Techniques of Digital Holography For Microscopy and Metrology

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DHML: Digital Holography and Microscopy Laboratory

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outline

- DHM: Digital Holographic Microscopy
- OPU: Optical Phase Unwrapping
- TIRHM: Total Internal Reflection Holographic Microscopy
- DHM of Laser Microsurgery
- Some current projects
- Optical Tomography and Topography
- Conclusions

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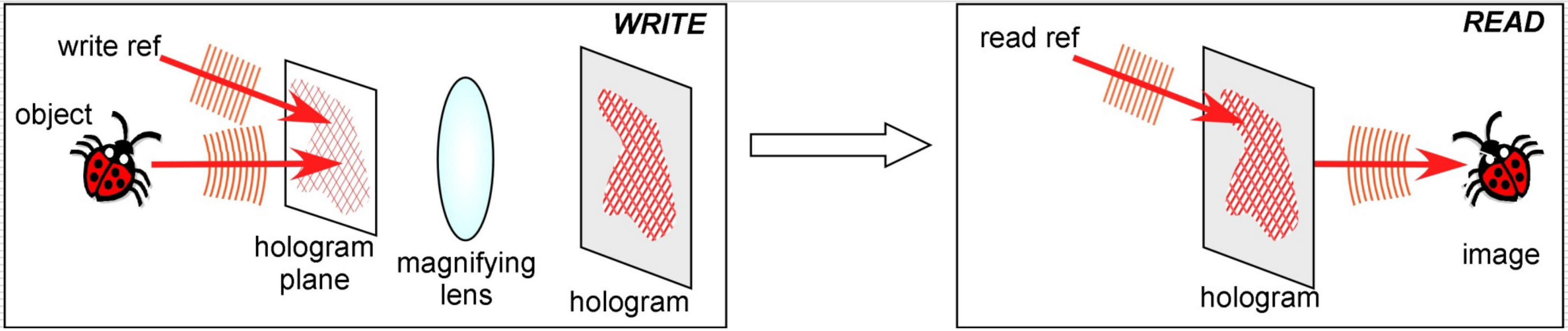
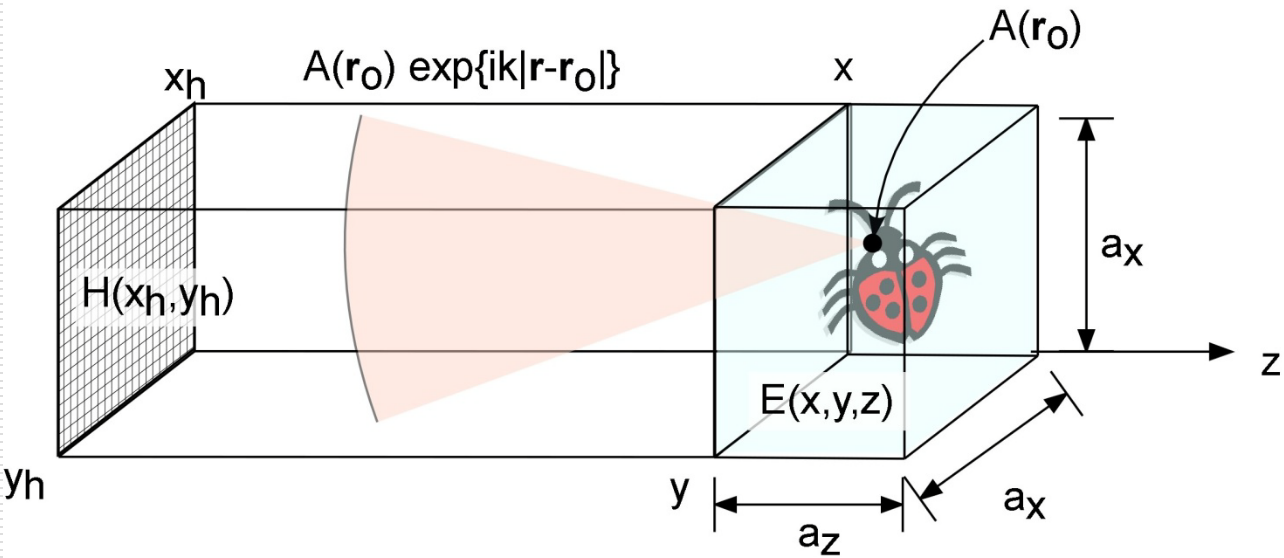
Holography



- Generation of holographic interference by superposition of object and reference waves
- Reconstruction of holographic image by illumination of the hologram with another reference wave

- Real space holography: optical generation & optical reconstruction
- Computer generated holography (CGH): numerical generation & optical reconstruction
- Digital holography (DH): optical generation & numerical reconstruction

digital holography: general geometry



$$E(x,y) = -\frac{ik}{2\pi} \iint_{\Sigma} dx_0 dy_0 E_0(x_0, y_0) \frac{e^{ikr}}{r}$$

M. K. Kim, "Principles and techniques of digital holographic microscopy," SPIE Reviews 1, 50 (2010).

numerical diffraction : angular spectrum method

- Angular spectrum at z = 0

$$A(k_x,k_y;0) = \mathfrak{F}\left\{E_0(x_0,y_0;0)\right\} = \iint E_0(x_0,y_0;0)\exp[-i(k_x x_0 + k_y y_0)]dx_0 dy_0$$

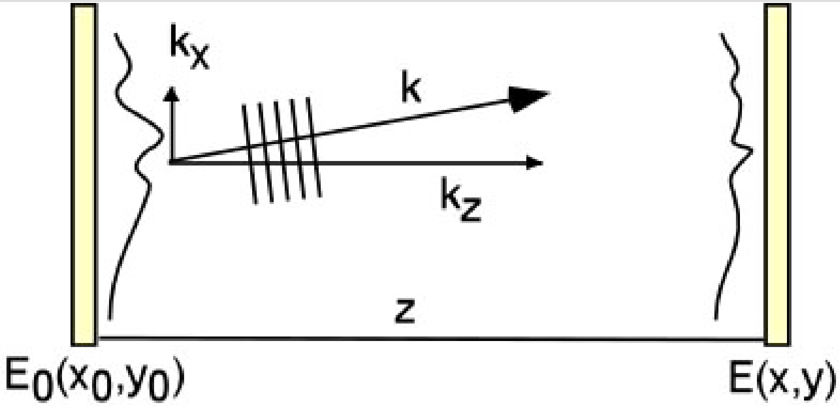
- Optical wave at z = 0

$$E_0(x_0,y_0;0) = \mathfrak{F}^{-1}\left\{A(k_x,k_y;0)\right\} = \iint A(k_x,k_y;0)\exp[i(k_x x_0 + k_y y_0)]dk_x dk_y$$

- Optical field at z

$$E(x,y;z) = \iint A(k_x,k_y;0)\exp\left[ik_z z\right]\exp[i(k_x x + k_y y)]dk_x dk_y$$

$$= \mathfrak{F}^{-1}\left\{\mathfrak{F}\left\{E_0\right\}\exp\left[ik_z z\right]\right\}$$

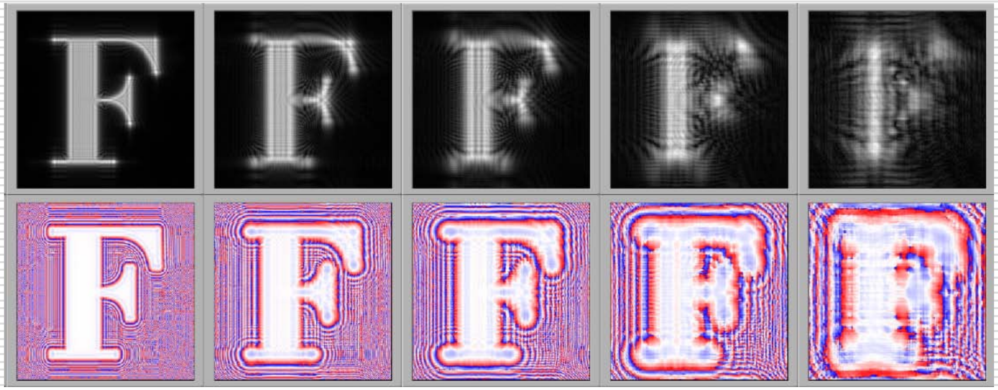


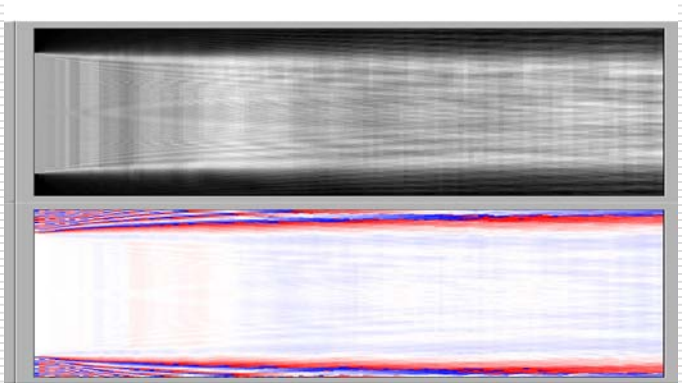
$$k_z = \sqrt{k^2 - k_x^2 - k_y^2}$$

angular spectrum method

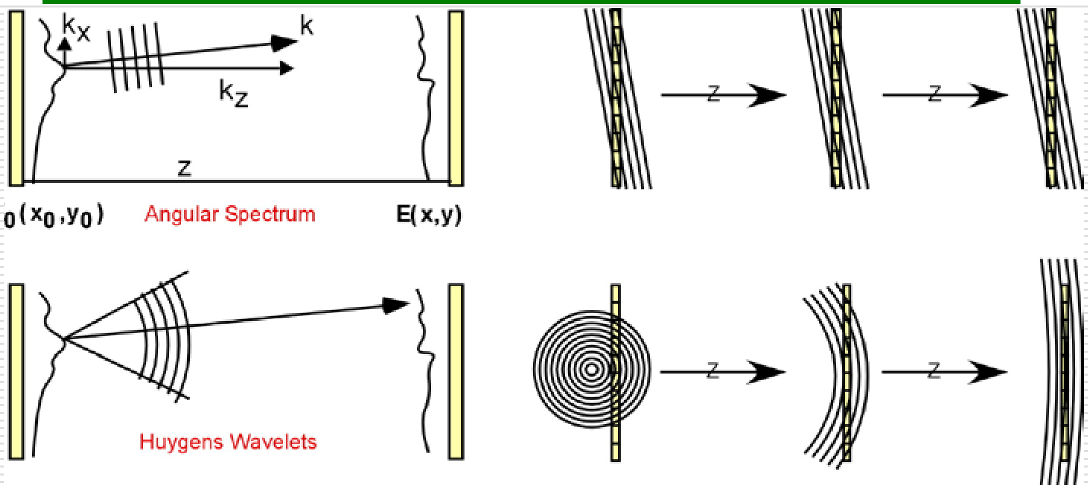
- plane wave decomposition
- two (2) Fourier transforms
  - one at z = 0, and
  - one at any arbitrary z
- no minimum distance requirement

- 3 peaks for off-axis holography
  - 0-order term: ref. and obj.
  - +/- 1st order: twin image terms
- numerical filtering of angular spectrum to suppress zero-order and twin image
  - noise control
- physical filtering by placing an aperture at a Fourier plane of hologram



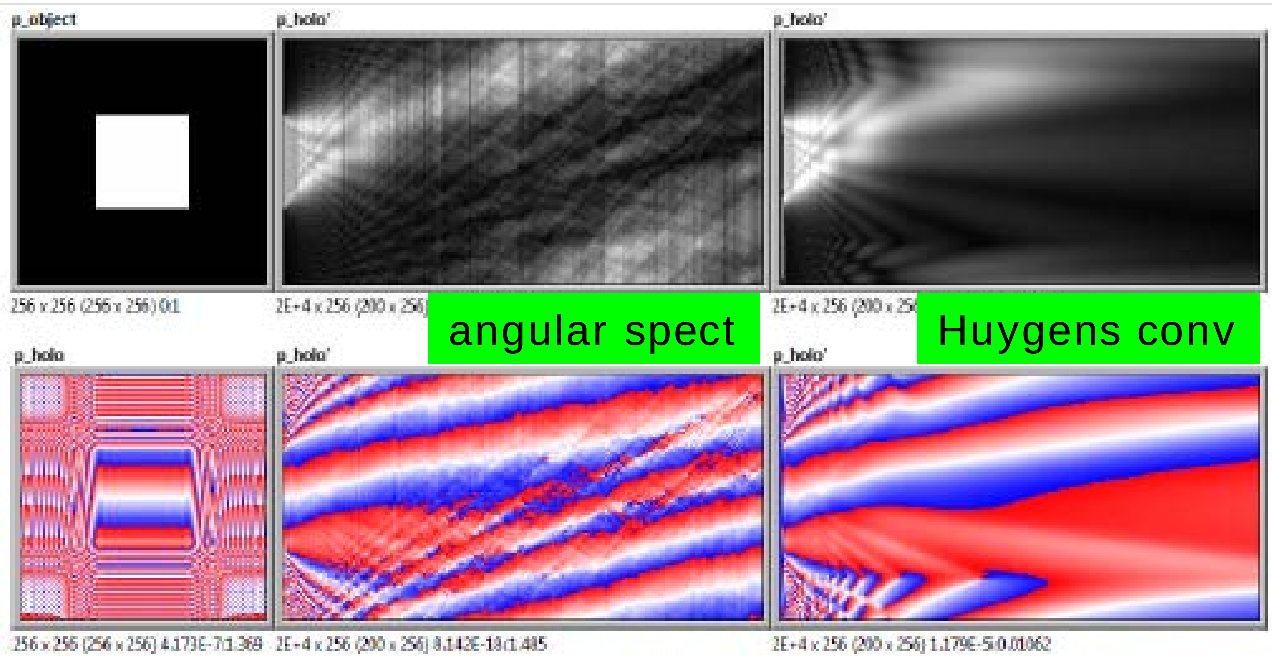


minimum & maximum distances



$$z_{\min} = X_0 \sqrt{\left(\frac{2\delta x}{\lambda}\right)^2 - 1}$$

$$z_{\max} = \frac{X_0^2}{2\lambda}$$

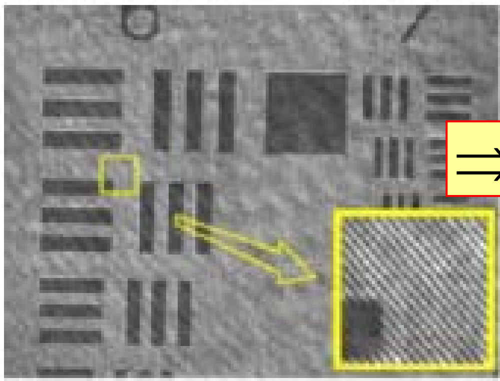


$z = 0 \sim 20{,}000\ \mu\text{m}$

digital holography procedure

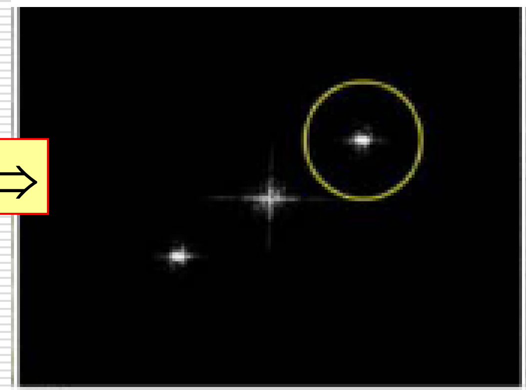
- HH': hologram
- FF: angular (Fourier) spectrum
- multiply propagation factor
- EEa: amplitude image
- EEp: phase image

$$E(x, y; z) = \mathfrak{F}^{-1}\left\{\mathfrak{F}\left\{E_0\right\}\exp\left[ik_z z\right]\right\}$$



