ELM: Europa Luminescence Microscope

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ELM: Europa Luminescence Microscope

(Micro)fluidics-based fluorescence and bright/dark field imaging microscope with sub-micron resolution

3 Operational Modes:

1) **Utilizing fluorescence stains specific to key structural biomarkers, i.e., typical membrane constituents such as fatty acids, phospholipid bilayers, and membrane proteins.**
2) **Using DUV and visible light-emitting diodes (LEDs) for the excitation of native luminescence in the samples; mineralogical and biological.**
3) **Dark field imaging for visual characterization and context - unscattered light excluded, providing dark background.**

ABSTRACT

The Europa Luminescence Microscope (ELM) is an automated fluorescence and bright-field microscope designed to meet key objectives defined in the 2016 NASA Europa Lander Study Report, including the identification and characterization of morphological biosignatures. ELM's heritage stems from a 2U cubesat fluorescence microscope, the Fluorescence Analysis for In situ Research imager, designed and built at NASA Ames Research Center, for the autonomous study of microbial biology in low Earth orbit. For ELM implementation, a sample is autonomously manipulated with a microfluidic system using in-line 10, 1.0, and 0.2 µm pore-size filters to capture successively smaller particles for imaging. For bright-field imaging, ELM uses deep-ultraviolet, ultraviolet and visible light to image organic and inorganic structures with submicron resolution. The ability to detect biosignatures as small as 0.2 µm in size is achieved by imaging native fluorescence and using fluorescence microscopy stains to identify key structural and functional indicators of microbial life (proteins, lipids, nucleic acids). For fluorescence imaging, ELM uses 265, 370, 470, and 530 nm LEDs with five emission bands. The use of multiple excitation and emission wavelengths for native fluorescence imaging enables the detection of a wide range of molecular species and their rough classification. Excitation at 265 nm allows for the detection of smaller polycyclic aromatic hydrocarbons (PAH), aromatic amino acids, and proteins with little to no interference from mineral fluorescence, given proper emission band selection. 370 and 470 nm light excites increasingly larger PAH structures and larger aromatic biomolecules that may be present (e.g., protective pigments). Similarly, inorganic fluorescence can be characterized and separated from organic fluorescence, allowing the recognition and in some cases classification, of minerals and other abiotic particles. ELM is based upon work supported by the NASA COLDTech and ICEE-2 programs.

Technology Heritage

Fluorescence Analysis for In-situ Research on Nanosatellites (FLAIR) Imager

Part of the Microsatellite in-Situ Technologies (MisST) project

- 2U (10 cm x 20 cm) CubeSat payload
- Designed for biological investigations in low-earth orbit
- Autonomous microfluidics and imaging
- Dual-wavelength LED fluorescence excitation (blue & amber)
- Dual-bandpass emission filter
- White LED dark field imaging (<4 µm resolution with LEO qualified imager; 2 µm resolution w/upgraded imager)
- Pressure, Temperature, RH Sensors

Science Objectives

Designing to broadly and specifically address the Europa Lander Study Report

Report Defined Goals:

- Search for evidence of life on Europa
- Assess the habitability of Europa

Objectives:

1) ***Identify and characterize morphological, textural, indicators***

   ELM: Sub-micron dark field imaging; Spatial Fluorescence

2) ***Detect and characterize inorganic indicators***

   ELM: Native Fluorescence - mineralogy

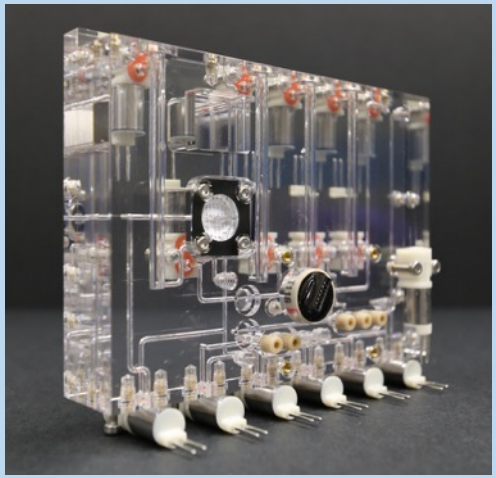
3) ***Detect and characterize any organic indicators***

