FLUMIAS – high-resolution live-cell imaging on-board the ISS: measuring gravity-mediated dynamic changes in astrocyte reactivity and cytoskeletal remodeling

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Introduction

Microgravity affects all biological systems with effects that are nearly impossible to predict. The ISS provides a constant and prolonged microgravity environment excellent for cellular gravitational experiments. FLUMIAS (FLUorescence MIcroscopic Analysis in Space) is a newly developed research platform, which combines an automated microscope with a life support system and a centrifuge to apply variable gravitational loads up to 1*g* onboard the ISS. The project LAARA (Live Assessment of Astrocytic Reactivity under Space Conditions) aims to investigate how astrocytes, the most prominent type of glial cell in the brain, adapt to altered gravity. Astrocyte reactivity is thought to play an important role in brain physiological changes in prolonged space missions. This hypothesis originates in the role of reactive astrogliosis as a physiological mechanism of the brain following injury or infection. FLUMIAS enables live visualization of astrocytic adaptation processes and sensitivity thresholds to altered gravity. To increase the knowledge on astrogliosis and the impact of space conditions on the reactive state of astrocytes, cytoskeletal dynamics and mitochondrial activity will be investigated.

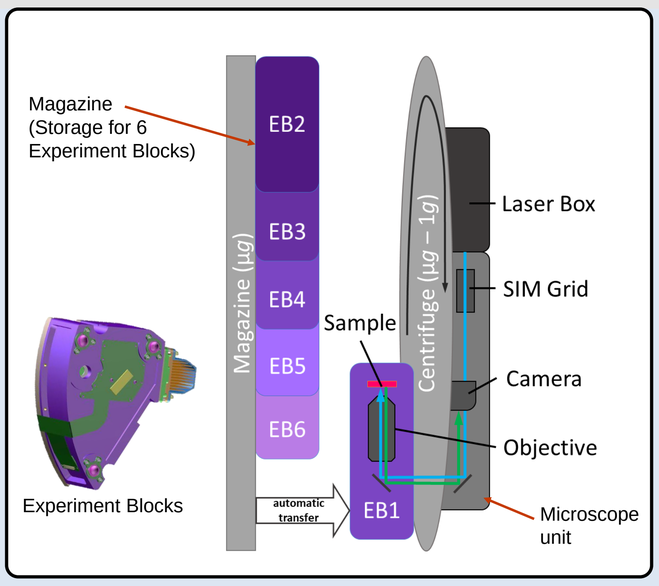

Fig. 1: The FLUMIAS Facility consists of a structured-illumination fluorescence (SIM) microscope equipped on a centrifuge and a storage magazine with life-support system housing 6 temperature-controlled Experiment Blocks. One EB is transferred onto the centrifuge and the sample is scanned by the excitation laser (blue) which was guided through the SIM grid to create the SIM structure. The light is focused onto the sample plane and fluorescent light (green) is emitted. The fluorescence is guided back and detected by a camera inside the microscope. *Lichterfeld, DLR Cologne and FLUMIAS ISS hardware description by Airbus*

FLUMIAS Payload Properties

- Ibidi 1- or 4-channel slide as sample holder
- Near-confocal resolution with lateral resolution <350nm and 4fps (x7 in line mode)
- Field of View: 400µm x 350µm
- Air Objective: 20x or 40x, NA=0.95
- Laser lines: 405nm, 488nm, 561nm, 640nm
- Life-support system for continuous medium supply and staining/stimulation 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*g* increments

Before a space mission is feasible, experimental parameters need to be verified by ground-testing. The FLUMIAS Science Reference Model in combination with the DLR Life Science Space Simulation Center (LSSC) in Cologne, Germany allow to verify and control essential parameters as a preparation for smooth experiment execution.

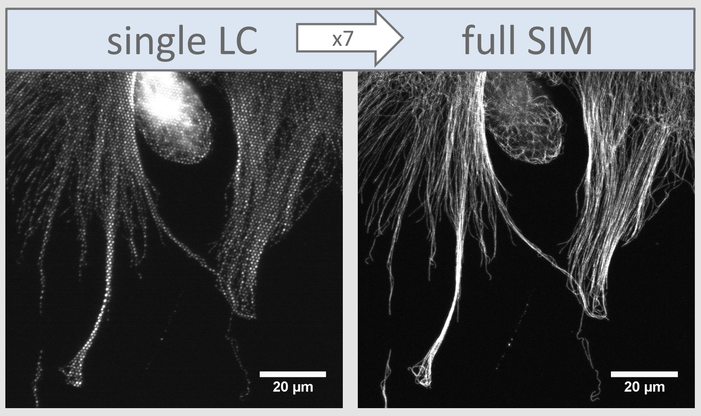

Fig. 2: Microtubules in astrocytes. The left picture shows the single LC mode, used for focus finding or quick overview images. The SIM-grid is visible since this mode features 1 of the 7 SIM raw images, shortening the image acquisition time but reducing image quality compared to a full SIM picture, as seen on the right. *FLUMIAS SRM Testing 2022, DLR Cologne*

