1996) and do not require calibration.

Also, magnetic traps achieve a constant force due to their magnetic field being uniform, whereas the Atomic Force Microscope and optical tweezers have to rely on force-feedback control to achieve this; and as force-feedback control is a limiting technique because their working frequency is of a few kHz, it does not allow for dynamical detection of processes faster than milliseconds.

Magnetic traps have been used extensively to study the elastic and torsional properties of DNA and have revealed the essential role played by DNA‘s mechanical properties in its interactions with proteins.

2.3.2 Measurement Mediated By Molecular Recognition Events

Indirect methods include those whose measurement is mediated by molecular recognition events. They can use either added reagents to add a labelled component that results in the analytical readout event (label formats) or have no need for added assay reagents (labeless). These methods enable visualisation of processes in real time.

2.3.2.1 Labelled techniques

Labelled techniques include those that use an added reagent such as fluorophores to mark and detect specific molecules. Fluorescence occurs by repeatedly exciting the fluorophores of interest and then detecting and analysing the emitted fluorescence photons. However, due to the rate and number of photons emitted by a single fluorophore being limited, fluorescence detection at single molecule resolution needs to use high-efficiency and low-background photon detection, as well as bright fluorophores.

The first detection of single fluorophores was made at liquid helium temperature via absorption by Moerner and Kador (1989) and by fluorescence by Orrit and Bernard (1990). Later this was achieved too at room temperature by Biezig and Chichester (1993), which enabled the technology to be used in biological applications. Single molecule fluorescence uses the fluorescence of a molecule to detect information of its environment, structure and position. Protein folding kinetics, molecular transport and DNA replication have been successfully studied using fluorophores.

High sensitivity can be achieved with various forms of selection: spatial, spectral and temporal. Introducing a labelled component allows an analytical readout to attain single molecule detection by using techniques such as fluorescently labelled assays (antibody, protein receptor, nucleic acid or biomimetric systems).

Optical fluorescence microscopy is an important tool for cell biology as light can be used to non-invasively probe a sample with relatively small perturbation of the specimen. This enables a dynamical observation of the motions of internal structures in living cells.

Fluorescence Correlation Spectroscopy

In the optical domain, fluorescence emission of single molecules can be imaged by advanced optical microscopes in some condensed based environments such as scanning confocal microscopy or near-field scanning optical microscopy. At low temperatures, imaging can also be achieved by making use of a frequency selective technique that uses the fact that the sharp optical transition frequencies of doping molecules are different because of the imperfections of the environment.

Fluorescence Correlation Spectroscopy (FCS) exploits temporal fluctuations in the fluorescence emission of small numbers of molecules in an open excitation volume defined by confocal optics. Fluctuations arise from changes in the number of molecules observed as they diffuse in and out of the volume element, as well as by the fluorescence intensity. An analysis of the signal fluctuations provides information about different states and lifetimes of the molecule but care must be taken to avoid induced surface perturbations.

It is important to note that molecular studies distinguish between molecules that are immobilised or freely diffusing in solution (most studies are carried out on immobilised molecules on a surface in order to acquire a long fluorescence signal). Also, while immobilisation of RNA and DNA molecules is adequately achieved, protein immobilisation methods are still unsatisfactory.

Förster Resonance Energy Transfer (FRET)

Förster Resonance Energy Transfer describes the energy transfer between two chromophores (*i.e.,* the part of the molecule responsible for the selective light absorption that gives it a colour) as the non-radiative dipole-dipole coupling energy transfer happening between a donor and an accepting chromophore in close proximity (<10nm); hence, it quantifies distances between molecules.

FRET is a useful tool to quantify molecular dynamics such as protein-protein interactions, protein-DNA interactions, and protein conformational changes. In order to monitor the complex formation between two molecules, one of them is fluorescently labelled with a donor and the other with an acceptor. They are then mixed so that when they dissociate the donor emission is detected upon the donor excitation. However, to monitor protein conformational changes, the target protein is labelled with a donor and an acceptor at two loci; thus, when a twist or bend of the protein brings the change in the distance or relative orientation of the donor and acceptor, FRET change is observed.

FRET is being used to study living cells with fluorescently tagged Myosin in the Sarah Rice Laboratory (2009), the Rock Lab (2009), the Purcell Lab (2009) and the Spudich Lab (2009). In the Zhuang Research Laboratory (2009), single molecule fluorescence imaging and spectroscopy techniques are being used to study the interactions of proteins with nucleic acids such as DNA replication, transcription, messenger RNA editing and protein synthesis.

Total Internal Reflection Fluorescence Microscopy (TIRFM)

Total internal reflection occurs at the interface between an optically dense medium such as glass and an optically less dense medium such as an aqueous solution. At a large angle of incidence, the excitation beam reflects back into the glass and generates an evanescent wave at the interface with water. The evanescent wave has maximum intensity at the surface and exponentially decays with the distance from the interface. Only molecules that are at the TIRF surface are excited and fluoresce, while molecules in the bulk of solution, at distances larger than 100-200nm are not excited and hence do not fluoresce.

Therefore, TIRF efficiently rejects background signals from the bulk of solution and allows for single molecule detection. It is a real-time, low volume (< 0.1 µl), *in situ* technique also capable of performing parallel analysis, allowing for thousands of different assays to be microarrayed on the surface of a single TIRF slide and thousands of DNA/RNA and protein targets simultaneously detected in a small sample. Additionally, exceptional surface selectivity permits TIRF to analyse scattering and turbid samples, such as whole blood, with no or minimum sample preparation. Detection is normally carried out using a CCD camera that acquires real-time images of immobilised or slowly diffusing species that can be in live cells.

A range of patents exist within this technique, such as the patented Flow Cell System based on TIRF developed by Williams *et al.* (2009). Also, companies such as TIRF Technologies (2009) offer a broad range of TIRF and TIRF-electrochemical (TIRF-EC) systems for detection and control of biomolecular interactions such as protein-protein, receptor-ligand, protein-DNA and DNA-DNA, protein-membrane. They are also developing a hand-held device based on TIRF-EC biosensors, which are fast, highly sensitive, selective, work in real-time and in parallel with no or minimum sample preparation. This latter TIRF application, a hand-held concept, makes this technology become desirable for Space use.

Superresolution Techniques

Optical microscopy is one of the most widely used imaging methods in biomedical research. It is a powerful tool for cell, tissue and animal imaging due to its high molecular specificity, relatively fast time resolution and non-invasive imaging nature. However, the spatial resolution of far-field optical microscopy, classically limited by the diffraction of light to a few hundred nanometres, is substantially larger than typical molecular length scales in cells.

Single molecule epifluorescence microscopy achieves nanometre-scale resolution by taking advantage of the fact that the point spread function of an isolated nanoscale emitter can be fit to a precision far greater than the standard diffraction limit. Over the past few years, this technique has been used for biological experiments at room-temperature using photoactivation to control the emitting concentration of single nanoscale fluorescent labels. This is used by the PALM (Photo-Activation Localisation Microscopy), F-PALM (Fluorescence Photo-Activation Localisation Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy) techniques, used in various laboratories around the worlds such as the Ting, Moerner and Zhuang Labs (2009).

All superresolution techniques are based on the critical requirement of imaging nanometre sized single molecule emitters and on the use of an active control mechanism to produce sparse sub-ensembles. These techniques are known as Single-Molecule Active-Control Microscopy (SMACM). In SMACM experiments, structures labelled by an ensemble of photoactivatable fluorophores too dense to be imaged simultaneously are resolved over repeated cycles in each of which only a sparse subset of the fluorophores is activated. The final superresolution image is reconstituted from a superposition of single molecule images.

STORM uses photo-switchable fluorescent probes to temporally separate the otherwise spatially overlapping images of individual molecules, allowing the construction of high resolution images. Hence three-dimensional, multicolour fluorescence imaging of molecular complexes, cells, and tissues with ~20nm lateral and ~50nm axial resolutions can be achieved. This new form of fluorescence microscopy allows molecular interactions in cells and cell-cell interactions in tissues to be imaged at the nanometre scale and it is being further developed to allow molecular detection.

PALM is very similar to STORM, as it optically resolves selected subsets of photoactivatable fluorescent probes within cells at mean separations of <25 nanometres. It does this by serially photoactivating and subsequently photobleaching numerous sparse subsets of photoactivated fluorescent protein molecules. Therefore, individual molecules are localised at near molecular resolution by determining their centres of fluorescent emission via a statistical fit of their point-spread function (Patterson, 2007). The position information from all subsets is then assembled into a superresolution image, in which individual fluorescent molecules are isolated at high molecular densities.

Magnetic Bead Microassays and Nanoshells

Magnetoresistive sensors in the form of magnetic bead microassays have been used as detection components in biological devices such as high sensitivity biosensors based on magnetic labelling.

This technique involves controlling the movement of magnetically labelled biomolecules by on-chip currents and using spin valve sensors in nanopatterned surfaces or nanoshells to detect the magnetic labels (*i.e*. the biomarkers attached to their surface). Detection is performed with SERS-based detection and other advanced optical techniques such as fluorescence (Jiang, 2012).

Quantum Dot Labelling

Performing single molecule detection in living cells is difficult due to the background fluorescence provided by other macromolecules and fluorescent materials (such as porphyrins and flavins) in the intracellular environment compared to the clean conditions achieved *in vitro*. Also, even though the background noise is constant and hence it does not interfere significantly with single photons, these signals are weak and difficult to detect. Methods to avoid background noise and get clear signals include using many copies of fluorophores and using detection by immobilisation or ingeneous reduction of background fluorescence.

However, another way is to use very bright ultrasensitive Quantum Dot labels. Quantum Dots have exceptional photophysical properties (intense brightness and long photostability) that make them ideal for single molecule imaging. Therefore, they can be conjugated to molecules in order to track their motion.

Nevertheless, their large size, multivalency, membrane impermeability and the difficulty of targeting them to specific cellular proteins make them hard to use -although laboratories such as the Oak Ridge National Laboratory (2009) are currently developing new methods to use Quantum Dots that address these difficulties.

Nanobarcode

In contrast to Quantum Dots, for which fluorescence measurements are needed both to identify the particles and to quantify the analyte, the nanobarcode consists of striped metal nanoparticles that are encoded due to the difference in reflectivity of adjacent metal stripes.

Oxonica (2009) is developing an automated platform capable of providing high volume screening using metallic nanorods with built-in barcodes. Atoms of gold, platinum and silver are deposited on templates so that the resulting nanorods have distinct striped patterns of the three metals. Each distinct pattern of gold, platinum, and silver lines can be identified based on the different reflectivity of adjacent metal stripes. By forming just nine stripes of the three metals, investigators can create nearly 20,000 different barcodes. Proprietary software belonging to a company making use of nanobarcodes enables the identification of each distinct barcode using a conventional optical microscope.

The Atomic Force Fluorescence Microscope (AFFM)

A newly developed technology platform combining atomic force and optical microscopy with single molecule resolution (the atomic force fluorescence microscope, AFFM) is being used by MESA+ University of Twente (2009) to explore the limits of dip-pen nanolithography for patterning and investigating molecular assemblies.

Energy transfer and intra-molecular energy redistribution in molecular systems occur on a femtosecond time scale and so is hard to detect with single molecule sensitivity because detection of individual molecules normally relies on the detection of the background-free fluorescence, which only occurs on a nanosecond time scale. The AFFM is able to circumvent the normal time limitation by using two intense femtosecond pulses with a variable delay to study the initial redistribution of the excitation energy over the different vibrational modes within the molecule.

Nanoarrays

A nanoarray, microarray or biochip usually consists of a specifically coated glass slide that contains up to several thousand microscopically small spots with different binders. In the case of DNA microarrays or DNA chips, each of these spots holds a large amount of copies of a certain DNA sequence (*e.g*. a particular gene) which act as capture probes for the target DNA or RNA that is to be analysed. These target molecules are usually labelled by fluorescent dyes before applying them onto the DNA chip, which later allows quantifying the amount of specific binding to each probe by a fluorescence scanner.

The technique can easily be added to existing biochip applications and allows universal operation in the field of molecular diagnostics. Microarrays represent a major technological leap for detecting genes and gene defects, for gene expression analysis and for understanding gene functions.

Companies such as NanoString Technologies' nCounter detector (2009) and Fluidigm's BioMark detector (2009) use single molecule arrays as their commercialised technology. A random array format with a decoding scheme for targeted multiplex digital molecular analyses is used. The random array is generated by immobilising all amplified single molecules on a microscopy glass slide. The amplified single molecules are then identified and counted through serial hybridisations of small sets of tag probes, according to a combinatorial decoding scheme. A similar method for flow cytometry is currently being commercialized by Q-Linea (2009) for the defence industry.

Piciu *et al.,* (2005), and the Delft University Lab (2009) are working on an optical device for qualitative and quantitative detection of biomolecules that is able to perform a rapid and accurate multiplex assay in a single experiment. The detection method is based on the optical effect that appears at the transmission of light through small holes in a thin metallic film. The device will be an atto-liter titer plate with up to several millions of reaction wells per square centimetre of chip area. This high density of very small reaction chambers will provide a low cost molecular analysis device, which uses reduced amount of reagents and samples with increased efficiency of the reactions. The device will support different types of molecule recognition (*e.g.* nucleic acids and proteins), being suitable for a large spectrum of applications such as gene expression analysis, medical diagnosis (hereditary diseases, cancer detection) or treatment monitoring.

Naïve Array

A technique that could be useful for Space applications (in particular when looking for an unknown amount of an unknown target –*i.e.*, "unknown unknowns") would be the use of naïve arrays. Antigen-specific antibodies are normally isolated by subjecting them to a naïve repertoire of antibodies to alternate rounds of selection by binding the target antigen and proliferation of binding clones. This selection can then be *in vivo* (by injecting an animal to trigger an immune response) or *in vitro*. However, selective techniques that apply selective pressure often exert biases; therefore only a small portion of binding antibodies are isolated from the starting array (Holt *et al.* 2000).

On the other hand, a naïve array avoids selection problems as there is no selection step.

Surface Plasmon Resonance Spectroscopy (SPRS)

An early labeless technique was based on Surface Plasmon Resonance (SPR), which was first used by Liedberg et al. in 1983 to detect protein absorption and antibody binding to absorbed antigens (Gizeli and Lowe, 2002). This technique works by detecting the polarised light from its laser light, which is reflected in a gold film on the sensor chip and detected by a diode array position sensitive detector. It is an optical method based on the detection of changes in the refractive index at the sensor chip surface, where SPR is observed as the decrease in light intensity for a specific angle of incidence.

Therefore, by directing plane-polarised light onto the sensor chip at an angle greater than the defined critical angle and monitoring the intensity of the reflected light using a position sensitive detector, SPR can be observed as a decrease in light intensity for its specific angle of incidence - the SPR angle. The angle at which the decrease in light intensity occurs is proportional to the refractive index at the sensor chip, which is proportional to surface mass changes as explained by Raghavan and Bjorkman (1995). In other words, if large molecules are present on the immobilised ligand of the sensor chip, the angle of reflectance will be shifted compared to when no molecules are present. This allows labeless real-time detection of mass changes within a surface.

However, because the component needs to be labelled in order to be able to identify it, and as both detecting and identifying is a requisite for this study, this technology has to be regarded as a labelled technique.

SPRS technology is the basis of the Biacore (2009) biosensor chip which is currently used for a diverse range of applications such as identification of specific protein-protein interactions, mapping of binding sites and investigations of kinetic parameters for previously characterised interactions.

Quartz Crystal Microbalance (QCM)

Another conventional labeless technique is Quartz Crystal Microbalance (QCM). It is an electro-acoustic detector typically consisting of a megahertz piezoelectric quartz crystal in between two gold electrodes. It measures a mass per unit area by detecting the change in frequency that happens when a foreign material is deposited on its surface. The deposition causes an increase in the plate thickness which causes a change in the resonant frequency.

Again, because the component needs to be labelled in order to be able to identify it, and as both detecting and identifying is a requisite for this study, this technology has to be regarded as a labelled technique.

A variation of it, Quartz Crystal Balance with Dissipation Monitoring (QCM-D), is used to detect viscoelasticity of absorbed protein layers at the solid-water interface by measuring the energy loss (dissipation) of the shear movement of the crystal in the water. QCM-D drives the crystal with an A/C current at its resonant frequency and then stops, producing a damped sinusoidal wave which is a pulse assisted way of discriminating between frequency and dissipation. This technique allows simple analysis of the protein layers and gives information about the hydrodynamic conductivity of the protein layers and the surrounding water. Therefore, very small structural and orientation changes of an absorbed protein layer (as well as chemical cross-linking) can be monitored with high accuracy. Q-sense (2009) commercialises this technology for characterisation of biointerfaces.

Surface Acoustic Wave (SAW) Devices

A surface acoustic wave (SAW) is created by the conversion of electrical energy into mechanical energy by a piezoelectric transducer. The wave travels along the surface of a material with an amplitude that decays exponentially with depth into the substrate. SAWs are not particularly new, having first been explained by Lord Rayleigh in 1885. The surface acoustic mode of propagation results in Rayleigh waves, which have a longitudinal and a vertical shear component that can couple with any media in contact with the surface (Friend *et al.,* 2008). This coupling strongly affects the amplitude and velocity of the wave, allowing SAW sensors to directly sense mass and mechanical properties.

The Space and Naval Warfare System Centre presented in 2003 their Surface Acoustic Wave Resonator (SAWR) as a sensor capable of detecting and identifying single gas molecules. Traditionally, SAW gas sensing only yields the total absorbed mass gas; however, fluctuation-

enhanced SAW sensing is able to analyse the total absorbed gas mass as well as the diffusion coefficients.

Also, by using a multi-component analysis to analyse a gas mixture a single sensor can be used as a complete electronic nose.

Microcantilevers

Microcantilevers can be used as a labeless method for very sensitive, simple and inexpensive biochemical sensing in ambient and aqueous environments although they again need a labelled agent in order to identify the molecule. These cantilever-based assays perform quantitative detection of molecular binding by measuring changes in the biomass of the cantilever. They consist of silicon strips which are treated to have a capture molecule such as an antibody or a protein bound to one surface in order to detect analyte binding by either measuring the bending or the mechanical resonant frequency of the cantilever. Techniques that detect bending allow analytes to bind only on one side of the cantilever either by exposing only one side to the analytes or by treating only one side of the cantilever for binding.

Hence, once binding occurs, tensile or compressive stresses are created on the surface, causing the cantilever to bend. This phenomenon can then be detected by analysing the deflection of an optical beam or by a change of electrical resistance in a piezoelectric film on the cantilever which allows electrical excitation of the cantilevers and their vibration. Microcantilevers have been used to detect DNA hybridisation as well as various types of protein recognition by making arrays of up to 10 cantilevers which offer a method to develop compact biosensors with a simple read-out scheme.

Studies are being pursued at the Anja Boisen Lab (2009) to achieve hybrid system integration so that the sensor unit is packaged for handheld diagnostic applications.

2.3.2.2 Labeless techniques

Labeless techniques include assays that have no need of reagents so that binding to the target occurs directly. The binding of the analyte molecules to the transducer surface results in a minute change of the refractive index at the surface and this can be detected using optical techniques such as evanescent wave methods, for example a waveguide or Surface Plasmon Resonance Spectroscopy, or by reflection/interference techniques such as ellipsometry (where the complex refractive index or dielectric function of thin films are studied) as opposed to measuring the intensity of light emitted by fluorophores.

Other labeless novel techniques being developed include carbon nanowires, nanotubes, natural or bioengineered ion-channels and using optical resonators.

Carbon Nanowires/Nanotubes

Nanofibres, nanoparticles, nanopatterned surfaces, nanowires or magnetic nanoparticles have been developed with microfluidics that have nanostructures dedicated to molecular sensing. Nanosensors can form microarrays with immobilised bioreceptor probes that are selective for target analytes that are label free. Nanosensor technologies include recombinant ion-channel protein receptor concepts, molecular receptor modified nanowire conductivity sensors as well as impedance sensors and molecular receptor modified nanomechanical sensors (Wang *et al.,* 2007).

Nanowires are treated and the conductance of the nanowire changes as the target molecules bind to the nanowires. It has been used to detect single virus particles and small molecules binding to proteins. Arrays can be made with thousand of independent nanowire sensors due to the small sensor size (tens of nanometres) and lengths (micrometers).

Ion Channels/Nanopores

Nanopores (or ion channels) detect single molecules by manipulating individual unlabelled molecules. These defined nanopores of 2 to 6nm are embedded in thin insulating membranes and probe the intermittent changes in ion current flow through them. Therefore by detecting an interruption in the flow they can localise and detect the passage of individual biopolymers such as DNA, RNA and polypeptides (Tartagni, 2005). They can then reveal the biopolymer's length, sequence and orientation during translocation. The translocation dynamics also reveal structural information. Using affinity techniques based on generic antibodies can fractionate the sample and isolate different types of molecules. Conformational changes and DNA-protein interactions can also be measured by using the nanopores as force spectrometers.

Nanopores can be made out of natural proteins or fabricated holes in a thin material and electrical current through the pore can be used to detect transport of DNA and other molecules driven by electrical force.

However the use of biological channels for single molecule analysis is not effective due to the fact that the lipid membranes that they are embedded in are fragile, with a lifetime of minutes to hours and they also display self-gating; where current fluctuations are created due to conformation changes in the channel. Hence research branches off into using protein nanopores and synthetic nanopores.

Optical Microcavities

Optical microcavities are a powerful method to achieve detection of single molecules because the resonant recirculation of light within a microcavity allows the light to sample target molecules many times. Armani et al. (2005) have developed a highly specific and sensitive optical sensor based on an ultrahigh quality factor ($Q > 10^8$) whispering-gallery microcavity. The resonant recirculation of light within a microcavity allows the light to sample target molecules more times than in a simple optical waveguide sensor (where the input light only has one opportunity to interact with the target molecule). By using the planar microcavity developed, a molecule is sampled more than 100,000 times. The silica surface of the resonator is functionalised to recognise the target molecule in order to detect the unlabelled target molecules by observing shifts in the resonant frequency.

2.3.3 Literature Review Conclusion

Demonstration, development and use of single molecule detection technologies have proliferated over recent years and a wide and diverse variety exist, ranging from the direct measurement of optical, mechanical or electrical properties to the detection of molecular recognition events both by using labelled components or using labeless technologies.

The techniques identified in this section can be categorised into evolutionary versus revolutionary techniques in order to further analyse them for Space application contexts. Evolutionary techniques include those that are evolutions of traditional analytical techniques and that with development of enabling technologies have become single molecule detection methods (*e.g*. fluorescence-based confocal systems). Whereas revolutionary techniques are those representing novel approaches and have no significant analytical heritage. Typically the revolutionary techniques are methods of detection with low maturity, (*e.g.* recombinant ion-channel receptors, receptor modified nanowires and some nanomechanical systems).

Suitability for miniaturisation is a key characteristic for possible Space applications, where low mass and volume are key constraints. Methods that rely upon miniaturisation of enabling components, such as many spectroscopic systems and mass spectrometers, are potentially less suitable than those that fundamentally operate in the microscopic and smaller domains, such as

nanomechanical sensors. However, in the foreseeable future a miniaturised single molecule mass spectrometer, X-ray or NMR instrument might become available.

The trend of miniaturisation is a common theme within the analytical sciences and this includes single molecule detection and counting applications; for example lab-on-chip type-technologies would be ideal for Space applications as microfluidics-based miniaturised platforms can handle small volumes, present high levels of integration, high autonomy, low power and low reagent consumption.

To check the level of maturity with respect to single molecule detection, a three step process was followed: first the amount of different peer-reviewed publications was assessed, then the amount of applications on the market, and finally, the size of the existing market was intended to be assessed (but in all cases the market was insufficiently developed to justify this approach).

To assess the amount of publications a simple test was performed by inputting each technology into the Topic and adding “molecule” to the search term in ISI Web of Knowledge Web of Science. The technologies were then given a value according to their number of hits, *i.e.,* less than 100 hits gave the technology a low maturity level; more than 100 but less than 200 gave the technology a medium maturity, and more than 200 hits gave the technology a high value. For example, Surface Enhanced Raman Scattering achieved 632 hits, whereas Nanobarcodes achieved only 1, demonstrating that Surface Raman Scattering Technologies and more widely used/researched than Nanobarcodes.

The technologies which achieved a high score of maturity were:

- Quantum Dots (with a score of 698), SERS (632), and Ion channels (530) and the Atomic Force Microscope (525).

Those which achieved a medium score were:

- QCM (268), Optical Nanofibres (120) and TIRFM (109).

Those which achieved a low score were:

- FRET (90), Optical Traps (42), Carbon Nanowires and Nanotubes (37), Nanoshells (35), SPRS (23), Nanoarrays (21), Superresolution Techniques (17), Optical Microcavities (13), Microcantilevers (12), SERRS (9), SAW (7), Magnetic Traps (5), CARS (2), Nanobarcodes (1), AFFM (1) and Naive Arrays (0).

It can be clearly seen that more technologies achieve a low score than a medium or high score, this is due to the recent advances in single molecule detection and the revolutionary nature of most of the technologies achieving a low score (such as Nanobarcodes and Optical Microcavities).

Maturity was then subjectively assessed making use of the knowledge gathered during the International Workshop on Single Molecule Detection and Counting Technologies for Space Applications and the literature review.

The following table summarises the output of the review including the maturity assessment.

Table 2–4: *Summary of the Maturity Levels of the Technologies Identified.*

Field	Technology	Research Activity (hits)	Subjective View of Maturity	Inherently Low Mass?
Optical	Surface Enhanced Raman Scattering	High (632)	High, commercially available sub-systems.	No
	SE Resonant Raman Spectroscopy	Low (9)	High, commercially available sub-systems.	No
	Coherent AntiStokes Raman Scattering	Low (2)	High, commercially available sub-systems.	No
	Optical Traps	Low (42)	Medium, some commercial availability.	No
	Optical Nanofibres	Medium (120)	Low, little or no commercial availability.	Yes
Mechanical & Electrical	Magnetic Traps	Low (5)	Medium, some commercial availability.	No
	Atomic Force Microscope	High (525)	High, commercially available instrument.	No
Molecular Recognition (Labelled)	Forster Resonance Energy Transfer	Low (90)	High, commercially available instrument.	No
	Total Internal Reflection Fluorescence Microscopy	Medium (109)	High, commercially available instrument.	No
	Superresolution Techniques	Low (17)	Medium, some commercial availability.	No
	Nanoshells	Low (35)	Low, little or no commercial availability.	Yes
	Quantum Dots	High (698)	Medium, some commercial availability.	Yes
	Nanobarcodes	Low (1)	Medium, some commercial availability.	Yes
	Atomic Force Fluorescence Microscope	Low (1)	High, commercially available instrument.	No
	Nanoarrays	Low (21)	Low, little or no commercial availability.	Yes
	Naïve Arrays	Low (0)	Low, little or no commercial availability.	Yes
	Surface Plasmon Resonance Spectroscopy	Low (23)	High, commercially available instrument.	Yes
	Quartz Crystal Microbalance	Medium (268)	High, commercially available instrument.	Yes
	Surface Acoustic Wave	Low (7)	High, commercially available instrument.	Yes
	Microcantilevers	Low (12)	High, commercially available instrument.	Yes
Molecular Recognition (Labeless)	Carbon Nanowires AND Nanotubes	Low (37)	Low, little or no commercial availability.	Yes
	Ion Channels	High (530)	Low, little or no commercial availability.	Yes
	Optical Microcavities	Low (13)	Low, little or no commercial availability.	Yes

2.4 Trade-Off Analysis

A trade-off analysis had to be performed as part of the ESA study in order to propose the most suitable technology for Space applications out of all the technologies identified in the literature review in an unbiased and analytical manner.

It is important to note that during the international workshop on Single Molecule Detection for Space applications run at Cranfield University, UK during March 30th, 31st and April 1st 2009 to achieve peer-reviewing of the study, it was decided that a confocal microscope encompassed the technology needed to conduct cell biology experiments.

Therefore, as there was a very clear consensus for the proposal of a confocal microscope for cell biology while there was no clear consensus for astrobiology applications, it was decided that a trade-off study was only needed for astrobiology scenarios.

Consequently, cell biology applications were not formally being considered for the trade-off analysis.

2.4.1 Pre-Trade-Off Analysis

The trade-off procedure is used to identify the most suitable concept for a set of requirements. It is done by assigning weightings to specific parameters relating to the requirements and then assessing each concept for each parameter individually and objectively.

As the concepts being analysed by the trade-off have to comply with the formal assessment and design requirements, a pre-trade-off analysis is needed first in order to quickly identify unsuitable technologies that should not even be considered in the trade-off.

2.4.1.1 Pre-Trade-Off analysis methodology

The technologies identified in the review already comply with the assessment requirements but not necessarily with the design requirements. Therefore, in order to trade-off the technologies to identify which one is the most suitable for Space applications, a pre-selection process was performed to make sure that all the technologies identified complied with the formal requirements. The technologies that passed this initial process were then further analysed during the trade-off procedure to identify the best one.

On the other hand, the technologies that were found to not comply with the requirements were ruled out of the study and not considered further.

Assessment requirements

As previously discussed in Section 2.2 “Aims, Objectives, Requirements and Targets for a Single Molecule Life Detection Instrument”, the assessment requirements that ESA propose in the Statement of Work (14/05/2008) had to be complied with.

The technologies identified in the literature review comply with these assessment requirements; however, they are not necessarily able to detect, identify and count at least one representative type of astrobiology biomarker as well as one representative type of cell biology biomarker.

Nonetheless, the unlikelihood of identifying a single detection concept that can cover the very broad range of targets listed was noted and confirmed during the International Workshop held at Cranfield University, UK.

Table 2–5: Assessment *Requirements.*

Reference	Requirement
AR1	The measurement techniques or principles shall be able to identify and count non-stochastic biomarkers.
AR2	Astrobiology biomarkers to be detected according to EXO-ESA-MO-11001 (Vago, 2005) for specific biomarkers on Mars. The following examples of astrobiology targets have been identified: • Life building blocks or traces of life (past or present), such as amino-acids and their chirality, nucleobases and sugars. • Molecules from metabolic pathways. • Presence of functioning metabolic pathways. • Chemical fossils, building blocks such as, but not only, hopanes, sterane, porphyrins, chlorophyll, and similar pigments. • Molecules that could be building blocks of life forms not recognisable by the classical biochemistry, immunochemistry or nucleic acid methods currently used on Earth – for example deviations from the expected patterns of decreasing occurrence with increased complexity in homologous series of organic molecules.
AR3	Cell biology biomarkers to be detected as mediators in cell biology for cell signalling mechanisms and/or transmission of neurosignals. • However, as already mentioned, during the international workshop there was a very clear consensus for the need of a confocal microscope to detect cell biology biomarkers and so no other technology needs to be considered.
AR4	The selected technologies shall be able to detect, identify and count at least one representative type of biomarkers in the area of cell biology and at least one in the area of exobiology.

Design and Operation requirements

As mentioned before, all the technologies proposed comply with the assessment requirements, but not necessarily with the design requirements.

Therefore, the design requirements set by ESA were analysed and key questions to consider during the assessment of the technologies were noted along with their possible answers which would help filtering out non-compliant technologies.

Table 2–6: *Design Requirements.*

Reference	Requirement
DR1	Be able to detect and recognise biomarkers. • *i.e.,* is it able to both detect AND identify its target? • Possible answer: “yes, it both detects and identifies”. (Note that a “no” would immediately rule out the technology for failure to comply with DR1).
DR2	Include selected technologies that shall be compact and integrate as much as possible in order to become part of a system. • *i.e.,* would the technology be implemented easily into a system? • Possible answers: “very easy”, “easy”, “medium” or “difficult” implementation.
DR3	Be able to detect concentrations in a deterministic (module counting) manner. • *i.e.,* can it count single molecules? • Possible answer: “yes it has single molecule counting potential”. (“No” would immediately rule out the technology for failure to comply with DR3).
DR4	Sample preparation and use of reagents shall be minimal. • *i.e.,* does it need to purify the sample first? • *i.e.,* does it require solvents or can it perform analysis of the sample in its powdered form? • Possible answers: “no need for purification and no sample solvent needed”; or, “need for purification but not of solvents”; or, “need of both purification and solvents”.
DR5	Be able to reach at least part per trillion (design aim) level sensitivity. • *i.e.,* what is its lowest sensitivity limit? (If the sample is 1g, then the detection requirement is for 10^{-12}g of the target). • Possible answer: “yes it has ppt sensitivity”. (“No” would immediately rule out the technology for failure to comply with DR5).
DR6	Have a mass of less than 5kg. • *i.e.,* is it inherently low mass? • i.e., is a low mass concept currently being developed? • Possible answers: “yes, it is inherently low mass” (*i.e.* nanotechnology concepts); or, “no, but a low mass concept is currently being developed (*i.e.* hand-held portable device) and can easily be envisaged with existing technology components”; or “no, and no concept is being developed”. The latter would immediately rule out the technology.

It shall also have the following operation requirement:

Table 2–7: *Operation Requirement.*

Reference	Requirement
OR1	To be operational in human, autonomous and tele-operated modes. • *i.e.,* is it automatable or does a human need to physically manipulate the technology and/or the sample? Does it have a large number of degrees of freedom that require adjustment and optimisation during a measurement cycle? • Possible answer: "yes it is automatable". ("No" would immediately rule out the technology for failure to comply with OR1)

It is worth noting that during design analysis procedures failure of compliance with one requirement is an absolute failure and a technology that does not comply with all the requirements is not considered further. However, initial flexibility with the pre-analysis carried out was needed when considering "ease of implementation" and "minimal sample preparation" as these requirements were more subjective, requiring further explanation than an explicit "yes" or a "no". This is due to these requirements being characteristics that could be improved upon more realistically than the rest of the requirements.

Therefore, it was decided that the main requirements that absolutely had to be complied with were: detecting AND identifying biomarkers; being able to count molecules; achieving part per trillion sensitivity; ability to have a low mass concept; and ability to be automatable.

"Ease of implementation" and "minimal sample preparation" are taken into account but do not rule out technologies. For example, a technology that has no need for purification but does need solvents is not ruled out now but will be considered and analysed in more detail during the trade-off procedure. Nonetheless, a technology that has no need for purification or solvents will always be more suitable and will win during the trade-off procedure over a technology that does need purification and/or solvents.

Once the methodology was established, the pre-trade-off analysis was carried out and the results are detailed as follows.

2.4.1.2 Pre-Trade-Off analysis results

Each technology identified in the literature review was analysed according to the methodology described in Section 2.4.1.1 "Pre-Trade-Off analysis methodology".

Direct measurement of inherent physical properties techniques

There are various techniques that directly measure inherent physical properties: optical, electrical and mechanical. These techniques do not introduce a labelled component.

Optical Techniques

These methods include Raman techniques (SERS, SERRS and CARS), optical traps and optical nanofibres.

Surface Enhanced Raman Scattering (SERS)

SERS is a technology that both detects and identifies, has parts per trillion sensitivity and is able to count single molecules. Also, its sample preparation needs are minimal, as there only needs to be an extraction step where a solvent is added -however, no complex steps such as purification or

introduction of a label are needed, making automation a lot less complex than with those technologies that do. Overall, the implementation of SERS is easy.

On the other hand, as SERS is not inherently low mass and this is a vital requirement for planetary exploration missions, it has to be ruled out of the study if miniaturisation is considered an overly complex task. However, as it is envisaged that a low mass implementation could be easily achieved with off-the-shelf components, it was recommended for further study.

Surface Enhanced Resonance Raman Spectroscopy (SERRS)

SERRS is very similar to SERS, although it identifies and detects a lower range of targets than SERS (but has a lower limit of sensitivity for that reduced range). Unlike SERS though, its sample preparation needs are more complex as purification is needed, which makes implementation harder.

However, compared to other technologies, one of the main advantages of SERRS over techniques such as fluorescence is that SERRS spectra contain sharp vibrational bands, giving fingerprint spectra which are molecularly specific while fluorescence spectra are broad and not unique to the analyte of interest. Hence, SERRS can be used to easily discriminate between analytes in a mixture without lengthy separation steps like those needed in fluorescence techniques.

Again, even though SERRS is not inherently low mass, it is envisaged that a low mass implementation could be easily achieved with off-the-shelf components; and hence it was recommended for further study.

Coherent AntiStokes Raman Scattering (CARS)

CARS is a technology with very difficult implementation due to its sample preparation needs as well as its working characteristics. Moreover, in order to count single molecules it needs to be coupled with a surface enhanced (SE) technique which casts further doubts on the ability to miniaturise this overly complex technology.

Therefore, as low mass concept is not being developed due to its complexity, and coupling it with an SE technique to achieve single molecule sensitivity would establish a dependency on other technology to be miniaturised as well, it was not considered further.

Optical Traps

Optical traps detect only, and need to be coupled with imaging techniques in order to identify as well. They are not overly difficult to implement as their sample preparation needs are not unreasonable; however, as optical traps are not automatable they were not considered further. Also, their miniaturisation was considered too complex to undertake.

Optical Nanofibres

Optical Nanofibres can both detect and identify targets, can count single molecules, have reasonable implementation requirements (such as needing sample purification), can be automated and are a low mass concept. Therefore, they were proposed for further study.

Hence, out of the optical techniques SERS, SERRS and Optical Nanofibres were considered further; while CARS and Optical Traps were not.

Mechanical and electrical techniques

These techniques include the AFM and Magnetic Traps

Force Probes/Atomic Force Microscope (AFM)

The AFM can be low mass and automatable as it has already been flown in the NASA Phoenix mission (2008); however, it is unsuitable for single molecule detection applications as a more sophisticated instrument would be required, and consequently, an overly complex automation.

Magnetic Traps

Magnetic traps are not automatable and a low mass concept has not been yet considered, making them unsuitable for planetary exploration missions.

Out of the mechanical and electrical techniques, none were recommended for further study.

2.4.1.3 Measurement mediated by molecular recognition events

These technologies can either use of label to aid detection (labelled techniques) or labeless.

Labelled techniques

These techniques need to introduce a label in order to identify the biomarkers, which make their sample preparation more complex.

Fluorescence Correlation Spectroscopy (FCS)

FCS based technologies include FRET and TIRFM.

Förster Resonance Energy Transfer (FRET)

FRET, as labelled technique, needs more sample processing steps. However, this added complexity does not make the technology unsuitable.

Its weakness however, is that it is not a low mass concept. But again, like SERS and SERRS, miniaturisation could easily be achieved with off-the-shelf components so it is recommended for further analysis.

Total Internal Reflection Fluorescence Microscopy (TIRFM)

Although TIRF microscopy is not low mass, the current development of a handheld device allows this technology to go forward to the trade-off stage.

However, as it needs a labelled component, during the trade-off analysis it will be less suitable compared to other promising technologies which are labeless and take measurements directly.

Superresolution Techniques

There is no low mass concept currently being developed for superresolution techniques and they are not considered automatable either due to their complexity. Therefore they were ruled out of the study.

Magnetic Bead Microassays/Nanoshells

Magnetic bead microassays comply with all requirements even though they have a dependency on other technologies because the technologies they depend on are either SERS based or fluorescence based techniques which also comply with the requirements. Therefore, Magnetic Bead Microassays were approved for further study.

Quantum Dot Labelling

Quantum dot labelling is a method that aids imaging, and hence is dependent on imaging technologies -which would indicate a possible dependency flaw. However, imaging methods could be easily implemented as low mass versions too and hence quantum dots comply with all the requirements and were recommended for further study.

Nanobarcodes

Nanobarcodes do not directly detect as they are used as an adjunct technology (like quantum dots), but they do aid identification. However, their implementation is considered to be overly complex due to the difficulty in reading the nanobarcodes, hence they were not considered further.

The Atomic Force Fluorescence Microscope (AFFM)

The AFFM is not a low mass concept and it does not have a low mass concept being developed (such as a handheld device); moreover, it is not automatable. Therefore, it was ruled out of the study.

Nanoarrays

Nanoarrays need a labelled component to count molecules; hence, although in order to count the molecules a fluorescence scanner (not potentially low mass but could be designed to be) is needed, it is thought that at this point in the assessment nanoarrays should be put forward for further study because of their compliance with all the other requirements.

Naïve Arrays

As with Nanoarrays, Naïve Arrays were also not ruled out at this time even though their possible dependency on other technologies could prevent them from being considered.

Further study of both Nano and Naïve arrays will eliminate this ambiguity during the trade-off analysis.

Surface Plasmon Resonance Spectroscopy (SPRS)

SPRS technology is the basis of the Biacore (2009) biosensor chip which is currently used for a diverse range of applications such as identification of specific protein-protein interactions, mapping of binding sites and investigations of kinetic parameters for previously characterised interactions. As the use of SPRS technology complies with all the requirements (in its labelled form), it was recommended for further study.

Quartz Crystal Microbalance (QCM)

QCM (with a labelled component to allow ppt sensitivity) is also recommended for further study due to its easy implementation, low mass and ability for automation.

Surface Acoustic Wave (SAW) Devices

SAW devices, like QCM devices, need to introduce a label for identification of biomarkers but comply with all requirements and hence were considered further.

Microcantilevers

Microcantilevers are relatively fragile as they need to avoid vibration to take the measurement (making their implementation more difficult); however, they comply with all the requirements and so were recommended for the next stage of the analysis.

Out of the labelled techniques, the following were considered further: Förster Resonance Energy Transfer (FRET), Total Internal Reflection Fluorescence Microscopy (TIRFM), Magnetic Bead Microassays and Nanoshells, Quantum Dot Labelling, Naïve arrays, Nanoarrays, Microcantilevers, Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM) and Surface Acoustic Wave (SAW) devices.

Labeless techniques

Labeless techniques have an easier implementation than those that are labelled because they do not need an extra sample preparation step where the label gets introduced.

Carbon Nanowires/Nanotubes

Carbon Nanowires/Nanotubes comply with all the requirements (although need to be functionalised, *i.e.,* labelled, to identify the targets). They are also inherently low mass, which is a very desirable feature that other technologies do not possess. Therefore, they were proposed for further analysis.

Ion Channels/Nanopores

Ion Channels/Nanopores can have a difficult implementation, but they were recommended for further analysis as it was not clearly known at this stage how difficult it would actually be to implement them. As they comply with all the other requirements, they were put forward to the next part of the study.

Optical Microcavities

Optical microcavities conform to all the design requirements, hence were also recommended for further analysis.

As was to be expected, all of the labeless techniques were recommended for further study. This is due to their inherently low mass features and their ease of implementation compared to that of the labelled techniques. However they still needed to be investigated further to understand if they were suitable for the constraints imposed in planetary exploration missions.

Pre-Trade-Off results

A summary of the single molecule detection and counting techniques assessed in the pre-trade-off analysis is presented below.

Table 2–8: *Pre-Trade-Off Analysis Summary.*

Technology	Detects AND Identifies	Implemen-tation	Counts Single molecules	Sample Preparation	PPT Sensitivity	Low Mass	Automatable
SERS	Y	Easy	Y	Easy	Y	Envisaged	Y
SERRS	Y	Medium	Y	Easy	Y	Envisaged	Y
CARS	Y	**Very Difficult**	Y	Easy	Y	**N**	Y
Optical Traps	Y	Medium	Y	Easy	Y	**N**	**N**
Optical Nanofibres	Y	Medium	Y	Easy	Y	Y	Y
AFM	Y	Medium	Y	Easy	Y	**N**	**N**
Magnetic Traps	Y	Medium	Y	Easy	Y	**N**	Y
FRET	Y	Medium	Y	Difficult	Y	Envisaged	Y
TIRFM	Y	Medium	Y	Difficult	Y	Hand-held	Y
Super-resolution Techniques	Y	Medium	Y	Difficult	Y	**N**	**N**
Magnetic Beads.	Y	Medium	Y	Difficult	Y	Y	Y
Quantum Dots	Y	Medium	Y	Difficult	Y	Y	Y
Nano-barcodes	Y	**Very Difficult**	Y	Difficult	Y	Y	Y
AFFM	Y	Difficult	Y	Difficult	Y	**N**	**N**
Nanoarrays	Y	Medium	Y	Difficult	Y	Y	Y
Naïve arrays	Y	Medium	Y	Difficult	Y	Y	Y
SPRS	Y	Easy	Y	Difficult	Y	Y	Y
QCM	Y	Easy	Y	Difficult	Y	Y	Y
SAW	Y	Easy	Y	Difficult	Y	Y	Y
Micro-cantilevers	Y	Medium	Y	Difficult	Y	Y	Y
Carbon Nanowires	Y	Medium	Y	Medium	Y	Y	Y
Ion Channels/ Nanopores	Y	Medium	Y	Medium	Y	Y	Y
Optical Micro-cavities	Y	Medium	Y	Medium	Y	Y	Y

Therefore, the technologies that were recommended for further study and those that were not are detailed in the table below.

Table 2–9: *Conclusions of the Pre-Trade-Off.*

Recommended Technologies	Disregarded Technologies
SERS.	CARS.
SERRS.	Optical Traps.
Optical Nanofibres.	AFM.
FRET.	Magnetic Traps.
TIRFM.	Superresolution Techniques.
Magnetic Bead Microassays/Nanoshells.	Nanobarcodes.
Quantum Dots.	AFFM.
Nanoarrays.	
Naïve arrays.	
SPRS.	
QCM.	
SAW devices.	
Microcantilevers.	
Carbon Nanowires/Nanotubes.	
Ion Channels/Nanopores	
Optical Microcavities.	

During the pre-trade-off, it was expected to find that nanotechnology based techniques would pass the pre-trade-off analysis due to their inherent low mass. On the other hand, direct measurement and imaging techniques were envisaged to struggle with this requirement; however, some were still found to pass due to the possibility of miniaturisation.

It was also expected to find that labelled techniques introduced complex integration needs due to the necessary introduction of a label which would add complexity to the sample preparation procedures needed. However, although it is assumed labelled techniques will be ruled out during the trade-off analysis due to this inherent complexity, thorough assessment during the trade-off analysis needed to be performed.

Comparison of results with the International Workshop

It is interesting to compare the technologies that were preliminarily proposed during the International Workshop conducted in Cranfield University documented in Appendix A, with the technologies that passed the assessment documented in this section. The technologies that were recommended for further study during the International Workshop as well as the ones that were disregarded are detailed in the table below.

Table 2–10: *Conclusions of the Preliminary Technology Assessment During the International Workshop.*

Recommended Technologies	Disregarded Technologies
SERS.	SERRS.
Imaging technologies.	CARS.
Mass spectrometers.	SPRS.
Enzymatic methods.	Evanescence.
Single molecule mass spectrometry.	Cantilever Arrays.
Single molecule X-ray.	QCM.
Single molecule NMR.	SAW devices.
Naïve arrays.	Nanowires.
	Nanopores.
	Optical traps.
	Optical nanofibres.
	Magnetic traps.
	AFM.
	FRET.
	AFFM.

The main discrepancies are that in the assessment described in this document, single molecule X-Ray, Mass Spectrometry and NMR were not considered for the pre-trade-off analysis as they were considered as possible complementary technologies for the novel nanotechnologies studied rather than as the main technologies to use. Therefore, nanotechnologies were studied in much more depth in this study than during the workshop because time constraints prevented the workshop delegates from fully assessing these during the two day conference. The notes of the workshop also stated as a clear conclusion that a confocal microscope was needed for cell biology applications and for astrobiology questions the hyphenated techniques such as Tandem Mass Spectroscopy (MS/MS)-SERS, Naïve and Nano Arrays would be suitable.

Another point stated during the workshop was that technologies such as SPRS, CARS, QCM and SAW were classified as unsuitable for their alleged inability to detect single molecules. However, detailed study of these has been performed in this document and research showed these technologies do achieve single molecule sensitivity.

Nonetheless, CARS was ruled out due to its high mass -but SPRS, QCM and SAW passed this assessment stage and were recommended for further study.

2.4.2 Trade-Off Analysis

As the pre-trade-off analysis ruled out the technologies that did not comply with the assessment and design requirements, the requirement compliant technologies that were proposed now had to be prioritised during the trade-off process in order to understand which one was the most suitable.

2.4.2.1 Trade-Off Analysis Methodology

The following technologies were selected by the pre-trade-off procedure to undergo the trade-off analysis:

- *Direct optical measurement based:* Surface Enhanced Raman Scattering (SERS), Surface Enhanced Resonant Raman Spectroscopy (SERRS) and Optical Nanofibres.
- *Indirect, labelled, measurement mediated by molecular recognition events based:* Förster Resonance Energy Transfer (FRET), Total Internal Reflection Fluorescence Microscopy (TIRFM), Magnetic Bead Microassays and Nanoshells, Quantum Dot Labelling, Naïve arrays, Nanoarrays, Microcantilevers, Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM) and Surface Acoustic Wave (SAW) devices.
- Indirect, labeless, measurement mediated by molecular recognition events based: Carbon Nanowires and Nanotubes; Ion Channels and Optical Microcavities.

Selection of parameters for the trade-off analysis

The parameters have to be selected adequately in order to make sure valuable information about the technologies is obtained from them. Therefore, they have to enquire about specific characteristics of the technologies to find both their flaws and their strengths which will, according to the specific weightings given to the parameters during the trade-off procedure, inform about the suitability of each technology.

Parameters used during the International Workshop

During the International Workshop a trade-off was also performed; therefore, the parameters used were analysed to understand their value for the in depth trade-off to be performed in the study. The following parameters were used during the International Workshop:

- Spatial resolution.
- Temporal resolution.
- Mass resolution.
- Dynamic range.
- Data volume.
- Analytical flexibility (can it accept a wide range of sample types?)
- Sample flexibility (can it detect a wide range of targets?)
- Freedom from preconceptions (can it detect unknown unknowns?)
- What does it detect? (Chirality? Chemical group or bond type?)
- Compatibility with target markers (*i.e.,* compliance with assessment requirement AR2).
- Does it perform detection or identification? (i.e., compliance with design requirement DR1).
- Maturity of technology in non-Space applications.

- Does it destroy the sample?
- Single molecule detection potential (*i.e.,* compliance with design requirement DR3).
- Number of molecules required for detection.
- Ability to measure components with 50Da (Daltons), 200-500Da, >500Da.
- Sample preparation complexity (*i.e.,* compliance with design requirement DR4).
    - Needs to be split into further parameters to be more relevant at this stage, such as:
        - Need for purification?
        - Need for solvents?
        - Need for label?
        - How sensitive will the sensor be to physical contamination?
        - Need to have liquid/gas environment or solid surface?
        - Use of separation techniques to isolate sample components of interest?
        - What consumables are needed?
- Lifetime:
    - How many samples can the technology analyse over its lifetime?
    - Reusability (does it require reagents or cleaning for re-use? Or is it single use/disposable?)
- Protection from extreme environment:
    - For lab use and for Space use?
    - Need to know the ionic strength, or pH of the environment?
    - Planetary Protection and Contamination Control issues?
    - Possibility to ruggedise fluidics and sample preparation system?
    - Needs radiation shielding/protection?
    - Needs vibration protection?
    - Needs temperature, pressure and atmosphere stability over measurement lifetime?
- Fault tolerance:
    - What is the stable (zero reading) baseline? How do you get a stable reading, without knowing exactly the environment, or having a dramatic shift in vapour pressure, or temperature?
- Miniaturise/Integrate the electronics (*i.e.,* compliance with design requirement DR6).
    - 'Automatable' encompasses all techniques in principle miniaturisable to fit <5kg limit (however, optical path lengths are a potential issue against miniaturisation).
    - Generally need low power/low voltage/reasonable frequency electronics.
- Automation (*i.e.,* compliance with operation requirement OR1).
    - It was noted that for imaging or confocal devices, this became difficult as need to automate alignment and focusing steps to obtain 100nm resolution images.
- Ease of implementation (*i.e.,* compliance with operation requirement DR2).

These parameters aided the selection of the parameters for the trade-off analysis, identifying two main areas of study for the technologies: parameters that identify and aid to quantify the complexity of the technology (such as if it destroys the sample and how complex the individual sample preparation steps are) and parameters that relate to the working characteristics of the technology (such as spatial and temporal resolution).

Proposed final parameters for the trade-off analysis

Making use of the workshop parameters that were found to be suitable for the trade-off analysis to be performed in this study, a final list of parameters is proposed.

Note that due to the ambiguity identified in the pre-trade-off analysis, where “ease of implementation” and “sample preparation” were found to be very subjective parameters, a thorough analysis needed to be carried out during the trade-off.

Therefore the trade-off was split into two stages: a first stage to clearly assess the complexity of the technology (dependent on “ease of implementation” and “sample preparation” as well as other measures of system complexity); and a second stage where the technologies were assessed according to their working characteristics.

Parameters for the first stage of the trade-off analysis

These parameters aimed to identify how complex the use of a particular technology would make the system. “Ease of implementation” and “sample preparation” both feed into the system’s complexity and so their ambiguity was addressed at this stage.

The chosen parameters for the first stage of the trade-off analysis are analysed as follows:

- Maturity of technology in non-Space applications.
    - How reliable is the technology?
    - Has it been “tried and tested”/validated by the expert community extensively?
    - How reproducible are its results?
- Sample preparation simplicity (number of sample preparation steps needed):
    - Need for purification?
    - Need for solvents?
    - Need for label?
    - Sampling aerosol plumes, small organics in water, soil/rock/ice matrices, cellular material?
    - Need to have liquid/gas environment or solid surface?
    - Use of separation techniques to isolate sample components of interest?
    - Consumables needed?
    - Diffusion time for low concentrations?
- Lifetime:
    - How many samples can it analyse over its lifetime?
    - Reusability (does it require reagents or cleaning? Or is it single use/disposable?)

- Protection from extreme environment:
  - For lab use and for Space use?
  - Need to know the ionic strength, or pH of the environment?
  - Planetary Protection and Contamination Control issues?
  - Possibility to ruggedise fluidics and sample preparation system?
  - Needs radiation shielding/protection?
  - Needs vibration protection?
  - Needs temperature, pressure and atmosphere stability over measurement lifetime?
- Automation complexity (integration of electronics and other components):
  - Generally need low power/low voltage/reasonable frequency electronics.

Therefore the parameters for the first stage of the trade-off analysis were:

- Maturity.
- Sample Preparation.
- Lifetime.
- Protection.
- Automation.

Parameters for the second stage of the trade-off analysis

These parameters addressed the specific features of each technology with respect to their working characteristics. They were first selected for the second stage but were later re-assessed as it became evident once the second trade-off commenced that some were irrelevant. For completeness, the original list of parameters for the second stage is included below:

- Spatial resolution (*i.e.,* the ability to separate the images of closely adjacent objects).
- Temporal resolution (*i.e.,* the precision of the measurement with respect to time).
- Low mass detection (*i.e.,* ability to measure components with 50Daltons).
- Dynamic range (ratio between smallest and largest values).
- Analytical flexibility (can it accept a wide range of sample types? *i.e.,* powdered, liquid or gaseous).
- Sample flexibility (can it detect a wide range of targets?)
  - Freedom from preconceptions (note that out of the chosen technologies to be traded-off, only the naïve arrays can detect “unknown unknowns”).
  - What does it detect? Need to distinguish between astrobiology and cell biology applications.
- Sample Destruction.
- Analytical Reliability (robustness, how well does analytical performance vary with different samples and different environments?)

However, as previously stated, they were analysed again and the following parameters were discarded:

- Spatial resolution.
    - Not relevant as there is no requirement for high spatial resolution.
- Temporal resolution.
    - Not relevant as there is no requirement for high temporal resolution.
- Data volume.
    - Not relevant as there is no requirement for high/low data volumes.
- Freedom from preconceptions.
    - Defines sample flexibility, hence not a parameter on its own.
- What does it detect?
    - Defines sample flexibility, hence not a parameter on its own.
- Number of molecules required for detection.
    - Not necessary (all technologies can detect single molecules).
- Ability to measure components with: 50Da (Daltons), 200-500Da and >500Da.
    - Defines analytical flexibility, hence not a parameter on its own.

Therefore, “freedom from preconceptions” was now not a parameter on its own as stated in the original list, but one of the defining characteristics of the “sample flexibility parameter”. This was also the case for “what does it detect?”

On the other hand, mass resolution (the ability to measure components with <50, 200-500 and >500Da) was added to “analytical flexibility” as a subset. This was because it is a quantifiable way of analysing the technologies as an instrument that can detect both large and small targets as well as all target types is needed.

“Number of molecules required for detection” was excluded because all the technologies can go down to single molecule detection level. Also, “data volume” was excluded because it was not a defining parameter as there was no indication in the requirements that a technology producing a high or low data volume should be penalised either way.

“Temporal resolution” was also not considered anymore because no fundamental technology or science drivers that required significantly less than 1 second time resolution had been noted. However, if this had been the case, the technologies would have been differentiated as those with seconds, microseconds and nanoseconds of resolution.

“Spatial resolution” was not considered either as cell biology applications were not pursued; hence there were no science or technological drivers requiring less than 1mm resolution. Again, if that had been the case, the technologies would have been differentiated as those who have millimetres, micrometres and nanometres of resolution.

Therefore, the main parameters that will act as differentiators for the second stage of the trade-off were: dynamic range, analytical flexibility, sample flexibility, sample preservation/re-use and analytical reliability.