HH'

$$\Rightarrow FFT \Rightarrow$$

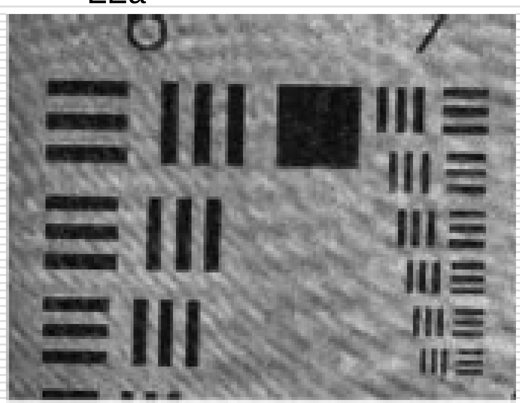


FF

$$\times \exp\left(ik_z z\right)$$

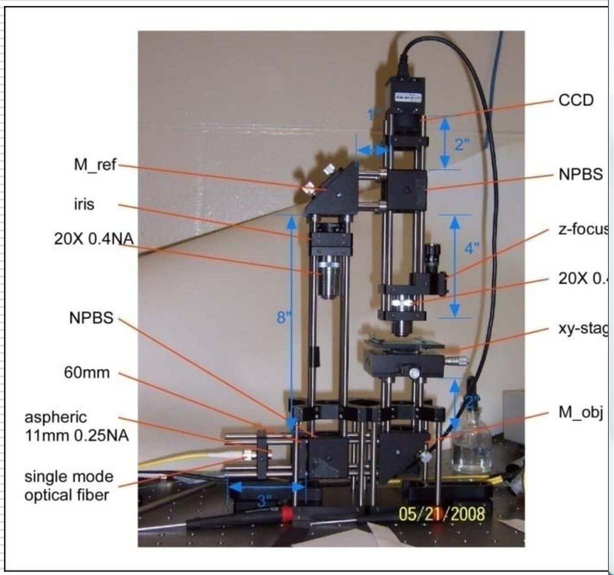
$$\Rightarrow FFT^{-1} \Rightarrow$$

EEa

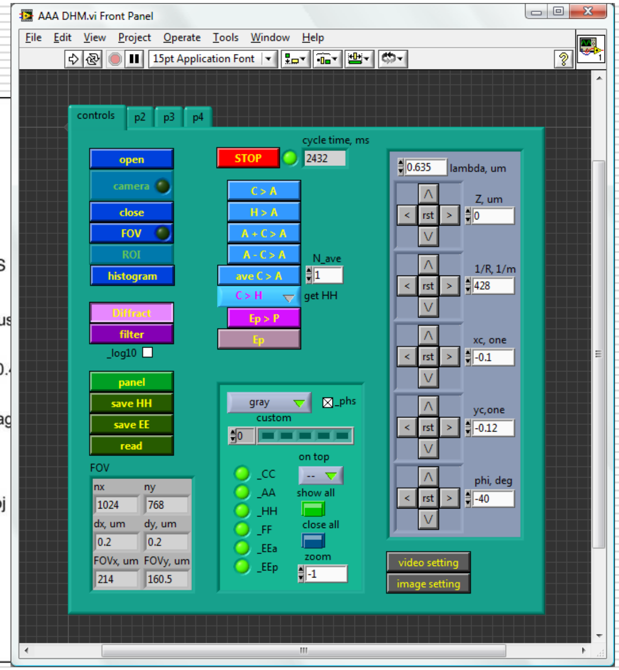


EEp

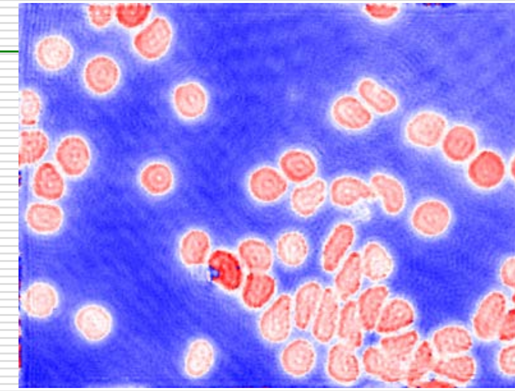
DHM.vi demo

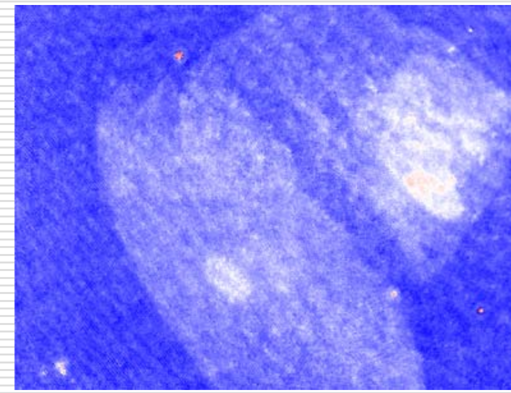


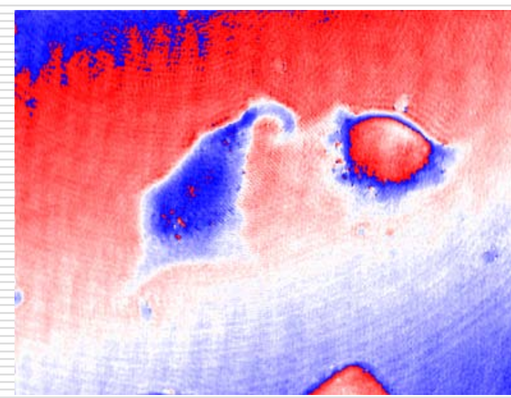
CCD
NPBS
z-focus
M_obj
M_ref
iris
20X 0.4NA
NPBS
60mm
aspheric
11mm 0.25NA
single mode
optical fiber
2"
4"
8"
3"
05/21/2008

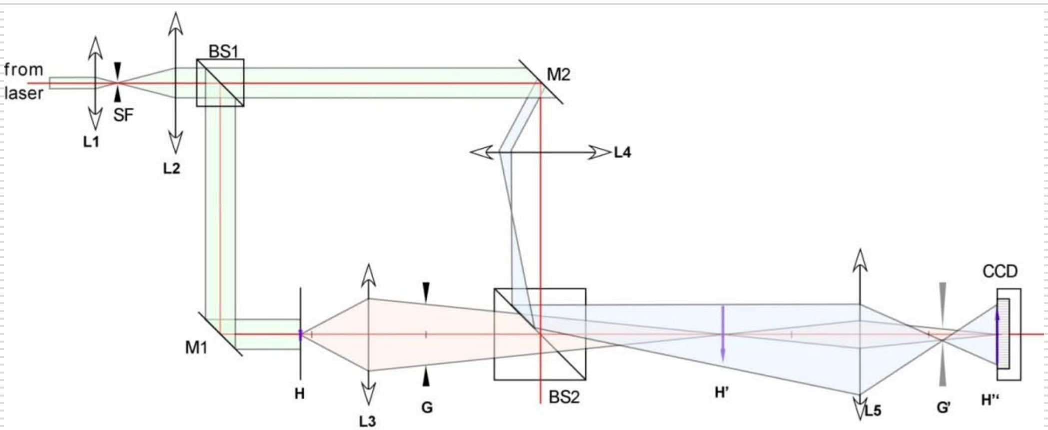


AAA DHM.vi Front Panel
File Edit View Project Operate Tools Window Help
15pt Application Font
controls p2 p3 p4
open
camera
close
FOV
ROI
histogram
Diffract
filter
_log10
panel
save HH
save EE
read
FOV
nx ny
1024 768
dx, um dy, um
0.2 0.2
FOVx, um FOVy, um
214 160.5
STOP
cycle time, ms
2432
C > A
H > A
A + C > A
A - C > A
N_ave
ave C > A
1
C > H
get HH
Ep > P
Ep
gray
_phs
custom
0
on top
_CC
_AA
_HH
_FF
_EEa
_EEp
show all
close all
zoom
-1
0.635
lambda, um
Z, um
0
1/R, 1/m
428
xc, one
-0.1
yc,one
-0.12
phi, deg
-40
rst
video setting
image setting

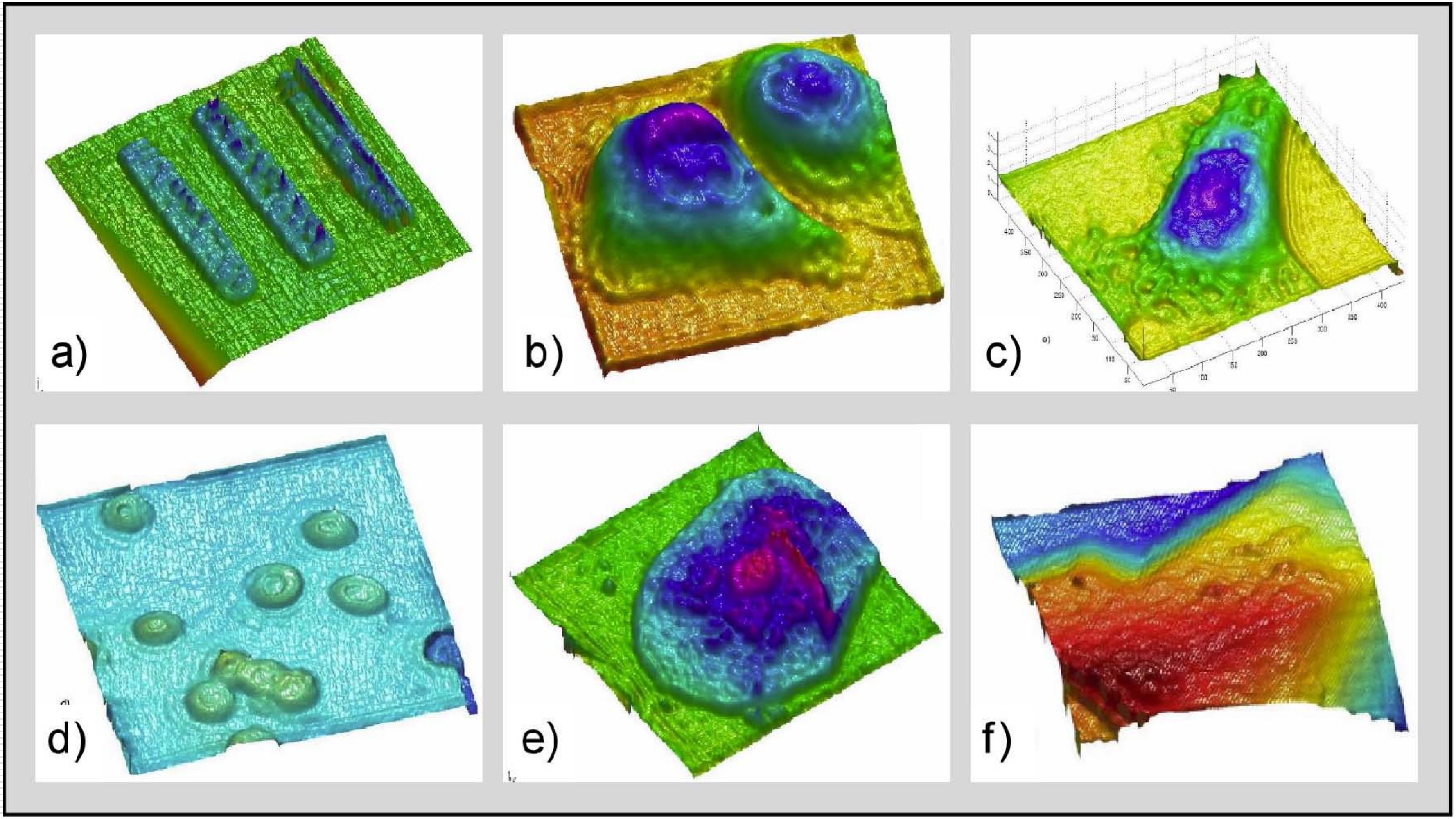








from
laser
L1
SF
L2
BS1
M2
L4
M1
H
L3
G
BS2
H'
L5
G'
H''
CCD



C.J. Mann, L.F. Yu, C.M. Lo, and M.K. Kim, "High-resolution quantitative phase-contrast microscopy by digital holography". Optics Express, 13: p. 8693-8698 (2005).

outline

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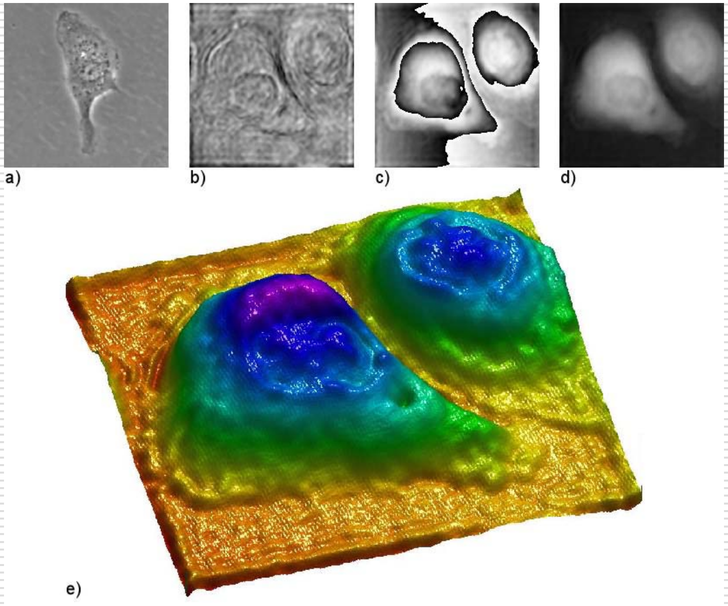
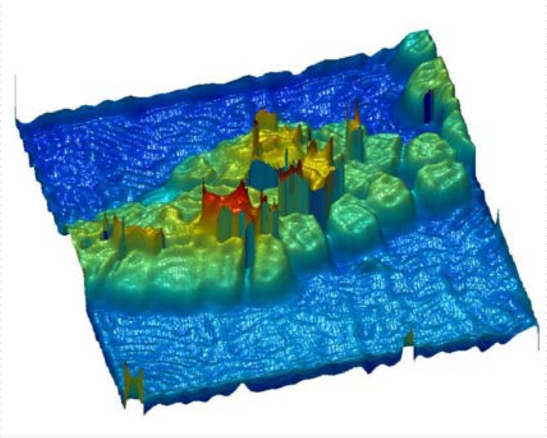
Unwrapping of 2pi-discontinuities in phase images

