Optical Imaging for Biological Sciences

Kai Wang

2018

Outline

About the light and imaging

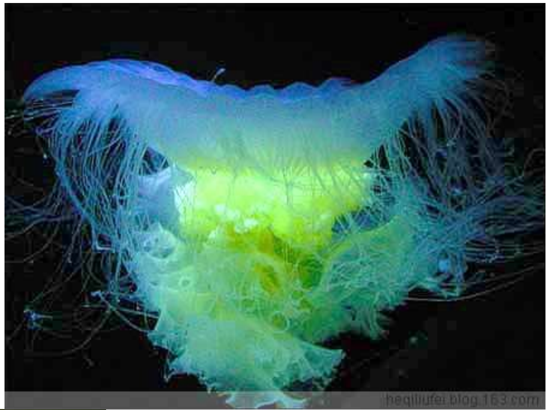
Optical imaging

- Basics
- Optical Neuroimaging

About the light

Energy

& Signal

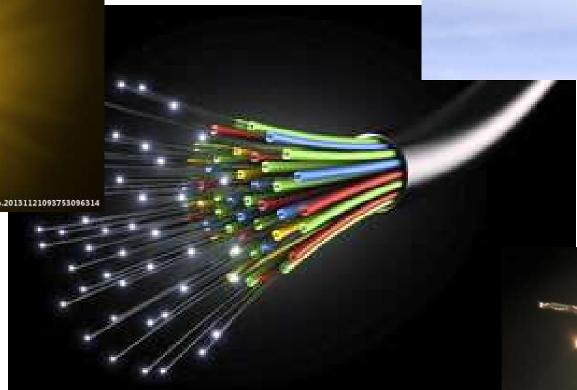


About the light

firework

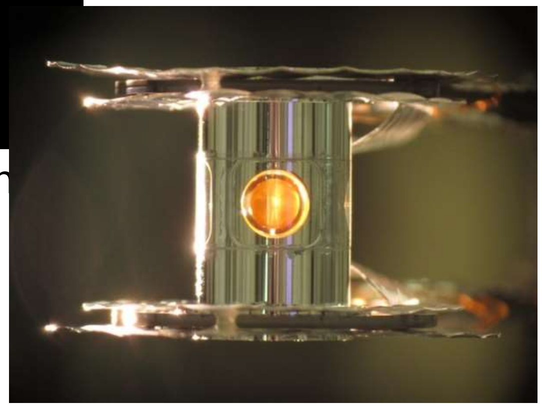
illumination

Laser weapon



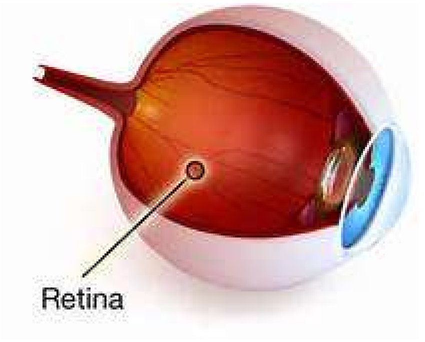
Optical communication

display

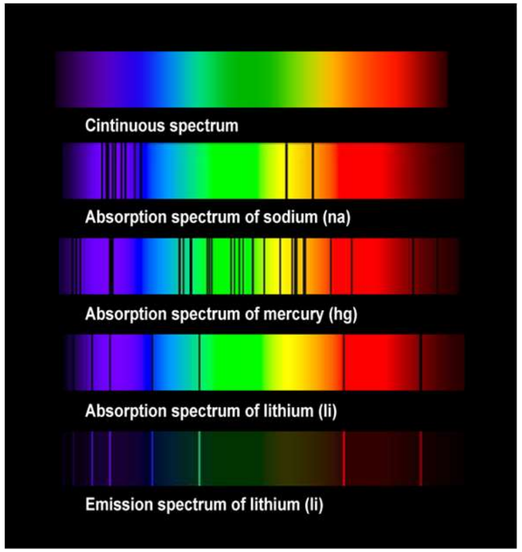


Laser induced nuclear fusion

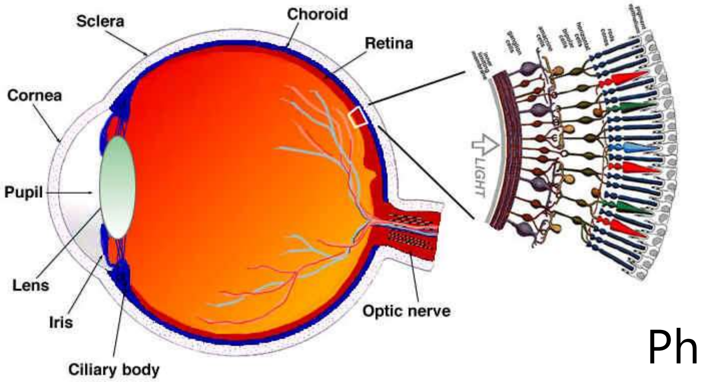
About the light



Colors & Contrast



Absorption spectrum

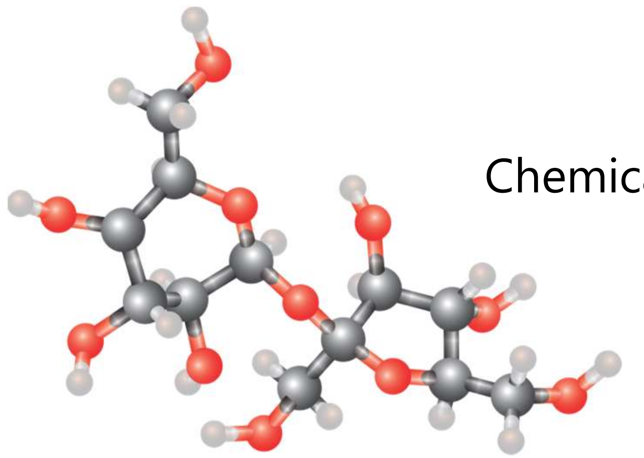


Photoreceptor

About the light

Why light is so important and everywhere in our lives?

Light is electromagnetic wave and can mediate electromagnetic interaction, which is one of the four fundamental interactions we know so far.



Chemical bond and reaction

About the light

Is there any other better means?

No!

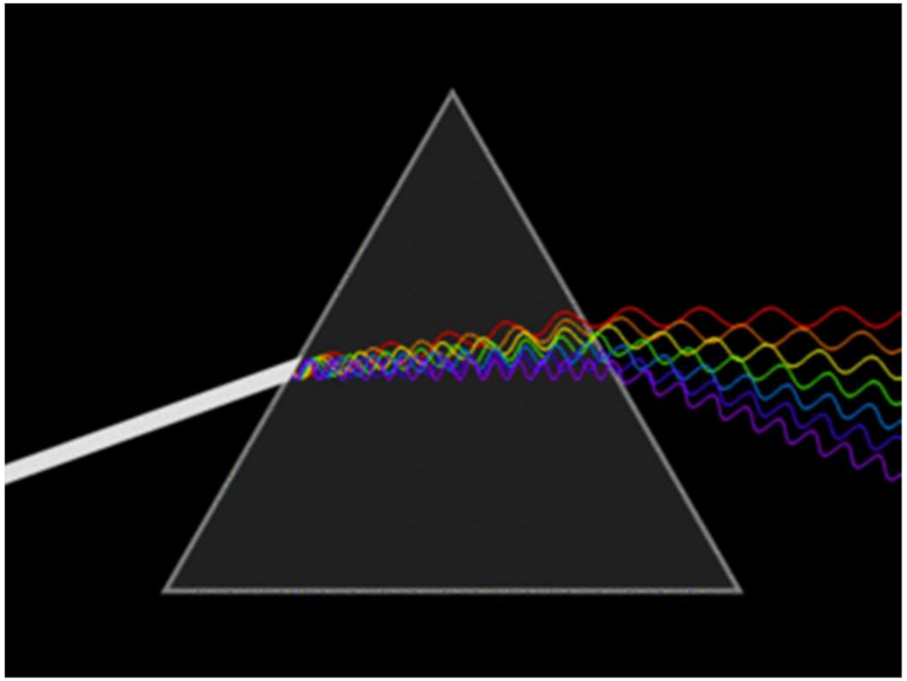
Stick to light, there is no other way out!!!

About the light

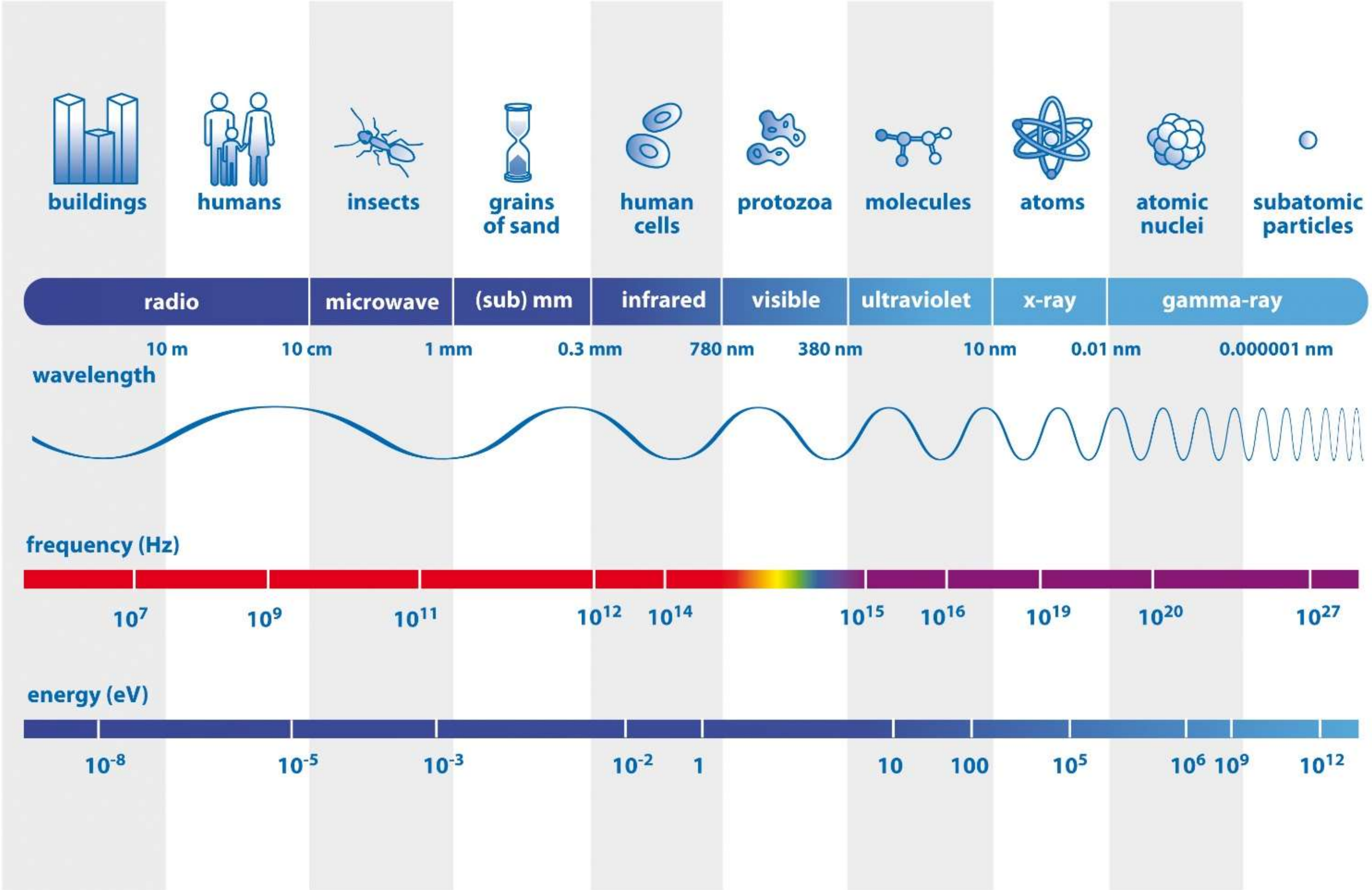
Physical Properties of Light

- Wavelength
- Photon energy

$$E = \hbar\nu$$

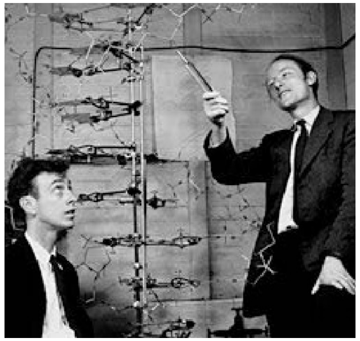


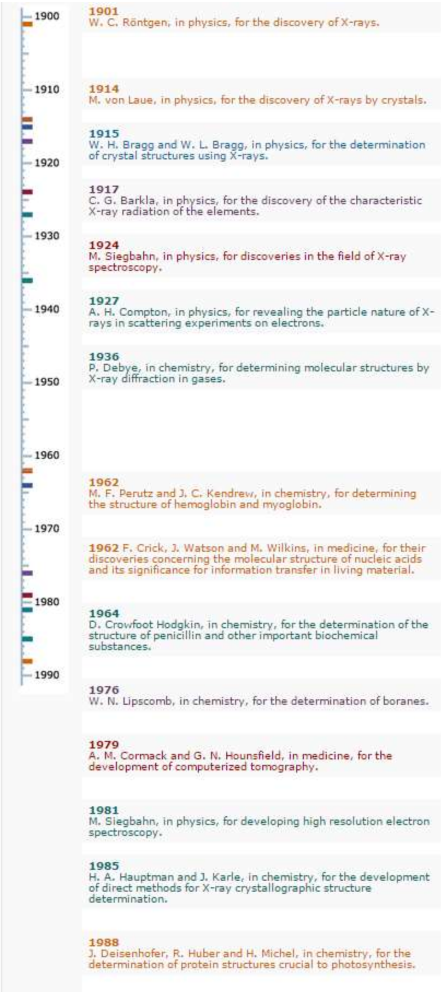
About the light



X Ray

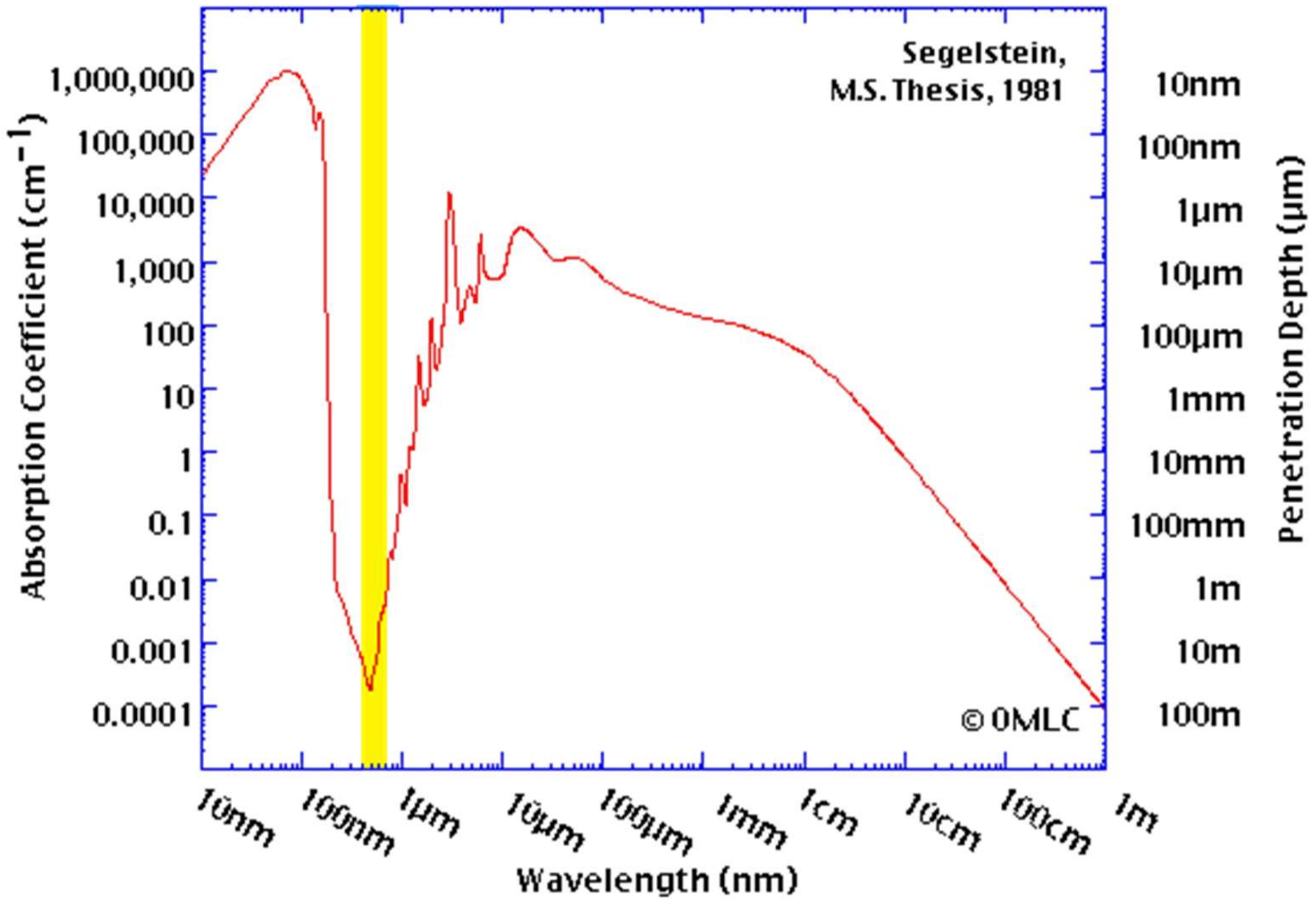
First Nobel Prize in Physics



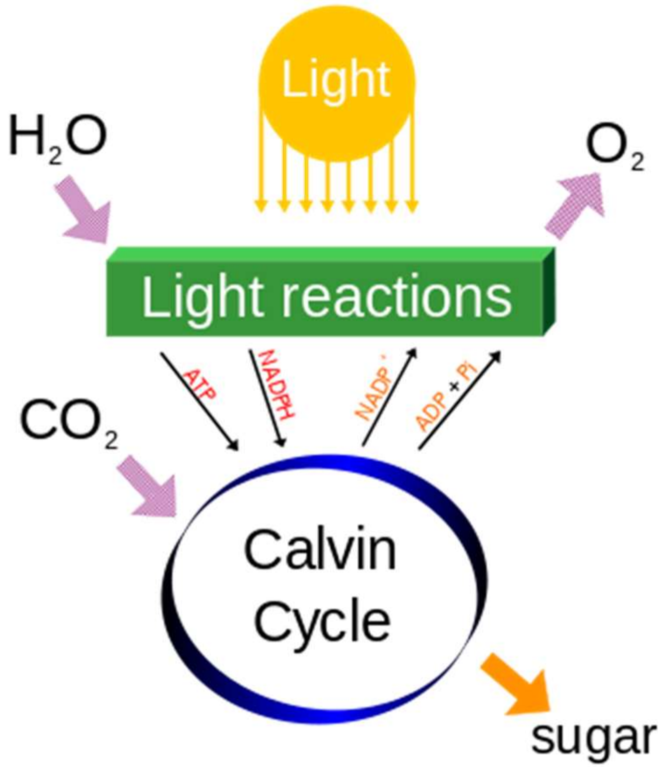


Visible light can penetrate deep into water

Water absorption spectrum



Photosynthesis



$$\underset{\text{Carbon dioxide}}{6CO_2} + \underset{\text{Water}}{6H_2O} \xrightarrow{\text{Light}} \underset{\text{Sugar}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6O_2}$$

From wiki

Visible light

Visible light is bio-compatible.

