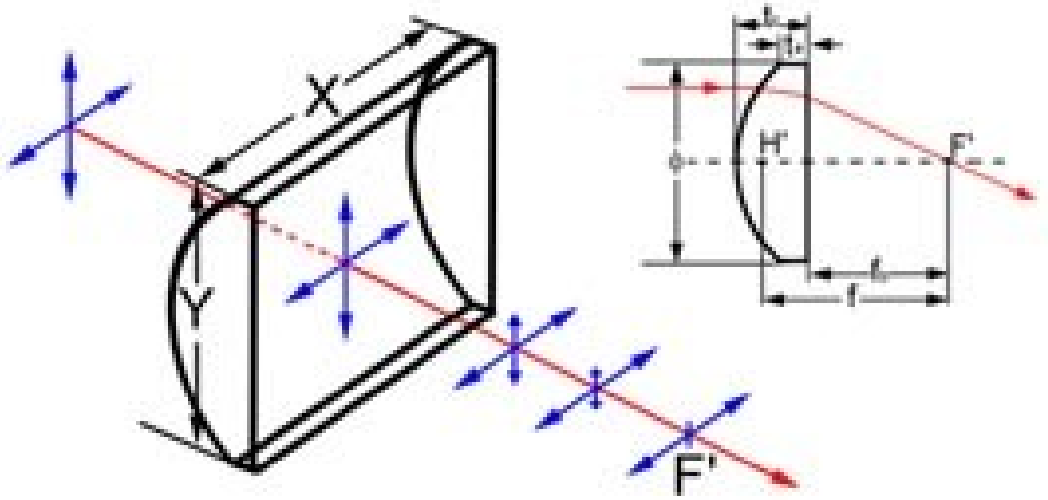
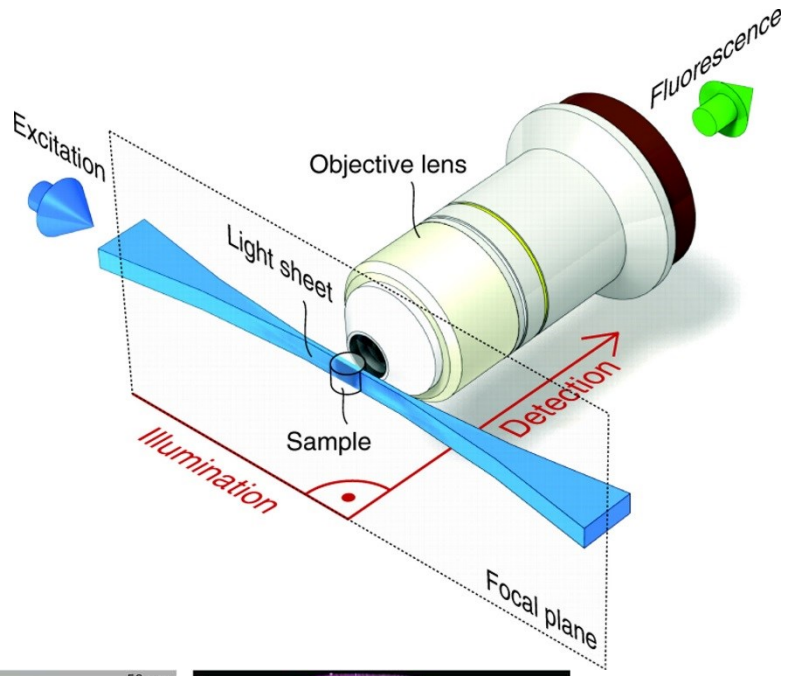
More fancy SPIM, Even fancier SPIM

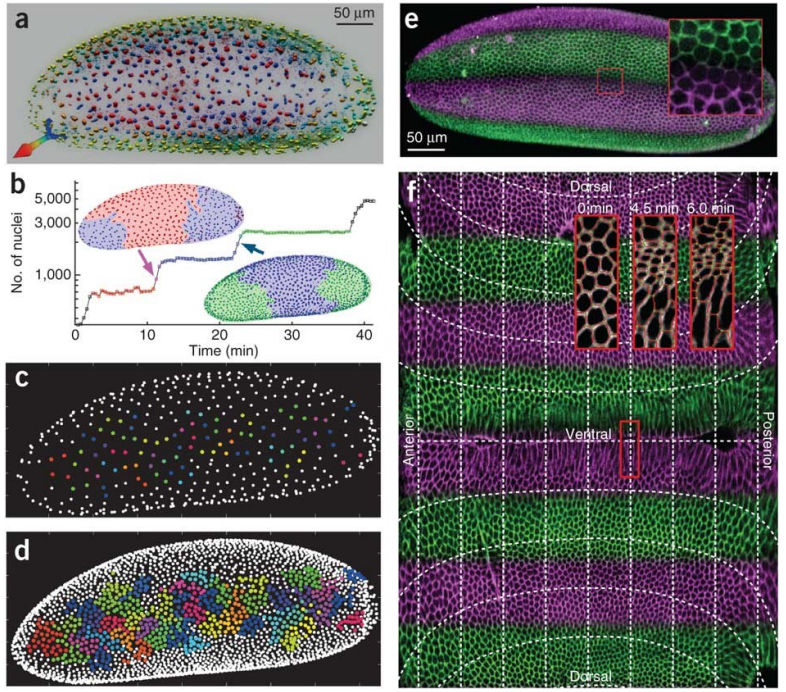
- Last class
  - Light sheet microscopy
  - Fancy SPIM (iSPIM, dSPIM, etc…)
- This class
  - Multi camera SPIM
  - SIM SPIM
  - Bessels





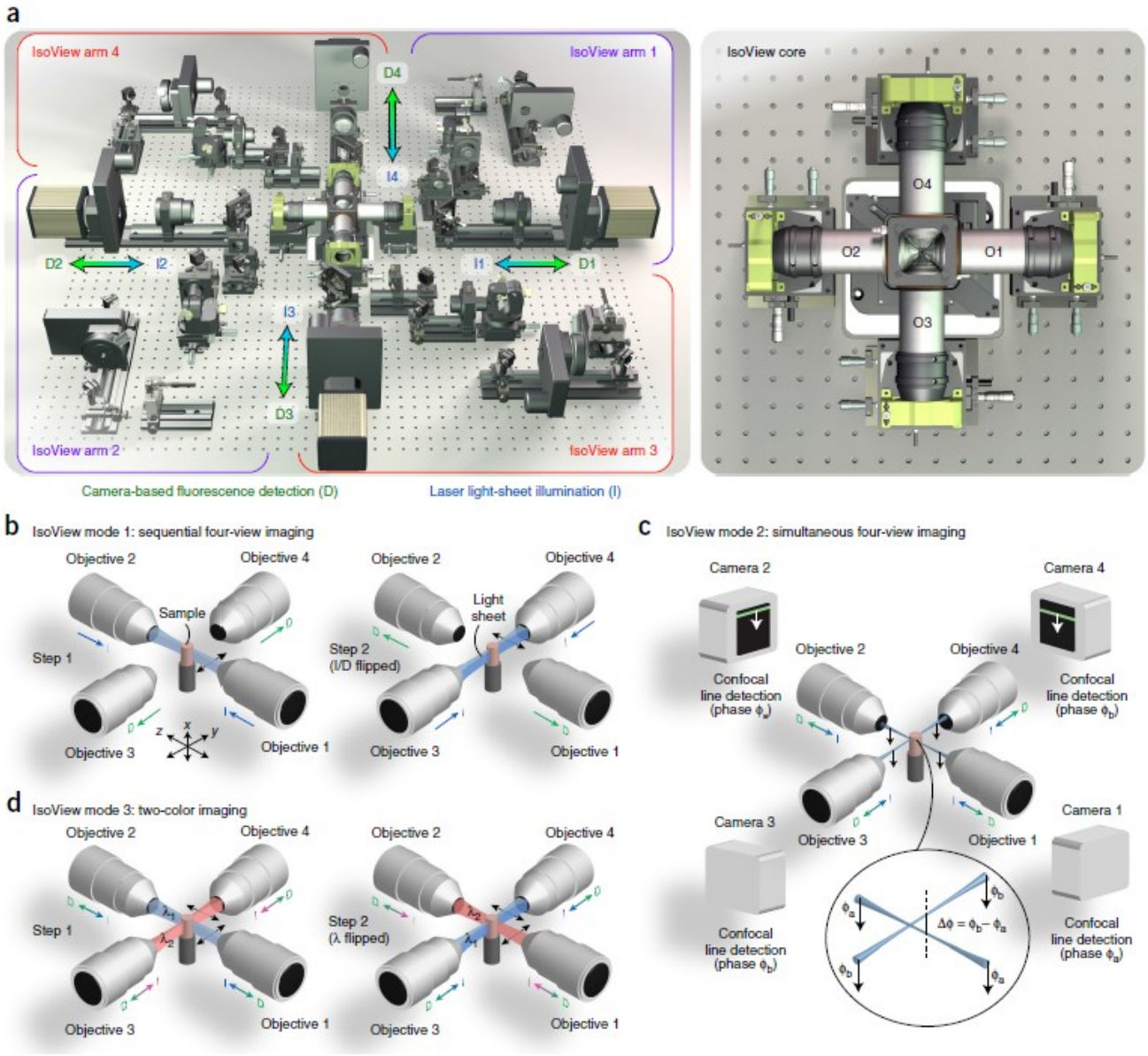
$$d_{x,y} = \frac{\lambda_{em}}{2 * NA}$$

$$d_z = \left(\frac{2 * NA}{\lambda_{ex}} + \frac{n(1 - cos\theta}{\lambda_{em}}\right)^{-1}$$

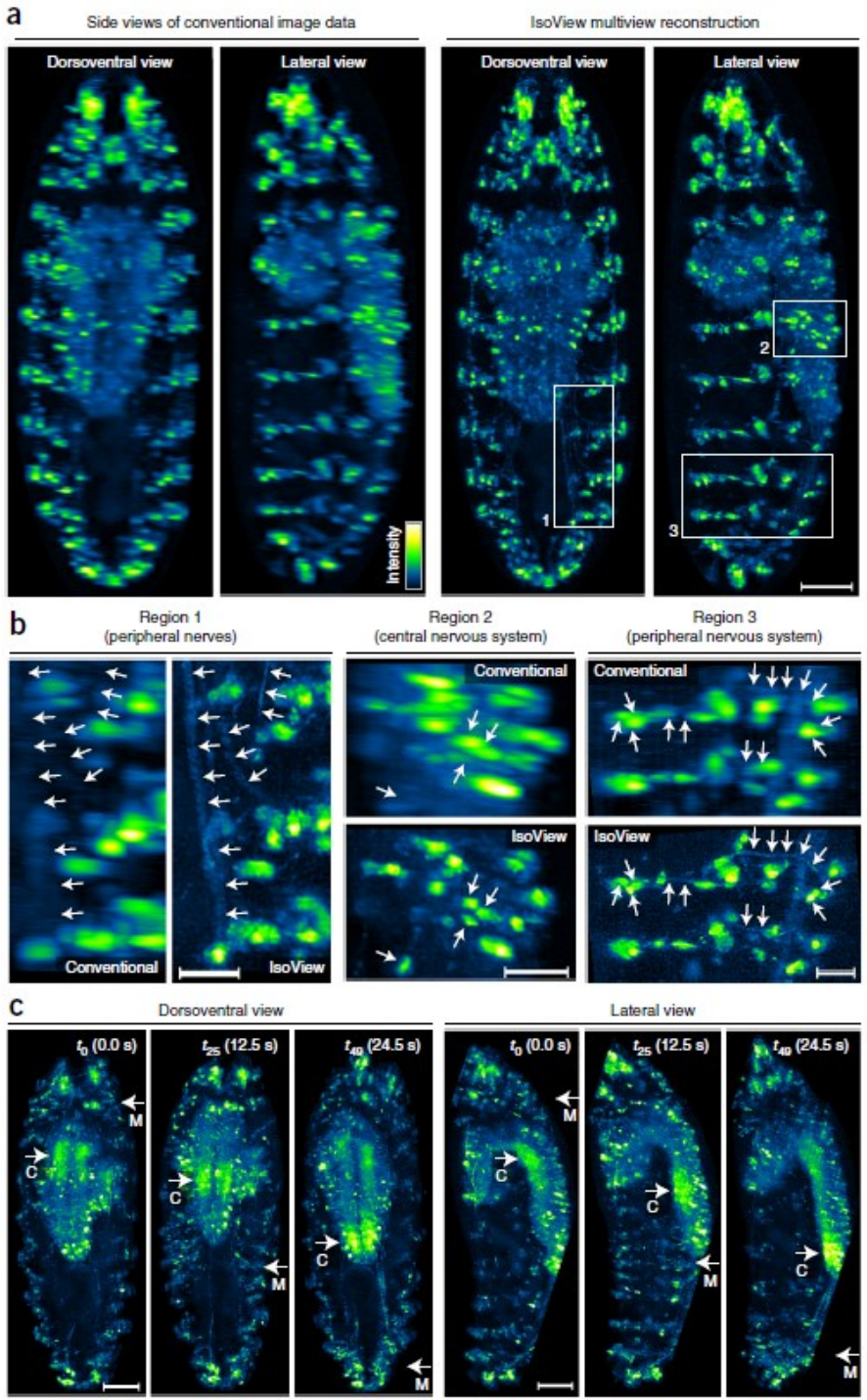


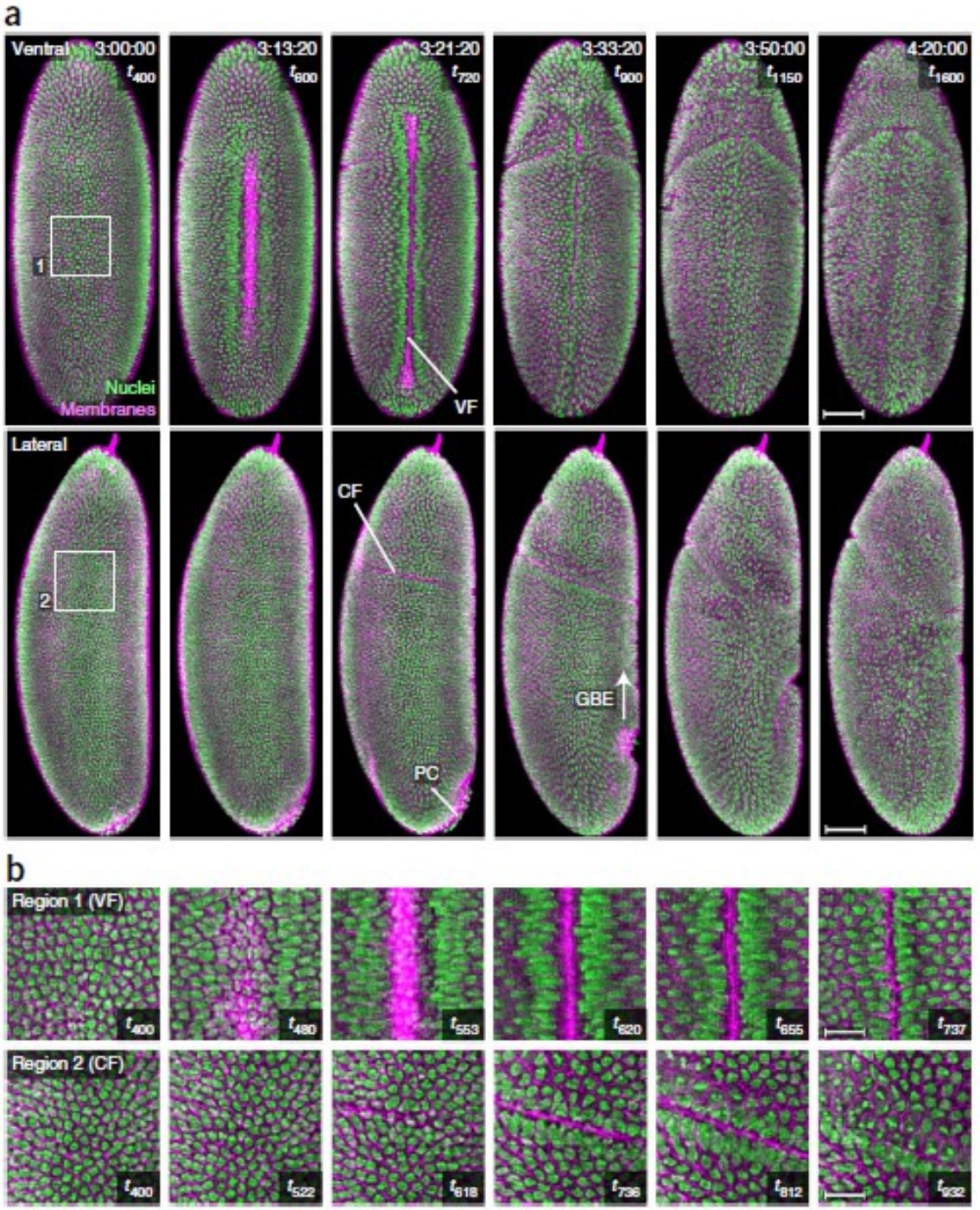
IsoView SPIM – 4x the cameras, 16x the alignement