Assigning weightings to the parameters

To assign weightings to each parameter it must be first understood which parameters are the most important and rate them in comparison to the others. Parameters are assigned a weighting from 1 to 5 and the actual score a technology gets for the different parameters ranges from 1 to 10. The higher the weighting, and the higher the score, the more appropriate the technology is for Space applications.

Weightings for the first stage of the trade-off analysis

The parameters assigned for the first stage of the trade-off were concerned with the system's complexity:

- Maturity of technology in non-Space applications.
- Sample preparation.
- Lifetime.
- Protection from extreme environment.
- Automation complexity.

As maturity entails reliability, and an unreliable instrument cannot even be considered, this parameter carried the highest weighting, a 5. It is followed by sample preparation and automation, which are formal ESA requirements, so they were awarded a 4 out of 5 weighting.

Lifetime was also important, because a reduced lifetime due to a technology not being reusable adds complexity to the system; however it is awarded a 3 because it is not as important as the requirements, and yet it is more important than being inherently protected from the extreme environment (which gets a weighting of 2). This is because all technologies will need protection from the environment as they will not have the radiation, vibration, shock and thermal standards required for Space applications. Nonetheless, if a technology is too fragile, it had to be penalised for it. An example table of how the trade-off will be carried out for each technology is shown in the following table:

Table 2–11: *First Stage Trade-Off Analysis.*

	Maturity	Sample Preparation	Lifetime	Protection	Automation
Weight	5	4	3	2	4
Score (Total: x)	1 to 10	1 to 10	1 to 10	1 to 10	1 to 10

A score of 4 or below implied the technology failed for that particular parameter as it was unsuitable for Space applications, in which case the technology should not be considered further. Realistically, all the technologies in the study had already been filtered and those that were not suitable have been excluded from the study. Consequently, none of the technologies were envisaged to score a 4 or below. It was also foreseen that none of the technologies would score a 10 in any of the parameters either as this would imply a particular technology was perfectly suited in a parameter, which was almost impossible. Therefore, practically, the scores assigned would be within the range of 5 to 9.

Table 2-36: "Scores for First stage Trade-Off Analysis" explains how the scores are assigned specific characteristics.

Table 2–12: *Scores for First Stage Trade-Off Analysis.*

Score	Maturity	Sample Preparation	Lifetime	Protection	Automation
4 or less	The technology has not reached a basic level of maturity to be used in Space applications.	The technology has overly complex sample preparation needs to be used in Space applications.	The technology has such a low lifetime it would be unusable in the Space applications envisaged.	The technology is so fragile it cannot be used in Space applications.	The technology cannot be used without a human operator.
5	Using the technology for single molecule detection exists only as a concept.	The technology needs to use solvents, needs to purify the sample, needs to introduce label.	The technology is not reusable.	The technology is sensitive to organics contamination. Affected by vibrations and radiation.	Possible automation but has not been tried yet.
6	Technology, though not widely used, has been used for single molecule detection applications.	Needs to use solvents, needs to purify the sample. Does not introduce label.	The technology is not reusable unless surface is regenerated or replaced and it can only be used a few times.	Sensitive to organics contamination. Affected by vibrations or radiation only while taking measurement.	Possible automation and components exist for it but has not been tried yet. Also it needs to introduce a label, making automation more difficult.
7	Technology has been widely used for single molecule detection applications.	Needs to use solvents, but it does not need to purify the sample and it does not introduce a label.	Not completely reusable as surface needs to be regenerated/ replaced and needs to immobilise target using traps to analyse it or needs organics. Lifetime is good.	Sensitive to organics contamination. Affected by either vibrations or radiation (but not both) only while taking measurement.	Possible automation and off-the-shelf components exist but has not been tried yet. Introduces no label.
8	Technology has been widely used for single molecule detection and has commercial applications.	Needs no solvents, purification, or labels but still has some basic sample preparation requirements such as grinding the sample.	Not completely reusable as surface needs to be regenerated/ replaced. Can analyse a high number of samples over its lifetime.	Sensitive to organics contamination. Not affected by vibrations or radiation at all.	Has been completely automated and tried without a human operator.

9	Technology has been widely used for single molecule detection, has commercial applications and a hand-held system device is being developed.	Has very minimal sample preparation.	Surface does not need to be regenerated or replaced and it can be used a high number of times.	Not sensitive to organics contamination and its measurement is not affected by radiation or vibration but still needs basic protection from extreme environment.	Has been completely automated and is widely used without a human operator.
10	Technology has been extensively used for single molecule detection, has commercial applications and a hand-held device has been widely used.	Needs no sample preparation or extraction.	Completely reusable and can be used a very high number of times.	Does not need protection from extreme environment (*i.e.* withstands extreme temperatures, pressures, vibrations and radiation).	Is completely automated and extensively used without a human operator in Space applications already.

Weightings for the second stage of the trade-off analysis

These parameters addressed the specific features of each technology with respect to their working characteristics: dynamic range, analytical flexibility, sample flexibility, sample preservation/re-use, analytical Reliability.

Analytical reliability, as a further expansion of the parameter used in the first trade-off (maturity) was essential as a technology needs to be very robust in order to be able to work in extreme environments. Moreover, if the probability of getting a false positive or a false negative is very high, *i.e.*, if the analytical reliability is very low, then it is not feasible to send the technology on a mission. Therefore as analytical reliability was the most important parameter it had a weighting of 5.

On the other hand, analytical and sample flexibility had a weighting of 4 as there is a need for an instrument that can be used in multiple mission scenarios, and most science questions are addressed by understanding a wide range of molecules/targets.

Dynamic range was not critical but still desirable so it was awarded a weighting of 3, and it was scored within this weighting by the dynamic range being at an ultra-low lower limit level of detection. Sample preservation was not as important as all the other parameters as this parameter is only critical for sample return missions; however, technologies that destroy the sample still had to be penalised.

Table 2–13: *Second Stage Trade-Off Analysis.*

	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation	Analytical Reliability
Weight	3	4	4	2	5
Score (Total: 125)	1-10	1-10	1-10	4,6,8,10.	1-10

As it is difficult to differentiate six levels of sample preservation, the scores were banded onto only 4 levels (a score of 4, 6, 8 or 10) that map onto obvious hierarchical categories.

The table below explains how the scores are calculated according to a technology's characteristic.

Table 2–14: *Scores for Second Stage Trade-Off Analysis.*

Score	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation/ re-use	Analytical Reliability
4 or less	Extremely Low	Extremely Low	Extremely Low	Destroys sample, sample cannot be salvaged at all (*e.g.* mass spectrometer) and waste products have no science value.	Reliability is too low to trust measurement.
5	Very Low	Very Low	Very Low	Not applicable.	Unreliable. There is a high risk of false positives/negatives.
6	Low	Low	Low	Sample modified but residue still usable by other technologies to answer science questions.	Low reliability, risk of false positives/negatives.
7	Medium	Medium	Medium	Not applicable.	Reliable. Includes receptor based labeless technologies.
8	Wide	Wide	Wide	Sample is modified or separated but completely recoverable by unbinding.	Very reliable. Receptor based labelled techniques have a clear cut analytical signature, so any other targets that do not interact with the label do not interact with signal.
9	Very wide	Very wide	Very wide	Not applicable.	Highly reliable. Includes direct technologies (which are more robust and hence more reliable than molecular recognition based technologies).
10	Extremely Wide (can detect)	Extremely Wide (accepts all target types and all sizes).	Extremely Wide (accepts all sample types: powdered, liquid or gaseous, from different sources).	Does not destroy sample, it stays unmodified (*e.g.* Raman spectrometer).	Infallible, has no error.

Once the parameters (along with their weightings and associated scores) were finalised, the trade-off analysis was performed.

2.4.2.2 First trade-off of single molecule detection technologies

During this first trade-off analysis, the technologies were scored for their complexity. The technologies that are ruled out were not considered for the second stage of the trade-off analysis.

Direct measurement of inherent physical properties techniques (Optical only)

Electrical and mechanical techniques were ruled out during the pre-trade-off analysis due to their non-compliance with the requirements and hence only optical techniques were considered for the trade-off.

Surface Enhanced Raman Scattering (SERS) and Surface Enhanced Resonance Raman Spectroscopy (SERRS)

As SERS is a technology with high maturity, it scored 8 out of 10 in maturity (it did not achieve 9 because it is not as mature for single molecule applications). It needs to use solvents, but it does not need to purify the sample, giving it a 7 for sample preparation. Its lifetime is good, although it is not completely reusable as the surface needs to be regenerated or replaced; but it can analyse a high number of samples over its lifetime, so it achieved an 8.

It is not a fragile technology that needs extra protection but it is sensitive to contamination with organics, as that is what it needs to detect. Also, it is not affected by vibration and it does not use organics for detection (so they do not make SERS sensitive to radiation), hence achieving an 8 for protection. It could also be easily automated with off-the-shelf components, hence a score of 7 for automation.

As SERRS is very similar to SERS, it scored the same for each parameter.

Optical Nanofibres

Optical Nanofibres are not widely used, so they scored a 6 for maturity. They need to use solvents, but do not need to purify the sample, giving them a 7 for sample preparation. Their lifetime is very good as they are completely reusable, so they obtained a 9 for lifetime. They are sensitive to organics contamination (like all technologies that detect organics); however they are not affected by vibration; and as they do not use organics for detection, they are not sensitive to radiation - hence achieving an 8 for protection. Also, as they could be easily automated with off-the-shelf components, they achieved a score of 7 in automation.

Out of the optical methods, SERS and SERRS score the highest, with a 136, followed by Optical Nanofibres with a score of 129.

Table 2–15: *Summary of First Trade-Off of Optical Techniques.*

	Maturity	Sample Preparation	Lifetime	Protection	Automation
Weight	**5**	**4**	**3**	**2**	**4**
SERS and SERRS scores (Total: 136)	8	7	8	8	7
Nanofibres scores (Total: 129)	6	7	9	8	7

Measurement mediated by molecular recognition events

Indirect methods include those whose measurement is mediated by molecular recognition events. They can use either added reagents to add a labelled component that results in the analytical readout event (label formats) or have no need for added assay reagents (labeless). These methods enable the visualisation of processes in real time.

Labelled techniques

The labelled techniques assessed during the first trade-off were FRET, TIRFM, Magnetic Bead Microassays/Nanoshells, Quantum Dots, Nanoarrays, Naïve Arrays, SPRS, QCM, SAW and Microcantilevers.

Förster Resonance Energy Transfer (FRET)

As FRET is a technology with high maturity, it scored 8 out of 10. However, it needs to use solvents and it needs to introduce labels in order to identify the biomarker, giving it the low score of 5 for sample preparation.

Also, as the surface needs to be regenerated and it needs to immobilise the target in order to analyse it, it achieved a lifetime score of 7.

For protection it scored a 6 as it is not a fragile technology that needs extra protection and has no alignment issues (but does have organics that are sensitive to radiation). As it needs to introduce a label autonomously, it obtained a lower score for automation than the labeless techniques, a 6.

Total Internal Reflection Fluorescence Microscopy (TIRFM)

As TIRFM is a technology with high maturity and a handheld device is also being developed (on the contrary to SERS and SERRS), it scored 9 out of 10 for maturity. However, it needs to use solvents and introduce labels in order to identify the biomarker, giving it a low score of 5 for sample preparation. The surface needs regenerating and it has organics, thus giving it a 7 for lifetime. However, it needs to avoid vibrations to perform the measurement as it can have alignment errors and the organics are affected by radiation, hence a 5 for protection. Also, it could be automated but needs to introduce a label, hence a score of 6 for automation.

Magnetic Bead Microassays/Nanoshells

Magnetic bead microassays are not widely used but have been used for single molecule applications so achieved a 6 for maturity. They need to use solvents, purify the sample and introduce a label, giving them a 5 for sample preparation. Their surface needs to be regenerated and needs organics to be restored so they obtained a 7 for lifetime. As these organics are sensitive to radiation they scored a 6 for protection, and as the receptors need to be manipulated, which makes automation harder, they scored of 6 for automation.

Quantum Dot Labelling

As Quantum Dots are commercially available reagents that have been widely used they got an 8 for maturity; however they need to use solvents and introduce a label, giving them a 5 for sample preparation. Quantum Dots have an organic shell so they got a score of 7 for lifetime (due to not being completely reusable) and because of these organics, which are sensitive to radiation, they obtained a 6 for their inherent weakness when dealing with environmental protection issues. Also, a label makes automation more difficult hence a score of 6.

Nanoarrays

As Nanoarrays are not widely used they scored a 6 in maturity. They need to use solvents, purify the sample and introduce a label, giving them a 5 for sample preparation. They also need to regenerate the surface organics in order to enable re-use so got a 7 for lifetime. Because of these organics they obtained a 6 for protection as these would be susceptible to radiation.

Also, automation will be difficult when introducing a label giving it a score of 6.

Naïve Arrays

As Naïve Arrays are like Nanoarrays, they had the same scores for every parameter except for maturity, where Naïve arrays score lower due to, so far, only being considered as a concept.

This gave them a total low score of 102, practically ruling them out of the study.

Surface Plasmon Resonance Spectroscopy (SPRS), Quartz Crystal Microbalance (QCM) and Surface Acoustic Wave (SAW) Devices

SPRS is very mature but it is not a mature technology within single molecule detection applications so it scored a 5. It also needs to use solvents and introduces a label in order to perform single molecule detection, giving it a 5. Its lifetime is very good and it is reusable, giving it a 7.

It needs to avoid vibrations to perform the measurement so it scored a 6 for protection. And its complex system makes automation difficult, hence another score of 6.

Microcantilevers

Microcantilevers are widely used for single molecule detection and commercially available systems exist, although they do not offer a hand-held system, so they achieve a good score for maturity (a 7). However, they need to use solvents and introduce a label, giving them a 5 for sample preparation.

Their lifetime is affected by their organics which call for the need of regenerating the surface before re-use, giving them a 7. They are also very susceptible to vibrations and hence score a 5 for protection. And as they need to introduce a label they scored a 6 for automation.

Out of the labelled techniques, TIRFM scored the highest with 120, followed by FRET as well as quantum dots with 117. Microcantilevers scored lower, 110, followed by Nanoarrays with 107; and the lowest scoring were Naïve Arrays, SPRS, QCM and SAW with 102.

Table 2–16: *Summary of First Trade-Off of Labelled Techniques.*

	Maturity	Sample Preparation	Lifetime	Protection	Automation
Weight	**5**	**4**	**3**	**2**	**4**
FRET scores (Total: 117)	8	5	7	6	6
TIRFM scores (Total: 120)	9	5	7	5	6
Magnetic Beads scores (Total: 107)	6	5	7	6	6
Quantum Dots scores (Total: 117)	8	5	7	6	6
Nanoarrays scores (Total: 107)	6	5	7	6	6
Naïve arrays scores (Total: 102)	5	5	7	6	6
SPRS, QCM and SAW scores (Total: 102)	5	5	7	6	6
Microcantilevers scores (Total: 110)	7	5	7	5	6

Labeless techniques

Carbon Nanowires/Nanotubes, Ion Channels/Nanopores and Optical Microcavities were analysed during the first trade-off.

Carbon Nanowires/Nanotubes

Nanotubes are not widely used, so they scored a 6 in maturity. They need to use solvents and need to functionalise the surface to identify targets, giving them a 6 for sample preparation. Their lifetime is good, but to re-use the surface it first needs to be regenerated, so they achieved a 7 for lifetime. Those same organics that need to be regenerated are susceptible to radiation so they scored a 6 for protection. Also, they could be easily automated because though they do need a fluidics system they do not use labels, scoring a 7.

Ion Channels/Nanopores

Ion Channels are not a new concept but they are difficult to implement, so they scored a 6 in maturity. They need to use solvents and need to functionalise the surface to identify targets, giving them a 6 for sample preparation. To re-use the surface, organics have to be replaced, hence a 7 for lifetime. Moreover, it is not a fragile technology that needs extra protection but the organics will be affected by radiation so it scores a 6; and they could be easily automated hence a score of 7.

Optical Microcavities

Optical Microcavities are not widely used yet, so they scored a 6 in maturity. They need to use solvents and need to functionalise the surface to identify the target, giving them a 6 for sample preparation. Their lifetime is good, but to re-use the surface it needs to be regenerated, so they

scored a 7. Those same organics are susceptible to radiation so they achieved a 6 for protection. As they could be easily automated because they need no labels, they scored a 7.

The labeless technologies that scored the highest were Carbon Nanowires and Ion Channels with 115, followed by the Optical Microcavities with 111.

Table 2–17: *Summary of First Trade-Off of Labelless Techniques.*

	Maturity	Sample Preparation	Lifetime	Protection	Automation
Weight	**5**	**4**	**3**	**2**	**4**
Carbon Nanowires scores (Total: 115)	6	6	7	6	7
Ion Channels scores (Total: 115)	6	6	7	6	7
Optical Microcavities (Total: 111)	6	6	7	6	6

Results from First Stage Trade-Off Analysis

The highest scoring technologies were the direct measurement optical technologies (SERS, optical nanofibres and SERRS). This was to be expected, because they are mature technologies that do not need to introduce labelled components or functionalisation in order to identify the biomarkers.

Overall, the highest scoring technology was SERS with 136 (out of 180), and the lowest were the labelled receptor based technologies SPRS, QCM, SAW and Naïve Arrays with 102. Therefore it was decided that any technology with a score lower than 110 should not be considered further. This therefore ruled out from the study Magnetic Bead Microassays, Nanoarrays, Naïve Arrays, SPRS, QCM and SAW and Microcantilevers and were not considered for the second and final stage of the trade-off.

2.4.2.3 Second Trade-Off of Single Molecule Detection Technologies

For the second stage, only the technologies that had passed the first stage were considered. These were: SERS, SERRS, Optical Nanofibres, TIRFM, FRET, Quantum Dots, Nanotubes, Ion Channels, Optical Microcavities and Microcantilevers.

Direct Measurement of Inherent Physical Properties Techniques

Only optical techniques are considered within the direct measurement technique category.

Surface Enhanced Raman Scattering (SERS)

SERS has a wide dynamic range, which scored it an 8. It also scored an 8 in analytical flexibility due to its wide target acceptance; plus its sample flexibility is very wide so it scored a 9 because it can deal with volatiles, powdered forms and direct liquid (which could be melted iced flowed over the SERS surface). However, SERS needs to get the targets onto its surface, so it modifies the sample and it cannot be reconstituted (although the residue is usable by other technologies to address other science questions); so it scores a 6 in sample preservation. It is a very reliable direct technology, not based on molecular recognition events, and so scored a 9 in analytical reliability.

Surface Enhanced Resonance Raman Spectroscopy (SERRS)

SERRS has a higher dynamic range than SERRS, as it has a lower limit of detection, so scored a 9. However, it detects a narrower range of molecules so obtained a 7 in analytical flexibility. SERS and SERRS have the same sample flexibility, sample preservation and analytical reliability characteristics so they have the same scores of 9, 6 and 9 respectively.

Optical Nanofibres

Optical Nanofibres have the same dynamic range and analytical flexibility as SERS, so scored an 8 in both. They also have the same sample flexibility, sample preservation and analytical reliability characteristics as SERS and SERRS so they achieved the same scores of 9, 6 and 9 respectively giving them a total of 149.

Out of the optical methods, SERS and Optical Nanofibres scored the highest (149) followed by SERRS with 148.

Table 2–18: *Summary of Second Trade-Off of Optical Techniques.*

	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation	Analytical Reliability
Weight	**3**	**4**	**4**	**2**	**5**
SERS score (Total: 149)	8	8	9	6	9
SERRS score (Total: 148)	9	7	9	6	9
Optical Nanofibres scores (Total: 149)	8	8	9	6	9

Measurement Mediated by Molecular Recognition Events

These included both labelled and labeless methods.

Labelled techniques

Included only FRET, TIRFM, Quantum Dots and Microcantilevers for the second trade-off analysis.

Förster Resonance Energy Transfer (FRET)

The dynamic range for FRET is dependent on the photodetector and the binding properties of the receptor; therefore it achieved a 7. For analytical flexibility, assuming antibody based FRET assays are used, (as they limit the range of antibody targets), it scores lower than general antibody assays (which have a wider range) and hence scored a 6.

FRET needs more sample processing than the direct technologies, as it may need to concentrate the sample (for example to get gases into liquid form) and so scored an 8 in sample flexibility. As the sample can be released again after unbinding and reconstituting, it scored an 8 for sample preservation. It also scored an 8 for analytical reliability due to being a labelled technique (more robust than those that are labeless, but less robust than the direct techniques).

Total Internal Reflection Fluorescence Microscopy (TIRFM)

TIRFM has a wide target range, but it is also dependant on the photodetector and the binding to the receptor, so obtained a 7 for its dynamic range, like FRET. However, unlike FRET, it is not restricted by two antibodies and so achieved a 7 for analytical flexibility.

Sample flexibility, sample preservation and analytical reliability achieve the same scores as FRET due to having similar characteristics.

Quantum Dot Labelling

Quantum dots were now obviously seen not as a standalone technique, but as a materials component of fluorescence labelled assays, such as TIRFM. If TIRFM won the trade-off, Quantum Dots would be considered in their use as a label for the TIRFM instrument; however, they were not considered further on their own.

Microcantilevers

Microcantilevers have the same dynamic range as FRET and TIRFM (restricted by the binding of the receptor) and the same analytical flexibility as TIRFM due to not being restricted by two antibodies. Sample flexibility, sample preservation and analytical reliability again achieved the same scores as FRET and TIRFM due to having similar characteristics.

Out of the labelled techniques TIRFM and Microcantilevers score 137 followed by FRET with 133.

Table 2–19: *Summary of Second Trade-Off of Labelled Techniques.*

	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation	Analytical Reliability
Weight	**3**	**4**	**4**	**2**	**5**
FRET scores (Total: 133)	7	6	8	8	8
TIRFM scores (Total: 137)	7	7	8	8	8
Microcantilevers scores (Total: 137)	7	7	8	8	8

Labeless techniques

No labeless techniques were discarded by the first trade-off and hence they were analysed again during the second trade-off.

Carbon Nanowires/Nanotubes

Carbon Nanowires have the same dynamic range as TIRFM (limited by the receptor binding), same analytical flexibility (not restricted by two antibodies like FRET), same sample flexibility (may need to concentrate sample) and same sample preservation (the sample can be released again after unbinding and reconstituting). However they are a labeless, molecular recognition based technique and hence score lower in analytical reliability, a 7.

Ion Channels/Nanopores

The dynamic range of the Ion Channels is lower than that of the Nanowires and hence they scored less (a 6). The same happened with analytical flexibility as ion channels have a limited range of receptors and hence again scored low (another 6). On the other hand, Ion Channels scored the same as the Nanowires for sample flexibility (may need to concentrate sample) and sample preservation (the sample can be released again after unbinding and reconstituting) and as it is a labeless, molecular recognition based technique it scored a 7 for analytical reliability.

Optical Microcavities

Optical Microcavities scored the same as the Nanowires in all the parameters: dynamic range (limited by the receptor binding), analytical flexibility (not restricted by two antibodies like FRET), sample flexibility (may need to concentrate sample), sample preservation (the sample can be released again after unbinding and reconstituting) and analytical reliability (they are a molecular recognition based labeless technique, making them less reliable). However, as an evanescent wave guide system, it is very sensitive -thus having a dynamic range with a lower range, giving it an 8.

Out of the labeless techniques, Carbon Nanotubes and Optical Microcavities score 132 followed by the Ion channels with 125.

Table 2–20: *Summary of Second Trade-Off of Labeless Techniques.*

	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation	Analytical Reliability
Weight	**3**	**4**	**4**	**2**	**5**
Carbon Nanowires scores (Total: 132)	7	7	8	8	7
Ion Channels scores (Total: 125)	6	6	8	8	7
Optical Microcavities scores (Total: 135)	8	7	8	8	7

Results from Second Stage Trade-Off Analysis

Out of the direct measurement techniques, SERS scored the highest with 149 (out of 200) along with Optical Nanofibres. While out of the molecular recognition based labelled techniques, TIRFM and the Microcantilevers won; and out of the molecular recognition based labeless techniques, the Nanowires and the Optical Microcavities. On the other hand, the Ion Channels were the lowest scoring technologies, with a 125.

2.4.3 Discussion of Trade-Offs

The techniques identified in this document had to comply with the design requirements detailed in Table 2–2: *Design Requirements.* to determine their suitability for Space applications; hence, thorough assessment of each technology was performed and if they were found to not comply with the requirements, they were disregarded for further study.

By dividing the trade-off into two separate stages, different characteristics of the technologies were addressed. During the first trade-off the technology relative to its system complexity was assessed, while during the second stage its individual working characteristics were scrutinised.

2.4.3.1 Sensitivity analysis of the Trade-Offs

At the end of the first stage of the trade-off SERS and SERRS were identified as the winners with a score of 136 out of 180. On the other hand the lowest scoring were Magnetic Bead Microassays, Nanoarrays, Naïve Arrays, SPRS, QCM and SAW. These technologies were ruled and not considered for the second stage of the trade-off.

Table 2–21: *Summary of First Trade-Off.*

	Maturity	Sample Preparation	Lifetime	Protection	Automation	FINAL RESULTS
Weight	5	4	3	2	4	-
SERS	8	7	8	8	7	**136**
SERRS	8	7	8	8	7	**136**
Optical Nanofibres	6	7	9	8	7	**129**
TIRFM	9	5	7	5	6	**120**
FRET	8	5	7	6	6	**117**
Quantum Dots	8	5	7	6	6	**117**
Carbon Nanowires	6	6	7	6	7	**115**
Ion Channels	6	6	7	6	7	**115**
Optical Microcavities	6	6	7	6	6	**111**
Microcantilevers	7	5	7	5	6	**110**
Magnetic Bead Microassays	6	5	7	6	6	**107**
Nanoarrays	6	5	7	6	6	**107**
Naïve Arrays	5	5	7	6	6	**102**
SPRS	5	5	7	6	6	**102**
QCM	5	5	7	6	6	**102**
SAW	5	5	7	6	6	**102**

A sensitivity analysis was carried out to understand if these results were conditioned by the choice of weightings for the most important parameters, in order to understand if SERS would have been the overall winner regardless.

Therefore, all the weightings were lowered in order to understand under what scenario would another technology win; and only by reducing all of them to a value of 1 except the weighting of "lifetime" which was increased to the maximum value of 5 (as this was the only parameter for which another technology had a higher weighting than SERS and SERRS) did another technology win: Optical Nanofibres (with a score of 81 in contrast with a score of 70 for SERS and SERRS).

However, this case would be unfeasible as the maturity of a technology is always going to be more critical than its lifetime because a technology that is not ready to be flown cannot be flown – regardless of how the long-lasting technology allows for more analyses due to having a higher lifetime. Therefore, SERS' flexible as well as robust system integration qualities made it win the first trade-off.

Table 2–22: *Sensitivity Analysis of First Trade-Off.*

	Maturity	Sample Preparation	Lifetime	Protection	Automation	SENSITIVITY ANALYSIS RESULTS
Weight	1	1	5	1	1	-
SERS	8	7	8	8	7	**70**
SERRS	8	7	8	8	7	**70**
Optical Nanofibres	6	7	9	8	7	**73**
TIRFM	9	5	7	5	6	**60**
FRET	8	5	7	6	6	**60**
Quantum Dots	8	5	7	6	6	**60**
Carbon Nanowires	6	6	7	6	7	**60**
Ion Channels	6	6	7	6	7	**60**
Optical Microcavities	6	6	7	6	6	**59**
Microcantilevers	7	5	7	5	6	**58**
Magnetic Bead Microassays	6	5	7	6	6	**58**
Nanoarrays	6	5	7	6	6	**58**
Naïve Arrays	5	5	7	6	6	**57**
SPRS	5	5	7	6	6	**57**
QCM	5	5	7	6	6	**57**
SAW	5	5	7	6	6	**57**

The same sensitivity analysis procedure was applied to the results of the second trade-off, where SERS and Optical Nanofibres were the winners of the trade-off, leaving SERRS behind.

Table 2–23: *Summary of Second Trade-Off.*

	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation	Analytical Reliability	FINAL RESULTS
Weight	3	4	4	2	5	-
SERS	8	8	9	6	9	**149**
SERRS	9	7	9	6	9	**148**
Optical Nanofibres	8	8	9	6	9	**149**
TIRFM	7	7	8	8	8	**137**
FRET	7	6	8	8	8	**133**
Carbon Nanowires	7	7	8	8	7	**132**
Microcantilevers	7	7	8	8	8	**137**
Optical Microcavities	8	7	8	8	7	**135**
Ion Channels	6	6	8	8	7	**125**

Therefore, again in order to understand if other technologies would have been selected if the weightings had been different as well as to instil confidence on the results of the trade-offs, all the critical weightings (“dynamic range”, “analytical flexibility”, “sample flexibility” and “analytical reliability”) were lowered to the lowest weighting value of 1 while the least important (“sample preservation”) was raised to the maximum weighting value of 5. This caused TIRFM and Microcantilevers and Optical Microcavities to win with a score of 70, leaving SERS behind with a score of 64.

However, again this case would be unfeasible as the sample preservation capabilities of a technology are irrelevant if the technology is not going to reliably detect and analyse the sample. Hence, the reliability of SERS is preferable to its reduced capabilities of sample preservation compared to other technologies as it is of course better to destroy the sample after reliable detection rather than preserving the sample for further analyses with a reduced level of confidence in these analyses.

Therefore, SERS’ reliable analytical capabilities made it also win (jointly with Optical Nanofibres) the second trade-off.

Table 2–24: *Sensitivity Analysis of Second Trade-Off.*

	Dynamic Range	Analytical Flexibility	Sample Flexibility	Sample Preservation	Analytical Reliability	FINAL RESULTS
Weight	1	1	1	5	1	-
SERS	8	8	9	6	9	**64**
SERRS	9	7	9	6	9	**64**
Optical Nanofibres	8	8	9	6	9	**64**
TIRFM	7	7	8	8	8	**70**
FRET	7	6	8	8	8	**69**
Carbon Nanowires	7	7	8	8	7	**69**
Microcantilevers	7	7	8	8	8	**70**
Optical Microcavities	8	7	8	8	7	**70**
Ion Channels	6	6	8	8	7	**67**

As SERS won the first trade-off as well as the second trade-off jointly with Optical Nanofibres (while Optical Nanofibres only won the second trade-off jointly with SERS), SERS was the overall winner of the trade-off analysis.

The scores of both trade-offs were normalised to a total score of 200, added and presented in the table below.

Table 2–25: *Total Scores for the Complete Trade-Off Analysis.*

Technology	Score out of 400
SERS	**300**
SERRS	299
Optical Nanofibres	292
TIRFM	270
FRET	263
Carbon Nanotubes/Nanowires	260
Microcantilevers	259
Optical Microcavities	255
Ion Channels	253

2.5 Stand-Alone SERS *vs.* Coupled SERS

Although Mass Spectrometry was not considered in the trade-offs, it was considered to be a key technology with both terrestrial and Space heritage during the International Workshop held at Cranfield University in March 2009 for detecting “unknown unknowns” in their Tandem form.

Therefore, it was envisaged that the instrument design of the winning novel technology from the trade-offs (SERS) should be considered both as a Stand-Alone system and as part of a Tandem Mass Spectrometry (MS/MS)-SERS system.

In order to recommend a suitable design for both systems a suitable analysis had to be performed: first a systems level analysis was carried out followed by a component level analysis in order to propose the final design of the system.

2.5.1 Design Concept Process Methodology

The following strict process methodology was performed:

System Level Analysis:

- An overview of the system was analysed to understand what components were needed and their interdependencies.
- A background to the system and its components was researched and the final components were proposed.

Component Level Analysis:

- During the component analysis each component was appropriately described, with its inputs, outputs and defining parameters to perform its function in the system.
- Specific component examples were researched in single molecule/ultra low lower limit detection peer-reviewed literature that could be used to build the system.

The strategy to find specific components for Stand-Alone SERS and MS/MS-SERS was to:

1. Identify the key components that have to be found for each technology, *i.e.,* components, specific working ranges, etc.
2. Review the documents compiled for the creation of literature review.
3. Find the companies mentioned in the literature review or if no companies had been mentioned in the references, relevant companies were found by searching for the specific components being sought after using general internet sources, *i.e.* Google Search Engine general Web search, to find the websites of the companies providing relevant components or extra information on specific components.

2.5.2 In-Depth Background of MS, MS/MS and SERS Systems

In order to propose a suitable design for MS/MS-SERS, a more in-depth background to MS, MS/MS and SERS had to be performed first to understand the best way of coupling the technologies.

2.5.2.1 In-depth background to MS

Mass spectrometry comprises a wide range of technology implementations that act as analytical tools to determine for, a given sample, one or more of the molecular mass of molecular components, the structure of a molecular components and the concentration of molecular components. The molecular masses of large samples such as biomolecules can be measured to within an accuracy of 0.01% of the total molecular mass of the sample, *i.e.* within a 4 Daltons (Da) or atomic mass units (amu) error for a sample of 40,000 Da. This is sufficient to allow minor mass changes to be detected, for example during the substitution of one amino acid for another or a post-translational modification. On the other hand, molecular masses for small organic molecules can be measured to within an accuracy of 5ppm or less, which is sufficient to confirm the molecular formula of a compound.

Each type, or technological implementation, of a mass spectrometer can be divided into four fundamental parts: the inlet sample system, the ionisation source, the mass/charge ratio analyser and the detector. The analyser and detector of the mass spectrometer, and often the ionisation source, are maintained at low gas pressures to allow the ions to travel from one end of the instrument to the other without any hindrance from background gas molecules.

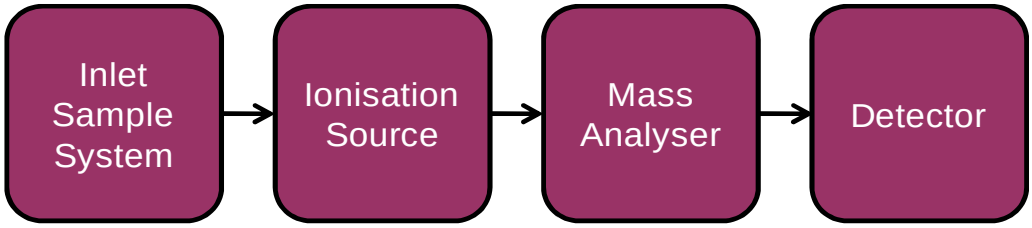


Figure 2-1: *Top-Level MS System.*

The components of a mass spectrometer are now summarised.

The inlet sample system

All 3 types of common samples (gases, liquids and solids) have to be introduced into the system as gas-phase species; each type of sample is introduced according to different techniques. The following are examples of direct inlets:

- Gas samples: if the gas is at atmospheric pressure, it can be leaked into the reduced pressure system through a restriction to maintain a low pressure internal operational environment.
- Liquid samples: these need to be introduced in such a way that they are rapidly vaporised -for example bleed into the reduced pressure system through a capillary (restricted port) and in which the sample liquid volatilises.
- Solid samples: these need to be introduced by vaporising into gaseous ions -for example by using electrical discharges of high frequency and high power (e.g. 100kW at 100MHz).

For routine analytical use, MS techniques are often hyphenated to chromatographic or other separation techniques to separate complex sample mixtures into individual components as a

function of time residency within the separation system and thereby easing the subsequent MS analysis of the sample components.

Common examples of separation techniques are:

- Gas Chromatography (GC): a gas chromatograph is used to separate different compounds in the gas-phase and the resulting separated stream of separated compounds is fed into the ion source.
- Liquid Chromatography (LC): in LC-MS / HPLC-MS (High Pressure Liquid Chromatography-MS), a liquid chromatograph separates compounds in liquid phase (usually a mixture of water and organic solvents) before they are introduced into the ion source.
- Ion Mobility (IM): gas-phase ions are separated by a drift time through a neutral gas under an applied electrical potential prior to input into a MS.

It is worth noting that for some ionisation sources such as MALDI and Electrospray, the functions of the inlet sample system and the ionisation source are intimately combined and should not be treated as two physically separate components.

The ionisation source

Once a sample has been introduced into the ionisation source of the instrument, the sample molecules are ionised to enable their subsequent handling in the mass/charge ratio analyser component. As briefly mentioned above, the method of sample introduction to the ionisation source often depends on the ionisation method being used, as well as the type and complexity of the sample. The sample can either be inserted directly into the ionisation source, or undergo a time-domain separation before entering the ionisation source. This latter method of sample introduction usually involves the mass spectrometer being coupled directly to a High Pressure Liquid Chromatography (HPLC), Gas Chromatography (GC) or Capillary Electrophoresis (CE) separation column, and hence the sample is separated into a series of components which then enter the mass spectrometer sequentially for individual analysis.

With most ionisation methods there is the possibility of creating both positively and negatively charged sample ions, depending on the proton affinity of the sample. Before starting an analysis, the user must decide whether to detect the positively or negatively charged ions.

Ionisation techniques are typically carried out in vacuum (reduced pressure) or atmospheric pressure. The following are vacuum ionisation techniques:

Electron Ionisation (EI):

- This is the most common method used for analysis of gases and organic volatile compounds. Energetic electrons interact with gas phase atoms or molecules to produce ions by colliding the neutral molecules of the sample with electrons emitted from a filament (made of carbonised tungsten, tantalum or coated with oxide) by a thermionic process. Electrons are drawn off by a pair of positively charged slits to pass into the body of the chamber (Khandpur, 2007).
- Ejection of the most weakly held electrons leads to positive ions. Negative ions are formed in smaller quantity and by a different mechanism; therefore this technique is used in the positive ion mode.
- The standard energy of ionisation (for example 70eV) is defined by the potential difference between the filament and the source housing and its efficiency is around 1 ion produced for every 10,000 molecules. To reduce fragmentation, the electron energy can be chosen according to the ionisation potential of the neutral molecule (*e.g.,* 10-15eV for organic molecules).

Chemical Ionisation (CI):

- This is a lower energy process than electron ionisation therefore it yields less fragmentation and typical CI spectra have an easily identifiable molecular ion.
- Ions are produced through the collision of the analyte with ions of a reagent gas (*e.g.,* methane, ammonia and isobutene) that are present in the ion source. As inside the ion source the reagent gas is present in large excess compared to the analyte, electrons entering the source will ionise the reagent gas. The resultant collisions with other reagent gas molecules create an ionisation plasma and positive and negative ions of the analyte are formed by reactions with this plasma.

Fast Atom Bombardment (FAB):

- The material to be analysed is mixed with a non-volatile chemical protection environment matrix typically made of glycerol, thioglycerol or 3-nitrobenzyl alcohol and is bombarded under vacuum with a high energy (*e.g.,* 4000 to 10,000eV) beam of non-ionised heavy atoms from an inert gas such as argon or xenon.
- As the sample on the probe is ionised when bombarded by the fast atom beam, this technique causes ionisation of the matrix which leads to a large amount of background noise. This makes the study of small ions impossible.

Matrix-Assisted Laser Desorption Ionisation (MALDI):

- This is a soft ionisation technique that allows the analysis of biomolecules and large organic molecules which tend to be fragile and fragment when ionised by conventional ionisation methods. It is similar to EI both in relative softness and ions produced, although it causes fewer multiply charged ions.
- Ionisation is triggered by a laser beam (*e.g.* a nitrogen laser) hence a solid matrix (*e.g.,* dihydroxybenzoic acid) is used to protect the biomolecule from being destroyed by the direct laser beam. The laser energy strikes the crystalline matrix to cause its excitation and the subsequent ejection of analyte species which are desorbed and ionised in the gaseous state.
- Pulsed ionisation is used with Time-Of-Flight mass analysers for bio-macromolecules but analysis of small molecules of less than 500Da is limited as the matrix decomposes upon absorption of the laser radiation –although to overcome this disadvantage porous silica can be used as the matrix.
- MALDI can be coupled with Electrospray ionisation to create the MALDESI technique.

The following are atmospheric pressure ionisation (API) techniques:

Thermal Ionisation (TI) or surface ionisation:

- Chemically purified material is loaded onto a filament which is heated to cause some of the material to be ionised as it boils off the hot filament.
- Filaments are generally flat pieces of metal around 1-2mm wide, 0.1mm thick, bent into an upside down U shape and welded to steel posts that supply the current.
- The hot filaments reach a temperature of less than 2500°C, leading to the inability to create atomic ions of species with high ionisation energy such as Osmium and Tungsten.

Electrospray Ionisation (ESI or ES):

- The analytes of interest are dispersed by Electrospray into a fine aerosol as a high voltage is applied to a liquid to form small and highly charged liquid particles which are radially dispersed due to Coulomb repulsion.
- This technique is normally used with LC-MS. The use of volatile solvents and buffers in HPLC provides a powerful separation technique which is compatible with ES-MS (LC-MS) and ES-MS/MS (LC-MS/MS). The use of LC-ES-MS/MS can often offer significant improvement in selectivity, specificity and sensitivity during quantitative analyses over similar LC-ES-MS applications.

Photoionisation (PI):

- Incident photons eject one or more electrons at around 10eV and a UV lamp is used for the photoionisation detection.
- As this technique yields few fragments, it is only interesting for use with molecules of low polarity.

Thermospray:

- Ionisation is achieved by passing a pressurised solution through a heated tube which partially vaporises the effluent to generate a spray prior to entering the ion source. Droplets from the spray contain a statistical imbalance of charges originating from charged solutes present in the solution.
- The droplets gradually decrease in size by evaporation of the neutral solvent molecules until the droplet reaches a size at which the charge repulsion forces overcome the cohesive forces of the droplet.

Atmospheric Pressure Chemical Ionisation (APCI):

- APCI allows for the high flow rates typical of HPLC to be used directly (without having to divert the larger fraction of volume to waste). It is a less soft technique than ESI as it generates more fragment ions relative to the parent ion.
- Typically the mobile phase containing the analyte is heated to high temperatures (*e.g.*, 400°C) and sprayed with high flow rates of nitrogen. The entire aerosol cloud is subjected to a corona discharge that creates ions.

Field Desorption (FD) or Field Ionisation (FI):

- Desorption techniques provide chemical information through rapid, definitive analysis. A high potential electric field is applied to an emitter with a sharp surface such as a razor blade or a filament from which tiny whiskers have been formed. This results in a very high electric field which can result in ionisation of gaseous molecules of the analyte. Mass spectra produced by FI have little or no fragmentation and it is dominated by molecular radical cations and protonated molecules.
- DESI (field desorption with ESI) allows ambient ionisation of a trace sample at atmospheric pressure with little sample preparation. Ionisation takes place by directing an electrically charged mist to the sample surface that is a few millimetres away. The Electrospray mist is attracted to the surface by applying a voltage on the sample holder and after ionisation the ions travel through air into the atmospheric pressure interface which is connected to the mass spectrometer.

Direct Analysis in Real Time (DART):

- DART is another desorption technique that provides the ability to analyse chemicals of wide-ranging volatilities by positioning a surface near the sampling inlet of an API-equipped MS.
- DART can instantaneously ionise gases, liquids and solids in open air under ambient conditions and has no need for sample preparation. It also allows for ionisation to occur directly on the sample surface as collision between electronically or vibronically excited atoms and the source of the sample provokes an energy transfer which turns the excited gas molecule into neutral form while an electron is released from the sample.
- DART is a very versatile technique as it can analyse a wide range of chemicals from warfare agents to pharmaceuticals, peptides, explosives and drugs.

In summary, many ionisation methods are available and each has its own advantages and disadvantages. The ionisation method to be used depends on the type of sample under investigation and the mass spectrometer available. The ionisation methods used for the majority of biochemical analyses are Electrospray Ionisation (ESI) and Matrix Assisted Laser Desorption Ionisation (MALDI) because very little fragmentation is produced.

Also, the compatibility of different analysers with different ionisation methods varies. For example, quadrupoles, TOF analysers, magnetic sectors, and both Fourier transform and quadrupole ion traps can be used in conjunction with Electrospray ionisation, whereas MALDI is not usually coupled to a quadrupole analyser.

Table 2–26: *Sample Types Compared with Chromatographic Interfaces, Ionisation Methods and Mass Spectrometers* (Ashcroft, 1997).

Sample classes	*Chromatographic interfaces*	*Ionization methods*	*Mass spectrometer*[a]
proteins, peptides, oligonucleotides, oligosaccharides	LC, CE	ES	M, Q, TOF
	low flow LC, CE	FAB/FIB/LSIMS	M, Q
	none	MALDI	TOF
polar organic compounds	GC	CI	M, Q
	LC, CE	ES	M, Q
	LC	APCI	M, Q
	low flow LC, CE	FAB/FIB/LSIMS	M, Q
	none	FD/FI	M, (Q)
	LC	TSP	M, Q
non-polar organic compounds	GC, particle beam LC	EI	M, Q
	GC	CI	M, Q
	none	FD/FI	M, (Q)
synthetic polymers	none	FD/FI	M, (Q)
	none	MALDI	TOF

[a] M = magnet; Q = quadrupole; TOF = time-of-flight.

The analyser

Once the ions are extracted into the analyser region of the mass spectrometer, they are separated according to their mass to charge ratios (m/z). There are a number of mass analysers currently available and they have different features, including the m/z range that can be covered, the mass accuracy, and the achievable resolution.

The different analyser techniques are as follows:

Magnetic Sector:

- Uses an electric and/or magnetic field to affect the path and/or velocity of the charged particles therefore bending the trajectories of the ions as they pass through the analyser. Deflection occurs according to the m/z ratios of the ions, as the lighter the ions are the more deflected they will be. This instrument can be used to select a narrow range of m/z ratios or to scan through a range of m/z ratios to catalogue the ions present.

Time-Of-Flight (TOF):

- Uses an electric field to accelerate ions through a potential and measures the time they take to reach the detector. If the particles have the same charge, the kinetic energies will be identical and their velocities dependant only on the mass; therefore, the lighter ions reach the detector first. MALDI is a readily compatible ionisation technique used with TOF.

Quadrupole:

- Uses oscillating electrical fields to selectively stabilise or destabilise the paths of ions passing through a radio frequency quadrupole field. Therefore, only a single m/z ratio is passed through the system at any time but changes to the potentials on magnetic lenses allow a wide range of m/z values to be swept rapidly, either continuously or in a succession of discrete hops. The instrument acts as a mass selective filter and is closely related to the Quadrupole ion trap (QIT) but it is designed to pass un-trapped ions rather than collect the trapped ones; thus it is called a Transmission Quadrupole.
- Triple quadrupole is a common variation; it has three quadrupoles arranged in series to incoming ions. The first quadrupole acts as a mass filter, the second as a collision cell where selected ions are broken into fragments, and the third scans the resulted fragments (note an example of a tandem MS system to be discussed later)

Quadrupole Ion Trap (QIT):

- This technique has the same physical principles as a quadrupole mass analyser, but ions are trapped and sequentially ejected.
- Ions are created and trapped in a mainly quadrupole RF potential and separated by m/z, non-destructively or destructively. There are many m/z separation and isolation methods but mass instability is the most commonly used; *i.e.*, the RF potential is ramped so that the orbit of ions with a mass a>b are stable while ions with mass b become unstable and are ejected on the z-axis detector. Ions can also be ejected by the resonance excitation methods, where a supplemental oscillatory voltage is applied to the endcap electrodes and the trapping voltage amplitude and/or excitation voltage frequency is varied to bring ions into a resonance condition in order of their m/z ratio.
- Derivatives include the cylindrical ion trap (CIT) mass spectrometer, which has a cylindrical rather than a hyperbolic ring electrode configuration; and the linear quadrupole ion trap (LQIT), which traps ions in a two dimensional quadrupole field rather than in a three dimensional field.

Fourier Transform Ion Cyclotron Resonance (FTICR):

- This instrument measures mass by detecting the image current produced by ions cyclotroning in the presence of a magnetic field. It does this by measuring the ions injected into a Penning trap (a static electric/magnetic ion trap). It has high sensitivity since each ion is counted more than once and much higher resolution and precision.
- Detectors at fixed positions in space measure the electrical signal of ions which pass near them over time, producing a periodic signal. Since the frequency of an ion's cycling is determined by its m/z ratio, this can be de-convoluted by performing a Fourier transform on the signal. Therefore, ions are detected when they pass near the detectors, rather than by hitting them.
- Ion cyclotron resonance is an older mass analysis technique similar to FTMS except that ions are detected with a traditional detector. Ions trapped in a Penning Trap are excited by an RF electric field until they impact the wall of the trap, where the detector is located. Ions of different mass are resolved according to impact time.

Orbitrap:

- Ions are injected tangentially into the electric field between the electrodes and trapped as their electrostatic attraction to the inner electrode is balanced by centrifugal forces. This makes the ions cycle around the central electrode in rings.
- Ions of a specific m/z ratio move in rings which oscillate along the central spindle and the frequency of these harmonic oscillations is independent of the ion velocity and is inversely proportional to the square root of the m/z ratio. By sensing the oscillation similarly to FTICR-MS, the trap can be used as a mass analyser.
- Orbitraps have a high mass accuracy (1-2ppm), high resolving power (up to 200,000) and a high dynamic range (around 5000).

The detector

The detector monitors the ion current, amplifies it and the signal is then transmitted to the data system where it is recorded as mass spectra. The m/z values of the ions are plotted against their intensities to show the number of components in the sample, fragmentation, the molecular mass of each component, and the relative abundance of the various components in the sample.

The type of detector is supplied to suit the type of analyser; the more common ones are the photomultiplier, the electron multiplier and the micro-channel plate detectors.

Electron Multipliers:

- These are comprised by a vacuum tube that multiplies incident charges due to the secondary emission created by a single electron hitting a secondary emissive material (which roughly produces 1 to 3 electrons).
- If an electric potential is applied between the plate with the secondary emissive material and another, the emitted electrons will accelerate to the next metal plate and induce secondary emission of more electrons again. This can be repeated, producing an avalanche of electrons collected by a metal anode. Detection occurs as the avalanche is triggered by any charged particle hitting the starting electrode with sufficient energy to cause secondary emission.

Faraday Cups:

- These are metal cups designed to catch charged particles in vacuum. The resulting current can then be measured and used to determine the number of ions or electrons hitting the cup.

Daly Detector:

- These are gas phase ion detectors that consist of a metal knob, a scintillator (a phosphor screen) and a photomultiplier. Therefore the ions that hit the knob release secondary electrons and a high voltage between the knob and the scintillator accelerates the electrons onto the scintillator where they are converted to photons. These photons are then detected by the photomultiplier.

Microchannel Plate Detectors:

- These are closely related to electron multipliers as both intensify single particles or photons by the multiplication of electrons via secondary emission.
- A micro-channel plate is made from highly resistive material in which each micro-channel is a continuous-dynode electron multiplier.

Mass Spectrometer Examples in Space Exploration

Mass spectrometers have been widely used for analysis in planetary exploration missions, examples include:

- For the Mars Viking 1 and Viking 2 missions, GC-MS (magnetic sector) instruments were carried on-board to detect small concentrations of organic compounds in soil samples (Biemann, 1977).
- Cassini-Huygens (2005) delivered a specialised GC-MS composed of an Ion and Neutral Mass Spectrometer (INMS) through the atmosphere of Titan on-board the Huygens probe as well as having an INMS on-board the Cassini-Huygens spacecraft taking measurements of Titan's and Enceladus' atmospheric composition (NASA, 2008).
- The Rosetta Spectrometer for Ion and Neutral Analysis (ROSINA) is the main mass spectrometer on the Rosetta mission to explore Comet 67P/Churyumov-Gerasimenko when it enters its orbit in 2014. It uses RTOF (Reflectron Time-Of-Flight) as TOF instruments have the inherent advantage that the entire mass spectra is recorded at once without the need of scanning the masses by varying some particular instrument parameter, *e.g.*, the magnetic field (Korth and Mall 2006). The MODULUS PTOLEMY is an Ion Trap MS on-board the lander that will contribute to the characterisation of the solid and volatile cometary materials.
- Beagle 2 carried a 90 degree Magnetic Sector instrument designed to distinguish between the two stable isotopes of carbon and quantify their ratios (Beagle2, 2004).
- ExoMars' GC-MS Mars Organic Molecular Analyser (MOMA) instrument has the task to detect and identify as many molecular species as possible at low concentrations (ppb to ppt) with high analytical specificity of, for example, volatile molecules in atmospheric and sedimentary material. The MS is an Ion Trap Mass Spectrometer (ITMS) which uses electron ionisation (ESA, 01/02/2008).

2.5.2.2 In-depth background to MS/MS

Tandem mass spectrometry (MS/MS), sometimes termed multidimensional mass spectrometry (MS^n), is any mass spectrometry method involving two or more stages of mass analysis. Additionally between analysis stages, additional fragmentation/dissociation/chemical reaction processes occur to modify the output of the preceding stage as input into the proceeding stage. For the current application, this approach enables relatively complex mixtures of sample components to be analysed directly by MS as the first MS stage can act as a mass selector / separator outputting specific parent ions into a second stage for identification. This approach enables avoidance of a traditional chromatography front-end to a single stage MS.

The two common approaches to tandem MS occur are:

- Performance in *space* (by linking two physically discrete mass analysers and separated by a further ionisation/fragmentation/chemical reaction chamber).
- Performance in *time* (by utilising a single mass analyser -typically an ion trap based analyser- to perform in a single volume the multiple stages of ionisation/fragmentation/chemical reaction and mass analysis separated in the time domain).

Simplistically, performance in time is the desirable approach for Space applications as only a single mass analyser would be flown.

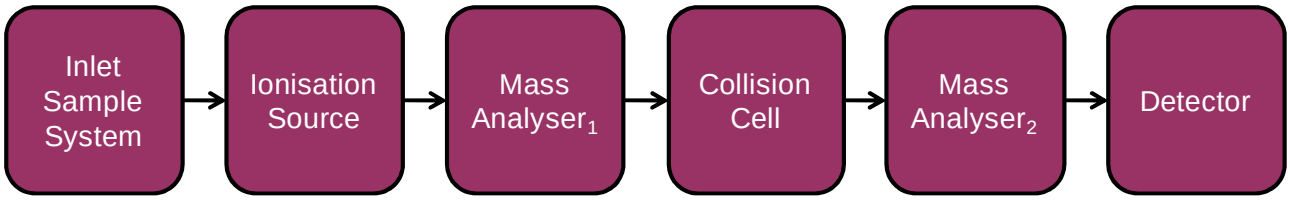


Figure 2-2: *Top-Level MS/MS System in Space.*

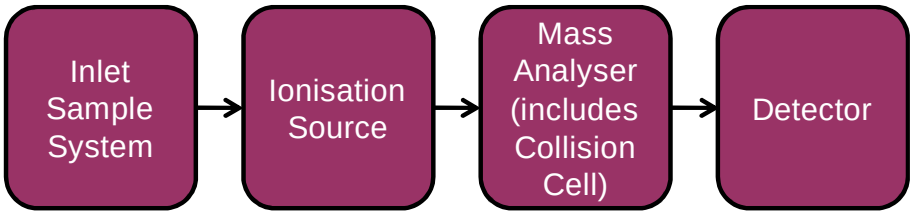


Figure 2-3: *Top-Level MS/MS System in Time.*

The basic modes of data acquisition for tandem mass spectrometry experiments are as follows:

Product or daughter ion scanning:

- The first mass analysis step is used to select user-specified sample ions arising from a particular component. These are then further processed -*e.g.* by bombardment by additional gas molecules, which cause fragment ions to be formed. Then, after a second mass analysis step, all the fragment ions arise directly from the precursor ions specified in the experiment, and thus produce a fingerprint pattern specific to the compound under investigation.
- This type of experiment is particularly useful for providing structural information concerning small organic molecules and for generating peptide sequence information (*i.e.,* this would be a key analysis mode for the current study).

Precursor or parent ion scanning:

- The first mass analysis step allows the transmission of all sample ions, whilst the second mass analysis step is set to monitor specific fragment ions, which are generated by appropriate processing of the first analysis step.
- This type of experiment is particularly useful for monitoring groups of compounds contained within a mixture which fragment to produce common fragment ions.

Constant neutral loss scanning:

- This involves both mass analysis steps scanning and collecting data across the whole m/z range, but the two analysis steps are off-set so that the second analysis step allows only those ions which differ by a certain number of mass units (equivalent to a neutral fragment) from the ions transmitted through the first analyser.
- For example, this type of experiment is used to monitor all of the carboxylic acids in a mixture as carboxylic acids tend to fragment by losing a neutral molecule of carbon dioxide, which is equivalent to a loss of 44Da. As all ions pass through the first analyser into the collision cell, the ions detected from the collision cell are those from which 44Da have been lost.

Selected/multiple reaction monitoring:

- Both of the mass analysis steps are static as user-selected specific ions are transmitted through the first mass analysis step and user-selected specific fragments arising from these ions are measured by the second mass analysis step. The compound under scrutiny must be known and have been well-characterised previously before this type of experiment is undertaken.
- This methodology is used to confirm unambiguously the presence of a compound in a matrix *e.g.* drug testing with blood or urine samples. It is not only a highly specific method but also has very high sensitivity.

