

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



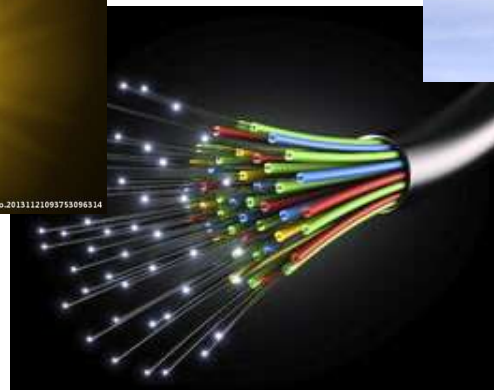
firework



illumination



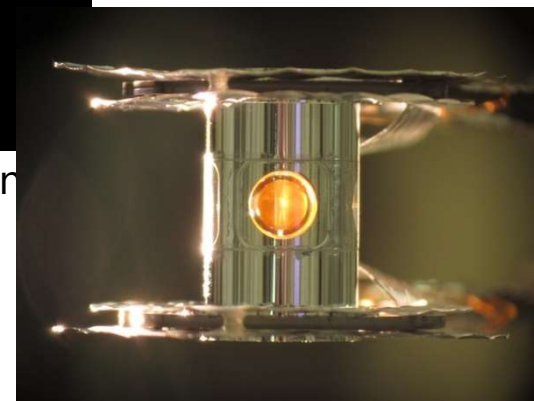
Laser weapon



Optical communication

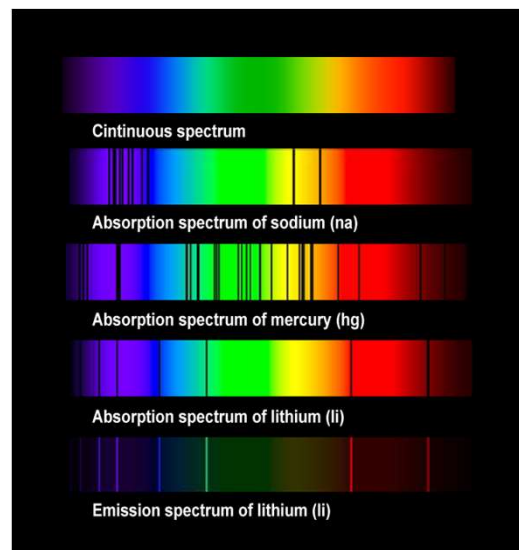
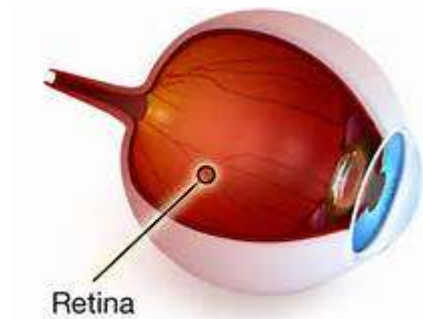


display



Laser induced nuclear fusion

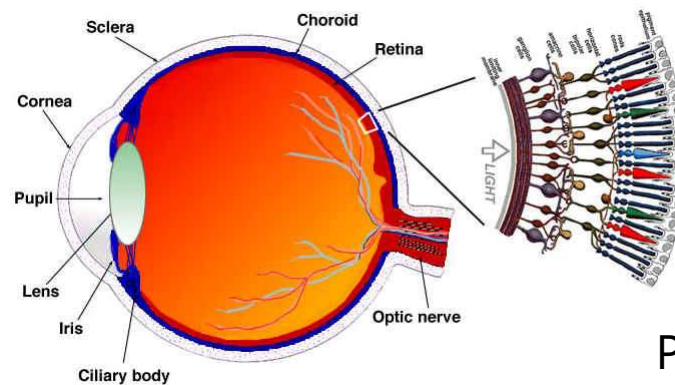
About the light



Absorption spectrum



Colors & Contrast

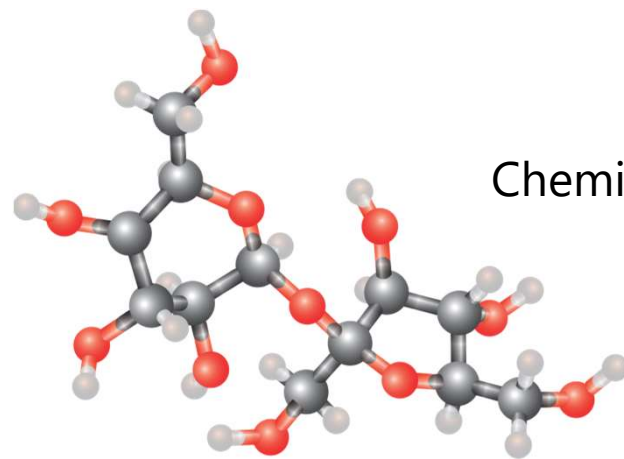


Photoreceptor

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!

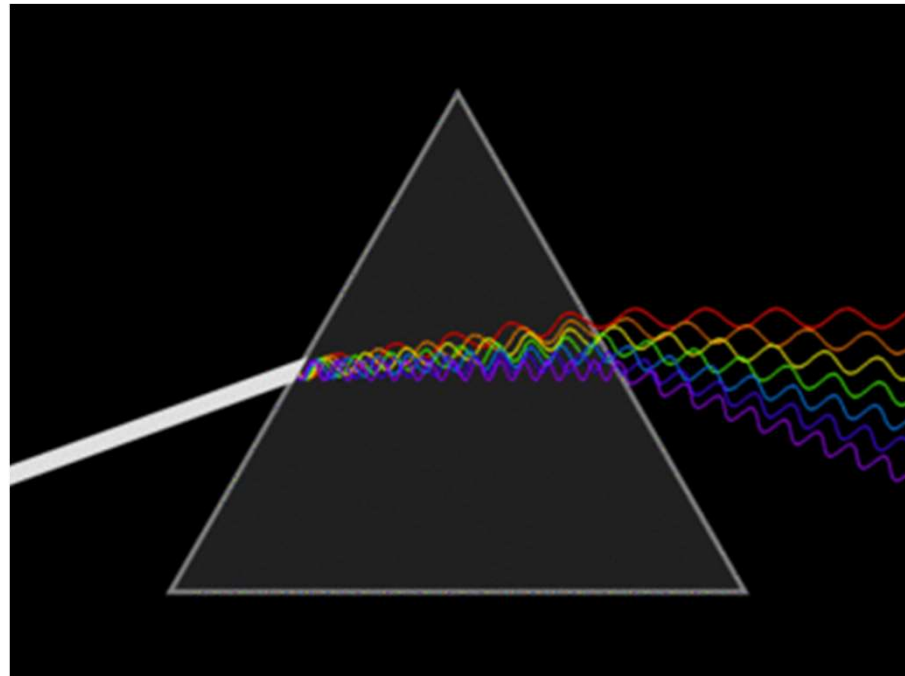
Stick to light, there is no other way out!!!

About the light

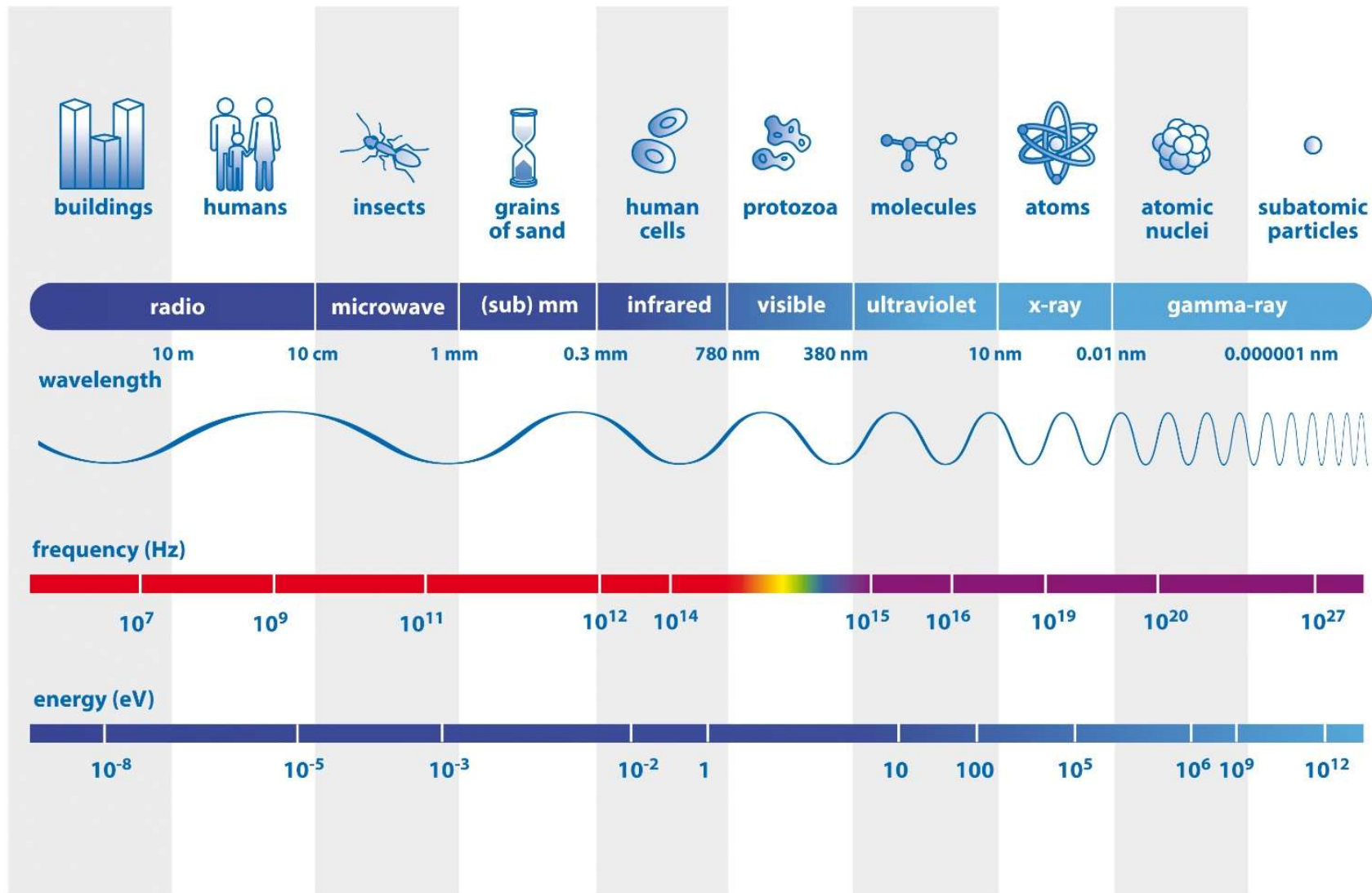
Physical Properties of Light

- Wavelength
- Photon energy

$$E = \hbar \nu$$

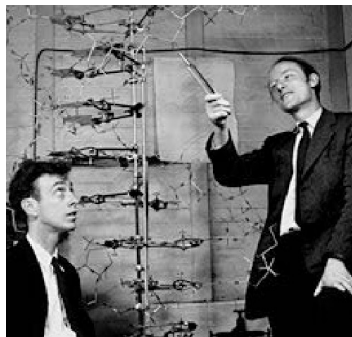


About the light





X Ray


First Nobel Prize in Physics





1900	1901 W. C. Röntgen, in physics, for the discovery of X-rays.
1910	1914 M. von Laue, in physics, for the discovery of X-rays by crystals.
1920	1915 W. H. Bragg and W. L. Bragg, in physics, for the determination of crystal structures using X-rays.
1930	1917 C. G. Barkla, in physics, for the discovery of the characteristic X-ray radiation of the elements.
1940	1924 M. Siegbahn, in physics, for discoveries in the field of X-ray spectroscopy.
1950	1927 A. H. Compton, in physics, for revealing the particle nature of X-rays in scattering experiments on electrons.
1960	1936 P. Debye, in chemistry, for determining molecular structures by X-ray diffraction in gases.
1970	1962 M. F. Perutz and J. C. Kendrew, in chemistry, for determining the structure of hemoglobin and myoglobin.
1980	1962 F. Crick, J. Watson and M. Wilkins, in medicine, for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material.
1990	1964 D. Crowfoot Hodgkin, in chemistry, for the determination of the structure of penicillin and other important biochemical substances.
	1976 W. N. Lipscomb, in chemistry, for the determination of boranes.
	1979 A. M. Cormack and G. N. Hounsfield, in medicine, for the development of computerized tomography.
	1981 M. Siegbahn, in physics, for developing high resolution electron spectroscopy.
	1985 H. A. Hauptman and J. Karle, in chemistry, for the development of direct methods for X-ray crystallographic structure determination.
	1988 J. Deisenhofer, R. Huber and H. Michel, in chemistry, for the determination of protein structures crucial to photosynthesis.


 The Nobel Prize in Physics 1901 - Wilhelm Conrad Röntgen »


 The Nobel Prize in Physics 1914 - Max von Laue »


 The Nobel Prize in Physics 1915 - Sir William Henry Bragg »


 The Nobel Prize in Physics 1915 - William Lawrence Bragg »

 The Nobel Prize in Physics 1917 - Charles Glover Barkla »

 The Nobel Prize in Physics 1924 - Karl Manne Georg Siegbahn »


 The Nobel Prize in Physics 1927 - Arthur Holly Compton »


 The Nobel Prize in Chemistry 1936 - Petrus (Peter) Josephus Wilhelmus Debye »


 The Nobel Prize in Chemistry 1962 - Max Ferdinand Perutz »


 The Nobel Prize in Chemistry 1962 - John Cowdery Kendrew »


 The Nobel Prize in Physiology or Medicine 1962 - Francis Harry Compton Crick »


 The Nobel Prize in Physiology or Medicine 1962 - James Dewey Watson »


 The Nobel Prize in Physiology or Medicine 1962 - Maurice Hugh Frederick Wilkins »


 The Nobel Prize in Chemistry 1964 - Dorothy Crowfoot Hodgkin »


 The Nobel Prize in Chemistry 1976 - William N. Lipscomb »


 The Nobel Prize in Physiology or Medicine 1979 - Allan M. Cormack »


 The Nobel Prize in Physiology or Medicine 1979 - Godfrey N. Hounsfield »


 The Nobel Prize in Physics 1981 - Kai M. Siegbahn »

 The Nobel Prize in Chemistry 1985 - Herbert A. Hauptman »

 The Nobel Prize in Chemistry 1985 - Jerome Karle »

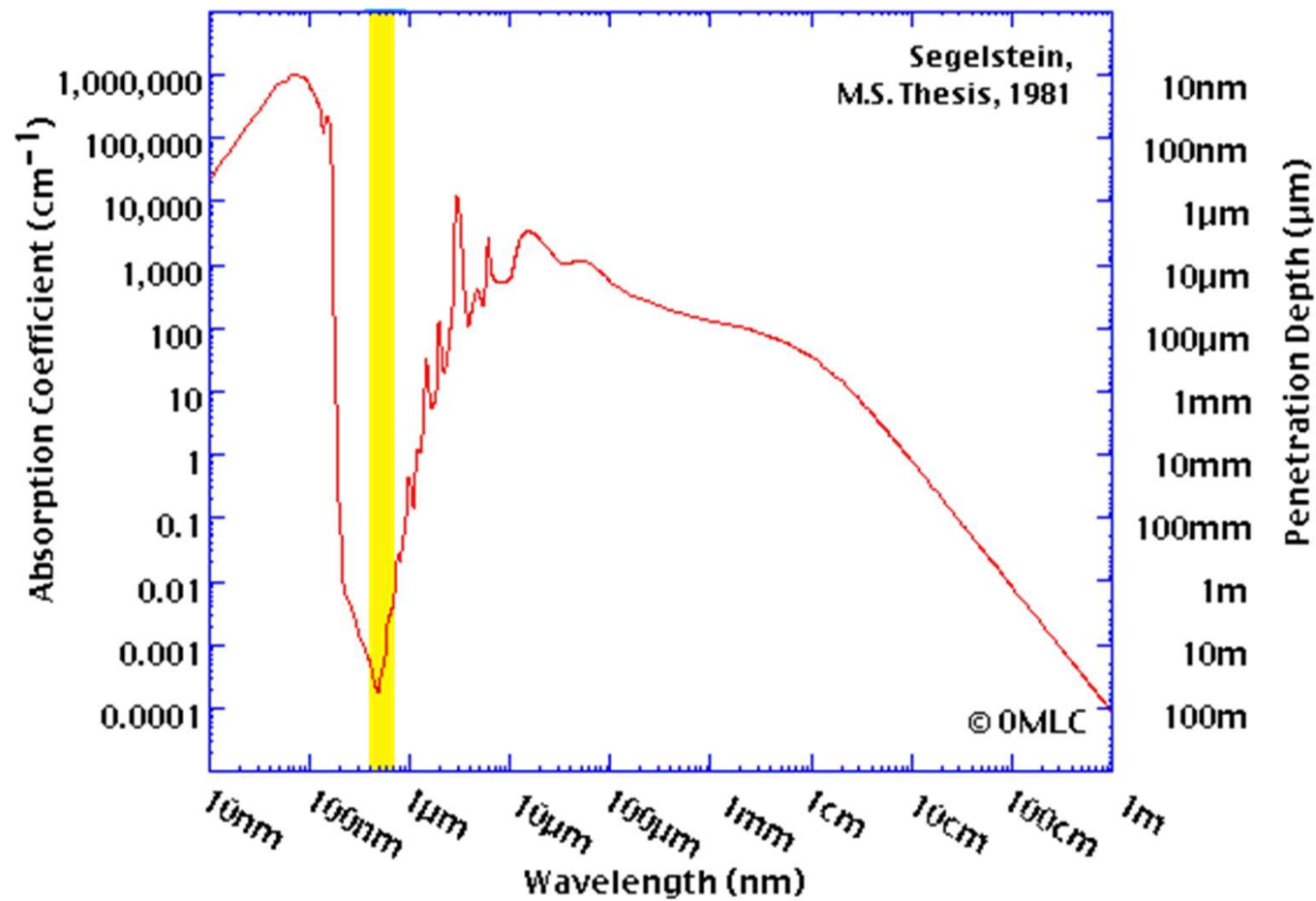
 The Nobel Prize in Chemistry 1988 - Johann Deisenhofer »

 The Nobel Prize in Chemistry 1988 - Robert Huber »

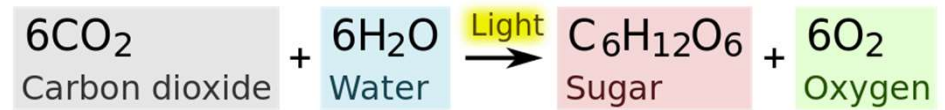
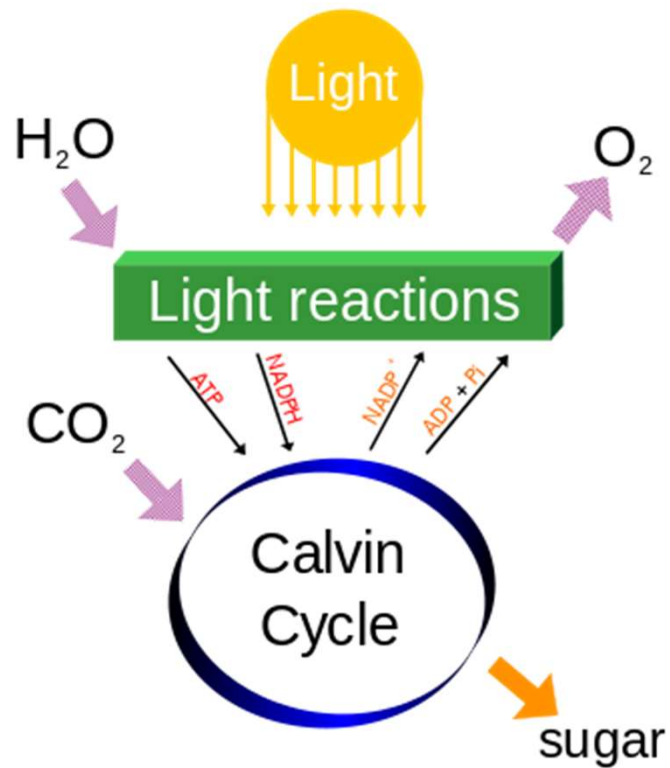
 The Nobel Prize in Chemistry 1988 - Hartmut Michel »

Visible light can penetrate deep into water

Water absorption spectrum



Photosynthesis



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?

