ESA’s ISS 3D biosystem

Info-session for scientists
January 2024

ESA SciSpacE team
REDWIRE Space Europe

Agenda

- SciSpacE introduction
- Information on ESA’s ISS 3D Biosystem
- Information on Announcement of Opportunity (AO) using the 3D Biosystem

SEEKING KNOWLEDGE AND SERVE SOCIETY

Science in a Space Environment (SciSpacE)

What

SciSpacE delivers the science for the exploration programme.

Why

SciSpacE returns practical benefits to society back on Earth, and helps us understand how space affects fundamental physical and biological processes, and to find ways to explore and live sustainably in space.

Where

International Space Station, ground analogues of space, the Moon, and Mars.

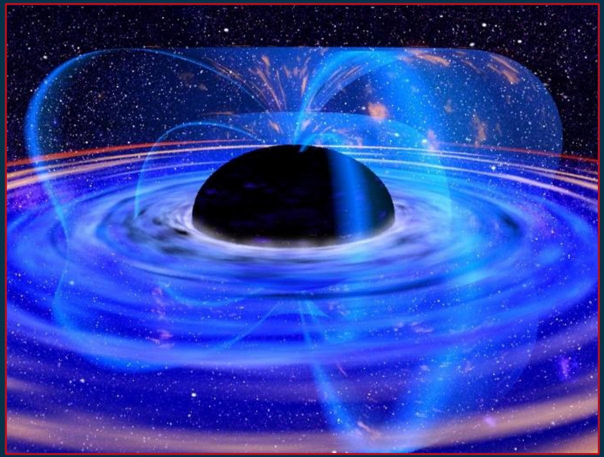
SciSpacE SPOTLIGHTS

HUMANS LIVING ON MOON & MARS

ASTRONAUT 2.0

SPACE TRAVEL AND TRANSPORT

EXTRATERRESTRIAL LIFE



FUNDAMENTALS OF NATURE

NATURE OF EXPLORATION DESTINATIONS

International Space Station (ISS)

GAINING KNOWLEDGE AND CLOSING GAPS

SciSpacE

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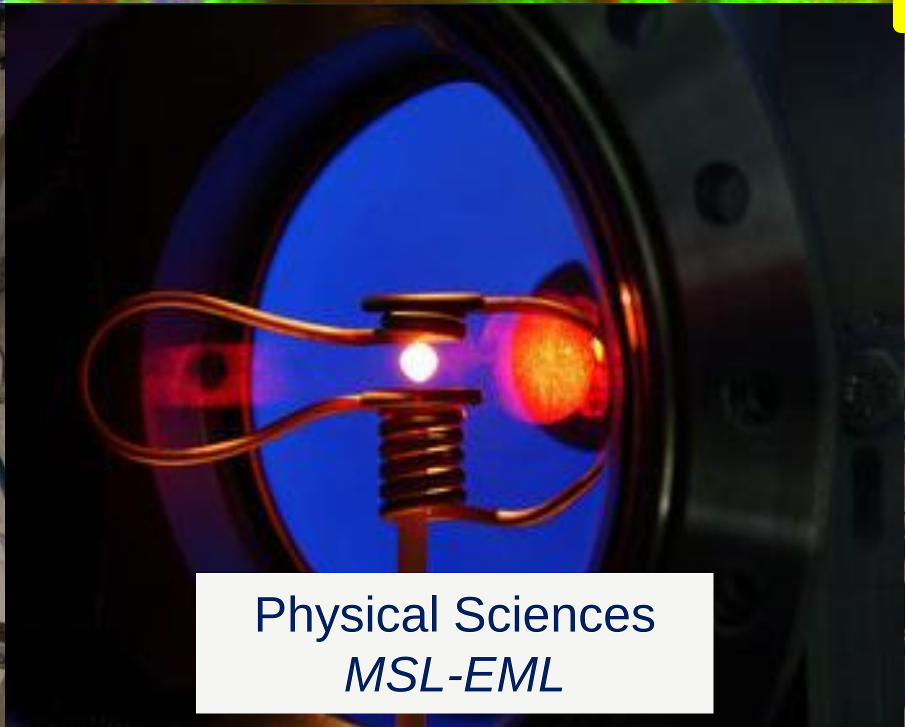
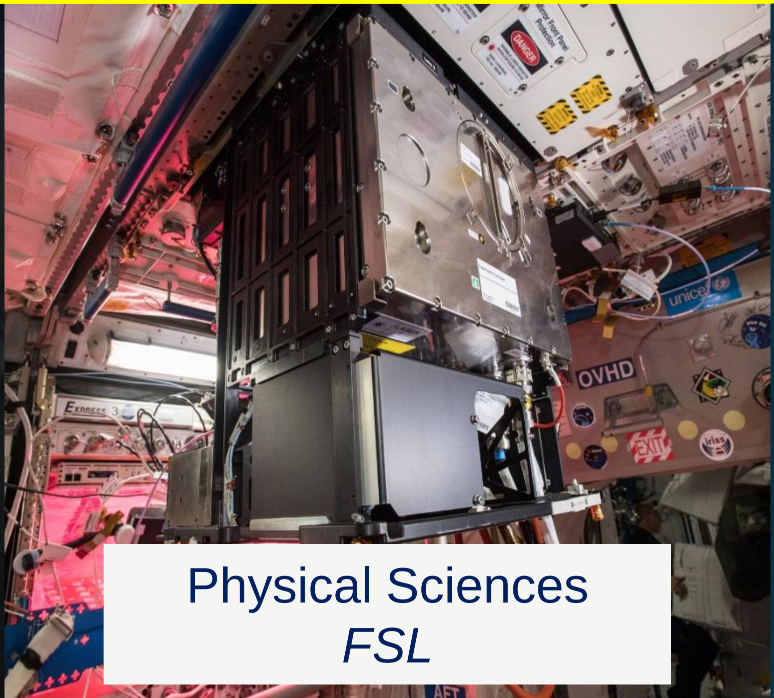
Planned:
Exobiology facility


Planned: Life Sciences
ESA-DLR Live Cell Imaging


Planned: Life Sciences
3D Biosystem


Life Sciences
KUBIK


Physical Sciences
MSL-EML



Physical Sciences
FSL

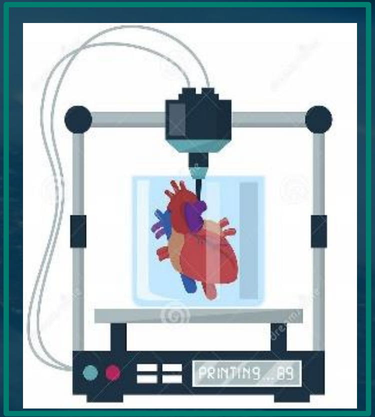
Biology Research in Space – Engagement with Community

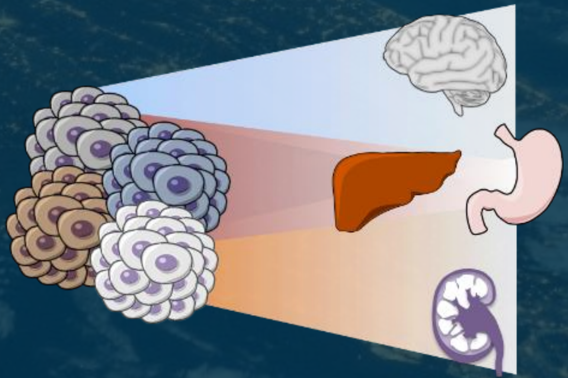
2017-2019: ESA 3D Printing of Living Tissue for Space Exploration project

2019-2020: Biology research community consultation and Call for Ideas for new Biology Research enabling capabilities

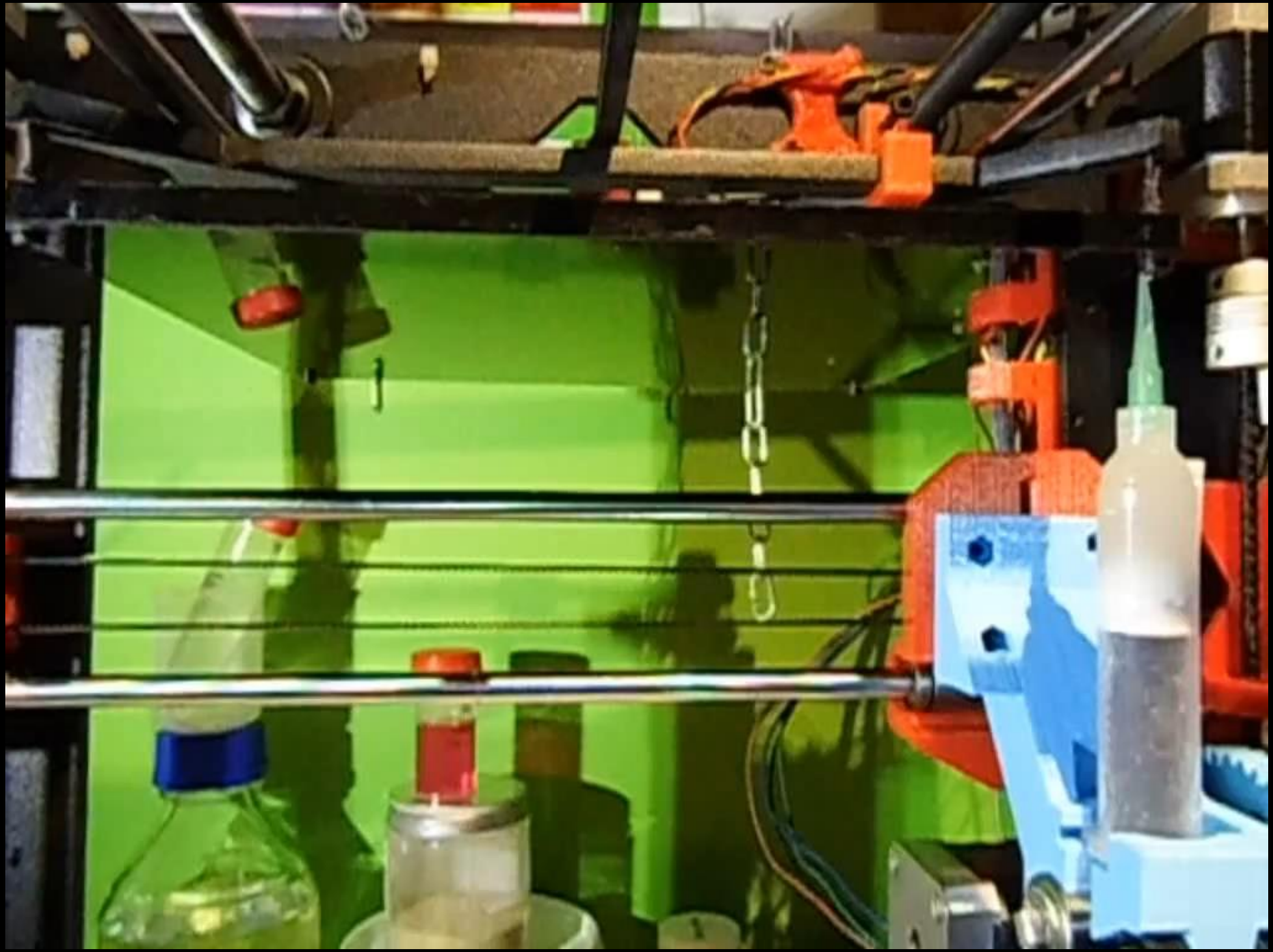
2020-2021: Update of SciSpacE Science Community White Papers