The most common usage of MS/MS in biochemical areas is the product or daughter ion scanning experiment which is particularly successful for peptide and nucleotide sequencing. Tandem mass spectrometry also enables specific compounds to be detected in complex mixtures on account of their specific and characteristic fragmentation patterns. Allain (2001) declares that MS/MS has been used for many years to identify and measure carnitine esters in blood and urine of individuals suspected of having a metabolic disorder and allows for improvement in and consolidation of methods for detecting amino acid disorders. Hannon (2001) describes how MS/MS is widely used for newborn screening since the 1990s due to its high specificity, sensitivity and less laborious sample preparation time than traditional newborn screening methods. Also, tandem mass spectrometry is estimated to reduce the false positive rate seen with traditional testing methodologies from about 1.5% to about 0.26%.

Two, three and four analysers have all been incorporated into commercially available tandem instruments (not necessarily of the same type), *i.e.* hybrid instruments can be developed. More popular tandem mass spectrometers include those of the Quadrupole-Quadrupole, Magnetic Sector-Quadrupole, Magnetic Sector-Magnetic Sector, Ion Trap-Ion Trap and more recently, Quadrupole-Time-Of-Flight geometries. Therefore, a separate analysis to understand which Tandem MS system would be more suitable for Space applications had to be carried out.

MS/MS for Space Applications

As there are many combinations of tandem MS instruments, in order to clearly propose an MS/MS-SERS instrument for Space applications requirements were set to understand how the MS/MS component had to be designed.

Table 2–27: MS/MS *Requirements.*

Reference	Requirement
MSMSR1	The experiment shall have a wide m/z range. • This is due to need for detection of organic molecular biomarkers and organic molecular markers of abiotic and prebiotic chemistry (typically from few amu to 1000 amu).
MSMSR2	The experiment shall have an ultra-low lower limit of detection. • Due to the need to detect very low concentrations of biomarkers (typically ppt levels in original sample).
MSMSR3	The experiment shall be compatible with development into a Space flyable instrument. • Therefore, the MS/MS system must be compatible with a portable implementation, *i.e.,* it has to be compatible with non-laboratory/field use as well as complying with PP&CC requirements (although this can be implemented further along in the design of the instrument). • Also, as was suggested in the Single Molecule Detecting and Counting for Space Applications International Workshop held in March 2009, Tandem MS should be used as opposed to using a separation technique coupled with MS.
MSMSR4	The experiment shall be based upon existing practical demonstrations due to maturity arguments. • *e.g.,* there should be published peer-reviewed articles that demonstrate the particular MS/MS format in at least a breadboard, or ideally in fieldable prototype, form.

It was immediately apparent that MSMSR1 and MSMSR2 are readily complied with by the majority of MS/MS systems and requirement MSMSR4 allows for all demonstrated MS/MS technologies.

However, requirement MSMSR3, “the MS/MS instrument must be compatible with development into a Space flyable instrument” was the most restrictive of all and hence the requirement that could filter out non-relevant technologies.

Therefore, portable MS/MS in peer-reviewed literature had to be researched.

Portable MS/MS in Peer-Reviewed Literature.

Portable MS/MS is becoming popular for applications such as the direct detection and quantification in real-time of toxic chemical compounds in air (both for industrial and security threats). The instruments for these applications need to have a wide range of detection for as many chemicals as possible, be able to operate in ambient conditions, ideally with no sample preparation, with quick analysis and very low probability for false positives and especially false negative responses.

As MS/MS instruments have high sensitivity, selectivity and broad applicability, they are ideal fieldable devices. Although much more needs to be done for miniaturising and ruggedising the instruments, significant effort has gone into miniaturising quadrupole ion traps, ion cyclotron resonance, TOF, magnetic sector and linear quadrupole devices. Normally though, only the

analyser can be miniaturised rather than the whole mass spectrometer due to the fact that the vacuum system tends to occupy a lot of volume and mass.

Many companies have released portable instruments in the form of GC-MS where the MS tends to be an EI-CIT, TOF or a quadrupole analyser. However, as has been mentioned before, separation techniques will not be considered as they add more complexity, volume and mass to the system. Also, higher analysis speeds can be achieved by using MS/MS and performing CID: Wells (2008) summarises that the confidence associated with true MS/MS (isolation followed by on-resonance excitation and dissociation) is the same as that provided by GC-MS but with 100-fold shortened analysis times.

Mulligan (2006) declares that MS/MS based both on collision-induced dissociation and on selective atmospheric pressure ion/molecule reactions can be used to increase selectivity and sensitivity. And as ion traps are the only ones that so far adequately meet portability requirements due to small analyser size, simple ion optics, ultra high sensitivity and selectivity, these are the ones that are currently used for fieldable MS/MS instruments. Therefore, for this study, only ion traps will be considered further and other analysers are ruled out.

Of the ion traps, three types of ion traps were considered: Quadrupole Ion Trap (QIT), Linear Quadrupole Ion Trap (LQIT) and Cylindrical Ion Trap (CIT).

It must be noted that although lab-based ion traps are smaller than other mass spectrometers, the size, weight and power consumption is determined by the large, resonantly tuned RF coil and power amplifier circuitry that provides the primary ion-trapping voltage (Lammert, 2001). Therefore, even if reductions in the RF power requirements offer small reductions in the size of the ion trap as smaller ion traps require significantly lower voltages and less power than larger traps (and this allows for simplification and miniaturisation of the instrumental electronics); as the volume of the ion trap decreases, the number of ions that can be stored decreases (although this problem can be alleviated by using arrays of traps).

In order to select which ion trap would be more suitable for the MS/MS system for Space applications, another trade-off needed to be performed. Therefore, analysis of the requirement for portability was further explored by the following parameters:

- Consumables needed
- Use of bleed gas?
- Vacuum requirements.
    - Need low mass, high performance vacuum?
    - Tolerant to crude vacuums?
- Extent of non-lab use in MS/MS form.
    - Is there a portable example of the technology?
- Sample utilisation efficiency.
    - Can it hold and selectively remove molecules as needed?
    - Captures total sample and detects as function of time?
    - Or wastes sample?
- Ionisation source needed.
    - Is it too complex for portable applications?

To conduct a trade-off, weightings are assigned to each parameter in order to select the appropriate technology for Space applications. However, initial analysis of the parameters yielded that "Expanse of non-lab use" was the most important to quantify portability. Therefore, if CIT, QIT or LQIT were found to not have any portable examples, they had to be immediately ruled out for non-compliance. In other words, only if 2 or more of these technologies were found to be portable would the full trade-off have to be performed to further analyse the most suitable instrument for Space applications.

MS/MS System Selection

Several groups have investigated the performance of miniature Ion Trap mass analysers; a summary of developments for each type of Ion Trap is presented below.

QIT

- The first miniature quadrupole ion trap (QIT) was built in a collaborative project between Riter (2002) and Los Alamos National Laboratory where 2.5mm inner radius ion traps were constructed to extend the mass-to-charge range, and ions up to 70,000m/z were observed (although with poor mass resolution).
- Therefore Riter (2002) declared that because of the demanding machining requirements of the hyperbolic surfaces, QITs were difficult to reduce in size any further.
- No examples of fieldable QIT instruments were found.

LQIT

- LQITs take advantage of the fact that ions are forced to the centre of the quadrupole assembly and the ion cloud is retained focused to a line (rather than a point, like in 3D quadrupole traps).
- This gives higher ion storage volume but as the ions are dispersed, they do not theoretically allow for mass analysis - although Bier and Syka (1985) filed a patent where this problem was solved by ejecting the ions through a slit in one of the rods onto a linear multichannel plate detector, (patent commercialised in 2002).
- Since then, various geometries have been investigated including the "race-track" and Toroidal geometries.
- However, no evidence a fieldable LQIT example was found.

CIT

- Kornienko (1999) reported the building of CITs with radii of 0.5mm, Meaker (2001) reported CITs with a radius of 3.2mm and then Mulligan (2006) developed a portable instrument with a mass range of ~m/z 450 suitable for monitoring organic compounds in ambient air.
- This instrument, coupled with ion sources that can operate at atmospheric pressure, gives the ability for quadrupole ion traps to tolerate higher pressures than other typical mass analysers (~10^{-5} Torr), which helps reduce the demands of the vacuum system (which then allows for miniaturisation).
- In 2002 Riter also used a portable CIT-MS/MS and even CIT-MS/MS/MS to detect p-Nitrotoluene, Acetophenone, Methyl Salicylate and DMMP.
- Wells (2008) also used CIT-MS/MS to detect explosives with a portable instrument.

As portability was the driver, examples of already fieldable and useable Ion Traps were researched, and as CIT appeared to be the only reported example of a fieldable implementation, it was the type of mass spectrometer selected for Space applications.

The ionisation source for the tandem MS CIT system had to then be defined and peer-reviewed literature was searched again to find examples. With this search, examples of the soft ionisation technique Electro Spray was found (for liquid samples), as well as APCI, Thermal Ionisation, DESI and DART. As differing analysis require differing ionisation sources for the MS, the ionisation source needed to be further investigated for its use with CIT-MS/MS.

Due to the fact that the ions can be held for long periods of time in the ion traps, they give an easy opportunity to fragment the parent ions formed from the soft ionisation technique of ES (for liquid samples). However, Mulligan (2006) describes how the best technique for a portable CIT is APCI due to its low molecular fragmentation, beneficial for specificity and for analysis of complex mixtures.

Yet on the other hand, the study on API ionisation sources that Wells (2008) performed concludes that out of ESI, DESI and DART, DESI and DART are the most suitable for the CIT fieldable instruments. This is due to the fact that, as desorption techniques, both provide the ability to analyse chemicals of wide-ranging volatilities by simply positioning a surface near the sampling inlet of an API-equipped MS and allow analysis with no sample preparation immediately after collection. As another example, Riter (2002) uses thermionic emission (thermal ionisation, TI) from a rhenium filament as the ionisation source for the CIT-MS/MS instrument.

Therefore, it can be seen how differing analysis require differing ionisation sources for the MS, and it can only be concluded that the ionisation source needs to be further investigated with use of CIT-MS/MS. However, it must be noted that Johnson (2007) warns that the large presence of salts on the Martian surface must be considered for selection of the ion source as large amounts of solvated ions can interfere with Electrospray ionisation of analytes. Therefore, this rules out ES, leaving DART and TI as the only possible sources.

As the most recent portable MS/MS (CIT) instrument study Wells (2008) strongly supports the use of DART for its portability, fast response and lack of sample preparation, this is the ionisation source considered further in this study.

2.5.2.3 Background to SERS

As detailed in Section 2.3: “Literature Review of Single Molecule Detection and Counting Technologies”, when a light source excites the molecules on the SERS surface, the surface plasmons created by the incident light on the metal surface oscillate and create scattering when the oscillation is perpendicular to the surface plane (which is achieved by roughening the metal surface).

A typical SERS system consists of a light source exciting a SERS surface, a light detector with optics to filter unwanted frequencies, and a light analysis instrument to interpret the received signals.

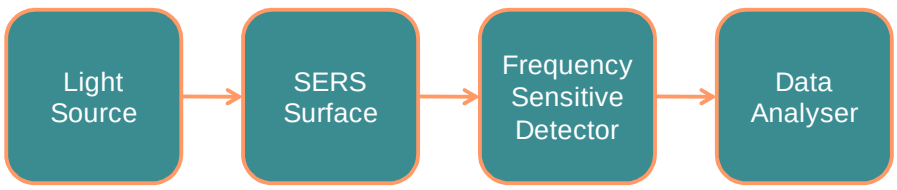


Figure 2-4: *Top-Level SERS System.*

2.5.3 Design of the MS/MS-SERS System

The underlying assumption is that by combining both MS/MS and SERS both structural information (*i.e.* mass/charge ratio and fragmentation pattern information) about the molecule(s) from the MS/MS output and chemical bonding information of the molecule(s) from the SERS output will be obtained. MS/MS-SERS is envisaged to be used for the detection of astrobiology biomarkers in Martian regolith (or ground rock and possible ice), Europan ice (and possible water) and samples from Titan's hydrocarbon lakes.

The system can be split into 3 blocks: the first MS instrument yielding predominantly the parent ion mass to charge ratios, the second MS instrument yielding fragmentation information and the SERS instrument yielding further information on the structure of the fragments (*i.e.,* chemical bond information).

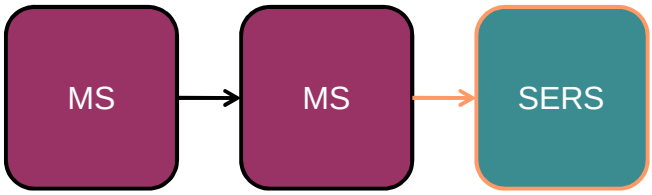


Figure 2-5: *Top-Level MS/MS-SERS System.*

MS/MS and SERS are the two components forming the MS/MS-SERS system; however, it must be remembered that each component is a system in itself and hence has its own components. Therefore MS/MS and SERS had to be considered individually as these two components in turn behave as systems with their own components: for example, the SERS system is comprised of a light source, a SERS surface, a frequency sensitive detector and a data analyser. And again, each component has its own components: for example, a frequency sensitive detector has a spectrometer and a detector.

2.5.3.1 System Level Analysis of MS/MS-SERS

For the system level analysis, MS/MS components and SERS components were considered separately. MS/MS is further split into one CIT-MS component with its detector.

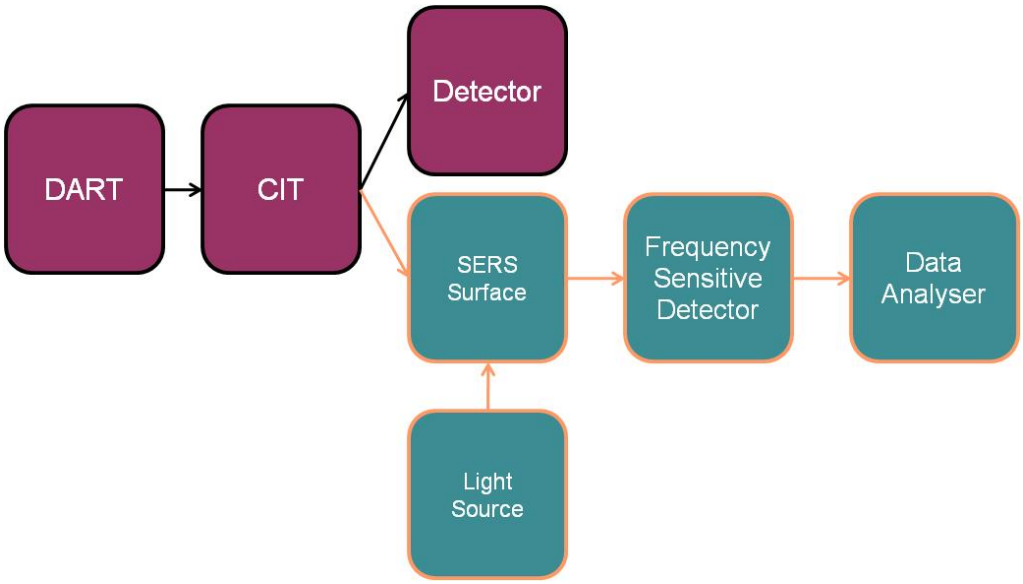


Figure 2-6: *DART-CIT-MS/MS-SERS*

The default/minimum sequence of operations for the proposed CIT-MS/MS-SERS instrument is as follows:

Molecular ion MS acquisition:

1. Sample introduction into Tandem MS (CIT) system via DART (including ionisation).
2. Molecular ion mass scanning in CIT.
3. Signal detection of molecular ion spectra by MS detector.

Daughter ion MS acquisition:

1. Sample introduction into tandem MS (CIT) system via DART (including ionisation).
2. Molecular ion mass selection in CIT.
3. Collision induced dissociation in CIT.
4. Signal detection of daughter ion spectra by MS detector.

SERS acquisition:

1. Sample introduction into tandem MS (CIT) system via DART (inc. ionisation).
2. Molecular ion mass selection in CIT *or* molecular ion mass selection in CIT followed by collision induced dissociation in CIT.
3. CIT output deposited on SERS surface.
4. SERS signal detection.

Therefore, by default, three spectra would be obtained: MS spectra of molecular ions, MS spectra of daughter ions and SERS spectra of molecular ions OR SERS spectra of daughter ions.

As the MS/MS-SERS system was defined, the components were looked at in detail.

2.5.3.2 Component Level Analysis of CIT-MS/MS-SERS

Each component has a number of parameters that define the suitability of the component for the system. Inputs, outputs and defining parameters are specified for each component and these parameters were used later on in the study to identify specific components for the system.

MS/MS Component Definition

All components identified have been found in the literature to be used for portable single molecule detection experiments.

The inlet sample system and ionisation source (DART component):

It is worth noting once more that by using DART, the sample is sampled directly without the need for a separate inlet sample system -hence simplifying the system.

Table 2–28: D Inputs, Outputs and Defining Parameters of the Inlet Sample System.

Inputs:	Outputs:	Defining Parameters:
• *Sample:* The sample.	• *Molecular_ions:* The sample coming from DART which has been ionised into molecular ions.	• *Sample_Type:* The sample (sample$_1$) can be either gas, liquid or solid. • Charge_Type: The charge (either positive or negative) that the ions need to be converted into.

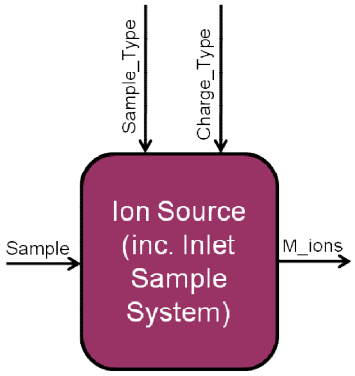


Figure 2-7: *The Inlet Sample System Block Diagram*

As an example, the DART ionisation source used by Wells (2008) was a commercial source from IonSense. It was placed at 1.5cm from the inlet capillary pointing slightly off-axis to reduce the gas load on the vacuum stage from the high flow of helium (2 litres/min at a temperature of 150°C). The sample was held near the edge of the interface between the DART source and the CIT inlet for optimal signal levels.

The analyser including the collision cell (CIT component):

The miniature mass spectrometer designed and constructed by Riter (2002) had a radius of 2.5mm and was operated with an RF drive frequency of 2MHz and a maximum voltage of 1000V. The resulting upper mass/charge limit of the instrument under these operating conditions was 250 Da. Upon entering the trap, electrons can take up kinetic energy from the RF field and ionise neutral molecules. The resulting ions are trapped, mass selected, and manipulated using the normal ion trap operations available in instruments capable of resonance ejection and excitation. Resonant ejection from the CIT was performed at 700 kHz. MS/MS spectra can be acquired manually by selecting the mass of the parent ion to be fragmented and the amount of collisional energy.

Product ions resulting from collision-induced dissociation (CID) are recorded in a mass-selective instability scan. In cases where MS/MS/MS experiments are recorded, a second sequence of isolation, cooling and activation steps has to be inserted before the mass analysis scan. The amplitude of the applied waveforms has to be varied in each experiment to optimise the efficiency of isolation and the degree of dissociation.

Table 2–29: Inputs, Outputs and Defining Parameters of the Analyser.

Inputs:	Outputs:	Defining Parameters:
• *Molecular_ions:* Molecular ion mixture coming from the ion source.	• *Molecular_ions(MS):* Categorised molecular ions from the first MS run of the analyser. • *Daughter_ions(MS/MS):* Categorised daughter ions from the second MS run of the analyser.	• *m/z range or upper mass limit:* the maximum value the analyser can determine the m/z ratio for. The greatest mass (in Da) will depend on the number of charges that the ion carries. • *Mass Resolution or Resolving Power:* the smallest observable change in a mass interval. • *Sensitivity:* the amount of sample consumed per second (*e.g.,* a few femtomole/s) in order to obtain a signal of normalised intensity. • *Throughput:* number of samples it can analyse (*i.e.,* is it limited by the consumables it needs?) • *Fragmentation_Type:* Mass of the parent ion to be fragmented by CAD.

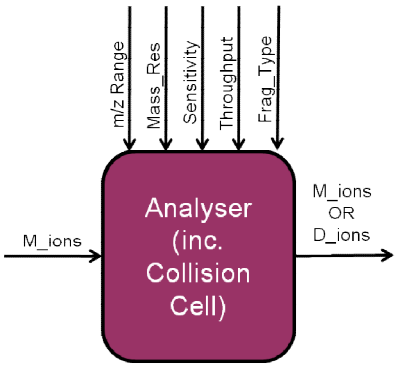


Figure 2-8: *The Analyser Block Diagram.*

The Detector (Electron Multiplier):

Examples of the detector include an electron multiplier (model 7505MH1 from K & M Electronics) used by Riter (2002). The detector signal was amplified and then collected using an embedded PC-based data acquisition system (a Pentium III 800 MHz ATX computer, a National Instruments 6070E multifunctional I/O card, a National Instruments 6602 timer card, and two arbitrary waveform generator cards). Wells (2008) also used an electron multiplier (model 328 conversion dynode/electron multiplier from Detector Technology).

Table 2–30: Inputs, Outputs and Defining Parameters of the Detector.

Inputs:	Outputs:	Defining Parameters:
• *Molecular_ions(MS):* The molecular ions (product from the first MS run of the analyser) that need to be detected. • *Daughter_ions(MS/MS):* The daughter ions (product from the second MS run of the analyser) that needs to be detected.	• *M_Spectra(MS):* The mass spectra of the molecular ions. • *D_Spectra(MS/MS):* The mass spectra of the daughter ions.	• *m/z lookup table:* Used to map the incidence of the ions to the mass spectra.

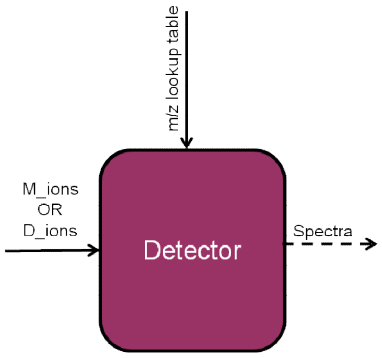


Figure 2-9: *The Detector Block Diagram*

Summary of the MS/MS system's inputs, outputs and defining parameters:

The components of the MS/MS system, with their inputs, outputs and defining parameters, are summarised in the figure below.

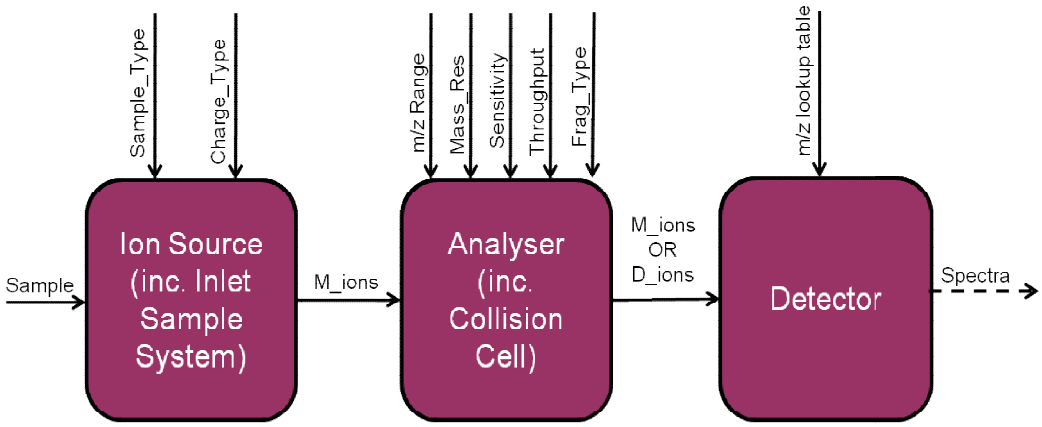


Figure 2-10: *MS/MS System Top Level Block Diagram.*

SERS Component Definition

The SERS system is composed of 4 major components: the light source, the SERS surface, the frequency sensitive detector and the data analyser. Note that SERS implemented into the MS/MS-SERS system differs from Stand-Alone SERS as in MS/MS-SERS SERS receives the output of MS/MS onto its surface.

The light source (Laser):

Typically consists of a laser, either continuous or pulsed. The laser excitation frequency is the most important determinant of the information content of a Raman spectral signal.

Maximum enhancement of the normal Raman cross-section occurs when both the incident and Stokes scattered fields are equally enhanced. Therefore, there is a need to choose a laser excitation frequency such that this frequency and the Raman Stokes-shifted frequency straddle the maximum extinction of the localised surface plasmon resonance (LSPR). Hence, the frequency of laser excitation should be one-half of the Raman shift higher in energy than the LSPR spectral maximum (Camden et al, 2008).

Table 2–31: Inputs, Outputs and Defining Parameters of the Light Source.

Inputs:	Outputs:	Defining Parameters:
• *Power:* the power required to operate the light source.	• *Photon output spatial distribution (psd_1):* how broadly or focused the light emitted is, *i.e.,* it depends on the nature of the light source (λ, BW, I, λ_s).	• *Wavelength (λ):* The spatial period of the wave (λ = v/f). • *Bandwidth (BW):* The width of the frequency band (Hertz). • Intensity/Irradiance (I): W/m^2 power incident on a surface. • *Stability of wavelength (λ_s): H*ow the wavelength gets affected by the environment. • *Thermal Control (TC):* How cool does the laser need to be? Thermal issues affect the stability of the frequency. Related to stability of wavelength. • *Power Requirement (P):* How much power the laser needs to function correctly.

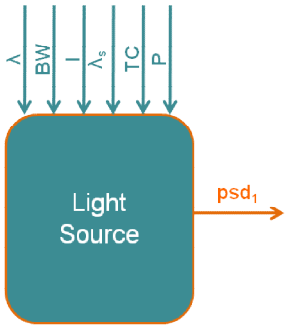


Figure 2-11: *The Light Source Block Diagram.*

Wilson et al. (2007) used a 532nm solid state laser passed through a neutral density filter and a Thorlabs laser line filter to clean up excitation radiation; Etchegoin et al. (2009) used a 633nm HeNe laser to match it to their RH800 silver (citrate-reduced) Lee-Meisel colloid which has an adsorption maximum at ~685nm with the next vibronic peak being at ~626nm in order to create almost resonance with the 633nm laser -hence producing Surface Enhanced Resonant Raman Scattering (SERRS). This was similar to Zhang et al's (2009) experiment, where they used a HeNe laser of 632.8nm. Similarly, Pieczonka et al. (2009) used a 514.5nm laser to be highly resonant with the surface plasmon adsorption of the Ag nanoparticles used in their experiment.

The SERS surface (Roughened Silver):

The SERS active surface is made of a roughened metal (silver, gold or copper, although typically, silver is used). The largest enhancements are achieved with 10nm rough surfaces.

The optical chamber configuration is normally made of a simple disposable microfluidic system; *e.g.*, in Wilson's (2007) set-up, a glass capillary tube with an outer diameter of 470micron and inner diameter of 320micron constrained within a polydimethylsiloxane (PDMS) holder connected to the SERS chamber.

Table 2–32: Inputs, Outputs and Defining Parameters of the SERS Surface.

Inputs:	Outputs:	Defining Parameters:
• *Photon output spatial distribution (psd_2):* How broad or focused the light that has been transported by the excitational light conduit is when it arrives at the SERS surface. Hence psd_2 depends on psd_1 and the light conduit's properties.	• *Photon output spatial distribution (psd_3):* How broad or focused the light scattered by the SERS surface is. Hence, psd_3 depends on the SERS surface (s_t and s_m) and psd_2.	• *Surface topography (s_t):* How the surface is, *i.e.*, roughened, colloid, coated beads. • *Surface metal (s_m):* What the surface is made of (*i.e.*, silver, gold or copper).

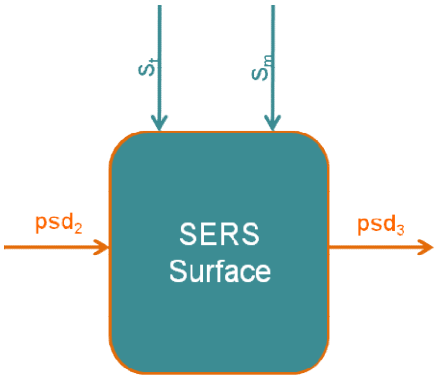


Figure 2-12: *The SERS Surface Block Diagram.*

Frequency sensitive detector (Raman Spectrometer):

Photomultipliers were typically used before Charge Coupled Detectors (CCDs), but now CCDs are typically the detectors of choice to collect the scattered light from the UV to the near IR spectral region. Filters are used to remove the Rayleigh (inelastically scattered) photons as their unattenuated entry in the detector would obscure all or part of the Raman spectrum. Therefore, the Raman spectrometer rejects Rayleigh scattered light and disperses Raman scattered light into its component frequencies for detection.

Table 2–33: Inputs, Outputs and Defining Parameters of the Detector.

Inputs:	Outputs:	Defining Parameters:
• *Photon output spatial distribution (psd_4):* How broadly or focused the light scattered from the SERS surface and transported through the emitted light conduit is when it arrives at the detector. Hence, psd_4 depends on the detector (e_{elr}) and the light conduit.	• *Raw Data:* Counts/Pixel.	• *Spectral resolution (r_s):* The range of wavelengths seen by the sensor, the smaller the range, the more specific the information the sensor can provide. • *Overall efficiency (e_o):* The percentage of the photons coming in that generate the Raman signal. • *Stray light level (SLL):* The amount of false signal noise. • *Excitation light rejection efficiency (e_{elr}):* How well the filter rejects the excitation light.

Wilson et al's (2007) experiment setup collected the scattered light from the SERS surface via a 650µm core optical fibre passed through a long-pass filter and then fed into 650µm core patch fibre to an Ocean Optics Inc. QE65000 scientific grade spectrometer. On the other hand, Zhang et al. (2009) used the Renishaw RM-1000 Raman microscopic spectrometer composed of an optical microscope, a CCD and a spectrometer. Also, the ALICE instrument on the LCROSS Lunar mission to confirm the presence or absence of water on the Moon was based upon a modified Ocean Optics QE65000 spectrometer, (Ocean Optics, 2010 B).

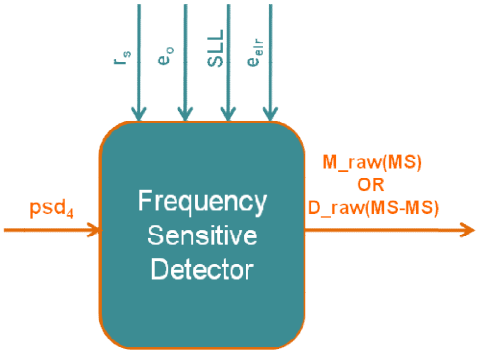


Figure 2-13: *The Detector Block Diagram.*

The data analyser:

The data analyser gets the raw data from the detector and displays the data set as a plot, *i.e.*, it is not an extra hardware component; it is the software component that interprets the data from the detector.

Table 2–34: Inputs, Outputs and Defining Parameters of the Analyser.

Inputs:	Outputs:	Defining Parameters:
• *M_Raw(MS):* The raw signal from the molecular ions (Counts/Pixel). • *D_Raw(MS/MS):* The raw signal from the daughter ions (Counts/Pixel).	• *M_Spectra(MS):* The spectra of the molecular ions *(*Frequency/Counts). • *D_spectra(MS/MS):* The spectra of the daughter ions *(*Frequency/Counts).	• *Pixel detection efficiency* (e_{pd})*:* How well the pixels detect incident light. • *Pixel conversion parameters* (p_{cp})*:* Used to convert the raw data in terms of pixels to spectra (intensity Vs λ).

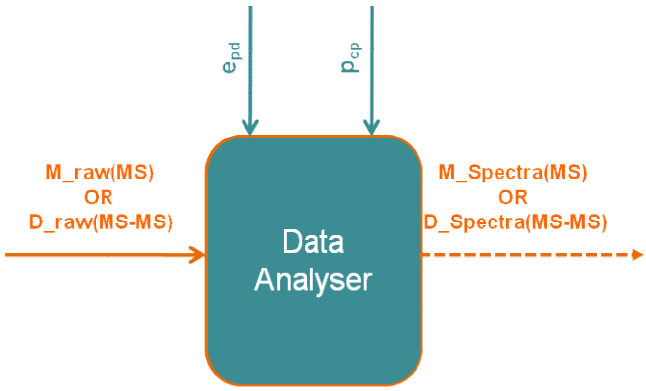


Figure 2-14: *The Analyser Block Diagram.*

SERS in MS/MS-SERS

In an MS/MS-SERS system, the sample deposited onto the SERS surface has already undergone one or two analyses and it will be either the molecular ions from the first stage of the MS or the daughter ions from the second stage of the MS. This differs from a Stand-Alone SERS system, where the "raw" sample would be deposited straight onto the SERS surface without any previous analyses by other techniques.

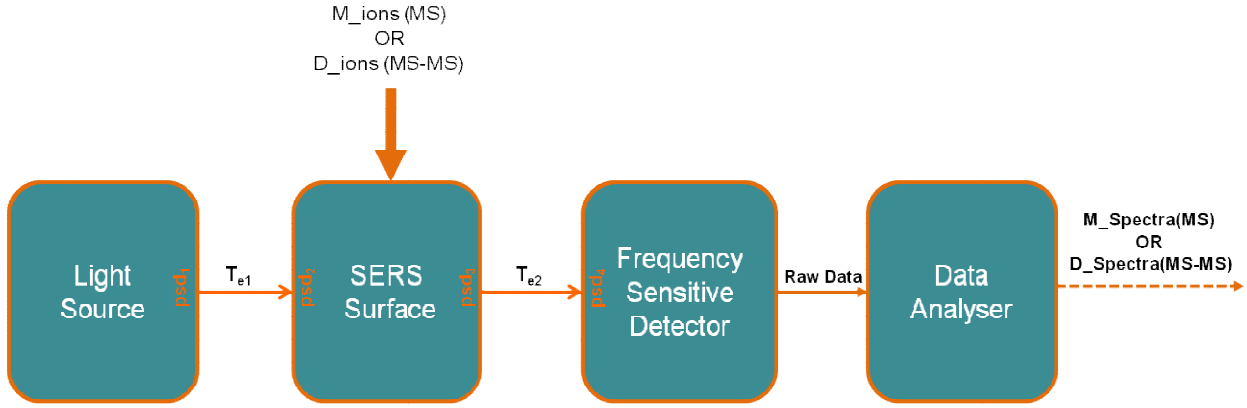


Figure 2-15: *SERS in MS/MS-SERS System Block Diagram.*

Please note that T_{e1} *(Excitational Light Conduit Photon Transfer Efficiency):* quantifies how efficient the light conduit is, *i.e.,* how psd_1 is affected; and T_{e2} *(Detection Light Conduit Photon Transfer Efficiency):* quantifies how much psd_3 is affected by the light conduit.

2.5.3.3 The MS/MS-SERS top level system

Figure 2-16: *The DART-MS/MS (CIT)-SERS System Block Diagram.*, shows the flow of a DART-CIT-MS/MS-SERS system: the sample would be introduced into the system via DART, which would also ionise the sample. Once ionised, these molecular ions would be analysed by the CIT and the SERS system. Or instead of being deposited on the SERS surface after the first stage of MS/MS, the molecular ions could be deposited on the SERS surface after the second stage of MS/MS, after they have been fragmented into daughter ions. Therefore, three spectra will be produced, the first from the molecular ions from first stage of the MS/MS system, the second from the daughter ions from the second stage of the MS/MS system, and the third from the molecular ions or daughter ions analysed by the SERS system.

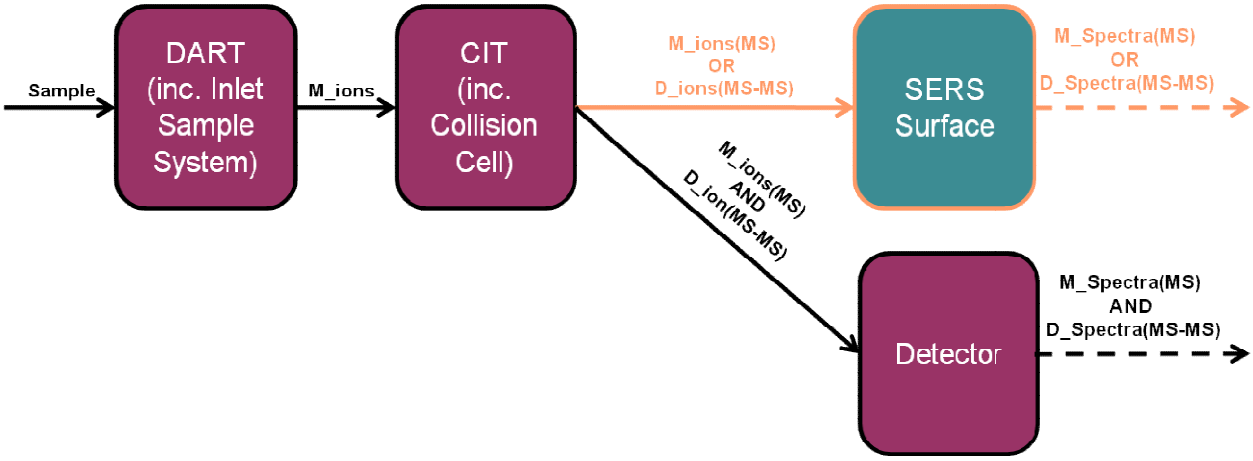


Figure 2-16: *The DART-MS/MS (CIT)-SERS System Block Diagram.*

2.5.4 Design of the Stand-Alone SERS System

The only difference between Stand-Alone SERS and MS/MS-SERS is that the sample deposited onto the surface in the latter is the output of the second mass analyser in the MS/MS-SERS system; while the sample deposited on a Stand-Alone SERS system would be a direct raw sample (having had no sample extraction and processing phases). Stand-Alone SERS is envisaged for the detection of biomarkers in Enceladean plumes, Venusian clouds and cometary coma.

2.5.4.1 The Stand-Alone SERS top level system

As described previously, a SERS system consists of a light source, a SERS surface, a frequency sensitive detector and a data analyser. The parameters for the Stand-Alone SERS system would be the same as for the SERS component within an MS/MS-SERS system although with different ranges determined by the different sample types encountered in the different scenarios these two SERS systems would encounter. In other words, the SERS component in a Stand-Alone SERS system would have different requirements for an Enceladean plume fly-through mission compared to the SERS component within an MS/MS-SERS system for a Mars regolith mission. To read more on SERS components, please refer to Section 2.5.3.2: “Component Level Analysis of CIT-MS/MS-SERS”.

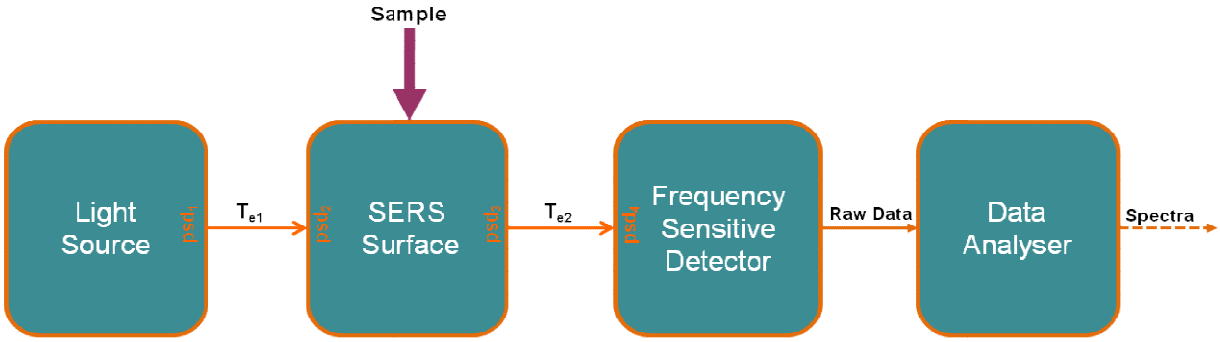


Figure 2-17: *SERS in Stand-Alone SERS System Block Diagram.*

Note that T_{ex}: Light Conduit Photon Transfer Efficiency and psd_x: Photon Output Spatial Distribution.

2.6 Development of the Systems

In this study, the practical development of the Stand-Alone SERS and MS/MS-SERS systems did not have to be pursued. However, a development plan as well as a test plan was proposed.

2.6.1 MS/MS-SERS Breadboard Development Plan

The development plan consists in designing the breadboard (representative of the flight model), sourcing the components, building the breadboard and testing it. In order to guide these activities, aims, objectives and requirements were set.

2.6.1.1 Aim, objectives and requirements for MS/MS-SERS breadboard

The aim of the bread-board is to enable an early stage de-risking of the MS/MS-SERS concept to assess further the viability of the concept for more detailed development leading to a flight model.

In order to constrain the breadboard to a specific environmental scenario for which the test plan would be geared, a mission to Mars was considered.

Objectives

- MSMSSERSO1: To use the breadboard to implement a pre-defined test plan to assess analytical performance.
- MSMSSERSO2: To build an MS/MS-SERS breadboard with support equipment able to simulate key identified features of the Martian environment.
- MSMSSERSO3: To assess critical items, risks and sub-systems of the MS/MS-SERS concept that have been identified in earlier studies.

Requirements

Table 2–35: *MS/MS-SERS Breadboard Requirements.*

Reference	Requirement
MSMSSERSR1	[Derived from MSMSSERSO1]: Be compatible with ultra-low lower limits of detection.
MSMSSERSR2	[Derived from MSMSSERSO1]: Be able to detect a-biotic, pre-biotic and biotic (extant and extinct) organic molecular targets.
MSMSSERSR3	[Derived from MSMSSERSO1]: Be compatible with Martian representative sample matrices (i.e., a range of mineral types known to be present on Mars and in a range of physical formats: from powders, *e.g.,* regolith, to rock outcrops).
MSMSSERSR4	[Derived from MSMSSERSO2 and MSMSSERSO3]: Have a sampling front-end that is compatible with the typical range of Martian surface environment conditions (i.e., temperature, pressure and atmospheric composition).
MSMSSERSR5	[Derived from MSMSSERSO3]: Have key components/subsystems that are available COTS to allow development within appropriate temporal and financial resource contexts.

2.6.1.2 MS/MS-SERS breadboard design

The components mentioned in this section are indicative of the values needed to build a breadboard. Note that to conform to requirement MSMSSERSR5 “Have key components/subsystems that are available COTS to allow development within appropriate temporal and financial resource contexts” all the components stated are COTS.

The support equipment for both MS/MS-SERS and Stand-Alone SERS is not explicitly mentioned in this study as it includes all the equipment that the spacecraft will provide the instrument with during the mission (*e.g.,* power, data transmission, etc) and that will be provided for during the development in the system.

Indicative components for MS/MS

Components for the MS/MS system (an ionisation source, analyser and detector) are now proposed.

Indicative ion source (DART):

The DART-SVP from IonSense was the DART source that was coupled to the Griffin MS/MS (CIT) by Wells (2008). It has an estimated cost of £50,000 and its characteristics are summarised below.

Table 2–36: *DART Specifications (IonSense, 2010).*

Specifications	Details
Sample Types:	Can analyse gases, liquids, solids and materials on surfaces. Although note that dust tends to be blown away partially during the ionisation procedure, hence perhaps needing some sample preparation to adhere the dust grains first (conforms to requirement MSMSSERSR3: The system must be compatible with Martian representative sample matrices).
Methods of Sample Introduction:	It requires no sample preparation or chromatographic separation (conforms to requirement MSMSSERSR3).
Vacuum Requirements:	Non-contact surface-sampling technique that operates at atmospheric pressure (conforms to MSMSSERSR4: "The system must have a sampling front-end that is compatible with the typical range of Martian surface environment conditions)."
Atmospheric Conditions:	Default conditions are ambient pressure, laboratory based (2bar/35psi). It is envisaged that in reduced pressure conditions (such as in a Martian atmospheric pressure of 10mbar), the DART source will be more efficient (therefore, conforms to MSMSSERSR4).
Ionisation Technique:	Based on Penning ionisation, the sample is ionised directly by energy transfer from the metastable ions.
Operating Temperature:	DART gas temperature can be varied from ambient to 500°C. Typically runs at 250°C.
Dimensions:	30 x 23 x 11.5 cm; controller can operate in either vertical or horizontal orientation; maximum footprint: 30 x 23 cm2, minimum footprint: 30 x 11.5 cm2 (could be reduced for Space applications).
Mass:	5 Kg.
Transportability:	A ruggedised version is envisaged to be feasible.
Consumables Required:	2 gases are needed for the operation of the DART source (Helium of at least 4.7 grade 99.997% purity and Nitrogen of at least 4.8 grade 99.998% purity), the minimum pressure required for both is 5.5 bar (80 psi) and the flow rate is of 200ml per minute. Note that the flow rate is dictated by the size of the source so this value could be reduced for a Space applications instrument. It is recommended that the gas supply should include 10 micron in-line particulate filters between the gas supply and the DART controller to eliminate possible contamination from gas supplies.
Power:	Heating of the gas is required; therefore the DART operates at 110-240VAC at 50/60Hz, 2.3A maximum. However, as this DART has not been built to be power efficient, it is envisaged a lower power version could be built for Space applications.
Adaptability:	Can be fitted to any MS with an ESI/APCI source by use of an adapter; ionising gas position can be varied in x, y and z; motorised rail for automatic operations.
Software:	I-Pod graphical software user interface.

Indicative mass analyser (CIT):

The Griffin series CIT instruments (from ICx Technologies), is the CIT proposed for development as it was found to be used in the peer-reviewed articles. However, as the instrument specifically used by Wells (2008) is not yet commercially available, and in order to have some estimates of the specifications of a portable MS/MS (CIT), the specifications for their COTS instrument, the Griffin 400, are detailed in Table 2–37: Griffin 400 Specifications (ICx Technologies, 2009*).*

Table 2–37: *Griffin 400 Specifications (ICx Technologies, 2009).*

Specifications	Details
Resolution:	Unit Mass Resolution. Therefore, it conforms to requirement MSMSSERSR1.
Mass Range:	40-425 m/z, configurable depending on application. Therefore, conforms with requirement MSMSSERSR2
Analyser:	Cylindrical Ion Trap (CIT) Technology, MS/MS capable.
Vacuum System:	Miniature turbo molecular pump and miniature quad diaphragm (contained within instrument, no external pump required).
Detector:	Electron multiplier.
Ionisation Source:	Internal Electron Ionisation (EI).
Operating Temperature:	5°C to 35°C.
Operating Humidity:	Less than 85% relative humidity.
Dimensions:	48.8cm x 48.8cm x 45.7cm -including pumps.
Mass:	37.2kg, including pumps.
Transportability:	Ruggedised chassis and internal shock mounting system for rugged transportation.
Consumables Required:	GC Carrier Gas (choice of He or H_2) available from many sources.
Methods of Sample Introduction:	Split/Splitless Injector for Sampling via Direct Syringe Injection, SPME Fibre, Headspace Sampler (optional accessory) or Autosampler (optional accessory).
Power:	Input voltage of 100–120/220–240VAC, 50/60Hz, and 15A. May also be powered by 24VDC (+/- 5%, 25A, 600W), fuse protection (30A minimum).
Accessories:	Autosampler (optional), Headspace Sampler (optional) and has optional separation techniques: LTM-GC, user-selected columns, Fast-GC operation, temperature programmable from 40-300°C/min (max temperature of 300°C), and temperature ramp rate of up to 100°C/min (Column dependent).
Software:	Griffin System Software (GSS). Multi-level software available to meet operator skill and analysis requirements.

It is worth noting that ICx Technologies quoted the MS/MS(CIT) currently being developed to be valued at an estimated £150,000 when a commercially available version is released (estimated to be by the end of 2010). Also, even though a Gas Chromatograph is coupled with Electron Ionisation in the Griffin 400, this could be removed as it is unnecessary for Space applications (due to the foreseen use of DART and MS/MS). Moreover, the detector used is within physical reach and could potentially be modified to allow some of the sample ions to be carried onto the SERS surface rather than be all detected by the MS detector.

As mass and power are critical for the final flight model and hence will have different requirements than the breadboard model being considered now, the standard pump supplied with the COTS system will be used for the breadboard; *i.e*. a miniature turbo molecular pump and miniature quad diaphragm -as in the case of the Griffin 400 (ICx Technologies, 2009).

Indicative detector:

As the detector is considered to be a non-critical element, the standard detector provided with the Griffin 400 (ICx Technologies, 2009) is recommended, *i.e.,* an electron multiplier.

Indicative components for SERS

COTS components for the SERS instrument part of the MS/MS-SERS system are now detailed.

Indicative laser:

At present it is expected that a long wavelength laser in the near infra-red wavelength will be used (to avoid inherent fluorescence issues). More details on the laser would be decided in follow-on studies; however, an indicative source could be Ocean Optics' Laser-785 (Ocean Optics, 2010).

Table 2–38: *Ocean Optics Laser-785 for Raman Spectroscopy (Ocean Optics, 2010).*

Specifications	Details
Laser:	Laser-785 for Raman Spectroscopy with integrated laser drivers and thermoelectric coolers.
Noise:	<0.5% RMS
Output Fibre:	100µm @ 0.22 NA
Operating Temperature:	-10°C to 40°C.
Dimensions:	110mm x 89mm x 53mm
Mass:	600g
Transportability	Compact and hermetically sealed.
Warm-up Time:	15 minutes
Stability	<3% peak-to-peak in 8 hours
Humidity	5-95% non-condensing
Power:	3A @ 5VDC.
Power Output (CW)	>500mW
Peak Wavelengths	785 +/- 0.3nm
Spectral Line Width	0.2nm (typically)
Rise Time	<500msecs
Laser Life:	10,000 hours
Control:	TTL modulation (0 to 100kHz)

Indicative SERS surface:

Ocean Optics offer Klarite™ (Ocean Optics, 2010A) substrates for trace level molecular analysis using Surface Enhanced Raman Spectroscopy. These substrates provide signal reproducibility and have a gold-coated silicon surface with sub-micron scale patterning. They also help control the surface plasmon effects and highly enhance the Raman signal.

Applications of Klarite™ SERS technology include explosive detection, where trace level sensitivity is required (without direct contact with suspected materials), and vapour phase detection, where in the event of exposure to a hazardous airborne chemical agent, vapour phase detection is used to detect and positively identify the compound. They are compatible with standard Raman spectrometers and a set of 5 slides mounted or un-mounted on glass sliders costs $525.

Note that according to requirement MSMSSERSR4, "The system must have a sampling front-end that is compatible with the typical range of Martian surface environment conditions (*i.e.,* temperature, pressure and atmospheric composition)". Therefore, the SERS surface must be encapsulated in a vacuum chamber in order to accommodate for the reduced Martian pressure.

Indicative detector and analyser:

To conform with requirement MSMSSERSR2 "The system must be able to detect a-biotic, pre-biotic and biotic (extant and extinct) organic molecular targets" an excitation wavelength of 785nm would be needed in order to detect polyaromatic hydrocarbons, PAHs, (Peron et al, 2009; Costa et al, 2006; Schmidt et al, 2004).

The Ocean Optics QE65000-Raman scientific-grade spectrometer is a preconfigured system which can be configured for this application. It would consist of a Raman Spectrometer with an H6 grating and a 50 micron slit aperture, optical bench electronics, a fibre probe for 785 nm and a 785 nm laser diode which, tuned to start from 780nm would give a spectral range of 150-2100 cm^{-1} and a resolution of approximately 6 cm^{-1} FWHM. In case the Raman signals are weak, the aperture size could be increased to 100 microns but the resolution would be reduced to 8 cm^{-1} (but the spectral range would remain the same). This set-up has a quoted value of £15,539 with taxes (Ocean Optics, 2010B).

A modified version of the QE65000 (to withstand the harsh environmental conditions as well as shock and vibration) was flown in the recent NASA's Lunar Crater Observing and Sensing Satellite (LCROSS) mission to detect if water and other substances were present on the moon (NASA, 2009). It measured the visible (263-650 nm) emission of the vapour plume and ejecta cloud created on impact, lunar grain properties and H_2O vapour disassociation.

The components would then be procured and building would commence followed by the test plan.

Table 2–39: *Ocean Optics QE65000-Raman Specifications (Ocean Optics, 2010 B).*

Specifications	Details
Analyser:	QE65000 Spectrometer with H6 grating (starting at 780nm) and 50µm slit: • Wavelength range: 780-1100 nm (grating dependant). • Optical resolution: ~0.14-7.7 nm FWHM. • Conforms to requirement MSMSSERSR2 "The system must be able to detect a-biotic, pre-biotic and biotic (extant and extinct) organic molecular targets". • SNR: 1000:1 at full signal. • Dynamic range: 7.5 x 109 (system), 25000:1 for a single acquisition. • Integration time: 8 ms to 15 minutes. • Stray light: <0.08% at 600 nm; 0.4% at 435 nm. • Corrected linearity: >99.8%
Detector:	Hamamatsu S7031-1006, range: 200-1100 nm (conforms to requirements MSMSSERSR1 and RMSMSSERS2. • 1024 x 58 pixels (1044 x 64 total pixels) with 24.576 µm^2 pixel size and 100Ke-pixel well depth. • ~0.065 counts per e- sensitivity with rapid signal processing speed. • 90% peak quantum efficiency: 65% at 250 nm.
Optical Bench:	• f/4, symmetrical crossed Czerny-Turner with 101.6 nm input and output focal length: • Entrance aperture of 50µm wide slit with HC6 grating which provides 123-170 nm range. • Fibre optic connector: SMA 905 to 0.22 numerical aperture single-strand optical fibre. • Standard only collimating and focusing mirrors, no detector collection lens option and no UV enhanced window.
Operating Temperature:	0°C to 50°C, no condensation (+/- 0.1°C off-set temperature in <2 minutes stability). Set point: software controlled, lowest set point is 40°C below ambient.
Dimensions:	182mm x 110mm x 47mm.
Mass:	1.18kg (without the power supply).
Transportability:	Ruggedised version created for LCROSS mission.
Power:	500 mA, 5 VDC (no TE cooling) or 3.5A, 5VDC (with TE cooling). Quick power up time (<5 seconds).
Accessories:	HR4-BREAKOUT Breakout Box separates signals from the spectrometer's 30 pin port to an array of standard connectors and headers.
Software:	SpectraSuite Spectroscopy Operating Software.

2.6.2 Stand-Alone SERS Breadboard Development Plan

The parameters for the Stand-Alone SERS system would be the same as for the SERS component within an MS/MS-SERS system although with different ranges determined by the different sample types encountered in the different scenarios these two SERS systems would encounter. In other words, the SERS component in a Stand-Alone SERS system would have different requirements for an Enceladean plume fly-through mission compared to the SERS component within an MS/MS-SERS system for a Mars regolith mission.

2.6.2.1 Aim, objectives and requirements for Stand-Alone-SERS breadboard

The aim is to enable an early stage de-risking of the Stand-Alone SERS concept to assess further the viability of the concept for more detailed development that could lead to a flight model.

Objectives

- SERSO1: to use the breadboard to implement a pre-defined test plan to assess analytical performance.
- SERSO2: to build a Stand-Alone SERS breadboard with support equipment able to simulate key identified features of the Enceladean environment.
- SERSO3: to assess critical items, risks and sub-systems of the Stand-Alone SERS concept that have been identified in earlier studies.

Requirements

The Stand-Alone SERS breadboard was planned against the scenario of an Enceladean plume fly-through mission and hence its requirements differed from those imposed on the SERS system within the MS/MS-SERS breadboard (which was set against a Martian regolith scenario).

Table 2–40: *MS/MS-SERS Breadboard Requirements.*

Reference	Requirement
SERSR1	[Derived from SERSO1]: Be compatible with ultra-low lower limits of detection.
SERSR2	[Derived from SERSO1]: Be able to detect a-biotic, pre-biotic and biotic (extant and extinct) organic molecular targets.
SERSR3	[Derived from SERSO1]: Be compatible with Enceladean plume representative sample matrices (*i.e.,* a range of particles and gases known to be present on Enceladean plumes and in a range of physical formats).
SERSR4	[Derived from SERSO2 and SERSO3]: Have a sampling front-end that is compatible with the typical range of Enceladean plume environment conditions (*i.e.*, temperature, pressure and plume composition). Note that, therefore, it must have a SERS surface that can be implemented into a vacuum chamber for Enceladean environment simulation.
SERSR5	[Derived from SERSO3]: Have key components/subsystems that are available COTS to allow development within appropriate temporal and financial resource contexts.

2.6.2.2 Stand-Alone SERS breadboard design

The components described in the previous section “2.6.1.2: MS/MS-SERS breadboard design” apply for Stand-Alone SERS as the SERS module for the MS/MS-SERS system would be the same.

2.6.3 Test Plan for MS/MS-SERS

The overall objective of the test plans is to use MS/MS-SERS and Stand-Alone SERS instrument breadboards to de-risk the concepts for use within a relevant Space mission scenario. In order to add a defined Space scenario context, a mission to Mars is set for MS/MS-SERS and an Enceladean plume fly-through mission for Stand-Alone SERS.

