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A Novel In Situ Microscope for Studying Benthic Organisms

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A Novel *In Situ* Microscope for Studying Benthic Organisms

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1. Narrative

Introduction

Coastal marine ecosystems such as coral reefs, kelp forests, sea grass beds and mangroves have significant societal value. As economic resources they support tourism, fisheries and storm protection while ecologically they host high biological diversity and productivity. Many basic biological, chemical, and physical processes taking place in these marine ecosystems occur at 'micro-scales' less than a millimeter in size. Examples of significant micro-scale activities include: coral bleaching of their single-celled symbiotic algae, larval settlement and attachment, competition between sessile organisms along thin interfaces, and fluxes of particles to and from the seafloor.

It is also important to consider that these shallow ocean environments are complex and dynamic. The physical environment is in continuous flux with variations in conditions such as pH, temperature, and fluid motion. Simultaneously, organisms interact with each other in non-linear ways across a wide range of scales. It is therefore difficult or impossible to fully replicate these natural systems in the lab. A distinct need thus exists to make in situ observations under natural conditions. Such observations are of interest to scientist across diverse disciplines such as physiology, ecology, biomechanics, and marine geology. Previous calls for technology to perform such micro-scale imaging in the ocean include that of V. Smetacek who asked "Could such an instrument (an in situ computerized telemicroscope) do for microbial ecology what Galileo's telescope did for astronomy?"(Nature, 2002).

My work seeks to address existing observational deficiencies by developing and applying underwater microscopic imaging techniques for studying benthic ocean environments at scales of less than a millimeter. Specifically, I have designed and built the Benthic Underwater Microscope (BUM), this is the first system to perform non-invasive imaging of seafloor environments *in situ* at nearly micrometer resolution. Funding from the Link Fellowship provided the means to complete the development and validation of the BUM. Additionally, I prepared a manuscript describing the BUM that is currently in submission for scientific publication. During my funding from the Link Foundation I also began work to further enhance the BUM's capabilities by incorporating micro-Particle Image Velocimetry (µPIV). This is flow visualization technique for measuring micro-scale fluid velocity fields, which are important to mass exchange and a variety of biological and geochemical processes.

Results

Microscopic imaging in the underwater environment presents several challenges including precise focusing, imaging live three-dimensional organisms, not interfering with natural processes, application in unstable conditions, and operation by a diver. The BUM meets these challenges through the application of three principle optical components: a long working distance microscopic objective lens, a shape changing Electrically Tunable Lens (ETL), and focused LEDs used for reflectance illumination. These elements are integrated into a compact, underwater imaging system that also includes a camera, electronics, and user controls. The system acquires high magnification images with very short exposures. Additionally it provides at least 60 mm between the subject and instrument so that natural processes are minimally disturbed, and is capable of electronically controlled focusing.

The BUM has been successfully deployed in the ocean by a scientific dive team. During fieldwork on coral reefs, we were able to distinguish individual single celled zooxanthellae, (6-13 µm diameter) inside live coral polyps. The system was also used to collect time-series recordings (for up to 8 hours on the reef) in order to observe ongoing organism behavior and ecological interactions such as inter-species coral competition. Testing in the lab showed the instrument has a resolution of up to 2.2 um with a field of view of 1.62 x 1.36 mm.

The second major development component of my work is further enhancing the BUM to perform Micro-Particle Image Velocimetry (µPIV). PIV is a quantitative flow visualization technique that uses images of tracer particles to study fluid dynamics. The displacement of tracer particles between successive image frames is used to calculate a two-dimensional field of fluid velocity vectors. µPIV is a specialized application of this technique for studying flows at scales of less than a millimeter. The µPIV system is currently in lab testing, upon completion it will be the first *in situ* instrument capable of measuring fields of two-dimensional fluid velocities at the micro-scale.

