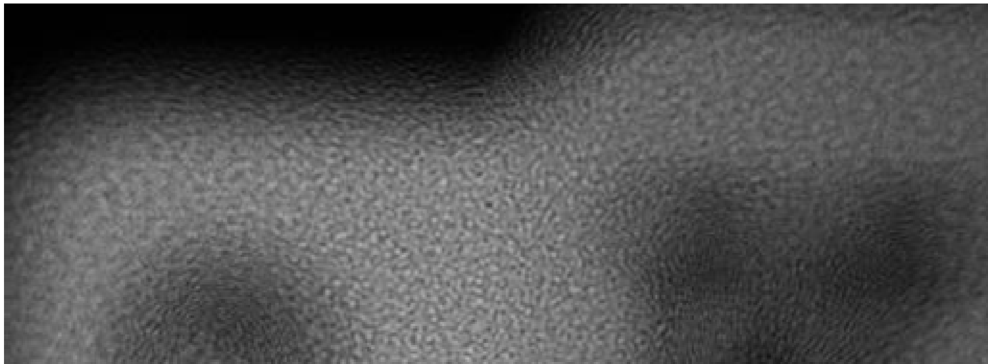
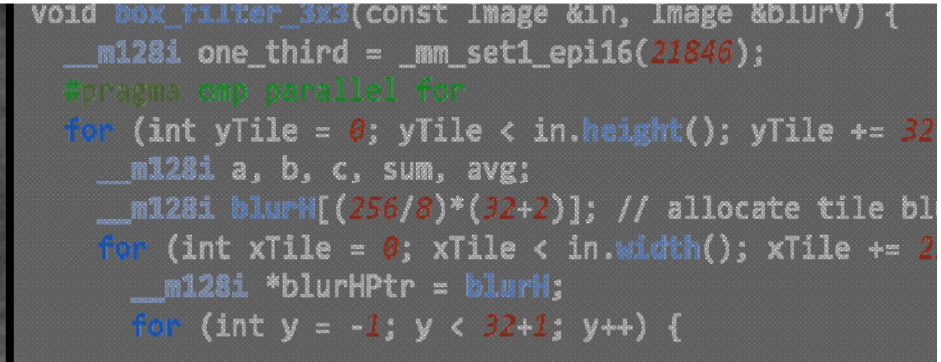
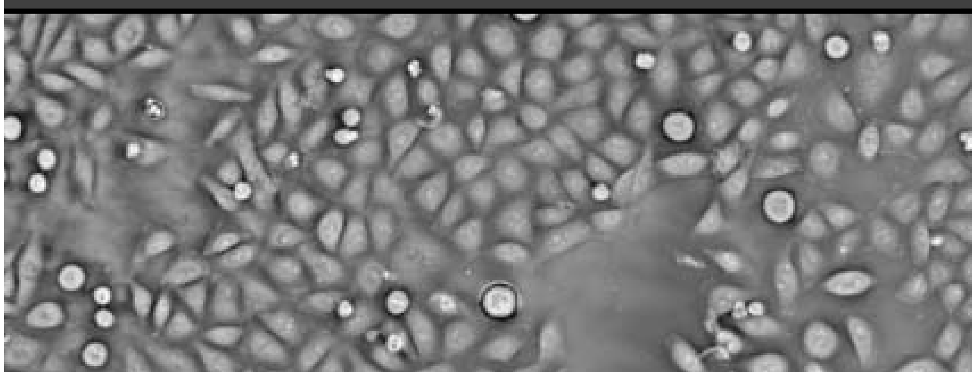
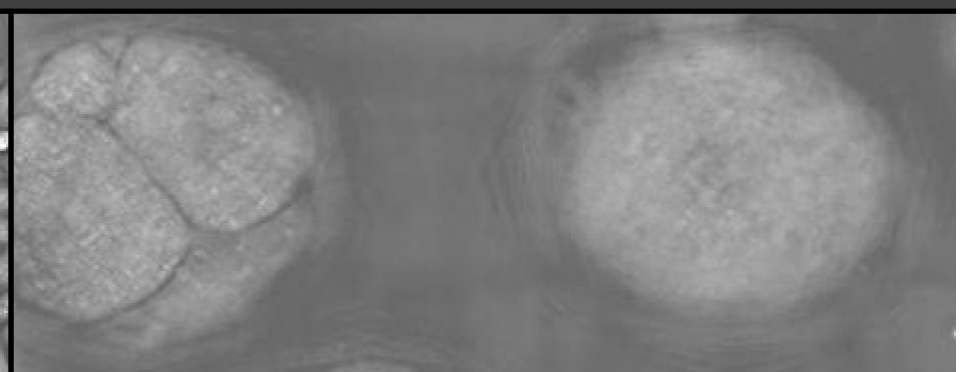




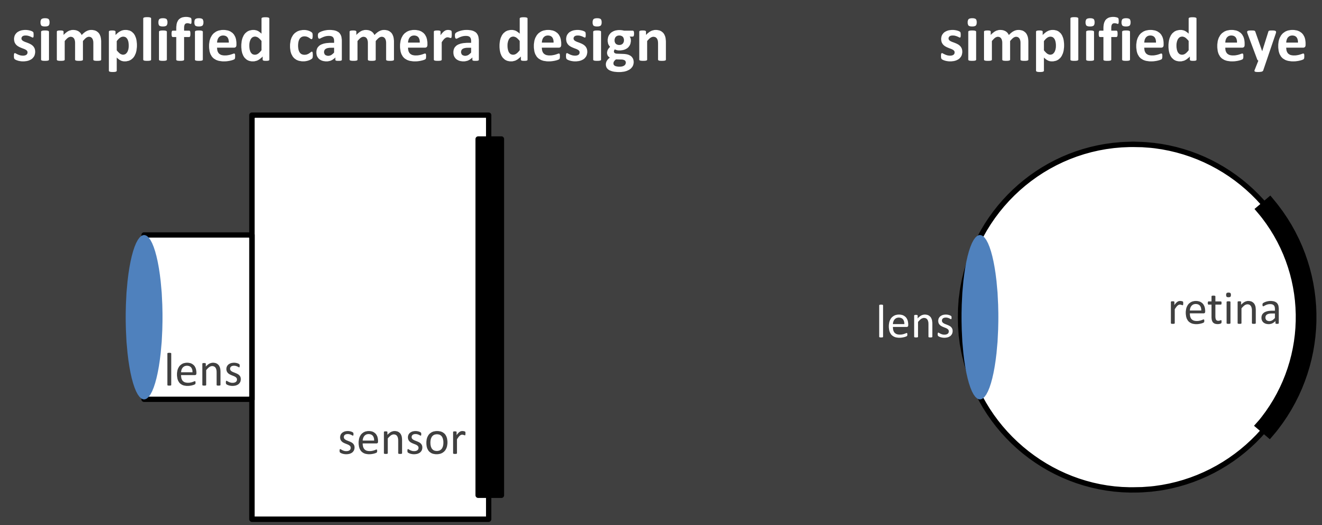
Computational Microscopy
for 3D fluorescence imaging

Laura Waller
Ted Van Duzer Associate Professor
Electrical Engineering and Computer Sciences
UC Berkeley



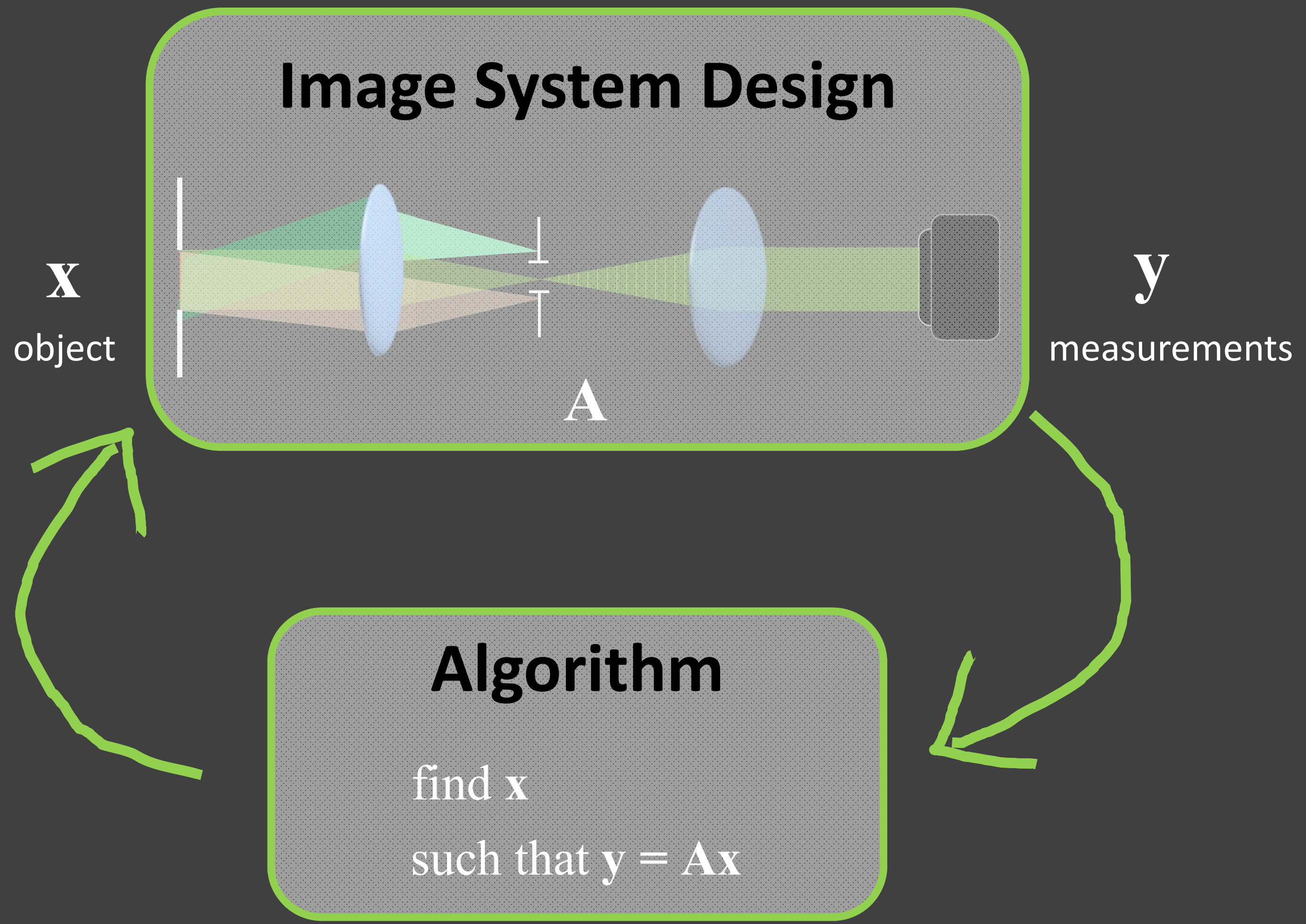


Traditional imaging systems are boring

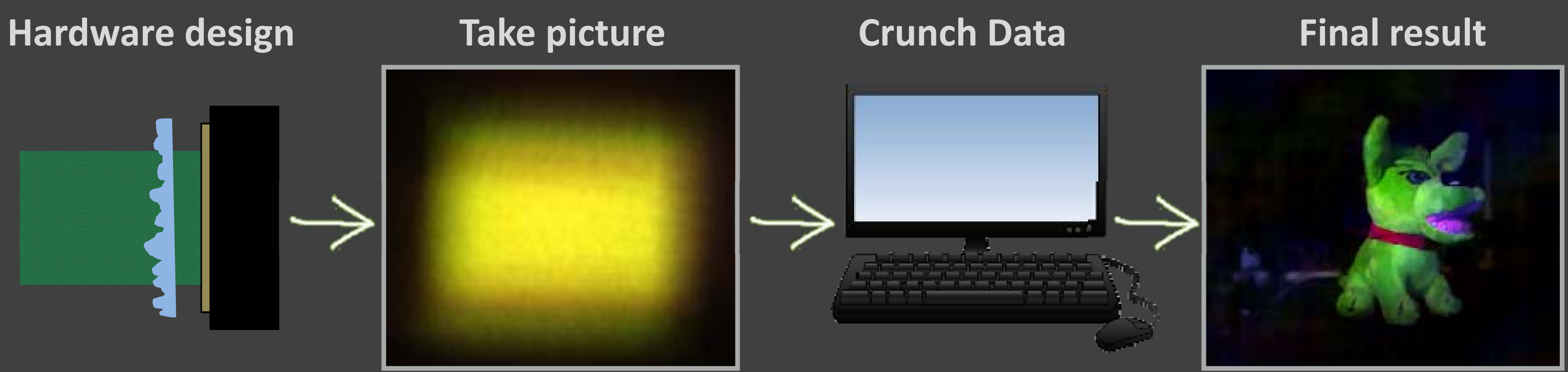


Coincidence? bio-mimetic? lack of creativity?

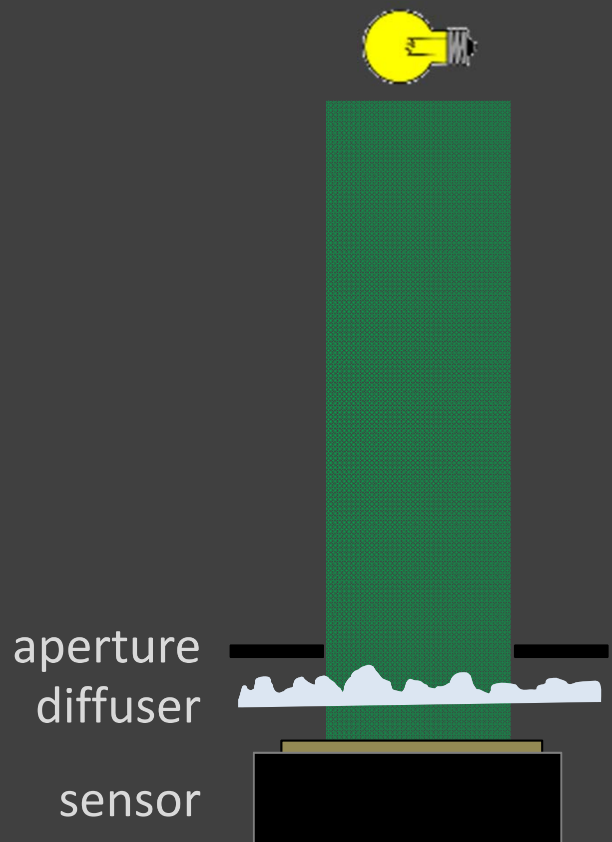
Joint design of hardware and software

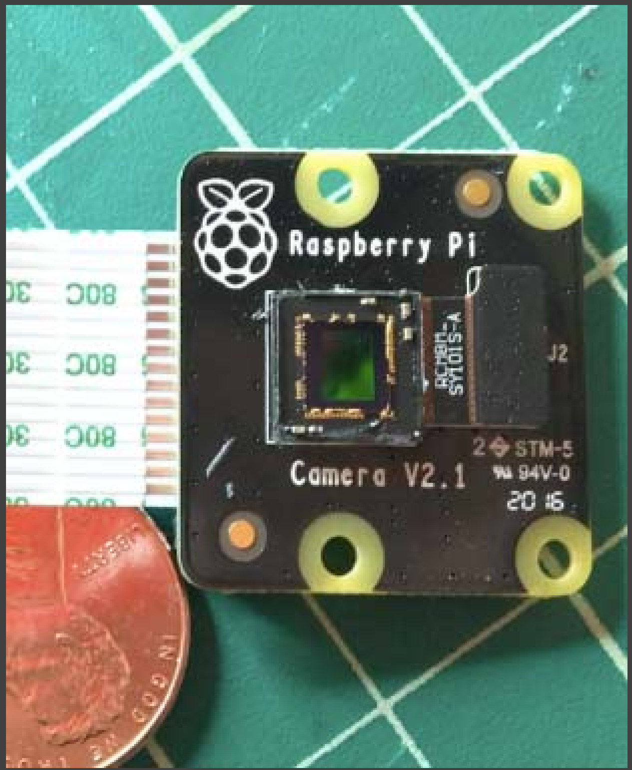


Computational imaging pipeline



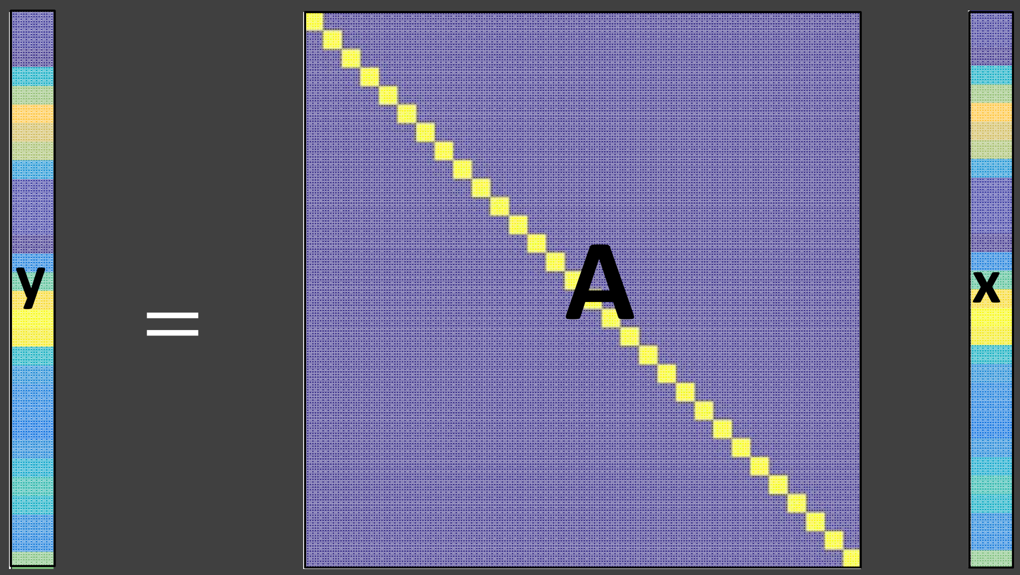
DiffuserCam: tape a diffuser onto a sensor



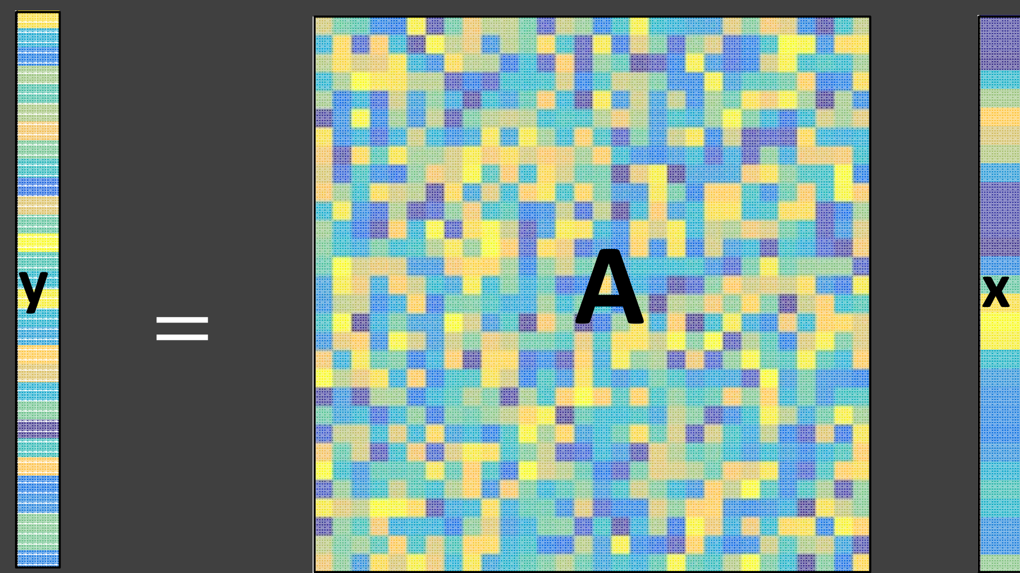


Grace Kuo
Nick Antipa

Traditional cameras take direct measurements



Computational cameras can multiplex

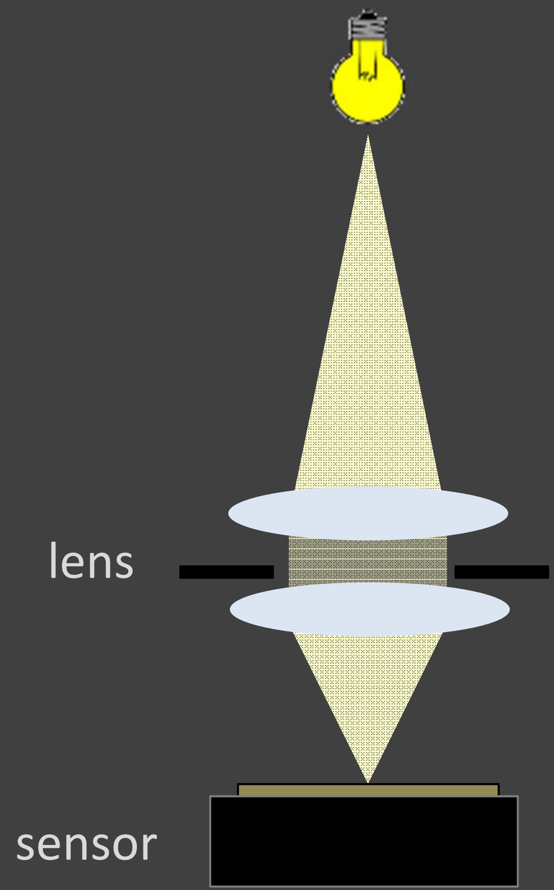


Forward model is:
- measured
- a physical model

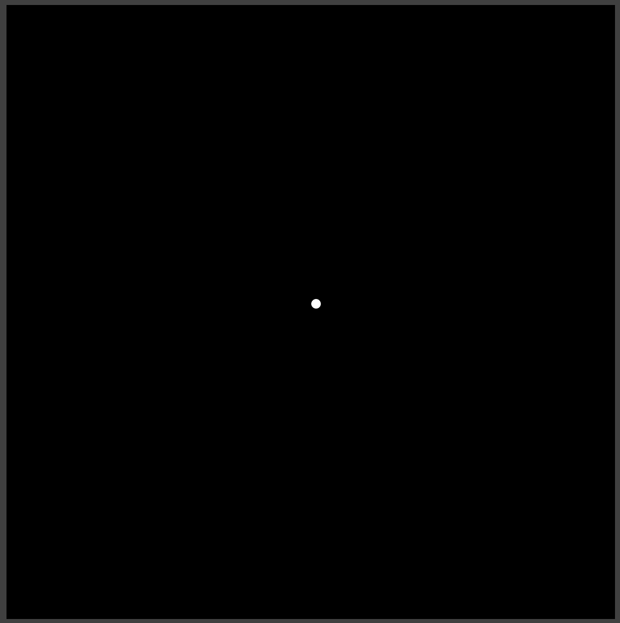
measurement

object

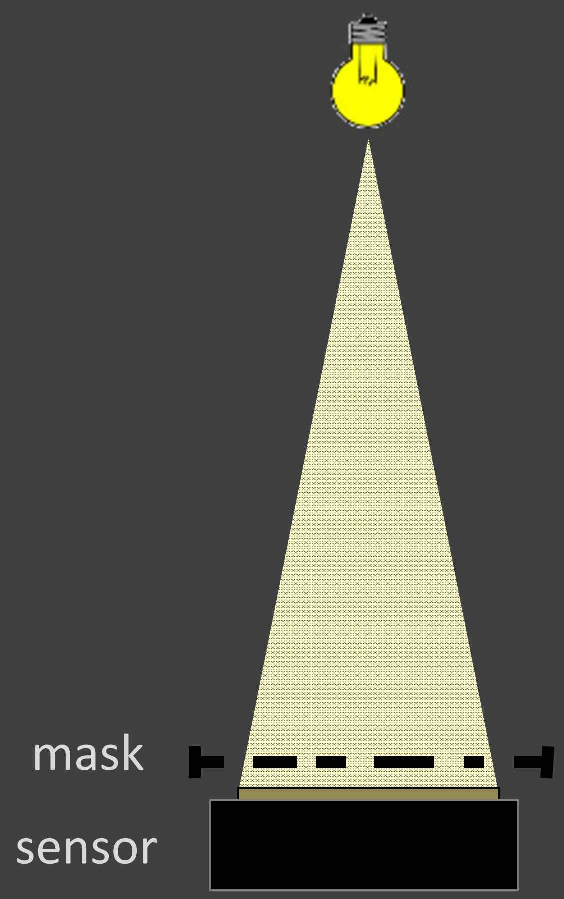
Lenses map points to points



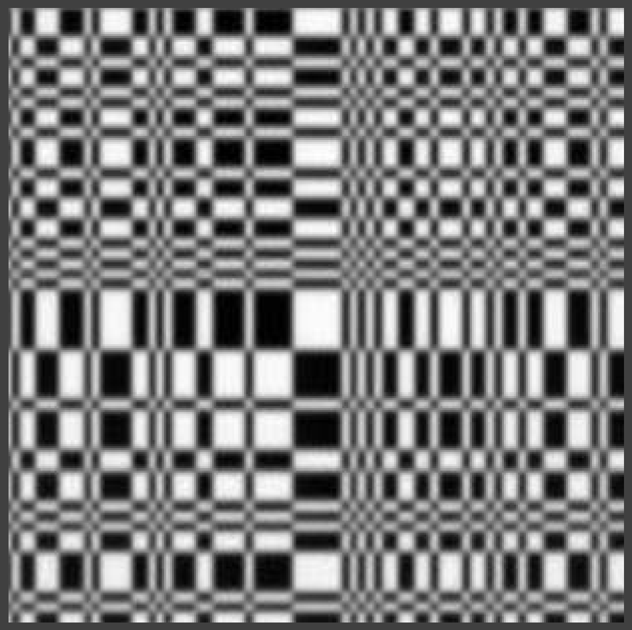
Point Spread Function (PSF)