	The world is 3D.
Objective	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



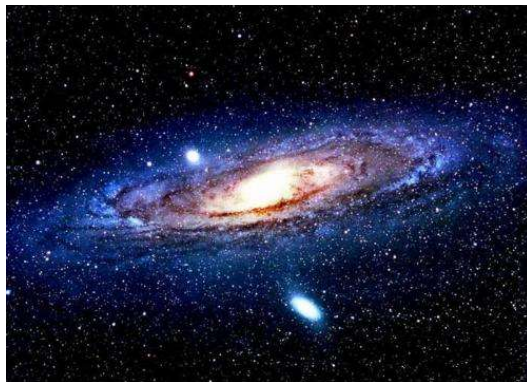
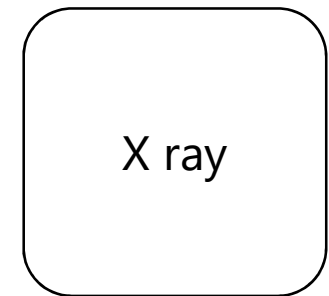
Galileo Galilei 1564 – 1642

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

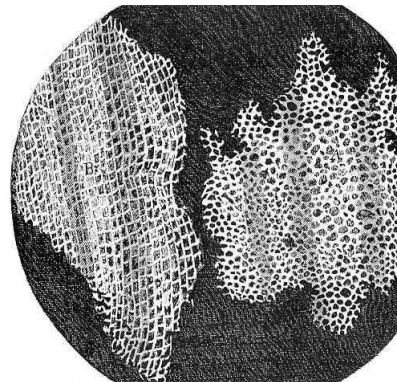
“In 1609, Galileo was, along with Englishman Thomas Harriot and others, among the first to use a refracting telescope as an instrument to observe stars, planets or moons.”

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

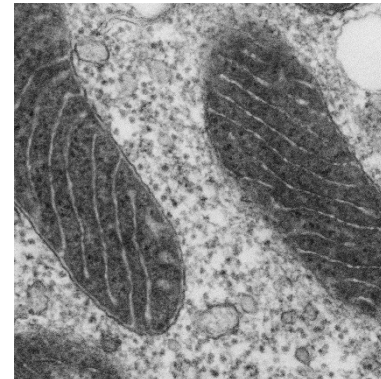
Seeing is believing



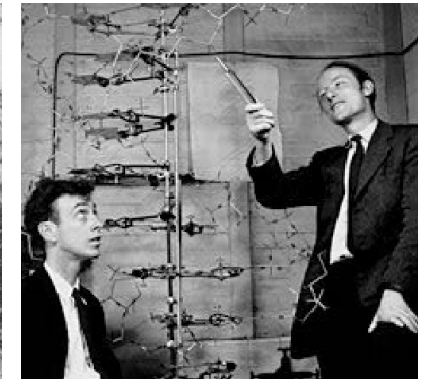
10^{26}m



10^{-6}m



10^{-9}m



10^{-11}m

Outline

About the light and imaging

Optical imaging

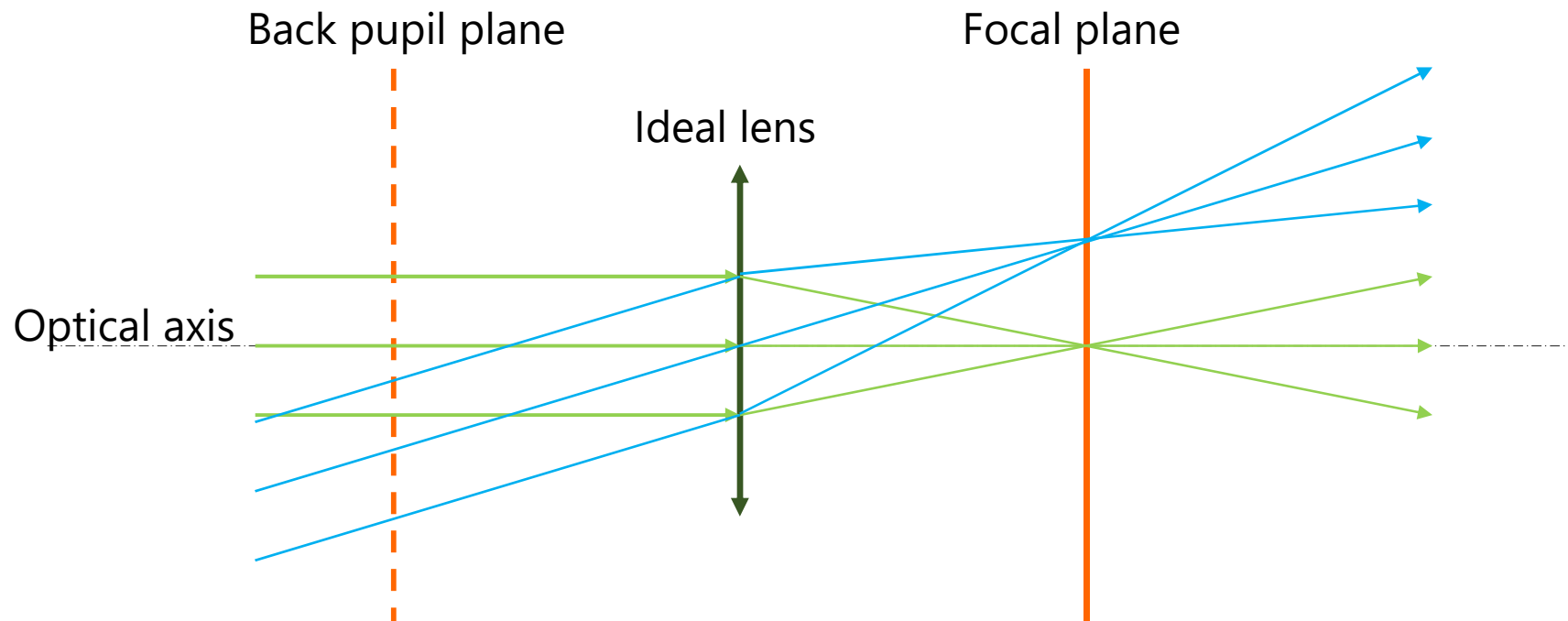
Basics

Optical Neuroimaging

Basics of optical imaging

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

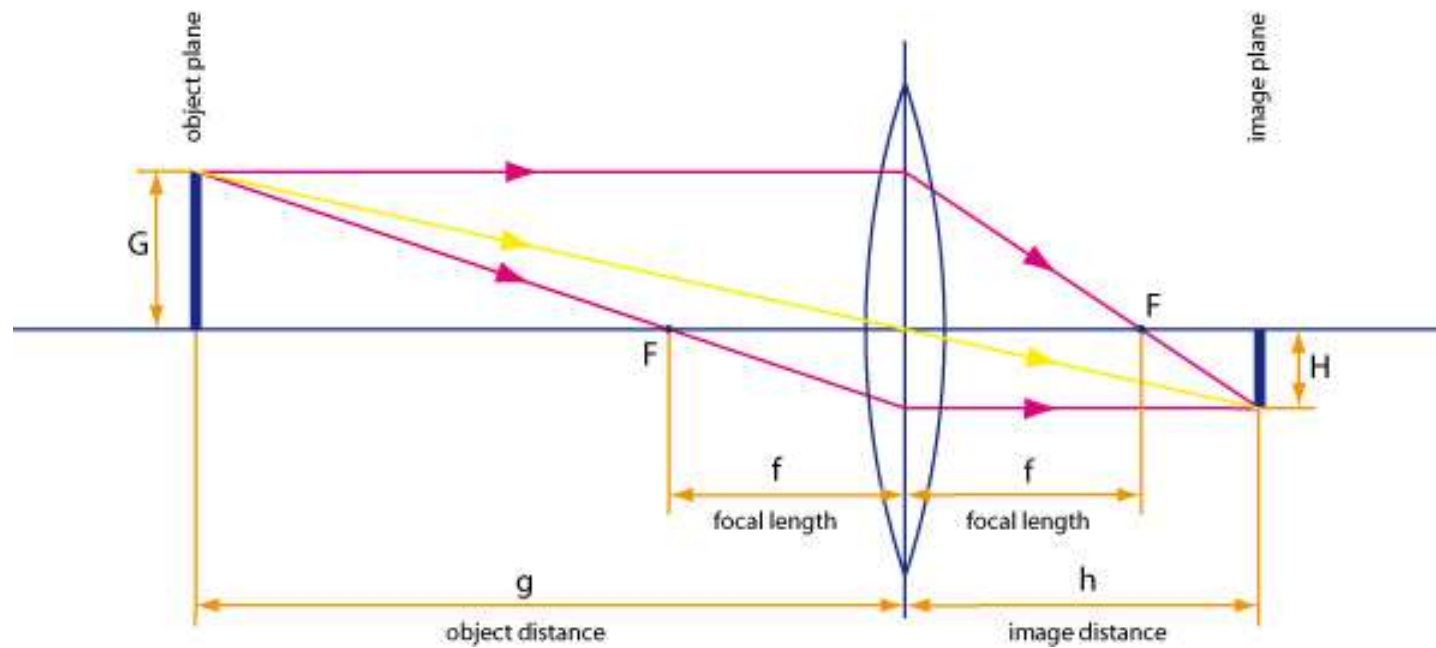
Light ray model of an ideal lens



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

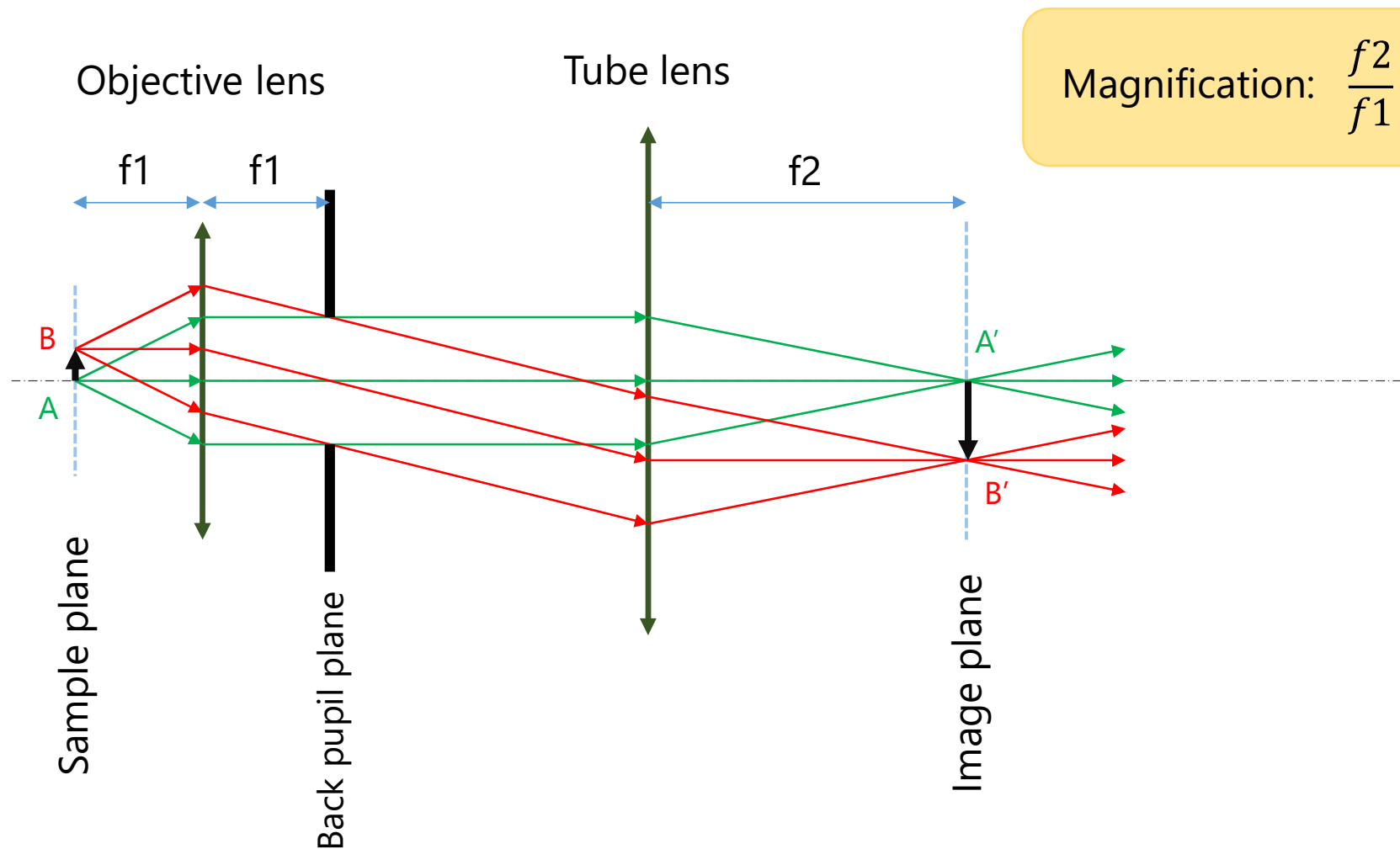
Rule 2: Parallel light rays will be focused into a single spot on focal plane

Imaging system: Light ray model



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

Infinity corrected imaging system



Objectives' parameters

Correction spec:

Magnification:

Numerical Aperture (NA):

Working Distance (WD):

Immersion medium:



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

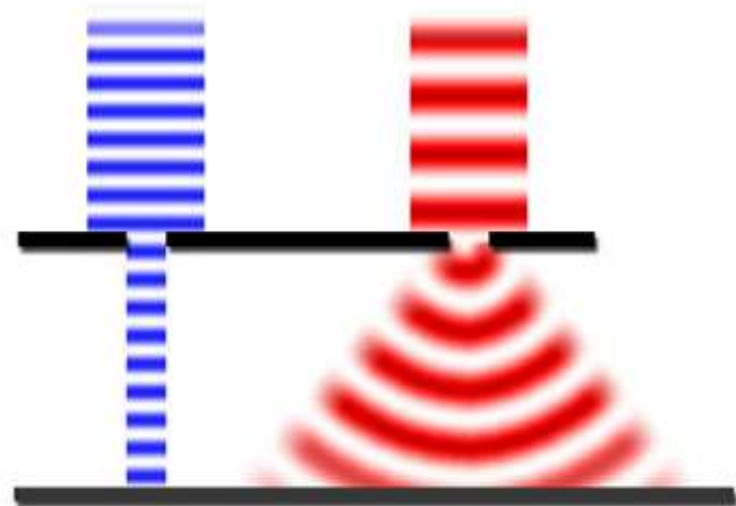
- Light Ray Model
- Light wave model
- Frequency domain model

