Digital Holographic Microscopy

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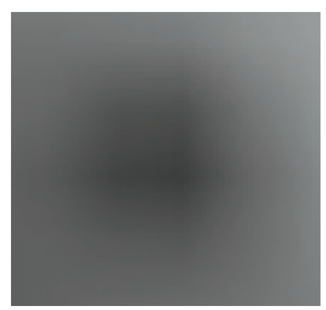
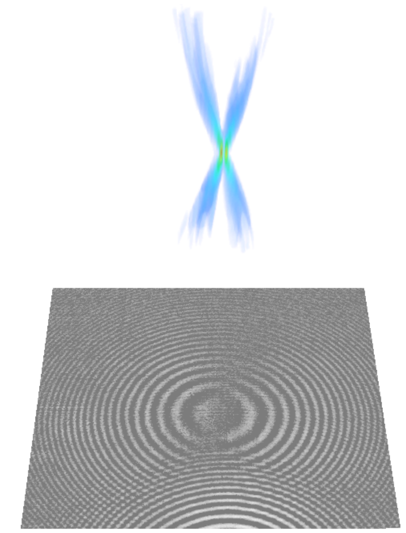
Digital Holographic Microscopy

The present...



Bright-field image of a gold nanoparticle

The world of tomorrow!

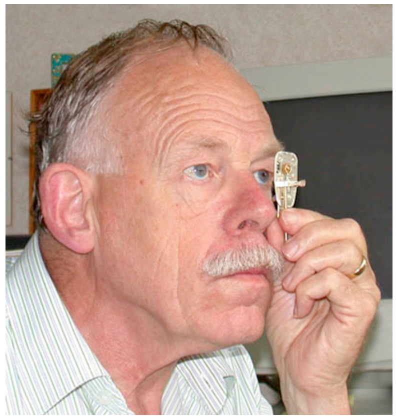


Hologram of, and 3D electromagnetic field around, a gold nanoparticle

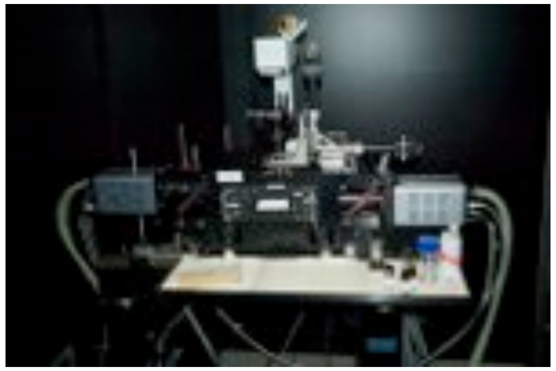
The Problem with Microscopy

- Most microscopes are limited to 2D snapshots of the intensity in a single focal plane

van Leeuwenhoek

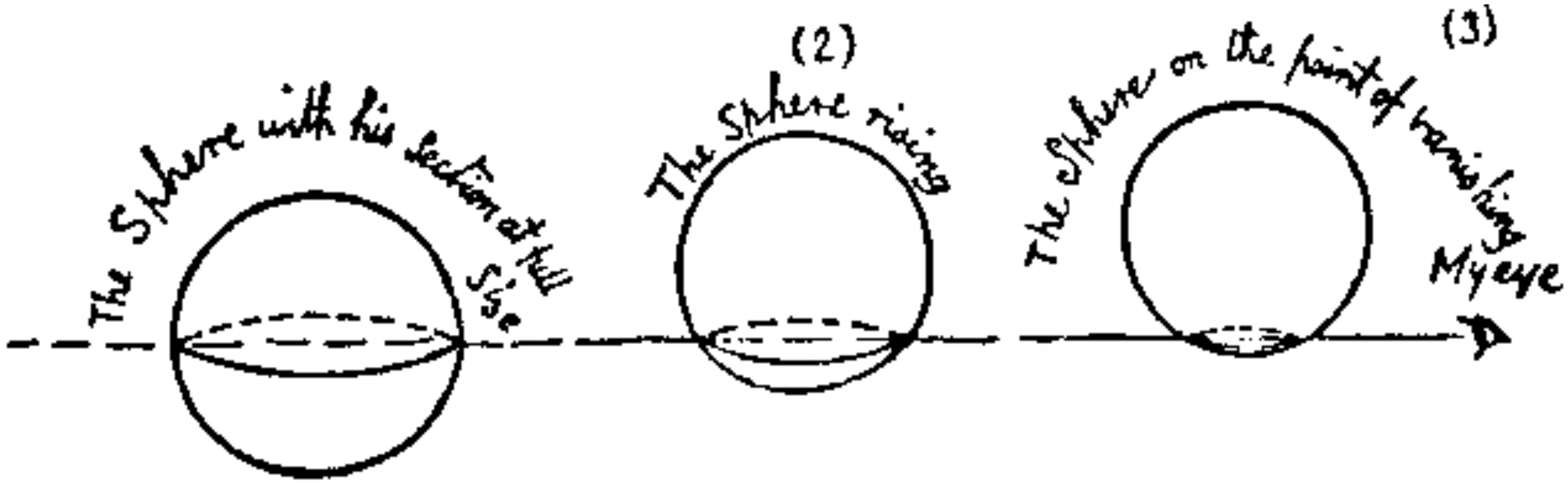


Microscopy in the 17th century



Microscopy today

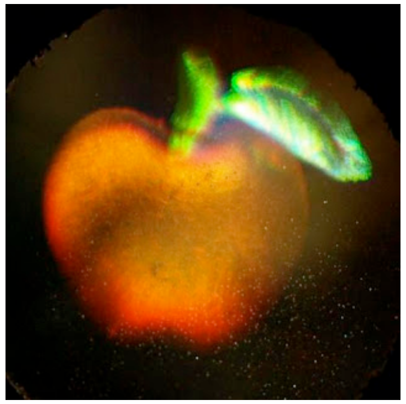
Beyond Flatland



Holography

- Holography is able to reconstruct in 3D the electromagnetic field scattered off an object