Mask-based cameras multiplex

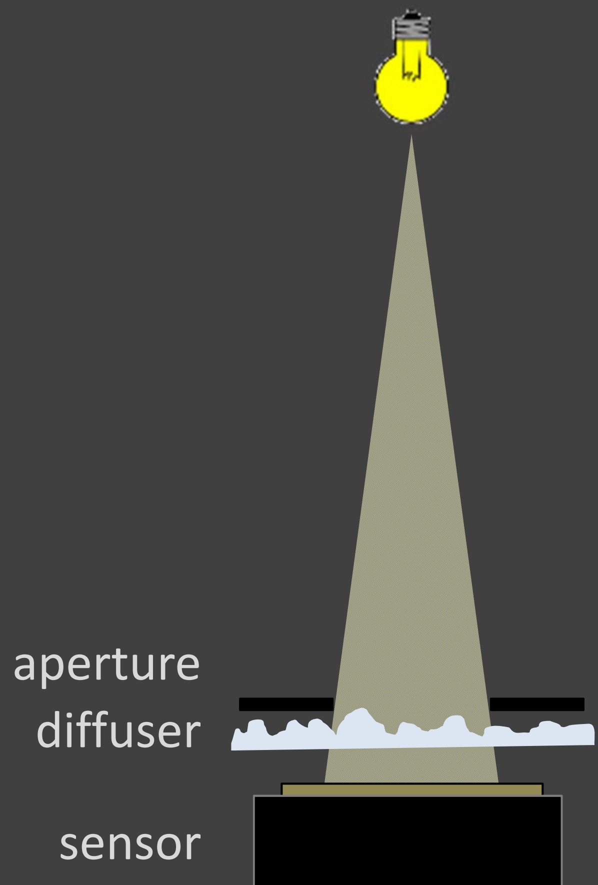


Point Spread Function (PSF)

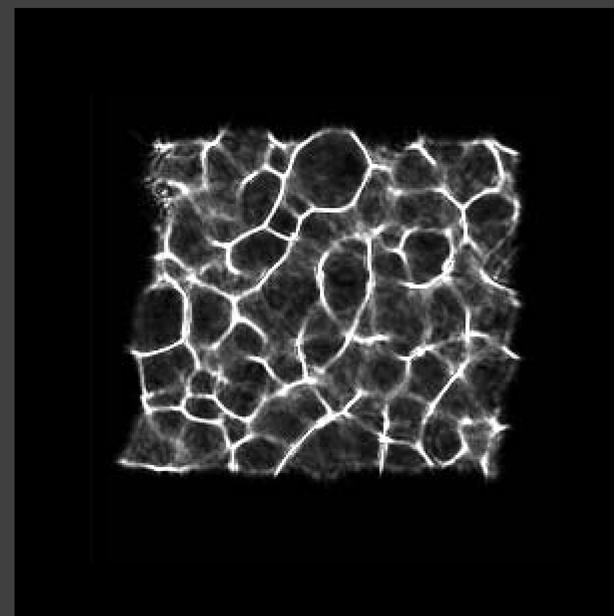


M. S. Asif, et al. *ICCVW* (2015)
J. Tanida, et al. *Applied optics* (2001)
K. Tajima, et al. *ICCP* (2017)
D. G. Stork, et al. *Int. J. Adv. Systems and measurements* (2014)

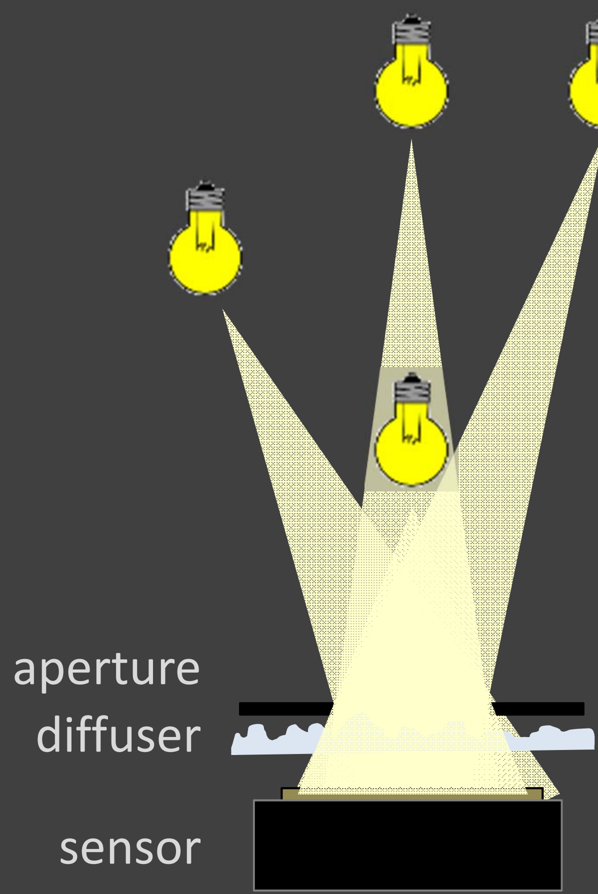
Diffuser indirectly encodes information

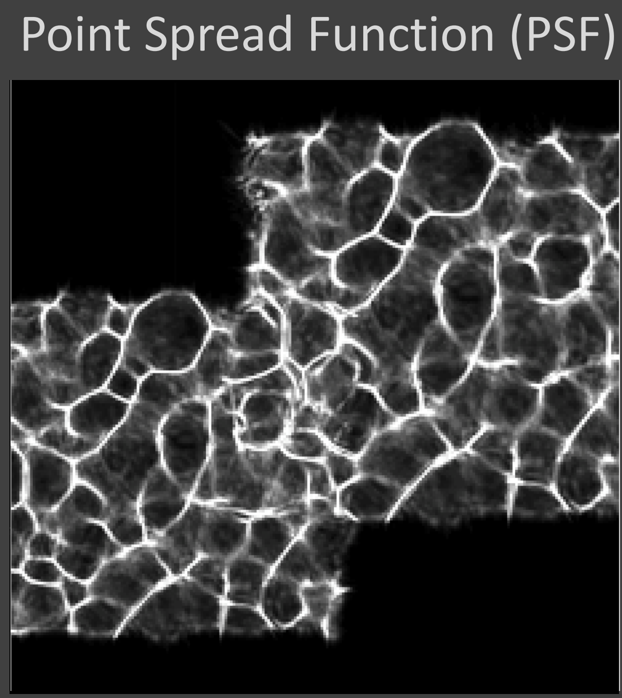


Point Spread Function (PSF)

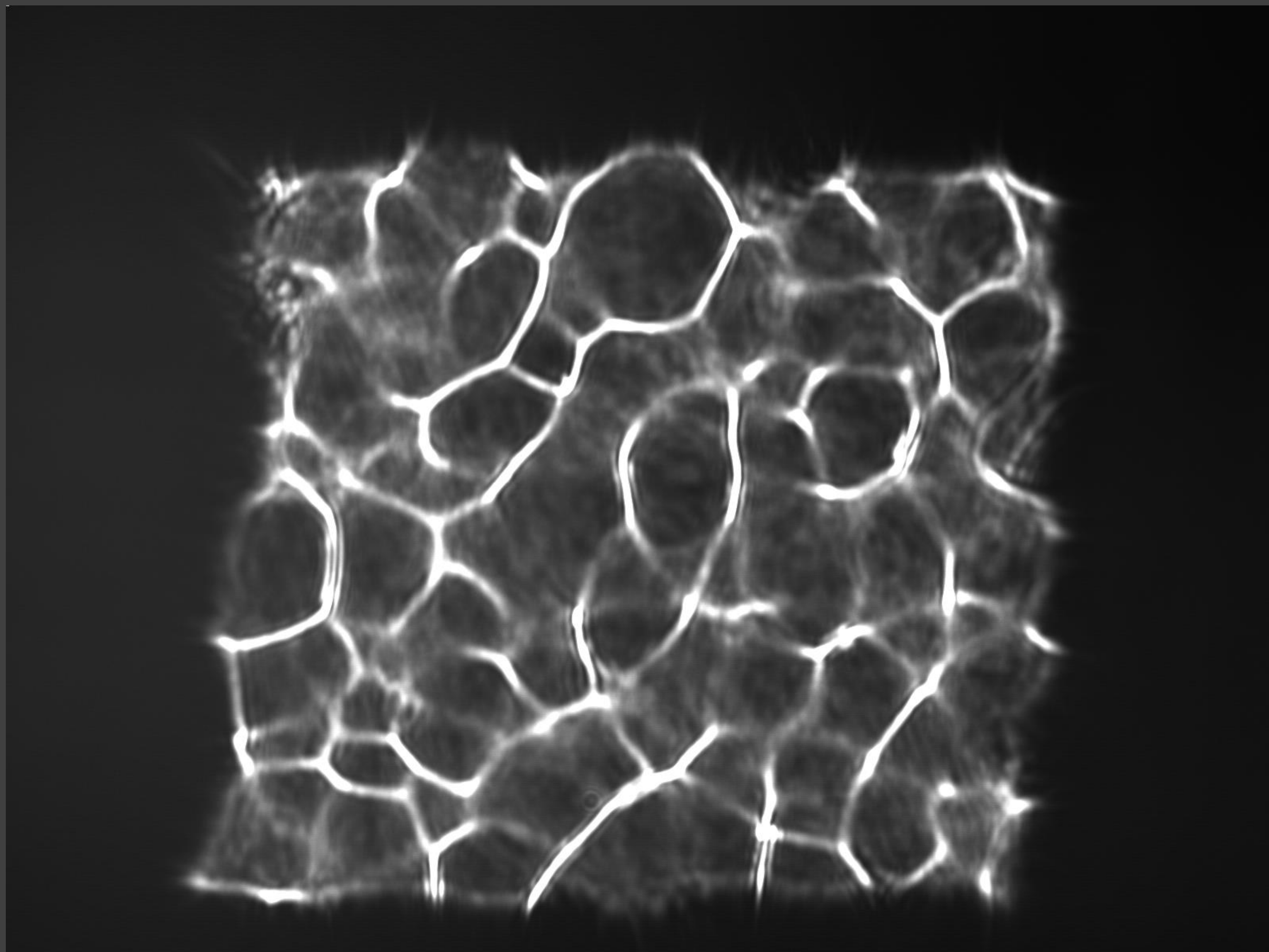


Diffuser indirectly encodes information

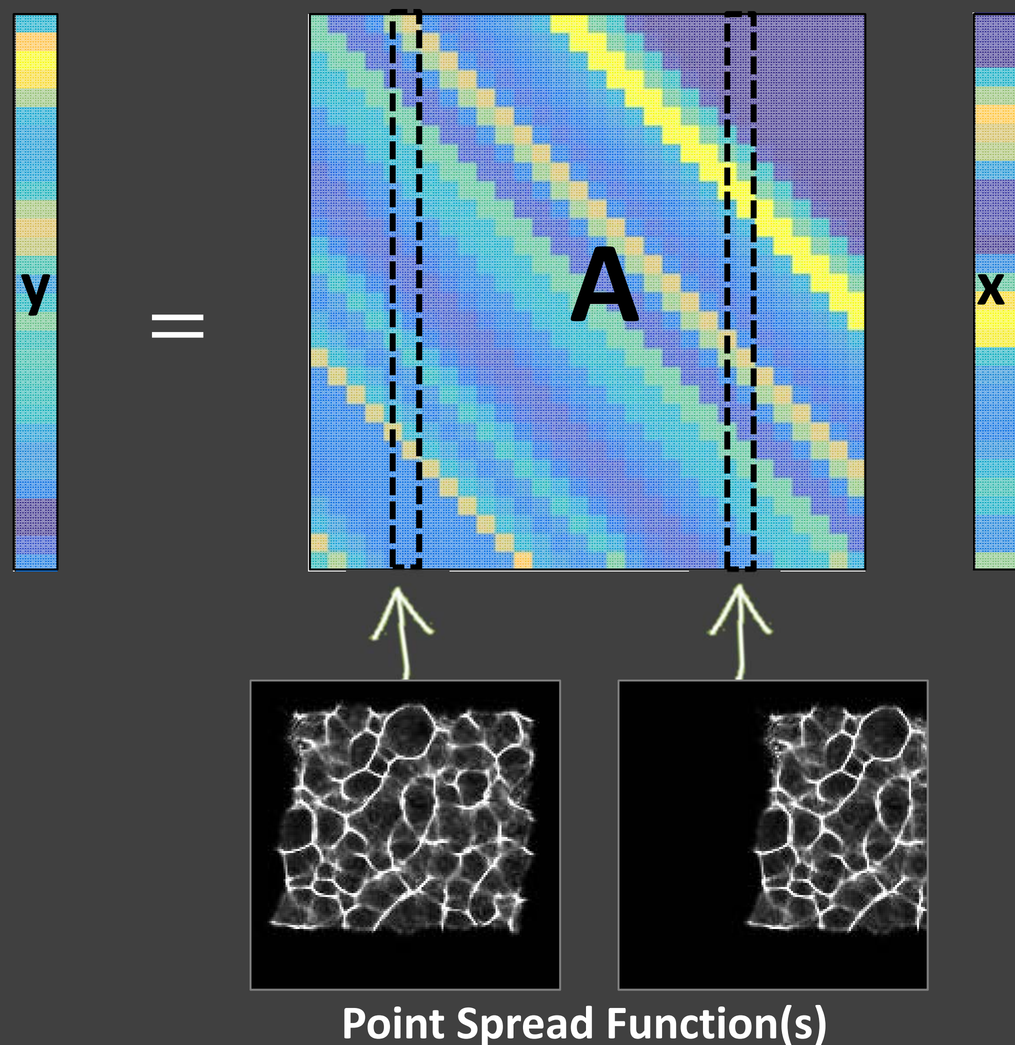




Point spread function shifts with object



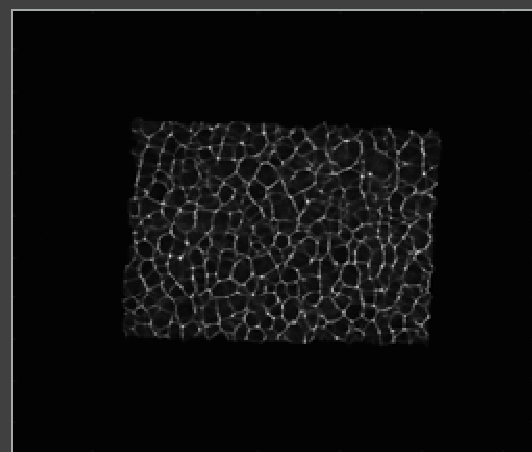
DiffuserCam forward model is a convolution



2D Photography Forward Model

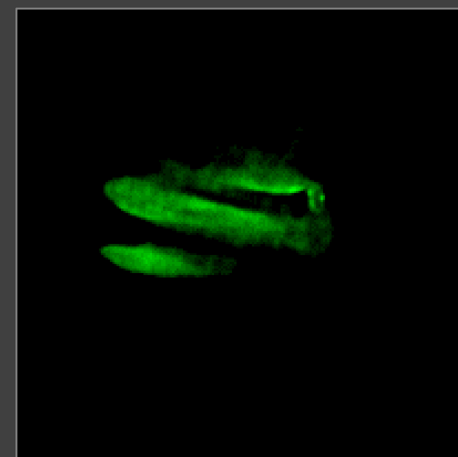
Measurement

=

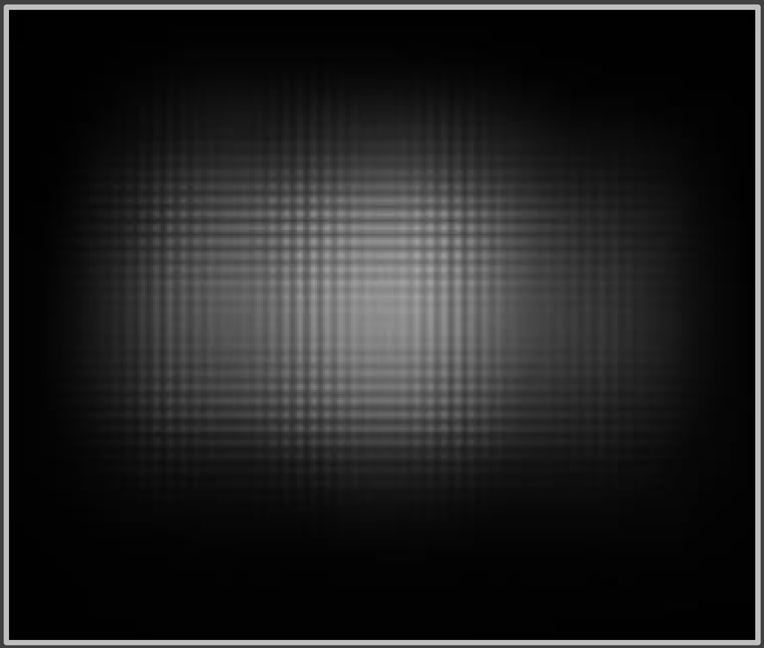


Point Spread Function

*



Object



raw sensor data

recovered scene

*solver is ADMM with TV reg in Halide

raw sensor data

recovered scene

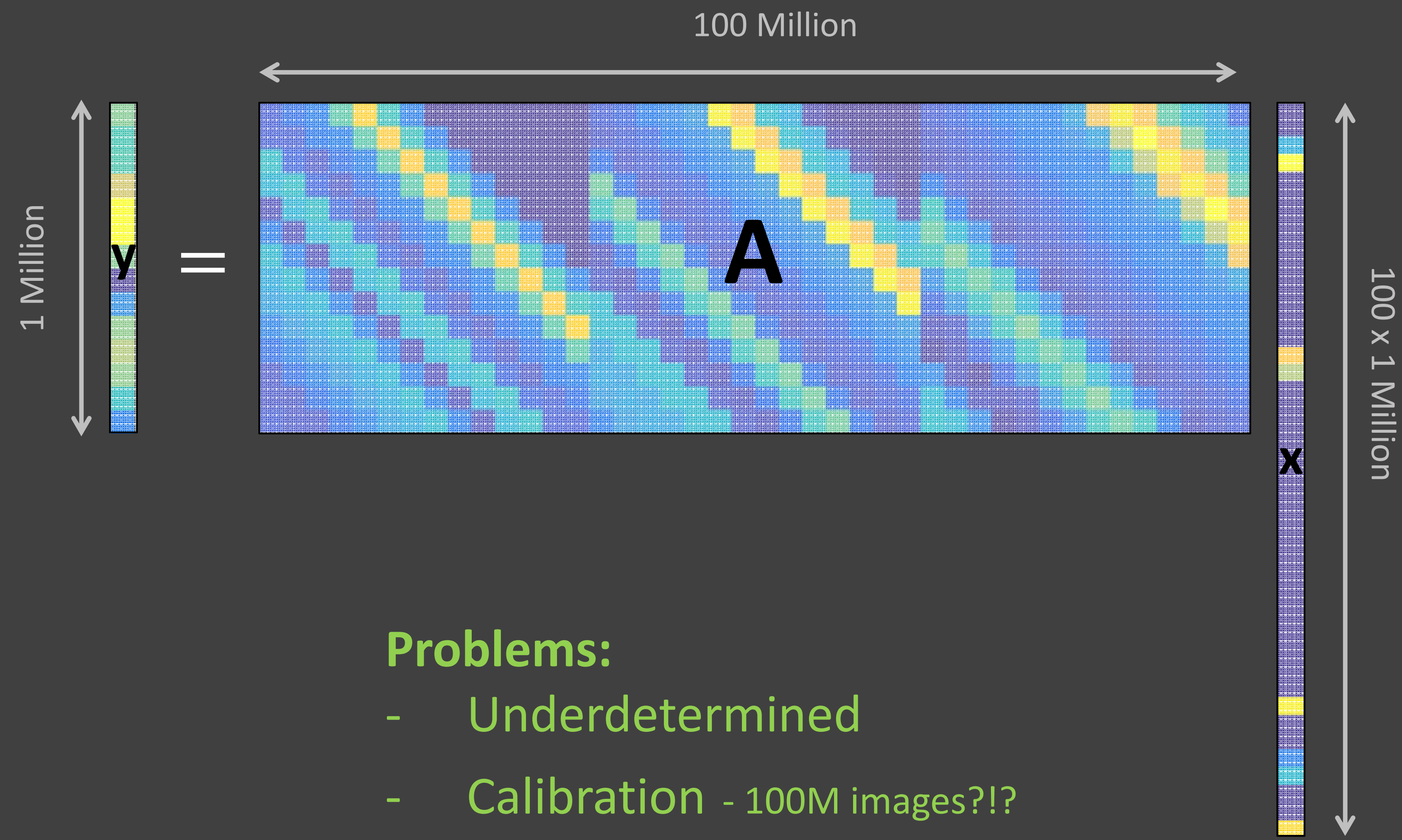
*solver is ADMM with TV reg in Halide

raw sensor data

recovered scene

*solver is ADMM with TV reg in Halide

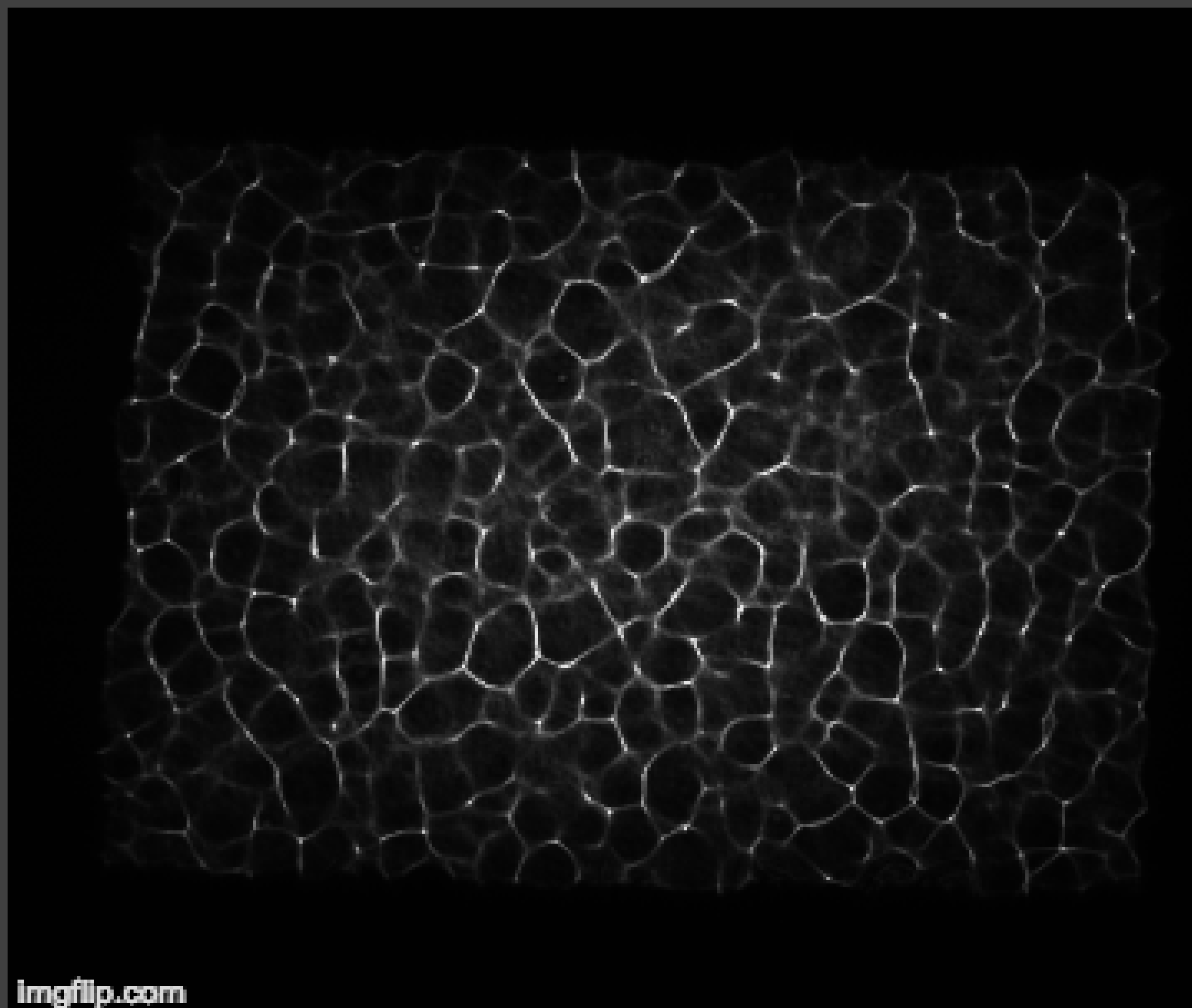
3D is not so easy



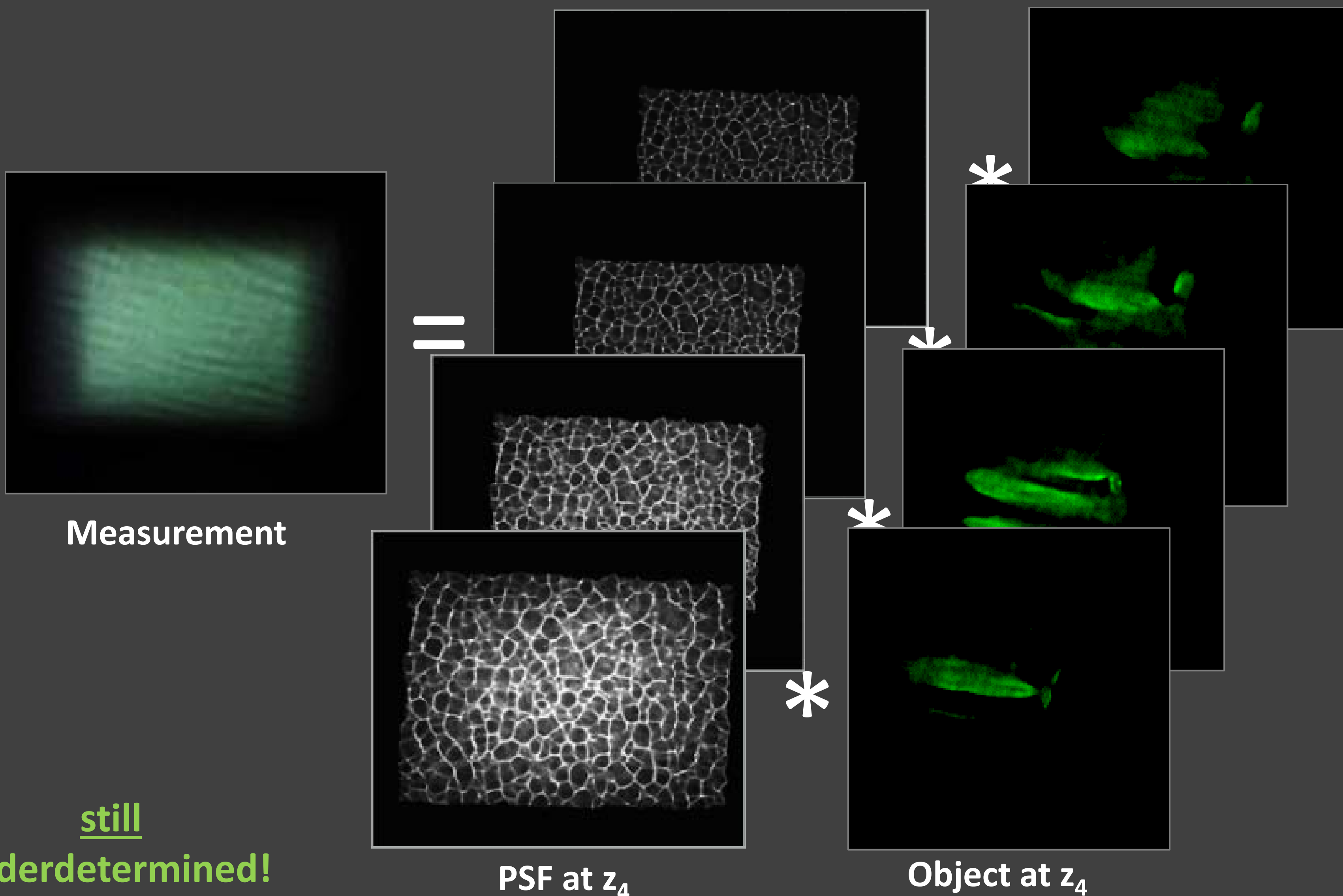
Problems:

- Underdetermined
- Calibration - 100M images?!?

The PSF changes with depth



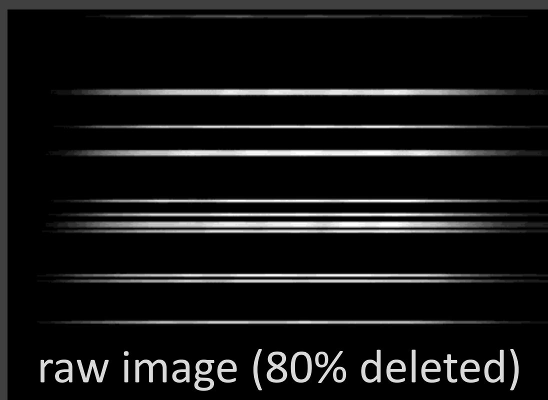
3D Forward Model: Sum of Convolutions



still
underdetermined!

Compressed sensing

solves under-determined problems via sparsity prior


raw image (80% deleted)

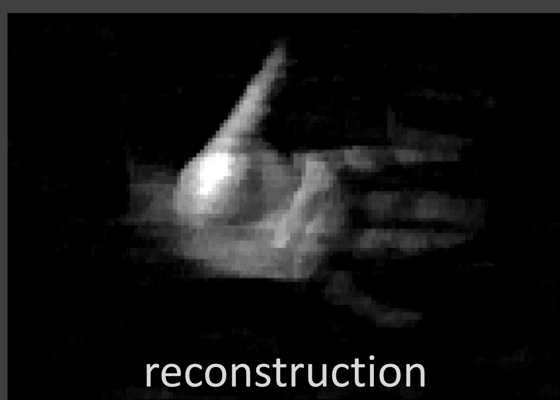
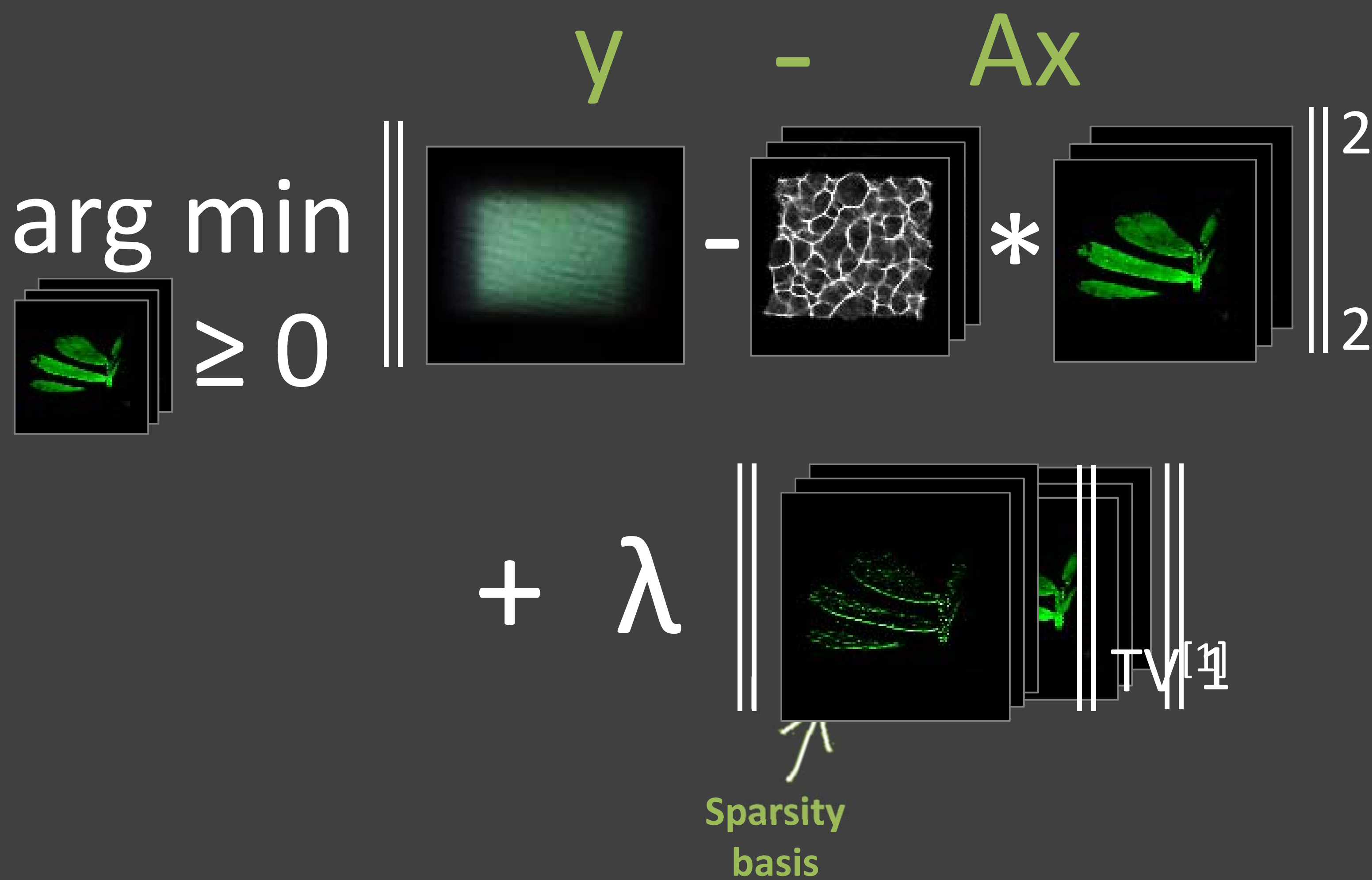
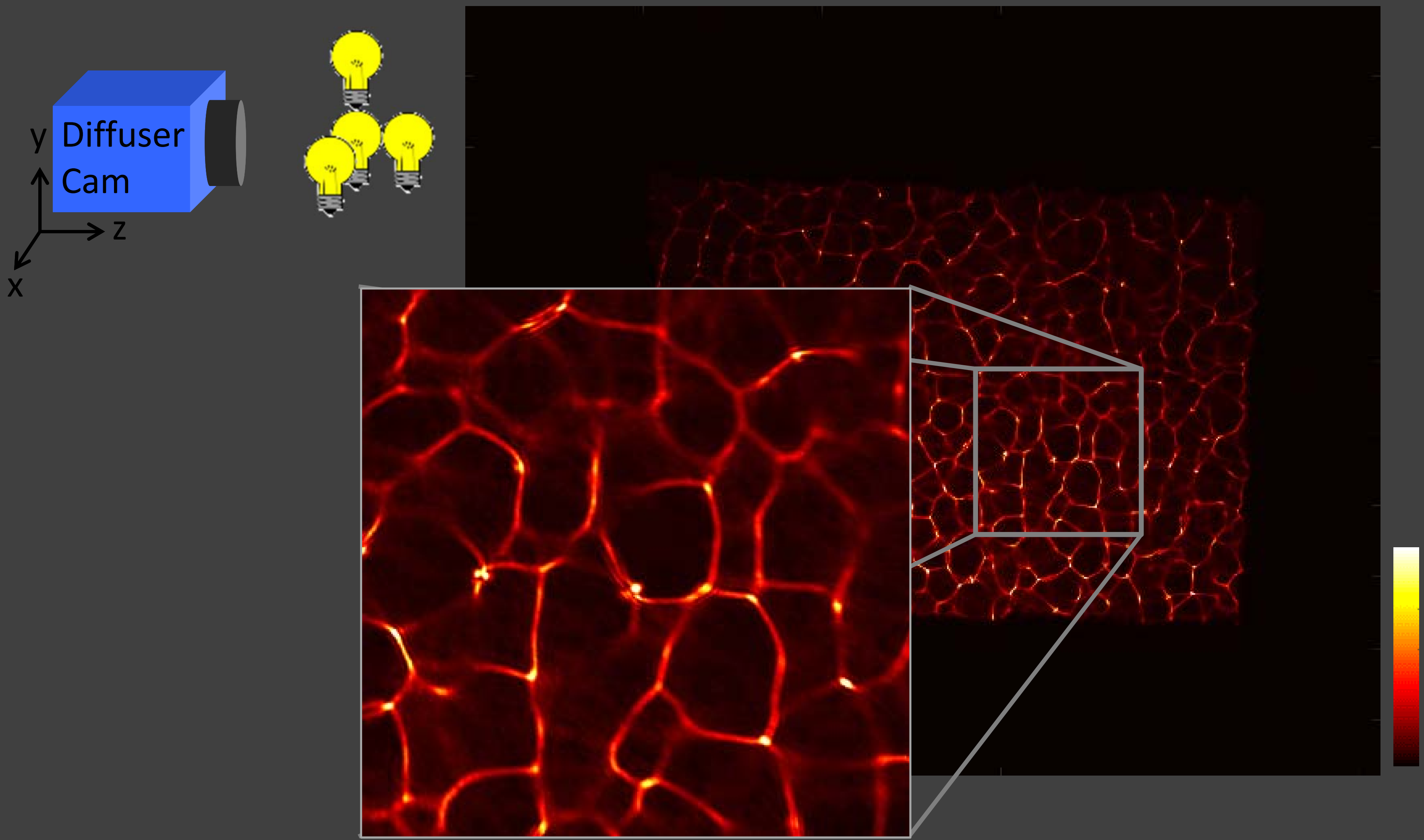

reconstruction

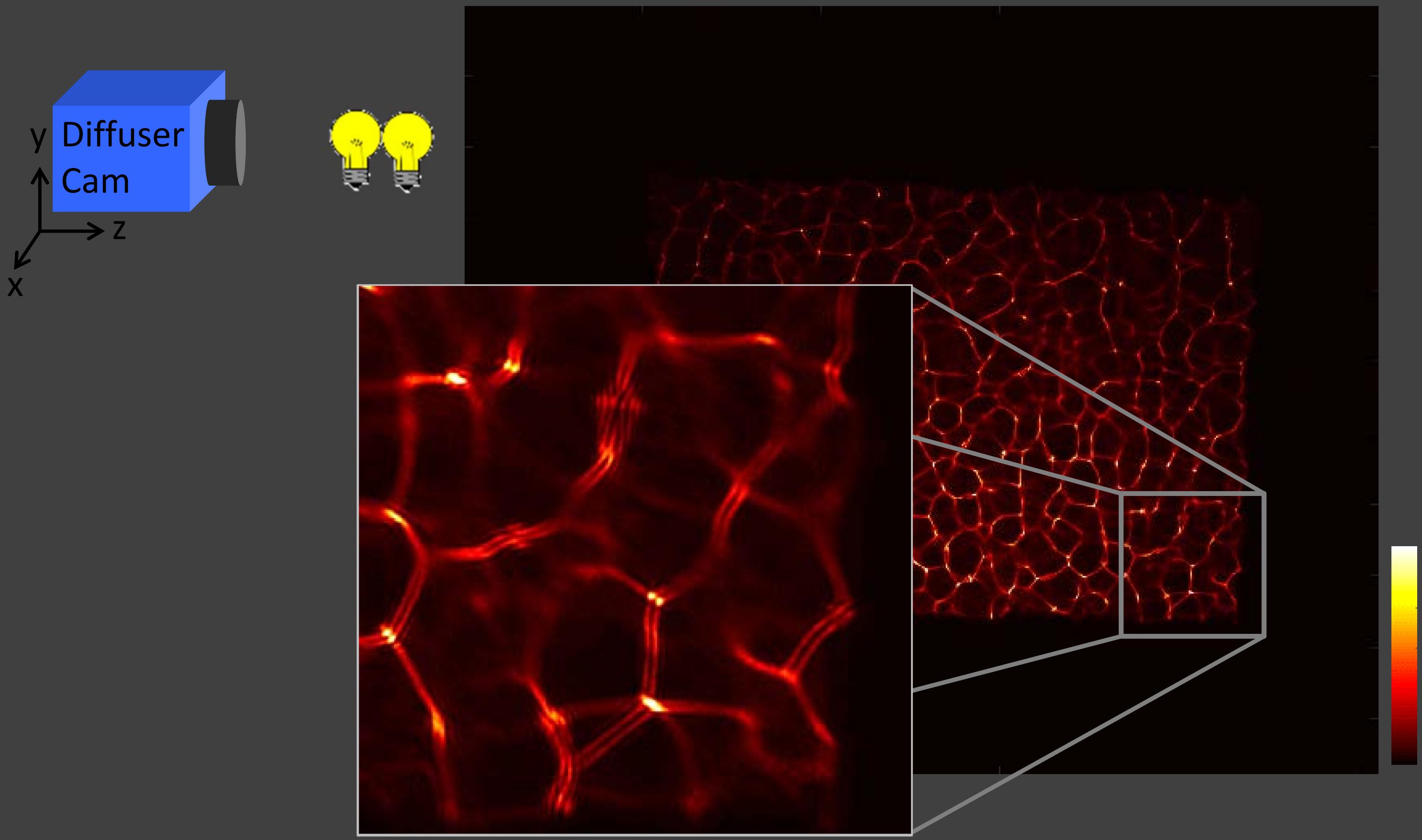
Image Reconstruction with Sparsity Prior



