Tomographic Phase Microscopy: a marker-free platform for 3D cell imaging

Prof. Renjie Zhou

Laser Metrology and Biomedical (LAMB) Lab

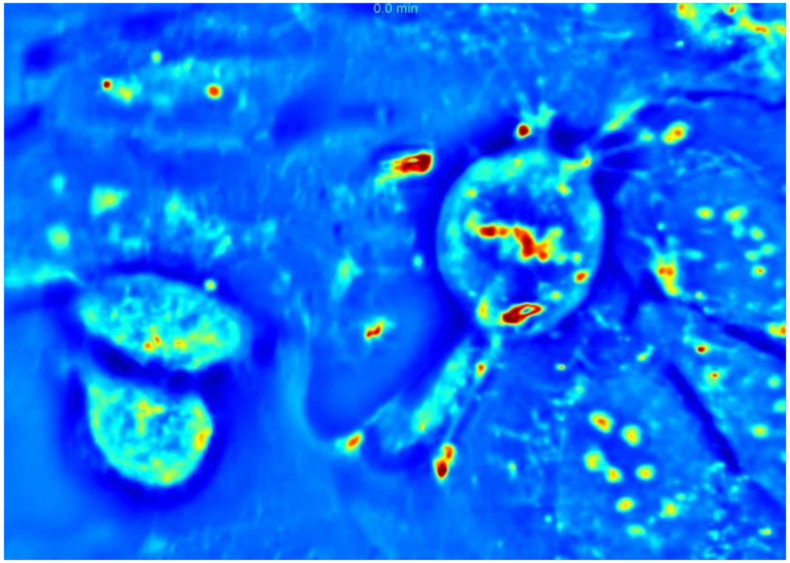
Department of Biomedical Engineering

The Chinese University of Hong Kong

HKPFSSW Promotional Talk
2:35 pm (ERB1009), July 9, 2018

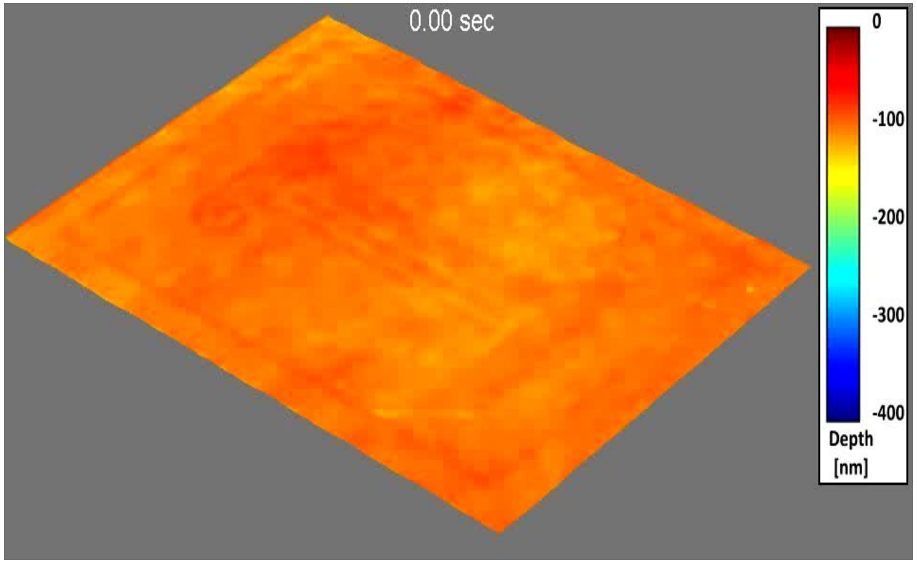
Quantitative Phase Microsocpy

Biological imaging



- Label-free
- Noninvasive
- Endogenous contrast
- Tomographic imaging

Material metrology

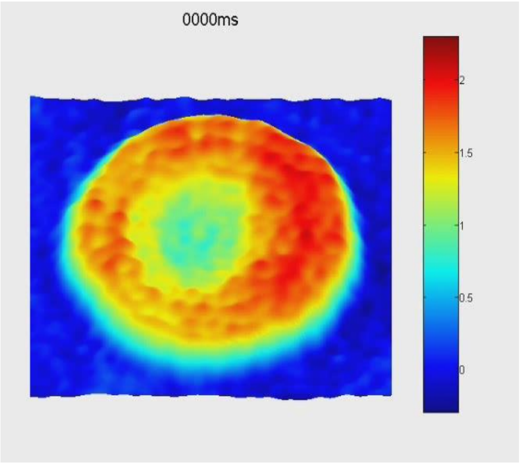


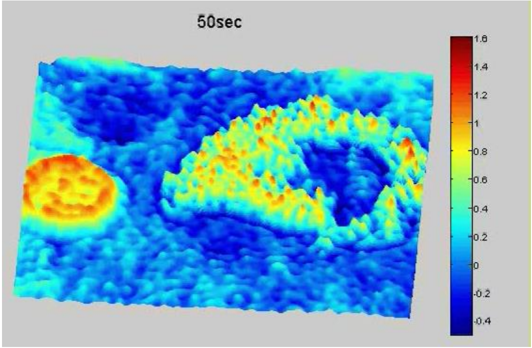
- Noncontact
- Full field technique
- High sensitivity
- Microscopic resolution

Imaging Applications

Hematology applications: red-blood cell (RBC) physiology and diseases

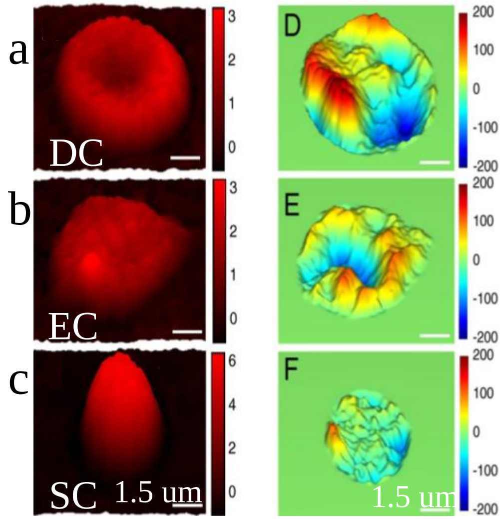
RBC dynamics

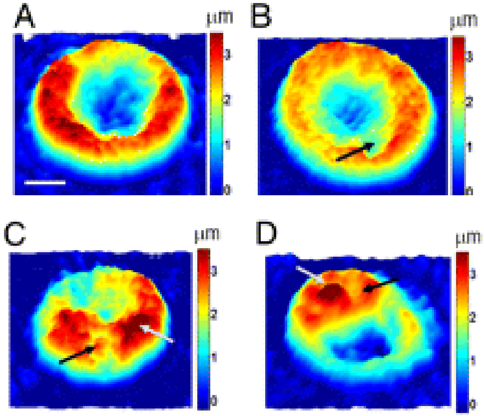




Y. Park *et al.*, *Opt. Express.* **18** (2006)

RBC morphology

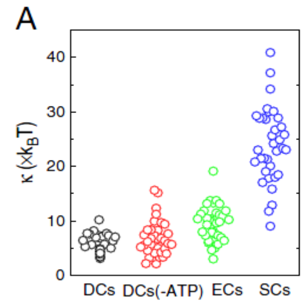




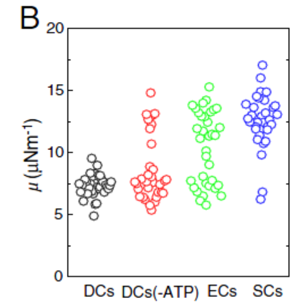
Y. K. Park *et al.*, *PNAS* **105** (2008)

