Executive Summary Report

4000126303/18/NL/KML – Mini Fluorescence Microscope (MFM)

MFM project

Document No.

ASRO-MFM-ESR-001

Issue 1, Rev. 1

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Revision History

Date	Issue	Revision	Section / paragraph	Reason for change
23 September 2022	1	0	All	New document
2 December 2022	1	1	All	Minor corrections to text
			Section 4	Figure 4-4 added
			Section 5	Table 5-1 and Figure 5-1 added

Contents

1 Introduction

1.1 Scope of the document

This document gives an overview of the Mini Fluorescence Microscope (MFM) project and summarises its findings. Background is given in Chapter 2. Aim of the project is explained in Chapter 3, and the resulting MFM breadboard instrument is presented in Chapter 4. Conclusions are presented in Chapter 5.

1.2 Acronyms and abbreviations

ASRO	Aboa Space Research Oy
CCC	Cell Culture Chamber
CMOS	Complimentary Metal-Oxide-Semiconductor
EGSE	Electrical Ground Support Equipment
ESA	European Space Agency
FPGA	Field-Programmable Gate Array
ISS	International Space Station
LED	Light-Emitting Diode
MFM	Mini Fluorescence Microscope
PCB	Printed Circuit Board
UTU	University of Turku

2 Background

The space radiation environment, together with microgravity or partial gravity, form a complex and dynamic environment that cannot be fully simulated on the ground. The health threats for humans in space are more or less characterized, but the underlying cellular mechanisms are poorly understood. For example, effects of high linear energy transfer radiation and synergistic impact of space radiation and microgravity on cells are not well characterized. To be able to realistically estimate the health risks of future manned missions, and to develop effective countermeasures, more research in the space environment is needed. Understanding the functionality of cellular mechanisms in space also has relevance for Earth applications, such as cancer and pharmaceutical research. Furthermore, studying the survivability of microorganisms in the space environment would be relevant for planetary protection policies, human space travel and more.

During the past couple of decades, several full-size microscopes have been developed for life sciences in space. However, these previously developed microscopes are bulky and often integrated parts of the facilities, which prevent versatile use. Particularly, they cannot always provide different g levels that are needed in gravity related research. There is a need for a more independently functioning and compact microscope that could be used in the platforms that enable real-time in-situ research in space. Development of this kind of instrument would advance radiation and gravity related biological research in space.

3 Aim of the project

The Mini Fluorescence Microscope (MFM) is an ESA project, implemented by Aboa Space Research Oy (ASRO) in collaboration with University of Turku (UTU), aiming to respond to the growing need of instrumentation for life science research in space. The objectives of the project were to assess the feasibility of developing and manufacturing the smallest possible fluorescence microscope to perform live cell imaging in space, and to build a breadboard model of the microscope to test and verify the concept.

The purpose was to reach the limits of feasibility while providing the best possible performance for scientific application. The application of MFM is for exploration programs dedicated to radiation, partial and microgravity related research as well as for use in ground-based facilities to prepare for and support flight experiments. The instrument is primarily developed for KUBIK, which is an incubator on-board the International Space Station (ISS) that includes a centrifuge. However, the possibility of further developing the microscope for other platforms, such as Biolab and ICE Cubes on the ISS, CubeSats, rovers or the ESA's upcoming uncrewed robotic laboratory Space Rider, was considered throughout the development.

