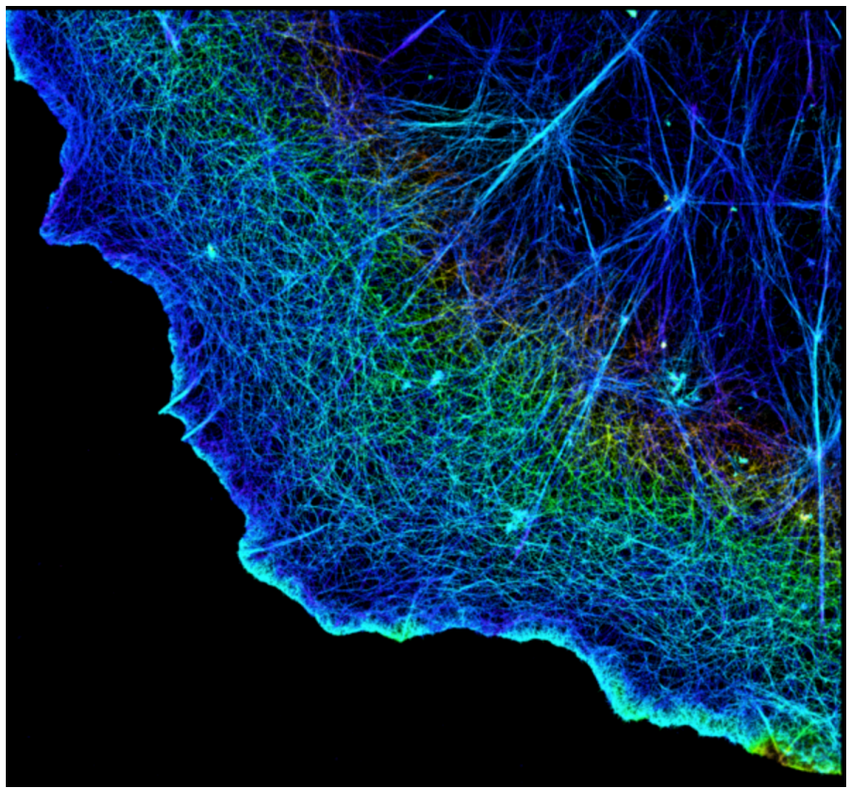
Super-resolution Microscopy



James Oakley
Literature Talk
02/02/2020

Presentation Overview

I. Introduction

- **Light microscopy in biology**
- **Scales of biological systems**

II. The Diffraction Barrier

- **Diffraction**
- **The Point Spread Function**
- **The Abbe Equation**

III. Stimulated Emission Depletion Microscopy

- **STED Theory**
- **Breaking the Diffraction Barrier**
- **Selected Publications**

IV. Single Molecule Localization Microscopy

- **Super localization**
- **SMLM Theory**
- **PALM**
- **STORM**
- **Selected Publications**

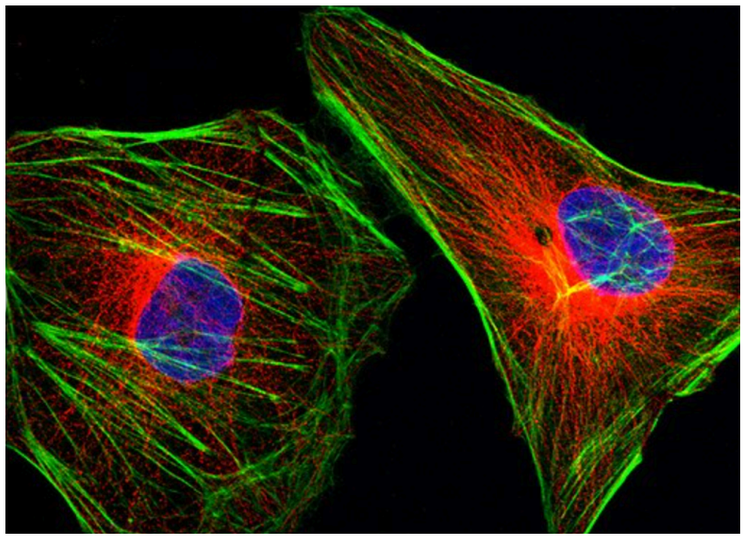
V. Outlook

Clathrin dynamics in fish larva expressing dsRED-clathrin light chain A

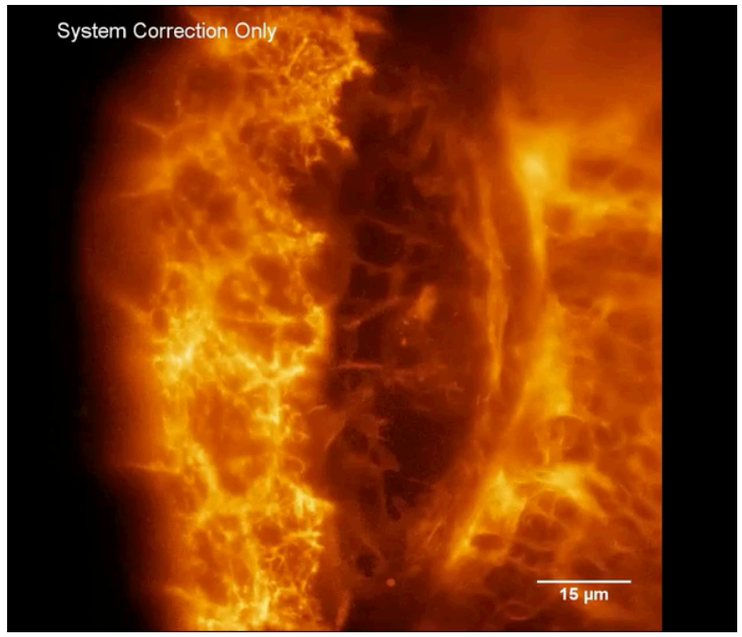
Light Microscopy in Biology

Light microscopy enables direct observation of biological systems

Unraveling of cellular processes and structural organization



Confocal image of HeLa cervical cancer cells

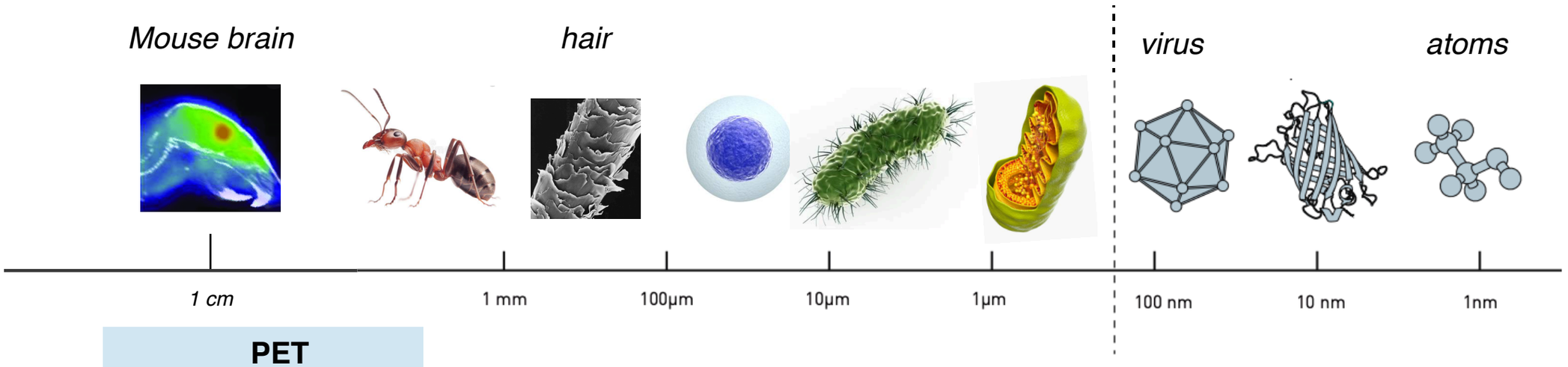


Immune cell migration in perilymph of zebrafish embryos

Advantages of light microscopy in biology

- *Non-invasive*
- *Real time and 3D imaging*
- *protein-specific contrast*
- *single molecule imaging*

Distance Scales of Biological Systems

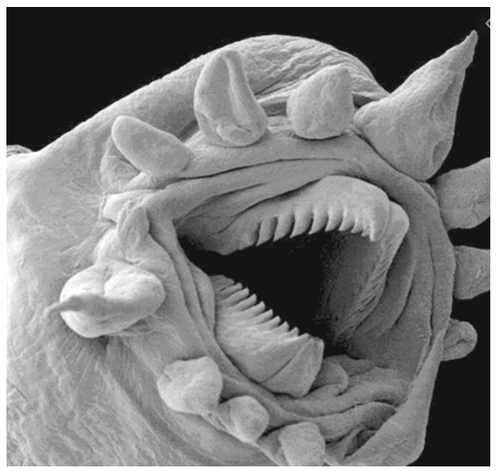


PET

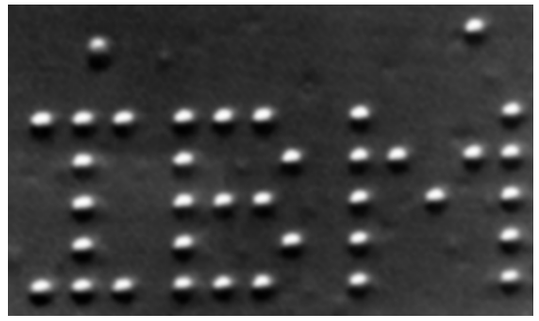
MRI and ultrasound

X-ray crystallography, NMR

electron microscopy

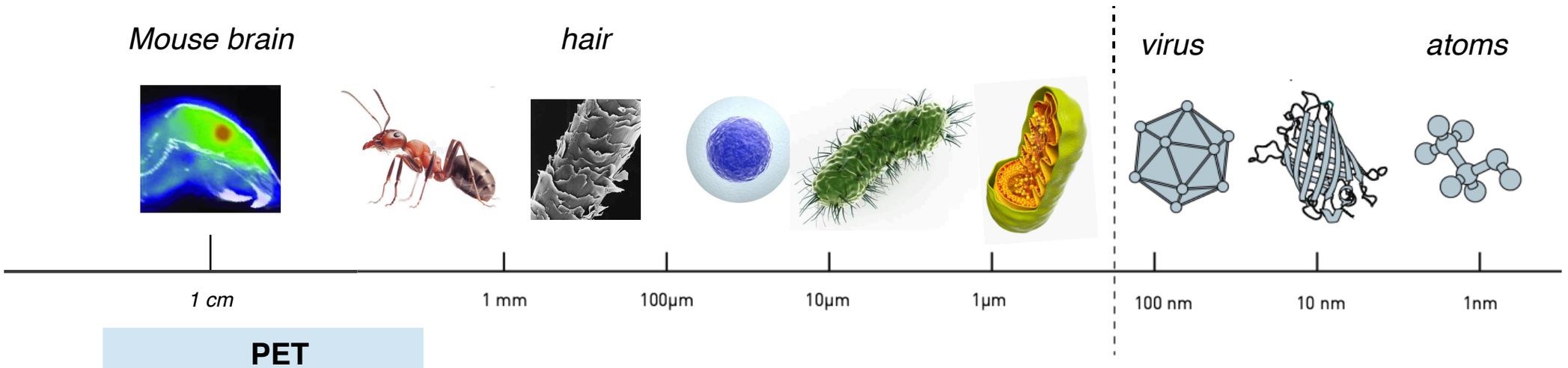


SEM image of hydrothermal worm (2011)



STM image of “IBM” written in xenon atoms (1989)

Distance Scales of Biological Systems



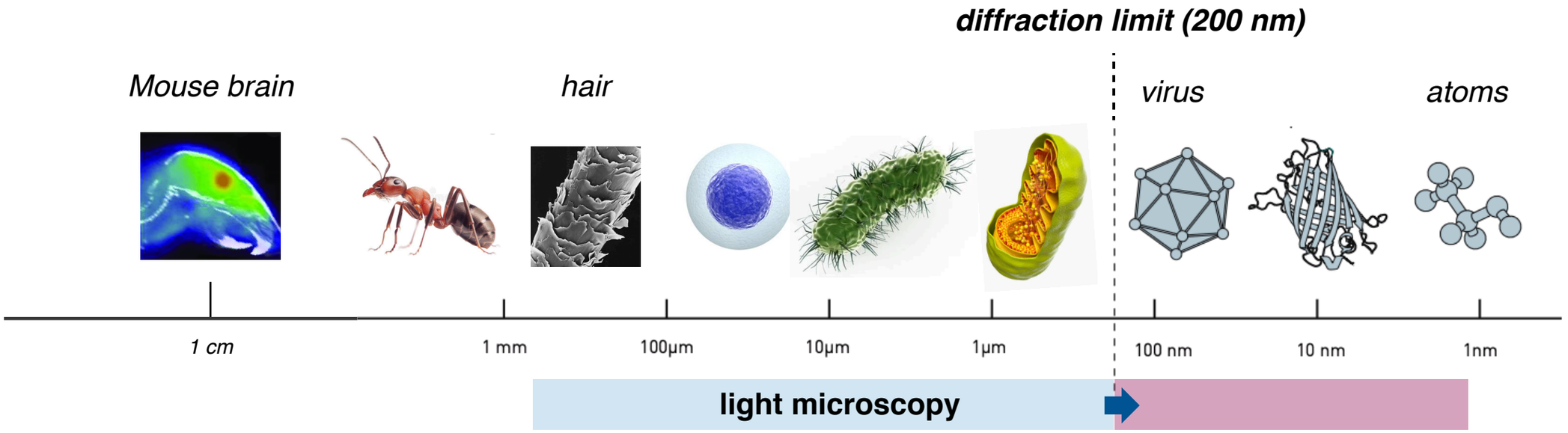
Live Cell Electron Microscopy Is Probably Impossible

Niels de Jonge*,†,‡ and Diana B. Peckys§

†INM—Leibniz Institute for New Materials and ‡Department of Physics, Saarland University, D-66123 Saarbrücken, Germany
§Department of Biophysics, Saarland University, D-66421 Homburg/Saar, Germany

Jonge, N.; Peckys, D. B. *ACS Nano* **2016**, 10, 9061-9063

Distance Scales of Biological Systems



■ **Resolution of light microscopy is limited by diffraction**

Breaking light microscopy diffraction barrier would greatly enable biological discovery

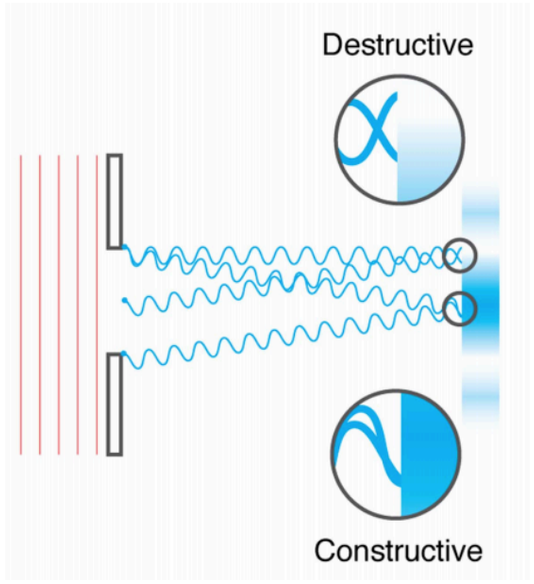
Diffraction of Light

- ***Wave nature of light produces diffraction patterns***

diffraction grating of a CD

“silver lining”

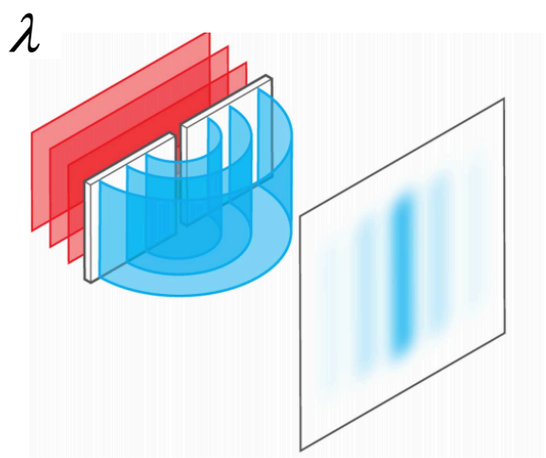
Diffraction pattern of light passing through a slit



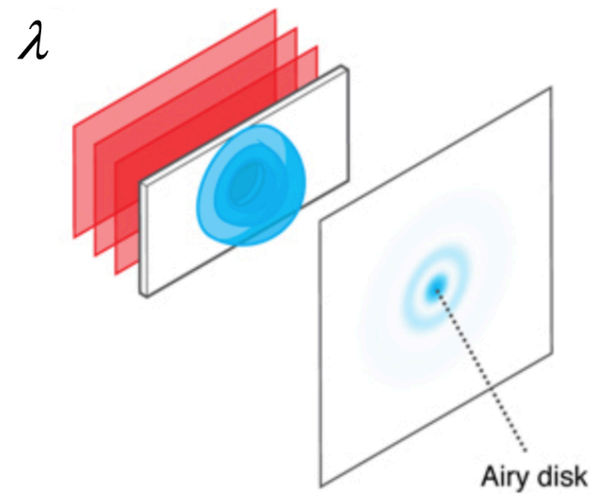
- *constructive and destructive interference generates diffraction pattern*