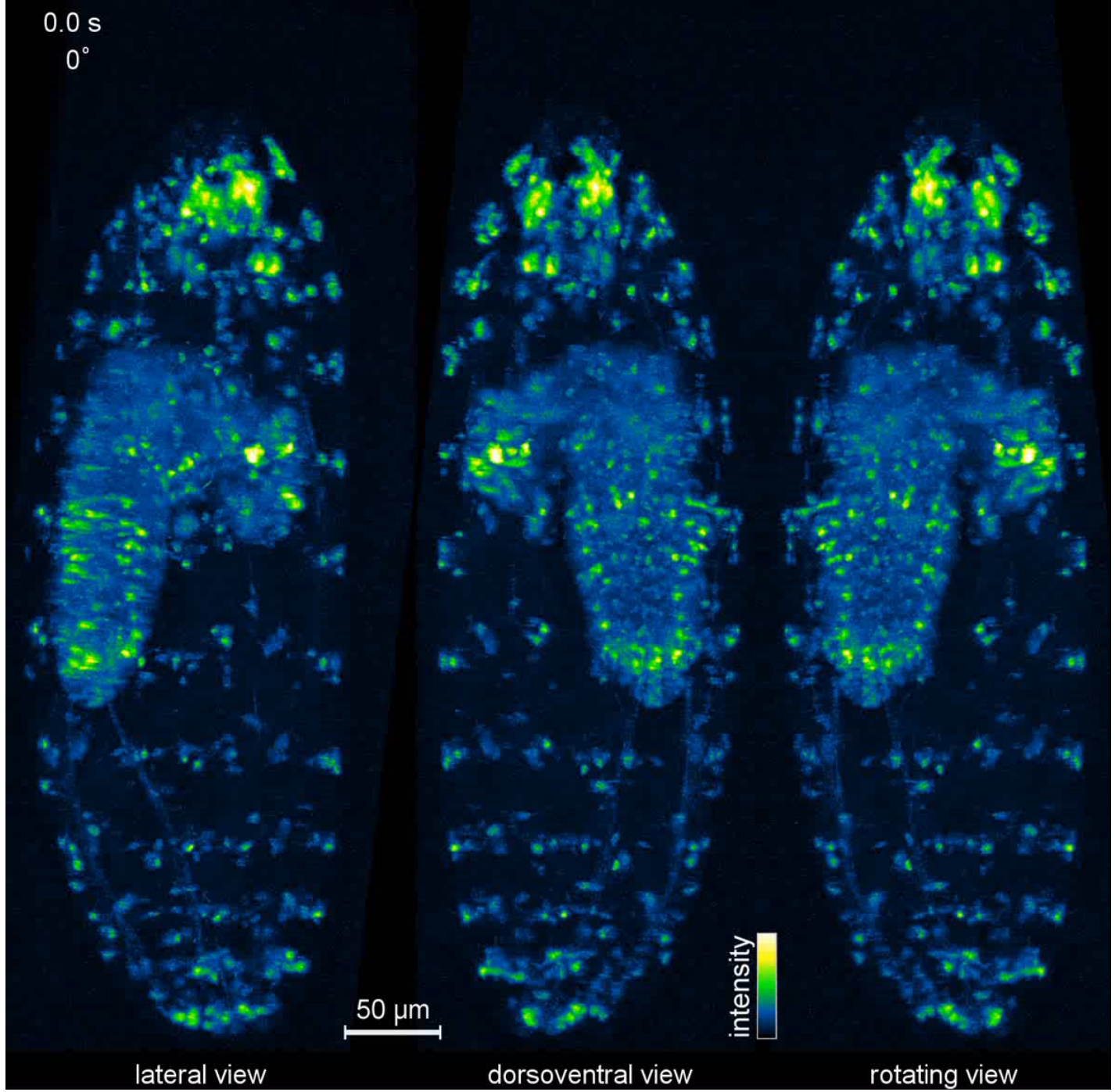
- IsoView – use lasers and cameras across all 4 axes
- Isotropic resolution in all dimensions
- Extremely rapid sectioning of thick specimens
- 2Hz imaging of drosophila larva at 1 μm resolution
- Multicolor imaging at .25 Hz
- 400 x larger specimen sizes



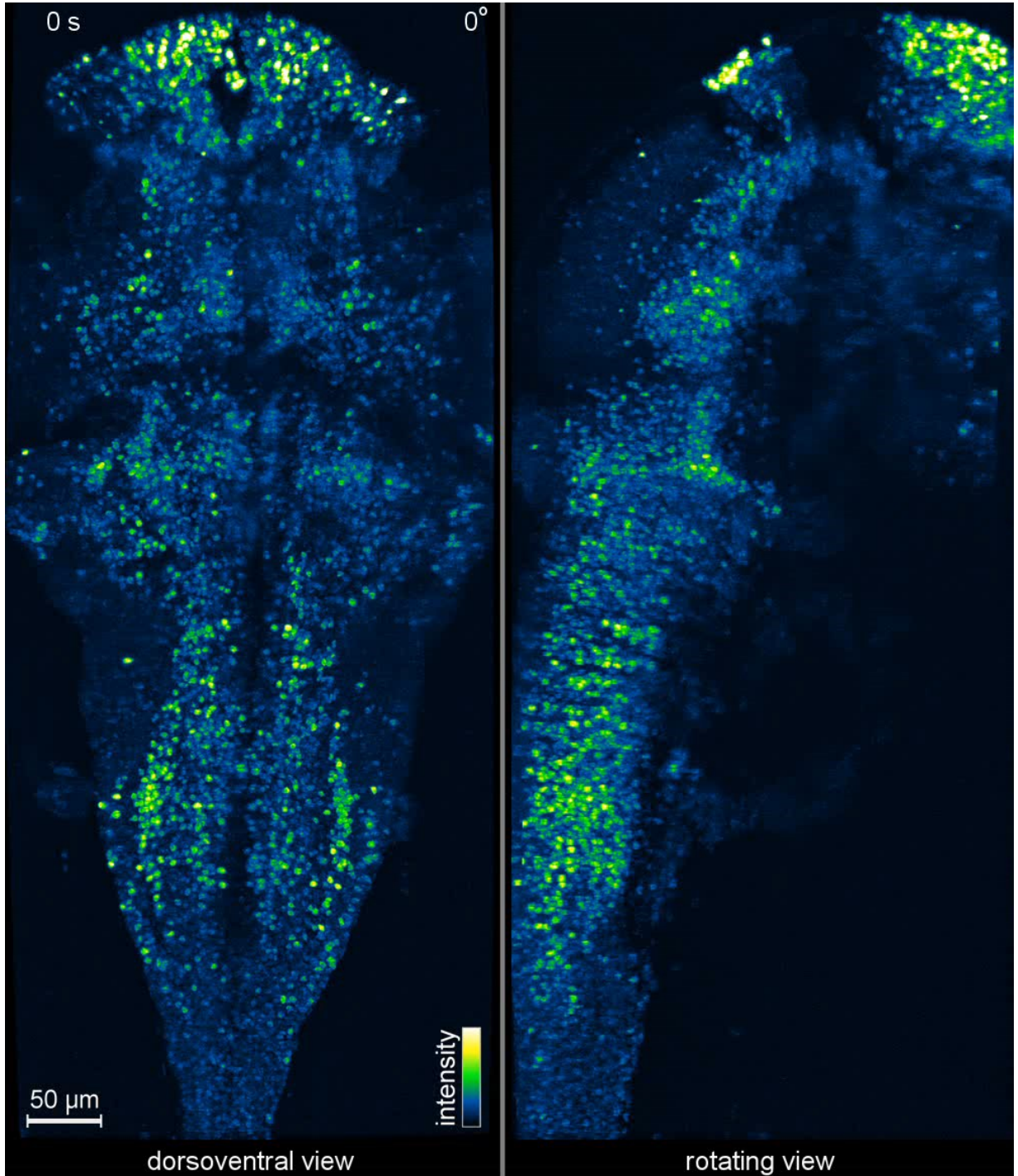
IsoView Improvements





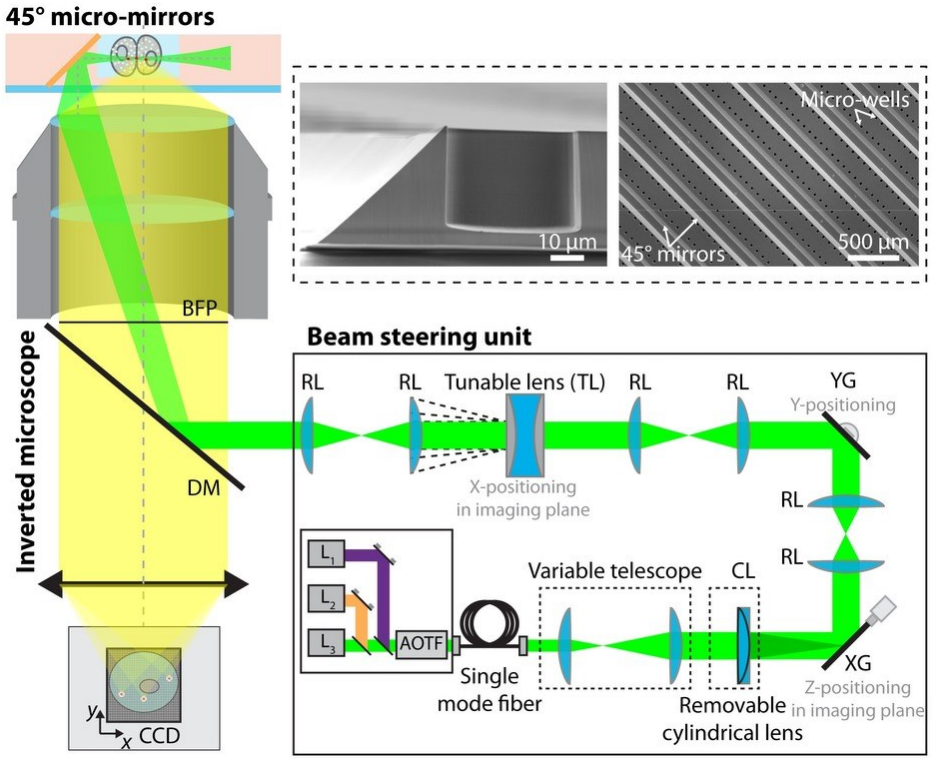

0.0 s
0°
50 µm
intensity
lateral view
dorsoventral view
rotating view

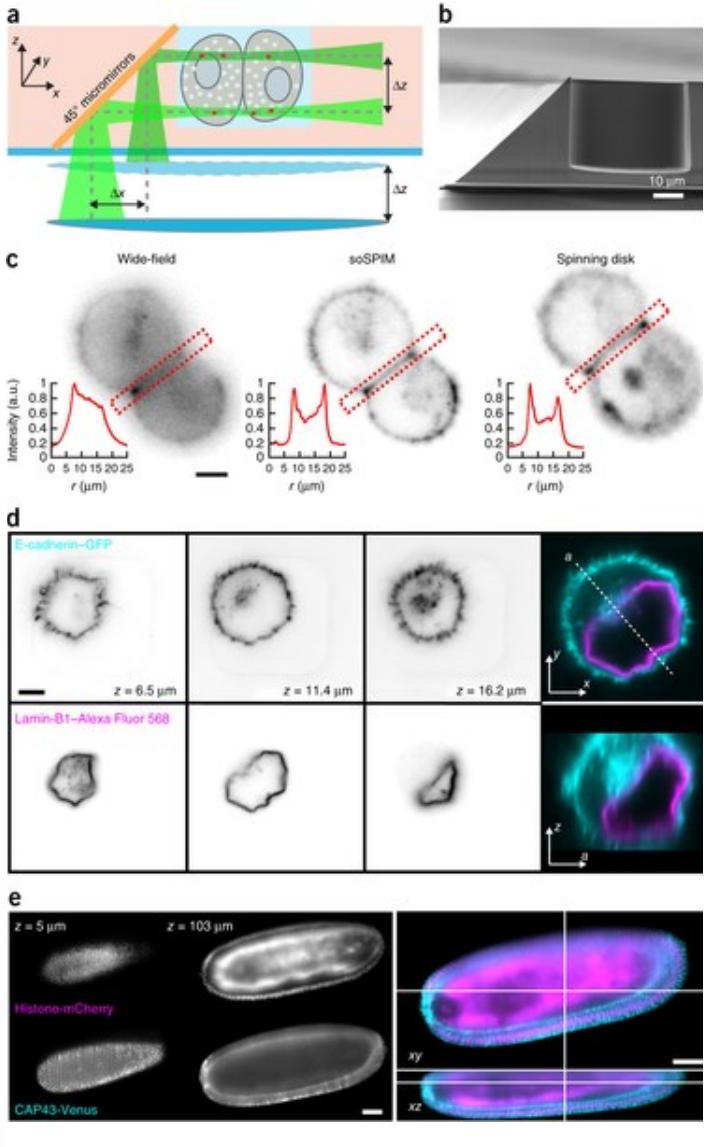

03:00:00
0°
nuclei
membranes
50 µm
ventral view
dorsal view
lateral-left view
lateral-right view
rotating view

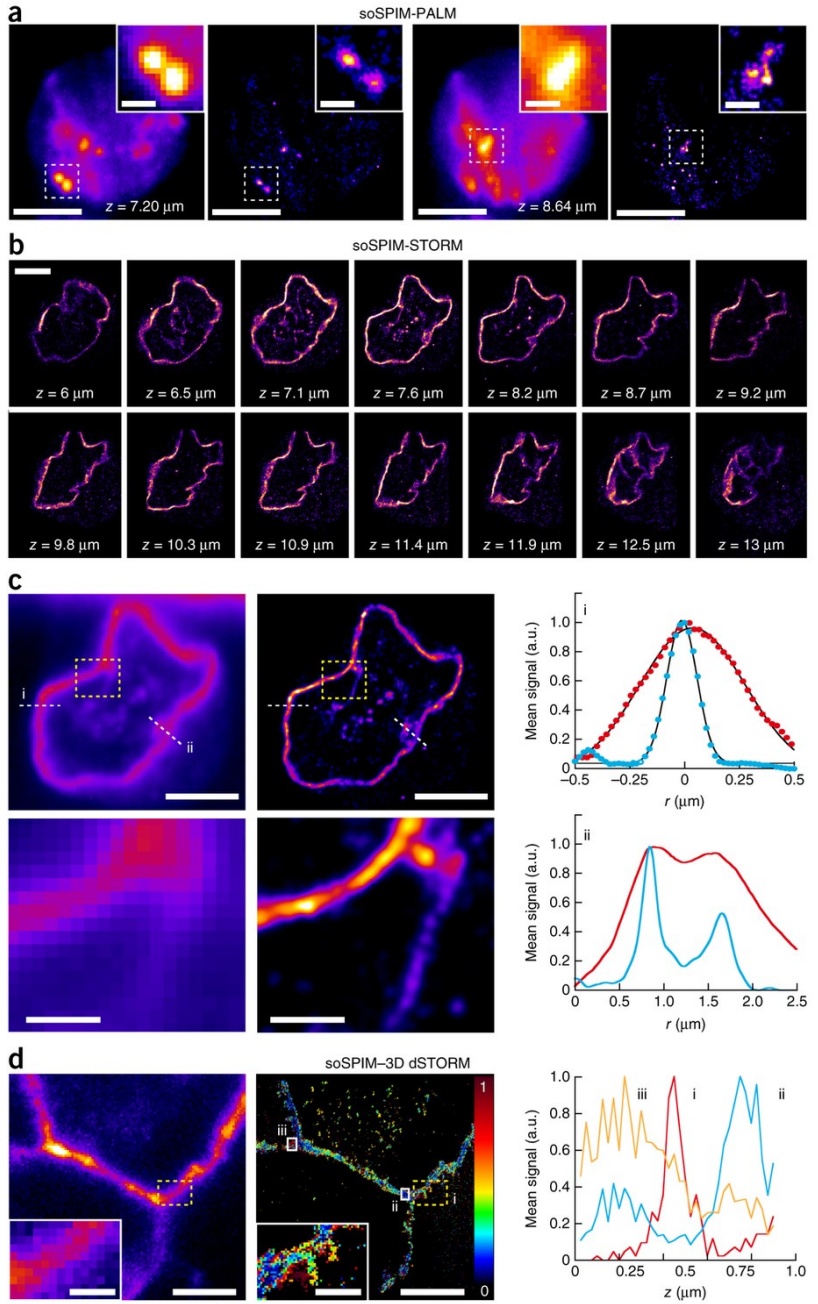

0 s
0°
50 µm
intensity
dorsoventral view
rotating view

SPIM STORM

- Use SPIM to confine in 3rd dimension
- Use improved signal to localize single molecules

