超分辨显微, 至极至美

——2014年诺贝尔化学奖解读

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2014-10-19，北京

Nobelpriset i kemi 2014

Eric Betzig
Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA

Stefan W. Hell
Max Planck Institute for Biophysical Chemistry, Göttingen, German Cancer Research Center, Heidelberg, Germany

William E. Moerner
Stanford University, Stanford, CA, USA

"för utveckling av superupplöst fluorescensmikroskopi"
"for the development of super-resolved fluorescence microscopy"

Seeing is believing.
Definitely so in biology!

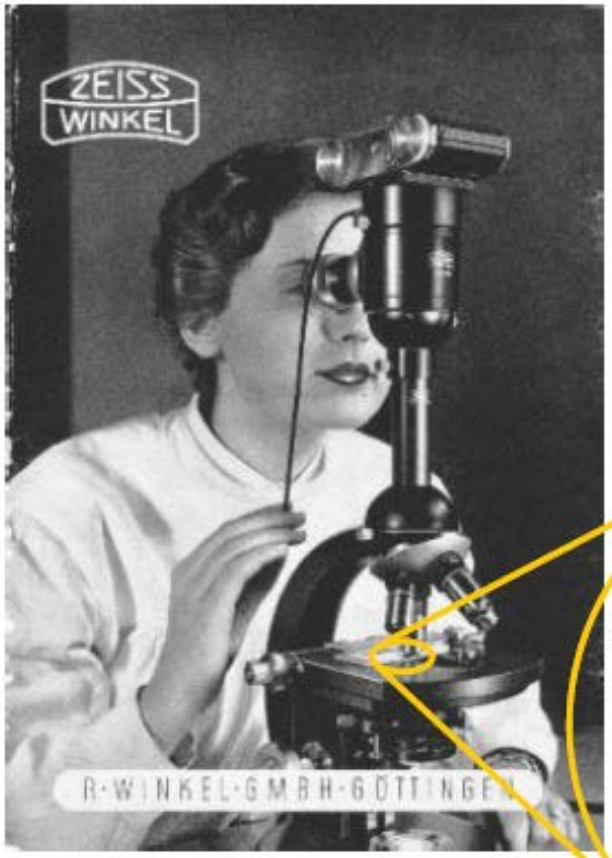
Feynman告诉你怎么做生物物理

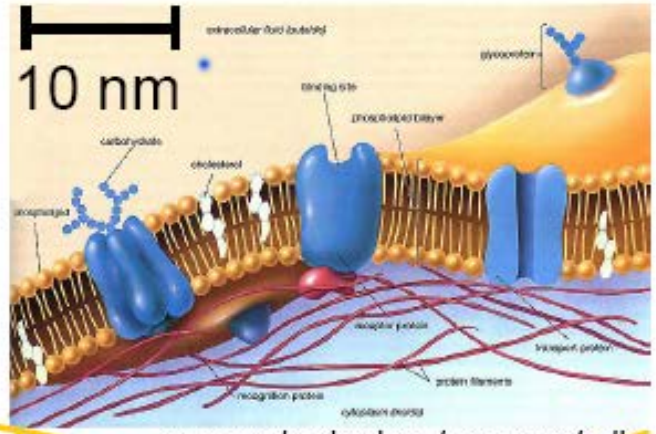
"It is very easy to answer many of these fundamental **biological** questions; you just look at the thing!"

from R. P. Feynman's talk

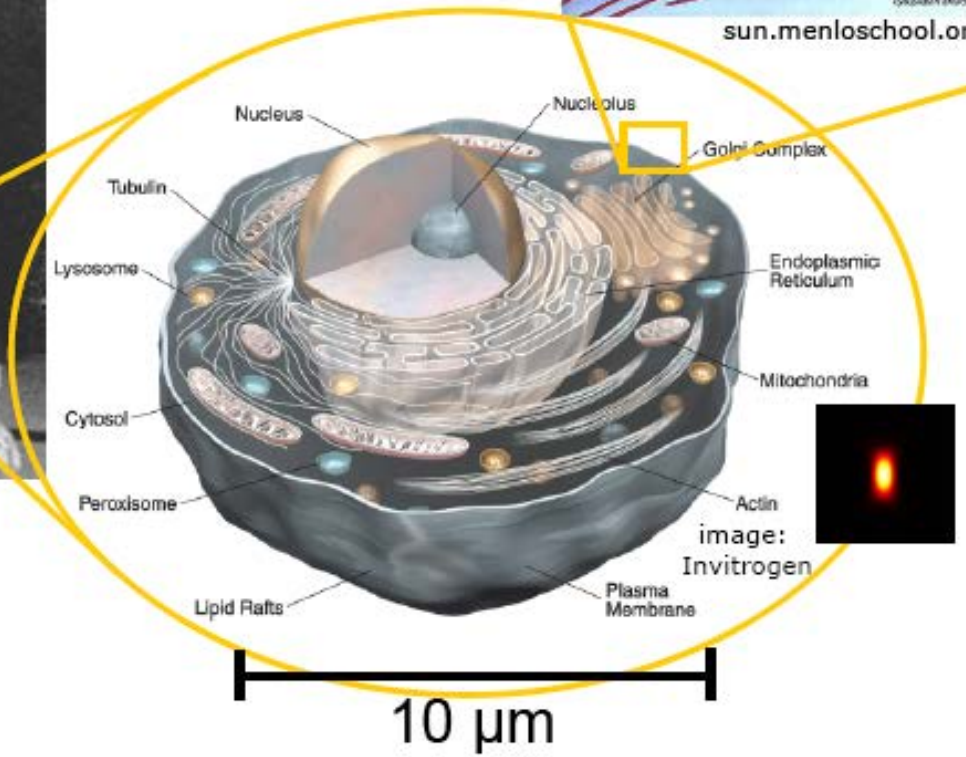
There's plenty of room at the bottom. Dec. 29th, 1959

光学显微镜的任何改进都会让生物学家如获至宝!





sun.menloschool.org/~cweaver/cells



1590 – Dutch lens grinders Hans and Zacharias Janssen make the first microscope by placing two lenses in a tube.

1667 – Robert Hooke studies various object with his microscope and publishes his results in Micrographia. Among his work were a description of cork and its ability to float in water.

1675 – Anton van Leeuwenhoek uses a simple microscope with only one lens to look at blood, insects and many other objects. He was first to describe cells and bacteria, seen through his very small microscopes with, for his time, extremely good lenses.

1903 – Richard Zsigmondy develops the ultramicroscope and is able to study objects below the wavelength of light.
The Nobel Prize in Chemistry 1925 »

1932 – Frits Zernike invents the phase-contrast microscope that allows the study of colorless and transparent biological materials.
The Nobel Prize in Physics 1953 »

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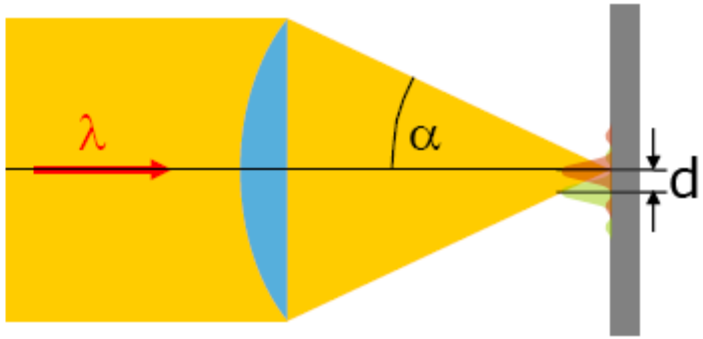
Resolution

Ernst Abbe
(1872)

diffraction limit

structures smaller than half a wavelength cannot be resolved.



$$d_{min} \approx 0.61 \frac{\lambda}{n \sin \alpha}$$

wavelength (λ)

refractive index (n)

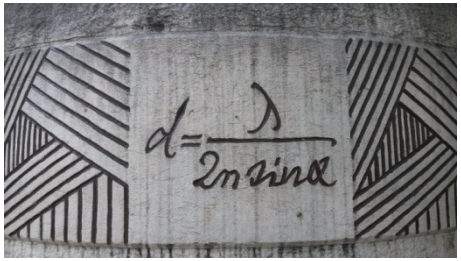
numerical aperture (NA) ($n \sin \alpha$)

aperture angle (α)

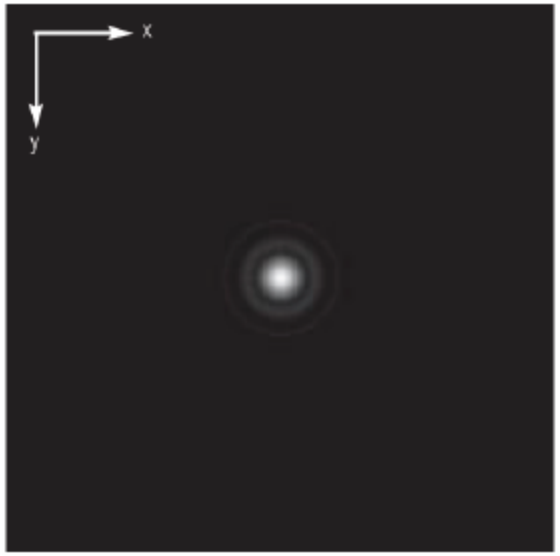
source	λ	d_{min}
light	~ 500 nm	~ 250 nm
X-ray	~ 2 nm	~ 25 nm
electron	~ 0.001 nm	~ 0.1 nm (>2 nm)

(size of a cell ~ 10 µm)


ERNST ABBE
1840-1905
FRIEDRICH SCHILLER
UNIVERSITÄT JENA

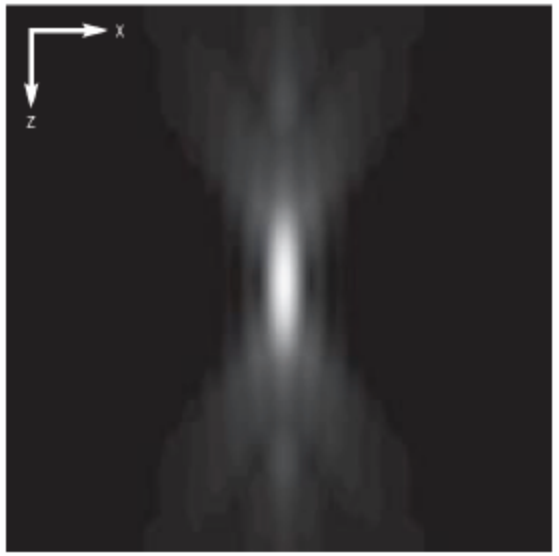

d = λ / 2n sin α

Three-dimensional point-spread function



Lateral:

$$FWHM_{ill,lateral} = .61 \frac{\lambda_{exc}}{NA}$$

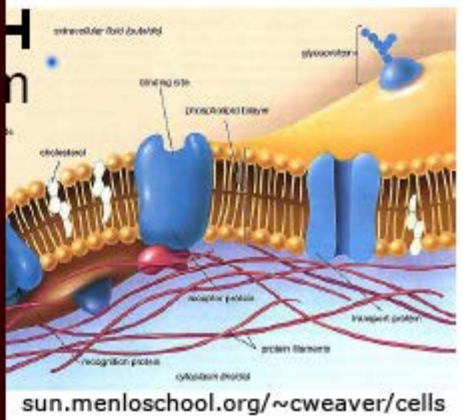


Axial:

$$FWHM_{ill,axial} = \frac{0.88 \cdot \lambda_{exc}}{(n-\sqrt{n^2-NA^2})}$$

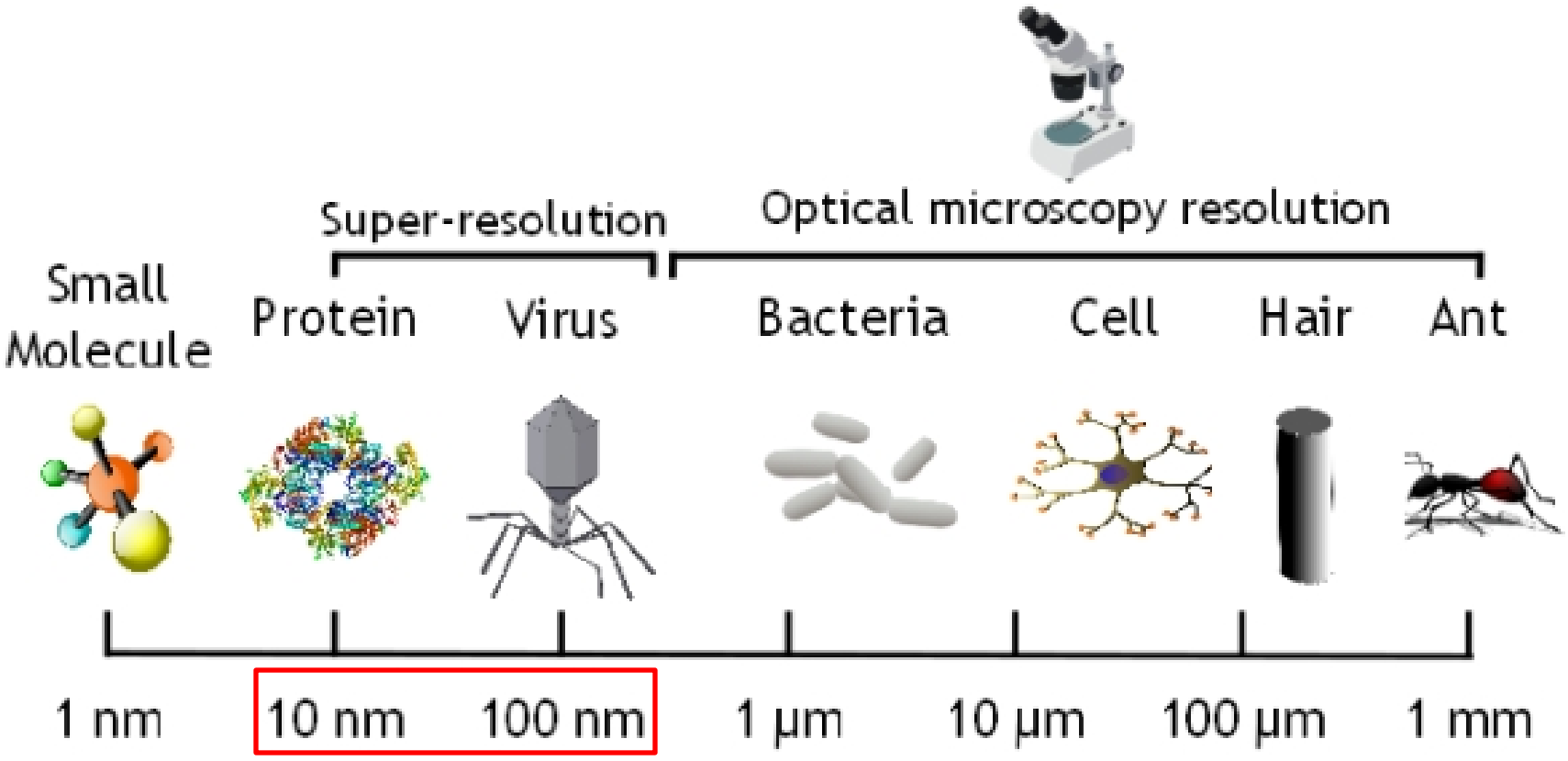
n = *refractive index of immersion liquid,*
NA = *numerical aperture of the microscope objective,*
λ_{exc} = *wavelength of the excitation light*

Ref: Carl Zeiss, *Confocal Laser Scanning Microscopy*

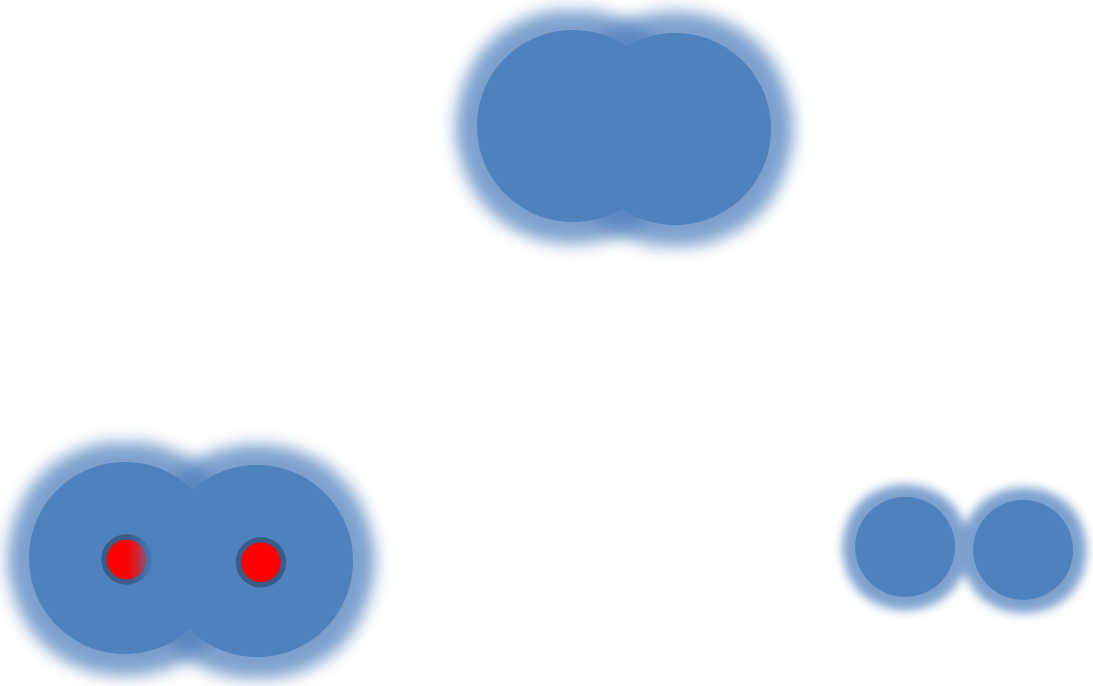


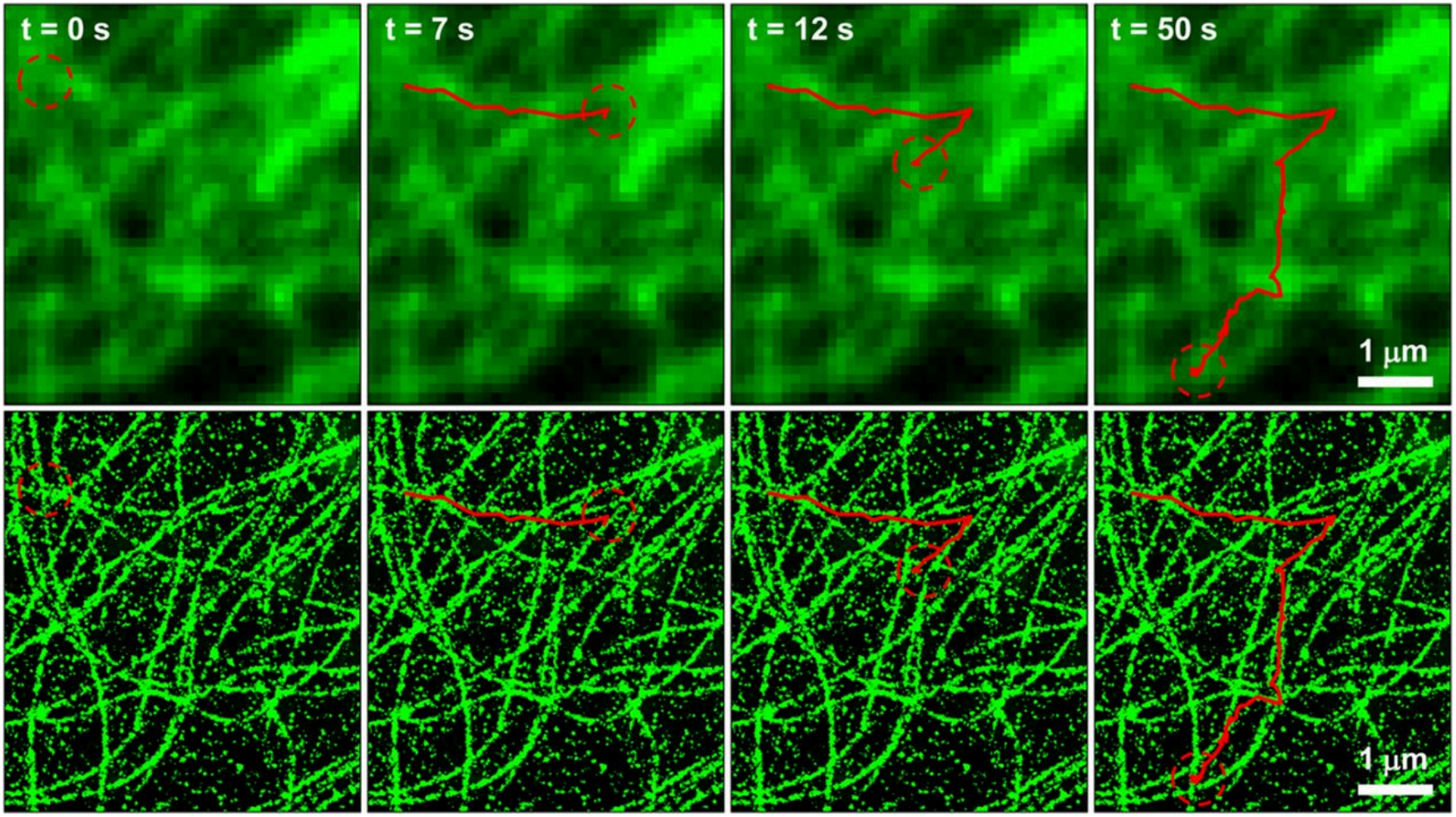
Just too large !