Diffraction of Light

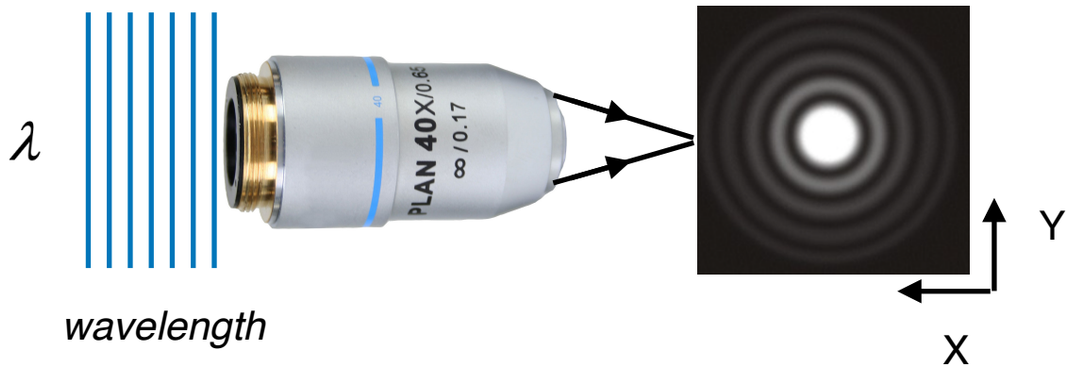
Diffraction pattern through a slit



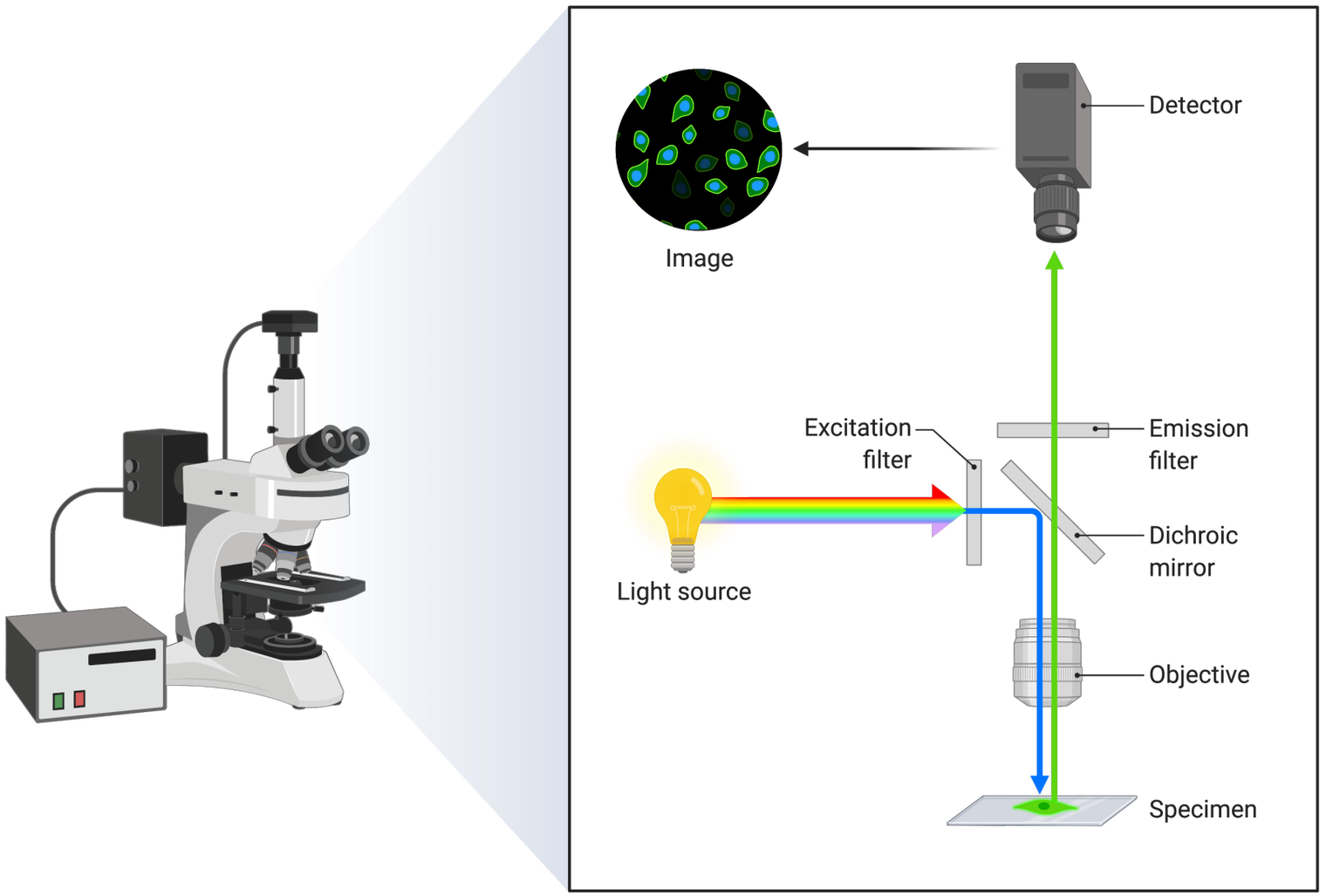
Diffraction pattern through a circular aperature



■ ***diffraction through an objective lense creates Airy disk diffraction pattern***



Anatomy of a Fluorescence Microscope

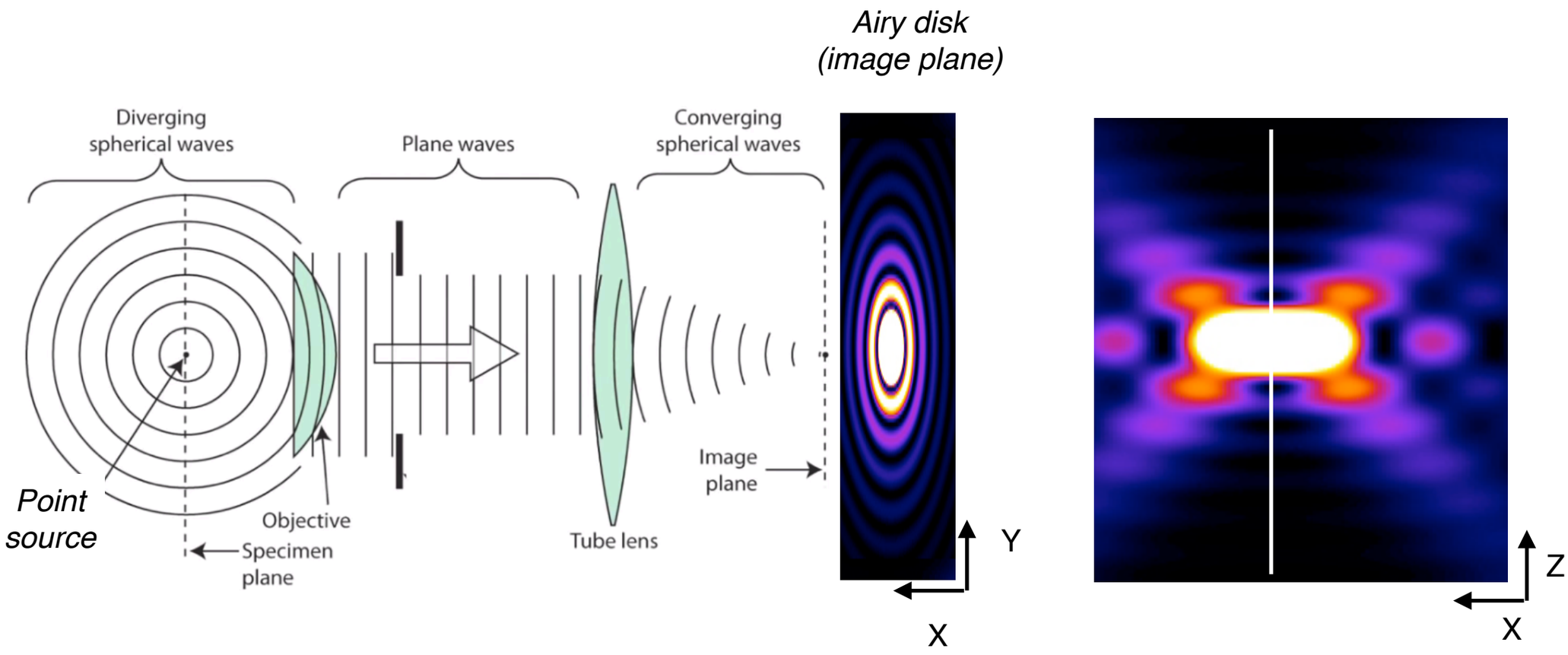


- ***Fluorescence of sample is captured to produce an image***

- ***Excitation and emission light is separated***

Point Spread Function (PSF)

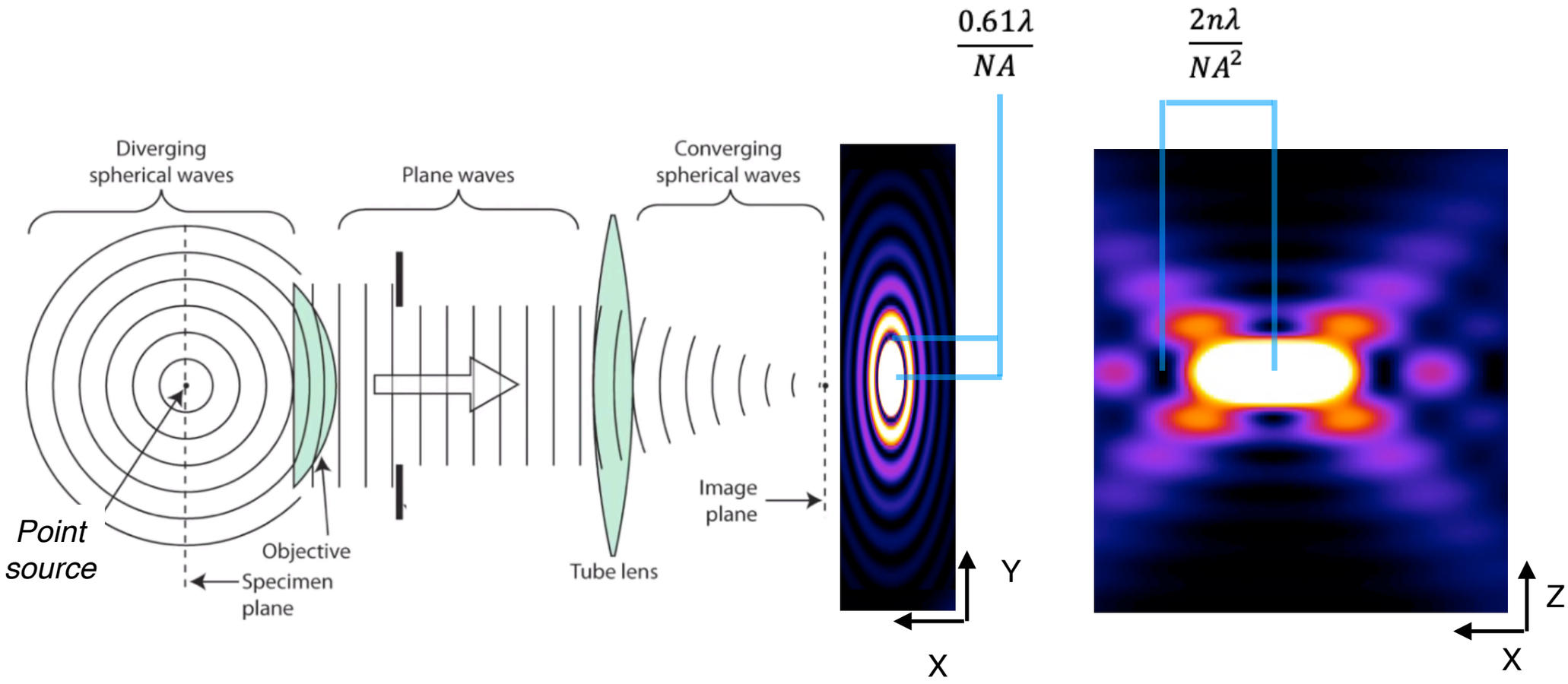
- ***Propagation of light waves through a microscope***



- *Point source of light generates a 3D geometric pattern*

Point Spread Function (PSF)

- ***Propagation of light waves through a microscope***



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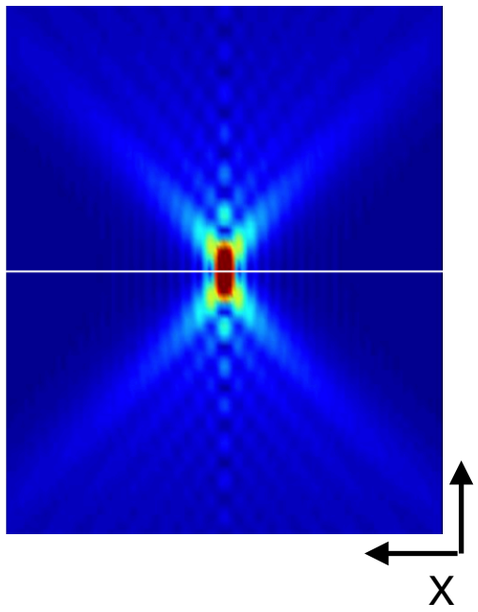
Point Spread Function (PSF)

point spread function

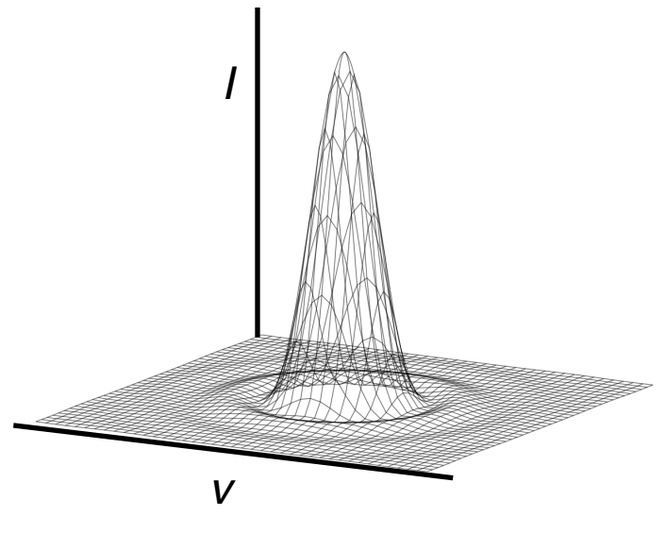


- ***Intensity distribution in the focal plane is described by the PSF***

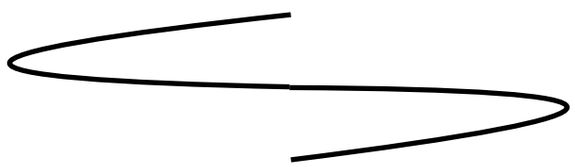
$$I_{exc}(v) = C\left|2\frac{J_1(v)}{v}\right|^2$$

where,

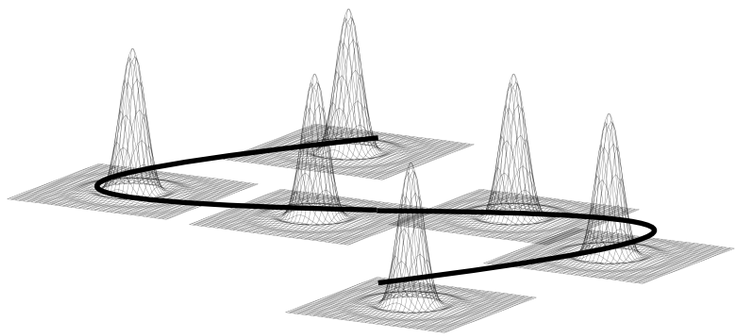
$$v = \frac{2\pi r\ NA}{\lambda_{exc}}$$



PSF is the fundamental unit for an image



object

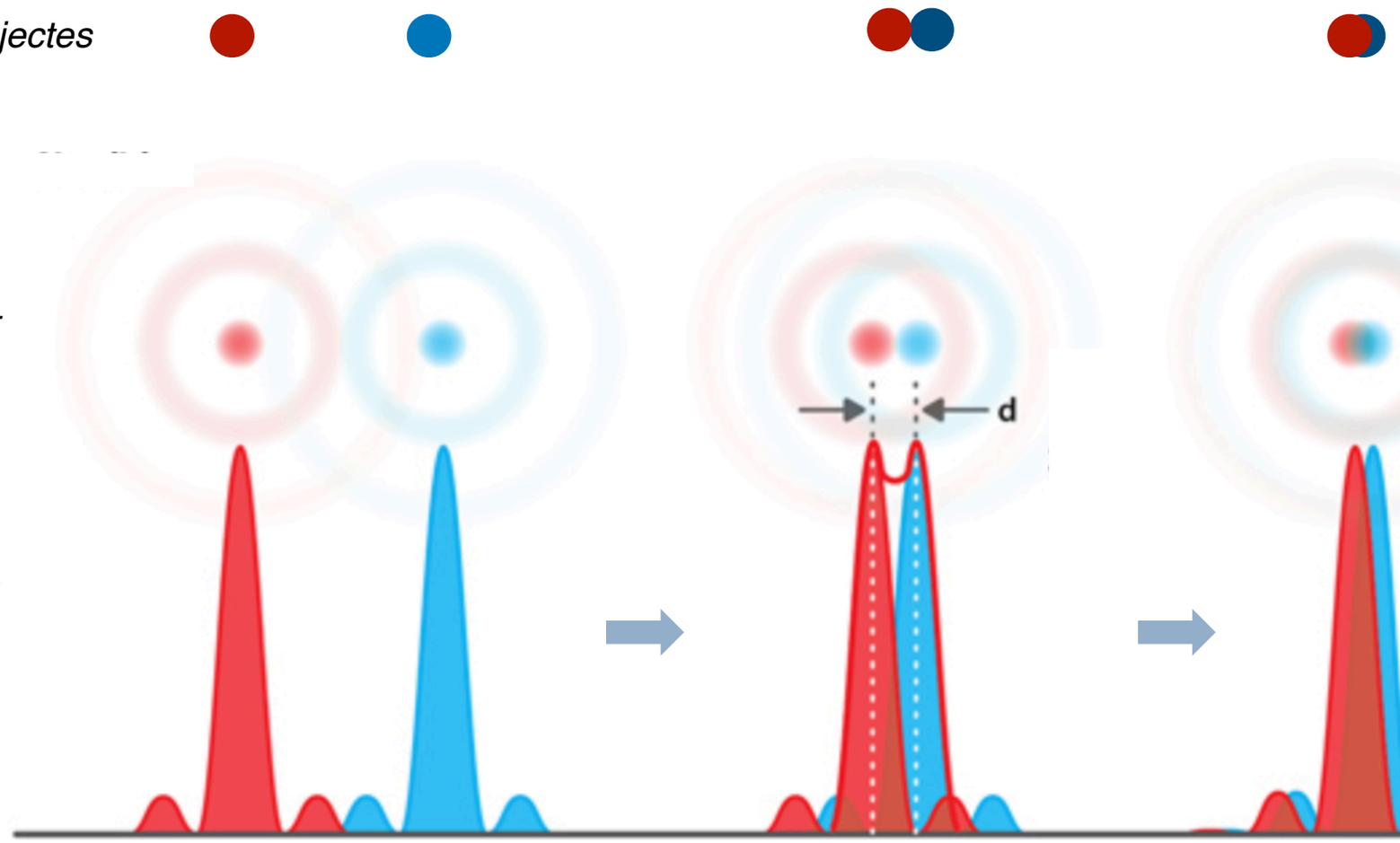


Image

Resolution

Resolution: The ability to discern between two point objects

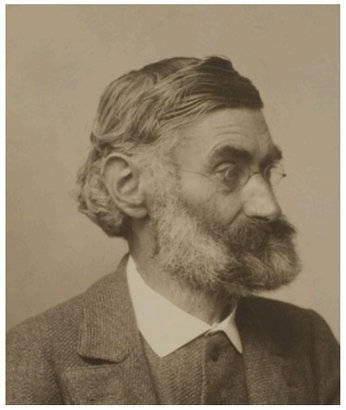
Point objectes



- ***minimum distance between point objects to resolve signals:*** $d = \dfrac{\lambda}{2n\sin\alpha}$

