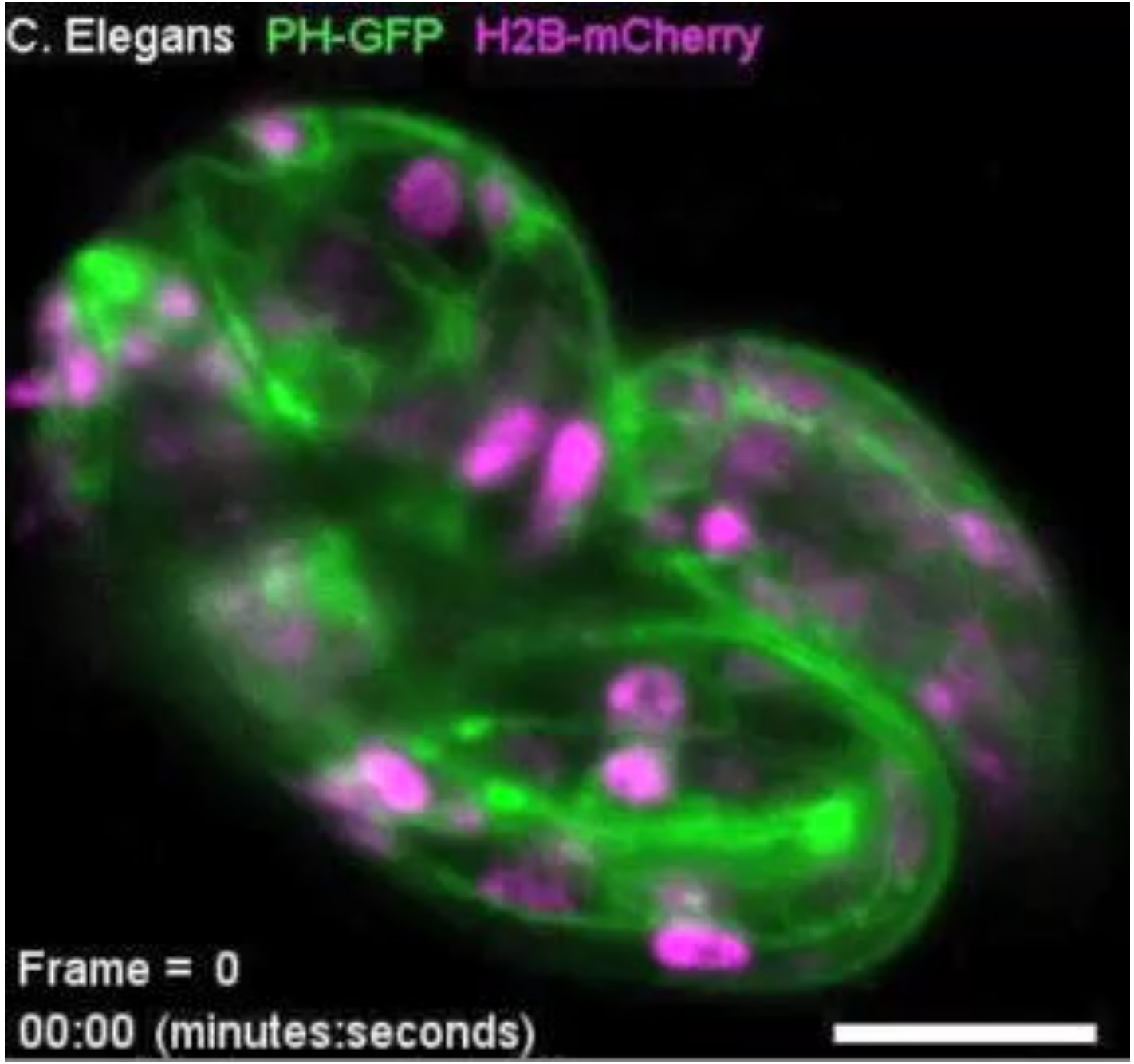
Lattice Light Sheet Microscopy

Seminar on Biophysics of Systems

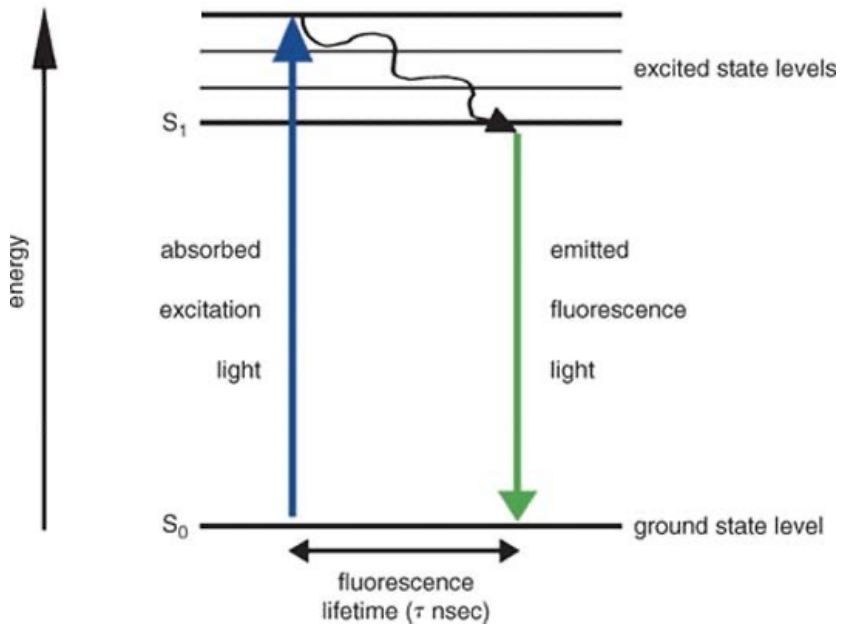
Isabella Tepfenhart, Lara Kunze

06.12.2021


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

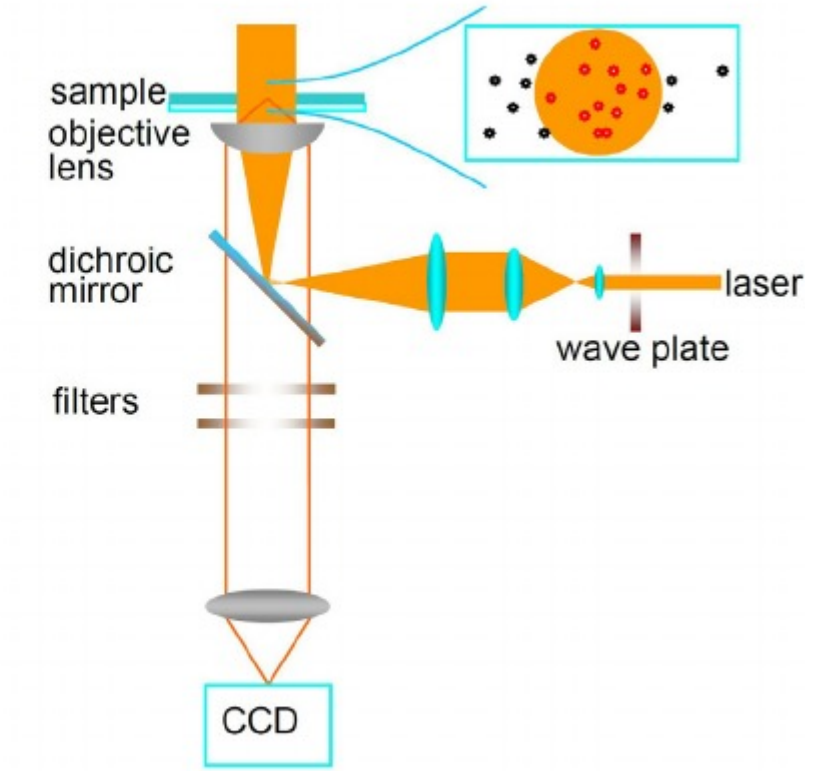
Fluorescence Microscopy

Energy state transitions leading to fluorescence



Lleres et al., *Current protocols in cytometry* (2007)