Software-unwrapping

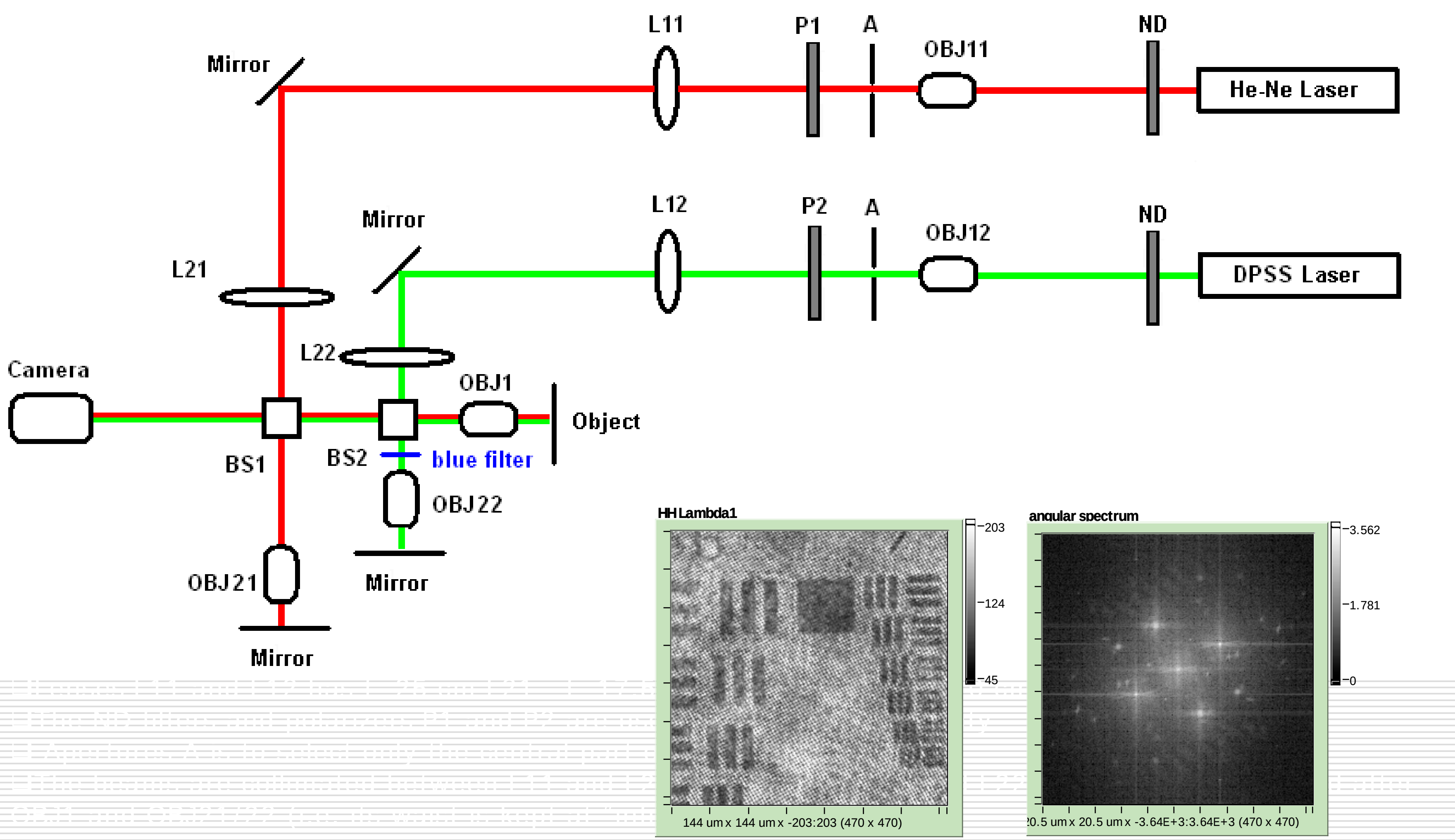
- Detection of discontinuities & addition of integer-multiples of 2pi
- Subjective and ambiguous topologies
- Very computation intensive: ~5 minutes per image for images presented here



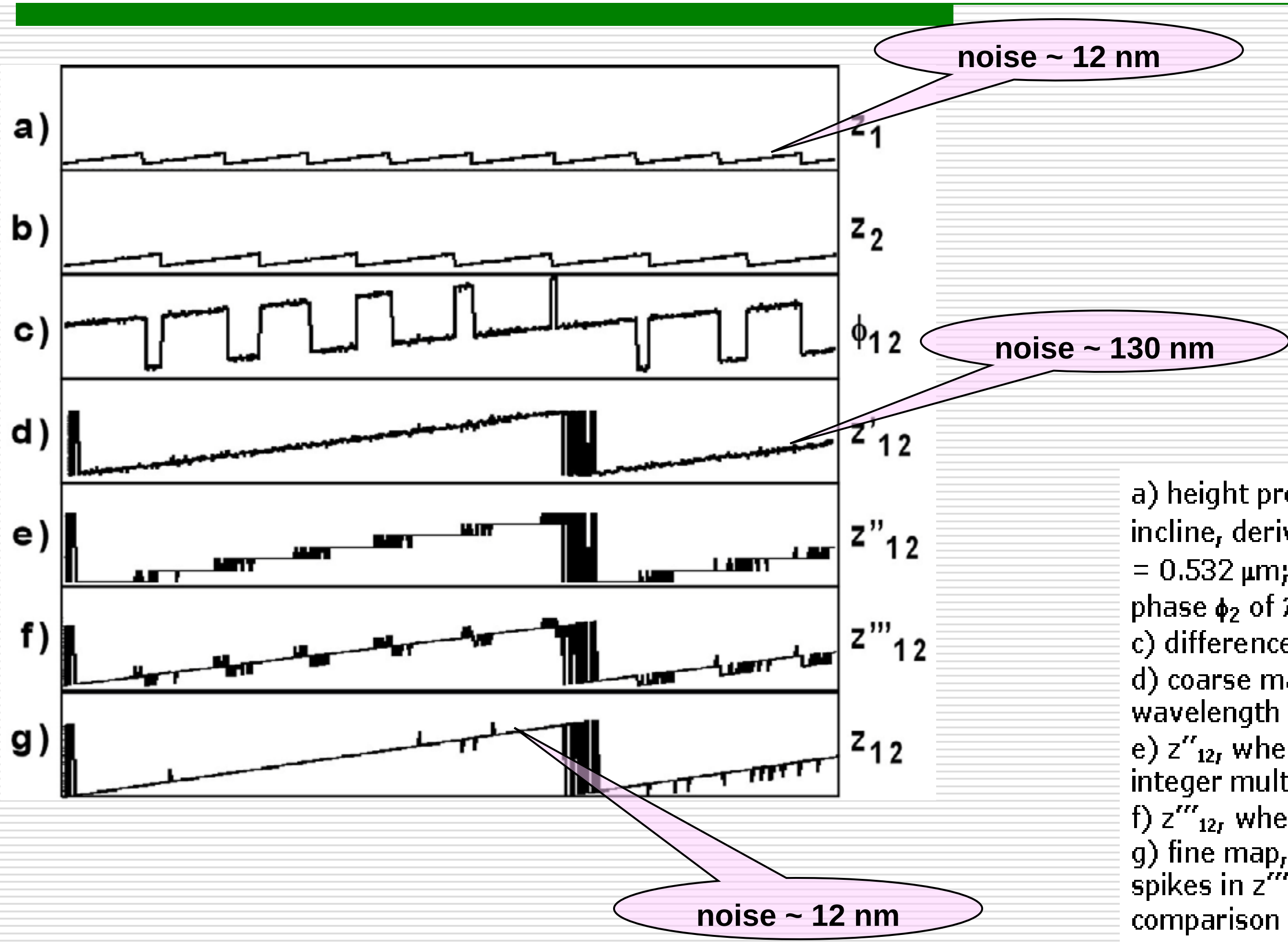


- **Optical phase-unwrapping**
- Two- or three-wavelength interference technique
- Completely deterministic
- Very fast ~100 ms
- Light computational load

Multi-wavelength Digital Holography Apparatus



Dual-wavelength phase imaging digital holography



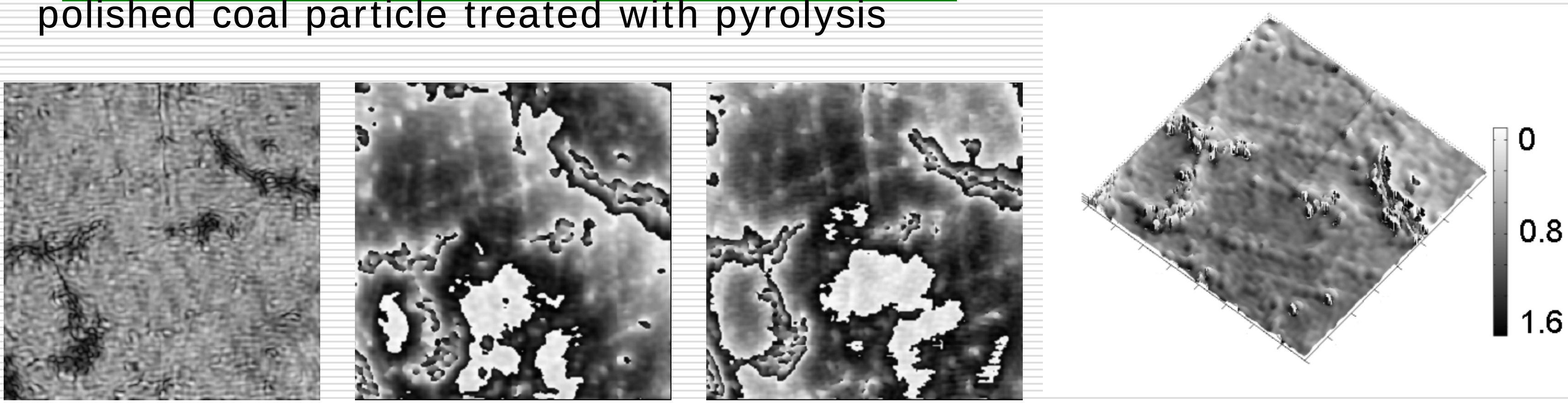
a) height profile z_1 of a 10 μm high incline, derived from phase ϕ_1 of λ_1 = 0.532 μm; b) z_2 derived from phase ϕ_2 of λ_2 = 0.633 μm
c) difference phase map $\phi_{12} = \phi_1 - \phi_2$
d) coarse map, z'_{12}, with beat wavelength Λ = 3.33 μm
e) z''_{12}, where z'_{12} is divided into integer multiples of λ_1
f) z'''_{12}, where z_1 is pasted on z''_{12}
g) fine map, z_{12}, where most of the spikes in z'''_{12} are removed by comparison with z'_{12}.

The vertical axis is 5.0 μm full scale in every panel, except for c) where the vertical range is -2π to $+2\pi$.

$$\Lambda_{12} = \frac{\lambda_1 \lambda_2}{|\lambda_1 - \lambda_2|}: \text{ beat wavelength(Axial range)}$$

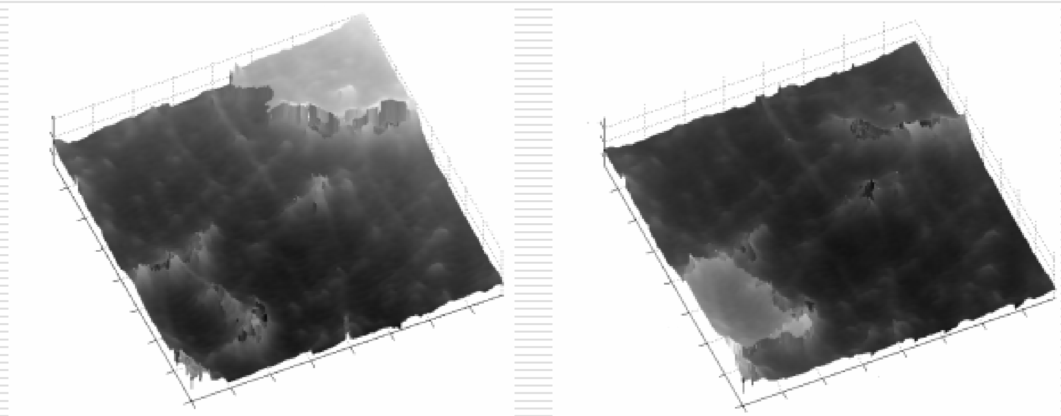
QPM by DH: - OPU vs. software unwrapping

polished coal particle treated with pyrolysis



(a) (b) (c) (d)

(a) amplitude image; phase maps reconstructed with (b) 633 nm and (c) 532 nm; (d) 3D rendering of the dual-wavelength phase map (vertical scale in microns);
software unwrapped phase maps reconstructed with (e) 633 nm and (f) 532 nm for comparison. All images are 78x78 microns (256x256 pixels).

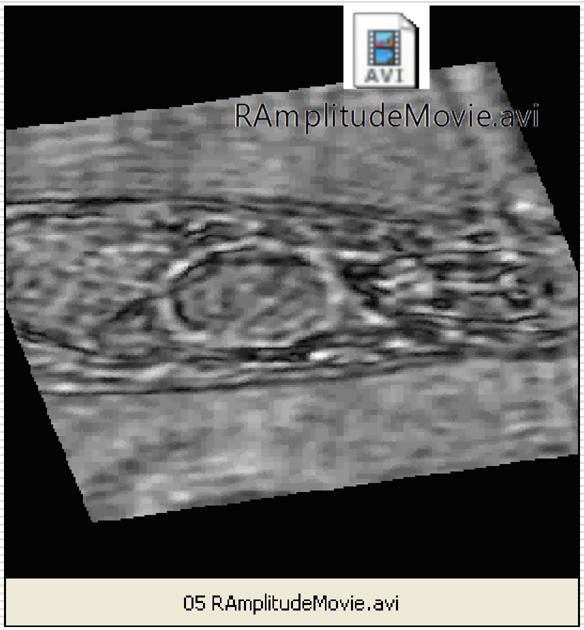


(e) (f)

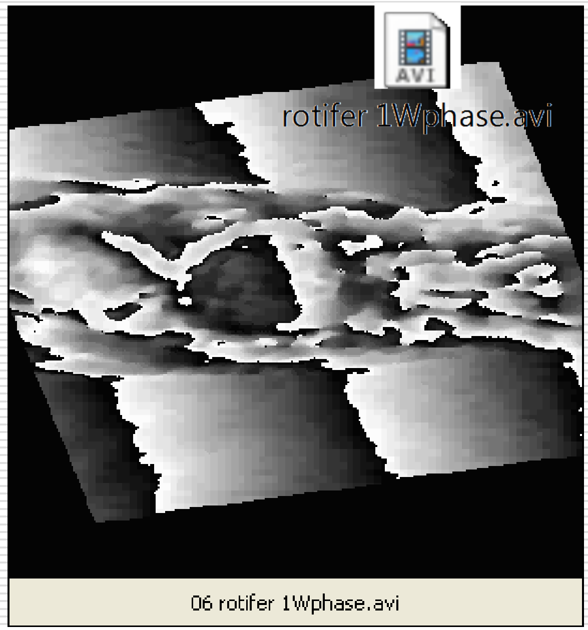
A. Khmaladze, A. Restrepo-Martinez, M. Kim, R. Castaneda, and A. Blandon, "Simultaneous dual-wavelength reflection digital holography applied to the study of the porous coal samples". Applied Optics, 47: p. 3203-3210 (2008).

