



In Situ Biology and Mineralogy Capabilities of Holographic Microscopy

Chris Lindensmith¹, Eugene Serabyn¹, J. Kent Wallace¹,
Kurt Liewer¹ Manuel Bedrossian², Stephanie Rider², Tae
Woo Kim², Max Schadeegg³, Iulia Hanczarek³, Niko
Ranta³, Jay Nadeau³

¹Jet Propulsion Laboratory, California Institute of Technology

²GALCIT, California Institute of Technology

³Portland State University

The work described in this presentation was performed in part at the
Jet Propulsion Laboratory, California Institute of Technology under
grant GBMF-4038 and GBMF-4037 from the Gordon and Betty Moore
Foundation. Government sponsorship acknowledged.

Team

JPL

- Chris Lindensmith
- Gene Serabyn
- Kent Wallace
- Scott Perl
- Lukas Mandrake
- Brian Bue
- Gary Doran
- Steven Lu
- Kurt Liewer
- Jonas Kühn (now at EPFL)
- Eric Zhong (summer intern)

Portland State University

- Jay Nadeau
- Max Schadegg
- Niko Ranta
- Iulia Hanczarek

McGill University

- Bimo Niraula
- Sara Najem
- Laurent René de Cotret
- Marwan Elkholy

University of Washington

- Jody Deming
- Max Showalter
- Brett Morris

University of Maine

- Zach Marin (continuing with project at Yale with Jörg Bewersdorf)

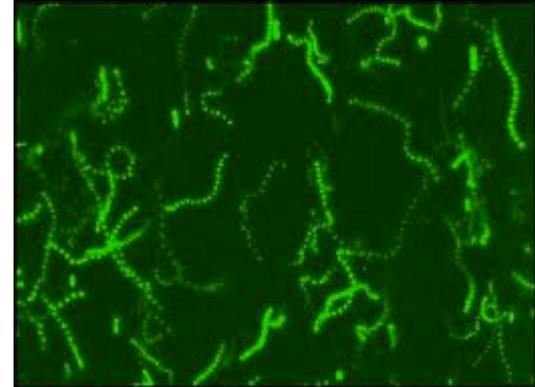
Caltech

- Stephanie Rider
- Manuel Bedrossian
- Tae Woo Kim
- Mory Gharib

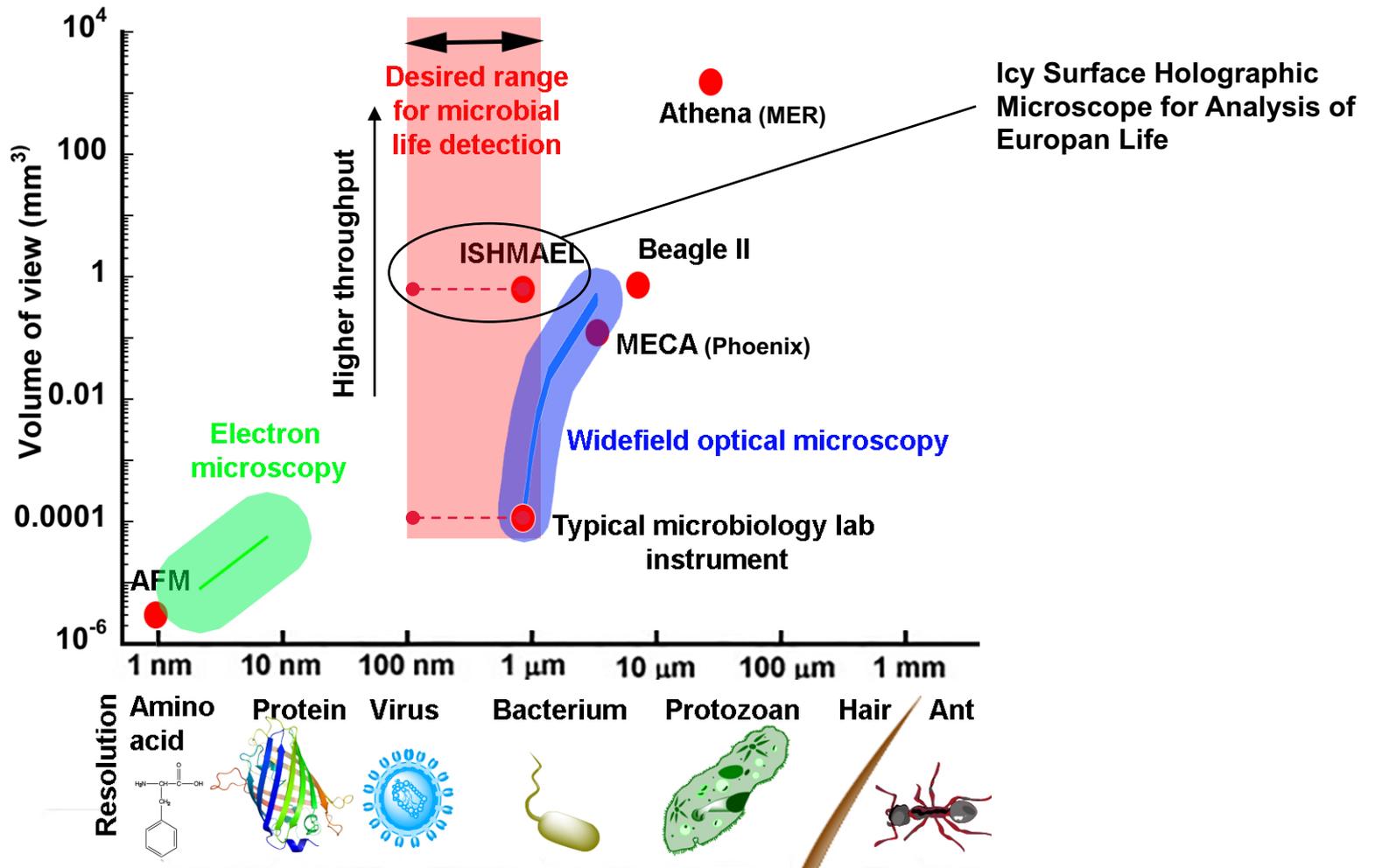
Background & Motivation

- Extant life on Ocean Worlds is almost certainly microbial
- All known life consists of isolated units (*cells*)
- **Thermodynamic estimates for Europa: sizes of 0.2 – 2 μm , $\sim 0.1 - 100$ cells/mL***
- *Activity* is a feature of life that can be detected with a microscope
- Direct imaging is complementary to other science instruments (e.g. capillary electrophoresis, mass spectrometry)
- Everyone wants to see a squid on another planet