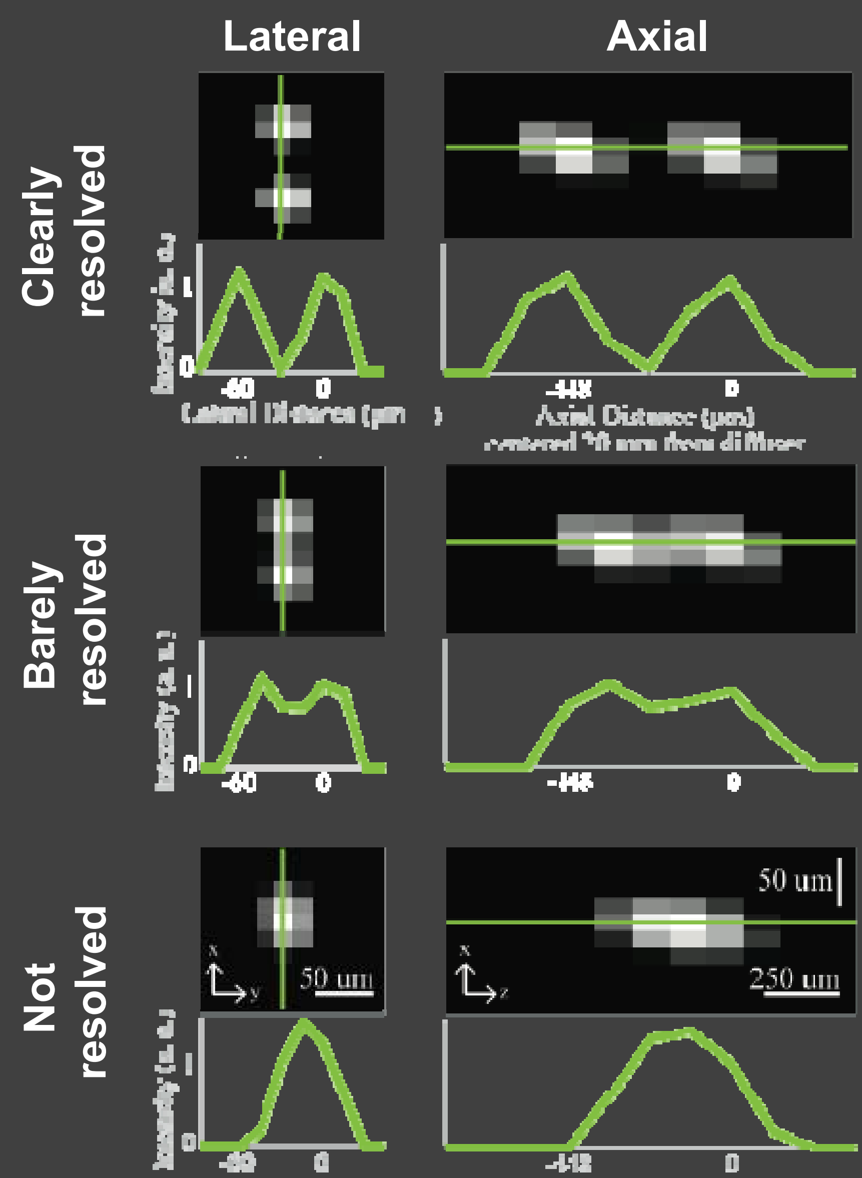
High frequencies define resolution

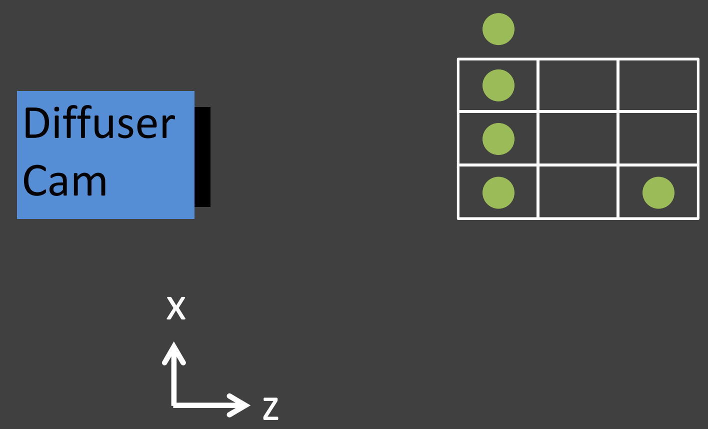


High frequencies define resolution

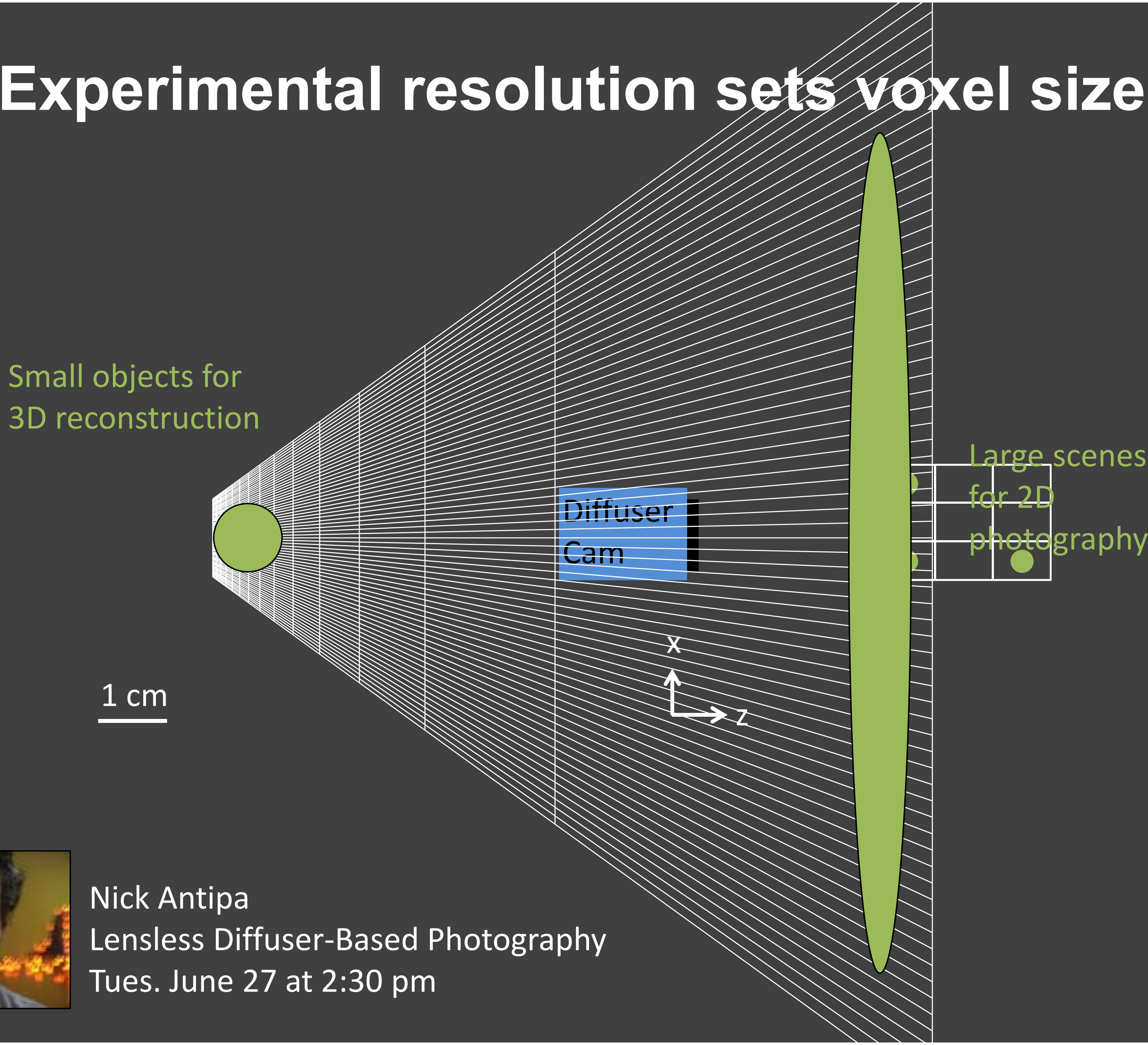


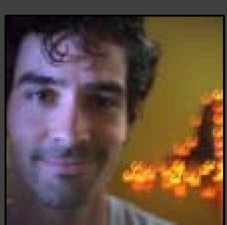
Experimental resolution sets voxel size



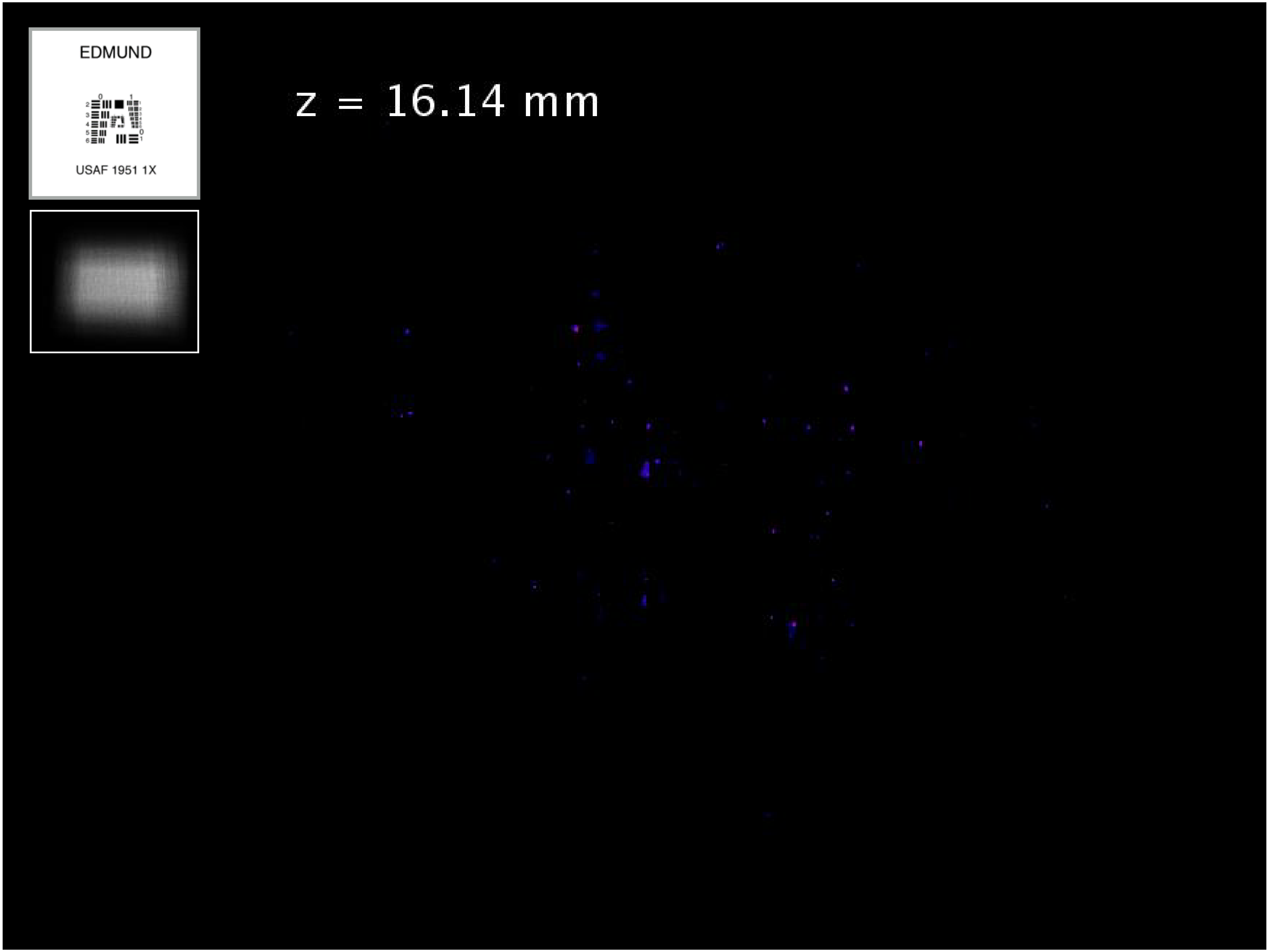


Experimental resolution sets voxel size





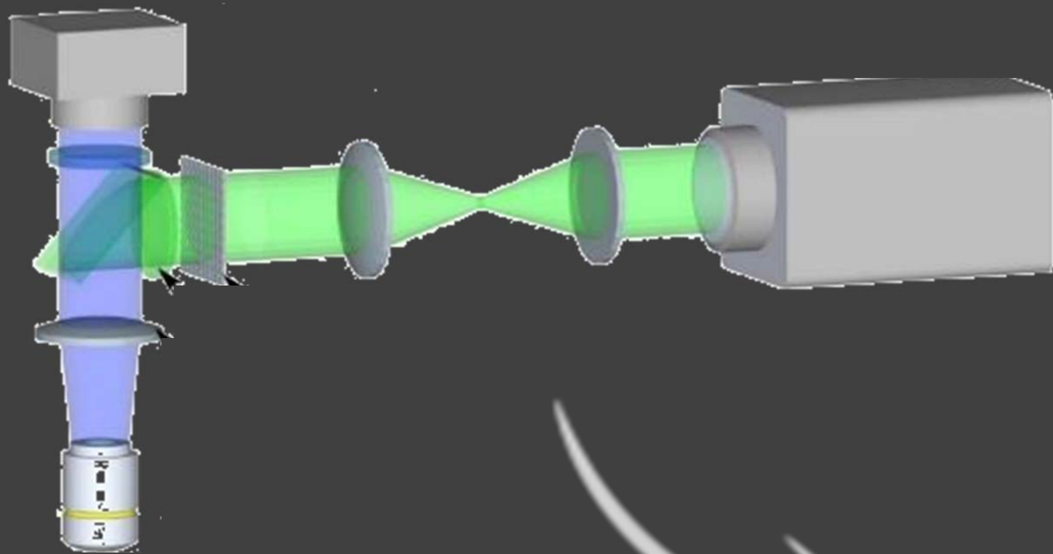
Nick Antipa
Lensless Diffuser-Based Photography
Tues. June 27 at 2:30 pm


EDMUND
USAF 1951 1X
z = 16.14 mm

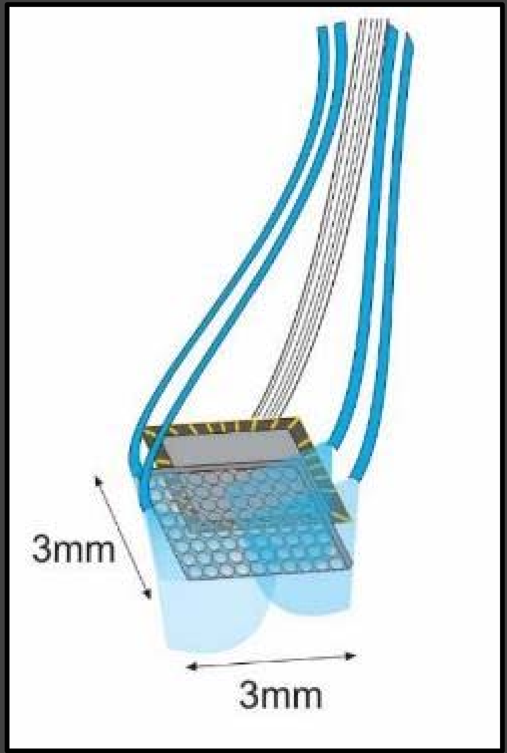

EDMUND
USAF 1951 1X
128x more pixels for FREE!


128x more pixels for FREE!

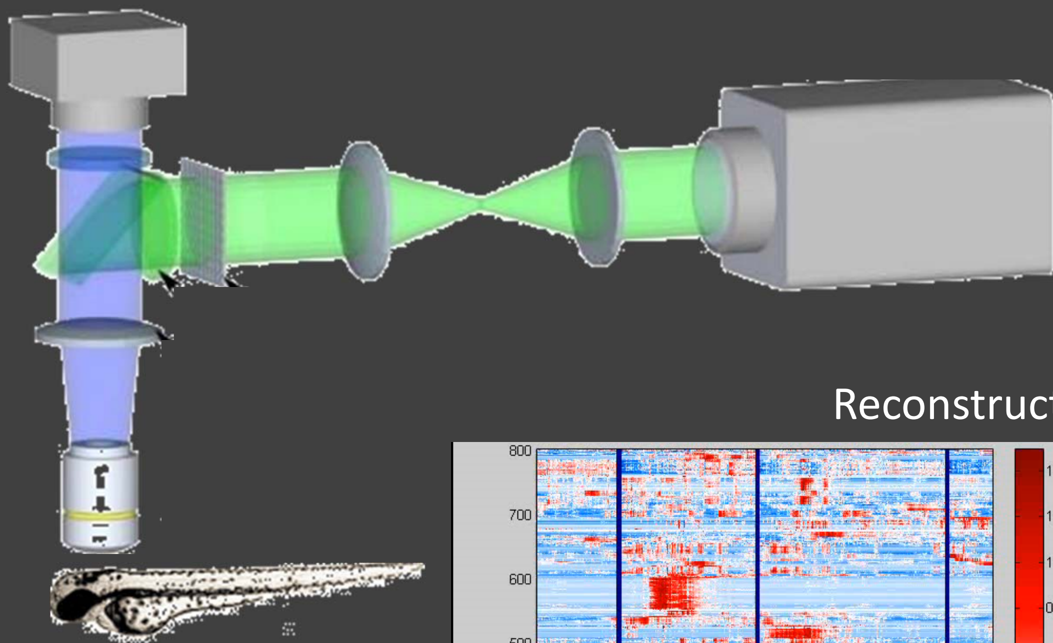
Towards lensless 3D microscopy



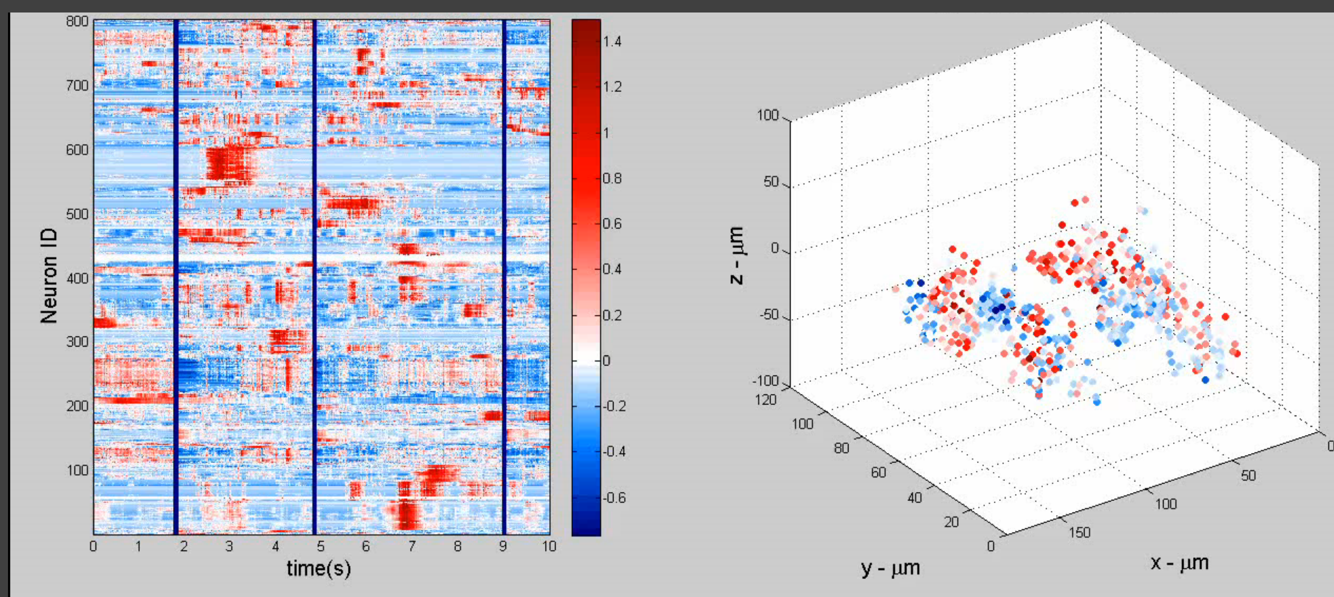
Lensless imager:
- small
- inexpensive
- enables tiling



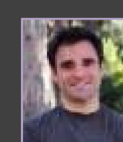
3D imaging of brains



Reconstructed neural activity



with Adesnik Lab



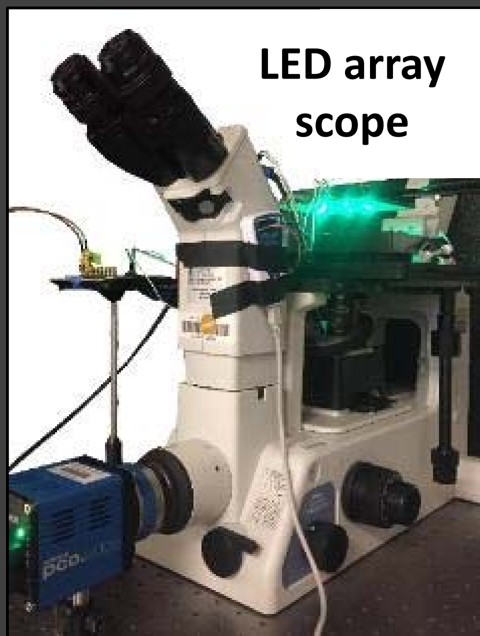
N. Pegard et al, *Optica* 2016

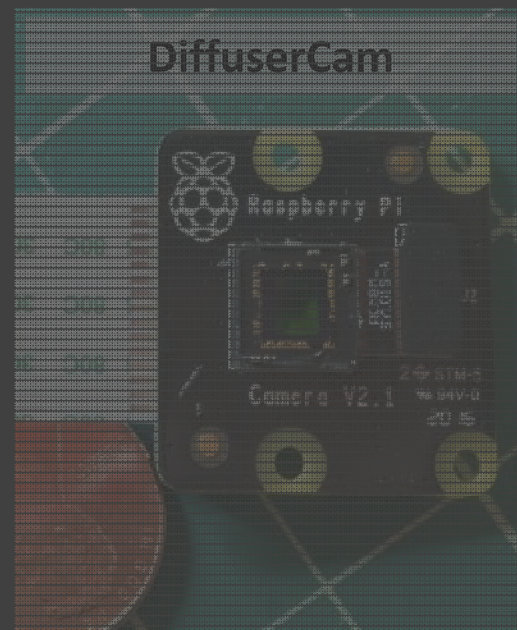
Scanning **vs.** **Compressed Sensing**

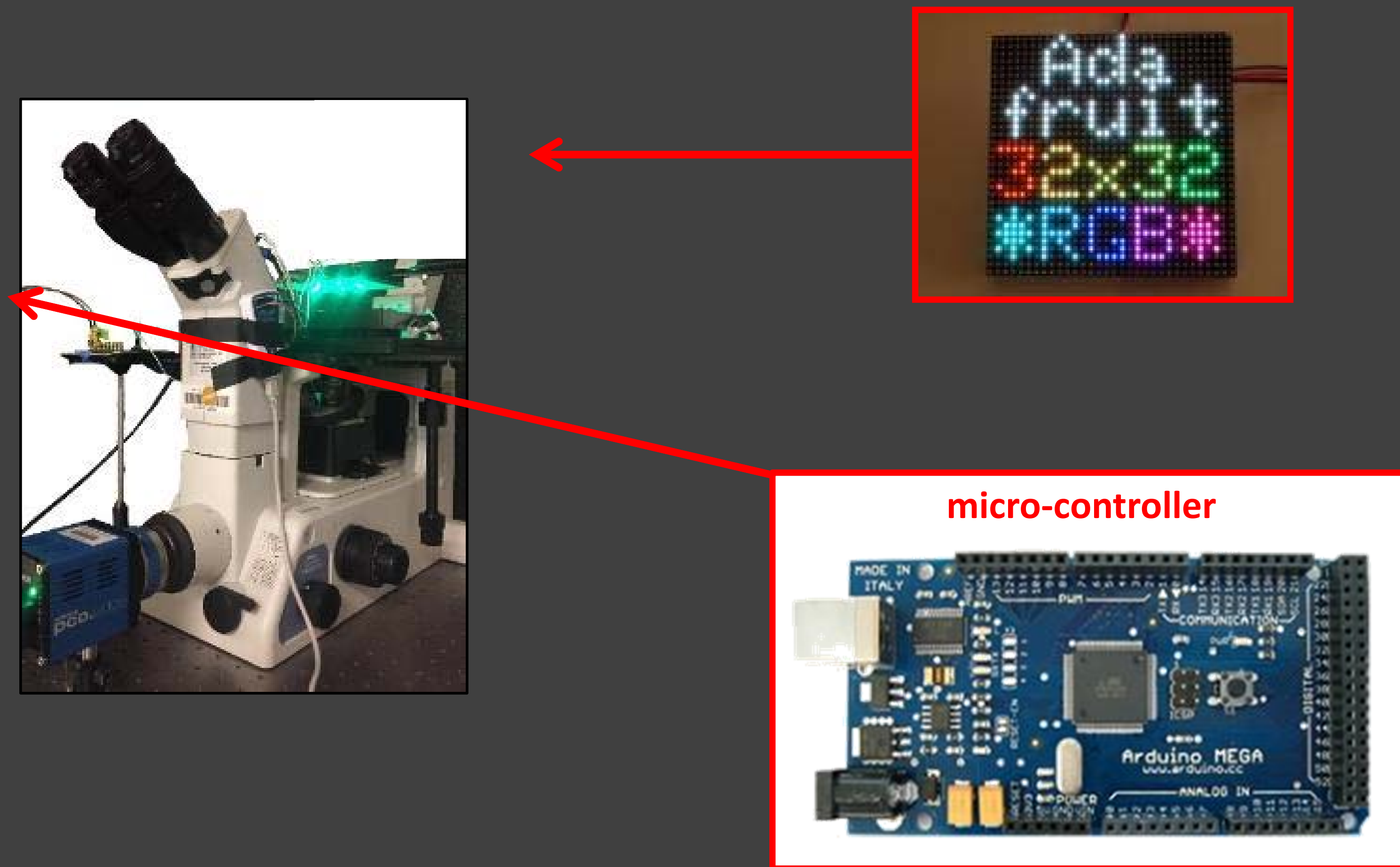
speed scales with # voxels in image

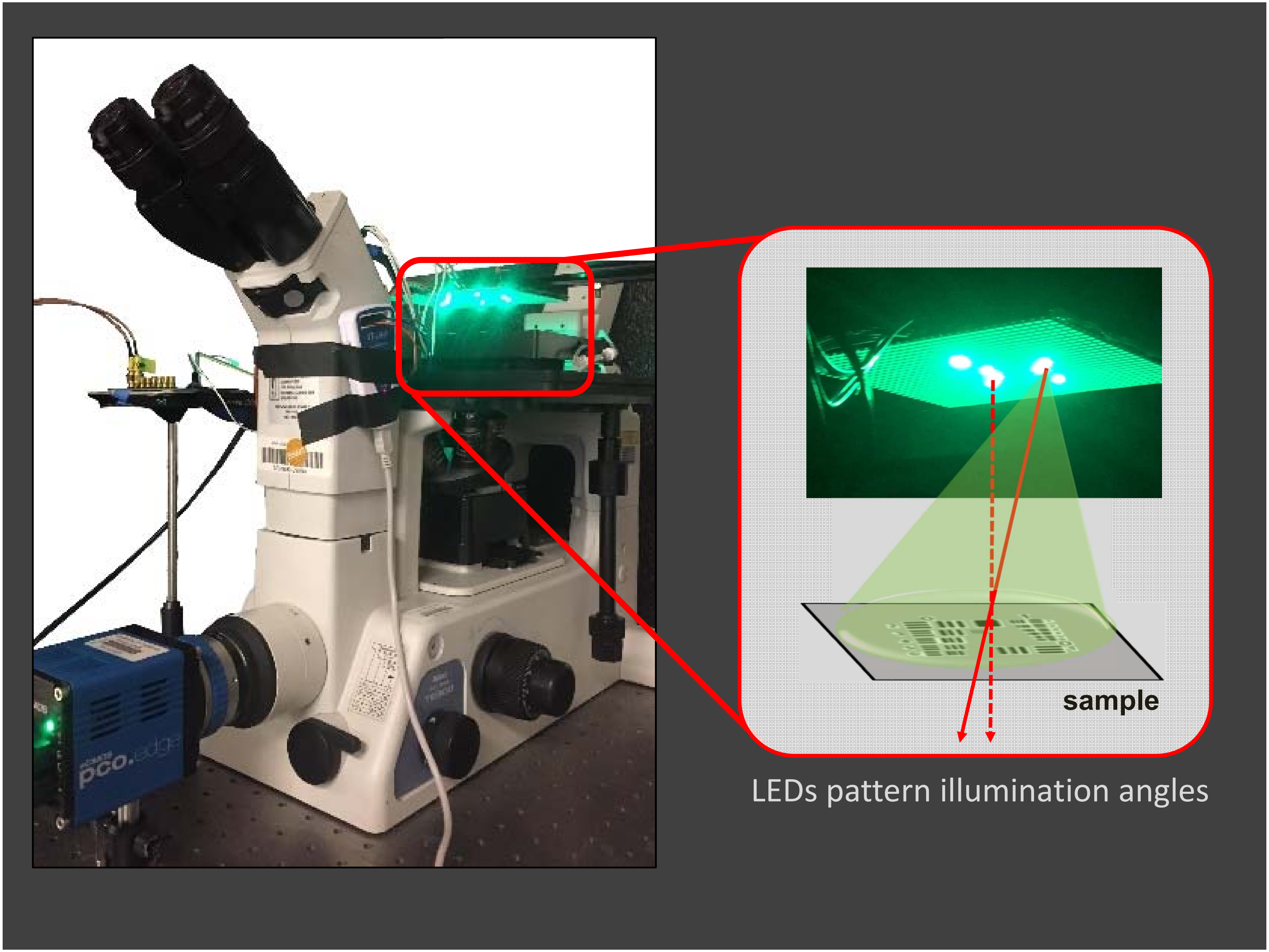
speed scales with *sparsity* of sample

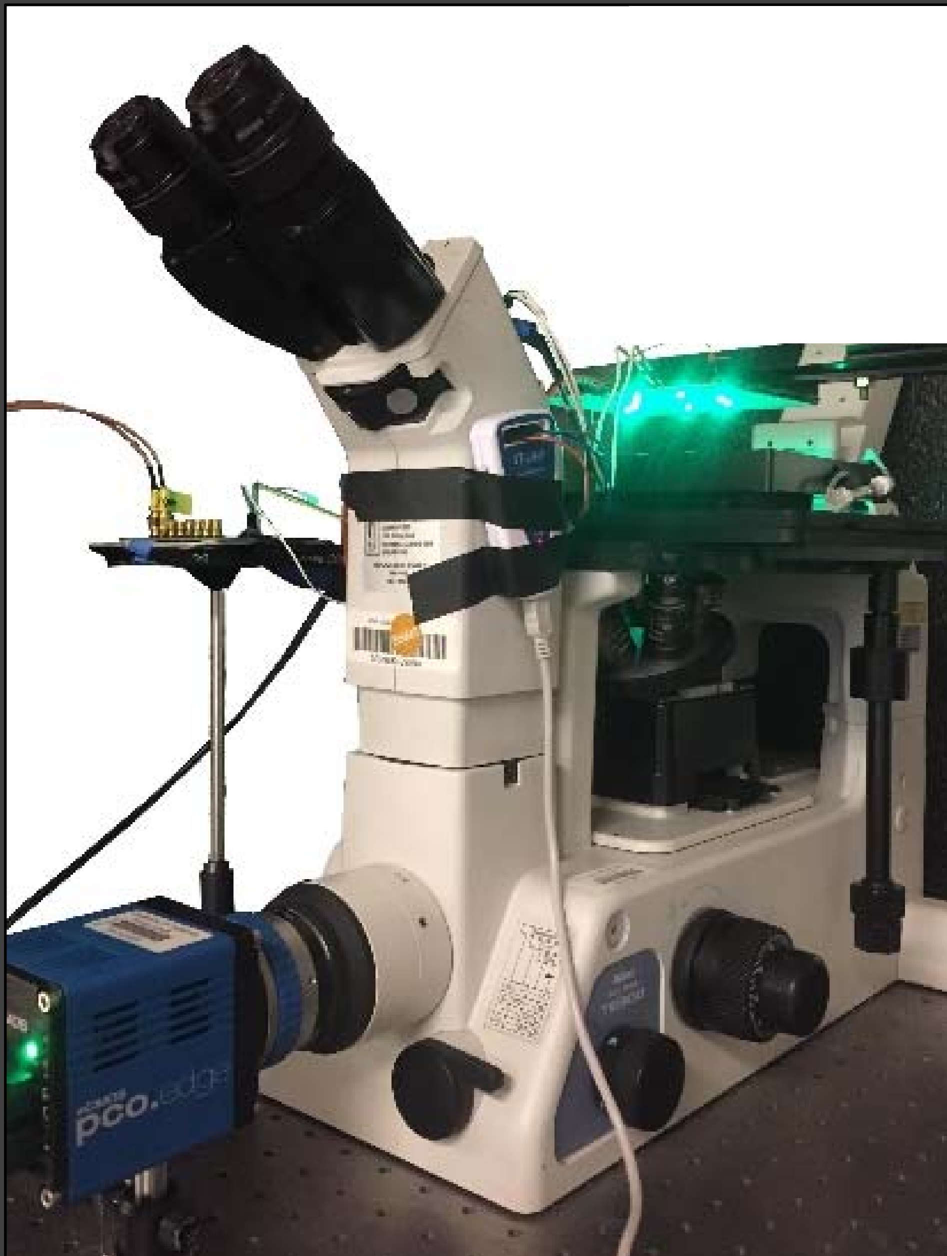
This talk:

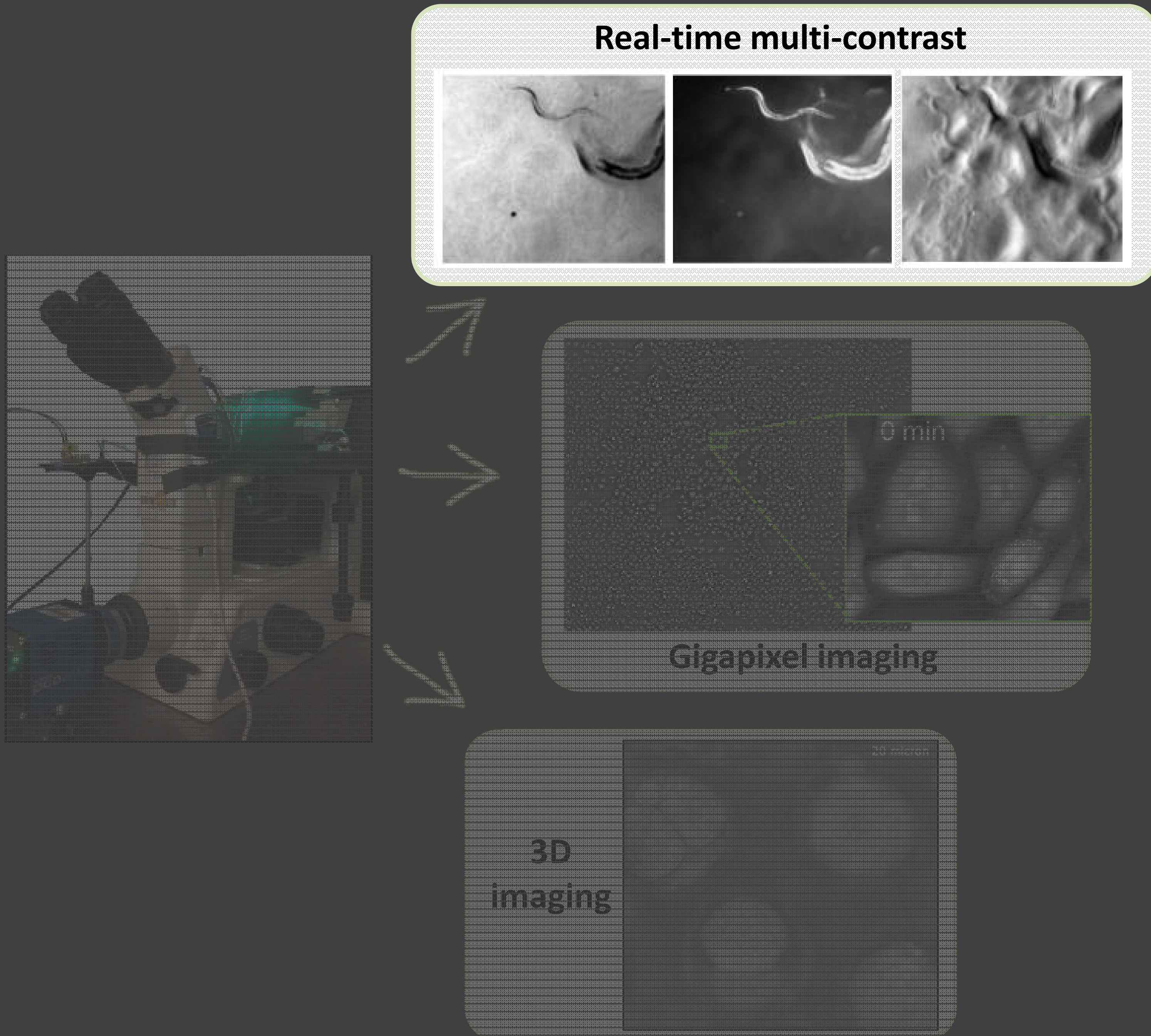




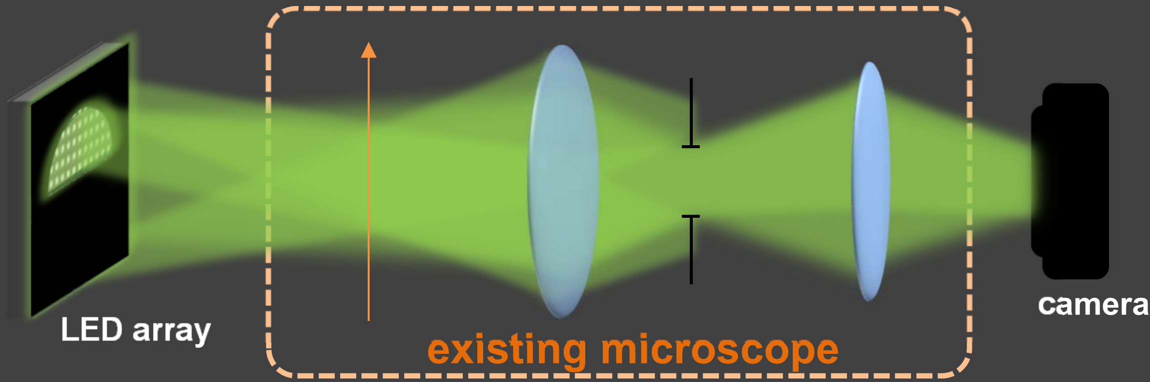

Ada
fruit
32x32
RGB
micro-controller
Arduino MEGA
www.arduino.cc

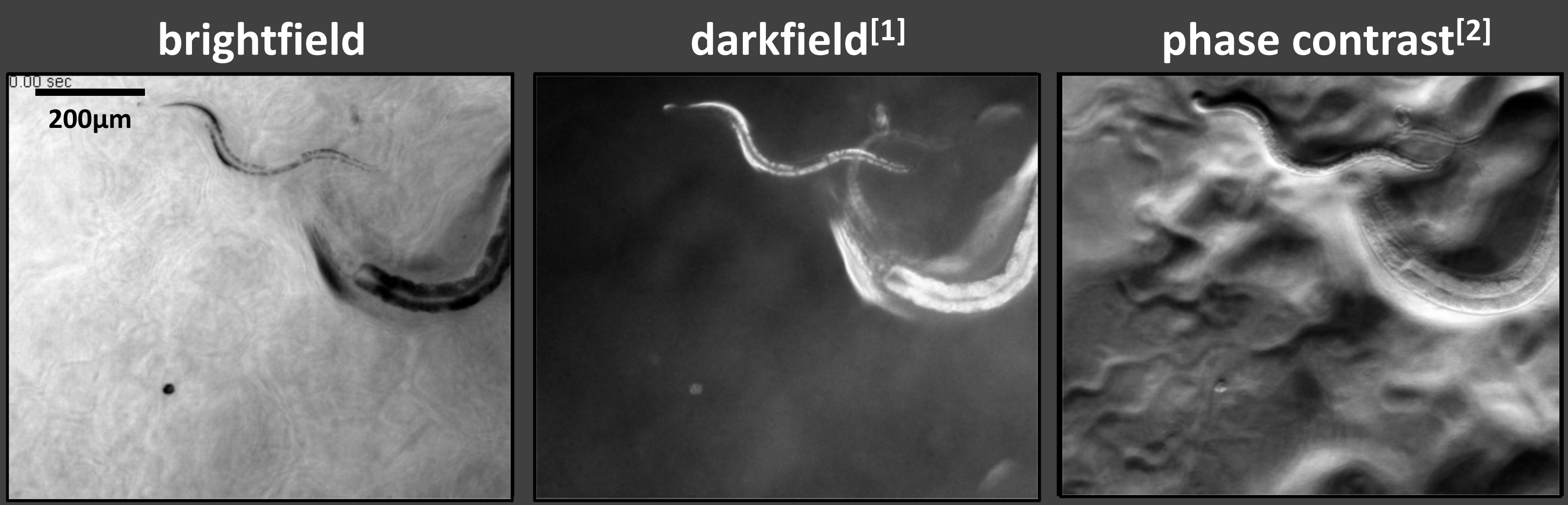

sample
LEDs pattern illumination angles


pco.edge


Real-time multi-contrast
0 min
Gigapixel imaging
20 micron
3D
imaging

Multi-contrast with an LED array microscope

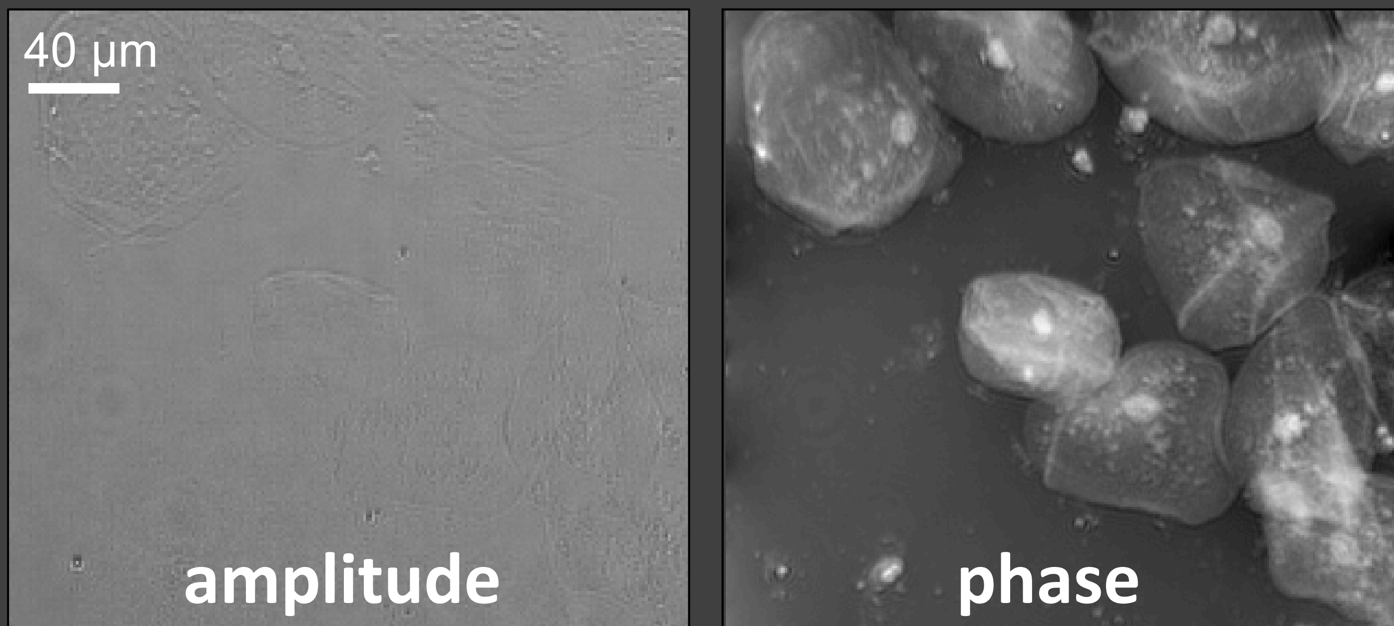




[1] G.Zheng, C. Kolner, C. Yang, *Opt. Lett.*. (2011).
[2] L. Tian, J. Wang, L. Waller, *Opt. Lett.* (2014).

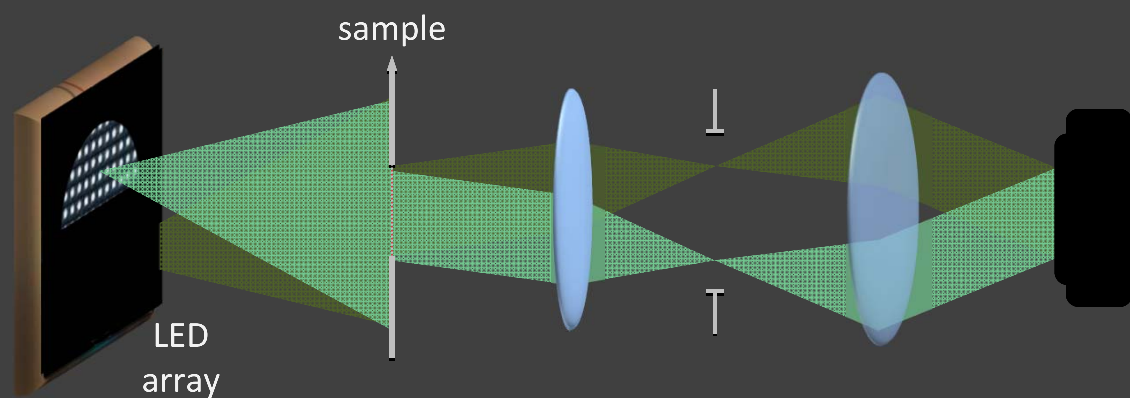
Computational Phase imaging

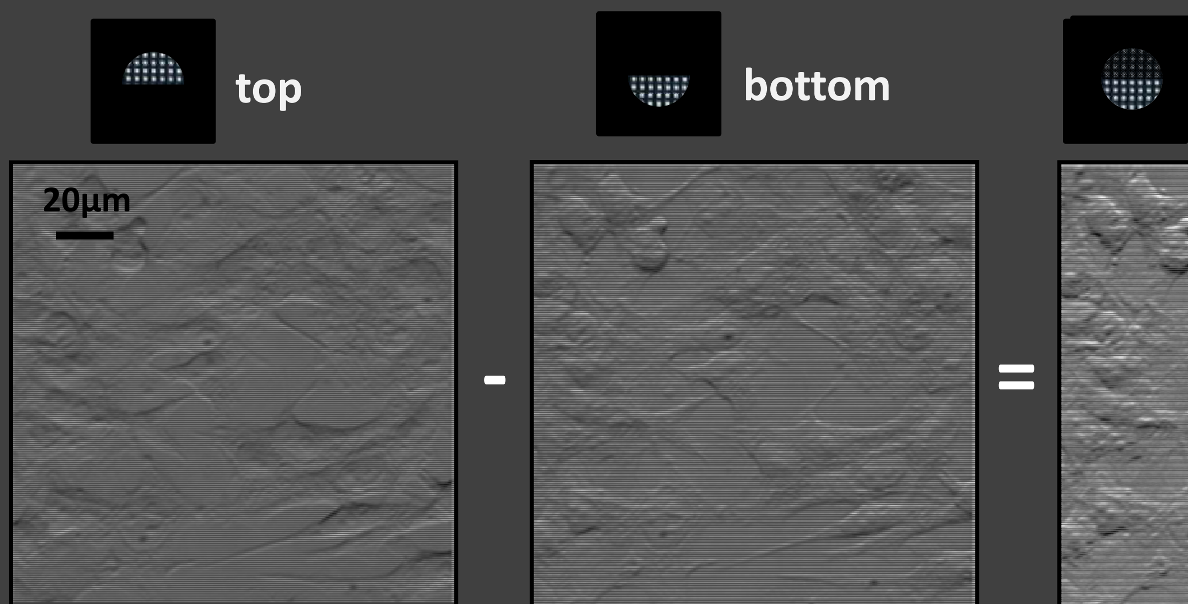
phase imaging *must* be computational



We can only measure intensity $\mathbf{y} = \left|\mathbf{A}\mathbf{x}\right|^2$

Differential Phase Contrast (DPC)



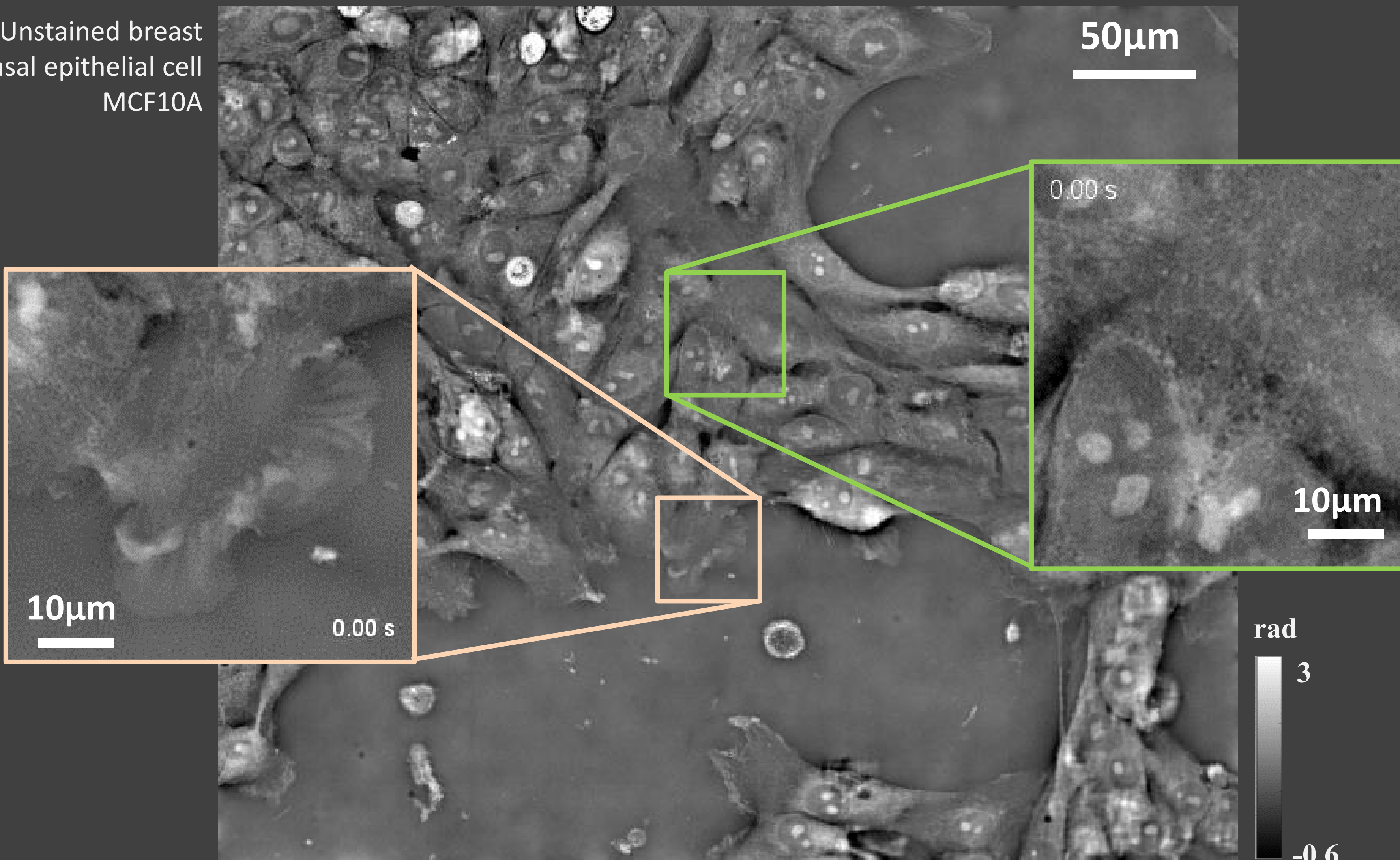


$$\text{DPC} = \frac{\text{top} - \text{bottom}}{\text{top} + \text{bottom}}$$

Kachar, Science 227, 27 (1985).
Ford, Chu, Mertz, Nat. Methods 9, 1195 (2012).
Mehta, Sheppard, Opt. Lett. 34, 1924 (2009).
Tian, Waller, *Opt. Express* 23(9), 11394-11403 (2015).

