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

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. Based on these meetings, the project team has finalized the system design (Figure 1). Project investigators have also met to define, discuss and test custom software and analytical techniques needed for analysis of holographic images.

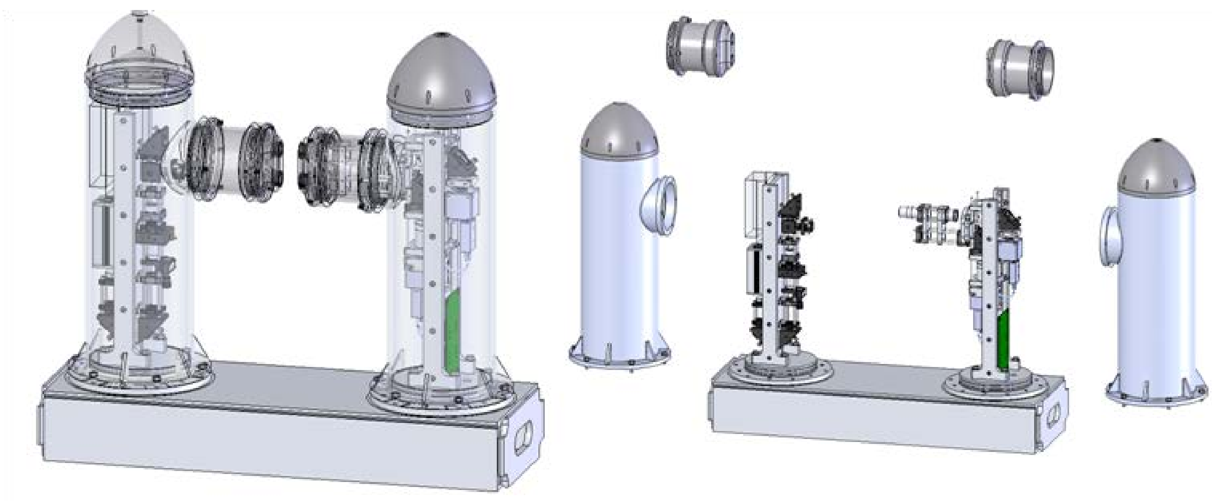

Figure 1. Mechano-optical design of the in-situ HOLOCAM. Design allows simplified field disassembly for service and optical alignment. Technical details are omitted due to proprietary design.

2. Acquired components.

The internal optical - electrical components needed for two submersible HOLOCAM systems have been acquired (lasers, cameras, optical rails, lenses, filters, etc.). Two custom digital video recording (DVR) and data storage systems needed to acquire holographic images from the HOLOCAMs were obtained from Boulder Imaging (Louisville, CO). These systems have 16 TB storage capacity and 400 MB/sec data throughput to disk. The DVR systems have custom camera link software optimized to the cameras used in the HOLOCAM and high-performance image acquisition and playback software. A high performance data processing computer for reconstruction and post-processing of holograms was also acquired. This 64 bit computer has 12 CPUs running at 3.5 GHz, 16 GB RAM and dual high performance NVIDIA graphics cards.

3. Control electronics.
It was determined that two custom electronics boards were needed for the HOLOCAM. The control and power supply unit supplied by the manufacturer for the HOLOCAM laser was large and designed to operate on AC power. A small, custom DC based power supply was designed by WET Labs to replace the original unit. A second board was required for the trigger circuits of the cameras and laser. In consultation with partners from JHU, WET Labs has finished the schematic and PCB layout of this board and all breadboard electronics are complete.

4. Optical layout and data acquisition testing.
A full bench-top layout and testing of the in-situ HOLOCAM system components has been initiated at WET Labs. This includes the laser and cameras, beam alignment and transfer optics, electronic breadboard controls and DVR data acquisition systems. A second set of HOLOCAM cameras and a DVR system were sent to our JHU partners to conduct concurrent testing of the data acquisition and storage system.

5. HOLOCAM construction.
Initial mechanical build of the in-situ HOLOCAM has begun at WET Labs. Specification for pressure housings and windows have been completed and quotes have been requested from external suppliers. It is anticipated that the assembly of the first HOLOCAM system will occur during Nov.- Dec. 2011.

