

Development of two-color fluorescent imager and integrated fluidic system for nanosatellite biology applications

Diana Wu *et al*

NASA Ames Research Center

ASGSR

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Technologies (MisST)

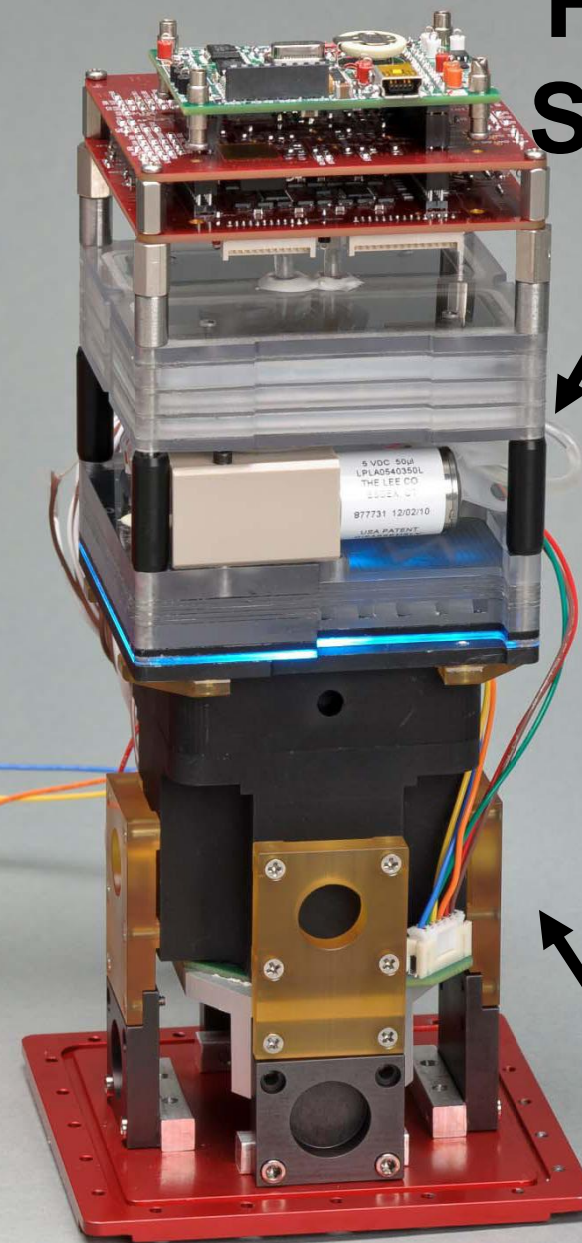
NanoSatellite Science Payload

22 cm

10 cm

10

Fluidic
System



2 color Fluorescence

Overview

Objective: Technology demonstration of 2-color
cent imager for space biology applications

Model organisms, resolve major features/organs,
interest & control

Configuration: 2-cube integrated culture/imaging
environment

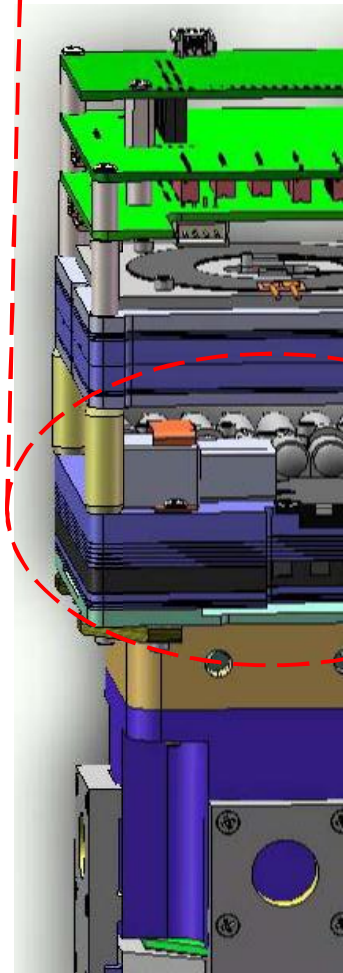
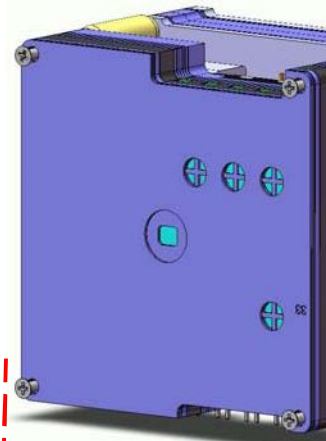
Model organism: *C. elegans*

Fluidics: culture wells, imaging zone, reagent storage

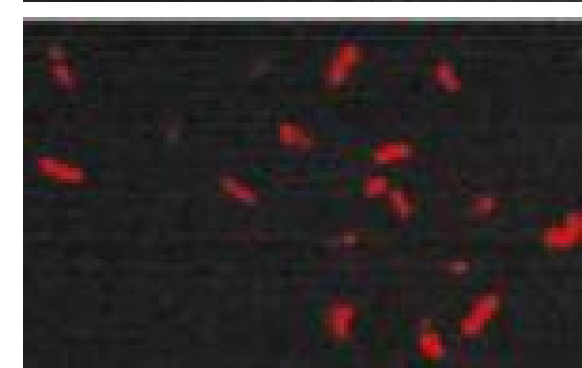
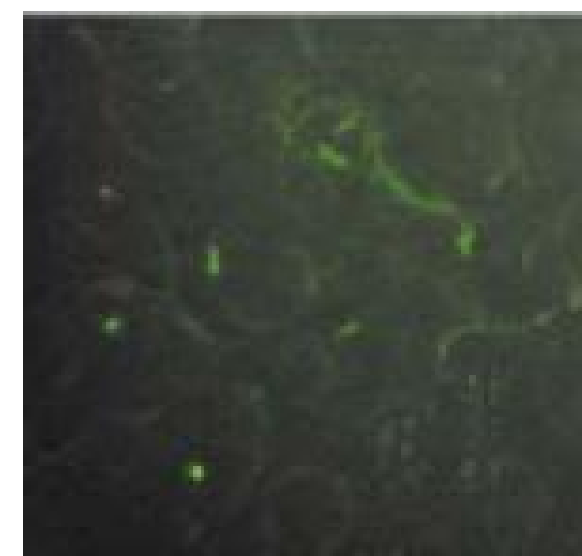
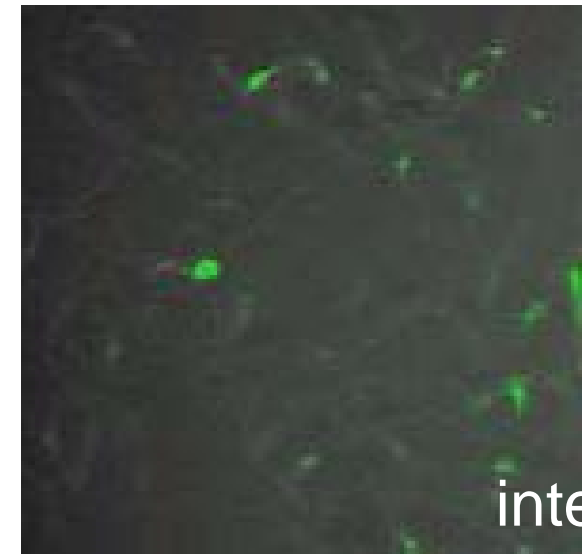
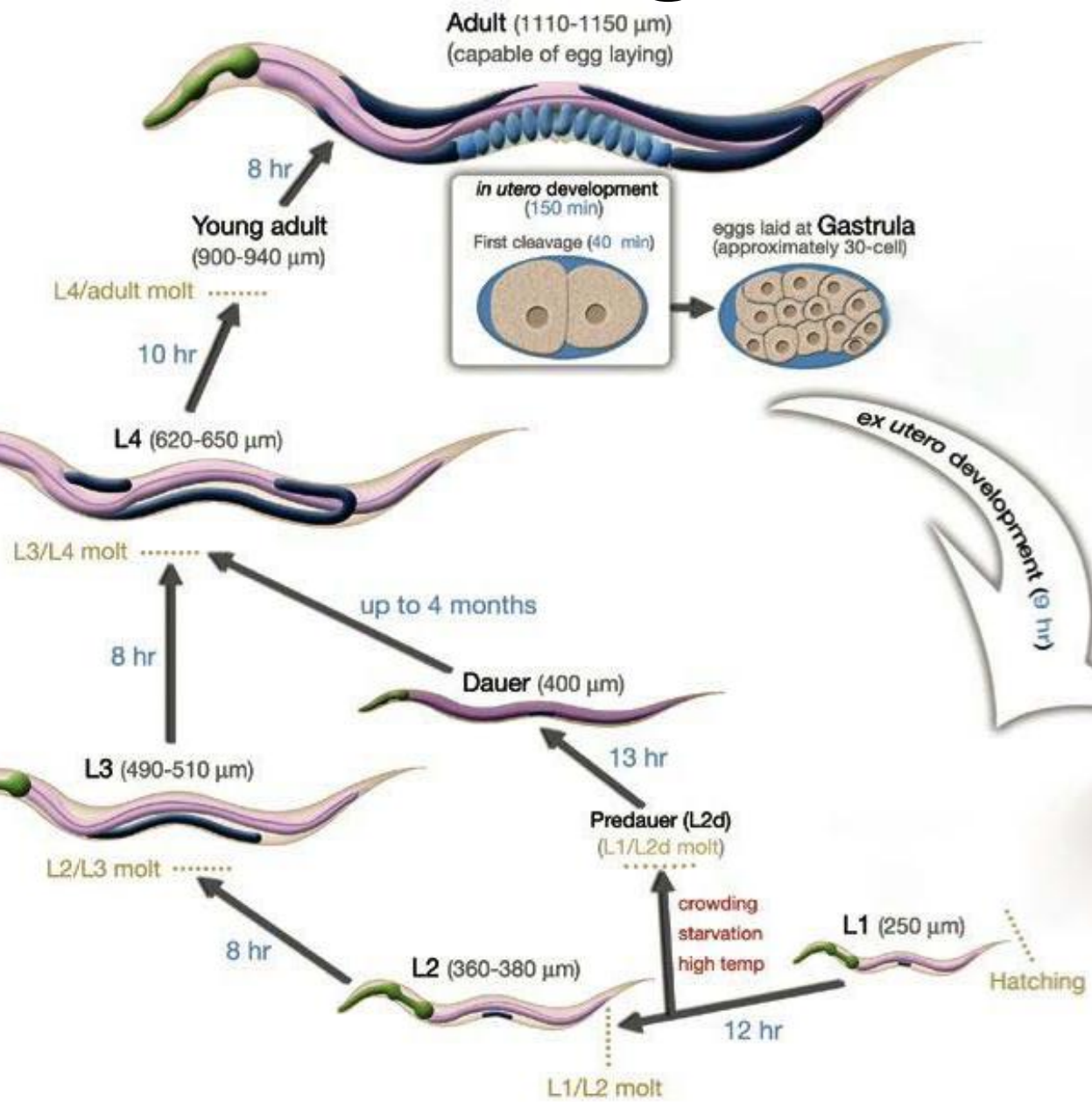
Imager: fixed focus, 300 kpixel CMOS chip

Image storage: 2 GB/6,000 images/8 bits per color
(telemetry of 1 - 20 images)

Hermetic containment vessel



C. elegans Overview



...ding and short life cycle make it inexpensive to
...and ideal for research

...study spaceflight effects and experiments using *C.*

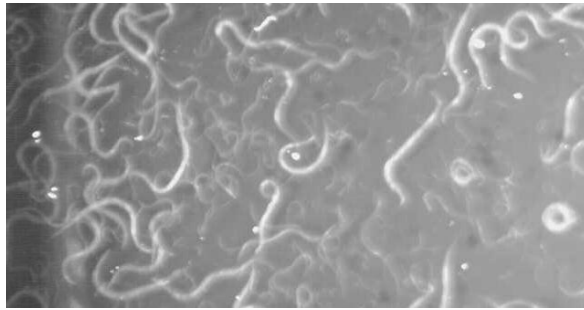
C. elegans Imaging

White

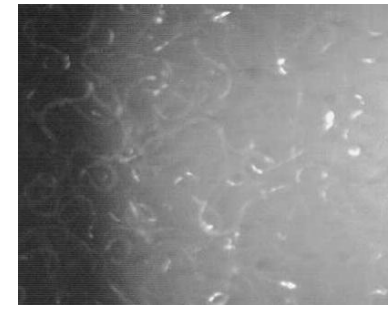
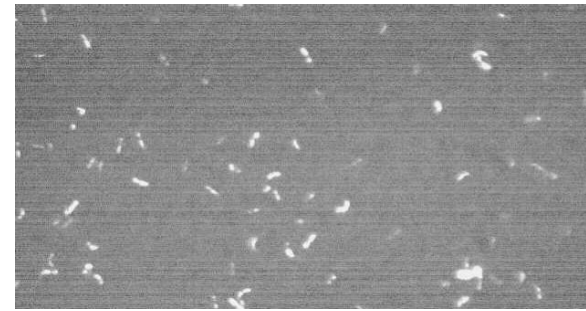
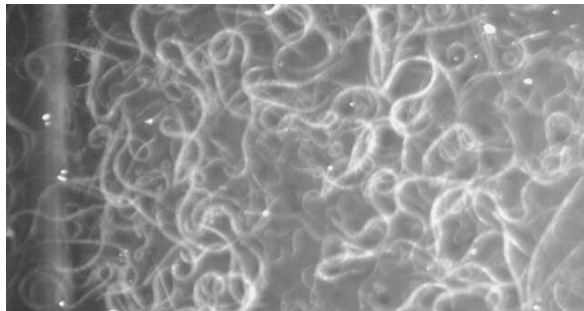
Amber

Blue

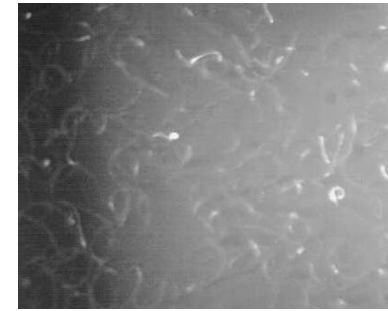
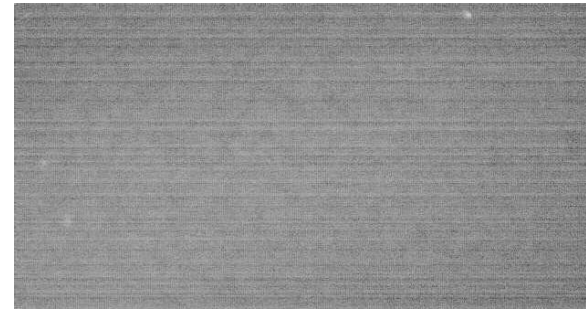
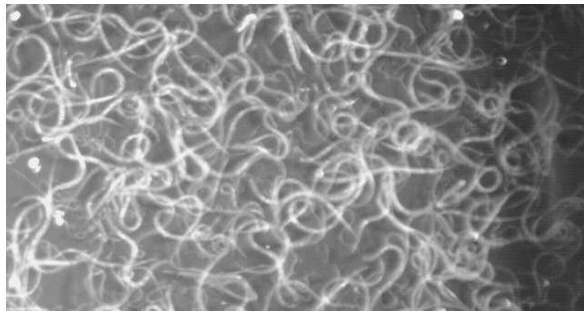
t)
rescent



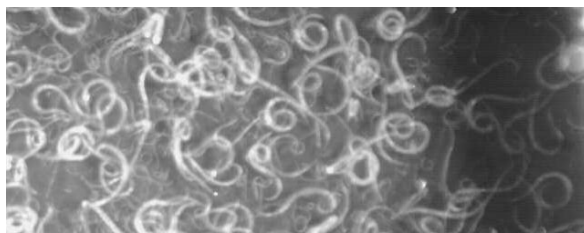
nato
muscle cells



4
nerve cells



42
intestinal cells

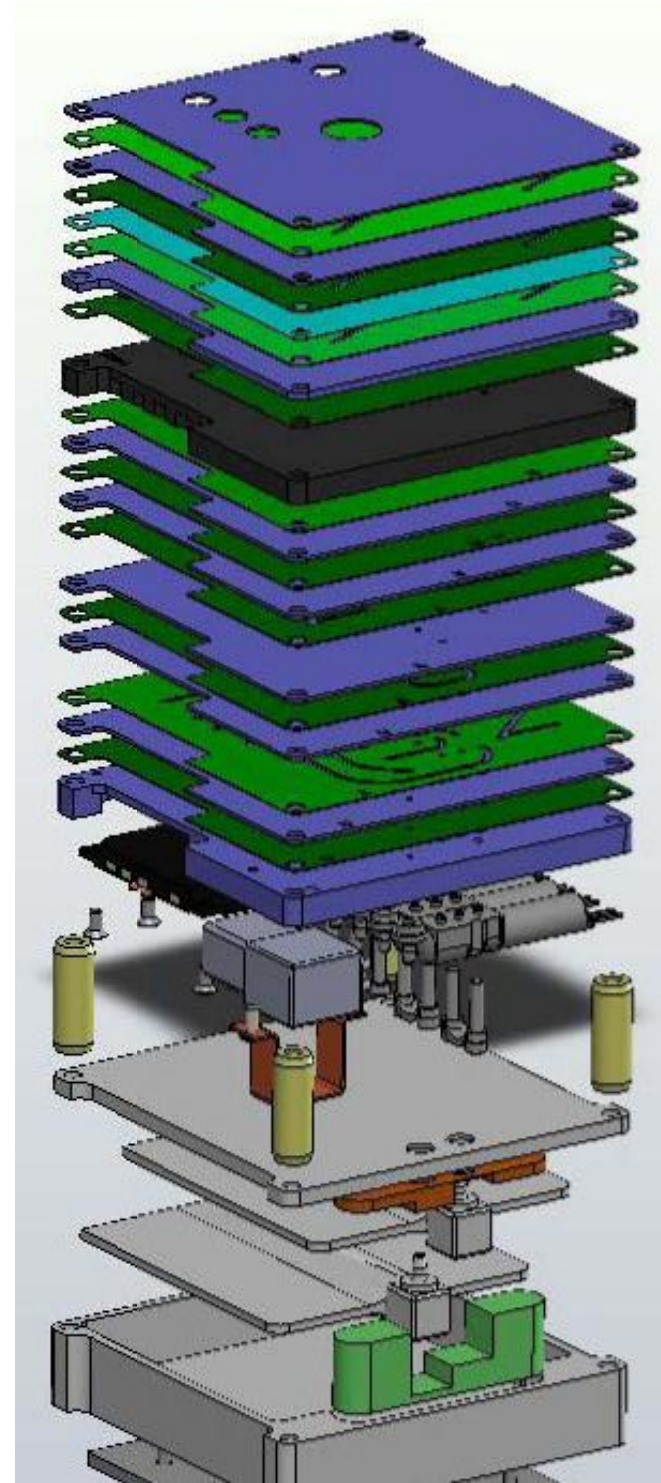


Fluidics Overview

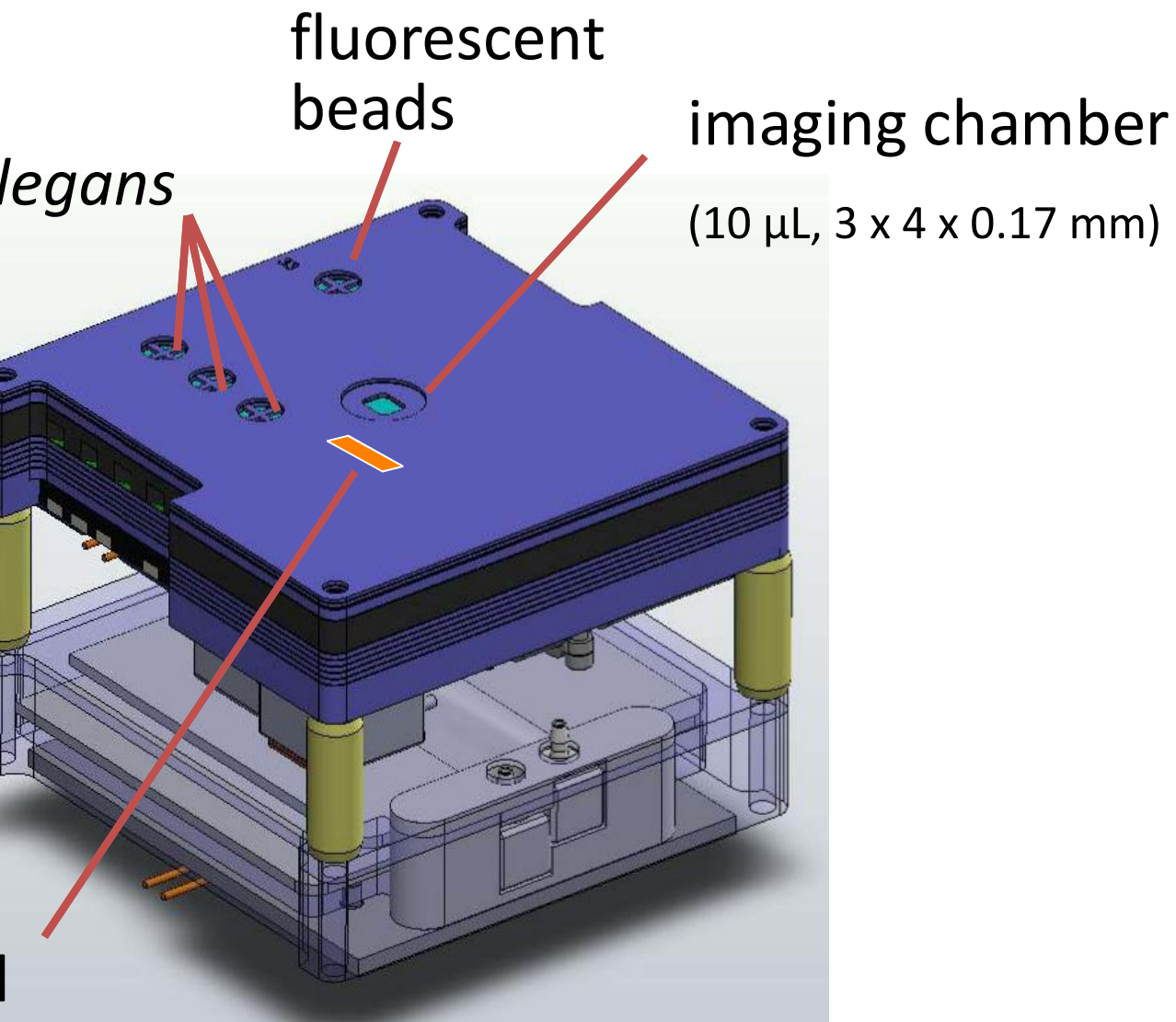
Each: Multilayer polymer microfluidic card

- Multi-layer lamination to form 3-D fluidic network
- Laser-cut fluidic channels in pressure-sensitive adhesive (PSA)
- Lamination of machined polycarbonate sheets with via holes, filters, polystyrene using PSA

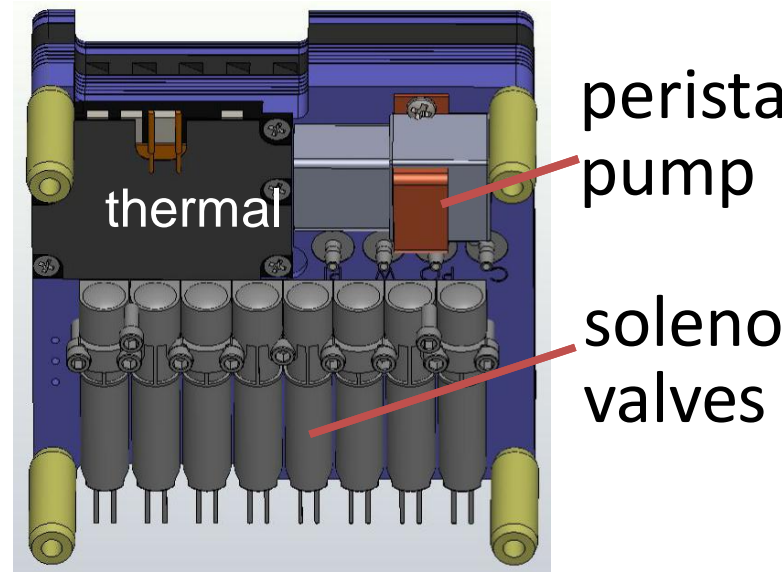
ers, adhesives, sterilization, biocompatibility,
all have GeneSat, PharmaSat, O/OREOS-
EcAMSat heritage