Wave nature of light

Water ripple



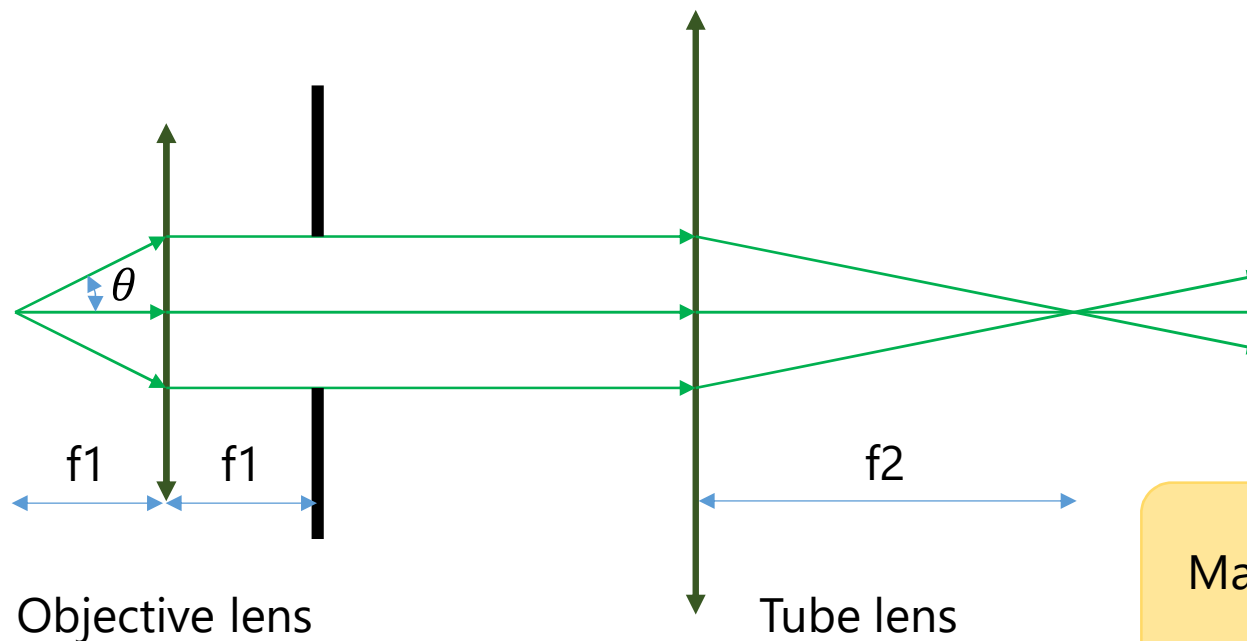
diffraction



Light wave model of optical imaging system

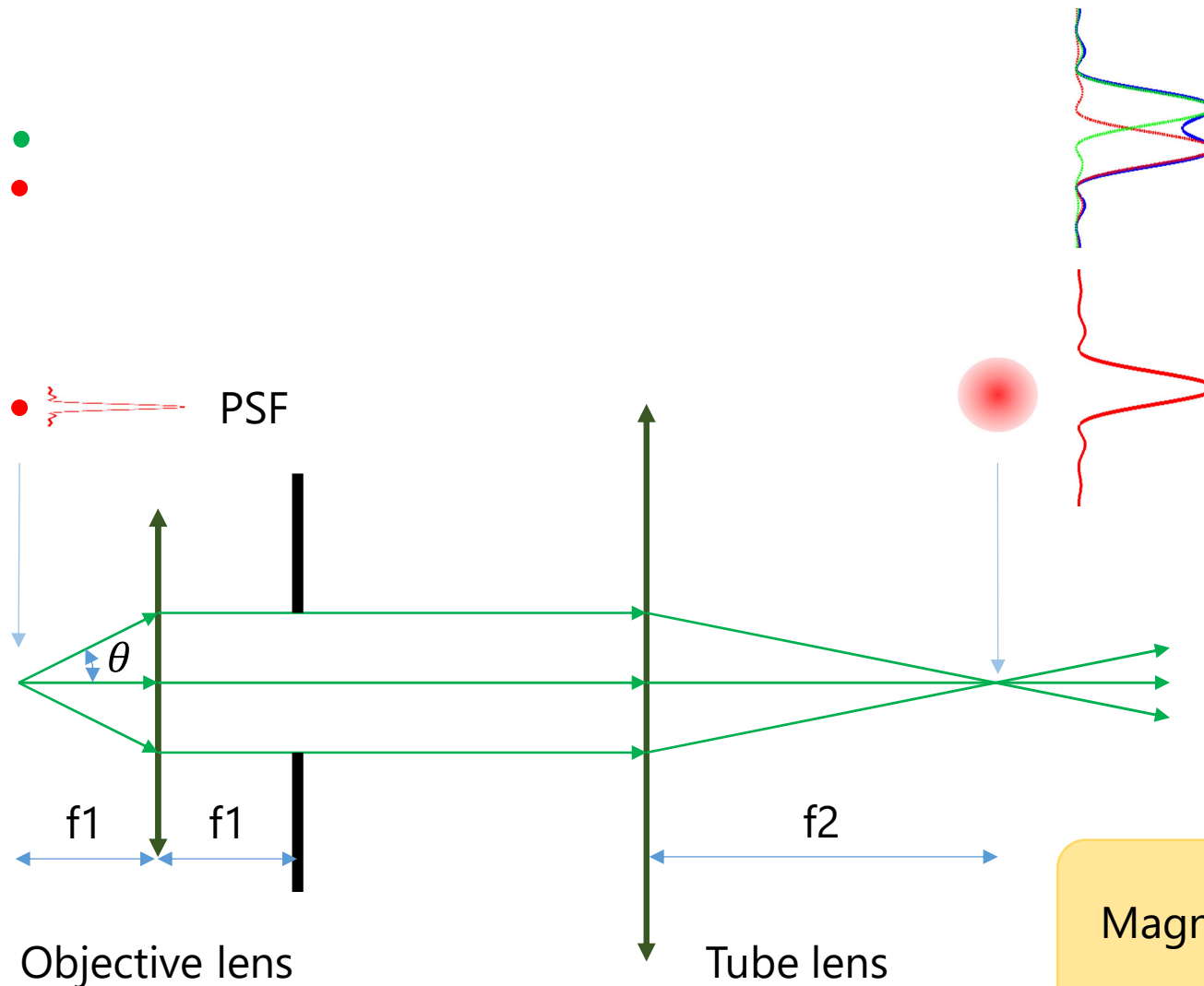
What's the resolution limit of the optical imaging system?

Point spread function (PSF):

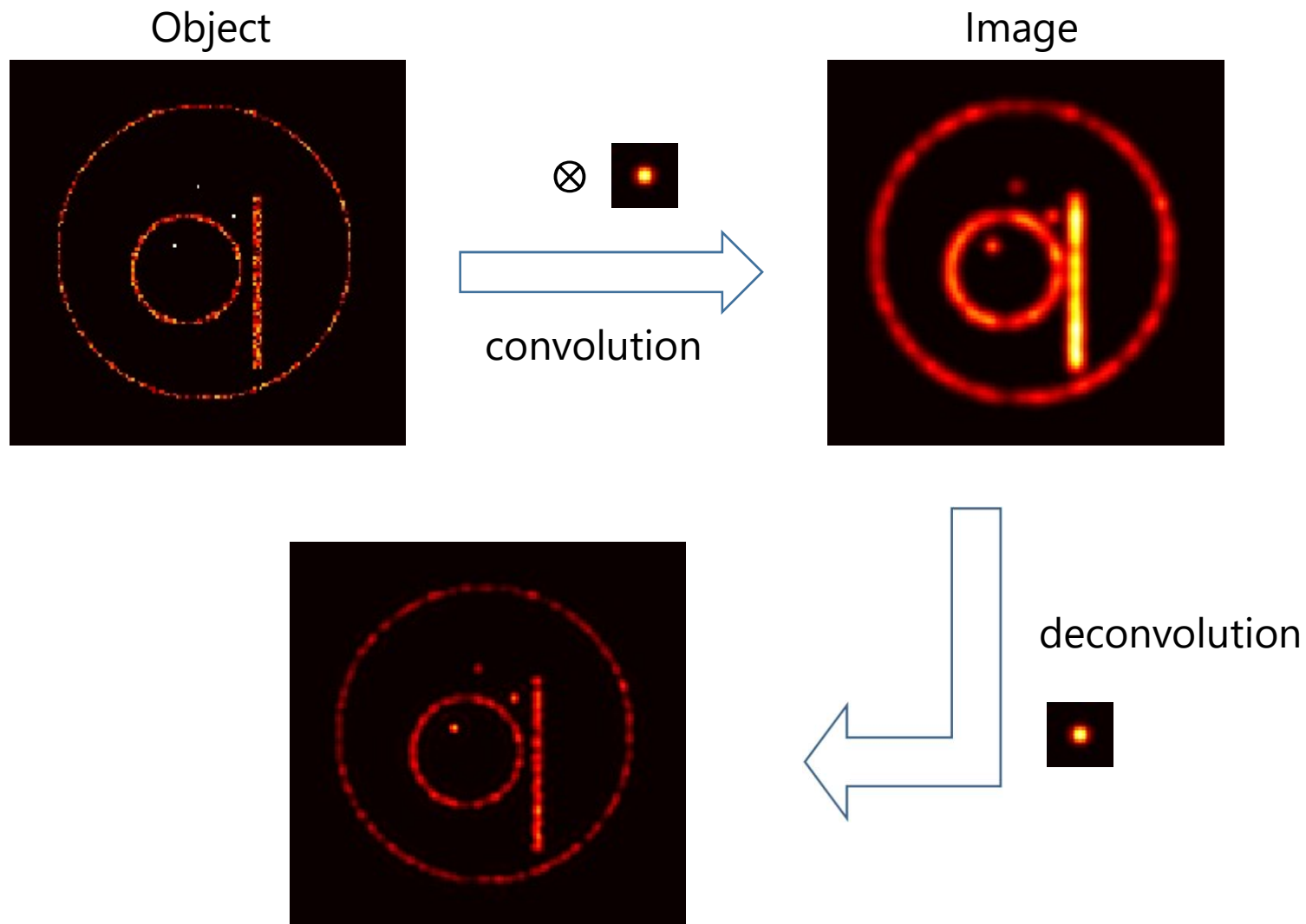


Magnification: $\frac{f_2}{f_1}$

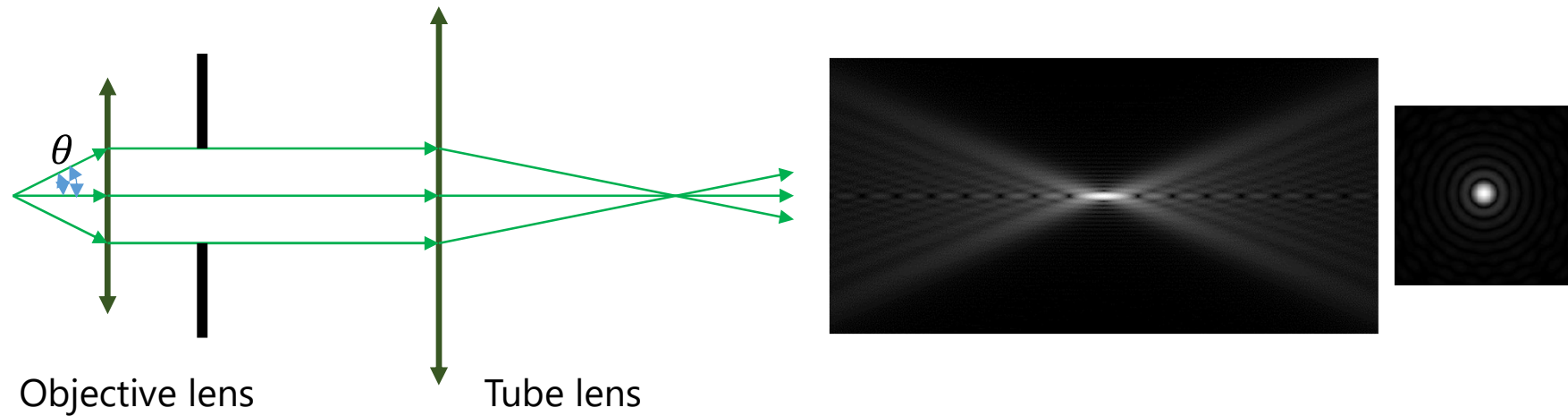
Light wave model of optical imaging system



Optical imaging system is linear & spatial invariant



Point spread function



Fraunhofer diffraction pattern of a circular aperture (Airy disk)

$$I(x) \sim \left(\frac{J_1(kx)}{kx} \right)^2 \quad k = \frac{2\pi}{\lambda} n \sin \theta = \frac{2\pi}{\lambda} NA$$

$$NA = n \sin \theta$$

Numerical Aperture

Point spread function & Resolution



Ernst Karl Abbe



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

Numerical Aperture:
$$NA = n \sin \theta$$

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

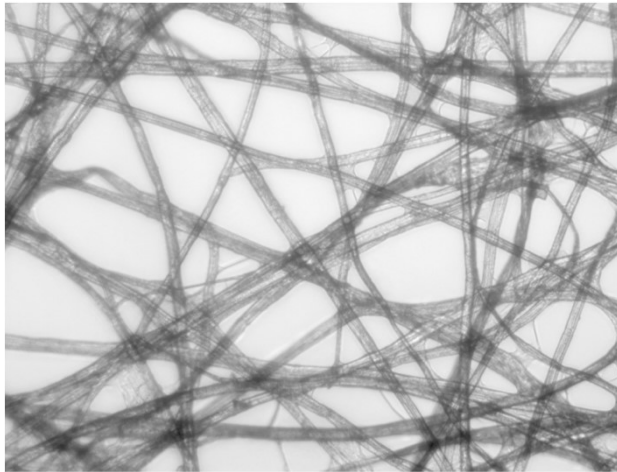
Olympus objective focal length: $180 \text{ mm} / 60 = 3 \text{ mm}$

Magnification: $200 \text{ mm} / 3 \text{ mm} = 66.7$

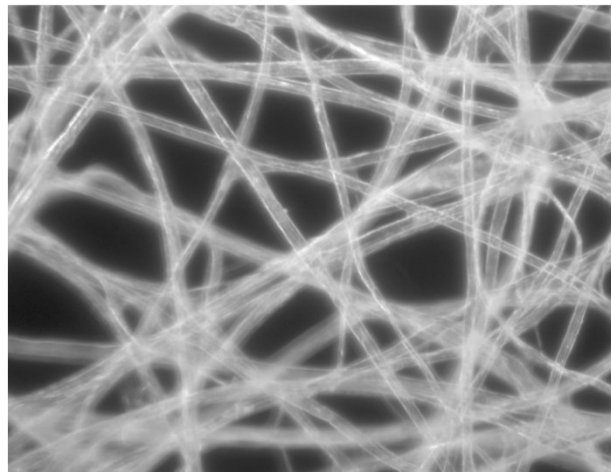
$$\text{Resolution: } \frac{\lambda}{2NA} = \frac{510 \text{ nm}}{2 \cdot 1.27} = 200.8 \text{ nm}$$

Conventional microscope

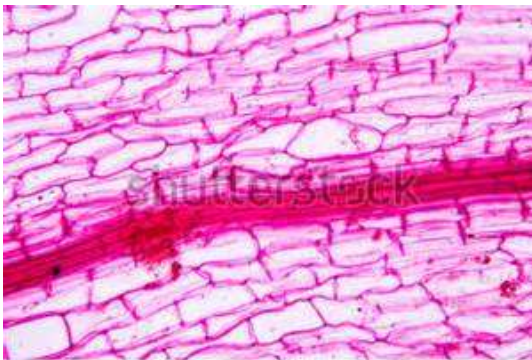
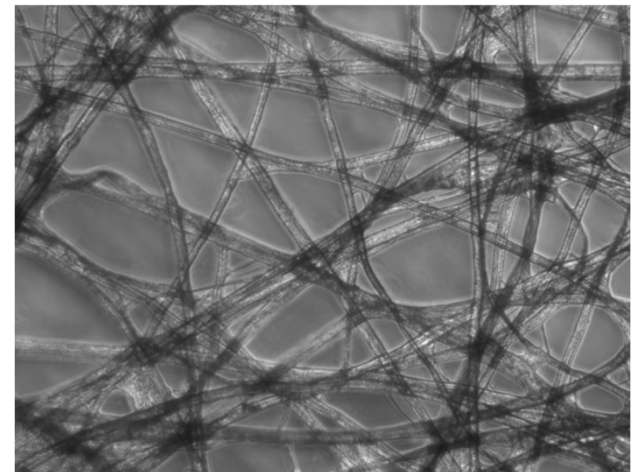
Bright Field



Dark Field

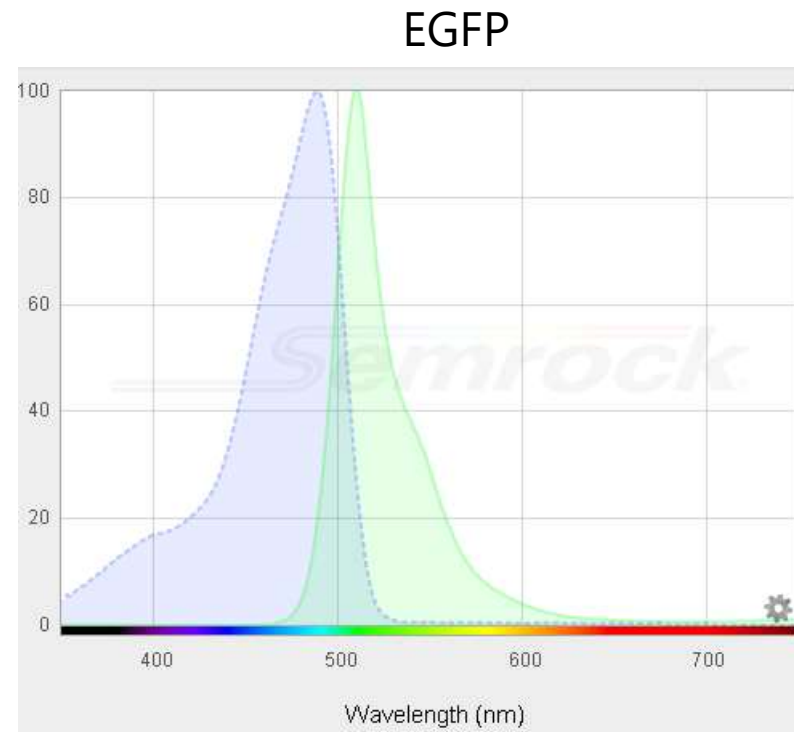
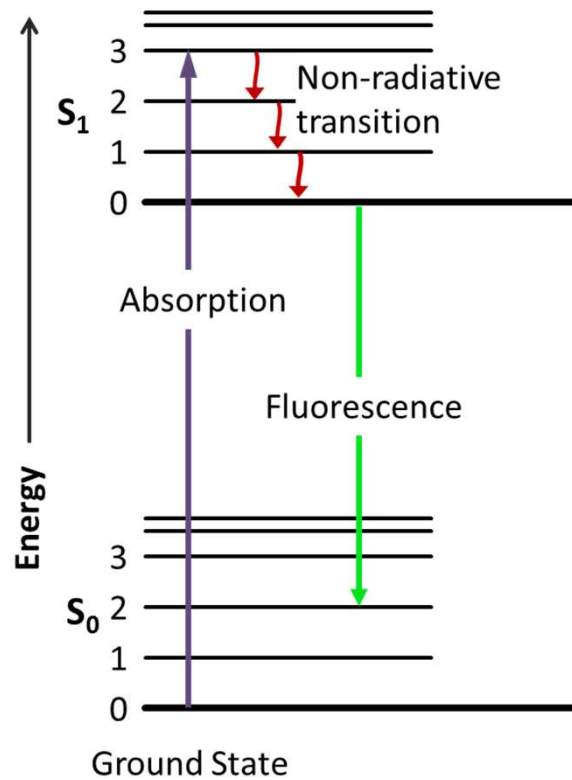


Phase Contrast/
Different Interference
Contrast (DIC)



