FLUMIAS - live-cell imaging fluorescence microscopy on a centrifuge for research on gravity-sensitive cellular dynamics on-board the ISS

Theresa Schmakeit

T. Schmakeit[1], Y. Lichterfeld[1], L. Kalinski[1], M. Braun[2], A. Carstens[2], S. Daali[2], R. Sütterlin[3], S. Herbert[3], R. Hemmersbach[1], and C. Liemersdorf[1]

[1]Institut für Luft- und Raumfahrtmedizin, Deutsches Zentrum für Luft- und Raumfahrt (DLR) Linder Hoehe, D-51147 Köln, Germany
[2]Organisation / Raumfahrtagentur, Forschung unter Weltraumbedingungen, Deutsches Zentrum für Luft- und Raumfahrt (DLR) Königswinterer Str. 522-524, D-53227 Bonn, Germany
[3]Dept. for Science and Life Support Missions, Airbus Defence and Space GmbH, D-88090 Immenstaad, Germany

Introduction

Since the dawn of space-research microgravity was one of the most important environmental factors affecting biological systems in space from humans and plants to single cells. Several biological research questions can be targeted on platforms providing short-duration microgravity conditions such as parabolic flights or sounding rockets. For a multitude of questions, prolonged time frames are necessary to observe processes such as cellular differentiation, maturation, tissue development or gravity adaptation. The ISS provides constant high quality microgravity and is therefore an excellent platform for gravity-related research. FLUMIAS-ISS will implement the first fluorescent live-cell microscope to grant true insight into dynamic changes or adaptive processes on a cellular level and induced by controlled changes between 1*g* and microgravity (0*g*). Identifying gravity-sensitive signaling pathways will further enhance the development of countermeasures for health risks of manned space flight.

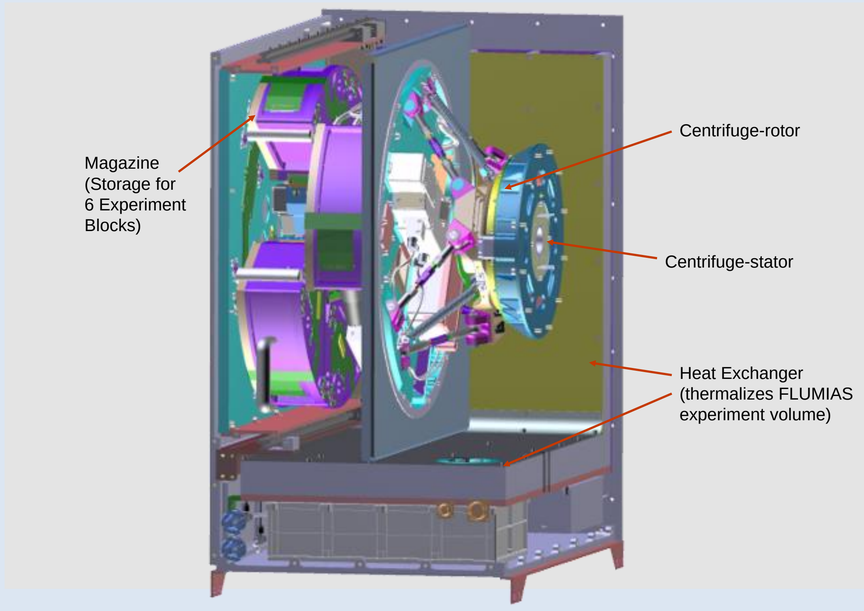

Fig. 1: The FLUMIAS Facility consists of a structured-illumination fluorescence microscope on a centrifuge and a storage magazine housing 6 temperature-controlled Experiment Blocks (EBs) allowing for medium exchange and incubation. Accelerations between 0*g* and 1*g* can be applied alternated during image recording. The sample holder can be adapted to house bacteria, algae, small animal and plant organisms, cells and tissues as well as physical science and material science samples. *From FLUMIAS ISS hardware description by Airbus*