RBC mechanics

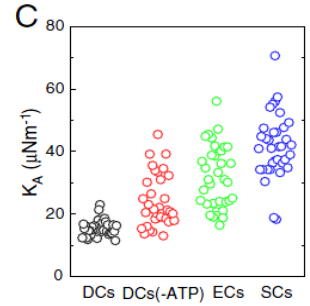
Bending modulus

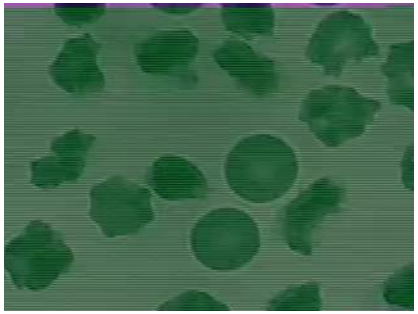


Shear modulus



Area compression modulus

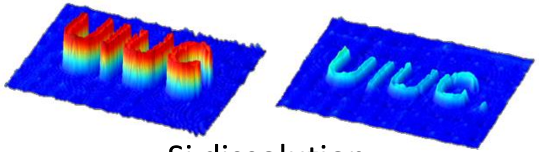




Y. K. Park *et al.*, *PNAS* **107** (2010)
P. Hosseini *et al.*, *PNAS* **113** (2016)

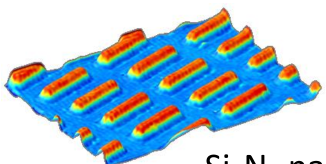
Metrology Applications

Dissolution of Biodegradable Electronic Materials



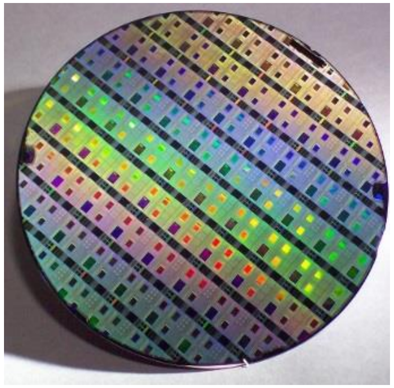
Si dissolution
in Bovine serum

Self-assembly of Nanotubes



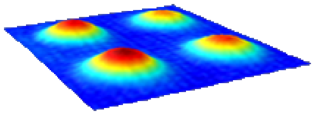
Si_3N_4 nanotubes
during formation

Wafer defect metrology

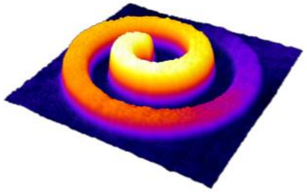


Digital Projection Photochemical Etching

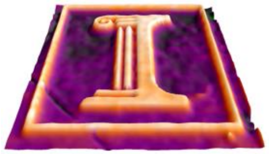
Microlens array



Archimedean spiral

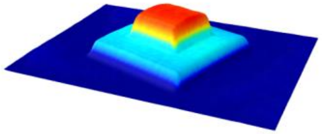


Etching Dynamics

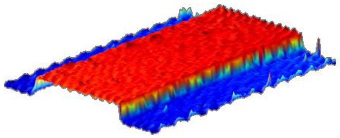


Watching semiconductors etch

Expansion and Deformation of Materials



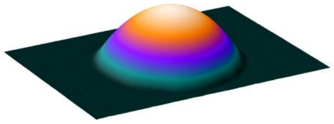
Pt stacked cubes
expanding in H_2 gas

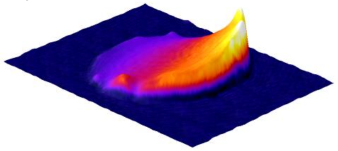


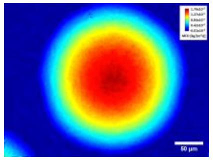
Pd ridge in H_2 gas

Surface Wetting and Evaporation

Evaporating
Microdroplet







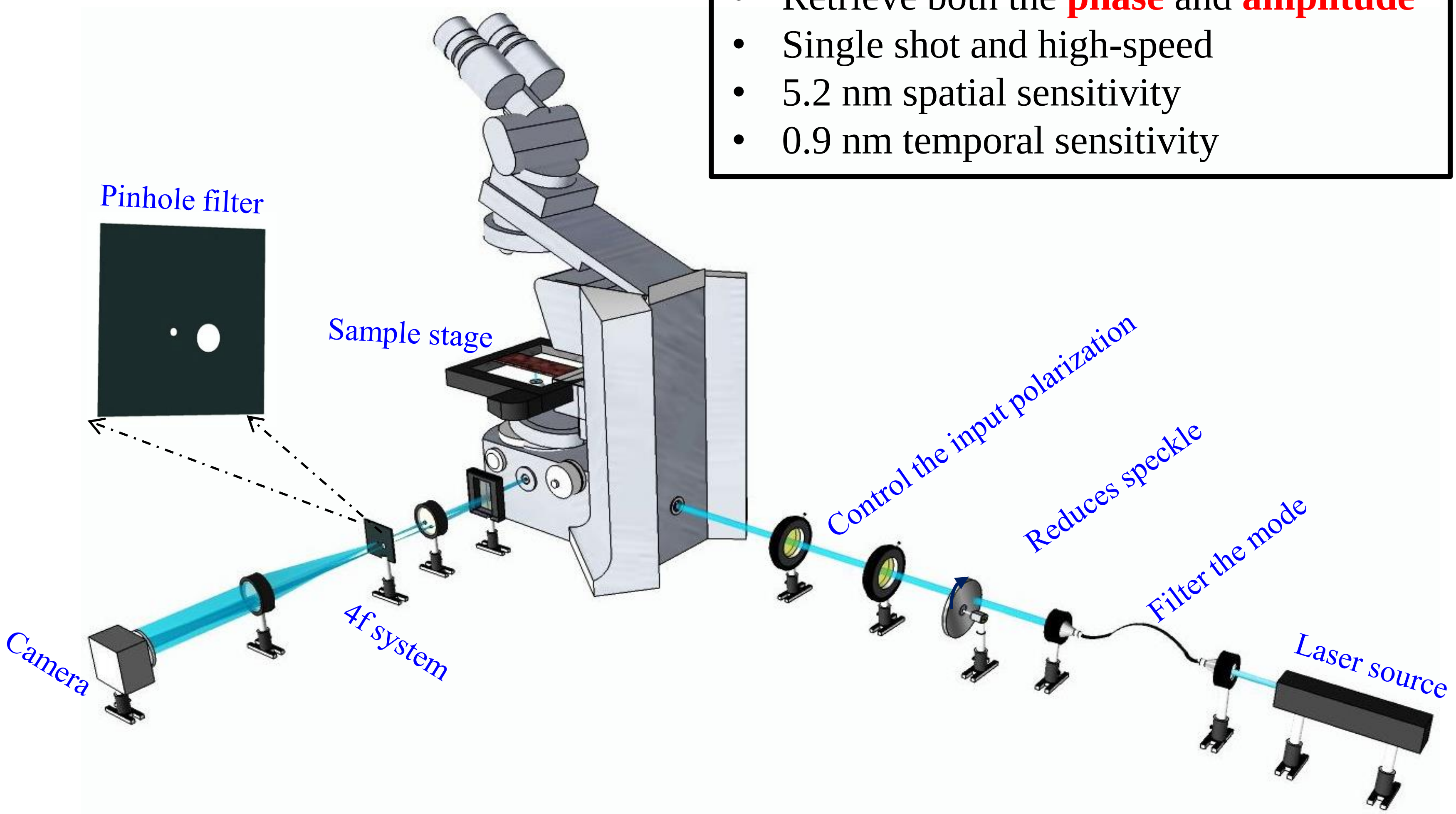
Mass Flux
Density

C. Edwards *et al.*, *Light: Science & Appl.* **1**(2012); R. Zhou *et al.*, *Nano Lett.* **13** (2013); S. –W. Hwang *et al*, *ACS Nano.* **8** (2014)
R. Zhou *et al.*, *Proc. SPIE.* **8681** (2013); R. Zhou *et al.*, *Proc. SPIE*, **9050** (2014); R. Zhou *et al.*, *Proc. SPIE*, **9336** (2015)

Common-path Quantitative Phase Microscopy

Epi-illumination diffraction phase microcopy (epi-DPM)

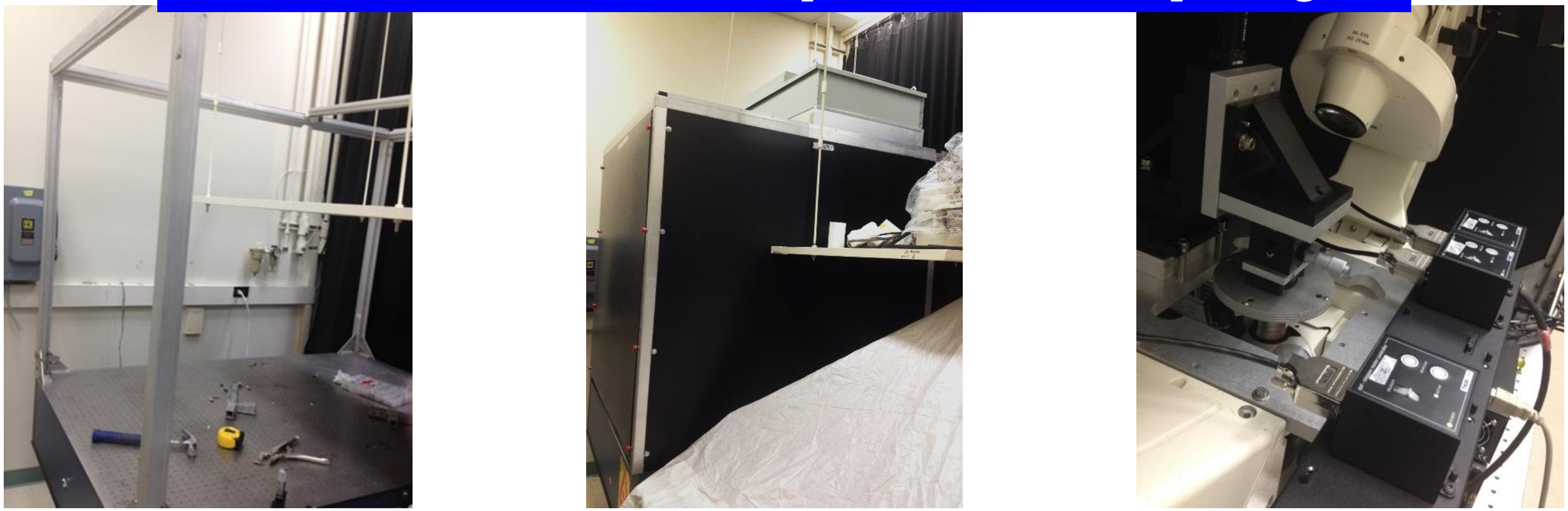
- Retrieve both the **phase** and **amplitude**
- Single shot and high-speed
- 5.2 nm spatial sensitivity
- 0.9 nm temporal sensitivity



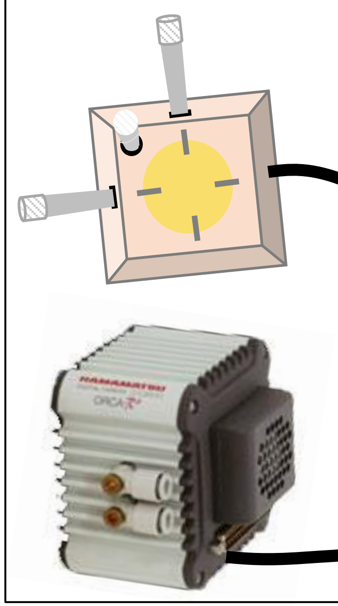
G. Popescu *et al.*, *Opt. Lett.* **31** (2006); C. Edwards et al., Light: Sci & App **1** (2012); R. Zhou *et al.*, *Nano Lett.* **13** (2013)

