Digital In-line Holographic Microscopy: design, advantages, challenges, and future integration into autonomous platforms

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Bathypelagos: Cross-correlations with oxygen deficits suggests that a large fraction of > 500 µm particles don’t sink

obs....optical backscatter (colloidal size particles)
video...particles > 500 µm

Bochdansky et al. (2010) PNAS 107
Various methods to visualize particles

- Underwater Vision Profiler
- Video Plankton Recorder
- Scripps Plankton Camera
More specifically for plankton (disrupting fragile amorphous particles)
Keck In-Situ Underwater Microscope

Underwater Imaging Lab

Jules Jaffe, Scripps
In-situ Holography
History

- Holographic microscopy since 1960ies (e.g., Knox 1966, Pennington 1968)

- First plankton application using glass photographic plates (1970)

  "... in a hologram the ability to adjust the depth of focus is retained in the reconstruction which is not possible with conventional incoherent illumination" (Beers, Knox, Strickland 1970)

Underwater holography

Until recently a “novelty” with limited routine use in oceanography

Goal: to integrate holography with routine CTD deployments and autonomous vehicles and combine them with other instruments (optical backscatter, transmissometer, fluorometer) to provide permanent records of high-resolution plankton distributions
Advantage of holography: Much larger volumes can be surveyed in one image than with the restricted depth of field for conventional lens-based technology.

- Large depth of field (many cm):
  - -> large volume surveyed: > 300,000,000 times volume
Types of holography

- Off-axis configuration:
  real 3D representation

- In-line configuration:
  (a) Parallel beam (LISST-HOLO, Sequoia) and (b) expanding beam (4-Deep) configurations
Commercial in-line holography instruments

Sequoia LISST-Holo

4-Deep Holographic Inwater Imaging

But not yet for the deep sea (except for ODU prototype)
HoloSub: Dual-view holography
(2 inline recordings set apart 90 degrees)
Full 3-D reconstruction (avoids shading)
Off-axis configuration

Lindensmith et al. (2016)
In-line and off-axis combination

Watson et al. (2001)
Digital in-line expanding-beam holographic microscopy

Sample Volume (variable width)

Laser
Pinhole
Camera chip (no lens)

Pinhole (1-10 μm) is “point source”

SIMPLICITY!
Forward scatter much brighter than side scatter
Principle of expanding beam DIHM

- Spherical waves travel from pinhole to screen
  Solid lines: wave fronts of main beam
  Dashed lines: wave fronts of scattered portions of the beam
- Interference pattern = hologram on screen (in digital holography CCD chip replaces photographic emulsion)

Xu et al. (2001)
DIHM reconstruction
Xu et al. (2001) PNAS 98: 11301-11305

Equation: \[ K(r) = \int d^2 \xi |I(\xi)\exp[2\pi \xi \cdot r/(\lambda \xi)] | \]

|K(r)|...plot of function on a 2-D plane perpendicular to optical axis = “reconstruction”,
\(\xi\)...coordinates (X,Y,L) where L is distance from the point source,
\(I(\xi)\)...contrast image of hologram (difference of images with and without object)
Software: Octopus by 4-Deep, Halifax, Canada
Image reconstruction of *Trichodesmium* sp.

Unreconstructed hologram

Reconstruction

#15 17Oct2010
Reconstruction video
DIHM for the deep sea (ODU)
Bochdansky et al. 2013 L&O Methods

- 6000 m pressure casing (2 cm thick walls, sapphire widows)
- Single mode fiber (9 µm diam.) as point source
- 640 nm Diode laser (red)
- 4 Mpixel GigE Camera with 1/16,000 sec shutter speed,
  7 frames sec\(^{-1}\)
- Embedded computer (small, 750 Gb SATA drive)
- 7 cm gap
- Vertical speed through water 1 – 1.5 m sec\(^{-1}\)
- Total imaged volume at 4000m: 1.8 ml x 7 frames s\(^{-1}\) x 4000m = 50 Liter
DIHM mounted on CTD rosette
DIHM mounted on CTD rosette

Length: 1.04 m
Diameter: 15 cm
Weight: 70 kg
ca. 7 cm gap
Metazoan plankton: copepods
Metazoan plankton: copepods

5 µm diam

12Oct 2011 #22
Metazoan plankton: copepods

20Oct2010 #18

20Oct 2011 #63
Comparison with light microscopy
Metazoan plankton: Siphonophores (Hydrozoa, Cnidaria)

http://www.obs-vlfr.fr/Mam/images/missions/pages/Stareso-siphonophore-3cm.htm
Metazoan plankton: *Sagitta* sp. (Chaetognatha)
Metazoan plankton: Appendicularia (Tunicata, Chordata)
Appendicularia (Larvacea)
*Trichodesmium* sp. colonies (tufts and puffs) (Cyanobacteria)
Protists: Tintinnida (ciliate in lorica)

[Image of a phase contrast image of a Tintinnida organism]

www.micro*scope.com (MBL)
Phase contrast image
Acantharia

www.micro*scope.com (MBL)
Phase contrast image
Protists: *Ceratium* sp. (2 individuals) (Dinoflagellata)
Protists: chain-forming diatoms

http://sanctuarysimon.org/monterey/sections/openOcean/project_info.php?projectId=100173&sec=oo

*Pseudonitzschia* sp.
Diatom chain with vorticellid ciliate parasites

DIHM analysis deep sea (> 2000 m):
3 categories: Others (top row), fecal pellets (center row), marine snow (bottom row)

Figure 1. Examples of three categories of particles: marine snow (bottom row), faecal pellets (centre row) and “others” (top row). The “others” category includes all recognizable planktonic organisms (alive and carcasses) and optically dense debris that does not classify as marine snow or faecal pellets. For each image, the size (μm) and depth sampled (m) are given.

