

Principles of Light-Sheet Microscopy

Alon Greenbaum, PhD
Gradinaru Lab
Antti Lignell, PhD

Overview

- Different imaging modalities pros and cons
- Introduction to light-sheet microscopy
- Light-sheet issues
- Light-sheet systems
 - SCAPE
 - AutoPilot light-sheet
 - Lattice light-sheet

Every Biologist Would Like to Image

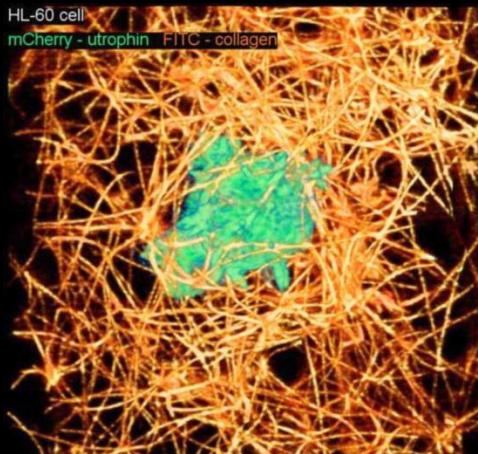
A biological sample with high-resolution, deep tissue penetration, fast acquisition time, and minimal photo bleaching

- In reality large trade-offs, no one size fits all solution

One cell

Very high resolution

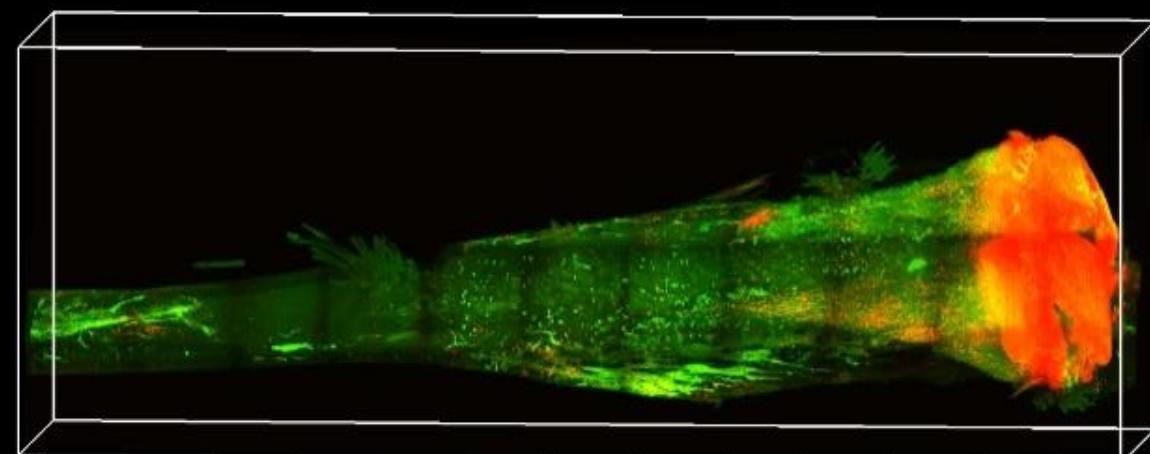
Live imaging



One organ

Micrometer resolution

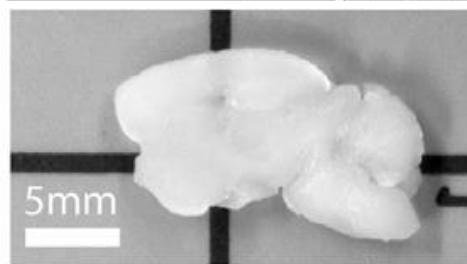
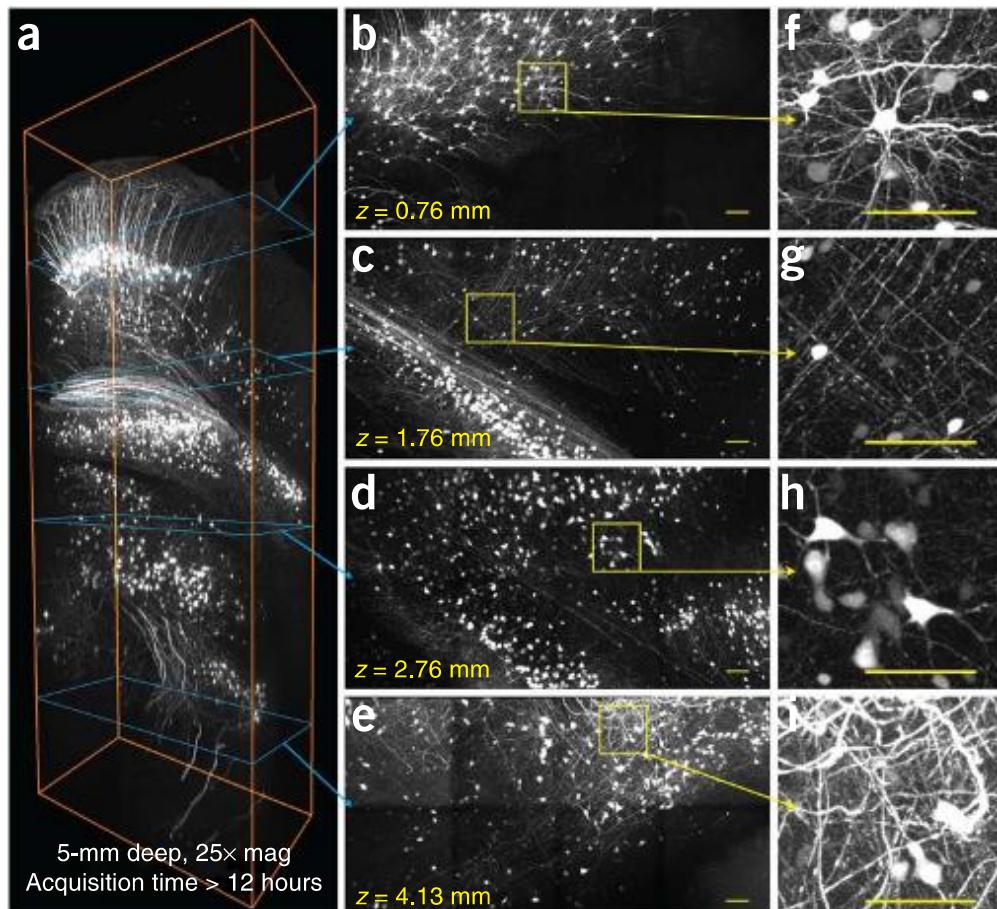
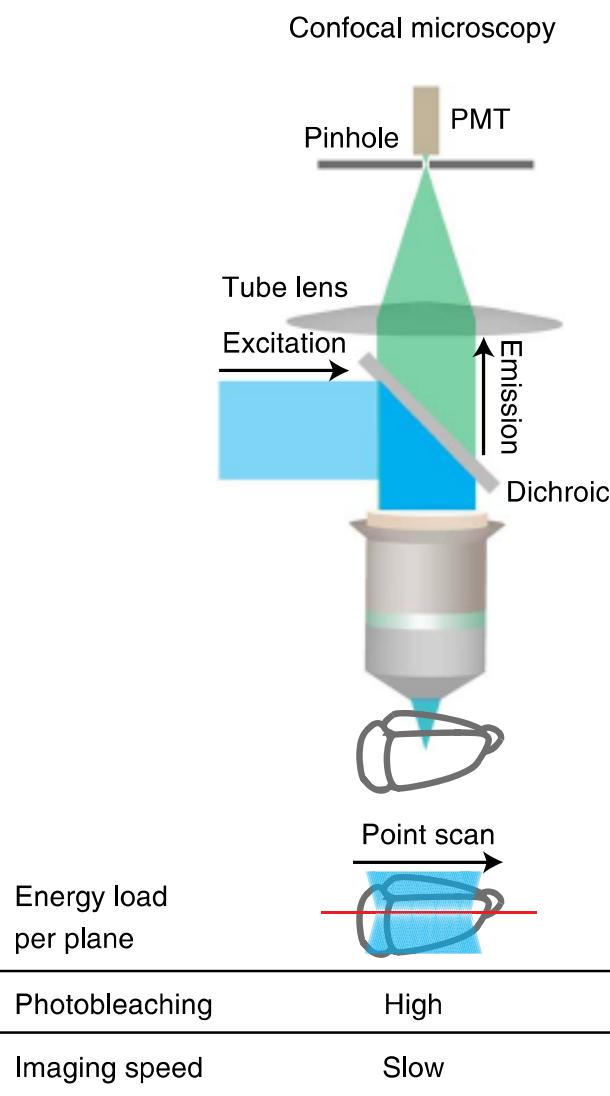
Snap shot



Comparison between imaging modalities



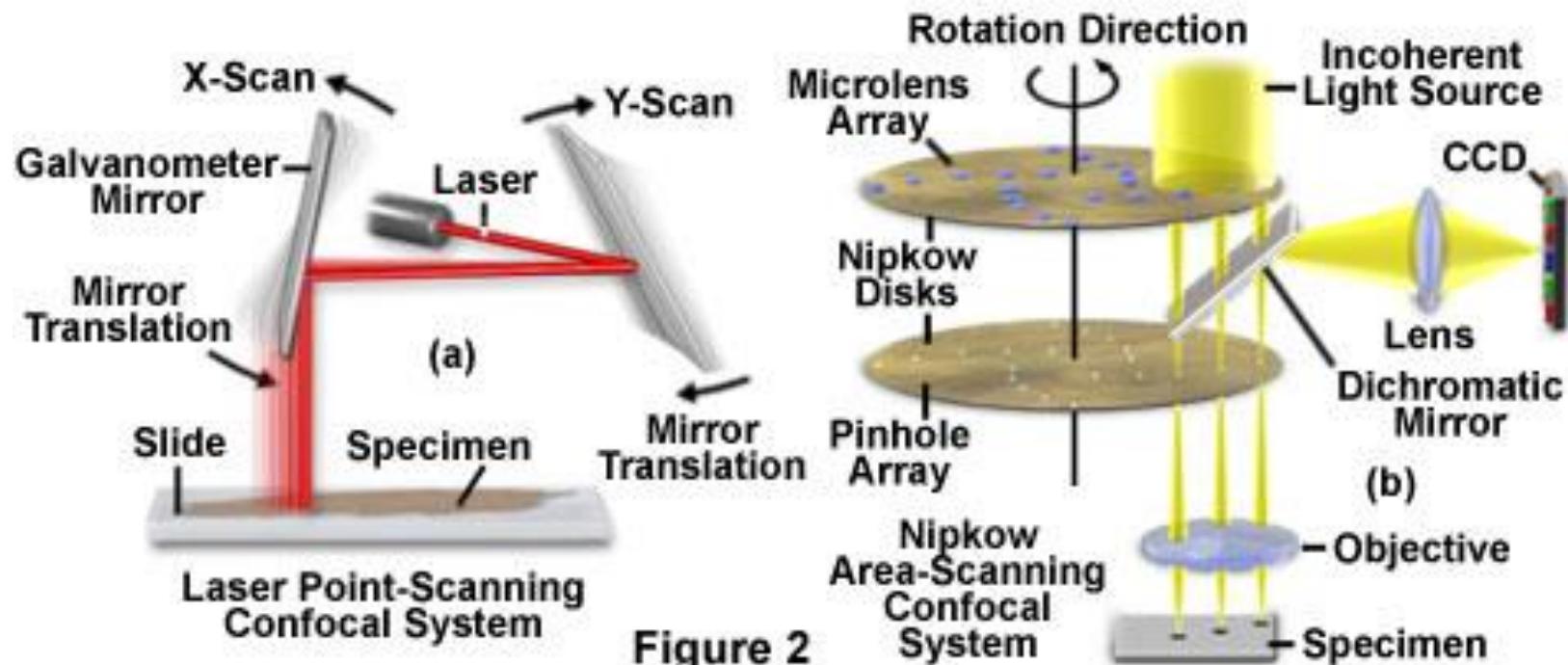
Confocal microscopy



FOV $1.84 \times 0.96 \times 5 \text{ mm}$
 $\sim 12 \text{ h}$

Spinning disc confocal:

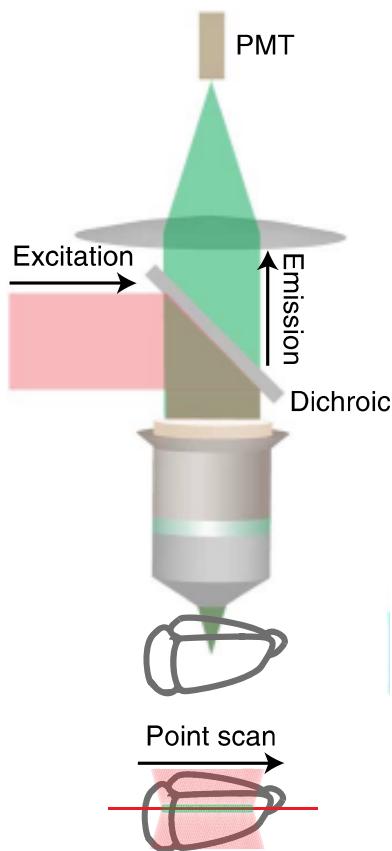
Point and Area-Scanning Confocal System Configurations