“At the present stage of technical development a visual search is probably too complex; it is nevertheless the most rapid and effective method of life recognition in terms of orderliness outside the bounds of random assembly” – Lovelock, Nature (1965)



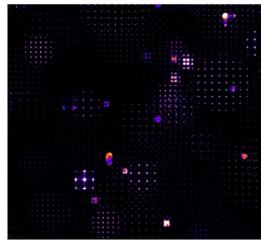
*Hand, K. P., et al. *Report of the Europa Lander Science Definition Team*. NASA-JPL. (2017)



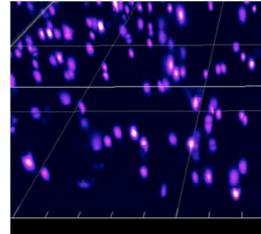
Combined Volumetric Microscope

Volumetric Fluorescence Imaging

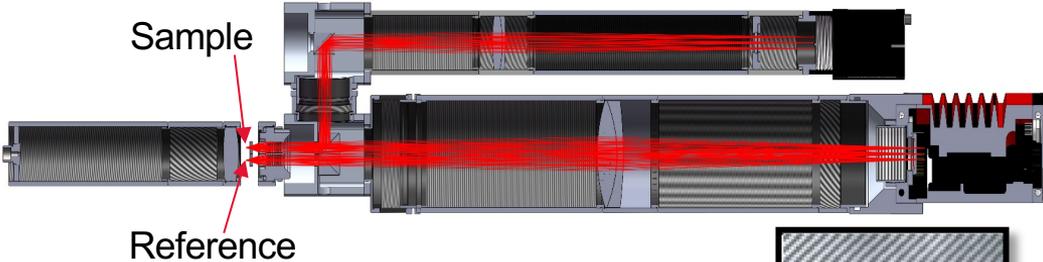
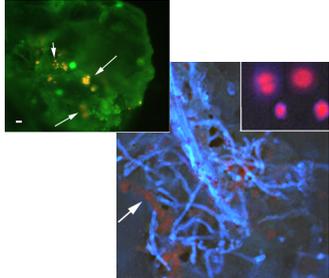
Light Field Images



Chemical Composition



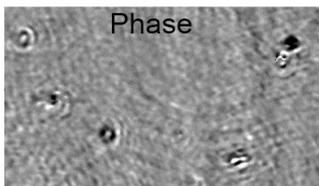
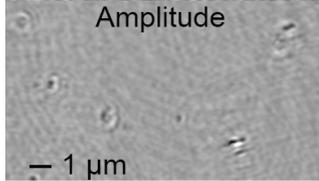
Cellular chemistry



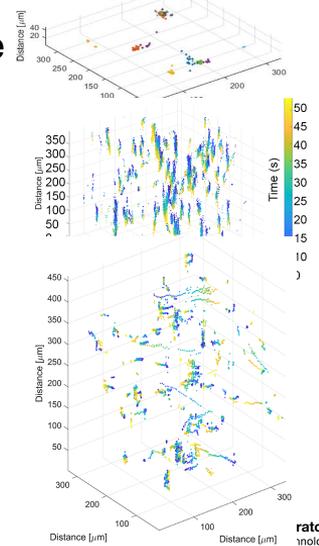
Sample

Reference

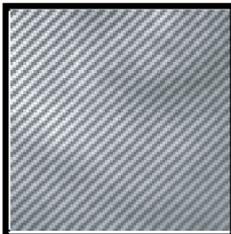
High Resolution Structure and optical properties



Mass, Density, and Activity

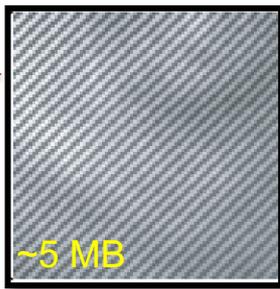
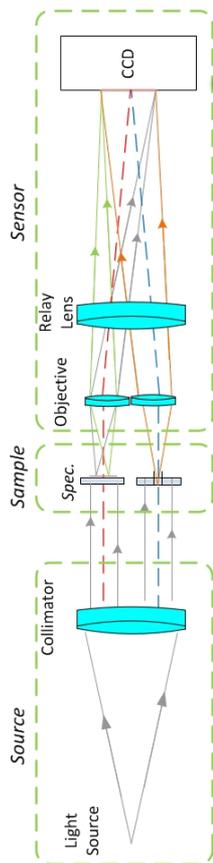


High Resolution Holographic Imaging



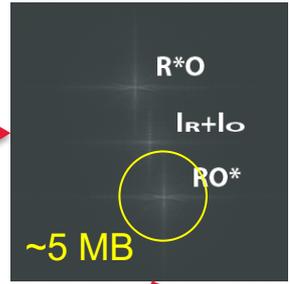
Hologram

Off-Axis Digital Holographic Microscope



Hologram h

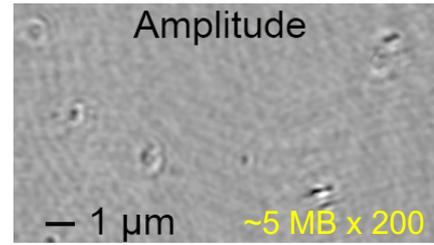
Fourier Transform



$\mathcal{F}(h)$

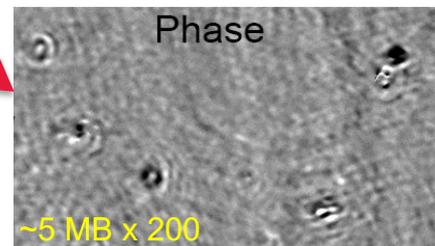
$$\Gamma = \mathcal{F}^{-1}\{\mathcal{F}(h \cdot R) \cdot G\}$$