ESA 3D BIOSYSTEM FOR ISS





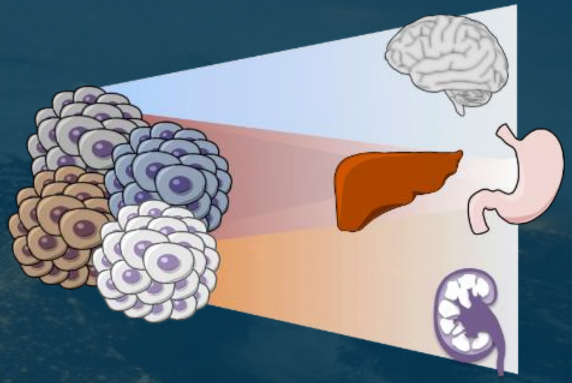
3D BIOPRINTING IN SPACE

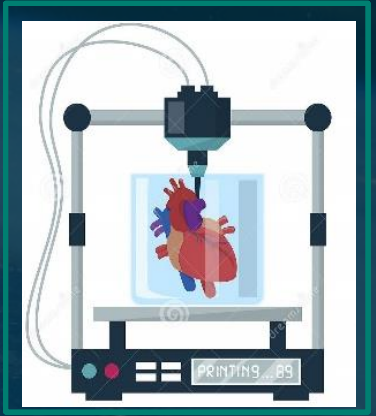


3DBioprinting upside-down

Background - 3D bioprinting in Space

- (3D) Bioprinting as enabling technology for space exploration missions:
  - Medical application
  - Bioreactors & closed loop life-support systems
  - Food production
  - Biomining
  - Bioconstruction
  - …





- ESA is in the process of developing a 3D Biosystem for ISS, which includes a 3D bioprinter to make use of this technology for research in space and equipment to culture, stimulate, and analyse samples

Bioprinting → Incubation and culturing (and *in-situ* stimulation) → *In-situ* analysis

General objectives for 3D bioprinting and 3D Cell Cultures in Space

Biomaterials for biology research

- Fundamental research in tissue engineering, biotechnology, system and synthetic biology
- Fundamental research for optimisation of 3D Bioprinting materials and processes
- Applied research for regenerative and personalised medicine
- Applied research for radiation mitigation strategies on specifically produced/cultured tissues
- Applied research for pharmaceutical and cosmetics industries

Biomaterials for clinical cases in different space mission scenarios

- Evaluation of the medical application of 3D bioprinting in support of crewed space exploration missions: e.g. Intervention in case of Bone/Skin incidents
- 3D printing of medical orthosis or appliances
- In-space manufacturing of 3D tissues

Innovative and impactful research for science and medicine

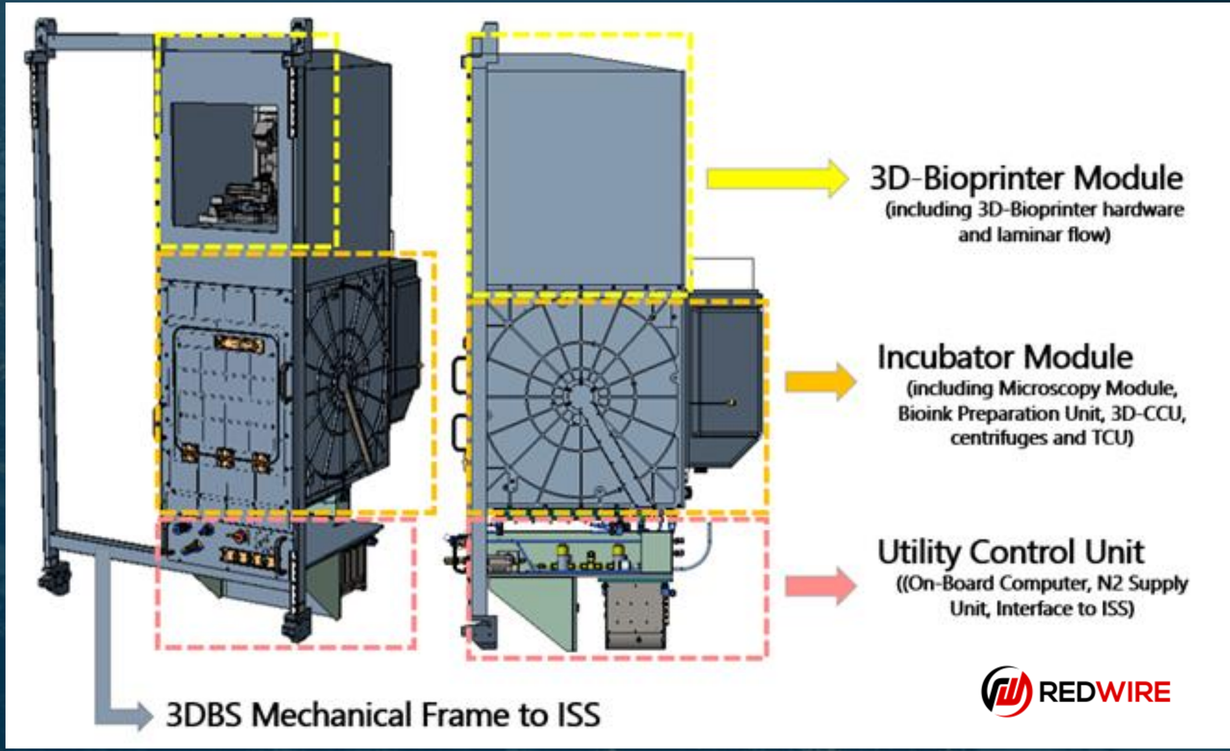
From incubator to *in-situ* capabilities on the ISS

BIOLAB

- Incubator
- Centrifugation and µg conditions
- Biological samples housed in standard ''Experiment Containers'' within the facility
- Operational since 2008