Two-photon microscopy

Two-photon microscopy



Energy load
per plane

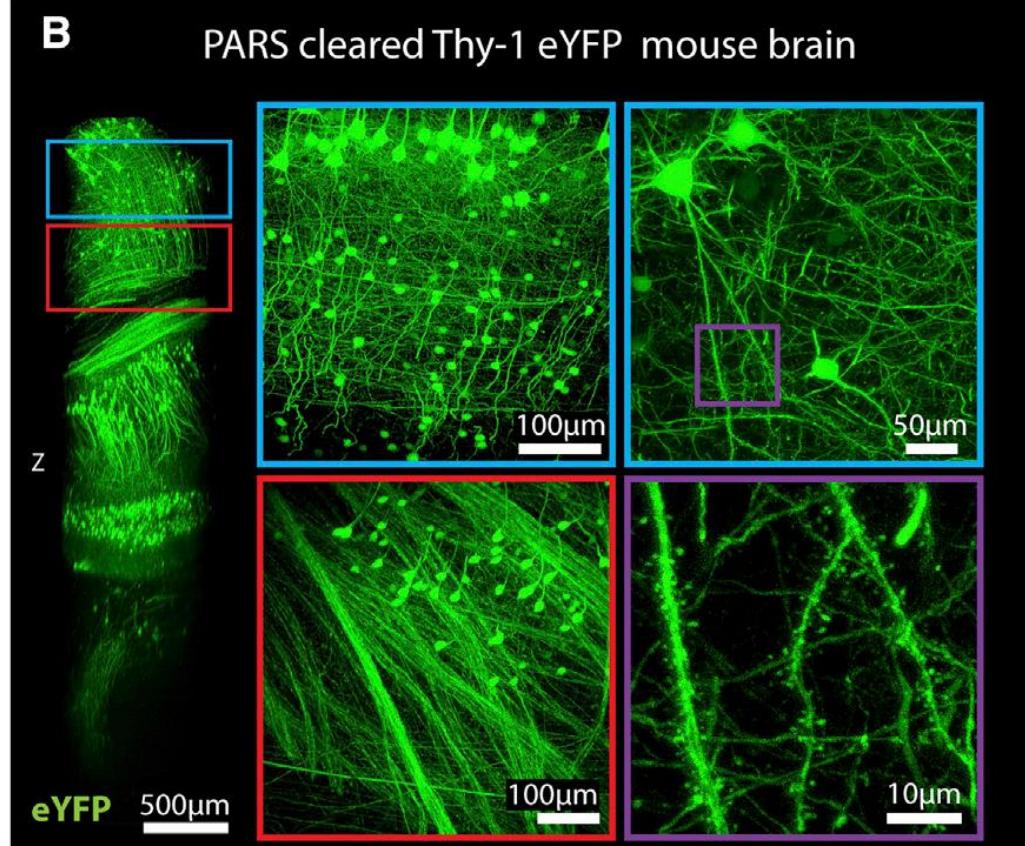
Photobleaching

Medium

Imaging speed

Slow

R. Tomer et al., **Nature Protocols** (2014)

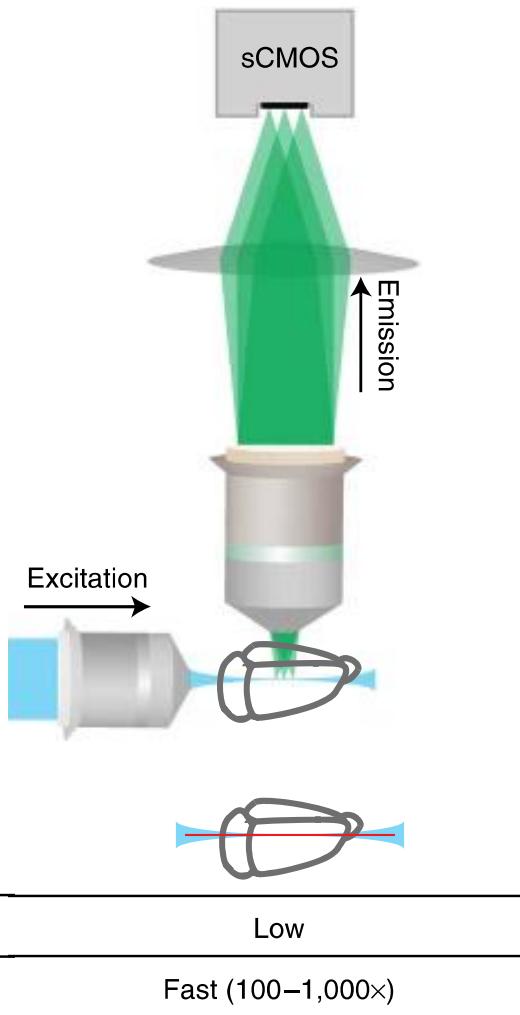


- Slow, point by point scan
- Expensive

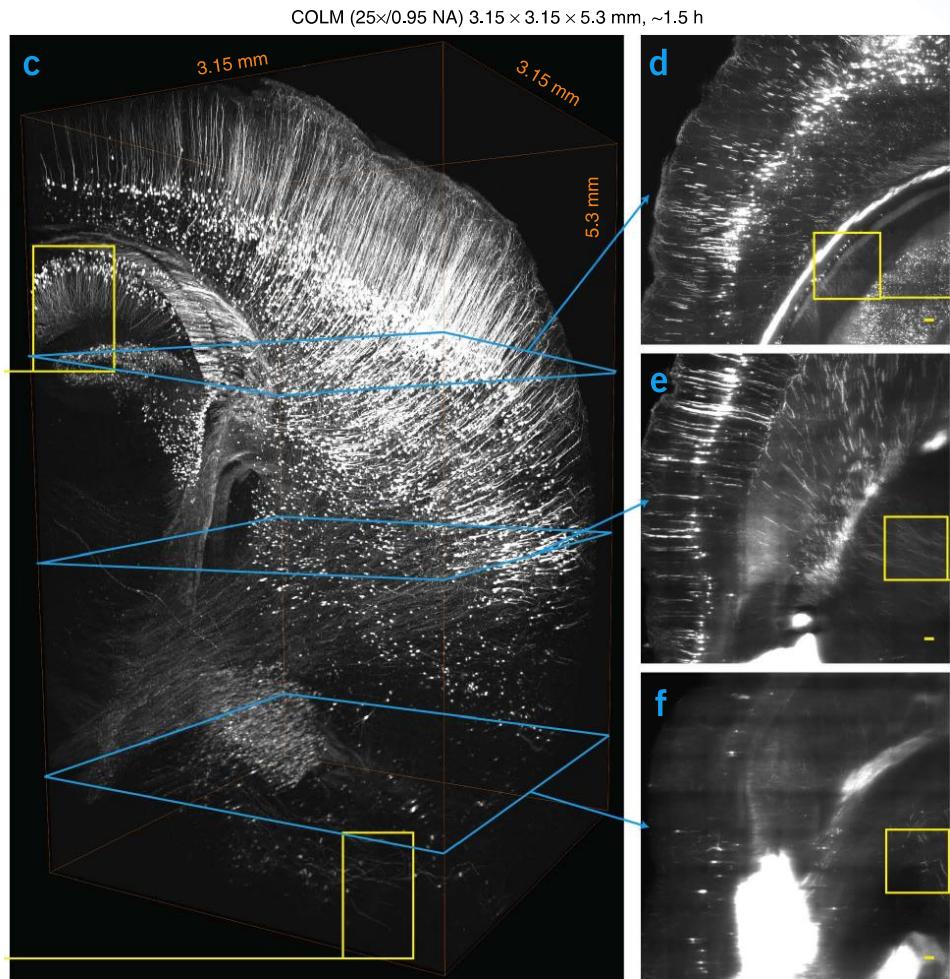
CLARITY optimized light-sheet microscopy (COLM)



Light-sheet microscopy



R. Tomer et al., *Nature Protocols* (2014)

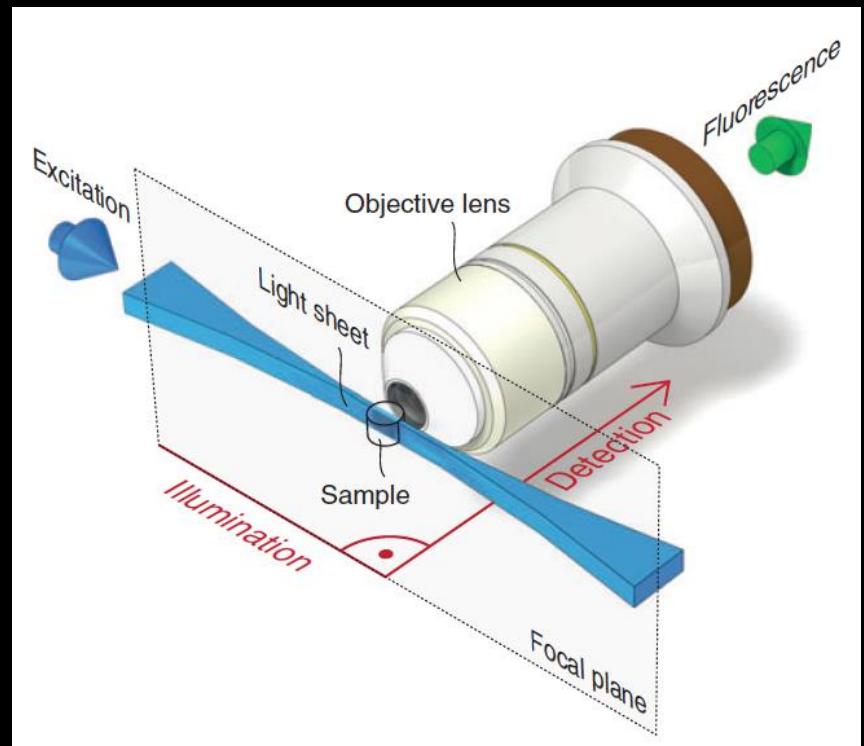


$3.15 \times 3.15 \times 5.3 \text{ mm}$, $\sim 1.5 \text{ h}$, ~ 25 times faster than confocal microscope

Light-sheet basics

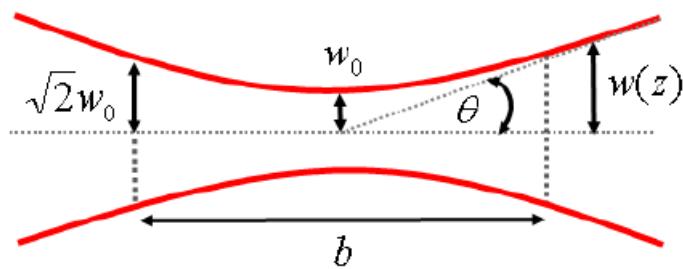
Light-Sheet Microscopy

- Optical sectioning of a sample with laser-light sheet
- Illumination light sheet thickness 1-10 μm
 - Comparable to the depth of field an objective
- Fast detection of an entire 2D FOV with a single shot
 - 3D images by moving a sample or light sheet z-axis
 - Dynamics
- Minimal photo bleaching
- Commercial systems
 - Zeiss Z.1
 - LaVisionBioTech



Huisken and Stainier development 2009

Basics of a light-sheet scope



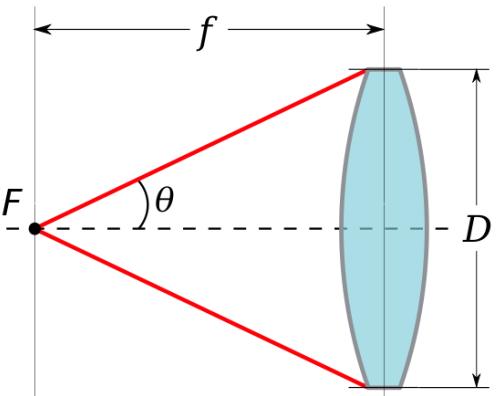
NA	$2w_0 [\mu\text{m}]$	$b [\mu\text{m}]$
0.5	0.68	1.81
0.3	1.13	5.02
0.2	1.70	11.29
0.1	3.39	45.15
0.05	6.79	180.61

For 533nm in water

$$w_0 = \frac{l}{\rho \cdot NA}$$

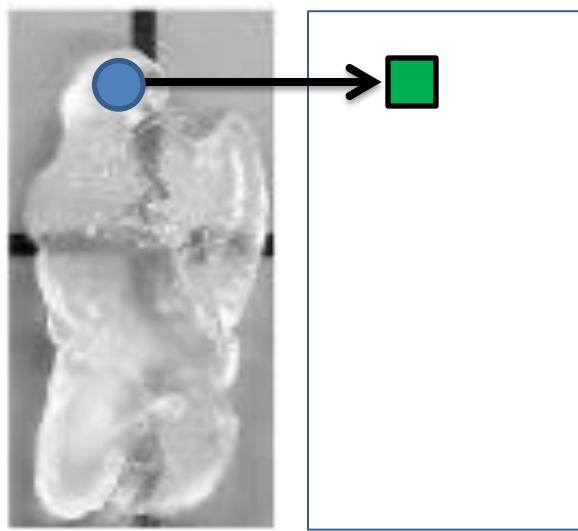
$$b = \frac{2n l}{\rho \cdot (NA)^2} \quad NA = n \sin Q \gg n \frac{D}{2f}$$

$$b = \frac{2n}{NA} w_0$$

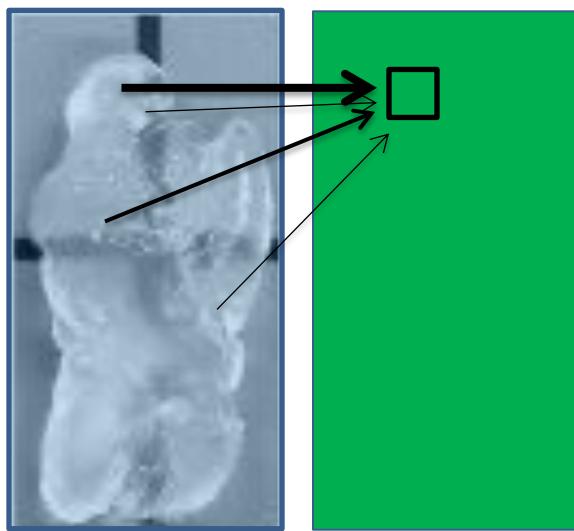


Signal to noise ratio

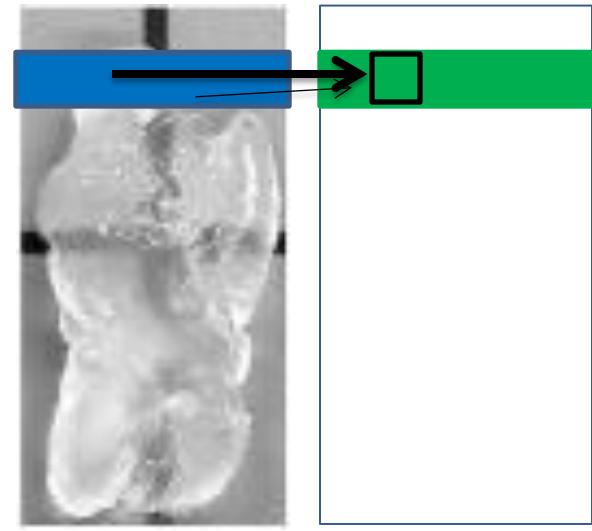
Ideal case,
confocal microscope



Regular case the entire
sample is illuminated

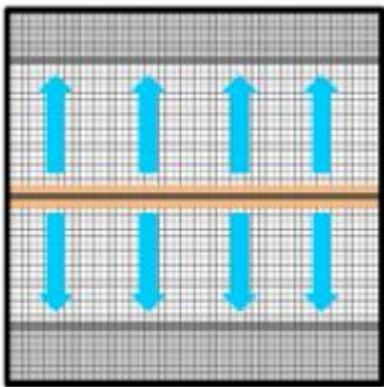


Light sheet mode

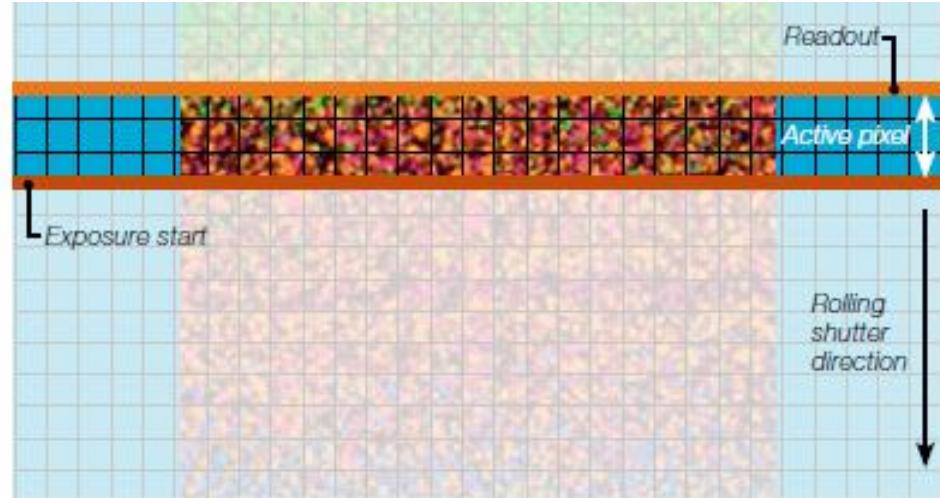
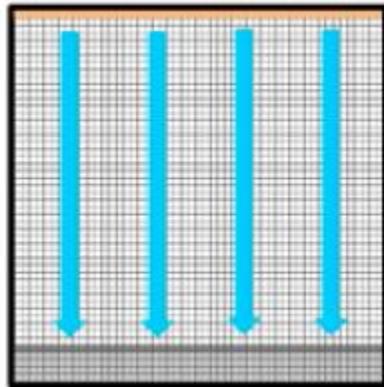