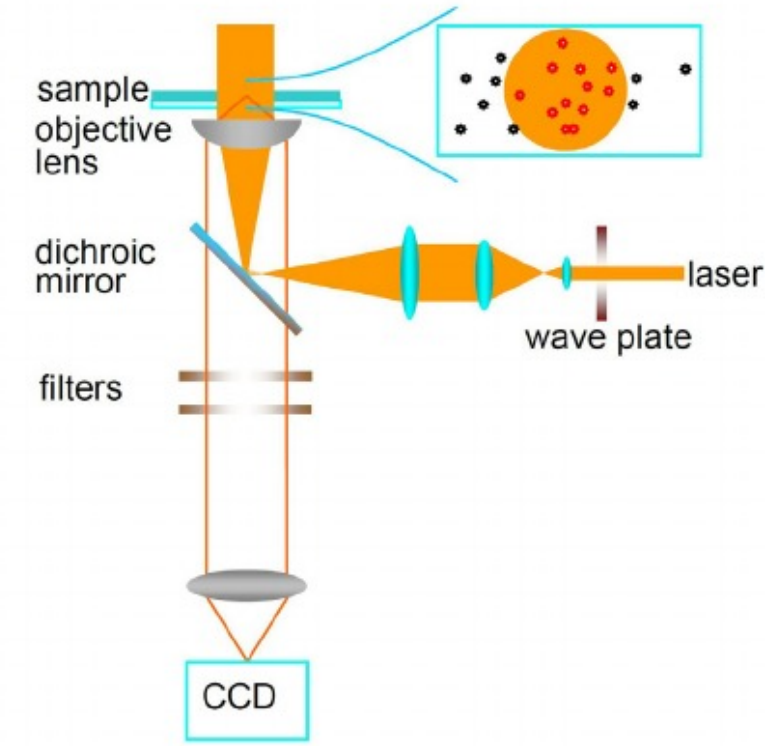
Fluorescence microscopy setup



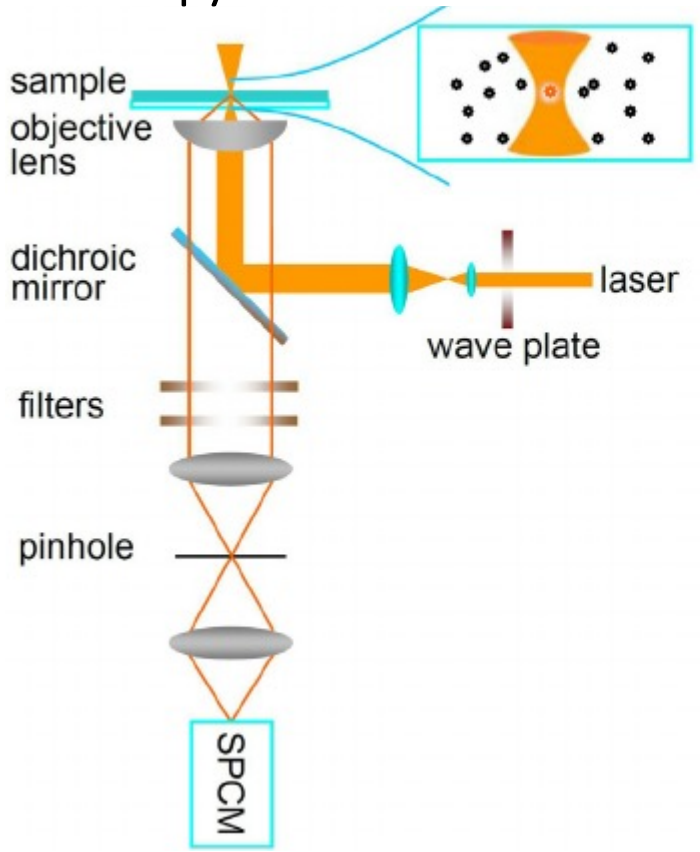
Chen et al., Sensors (2014)

Confocal Fluorescence Microscopy

Setup for widefield fluorescence microscopy



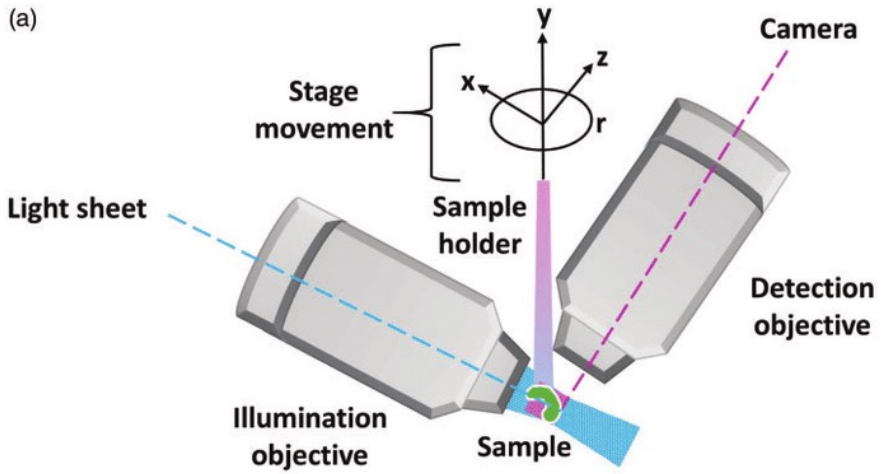
Setup for confocal fluorescence microscopy



Chen et al., Sensors (2014)

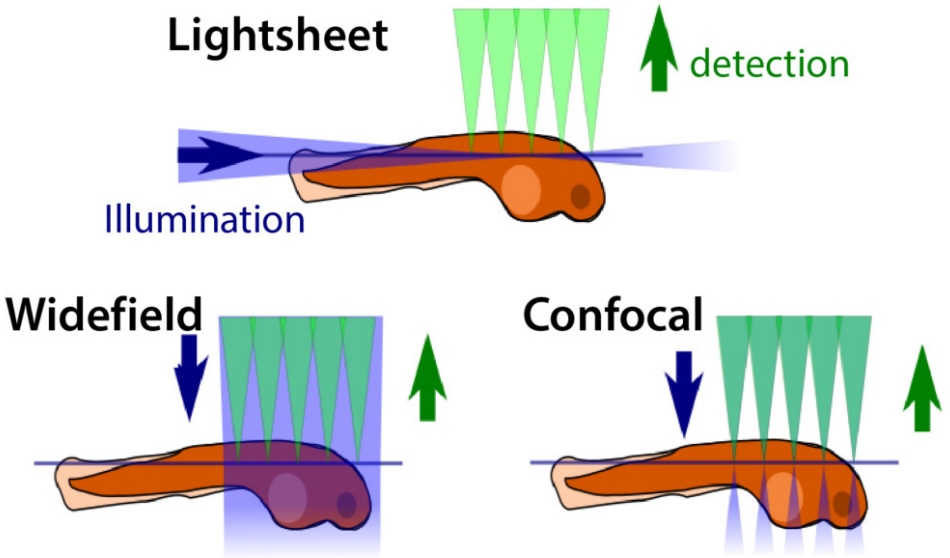
Light Sheet Microscopy

Arrangement of illumination and detection objective in light sheet microscopy



Chatterjee et al., Applied Spectroscopy (2018)

Different illuminations in fluorescence microscopy

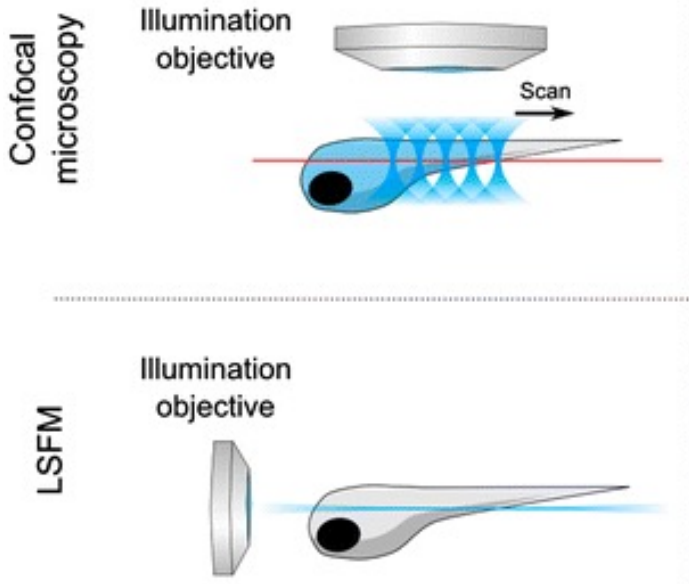


Krieger, *Wikimedia Commons* (2013)

Light Sheet Microscopy

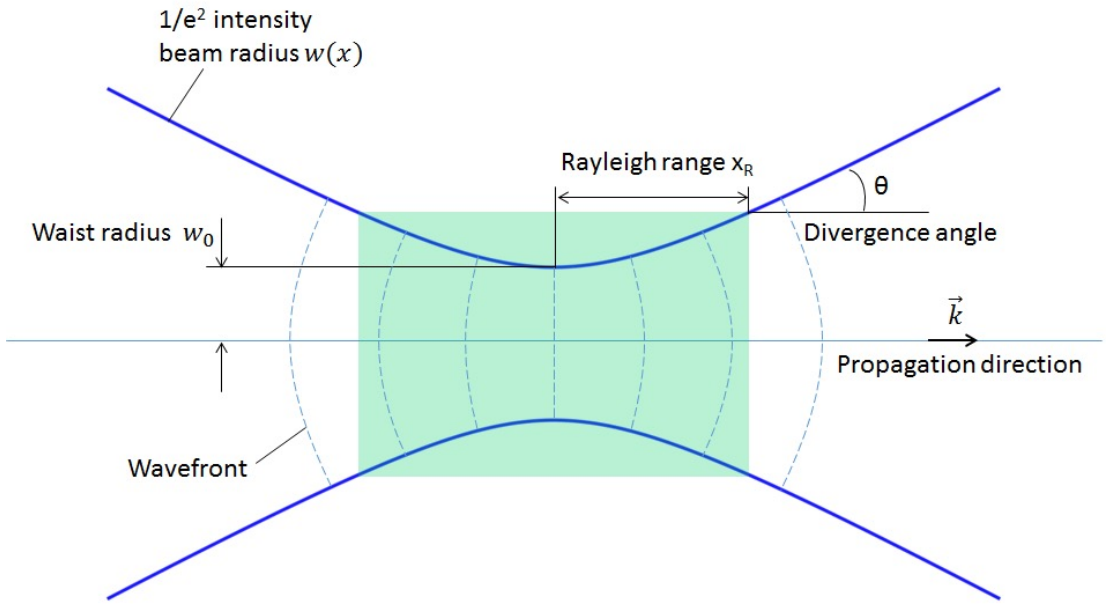
Advantages of Light sheet microscopy:

- Optical sectioning
- Speed
- Reduction of photobleaching and phototoxicity

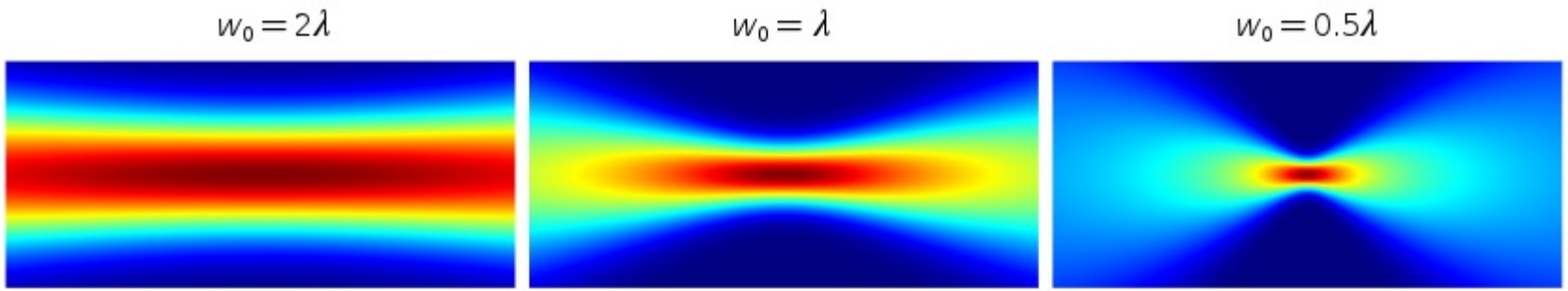


Optogenetics: *Light-driven Actuators and Light-emitting Sensors in Cell Biology* (2018)