A Novel and Compact µ*g* SIM Microscope

FLUMIAS (FLUorescencent MIcroscopic Analysis in Space; developed by the German Space Agency at DLR as a national contribution to the ESA SciSpace Program) is a newly developed research platform, which combines incubation of biological samples in varying gravity levels with laser-excited structured illumination microscopy (SIM) on a centrifuge. FLUMIAS houses 6 Experiment Blocks (EBs). Each EB contains a single sample slide, life-support system and an air objective. One EB can be transferred to the microscope on the centrifuge at a time (Fig. 1). Continuous imaging is possible even during acceleration from µ*g* to 1*g* at 0.1*g* increments.
This new variant of SIM technique enables spatial and temporal high-resolution fluorescent live-cell imaging. One SIM image is generated from 7 single raw images, resulting in images with a resolution comparable to confocal microscopy (Fig. 2) while the hardware was considerably reduced in size.

Astrocyte Reactivity as induced by Neuro-degenerative Diseases is Gravity-Sensitive

In space environments, effective cognitive function mediated by undisturbed neuronal integrity is a prerequisite for effective human performance. One key cell type in neuronal function are astrocytes which can become reactive in consequence to neurodegeneration, injury, infection, etc. Increased astrocyte reactivity could be the cause of cognitive and fine motor impairments in space and is also of interest for neurodegenerative diseases on Earth.

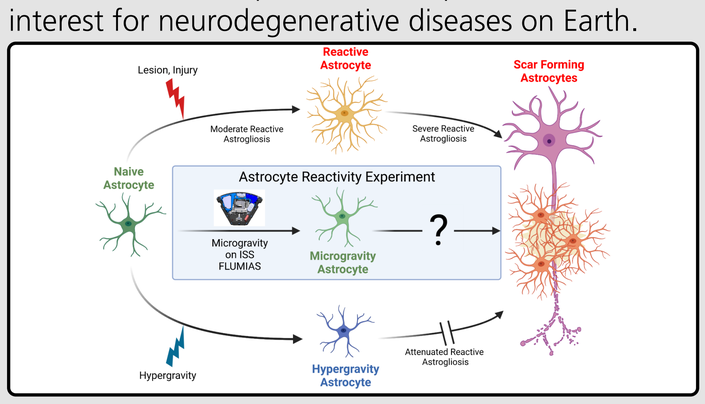

Fig. 3: Pre-studies identified a gravity-dependency of morphology and signaling in Astrocytes. The LAARA project aims to confirm these effects in prolonged timeframes of microgravity. *LAARA ESR, Lichterfeld, DLR Cologne*

Astrocytes have been shown to react gravity-sensitive in studies employing ground-based facilities (Fig. 3). Thus, their function and reactivity will be assessed employing FLUMIAS within the LAARA project. Prolonged periods of altered gravity are thought to influence murine astrocytes with regards to various pathways, morphological and behavioral changes, as well as adaptations of cytoskeletal structures and dynamics, mitochondrial activity and astrocytic reactivity. Reduction of cell area, polarization and migration rate have been shown in hypergravity (Lichterfeld et al., 2022). Simulated microgravity induced reduced cell spreading, increase in reactivity marker (GFAP) levels and accelerated migration (Lichterfeld et al., in preparation 2023). LAARA aims to utilize FLUMIAS for the visualization of (re–)adaptation processes of the major cytoskeletal networks (actin and tubulin as pictured in Fig. 6) and mitochondrial activity (Fig.5) of astrocytes to different gravity levels (i.e., microgravity, Earth, Moon, Mars) (Fig. 4).

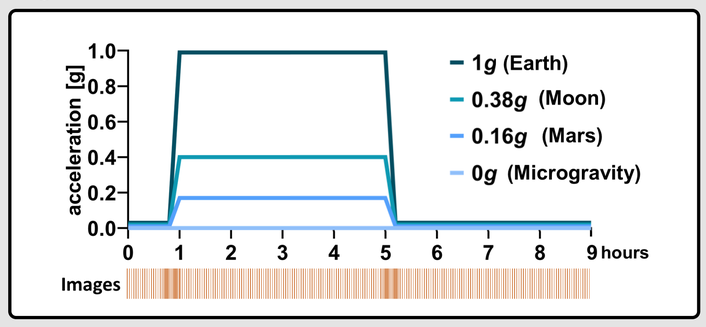

Fig. 4: LAARA timeline on the FLUMIAS facility. The EB will be imaged for 7 days with varying gravitational loads of µg, Moon, Mars and Earth. Images will be taken over a period of 9 hours, with rapid imaging during *g*-level transition. *LAARA ESR, Lichterfeld, DLR Cologne*