Light-sheet mode camera

Standard
camera



Light-sheet
camera



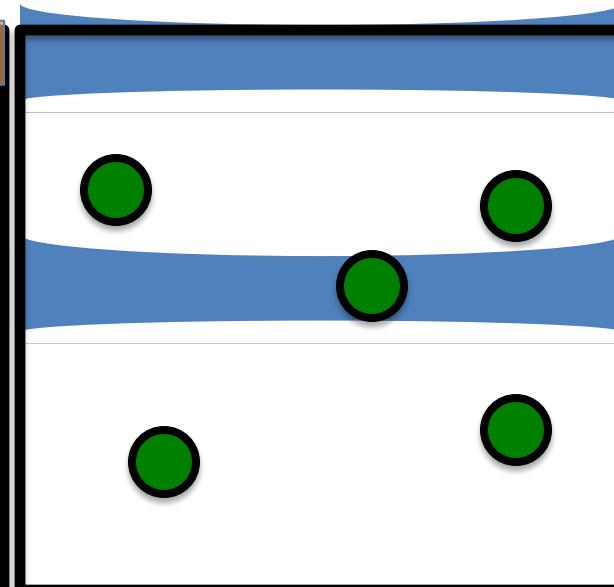
http://www.andor.com/pdfs/literature/Andor_sCMOS_Brochure.pdf

Camera

Exposure
window

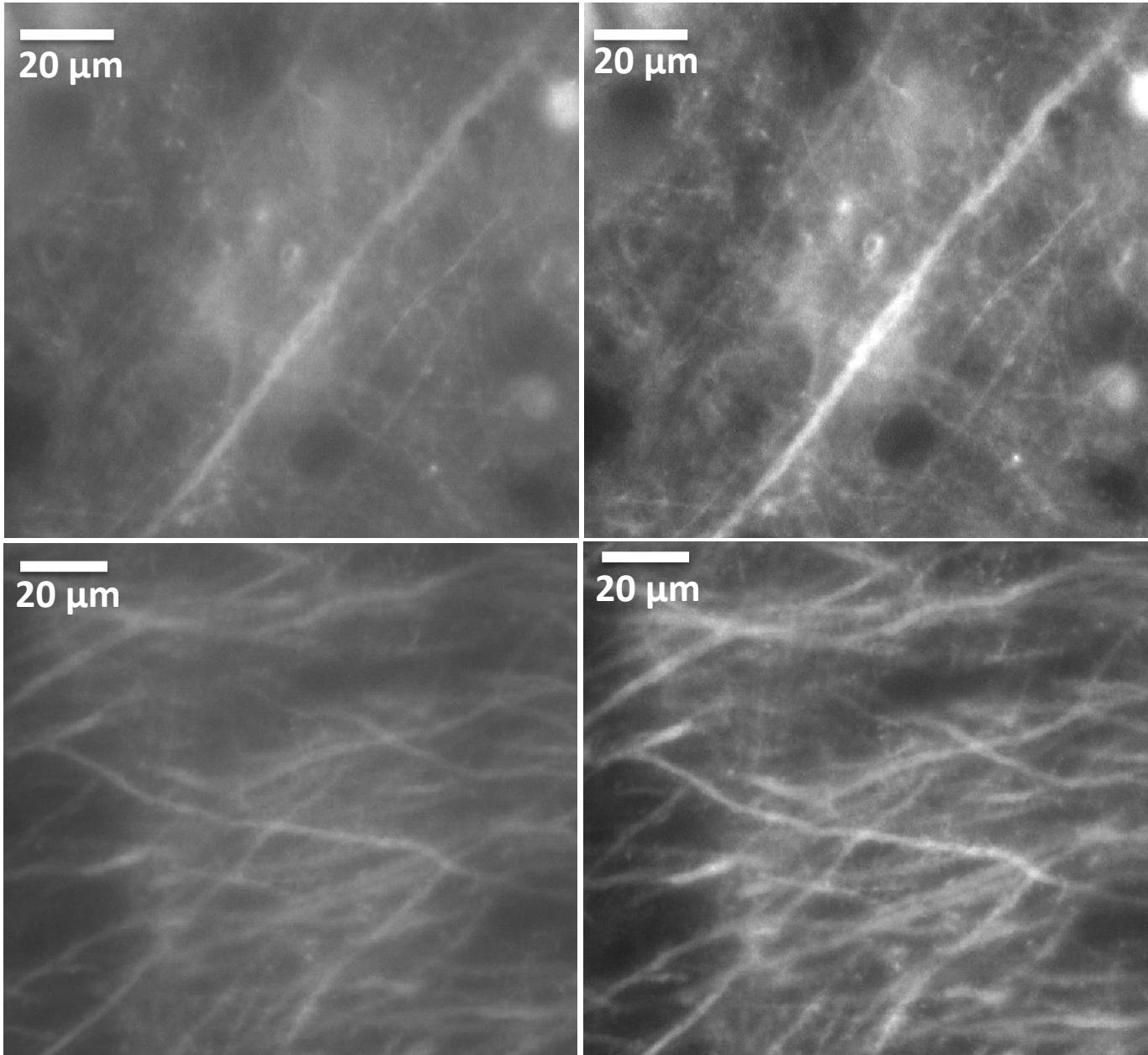


Sample



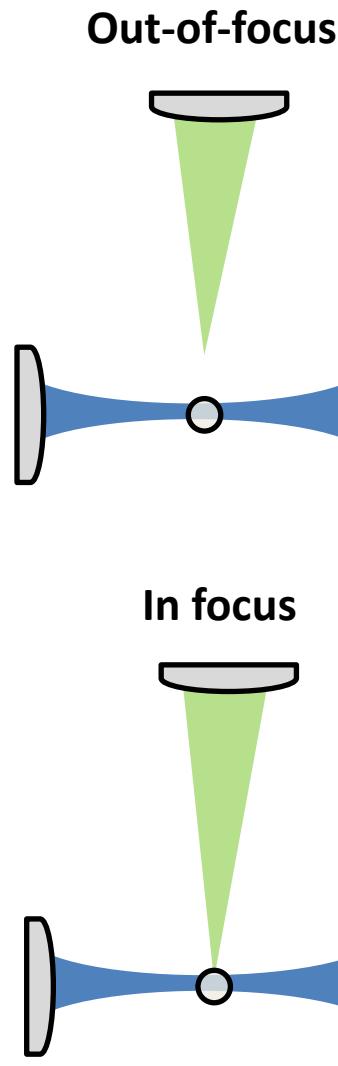
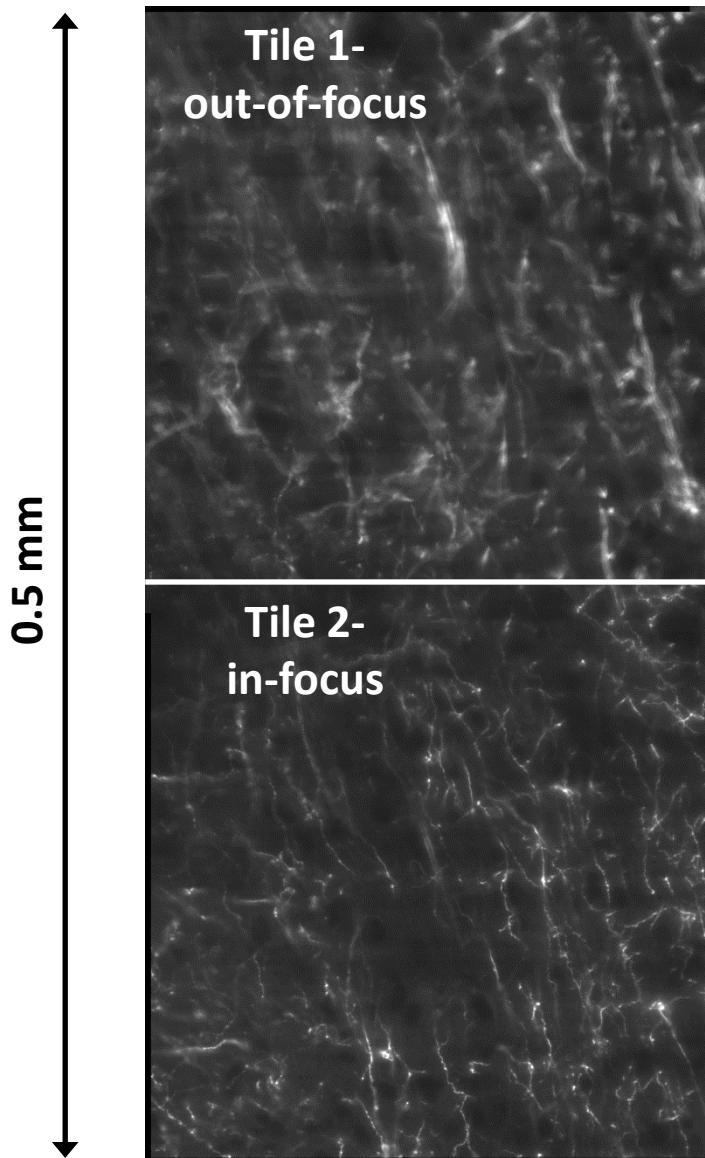
Light
Sheet

Continuous scan vs. light-sheet mode

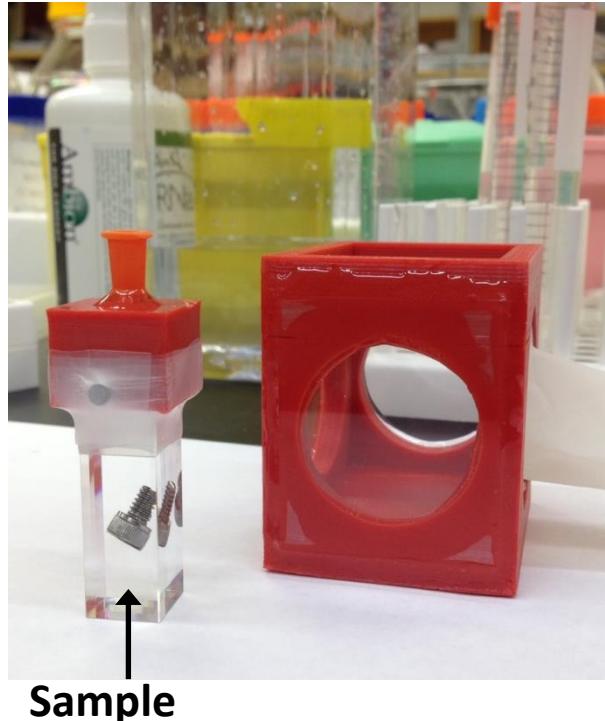


Light-sheet issues

Focusing in light-sheet microscopy

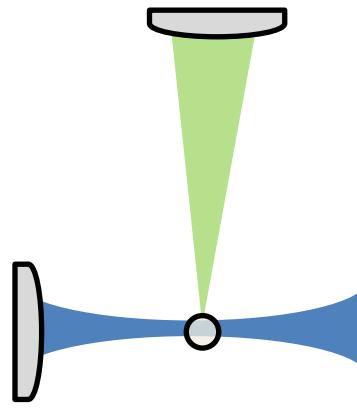


Index of refraction changes along the scan

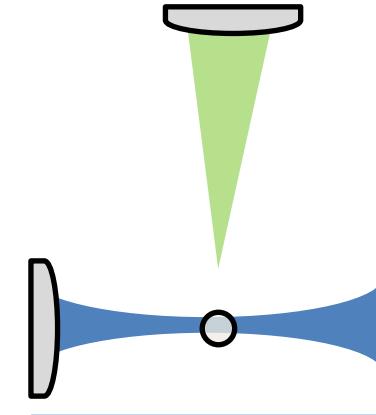


Variations in refractive index as a function of depth cause the detection objective to get out-of-synchronization with the light sheet