Point Spread Function

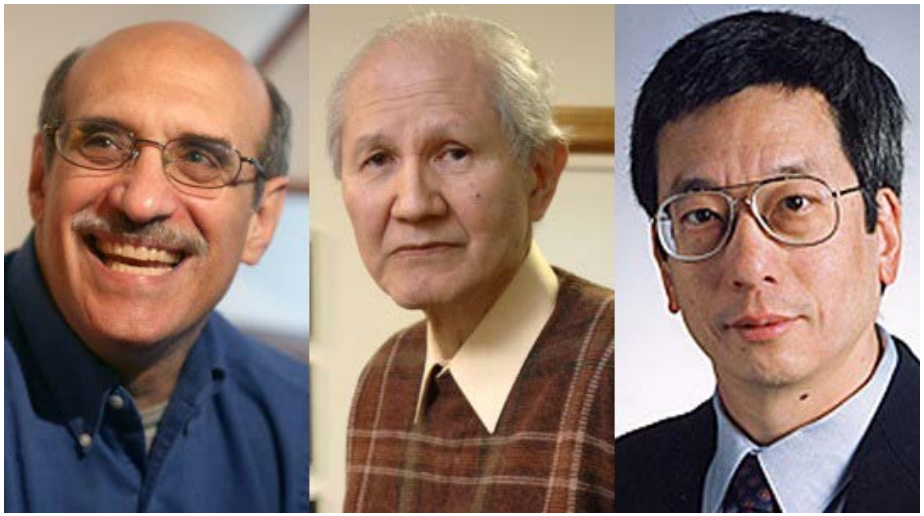

Super-resolution
Optical microscopy resolution
Small Molecule
Protein
Virus
Bacteria
Cell
Hair
Ant
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm

Super-resolution, beyond the Abbe limitation



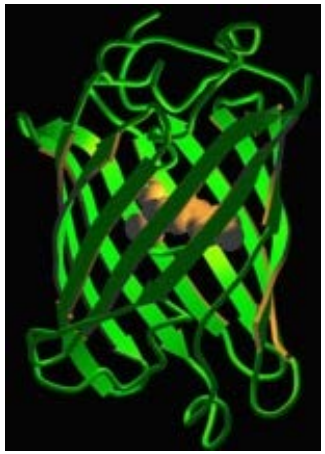

t = 0 s
t = 7 s
t = 12 s
t = 50 s
1 μm
1 μm

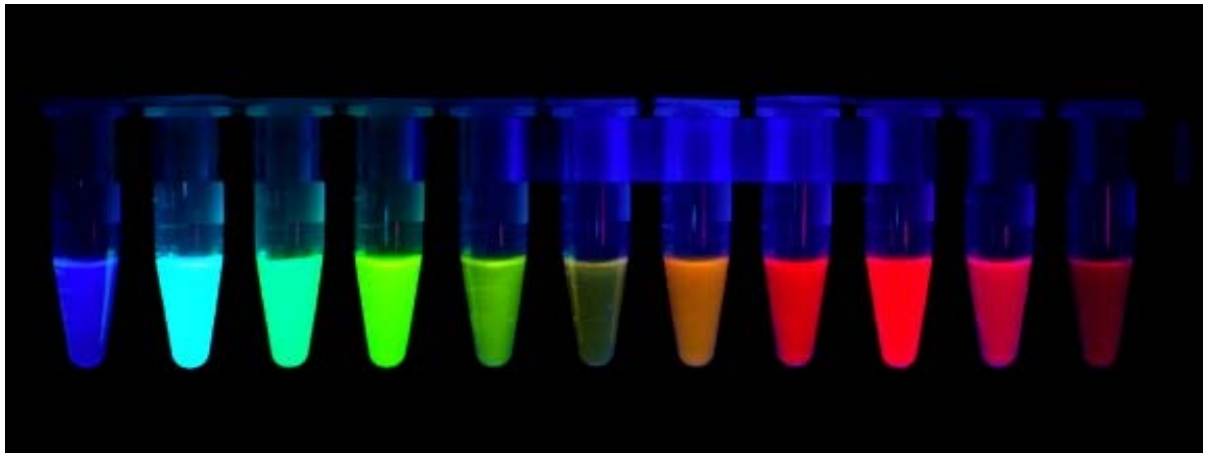
2008 Nobel Prize in Chemistry

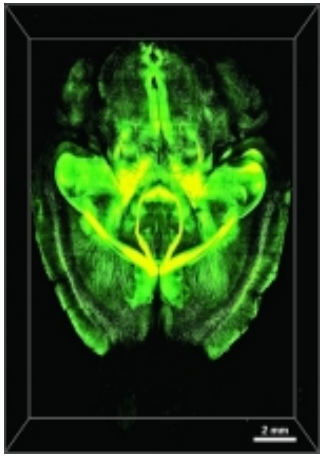


Martin Chalfie, Osamu Shimomura, and Roger Tsien,

“for the discovery and development of the green fluorescent protein, GFP”







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"för utveckling av superupplöst fluorescensmikroskopi"
"for the development of super-resolved fluorescence microscopy"

What have you done, W.E. ?!

WILLIAM E. MOERNER

Born 1953 in Pleasanton, CA. Ph.D. 1982 from Cornell University, Ithaca, NY.
Professor in Chemistry and Applied Physics at Stanford University.

First single molecule detection in solid

Moerner WE and Kador L (1989) Optical detection and spectroscopy of single molecules in a solid. *Phys. Rev. Lett.* 62:2535-2538.

1. Photoelectric Effect (March 1905)
On a Heuristic Point of View Concerning the Production and Transformation of Light
(Übereinen die Erzeugung und Verwandlung des
LichtesbetreffendenheuristischenGesichtspunkt)
AnnalenderPhysik 17:132-148

2. Doctoral Dissertation (completed April 30, 1905)
The Determination of Molecular Dimensions
(EineneueBestimmungderMoleküldimensionen).
Submitted to the University of Zurich on July 20, 1905.
AnnalenderPhysik 19:289-305

3. Brownian Motion (May 1905)

On the Movement of Small Particles Suspended in Stationary Liquids Required by the Molecular-Kinetic Theory of Heat
(Über die von dermolekularkinetischenTheoriederWärmegeforderteBewegung von in ruhendenFlüssigkeitensuspendiertenTeilchen)
AnnalenderPhysik 17:549-560

4. Special Relativity (June 1905)
On the Electrodynamics of Moving Bodies
(ZurElektrodynamik begetter Körper)
AnnalenderPhysik 17:891-921

英雄榜 I

Moerner WE and Kador L (1989) Optical detection and spectroscopy of single molecules in a solid. *Phys. Rev. Lett.* 62:2535-2538.

4K

Orrit M and Bernard J (1990) Single pentacene molecules detected by fluorescence excitation in a p-terphenyl crystal. *Phys. Rev. Lett.* 65:2716-2719.

Shera EB, Seitzinger NK, Davis LM, Keller RA and Soper SA (1990). Detection of single fluorescent molecules. *Chem. Phys. Lett.* 174:553-557.

Rigler R and Widengren J (1990) Ultrasensitive detection of single molecules by fluoresc correlation spectroscopy. *Bioscience* 3:180-183.

300K

Betzig E and Chichester RJ (1993) Single molecules observed by near-field scanning optical microscopy. *Science* 262:1422-1425.

Ambrose WP, Goodwin PM, Keller RA and Martin JC (1994) Alterations of single molecule fluorescence lifetimes in near-field optical microscopy. *Science* 265:364-7.

dynamics

Xie XS and Dunn RC (1994) Probing single molecule dynamics. *Science* 265:361-364,

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Nie S, Chiu DT and Zare RN (1994) Probing individual molecules with confocal fluore microscopy. *Science*. 266:1018–1021.

diffusion

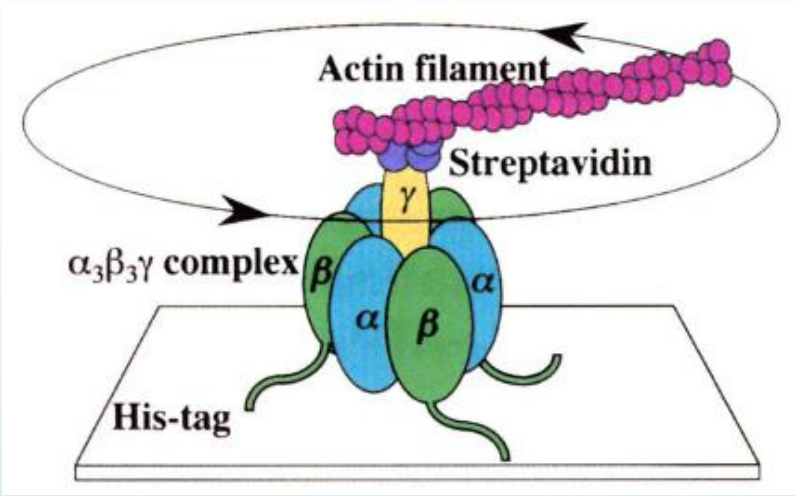
Funatsu T, Harada Y, Tokunaga M, Saito K, Yanagida T (1995) Imaging of single fluc molecules and individual ATP turnovers by single myosin molecules in aqueous solution. *Nature* 374:555-559.

Bio-motor

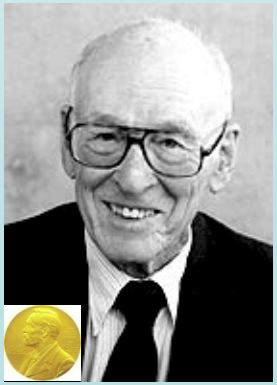
曾经做过嫁衣裳

F_1-ATPase: 120° step

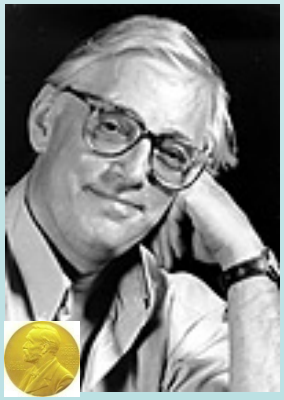
K.Kinosita, Jr



1997 Nobel Prize in Physiology or Medicine



Paul D.Boyer proposed binding change mechanism in 1973 and won Nobel Prize in 1997.
PNAS, **70**(1973)2837



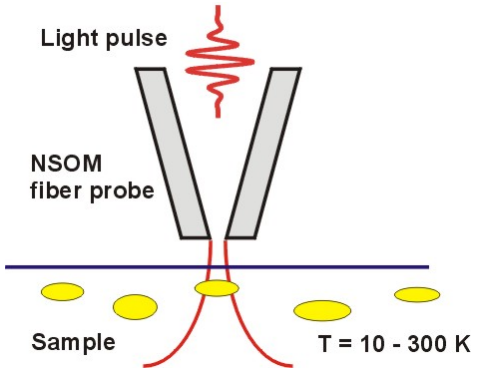
John E.Walker analyzed the structure of bovine F_1-ATPase in 1994 and won Nobel Prize in 1997.
Nature, 3**70**(1994)621

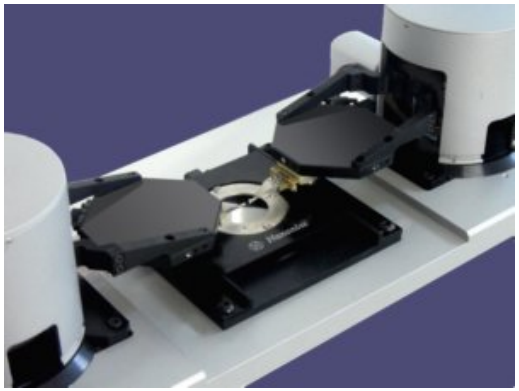
I did not feel at home in academia….But now!

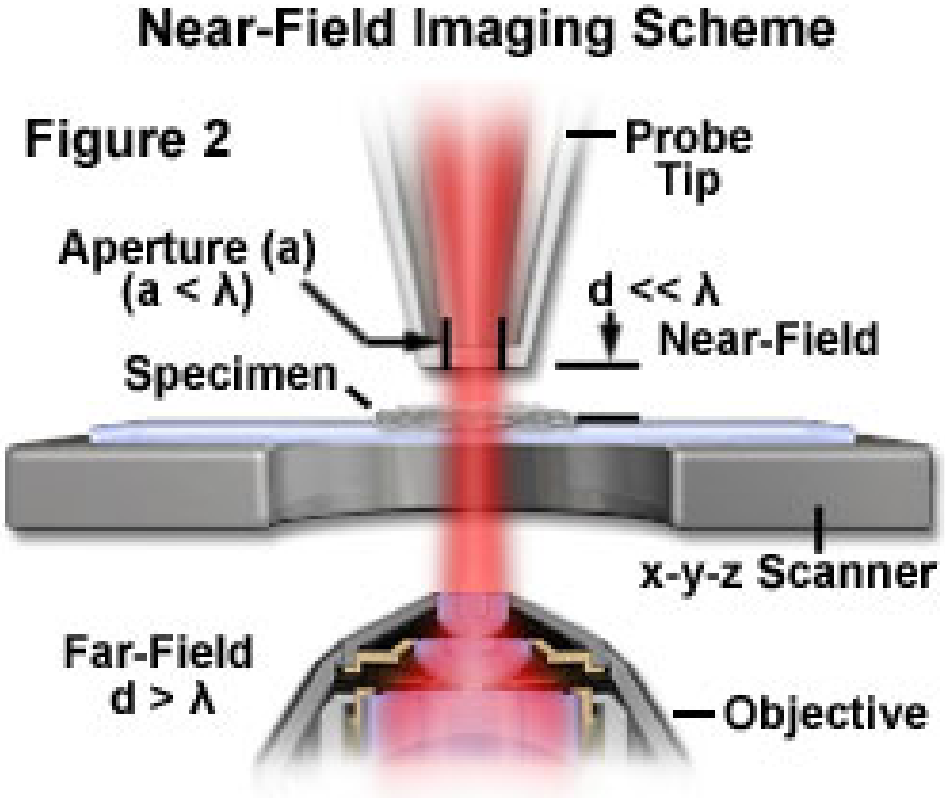
ERIC BETZIG

Born 1960 in Ann Arbor, MI. Ph.D. 1988 from Cornell University, Ithaca, NY.
Group Leader at Janelia Research Campus, Howard Hughes Medical Institute.

Scanning Near-Field Optical Microscopy (SNOM), hopeless?







Betzig E and Trautman JK (1992) Near-field optics: microscopy, spectroscopy and surface modification beyond the diffraction limit. *Science* 257: 189-195.

Betzig E and Chichester RJ (1993) Single molecules observed by near-field scanning optical microscopy. *Science* 262:1422-1425.

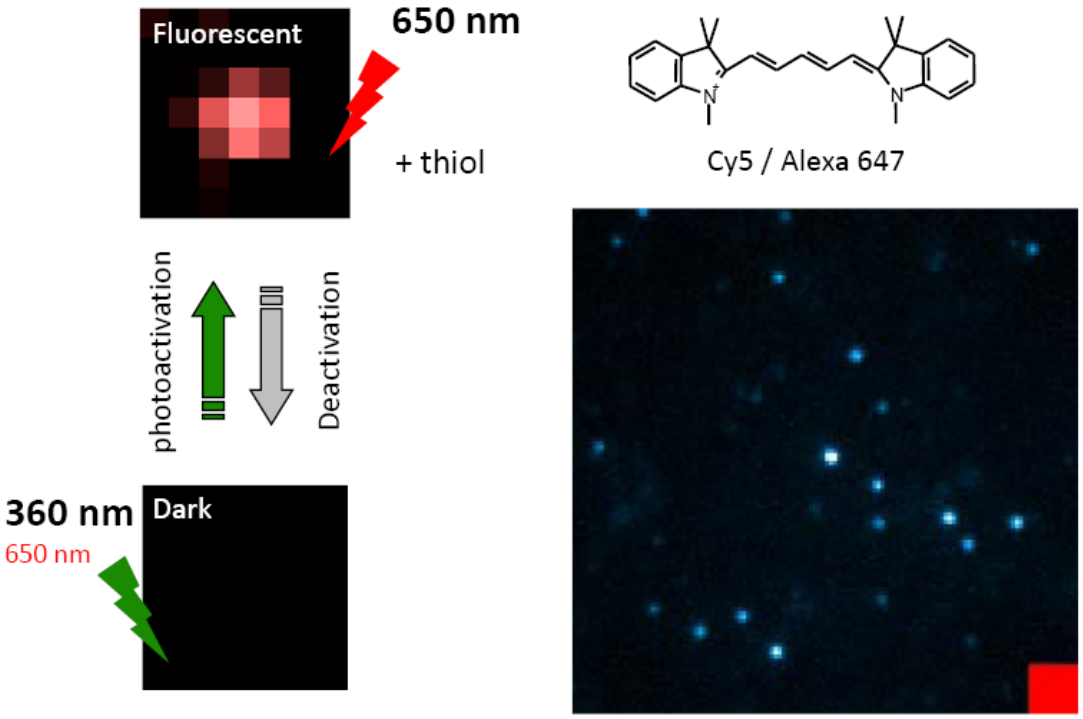
Betzig E (1995) Proposed method for molecular optical imaging. *Opt Lett.* 20:237-239.

Dickson RM, Cubitt AB, Tsien RY and Moerner WE (1997) On/off blinking and switching behaviour of single molecules of green fluorescent protein. *Nature* 388:355-358.

Switching Effect of Fluorophores

Dickson RM, Cubitt AB, Tsien RY and Moerner WE (1997) On/off blinking and switching behaviour of single molecules of green fluorescent protein. *Nature* 388:355-358.

