The LIFT module: A multi-cell type platform for biochemical analyses under altered gravity conditions

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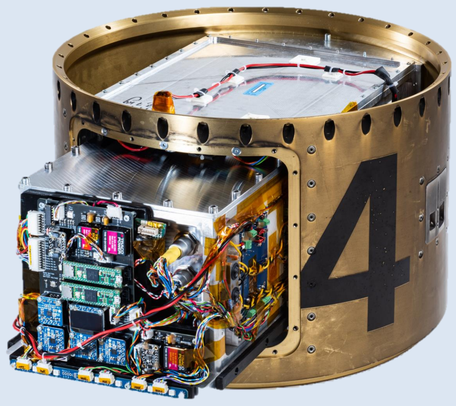
Introduction

The Laminar Inflight Fixation Technology (LIFT) module is a frequent-flyer payload designed to enable a fast and reliable chemical fixation of biological samples during different phases of a sounding rocket flight for subsequent biochemical analysis.

The modularity and reliability of the LIFT module were already proven during the rocket campaigns MAPHEUS-13 and -14 by chemically fixating different neuronal cell types, i.e. human-induced pluripotent stem cell (hiPCS) derived motor neurons, and primary murine cortical astrocytes. Analysing the effects of microgravity on these neuronal cell types will further our understanding of microgravity exposure on the brain and central nervous system of astronauts on a cellular level.



The LIFT module

The platform enables the analysis of different adherent cell types which can be either cultured on glass or polymer slides in the format of 76 x 26 x 1 mm. The timepoint of the chemical fixation is variable and can be chosen at different acceleration stages.

On the rocket flights MAPHEUS-13 and -14 the biological samples were chemically fixated directly after the launch (hyper-*g*) and following the microgravity phase.

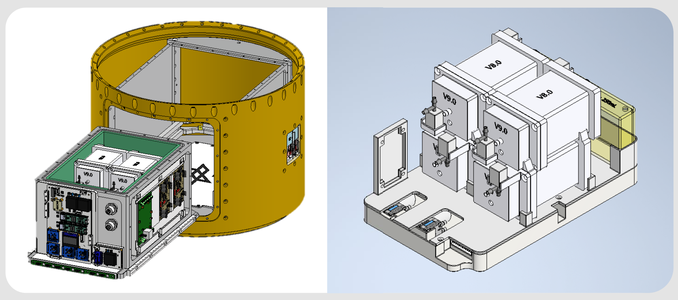


Fig. 1 | Overview LIFT insert for MAPHEUS sounding rocket (left) and inner payload with the reactor envelopes for cell containment (right).

The LIFT module comprises four containers (Fig. 1) with up to 32 microscope slides each, which are integrated back-to-back into custom-designed cultivation vessels (Fig. 2).

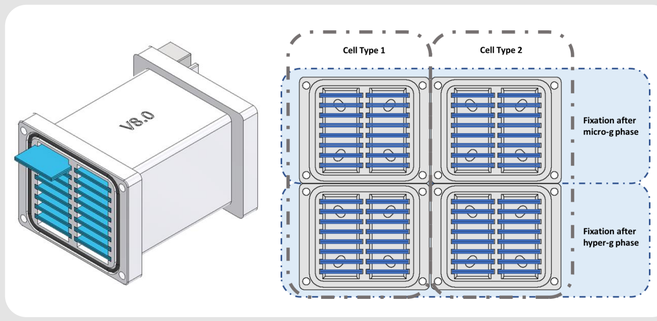


Fig. 2 | LIFT reactor filled with slides (left) and cross section of the distribution of samples with different cell types and acceleration states (right). In total 128 slides can be fixed by the LIFT module.

Fixation takes place under controlled conditions in a very short time with comparatively low shear forces. The samples are cultivated at physiological conditions in a pressure-tight chamber to ensure undisturbed environmental conditions during spaceflight (Fig. 3).

Corresponding Ground Controls

In order to validate the collected data, following controls were conducted:

- **Test-countdown** (1*g*): same procedure as Hot-countdown without lift-off
- **Campaign control** (1*g*): standard lab conditions during the campaign
- **Home-laboratory control** (1*g*): standard lab conditions in the home laboratory