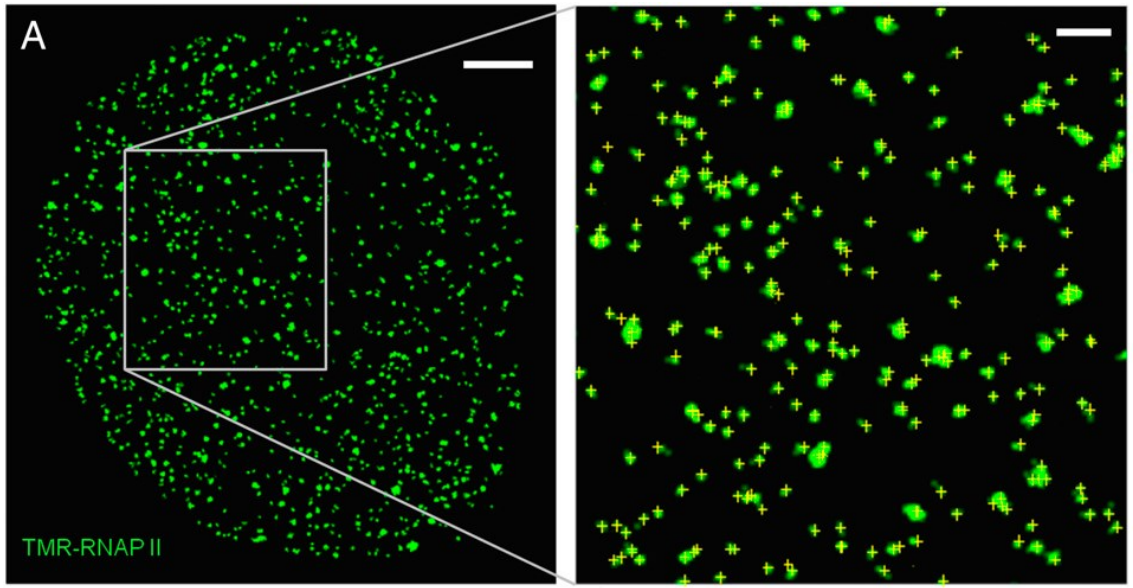


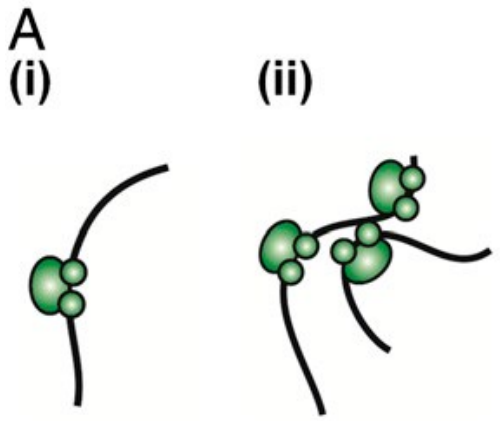


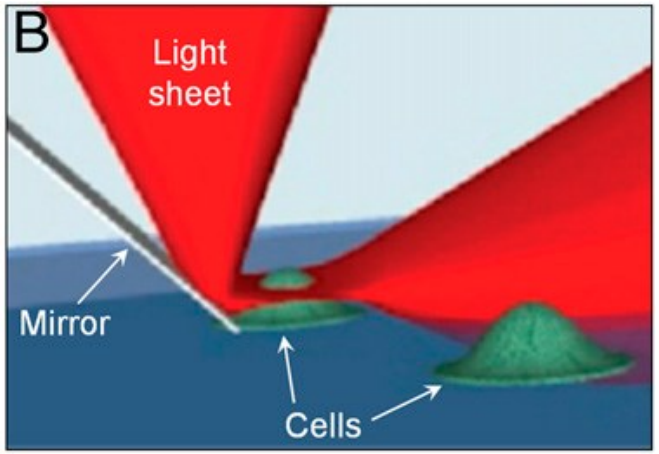


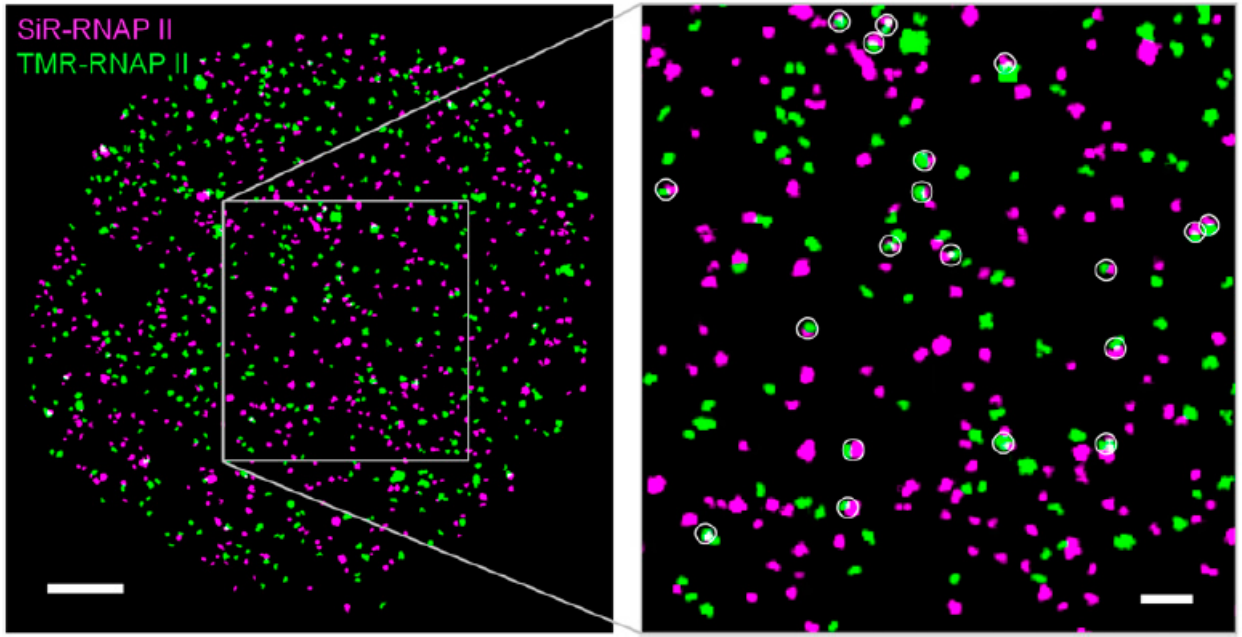
SPIM STORM 2

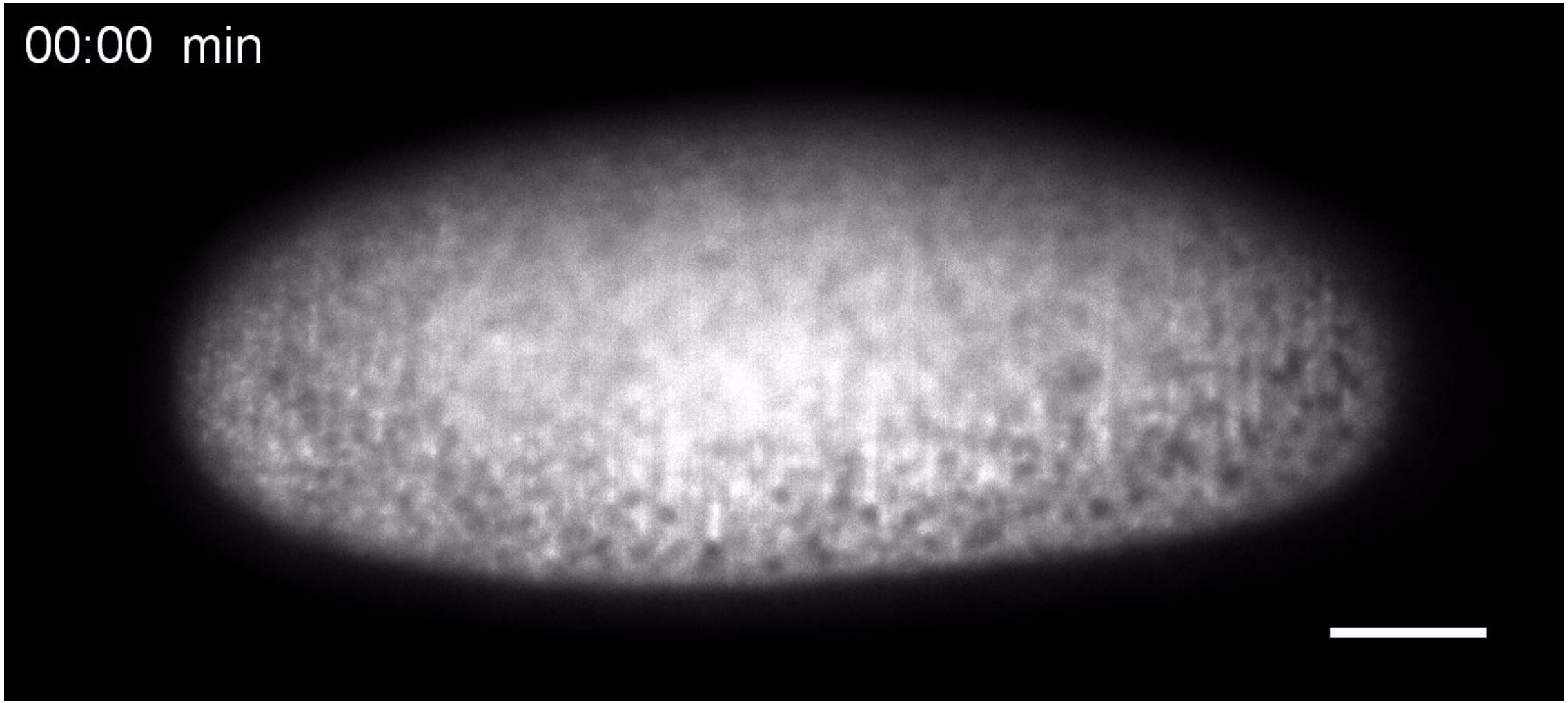
- Use an AFM tip as mirror
- Image single copies of RNA polymerase





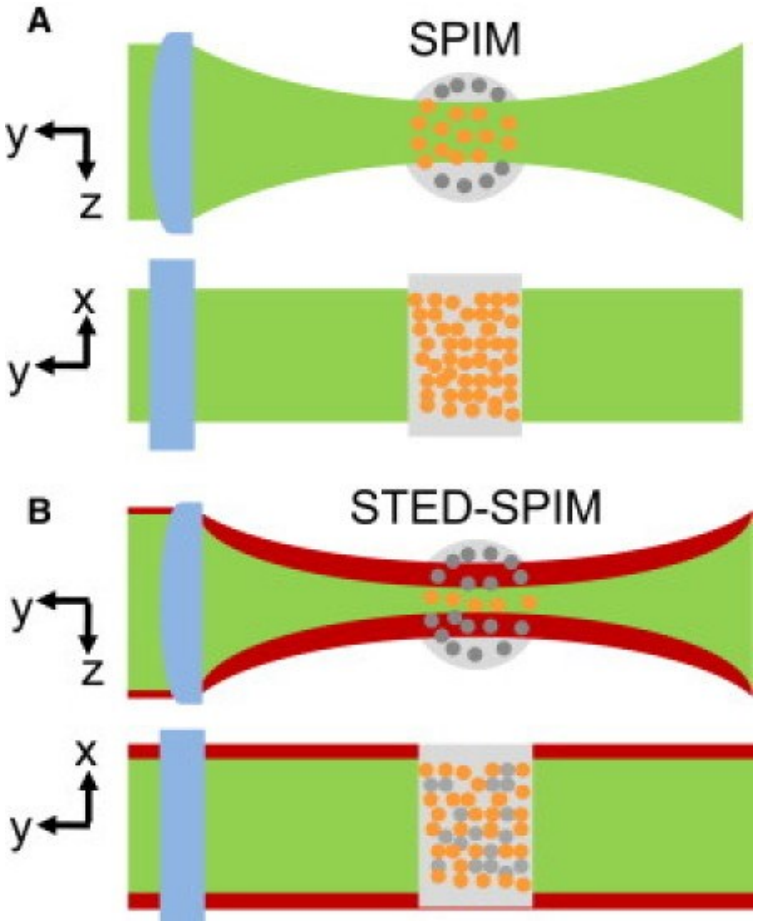


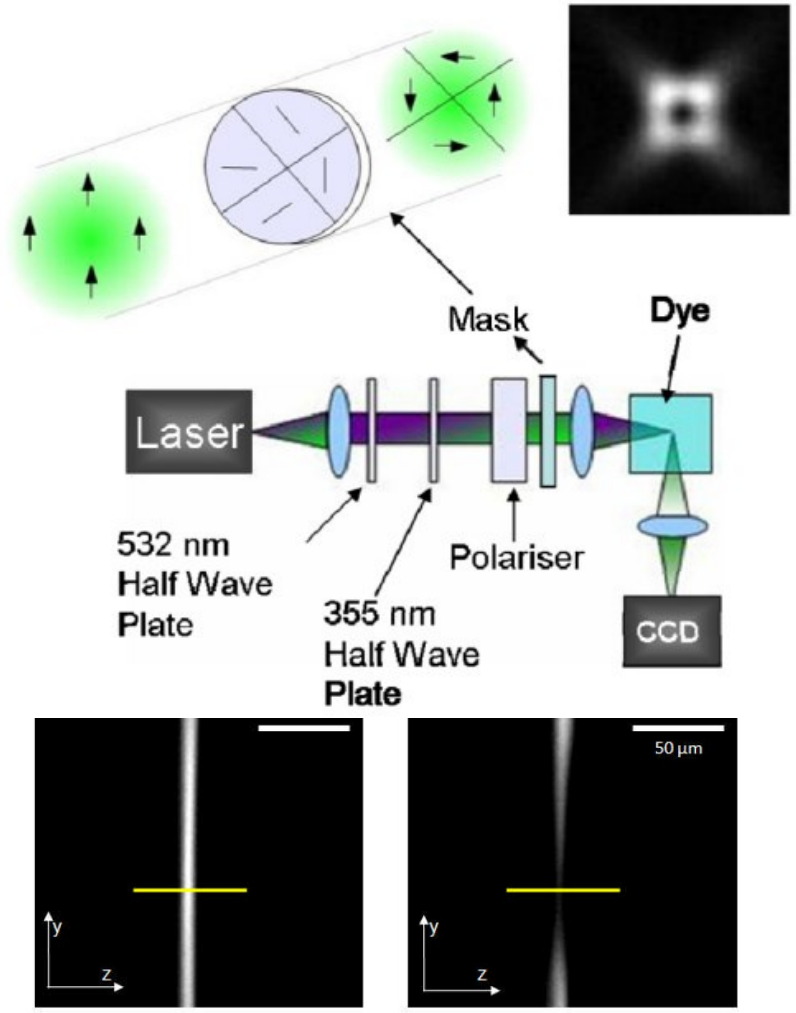



00:00 min

SPIM STED

- Pattern STED beam around axial dimensions of SPIM beam
- Illuminate thinner axial slice
- Improved axial resolution
- Only a few publications, probabl not really useful





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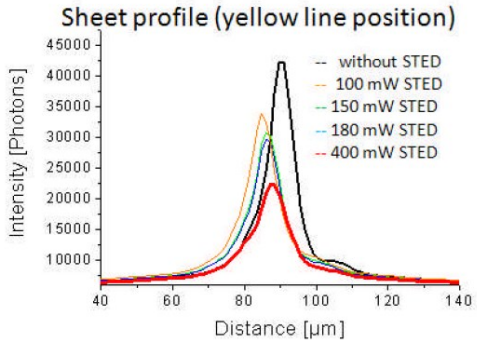
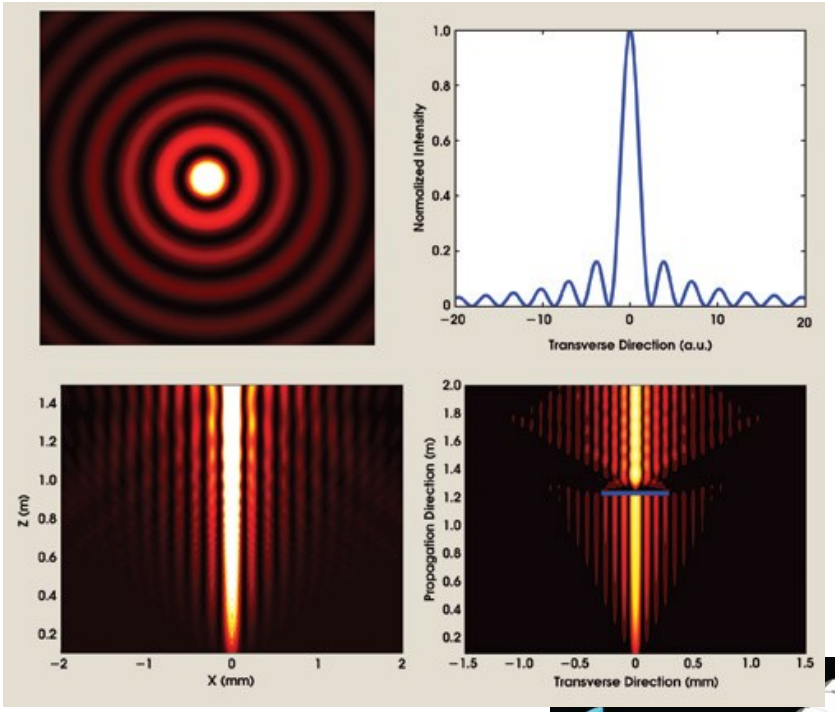


Figure S6.