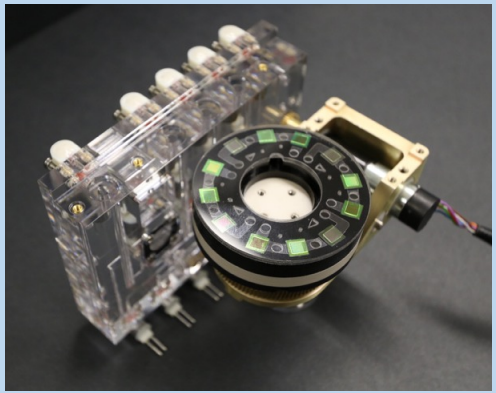
   ELM: Proteins, polypeptides (i.e. amine-containing compounds), Lipids in organized structures (e.g. membranes), Nucleic Acids

4) ***Determine the presence of environmental factors essential for life (habitability)***

   ELM: Imbedded sensors (pH, Eh, Conductivity)

ELM Microfluidics Subsystem





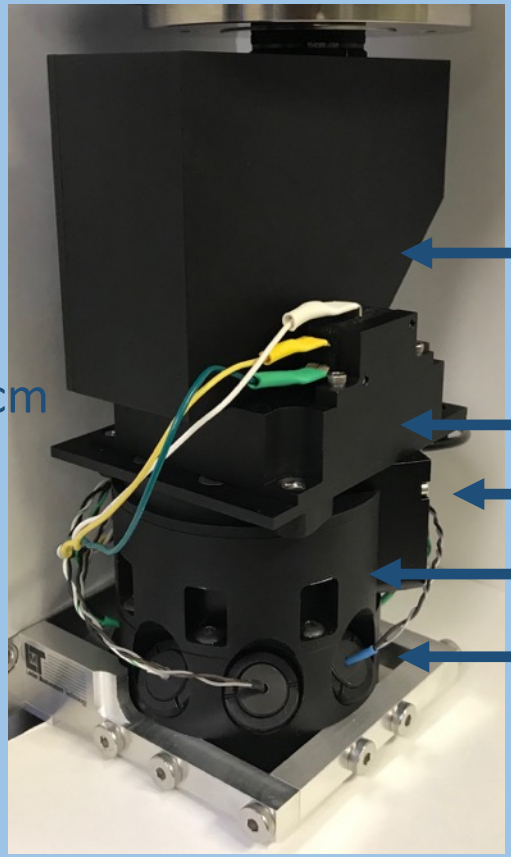
Key Features

- 8 cm x 10 cm Monolithic Fluidic Manifold
- Space Biology Lineage
- Radiation Tested to > 300 krad
- pH, ORP and Pressure Measurements with feedback control
- Fluorescent Stains: Porous Polymer Stabilization and Storage

Silicon Nitride Membrane Particle Filtering

- Three stage filtering 10 µm; 1 µm; 0.2 µm
- 5 mm x 5 mm Si Frame
- 3 mm x 3 mm SiN Filter Collection Area
- 14 X 50 µm x 3 mm collection fields
- Provides optimized imaging efficiency