Bochdansky et al. 2016 Sci Rep
Figure 2. Size frequency distribution of three categories of particles in five different water masses: marine snow (bottom row), faecal pellets (centre row), and other particles including planktonic organisms (top row). AABW: Antarctic Bottom Water (n = 6,213), NADW: North Atlantic Deep Water (n = 13,824), LDW: Lower Deep Water (n = 1,408), NEADW: Northeast Atlantic Deep Water (including some mixed-in Labrador Sea Water, n = 3,610), NSDW: Norwegian Sea Deep Water (n = 440). The relative volume contribution for each of the particle types is given as a percentage: $V_{ms}$ (marine snow) + $V_{fp}$ (faecal pellets) + $V_o$ (others) = 100%.
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Figure 4. Examples of dragon-king particles with little apparent ballast (a–c) and a ballasted stringer-type particle (d). Particles are held together by a large amount of transparent exopolymers. White scale bars = 1 mm.
Fecal pellets and fecal strings

21Oct 2010 #27
1000 µm

21Oct 2010 #50
200 µm

23Oct 2010 #18
100 µm

20Oct 2010 #71

20Oct 2010 #75
1000 µm

20Oct 2010 #52
500 µm
Marine snow: mineral particles (a,c), stringer (b), ballast (d)
Marine snow: amorphous without ballast
Number spectrum of particles: 2000 – 6000 m
North Atlantic / Arctic

- Slope = -3
- 95% prediction intervals
- ~400 µm
- "dragon kings"
- Junge power law slope = -4

Bochdansky et. al. 2016 Sci Rep
Mosaic of epifluorescence microscopy images: typical dragon king particle
Each dot represents DNA of a single microbe
Major problems in deriving particle flux estimates from optical particle inventory:

1) Particles we see in the water column ≠ Particles that sink

2) Stokes Law does not apply (not even approximately)
Why care about neutrally buoyant or slowly sinking particles in the deep sea if they don’t contribute much to vertical flux?
Dragon King
North Atlantic
Depth: 4118 m
length: 3.2 mm
Dragon King
North Atlantic
Depth: 4118 m
length: 3.2 mm

Different communities of microbes
(different ecosystem function)

Eukaryotes dominate biomass
(incl. fungi, labyrinthulomycetes)

Oxygen gradients
Diversity of metabolisms

Diffusion limitation for enzymes
and substrates

Extremely high biomass concentrations
10,000 x ambient

Passing metabolites and signal molecules

Quorum sensing

Resource

Process (rate)

threshold
Integration into autonomous vehicles
Miniature diode lasers, camera & video recorder

Miniature diode lasers, camera & video recorder

2 Megapixel, progressive scan camera with up to 1/50,000 second shutter speed

Components < $1000

Precision pinholes

Miniature digital recorder for drones
Glider at BATS

Figure 4. (a) Schematic of the Carbon Flux Explorer (CFE). CFE represents the integration of the Optical Sedimentation Recorder (OSR, engineered at Berkeley Lab) and the Sounding Oceanographic Lagrangian Observer (SOLO; Sippri) profiling float. (b) Optical Sedimentation Recorder (OSR). This instrument was designed to quantify carbon sedimentation on hourly time scales for seasons. SOLO communicates its dive status and pending actions to OSR and OSR communicates reduced data to SOLO for relay to Iridium satellites.
High-bandwidth downloads of DIHM images

In seawater:
7 Gbps at 7 m distance

Maybe faster in deep sea with fewer impurities

Tsai-Chen et al. (2016) Scientific Reports
Summary

• DIHM useful tool for exploration of relatively large volumes of seawater
• Produce permanent record: detailed analysis can be done post-hoc depending on target particle (“shoot first, ask questions later”)
• Explore fine-scale distribution of plankton and particles in unaltered state
• Simplicity of optical setup makes it highly flexible for a wide range of platforms
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Holography | Art
Follow-up analysis of macroscopic deep-sea particles
Bochdansky et al. (2016) ISME J

High TEP content
  a) Alcian Blue stained TEP
  b) DAPI stained prokaryotes in TEP matrix (hole = 30 µm)

Highly enriched saprotrophic eukaryotic microbes
(fungi, labyrinthulomycetes)

d: Labyrinthulomycete, CARD FISH
e-h: fungal cells, CARD FISH

High TEP content, lack of ballast material, development of a unique microbial community suggest the existence of a large proportion of non-sinking or slowly sinking macroscopic particles in the pelagic deep sea.