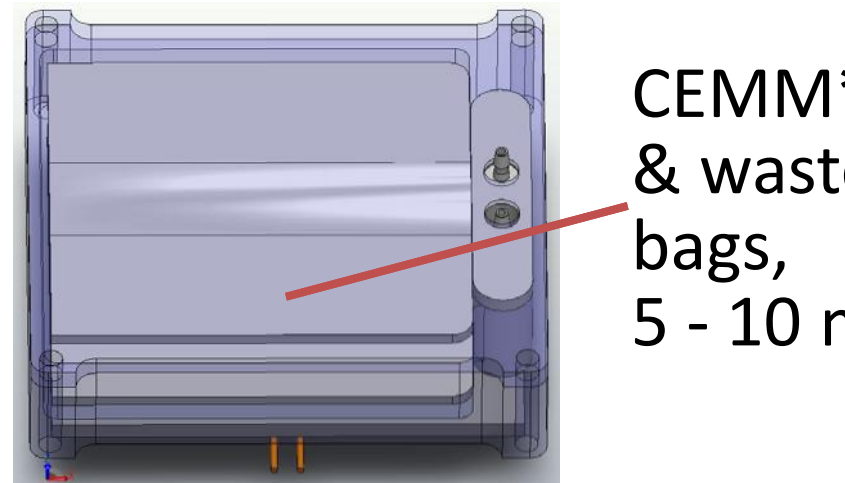
Fluidic Card and Reservoir



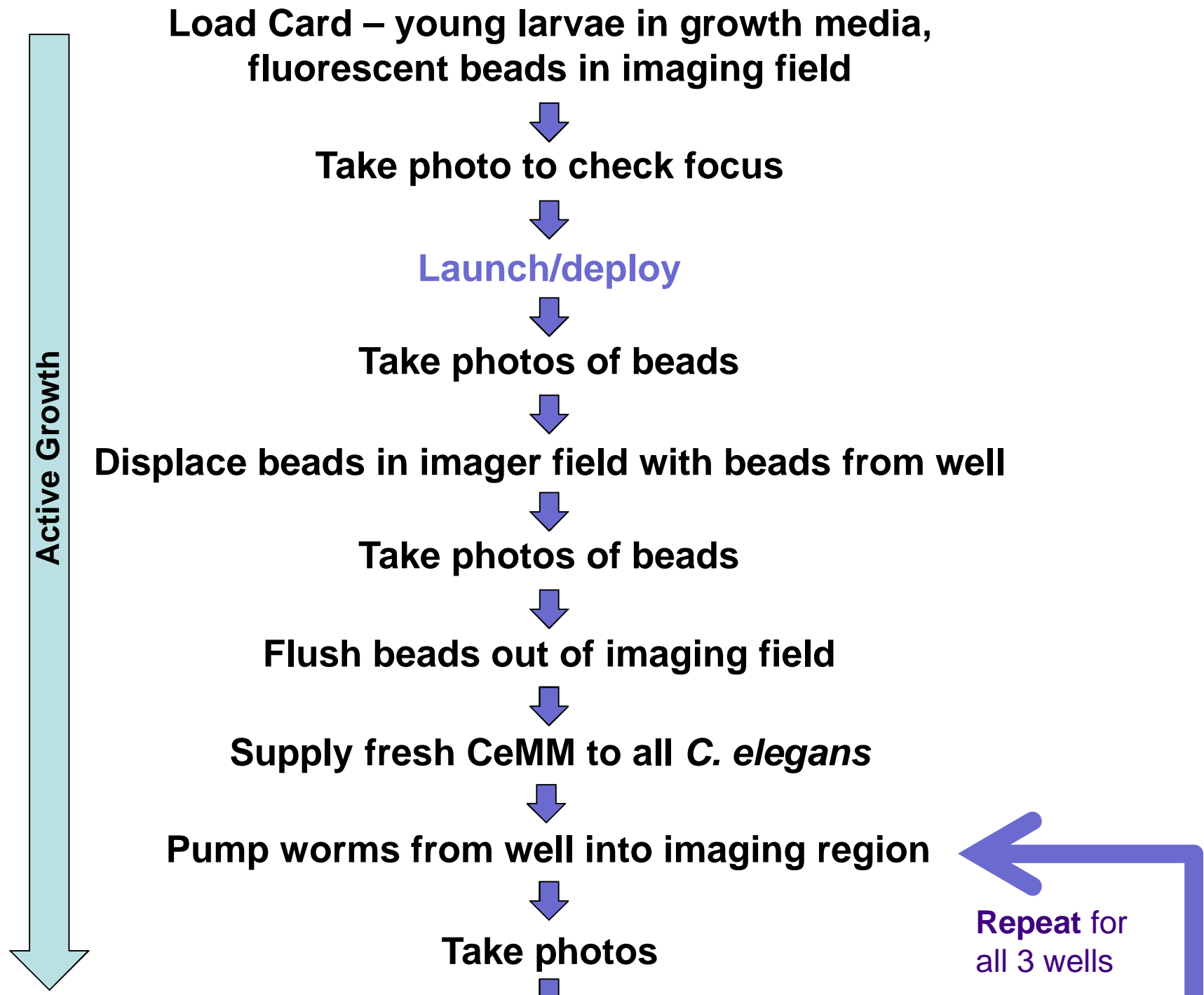
Fluidic Card



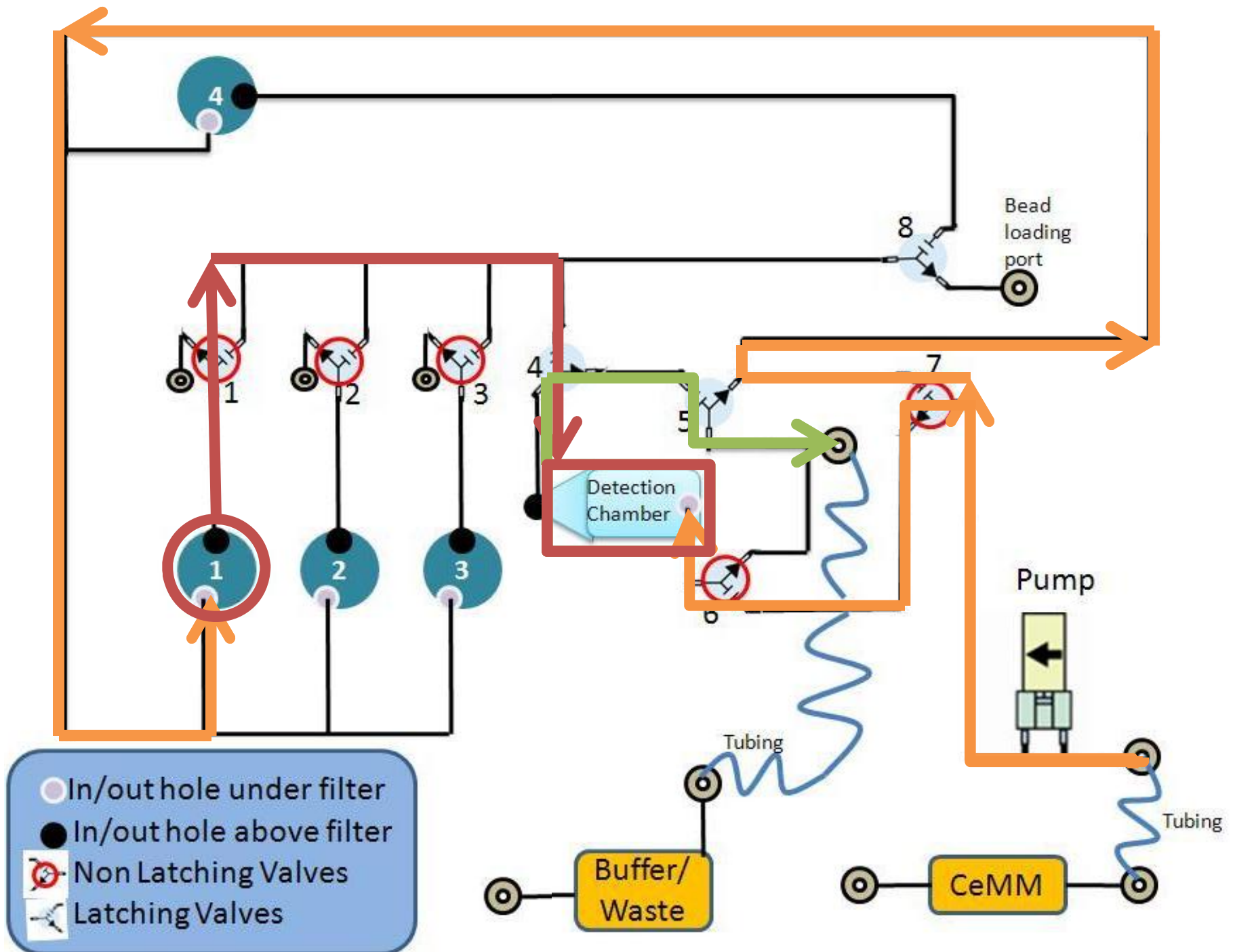
Reservoir



Experimental Timeline



Fluidic Sequence- Worms



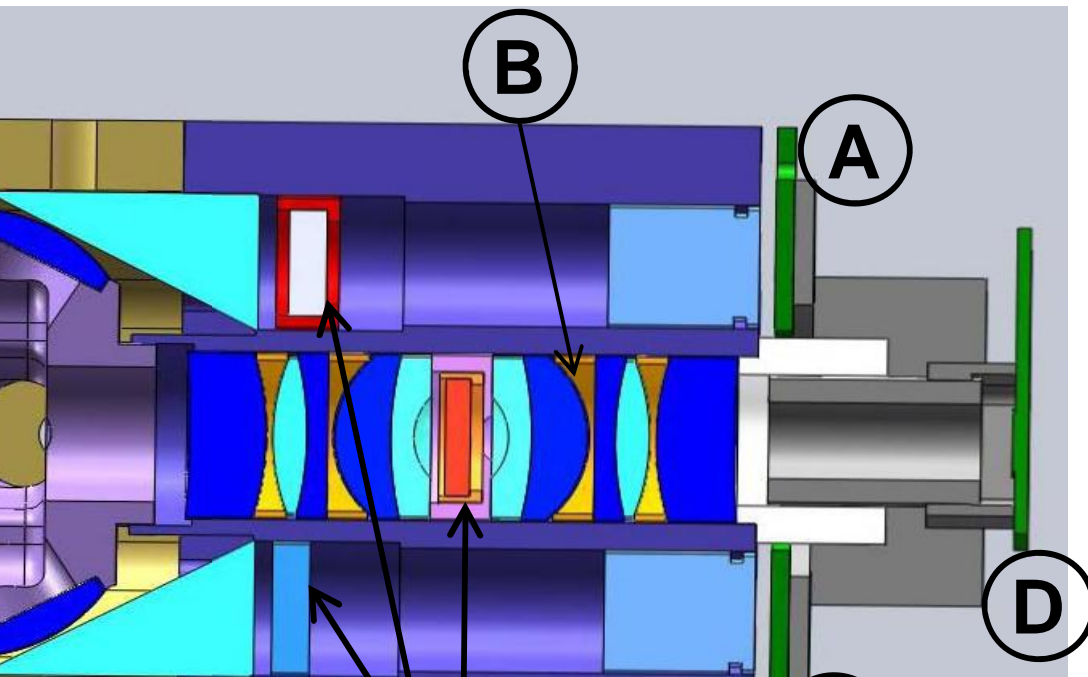
Fluorescence Imager

Imager Components:

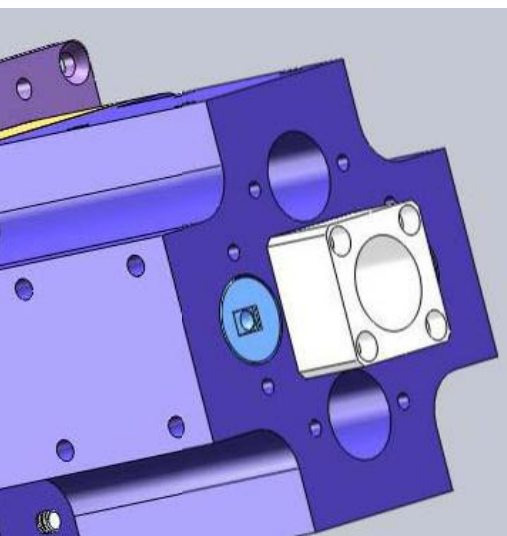
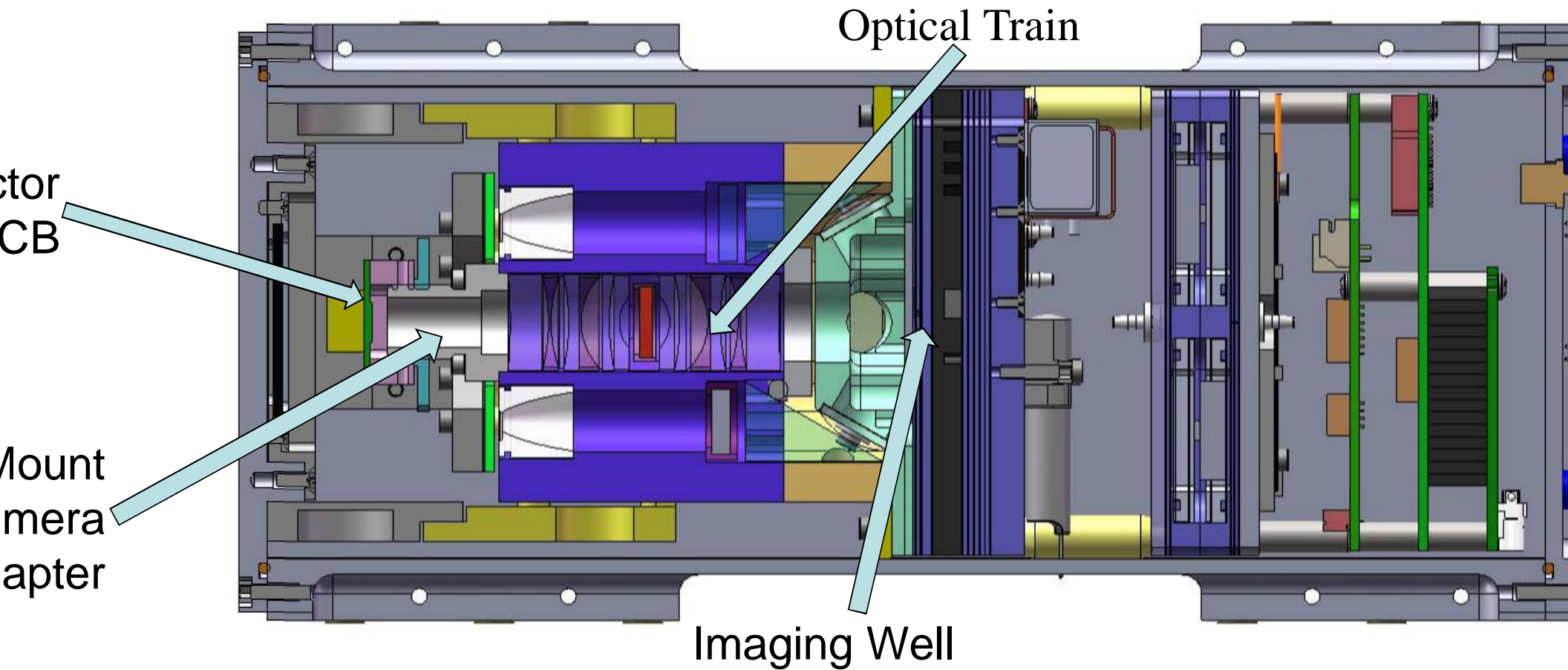
- A** Luxeon Rebel LEDs:
2 amber, 1 blue, 1 white
- B** Edmunds relay lens
- C** Semrock filters:
blue excitation, amber excitation,
dual band-pass emission
- D** Camera:
COMedia C328-7221

Imager Specifications:

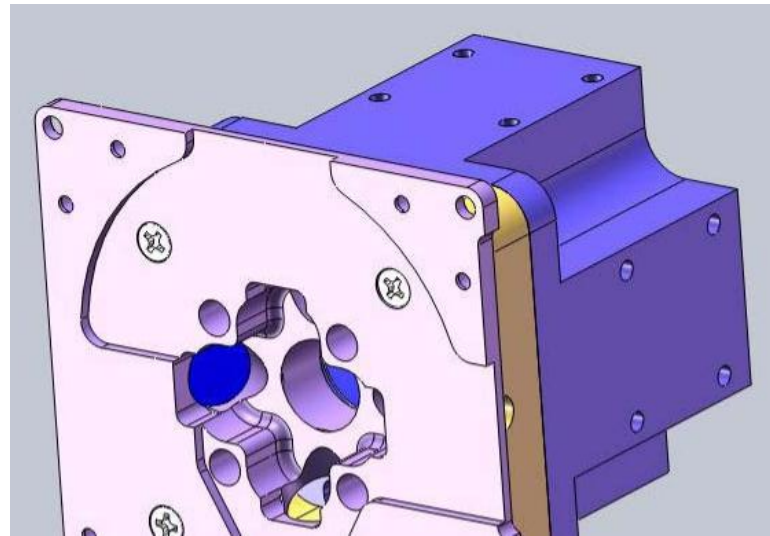
- 2-color fluorescence:
Green excite/emit peaks at: 487/
Red excite/emit peaks at: 587/61
- Cool white for standard imaging
- Lateral resolution: $\sim 8 \mu\text{m}$
- Magnification: 1:1
- Field of View: $\sim 3 \times 4 \text{ mm}$
- Depth of Focus: $\sim 150 \mu\text{m}$



Fluorescence Imager



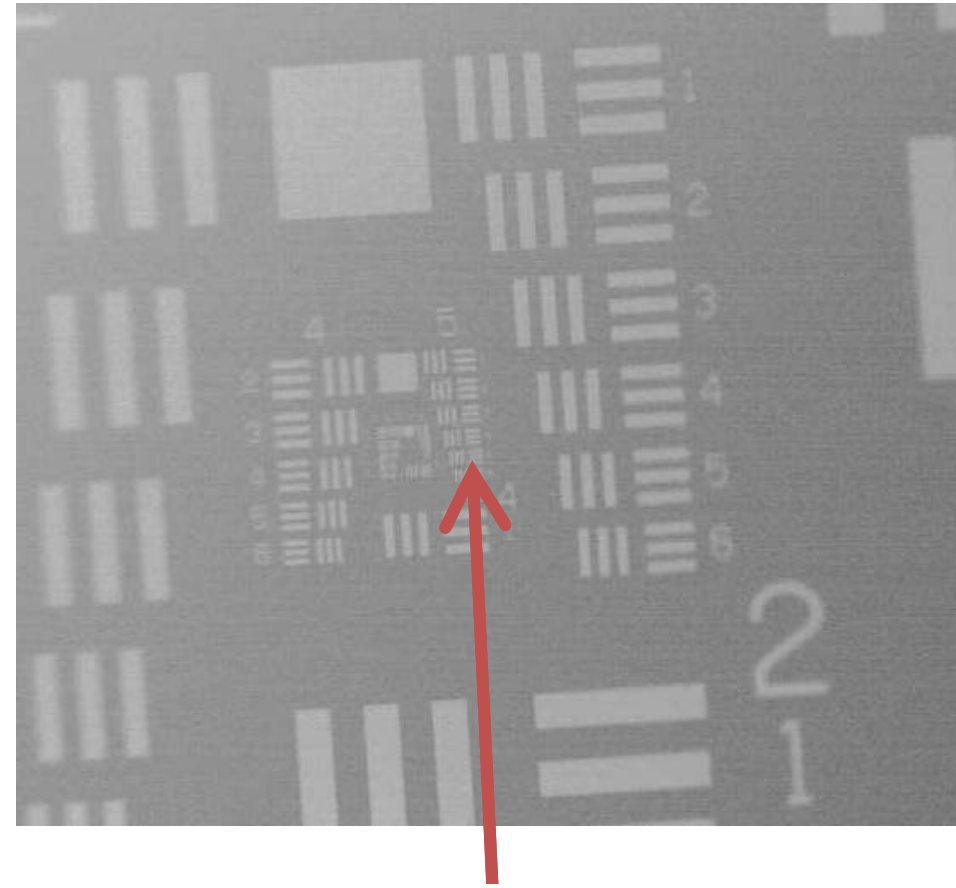
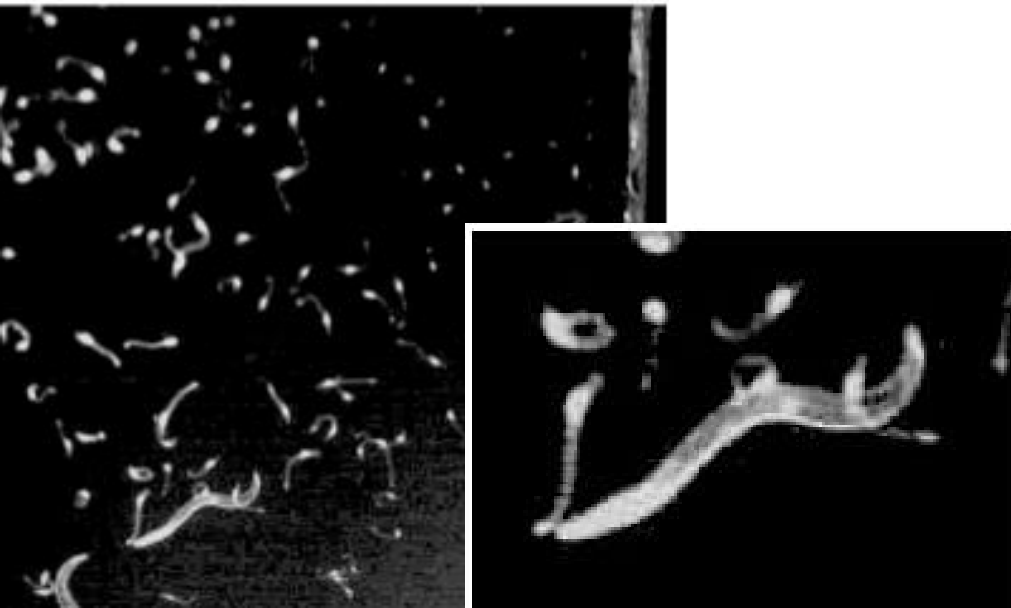
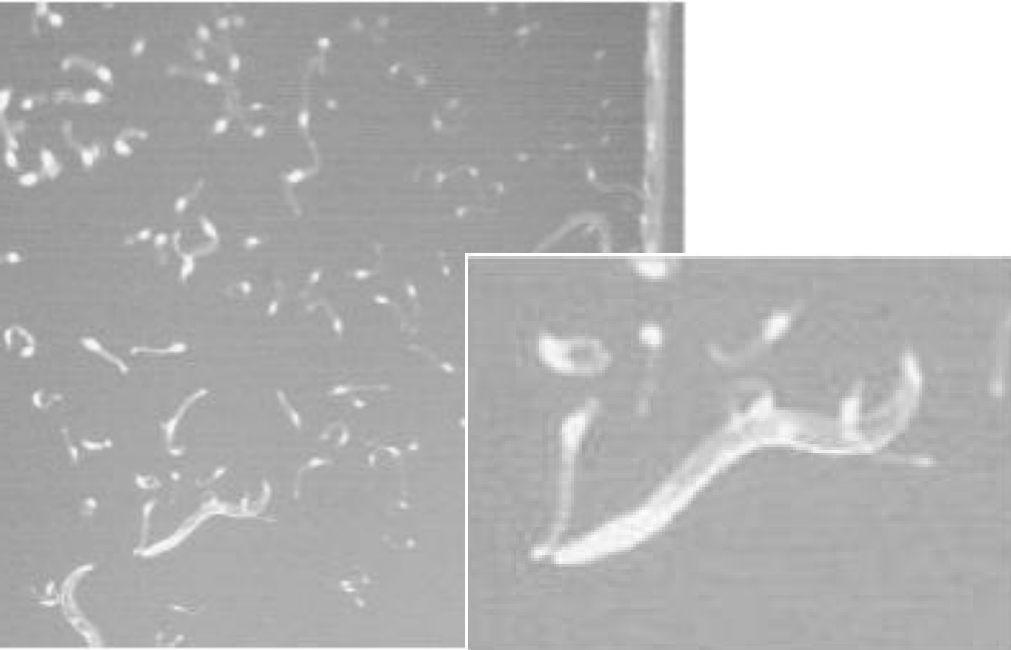
**Imager
Assy.**



- Fluidic well depth: **0.17 mm**
- Fine pitch thread of S-mount (**0.5mm/thread**)
- Camera adjustment **±5 mm**
- Locknut/set screw

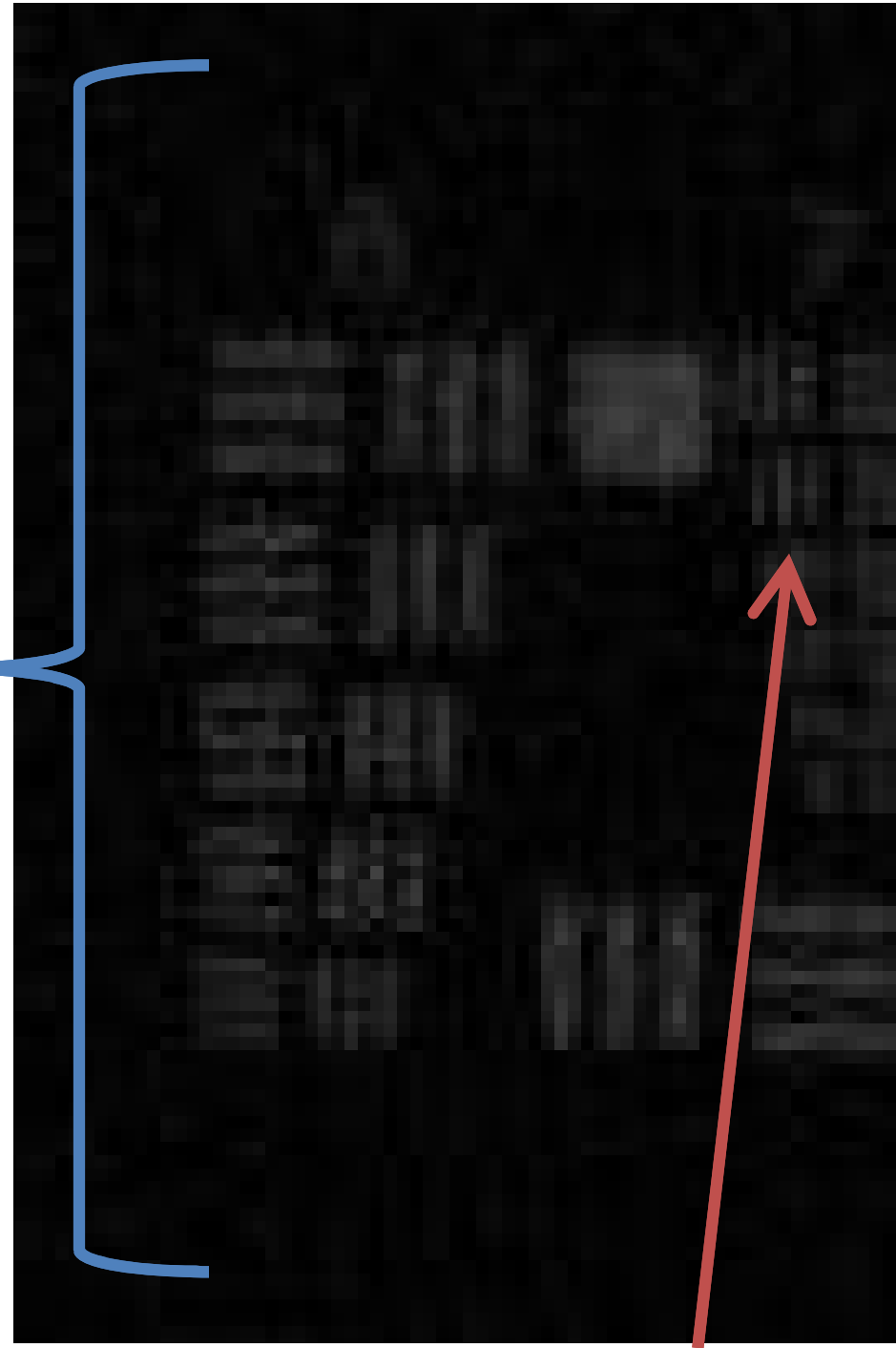
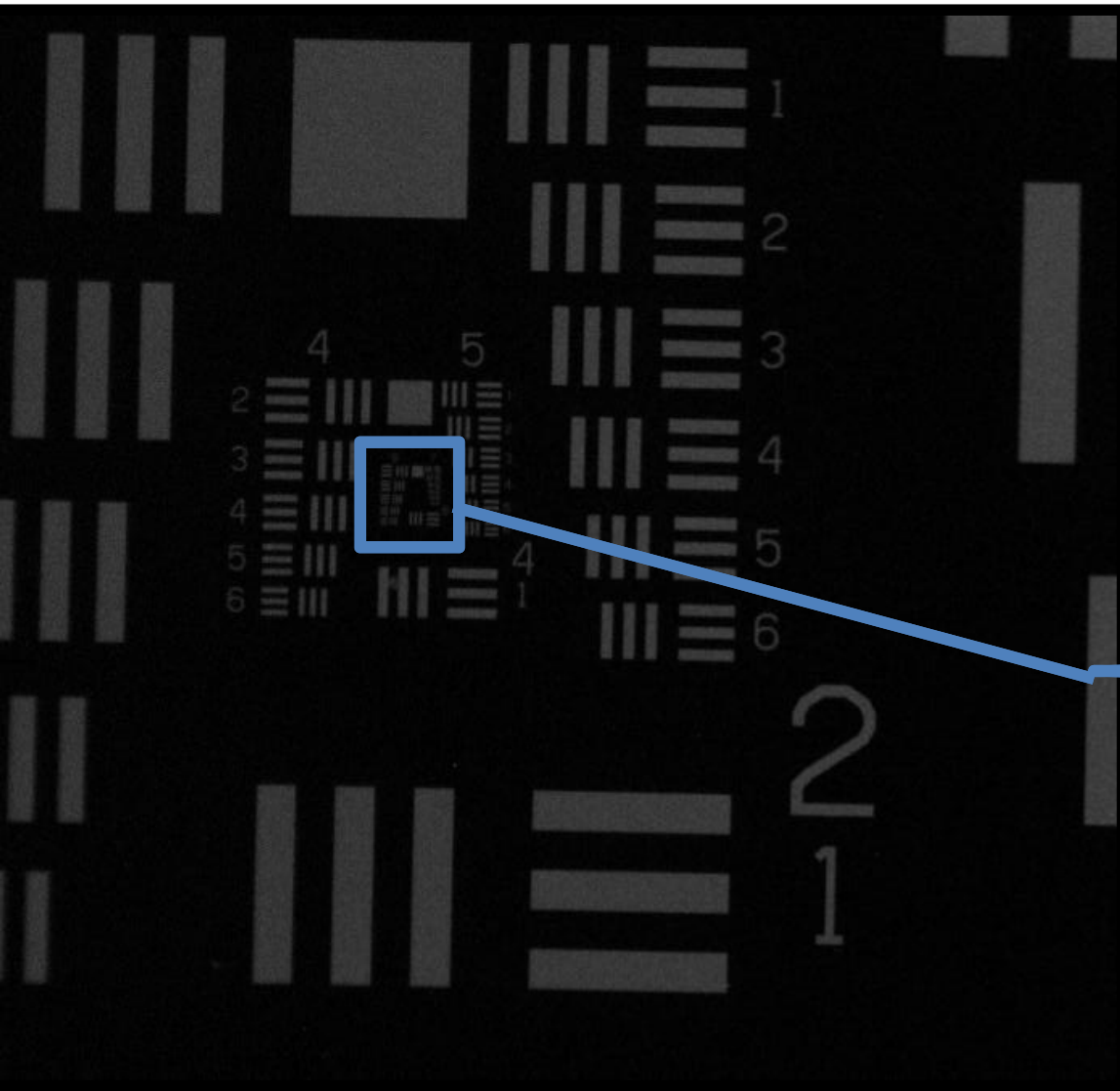
Camera chip

Fluorescently labeled *C. elegans*



Resolves $\sim 8 \mu\text{m}$ feature size

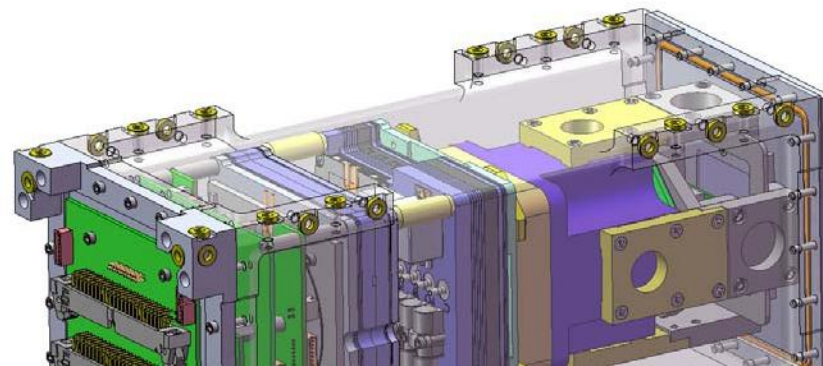
CCD imaging chip



Camera options are compatible with
and lens system

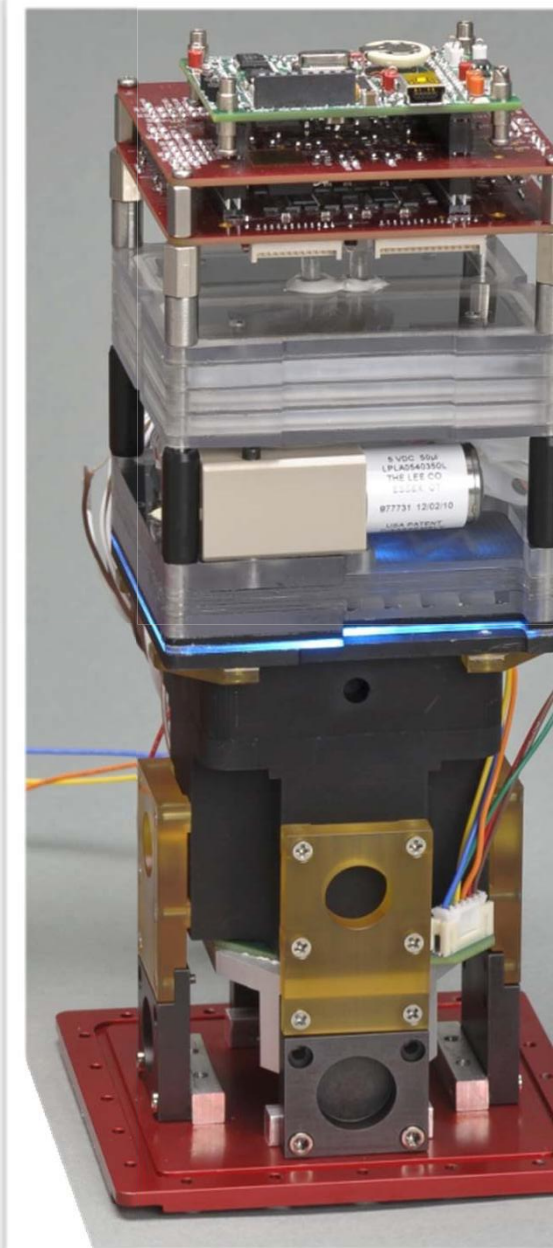
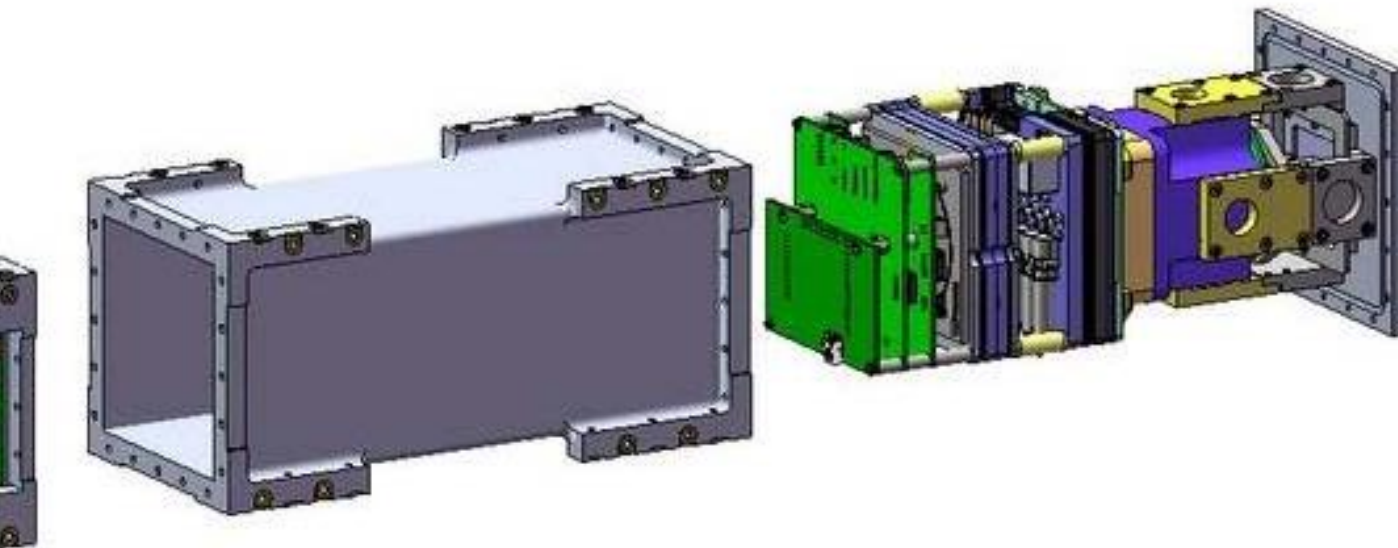
Payload Functional Overview

Mass	~1.5 – 2kg
Sensors	Pressure : accurate to within 2% of 1 atm. Humidity: accurate to within 4% from true value after temp. correction Temperature accurate to within +/- 0.5°C.
Processor	PIC 32
Temperature Control	Kept at 4°C to 30°C during the experiment phase.
Environmental Chamber	Maintains an atmospheric pressure in the pressurized payload chamber volume within 11.7 to 15.7 psia.
Material Selection	Material selections have heritage to past missions for ability to be sterilized and low outgassing.



