A submersible holographic camera for the undisturbed characterization of optically relevant particles in water (HOLOCAM).

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LONG-TERM GOALS

Our long-term goal is to develop novel oceanographic instrumentation to address fundamental questions in ocean optics. The primary goal of this project is to develop a holographic instrument capable of imaging and characterizing natural (i.e. undisturbed) particle fields in the ocean. The long-term science goal is to understand the link between suspended particles and the bulk scattering properties of natural waters. We believe in-situ digital holographic microscopy, recently developed and employed for both fluid dynamics and biological studies, has the capability to obtain critical data relevant to this goal.

OBJECTIVES

Our overall objective is to develop an in-situ profiling digital holographic microscopy system (HOLOCAM) capable of characterizing the properties of optically relevant particles within a size range of < 1 to 1000 µm. Our team will design, fabricate and characterize the HOLOCAM with the goal of commercialization. The HOLOCAM will be compact, submersible, capable of vertical profiling of undisturbed volumes of water, and with real-time visualization. It will quantify particle number, size and shape (e.g. cross-sectional area, surface area, aspect ratio, sphericity) and the 3-D spatial structure of the particle field (e.g. nearest neighbor distances). Identification of particles with unique shape characteristics (e.g. bubbles) and orientation should be achievable. The proposed HOLOCAM will extend the size range of particles currently resolvable by an existing system from a minimum of ~10 µm down into the sub-micron range (thus capturing the full range of optically relevant particles).

APPROACH

We have assembled a team of established experts from the oceanographic community to help achieve our objectives, including investigators from a business and two academic entities: J. Sullivan and M. Twardowski from WET Labs Inc., J. Katz from the Johns Hopkins University (JHU) and P. Donaghay from the University of Rhode Island (URI). In addition to HOLOCAM design and fabrication, this research team will evaluate the sensor's performance in the laboratory, assess absolute resolution and uncertainties, deploy it in the field, and develop custom software to analyze particle characteristics.

WORK COMPLETED

Our approved contract was not in place until late in the fiscal year (April 2010), thus, as of the writing of this report, we are only between 5 and 6 months into the project. However, the project is entering an intense period of activity. During the first 5 months of the contract, we have completed the following:

1. Project design meetings.
Project investigators and lead engineers have met several times to discuss the HOLOCAM system design. Based on options laid out in our original proposal, a number of critical design decisions and development paths required discussion. Among these were system layout, system magnifications, CCD camera choices, laser color and pulse specifications and possible lower cost laser alternatives, real-time data acquisition and on-board data storage, and the critical particle characterization suite to integrate into custom analytical software. Based on these meetings, the project team has finalized the prototype system design. The components needed to begin construction of the first submersible HOLOCAM system should be acquired in the next month. Meetings have also taken place to address Intellectual Property (IP) agreements between JHU and WET Labs

2. Fabrication of a prototype bench-top HOLOCAM.
Concurrent with discussions of the final system design, team members from WET Labs, in consultation with partners from JHU, have completed assembly of a bench-top HOLOCAM test bed (Figure 1 and 2) to evaluate parameters stipulated in the concept design.