SPIM SIM, with a twist

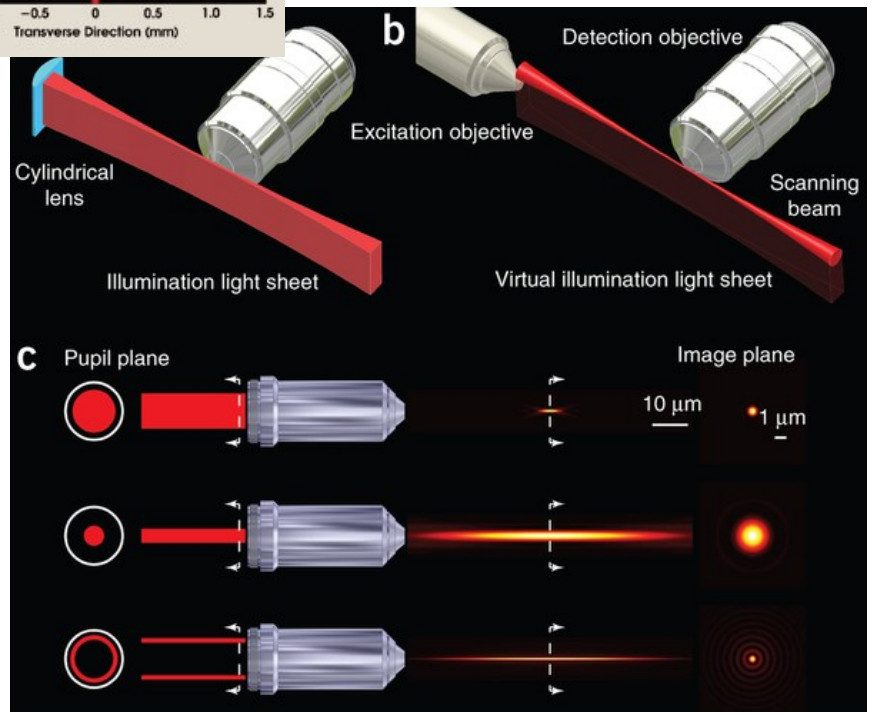
Bessel beams

- Use pencil rather than sheet
- Bessel beams are perfectly non-diffracting and self reconstructing
- Can maintain a pencil of illumination across very long distances
- Like one section of the sheet
- Formed by imaging annulus at the back aperture of the objective
- Larger side lobes than Gaussian beam



Infinitely thin annulus will create perfect Bessel beam

$$d = \frac{\lambda_{ex}}{2NA_{ex}}$$



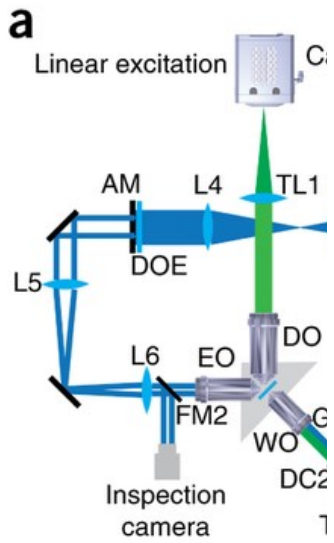
Two photon Bessel imaging

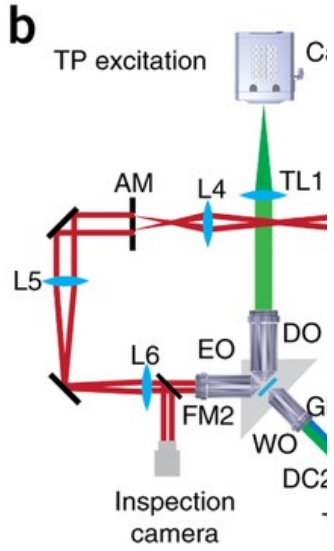
- Bessel beam excitation enforces isotropic resolution, but side lobes remain
- Since side lobes can cause a large problem, we can get rid of them by requiring 2 photon excitation
- Lower intensity side lobes don't have enough energy to excite fluorescence
- Typical limitations of 2 photon – very expensive and hard to image 2 colors

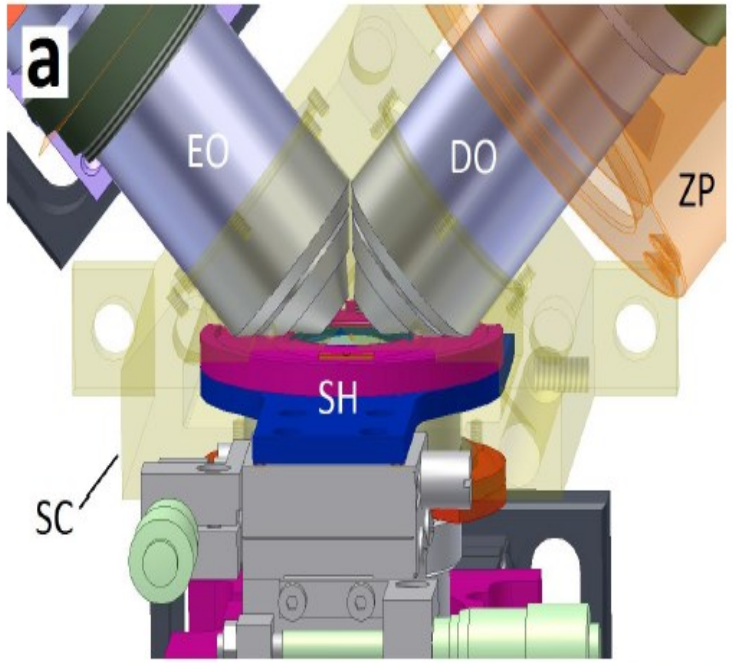
Operation modes	Bessel beam plane illumination	TP Bessel beam plane illumination
Illumination pattern (*xz* cross-section)		
Typical spatial resolution	*x* ~230 nm	*x* ~230 nm
	y ~230 nm	*y* ~230 nm
	z ~600 nm	*z* ~450 nm
Optical sectioning capability	Fair	Excellent
Typical imaging speed	~40 planes/s	~40 planes/s
Multicolor imaging	Yes	No
Fluorophore brightness requirement	Low	High
Photodamage	Low	Low

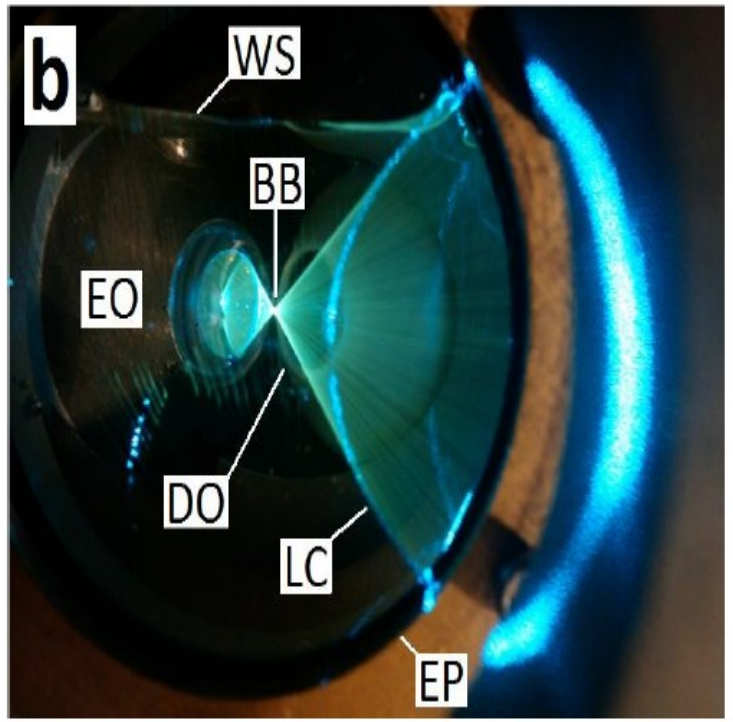
Bessel microscope setup

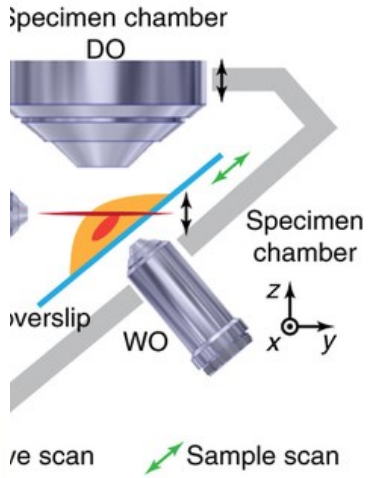
- Not easy to align
- No commercial vendor of Bessel scopes, although Zeiss has licensed the IP
- Even placing the sample in the chamber becomes a challenge

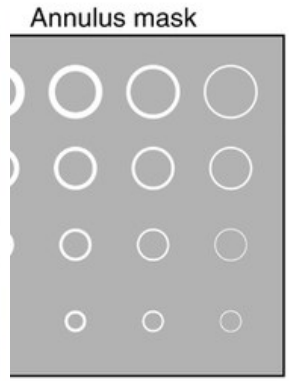






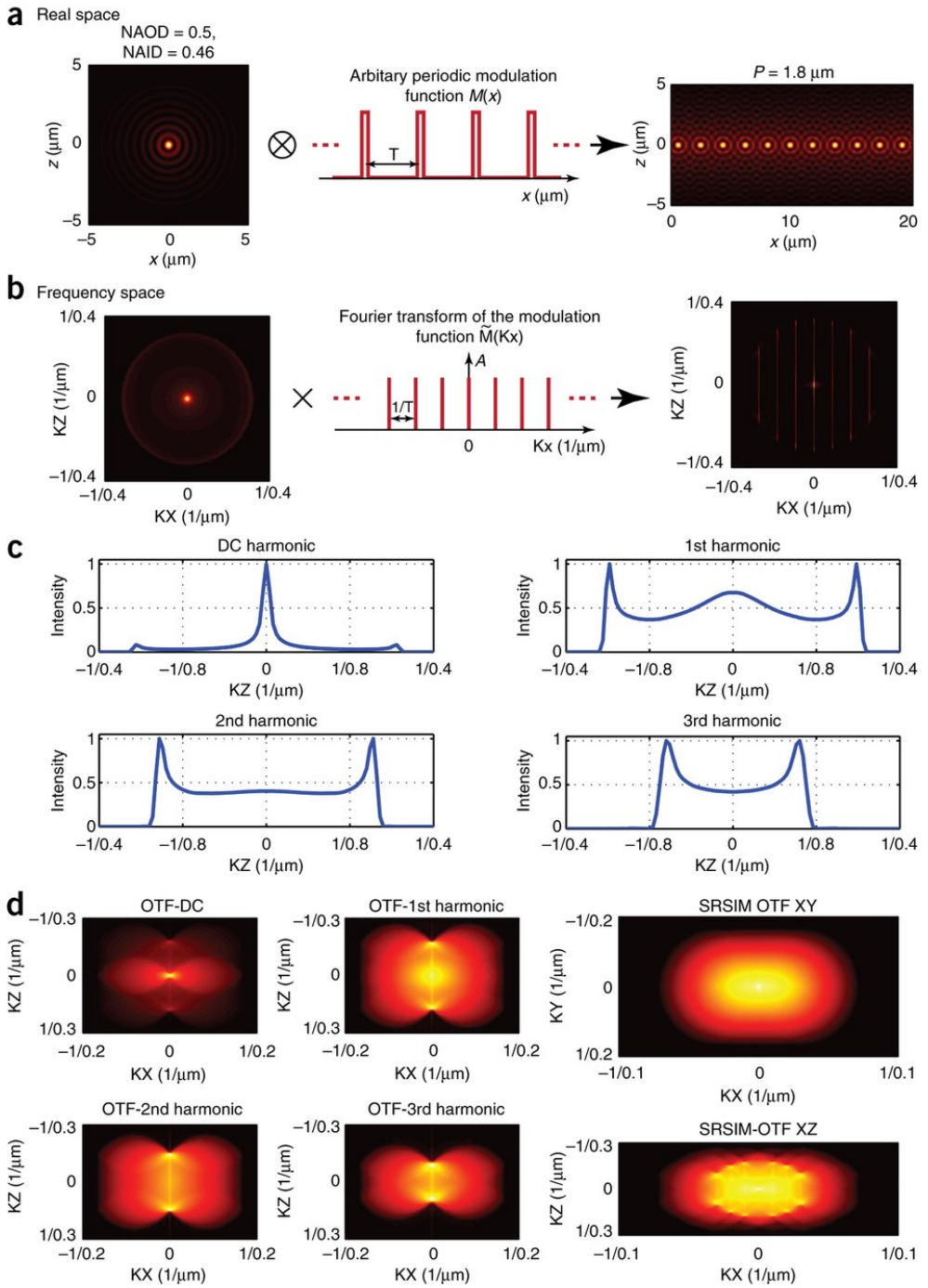






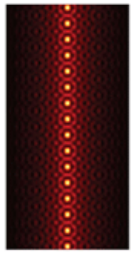
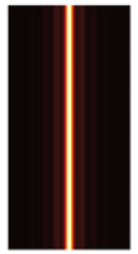
SIM SPIM (with Bessel beams)

- The Bessel beam enables periodicity
- Rather than scanning through sample rapidly, illuminate discrete spots in time
- You can illuminate an effective grating
- Reconstruct as per normal SIM experiment
- Unlike normal SIM, typically need 7-9 illuminations per sample plane
- Can still work faster than normal SIM imaging

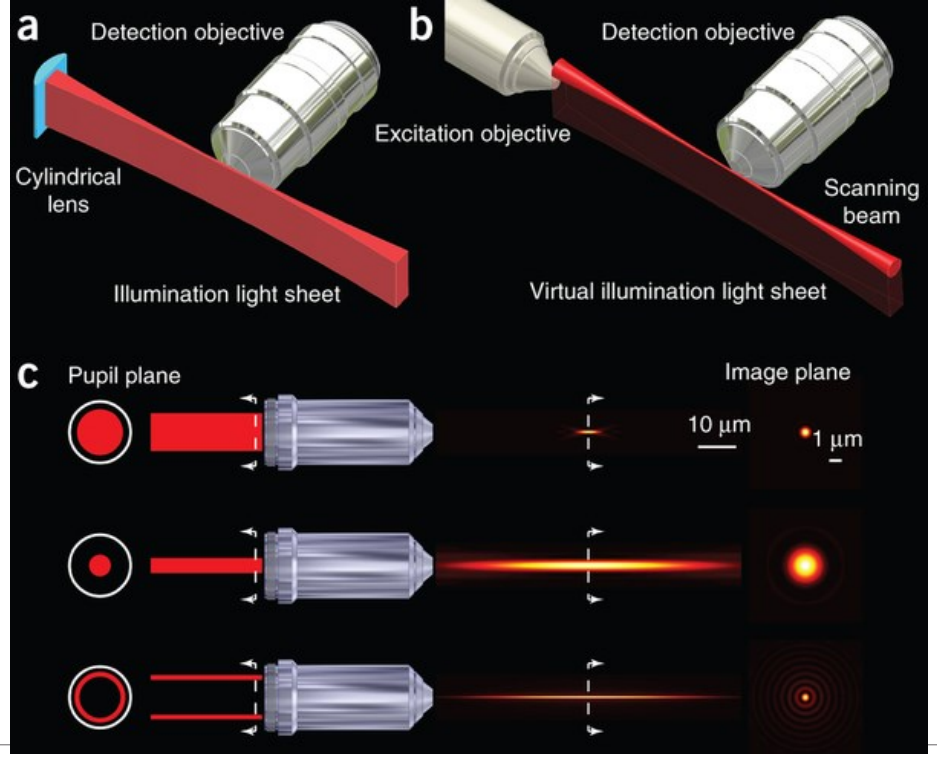


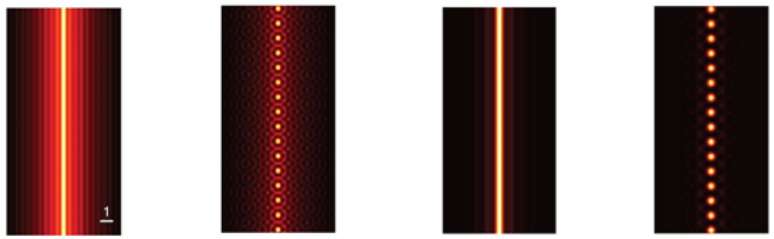
SIM SPIM

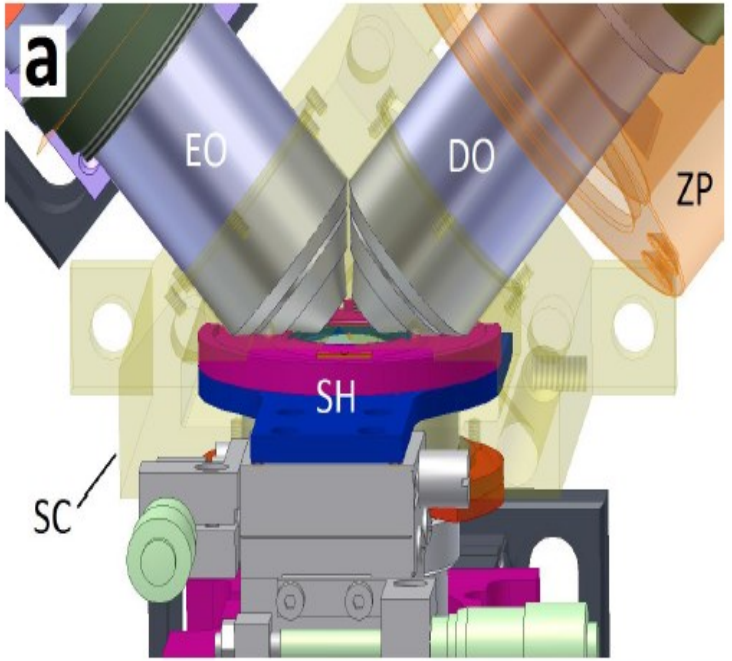
- Also possible to use high intensities to achieve SSIM
- Just like normal SSIM, have to take more individual frames per SIM frame
- Also very easy to do 2 photon SIM SPIM
- Tradeoffs in time, photobleaching, and resolutions