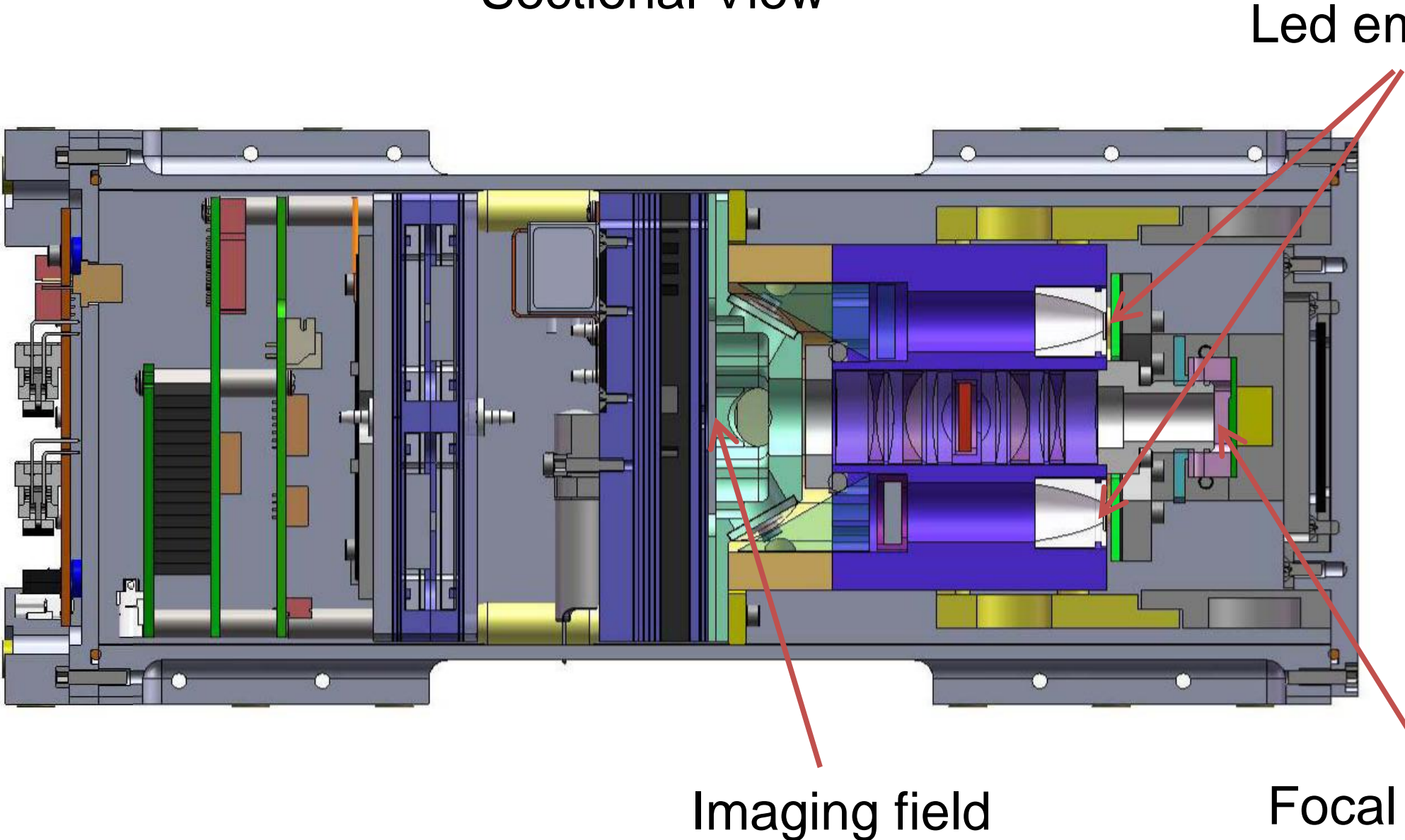
Payload Design Overview

Integrated Payload - Exploded View -

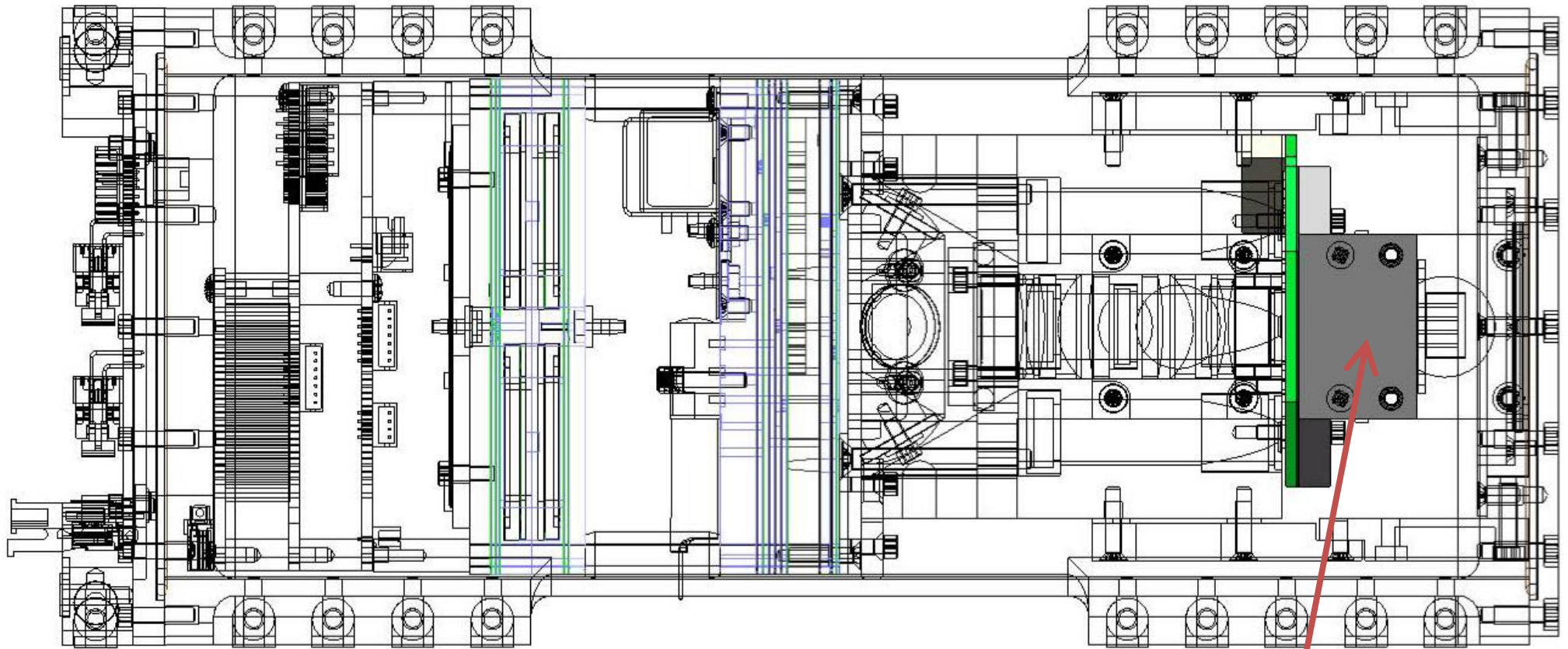


Integrated Payload

- Sectional View -

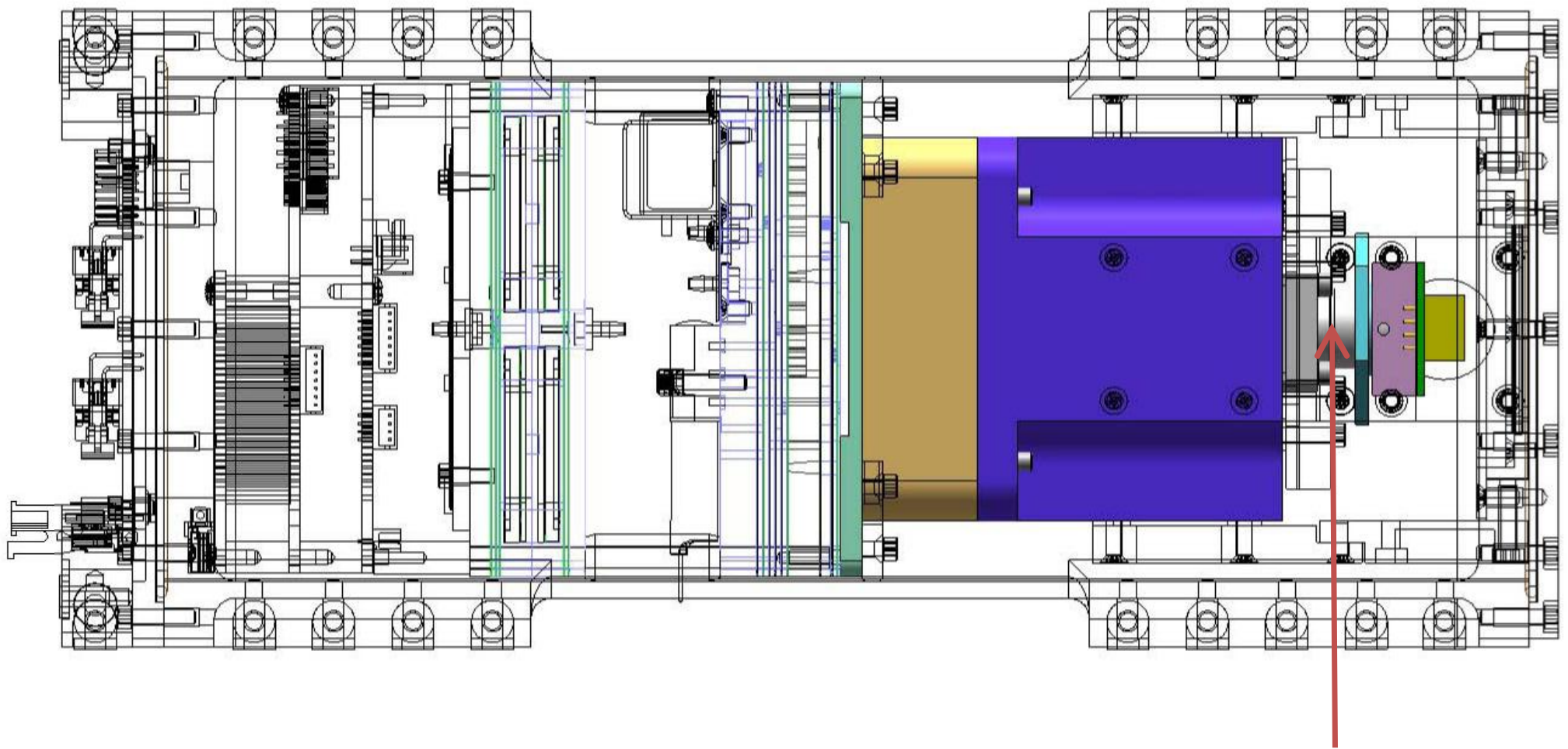


LED Assembly



LED heat sink

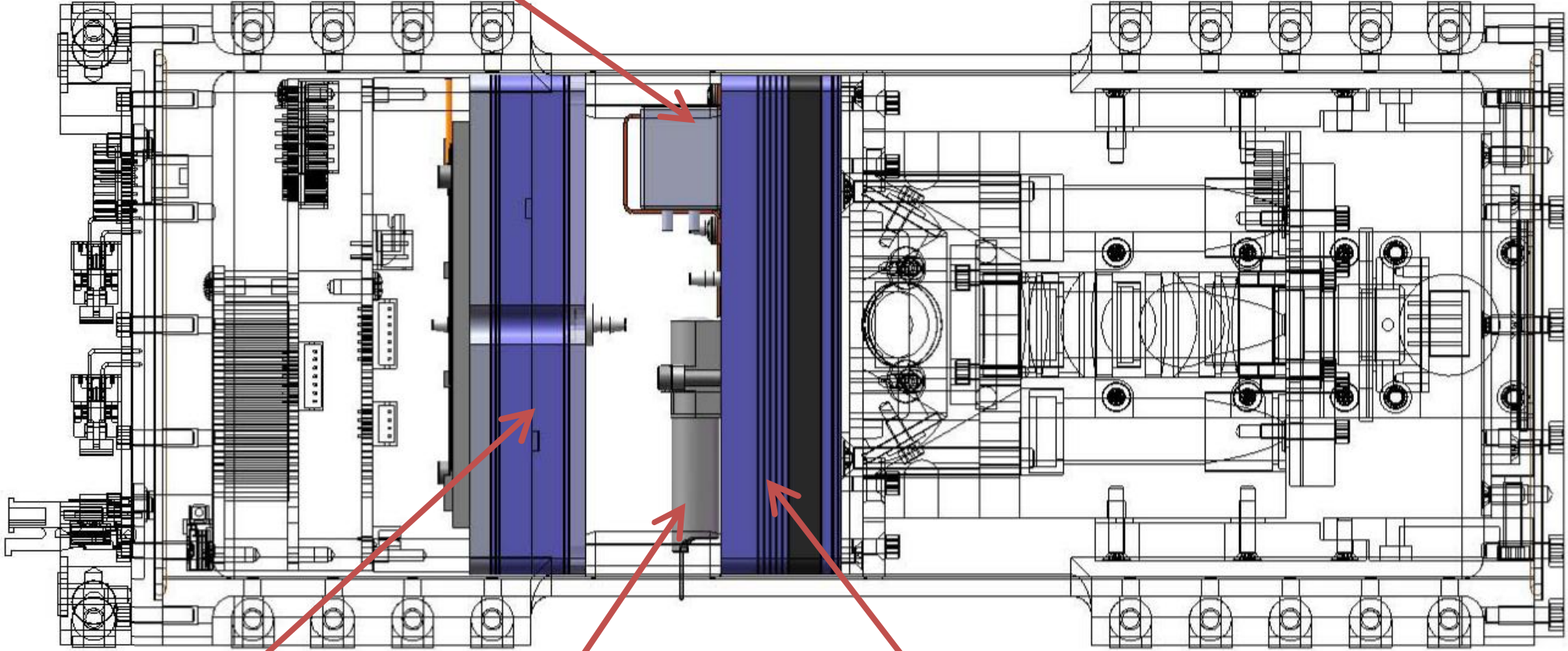
Camera Assembly



Camera Adapter/Focus Adjuster

Fluidics Assembly

Dolomite
Pump

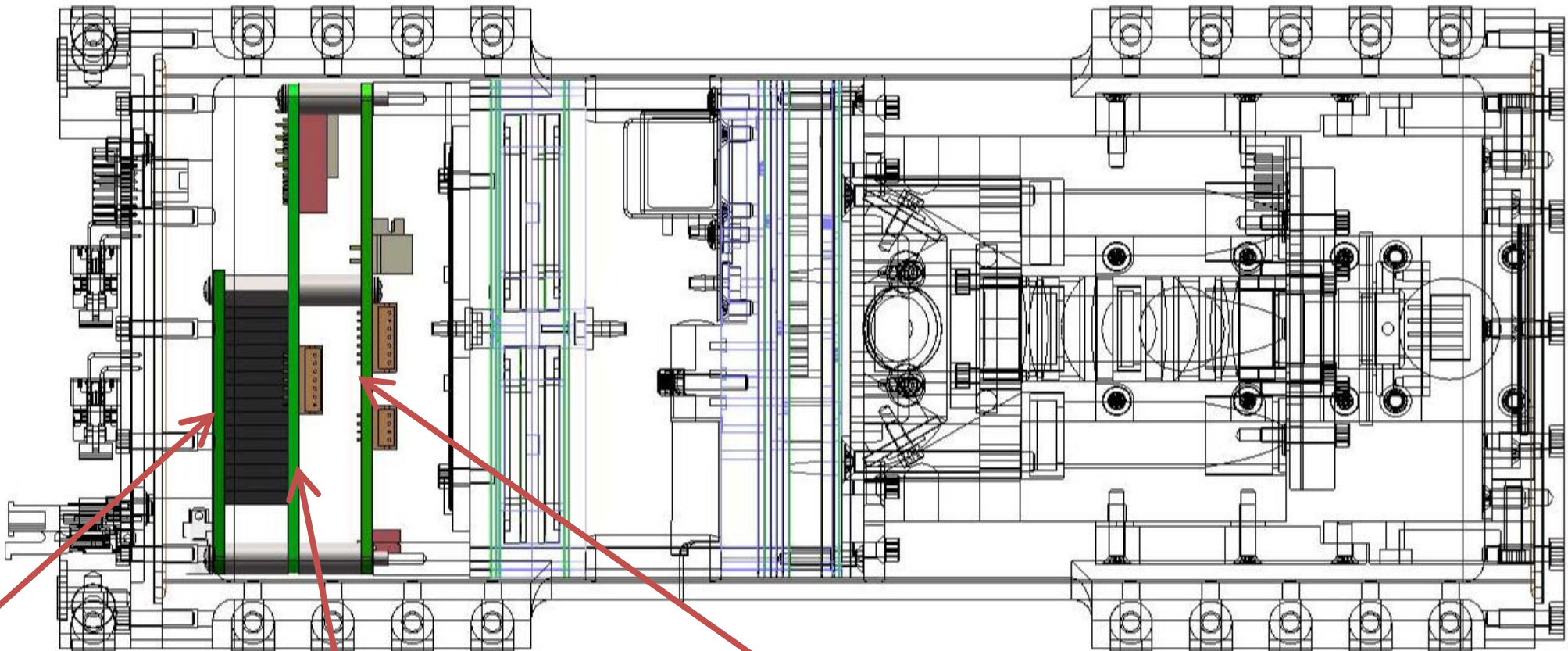


Reservoir

Lee Co. Surface

Fluidics Block

Electronics Assembly



• Sensor PCB

• Analog PCB

• Digital PCB

- LED
- Thermal

- Camera

• V...

Conclusion

Technology built:

- integrated fluidics system that maintains *C.elegans* viability and supports growth
- fixed-focus imager with fluorescence and scattered-light imaging capabilities

Biocompatibility testing complete

Waiting to partner with a principle investigator and launch

Acknowledgements

edics: Ming Tan, Matthew Piccini

ogy: Matthew Lera, Macarena Parra

ging: Linda Timucin

chanical: Abraham Rademacher, Giovanni Minelli, Chris Beasley

trical: Aaron Schooley

agement: Andres Martinez

anology: Antonio Ricco

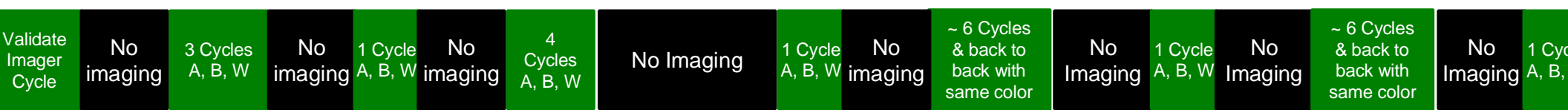
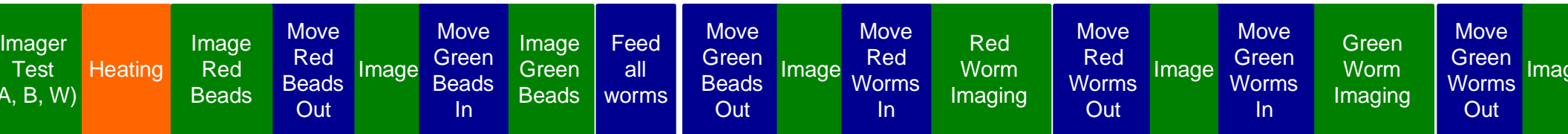
ding: NASA/Exploration Systems Mission Directorate

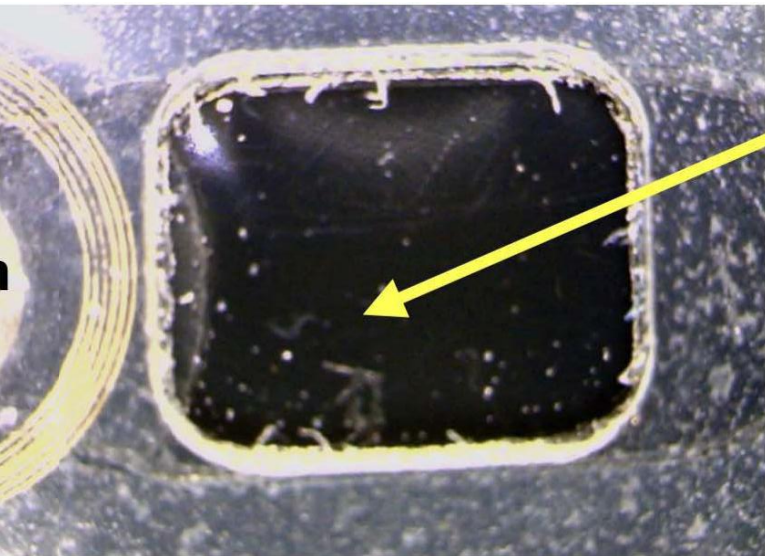


Thank You

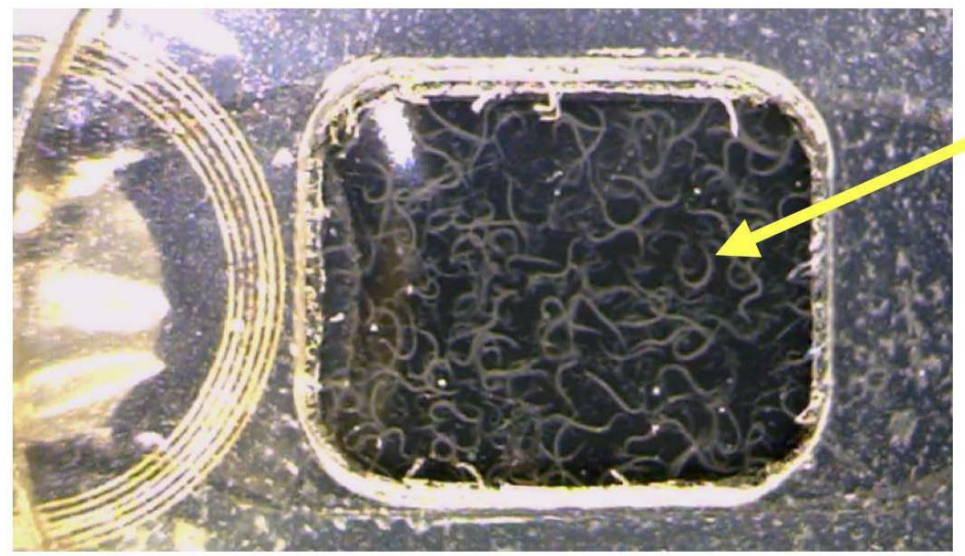
Questions?

ConOps

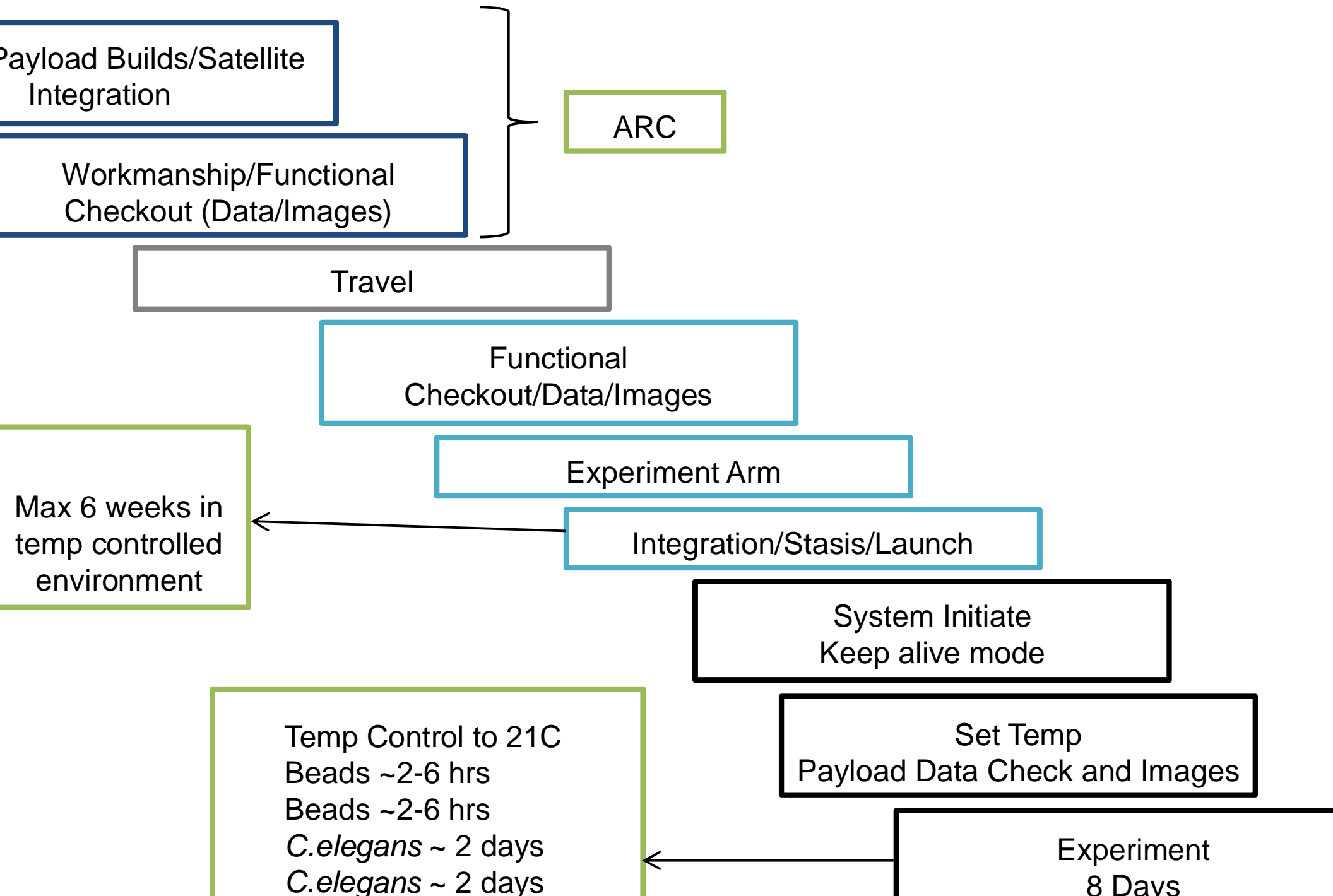




**After
pumping
worms
into
imaging
chamber**



Payload ConOps



contact with *C. elegans*

b. (machined)

b. porous membrane

b. urethane sensitive adhesive (PSA) (cut edges only)

b. acrylic adhesive, polyester carrier

b. laser-cut edges

b. polyethylene gas-permeable cover film

contact with growth medium only (reservoir/tubing)

b. ethylene vinylacetate) [EVA] bag

b. polyethylene barbed ports

b. polyethylene tubing

contact with *C. elegans* and growth medium

b. wetted valve materials

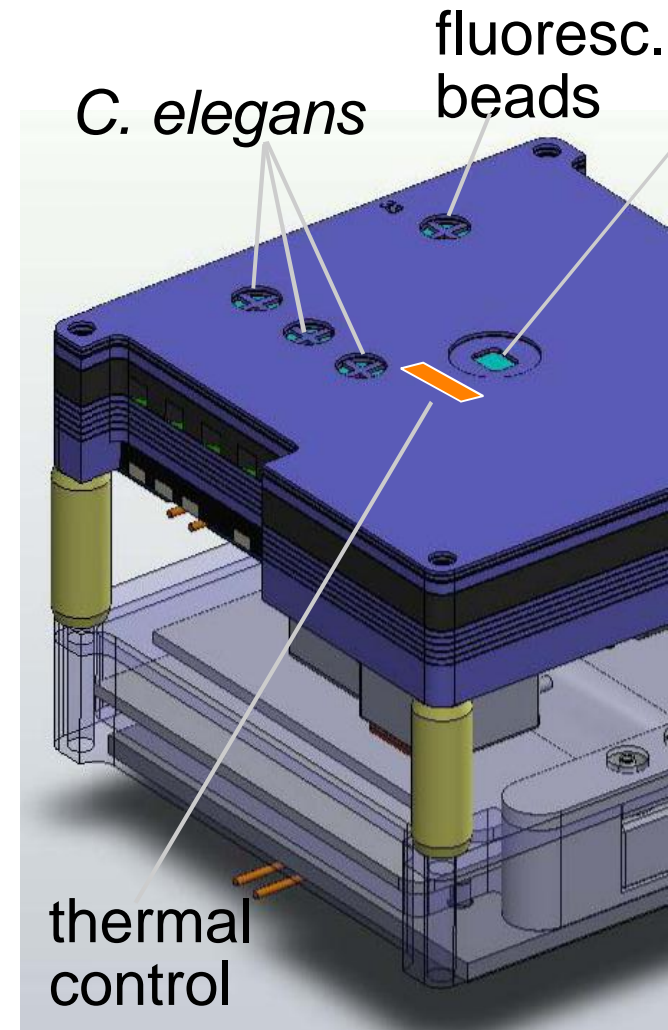
b. PPS = Polyphenylene sulfide (“Fortron”)

