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

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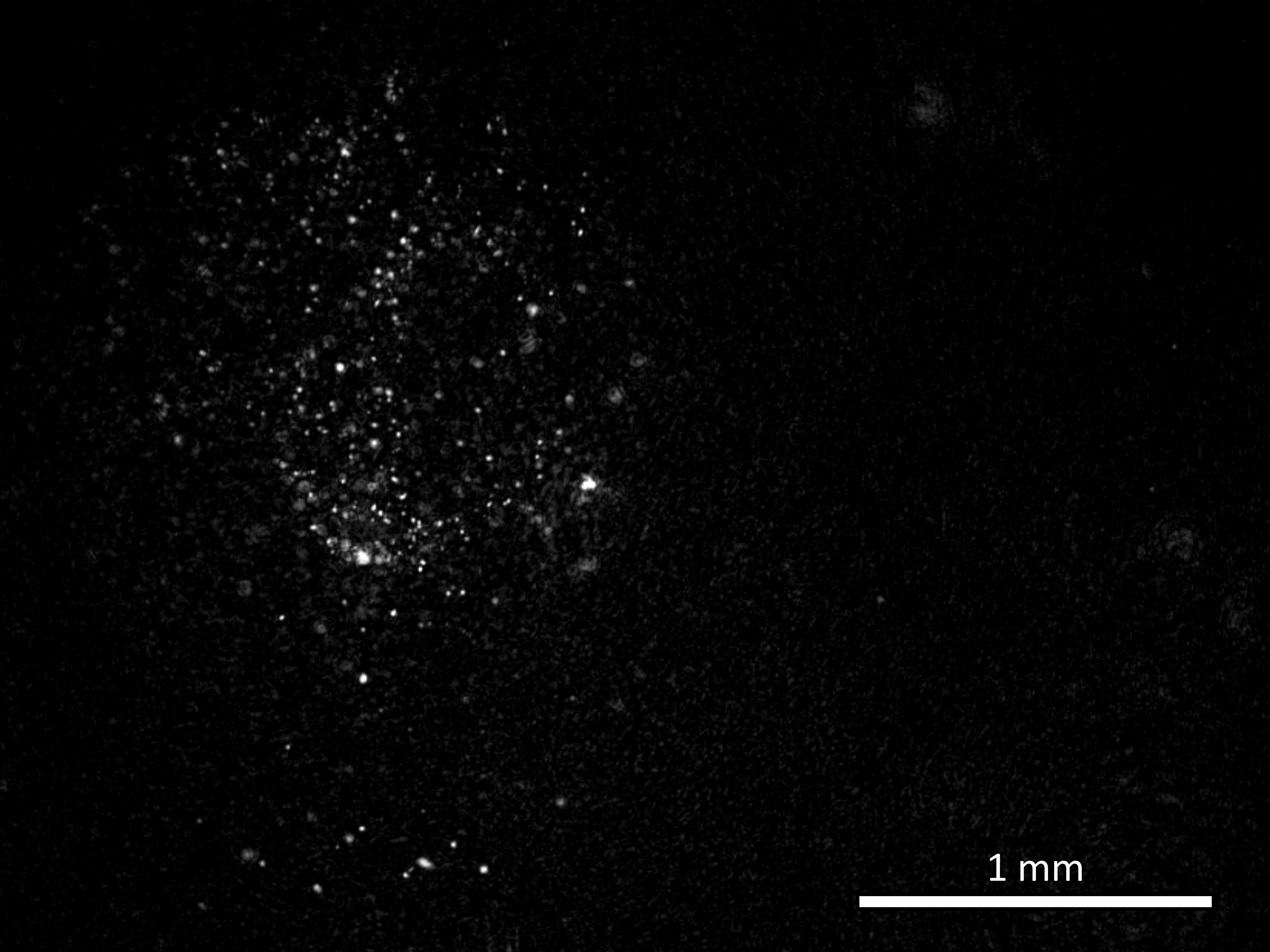
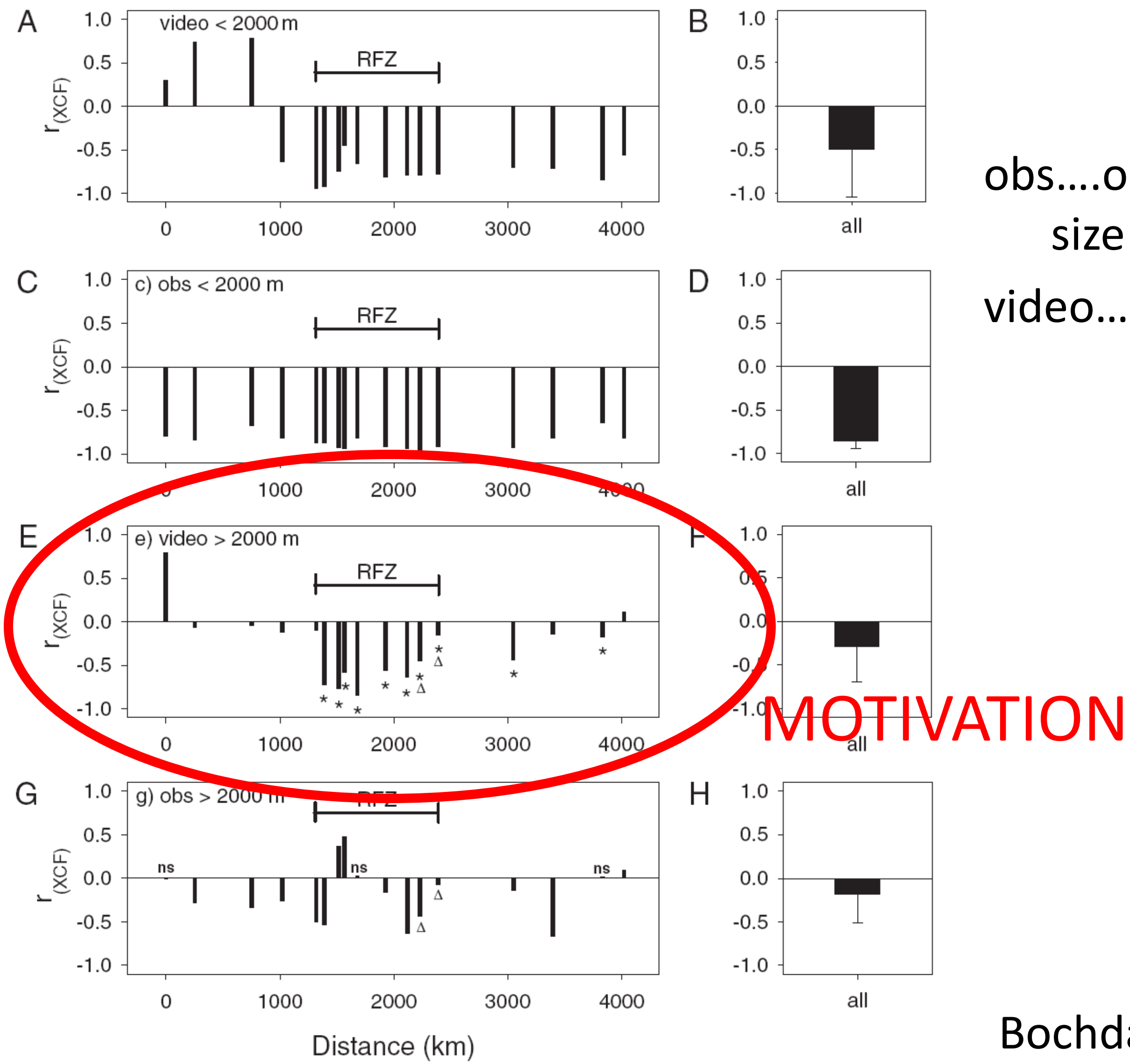
Biological Oceanography


1 mm

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

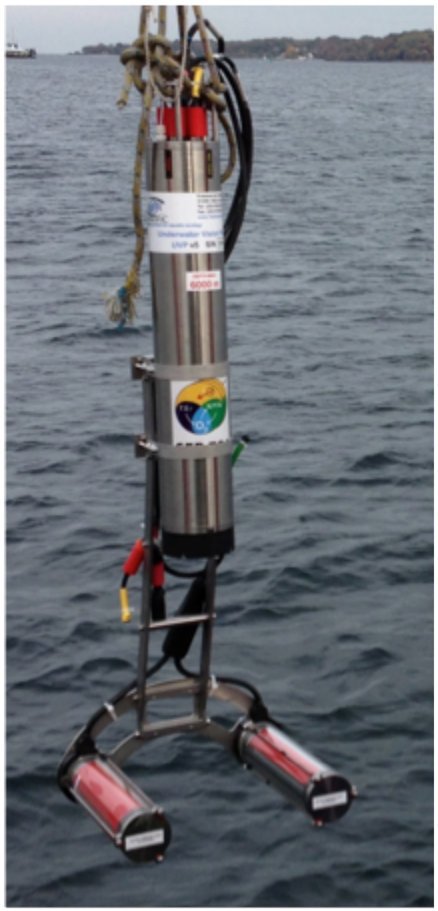


obs….optical backscatter (colloidal size particles)

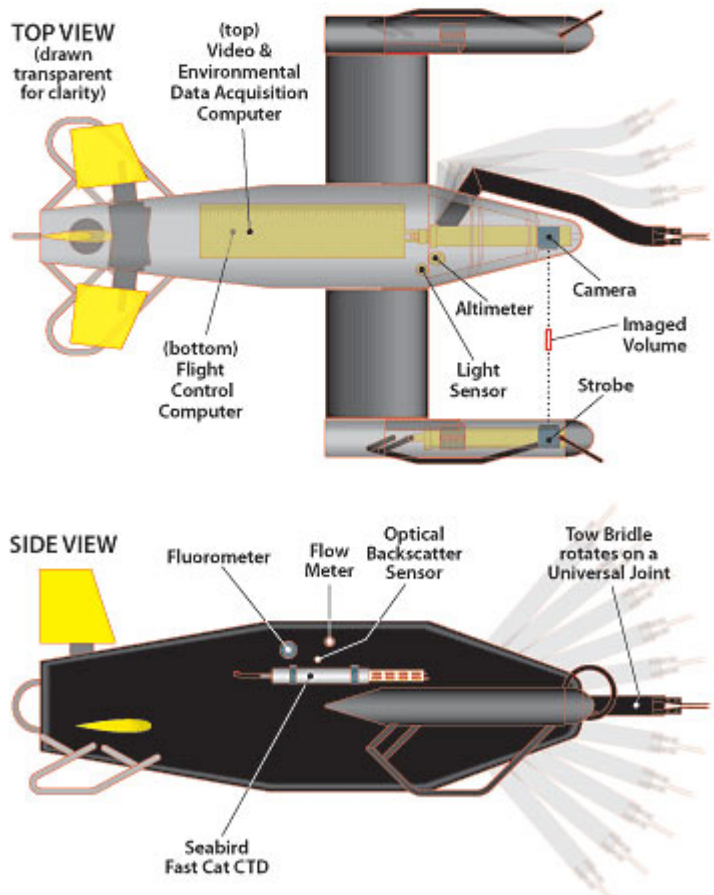
video…particles > 500 μm

Bochdansky et al. (2010) PNAS 107

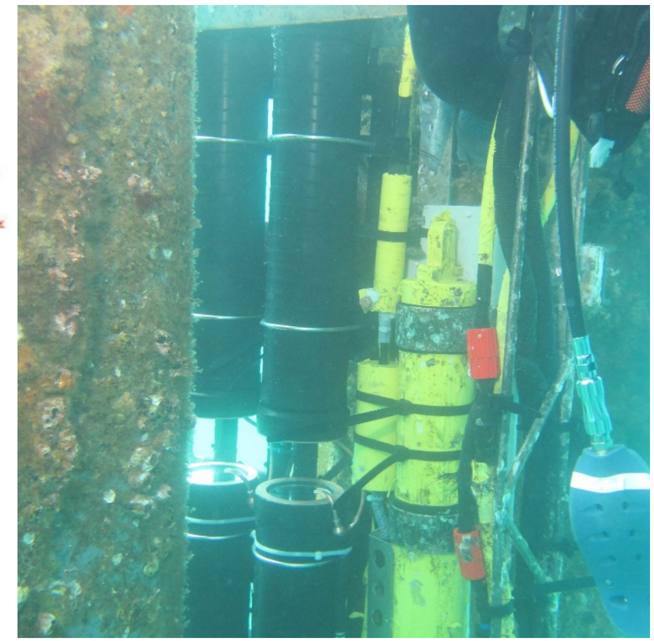
Various methods to visualize particles



Underwater Vision Profiler



Video Plankton Recorder

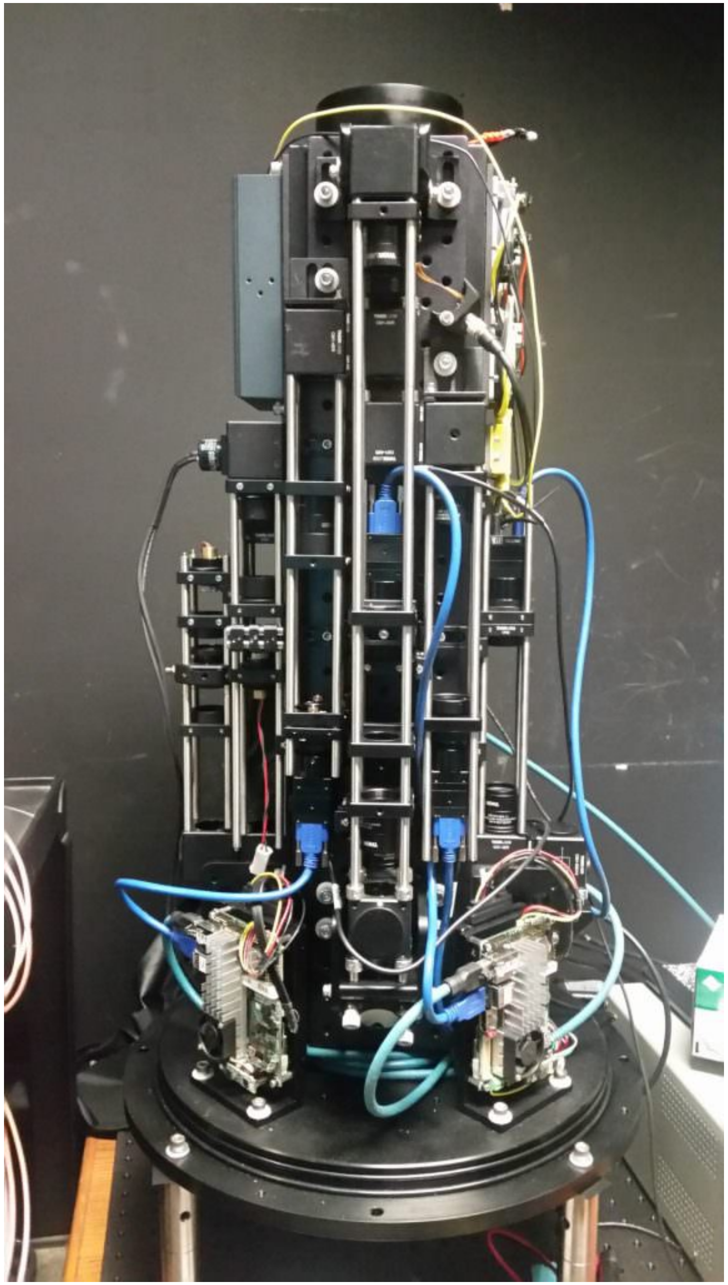


Scripps Plankton Camera

More specifically for plankton (disrupting fragile amorphous particles)

Flow Cam

Flow Cytobot

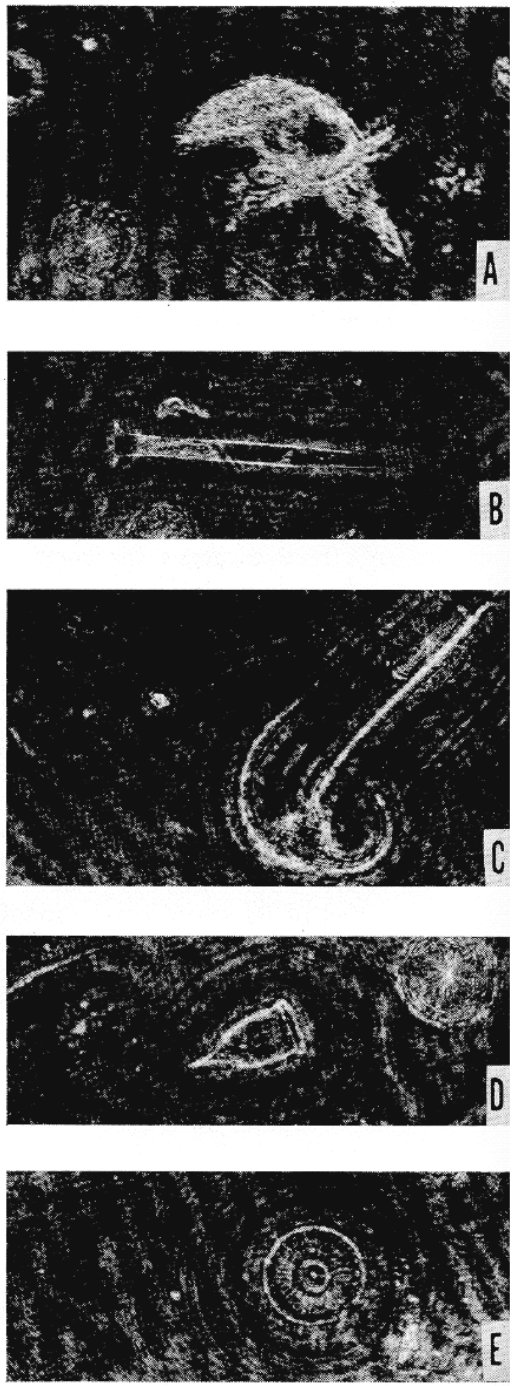


Keck In-Situ Underwater Microscope

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)

- Further developments: Malkiel et al. 1999, 2006; Watson et al. 2003, Watson 2005, Sun et al. 2007; Jericho et al. 2006, 2010; Lewis et al. 2006; Xu et al. 2001, Nimmo Smith WAM

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

Depth of Field and Image Depth Nikon MicroscopyU

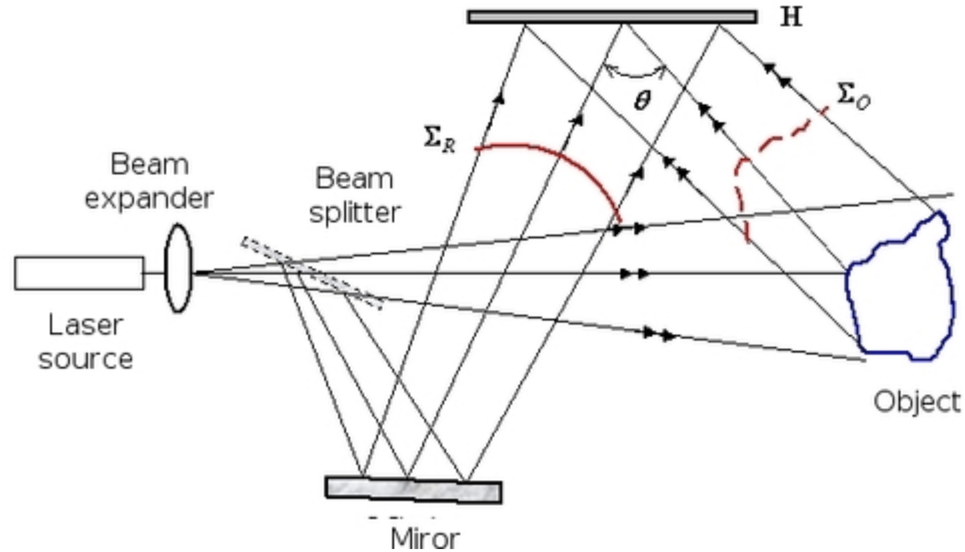
Magnification	Numerical Aperture	Depth of Field (µm)	Image Depth (mm)
4x	0.10	55.5	0.13
10x	0.25	8.5	0.80
20x	0.40	5.8	3.8
40x	0.65	1.0	12.8
60x	0.85	0.40	29.8
100x	0.95	0.19	80.0

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

Types of holography

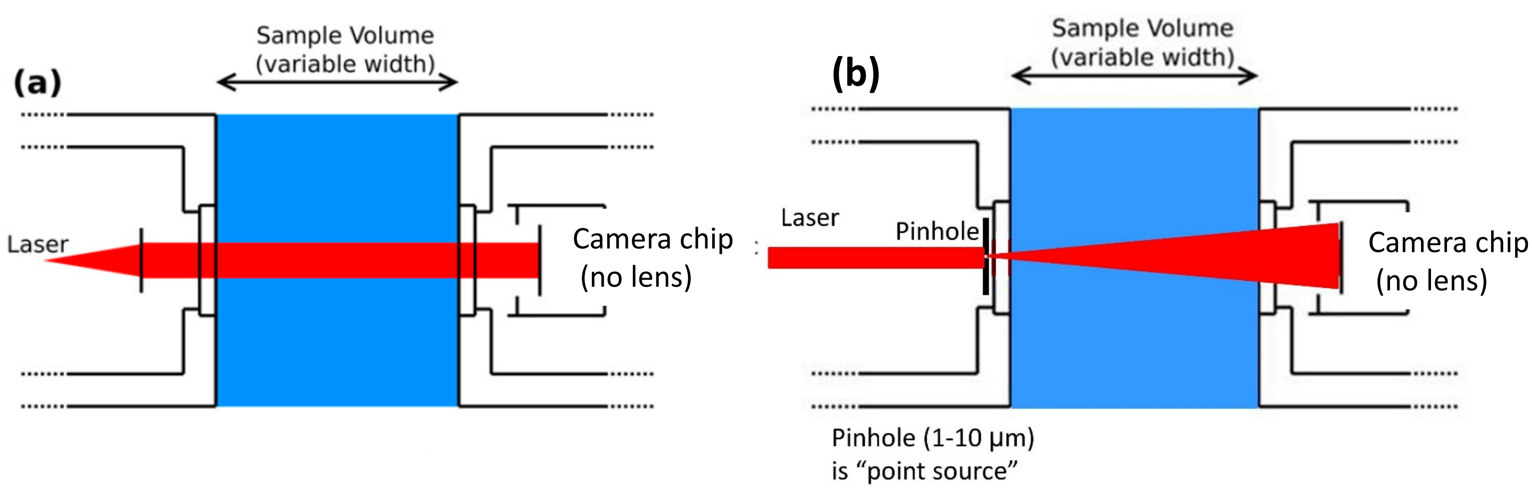
- Off-axis configuration:

  real 3D representation



- In-line configuration:

(a) Parallel beam (LISST-HOLO, Sequoia) and (b) expanding beam (4-Deep) configurations



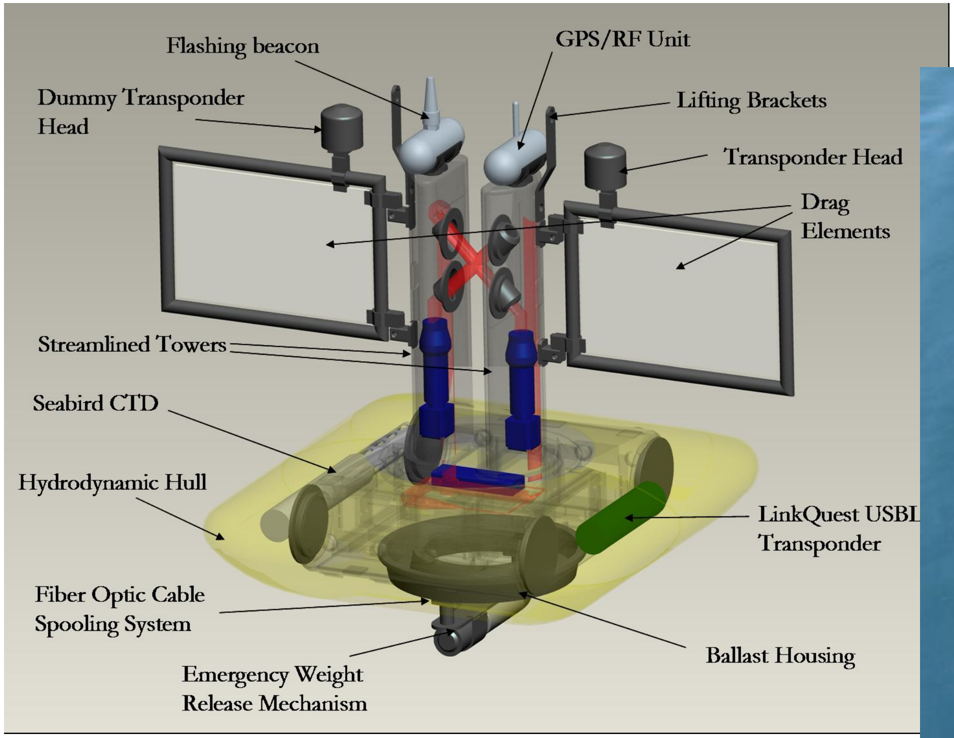
Commercial in-line holography instruments