Gaussian light sheet



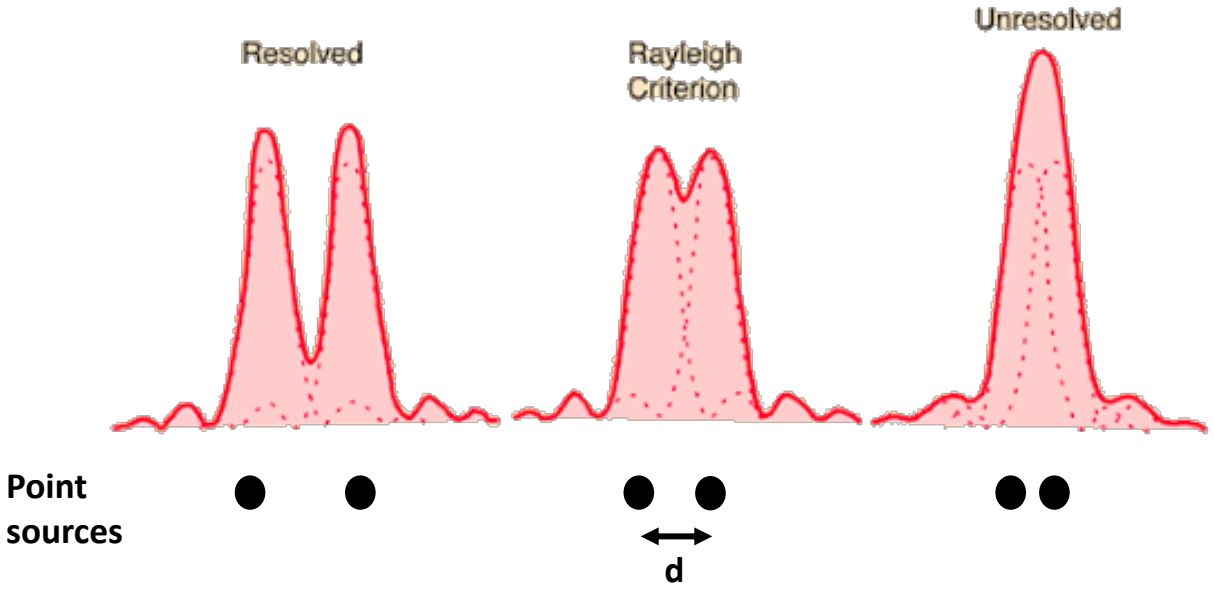
Profile of a Gaussian light sheet



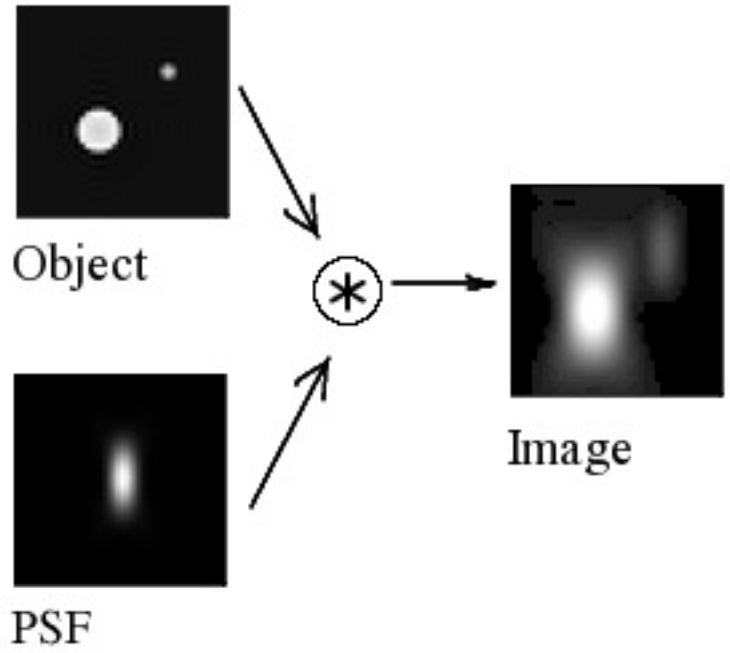
Mizuyama, *COMSOL Blog* (2016)

Resolution

Resolution limit: minimal distance between two distinguishable radiating points

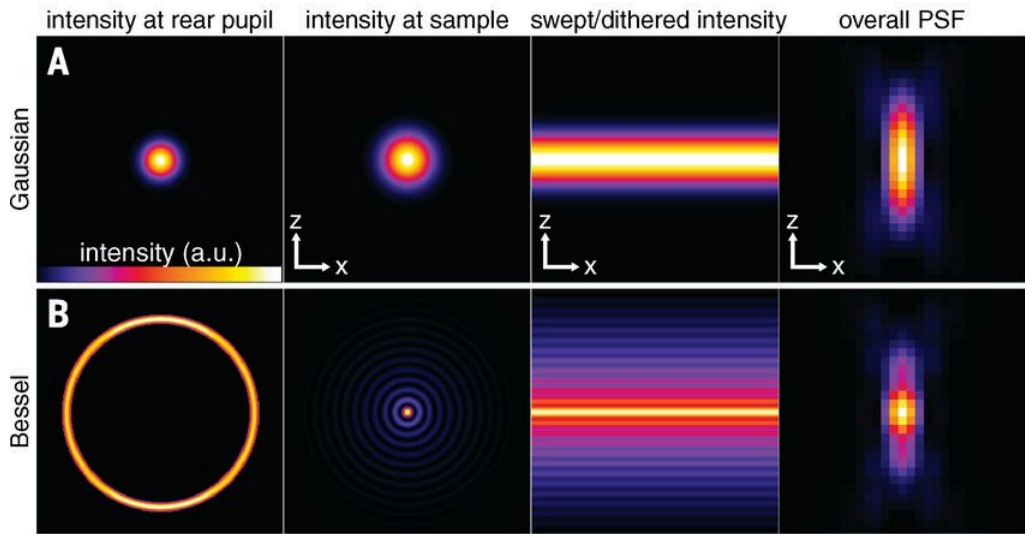


Point spread function: response of an optical system to a point source



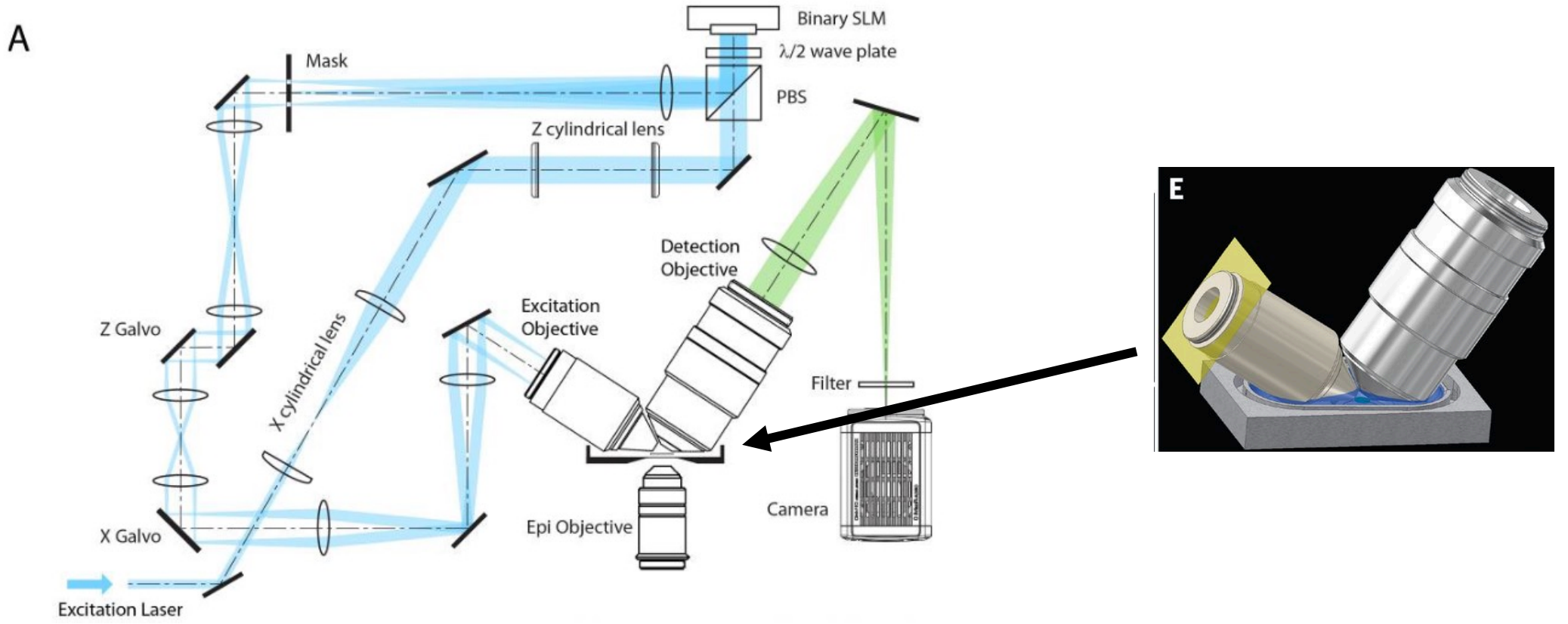
Latychevskaia, *Applied Optics* (2019)