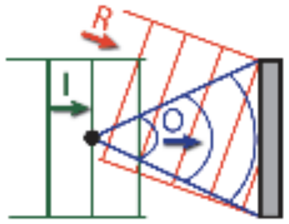
Gabor



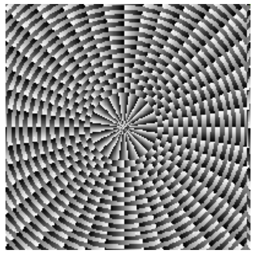
Not Holograms

What Holography Actually Is

- Holography is about interference



Interference



Hologram

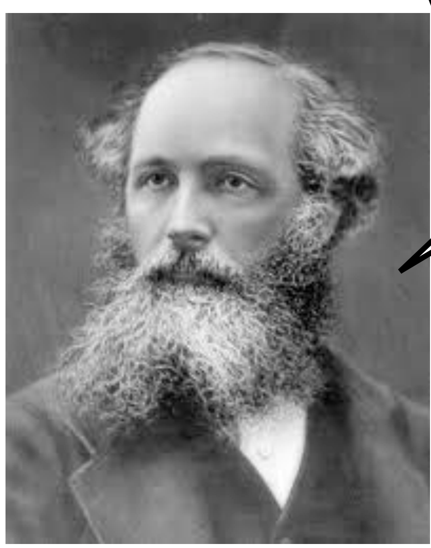
- To record a hologram, interfere the light scattered by an object with a reference beam

- To reconstruct a hologram, shine a copy of the reference beam on the recording (classical holography) or numerically propagate the scattered light backwards (digital holography)

Holography in One Slide

- If we know how light propagates in a medium, then the 3D electromagnetic field can be reconstructed from a single 2D slice

$$
\begin{aligned}
\nabla \cdot \mathbf{D}(\mathbf{r}, t) &= \rho(\mathbf{r}, t) \\
\nabla \times \mathbf{E}(\mathbf{r}, t) &= -\frac{\partial}{\partial t}\mathbf{B}(\mathbf{r}, t) \\
\nabla \cdot \mathbf{B}(\mathbf{r}, t) &= 0 \\
\nabla \times \mathbf{H}(\mathbf{r}, t) &= \frac{\partial}{\partial t}\mathbf{D}(\mathbf{r}, t) + \mathbf{j}(\mathbf{r}, t)
\end{aligned}
$$



Maxwell

Monochromatic wave

Homogeneous and isotropic medium

$$\nabla^2 U(\mathbf{r}, t) + k^2 U(\mathbf{r}, t) = 0$$

$$U(x, y, z) = \int g(x - x', y - y', z) U(x', y', 0)\, dx' dy' \quad \text{with} \quad g(x, y, z) = \frac{z e^{ikr}}{r^2}\left(\frac{1}{2\pi r} - \frac{i}{\lambda}\right)$$

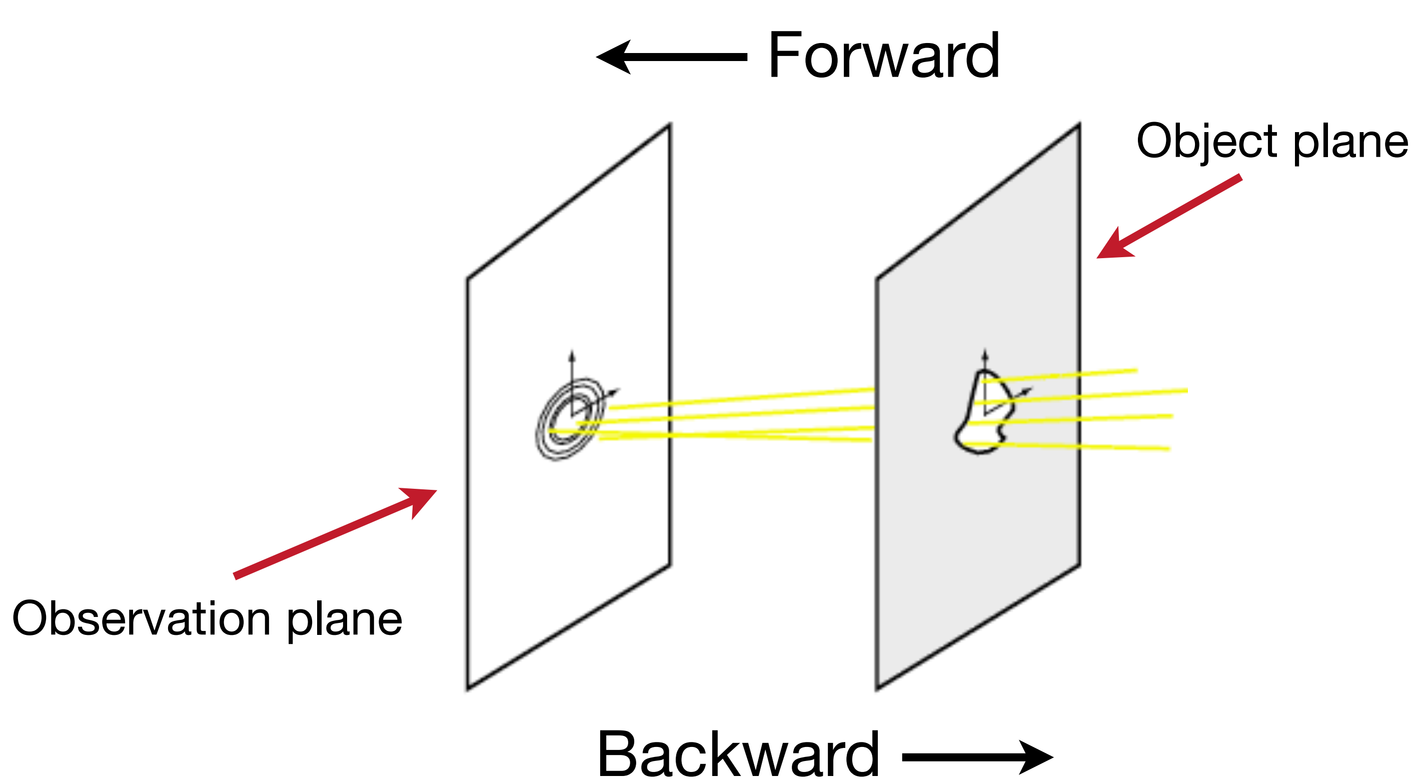
Rayleigh-Sommerfeld Diffraction

$$U(x,y,z) = \int g(x-x',y-y',z)U(x',y',0)\,dx'dy' \quad \text{with} \quad g(x,y,z) = \frac{ze^{ikr}}{r^2}\left(\frac{1}{2\pi r} - \frac{i}{\lambda}\right)$$