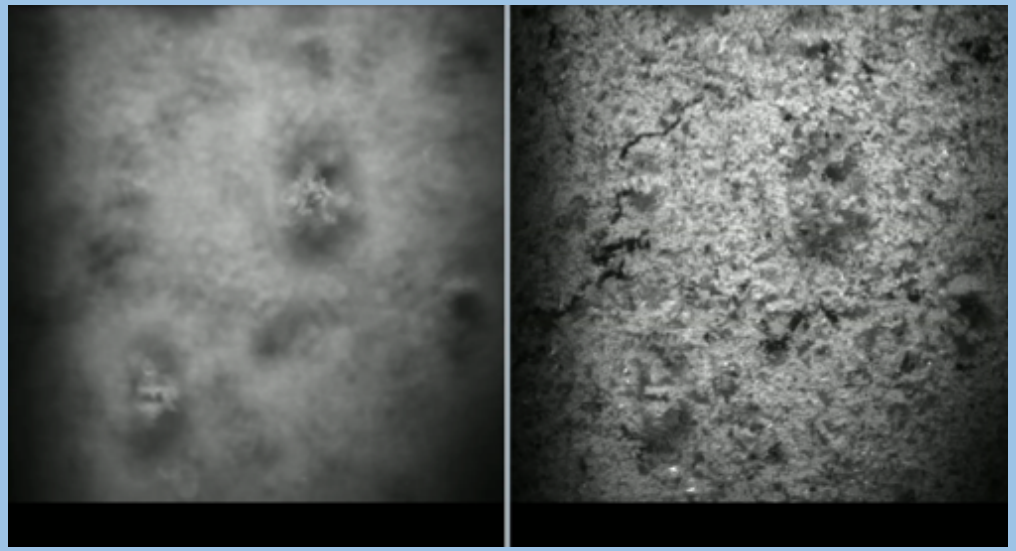
ELM Microscope Subsystem



Field-of-View	761 x 761 µm
Spatial Resolution	< 0.8 µm
Piezo Z-motion	up to 400 µm
Piezo Positioning	1 nm accuracy
LEDs 1 & 5	275 nm
LEDs 2 & 6	375 nm
LEDs 3 & 7	470 nm
LEDs 4 & 8	525 nm
Emission Filters	334, 470, 529, 579

Components have passed radiation testing to 300 krad, including: bandpass filters (single- and multi-band); LED modules; lenses and lens materials.