Staining

Fluorescent indicator



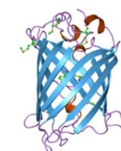
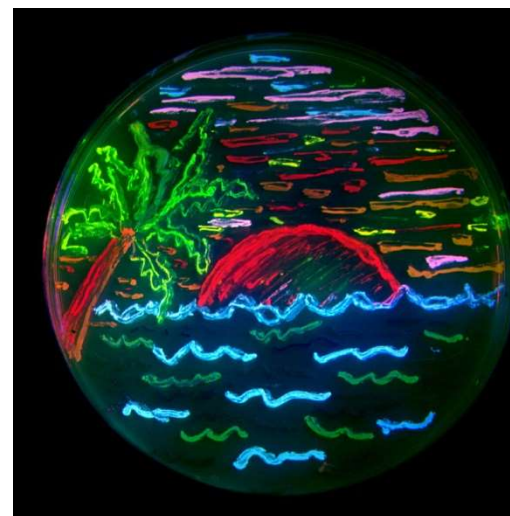
➤ Single molecule sensitivity

Fluorescent indicator

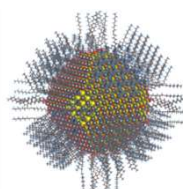
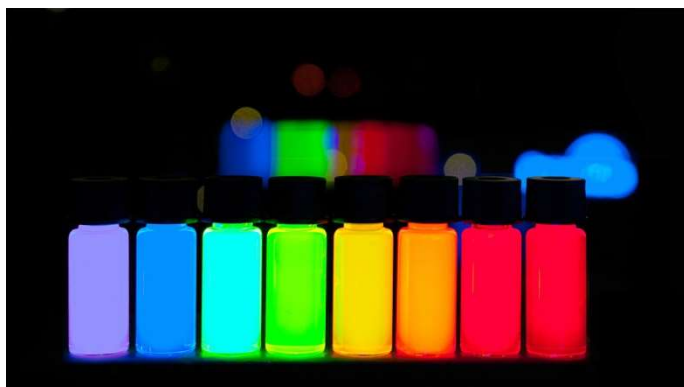
Dye



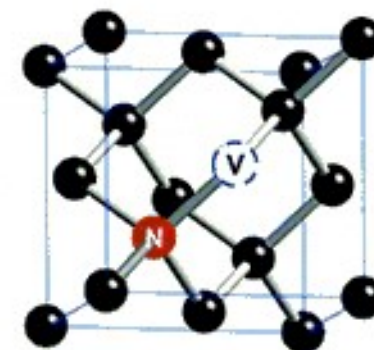
Protein



Quantum dot



Nano Diamond



Hot topics in optical imaging

Fluorescence imaging

3D imaging

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

Higher resolution

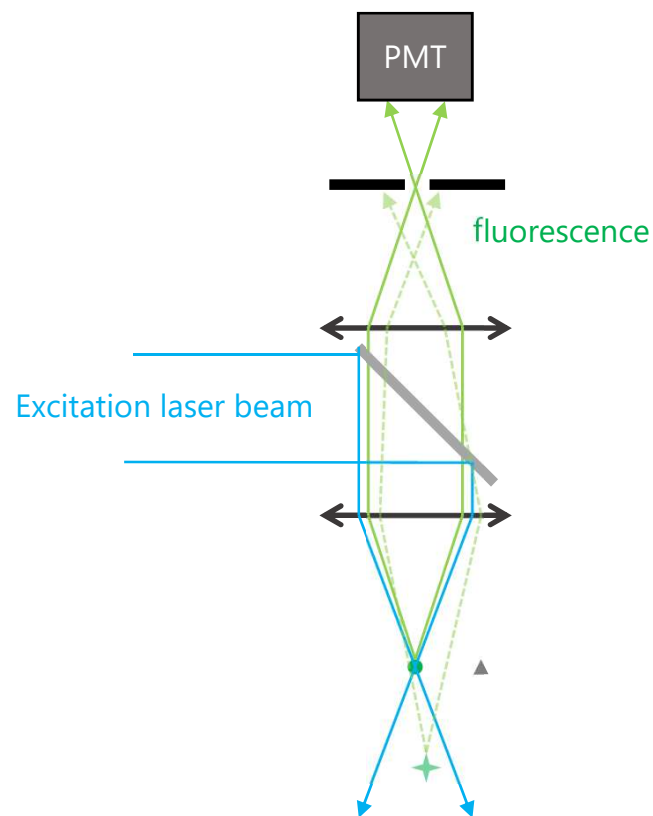
- PALM/STORM
- STED
- Super-resolution SIM
- Expansion microscope

3D Imaging

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

Confocal Scanning Microscope

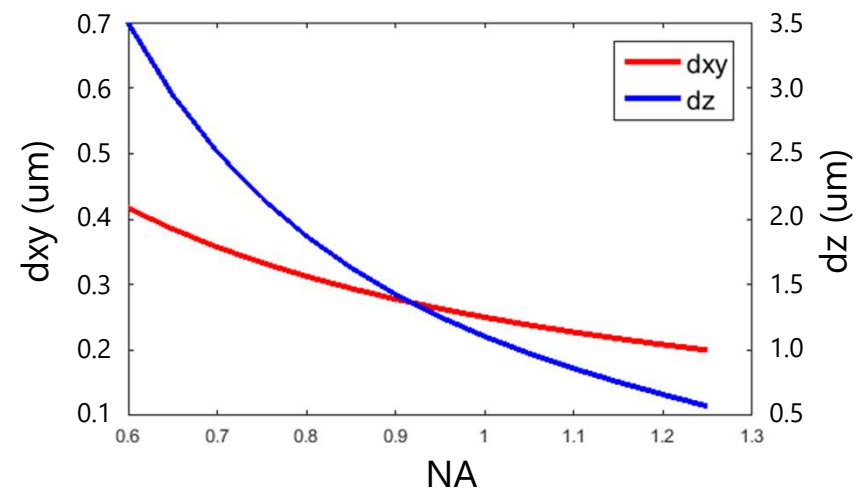
In 1940 Hans Goldmann, ophthalmologist in Bern, Switzerland, developed a slit lamp system to document eye examinations. This system is considered by some later authors as the first confocal optical system.



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

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

Imaging resolution of GFP using water objective of different NA

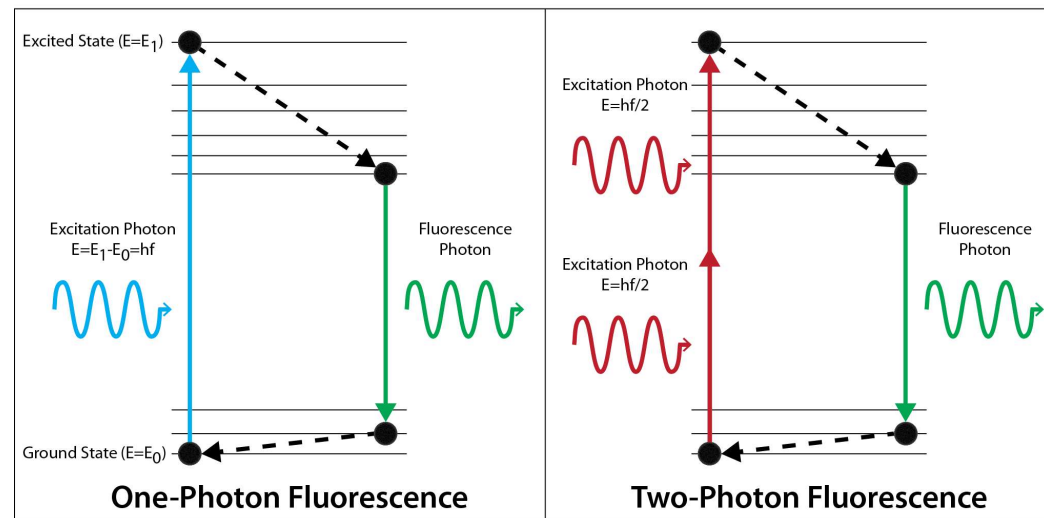


Two photon microscope

Theory of two photon absorption:

Maria Goeppert-Mayer

1 GM = 10^{-50} cm⁴ s/photon



Wide field: 10^4 W/m²

10^{16} W/m²

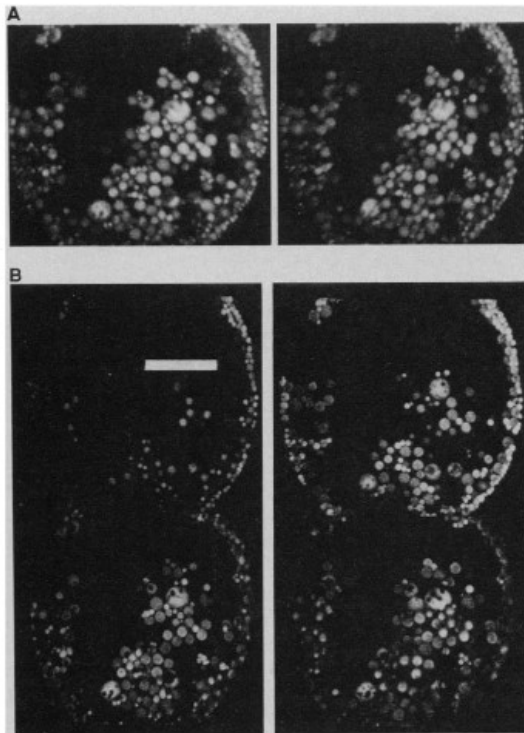
Two photon microscope

Two-Photon Laser Scanning Fluorescence Microscopy

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

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

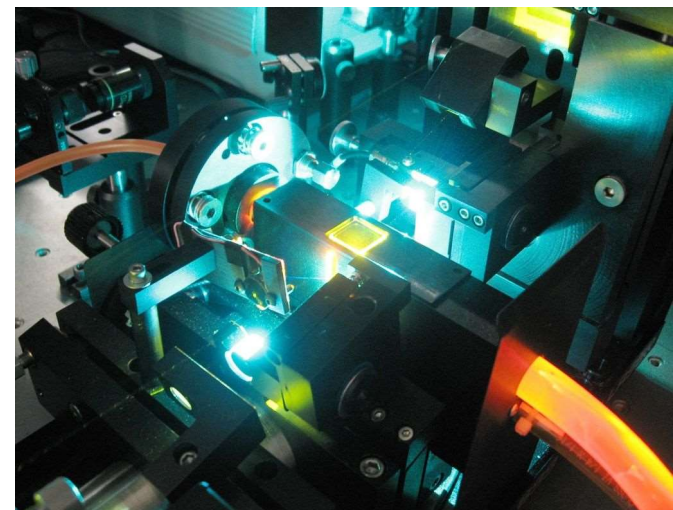
Fig. 1. (A) A stereo image pair is synthesized from a stack of six cross sections (xy sections) with an axial (z) increment of $3\ \mu\text{m}$. Blue ($380\ \text{nm} \leq \lambda \leq 445\ \text{nm}$) fluorescence excited by two-photon ($630\ \text{nm}$) absorption was detected to record these images 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 $415\ \text{nm}$, 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 $2\ \text{min}$. **(B)** The topmost four of the images, xy sections, used to synthesize the stereo pair in **(A)**. Scale bar, $50\ \mu\text{m}$.



Femto second dye laser

80MHz, 100fs pulse width

→ peak power is 125000 times of
the average power



Laser source

Ti: Sapphire Laser

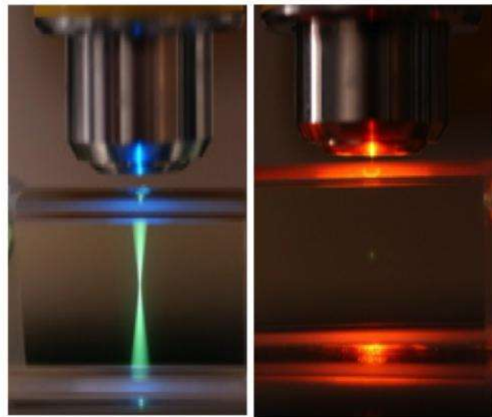


Coherent Chameleon family



Two photon microscope

1-photon vs. 2-photon



Photos by Steve Ruzin

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

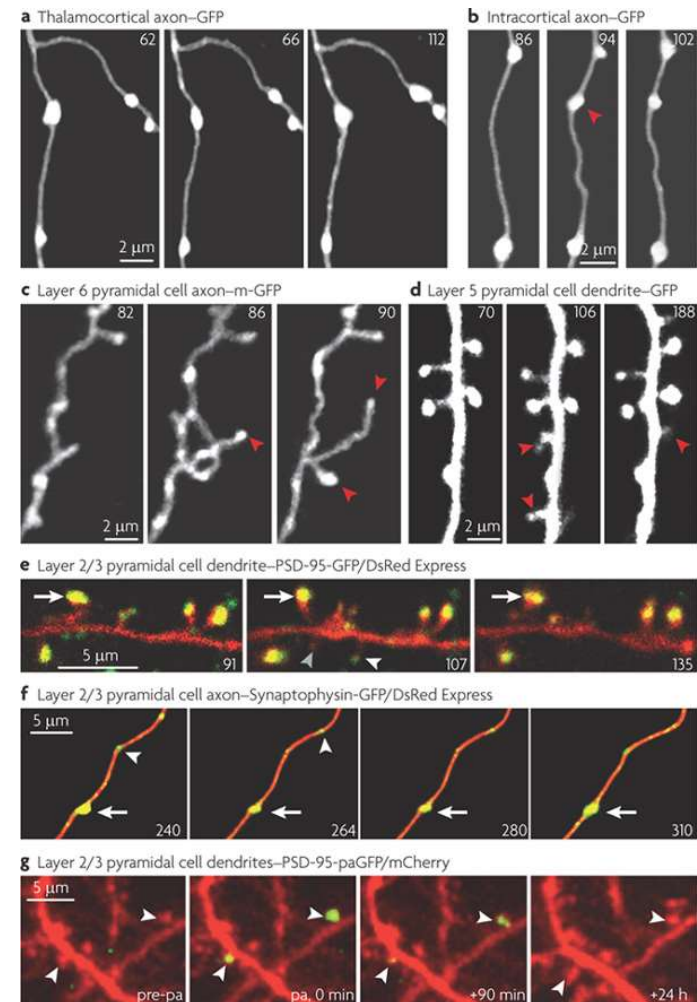
Brain Prize 2015



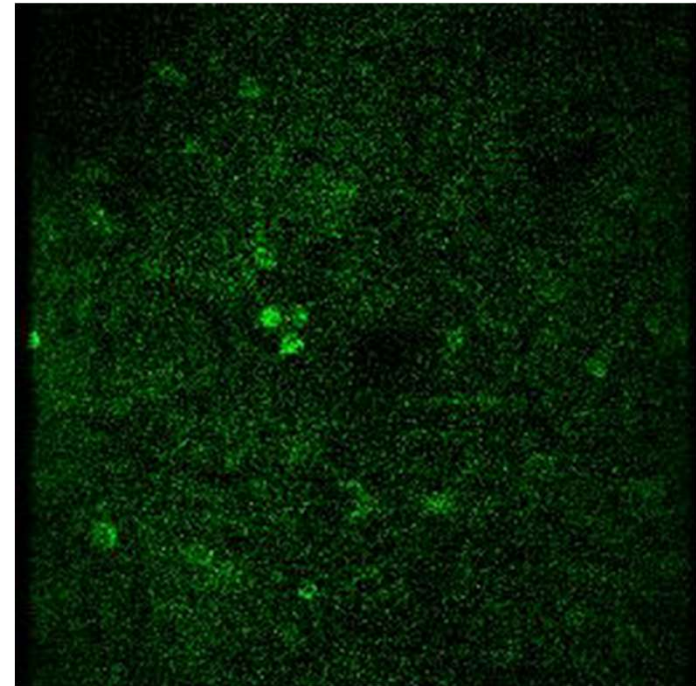
"invention, refinement and use of two-photon microscopy to provide detailed, dynamic images of activity in individual nerve cells, dendrites and synapses, thereby transforming the study of development, plasticity and functional circuitry of the brain."

Time lapse two photon imaging of neuron plasticity

Behavioral Experiments



Two photon functional imaging



Light sheet microscope & whole brain imaging



Richard Adolf Zsigmondy

Nobel Prize in chemistry in 1925

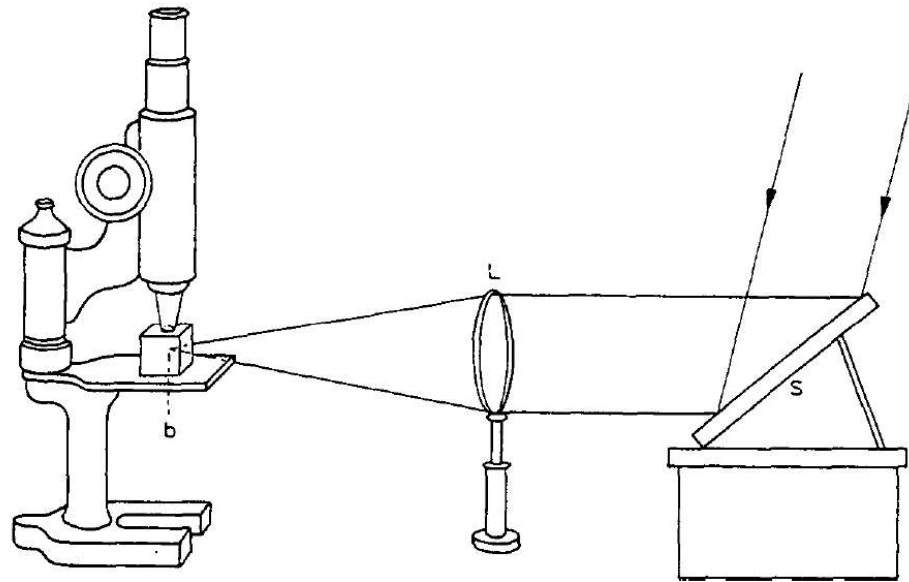
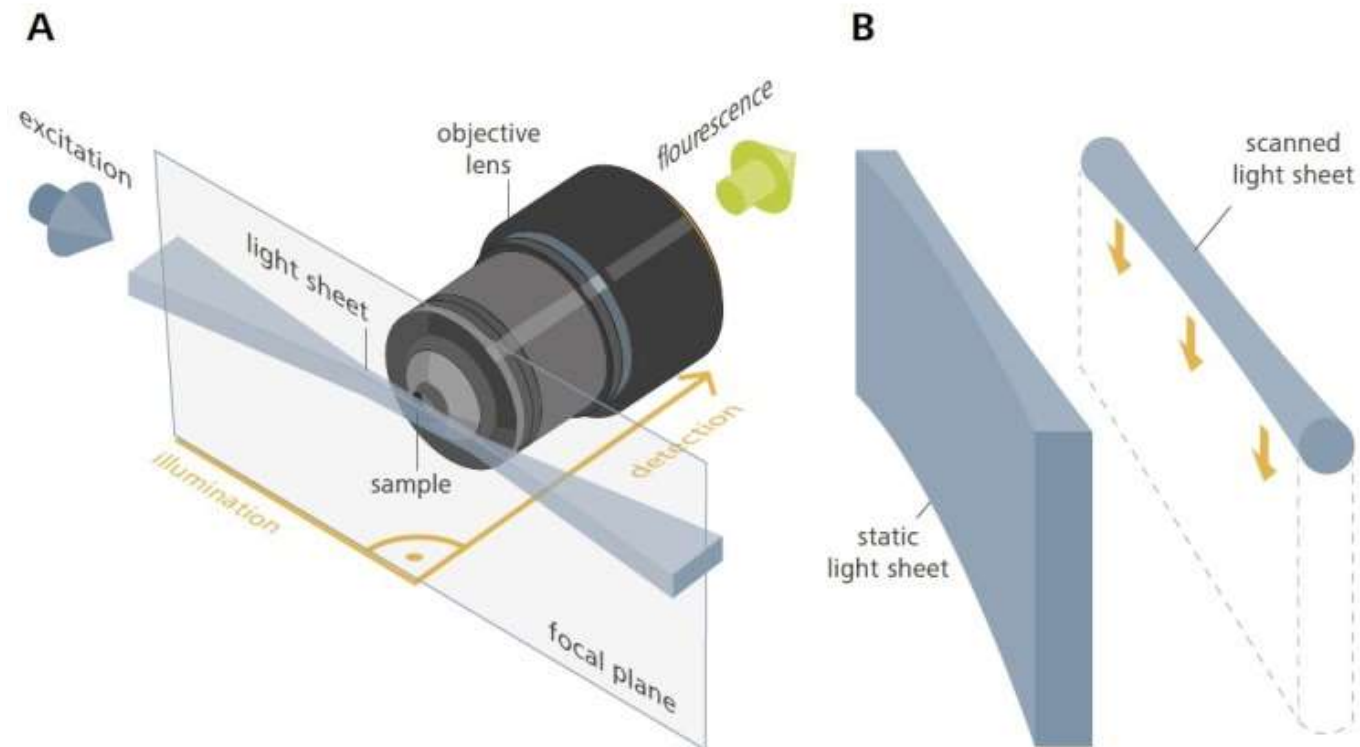


Fig. 1. The first arrangement for making ultramicroscopic particles visible.