Field at z

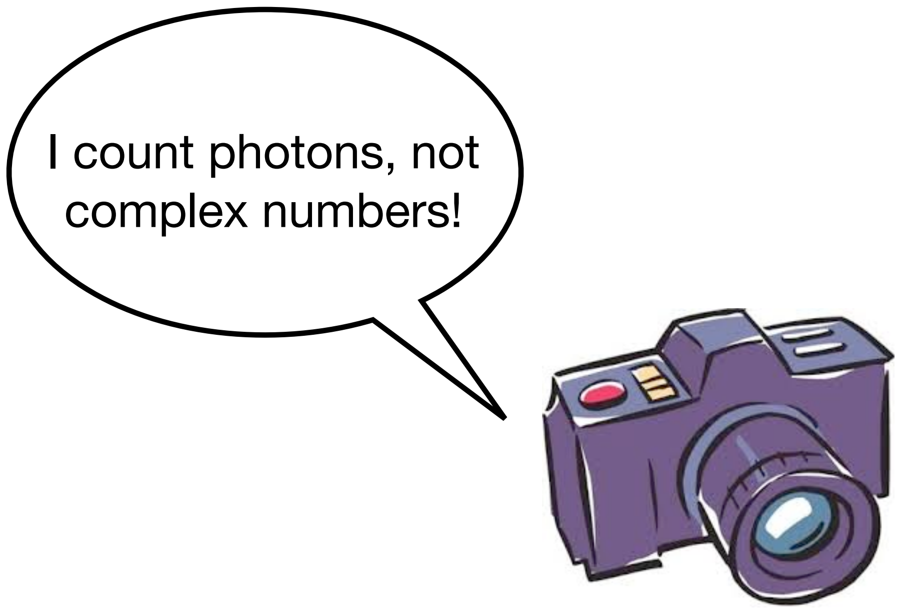
Propagator

Field at z = 0



How to Record a Complex Field?

- A hologram encodes the amplitude and phase of an electromagnetic wave



Reference wave

Reference intensity

$$I(x,y) = |R(x,y) + O(x,y)|^2 = |R(x,y)|^2 + |O(x,y)|^2 + R(x,y)O(x,y)^* + R(x,y)^*O(x,y)$$

Hologram

Object wave

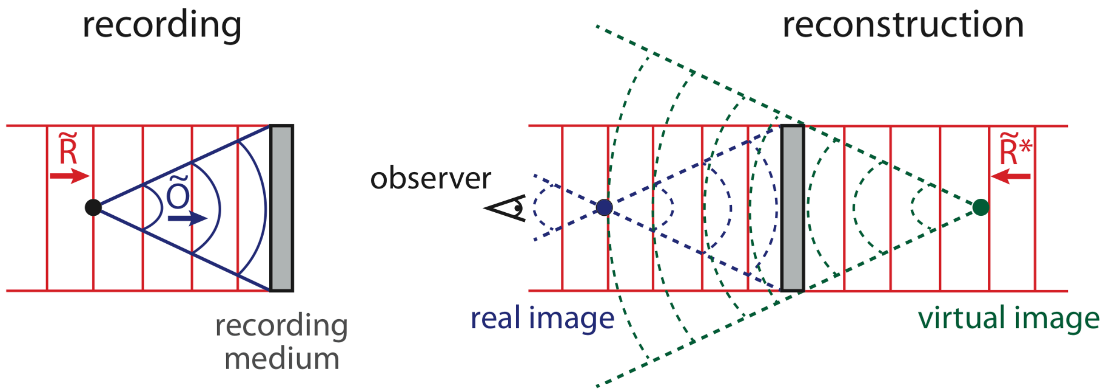
Object intensity

What we really want...

$$\frac{I(x,y)}{|R(x,y)|^2} = 1 + \left|\frac{O(x,y)}{R(x,y)}\right|^2 + \frac{e^{i\phi(x,y)}O(x,y)^* + e^{-i\phi(x,y)}O(x,y)}{|R(x,y)|} \quad \text{with} \quad R(x,y) = |R(x,y)|e^{i\phi(x,y)}$$