Sequoia LISST-Holo

4-Deep Holographic Inwater Imaging

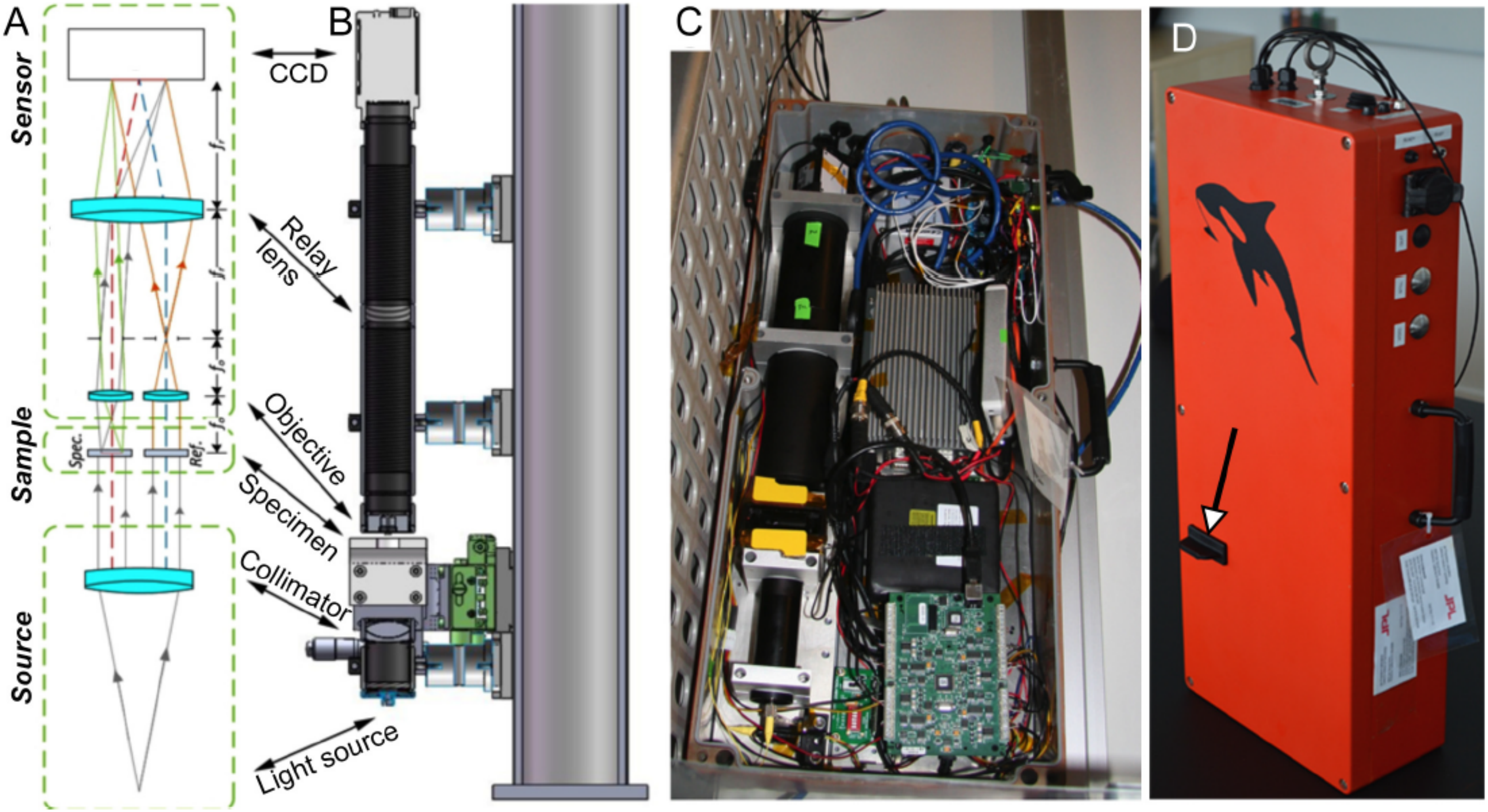
But not yet for the deep sea (except for ODU prototype)

HoloSub: Dual-view holography (2 inline recordings set apart 90 degrees) Full 3-D reconstruction (avoids shading)



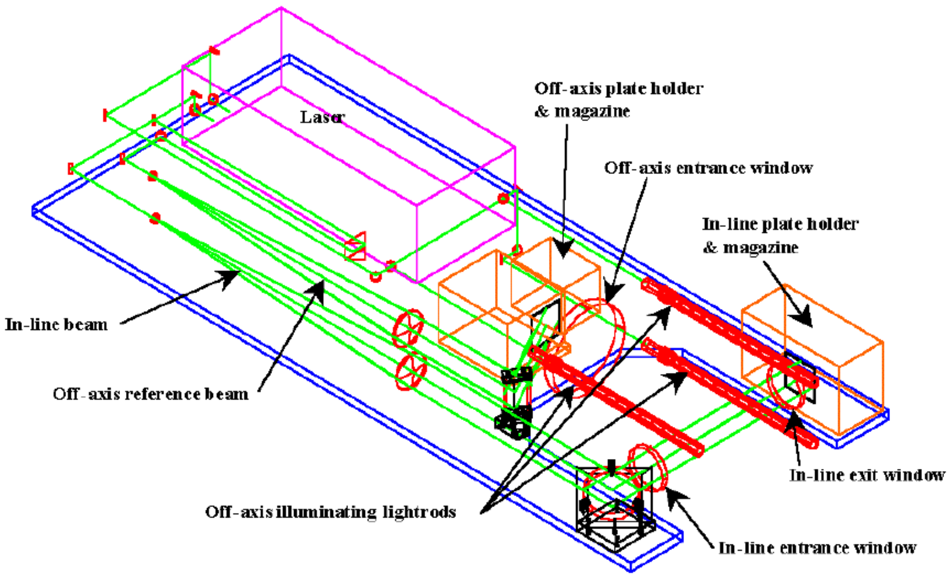
Malkiel, Katz – Johns Hopkins

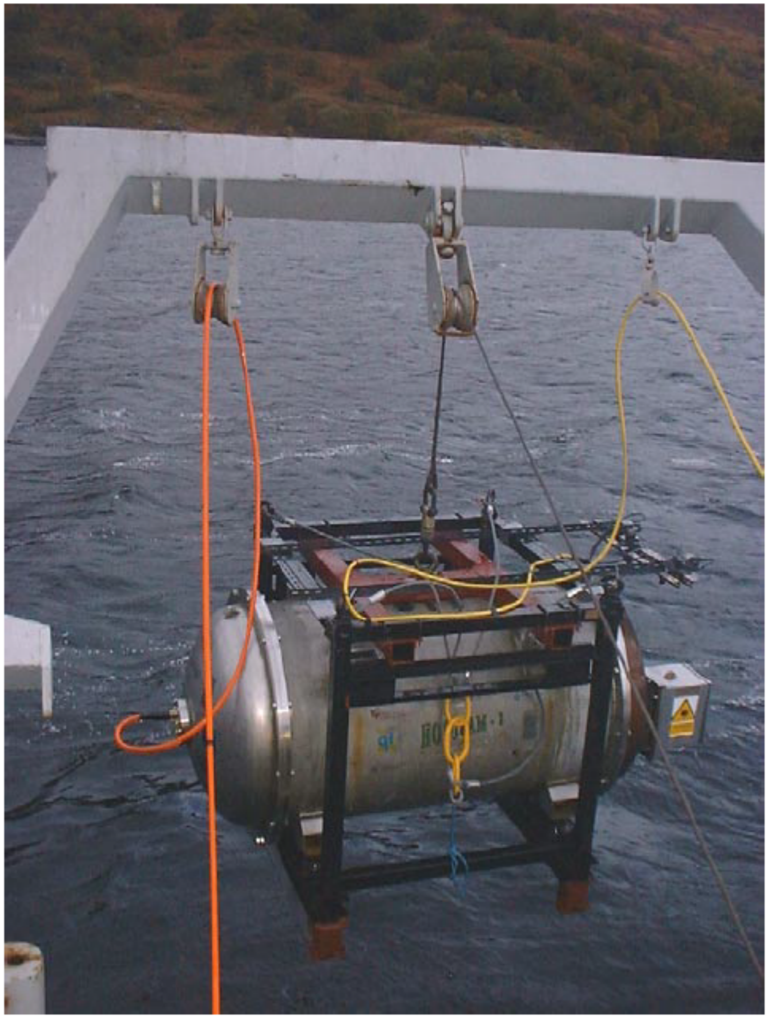
Off-axis configuration



Lindensmith et al. (2016)

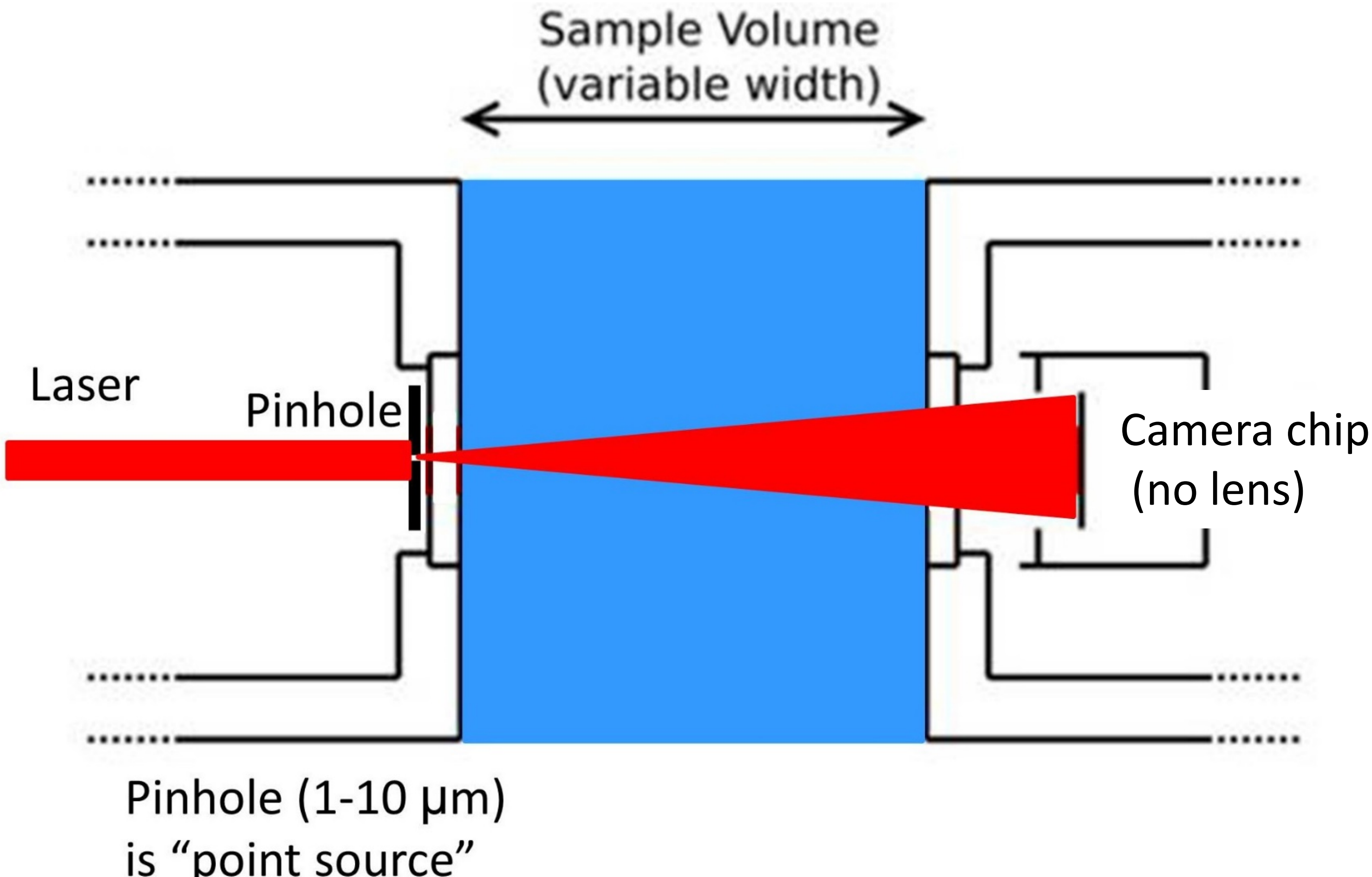
In-line and off-axis combination





Watson et al. (2001)

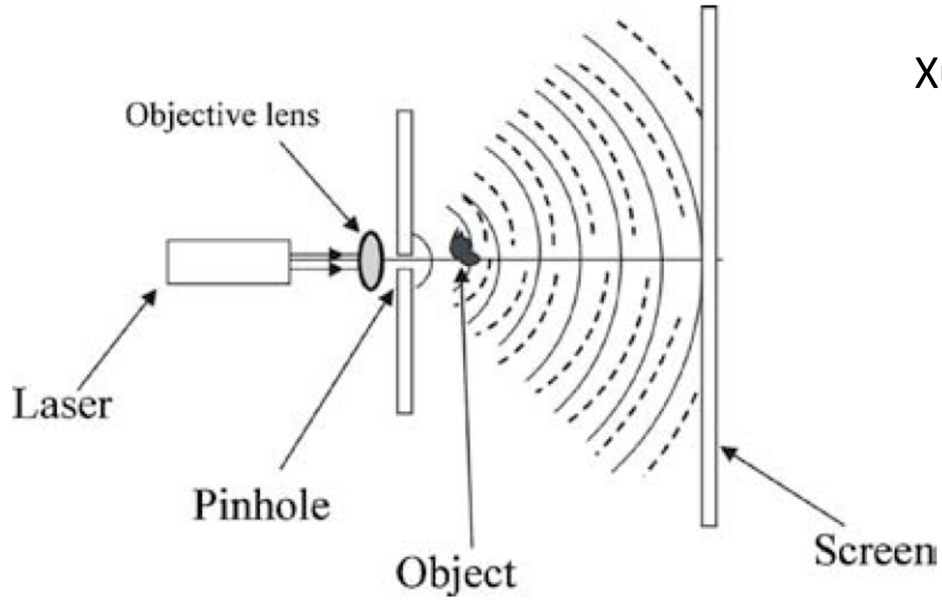
Digital in-line expanding-beam holographic microscopy



SIMPLICITY!

Forward scatter much brighter than side scatter

Principle of expanding beam DIHM



Xu et al. (2001)

- 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)

DIHM reconstruction

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

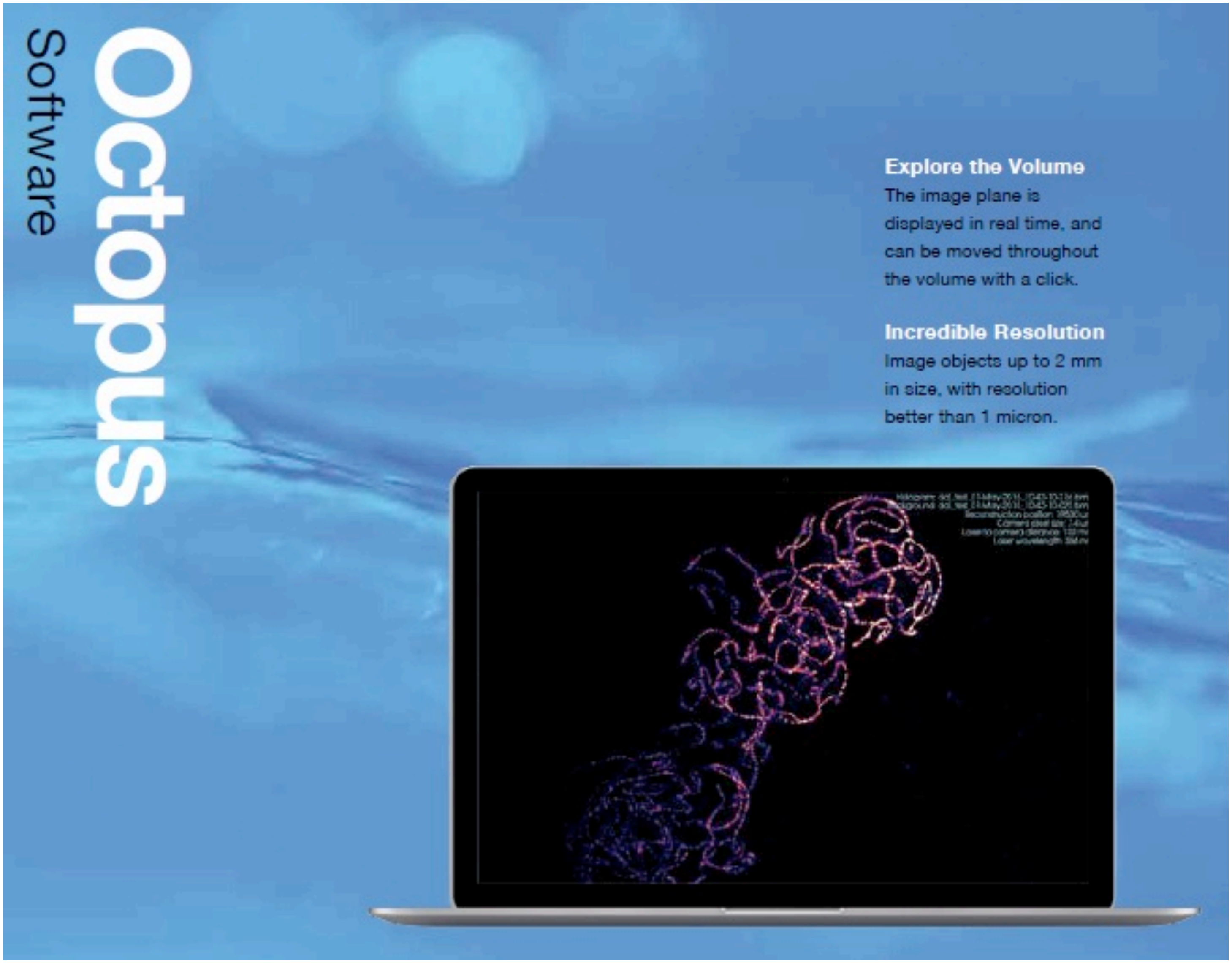
Equation: $$K(\mathrm{r}) = \int_S d^2\xi I(\xi)\exp[2\pi\xi\cdot\mathrm{r}/(\lambda\xi)]$$

|K(r)|…plot of function on a 2-D plane perpendicular to optical axis = "reconstruction" ,

ξ…coordinates (X,Y,L) where L is distance from the point source,

I(ξ)…contrast image of hologram (<u>difference of images with and without object</u>)

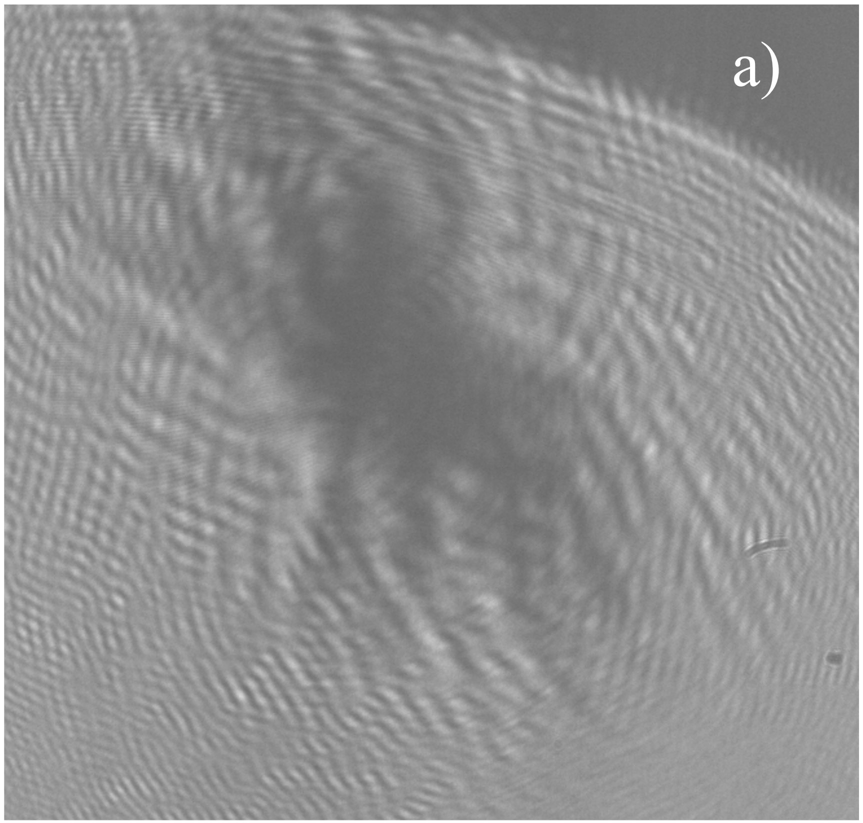
Image reconstruction software



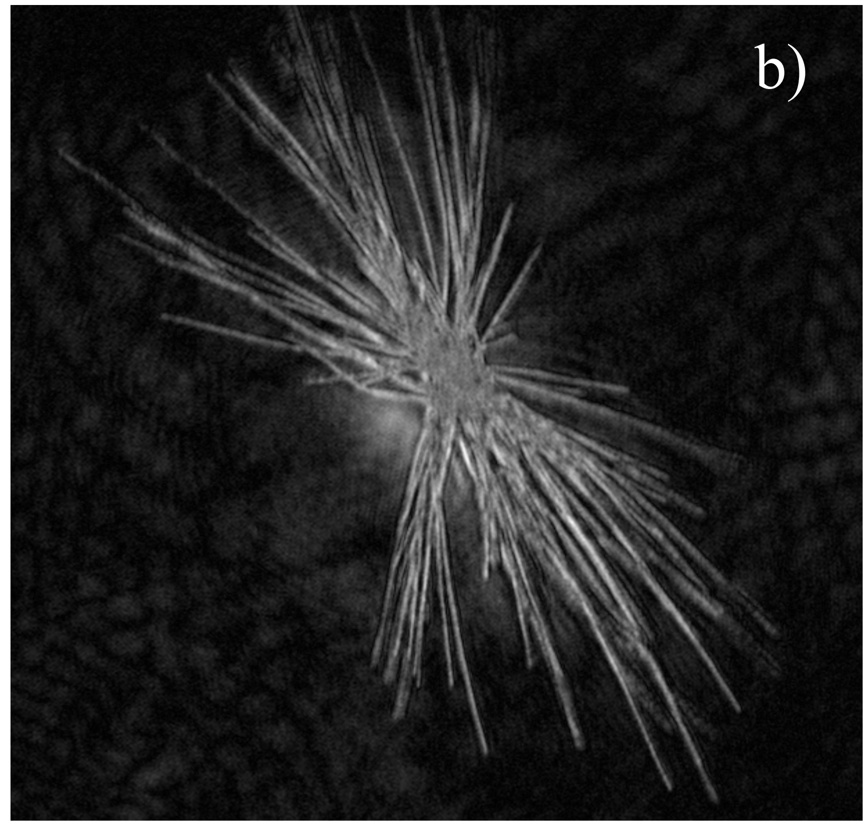
Software: Octopus by 4-Deep, Halifax, Canada

Image reconstruction of *Trichodesmium* sp.