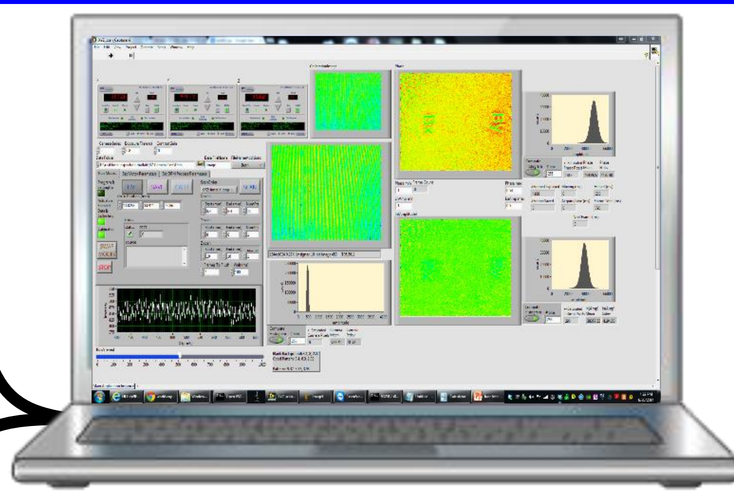
System Deployment

Construction: DPM + Tabletop cleanroom + Sample stage

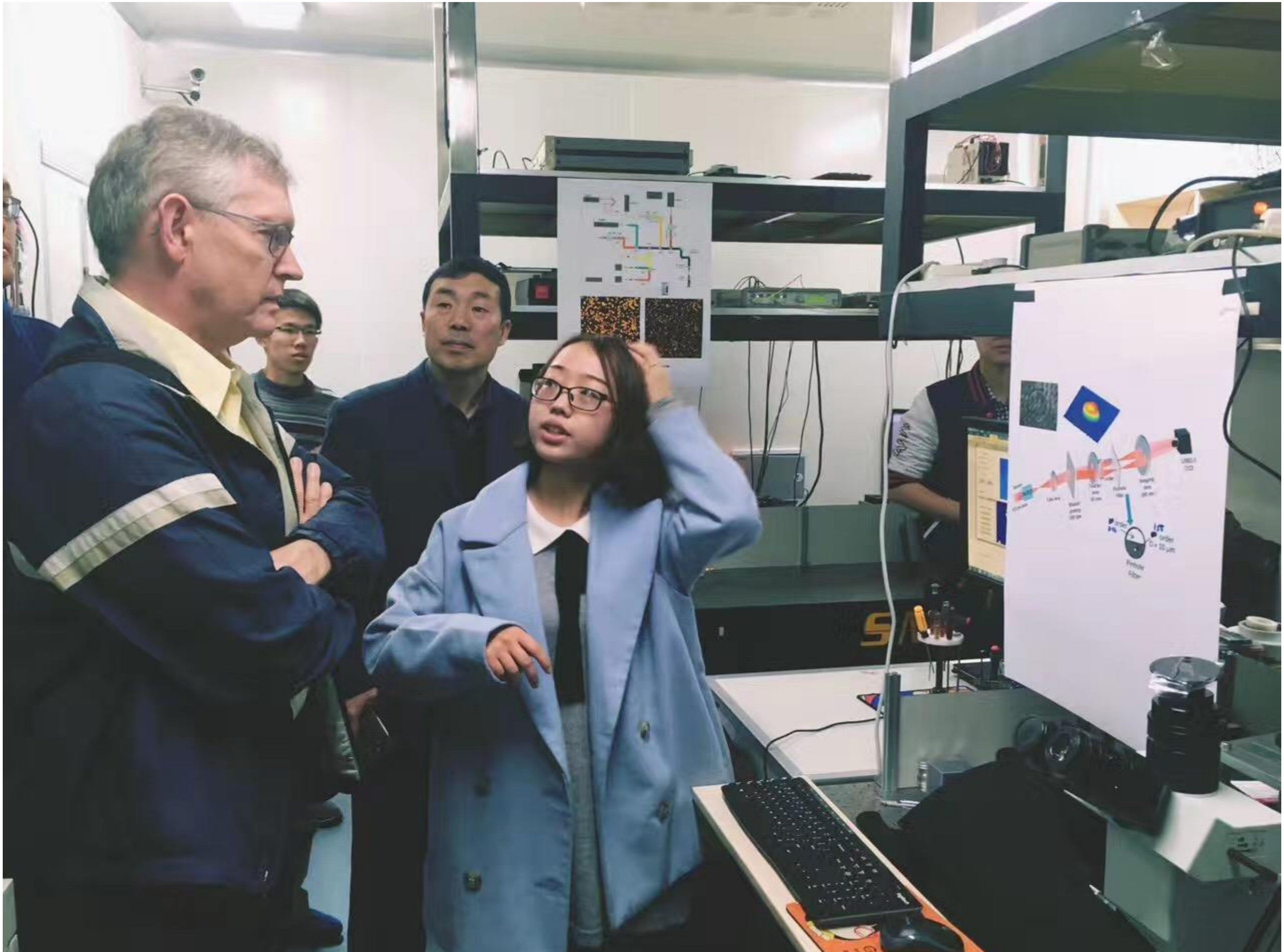


Control software: CUDA + Labview



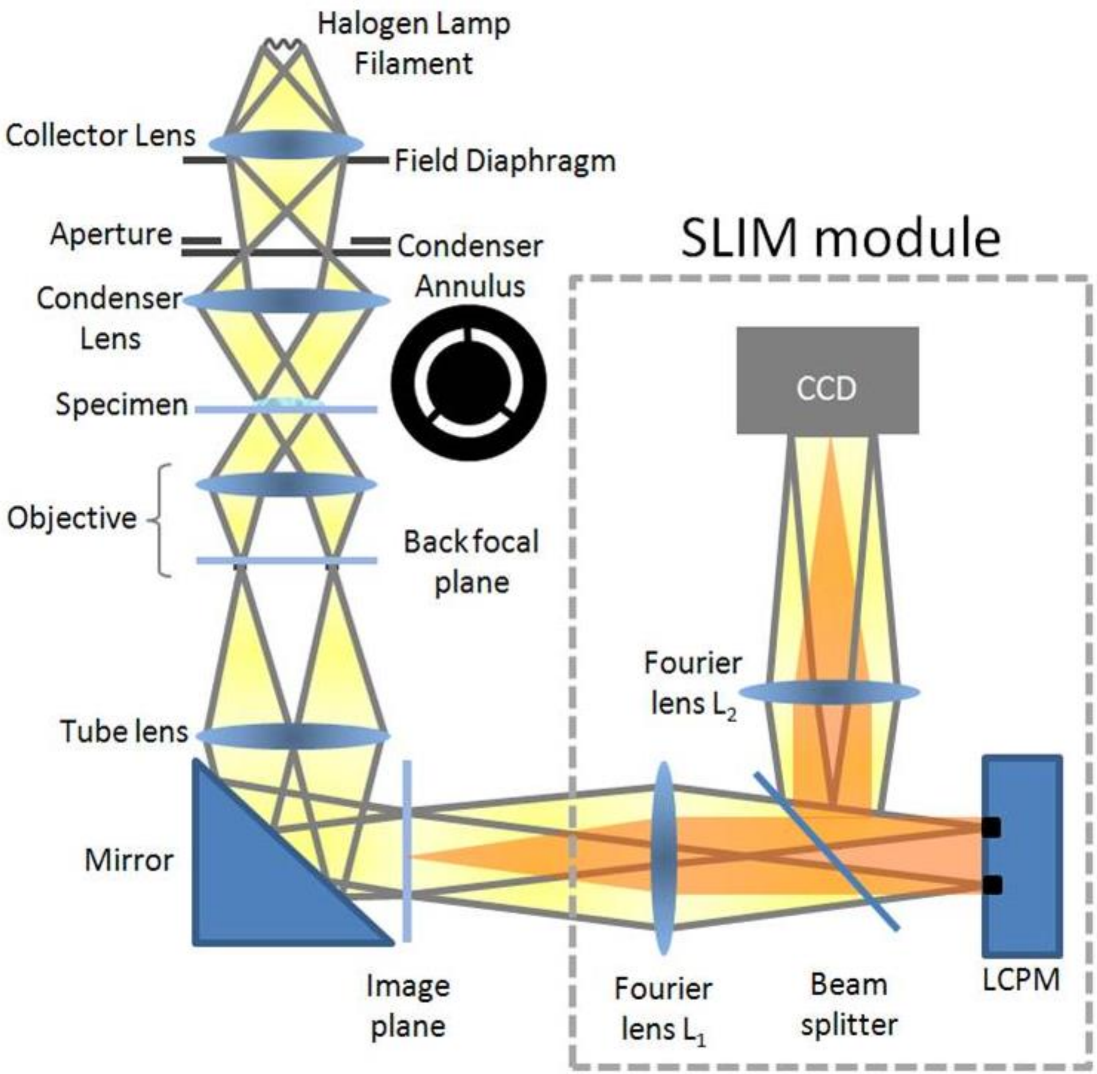


R. Zhou *et al.*, *SRC Techcon* (2014)



Spatial Light Interference Microcopy

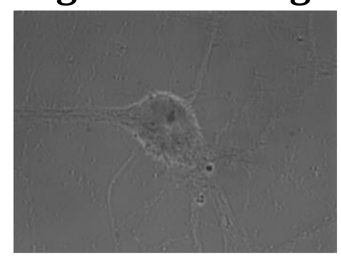
Add a phase-shifting module to phase-contrast microscopy



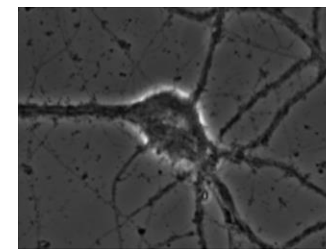
SLIM is a white-light QPI

- 0.3 nm spatial sensitivity
- 0.03 nm temporal sensitivity

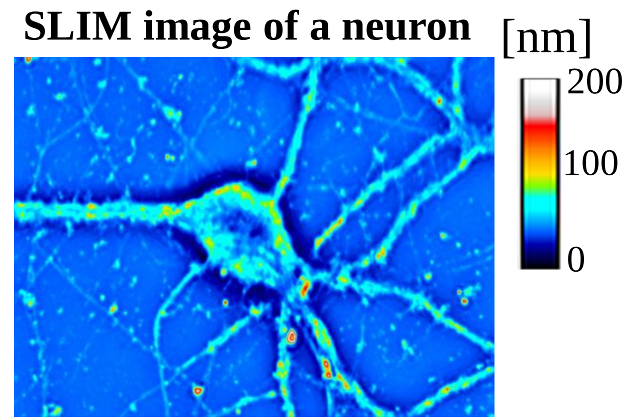
Bright-field image



Phase contrast image

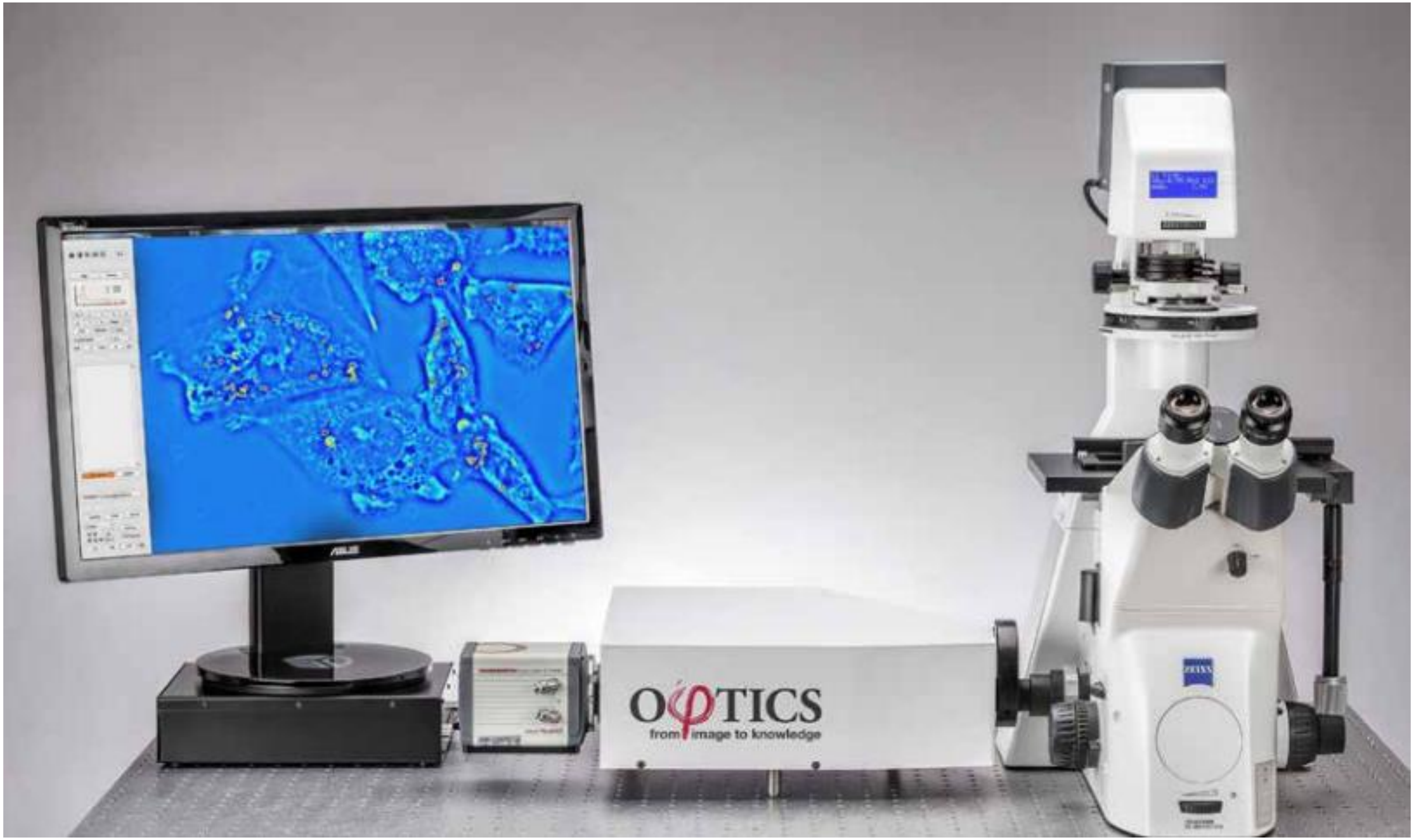


SLIM image of a neuron



Z. Wang *et al.*, *Opt. Exp* **19** (2011)

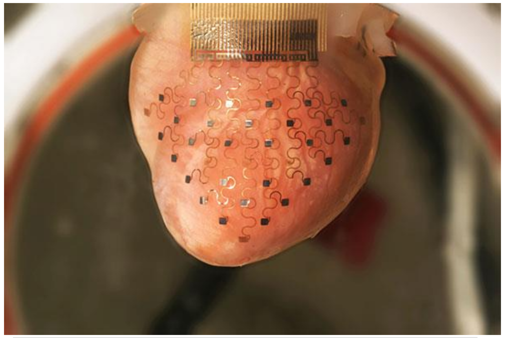
SLIM Module for Phase-Contrast Microscopes