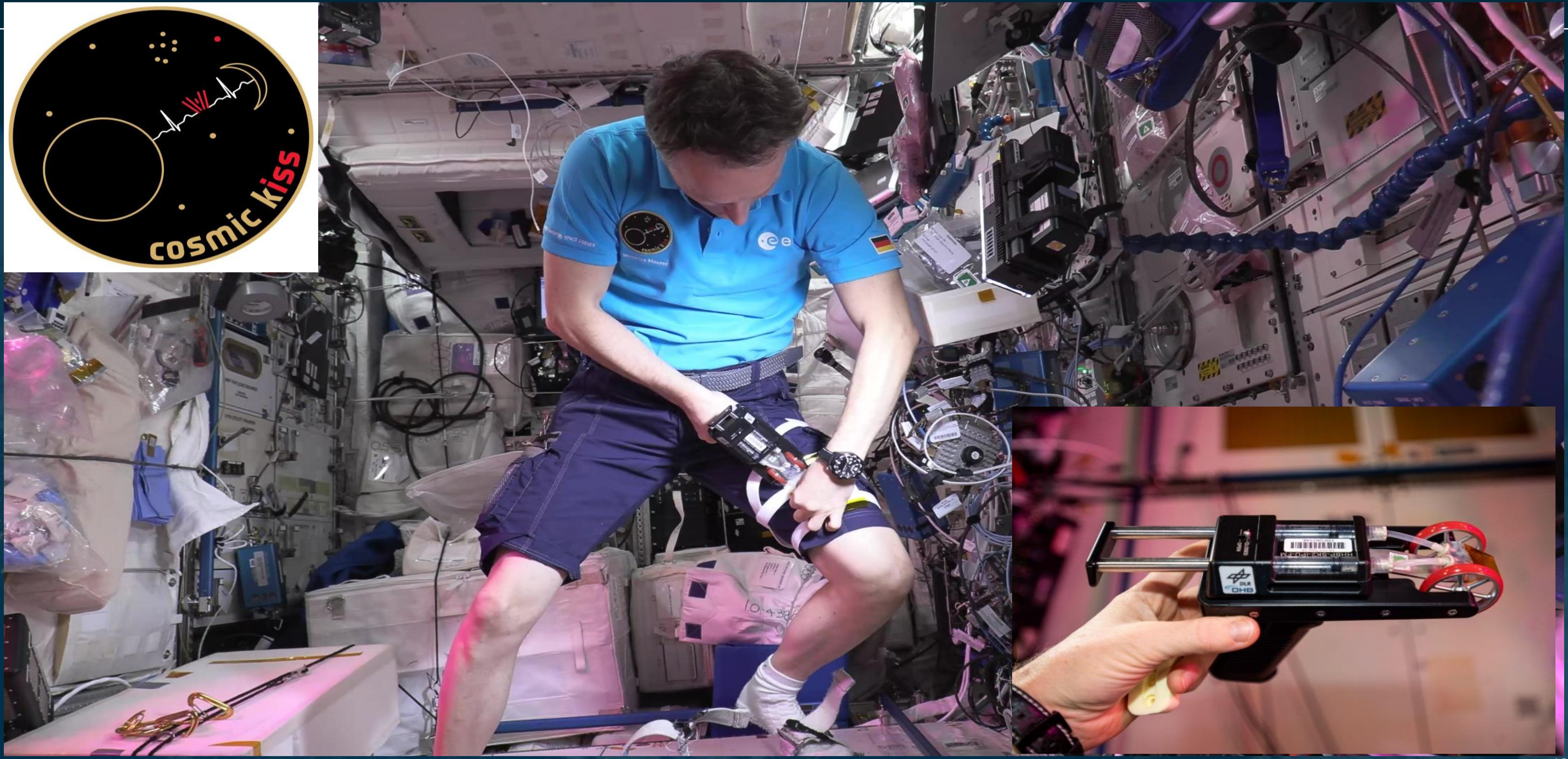
Upgrade

3D Biosystem



- In-situ analysis
- In-situ incubation and stimulation
- 3D bioprinting + bioink preparation in orbit
- Planned integration on ISS in end of 2026

ESA's 3D bioprinting and culturing initiative



3D Biosystem operational concept

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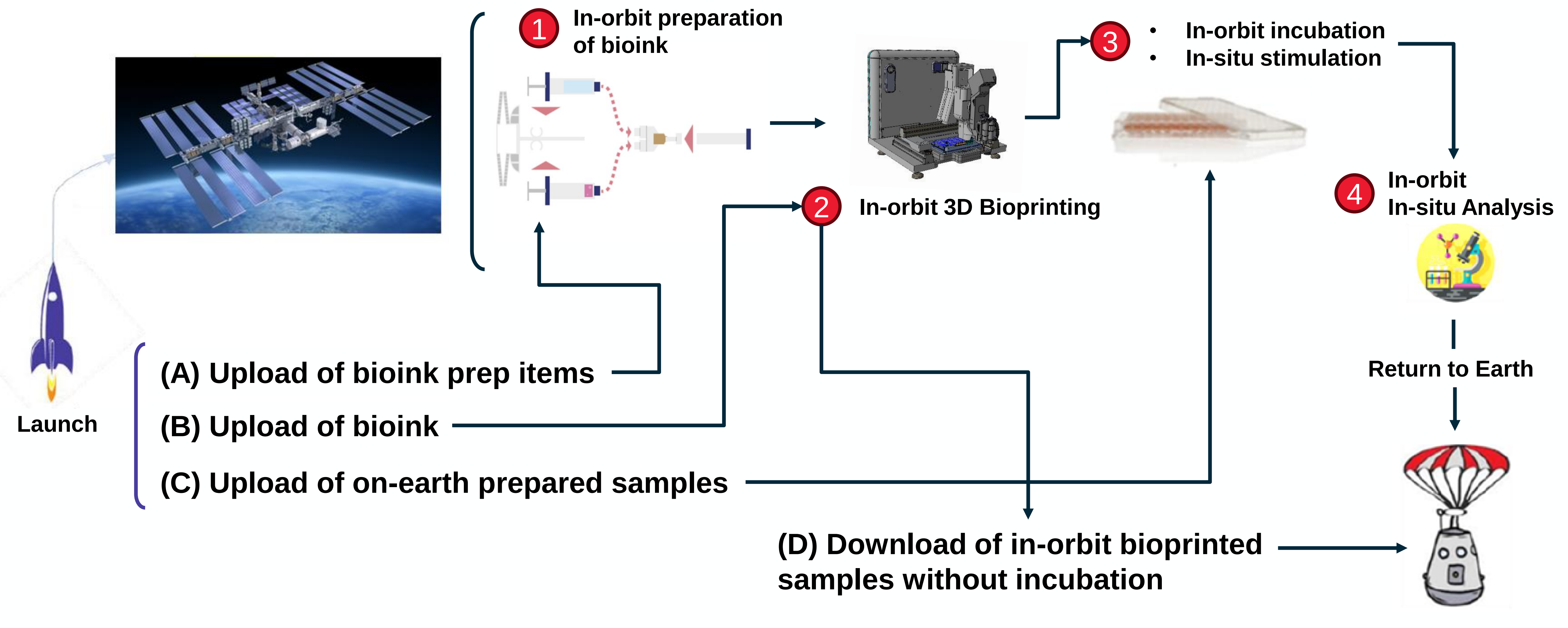
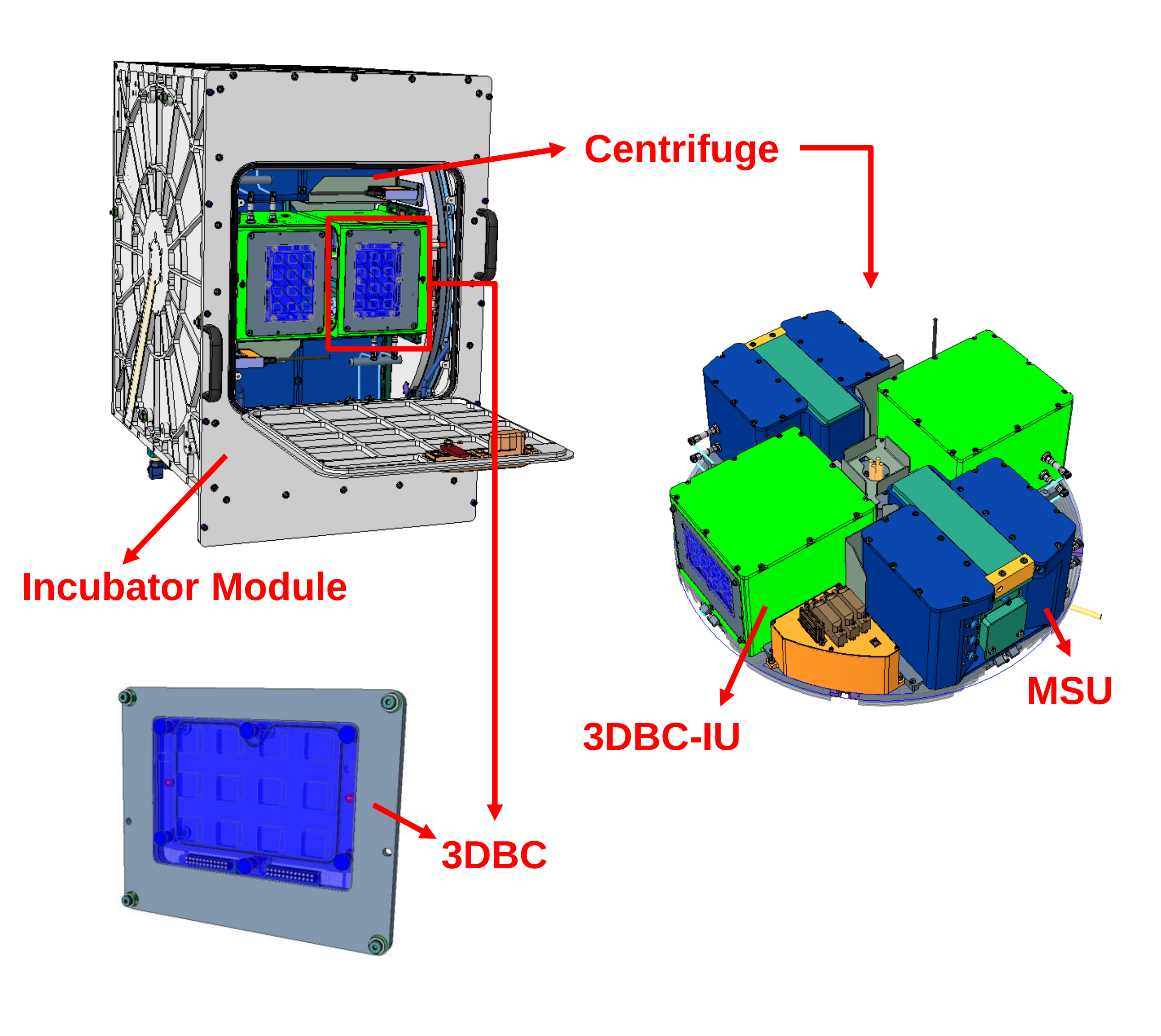


Incubator Module Main Capabilities

Specifications will be tested with scientific samples during the development and could change depending on the testing results

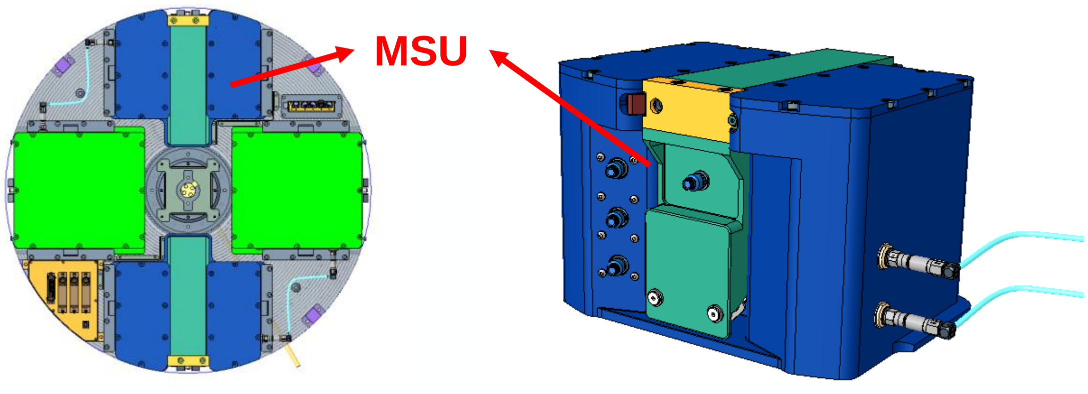
- Incubator able to accommodate **multiple samples** in the 3D Bioprinting Chamber (3DBCs) transferred from the 3D-Bioprinter or uploaded from ground
- Possibility to run experiments exposed to **microgravity,** but also exposed to **artificial gravity (up to 2g)** by placing the samples on rotating centrifuges using the 3D Cell Culture Units (3D-CCUs)
  - 4 slots for well-plates across 2 centrifuges
- The 3D-CCUs includes an Interface Unit to the 3DBC (3DBC-IU) and a fluidic system for culture medium exchanges, growth factors, fixatives, agent, etc. injections, named Media Supply Unit (MSU, *more information on the next slide)*
- Possibilty to take a sample of the exhausted culture medium
- Measure (temperature, oxygen, CO_2, radiation doses, pH) and control (temperature, oxygen, CO_2) environmental conditions