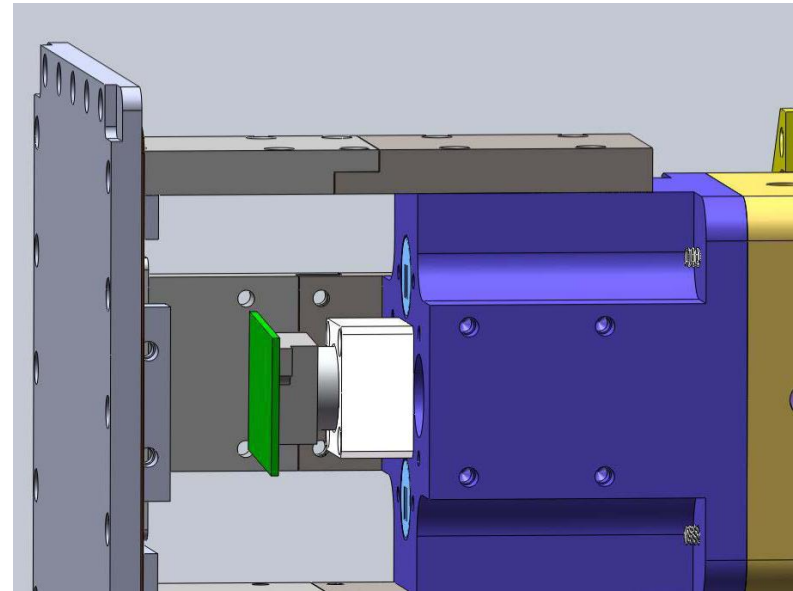
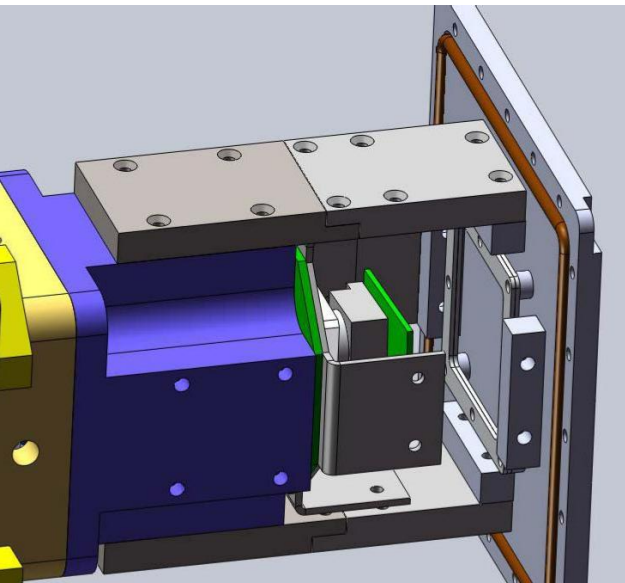
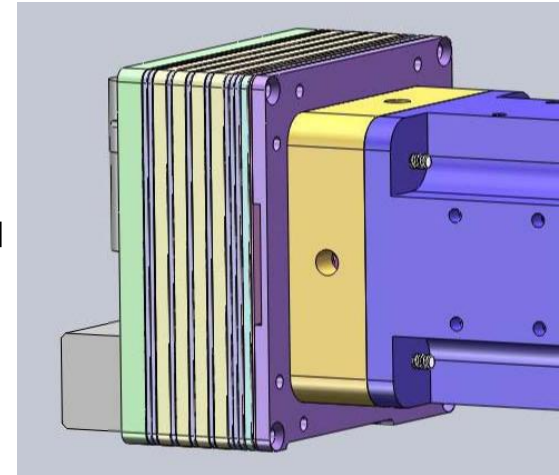
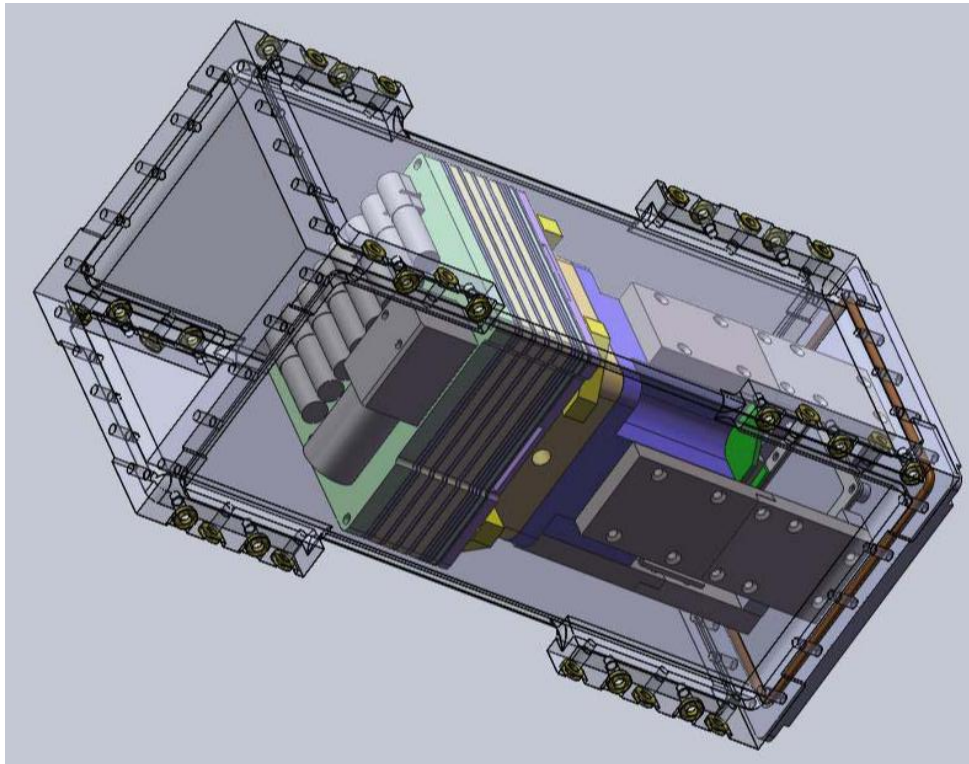
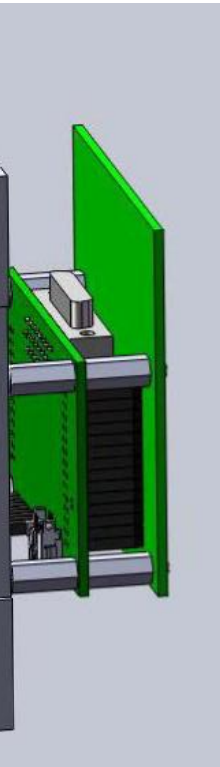
b. PBT = Polybutylene terephthalate (“Valox”)

b. 316 SS

b. FeCr Alloy

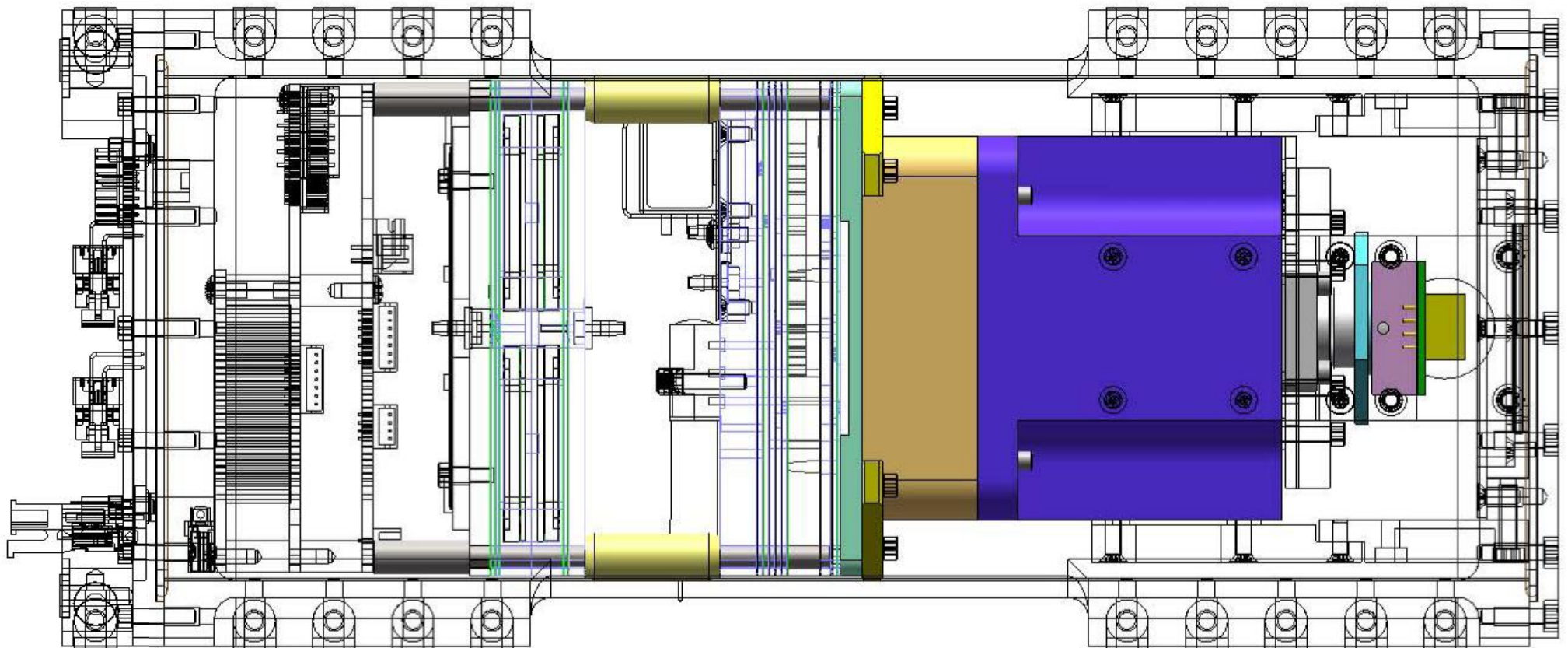
b. Silicone Rubber





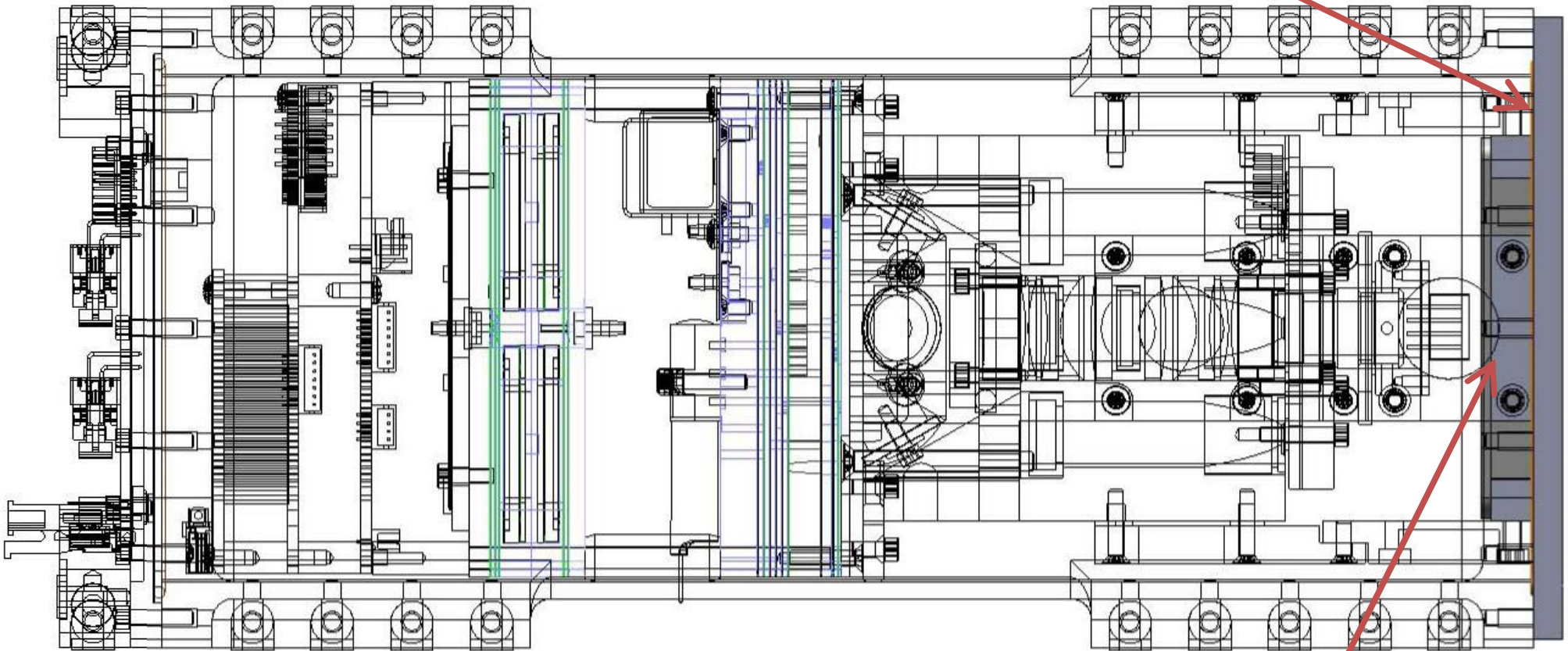
Fluidics Stack Pins

- Detail -



Back-Cover Assembly

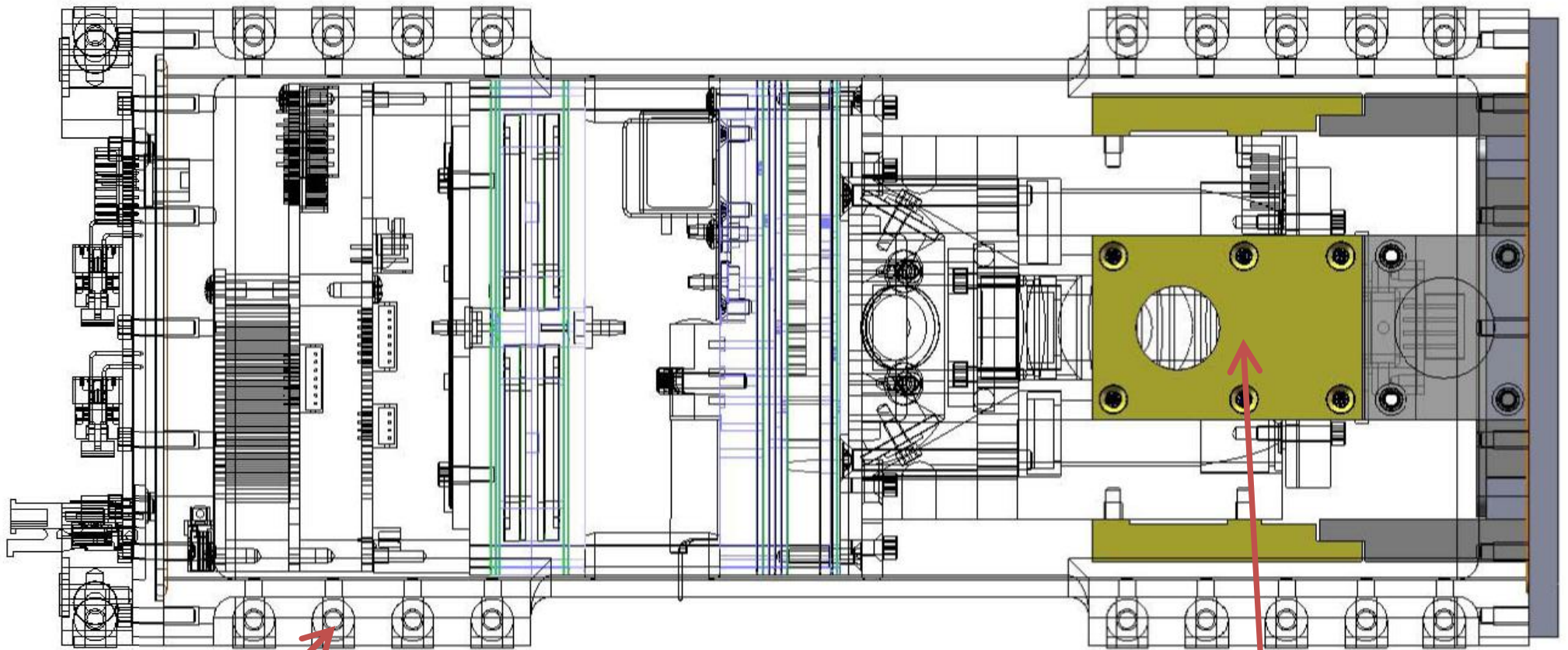
Viton O-Ring



Activated Carbon

Camera Mount

- Mated to Back Panel -

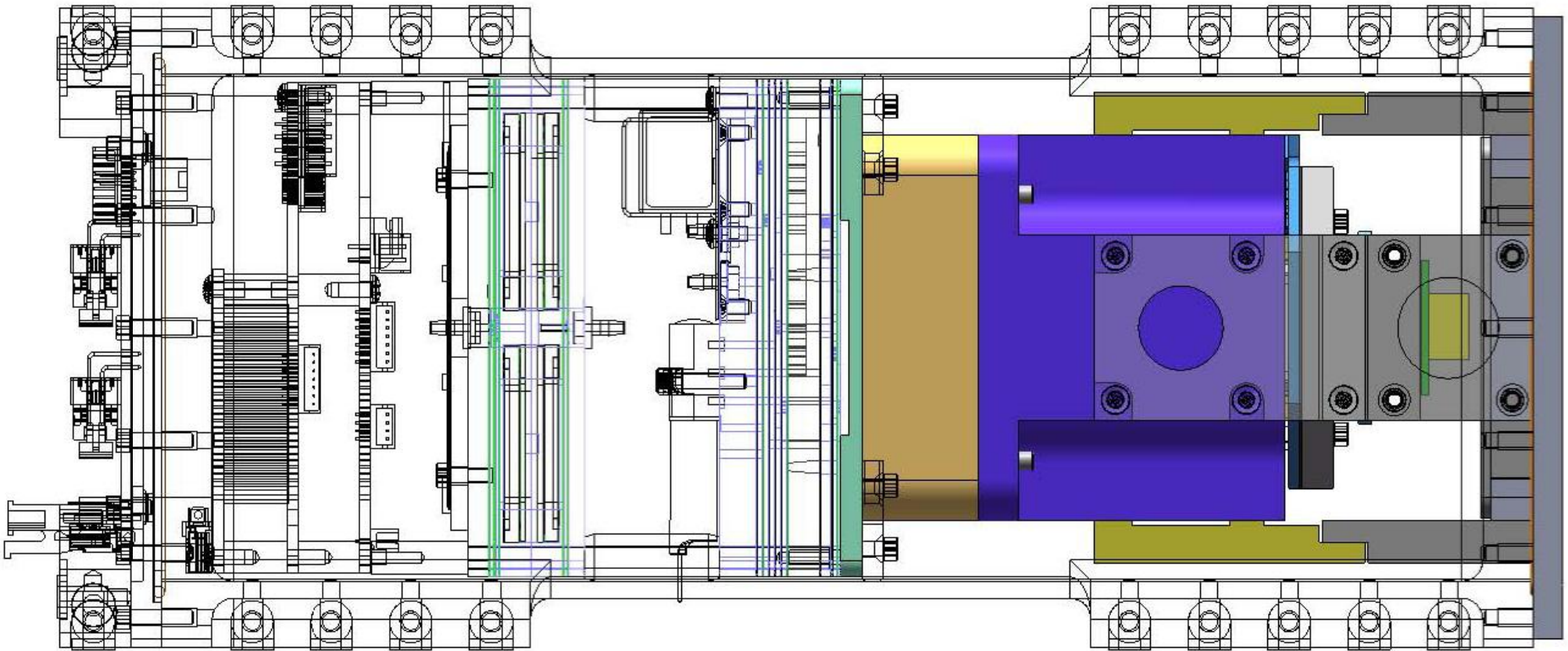


Ultem Washers

Ultem Mount

Camera Assembly

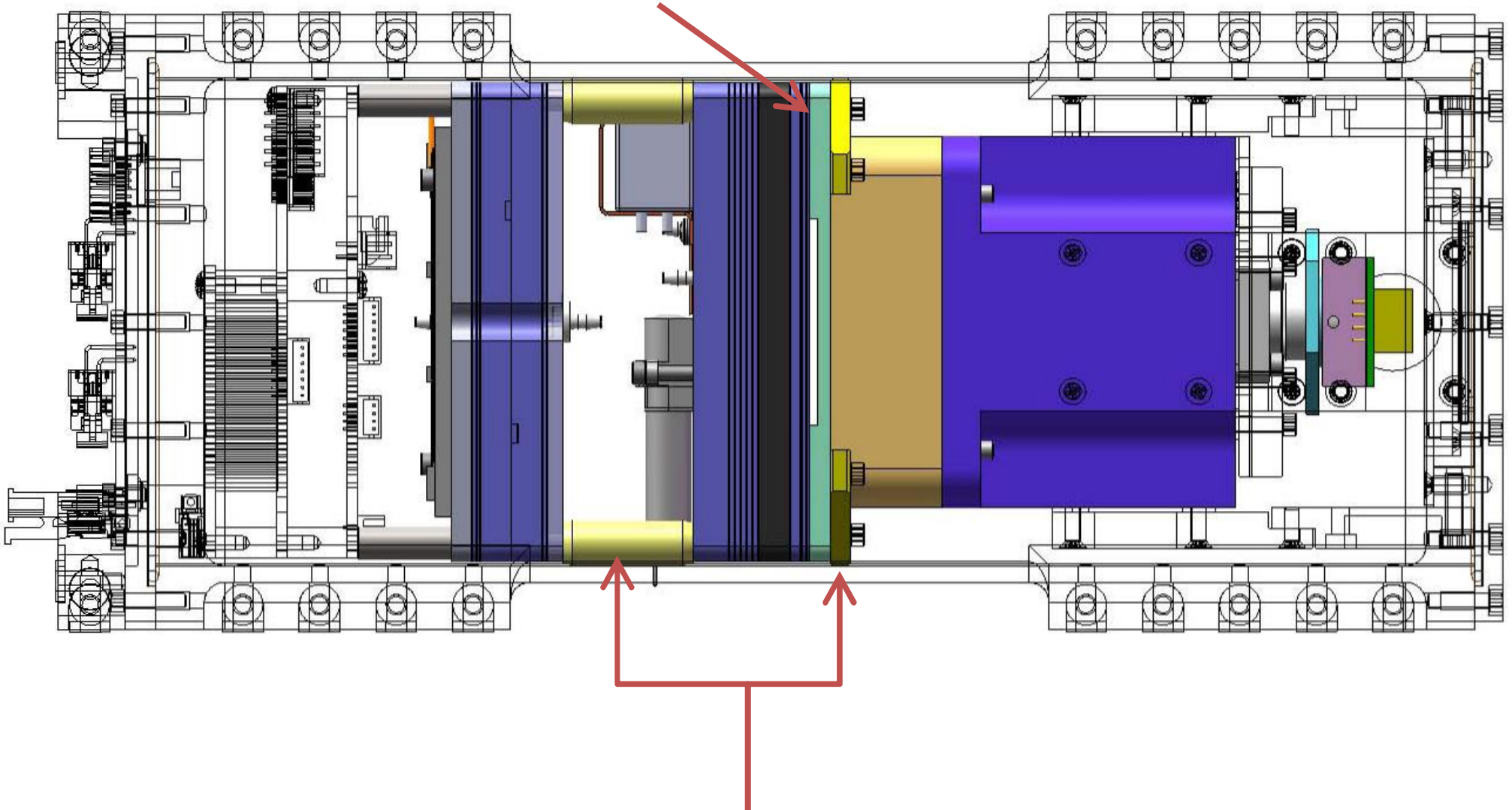
- Mated to Camera Mount -



Fluidics Assembly

- Mated to Camera Assembly -

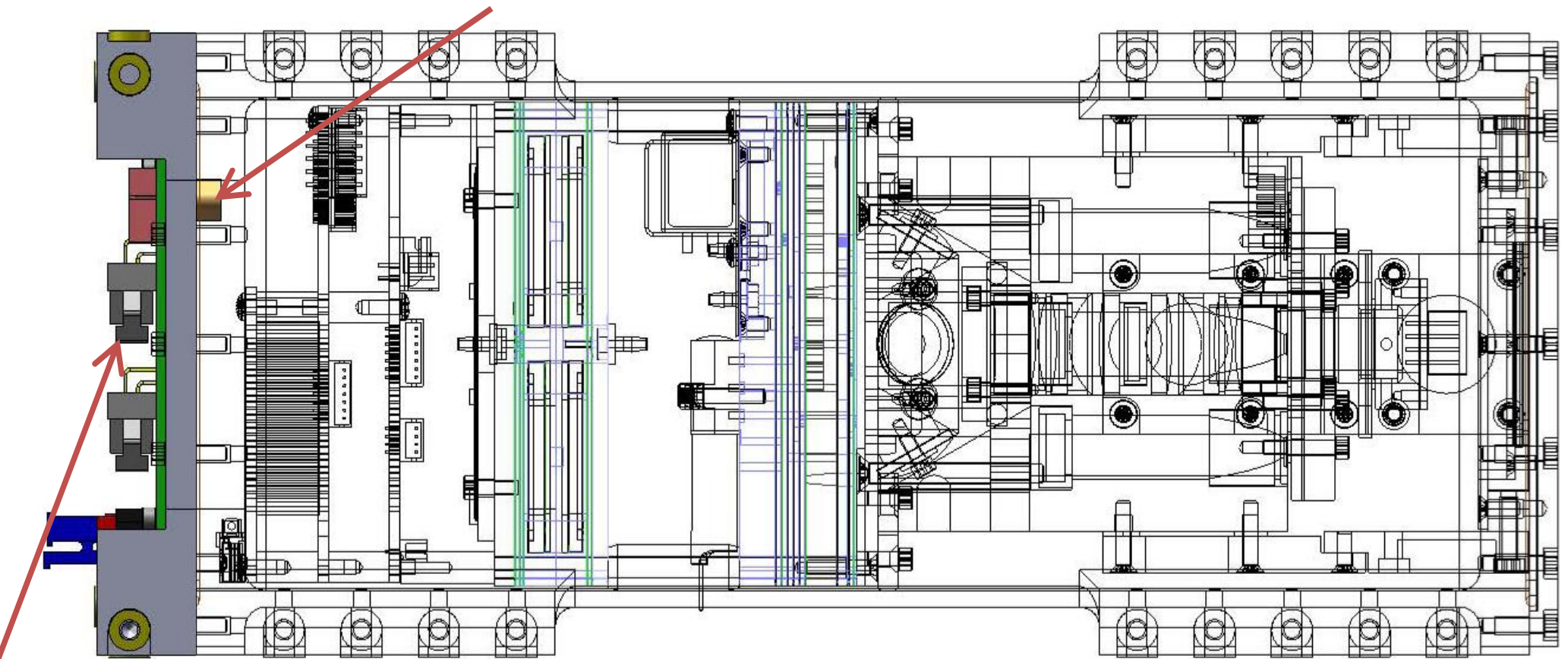
PSA Layer



Front Panel Assembly

Hermetic Connector

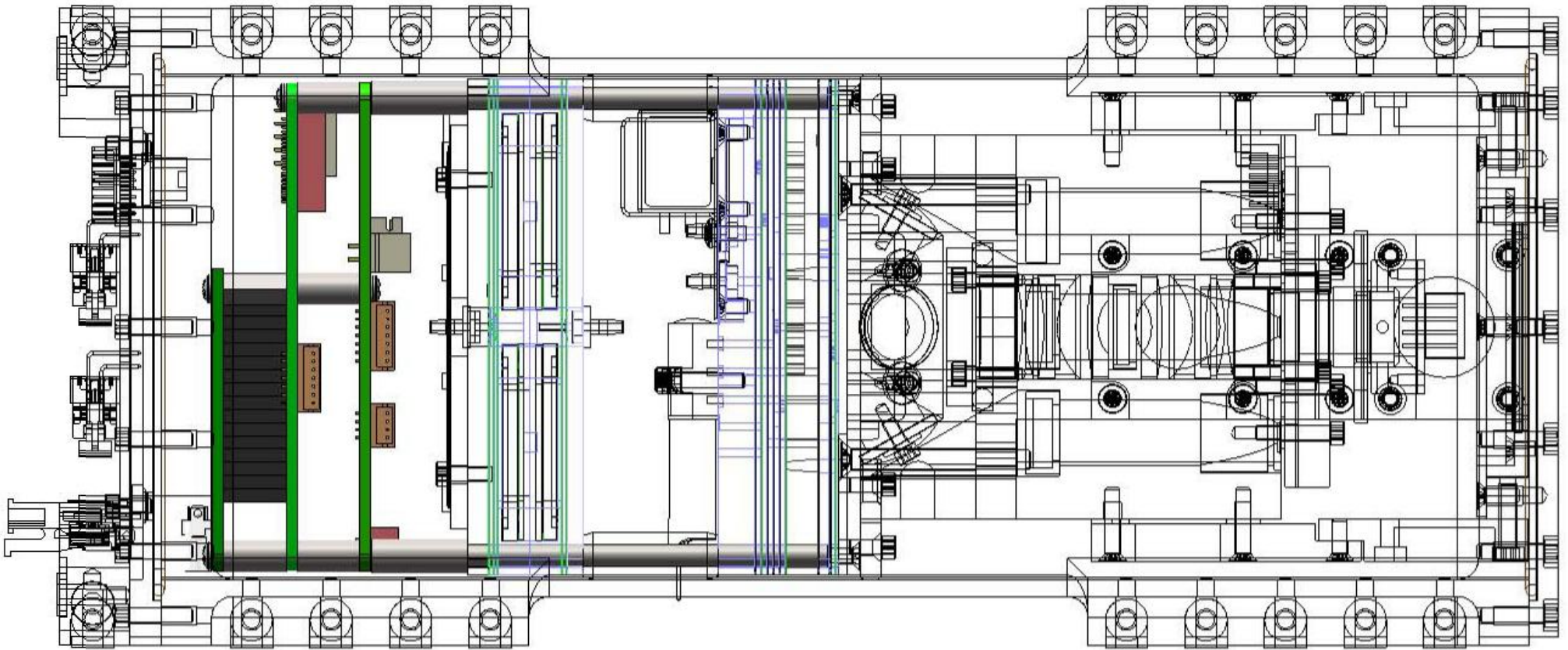
- Laser Welded



er Interface Board

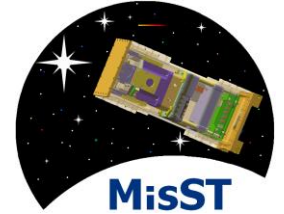
Electronics Assembly

- Mated to Fluidics Stack Pins -



Payload Electrical Overview

	Description
0 Imager Interface Board	Translates the bus 50pin cable to the hermetic connector on the payload can. Also has connection for the remove before flight “kill switch”
0 Imager Payload Analog	Contains all analog payload circuits. Temperature sensor, pressure sensor, LED current drivers, RH sensor, pressure sensor circuitry, Heater circuitry
2 Imager Payload Digital	Contains all digital and inductive load circuits. Valve driver bridges, motor switches, memory chips, camera interface
0 Imager LED	Contains the 4 high power imager illumination led’s. All are mounted to a heat sink and devices are properly thermally sunk for continuous use.
0 Imager Processor	Contains the payload microprocessor and associated components. uC LDO, RTC, FRAM, ADC reference

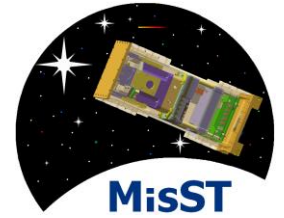


Development of two-color fluorescent imager and integrated fluidic system for nanosatellite biology applications