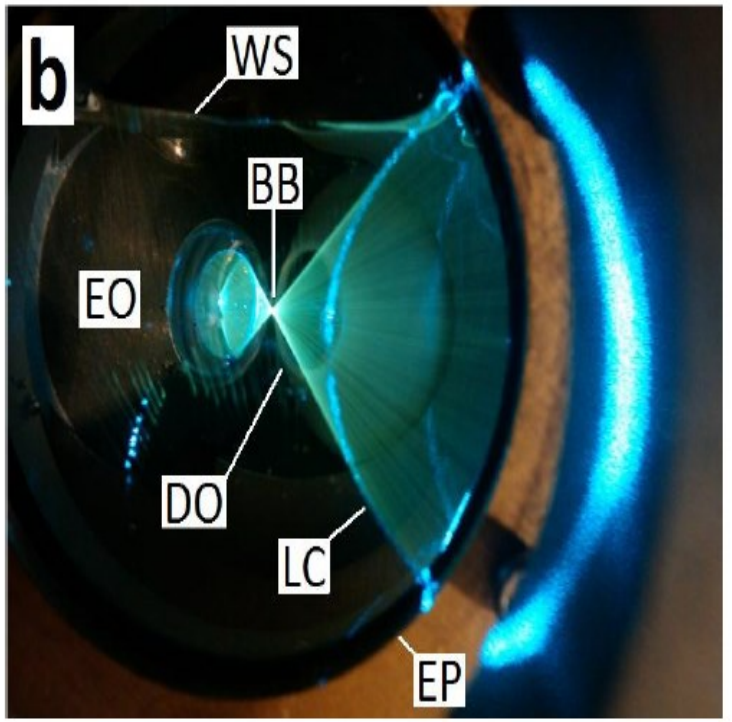
Operation modes	Bessel beam plane illumination	Bessel SIM	TP Bessel beam plane illumination	TP Bessel SIM
Illumination pattern (*xz* cross-section)				
Typical spatial resolution	*x* ~230 nm *y* ~230 nm *z* ~600 nm	*x* ~180 nm *y* ~230 nm *z* ~350 nm	*x* ~230 nm *y* ~230 nm *z* ~450 nm	*x* ~180 nm *y* ~230 nm *z* ~450 nm
Optical sectioning capability	Fair	Good	Excellent	Excellent
Typical imaging speed	~40 planes/s	10–20 planes/s	~40 planes/s	10–20 planes/s
Multicolor imaging	Yes	Yes	No	No
Fluorophore brightness requirement	Low	Low	High	High
Photodamage	Low	Fair	Low	High

Imaging Modality	Theoretical FWHM	FWHM from 100 nm bead	Averaged FWHM from 40 microtubules
widefield (0.8 NA)	1.76 μm	1.85 μm	--
point scan confocal (LSM 510, 1.2 NA)	0.44 μm	0.68 μm	(1.41 ± 0.13) μm
line scan confocal (LSM 5 LIVE, 1.2 NA)	0.44 μm	0.76 μm	(1.88 ± 0.23) μm
DSLM	1.02 μm	1.20 μm	(1.42 ± 0.16) μm
Bessel swept sheet	0.47 μm	1.02 μm	(2.12 ± 0.21) μm
Bessel TPE sheet	0.45 μm	0.49 μm	(0.57 ± 0.09) μm
Bessel TPE SI, 5 phases	0.47 μm	0.53 μm	--
single harmonic Bessel SI (3 phases)	0.26 μm	0.27 μm	(0.37 ± 0.05) μm
multi-harmonic Bessel SI (9 phases)	0.27 μm	0.29 μm	(0.47 ± 0.10) μm

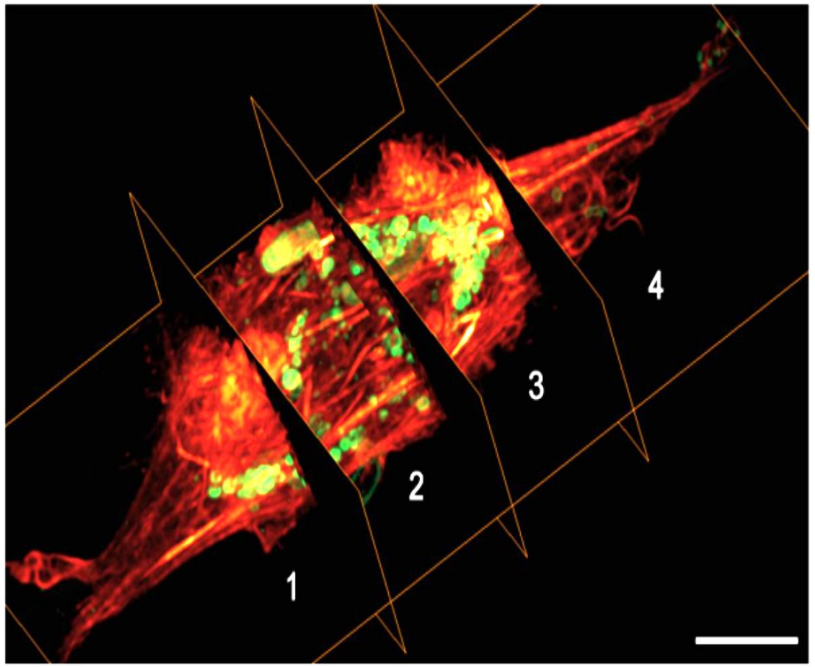


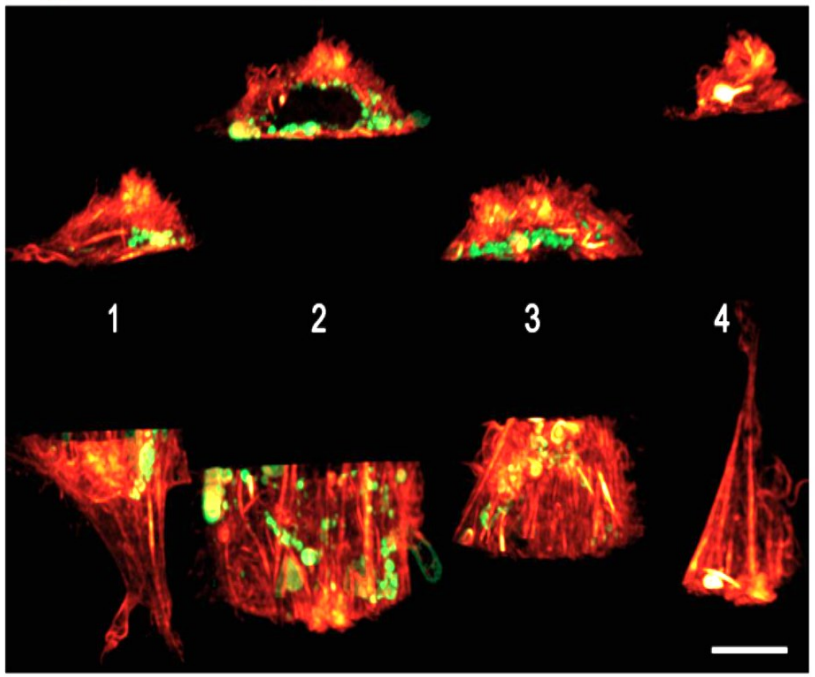
Operation modes	Bessel beam plane illumination	Bessel SIM	TP Bessel beam plane illumination	TP Bessel SIM
Illumination pattern (*xz* cross-section)				
Typical spatial resolution	*x* ~230 nm *y* ~230 nm *z* ~600 nm	*x* ~180 nm *y* ~230 nm *z* ~350 nm	*x* ~230 nm *y* ~230 nm *z* ~450 nm	*x* ~180 nm *y* ~230 nm *z* ~450 nm
Optical sectioning capability	Fair	Good	Excellent	Excellent
Typical imaging speed	~40 planes/s	10–20 planes/s	~40 planes/s	10–20 planes/s
Multicolor imaging	Yes	Yes	No	No
Fluorophore brightness requirement	Low	Low	High	High
Photodamage	Low	Fair	Low	High

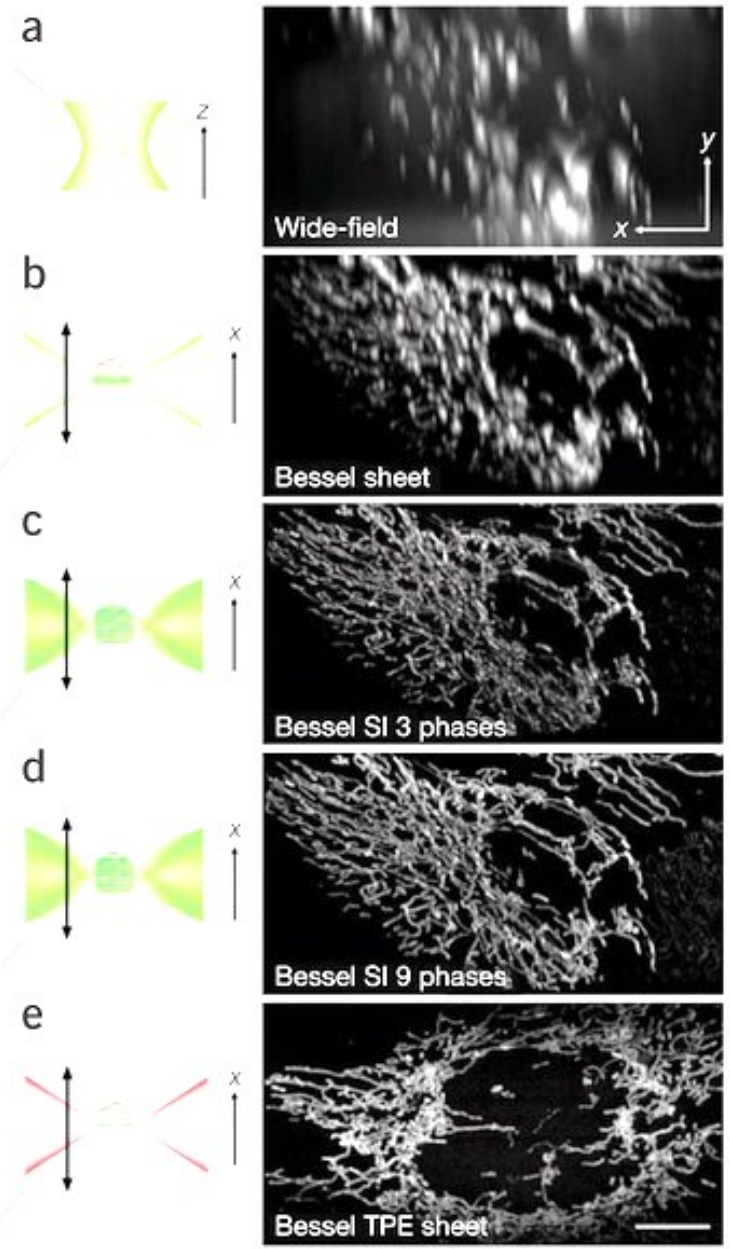


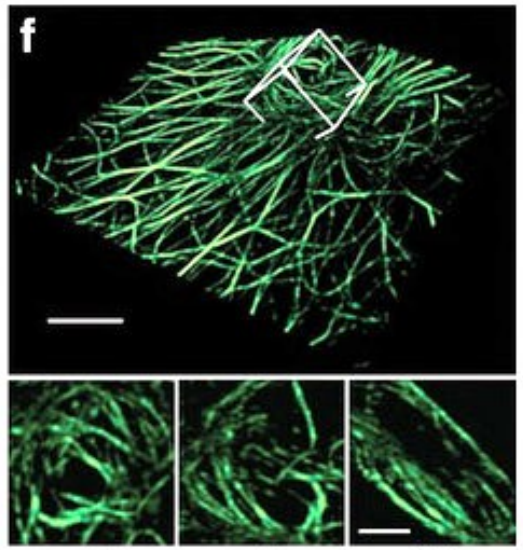


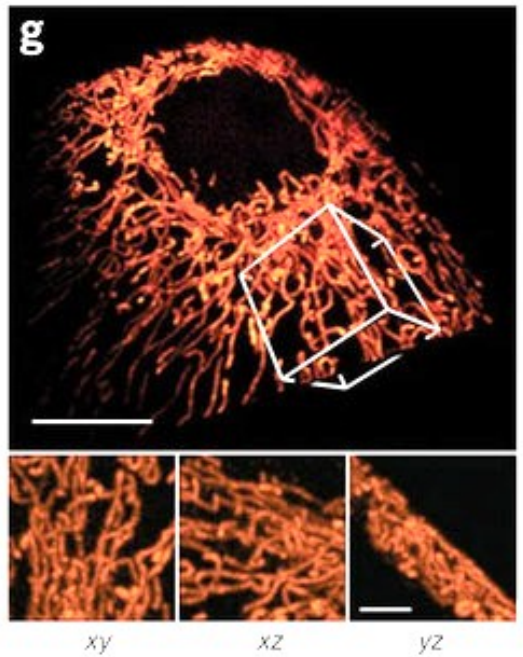
Two color HeLa cell with actin and connexin

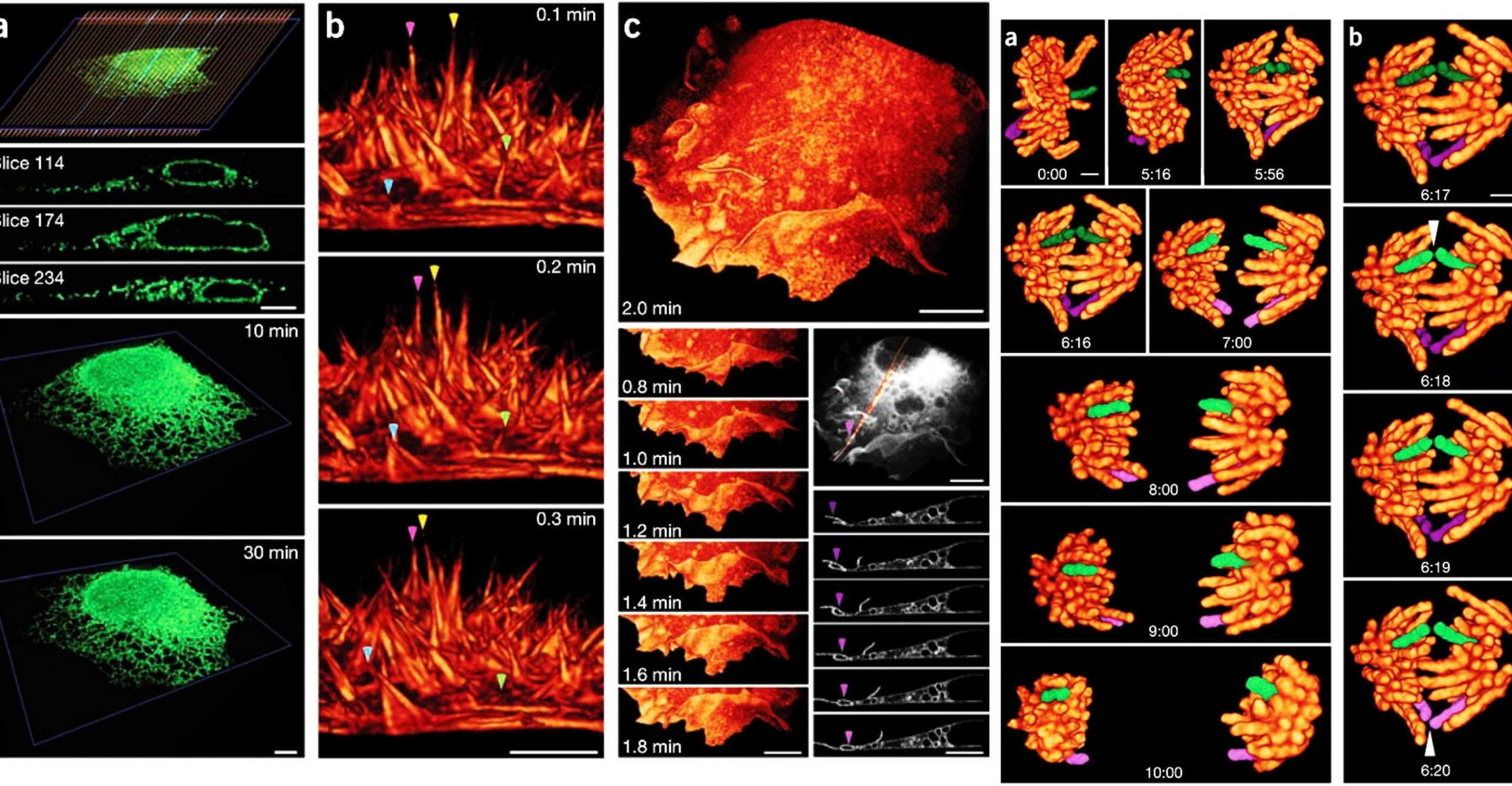






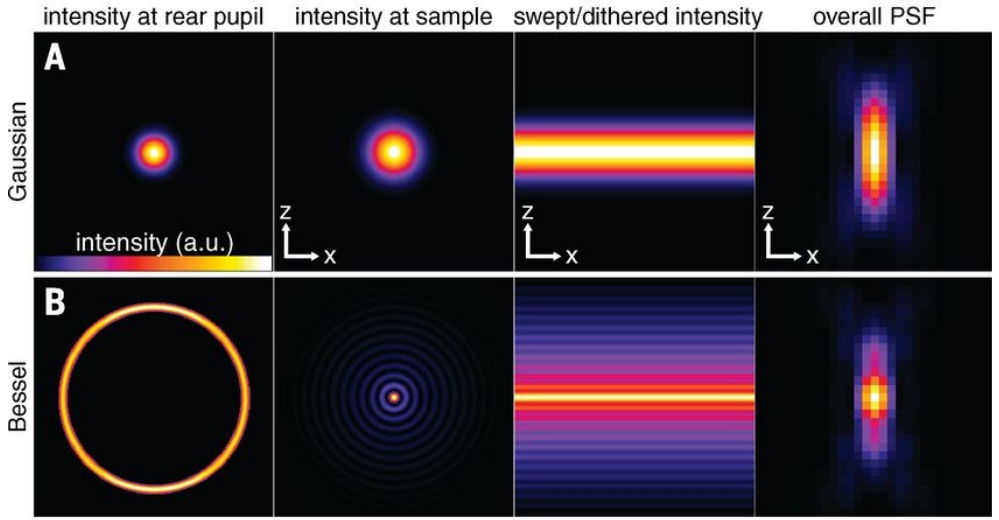





a
lice 114
lice 174
lice 234
10 min
30 min
b
0.1 min
0.2 min
0.3 min
c
2.0 min
0.8 min
1.0 min
1.2 min
1.4 min
1.6 min
1.8 min
a
0:00
5:16
5:56
6:16
7:00
8:00
9:00
10:00
b
6:17
6:18
6:19
6:20