Complex field at selected reconstruction plane



Amplitude Images are regular photos

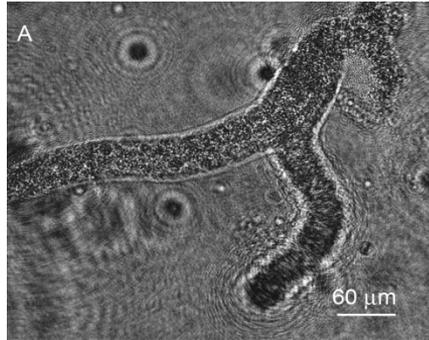
$$|\Gamma|^2$$



Phase Images give index of refraction times thickness

$$\arctan(\text{Im}[\Gamma]/\text{Re}[\Gamma])$$

Structure and Motion

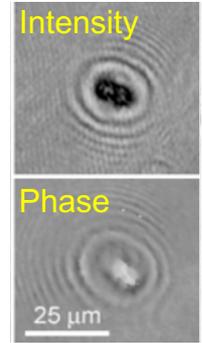


Ameba from pond water

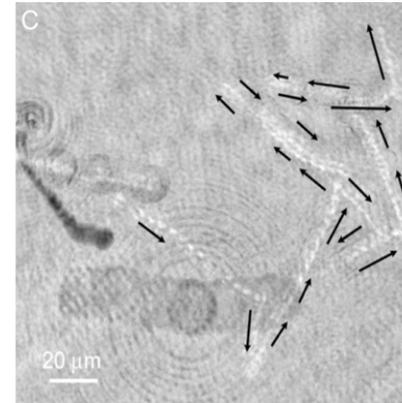
- DHM can observe objects at many scales simultaneously without adjustment
- Focus on each object is done in post processing



Diatom



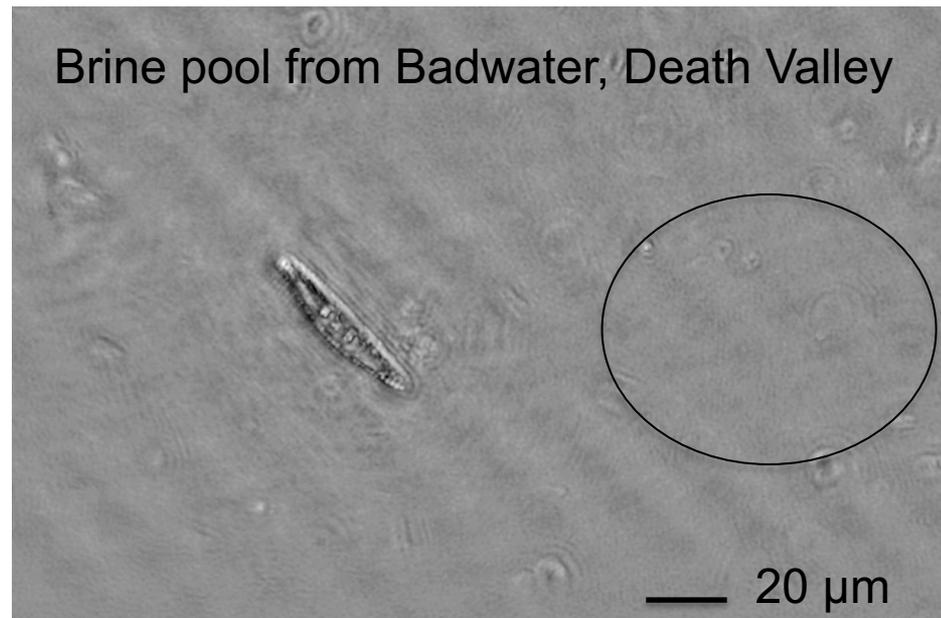
Prokaryote



Motion helps in identifying sub-micrometer objects

Instrument Dynamic Range

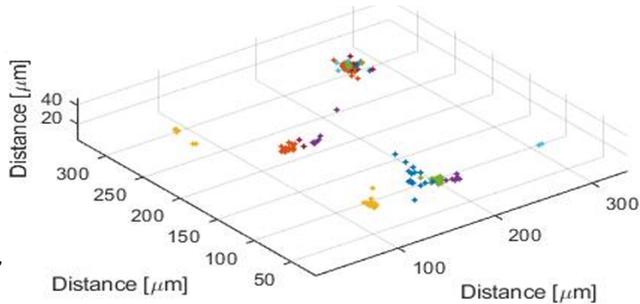
Simultaneous observation of
large diatoms and micron scale
bacteria