Motivation

Bridging the gap in 3D surface testing

Flexible Electronics Testing



Dynamic, Precision, Low cost

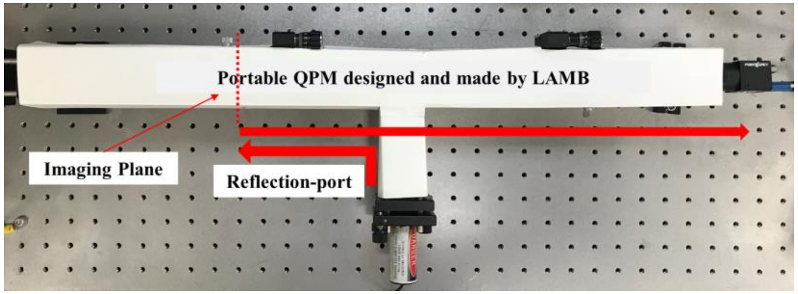
Mechanical Surface Testing

Portable, Precision

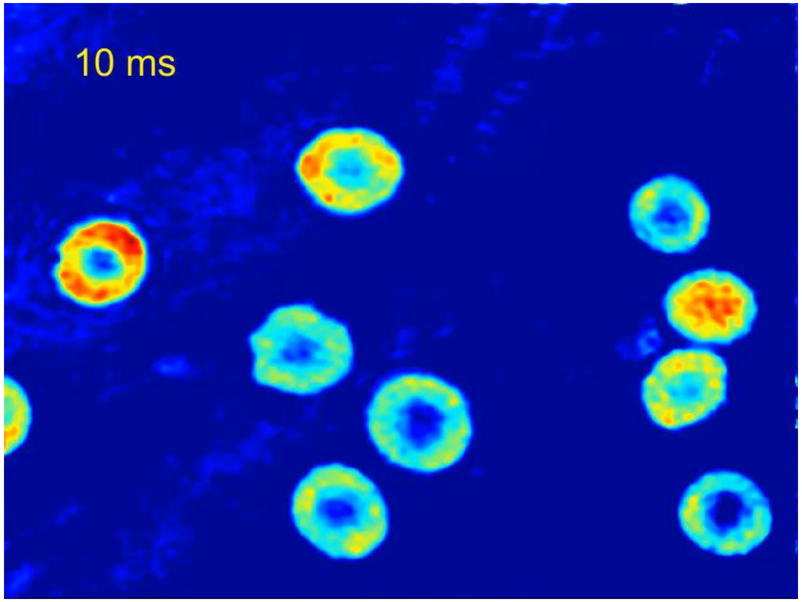
Blood Testing

Simple, Precision, Low cost

2nd Prototype Design



SPIE Startup competition semifinal 2017

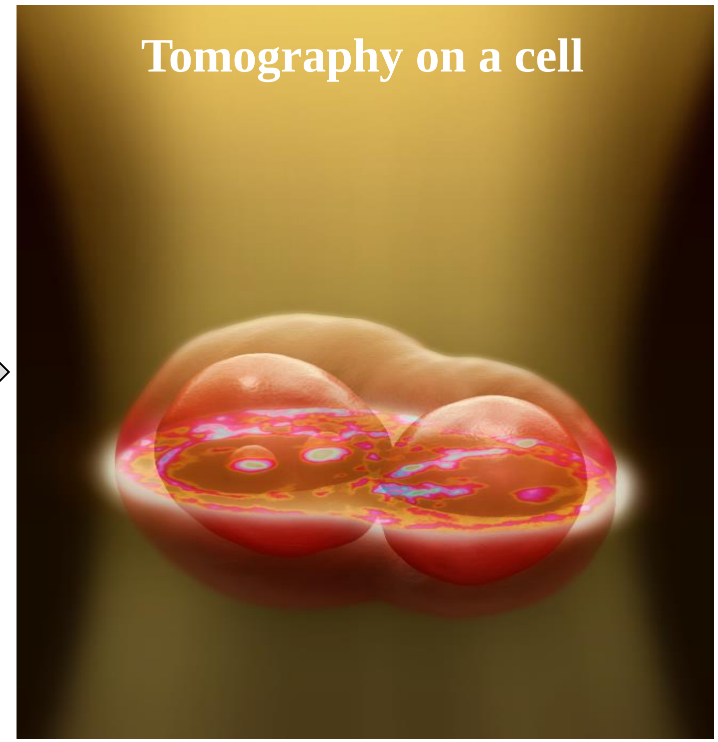


Tomography Phase Microscopy (TPM)

Mapping cell refractive index in 3D

$$\phi(x,y)=\frac{2\pi}{\lambda}\int \Delta n(x,y,z)\,dz \neq \frac{2\pi \Delta n h\left(x,y\right)}{\lambda}$$

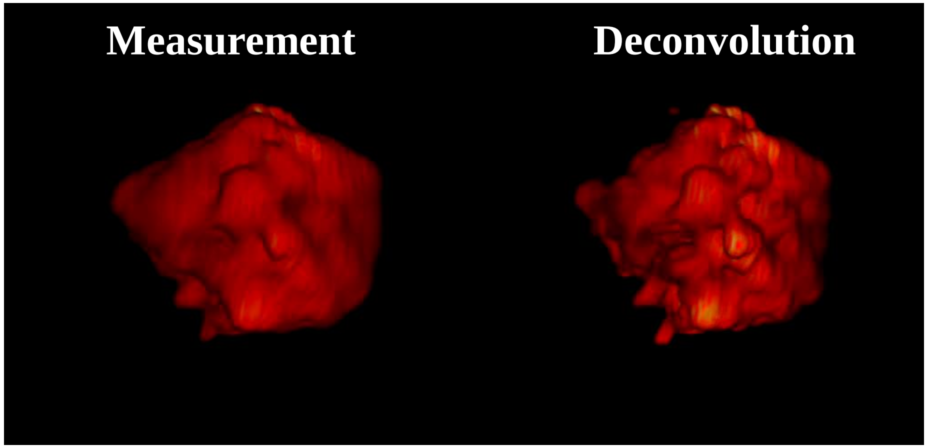
X-ray computed tomography (CT)

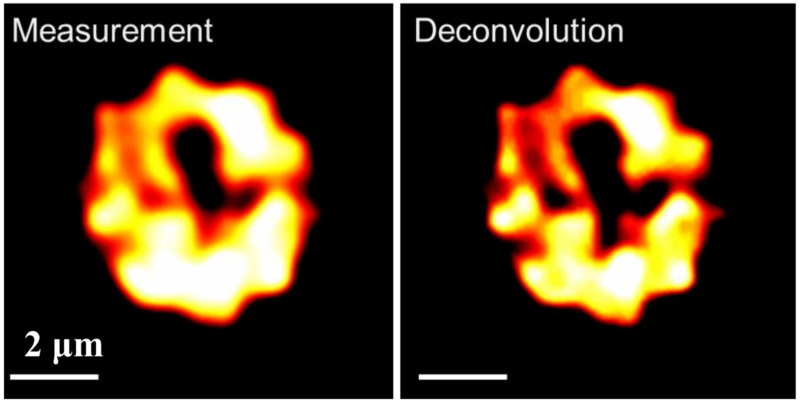


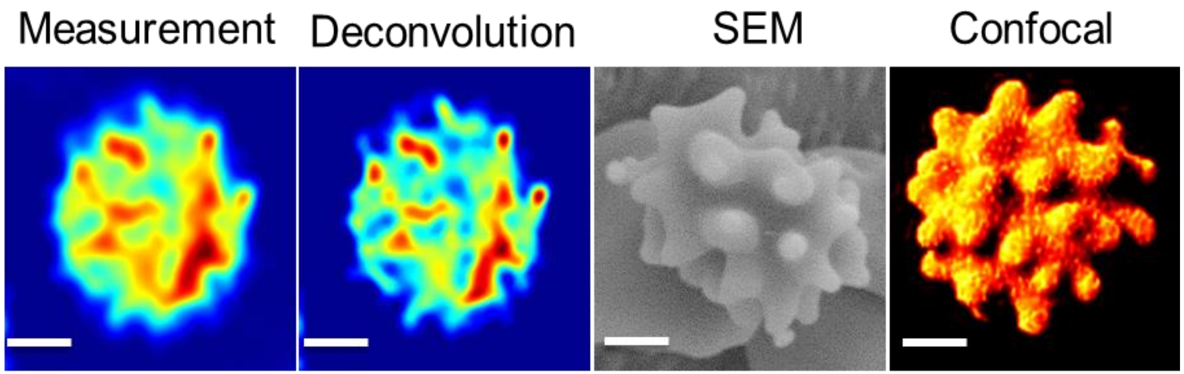
T. Kim[+], R. Zhou[+] *et al*, *Nat. Photon*. **8** (2014)