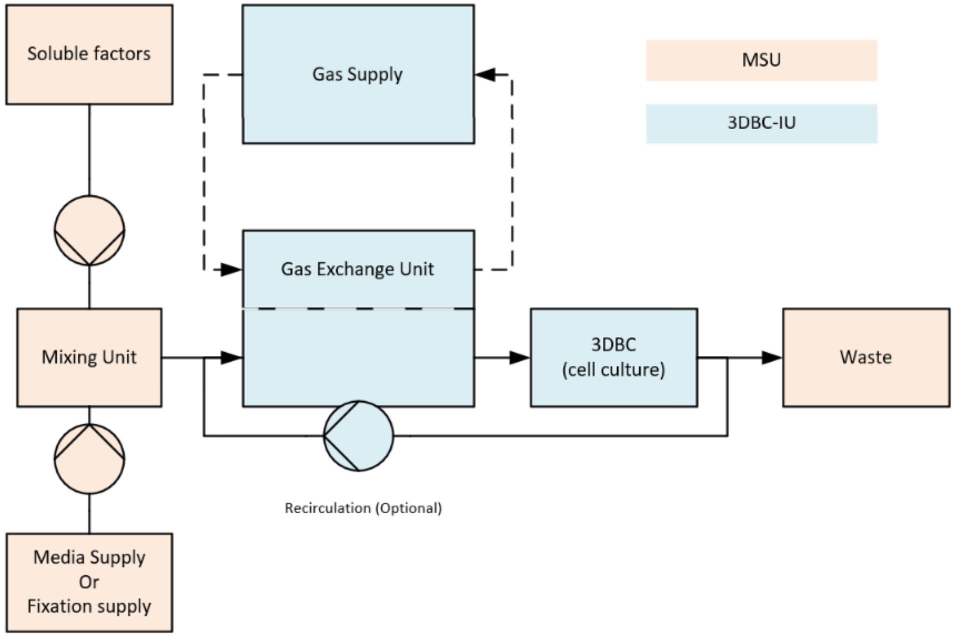


3D Cell Culture Units Main Capabilities: Fluidic system

Specifications will be tested with scientific samples during the development and could change depending on the testing results

- Fluidic system for culture medium exchanges, growth factors, fixatives, agent, etc. injections, named Media Supply Unit (MSU)
- O2 concentration (between 3 and 20%) and CO2 (at 5%) control done by conditioning the medium via the gas exchange unit upstream of the 3DBC
- Medium can be supplied batch-wise, or continuously perfused in either linear or recirculating mode
  - 250 mL supply capacity for medium (2 bags of 125 mL each), exchangeable to increase capacity
  - 3 bags of 20 mL capacity for factor addition (one type per time)
- Ppossibility to take a sample of the exhausted culture medium

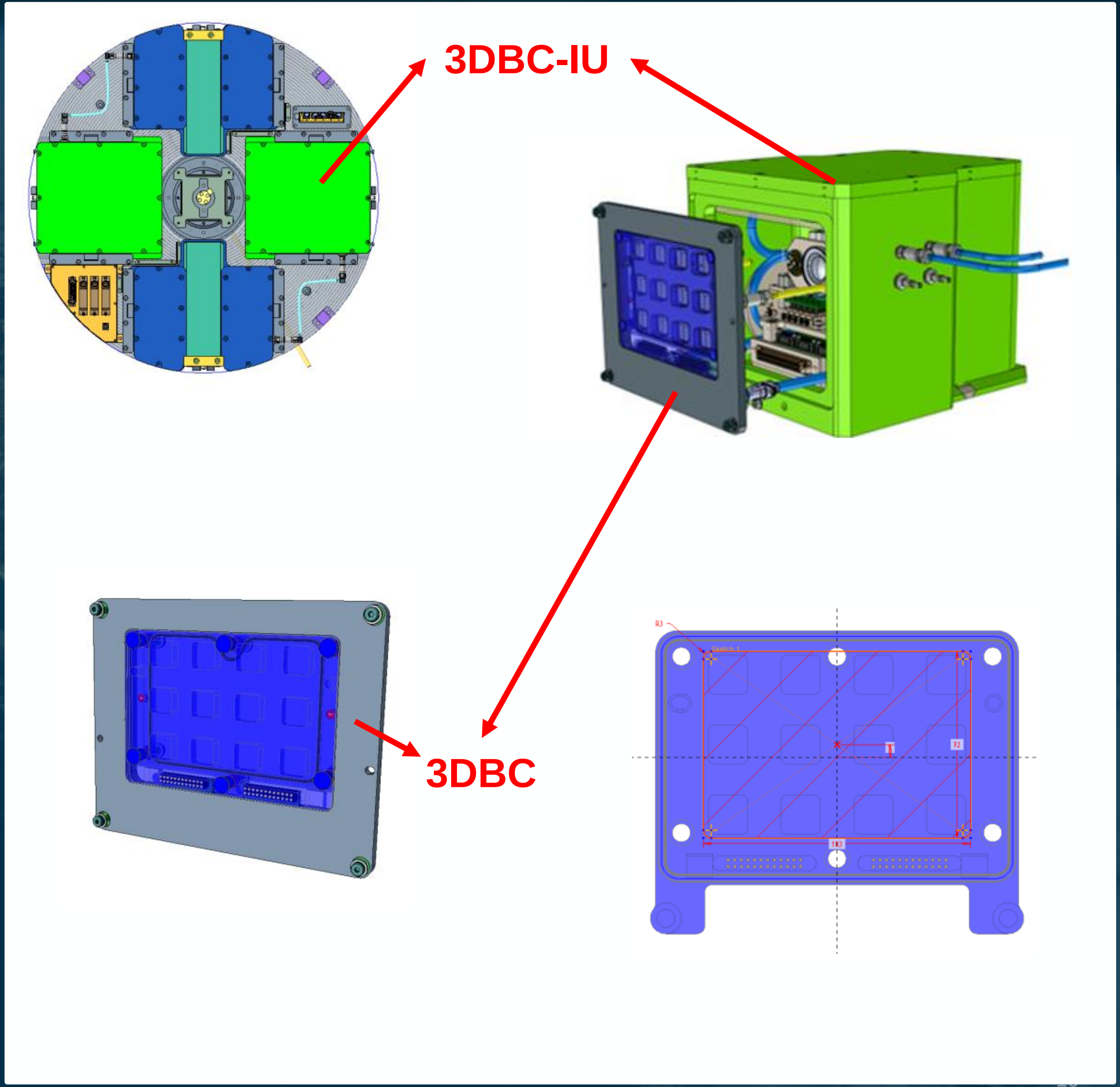




3D Cell Culture Units Main Capabilities

Specifications will be tested with scientific samples during the development and could change depending on the testing results