Use visible light for in *vivo* biological studies!

Optical imaging

Why imaging?

Seeing is believing!

imaging

wording

Sorry, I can't do it ! (>_<)

Why imaging?

What’s is understanding?

Objective	The world is 3D. The world is physically there. This is how the world works, despite what you think
Subjective	Our understanding is built on our collected information and experience, based on which we can predict.

Vision is our major sense to collect information, so seeing leads to understanding!!!

Early times of optical imaging

Galileo Galilei 1564 – 1642

- “Father of observational astronomy”
- “Father of modern physics”
- “Father of scientific method”
- “Father of science”
- “Father of modern science”, by Albert Einstein

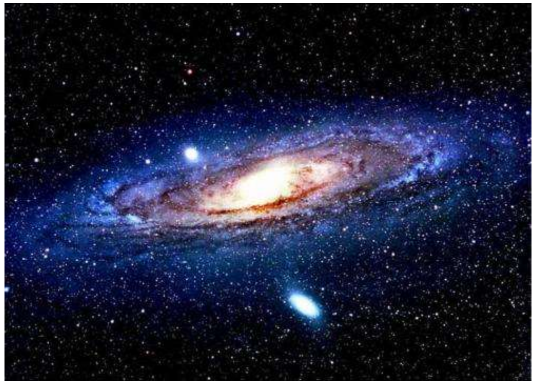
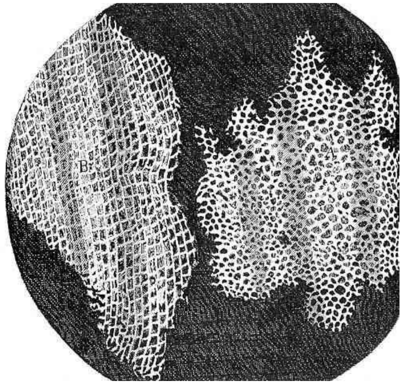
“In 1609, Galileo was, along with Englishman Thomas Harriot and others, among the first to use a refracting telescope as an instrument to observe stars, planets or moons.”

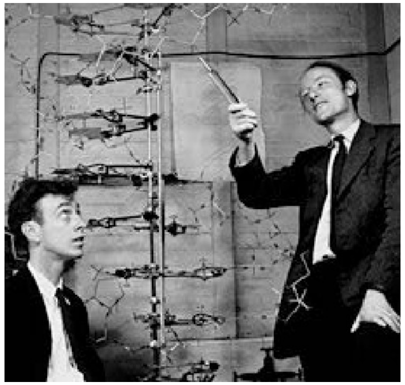
“In 1610, he used a telescope at close range to magnify the parts of insects.”

Seeing is believing



X ray



10^{26}m

10^{-6}m

10^{-9}m

10^{-11}m

Outline

About the light and imaging

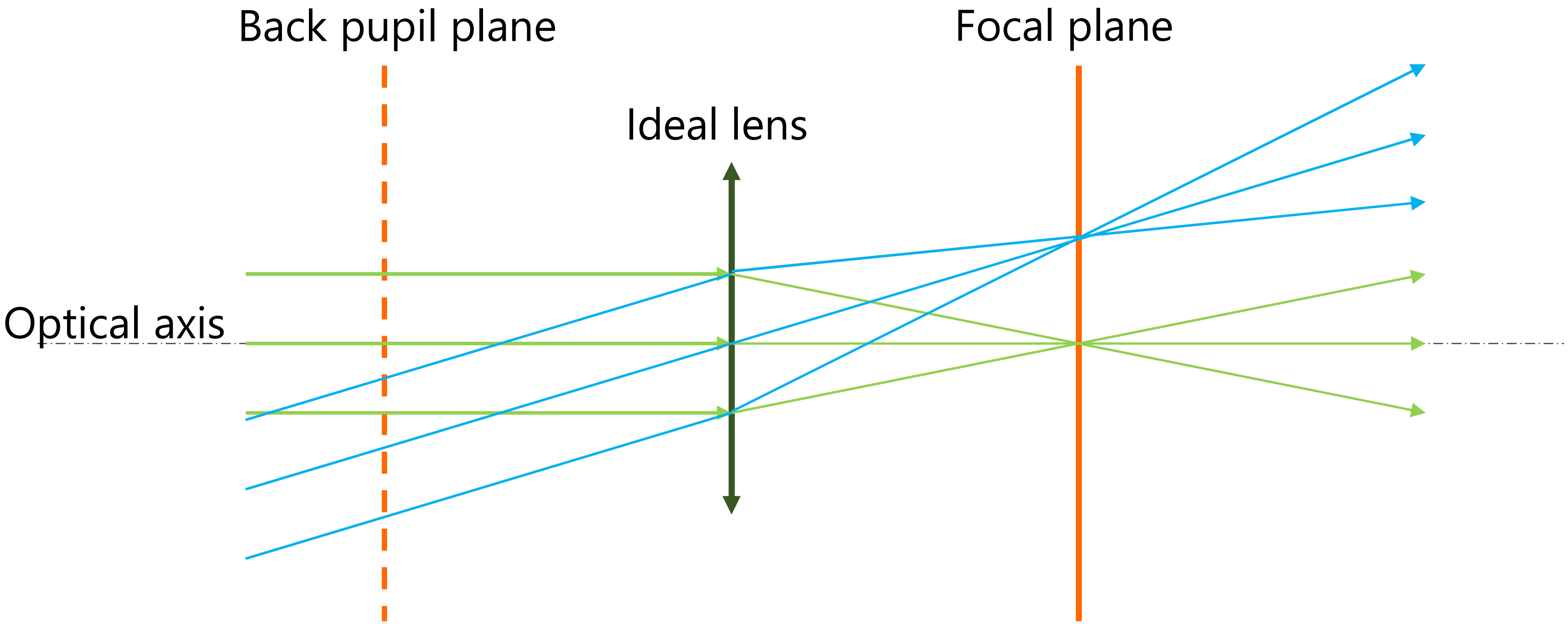
Optical imaging

- Basics
- Optical Neuroimaging

Basics of optical imaging

- Light Ray Model
- Light wave model
- Frequency domain model

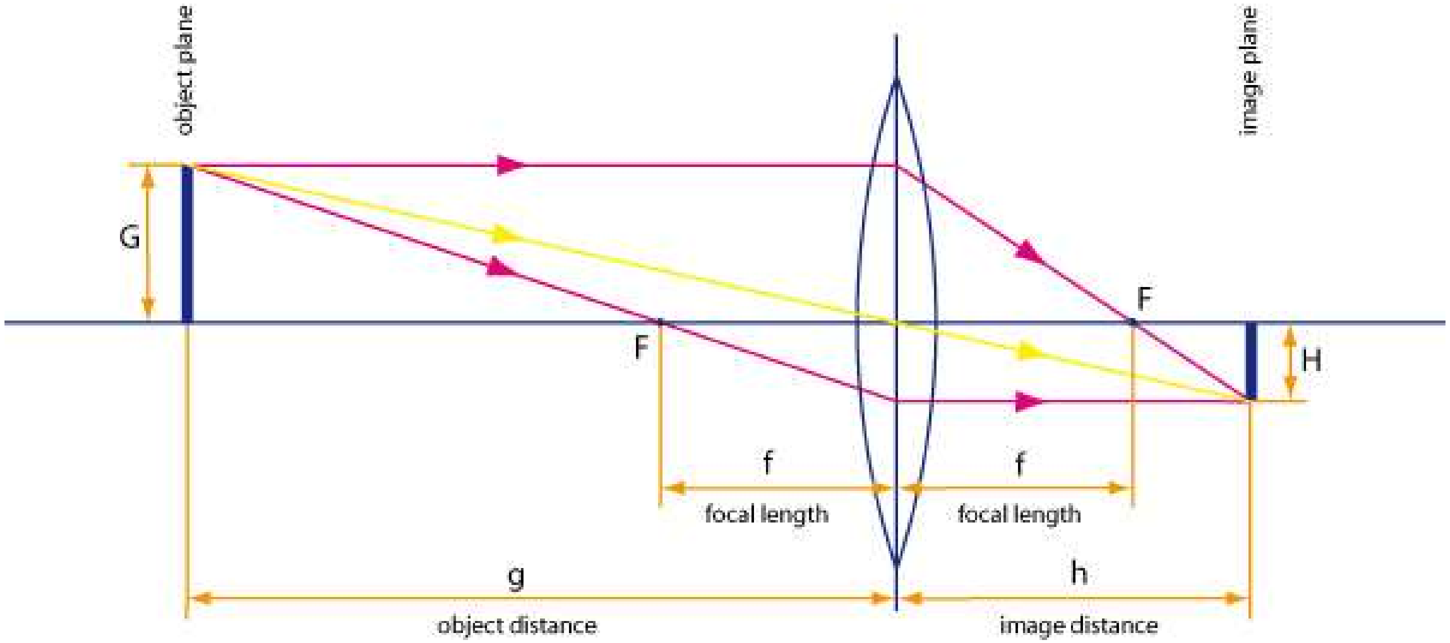
Light ray model of an ideal lens



Rule 1: Light ray will not be deflected when passing through the center of lens

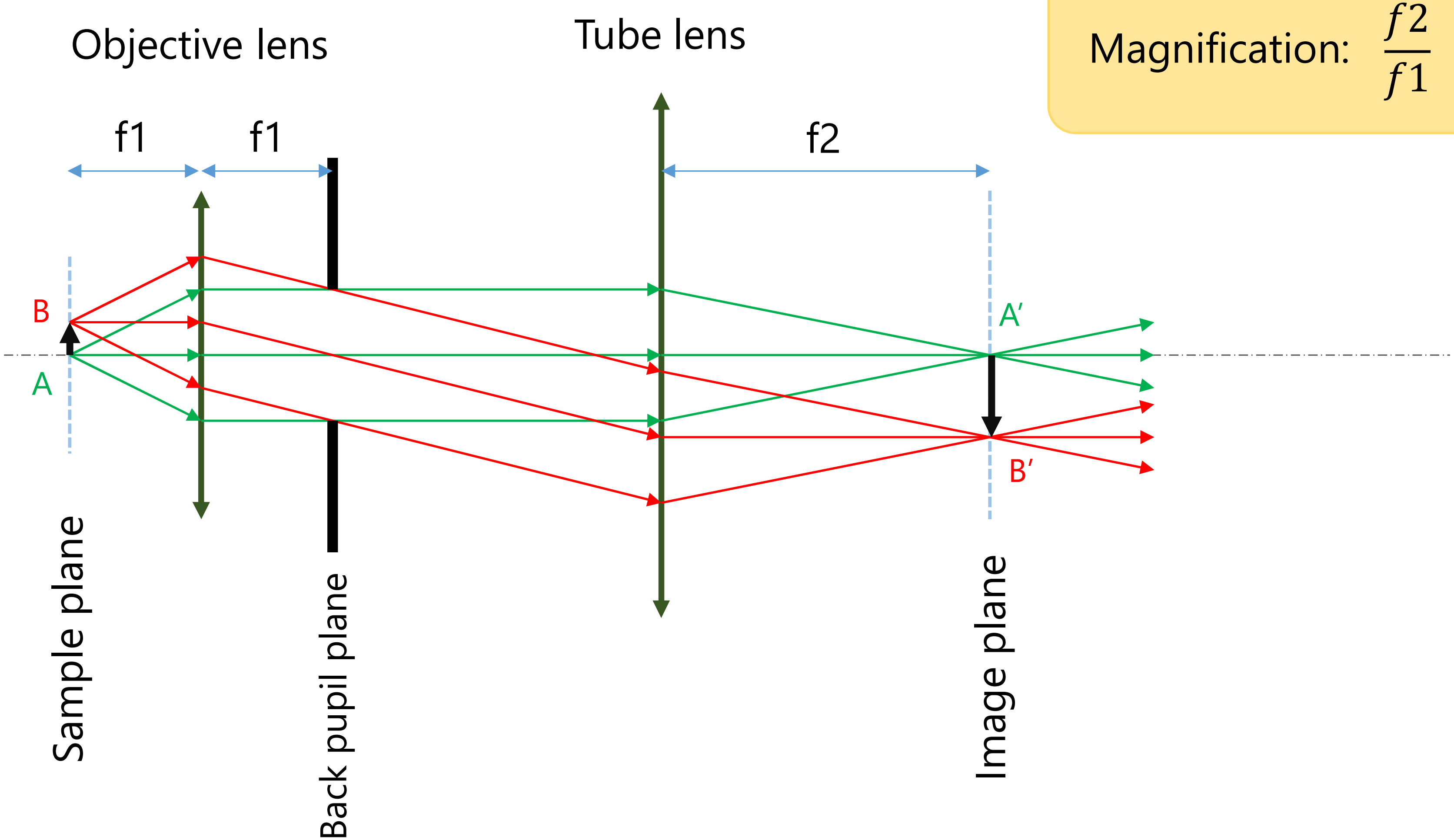
Rule 2: Parallel light rays will be focused into a single spot on focal plane

Imaging system: Light ray model



$$\frac{1}{d_o} + \frac{1}{d_i} = \frac{1}{f}$$

Infinity corrected imaging system



Magnification: $\frac{f2}{f1}$

Objectives' parameters

Correction spec:

Magnification:

Numerical Aperture (NA):

Working Distance (WD):

Immersion medium:

Figure 1

Objective Correction for Optical Aberration

Objective Type	Spherical Aberration	Chromatic Aberration	Field Curvature
Achromat	1 Color	2 Colors	No
Plan Achromat	1 Color	2 Colors	Yes
Fluorite	2-3 Colors	2-3 Colors	No
Plan Fluorite	3-4 Colors	2-4 Colors	Yes
Plan Apochromat	3-4 Colors	4-5 Colors	Yes

Objectives' parameters

Microscope Optical Train Components

Manufacturer	Tube Lens Focal Length (Millimeters)	Parfocal Distance (Millimeters)	Thread Type
Leica	200	45	M25
Nikon	200	60	M25
Olympus	180	45	RMS
Zeiss	165	45	RMS

Example:

100x objectives of different brands have different focal length:

Leica & Nikon Objectives: 200/100=2 mm

Olympus Objective: 180/100 = 1.8 mm

Zeiss Objective: 165/100 =1.65 mm

Summary of light ray model

- Lens focuses parallel light rays of different directions into spots at different positions on the focal plane.

- Modern imaging system (infinity corrected imaging system) consists of two lens. Light from a point source is converted into plane wave, then back to a spot.

- The magnification of the imaging system can be calculated as:

$$M = \frac{f_{tube\ lens}}{f_{objective\ lens}}$$

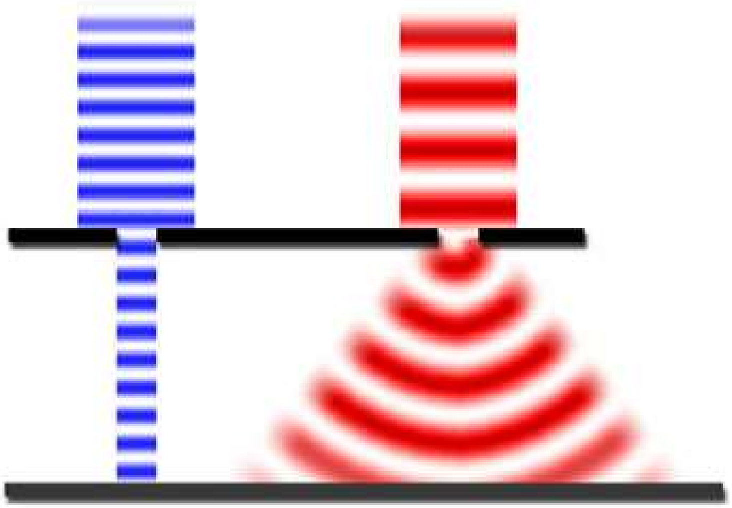
Basics of optical imaging

- Light Ray Model
- Light wave model
- Frequency domain model

Wave nature of light

Water ripple

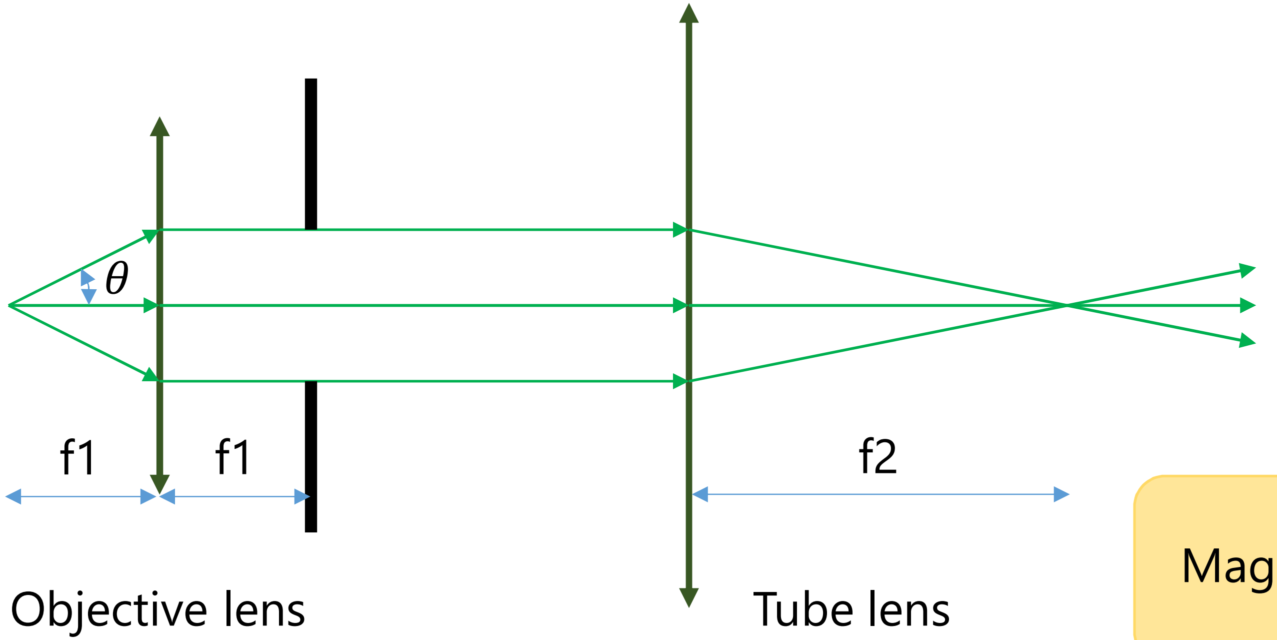
diffraction



Light wave model of optical imaging system

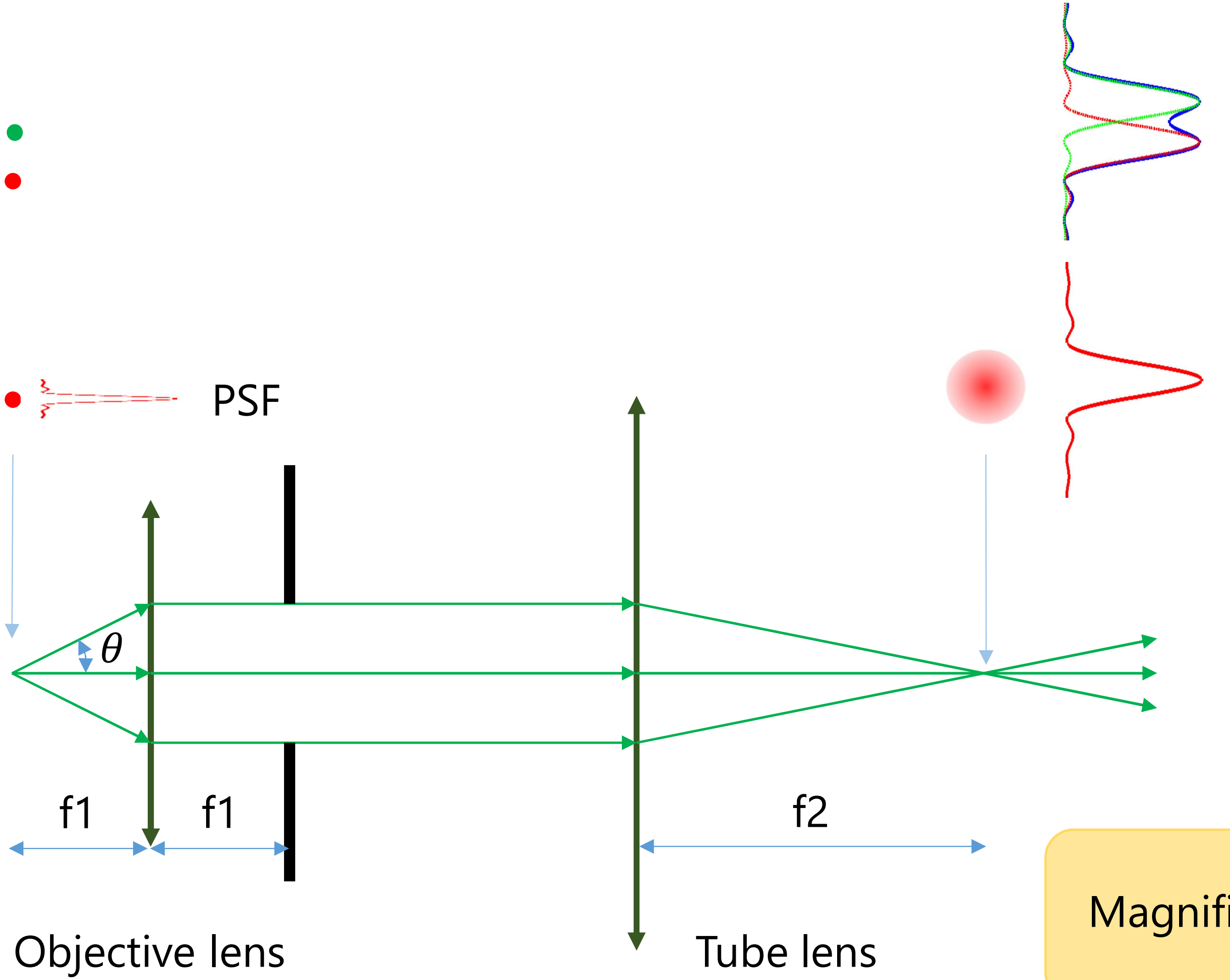
What's the resolution limit of the optical imaging system?

Point spread function (PSF):



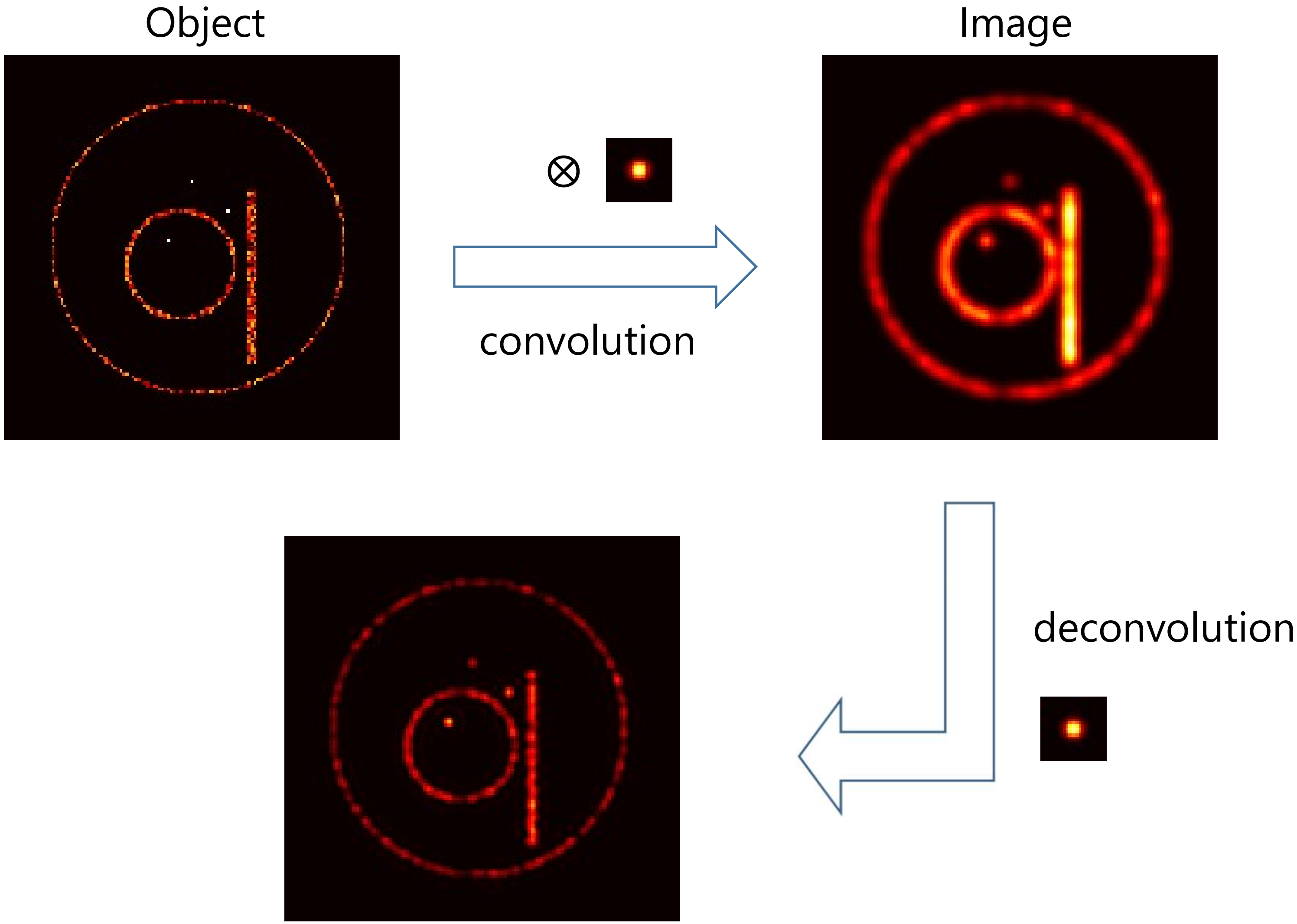
Magnification: $\frac{f2}{f1}$

Light wave model of optical imaging system

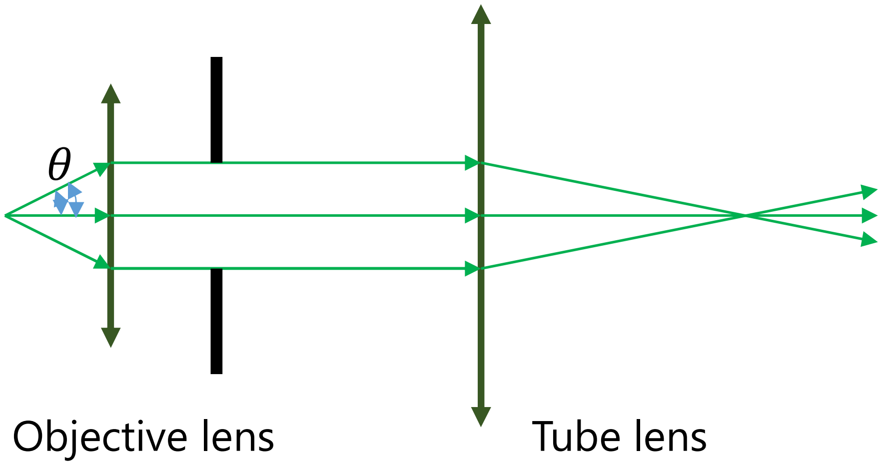


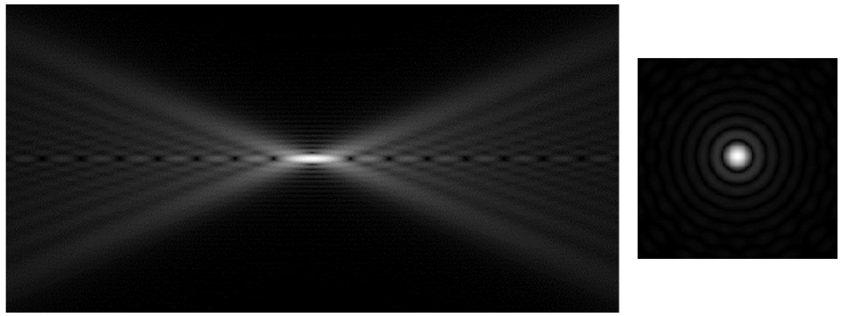
Magnification: $\frac{f2}{f1}$

Optical imaging system is linear & spatial invariant



Point spread function





Fraunhofer diffraction pattern of a circular aperture (Airy disk)

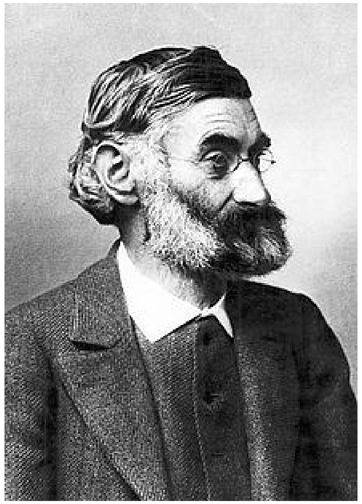
$$I(x) \sim \left(\frac{J_1(kx)}{kx}\right)^2$$

$$k = \frac{2\pi}{\lambda} n sin\theta = \frac{2\pi}{\lambda} NA$$

$$NA = n sin\theta$$

Numerical Aperture

Point spread function & Resolution



Ernst Karl Abbe

Resolution: $d = \frac{\lambda}{2nsin\theta} = \frac{\lambda}{2 * NA}$

Numerical Aperture: $NA = nsin\theta$

Point Spread Function & Resolution

Example:

An Olympus Plan Apochromat, 60X, NA 1.27, water objective is mistakenly installed in a Nikon microscope, please calculate the magnification and expected resolution when imaging a GFP labelled cell.

Olympus tube lens focal length: 180 mm
Nikon tube lens focal length: 200 mm
Emission wavelength of GFP: 510 nm

Olympus objective focal length: 180 mm/60 =3 mm
Magnification: 200 mm/3 mm = 66.7

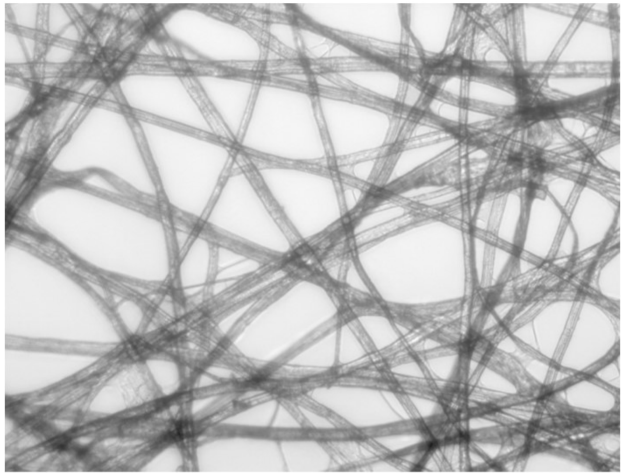
Resolution: $\frac{\lambda}{2NA} = \frac{510\ nm}{2*1.27} = 200.8\ nm$

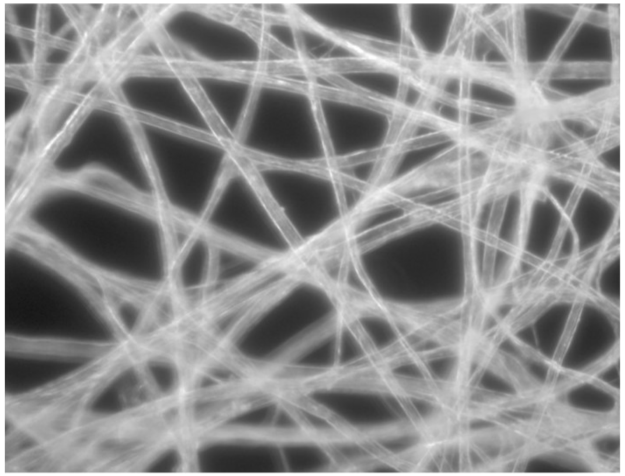
Conventional microscope

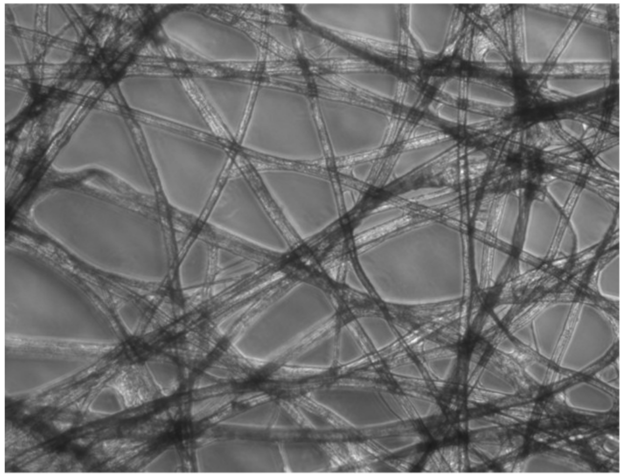
Bright Field

Dark Field

Phase Contrast/
Different Interference
Contrast (DIC)