2.6.3.1 Test Plan Methodology

Three tests are proposed to assess the suitability of MS/MS-SERS and Stand-Alone SERS: a functional test, a basic analytical test and a Space scenario analytical test. Each test can be described as follows:

Functional Test:

- Needed to confirm that the hardware meets the original design criteria.
- The inputs, outputs and requirements are described to conduct the test.

Basic Analytical Test

- Needed to confirm that the prototype(s)/breadboard(s) can perform appropriate molecular detection (either single molecule detection or ultra-low lower level of detection) independent of any Space application context.
- The inputs, outputs and requirements are described to conduct the test.

Space Scenario Analytical Test

- Needed to confirm that the prototype (breadboard) can perform single molecule detection in a representative Space mission scenario.
- The inputs, outputs, requirements, Space scenarios, sample matrix, targets, Space environment constraints are described to conduct the test.
- Specific components that need to be de-risked and the tests that could be performed to do so are also recommended.

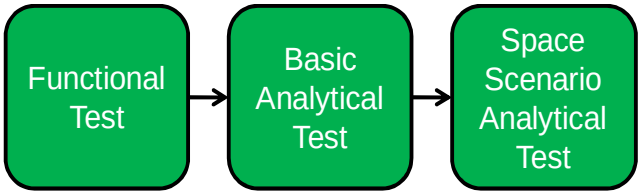


Figure 2-18: *Test Flow Process.*

2.6.3.2 The MS/MS-SERS test plan

Objectives, inputs, requirements and expected outputs are described for each test.

Functional test

This test ensures the hardware works according to its specified characteristics.

Functional test objectives:

- To confirm that the hardware meets the original design criteria.
- Note that this test is a standard hardware check; it is not an analytical test and hence no sample is introduced.

Functional test inputs:

- The inputs would be based upon the specific hardware design, for example the requirements for the power and thermal subsystems would be tested based upon pre-defined specifications.

Functional test requirements:

- To confirm basic electronic, mechanical, optical, environment and data handling function for breadboard and associate support equipment.

Functional test outputs:

- Like the inputs, the outputs would be based upon the specific hardware design.

Basic analytical test

This test ensures the instrument can perform appropriate molecular detection independent of any Space application context.

Basic analytical test objectives:

- To confirm that the prototype(s)/breadboard(s) can perform appropriate molecular detection (either single molecule detection or ultra-low lower level of detection) independent of any Space application context.

Basic analytical test inputs:

- The approach will be to select well established examples of analytes from the literature and their interfacing to the analytical technique to demonstrate single molecules and/or ultra-low lower levels of analyte detection. No relevance to Space applications will be considered, *i.e.* the test can be performed at room temperature and under ambient laboratory atmosphere.
- For SERS: there are very varied examples of target analytes in the literature, *e.g.,* Dieringer et al. (2008) studied Rhodamine 6G, Pieczonka et al. (2009) studied phospholipids, Zhang et al. (2009) studied 4-dimethylaminoazonbenzene and Gu and Suh (2009) studied p-aminothiophenol – in current context these could be transferred by DART into the CIT and then onto the SERS surface as an un-fragmented molecular ion.
- For Tandem MS: the preceding SERS relevant targets are also relevant to detection via Tandem MS (CIT).

Basic analytical test requirements:

- Confirm ability to detect spectra from target molecules identified from established examples from the peer-reviewed literature. Two example target molecules to be used in separate tests.
- Confirm limits (lower and upper) of detection for given demonstration target molecules in isolation with lower detection limit to be compatible with peer-reviewed literature examples of single molecule detection and/or ultra-low lower limits of detection.
- Confirm ability to detect mixtures of two demonstration target molecules.
- Perform appropriate control experiments to confirm appropriate interpretation of findings.

Basic analytical test outputs:

- Collected MS and SERS spectra
- Comparison and interpretation of collected MS and SERS spectra that show agreement with known examples of single molecule detection and/or ultra-low lower-limit of analyte detection published in the peer-reviewed literature.

Space scenario analytical test

This section relates to the demonstration of the analysis using one or more Space relevant scenarios and comprising:

- A particular scenario relevant sample matrix.
- Related analytical targets relevant to the given sample matrix.
- Suitable replication of key components of the mission/Space scenario environment in which a measurement would take place.

Space scenario analytical test objectives:

- Confirm that the prototype (breadboard) can perform single molecule detection in a representative Space mission scenario.

Space scenario analytical test inputs:

It is worth noting that when MS/MS-SERS is used to analyse Mars regolith, two options arise:

- An established, low risk approach requiring solvent based sample extraction and preparation and thus a separate sample extraction system would have to be analysed in detail separately.
- Via the use of the DART sample acquisition approach into the CIT-MS that offers the potential for direct analysis of regolith without extraction.

Space mission scenario:

- Presence of organic molecular biomarkers in Mars regolith and/or rocks as well as ice rich samples from Europa/comets. Note that this test plan only describes in detail the Mars scenario.
- Titan was considered for its complex organics and macromolecules. However, Titan's atmosphere could not be easily duplicated therefore it is discounted because of the complexity of the atmospheric matrix.

Sample matrix:

- Martian regolith/rock simulant, *i.e.* JSC Mars-1.
- Crushed and or sieved to given particle size distribution (details TBD).
- Furnace treat (pyrolysis) to remove all indigenous organic molecules.
- Sample spiked with target (biomarker) examples to a given concentration (*i.e.* TBD ppb/t levels).
- Note: as an intermediate step, biomarkers directly spiked into extraction solvent can be used to avoid sample extraction issues and complexity.

Analytes/Targets on Mars:

- A wide range of possible analytes exist and include amino acids, carboxylic acids, fatty acids, sugars, pigments, and cell membrane constituents/derivatives (Parnell, 2007).
- Specific targets could be:
  - Abiotic organics derived from meteoritic in-fall: α-amino isobutyric acid, fluorene.
  - Martian processed (oxidised) abiotic polyaromatic hydrocarbons: mellitic acid.
  - Geologically stable/processed Earth-like life cell membrane biomarkers: phytane (isoprenoid), octadecane, and hopanes.
  - Note: above target represent low molecular weight polar and apolar targets.
- Target concentrations:
  - Parts per trillion levels in an original (simulant) sample.

Martian environment:

- Mars (liquid extraction):
  - Temperature: as liquid extraction is required, expect internal instrument conditions to be significantly above Mars ambient temperature to enable solvents including water-based (TBC) solvent to be used; therefore assume breadboard operation at +20°C as relevant to Mars operation.
  - Pressure: as liquid extraction is required, expect internal instrument conditions to be significantly above Mars ambient pressure to enable water-based solvent to be used; therefore assume breadboard operation at 1 bar pressure as relevant to Mars operation
  - Radiation: relatively benign and therefore not considered as relevant to current test plan.
- Mars (DART interface):
  - Temperature: as heat gas stream (>> +20°C) to be used, therefore assume breadboard operation at +20°C as relevant to Mars operation.
  - Pressure: the efficiency of the DART interface is likely to be dependent upon ambient pressure and gas composition, therefore assume breadboard operation at approximately 5 mbar pressure and carbon dioxide based simulation of Mars atmosphere.
  - Radiation: relatively benign and therefore not considered as relevant to current test plan.

Space scenario analytical test requirements:

- To confirm basic ability to detect spectra from individual Mars target molecules under non-Space relevant conditions and without reference to concentrations relevant to Mars context (only if non-Space relevant conditions are appropriate).
- To confirm ability to detect spectra from individual Mars target molecules under non-Space relevant conditions at concentrations relevant to Mars context (only if non-Space relevant conditions are appropriate).
- To confirm ability to detect spectra from mixtures of Mars target molecules under non-space relevant conditions at concentrations relevant to Mars context (only if non-Space relevant conditions are appropriate).
- To confirm ability to detect spectra from individual Mars target molecules under Space relevant conditions and in a Mars relevant sample matrix.
- To confirm ability to detect spectra from individual Mars target molecules under Space relevant conditions at concentrations relevant to Mars context and in a Mars relevant sample matrix.
- To confirm ability to detect spectra from mixtures of Mars target molecules under Space relevant conditions at concentrations relevant to Mars context and in a Mars relevant sample matrix.
- To perform appropriate control experiments to confirm appropriate interpretation of findings.

Space scenario analytical test outputs:

- Collected MS and SERS spectra.
- Comparison and interpretation of collected MS and SERS spectra that show agreement with known examples of single molecule detection and/or ultra-low lower-limit of analyte detection published in the peer-reviewed literature.

Early stage de-risking of fundamental components with no Space heritage:

There are a number of key aspects of the MS/MS-SERS approach that can be identified for which little or no data exists and which could be considered as possible “show stoppers” within a Space/Mars context. These key aspects should be independently tested alongside the main test plan and are described below.

- For DART sampling:
  - The volumetric gas flow rate and related power requirements to heat the gas flow need to be considered within a space flight scenario in terms of required flown gas mass and power requirements.
  - The ability for DART to operate under Mars atmospheric pressure and gas constitution is an early stage de-risking requirement.
  - The sampling efficiency in terms of sampling volume/area and related effects on detection limits and efficiency -*e.g.* volume of gas and its collection efficiency of transfer into the MS inlet- is again an early stage de-risking requirement.
- CIT MS to SERS transfer:
  - The ability to collect ions ejected from the CIT by a SERS surface is not established and should form an early stage de-risking work package.

2.6.3.3 Test plan for Stand-Alone SERS

The overall objective of the test plan is to use a Stand-Alone SERS instrument breadboard system to de-risk the concept of the use of a Stand-Alone SERS method within a relevant space mission scenario to the point that basic questions concerning its performance can be answered. Suitable outputs would be used to further develop the case for the use of a Stand-Alone SERS instrument within future missions.

Functional test

Functional test objectives:

- To confirm that the hardware meets the original design criteria.
- Note that this test is a standard hardware check; it is not an analytical test and hence no sample is introduced.

Functional test inputs:

- The inputs would be based upon the specific hardware design, for example the requirements for the power and thermal subsystems would be tested based upon pre-defined specifications.

Functional test requirements:

- Confirm basic electronic, mechanical, optical, environment and data handling function for breadboard and associate support equipment.

Functional test outputs:

- Like the inputs, the outputs would be based upon the specific hardware design.

Basic analytical test

Basic analytical test objectives:

- Confirm that the prototype(s), *i.e.,* the breadboard(s), can perform appropriate molecular detection (either single molecule detection or ultra-low lower level of detection) independent of any Space application context.

Basic analytical test inputs:

- The approach will be to select well established examples from the literature of analytes and their interfacing to a SERS surface to demonstrate single molecules and/or ultra low lower levels of analyte detection. No relevance to Space applications will be considered, i.e. the test can be performed at room temperature and under ambient laboratory atmosphere.
- There are very varied examples of target analytes in the literature, *e.g.,* Dieringer et al. (2008) studied Rhodamine 6G, Pieczonka et al. (2009) studied phospholipids, Zhang et al. (2009) studied 4-dimethylaminoazonbenzene and Gu and Suh (2009) studied p-aminothiophenol.

Basic analytical test requirements:

- Confirm ability to detect spectra from target molecules identified from established examples from the peer-reviewed literature. Two example target molecules to be used in separate tests.
- Confirm limits (lower and upper) of detection for given demonstration target molecules in isolation with lower detection limit to be compatible with peer-reviewed literature examples of single molecule detection and/or ultra-low lower limits of detection.
- Confirm ability to detect mixtures of two demonstration target molecules.
- Perform appropriate control experiments to confirm appropriate interpretation of findings.

Basic analytical test outputs:

- Collected SERS spectra.
- Comparison and interpretation of collected SERS spectra that show agreement with known examples of single molecule detection and/or ultra-low lower-limit of analyte detection published in the peer-reviewed literature.

Space scenario analytical test

This section relates to the demonstration of the analysis using one or more Space relevant scenarios and comprising a particular scenario relevant sample matrix, related analytical targets relevant to the given sample matrix and suitable replication of key components of the mission/Space scenario environment in which a measurement would take place.

- For Stand-Alone SERS, the elimination of a sample processing/extraction requirement (*e.g.* required for analysis of a Mars regolith sample) enables a low mass implementation to be considered. Additionally, operation in an ultra low pressure environment allows the re-generation of SERS surfaces for repeated measurement (i.e. by in situ metal deposition by evaporation or sputtering). Two obvious examples exist within these contexts: an Enceladean plume fly-by and a cometary coma fly-by.

Space scenario analytical test objectives:

- Confirm that the prototype (breadboard) can perform appropriate molecular detection (either single molecule detection or ultra-low lower level of detection) within one or more specific Space application contexts.

Space scenario analytical test inputs:

Space mission scenario:

- Detect the presence of simple organic molecules in Enceladean fly-through.
- This example is chosen to demonstrate a test plan due to (i) recent data the defines the organic molecular contents of the plume and (ii) the current interest in understating the history, present and future of Enceladus and especially in the context of the abiotic/pre-biotic and potentially biotic chemistry.
- Note: a similar mission scenario would be a cometary coma fly-through mission, *i.e.* an ultra-low pressure environment containing water/water ice and associated trace organic molecules.

Sample matrix:

- Enceladean plume (no sample extraction, sample interacting directly with the SERS active surface). From recent Cassini and ground-based telescope studies of Enceladus' plumes and the associate Saturn E-ring, plume is defined within the context of this study as:
  - Particles*:* ice grains are dominated by water ice but 6% of them contain 1.5% of a mixture of sodium chloride, sodium carbonate and sodium bicarbonate (Spencer, 2009). Particles predominantly have a radius of 0.1-1µm.
  - *Gases:* mainly water vapour, produced at a rate of ~150-300kg s^{-1} (Postberg, 2009).
  - *Trace inorganics*: dilute salt solutions (Na/H_2O $<10^{-7}$) (Postberg, 2009).
  - *Trace organics:* organic compounds and/or silicate minerals are identified as impurities in the icy particles (Postberg, 2007).
  - Note: the duration of a fly-through an Enceladean plume is typically (for the Cassini spacecraft travelling at 8 kilometres per second) of 1 minute of duration (Buratti, 2009).
- Enceladus (model system implementation):
  - Ultra-pure water spiked with target examples to a given concentration (*i.e.* target volume fractions referenced to water content). Small volumes injected into a reduced pressure chamber (*e.g.* SERS surface mounted on a cryogenic plate in a UHV chamber) thereby generating a "plume" of water and solute molecules (*i.e.* molecules with mean free paths lengths similar to the dimensions of the chamber).

Analytes/Targets for Enceladus:

- The targets for Enceladus are based upon recent Cassini Enceladus plume fly-through data (Waite, 2009). Specific targets could be ammonia; methanal (formaldehyde); hydrogen cyanide; and methanol (these represent examples of organic molecule targets with higher abundances in the plume).
- Target concentrations (as volume fractions referenced to water) are based upon Cassini data (Waite, 2009) based upon mixing ratio with water and therefore would be (*i.e.* levels in spiked water injected into reduced pressure chamber): ammonia ($8x10^{-3}$); methanal (formaldehyde) ($3x10^{-3}$); hydrogen cyanide ($7x10^{-3}$); methanol ($2x10^{-4}$).

Enceladean environment:

- *Temperature:* the critical aspect of temperature, within a vacuum system, is the temperature of the SERS surface due to the uncertainty in the negative or positive effects of low/cryogenic ambient temperature on the ability of the SERS surface to interact with sample matrix components and targets. This feature should be able to be changed, *e.g.* ranging from deep Space (*e.g.* this temperature can be pragmatically obtained via thermoelectric cooling or liquid nitrogen) to +25°C (laboratory ambient).
- *Pressure:* the low pressure environments of an Enceladus fly-through can be simulated using a high vacuum (10^{-2} to 10^{-4} Pa, note this is prior to injection of water matrix into the chamber). This can be for example by using a vacuum chamber and vacuum pump.
- *Radiation (energetic electrons, protons and other ions trapped in Saturnian magnetosphere):* this will not be considered within the central test plan (Note: a separate test to de-risk the radiation effects on the surface enhancement ability of the SERS are described later).
- *Gravity*: will not be considered in the current test plan as no fundamental aspect of the test will be significantly influenced by 1g versus 0g; therefore, for pragmatic reasons 1g will be used.

Space scenario analytical test requirements:

- Confirm basic ability to detect spectra from individual Enceladean target molecules under non-Space relevant conditions and without reference to concentrations relevant to Enceladean context (only if non-Space relevant conditions are appropriate).
- Confirm ability to detect spectra from individual Enceladean target molecules under non-Space relevant conditions at concentrations relevant to Enceladean context (if appropriate).
- Confirm ability to detect spectra from mixtures of Enceladean target molecules under non-Space relevant conditions at concentrations relevant to Enceladean context (if appropriate).
- Confirm ability to detect spectra from individual Enceladean target molecules under Space relevant conditions and in a relevant sample matrix.
- Confirm ability to detect spectra from individual Enceladean target molecules under Space relevant conditions at concentrations relevant to Enceladean context and in a relevant sample matrix.
- Confirm ability to detect spectra from mixtures of Enceladean target molecules under Space relevant conditions at concentrations relevant to Enceladean context and in an Enceladean relevant sample matrix.
- Perform appropriate control experiments to confirm appropriate interpretation of findings.

Space scenario analytical test outputs:

- Collected SERS spectra.
- Comparison and interpretation of collected SERS spectra that show agreement with known examples of single molecule detection and/or ultra-low lower-limit of analyte detection published in the peer-reviewed literature.

Early stage de-risking of fundamental components with no space heritage:

The key aspects that should be independently tested alongside the main test plan and are described as follows:

- SERS surface interaction issues:
    - *Radiation:* within a Saturnian/Enceladean mission, a significant local source of radiation is energetic water group ions (*e.g.* O^+, H_2O^+, OH^+, H_3O^+, HO_2^+, O_2^+ and H^+) produced from the Enceladean plumes. The effect of such ions on a SERS surface is uncertain and requires early stage de-risking. The use of water plasma in a vacuum chamber containing the SERS surface is a possible approach to implement such as study.
    - *Temperature:* it is uncertain whether the cryogenic temperatures that are likely to be the default situation for operation of a SERS instrument in an Enceladus fly-by scenario will have a negative, neutral or positive effect on the ability of the SERS surface to interact with sample matrix components and targets and produce detection/spectra. Preliminary studies are required to determine the effect of SERS surface temperature on the ability to interact and generate detection signals/spectra for targets and with the temperature range encompassing typically cryogenic temperatures up to typical temperatures that the majority of published SERS data in the peer-reviewed literature (*i.e.* +25°C).

- *In situ* SERS surface re-generation (the following points are noted when considering the use of SERS in an Enceladus fly-by mission context):
  - There is a desire to perform multiple measurements.
  - A SERS surface is normally considered a single use item, *i.e.* after measurement of sample the surface is discarded.
  - A SERS surface is prone to contamination during instrument AIV, ground handling, storage and flight/cruise.
  - A mechanical approach to replacing a SERS surface is undesirable due to mass and complexity issues.
  - Therefore, a task is required to demonstrate an approach to *in situ* SERS surface regeneration compatible with the above situation (specific requirements TBD).
  - It is anticipated that due to use within an ultra high vacuum environment, there is the potential for repeated *in situ* deposition of SERS active surfaces (*e.g.* silver film) by thermal evaporation or sputtering (possibly sandwiched with evaporation or sputtering dielectric layers to enable silver island films to be repeatedly formed). It is anticipated that repeated deposition of a suitable metal on a suitable SERS surface will overlay and mask any build-up of contamination and/or sample thereby generating a new clean SERS surface and enabling multiple measurements
  - Key functions to be considered/tested include:
    - Minimum thickness of metal to be deposited to avoid cross-talk of spectroscopic signals between coatings.
    - Number of coatings possible before loss of SERS efficiency due to lack of appropriate roughness and/or the inherent roughness of multiply recoated layers.
    - Power requirements.
    - Initial consideration of a system design that allows SERS spectroscopy and recoating of SERS surface without requirement for mechanical intervention/mechanisms.
    - Inclusion of a method to *in situ* monitor the re-coating processes; *e.g.* use of SERS surface supported on a gravimetric sensor such as a Quartz Crystal Microbalance or Surface Acoustic Wave device.

2.7 Discussion and Analysis of the Proposed Systems

In order to recommend a suitable design for a single molecule/ultra-low lower limit of detection instrument for Space applications an assessment of its suitability needed to be performed.

However, instead of proposing a technology at the beginning of the study and now having to consider its feasibility, it is worth re-iterating that the technologies considered throughout the study have been already assessed. In other words, throughout the study all the technologies considered were assessed in order to understand which one would be the most suitable for Space applications, and the technologies stated to be suitable (MS/MS-SERS and Stand-Alone SERS) were the conclusion of that assessment.

2.7.1.1 Comparison between the two systems

In the aid of assessing the output of the study, the salient features of the two systems identified are summarised in the table below in the context of an expected flight instrument for the two envisaged mission scenarios (Mars for MS/MS-SERS and Enceladus for Stand-Alone SERS).

Table 2–41: *Comparison between MS/MS-SERS and Stand-Alone SERS in Context of an Expected Flight Instrument.*

Parameters	MS/MS-SERS	Stand-Alone SERS
Technology	Tandem mass spectroscopy (CIT) with "direct" sample input/ionisation and mass analyser output option to SERS.	Surface Enhanced Raman Spectroscopy (SERS).
Mission scenario	Mars.	Enceladus.
Science scenarios	• *Primary scenario*: search for evidence of life -extinct and extant. • *Secondary scenario*: search for pre-biotic/a-biotic organic inventory.	• *Primary scenario*: search for a-biotic/pre-biotic organic inventory. • *Secondary scenario*: search for evidence of life -extinct and extant.
Molecular targets	• *Primary targets*: higher-level molecular organic biomarkers. • *Secondary targets*: simpler organics including meteoritic in-fall.	• *Primary targets*: simple organics. • *Secondary targets*: higher-level molecular organic biomarkers (details TBD).
Sample matrix	Low target levels in low organics background in regolith samples.	Ultra low pressure volatile plume primarily of water ice with trace levels of organic molecules.
Measurement environment	Low radiation, 10mbar CO_2.	High radiation, ultra-high vacuum.
Size	Medium, as volume is envisaged to be <10 litres in order to accommodate for the following components: • For the MS/MS-CIT system: Sampling and ionisation system, vacuum system (depending on environment) and mass analyser (Cylindrical Ion Trap).	Low, as volume is envisaged to be <5 litres in order to accommodate for the following components: Optical spectrometer and detector, solid-state laser, SERS surface and *in situ* SERS surface regeneration.

	• For the SERS system: optical spectrometer and detector, solid-state laser, SERS surface and *in situ* SERS surface regeneration.	
Consumables required	• SERS surface and DART gas.	• SERS surface.
Mass	Medium, as mass is envisaged to be a total of <10kg to accommodate for the following components: For the MS/MS-CIT system: Sampling and ionisation system, vacuum system (depending on environment) and mass analyser (Cylindrical Ion Trap). • Plus for the SERS system: optical spectrometer and detector, solid-state laser, SERS surface and *in situ* SERS surface regeneration.	Low, as mass is envisaged to be a total of <5kg for the following components: Optical spectrometer and detector, solid-state laser, SERS surface and *in situ* SERS surface regeneration.
Power	Medium, as the power requirements needed to service both MS/MS-CIT and SERS systems are envisaged to be higher than just powering the SERS system.	Low, as the power requirements are only needed to service the SERS system.
Data budget	Low (only multiple 2D spectra and general house-keeping data need to be transmitted).	• Low (only multiple 2D spectra and general house-keeping data need to be transmitted).
Current development status	• CIT tandem mass spectrometers established in research laboratories. • Flight model CIT mass spectrometers developed (MODULUS PTOLEMY for Rosetta) and under development (MOMA for ExoMars). • Direct atmospheric pressure sampling and ionisation systems established in research laboratories under ambient Earth conditions. • No demonstration of coupling MS with SERS.	• Single molecule/ultra-low limit of detection SERS well established in research laboratories. • Flight model Raman (not ultra-low limit of detection SERS) instrument under development for ExoMars.

Identified current risks	For DART sampling: • The volumetric gas flow rate and related power requirements to heat the gas flow need to be considered. • The ability for DART to operate under Mars atmospheric pressure and gas constitution. • The sampling efficiency in terms of sampling volume/area and related effects on detection limits and efficiency. For CIT-MS to SERS transfer: • The ability to collect ions ejected from the CIT by a SERS surface is not established.	For SERS: • The effect of radiation on a SERS surface is uncertain and requires early stage de-risking. • It is uncertain whether the cryogenic temperatures in an Enceladus fly-by scenario will have a negative, neutral or positive effect on the ability of the SERS surface to interact with sample matrix components and targets and produce detection/spectra. • Requirement for in situ SERS surface regeneration for optimal science return (maximise number of samples analysed) and instrument mass efficiency.
Initial development resource requirements	• Higher required number of core components for de-risking and breadboard studies, and of average higher cost. • Higher number of tasks required to de-risk concept (see “Identified current risks”).	• Lower required number of core components for de-risking and breadboard studies, and of average lower cost. • Lower number of tasks required to de-risk concept (see “Identified current risks”).

2.7.1.2 MS/MS-SERS and STAND-ALONE SERS additional considerations

In addition to the above parameters, there are also a number of other obvious parameters that flight instruments would need to survive. The following is a simple assessment of these parameters for the MS/MS-SERS system, where MS/MS and SERS systems are looked at separately for each parameter.

Environmental Compatibility

Compatibility with radiation, vibration/shock, thermal extremes, pressure and atmospheric composition are explored.

Radiation:

Mass spectrometers have commonly flown in Space exploration missions, including CITs (MODULOUS PTOLEMY for the Rosetta mission and MOMA for ExoMars -to launch in 2018) and their previous use has de-risked the ability to fly these instruments in Space radiation environments/contexts. For SERS, the stability of the SERS metal surface to various radiation environments is a current unknown.

Vibration/Shock

For mass spectrometers, the same argument as used for radiation in is applicable. For SERS, which involves optical spectroscopy, this has been de-risked by the many other optical spectrometers that have been flown in various Earth observation and other Planetary Exploration missions.

Thermal

Again, for mass spectrometers, the same argument as used for radiation is applicable. For SERS, the crucial step is the interaction of the sample molecular targets with the SERS active surface; i.e., for low temperatures it is envisaged that molecular targets/SERS surface interactions will be stabilised but that the energy of molecular targets and the SERS surface would be low and that might impede adsorption of the target molecules to allow formation of the molecular target/SERS surface combination.

Pressure

For mass spectrometry this affects the resources required to deliver the needed vacuum levels at various stages within the in situ measurement process. Therefore, for a Europa context, the presence of an ultra high vacuum like ambient environment minimises the mass and power requirements for MS associated vacuum systems. Whereas in a environment such as the Venusian upper atmosphere (1 bar) and on the surface of Titan, more mass and power resources would be required to achieve MS appropriate vacuum levels. For SERS, atmospheric pressure does not appear to pose any significant issues.

Atmospheric composition

For Titan the high organics content of the atmosphere would be expected to cause background noise/signal in both mass spectrometry and SERS applications, making the detection of trace levels of biomarkers more difficult without resorting to further sample processing.

However, the composition of ultra-low pressure atmospheres of Europa and related icy moons do not appear to pose any significant issues to mass spectrometry and SERS.

While the relatively inert carbon dioxide based atmosphere of Mars does not appear to cause any significant issue to mass spectrometry and SERS (although the compatibility of the proposed DART ionisation source with a carbon dioxide based atmosphere, is not currently known -but not expected to be a significant issue). On the other hand, for Venus, the atmospheric composition does not appear to pose any fundamental issues for mass spectrometry whereas for SERS, the acid content may be problematic for certain types of SERS surface (for example, the commonly used silver surface is not expected to be compatible with a sulphuric acid rich sample environment).

Spaceflight Compatibility

Compatibility with typical spaceflight mass, volume, power and data budgets are noted, as well as PP&CC requirements assessed.

Mass, volume, power and data budgets

For the proposed tandem MS system, the CIT basis of the system is broadly similar to existing designs that are either in flight (Modulus Ptolemy) or being prepared for flight (MOMA) and therefore these examples demonstrate at the very top level that CIT based mass spectrometers can be built within planetary exploration mass, volume, power and data budget requirements.

For the proposed SERS system, if it is considered to be an optical spectrometry based instrument, then there are a large number of other optical spectrometry instruments that have been used Earth observation and planetary exploration missions and hence demonstrate that, at this simplistic level, the mass, volume, power and data budget requirements would be complied with.

PP&CC requirements

For Planetary Protection, it is not envisaged that any components of the proposed MS and SERS systems will pose a problem in sterilisation steps as all materials are expected to have been used in previous or existing flights instruments that have undergone sterilisation protocols.

For Contamination Control, the flight of ultra-low lower limit of detection instruments poses a significant problem as this places more stringent requirements on the cleanliness in terms of levels of contaminating biomarkers and other instrument targets. Therefore, this may require the development of alternative or more advanced methods of instrument cleaning and the verification of cleaning during instrument and spacecraft AIV.

2.7.1.3 The complete system

The complete system is based on an IonSense DART Ion Source coupled on to a Griffin 400 CIT from ICx Technologies followed by Klarite™ SERS surface, an optical bench with a laser and an Ocean Optics Spectrometer.

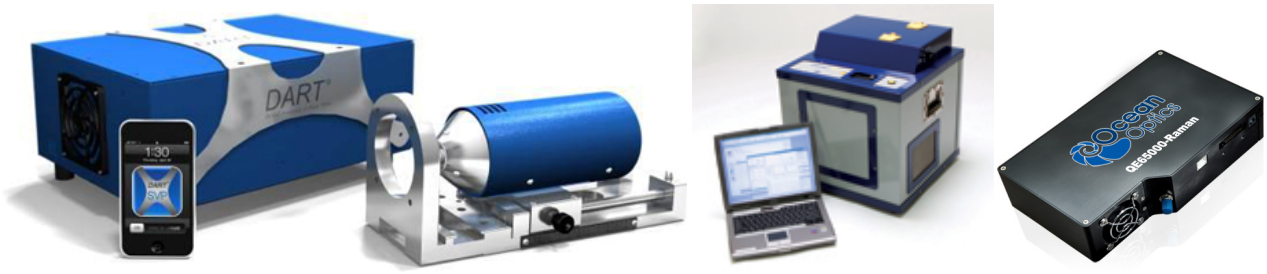


Figure 2-19: *(Left) DART Ion Source (IonSense, 2010); (Middle) Griffin 400 CIT (ICx Technologies 2009); (Right) Ocean Optics Spectrometer (Ocean Optics 2010B).*

2.8 Conclusions and Future Work

The aim of this research was to consider current and near-future single molecule detection (ultra-low lower limit of detection) analytical techniques that would be compatible with development into a Space qualifiable *in situ* analytical instrument for the detection of biomarkers in a planetary exploration context.

In order to achieve this, a desk-based European Space Agency study was carried out which, according to the European Space Agency (14/05/2008) Statement of Work, entailed producing a literature review on single molecule detection technologies that had to be validated by the expert community. This was done by organising an International Workshop on Single Molecule Detection Technologies for Space Applications in March 2009 at Cranfield University, UK. The approved technologies then had to be analysed with standard analytical techniques (*i.e.*, trade-offs) in order to propose a specific technology for development and present its breadboard implementation and test plans at the end of the study.

2.8.1 Conclusions

This study considered, and thus recommends at a design level stage, an MS/MS-SERS system as well as a Stand-Alone SERS system for different Space applications; *i.e.,* MS/MS-SERS is proposed for the detection of astrobiology biomarkers in Martian regolith (or ground rock and possible ice), Europan ice (and possible water) and samples from Titan's hydrocarbon lakes. While a Stand-Alone SERS system is envisaged for the detection of biomarkers in Enceladean plumes, Venusian clouds and cometary coma.

Both systems could be developed by summarising the procedures that lead to the selection of these specific systems (which, by following the analytical process of trade-offs, ensured the systems were feasible). Specific components to build breadboards for the systems, test plans to be conducted to demonstrate the suitability of the technologies, and items that need to de-risked to ensure reliable performance in Space applications are also proposed.

The figure below shows the flow of the proposed Tandem MS (CIT)-SERS system. The sample would be introduced into the instrument via the DART ionisation system which would also ionise the sample targets. Once ionised, the molecular ions would be analysed by the CIT and sent to the SERS surface or fragmented to be analysed again by the CIT and then sent to the SERS surface.

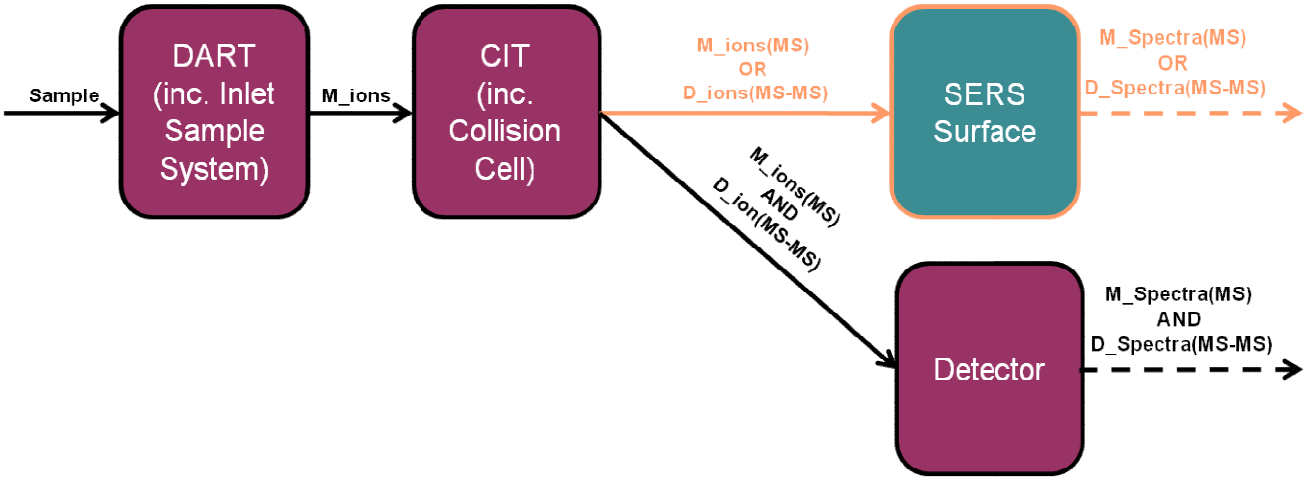


Figure 2-20: *The MS/MS (CIT)-SERS System.*

Therefore, in default mode, the system will produce three spectra: spectra of the molecular ions from first stage of the MS, spectra of the daughter ions from the second stage of the MS/MS, and spectra of the molecular ions ***or*** daughter ions from the SERS system.

Whereas in a MS/MS (CIT)-SERS system the sample deposited onto the SERS surface arrives as the output from the CIT, in the Stand-Alone-SERS system the sample will be deposited directly onto the surface, *e.g.* from a fly-through of an Enceladean plume.

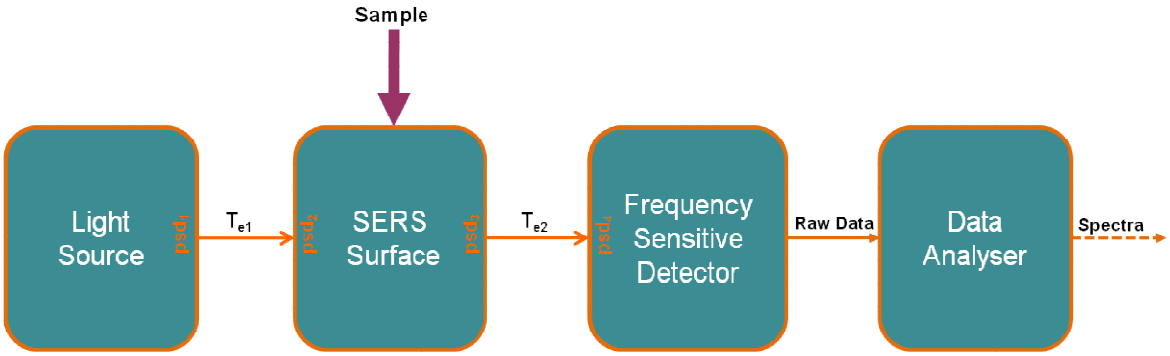


Figure 2-21: *The Stand-Alone SERS system.*

Note that T_{ex} is the *light conduit photon transfer efficiency* and psd_x the *photon output spatial distribution.*

2.8.2 Future Work

Immediate work would include:

- Building the instruments proposed according to the development plans in order to identify the components needed to build the system.
- Testing the system according to the test plans in order to identify the reliability of the instruments proposed (especially in Space simulating environments).

Long-term work would include de-risking the items identified:

- Clarifying the operating constraints of the DART sampling system under Mars atmospheric pressure.
- Coupling the MS/MS output to the SERS input for the MS/MS-SERS system.
- Understand the effects of radiation and temperature on the SERS surface.
- Explore the possibility of SERS surface regeneration.

Therefore, novel instrumentation has been proposed that could be used for future planetary exploration life detection missions along with set plans to carry the work forward towards a full system that could be proposed in the next call for opportunities for a specific planetary exploration astrobiology mission.

3. Design, Implementation, Testing and Validation of a Sample Return Instrument for a Stratospheric Balloon Mission Instrument for Life Detection

The aim of this research was to practically consider the consequences of Planetary Protection and Contamination Control on the development of sample return instrumentation in a planetary exploration context.

3.1 Introduction

This chapter describes, in the context of setting a PP&CC practical sample return example, how the Cranfield Astrobiological Stratospheric Experiment (CASS•E), a sample return payload on-board a stratospheric balloon, was realised.

3.1.1 Science and technology background

Searching for life in the Stratosphere is a vital study needed to understand possible paths for microbial dispersion from one part of the Earth to the other as well as to test the hypothesis of panspermia*; i.e.,* the possibility of microbial transport through Space seeding life on other planets (Imshenetsky, 1978). In addition to this, the study of life in extreme environments on Earth, including the Stratosphere, contributes to our understanding of the possibility of life elsewhere in the Universe.

3.1.1.1 Searching for Life in the Stratosphere

A small number of experiments have previously been conducted in an attempt to detect microbial life in the Stratosphere using balloon platforms (including organisations such as the Japanese Space Agency and the Indian Space Research Organisation) as well as meteorological rockets and high altitude aircraft. These experiments attempted to define the presence of microorganisms in the Stratosphere and the findings were documented in papers such as those published by Imshenetsky (1978), Narlikar (2003) and Griffin (2004). These experiments addressed, to varying levels, the issue of contamination with non-stratospheric microorganisms that may occur pre- and post-stratospheric flight phases. However, it has proven difficult to convince the wider scientific community that resultant claims of stratospheric life collection are not simply ground or other tropospheric derived contamination.

Therefore, the Cranfield Astrobiological Stratospheric Sampling Experiment (CASS•E) used the BEXUS stratospheric balloon platform to attempt a first implementation of a stratospheric particle detection experiment that included space-sector developed PP&CC protocols as well as additional features to control and understand contamination. The hypothesis for this study is that the implementation of space-sector developed PP&CC protocols and related design approaches to stratospheric balloon experiments will help to convince the majority of the scientific community that any detected microorganisms are unlikely to be ground or other tropospheric derived contamination.

3.1.1.2 REXUS/BEXUS

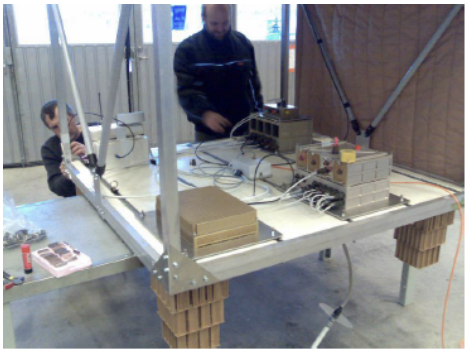
The REXUS/BEXUS programme allows students from universities and higher education colleges across Europe to carry out scientific and technological experiments on research rockets and balloons. Each year, two rockets and two balloons are launched, carrying up to 20 experiments designed and built by student teams. The programme is realised under a bilateral Agency Agreement between the German Aerospace Center (DLR) and the Swedish National Space Board (SNSB). The Swedish share of the payload is made available to students from other European

countries through a collaboration with ESA. EuroLaunch, a cooperation between the Esrange Space Center of the Swedish Space Corporation (SSC) and the Mobile Rocket Base (MORABA) of DLR, is responsible for the campaign management and operations of the launch vehicles at the Esrange Space Center in northern Sweden (REXUS/BEXUS, 2010).

REXUS experiments are launched on an un-guided, spin-stabilised rocket powered by an Improved Orion Motor with 290 kg of solid propellant. It is capable of taking 40 kg of student experiment modules to an altitude of approximately 100 km. BEXUS experiments are flown on a balloon with a volume of 12000 m³ to a maximum altitude of 35 km, depending on the total experiment mass (40-100 kg). The flight duration is 2-5 hours.

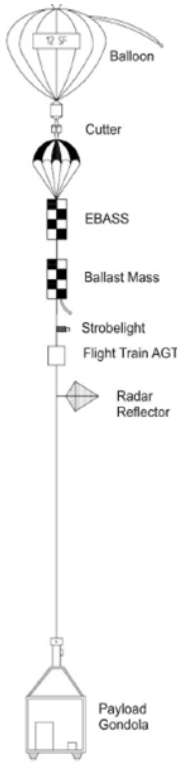
The BEXUS platform

The BEXUS system mainly consists of the balloon, a parachute system, a cutter, the Esrange Balloon Service System (EBASS) and the flight train with the Argos GPS, ATC transponder, radar reflector and the experiment gondola. The total length of the system is approximately 75m.



(a)

(b)



(c)

Figure 3-1: *(a) BEXUS Experiment Gondola; (b) Hercules Launch Vehicle with Gondola; (c) the BEXUS System (Persson, 2009).*

CASS•E won the opportunity to fly on-board the BEXUS platform in 2010, flying both on the BEXUS-10 and BEXUS-11 balloons.

Flight sequence

The balloon is launched from Esrange in Kiruna, Sweden. The ascent speed is nominally 5 m/s and the ascent phase can take up to 2 hours depending on the floating altitude. The float phase lasts up to four hours at an altitude between 25 and 35 km. The descent phase is initiated by activating the cutter, thus ripping the balloon from the rest of the system via the flight train. A parachute is deployed which lands the separated system at 8 m/s. Shock absorbing material at the

bottom of the gondola reduces the shock load during landing, which is planned to be in a sparsely-populated area with no lakes.

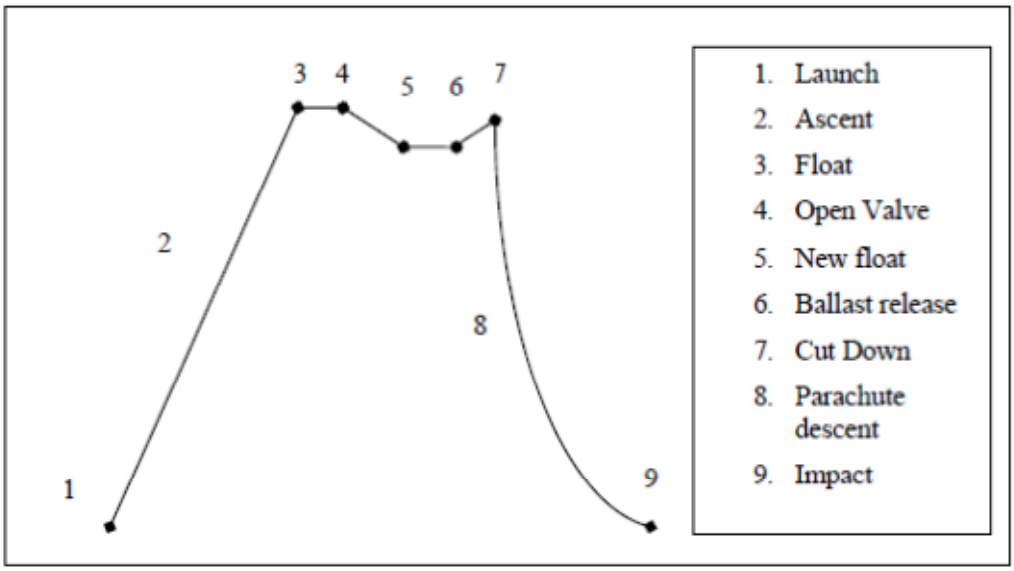


Figure 3-2: *BEXUS flight profile (Persson, 2009).*

3.1.1.3 The stratospheric environment

The Stratosphere is a major layer in the Earth's atmosphere found after the Troposphere and before the Mesosphere, at an altitude between 10km to 50km (between 8km to 50km at the poles) with very low temperatures and near vacuum conditions.

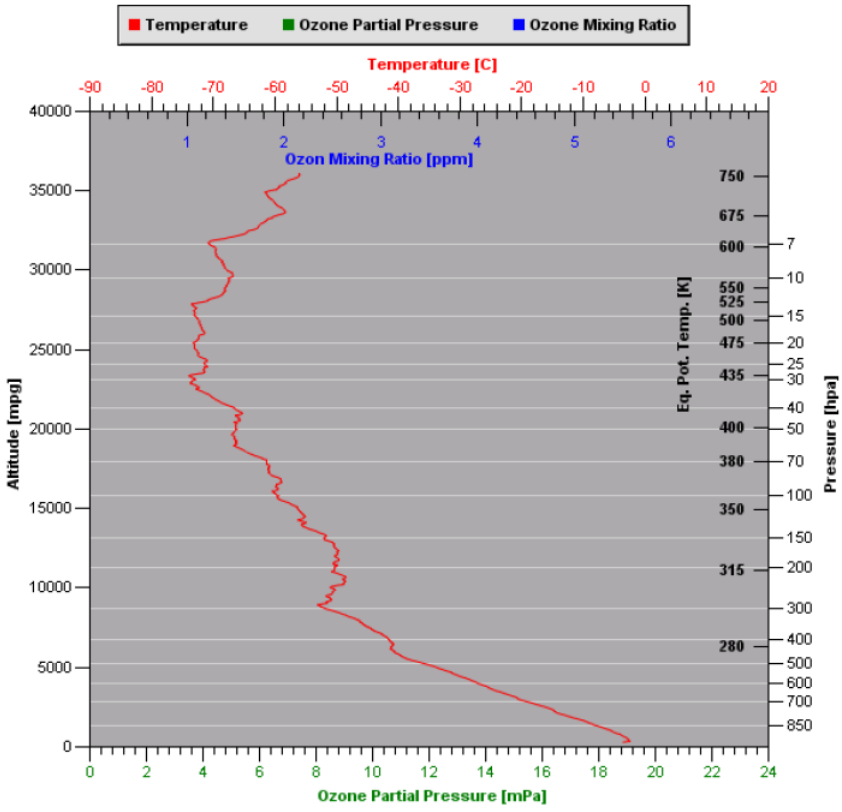


Figure 3-3: *BEXUS Temperature and Ozone Graph from 18/10/2004 Flight (Persson, 2009).*

However, in contrast to the conditions observed in the Troposphere, the higher the altitude in the Stratosphere the higher the temperature; this phenomenon begins at the Tropopause, the border between the Troposphere and the Stratosphere. This occurs due to the Ozone (O_3) content in the Stratosphere, which absorbs UVB and UVC energy waves from the Sun to create O_2 and O. Due to the lack of convection between the layers, the Stratosphere is dynamically stable.

As seen in "Figure 3-3: BEXUS Temperature and Ozone Graph from 18/10/2004 Flight (Persson, 2009)" the thermal environment of the BEXUS flights can experience temperatures down to -90 °C and pressures of 7mBar.

3.1.1.4 The CASS•E Team

The live project required interaction and cooperation between various engineering and scientific disciplines, in addition to the systems engineering aspects presented in this report. Hence, the project was divided into five main subsystems handled by six team members as follows:-

- Team Leader and Electronics subsystem: Handled by Ms. Clara M. Juanes Vallejo, PhD student from Cranfield Health, Cranfield University (author of this document). Was responsible for correspondence with the BEXUS group, sponsors, arranging group meetings, approving all engineering and scientific decisions in order to control the design of the system, integrating and verifying the SED (student experiment document) submitted to the BEXUS group and managing the team in order to execute the mission successfully. As well as designing the electronics subsystem, manufacturing and testing the PCBs and constructing the electrical harness.
- Mechanical & Thermal subsystem: Handled by Mr. Vinay Visweswara Grama, MSc Astronautics and Space Engineering student from the School of Engineering, Cranfield University. Was responsible for the configuration design, generation of CAD models, manufacturing drawings, structural and thermal finite element analysis, manufacturing (simple parts) and assembly. This also included the responsibility of identifying components and ordering parts related to the mechanical subsystem.
- PP&CC subsystem: Handled by Ms. Carla Rato & Ms. Catherine Rix, PhD students from Cranfield Health, Cranfield University. Ms. Carla Rato was in charge of project planning, cleaning and sterilisation of the components. Ms. Catherine Rix was in charge of preparation of fluorescent beads and analysis of the filters in the post flight stage.
- Software subsystem: Handled by Mr. Ioannis Katramados, PhD student from Applied Mathematics and Computing Group, School of Engineering, Cranfield University. He is responsible for the development of the software for ground control and autonomous operation of the experiment.
- Verification and testing: Handled by Ms. Clara M. Juanes-Vallejo (author), Mr. Vinay V. Grama and Mr. Lolan Naicker MSc Astronautics and Space Engineering student from the School of Engineering, Cranfield University. Mr. Lolan Naicker was responsible for the documentation of test procedures, performing tests, manufacturing and assembly along with Mr. Vinay V. Grama and Clara M. Juanes-Vallejo. Mr Lolan Naicker was also responsible for outreach and media correspondence for the project.

3.2 Aims, Objectives, Requirements and Targets for a Stratospheric Sample Return Instrument

The objective of CASS•E's implementation and flights was to better understand the issues of incorporating PP&CC and additional features to control and understand contamination in stratospheric particle collection experiments rather than a definitive collection of stratospheric samples for life detection. Therefore, CASS•E's primary objective was to demonstrate approaches to implement PP&CC strategies to reduce the levels of contamination within a stratospheric balloon experiment designed to collect microorganisms from the Stratosphere (to a level that would satisfy the scientific community of the validity of the findings).

3.2.1 Aims and Objectives

Primary Objectives

- To design, build and fly an experiment that is capable of collecting microorganisms in the Earth's Stratosphere.
- To ensure that microorganisms collected are truly stratospheric rather than contamination introduced during payload assembly, integration, ground handling, launch, flight and recovery through the implementation and assessment of appropriate PP&CC protocols.

Secondary Objectives

- To expand understanding of the nature and limits of life in Earth's Stratosphere.
- To detect stratospheric microorganisms.
- To increase the profile of stratospheric balloon experiments within Cranfield University and beyond.
- To improve the understanding of Planetary Protection and Contamination Control (PP&CC) implementation in life detection experiments for stratospheric and broader astrobiology missions.
- To train and give experience of PP&CC requirements to early career Space scientists and engineers.

3.2.2 Design requirements and targets

Two types of requirements were imposed, those constraining the experiment to the requirements of the BEXUS balloon platform (*i.e.,* considering the implications of having the experiment on-board) and those pertaining the CASS•E experiment itself (*i.e.*, the requirements to meet the experiment's scientific objectives).

3.2.2.1 BEXUS requirements

According to the BEXUS User Manual (Persson, 2009), the experiment needs to have the following requirements:

Table 3–1: *BEXUS Requirements.*

Reference	Requirement
BEXUSR1	The experiment support frame shall withstand -10g vertical and ±5g horizontal loads.
BEXUSR2	The experiment shall operate at an ambient pressure of 20mbar and ambient temperature of -56.5°C.
BEXUSR3	The batteries in the experiment shall be accessible from the outside within one minute in order to safeguard the experiment from an explosion.
BEXUSR4	The experiment shall have sufficient mounting provisions to facilitate a rigid connection between the experiment and gondola.
BEXUSR5	The experiment shall have mounting provisions to interface on to both Egon and S-Egon gondolas.
BEXUSR6	The front panel connector for the E-Link must be an RJF21B socket on the experiment housing.
BEXUSR7	The experiment shall survive temperatures of -15°C for several hours (temperature on launch pad).
BEXUSR8	The experiment (specifically the re-sealed biobarrier) shall survive temperatures of -15°C for up to 48 hours (conditions of gondola whilst awaiting recovery)
BEXUSR9	The experiment shall be designed in such a way that it shall not disturb or harm the gondola.

3.2.2.2 CASS•E Requirements

Functional requirements ensure the experiment functions as required.

Table 3–2: *CASS•E Functional Requirements.*

Reference	Requirement
FR1	The instrument shall pump air from the Stratosphere through a collection filter in order to collect microorganisms for the duration of the float phase of the balloon.
FR2	The instrument shall confirm, record and relay to Ground that while the experiment is running the pumps achieve flow.
FR3	The instrument should measure, record and relay to ground station external ambient temperature, temperature of the pumps, temperature of the batteries and temperature of the PCB during pre-launch testing, launch and flight.
FR4	The instrument shall measure, record and relay pressure inside the experiment to Ground during pre-launch testing, launch and flight.
FR5	The instrument shall be clean to ensure microorganisms collected are stratospheric rather than contamination.

Performance requirements describe the specific values to evaluate the performance of the experiment.

Table 3–3: *CASS•E Performance Requirements.*

Reference	Requirement
PR1	The pump *should* be capable of pumping 10,000 litres of air (at stratospheric conditions) during the float phase of the balloon.
PR2	The UCZ *should* meet the requirements of a COSPAR category IVa mission *i.e.* bioburden at launch should be no greater than 300 spores per m^2 pre-sterilisation.

Design requirements specify particular design details that will ensure the experiment can perform its mission.

Table 3–4: *CASS•E Design Requirements.*

Reference	Requirement
DR1	The experiment module must either be gas tight or equipped with venting holes.
DR2	The CASS•E box shall be supplied with a sufficient number of brackets or a bottom rail plate to facilitate safe mounting of the experiment.
DR3	The CASS•E box shall have mounting provision to interface on to both EGON and S-EGON gondolas.
DR4	The CASS•E experiment (including the UCZ) shall withstand the following loads: -10 g vertically, +/-5 g horizontally
DR5	The experiment shall have a 4 pin connector type MIL-C-26482P series 1 on the outside of the CASS•E box to access BEXUS' power bus.
DR6	The front panel connector for the E-Link must be the RJF21B, the cable mating connector must be the MIL-C-26482-MS3116F-12-10P and the cable should be at least 3 metres long.
DR7	The experiment batteries shall be qualified for use on a BEXUS balloon.
DR8	The experiment batteries shall either be rechargeable or shall have sufficient capacity to run the experiment during pre-flight tests, flight preparation and flight, *i.e.,* for a maximum of 8 hours.
DR9	The batteries in the gondola-mounted experiment shall be accessible from the outside within 1 minute.
DR10	The design shall neutralise the pressure difference experienced during flight.
DR11	The experiment shall survive temperatures of -15°C for several hours (temperature on launch pad).
DR12	The experiment (including the biobarrier) shall survive for the duration of the flight at temperatures down to -90°C.
DR13	The experiment shall be designed in such a way that it shall not disturb or harm the gondola.
DR14	The experiment shall be designed to operate in the vibration profile of the BEXUS balloon (especially for shocks).
DR15	The pore size of the filter shall be 0.2µm, to ensure the collection of microbes.
DR16	The filter shall withstand the vibrations created by the pumps.

DR17	The UCZ *should* meet the requirements of a COSPAR category IVa mission *i.e.* bioburden at launch should be no greater than 300 spores per m^2 pre-sterilisation -this can be assessed by swabbing and culturing (Pillinger, 2006).
DR18	All components inside the UCZ shall be compatible with 70% isopropyl-alcohol (IPA) for immersion and/or wiping for cleaning.
DR19	All components outside the UCZ should be compatible with IPA wiping for cleaning
DR20	All components contained within the UCZ shall be compatible with Dry Heat Microbial Reduction (DHMR), *i.e.,* 110°C for a minimum of 32 hours -as per the requirements for a 10^4 reduction in bioburden by DHMR on free and mated surfaces (ESA, 2008).
DR21	The UCZ shall be protected using a biobarrier to ensure it remains clean after sterilisation and during assembly, testing and integration.
DR22	The re-sealed biobarrier shall retain 99.7 % of all particles or organisms greater than 0.3µm in size -as per NASA planetary protection standards for sealing (this can be assessed by spraying the sealed biobarrier with fluorescent beads).
DR23	The experiment (specifically the re-sealed biobarrier) shall withstand landing shocks of up to 35g.
DR24	The experiment (specifically the re-sealed biobarrier) shall withstand landing in water.
DR25	The experiment (specifically the re-sealed biobarrier) shall survive temperatures of -15°C for up to 48 hours (conditions of gondola whilst awaiting recovery)
DR26	The control software shall be fault tolerant to software errors and resistant to signal noise.
DR27	The software real-time performance and task scheduling shall be predictable.
DR28	The software shall be able to recover from critical failures including multiple component failure and temporary power loss.
DR29	In case of a critical failure the software shall continue/restart in safe mode. If no command from the ground station is received within 10 minutes of the critical failure then the system may continue operating in autonomous mode.
DR30	All transmitted information from the control board and the ground station shall be logged with the associated time-stamps.