White-light Diffraction Tomography

Red blood cell with Echinocyte

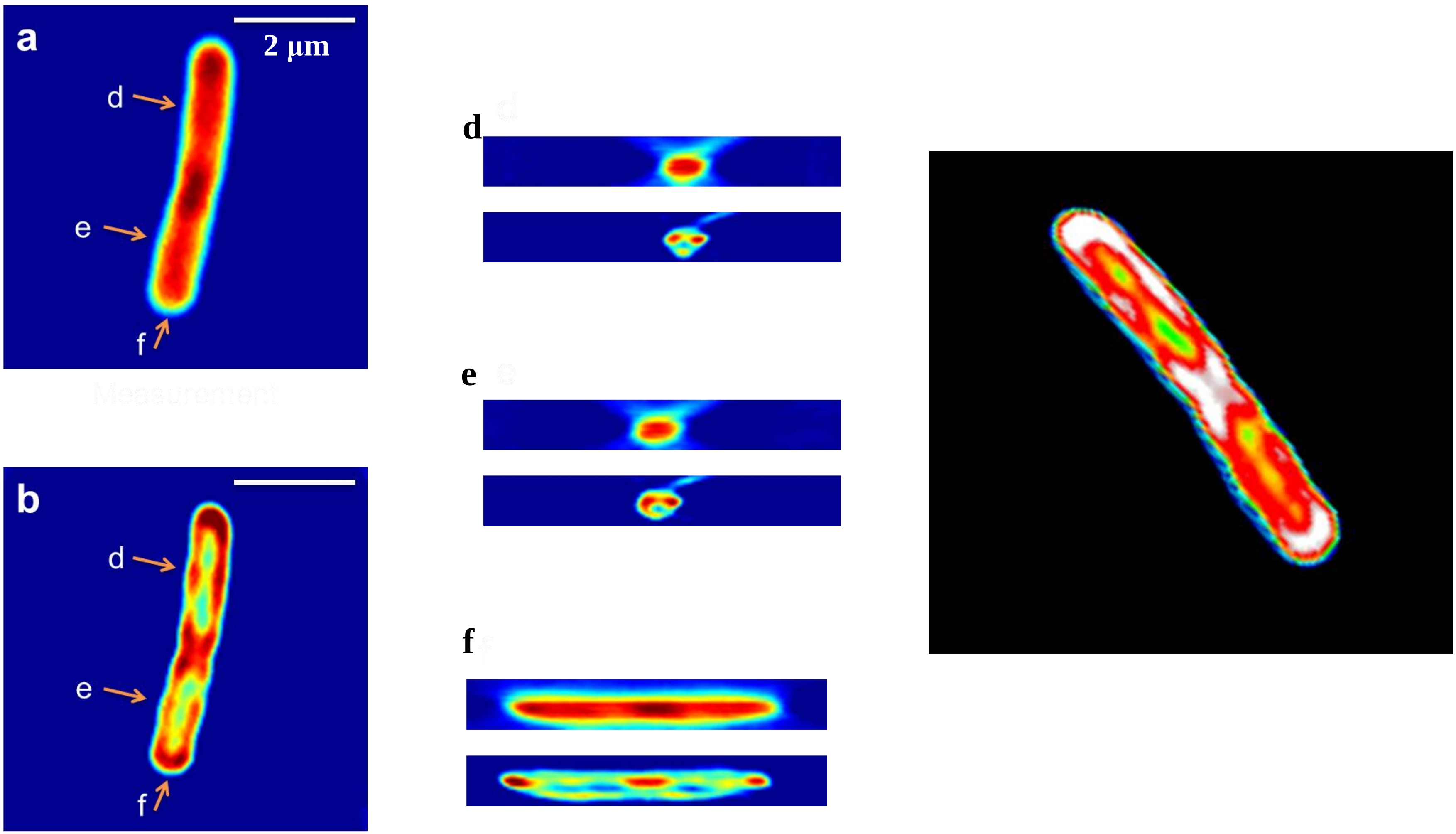






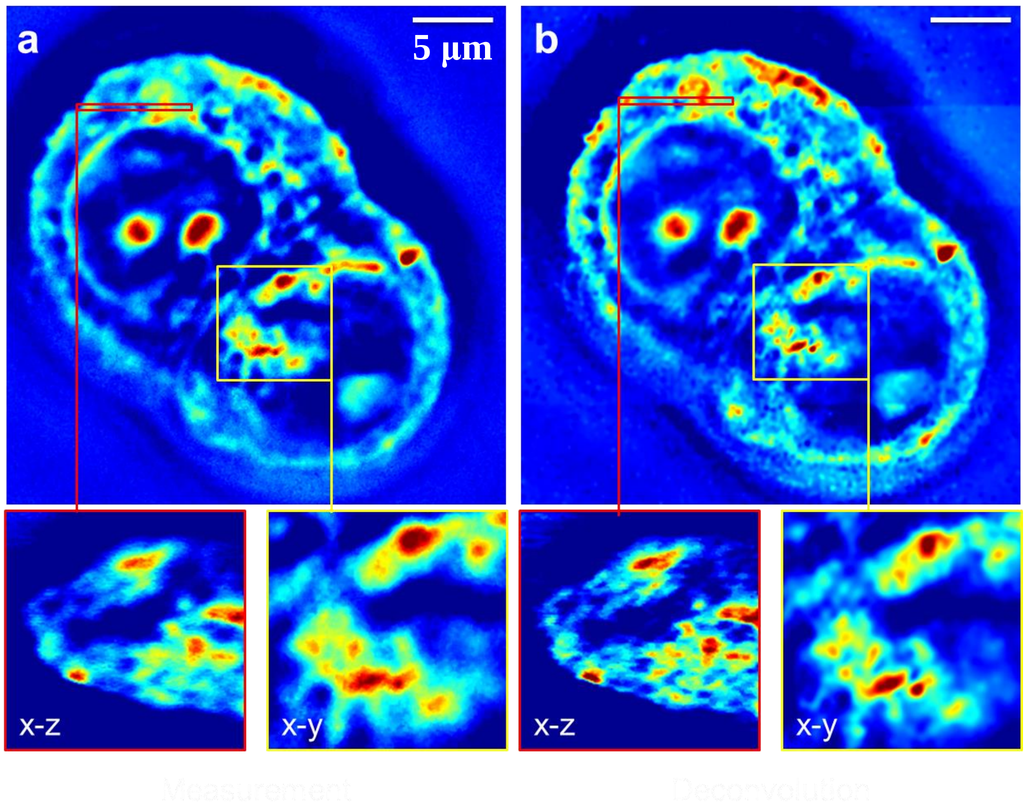
E. Coli Cell in 3D

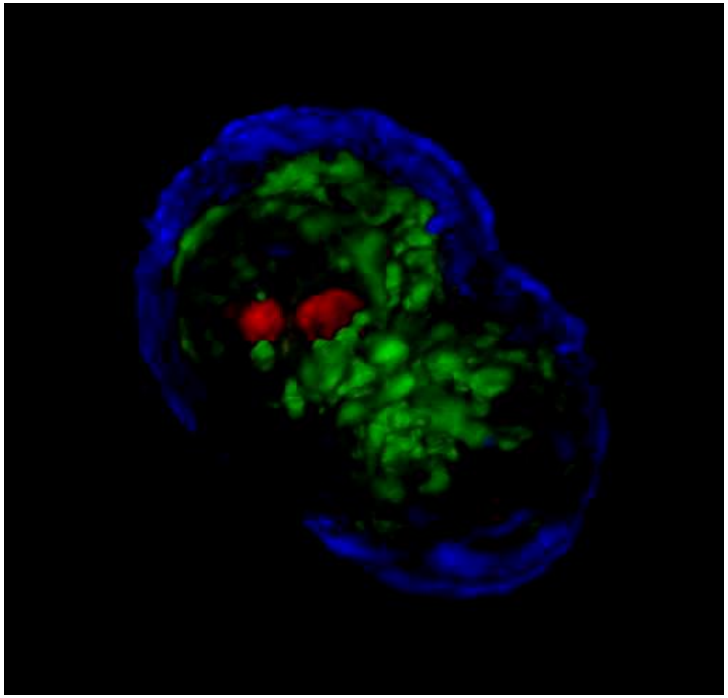
Revealing the helical structure



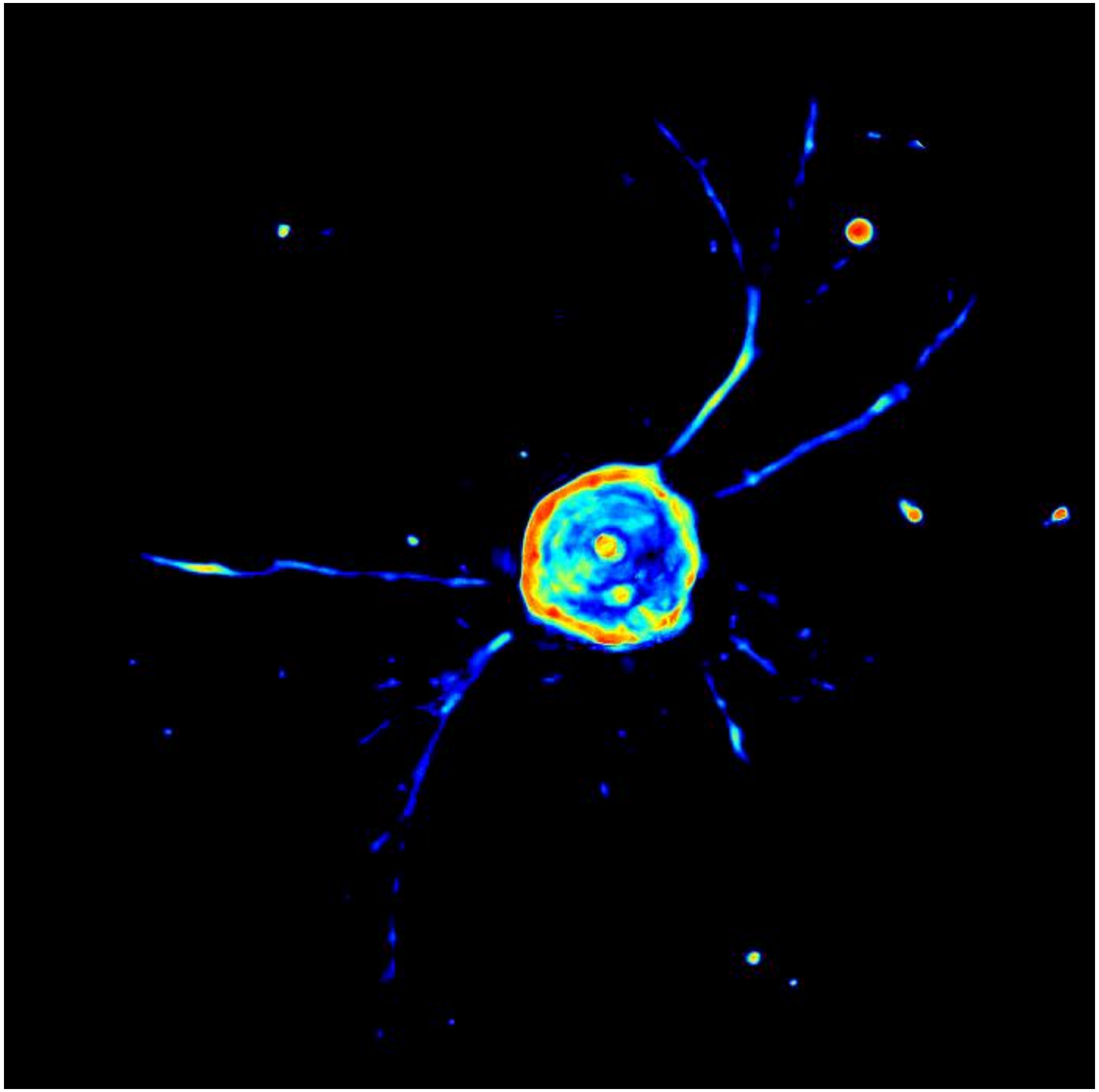
HT-29 Cell in 3D

Image larger mammalian cells





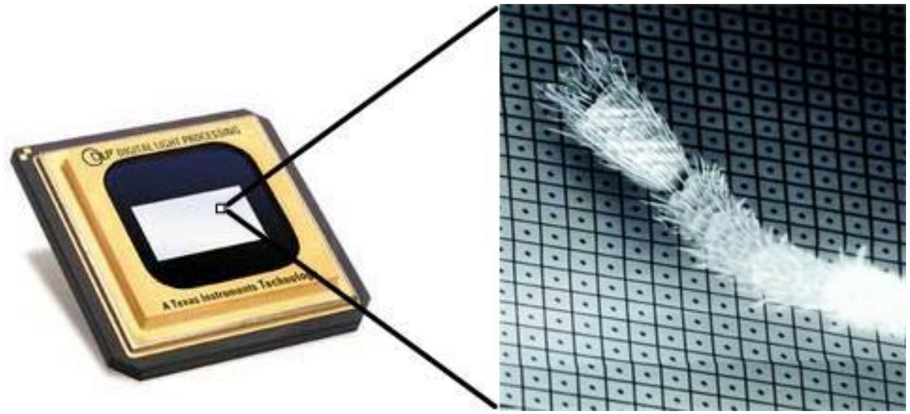
Neuron in 3D

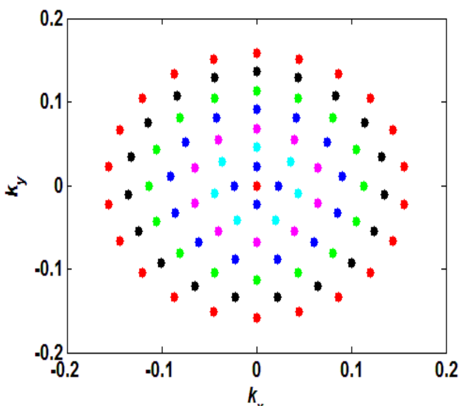


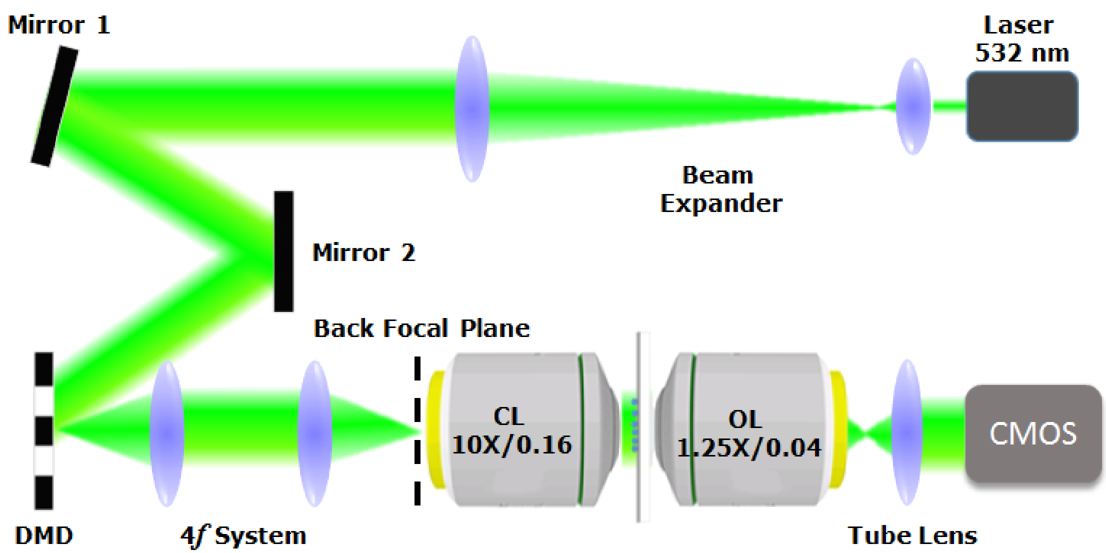
T. Kim[+], R. Zhou[+] *et al.*, *Lasers & Photonics Rev.* **10** (2016)