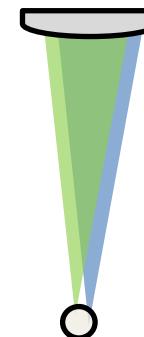
In focus @ $z = 100 \mu\text{m}$



Out-of-focus @ $z = 1010 \mu\text{m}$



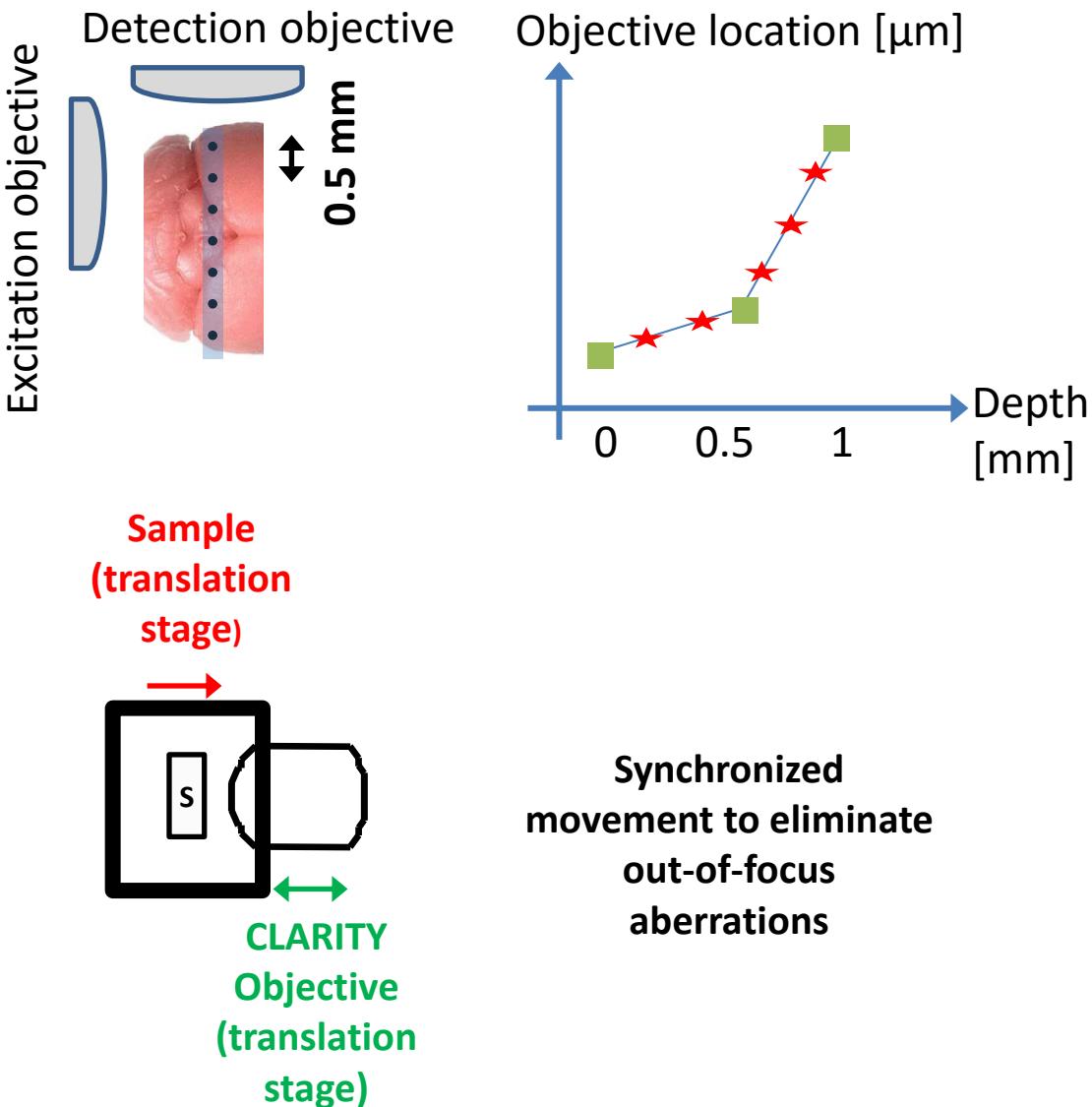
Confocal



The sample chamber is filled with Glycerol ($\text{RI} = 1.47$) , while the Quartz cuvette ($\text{RI} = 1.46$) is filled with RIMS ($\text{RI} \sim 1.467$).

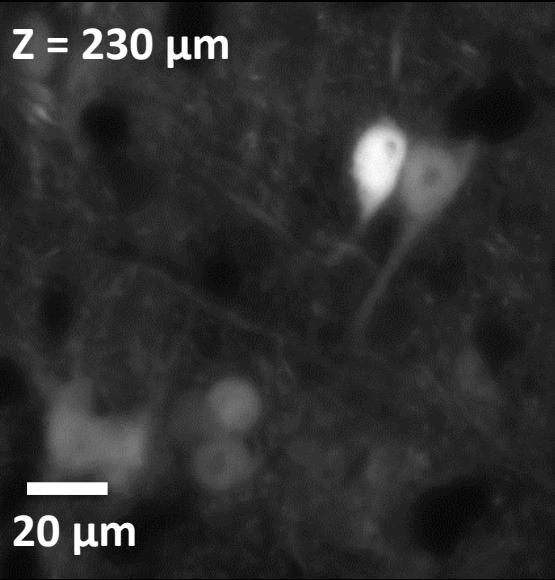
Auto-focus in different depths

- Auto-focus calibration step
- Scattered light/emitted light
- Focus measures:
 - Tamura coefficient
 - Variance of gradient magnitudes
 - Stop galvo scan and minimize FWHM of light-sheet
- No “one size fits all” solution
- Trade-off light-sheet FWHM and signal to noise ratio

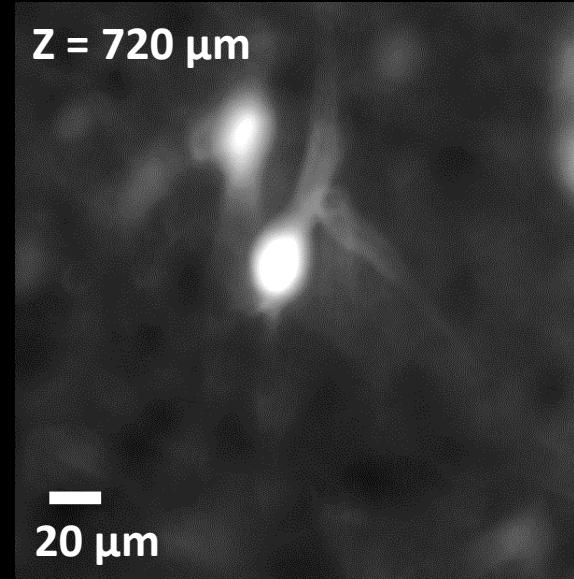
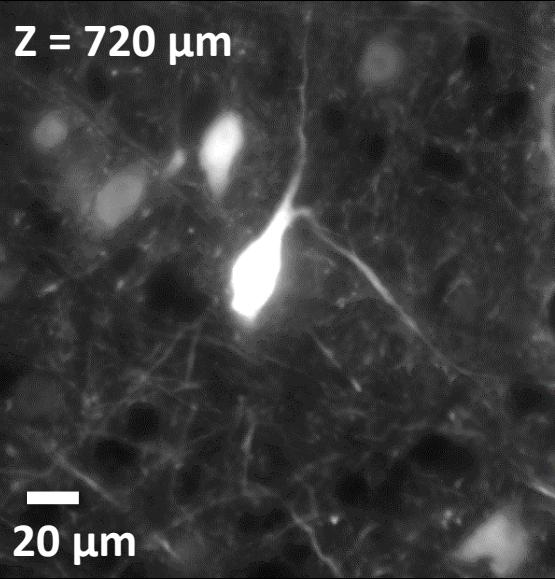
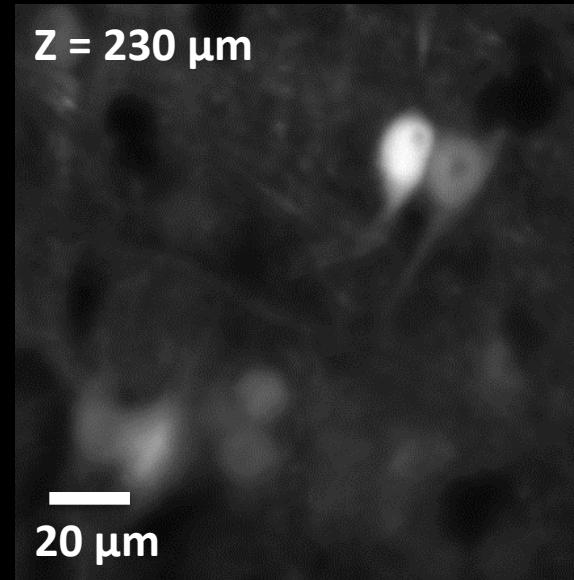


Auto-focus in different depths

With correction

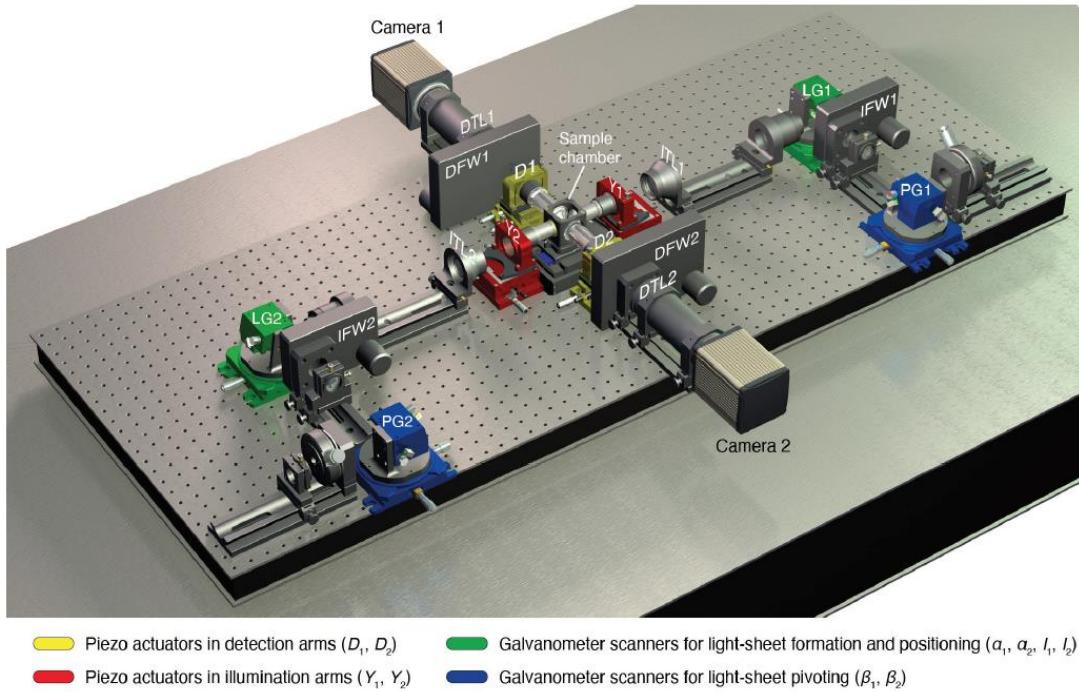


Without correction

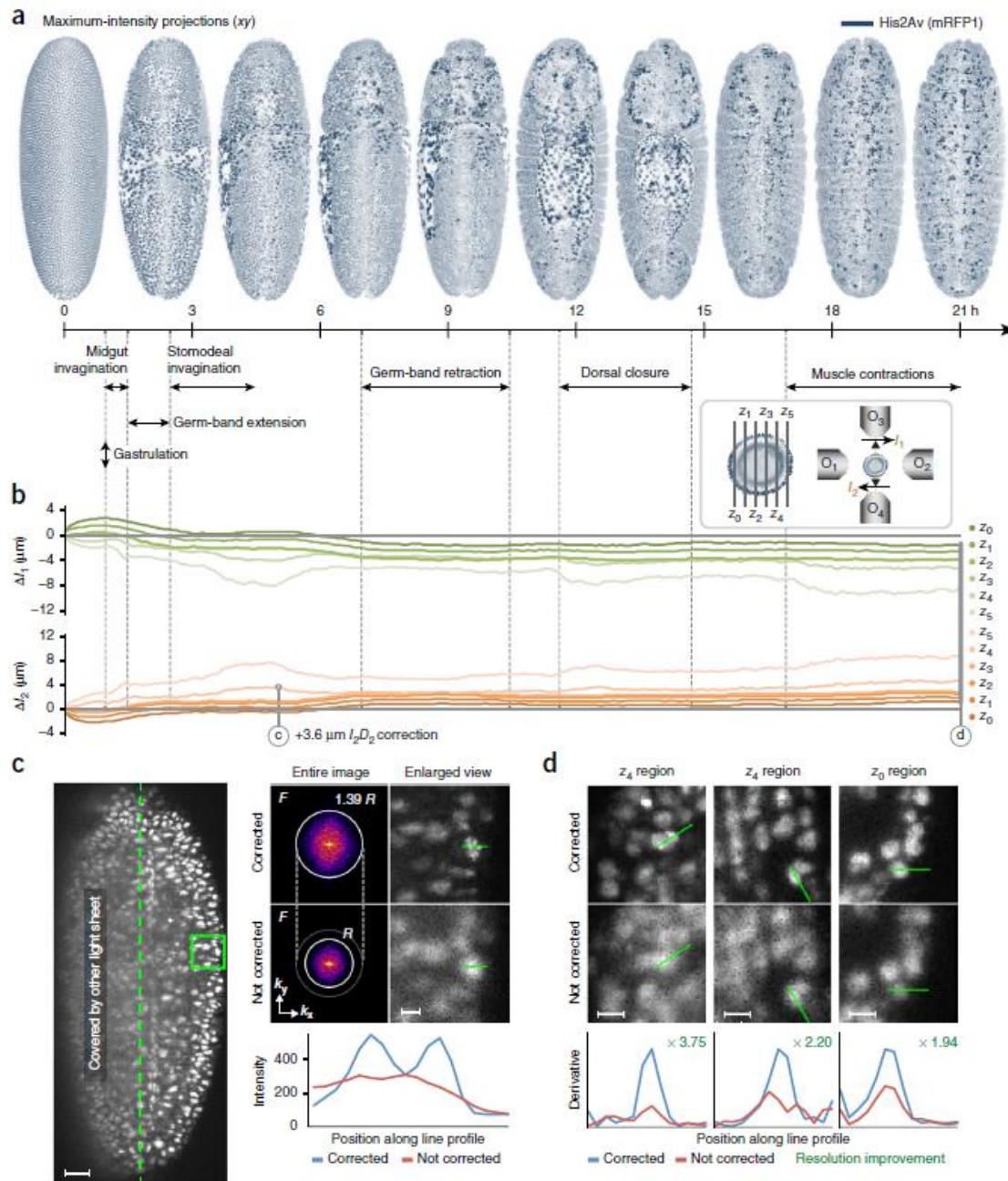


Adaptive light-sheet microscopy for long-term, high-resolution imaging in living organisms

Loïc A Royer^{1,2}, William C Lemon¹, Raghav K Chhetri¹, Yinan Wan¹, Michael Coleman³,
Eugene W Myers² & Philipp J Keller¹