6. Improvements to the prototype bench-top HOLOCAM.
Concurrent with the final system design, team members have completed assembly of two bench-top HOLOCAM test beds (Figure 2). These systems are used to evaluate parameters stipulated in the design and to test data processing techniques. In order to closely mimic the HOLOCAM design, the same spatial filter specified for the in-situ HOLOCAM was recently integrated into the bench-top test bed.

7. Software development/refinement for reconstructing digital holograms and particle analysis.
The project team has continued to define desired particle characterization suites, e.g. size, number, shape metrics, spatial distribution, etc. JHU and WET Labs software programmers have been testing and integrating these metrics into custom software for hologram numerical reconstruction and analysis of particle characteristics.

8. Continued diagnostic lab testing with bench-top HOLOCAM test-bed.
Team members from WET Labs, URI and JHU have continued 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. The bench-top HOLOCAMs also provide working holographic images that are used in the analytical software development described in the sections above and below.

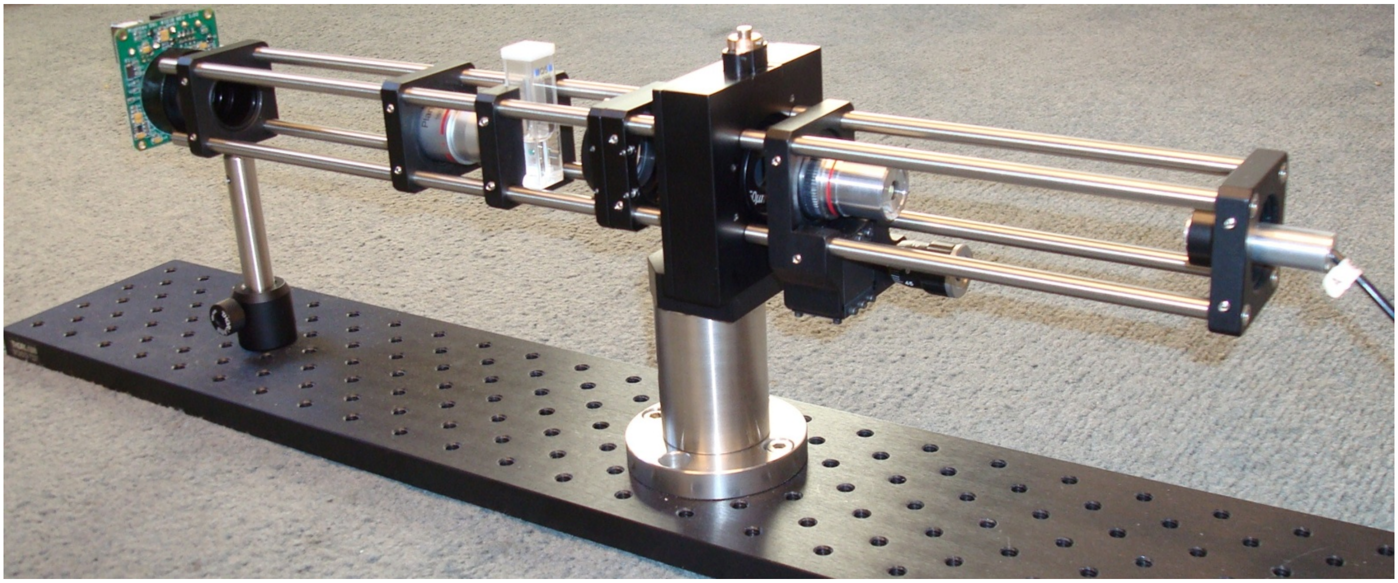

Figure 2. Bench-top HOLOCAM (~ 1 ft. in length) constructed at WET Labs. Starting from the far left is the CCD high resolution board camera, followed by a microscope objective and sample holder with cuvette, followed by a new spatial filter assembly, and finally on the far right is a small CW laser.

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 Figures 3 and 4 for examples). Validation using other independent characterizations of particles has been accomplished using SEM, Coulter counters, flow cytometry and light microscopy techniques.

10. Continued field measurements.
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 third field experiment is planned in late 2012 and it is hoped that the in-situ HOLOCAM will be deployed as part of this experiment.