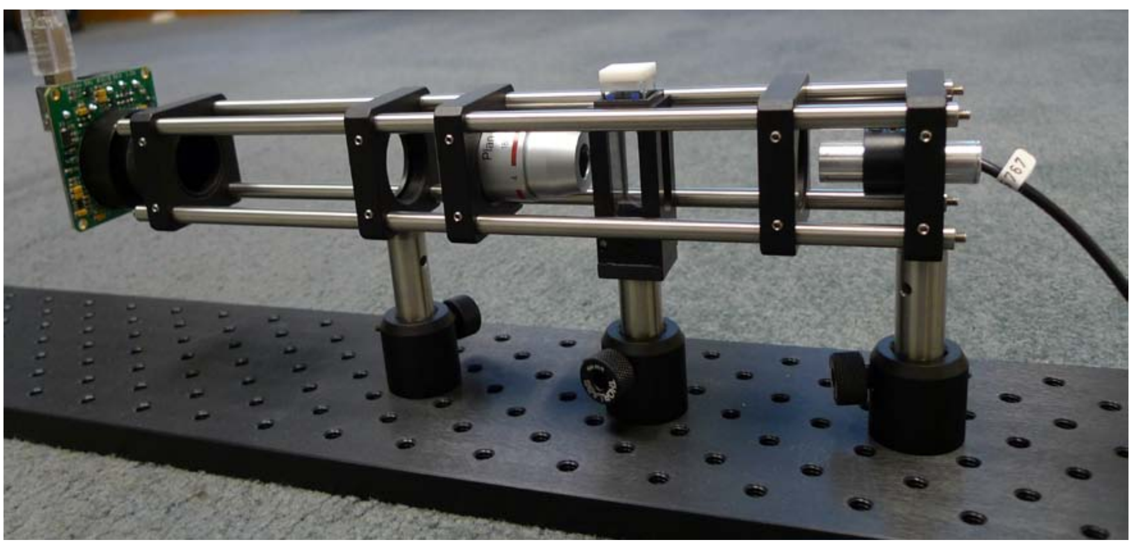

Figure 1. Bench-top HOLOCAM (~ 1 ft. in length) constructed at WET Labs. On the left is the CCD high resolution board camera, in the middle is the microscope objective and sample cuvette, and on the right is a small CW laser and neutral density filter.

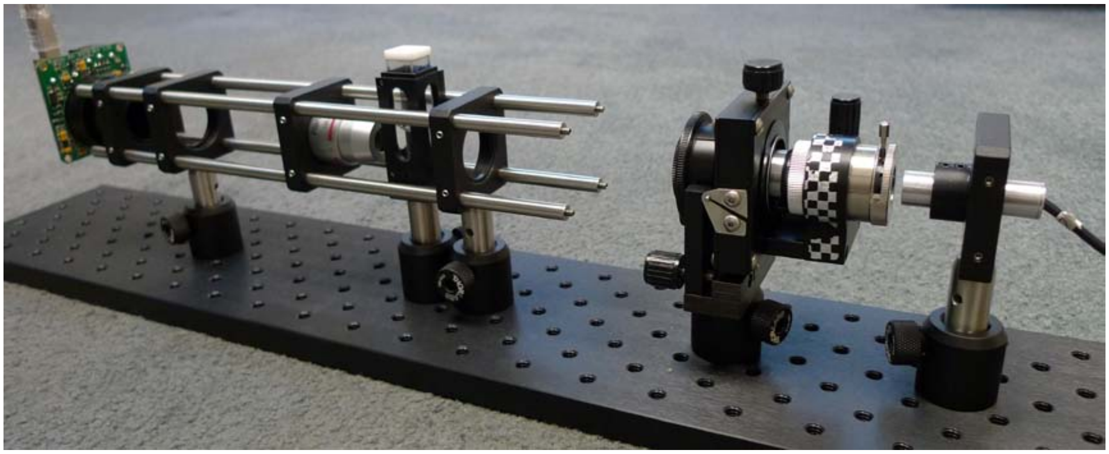

Figure 2. Bench-top HOLOCAM with spatial filter added to improve hologram image quality.

3. Software development/refinement for reconstructing digital holograms and particle analysis.
The project team has begun to define desired particle characterization suites, e.g. size, number, shape metrics, spatial distribution, etc. JHU and WET Labs programmers will integrate these metrics into software for hologram numerical reconstruction and analysis of particle characteristics.

4. Analytical software training and transition.
The expertise in both the development and use of holography post-processing tools is with our academic partner at JHU. In order for WET Labs to transition this expertise, on-site training of WET Labs personnel has occurred at JHU and is ongoing. Experience with the use of this software is a fundamental requirement to facilitate field and laboratory testing of HOLOCAM prototypes by WET Labs.