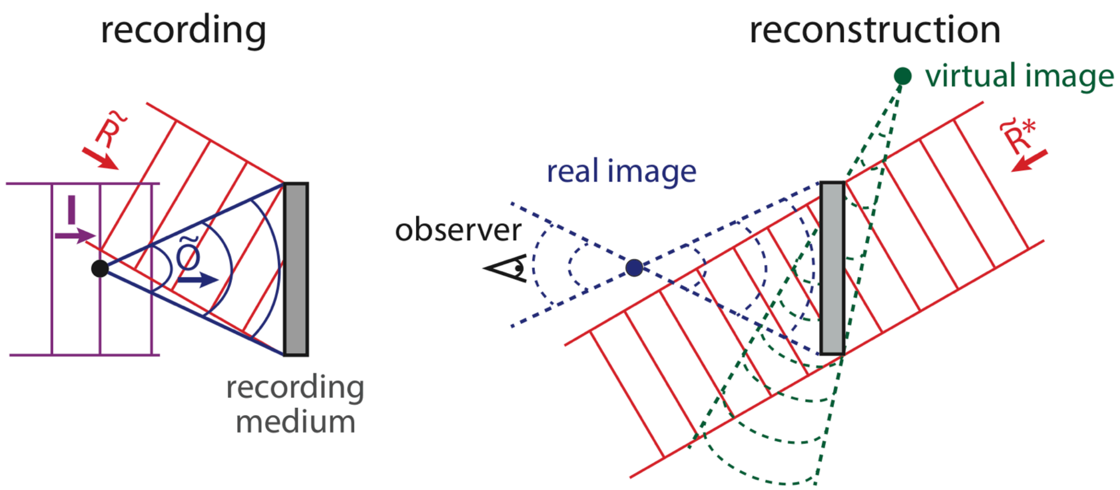
Flavours of Holography

Inline holography



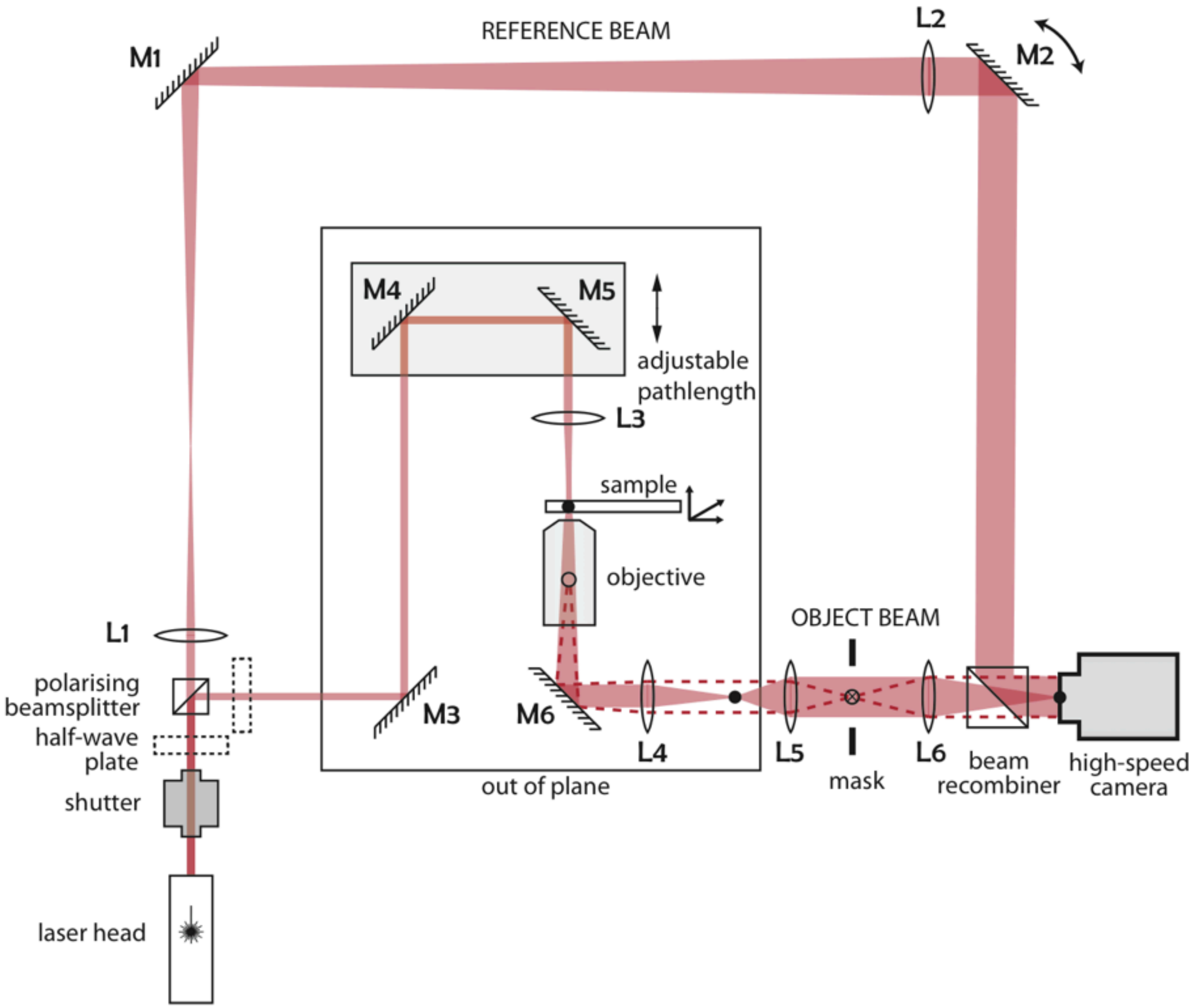
$$R(x,y) = |R(x,y)|$$

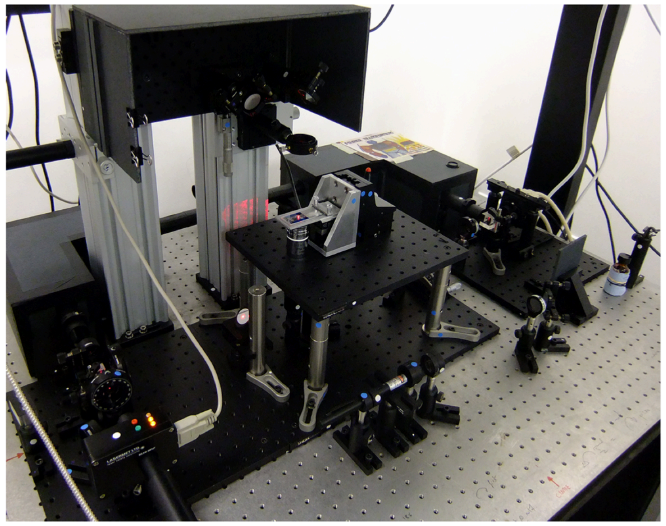
Off-axis holography



$$R(x,y) = |R(x,y)|e^{-ikx\sin\theta}$$

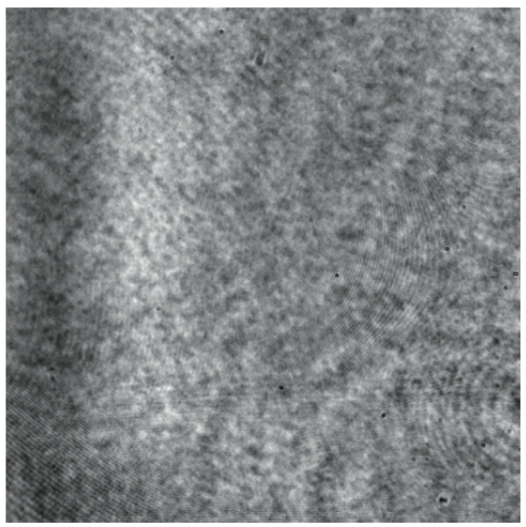
A Digital Holographic Microscope





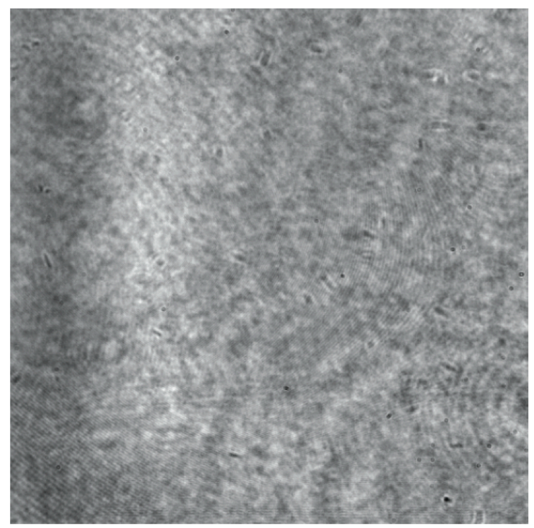
- ~60 nm spacing in x and y
- 2000 FPS at 512 px by 512 px