4 Results

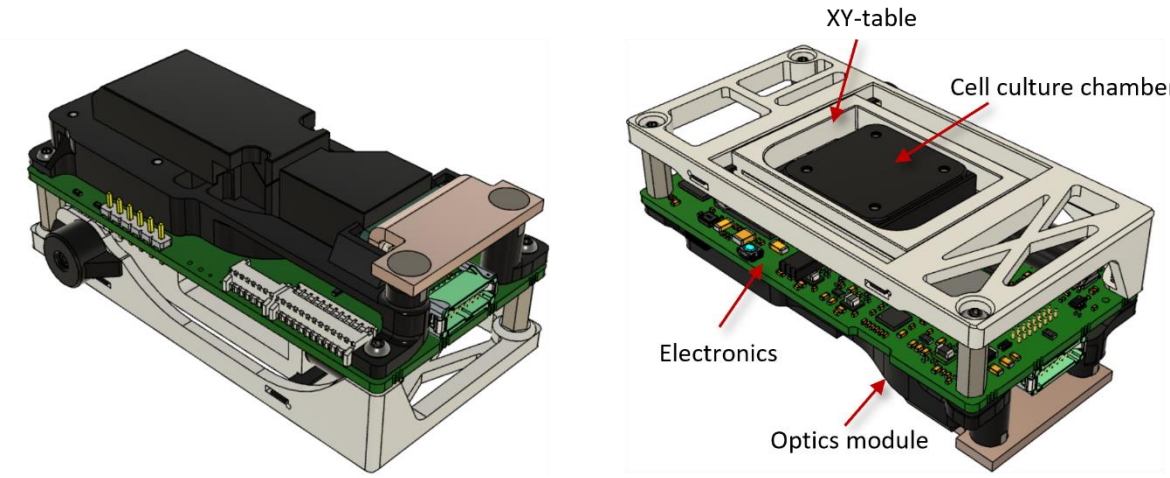

Figure 3-1. The MFM breadboard. Right: The MFM breadboard is based on a solid frame supporting the four subsystem modules, which are the optics module, electronics, XY-table and cell culture chamber. Left: The instrument viewed from the other direction.

The project started with a literature review on the state-of-the-art in miniaturized microscopes, followed by a trade-off analysis. The benefits and feasibility of many microscope features were reviewed. However, already at the early stages of the project, the miniaturization in the scale of KUBIK's experiment containers (extended container has dimensions of 82 x 42 x 31 mm^3) turned out to be challenging. A motorized XY-table was considered necessary to identify areas of interest and select several fields of views within the cell culture. Other additional features (such as fluidic system for media exchange) were discarded in order to provide more space for the optics development. The design and manufacturing phases were carried out in an iterative way, meaning that subsystems were tested separately and improved before the manufacturing and integration of the whole instrument. The resulting design is realized in a modular way, providing the possibility to, for example, change the whole optics module to match other kinds of fluorophores.

The resulting MFM breadboard is based on a solid frame supporting four subsystems modules, which are the optics module, cell culture chamber (CCC), XY-table and electronics. Assembled unit is shown in Figure 4-1. The MFM optics module is presented in Figure 4-2. The optical path is designed in epi-fluorescent configuration. Two-colour imaging is achieved by two different LEDs, which are on a single excitation LED PCB that is attached to a heatsink. The light beams are combined into one optical path with a dichroic mirror (DM1), and a filter (F1) is used to limit the broad spectra of the LEDs. Separation of the excitation and emission light paths is performed with the use of another dichroic mirror (DM2). Two filters (F2-1 and F2-2) are used to guarantee that only emitted light is delivered to the camera sensor (CS), which is a monochrome CMOS sensor. Silver mirrors (M1-M3) are used to direct the light along the optical path. The optical components are assembled into a 3D-printed optics module.