Operational requirements ensure the experiment remains operational during its working life.

Table 3–5: *CASS•E Operational Requirements.*

Ref	Requirement
O1	The experiment shall be able to function autonomously in the event that contact with Ground is lost.
O2	The experiment shall only be handled by operators wearing nitrile gloves during integration and launch.
O3	The part of CASS•E exposed to the exterior of the Gondola shall be protected with a *remove before flight cover.*
O4	The *remove before flight cover* shall be removed before flight.
O5	The UCZ shall open once the balloon has reached the Stratosphere (at 20km above sea level) and not before.
O6	The UCZ shall be re-sealed prior to the descent phase of the balloon.

3.2.2.3 Targets

It is assumed that any microbes in the Stratosphere will be spores, since these can survive in hostile environments for long periods of time. Spores are formed within vegetative cells in response to adverse changes in the environment (Yung, 2006).

Yang (2005) and Wainwright (2002) reported the presence of microorganisms at stratospheric altitudes: fungi, endospore-forming bacteria and high UVC_{254nm}-resistance strains

Table 3–6: *Microbial Sampling at High-Altitudes* (Yang, 2005).

Year	Country	Mission	Altitude (km)	Microbial Collection	Reference
1936	USA	Balloons	11-21	**5 *Bacillus* sp.** *Macrosporium* sp., *Rhizopus* sp., *Penicillium* sp. and 2 *Aspergillus* sp.	Rogers and Meier, 1936
1962, 1963, 1965	USA	Balloons	9-27	(Predominant isolates) **Micrococci and spore-forming rods** *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp. and *Cladosporium* sp.	Greene *et al.*, 1964; Bruch, 1967
1975	Russia	Meteorological rockets	48-77	***Mycobacterium* sp., *Micrococcus* sp.** *Circinella* sp., *Aspergillus* sp., *Papulaspora* sp. and *Penicillium* sp.	Imshenetsky *et al.*, 1976
2001	India	Balloon	41	**5 *Bacillus* sp. and *Staphylococcus* sp.** *Engyotontium* sp.	Wainwright *et al.*, 2003; Suresh *et al.*, 2004
2003	USA	High-altitude aircraft	20	**2 *Bacillus* sp.** *Penicillium* sp.	Griffin, 2005
2004	USA	High-altitude aircraft	20	***Micrococci*, *Microbacteria*,** ***Staphylococcus* sp., *Brevibacterium* sp.**	Griffin, 2008

Bacterial species are boldfaced. Other species are fungi.

3.3 Literature Review of Stratospheric Sampling Experiments

Due to having the opportunity to fly on-board the BEXUS balloon -and thus only a year for design, build, test and flight- it was deemed that *in situ* technologies would be too complex and thus only sample return technologies were considered. A review of the existing peer-reviewed literature was conducted.

3.3.1 Sample Return Technologies

As mentioned in Section 3.1.1.1 “Searching for Life in the Stratosphere”, there are many examples of attempts to classify airborne microorganisms. Greene (1962) documented flights from 1873 until 1962 using kites, balloons and aeroplanes up to a maximum of 21km with the following diverse sample collection mechanisms: sticky slides, impaction on nutrient plates, oiled slides, spore traps, release of a sterile tube sampler by parachute, volumetric filtration.

Therefore there are two types of sample return technologies, those relying on active collection (for example by the use of pumps to filter air through collection filters) and those based on passive collection by simply exposing a surface to the stream of air against the direction of travel.

In recent years, high-altitude aircraft, rockets and balloons have been used; the payloads, although more sophisticated, follow the same principles of active or passive collection. Yang et al. (2005)

conducted an experiment for JAXA using volumetric filtration filtering a total of 32 litres at altitudes of 12km to 35km, while Wainwright et al. (2002) used 16 cryosamplers with a volume of 0.35L each that sampled from 30km to 41km. Both claimed to have found examples of bacterial and fungal species in the Stratosphere.

3.3.2 Principles of Planetary Protection and Contamination Control

It is assumed that the level of stratospheric microorganisms will be very low compared to the potential for ground based contamination. For this reason, in order to have any confidence in the results of this type of experiment, it is essential that rigorous cleaning and sterilisation procedures are implemented.

A concern about Earth-derived microbial contamination in life detection experiments and spacecraft for planetary exploration missions has lead to the development of Planetary Protection and Contamination Control (PP&CC) protocols to address these concerns; *i.e.* to minimise the potential for contamination during build, assembly, verification and handling of instrumentation and spacecraft. Examples of protocols include thorough cleaning to minimise the level of contamination with viable microorganisms (termed “bioburden”), measurement of the achieved level of bioburden and then followed by the use of Dry Heat Microbial Reduction (DHMR) involving the heating of items under controlled humidity (< 1.2 g/m^3 water) for a given length of time. The time and temperature required in order to achieve a $x10^4$ to $x10^6$ reduction in bioburden vary depending on the nature of the item to be sterilised. Therefore, such protocols are intended to achieve a final level of bioburden that is compatible with a given acceptable risk of contamination (Moissl, 2007).

Due to the ability of microbes (particularly spores) to survive under extreme conditions, bioburden reduction procedures are rigorous and materials used must be compatible with the selected method of cleaning and sterilisation as well as mission conditions. At the present time the only approved process for bioburden reduction is Dry Heat Microbial Reduction at low humidity for an extended period of time; although other methods, such as hydrogen peroxide vapour, have also been investigated (Salinas, 2006).

Planetary protection requirements are based on the category of the mission, which is determined by the destination and mission type (*i.e.*, obiter, lander, rover) and include the need for bioburden reduction to specified levels, microbiological controls, cleanroom assembly, re-contamination prevention and organic material inventories (ESA, 2008).

Of the lessons learned from the implementation of PP&CC on the Beagle 2 mission, one of the most critical was that “Planetary Protection issues should be addressed early in mission concept design in order to avoid increased complexity in aseptic assembly” and that “training of personnel involved in AIT/AIV is of invaluable benefit in integrating engineers with the planetary protection function” (Pillinger, 2006). For the CASS•E experiment, it is the intention that Planetary Protection will be a major consideration in the design of the instrument and that the engineers will work closely together with the Planetary Protection personnel from the outset of the project. Even though CASS•E is a life detection mission on Earth, PP&CC protocols for a Category IVb mission will be followed.

A critical aspect of the use of PP&CC protocols are approaches to maintain, after treatment, the achieved levels of bioburden whilst performing subsequent pre-flight handling and the flight of the experiment or spacecraft. Physical barrier approaches to stop re-contamination with microorganisms are often called ‘biobarriers’. The biobarrier design will be based upon one of the approaches investigated for the robotic arm on the NASA Phoenix Lander (Salinas, 2006); where the biobarrier covered the clean robotic arm with Tedlar and would be breached by releasing a pyro-actuated pin puller which then retracted using springs.

3.3.3 Literature Review Conclusion

As various types of sampling mechanisms were noted, a trade-off analysis to investigate which would be the most suitable one for CASS•E had to be carried out. However, due to the short timescale imposed by the BEXUS opportunity (one year to design, build, test and deliver the experiment); the fact that pumps are readily available COTS components; the possibility of sealing the sample post-sampling; the heritage that Cranfield Health had due to the Cranfield/Team CGN Lucas experiment (where a small 500g payload comprising a small pump and a filter holder was flown on the Cambridge University Space Flight stratospheric balloon platform); as well as the previous BEXUS heritage with Stratospheric CENSUS, an experiment flown on-board BEXUS 7 which filtered air in order to characterise particles in the Stratosphere (Rudolph, 2009) and JAXA's 2005 experiment; it was decided at proposal stage that the technique with most heritage and therefore least risk would be volumetric filtration.

Therefore, in order to collect stratospheric microorganisms, CASS•E would draw a volume of stratospheric atmosphere through a collection filter once the BEXUS balloon reached the Stratosphere.

Due to the flow rates achievable with pumps compatible with the size and mass requirements of the BEXUS platform, it was statistically difficult for any stratospheric microorganisms to be collected and therefore, as indicated previously, it was not a primary objective of the CASS•E experiment. The intention was for scientifically rigorous collection of stratospheric organisms to occur in a future larger version of CASS•E, which will benefit from the protocols to be developed and established by the CASS•E experiment on BEXUS.

Therefore, due to the necessary implementation of Planetary Protection and Contamination Control procedures in order to comply with the aim of the research, PP&CC requirements had to be implemented from the outset, starting at the design phase.

The approach to implement PP&CC strategies will be to use protocols already established within the planetary exploration community. This will include bioburden reduction by dry heat microbial reduction (DHMR) and maintenance of cleanliness using biobarriers. The biobarrier design will be based upon one of the approaches investigated for the robotic arm on the recent NASA Phoenix Lander as documented by Salinas (2006); where the biobarrier was covered using Tyvek^{TM}, released by a burn wire and retracted via tension springs. Bioburden will be assessed via standard swabbing techniques, but due to resourcing issues, standard culture based methods will only be used for assessment of critical areas of the experiment, where resources and budget allow. Instead, the less resource intensive, but not so well established ATP bioluminescence method will be utilised (Davidson, 1999).

Additionally, to better understand contamination pathways, 1µm and 0.2µm diameter fluorescent beads were used as easily detectible proxies of microorganisms. To differentiate between paths of contamination, different coloured beads will be used to deliberately contaminate regions of the experiment. The post-flight detection of fluorescent beads on the filter would allow us to estimate contamination levels and identify their source. The use of fluorescent beads as a proxy for microbial contamination was investigated during an MSc project carried out at Cranfield University in 2009 by Monaghan (2009).

3.4 Experiment Design

To be able to recommend a suitable design for a stratospheric sample return instrument, it was very important to understand the effect of implementing PP&CC requirements to the design from the outset. Therefore, first a systems level analysis was carried out followed by a component level analysis in order to propose the final design of the system.

3.4.1 Design Concept Process Methodology

As with the research described in Section 2 “Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions” the design of the system was performed by analysing the system at system level to understand the dependencies between the components and make initial proposals to then start the component level analysis where each component was analysed and sourced according to its requirements.

3.4.1.1 Research of components methodology

As with the research described in Section 2 “Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions”, the strategy to find specific components for CASS•E was to:

- Identify the key components that have to be found for each technology, *i.e.,* components, specific working ranges, etc.
- Review the documents compiled for the creation of literature review.
- Find the companies mentioned in the literature review or if no companies had been mentioned in the references, relevant companies were found by searching for the specific components being sought after using general internet sources, *i.e.* Google Search Engine general Web search, to find the websites of the companies providing relevant components or extra information on specific components.

3.4.2 Design of the CASS•E System

It was important to first understand the key areas of the system and their interdependencies before analysing the specific components needed in each one.

3.4.2.1 System level analysis

The experiment design had to ensure that the sample collection filters remained in a sealed and sterile area. This area was quickly defined as the Ultra Clean Zone (UCZ) and all components inside this area would have to be assembled in a cleanroom and undergo cleaning and sterilising procedures. For this reason, the vacuum diaphragm pumps allowing the passage of stratospheric air through the filters would have to be housed outside the UCZ in order to avoid undergoing DHMR.

It was also critical to isolate the batteries in order to avoid damage to the rest of the experiment in case of an explosion. Therefore, three main areas were defined:

- The Ultra-Clean Zone.
- The battery housing area.
- Housing for the pumps and the rest of the electronics.

The battery housing area would have to be connected to the UCZ and the pumps plus the rest of the components in order to feed power, while the pumps would have to be connected to the UCZ in order to be able to sample air.

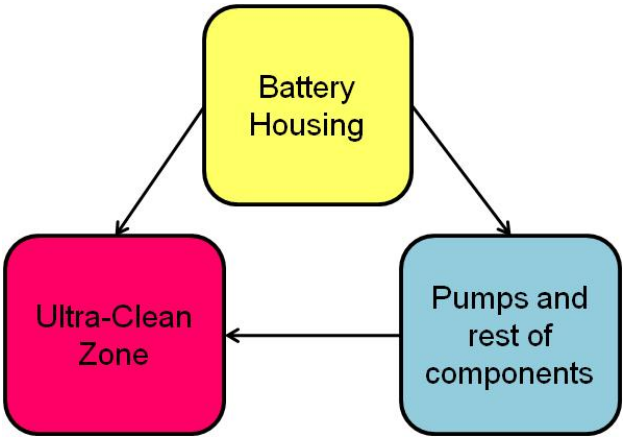


Figure 3-4: *CASS•E Areas.*

Due to the physical separation of these areas, CASS•E's design naturally evolved as being modular.

3.4.2.2 Component level analysis

The table below describes the components that needed to be housed within each area of the system.

Table 3–7: *CASS•E Areas and Components.*

UCZ	Battery Housing	Pumps and Rest of Electronics
Sample collection filters	Battery packs	Pumps
Back-contamination filters to prevent back-contamination from the pumps		Temperature sensors and heaters to maintain working temperatures of components
Temperature sensors and heaters to main working temperatures of components		Main board to control experiment
Biobarriers to protect sampling tubes exposed to outside air		GPS sensor to detect stratospheric arrival
Valves to maintain seal		Communication link with experiment and Ground

Once these main components were set, the system started to take form with each main component being a system in itself with its own components. The final system had the following key features:

Redundancy

In order to build redundancy into the system, as well as offer the potential of two semi-independent measurements, it was proposed to fly a two channel system comprising two pumps and filter units, with an additional third 'control' channel (consisting of a valve, tubing and filter but not connected to the pump), which will not be exposed to the Stratosphere and act as a 'flight control'.

Therefore, two independent sampling lines each connected to a pump were implemented into the design to achieve redundancy as without it, one of the single point failures of the experiment would be the failure of a pump. Furthermore, the pumps were also cross-linked in order to make sure that one pump could take on the load of the other if one were to fail.

Each pump also had its own battery pack, therefore isolating the pumps' power systems to achieve more redundancy.

The Ultra Clean Zone

The UCZ contained the sample collection filters in a sterile environment. Normally Closed (NC) solenoid pinch valves remained closed until the Stratosphere was reached, preventing Ground contamination into the UCZ. They are closed before the Cut-Off of the balloon in order to prevent contamination during landing and recovery.

The biobarriers

The biobarriers covered the projecting inlet tubing line from which the air was sampled. They had to open only once the Stratosphere had been reached, thus preventing contamination from Ground and the Troposphere.

The design of the biobarrier aimed to minimise the risk of contamination after biobarrier opening by pulling the opened biobarrier back and away from the collection tubes. The tubing protrudes 100mm from the edge of the gondola.

Ideally, longer tubes would be preferred to reduce the risk of contamination from the gondola itself, previous balloon experiments have used tubing that protrudes as much as 2m from the edge of the gondola (Wainwright, 2002) but this was not possible on the BEXUS balloon platform.

The two sets of inlet tubing projecting out of the Ultra Clean zone will be each covered by a biobarrier. This provides redundancy since, if one inlet biobarrier fails to open, the experiment fails to meet its objectives. These inlet biobarriers are an extension of the Ultra Clean Zone. They have three main functions:

- To restrict the contamination of the projecting inlet tubing a "remove before flight" cover was placed over the inlet biobarriers to prevent any damage during handling and shipping.
- To provide access to the stratospheric air by opening only in the Stratosphere.
- To minimise contamination to the inlet region of the tube from the outer exposed surface of the biobarrier by retracting.

Also, the farther the biobarrier is pulled back, the longer the path of contamination will be for the microbes to migrate to the inlets of the tubing. Therefore, the biobarrier shall open all along the tubing (making the biobarrier at least 120mm long, as this is the length of the tubing).

The opening of the inlet biobarrier was detected through a micro camera installed to record the opening mechanisms of the biobarriers (video was streamed directly over a separate Ethernet channel). Micro switches were installed in the biobarriers to be able to detect their breaching.

Quick couplings were implemented in order to be able to run the pumps during pre-flight testing (the biobarrier, though porous, did not admit a high flow rate and this would cause the pressure drop across the pump to rise, stressing the diaphragm.). Hence, biomedical quick couplings with a built in valve enabled quick disconnection of the inlet line from the UCZ for pre-flight tests.

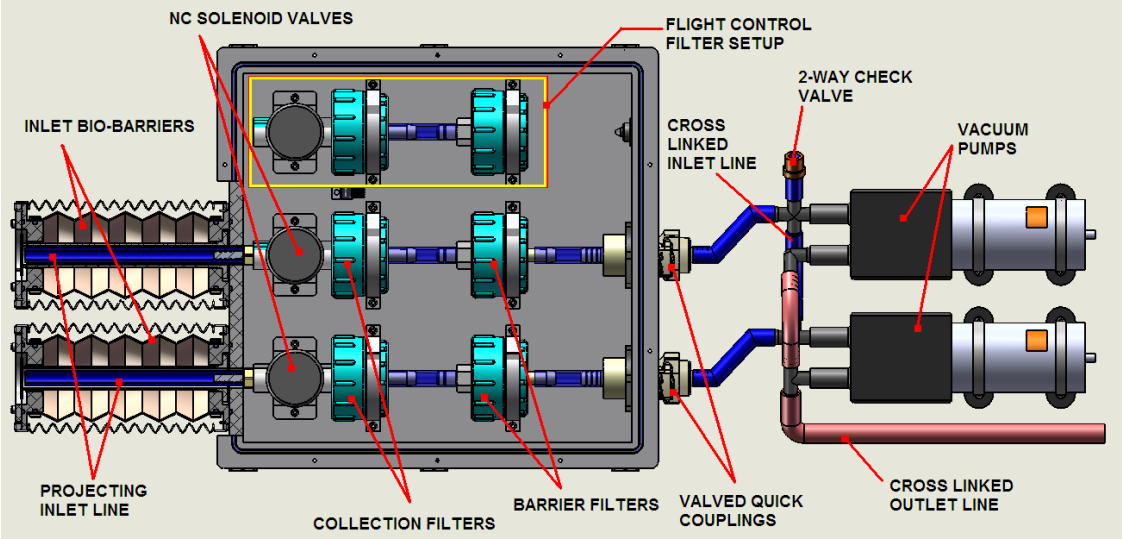


Figure 3-5: *UCZ Layout and Pumps Assembly (Grama, 2011)*

PP&CC control

A flight control filter, with the same valves, filter holder and filters as the sampling lines was implemented. It was not connected to a sampling line and its inlet valve was not to be powered, since it had to remain closed for the duration of the flight.

In addition to this, further controls were included in the experiment: two shipping controls that would undergo DHMR to assess whether contamination has been introduced during shipping, and a storage control will also be prepared at Cranfield to check for contamination during clean room handling.

There was a possibility that microbes may deposit on the internal surfaces of the inlet piping en-route to the filter through mechanisms such as charge interaction and interaction with condensation. As microbe concentration is determined based on the volume of air filtered and microbe count between both biobarrier valves, there is a possibility of underestimation should microbes adhere to the piping between the inlet pipe and the first biobarrier valve encountered by the flow. Further, to reduce deposition of microbes in the tubing and for proper operation of the experiment, the length of tubing between the inlet and the first biobarrier valve was made as short as possible to reduce the deposition area.

Thermal control

Temperature sensors controlled the temperature of critical components and a foil heater maintained the temperature of these components in their optimal range (except for the batteries as they were expected to work at the temperatures faced during flight due to their heritage as the official batteries for the BEXUS EBASS system.

Environmental monitoring

The rest of the sensors monitor environmental parameters that cannot be controlled. These include: a real time flow sensor as well as a mass flow sensor monitor the flow at the outlet of the pumps; and a GPS receiver to monitor altitude.

Pressure equalising

It was vital to ensure that the pressure difference within the inlet piping was neutralised during the ascent phase, this was achieved with the help of a check valve connected to the inlet line. The pressure was neutralised during the descent phase via a normally closed pinch valve connected to the inlet line. Since this was connected close to the inlet before the UCZ, it did not lead to any contamination in the UCZ.

3.5 Development of the system

The main assembly of the experiment is subdivided into the support frame assembly, Ultra Clean Zone assembly, electronics housing assembly, inlet bio barrier assembly and battery housing assembly.

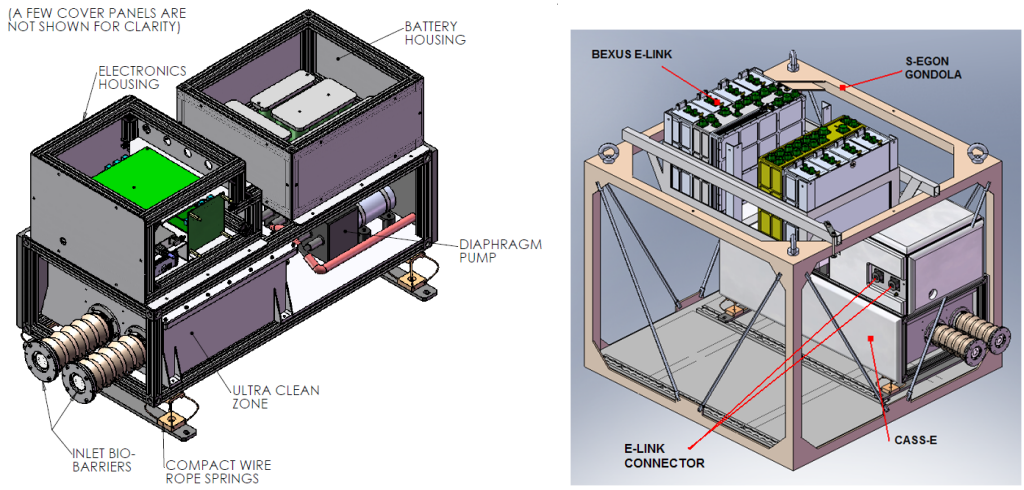


Figure 3-6: *CASS•E Assembly (Grama, 2010).*

Components are split into two categories: UCZ components which as far as possible will only be handled inside the cleanroom (except where they are protected from contamination using biobarriers for example during testing, transportation, DHMR and flight), and other components of the experiment, which can be handled outside the cleanroom, but will be subject to some level of cleaning in order to minimise overall bioburden. CASS•E was developed with the following specific components.

3.5.1 Mechanical components

The pumps were mounted on a 5mm thick aluminium alloy plate. The experiment was mounted on to the gondola via the gondola attachment plate through four CR6-100 Enidine® compact wire springs. The attachment plate was designed to have interfaces onto both EGON and S-EGON gondolas. The IP camera was mounted outside on the front frame cover plate overlooking the biobarriers and the GPS antenna was mounted on the gondola.

A “Remove before flight” cover protects the biobarrier mechanism. This currently obstructs one of the tie-rods. This is only during the pre-flight phase, i.e., there is no obstruction during the flight phase since the cover is removed.

The experiment is mounted on four Enidine® Compact wire rope springs (CR6-100) to reduce the forces experienced during landing.

3.5.1.1 The Ultra Clean Zone (UCZ)

The Ultra Clean Zone housing was made of aluminium alloy. It housed the sample collection filters, barrier filters, temperatures sensors, foil heater and solenoid valves. All components conformed to PP&CC requirements (*i.e.,* were compatible with cleaning and sterilisation methods).

The sealing of the UCZ was achieved with silicone o-rings and flange connections. The top face of the Ultra Clean Zone housing was sealed with Tyvek® and a 2.4mm diameter silicone o-ring with a flange connection. Since a pressure difference did not exist due to the Tyvek® being permeable (and yet still a barrier for particles bigger than 0.3 microns), standard 2.4mm diameter silicone o-rings were selected. Also, the Tyvek® had a moulded transparent polypropylene sheet window which provided visual access to the contents in the UCZ.

The UCZ housing had two bulkhead wall-mounted bio-medical quick release couplings with self-sealing valves connected to the barrier filter. The couplings were mounted with silicone gaskets to seal the gap between the housing and the coupling. Also, an IP68 bio-medical electric panel mounted connector provided sealing and electric connection for the solenoid valves, temperature sensors and foil heaters inside the Ultra Clean Zone.

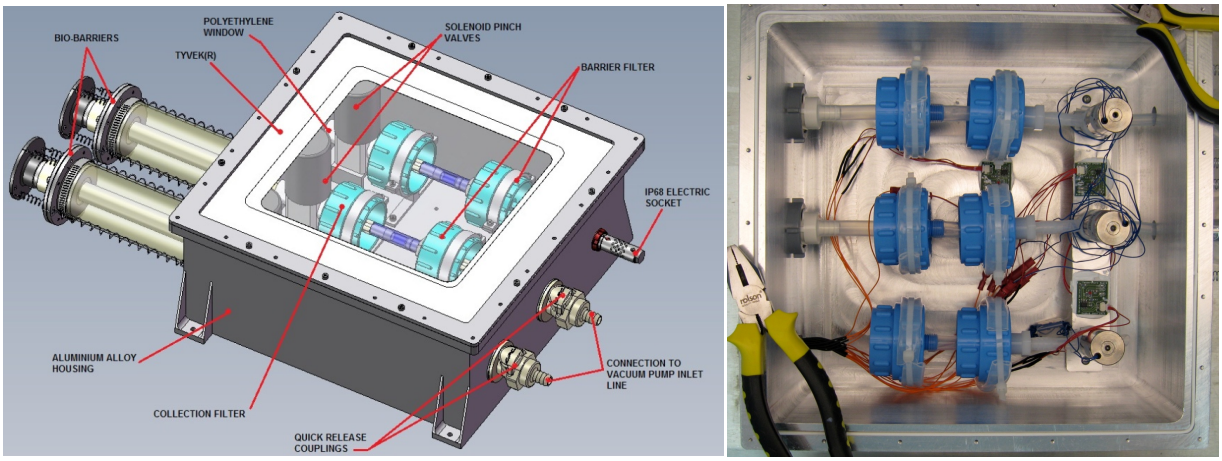


Figure 3-7: *(Left) The UCZ (Grama, 2011); (Right) The UCZ Assembly in the Cleanroom.*

The UCZ could be easily dismantled from the experiment housing to enable the replacement of the flight UCZ with the flight-spare UCZ should the flight UCZ become breached before launch.

3.5.1.2 The inlet biobarriers

The biobarriers were the only component of CASS•E that were not Commercial Off-The-Shelf (COTS). For this reason, they had to be designed carefully, developed and tested thoroughly.

The inlet biobarriers were an extension of the UCZ and had two main functions: to prevent contamination of the projecting inlet tubing; and to provide the UCZ with controlled access to stratospheric air. Due to difficulties sourcing medical grade silicone bellows, a sterilized polyethylene sheet was manufactured to cover the inlet mechanism. The bellows were sealed using stainless steel vacuum hose clamps and o-rings.

Two Tyvek® “sealing” discs sealed the front of the inlet piping against contamination. Due to the permeability of Tyvek®, essentially allowing it to act as a filter, any over-pressure or vacuum condition that may have occurred in the tube space leading to the inlet valves was prevented. This reduced the risk of air ingress to the filters under a closed inlet valve condition and changing altitude. Two springs provided tension which allowed the Tyvek® sealing discs, mounted within the

outer movable flange, to rest on the end of the protection pipes. All flanges were made of 304 Stainless Steel.

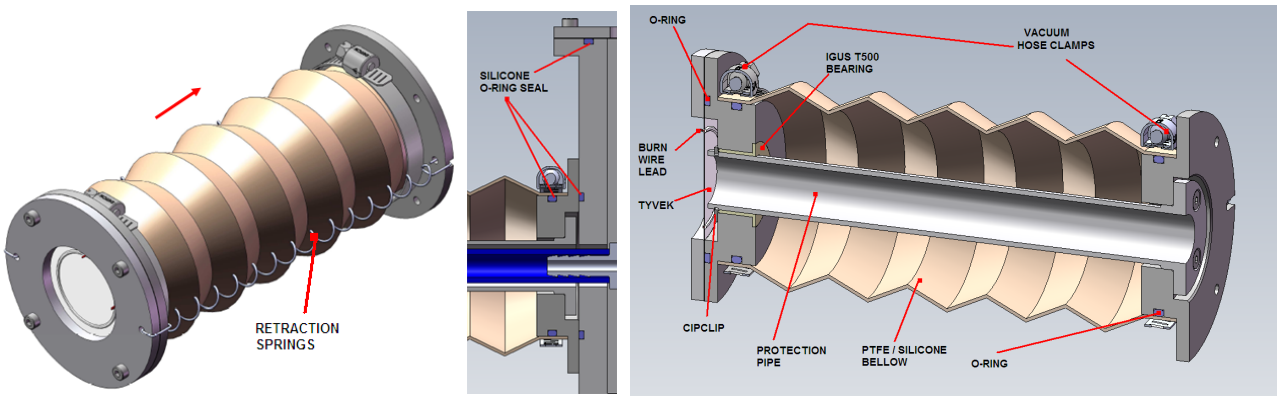


Figure 3-8: *Inlet Biobarrier Design (Grama, 2011).*

The biobarrier movable flange retracted around the central protection pipe under the tension of two springs after the Tyvek® sealing discs had been ruptured. This part of the UCZ was protected with a temporary “remove before flight” cover to prevent damage during handling and transport.

The burn wire for the 9th of October 2010 flight was made out of three 5Ω resistors that would burn up at 12V 0.8A. It is worth noting that in addition to a burn wire made of resistors, other metals were also investigated. Tungsten had too high a resistance (283Ω) and other metals had either too high a resistance, needing higher current, or too low a resistance (even after coiling 1m of wire around itself) and would not burn. Therefore using the resistors in series continues to be the most suitable idea.

Figure 3-9: *Inlet Biobarrier Prototype (Juanes-Vallejo et al, 2011).*

However, this biobarrier design failed during the October 2010 flight and hence the design had to be reconsidered.

Revised biobarrier design

During the BEXUS-10 the biobarriers failed to open, this was due to a lack of contact between the Tyvek® and the resistors and hence not enough heat transfer to enable melting the Tyvek® and breaching the biobarrier.

In order to fix the contact issue between the resistors and the Tyvek®, both the burn wire mechanism and the contact between the burn wire and the Tyvek® had to be improved.

To improve the burn wire, 10 resistors of 1.5Ω rather than 3 resistors of 5Ω were tested and they achieved a wider and faster burn (45 seconds rather than 1 minute) of the Tyvek®, making the ideal horse-shoe shape needed for the biobarrier mechanism to retract.

To improve the contact between the resistors and the Tyvek®, the contact with the Tyvek® was forced from the outside by using a hollow cylinder with the burn-wire mounted circumferentially on one end rather than having the burn-wire sandwiched between three layers of Tyvek®.

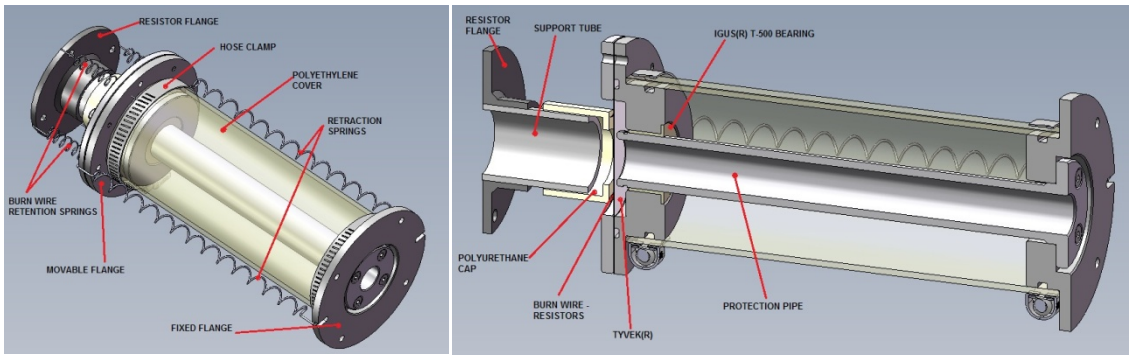


Figure 3-10: *The Revised Biobarrier Design Flown on BEXUS 11 (Juanes-Vallejo et al, 2011)*

Therefore, for the BEXUS-11 flight, the burn-wire was mounted to the surface of a circular disk which was held under spring tension against the outside face of the Tyvek® sealing discs. This allowed the burn-wire to press against the Tyvek® sealing disks and ensured contact throughout the burn.

During testing, a voltage of 12 V and current of 0.8 A was supplied to the burn-wire allowing it to burn a hole in the Tyvek® sealing discs, within two minutes (as tested at the combined conditions of -3°C and 0.5kPaA). For the actual flight on BEXUS 11, five minutes were allowed for each biobarrier burn.

Figure 3-11: *Revised Inlet Biobarrier Prototype (Juanes-Vallejo et al, 2011).*

3.5.1.3 Electronics and battery housing

The electronics compartment housed the main control board, the serial-to-Ethernet converter to enable communication over the E-link with the Ground Station, the GPS module and the IP camera electronics. Four separate sets of batteries (for each pump, the IP camera and the rest of the electronics) were housed inside battery compartment. The top cover had a single 22 pin Amphenol® bayonet connector mounted on the housing which enabled it to be easily detached from the main assembly. Venting holes were implemented in case of battery explosion.

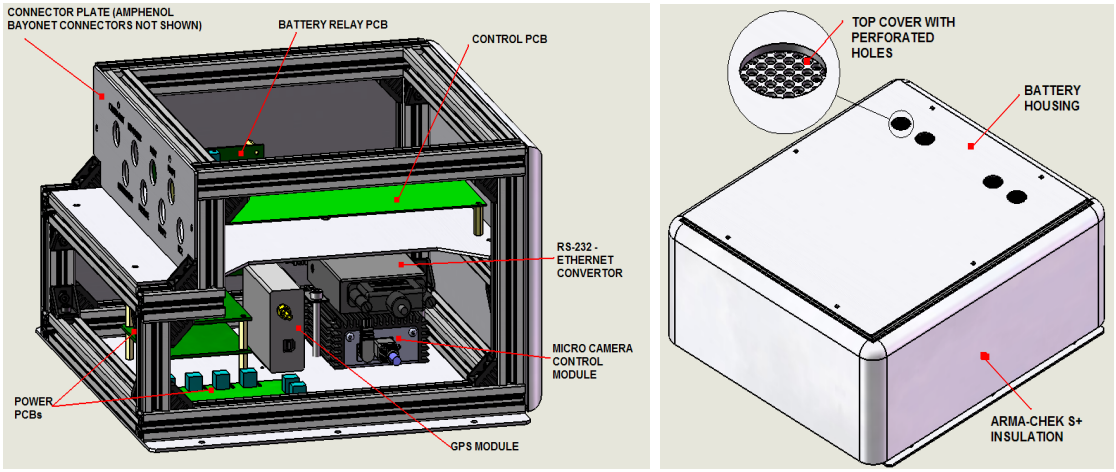


Figure 3-12: *CASS•E Electronic Housing Assembly (Grama, 2010)*.

3.5.1.4 Thermal Design

The experiment was expected to experience very low temperatures during the pre-launch and flight phase. Therefore, the housing of the experimental setup was thermally insulated with Arma-Chek S+ on all cover plates. This prevented significant heat loss during the transportation and flight phase.

The target was to maintain the internal experiment temperature close to the operational temperature range of the components. Polyimide Thermofoil flexible heaters, or Kapton heaters, are ideal for applications with space and weight limitations, or where the heater will be exposed to vacuum, oil, or chemicals. The foil heaters kept the temperature of the vacuum pumps and batteries close to room temperature. These were to be switched on and off receiving feedback from the temperature sensors to ensure the right temperature was maintained and no overheating occurred.

3.5.2 Hardware and Software Components

Both hardware components and its implementation with the software had to be considered.

3.5.2.1 Hardware

Hardware included both mechanical and electronic components.

Piping

Piping material was selected based on compatibility with the pump inlet and outlet ports which were made of polypropylene and hence, to create a good seal, the process piping could not be metallic. Therefore, Silicone Select™ platinum cured biopharmaceutical silicone tubing was used.

This tubing was compatible with Dry Heat Microbial Reduction and had an operating temperature from -62°C to +260°C. All tubes had an internal diameter of 0.8cm.

Filters

Both the barrier filter and main filter were disk type 0.2µm pore-size Millipore GSWP047 mixed cellulose membrane filters. Polypropylene filter holders with 1/4" NPT ports were used to house the filter disks. The filter holders had built-in o-rings at various locations which sealed the filter.

Pumps

To satisfy the objectives, it was sufficient that non-zero flow was achieved *i.e.* that air passed through the biologically protected region. This placed a lower bound of >0litres/min and a target of 10,000 litres which, over the flight time of 2 hours, is equivalent to ~80 litres/min at stratospheric conditions. The pumps chosen for this experiment were two BOXER® 7502 positive displacement diaphragm pumps (an upgrade from the smaller BOXER® 5100 pump used for the smaller Cranfield University-Cambridge University flights). The diaphragm provided a seal between the pump motor and process gas thus eliminating a major contamination pathway. Each pump provided 32 litres/min at NTP. The pumps were cross-linked for redundancy so in the best-case scenario, with both operating in parallel, a flow rate of 64 litres/min NTP could be achieved. The pumps were tested in a vacuum chamber in order to characterise them adequately and they were found to have a flow rate of approximately 0.8L/min at 5.5 KPa. Their nitrile rubber diaphragms and valves were replaced with silicone diaphragm and valves to give better resistance to extreme temperatures.

Flow sensing

A flow sensor was constructed consisting of a Light Emitting Diode emitting light continuously through an in-line impeller connected to the outlet tubing. The impeller spun due to the air flow from the discharge ports of the diaphragm pumps, and in so doing blocked light intermittently to a phototransistor. This phototransistor passed a fluctuating voltage signal to the electronics board indicating impeller rotation and therefore flow. However, due to the higher float altitude expected on-board BEXUS-11 (compared to BEXUS-10), and therefore a lower air mass flowrate to drive the impeller, a second more sensitive flow indicator (the Honeywell AWM43300V) was installed in parallel for redundancy.

The Honeywell AWM43300V microbridge mass airflow sensor operates on the theory of heat transfer by detecting the rate of cooling of its sensing line to understand the rate of flow. A parallel installation was ideally required with the ability to actively direct flow between the two flow indicators, depending on altitude related performance. However, the choice of the parallel, split-flow, installation which flew on BEXUS-11 was motivated by the time constraints to prepare the experiment for re-flight soon after the BEXUS-10 flight. The device was powered separately and its analogue voltage output was recorded using a TinyTag® TK-4703 data logger that was retrieved post-flight.

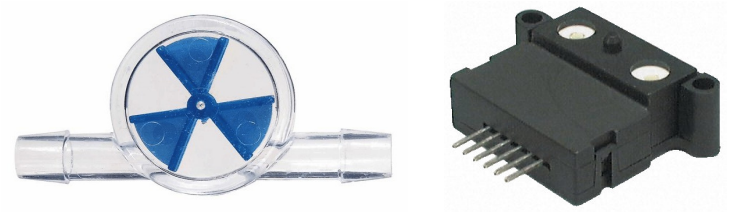


Figure 3-13 *(Left) In-Line Flow Indicator Based on Impeller Rotation Rate (Cole Palmer, 2010). (Right) Honeywell AWM43300V Microbridge Mass Airflow Sensor (Honeywell, 2010).*

Valves

The Bio-Chem 100P2NC12-06S is a normally closed pinch valve capable of sealing a 6.4mm internal diameter silicone tubing. The valve is rated for a maximum pressure of 10 psi (69 KPa). Due to being housed in the UCZ and therefore having to be subjected to DHMR, the valve was tested prior to implementation by subjecting it to a temperature of +120°C for 61 hours. It was then allowed to cool to room temperature and connector to a power supply and tested. The valve operated properly by opening and closing the tubing.

The Control Board

The electronics design revolves around the microcontroller, which monitors and controls the other components. The environmental sensors were monitored via the I2C interface as they do not require direct control. However the pumps, valves, biobarrier opening mechanisms and heaters were controlled via power MOSFETS (as the microcontroller could not handle the high voltage and current required to run these).

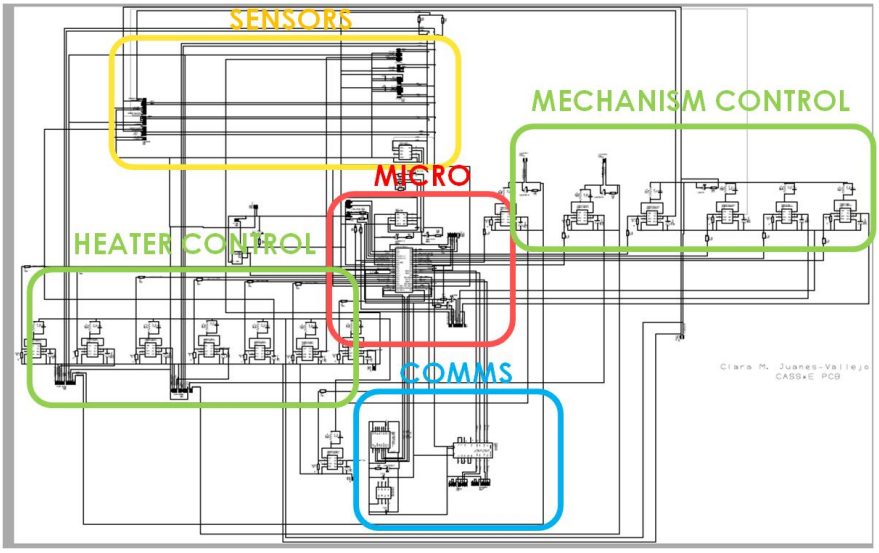


Figure 3-14 *Schematic of Electronics Design for CASS•E with Main functional Areas Noted (Juanes-Vallejo et al, 2011).*

The GPS receiver sent its data to the microcontroller via one of the serial ports and the microcontroller connected to Ground via its second serial port which had its signal passed on to the Ethernet converter board to be downloaded via the E-link (and vice versa for the uplink from Ground into the microcontroller). On the other hand, the IP camera is treated as a stand-alone system with its own power supply and own E-link channel so that real-time video could be streamed down to the ground station.

All the telemetry data was envisaged to be saved onto an SD card connected to the SPI bus; however, due to time constraints this functionality was never implemented.

Each electronic component in the control board is now described.

PIC18F46K22 Microcontroller

The PIC18F48K22 is an 8 bit CMOS microcontroller (Microchip Technology Inc., 2010), that belongs to a family of low-power, high-performance microcontrollers with nanoWatt^{TM} XLP Technology with very low power consumption during sleep mode. The PIC18F48K22 has 64kB of

Flash, 1024Bytes of EEPROM, 36 programmable I/O lines 30 10-bit A/D channels, 2 USARTs (one needed to communicate with Ground and the other with the GPS sensor) as well as I2C and SPI buses (needed to host the environmental sensors) and an operating temperature range from -44ºC to +125ºC.

An external powered oscillator provided the external clock signal (20MHz) for the microcontroller.

High Speed 6A Single MOSFET driver MAX4420EPA+

Logic drive N-channel power MOSFETs were used to control the operation of the heaters, biobarriers, pumps and valves by acting as switches. The MAX4420EPA+ has: V_{DS} range from 4.5V to 18V, R_{ON} max of 2.5Ω, maximum power dissipation of 727mW, 40ns delay time, I_D of 6A and a temperature range of -40ºC to +80ºC.

Therefore making them ideal to deal to drive the 12V 3A pumps, 24V 0.54A valves, 12V 0.8A biobarriers and heaters 12V 0.19A (MAXIM, 2010).

WatchDog Timer IC

The MAX6369 is a watchdog timer that supervises microprocessor activity and signals when a system is operating improperly. During normal operation, the microprocessor will repeatedly toggle the watchdog input before the selected watchdog timeout period elapses to demonstrate that the system is processing code properly. If the microprocessor does not provide a valid watchdog input transition before the timeout period expires, the supervisor asserts a watchdog output to signal that the system is not executing the desired instructions within the expected time frame. The watchdog output pulse can be used to reset the microprocessor or interrupt the system to warn of processing errors (MAXIM, 2010A).

MAX7500MSA+ Digital Temperature Sensor

The MAX7500 temperature sensors accurately measure temperature and provide an over-temperature alarm/interrupt/shutdown output. The MAX7500MSA+ has an I2C serial interface and integrates a timeout feature that offers protection against I2C bus lockups. Features three address select lines in order to have more than one sensor on the same bus, has a 3.0V to 5.5V supply voltage range and low 250µA supply current, and can operate over a -55°C to +125°C temperature range (MAXIM, 2010B).

The temperature sensors were used to read the temperatures of the pumps, the main board, the electronics box, the IP camera, the valves and the UCZ.

Ublox AMY-5M GPS Receiver and Taoglas GPS Antenna

This GPS receiver measures only 8 x 6.5 x 1.2mm, has very low power consumption using 3.6V and 10mA, has UART and USB connections and has high sensitivity (-159dBm, tracking) (ARM 2010). It also comes with SuperSense™, providing ultra-fast acquisition/reacquisition and exceptional tracking sensitivity.

It works up to an altitude of 50,000m making it ideal for a stratospheric flight. The development board of the Ublox AMY-5M was used as it came enclosed with its own electronics and serial socket.

The Taoglas GPS antenna was coupled with the AMY-5M GPS receiver due being recommended by the application notes of the AMY-5M. It was specified to have low noise (1.5db max), low current consumption 19 ± 2mA (at 3~5Vdc), UV resistant housing, input voltage: 2.7~12V, and operating temperature ratings of -30ºC to +80ºC as well as being waterproof rated to ip67 (Taoglas, 2010).

LANTRONIX UDS1100 Serial to Ethernet Device

The UDS1100 is a gateway module that converts RS-232 protocol into TCP/IP protocol. It enables remote gauging, managing and control of a device through the network based on Ethernet and TCP/IP by connecting to the existing equipment with RS-232 serial interface transparently (LANTRONIX, 2010)

It is powered by 9-30VDC through a barrel connector with 1.5W maximum consumption or 9-30VDC on DB25F serial interface. Its data rate has a software-selectable baud rate range from 300 to 230 KBaud and it can operate from 0ºC to 60ºC.

IP Camera MCHIP1-POE-20A (Audio)

An IP camera is ideal as no video processing would be needed on-board because the camera would just directly transmit the video over a second dedicated E-link channel.

The camera, a 1/4-inch colour CMOS, requires a mere 1/16-inch viewing window and has a bit rate of 28K ~3Mbps (Aigis Mechtronics, 2009). The IP Micro Cam System is a self-contained covert surveillance system consisting of a camera and cable, a small 2.74 inches wide x 1.29 inches high and 3.6 inches long aluminium housing with a built-in video server and a RJ45 Ethernet connection for power and data.

3.5.2.2 Software

The embedded software design is broken down into three software modes that reflect different levels of system autonomy. In addition, the whole mission is divided into a number of discreet states that facilitate the definition of the system's behaviour during different parts of the mission.

Software Operating Modes

The software had 3 modes: Autonomous Mode (default mode that monitors all sensors and relays data back to Ground. Functions autonomously); Ground-ACK Mode (entered from autonomous mode when a command and/or sensor reading needs to be confirmed by Ground. If no response from Ground is received (*e.g.,* link is broken) it enters Autonomous Mode after a set time wait (10 minutes); and Manual Override Mode (Ground interrupts Autonomous Mode to force a command in case the Autonomous Mode has failed to act due to a malfunctioning or faulty sensor reading (*e.g.,* temperature of pumps is too hot and the foil heaters have not been triggered to turn off by the Microcontroller).

Software Functions

The five main functions of the control software were:

- To monitor the thermal environments of the sensitive components and control them with the foil heaters.
- To monitor the altitude readings so that the biobarriers open in the Stratosphere, the valves open and the pumps start running.
- To monitor the altitude readings so that the valves close before Cut-Off and the pumps stop running.
- To maintain communication with the ground station.
- To store critical data in internal memory (EEPROM).

The CASS•E electronic control unit (ECU) performs four major types of functions: monitoring, controlling, communicating, house-keeping.

Software Control States

The mission control software is divided into a set of discrete system states that facilitated the design of the embedded software and allowed the ground station operator to accurately monitor the progress of the experiment.

The control board software tasks were managed by a periodic scheduler with a period of 1.5 seconds (no operating system was required as the software was designed for the sequential execution of tasks). In each control state, the scheduler executed the required subset of functions and ensured that the system functions were completed within the required deadlines.

The figure below describes the transitions between states. These were made if conditions (in gray font) were satisfied. Conditions with “GS” prefix depend on acknowledgement from the ground station.

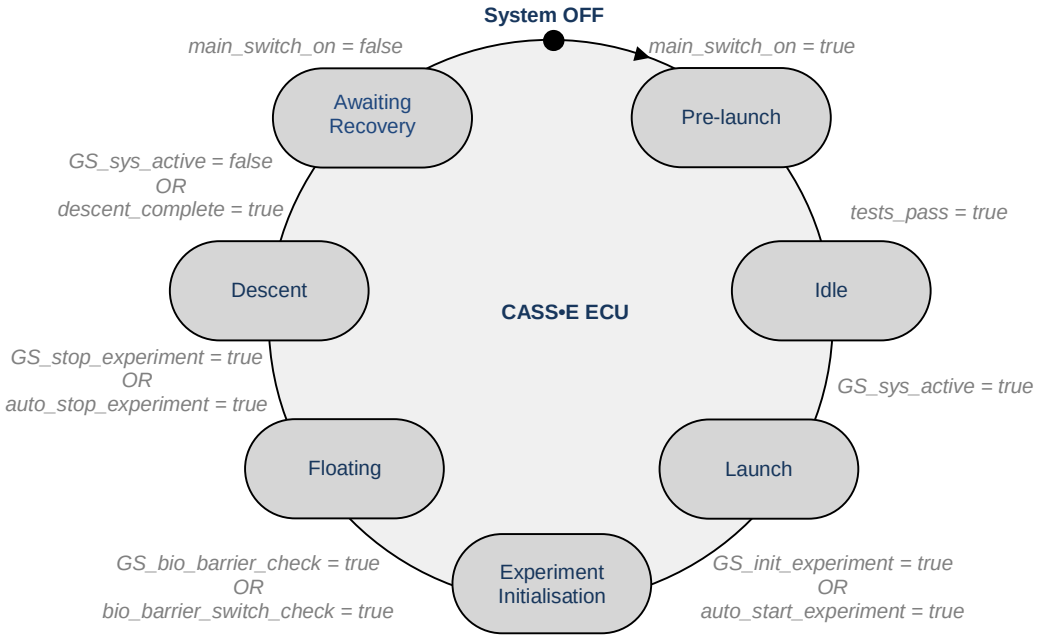


Figure 3-15: *Mission Control State Diagram.*

The following analyses the system behaviour for each control state.

Pre-launch:

- Input: Main power switch was turned on (main_switch_on = true)
- Output: All tests pass (tests_pass = true)
- Description: In the “pre-launch” state the CASS•E ECU performs a series of initialisation tests to ensure that all system sensors and actuators are operating correctly. At this stage, the ECU operates in “MANUAL OVERRIDE” mode, while the ground-station monitors the progress of each test through E-link.

Idle:

- Input: All system initialisation tests have passed (tests_pass = true)
- Output: Ground-station makes request for launch (GS_sys_active = true)
- Description: In the “idle” state the CASS•E ECU is on a low-power consumption mode, while maintaining communication with the ground station. All the sensors are continuously monitored and their status transmitted through E-link. All the system actuators are turned off at this stage and no data is logged. The ECU remains in this state until the ground-station makes a request for launch. The ECU operating mode is set to “MANUAL OVERRIDE”.

Launch

- Input: Ground-station makes request for launch (GS_sys_active = true)
- Output: System has reached stratosphere (GS_init_experiment = true OR auto_start_experiment = true)
- Description: In the “launch” state the CASS•E ECU continuously measures the altitude. The sensors are continuously monitored and their status transmitted through E-link to the ground station, where all the data is logged. The on-board heaters are turned on while the valves, pumps and biobarrier remain inactive. During launch the ECU operates in “AUTONOMOUS” mode until it reaches the stratosphere (altitude ≥ 20km). Then the ECU requests the ground station to validate the GPS data and turns to “GROUND_ACK” mode. If the acknowledgement is positive, or no reply has been received within 10 minutes, then the system advances to the “Experiment Initialisation” state.

Experiment Initialisation

- Input: System has reached stratosphere (GS_init_experiment = true OR auto_start_experiment = true)
- Output: System has reached stratosphere (GS_bio_barrier_check = true OR bio_barrier_switch_check = true)
- Description: In the “experiment initialisation” state the CASS•E ECU opens the biobarrier using a burn-wire mechanism. The sensors are continuously monitored and their status transmitted through E-link to the ground station, where all the data is logged. The on-board heaters are turned on while the valves and pumps remain inactive. During “experiment initialisation” phase the ECU operates in “AUTONOMOUS” mode until the biobarrier opening operation has been completed. Then the mode changes to “GROUND_ACK”, while the ground operator performs visual inspection of the biobarrier. If no acknowledgement is received within 10 minutes then the system advances to the “Floating” state.

Floating

- Input: System has reached stratosphere (GS_bio_barrier_check = true OR bio_barrier_switch_check = true)
- Output: Experiment completed, system ready for descent (GS_stop_experiment = true OR auto_stop_experiment = true)
- Description: In the “floating” state the CASS•E ECU collects samples from the stratosphere. This is the longest phase of the experiment (up to 5 hours), where all the sensors and actuators are operating. The system is continuously monitored and its status transmitted through E-link to the ground station, where all the data is logged. The ECU operates in “AUTONOMOUS” mode and remains in this state as long as the altitude is higher than 18km. It is expected that under normal circumstances the ground operator will terminate the experiment before descent starts.

However, if communication fails, then the ECU will automatically switch to “Descent” state as soon as the balloon falls below 18km.

Descent

- Input: Experiment completed, system ready for descent (GS_stop_experiment = true OR auto_stop_experiment = true)
- Output: Balloon Landed (GS_sys_active = false OR descent_complete = true)
- Description: In the “descent” state the CASS•E ECU prepares the system for landing by turning off the pumps and sealing the valves. The sensors are continuously monitored and their status transmitted through E-link to the ground station, where all the data is logged. During “descent” phase the ECU operates in “AUTONOMOUS” mode. Once the balloon has landed the system advances to the “awaiting recovery” state.

Awaiting recovery

- Input: Balloon Landed (GS_sys_active = false OR descent_complete = true)
- Output: Main power switch is turned off (main_switch = false)
- Description: The “awaiting recovery” state is similar to the “descent” state except that datalogging stops to allow data back-ups to be performed at the ground station. Once recovered, the system switch will be turned off and the mission will be completed.

Ground Station Software

All sensory data (*i.e.,* temperature, pressure and altitude values) and other health check parameters such as monitoring the control board is functioning correctly will be transmitted to the ground station and displayed via a graphical user interface as illustrated in the figure below.

This interface also allowed the user to tweak certain system parameters such as turning the heaters, the camera, the valves, the pumps and the biobarriers on or off.

Figure 3-16: *The CASS•E Ground Station with GPS Antenna on the Left.*

The live video stream from the IP camera was shown on another computer which would also log all the data exchange between the ground station and the control board.

3.5.3 PP&CC plan

Throughout the design phase of the experiment, planetary protection was given high priority and heavily influenced component selection and experiment design within the budgetary and time constraints of the project.

As described previously CASS•E was designed so that all components that directly come into contact with the stratospheric sample were housed within the UCZ. Components within the UCZ were selected to be compatible with bioburden reduction methods and to facilitate cleaning (*e.g.*, pinch valves were selected in order to impede the sampled air coming into contact with any surfaces of the valve which could be difficult to effectively clean). The UCZ would breach only after the BEXUS balloon had ascended to the Stratosphere and the biobarriers had opened to allow the flow path of the sampled air. The flow path was closed and the UCZ re-sealed prior to the descent phase of the balloon by closing the valves to protect the collected sample from contamination during landing and transportation back to Cranfield University.

3.5.3.1 Sources of Contamination

As far as possible the UCZ was protected from contamination during flight and landing by the biobarriers and the valves. However it was important to consider contamination that might occur before the flight took place.

The main sources of contamination from the ground were likely to be environmental (from the environments in which the components are manufactured and handled) and human sources during AIT. For this reason AIT was carefully planned so as to minimise opportunities for contamination and to clean and monitor hardware cleanliness regularly.

3.5.3.2 Cleaning and sterilisation

For the CASS•E experiment it was important that the presence of both viable and dead microorganisms inside the UCZ be minimized as the post-flight detection methods used on the filters may not discriminate between the two. This minimization was achieved by cleaning and sterilization during assembly and prior to launch. Cleaning is a physical or chemical process that reduces bioburden but does not eliminate it, whereas sterilization is a process that destroys or eliminates all forms of life. The cleaning method of choice for CASS•E was immersion in 70% Isopropyl Alcohol (IPA) with sonication, which both reduces bioburden through the physical process of immersion and sonication, and acts as a sterilization agent. Where components were incompatible with IPA immersion, wiping was used as an alternative technique. In addition to this, all components within the UCZ were cleaned by either IPA immersion or wiping, prior to the sealing of the UCZ, and Dry Heat Microbial Reduction (DHMR) after the UCZ had been sealed.