Unreconstructed hologram



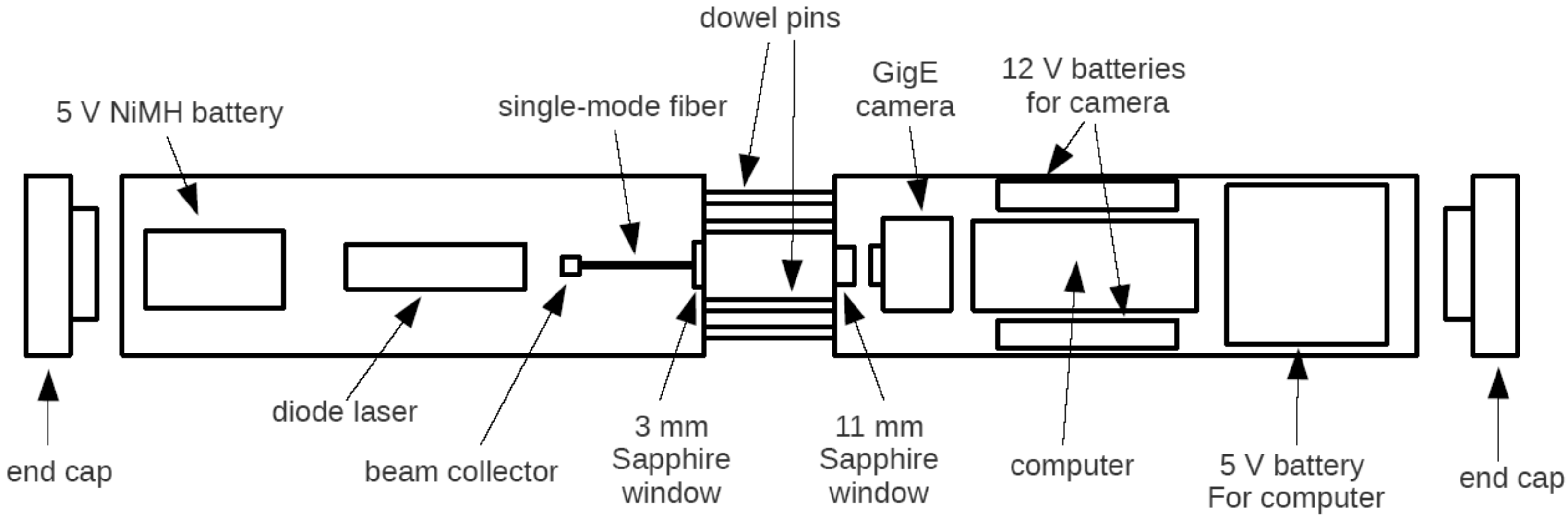
Reconstruction



#15 17Oct2010

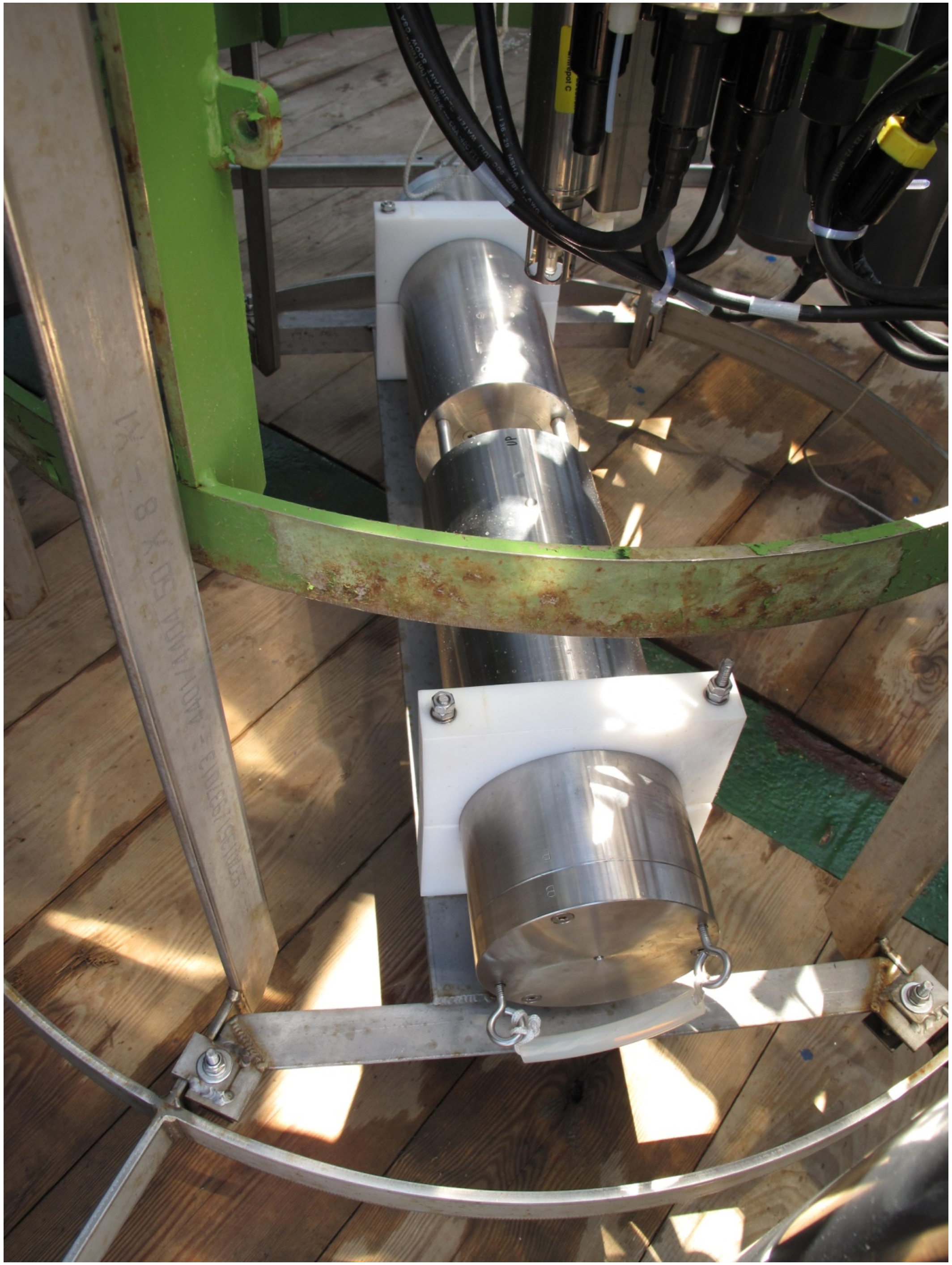
Reconstruction video

DIHM for the deep sea (ODU)
Bochdansky et al. 2013 L&O Methods



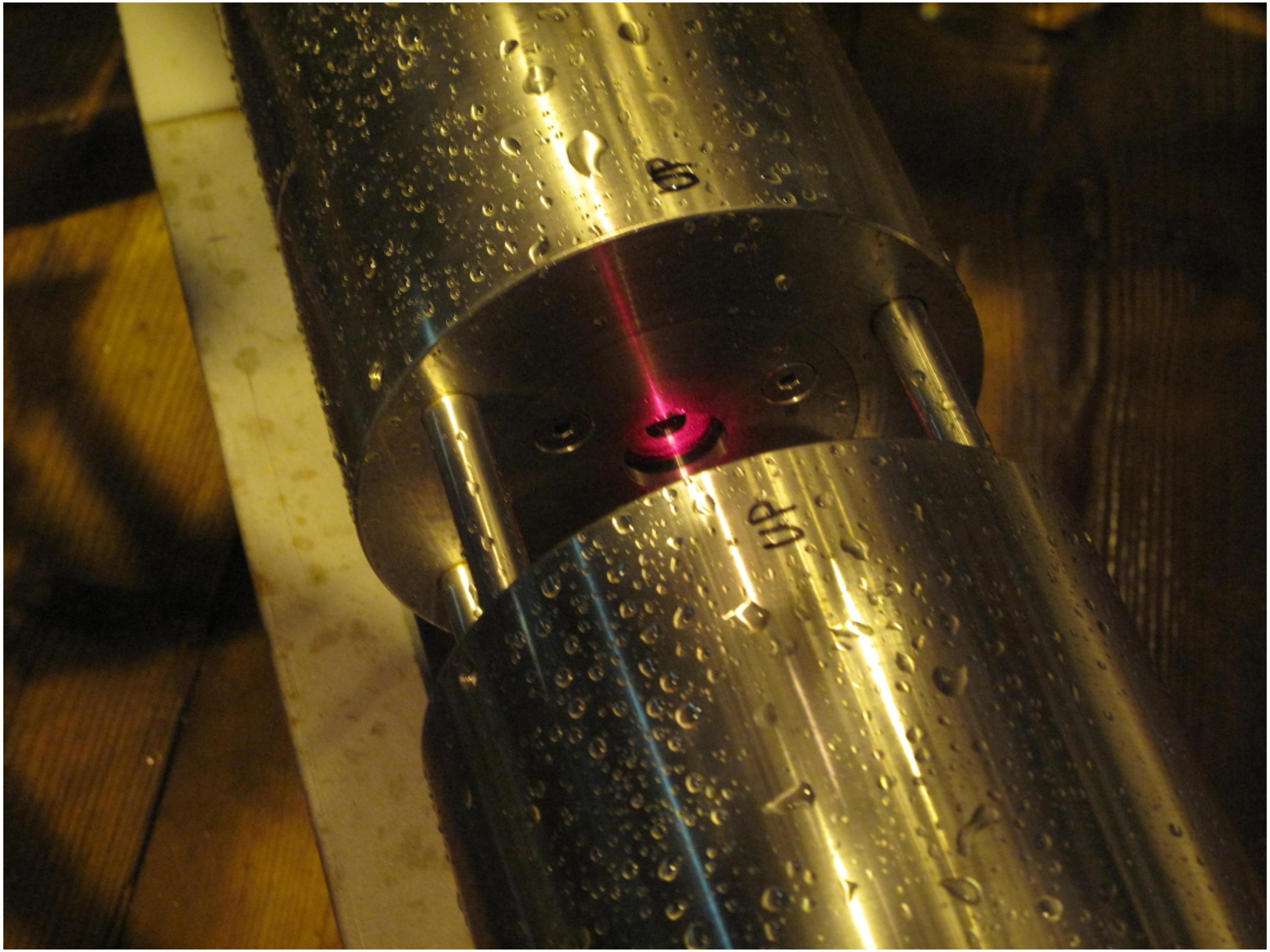
- 6000 m pressure casing (2 cm thick walls, sapphire widows)
- Single mode fiber (9 μm diam.) as point source
- 640 nm Diode laser (red)
- 4 Mpixel GigE Camera with 1/16,000 sec shutter speed,
- 7 frames sec^{-1}
- Embedded computer (small, 750 Gb SATA drive)
- 7 cm gap
- Vertical speed through water 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



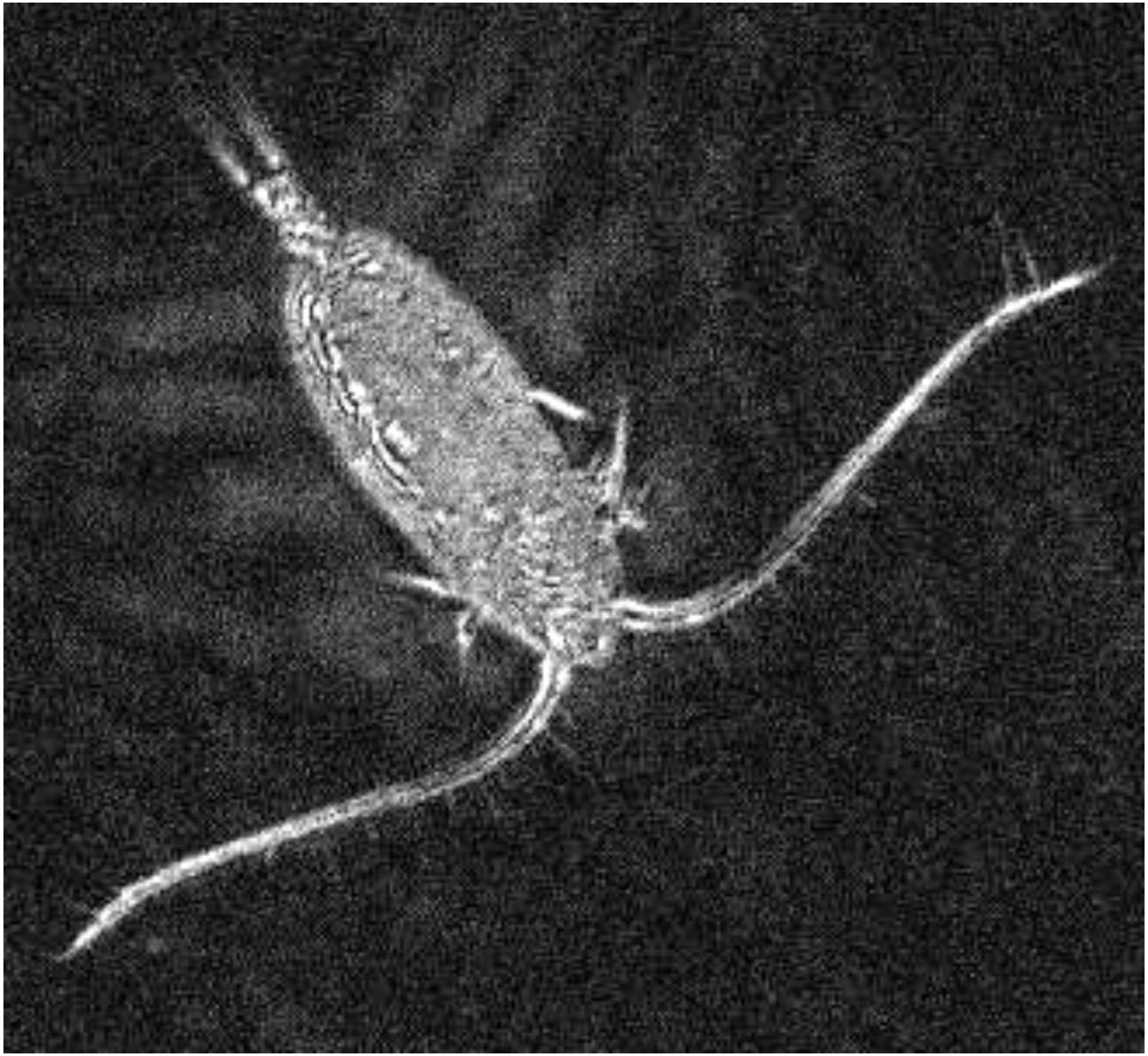
DIHM mounted on CTD rosette

Length: 1.04 m
Diameter: 15 cm
Weight: 70 kg



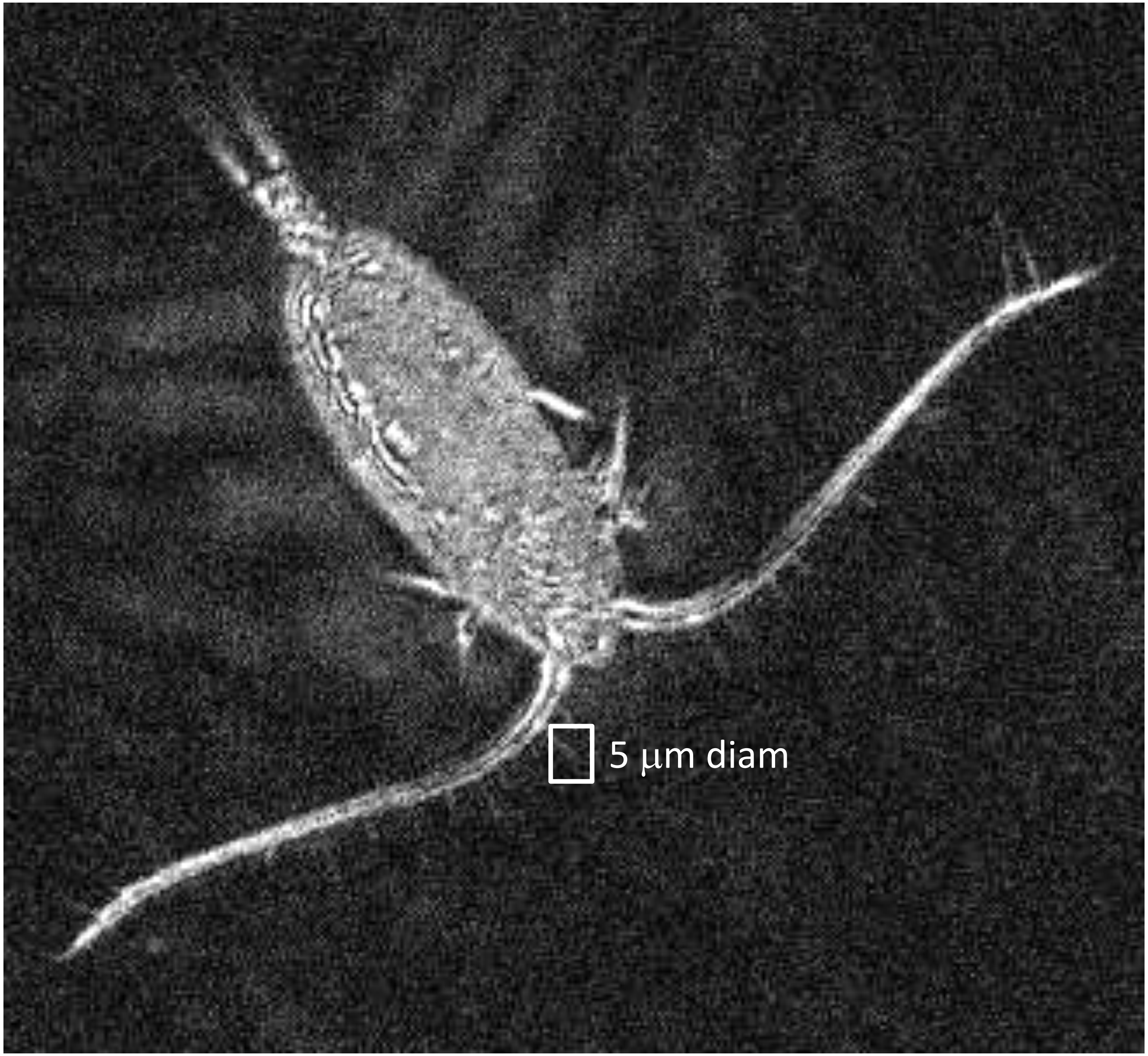
ca. 7 cm gap

Metazoan plankton: copepods



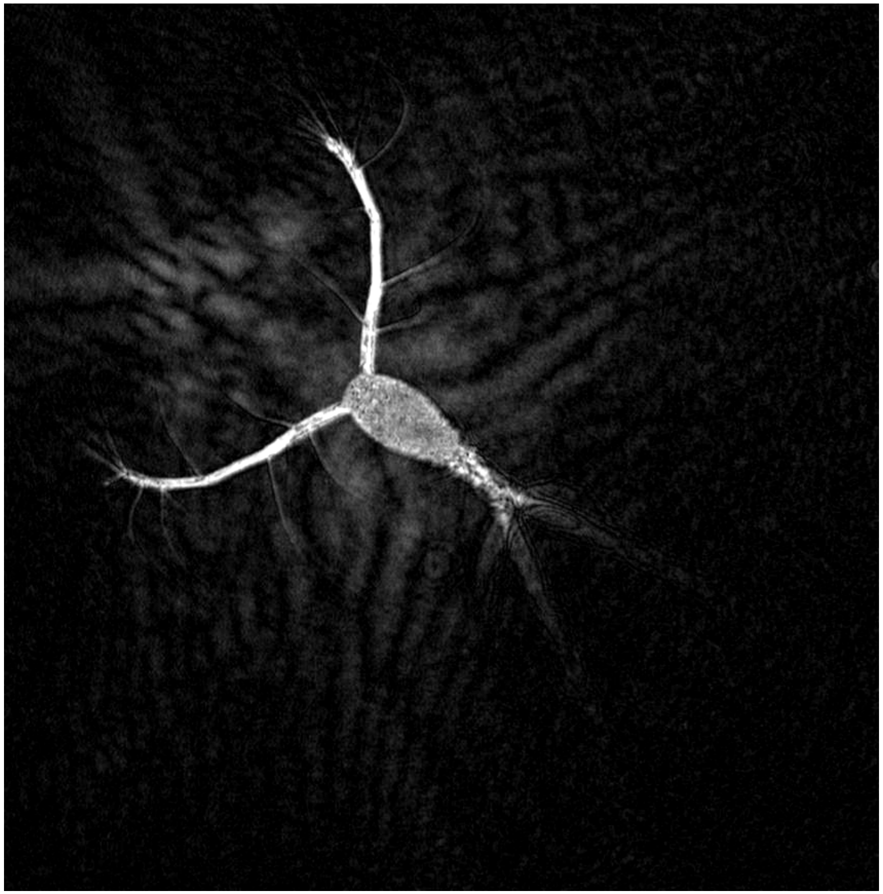
12Oct 2011 #22

Metazoan plankton: copepods



12Oct 2011 #22

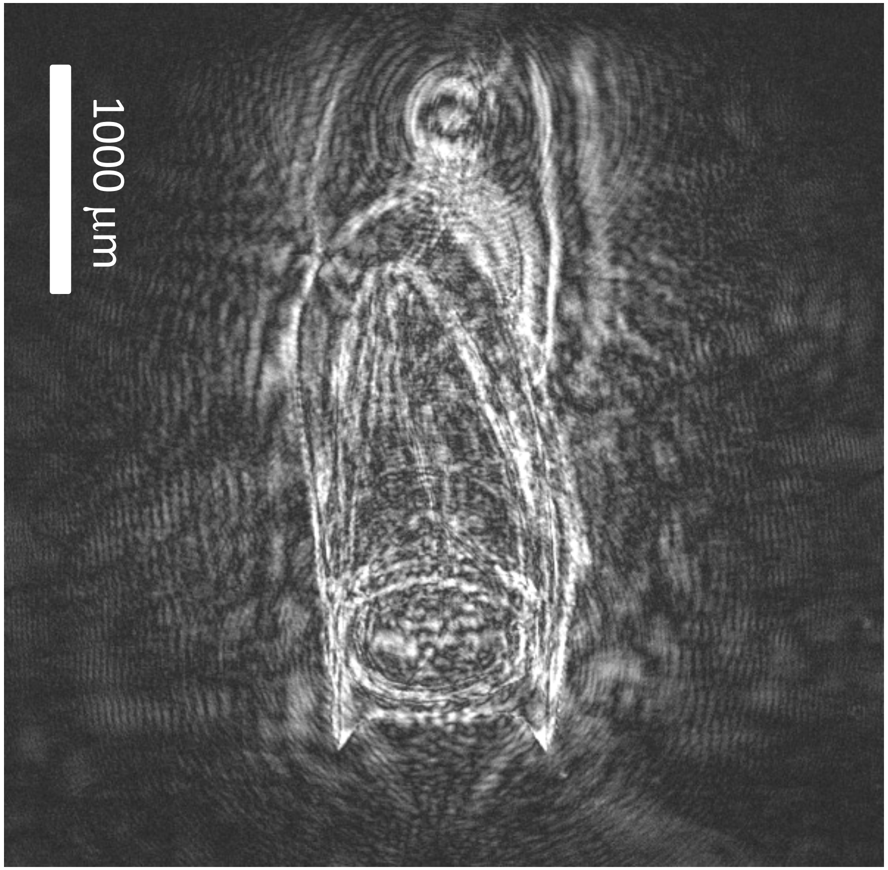
Metazoan plankton: copepods



20Oct2010 #18

20Oct 2011 #63

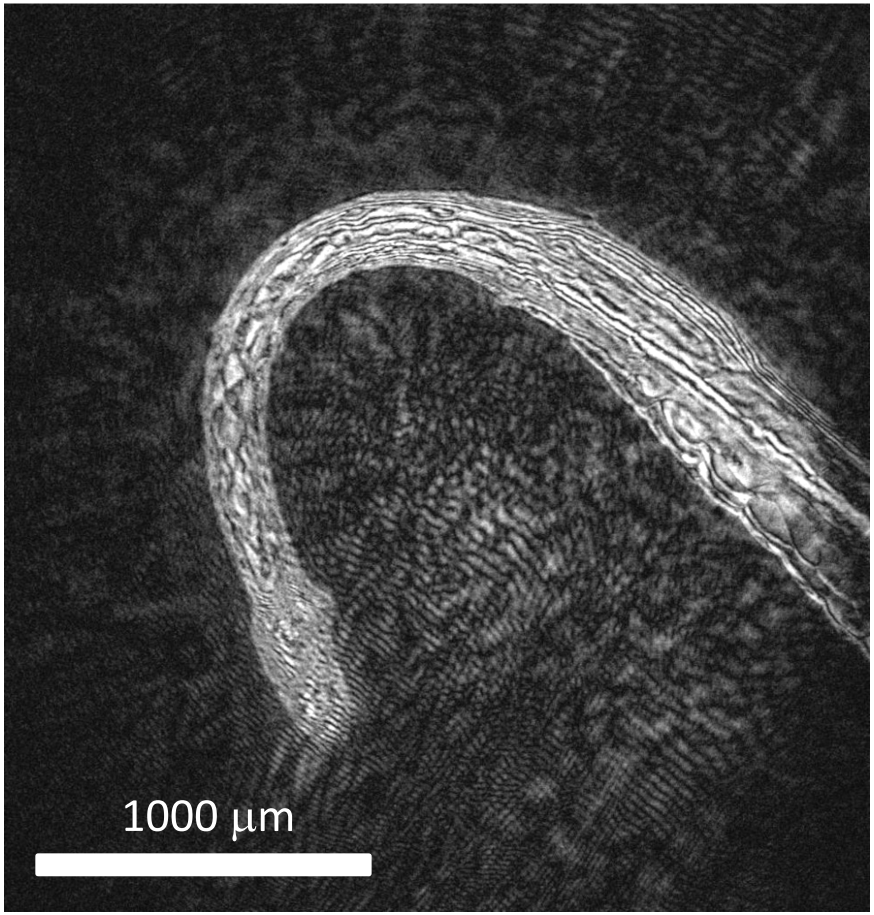
Comparison with light microscopy
Metazoan plankton: Siphonophores (Hydrozoa, Cnidaria)



