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Science for the benefit of humanity

Light Sheet Fluorescence Microscopy

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https://mbo.rockefeller.edu

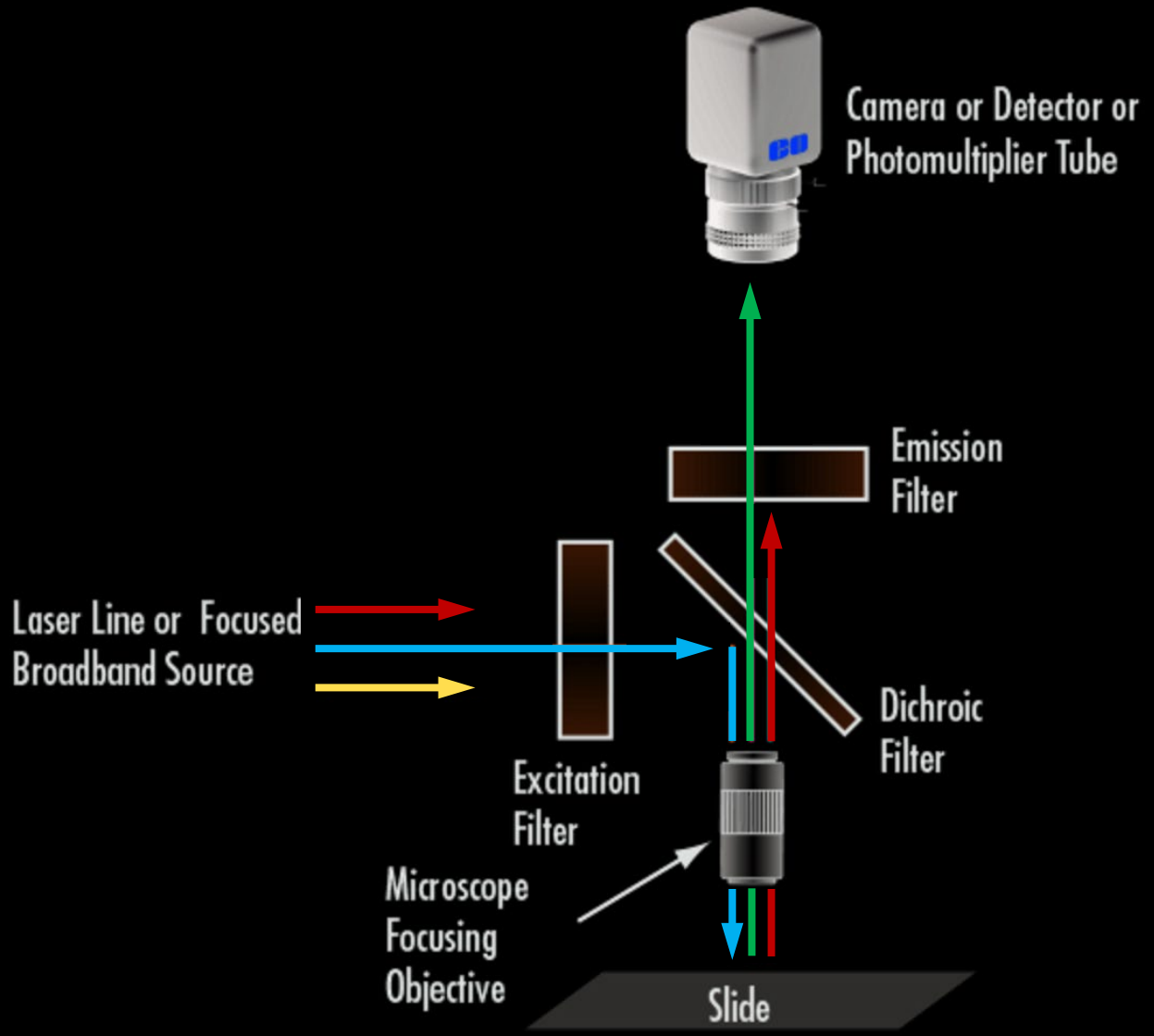
May 28, 2026

Session 5: Fundamentals in Microscopy Workshop

Agenda

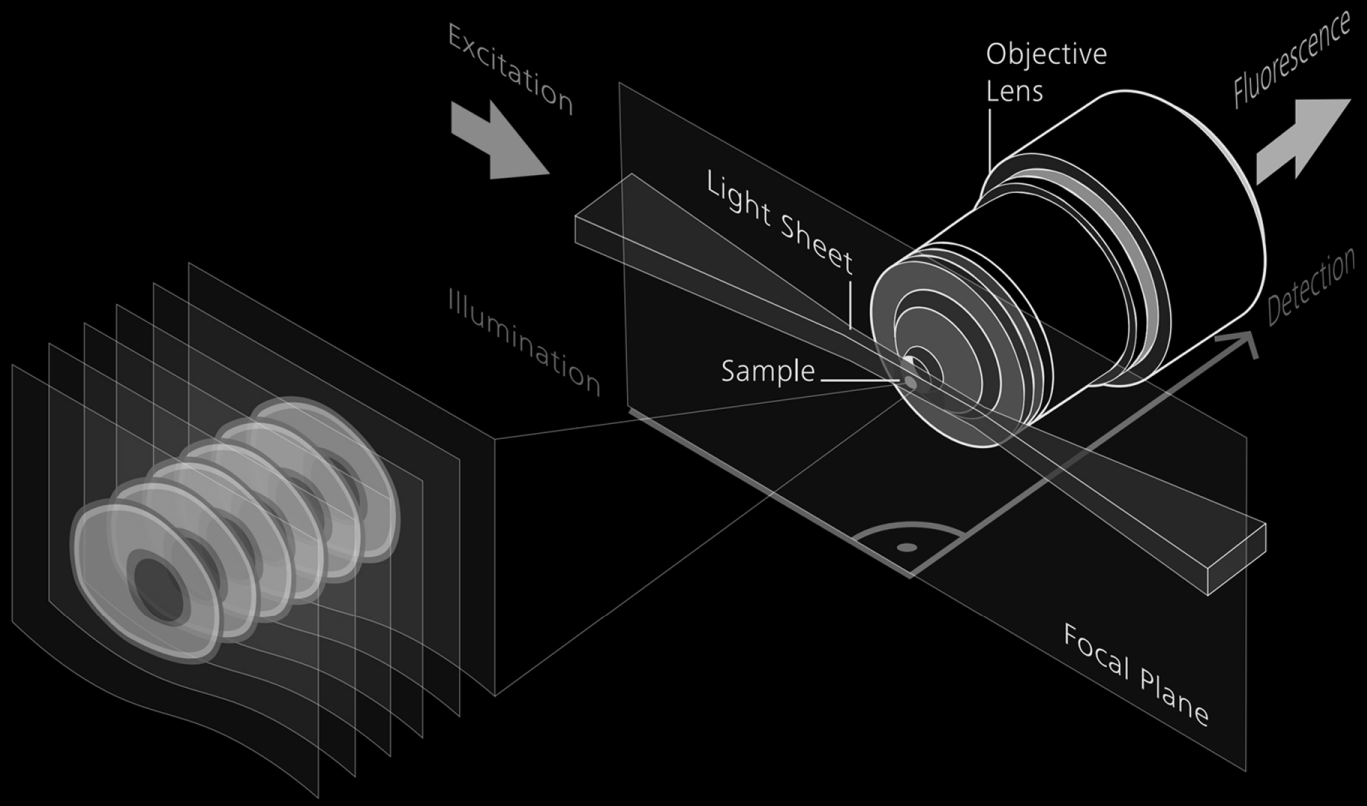
- Basics of light sheet microscopy
- Key concepts: optical sectioning, optical resolution
- What instrument to use?
- Specialized systems accessible to Rockefeller scientists

Basic Setup- Fluorescence Microscopy



Adapted From Edmund Optics Application Notes

Light sheet fluorescence microscopy



Adapted from Zeiss, Light sheet microscopes

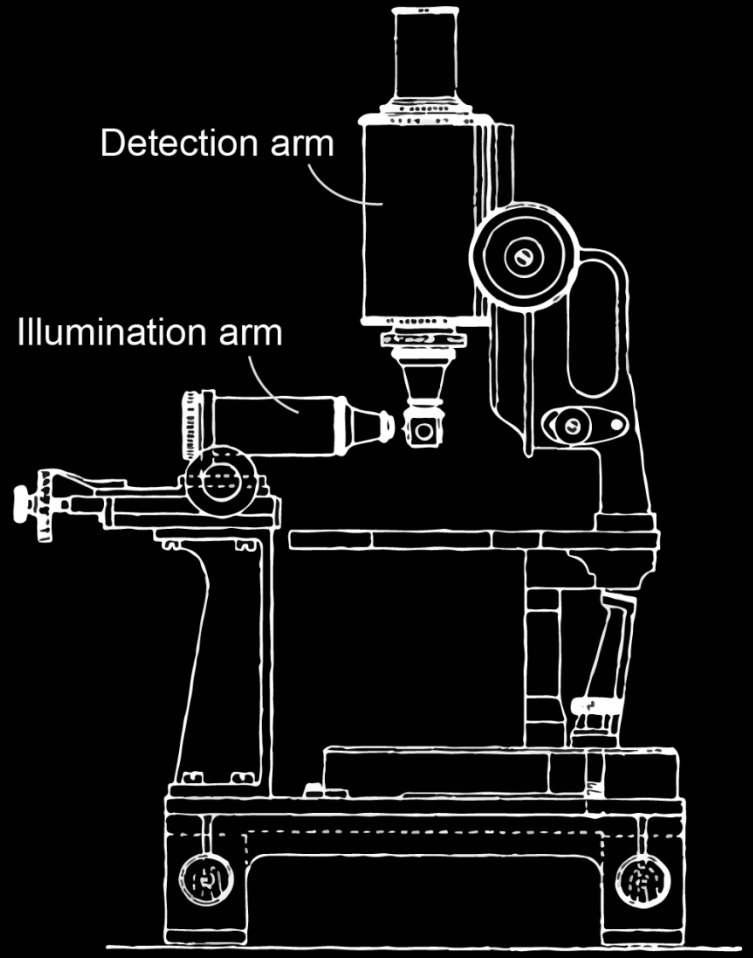
Ultramicroscope

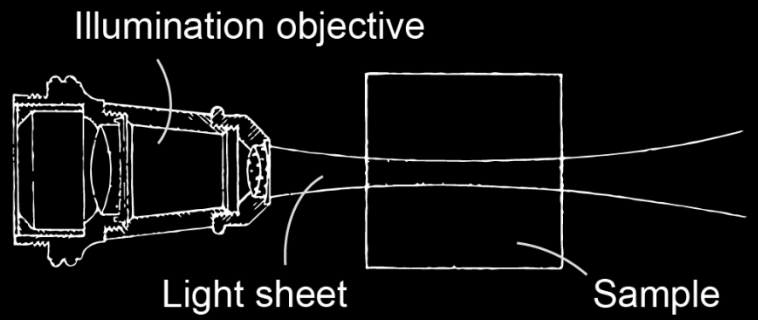
Siedentopf and Zsigmondy 1903

- Light-sheet to inspect colloidal gold

Timelapse: Ultramicroscope microcinematography, Zeiss 1930s

- Microbiology, Parasitology, Plant biology



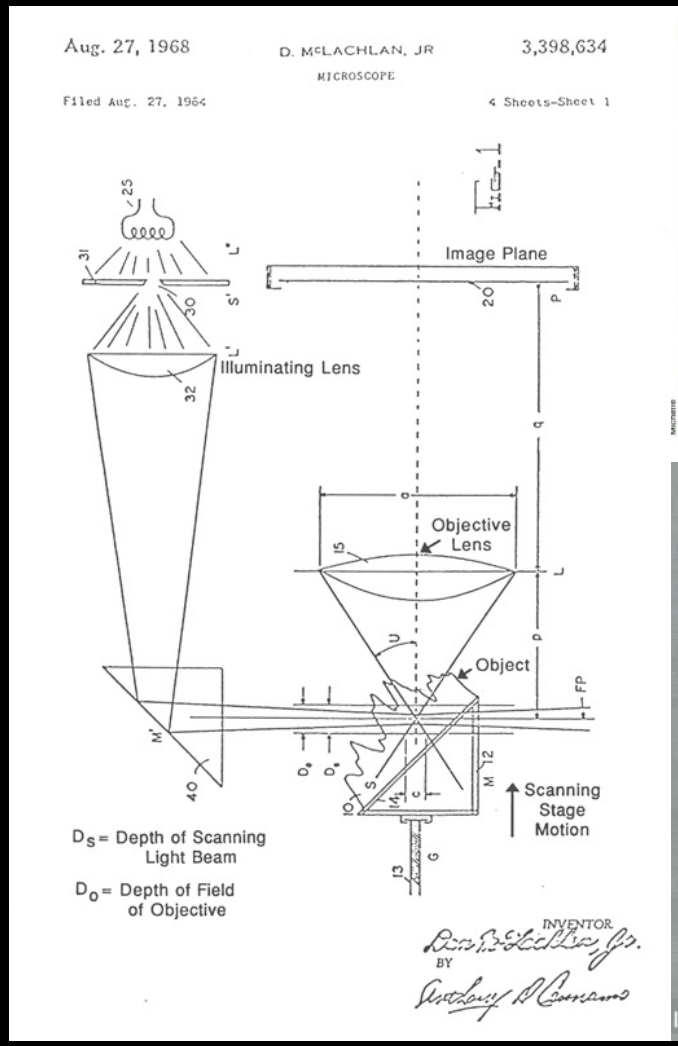


Adapted from H. Siedentopf and R. Zsigmondy (1903), *Annalen der Physik*

Light Scanning Photomacrography (LSP)

'Dynaphot,' Irvine Optical Corporation 1964

- Light-sheet to photograph the surface of 3D objects – shells and insects



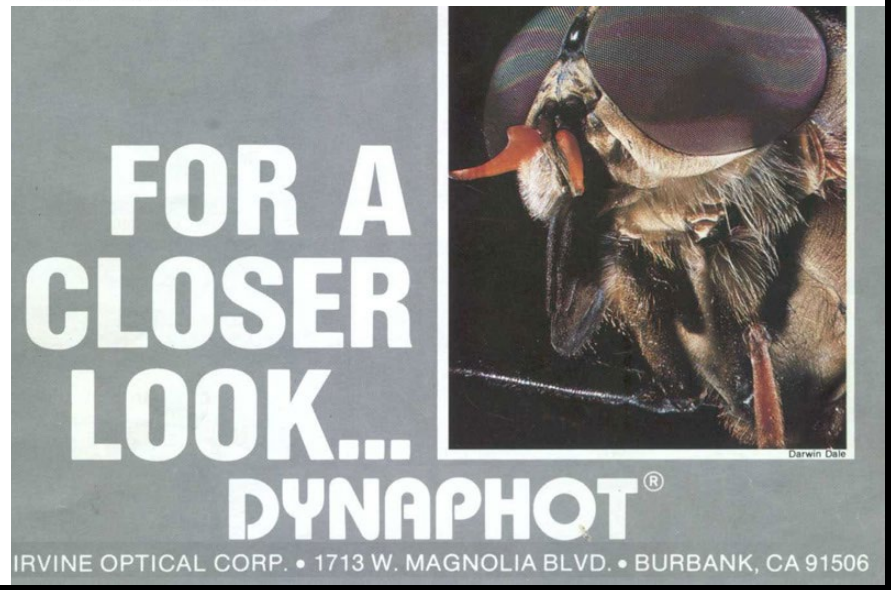

Dynaphot® with Wild M400 Makroskop

DYNAPHOT® Light Scanning Photographic Systems

— A precision light scanning system used to produce photomacrographs of unlimited depth of field. The Dynaphot® functions in conjunction with 4x5 format bellows camera systems, 35mm SLR's and the Wild M400 Makroskop.

— The Dynaphot® is designed to photograph various 3-dimensional objects, using one to four light sources depending on sample size.

— The magnification range is 2x to 40x. Photos can be taken in color as well as black & white. This provides clear sharp details of all hard to focus samples that standard photomacrographs and SEMs over look.