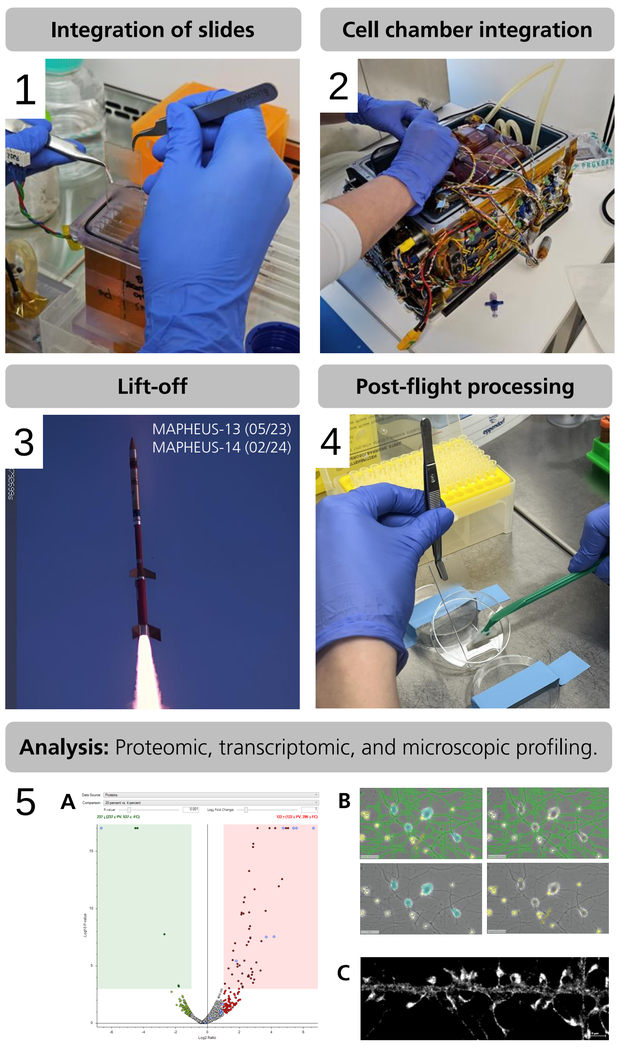


Fig. 3 | LIFT procedure during hot-countdown. **(1)** Integration of slides into custom-designed cultivation vessels. **(2)** Cell chamber integration into pressure stable vessel. **(3)** MAPHEUS-14 sounding rocket. **(4)** Post-flight processing. **(5)** Proteomic, transcriptomic, and microscopic analysis. **(A)** Proteomic profiling of human motor neurons, **(B)** morphological assessment with self-trained deep learning segmentation, **(C)** super-resolution imaging of potential ultrastructural changes on a synaptic level.

Results

The LIFT module continuously recorded environmental data during the flight including acceleration, absolute pressure, voltage level, and temperature. Culturing media temperatures were recorded in each reactor (Fig. 4), as well as the temperature inside of the pressure vessel.

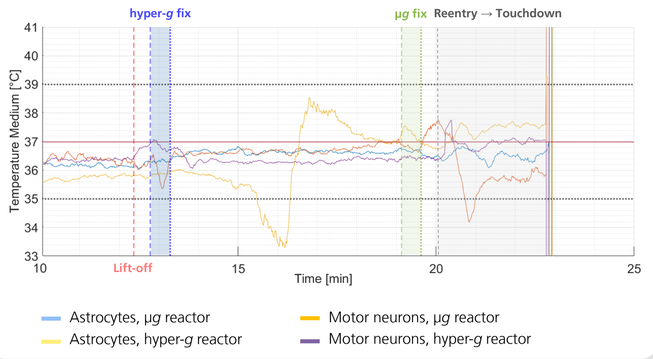


Fig. 4 | Medium temperature measurements during MAPHEUS-14 (the injected PFA was not adjusted to 37°C).

We were able to verify sample integrity and successful fixation performance of the LIFT module during post-flight analysis (Fig. 5). The samples exposed to real microgravity (µ*g*) on the rocket campaigns MAPHEUS-13 and -14 and their corresponding ground controls have been subjected to preliminary analysis and are now analysed further in regards of e.g. cell morphology, gene expression, as well as protein content.

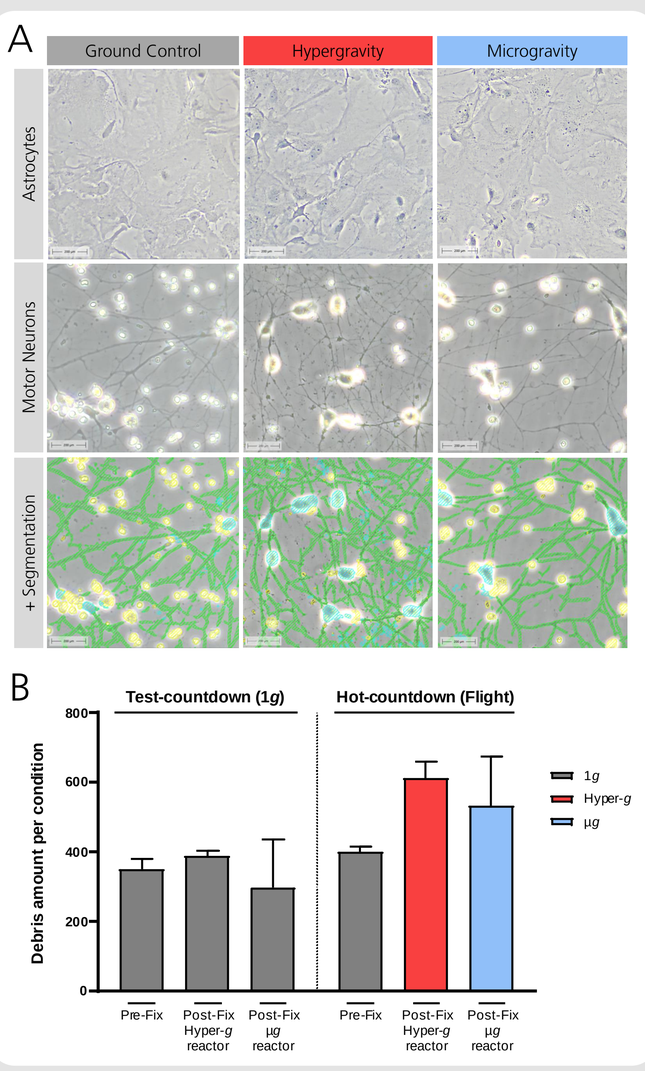


Fig. 5 | (A) Post-flight phase-contrast microscopy of motor neurons and astrocytes. For preliminary morphological analysis a self-trained deep learning segmentation (blue: soma; green: neurites; and yellow: debris) was applied. **(B)** Amount of debris per image of motor neurons during test-countdown (1*g*) and hot-countdown measured before and after PFA fixation.

Conclusion

The modularity and reliability of the LIFT module were proven with motor neuron and astrocyte cultures during two rocket launches. We are currently investigating potential alterations of motor neurons induced by altered gravity conditions. The technology will be used to further investigate neuromuscular models and more complex co-culture systems.

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