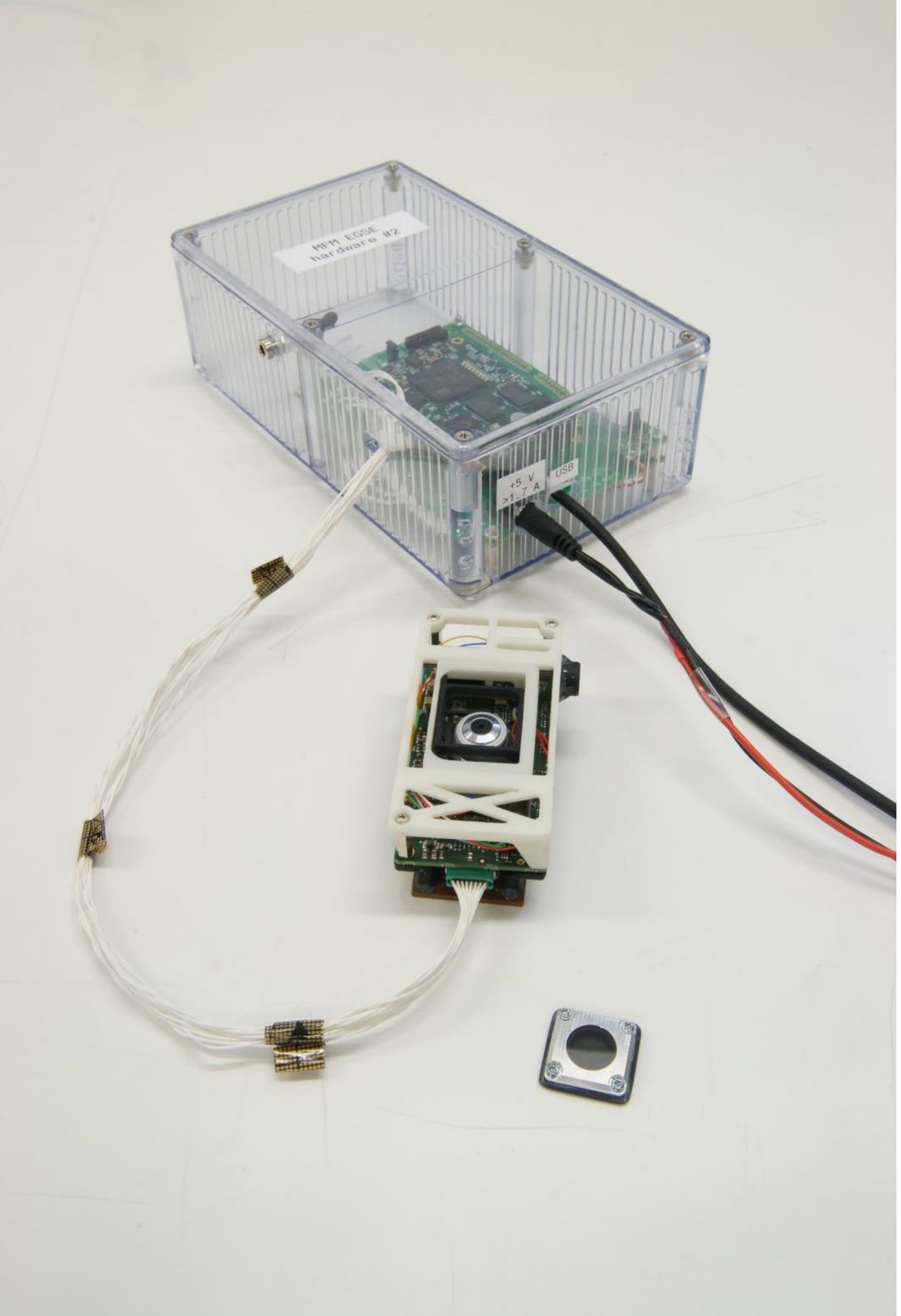


MINI FLUORESCENCE MICROSCOPE – MFM (AO9412)

Final Presentation

23rd November 2022

Agenda

1. Project overview
2. Performed work
3. Results
4. Recommendations
5. Conclusions

MINI FLUORESCENCE MICROSCOPE – MFM

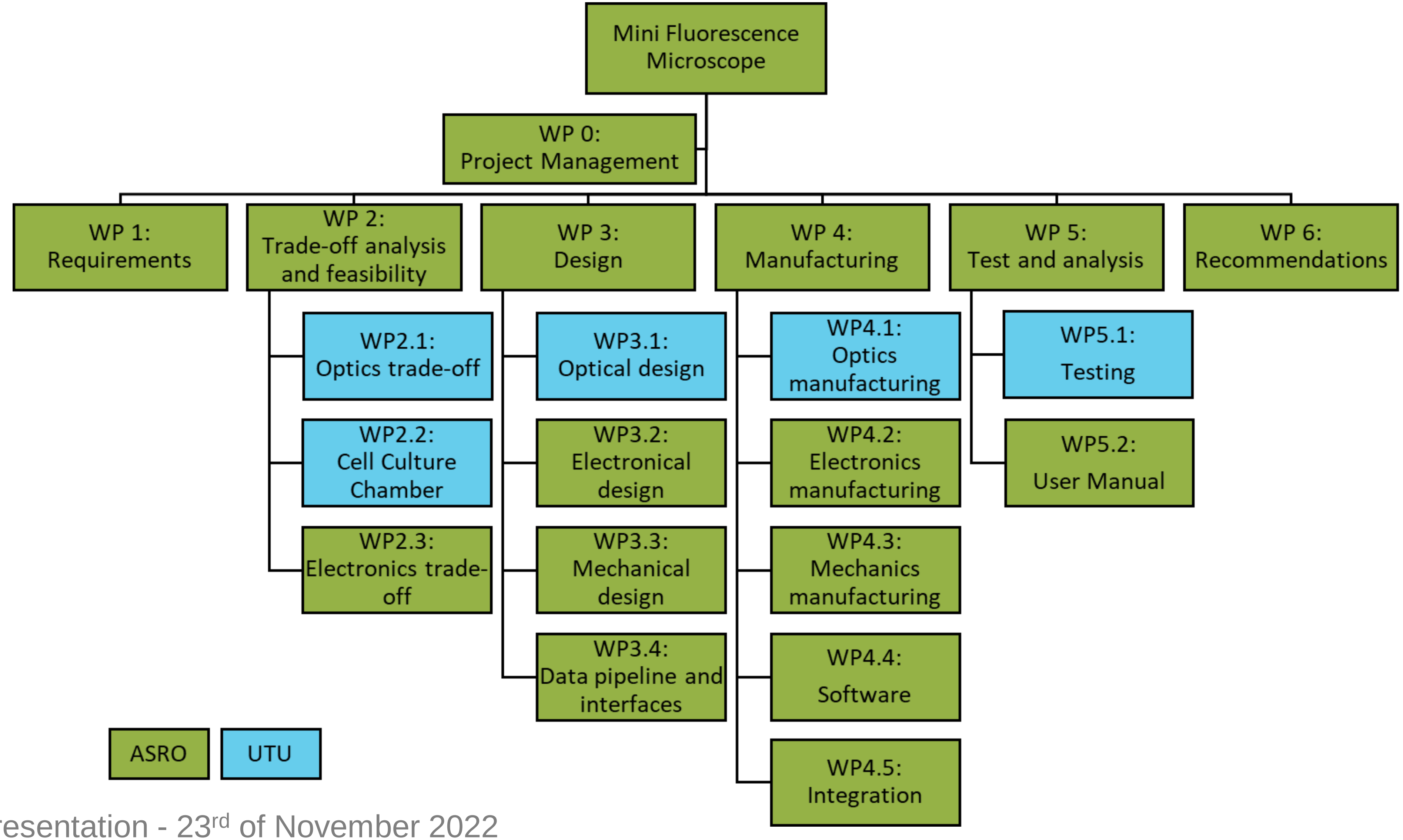
Overview of the activity

- OBJECTIVES
  - Assess the feasibility of developing and manufacturing the smallest possible fluorescence microscope to perform live cell imaging in space
  - Build a breadboard model of the microscope to test and verify the concept
  - Developed for KUBIK, but other platforms were considered throughout the development
  - The domain of scientific applications for an MFM are diverse, for example:
    - Study of flux of proteins in cells resulting from radiation exposure,
    - Explore in detail the morphological changes in cells;
    - Cytoskeleton examinations,
    - Membrane mechanosensitive channels or any other protein that can be tagged with a fluorescent molecule like e.g. GFP (green fluorescent protein) or YFP (Yellow Fluorescent Protein) and others.
- PROJECT DURATION AND COSTS
  - 16 months
  - FFP 350 k€

Work packages

- TASK 0 - PROJECT MANAGEMENT
  - Project management per SoW requirements
- TASK 1 – REQUIREMENTS
  - Review of requirements and analysis of the State of the Art for mini fluorescent microscopes.
- TASK 2 – TRADE-OFF ANALYSIS AND FEASIBILITY
  - Perform a trade-off analysis between possible microscope concepts and assess the feasibility to design and manufacture the down selected MFM concept
- TASK 3 – DESIGN
  - Develop a detailed design of the down-selected MFM concept, chosen in the trade-off exercise.
- TASK 4 – MANUFACTURING
  - Manufacture a breadboard model of the MFM and elaborate a corresponding test plan to verify its performance.
- TASK 5 – TEST AND ANALYSIS
  - Test the MFM, and analyse the results, against performance expectations.
- TASK 6 – RECOMMENDATIONS
  - Draw conclusions from the tests and make recommendations for the space application of MFM.