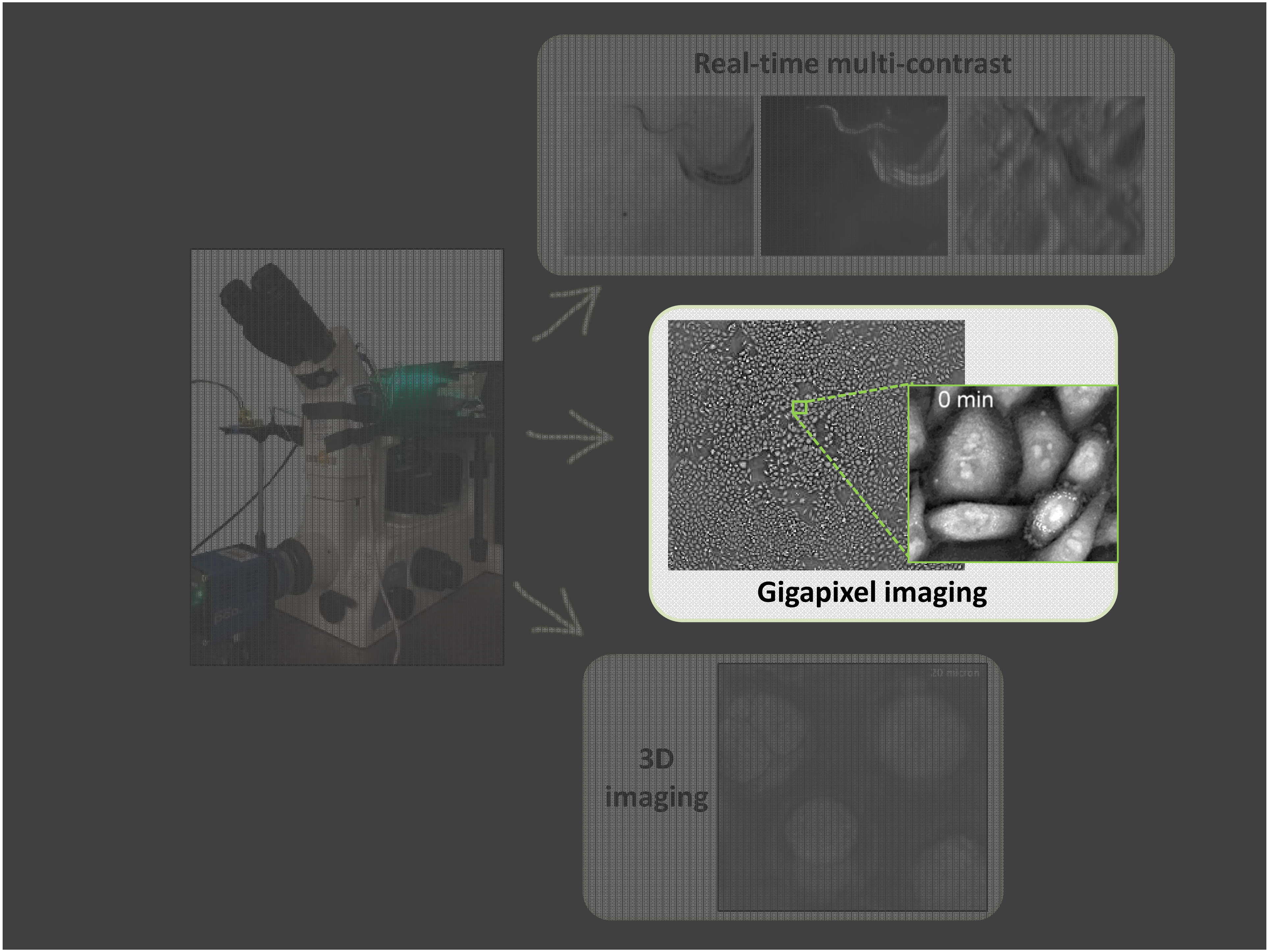
Real-time phase *in vitro*

Unstained breast basal epithelial cell MCF10A

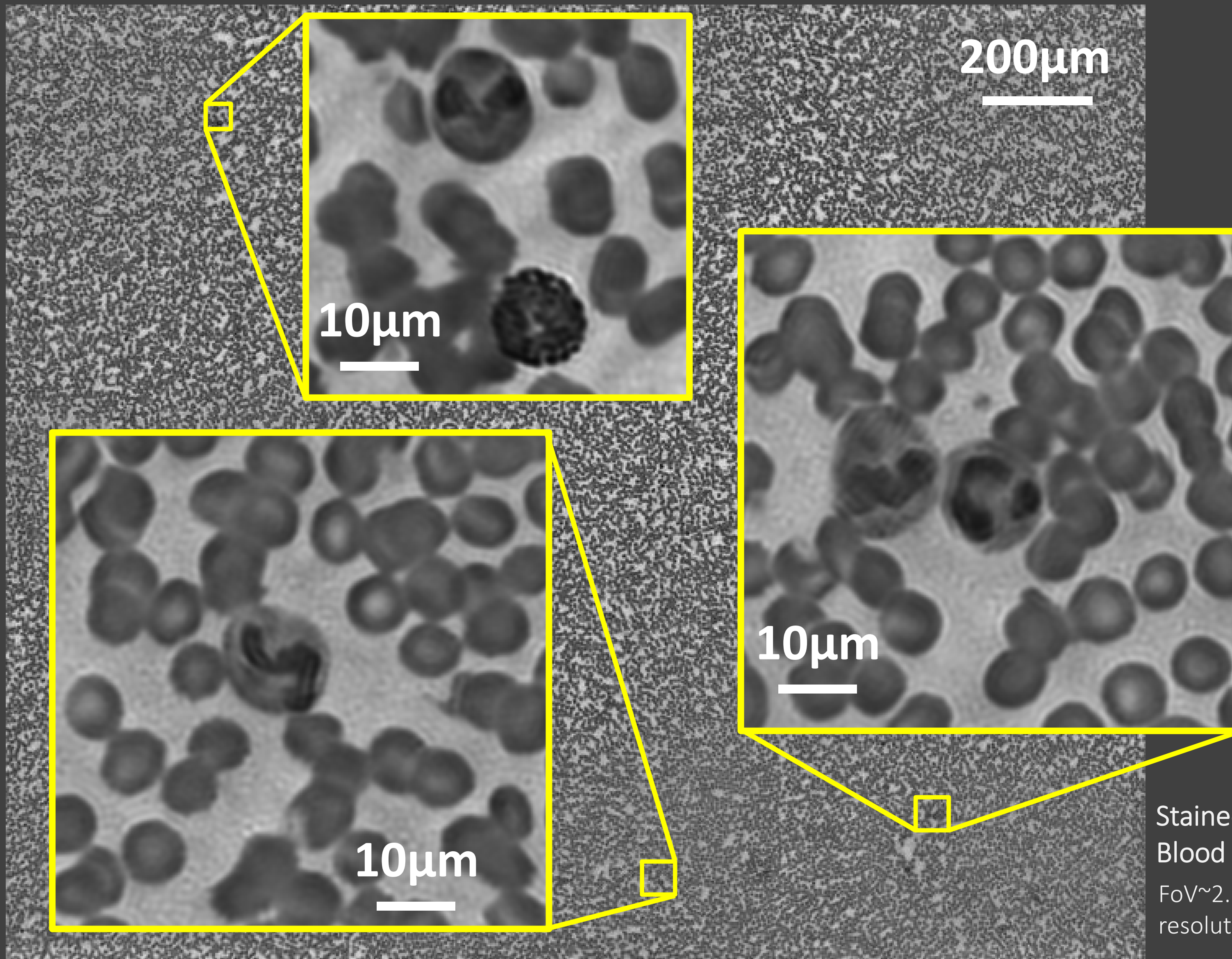


L. Tian and L. Waller, *Opt. Express* 23(9), 11394-11403 (2015).

10 Hz, NA 0.8


Real-time multi-contrast
0 min
Gigapixel imaging
3D imaging

Gigapixel imaging for disease screening



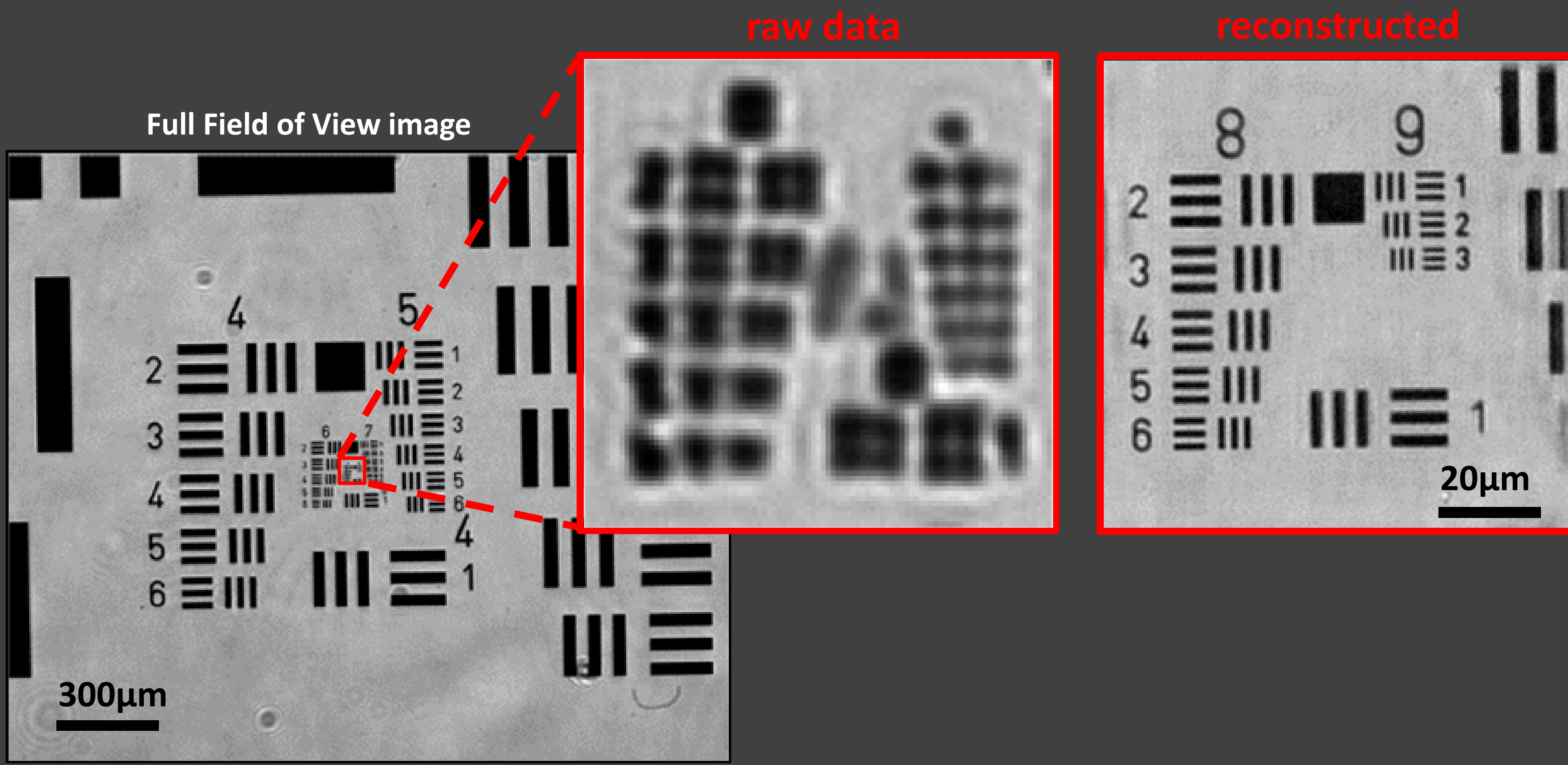
Stained Human Blood Cells

FoV~2.1mmx1.7mm
resolution ~0.4um

26k x 22k pixels

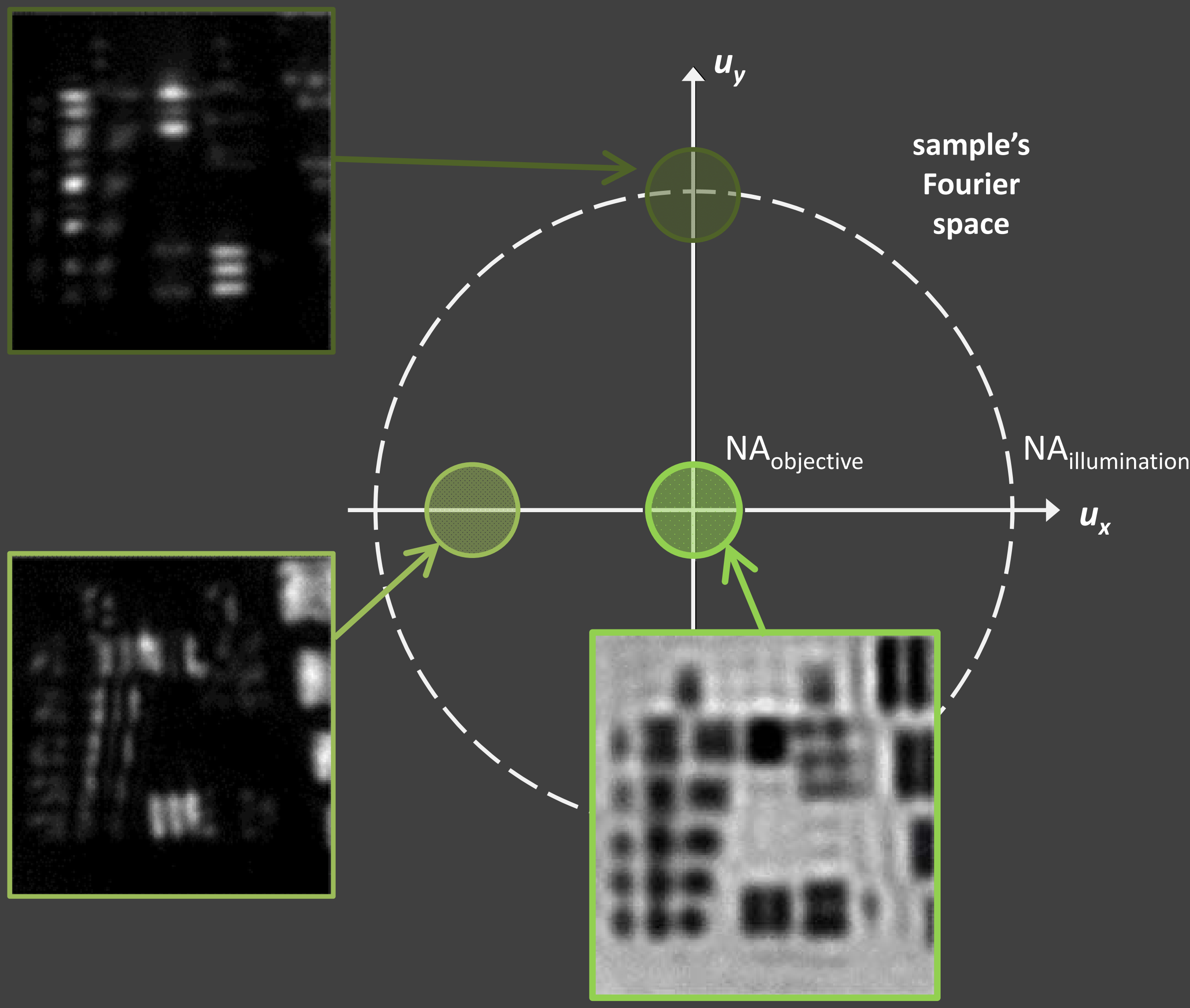
Our version of: G.Zheng, R. Horstmeyer, C. Yang, *Nat. Photon.* (2013).
L.Tian, X.Li, K.Ramchandran, L. Waller, *Biomed. Opt. Express* (2014).

Gigapixel imaging by Fourier Ptychography



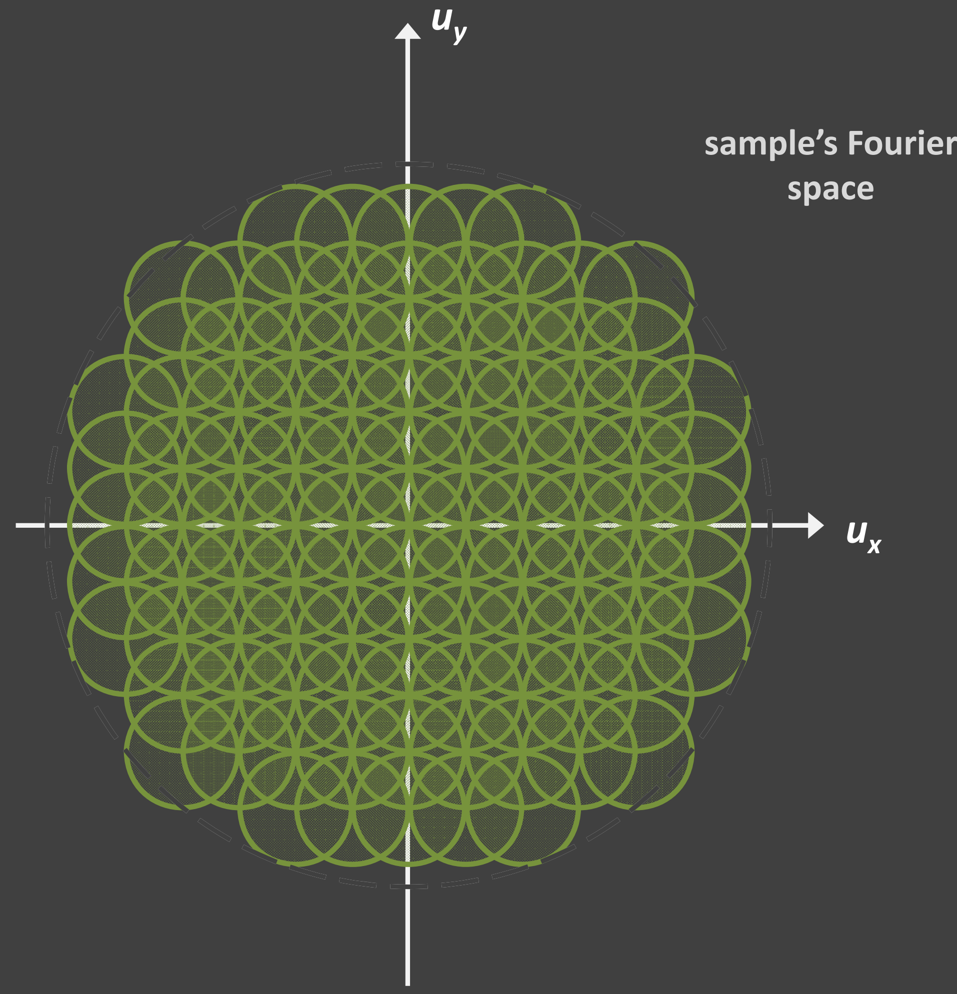
G.Zheng, R. Horstmeyer, C. Yang, *Nat. Photon.* (2013).
L.Tian, X.Li, K.Ramchandran, L. Waller, *Biomed. Opt. Express* (2014).

Darkfield images give super-resolution



Our version of ideas in:
G.Zheng, R. Horstmeyer, C. Yang, *Nat. Photon.* (2013).

Darkfield images give super-resolution



But we have *intensity-only* measurements?

Inverse problem uses nonlinear optimization

Forward model:

$$\mathbf{y} = \left|\mathbf{A}\mathbf{x}\right|^2$$

measurements

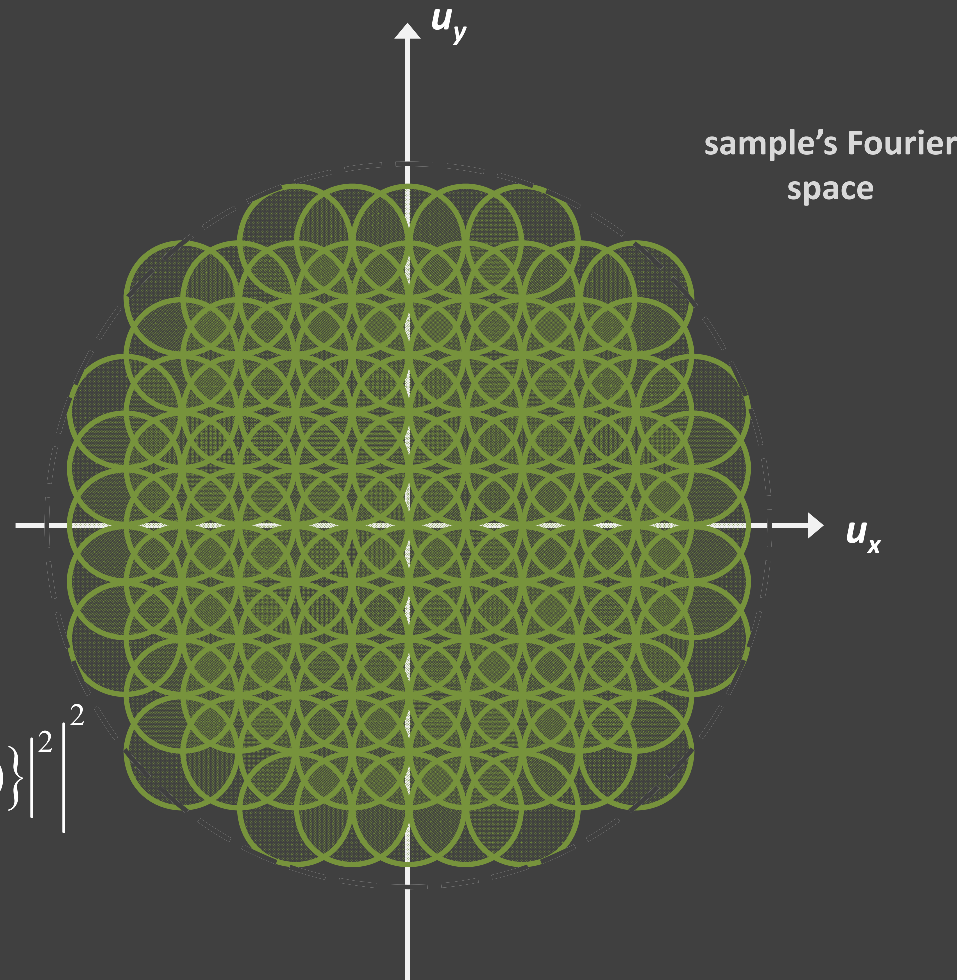
system matrix

object

Inverse problem:

$$\min_{\mathbf{O}(\mathbf{u})} \sum_n \sum_r \left| \mathbf{I}_n(\mathbf{r}) - \left| \mathfrak{I}^{-1}\left\{ \mathbf{O}(\mathbf{u}-\mathbf{u}_n) \bullet \mathbf{P}(\mathbf{u}) \right\} \right|^2 \right|^2$$

Solve for me!

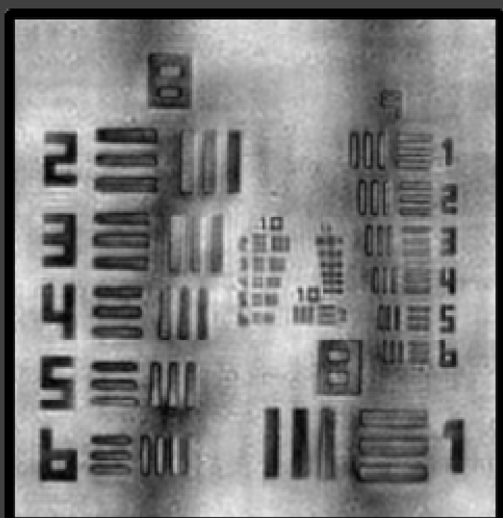


J. Rodenburg, H. Faulkner, *Appl. Phys. Lett*. 85 (2004).
M. Guizar-Sicairos, J. Fienup, *Opt. Express* 16 (2008).
L.Tian, X.Li, K.Ramchandran, L. Waller, *Biomed. Opt. Express* (2014).
S. Alexandrov, T. Hillman, T. Gutzler, D. Sampson, *Phys. Rev. Lett.* 97 (2006).

A. Kirkland, et al., *Ultramicroscopy* 57 (1995).
G.Zheng, R. Horstmeyer, C. Yang, *Nat. Photon.* (2013).
O. Xu, C. Yang, *Opt. Express* (2013).
P. Thibault, et al., *Ultramicroscopy* 109 (2009).
T. Hillman et al., *Opt. Express* (2009).

2^{nd} order optimization is better

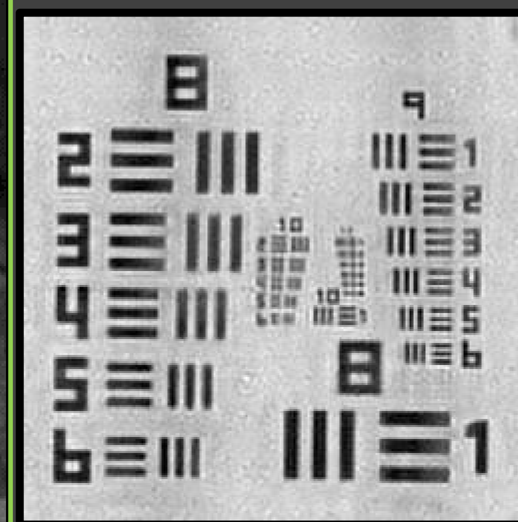
1st order (Gerchberg-Saxton)



3 seconds

quasi 2nd order

2nd order (Newton)



100 seconds

Li-Hao Yeh

L. Yeh, Dong, Zhong, Tian, Chen, Tang, Soltanolkotabi, Waller, *Opt. Express* (2015)

Algorithmic self-calibration

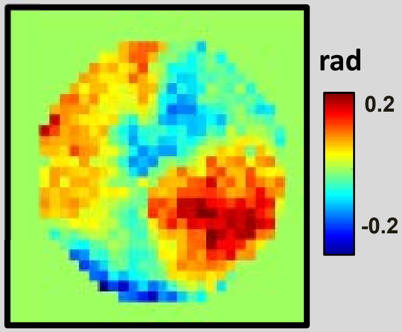
$$y = |Ax|^2$$

measurements

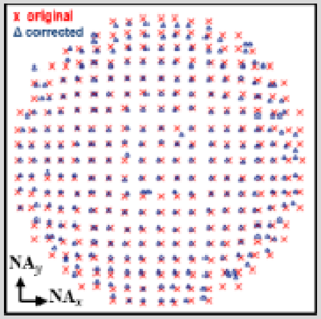
system matrix

object

Experimental errors:

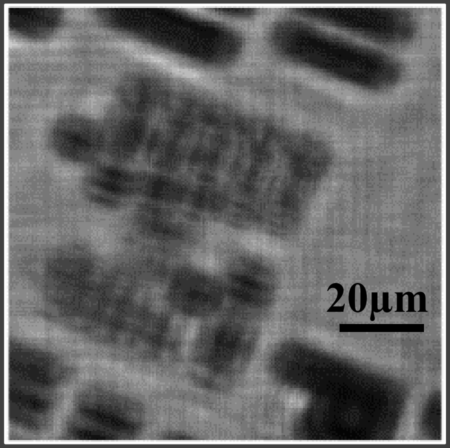


Aberrations

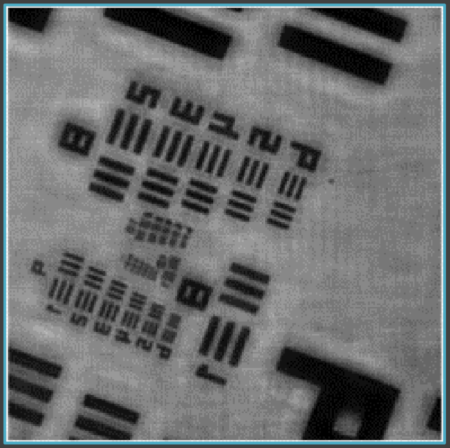


LED positions

not calibrated



calibrated



calibration parameters

$$A \rightarrow A(\theta)$$

Algorithmic self-calibration

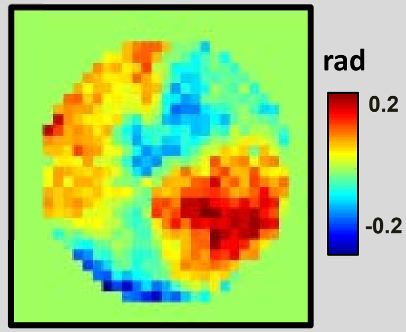
$$\mathbf{y} = \left|\mathbf{A}\mathbf{x}\right|^2$$

measurements

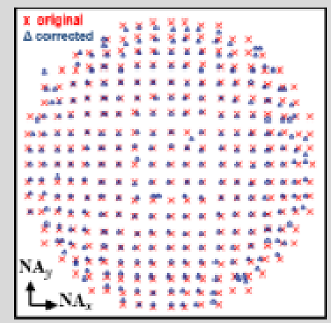
system matrix

object

Experimental errors:



Aberrations



LED positions

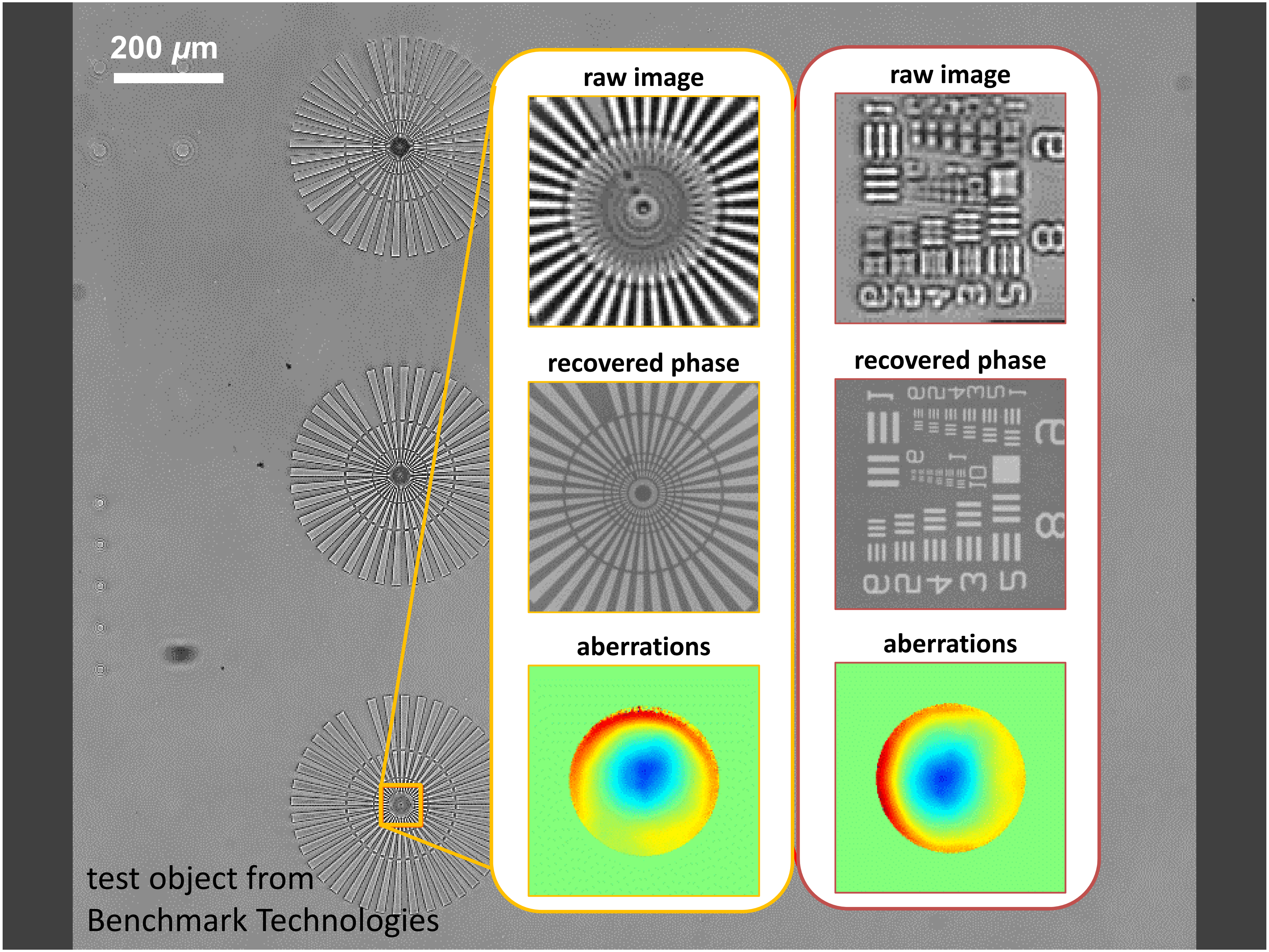
Learn more:

Tues 5pm (JTu5A.2)

But I don't want to calibrate!

$$\min_{\mathbf{x},\boldsymbol{\theta}} \left\| \mathbf{y} - \left|\mathbf{A}(\boldsymbol{\theta})\mathbf{x}\right| \right\| + \lambda_1 \left\|\mathbf{x}\right\|_1 + \lambda_2 \left\|\boldsymbol{\theta}\right\|_2$$

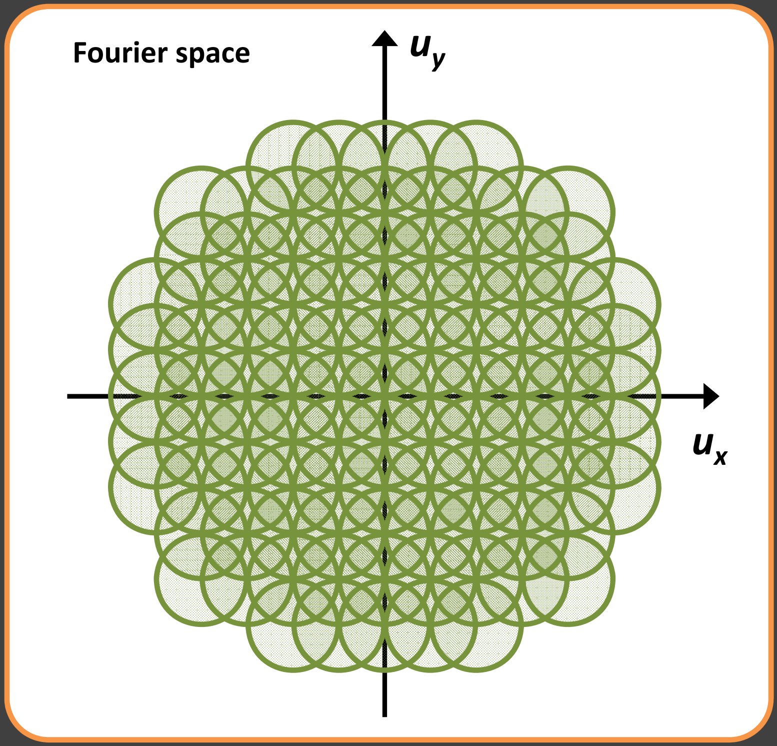
calibration parameters



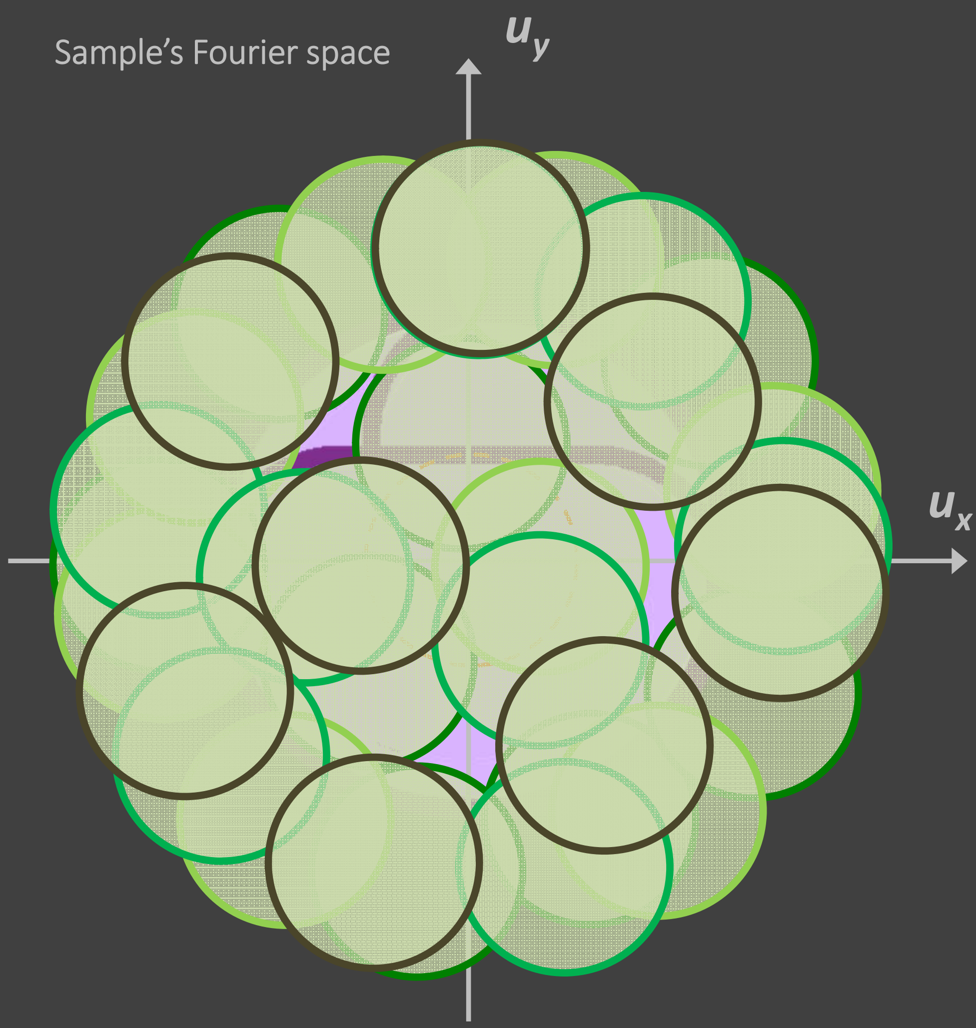
200 μm
raw image
recovered phase
aberrations
raw image
recovered phase
aberrations
test object from
Benchmark Technologies

Redundancy is necessary, but inefficient…

requires **~10x** more data collected than reconstructed



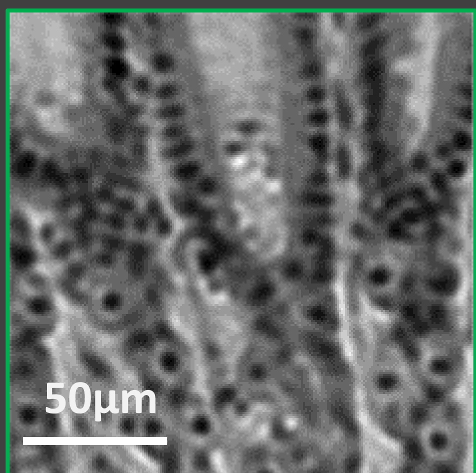
Multiplexed measurements are faster



L.Tian, X.Li, K.Ramchandran, L. Waller, *Biomed. Opt. Express* (2014).

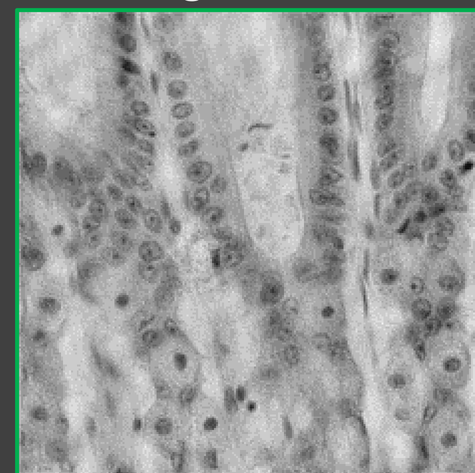
Multiplexing reduces time *and* data size

low resolution zoom-in



Original method
293 images, Time ~10min

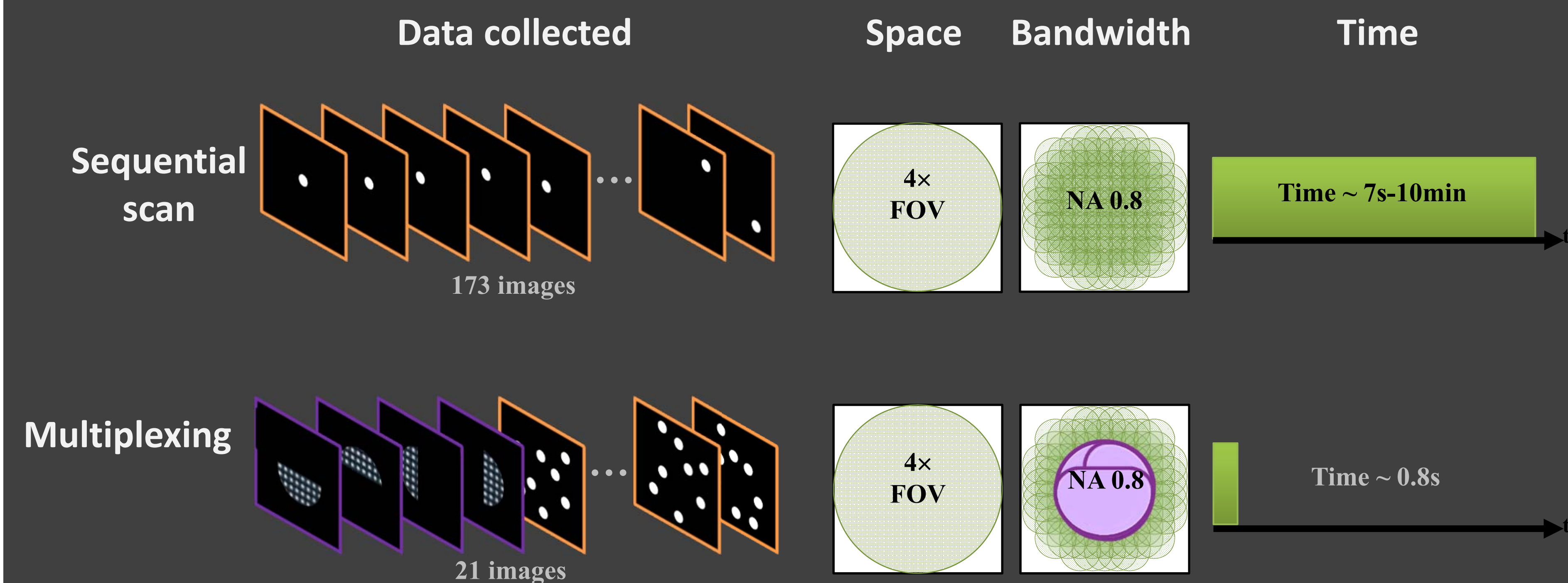
Multiplexing
40 images, Time 0.4s

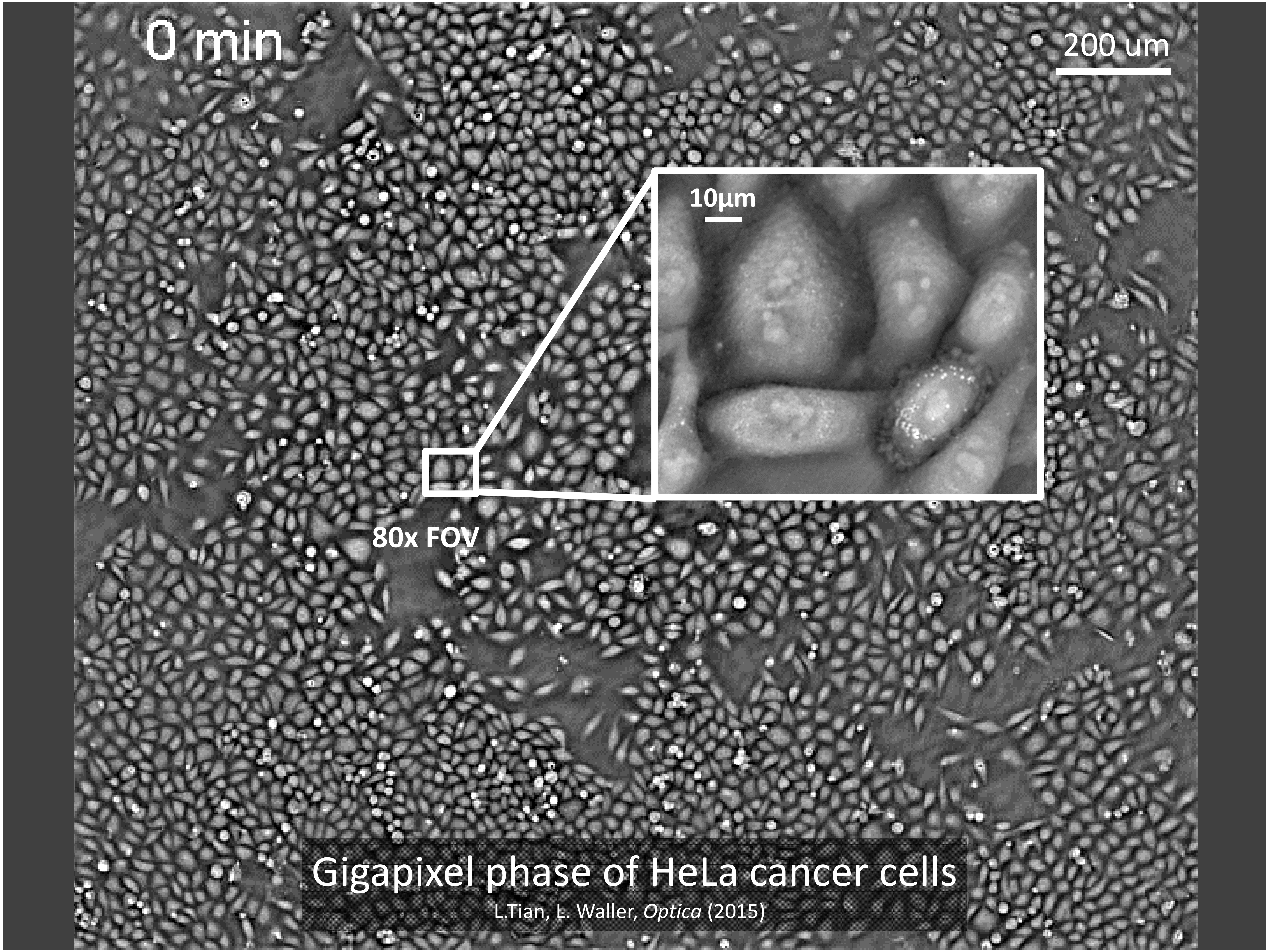


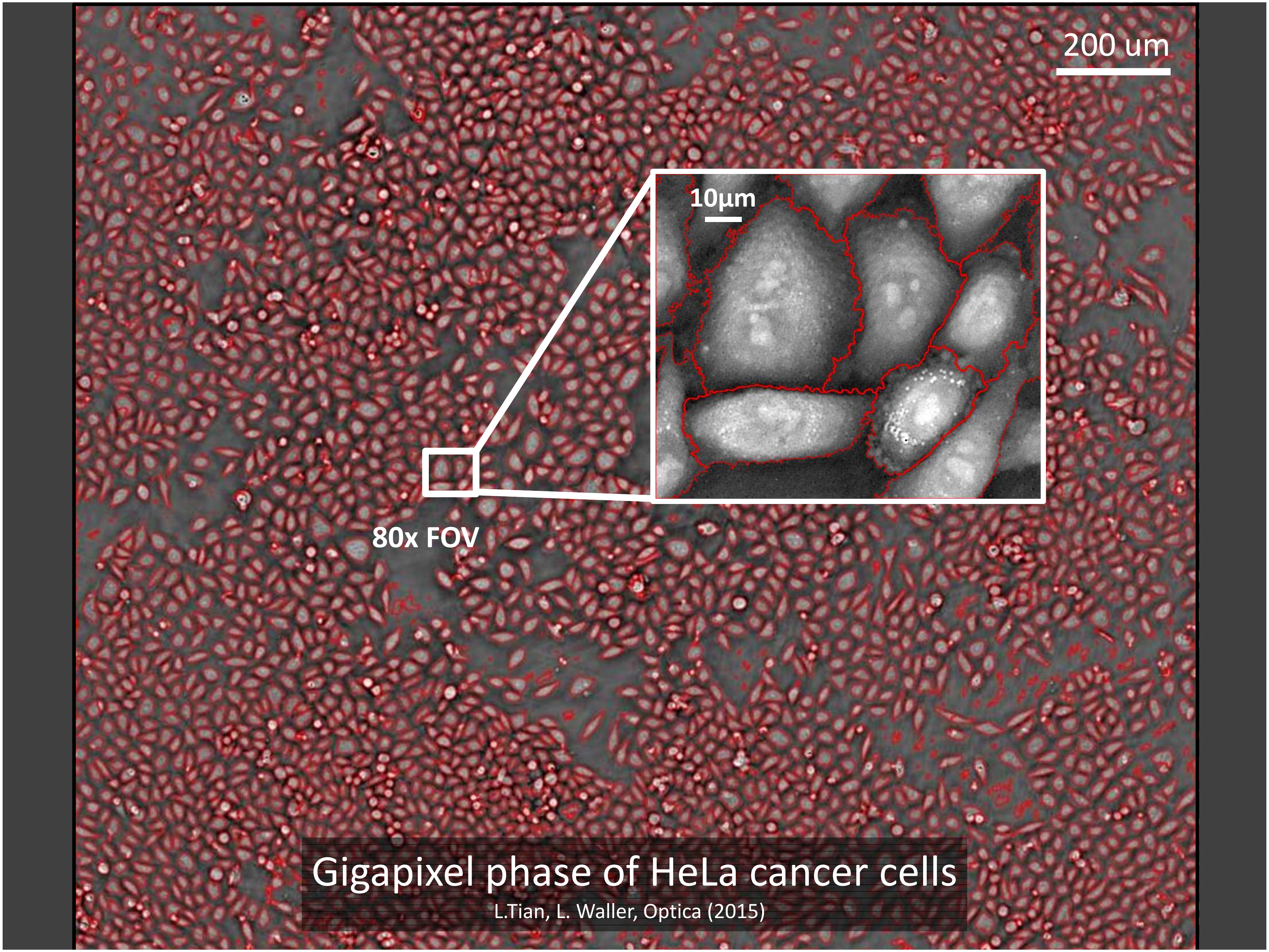
Only uses 17% of data!

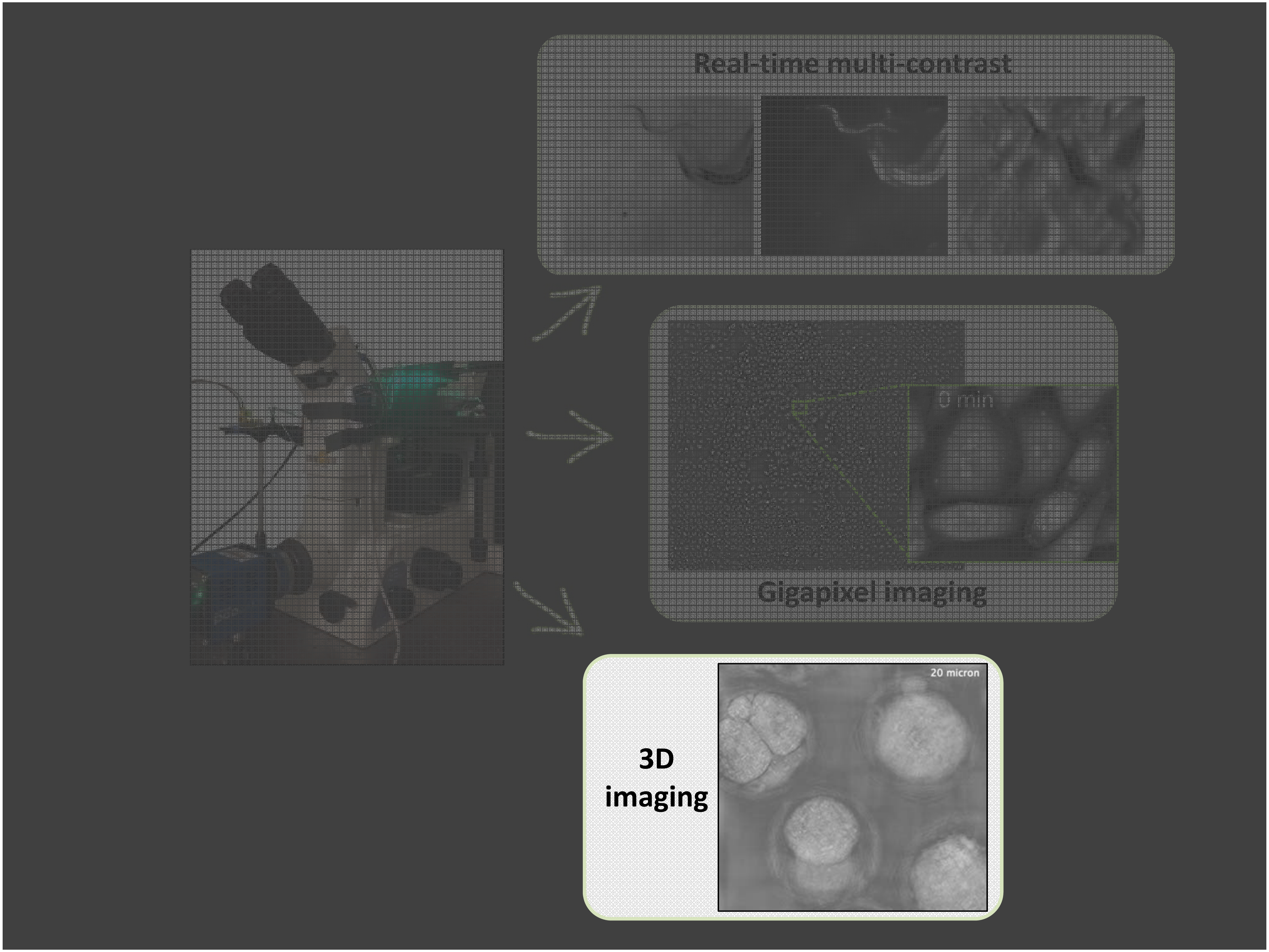
L.Tian, X.Li, K.Ramchandran, L. Waller, *Biomed. Opt. Express* (2014).