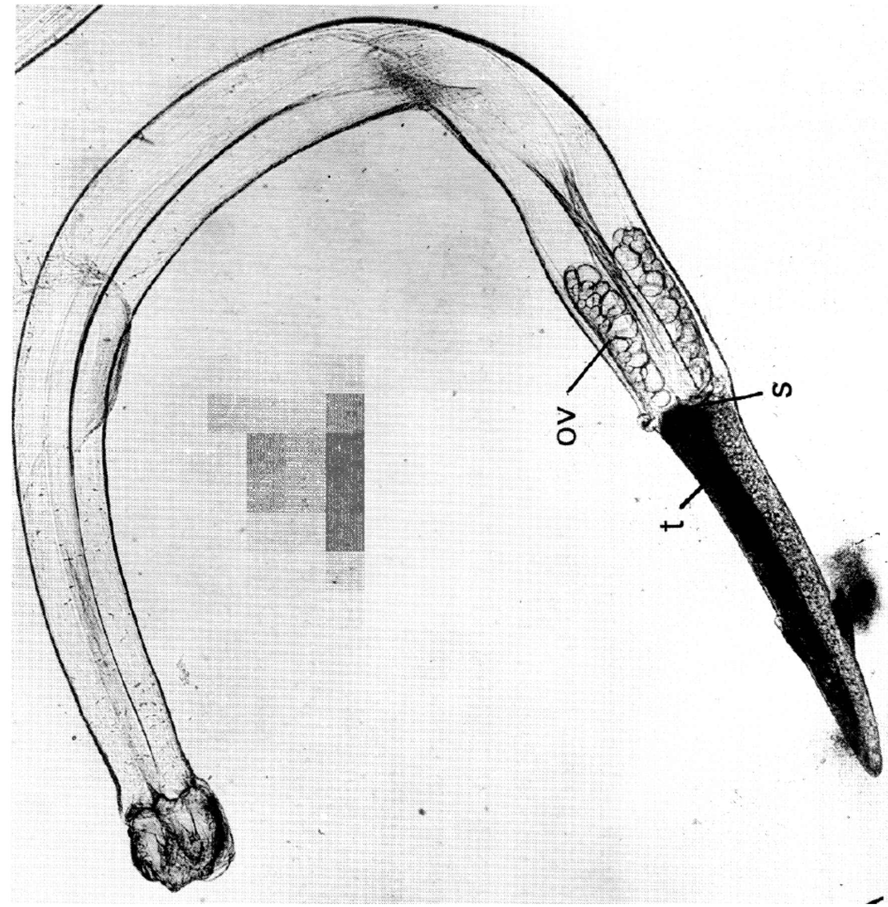
20Oct2010 #66

http://www.obs-vlfr.fr/Mam/images/missions/pages/Stareso-siphonophore-3cm.htm

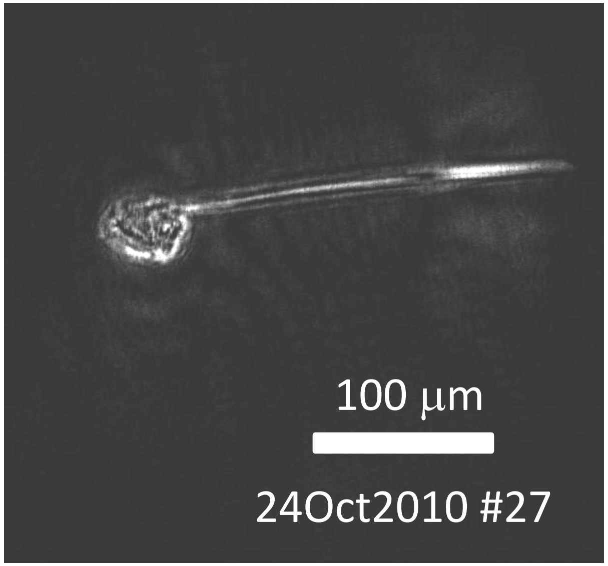
Metazoan plankton: *Sagitta* sp. (Chaetognatha)

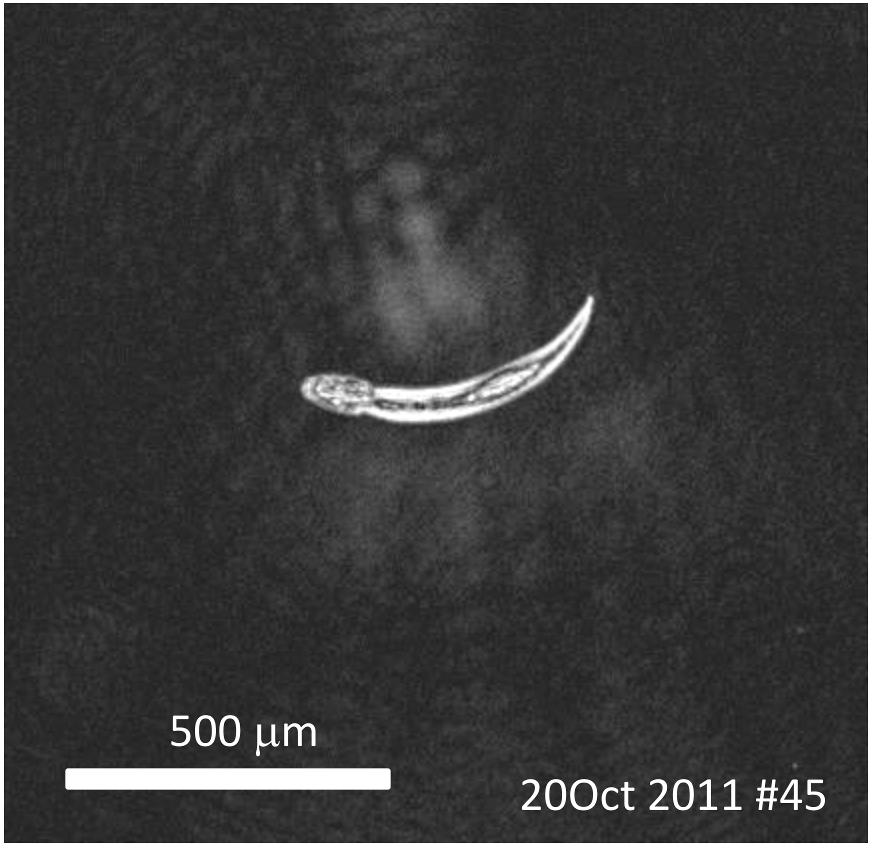


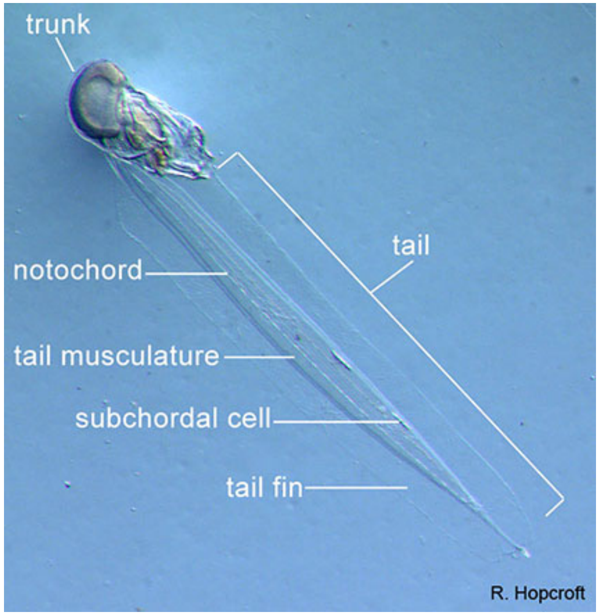
21Oct 2011 #39



Metazoan plankton: Appendicularia (Tunicata, Chordata)





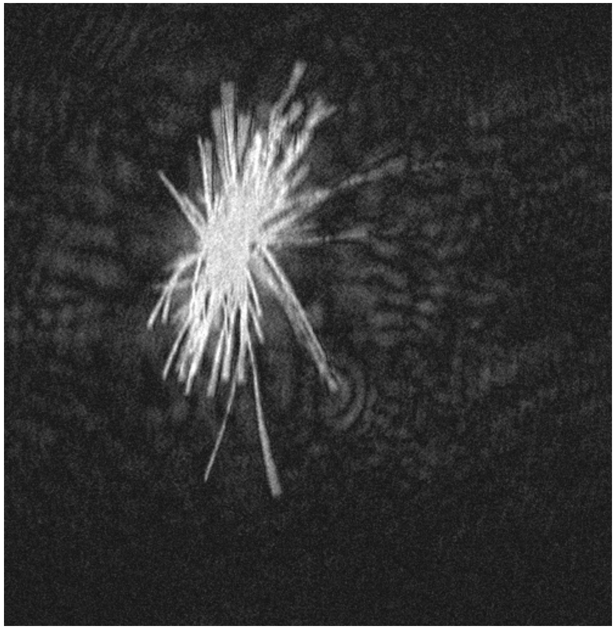


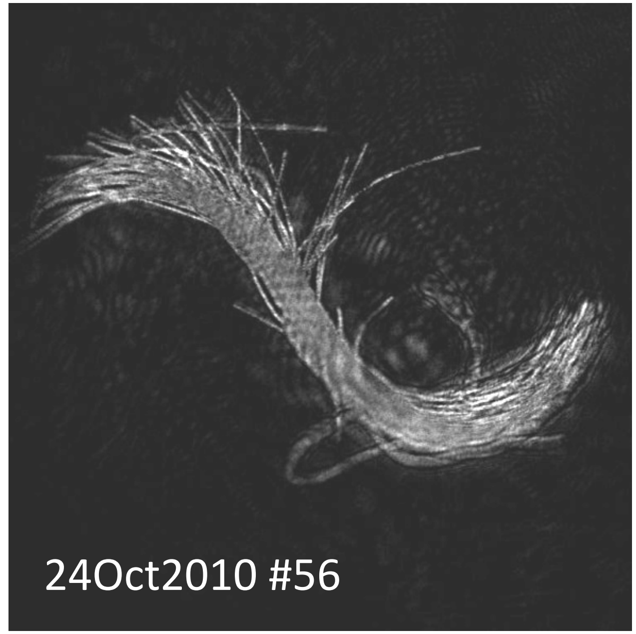
Appendicularia (Larvacea)

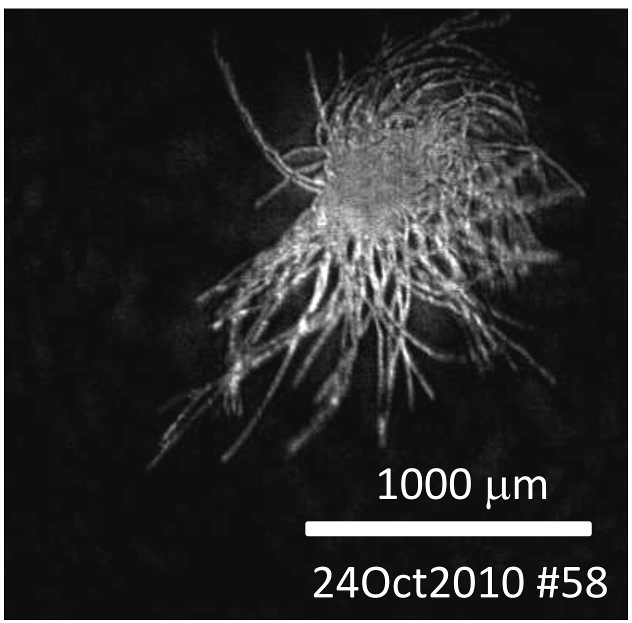
Ross Sea

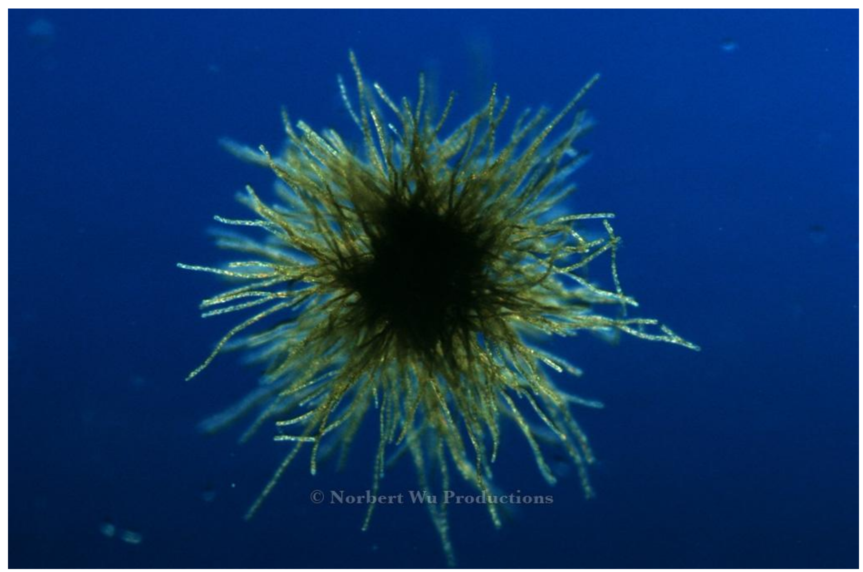
Newfoundland

Trichodesmium sp. colonies (tufts and puffs) (Cyanobacteria)



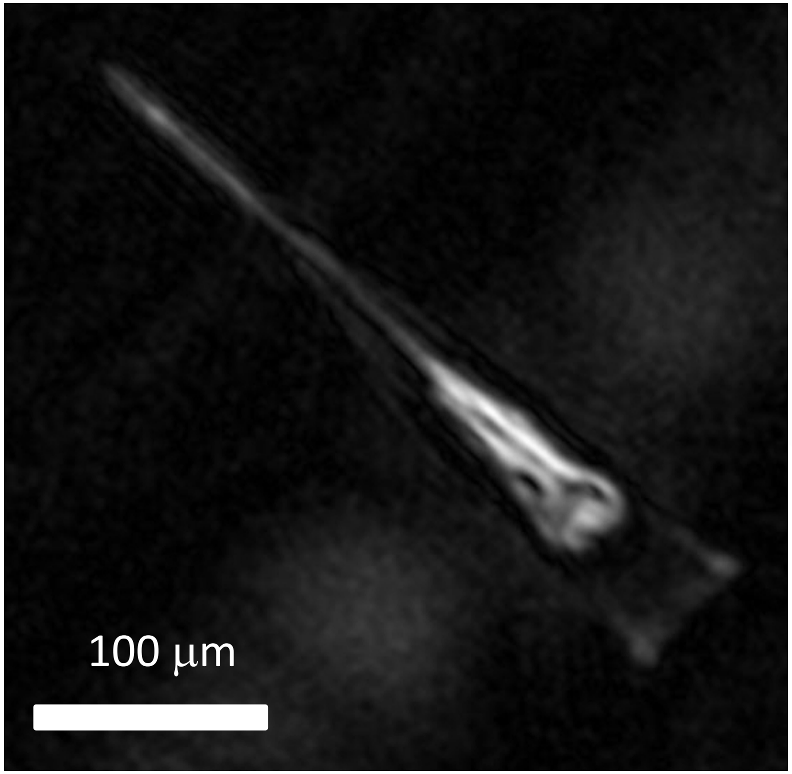


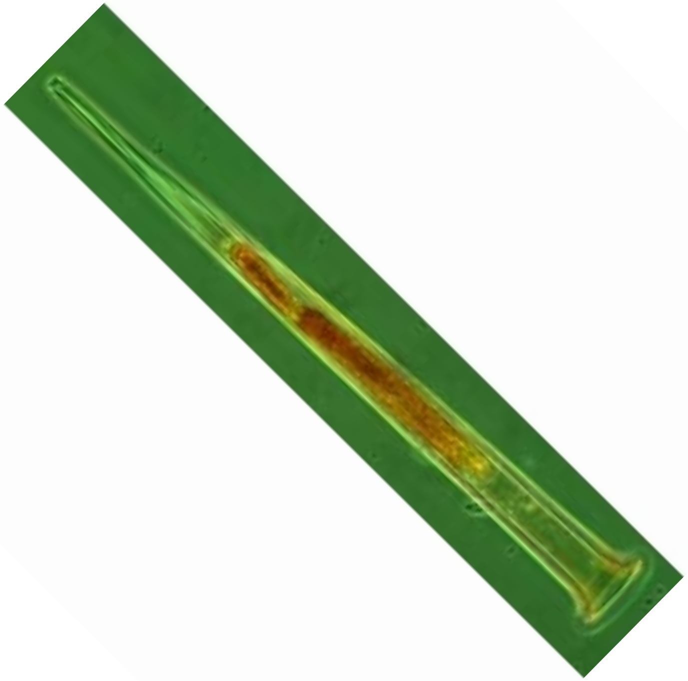




http://www.norbertwu.com/lightbox/index/module/media/category/gallery|PPK/start/72

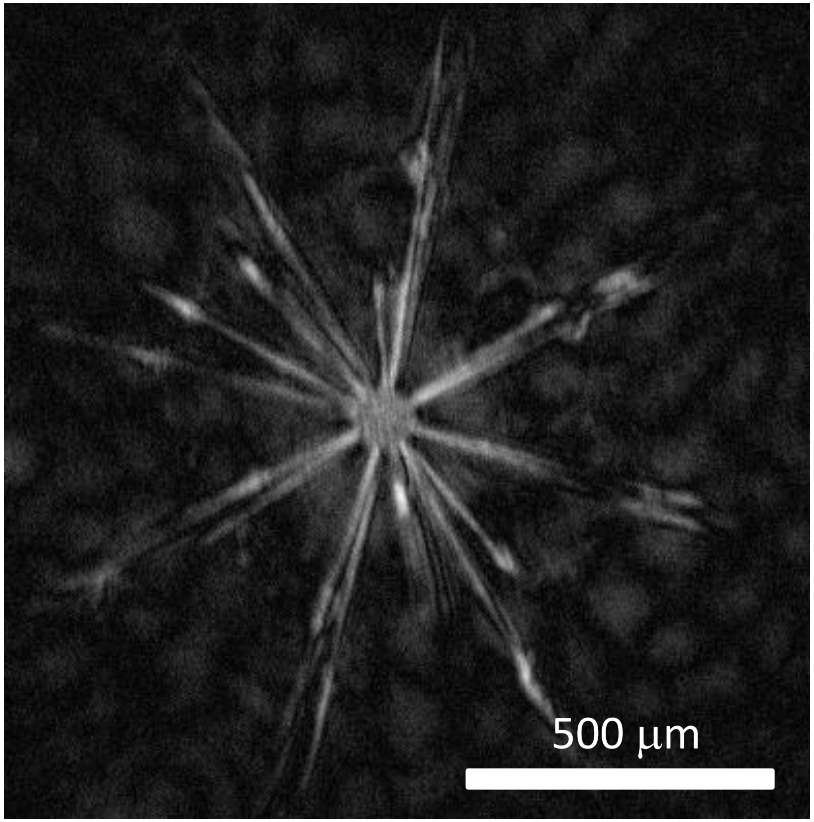
Protists: Tintinnida (ciliate in lorica)