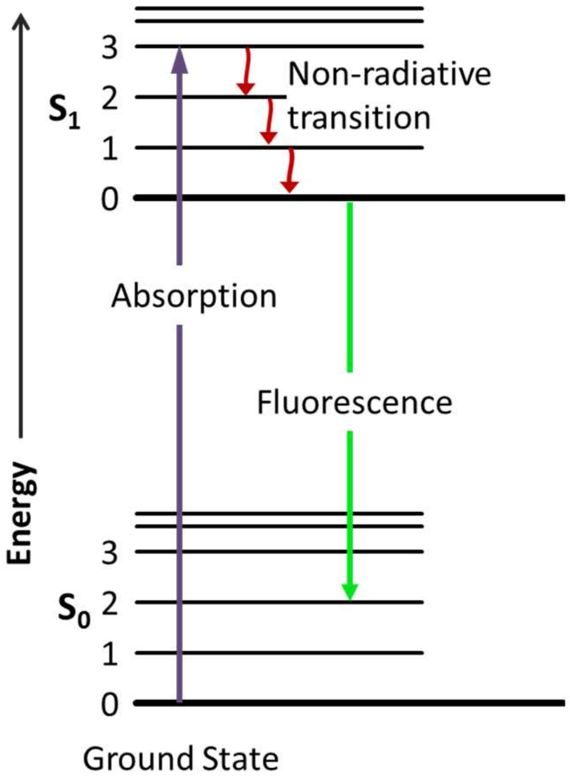




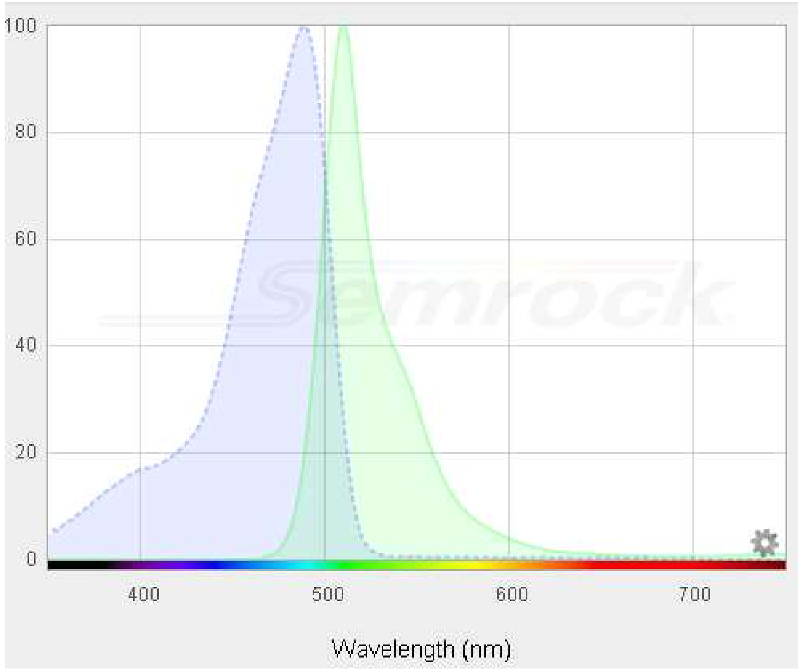


Staining

Fluorescent indicator



EGFP

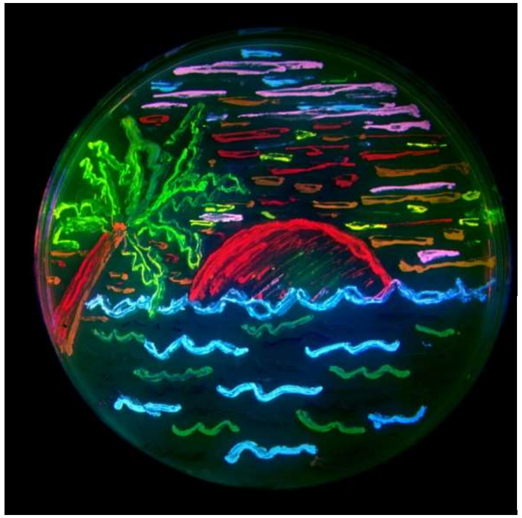


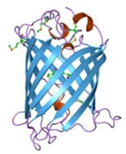
- Single molecule sensitivity

Fluorescent indicator

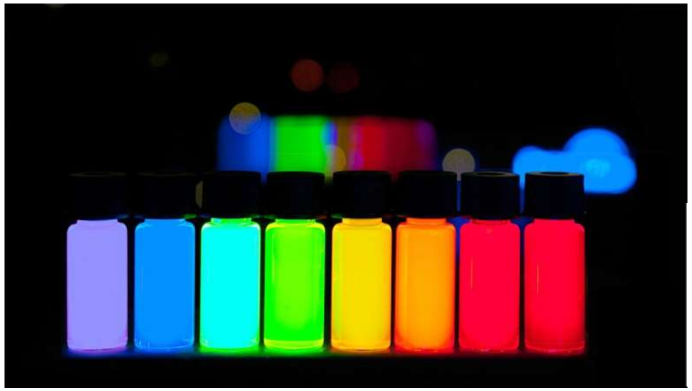
Dye

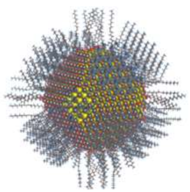
Protein



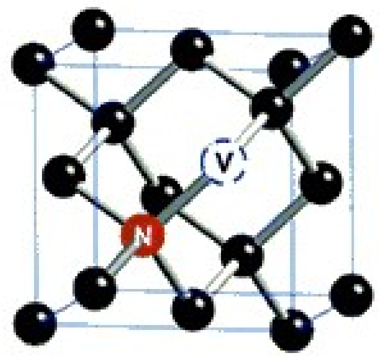


Quantum dot





Nano Diamond



Hot topics in optical imaging

Fluorescence imaging

3D imaging

- Confocal
- Multiphoton
- Light sheet
- Light field
- Sectioning SIM

Higher resolution

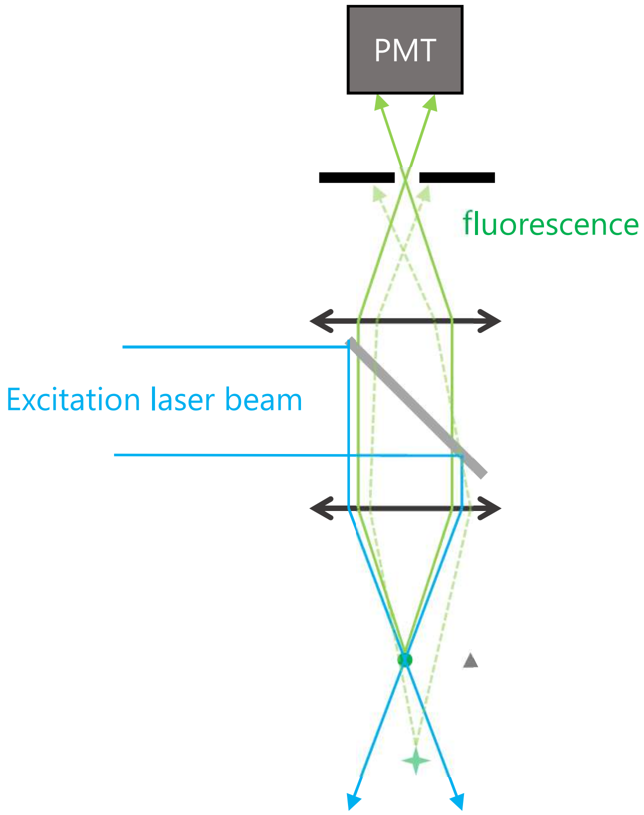
- PALM/STORM
- STED
- Super-resolution SIM
- Expansion microscope

3D Imaging

- Confocal microscope
- Two photon microscope
- Light sheet microscope
- Light field microscope

Confocal Scanning Microscope

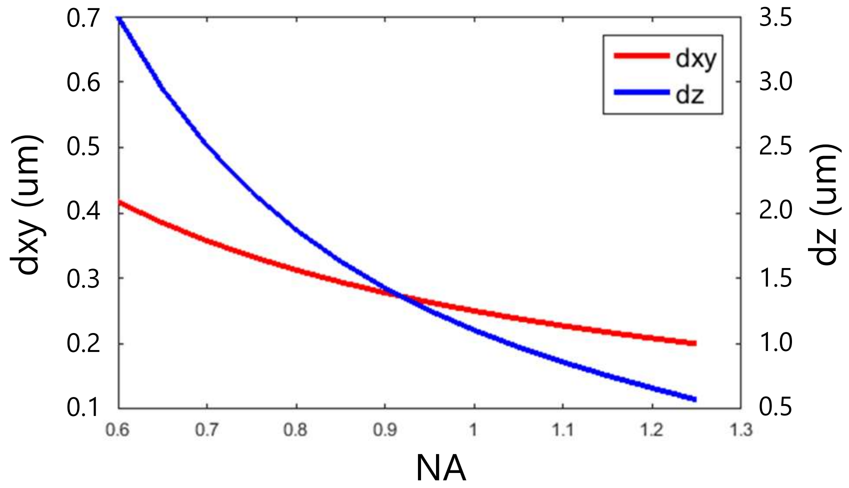
In 1940 Hans Goldmann, ophthalmologist in Bern, Switzerland, developed a slit lamp system to document eye examinations. This system is considered by some later authors as the first confocal optical system.



Resolution: $$d_{xy} = \frac{\lambda}{2 * NA}$$

$$d_z = \frac{\lambda}{n - \sqrt{n^2 - NA^2}}$$

Imaging resolution of GFP using water objective of different NA

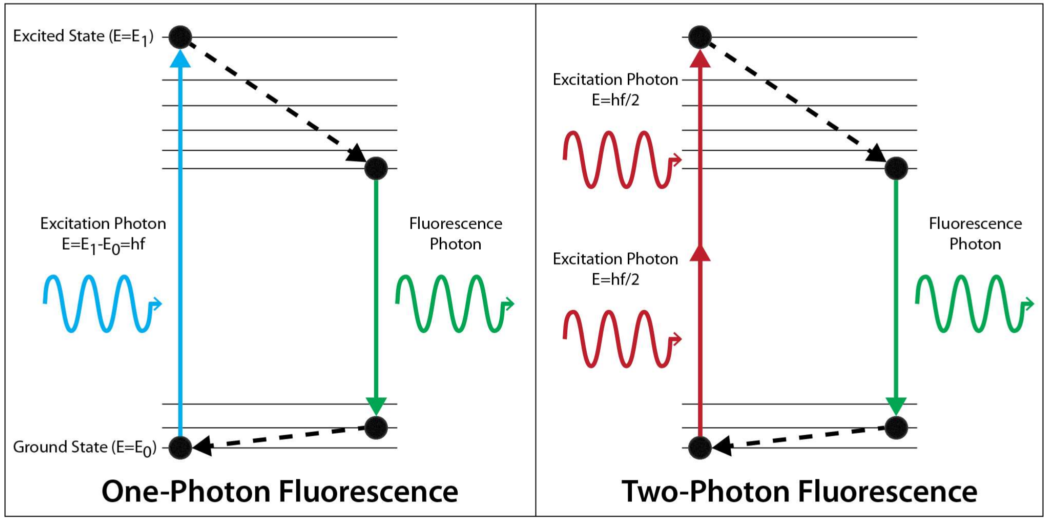


Two photon microscope

Theory of two photon absorption:

Maria Goeppert-Mayer

1 GM = 10e-50 cm4 s/photon



Wide field: 1e4 W/m^2 1e16 W/m^2

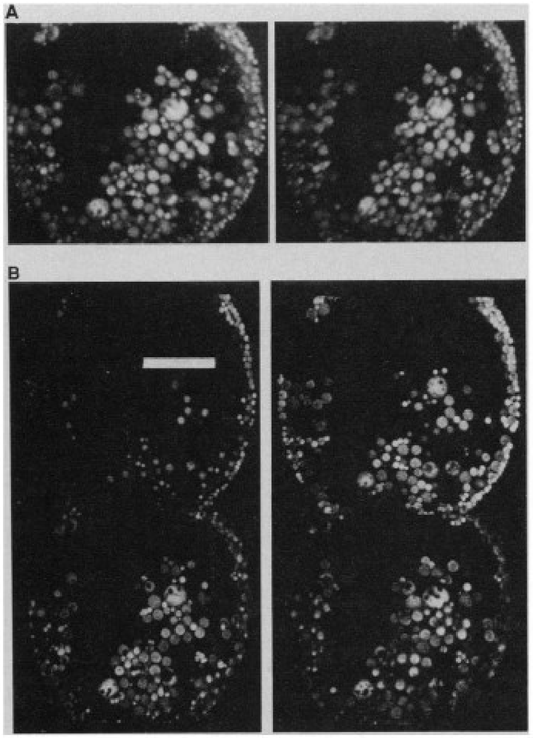
Two photon microscope

Two-Photon Laser Scanning Fluorescence Microscopy

WINFRIED DENK,* JAMES H. STRICKLER, WATT W. WEBB

Science, Vol. 248, Issue 4951, pp. 73-76 (1990)

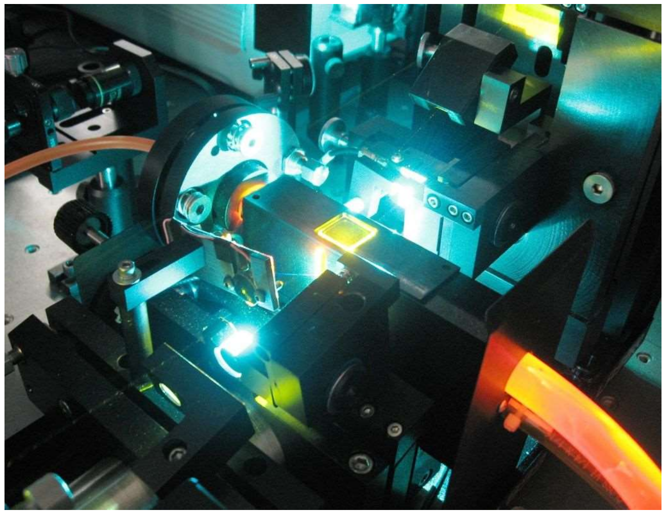
Fig. 1. (**A**) A stereo image pair is synthesized from a stack of six cross sections (*xy* sections) with an axial (*z*) increment of 3 μm. Blue (380 nm ≤ λ ≤ 445 nm) fluorescence excited by two-photon (630 nm) absorption was detected to record these images of a cluster of fluorescent beads with an LSM but with its confocal pinhole fully opened. The latex beads are volume-stained with the dye Coumarin 138 and have their measured absorption and emission maxima at 365 and 415 nm, respectively. The data comprise ten averages for each section with no background subtraction or image enhancement. The total time to acquire the data was less than 2 min. (**B**) The topmost four of the images, *xy* sections, used to synthesize the stereo pair in (A). Scale bar, 50 μm.



Femto second dye laser

80MHz, 100fs pulse width

→ peak power is 125000 times of the average power



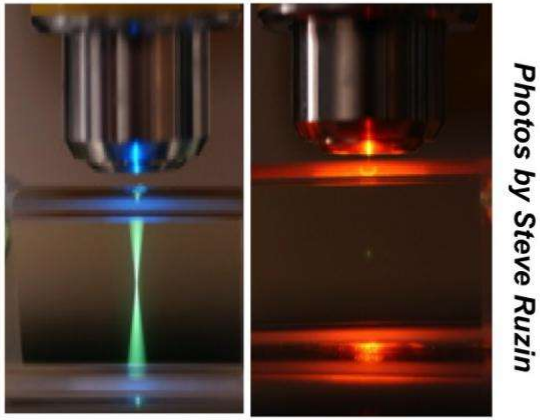
Laser source

Ti: Sapphire Laser

Coherent Chameleon familiy

Two photon microscope

1-photon vs. 2-photon



Fluorescence from out of focus planes **Fluorescence from focal spot only**

	1-photon	2-photon
Thin sample	Low laser intensity Higher resolution	
Thick sample		Less scattering Low photo damage Low photo bleaching

Problem: speed!!!!!!

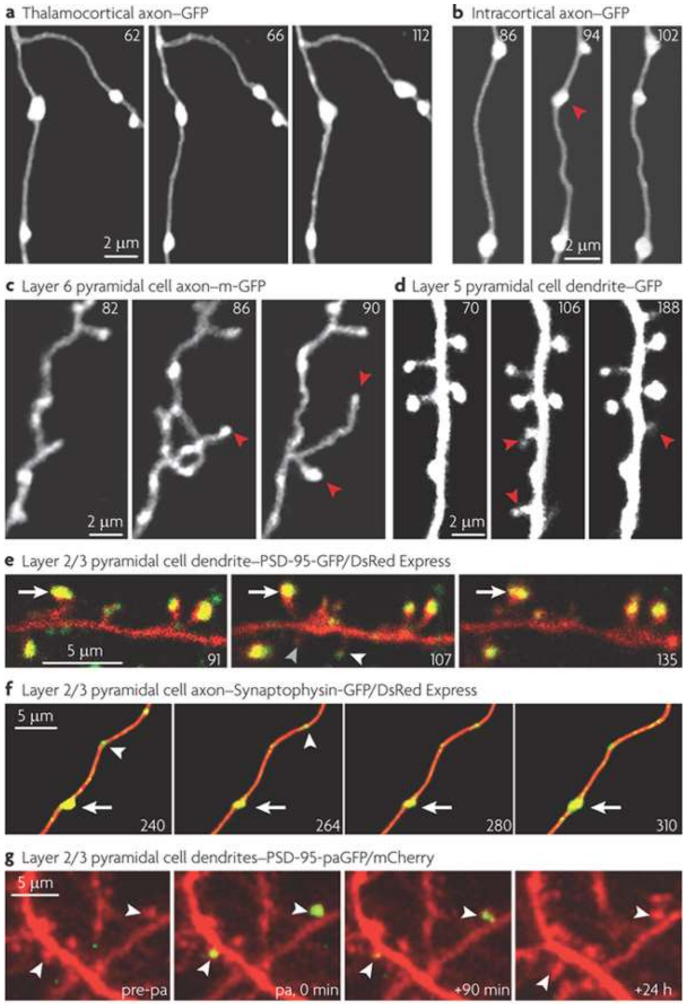
Two photon microscope

Brain Prize 2015

"invention, refinement and use of two-photon microscopy to provide detailed, dynamic images of activity in individual nerve cells, dendrites and synapses, thereby transforming the study of development, plasticity and functional circuitry of the brain."

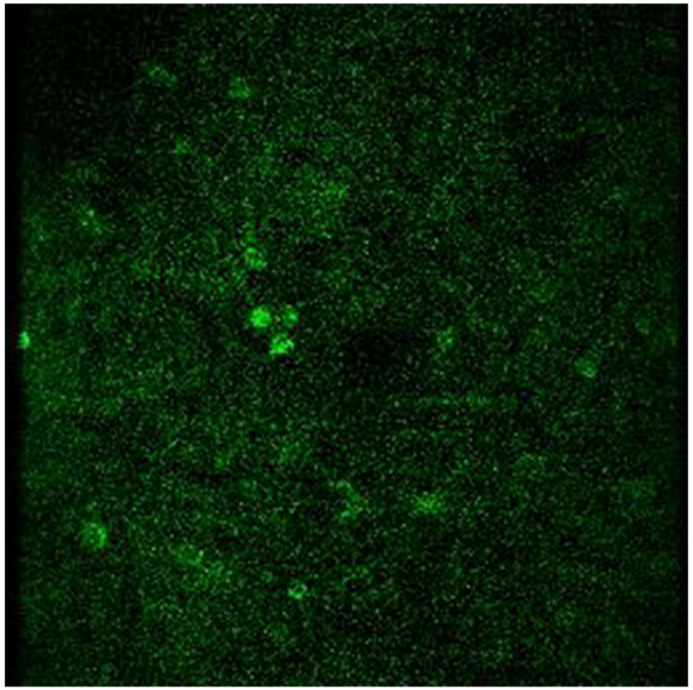
Time lapse two photon imaging of neuron plasticity

Behavioral Experiments



Nature Reviews | Neuroscience

Two photon functional imaging



Light sheet microscope & whole brain imaging

Richard Adolf Zsigmondy

Nobel Prize in chemistry in 1925

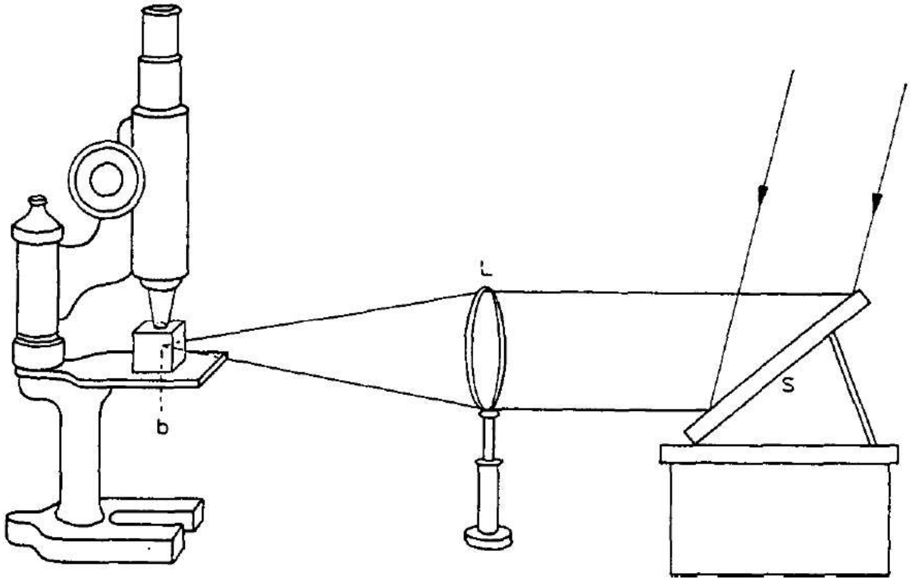
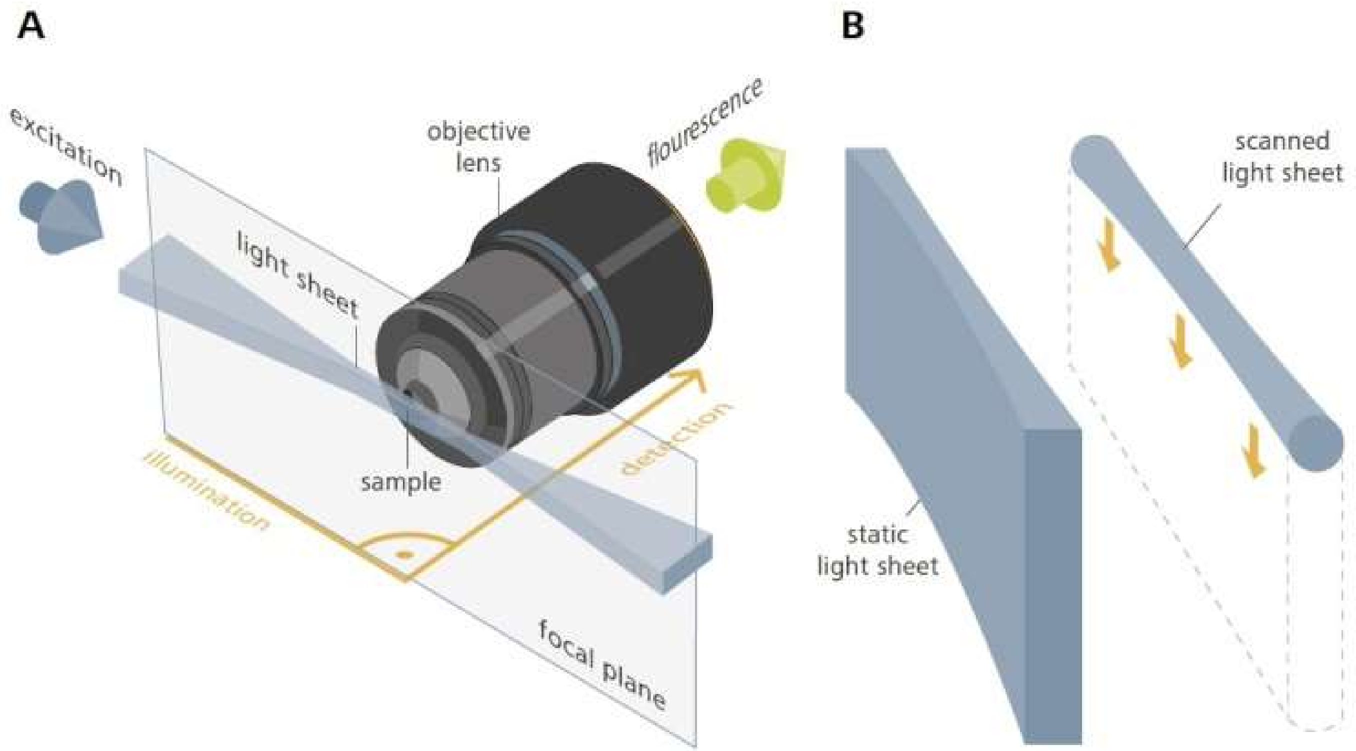


Fig. 1. The first arrangement for making ultramicroscopic particles visible.