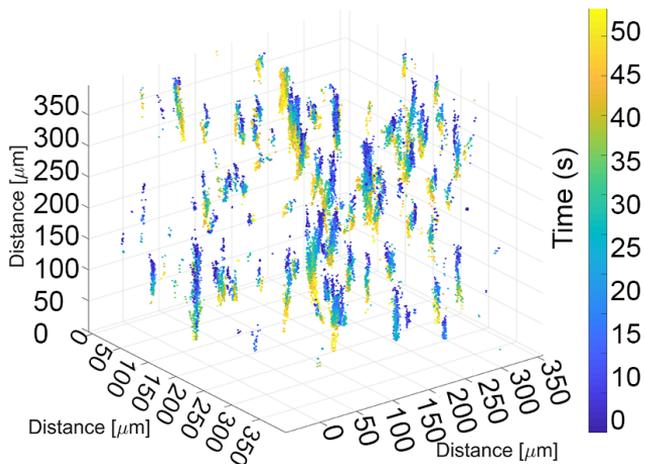
Reconstruction of a single plane

Time Series Tracking Provides Information About Objects

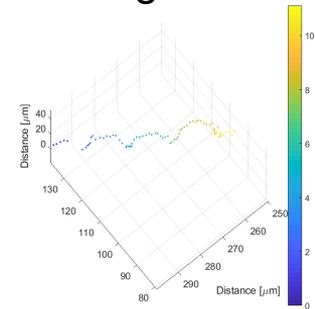
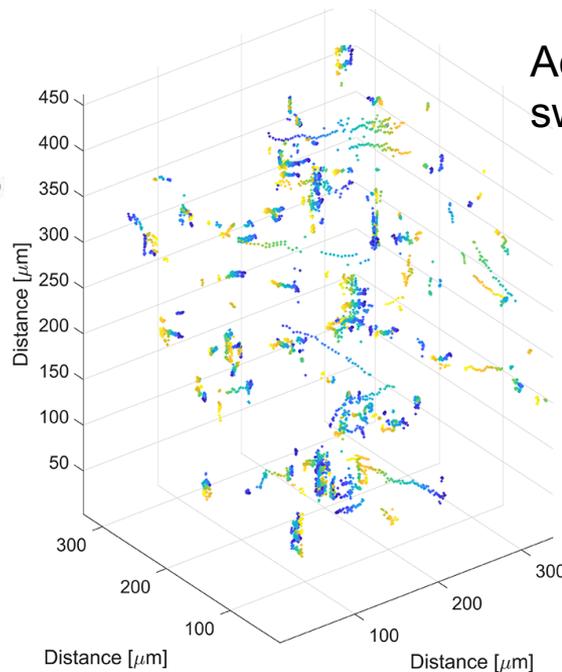
Brownian motion of neutrally buoyant objects (e.g. non-motile or dead cells)



1.3 μm Al_2O_3 beads sinking



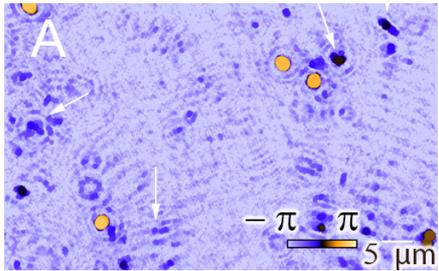
Actively swimming cells



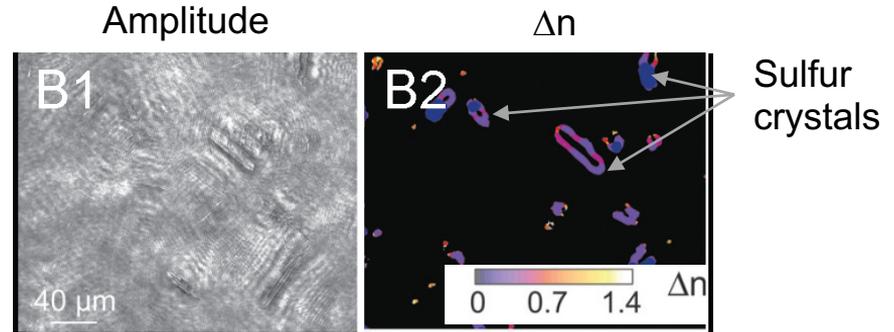
Each less than 1 minute of data

Refractive Index Information

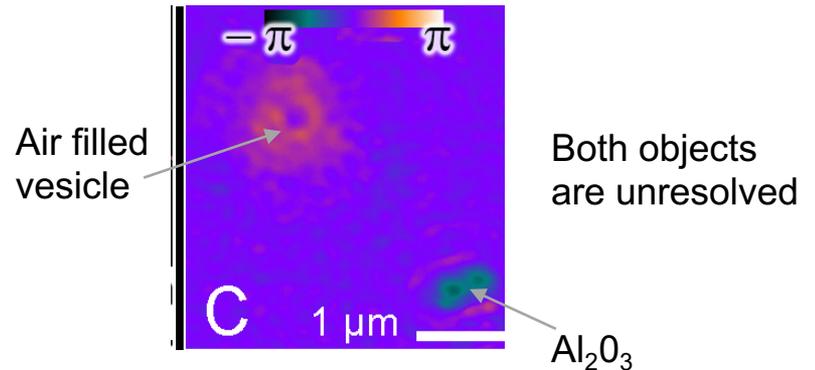
Phase shift:
$$\Delta\phi = 2\pi \frac{t\Delta n}{\lambda}$$



E. coli (arrows) and 1.3 μm Al_2O_3 beads ($n=1.79$)

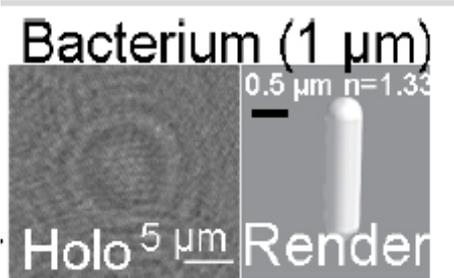
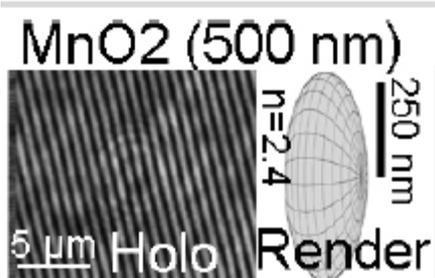
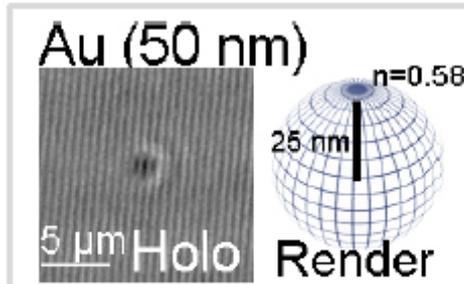