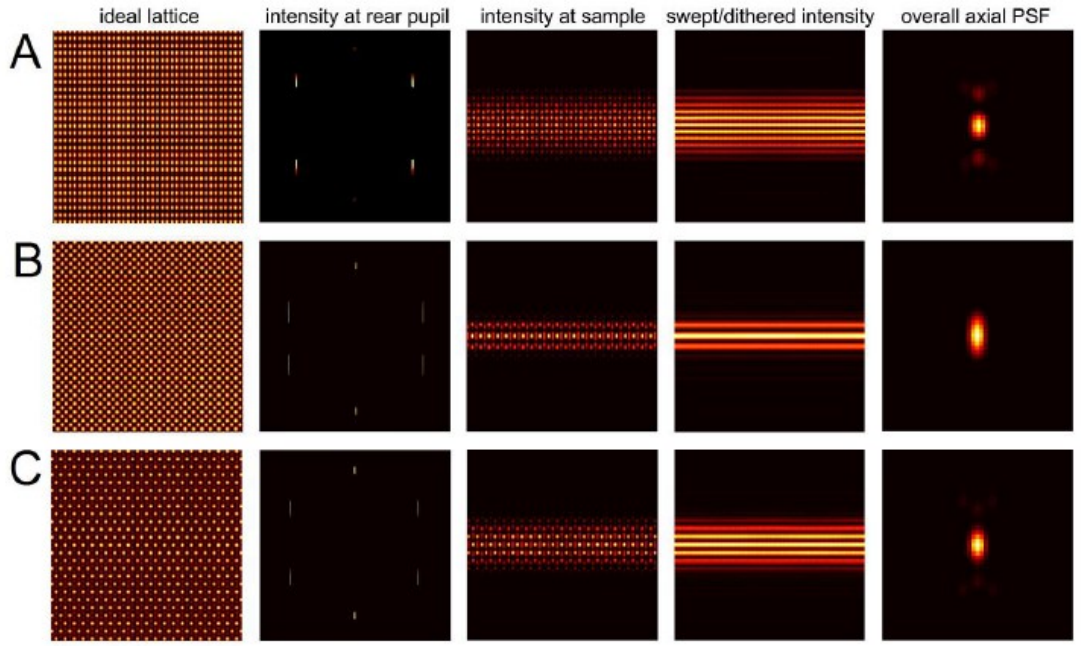
Lattice sheet – the (current) ultimate imaging

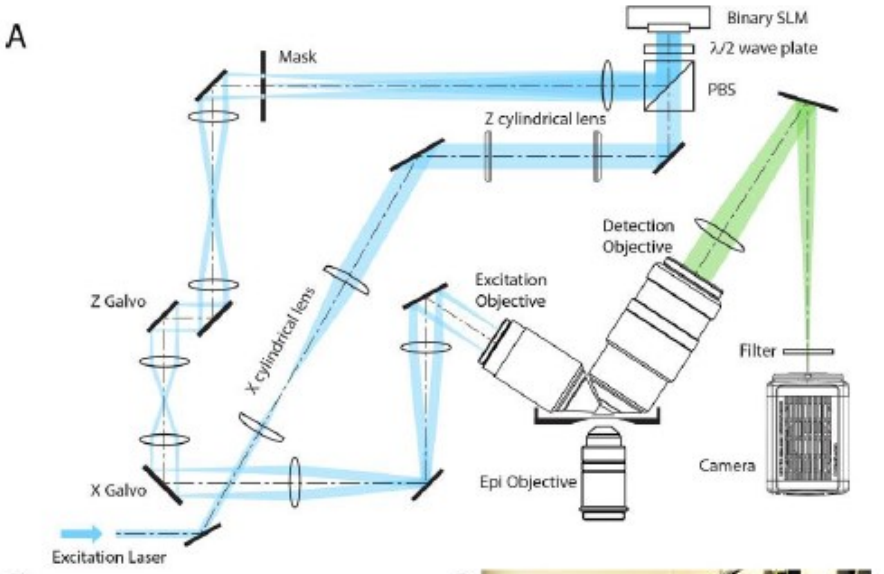
- A combination of Bessel beams and a SIM like illumination
- By playing with the intensity in the Fourier space, it is possible to make lattices of light in the image plane
- Sweep lattices around in space to complete entire 3D image
- Requires opposing excitation and detection objectives just like SPIM

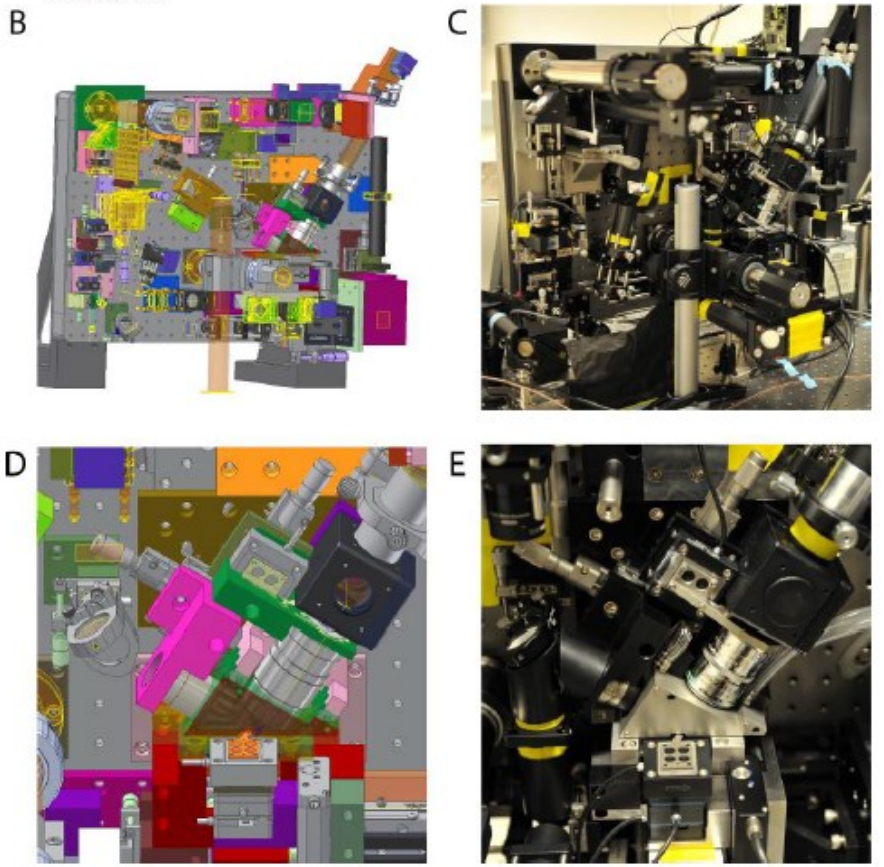


Lattice sheet is hard to make

- Optics are laid out vertically
- Requires spatial light modulator and annulus mask to pattern light at objective
- Sample chamber must be waterproof, and objectives must be realigned often