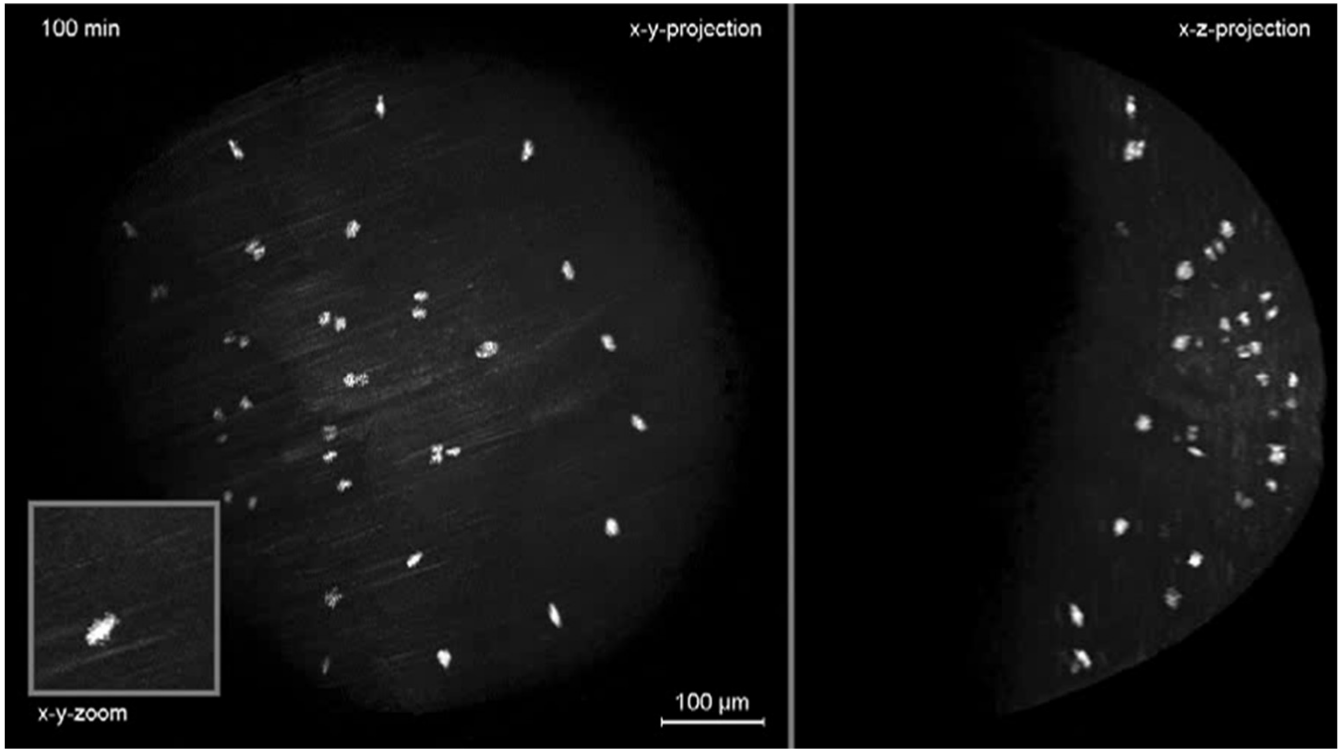
Light sheet microscope



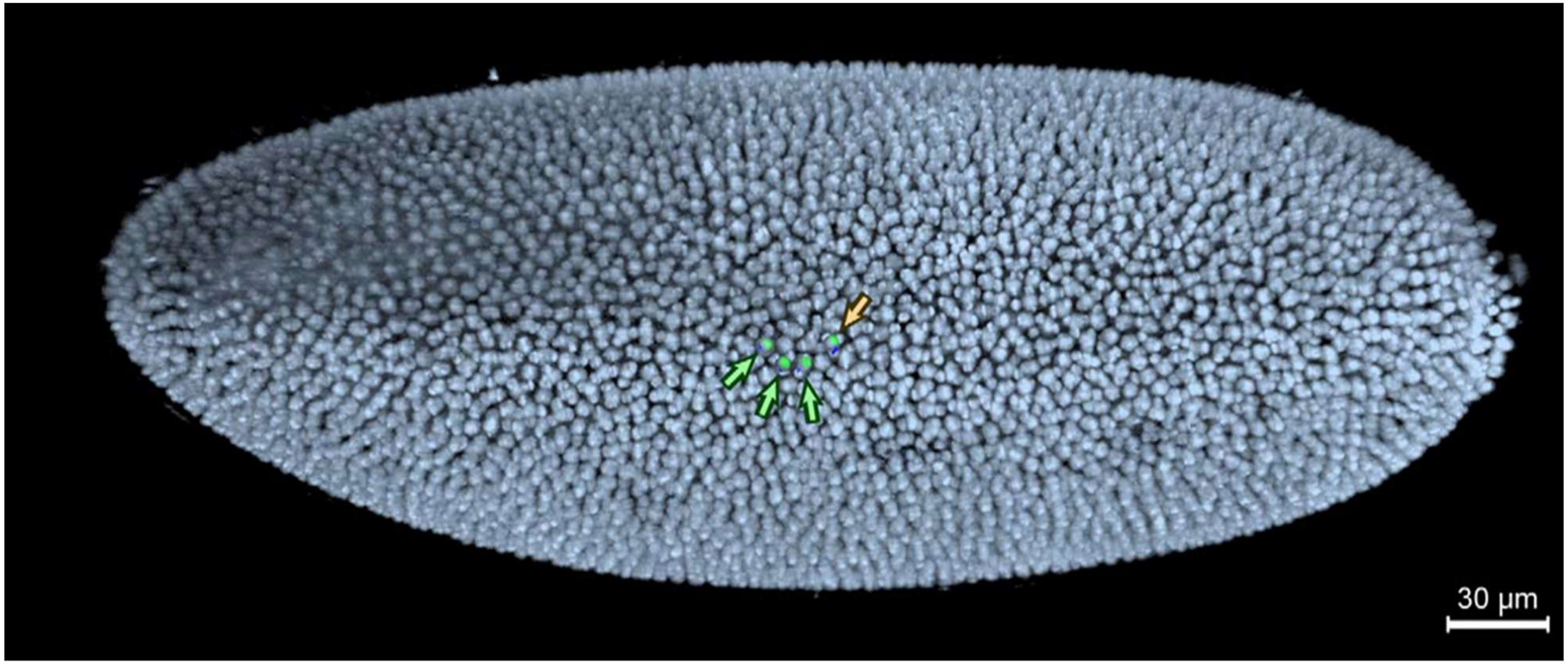
Light sheet microscope

Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy

Philipp J. Keller,[1,2]* Annette D. Schmidt,[2] Joachim Wittbrodt,[1,2,3,4]* Ernst H.K. Stelzer[1]



Light sheet microscope for development biology

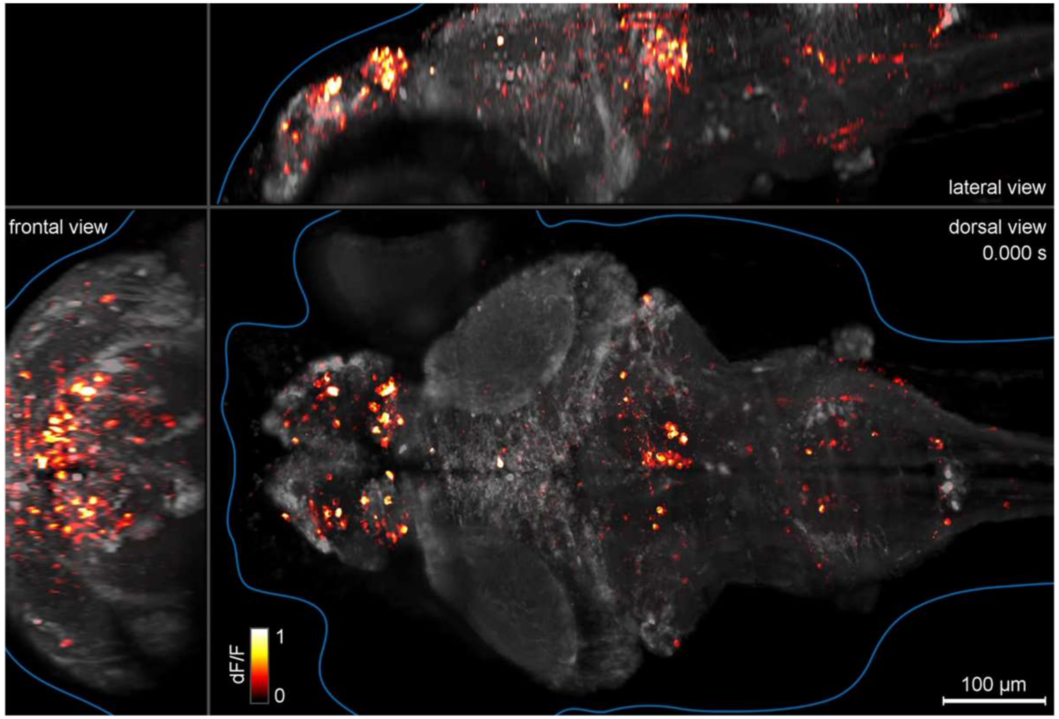


Tomer, R. et al. Nature Methods 9, 755-763 (2012)

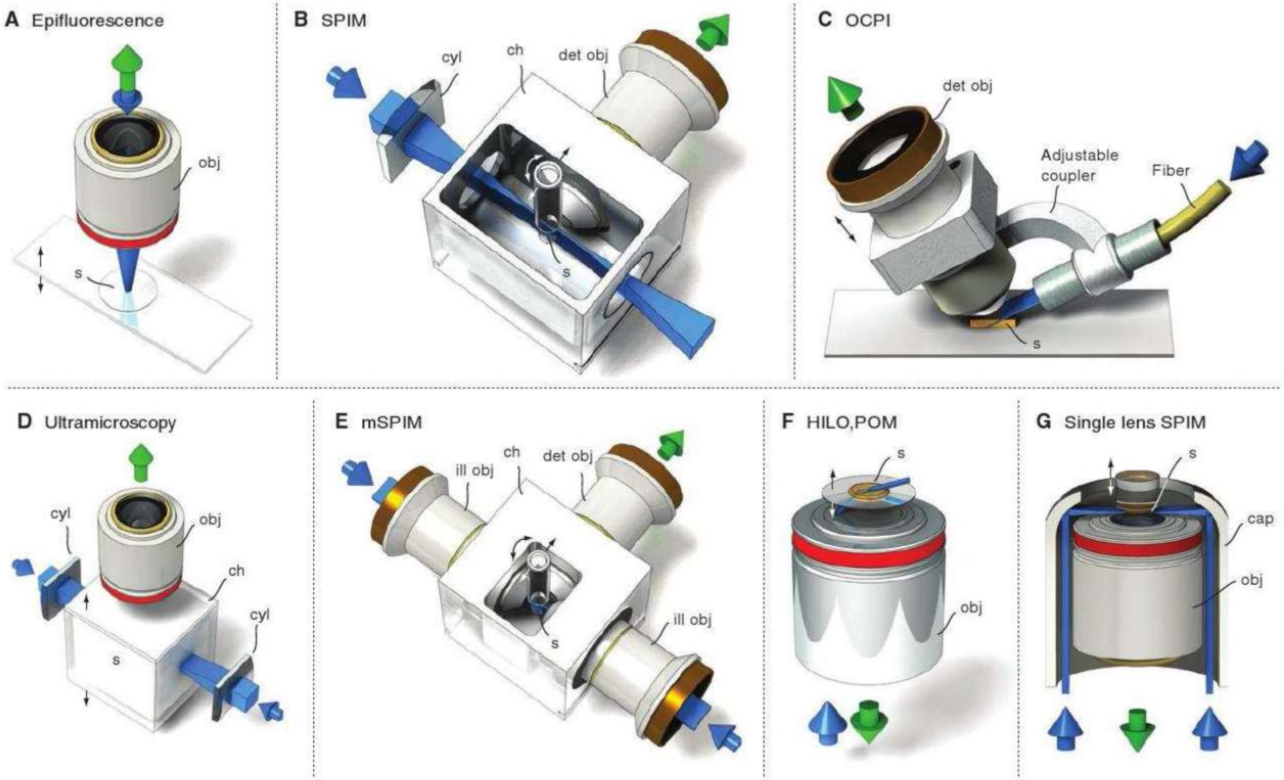
Light sheet microscope

Whole-brain functional imaging at cellular resolution using light-sheet microscopy

Misha B Ahrens[1], Michael B Orger[2], Drew N Robson[3], Jennifer M Li[3] & Philipp J Keller[1]