Dual-wavelength PIDH: movie of live rotifer

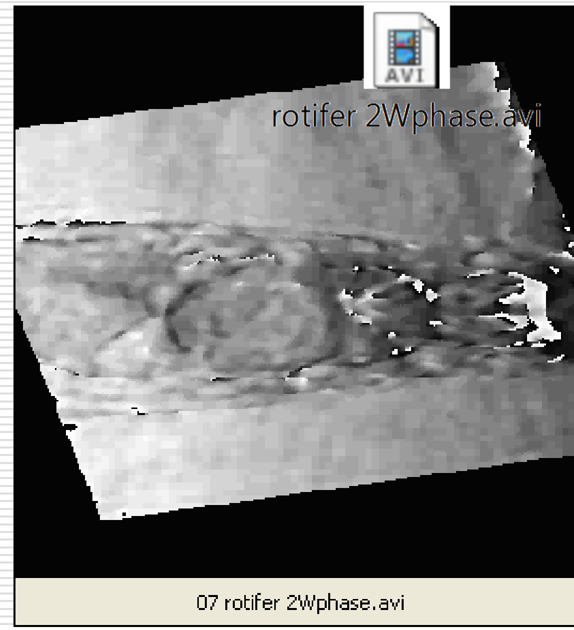
amplitude



1W PIDH



2W PIDH



area = 70µm x 70µm
pixels = 360 x 360
z = 79~80µm

C.J. Mann, L.F. Yu, and M.K. Kim, "Movies of cellular and sub-cellular motion by digital holographic microscopy". Biomed. Eng. Online, 5 (2006).

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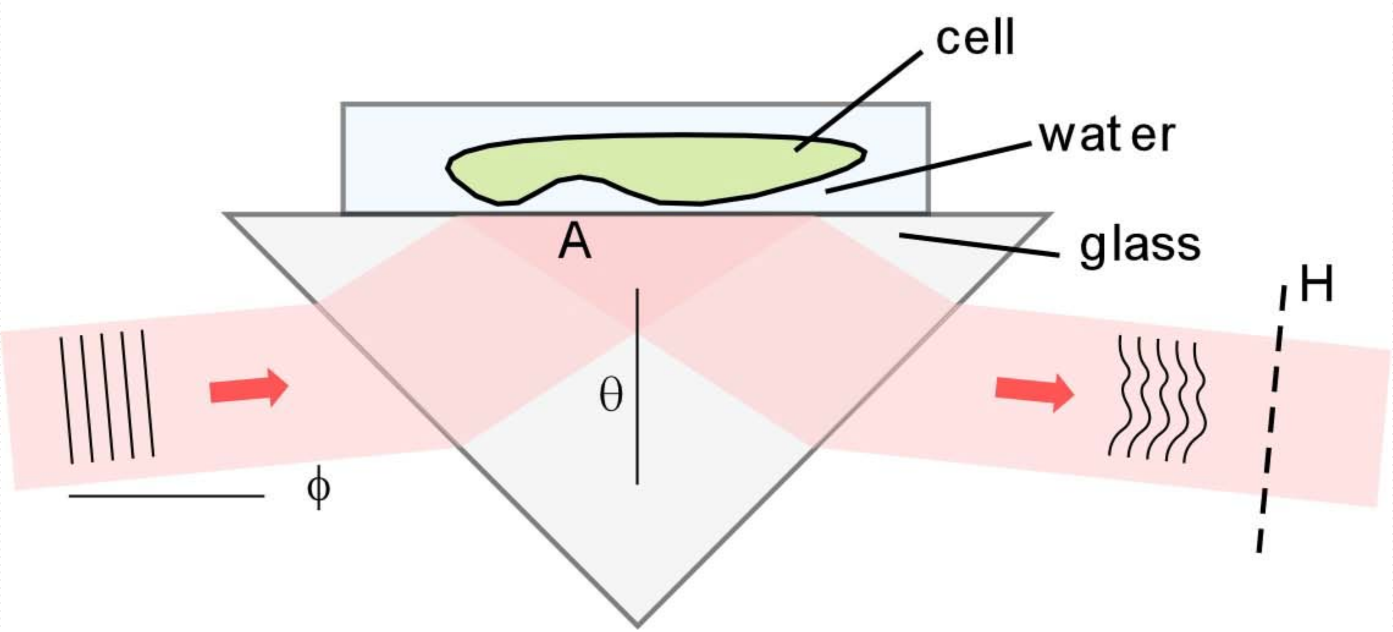
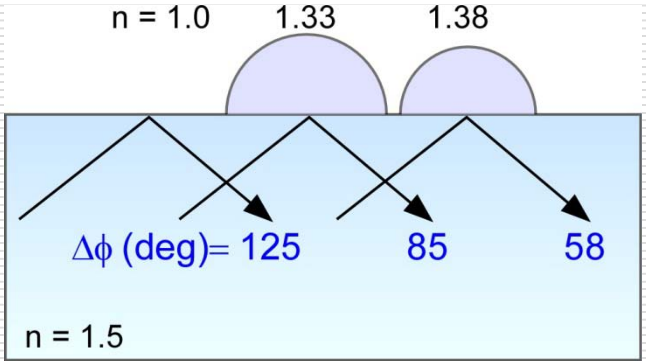
TIRHM: total internal reflection holographic microscopy

- modulation of TIR wavefront by variation of interface
- detection and imaging of modulated wavefront by DH
- quantitative profiling of interface

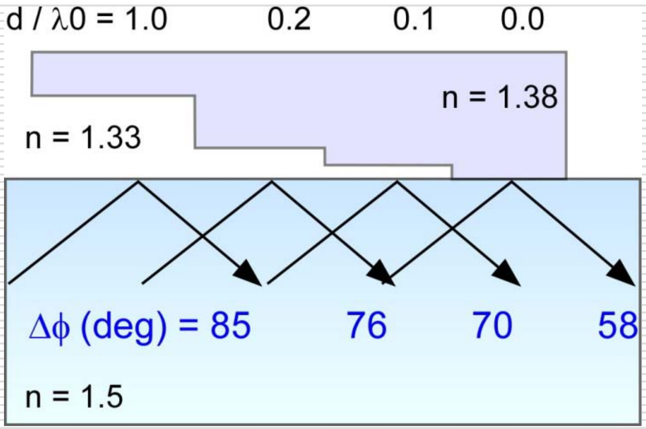


W. M. Ash, and M. K. Kim, "Digital holography of total internal reflection," Optics Express 16, 9811-9820 (2008).

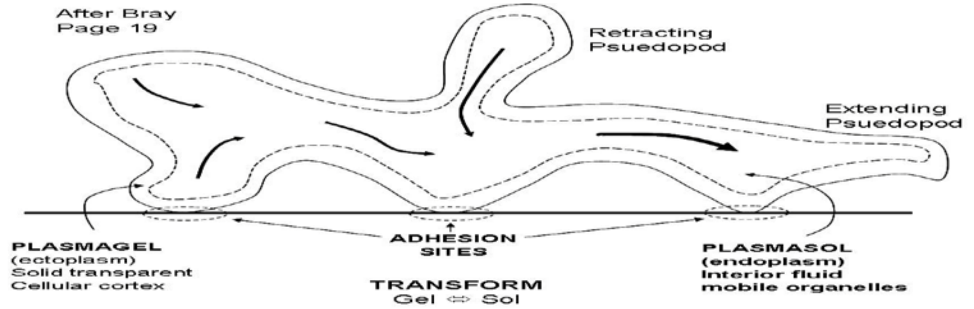
TIRHM imaging modes



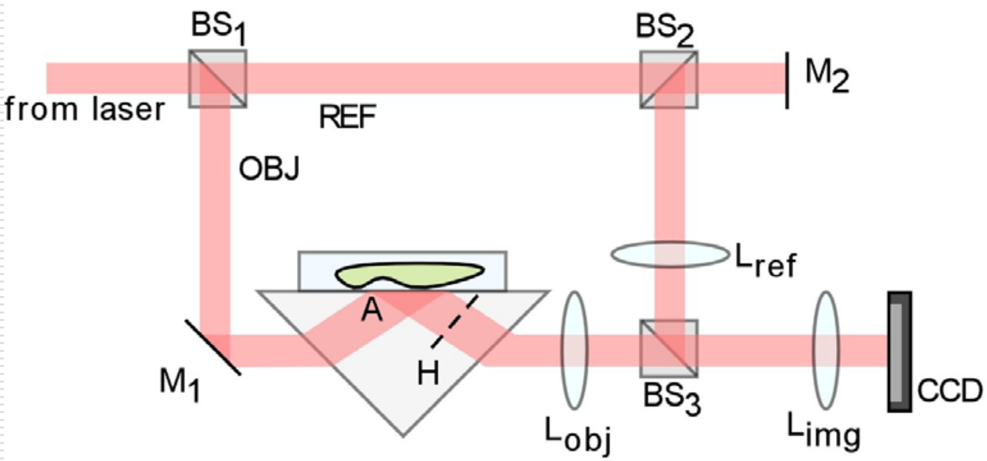
index profile

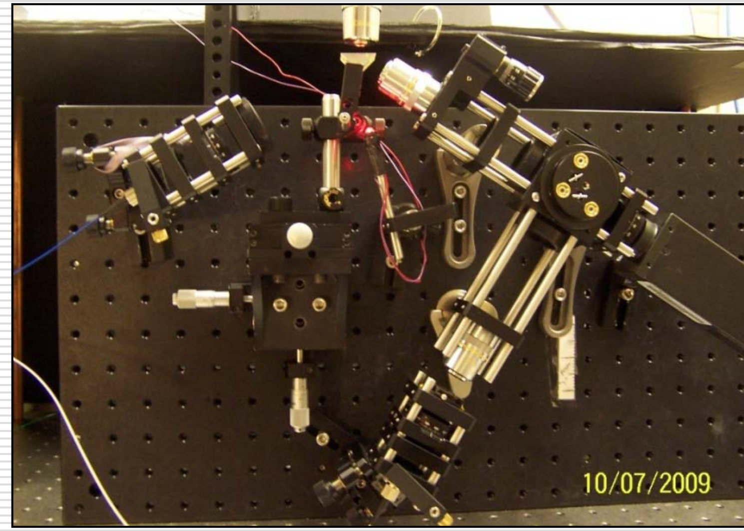


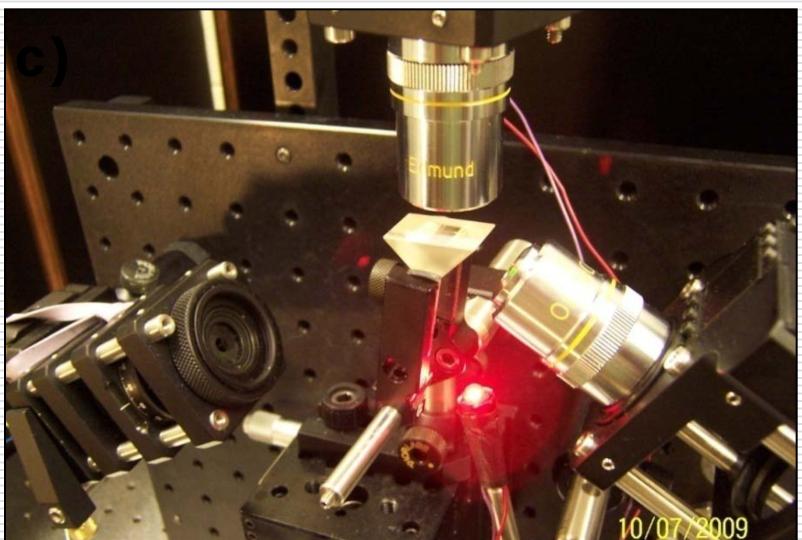
thickness profile



TIRHM Apparatus





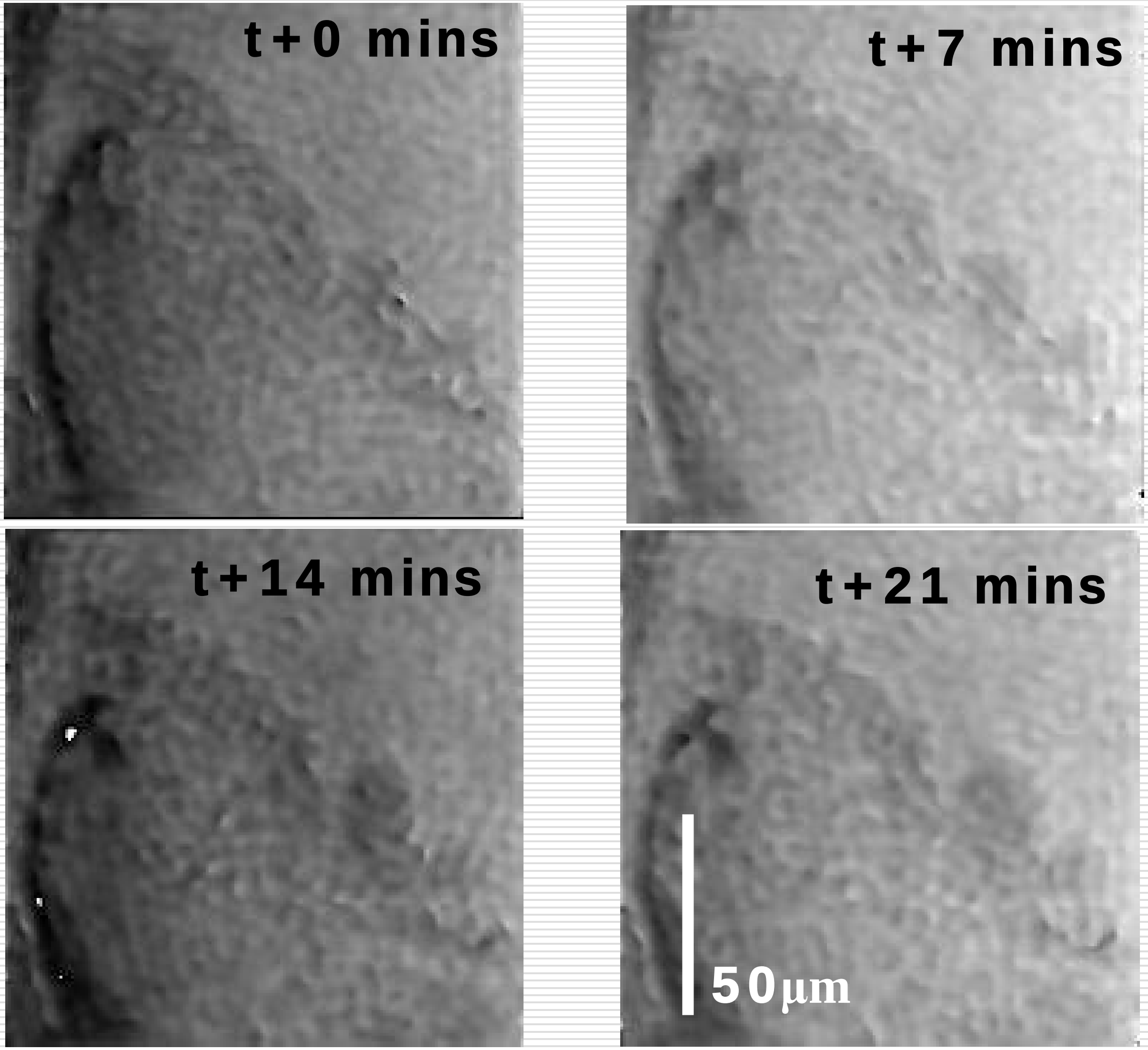


a) Schematic
- Laser- DPSS Nd:YAG @ 532nm
- Camera- Sony Firewire CCD

b) Operational system

c) Close-up of TIR prism
- w/ 10x microscope objectives

Amoeba Sample: Time lapse phase imagery



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laser cellular microsurgery apparatus