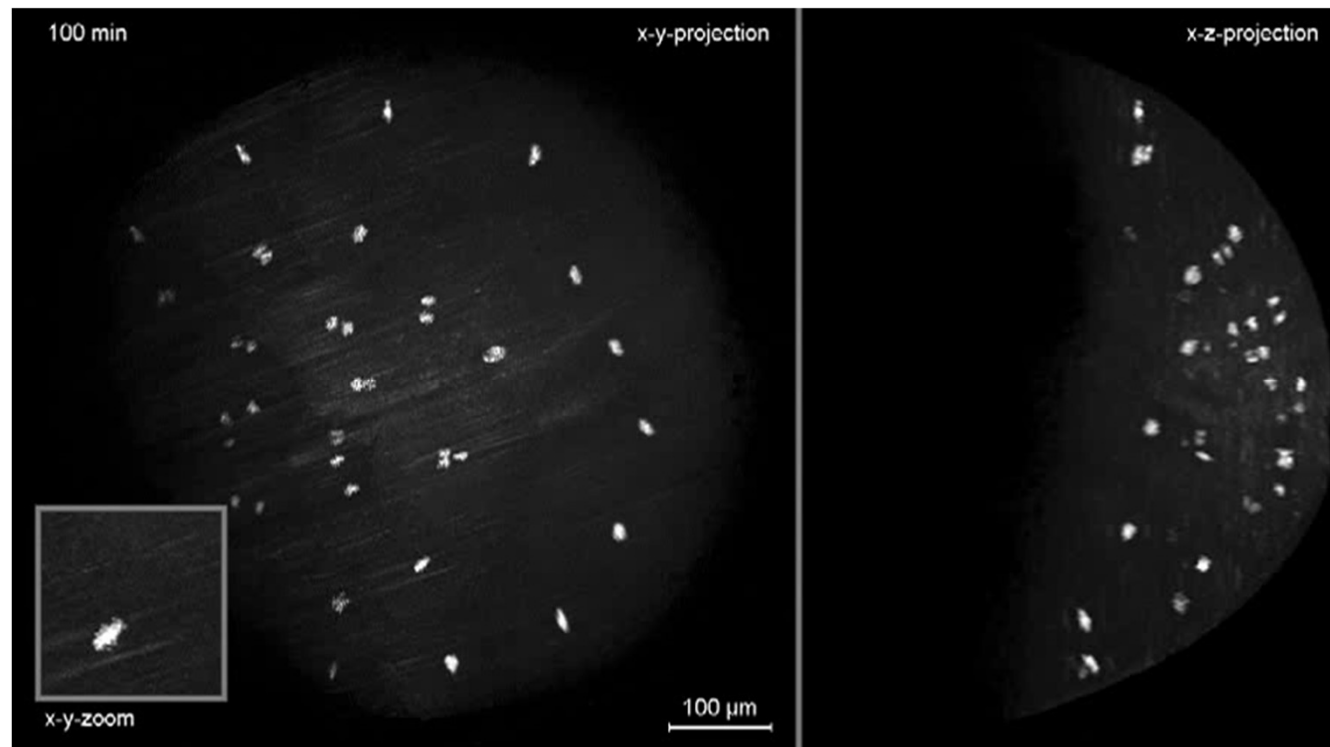
Light sheet microscope



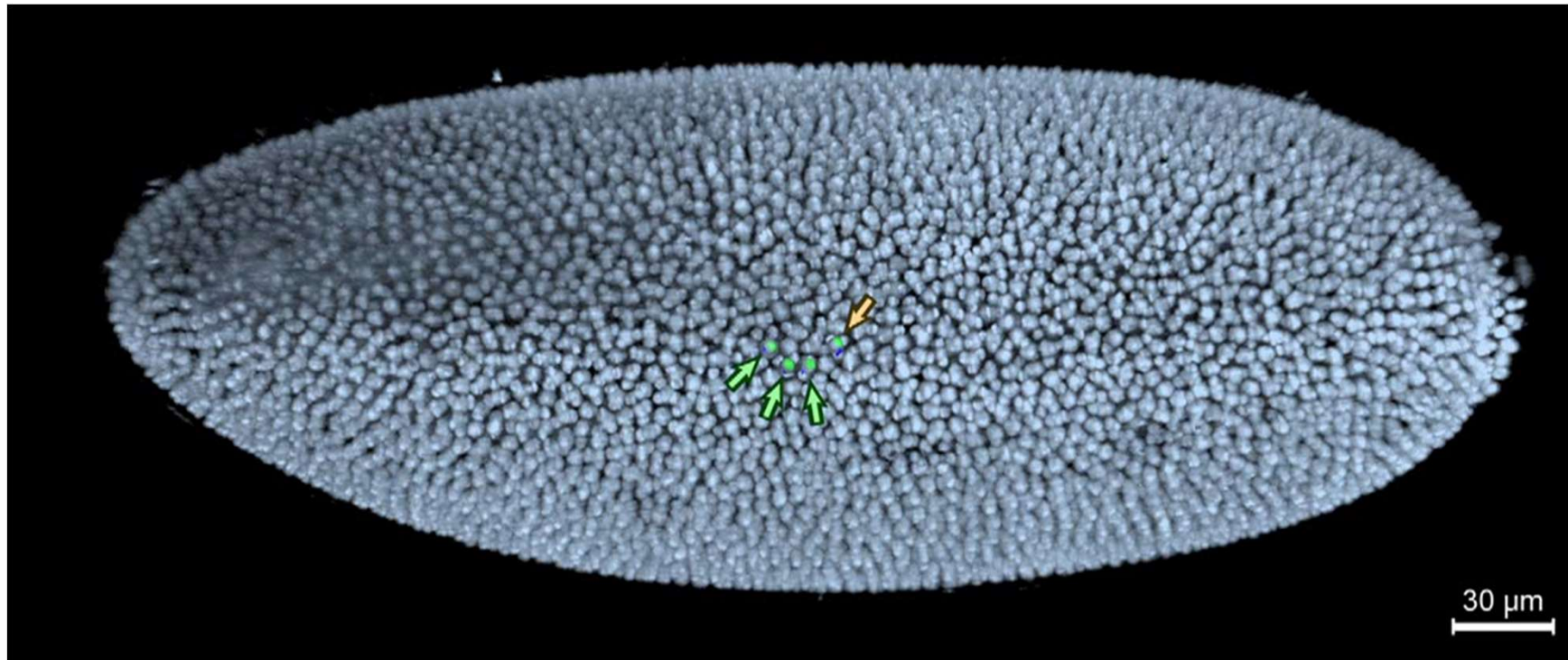
Light sheet microscope

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. Stelzer¹



Light sheet microscope for development biology

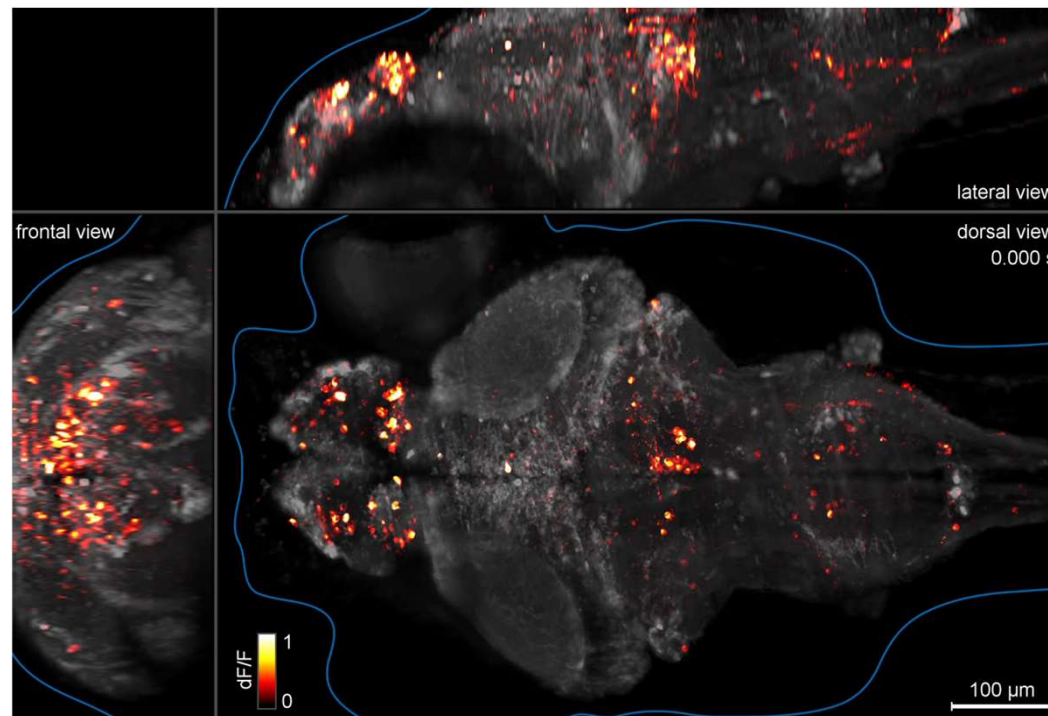


Tomer, R. et al. Nature Methods 9, 755-763 (2012)

Light sheet microscope

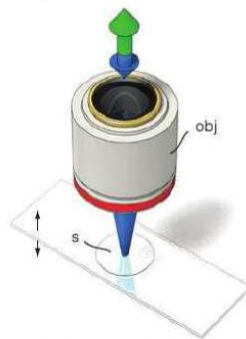
Whole-brain functional imaging at cellular resolution using light-sheet microscopy

Misha B Ahrens¹, Michael B Orger², Drew N Robson³, Jennifer M Li³ & Philipp J Keller¹

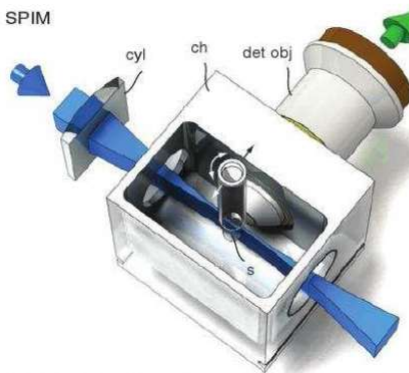


Light sheet microscope

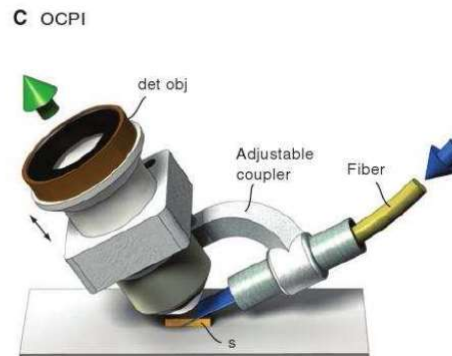
A Epifluorescence



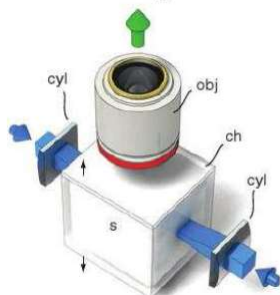
B SPIM



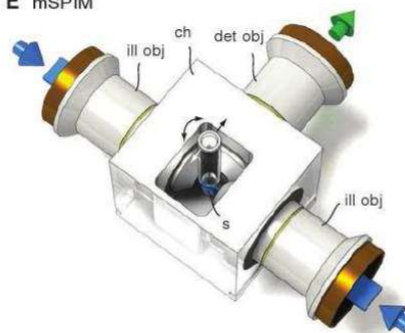
C OCPI



D Ultramicroscopy



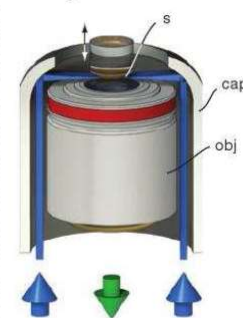
E mSPIM



F HILO, POM



G Single lens SPIM

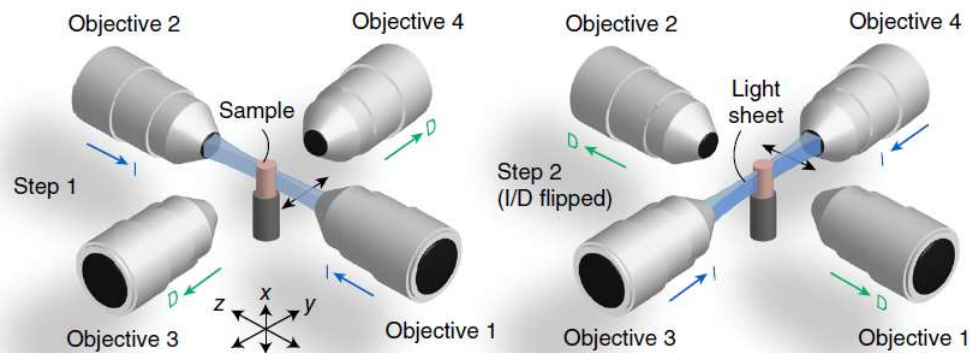


Light sheet microscope

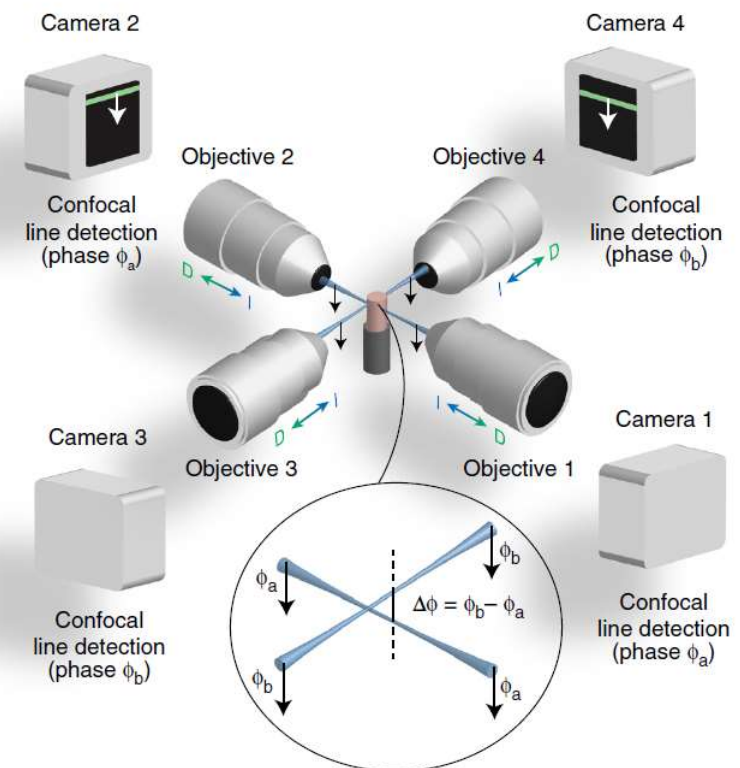
Whole-animal functional and developmental imaging with isotropic spatial resolution

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

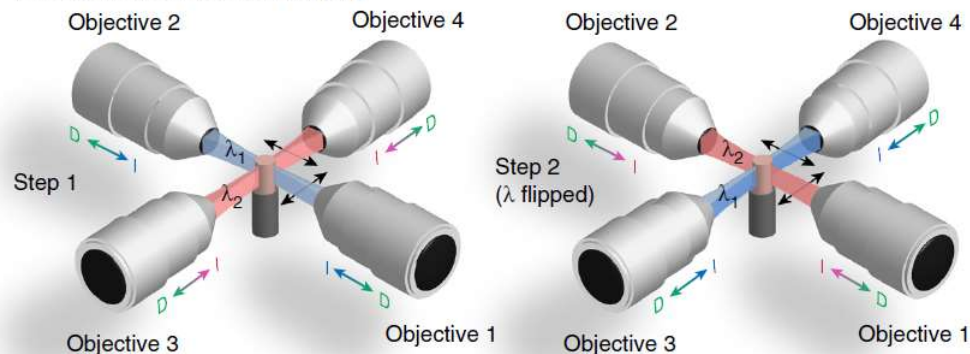
b IsoView mode 1: sequential four-view imaging