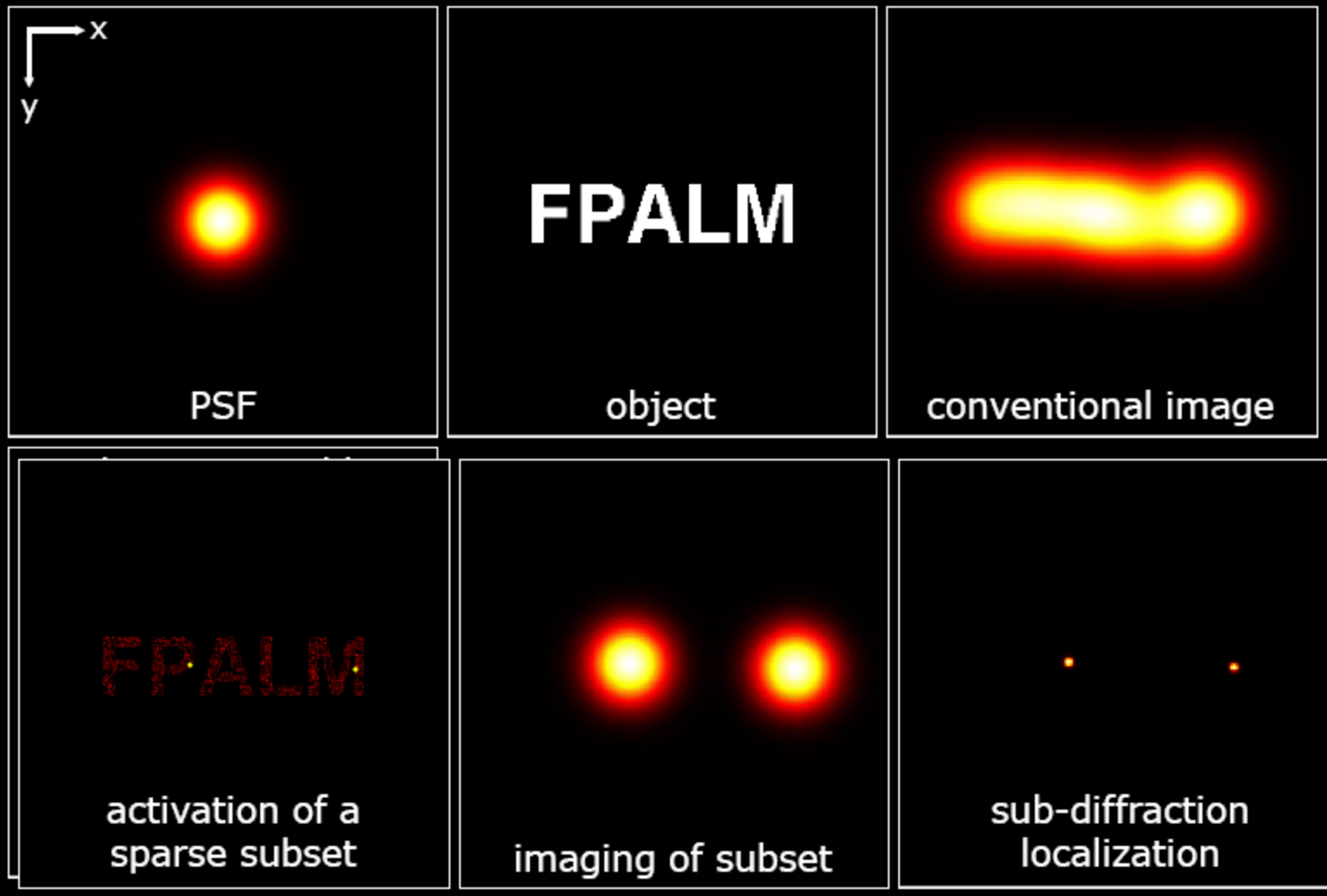
Photoswitching of red cyanine dyes



Bates eta l., *PRL* 2005, Bates et al., *Science* 2007, Dempsey et al., *JACS* 2009

Fluorescence Photoactivation Localization Microscopy

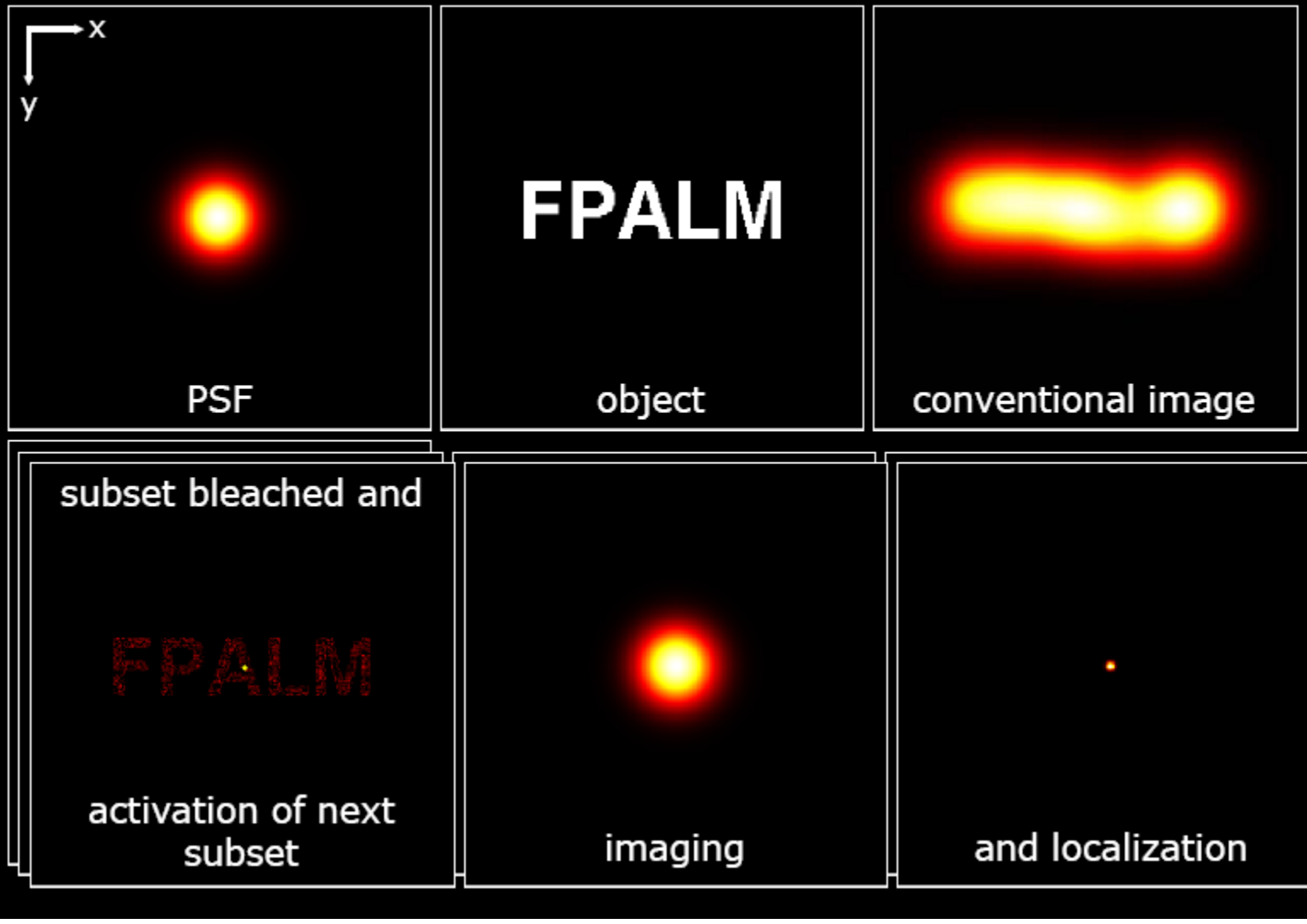
FPALM

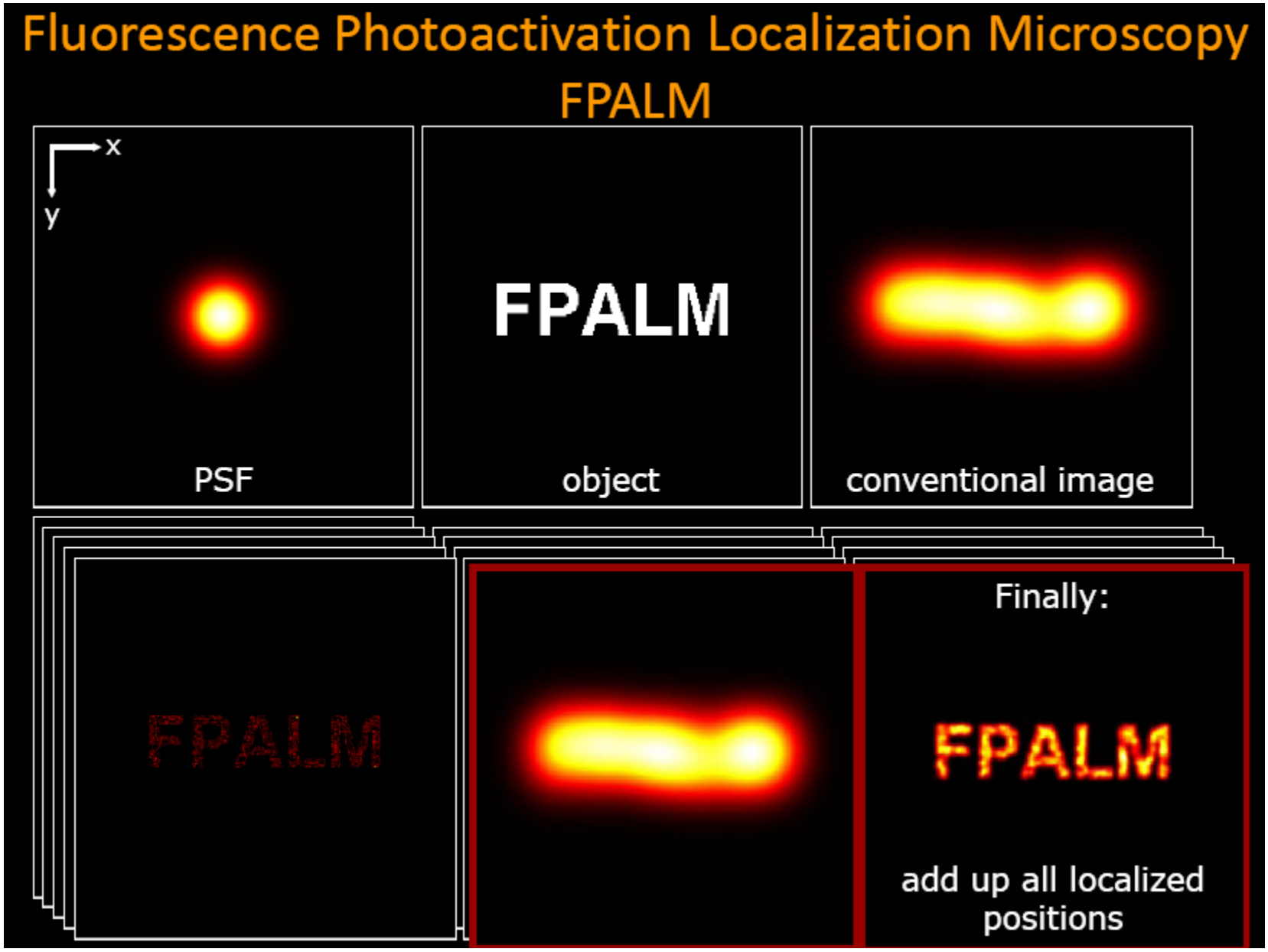


Sam Hess *et al.* Biophys. J. 2006

Fluorescence Photoactivation Localization Microscopy

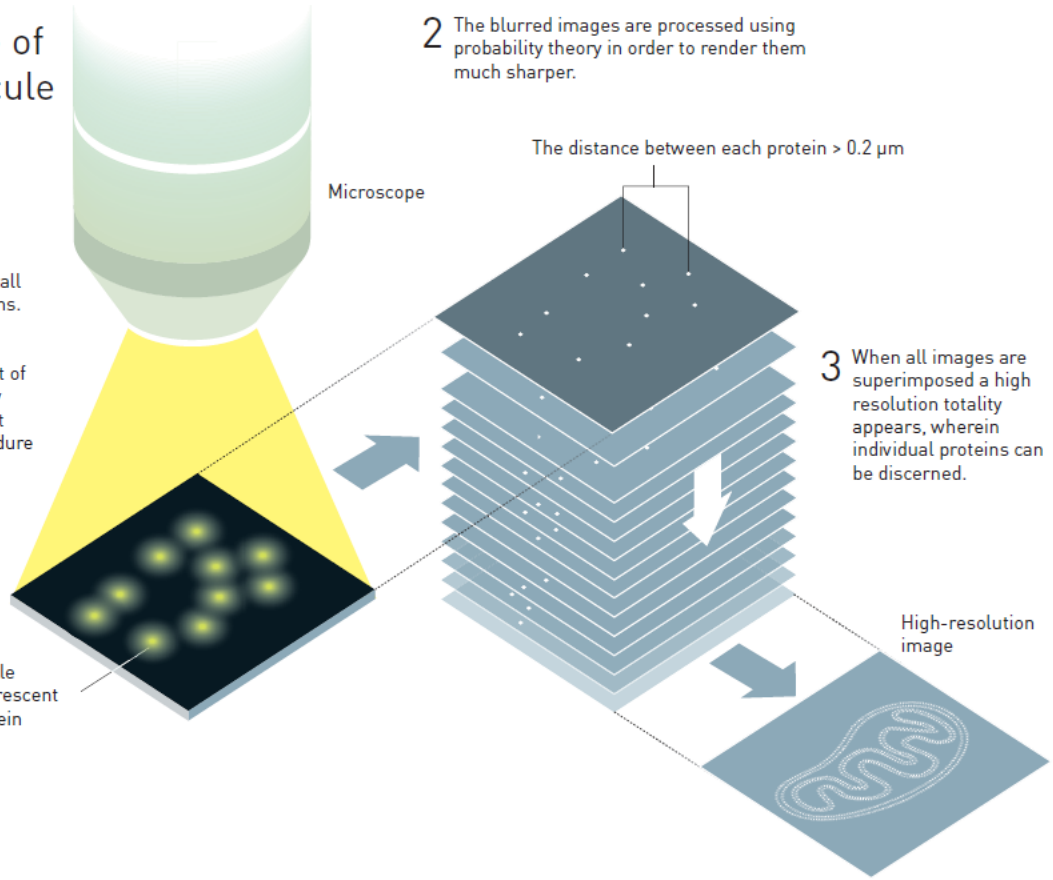
FPALM




Fluorescence Photoactivation Localization Microscopy
FPALM
x
y
PSF
FPALM
object
conventional image
FPALM
Finally:
FPALM
add up all localized positions

The principle of single-molecule microscopy

1 A weak light pulse activates a fraction of all the fluorescent proteins. The distance between them is greater than Abbe's diffraction limit of 0.2 micrometres. They glow until bleached, at which point the procedure is repeated on a new subgroup of proteins.



2 The blurred images are processed using probability theory in order to render them much sharper.

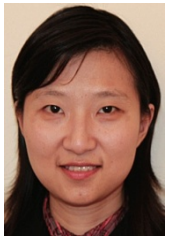
3 When all images are superimposed a high resolution totality appears, wherein individual proteins can be discerned.

英雄榜 II

W. Heisenberg, The Physical Principles of the Quantum Theory (University of Chicago Press, Chicago, 1930).

Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF. Imaging intracellular fluorescent proteins at nanometer resolution. Science. 2006 Sep 15; 313(5793): 1642-5
Received 13 March 2006; accepted 2 August 2006

Rust MJ, Bates M,Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nat Methods. 2006 Oct;3(10):793-5.
Received 7 JULY; accepted 31 JULY



Samuel T. Hess, Thanu P. K. Girirajan,yz and Michael D. Mason, Ultra-High Resolution Imaging by Fluorescence Photoactivation Localization Microscopy. Biophys. J. 2006 Dec; 91: 4258–4272
Submitted June 12, 2006; accepted August 28, 2006

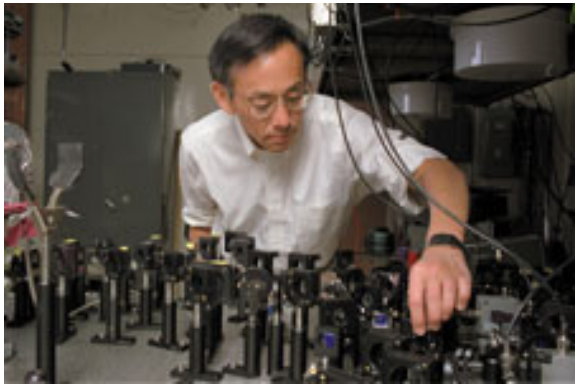
At the time of publication, a related work by E. Betzig et al. had recently appeared online in the journal *Science* (56).

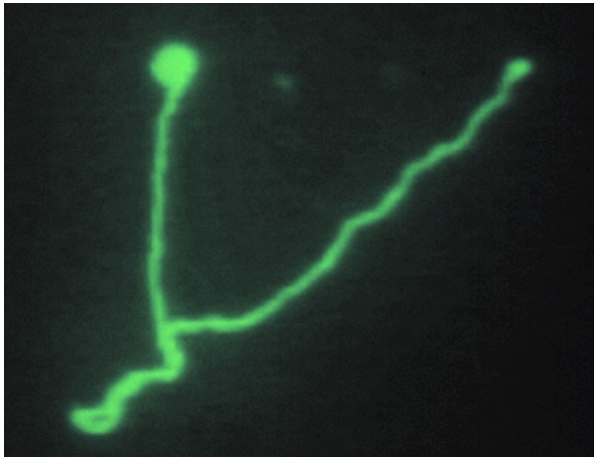
Subnanometre single-molecule localization, registration and distance measurements

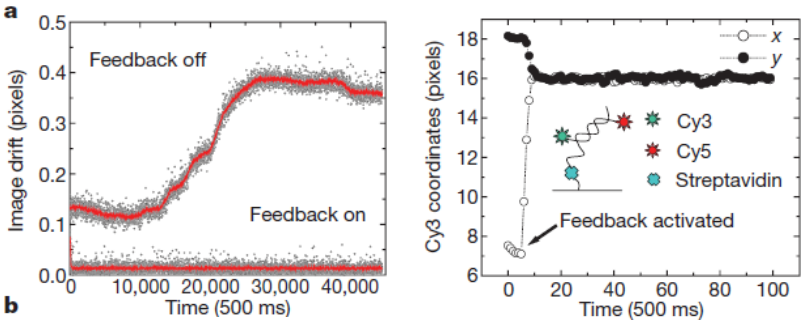
Alexandros Pertsinidis[1,2], Yunxiang Zhang[1,2] & Steven Chu[1,2,3,4]†