Automated Image Z-Stacking

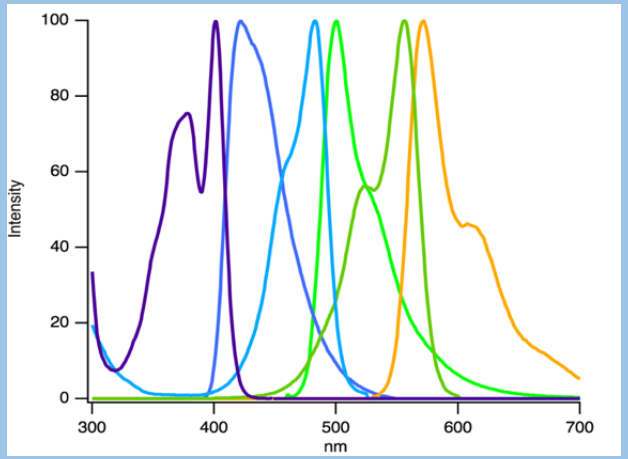


Single Image Slice

In focus Z-stacked Image

Fluorescent Probes

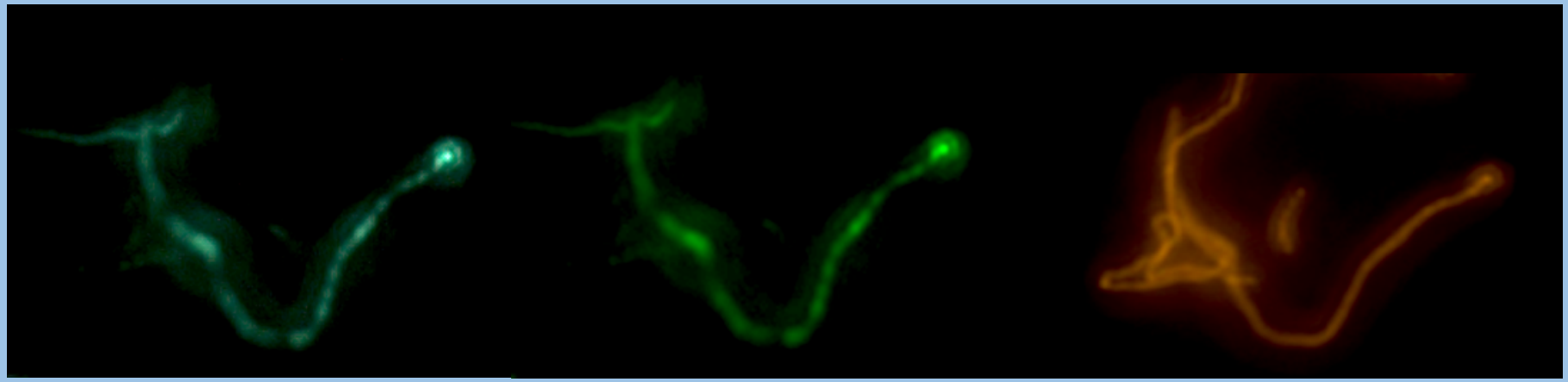
Alexa405 SYTO9 Cell Mask



- 3-Stain Combination – Targets – Proteins, Lipids, Nucleic Acids
- No loss of fluorescent intensity for 3-dye mixture vs. individual dyes
- All targets stained simultaneously after capture on SiN filters
- Detection of stained structures below spatial resolution limit