Source: https://www.mccrone.com/mm/scanning-light-photomacrography-system/

Light sheet **fluorescence** microscopy

Orthogonal-plane fluorescence optical sectioning (OPFOS)

Arne Voie, Francis Spelman, University of Washington 1993
- To inspect **cleared** guinea pig cochlea

Selective plane illumination microscopy (SPIM)

Jan Huisken, Ernst Stelzer, EMBL 2004
- Started the current interest in light-sheet microscopy

Digitally scanned light sheet microscopy (DSLM)

Philipp Keller, Ernst Stelzer, EMBL 2008
- "Digital embryo," a database of cell positions, divisions, and migratory tracks

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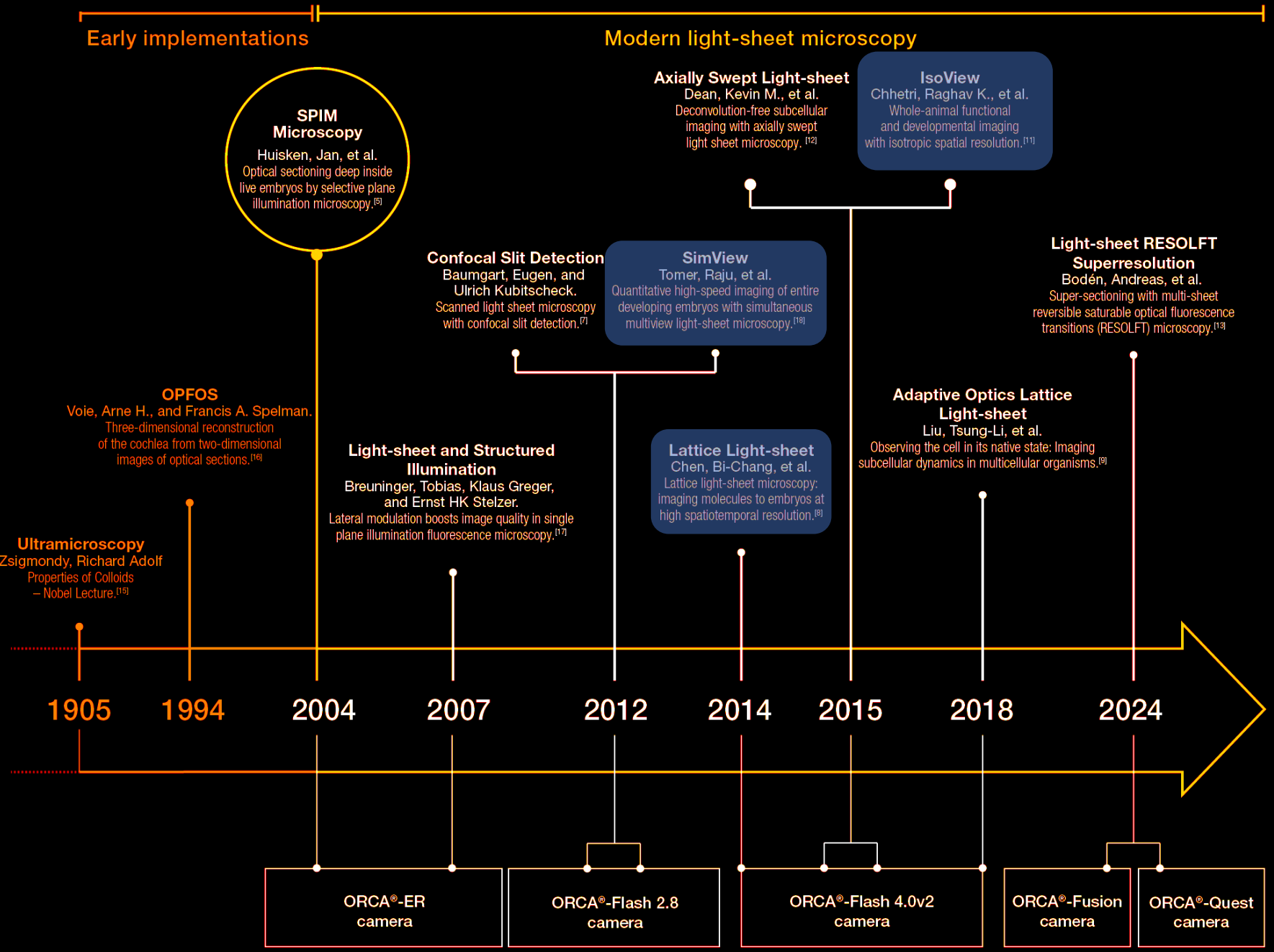
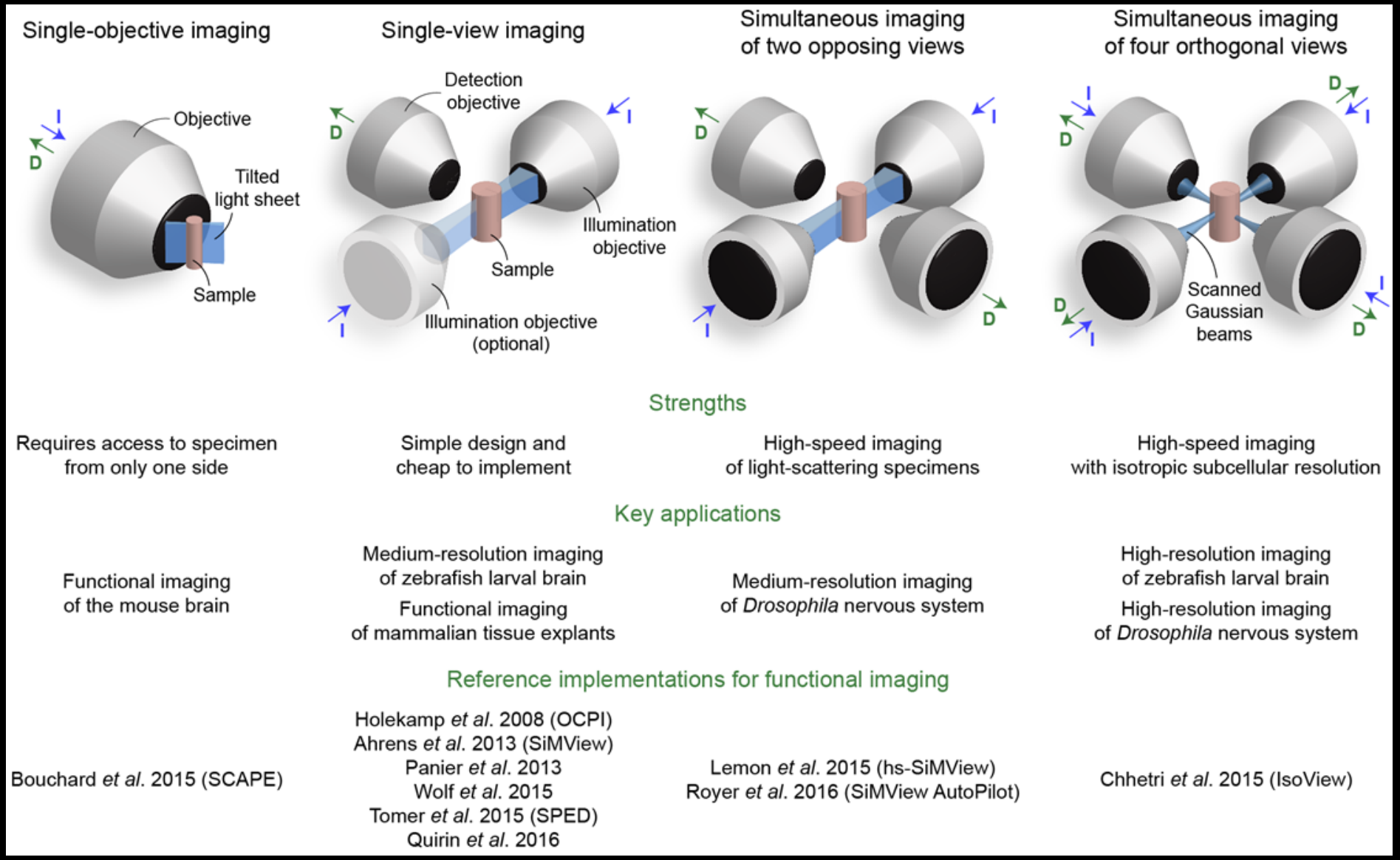


Adapted from Hamamatsu Featured products & technologies, 2025

Acronym overload

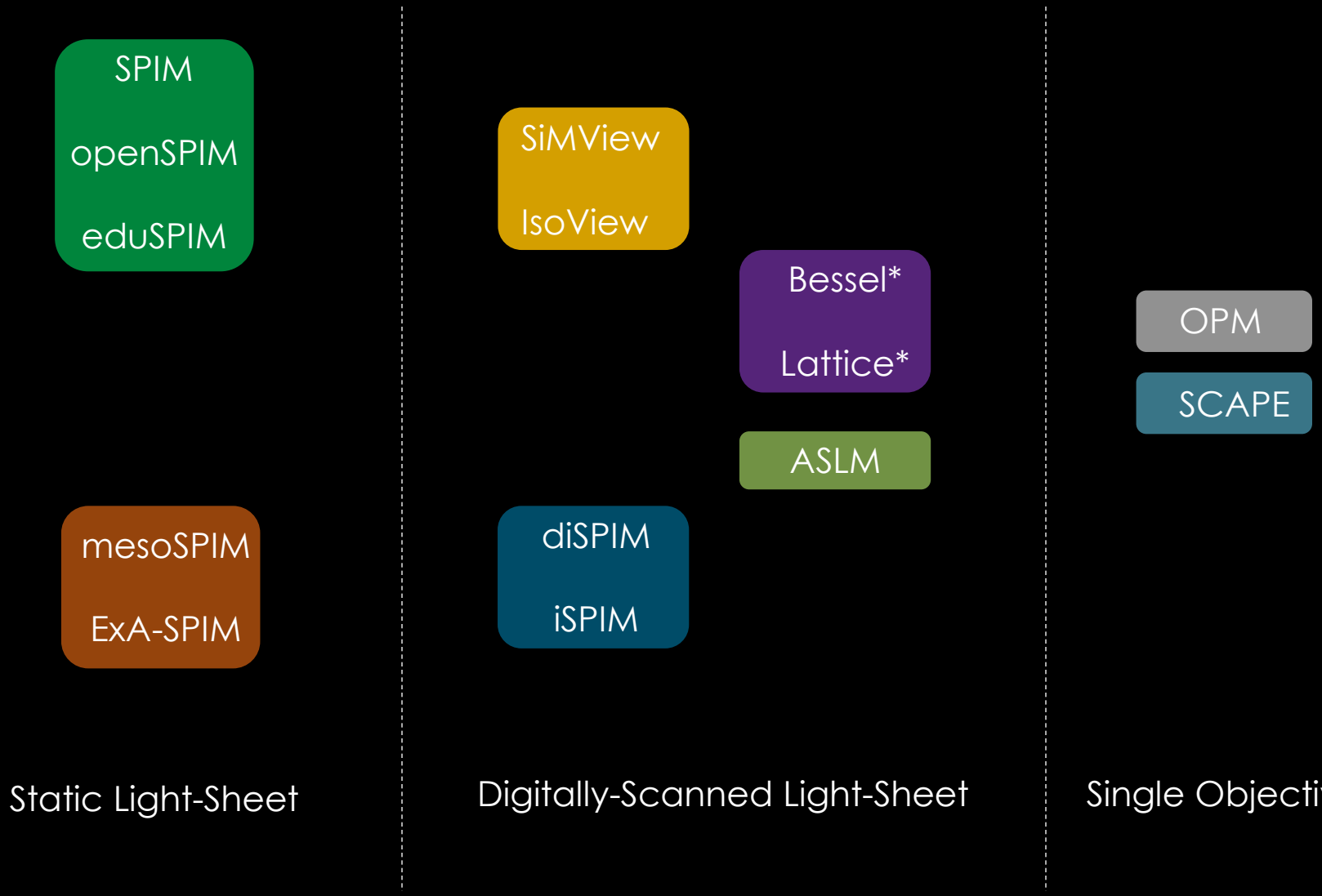
2P-DSLM - 2P3A-DSLM - 2P-SPIM - 2PE-SPIM - 2PE-iSPIM - 2PLS-SOFI - 3D LSM -3P LSFM - Adaptive SPIM - AFM-LS - aLSFMM - AO-LSFM - AO-SPIM - ASLM - BBPI - c-LSFM - csiLSFM - CLSM - COLM - compactLSFM - CTLS - DC LSF IFC - DiaSLM - di2CLSFM - diSPIM - DIY-SPIM - doSPIM - DSLM - DSLM-SI - Edu-SPIM - EzDSLM - FL-DSLM - FLIM-SPIM - HILO - hLCCT - HLTP - HR-OPFOS - IML-SPIM - InVi-SPIM - InVi-SPIM PM - InVi-SPIM PM LS - iSPIM - LEGOLISH - LEMOLISH - L-OpenSPIM - Lattice SPIM - LCS-SPIM - LLS - LLSFM - LightSheet Z.1 - LightSheet 7 - LISH-M - LSBM - LLSDM - LSF IFC - LSFM - LSLFM - LSP - LST - MesoSPIM - miniSPIM - MISERB - MLSM - mSPIM - muSPIM - MuVi-SPIM - MuviSPIM LS - Muvi SPIM LS PM - Muvi SPIM CS - Muvi SPIM CS PM - OCPI - OpenSPIM Farm - Open Top SPIM - OPFOS - OPM - OPTiSPIM - oSPIM - OTLS - πSPIM - PIP - PCLSM - PLIF - pLSFM - PLST - QuviSPIM - RESOLFT - RLSM - SCAPE - sideSPIM - SiMView - SLM-SPIM - SPIM selective - SPIM single - SPIM-FCS - SPIM-FCCS - SPIM-fluid - SPIM- µPIV - soLSM - SOPi - soSPIM - socSPIM - SPIDDM - SPOT - STED-MISERB - STED-SPIM - sTSLIM - SVI-LFM - T-OpenSPIM - TC-LSFM - TILT3D - TLS-SPIM - TLSM - TSLIM - TruLive3D - Ultramicroscope – BTLSM - UltraMicroscope II – BTLSM - WAO-SPIM - X-OpenSPIM

Flavors of light-sheet microscopes



R. K. Chhetri, P. J Keller (2020), Handbook of Neurophotonics

Custom-built light-sheet systems

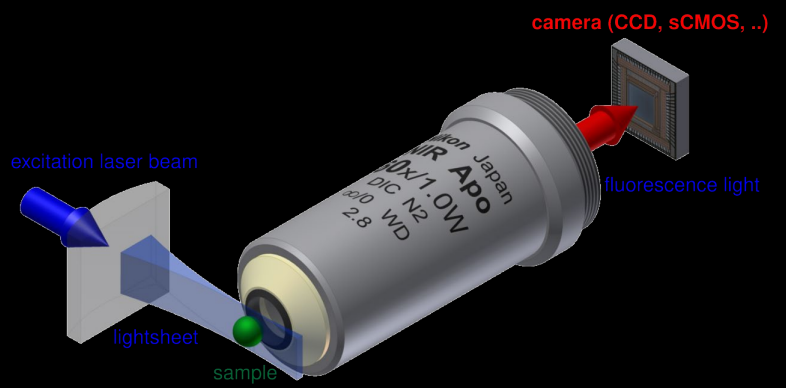


Not exhaustive!
Only listing a few sustained, active development

* Non-Gaussian Beam

Static light-sheet

A sheet of light shaped with a cylindrical lens



Selective Plane Illumination Microscopy (SPIM)

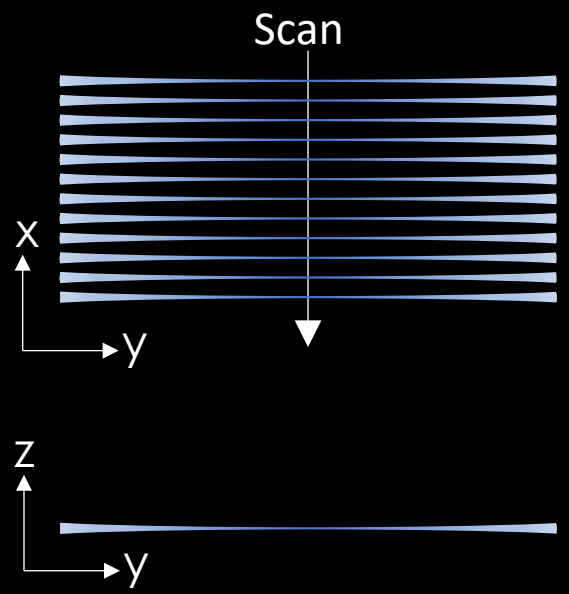
Simple and cost-effective

Light-sheet height adjusted with an aperture

Beam non-uniformity along X results in distinct intensity gradients

Digitally-scanned light-sheet

A low NA beam scanned (along X) to generate a virtual sheet of light



Forms images with uniform intensity

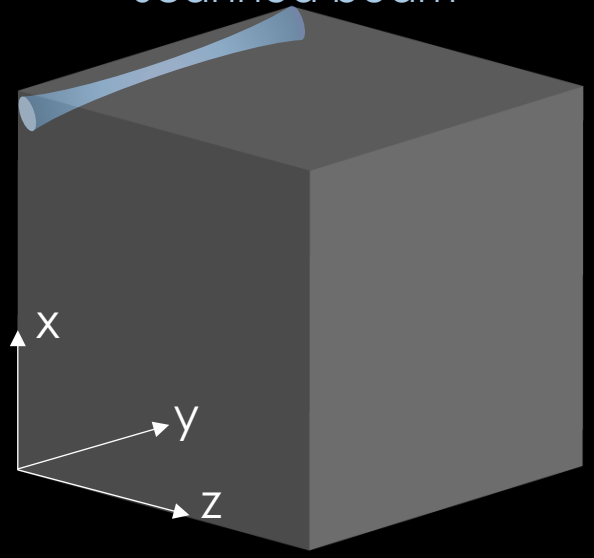
Light-sheet height (along X) digitally adjustable