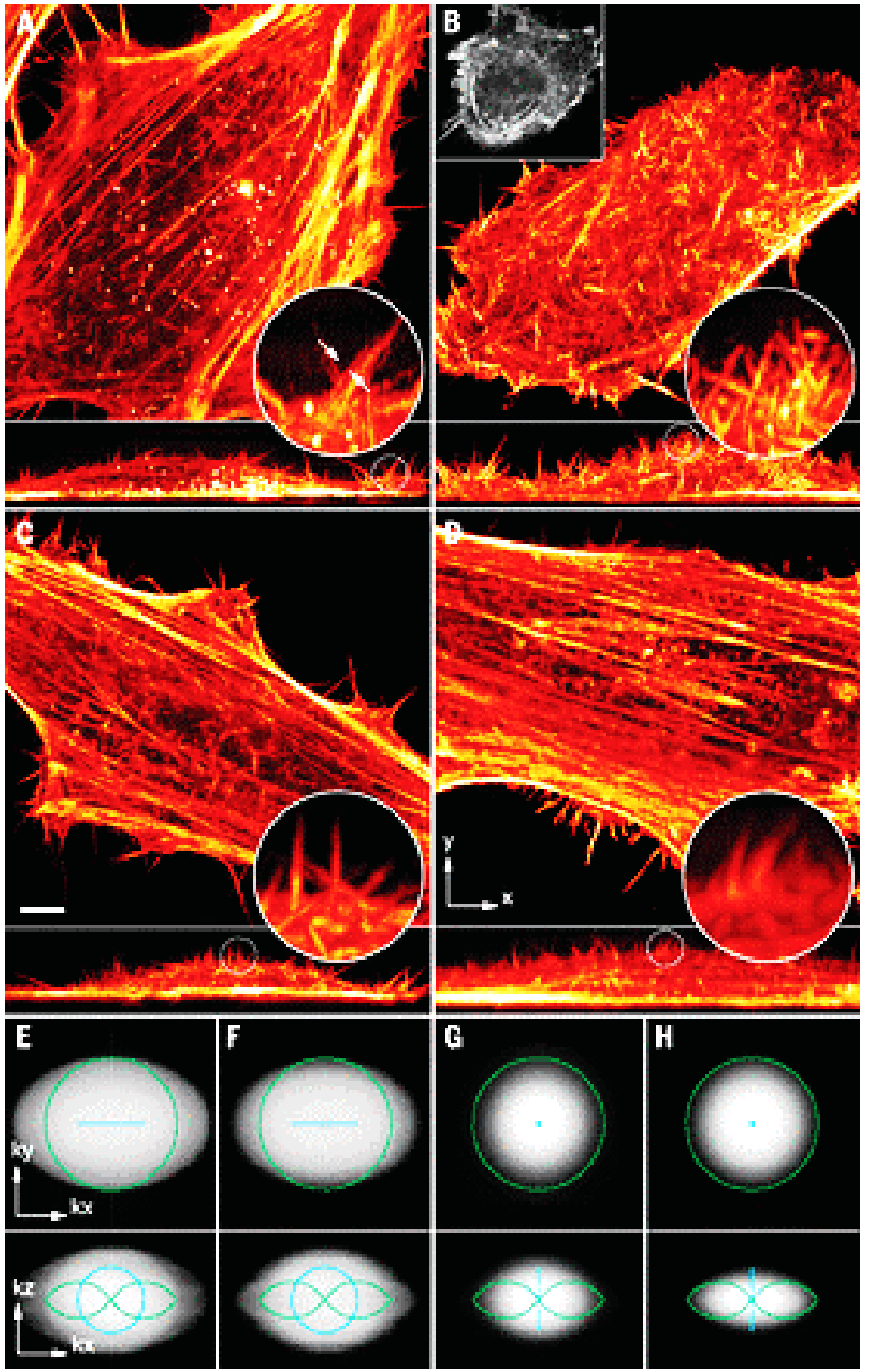


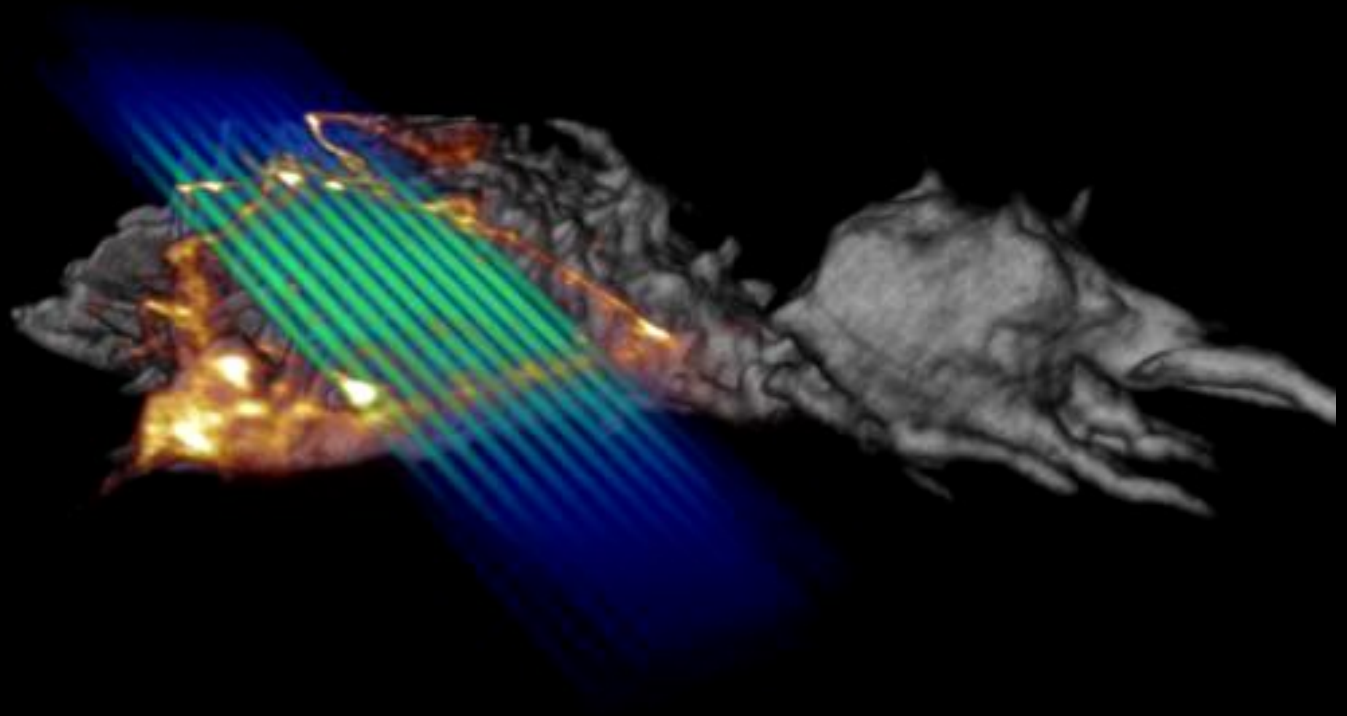


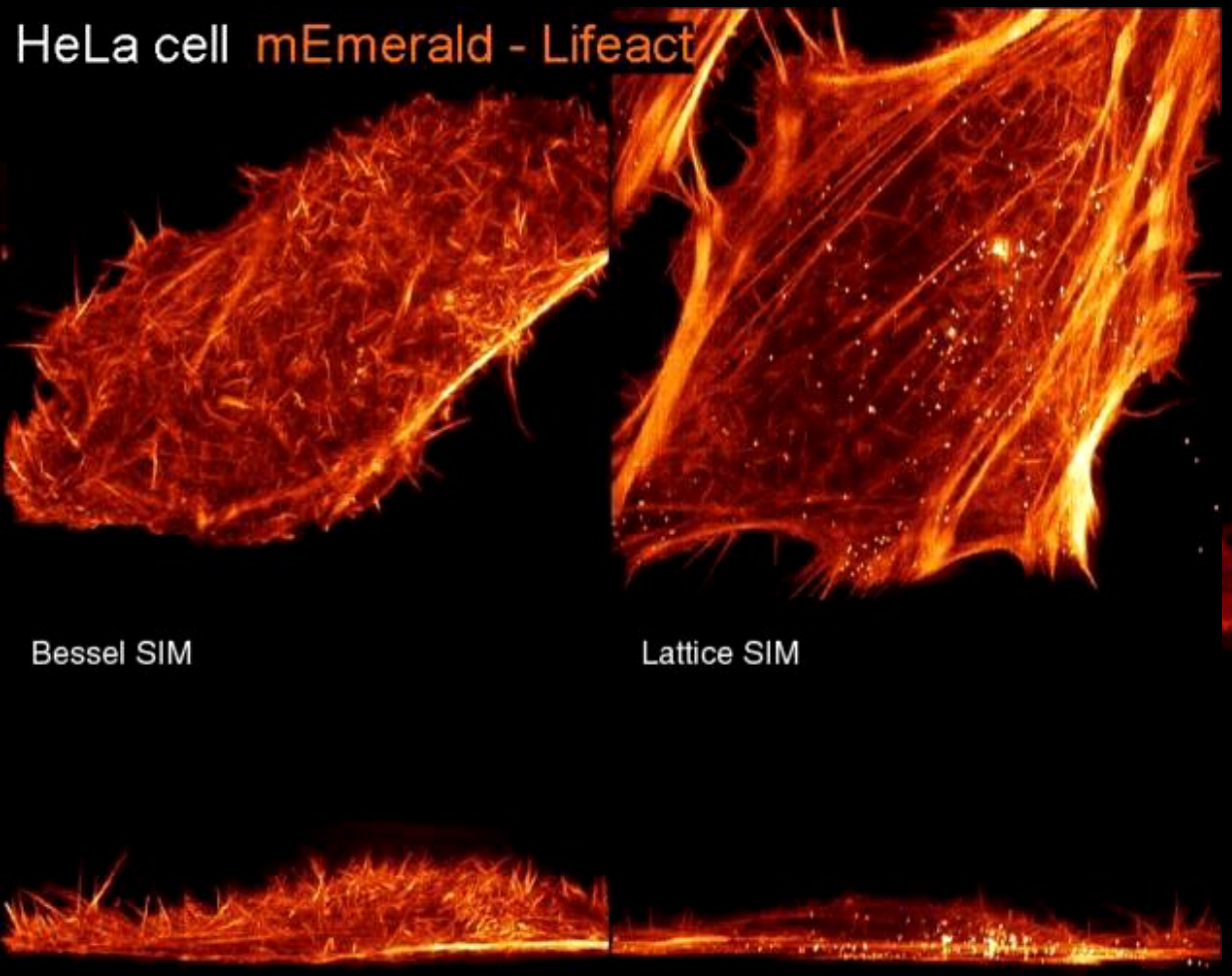
Lattice advantages

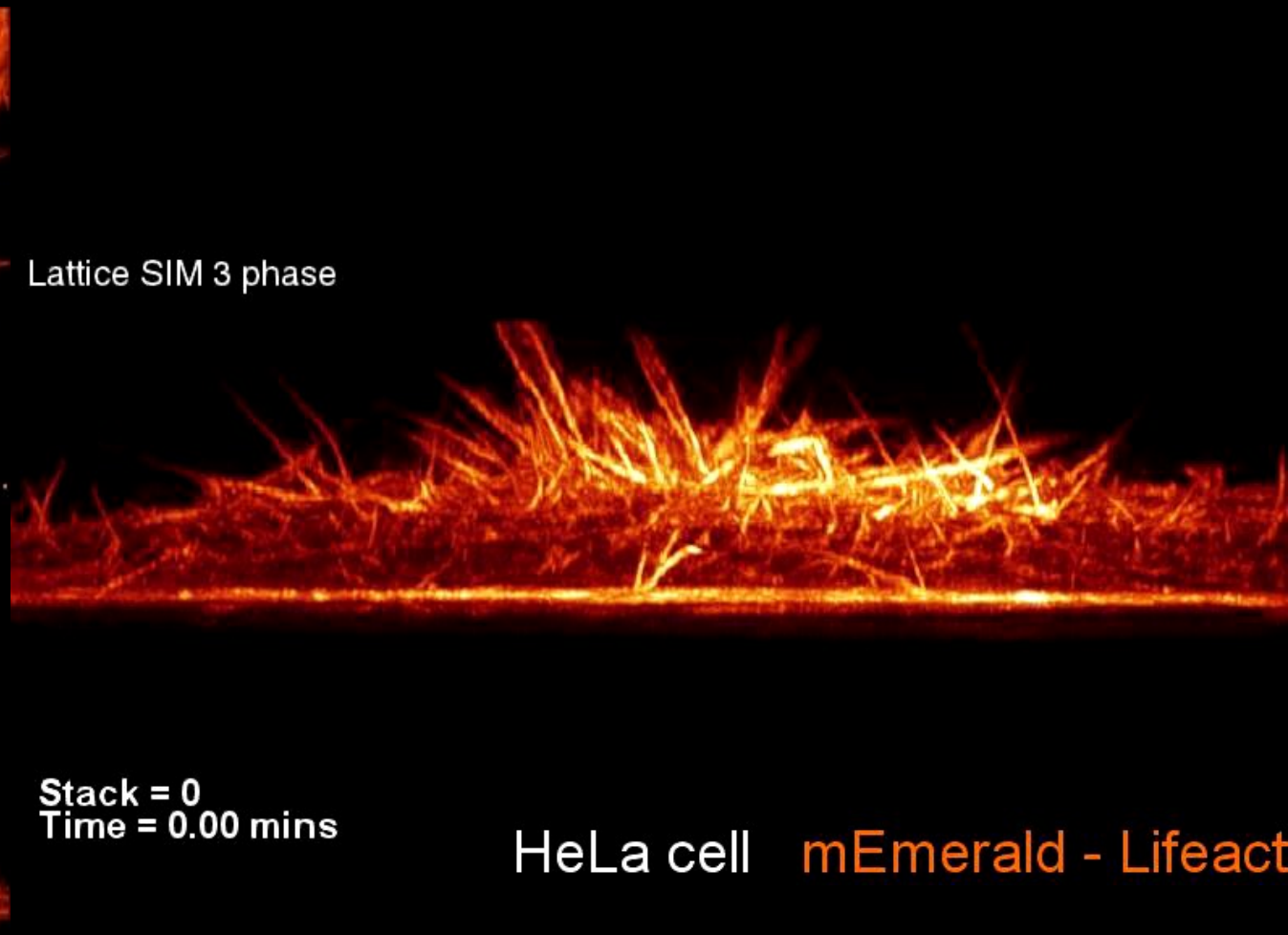
- High spatial resolution and high temporal resolution
- Each Bessel lattice is close to diffraction limited beam
- Multiple Bessel beams MUCH less phototoxic than confocal or even SPIM, even with same power delivered to sample
- Similar tradeoff between thinness of sheet and effective field of view
- Can do SIM exactly the same way as the single Bessel sheet

HeLa with labeled actin

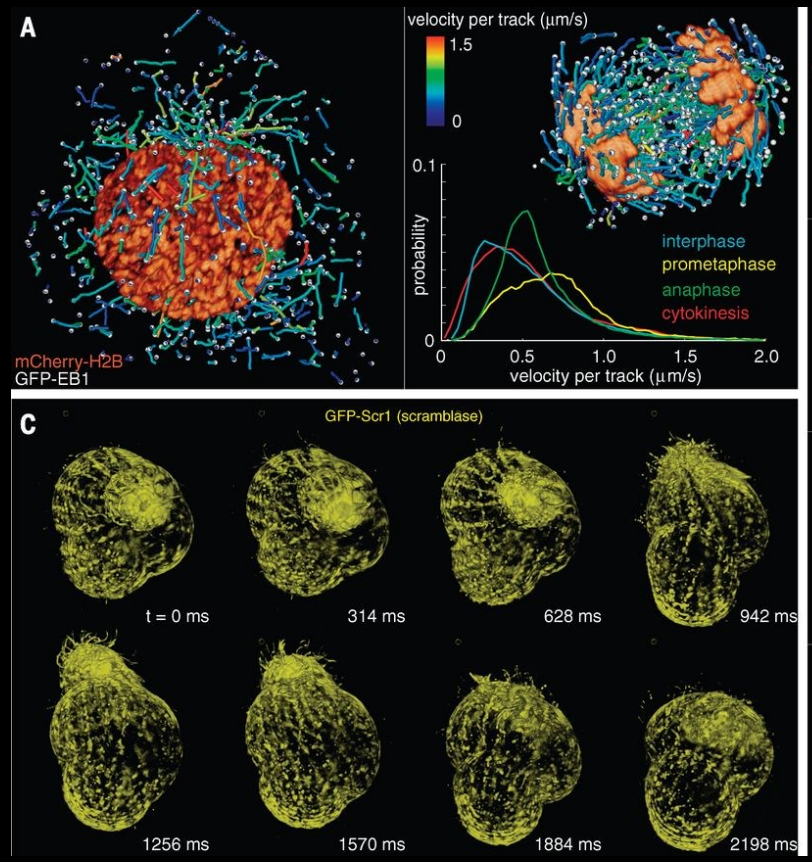


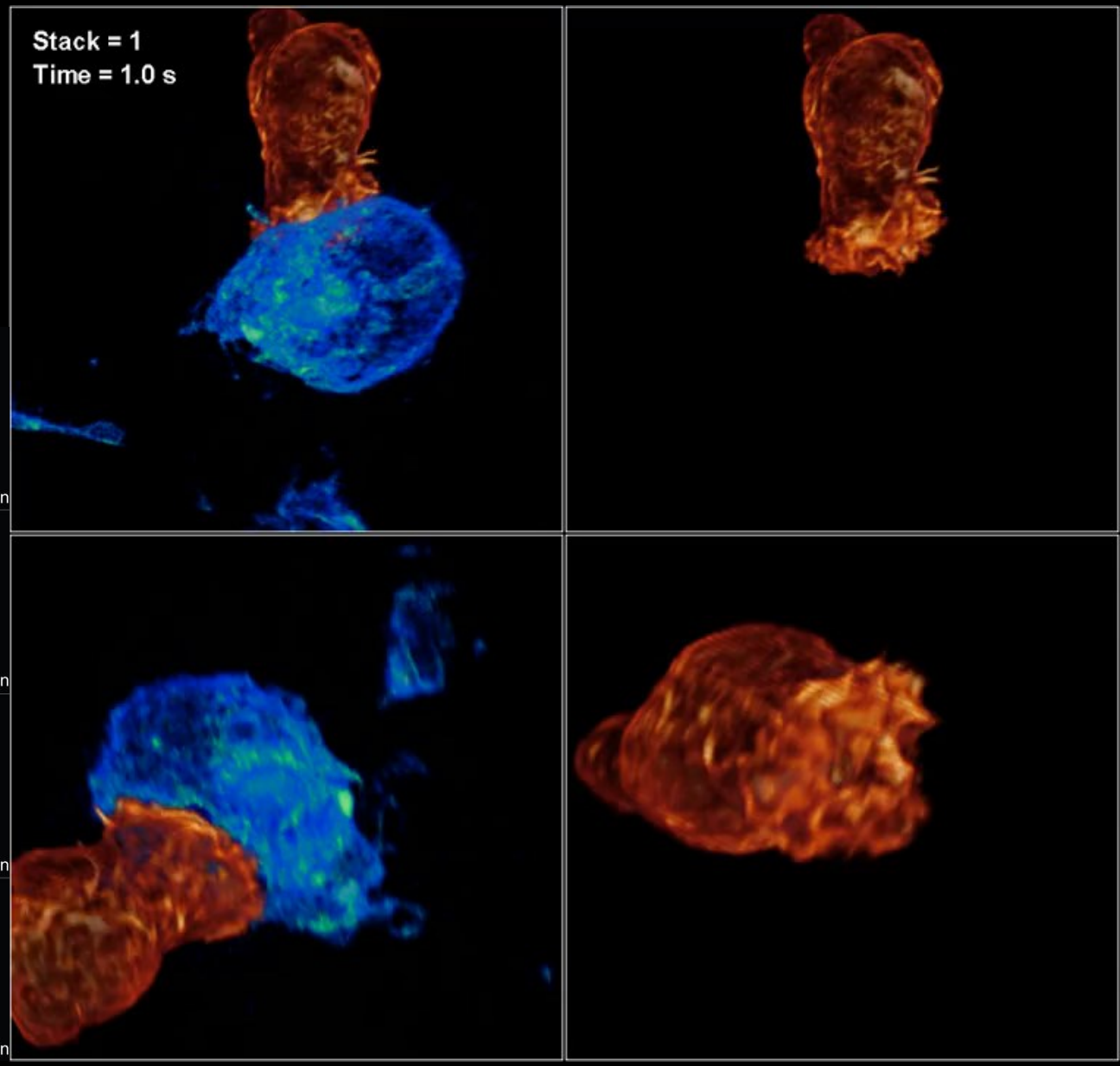


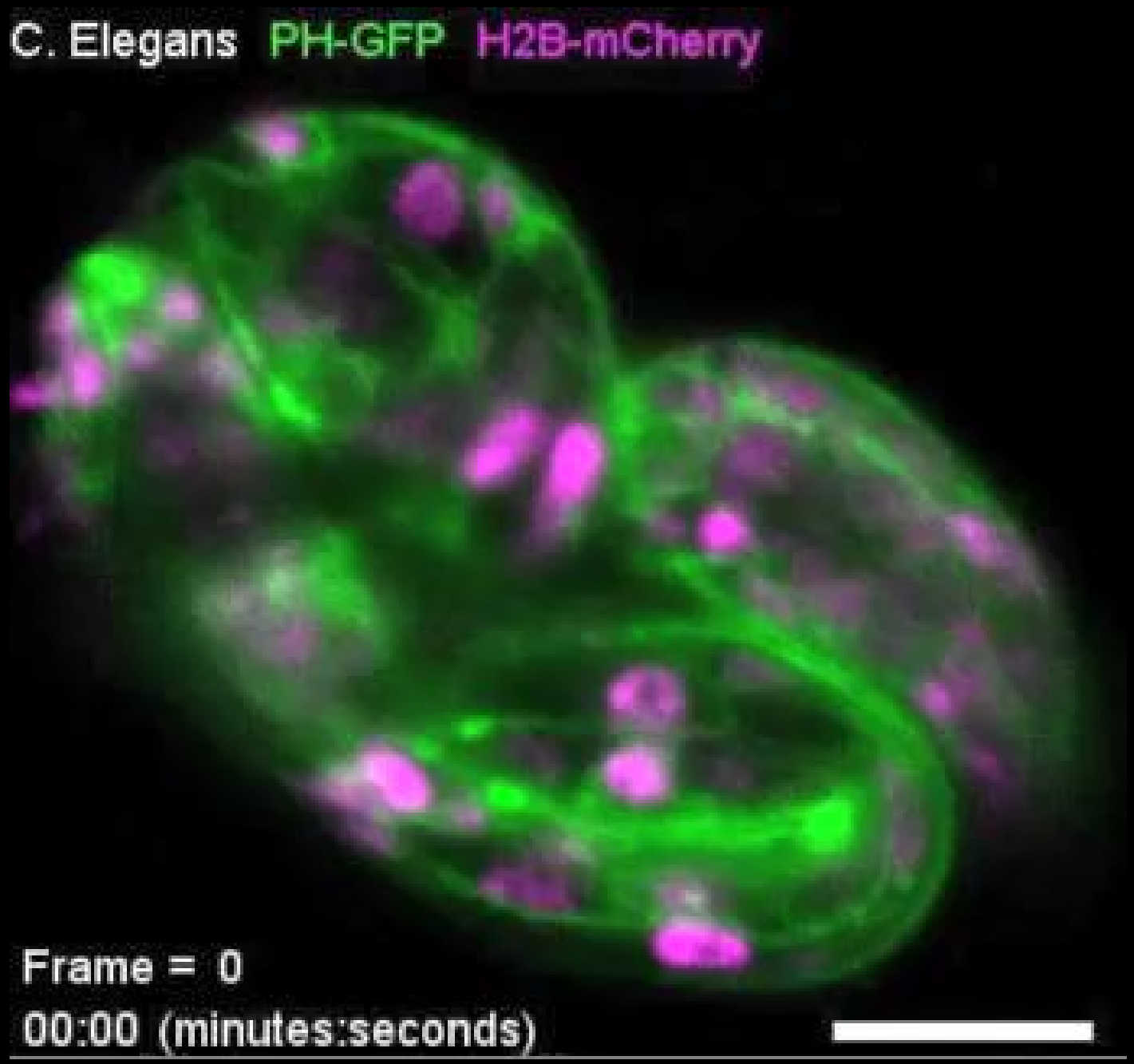

HeLa cell mEmerald - Lifeact
Bessel SIM
Lattice SIM
Stack = 0
Time = 0.0 min
Stack = 0
Time = 0.0 min
2 um