In order to perform µPIV, we couple the BUM with a dark field transmission illuminator, and inject ~10 µm tracer particles into the flow. We then use precise timing to acquire a rapid pair of images, which record the tracer particle's locations. Finally we can compare these images, using cross-correlation techniques, to determine fluid motions. To date we have designed the dark field illuminator and successfully recorded images of tracer particles in the lab. Testing has demonstrated the system's ability to resolve tracer particles, and meet PIV timing constraints. Currently I am writing code to improve the camera and lighting synchronization and accuracy. I am also research image analysis technique. Once we have a lab system working we will then bring the instrument into the field. We expect to be able to measure a field of two-dimensional fluid motion at a spatial resolution of 10's of micrometers.

Significance and impact

The new scientific viewpoint provided by the BUM is significant as small-scale activities in ocean systems often drive large-scale changes in ecosystem health and function. Existing laboratory studies of such processes obscure many environmental complexities and interactions. *In situ* observations thus have significant potential to reveal new phenomena and connect existing lab work to the real world. The BUM provides the first direct observations of natural benthic environments at close to micrometer resolution. This perspective is relevant to a wide range of basic and important scientific topics, including issues related to climate change and habitat loss such as coral bleaching and coral-algal competition.

The underwater µPIV system described here will provide the first means to measure two-dimensional micro-scale fluid dynamics in the natural ocean environment. This is significant because current µPIV studies of ocean organisms are conducted in micro-cuvettes chambers in the lab. These highly modified environments alter fluid movements and interfere with natural behavior. Fluid motions at scales of a millimeter or less are relevant to many significant processes in coastal oceans, including boundary layer dynamics, mass transport, and organism swimming mechanics.

Where might this lead?

Once the *in situ* µPIV system is operational we plan to use it to study micro-fluid dynamics surrounding coral polyps in the natural environment. In order to sustain basic metabolic activities, corals must continuously exchange-dissolved solutes such as oxygen, carbon dioxide, and calcium carbonate with the surrounding water. The flow environment significantly impacts these exchanges and may effect a variety of health factors. Our work will examine mechanisms of fluid mixing and the ability of corals to modulate their flow environments. This is relevant for understanding the coral's ability to cope with increasing environmental stresses such as ocean warming and acidification.

In addition to this specific planed work we are hopeful that the BUM will spur a new frontier of *in situ* measurements As a versatile tool we believe this instrument can enable important studies on a variety of basic marine science questions. Additionally, this instrument offers a platform for implementation of a variety of enhanced imaging methods that may be used to further investigate micro-scale physiological and physical phenomena. In addition to µPIV such techniques include: variable chlorophyll fluorescence to study photosynthetic efficiency, chemical sensing using optical indicators, three-dimensional modeling of subjects using the scanning lens focal stacks.

2. Publications, Presentations, and Other Outputs.

Presentations

A.D. Mullen, T. Treibitz, J.S. Jaffe P.L.D. Roberts and B. Laxton, "Benthic Underwater Microscope: A Novel Tool for In Situ Micro-Scale Imaging," *Ocean Optics XXII*, Oct. 2014, Portland, Maine.

A.D. Mullen, T. Treibitz, J.S. Jaffe P.L.D. Roberts and B. Laxton, "An Underwater Microscope for In Situ Imaging of Coral Reefs," *Scripps Student Symposium*, Aug. 2014, La Jolla, California.

Publications

A.D. Mullen, T. Treibitz, P.L.D. Roberts, and J.S. Jaffe, "Underwater Microscopy for *In Situ* Studies of Coastal Ecosystems," - in submission

"A Diver Deployed Micro-PIV System" – expected future publication

3. Future Plans and How did the fellowship make a difference?

The Link Fellowship provided me the freedom and resources to move forward in the best direction for both this project and my career. With the fellowship funding, I had the time required to learn new skill set in order to implement µPIV. I was also able to take a course in the Spring titled "Sea Technology in Biological Oceanography" during which the class spoke to the a variety of leaders in the ocean technology world, from institutions such as MBARI, WHOI, SIO, and UW. With the fellowship's support I had the time and resources to build important skills I will need for a future career building instruments to study and explore the ocean.