Higher background suppression

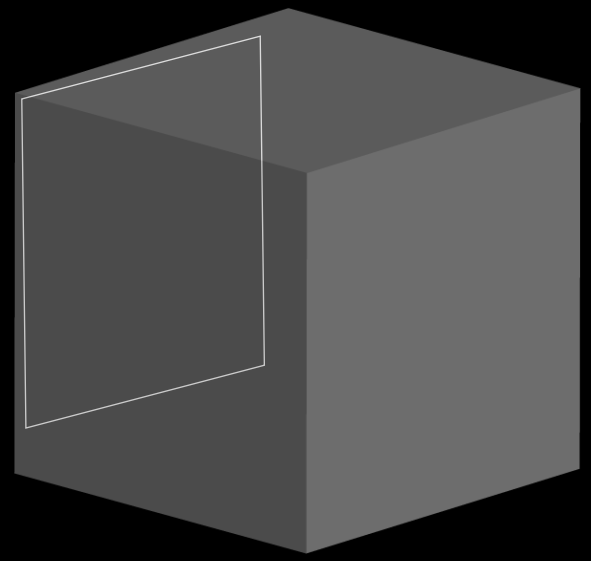
Digitally-scanned light-sheet: volumetric acquisition

Axial scan of the detection objective

Scanned beam



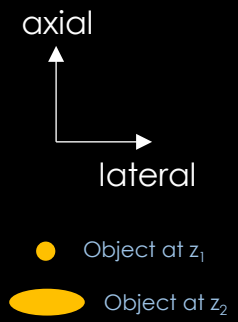
Sample volume

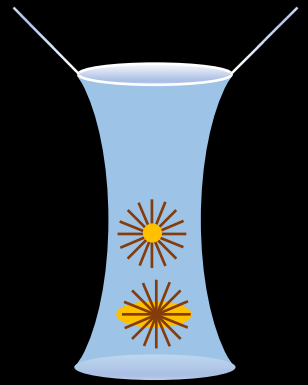


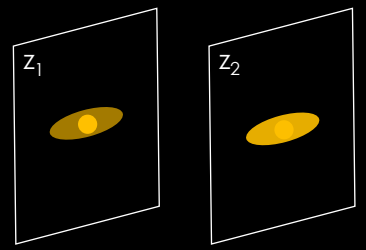
Optically sectioned image

Optical sectioning

Wide-field



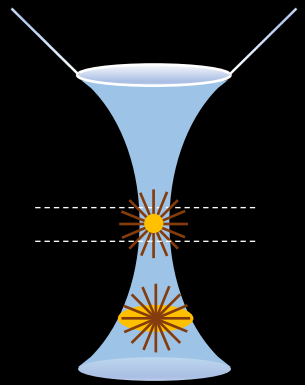


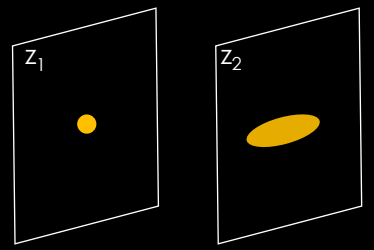


Images at different depth

Lacks inherent optical sectioning

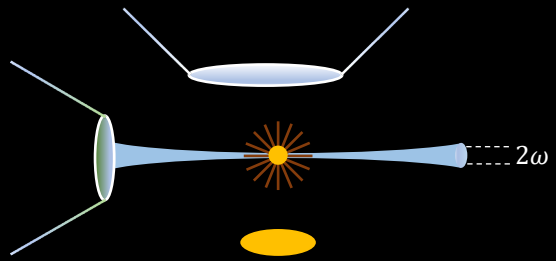
Confocal

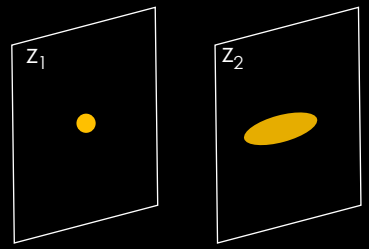




Optical sectioning via the size of the pinhole

Light-sheet

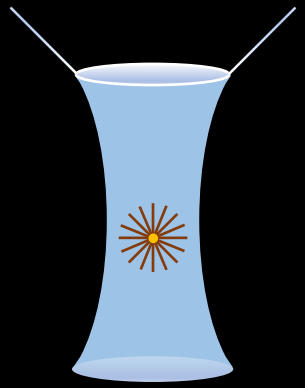




Optical sectioning via the thickness of the light-sheet

Optical resolution

Wide-field



Axial

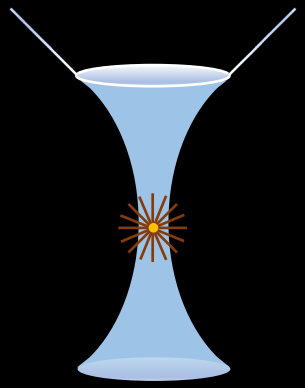
Lateral

- Sub-diffraction point source
- Image of the point source

$$R_{xy} = \frac{0.61\,\lambda}{NA}$$

$$R_z = \frac{2\,n\,\lambda}{NA^2}$$

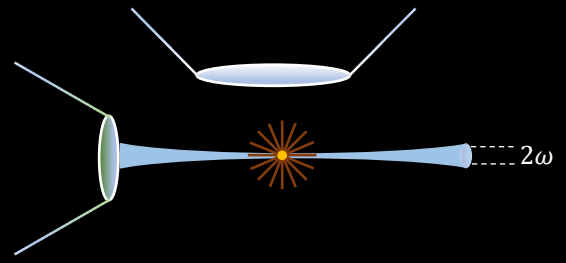
Confocal



$$\geq \frac{R_{xy}}{\sqrt{2}}$$

$$\geq \frac{R_z}{\sqrt{2}}$$

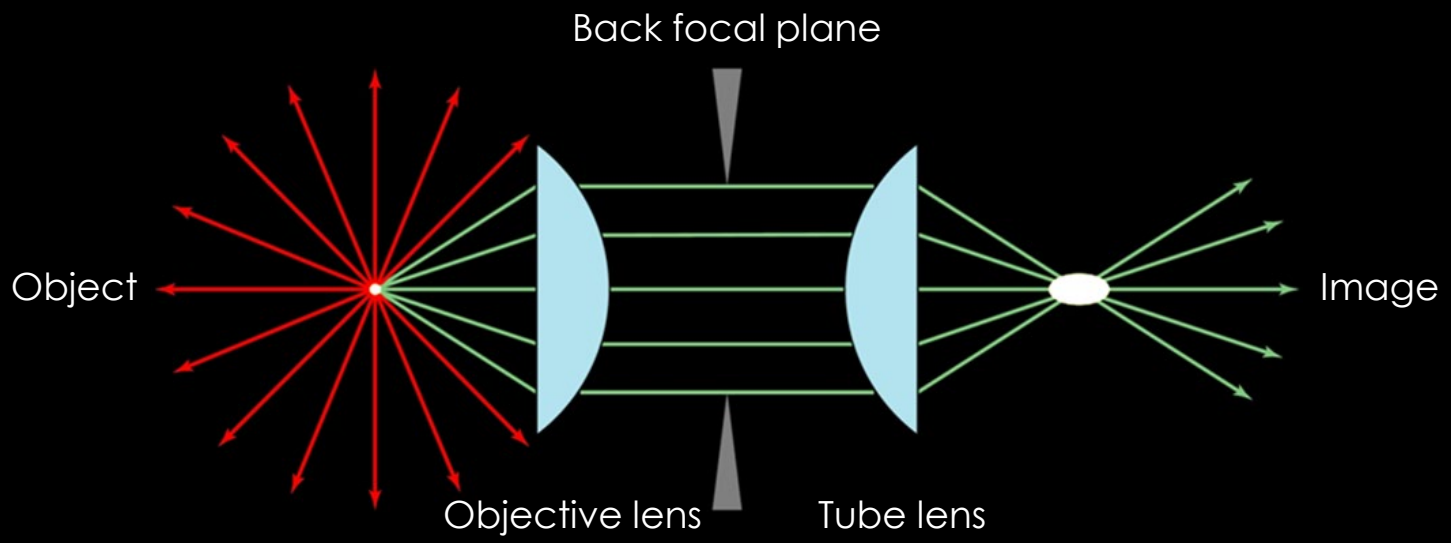
Light-sheet



$$R_{xy}$$

$$\sim \left(\frac{1}{R_z} + \frac{1}{2\omega}\right)^{-1}$$

Resolution anisotropy in 3D image data



Lateral Resolution

$$R_{xy} = \frac{0.61\,\lambda}{NA}$$

Axial Resolution

$$R_z = \frac{2\,n\,\lambda}{NA^2}$$

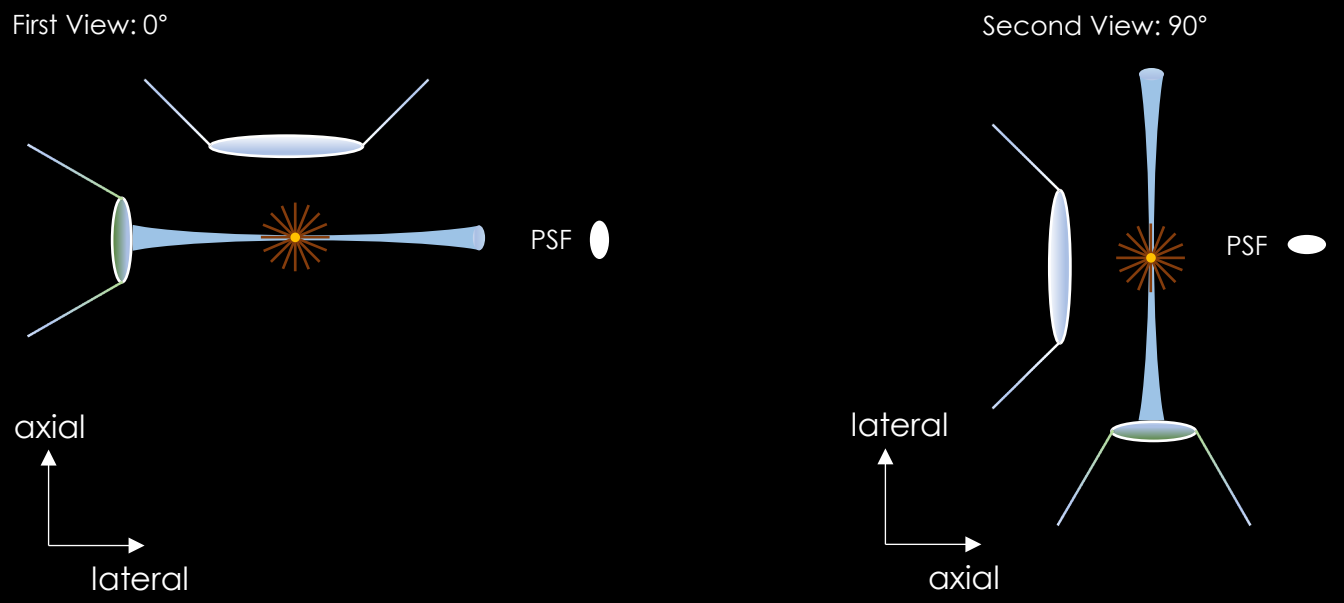
NA			Resolution Anisotropy
0.3	1.1 µm	15.6 µm	14x
0.8	420 nm	2.2 µm	5.2x
1.0	320 nm	1.4 µm	4.4x

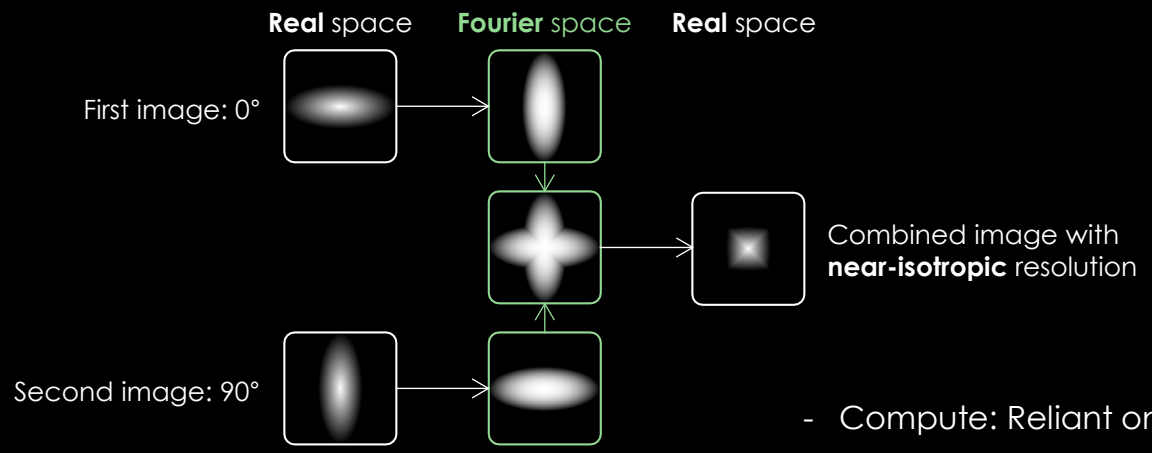
- Emission wavelength of 525 nm
- In water, refractive Index of 1.333
- Light-sheet thickness not taken into consideration

Isotropic resolution: Acquisition from multiple views

IsoView

diSPIM





Limitation:

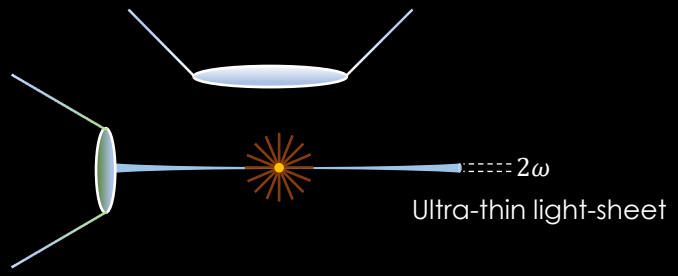
- Compute: Reliant on computational registration and deconvolution

J. Swoger *et al*, Multi-view image fusion improves resolution in three-dimensional microscopy, *Optics Express* **15**, 8029 (2007).