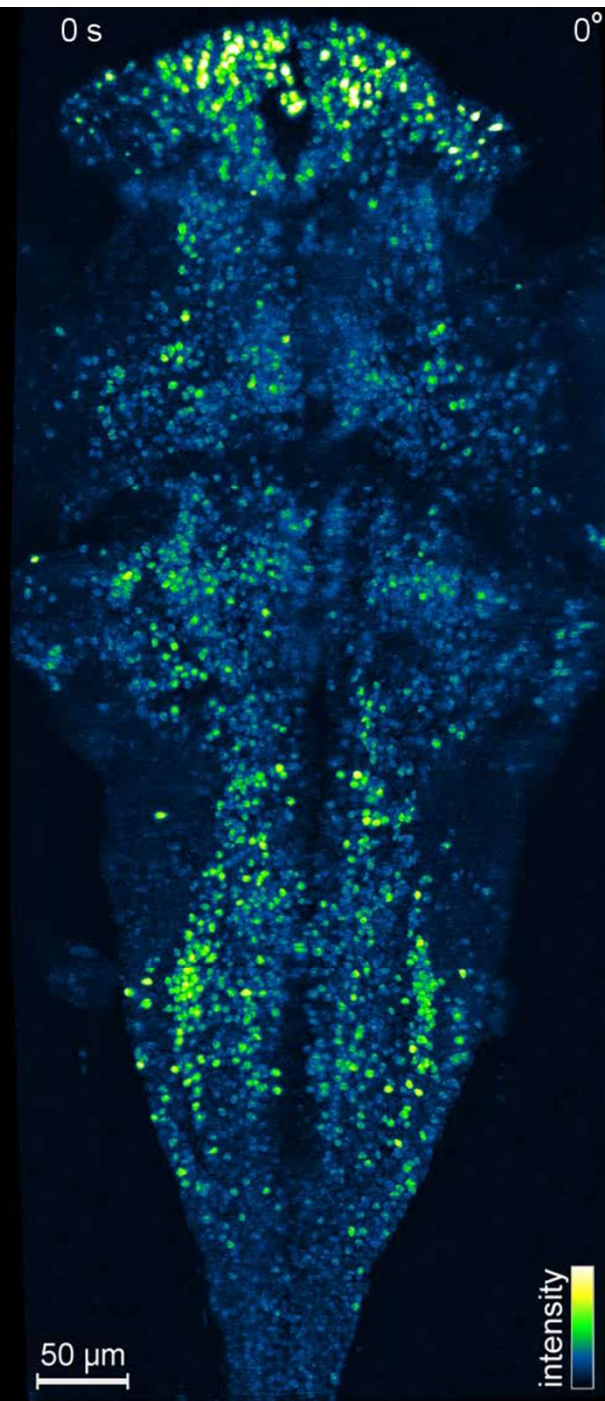


c IsoView mode 2: simultaneous four-view imaging

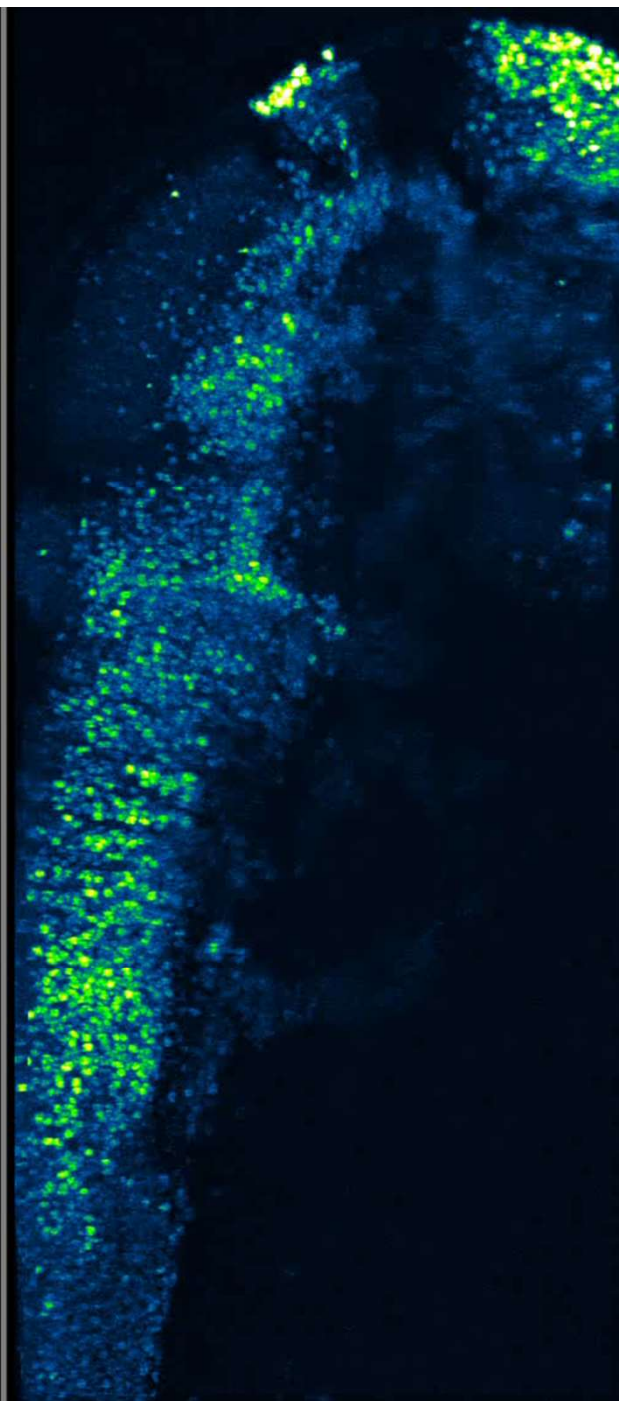


d IsoView mode 3: two-color imaging





dorsoventral view

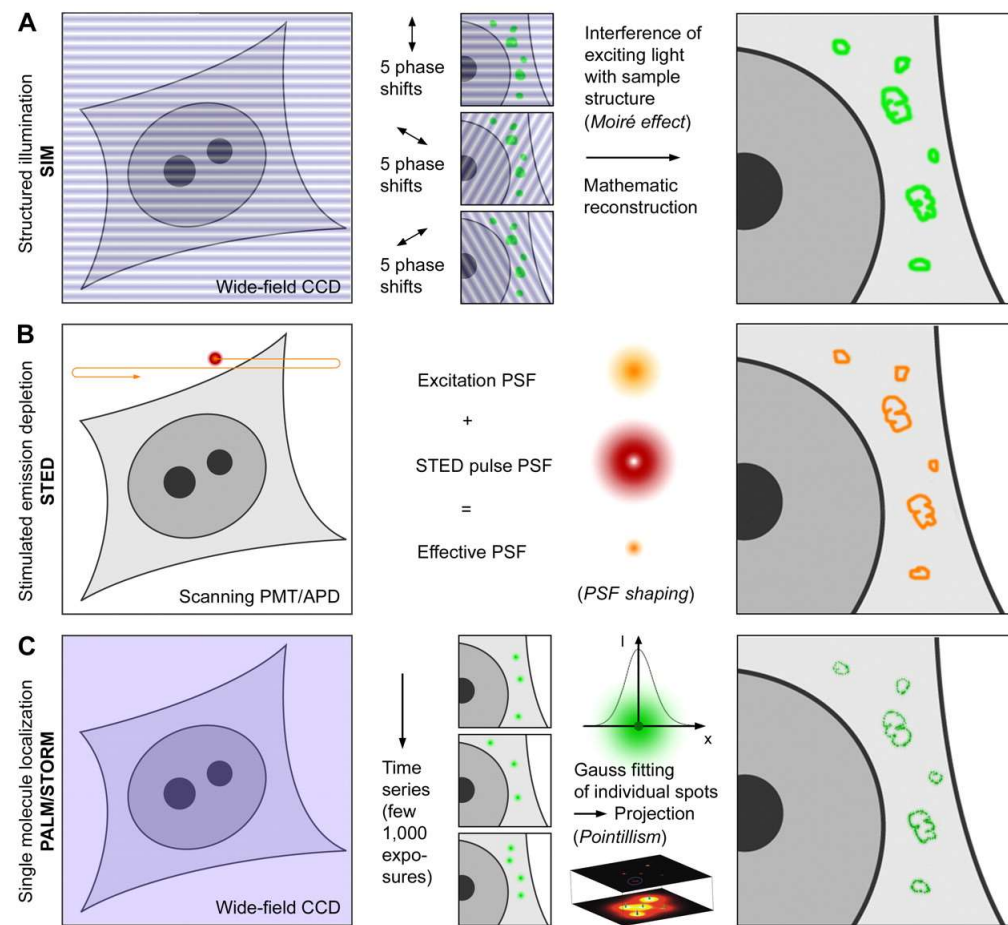


rotating view

Super resolution microscope

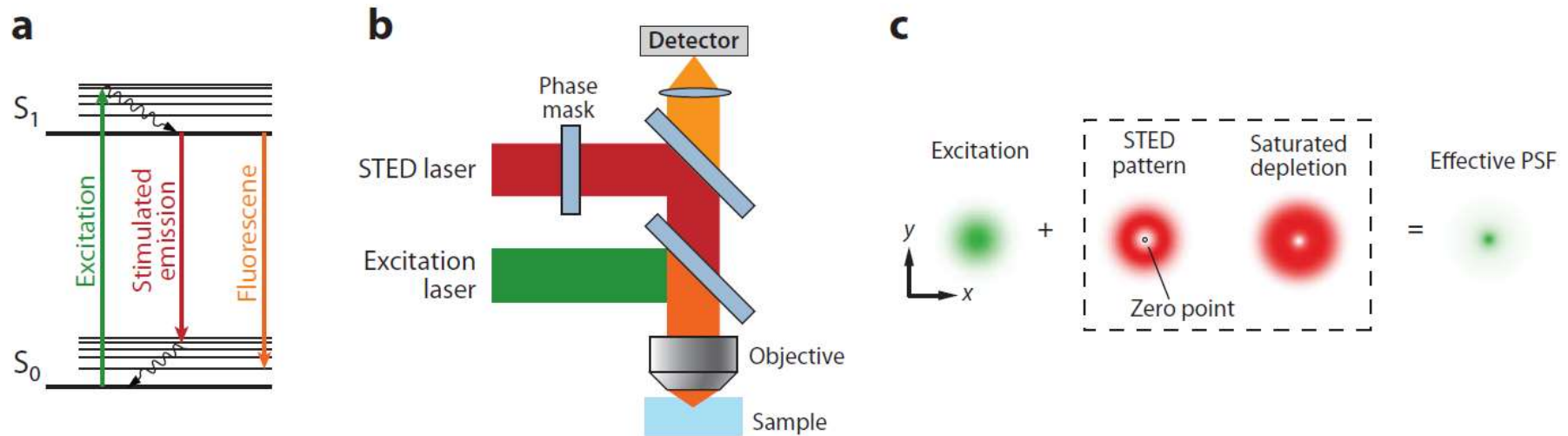
- STED Microscope
- Localization Microscope: PALM/STORM
- Structured Illumination Microscope (SIM)

Super-resolution microscope

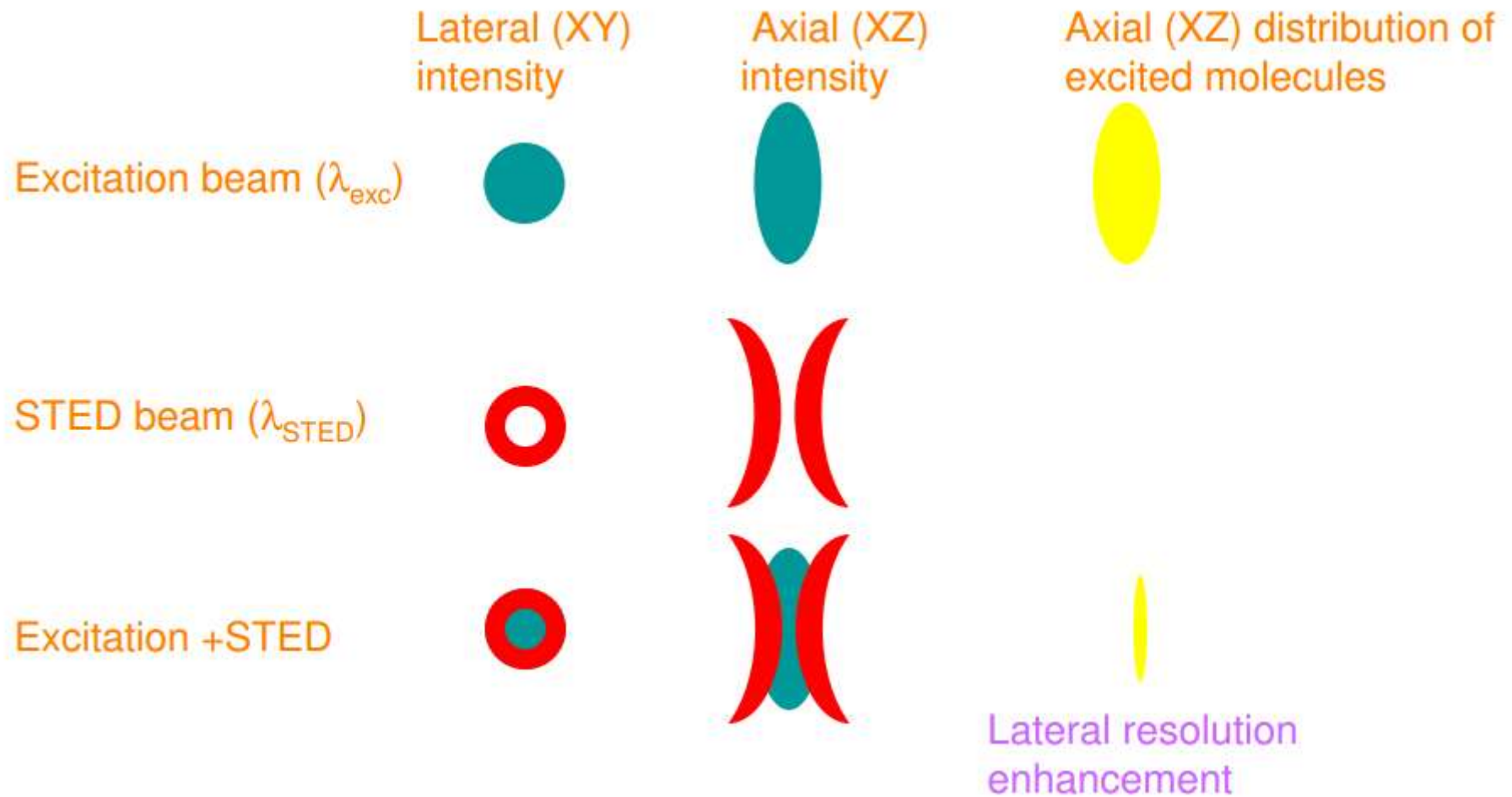


Lothar Schermelleh et al. J Cell Biol 2010;190:165-175

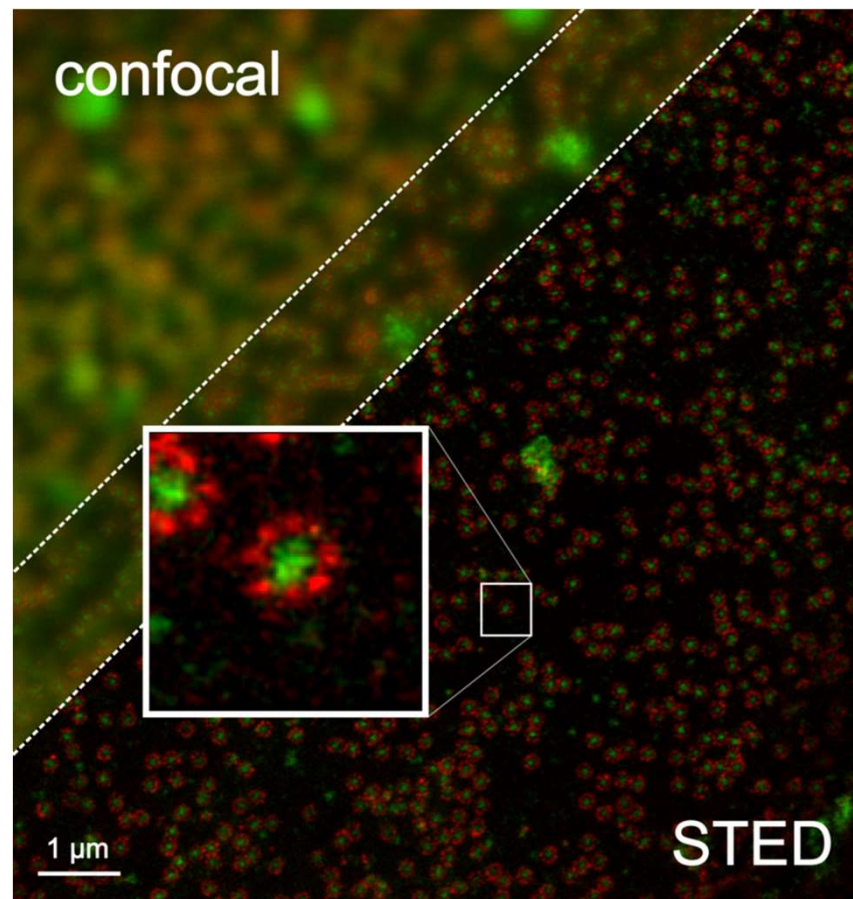
STimulated Emission Depletion (STED) Microscopy



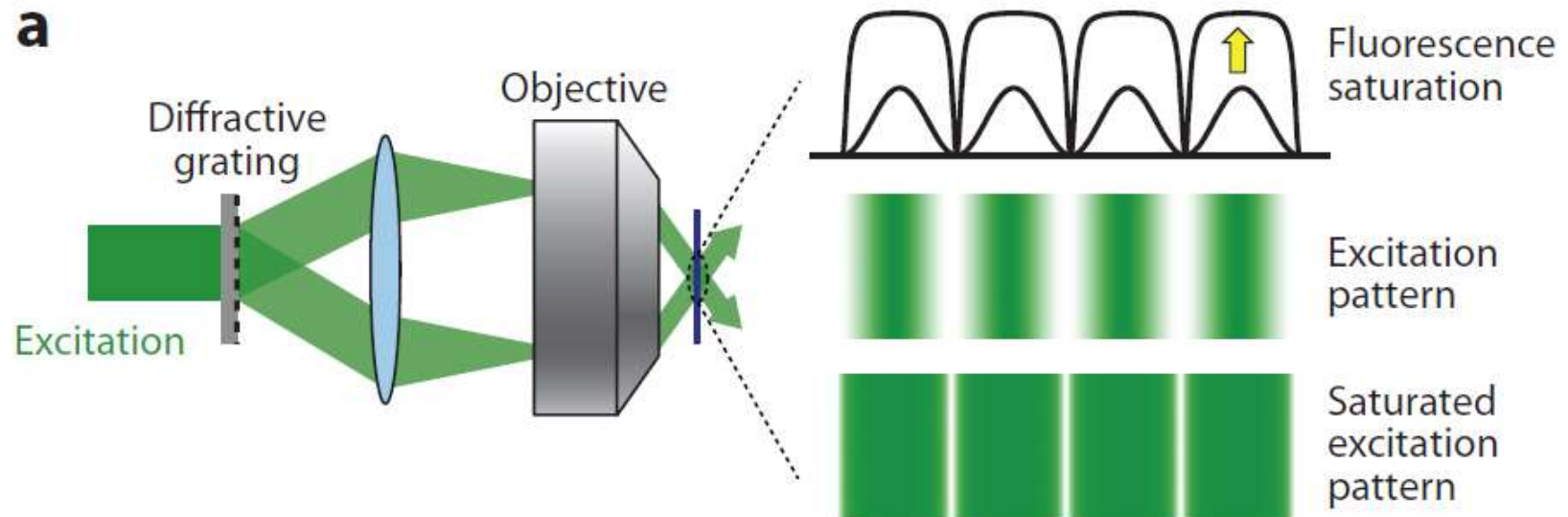
STimulated Emission Depletion (STED) Microscopy