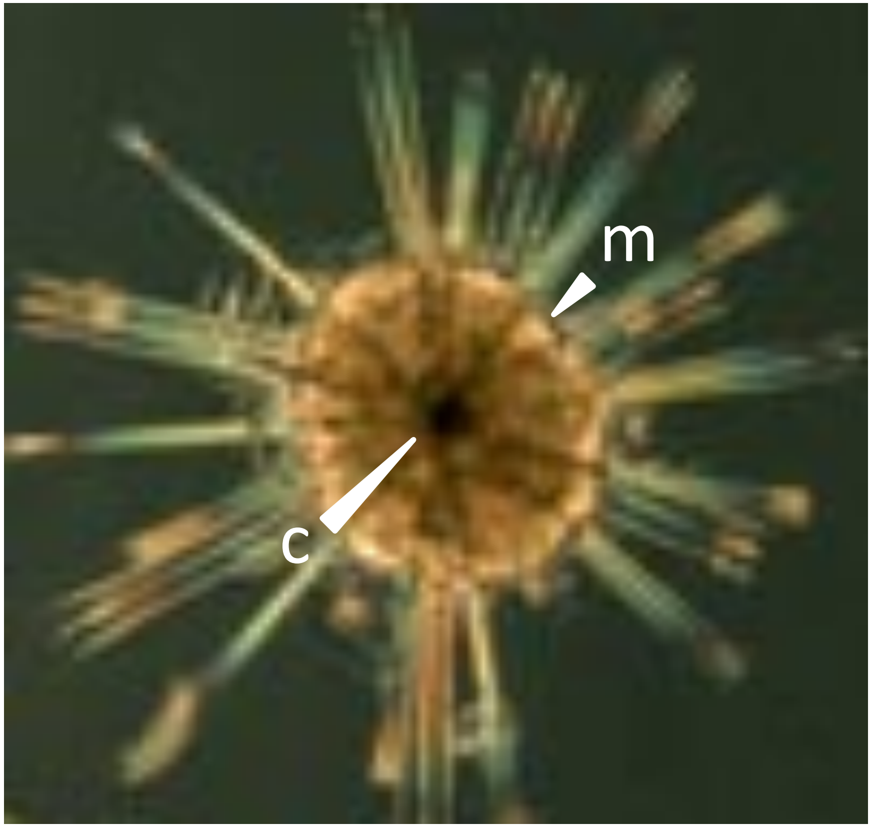




www.micro*scope.com (MBL)
Phase contrast image

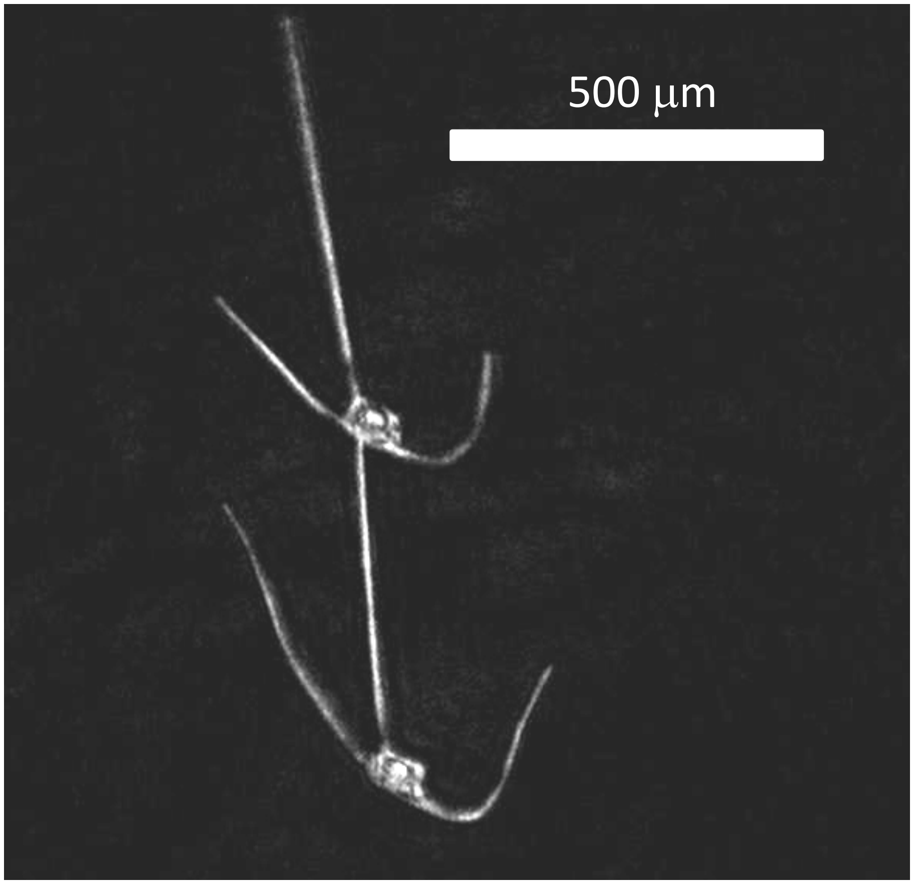
Acantharia





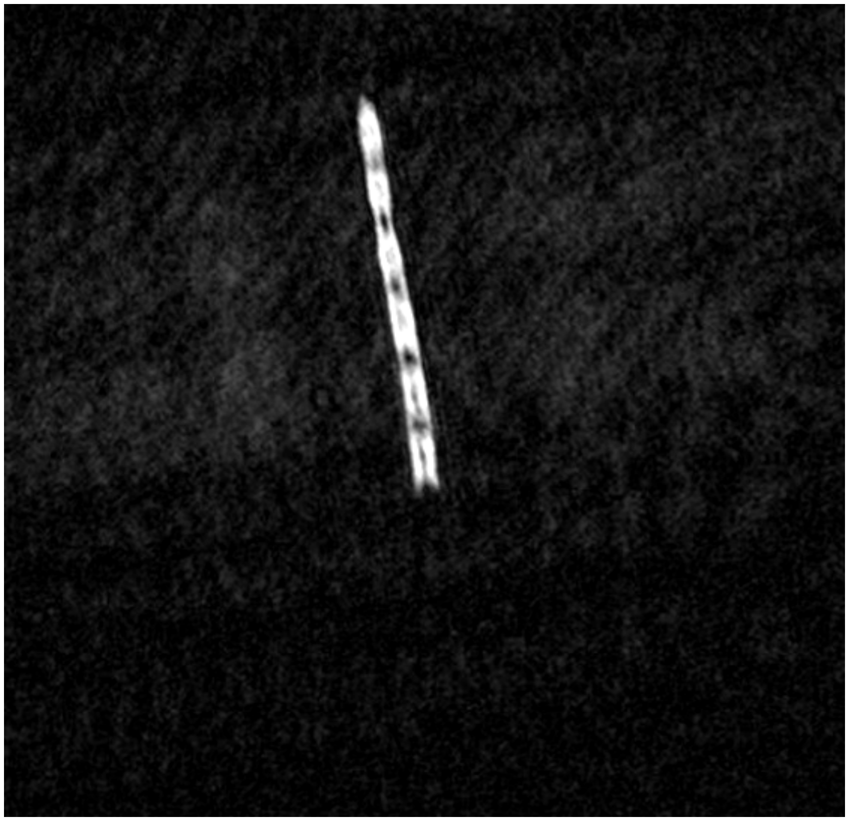
www.micro*scope.com (MBL)
Phase contrast image

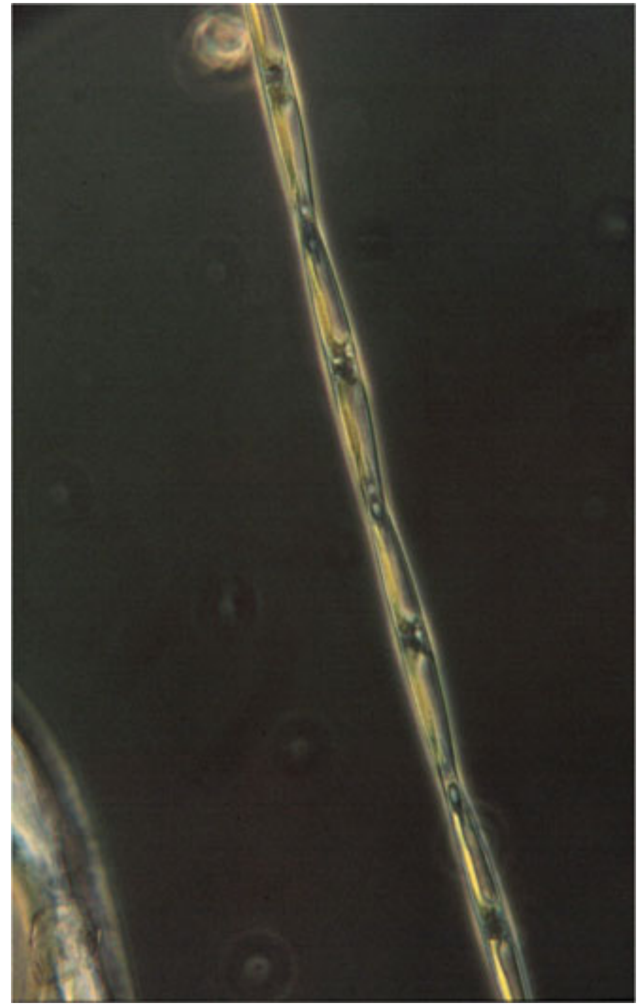
Protists: *Ceratium* sp. (2 individuals) (Dinoflagellata)



28Oct2010 #8

Protists: chain-forming diatoms

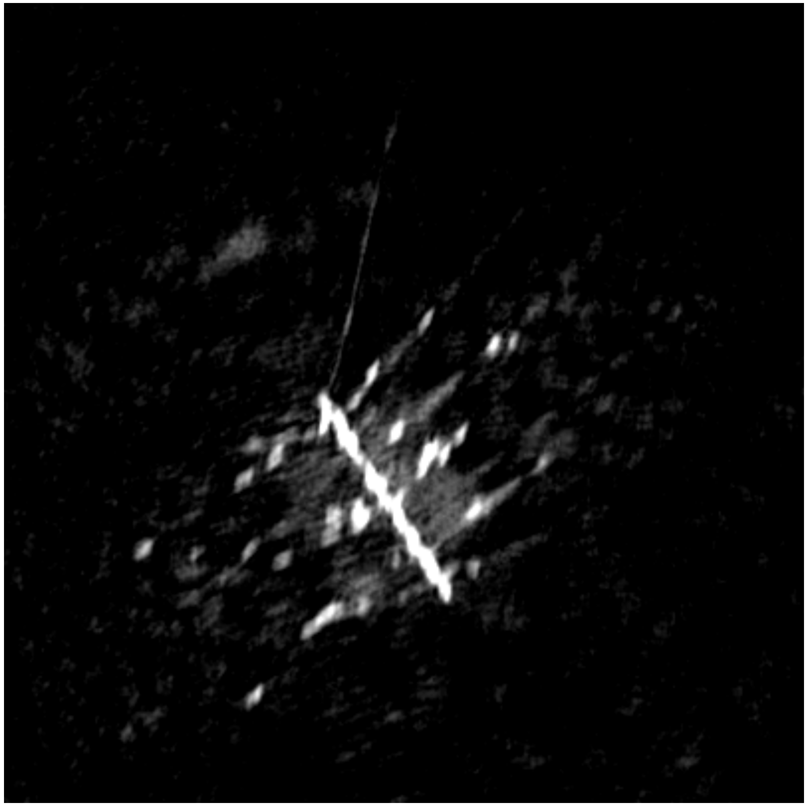




Pseudonitzschia sp.

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

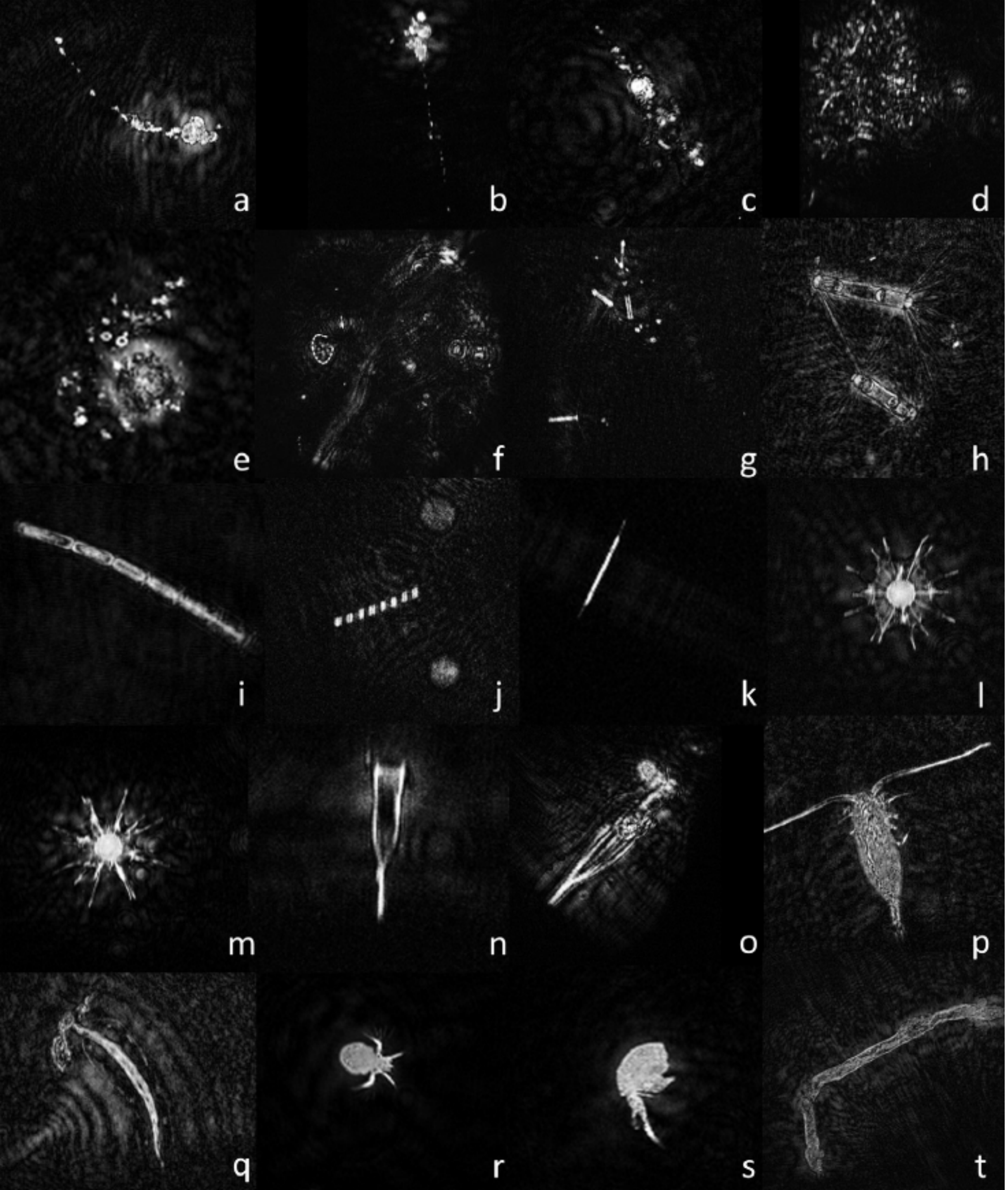
Diatom chain with vorticellid ciliate parasites



23Oct2010 #48

http://www.odec.ca/projects/2007/thom7h2/typesofphyto.htm

Ross Sea



Bochdansky, Clouse, Hansell (2017) J Mar Syst (Ross Sea Special Issue)

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

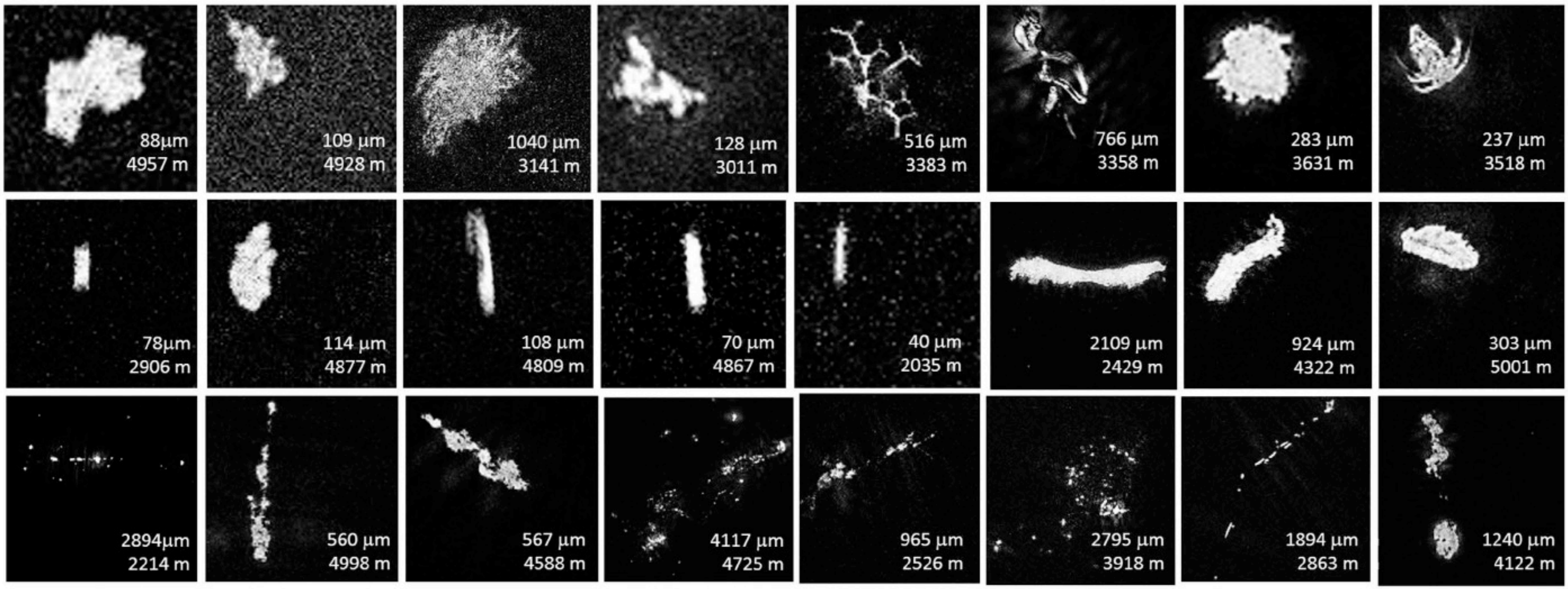


Figure 1. Examples of three categories of particles: marine snow (bottom row), faecal pellets (centre row) and "others" (top row). The "others" category includes all recognizable planktonic organisms (alive and carcasses) and optically dense debris that does not classify as marine snow or faecal pellets.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

Bochdansky et al. 2016 Sci Rep

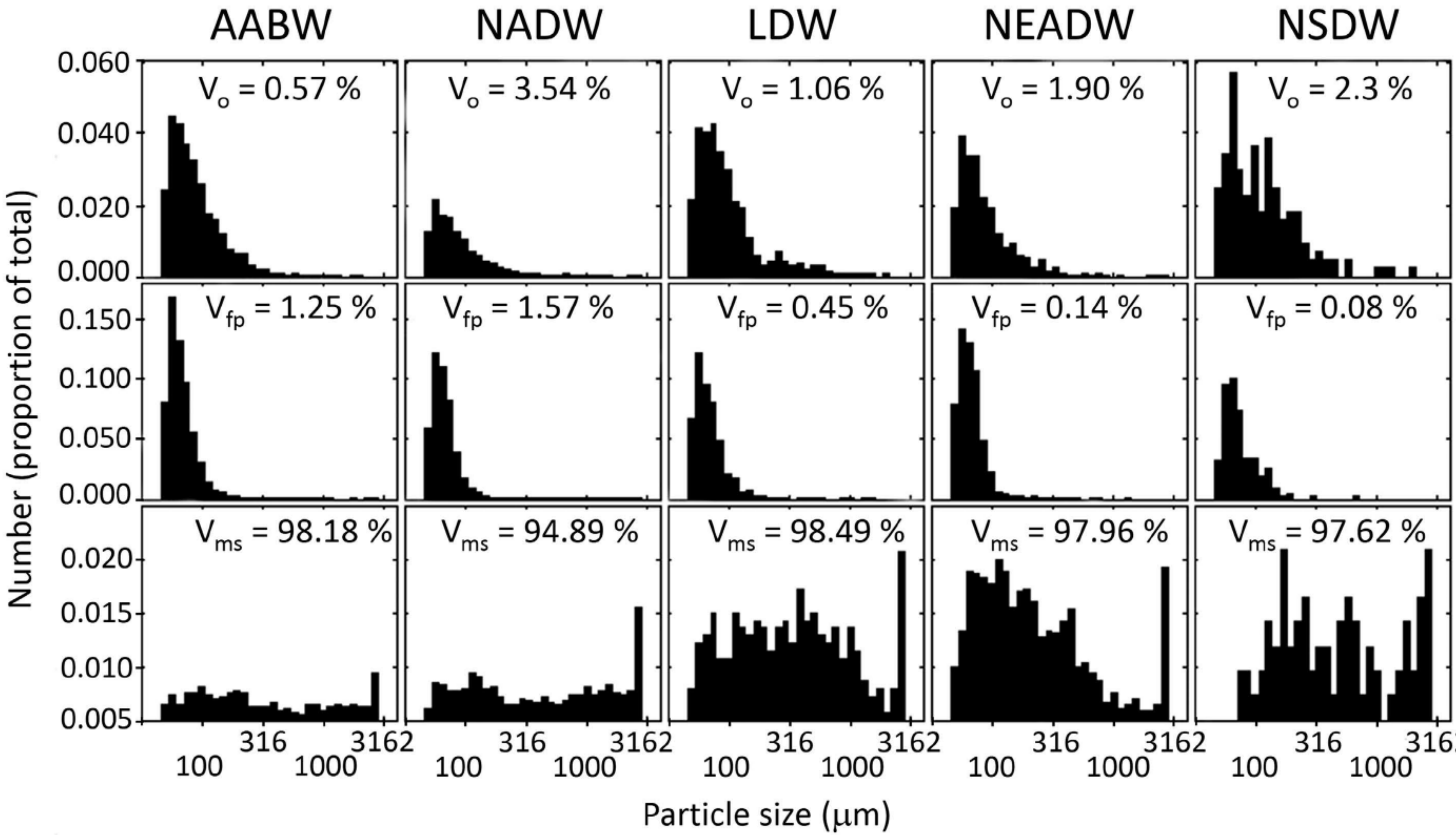
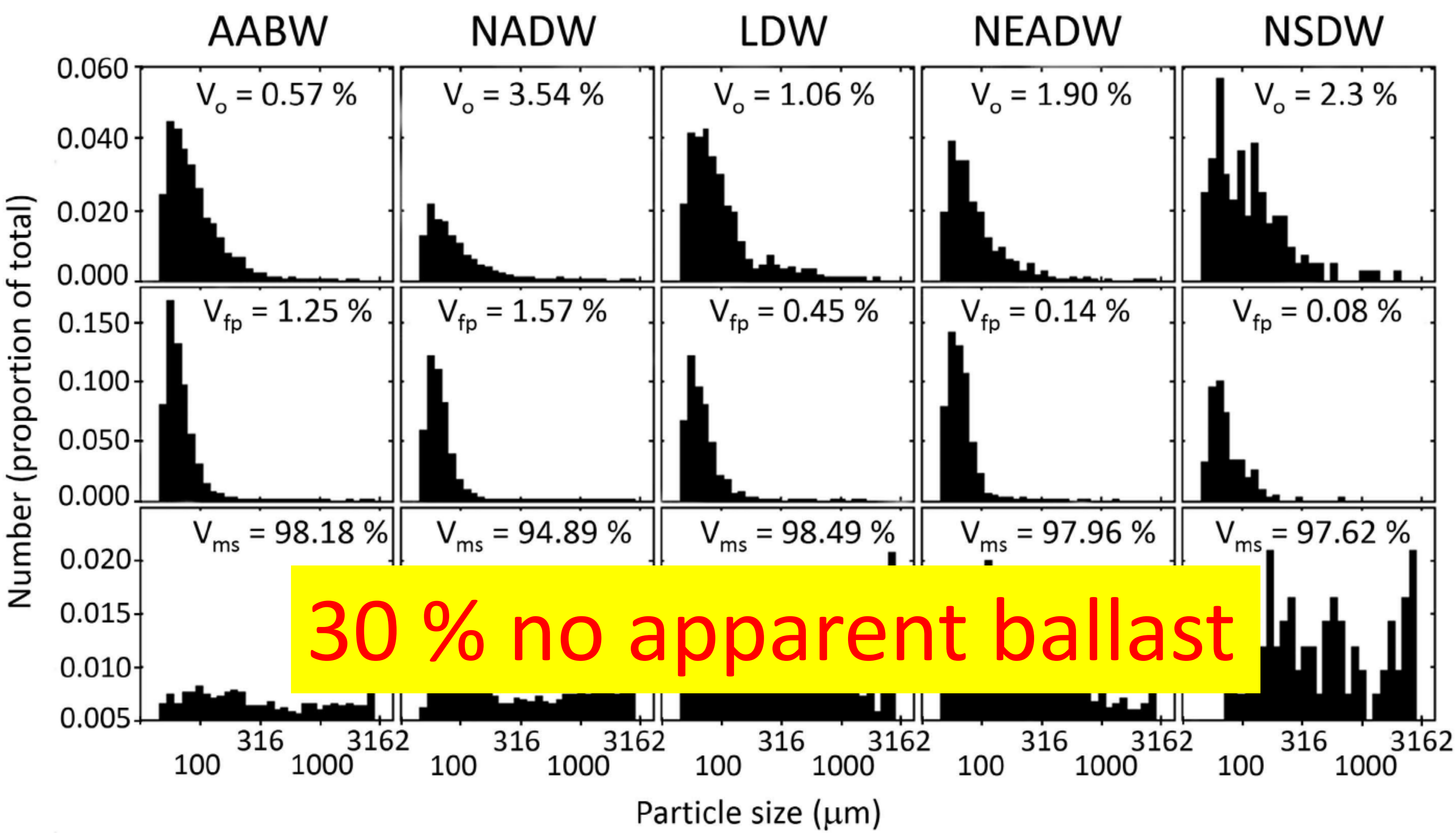


Figure 2. Size frequency distribution of three categories of particles in five different water masses: marine snow (bottom row), faecal pellets (centre row), and other particles including planktonic organisms (top row). AABW: Antarctic Bottom Water (n = 6,213), NADW: North Atlantic Deep Water (n = 13,824), 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)[47]. 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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DIHM