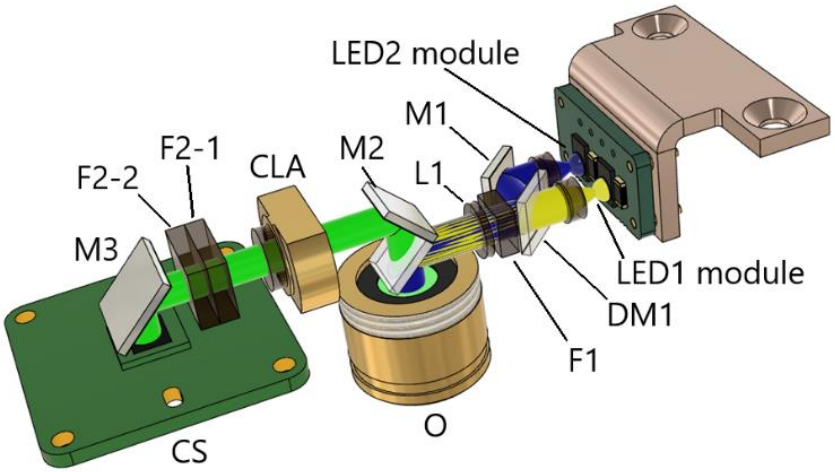

Figure 4-2. The MFM optics module. LED1 and LED2 modules - excitation LEDs, DM1-DM2 - dichroic mirrors, M1-M3 - silver mirrors, L1 - lens, F1 - excitation filter, O - objective, CLA - correction lens assembly, F2-1 and F2-2 - emission filters, CS - camera sensor.

The MFM CCC has a volume of 335 µl, and the chamber is built by two parts which are screwed together by four screws. Cover glass and O-ring sealing are squeezed between chamber body and lock. The CCC is placed on top of a XY-table that allows the movement of the sample. The table is operated by two stepper motors, which enable the scanning of the CCC in order to find the area of interest before imaging or running a grid imaging sequence.

The electronics module consists of a custom-made PCB with a field-programmable gate array (FPGA) interfacing to the camera sensor. The electronics module (the main PCB) is located between the optics module and the XY-table, and it has a hole in the middle, allowing the objective to go through it and thus, maximizing the critical dimension for the objective design.

An electrical ground support equipment (EGSE) is used together with the MFM breadboard. With the EGSE, it was possible to simplify the MFM internal design by moving data conversion to an external unit. The EGSE consists of a PC with MFM software, the MFM EGSE hardware, power supply unit and cables. The MFM EGSE hardware is based on a commercial FPGA integration module, and it has dimensions of 120 x 80 x 21 mm^3 without casing.

All advanced image processing is done on PC, with the MFM µManager software. µManager is free and open-source software for control and automation of microscope hardware. For the MFM to be controllable from the µManager, a specific device adapter C++ class has been written, which implements the abstract µManager interface to link the functionality of the device to the parameter setting fields on the graphical user interface, as well as processes the raw image data. The parameters that the user can set include gain, exposure time, XY-position, and selection of the LED and its brightness. µManager offers some tools for image processing, such as changing the contrast, but main image processing and analysis tools are available in the ImageJ, which is automatically installed alongside the µManager. With ImageJ and its plugins, it is possible to, for example, reconstruct large images from an arbitrary number of

tiled input images. This enables the creation of large field-of-view images with high accuracy from the specimen inside the CCC by taking adjacent images by incrementally moving the XY-table.

The resulting MFM breadboard has dimensions of 83.2 mm x 42.2(+5) mm x 33.0 mm. The +5 mm means the stepper motor lead screw that do not fit inside the envelope (see Figure 4-1, left side). Thus, the resulting breadboard would not fit inside the KUBIK experiment container as it is, but solutions for reducing the volume already exist. The stepper motor used in the breadboard was selected because it was a standard size from the supplier, and therefore, available with considerably shorter lead time. However, it is possible to order stepper motors that fit inside the KUBIK experiment container, if needed in the future projects.

The breadboard of the microscope was built to test and verify the concept. The functionality of the breadboard was tested, for example, by taking fluorescence images of different samples. A screen capture of the µManager user interface showing this example test image of Convallaria root, taken with the breadboard MFM, is shown in Figure 4-3. Another example test image, with Vimentin structures stained for blue, is presented in Figure 4-4.