Work breakdown structure

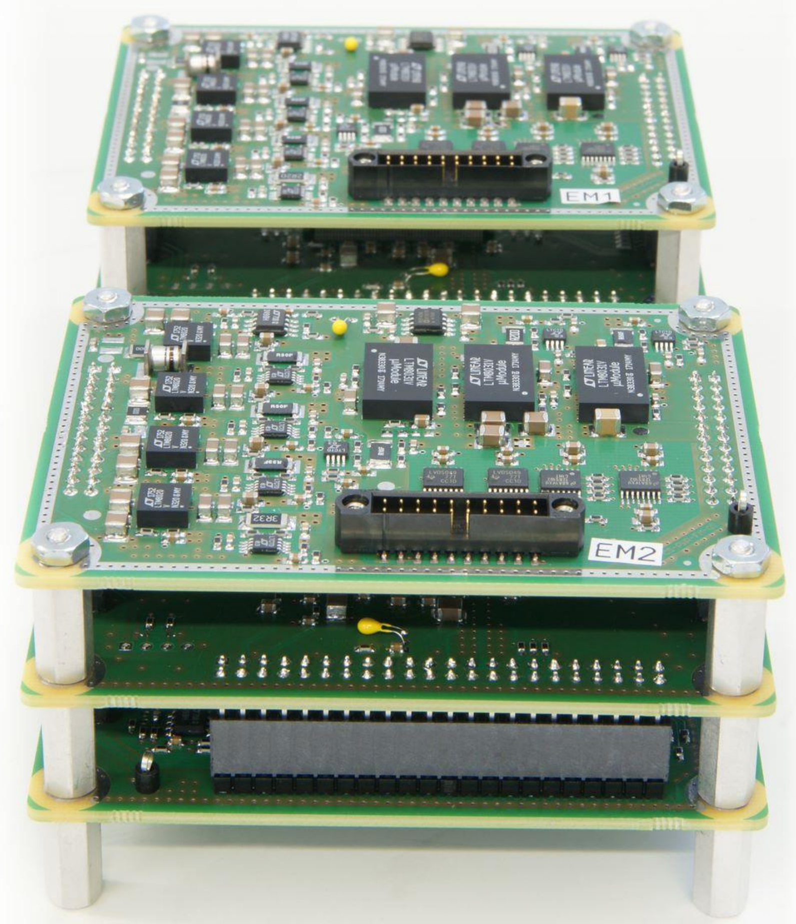


Members of the consortium

Member	Role	Tasks	Resources
Aboa Space Research Oy (ASRO)	Prime contractor	• Management • Overall design • Electronics • Imaging	1) Project manager 2) SW developer 3) Mechanical engineer 4) Electronics designer
University of Turku (UTU)	Optics developer	• Optics development / Imaging • Fluorescence expertise	1) Optics designer 2) Cell culture chamber designer

Members of the consortium - ASRO

- Research and design agency based in Turku, Finland
- Founded 1999 and has over 20 years of experience in international space technology projects
- Specialized in development of high-quality electronics systems for instrumentation projects
- Previous and current projects:
  - Space weather/radiation instruments for SOHO (ERNE), BepiColombo (SIXS), Solar Orbiter (LET), NOAA Space Weather Next L1 (XFM) and CubeSats (RADMON, XFM-CS)
  - Dosimeters for ISS, Artemis 1 and Lunar Gateway
  - Optical filter development for ALTIUS
  - Space debris observation technology projects



PERFORMED WORK

Introduction

PROJECT

- Duration March 2019 -> November 2022.
- Two main challenges: Covid and lack of components
- The target was the flight version from the beginning. No shortcuts in the design approach which caused several unsolved small details in the design due to limited time and budget. Approach gave better overall understanding of the complexity of the system.
- Miniaturization was pushed to the limits. Compact and complex design required several iterations between mechanics, electronics and optics.

WP1 – Literature review and requirement updates

LITERATURE REVIEW

- State-of-the-art in miniaturised microscopes
  - → Smallest microscopes are used in animal brain research (not integrated instruments or not very good resolution)
  - → Small microscopes used in other biomedical applications (large in this project's scale)
  - → Microscopes used in space are large in this project's scale

- Components and their availability
  - → B/W CMOS sensor was selected over colour sensor due to better resolution
  - → Challenges with size requirement and component minimum order quantities

REQUIREMENT UPDATES

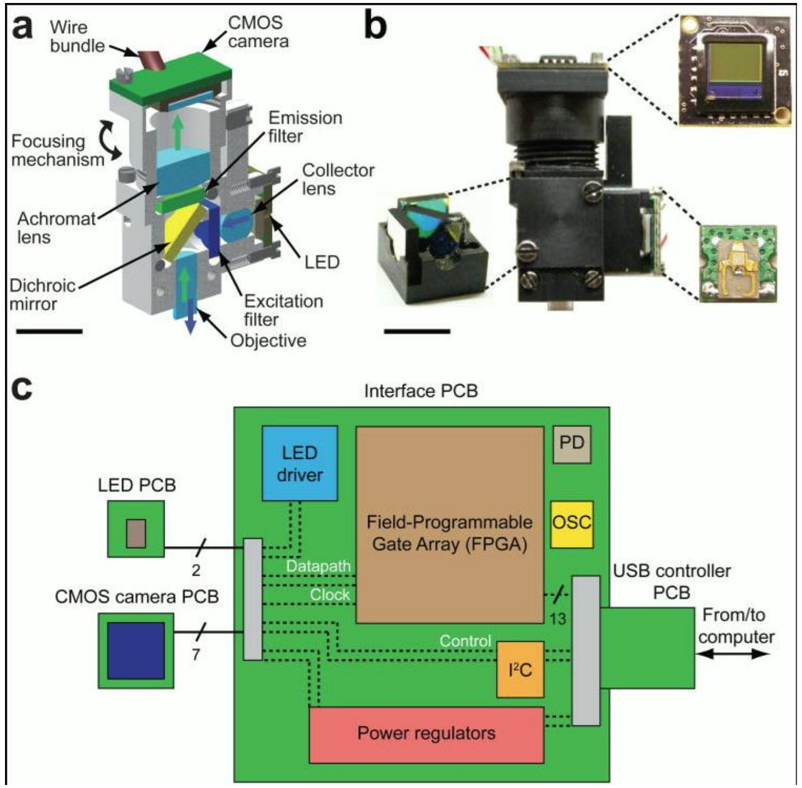
- Requirements were commented and some updated
- Most requirements were kept as is, as the objective was to assess the feasibility

WP1 – Literature review and requirement updates

Microscope	Ghosh	Liberti	Zhang	Forcucci	FLUMIAS-DEA	Jacob	Barbera
Reference	[RD-17]	[RD-18], FinchScope	[RD-25]	[RD-27]	[RD-30]	[RD-22], CHEndoscope	[RD-21], Miniscope
Sensor type	640x480 / 0.3 MP / Aptina Imaging MV9V021	640x480 / 0.3 MP / OmniVision OV7960	1280 × 1024 / 1.31 MP CMOS webcam / Logitech C160m	4096 x 2160 / 8.8 MP / Sony IMX121	4112 x 3008 / 12.3 MP / Sony IMX253	2592 x 1944 / 5 MP / APTINA MT9P031	752 x 480 / / 0.35 MP / OnSemi MT9V022
Pixel size	5.6 µm	6.0 µm	N/A	1.55 µm	3.5 µm	2.2 µm	6.0 µm
Resolution	2.5 µm (Nyqvist) 1.5 µm (Optical)	<10 µm	<2 µm	N/A	Resolution xy (theory) 0.3 µm, super resolution xy 230 nm	N/A	Cellular spatial resolution
Color / B&W	Color	Color	Color	Color	Color	Color	Monochrome
Field of View	600 x 800 µm	600 x 800 µm	1060 x 850 µm – 130 x 105 µm	diameter of 1.2 mm	N/A	500 mm across	1.1 mm × 1.1 mm
Optical Magnification	5x	~5x	8-60x	4.5x	40x	N/A	N/A
Frame rate	36 Hz (full frame) 100 Hz (ROI: 300 x 300 / 0.09 MP)	50 fps	30 fps	21 fps	69 fps	20 Hz for 648 x 486 pixels	10 Hz with a 400 × 400 pixels FOV
Image storage	No data storage	No data storage	No data storage	N/A	2 TB M.2 SSD 960PRO hard drive	No data storage	No data storage
Data interface	USB	USB	USB	N/A	N/A	USB	USB
Total volume	8.4 mm × 13 mm × 22 mm (optics	~10 mm x 10 mm x 30 mm (optics)	form factor: 4.2 x 5.5 lens to sensor: 5 - 48 mm	length of the optical system: 78.95 mm	400 mm x 200 mm x 90 mm	15.9 mm × 17 mm × 32.5 mm	12 mm × 12 mm × 20 mm

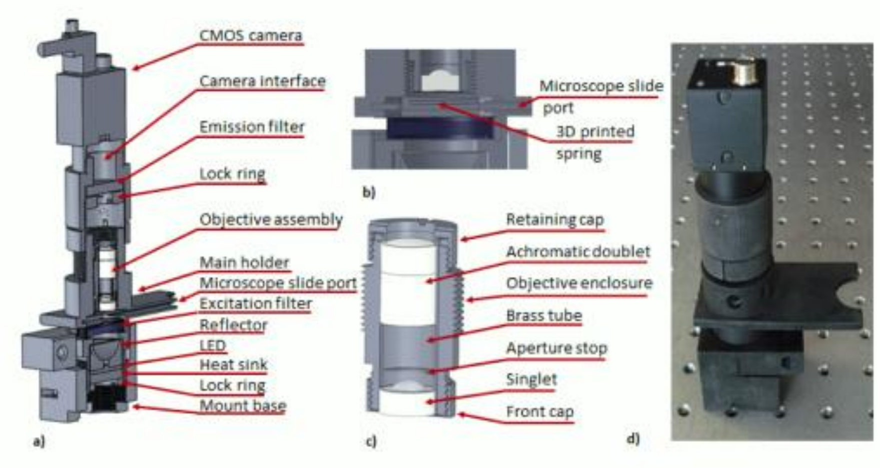
WP1 – Literature review and requirement updates

Ghosh, et al., Miniaturized integration of a fluorescence microscope, Nature Methods, 8, 871–878 (2011)



Thiel, *et al.,* Real-Time 3D High-Resolution Microscopy of Human Cells on the International Space Station, International Journal of Molecular Sciences, 8, 2033 (2019)

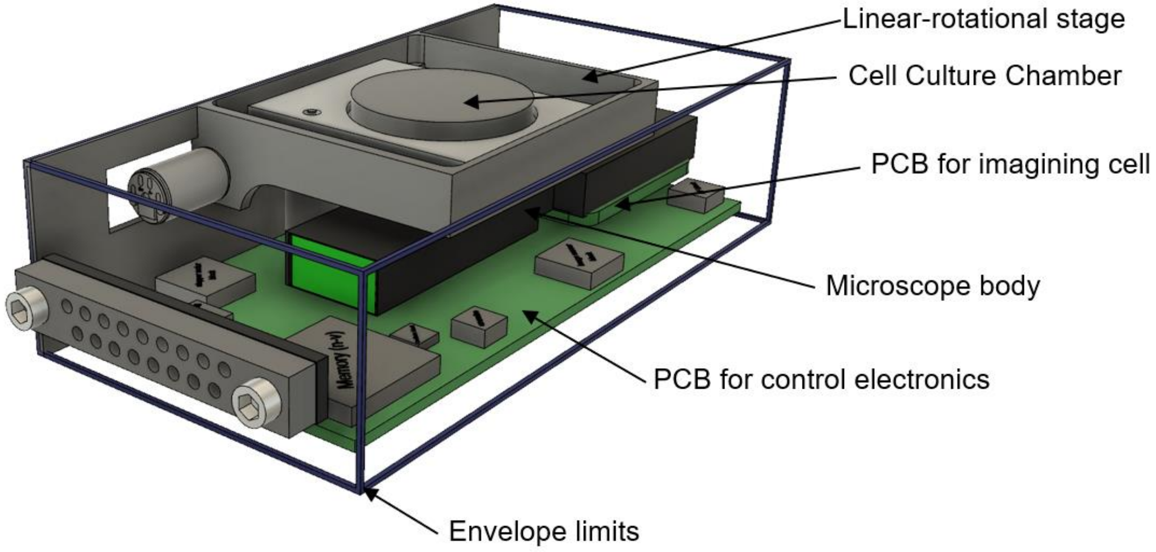
A. Forcucci, et al., All-plastic, miniature, digital fluorescence microscope for three-part white blood cell differential measurements at the point of care, Biomedical Optics Express, 6, 4433-4446 (2015)