Inline Recording



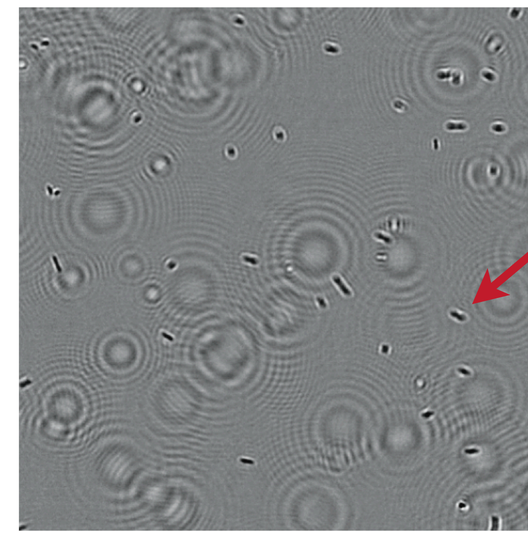
Raw hologram

$\div$

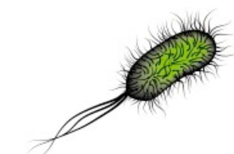


Background hologram

$=$



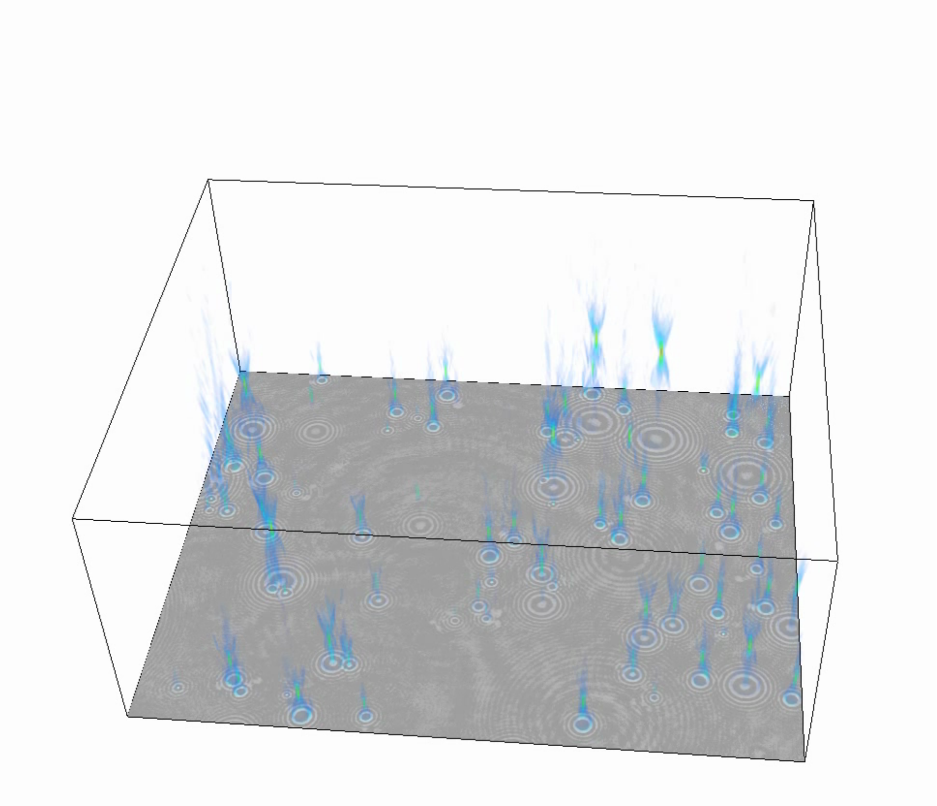
Normalized hologram



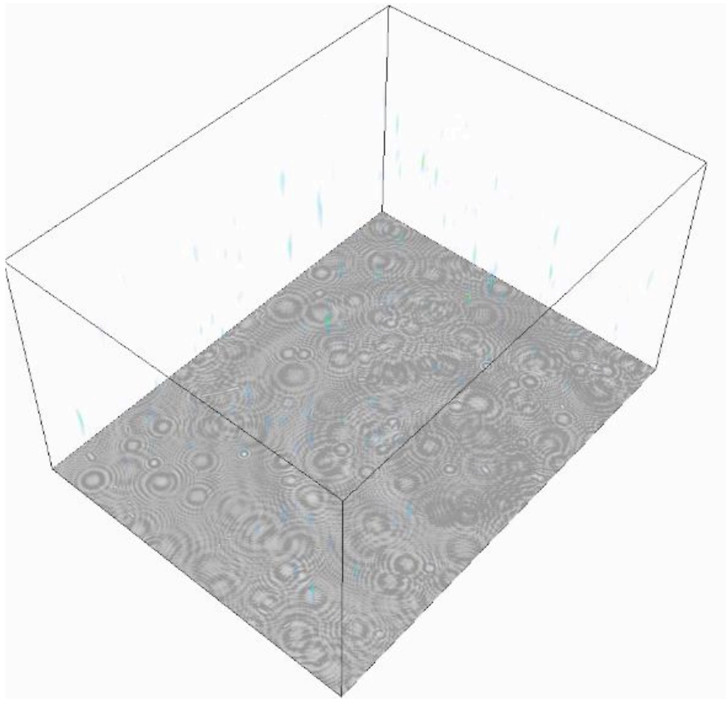
E. coli