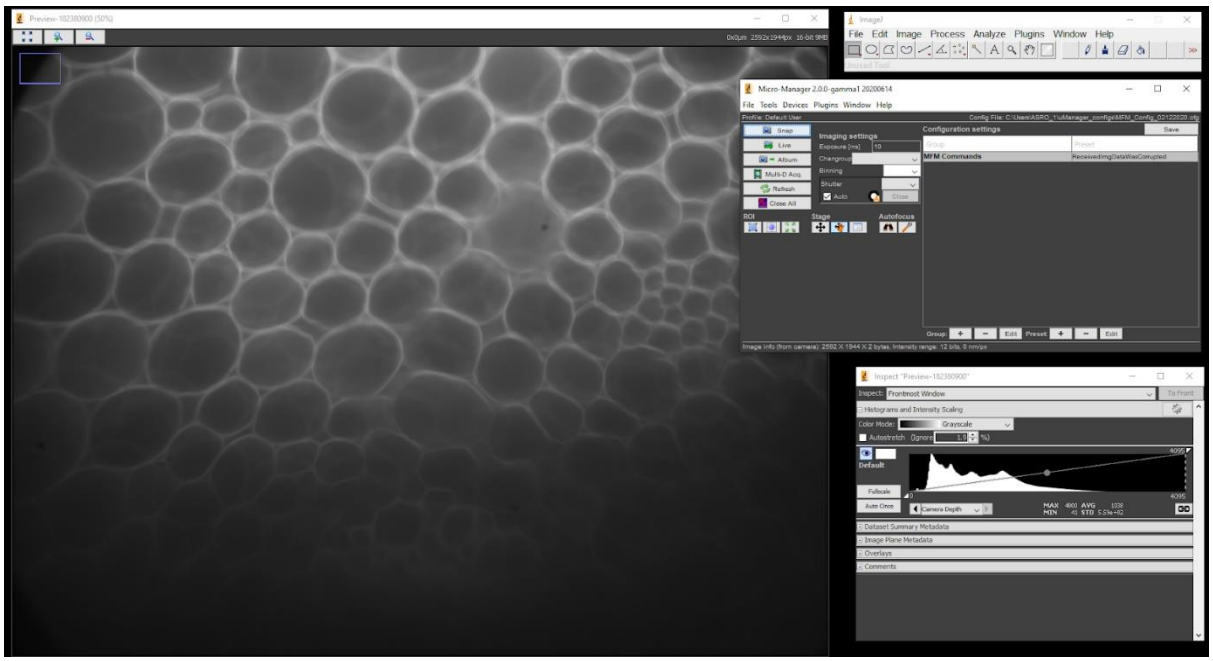

Figure 4-3. Test image taken with the breadboard MFM and shown in the µManager user interface. Test target is Convallaria rhizome training slide, imaged with blue LED. Image shown on the µManager interface screen is 50 % scaled version of the original 2592 x 1944 px image. Image covers 516 µm x 387 µm area of the sample.