Abbe Diffraction Limit

- ***Resolution of optical instruments in fundamentally limited by the diffraction of light.***



Ernst Abbe

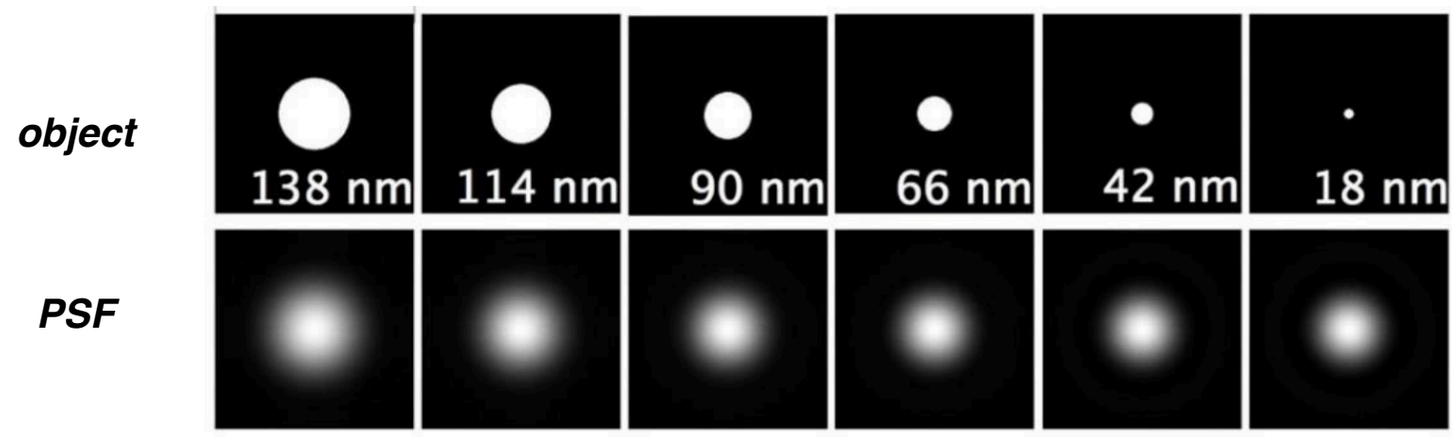
The Abbe equation (1873)

$$d = \frac{\lambda}{2n\sin\alpha}$$

Best resolution under ideal conditions ~ 200 nm

Considered a fundamental, unbreakable rule

- ***Beyond the diffraction barrier, true object size is unresolvable***



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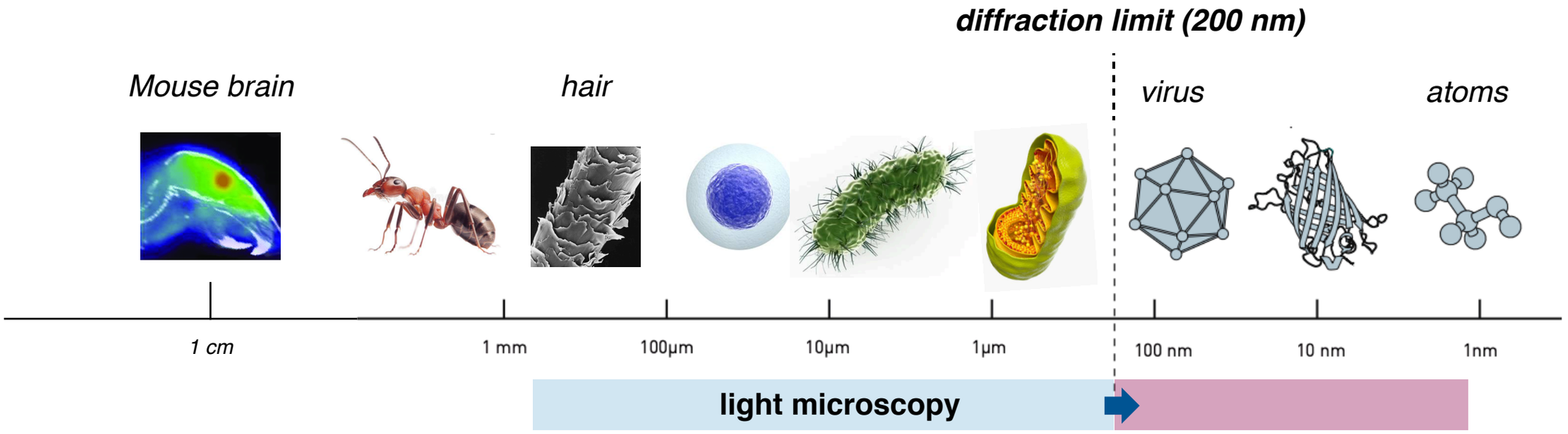

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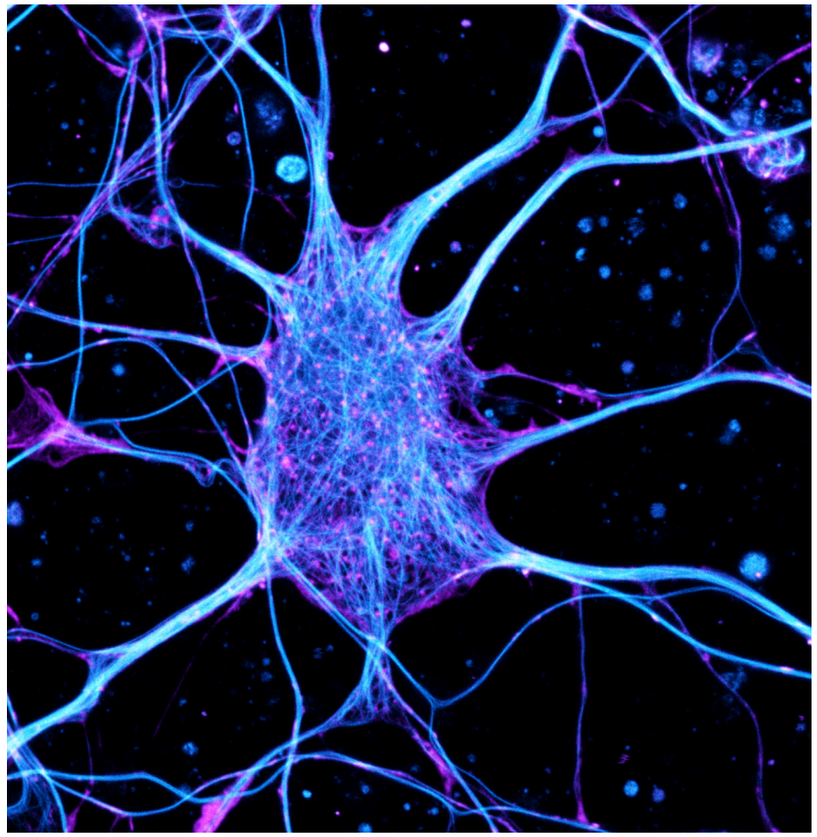

STED image of a dissociated hippocampal neuron stained for actin and microtubules

Breaking the Diffraction Barrier

1933: Conception of STED microscopy

"…separating features via the molecular states of the sample, rather than tackling diffraction itself."

Stefan Hell
Max Planck institute

Breaking the Diffraction Barrier

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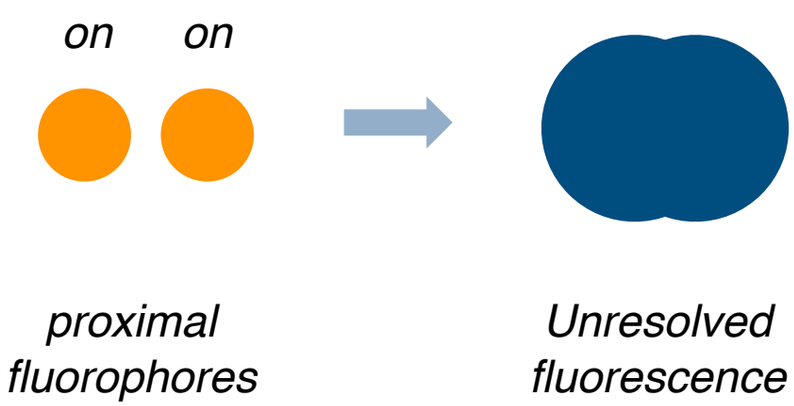

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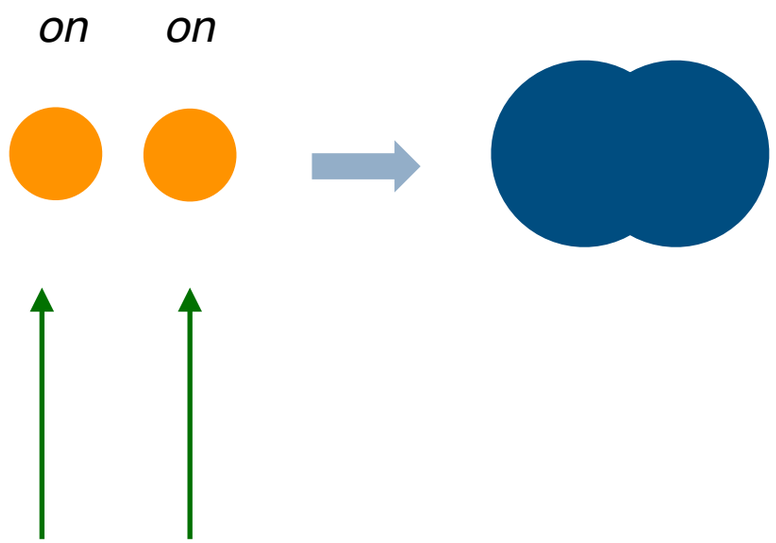
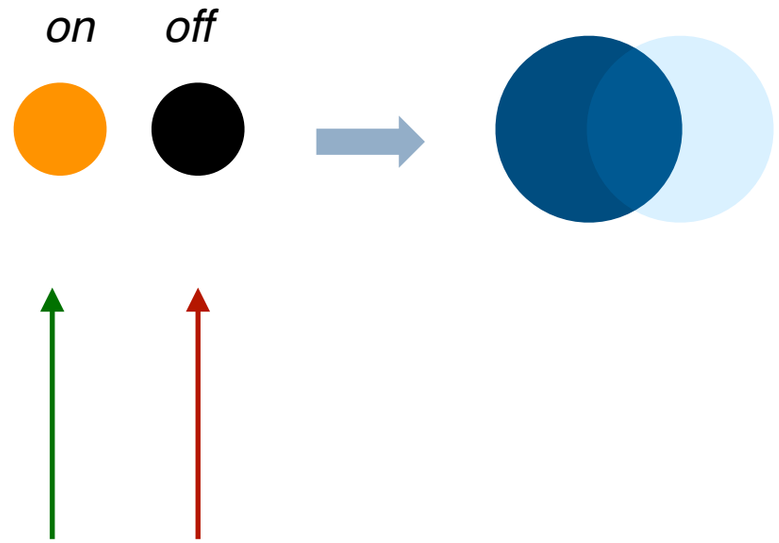
Stimulated Emission Depletion Microscopy



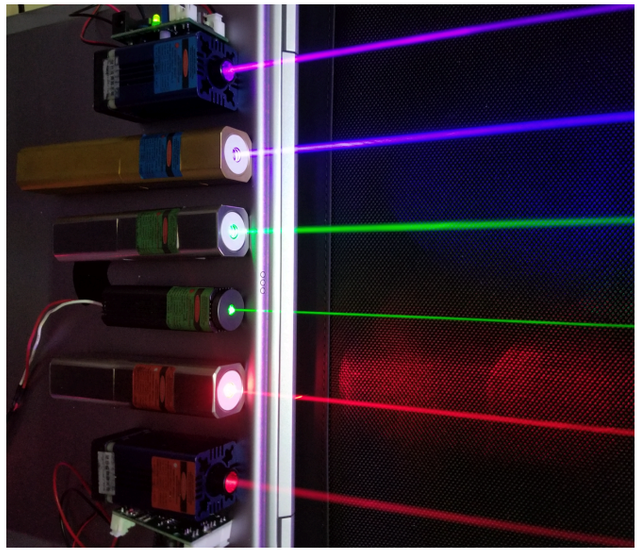
Excitation beam to turn fluorescent state on

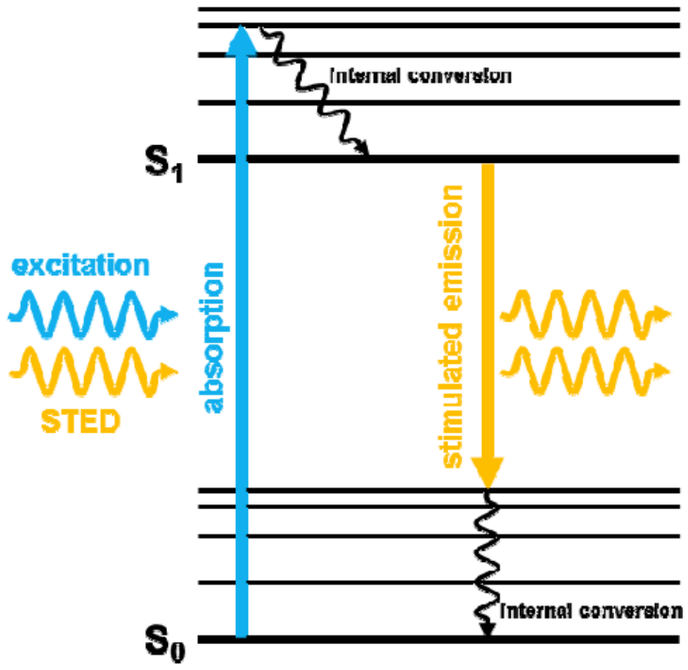


second light source to turn fluorescent state off

Stimulated Emission Depletion Microscopy

Light amplified stimulated emission





Fluoresence

on

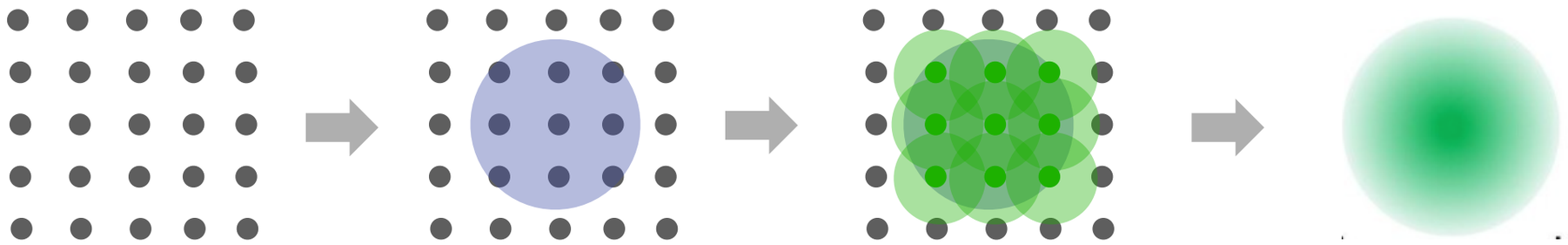
Stimulated emission

off

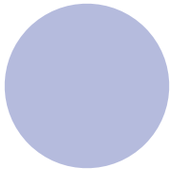
Stimulated emission as a toggle switch for fluorescence