5. Diagnostic lab testing with bench-top HOLOCAM test-bed.
Team members from WET Labs and JHU have started (and will continue) to test critical components and layouts to specify final design elements for the submersible HOLOCAM. These tests are designed to 1) optimize the balance between camera resolution and magnification in determining the size ranges sampled and sample volumes, 2) optimize the effective sampling rate and data volume flux, and 3) determine the level of flexibility that can be accommodated in the submersible version.

6. Lab validation of particle characteristics (e.g. PSDs) using bench-top HOLOCAM test bed.
The entire project team has begun testing the performance of the bench-top HOLOCAM with defined particle fields using different concentrations and sizes of NIST traceable micro-spherical beads and poly-dispersed particle standards (e.g. AZRD), phytoplankton cultures and natural ocean water (see Figure 3 for example). Validation using other independent characterizations of particles will be accomplished using SEM, Coulter counters, flow cytometry and light microscopy techniques.

7. Field measurements.
An initial field test/deployment took place during May of 2010 in East Sound, WA. This test was coordinated with existing ONR funded field experiments designed to validate airborne LIDAR (PIs: Donaghay, Churnside, Sullivan & Rines) and in-situ flow cytometry to (PIs: Donaghay, Rines & Sullivan). As part of these pre-existing programs, vertical profiling and discrete sampling was

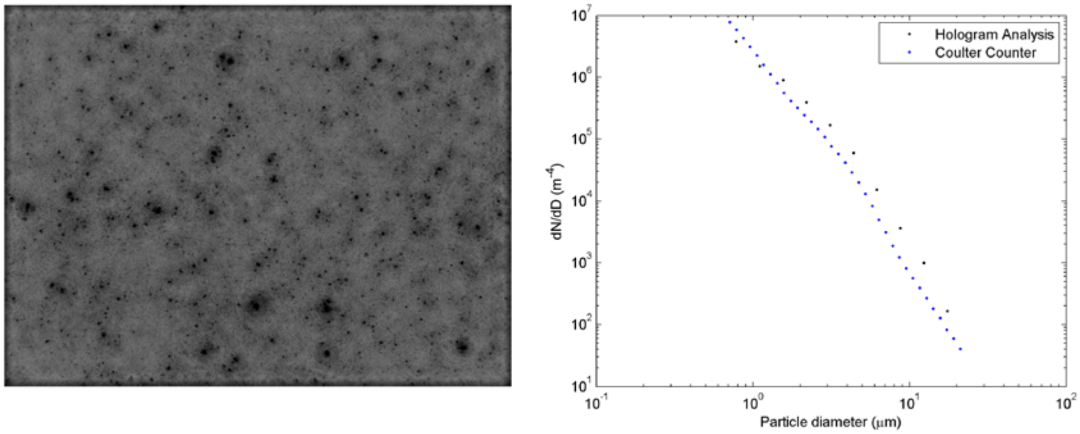

Figure 3. Bench-top HOLOCAM 2-D image of an AZRD polydispersed particle standard (left panel) and resulting PSDs compared between a Coulter counter and the holographic analysis (right panel).

conducted to characterize particle populations and bulk optics. Academic partner Katz's existing in-situ holographic instrument (HoloSub), which was modified to be similar to the proposed HOLOCAM (but at lower system magnifications), was deployed concurrently. Discrete water samples were also analyzed using the bench-top HOLOCAM.

RESULTS

1. After several design meetings, the research group determined the best development path to follow for the initial prototype submersible HOLOCAM will be similar to that outlined in the original proposal. This design allows for future velocity measurements using the low magnification system, and the highest possible particle resolution for the high magnification system. Diligent research into other potential laser sources concluded that the originally specified red wavelength pulsed laser was best for the technical objectives of the project and provided the highest flexibility in future deployment options. On-board flash drive data acquisition was deemed an important development path to continue investigating given it would allow the HOLOCAM to be used in mooring situations and could eliminate the use of fiber optic cables. However, a full DVR system for real-time recording of holograms (at least in the prototype testing system) will be developed.