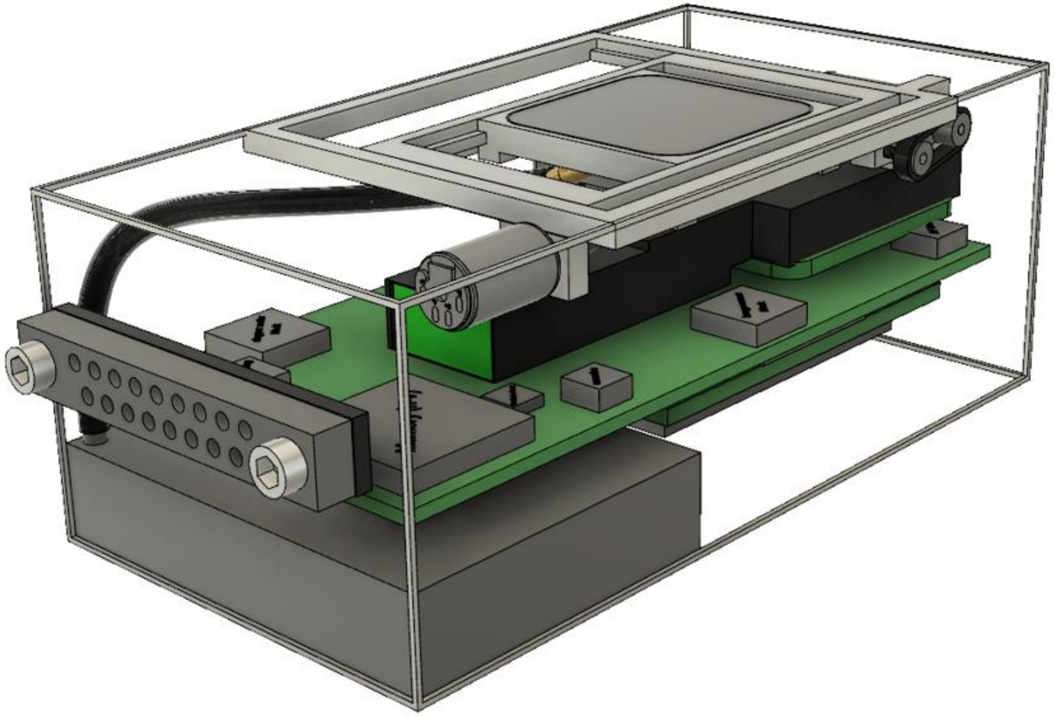


WP2 – Trade-off analysis

CONCEPTUAL DESIGNS

- Further research and trade-off on possible solutions
- Two conceptual designs were prepared, volume optimized version for KIC-SL and performance optimized version including microfluidic system for KIC-SL-E





WP2 – Trade-off analysis

SUBSYSTEM TRADE-OFF

- COTS control electronics → not small enough → Custom electronics
- Sensor selection → Sony IMX335 (best availability and performance)
- Cell Culture Chamber → no suitable COTS available → Custom made CCC
- Stages for sample moving → manual not possible → COTS stages not small enough → Custom made stage with two stepper motors

CONCEPT SELECTION

- In the end, neither of the concepts (Volume / Performance optimized) were selected as is
- Optical performance was preferred
- Decisions made in the WP2 review meeting:
  - → Volume requirement was changed to KIC-SL to KIC-SL-E
  - → Microfluidic system was discarded in order to provide more space for optics
  - → Requirement for dark field imaging was added

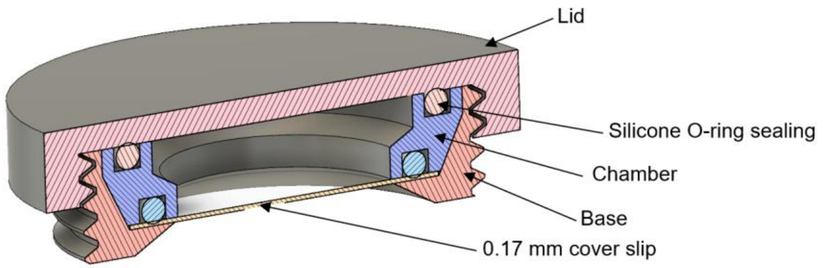
WP3 – Detailed design

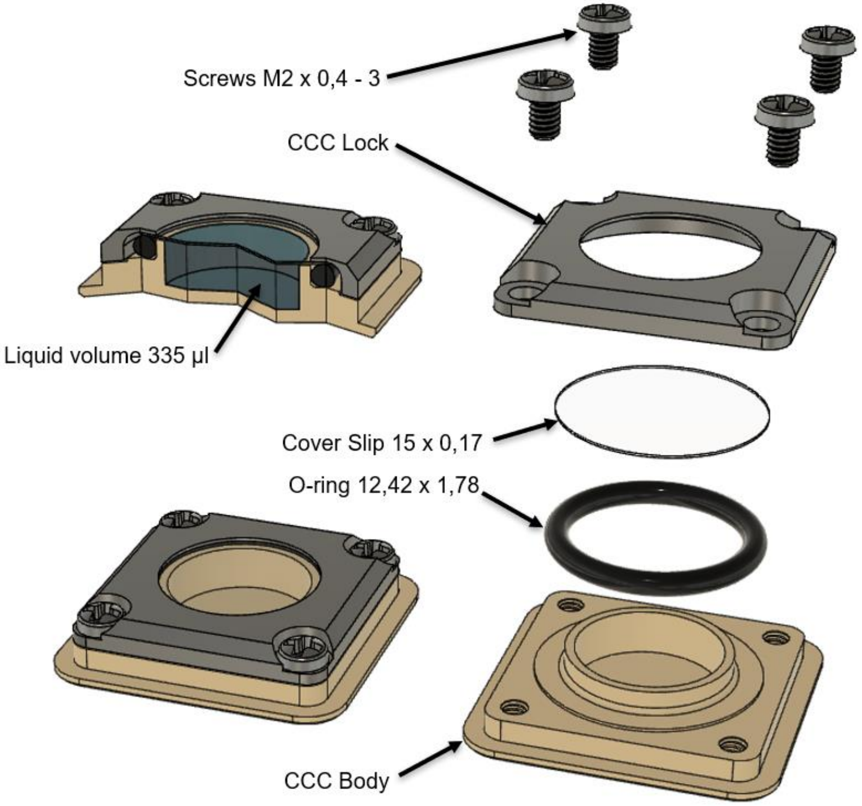
ITERATIVE DEVELOPMENT PROCESS

- Concept selected in WP2 review meeting as the baseline
- Subsystems were tested as stand-alone units and design was improved based on the results
  → Optics tested in a test bench, different CCC materials were tested, tests on mechanics and critical electronics
- Development of the MFM electrical ground support system (EGSE)
- Preliminary evaluation of the compatibility of MFM with other platforms

