Microscopes on ISS
Present and Future Possibilities

2018 Microgravity Molecular Crystal Growth Workshop

Presented by:

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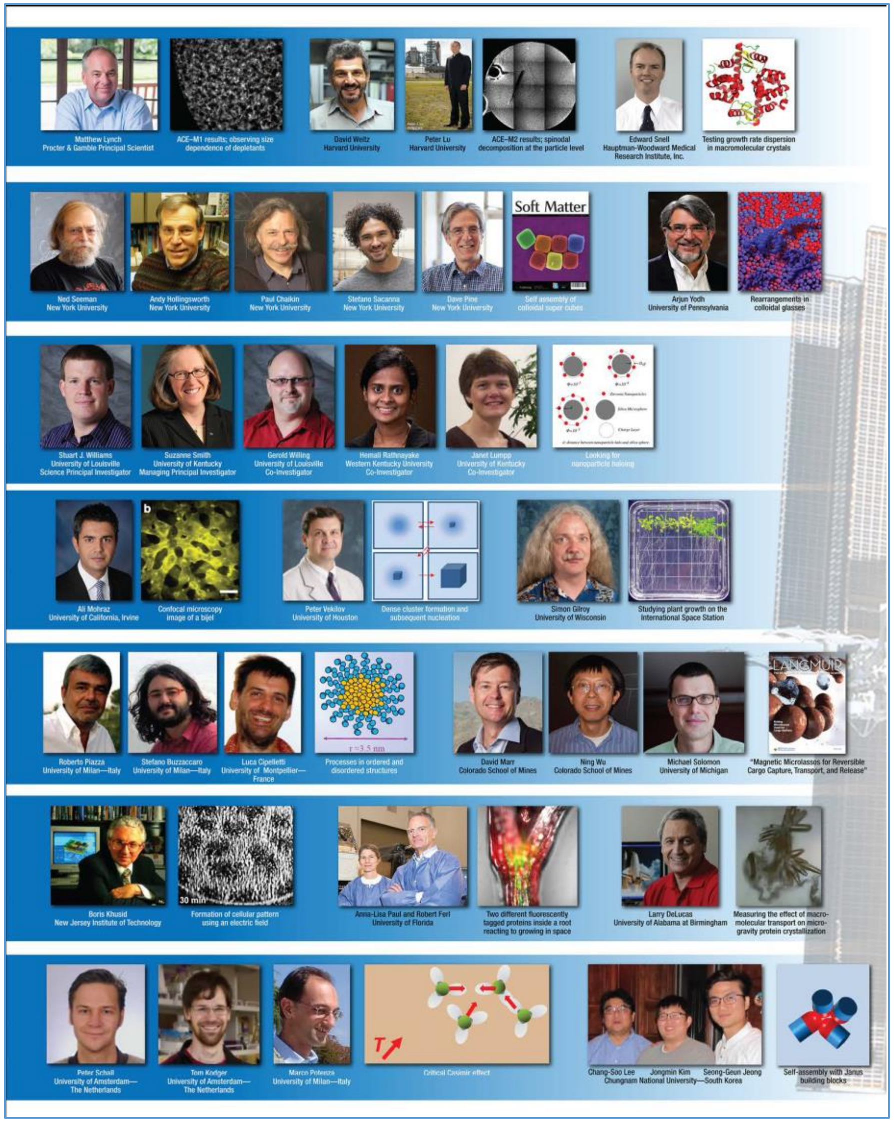
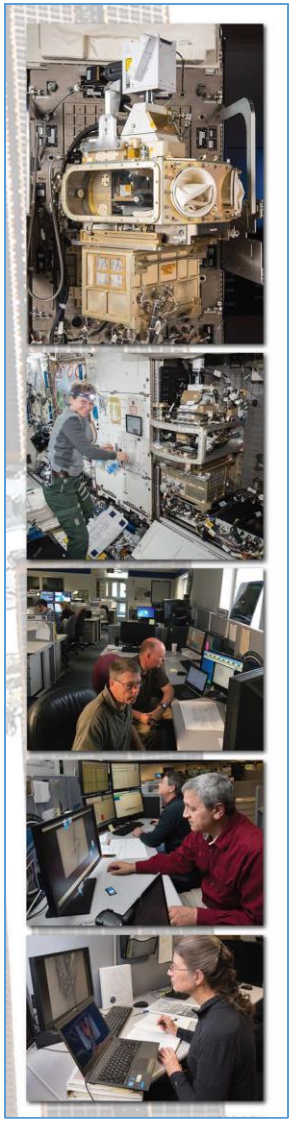
Light Microscopy Module (LMM)