Arctic biofilm stained with fluorescent dyes
 $n \sim 2.0$ for the minerals



Unresolved Objects

- Discrete Dipole Approximation to generate scattering models to compare to diffraction patterns
- Extracts geometry and refractive index for objects well below the diffraction limit
- Examples on the right from our DHM using open source HoloPy code from Manoharan lab



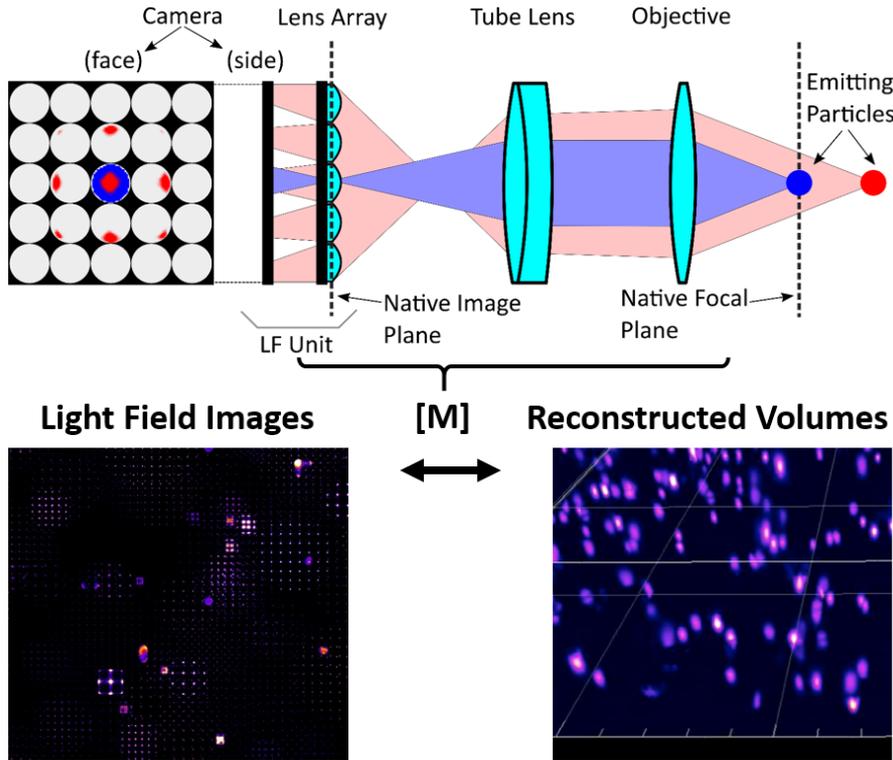
<https://holopy.readthedocs.io/en/latest/#>

E.M. Purcell and C.R. Pennypacker ApJ 186:705 (1973)