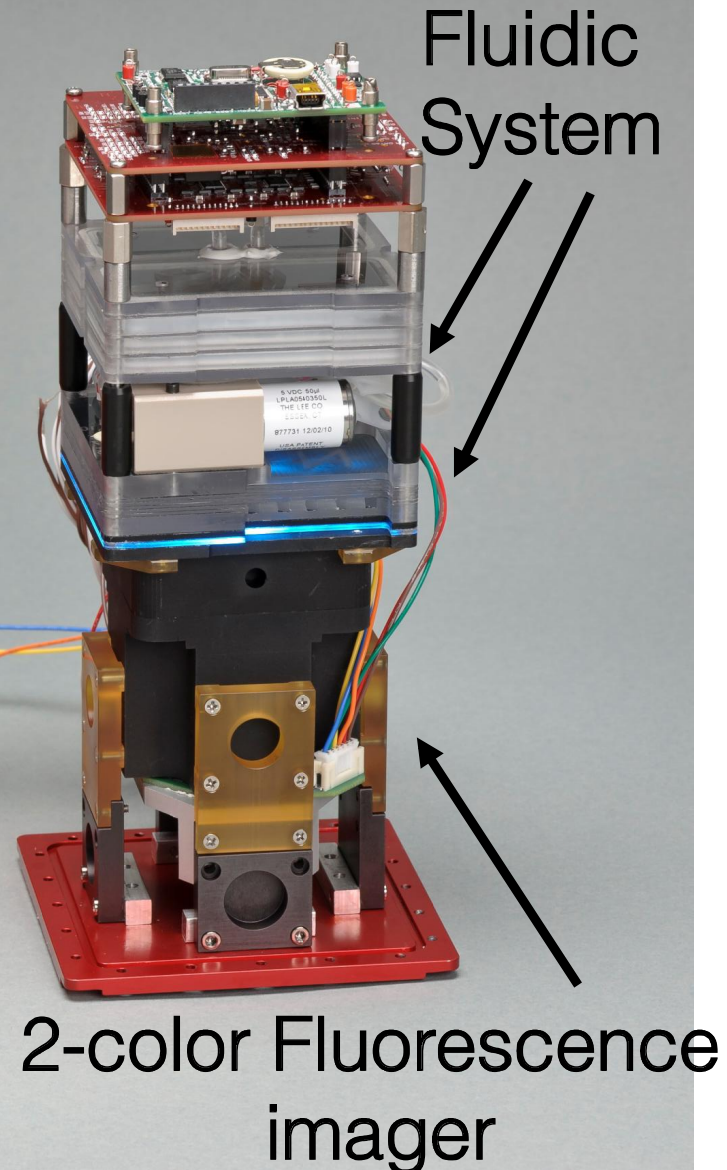
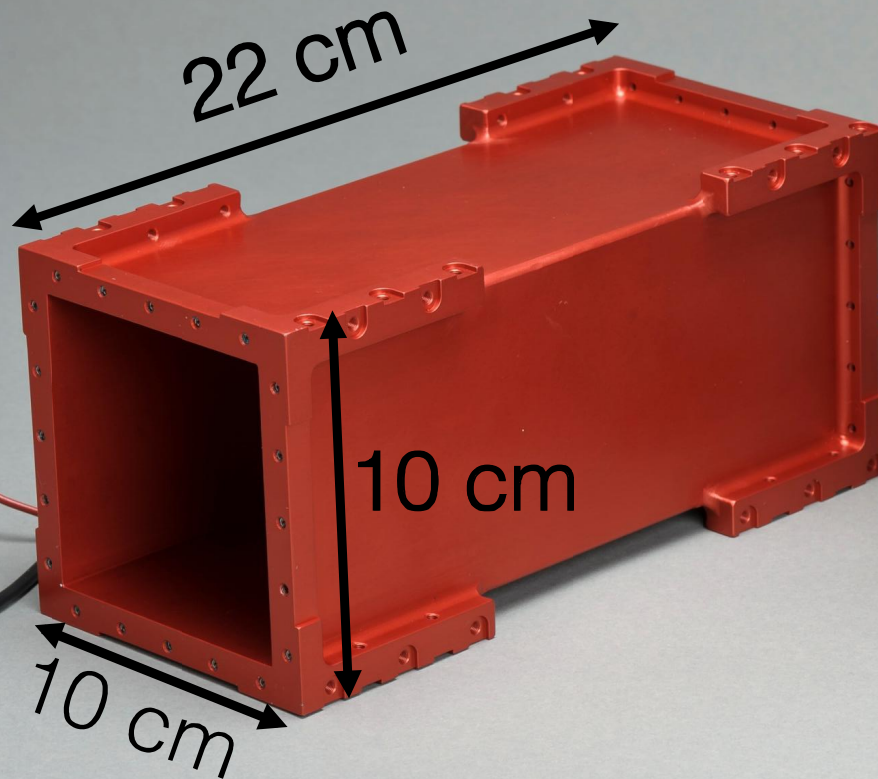
Diana Wu *et al*
NASA Ames Research Center
ASGSR
December 1, 2012



Microsatellite *in-situ* Space Technologies (MisST)

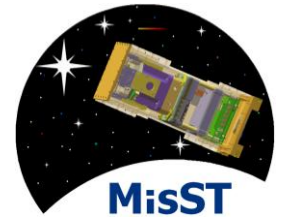


NanoSatellite
Science Payload





Payload Science & Technology Overview

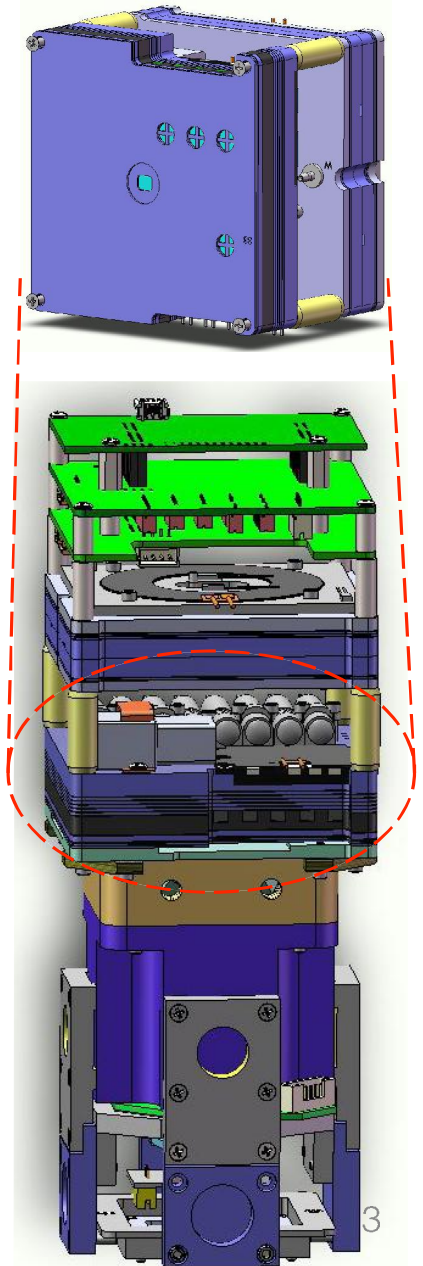


Objective: Technology demonstration of 2-color fluorescent imager for space biology applications

Target: model organisms, resolve major features/organs, gene of interest & control

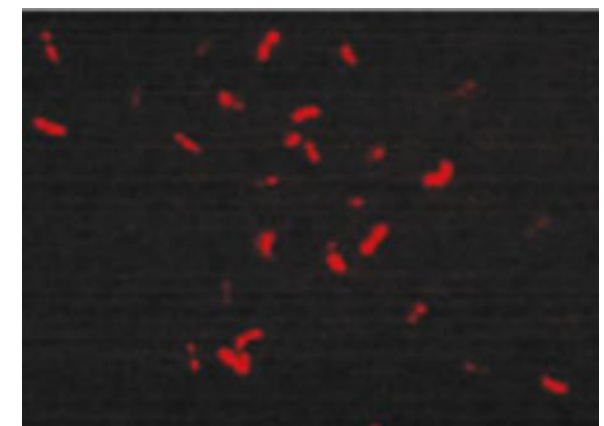
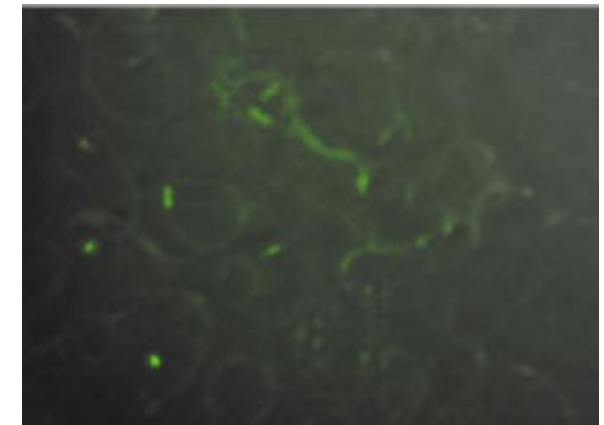
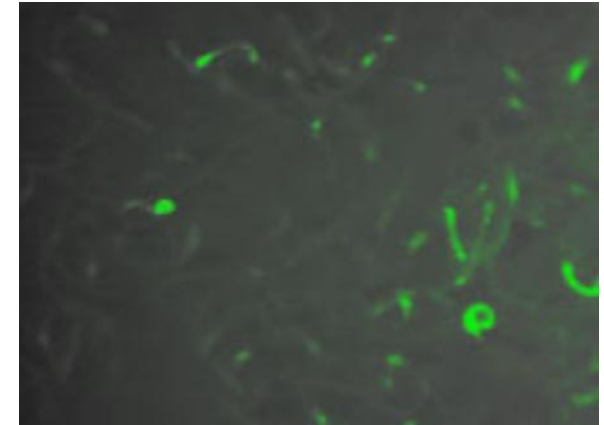
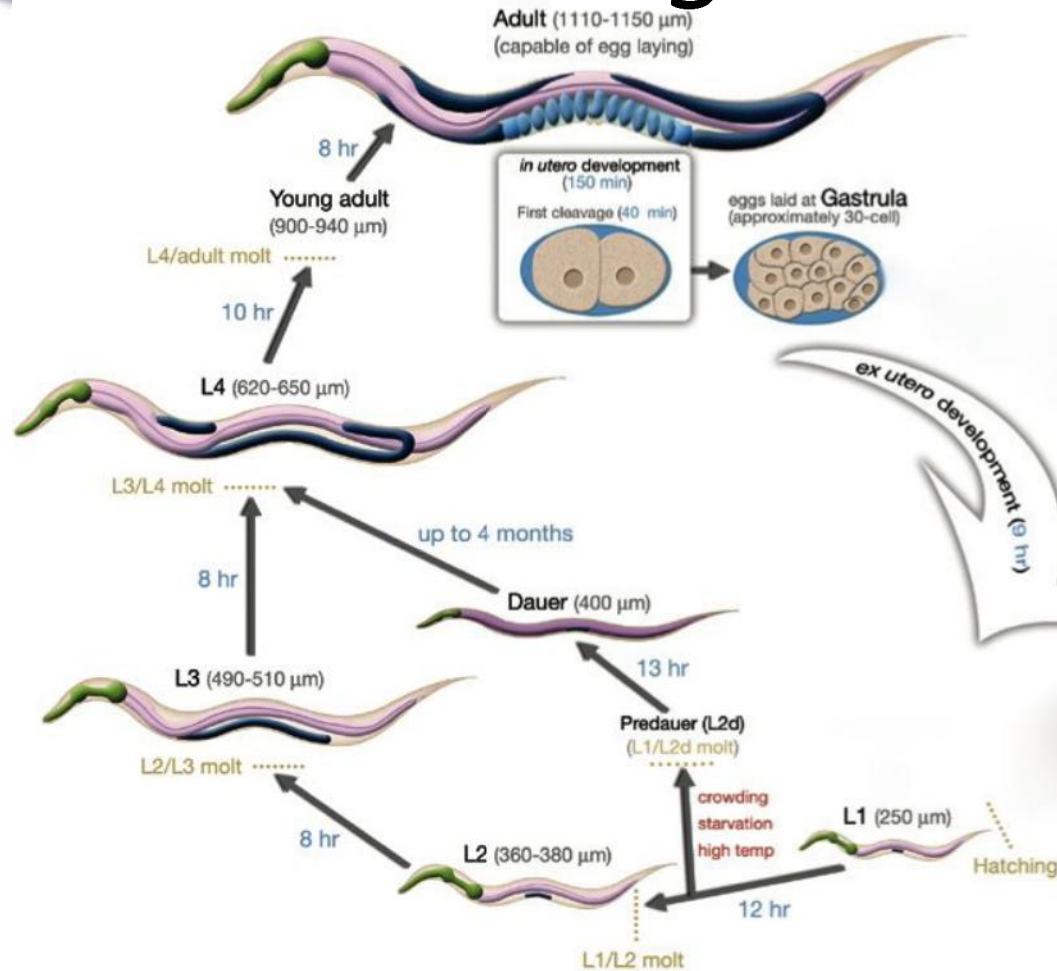
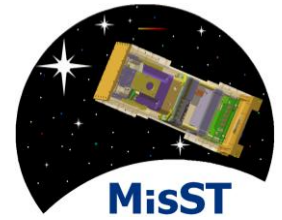
Implementation: 2-cube integrated culture/imaging instrument

- C. elegans model organism
- Fluidics: culture wells, imaging zone, reagent storage
- Imager: fixed focus, 300 kpixel CMOS chip
- Image storage: 2 GB/6,000 images/8 bits per color (telemetry of 1 - 20 images)
- Hermetic containment vessel





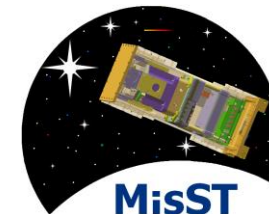
C. elegans Overview



- Easy feeding and short life cycle make it inexpensive to maintain and ideal for research
- Used to study spaceflight effects and experiments using *C. elegans* have flown numerous times including on STS-107.



C. elegans Imaging

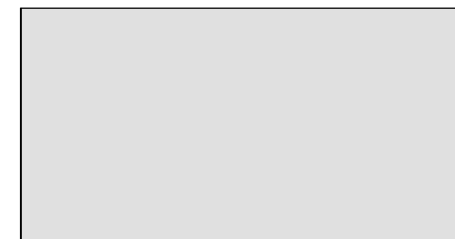
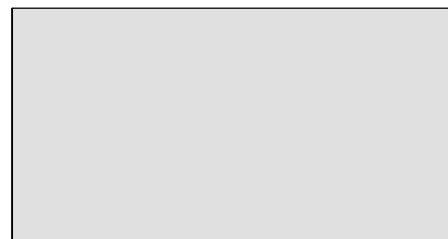
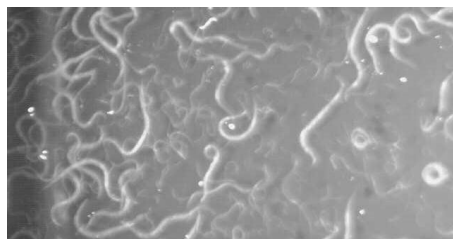


White

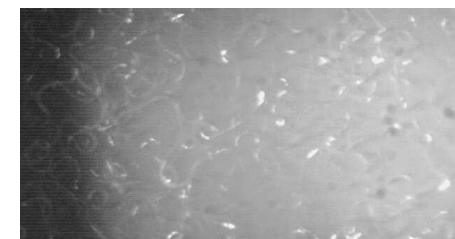
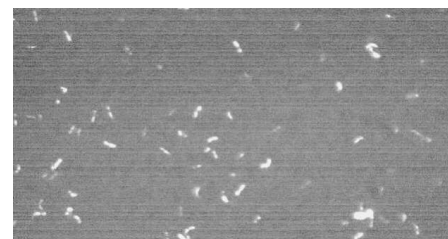
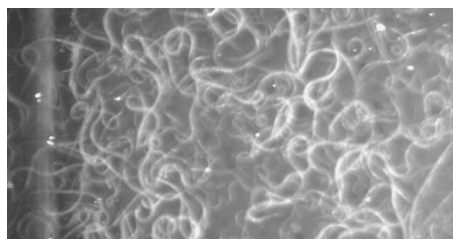
Amber

Blue

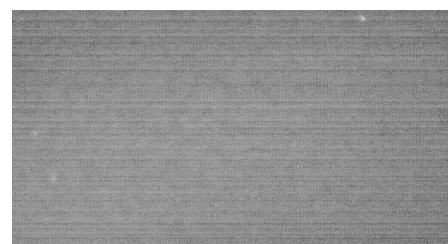
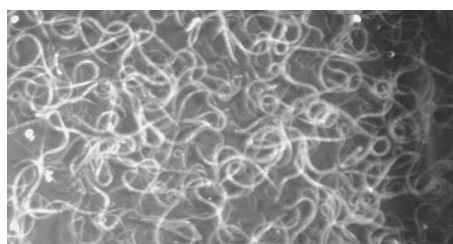
N2 (wt)
not fluorescent



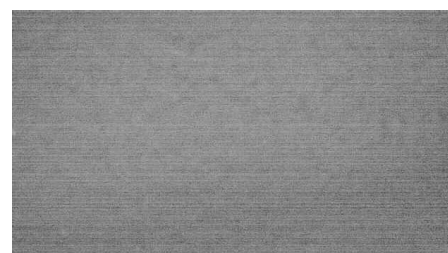
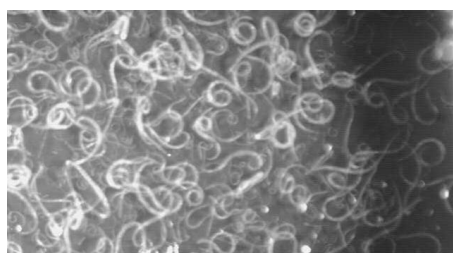
tdTomato
red – muscle cells



IM324
green – nerve cells

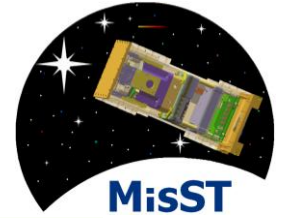


MR142
green – intestinal cells





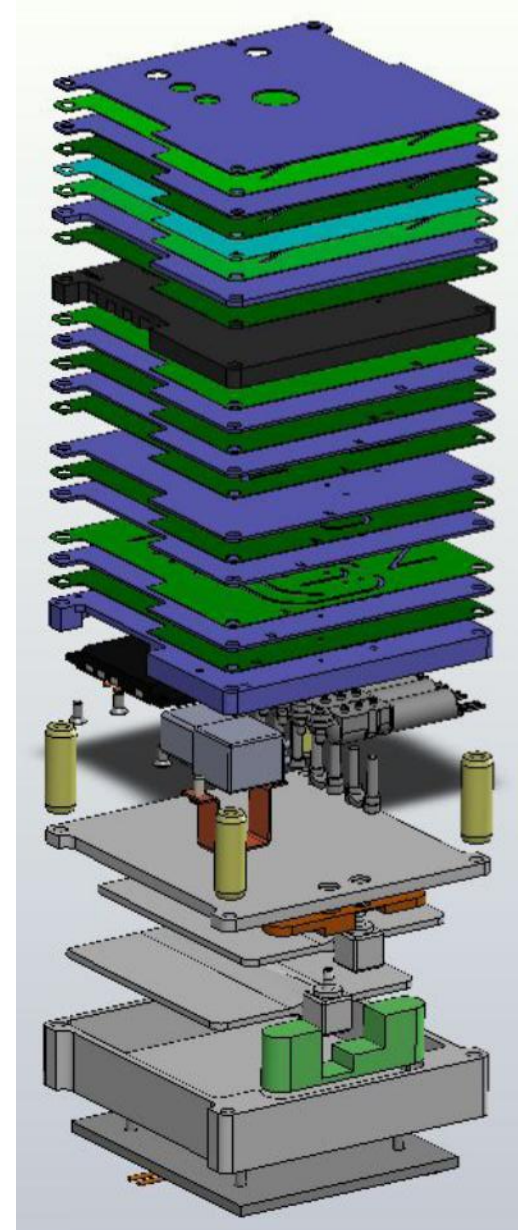
Fluidics Overview



Approach: Multilayer polymer microfluidic card

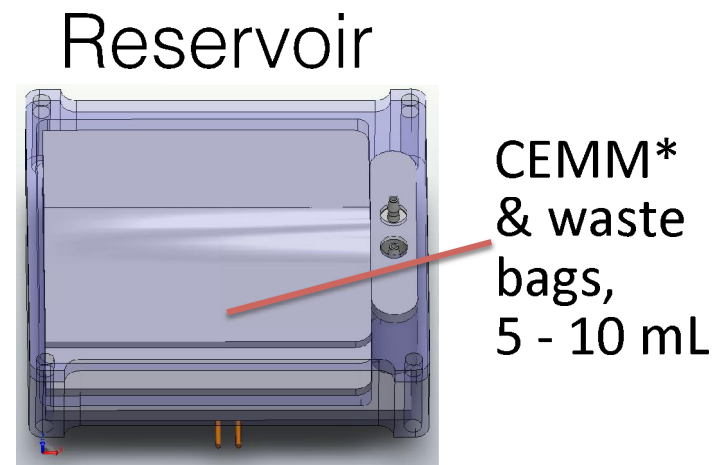
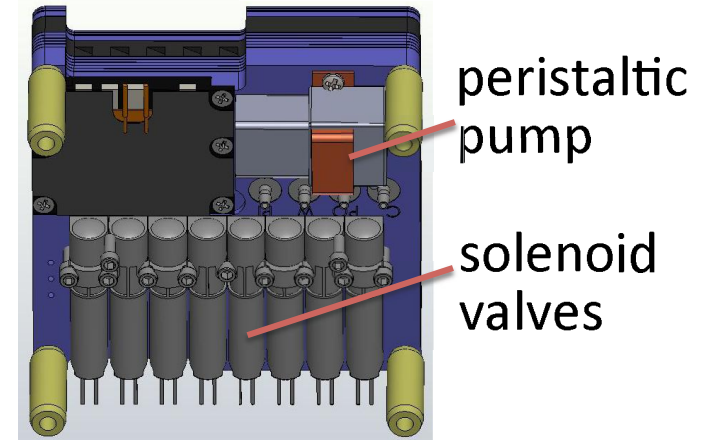
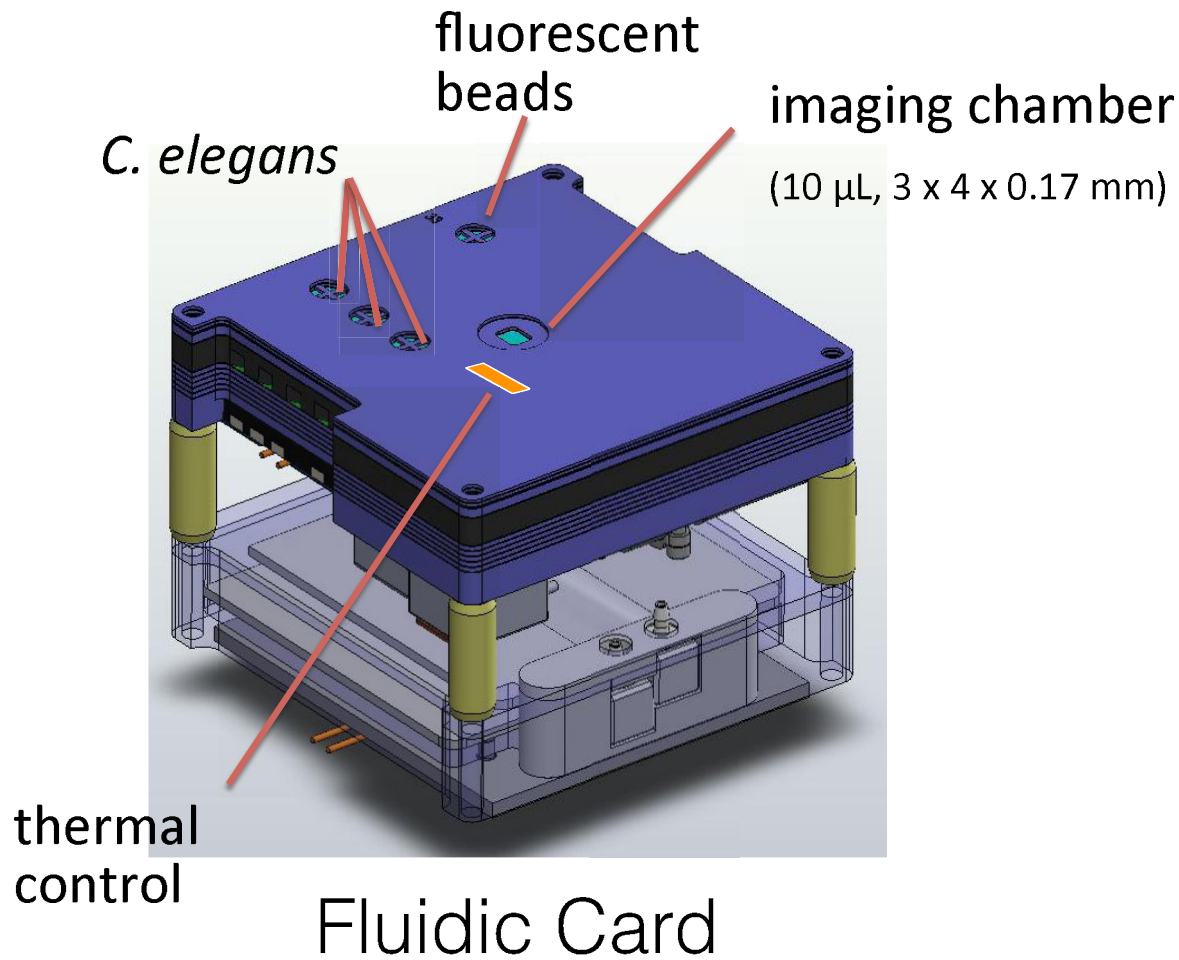
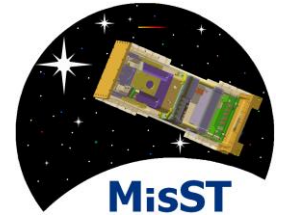
- Multi-layer lamination to form 3-D fluidic network
- Laser-cut fluidic channels in pressure-sensitive adhesive (PSA)
- Lamination of machined polycarbonate sheets with via holes, filters, polystyrene using PSA

Polymers, adhesives, sterilization, biocompatibility, & fab. all have GeneSat, PharmaSat, O/OREOS-SESLO, EcAMSat heritage





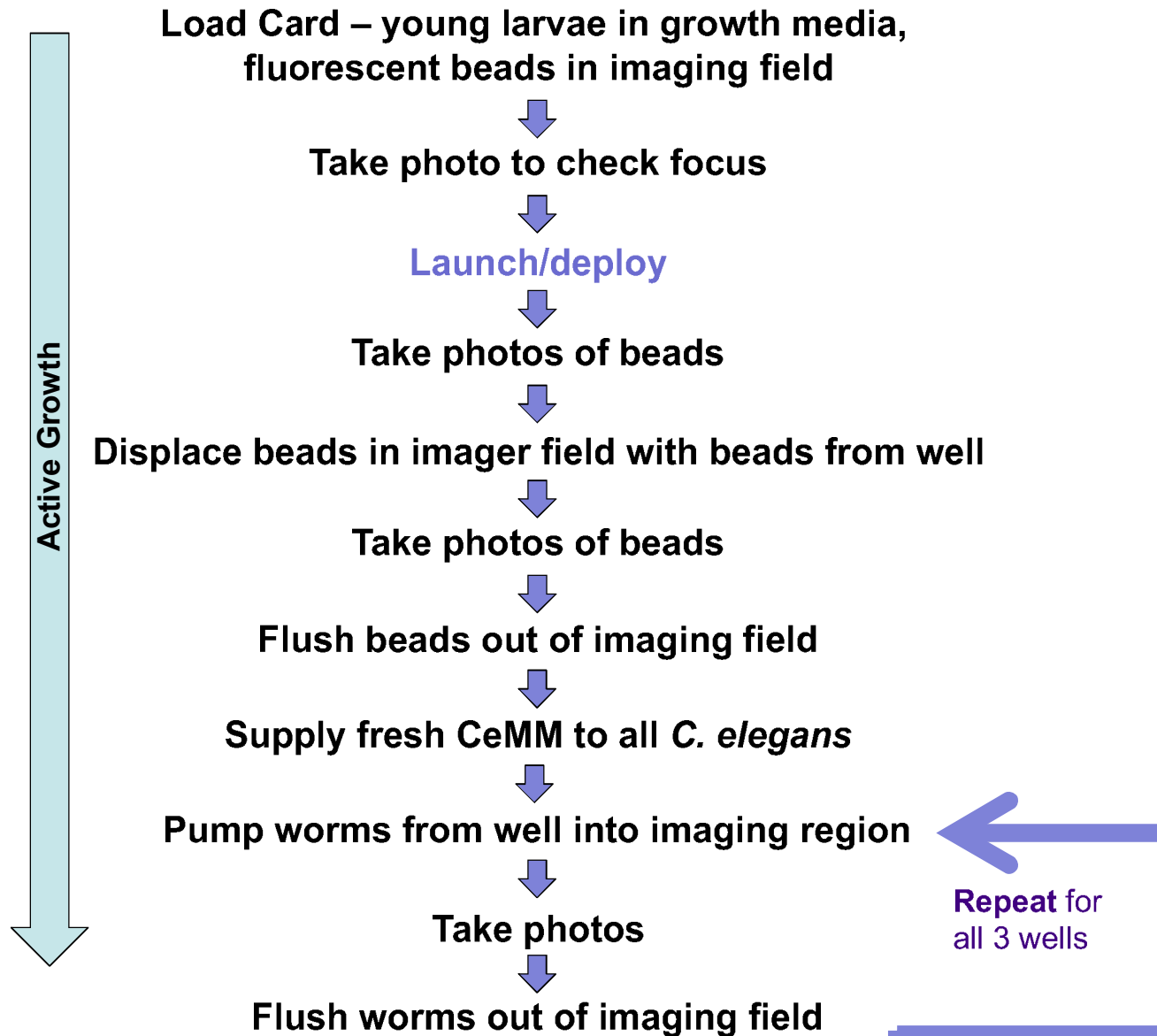
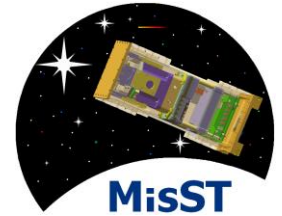
Fluidic Card and Reservoir