A microgravity microscope on the International Space Station (ISS) with 3D imaging capabilities

Present capabilities (science)





Background – LMM vs Requirements
Examples

Parameter/Capability	Colloid Investigation Requirements	Macromolecular Biophyics Requirements	Space Biology Requirements
Microscopy			DRAFT DOCUMENT OF REALISTIC UPGRADE
Magnification			
Dry	10x, 20x, 40x, 50x, 63x, 100x High magnification - assume 100x Ref: Colloidal particle size (0.5-2.0 µm)	10x to 63x	Multiple objectives- 1-2x, 10x, 20x, 40x, 50x, 63x, 100x High magnification - assume 63-100x Ref: Zoom out of specimen at 1-2x to 10-40x for cells to subcellular features of 0.5-2.0 µm)
Oil	100x NA 1.4		
Field of view (mm)	at 100x: 100 µm x 100 µm at 10x: 6 mm x 6 mm		
Aperture			
X-movement (range & resolution)	77 mm ± TBD		8 cm for petri dish at 0.5-2.0 µm
Y-movement (range & resolution)	44 mm ± TBD		8 cm for petri dish at 0.5-2.0 µm
Z-movement (resolution)	200 nanometers		approx 150 µm for confocal
Area to view		Sample chamber must have movement in X, Y, Z directions to visualize all area within chamber (Chambers must be at least 1.0mm per side, maximum of 10mm X 10mm X 5 mm)	Check with Ames as to what is currently practical.
Location repeatability			If there is a specific group of cells of interest, the camera must be able to return to the exact same ROI to monitor the same group of cells over time to see cell response to environment or factor treatment over time.
Illumination methods			
Kohler Illumination	Strongly preferred for dynamic differential microscopy		KOHLER ILLUMINATION ACCEPTABLE
Epi-illumination	Yes		Yes
Transillumination	Strongly preferred for dynamic devirational microscopy	Desired for brightfield	Desired for brightfield
Imaging techniques			
Brightfield microscopy	Can use White light (epi-illumination) Prefer Kohler illumination with uniform, broadband lighting	Epi-illumination can be used	Epi-illumination; Kohler illumination good
Darkfield microscopy	View crystal morphology from Bragg scattering		Useful for blood cell imaging; invertebrates such as shrimp. Otherwise not so commonly used.
Phase contrast microscopy	View index matched samples		Commonly used in cell culture and imaging of small plants and animals
Differential interference contrast (DIC) microscopy			Also important method in biology.

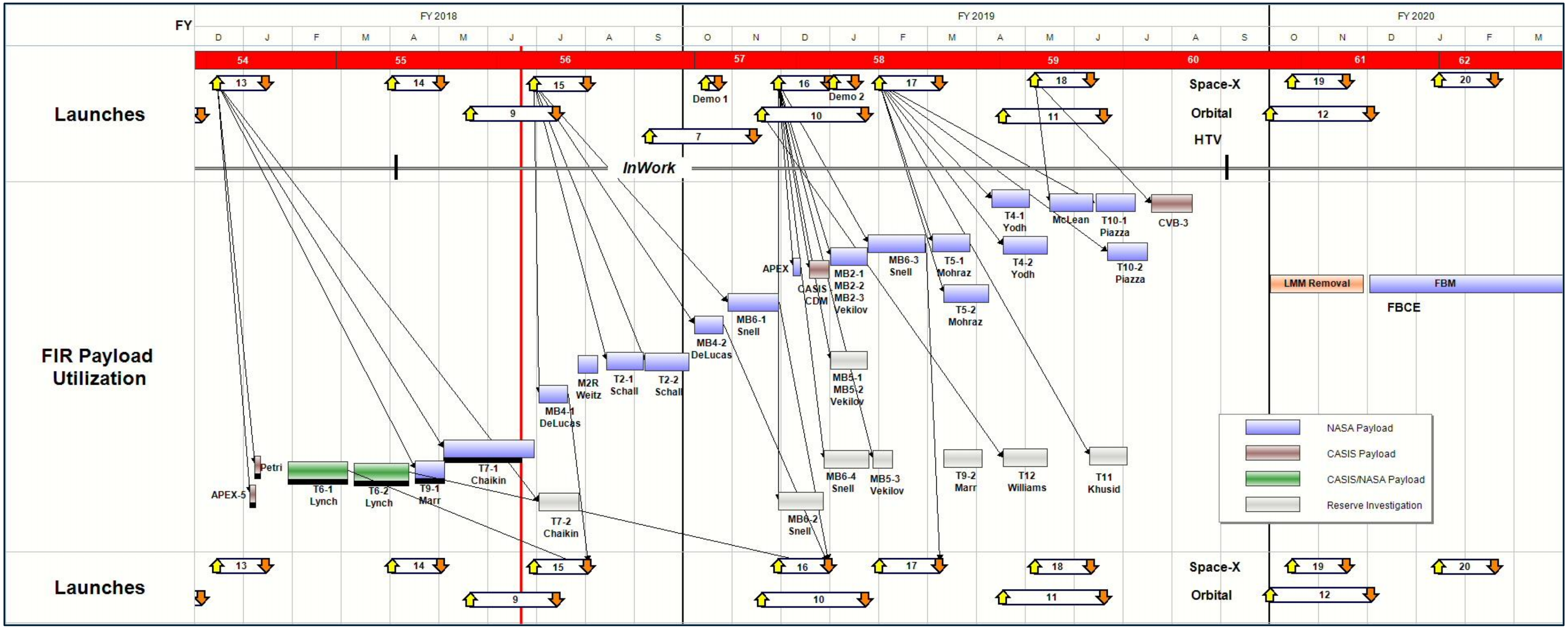
Light Microscopy Module Utilization Table