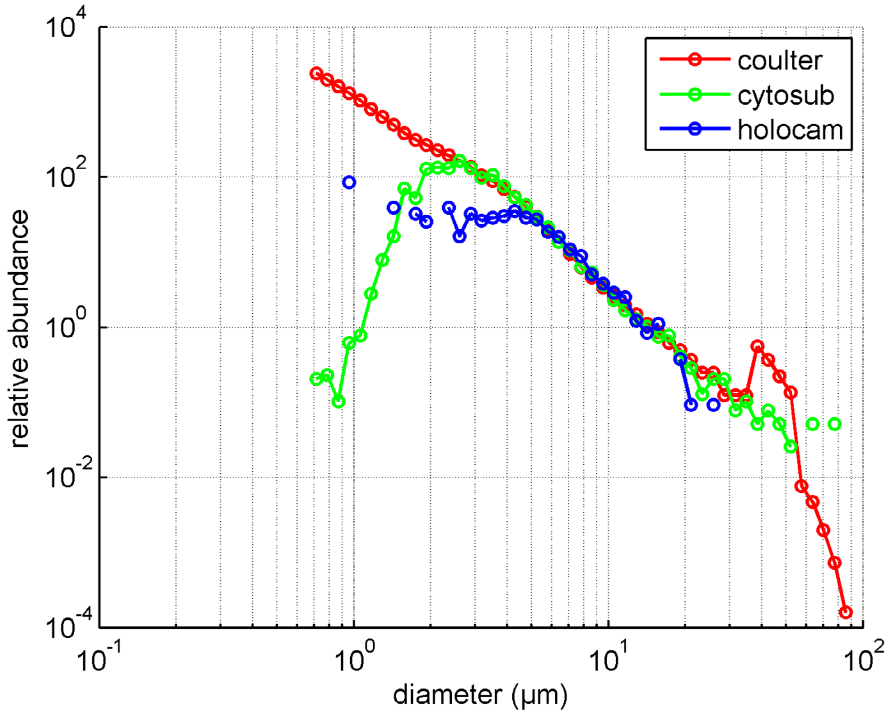

Figure 3. Particle size distribution (PSD) slope comparison between the bench-top HOLOCAM, Cytosub flow cytometer and a Coulter counter using an AZRD polydispersed particle standard (A1 Ultrafine, Powder Technology Inc.). The bench-top HOLOCAM is optimized for particle sizes > ~ 4 to 5 μm and the Cytosub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