Space-bandwidth-time product

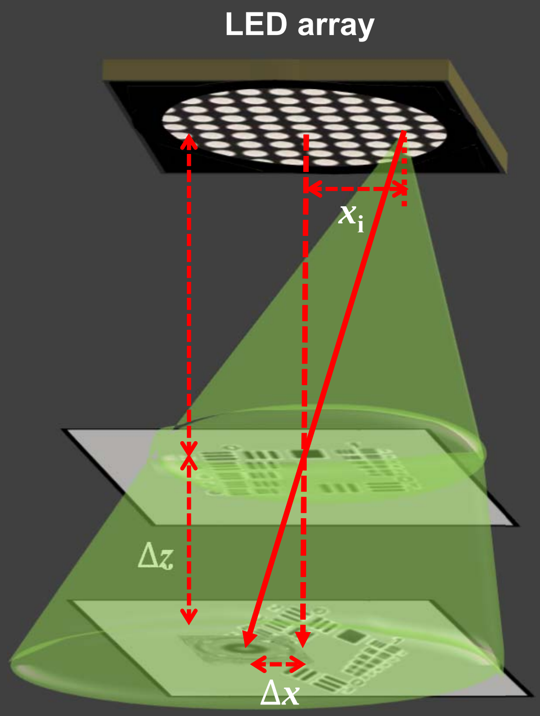



0 min
200 um
10μm
80x FOV
Gigapixel phase of HeLa cancer cells
L.Tian, L. Waller, *Optica* (2015)

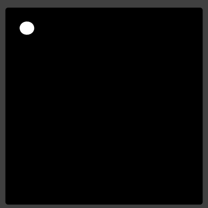

200 um
10µm
80x FOV
Gigapixel phase of HeLa cancer cells
L.Tian, L. Waller, Optica (2015)

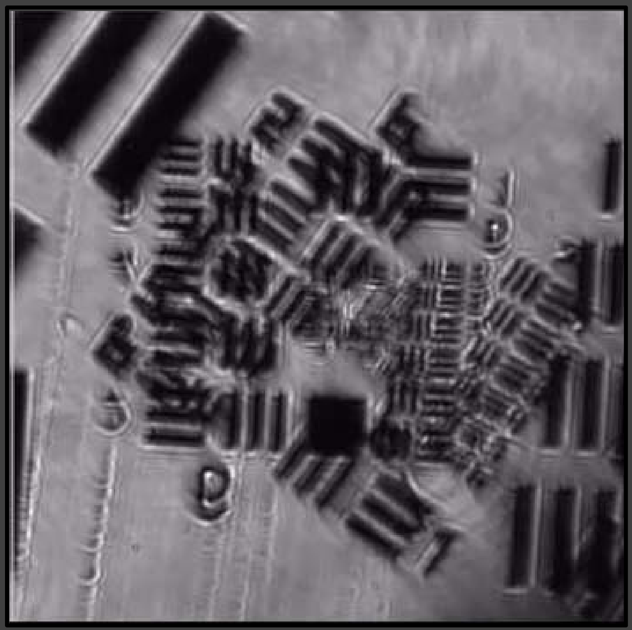

Real-time multi-contrast
0 min
Gigapixel imaging
20 micron
3D
imaging

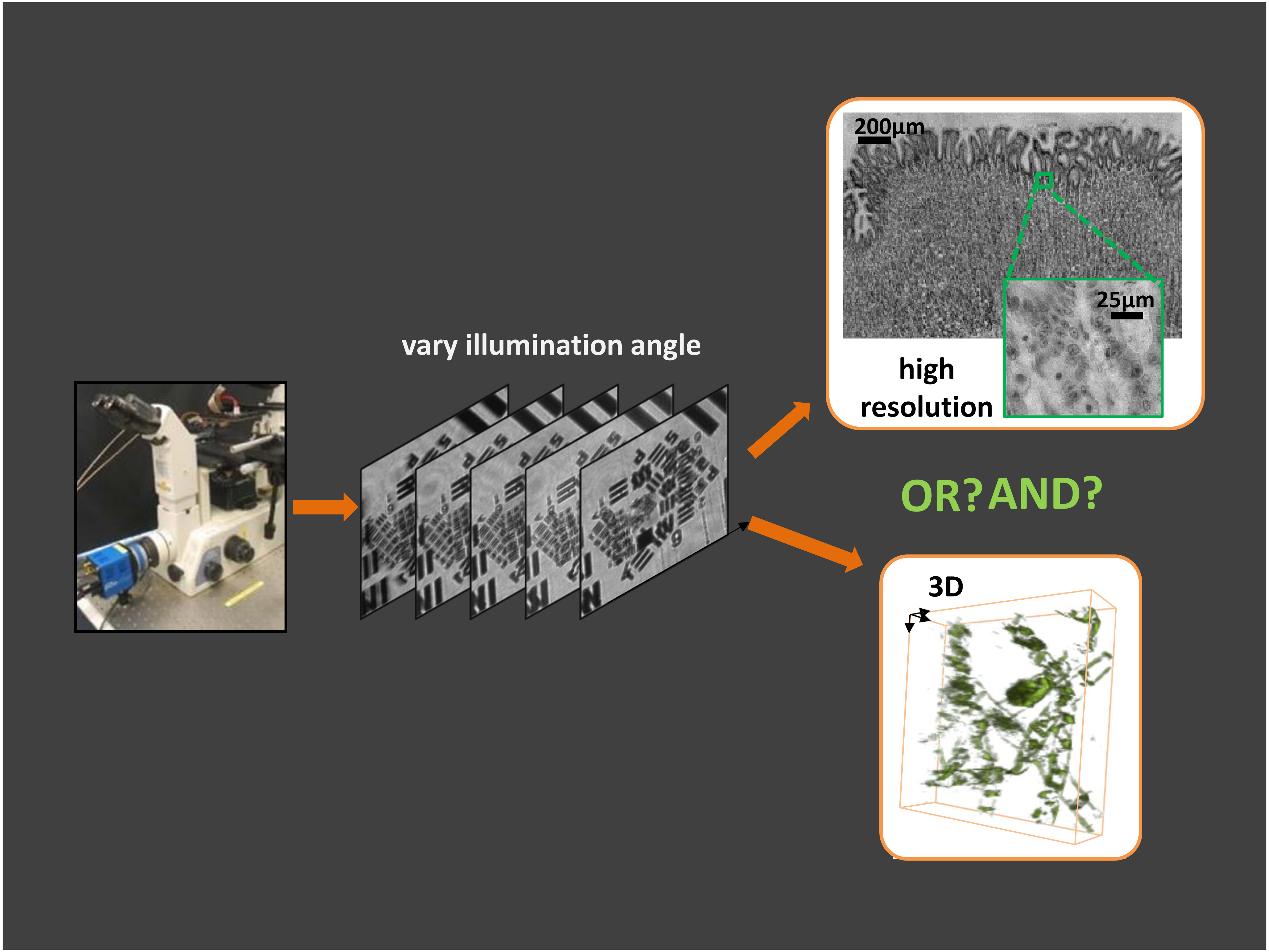
Angle scanning gives 3D information



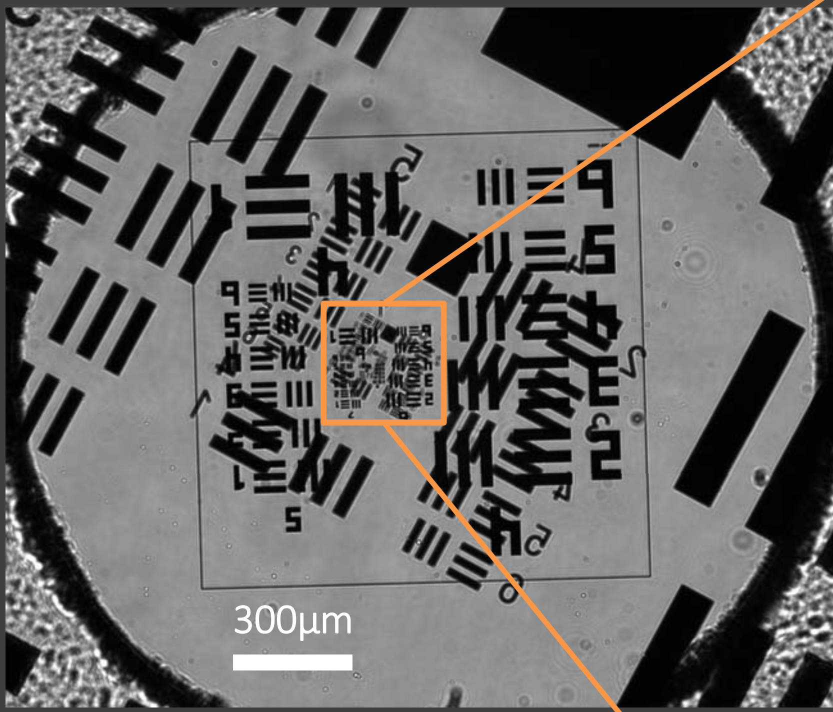
scan illumination
in (θ_x, θ_y)



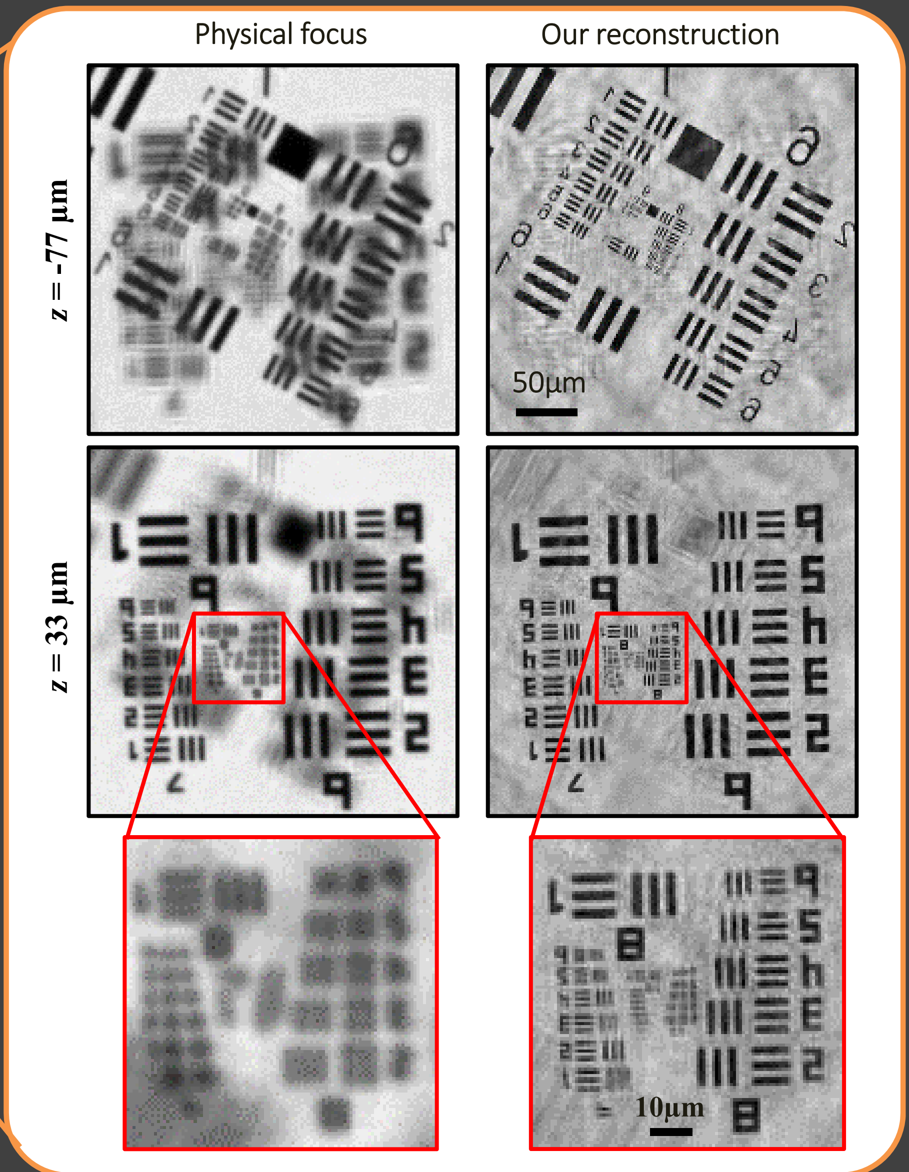



vary illumination angle
200µm
25µm
high
resolution
OR? AND?
3D

Can we super-resolution *and* 3D?

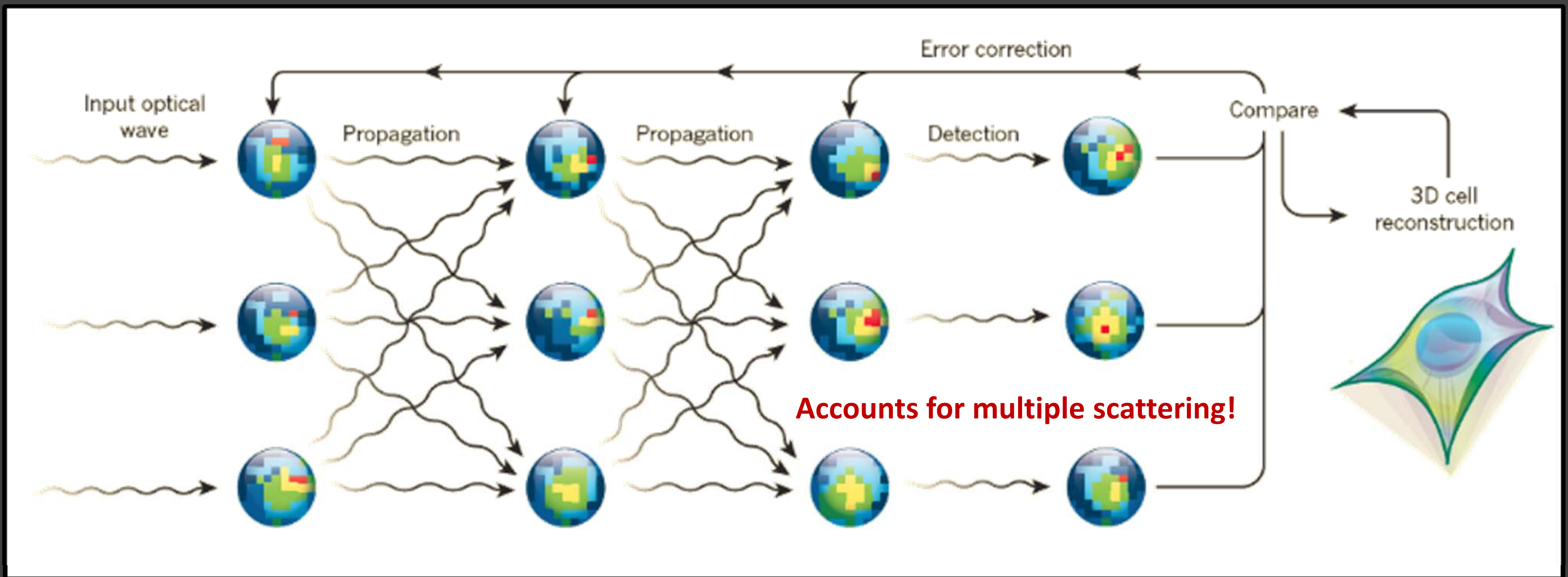


Low-resolution full FoV image
from 4× 0.1 NA objective



L. Tian, L. Waller, *Optica* (2015).

3D phase imaging as a neural network

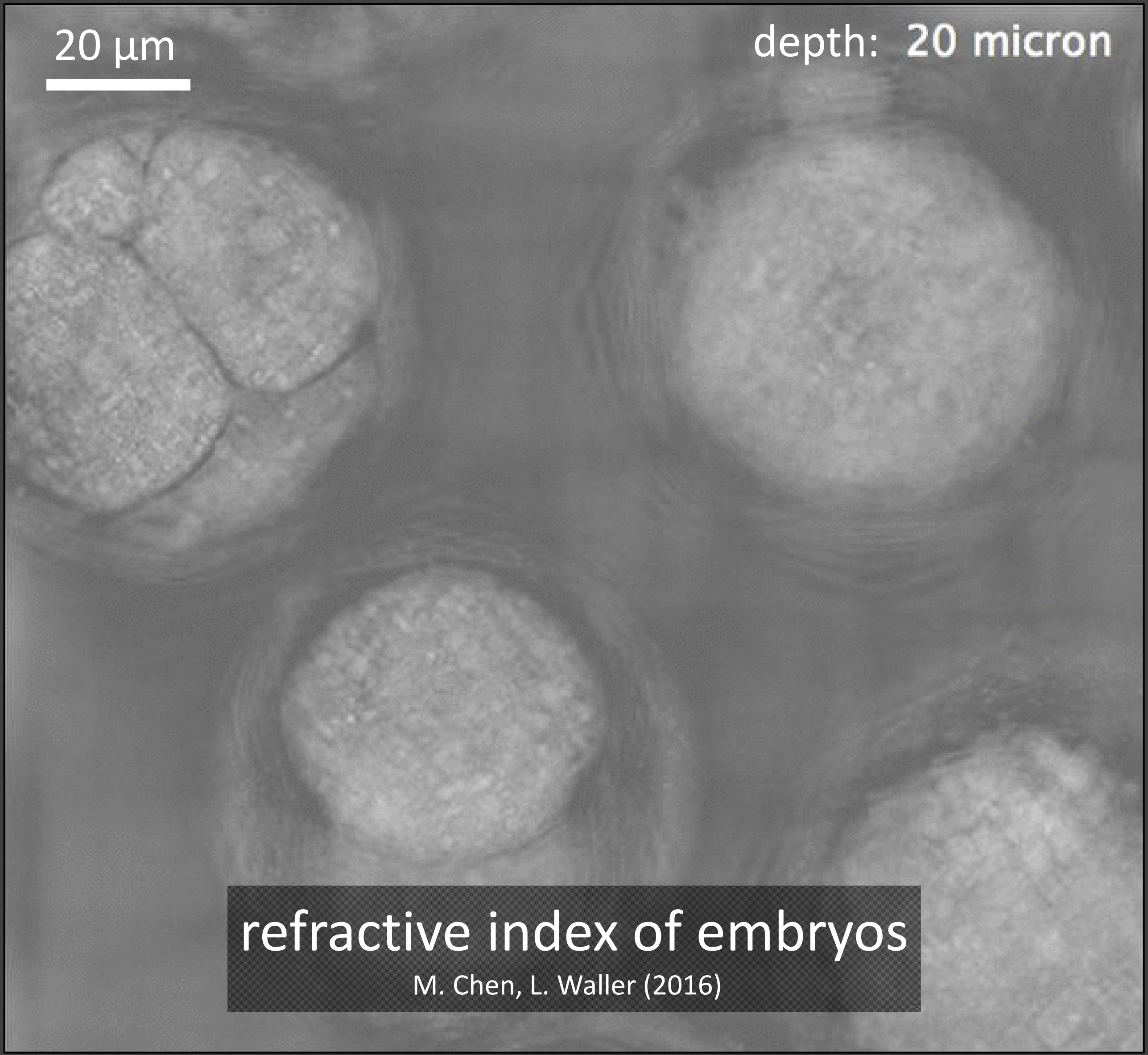


Nonlinear, nonconvex…
so will it converge?

Van Roey, van der Donk, Lagasse, *J. Opt. Soc. Am.*(1981)
Cowley, Moodie, *Acta Crystallogr.* (1957).
Maiden, Humphry, Rodenburg, *J. Opt. Soc. Am. A* (2012).
Tian, Waller, *Optica* (2015)
Van den Broek, Koch, *Phys. Rev. Lett.* (2012)
Van den Broek, Koch, *Phys. Rev. B* (2013)
Kamilov, Papadopoulos…, Psaltis, *Optica* (2015)
Waller, Tian, *Nature* (2015).

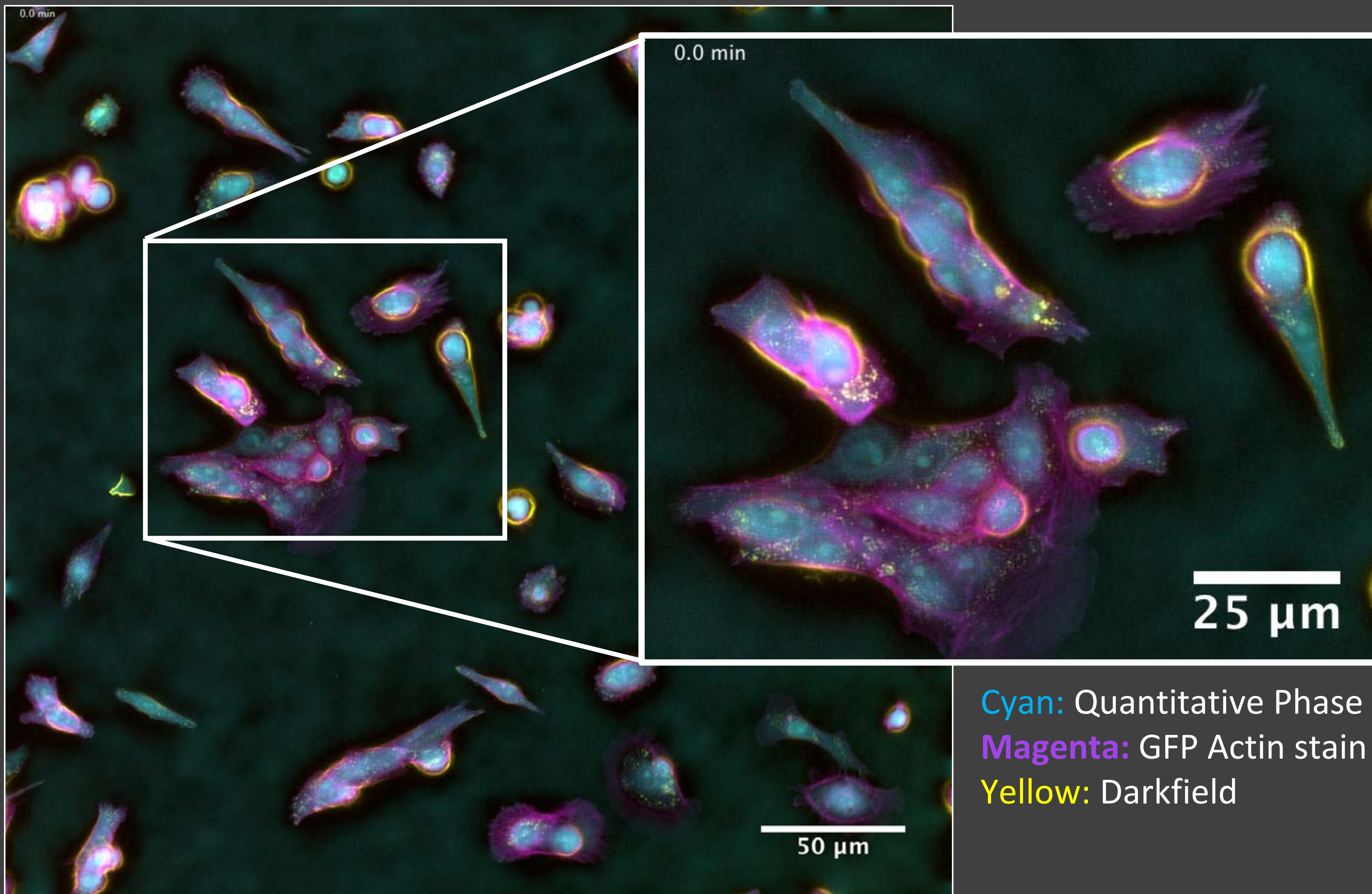
Analogy to Artificial Neural Networks

3D refractive index measurement



Michael
Chen

All together: phase + darkfield + fluorescence

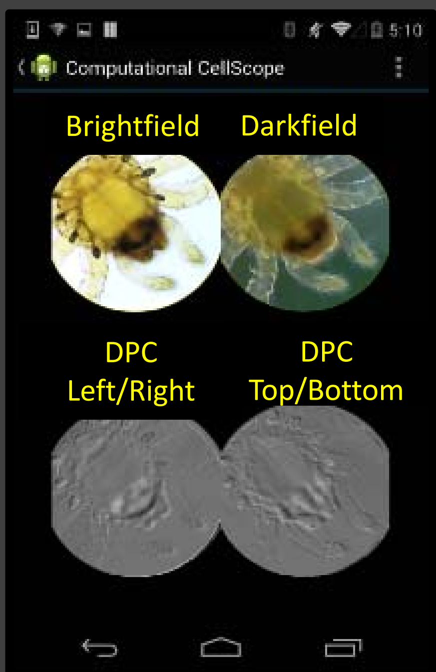


Cyan: Quantitative Phase
Magenta: GFP Actin stain
Yellow: Darkfield

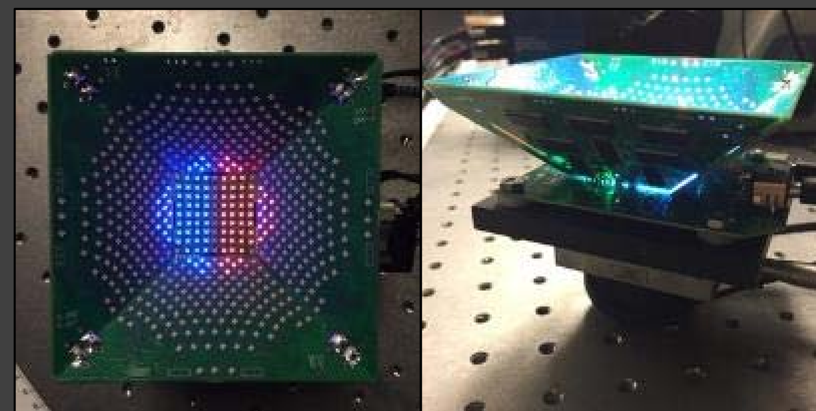
20x / 0.5NA | Mouse Kidney Cells |Courtesy of Marine Biology Lab (Woods Hole)

Open-source hardware + software

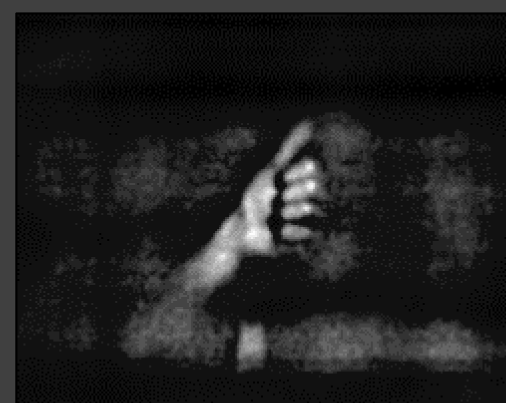
Computational CellScope



Quasi-dome



ScotchTape Cam



www.laurawaller.com/opensource

Outlook

Hardware Toolbox

Computational Toolbox

Computers + Optics should talk more!

Collaborators:
Hillel Adesnik (Neuro)
Ben Recht, Ren Ng (EECS)
David Schaffer, Lydia Sohn, Dan Fletcher (BioE)

GigaPan: WallerLab_Berkeley
Open-source : www.laurawaller.com
Twitter: @optrickster
Github: Waller_Lab