7/19/18

Chaikin	NYU	Colloids-01	Motility, Self-assembly	ACE	M-3		T-7		
Weitz	Harvard	Colloids-02	Critical Point	ACE	M-2R				
Yodh	U. Penn	Colloids-03	Polydispersity	ACE		H-1	T-4		
Lynch	P&G	Colloids-04	Stabilizers	ACE	M-1		T-6		
Lee	CNU, S. Korea	Colloids-05	Anisotropic Particles	ACE			T-1		
Schall	UvA	Colloids-06	Critical Casimir Effect	ACE			T-2		
Piazza	Milan	Colloids-07	Depletion gels	ACE			T-10		
Marr	CSM	Colloids-08	Applied External Fields	ACE			T-9		
Mohraz	UC Irvine	Colloids-09	Bijels	ACE			T-5		
Khusid	NJIT	Colloids-10	Hard Spheres	ACE			T-11		
Williams	UL	Colloids-11	Nanoparticle haloing	ACE		H-2	T-12		
Delucas	UAB	Biophysics-01	Macromolecular	MMB				MB-1/4	
Vekilov	UH	Biophysics-02	Nucleation	MMB				MB-5	
Snell	HWMRI	Biophysics-03	Growth Rate Dispersion	MMB				MB-3/6	
Plawsky	RPI	Fluid Physics-01	Transport Phenomenon	CVB					CVB-1,2,3
Ferl & Paul	Florida	Space Biology	Arabidopsis	ACE					Petri, APEX3
McLean									