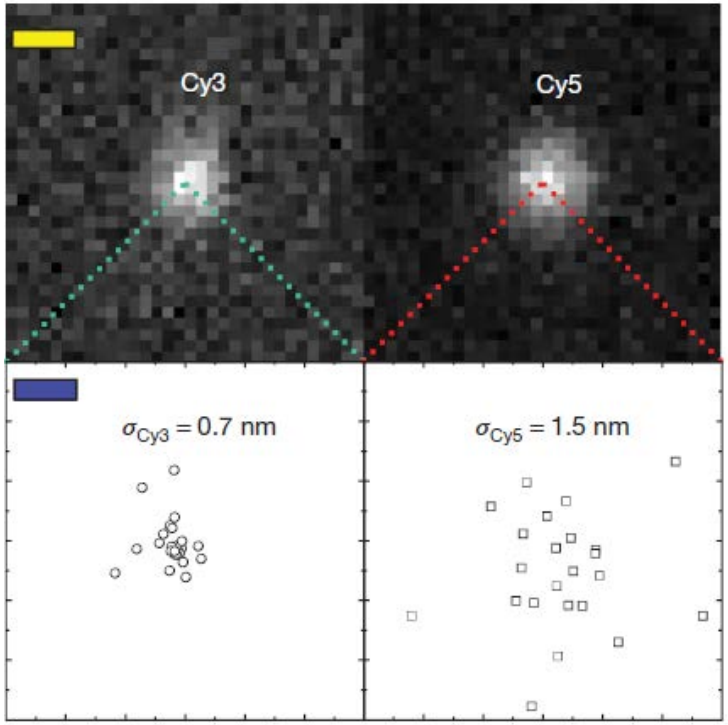
Vol 466|29 July 2010|doi:10.1038/nature09163

world record

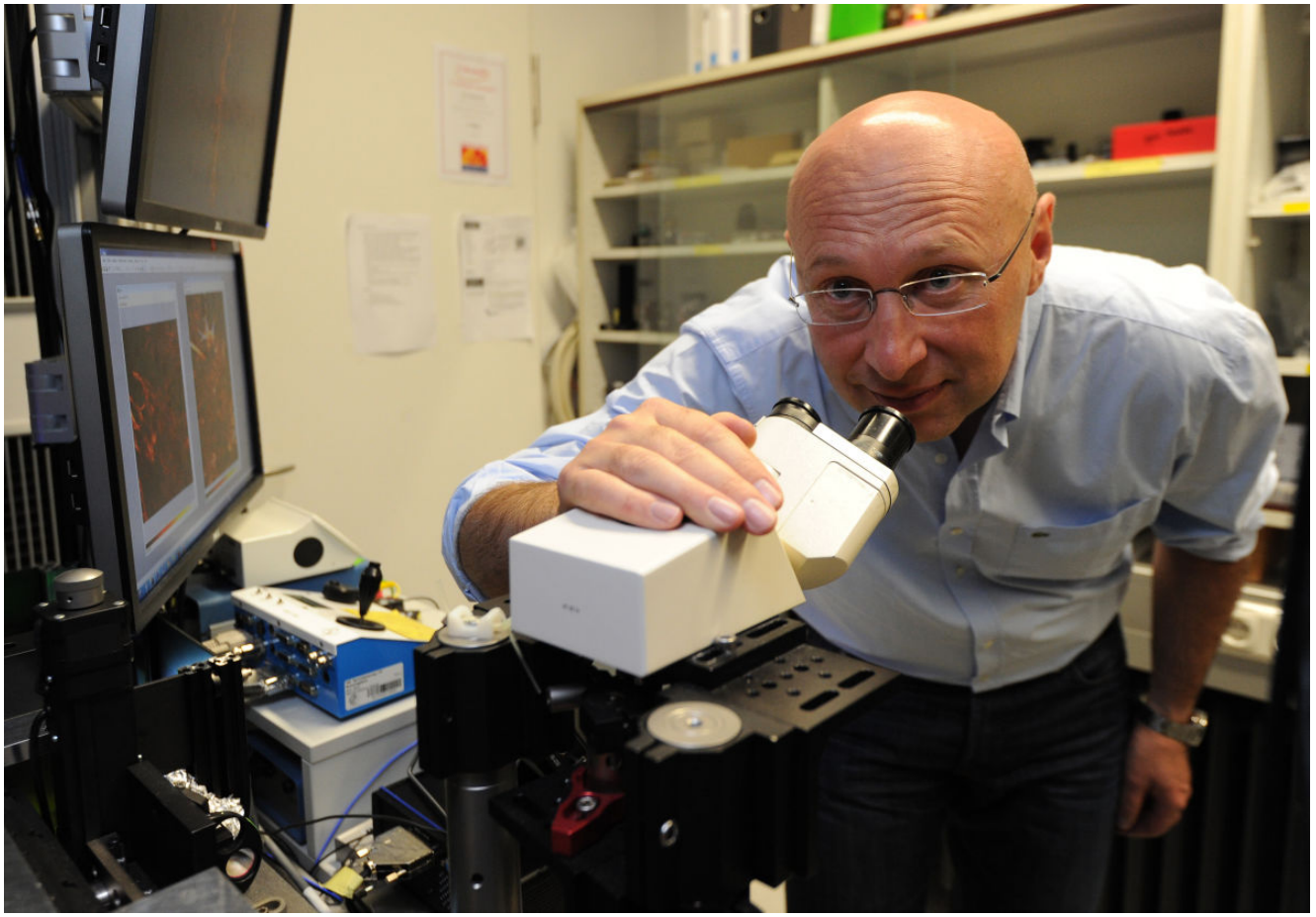






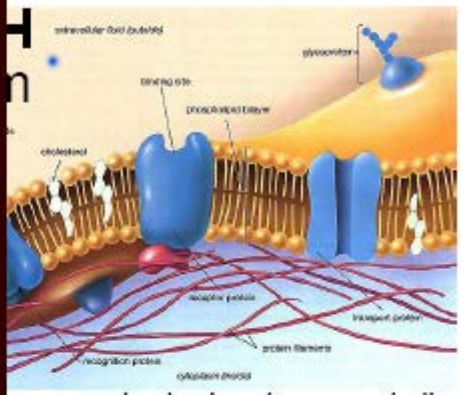


What the HELL is STED?



STEFAN W. HELL

Born 1962 in Arad, Romania. Ph.D. 1990 from the University of Heidelberg.
Director at the Max Planck Institute for Biophysical Chemistry, Göttingen.

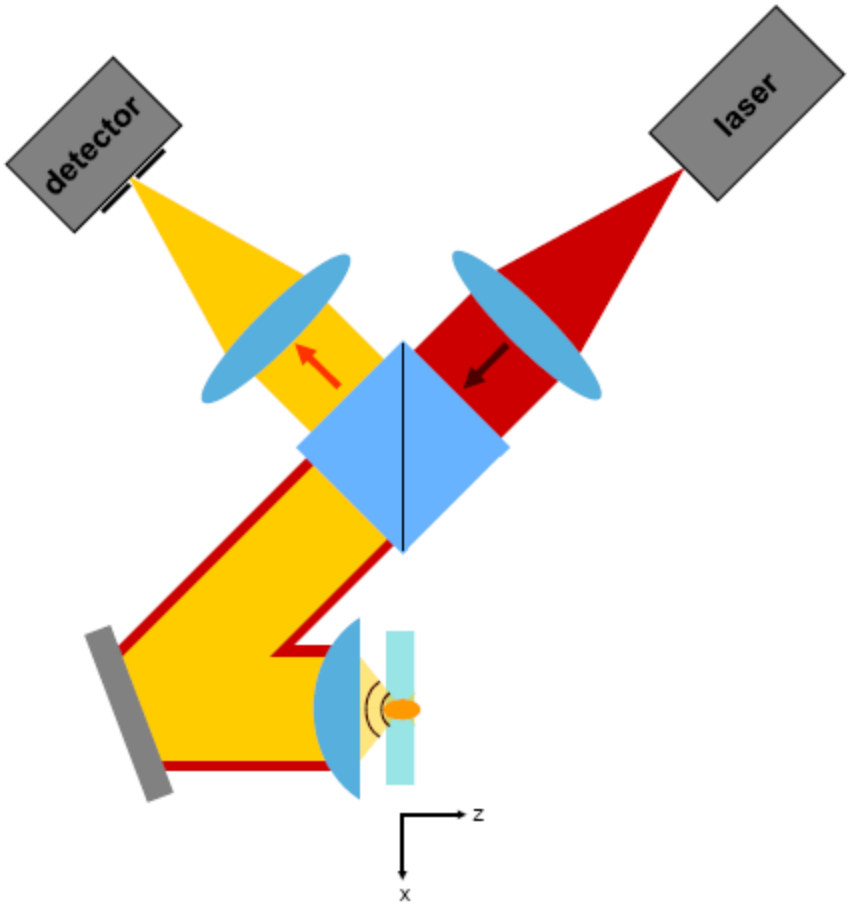


sun.menloschool.org/~cweaver/cells

$$\delta z_{\min} \approx \frac{\lambda}{2n(\sin\alpha)^2}$$

Point Spread Function

Confocal Microscopy



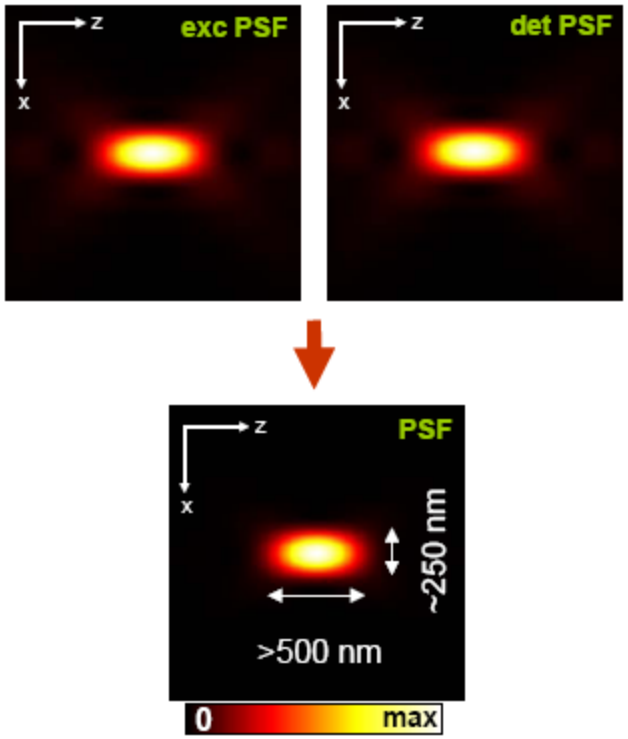
diffraction limit:

$$d_{\min} \approx 0.61 \frac{\lambda}{n \sin \alpha}$$

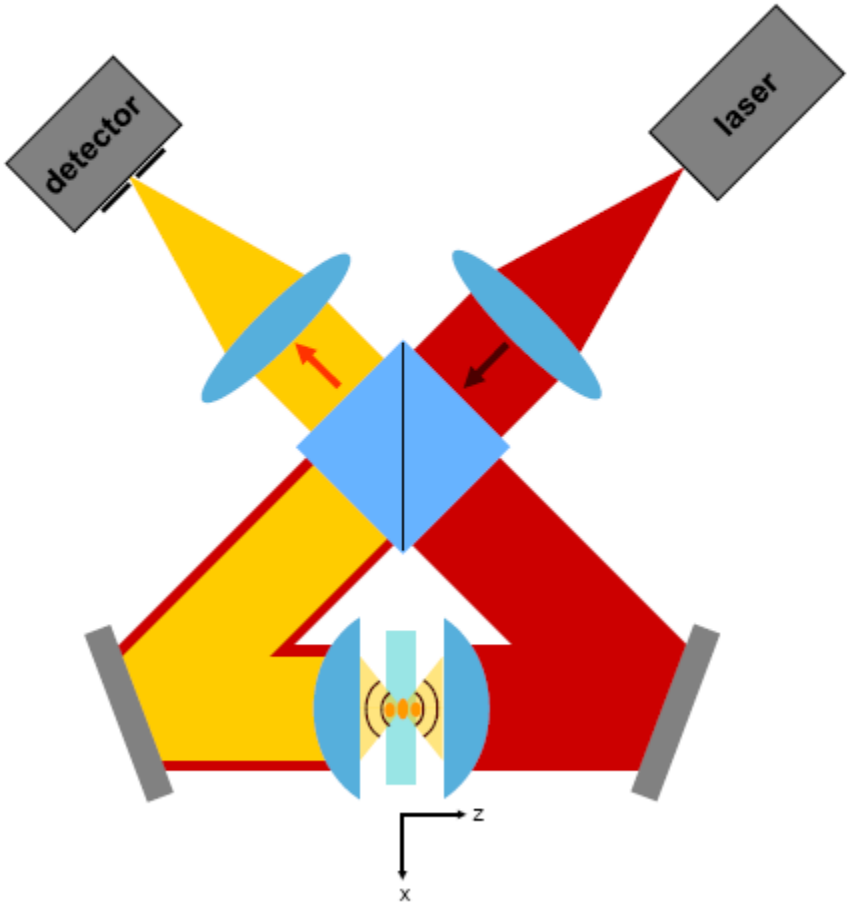
wavelength

numerical aperture

aperture angle

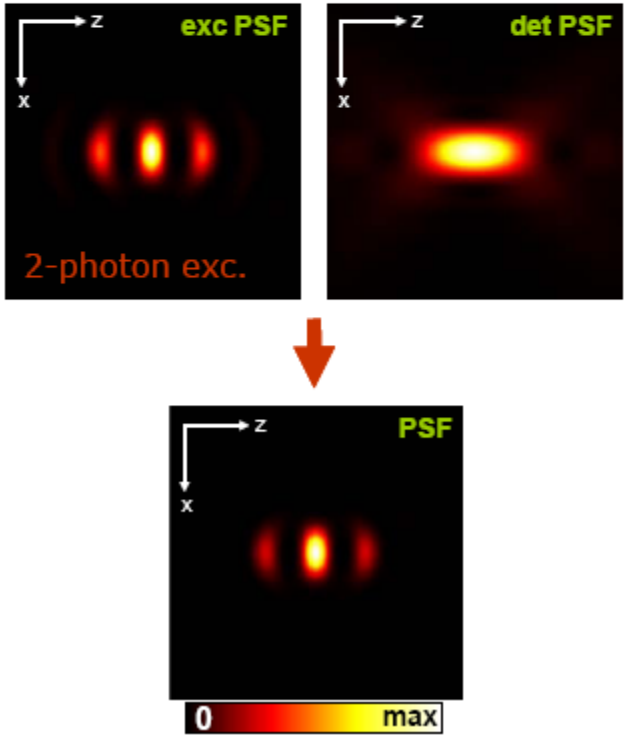


4Pi Microscopy



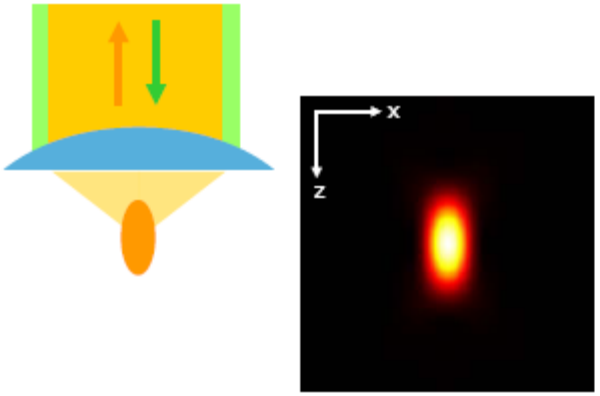
Stefan Hell 1990, 1992

Coherent excitation with two opposing objectives

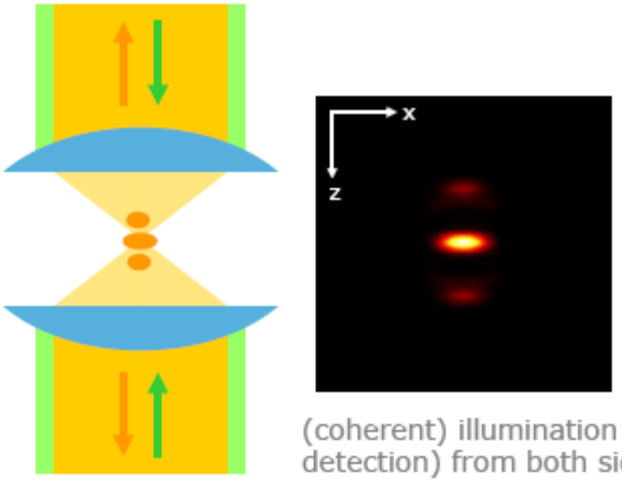


4Pi Microscopy Enhances the Depth Resolution

(confocal) Laser Scanning Microscope

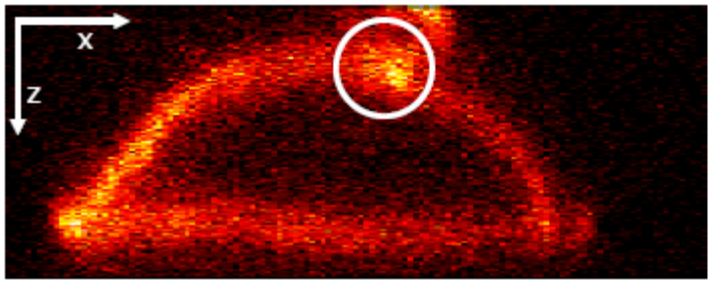


4Pi Microscope

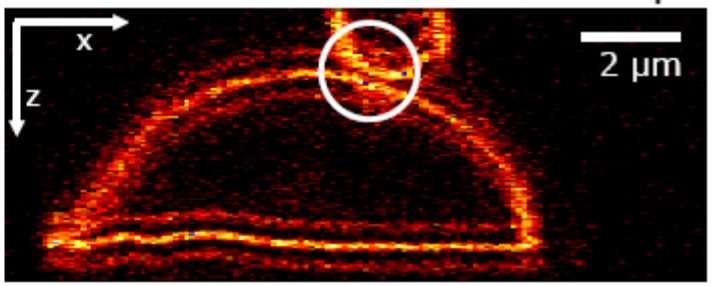


(coherent) illumination (and detection) from both sides

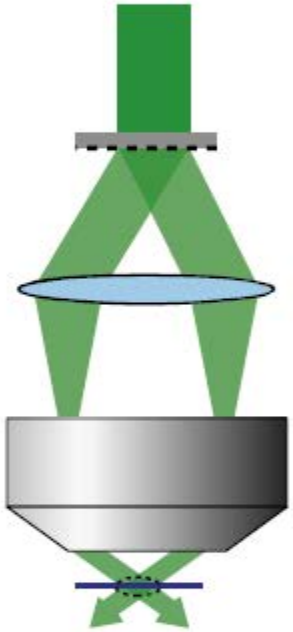
Depth resolution ca. 600 nm
Lateral resolution ca. 250 nm

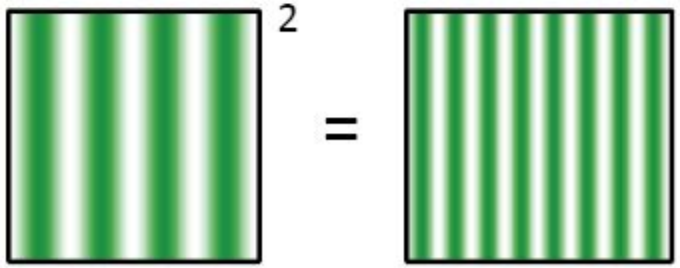


Depth resolution ca. 100 nm - 6x better
Lateral resolution ca. 250 nm - equal

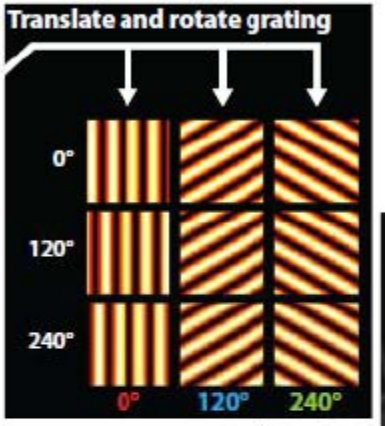


Structured Illumination Microscopy (SIM)