Comparison of different light-sheets



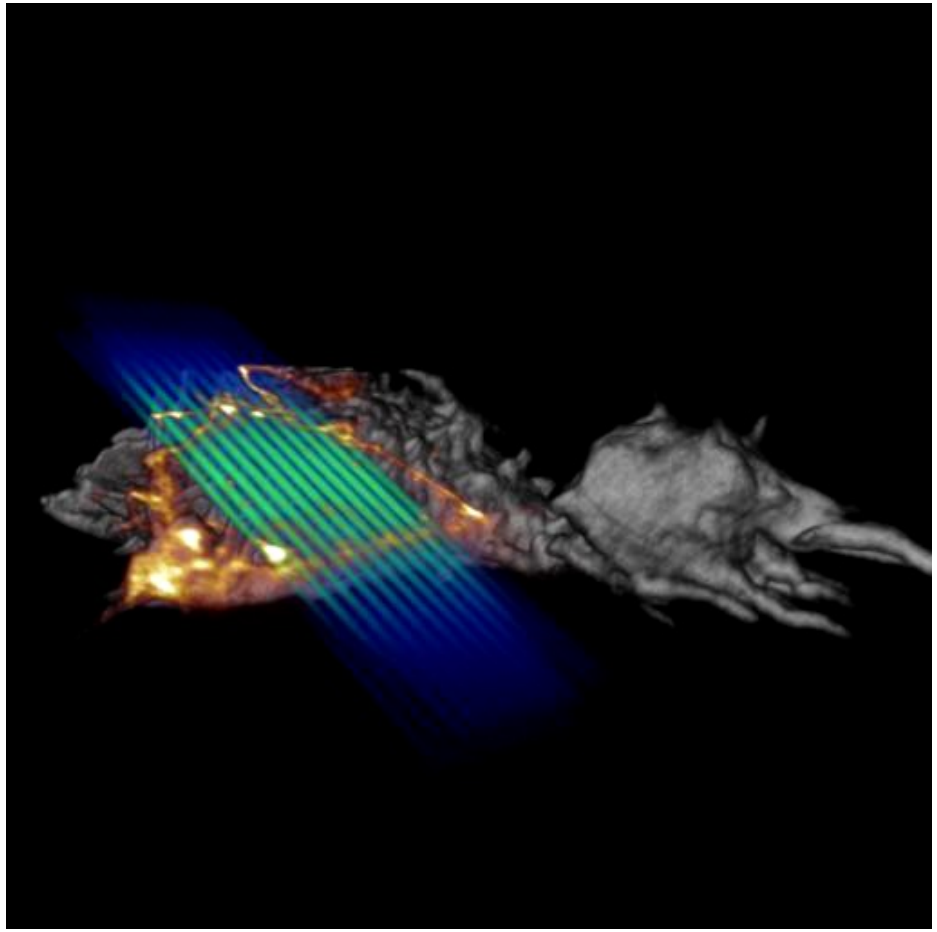
Chen et al., *Science* (2014)

Setup



Chen et al., *Science* (2014)

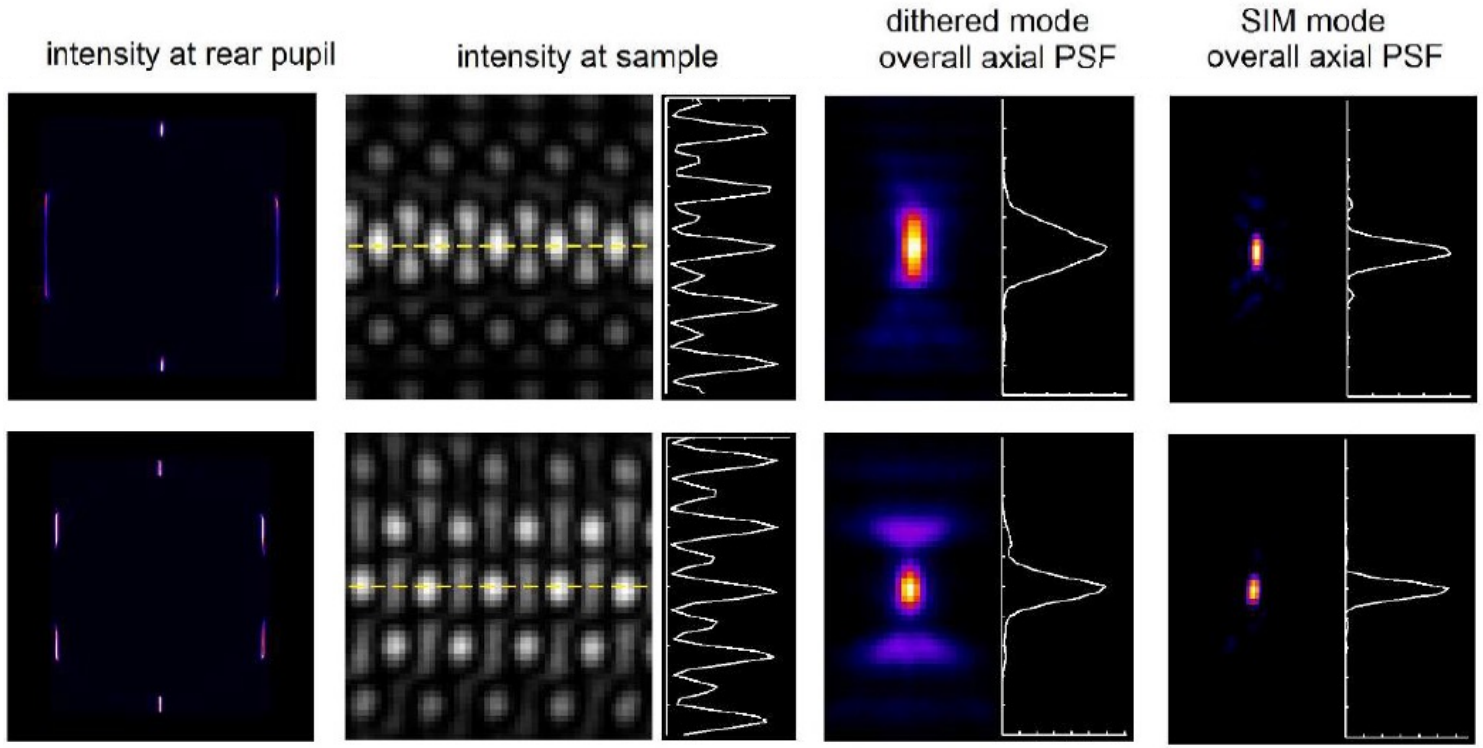
Aquisition of 3D images



Chen et al., *Science* (2014)

Superresolution Structural Imaging Microscopy

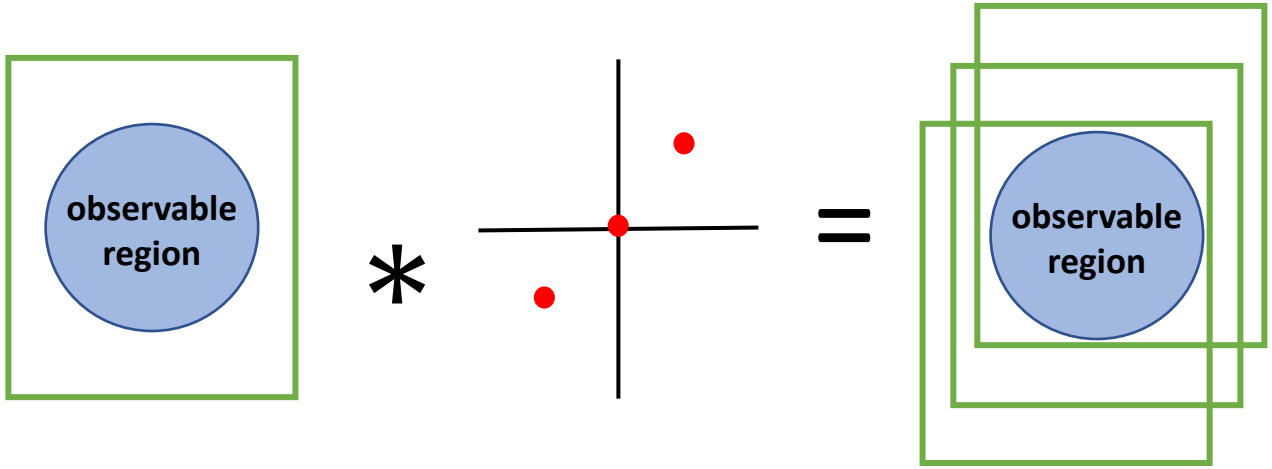
Comparison of point spread function in dithered mode and SIM mode:



Chen et al., *Science* (2014)

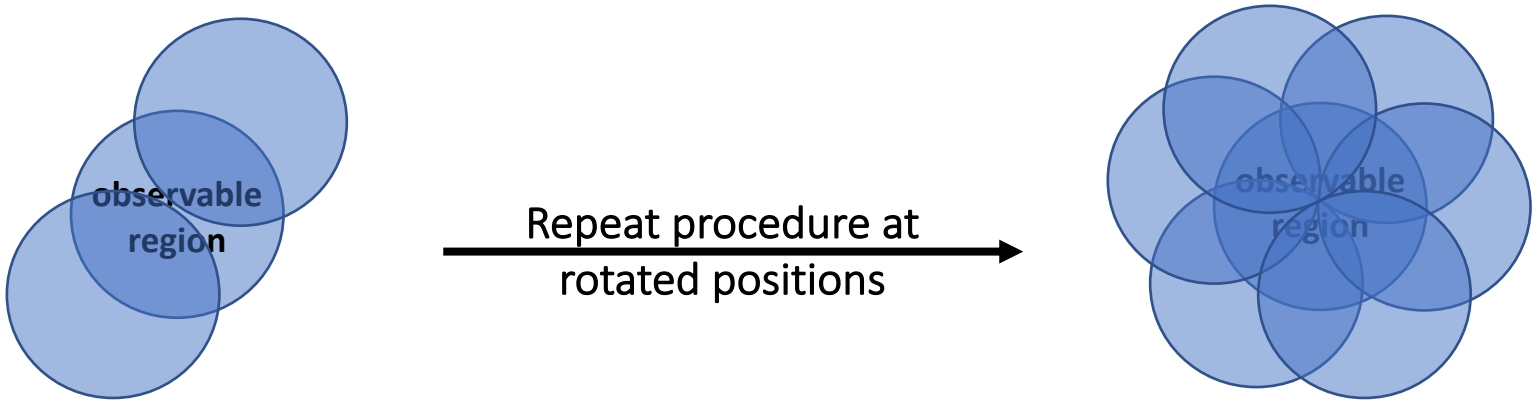
Superresolution Structural Imaging Microscopy

Theoretical background of SIM



FT(object) FT(illumination) FT(emission)