GRC ISS Physical Sciences Research
FIR 2 Year Schedule

06/21/18

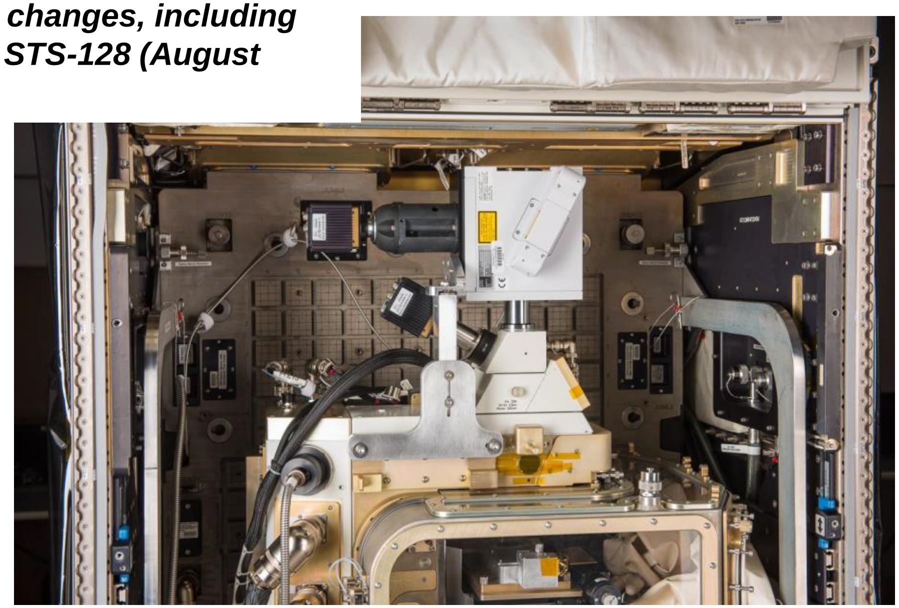


Background - Overview
Fluids Integrated Rack - FIR

The Fluids Integrated Rack (FIR) is a fluid physics research facility designed to host investigations in areas such as colloids, gels, bubbles, wetting and capillary action, and phase changes, including boiling and cooling. Hardware was delivered on STS-128 (August 2009) to ISS and installed in the USLAB.

ISS021E033218

Astronaut Bob Thirsk completing install of the FIR/LMM prior to FIR activation in December 2009.



Key Events	Date
Design Phase	1999
Launch (STS-128; 17A)	Aug 2009
LMM Science	Jan 2010 - Aug 2017
LMM Enhancements	Aug 2017 - Sept 2019
Return (refurbish ?)	Dec 2019 (begin ?)

Fluids Integrated Rack (FIR)
Light Microscopy Module (LMM) Overview

Auxiliary Fluids Container

The AFC provides one level of containment.
Two sealed glove ports, electrical pass through,
1/4" Lexan© windows with Viton© seals.
Working Volume = 0.054 cu. Meters

Core Microscope

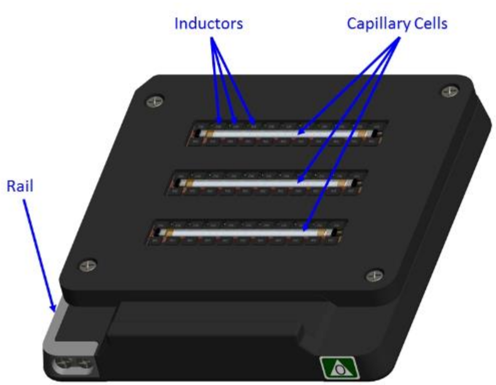
- The LMM is a script driven, on-orbit microscope
- The imaging techniques of high resolution color video microscopy, bright field and fluorescence are are currently available.
- Currently available objectives: 2.5x, 10x, 20x, 40x, 63x air, 63x oil, 100x oil
- Infinity optics (Leica aberration corrections)
- Confocal microscopy hardware details on next slide

Confocal Camera

ImperxBobcat B2020 Camera
Resolution 2048 x 2048
Sensor Progressive Scan CCD
2048 x 2048 pixels
15.2mm (H) x 15.2mm (V)
4/3" optical format
Frame Rate 16 fps,
32 fps binned
Max. Frame Rate 153 fps
Minimum S/N Ratio 60dB
ADC: 8, 10, 12, 14-bit

Observation Camera

Basler ACE model acA1600-20gc
•Sony ICX274 Progressive Scan CCD array
•1600 x 1200 pixels at 20 fps
•Color, 1/1.8" format
•GigE Vision interface
•FOV 51 mm x 42 mm (2" x 1.7") at 35 microns/pixel sampling, at Working Distance of 75 mm
•Schneider Xenoplan 1.4/23 mm lens
•Fits in AFC with Schneider lens (60 x 60 x 77 mm3(W x H x L; 0.4 kg)



Temperature controlled sample cells

- Ambient (~23C) to 60C +/- 0.1C over length of capillary cell.
- Linear with a gradient temperature control, delta up to 17C.
- In-situ mixing.

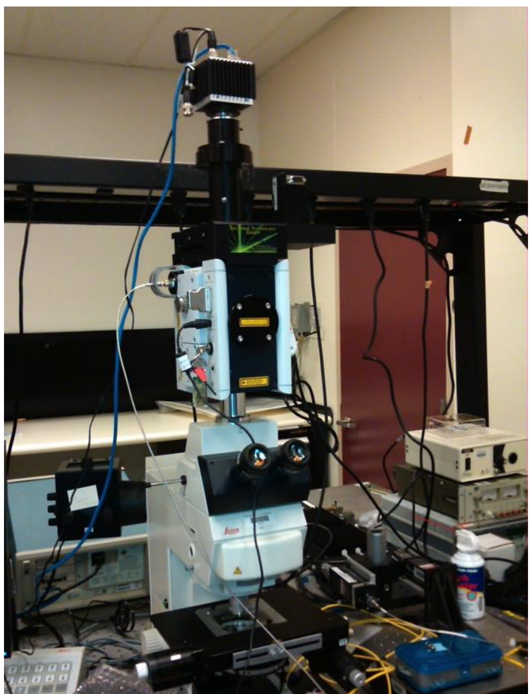
X-Y Stage

Leica COTS part
Mounts to microscope
Integrated Cold Plate
Water hoses connect to AFC

