Microscopy Methods for Life Detection on Ocean Worlds

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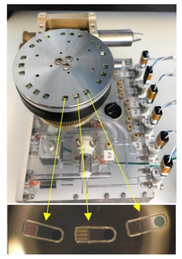
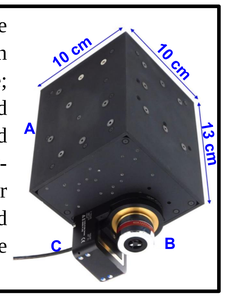
The Luminescence Imager for Exploration (LIfE)

Light microscopy is commonly used in microbiology to visualize the morphology of cells and correlate this with chemical information about their composition; this makes it a key technology for life detection missions.[1] The Luminescence Imager for Exploration (LIfE) is a brightfield and epifluorescence microscope with an integrated fluidic sample processing system designed for the *in-situ* search for microbial of life on ocean worlds.

Fluidics subsystem. Rotary sample filtering stage (5 cm dia.) and fluidics manifold (9 x 8 x 4 cm). Functionality includes pressure, pH, conductivity measurements; sample size sorting using in-line 10, 1, and 0.25 µm silicon nitride filters; fluorescent stain dry storage; metering pump and valves for sample manipulation and processing. The lower image shows a detail of the three-stage silicon nitride particle filters.



Optics subsystem. A) microscope box with excitation LEDs, emission filters, folded optics; B) objective; C) piezo stage. The optics and fluidics subsystems have passed environmental testing (GSFC-STD-7000A). All all lens, filter, mirror and coating materials have passed 300 krad total ionizing dose gamma-radiation testing.



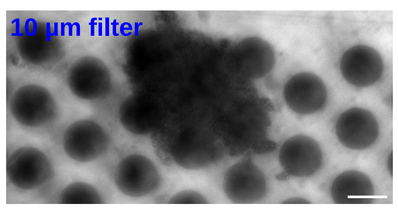
Brightfield and Fluorescence Imaging

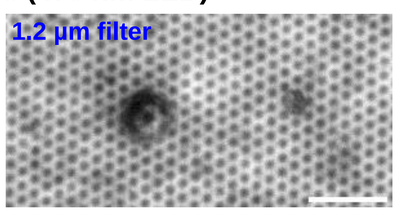
LIfE is a high-resolution optical microscope that can spatially resolve structures smaller than 0.5 µm in size using bright-field imaging. LEDs with wavelengths centered near 275, 375, 470, and 525 nm are used to excite autofluorescence. The use of multiple excitation wavelengths not only allows for the detection of different molecular species, but also their rough classification. For example, excitation at 275 nm enables the detection of smaller polycyclic aromatic hydrocarbons (PAHs; 1-5 rings), and proteins containing aromatic amino acids. 370 and 470 nm light excites increasingly-larger PAH structures (e.g., coronene) and larger aromatic biomolecules that may be present (e.g., protective pigments).

Natural Samples from Lake Untersee

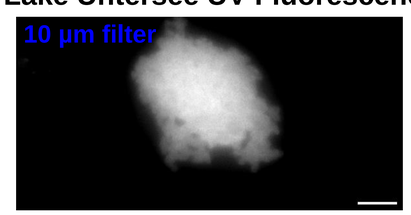
Microscopy methods in life detection missions will require the analysis of complex natural samples. Lake Untersee is a glacial lake in Antarctica perennially sealed from the outside environment by a layer of ice. Shown below are images of a sample collected from the bottom of lake Untersee's south basin is anoxic and high in methanogenic organisms with the main sources of energy being chemical in origin[2].

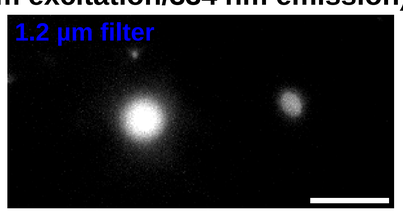
Lake Untersee Brightfield Images (470 nm LED)





Lake Untersee UV Fluorescence Images (275 nm excitation/334 nm emission)

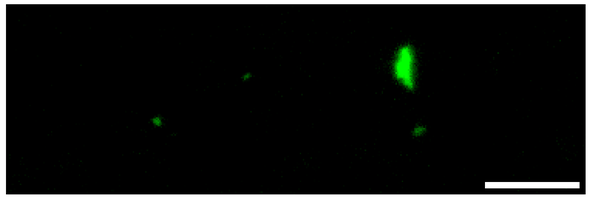


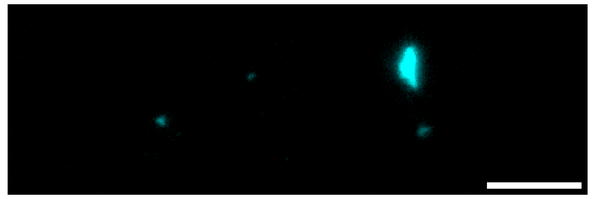


Samples collected at 80m of depth from lake Untersee's south basin imaged in brightfield and autofluorescence on LIfE. Scale bars are 10 µm

Colocalization of Structural Biosignatures

LIfE is a powerful tool for the characterization of sample morphology, texture, and autofluorescence. Using selective fluorescent stains, additional information on potential spatial co-location of structural biomarkers (lipids, proteins) can be achieved.





A Lake Untersee sample simultaneously co-stained with dyes selective for proteins and lipids. *Left:* Proteins stained using Alexa Fluor 488 and imaged with 470-nm excitation and 595/31-nm emission bandpass. *Right:* Lipids stained using Primuline and imaged with 375-nm excitation and 432/36-nm emission bandpass. Scale bars are 10 µm.

Image Data Compression

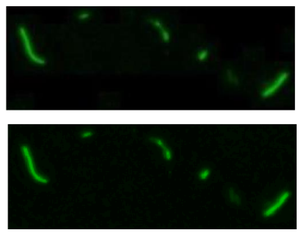


Image compression is achieved through on-instrument "z-stacking;" a custom script stacks up to 60 images together to create a single, in-focus image. Images are captured in 16-bit grayscale for high dynamic range and then converted to 8-bit grayscale. Additional compression of fluorescence images is achieved by autonomously identifying regions of interest. These regions are then cropped out of the original z-stacked image and saved with the metadata, from which an image can be reconstructed after downlink against a black background. Final data reduction comes from JPEG compression of the resulting images.

Top: Uncompressed image of *B. megaterium* stained with AlexaFluor 488. *Bottom:* the image on the left cropped, reconstructed, and JPEG compressed

Microscope Technical Specifications

Resolution	<0.5 um
LED 1 excitation	275 nm
LED 2 excitation	375 nm
LED 3 excitation	470 nm
LED 4 excitation	525 nm
Emission filter 1	334/40 nm
Emission filter 2	432/36 nm
Emission filter 3	515/30 nm
Emission filter 4	595/31 nm

References

1. Hand, et al. (2017) Report of the Europa Lander Science Definition Team
2. McKay, et al. (2017) *Polar J.* **7**, 303–318.

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