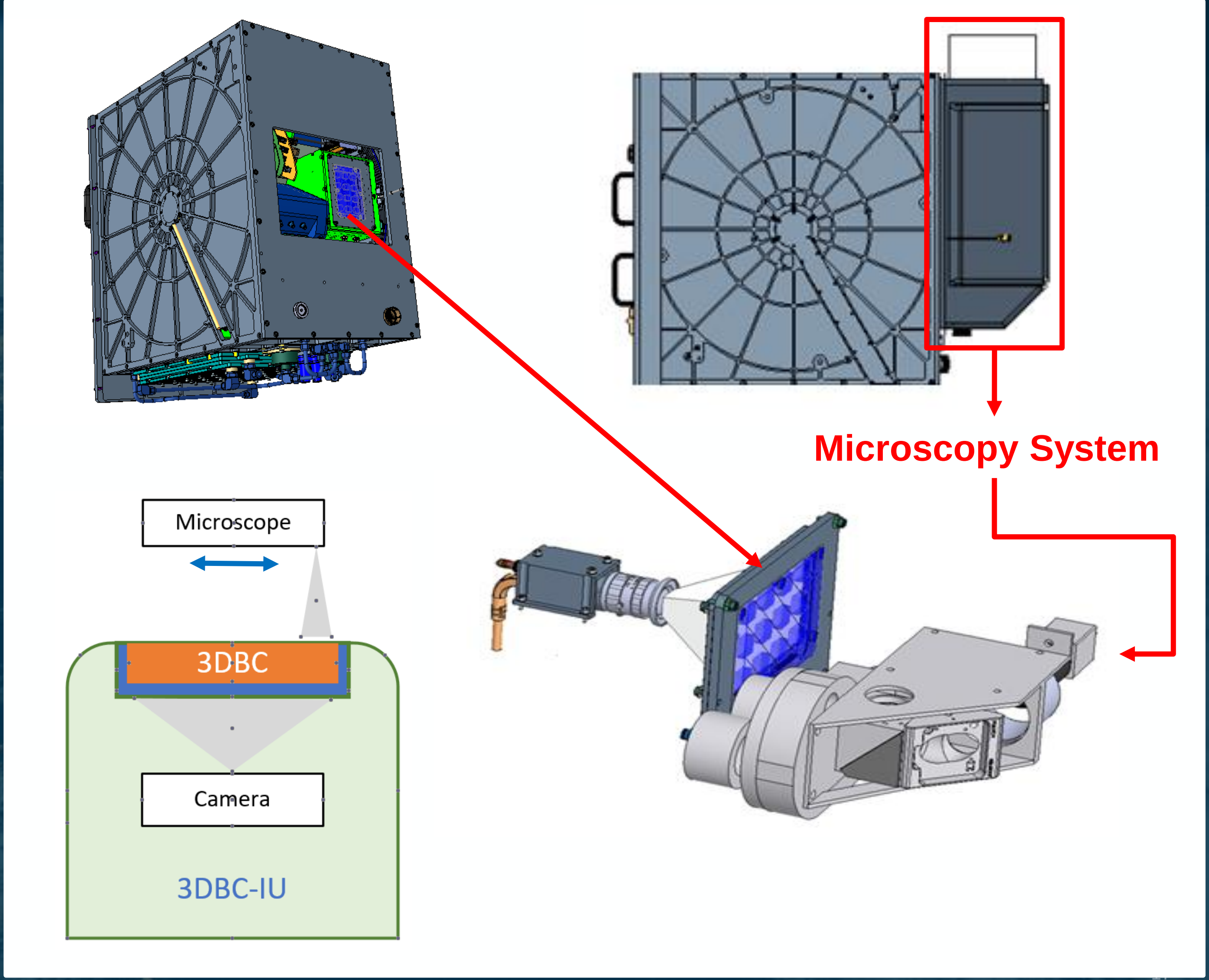
- The cells are 3D bioprinted in the 3D bioprinting chambers (3DBCs).
  - The printing width is limited to 72mm
  - The printing length is 103 mm
  - The printing height is 20 mm
- The maximum area available for the printed samples (1, 6 samples, 12 samples or 24 samples) on each **3DBC well-plate is 103 x 72 mm**
- The standard multi-well give the possibility to print 6 samples, 12 samples or 24 samples per plate (equipped also with impedance measurements)
- **In-situ stimulation** by using specific 3DBCs and dedicated well plates installed inside the 3D-CCU:
  - Mechanical loads application (tension on ex-vivo tissue and tension and compression on 3D-printed construct)
  - Shear stress application
  - Electrostimulation



3D Cell Culture Units Main Capabilities: *In-situ* diagnostics

Specifications will be tested with scientific samples during the development and could change depending on the testing results

- **In-situ diagnostics**:
  - Optical microscopy (with scanning capabilities):
    - **Fluorescence microscope** with Z-Scan for 3D imaging
    - Inspection through **darkfield microscope**
  - **Colour camera** (fixed inside the 3DBC-IU) for pictures taking and video recording
    - Resolution of at least 1280 x 960 pixels for image acquisition
    - Resolution of at least 1280 x 960 pixels (at 15 frames/second) for video recording
  - Electrochemical impedance on samples within the multi-wells

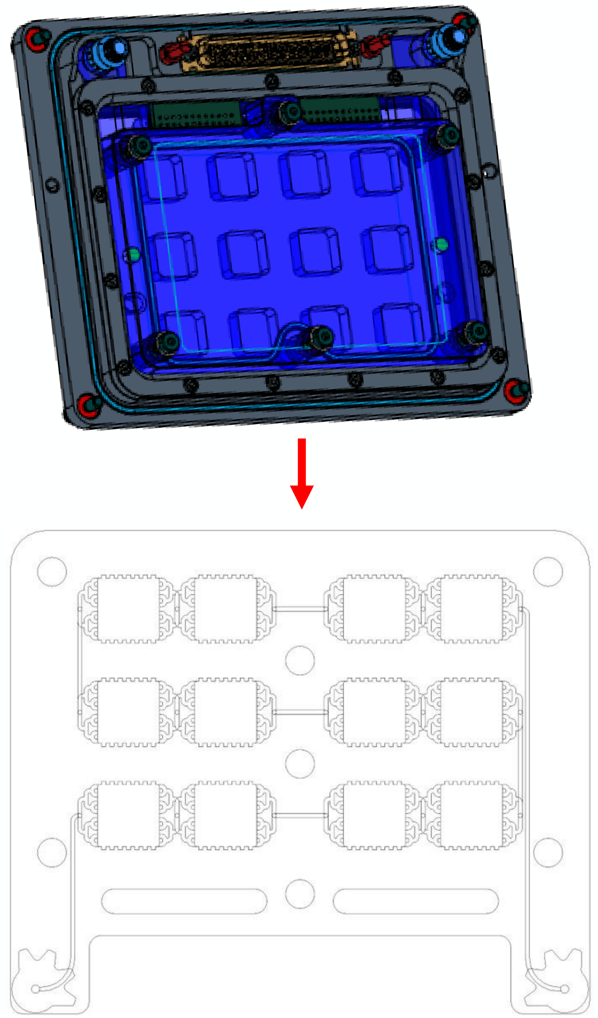


3D Cell Culture Units Main Capabilities: Multi-wells

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Standard multi-wells

- **6, 12** (example on the right), or **24 wells** per plate
- Samples of 5mm or 10mm height (well-plate dependent) can be printed, with a surface area up to 20mm x 20mm (rectangular) or up to 30mm in diameter (cylindrical)
- Current foreseen well-plate material: PMMA
- Option: **electrical impendence spectroscopy** with up to 24 selectable separate measurement points (depending on experiment's needs)
  - selectable frequency value within the range of 500 Hz - 1 MHz, 50 Hz step, and an accuracy of ± 50 Hz
  - Set signal intensity within the range of 50 mV - 500 mV with a step of 50 mV and an accuracy of ± 50 mV, in 25 frequency points logarithmically spaced across the intensity range

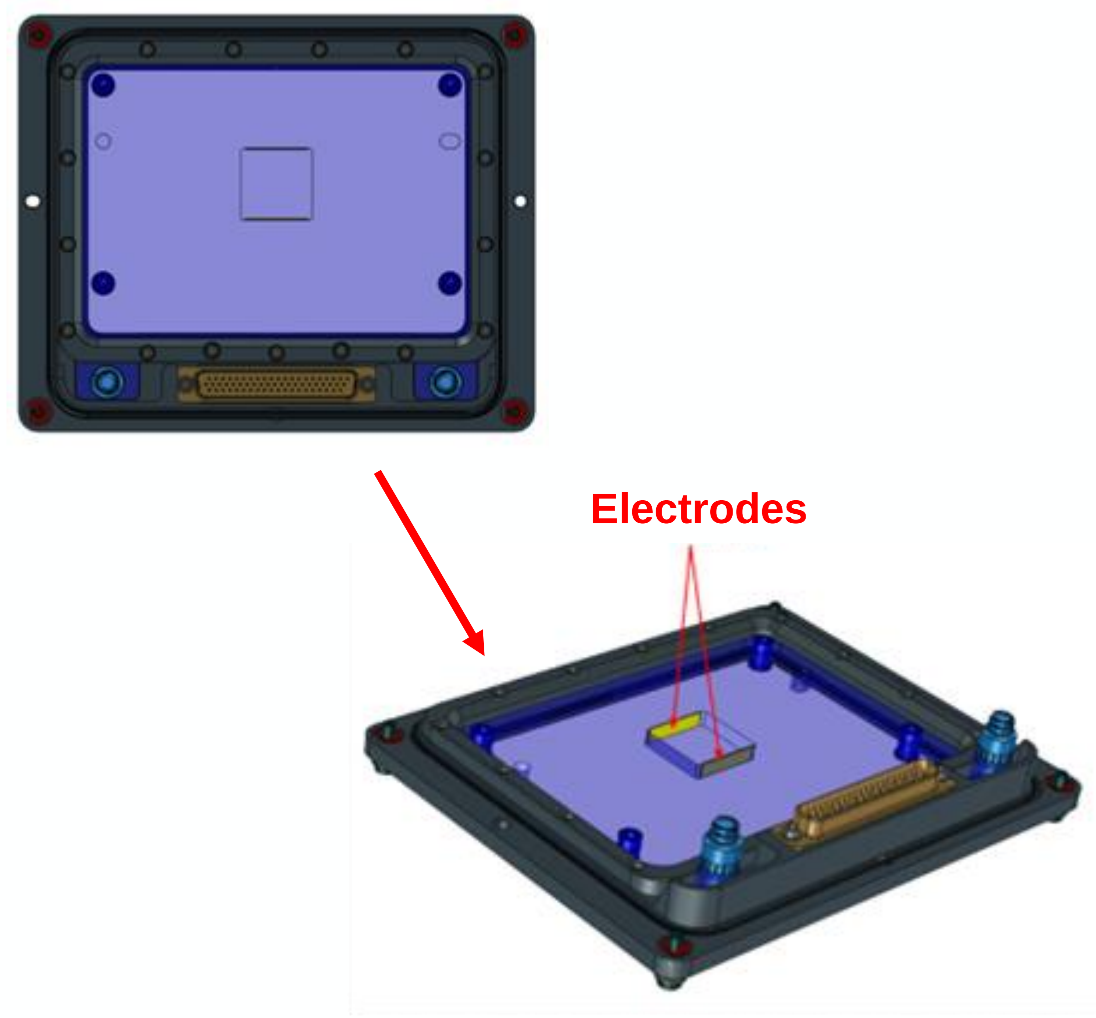


3D Cell Culture Units Main Capabilities: Electrostimulation

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Electrostimulation on a 1-well plate