LMM Enhancements
Confocal Overview

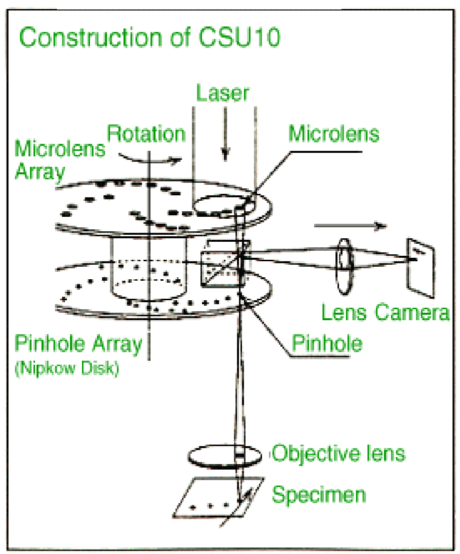
Confocal Microscopy: *Enables 3-D interrogation of structures*

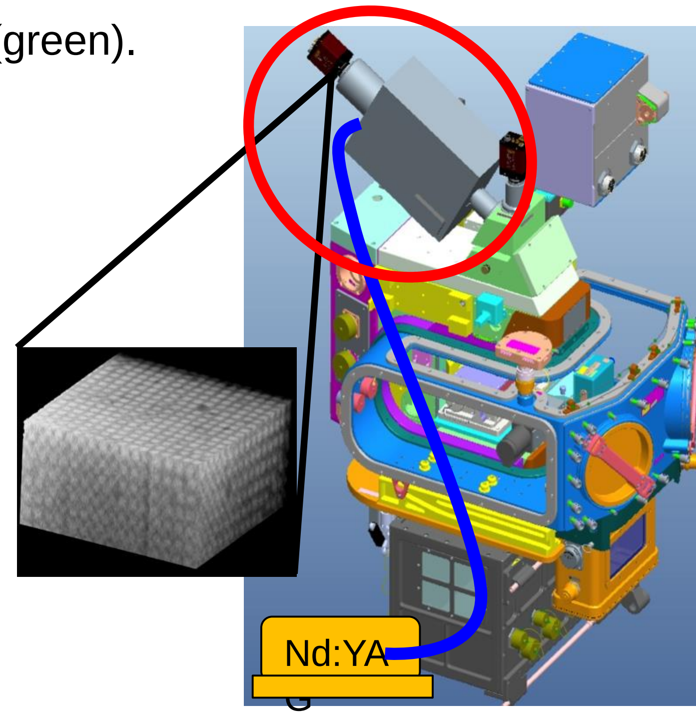
- Image sectioning enhancement; add-on to LMM - mounts to camera port.
- Requires external camera connected through IPSU-G.
- Requires fiber-coupled laser – single color, 532nm (green).



Confocal Unit in GRC's LMM Lab

Schematic of CSU-X1





LMM Options Available with Funds

The present LMM has the "hooks & scars" built-in to accommodate:

1. An impressive laser tweezers package (several dozen parallel programmable spots) using a modified objective turret, like David Grier's (NYU) holographic optical tweezers, which are compact and were tested with a different brand of microscope. It would also require the additional of a fiber optic coupled laser – not hard, but worth mentioning. The slightly different hardware (modified objective turret) needed to add laser tweezers to the present Leica (LMM) microscope was proposed a few years ago for inclusion in the LMM via a Phase 2 SBIR by Boulder Nonlinear Systems (BNS) Lafayette, CO 80026.
2. A multi-color light source like that flown on the JAXA microscope in the Japanese Experiment Module (JEM) on the ISS. Allows multiple fluorophores needed for biology and to easily distinguish different particle sizes (which have been tagged with different fluorescent dyes).
3. Trans-illumination, lighting from below with a Kohler light source. Light mostly forward scatters in the Mie Regime (when particles are bigger than 1/10 the wavelength of light. Supports DIC, darkfield, phase contrast, …
4. Heated oil objective or thermally isolated oil objective (ceramic spacer) to avoid need to adjust temperature of sample to avoid Soret diffusion caused by possible temperature gradient. Metal microscope conducts heat from electronics through its body, through the objective, though the oil, into the sample. Sample must presently have same temperature as immersion objective to not induce particle movement from Soret diffusion.