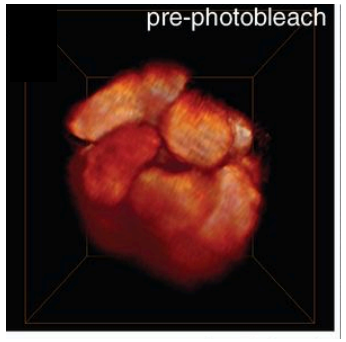
Resulting observable region:



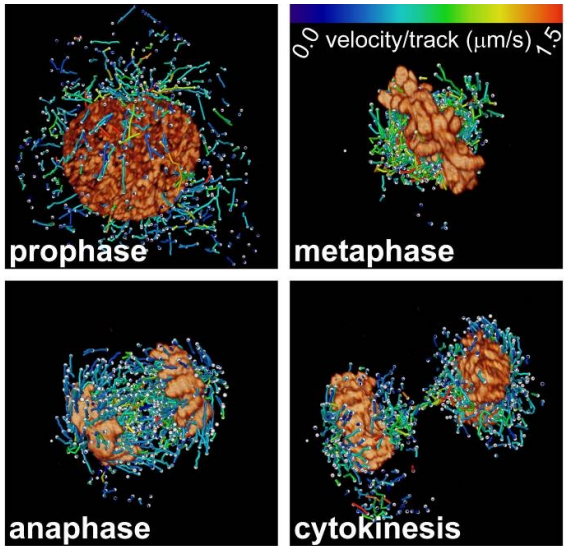
Application of the Lattice Light Sheet Microscopy

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

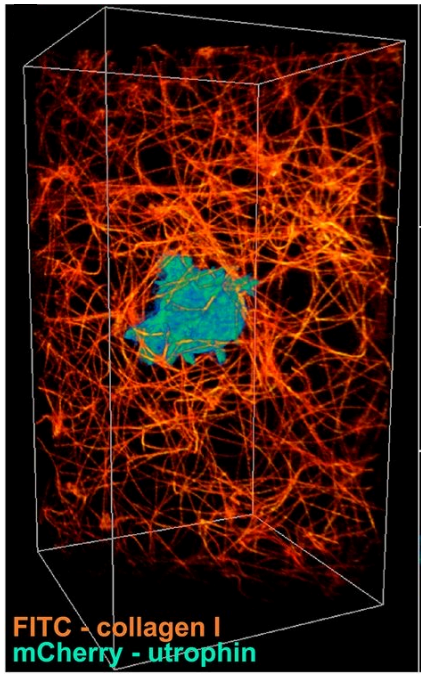
Bi-Chang Chen et al., *Science* 346, 2014.



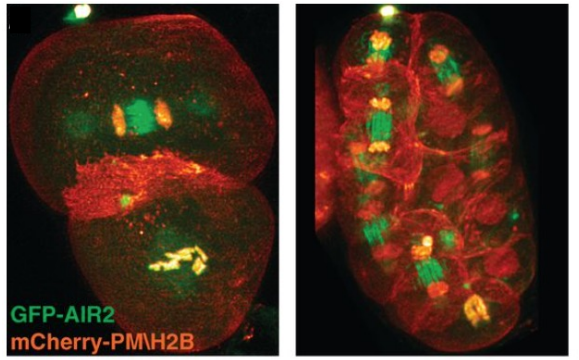
Spheroid of mouse embryonic stem cells.



Histones and 3D tracks of growing microtubule ends.



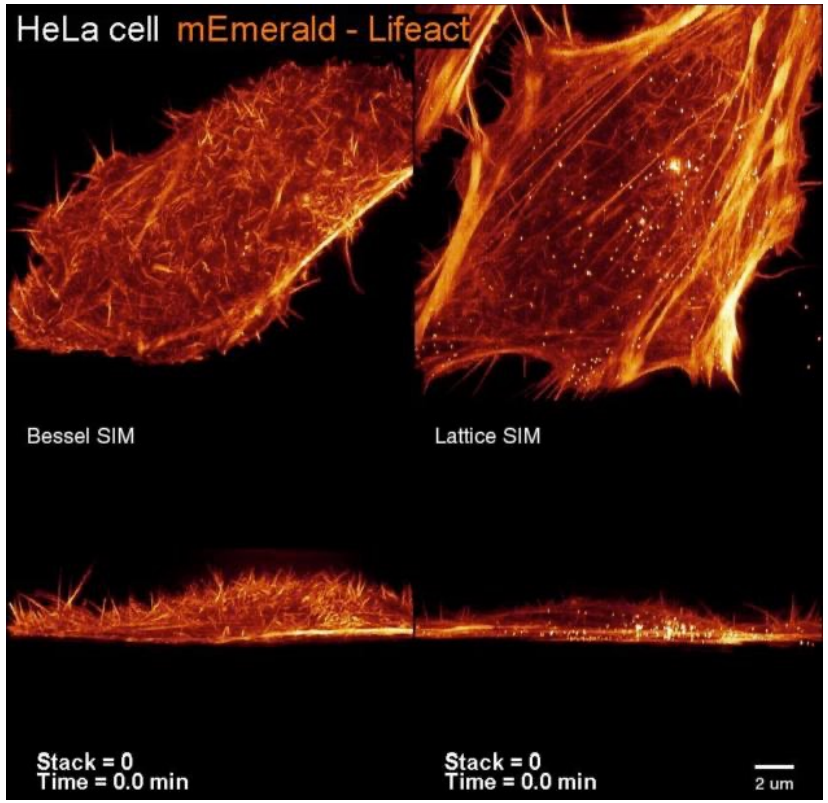
Neutrophil-like human HL-60 cell in collagen matrix.



Distribution of AIR-2 relative to plasma membranes and histones in C. elegans.

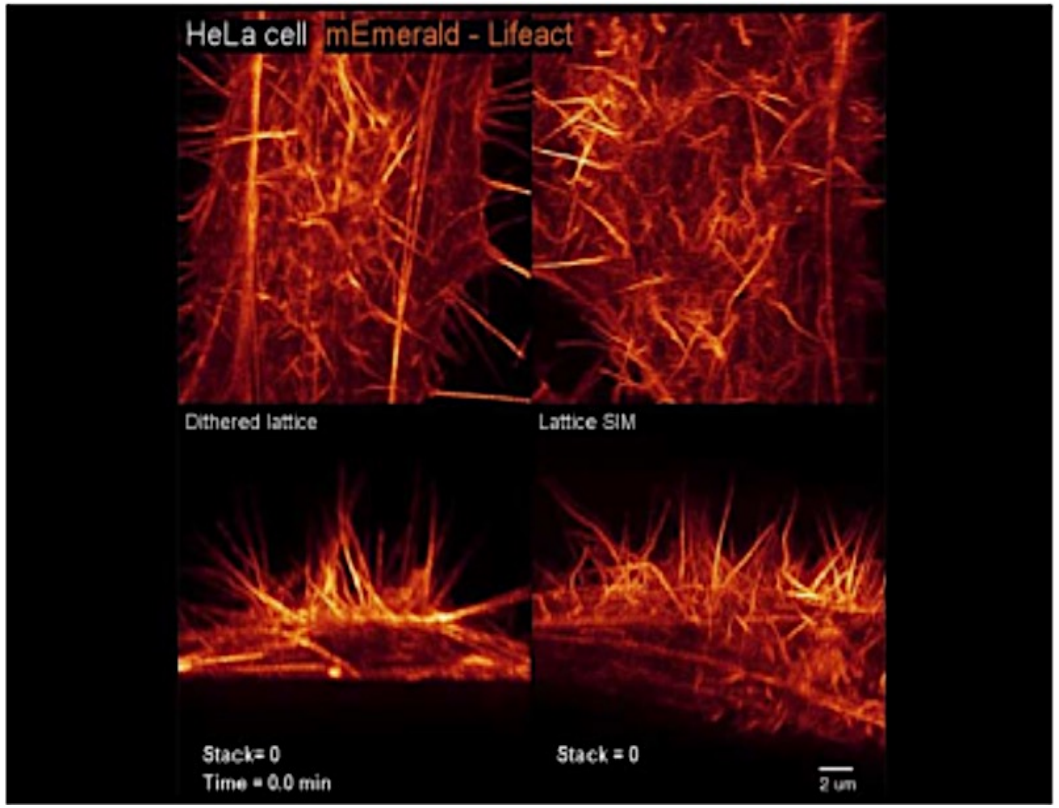
Dynamics of filopodia on HeLa cells

Lattice Light Sheet Microscopy
in comparison with
Light Sheet Microscopy using a Bessel Beam



Top and side view volume renderings of HeLa cells expressing mEmerald-Lifeact.

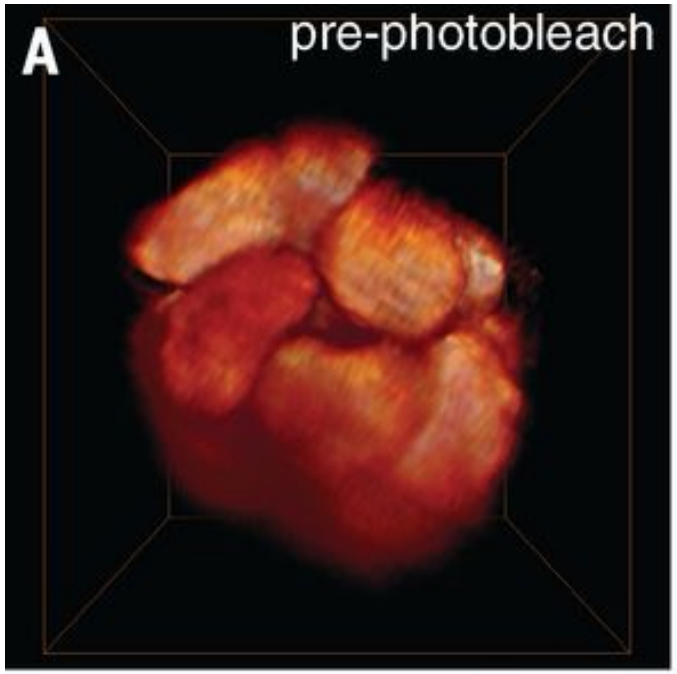
Dithered and SIM mode
of Lattice Light Sheet Microscopy