- Automatic **selectable electric stimulation via bipolar square wave pulses** with a set electrical field value in the range of 50 V/m - 3000 V/m with a step of 1 V/m and an accuracy of ± 1V/m
- Automatic and/or **selectable electrical stimulations with a set current pulse** in the range of 1 mA - 10 mA, with a step of 1 mA and an accuracy of ± 1 mA
- Pulse duration: selectable in the range of 200 µs - 10000 µs with a step of 50 µs and an accuracy of ± 50 µs
- Pulse repetition: selectable in the range of 5 ms - 2000 ms with a step of 5 ms and an accuracy of ± 5 ms but will be extended when charge balancing is not met at end of period
- Pulses can be asymmetric in time and amplitude as long as charge balance is maintained. Negative pulse duration will be driven by the charge balance
- Electrodes will be immersed in medium and positioned at opposite ends of the well, covering well height

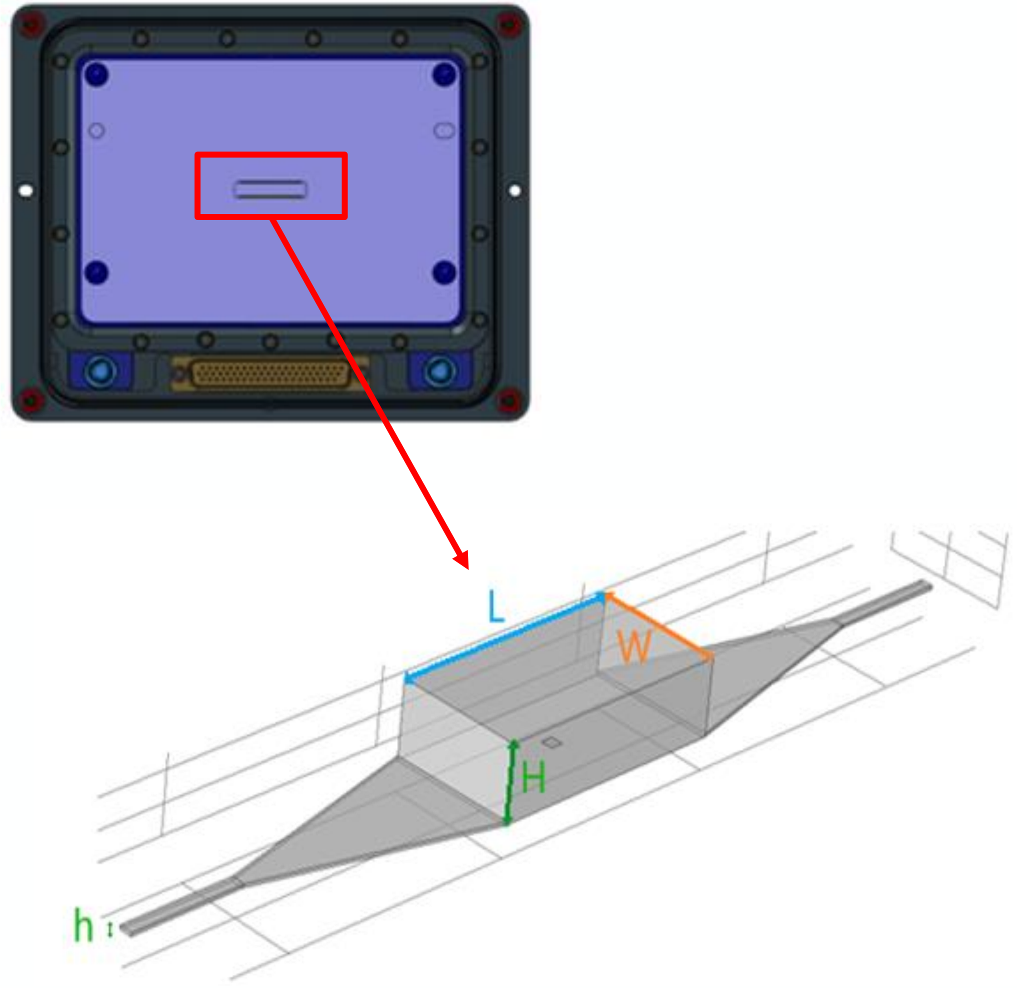


3D Cell Culture Units Main Capabilities: Shear application

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Shear stress application

- 3DBC equipped with 1-well plate design (well dimensions: 1 (H) x 2 (W) x 30 (L) mm)
- **Laminar flow** with shear stresses of 1 dynes/cm2 to 80 dynes/cm2
- Laminar flow can be **pulsated** at a set frequency in the range of 0.5 Hz - 1.5 Hz, with a step of 0.5 Hz and an accuracy of ± 0.5 Hz
- Flow rate between 0 and 300ml/min, corresponding to 0 to 250 cm/s as average speed in the well

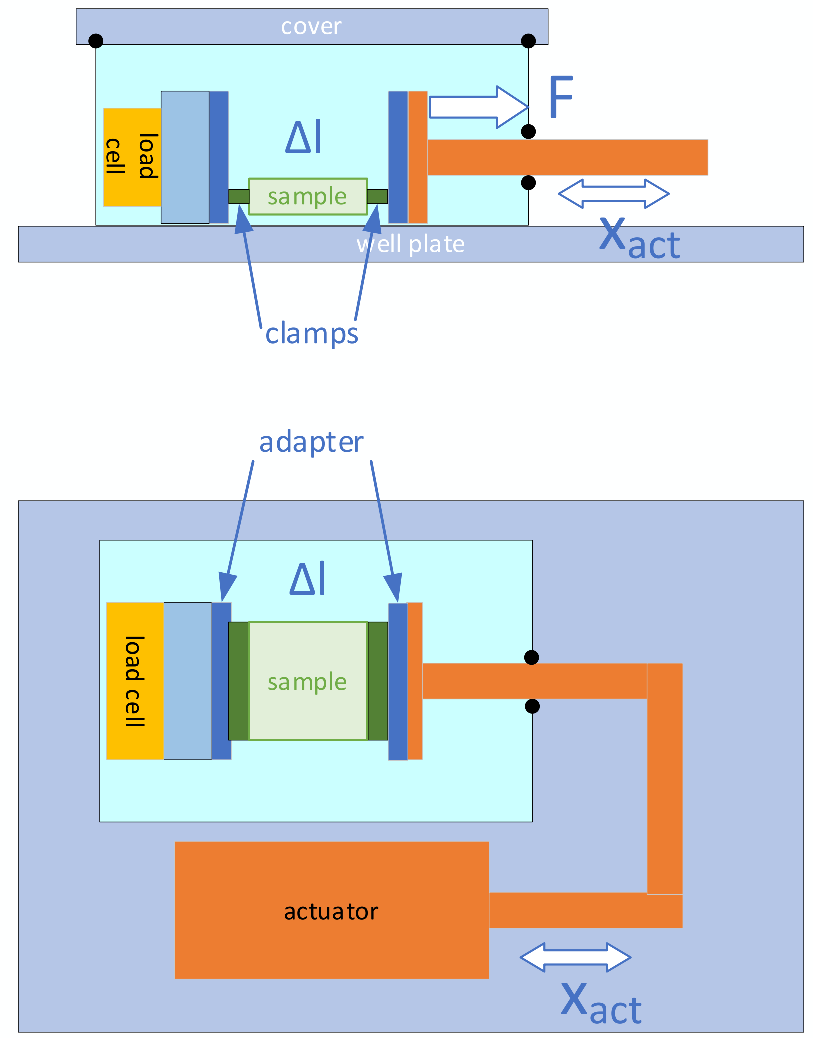


3D Cell Culture Units Main Capabilities: Tension

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Tension on *ex-vivo* constructs

- The mechanical stimuli system applies a known deformation in tension on the sample in the range 0-10mm at a set frequency within the range of 0.1 Hz - 30 Hz, with a step of 0.1 Hz and an accuracy of ± 0.1 Hz.
- Feedback and limitation of the applied force (0-20N, accuracy 10mN) will be monitored

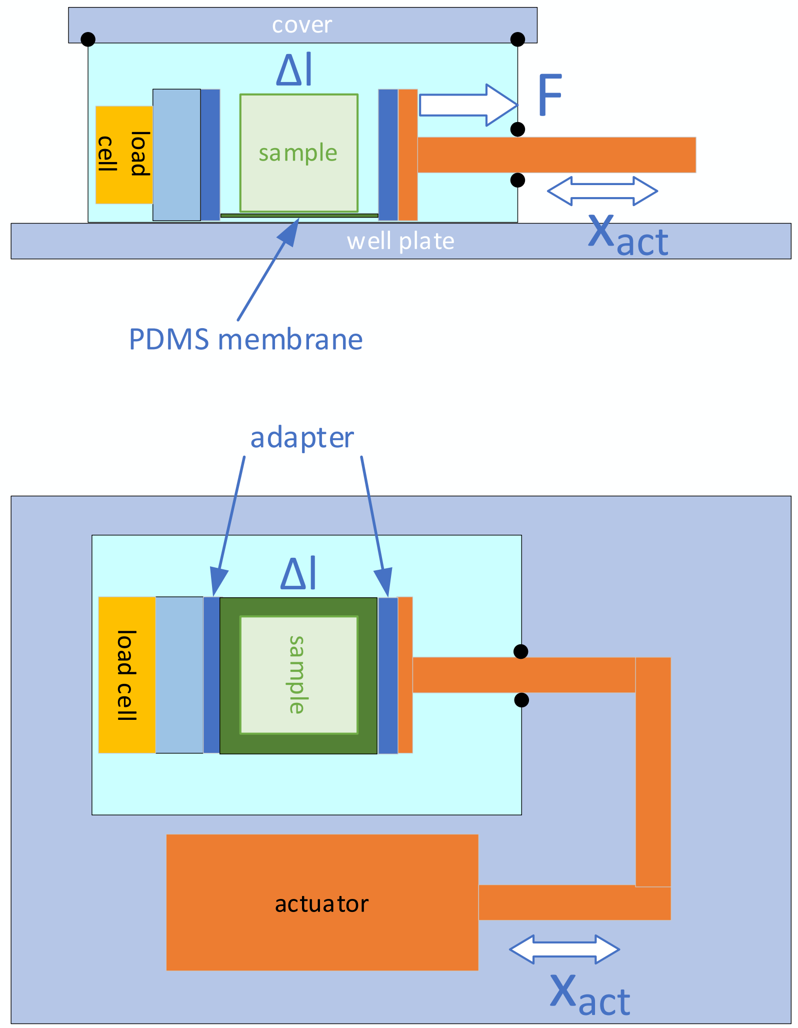


3D Cell Culture Units Main Capabilities: Tension

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Tension on 3D-printed constructs