DMD-based TPM

DMDs are fast and stable with > 10 kHz patterning speed



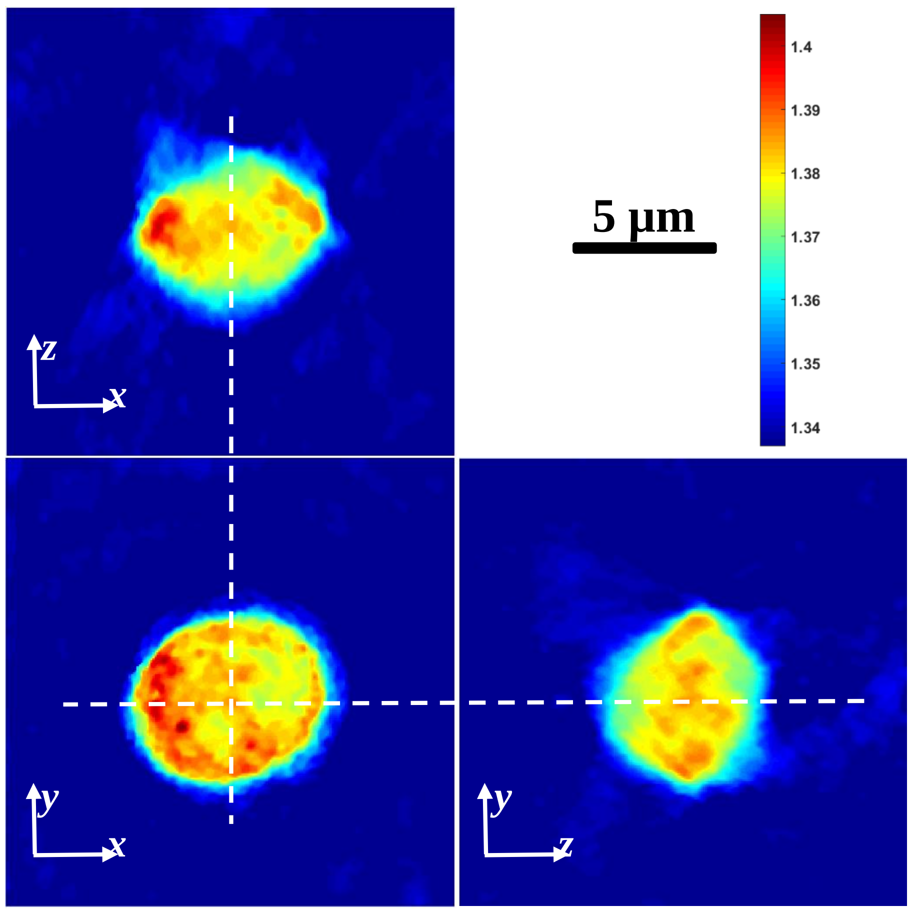




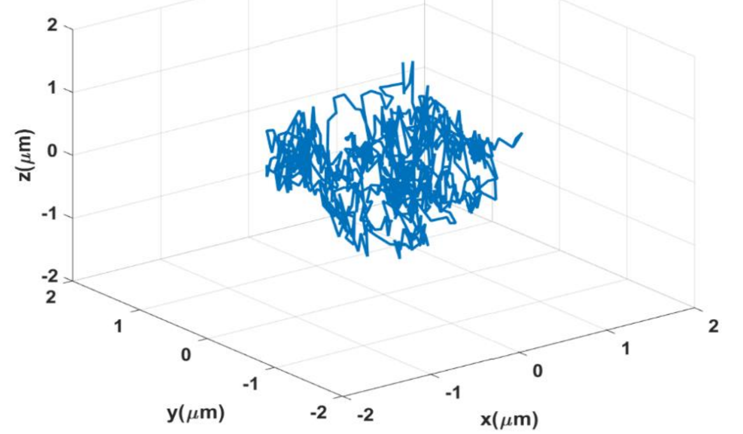
C Kuang, Y Ma, R Zhou* *et al.*, *Opt Exp.* **23** (2015)

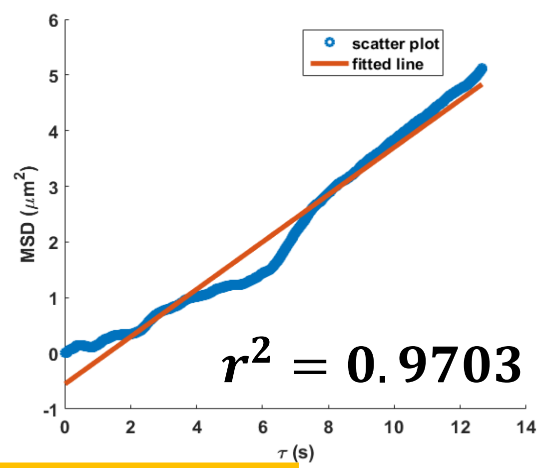
3D Imaging Results

HeLa cell



3D trajectories of a bead





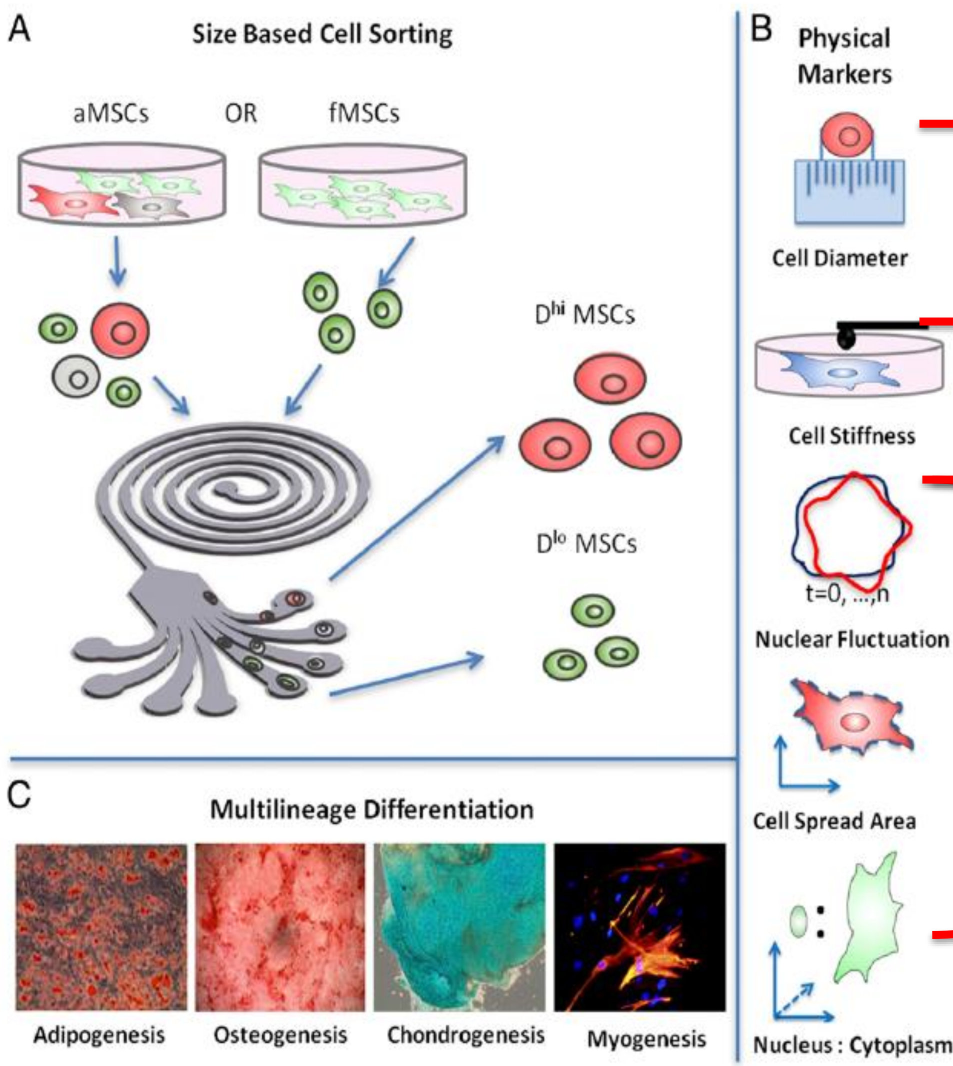
Throughput

- Speed > 30 tomogram/sec
- > 300 cells per second

D. Jin, R. Zhou*, *el al.*, *Opt. Exp.* **26** (2018)

Outlook 1: High Speed 3D Imaging Cytometry

Stem cell therapies, circulating cell detection, embryo selection, etc.



Efficient isolation of the stems

Krystyn Van Vliet's group at MIT, *PNAS* **111** (2014)

Current methods

- Spiral microchannel devices
  Limitation: Only cell size groups
- Silicon nitride AFM cantilever
  Limitation: Low speed
- Confocal images
  Limitation: Fluorescence staining

Our goal

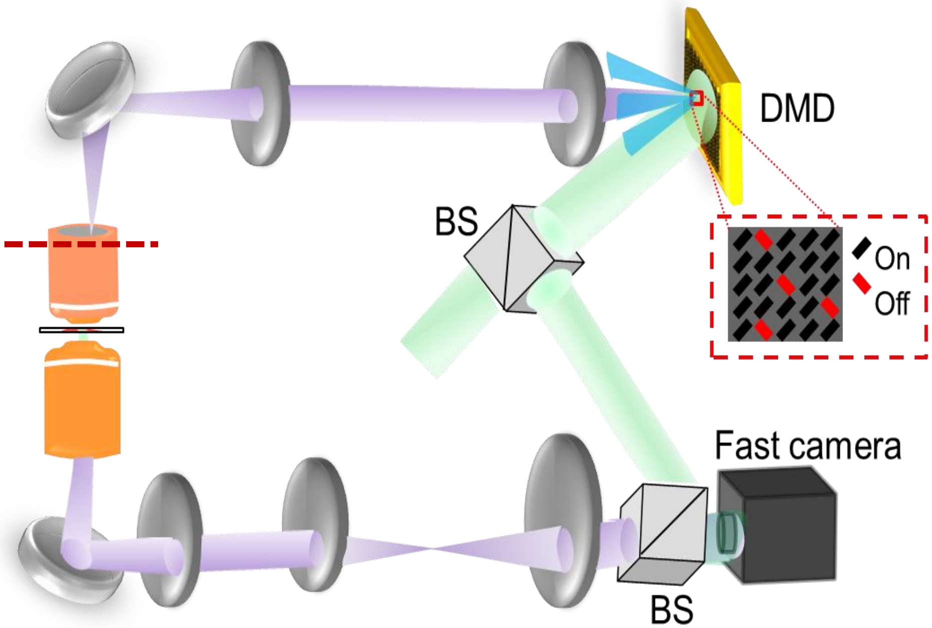
Develop a label-free method that can:

- Obtain all the possible biophysical
- Perform multivariate identification
- Long-term monitor cell changes
- High throughput imaging
- Measure mechanical properties