A. Wang *et al.*, *Journal of Quantitative Spectroscopy & Radiative Transfer*, vol. 146, p. 499 (2014)

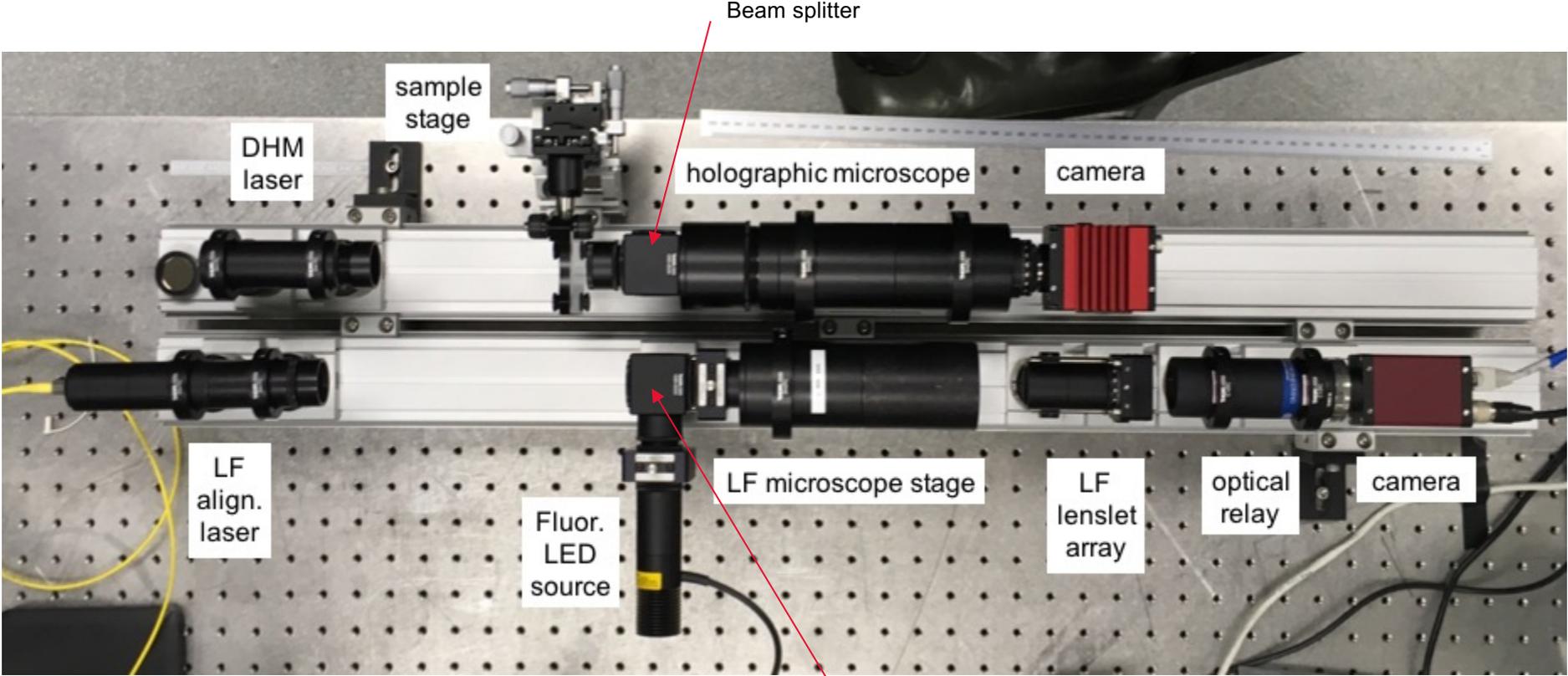
AbSciCon 2019 - Lindensmith *et al.*

Lightfield Imaging



- The lightfield imager is similar to a conventional imager, but the focal plane is replaced by a lenslet array, and the detector is placed at the focus of the lenslet array.
- The lenslets disperse the image onto pixel subarrays in a way that encodes the distance of objects from the native focal plane.
- Software can be used to reconstruct in-focus images at many planes away from the native focus
- **Fluorescent dyes can be added to detect chemical features, even below the resolution limit**

Benchtop integrated system



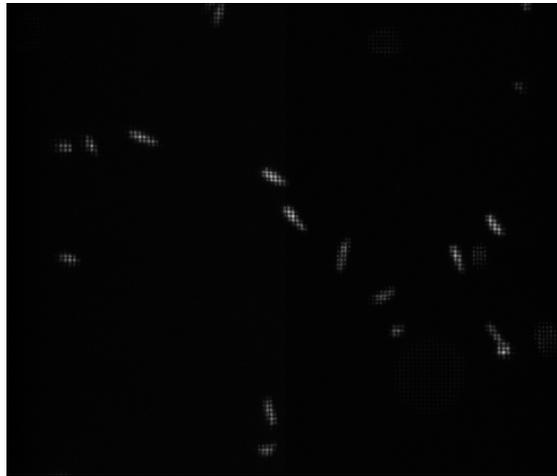
Fold mirror

Lightfield Imaging of Euglena

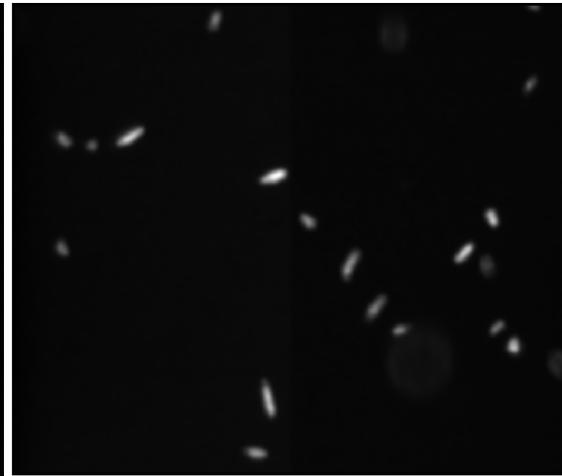
(using chlorophyll autofluorescence)

Z=0
(near system focus)

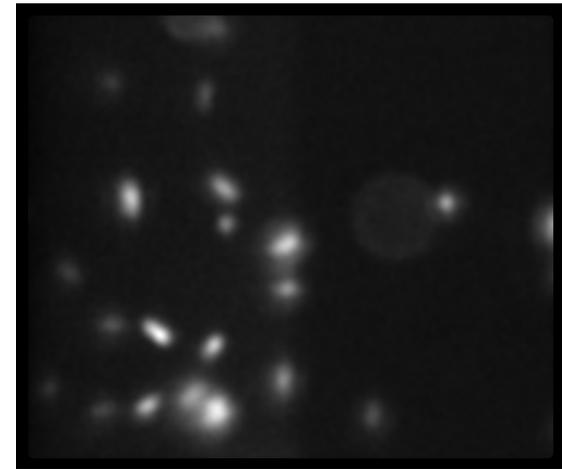
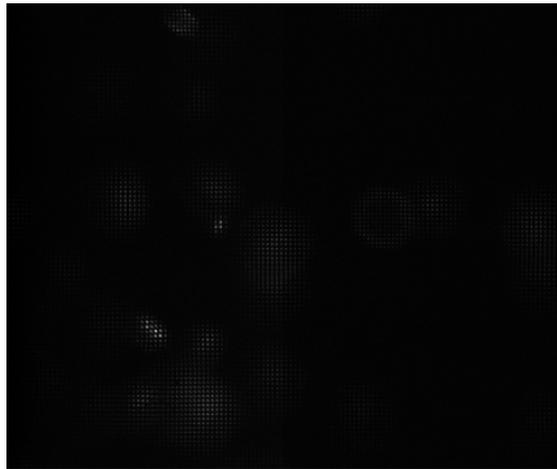
Raw Detector Output