- 3DBC equipped with 1-well plate design
- Tension set-up: deformation applied using a PDMS gas permeable membrane on which the sample is printed, and the force applied on the membrane shall be measured with an accuracy of 10mN

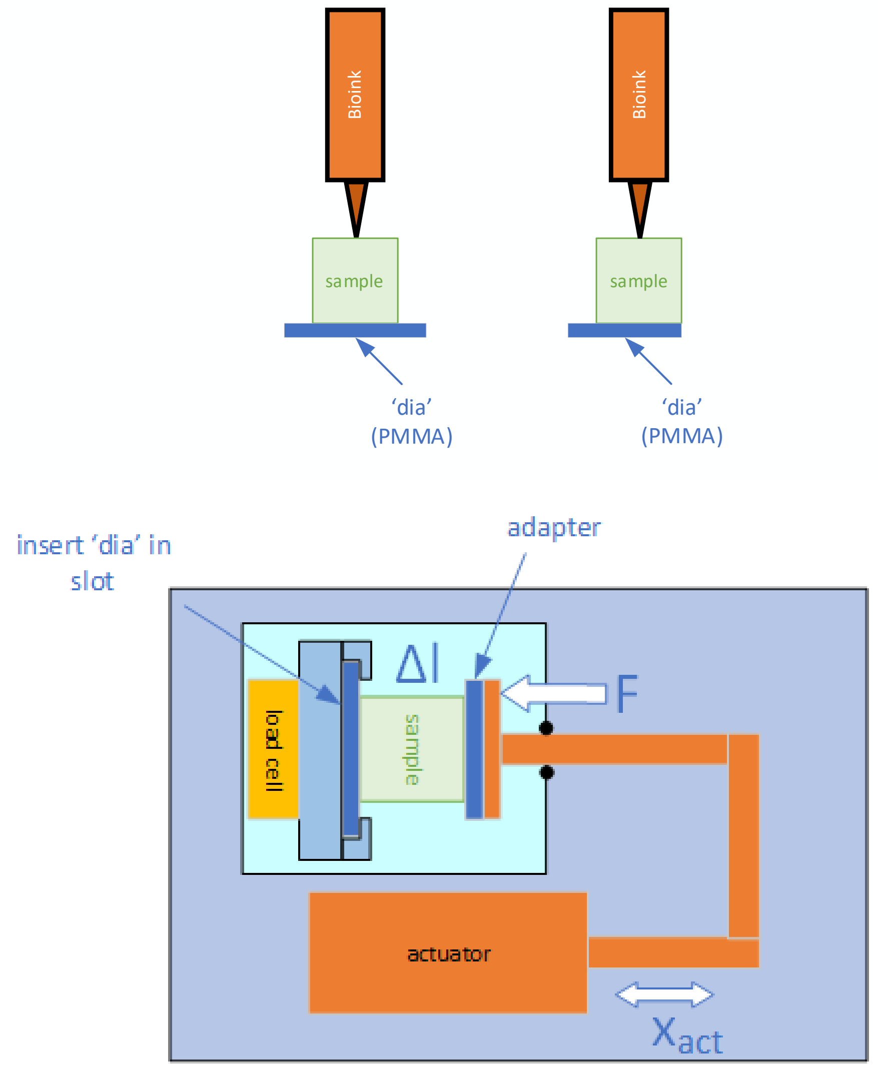


3D Cell Culture Units Main Capabilities: Compression

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Compression on 3D-printed constructs

- 3DBC equipped with 1-well plate design
- Compression set-up: application of a known deformation on the sample in the range of 0-10mm at a set frequency value in the range of 0.1 Hz - 30 Hz, with a step of 0.1 Hz and an accuracy of ± 0.1 Hz.
    - The force range to be applied and measured on the sample is in the range of 1-100mN, with an accuracy of 10mN (for 3D-printed samples)



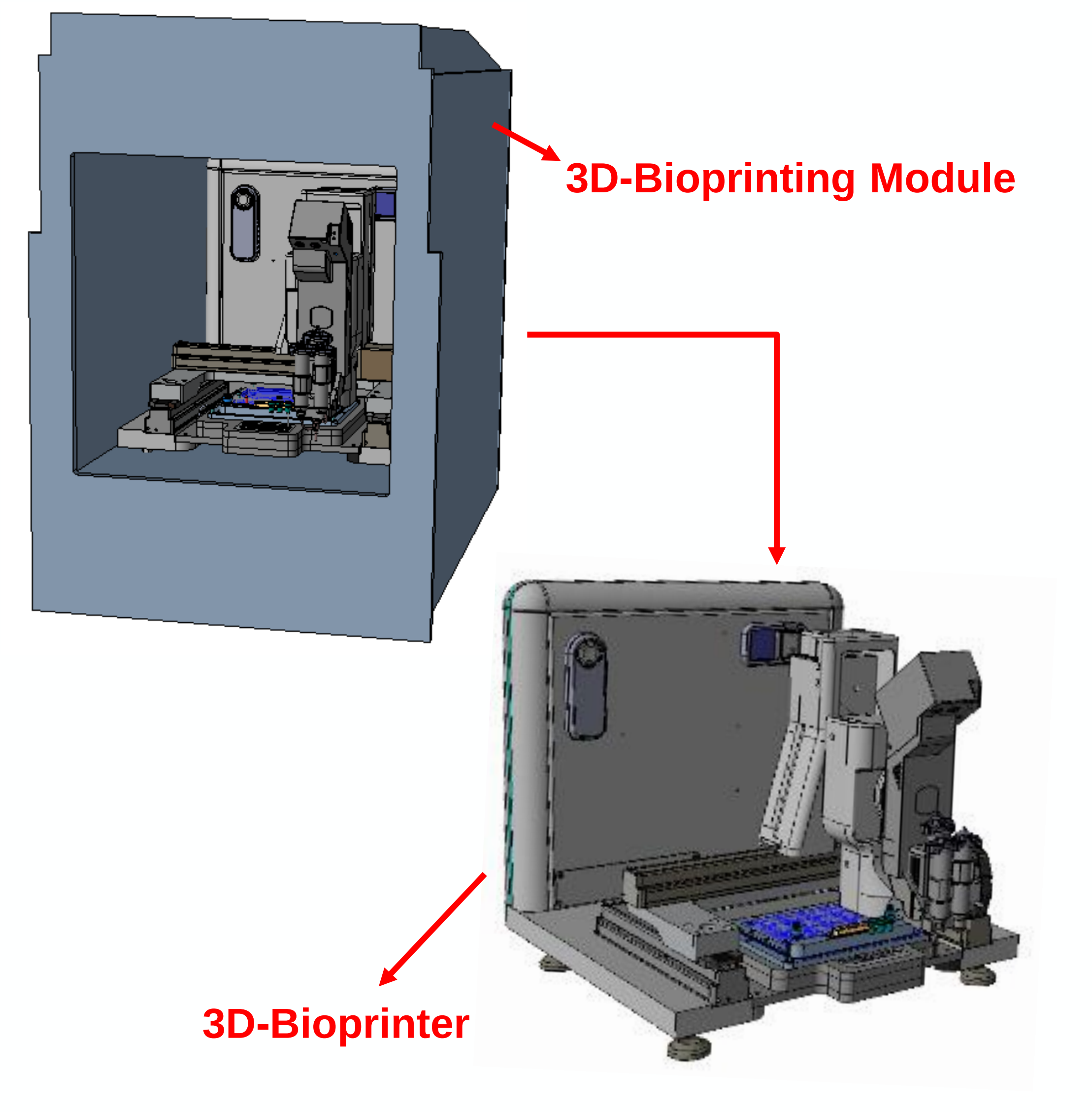
3D Bioprinter Main Capabilities

Specifications will be tested with scientific samples during the development and could change depending on the testing results

Combination of two 3D bioprinting techniques:

- **Microextrusion**
  - Two printhead channels connectable to a coaxial nozzle, allowing setting of pressure for the channels, with the possibility to illuminate the cell constructs while it is being extruded or after layer deposition
  - Printheads nozzles diameters of 200 - 800 µm
- **Drop-on demand/ink-jet**
  - At least one printhead channel, with the possibility to illuminate the cell constructs for photo-crosslinking after it has been printed
  - Printhead will be able to print droplet volumes of 20nL – 5µL with a step and an accuracy of ± 10% of the set volume

- Possibility to 3D bioprint **multiple samples** within one printing process.
- Cells will be bioprinted in a 3D bioprinting chamber (3DBC) to allow handling of the samples by the crew in orbit while maintaining sterility.
- Samples prepared in the 3D-Bioprinter will be further processed in orbit with **no exposure to gravity**.
- Samples of 5mm or 10mm height (well-plate dependent) can be printed, with a surface area up to 20mm x 20mm (rectangular) or up to 30mm in diameter (cylindrical)
- Printing will be possible with an x, y positioning axes' spatial resolution of 10 µm for both the drop-on demand/inkjet and microextrusion printheads
- Printing will be possible with a z positioning axis' spatial resolution of 10 µm



Planning your experiment

Considerations

Upload of bioink/structures/reagents

- Time between handover of samples from scientist to launch authority and start of experiment in orbit could be as long as **7 days**
- Limited capacity for **transporting biological material** at cold/set temperatures during transit (consider volumes, temperature requirements)
- **Medium exchange** during upload will not be possible

Volume of medium required for experiment

- Limited stowage for spare **medium** (consider length of experiment + number and volumes of medium exchanges required)
- Limited **cold stowage** available on ISS for storing samples for return to Earth
- Limited capacity to upload/store on-board **large quantities** of bioink containers

