THE UNIVERSITY OF RHODE ISLAND
GRADUATE SCHOOL OF OCEANOGRAPHY

Assessment of holographic microscopy for quantifying marine particles

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1. Why use holographic microscopy?

Introduction. Plankton and other marine particles can reveal much about biogeochemical cycles, carbon export, and the supply of food for higher trophic levels. However, characterizing marine snow and phytoplankton communities in sparse, highly variable oceanic environments remains a methodological challenge. Laser holographic microscopy has been proven to work well in the laboratory, and has been widely used in cytobiology for over a decade. Digital Inline Holographic Microscope (DIHM) units are now available in a depth-rated housing for deployment in seawater, providing 3D imagery of plankton and marine snow[5]. Compared to similar in situ imaging systems, holographic microscopy improves sampling volume, resolution, and autonomy.

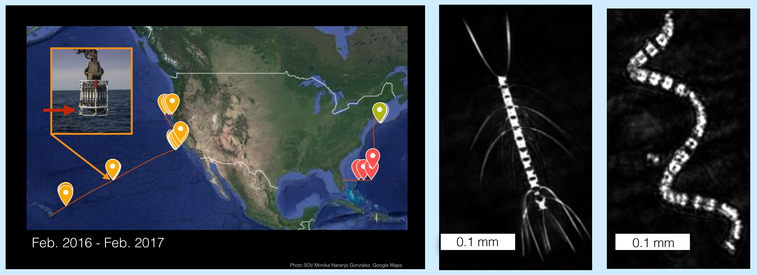


Figure 1. Since Feb. 2016, our group has completed over 70 deployments (profiles) of the holographic microscope in the New England Shelf Break, Sargasso Sea, and North Pacific. Left: Deployment map. The current deployment configuration, from the bottom of a CTD Rosette, uses battery power and autonomous data logger to sample for up to 2 hours. Middle and Right: Examples of reconstructed holographic images of two chaetoceros diatom chains.

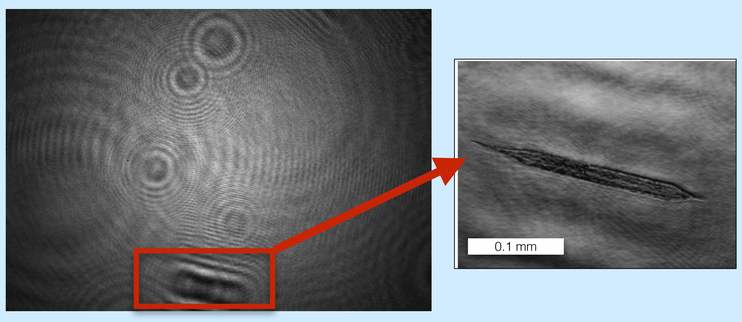


Figure 2. The holographic microscope uses fourier reconstruction to focus objects, which enables a large sample volume: 0.005 mm particles can be resolved across a 13 mm focal depth. Imaging requires no lenses or mechanical components, which increases autonomy through decreased power requirements and maintenance. Top: A raw holographic image can be mathematically focussed after the image has been taken. This example shows a pennate diatom.

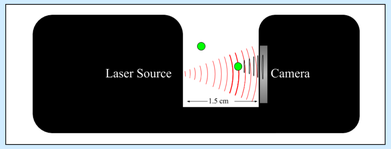


Figure 3. Schematic drawing of the 4Deep Digital Inline Holographic Microscope (DIHM). Not to scale. Objects that intercept the spherical laser wave source form diffraction patterns which are imaged by the DIHM camera at up to 16 fps.

2. How are quantitative measurements from holographic microscopy acquired?

Methods. The millions of images generated by the holographic microscope are analyzed by computer vision techniques. A modified edge-detector was used to find regions of interest (ROI) and isolate them from the surrounding data. A probability distribution of particle concentration (for a given XYZ position) was empirically determined (Figure 5) and used to correct for biases in non-uniformity in object detection in the holographic microscope conical beam. This allowed us to accurately compute the effective volume sampled by the DIHM.

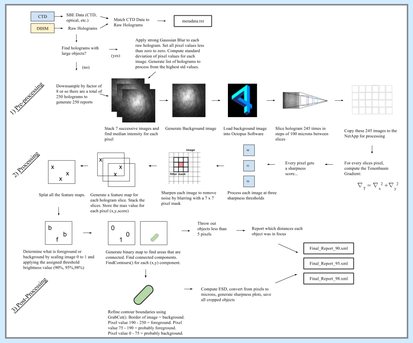


Figure 4. The holographic image processing pipeline automates particle size and abundance measurements. Background subtraction is applied to raw holographic images before mathematical hologram reconstruction using the 4Deep Octopus software. The Octopus software uses the Kirchhoff- Helmholtz transform to solve point source wave front intensities at the object focal plane (Xu et al., 2002). The Octopus software saves slices of the hologram focal planes in 100 micrometer increments between the point source and the camera. OpenCV image processing libraries are used to detect, crop, and measure the objects with the highest sharpness score. Imaging artifacts were determined empirically using object ESD, sharpness score, and position. Left: Schematic of the hologram processing pipeline.