2. We concluded that hardware based DSP hologram reconstruction was a critical path to focus resources. Since a quad-4 computer requires about 1 second to reconstruct a single image plane, and a typical hologram is sliced into 3000 planes, complete reconstruction of a single hologram takes ~ 1 hr. Since we will acquire 15 holograms per second during a vertical profile that may last 15 to 30 minutes, the storage space, computing power, and most importantly, time, needed to reconstruct a single vertical profile is overwhelming. This is before any detailed particle analysis has begun. To address this problem, we are working with outside sources to develop a new generation of DSP boards to run hologram reconstruction code in hardware. If we can successfully integrate this technology, we may attain near-real time reconstruction times. This technology is critical to making the HOLOCAM a viable, near-real time research tool.

3. The construction of a bench-top HOLOCAM test bed was completed. Because this device uses an enclosed cuvette with low particle motion, an inexpensive Continuous Wave (CW) laser was found to be adequate as a source. However, it was determined after both lab and field-testing that a spatial filter was required to clean up the source beam from the CW laser (Figure 2).

4. Transitioning of the expertise required for reconstruction and analysis of holograms to WET Labs has been ongoing. WET Labs personnel have received training in the steps and programs required. Briefly, the first processing step requires subtracting background noise out of holograms and then re-scaling the 8-bit images. The background subtracted holograms are reconstructed using an executable C-based GUI. Reconstructed holograms are then processed to smooth out the particles from the background and diffraction patterns using image processing filters. The processed reconstructed image planes from the 3-D volume are then collapsed into a single 2-D image of all in-focus particles using Matlab. Image processing of the 2-D images can then be performed to yield desired particle characterizations. The steps above are a simplification of many complex operations. We have concluded that a number of these steps could lead to the introduction of artifacts or uncertainties in particle characterizations if not carefully implemented. We will continually assess how to quantify and minimize these issues during analytical software development.

5. The HoloSub was modified from the single magnification, crossing-path holography system, to a system similar to the proposed HOLOCAM, but at an overall lower system magnification (the HoloSub also samples undisturbed water). This in-situ system was deployed, along with concurrent sampling using a bench-top HOLOCAM, as part of initial project testing in May of 2010 in East Sound, WA. Tens of thousands of holograms were collected. Initial analysis of just a single vertical profile revealed a dramatic shift in particle types and orientation within the undisturbed water column (Figure 4). Within this vertical profile, there was an intense optical scattering and particle peak at ~ 2.25 m (Figure 4, top right). The most common particles in this peak were colonies of the diatom *Chaetoceros socialis* (large round particles ~ 1 mm in size). Within this particle peak, there was also true orientation of linear diatom chains, where the calculated mean inclination angle was significantly different from the rest of the water column. A distinct shift from long linear diatom chains and *Chaetoceros socialis* colonies in the surface waters, to more detrital particles, broken up diatom chains and smaller dense (dark) background particles in deeper waters was evident. Also evident was the large size of undisturbed particles in the upper water column, where most phytoplankton colonies were several millimeters (or more) in size. Detailed data analysis from this field exercize is continuing and will involve comparing concurrent IOP measurments and independent particle characterizations (e.g. SEM and flow cytometry PSD estimates) to those extracted from both the bench-top and in-situ holography.

IMPACT AND APPLICATIONS

Quality of Life

The HOLOCAM could be used in a number of applications relating to ecosystem health and coastal resource management. For example, it could be used to monitor and detect oil droplets in the water column. It could also be used for sediment load monitoring and assesment. The HOLOCAM could provide critical data to any science/management question that requires an understanding of particle size, shape, fine-scale distribution, and/or short time-scale dynamics.