Robustness of cells/constructs for upload and download

- Transport to launch site vs culturing at launch site
- Upload condition (extreme g-values)
- On-orbit processing (e.g. bioink generation)
- Samples will **experience temperature changes** before experiment start (e.g. from room temperature(18-29˚C) to incubation)
- **storage time** of fixed sample (cold stowage on board very limited, especially -20 and -80˚C freezers)

Number of cells required for bioink generation in-orbit

- consider volume limitations
- consider cell survivability

3D BS designed to accommodate THL-1 and BSL-2M reagents/samples

- Example fixatives:
  - RNAlater
  - Formaldehyde (concentrated as low as possible)
  - NoToxHisto
- Embedding agents:
  - Water soluble glycol/resin
- Organisms that are associated with mild disease:
  - Staphylococcus aureus
  - Mycobacterium
  - Salmonella choleraesuis

More information:

THL: https://www.nasa.gov/wp-content/uploads/2023/03/jsc-26895-rev1-final.pdf

BSL: Biosafety Review Board (BRB) - NASA

Facility development timeline/process

esa

3DBS design and development milestones

1. System requirements review (SRR) **Completed**

- Complete set of requirements is agreed with industry
- Upon successful review, reduced models (RM) will be built for science teams to start testing functionalities

2. **Preliminary design review (PDR)** **May 2024**

- Industry presents preliminary design, supported by results of functional and scientific testing on RM

3. **Critical design review (CDR)** **Q2 2025**

- Industry presents complete design, complemented by results of testing on Science Reference Models (SRMs)

4. **Flight Acceptance review (FAR)** **mid-2026**

- Industry presents the built and verified model

5. **Launch** **end of 2026**

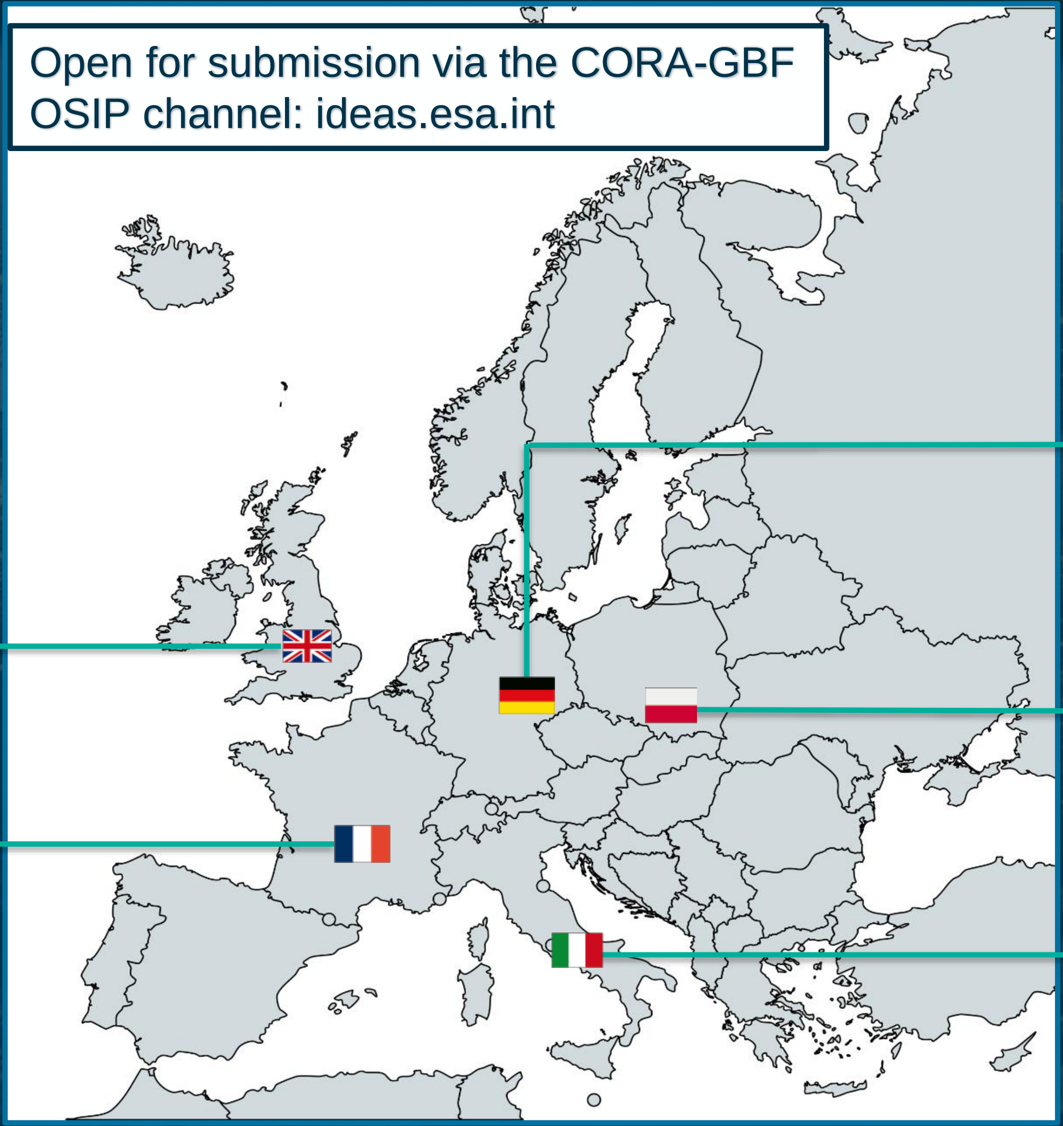
6. **On-Orbit Commissioning Review (OOCR)** **Beginning of 2027**

- HW successfully integrated on orbit and all elements work as intended

European 3D Bioprinting Ground Consortium

Providing scientists in Europe ground 3D bioprinting facilities to prepare for flight

Open for submission via the CORA-GBF OSIP channel: ideas.esa.int



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Biofabrication Laboratory of Biomaterials Group, Warsaw University of Technology, PL [W. Swieszkowski]

Biofabrication Laboratory, University of Pisa, IT [G. Vozzi]

Ground research to prepare for flight

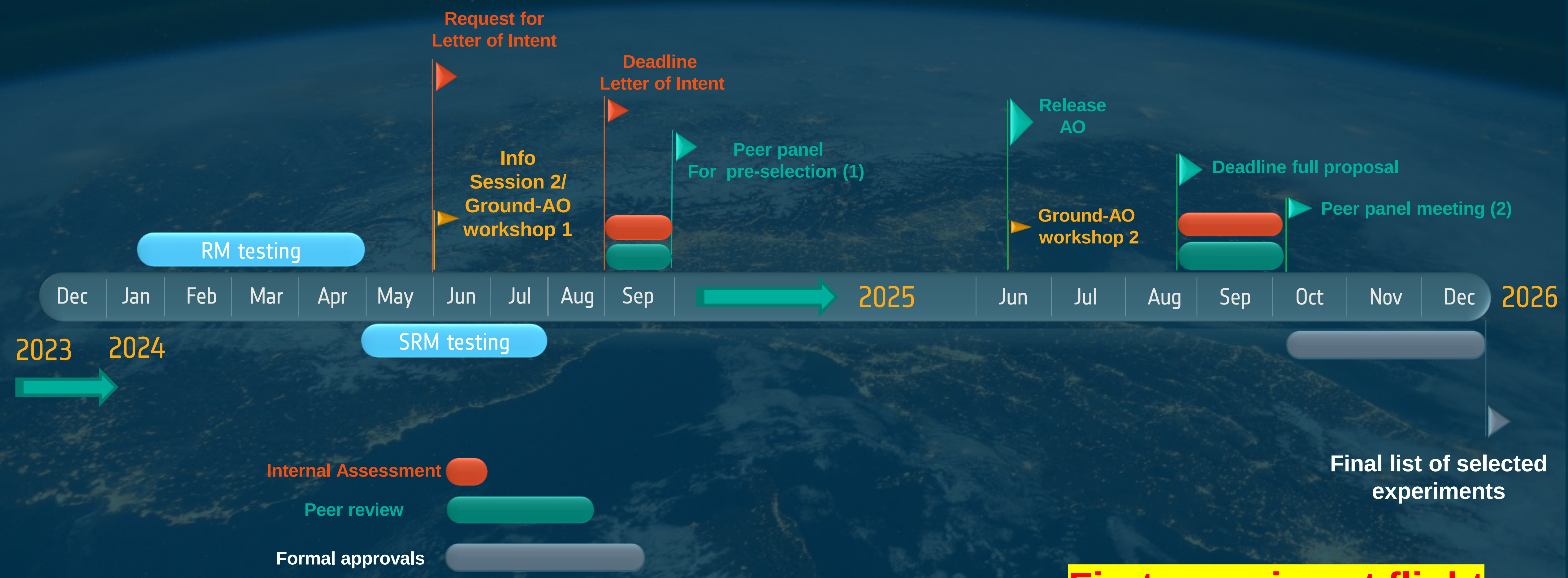
- ESA solicited for precursor and/or preparatory ground-based activities experiments focusing on closing some of technology or knowledge gaps to facilitate or reduce the risks of the planned in-orbit activities

6 projects were selected and are in progress

- Development of 3D printed skeletal muscle constructs to test how microgravity affects myofibre development
- Biofabrication of a 3D bone-on-a-chip by inkjet printing for microgravity research
- Making bioprinting in microgravity possible on Earth
- Paving the way for bioprinting of skin in space
- Core-Shell Bioprinting of Skeletal Muscle Motoric Units
- Robust extrusion bioprinting of mesoscopic tissue constructs: optimising flow and transport regimes

- Workshop with selected projects will take place at release of AO to inform applicants on preliminary results

3D biosystem – <u>tentative</u> plan for proposal submissions



First experiment flight NET 2027

<u>Schedule is preliminary and subject to change</u>

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- Rodrigo Coutinho de Almeida
- Lina Cumps

THANK YOU! – QUESTIONS?

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