Different developmental stages – change in the embryo properties



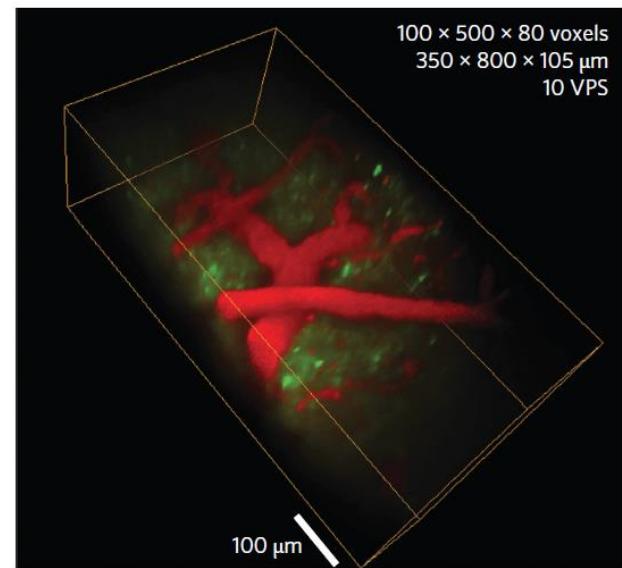
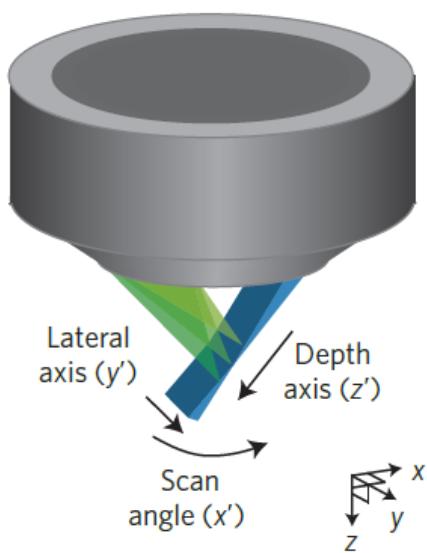
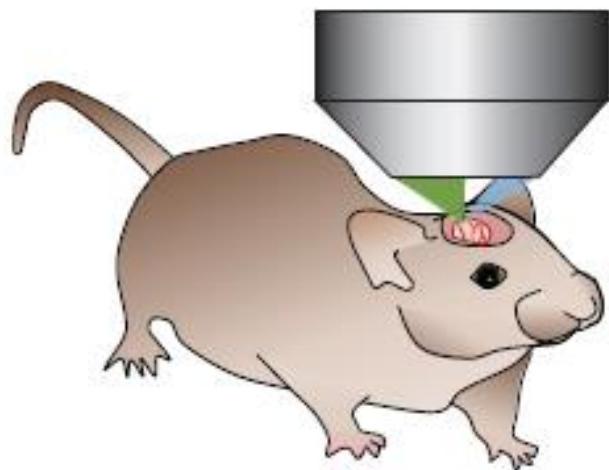
The autopilot correction graphs

Comparison of corrected versus uncorrected

Recent developments in light-sheet microscopy

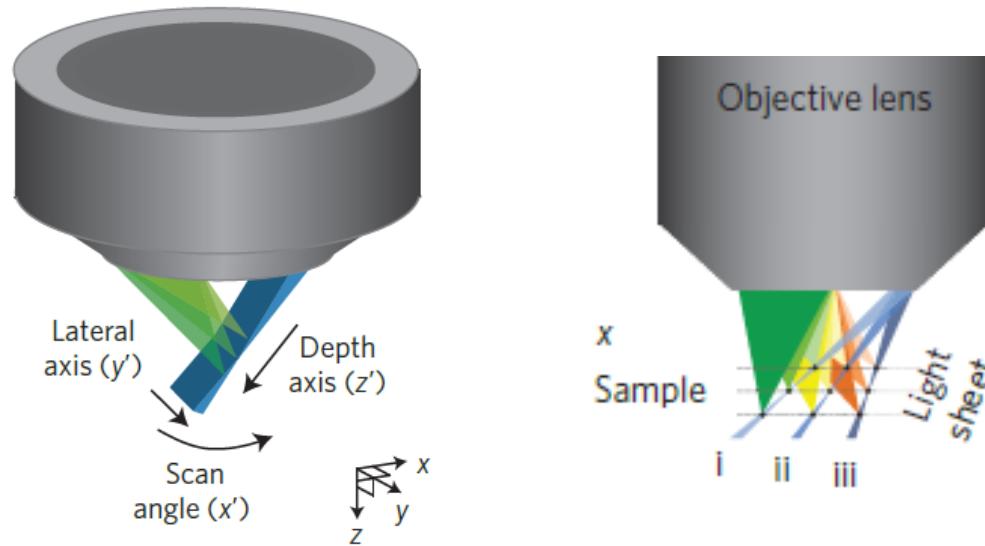
Swept confocally-aligned planar excitation (SCAPE) microscopy for high-speed volumetric imaging of behaving organisms

Matthew B. Bouchard¹, Venkatakaushik Voleti¹, César S. Mendes², Clay Lacefield³, Wesley B. Grueber⁴, Richard S. Mann², Randy M. Bruno^{3,5} and Elizabeth M. C. Hillman^{1,5*}

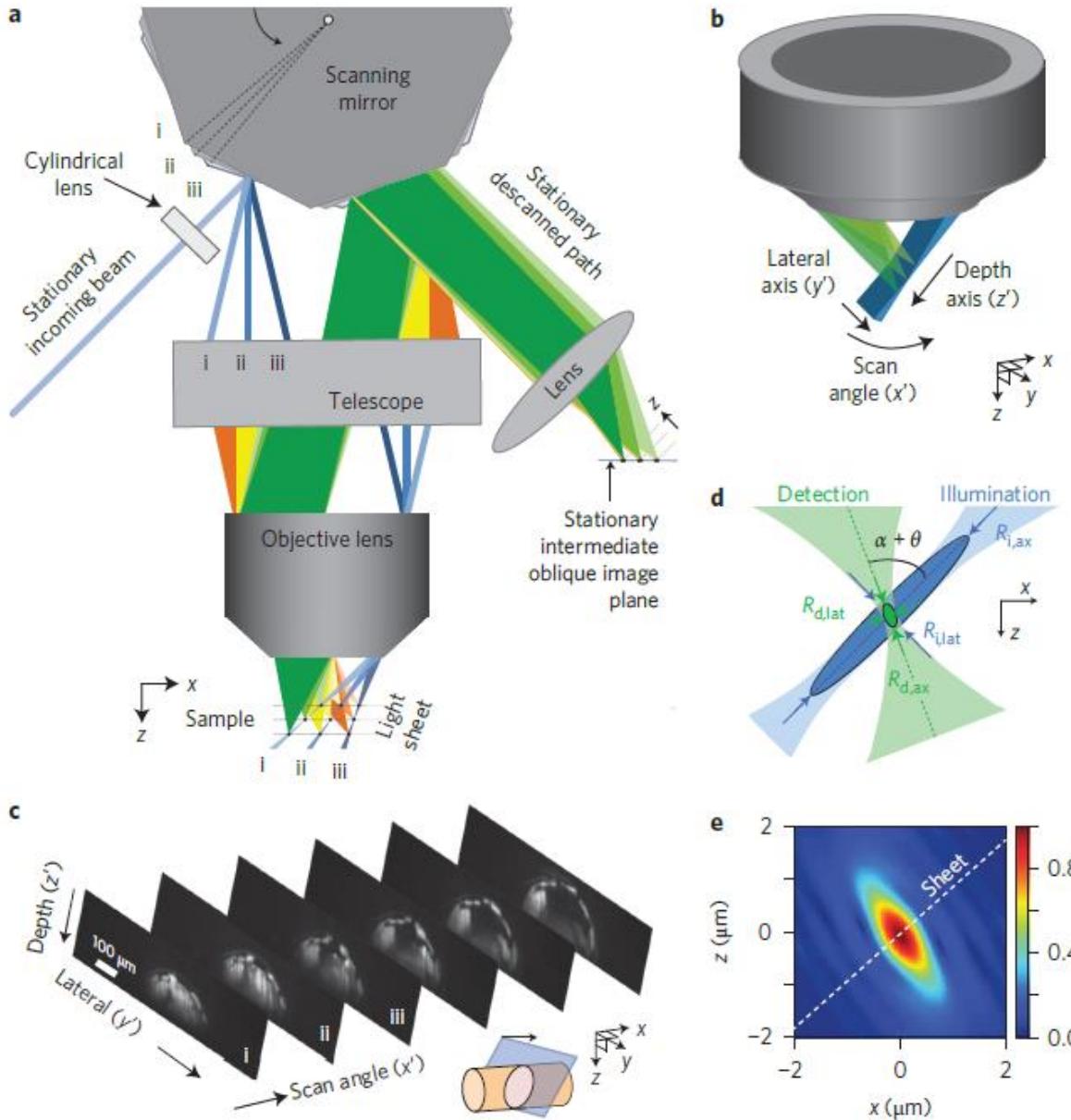


How to image spontaneous neuronal firing in the intact brain of awake behaving mouse ?

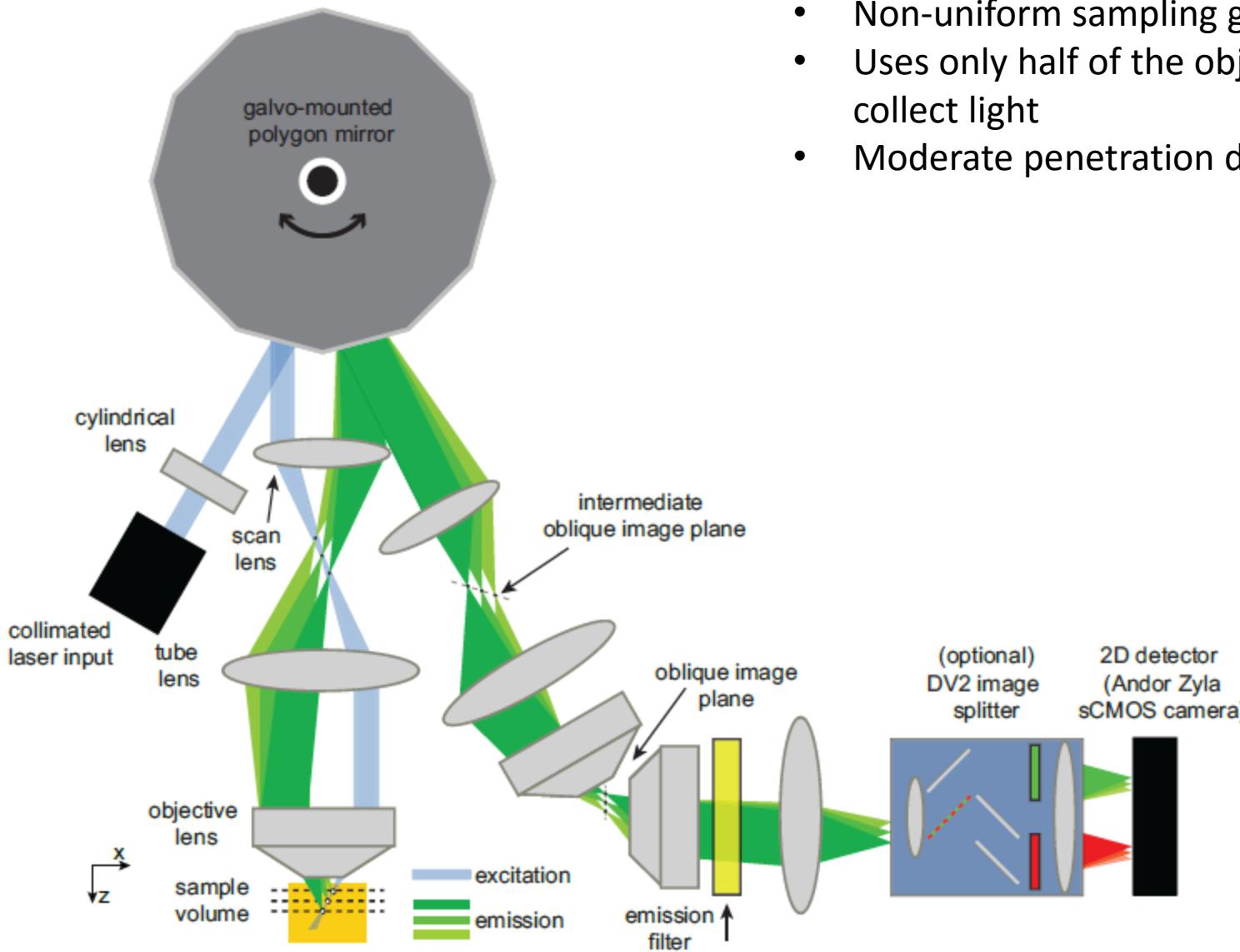
The solution: Carnial window and creating light sheet using only one lens



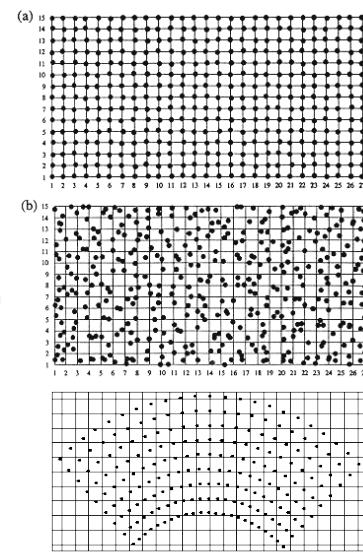
SCAPE imaging geometry and image formation