- Nd:YVO4 laser for microsurgery: 0.4uJ/pls, 12ns, 20kHz, 10e9W/cm2
- LD for holographic microscopy

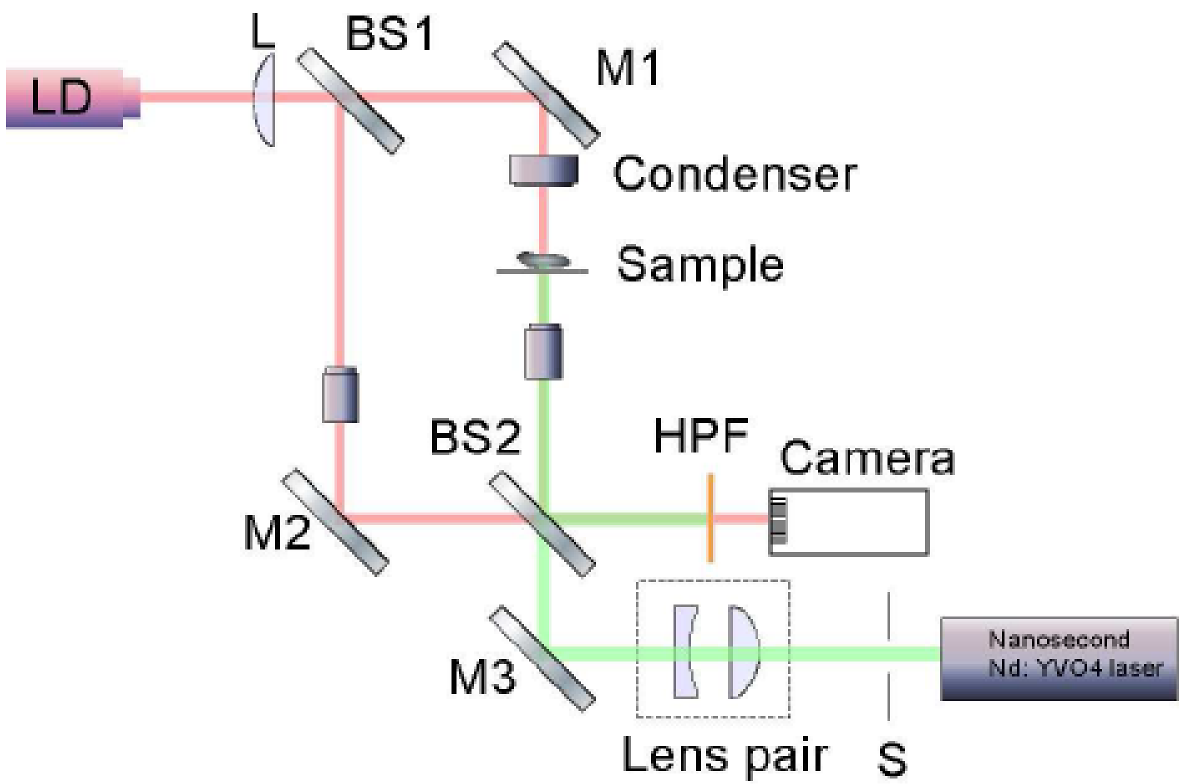
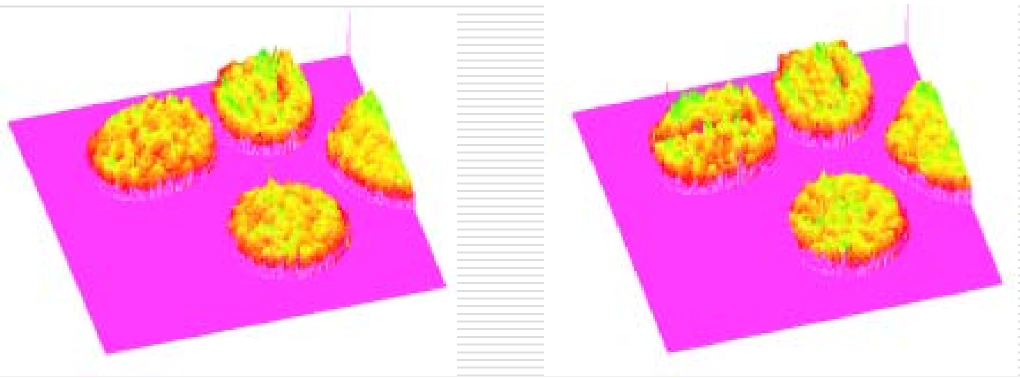


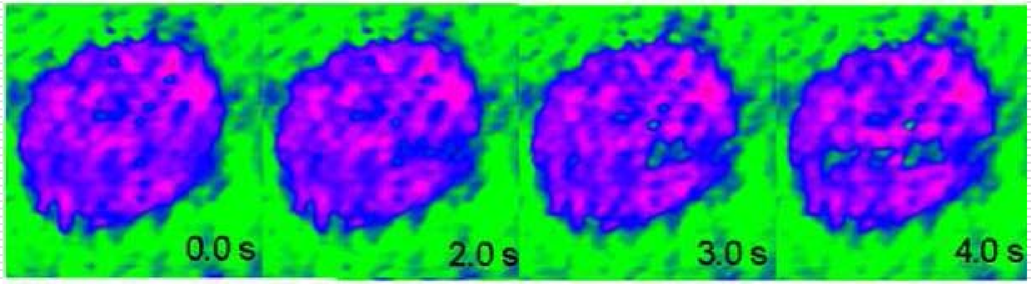
Fig. 1. Schematic of the experimental setup. LD is the 675nm laser diode; BS1 and BS2 are beam splitters; Ms are mirrors; S is a shutter; L is a lens; HPF is a high-pass filter for red light.

quantitative phase microscopy of cellular laser microsurgery

- laser microbeam
  - study cell structure and function
  - injection of exogenous materials
  - free the internal constituents
  - study developing organisms—such as in the formation of the nervous system
  - reduce the thickness of the zona pellucida layer of the ovum to improve human in vitro fertility

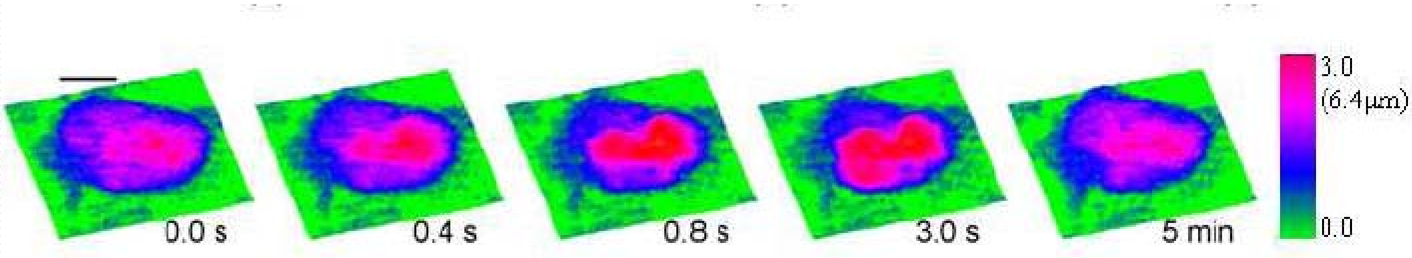
- quantitative phase evaluation by DHM during laser incision of red blood cells





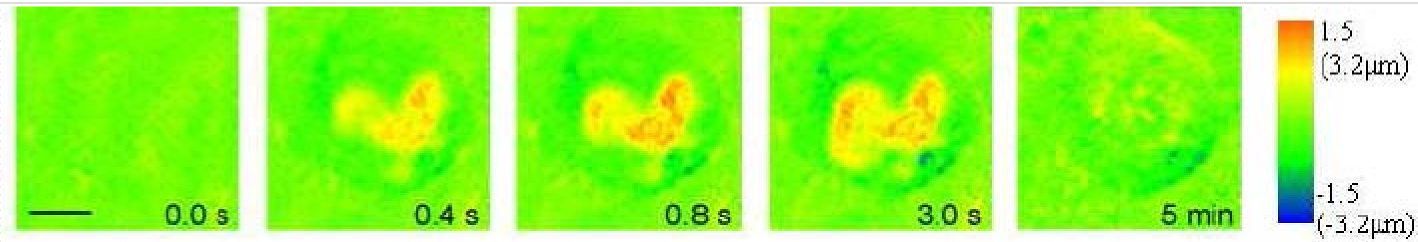
DHM movies of laser microsurgery

- rat kangaroo kidney epithelial (PTK2) cells:



movie1.mov

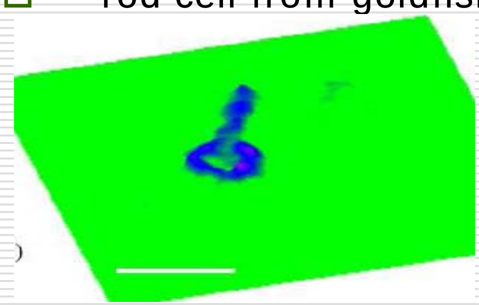
phase image movie during surgery



movie2.mov

phase change movie : swelling of chromosomes during ablation, with subsequent recovery after laser is removed.

- rod cell from goldfish retina: drastic morphological change following a slight damage of one end of cell



movie5.mov

L Yu, S Mohanty, J Zhang, S Genc, MK Kim, MW Berns, Z Chen, "Digital holographic microscopy for quantitative cell dynamic evaluation during laser microsurgery," Opt. Express 17, 12031-12038 (2009)

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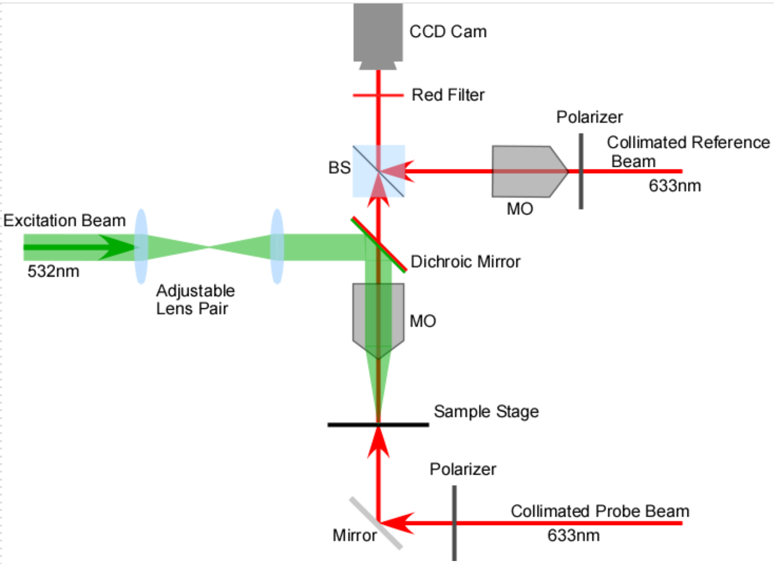
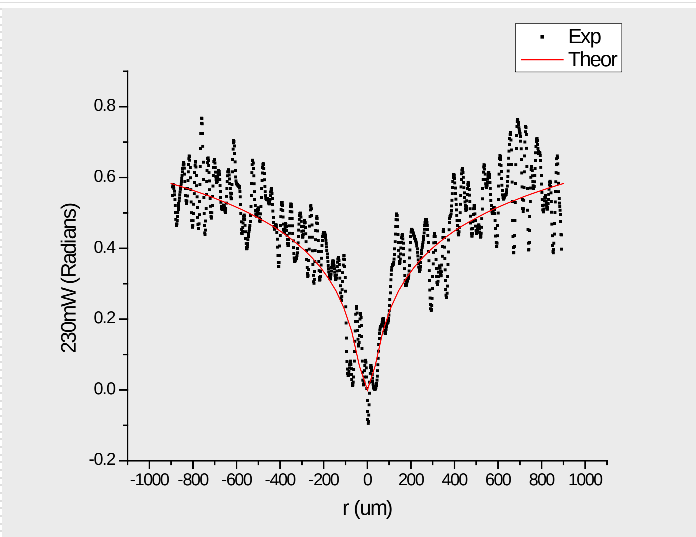
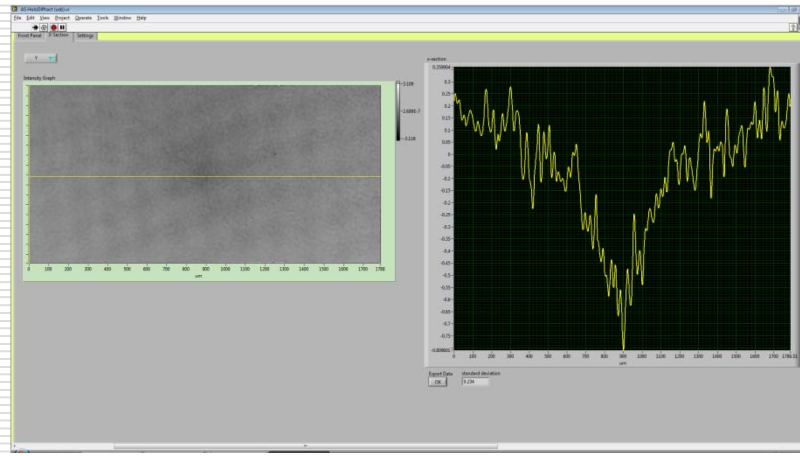
Thermal Lensing and Absorption Coefficient



$$\phi = \theta \int_0^t \frac{1}{1+2t'/\tau}\left[1-\exp\left(-\frac{2r^2/w^2}{1+2t'/\tau}\right)\right]\frac{dt'}{\tau}$$

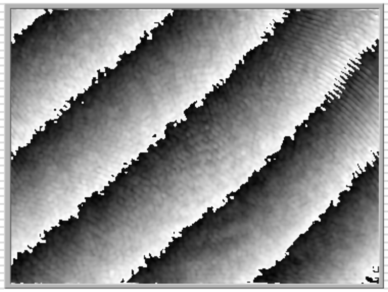
$$\theta = -\frac{P\alpha l(dn/dT)}{\kappa\lambda}$$



