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 AND WORK PLAN

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. Our work plan for the next year is to complete assembly of two HOLOCAMs and begin rigorous field testing.

WORK COMPLETED

1. Project design and status meetings.
Project investigators and lead engineers have met numerous times to discuss the evolution of the HOLOCAM system design and control electronics. The primary focus of meetings have included overall mechanical design of the HOLOCAM, designing control systems for synchronous firing of laser and cameras, redesign and repackaging of laser power electronics, building flexibility into the optical layout of the holography system, data acquisition and storage system requirements, and optimizing the external housing design to minimize turbulence-shear in sample volumes. During this year, the system design was finalized and the build of the first in-situ system was completed (Figure 1). Additional build meetings were held with the group as the system was constructed to address any engineering issues and to bench-top test the prototype system.

Figure 1. Internal electro-optical structure of the first in-situ HOLOCAM prototype (left panel). Pressure housings being assembled onto the HOLOCAM (right panel).

2. Acquired components.
While the bulk of components required to build the systems were acquired last year, additional components were needed and acquired. Electro-optical sea cables were purchased for each system. Several modifications were determined to be needed during the build of the first system and the additional required parts (neutral density filters, mounting hardware, etc.) were obtained. Some components were also found to be defective during the build/testing and required warranty repairs.

3. Control electronics.
Custom DC based power supplies (350 V) and electronic trigger circuits of the cameras and laser were built and tested with prototype system. It was determined during testing that modifications to the control electronics were required for accurate firing of the laser and synchronization to the dual camera system. This was a substantial effort during this year.

4. Optical layout and data acquisition testing.
Full bench-top testing of the in-situ HOLOCAM system components were completed at WET Labs. This included testing of the laser and cameras, beam alignment and transfer optics, electronic breadboard controls and DVR data acquisition systems. Several modifications of the system were required and completed after testing.

5. HOLOCAM construction.
The full mechanical build of the first in-situ HOLOCAM was completed at WET Labs. It has been pressure and submersion tested (Figure 2). Build of the second system is awaiting repairs to the second laser (see below).

Figure 2. Submersion and system testing of the HOLOCAM at WET Labs.

6. Prototype system testing.
It was discovered during testing of the lasers acquired for the two HOLOCAM systems that one of the lasers did not meet the firing frequency specification of the other. This required sending the laser back to the manufacturer for modification to make it identical to the other laser. The build of the second HOLOCAM system is on hold until this laser is returned from service. As of the writing of this report, these repairs should be completed within the next few weeks.

7. Software development/refinement for reconstructing digital holograms and particle analysis.
Custom software for hologram numerical reconstruction and analysis of particle characteristics is mostly complete. Improvements to these programs from both JHU and WET Labs software programmers will be addressed during in-situ HOLOCAM testing. System testing in both the laboratory and field is currently underway.

8. Continued diagnostic lab testing with bench-top HOLOCAM test-bed.
The small bench-top HOLOCAMs built to provide working holographic images used in the analytical software development described in the sections above and below are also being used in comparisons to data collected by the in-situ HOLOCAM system.

9. Lab validation of particle characteristics (e.g. PSDs) using bench-top HOLOCAM test bed.
The entire project team has continued 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 has been accomplished using SEM, Coulter counters, flow cytometry and light microscopy techniques.

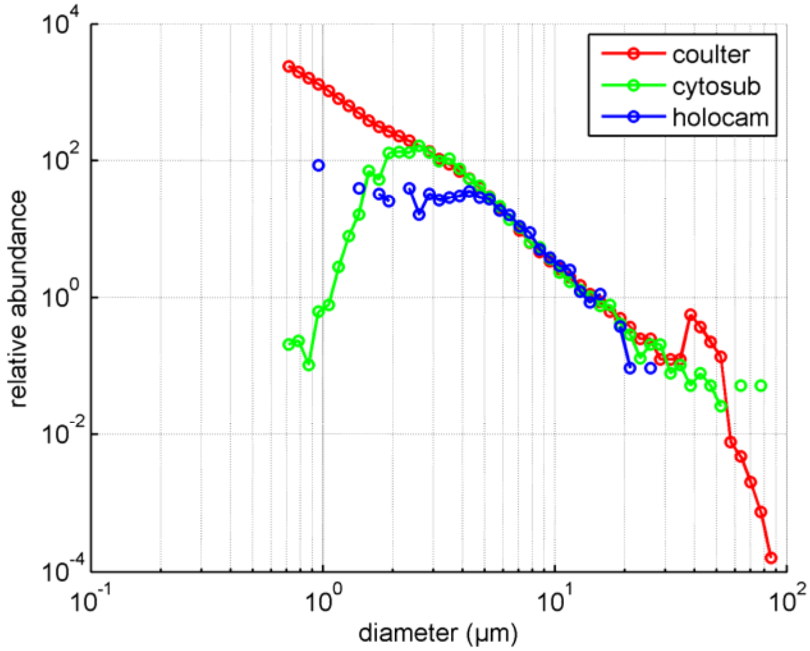

Figure 3. Particle size distribution (PSD) slope comparison between the bench-top HOLOCAM, Cytosub flow cytometer and a Coulter counter using an AZRD polydispersed particle standard (A1 Ultrafine, Powder Technology Inc.). The bench-top HOLOCAM is optimized for particle sizes > ~ 4 to 5 μm and the Cytosub for particles > ~ 2 μm. The PSD slopes compare very well within the working measurement ranges of these devices.