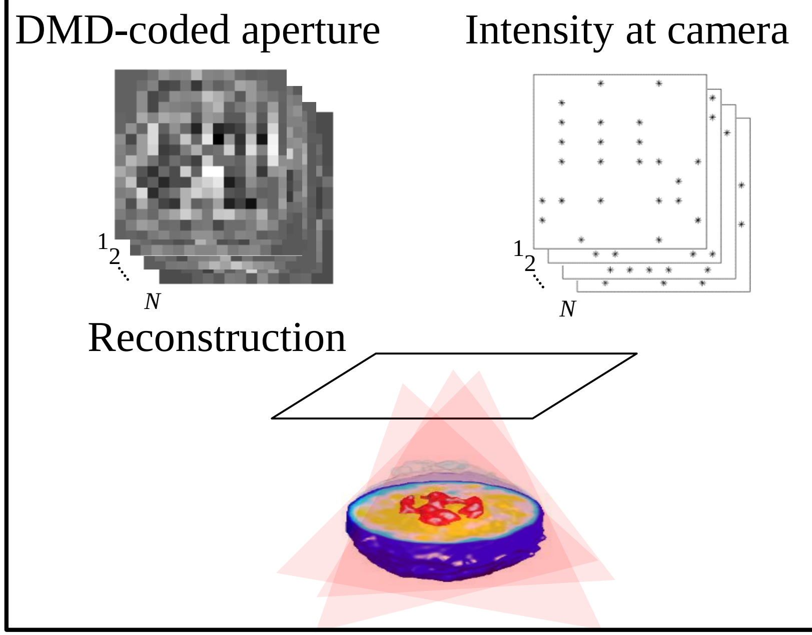
Proposed 3D Imaging Cytometry Design

Compressive-sensing optical diffraction tomography

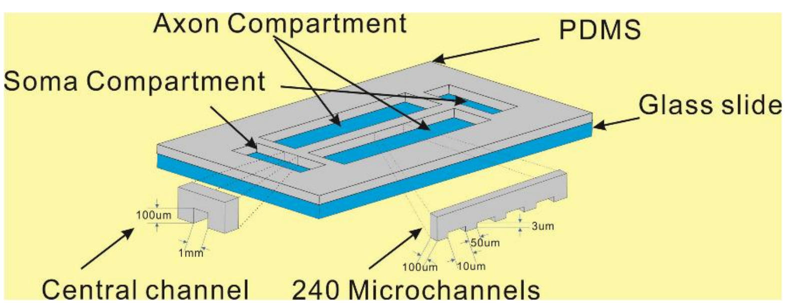
Tomographic Phase Microscopy System



Compressive sensing

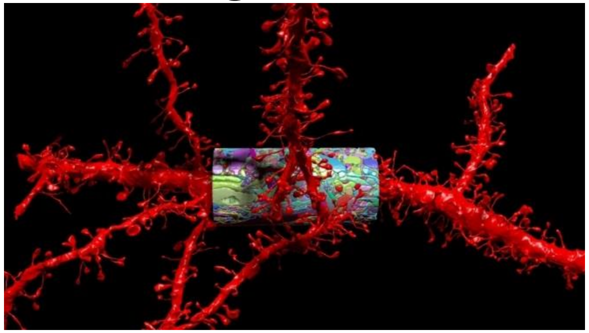


Microfluidic channel network



Ellis Meng group at USC

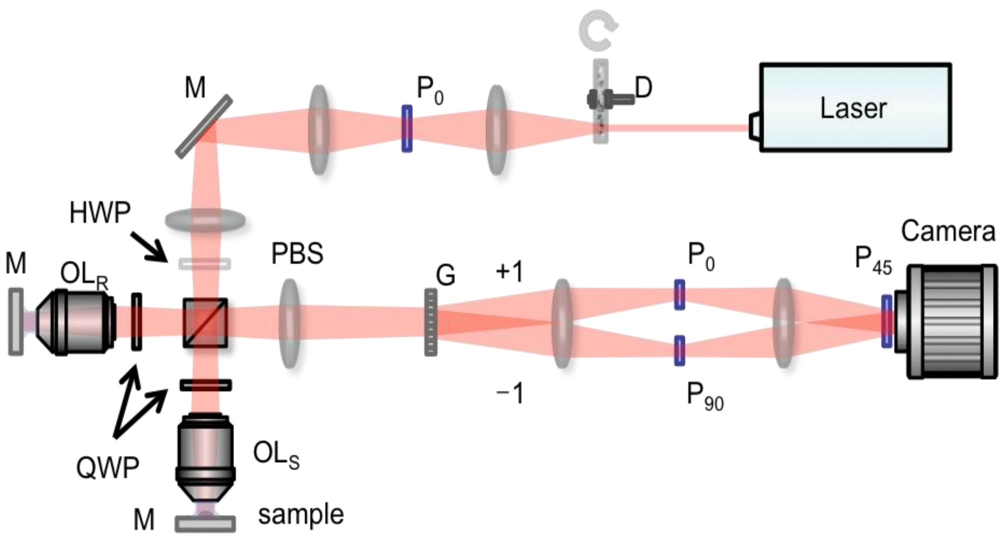
Segmentation

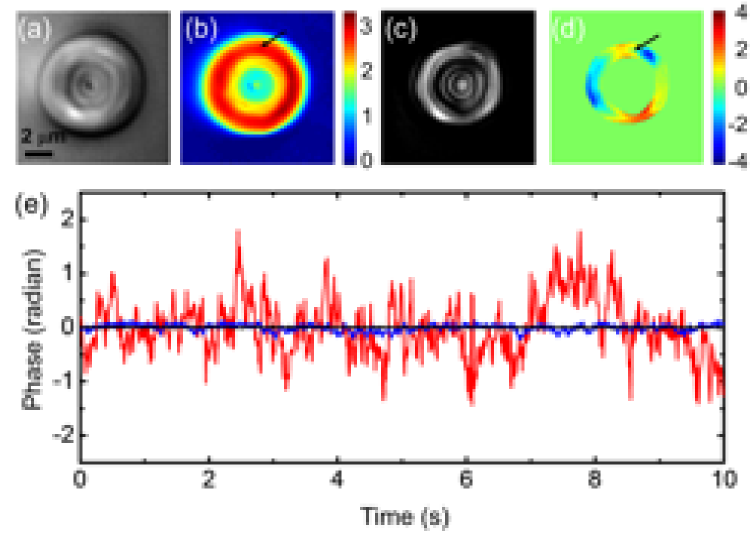


Jeff Lichtman group at Harvard

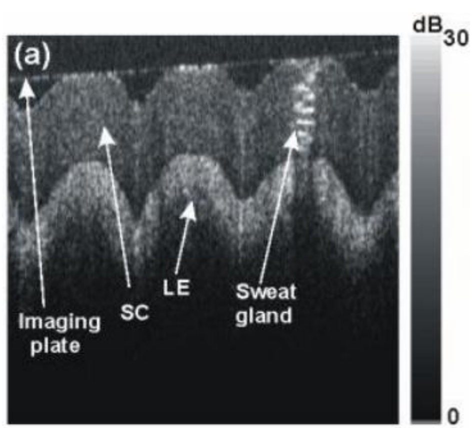
Temporal-coherence gated TPM

Similar to full-field OCT, SDT uses the coherence-gating effect





Y. Choi *et al.*, *Opt. Lett* **39** (2014); R. Zhou *et al.*, *Opt Exp.* **25 (2017)**

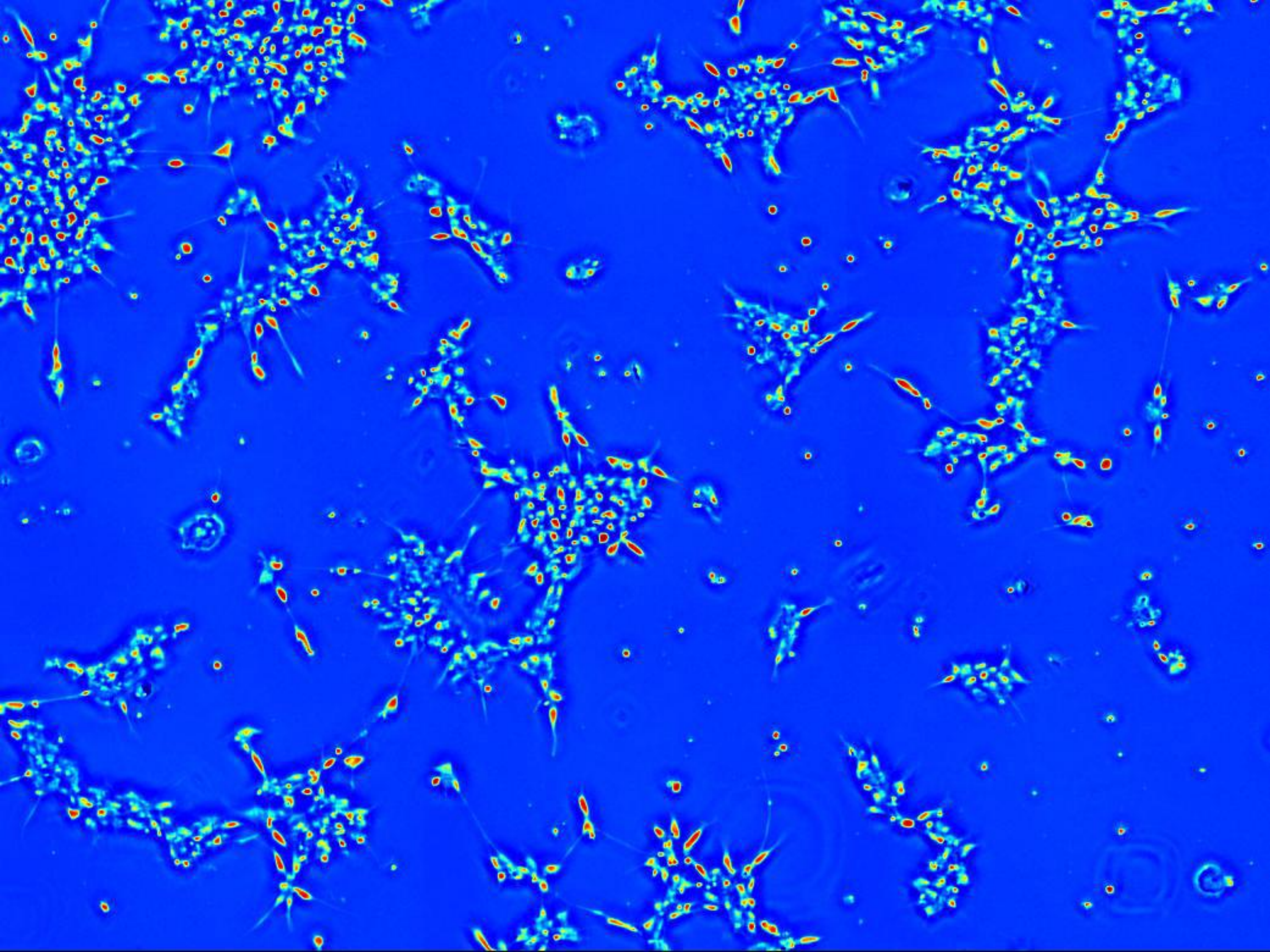


B.F. Kennedy *et al.*, Opt. Exp. **19** (2011)