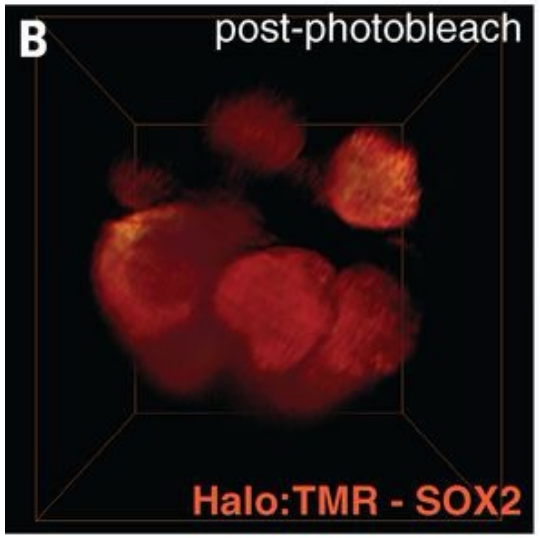


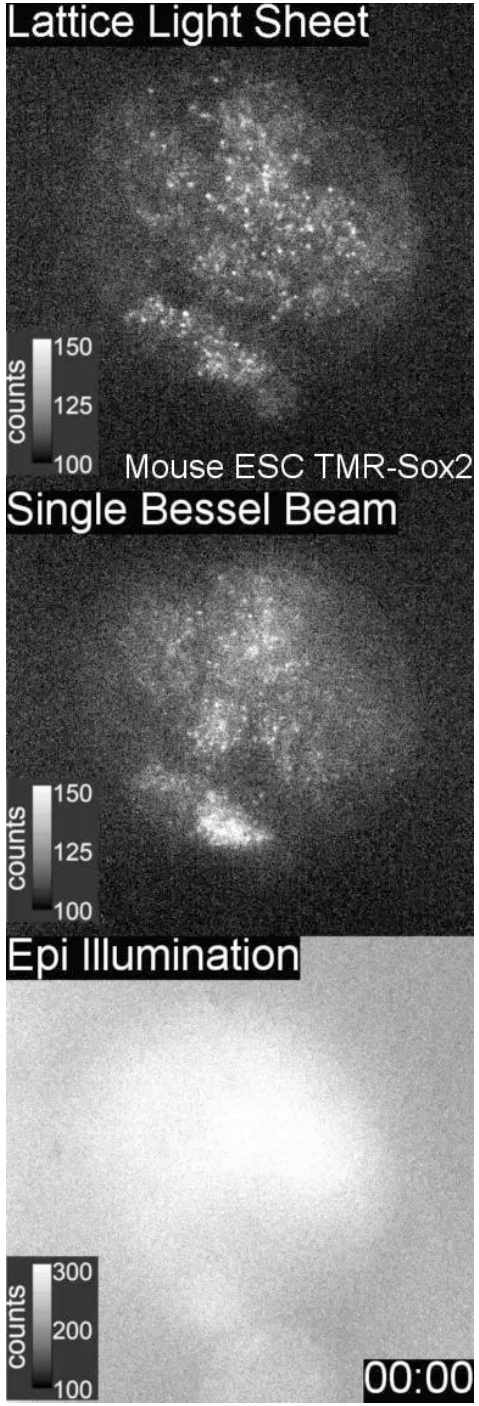
Chen et al., *Science*, 2014.

Single molecule tracking

Investigation of diffusion and binding kinetics of SOX2 transcription factors *(densely labeled with TMR HaloTag),*
across a 35µm-diameter spheroid of mouse embryonic stem cells.



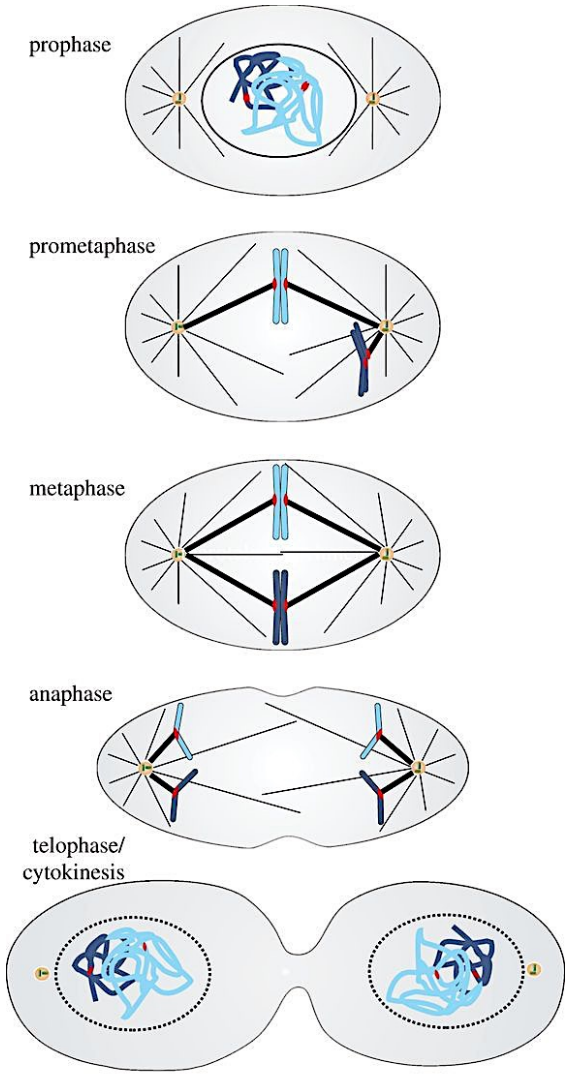


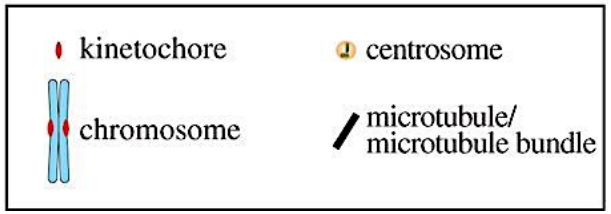


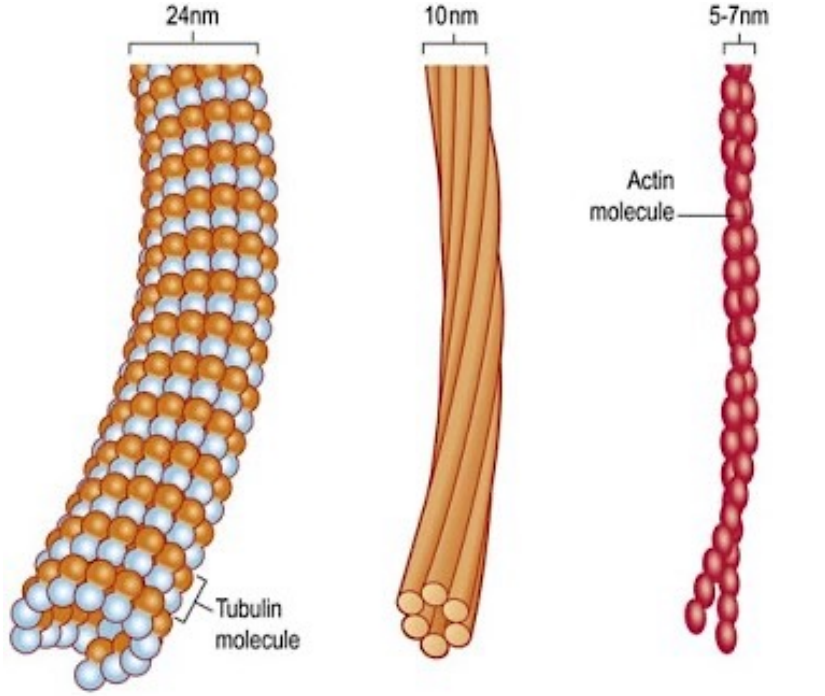
Chen et al., *Science*, 2014.

Non-invasive 3D imaging of intracellular dynamics

Schematic representation of mitosis





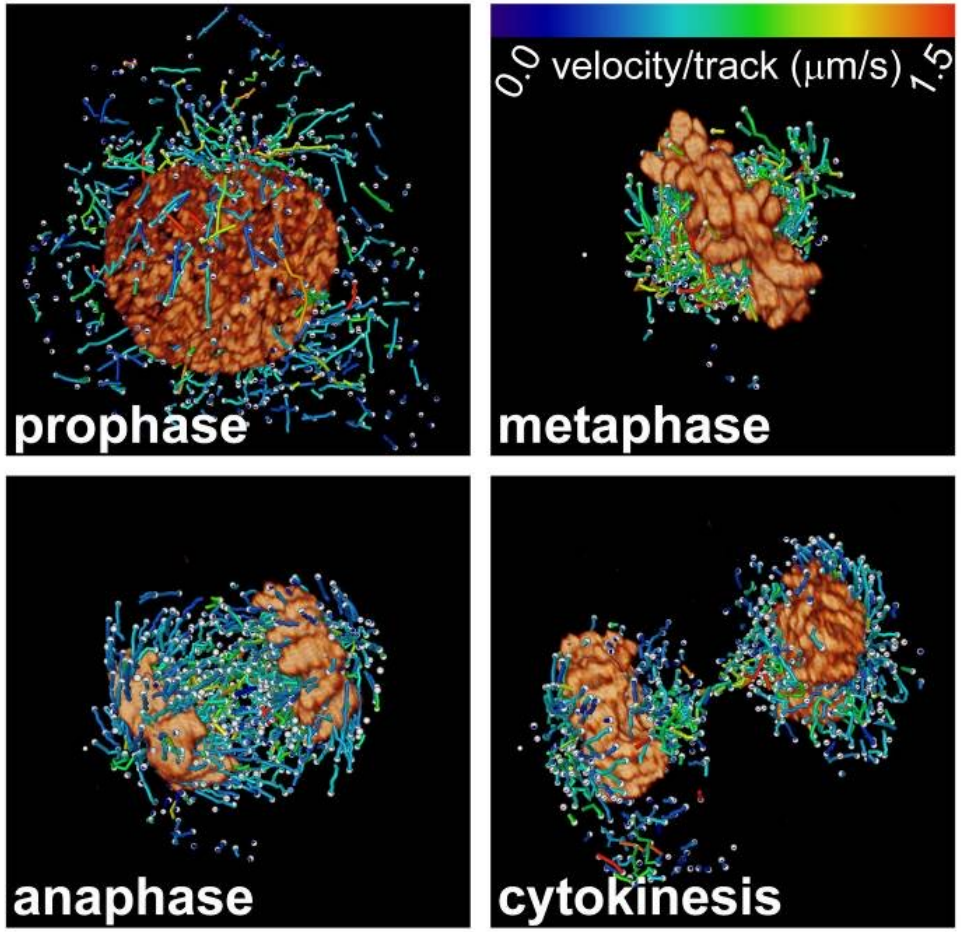


Microtubule *Intermediate Filaments* *Aktinfilaments*

G.Civelekoglu-Scholey, D.Cimini, *The Royal Society*, 2014.