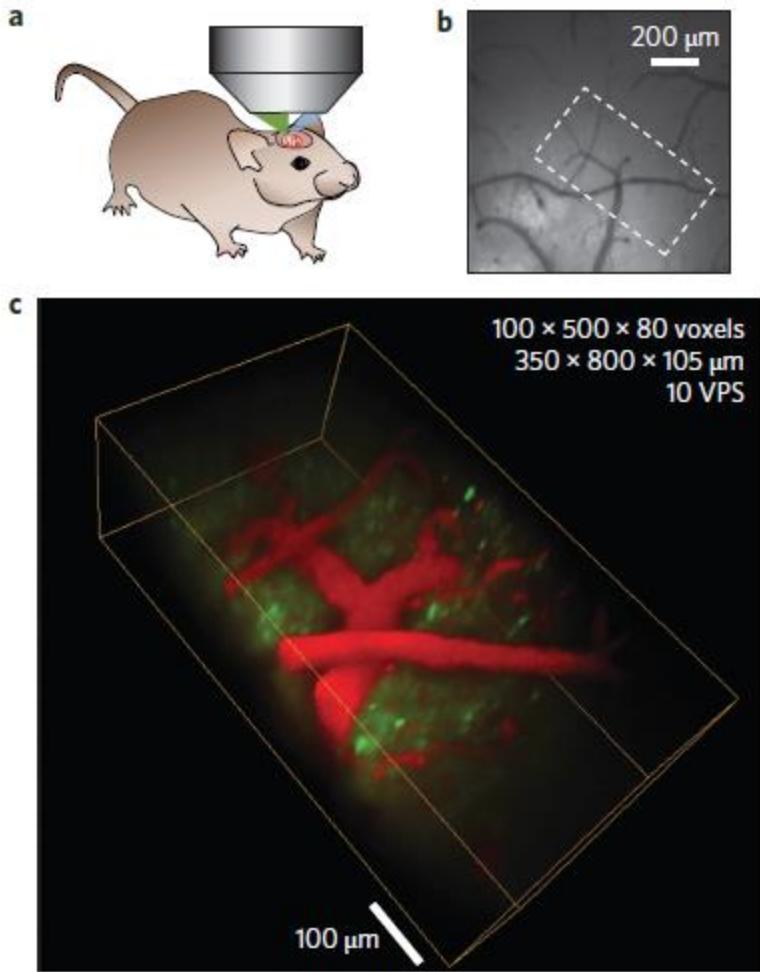
Full schematic of current SCAPE system



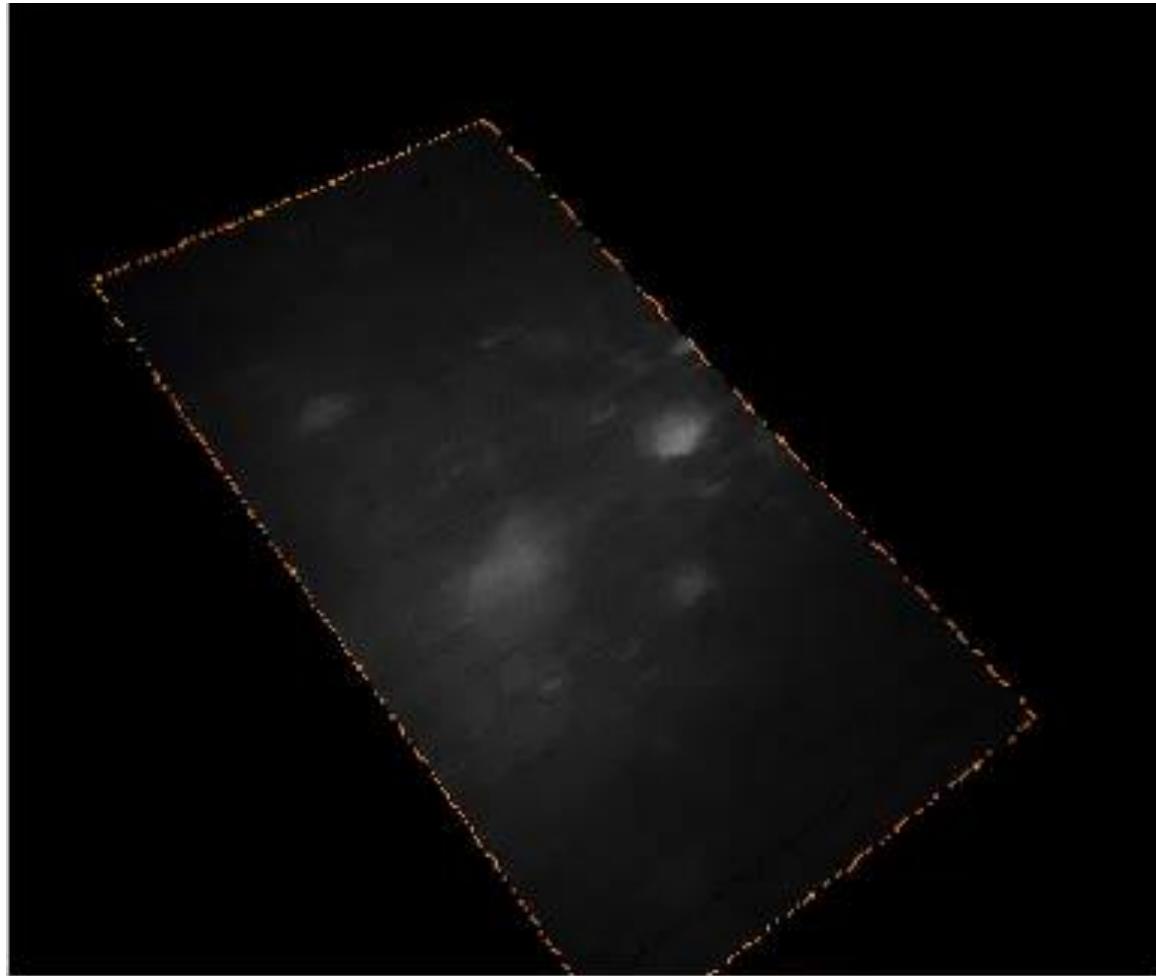
- Non-uniform sampling grid.
- Uses only half of the objective to collect light
- Moderate penetration depth



SCAPE microscopy in mouse brain

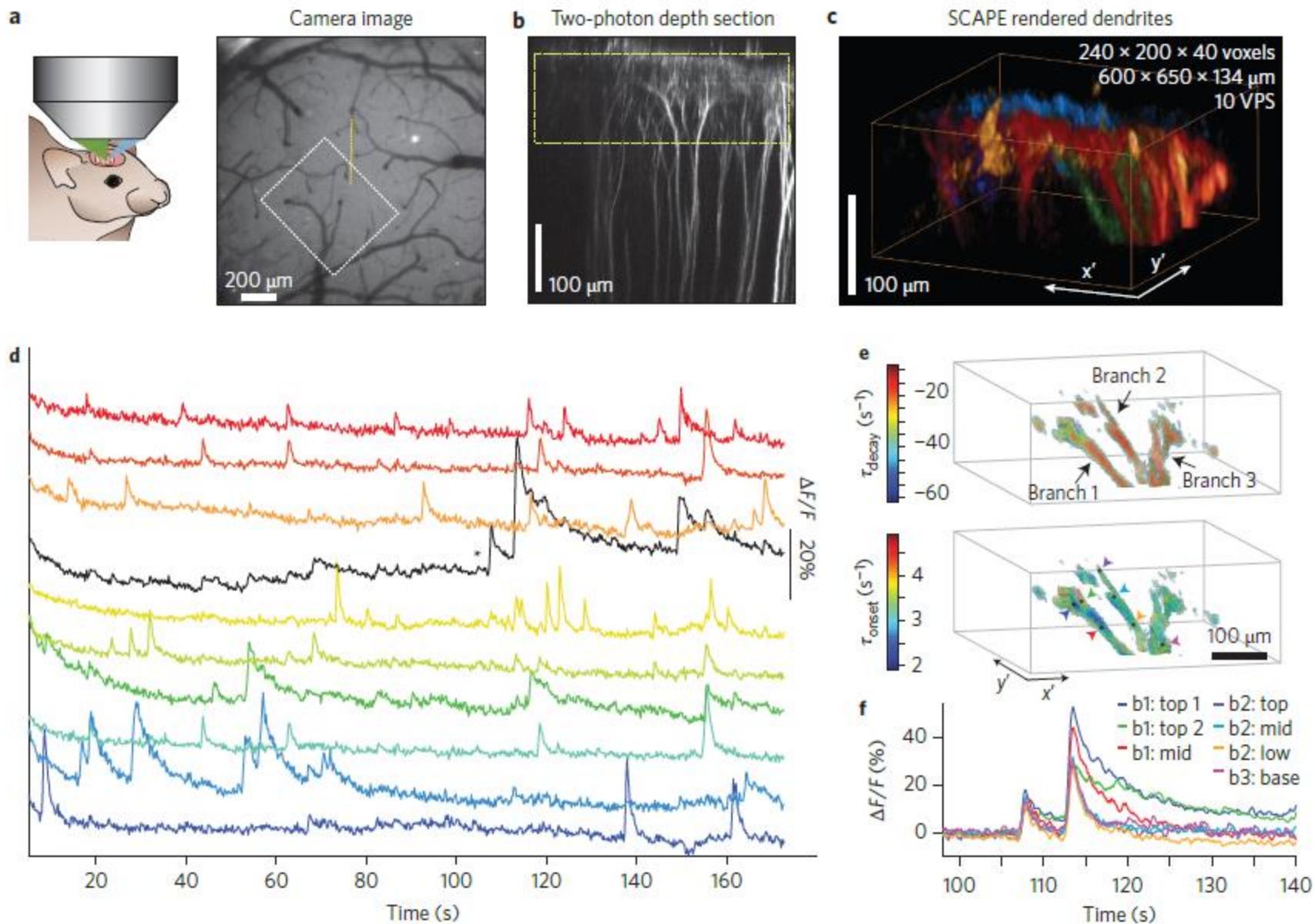


- Intravascular Texas red dextran
- Cre-recombinase in cortical 5 pyramidal neurons
- Cortical injection of adeno virus (AAV2:hSyn:FLEX:GCamp6f)



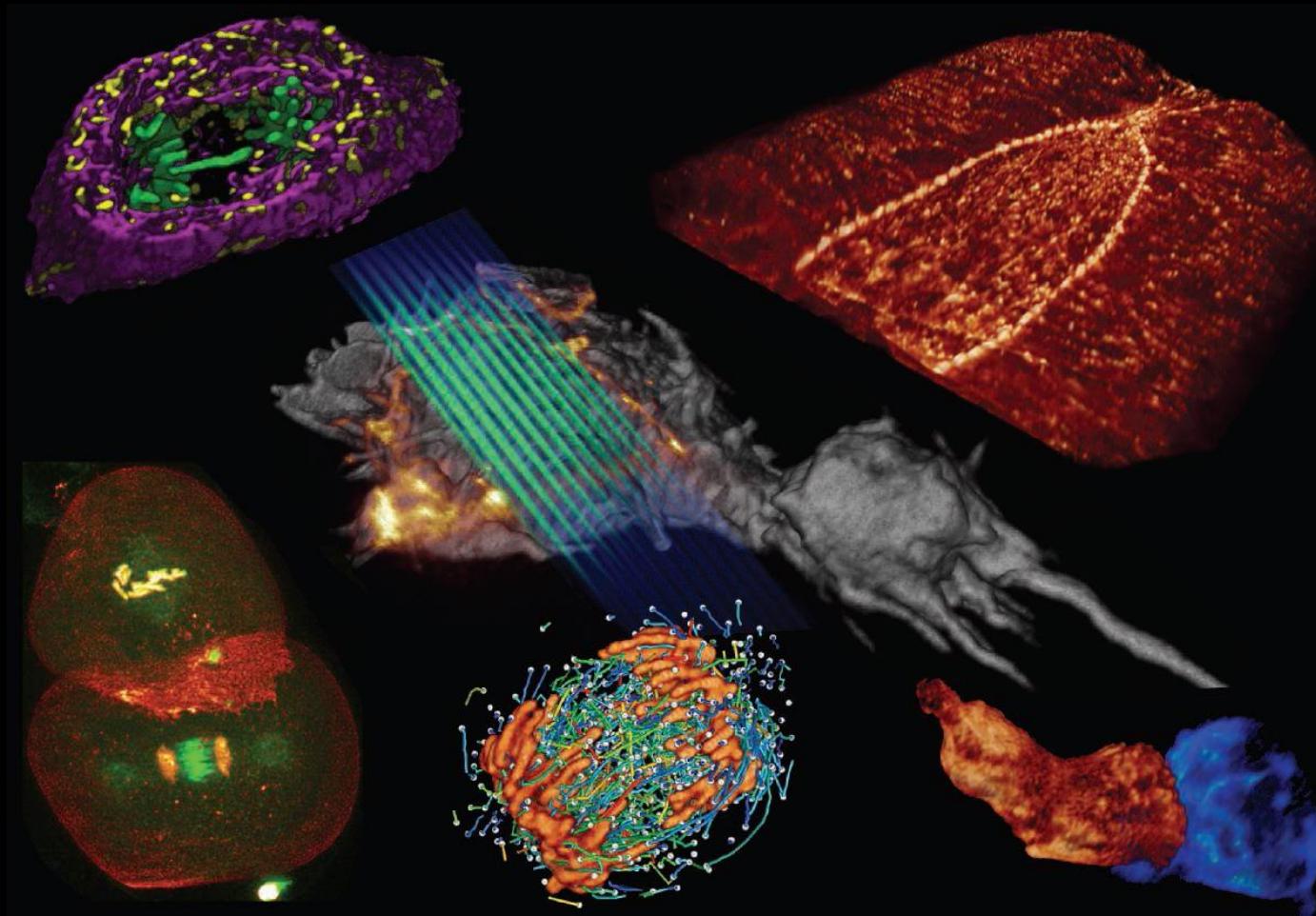
Supplemental Movie 1. 3D volume rendering of SCAPE data acquired in an awake, behaving mouse expressing GCaMP6f in apical dendrites and with Texas red dextran in its vasculature. Movie shows orthogonal slices through a single volume, and then 4D dynamic vascular and neuronal activity at 2x real time. Imaging parameters: 2 color, 350 x 800 x 106 micron volume (100 x 500 x 80 voxels x'-y'-z'), imaged at 10 VPS. See Figure 2 for details.