Stimulated Emission Depletion Microscopy

Diffraction-limited light microscopy



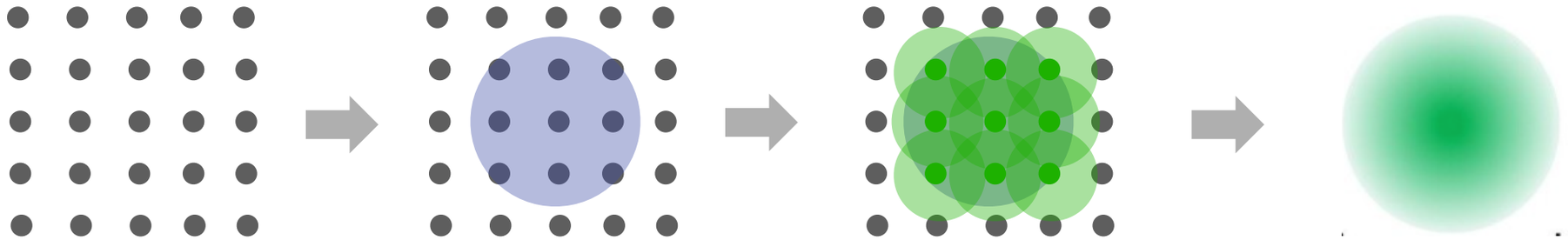
molecular ensemble *excitation* *overlapping fluorescence* *PSF*



excitation beam

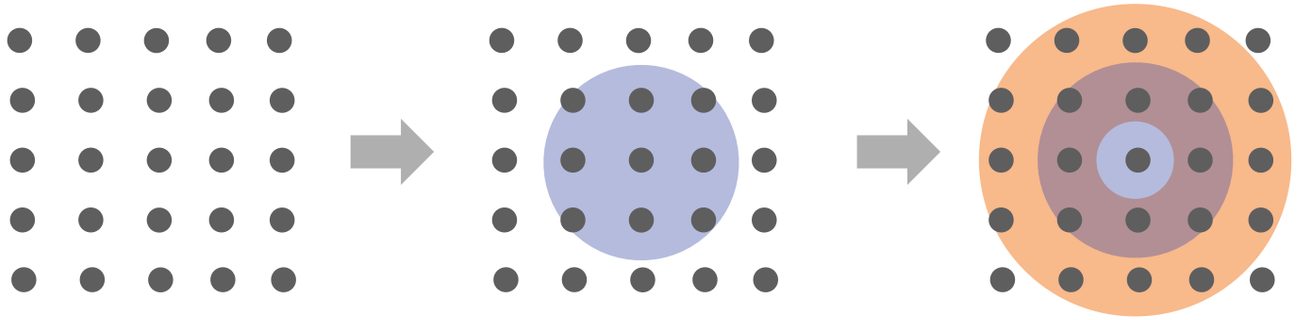
Stimulated Emission Depletion Microscopy

Diffraction-limited light microscopy

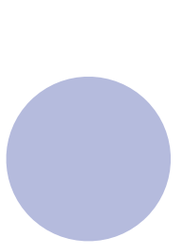


molecular ensemble *excitation* *overlapping fluorescence* *PSF*

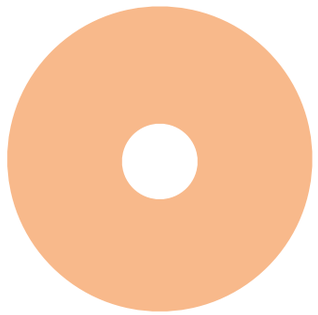
STED microscopy



Beams are co-aligned



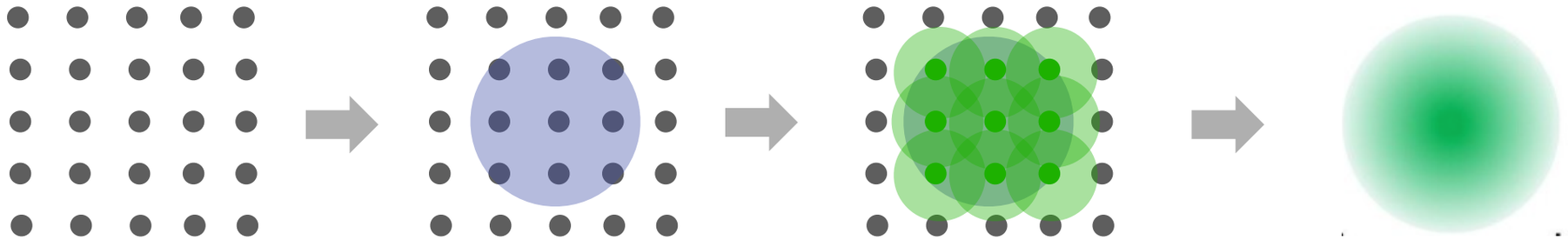
excitation beam



stimulated emission beam (STED)

Stimulated Emission Depletion Microscopy

Diffraction-limited light microscopy



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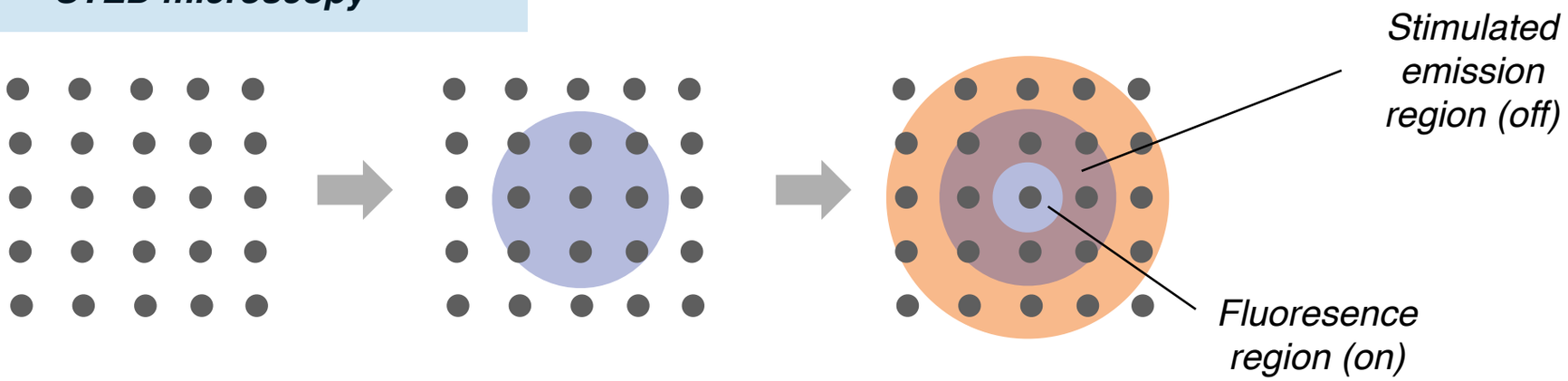
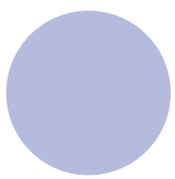
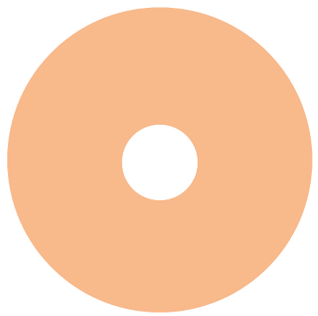




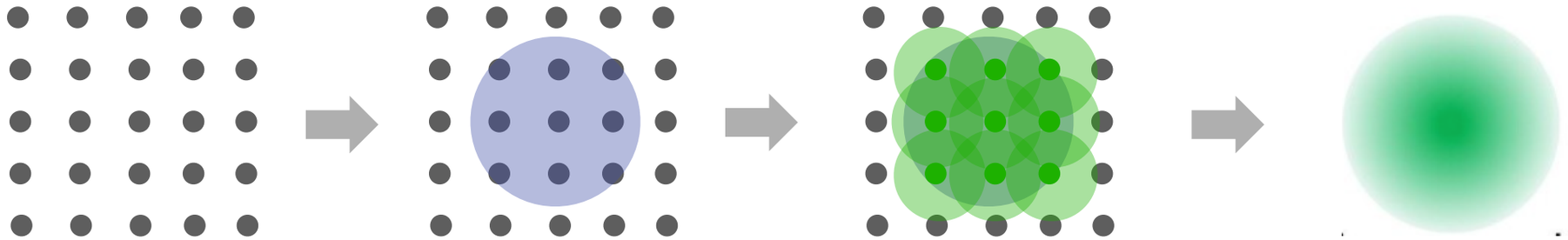
excitation beam



stimulated emission beam (STED)

Stimulated Emission Depletion Microscopy

Diffraction-limited light microscopy



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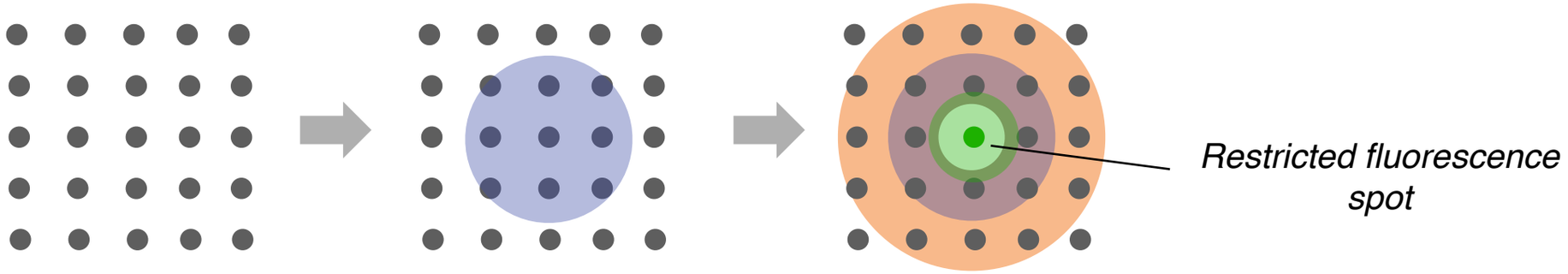
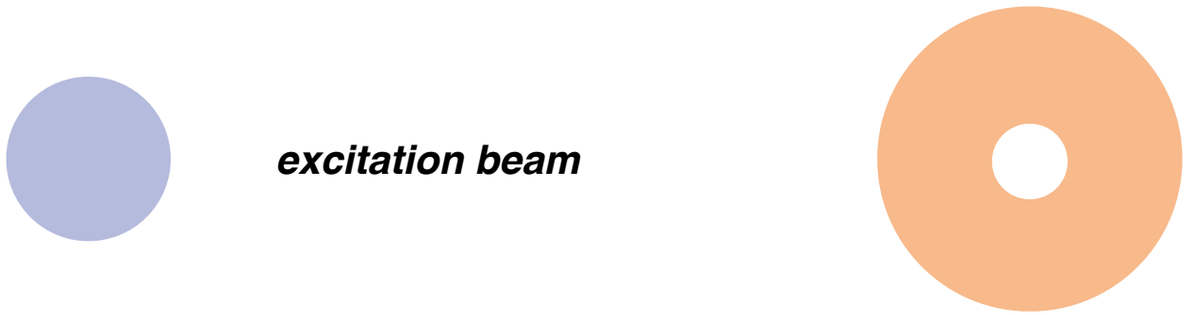
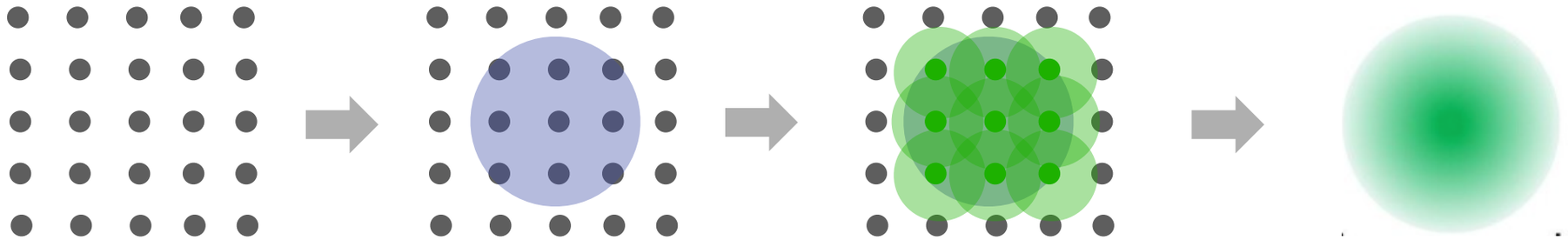




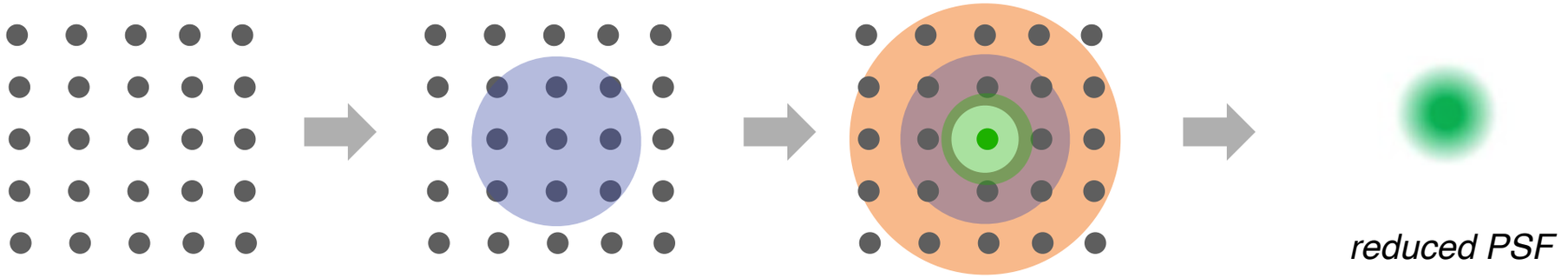
Stimulated Emission Depletion Microscopy

Diffraction-limited light microscopy



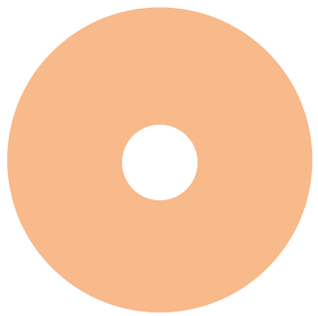
molecular ensemble *excitation* *overlapping fluorescence* *PSF*

STED microscopy



reduced PSF

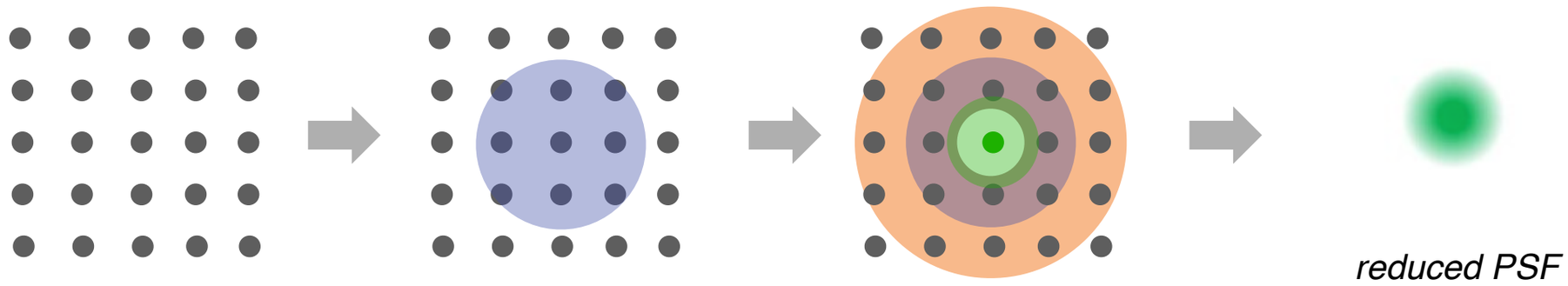
excitation beam

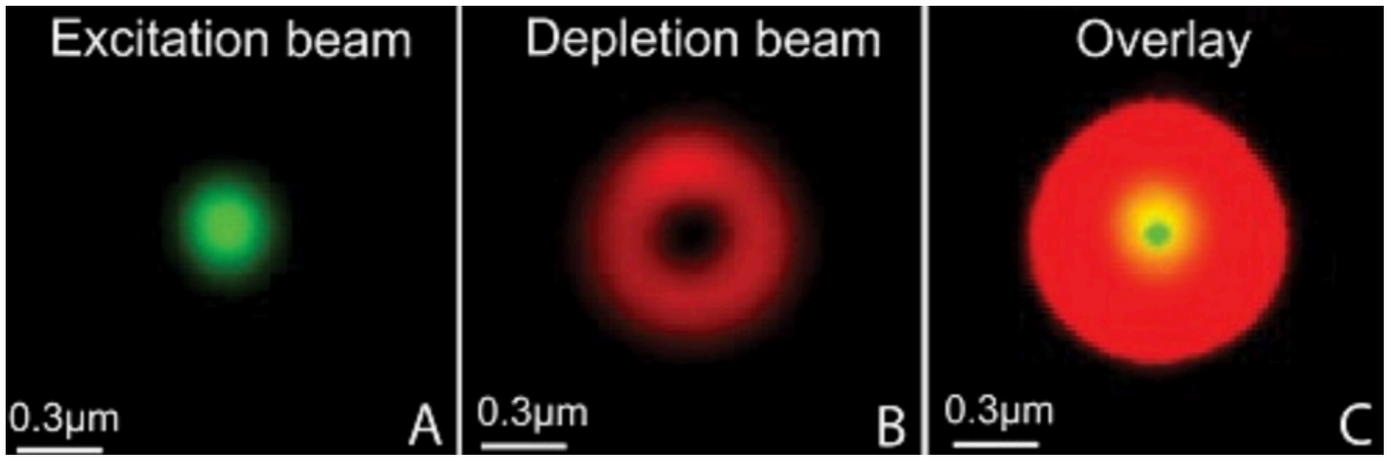


stimulated emission beam (STED)