$$\frac{I(x,y)}{|R(x,y)|^2} = 1 + \left|\frac{O(x,y)}{R(x,y)}\right|^2 + \frac{O(x,y)^* + O(x,y)}{|R(x,y)|} \quad \text{with} \quad R(x,y) = |R(x,y)|$$

Inline Reconstructions

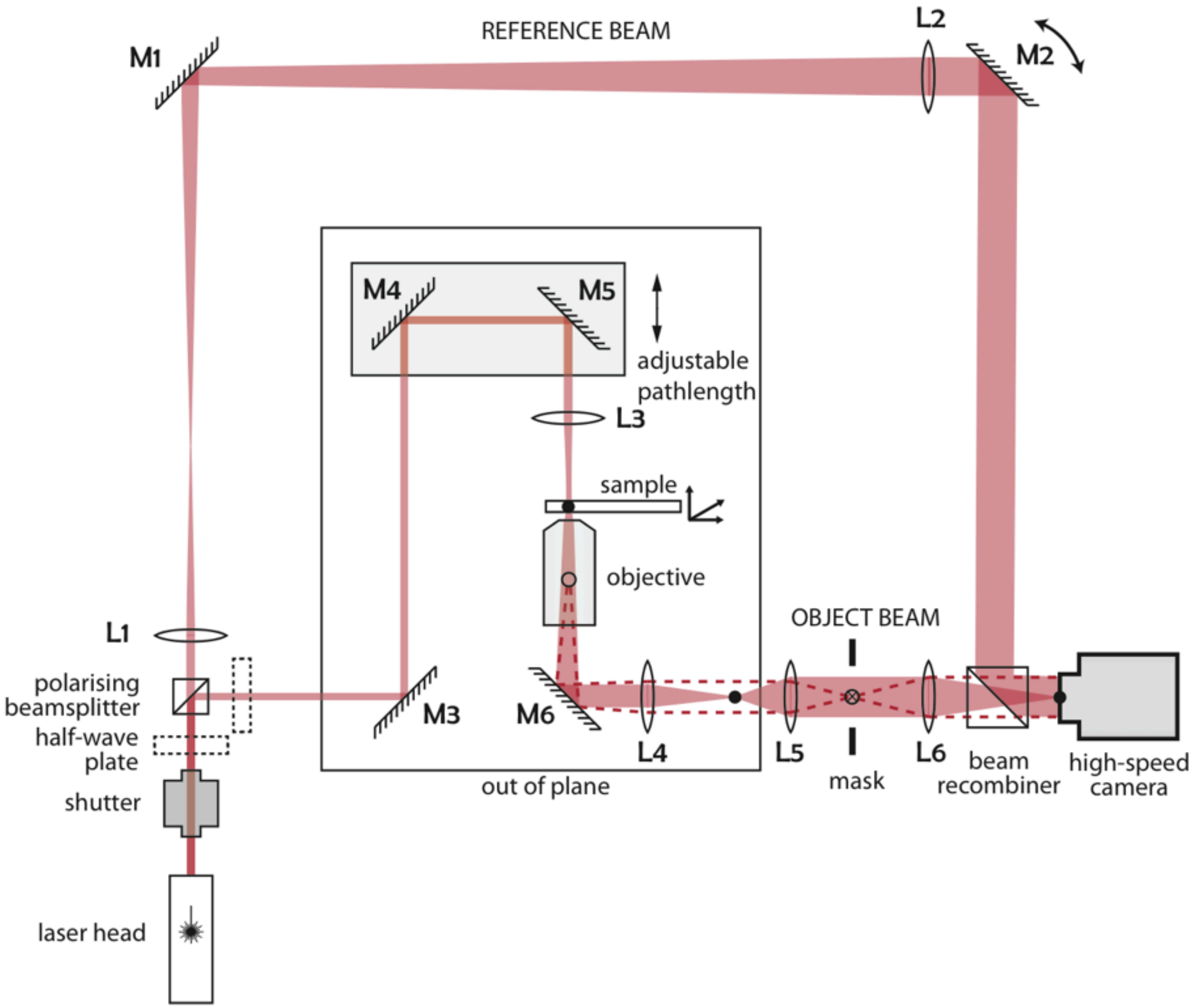


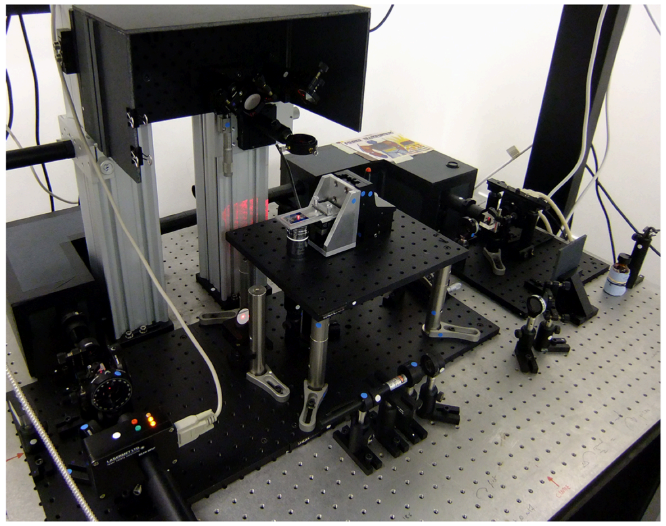
Diffusing 1 μm particles in a volume of 180 μm by 140 μm by 70 μm