Additional Microscope Options for Science Experiments – ISS Facilities

NASA SLPSRA – Liquid Crystal Facility)	Jim Kolibas	ZIN Technologies	james.kolibas@nasa.gov (216) 433-5103
CASIS/ISS – MVP and ADSEP-2 Facilities	Rachel Ormsby	techshot	rormsby@techshot.com (812) 728-8122
ISS Program IDIQ – SCORPIO-V SUITE OF SPACELABS	Caitlin O'Connell Devin Ridgley	Hnu Photonics	coconnell@hnuphotonics.com dridgley@hnuphotonics.com (808) 244-7800

MVP Experiment Module (Cell Module) - Available Now

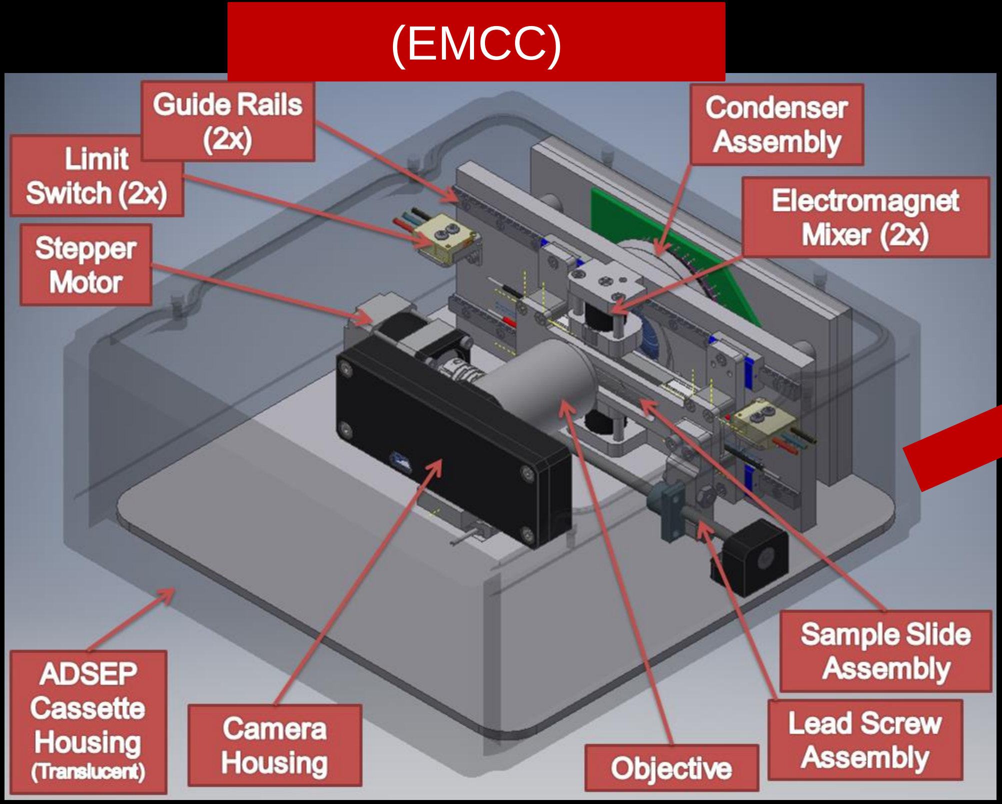
Sample Chamber

Microscope Objective
(Olympus 20X shown)

Camera

ADSEP-2 Facility and the Electromagnet Mixer Colloid Cassette (EMCC)

Available March 2020



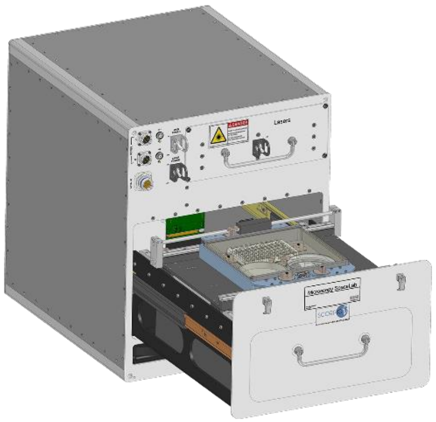
Techshot Facilities with Microscope Capabilities