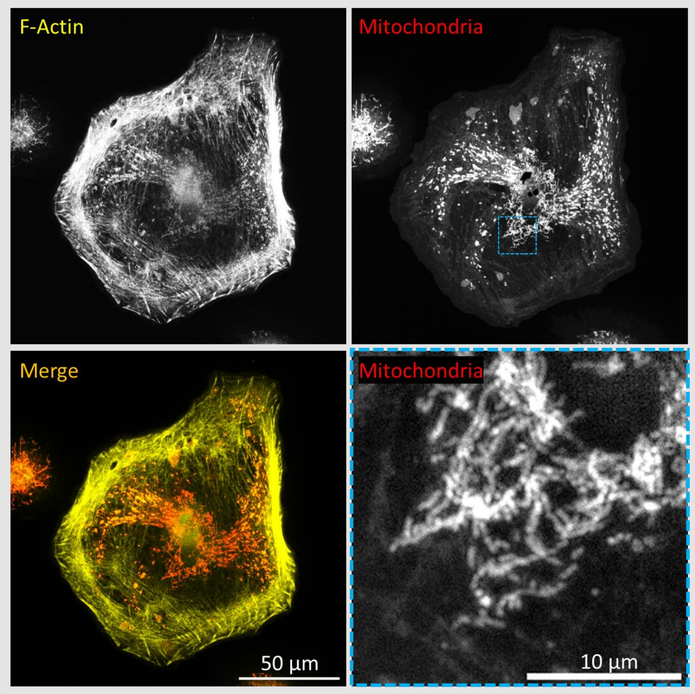

Fig. 5: Assessment of astrocyte activity changes by mitochondrial morphology and dynamics imaging. The cells were cultured and stained in Ibidi µ-Slides. Single channel (top) and the merged image (bottom left) are shown. Live cell imaging grants insights to the dynamics and distribution of actin fibers and mitochondria. *FLUMIAS SRM Testing 2023, DLR Cologne*

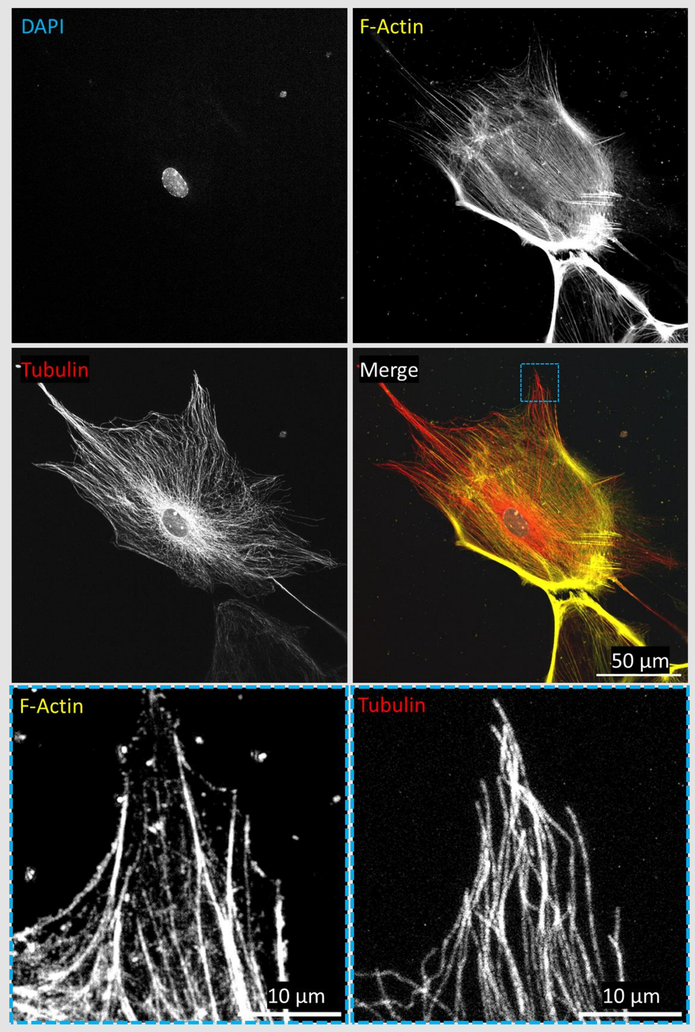

Fig. 6: Primary murine astrocytes imaged in the FLUMIAS breadboard model. Live Cell Imaging of astrocytes, stained: DAPI (blue), F-actin (yellow), tubulin (red) and a merge. The nucleus, as well as the actin and tubulin cytoskeleton are imaged in a confocal-like resolution. Single microtubules were clearly discernible. For cytoskeletal analysis, individual microtubules and F-actin fiber meshworks could be clearly discerned as shown in the bottom row. A similar microscopy quality was confirmed on the FLUMIAS EM. *FLUMIAS SRM Testing 2023, DLR Cologne*

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. The SciSpacE Research programme is organized via Announcements of Opportunities and international usage will be possible in future opportunities as well.

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