STimulated Emission Depletion (STED) Microscopy

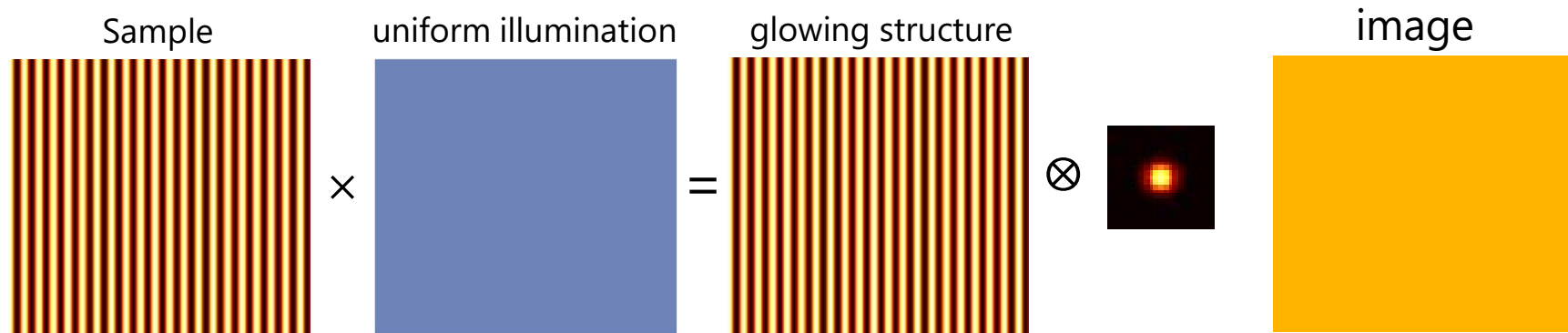


Structured Illumination Microscope (SIM)



Structured Illumination Microscope (SIM)

epi fluorescence imaging

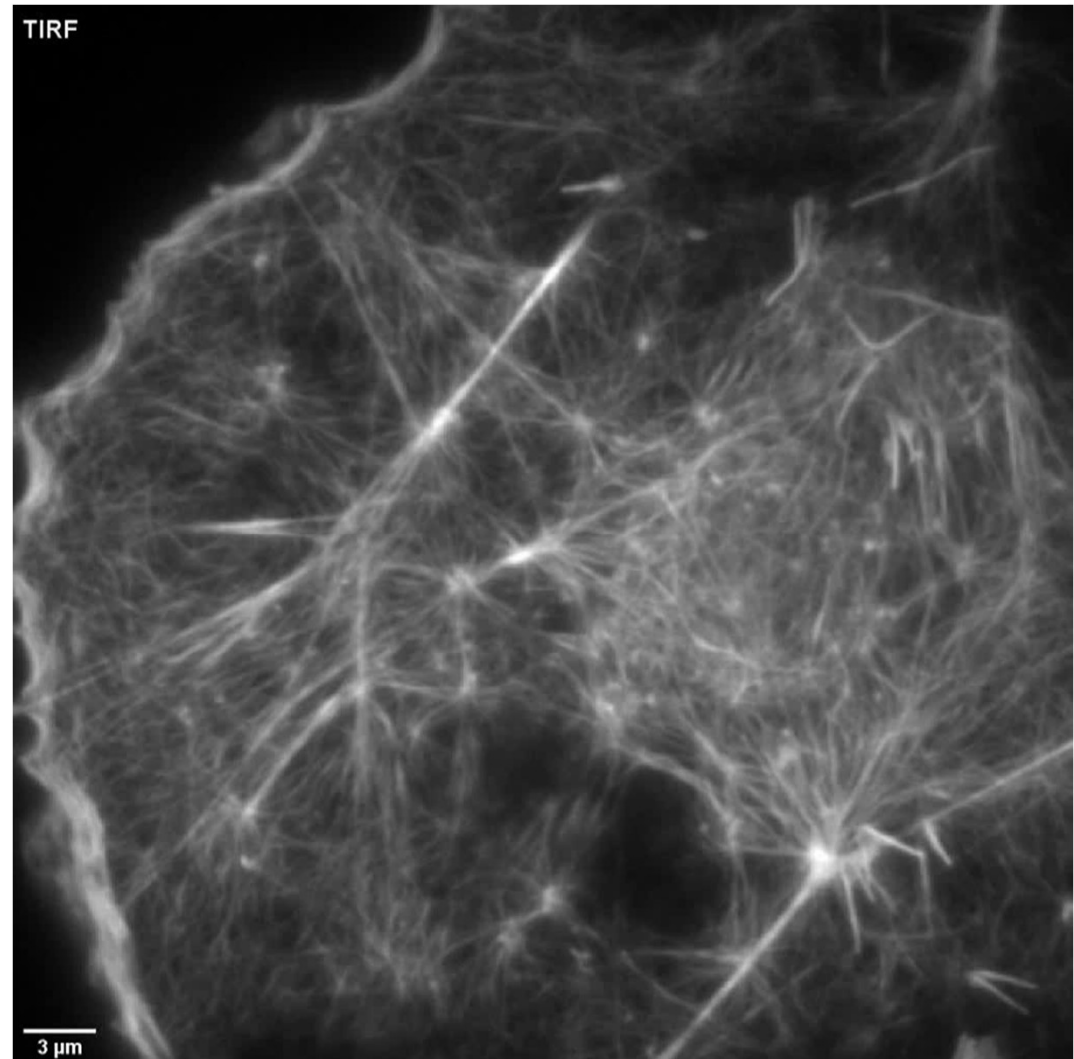


Structured Illumination Microscope (SIM)

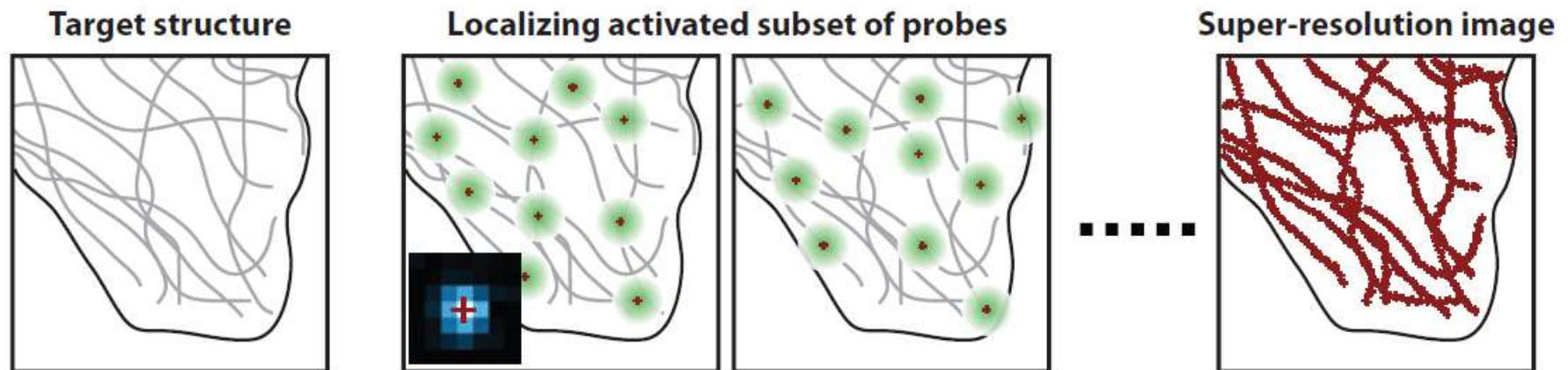
Live imaging demonstrated

	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
1 st nonlinear SIM	-	-	60 nm
2 nd nonlinear SIM	-	-	45 nm

D. Li et al. *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

Single-Molecule Superresolution Microscopy for Precise Localization

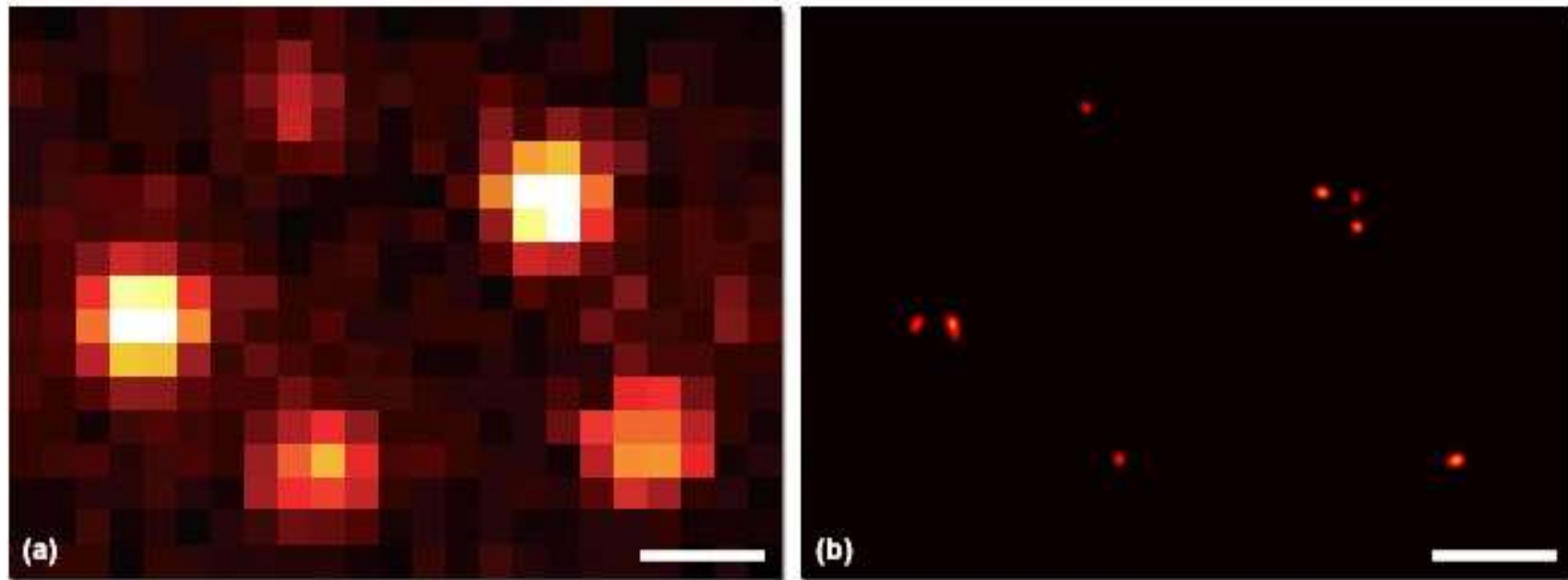
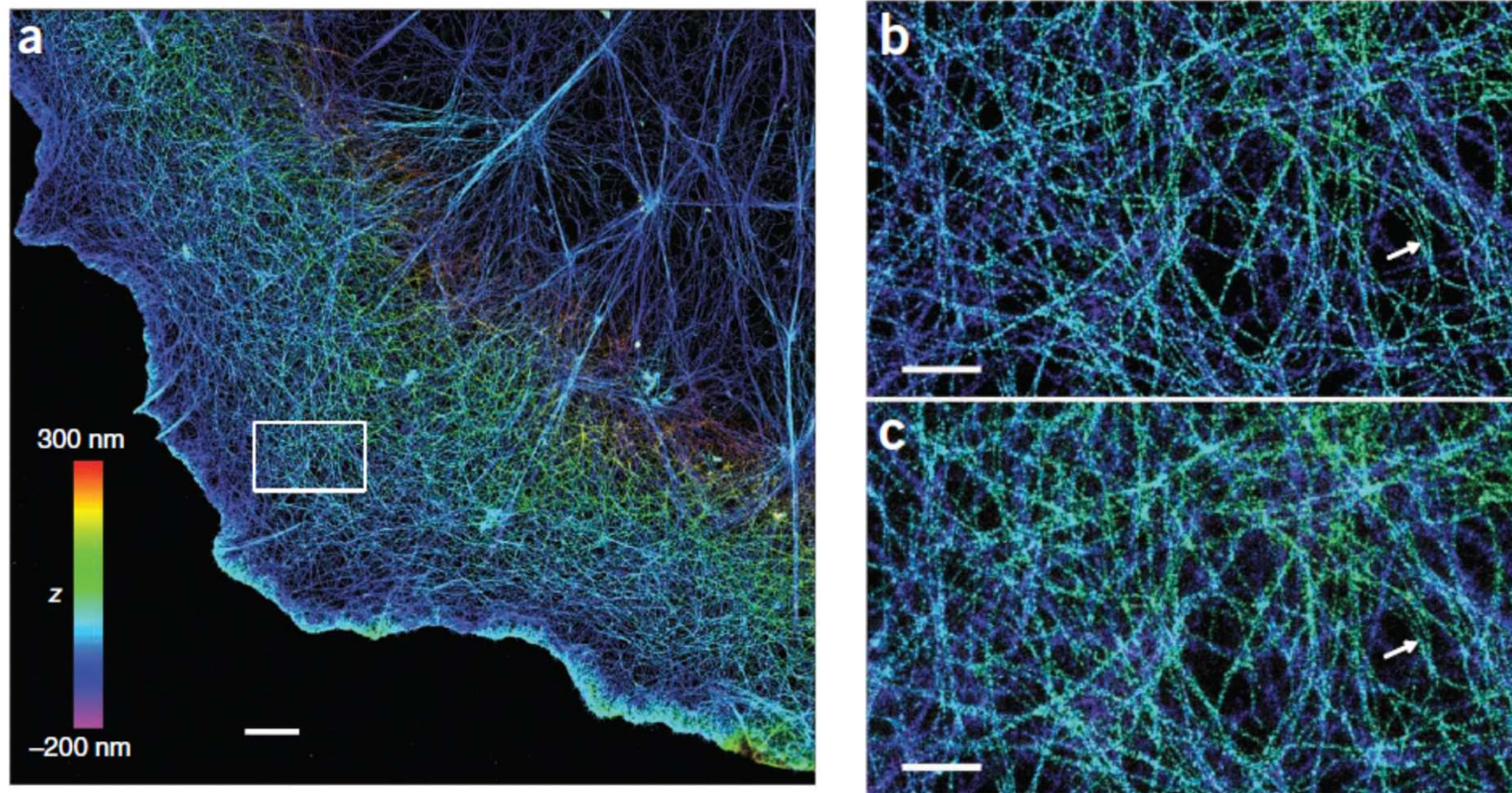