WP3 – Detailed design

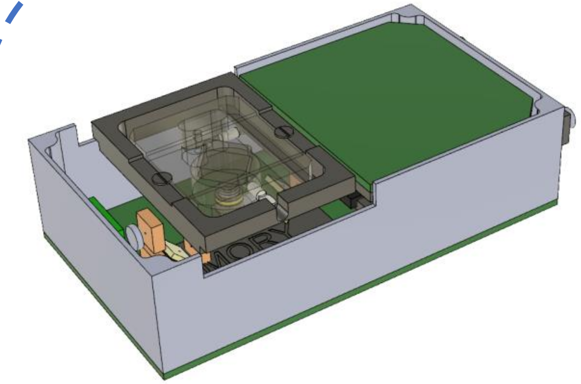
CONCEPTUAL DESIGNS of CCC

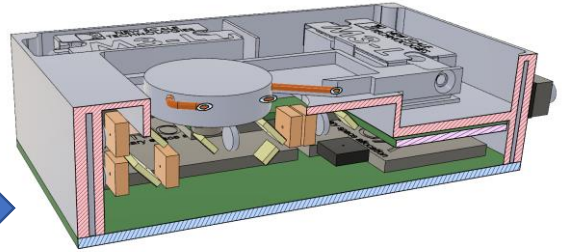


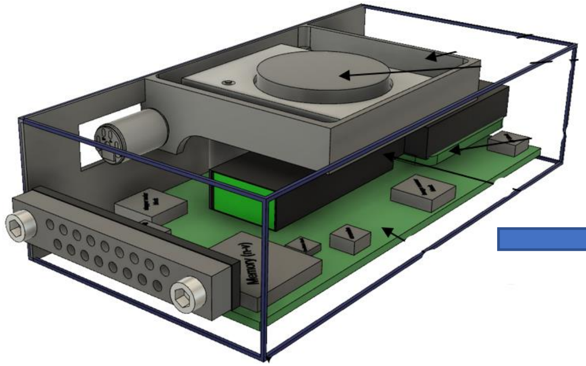


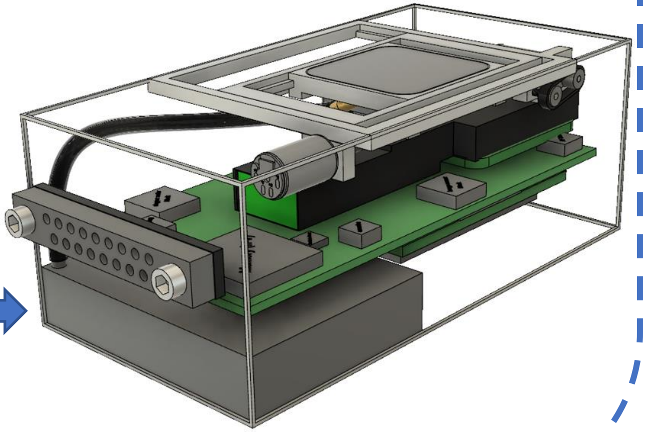
WP3 – Detailed design

CONCEPTUAL DESIGNS of MFM

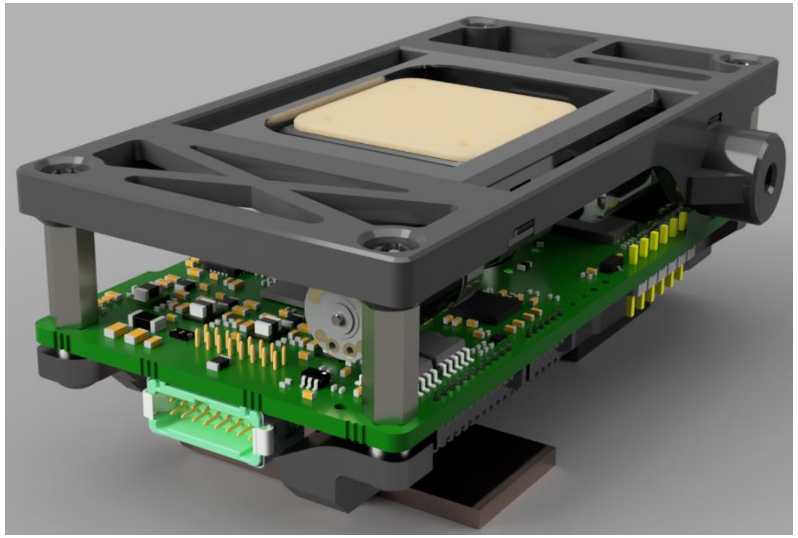








Conceptual designs from WP2



WP4 – Manufacturing

MANUFACTURING

- Iterative style was continued:
  - → Manufacturing and preliminary testing of breadboard version 1
  - → Adjustments to the design (e.g., updates on optics filters and heatsink)
  - → Manufacturing of the breadboard version 2

USER INTERFACE

- Development of the µManager user interface

TEST PLAN

- Test plan was prepared as part of WP4

WP5 – Test and analysis

TESTING

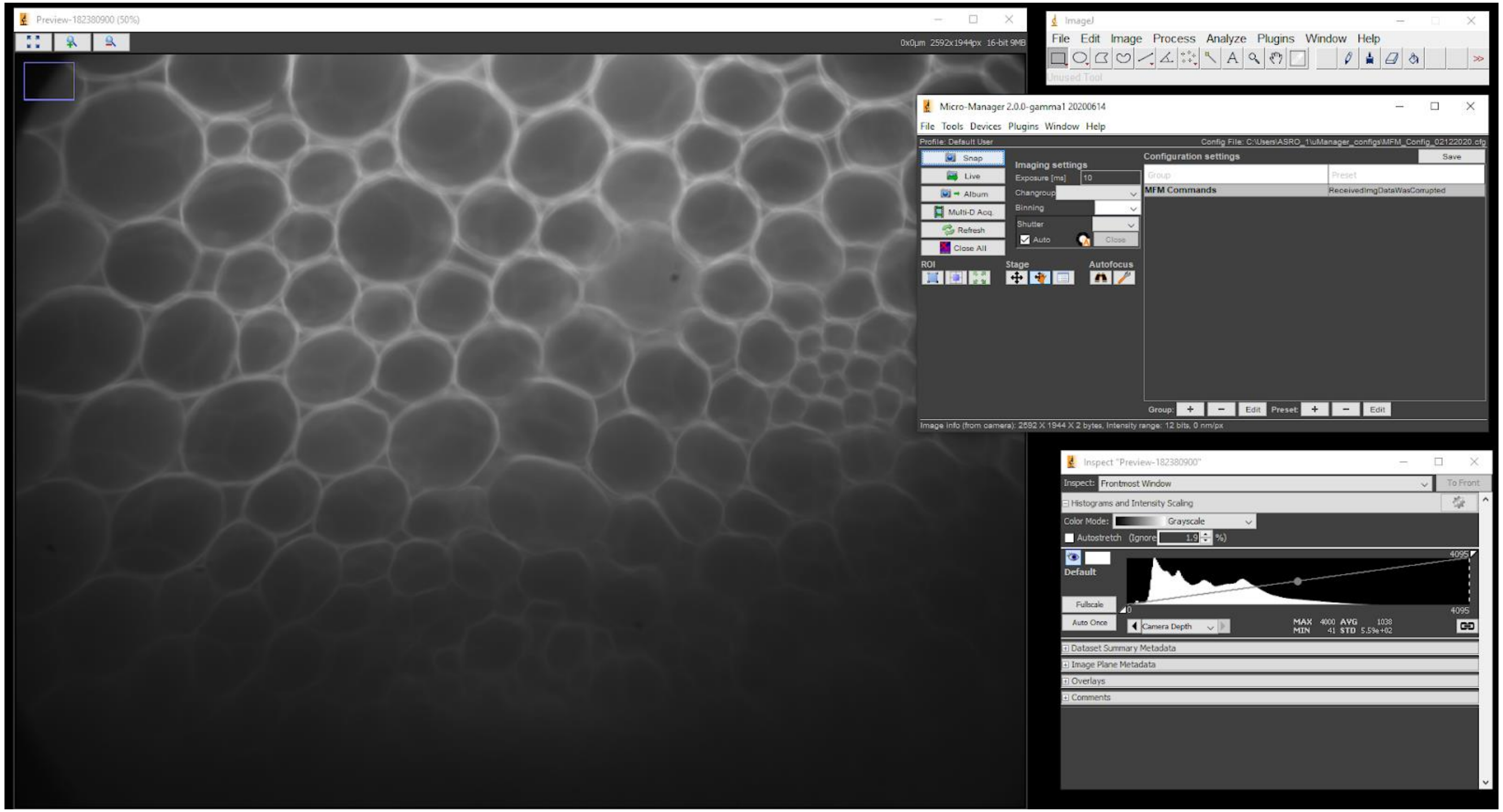
- Testing was divided into two levels
    1) Subsystem level
        1) Electronics tests
        2) Optical tests
        3) Mechanical XY-stage
        4) Cell viability tests
    2) Integrated instrument (physical properties and functional and performance tests)
- Verification Control Document was created
- EGSE HW and Micromanager SW testing

USER MANUAL

- MFM user manual was written as part of WP5

WP5 – Test and analysis

Test image taken with the MFM breadboard and shown in the µManager user interface



WP6 – Recommendations

POTENTIAL PLATFORMS

- Potential platforms for a space-qualified MFM were researched, including platforms on-board the ISS (KUBIK, Biolab, ICE Cubes), CubeSats, rovers and the Space Rider

LESSONS LEARNED AND RECOMMENDATIONS

- Lessons learned and improvement ideas were listed together with recommendations for space application

ROADMAP FOR SPACE APPLICATION

- A roadmap for developing a space-qualified MFM for the KUBIK platform was created, including main development areas, timeline and estimate of costs

Deliverables - Documents

DELIVERED DOCUMENTS

- TN1 – State of the art in miniaturised microscopes and updated requirements (Issue 1, Rev. 1)
- TN2 – Trade-off and MFM options (Issue 1, Rev. 2)
- TN3 – Detailed design of MFM (Issue 2, Rev. 0)
- TN4 – Breadboard manufacture report and test plan (Issue 3, Rev. 1)
- TN5.1 – Test report of MFM (Issue 1, Rev. 1)
- TN5.2 – MFM user manual (Issue 1, Rev. 1)
- TN6 – MFM conclusion and roadmap for space application (Issue 1, Rev. 0)
- MFM Verification Control Document (Issue 4.0)
- Executive Summary Report (Issue 1, Rev. 0)
- Final Report (Issue 1, Rev. 0)

Deliverables - Hardware

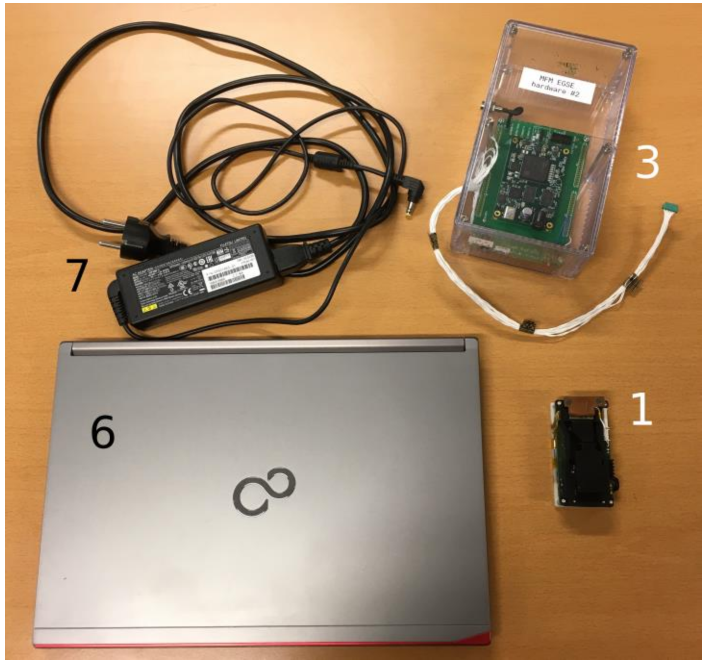
DELIVERED HARDWARE

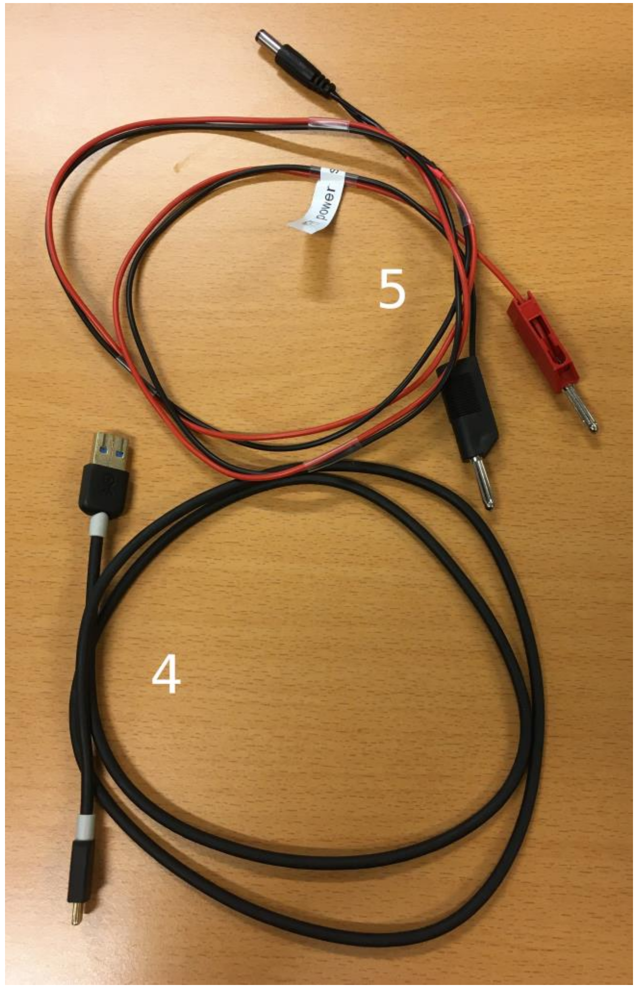
- Delivered hardware [numbers in brackets refers to markings on the images in the next page]
- 1x MFM breadboard instrument [1] (including 1x CCC locking bracket [2])
- 1x MFM EGSE H/W [3] with USB-cable [4] and power supply cable [5]
- 1x Laptop PC [6] (including power adapter [7]) with installed MFM EGSE S/W and MicroManager