- MVP and ADSEP-2 Facilities accommodates up to twelve Experiment Modules and 3 Cassettes respectively
- Installed and removed by the crew without tools. Electronic connects using blind mate connectors (no cables)
- 10 megapixel microscope camera, capable of high definition video or still images
- Magnification from 5x to 100x using standard RMS objectives
- Facilities can record and/or downlink images for near to real-time viewing (bandwidth permitting)
- Experiment modules and Cassettes can achieve 3 levels of containment and may be opened by crew (without tools) inside a glove box/bag
- EMCC capable of light-field and dark-field illumination from LED substage condenser
- EMCC uses piezoelectric stage for focusing

THE SCORPIO-V SUITE OF SPACELABS

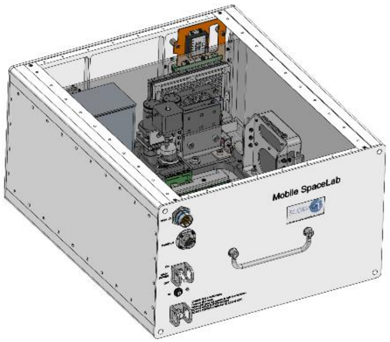
Automated, high-throughput microgravity research platforms for life and physical sciences

HNu Photonics has developed several portable, fully automated microfluidic platforms, for biological, protein crystallization and material science microscopy investigations on the International Space Station (ISS), Sub-orbital and ISS resupply vehicles.



Microscopy SpaceLab (Q1 2020)

BioChip SpaceLab (Q3 2019)



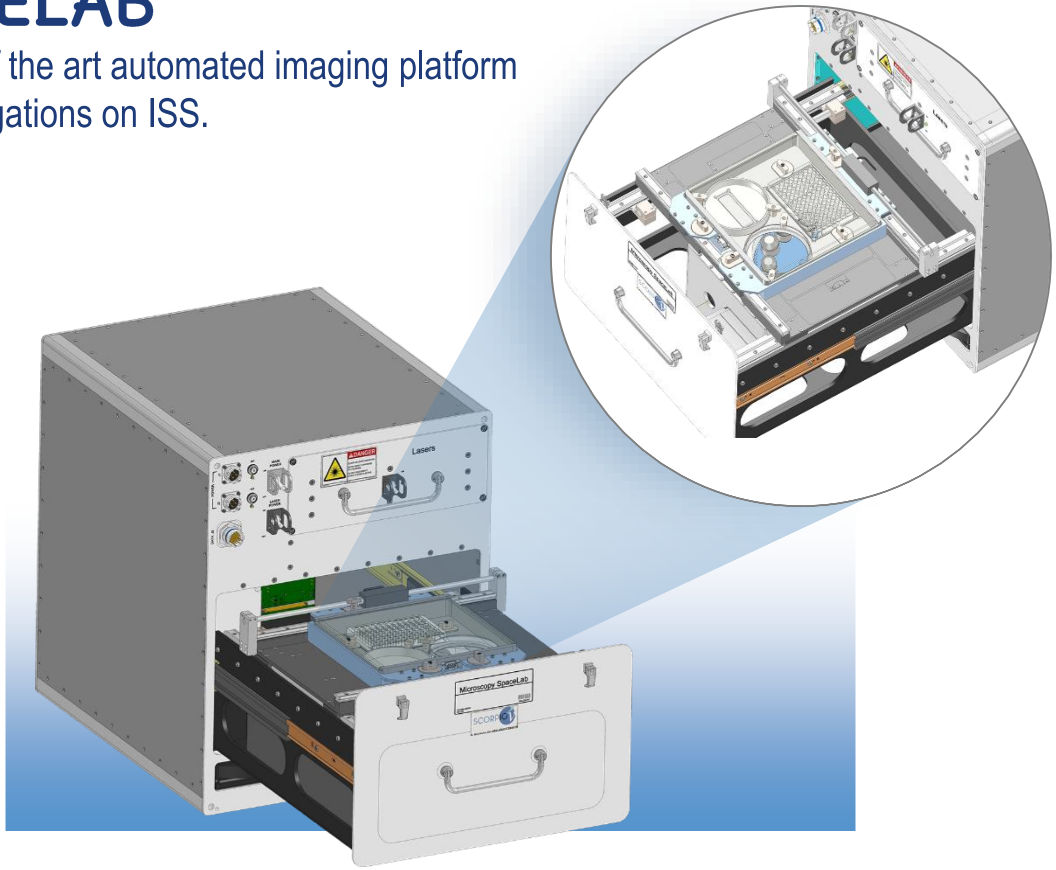
Mobile SpaceLab (Q1 2019)

MICROSCOPY SPACELAB

Microscopy SpaceLab is a versatile, state of the art automated imaging platform facilitating physical and life sciences investigations on ISS.