Stimulated Emission Depletion Microscopy

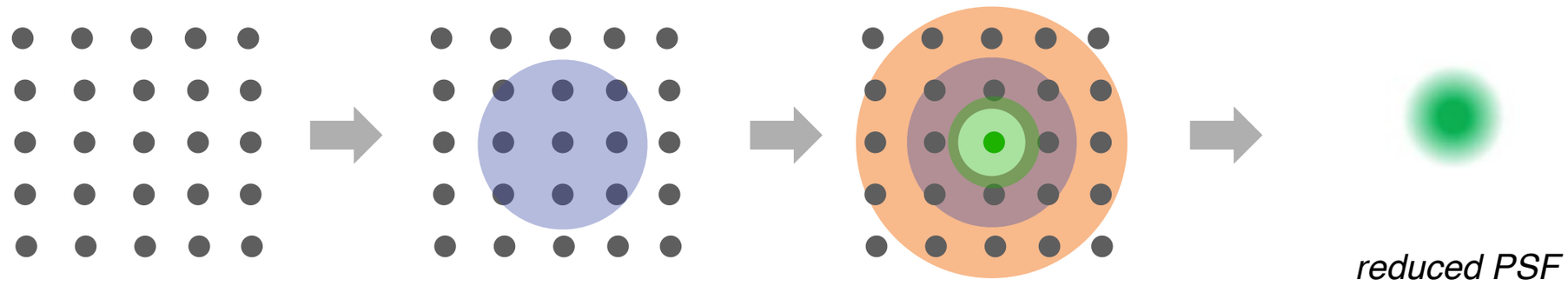
STED microscopy

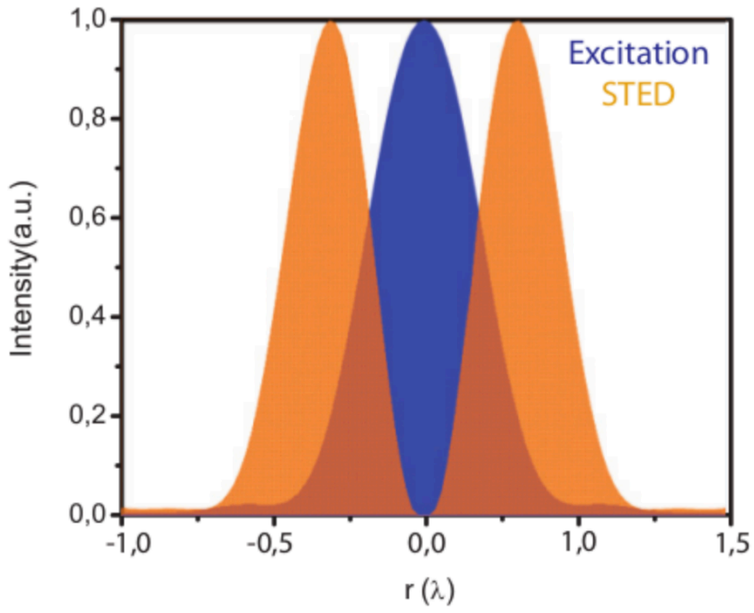


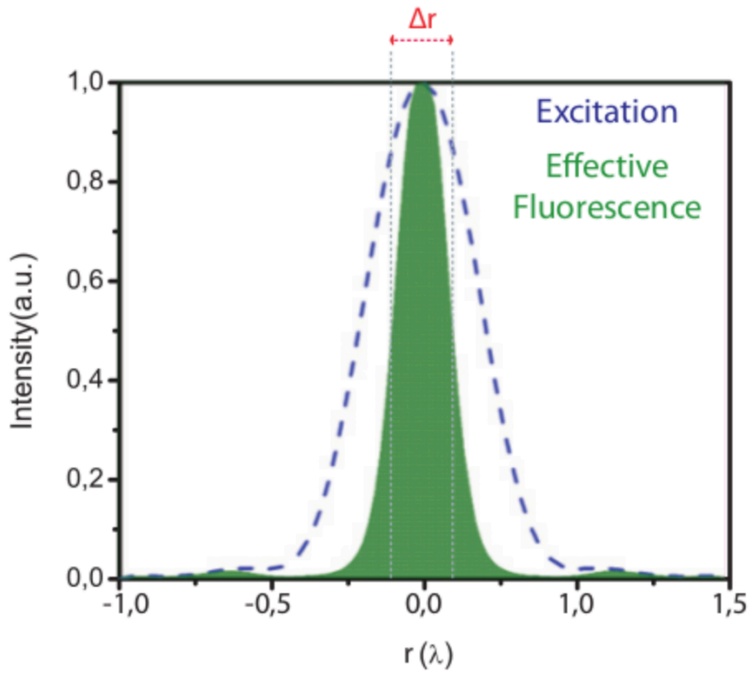


Stimulated Emission Depletion Microscopy

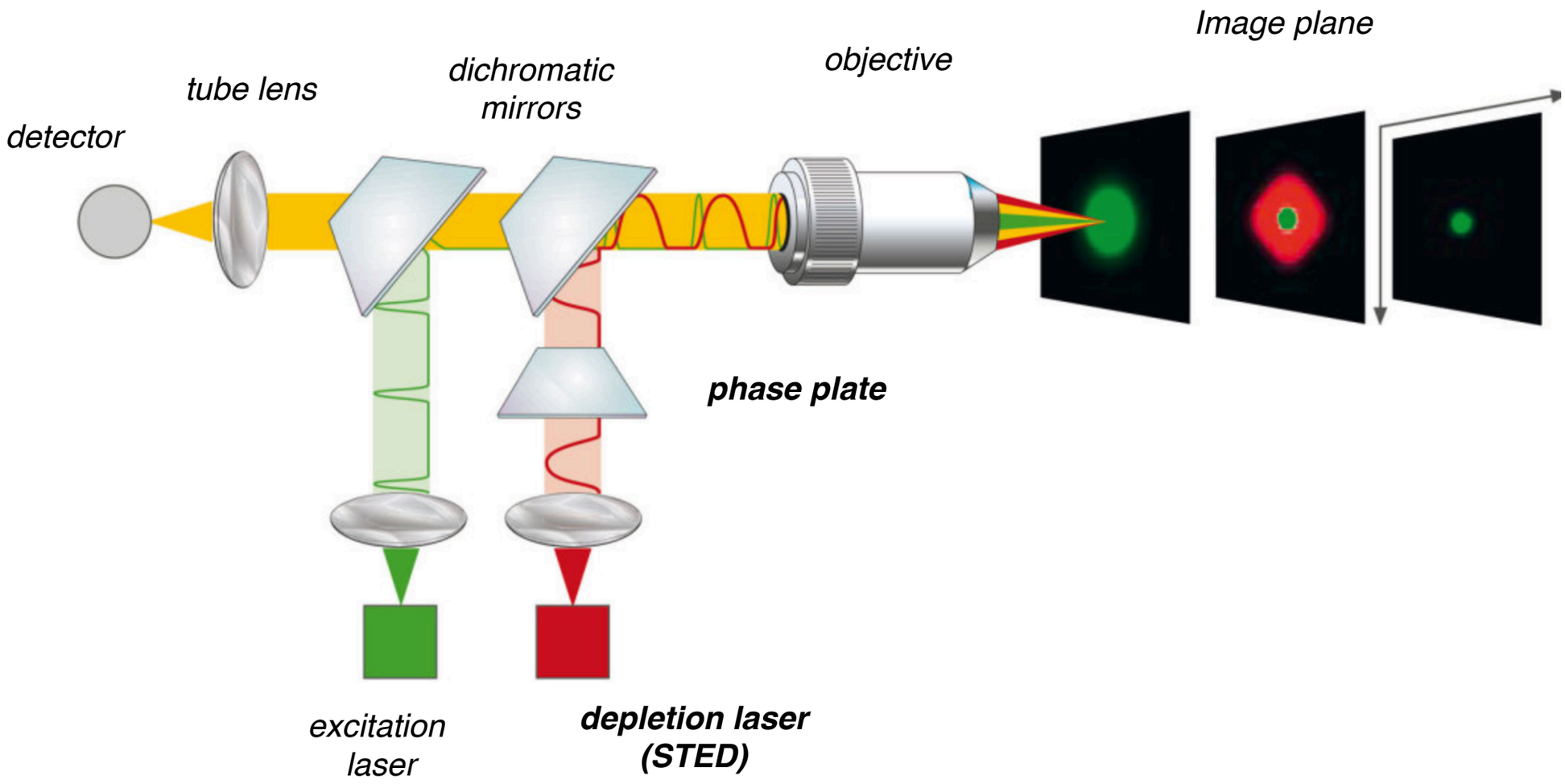
STED microscopy







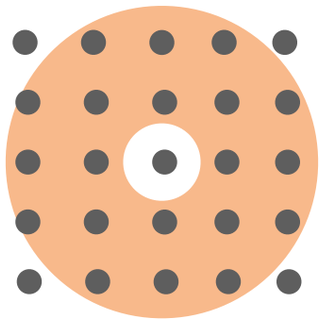
Anatomy of a STED Microscope



- *Phase plate produces donut-shaped depletion beam*
- *STED laser pulsed after excitation laser*

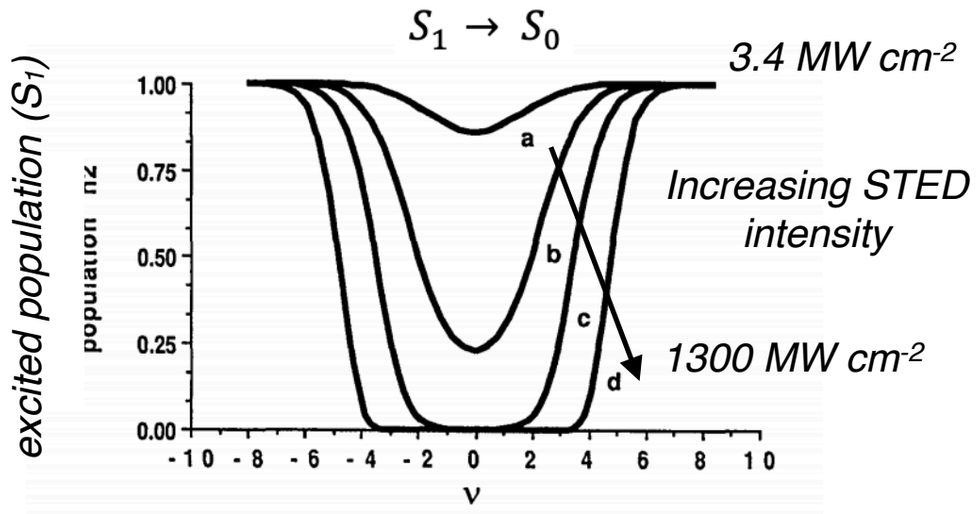
Westphal, V.; Hell, S. W. *Phys. Rev. Lett.* **2005**, *94*, 143903

STED Laser Intensity and Depletion

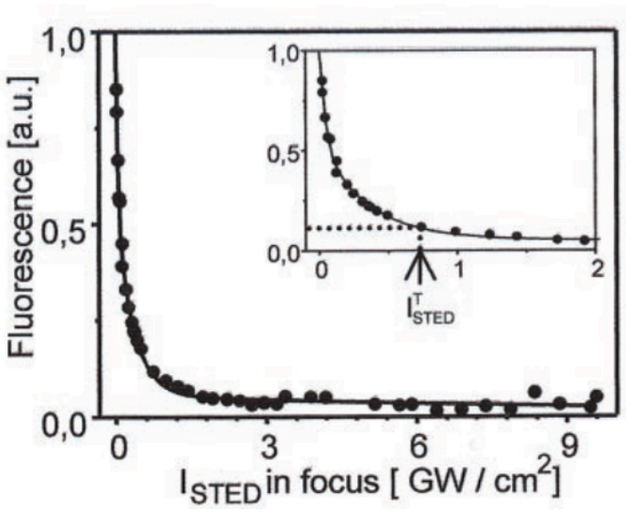


$$S_1 \rightarrow S_0$$

$$k_{STED} = \sigma I_{STED}$$



- ***Greater S_1 depletion at higher STED intensity***



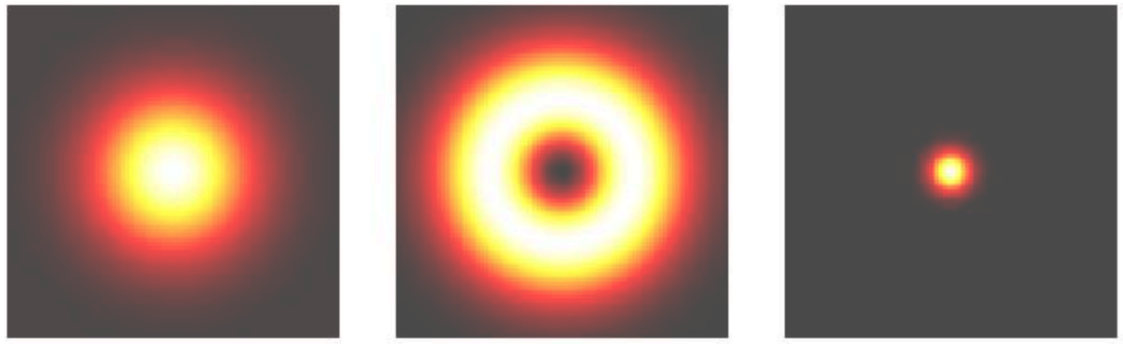
- *Saturate the off state within STED region*

Only molecules within inner region emit

Wichmann, J.; Hell, S. W. *Opt. Lett.* **1994**, 19, 780-782

Westphal, V.; Hell, S. W. *Phys. Rev. Lett.* **2005**, *94*, 143903

Breaking the Diffraction Barrier



Modification of Abbe's formula:

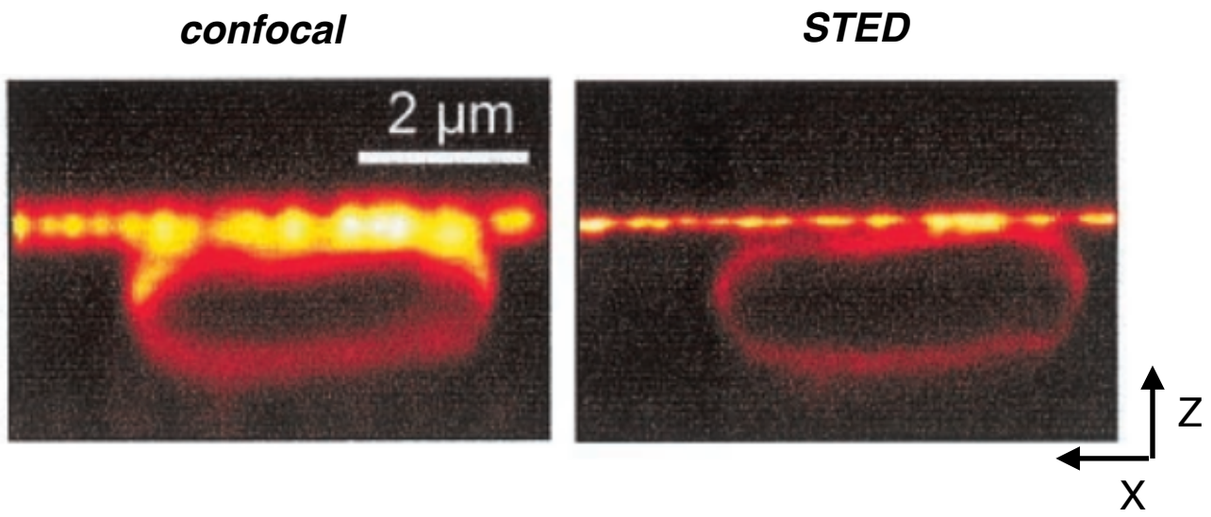
$$d = \frac{\lambda}{2n\sin\alpha} \quad \Rightarrow \quad d = \frac{\lambda}{2n\sin\alpha\sqrt{1 + \frac{I}{I_{sat}}}}$$

- *Resolution becomes a function of intensity*
- *Maximum resolution attainable is given by the largest practical intensity*