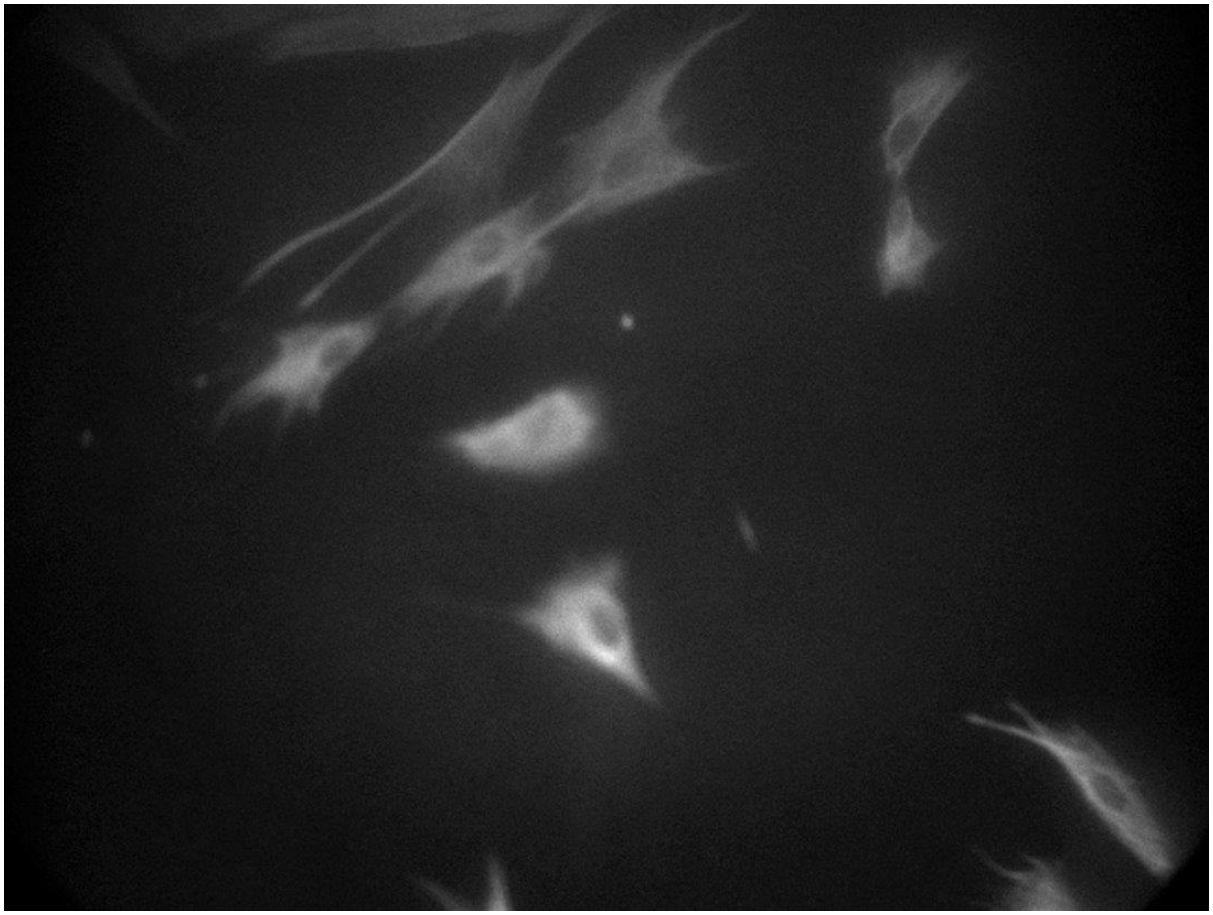

Figure 4-4. Example test image with Vimentin structures stained for blue.

5 Conclusions

The objective of the MFM project was to develop a breadboard model of a miniaturized fluorescence microscope that could perform live cell imaging in space. The resulting breadboard consists of four subsystem modules: the optics, cell culture chamber, mechanics and electronics. In addition, MFM electrical ground support equipment and µManager user interface were developed during the activity. Main characteristics of the resulting Mini Fluorescence Microscope are presented in Table 5-1. The resulting MFM breadboard, EGSE and cell culture chamber are shown in Figure 5-1.

The goal of the project was to reach the limits of feasibility and it can be concluded that miniaturization at the scale of the current design fulfilled that goal. Compact and complex design required several iterations between mechanics, electronics and optics. The project resulted in multiple valuable lessons learned, and solutions to the faced challenges. Therefore, the breadboard MFM provides a good basis for future developments of the microscope.

Potential platforms for a flight qualified MFM would be different facilities on-board the ISS, CubeSats, rovers or the ESA's upcoming uncrewed laboratory Space Rider. The platforms on-board the ISS, as well as the Space Rider are suitable for gravity related research. CubeSats could be used to study the synergistic impact of space radiation and microgravity, while rovers would be the platforms to search for extraterrestrial life.

Table 5-1. Characteristics of Mini Fluorescence Microscope.

Characteristics of the Mini Fluorescence Microscope	
Dimensions	83.2 mm x 42.2(+5) mm x 33.0 mm
Mass	95 g (without CCC)
Excitation LEDs	Blue and Amber (482/587 nm)
Resolution	530 nm
Field of view	516 µm x 387 µm (field of view of 1700 µm x 1450 µm can be achieved with automatic grid imaging sequence)
Scanning area	9.7 mm x 5.0 mm
Power consumption at +25 ˚C (nominal/peak)	1.91 W / 4.97 W
Power consumption at +40 ˚C (nominal/peak)	2.05 W / 5.10 W