Fluorescent Probes

P. boydii ~ 1 Micron Wide

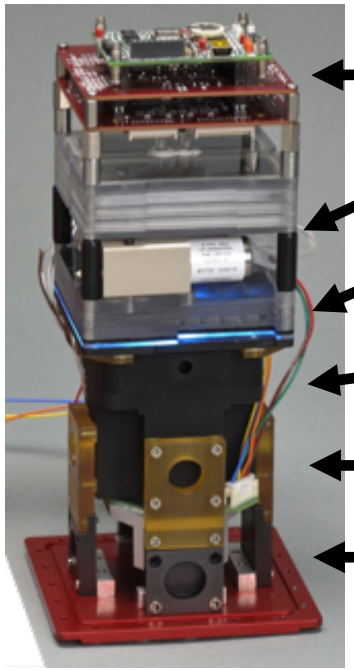


Alexa Fluor 405 - Membrane Proteins

SYTO9 – Nucleic Acids

Cell Mask – Membrane Lipids

Technology Readiness Migration Low Earth Orbit to Europa



2U → 3U

Electronics

Fluidics Manifold

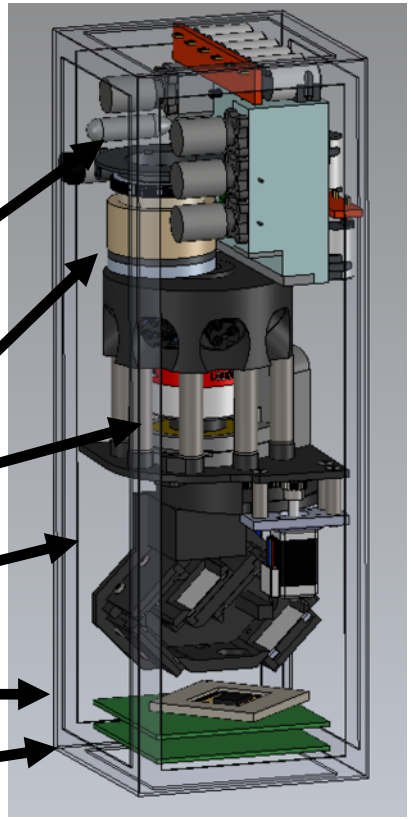
Fluidic Sample Stage

LED Excitation

Optics Assembly

Camera Board

Electronics

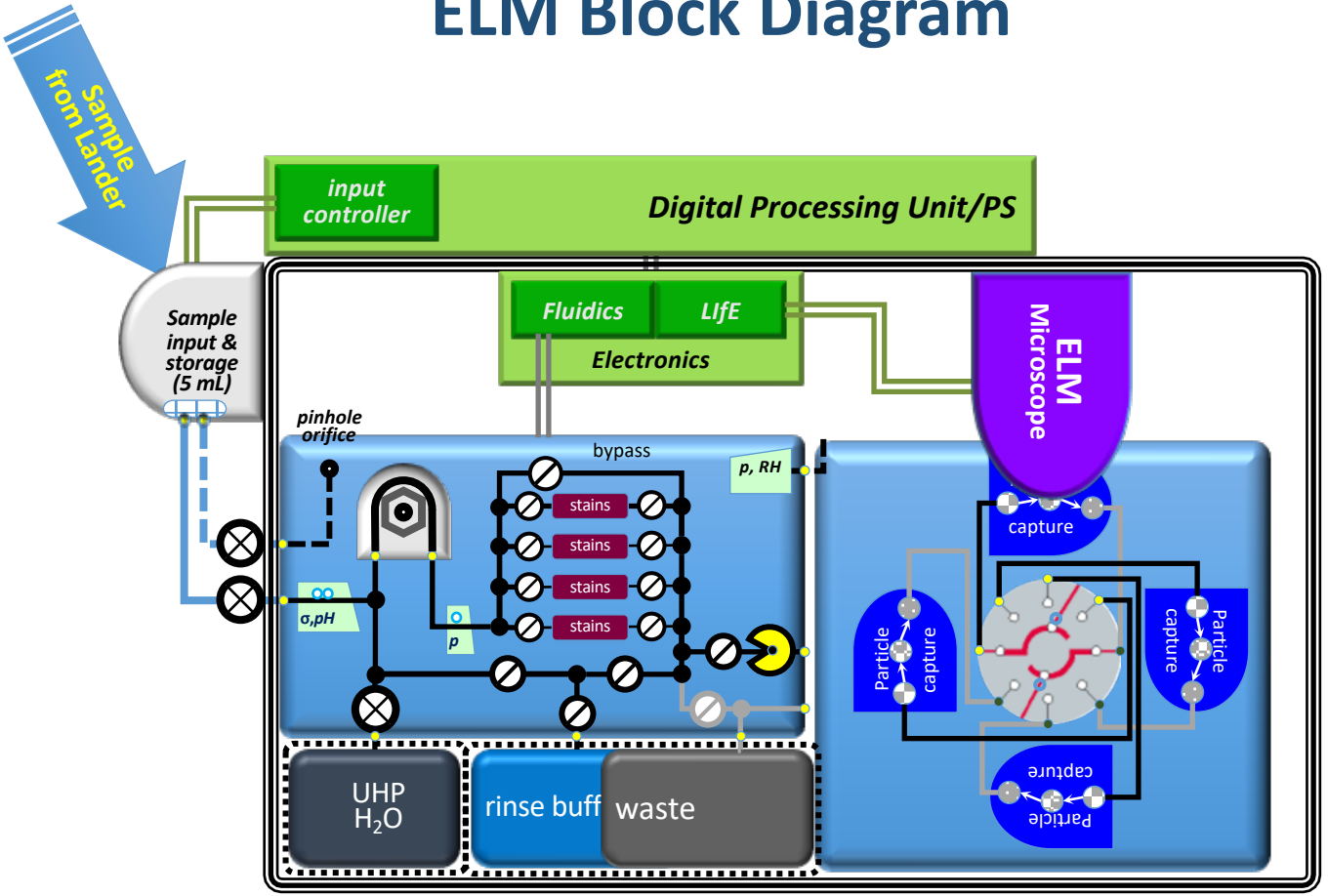


30 cm

FLAIR

ELM

ELM Block Diagram



3U Hermetic Container

Current Developments

- Optimize Sample Silicon Nitride Filters for Imager FOV
- Build Shock and Vibe Tolerant Objective
- Build Rad-Hard Image Sensor Board
- Sample Transfer Dock Brass Board

Acknowledgements

NASA Concepts for Ocean worlds Life Detection Technology Program

NASA Instrument Concepts for Europa Exploration-2 Program