Facility Capabilities:

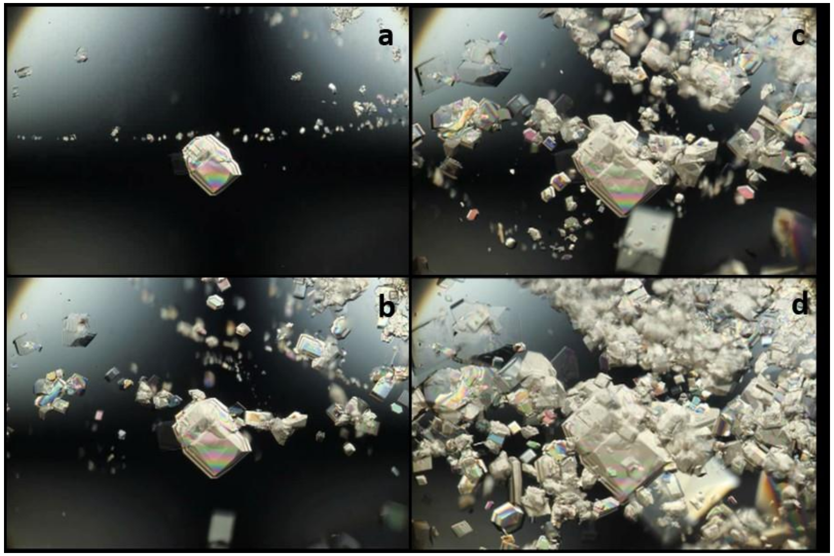
- Brightfield and darkfield imaging with transmitted and reflected light
- Minimum eight selectable objectives with magnifications ranging from 2X-100X
- Fluorescence Microscopy
    - 7 Selectable Lasers
- Confocal Microscopy
    - 2 Selectable Lasers
- 3D Microscopy
    - Multiple modes of Imaging and Analysis
- Polarization, DIC and Phase Contrast



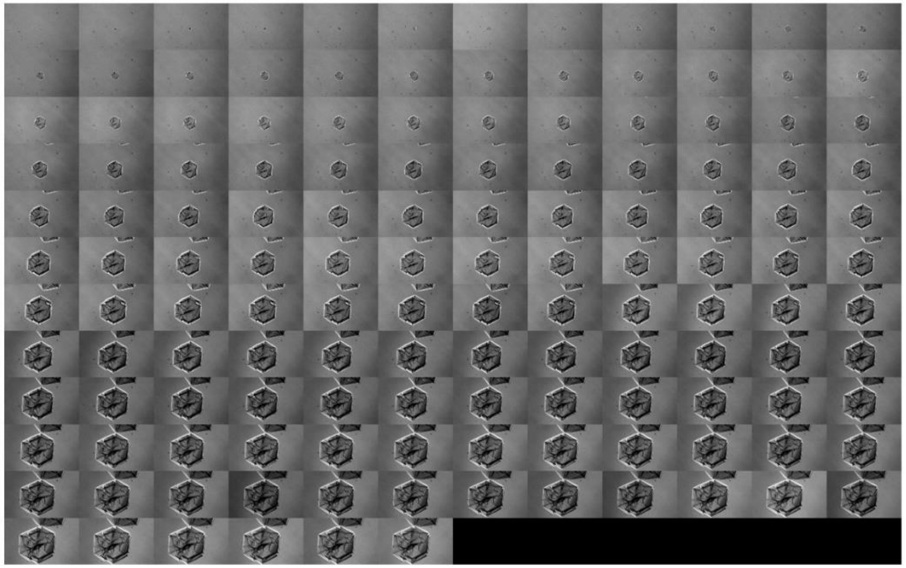
Microscopy SpaceLab Scheduled for Launch to ISS in the Spring of 2020

MICROSCOPY SPACELAB

Microscopy SpaceLab Crystallization Investigations



Time-Lapse images of sugar crystallizing over (a) 0, (b) 10, (c) 20 and (d) 30 minutes as observed with transmission polarized microscopy and a 4X lens.



Montage of insulin crystallization performed by adjusting the temperature of the solution between 8°C and 20°C at pre-defined times. The images were acquired by the SCORPIO-V team with a 4X lens over 66 hours with each frame taken at 30-minute intervals.

Microscopy - Observing Insulin Crystal Growth