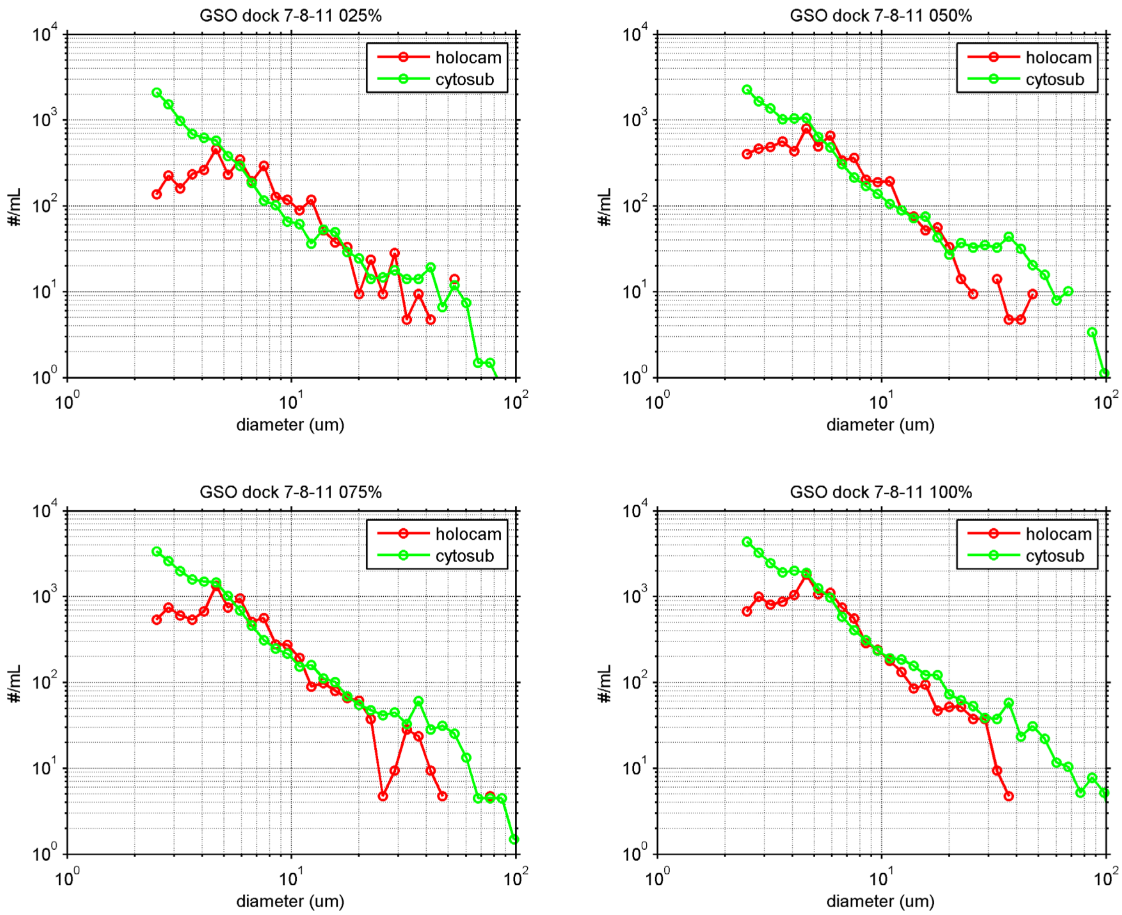

Figure 4. Particle size distribution (PSD) comparisons between the bench-top HOLOCAM and a Cytosub flow cytometer using four dilutions of a natural water sample from Narragansett Bay, RI. The bench-top HOLOCAM is optimized for particle sizes > ~ 4 to 5 µm and the Cytosub for particles > ~ 2 µm. The PSDs compare very well within the working measurement ranges of these devices.

RESULTS

1. After extensive design meetings, the research group has finalized the design of the submersible HOLOCAM (i.e. Figure 1). It will be very 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. All internal components needed to build two systems have been acquired, as well as two full DVR and data storage systems for real-time recording of holograms, and a high performance computer for post-processing of holographic images. External pressure housings and windows are in the process of being constructed. It is anticipated that the assembly of the first HOLOCAM system will occur during Nov.- Dec. 2011.

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. The construction of two bench-top HOLOCAM test beds was completed and these devices have been continually used in both laboratory validation experiments and field testing. It was determined after both lab and field-testing that a spatial filter was required to clean up the source beam from the laser. In order to make the bench-top similar in design to the submersible HOLOCAM, the same spatial filter assembly was integrated into the bench-top devices (i.e. Figure 2).

4. Transitioning of the expertise required for reconstruction and analysis of holograms to WET Labs has continued. WET Labs personnel have received training in the steps and programs required. Our JHU partners typically use both custom GUI software (C-based) and commercial software packages (e.g. Photoshop) to process holograms. WET Labs has taken these processing steps 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). Extensive testing of analytical image analysis techniques for particle analysis of holograms (e.g. 3-D deconvolution with point spread functions; thresholding at different spatial frequency ranges) has determined that variable band-pass filtering appears to work best in improving signal to noise for image processing. 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 (see section 5 below). We will continually assess how to improve both reconstruction and analytical software during development of the submersible system.

5. The JHU HoloSub was modified from the single magnification, crossing-path holography system, to a system design similar to the proposed HOLOCAM. 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, along with extensive optical data. These data have been analzed and written up in a paper that has been submitted to *Limnology and Oceanography* (Talapatra et al., see publications). This study was part of a collaborative effort to characterize planktonic thin layers and was sponsored by ONR. Key findings described in the paper were: (a) a prominent thin layer of non-motile small particles, many of them containing chlorophyll, formed in a region of near zero shear and low turbulence dissipation rates at the base of a strong pycnocline; (b) multiple thin layers of chlorophyll rich small particles also formed in unstratified low shear regions at several depths; (c) diatom chains exhibited horizontal alignment in the prominent thin layer and other low shear regions. Conversely, diatom chains were randomly oriented in other local shear rate maxima zones; (d) *Chaetoceros socialis* colonies dominated (by volume fraction) thin layers in and around the pycnocline; (e) zooplankton avoided the *C. socialis* peaks near the pycnocline, as well as other thin layers; (f) the PSD of small particles resembled a power law, while larger diatom chains and *C. socialis* colonies exhibited a log-normal size distribution.

IMPACT/APPLICATIONS

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.

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, 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.

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." *Limnology and Oceanography*, submitted.