Westphal, V.; Hell, S. W. *Phys. Rev. Lett.* **2005**, *94*, 143903

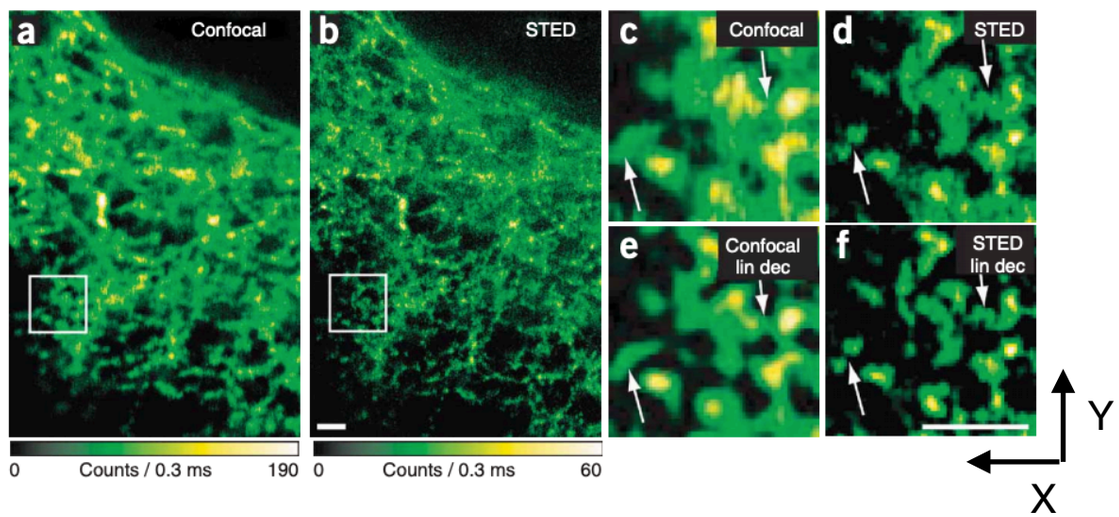
Landmark Publications

- ***STED imaging of RH-414 labeled E. coli (2000)***



Klar, T. A.; Jakobs,S.; Dyba, A.; Egner, S. *PNAS,* **2000***, 19*, 780-782

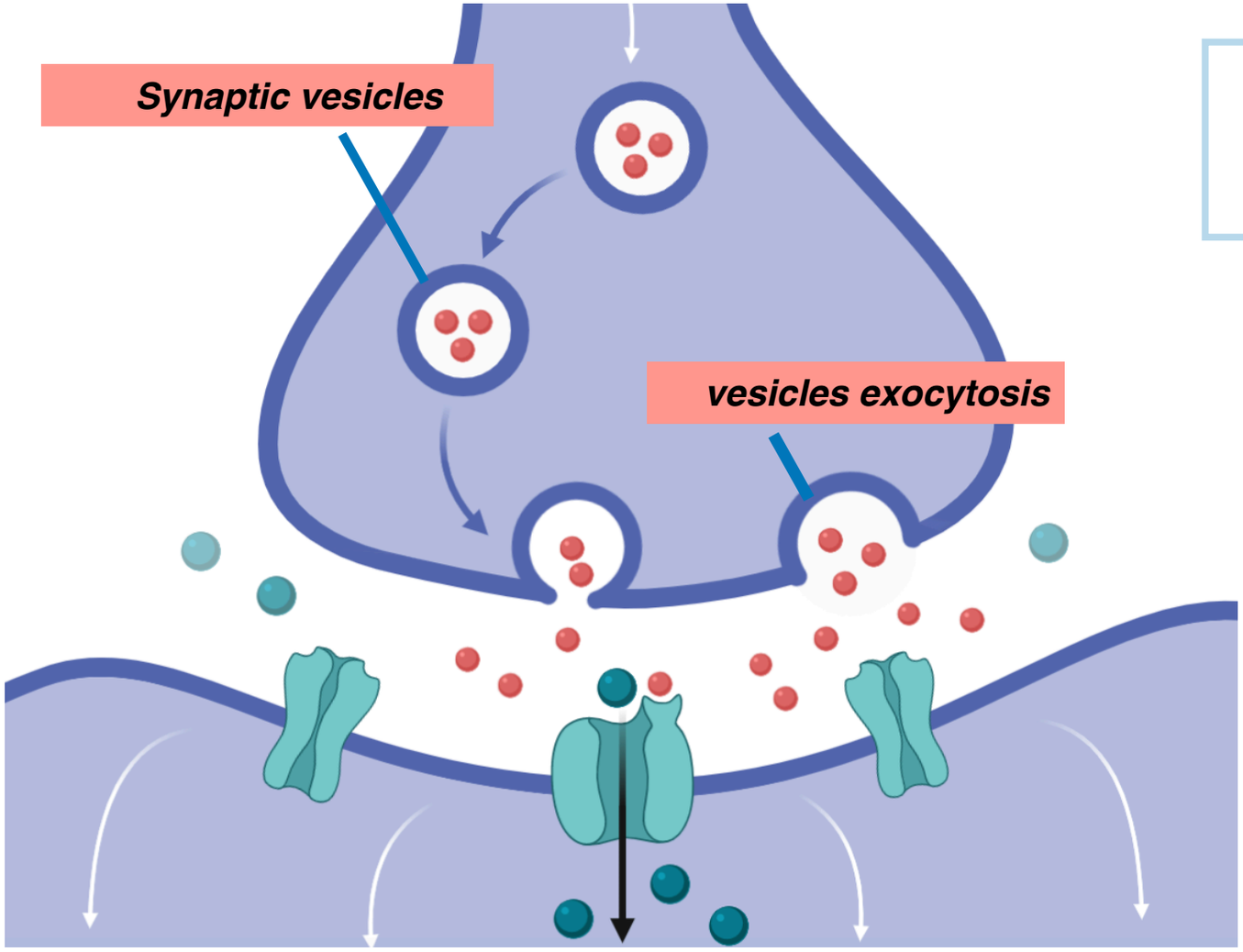
- ***STED imaging of GFP-labeled ER in PtK2 cells (2006)***



Willig, K. I.; Kellner, R. R.; Medda, B.; Hein, S. Hell, S. W. *Nat. Methods* **2006***, 3*, 721-723

STED Microscopy to Study the Neurosynapse

Neurotransmitter release at the neurosynapse

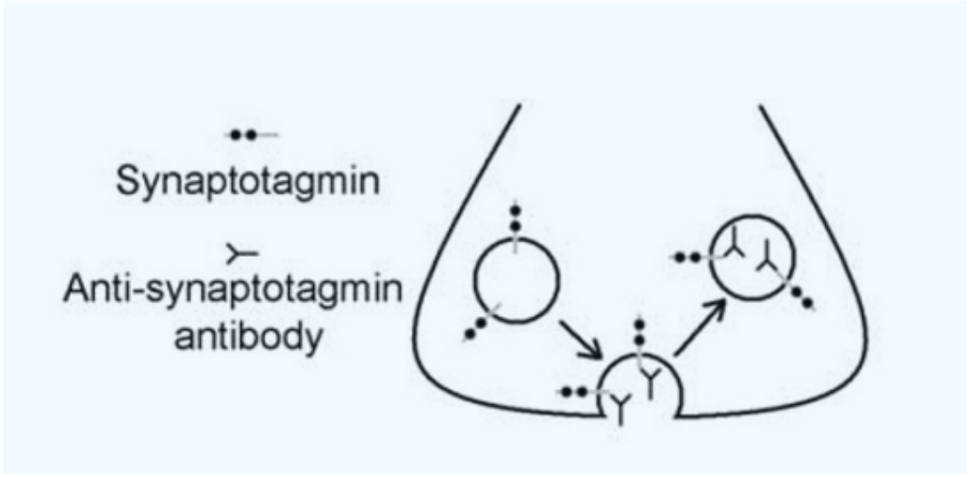


What happens to vesicle constituents after fusion?

- *small diameter (40 nm) and density pose imaging challenges*
- *hitherto unsuccessful with electron microscopy*

Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. *Nature,* **2006**, 440, 935

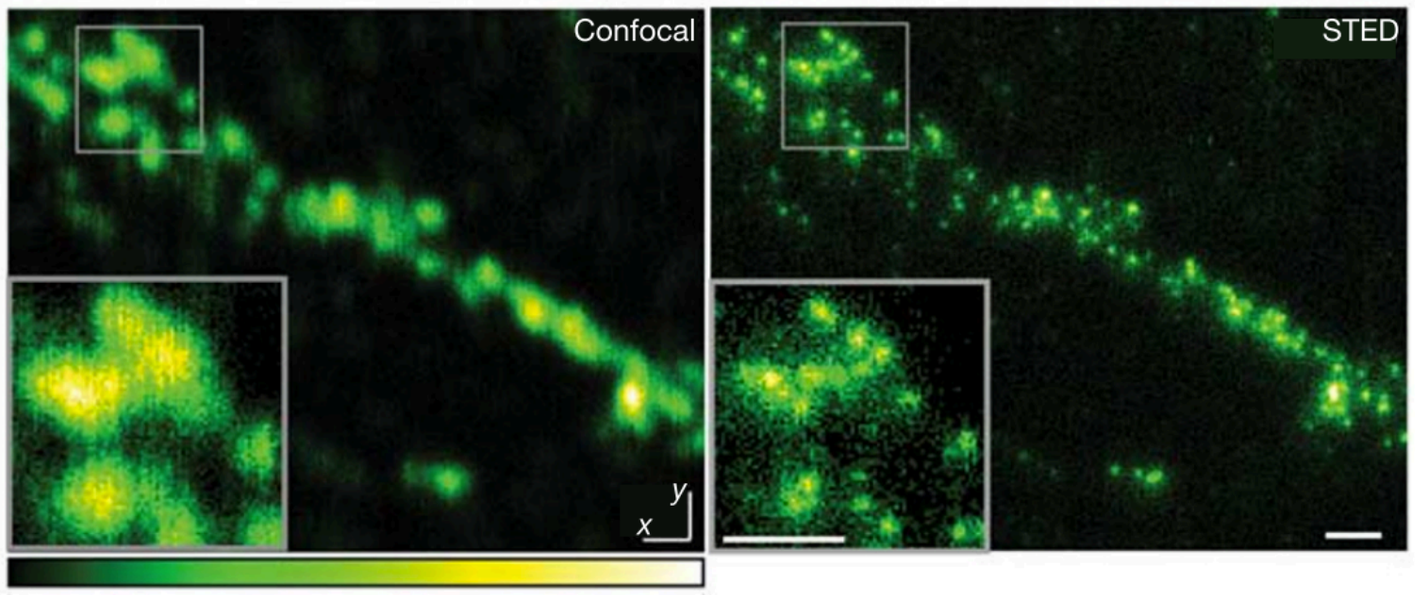
STED Microscopy to Study the Neurosynapse



- *Synaptotagmin is associated with vesicles*

Immunostain synaptotagmin to visualize vesicle distribution

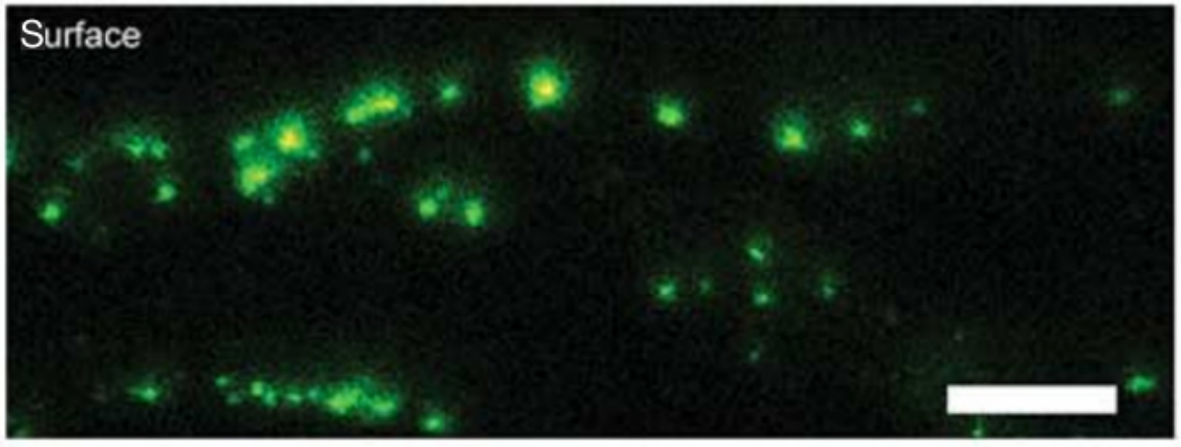
Confocal vs STED image of a rat neuron labeled with anti-synaptotagmin antibodies



- *Synaptigoamin remains clusters on membrane after exocytosis*

Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. *Nature,* **2006***,* 440, 935

STED Microscopy to Study the Neurosynapse



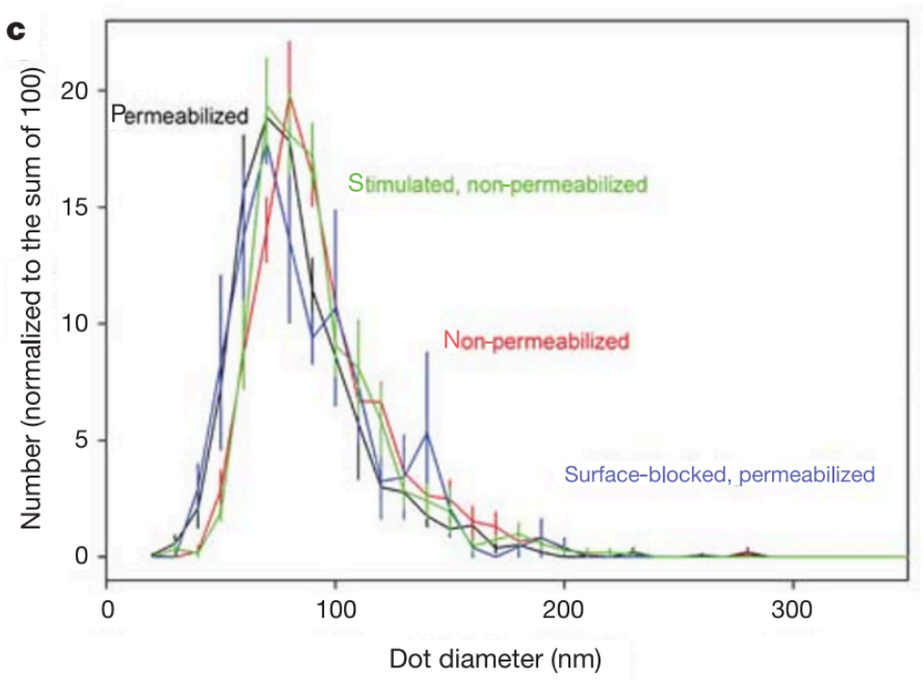
- Synaptotagmin remains clustered even at strong stimulation (70 mM KCl)

Are clusters individual vesicles or aggregates?

Majority of clusters 70-85 nm in diameter

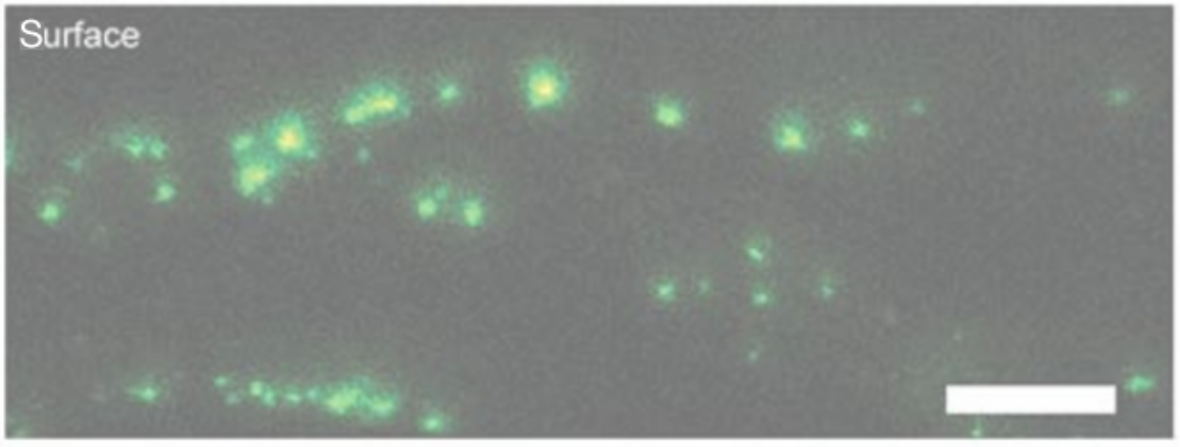
(35-40 nm observed with electron microscopy)

Surface and internalized patches are individual vesicles



Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. *Nature,* **2006**, 440, 935

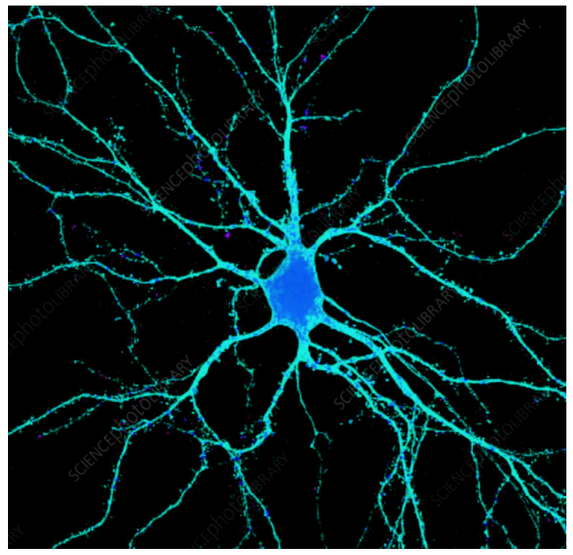
STED Microscopy to Study the Neurosynapse



- Synaptotagmin remains clustered even at strong stimulation (70 mM KCl)

Neurons function in networks

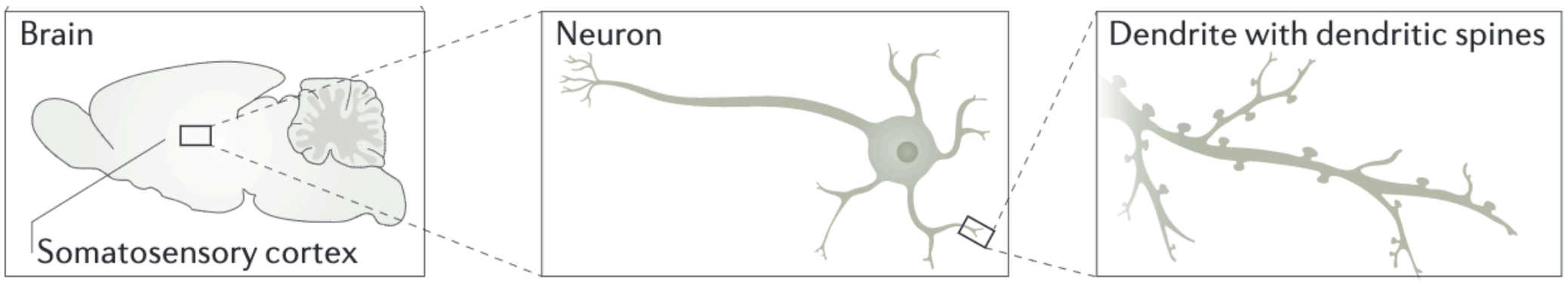
Unraveling of neuron dynamics requires in vivo imaging



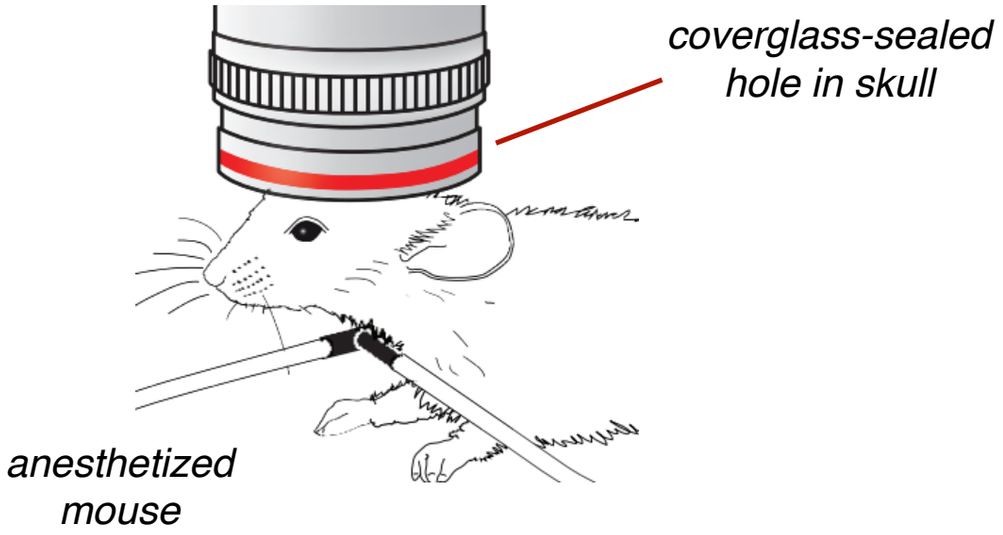
Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. *Nature,* **2006**, 440, 935