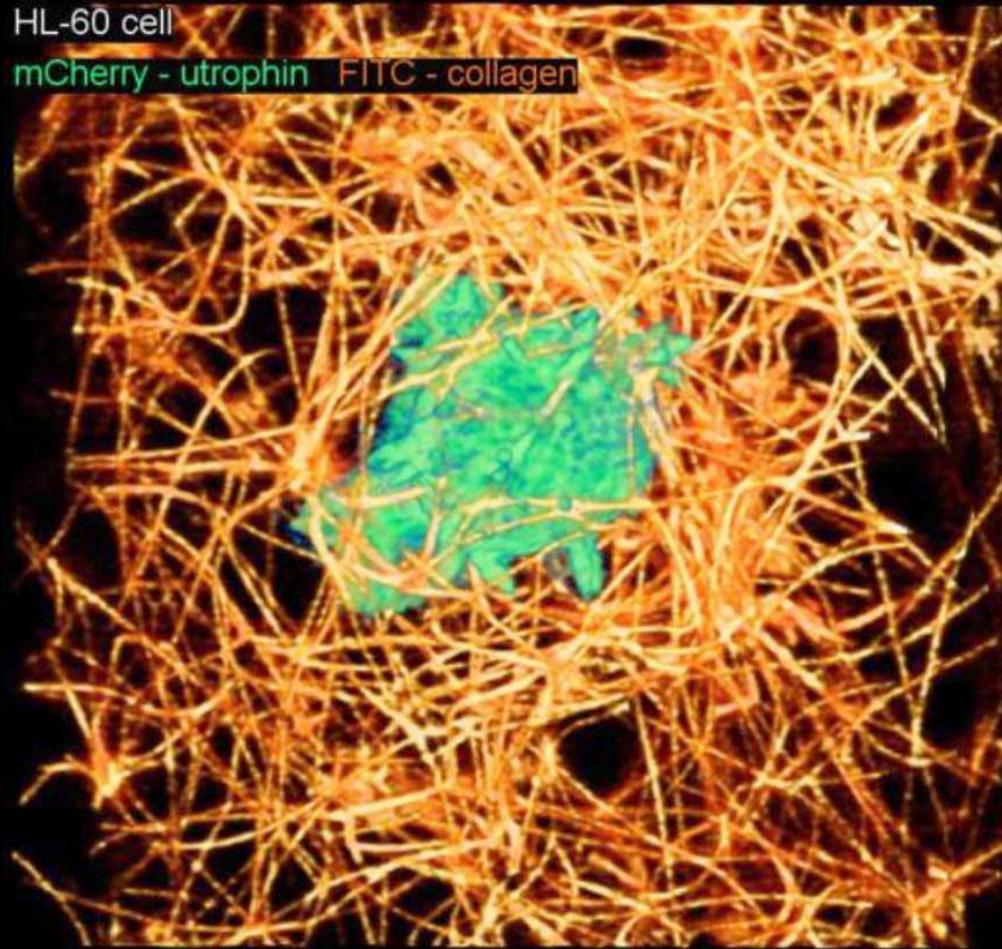
SCAPE microscopy of neuronal calcium dynamics in an awake mouse brain



Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution

Bi-Chang Chen, Wesley R. Legant, Kai Wang, Lin Shao, Daniel E. Milkie, Michael W. Davidson, Chris Janetopoulos, Xufeng S. Wu, John A. Hammer III, Zhe Liu, Brian P. English, Yuko Mimori-Kiyosue, Daniel P. Romero, Alex T. Ritter, Jennifer Lippincott-Schwartz, Lillian Fritz-Laylin, R. Dyche Mullins, Diana M. Mitchell, Joshua N. Bembenek, Anne-Cecile Reymann, Ralph Böhme, Stephan W. Grill, Jennifer T. Wang, Geraldine Seydoux, U. Serdar Tulu, Daniel P. Kiehart, Eric Betzig

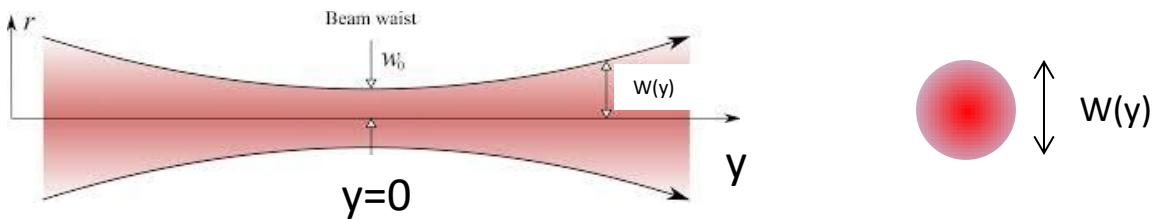




Movie 9 Cell movement through a matrix. Two-color volume rendering of a neutrophilic HL-60 cell expressing mCherry-utrophin migrating through a 3D collagen matrix labeled with FITC over 250 time points at 1.3-s intervals (compare with [Fig. 5, D to F](#)). [Play video](#)

Non-diffracting beams

Gaussian beam



The tighter the waist is the divergence will increase

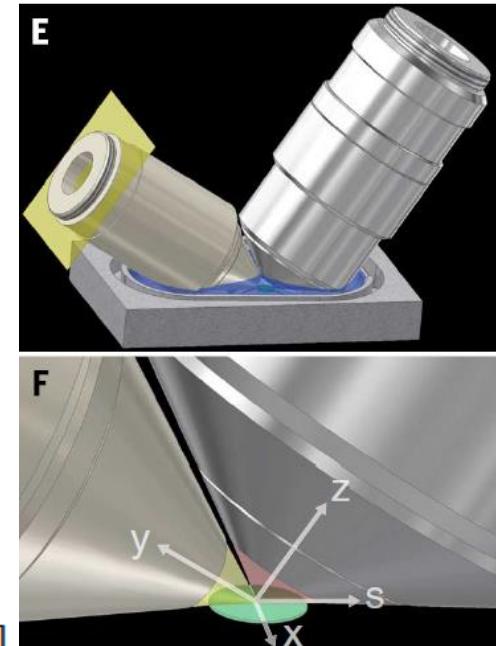
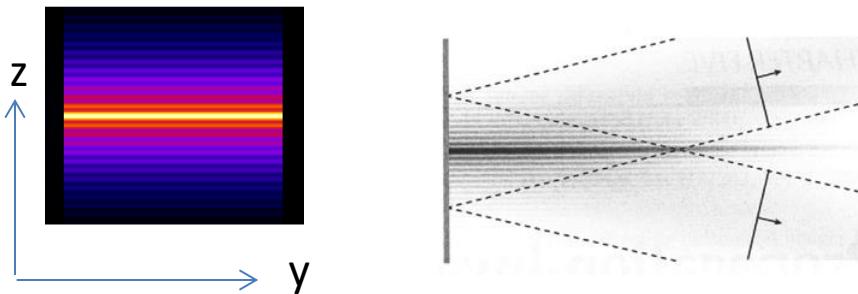
$$2\theta_0 = \frac{4\lambda}{\pi 2W_0}$$

For uniform illumination and resolution, we would like to have a non-diffracting beam

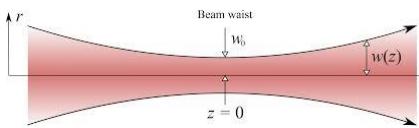
$$\mathbf{e}(\mathbf{x}, t) = \exp[i(k_y \cos \theta - \omega t)] \sum_{n=1}^N \mathbf{e}_n \exp[i((k_x)_n x + (k_z)_n z)] = \mathbf{e}(x, z) \exp[i(k_y \cos \theta - \omega t)]$$

The electric field of the light beam propagates in the 'y' direction without any change in its spatial distribution or amplitude in the XZ plane

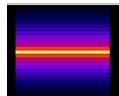
Bessel beam



Beam types and properties



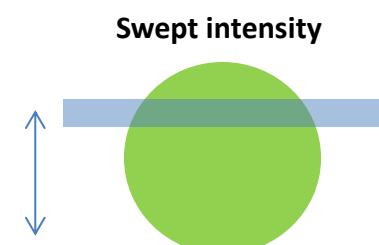
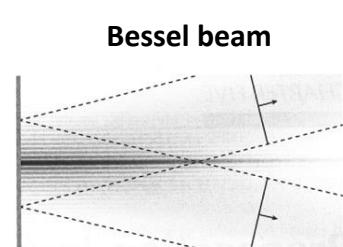
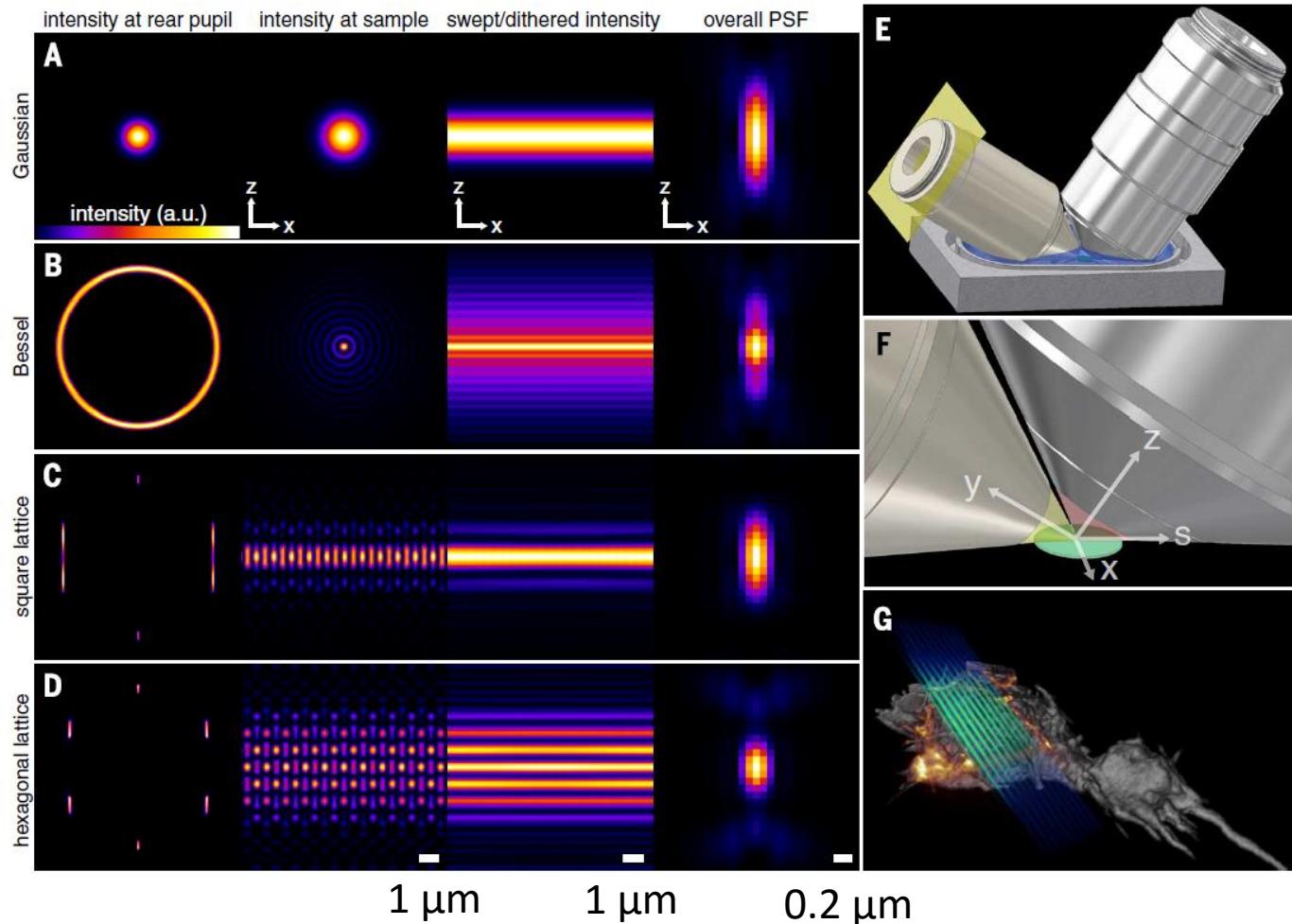
Issues: Divergence, axial resolution, thick



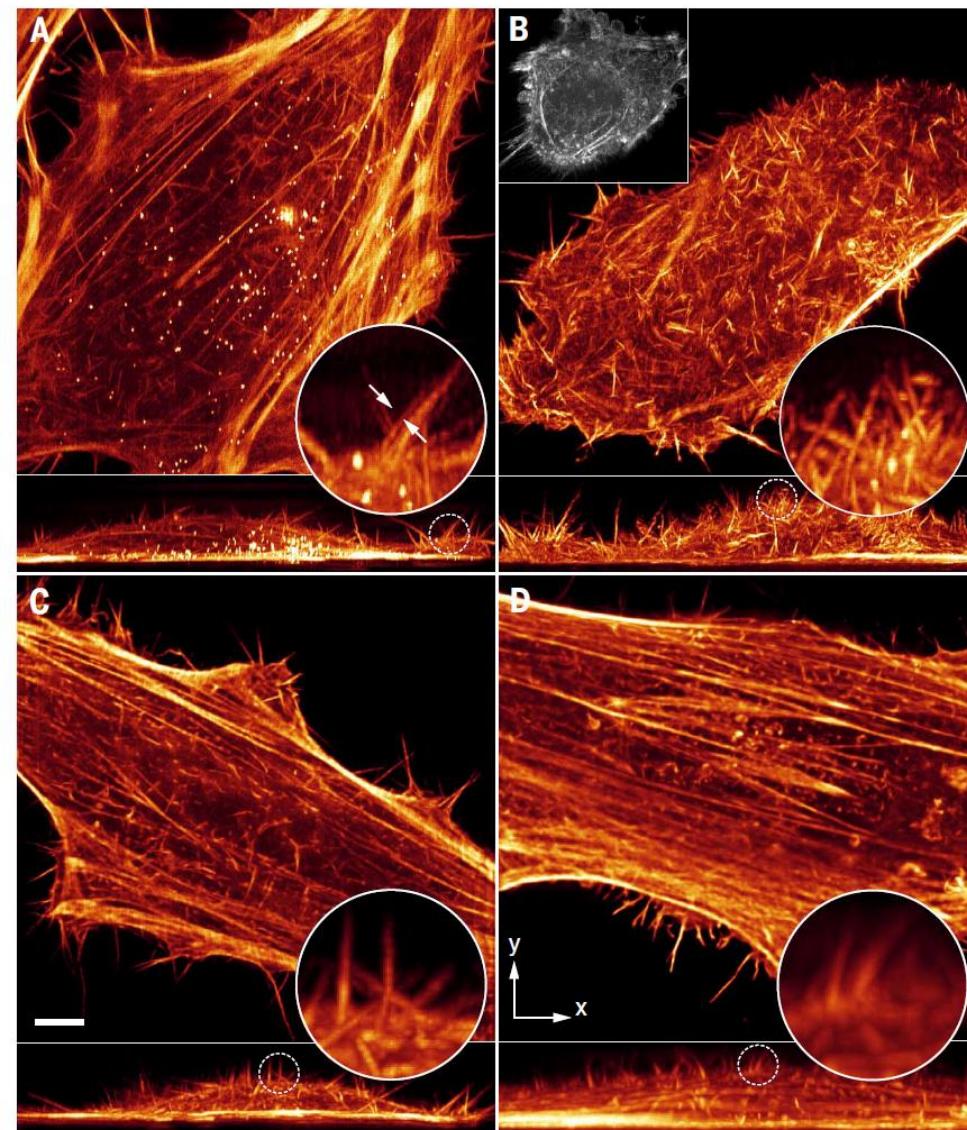
+: "Non-diffracting", $\sim 1\mu\text{m}$ diameter
-: Side lobes, axial resolution

+: "Non-diffracting", excitation confinement, minimal side lobes
high SNR, low photo-toxicity
-: Axial resolution