Swimming *E. coli* cells in a volume of 200 μm by 160 μm by 100 μm

A Digital Holographic Microscope



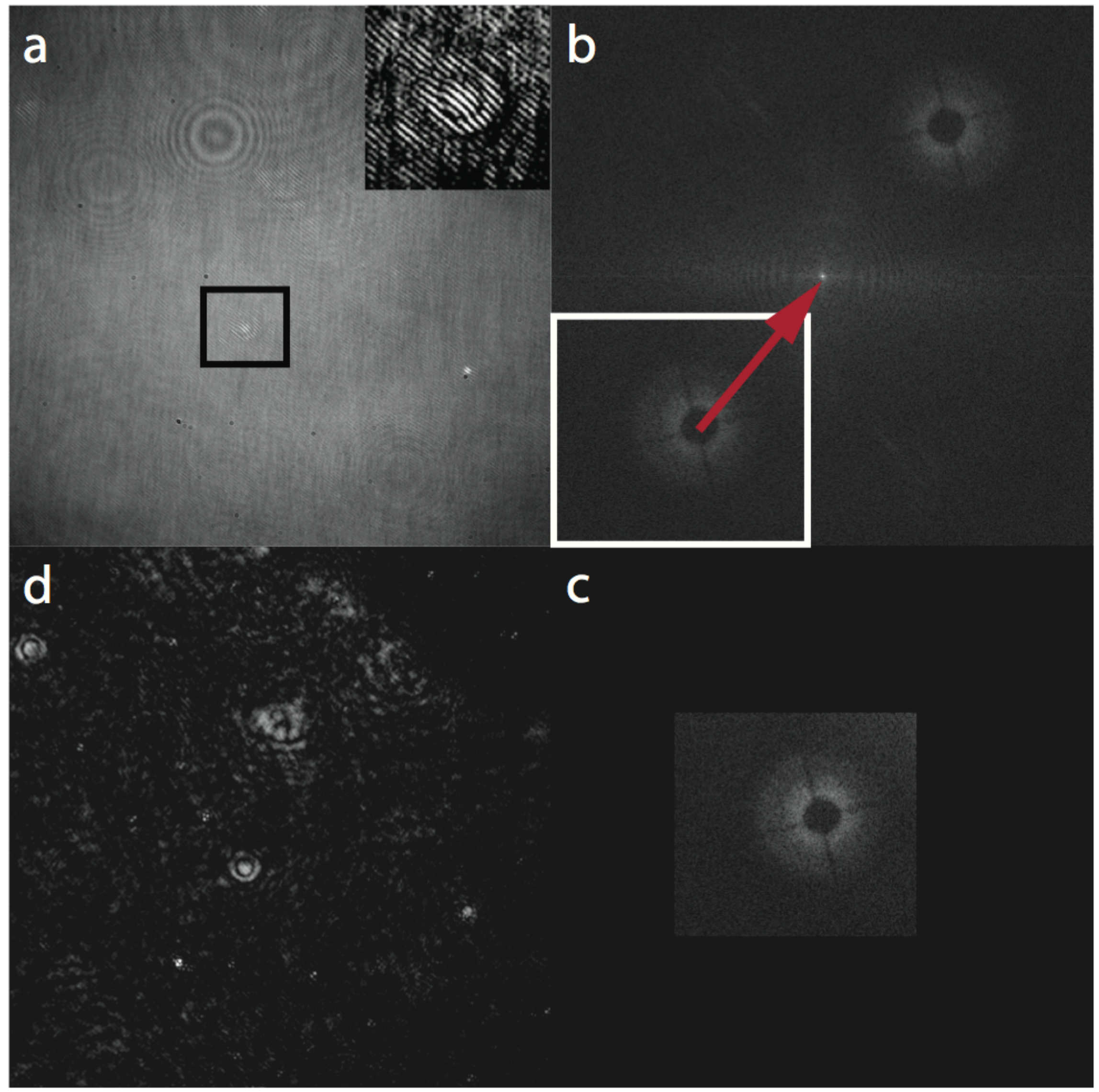


- ~60 nm spacing in x and y
- 2000 FPS at 512 px by 512 px

Off-axis Recording

Hologram

Fourier transform

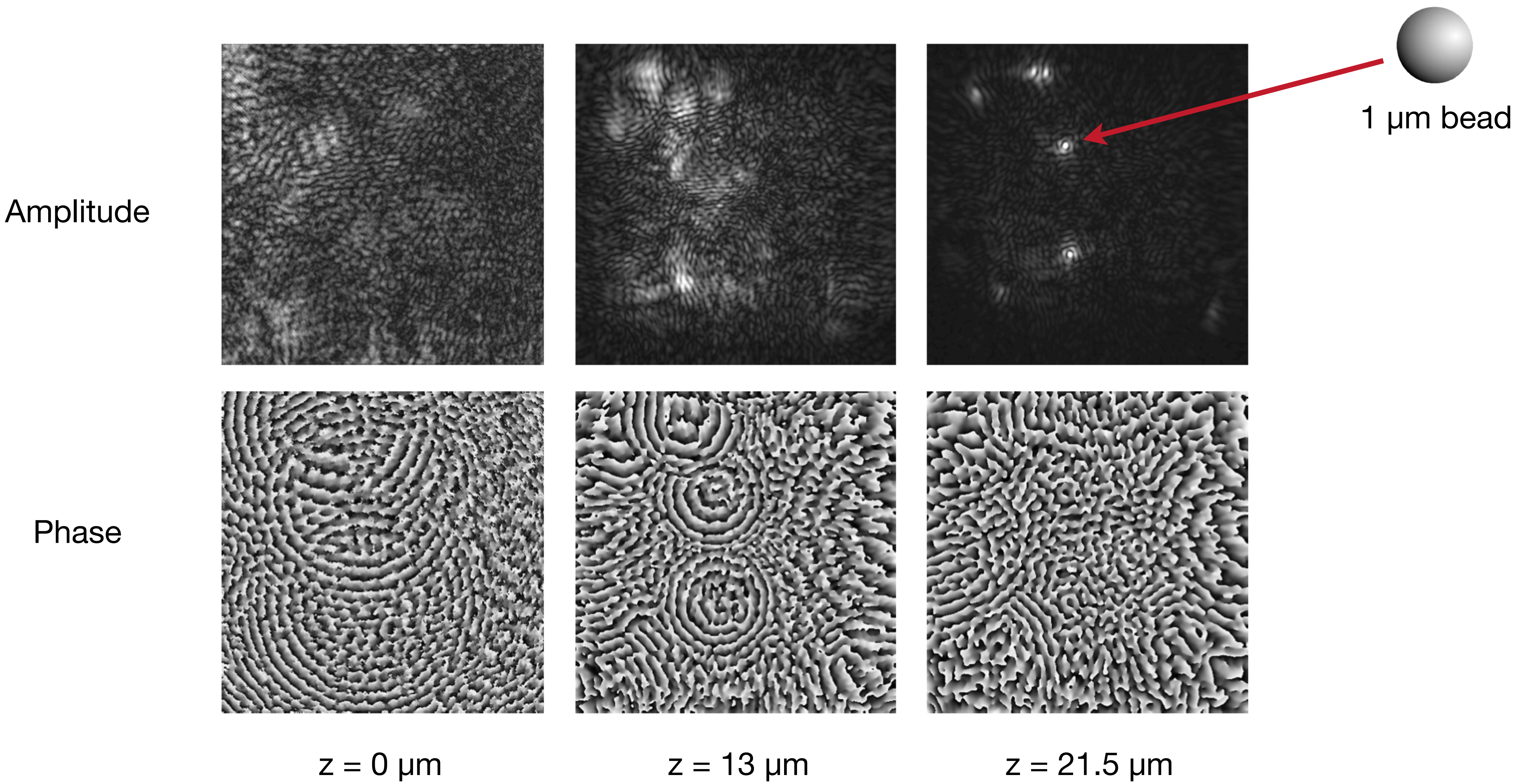


Filtered hologram

Cropped and shifted
Fourier transform

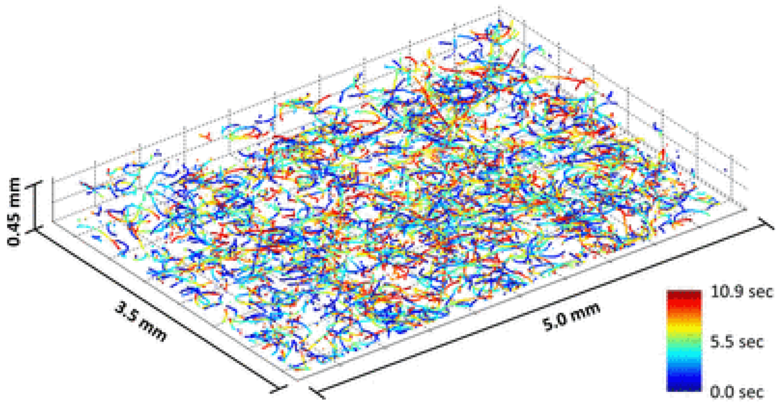
$$\frac{I(x,y)}{|R(x,y)|^2} = 1 + \left|\frac{O(x,y)}{R(x,y)}\right|^2 + \frac{e^{-ikx\sin\theta}O(x,y)^* + e^{ikx\sin\theta}O(x,y)}{|R(x,y)|} \quad \text{with} \quad R(x,y) = |R(x,y)|e^{-ikx\sin\theta}$$

Off-axis Reconstructions



Why You Should Bother...

Motility of human sperm



T.-W. Su *et al*, PNAS (2013)