Reconstructed Images

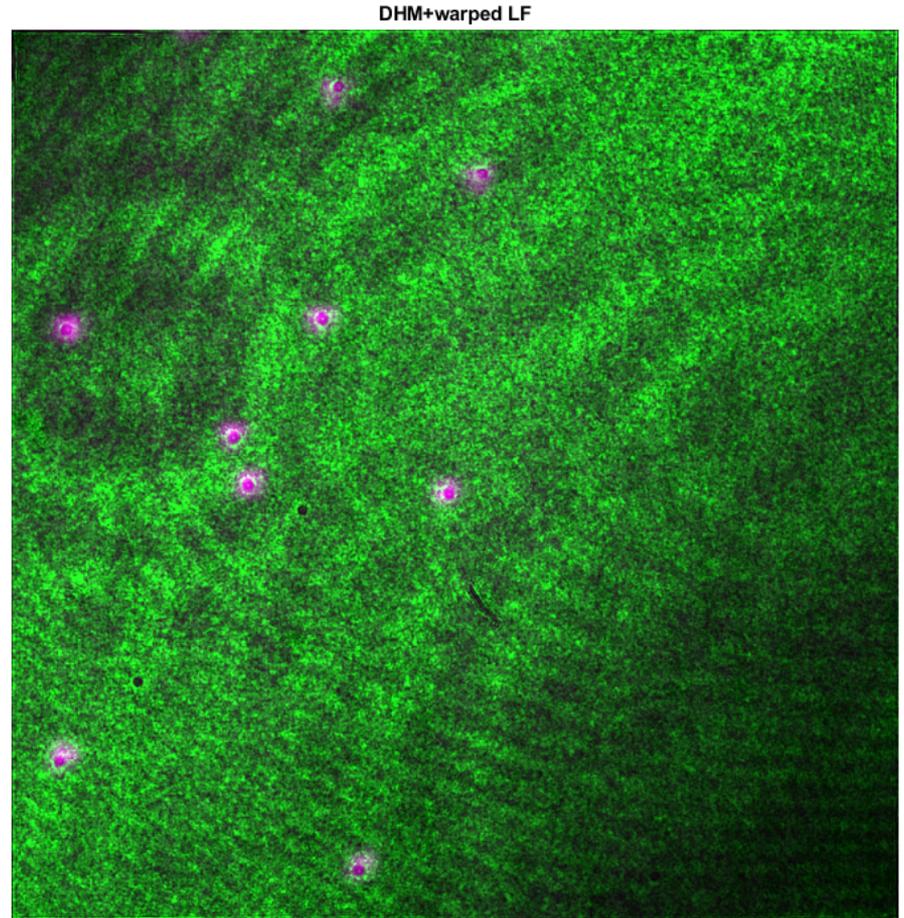


Z= 100 μm
(away from system focus)

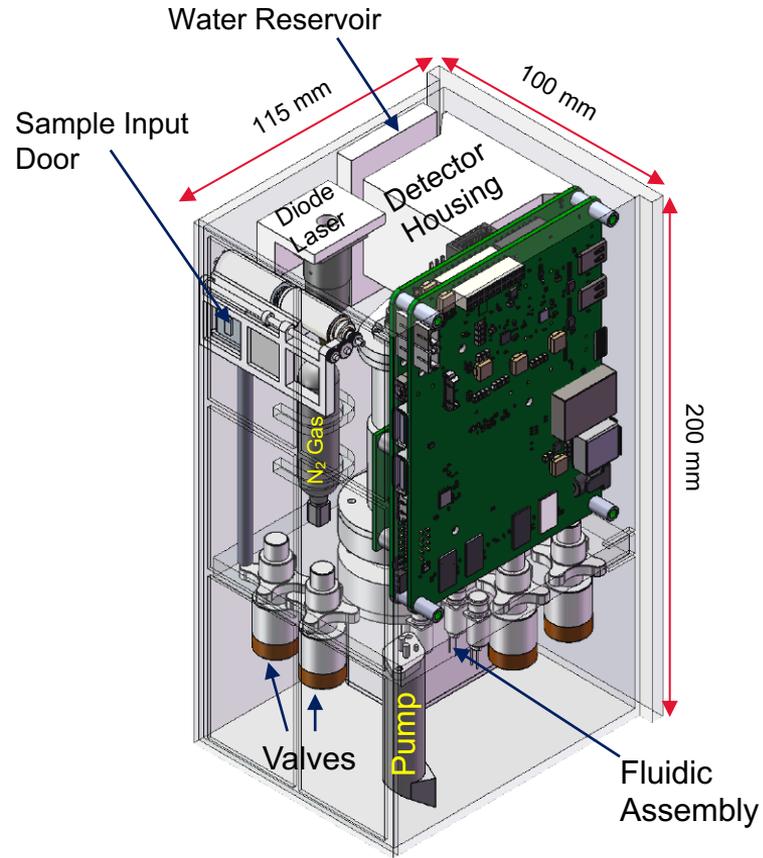


Combined Microscope output

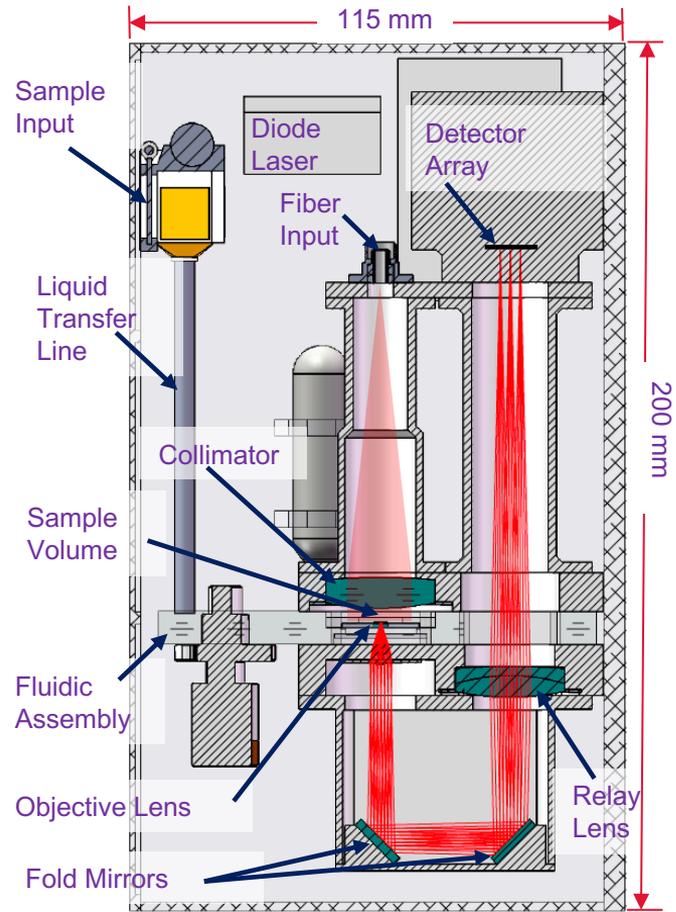
- Green is reconstructed DHM image
- Pink dots are fluorescent beads – image reconstructed from lightfield microscope
- For cellular imaging, dyes can be used to identify presence of molecular types, e.g.
 - Lipids
 - Proteins
 - Nucleic acids