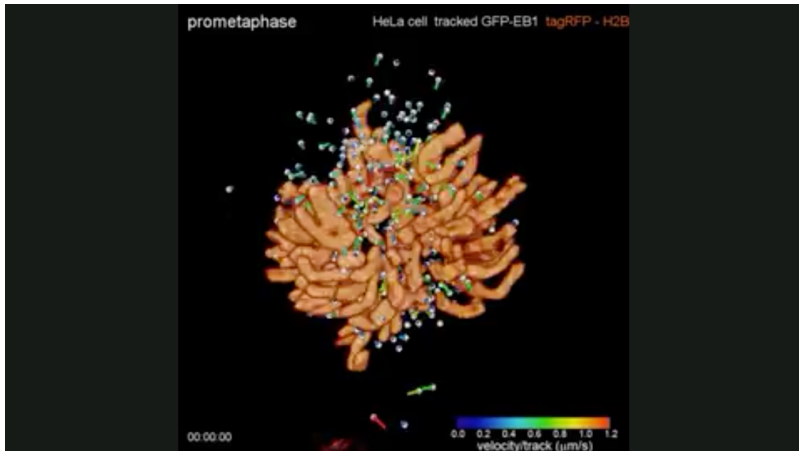
Non-invasive 3D imaging of intracellular dynamics

Trajectories of the endpoints of growing microtubules in HeLa cells

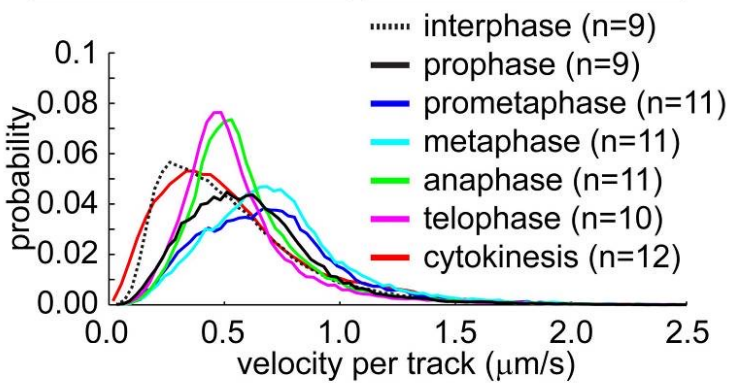


EB1 protein labeled with GFP.
HB2 protein labeled with mTagRFP.

Five different stages during the division of a single HeLa cell



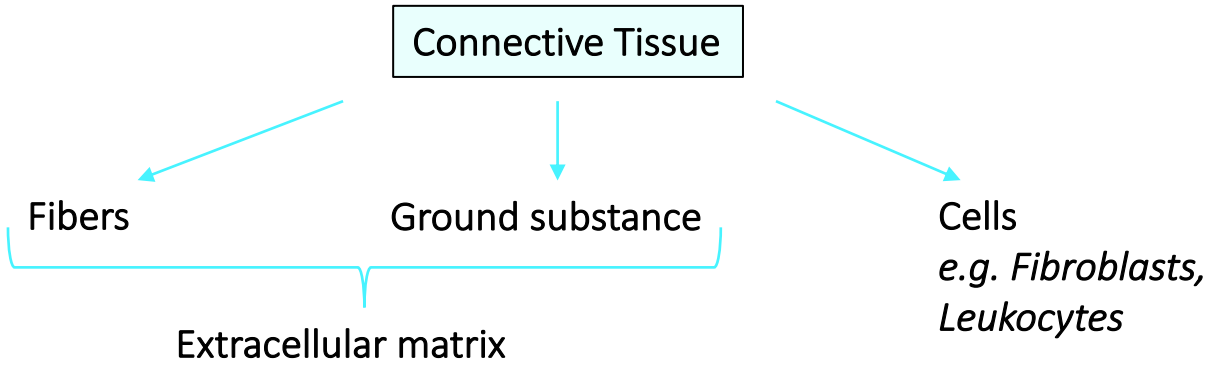
Distributions of the mean velocity of each growth track



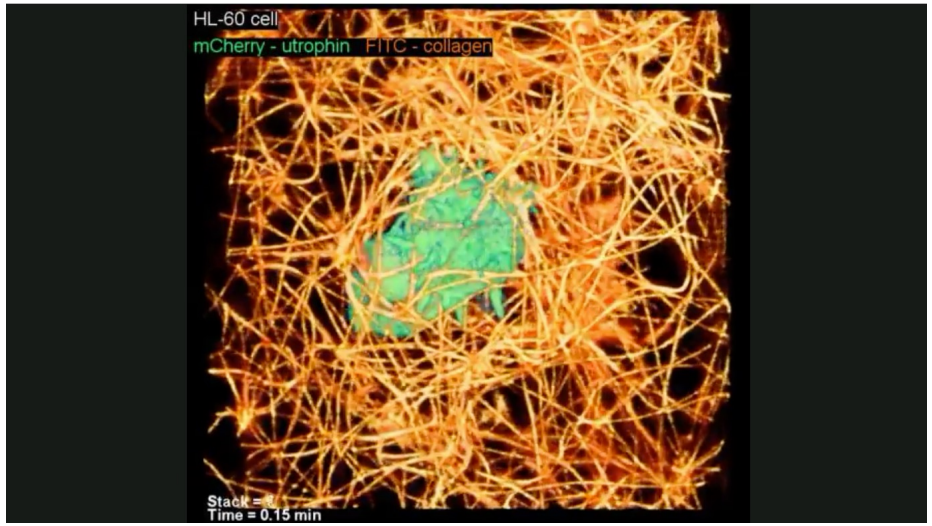
Collated over nine to eleven different cells at seven different stages before and during mitosis.

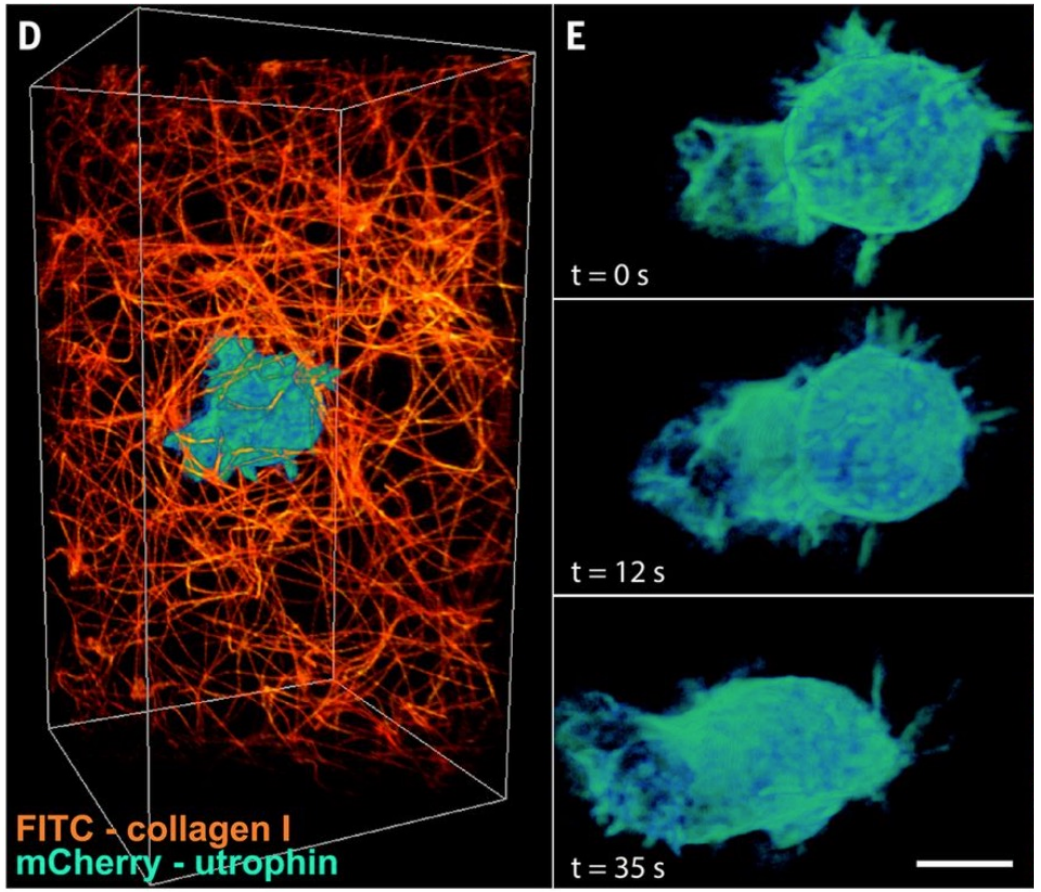
Chen et al., *Science*, 2014.