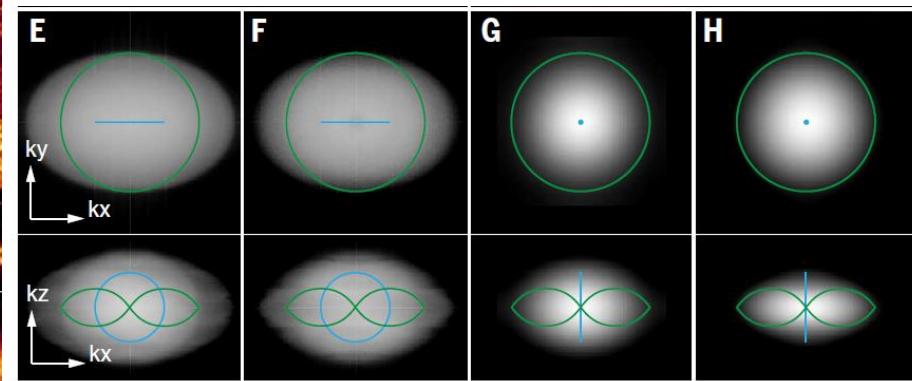
+: Optimized axial resolution, low photo-toxicity
-: SNR (not mentioned in the paper)



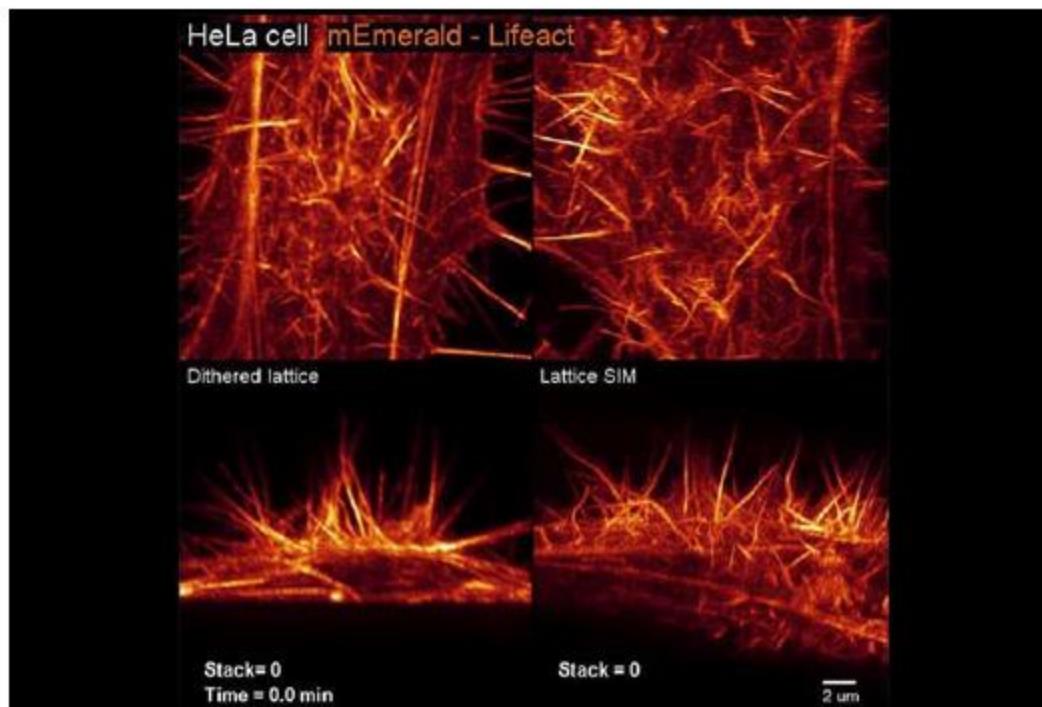
Results, Hela cell



- A) SIM mode, 5 phase (high resolution but slower), mEmerald-Lifeact (Actin). 150 nm X, Y 230 nm and Z 280 nm.
- B) SIM with stepped Bessel beam (previous approach), less beams, incoherent illumination => photo-toxicity. ~ 6 times slower compared to current method.
- C) Dithered mode, good axial resolution, very fast 100 frames per second. 230 nm res in X and Y, 370 nm in Z.
- D) Swept Bessel beam

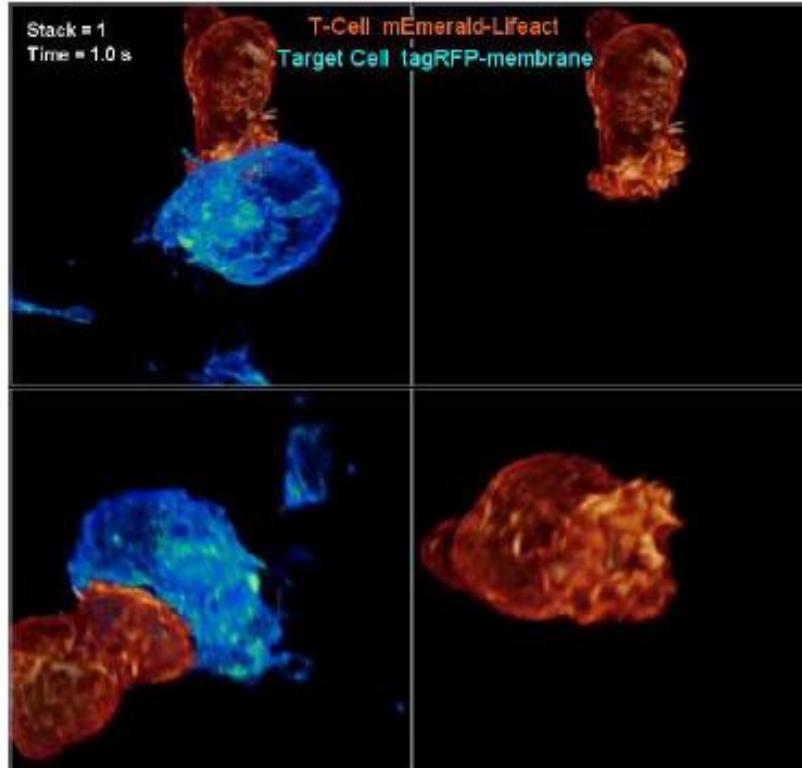


Movie 1



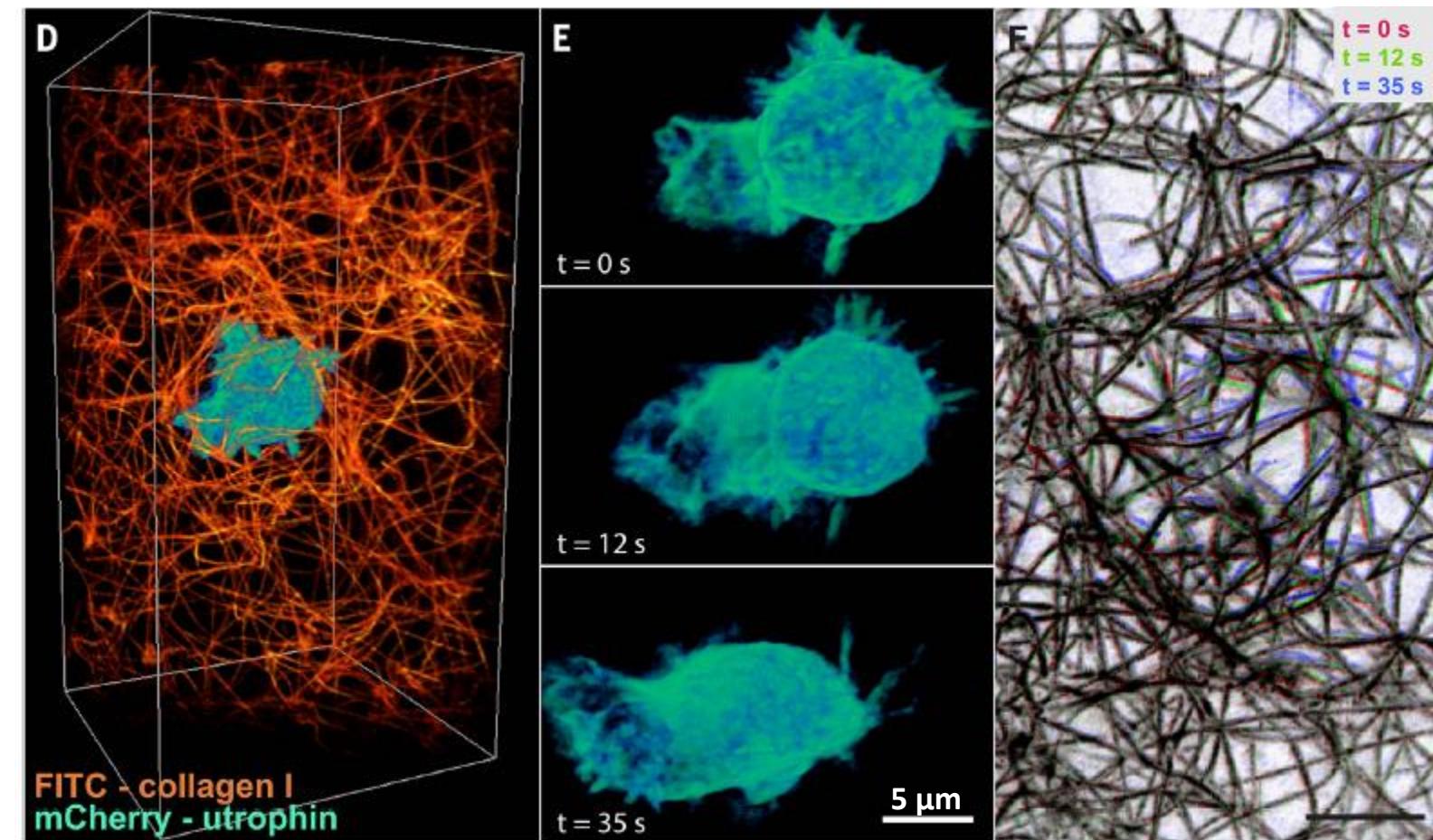
Movie 1. Top and side view volume renderings of filopodia in a HeLa cell expressing mEmerald-Lifeact. The high speed of the dithered mode of lattice light-sheet microscopy (**left**) is compared against the high resolution of the SR-SIM mode (**right**).

Movie 8



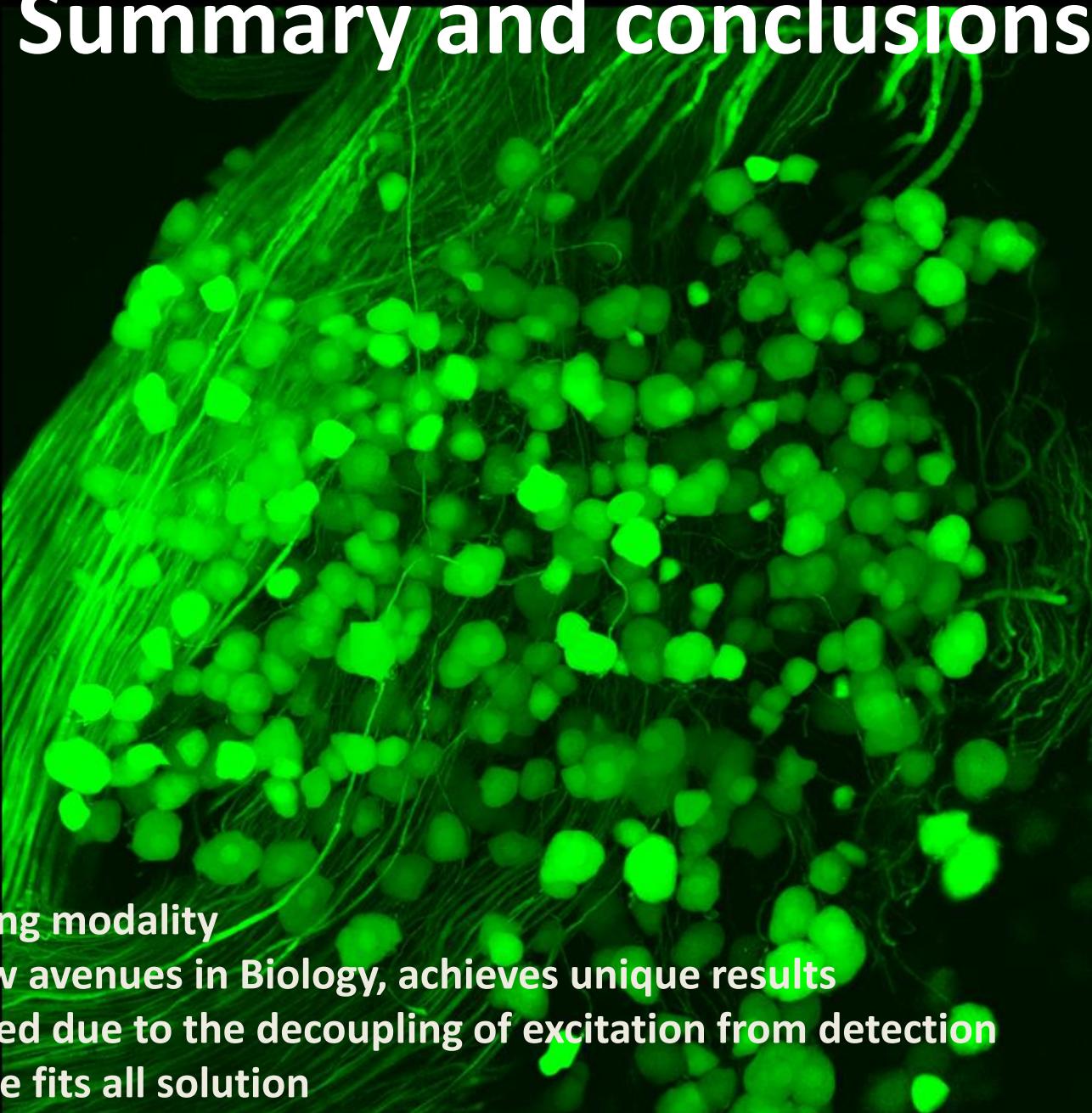
Movie 8. T cell and its target cell. Two-color volume rendering seen from two different orientations of the interaction of a T cell expressing mEmerald-Lifeact (orange) with a target cell expressing a plasma membrane marker fused to TagRFP (blue) over 430 time points at 1.3-s intervals. The target cell has been made invisible in the views in the right column (compare with Fig. 5, A to C, for a second example).

Cell-matrix interactions, dithered light-sheet



- 4D migration of cells in a 3D meshwork of ECM proteins.
- Fast moving neutrophils HL-60 cells (10 $\mu\text{m/sec}$), mCherry-utrophin (component of the cytoskeleton)
- Collagen displacement

Summary and conclusions



- Fast imaging modality
- Opens new avenues in Biology, achieves unique results
- Complicated due to the decoupling of excitation from detection
- No one size fits all solution
- How to manage the data ?