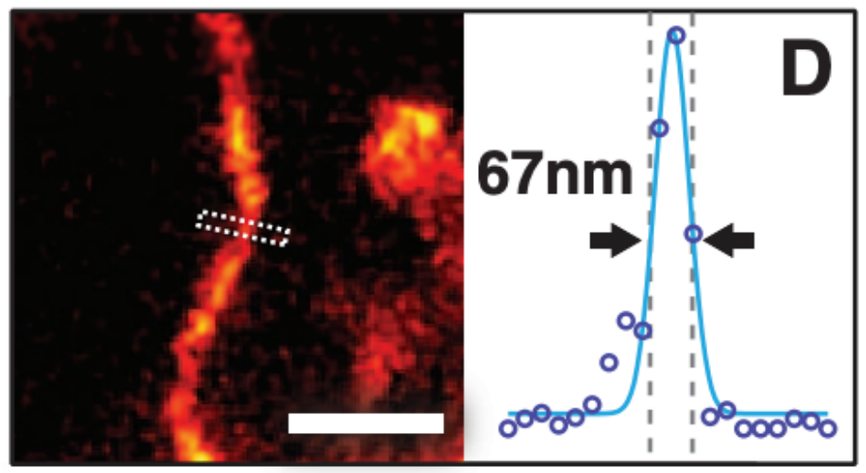
In vivo Optical Nanoscopy

2012: STED imaging of dendritic spines in a living mouse brain



Imaging of somatosensory context via transgenic expression of eYFP in neuronal cytoplasm

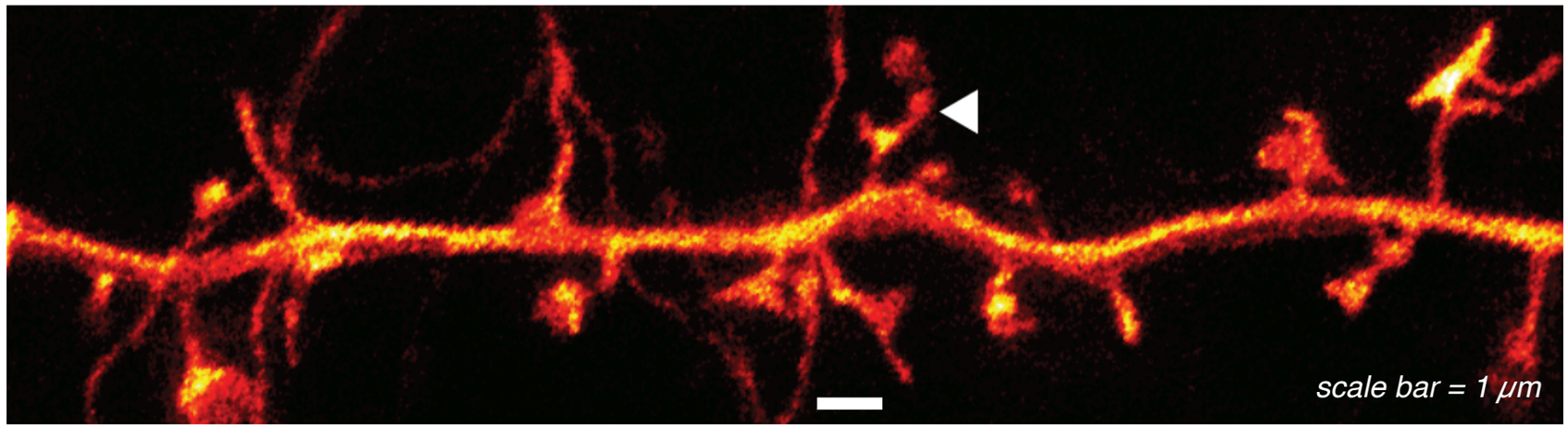




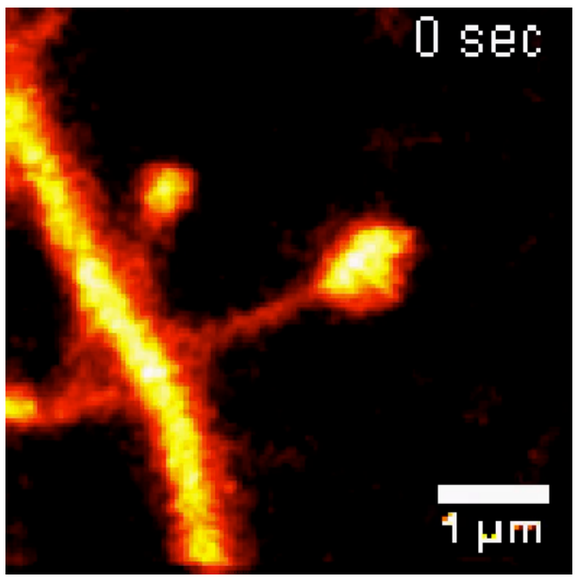
- **67 nm in vivo resolution observed**

Berning, S.; Willig, K. I.; Steffens, H.; Dibaj, P.; Hell, S. W. *Science* **2012**, 335, 551

In vivo Optical Nanoscopy



Clear visualization of dendritic structures on neurons



- ***dendritic spines undergo morphological changes***

⬇

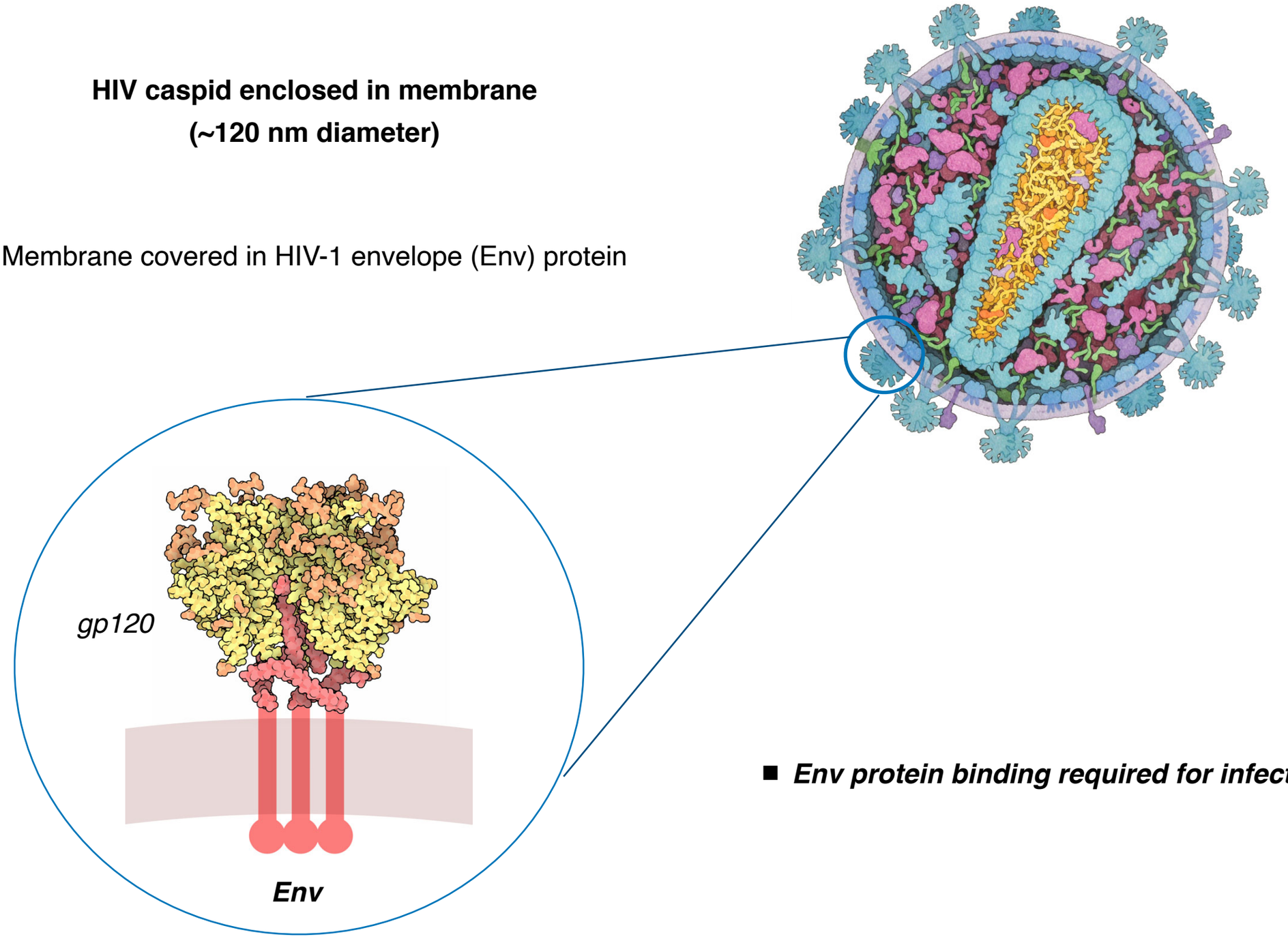
alteration in the neural network connectivity

Berning, S.; Willig, K. I.; Steffens, H.; Dibaj, P.; Hell, S. W. *Science* **2012**, 335, 551

STED to Elucidate HIV Viral Entry

HIV caspid enclosed in membrane
(~120 nm diameter)

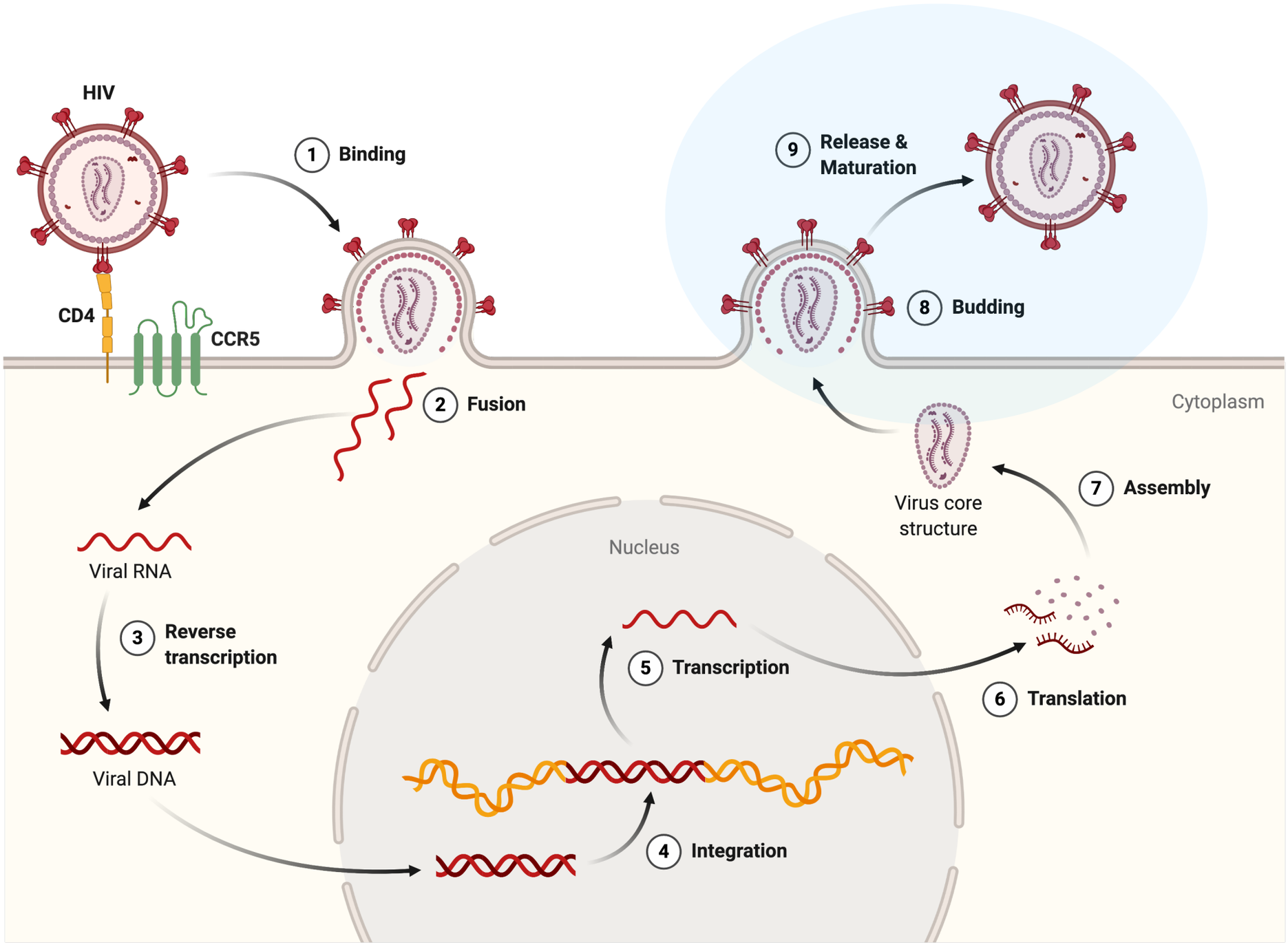
Membrane covered in HIV-1 envelope (Env) protein



- ***Env protein binding required for infectivity***

Chojnacki, J. *et al*. *Science* **2012**, 338, 524

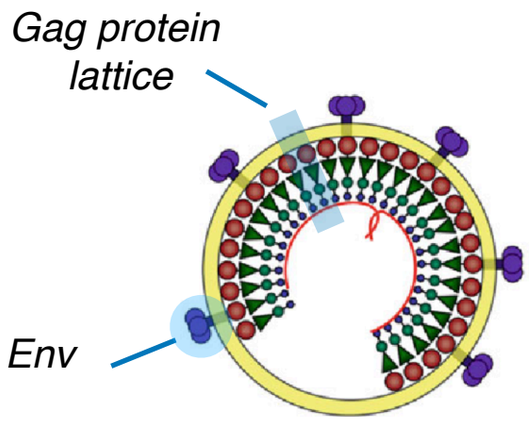
STED to Elucidate HIV Viral Entry



Created with BioRender.com

Chojnacki, J. *et al. Science* **2012**, 338, 524

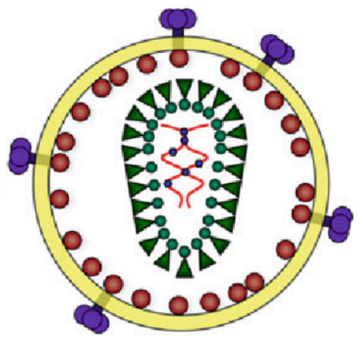
STED to Elucidate HIV Viral Entry



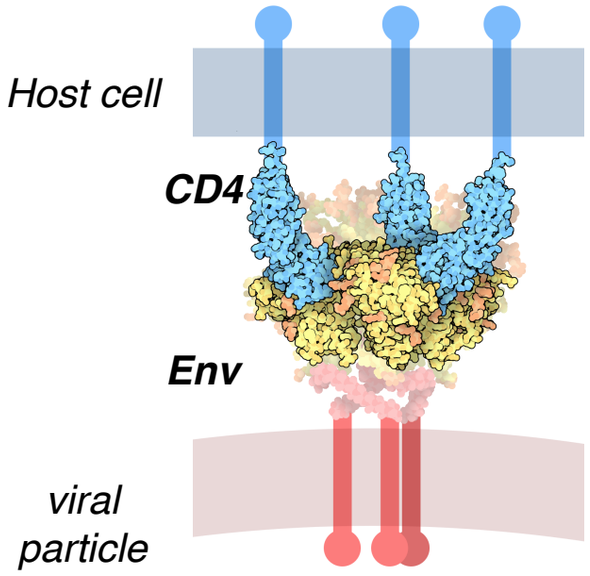
budding/immature viral particle

HIV protease cleaves Gag protein lattice

Morphological maturation



Mature HIV particle



- *immature particles show reduced infectivity*
- *no differences in Env structure or abundance in immature particles*

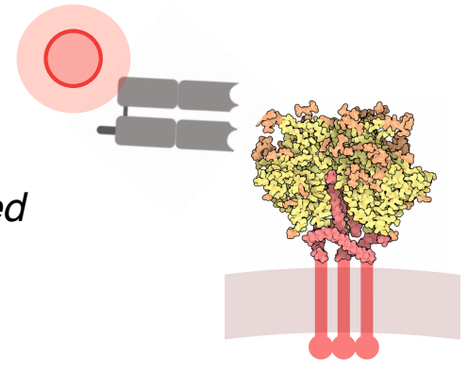
Does Env surface distribution account for this difference?

Chojnacki, J. *et al. Science* **2012**, 338, 524

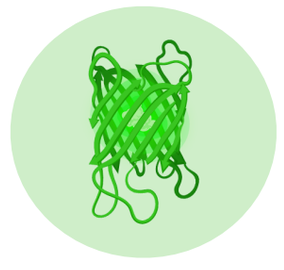
STED to Elucidate HIV Viral Entry

Does Env distribution differ in mature vs. immature viral particles?