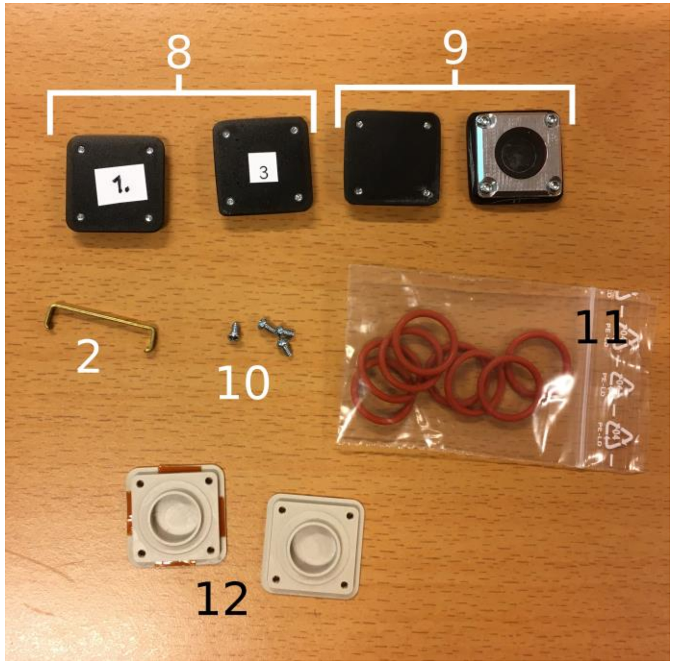
- 2x CCC [8], preassembled with fixed cell sample, on each: CCC body (3D-printing resin), O-ring, cover slip (with cell samples), CCC lock and 4 screws
- 2x CCC "kit" [9], on each: CCC body (3D-printing resin), O-ring, cover slip, CCC lock and 4 screws
- 4x spare screws [10]
- 8x spare O-rings [11]

- 2x CCC body [12] (PEEK, just for a material reference)

Deliverables - Hardware



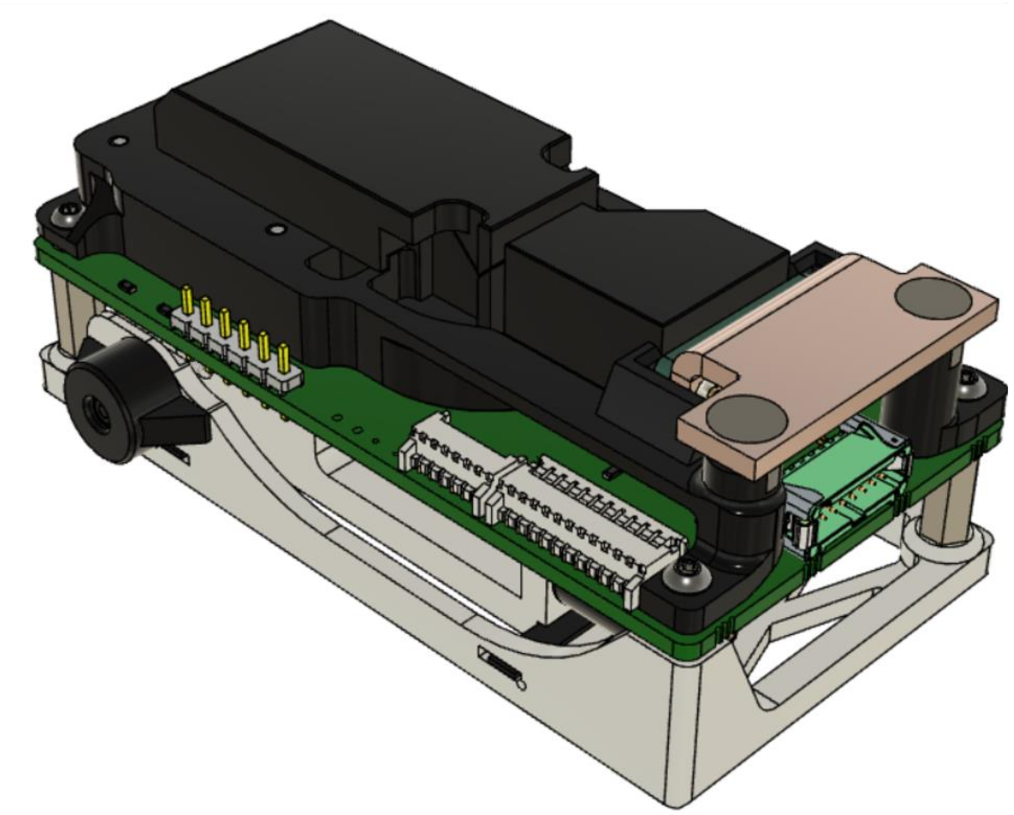


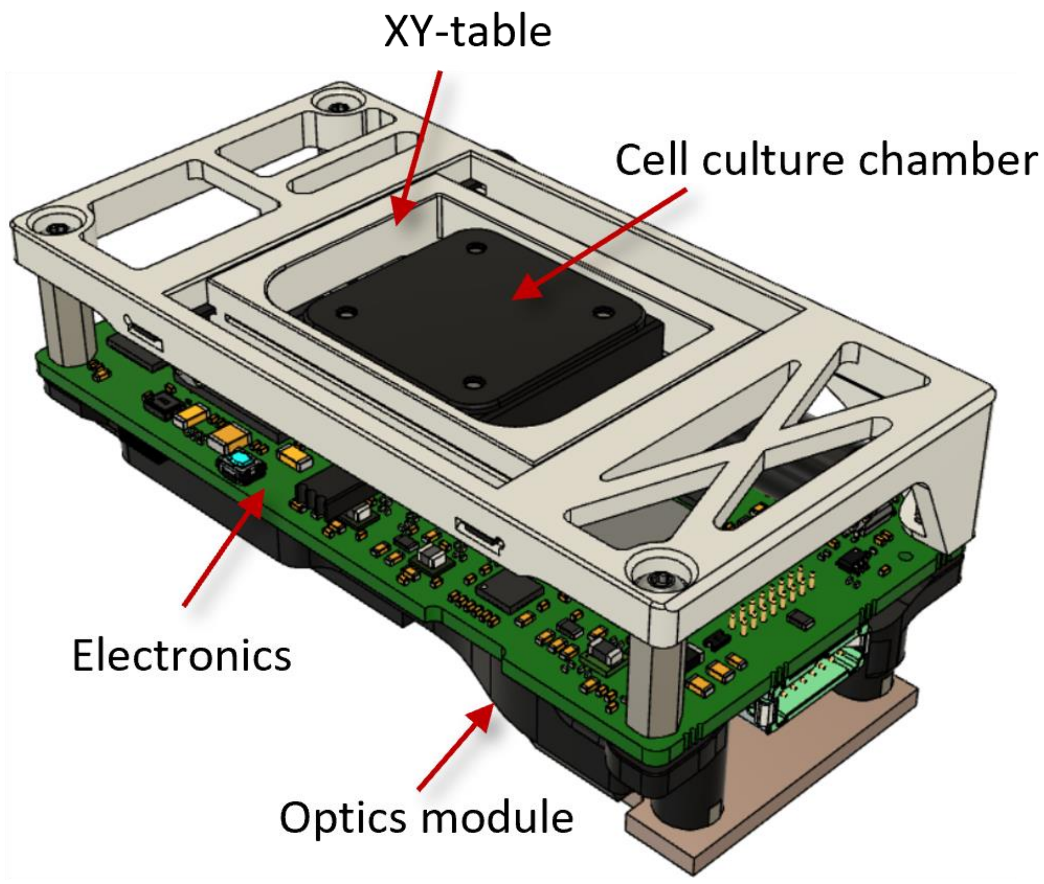


RESULTS

Overview of the design

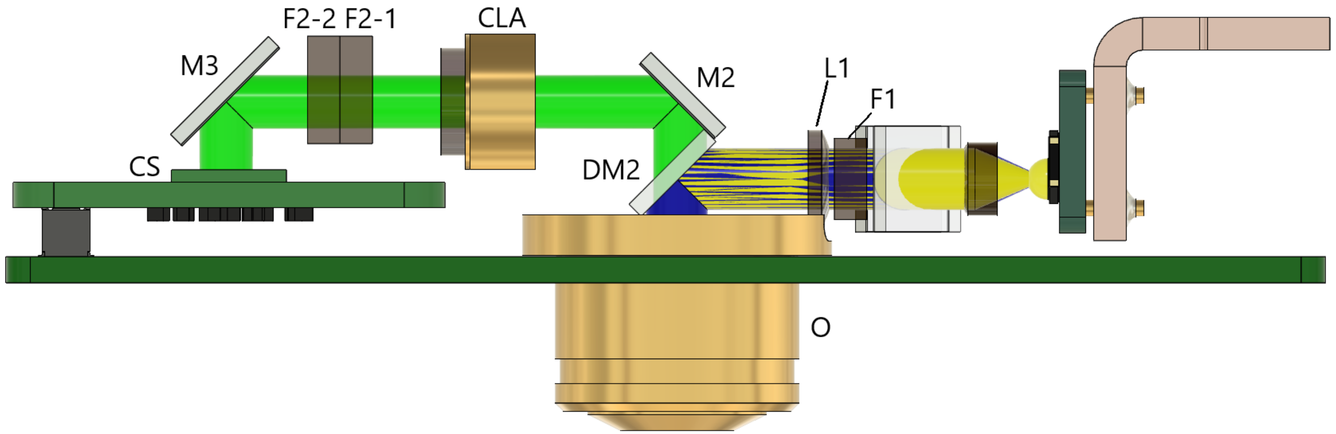
- MFM breadboard is based on a solid frame supporting four subsystem modules