Cell-matrix interactions



Cell movement through a matrix





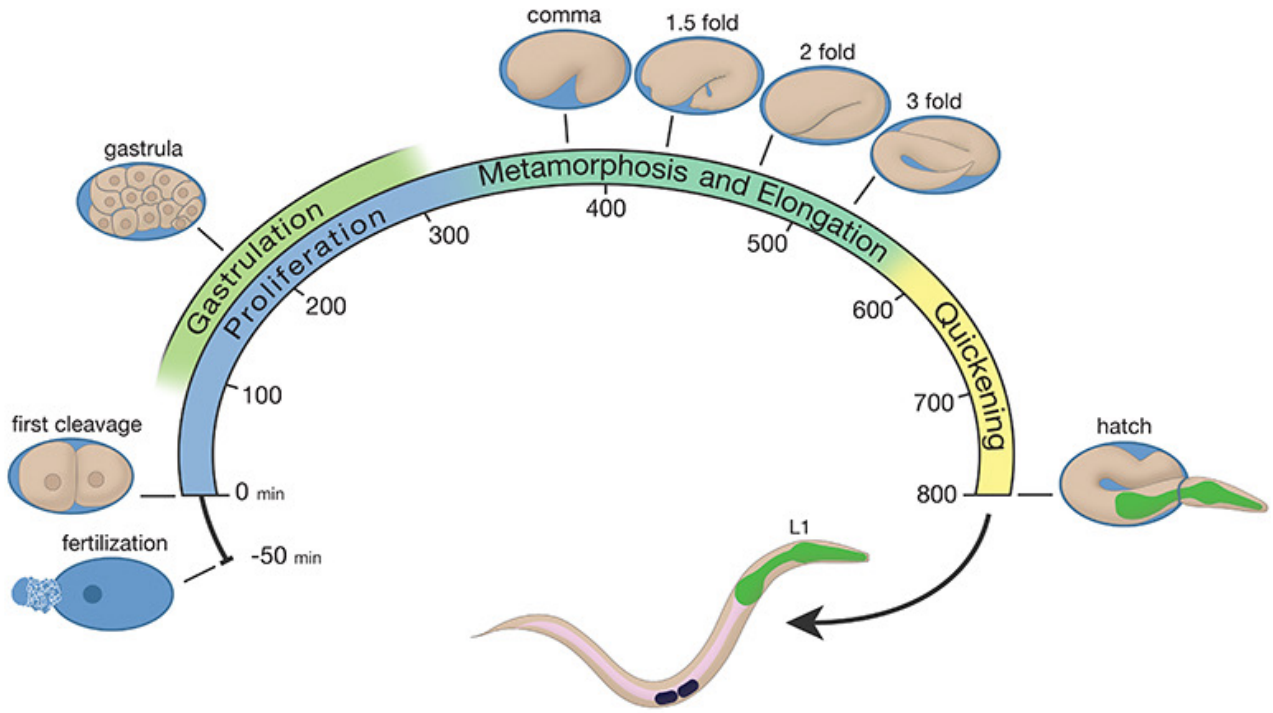
Neutrophil-like human HL-60 cell expressing mCherry-utrophin in a fluorescently labeled collagen matrix.

Volume renderings of the cell at three time points.

Chen et al., *Science*, 2014.

Subcellular physiology of developing cells

Embryonic stages of developement of Caenorhabditis Elegans



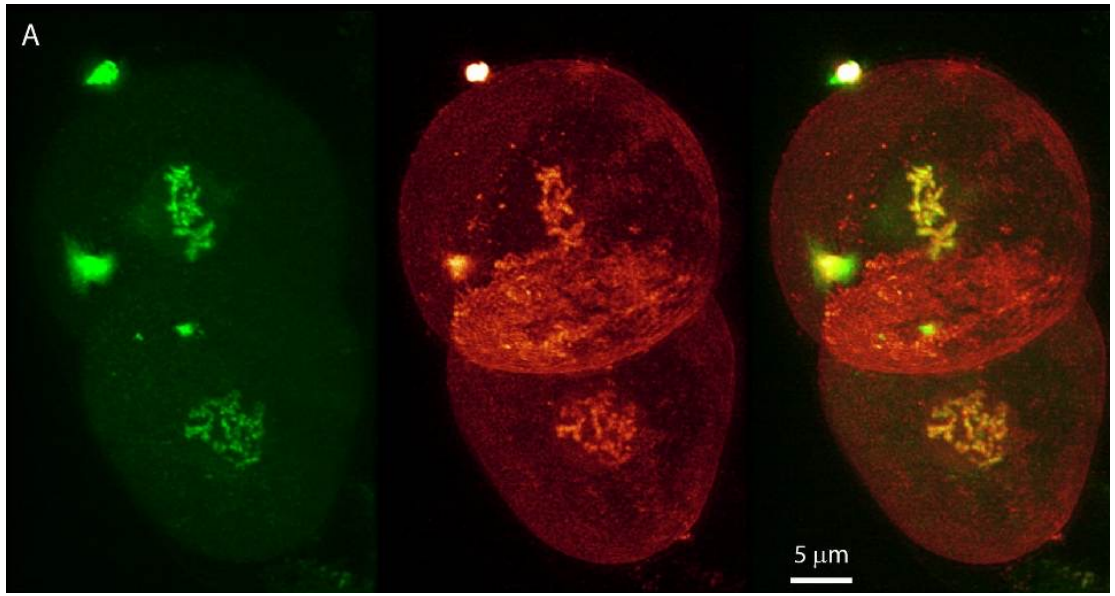
Challenge

Subcellular processes on time-scale of seconds to minutes in contrast to the hours lasting whole development.

D.H. Hall et al., *Introduction to C. elegans Embryo Anatomy*, WormAtlas.

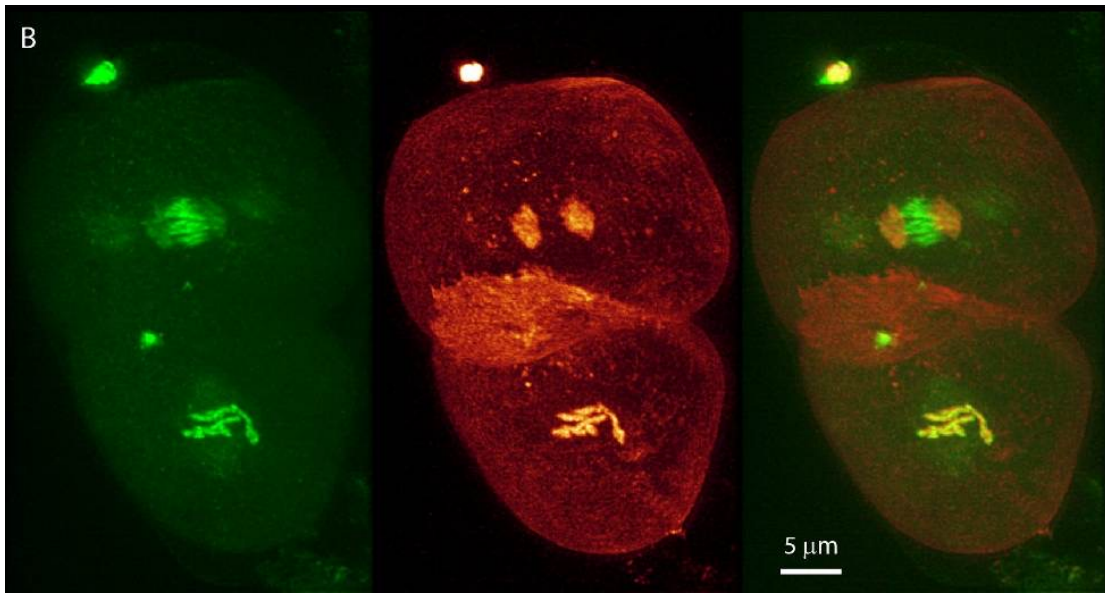
Subcellular physiology of developing cells

Imaging of the chromosomal passenger protein AIR-2 in C. Elegans embryos.



Volume renderings of GFP-AIR- 2, localized at condensed chromosomes during prophase and metaphase.

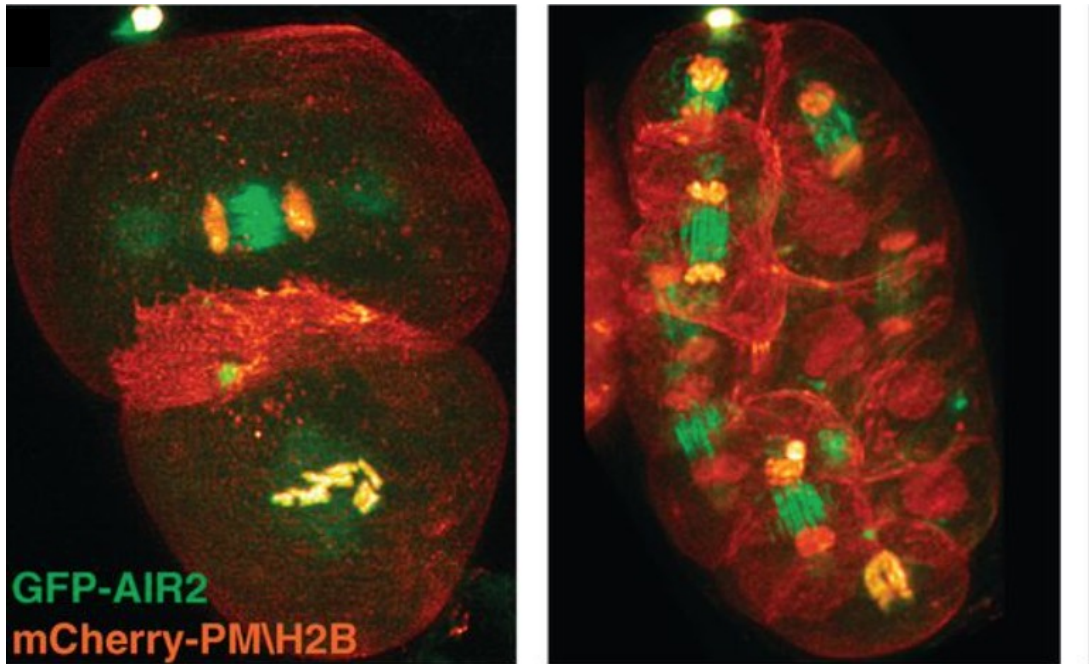
AIR-2 → Green channel
Chromosomes and plasma membrane → Red channel



Volume renderings of GFP-AIR- 2, present on microtubules in the spindle midzone of the AB cell during anaphase.

Chen et al., *Science*, 2014.

Subcellular physiology of developing cells



Two-cell to six-cell developmental stage.

Volume rendering of GFP-PH domains (green) and mCherry-H2B (orange) in *C. elegans* embryo at the three-fold stage, after the onset of muscle contractions.

Chen et al., *Science*, 2014.

Summary

- Two objectives (illumination and detection) arranged in 90° to each other
- Illumination with a light sheet, generated by a lattice
- Possibility of combination with superresolution techniques