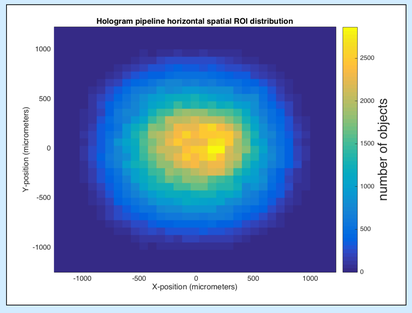
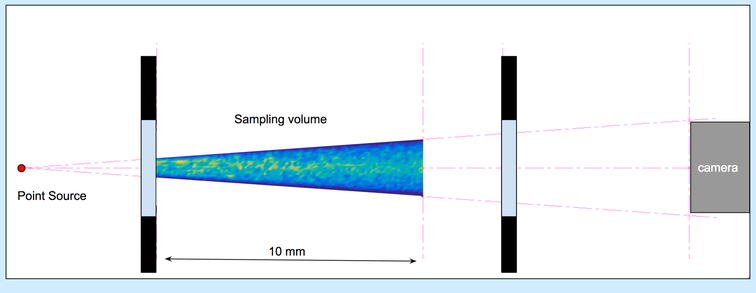


Figure 5. The non-uniform distribution of ROI positions in the horizontal plane (parallel to the laser source) suggests that the DIHM and hologram processing pipeline are biased by changes in the laser intensity, which decreases radially and from the laser to the camera. To correct for this bias, we created probability density distribution of object positions and normalized each sample by this spatial probability. Objects observed at the periphery are scaled as much as 3x the observed concentration. Left: The density of ROIs decreases radially. Bottom: Scaled geometric representation of the imaging volume with z-y position ROI histogram. Figure is to scale.



3. How does the data compare to that from other imaging tools?

Results. Quantitative assessment of the hologram processing pipeline shows promising results for particle concentration and size. Comparison with FlowCam, Imaging Flowcytobot and manual counts are correlated (Figure 6). In the future we plan to include taxonomic classification using machine learning tools.

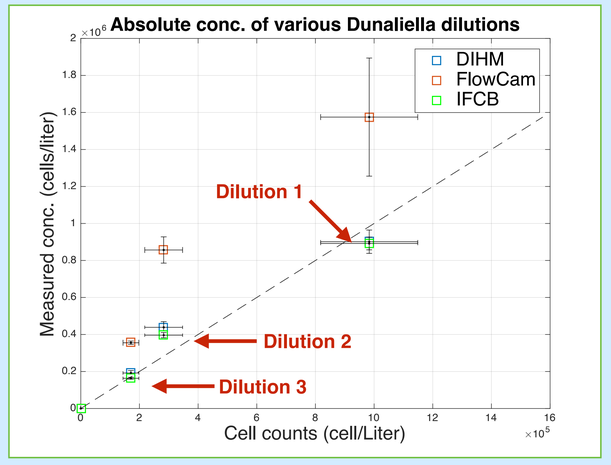


Figure 6. Absolute concentration of three dilutions of a Dunaliella culture quantified with the holographic microscope (DIHM), Imaging FlowCytobot (IFCB), FlowCam versus manual microscope counts. Three standardized dilutions of Dunaliella culture were processed by each particle counter: 100% concentration Dunaliella, ~50% dilution, ~20% dilution. A filtered seawater sample was prepared using underway seawater filtered through a 2 micrometer glass fiber filter as a control sample. Preliminary results between the DIHM, FlowCam, and manual microscope counts, show good correlation (Figure 2, $r^2 = 0.92$, $p < 0.05$).

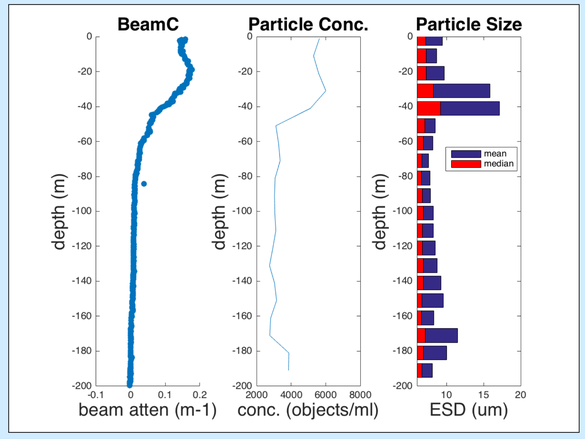


Figure 7. Example profile of data from a beam transmissometer (BeamC, left panel) compare well with the DIHM particle concentration (middle) and particle size (right).

1. University of Rhode Island Graduate School of Oceanography 2. Brown University Center for Computation and Visualization 3. NASA Goddard 4. University of Zagreb 5. Bochdansky, Alexander B., Manfred H. Jericho, and Gerhard J. Herndl. "Development and deployment of a point-source digital inline holographic microscope for the study of plankton and particles to a depth of 6000 m." Limnology and Oceanography: Methods 11.1 (2013): 28-40. 4.