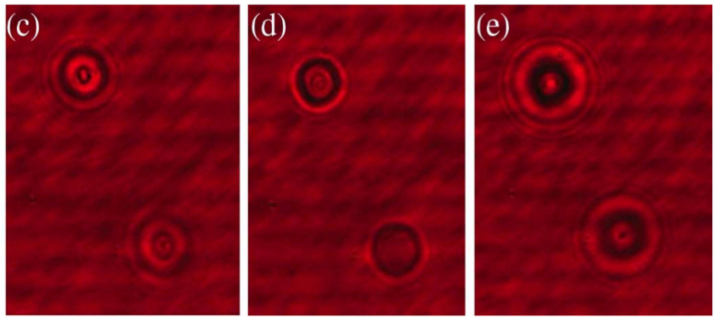
Fluid flow around a copepod

E. Malkiel *et al*, J. Exp. Bio. (2003)

3D imaging of biological samples

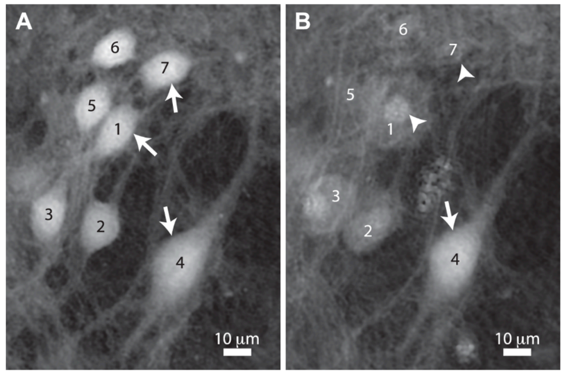
S. Bernet *et al*, Opt. Express (2011)

Differentiation of mature and immature blood cells



M. Mihailescu *et al*, Appl. Opt. (2011)

Detection of early neuron death



N. Pavillon *et al*, PLOS One (2012)

Summary

- 3D reconstruction of the amplitude and phase
- Objects are arbitrary
- As fast as your camera
- Simple and inexpensive (about £10K)

- Computing is slow (8 hours for 2000 frames at 512 px^3)
- Cannot get too dense (totally depends on scatterers)
- Fluorescence holography is embryonic (perhaps a reason to move into it...)