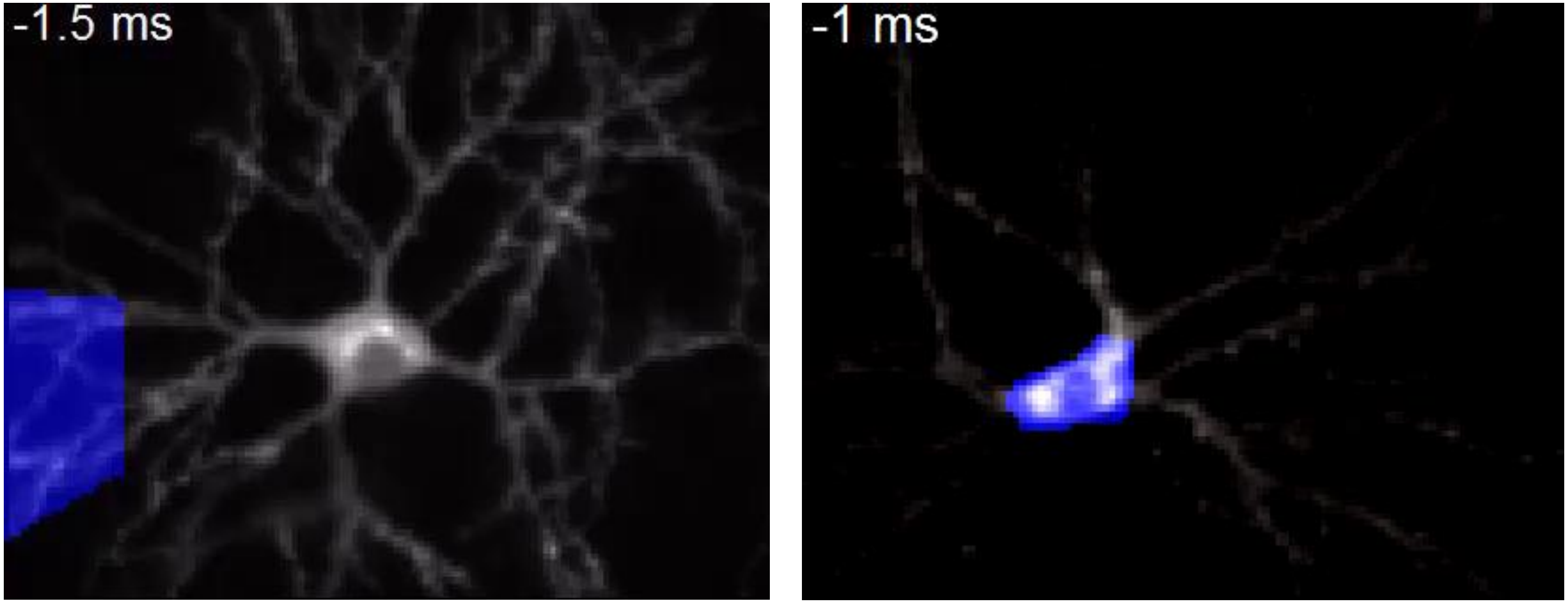
Table 2. OCT Optical Path Length Refractive-Index Measurements for *In Vitro* Human Tissue Specimens

Tissue Type	Measured n (mean ± std. dev.)	Previously Estimated Value of n^a
Dermis	1.40 ± 0.007	1.37 − 1.5
Left ventricular muscle	1.382 ± 0.007	1.40 (canine tissue)
Mesenteric adipose	1.467 ± 0.008	1.455 (bovine tissue)

G. J. Tearney *et al.*, *Opt. Lett.* **20** (2011)



Action-potential Imaging

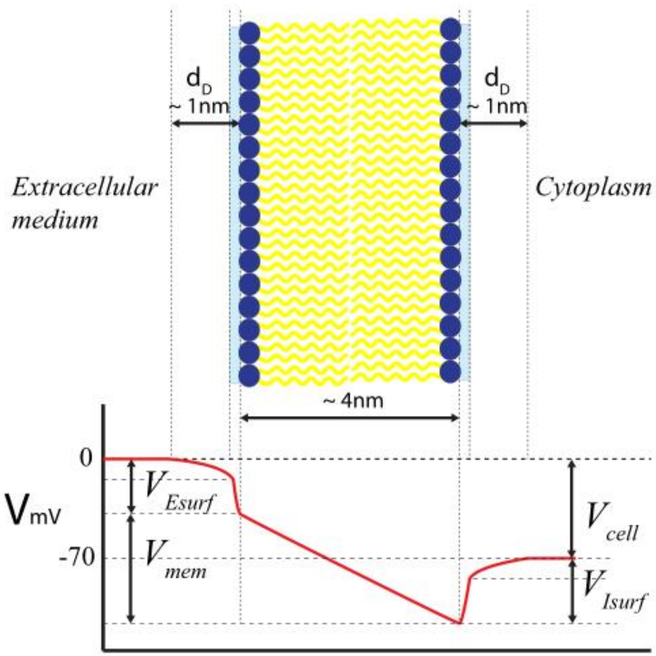


D. R. Hochbaum *et al.*, *Nat. Meth.* **11** (2014)

Platform 2: Super-sensitive Phase Microscopy

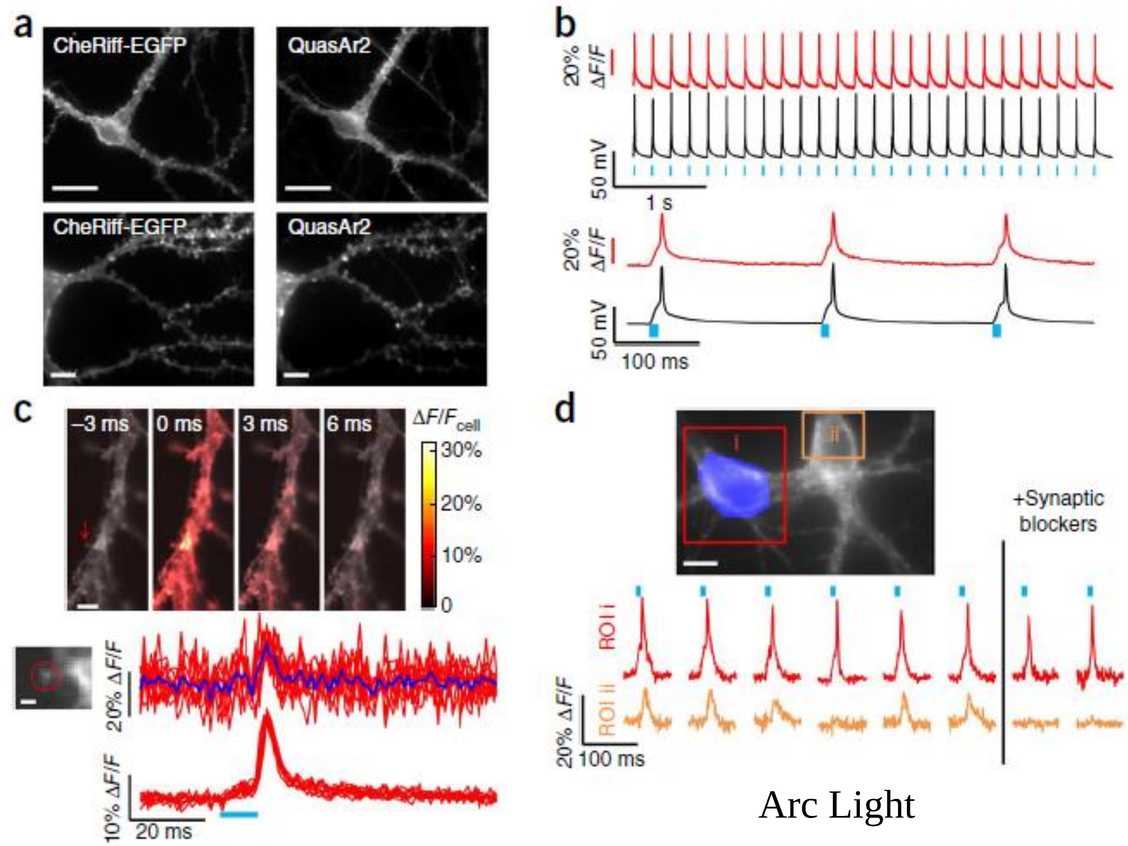
Measure highly dynamical activities on nanometer-scale membrane structures

Plasma membrane potential imaging in neurons



D. S. Peterka *et al.*, Neuron **69** (2011)

Fluorescence-based voltage sensing



Arc Light

D. Maclaurin *et al.*, *PNAS* **110** (2013)
D. R. Hochbaum *et al.*, *Nat. Meth.* **11** (2014)

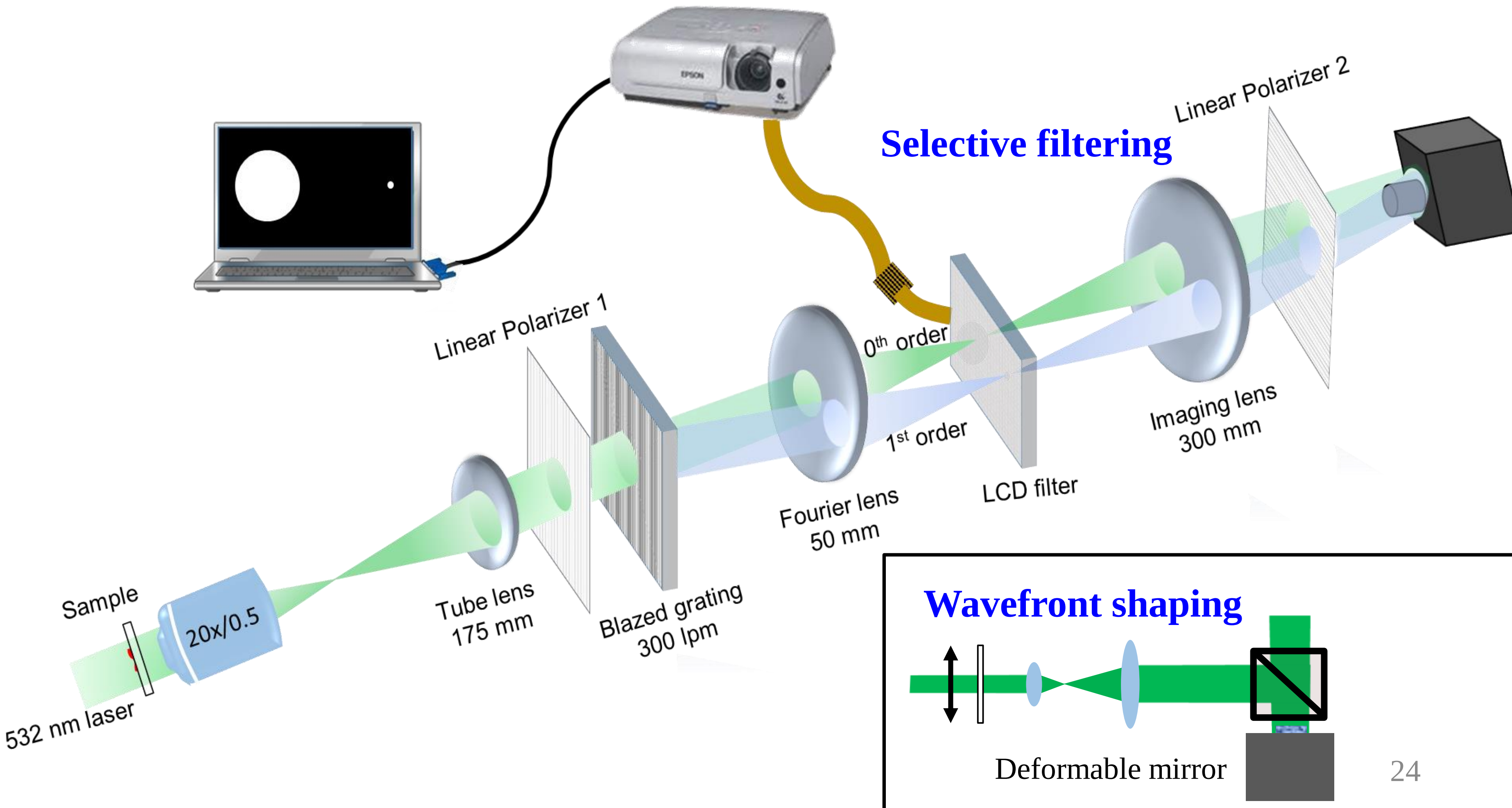
Fluorescence limitations:

1. Photobleaching limited recording length
2. Slow kinetics limit it to resolve closely spaced spikes
3. SNR is limited by probe concentration and fluorescence lifetime
4. Cannot be directly applied in human

Theory and Proposed System Design

Theory and instrumentation design to achieve 10^{-5} sensitivity with ~1ms temporal resolution

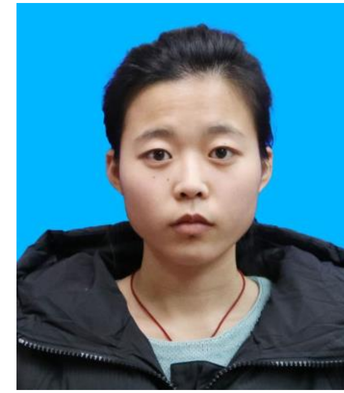
Phase limit: $\delta\varphi = 2\pi\sqrt{\frac{1}{N}}$ rad **Need a super-high well capacity camera**



Current LAMB Members







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