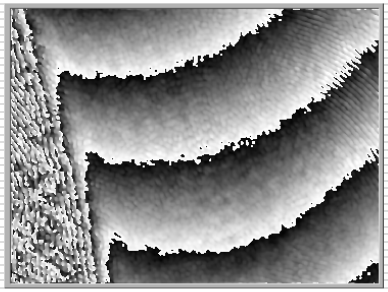


Absorption coefficients of 4.5 x 10^{-4} and 3.8 x 10^{-4} cm^{-1} (@532nm) were successfully determined for our samples of methanol and ethanol,

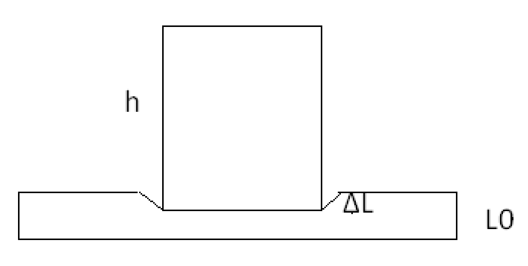
Deformation and Young's Modulus Measurement of Thin Film



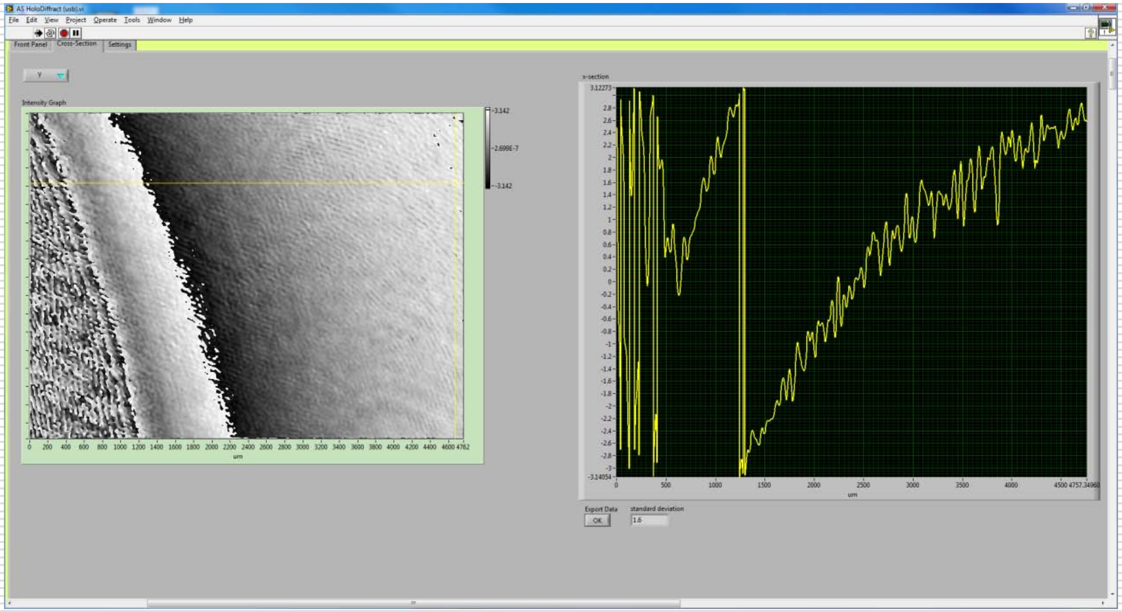
143.4 x 107.5 (1024 x 768) -3.142:3.142



143.4 x 107.5 (1024 x 768) -3.142:3.142



2 mm dia x 0.5 mm height brass plate (13.5 mg) on 1 mm thick polyacrylamide film



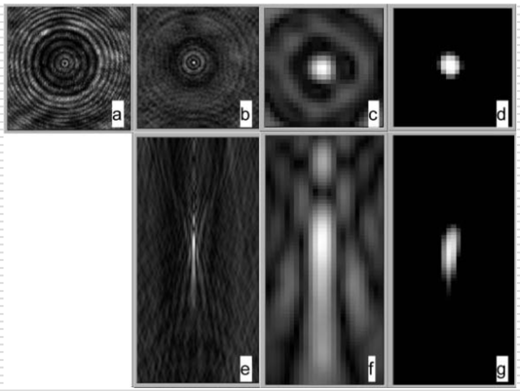
Young's modulus of polyacrylamide film measured to be 14.5 kN/m2

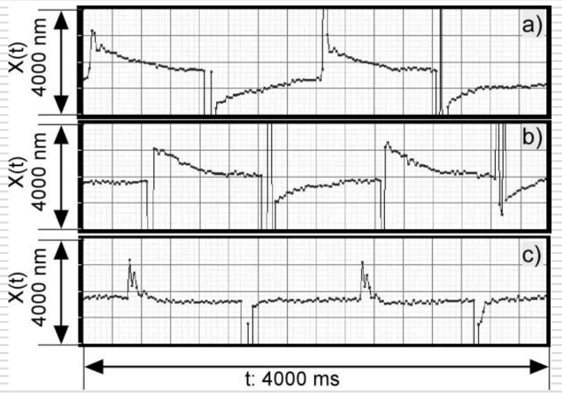
Applications:

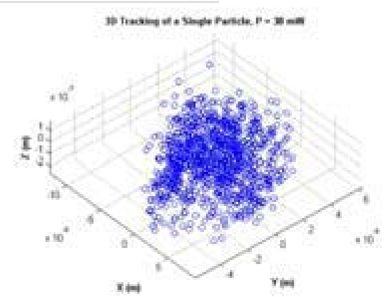
- Force measurement of cellular motility
- Characterization of polyelectrolyte multilayer film growth for tissue engineering

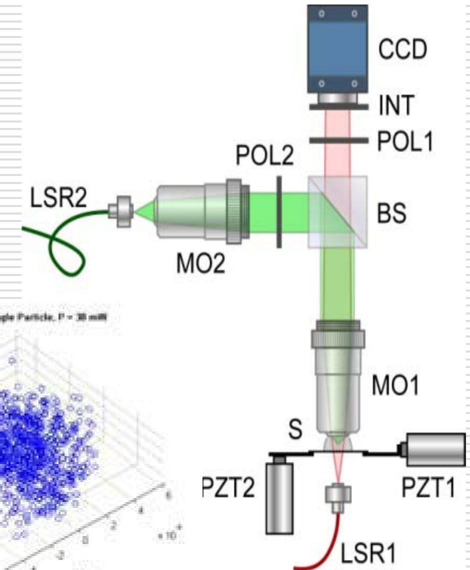
Digital Gabor holography for particle tracking

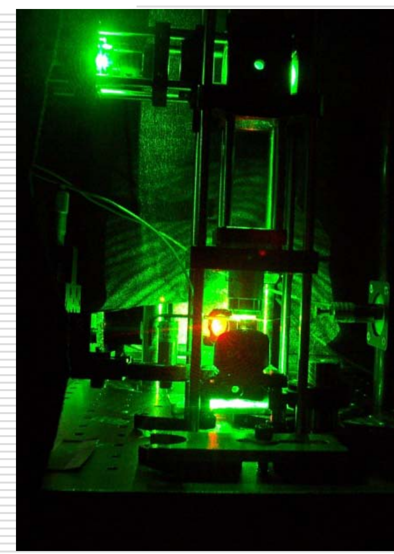
- polystyrene (10um) spheres fixed on cover slip
- piezo transducer drives for x- and z-movements
- sub-nanometer control of x,z-movements

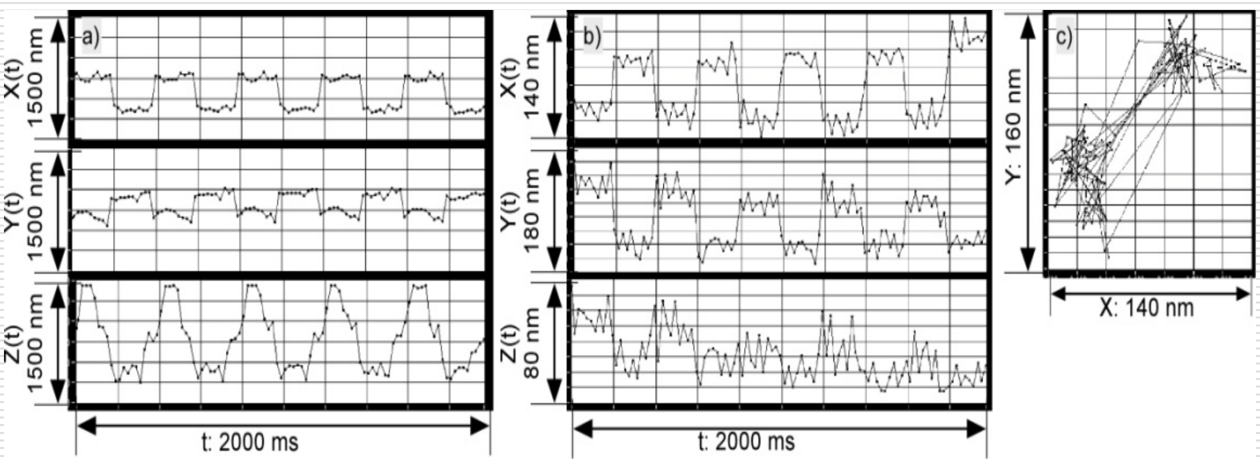






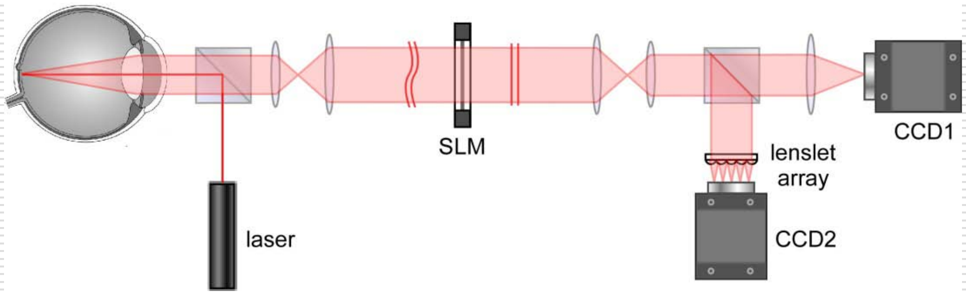


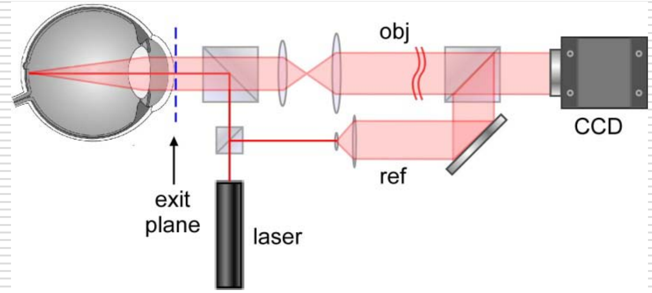




USF

Adaptive Optics without wavefront sensor or corrector





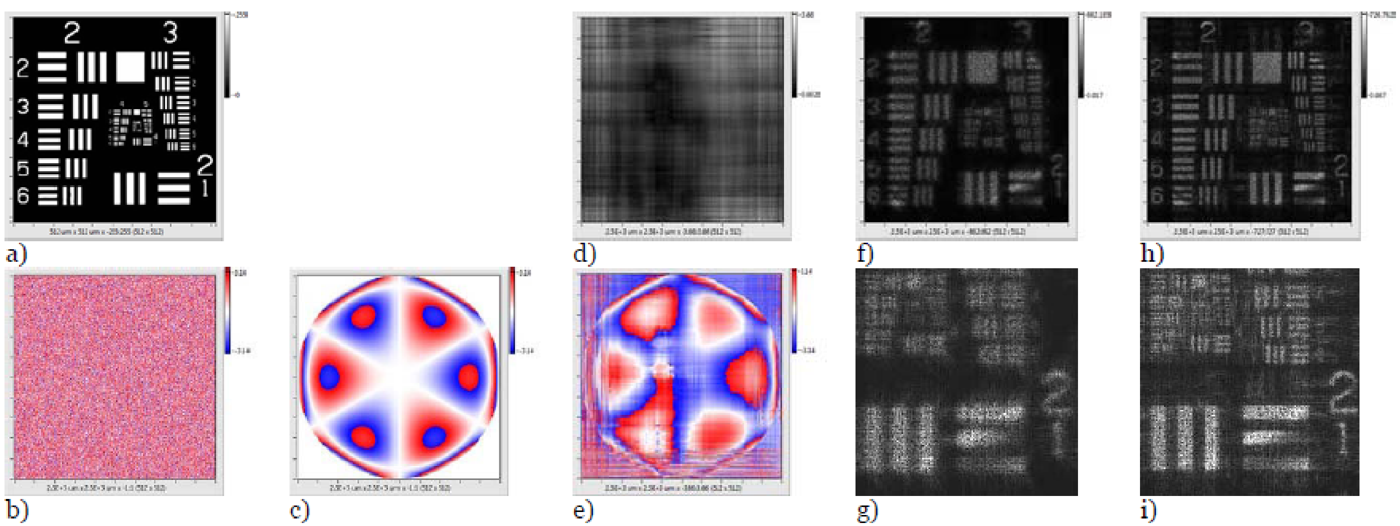
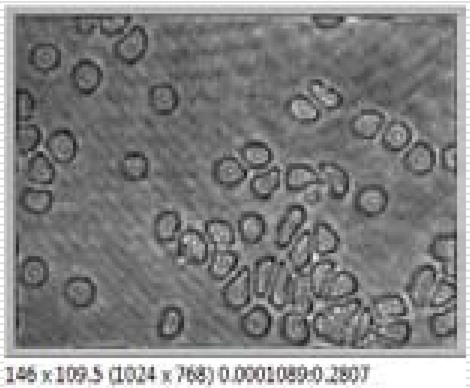


Fig. 2: Simulation of DHAO process. Amplitude image are shown in gray scale and phase images (b,c, & e) in blue-white-red color scale, representing the range of phase from −pi to +pi. a) Assumed amplitude pattern on retina; b) phase noise of retinal surface; c) assumed aberration of the eye; d) amplitude of exit field; e) phase of exit field, representing recovered aberration of the eye; f) uncorrected image of retina and g) its detail; and h) corrected image of retina and i) its detail.

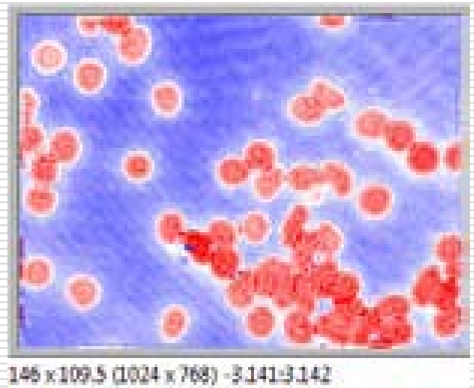
Multi-mode contrast generation from a single hologram

- Multi-mode contrast generation from a single hologram: a) amplitude contrast; b) quantitative phase contrast; c) dark field; d) Zernike phase contrast; e) DIC; f) spiral DIC.