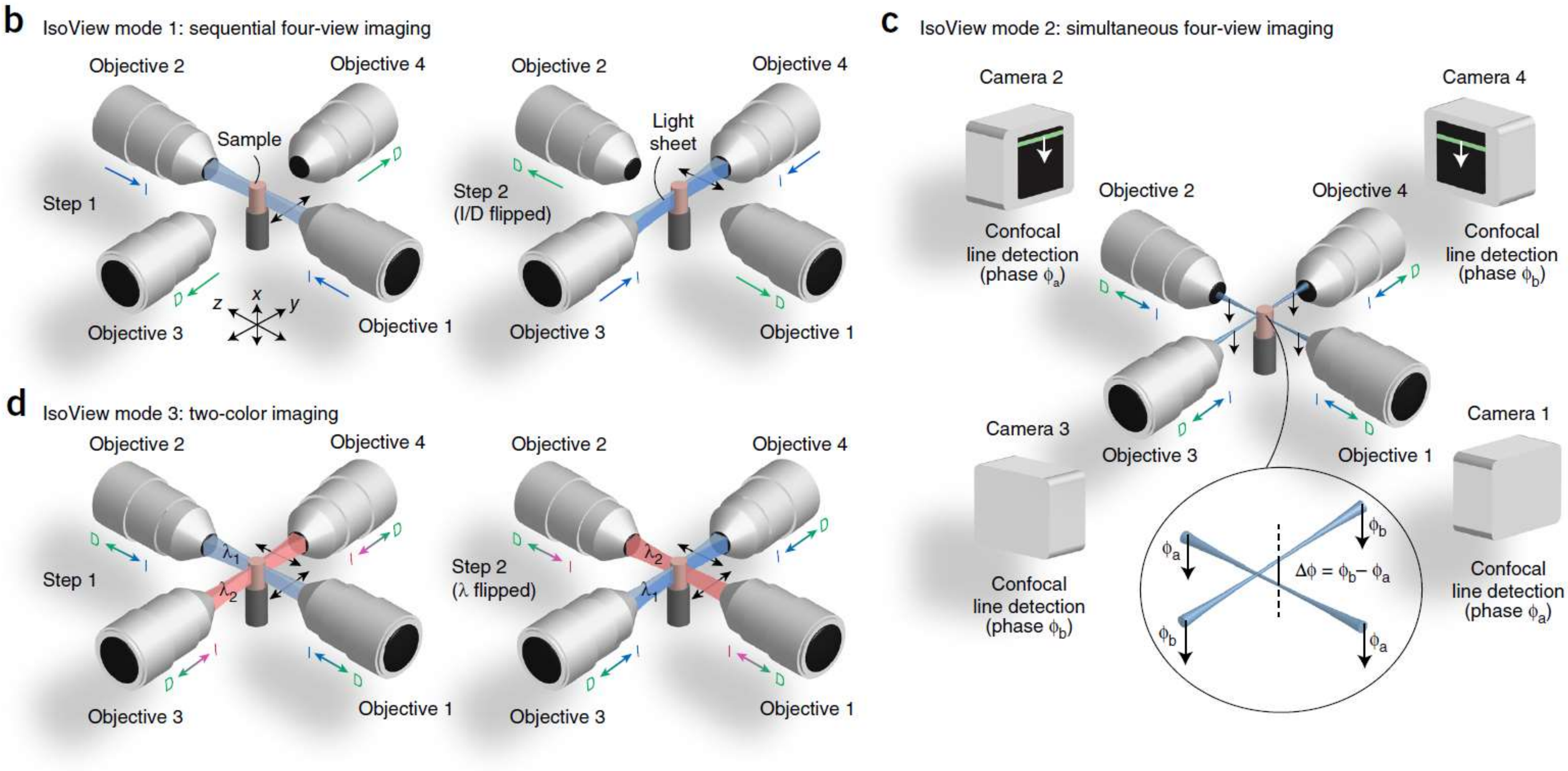
Light sheet microscope

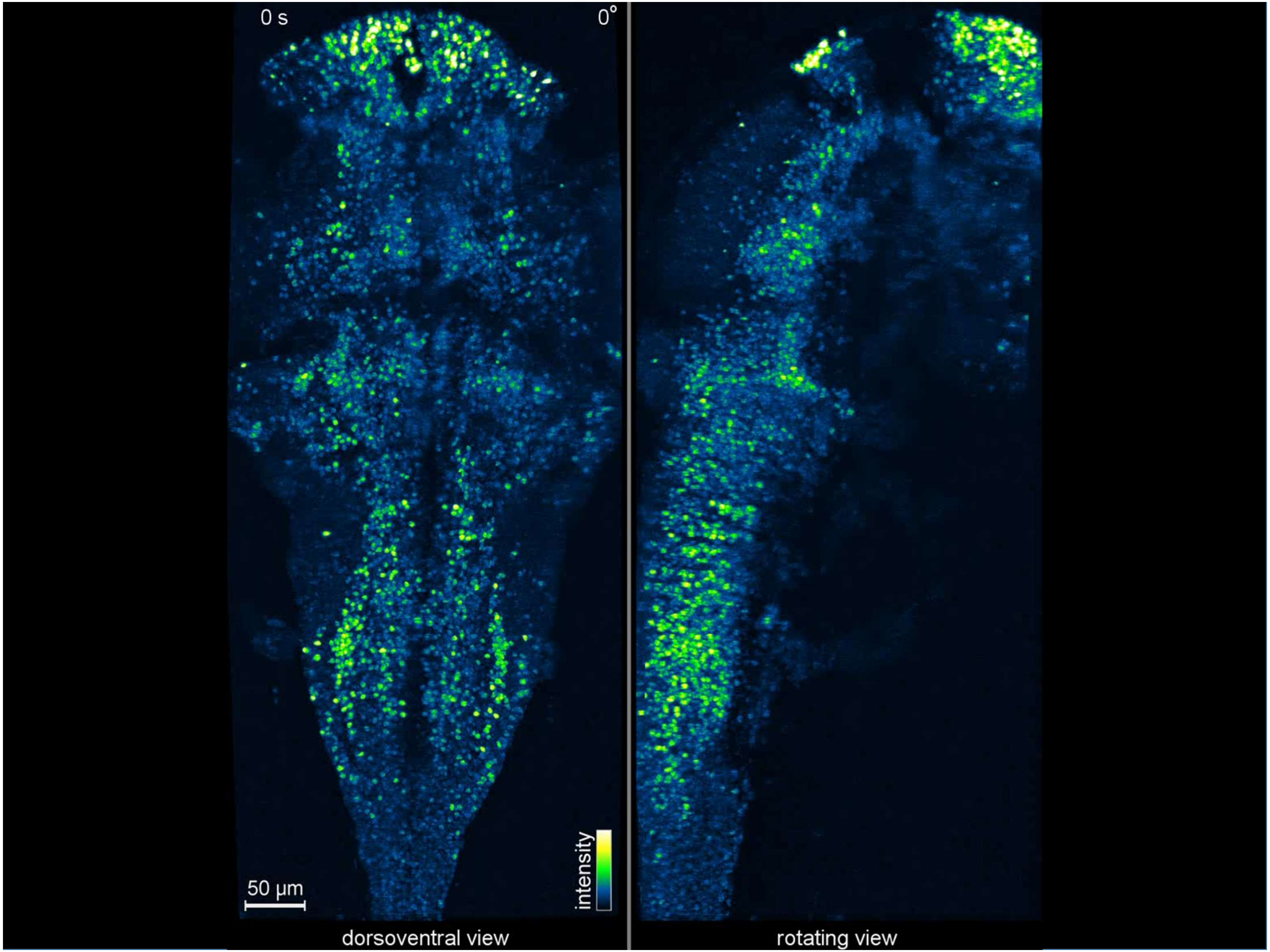


Light sheet microscope

Whole-animal functional and developmental imaging with isotropic spatial resolution

Raghav K Chhetri, Fernando Amat, Yinan Wan, Burkhard Höckendorf, William C Lemon & Philipp J Keller

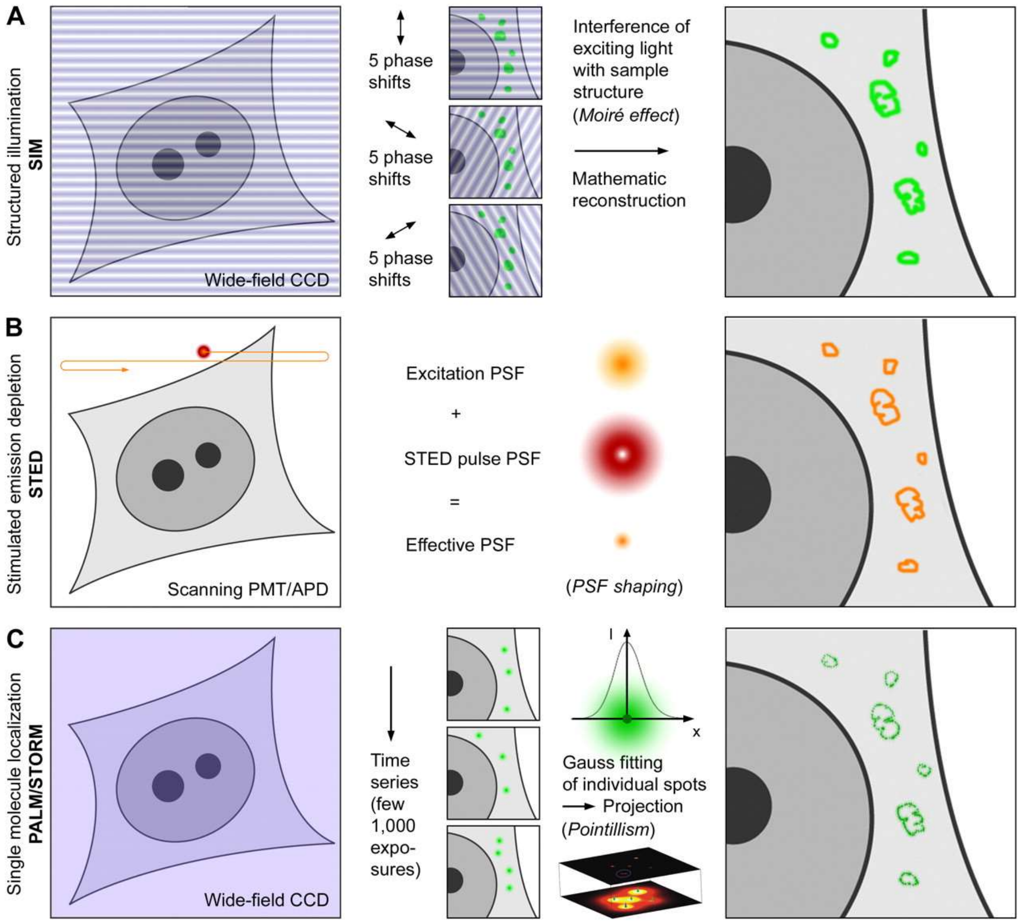



0 s
0°
50 µm
intensity
dorsoventral view
rotating view

Super resolution microscope

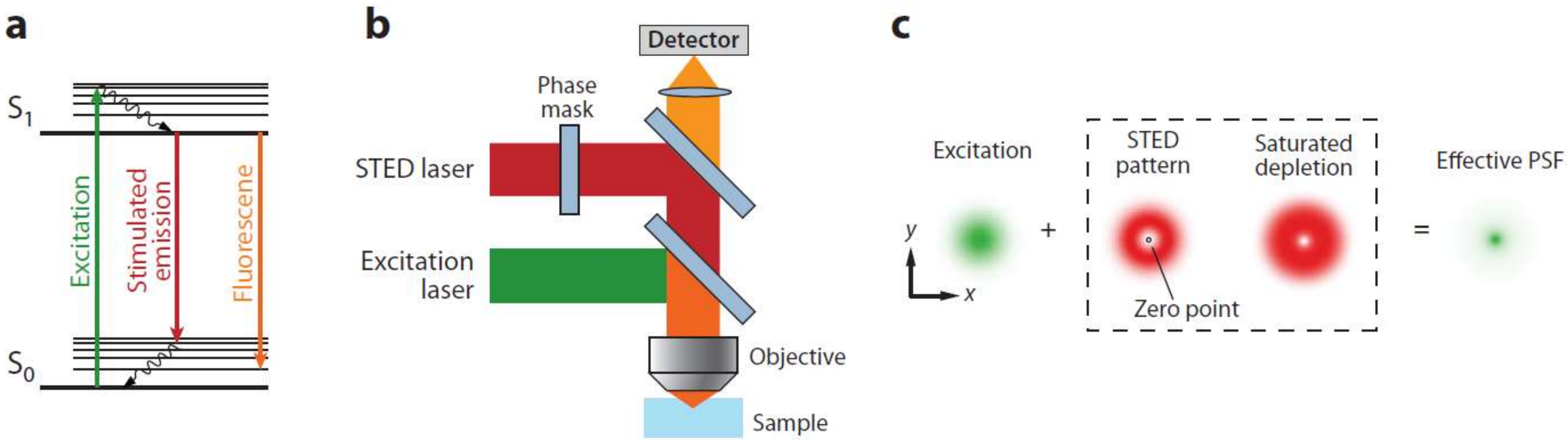
- STED Microscope
- Localization Microscope: PALM/STORM
- Structured Illumination Microscope (SIM)

Super-resolution microscope

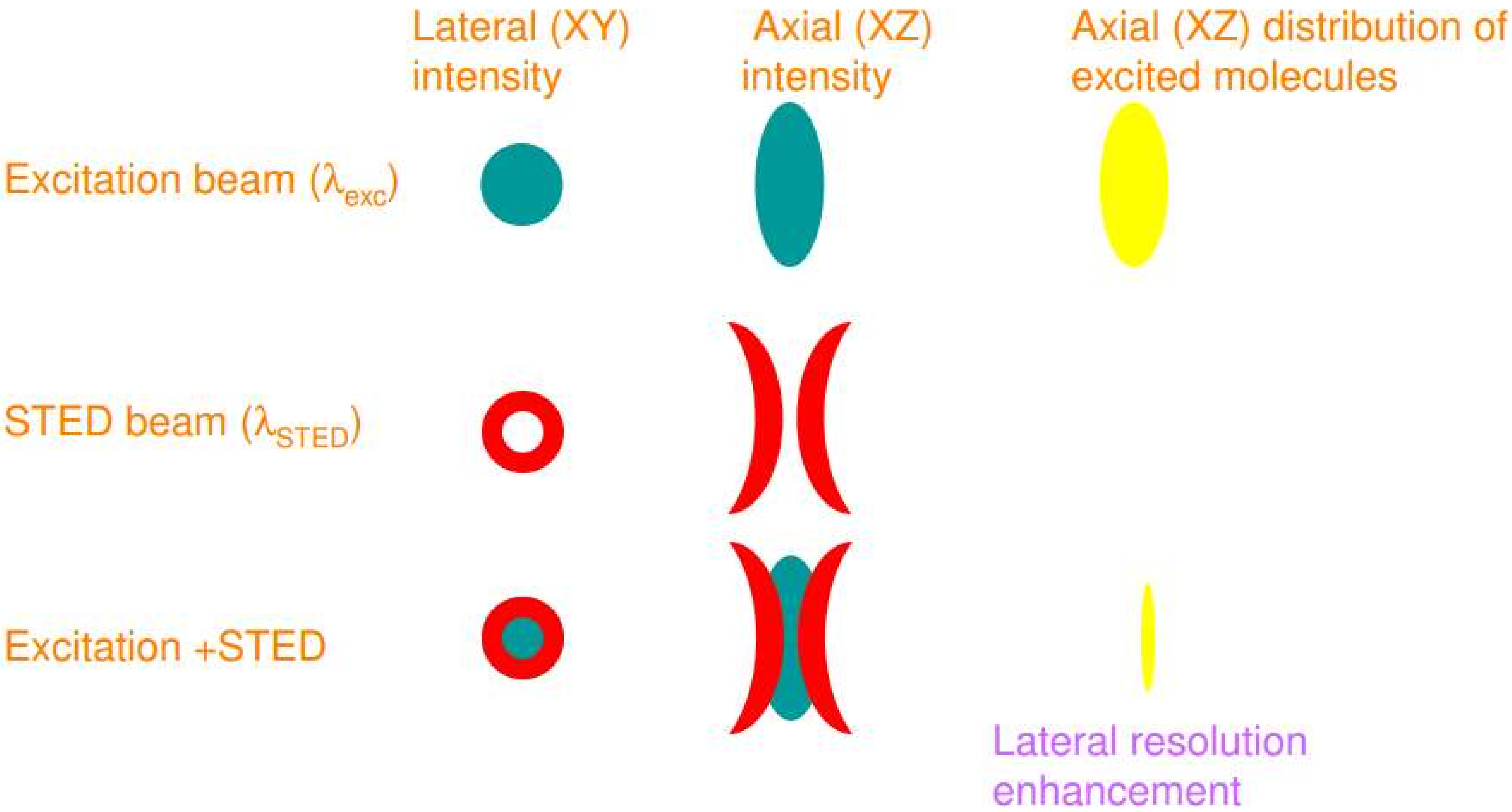


Lothar Schermelleh et al. J Cell Biol 2010;190:165-175

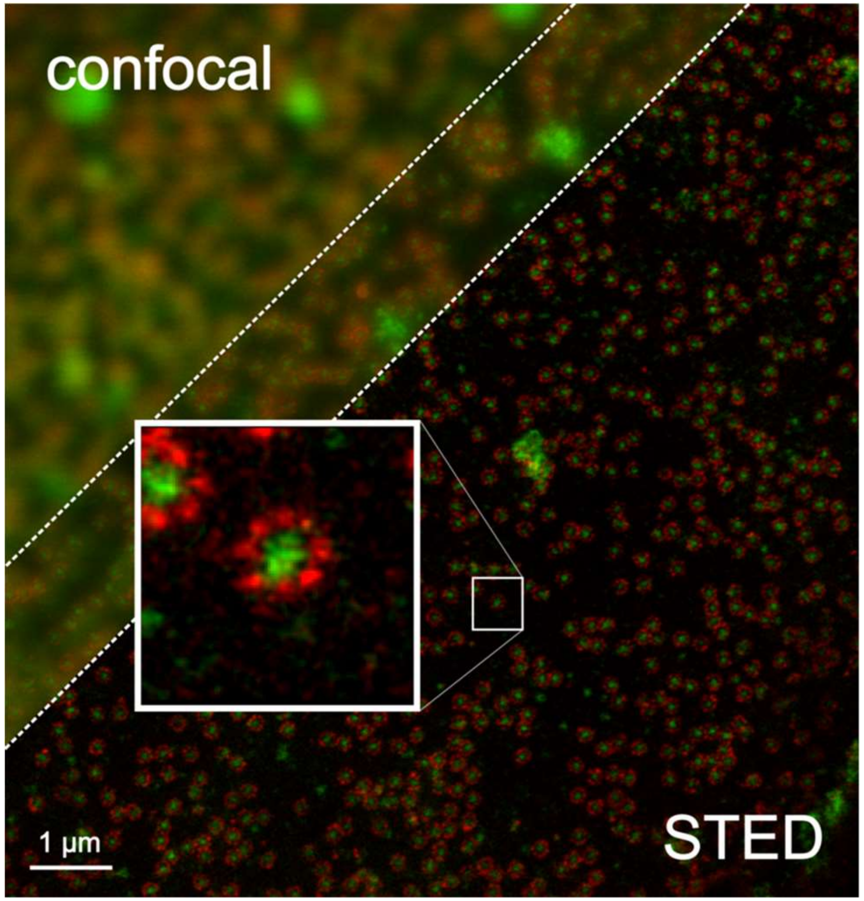
STimulated Emission Depletion (STED) Microscopy



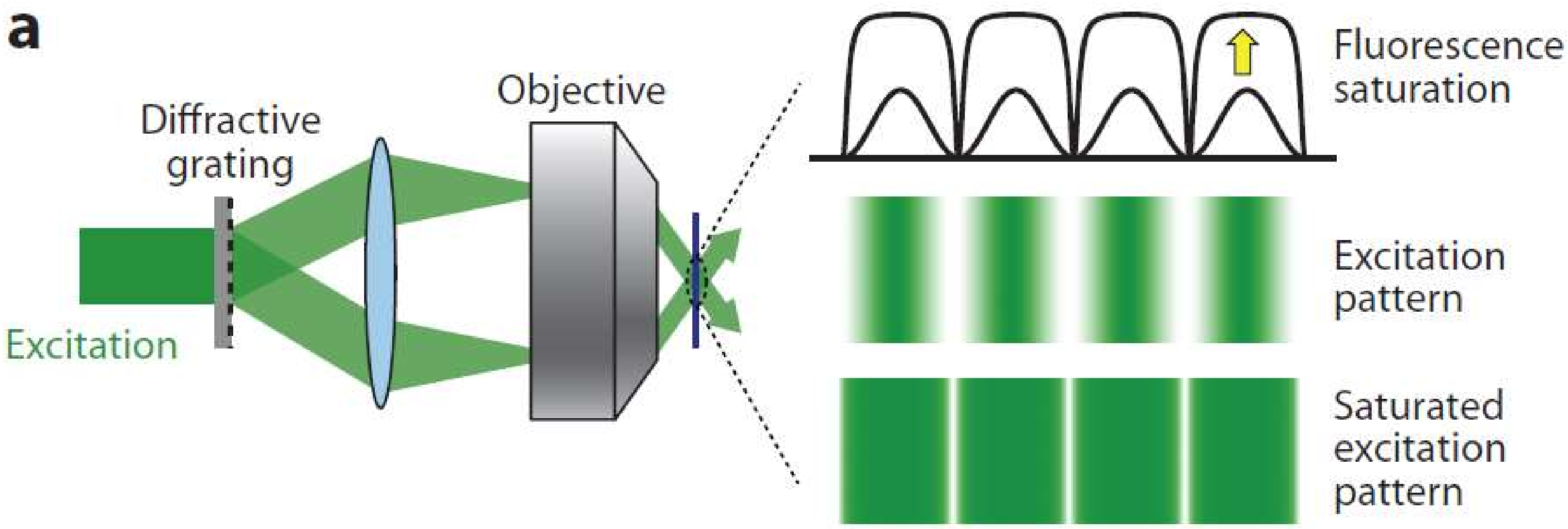
STimulated Emission Depletion (STED) Microscopy