Lattice SIM 3 phase
Stack = 0
Time = 0.00 mins
HeLa cell mEmerald - Lifeact

Multicolor lattice sheets

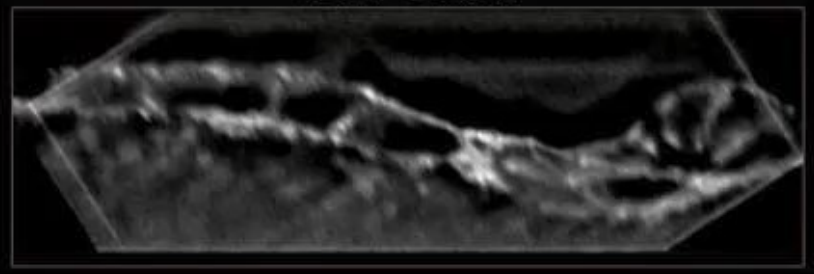




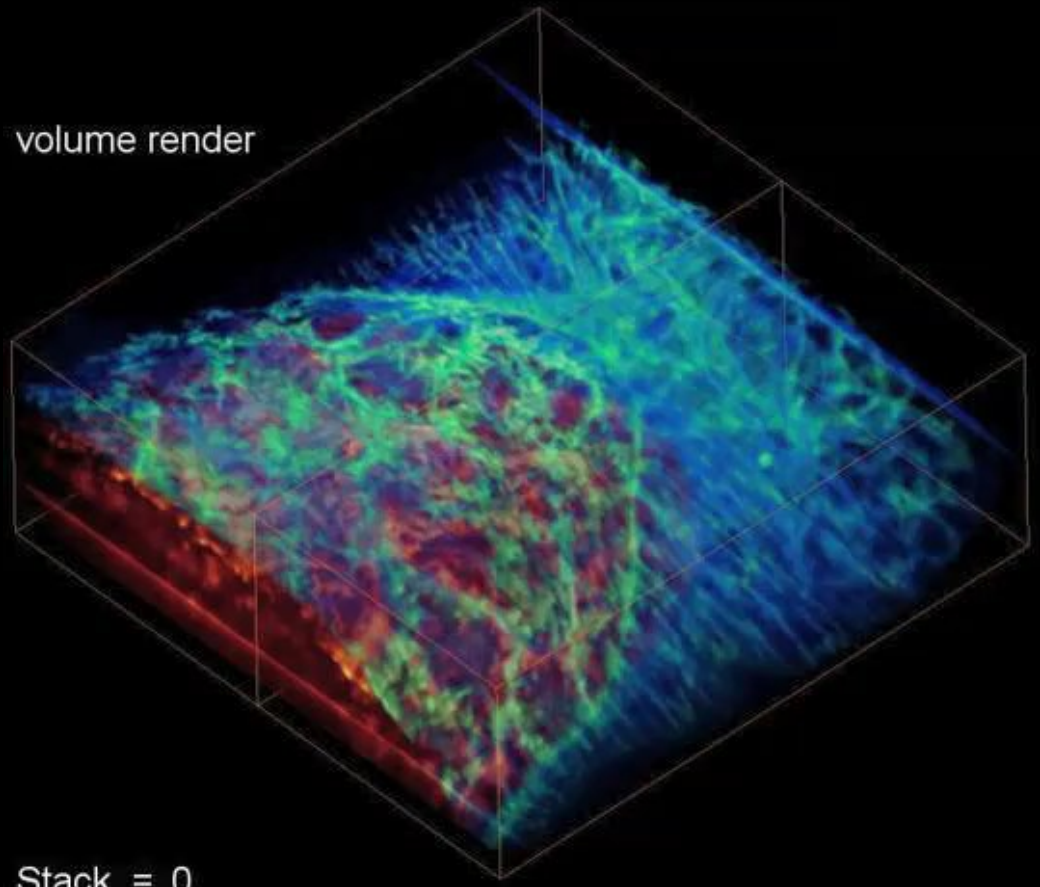

C. Elegans PH-GFP H2B-mCherry
Frame = 0
00:00 (minutes:seconds)

Drosophila Dorsal Closure, eGFP-sGMCA (actin)

cross-section

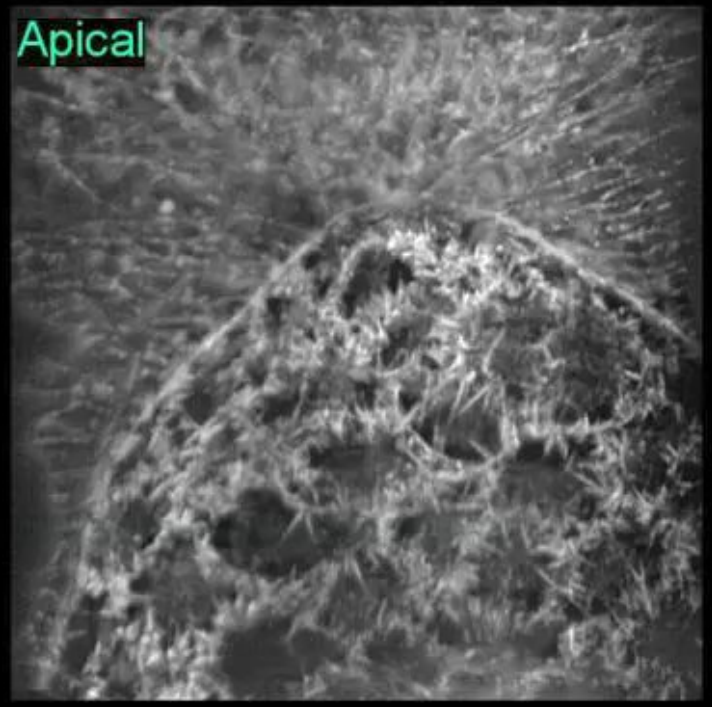


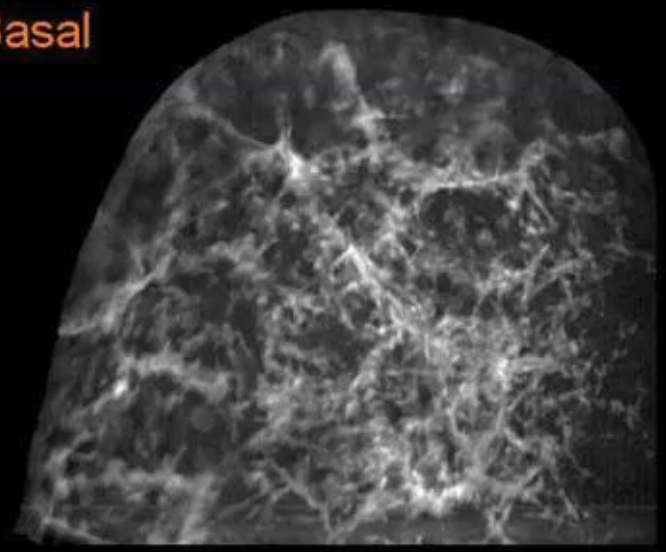
volume render



Stack = 0

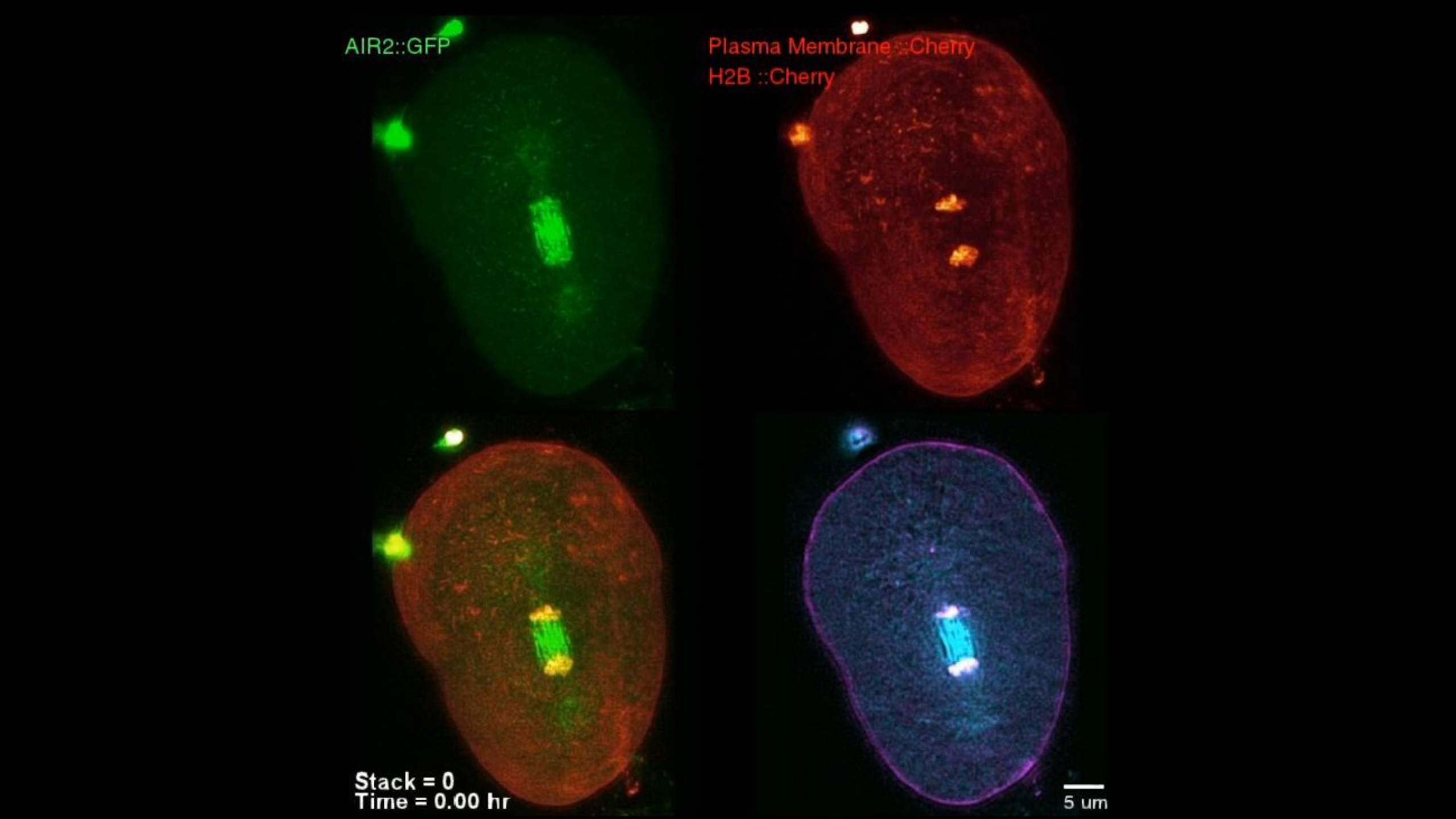
00:00 (min:sec)





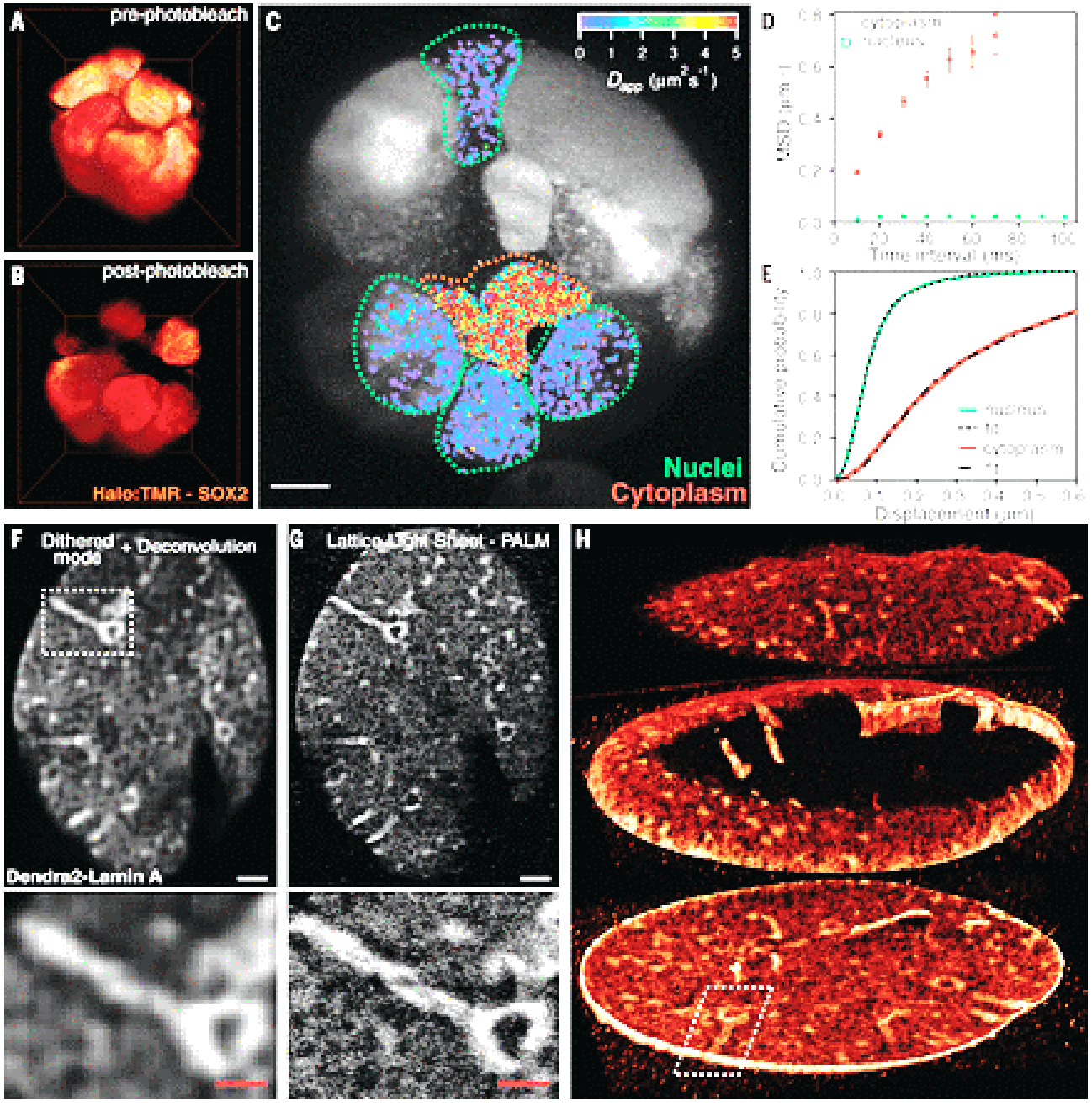
HL-60 cell

mCherry - utrophin FITC - collagen


AIR2::GFP
Plasma Membrane ::Cherry
H2B ::Cherry
Stack = 0
Time = 0.00 hr
5 um

Lattice STORM

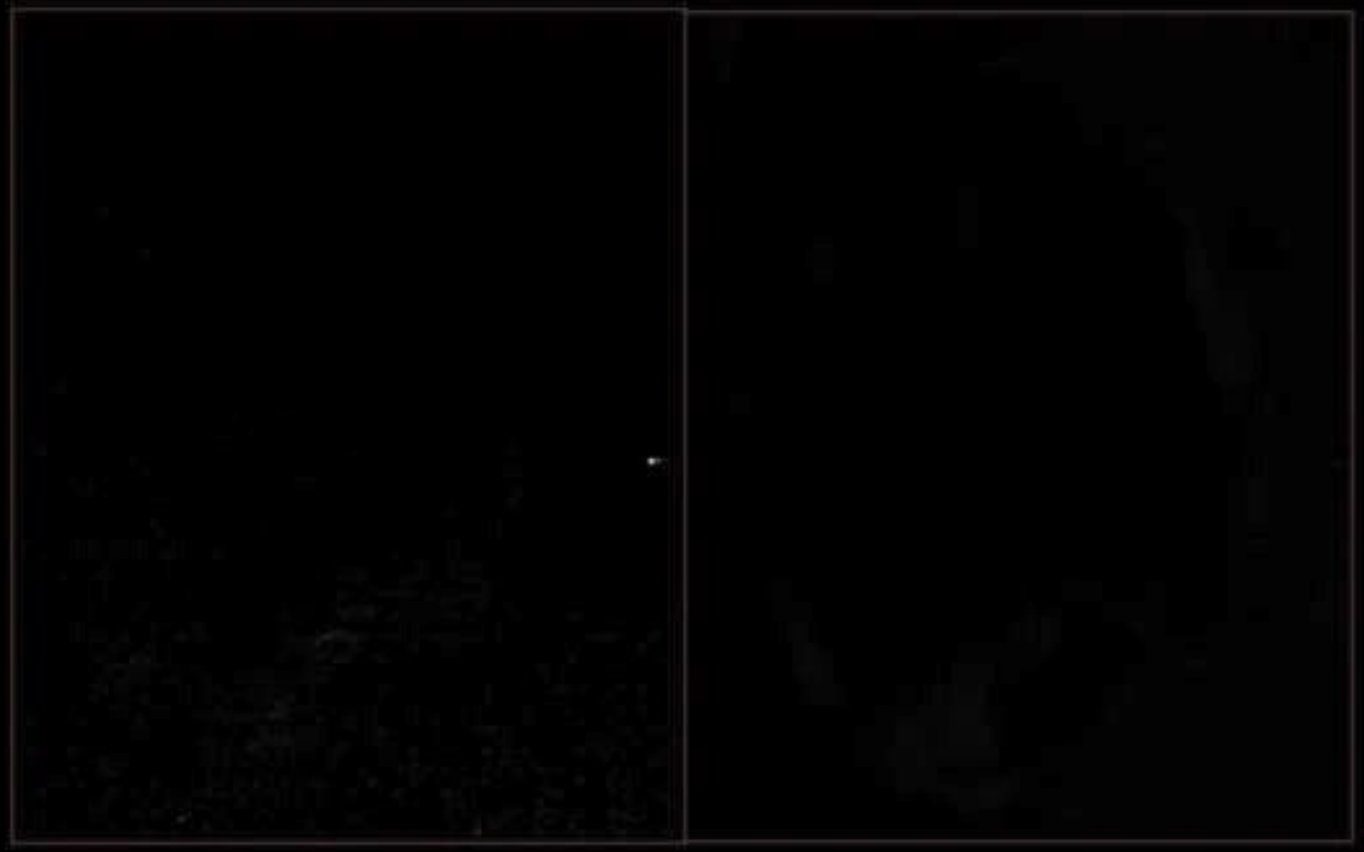
- Due to thinness of Bessel beams, it's possible to track and localize single molecules that are much more densely packed as compared to widefield
- High speed imaging allows tracking with high temporal and spatial resolution



U2OS cell Dendra2 - Lamin A

Lattice Light Sheet PALM

Dithered mode + Deconvolution



0.00 um

0.0 um

On to Matlab…