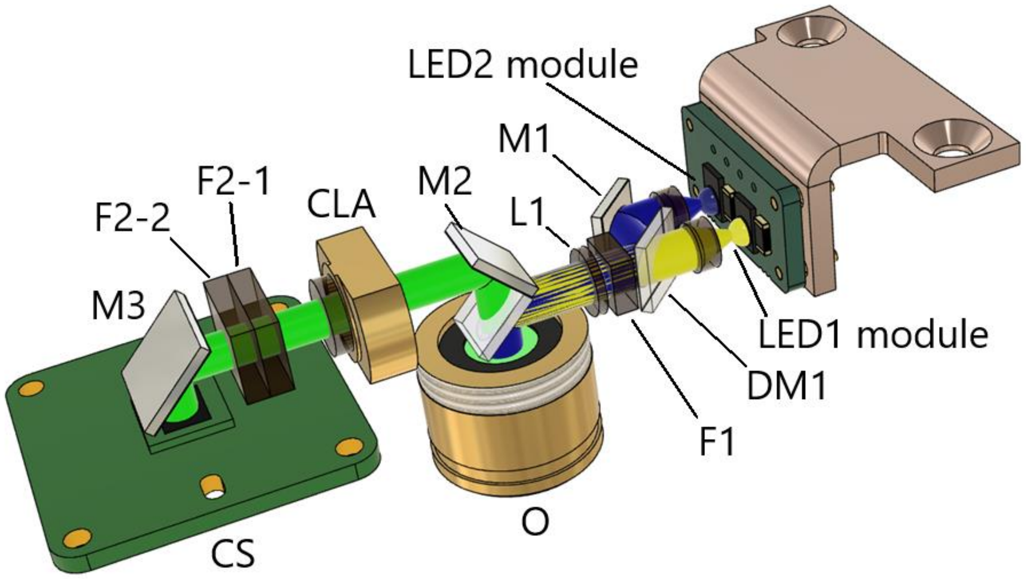




Optics

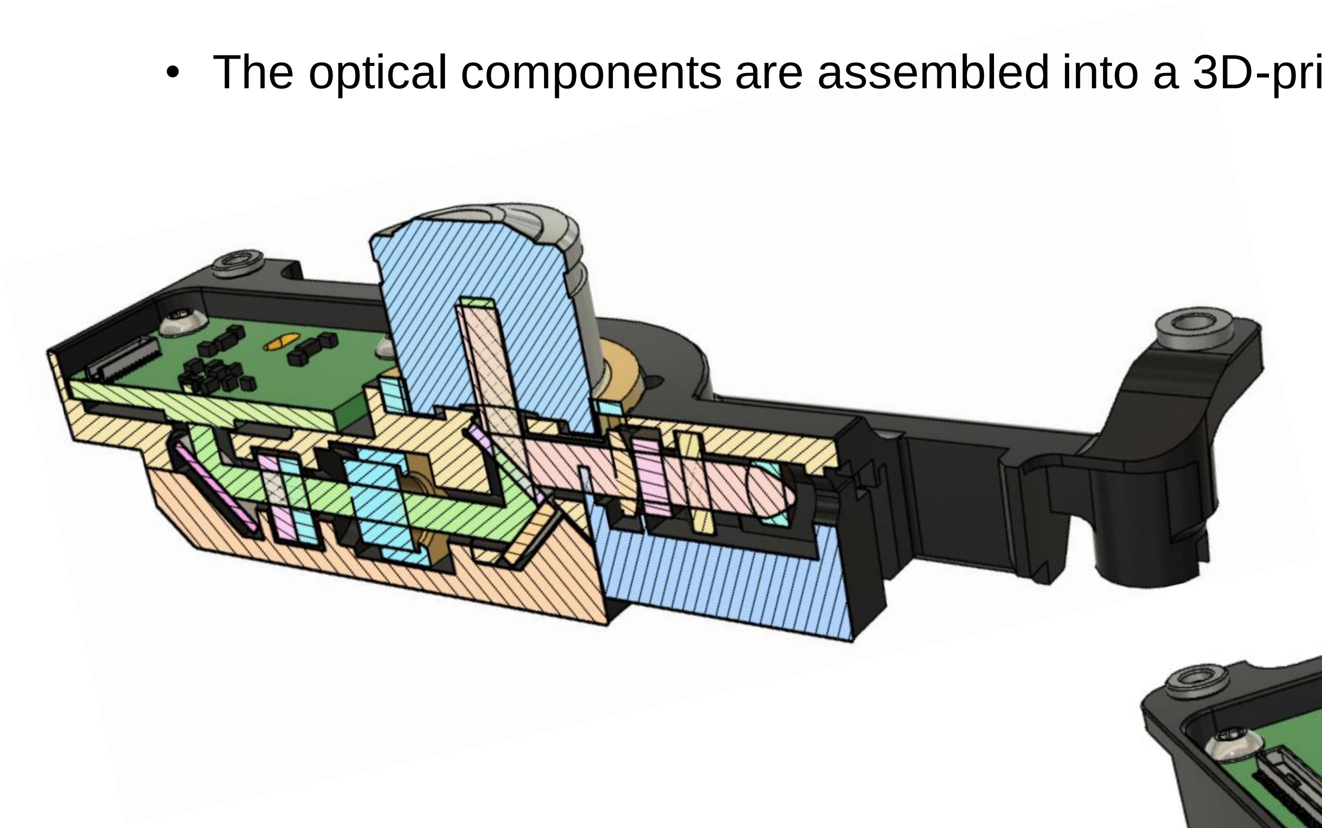
- Optical path is designed in epifluorescent configuration
- LED1 and LED2 modules -excitation LEDs, DM1-DM2 -dichroic mirrors, M1-M3 -silver mirrors, L1 -lens, F1 -excitation filter, O -objective, CLA -correction lens assembly, F2-1-F2-2 -emission filters, CS -camera sensor

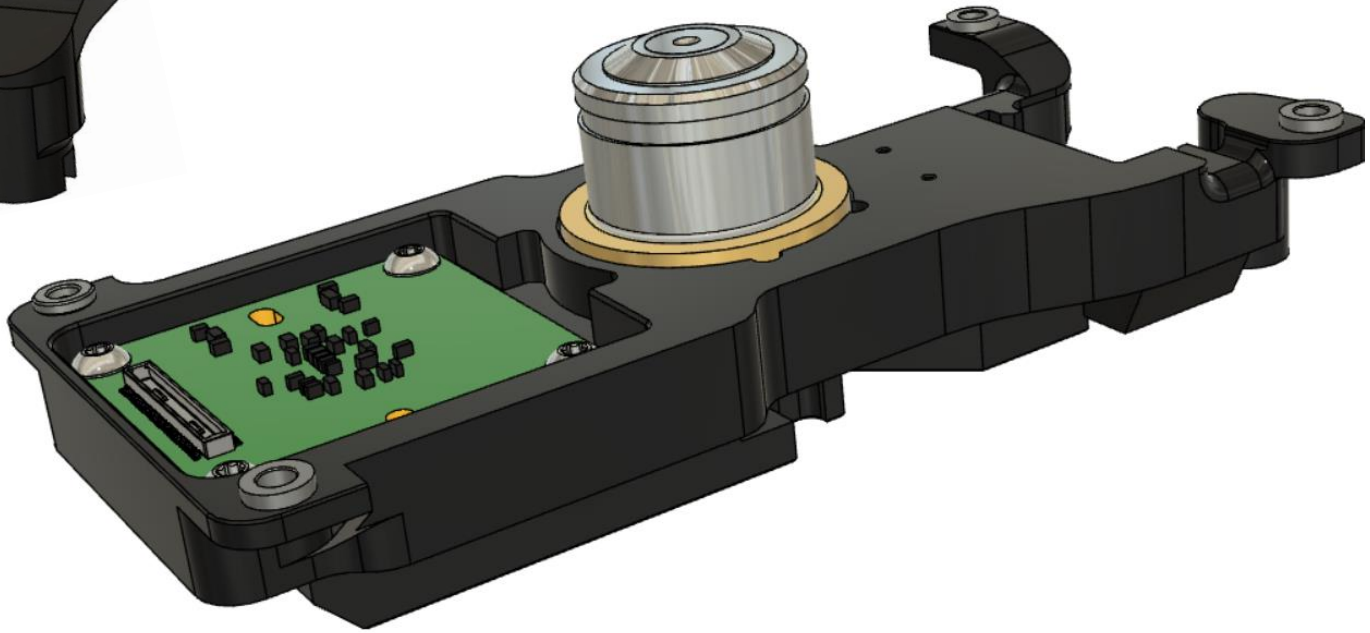




Optics

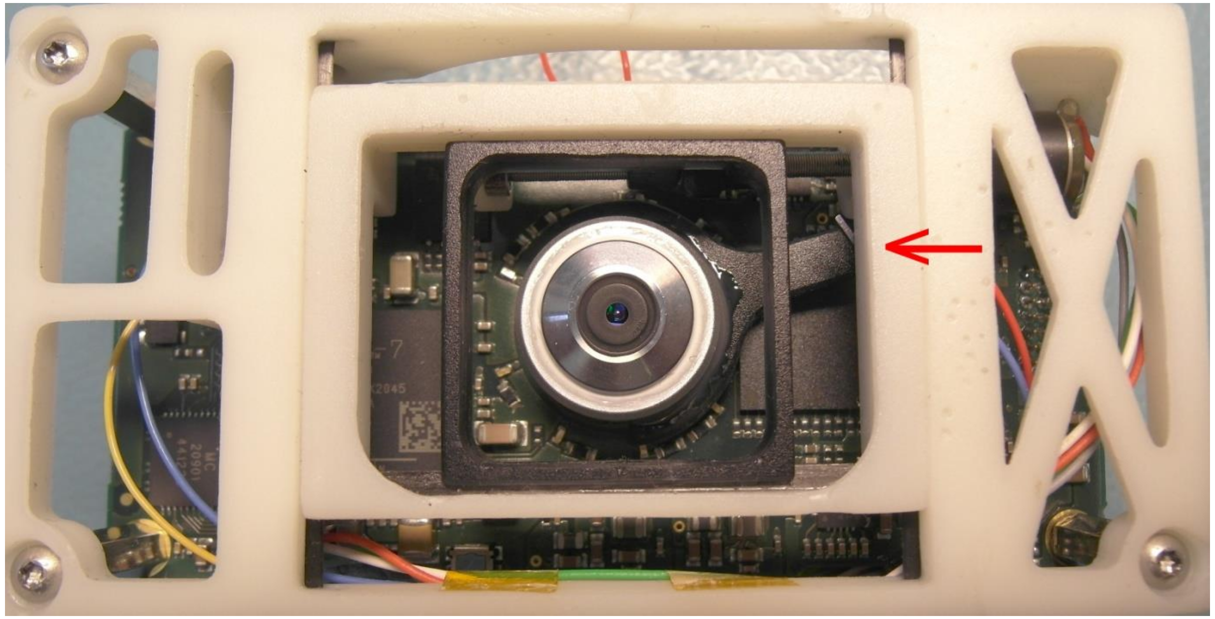
- The optical components are assembled into a 3D-printed optics module