Isotropic resolution: Ultra-thin light-sheet

Lattice light sheet

Axially swept light sheet



$$R_{\mathrm{xy}}$$

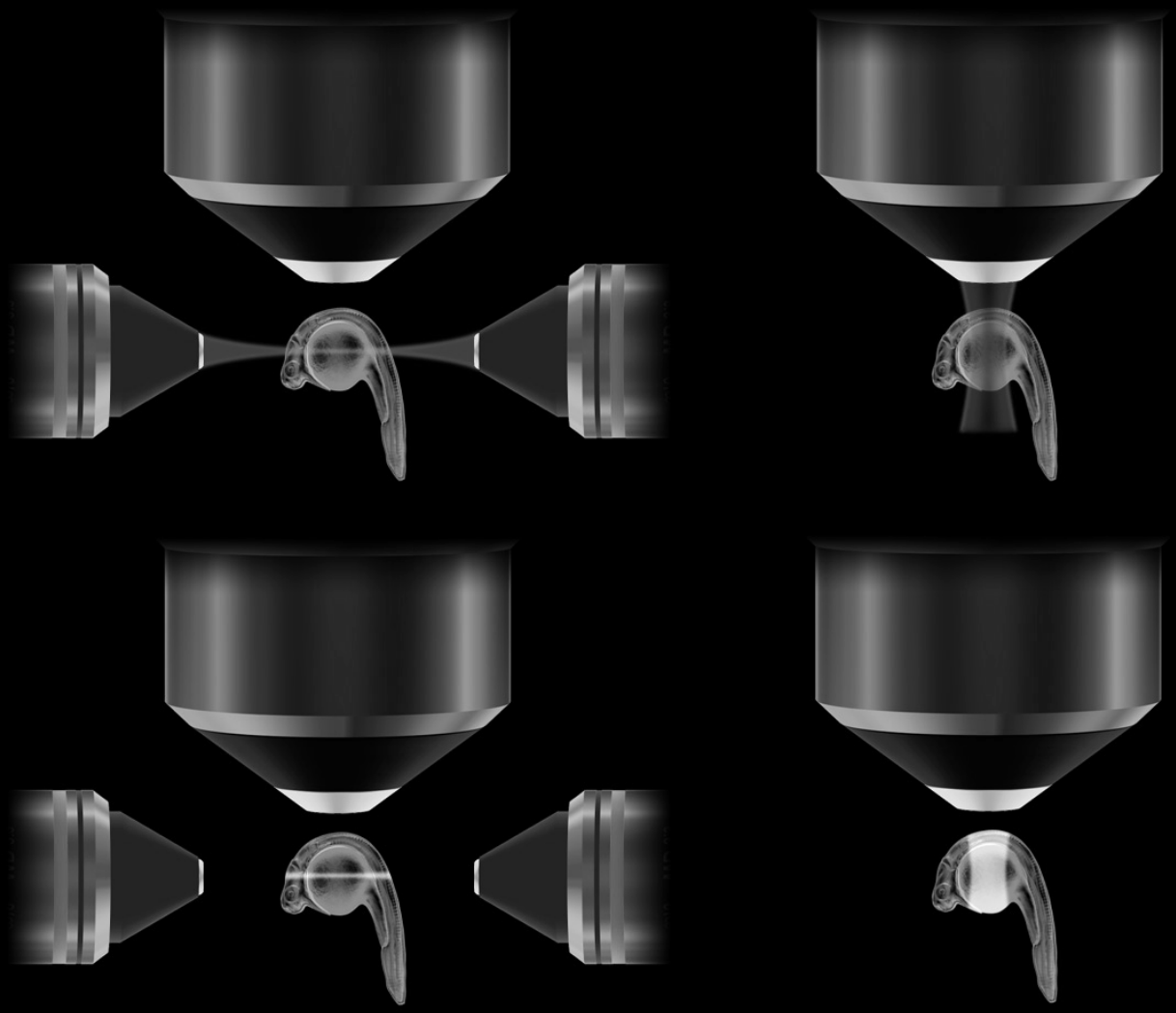
$$\sim \left(\frac{1}{R_z} + \frac{1}{2\omega}\right)^{-1}$$

Limitations:

- Restricted to a small field-of-view (Lattice)
- Reduced speed: sample scanning (Lattice), sweeping of a Gaussian focus (ASLM)
- Ultra-thin light-sheet: susceptible to out-of-focus motion artifacts

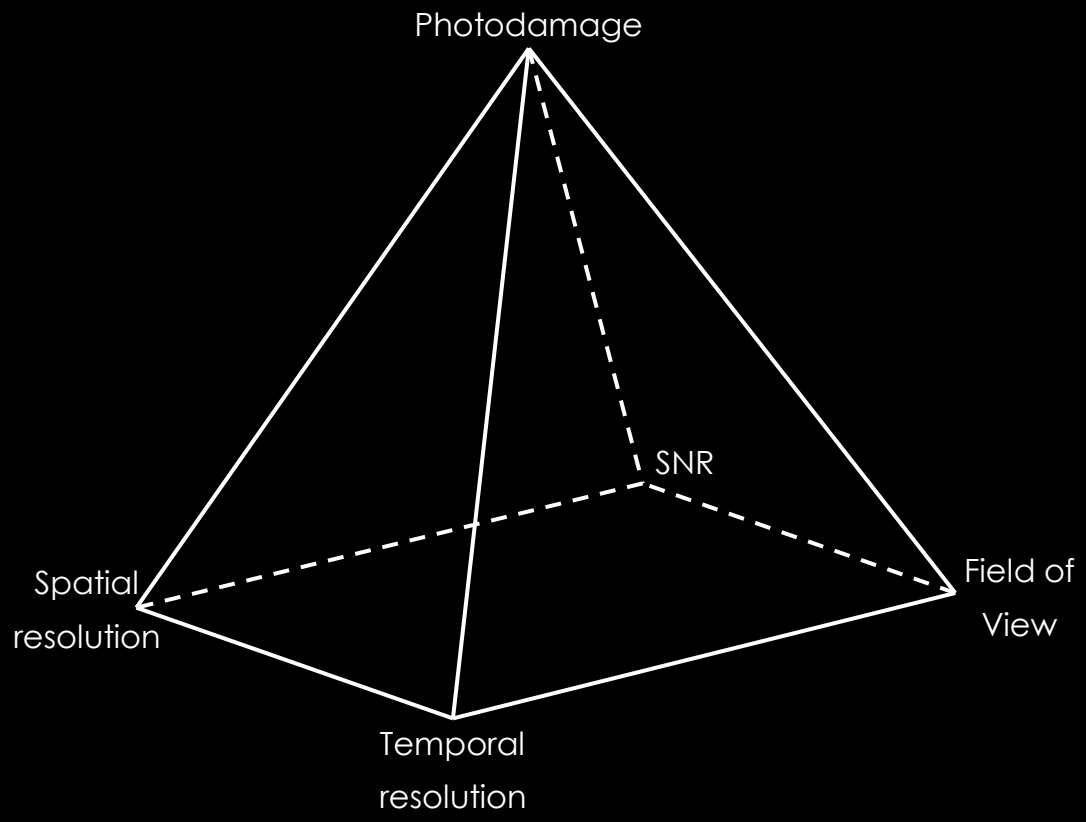
Light-sheet: gentle imaging

Minimal photo-damage with light-sheet imaging



Adapted from https://www.microscopyu.com/techniques/light-sheet/light-sheet-fluorescence-microscopy

Trade-offs



Light-sheet microscopy

- ✓ High Spatiotemporal resolution
- ✓ Large physical coverage
- ✓ Low Background
- ✓ Low power density
- ✓ Long-term imaging

Recap

	Wide-field	Confocal	Spinning Disk Confocal	Light-sheet
Diffraction-limited resolution	✓ *	✓	✓	✓
Optical sectioning	x **	✓	✓	✓
Rapid Volumetric Acquisition	✓	x	✓	✓
Low Photobleaching, phototoxicity	x	x	x	✓

* For thin samples, otherwise hindered by lack of contrast

** Relies on the depth of field of the detection objective

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What light-sheet instrument to use?

What do you want to see?

openSPIM
eduSPIM

Simple and customizable setups

diSPIM
iSPIM

Bessel*
Lattice*

Fast and high-resolution imaging of small, mostly flat samples (<100 µm in depth)

OPM
SCAPE

Fast imaging of samples (~100 µm in depth), single objective access to samples

mesoSPIM**
ExA-SPIM**

Imaging of large, centimeters-scale cleared samples

ASLM

High-resolution imaging of small samples (<100 µm in depth) or large, cleared samples

SiMView
IsoView

Fast and high-resolution imaging of large 3D samples (100 µm to >1 mm), cleared samples

Commercial options

** Utilizes ASLM

* Non-Gaussian Beam

Light-Sheet flavors accessible to Rockefeller scientists

Lattice

Fast and high-resolution imaging of small, mostly flat samples (<100 µm in depth)

SCAPE

Fast imaging of samples (~100 µm in depth), single objective access to samples

SiMView

IsoView

Fast and high-resolution imaging of large 3D samples (100 µm to >1 mm), cleared samples

Ultramicroscope Blaze

Fast imaging of multiple centimeter-scale cleared tissue samples

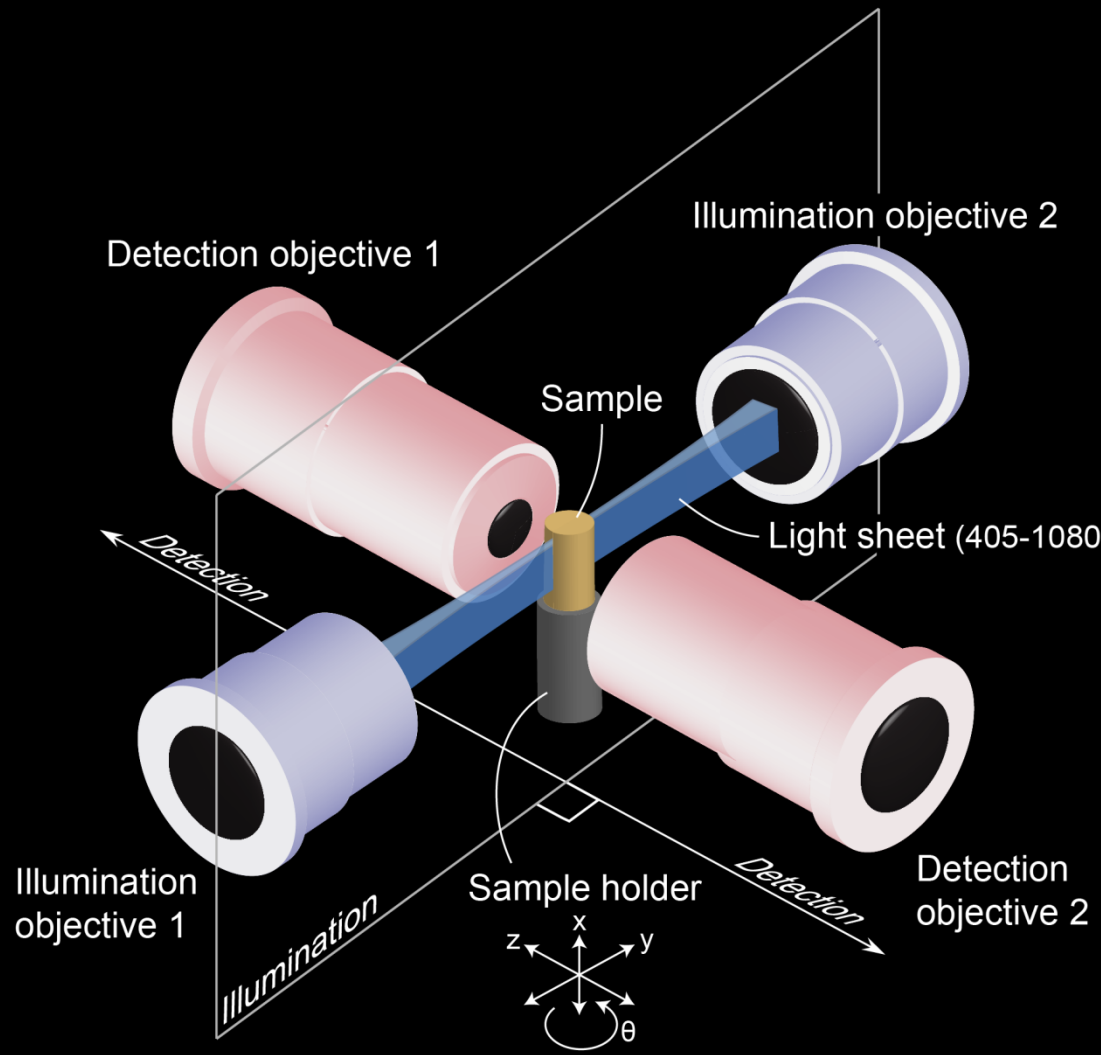
MuVi SPIM LS

MuVi SPIM CS

TrueLive3D Imager

Available at MSK

SiMView: Simultaneous multi-View light-sheet microscopy

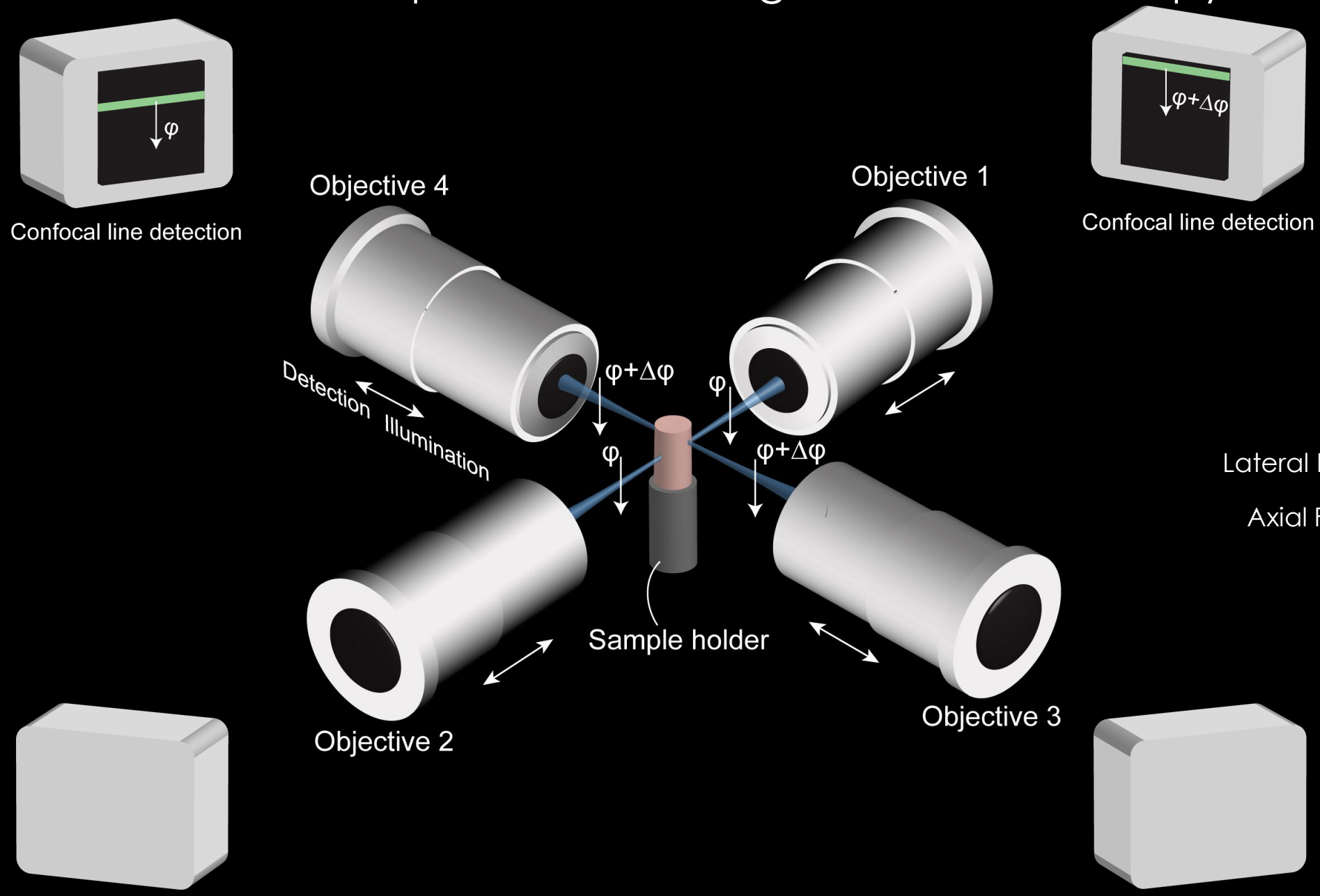


Lateral FWHM ~ 400 nm

Axial FWHM ~ 1.5 µm

Tomer, R., Keller, P.J. et. al., Quantitative high-speed imaging of entire developing embryos with simultaneous multiview light-sheet microscopy. Nat. Methods 9, 755–763 (2012).