**C. elegans* maintenance medium

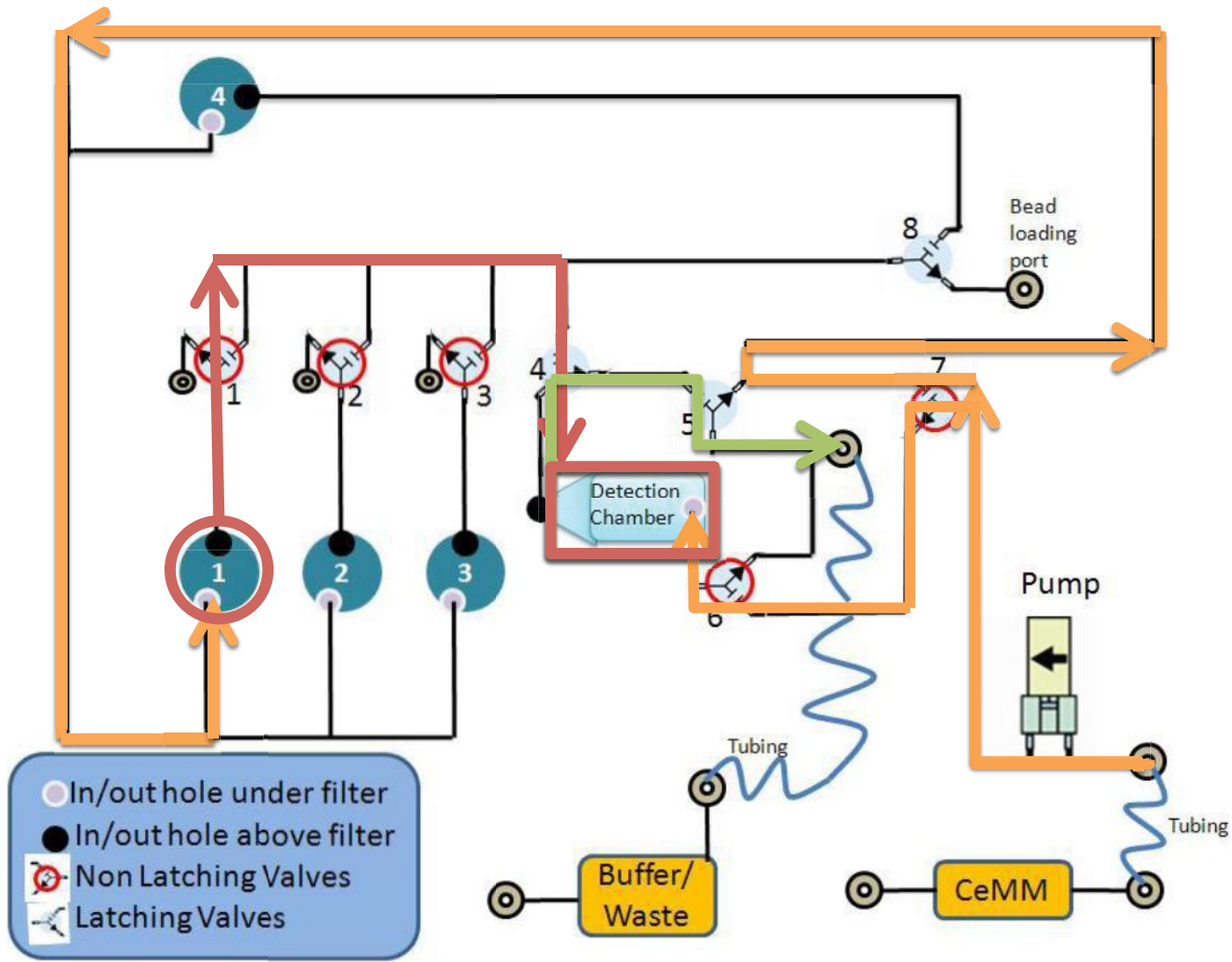
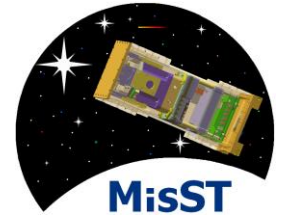


Experimental Timeline



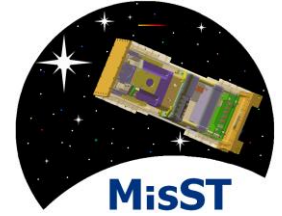


Fluidic Sequence- Worms



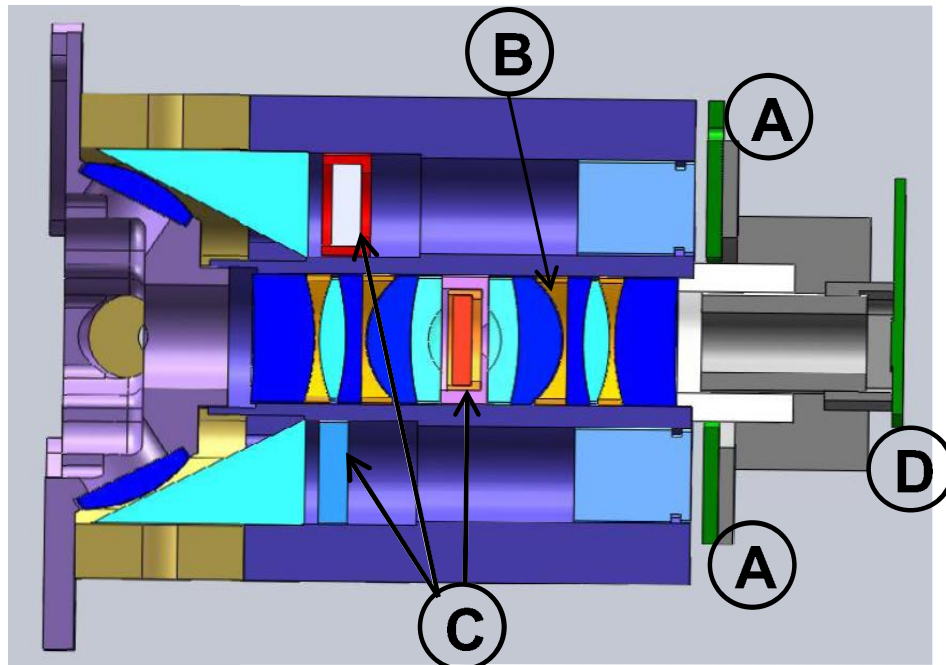


Fluorescence Imager



Imager Components:

- A** Luxeon Rebel LEDs:
2 amber, 1 blue, 1 white
- B** Edmunds relay lens
- C** Semrock filters:
blue excitation, amber excitation,
dual band-pass emission
- D** Camera:
COMedia C328-7221

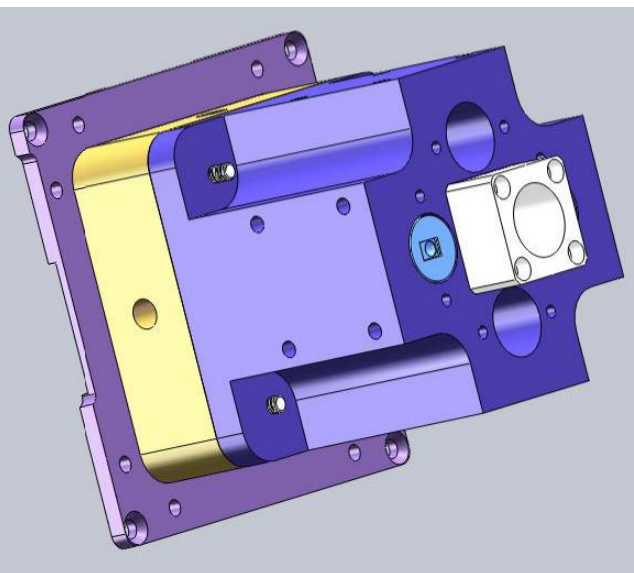
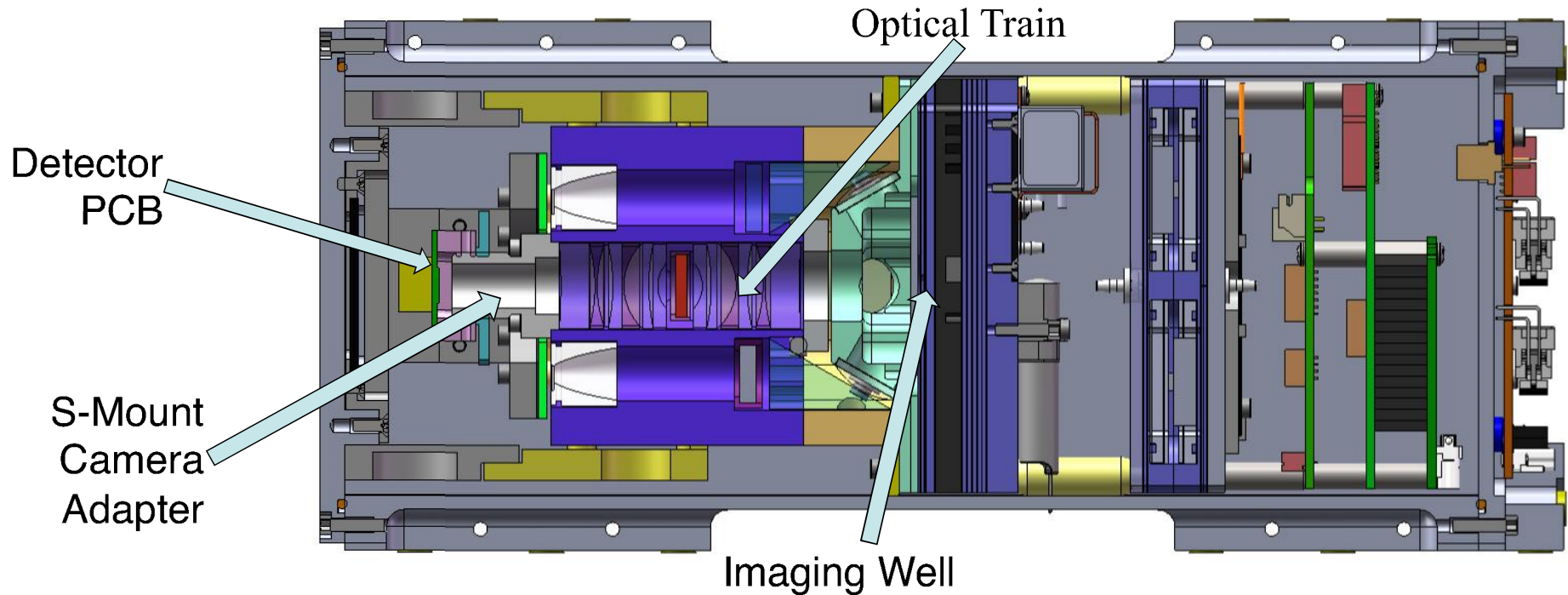
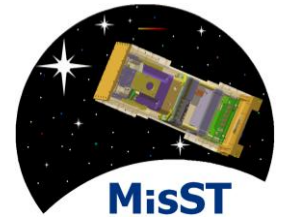


Imager Specifications:

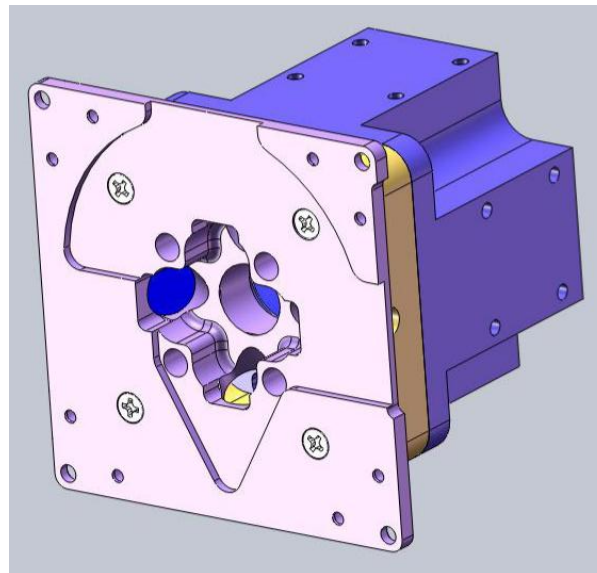
- 2-color fluorescence:
 - Green excite/emit peaks at: 487/509nm
 - Red excite/emit peaks at: 587/610nm
- Cool white for standard imaging
- Lateral resolution: $\sim 8 \mu\text{m}$
- Magnification: 1:1
- Field of View: $\sim 3 \times 4 \text{ mm}$
- Depth of Focus: $\sim 150 \mu\text{m}$



Fluorescence Imager



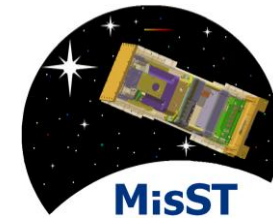
Imager Assy.



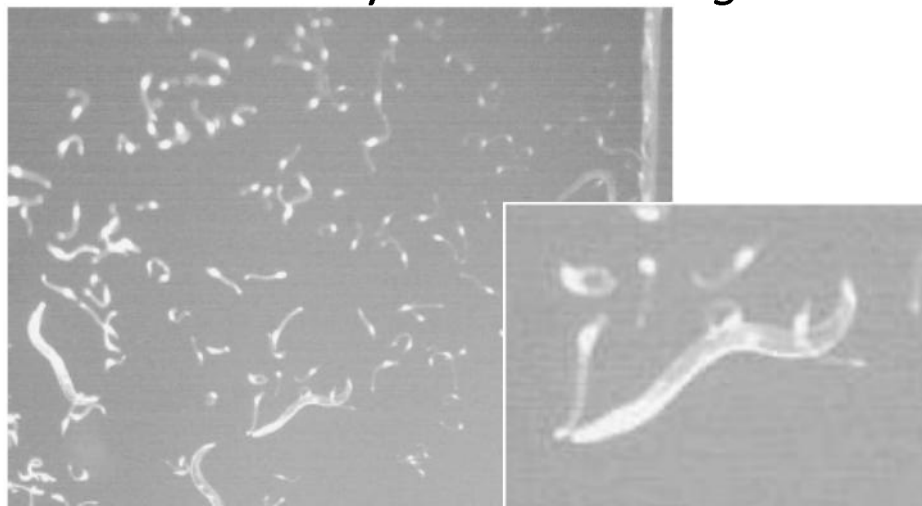
- Fluidic well depth: **0.17 mm**
- Fine pitch thread on S-mount (**0.5mm/thread**)
- Camera adjustment: **±5 mm**
- Locknut/set screws hold detector in place



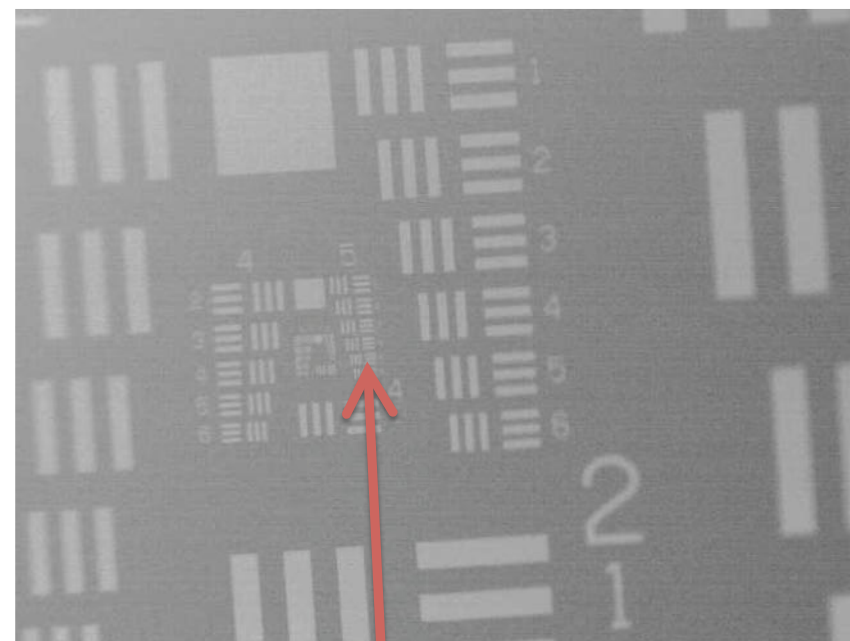
COMedia C328-7221 Camera chip



green fluorescently labeled *C. elegans*



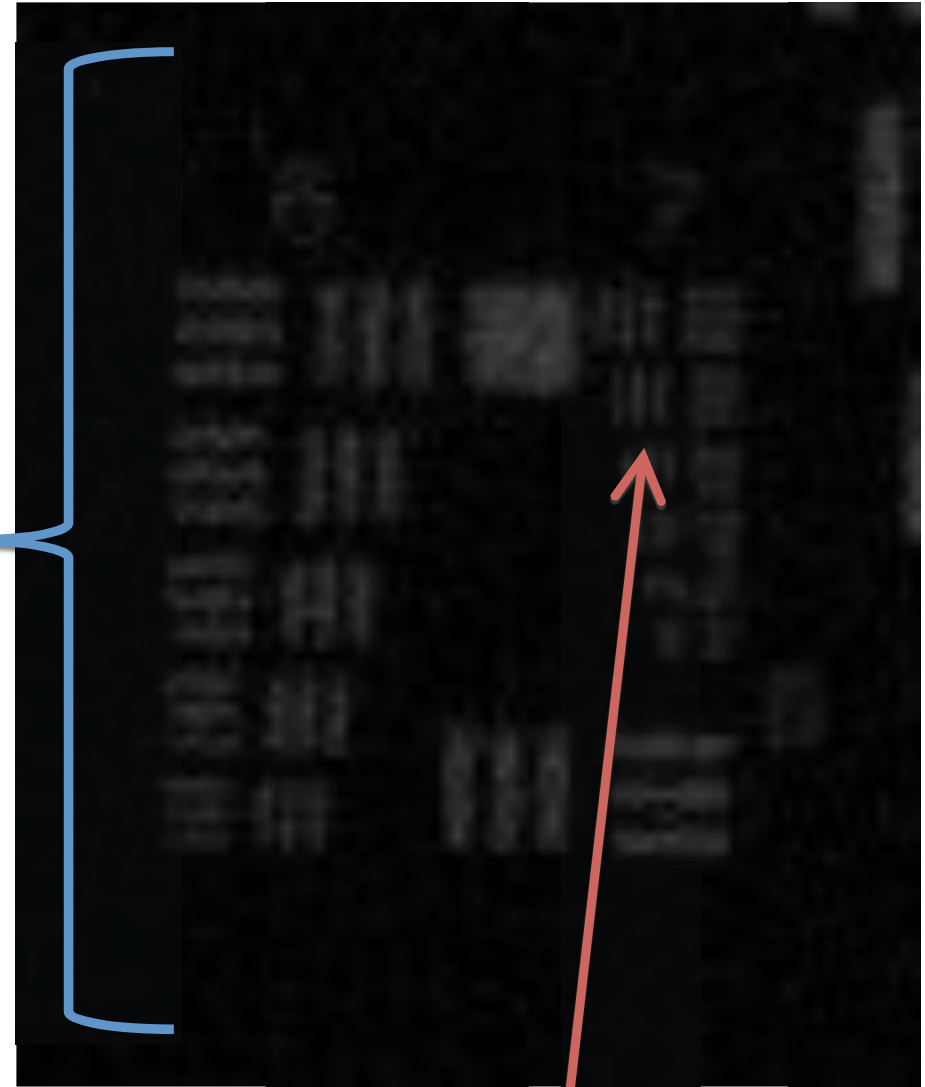
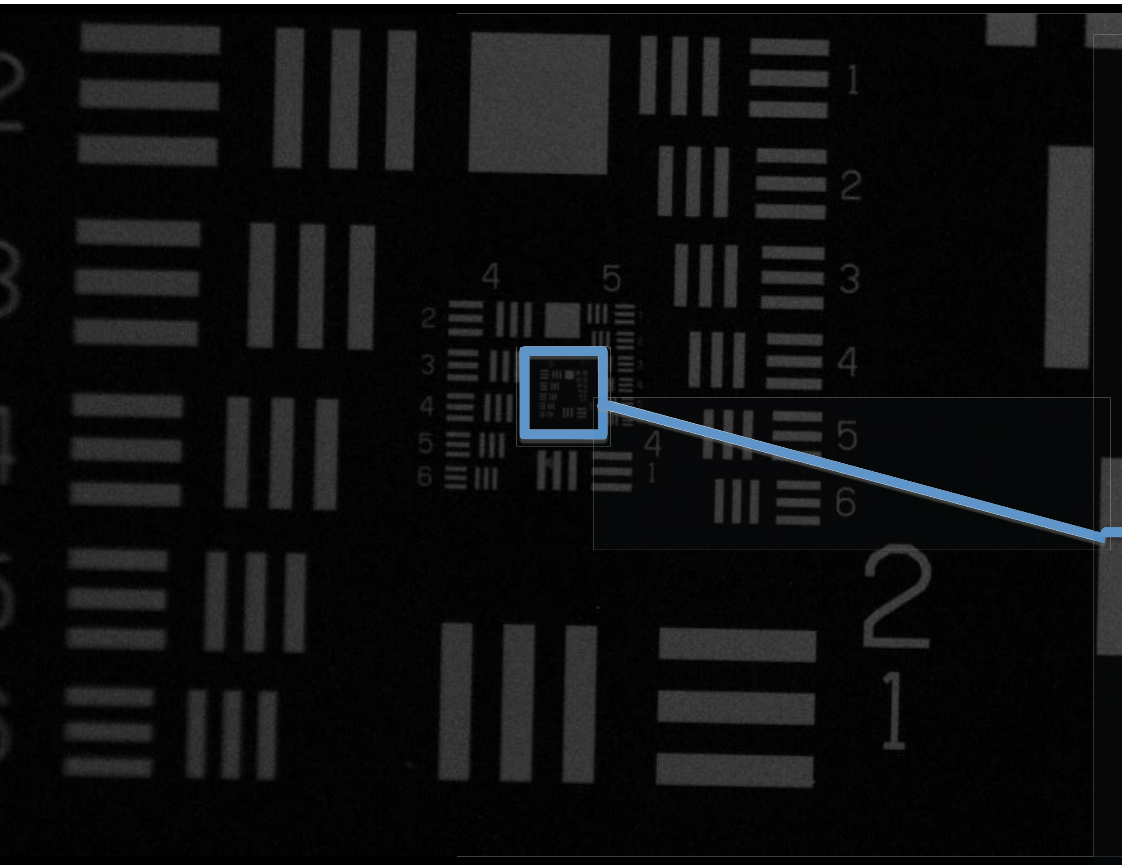
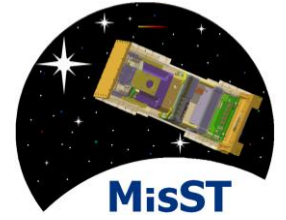
Post Processing [Contrast]



Resolves $\sim 8 \mu\text{m}$ feature size



CCD imaging chip

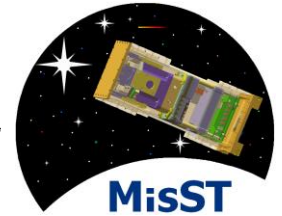


Future camera options are compatible with developed lens system (e.g., Omnivision 7141 CMOS)

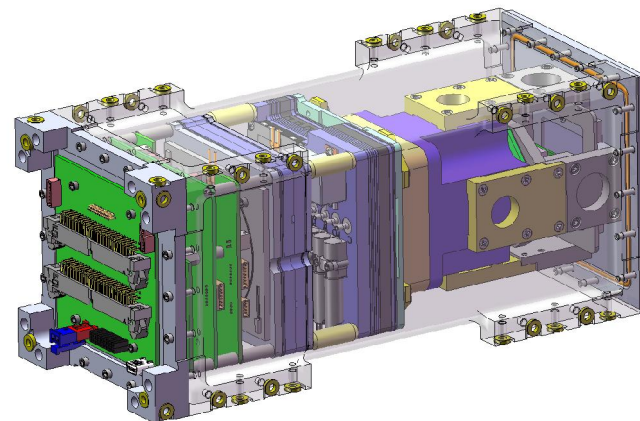
Resolves $\sim 4 \mu\text{m}$ feature size



Payload Functional Overview

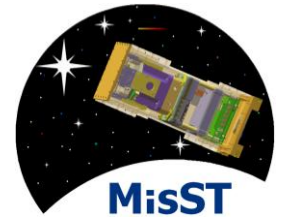


Mass	~1.5 – 2kg
Sensors	Pressure : accurate to within 2% of 1 atm. Humidity: accurate to within 4% from true value after temp. correction Temperature accurate to within +/- 0.5°C.
Processor	PIC 32
Temperature Control	Kept at 4°C to 30°C during the experiment phase.
Environmental Chamber	Maintains an atmospheric pressure in the pressurized payload chamber volume within 11.7 to 15.7 psia.
System Sterilization	Material selections have heritage to past missions for ability to be sterilized and low outgassing.

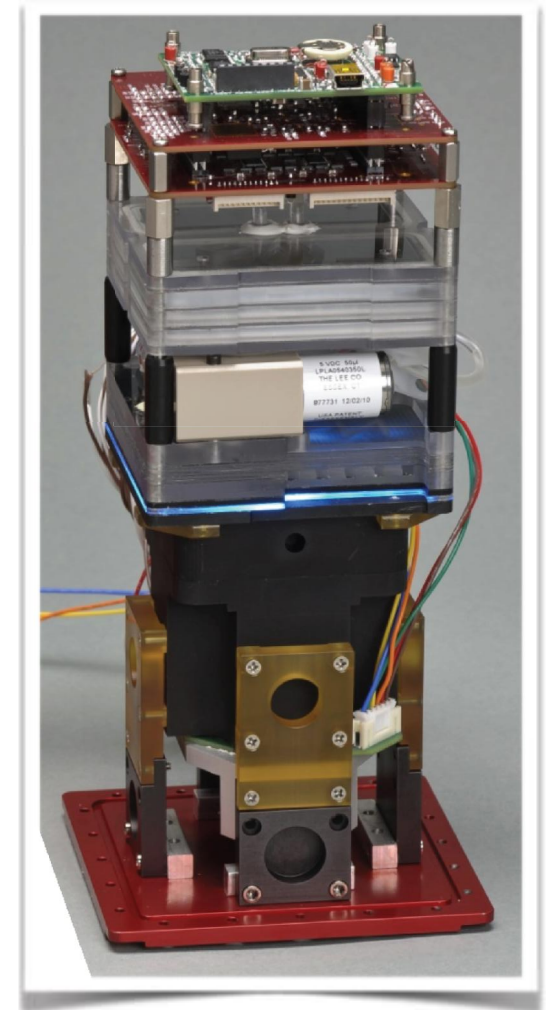
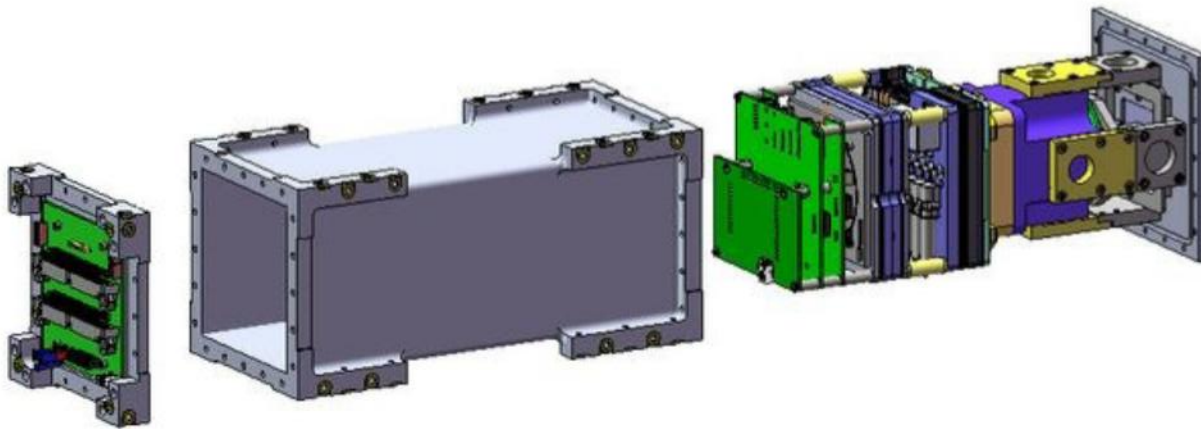