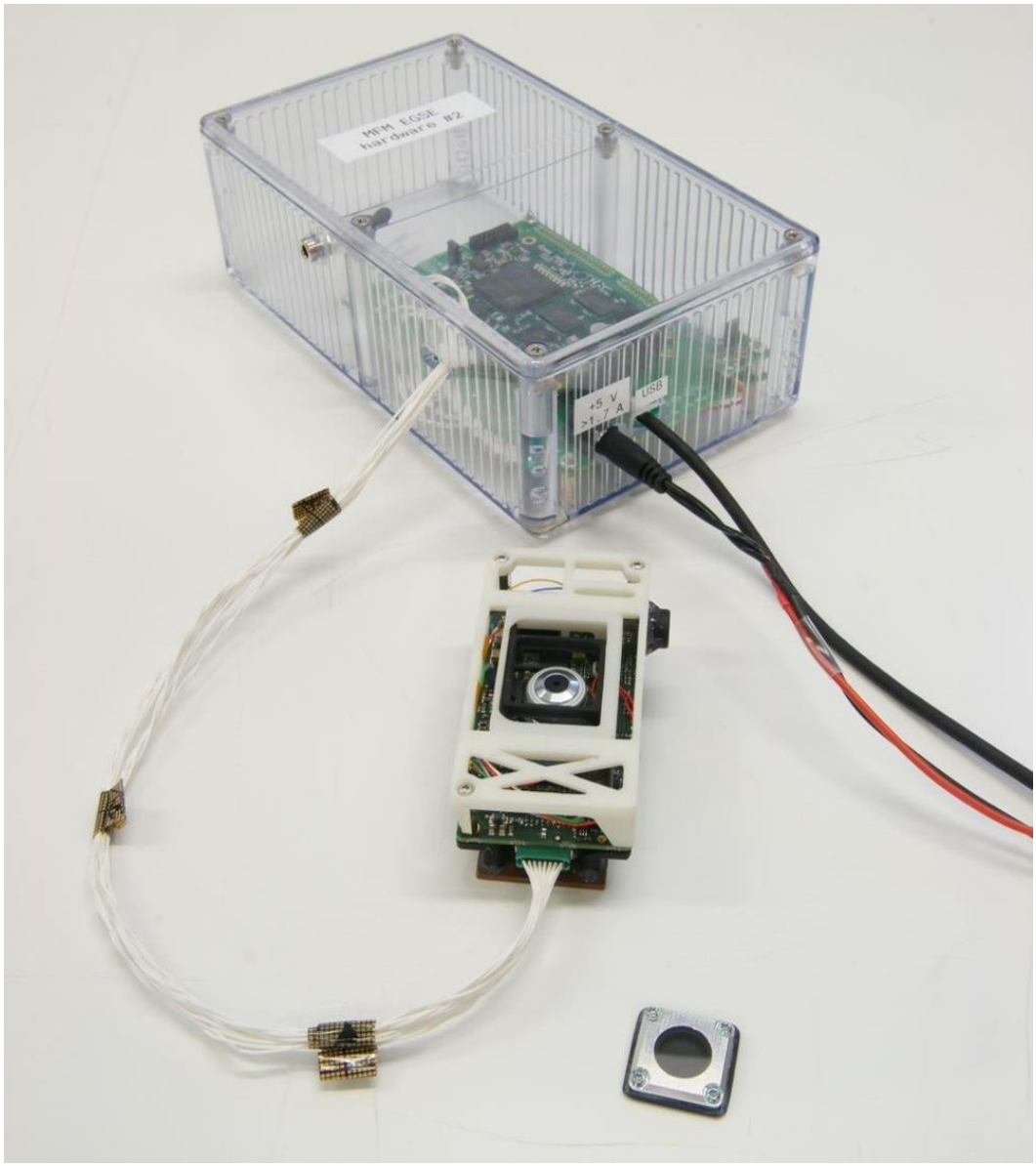

Figure 5-1. MFM EGSE, MFM breadboard and cell culture chamber.