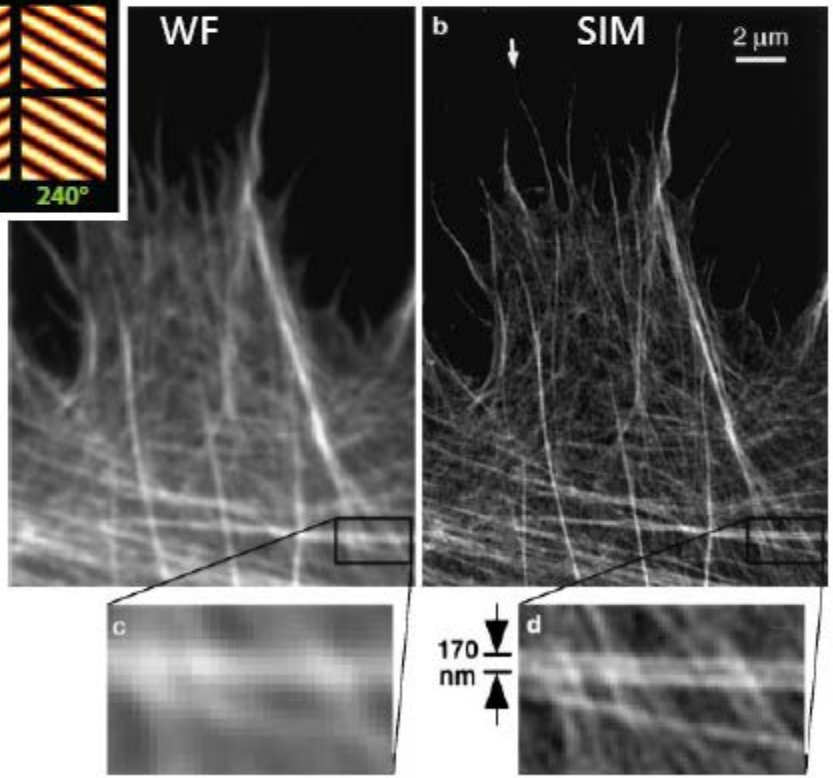




9 images



Reconstruction



Gustafsson, J Microscopy 2000

A brief history of STED

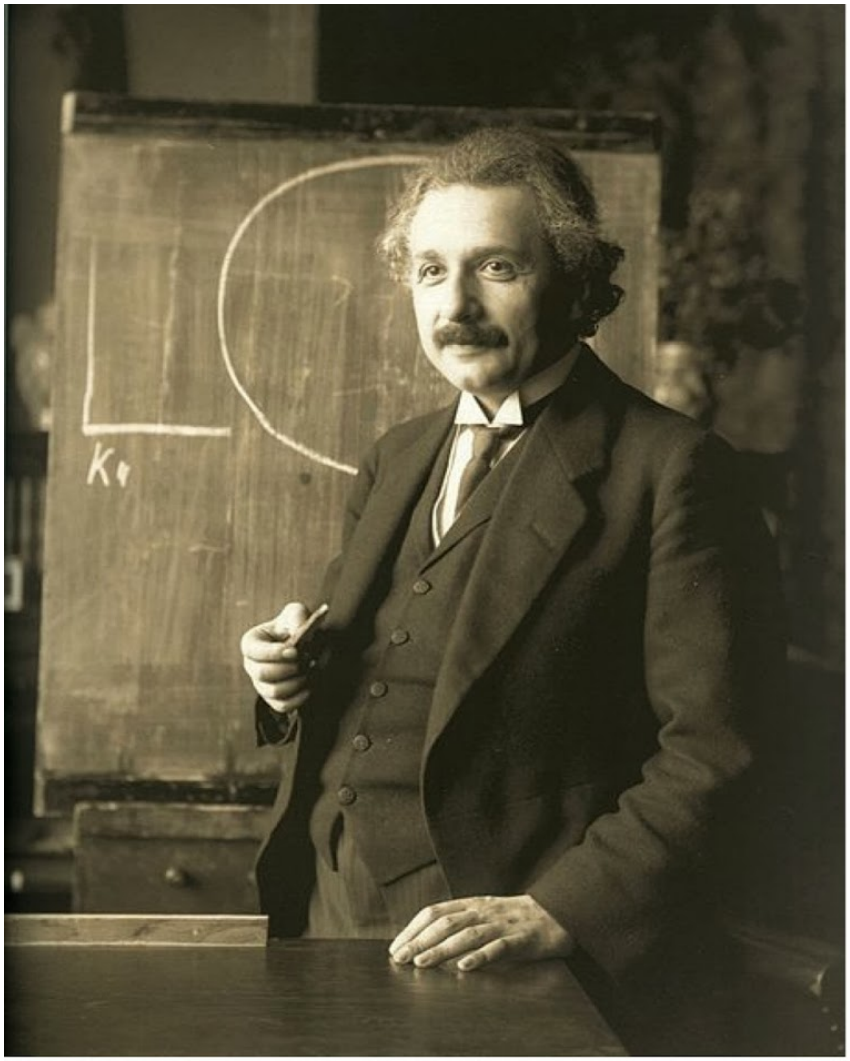
Stimulated Emission Depletion (STED)

Hell SW and Wichman J (1994) Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion-microscopy. *Opt. Lett.* 19:780-782.

Hell SW and Kroug M (1995) Ground-state depletion fluorescence microscopy, a concept for breaking the diffraction resolution limit. *Appl. Phys. B.* 60:495-497.

Klar TA, Jakobs S, Dyba M, Egner A and Hell SW (2000) Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission. *Proc. Natl. Acad. Sci. USA.* 97: 8206-8210.

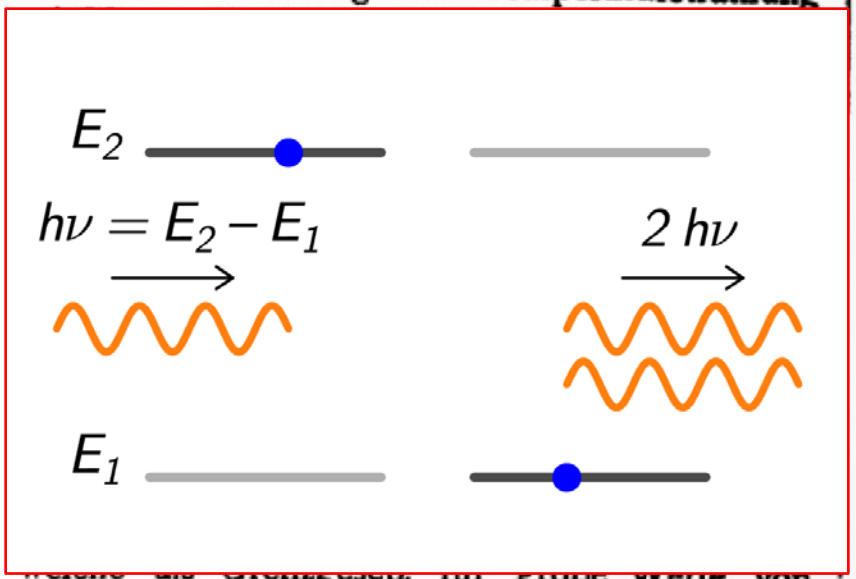
Stimulated Emission



Zur Quantentheorie der Strahlung.

Von A. Einstein[1])

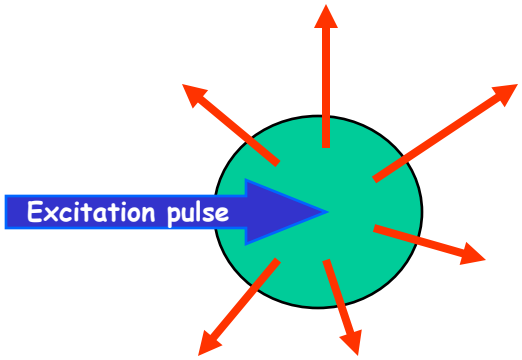
Die formale Ähnlichkeit der Kurve der chromatischen Verteilung der Temperaturstrahlung

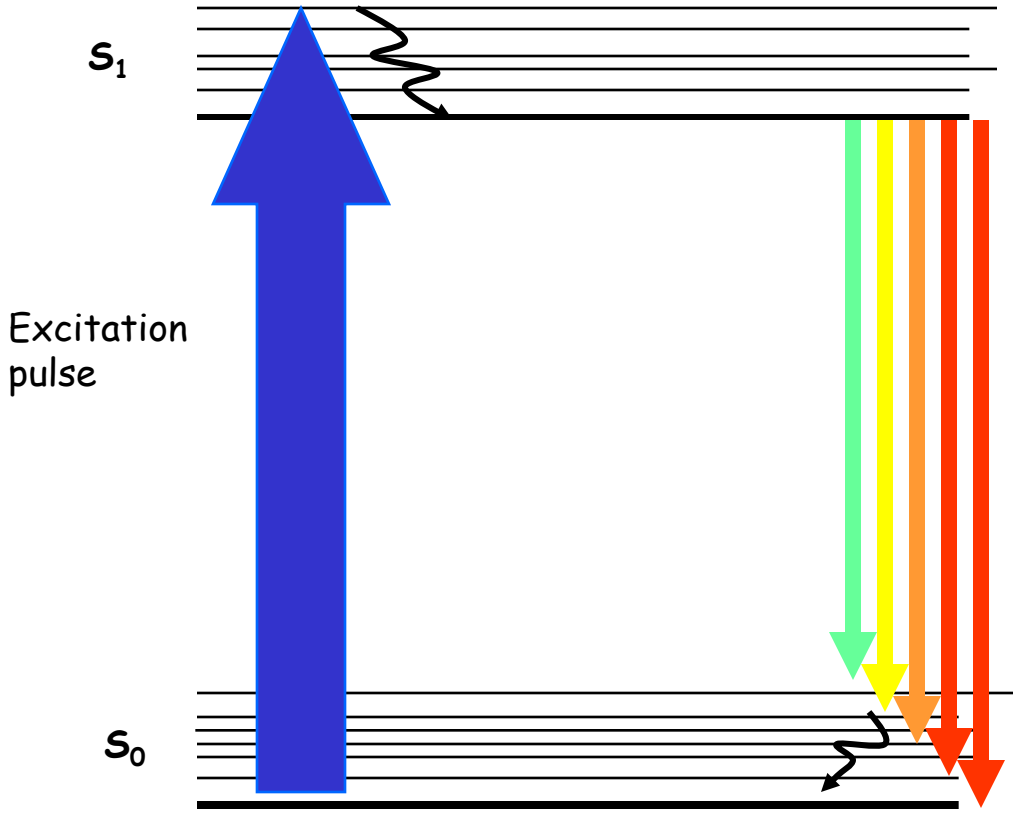


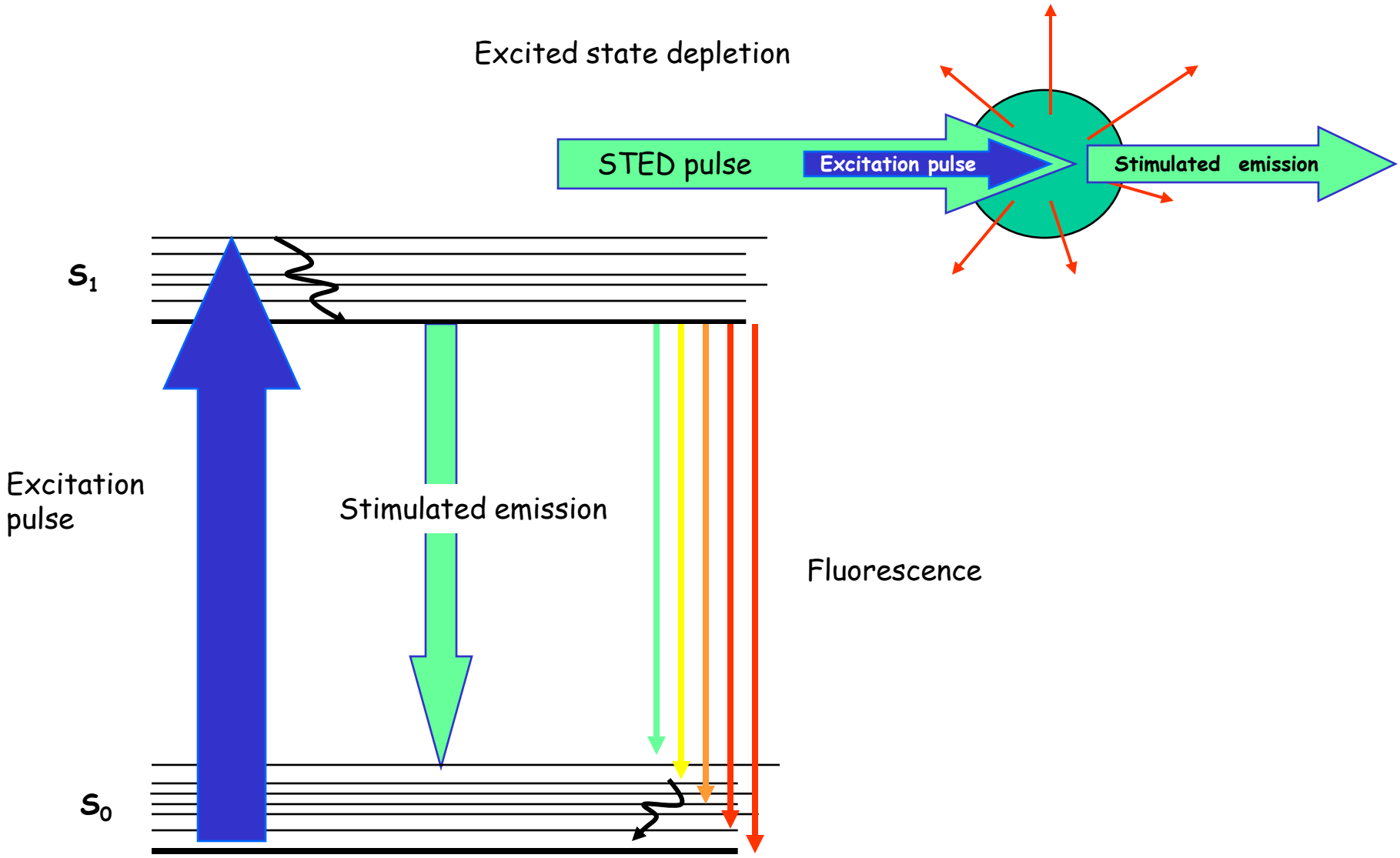
$\frac{\nu}{T}$ auch heute als richtig anerkannt wird (Wien-

1) Zuerst abgedruckt in den Mitteilungen der Physikalischen Gesellschaft Zürich. Nr. 18, 1916.

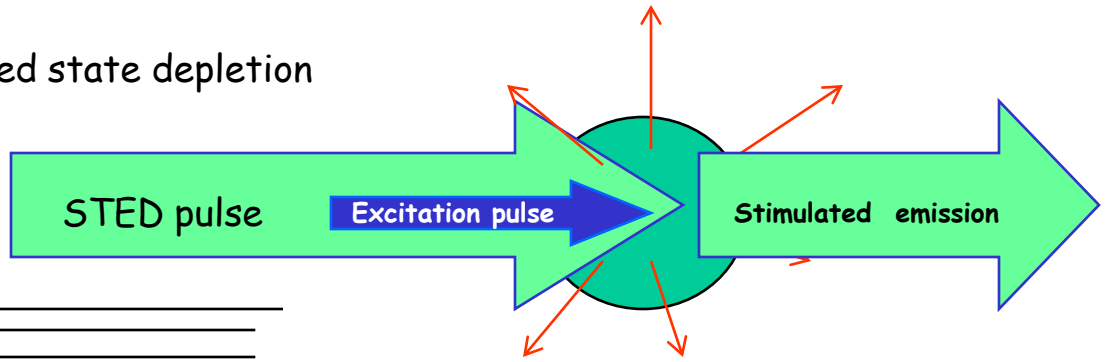
Excited state depletion

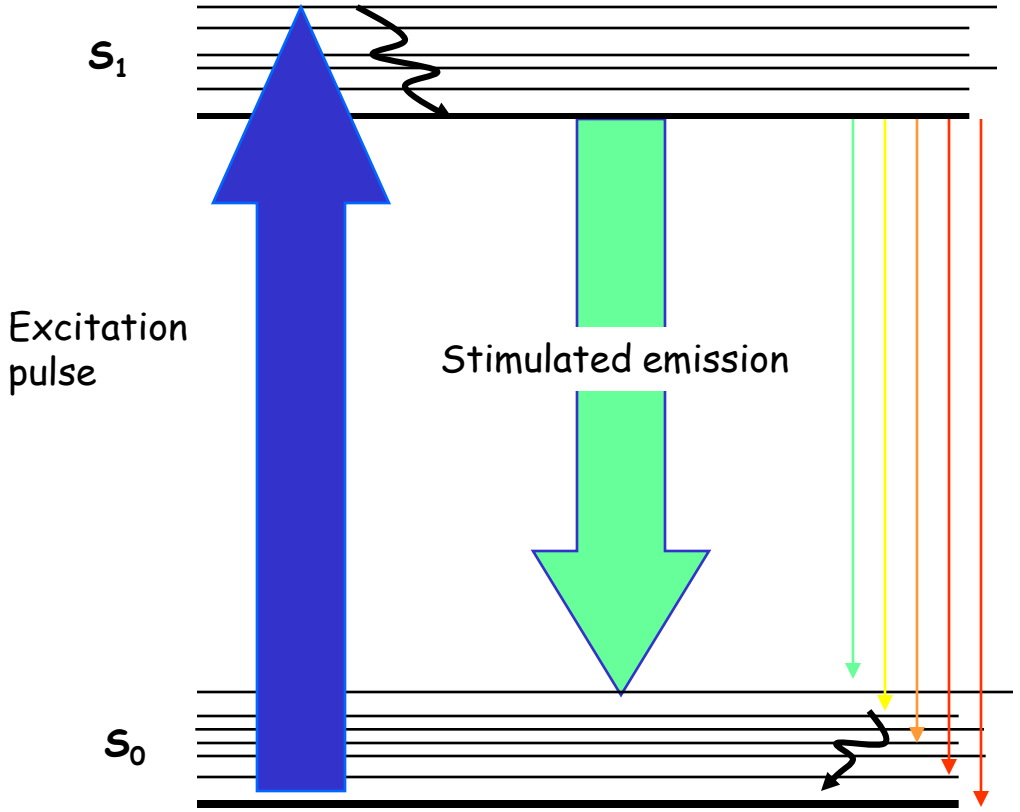





Excited state depletion
STED pulse
Excitation pulse
Stimulated emission
S_1
Excitation pulse
Stimulated emission
Fluorescence
S_0

Excited state depletion





Photons in STED pulse has lower energy to avoid excitation.

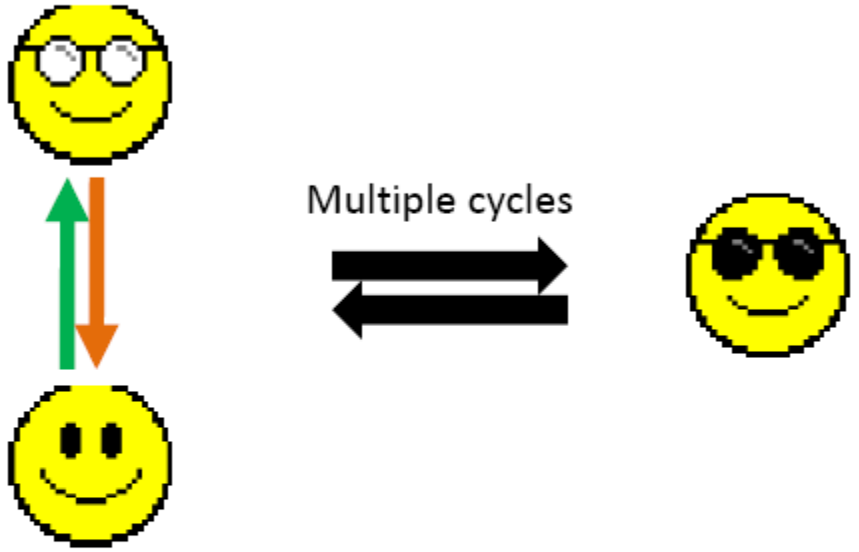
Pulse duration should much shorter then S_1 lifetime = $1/K_{fluores}$

$K_{internal\ relaxation} > K_{SM} >> K_{fluorescence}$

Fluorescence is completely suppressed by stimulated emission process.