Design choices were made such that all components within the UCZ were compatible with IPA immersion or wiping. Similarly to cleaning, there are a number of bioburden reduction techniques available, but DHMR is the only technique that has been qualified by NASA. DHMR involves heating of components under controlled humidity (< 1.2 g/m3 water) for a given length of time. Since there was free exchange of air between the UCZ and the atmosphere (filtered through the Tyvek® cover sheet), the surfaces inside the UCZ were considered as free or mated and so the time-temperature regime required for a 10^4 reduction of bioburden was 110°C for 32 hours. In order to ensure the efficiency of the sterilization procedure, temperature and pressure profiles were recorded during the sterilisation.

Table 3–8: *Time Temperature Regimes for Dry Heat Microbial Reduction (Juanes-Vallejo et al, 2011).*

	Time Needed at Specific Temperature			
Type of Surface	110 °C	115 °C	120 °C	125 °C
Free and Mated	32 hr	18 hr	11 hr	6 hr
Encapsulated	156 hr	90 hr	52 hr	30 hr

Since the surfaces inside the UCZ can be considered encapsulated the time temperature regime selected for sterilisation of the CASS•E UCZ is 110°C for 156 hours. Design requirement DR20 states that all components within the UCZ must be compatible with DHMR.

AIV procedures

In order to ensure the minimum possible level of contamination and to maintain cleanliness post sterilisation it was essential that the AIV process was carefully planned and that all operators were trained and aware of the principles of PP&CC.

- All assembly of UCZ components had to be carried out in a cleanroom and the level of cleanliness monitored throughout the AIV process.
- A further level of protection had to be introduced through the use of a laminar flow cabinet within the cleanroom for the handling of UCZ components.
- The verification of the experiment had to be planned carefully to avoid re-contamination as the UCZ, once sealed and treated by DHMR, could not be reopened until it reached the Stratosphere without compromising the experiment.
- Following assembly and verification, the experiment had to be shipped to Esrange where pre-flight tests and integration into the gondola was to be carried out. These activities were to be carried out in non-cleanroom environments at the Esrange launch site and thus the UCZ had to remain sealed and handling of it restricted to operators wearing gloves. The exterior of the experiment also had to be cleaned with 70% IPA wiping during this process to keep contamination levels as low as possible.

Cleanroom

In order to ensure the minimum possible level of contamination and to maintain cleanliness post-sterilisation, it was essential that the AIT process for the UCZ be conducted within a clean room. A further level of protection was introduced through the use of a laminar flow cabinet within the clean room for handing UCZ components. All handling and integration of UCZ components were carried out inside an ISO8 (Class 100 000) clean room situated at Cranfield University.

The number of personnel in the cleanroom had to be minimised. This meant that during operations inside the cleanroom one operator or two would carry out the procedure whilst a third operator would supervise from outside the cleanroom.

Rapid sterility level verification

The standard method used in the Space exploration community to verify that the cleaning procedures implemented have been successful is by swabbing and culturing; *i.e.,* surfaces are swabbed and the cells collected on the swabs are extracted and then cultured following a standard

protocol (NASA, 1980). The drawback of this method is the time involved in culturing, *i.e.* an analysis time of days meaning that timely feedback to the AIT process is not possible.

A method that has been proposed to allow rapid feedback to the AIT process is adenosine triphosphate (ATP) bioluminescence assays. Critically, assay times are minutes and lower limits of detection can reach a few hundred cells. It is important to realise that such a method at present is used simply as rapid indicator of gross level of contamination and reduction during cleaning and does not replace the culture based techniques as the definitive method to assess bioburden levels -for example the ATP bioluminescence approach is unreliable for the detection of microbial spores as these are dormant and therefore may not have detectable levels of ATP.

There are a number of COTS kits available for ATP detection, which is commonly used in the food and healthcare industries as a measure of cleanliness. ATP detection is rapid and relatively easy to carry out. The major point to be aware of is the detection limit achieved. The lower the value of ATP detected the better, as it represents the lowest presence of microorganisms present on the material. The detection limit depends on how the material is collected by the swabs and then biochemically reacted; for example, there are swabs where the biochemical reaction occurs immediately after the swab and others where the material swabbed needs to be transferred into a solution and then reacted. Moreover, the detection limit also depends on the luminometer sensitivity to measure the luminescence released in the reaction.

In the current study, only rapid ATP bioluminescence measurements were made whereby a component's level of ATP would be analysed after undergoing its assigned cleaning procedure, and, if still above a threshold (set by the level of cleanliness attained by the other components), the component would be cleaned again and its ATP level verified once more.

Positive Control for Pathways of Contamination

To better understand contamination pathways, a positive control for the presence of contamination pathways was implemented by using fluorescent polymer beads as readily detectable (via fluorescence microscopy) proxies of microorganisms.

For the present work a limited set of beads were used comprising different coloured fluorescence (red and green) as well as different size (1µm and 0.2µm). The detection of beads post-flight was via fluorescence microscopy of the particle filters and with corroboration via scanning electron microscopy (only using size and shape information). The appearance of a particular type of the bead (dye and size) on a particle collection filter would allow identification of what surface the contamination came from and therefore a potential contamination pathway for microorganisms from the same surface. Beads were suspended in a volatile solvent and sprayed using an aerosol system onto various surfaces of the instrument.

The use of the fluorescent beads therefore had two purposes, the first is to give information about sources of contamination and the second was to validate any results as if no beads were found to be present on the sample collection filters during the post-flight analysis, and microbes were to be found, confidence will be increased that the microbes are stratospheric.

The number of areas that could be contaminated was limited by the number of bead colours that could be distinguished using the available microscope and filter sets. Although the intention was to use two different colours, only one bead colour, available as polystyrene microspheres from Thermo Scientific, was distinguishable with the available filters. Areas that were deliberately contaminated included: outside the UCZ (exterior cover), exterior fabric on the gondola cover and other experiments on the gondola. Deliberately contaminating the balloon was considered unnecessarily complex due to the inability to access the balloon envelope pre-launch.

3.5.4 Test plan for CASS•E

CASS•E's components and integrated system had to be thoroughly tested in order to ensure its correct functioning during flight.

3.5.4.1 Verification matrix

A verification matrix was created to ensure the requirements were complied with. It stated the verification method needed to understand if the requirements had been complied with.

Please note that:

- T: denotes verification by test.
- I: denotes verification by inspection.
- A: denotes verification by analysis with analytical methods.
- R: denotes verification by review of the design.
- S: denotes verification by similarity with another component previously verified.

Table 3–9: *CASS•E Verification Matrix (Juanes-Vallejo et al., 2011)*.

Ref	Requirement	Verification
FR1	The instrument shall pump air from the stratosphere through a collection filter in order to collect microorganisms for the duration of the float phase of the balloon.	R
FR2	The instrument shall confirm, record and relay to Ground that while the experiment is running the pumps achieve flow	R, T
FR3	The instrument should measure, record and relay to ground station external ambient temperature, temperature of the pumps, temperature of the batteries and temperature of the PCB during pre-launch testing, launch and flight.	R, T
FR4	The instrument shall measure, record and relay external pressure to ground station during pre-launch, launch and flight.	R, T
FR5	The instrument shall be clean to ensure microorganisms collected are stratospheric rather than contamination	T
PR1/ FR1	The pump ***should*** be capable of pumping 10,000 litres of air (at stratospheric conditions) during the float phase of the balloon	R, I
DR1	The experiment module must either be gas tight or equipped with venting holes	R,I
DR2	The CASS•E box shall be supplied with a sufficient number of brackets or a bottom rail plate to facilitate safe mounting of the experiment	R,I
DR3	The CASS•E box shall have mounting provision to interface on to both EGON and S-EGON gondolas	R,I
DR4	The CASS•E experiment (including the UCZ) shall withstand the loads given below: -10 g vertically +/-5 g horizontally	A
DR5	The experiment shall have a 4 pin connector type MIL-C-26482P series 1 on the outside of the CASS•E box to access BEXUS' power bus	R, I

DR6	The front panel connector for the E-Link must be the RJF21B, the cable mating connector must be MIL-C-26482-MS3116F-12-10P and the cable should be at least 3 meters long	R, I
DR7	The experiment batteries shall be qualified for use on a BEXUS balloon	I
DR8	The experiment batteries shall either be rechargeable or shall have sufficient capacity to run the experiment during pre-flight tests, flight preparation and flight *i.e.,* for a maximum of 8 hours.	R, I
DR9	The batteries in the gondola-mounted experiment shall be accessible from the outside within 1 minute.	R, T
DR10	The design shall neutralise the pressure difference experienced during flight	T
DR11	The experiment shall survive temperatures of -15°C for several hours (temperature on launch pad)	T
DR12	The experiment (including the biobarrier) shall survive for the duration of the flight at temperatures down to -90°C	T
DR13	The experiment shall be designed in such a way that it shall not disturb or harm the gondola.	R
DR14	The experiment shall be designed to operate in the vibration profile of the BEXUS balloon (especially for shocks)	R, A
DR15	The pore size of the filter shall be 0.2µm, to ensure the collection of microbes	I
DR16	The filter shall withstand the vibrations created by the pumps	T
DR17	The UCZ *should* meet the requirements of a COSPAR Category IVa mission *i.e.* bioburden at launch should be no greater than 300 spores per m^2 pre-sterilisation	T, S
DR18	All components inside the UCZ shall be compatible with 70% isopropyl-alcohol (IPA) wiping/immersion for cleaning	I, T
DR19	All components outside the UCZ should be compatible with IPA wiping for cleaning	I, T
DR20	All components contained within the UCZ shall be compatible with heating to 110 °C for 154 hours (as per the requirements for a 10^4 reduction in bioburden by DHMR on encapsulated surfaces	I, T
DR21	The UCZ shall be protected using a biobarrier to ensure it remains clean after sterilisation and during assembly, testing and integration	R, I
DR22	The resealed biobarrier shall retain 99.7 % of all particles or organisms greater than 0.3 µm in size (as per NASA planetary protection standards for sealing)	T
DR23	The experiment (specifically the re-sealed biobarrier) shall withstand landing shocks of up to 35 g	T
DR24	The experiment (specifically the re-sealed biobarrier) shall withstand landing in water	T
DR25	The experiment (specifically the re-sealed biobarrier) shall survive temperatures of -15 °C for up to 48 hours (conditions of gondola whilst awaiting recovery)	T
DR26	The control software shall be fault tolerant and resistant to signal noise.	T
DR27	The software real-time performance and task scheduling shall be predictable.	T
DR28	The software shall be able to recover from critical failures including temporary power loss.	T

DR29	In case of a critical failure the software shall restart in safe mode. If no command from the ground station is received within 5 minutes of the critical failure then the system may continue operating in autonomous mode.	T
DR30	All transmitted information from the control board and the ground station shall be logged with the associated time-stamps.	T
O1	The experiment shall be able to function autonomously in the event that contact with the ground is lost	R, T
O2	The experiment shall only be handled by operators wearing gloves during integration and launch	I
O3	The part of CASS•E exposed to the exterior of the Gondola shall be protected with a *remove before flight cover*	I
O4	The *remove before flight cover* shall be removed before flight	I
O5	The UCZ shall open once the balloon has reached the stratosphere (at 20 km above sea level) and not before	R, T
O6	The UCZ shall be re-sealed prior to the descent phase of the balloon	R, T

3.5.4.2 Critical tests carried out

The critical components to be tested were the pumps, valves and biobarriers. As the biobarrier design was closely interlinked with testing, the biobarrier tests were already described in “Section 3.5.1.2 The inlet biobarriers”. The rest of the components were tested as they were procured and would only be used if confirmed to work according to the requirements of the verification matrix.

Pump components validation

The Boxer™ 7502 vacuum diaphragm pumps were supplied free of cost by the manufacturing company. The pumps were supplied in their original configuration with NBR (Nitrile butadiene rubber) diaphragms.

Test Procedure

The pumps and a temperature sensor were initially placed in a polyethylene bag. The electric wires to power the pump were passed through two holes made in the bag. The holes were sealed with Sellotape®. The air in the bag was evacuated manually by compressing the bag by hand and the bag then sealed to avoid additional condensation when removed for testing. The bag was placed in the -20°C freezer for 1 hour and removed for testing.

Results

The pumps were found to operate at their optimal rate only after the temperature reached +5°C. The pumps performed at their optimal rate when they drew a current of 3.6A at 12V DC. The pump motor and the diaphragm were suspected to be the reason causing the operational temperature to be +5°C. The diagnosis was performed by separately freezing the pump motor and the NBR diaphragm at -20°C for one hour. The motors were removed from the freezer and connected to the power supply. It was found that the motors operated immediately with the temperature being equal to -20°C.

The NBR diaphragms were removed from the freezer after one hour and bent by hand to check for flexibility. The NBR diaphragms were found to be very stiff up to a temperature of 0°C. The temperature was constantly measured using a probe from the temperature sensor.

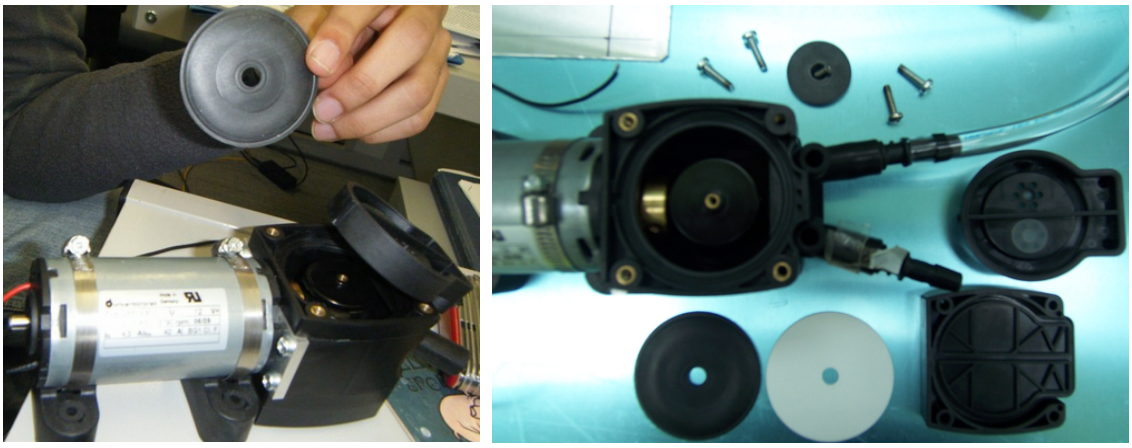


Figure 3-17: (Left) *Pump with NBR Diaphragm. (Right) Ready to Install Silicone (white) Diaphragm.*

Hence, it was discovered that the reason for the +5°C lower operating temperature of the pump was due to the stiffening of the NBR diaphragms at low temperatures. The NBR diaphragms were replaced with silicone diaphragms which are known to retain their flexibility at low temperatures.

A second test was performed following the same procedure. However, instead of placing the pump in the -20°C freezer, it was bagged and placed in the -86°C freezer for one hour. The pump was removed and connected to the power source for testing. The pump began to start operating at its optimal rate after placing it for 5 minutes at room temperature. The temperature measured on the pump was -15°C. It is assumed that the temperature of the pumps and the diaphragms are lower mainly due to the following reasons:

- The thermometer was discovered to have a very slow updating rate of the measured temperature, hence, when the thermometer began to work properly by providing a readable display, the measured value started from 0°C and finally read -15°C after 5 minutes.
- The probe was attached to the surface of the pump casing, hence it is assumed that the temperature of the diaphragms inside was lower.

Pump flow validation

The pumps had to be tested at stratospheric pressure to confirm the presence of flow. Also, knowing the flow rate at stratospheric pressure was considered beneficial.

Two main options were explored:

- Mass flow meter: The presence and measurement of the air flow can be confirmed by using a standard mass flow meter. However, the flow meters are fairly expensive and have to be calibrated to measure flow at the stratospheric pressure. Due to budget constraints, this option was ruled out.
- Bag inflation: This is a simpler method providing fairly accurate results. Essentially, the pump outlet is connected to a plastic bag of a known volume and inflated. A stopwatch is used to measure the time it takes to inflate the bag to determine the flow rate.

The bag inflation method was used to determine the flow rate. The presence of flow was confirmed by the impeller flow indicator.

Procedure

The flow test has been conducted in two stages. The first test (stage-1) has been conducted at STP conditions (standard temperature and pressure) to validate the method. The second test (stage-2) has been conducted in a vacuum chamber at The Open University, UK at stratospheric pressure.

Stage-1 test

A standard 16 litre capacity polyethylene bag was sealed with a barb tube reducer using duct tape. The pump inlet line was initially connected via PVC tube (Polyvinyl chloride) to the bag via the barb tube reducer. The pump was switched on to evacuate the bag. The bag was then folded to ensure only a minimum amount of air is present in the bag. The inlet line was disconnected and the outlet line was connected to the bag. The pump was switched on and the time recorded until the bag is fully inflated. The bag was considered to be fully inflated when no wrinkles remained. The experiment was repeated three times.

Table 3-10: *Flow rate measurement at STP.*

Bag size (litres, L)	Time (seconds)	Flow rate (L/min)	% error
16	28.20	34.04	6.375
16	28.52	33.66	5.187
16	28.43	33.76	5.500

The pumps are rated to provide a flow rate of 32 L/min. Hence, it can be concluded that the bag inflation method provides results with an error between 5-6%.

Stage-2 test

The second test has been conducted at room temperature (25°C) in a vacuum chamber at the following pressures:-

- 5.5KPa simulating an altitude of 20 km.
- 1.98KPa simulating an altitude of 26.5 km.

The bag was initially evacuated by connecting it to the inlet line of the pump. The pump outlet line was connected to the 16L polyethylene bag as described in section 0. The impeller flow indicator was connected between the pump outlet and the bag. The inlet line of the pump was connected to a two 0.2µm collection filters in the filter casings to simulated the process line in CASS•E.

The pump was switched on when the pressure in the vacuum chamber reached 5.5KPa. The inflation of the bags and the impeller flow indicator were visually monitored through the vacuum chamber glass window. The flow indicator confirmed the presence of air flow by the spinning of the impeller. The bag was fully inflated after 20 minutes. This corresponds to a flow rate equal to 0.8 L/min.

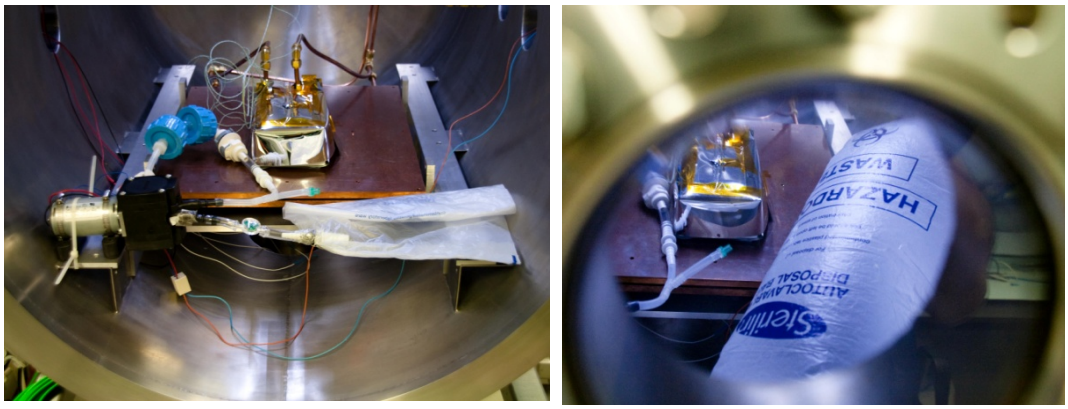


Figure 3-18: *Bag Inflation at 5.5 KPa in Vacuum Chamber.*

Flow indication validation

A calibration of the flow indicator was made in the Open University's (UK) thermo vacuum chamber.

Procedure

Temperature was reduced to -1°C (given the time available for testing in the thermo vacuum chamber this was all that was possible). Both pumps were turned on.

The speed of rotation of the flow indicator wheel was recorded while varying the pressure within the vacuum chamber.

Results

At pressures higher than 8 kPa (experienced during BEXUS 10), knowledge of the flow indicator FI1 impeller speed can provide information on the flowrate from the pumps. The trend line for the flow indicator reading extrapolates to 0 rpm as the pressure tends towards 0 kPa, however it should be noted that below 8 kPa, this flow indicator is unreliable

Due to the higher float altitude expected on BEXUS 11, and therefore a lower air mass flowrate to drive the impeller, a second more sensitive flow indicator, the Honeywell AWM43300V micro-bridge, was installed in parallel for redundancy (rather than in series, as the Honeywell AWM43300V created a bottle-neck effect when positioned at the inlet or outlet of the real time flow indicator).

Flow measuring

A secondary requirement was to obtain a flow rate reading during pump operation. Lab experiments were performed to determine what the expected flow rate would be at stratospheric conditions.

Procedure

One of the crude ways of determining flow rate in the lab is to meter the fluid pumped. This is analogous to the "bucket and stopwatch" approach used for crude flow rate calculations with liquid flows. In this method a known volume of the pumped liquid is collected in a container over a known period of time -dividing these two values yields flow rate.

The method can been adapted for a gas stream by collecting the pumped gas in a plastic bag of known volume over a known period of time until the measured pressure within the bag begins to

rise above ambient pressure (plastic is used as elastic considerations are avoided). This method was implemented at ambient conditions of 101kPa and 25°C. It was found to correctly predict the known pump flow rate (32l/min) to less than 2% error.

A repeat under varying levels of vacuum was performed to produce Figure 2. This produced an understanding of pump flow rate as a function of ambient pressure i.e. a pump curve. Given the ambient pressure, this curve is used to estimate the flowrate from the pumps in post-flight calculations.

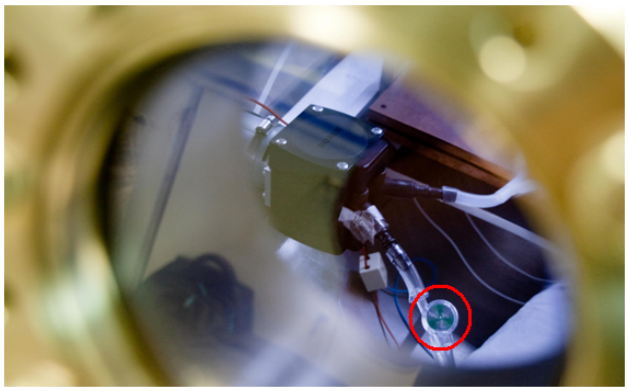


Figure 3-19: *Flow Indicator at 1.98KPa in Vacuum Chamber.*

The chamber was then pressurised and the bag evacuated by connecting it to the inlet line of the pump. During the depressurisation of the chamber in the second test it was observed that the bag was fully inflated at 60KPa. The reason for the inflation is most likely due to the out-gassing of the bag. The pressure was further reduced to 1.98KPa and the pumps switched on. The flow indicator showed the presence of air flow.

Results

It is difficult to confirm the flow rate measured at 5.5KPa due to the outgassing discovered during the 1.98KPa test. Hence, it is possible that the pump has a flow rate lower than 0.8L/min. However, the flow tests have confirmed the presence of flow at a pressure as low as 1.98KPa.

Solenoid Pinch Valve

Low temperature test procedure & results

The solenoid pinch valve with the silicone tubing was bagged and sealed and the bagged valve was then placed in the -20°C freezer. The valve was removed from the freezer after 1 hour and connected to the power supply. The openning and closing of the valve was visually checked. The valve functioned normally at -20°C.

A further test was then conducted by connecting the pump outlet to the silicone tubing after freezing the valve at -20°C for 1 hour. This was to check if the valve operated as required by allowing airflow when it is powered and seals when it is switched off. The valve functioned normally.

High temperature test procedure & results

Since the valve has to be sterilised, it has to meet the sterilisation requirements.

The test to qualify the valve to meet the sterilisation requirements is fairly simple. The valve with the tubing was placed in a laboratory oven at 120°C for 61 hours. The valve was then removed from the oven and placed outside until it attained a room temperature of 25°C. The valve was first

visually inspected and no cracks or any forms of damage were visible. Only a very mild discoloration of the exterior surface was observed. The valve was then connected to the power supply and the tubing to the outlet port of the pump. The valve was switched on and then the pump. The valve opened the tubing by drawing 12V, 0.08A as per factory specifications allowing the passage of air. The valves sealed the tubing when the power was switched off.

The Bio-Chem 100P2NC12-06S is a normally closed pinch valve capable of sealing a 6.4mm internal diameter silicone tubing. The valve is rated for a maximum pressure of 10 psi (69 KPa).

The valve has been tested by subjecting it to a temperature of +120°C for 61 hours (for DHMR). It was then allowed to cool to room temperature and connector to a power supply and tested. The valve operated properly by opening and closing the tubing.

The valve was also tested at low temperatures by freezing it to -20°C for one hour. It was then removed from the freezer and immediately connected to a power supply. The valve operated properly by opening and closing the tubing.

3.6 Discussion and Analysis of the System

CASS•E can be analysed in terms of its downlink and uplink budgets, as well as mass and power budgets before actually confirming and evaluating its performance during flight.

3.6.1 Downlink and Uplink Budgets

The microcontroller completes a cycle in 1.4 seconds, during which it reads all the sensors (temperature, pressure and GPS), performs required actions such as turning off and on heaters and sends the data down to Ground. Evaluating the LANTRONIX Serial-to-Ethernet module (LANTRONIX, 2010) the following can be ascertained:

- Uplink/Downlink Maximum Bandwidth = 28800bps (the theoretical maximum that can be achieved when the serial port transmits at 9600 bps).
- Uplink/Downlink Normal Bandwidth = Total_Byte_Count x 3 (to include TCP/IP overhead). *I.e.*, if 100 bytes per second are transmitted on the serial link, 300 bytes per second (approximately) are transmitted on the E-Link.
- Camera Link Max Bandwidth = 1.8Mbps (assuming a 320x240 pixels colour picture, uncompressed).

Table 3–11: *CASS•E Downlink Budget.*

Components	Quantity	Data load	Total Data load *3 (Kb/cycle)
Vacuum pump	2	8bit (on or off plus header sent only once)	4.80E-02
Valves	2	8bit (on or off plus header sent only once)	4.80E-02
Biobarrier mechanisms	2	8bit (on or off plus header sent only once)	4.80E-02
GPS receiver	1	Needs 32bit number for altitude, latitude and longitude	0.096
Pressure Sensors	1	8bit (on or off plus header)	2.40E-02
Foil Heaters	5	8bit (on or off plus header)	1.20E-01
Flow sensor	1	8bit (on or off plus header)	0.024
Temperature Sensors	6	480bit/min per sensor * 6 = 2880bit/min	0.144
Total sent/cycle	-	-	**0.552**
Total with 20% margin	-	-	**0.6624**

The camera is not included as it was in a separate system, transmitting 28K to 3Mbps real-time video (the settings are configurable) over a separate E-link channel.

The uplink includes the commands sent from Ground. This will only happen if manual override is needed and the worst case scenario would be if everything needed to be turned on/off by Ground in one go, *i.e.,* the pumps, valves, biobarriers and heaters all needed to be sent commands. This scenario would bring the total data load to 0.3Kb/cycle.

Table 3–12: *CASS•E Uplink Budget.*

Instructions	Quantity	Data load	Total Data load *3 (Kb/cycle)
To the vacuum pumps	2	8bit (on or off plus header). Pumps start once and stop once.	0.048
To the valves	2	8bit (on or off plus header). Valves open once and close once.	0.048
To the biobarrier mechanisms	2	8bit (on or off plus header). Only open once.	0.048
To the foil heaters	5	8bit (on or off plus header). Worst case, turn on or off all of them every cycle.	0.12
Total sent every 10 minutes	-	-	**0.264**
Total with 20% margin	-	-	**0.3168**

3.6.2 Power system and power budgets

As the pumps are a critical part of the system, as well as the most power consuming, each pump had its own battery pack. In case of failure from one of the packs, power would be re-routed to the pump left with no power and the remaining battery pack shared. The IP camera also had its own battery pack designed to keep it running for a day, and the control board plus the sensors and the valves and biobarrier opening mechanisms were powered from their own pack.

Therefore, 4 battery packs were used: for Pump1, for Pump2, for the IP Camera and for the sensors, heaters, control board, biobarriers and valves.

The batteries used were the 3.6V 13Ah (1.8A recommended current draw) lithium-thionyl chloride LSH20 SAFT primary batteries (SAFT, 2010) used by BEXUS, as their thermal range is very appropriate (they go down to -60°C) allowing the system to not have active thermal control of the batteries.

In order to allow for sufficient battery power, in all cases the calculations were made with the worst case scenario in mind, *i.e.,* 3 hours testing at launch pad and a 5 hour flight.

3.6.2.1 Pump1 Battery Pack

Each pump uses 3A, 12V for a total of 8 hours, giving a total of 24Ah.

Table 3–13: *Power Budget for Pump1 (12V, 3A).*

Components	Hours	Current (A)	Voltage (V)	Power (W)	Total Ah
Vacuum pump	8	3	12	36	24

3.6.2.2 Pump2 Battery Pack

Table 3–14: *Power Budget for Pump2 (12V, 3A).*

Components	Hours	Current (A)	Voltage (V)	Power (W)	Total Ah
Vacuum pump	8	3	12	36	24

Pump2 had the same requirements of Pump1 and used its own separate battery pack.

3.6.2.3 IP Camera Battery Pack

The IP camera uses 12V 0.4A and would be switched on for 1 day, using a total of 9.6Ah. Therefore it required a battery pack of four batteries in series providing 14.4V, 1.8A, 13Ah.

Table 3–15: *Power Budget for the IP camera (12V, 0.4A).*

Components	Hours	Current (A)	Voltage (V)	Power (W)	Total Ah
Camera	24	0.4	12	4.8	9.6

3.6.2.4 The control board, heaters, sensors, biobarrier opening mechanisms and valves battery pack

The following tables show the components grouped according to their voltage requirements in order to identify the power distribution.

Table 3–16: *Valves Power Budget (12V, 0.54A).*

Components	Quantity	Hours	Current (A)	Voltage (V)	Power (W)	Total (W)
Valves	2	8	0.18	24	12.96	25.92

Table 3–17: *Foil Heaters Power Budget (12V, 0.19A).*

Components	Quantity	Hours	Current (A)	Voltage (V)	Power (W)	Total (W)
Foil Heaters	5	8	0.19	12	2.28	11.4

Table 3–18: *Biobarriers Power Budget (12V, 0.8A).*

Components	Quantity	Hours	Current (A)	Voltage (V)	Power (W)	Total (W)
Biobarrier opening mechanism	2	0.02	0.8	12	9.6	19.2

Table 3–19: *Ethernet Board Power Budget (9V, 0.17A).*

Components	Quantity	Hours	Current (A)	Voltage (V)	Power (W)	Total (W)
Ethernet board	1	8	0.13	9	1.53	1.53

Table 3–20: *Control Board Power Budget (5V, 0.981A).*

5V Components	Quantity	Hours	Current (A)	Voltage (V)	Power (W)	Total (W)
PIC18F46K22 Microcontroller	1	8	0.02	5	0.10	0.10
WatchDogTimer	1	8	0.02	5	0.10	0.10
MOSFET	11	8	0.26	5	0.12	1.32
MAX233A RS232 IC	1	8	0.02	5	0.12	0.12
MAX7500 Temperature Sensors	7	8	0.17	5	0.12	0.84
MAXIM voltage regulators	3	8	0.07	5	0.12	0.36
Pressure Sensors	1	8	0.02	5	0.12	0.12
GPS receiver	1	8	0.10	5	0.50	0.50

Therefore, the power system needed to cater for the following cases:

- **CASE 1:** 12V and 5V devices ON, biobarrier opening mechanisms ON, valves OFF. This case requires a total of 0.06Ah.
- **CASE 2:** 12V and 5V devices ON, biobarrier opening mechanisms OFF, valves ON. This case requires a total of 13.9Ah.

Hence, an 8 battery, battery pack like the one used for the pumps was used, with a total of 14.4V, 3.6A and 26Ah. This brought the total of batteries in the battery bank to 28, and three battery packs were needed: a pack to test the system at Esrange before the launch, a flight battery pack and a flight spare battery pack.

Each battery pack needed to be regulated as they provided more voltage than required (14.4V instead of 12V or 5V). Therefore, the pump1 and pump2 battery packs needed to be regulated down to 12V; the IP camera battery pack needed to be regulated down to 12V; and the main battery pack needed to be regulated down to 12V for the biobarrier opening mechanisms, valves, heaters and Ethernet converter and 5V for the board and sensors.

Switching regulators are more efficient compared to linear voltage regulators although they create more noise; therefore, they were only used for high voltage steps (*i.e.,* for regulating 14.4V down to 12V or to 5V). Also, as the Ethernet converter needed to be powered from a different 12V power line than the heaters to avoid interference, a small voltage regulating Vero-board was built to regulate the 14.4V from the battery packs to 12V. The same was done for the IP camera. A pair of decoupling capacitors was added to each power line to reduce noise along with current limiting resistors to protect the system from current surges.

3.6.3 Mass Budget

The experiment was within the mass and volume budgets for the BEXUS gondolas and, although these were not restricted to a specific value, it was expected that an experiment would fit in a gondola while leaving space for other experiments to be mounted next to it.

Also, as the floating altitude of the balloon is directly proportional to the amount of mass carried, the total experiment mass had to be within 40kg to 100kg, meaning an experiment would be placed in a gondola depending on its final mass (*i.e.,* the bigger and heavier an experiment, the more difficult it would be to accommodate it).

Table 3–21: *Experiment Mass Distribution (Grama, 2010).*

Sub-assembly	Mass (kg)
Main frame assembly	3.2
Battery housing assembly	3.3
Electronics housing assembly	4.6
UCZ & Biobarrier assembly	6.9
Batteries	3.3
Pumps	3.6
Main assembly (total)	24.9

The heaviest sub-assembly was the UCZ due to having to be machined out of an entire block of aluminium in order to make it easier to clean while implementing PP&CC protocols.

The total experiment mass and volume is described in the table below.

Table 3–22: *Experiment Total Mass and Volume.*

CASS•E Mass and Dimensions	
Experiment mass (in kg):	24.9kg
Experiment dimensions (in mm):	808mm (L) x 395mm (W) x 455mm (H) (689mm (L) without Biobarriers)
Experiment footprint area (in m^2):	0.27m^2

3.6.4 Electrical Interfaces

The table below had to be forwarded to Esrange personnel for review prior to launch and acts as a summary of the analysis of the CASS•E experiment as it would only be approved for launch if it complied with the requirements imposed by the BEXUS platform.

Table 3–23: *Electrical Interfaces Applicable to BEXUS.*

BEXUS Electrical Interfaces		
E-Link Interface: E-Link required? Yes		
	Number of E-Link interfaces:	**2**
	Data rate E-Link1 (Experiment) downlink:	**0.7Kbit/1.5s**
	Data rate E–Link2 uplink	**0.3Kbit/1.5s**
	Data rate E-Link2 (Camera) downlink:	**28Kbit/s to 3MKb/s**
	Data rate E–Link2 uplink	**0**
	Interface type (RS-232, Ethernet):	**Ethernet**
Power system: Gondola power required? No		
	Peak power (or current) consumption:	**36W**
	Average power (or current) consumption:	**36W**
Power system: Experiment includes batteries? Yes		
	Type of batteries:	**Lithium Ion**
	Number of batteries:	**28**
	Capacity (1 battery):	**13Ah**
	Voltage (1 battery):	**3.6V**

3.6.5 The Complete System

The complete system consisted of an Ultra Clean Zone housing the sample collection filters with biobarriers protecting the inlet sampling lines, the pumps which drew stratospheric air through the sampling lines, the electronics box containing the electronics controlling and monitoring the system, and a separate battery compartment to house the four packs of batteries needed to power the system.

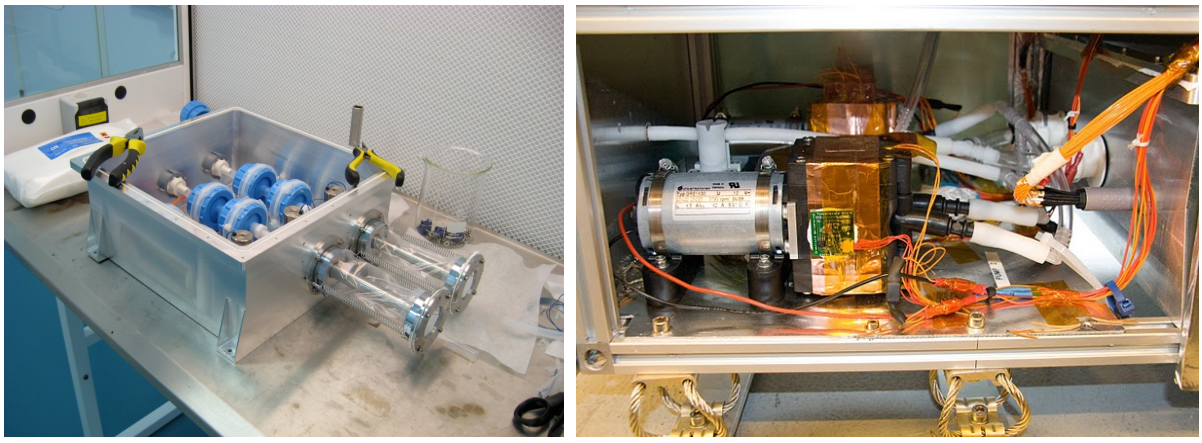


Figure 3-20: *(Left) The UCZ Assembled in the Cleanroom; (Right) The Assembled Pumps.*

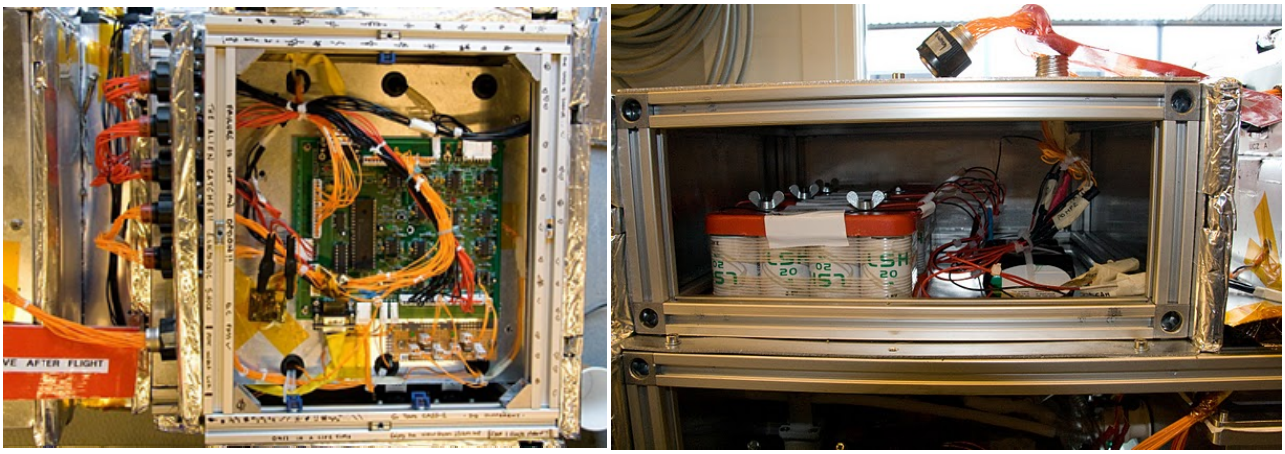


Figure 3-21: *(Left) Top View of the Electronics Box; (Right) The Battery Housing.*

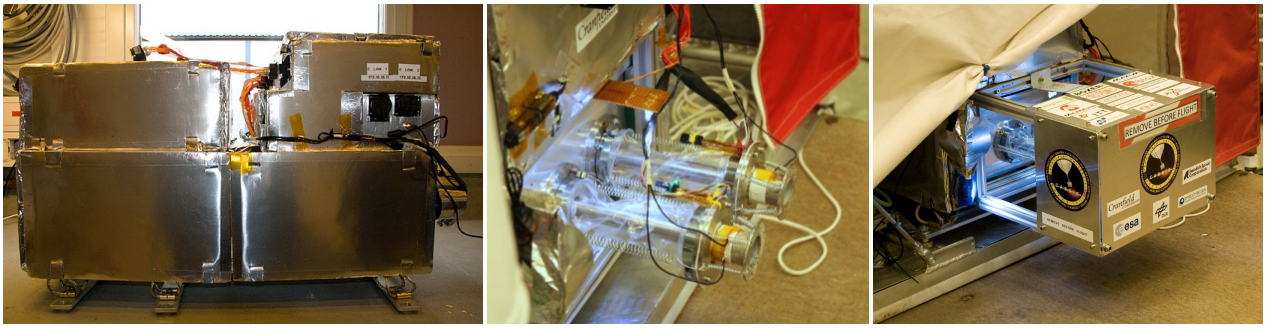


Figure 3-22: *(Left) The Full Experiment; (Middle) The Biobarriers Protruding Out of the Gondola; (Right) The Remove Before Flight Cover Protecting the Biobarriers On the Gondola.*

3.7 Flights and Results

CASS•E was flown on-board the BEXUS-10 and the BEXUS-11 stratospheric balloons from the Esrange base in Kiruna, Sweden. In October 2010 CASS•E flew to a height of 24km on-board BEXUS-10 but experienced a malfunction of the biobarrier mechanism (failure to open). It flew again in November 2010 on-board BEXUS-11 to a height of 33km and with a revised biobarrier mechanism that opened successfully. The UCZ was recovered from the BEXUS-11 flight and transferred to the cleanroom at Cranfield University where it was externally de-contaminated and the sample collection filters were recovered.

3.7.1 Flight Operations On-Board BEXUS-10

- Communication was successfully established and maintained from launch pad operations until approximately an altitude of 1.5km, during the descent phase.
- Data from temperature sensors mounted close to critical components of the experiment was continuously monitored. This allowed the decision to switch on or off heaters to maintain working temperatures.
- Due to the failure of the on-board camera during the RF test the 5th of October 2010 (which was to provide visual aid of the opening of the biobarriers), a backup checklist was made to conduct post-biobarrier opening tests to ascertain if breaching of the biobarriers had occurred. This involved switching on valves and pumps in a specified sequence to detect flow through the inlet lines using the flow indicator.
- These tests were initiated at an altitude of 21km, which was verified using an on-board GPS receiver, and confirmed with EuroLaunch. The tests yielded the following results:
    - Both pump1 and pump2 were working and their flow was recorded when allowing them to pump through the bypass line.
    - Both pump1 and pump2 had no flow detected when the bypass line was closed and their inlet line valves and biobarriers were assumed to be open.
    - These tests were repeated after longer biobarrier burn durations (up to 10 minutes), but still yielded no flow on the inlet lines.
    - As soon as CASS•E was delivered it was noted that it had survived the crash landing on trees in Finland reported by the EuroLaunch personnel.

Figure 3-23: *(Left) The BEXUS-10 Landing Site; (Right) The BEXUS-10 Gondola Landing.*

3.7.2 Post-BEXUS-10 Flight Recovery of the Experiment

- The first contact of the gondola with the trees at the landing site occurred very near the biobarriers as evidenced by the shearing off of biobarrier1.
- Landing impact bent the inlet stainless steel tube but there was no damage to the UCZ. Stainless steel tube was designed to be sacrificial and functioned as designed.
- Biobarrier1 was bent at the base of the inlet guard pipe. The Tyvek® sheet had been breached and the retraction mechanism had activated. The Tyvek® sheet showed evidence of spot burning and tearing; however the burning is considered insufficient to have allowed the mechanism to have retracted around the inlet guard pipe. Within the guard tube was a piece of tree bark indicating that the gondola impacted with the tree on Biobarrier1. This tearing would have allowed the retraction mechanism to activate.
- Biobarrier2 remained attached and un-retracted and showed minor burns on the Tyvek® compared to Biobarrier1. Burns occurred only at regions where the ceramic part of the resistor was in contact with Tyvek®.
- The rest of the UCZ was intact and in good condition.
- The wire rope springs were bent backwards probably due to the crash landing. However they remained functional and protected the experiment from transport vibrations.
- All electronics boards, cables and connectors looked intact and with no obvious damage. Battery integrity remained and all battery packs showed no damage although a strong chemical smell was noted.
- The pump mounting region had pine tree needles and twigs due to the crash landing in trees. However the piping looked intact and there was no visible damage on outside of the pumps.

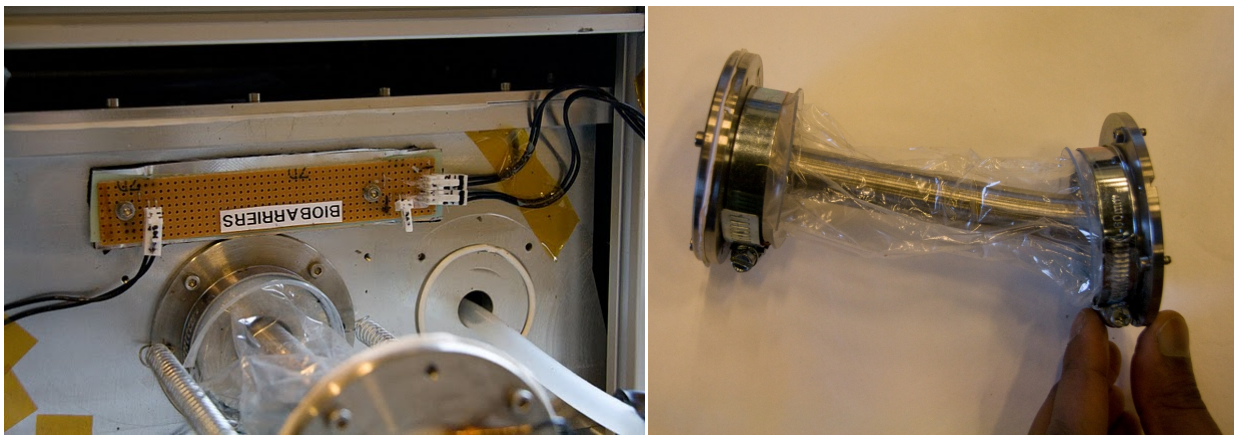


Figure 3-24: *(Left) Sheared Off Biobarrier1; (Right) Biobarrier2 Still Attached to the UCZ.*

Due to the IP camera malfunctioning during BEXUS-10 and hence no confirmation of the biobarriers opening during flight could be obtained, a hall effect sensor that would detect the breaching of the biobarrier due to being in contact with a magnet upon retracting was installed on each biobarrier. An MP3 player was also mounted onto pump2 to record the pumps running, as well as the mass flow sensor attached to the outlet lines to detect flow.

These extra components were run separately and were not read real time, thus not affecting the system (a critical decision was made to not disturb the system while waiting for the second launch).

3.7.3 Flight Operations On-Board BEXUS-11

The full checklist that was followed pre-, during and post- the BEXUS-10 flight can be found in "Appendix D: CASS•E Flight Checklists".

The following events occurred during the BEXUS-11 flight:

- Communication with the ground station on the first E-link channel was successfully established at launch pad operations and during flight until approximately an altitude of 10km, when the E-link was cut-off during the descent phase. However CASS•E was affected by the constant loss of E-link communications beginning at the ascent phase and continuing throughout the flight, provoking loss of data packets.
- Communications with the on-board IP camera on the second E-link channel was lost during the ascent phase at 6.5km (when E-link communications started to fail) and was never recovered.
- Data from temperature sensors mounted close to critical components of the experiment was continuously monitored. This allowed the decision to switch heaters on/off through the flight.
- Due to loss of communication with the on-board IP camera during the flight (which was to provide visual aid of the opening of the biobarriers), a backup checklist was used to conduct post-biobarrier opening tests to ascertain if breaching of the biobarriers had occurred. This involved switching on valves and pumps in a specified sequence to detect flow through the inlet lines. These tests were initiated at an altitude of 21km (before and after opening biobarrier1), and at 33km (after opening biobarrier1). Both altitude readings from the on-board GPS receiver were confirmed with EuroLaunch. The tests yielded the following results:
  - At 21km, before starting the burn of biobarrier1, both pump1 and pump2 were working and their flow was recorded when allowing them to pump through the bypass line.
  - At 21km, after burning biobarrier1 for 5 minutes, both pump1 and pump2 had flow detected when the bypass line was closed and the inlet line valves were open.
  - These tests were repeated after the second biobarrier was burnt at 32km for 5 minutes. Flow readings continued to be shown.

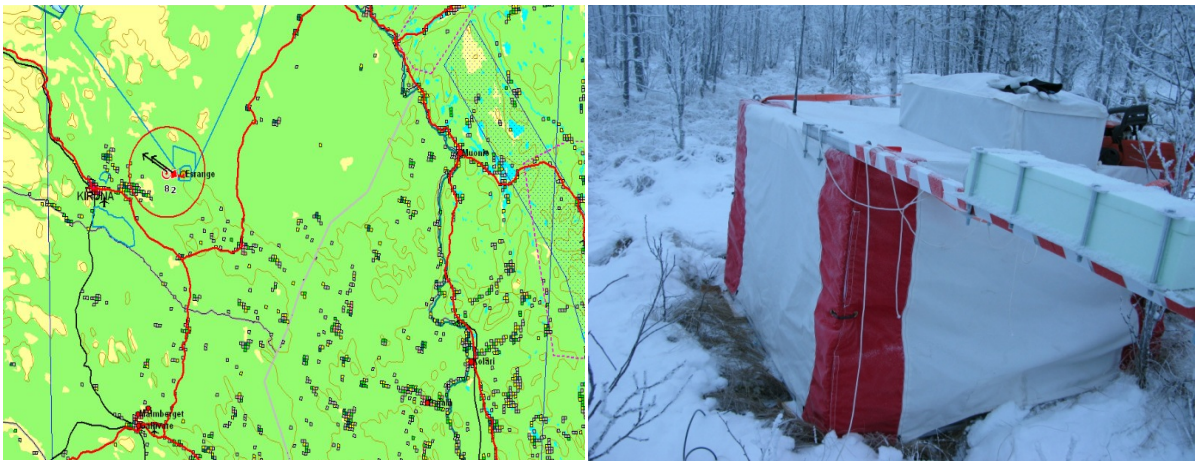


Figure 3-25: *(Left) The BEXUS-11 Landing Site; (Right) The BEXUS-11 Gondola Landing.*

3.7.4 Post-BEXUS-11 flight recovery of the experiment

CASS•E was recovered intact after landing in Finland, with the biobarriers visibly breached. No damage was sustained in spite of a landing force of 25g experienced.

3.7.5 Comparison between the BEXUS-10 and BEXUS-11 Flights:

BEXUS-10 (a 12,000m^3 balloon) was launched the 8th of October 2010 carrying a 112kg gondola with 3 experiments while BEXUS-11 (a 100,000m^3 balloon) was launched the 23rd of November 2010 lifting a 396kg gondola with 6 experiments. The first flight rose to an altitude of 21km while the second achieved an altitude of 33km. The table below gives a summary of the two CASS•E flights on-board BEXUS-10 and BEXUS-11.

Table 3–24: *Comparing BEXUS-10 and BEXUS-11 Flights.*

BEXUS-10 (9th of October 2010)	BEXUS-11 (23rd of November 2010)
The on-board IP camera did not pass the RF interference test as it was not plugged in correctly.	The on-board IP camera successfully transmitted clear video during ascent until loss of signal was reported at 6.5km due to E-link loss of communications. Communications with the camera was never recovered as the E-link was never fully restored.
Tests performed after biobarrier1 and biobarrier2 opened indicated there was no flow.	Tests performed after biobarrier1 opened at 21km indicated there was flow and tests following the opening at 33km of biobarrier2 showed there was still flow. Therefore, CASS•E pumped stratospheric air for 2 hours
The UCZ was recovered intact after landing but one biobarrier was un-breached and the other had broken off during landing.	The UCZ was recovered intact after landing, and the biobarriers were confirmed to have breached.
Partial success in implementation of PP&CC –protocols could never be proved.	Full success in implementation of PP&CC protocols, filters recovered and analysed.

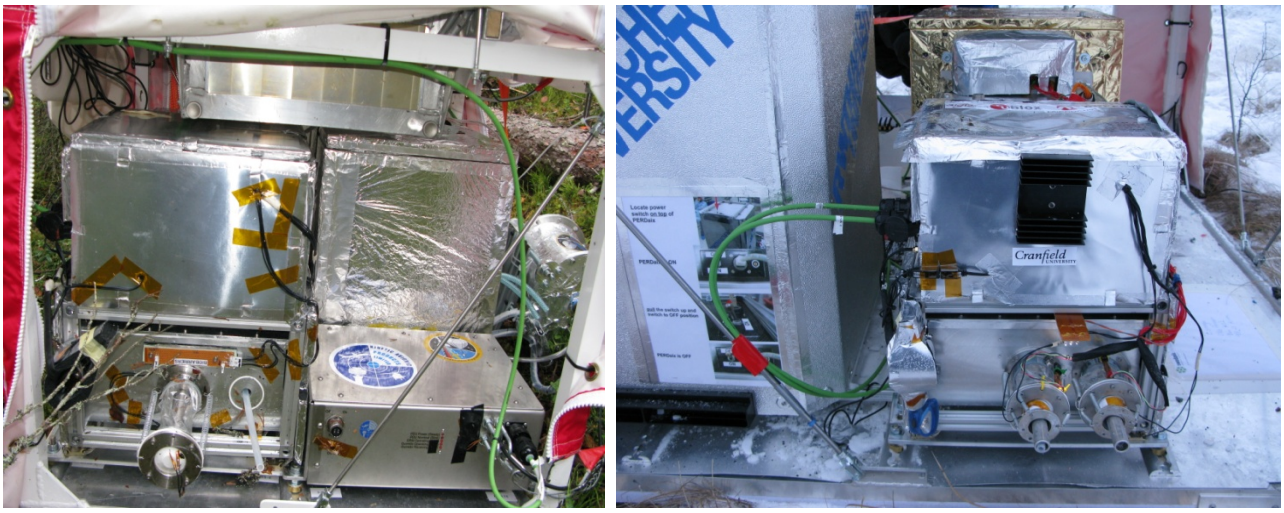


Figure 3-26: *(Left) The BEXUS-10 Gondola with CASS•E having Biobarrier1 Un-Breached and Biobarrier2 Missing; (Right) The BEXUS-11 Gondola with CASS•E Having Both Biobarriers Visibly Breached.*

3.7.6 BEXUS-11 Flight Results

Due to CASS•E suffering a biobarrier malfunction during BEXUS-10, which resulted in no stratospheric air being filtered through the sampling filters and hence deemed an unsuccessful flight with no flow data or samples collected, only the results of BEXUS-11 are discussed.

Figure 3-27: *The Launch of the BEXUS-11 Balloon.*

After the BEXUS-11 balloon cut-off and landing, the UCZ was recovered in Finland and transferred to the cleanroom at Cranfield University where it was externally de-contaminated and the sample collection filters recovered.

The engineering data collected is summarised as follows:

- GPS altitude profile was compared with the data of the GPS on-board BEXUS-11 and was found to be correct (therefore the altitude at which the biobarriers opened was confirmed by both the on-board GPS and BEXUS' GPS).
- The temperature inside CASS•E never dropped below -9°C.
- Flow data from the real time sensor and the HONEYWELL AMW33000 mass flow sensor indicate flow.
- An MP3 was attached to pump2 as a last minute addition to gain audio confirmation of the pumps running. When it was recovered, its recorded audio had the faint sound of the pumps running during the flight.
- Video footage from the IP camera was recorded until the E-link connection to it was lost.
- The biobarrier opening was not detected.

3.7.6.1 EBASS data

The figure below shows the EBASS GPS data correlated with the external pressure –clearly indicating the relationship between altitude and pressure. The pressure dropped during the ascent phase and reached 6mBar during the float phase. Only until after the balloon cut-off and beginning of the descent phase can the pressure be seen to rise again.