Science Education and Communication

Currently the link between the suspended particle field and the bulk scattering properties of natural waters is poorly known because: 1) adequate technology is lacking to fully characterize all the parameters of the particle field needed to compute the bulk optical properties (especially for ephemeral bubbles and aggregates); and 2) models are not currently available that can effectively compute bulk optical properties while taking into account the full complexity in the shape and structure of each relevant particle in the field. Accurate and detailed characterization of suspended particle fields is essential if one hopes to ever carry out the forward modeling problem without having to make expansive assumptions. When considering the incredible complexity and uniqueness of the particles found in natural waters, this is an exceedingly challenging prospect. While impractical to expect we may someday completely do away with all assumptions, the optics community scarcely knows which assumptions may be reasonably representative and which may not. For example, are particle fields in discretely collected samples, subjected to associated shear stresses and storage, representative of natural particle fields? Conceptually and practically are there "equivalent" homogeneous spherical particle populations with the same bulk optical properties as naturally occurring populations (i.e., what is the effective applicability of Mie theory or assuming idealized non-spherical geometrical shapes such as ellipsoids or cylinders)? Are particles captured on a microscope slide or in a particle counter the whole story or are ephemeral particle populations such as micro-bubbles or transparent exo-polymer (TEP) gels important? Can we reasonably assume all particles are randomly oriented in-situ? We do not have sufficient answers to any of these critical questions because the technology has not existed to sample optically relevant particles in a manner that will produce the necessary data. Holographic microscopy has the capability to obtain much of these critical data and answer these questions. The HOLOCAM will facilitate an improved understanding of the variability of inherent optical properties (IOPs, e.g. volume scattering), apparent optical properties (e.g. remote sensing reflectance, diffuse attenuation) and the performance of operational systems (e.g. LIDAR, laser line scanners).

TRANSITIONS

Economic Development

The HOLOCAM is being developed as a new commercial product for WET Labs, Inc. The design of the HOLOCAM requires WET Labs to integrate new engineering and manufacturing capabilities in the use of lasers, high-speed fiber optic data transfers, DSP programming and image analysis techniques.

RELATED PROJECTS

WET Labs and our partners on this project have several on-going ONR sponsored research projects that will both benefit, and be benefited by, the HOLOCAM project. Upon successful development and laboratory validation/characterization of the HOLOCAM, the sensor will be deployed in the field as part of an integrated HOLOCAM - optics profiler. This vertical profiling package will be equipped with state-of-the-art optical instrumentation including the HOLOCAM, CTD, WET labs AC-S, WET labs ECO-BB3, LISST-100 and the WET Labs MASCOT VSF device. The MASCOT is a prototype instrument that measures the in-situ VSF of monochromatic light (658 nm) from 10° to 170° at 10° intervals. Field deployments of the HOLOCAM – optics profiler will be used to further evaluate and validate the HOLOCAM while also providing data (in addition to that from laboratory experiments) to investigate science objectives relevant to these related projects. For example, Co-I Twardowski is PI for an existing ONR SBIR contract to develop and evaluate new drifters for the optical characterization of the surf zone. As part of this program, surf zone measurements and discrete sampling to characterize the particle populations using bulk optics (and perhaps acoustics) will occur. Including HOLOCAM deployments for particle and bubble characterizations would be highly synergistic with the surf zone work, while also providing further field testing and validation of the HOLOCAM. Holography is advantageous over traditional acoustic bubble detection methods, which have been successfully used for larger bubble sizes, but are difficult for bubble sizes < 20 µm in diameter. Detection of bubbles through holography is straightforward using established algorithms.