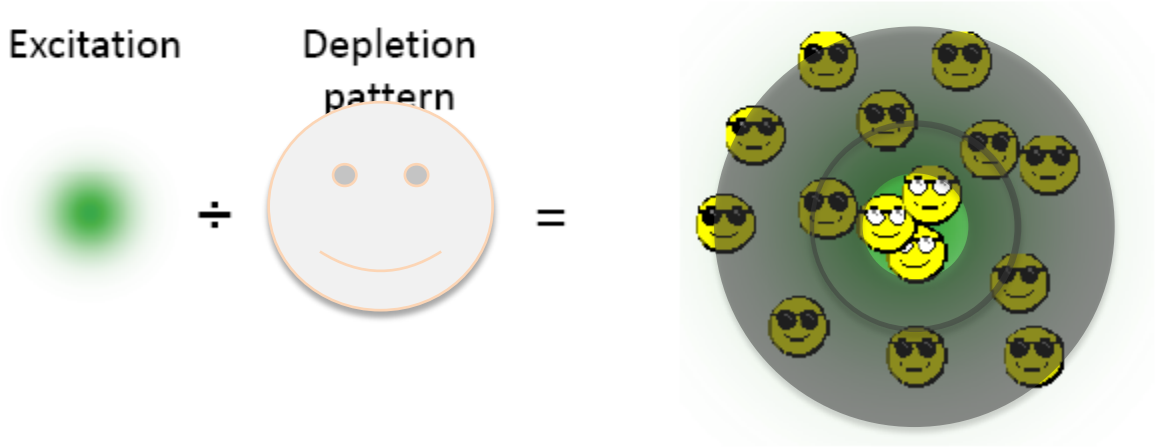
Suturation condition for STED pulse: $K_{SM}=K_{fluorescence}$; $I_{saturation}\sigma_{absorption} \sim 1\ ns^{-1}$

Stimulated Emission Depletion (STED)



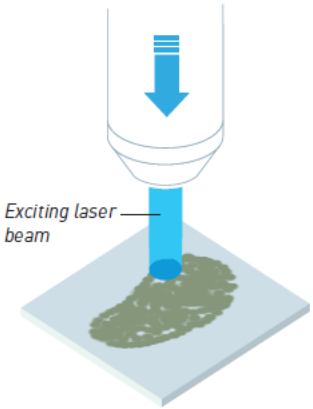
- Ground state
- Triplet state
- Isomerization

etc.



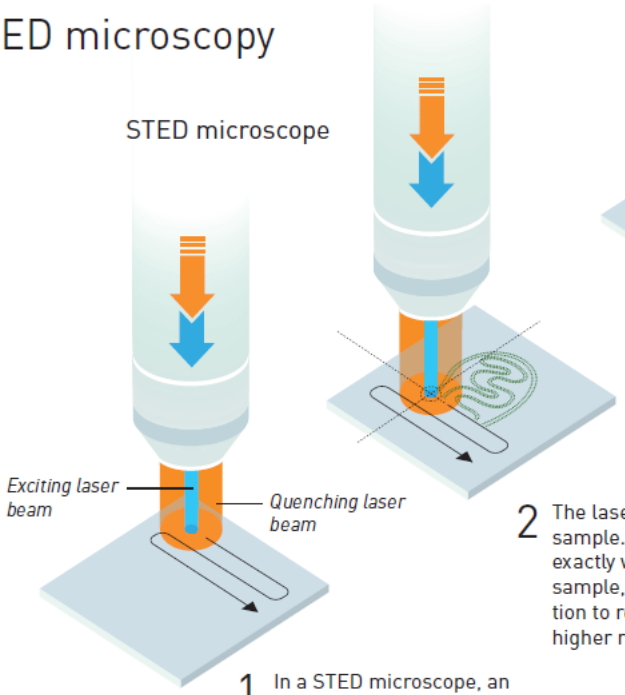
The principle of STED microscopy

Regular optical microscope



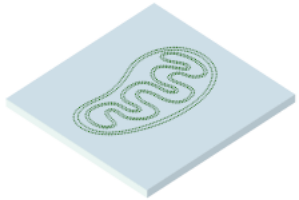
In a regular optical microscope, the contours of a mitochondrion can be distinguished, but the resolution can never get better than 0.2 micrometres.

STED microscope



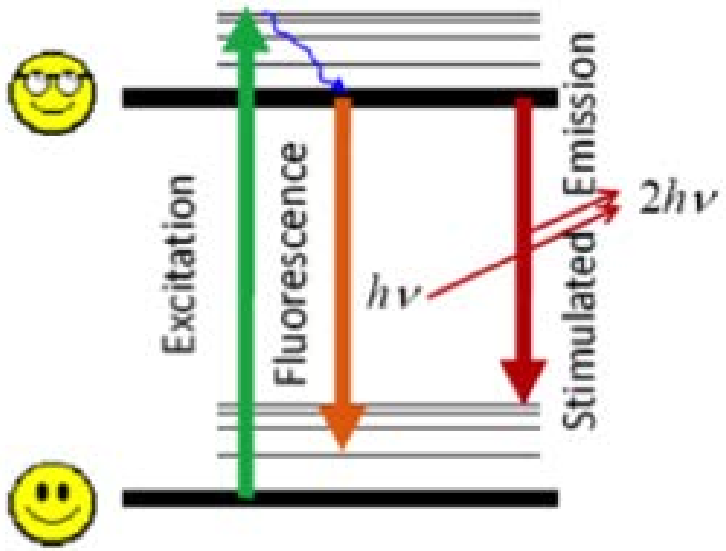
1 In a STED microscope, an annular laser beam quenches all fluorescence except that in a nanometre-sized volume.

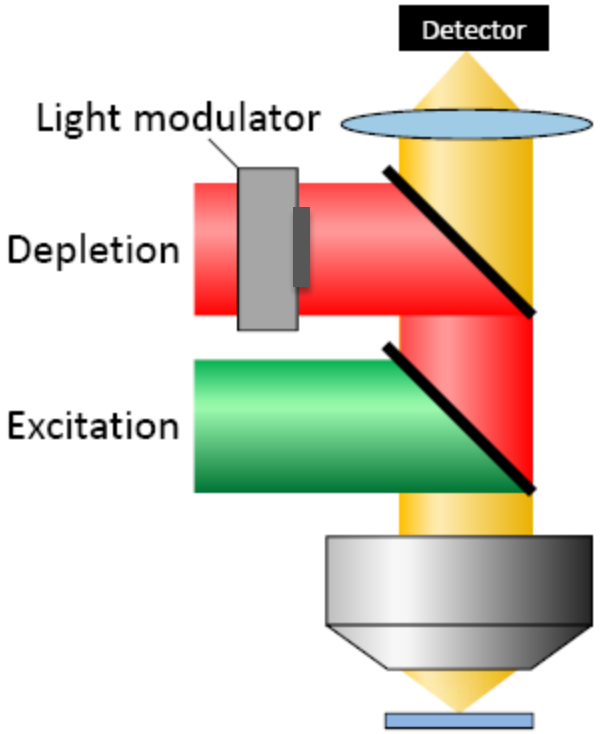
2 The laser beams scan over the sample. Since scientists know exactly where the beam hits the sample, they can use that information to render the image at a much higher resolution.

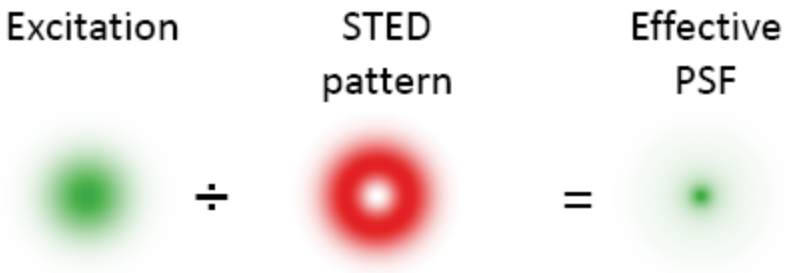


3 The final image gets a resolution that is much better than 0.2 micrometre.

STED microscopy

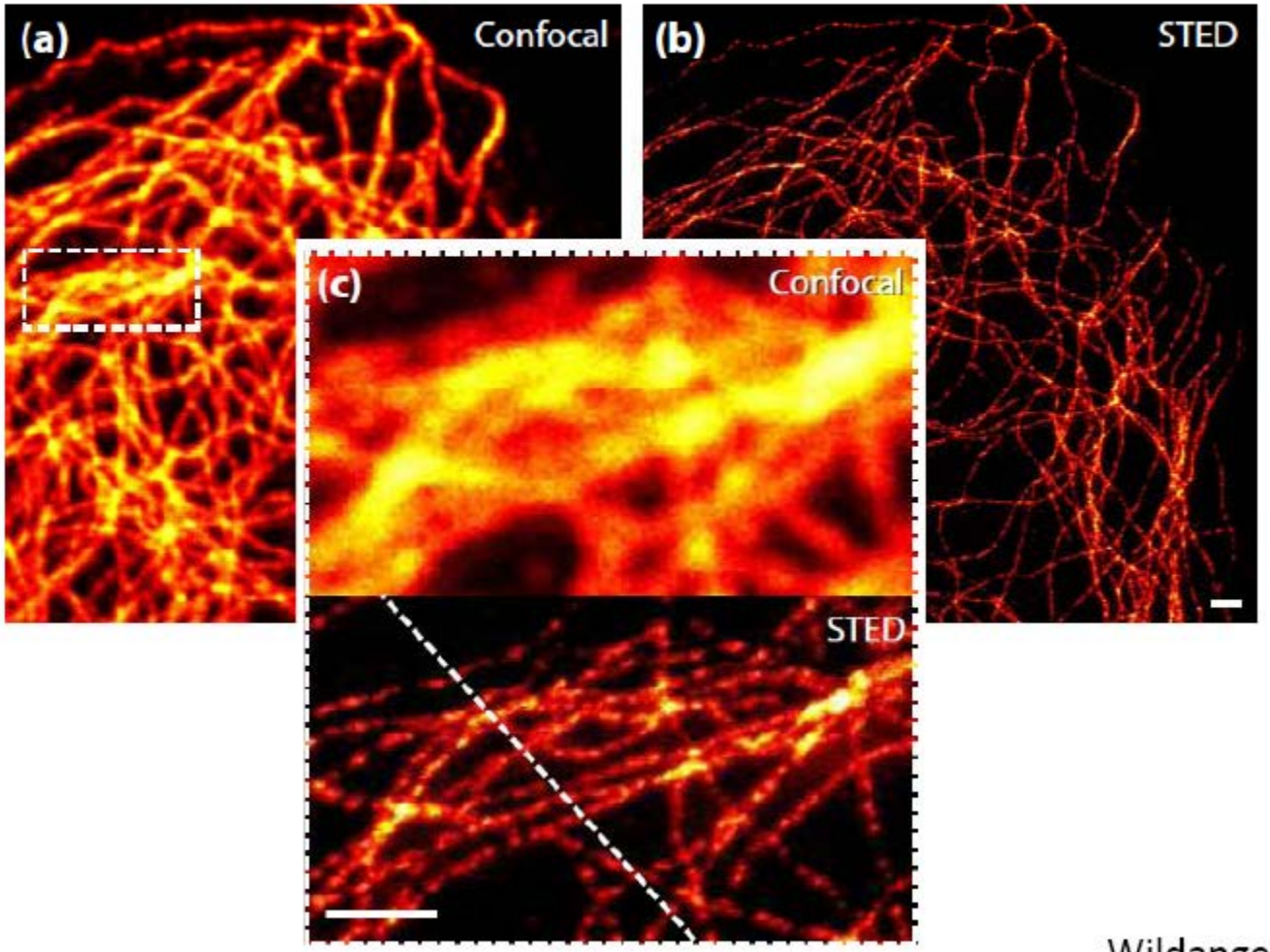






Hell 1994, Hell 2000

STED images of microtubules

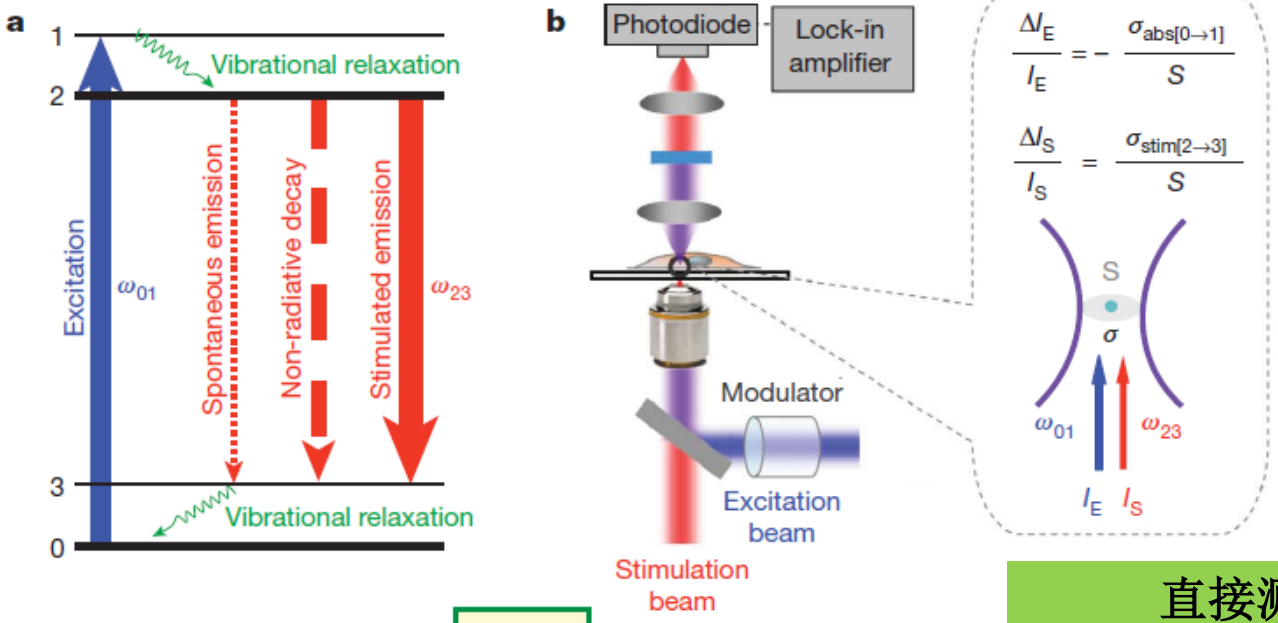


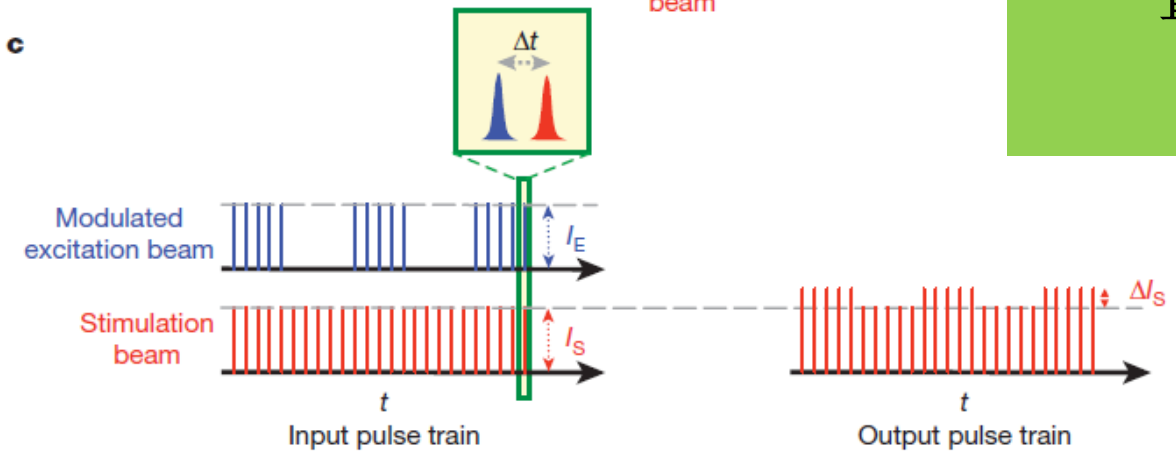
Wildanger et al., 2009

Imaging chromophores with undetectable fluorescence by stimulated emission microscopy

Wei Min[1]*, Sijia Lu[1]*, Shasha Chong[1], Rahul Roy[1], Gary R. Holtom[1] & X. Sunney Xie[1]

Vol 461|22 October 2009|doi:10.1038/nature08438



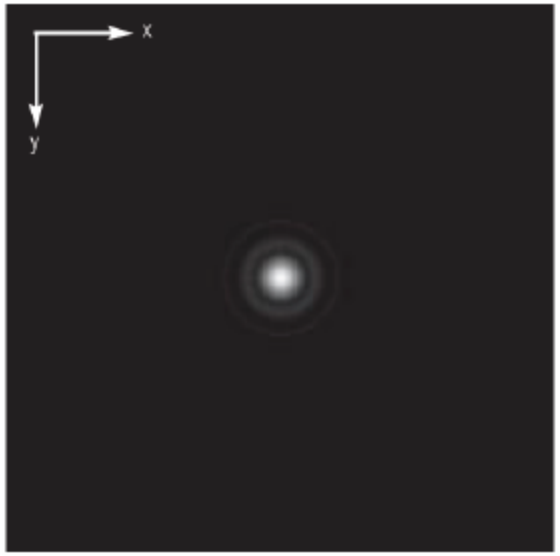


直接测量单个分子的受激辐射
10^-7的强度调制幅度测量
免标记；受激拉曼散射等

Shot-noise limited.
Any means to break it?
Squeezed light?

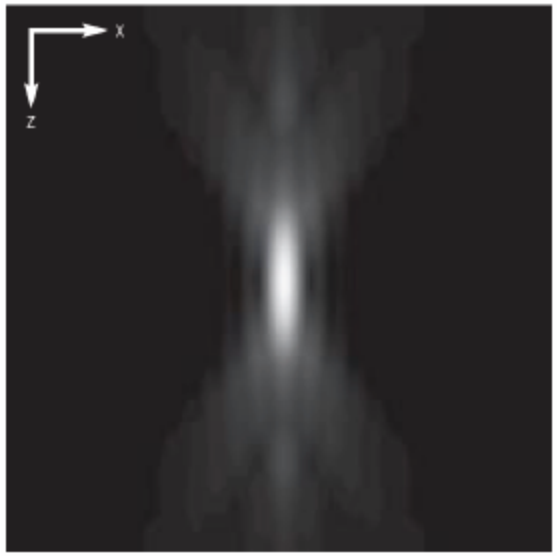
Let’s go to 3D!