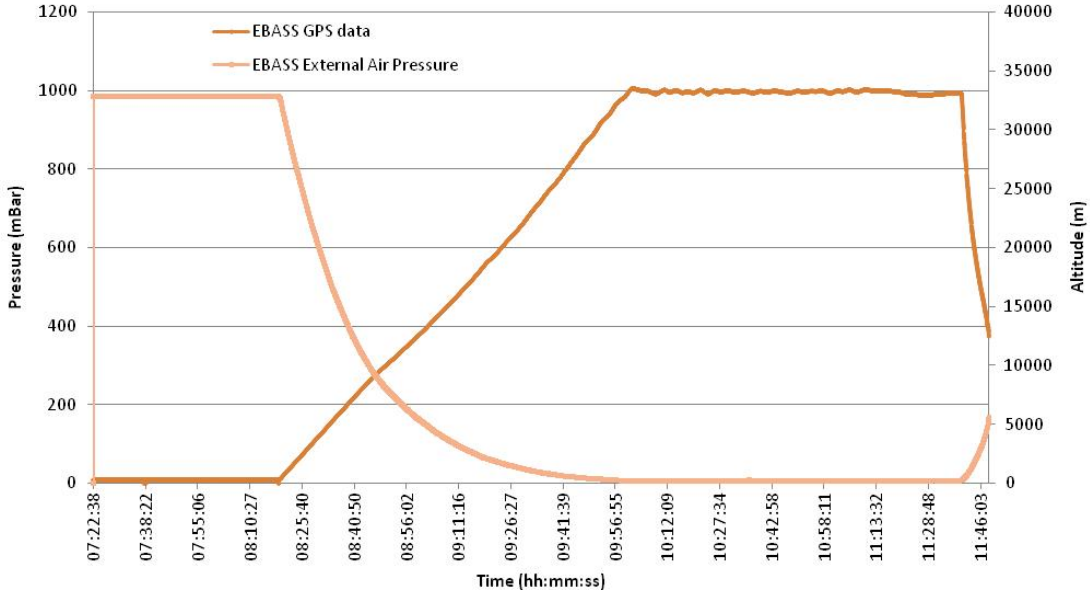


Figure 3-28: *EBASS GPS and External Pressure Data.*

The figure below shows the EBASS GPS data coupled with the external temperature. The temperature can be seen to drop during the ascent phase to -58°C and climb up to a minimum of -14°C during the float phase. After cut-off the temperature dropped to -62°C and started to increase just before landing.

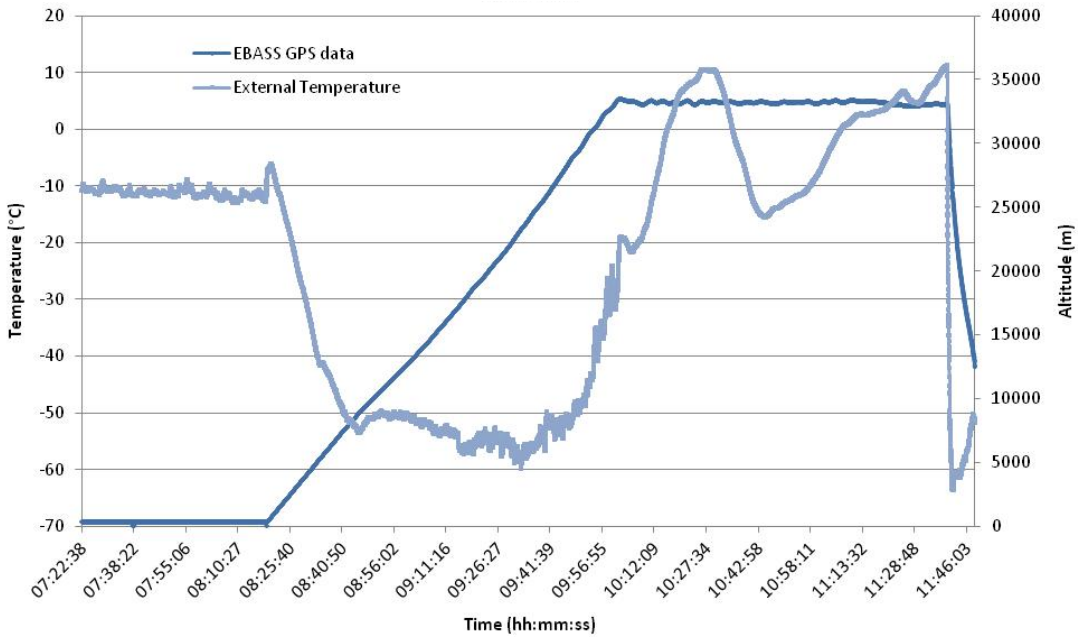


Figure 3-29: *EBASS GPS and External Temperature Data.*

3.7.6.2 GPS and CASS•E temperature data

The temperature observed inside CASS•E was within the working limits of all components as it was maintained by use of the on-board foil heaters. This meant that temperatures inside the electronics box (where the main electronics boards, voltage regulating boards and communications system were housed) never dropped below -10ºC. The temperature of the camera never dropped below -20ºC despite being located on the CASS•E frame and protected from the extreme outside temperatures only by being wrapped in a piece of Arma-Chek+ insulation and a foil heater.

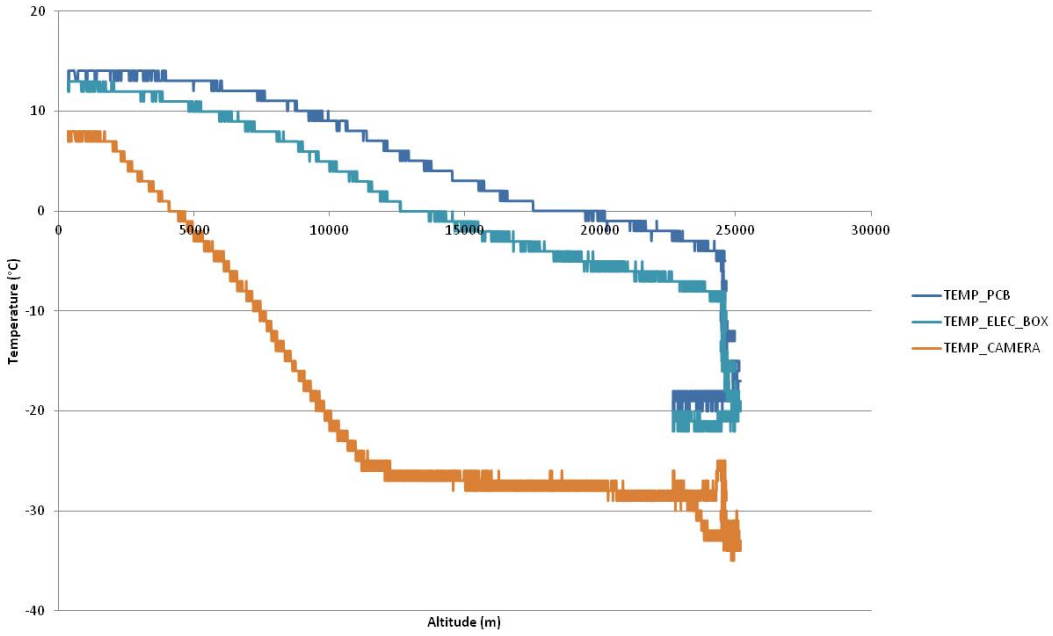


Figure 3-30: *CASS•E Camera, ElectronicsBox, PCB Temperatures during BEXUS-11 Flight.*

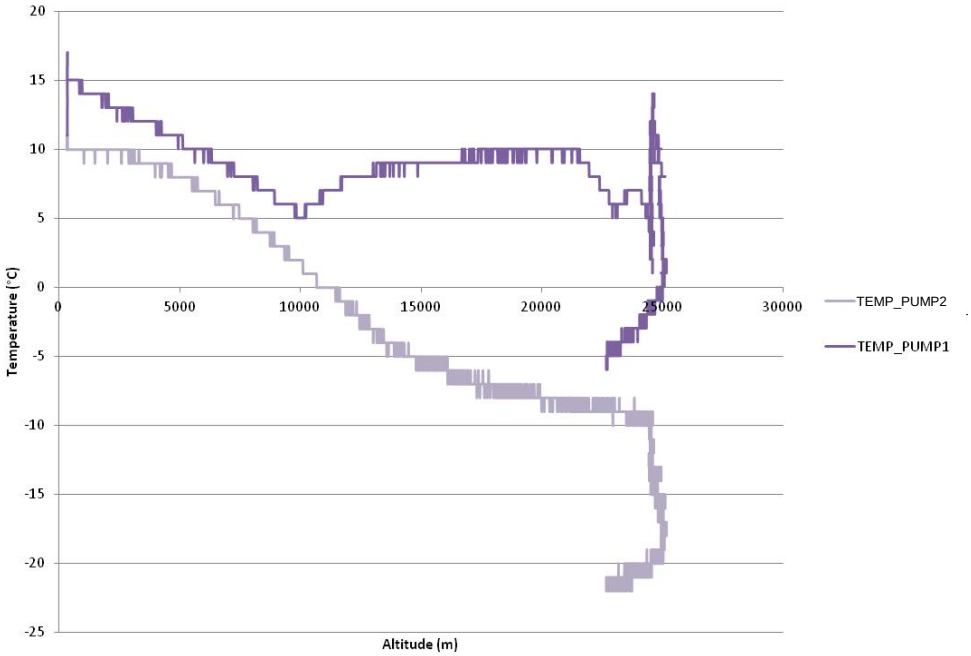


Figure 3-31: *CASS•E Pump1 and Pump2 Temperatures during BEXUS-11 Flight.*

The pumps had to be critically kept above -10ºC in order to avoid freezing of the diaphragm, these observed a minimum temperature of -2ºC (when the gondola was waiting on the launch pad) and then were kept between 5ºC and 21ºC.

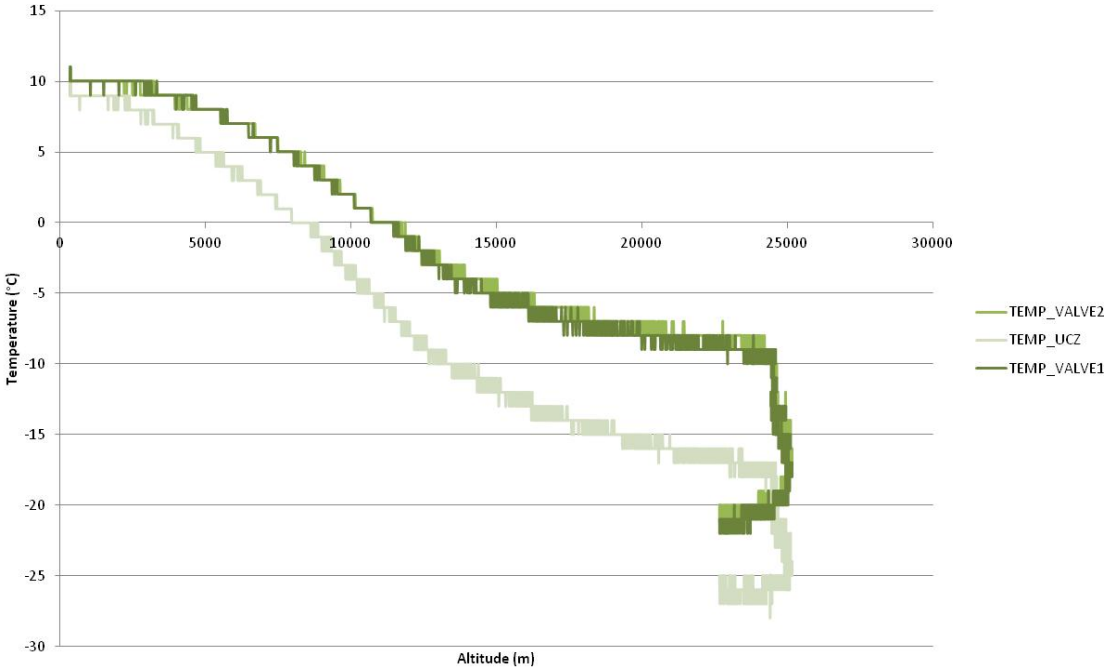


Figure 3-32: *CASS•E UCZ, Valve1 and Valve2 Temperatures during BEXUS-11 Flight.*

The UCZ was the component that got the coldest (-23ºC) as it did not have a bottom plate to protect it from the outside temperature due to the UCZ being a separate box that was inserted into the frame; however the valves inside it never dropped below -8ºC.

3.7.6.3 Pump flow rate

Flow data was collected by the real time sensor and was immediately down-linked to the Ground station while data from the mass flow sensor was recorded by the TinyTag™ data logger.

Gaps in the flight profile can be observed due to the loss of communications that occurred as a result of the E-link malfunctioning. Spikes denote flow during float phase (when the pumps were turned on); however, spikes can also be seen during the descent (thought to be due to the rush of wind entering the outlet of the flow indicator during the quick descent phase).

The GPS data obtained during the BEXUS-11 flight is shown in Figure 3-33: *Real Time Flow Sensor Flow Data Correlated with BEXUS-11 Flight Profile.* coupled with CASS•E's flow data. The flight profile was compared to that obtained from the EBASS system during the flight and was confirmed to be accurate.

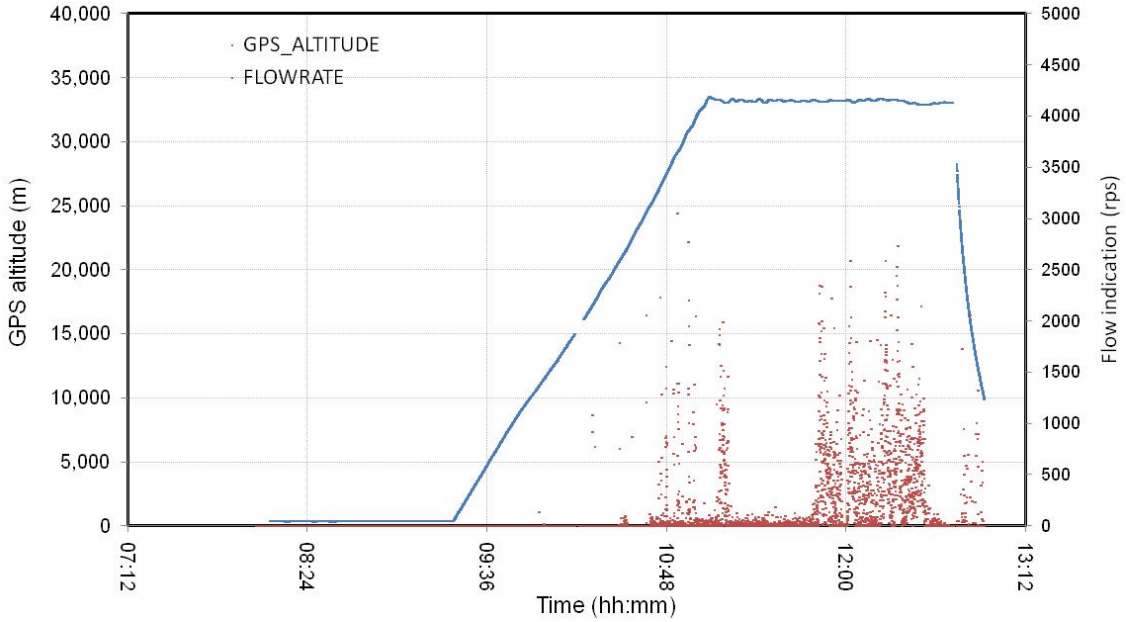


Figure 3-33: *Real Time Flow Sensor Flow Data Correlated with BEXUS-11 Flight Profile.*

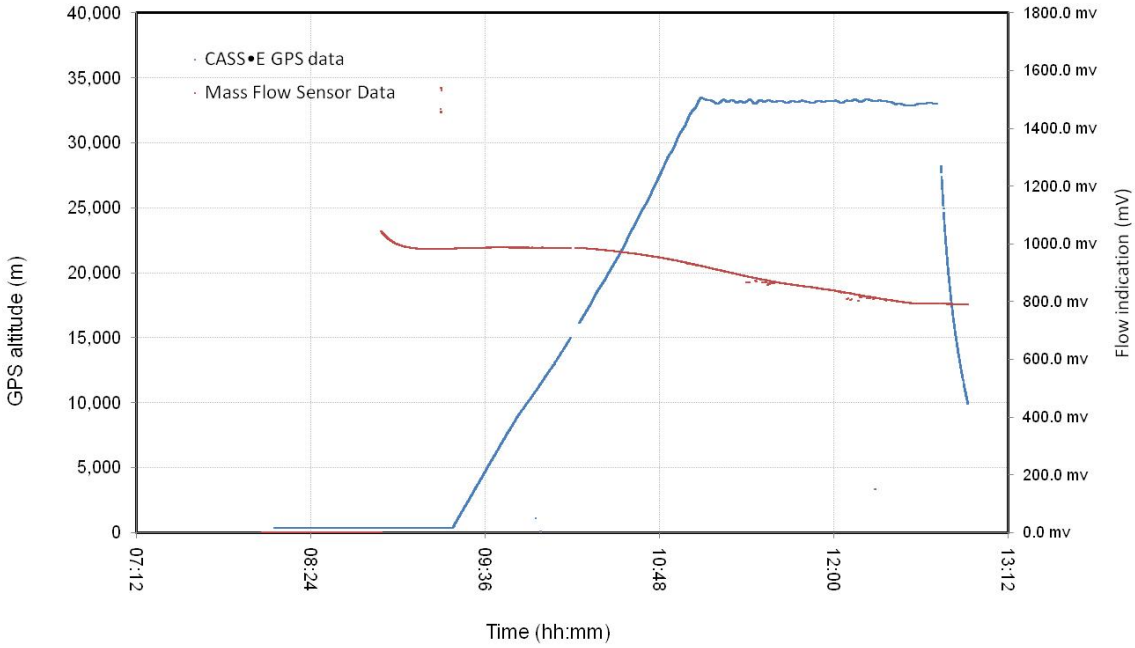


Figure 3-34: *HONEYWELL AWM43300V Mass Flow Sensor Flow Data Correlated with BEXUS-11 Flight Profile.*

Inverted spikes denote flow during float phase (when the pumps were turned on).

Future work would include increasing the sample collection capacity in order to attain more flow through the sample collection filters (*e.g.*, use of bigger and heavier vacuum pumps with increased performance and power) as well as using a more reliable real-time flow indicator.

3.7.6.4 Recorded Audio

4 hours after being turned on, the MP3 player recorded the pumps running during the pre-launch tests as well as general noise while the gondola was being transported to the launch site and while it was waiting for launch (including voices, the engine of the launch vehicle and bangs of the harness against the gondola). After 6 hours 46 minutes of recording, the engine of the launch vehicle cannot be heard anymore, denoting launch.

No discernable noise is heard until after 8 hours and 3 minutes of recording, when the pump2 is clearly heard to be turned on and off for the pre-biobarrier opening flow tests (pump1 cannot be heard due to the near vacuum conditions which only enabled recording of pump2 as the MP3 player was mounted on it). Pump2 then stays on from 8 hours and 5 minutes until 10 hours 4 minutes, confirming 2 hours of pumping. Minutes later, strong winds can be heard, denoting the descent of the balloon.

After 10 hours 32 minutes of recording, an explosion can be heard (corresponding with the explosion of the large bank of batteries on-board BEXUS-11). The MP3 recorder then recorded until its battery ran out.

3.7.6.5 Video footage

The Ground Station recorded live video from the IP camera from launch until the E-link was lost at 6.5km. Therefore, the video feed was lost before arriving to the Stratosphere and hence the Ground Station did not record the biobarriers opening.

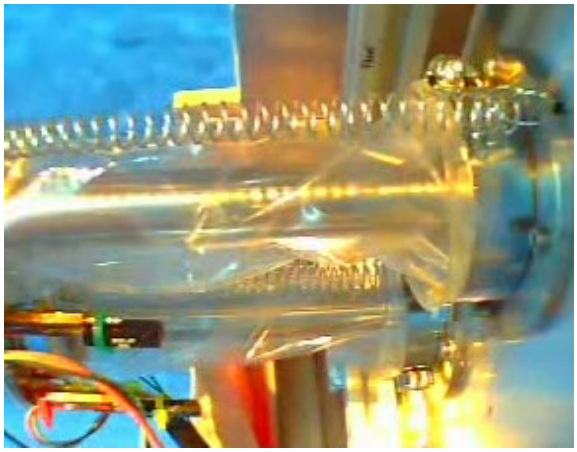


Figure 3-35: *Still Image from IP Camera Video Footage.*

3.7.6.6 Biobarrier detection mechanisms

The Hall Effect sensors gave inconclusive readings as to when they detected the breaching of the biobarriers. It is thought that the magnets and the sensors had been misaligned and/or that the Hall Effect sensors got too cold during the ascent phase and hence did not detect the biobarriers opening.

Future work would include using both a redundant biobarrier breaching mechanism in order to avoid a single point failure if the mechanism to breach the biobarriers fails (*e.g.*, use of a mechanical system that would tear the biobarriers if the burning mechanism failed); as well as a more reliable biobarrier opening detection mechanism in order to be able to confirm during flight that the biobarriers have been breached.

3.7.6.7 Hardware and software performance

As the experiment was designed to be robust and accommodate for failures through redundancy, the electronics remained functional and communicating, enabling its monitoring despite loss of communications with the E-link. Therefore, even though data packets were lost and sensor readings from the GPS and the temperature sensors were not recovered at various times throughout the flight, the main ground station would re-connect to the E-link when it was restored after each loss of signal.

The software performed well during both flights and was proven to be robust as it continued to work even during the loss of signal during the BEXUS-11 flight.

The hardware also performed well, although Valve2 was found to be malfunctioning during the ground operations of the BEXUS-11 flight.

3.7.6.8 Mechanical reliability

Post-flight visual inspection showed that even after an impact landing sustaining 25g, the experiment experienced minimum damage. This validated the design calculations made by Grama (2010) to withstand landing loads by implementing sacrificial joints on the biobarriers and shock absorbers to avoid breaching of the Ultra Clean Zone. Therefore the design of the instrument was proved to be robust and suitable for near Space flight on-board stratospheric balloons.

3.7.6.9 Sample analysis

After the BEXUS-11 flight the CASS●E UCZ was shipped back to Cranfield and opened inside the cleanroom. Each filter was removed from its holder and marked with a pen at several locations around the edge to record which side of the filter was exposed to stratospheric air. The filters were then cut into 6 pieces using metal scissors cleaned by IPA sonication and wiping.

Each segment of each filter was sealed inside a sterile *petri* dish and stored in the dark at room temperature until analysis took place. Handling of the filters was carried out using tweezers that had been IPA sonicated and wiped and was minimised to the edges of the filter (where the holder has been in contact with the filter surface) only.

The filters that were analysed are summarised in the table below.

Table 3–25: *The CASS•E Filters.*

Filter Name	Position	Function
Test Line 1	CASS●E line 1 collection filter	Stratospheric sample
Test line 2	CASS●E line 2 collection filter	Stratospheric sample
Flight control	CASS●E line 3 collection filter	Filter flown but not exposed to stratospheric sample
Shipping control1	In shipping case inside Tyvek pouch	Filter shipped, but not flown (negative control)
Shipping control2	In shipping case inside Tyvek pouch	Filter shipped, but not flown (negative control)

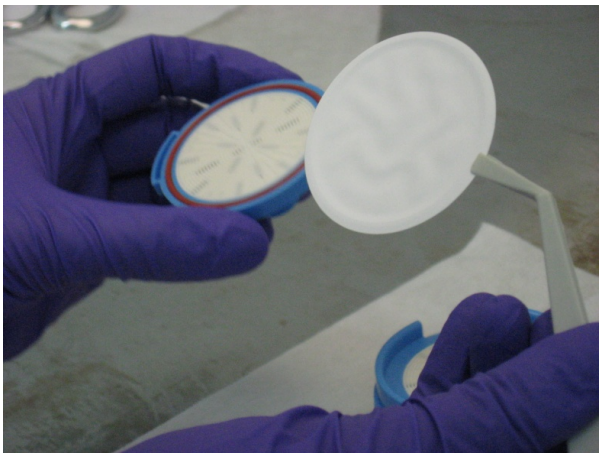


Figure 3-36: *Filter from the BEXUS-11 Flight Being Removed from its Filter Holder Using IPA Cleaned Tweezers in the Cleanroom.*

The results of the fluorescent analysis yielded evidence of low numbers of 1 µm diameter fluorescent beads (most of the beads detected were green in colour, which were those used to contaminate the outside of CASS●E, including the biobarriers, the shortest pathway of contamination) on the sample collection filters indicating that pathways for microbial contamination from other parts of the experiment/balloon existed.

This was confirmed by Environmental Scanning Electron Microscopy (E-SEM) analysis of the filters which also showed 1 µm diameter beads (assumed to be fluorescent beads) as well as particles other than fluorescent beads identified by size and shape. Note that for each filter the beads on one of its six segments were counted using E-SEM images taken from 9 points of each segment (data only exists for two of the filters, line 1 and line 3, as the images for line 2 were ambiguous).

Each of the 9 points imaged on each segment was approximately 250 µm x 180 µm in area = 4.5 x 10^{-8} m^2. As the total area of each filter is 0.0018 m^2, a very rough estimate of bead number per filter would yield the number of beads found in all 9 points on each segment (assuming the beads were evenly distributed, which they will not have been). Therefore:

- *For the collection filter line 1:*
  - 70 beads were counted for all 9 points x 40212 = 2814840 total number of beads.
- *For the collection filer line 3 (flight control).*
  - 58 beads were counted for all 9 points x 40212 = 2332296 total number of beads.

Also, during the BEXUS-10 flight the following concentrations of 1 µm beads were used.

- Concentration of stock 1 µm beads = 1.91 x 10^{10}/ml
- Solutions used for spraying (red and green beads) = 100 µl of stock beads in 25 ml ethanol = $(0.1*1.91x10^{10})/25$ = 7.64 x 10^{7}/ml
- Total volume of solution sprayed (inside and outside cathedral) = 8.5 ml
- Total number of beads sprayed = 6.49 x 10^{8} beads

Whereas for the BEXUS-11 flight, which used 0.2 µm beads, the following concentrations were used:

- Concentration of stock 0.2 µm beads = 2.4 x 10^{12}/ml
- Solutions used for spraying (red and green beads)= = 100 µl of stock beads in 25 ml ethanol = (0.1*2.4 x 10^{12})/25 = 9.6 x 10^{9}/ml
- Total volume of solutions used for spraying = 18.5 ml
- Total number of beads sprayed = 1.78 x 10^{11}

Using the estimated number of beads per filter, the contamination per filter is approximately 0.3 % of the total number of 1 µm beads sprayed.

Therefore, overall the number of beads found on all of the filters was low. This suggests that the implemented PP&CC protocols and the design of the experiment were successful in minimising introduction of non-stratospheric particles onto the collection filters. However there are limitations in the methods used to examine the filters, especially since it is hard to visualise small numbers of 0.2 µm beads and the majority of the beads detected appeared to be 1 µm in size and were therefore introduced during the BEXUS-10 flight, rather than BEXUS-11.

Also, some beads were present on the shipping and flight control filters which could have been introduced during pre-flight ground handling or shipping of the experiment after the flight. Most of the beads detected were found around the edges of the filter, close to where it was in contact with the filter holder (and where the filters were handled) supporting the idea that these beads may have been introduced during removal from the filter holders in the cleanroom.

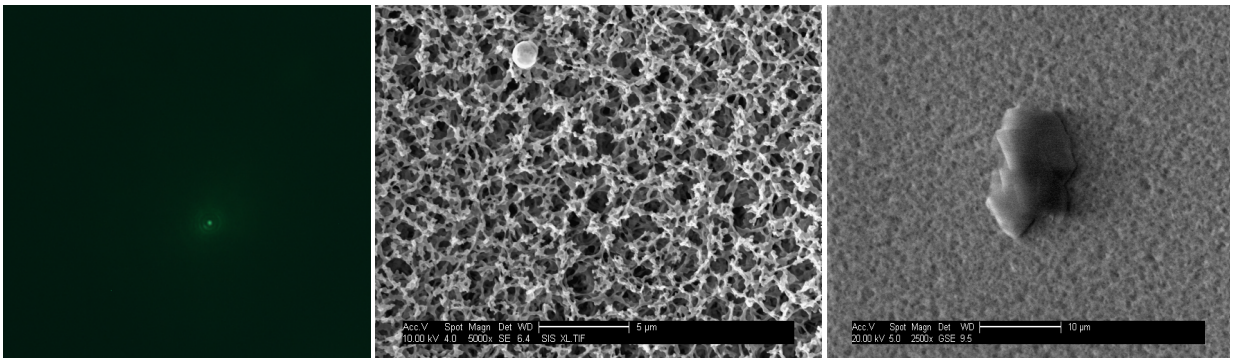


Figure 3-37: *(Left) Fluorescent Bead Analysis Result on Test Line 1 Filter (Middle) E-SEM Image of a of 1µm dia. Fluorescent Bead on Test Line 1 Filter, see top centre-left of image; (Right) E-SEM Image of ~10µm dia. Particle on Test Line 1 Filter.*

X-ray analysis was also conducted on the particles found. On collection filter line 3 an aluminium containing particle was found at the edge of the filter segment, where the holder contacts the filter surface (22 % aluminium, 4.1 % zinc by weight). It is possible that this may have come from the UCZ box which was made of aluminium and some evidence of metal shavings was noted when the UCZ was opened. On the other hand, on the collection filter from line 1, three particles were found with a different composition to any detected on the shipping or flight control filter which may be of stratospheric origin.

Table 3-26: *X-Ray Microanalysis of Particles Detected on BEXUS-11 Collection Filter 1 and 3.*

	Weight % of each element		
	Filter background	***Collection filter line 3 (flight control)***	***Collection filter line 1***
Carbon	42.55	27.71	44.08
Oxygen	56.17	45.03	50.02
Magnesium	-	-	
Aluminium	1.28	22.86	0.57
Silicon		0.26	0.59
Sulphur			0.45
Chromium			1.19
Zinc		4.14	

However, the implication of finding fluorescent beads on the sample collection filter is that a contamination pathway existed during the mission that enabled the fluorescent beads to migrate from a non-cleaned/sterilised surface to the filter; therefore demonstrating that a similar pathway for microbial contamination was present.

Future work would include understanding the contamination pathways which lead to the contamination of the sampling filters with fluorescent beads as well as developing further the use of positive controls for the presence of microbial contamination pathways by, for example, using beads of different materials rather than different colours so that they can be clearly distinguished during E-SEM X-ray analysis instead of depending on the fluorescent microscope optical filters.

3.8 Conclusions and Future Work

The aim of the research described in this chapter was to practically consider the consequences of Planetary Protection and Contamination Control on the development of sample return instrumentation in a planetary exploration context.

In order to achieve this aim, a sample return experiment implementing PP&CC constraints and protocols was designed, built, tested and flown on-board the ESA, Swedish Space Corporation (SSC), Swedish National Space Board (SNSB) and German Space Agency (DLR) BEXUS stratospheric balloon platform. The biological and engineering results obtained from the sample return flight were then analysed and lessons learnt obtained for future flights.

3.8.1 Conclusions

A stratospheric particle collection experiment (the Cranfield Astrobiological Stratospheric Sampling Experiment, CASS•E), that included a number of novel features, was built and flown on a stratospheric balloon. The key features are those intended to address concerns of the wider scientific community that any collected microorganisms are likely to be ground based contamination.

The results of this research provide an insight into the implementation of PP&CC requirements for an instrument to detect life. It has been clearly understood how the instrument's design must be driven by the requirements to be able to undergo cleaning and sterilisation and a method

comprising on the use of an Ultra Clean Zone coupled with biobarriers has been validated for a sample return life detection instrument for a stratospheric balloon platform.

Features adopted from the space-sector to minimise microbial contamination were: the use of Dry Heat Microbial Reduction, the use of biobarrier mechanisms, Ultra Clean Zone sub-systems and rapid contamination monitoring during cleaning and assembly by ATP bioluminescence assays. A key feature was the inclusion of a positive control comprising deliberate contamination of parts of the experiment with µm-sized fluorescent beads as proxies of microorganisms. Any appearance of fluorescent beads on the sample collection filters would indicate that a contamination transfer pathway existed.

CASS•E was flown on-board the BEXUS stratospheric balloon platform from the Esrange base in Kiruna, Sweden in October and November 2010. The first flight was unsuccessful due to a failure in the sampling mechanism; however the second flight of CASS•E allowed the collection of stratospheric samples.

The post-flight finding of fluorescent beads on the sample collection filters demonstrated that a contamination pathway was present. Therefore this feature allowed a clear interpretation of the experiment -*i.e.* contamination did occur, which would not have been achievable without.

3.8.2 Future Work

Immediate work would include:

- Clarifying the specific contamination pathways seen (including more detailed analysis of the sample filters and various control filters).
- Researching further the use of positive controls for the presence of microbial contamination pathways.

Long term future work would be to apply to a specific call for opportunities for sample return life detection in the Stratosphere or planetary exploration scenario analogues. This would entail the design, build, test and fly of a 2nd generation CASS•E incorporating lessons learnt.

Therefore, the following features would have to be included:

- Increased sample collection capacity to achieve more flow through the sample collection filters.
- A more reliable real-time flow indicator.
- A redundant biobarrier breaching mechanism in order to avoid a single point failure if the mechanism to breach the biobarriers fails.
- A more reliable biobarrier opening detection mechanism in order to be able to confirm during flight that the biobarriers have been breached.

4. An *In Situ* Ultra-Low Lower Limit of Detection Instrument for a Stratospheric Balloon Mission Searching for Life in Venus.

The aim of the research explored in this chapter was to consider the implications of flying an *in situ* instrument on-board a stratospheric balloon platform in order to apply them into a specific planetary exploration mission.

4.1 Introduction

Venus has always tantalised scientists due to its proximity to Earth and similarity in size and bulk density, earning it the name of "Earth's twin". However, Venus hosts extremely differing conditions to those on Earth, with temperatures that reach 450°C and pressures 92 times that on Earth on its surface as well as a permanent sulphuric acid cloud cover (VEXAG, 2011). And yet, it was in studying Venus that the greenhouse effect was first understood. Also, although Venus has no seasons or oceans transporting heat, thus allowing for a simpler meteorological model, the precursors to its climate have not been deciphered. Moreover, with no plate tectonics, the Venus model simplifies further; and yet no clear explanation has been found for the presence of hurricane force winds ubiquitous around the planet.

As already considered in "Section 1.2.2.2 Venus", the planet is considered to be a target in the quest for searching for life outside Earth. It is thought that life could have originated before the planet lost its oceans and then migrated to its clouds (where more benign conditions exist and water is present, albeit with high acidity).

Most importantly, the 6th of October 2011 ESA announced the discovery of a tenuous Ozone layer on Venus at an altitude between 92 and 120km that is a thousand times less dense than Earth's (ESA, 2011). This data was obtained using the Spectroscopy for Investigation of Characteristics of the Atmosphere of Venus (SPICAV) instrument on-board ESA's Venus Express spacecraft currently orbiting Venus. As the existence of Ozone indicates the existence of biogenic elements, this strengthens the case for a Venusian stratospheric mission with a life detection payload. It also provides a UV sunscreen -thereby allowing survival of existing microorganisms in the Venusian atmosphere.

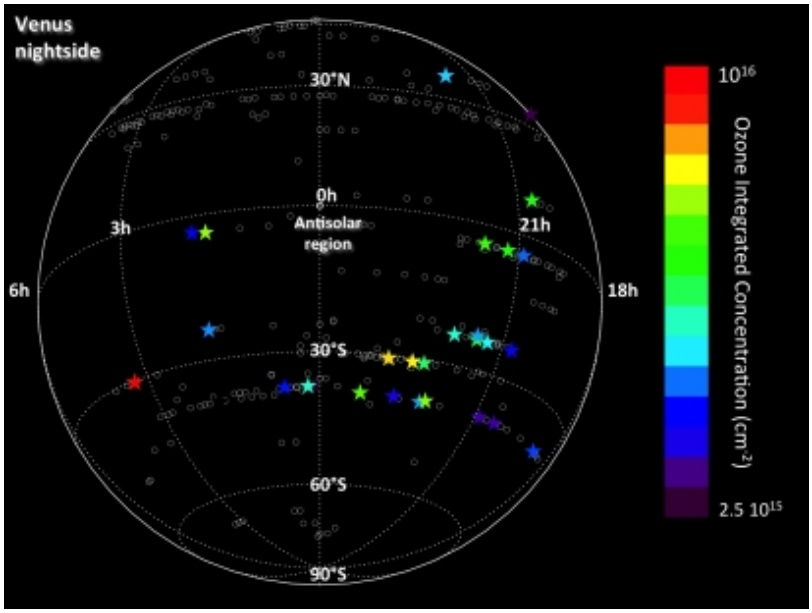


Figure 4-1: *SPICAV Venus Express Measurements of Ozone in Venus' Atmosphere (ESA, 2011).*

In this chapter, the possibility of searching for life in Venus with a SERS payload on-board a stratospheric balloon is explored.

4.1.1 Fifty Years Exploring Venus

Since 1961 Venus has had a plethora of missions from the Russian, American, Japanese and European Space agencies that included atmospheric probes, landers, orbiters and balloon missions. This produced a wealth of data that helped map and understand the planet as well as identify the key design requirements needed to survive the planet's environment.

Nowadays, ESA's Venus Express mission (the only spacecraft currently on orbit around Venus) is providing significant science contributions by measuring the planet's atmospheric dynamics and structure, composition and chemistry, cloud layers and hazes, radiative balance, plasma environment and escape processes as well as surface properties and geology through remote sensing (VEXAG, 2011). Unfortunately, the most recent mission to Venus, JAXA's Akatsuki (Planet-C, Venus Climate Orbiter) mission, failed to achieve Venusian orbit insertion on December 7, 2010 and at the time of writing was in orbit around the sun.

However, Akatsuki is expected to encounter Venus in 2016-2018 and, if its instrumentation is still in working order, will perform a fly-by.

Table 4–1: *Summary of Past, Present and Future Venus Missions (VEXAG, 2011).*

Spacecraft	Launch Date	Type of Mission
Venera 1 (Soviet Union)	1961	Fly-by (intended); telemetry failed 7 days after launch.
Mariner 2 (US)	1962	Fly-by; first to fly by Venus.
Zond 1	1964	Probe and main bus; entry capsule designed to withstand 60°C-80°C and 2-5 bar.
Venera 2 & 3 (Soviet Union)	1965	Probe and main bus; entered Venusian atmosphere; designed for 80°C and 5 bar.
Venera 4 (Soviet Union)	1967	Stopped transmitting at 25km; 93 minutes descent; first to descend through the Venusian atmosphere; designed for 300°C and 20 bar.
Mariner 5 (US)	1967	Fly-by.
Venera 5 (Soviet Union)	1969	Lander; 53 minute descent; stopped transmitting at ~20km (320°C and 27 bar).
Venera 6 (Soviet Union)	1969	Lander; 51 minute descent; stopped transmitting at ~20km (320°C and 27 bar).
Venera 7 (Soviet Union)	1970	First to transmit data from the surface; parachute failure, rough landing on the side; 55 min descent and 23 min on surface.
Venera (Soviet Union)	1972	Performed as designed; soft landing; 55 min descent and 50 min on surface.
Mariner 10 (US)	1973	Fly-by en route to Mercury.
Venera 9 (Soviet Union)	1975	Orbiter and lander; first to return photos of surface; 20 + 55 min descent and 53 min on surface.

Venera 10 (Soviet Union)	1975	Orbiter and lander; 20+55 min descent and 65 min on surface.
Pioneer-Venus 1 (US)	1978	Orbiter with radar altimeter; first detailed radar mapping of surface.
Pioneer-Venus 2 (US)	1978	Four hard-landers.
Venera 11 (Soviet Union)	1978	Fly-by, soft-lander; 60 min descent and 95 min on surface.
Venera 12 (Soviet Union)	1978	Fly-by, soft-lander; 60 min descent and 110 min on surface.
Venera 13 (Soviet Union)	1981	Orbiter, soft-lander; first colour images of surface; 55 min descent and 127 min on surface.
Venera 14 (Soviet Union)	1981	Orbiter, soft-lander; 55 min descent and 57 on surface,
Venera 15 & 16 (Soviet Union)	1983	Orbiter with suite of instruments, including radar mapper and thermal IR interferometer spectrometer.
Vega 1 & 2 (Soviet Union/International)	1984	Fly-by, atmospheric balloon probe.
Magellan (US)	1989	Orbiter with radar mapper (mapped 98% of surface); first high resolution global map of Venus.
Venus Express (Europe)	2005	Orbiter with suite of instruments (on-going ESA mission)
Venus Climate Orbiter "Planet C" (Japan)	2010	Venus orbit insertion failed in December 2010; possible return to Venus in 2016.
Venera-D (Russia)	2016	Orbiter with lander and balloons.

4.2 Aims, Objectives, Requirements and Targets for a Single Molecule Life Detection Instrument On-Board a Venusian Stratospheric Platform

4.2.1 Aims and Objectives

The aim of this research was to consider the conclusions of the first and second studies in order to apply them into a specific planetary exploration mission. Therefore, an in situ ultra-low lower limit of detection instrument had to be proposed for a stratospheric balloon mission.

As Venus is a prime target for a balloon stratospheric mission, the objectives were then:

- To identify possible stratospheric balloon missions to Venus.
- To identify the possible technology to fly on-onboard a stratospheric balloon in Venus.
- To devise a test plan for the technology
- Design a breadboard system.
- Consider implication of findings on systems aspects of instrument builds.
- To validate the concept by attending the Venus Exploration Analysis Group (VEXAG) 2011 conference for peer-reviewing.

4.2.2 Requirements and Targets

4.2.2.1 Requirements

The assessment requirements, design requirements and operation requirement obtained from ESA's Statement of Work (2008) and used in "Chapter 2: Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions" are valid for the current study as an ultra-low lower limit of technology was needed for detection of astrobiology biomarkers.

Table 4–2: Assessment *Requirements.*

Reference	Requirement
AR1	The measurement techniques or principles shall be able to identify and count non-stochastic biomarkers.
AR2	Astrobiology biomarkers to be detected according to EXO-ESA-MO-11001 (Vago, 2005) for specific biomarkers on Mars.
AR3	Cell biology biomarkers to be detected as mediators in cell biology for cell signalling mechanisms and/or transmission of neurosignals.
AR4	The selected technologies shall be able to detect, identify and count at least one representative type of biomarkers in the area of cell biology and at least one in the area of exobiology.

Table 4–3: *Design Requirements.*

Reference	Requirement
DR1	Be able to detect and recognise biomarkers.
DR2	Include selected technologies that shall be compact and integrate as much as possible in order to become part of a system.
DR3	Be able to detect concentrations in a deterministic (module counting) manner.
DR4	Sample preparation and use of reagents shall be minimal.
DR5	Be able to reach at least part per trillion (design aim) level sensitivity.
DR6	Have a mass of less than 5kg.

Table 4–4: *Operation Requirement.*

Reference	Requirement
OR1	To be operational in human, autonomous and tele-operated modes.

4.2.2.2 Astrobiology targets

Again, the astrobiology targets were deemed the same as in "Chapter 2: Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions":

- Life building blocks or traces of life (past or present).
- Molecules from metabolic pathways.
- Presence of functioning metabolic pathways.
- Chemical fossils and molecules that could be building blocks of life forms not recognisable by the classical biochemistry.
- Immunochemistry or nucleic acid methods currently used on Earth.

4.3 Literature Review of Stratospheric Missions to Venus

Several concepts proposed for a stratospheric balloon mission are considered to understand the features and constraints of a future mission to Venus.

4.3.1 The European Venus Explorer Mission (EVE)

While ESA's Venus Express mission is currently answering many questions about Venus and providing key data, several fundamental questions require *in situ* investigation, in particular relating to the noble gas isotopic signatures of its past history and its complex cloud-level atmosphere.

A fairly small *in situ* mission based on a single balloon probe, called the European Venus Explorer (or EVE) mission, was proposed by a European-led International team for ESA's 2015-2025 Cosmic Vision programme. Unfortunately, EVE was not selected for this programme -however, it is still considered in this study in order to understand possible future balloon missions to Venus.

4.3.1.1 Science objectives

The science goals for the EVE mission are the following (EVE, 2010):

- To elucidate the origin and evolution of Venus, and its relationship to Earth and Mars, that is:
  - To unambiguously determine which kind of accretion scenario gave rise to Venus ("gas-rich" or "gas-poor").
  - To establish the origin and eventual loss to space of volatile species over the history of Venus.
  - To constrain the degassing history of the planet, with an emphasis on early degassing.
  - To search for active volcanism, remnant magnetic field and other clues to solid planet history.
  - To trace back the evolution of the atmosphere with the main goal to constrain water and climate histories, to construct a unified scenario for the formation and evolution of the terrestrial planets.

- To investigate the current climate of Venus and its stability, with a focus on the cloud-level environment, that is:
  - To investigate the complex coupled chemical, radiative, and dynamical processes governing the Venus clouds.
  - To study the mean and transient dynamics of the cloud-level atmosphere, from small-scale to global scale.
  - To study the role of clouds in maintaining the enormous greenhouse effect of Venus.
  - To measure for the first time the electrical and magnetic environment at cloud level, including lightning.
  - To investigate the habitability of Venusian clouds.

Therefore, these science investigations would contribute to understand the origin and evolution of Venus and its climate, with relevance to terrestrial planets everywhere.

4.3.1.2 Key Features

The key features of the EVE mission are detailed in the table below.

Table 4–5: *Key Features of the European Venus Explorer Mission (EVE, 2010).*

Features	Description
System	The spacecraft would release the entry probe carrying the balloon system and gondola and then would be diverted to perform a flyby (and hence it would not become an orbiter). The balloon communicates with the spacecraft for 2 hours after the floating altitude is reached and is then monitored from Earth during the first four days of the mission. The Earth would then be visible again after ~7 days (however the balloon would be visible from the spacecraft after ~3 days during the whole period that it is not visible from Earth). Teflon coated (for sulphuric acid resistance) and metallised (for low solar heating) He balloon is inflated in 5 minutes.
Launch Date	April 2023 window, with a 6 month cruise to Venus.
Science Campaign	10 day mission at an altitude of~55 km.
Total Spacecraft Dry Mass	~1000 kg.
Launch Vehicle	Soyuz/Fregat2-1b from Kourou.
Cost	Low end flagship.

4.3.1.3 Science payload

The balloon gondola would carry (EVE, 2010):

- A GC-MS focused on atmospheric and aerosol composition (with heritage from the Cassini-Huygens mission).
- An Isotopic MS for the study of isotopic ratios.
- A polarising nephelometer to study the cloud particles in detail.
- An optical package to measure upward and downward radiation, in six spectral bands from UV to thermal IR, in order to characterise the greenhouse effect (and it would also have a lightning detection mode).
- A meteorological package including pressure and air temperature sensors as well as a sound detector to characterise the acoustic environment (including the search for thunder) and an accelerometer to determine pressure and density profiles during entry.
- An electrical & EM properties package to measure the electric field and the EM spectrum.
- An IR Attenuated Total Reflection (ATR) Spectrometer to obtain absorption spectra of the aerosols (note that ATR spectrometry is particularly important for identifying pre-biotic or biotic constituents).

4.3.2 The Venus Climate Mission (VCM)

The National Research Council's 2010 Planetary Decadal Survey Inner Planets Panel commissioned the NASA Goddard Space Flight Center (GSFC) and the California Institute of Technology Jet Propulsion Laboratory (JPL) to perform a design study on a Venus mission concept capable of studying the Venus climate within a New Frontiers cost range (*i.e.,* a low end flagship mission).

4.3.2.1 Science objectives

- To characterise the strong CO_2 greenhouse atmosphere of Venus, including variability.
- To characterise the dynamics and variability of Venus' super-rotating atmosphere.
- To characterise surface/atmosphere chemical exchange in the lower atmosphere.
- To search for atmospheric evidence of climate change on Venus.
- To determine the origin of Venus' atmosphere and the sources and sinks driving evolution of the atmosphere.
- To understand the implications of Venus' climate evolution for the long-term fate of Earth.

4.3.2.2 Key Features

The key features of the Venus Climate Mission Planetary Science Decadal Survey mission concept study are described in the table below:

Table 4–6: *Key Features of the Venus Climate Mission (VCM, 2010).*

Features	Description
System	Carrier spacecraft delivers and deploys the Entry Flight System and then acts as an orbiter. Gondola/balloon system carries a mini-probe and two sondes. Teflon coated (for sulphuric acid resistance) and metallised (for low solar heating) He balloon is inflated in 5 minutes.
Launch Date	November 2, 2021 with 5 month cruise to Venus.
Science Campaign	April 7, 2022 – April 28, 2022 at an altitude of 55.5km.
Total Spacecraft Dry Mass	~1000kg
Launch Vehicle	Atlas V 551.
Cost	Low end flagship.

4.3.2.3 Science payload

- The spacecraft would carry a Venus Monitoring Camera Visible-IR while the gondola/balloon system would carry a Neutral Mass Spectrometer (NMS), Tunable Laser Spectrometer (TLS), Atmospheric Structure Instrumentation (ASI), nephelometer and Net Flux Radiometer (NFR).
- The mini probe (which is released just after balloon inflation, at the lowest altitude of 53km) would carry an NMS, NFR and ASI.
- The drop sondes, deployed on command or at a predetermined time, would enclose an ASI and NFR.

4.3.3 Venus Flagship Design Reference Mission (VFDRM)

NASA Headquarters conducted a Venus Flagship mission study in 2008–2009 based on recommendations identified by the 2003 NRC Decadal Survey [3] and the 2006 NASA Solar System Exploration Roadmap [4]. This study was supported by a NASA-appointed Venus Science and Technology Definition Team (STDT), an international group of scientists and engineers from France, Germany, Japan, the Netherlands, Russia, and the United States. JPL supported this study with a dedicated engineering team and the Advanced Project Design Team (Team X).

This mission would revolutionise our understanding of the climate of terrestrial planets (including the coupling between volcanism, tectonism, the interior, and the atmosphere); the habitability of planets; and the geologic history of Venus (including the existence of a past ocean).

4.3.3.1 Science objectives

To understand the following three major questions:

- What does the Venus greenhouse tell us about climate change?
  - Addressed by characterising the dynamics, chemical cycles, and radiative balance of the Venus' atmosphere and by placing constraints on the evolution of the Venus atmosphere.
- How active is Venus?
  - Addressed by identifying evidence for active tectonism and volcanism in order to place constraints on evolution of tectonic and volcanic styles, characterising the structure and dynamics of the interior in order to place constraints on resurfacing, and by placing constraints on stratigraphy, resurfacing, and other geologic processes.
- When and where did the water go?
  - Addressed by identifying evidence of past environmental conditions, including oceans, and characterizing geologic units in terms of chemical and mineralogical composition of the surface rocks in context of past and present environmental conditions.

4.3.3.2 Key Features

The key features of the Venus Flagship Design Reference Mission are described in the table below:

Table 4–7: *Key Features of the Venus Flagship Design Reference Mission (VEXAG, 2010).*

Features	Description
System	Two launched spacecraft (one as the orbiter and the second to deliver two entry vehicles each with dual landers and balloons)
Launch Date	Two launches in 2020-2025 (the first launch to deliver the orbiter, and 3.5 months later the second launch to deliver the two landers and the two balloons).
Science Campaign	Orbiter: 2 years; landers: 1 hour atmospheric descent followed by 5 hours of operation on the surface; balloons: 1 month at an altitude of 55km.
Total Spacecraft Dry Mass	~2000kg.
Launch Vehicle	Two Atlas V launchers.
Cost	Flagship ($3B-$4B).

4.3.3.3 Science payload

- The orbiter would carry an extremely high-resolution radar and altimetry mapping suite of instruments (an Interferometric synthetic Aperture Radar, Vis-NIR Imaging Spectrometer, Neutral Ion Mass Spectrometer, Sub-mm Sounder, Magnetometer and Langmuir Probe) to explore Venus' surface at resolutions up to two orders of magnitude greater than was achieved with the Magellan mission.
- The balloons would circumnavigate the planet up to seven times while continually sampling gases and cloud aerosols and measuring the solar and thermal radiation within the clouds with a GC-MS, a nephelometer, a Vis-NIR camera, a magnetometer and an Atmospheric Science Instrument (ASI) with pressure, temperature and wind speed sensors.
- The landers would perform descent science (obtaining atmospheric measurements and taking images of the surface on the way down) as well as surface science (high-fidelity analyses of the elemental and mineralogical content of rocks and soils on and beneath the surface). Panoramic images of the landing sites at an order of magnitude higher resolution than achieved with previous landers would provide geologic context for the landing and sampling sites.
    - During the descent phase of 1-1.5 hours, an ASI, Vis-NIR cameras with spot spectrometry, GC-MS, magnetometer, net flux radiometer and nephelometer would be in operation.
    - During the landed phase of 5 hours, a microscopic imager, XRD/XRF, Heat Flux Plate, Passive Gamma Ray Detector, Microwave Corner Reflector and a drill to ~10cm with a sample acquisition, transfer and preparation system would provide data.

Note that the Venus Flagship Lite mission, with only one orbiter, one balloon and one lander (hence needing only one launch vehicle) was also proposed in order to save costs (for a total mission cost of $1.7B instead of $3B-$4B).

4.3.4 The Venera-D Mission

The Venera-D (Венера-Д) probe is a proposed Russian Venus space probe being considered for launch beyond 2016. Venera-D will serve as the flagship for a new generation of Russian-built Venus probes, culminating with a lander capable of withstanding the harsh Venus environment for more than the 1½ hours logged by the previous Russian probes.

In order to keep research and development costs down, the new Venera-D probe will resemble the previous Russian probes, but will rely on new technologies developed by Russia since its last Venus missions (Vega 1 and Vega 2 in 1985).

4.3.4.1 Science objectives

Venera-D's prime purpose is (VEXAG, 2011):

- To make a host of remote-sensing observations of Venus.
- To map future landing sites.

4.3.4.2 Key Features

The key features of the Venera-D mission are described in the table below:

Table 4–8: Key Features of the Venera-D Mission (VEXAG, 2011).

Features	Description
System	An orbiter, two atmospheric balloons, microprobes and a lander.
Launch Date	2016
Science Campaign	The two balloons would operate for 8 days at 55-60km.
Total Spacecraft Dry Mass	1080kg-2200kg.
Launch Vehicle	Proton booster or Angara rocket.
Cost	Flagship.

4.3.4.3 Science payload

- The orbiter would carry: a Fourier Imaging UV Spectrometer, a high resolution Limb Spectrometer, a wide-angle CCD camera, a radiometer and fields and particles sensors.
- The balloons would carry: a meteorological instrument suite, a Mini-Fourier Spectrometer, a nephelometer, a CCD camera and a radiometer.
- The lander would carry: a Neutral Mass Spectrometer, an instrument suite to characterise surface properties, a meteorological instrument suite, a Mini-Fourier Spectrometer, a CCD camera, a seismometer, a nephelometer and a radiometer.

4.3.5 Literature Review Conclusion

Various mission concepts have been proposed by NASA, ESA and Roscosmos to explore Venus' cloud layer. All include a balloon with a certain float time (from 8 days to 1 month) at an altitude of ~55km carrying gondolas with a suite of instruments capable of characterising Venus' weather, cloud composition and environment (its radiation, temperature and pressure).

Therefore, a life detection payload would be suitable for such a mission, where samples would be collected as the balloon floated through the Venusian clouds.

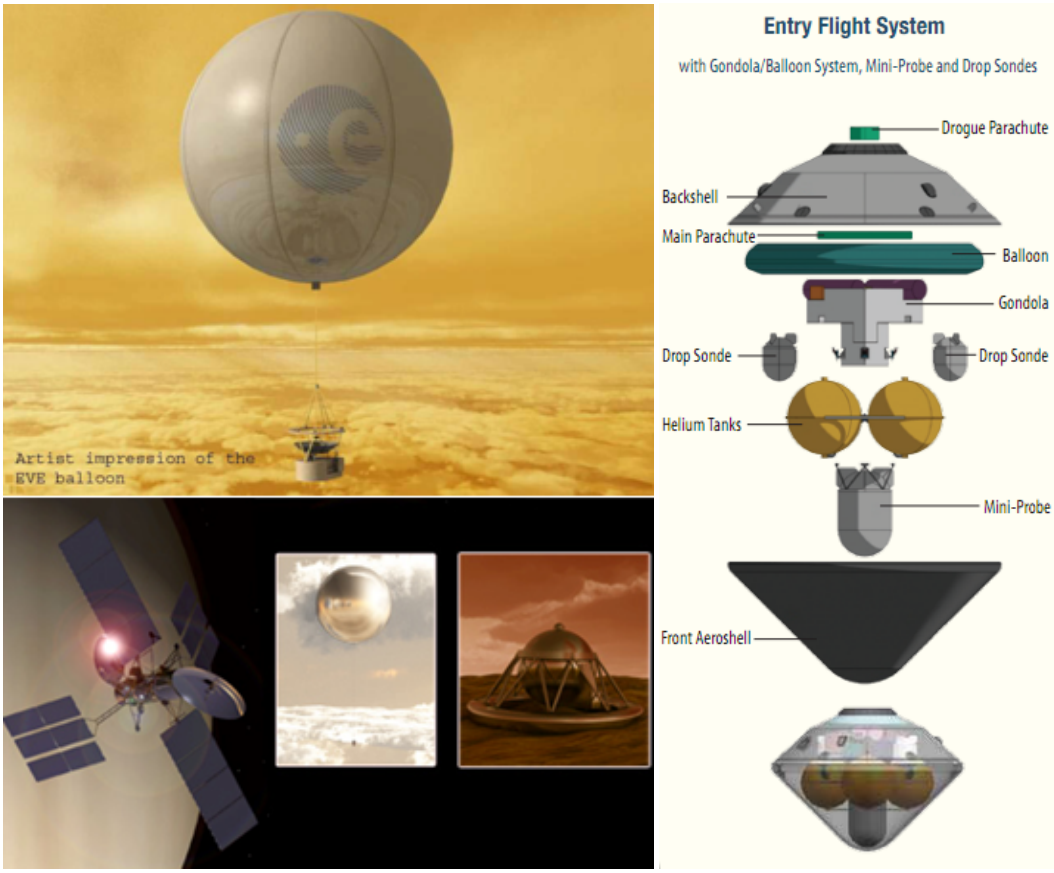


Figure 4-2: *(Top) European Venus Explorer (EVE, 2010); (Bottom) Venus Flagship Design Reference Mission (VEXAG, 2011); (Right) Venus Climate Mission Entry Flight System (VCO, 2010).*

4.4 The Design of the Payload

The design of the payload was already considered in Chapter 2 “Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions” the design of the system was performed as well as its analysis at system level to understand the dependencies between the components and make initial proposals to then start the component level analysis where each component was analysed and sourced according to its requirements.