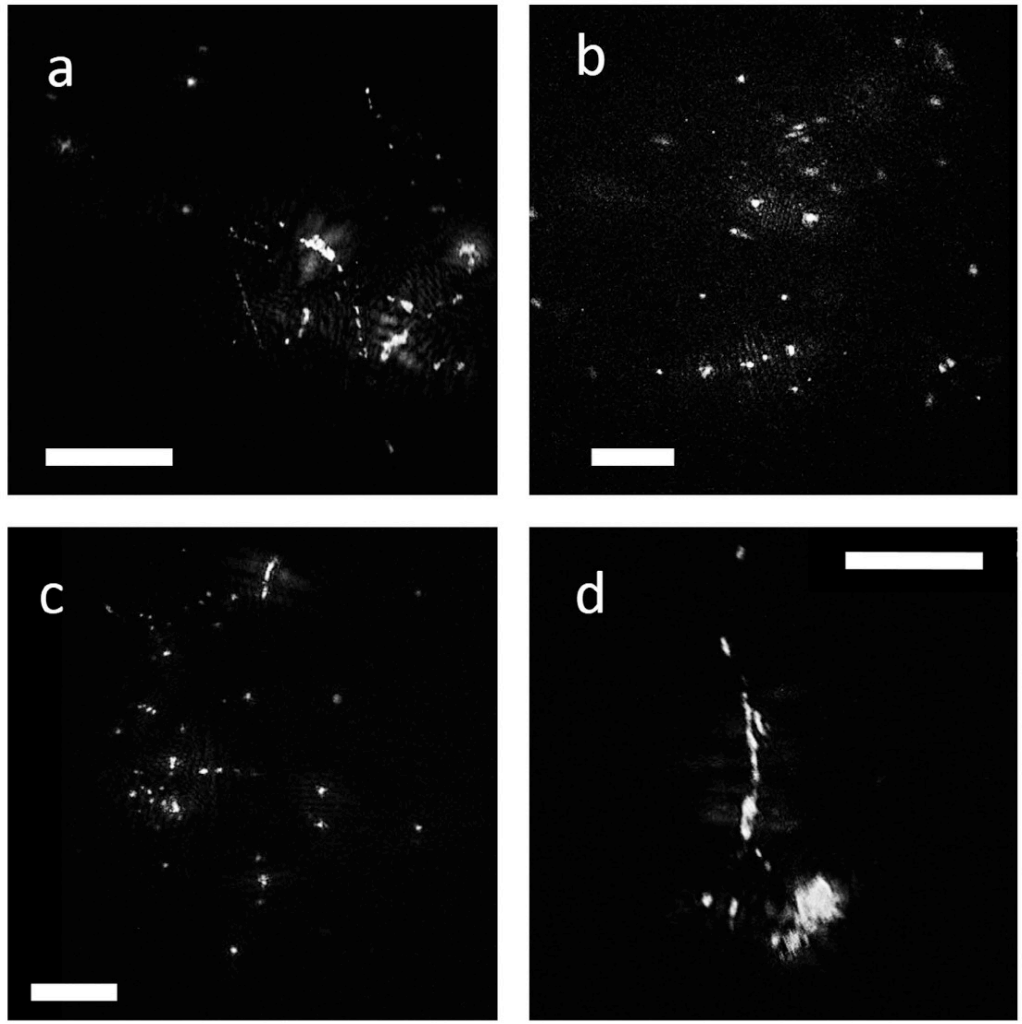
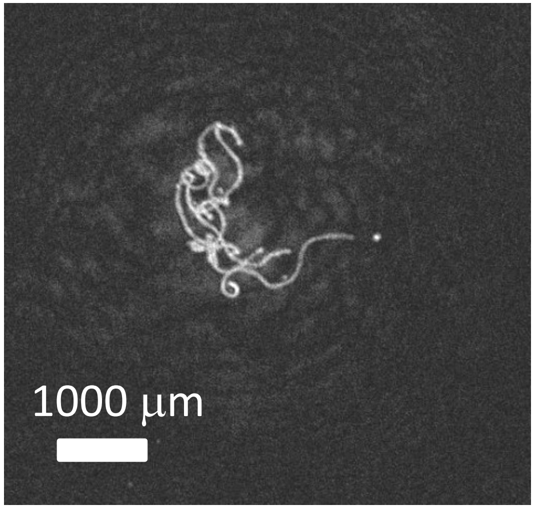


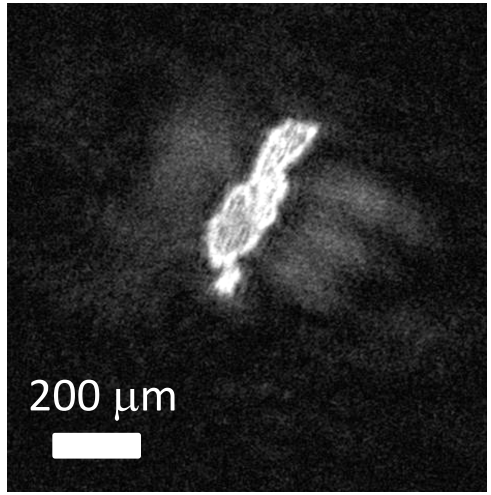
Figure 4. Examples of dragon-king particles with little apparent ballast (a–c) and a ballasted stringer-type particle (d). Particles are held together by a large amount of transparent exopolymers. White scale bars = 1 mm.

Bochdansky et. al. 2016 Sci Rep

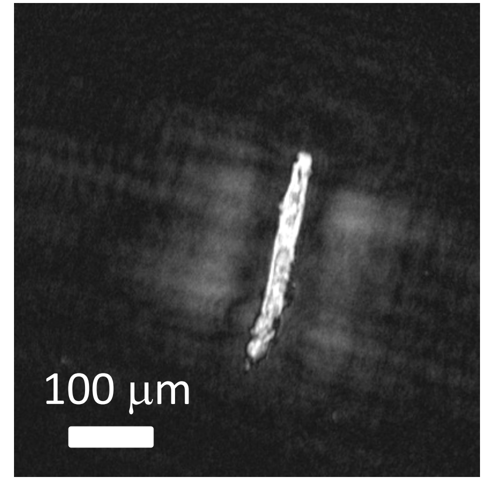
Fecal pellets and fecal strings



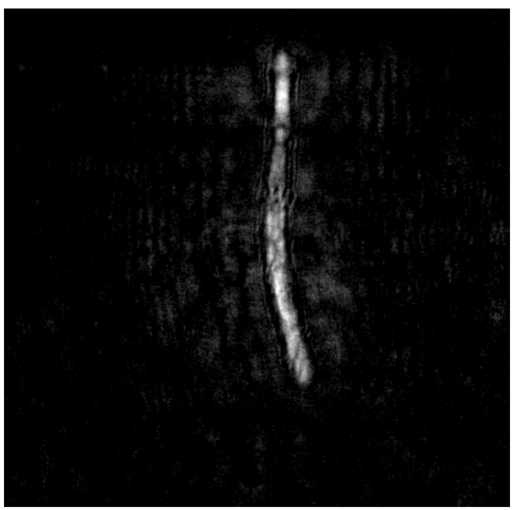
21Oct 2010 #27



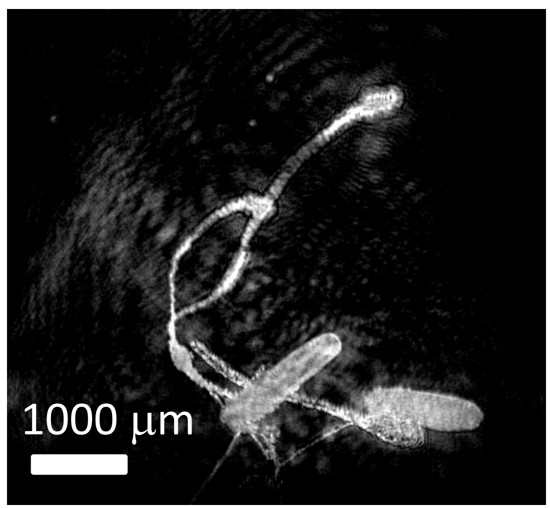
21Oct 2010 #50



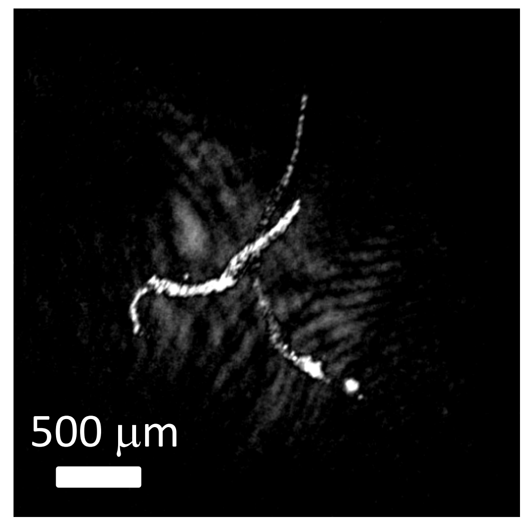
23Oct 2010 #18



20Oct 2010 #71

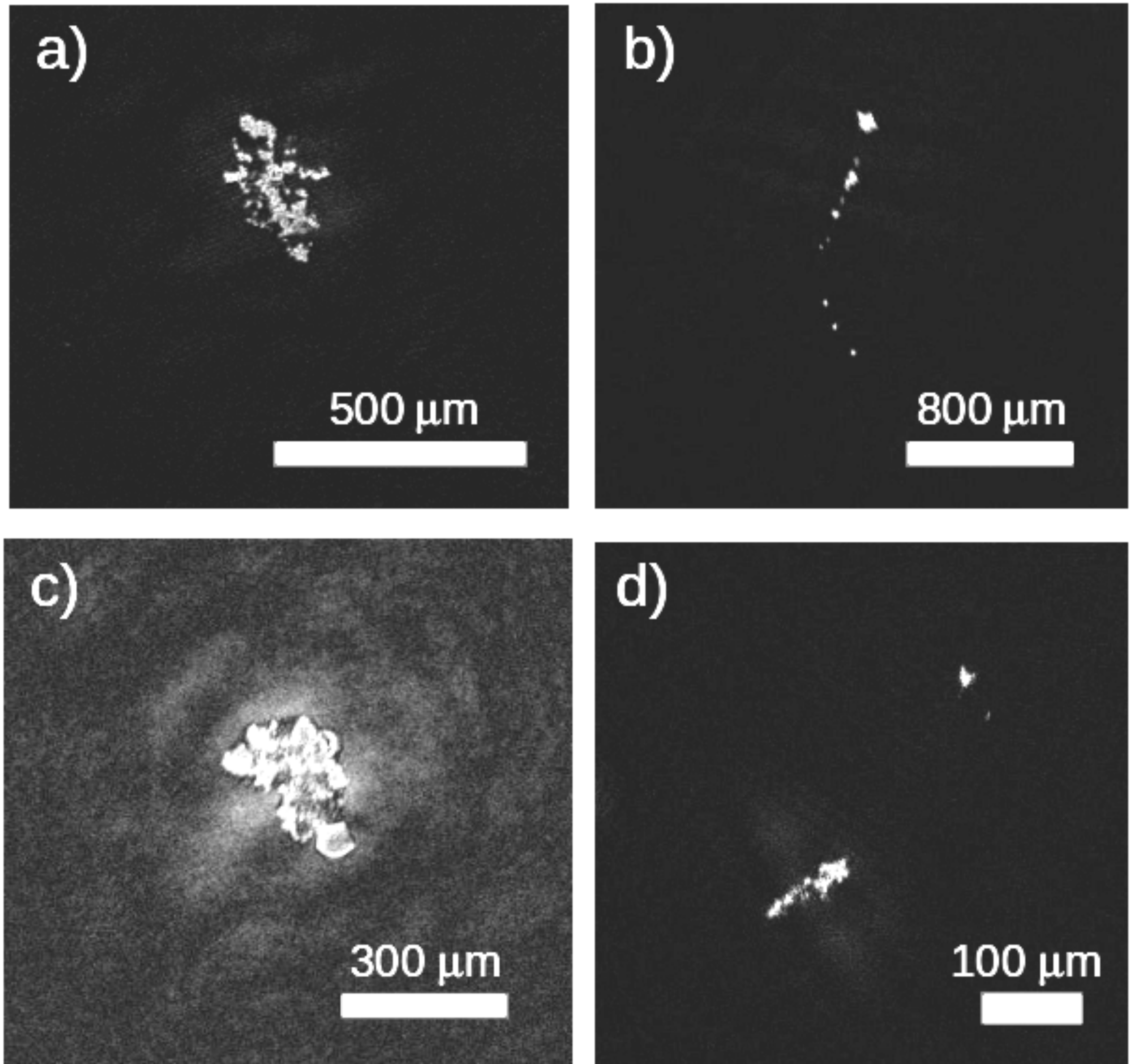


20Oct 2010 #75

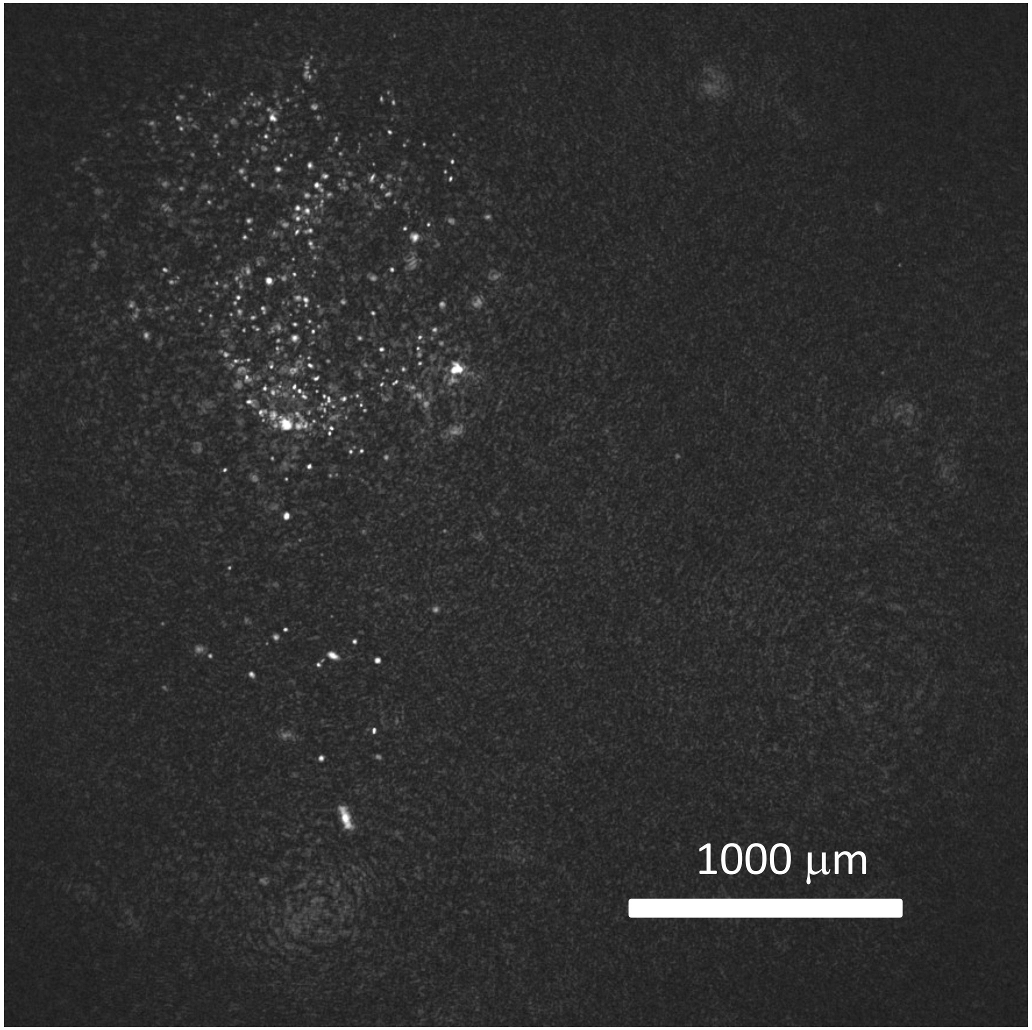


20Oct 2010 #52

Marine snow: mineral particles (a,c), stringer (b), ballast (d)