STimulated Emission Depletion (STED) Microscopy

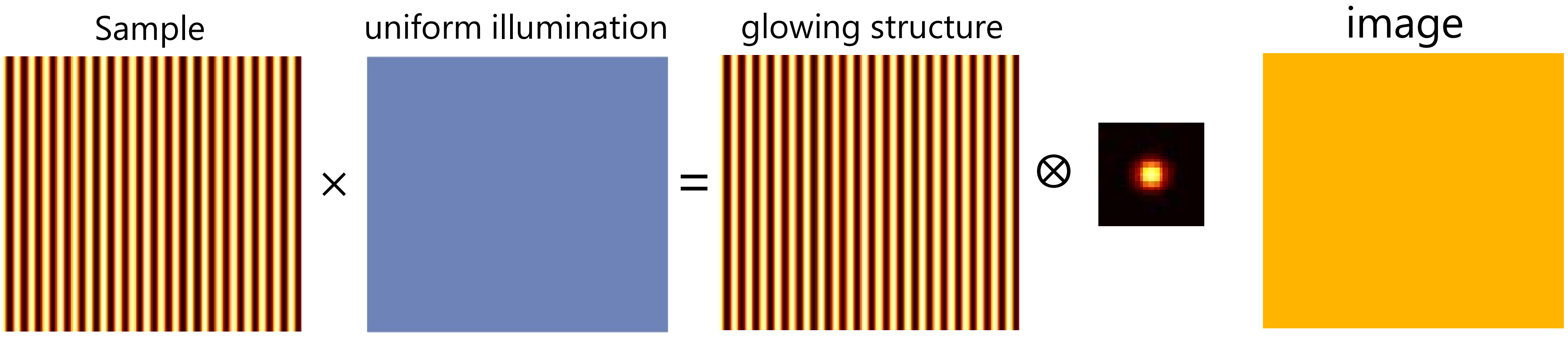


Structured Illumination Microscope (SIM)



Structured Illumination Microscope (SIM)

epi fluorescence imaging

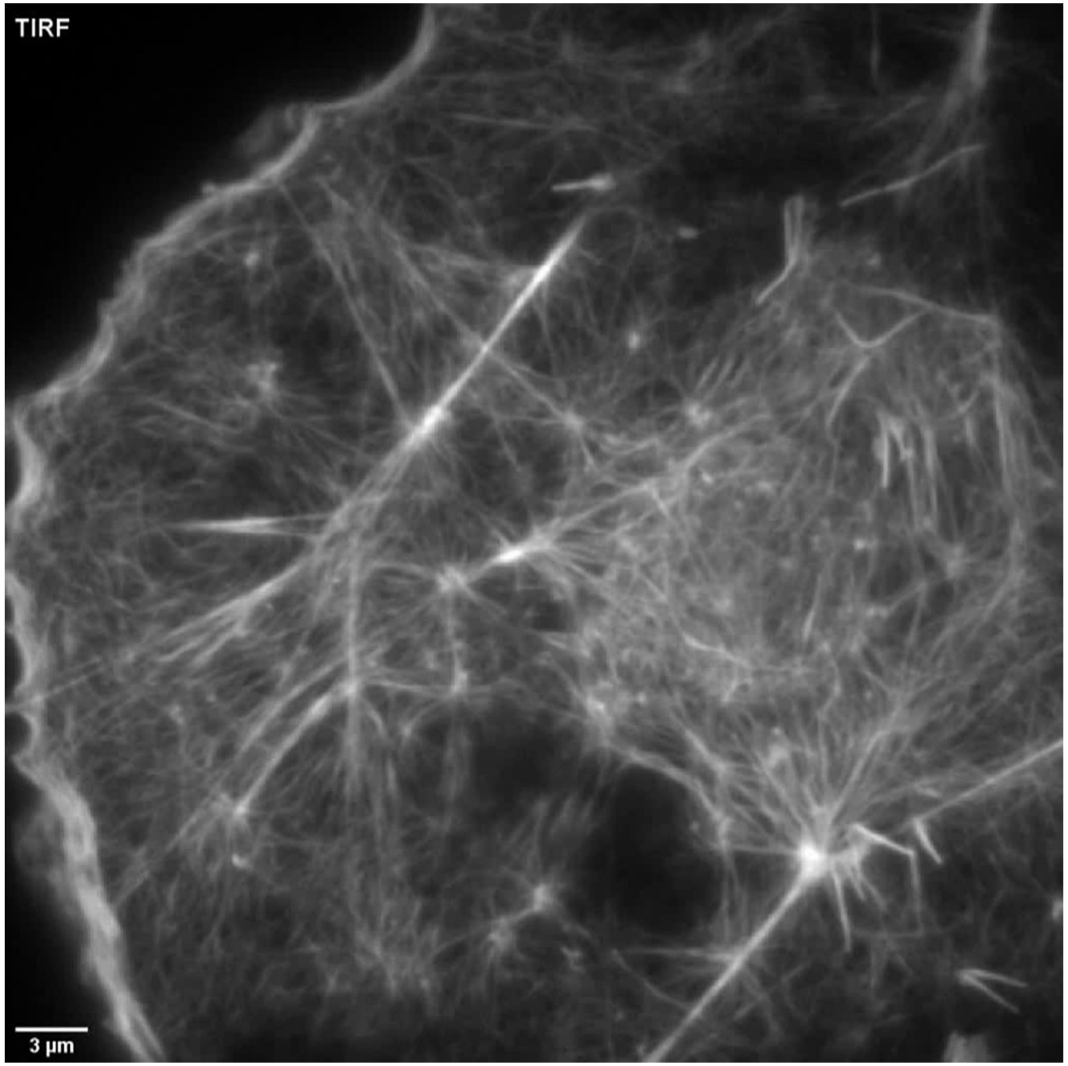


Structured Illumination Microscope (SIM)

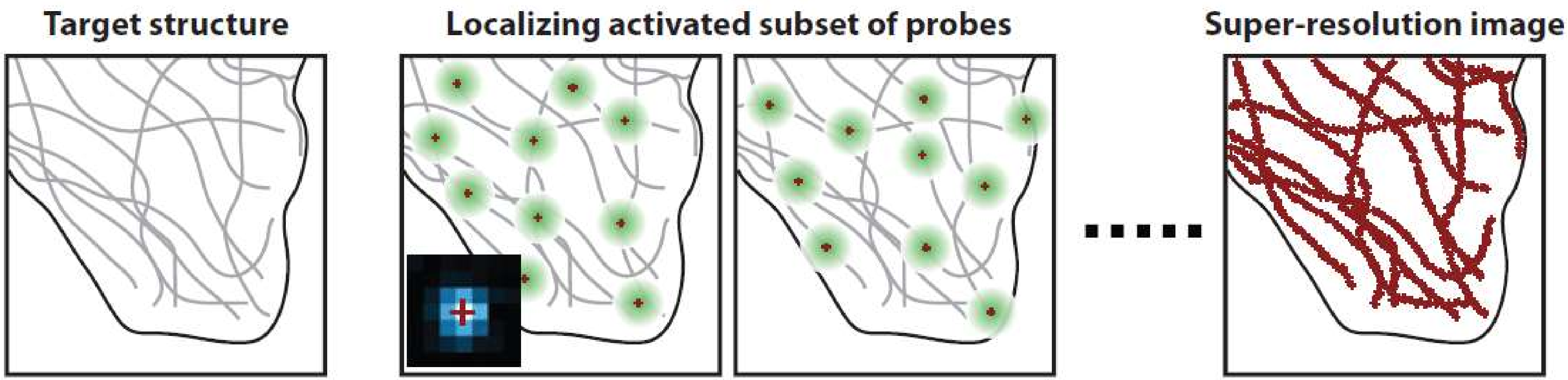
Live imaging demonstrated

	3D resolution		2D x-y resolution
	x-y	z	
Wide field	200 nm	600 nm	170 nm
Linear SIM	100 nm	300 nm	90 nm
1st nonlinear SIM	-	-	60 nm
2nd nonlinear SIM	-	-	45 nm

D. Li et a. *Science*, 2015; 349 (6251)



Localization microscope: PALM/STORM



Resolution is limited by labeling density and photon budget of each fluorophore

~10,000 images are required to reconstruct one image

Localization microscope: PALM/STORM

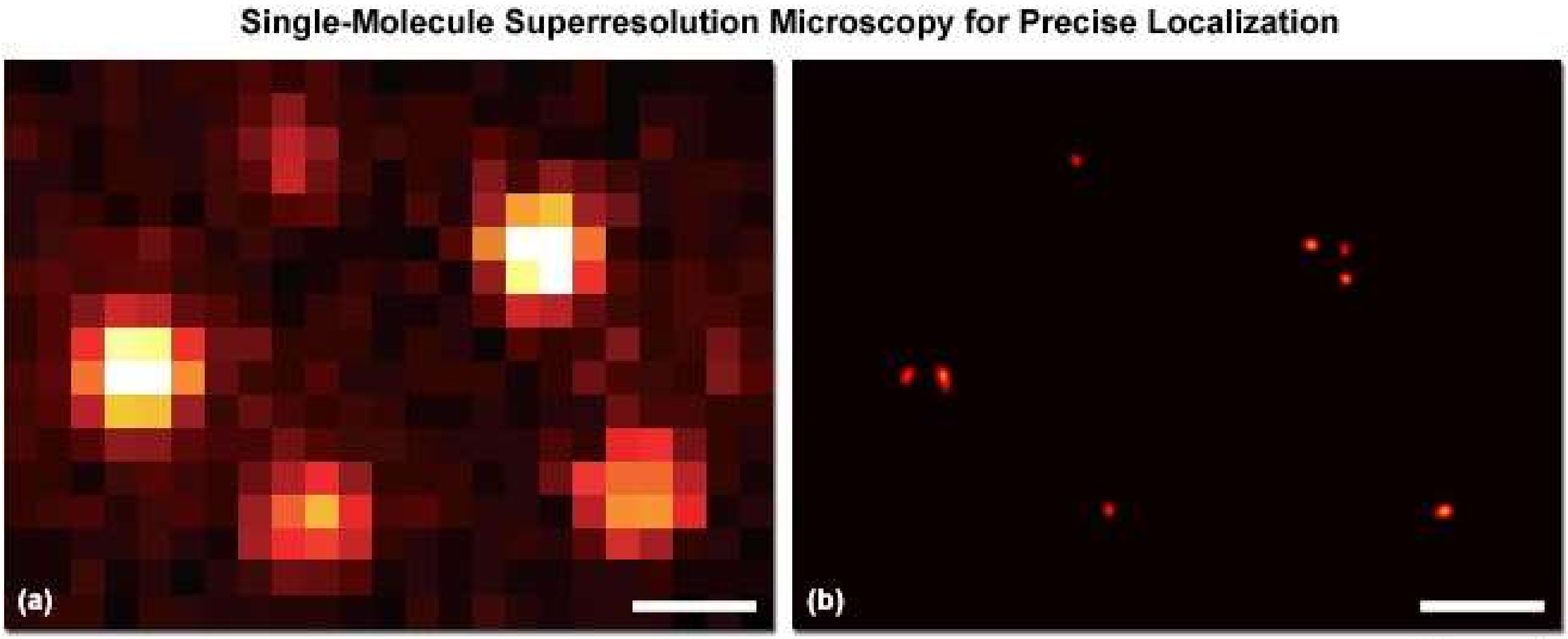
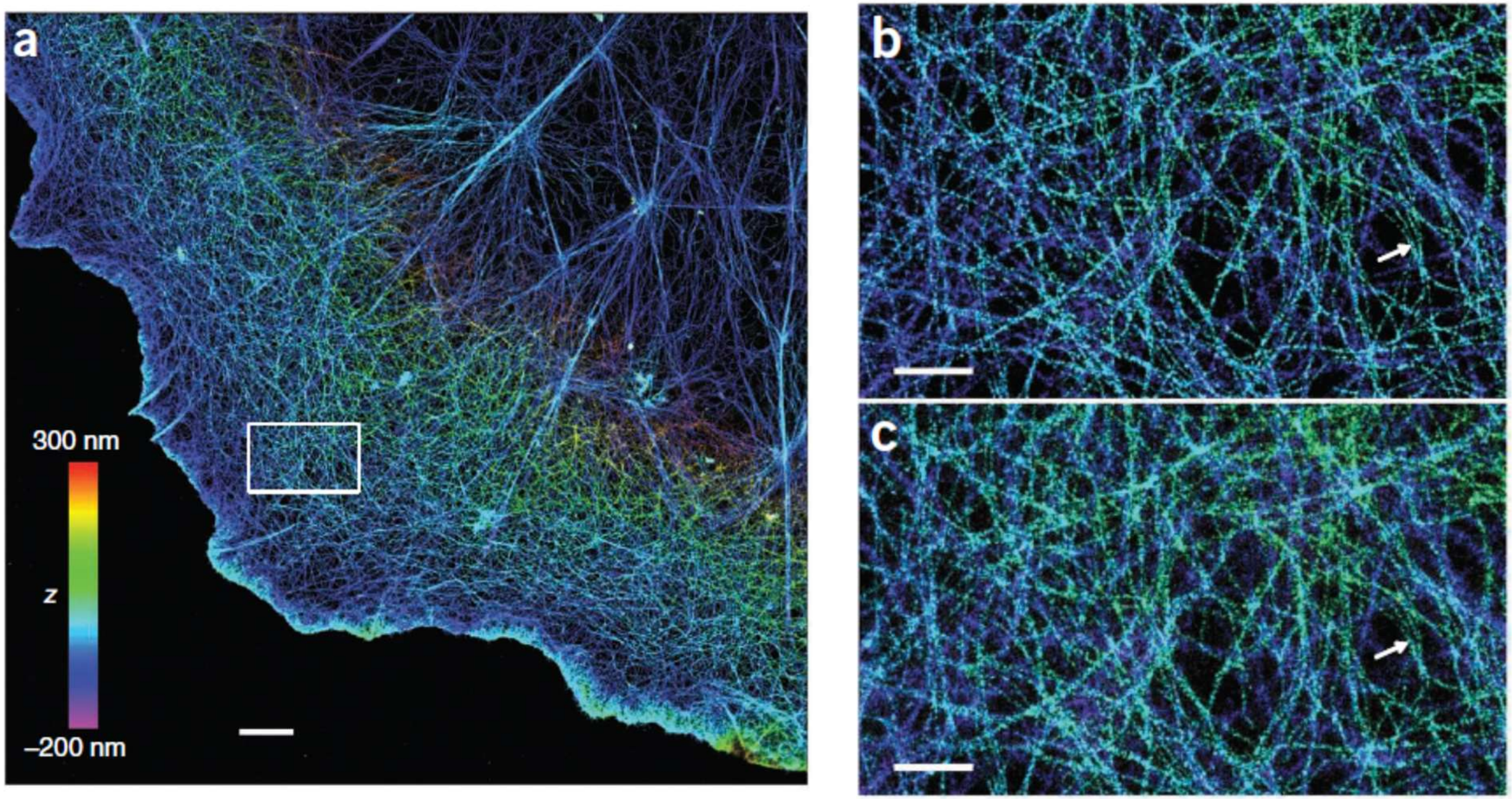


Figure 1

3D STORM



Ke Xu et al. Nature Methods 185-188 (2012)

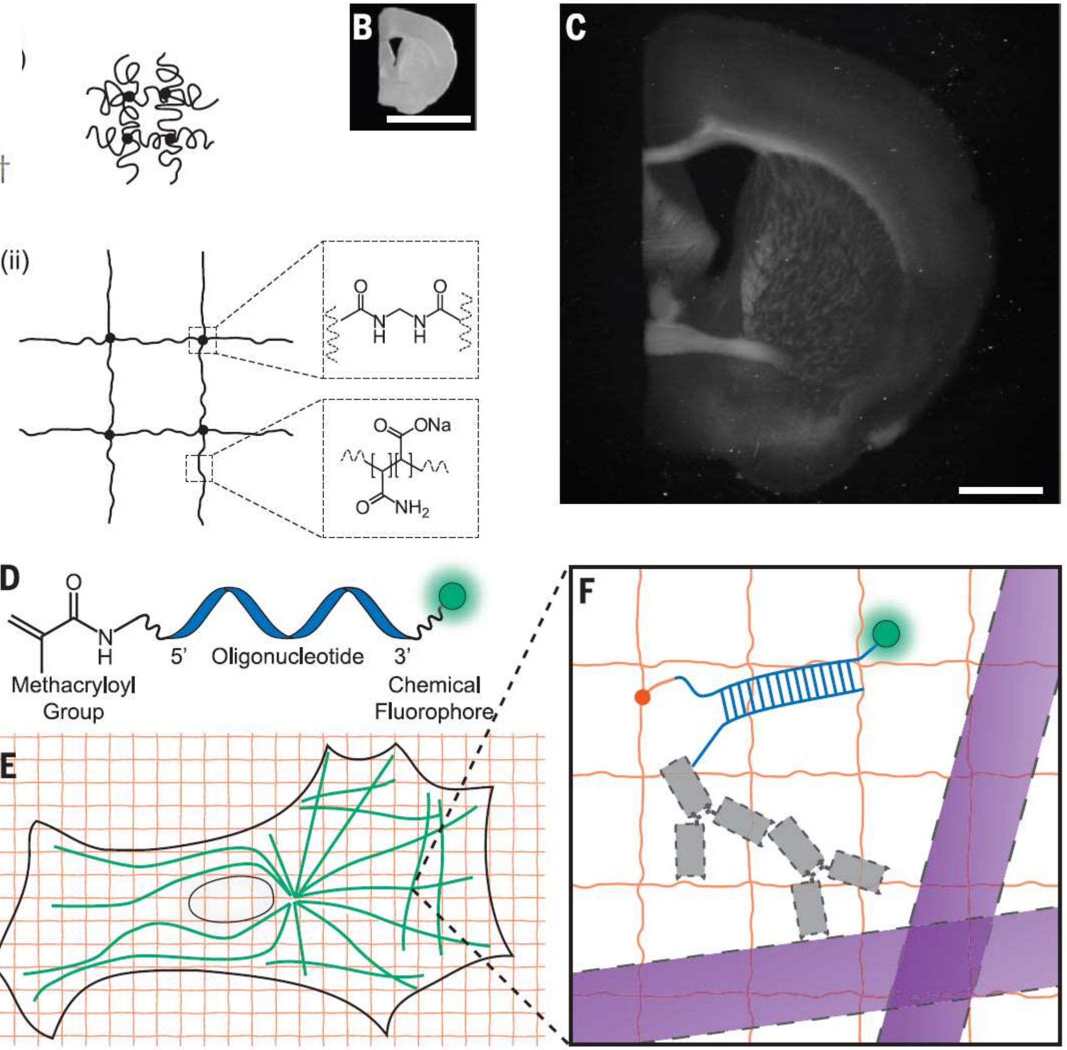
Expansion microscope

OPTICAL IMAGING

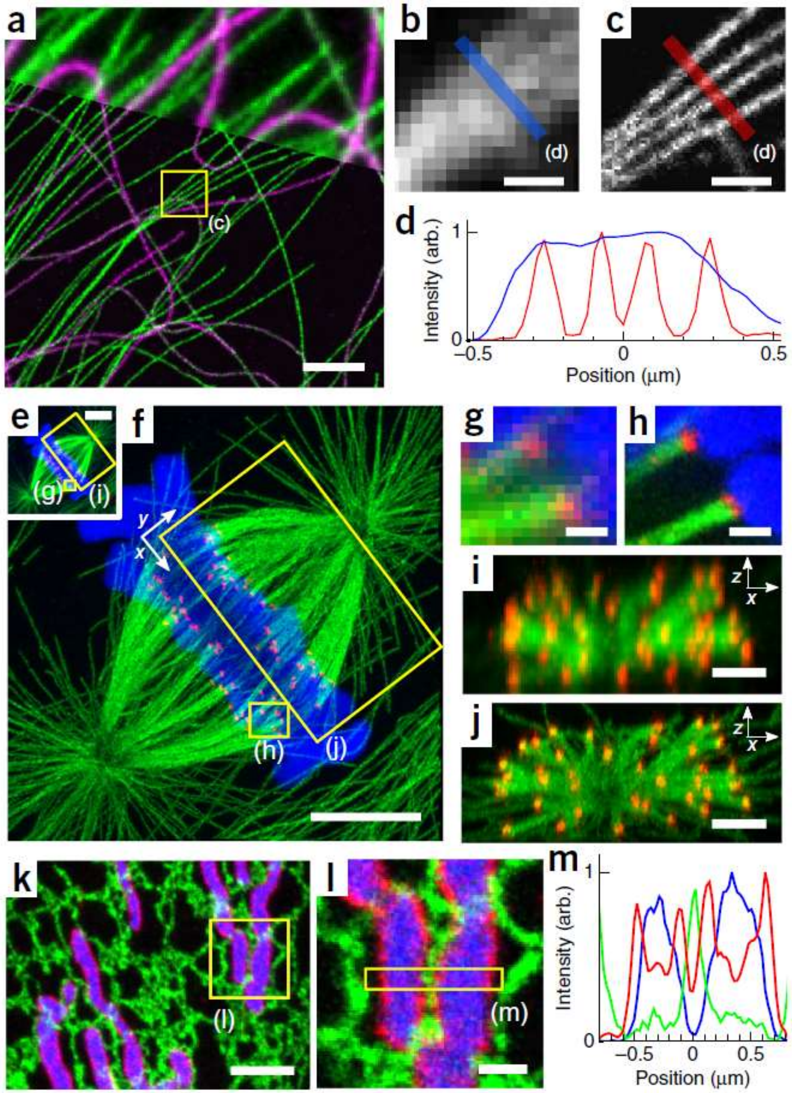
Expansion microscopy

Fei Chen,[1*] Paul W. Tillberg,[2*] Edward S. Boyden[1,3,4,5,6†]