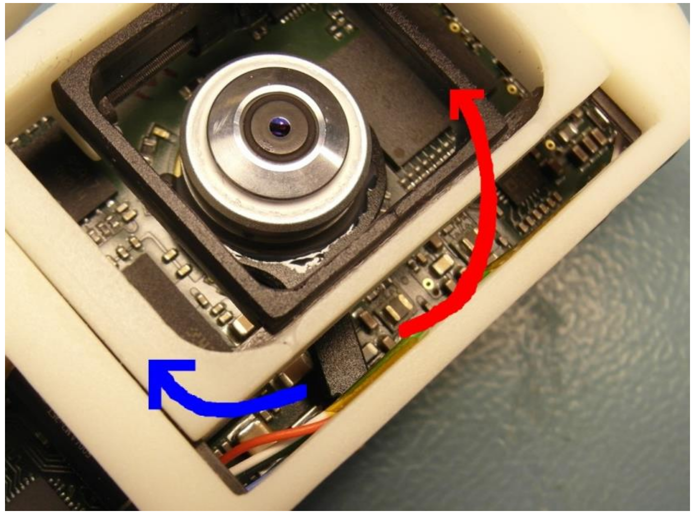




Optics

- Manual focus was introduced into the system. The tolerances of the system do not allow repeatable enough performance for fixed focus.
- Manual focus implemented via rotation of objective on its threads



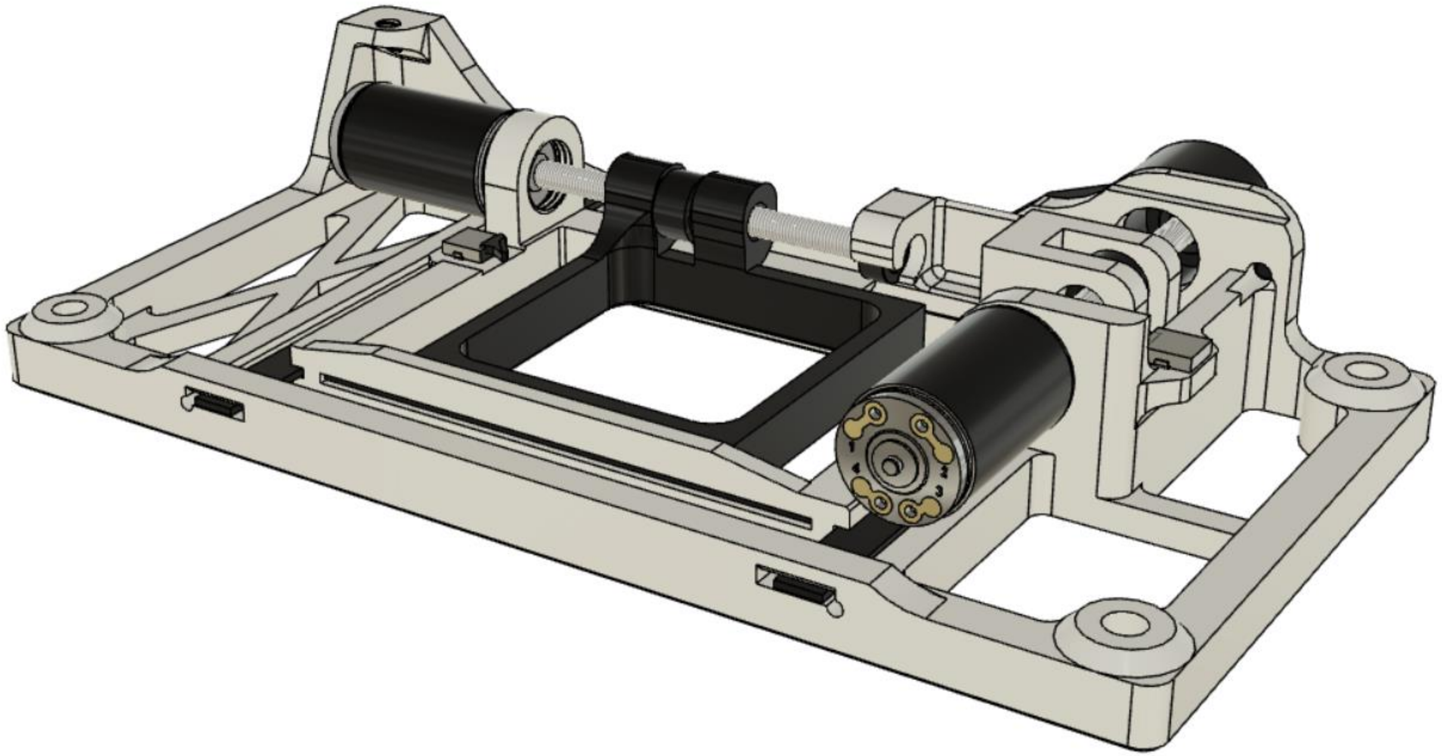


Cell culture chamber

- Volume of 335 µl
- Built by two parts which are screwed together
- CCC body material is PEEK, and O-ring is silicone rubber
  - →It was found out during testing that PEEK is autofluorescent and therefore, not suitable for fluorescence imaging
  - → Suggestion is to test polycarbonate as an alternative for PEEK

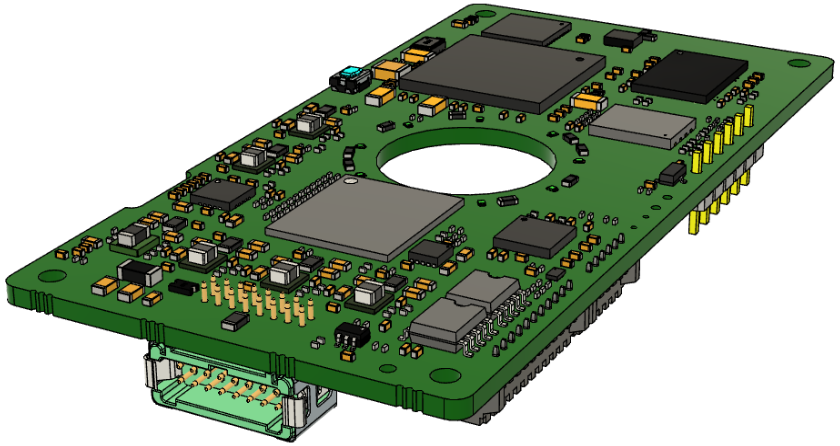
Mechanics

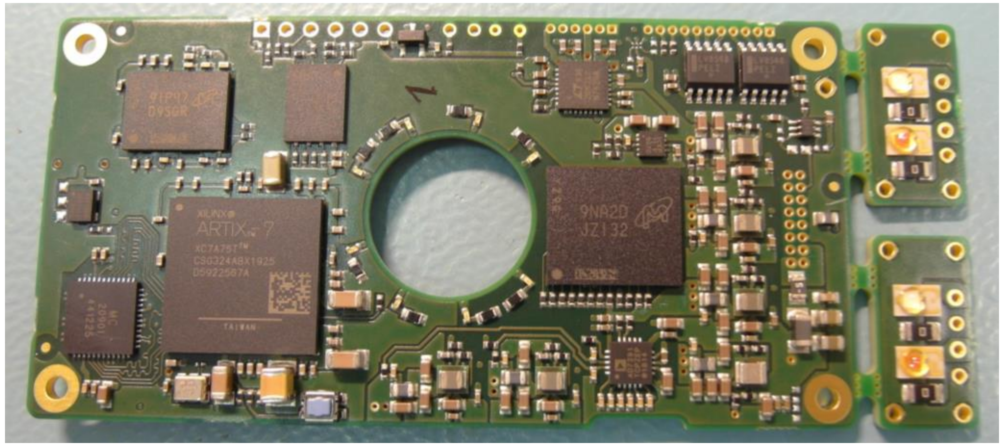
- The CCC is placed on top of a XY-table that allows the movement of the sample
- Table is operated by two stepper motors
- Movement range:
  - 9.7 mm in the X-axis
  - 5.0 mm in the Y-axis



Electronics

- Custom PCB with FPGA interfacing to the camera sensor
- The main PCB is located between the optics module and XY-table
- Hole in the middle to allow the objective to go through it, maximizing the critical dimension for the objective design



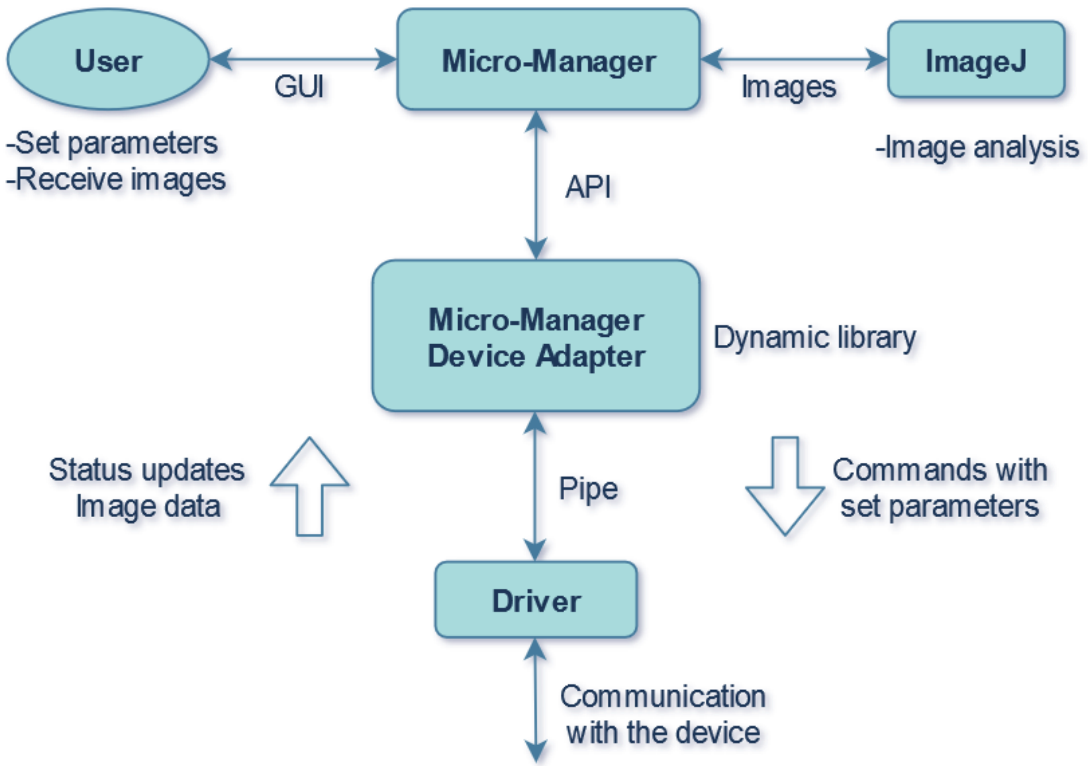


Electrical ground support equipment (EGSE)