Europa Lander sized package (DHM Only)

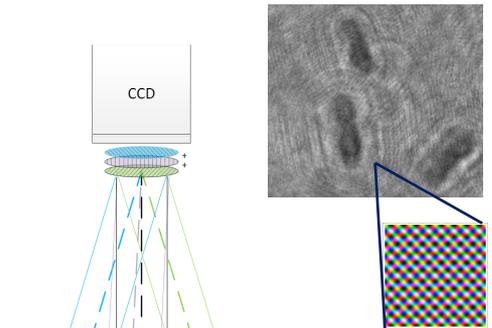


Concept Drawing

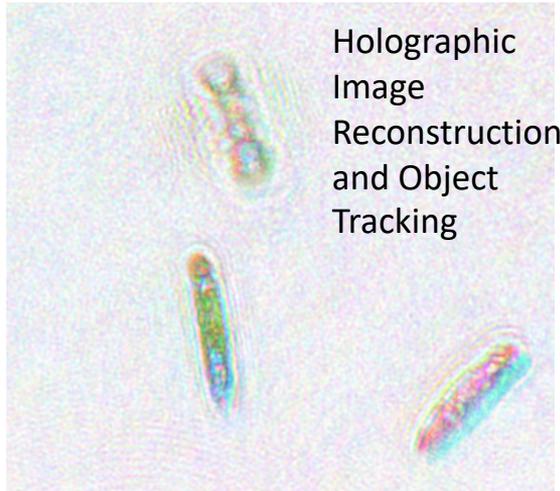
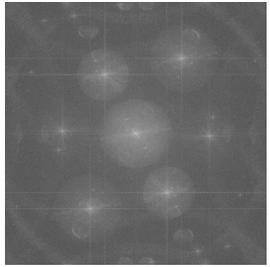


Concept Drawing

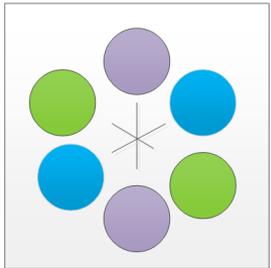
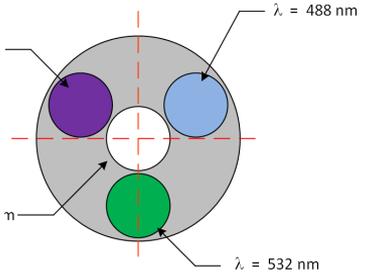
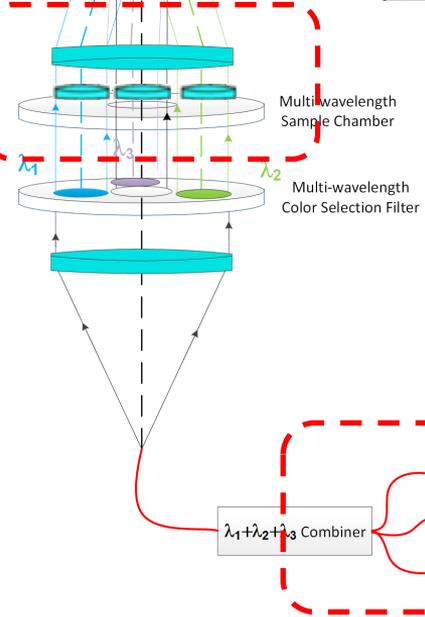
Multiwavelength Holography



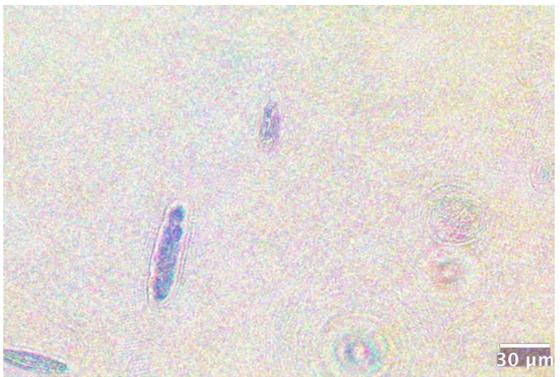
Fourier Transform of
 Image Plane Fringes



3-color reconstructed image
 (cropped, full image is 360 x 360 μm)



1. Clean Separation in Frequency Space
2. Simultaneous Sensing at Multiple Wavelengths



Summary & Future Work

- We have constructed and fielded a holographic microscope with performance consistent with Europa SDT microscopic imager requirements
 - Resolution suitable to detect the smallest predicted cell sizes
 - Throughput and detection capability $\sim 100/\text{mL}$ given $\sim 40 \mu\text{L}$ sample volume (95% confidence)^{1,2}
 - Simple: we have multiple working implementations with no moving parts and loose tolerances. We can use nearly any detector array.
 - Supports integrated stimuli to detect a variety of taxis modes to observe cellular activity
- Future work:
 - Field implementation of compact lensless version
 - Integrate with volumetric fluorescence imaging
 - Improve sample delivery system
 - On-board autonomous data classification (e.g. probable life, probable non-life)
 - Investigate super-resolution capability from time series data & scattering models
 - Polarization properties (full Stokes parameter measurement)
 - More field work!

1. Bedrossian Manuel, Lindensmith Chris, and Nadeau Jay L..
Astrobiology. July 2017, ahead of print.

2. Bedrossian, Barr, Lindensmith, Nealson, Nadeau, JoVE

Imaging Workshop Aug 2019



August 28- 30, 2019,
University of Michigan, Ann Arbor

<https://motility.research.pdx.edu/ImagingWorkshop.html>



Jet Propulsion Laboratory
California Institute of Technology