IsoView: Isotropic, multi-View light-sheet microscopy



Lateral FWHM ~ 400 nm

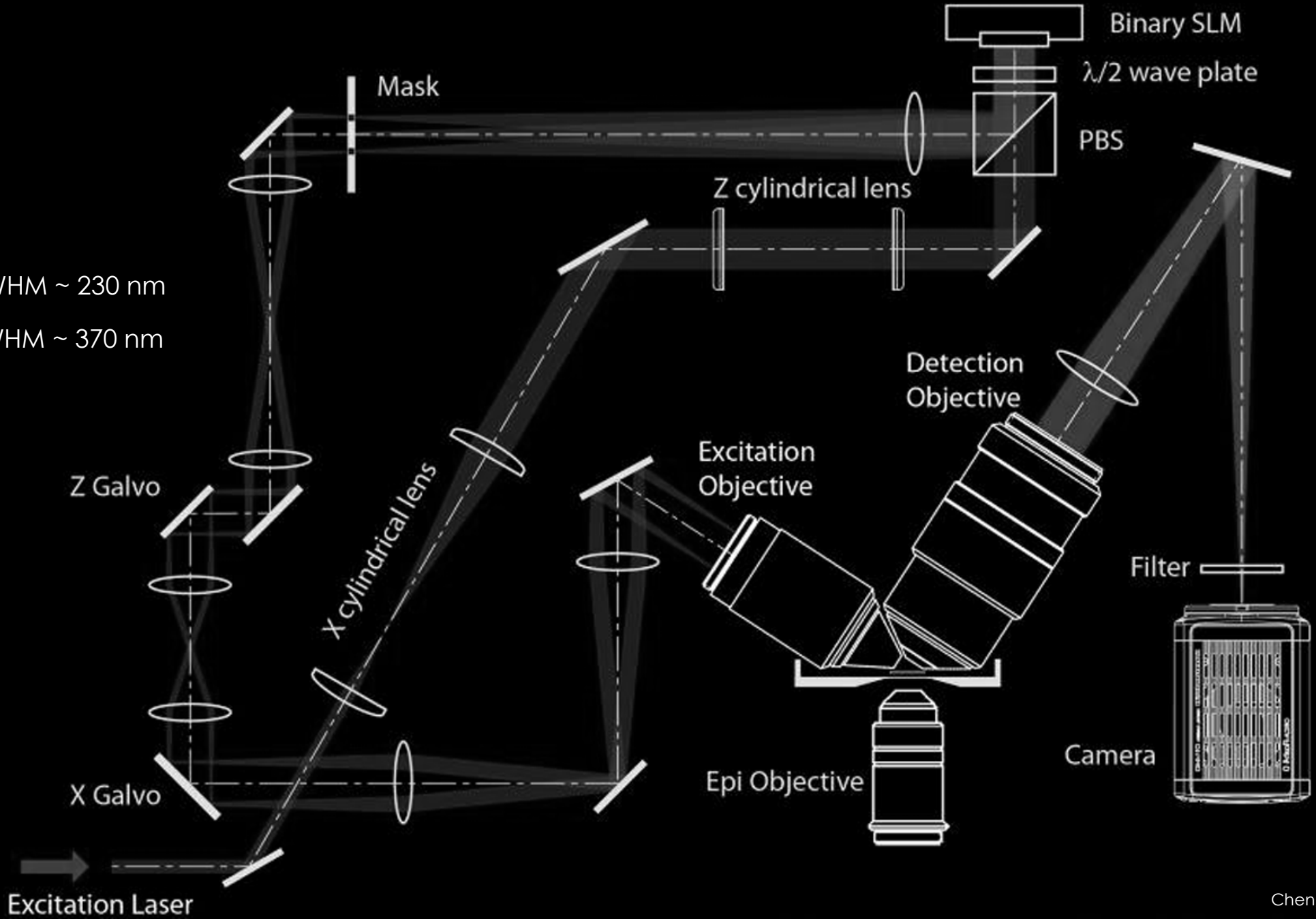
Axial FWHM ~ 400 nm

Chhetri, R. et al. (2015), Nature Methods

Lattice light-sheet microscopy

Lateral FWHM ~ 230 nm

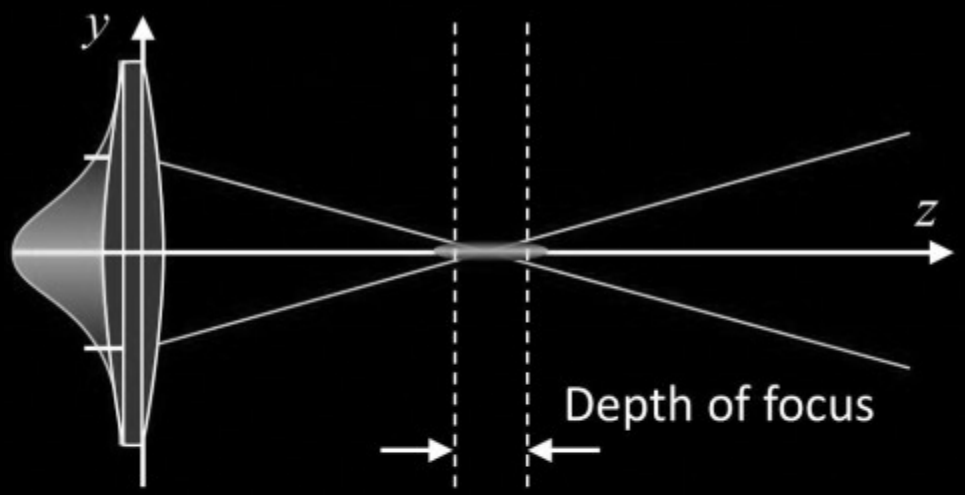
Axial FWHM ~ 370 nm

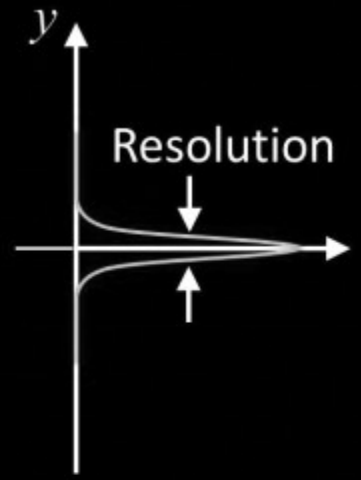


Chen, B., et al., Science 2014

Beam shaping

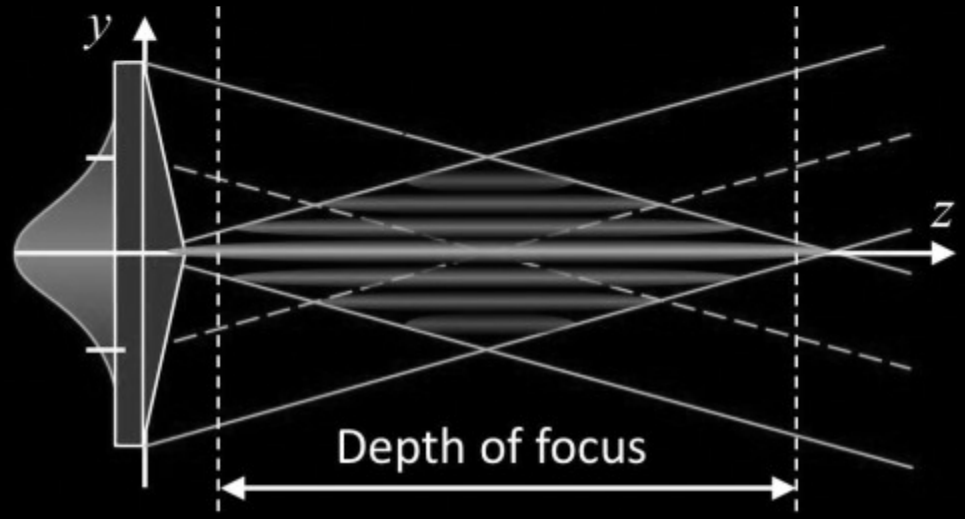
Input:
Gaussian Beam

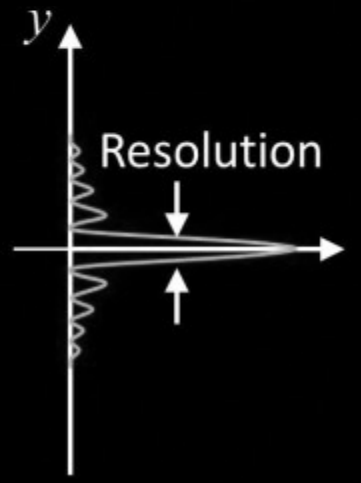




Output:
Gaussian Beam

Input:
Gaussian Beam

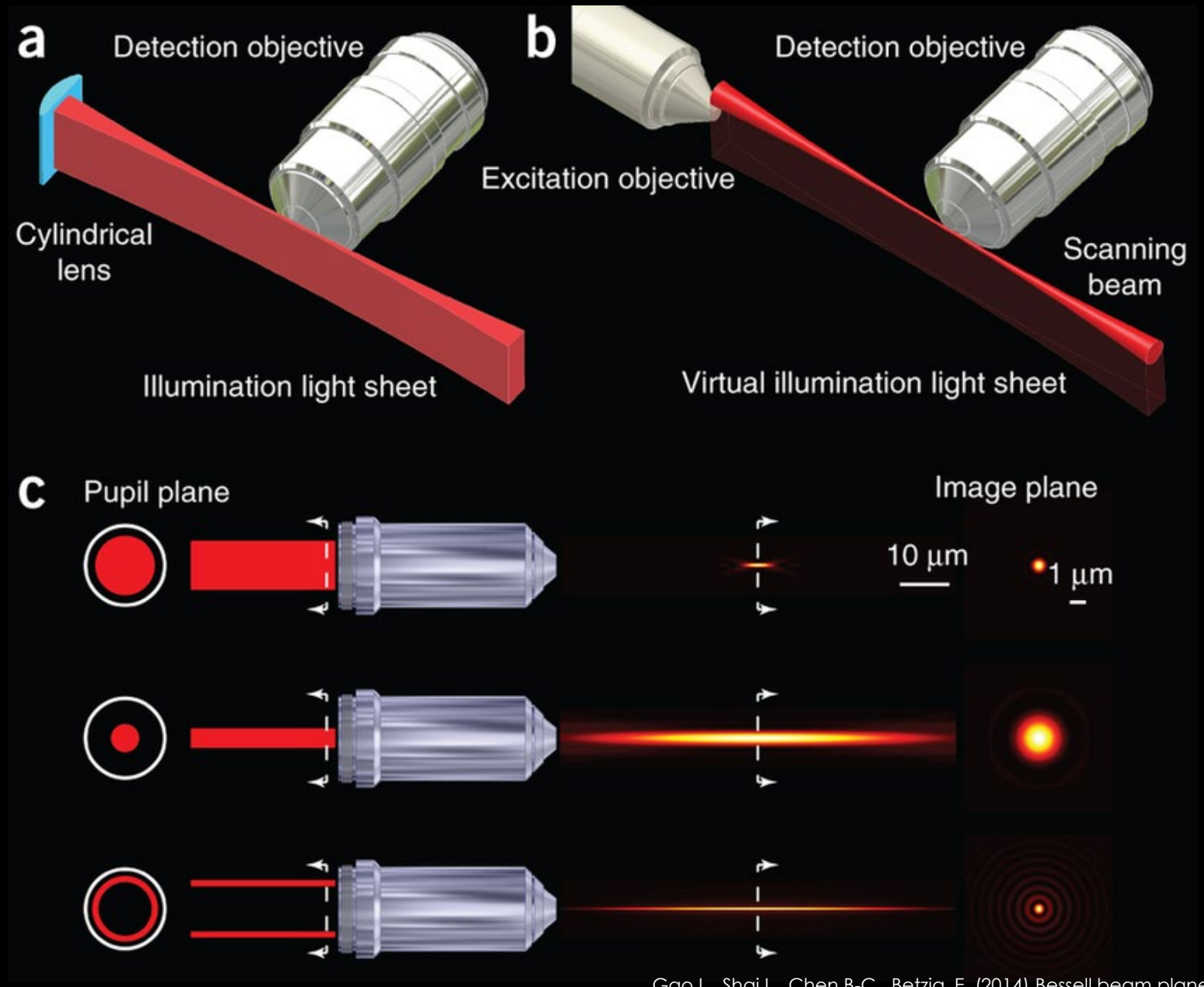




Output:
Bessel Beam

Source: https://obel.ee.uwa.edu.au/research/techniques/beam-shaping/

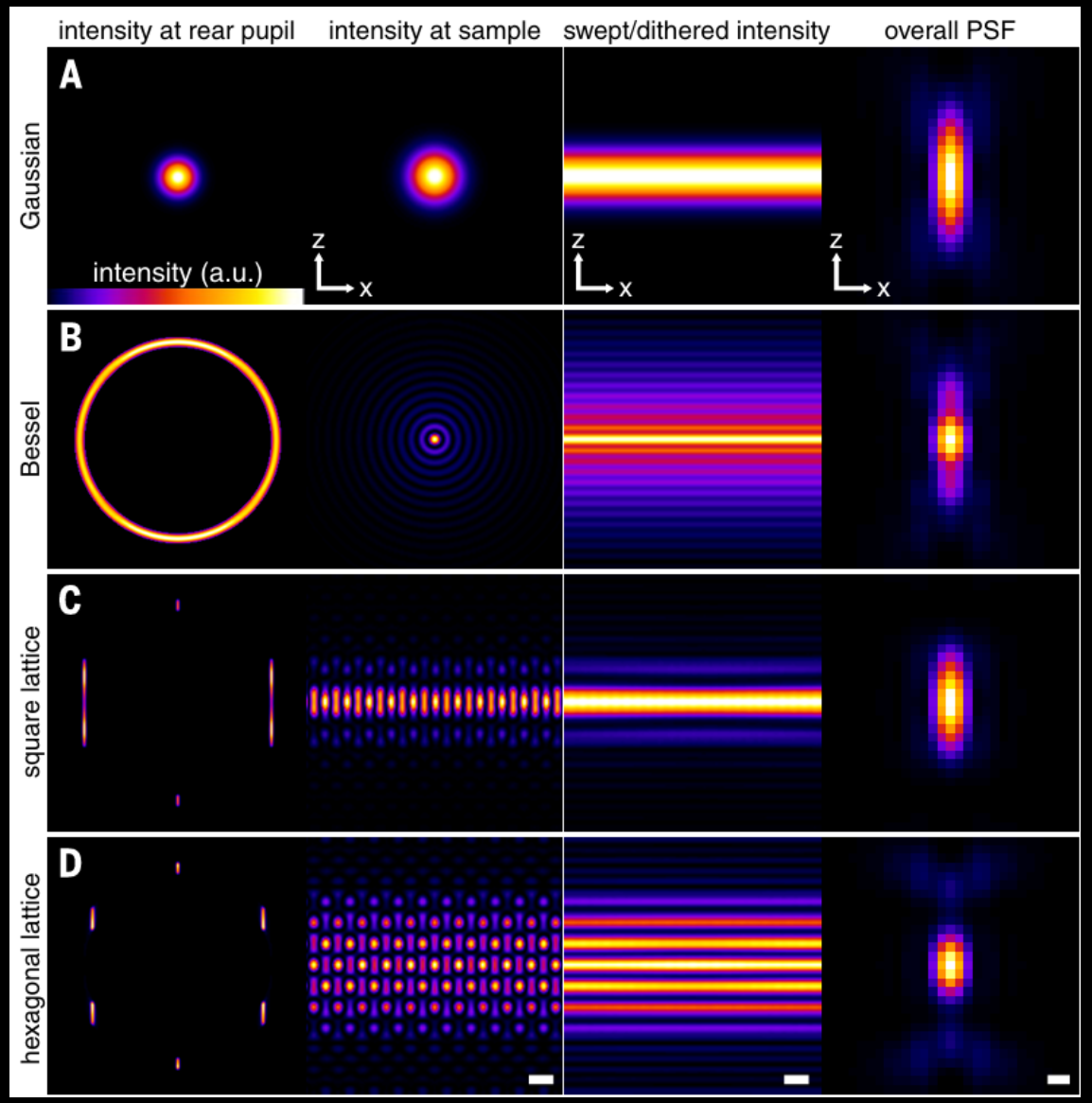
Beams at the objective back pupil



Gao L., Shai L., Chen B-C., Betzig, E. (2014) Bessell beam plane illumination microscopy, Nature Protocols