As described in Chapter 2: *Review, Analysis and Design of an In Situ Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions* a European Space Agency (ESA) supported activity was performed where a series of trade-off studies were implemented to identify new and emerging ultra-low lower limit of detection technologies that would be suitable for planetary exploration.

Due to Venusian clouds being considered as the sample matrix, the instrument proposed for this environmental scenario in “Chapter 2: Review, Analysis and Design of an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions”, *i.e.*, Stand-Alone SERS, was proposed for a mission to Venus. It is also considered ideal due to its small size and low mass which make it perfect as a payload that could easily accommodate into a balloon gondola (although, of course, integrating a life detection instrument would impose PP&CC requirements

that would unavoidably have effects on the rest of the instruments –although this could be minimised with the inclusion of a UCZ.

As a conclusion of the study, Surface Enhanced Raman Scattering (SERS) emerged as a technique with the potential to contribute to payloads for *in situ* detection of trace levels of organics. With appropriate implementation, detection of organic molecular sample components deposited on a SERS active surface can enable parts-per-trillion detection in a relevantly simple system. This is especially true for planetary applications that require minimal sample preparation and hence avoid the complexity of automating sample preparation.

One of the planetary exploration scenarios considered within the study was the detection of trace organic molecules, including biomarkers of life, in the upper atmosphere of Venus. Therefore the implementation on a Venusian atmospheric balloon platform is now considered.

As already considered in Section 2.5.4: *Design of the Stand-Alone SERS System* representative examples of the COTS components needed for a SERS system include:

- Klarite™ SERS surface.
- Ocean Optics Raman Spectrometer (which has Space heritage due to a ruggedised version being flown on the Lunar Observation LCROSS mission).
- Optical bench electronics and 785nm laser (such as a smaller version of the Ocean Optics Laser-785).

4.5 Development of the SERS Payload

The system would have to be developed considering PP&CC requirements from the outset, as well as the environmental conditions that would be encounter (especially as the SERS surface would be exposed).

4.5.1 Test Plan for the SERS Payload

The test plan for the SERS payload would accommodate for the extreme conditions of Venus.

4.5.1.1 Modelling the conditions on Venus

A Venus environmental test facility would enable testing instrumentation under Venusian conditions; this would allow:

- Understanding the chemistry above the top clouds.
- Understanding the physical and chemical properties of the sulphurous cloud layers.
- Understanding the composition of the atmosphere below the clouds.
- Understanding the rates of reaction of surface weathering processes.

Chambers that can maintain stable pressures and temperatures for longer durations are needed to study reaction rates.

Chambers are also needed to test and validate components, chambers such as the Venus Test Chamber at Goddard Space Flight Centre (VEXAG, 2011) is an example of a test facility that could be used for testing high pressure and high temperature electronics, efficient cooling mechanisms, sensors and transducers.

Table 4–9: *The Venus Test Chamber at Goddard Space Flight Centre (VEXAG, 2011).*

Parameters	Values
Volume	12.7cm diameter, 30.5cm depth.
Temperature Range	25°C-467°C
Pressure Range	1-95.6 bar
Gases	Pure CO_2 or N_2 or mixture (can include SO_2 at ppm levels)
Duration	Maintains high temperature and pressure for a minimum of 48 hours.
Features	Sapphire view ports. Feed-throughs for data/power/RF.
Software	LabView, automated data logging.

4.5.1.2 Test plan for Stand-Alone SERS

As described in "Section 2.6.3.3 Test plan for Stand-Alone SERS", the test plan is divided into the functional, analytical and scenario tests, each with their objectives and requirements.

Functional test

Functional test objectives:

- To confirm that the hardware meets the original design criteria (no sample introduction).

Functional test inputs:

- The inputs would be based upon the specific hardware design.

Functional test requirements

- Confirm basic electronic, mechanical, optical, environment and data handling function for breadboard and associate support equipment.

Functional test outputs

- Like the inputs, the outputs would be based upon the specific hardware design.

Basic analytical test

Basic analytical test objectives:

- Confirm that the prototype(s), *i.e.,* the breadboard(s), can perform appropriate molecular detection (either single molecule detection or ultra-low lower level of detection) independent of any Space application context.

Basic analytical test inputs:

- Select example target analytes
- No relevance to Space applications will be considered, i.e. the test can be performed at room temperature and under ambient laboratory atmosphere.

Basic analytical test requirements:

- Confirm ability to detect spectra from target molecules. Two example target molecules to be used in separate tests.
- Confirm limits (lower and upper) of detection.
- Confirm ability to detect mixtures of two demonstration target molecules.
- Perform appropriate control experiments to confirm appropriate interpretation of findings.

Basic analytical test outputs:

- Collected SERS spectra (compared and collected).

Venus scenario analytical test

Space scenario analytical test objectives:

- Confirm that the prototype (breadboard) can perform appropriate molecular detection (either single molecule detection or ultra-low lower level of detection) in a Venusian cloud environment.

Space scenario analytical test inputs:

Space mission scenario

- Detect the presence of simple organic molecules in Venusian clouds.

Sample matrix

- No sample extraction, sample interacting directly with the SERS active surface. Venusian clouds are believed to host (Schulze-Makuch, 2004):
  - Particles: the lower cloud layer contains non-spherical particles comparable in size to microbes.
  - *Gases:* H_2, O_2, H_2S, SO_2
- Venus (model system implementation):
  - Ultra-pure water spiked with target examples to a given concentration (*i.e.* target volume fractions referenced to water content).

Analytes/Targets for Venus

- The targets for Venus would be trace organics including biomarkers. Specific examples would need to considered once more in depth studies of the Venusian cloud layer are made available.

Venusian environment

The clouds of Venus (at 50 to 60km) have mild environmental conditions.

- *Temperature:* 148°C to 177°C.
- *pH:* 0.
- Pressure: 1 bar.
- *Radiation:* Venus has a negligible magnetic field and hence is subjected to cosmic radiation (believed to cause cloud-to-cloud lighting discharges).

Space scenario analytical test requirements:

- Confirm basic ability to detect spectra from individual Venusian target molecules under non-Space relevant conditions and without reference to concentrations relevant to Enceladus context (only if non-Space relevant conditions are appropriate).
- Confirm ability to detect spectra from individual Venusian target molecules under non-Space relevant conditions at concentrations relevant to Venusian context (only if non-Space relevant conditions are appropriate).
- Confirm ability to detect spectra from mixtures of Venusian target molecules under non-space relevant conditions at concentrations relevant to Venusian context (only if non-Space relevant conditions are appropriate).
- Confirm ability to detect spectra from individual Venusian target molecules under Space relevant conditions and in an Enceladus relevant sample matrix.
- Confirm ability to detect spectra from individual Venusian target molecules under Space relevant conditions at concentrations relevant to Venusian context and in a Venusian relevant sample matrix.
- Confirm ability to detect spectra from mixtures of Venusian target molecules under Space relevant conditions at concentrations relevant to Venusian context and in a Venusian relevant sample matrix.
- Perform appropriate control experiments to confirm appropriate interpretation of findings.

Space scenario analytical test outputs:

- Collected SERS spectra.
- Comparison and interpretation of collected SERS spectra that show agreement with known examples of single molecule detection and/or ultra-low lower-limit of analyte detection published in the peer-reviewed literature.

Early stage de-risking of fundamental components with no space heritage:

- The effects of radiation and temperature on the SERS surface are not understood (however, the SERS surface could be actively cooled).
- The effects of the highly acidic environment on the SERS surface need to be understood.

4.6 Discussion and Analysis of the Proposed Instrument

There are a number of parameters that flight instruments need to accommodate for in order to be qualified for planetary exploration missions. These include both environmental parameters and parameters defining Spaceflight compatibility. The following is a simple assessment of these parameters for the SERS payload.

Environmental Compatibility

Environmental compatibility parameters include the ability of an instrument to survive harsh radiation, vibration/shock, thermal, pressure and atmospheric composition conditions.

Radiation:

The stability of the SERS metal surface to various radiation environments is a current unknown.

Vibration/Shock

Optical spectroscopy has been de-risked by the many other optical spectrometers that have been flown in various Earth observation and other Planetary Exploration missions.

Thermal

It is not understood if the interaction of the sample molecular targets with the SERS active surface is affected by temperature. Further studies with mission relevant targets and environments are needed.

Pressure

Atmospheric pressure does not appear to pose any significant issues for SERS.

Atmospheric composition

The acid content in the Venusian atmosphere may be problematic for certain types of SERS surface (for example, the commonly used silver surface is not expected to be compatible with a sulphuric acid rich sample environment).

Spaceflight Compatibility

Spaceflight compatibility requirements relate to those needed for an instrument to be compatible with Spaceflight, *i.e.*, low mass, volume and power budgets and well as PP&CC requirements for astrobiological planetary exploration missions.

Mass, volume, power and data budgets

As the Ocean Optics spectrometer that would be used has been flown in the LCROSS mission to the Moon, it is expected that the system would be compatible.

PP&CC requirements

For Planetary Protection, it is not envisaged that any components of the proposed SERS systems will pose a problem in sterilisation steps as all materials are expected to have been used in previous or existing flights instruments that have undergone sterilisation protocols. However, for Contamination Control, the flight of ultra-low lower limit of detection instruments poses a significant problem as this places more stringent requirements on the cleanliness in terms of levels of contaminating biomarkers and other instrument targets.

Therefore, this may require the development of alternative or more advanced methods of instrument cleaning and the verification of cleaning during instrument and spacecraft AIV.

4.6.1.1 The SERS payload

The SERS payload would consist on the Klarite™ SERS surface, optical bench with a 785nm laser source for Raman spectroscopy such as the Ocean Optics Laser-785, and Ocean Optics spectrometer in the ruggedised version of their current COTS versions.

As previously stated, the Ocean Optics QE65000 Raman Spectrometer was used for NASA's LCROSS Lunar mission and it consisted of an FC optical fiber input (0.11 NA) feeding a 25 micron x 1 mm entrance slit, where light was diffracted by a 1-inch f/4 optical cross Czerny-Turner spectrometer (grating 600 lines/mm, blazed at 350 nm) with an oversized camera mirror (Ocean Optics B, 2010). It is assumed that a similar setup of the spectrometer (in its ruggedised form) would be built for the Venus mission but its housing and perhaps the choice of materials would have to be reconsidered for its suitability for the Venusian environment (as well as the cruise phase of the mission before arriving to Venus, when it will be exposed to radiation). Mass and volume would also have to be considered and, if needed, reduced.

The ~263-650 nm spectrum from the slit was imaged onto a 1044 x 64 pixel Hamamatsu CCD detector (the first 1024 pixels contained spectral data and the remaining 20 pixels provided temporally coincident but off-slit dark reference pixels) and the data co-added within the spectrometer, delivering a 16 bit, 1 x 1044 pixel spectra (Ocean Optics B, 2010). Its integration time (between 8 ms and 65.5 s) was configurable and it supported two operation modes: single spectra (acquiring a single spectra of the requested integration time) and bracket spectra (a three-spectra acquisition defined by a base integration time and a multiplier divided or multiplied by the base integration time to yield a shorter or longer exposure time).

Therefore, using the QE65000 on-board the LCROSS mission as an application example, taking 10 images per sampling would yield a data rate of 160bps. This data could even then be compressed to allow for lower data rates by a factor of 10.

As a future balloon mission to Venus such as EVE accommodates for 2kpbs for a Gamma spectrometer on-board the balloon (EVE, 2010) and, as another example, 135Mb are expected as the total gondola science return on-board the VCM mission (VCM, 2010), a payload with 0.16kbps could be easily integrated into the payload suite of instruments of a future mission.

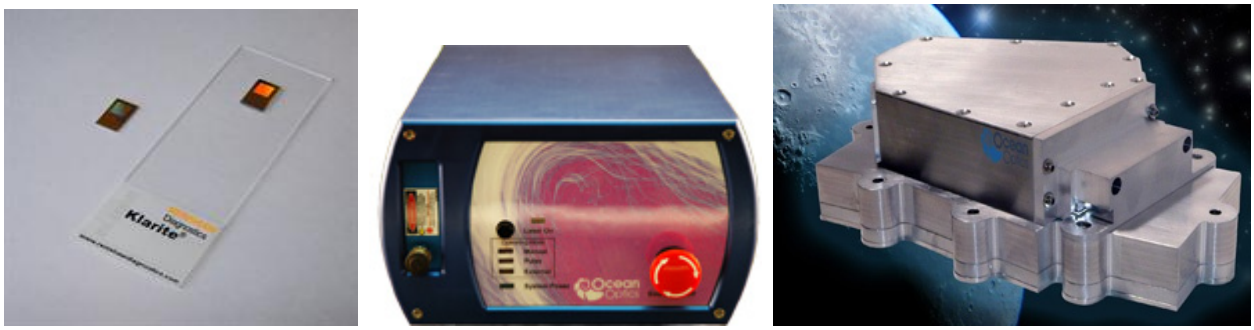


Figure 4-3: *(Left) Klarite™ SERS Surface; (Middle) Ocean Optics Laser-785; (Right) the Ruggedised Version of the Ocean Optics Raman Spectrometer Flown in the LCROSS Mission (Ocean Optics, 2010A and B).*

This payload was approved by experts during the Venus Exploration Analysis Group (VEXAG) conference held in August 2011 in Washington D.C, USA.

4.7 Conclusions and Future Work

The aim of the research described was to consider the implications of flying an *in situ* instrument on-board a stratospheric balloon platform in order to apply them into a specific planetary exploration mission.

To achieve this, another desk-based study was performed to research future stratospheric balloon platforms for the exploration of Venus' cloud layer. The *in situ* instrument previously proposed for the detection of biomarkers for planetary exploration missions was then put forward as a possible payload for a Venusian stratospheric balloon platform and approved by experts during the Venus Exploration Analysis Group (VEXAG) conference held in August 2011 in Washington D.C, USA.

4.7.1 Conclusions

The output of an ESA study was proposed as a SERS based payload for the detection of astrobiology biomarkers on-board a Venusian stratospheric balloon mission. The concept envisaged would enable parts-per-trillion detection in a relevantly simple system that could be accommodated into a balloon gondola due to its low mass and volume (~5kg).

Development and test plans with available COTS components have been proposed to enable future studies.

4.7.2 Future Work

Immediate work would include:

- Identifying a detailed science case from which analytical requirements can be identified (this would also enable identifying specific targets).
- Assembling a suitable breadboard system using primarily COTS components.

Long term future work would include applying for specific calls for proposals of payloads for a Venusian stratospheric balloon mission; this would then follow with the following work:

- Investigating suitable materials and implementation of the payload for Venusian conditions.
- Developing an upper Venusian atmosphere simulator in order to representatively test the proposed system.
- Consider implementations of SERS active surfaces compatible with a Venus mission.

Therefore, a novel ultra-low lower limit of detection instrument for *in situ* life detection on-board a stratospheric balloon with a focused planetary exploration scenario has been proposed.

5. Final Discussion, Conclusions and Future Work

5.1 Final Discussion

The aim of the research documented in this thesis was to explore issues associated with the development of instrumentation for life detection and characterisation in a planetary exploration context.

Within this aim, the following objectives had to be achieved:

- To consider current and near-future single molecule detection (ultra-low lower limit of detection) analytical techniques that would be compatible with development into a Space qualifiable in situ analytical instrument for the detection of biomarkers in a planetary exploration context.
- To practically consider the consequences of Planetary Protection and Contamination Control on the development of sample return instrumentation in a planetary exploration context.
- To consider the implications of flying an *in situ* instrument on-board a stratospheric balloon platform in order to apply them into a specific planetary exploration mission:

In order to achieve the objectives described above, the following work was pursued:

- A desk-based European Space Agency (ESA) study was carried out which entailed producing a literature review on single molecule detection technologies that had to be validated by the expert community. This was done by organising an International Workshop on Single Molecule Detection Technologies for Space Applications in March 2009 at Cranfield University, UK. The approved technologies then had to be analysed with standard analytical techniques (*i.e.*, trade-offs) in order to propose a specific technology for development and present its breadboard implementation and test plans at the end of the study.
- A sample return experiment implementing PP&CC constraints and protocols was designed, built, tested and flown on-board the ESA, Swedish Space Corporation (SSC), Swedish National Space Board (SNSB) and German Space Agency (DLR) BEXUS stratospheric balloon platform. The biological and engineering results obtained from the sample return flight were then analysed and lessons learnt obtained for future flights.
- Another desk-based study was performed to research future stratospheric balloon platforms for the exploration of Venus' cloud layer. The *in situ* instrument previously proposed for the detection of biomarkers for planetary exploration missions was then put forward as a possible payload for a Venusian stratospheric balloon platform and approved by experts during the Venus Exploration Analysis Group (VEXAG) conference held in August 2011 in Washington D.C, USA.

Therefore, three separate studies have been performed as part of the engineering design for life detection instrumentation research: the design of an ultra low lower limit of detection instrument (as part of an ESA study) and the design and implementation of a sample return instrument for a stratospheric balloon mission that flew on-board the BEXUS-10 and BEXUS-11 ESA, DLR and SSC balloon platform and the proposal of an *in situ* ultra-low lower limit of detection instrument for a focused planetary exploration scenario on-board a Venusian stratospheric balloon .

The results of the first study yield an instrument for *in situ* detection while the second part yield a sample return instrument, both aimed at detecting life. They have also both been validated, the ultra-low lower limit of detection instrument was approved by ESA and the sample return instrument was flown and its collected samples analysed.

As a concept study, the inclusion of the payload proposed in the first part of the study is proposed for a Venus stratospheric balloon mission.

5.2 Conclusions and Future Work

Therefore, all objectives of the research were met and future studies were proposed to follow on the work described here.

5.2.1 Conclusions and Future Work for an *In Situ* Ultra-Low Lower Limit of Detection Instrument for Planetary Exploration Missions

A novel instrumentation has been proposed that could be used for future planetary exploration life detection missions along with set plans to carry the work forward towards a full system that could be proposed in the next call for opportunities for a specific planetary exploration astrobiology mission. This study considered, and thus recommends at a design level stage, an MS/MS-SERS system as well as a Stand-Alone SERS system for different Space applications; *i.e.,* MS/MS-SERS is proposed for the detection of astrobiology biomarkers in Martian regolith (or ground rock and possible ice), Europan ice (and possible water) and samples from Titan's hydrocarbon lakes. While a Stand-Alone SERS system is envisaged for the detection of biomarkers in Enceladean plumes, Venusian clouds and cometary coma.

Immediate work would include:

- Building the instruments proposed according to the development plans in order to identify the components needed to build the system.
- Testing the system according to the test plans in order to identify the reliability of the instruments proposed (especially in Space simulating environments).

Long-term work would include de-risking the items identified:

- Clarifying the operating constraints of the DART sampling system under Mars atmospheric pressure.
- Coupling the MS/MS output to the SERS input for the MS/MS-SERS system.
- Understand the effects of radiation and temperature on the SERS surface.
- Explore the possibility of SERS surface regeneration.

5.2.2 Conclusions and future Work for a Sample Return Stratospheric Balloon Instrument for Life Detection

A stratospheric particle collection experiment (the Cranfield Astrobiological Stratospheric Sampling Experiment, CASS•E), that included a number of novel features, was built and flown on a stratospheric balloon. Features adopted from the space-sector to minimise microbial contamination were: the use of Dry Heat Microbial Reduction, the use of biobarrier mechanisms, Ultra Clean Zone sub-systems and rapid contamination monitoring during cleaning and assembly by ATP bioluminescence assays. A key feature was the inclusion of a positive control comprising deliberate contamination of parts of the experiment with μm-sized fluorescent beads as proxies of microorganisms. Any appearance of fluorescent beads on the sample collection filters would indicate that a contamination transfer pathway existed.

CASS•E was flown on-board the BEXUS stratospheric balloon platform from the Esrange base in Kiruna, Sweden in October and November 2010. The first flight was unsuccessful due to a failure

in the sampling mechanism; however the second flight of CASS•E allowed the collection of stratospheric samples. The post-flight finding of fluorescent beads on the sample collection filters demonstrated that a contamination pathway was present. Therefore this feature allowed a clear interpretation of the experiment -*i.e.* contamination did occur, which would not have been achievable without.

Immediate work would include:

- Clarifying the specific contamination pathways seen (including more detailed analysis of the sample filters and various control filters).
- Developing further the use of positive controls for the presence of microbial contamination pathways by, for example, using beads of different materials rather than different colours so that they can be clearly distinguished during E-SEM X-ray analysis instead of depending on the fluorescent microscope optical filters.

Long term future work would include applying to specific call for opportunities for sample return life detection in the Stratosphere or planetary exploration scenario analogues. This would entail the design, build, test and fly of a 2nd generation CASS•E incorporating lessons learnt. These include:

- Increased sample collection capacity to attain more flow through the sample collection filters (*e.g.*, use of bigger vacuum pumps with increased performance and power).
- Use of a more reliable real-time flow indicator.
- Use of a redundant biobarrier breaching mechanism in order to avoid a single point failure if the mechanism to breach the biobarriers fails.
- Use of more reliable biobarrier opening detection mechanism in order to be able to confirm during flight that the biobarriers have been breached.

5.2.3 Conclusions and Future Work for an *In Situ* Ultra-Low Lower Limit of Detection Instrument for a Stratospheric Balloon Mission Searching for Life on Venus

A novel ultra-low lower limit of detection instrument for *in situ* life detection on-board a stratospheric balloon with a focused planetary exploration scenario has been proposed as the output of an ESA study was proposed as a SERS based payload for the detection of astrobiology biomarkers on-board a Venusian stratospheric balloon mission. The concept envisaged would enable parts-per-trillion detection in a relevantly simple system that could be accommodated into a balloon gondola due to its low mass and volume (~5kg).

Immediate work would include:

- Identifying a detailed science case from which analytical requirements can be identified (this would also enable identifying specific targets).
- Assembling a suitable breadboard system using primarily COTS components.

Long term future work would include applying for specific calls for proposals of payloads for a Venusian stratospheric balloon mission; this would then follow with the following work:

- Investigating suitable materials and implementation of the payload for Venusian conditions.
- Developing an upper Venusian atmosphere simulator in order to representatively test the proposed system.
- Consider implementations of SERS active surfaces compatible with a Venus mission.

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Appendix A: Single Molecule Detection Review Methodology and Complimentary International Workshop Results

Approach to Review of Existing Public Domain Information on Single Molecule Detection Technologies

Research was conducted by making use of the following resources: specialised databases (ISI Web of Knowledge, PubMed and Science Direct); academic search engines (Google Scholar) and patent databases (FreePatentsOnline and PatentStorm).

Once a relevant document had been found, its contents and references were assessed and, if deemed relevant, cited references were obtained either as an abstract or, if readily available, as a full text document. If an identified resource was not readily available in a full text online version (using online resources available through Cranfield University), Cranfield University's library was then used to acquire reviews and identified books through interlibrary loans.

The strategy to perform a review of public domain information on single molecule detection and counting was as follows:

- Initially identify a number of review articles/books on single molecule detection and counting.
    - Achieved using an initial simple search for documents with the search term "single molecule". The review articles/books identified were then used to provide the background to define more specific search terms.
- Identify a number of databases to interrogate for more specific documents.
    - Peer-reviewed scientific literature:
        - o ISI Web of Knowledge is a readily available comprehensive research platform, with tools that allow searching, tracking, and measuring in the sciences, social sciences, arts and humanities fields. It only allows searching of titles, abstracts and keywords and not full-text but provides a resource that encompasses the majority of peer-review scientific and technical literature available.
        - o Science Direct is a database for scientific, technical, and medical research of Elsevier publications that allows full-text searches. As this database constitutes only approximately 10% of peer-reviewed scientific and technical literature, ISI Web of Knowledge is a more comprehensive source. However Science Direct does allow downloading of full-texts of the available publications.
        - o PubMed Central is a free digital archive of biomedical and life sciences journal literature. Note that in the current study it was used as a complementary resource for acquiring extra information on identified documents found using ISI Web of Knowledge and Science Direct when these did not provide sufficient information.
    - General Internet Sources:
        - o Google Search Engine general Web search was used to find full texts or extra information on specific technologies.
        - o Google Scholar, which provides a free search of scholarly literature across many disciplines and sources, including theses, books, abstracts and articles, was used to find full texts when these had not been obtained using the peer-reviewed scientific literature databases.

- Patent literature:
  - FreePatentsOnline, a complete international patent searching database and patent data analytics services provider was used to identify key new technologies that are either commercialised or with the potential to be.
  - Patents.com also provides international patent searching and hence was used to complement FreePatentsOnline.

- Develop and perform searches to identify the total literature relevant to single molecule detection and counting.
  - Used identified keywords: “single molecule” or “non-stochastic” combined with extra search terms with wild card usage “analy*”, “detect*” and “measure*”. Wild cards were used in order to avoid limiting to a specific term
- Perform data mining of the total literature identified.
  - Database reduction by reading the abstracts of the identified documents and selecting or rejecting documents as appropriate.
  - Sub-division into technology and application fields by analysis of the identified relevant documents.
  - Further expansion of information on individual techniques by revised and focused literature searching in the peer-reviewed scientific literature databases ISI Web of Knowledge and Science Direct.
  - When a relevant technology was identified, it was searched for in Google Web in order to find if companies were commercialising the said technology as well as searching through the patent websites FreePatentsOnline and PatentStorm.

Results from the Review conducted at the International Workshop on Single Molecule Detection Technologies at Cranfield University, UK.

Table A–1: *International Workshop Trade-Off Results*

Instrument Concept	Sample Spatial Res	Sample Time Res	Mass Res (too focused on MS)	Maturity of technology in non-space applications	Sample Destructive?	LLD Number of Molecs. Required	Sample Prep Complexity	Freedom from Precon-ceptions	Analytical Flexibility	Sample flexibility	Re-usability	Lifetime	Con-sumables	Data Vol.
MS/MS	N/A	N/A	High	High	Yes	1000s	Not Complex	Good	Broad limited by mass/charg e limits	High	High	Good	None for "laser ablation", required for extraction	Low
MS/MS-SERS	N/A	N/A	High	Medium	Yes	1000s	Not Complex	Good	Broad limited by mass/charg e limits	High	Medium	Medium	Required for extraction, SERS surface	Med.
Naïve Array, MS/MS-SERS	N/A	N/A	High	Low	Yes	1000s	Extraction	Good	Broad limited by mass/charg e limits	High	Poor-Medium	Medium	Required for extraction, SERS surface, naïve array	High
Imaging Confocal Raman	Yes microns	Yes Seconds	N/A	High	No depends on laser power	?	Minimal	Good dependant on wave number capability	Good dependant on wave number capability	Medium	High	Good	None (sample prep?)	Very high
SERS/ SERRS	N/A	N/A	N/A	High	Yes	10s	Extraction	Good	If Resonant System limited organics	High	Poor-Medium	Medium	Required for extraction, SERS surface	Med.
Naïve Array	N/A	N/A	?	Low	Yes	1000s	Extraction	Good	Good	High	Poor-Medium	Medium	Required for extraction, naïve array	Med.

Appendix B: CASS•E Risk Register

The risks of failure of the CASS•E experiment was assessed and documented in Table B-2: "CASS•E Risk Register".

Explanation of columns in the Risk Register:

- Risk ID (type of risk):
  - TC: technical/implementation.
  - MS: mission (operational performance).
  - SF: safety.
  - VE: vehicle.
  - PE: personnel.
  - EN: environmental.

- Probability, P (probability of the risk occurring):
  - A. Minimum: Almost impossible to occur.
  - B. Low: Small chance to occur.
  - C. Medium: Reasonable chance to occur.
  - D. High: Quite likely to occur.
  - E. Maximum: Certain to occur, maybe more than once.

- Severity, S (severity of risk):
  - 1. Negligible: Minimal or no impact.
  - 2. Significant: Leads to reduced experiment performance.
  - 3. Major: Leads to failure of subsystem or loss of flight data.
  - 4. Critical: Leads to experiment failure or creates minor health hazards.
  - 5. Catastrophic: Leads to termination of the project, damage to the vehicle or injury to personnel.

As can be seen in Table B-2: "CASS•E Risk Register", no "high" or "very high" risks were found for the CASS•E experiment. Even a failure of a biobarrier (which indeed did occur), was not considered high due to the redundancy implemented in having two separate biobarriers with two separate inlet lines. However, it was unfortunate that both failed and created a single point failure for the experiment.

Table B–1: *CASS•E Risk Register.*

ID	Risk and consequence (if not obvious)	P	S	P x S	Action
TC10	Biobarrier loses integrity prior to flight (experiment is contaminated, but filter is still protected by valve)	C	3	Low	Implement flight spare, ensure valves are closed during transportation
MS10	Biobarrier fails during flight prior to reaching stratosphere (experiment is contaminated, but filter is still protected by valve)	B	4	Low	Ensure valves are closed prior to flight
MS20	Valves fail to open, experiment fails to pump air through filter	C	4	Medium	Introduce redundancy
MS30	Pump fails during flight (air is not pumped through filter	C	4	Medium	Two pumps cross linked in case of failure
MS40	Valves fail to close before decent from stratosphere	C	4	Medium	Redundancy, two filters each with separate valve
TC20	Components within UCZ do not withstand DHMR	C	4	Medium	Select components known to be compatible, have alternatives available
TC30	Components within UCZ (with exception of filter) do not withstand 70% IPA wiping	A	3	Very low	
TC40	Components outside UCZ do not withstand IPA wiping	D	1	Low	
TC50	Required level of cleanliness is not achieved	C	2	Low	Control filters will allow this to be taken into consideration when analysing results
MS50	Biobarrier loses integrity on landing	C	4	Medium	Mitigate risk with testing
MS60	Filter breaks	C	4	Medium	Mitigate risk with testing
MS70	Contact lost with ground during flight	B	3	Low	Ensure experiment can operate autonomously
MS80	Batteries fail	A	4	Very low	
TC60	Components not delivered on time	A	3	Very low	
TC70	Critical component destroyed during testing	B	3	Low	Make spare components available
MS90	Biobarrier fails to open	C	4	Medium	Second biobarrier opening available as redundancy
MS100	Foil heaters fail to maintain temperature of experiment within operational parameters leading to component failure	C	3	Low	Redundancy, testing
PE10	Personnel unavailable unexpectedly	C	3	Low	Ensure all sub-systems staffed by 2 personnel Recruit back up resource
MS110	GPS fails, leading to failure of biobarrier to open automatically	B	3	Low	Override from ground Secondary parameter of pressure
MS120	Temperature sensors fail	B	3	Low	Redundancy
MS130	Software program in micro-controller fails during flight	C	3	Low	Watchdog checks for crashes and re-sets if necessary
MS140	Communication between the balloon and ground stations fails.	C	3	Low	System switches to fully autonomous mode, while trying to re-establish connection.
MS150	Temporary power failure leading to control board reset.	C	3	Low	Control software restarts in safe mode.

Appendix C: CASS•E PP&CC Protocols

The following protocols were used during the assembly and integration phases of the CASS•E experiment.

Three different protocols were needed: cleaning protocols for cleaning the majority of the components to be used in the assembly of CASS•E; 70% IPA wiping protocols for cleaning the components that could not be immersed as part of their cleaning procedures; and ATP detection protocols to monitor the levels of contamination during the assembly and integration phases.

Cleaning Protocols

Materials Needed:

- Gloves, IPA spray and towel paper, 70% IPA with 30% deionised (DI) water solution sterile, Sterilised 18MΩ.cm water, Laminar Flow cupboard, Aluminium foil sheets sterilised by DHMR (at 125°C for 6hr), Pyrex beaker.

Procedure:

- Wear gloves and disinfect the working area by spraying with IPA and wiping with towel paper.
- Place the Pyrex Beaker into the Laminar Flow cupboard.
- Place the components into the beaker and decant 70% IPA with 30% sterilised 18MΩ.cm water solution into the Pyrex beaker. Agitate the beaker to ensure all the components have been washed.
- Remove the 70% IPA solution.
- Fill the Pyrex beaker with sterilised 18MΩ.cm water. Agitate the beaker to ensure all the components have been washed.
- Empty the beaker and repeat.
- Empty the beaker of sterilised 18MΩ.cm water and let the materials drip-dry for a few seconds.
- Remove each component individually and place it on the sterilised aluminium foil sheet. Wrap each component in the foil.

70% IPA wiping

Materials Needed:

- Gloves, IPA spray and towel paper, 70% IPA with 30% DI water impregnated sterile wipes, sterilised 18MΩ.cm water, Laminar Flow cupboard, Aluminium foil sheets sterilised by DHMR (at 125°C for 6hr), Pyrex beaker.

Procedure:

- Wear gloves and disinfect the working area by spraying with IPA and wiping with towel paper.
- Place the Pyrex Beaker into the Fume cupboard.
- Wipe individually all the components carefully ensuring all surfaces have been wiped clean.
- Place the components into the beaker and fill it with sterilised 18MΩ.cm water. Agitate the beaker to ensure all the components have been washed with sterilised 18MΩ.cm water.
- Empty the beaker and repeat.
- Empty the beaker of sterilised 18MΩ.cm water and let the materials drip-dry for a few seconds.
- Remove each component individually and place it on the sterilised aluminium foil. Wrap each component in the foil.
- With the presented procedures the material is ready for DHMR procedure or to be moved into the cleanroom if the DHMR is not applicable. If moved into the cleanroom without DHMR, repeat the procedure before entering the clean room in a support area.
- Preferentially the components will be cleaned by immersion in 70% IPA solution, only the components that might be sensitive to immersion are to be wiped.

ATP detection

Materials Needed:

- Gloves, IPA spray and towel paper, ATP detection swabs, 70% IPA cleaned material, Luminometer, Sterile luminometer reading cuvettes, Laminar Flow cupboard.

Procedure:

- Wear gloves and disinfect the working area by spraying with IPA and wiping with towel paper.
- Place the luminometer after wiping in the Laminar Flow cupboard.
- Swab the material before and after being wiped and washed with DI water. The area required for the swab varies according to the manufacture.
- Allow the biochemical reaction to occur by breaking the Snap Valve and squeeze the bulb twice expelling all liquid down the swab shaft. Shake for 5-10 sec. (This also depends on the manufacturer instructions).
- Transfer the chemical solution to previously sterilised luminometer cuvettes.
- Run the assay by inserting the cuvette and pressing the OK button. Write down the luminometer displayed value.
- Repeat the IPA wipe cleaning procedure and the previous steps as many times as necessary to achieve a luminometer value representative of the lowest detection limit value.
- A standard ATP curve must be done previously for results interpretation.

Appendix D: CASS•E Flight Checklists

Timeline for Countdown and Flight

CASS•E flew on BEXUS-10 in October and BEXUS-11 in November; the checklists shown below are for BEXUS-11.

Adam Lambert (ESA contact) was shown as responsible for mechanical duties instead of Vinay Grama, Carla Rato in charge of fluorescent bead duties instead of Catherine Rix, Dave Cullen in charge of outreach instead of Lolan Naicker and Edwin Sanchez-Camilo in charge of the ground station instead of Ioannis Katramados due to a reduced team being able to travel to Esrange for the BEXUS-11 flight.

Table D–1: *CASS•E Pre-Flight Status BEXUS-11 Checklist.*

Pre-flight status	
	CASS•E powered off (battery connector disconnected)
	Flow path connected
	Remove before flight cover installed
	Biobarriers disconnected
	Camera mounted
	Flight Batteries installed and verified.
	MP3 player change battery and set to record.
	DATALOGGERS with flight batteries and turned on.
	CHANGE BIOBARRIER MOSFETS!

Table D–2: *CASS•E Pre-Flight Sequence BEXUS-11 Checklist.*

Flight Sequence/requirements		
Time	**Action details**	**Who**
T – 5H	Decision meeting	Eurolaunch
T – 4H45	Dilute fluorescent beads and prepare for spraying	Carla
T – 4H45	**CASS•E cathedral preparations (before E-Link access)** • DECISION – are hand-warmers required? Checklist (Clara) • Experiment visually checked for integrity (Edwin) • If hand-warmers used, put hand-warmers in experiment (Edwin)	Clara, Edwin
T – 4H30	**CASS-E cathedral preparations (with E-Link access)** Checklist (Clara): • Start video recording of the sequence (Dave) • Experiment visually checked for integrity (Edwin). • Switch on CASS•E, connect power cable (Adam) • Confirm E-Link contact with CASS•E (Edwin) • **REQUIREMENT: Low acoustic noise**	Clara, Dave, Edwin, Carla, Adam

- Starting Pre-launch Test (Edwin):
  - Turn on GPS (Edwin to acknowledge).
  - Turn on temperature sensors (Edwin to acknowledge, expect short delay).
  - Turn on VALVE1 (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn off VALVE1 (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn on VALVE2 (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn off VALVE2 (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn on PRESSURE VALVE (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn off PRESSURE VALVE (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn on again PRESSURE VALVE (Edwin to acknowledge/Adam to confirm acoustic signal).
  - Turn on PUMP1 and confirm flow (Edwin to confirm).
  - **Flow = _____ RPS.**
  - Turn off PUMP1 and confirm NO flow (Edwin to confirm).
  - Turn on PUMP2 and confirm flow (Edwin to confirm).
  - **Flow = _____ RPS.**
  - Turn off PUMP2 and confirm NO flow (Edwin to confirm).
  - Close PRESSURE VALVE (Adam to confirm acoustic signal).
  - Confirm GPS signal (Edwin to confirm).
- Notify Adam to wear gloves (Clara).
- Notify Carla to wear gloves (Clara)
- Remove “Remove Before Flight Cover” (Adam with Carla’s help).
- Confirm camera is still angled correctly (Edwin).
- **Adam to take over reading instructions.**
- Notify Clara to put gloves on (Adam).
- Notify Clara to ground herself (Adam).
- WARNING: ABOUT TO PERFORM CRITICAL TEST, FULL ATTENTION AND CONCENTRATION NEEDED.
- Starting Biobarrier ON Signal Test:
  - Connect biobarrier1 ground cable (Clara).
  - Connect biobarrier1 + cable to DMM set on 10A reading (Clara).
  - Turn ON biobarrier1 (Edwin).
  - Reading = _____A
  - As soon as reading is taken IMMEDIATELY TURN OFF BIOBARRIER1 (Edwin).
  - Connect biobarrier2 ground cable (Clara).
  - Connect biobarrier2 + cable to DMM set on 10A reading (Clara).
  - Turn ON biobarrier2 (Edwin).
  - Reading = _____A
  - As soon as reading is taken IMMEDIATELY TURN OFF BIOBARRIER2 (Edwin).

- Connect biobarrier1 and biobarrier2 (Clara).
- Confirm the biobarrier cables are secured in between the springs

	(Clara). • **Clara to again read out instructions.** • Collect 4 swab samples for ATP analysis (Carla): o BB left cover flange. o BB right cover flange. o BB left Tyvek. o BB right Tyvek. • Shut down ECU software (Edwin). • Switch OFF CASS-E (Adam). • **REQUIREMENT: No ignition sources in locality of CASS•E** • Cover sensitive experiments, connectors and cameras (Carla). • Notify Carla to wear safety spectacles (Clara). • **Notify all persons to move back (Clara)** • Spray CASS•E with fluorescent beads (red colour), inside flight cover and accessible CASS•E external panels (Carla). • Replace "Remove Before Flight Cover" (Adam, with Carla's help) • **"PAYLOAD MANAGER, EXPERIMENT CASS•E GO FOR LAUNCH"** (Clara)	
T – 2H30	Payload pick-up from Cathedral.	EuroLaunch
T – 1H45	Payload to launch position. Decision meeting Payload Operations.	Payload Manager and Team Captains
T-1H30	Decision meeting Balloon Operations.	EuroLaunch
T – 1H15	**REQUIREMENT: Access to CASS•E on launch pad** **Late access to CASS•E (on launch pad)** **(only 10 minute access)** Checklist (Clara) ☐ Start continuous video recording of ground station screen and team audio around ground station (Dave). ☐ Clara, Carla and Adam to go to launch pad. ☐ **REQUIREMENT: Wait until all other teams have finished their access** ☐ Photograph procedures (Clara). ☐ Switch on CASS•E –connect power cable (Adam). ☐ **"Confirm E-link contact"** order to be relayed to Launch Pad Manager to radio it to Payload Manager in Cathedral to ask Edwin. ☐ **"Set ECU software to IDLE state with thermal control ON"** order to be relayed to Launch Pad Manager to radio it to Payload Manager in Cathedral to ask Edwin. ☐ ☐ Collect 6 swab samples for ATP analysis (Carla): ☐ Canvas roll above BB. ☐ Canvas CASS•E side (front with BB). ☐ Canvas right-side. ☐ Scope. ☐ Perdaix. ☐ Canvas back-side. ☐ Canvas left-side. ☐ Cover BEXUS-11 camera and experiments (Carla). ☐ Notify Carla, Adam and Clara to wear safety spectacles (Clara).	Clara, Adam and Carla on launch pad, Edwin and Dave in Cathedral

	☐ Notify Launch Pad Manager to notify personnel to move back (Clara). ☐ Spray exterior of Gondola with green fluorescent beads (Carla). ☐ Uncover camera and experiments (Carla). ☐ Notify Adam to wear gloves (Clara). ☐ Take off "Remove Before Flight Cover" (Adam with Carla's help). ☐ Check biobarrier wires (Adam). ☐ **"Check camera view"** order to be relayed to Launch Pad Manager to radio it to Payload Manager in Cathedral to ask Edwin. ☐ **"Launch operations are complete"** (Clara).	
T – 0H45	Access to gondola on pad ends.	
T – 0H35	Balloon inflation starts.	EuroLaunch
T – 0H30	**Final CASS-E pre-flight operations** Checklist (Clara): ☐ Confirm ECU software is still on "IDLE" state (Edwin). ☐ Switch ON Thermal Control (Edwin).	Clara, Edwin
T - 0H10	Lift gondola.	EuroLaunch
T = OHO	Release balloon.	EuroLaunch
T + 0H15	**REQUIREMENT: Access to BEXUS-11 GPS data** **Immediate CASS•E post launch operations** Checklist (Clara): ☐ Confirm agreement of CASS•E GPS altitude readings on the ground station with BEXUS GPS readings (Edwin)	Clara, Edwin
T + ~1H00 (altitude = 21km – BEXUS11 target height =35 km)	**CASS-E late ascent and float phase operations** Note, all actions to be completed by Edwin. Checklist (Clara): ☐ Monitor CASS•E GPS altitude readings. ☐ Wait until GPS altitude reading = 21km. ☐ **Start Flow Tests and BB1 burn:** ☐ Open PRESSURE VALVE. ☐ Switch ON PUMP1 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP1 and confirm NO flow. ☐ Switch ON PUMP2 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP2 and confirm NO flow. ☐ Switch ON PUMP1 and PUMP2 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP1 and PUMP2 and confirm NO flow. ☐ Close PRESSURE VALVE. ☐ Requirement to have bandwidth for live video feed. ☐ Start recording program to record IP Camera live video feed. ☐ Ensure all heaters are OFF. ☐ Prepare timer. ☐ OPEN biobarrier1 and start timer. ☐ Time taken to breach biobarrier1= _____ ☐ Open VALVE1.	Clara, Edwin

	☐ Switch ON PUMP1 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP1 and confirm NO flow. ☐ Switch ON PUMP2 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP2 and confirm NO flow. ☐ Switch ON PUMP1 and PUMP2 and confirm flow. ☐ Flow rate = _____ RPS. ☐ **Record altitude at which flow rate is lost = _____km.** ☐ Wait until floating phase confirmed by EuroLaunch. ☐ Start Flow Tests and BB2 burn: ☐ Switch OFF PUMP1 and PUMP2 and confirm NO flow ☐ Close VALVE1. ☐ Requirement to have bandwidth for live video feed. ☐ Start recording program to record IP Camera live video feed. ☐ Ensure all heaters are OFF. ☐ Prepare timer. ☐ OPEN biobarrier2 and start timer. ☐ Time taken to breach biobarrier2= _____ ☐ Open VALVE2. ☐ Switch ON PUMP1 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP1 and confirm NO flow. ☐ Switch ON PUMP2 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Switch OFF PUMP2 and confirm NO flow. ☐ Switch ON PUMP1 and PUMP2 and confirm flow. ☐ Flow rate = _____ RPS. ☐ Set ECU software to FLOATING state. ☐ Ensure Thermal Control button is ON. ☐ **Record altitude at which flow rate is lost = _____km.**	
	<u>REQUIREMENT</u>: Need minimum 5 minute pre-warning from EuroLaunch of balloon cut-down **<u>CASS-E immediate pre-balloon cut-down operations</u>** Checklist (Clara): ☐ Wait for balloon cut-down warning from Payload Manager. ☐ Turn off PUMP1. ☐ Turn off PUMP2 and confirm NO flow. ☐ Close VALVE1. ☐ Close VALVE2. ☐ Open PRESSURE VALVE.	Clara, Edwin
T + ~4H00	Balloon cut-down	EuroLaunch
T + ~4H00	**<u>CASS-E descent phase operations</u>** All actions to be done by Edwin.	Clara, Edwin

Checklist (Clara):

- ☐ Monitor GPS signal to confirm balloon cut-down.
- ☐ Set ECU software to DESCENT state.
- ☐ Wait for E-link until communication is lost.
- ☐ Back up data logs.

Table D–3: *CASS•E Post-Flight Activities BEXUS-11 Checklist.*

Time	Action details	Who
After flight	Download data from camera/online storage	Edwin
After flight	Back up all data on external hard drives	Edwin
After flight	Review technical data	Clara, Edwin
After flight	Update website etc with latest news, photos and videos	Lolan
After recovery	Recover experiment	ESRANGE personnel
After recovery	Inspect for signs of damage	Clara
After recovery	Ensure the experiment is electrically and mechanically safe	Clara, Edwin
After recovery	• Start swabs collection procedure: o Right side CASS-E gondola. o Above CASS-E gondola. o Below CASS-E gondola. o BB left cover flange. o BB right cover flange. o BB left inlet tubing. o BB right inlet tubing. o Scope. o Perdaix front side. o Perdaix right side.	Carla
After recovery	• Start remove UCZ procedure: o Remove Pumps panel. o Remove UCZ panel. o Unplug UCZ elecs conn. o Unplug UCZ flow line 1 conn. o Unplug UCZ flow line 2 conn. o Remove frame beam next to BBs. o Remove UCZ. o Bag UCZ. o Secure UCZ in pelicase. • Replace beam to ensure the experiment is mechanically secured (Adam, Clara).	Adam, Edwin, Clara
After recovery	Take out of gondola	Adam, Edwin
After recovery	Remove batteries	Clara
After recovery	Package experiment and tools for shipping	Clara, Edwin
After recovery	Ship to Cranfield	Esrange personnel
After recovery	Move to cleanroom and perform analysis on the filter to look for collected microorganisms.	Catherine, Carla

Appendix E: CASS•E Circuits

As the circuits for CASS•E were fully designed and built by the author in her role as the Electronics Engineer of the CASS•E Team (as well as the Systems Engineer and Team Leader), they are included for completeness of work performed during the research period.

There were three main circuits: the main control board circuit (which included the design, development and testing of a test board to de-risk the main control board as well as a flight spare main board); the temperature board circuit needed to be able to place the temperature sensors on the different components (7 of them in total); and the power circuits.

The Control Board

The control board was split into three functional areas: the control circuit (*i.e.,* to control the heaters, pumps, biobarrier mechanisms and valves); the communications circuit (*i.e.,* the RS-232 serial and Ethernet circuits); and the sensors circuit (*i.e.,* the sensors on the I^2C and SPI buses).

The valves, biobarrier mechanisms, pumps and heaters have MOSFETS to control them, diodes for surge protection, resistors for current limiting and capacitors to dampen voltage spikes. LEDs were added to the circuit to have visual confirmation of “5V power in”, “12V power in”, “biobarrier1 open”, “biobarrier2 open”, “SD card power in” (not used), “pump1 power in”, “pump2 power in” and “WatchDogTimer reset” signals.

The Control Circuit

The two pumps, two UCZ valves, one pressure equalising valve and two biobarrier opening mechanisms were controlled via logic drive power MOSFETS which were controlled by the PIC. This allowed for high voltage and current to be switched by the MOSFET to the component that needed to be controlled hence isolating the rest of the circuit.

Due to the high currents needed by the valves, heaters, biobarriers and specially the pumps, the track width on the PCB was increased accordingly to avoid the tracks becoming “fuses” (the circuit would break when high current evaporates sections of the tracks).

Resistors in series with the microchip line were included to create a buffer to protect the microcontroller should the MOSFET fail. Capacitors were included to avoid voltage spikes and diodes protected from back-currents.

The heaters were also controlled by power MOSFETs that were driven by the PIC. These heaters were in a closed loop control with feedback from the temperature sensors. The heaters included:

- Heater1Pump1 and Heater2Pump1. Wired to the same microcontroller input signal as Heater2Pump1, but has its own MOSFET circuit to increase redundancy.
- Heater1Pump2 and Heater2Pump2. Wired to the same microcontroller input signal as Heater2Pump2, but has its own MOSFET circuit to increase redundancy.
- HeaterUCZ.
- HeaterIPcamera.
- HeaterElectronicsBoxTop and HeaterElectronicsBoxBottom.
    - Wired to the same microcontroller input signal as HeaterElectronicsBoxBottom, but has its own MOSFET circuit to increase redundancy.

The Communications Circuit

The communications circuit was comprised of the MAX RS232 driver and the two sockets for the plug-in of the Serial to Ethernet converter and of the GPS. Information was serially exchanged between these components and the PIC.

The Sensor Circuit (I2C and SPI Buses)

The I^2C bus enabled communications of the sensors with the PIC. In the figure below the I^2C bus can be seen with the on-board temperature sensor directly hooked up onto it as well as the connectors of the other sensors (the temperature sensors are not seen in the image as they are wired to dedicated pump system connectors not seen in this part of the schematic).

Note that the temperature sensors, as they are various instances of the same component, have their I^2C addresses hard wired by pulling their 5, 6, and 7 pins up or down.

The microcontroller

The microcontroller is shown in the figure below. The analogue battery temperature sensor connector, the exterior temperature sensor connector and the flow LED and flow PLEDs connector are connected to the A/D ports of the PIC. The biobarrier detection sensor connector is also shown in the diagram below, as well as the WatchDogTimer and the reset circuit.

A resistor parallel to R24 on the reset circuit is included in case R24 fails (as this would leave the reset circuit constantly pulled up high). Capacitors on the power lines of the microcontroller are included to avoid disturbances. A 20MHz powered oscillator (out of view in the figure below) provides the microcontroller with the clock signal.

The Test Board

The purpose of the test board was to de-risk the inevitable integrating issues between the hardware and the software. As the PIC18F4XK22 is a new chip it had to be de-risked and the drivers sought. Therefore, the functional areas that needed to be tested were the IO ports, RS-232, I2C and SPI. For the IO ports, LEDs were integrated to be able to visually confirm if the microcontroller could switch them on. For RS-232, D-9 connectors were added to connect to the GPS sensor and to the ground station. For I2C, two test pins were integrated to be able to hook sensors onto the bus, as well as having a temperature sensor on board. The SD card module was also integrated to be able to test SPI (although this was not used).

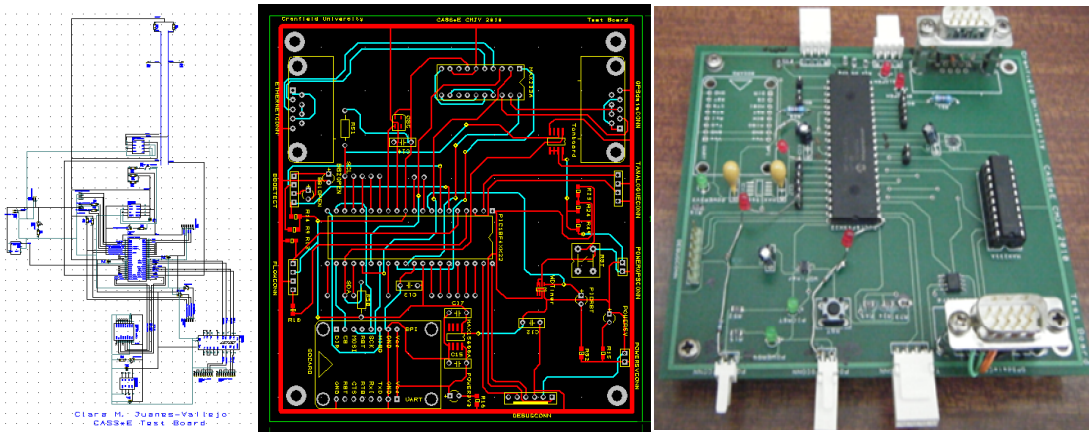

Figure E-1: *Schematic, Layout and Photo of the Test Board.*

The Temperature Boards

Due to the small size of the temperature sensors, mini-boards were created for them to be placed safely around the system to take temperature readings. An LED was included to enable visual confirmation of “power on”.

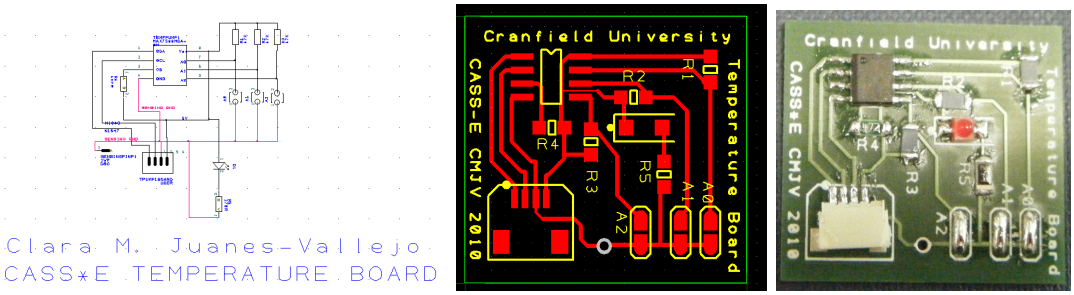

Figure E-2: *Schematic, Layout and Photo of the Temperature Board.*

In order to have a board that could be used for any of the sensors, the address lines had to be left unconnected in order to be able to set each sensor independently. Therefore, the 3 links are pulled to ground if left unconnected, and just by soldering across them they are pulled high, thus allowing the production of one board to suit all the temperature sensors.

The top layer of the layout has all the components as surface mount due to the bottom layer being a solid copper layer which acts both as the ground plane and the temperature sensing plane. A via connected both layers.

The Control Board

The final board is shown in the figure below along with its layout. The schematic can be found on the following page.

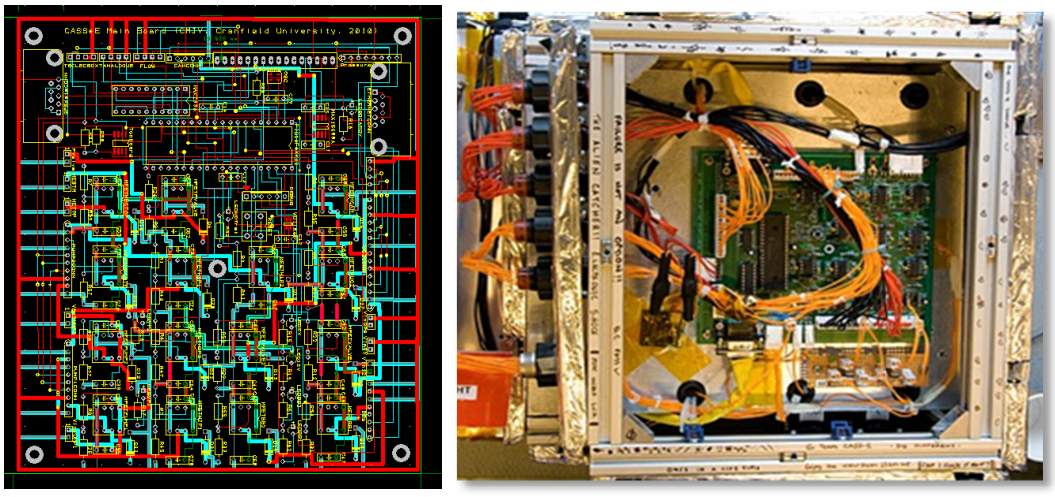

Figure E-3: *Layout and Photo of the Main Board Integrated into the Electronics Box.*

Figure E-4: *Schematic of the Electronics Control Board.*