10. <u>Continued laboratory and field measurements</u>.

The in-situ HOLOCAM system is built and initial laboratory and field testing has begun. As part of an ONR MURI project on the biological response to the dynamic spectral-polarized underwater light field (Sullivan, Co-PI), two field exercizes were conducted to measure and model (among other goals) the underwater spectral-polarized light field in distinct water types (oligotrophic and eutrophic). As part of this effort, water samples were taken from all environments sampled and the bench-top HOLOCAM was used to quantify the particle fields in these samples. The results will be used to help augment other in-situ optical data for the project. A final MURI field experiment is planned in 2013 and it is hoped that the in-situ HOLOCAM will be deployed as part of this experiment. The bench-top HOLOCAM has also recently been used in support of a U.S. Coast Guard sponsored project to develop new technologies to quantify and characterize oil emmulsions in seawater (Twardowski, PI).

RESULTS

1. After extensive design and build meetings, the research group has finalized the design of the submersible HOLOCAM and completed the build of the first system (e.g. Figure 1). The system is very similar to that outlined in the original proposal. This design still allows for future velocity measurements using the low magnification system, and the highest possible particle resolution for the high magnification system. All internal, external and deployment components needed to build two

complete systems have been acquired, as well as two full DVR and data storage systems for real-time recording of holograms.

2. Research into hardware based DSP hologram reconstruction has continued. We are working with outside sources to develop a new generation of DSP boards to run hologram reconstruction code in hardware. This technology will be critical to making the HOLOCAM a viable, near-real time research tool.

3. Two bench-top HOLOCAM test beds have been continually used in both laboratory validation experiments and field testing and will be used alongside the submersible system during its testing phase.

4. Transitioning of the expertise required for reconstruction and analysis of holograms to WET Labs from JHU has continued. WET Labs has taken holographic processing techniques outlined by our academic partners at JHU and adapted them all into a single programming language that is both widely used in the community and easily modified by end users (i.e. Matlab). Along with image analysis techniques, our JHU partners have developed software for detecting and measuring the size and spatial distribution of bubbles in reconstructed holograms utilizing edge detection and the unique shape of bubbles, and software to calculate in-situ velocity fields and gradients, as well as turbulence dissipation rates from pulsed time series of holograms. This software has been used successfully to process East Sound field data (Talapatra et al., see publications) and we will seek to transition these additional processing techniques into the core system software. We will continually assess how to improve both reconstruction and analytical software during testing of the submersible system.

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 and in fact, the bench-top HOLOCAM was used for this application in a recent Coast Guard project (see related projects below). 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. Such characterization should include the size, shape, and internal refractive index distribution of each relevant particle in the field. When considering the incredible complexity and uniqueness of the particles found in natural waters, this is an exceedingly challenging prospect. The most extensive studies in this respect have been able to characterize only a small fraction of the information required, and even those data carry substantial caveats due to the intrusive nature of the measurement technique(s). 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. Holographic images of undisturbed, optically significant particles will not only 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), but could also provide critical data to any science question that requires an understanding of particle size, shape, fine-scale distribution, and/or short time-scale dynamics.

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. 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, We were encouraged by ONR in 2012 to submit a full proposal to characterize the degree to which natural, undisturbed particle populations are preferentially oriented in coastal waters using several optical sensors including the HOLOCAM. If this proposal is funded, the HOLOCAM will be extensively used during a 2013 field effort to support this research. This research is critical because in virtually all optical measurements and models, the VSF is assumed to have no dependency on the direction of illumination or the azimuthal plane of scattered radiance. For this assumption to be true, all particles suspended in the water column must be randomly oriented. From simple physics of hydrodynamic flows for the nonspherical particles that comprise virtually all the particles in the ocean, this is not a reasonable assumption. Furthermore, direct evidence from the field is confirming ubiquitous preferential particle alignment in coastal waters, particularly within density gradients (e.g.

HOLOCAM field data from Talapatra et al., in-press). Consequently, it appears that our understanding of the geometry-dependent VSF, and thus light transmission through coastal waters is significantly biased, with errors expected to be > 20% and often possibly > 50% in particle layers found within density gradients with low turbulence.

In addition, phase-I work for a recent U.S. Coast Guard contract (Twardowski, PI) evaluating the feasibility of developing a compact, inexpensive, multi-angle scattering instrument to quantify the size distribution and abundance of emulsified oil droplets in water, and determine the refractive index of the oil to readily derive density and viscosity, has used the bench-top HOLOCAM to determine the accuracies in size distributions and concentrations determined with the scattering instrument.

PUBLICATIONS

Talapatra, S., J. Hong, M. McFarland, A. R. Nayak, C. Zhang, J. Katz, J. M. Sullivan, M. Twardowski, and P. Donaghay, "Characterization of organisms and particles in the water column using a free drifting, submersible, digital holography." *Marine Ecology Progress Series*. in press.