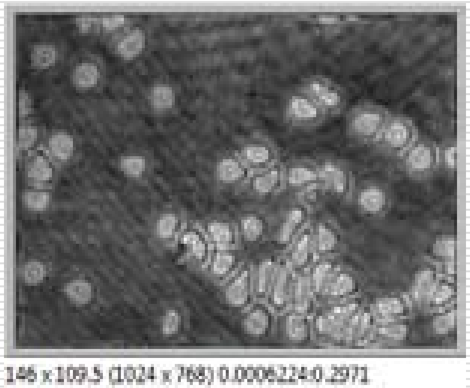
amplitude contrast



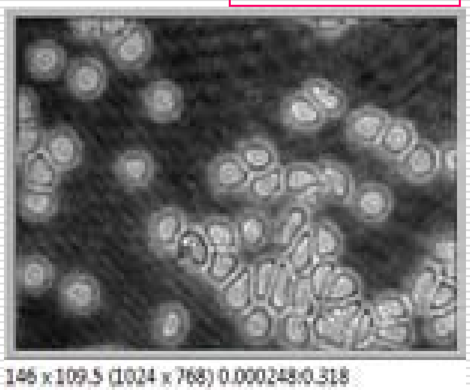
amplitude contrast



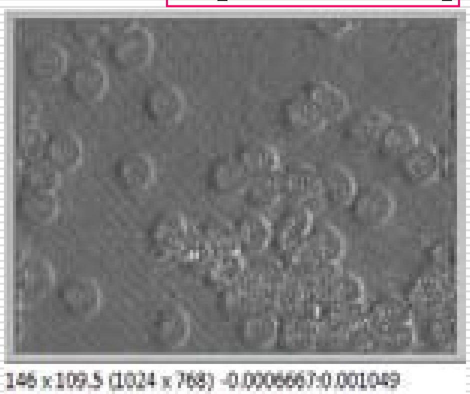
Dark field $1-\delta(f_x, f_y)$



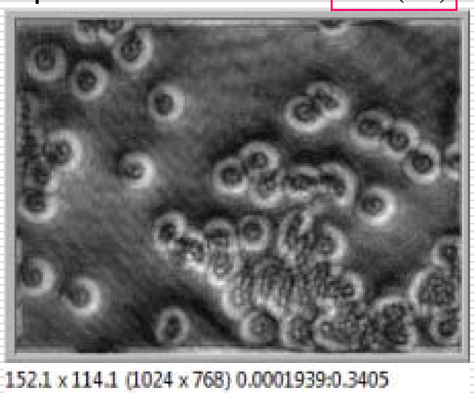
ZPC $1-(1-i)\delta(f_x, f_y)$



DIC $\exp\left[2\pi i\left(f_x\Delta_x + f_y\Delta_y\right)\right]$



spiral DIC $\exp(i\theta)$

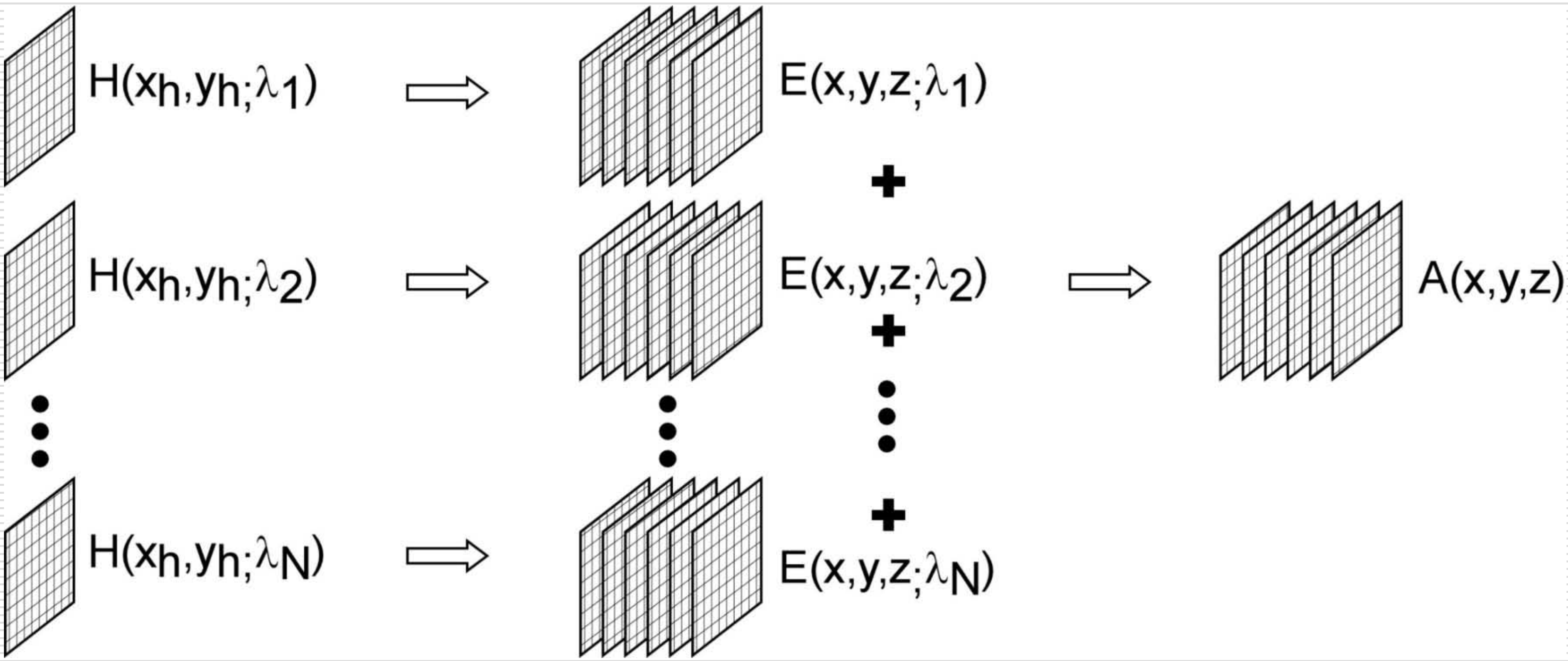


outline

- DHM: Digital Holographic Microscopy
- OPU: Optical Phase Unwrapping
- TIRHM: Total Internal Reflection Holographic Microscopy
- DHM of Laser Microsurgery
- Some current projects
- **Optical Tomography and Topography**
- Conclusions

Digital Interference Holography: the process

- acquire a hologram from camera
- calculate 2D optical field array at many equally spaced z-distances
  - this produces 3D volume of optical field for one wavelength
- step the wavelength
  - and calculate another 3D volume of optical field for the new wavelength
- calculate 3D volumes of optical field for many wavelengths
- numerically superpose all 3D volumes of optical field
- final single volume of optical field with tomographic peaks



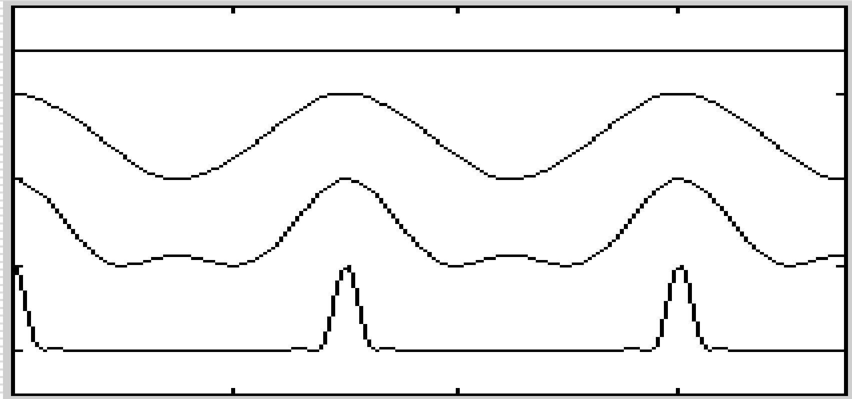
superposition of many wavelengths

1 wave

2 waves

3 waves

10 waves

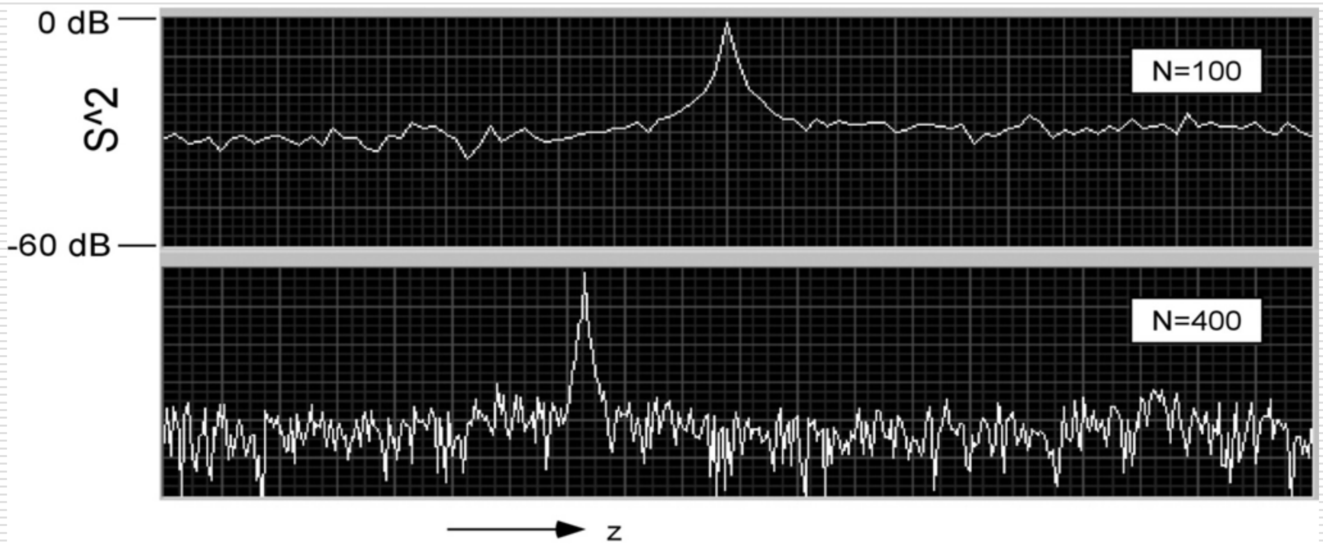


a) $\left|e^{ikz}\right|^2$

b) $\left|e^{ikz} + e^{i(k+\Delta k)z}\right|^2$

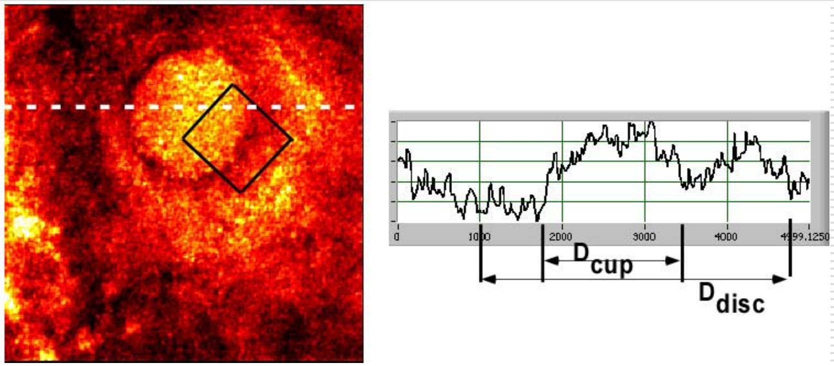
c) $\left|e^{ikz} + e^{i(k+\Delta k)z} + e^{i(k+2\Delta k)z}\right|^2$

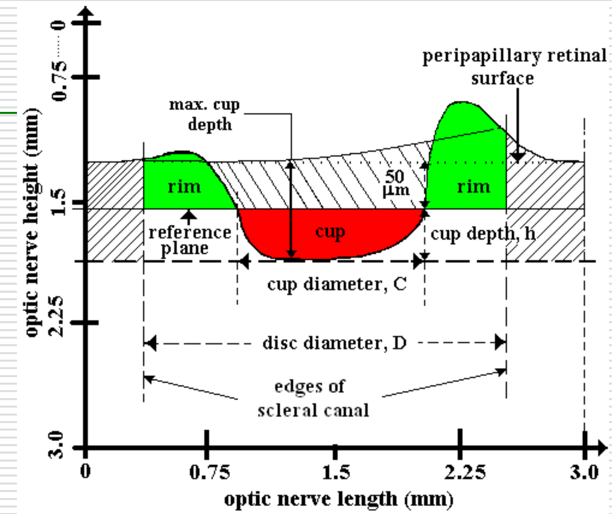
d) $|10 \text{ frequencies}|^2$

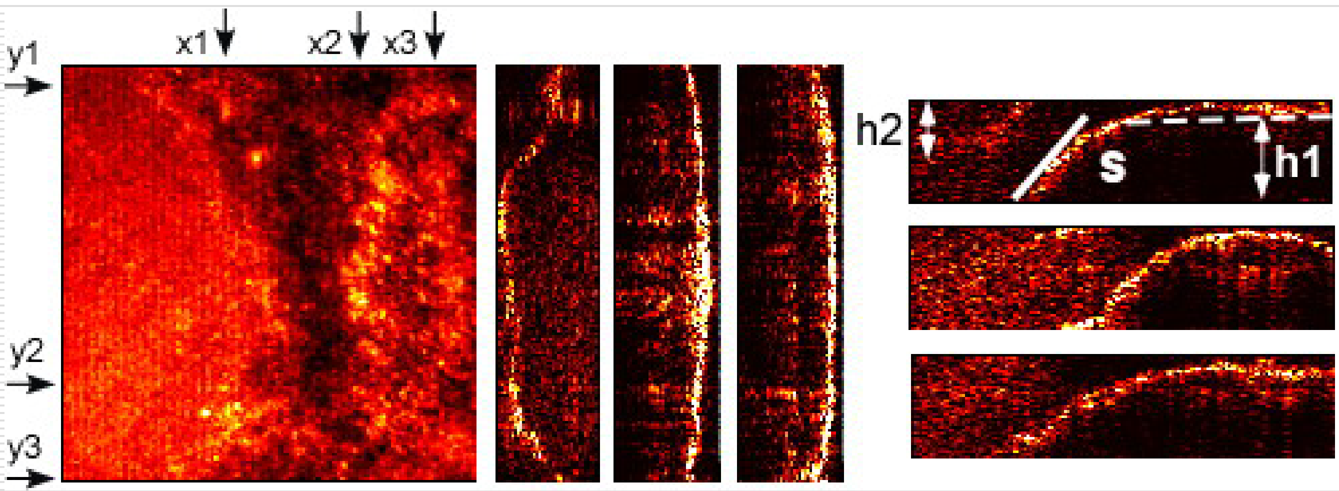


- axial range: $\Lambda = \lambda^2/\Delta\lambda$
- axial resolution: $\delta = \Lambda/N$