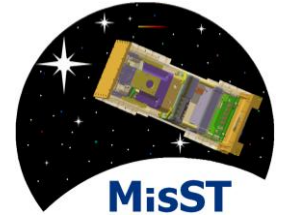


Payload Design Overview

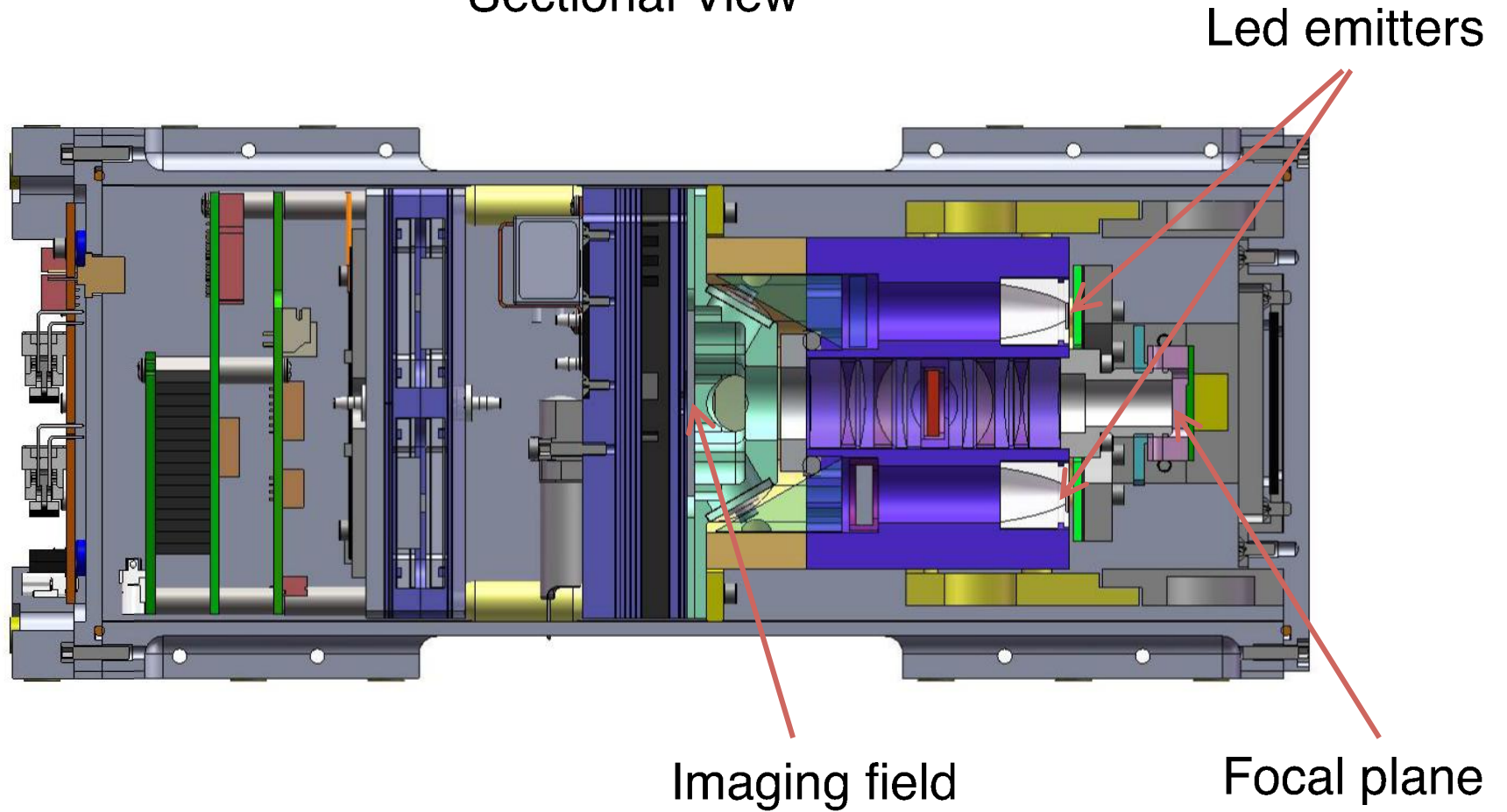


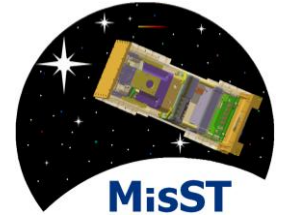
Integrated Payload - Exploded View -



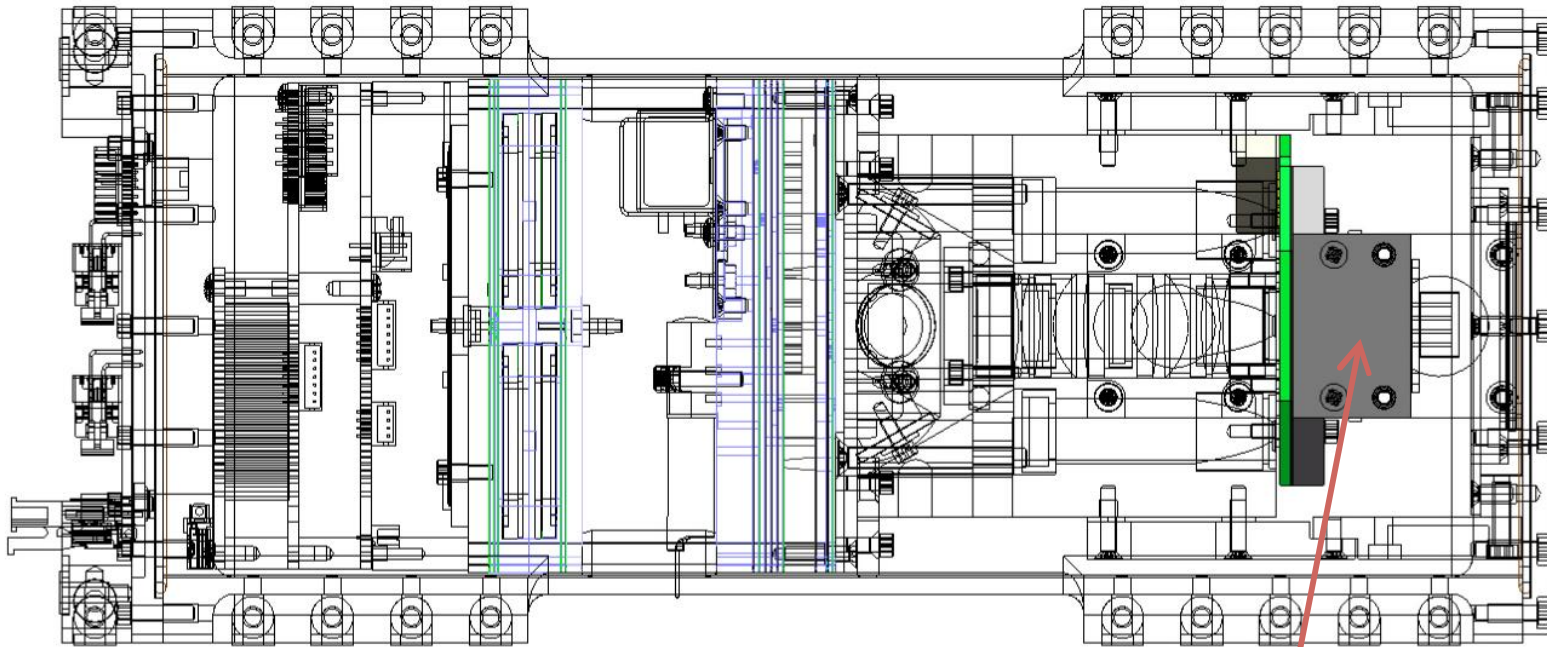


Integrated Payload - Sectional View -

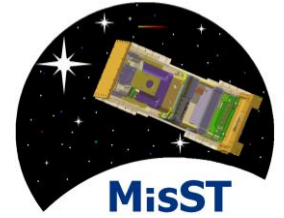




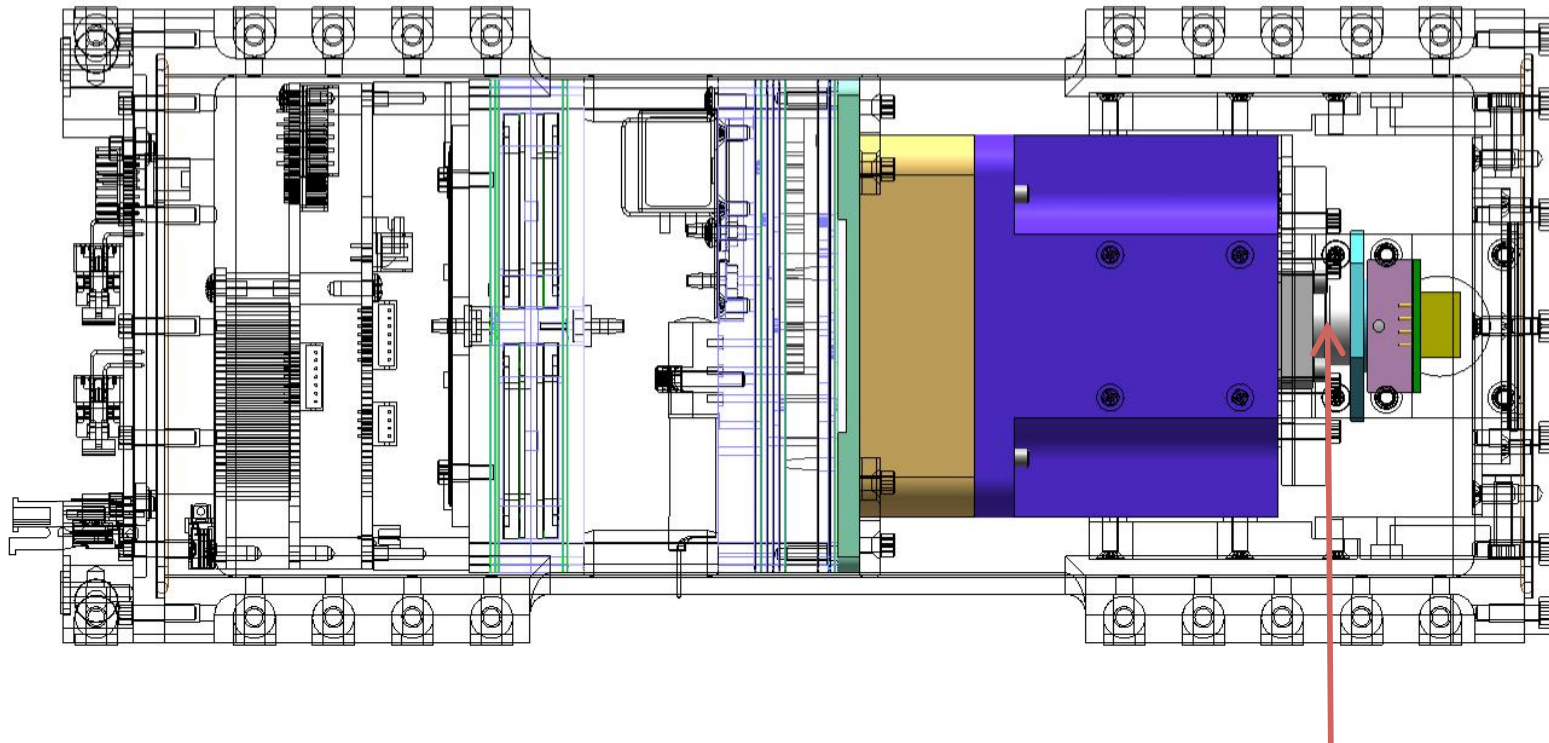
LED Assembly



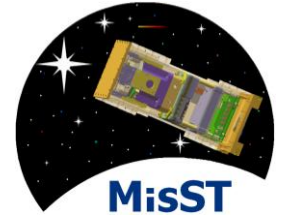
LED heat sink



Camera Assembly

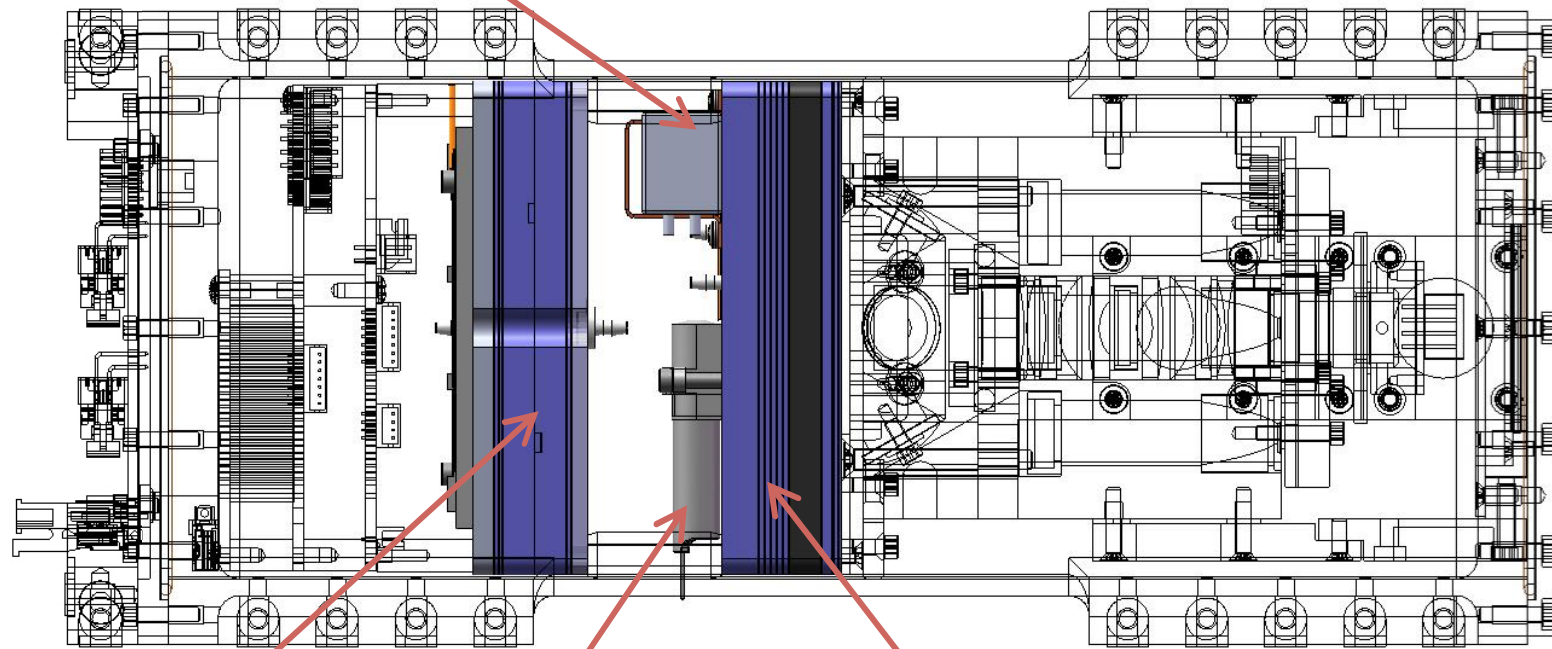


Camera Adapter/Focus Adjustment
With Locknut



Fluidics Assembly

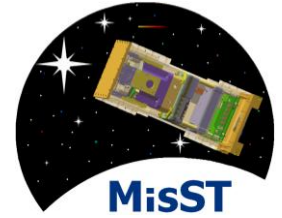
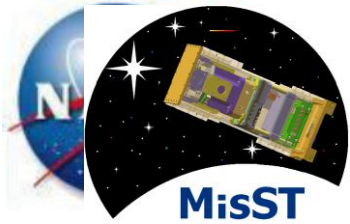
Dolomite Pump



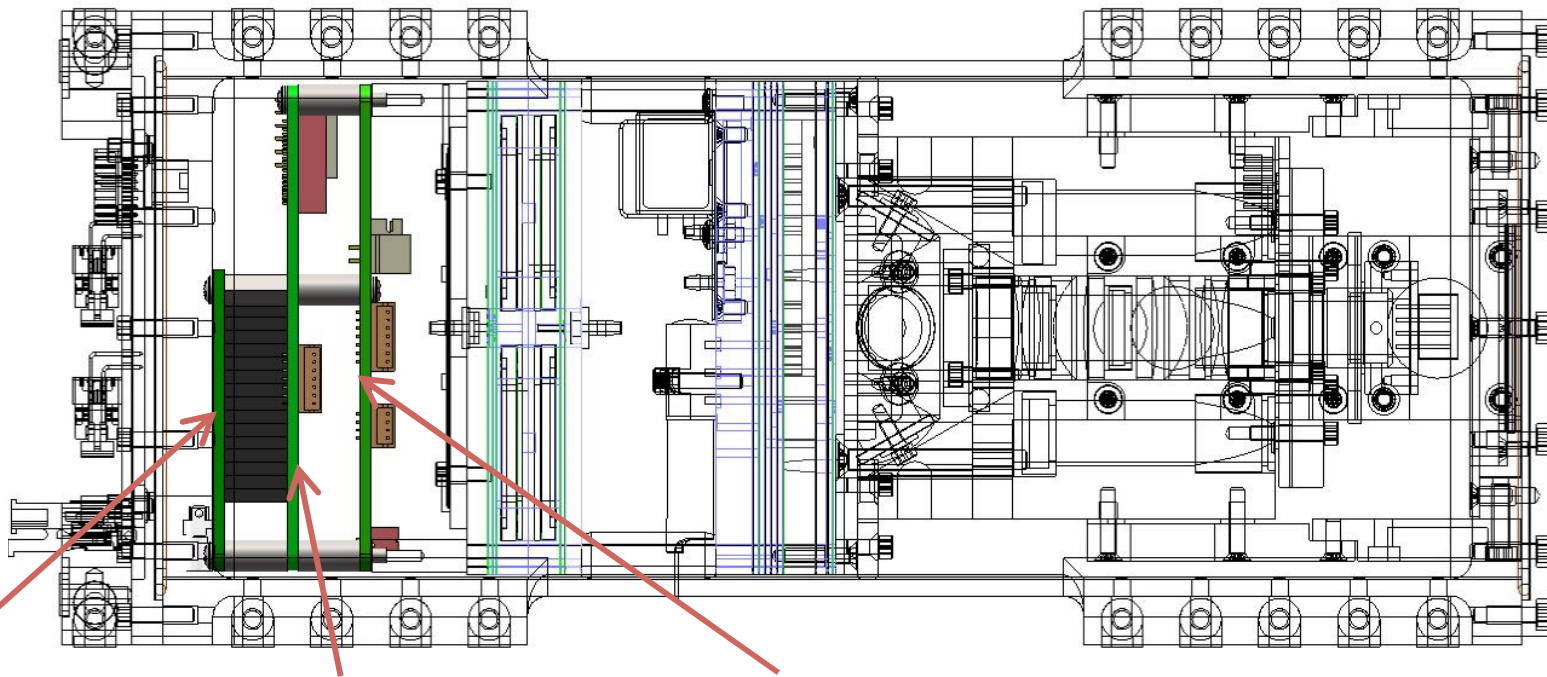
Reservoir

Lee Co. Surface Mount Valves

Fluidics Block



Electronics Assembly



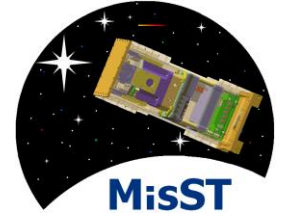
Processor PCB
•PIC 32

Analog PCB
•LED
•Thermal
•RH
•Pressure

Digital PCB
• Camera
• Valves
• Pump



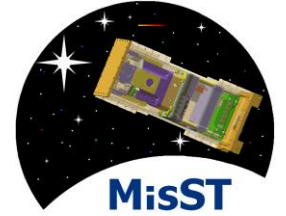
Conclusion



- Technology built:
 - integrated fluidics system that maintains *C.elegans* viability and supports growth
 - fixed-focus imager with fluorescence and scattered-light imaging capabilities
- Biocompatibility testing complete
- Waiting to partner with a principle investigator and launch



Acknowledgements



Fluidics: Ming Tan, Matthew Piccini

Biology: Matthew Lera, Macarena Parra

Imaging: Linda Timucin

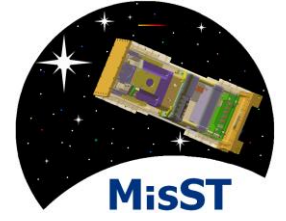
Mechanical: Abraham Rademacher, Giovanni Minelli, Chris Beasley

Electrical: Aaron Schooley

Management: Andres Martinez

Technology: Antonio Ricco

Funding: NASA/Exploration Systems Mission Directorate

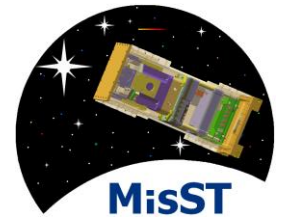


Thank You

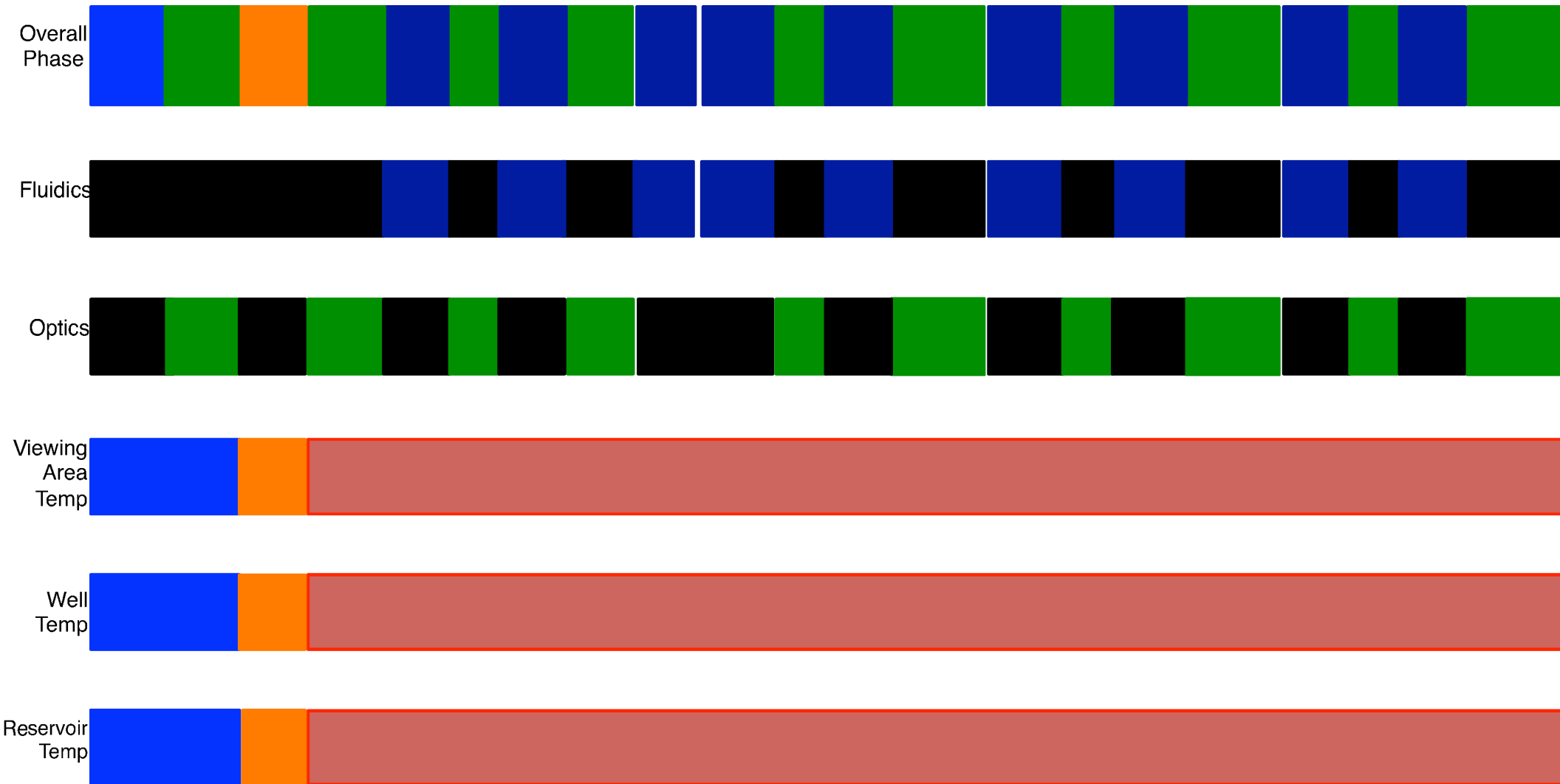
Questions?



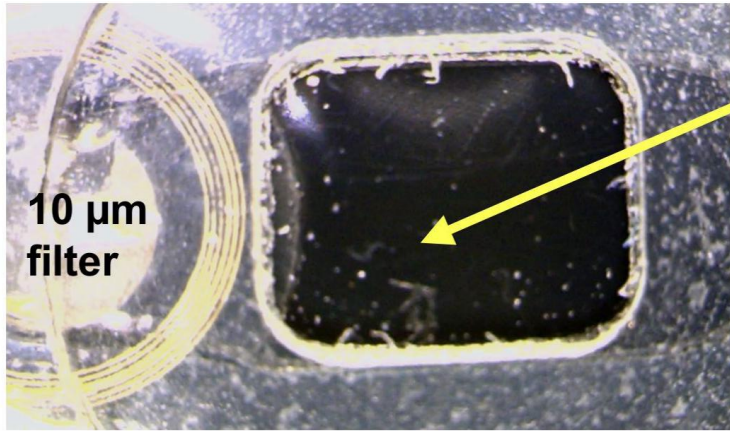
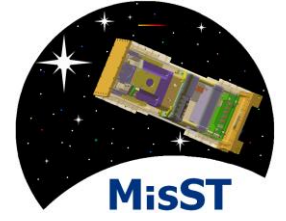
MisST Free-Flyer Payload ConOps



Deployment

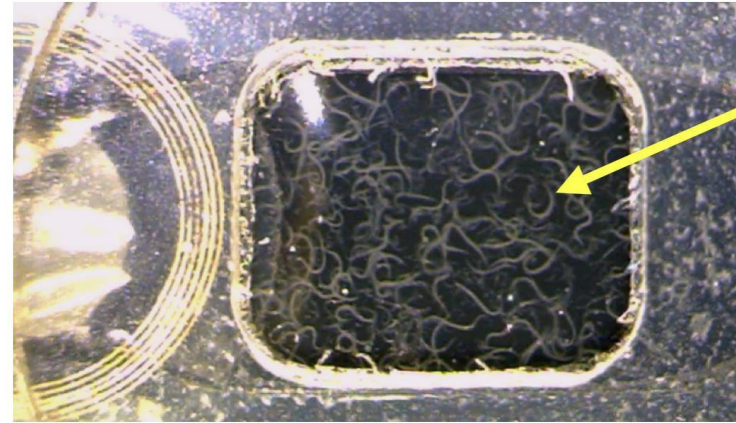


Note: A = amber, B = blue, W = white



10 µm
filter

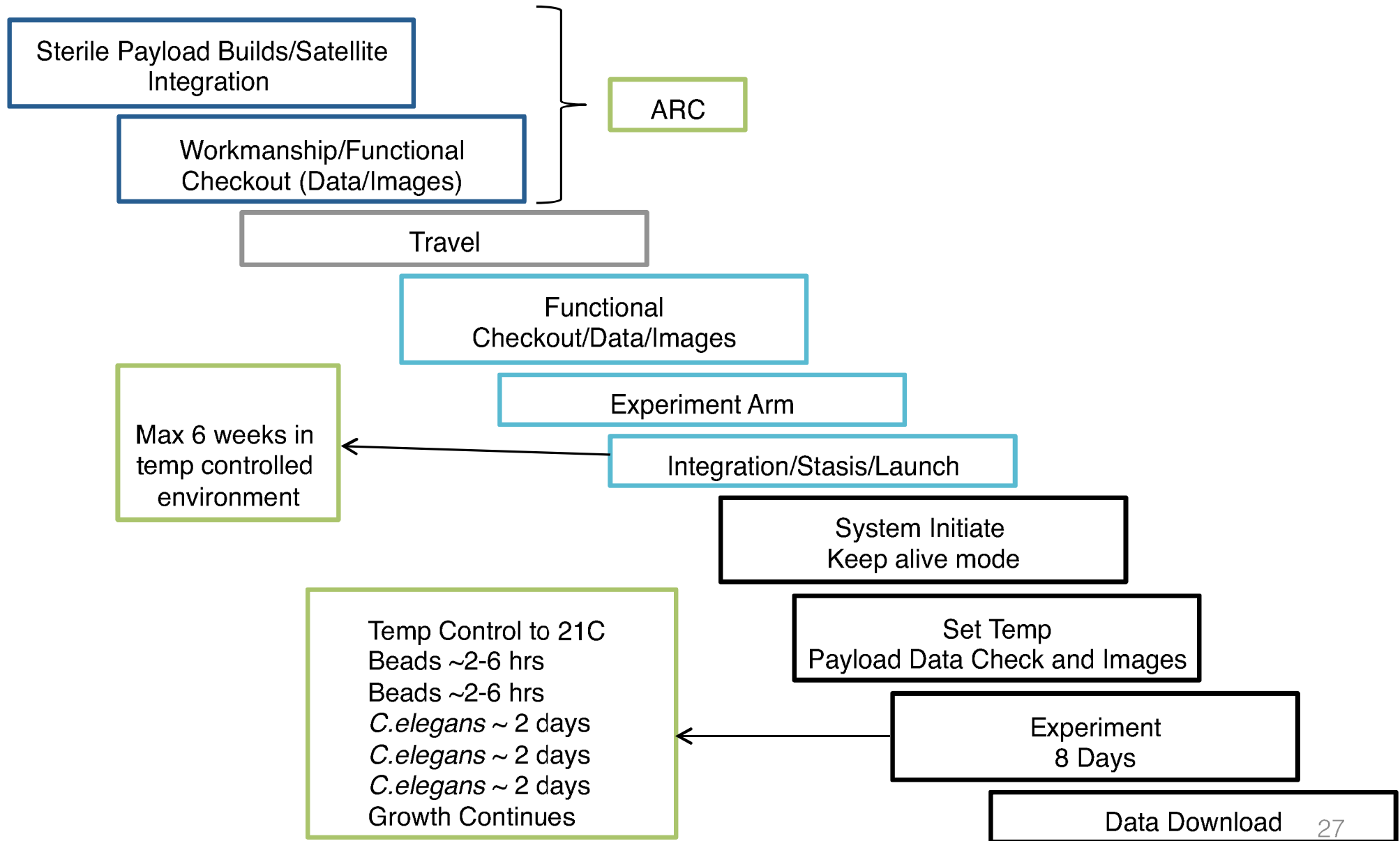
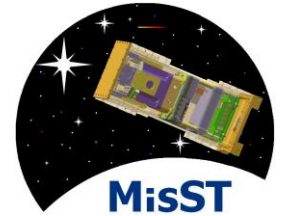
After
pumping
worms
into
imaging
chamber



C. elegans
growth
after 1
week

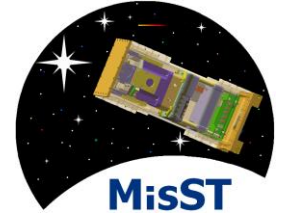


Payload ConOps





Payload 1 (Imager): *Fluidics materials*



Direct contact with *C. elegans*

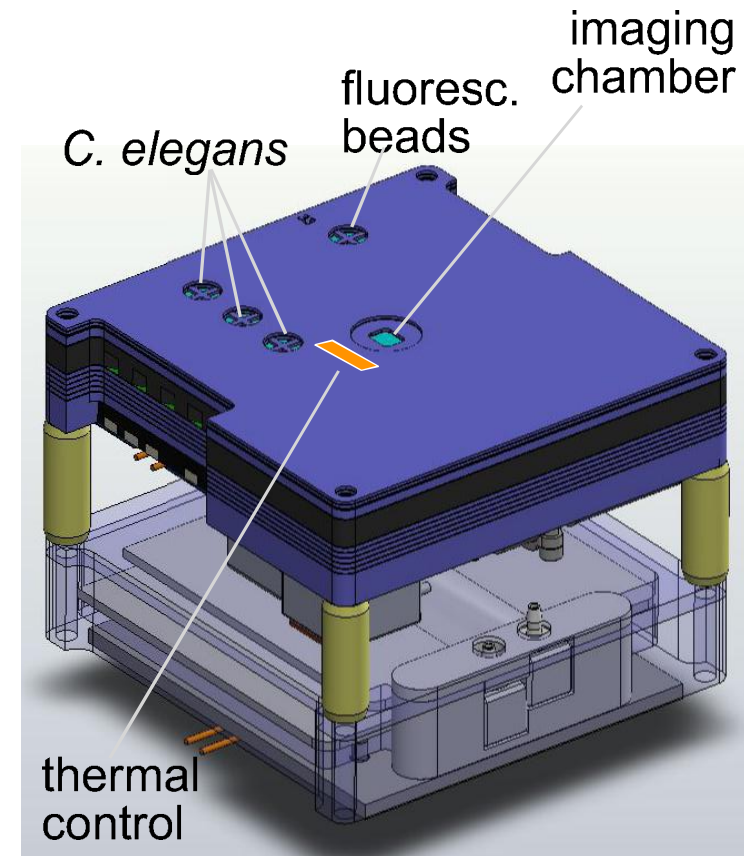
1. Polycarb. (machined)
2. Polycarb. porous membrane
3. Pressure-sensitive adhesive (PSA) (cut edges only)
 - acrylic adhesive, polyester carrier
 - laser-cut edges
4. Polystyrene gas-permeable cover film

