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ELVIS: A Correlated Light-Field and Digital Holographic Microscope for Field and Laboratory Investigations

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We are developing a unique multi-modal volumetric microscope capable of instantaneous imaging of sample volumes approximately 100-fold larger than those sampled by conventional fluorescence microscopy, with spatial resolution sufficient to detect the smallest prokaryotes (**Figure 1**). This instrument combines two modalities for the first time: Digital Holography Microscopy (DHM) and Fluorescence Light Field Microscopy (FLFM). The instrument will be packaged for field use in aquatic environments. It will be used in the lab, and also made available to researchers inside and outside the home institutions, enabling a wide array of environmental microbiology applications.

Instantaneous volumetric imaging has a wide variety of emerging and potential applications in cell, developmental, and organismal biology, as well as in the physical sciences (hydrology, materials science). However, conventional microscopy requiring sectioning (confocal or multiphoton) is too slow to capture a large number of kinetic events: bacterial swimming, heartbeats, blood flow, intracellular organelle movement, and many more [1, 2]. The limitations of conventional imaging modalities could be mitigated by recording the spatial information of an extended sample volume in a single camera snapshot. Such synchronous volumetric imaging capability not only removes the delicate focusing requirement of conventional microscopy, but also dramatically enhances the imaging throughput.

The instrument is tailored to two areas of research that have immediate needs for the combination of modalities that we propose: (1) Instantaneous multi-modal volumetric imaging; (2) *In situ* environmental microbiology of remote or extreme environments.

Some of the most remarkable findings in environmental microbiology relate to the extreme conditions under which living microorganisms may be found [3]. Recent technological developments, such as the Flow Cytobot, have revolutionized the study of microorganisms at the 10 µm scale or above, but micron-scale organisms (bacteria and archaea) remain a neglected size scale for which limited *in situ* imaging has been performed [4].

Specific target areas for ELVIS include the open ocean, sea ice, glacier ice, and hydrothermal vents. Although it may appear solid, sea ice is porous with an interior network of microscopic veins and channels filled with brine that contains living microorganisms, including bacteria and eukaryotes [5]. Viable prokaryotes and eukaryotes have also been found in permafrost [6] and in glacier ice, including ice many thousands of years old in both the Northern and Southern hemispheres. Hydrothermal vents are populated with organisms across the size scale, but technological difficulties have only recently permitted any form of *in situ* imaging of the deepest areas of the ocean [7]. It is known that some prokaryotes adapted to high pressures (barophiles) may die or lose activity when taken to the surface; current approaches mainly focus on creating pressure cells for imaging samples that have been returned to the surface [8, 9].

Bacteria and archaea from these target environments have been studied by DNA analysis, epifluorescence examination of fixed specimens, and culturing—but almost never by direct observation. However, many of these organisms are known to be viable. Once we have the technology, we can achieve important science goals: analysis of structure, motility, predation/grazing and other activity of organisms in their native environments; quantitative live/dead analysis; staining for specific cell wall or lipid compositions; analysis of enzymatic activity; and more.

The instrument is located within the Center for Life in Extreme Environments shared user facility at PSU. After training, certified users will receive the keys and a user account on the instrument computer. Permanent staff will be available to train users. Users who are external to PSU and who wish to use the laboratory instrument will become adjunct members of CLEE and receive access in the same fashion as internal users. The instrument will be available for laboratory use as soon as the DHM and fluorescence modules are integrated—no later than the end of 2019. The field instrument will become available in 2020. Lending of the instrument for field users will be handled on a first-come, first-served basis. All designs will be published in the open literature, and we will be eager to assist anyone in building a similar instrument. This research is funded under an NSF Major Research Instrumentation Development grant [10].

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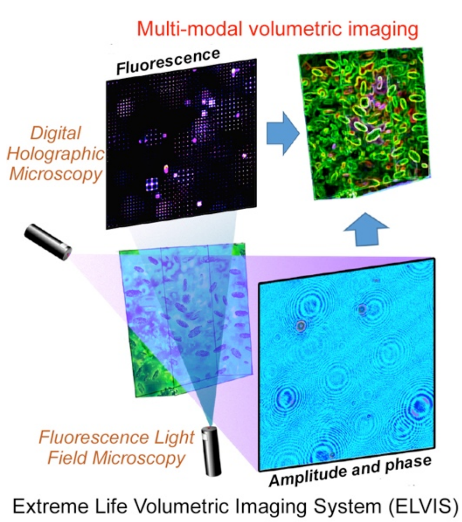

Figure 1. The ELVIS approach combines complementary optical technologies for robust volumetric imaging. This is ideal for applications in the laboratory and in the field at sites such as the open ocean, at hydrothermal vents, and in lakes and ponds.

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