FLUMIAS (FLUorescence MIcroscopic Analysis in Space) is a newly developed research platform, which combines incubation of biological samples in varying gravity levels with Structured Illumination Microscopy (SIM) and a rotating platform. The evolution of the SIM technique enables spatial and temporal high resolution fluorescent live-cell imaging. Samples including single cells, tissues, plants, microorganisms, small animals, 3D culture systems, *ex vivo* tissues or colloids can be investigated. The Experiment Blocks are designed to provide stable incubation temperatures between 25°C and 40°C and can be filled pre-launch with a desired gas concentration. The system allows for the addition of live staining agents or pharmacological stimulation while imaging with defined varying gravitational loads onboard the ISS.

FLUMIAS Capabilities

- Ibidi channel slides as sample holder
- Near-confocal resolution via SIM with 4fps and lateral resolution <350nm
- FoV of 400µm x 350µm
- Laser lines: 405nm, 488nm, 561nm, 640nm
- Life-Support system for medium supply in the range of 25°C to 40°C, initial gas filling possible
- 4 fluid tanks: 1x100ml, 3x5ml
- Gravity levels from 0*g* to 1*g* at 0.1 increments
- Fully automated EB processing with interaction from ground possible
- Applications include: Cell differentiation, maturation, activity changes, (re-)adaptation processes, Calcium-Imaging, pharmacokinetics, physical sciences, investigation of thresholds

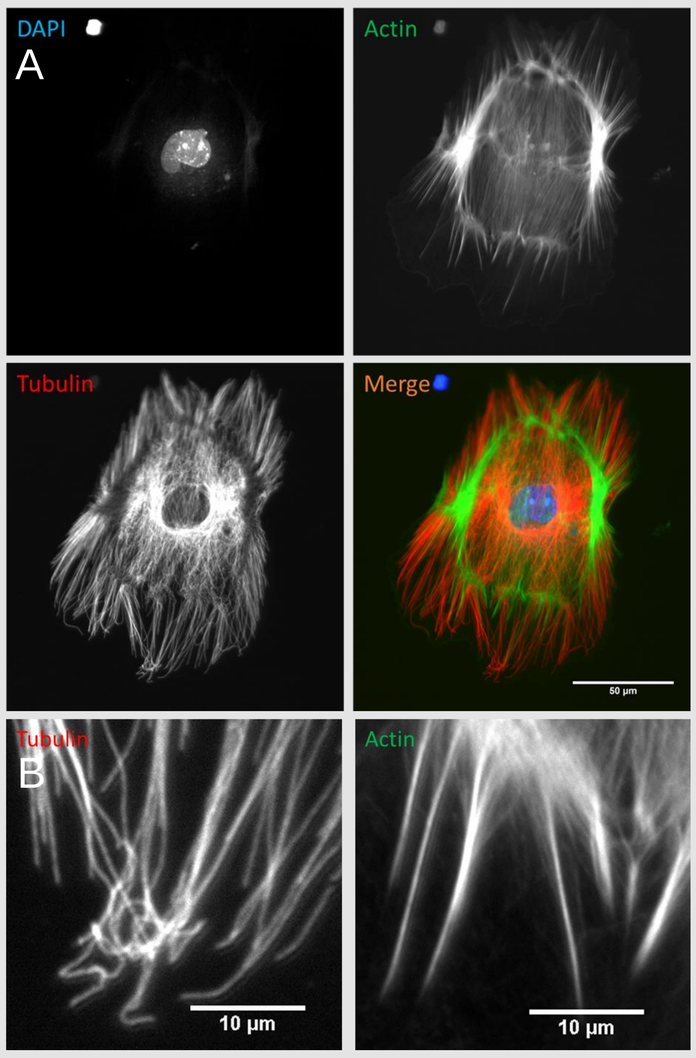

Fig. 4: Primary murine astrocytes imaged in the FLUMIAS breadboard model. (A) Formaldehyde fixed astrocytes, stained: DAPI (blue), actin (green), tubulin (red). The nucleus, as well as the actin and tubulin cytoskeleton could be imaged in a good resolution. Single microtubules were clearly discernible. The actin cytoskeleton, stained via Phalloidin was resolved nicely, with stress fibers as well as smaller actin filaments visible as shown in (B). *From FLUMIAS SRM testing 2022*