Lattice light-sheet microscopy

Shapes a Bessel beam to create a "lattice" of beams

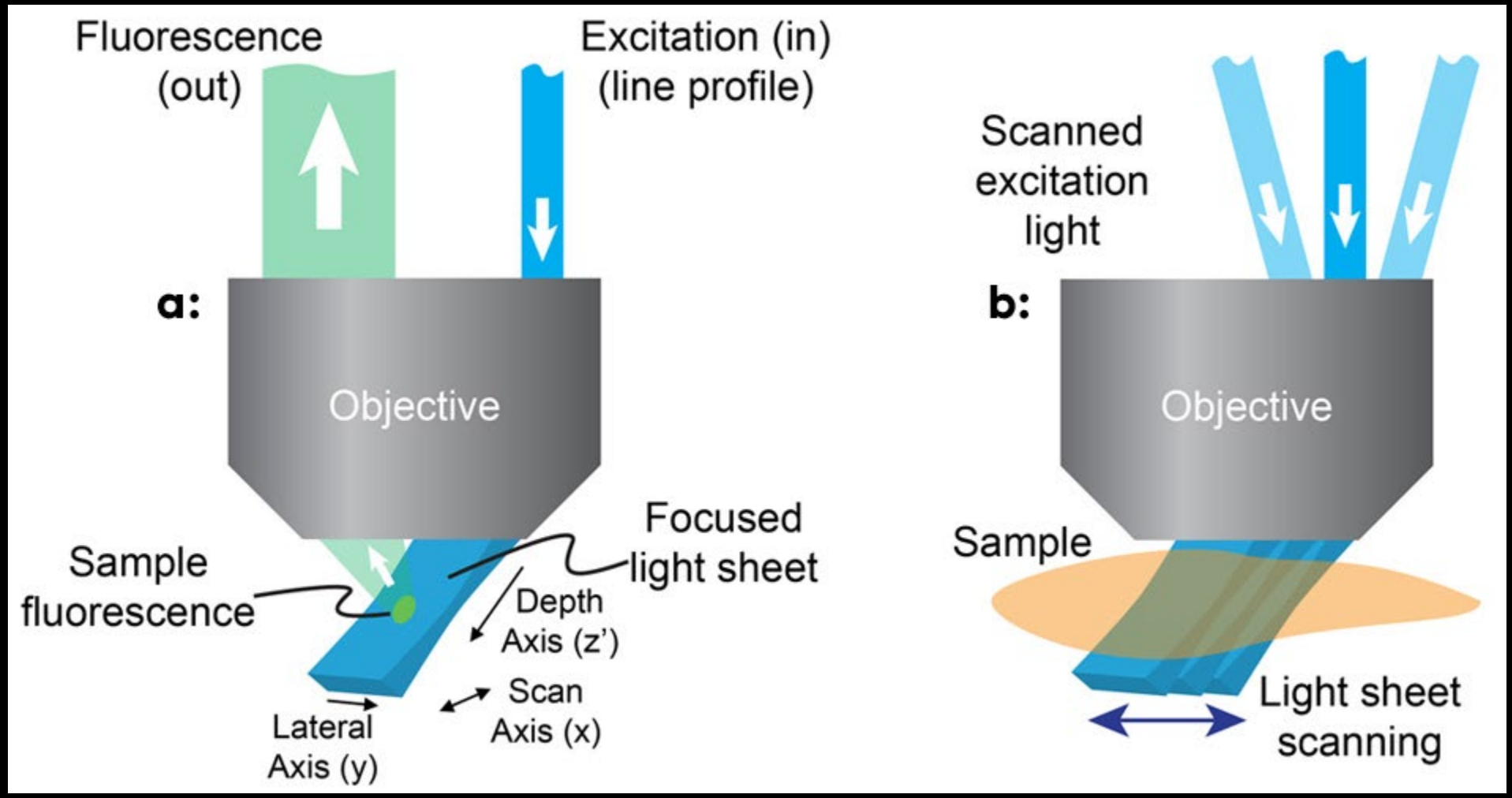


Lateral FWHM ~ 230 nm

Axial FWHM ~ 370 nm

Chen, B., et al., Science 2014

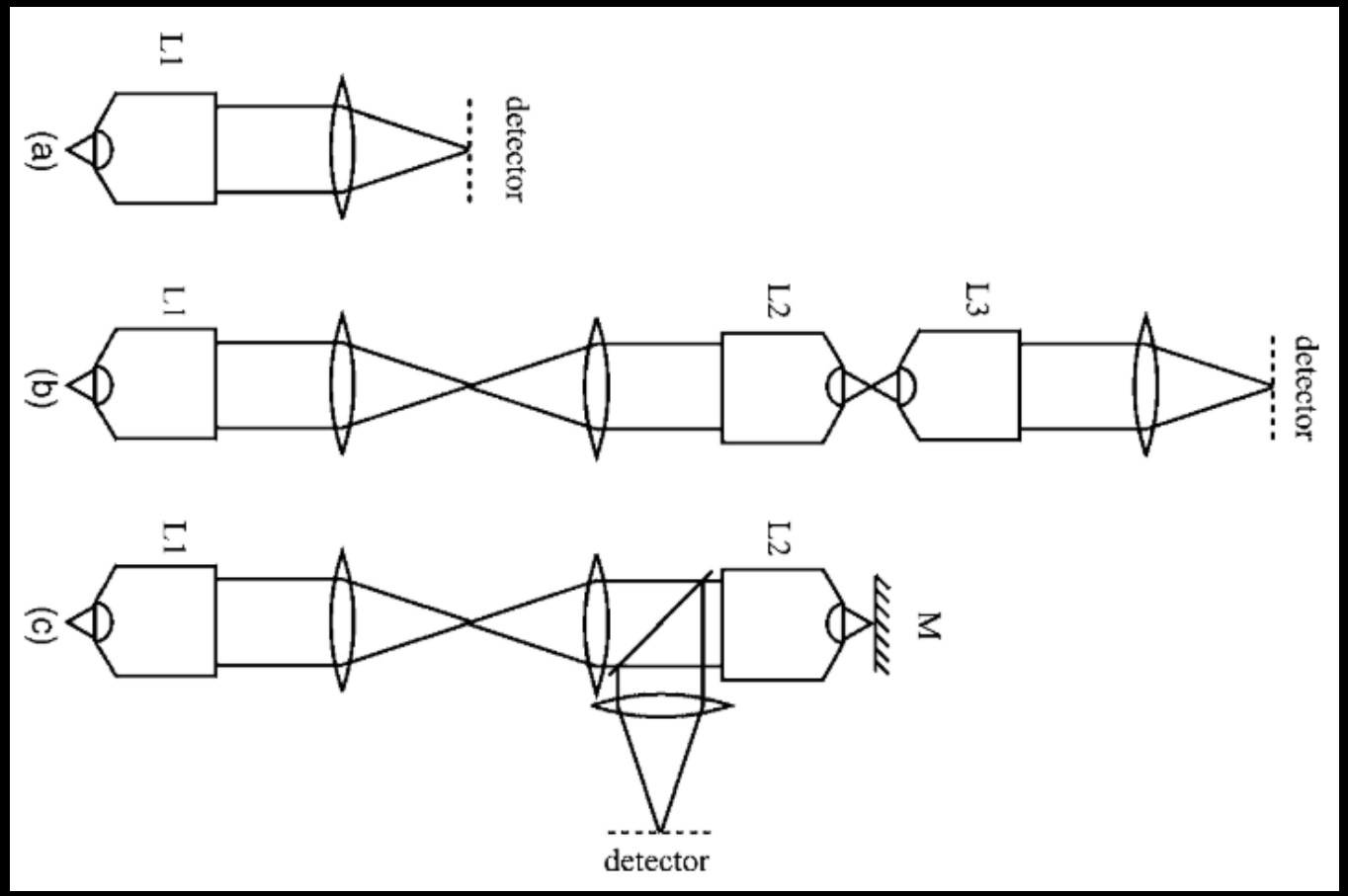
SCAPE: swept confocally aligned planar excitation microscopy



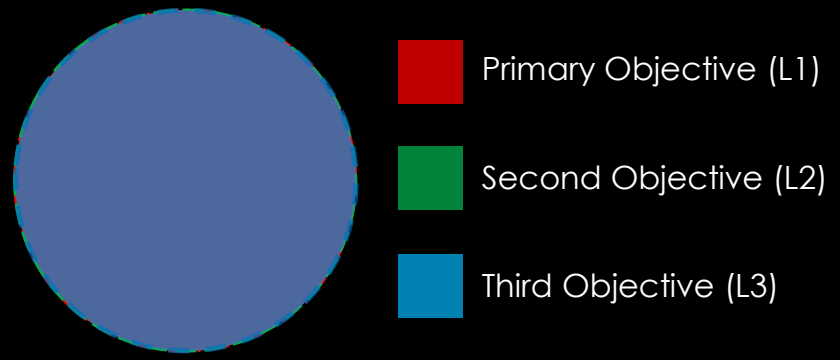
V. Voleti et al., (2019) Nature Methods

Remote focusing

Axial sectioning without the movement of the sample or the primary objective lens

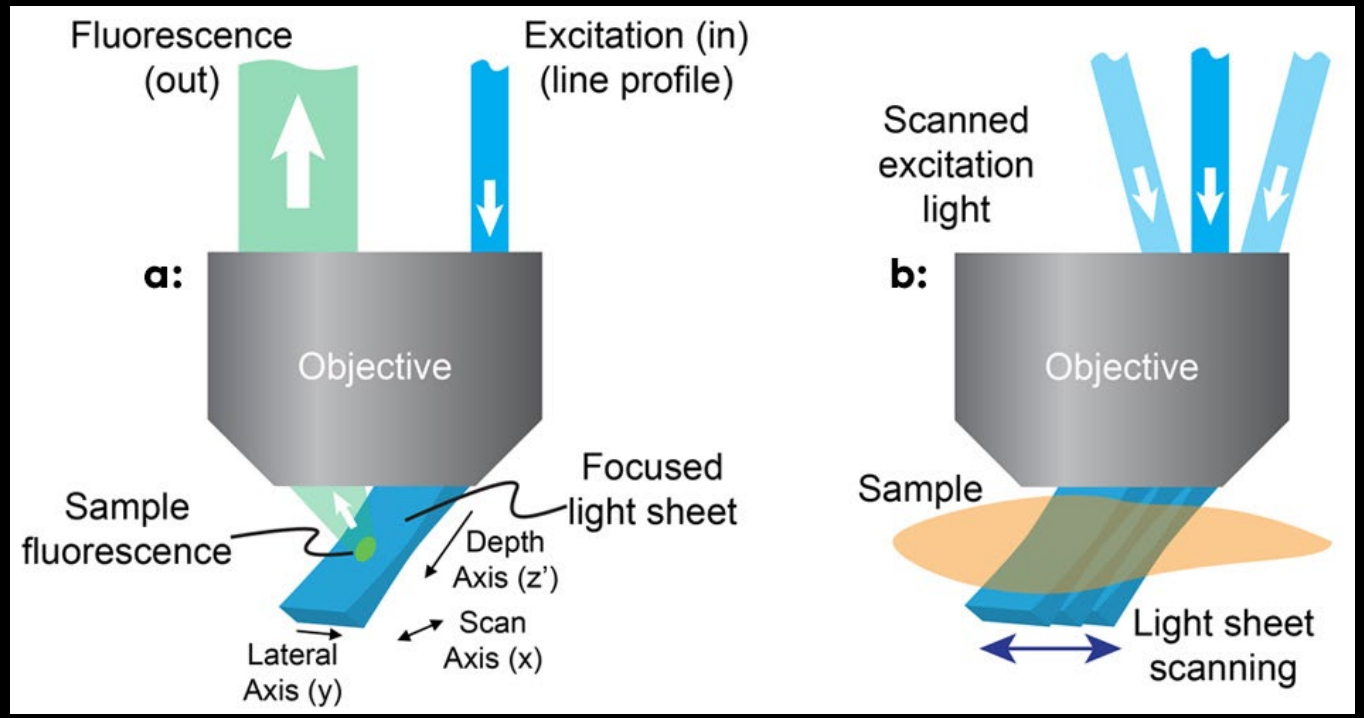


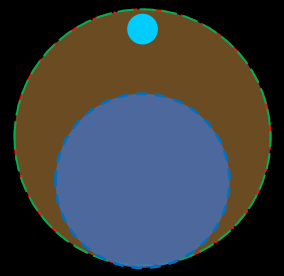
Back focal Planes



Botcherby EJ, Juskaitis R, Booth MJ, Wilson T (2007) Aberration-free optical refocusing in high numerical aperture microscopy. Opt Lett 32:2007

SCAPE: swept confocally aligned planar excitation microscopy





Primary Objective
Olympus 20x/1.0 water

Second Objective
Nikon 20x/0.75NA air

Tilted Objective
Edmund 20x/0.60NA (eff NA 0.35)

Lateral FWHM ~ 1µm

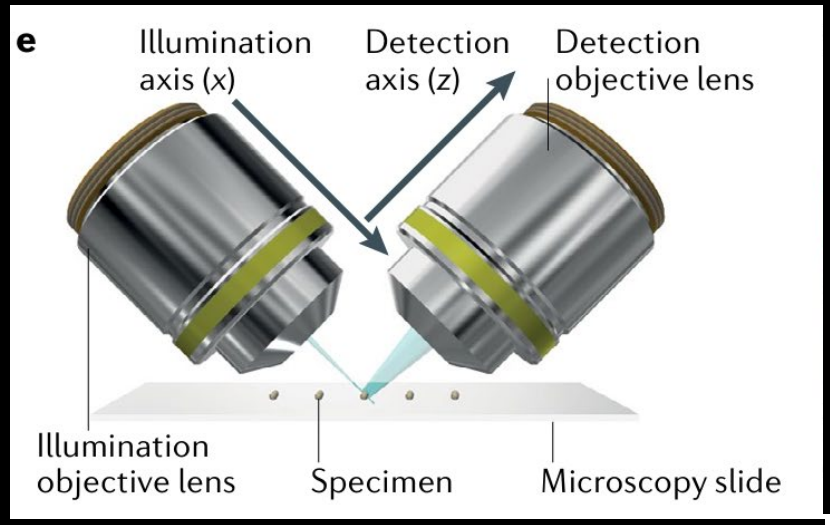
Axial FWHM ~ 3-4 µm

Effective NA of 0.35
Collection efficiency max = $(0.35/1.0)^2$ = 12.25%

V. Voleti et al., (2019) Nature Methods

What light-sheet instrument to use?

Sample geometry and mounting: flat

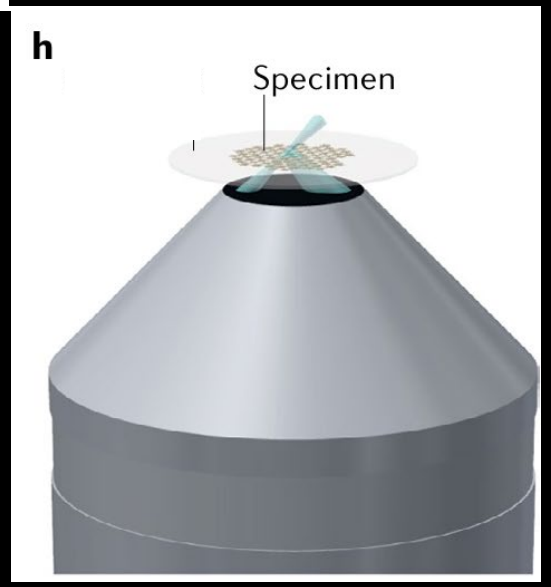


Lattice

Fast and high-resolution imaging of small, mostly flat samples (<100 µm in depth)

SCAPE

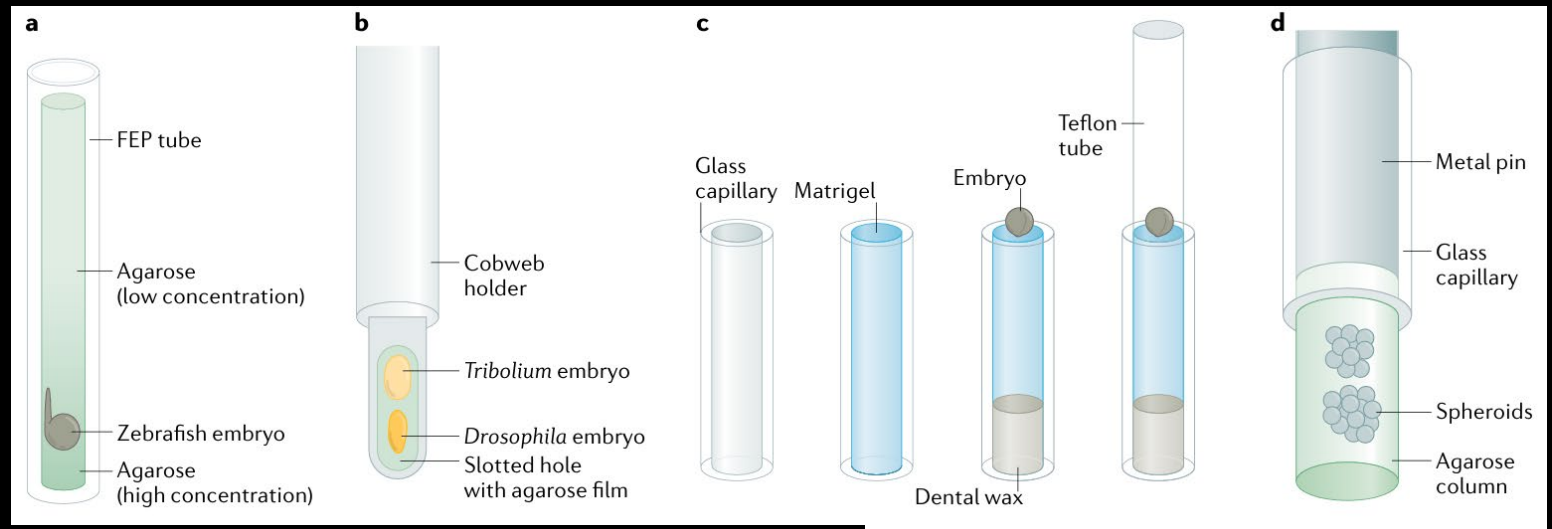
Fast imaging of samples (~100 µm in depth), single objective access to samples



Stelzer, E. H. et al. (2021). Light sheet fluorescence microscopy. Nature Reviews Methods Primers

What light-sheet instrument to use?