illumination. (**D**) Schematic of label that can be anchored to the gel at site of a biomolecule. (**E**) Schematic of microtubules (green) and polymer network (orange). (**F**) The label of (D), hybridized to the oligo-bearing secondary antibody top (top gray shape) bound via the primary (bottom gray shape) to microtubules (purple), is incorporated into the gel (orange lines) via the methacryloyl group (orange dot) and remains after proteolysis (dotted lines). Scale bars, (B) and (C) 5 mm. Schematics are not to scale.



Expansion microscope



Expansion microscopy with conventional antibodies and fluorescent proteins

Tyler J Chozinski[1,4], Aaron R Halpern[1,4], Haruhisa Okawa[2], Hyeon-Jin Kim[1], Grant J Tremel[1], Rachel O L Wong[2] & Joshua C Vaughan[1,3]

nature biotechnology

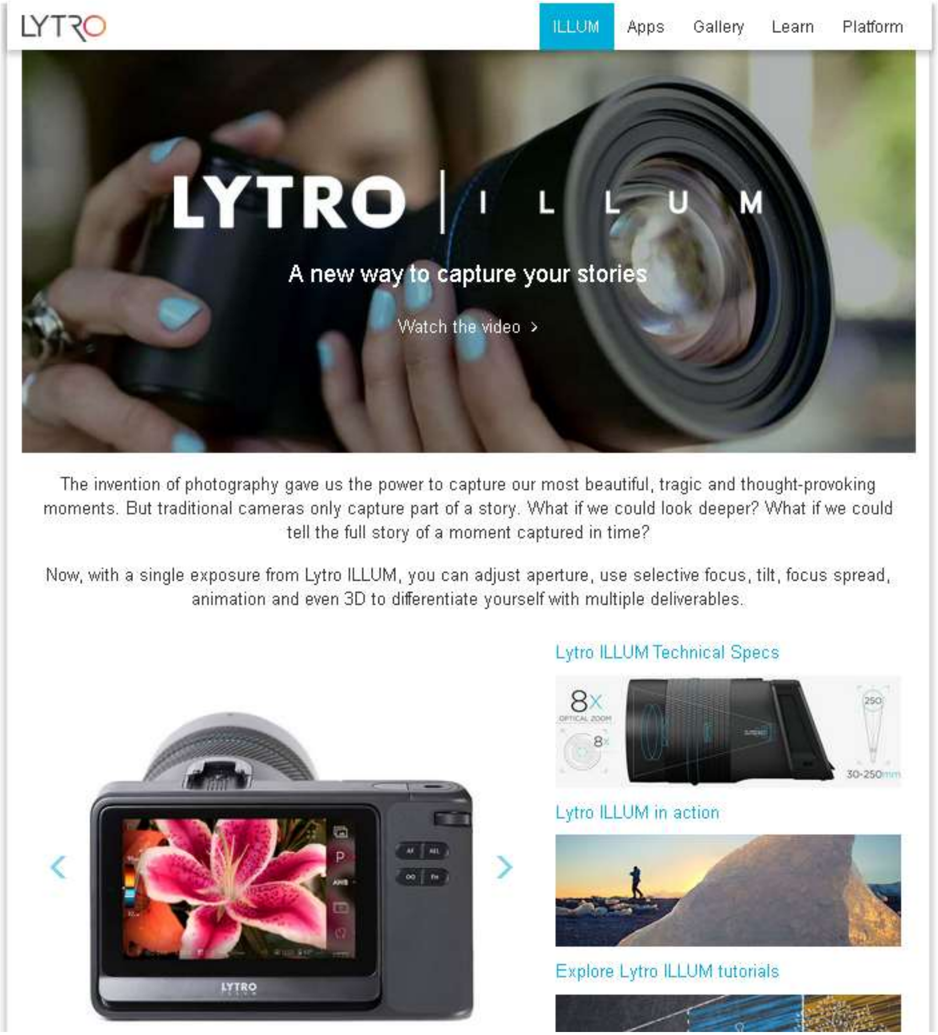
Protein-retention expansion microscopy of cells and tissues labeled using standard fluorescent proteins and antibodies

Paul W Tillberg[1,2,10], Fei Chen[2,3,10], Kiryl D Piatkevich[2], Yongxin Zhao[2], Chih-Chieh (Jay) Yu[2,3], Brian P English[4], Linyi Gao[3], Anthony Martorell[5], Ho-Jun Suk[2,6], Fumiaki Yoshida[7,8], Ellen M DeGennaro[5,8], Douglas H Roossien[9], Guanyu Gong[3], Uthpala Seneviratne[3], Steven R Tannenbaum[3], Robert Desimone[5,8], Dawen Cai[9] & Edward S Boyden[2,3,5,8]

Nanoscale imaging of RNA with expansion microscopy

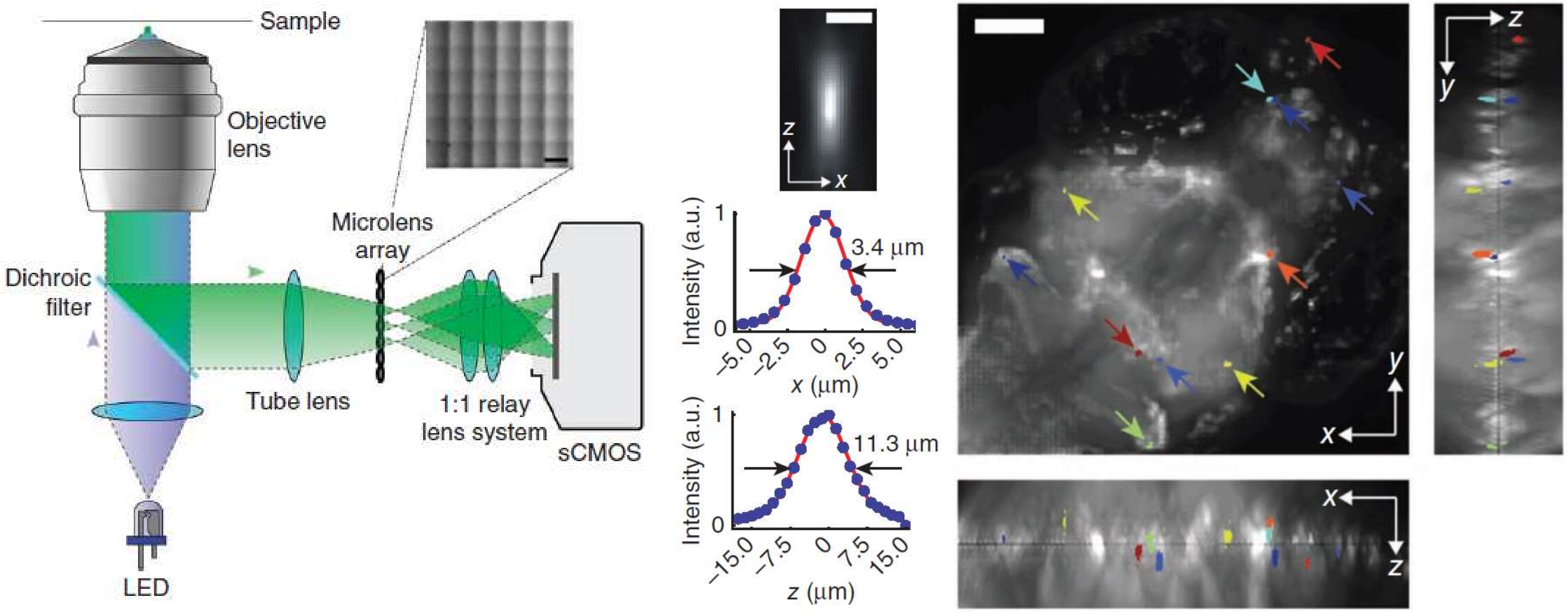
Fei Chen[1–3,10], Asmamaw T Wassie[1–3,10], Allison J Cote[4], Anubhav Sinha[5], Shahar Alon[2,3], Shoh Asano[2,3], Evan R Daugharthy[6,7], Jae-Byum Chang[2,3], Adam Marblestone[2,3], George M Church[6,8], Arjun Raj[4] & Edward S Boyden[1–3,9]

Special Topcis: Light field microscope



Light field microscope

Light field microscope



R. Prevedel, Nature Methods 2014

X-LFM X-Light Field Microscope

What's new?

- Resolving conflict between volume coverage and resolution by:
  - Decoupling between Numerical Aperture (NA) and Field of View (FOV)
  - Overlapping tolerance
  - Multifocal plane imaging
  - New reconstruction algorithm based on optical wave theory and maximum likelihood estimation

Free moving zebrafish whole brain imaging

XLFM X-Light Field Microscope

Improved z depth tracking system

丛林

王泽冠

杭苇

+

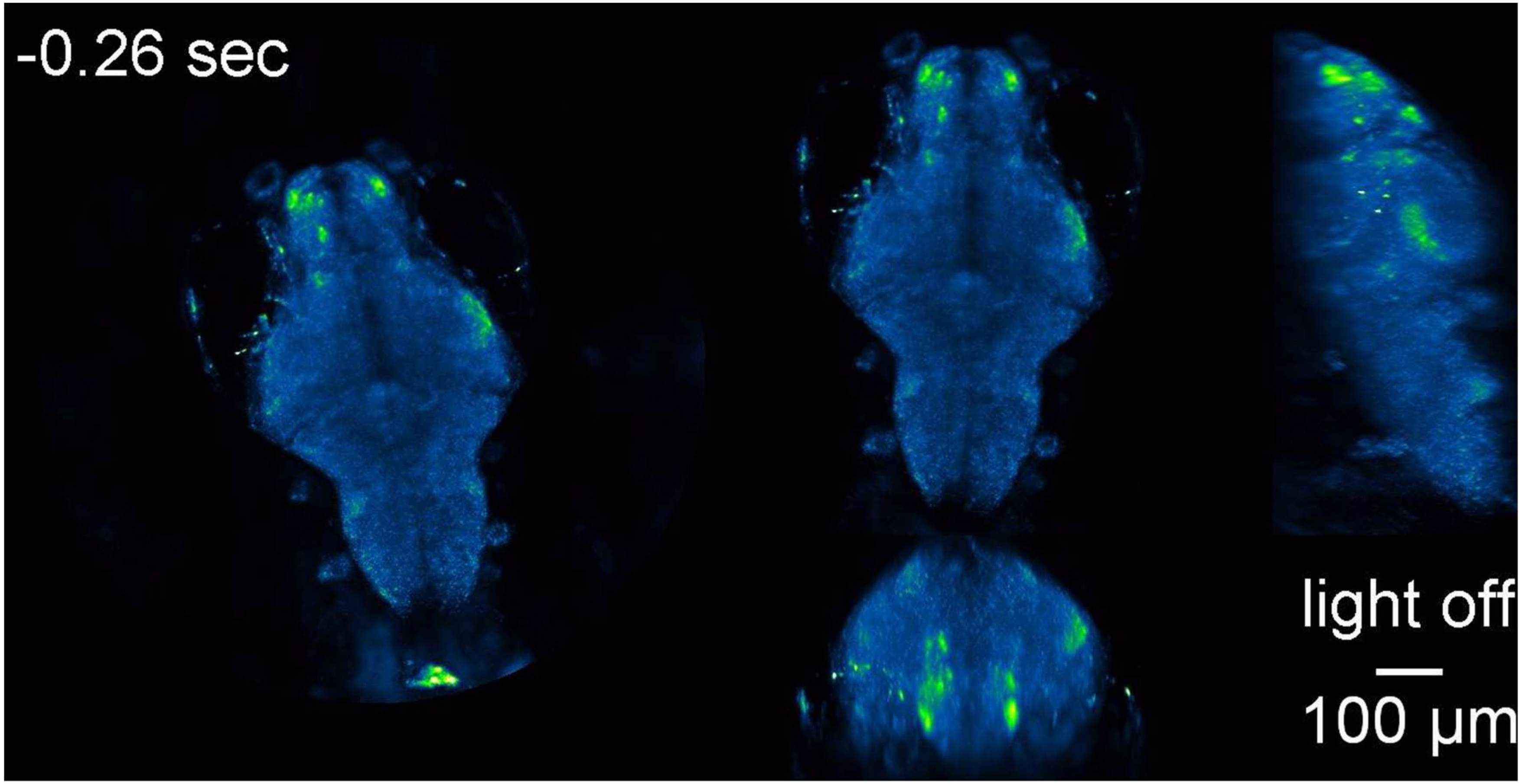
温泉

柴宇明

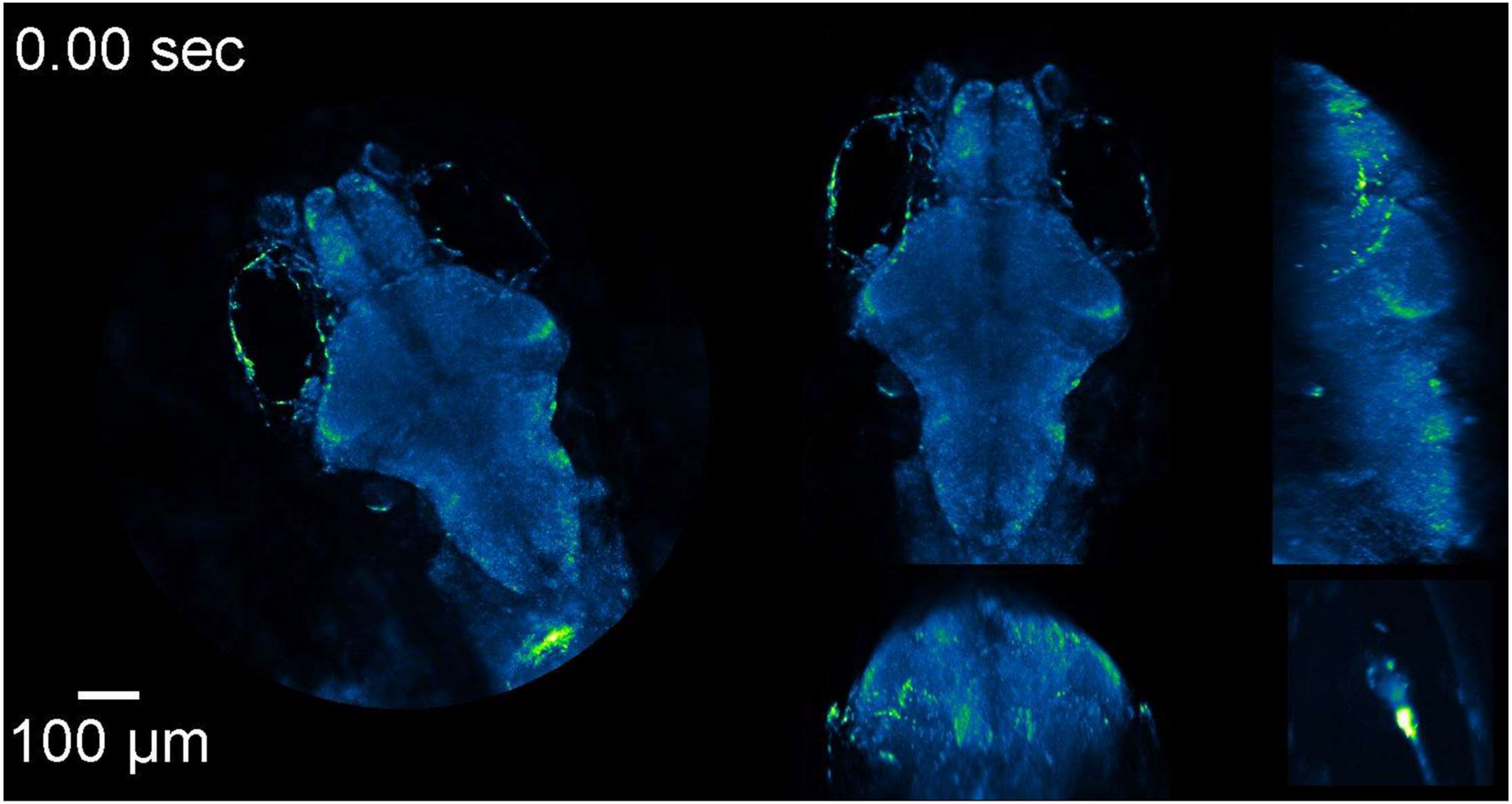
Free moving-zebrafish tracking

中科大温泉组

Whole brain functional imaging of neuronal activities in freely behaving larval zebrafish



Whole brain functional imaging of neuronal activities in freely behaving larval zebrafish



Whole brain functional imaging of neuronal activities in freely behaving larval zebrafish