3D human optic nerve disk



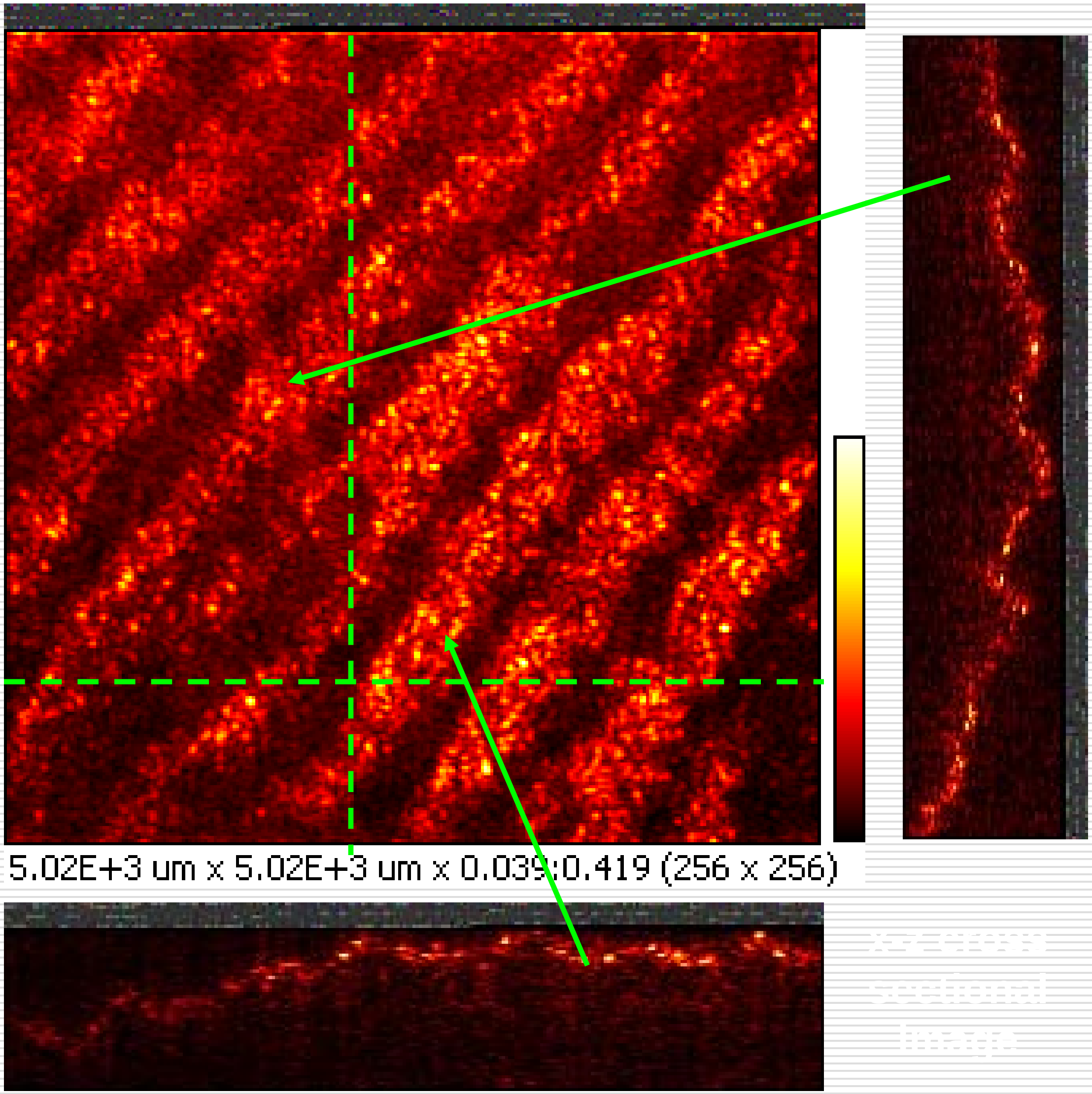




(a) x-y cross-section, FOV=1100 x 1100 µm^2; (b) y-z cross sections at various x values, 1100 x 280.35 µm^2, from left to right, x1, x2, and x3; (c) x-z cross sections at various y values, 280.35 x 1100 µm^2, from top to bottom, y1, y2, and y3; Z = 29.7 µm; NX= NY=256 pixels; NZ= 50 pixels; s = the slope; h1, h2: heights.

M.C. Potcoava, C.N. Kay, M.K. Kim, and D.W. Richards, "In vitro imaging of ophthalmic tissue by digital interference holography". Journal of Modern Optics, 57: p. 115-123 (2010).

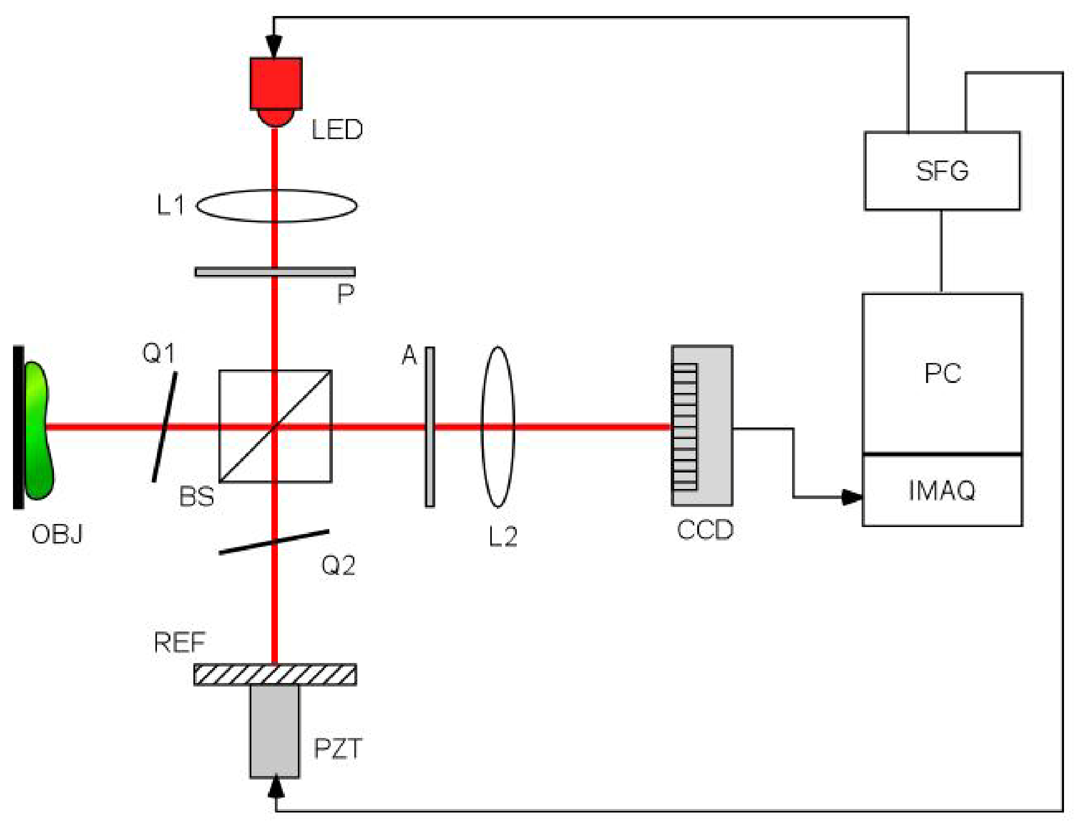
3D fingerprints by DIH

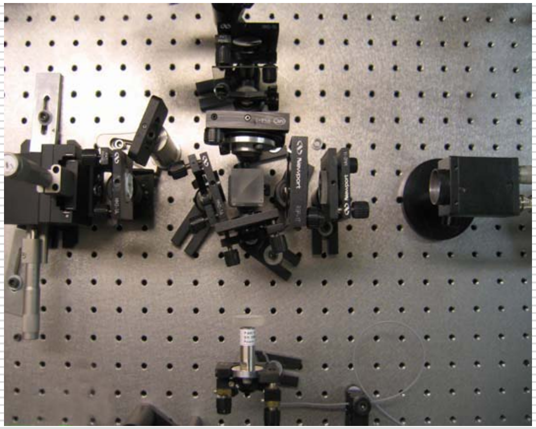


FOV =5 x 5 mm
$\Delta\lambda$: 0.560-0.600 μm
Z= 742.57 μm
Λ= 420.5 μm
dz= 8.41 μm
DX= DY= 19.6 μm
NX x NY x NZ= 256 x 256 x 50

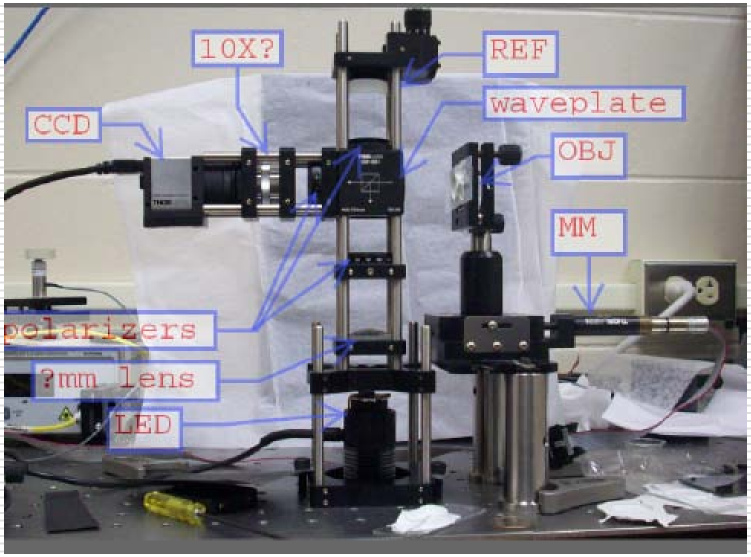
full-color wide-field OCT by PSIM

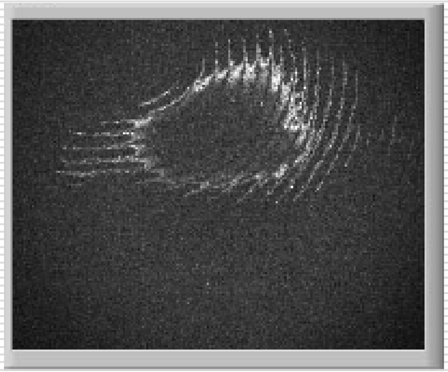
OCT: optical coherence tomography

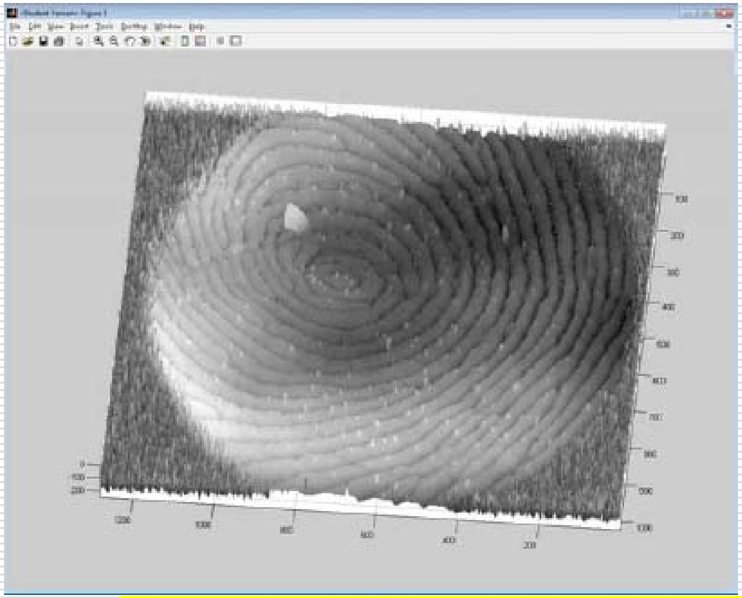


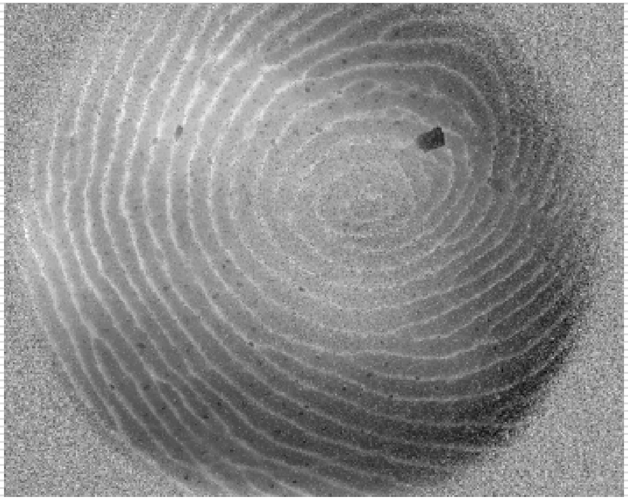


3D fingerprint by PSIM



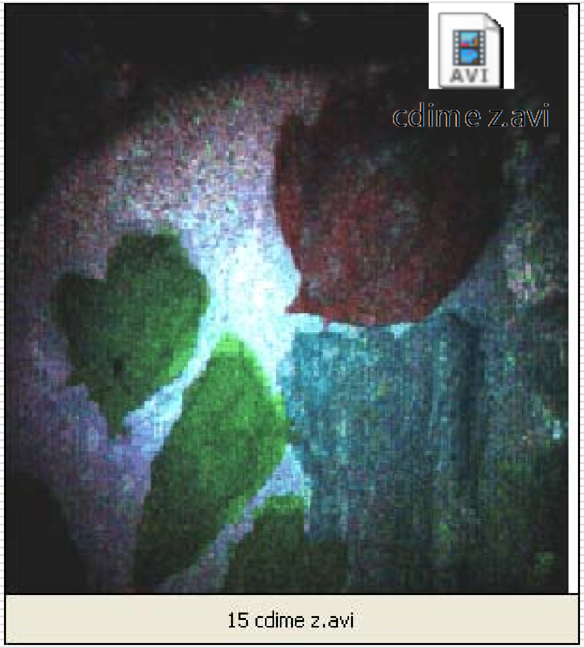




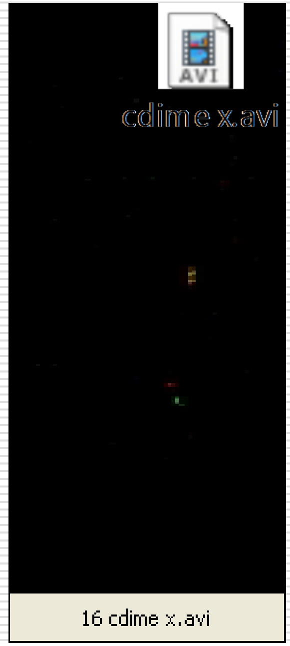


MC Potcoava & MK Kim, “Fingerprint biometry applications of digital holography and low-coherence interferography,” Appl. Opt. 48, H9-15 (2009)

color WFOCT: colored dime



X-Y



X-Z

3D

image volume = 7.2mm x 7.2mm x 335um
voxels = 480 x 480 x 67
voxel volume = 15um x 15um x 5um

L.F. Yu and M.K. Kim, "Full-color three-dimensional microscopy by wide-field optical coherence tomography". Optics Express, **12**: p. 6632-6641 (2004).

Conclusions: Capabilities of DHM

- High resolution quantitative phase microscopy
- Single-exposure image capture and numerical focusing
- Versatile manipulation of complex optical fields
  - Numerical focusing
  - Aberration,
  - Wave front curvature, etc.

- Quantitative label-free microscopy of live cellular and intra-cellular structures and processes
- Biomedical tissue tomography: ophthalmology
- Microstructure imaging and testing
- Particle imaging and tracking: cytometry
- Biomaterials characterization
- etc. ...

Thank You!