Three-dimensional point-spread function



Lateral:

$$FWHM_{ill,lateral} = 0.51 \frac{\lambda_{exc}}{NA}$$

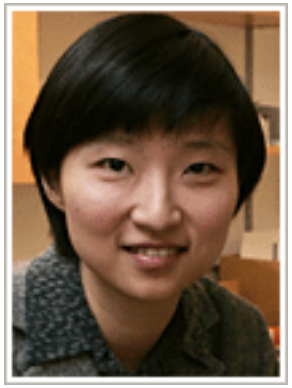


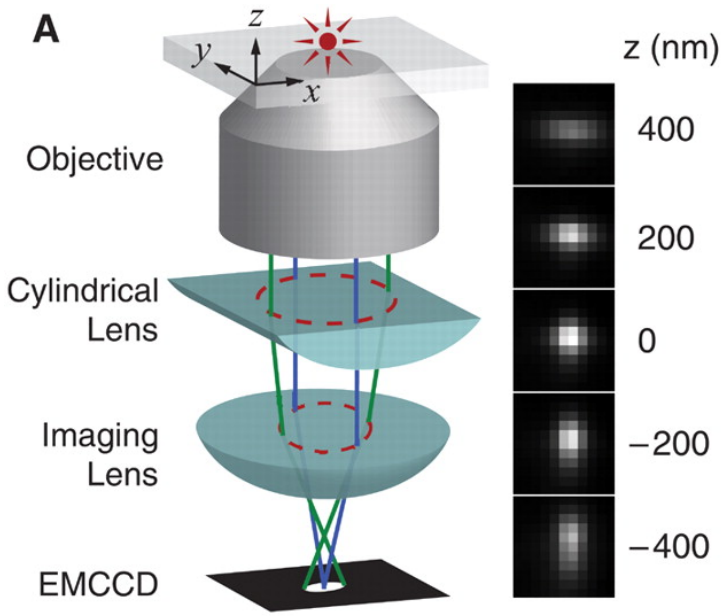
Axial:

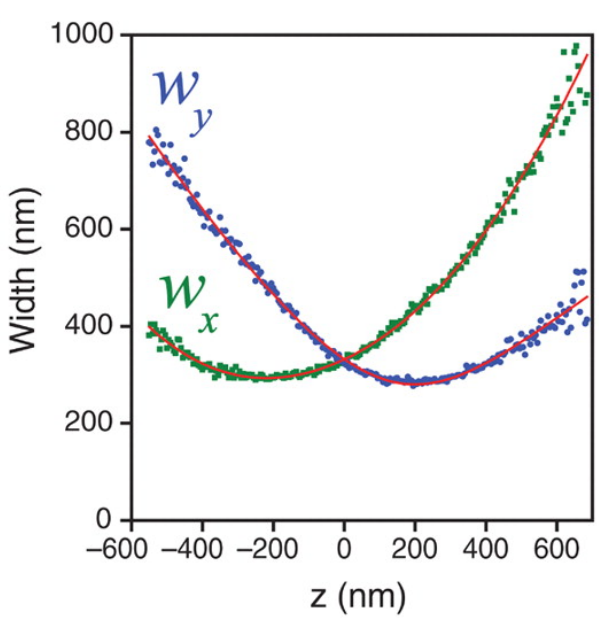
$$FWHM_{ill,axial} = \frac{0.88 \cdot \lambda_{exc}}{(n-\sqrt{n^2-NA^2})}$$

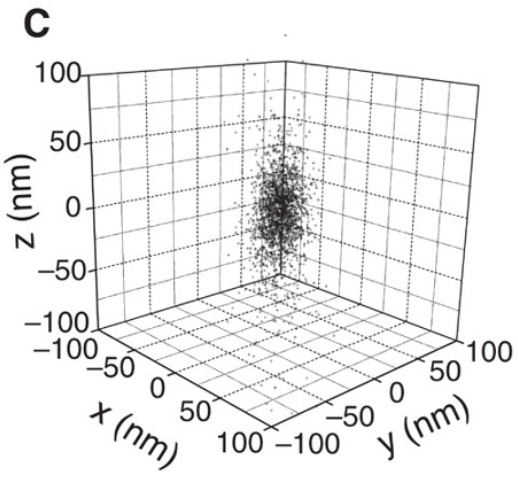
n = *refractive index of immersion liquid,*
NA = *numerical aperture of the microscope objective,*
λ_{exc} = *wavelength of the excitation light*

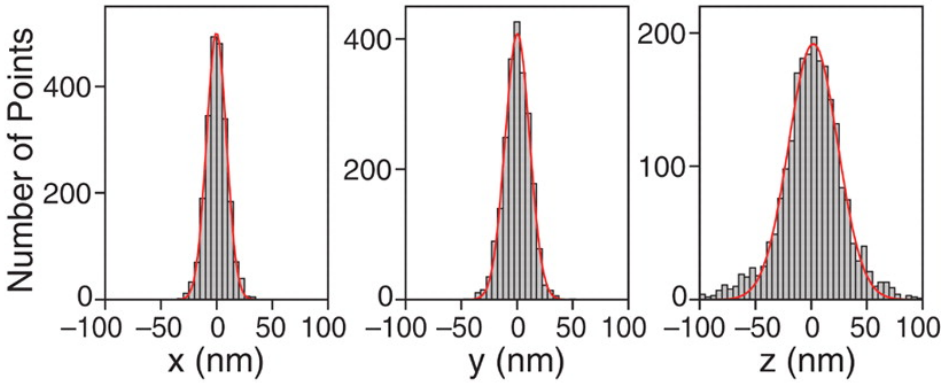
Ref: Carl Zeiss, *Confocal Laser Scanning Microscopy*

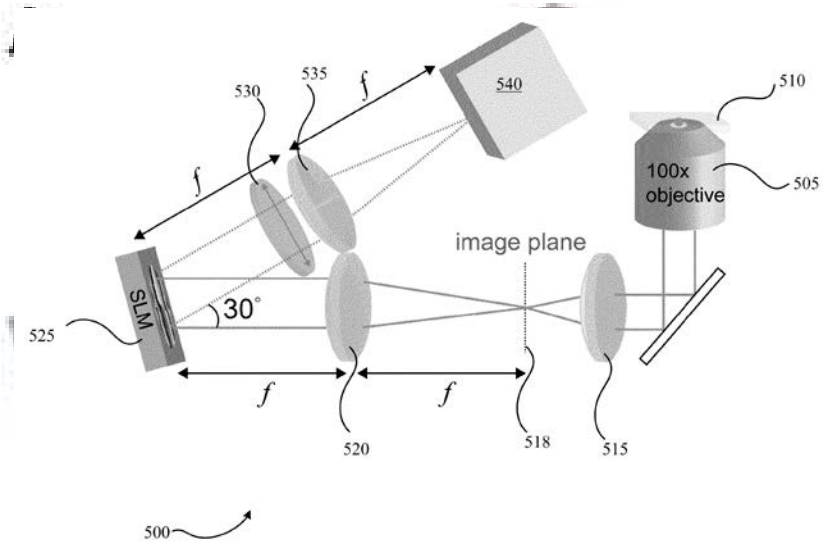


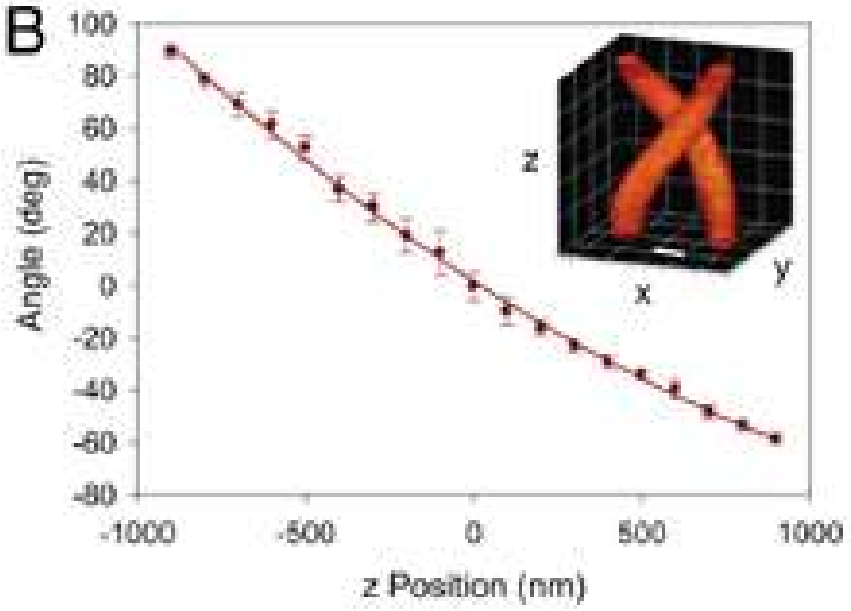

A
z
y
x
Objective
Cylindrical Lens
Imaging Lens
EMCCD
z (nm)
400
200
0
−200
−400

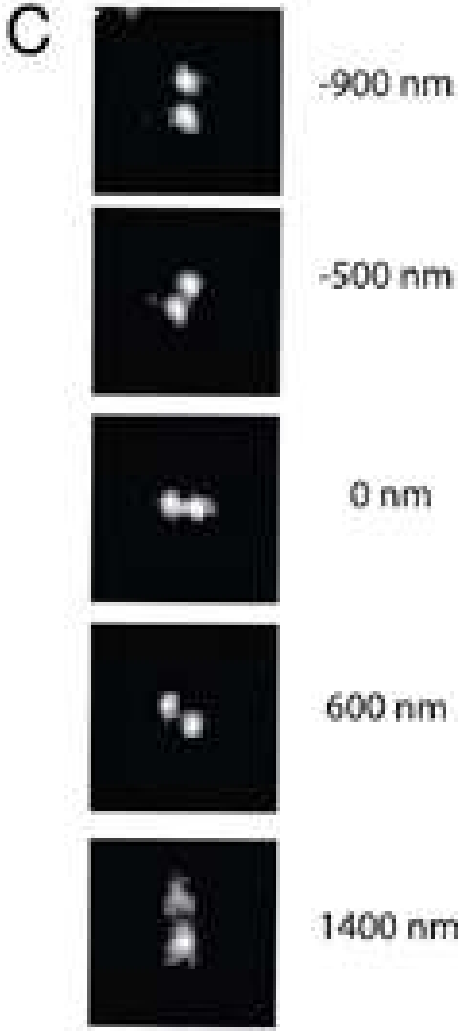

B
Width (nm)
1000
800
600
400
200
0
w_y
w_x
−600 −400 −200 0 200 400 600
z (nm)

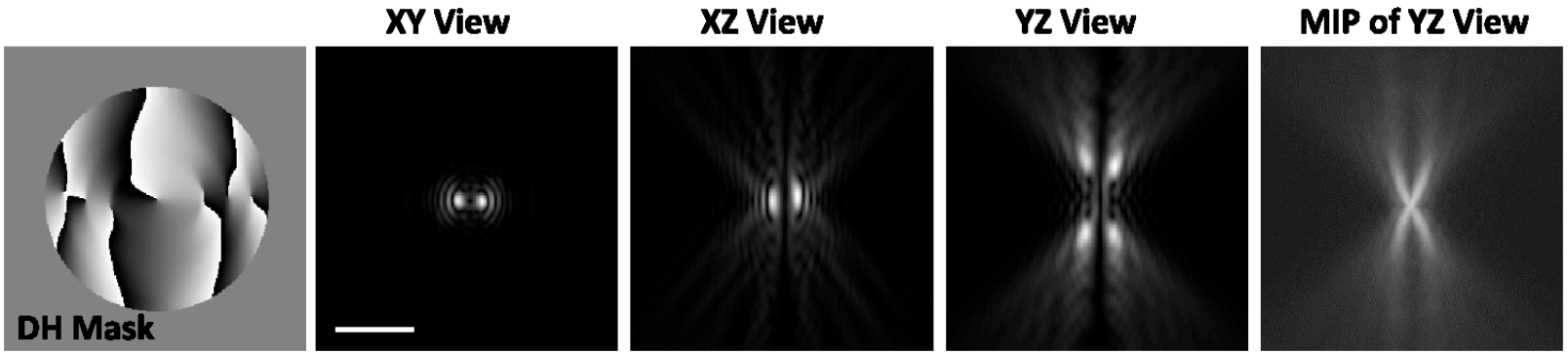

C
z (nm)
100
50
0
−50
−100
x (nm)
y (nm)


Number of Points
x (nm)
y (nm)
z (nm)

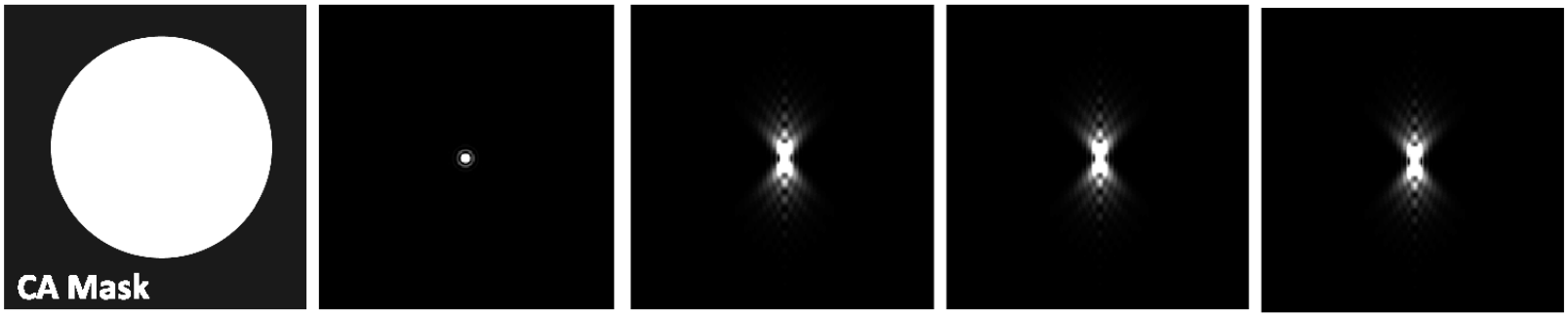

530
535
f
540
510
f
100x
objective
505
image plane
SLM
30°
525
f
f
520
518
515
500


B
Angle (deg)
100
80
60
40
20
0
-20
-40
-60
-80
-1000
-500
0
500
1000
z Position (nm)
z
x
y

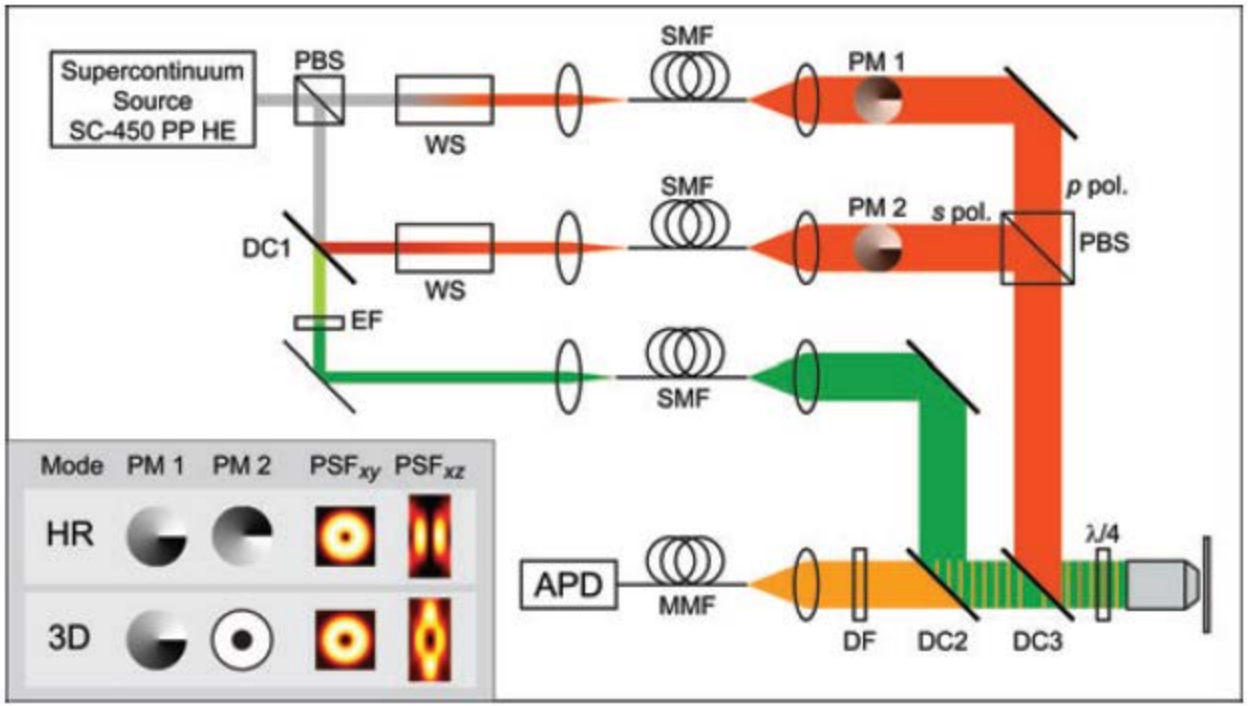

C
-900 nm
-500 nm
0 nm
600 nm
1400 nm

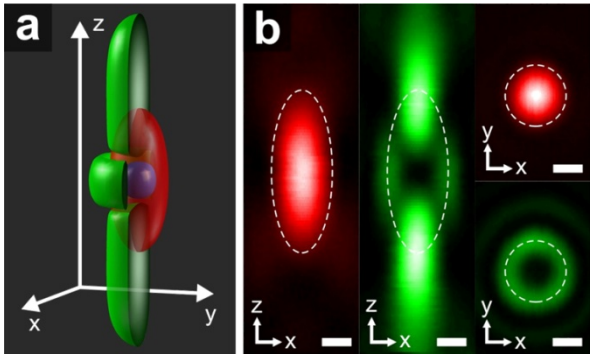


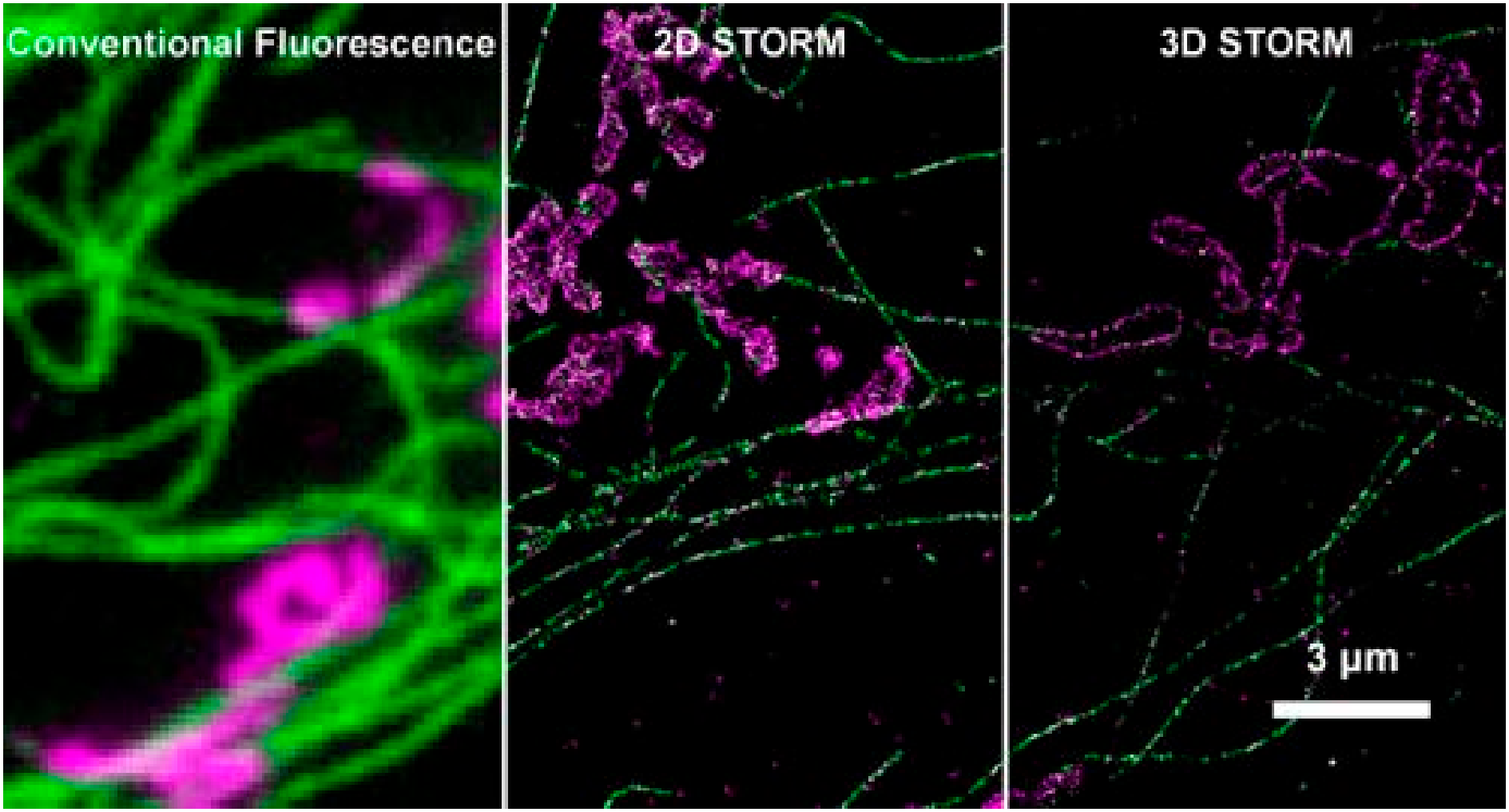
(a) Double-Helix PSF



(b) Conventional PSF


Supercontinuum Source SC-450 PP HE
PBS
WS
SMF
PM 1
p pol.
PM 2
s pol.
PBS
DC1
WS
SMF
EF
SMF
Mode
PM 1
PM 2
PSF$_{xy}$
PSF$_{xz}$
HR
3D
APD
MMF
DF
DC2
DC3
λ/4