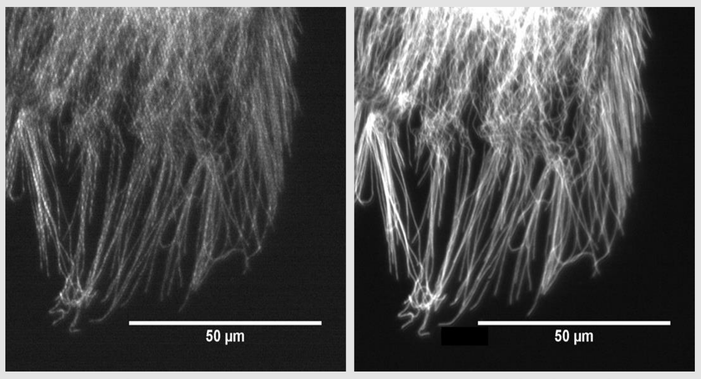

Fig. 2: The left picture shows the single LC mode, used for focus finding or for quick overview images. The SIM-grid is visible since in this mode only one of the 7 SIM raw images is shown, shortening the image acquisition time but reducing image quality of a full SIM picture, as seen on the right. *From FLUMIAS SRM testing 2022*

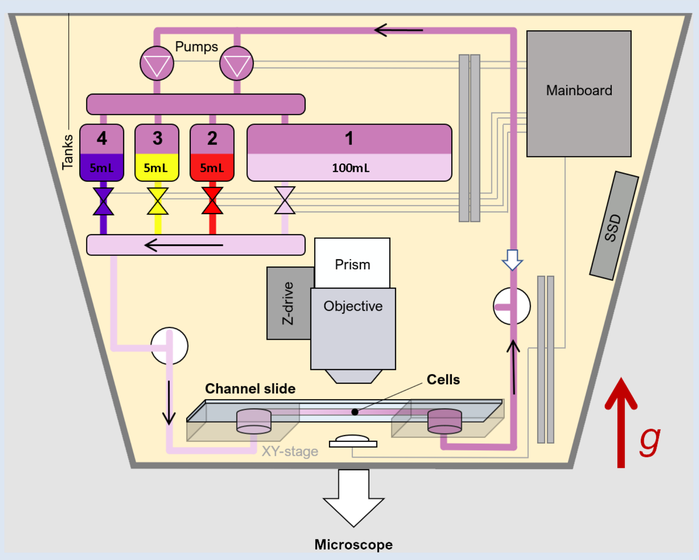

Fig. 3: Schematic EB-Overview depicting the pumps, fluid tanks, sample, electronics and objective. *From ESR CANCEROIDS, MTRM, Uni Magdeburg*

Fluorescent Live-Cell Imaging at near-confocal resolution by SIM technique

Ground-testing of the FLUMIAS Science-Reference-Model revealed the capabilities of the microscope comparable to confocal microscopy. Two different types of specialized Experiment Blocks (EBs) are currently being designed: for plants (EB-P) and for cells (EB-C). Within the EB-C, a 4-channel or 1-channel slide can be utilized. It will feature a closed-circuit liquid system for the perfusion of nutrient solutions. The EB-P will be equipped with a phyto-LED, local cooling at the sample down to 18°C and specialized slides for the imaging of the plant root or shoot.