- Purpose of the EGSE is to simplify the MFM internal design by moving data conversion to an external unit, and to supply power to MFM
- The MFM EGSE consists of:
  - PC with MFM software
  - MFM EGSE hardware
  - Power supply unit
  - Cables
- The EGSE H/W is based on commercial FPGA integration module
- Dimensions: 120 x 80 x 21 mm3 without casing

µManager user interface

- All advanced image processing is done on PC
- µManager is free and open-source software for control and automation of microscope hardware
- Specific device adapter C++ class has been written for MFM to be controllable from µManager
- 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

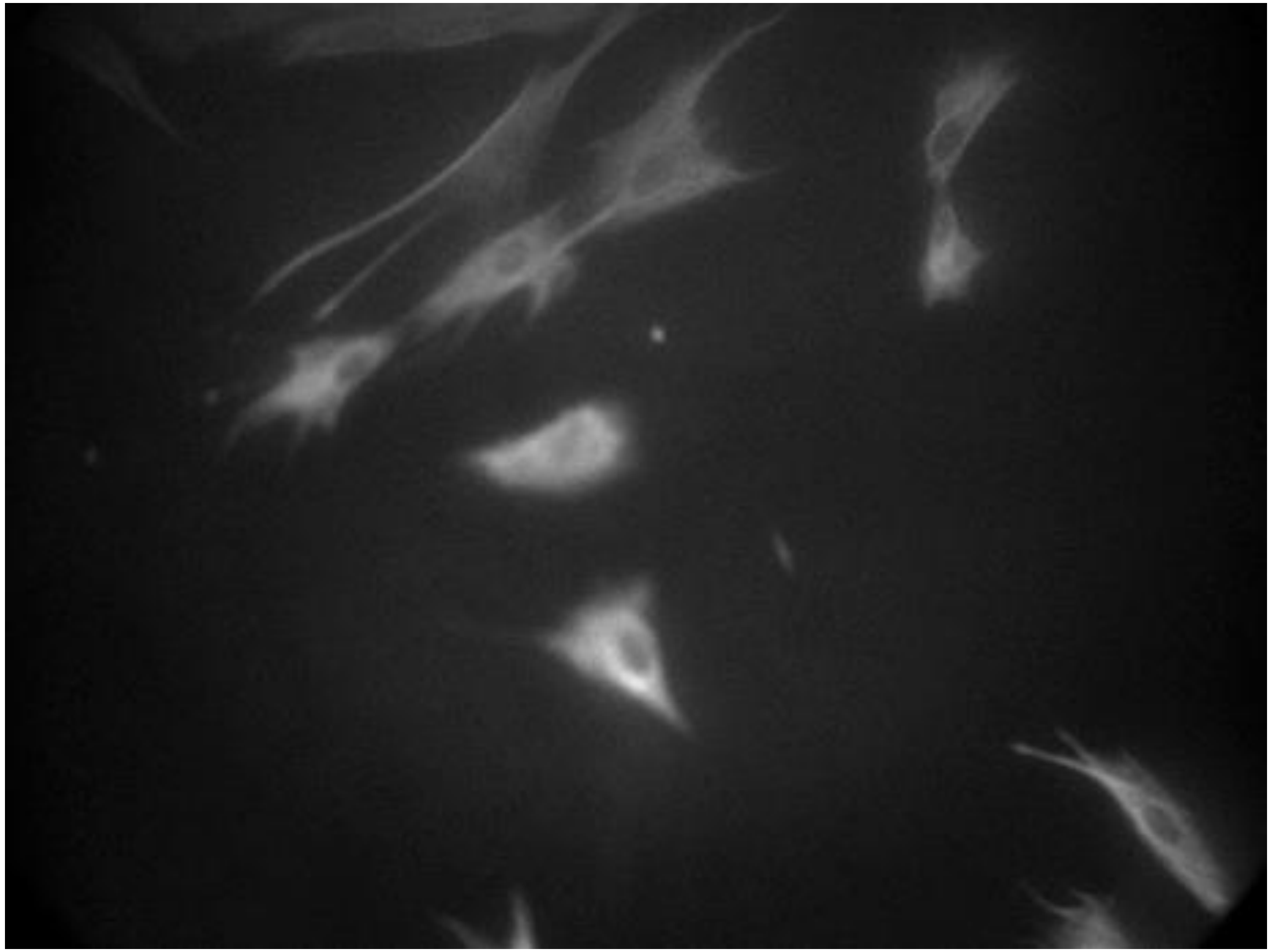


Main characteristics of the resulting instrument

Dimensions	83.2 mm x 42.2(+5) mm x 33.0 mm
Excitation LEDs	Amber and Blue
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

Example test image

Vimentin structures stained for blue



RECOMMENDATIONS

Recommendations

POTENTIAL PLATFORMS

- Potential platforms for a space-qualified MFM were researched
  - Platforms on-board the ISS (KUBIK, Biolab, ICE Cubes) & Space Rider → Suitable for microgravity research or in-orbit technology demonstrations
  - CubeSats → In higher orbits possible to study the synergistic effects of space radiation and microgravity
  - Rovers → Searching for biosignatures

ROADMAP FOR SPACE APPLICATION

- Roadmap for the development of a space-qualified MFM for the KUBIK platform was created, including main development areas, timeline and estimate of costs

  → Some improvements are required in order to fit the MFM into KUBIK and to improve system reliability and performance

Recommendations – KUBIK implementation

Challenge	Description	Possible solutions
Volume	Current version of MFM is slightly larger than the KIC-SL-E container. Some modifications are needed in order to fit the microscope inside the container envelope.	• Removal of the second stepper motor • Stepper motors with shorter lead screw • Optimisation of LED PCB heatsink (e.g., by using one-sided aluminium PCB which may not even need heatsink) • Finding a smaller solution for the objective (note: it might not be possible to find a smaller solution, at least without expensive custom optics)
Power consumption	Lower power consumption would be considered beneficial and also help with the heat generation challenge.	• By selecting a newer FPGA, a compromise can be made to remove the external memory and get along with only internal memory. This reduces the power consumption. • By selecting a newer FPGA with MIPI interface circuitry included, the external MIPI circuit can be left out. This reduces the power consumption. • Just by having newer technology can reduce power consumption from the current version.
Heat generation	Currently the electronics produce heat in the middle of the microscope envelope, and the heat is difficult to conduct away.	• Moving the electronics board to the edge would improve the possibility to dissipate extra heat on the interface to the host platform. • Lowering power consumption.

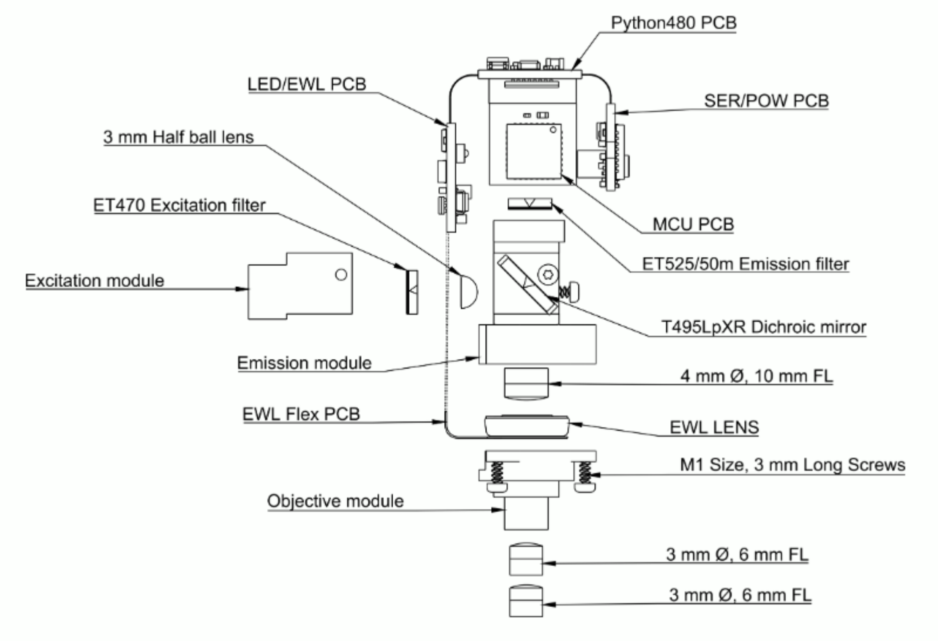
Recommendations – KUBIK implementation

Challenge	Description	Possible solutions
Focusing	Fixed focus is not a good enough solution to keep desired CCC / coverslip surface in focus.	• Adding autofocus (requires algorithm). •Fully autonomous algorithm might be complex. Easier first step solution would be electronically adjustable focus (without algorithm).
CCC material	PEEK is an autofluorescent material and therefore, not suitable for fluorescence imaging.	• Changing the CCC material. Suggestion is to test polycarbonate.
Overall complexity and reliability of the system	For space application, the reliability of the system needs to be increased. Currently the system is also fairly complex and reducing the complexity would be a way to simplify manufacturing and increase the reliability.	• Removing the dark field imaging option • By changing the FPGA to a newer one • Finding another solution for the objective • Using only one excitation LED • Removal of the second stepper motor • Integrating the sensor directly to the optics module • Simpler mechanics (machinable instead of 3D printing)

Recommendations

UPDATED TRADE-OFF ANALYSIS

- New developments for e.g., Miniscope, new autofocus elements, improved FPGA circuits, etc. Would be important to update the trade-off analysis.



CONCLUSIONS

Conclusions

OBJECTIVES

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.

RESULTS

→ Breadboard has been manufactured and tested

→ Compact and complex design required several iterations between mechanics, electronics and optics

→ Miniaturization was pushed to the limit

→ Some unsolved small details in the design due to limited time and budget, but good overall understanding of the complexity of the system was gained

→ Good basis for follow-up projects