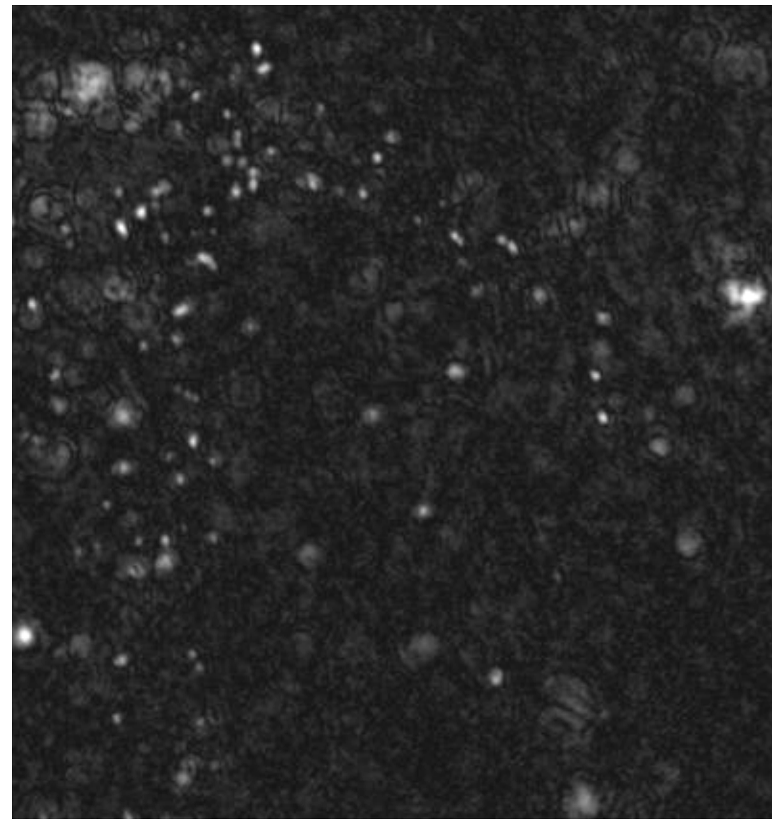


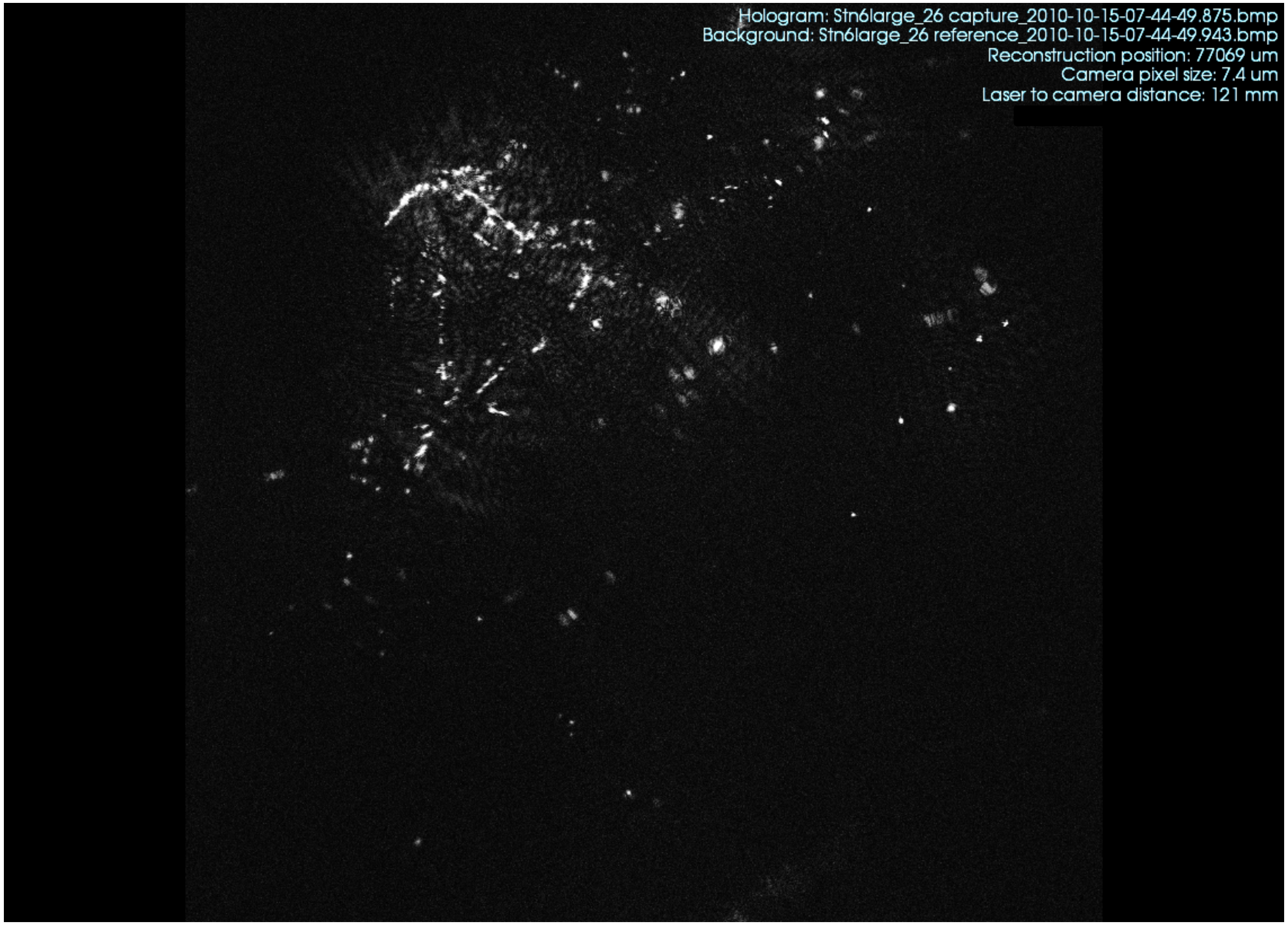
Marine snow: amorphous without ballast



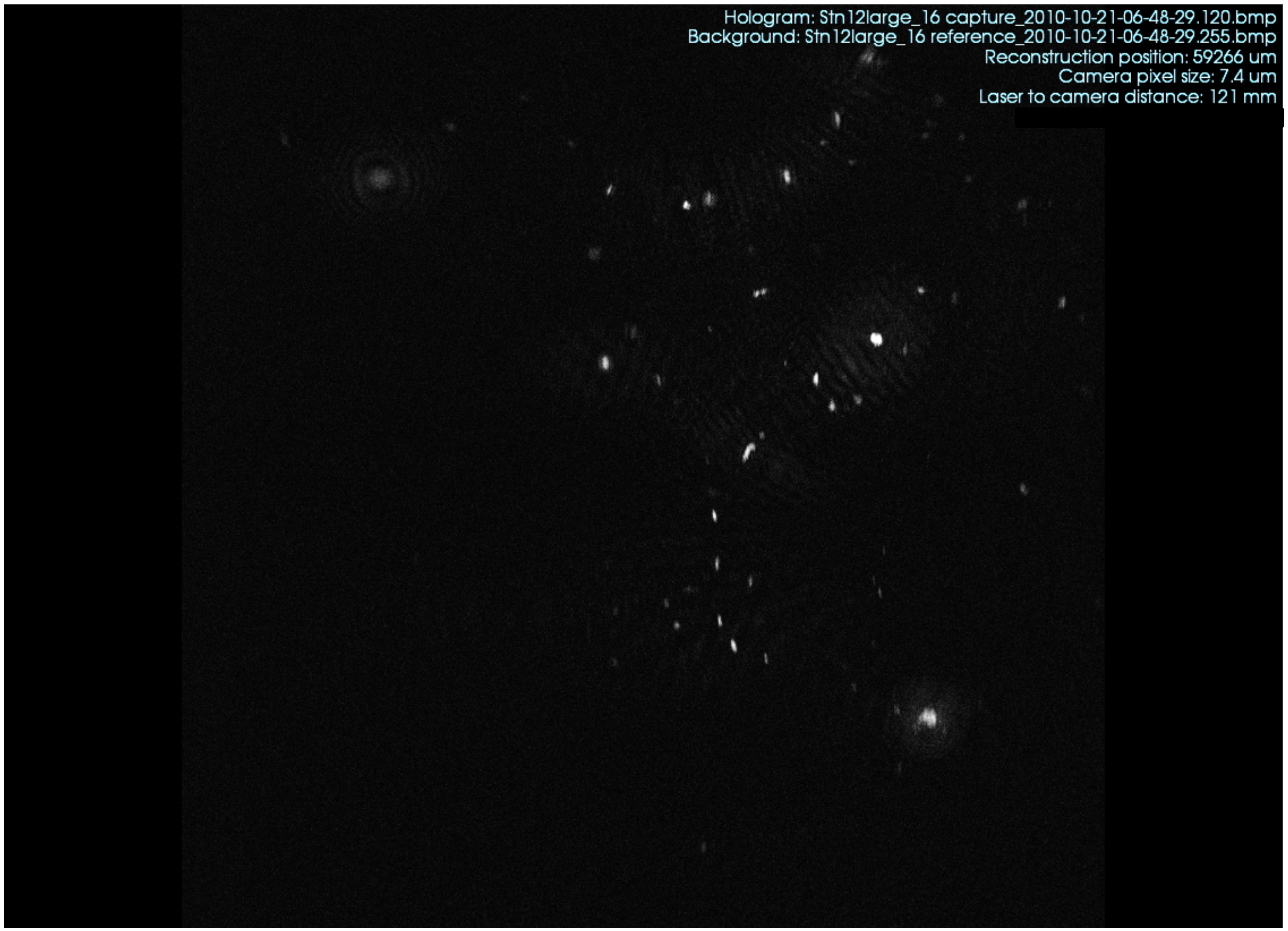
23Oct2010 #35

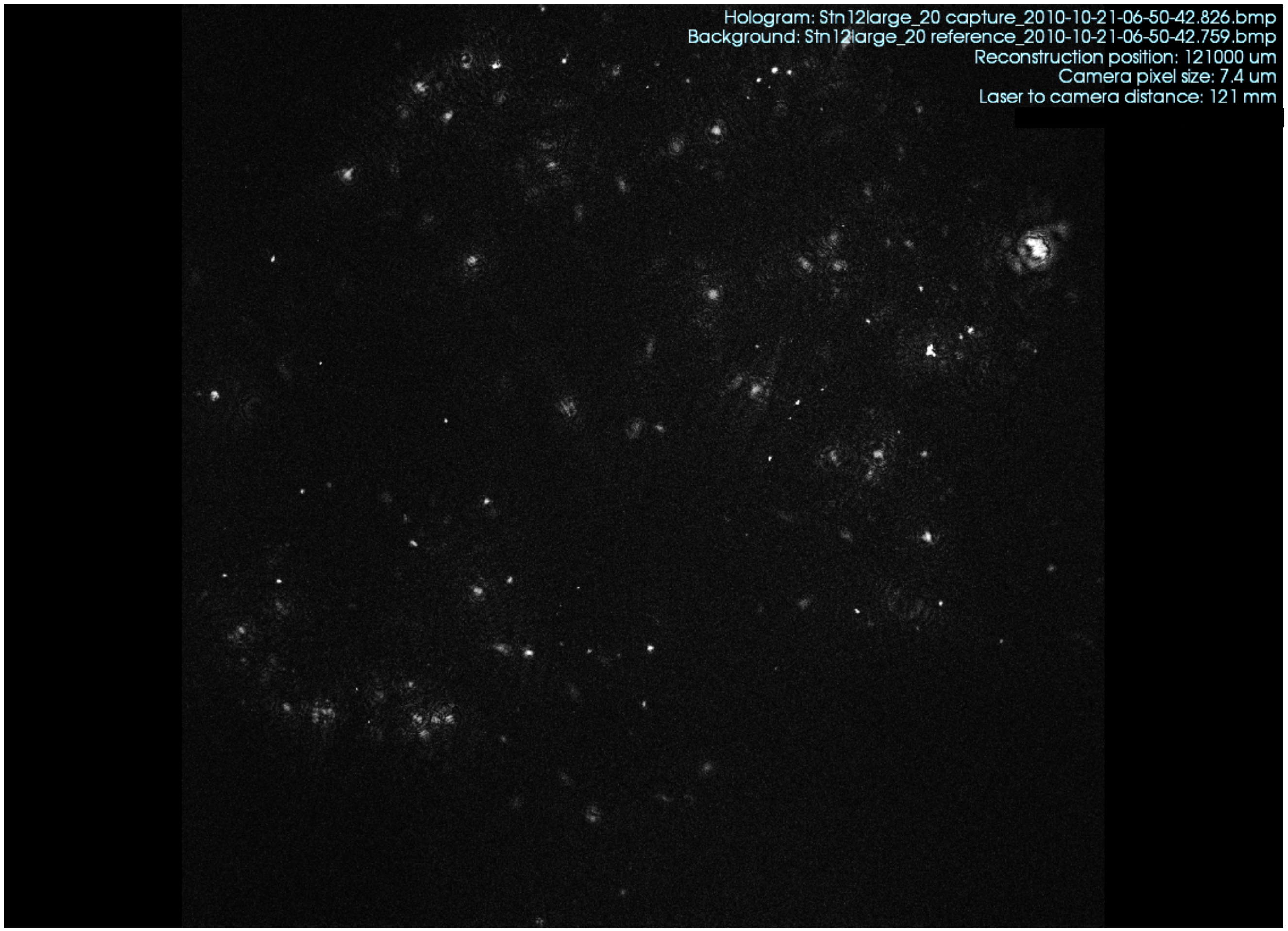
Detail:

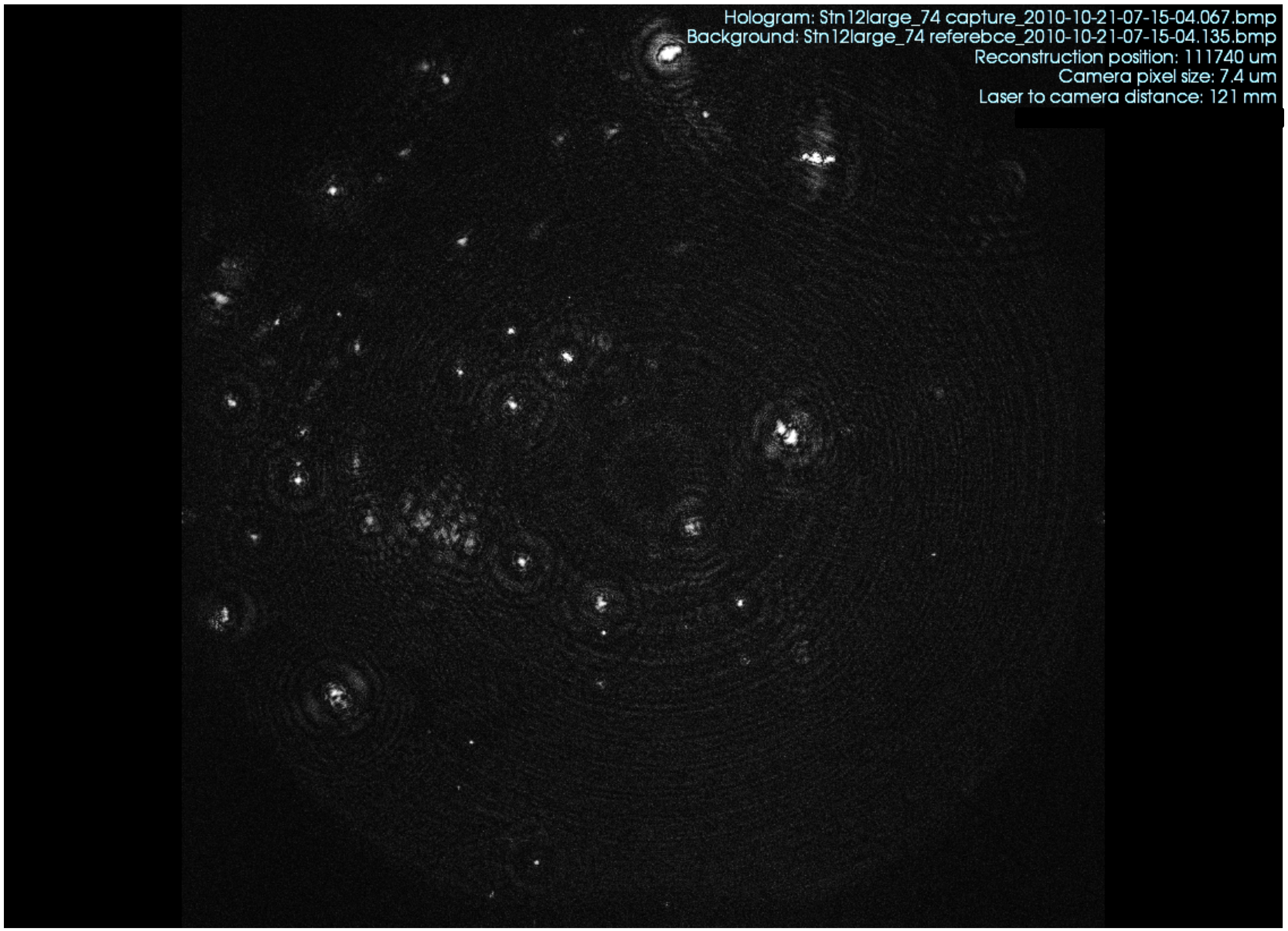


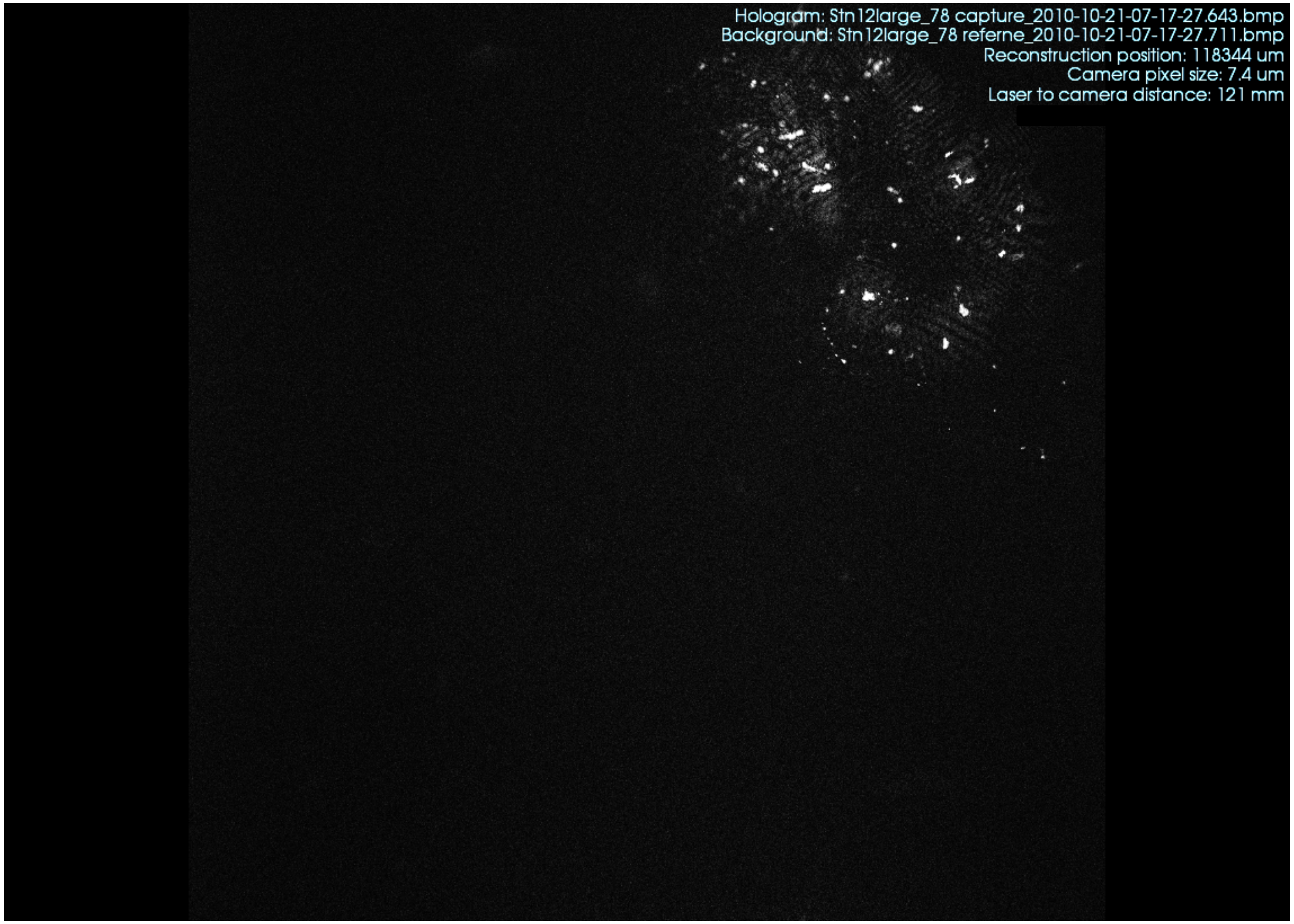

Hologram: Stn6large_26 capture_2010-10-15-07-44-49.875.bmp
Background: Stn6large_26 reference_2010-10-15-07-44-49.943.bmp
Reconstruction position: 77069 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm

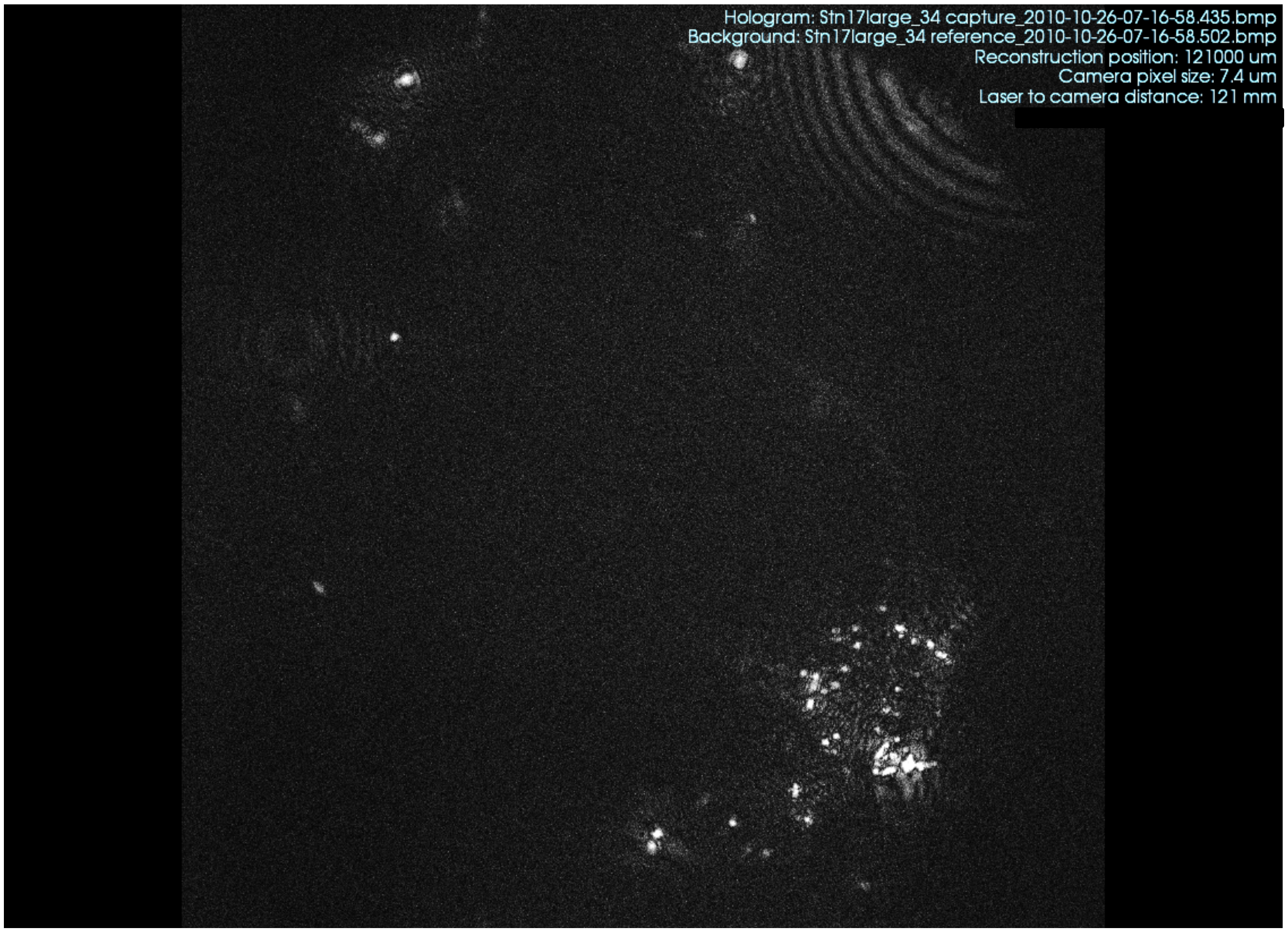

Hologram: Stn12large_9 capture_2010-10-21-06-47-00.771.bmp
Background: Stn12large_9 reference_2010-10-21-06-47-00.838.bmp
Reconstruction position: 91126 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm

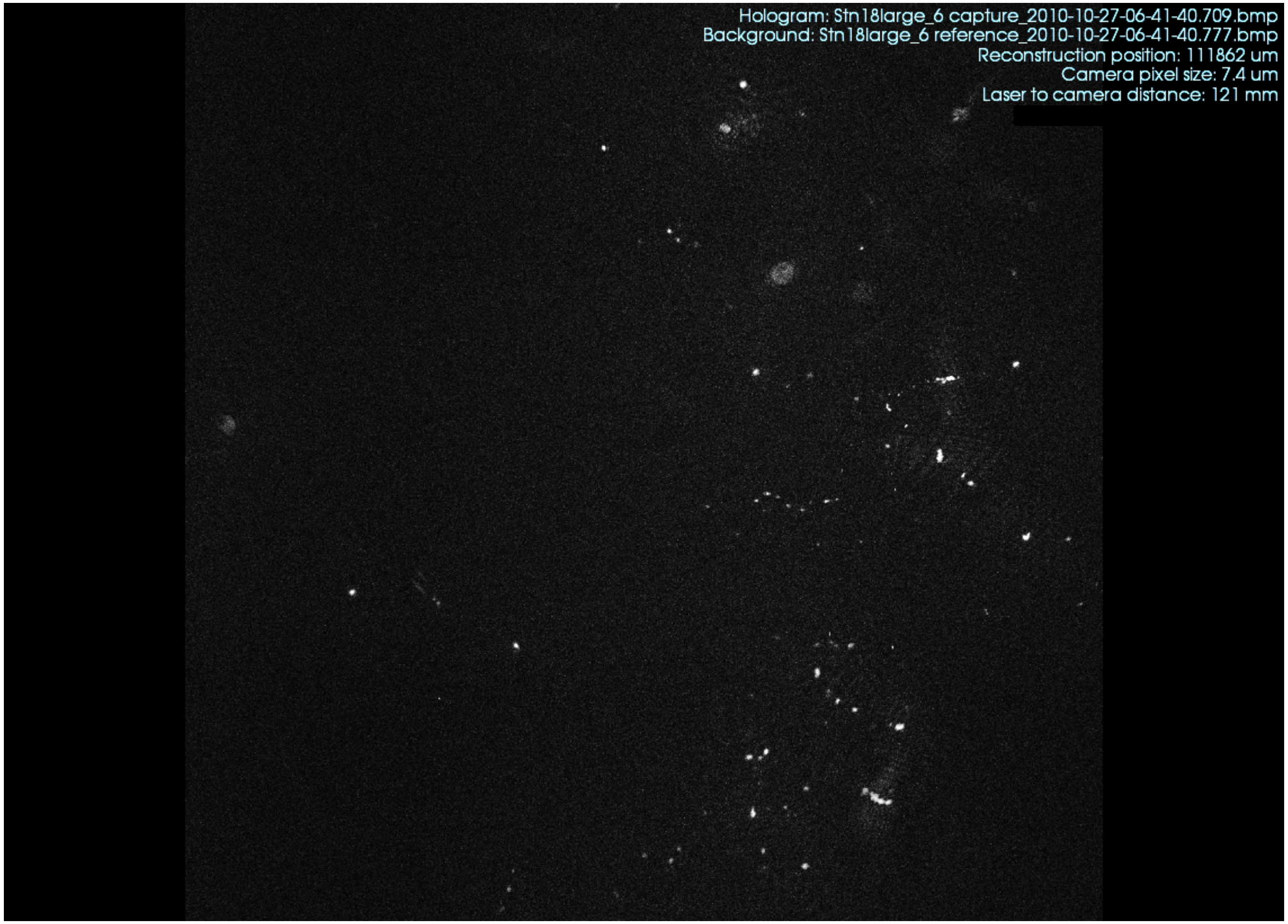

Hologram: Stn12large_16 capture_2010-10-21-06-48-29.120.bmp
Background: Stn12large_16 reference_2010-10-21-06-48-29.255.bmp
Reconstruction position: 59266 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm

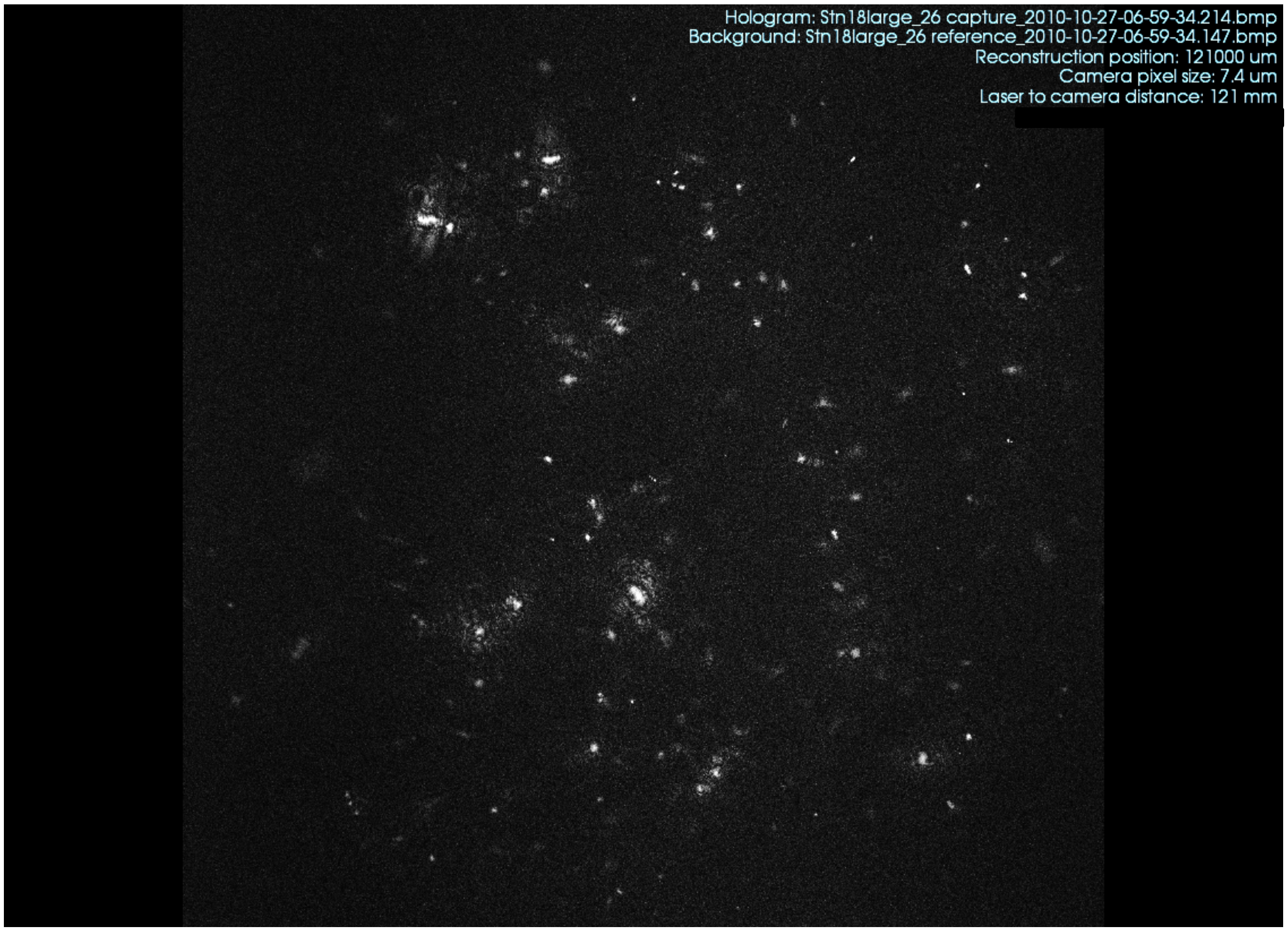

Hologram: Stn12large_20 capture_2010-10-21-06-50-42.826.bmp
Background: Stn12large_20 reference_2010-10-21-06-50-42.759.bmp
Reconstruction position: 121000 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm
[redacted]


Hologram: Stn12large_74 capture_2010-10-21-07-15-04.067.bmp
Background: Stn12large_74 referebce_2010-10-21-07-15-04.135.bmp
Reconstruction position: 111740 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm

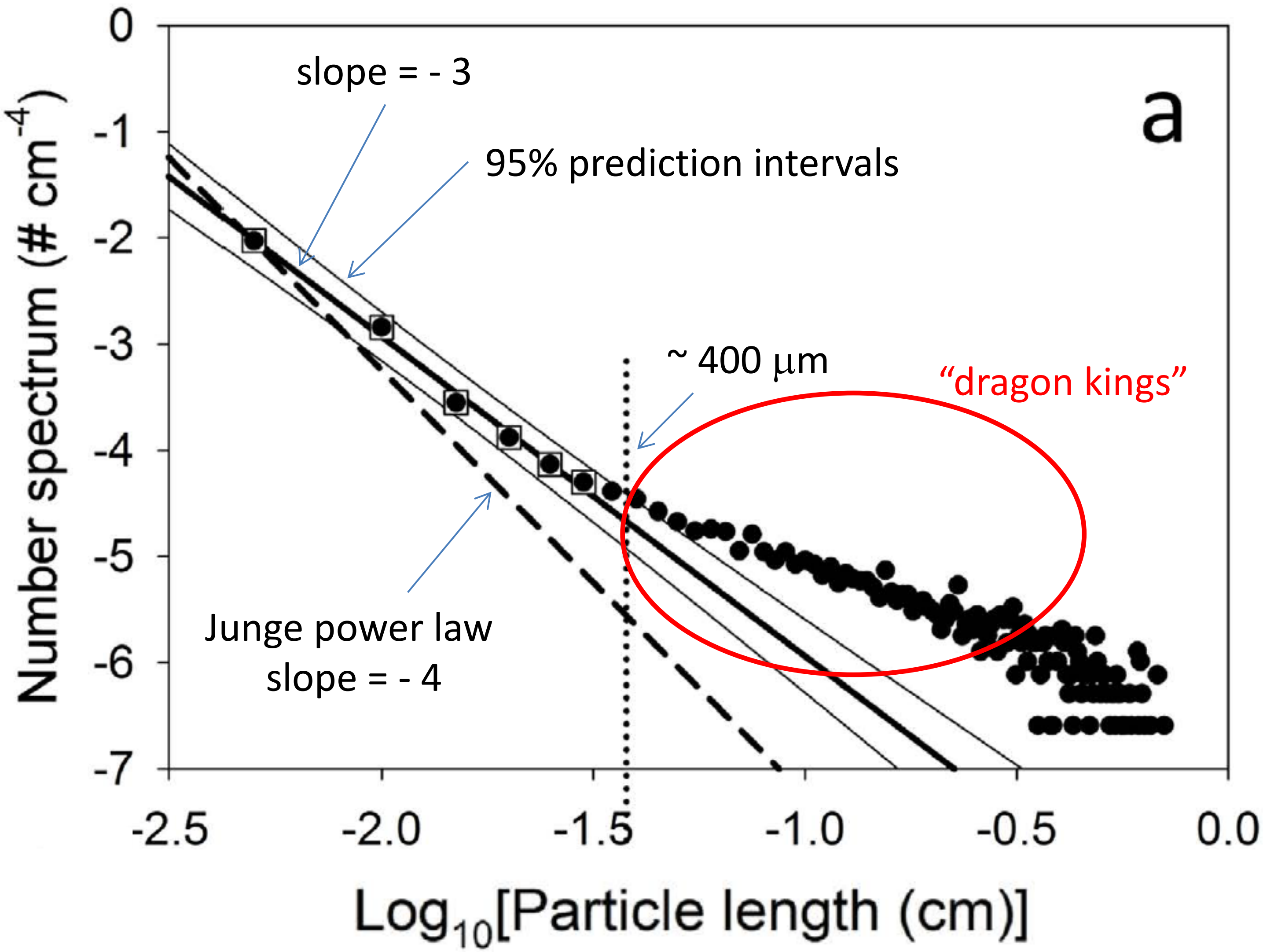

Hologram: Stn12large_78 capture_2010-10-21-07-17-27.643.bmp
Background: Stn12large_78 referne_2010-10-21-07-17-27.711.bmp
Reconstruction position: 118344 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm


Hologram: Stn17large_34 capture_2010-10-26-07-16-58.435.bmp
Background: Stn17large_34 reference_2010-10-26-07-16-58.502.bmp
Reconstruction position: 121000 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm
[redacted]


Hologram: Stn18large_6 capture_2010-10-27-06-41-40.709.bmp
Background: Stn18large_6 reference_2010-10-27-06-41-40.777.bmp
Reconstruction position: 111862 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm

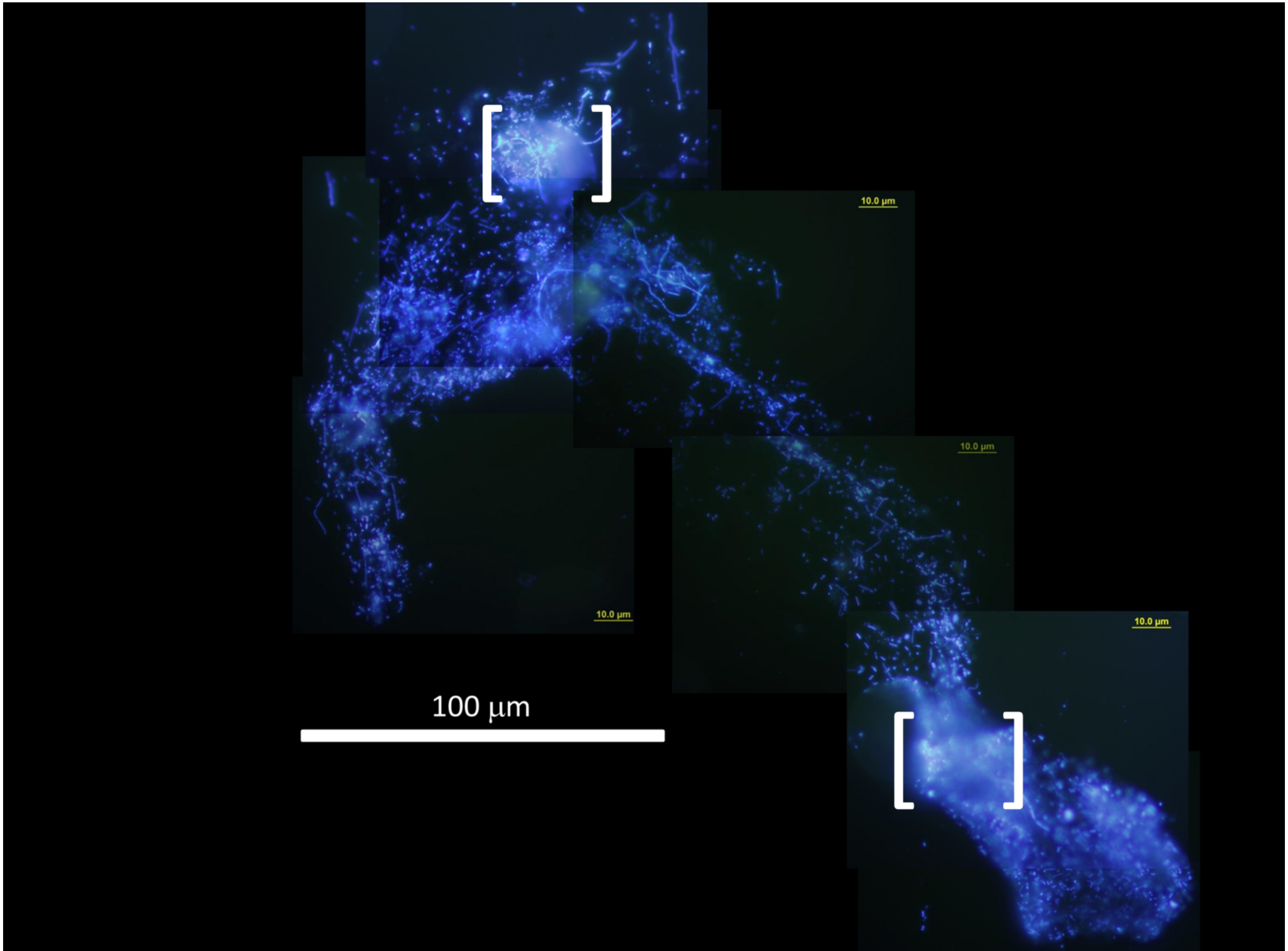

Hologram: Stn18large_26 capture_2010-10-27-06-59-34.214.bmp
Background: Stn18large_26 reference_2010-10-27-06-59-34.147.bmp
Reconstruction position: 121000 um
Camera pixel size: 7.4 um
Laser to camera distance: 121 mm

Number spectrum of particles: 2000 – 6000 m North Atlantic / Arctic



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

$\neq$

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

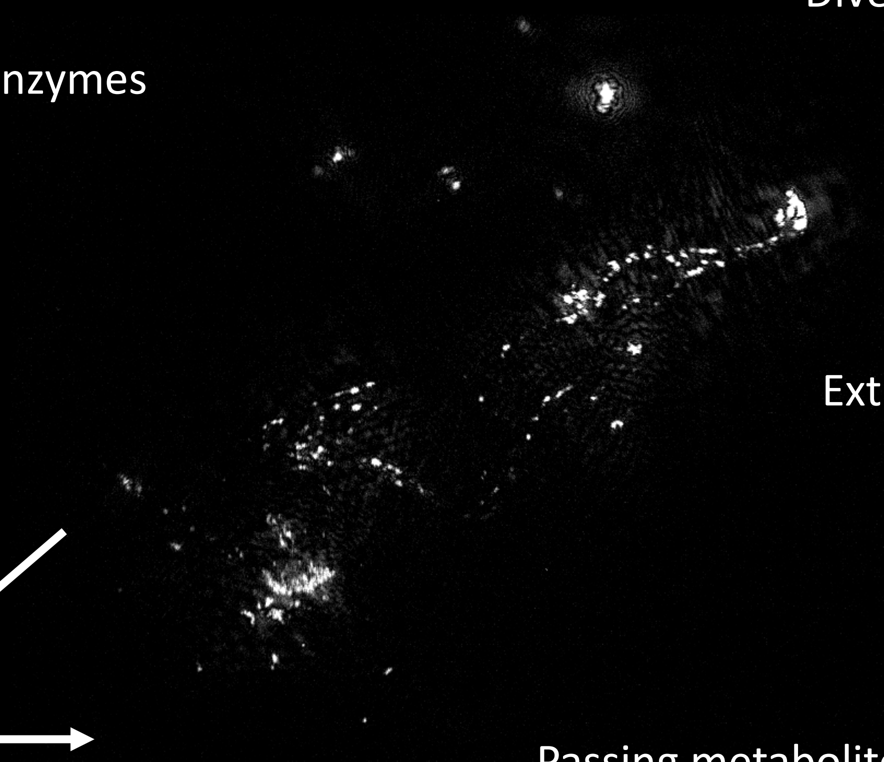
length: 3.2 mm

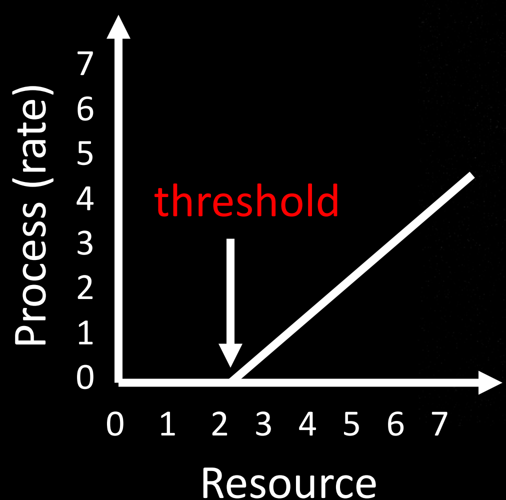
Different communities of microbes
(different ecosystem function)

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

Integration into autonomous vehicles

Miniature diode lasers, camera & video recorder

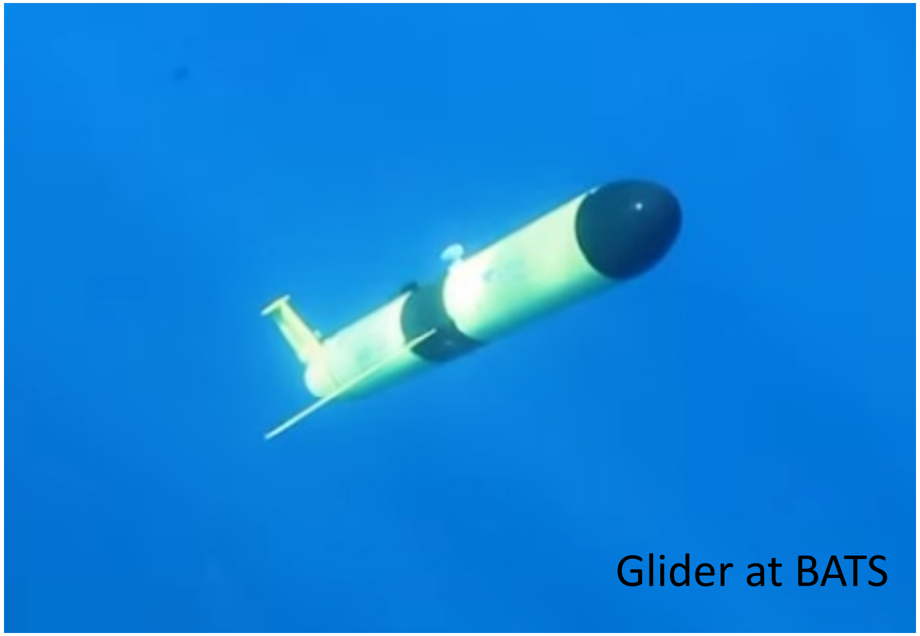
Miniature diode lasers

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

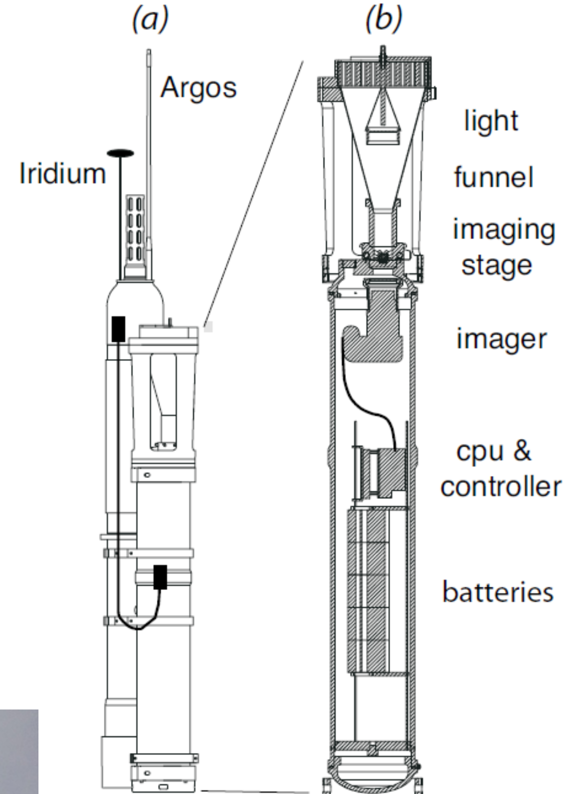
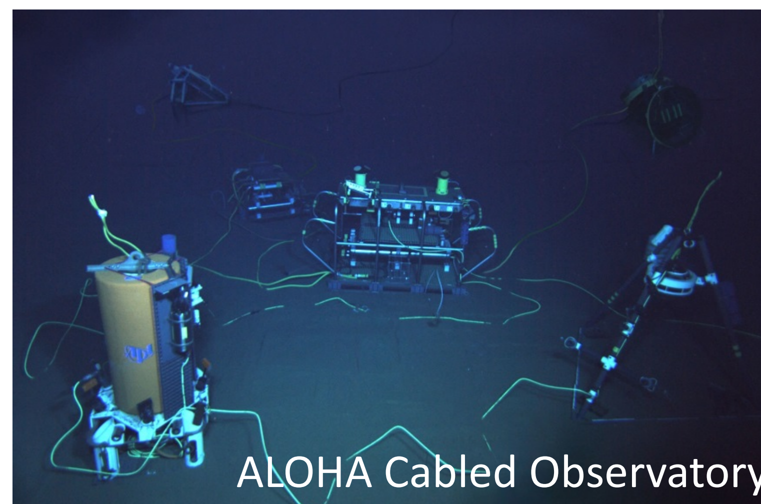


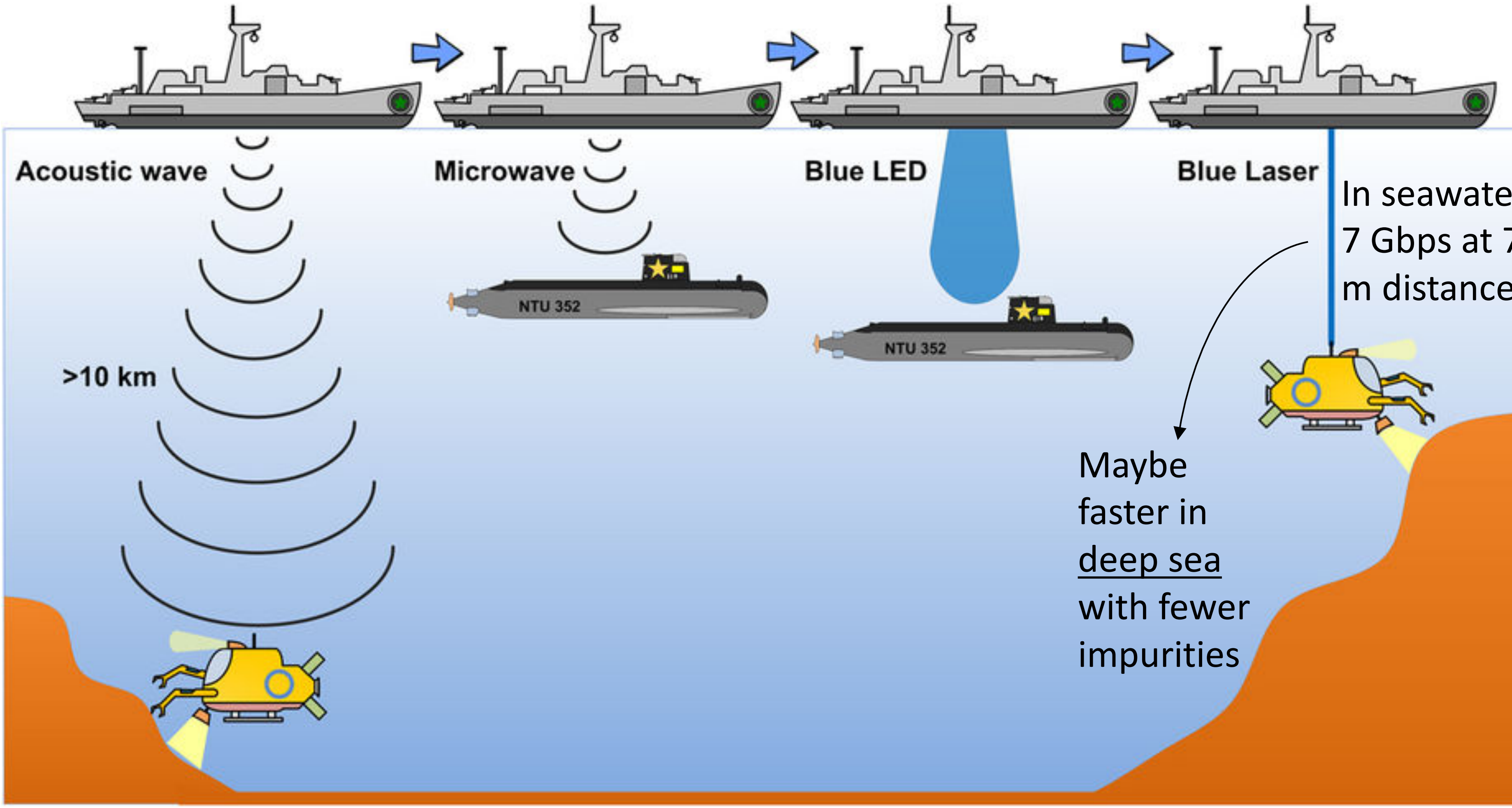
Figure 4. (a) Schematic of the Carbon Flux Explorer (CFE). CFE represents the integration of the Optical Sedimentation Recorder (OSR, engineered at Berkeley Lab) and the Sounding Oceanographic Lagrangian Observer (SOLO; Scripps) profiling float. (b) Optical Sedimentation Recorder (OSR). This instrument 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.

AUV Gulper MBARI



ALOHA Cabled Observatory

High-bandwidth downloads of DIHM images



In seawater: 7 Gbps at 7 m distance

Maybe faster in <u>deep sea</u> 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

Acknowledgements

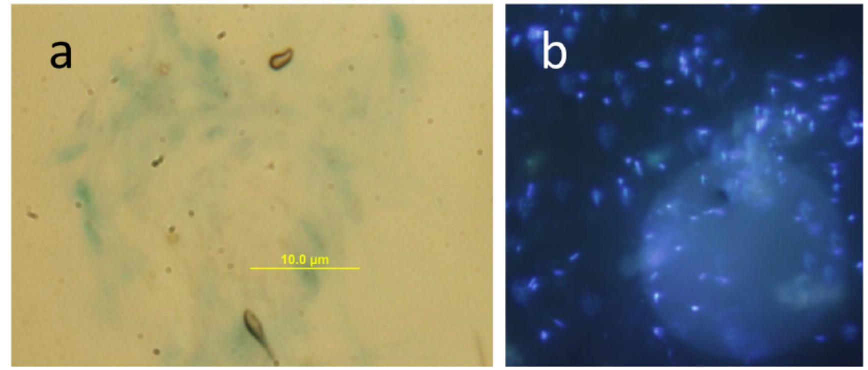
- Gerhard Herndl (University of Vienna, NIOZ Netherlands)
- Manfred Jericho (Dalhousie University, Halifax, Canada)
- Melissa Clouse (ODU)
- Crew of the RV Pelagia (NIOZ, Netherlands) & RV Nathaniel B Palmer

Biological Oceanography
& Polar Programs

Holography | Art

Follow-up analysis of macroscopic deep-sea particles

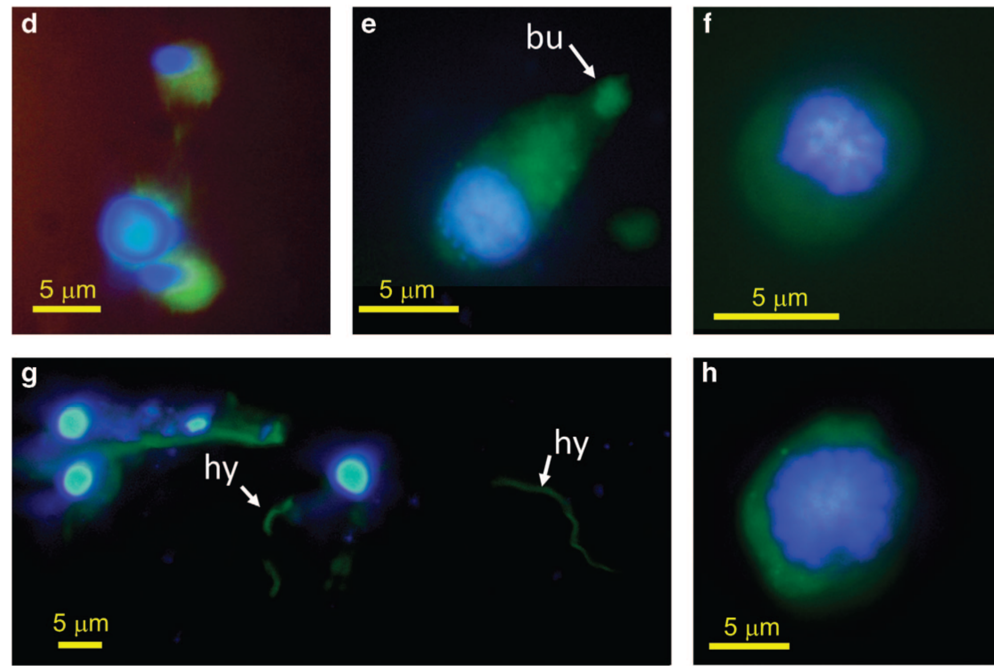
Bochdansky et al. (2016) ISME J



High TEP content

a) Alcian Blue stained TEP

b) DAPI stained prokaryotes in TEP matrix (hole = 30 µm)



Highly enriched saprotrophic <u>eukaryotic microbes</u>

(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.