Imaging parameters include a standard Field of View (FoV) of 400µm x 350µm (using a 40x air NA 0.95 objective), with multi-dimensional acquisition modes such as tile/mosaic imaging to create overview images of regions of interest or the whole slide, time lapse acquisition with a frame rate of up to 4 fps (in SIM mode), as well as z-stacks. The microscope supports brightfield and fluorescence illumination with 4 excitation lasers (405 nm/ 488 nm/ 561 nm/ 640 nm) at 10 mW power each. Each EB will have one air objective installed, in the range of 10-40x to be chosen from.

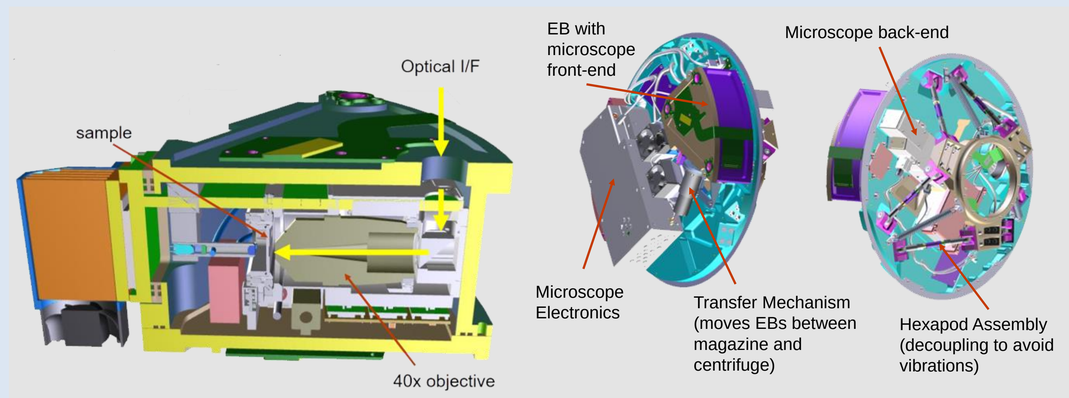

Fig. 5: On the left a cut through the EB shows the optical path from optical I/F to the objective and to the sample. The centrifuge-part on the right consists of the microscope and a transfer-mechanism from the magazine, which transfers one EB for centrifugation and imaging. *From FLUMIAS ISS hardware description by Airbus*

Incubation at Varying Gravity-Levels

FLUMIAS provides the ability to incubate various samples in adaptable Experiment Blocks (EBs) with automated medium exchange and a controlled temperature environment (25-40°C, 0.5°C increment). During the experiment run, perfusion of up to 4 different fluids is possible, e.g. nutrient solutions, pharmacological stimulants, or staining solutions (1x100ml, 3x5ml). The EBs can be transferred to the centrifuge which was designed for minimal vibration and will be capable to accelerate the experiments from 0*g* to 1*g* within 3 seconds and from 1*g* to 0*g* within 7 seconds.

FLUMIAS is a project of the German Space Agency at DLR (German Aerospace Center), developed by Airbus Friedrichshafen, based on an innovative microscope by TILL I.D., Martinsried. FLUMIAS will be handed over to ESA as national contribution to the SciSpacE programme for organizing European utilization and operation on ISS. SciSpacE Research programme is organized via Announcements of Opportunities and international usage will be possible in future opportunities as well.

27th ELGRA Biennial Symposium
September 06th – 09th , 2022
Lisbon, Portugal

Contacts DLR Space Agency
PD Dr. Markus Braun
Dr. Catharina Carstens

Email: anna.carstens@dlr.de
m.braun@dlr.de
phone: +49 0228 447 367

Contacts DLR Cologne
Dr. Christian Liemersdorf
Yannick Lichterfeld
Theresa Schmakeit
Email: christian.liemersdorf@dlr.de
yannick.lichterfeld@dlr.de
theresa.schmakeit@dlr.de
phone: +49 2203 601 5403

Deutsches Zentrum für Luft- und Raumfahrt
DLR
esa
I.D.
AIRBUS
Neuro Space

Gefördert durch:
Bundesministerium für Wirtschaft und Klimaschutz
aufgrund eines Beschlusses des Deutschen Bundestages