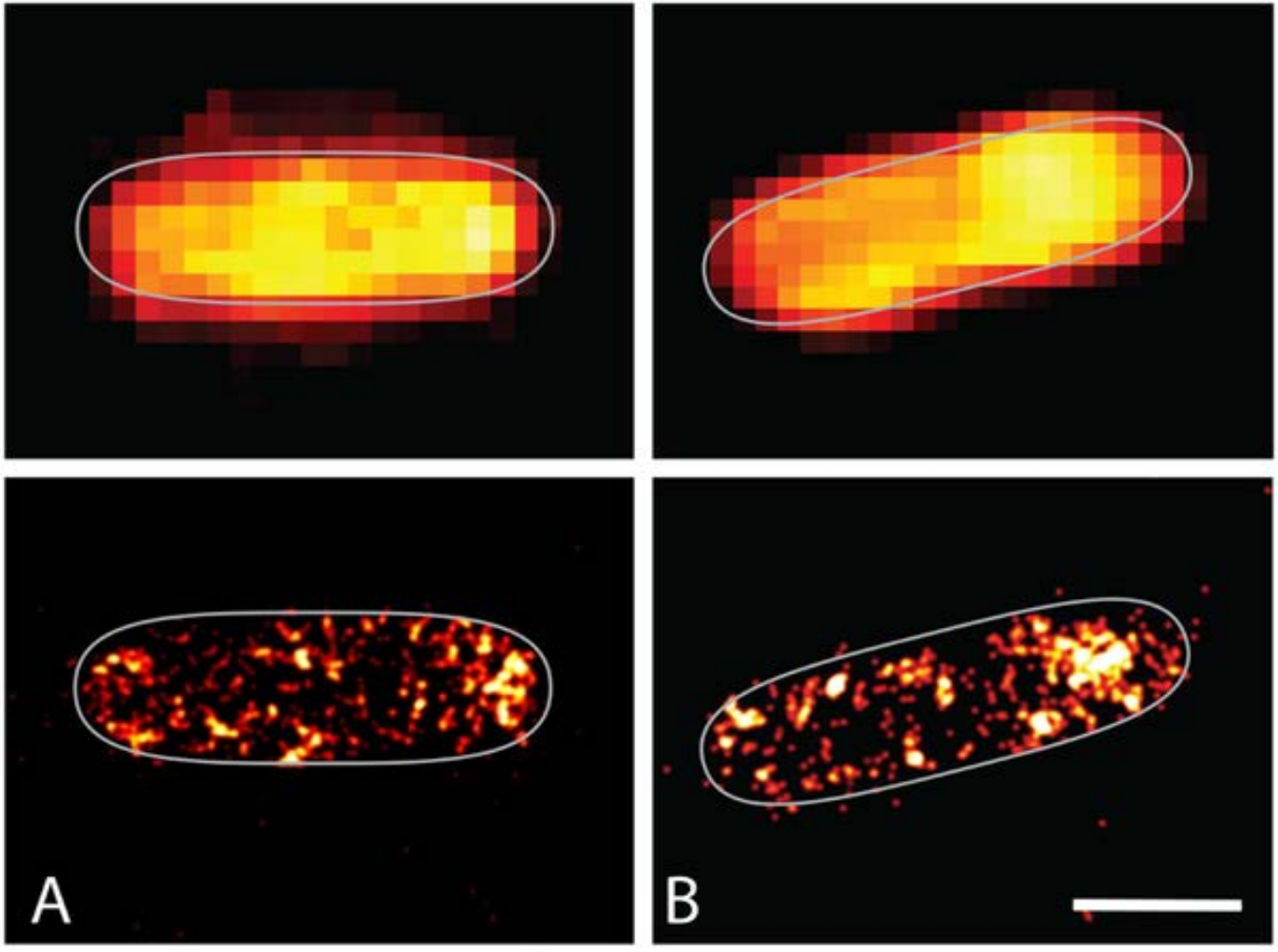

a
z
x
y
b
z
x
z
x
y
x
y
x



huanglab.ucsf.edu

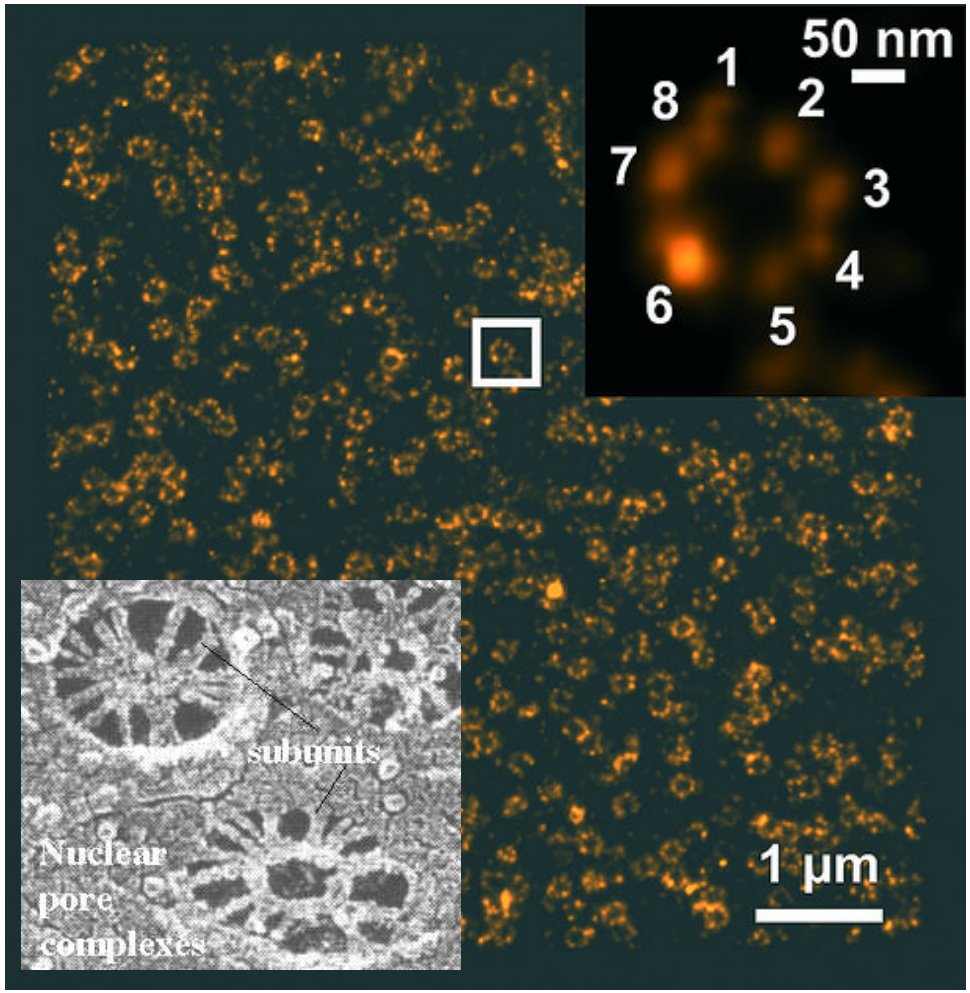
The Super-Resolution Gallery

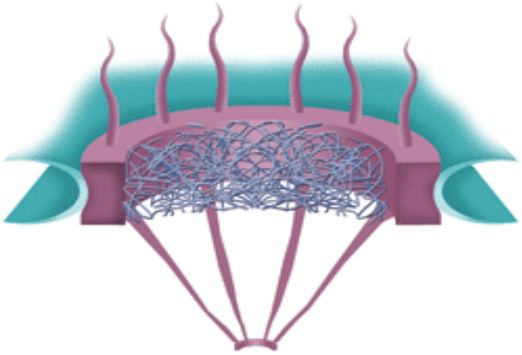
FPALM images of E. coli cells expressing wt YtvA.

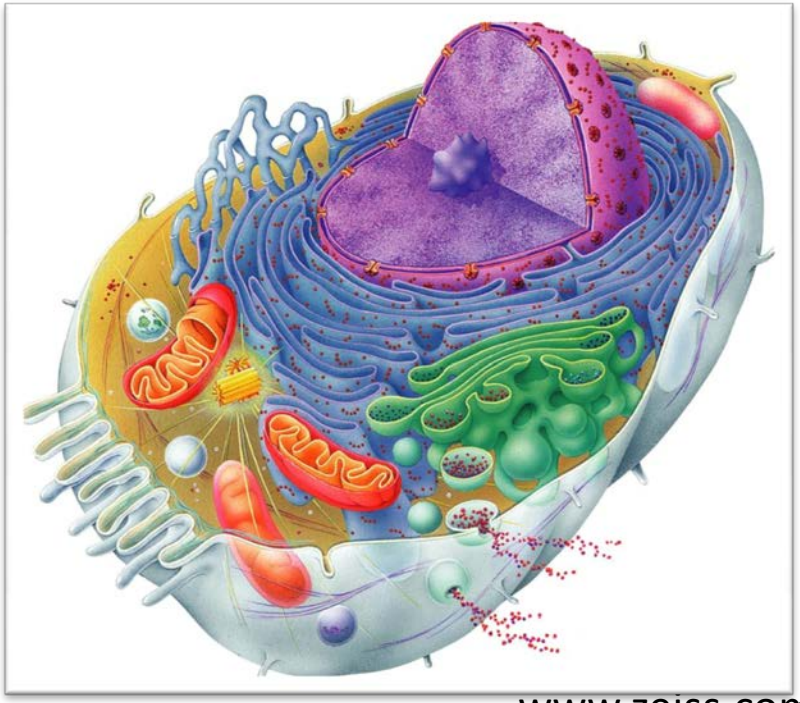


Pennacchietti F, Abbruzzetti S, Losi A, Mandalari C, et al. (2014) The Dark Recovery Rate in the Photocycle of the Bacterial Photoreceptor YtvA Is Affected by the Cellular Environment and by Hydration. PLoS ONE 9(9): e107489. doi:10.1371/journal.pone.0107489
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0107489

Nuclear pore complexes

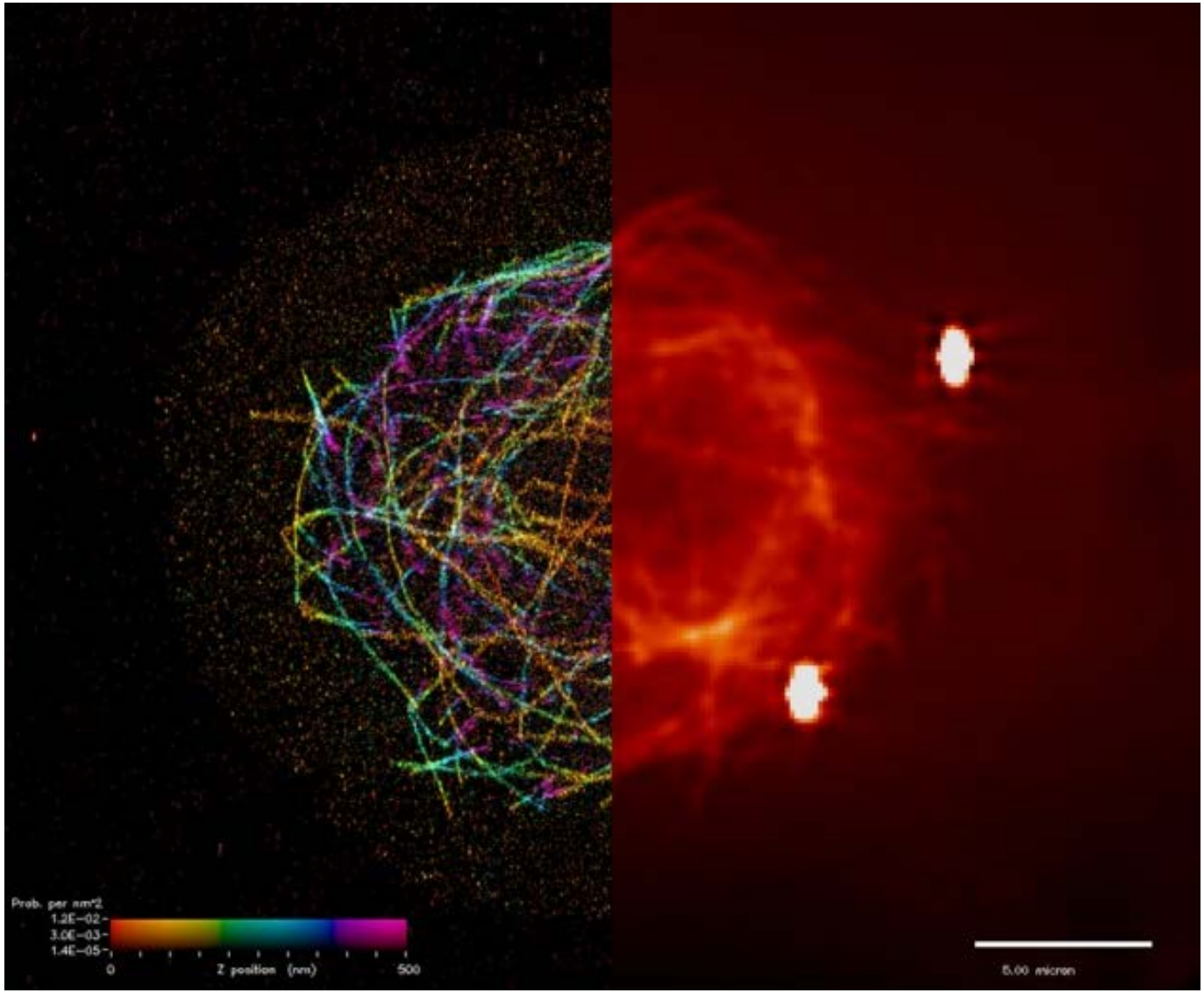






www.zeiss.com

Microtubules in cells from fruit flies



Jim and Cathy Galbraith, GlebShtengel, Harald Hess/HHMI/Janelia Research Campus

Their approaches

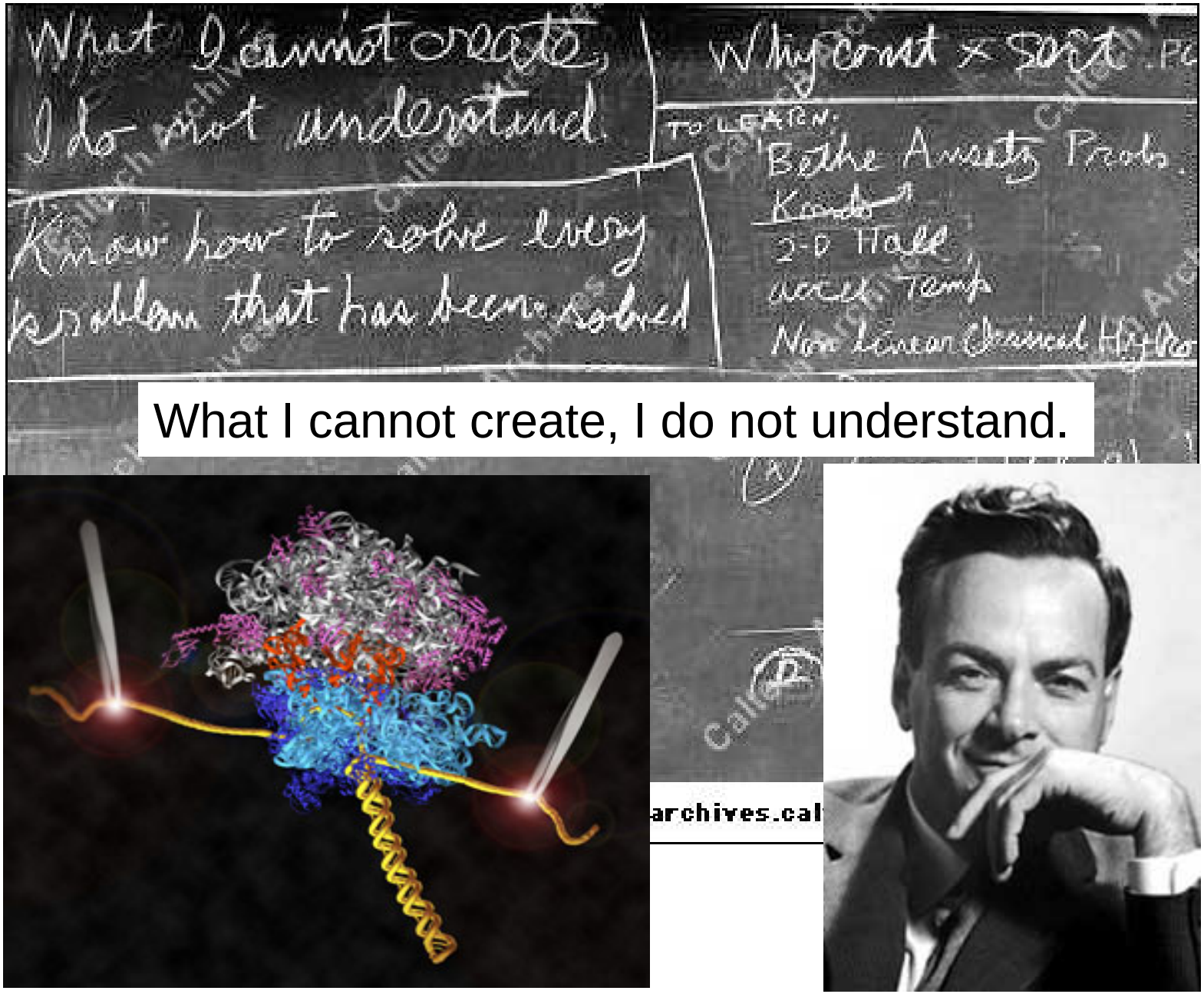
visualized the invisible,
enabled the incapable,

and is having impacts on life science.

The next challenge:

Identification, localization and manipulation of molecules with nanometer or even sub-nanometer precision in 3D in living cells, better time-resolved (<ms), and even label-free!

Do not say there is not a way!


What I cannot create, I do not understand.
archives.cal

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and published by the Cambridge University Press
(Cambridge, and Bentley House, London)
Agents for Canada and India: Macmillan

$$ih\frac{\partial\psi}{\partial t} = E\psi$$

Life Science Calls for Physicists!

WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

ERWIN SCHRÖDINGER

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Thank you!

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