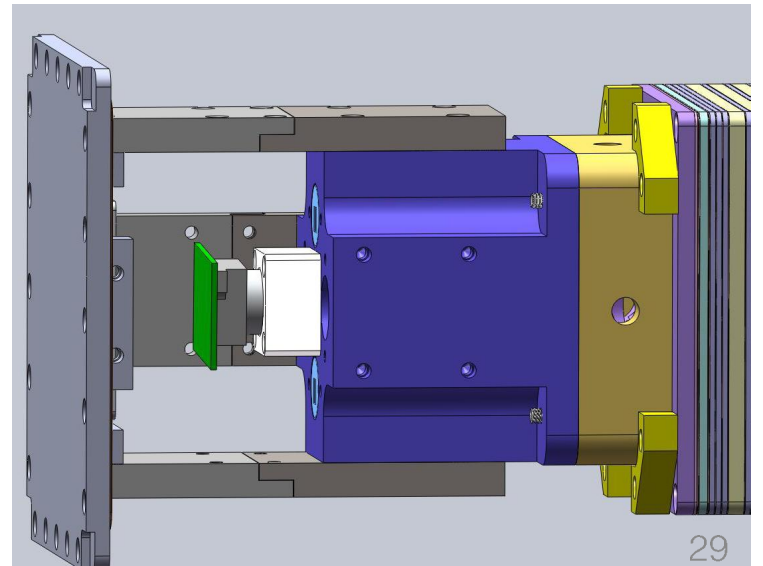
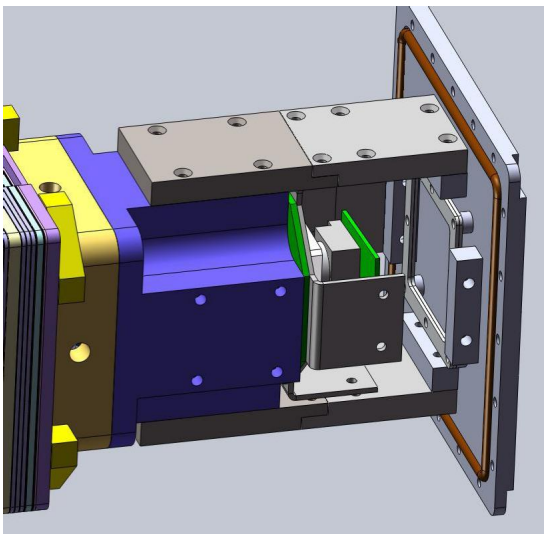
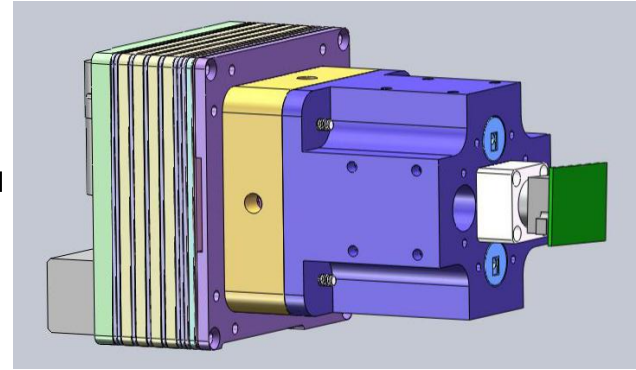
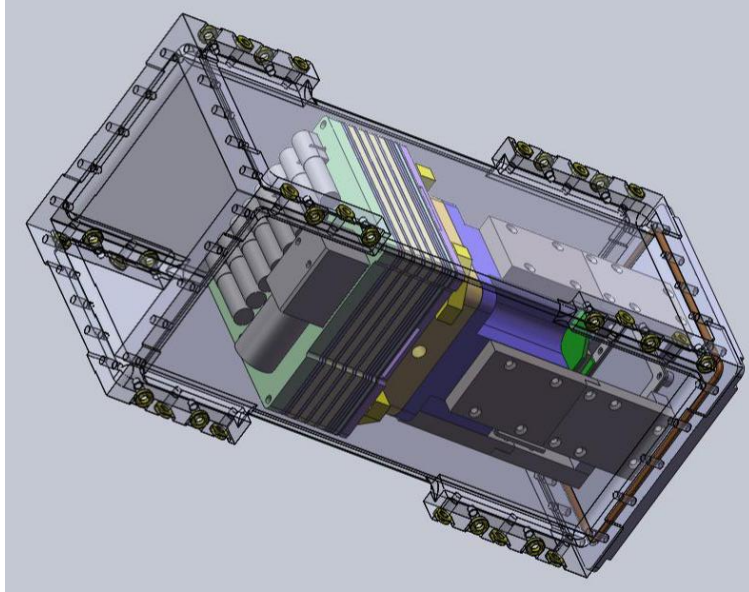
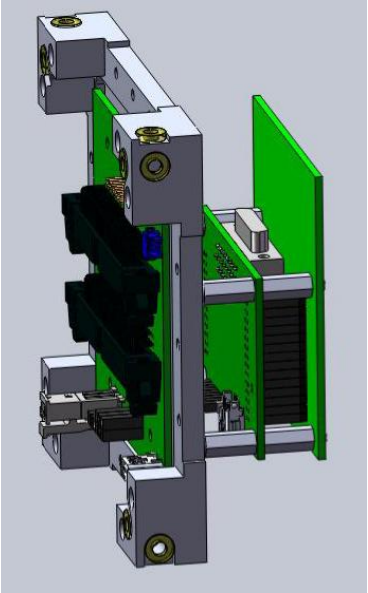
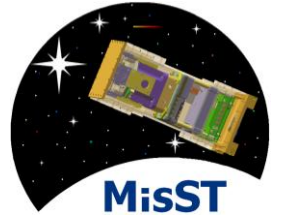
Contact with growth medium only (reservoir/tubing)

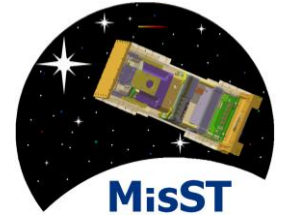
1. Poly(ethylene vinylacetate) [EVA] bag
2. Polysulfone barbed ports
3. Polyurethane tubing

Direct contact with *C. elegans* and growth medium

1. Internal wetted valve materials
 - 1.PPS = Polyphenylene sulfide (“Fortron”)
 - 2.PBT = Polybutylene terephthalate (“Valox”)
 - 3.316 SS
 - 4.FeCr Alloy
 - 5.Silicone Rubber

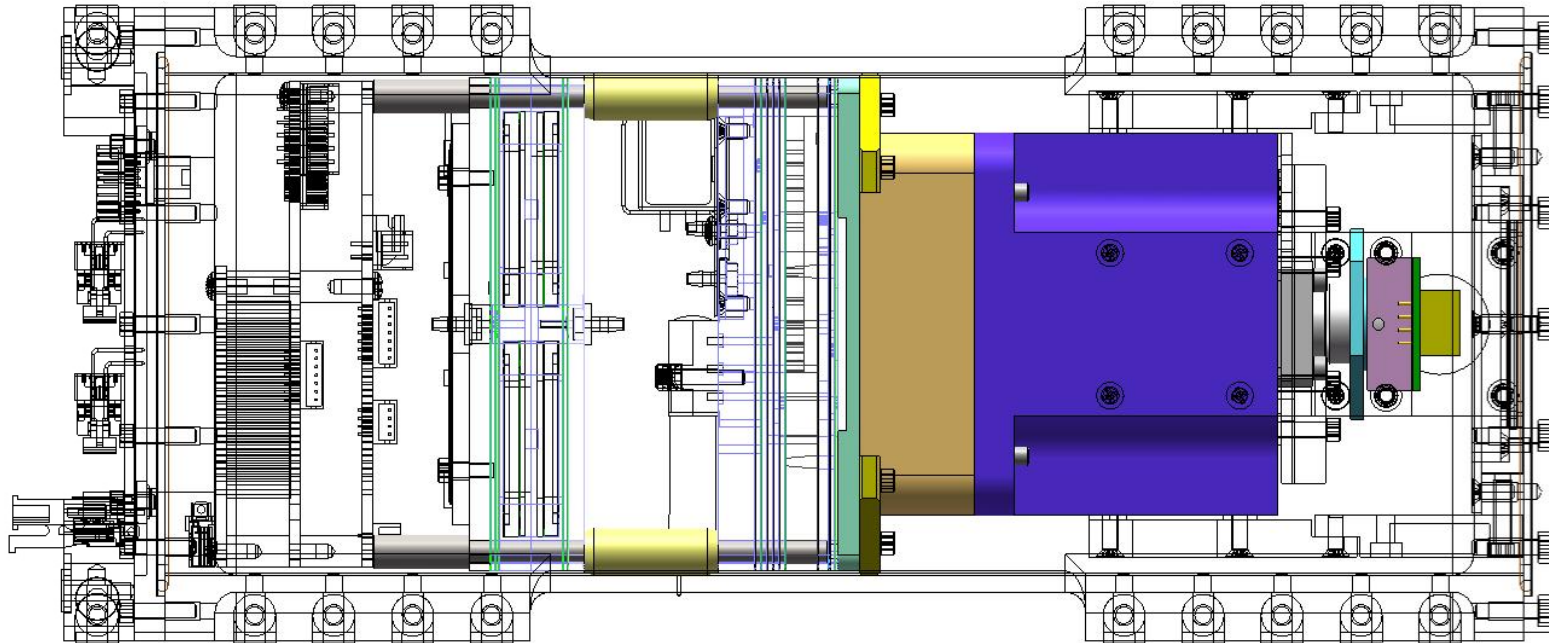


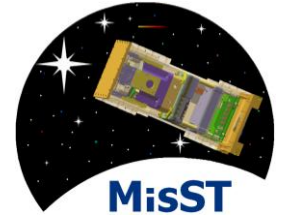




Fluidics Stack Pins

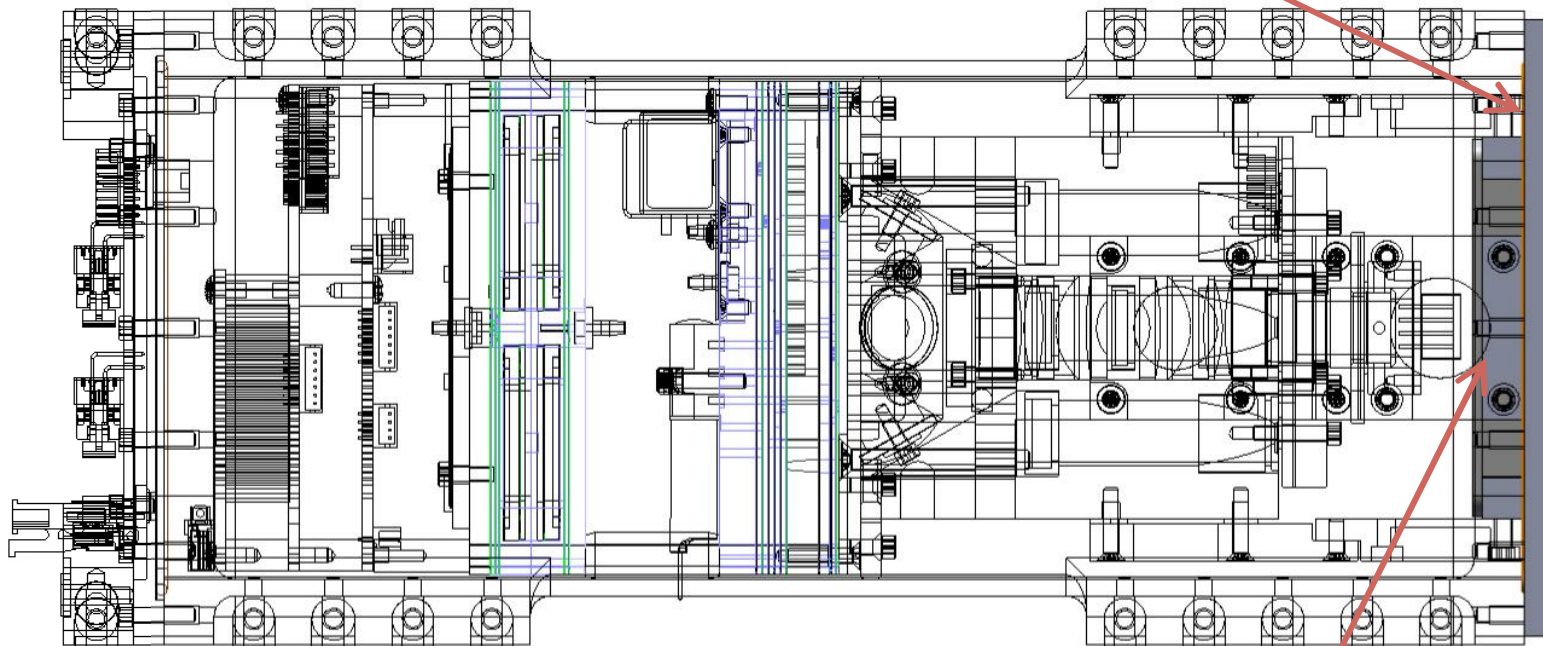
- Detail -



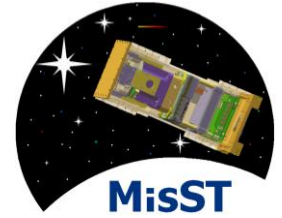


Back-Cover Assembly

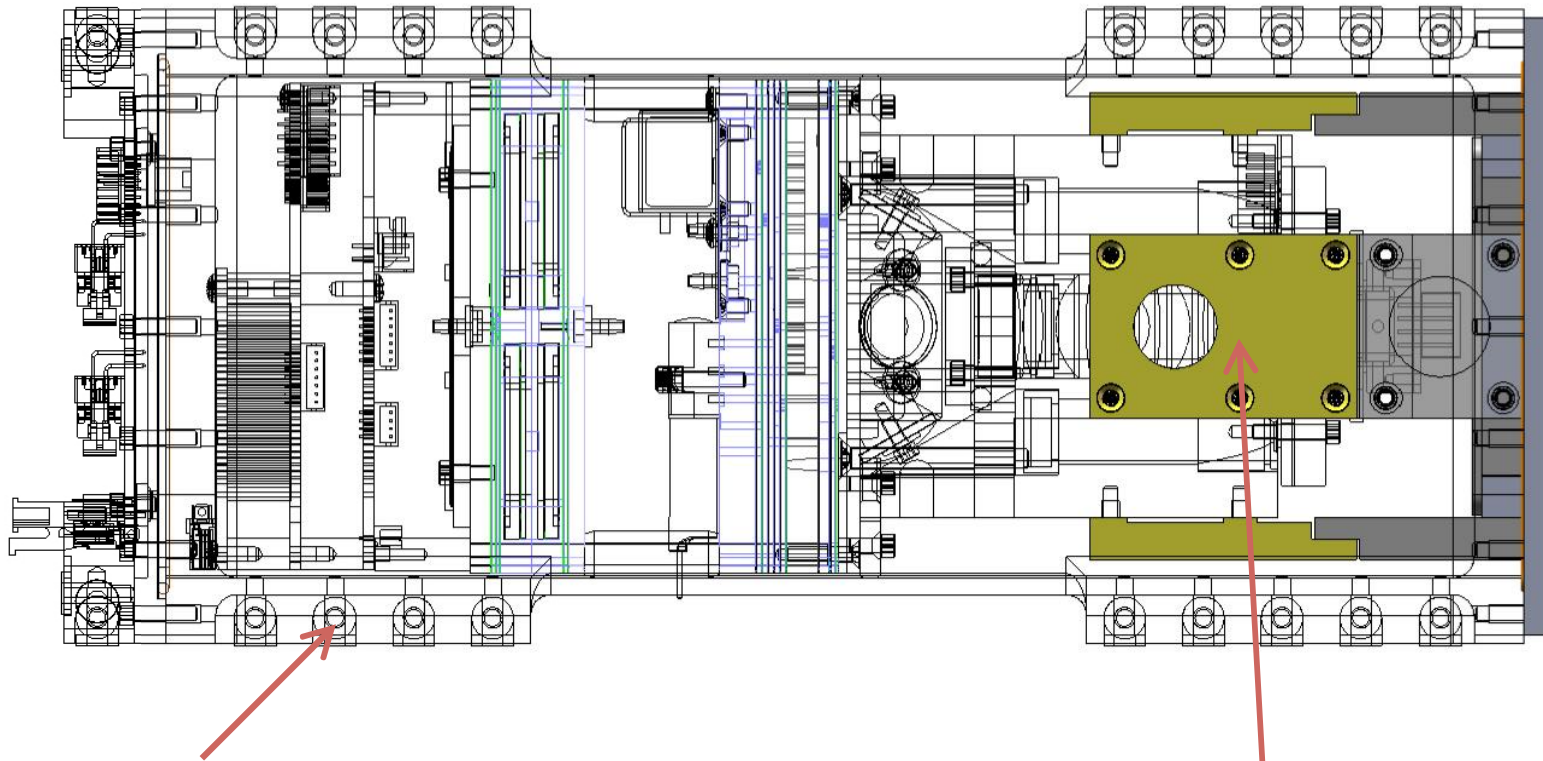
Viton O-Ring



Activated Carbon
Assembly

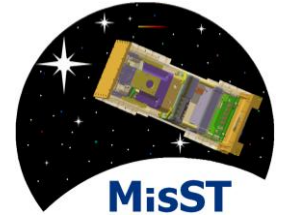


Camera Mount - Mated to Back Panel -



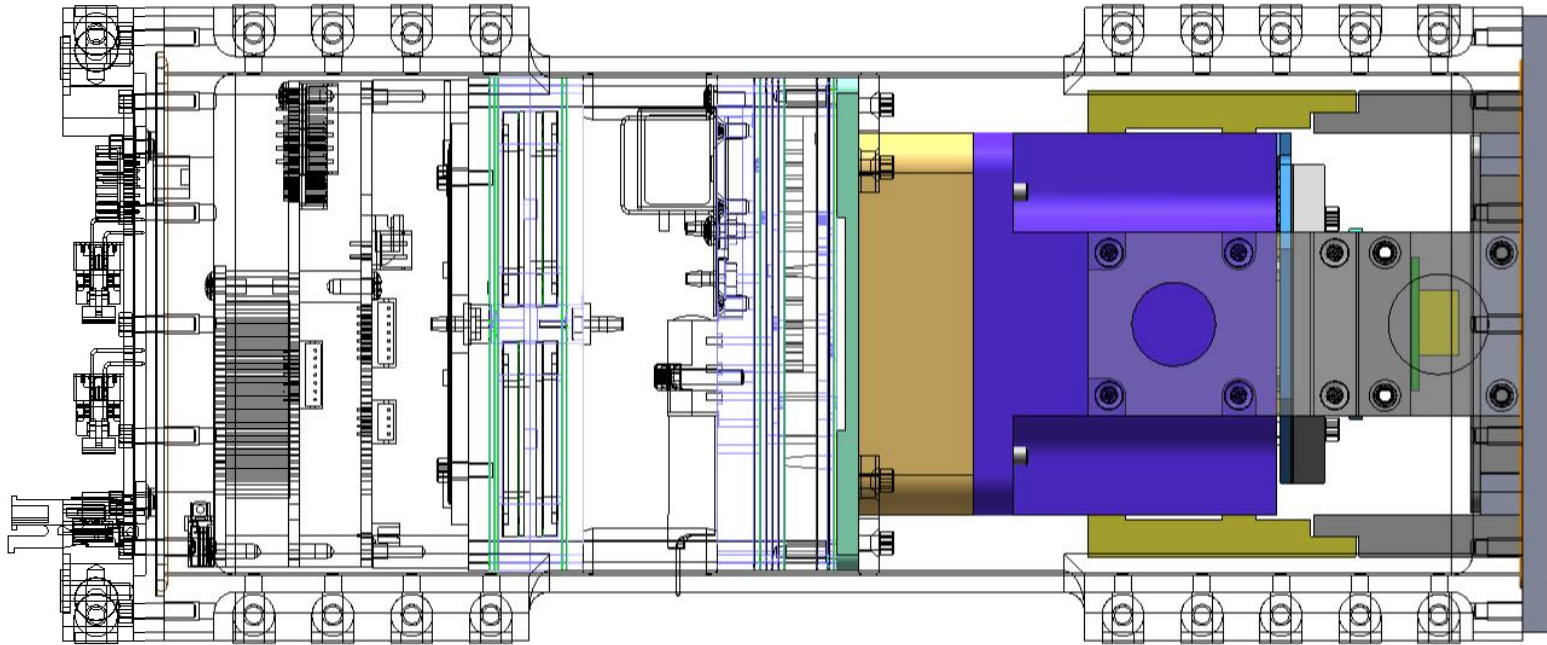
Ultem Washers
Enclosure Thermal Isolation

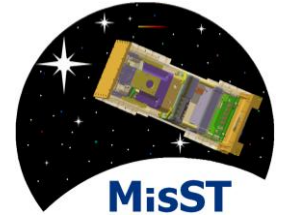
Ultem Mount
Payload Thermal Isolation



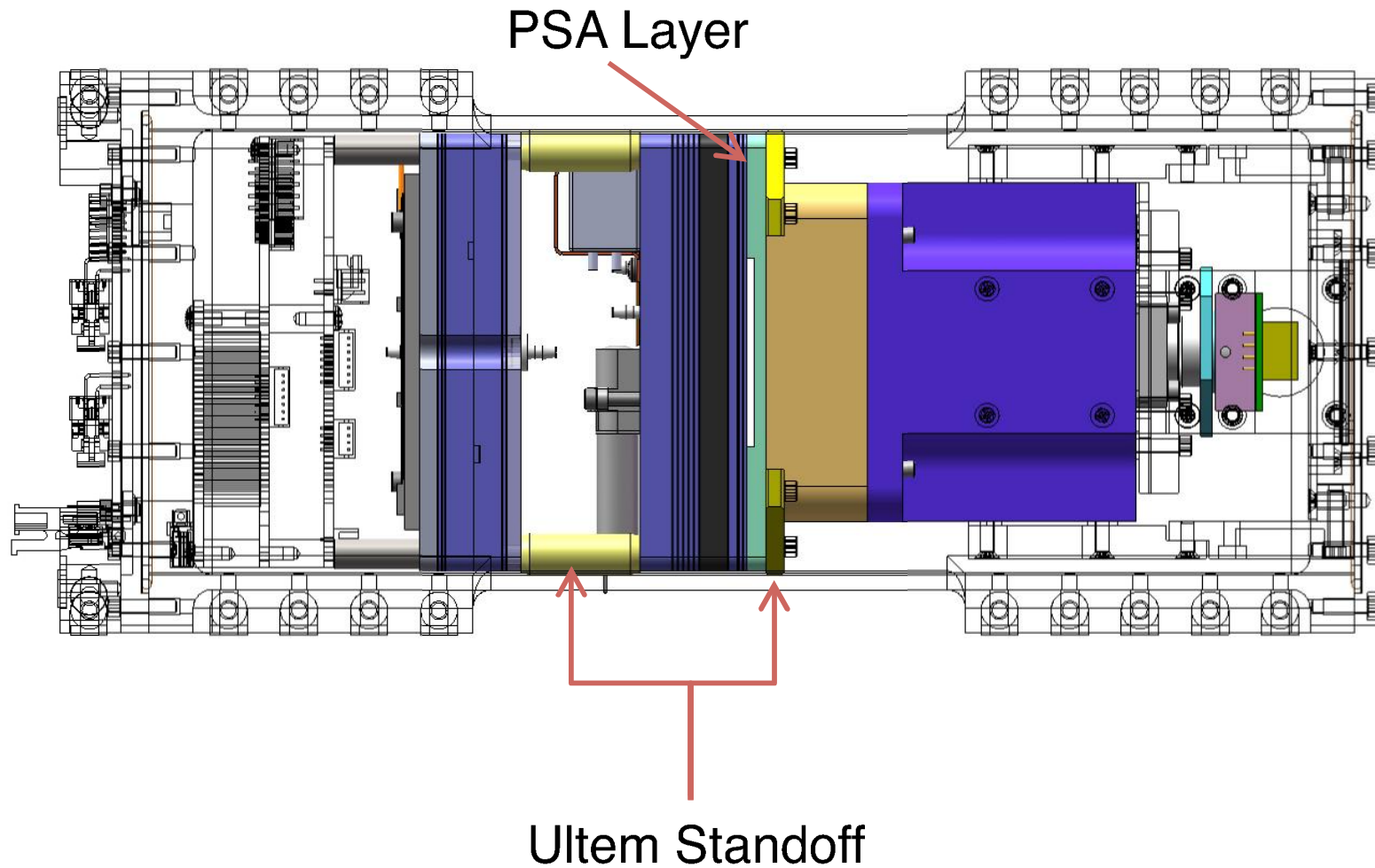
Camera Assembly

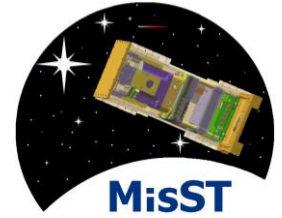
- Mated to Camera Mount -





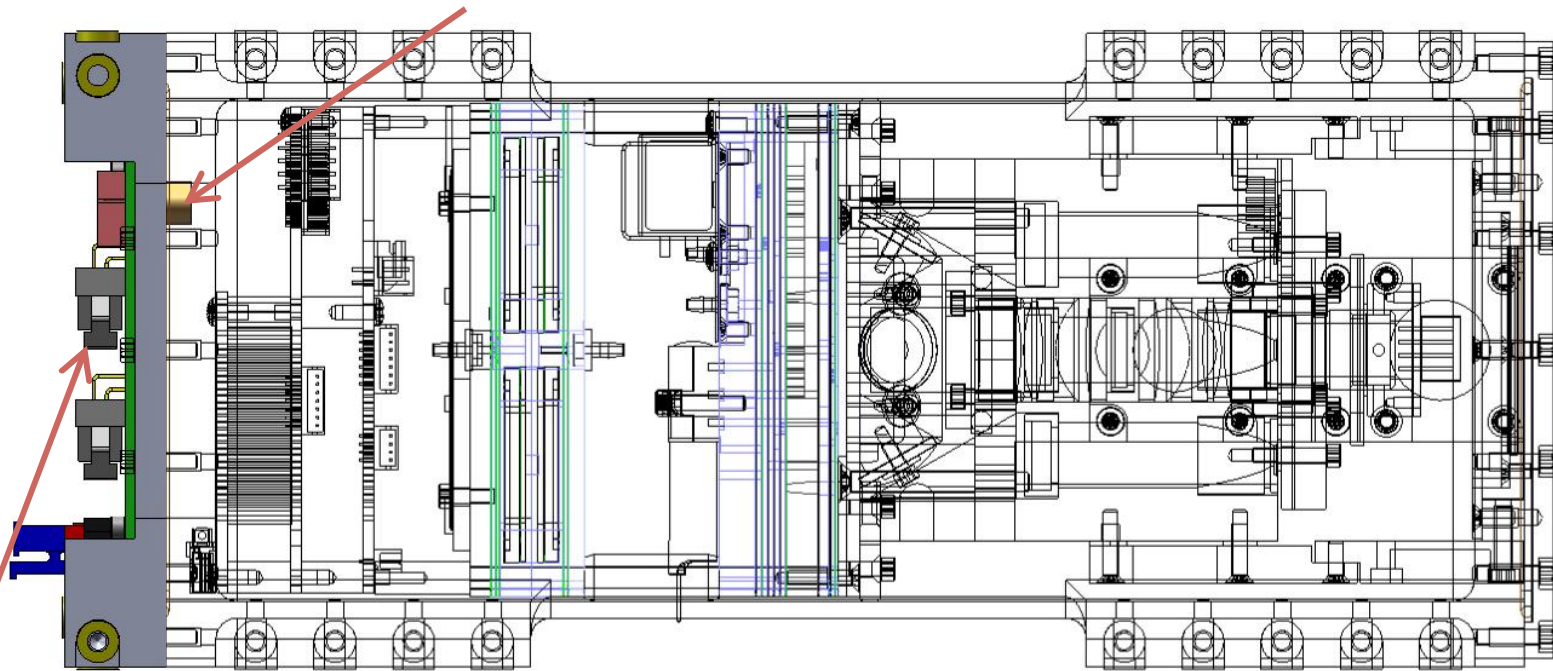
Fluidics Assembly - Mated to Camera Assembly -



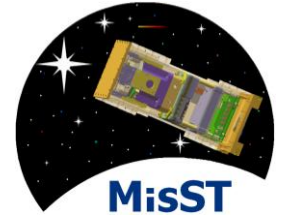


Front Panel Assembly

Hermetic Connector
•Laser Welded

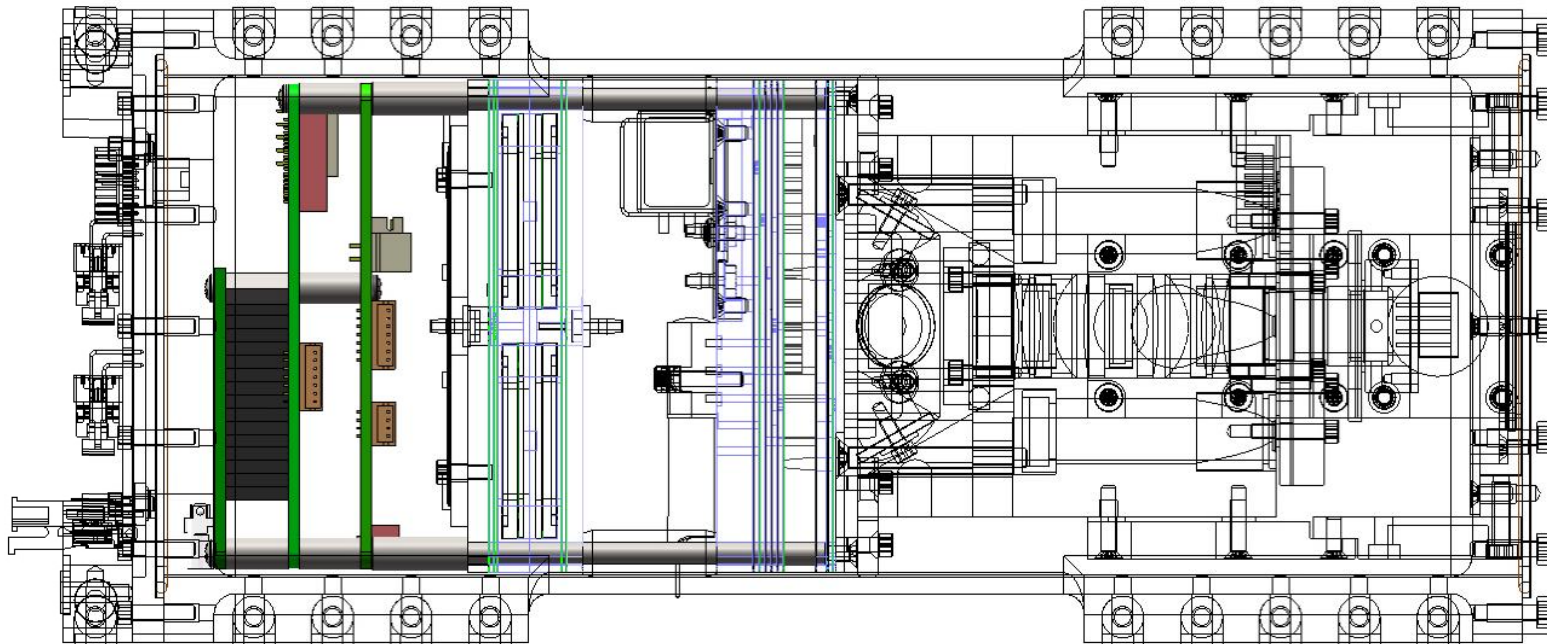


Imager Interface Board



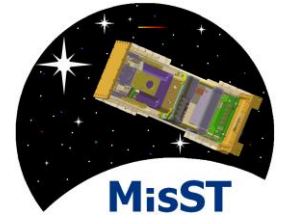
Electronics Assembly

- Mated to Fluidics Stack Pins -





Payload Electrical Overview



M410 Imager Interface Board	Translates the bus 50pin cable to the hermetic connector on the payload can. Also has connection for the remove before flight “kill switch”
M420 Imager Payload Analog	Contains all analog payload circuits. Temperature sensor circuits, LED current drivers, RH sensor, pressure sensor and circuitry, Heater circuitry
M422 Imager Payload Digital	Contains all digital and inductive load circuits. Valve H-bridges, motor switches, memory chips, camera interface.
M430 Imager LED	Contains the 4 high power imager illumination led’s Attached to a heat sink and devices are properly thermally sunk to allow for continuous use.
M450 Imager Processor	Contains the payload microprocessor and associated circuitry, uC LDO, RTC, FRAM, ADC reference