Sample geometry and mounting: 3D

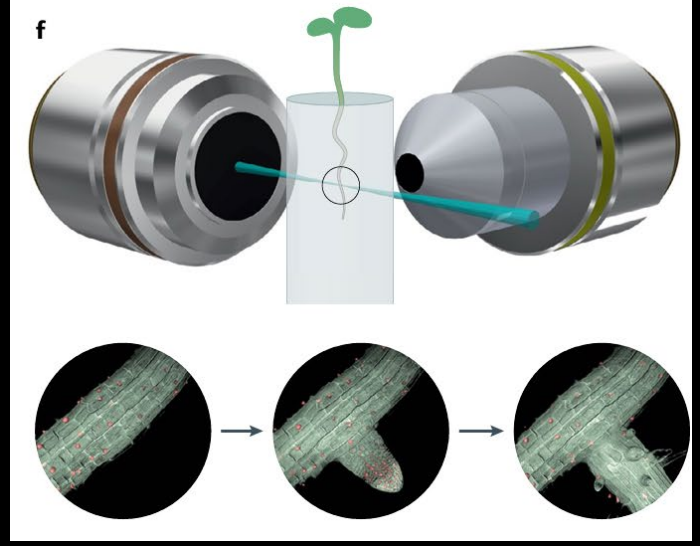


SiMView

IsoView

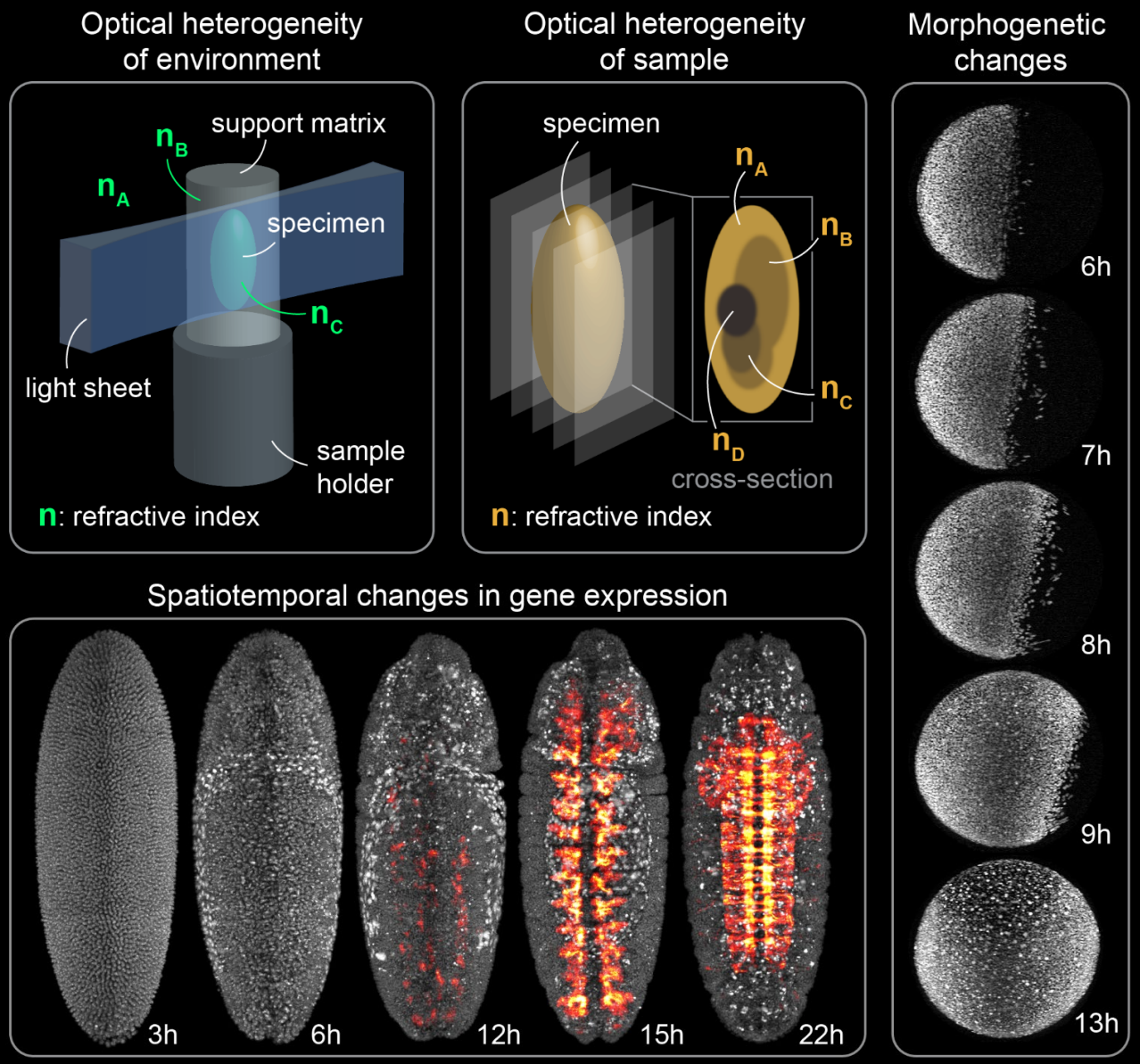
Fast and high-resolution imaging of large 3D samples (100 µm to >1 mm)

Cleared samples: 1 – 10 mm

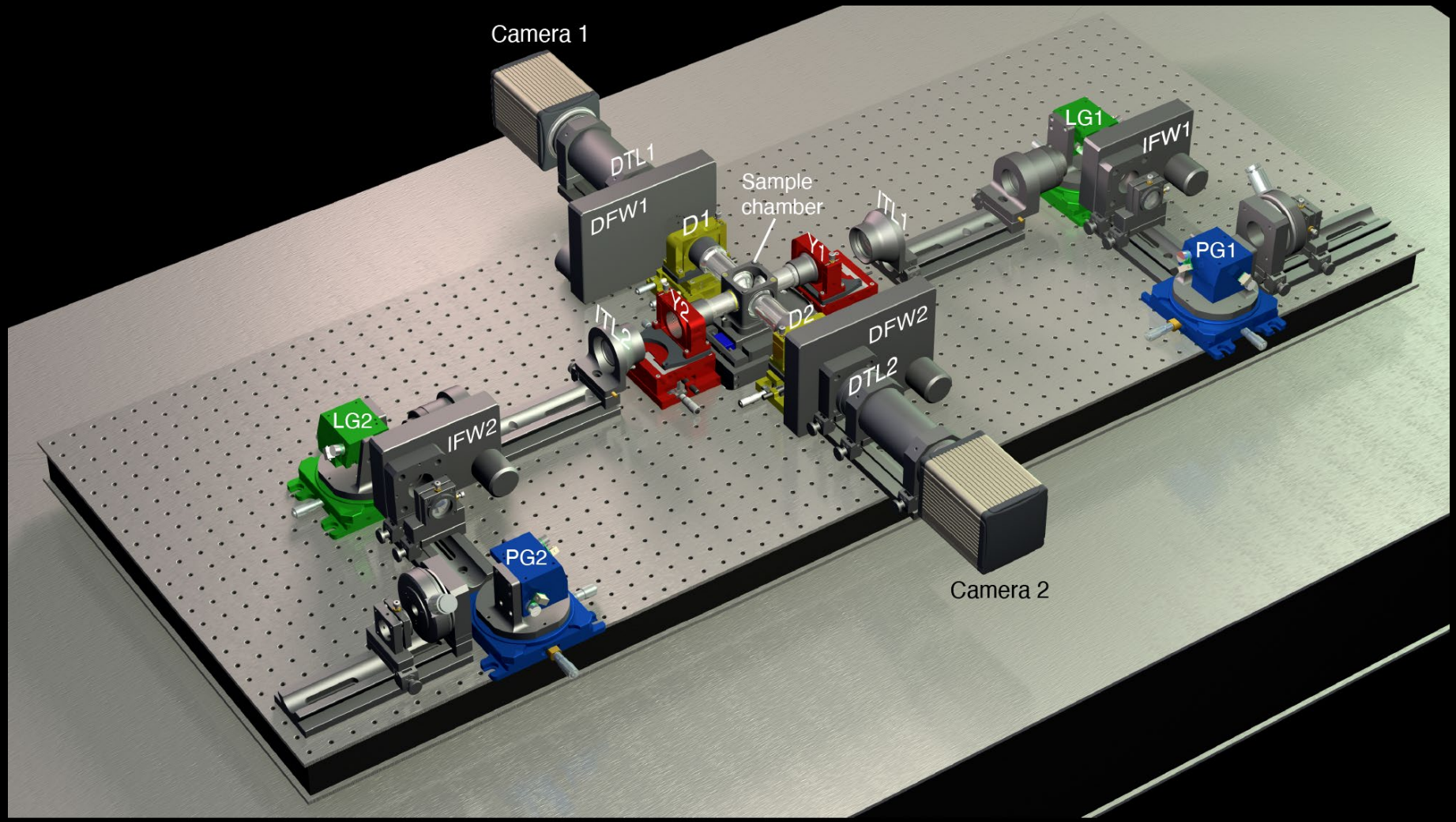


Stelzer, E. H. et al. (2021). Light sheet fluorescence microscopy. Nature Reviews Methods Primers

Challenges in light-sheet imaging of large specimens



SiMView with automated adjustment of light sheet geometry



Piezo actuators in detection arms (D_1, D_2)

Piezo actuators in illumination arms (Y_1, Y_2)

Galvanometer scanners for light-sheet formation and positioning (α_1, α_2, I_1, I_2)

Galvanometer scanners for light-sheet pivoting (β_1, β_2)

PJ Keller, RK Chhetri, L Royer, US Patent US12360353B2

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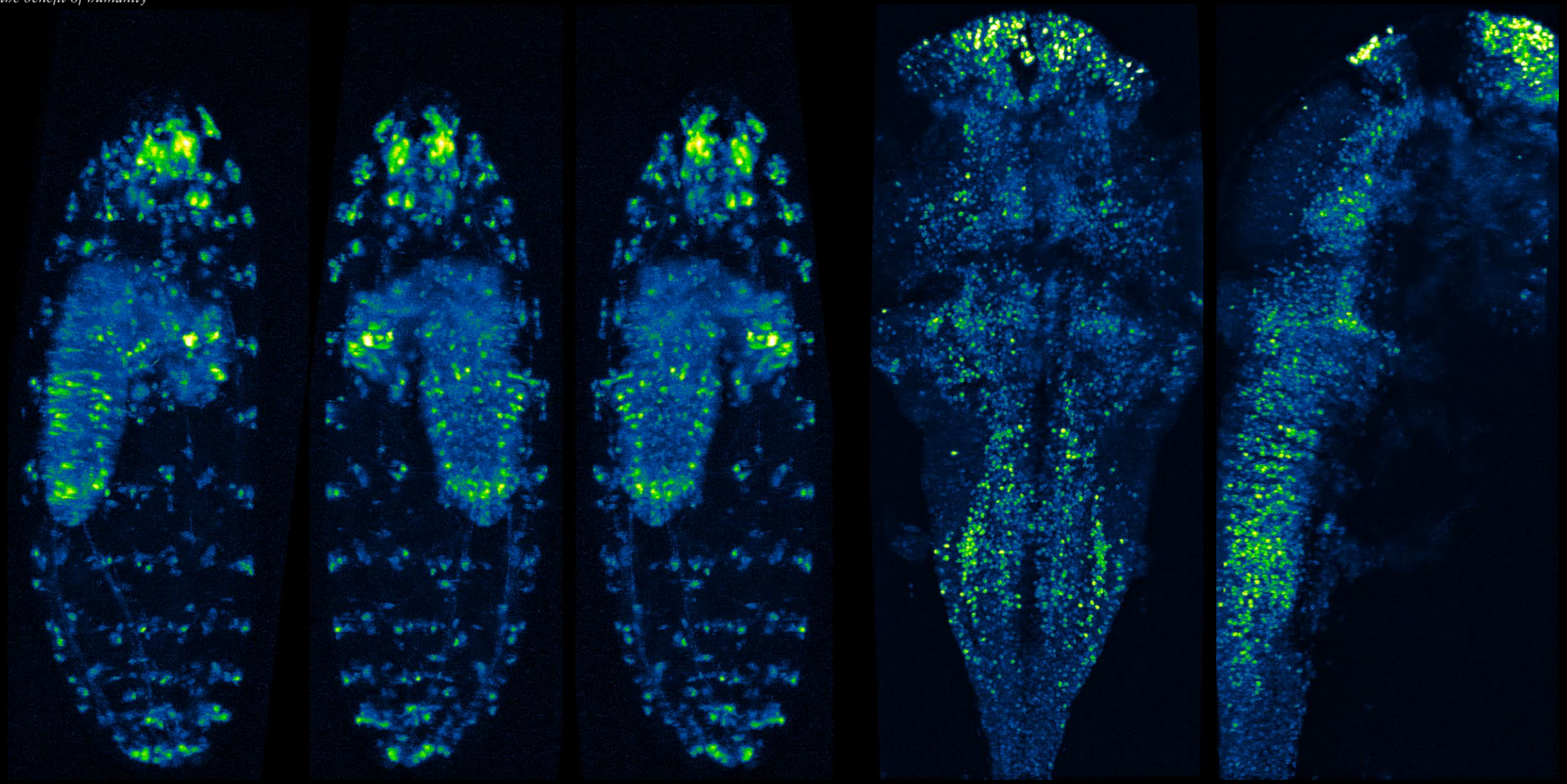
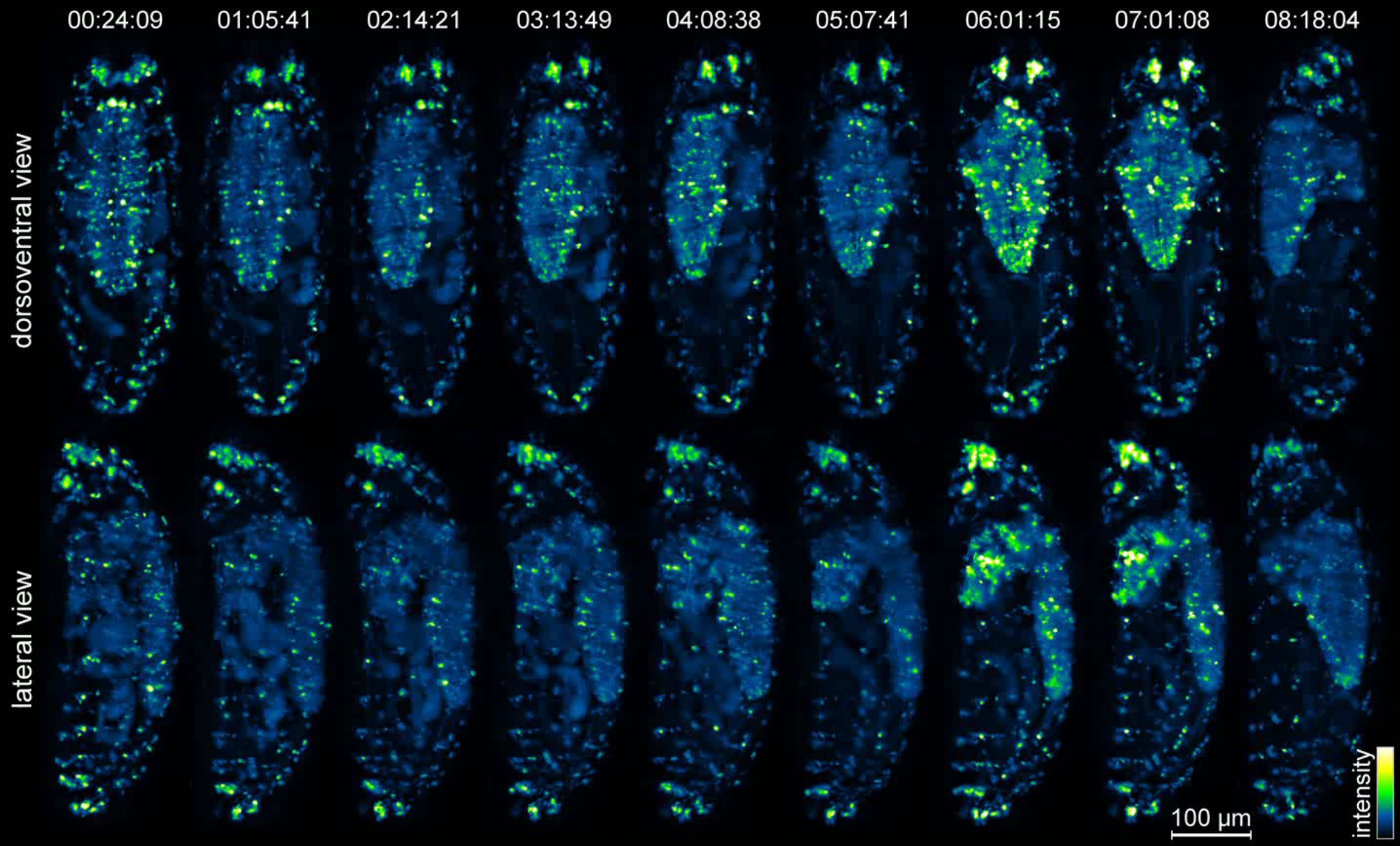
Science for the benefit of humanity