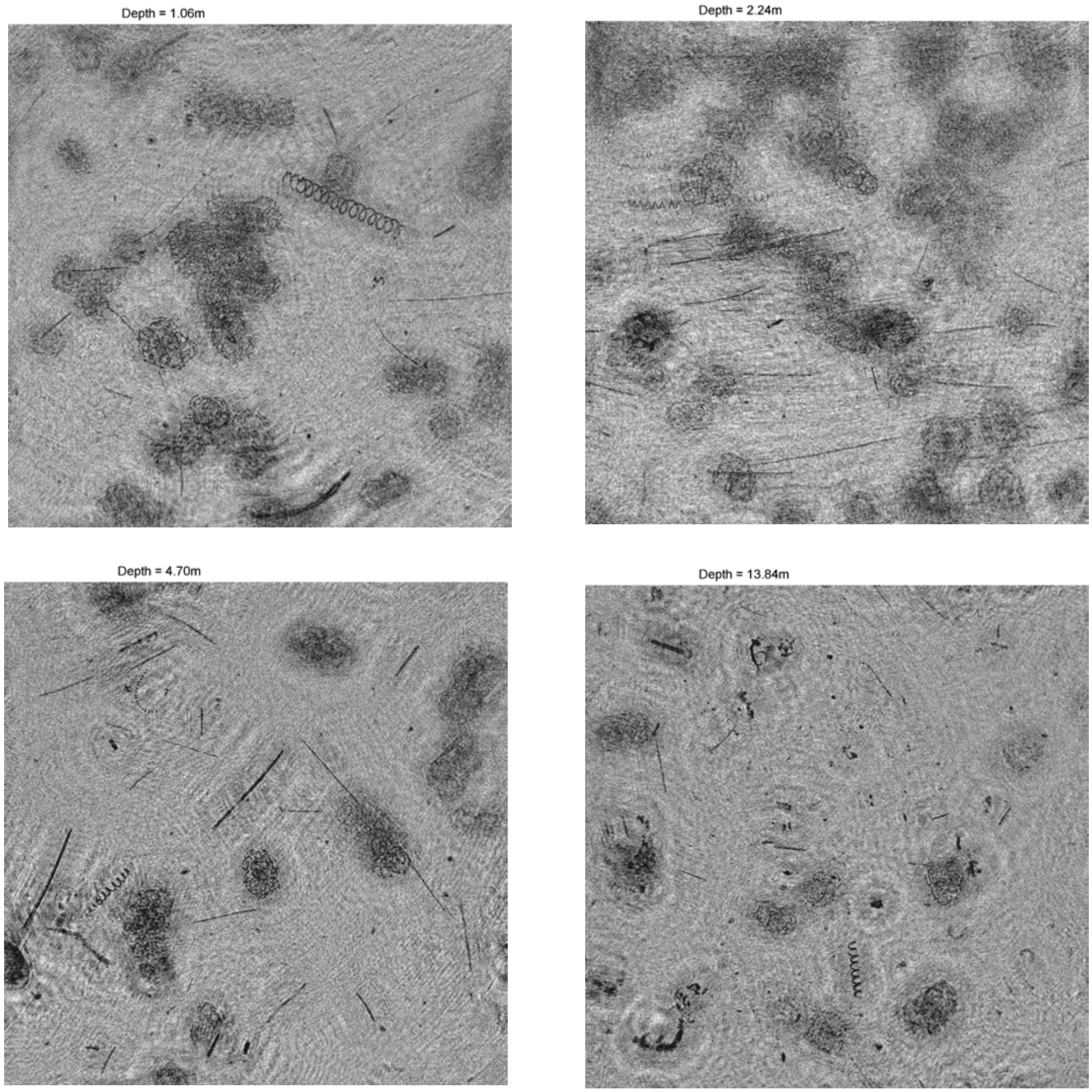

Figure 4. In-situ 2-D "splat" holograms of selected depths from a single vertical profile in East Sound, WA. These were processed by finding the in-focus image of each particle in the 3-D reconstructed hologram and compressing them all into a 2-D image. Thus, these images represent all the undisturbed particles in about a 6 ml volume (these holograms are ~ 1 cm x 1 cm by 10 cm in depth). The large round particles (~ 1 mm in size) are colonies of the diatom Chaetoceros socialis that were blooming in the Sound during the deployment. Notice in the top right panel (2.24 m depth), the consistent orientation of the long linear diatom chains. This depth was also the location of the strongest optical scattering in the water column.

Co-I Donaghay (URI) with Dr. Jan Rines and PI Sullivan have an on-going ONR project to examine the in-situ impact of episodic enhanced turbulent events on large phytoplankton. This project uses in-situ flow cytometry to detect changes in the individual particle characteristics of large non-spheroid phytoplankton under storm or other conditions. Leveraged co-deployment of the HOLOCAM during these field experiments could provide critical co-validation of these independent sensors, as well as additional scientific results that flow cytometry alone cannot provide.