Figure 1

3D STORM



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

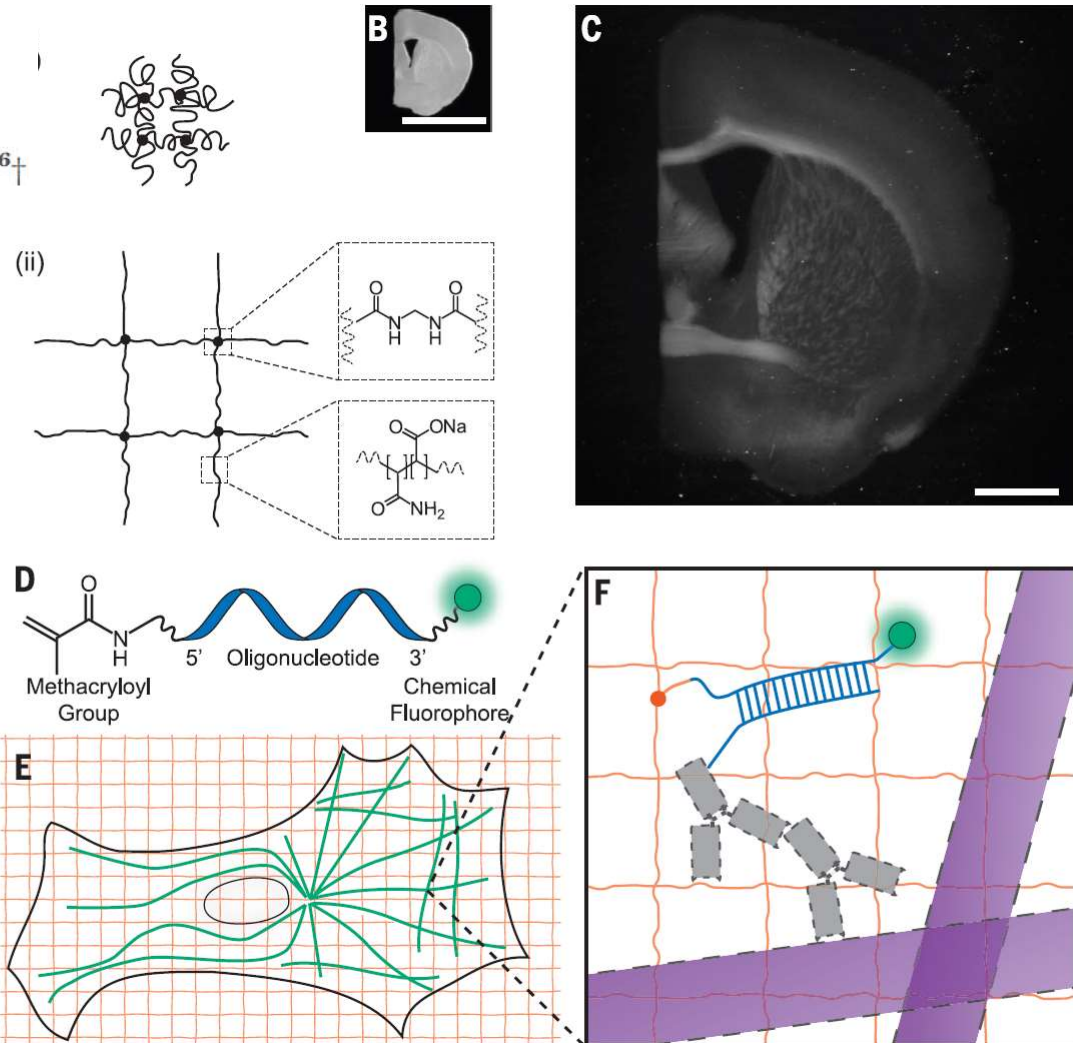
Expansion microscope

OPTICAL IMAGING

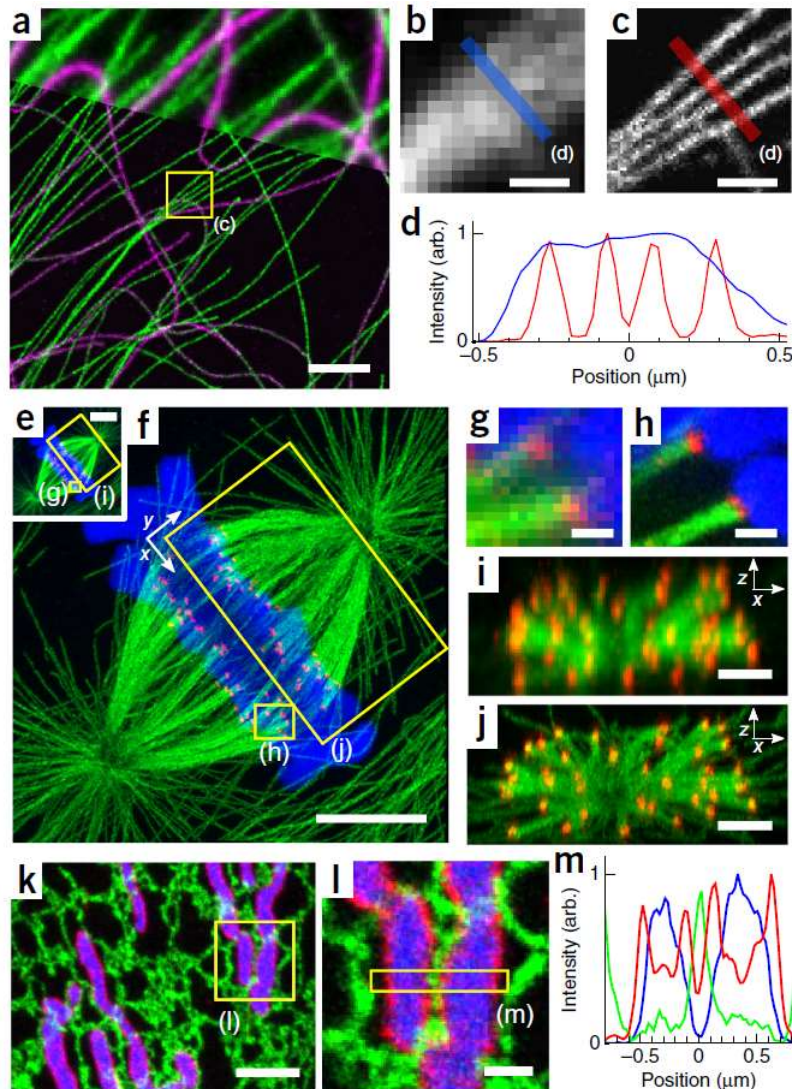
Expansion microscopy

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 Okawa², Hyeon-Jin Kim¹, Grant J Tremel¹, Rachel O L Wong² & Joshua C Vaughan^{1,3}

**nature
biotechnology**


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

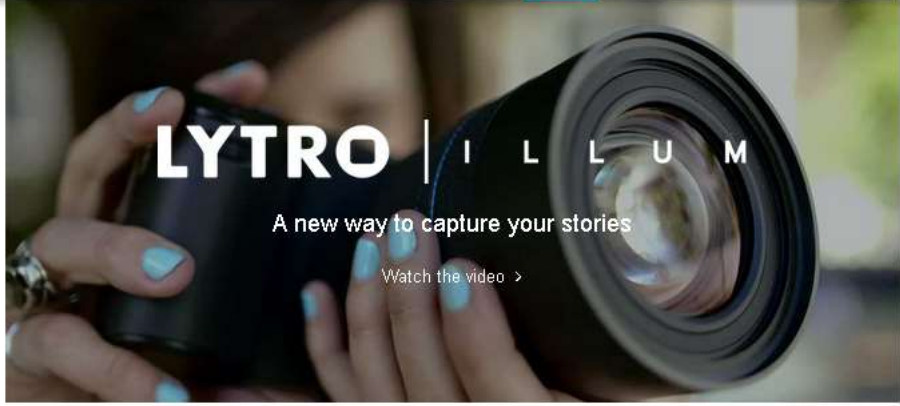
Paul W Tillberg^{1,2,10}, Fei Chen^{2,3,10}, Kiryl D Piatkevich², Yongxin Zhao², Chih-Chieh (Jay) Yu^{2,3}, Brian P English⁴, Linyi Gao³, Anthony Martorell⁵, Ho-Jun Suk^{2,6}, Fumiaki Yoshida^{7,8}, Ellen M DeGennaro^{5,8}, Douglas H Roossien⁹, Guanyu Gong³, Uthpala Seneviratne³, Steven R Tannenbaum³, Robert Desimone^{5,8}, Dawen Cai⁹ & 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 Cote⁴, Anubhav Sinha⁵, Shahar Alon^{2,3}, Shoh Asano^{2,3}, Evan R Daugherty^{6,7}, Jae-Byum Chang^{2,3}, Adam Marblestone^{2,3}, George M Church^{6,8}, Arjun Raj⁴ & Edward S Boyden^{1-3,9}

Special Topcis: Light field microscope

 [ILLUM](#) [Apps](#) [Gallery](#) [Learn](#) [Platform](#)





LYTRO | ILLUM


A new way to capture your stories


[Watch the video >](#)

The invention of photography gave us the power to capture our most beautiful, tragic and thought-provoking moments. But traditional cameras only capture part of a story. What if we could look deeper? What if we could tell the full story of a moment captured in time?

Now, with a single exposure from Lytro ILLUM, you can adjust aperture, use selective focus, tilt, focus spread, animation and even 3D to differentiate yourself with multiple deliverables.



[Lytro ILLUM in action](#)


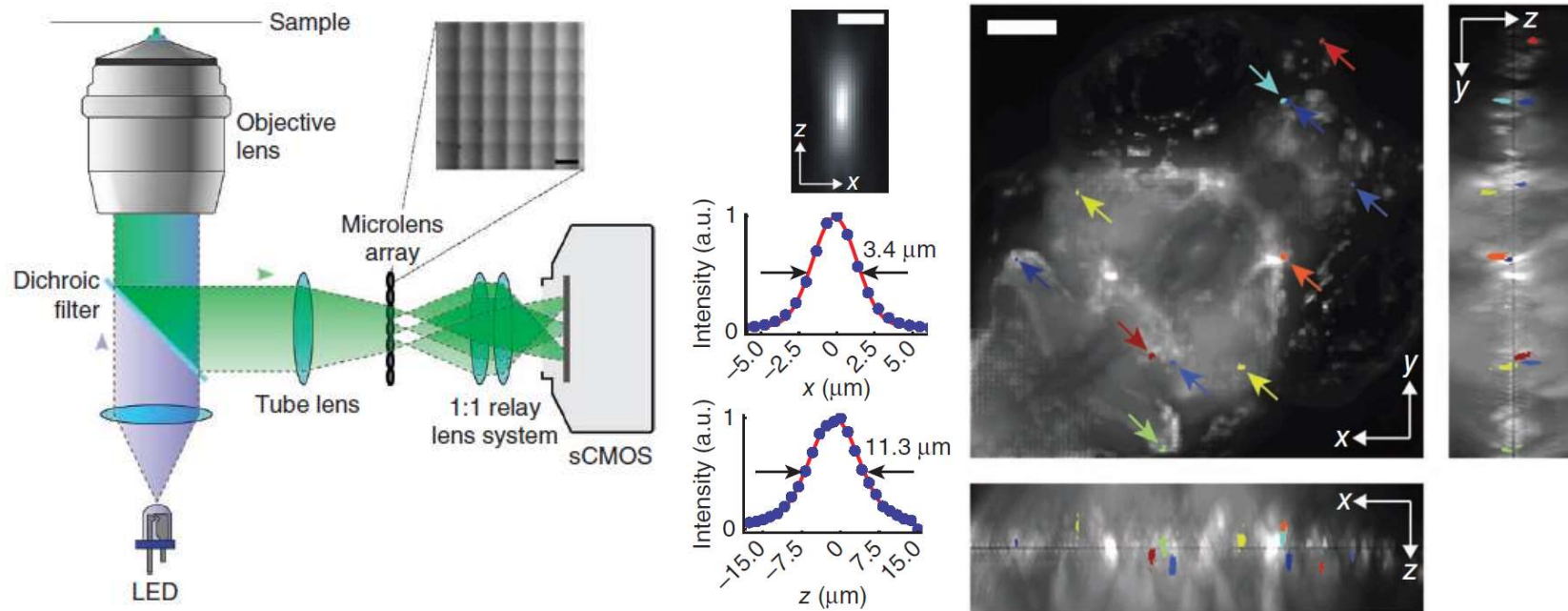
[Explore Lytro ILLUM tutorials](#)




Light field microscope

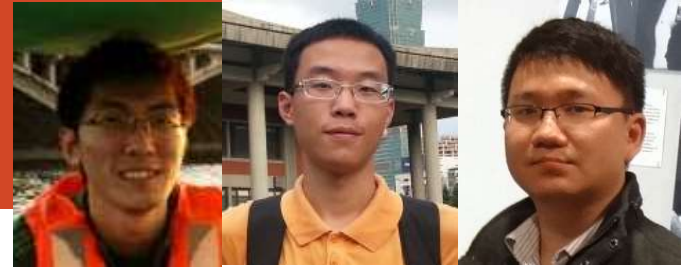


Light field microscope



R. Prevedel, Nature Methods 2014

X-LFM X-Light Field Microscope

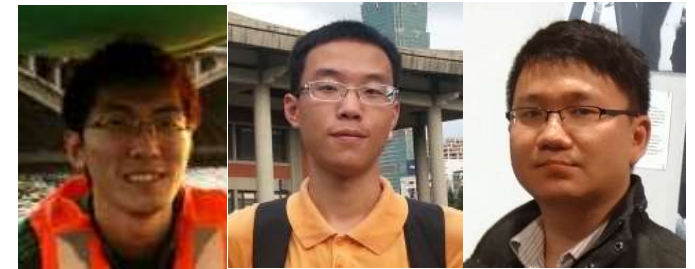


What's new?

- 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

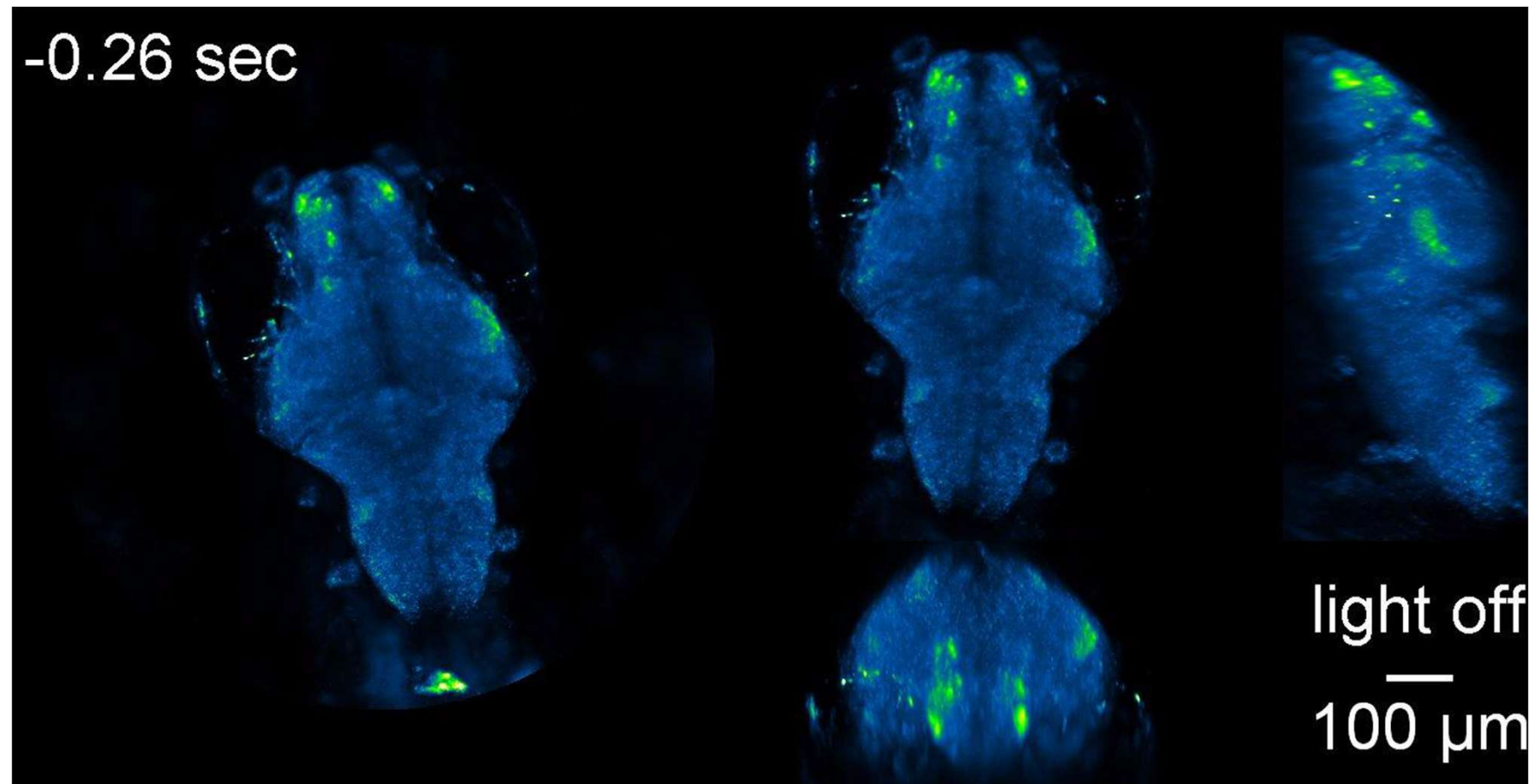


柴宇明

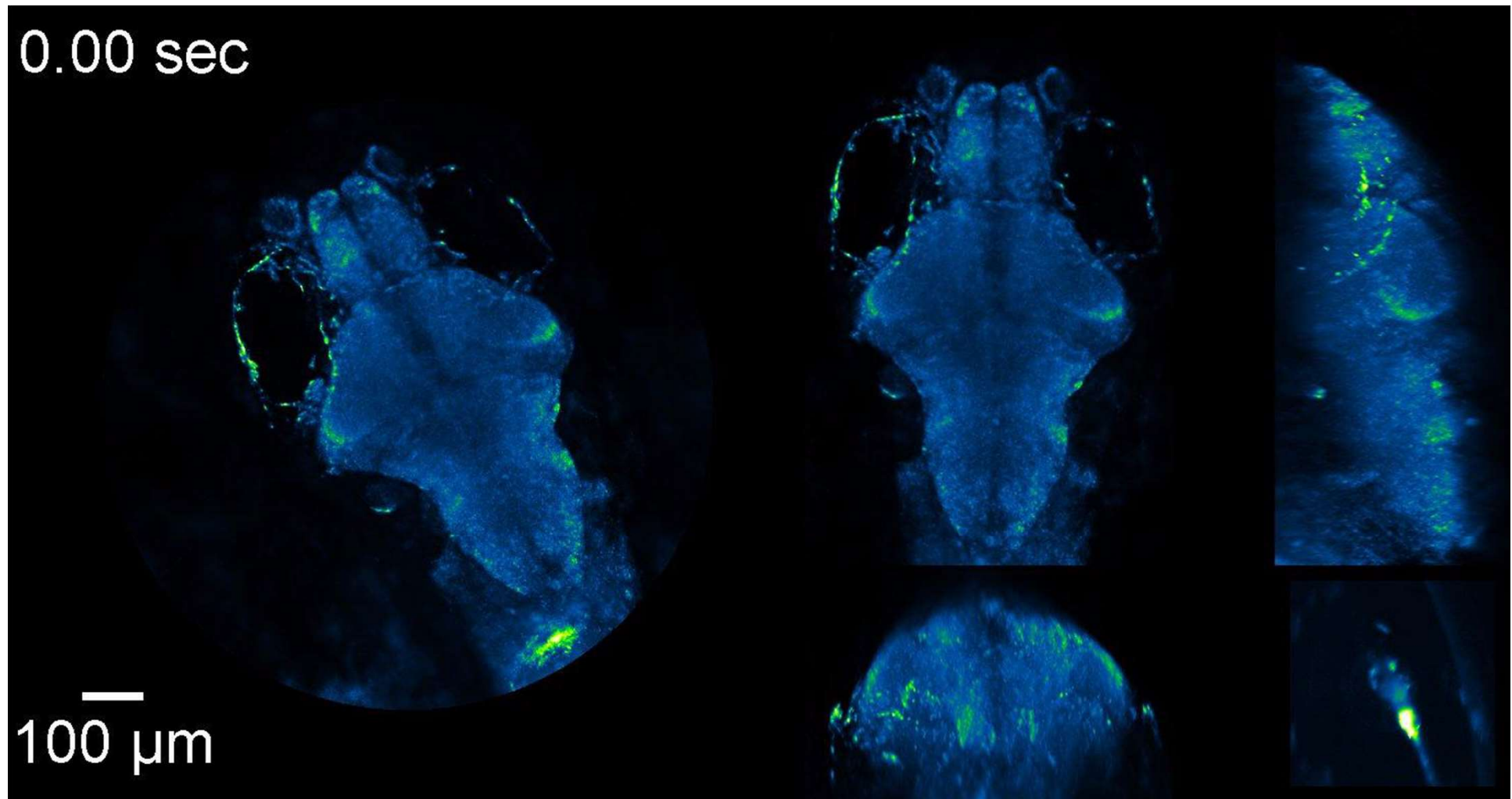
Free moving-zebrafish tracking

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