Left/right: 4.5/0.4 million images, 0.55/0.56 µJ per image; 500×500×200/800×400×400 µm^3 volume, 280/268 frames s^{-1}, 500/1,000 MB s^{-1}

Functional imaging throughout development



Long-term, whole-animal functional imaging from embryonic to larval stages

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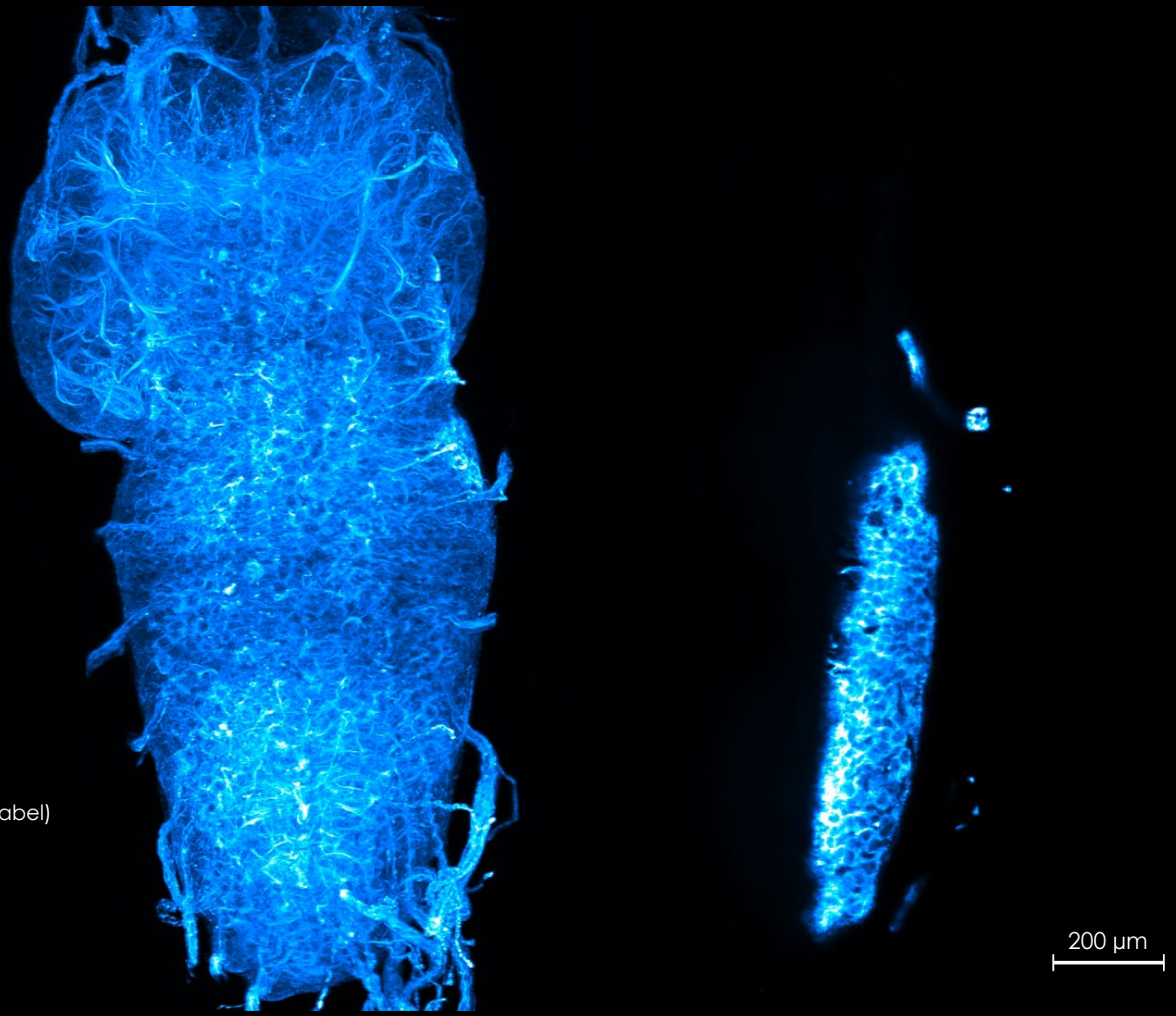
Expanded, cleared-tissue imaging

Rotating projection

Volume slicing



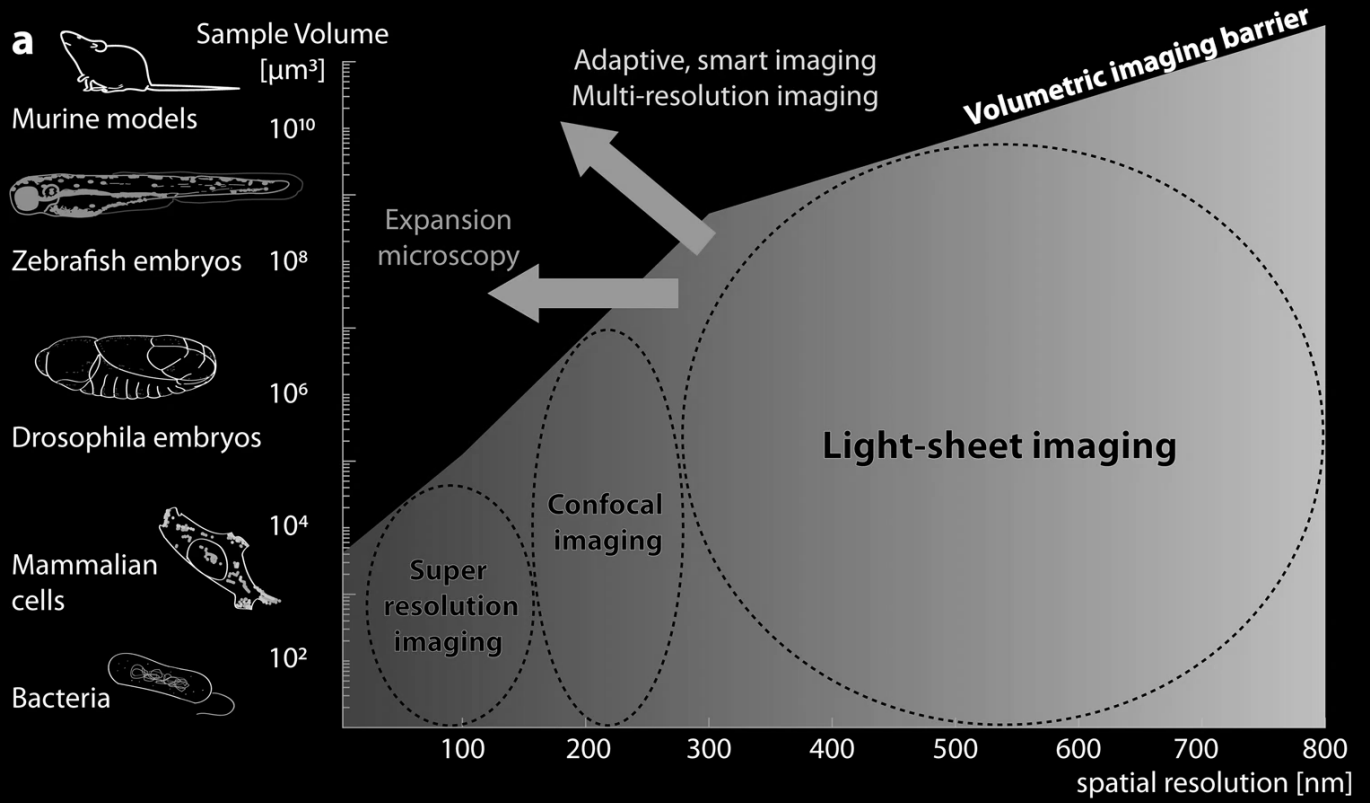
7.5-fold expanded *Drosophila* L1 larval CNS (tubulin label)

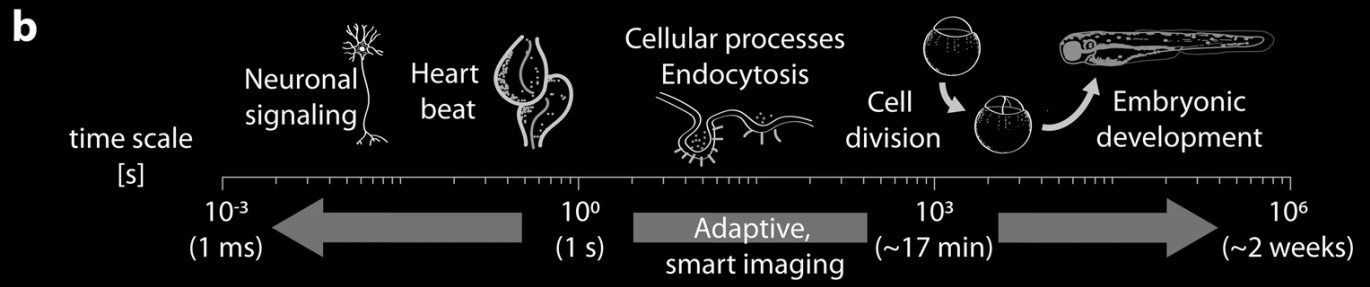
Effective 100 nm isotropic resolution

Entire volume imaged in 10 min

Hörl, D., Rusak F. R., Preusser F., Tillberg P., Randel N., Chhetri, R.K., et al. (2019), Nature Methods

Outlook for the future

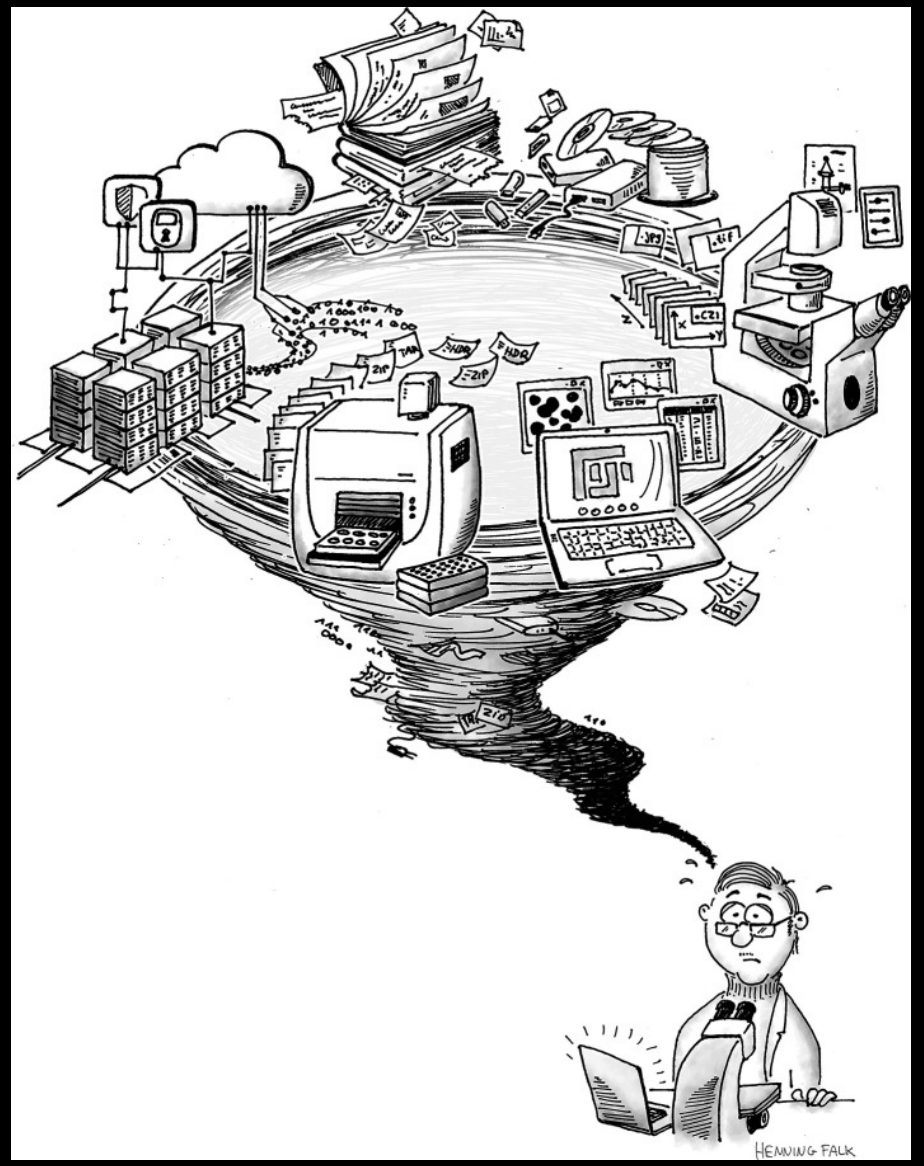




- Large-format optical components
- Parallelized imaging: Faster acquisition
- Event-driven, AI-embedded imaging
- Multi-modality integration
- Broader scientific, clinical applications

Daetwyler S., Fiolka R. P. (2023) Light-sheets and smart microscopy, Communications Biology

Bioimaging data vortex



Schmidt C., et al. Research data management for bioimaging: the 2021 NFDI4BIOIMAGE community survey

Survival guide: light-sheet data vortex

- Light-sheet data adds up quickly!
  100+ GB to a few TB per experiment

- Pre-study planning
  - Conduct exploratory imaging, mock session
    e.g., light-sheet thickness, multiple views
  - Identify necessary pre-processing steps
    e.g., de-skewing, multiview registration, deconvolution, motion-correction
  - Don't ignore your data representation
    e.g., bit depth, file type, int/float
  - Plan ahead for data transfer, storage, and processing platform (on-premise, HPC, cloud), visualization
  - Identify necessary quantitative analysis steps and available resources
    e.g., segmentation, tracking (Δx, Δy, Δz, $\Delta F/F$)

- Collect minimal data necessary first, then scale up

- Reach out for help, ask questions!

Questions?

Reminder: Please fill out the post-workshop survey

Light-sheet Reviews

Power, R. M., Huisken J. (2017), A guide to light-sheet fluorescence microscopy for multiscale imaging, Nature Methods

Chhetri R. K., Keller P. J. (2020), Functional imaging with light-sheet microscopy, Handbook of Neurophotonics

Stelzer, E. H. et al. (2021), Light sheet fluorescence microscopy, Nature Reviews Methods Primers

Comparison of Lattice and Gaussian light-sheets

Chang B.J., Dean K. M., Fiolka R. (2020), Systematic and quantitative comparison of lattice and Gaussian light-sheets