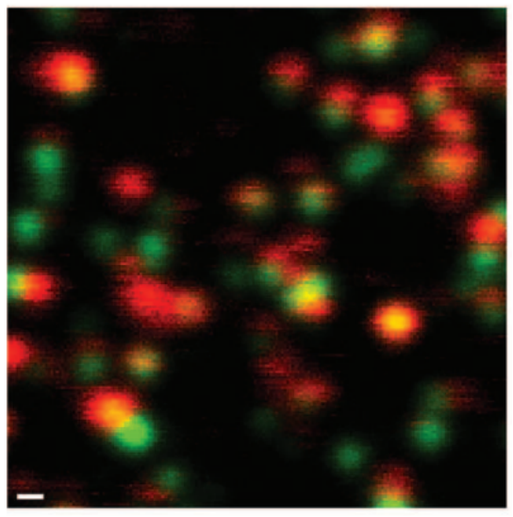
Atto 565-labeled 2G12 Fab (anti-gp120)



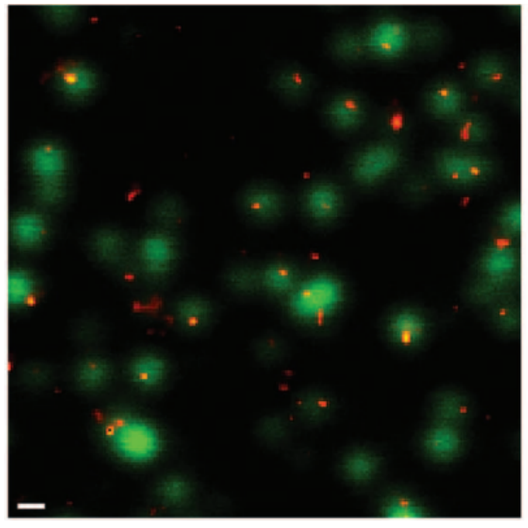
Viral particle counterstained with eGFP (via eGFP-Vpr fusion)



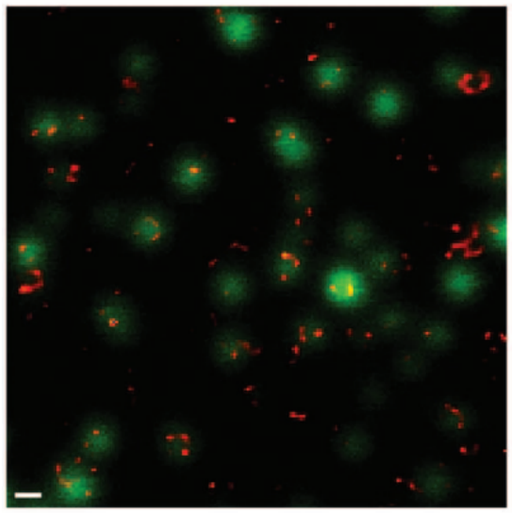
565 in confocal



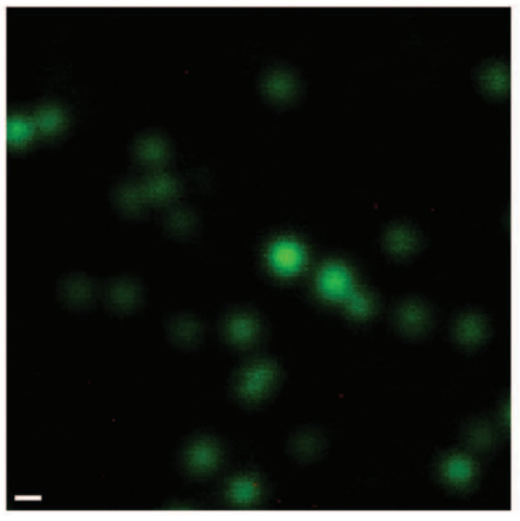
565 in STED



Immature virus



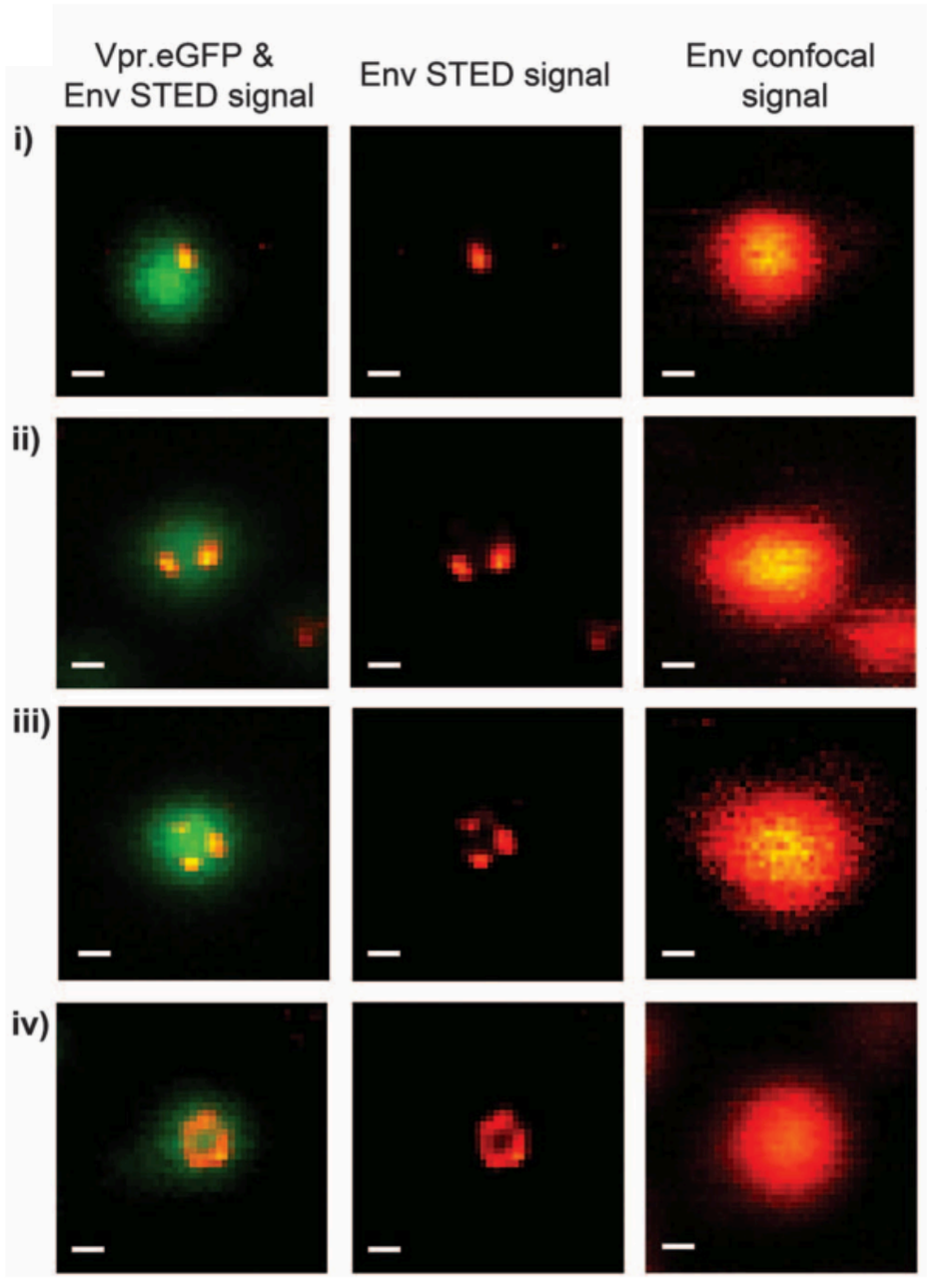
— Env



■ ***Single Env foci observed for mature while multiple foci for immature***

Chojnacki, J. *et al*. *Science* **2012**, 338, 524

STED to Elucidate HIV Viral Entry



- *Different Env localizations observed*

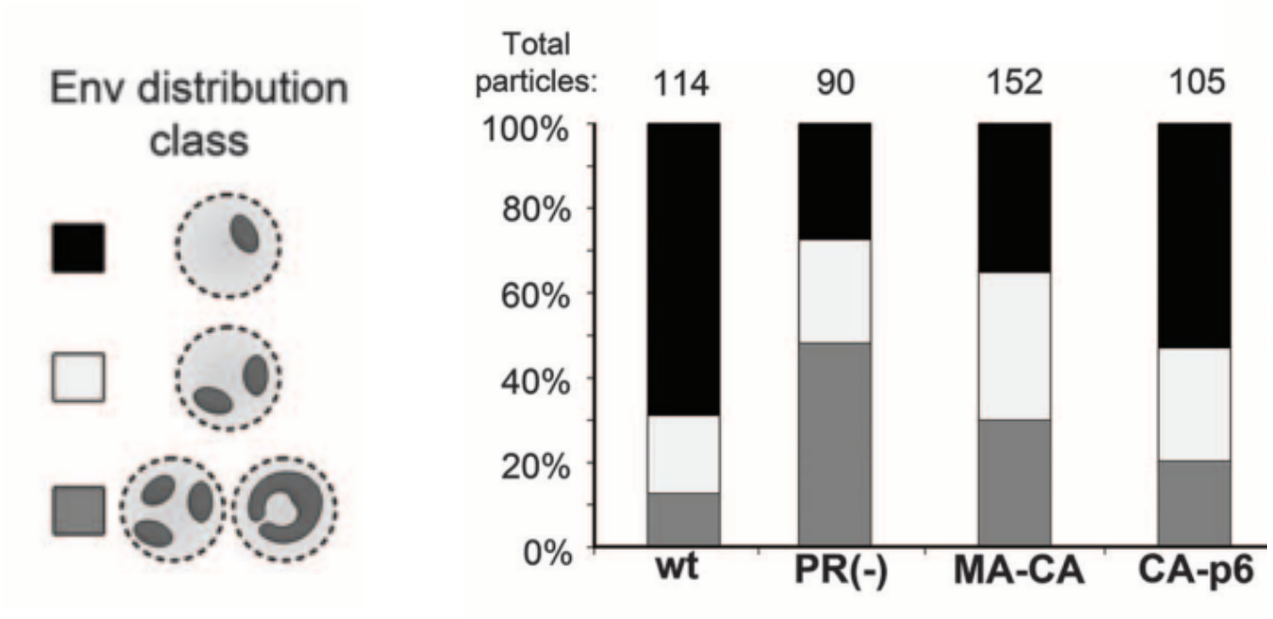
(i - iv)

How do the relative amounts of these distributions vary amongst particles?

Chojnacki, J. *et al*. *Science* **2012**, 338, 524

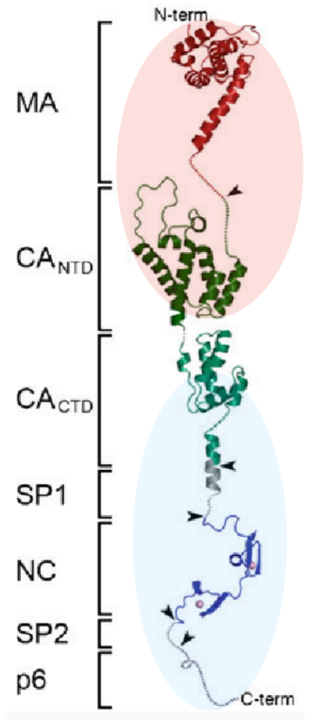
STED to Elucidate HIV Viral Entry

- ***Distribution of Env foci across viral particles***



Immature Particles *compromised Gag machinery*

Gag protein structure



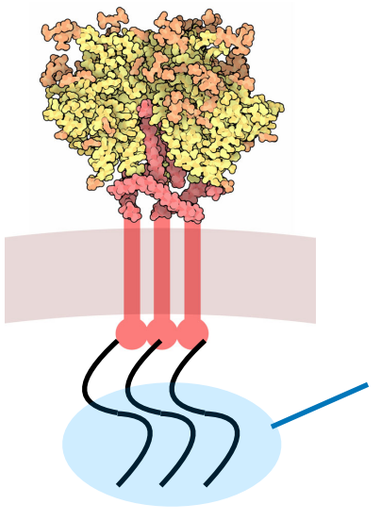
- ***70% of single foci found in mature particles***

- *less than 30% single foci in immature*

- *Surface distribution depends on Gag processing*

Chojnacki, J. *et al*. *Science* **2012**, 338, 524

STED to Elucidate HIV Viral Entry



Long C-terminal tail
(151 animo acids)

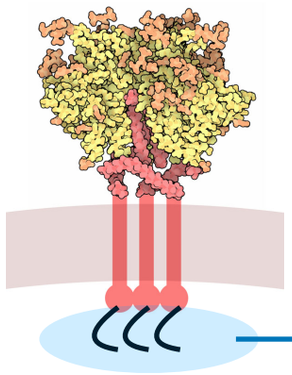
HIV protease cleaves
Gag protein lattice

→

Clustering of Env

Does Env C-terminal tail underly Env clustering?

*Mutant Env with C-terminal truncation **(EnvΔCT)***



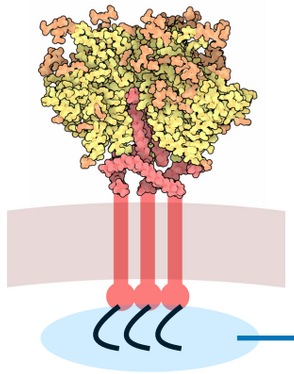
Truncated C-terminal mutant

How does C-terminal truncation influence clustering?

Chojnacki, J. *et al. Science* **2012**, 338, 524

STED to Elucidate HIV Viral Entry

*Mutant Env with C-terminal truncation **(EnvΔCT)***

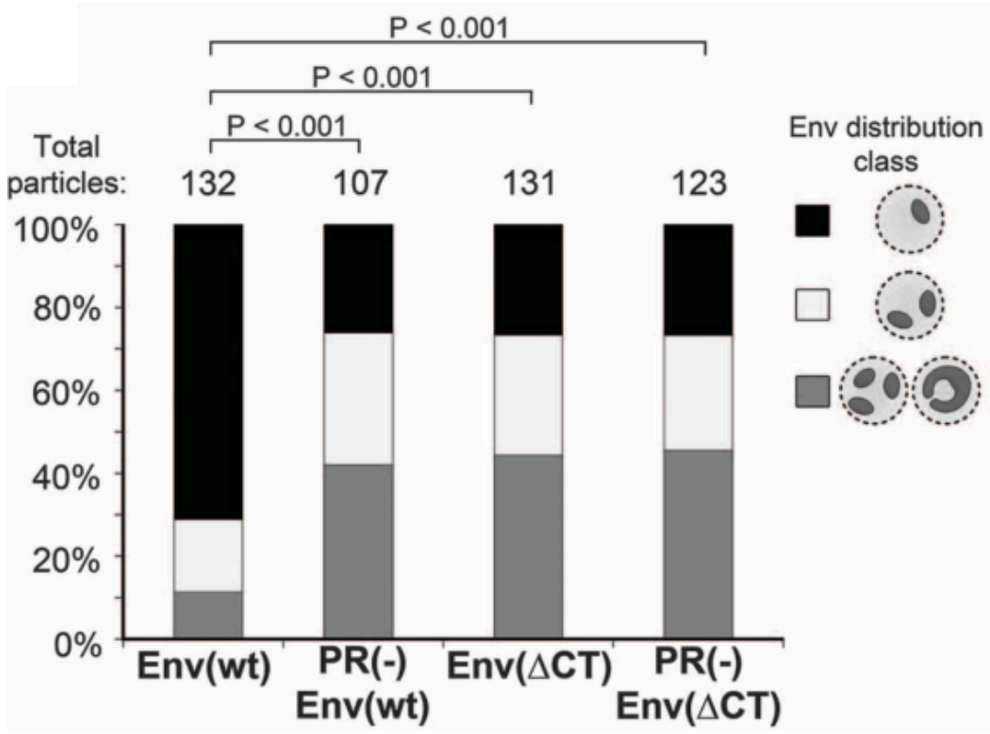


Truncated C-terminal mutant

How does C-terminal truncation influence clustering?

- No clustering observed in truncated mutants

Elongated C-terminal tail induces Env clustering



Chojnacki, J. *et al. Science* **2012**, 338, 524

STED to Elucidate HIV Viral Entry

- ***multi-color STED to image viral particle binding to host cells***

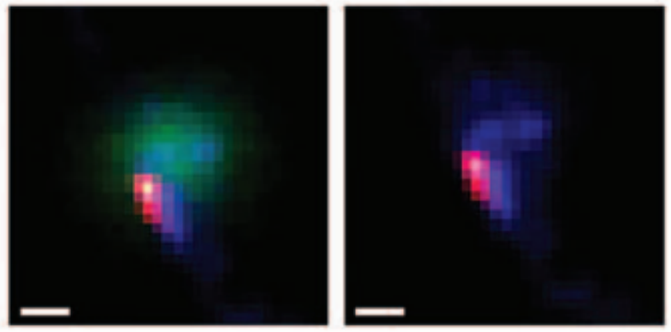
Viral particle

eGFP *Env*

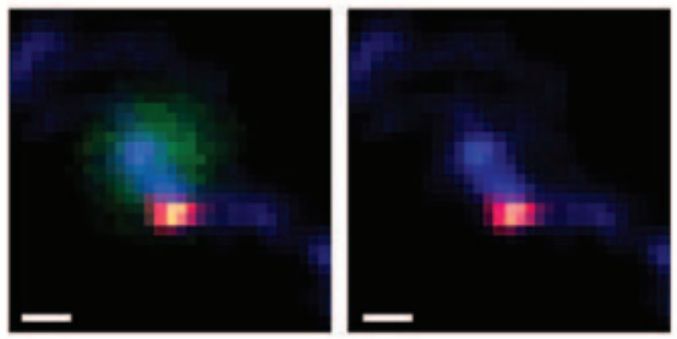
Immune cell

CD4 (host cell)

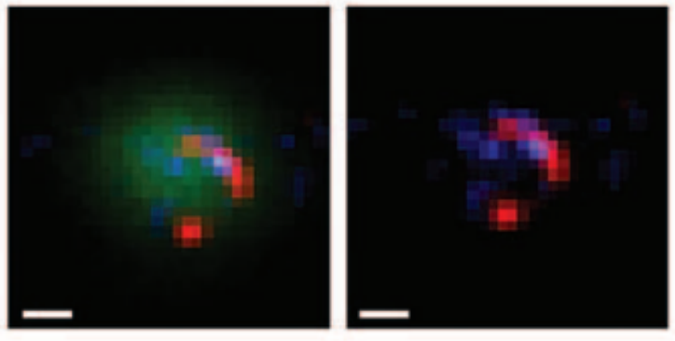
Mature Env(wt)



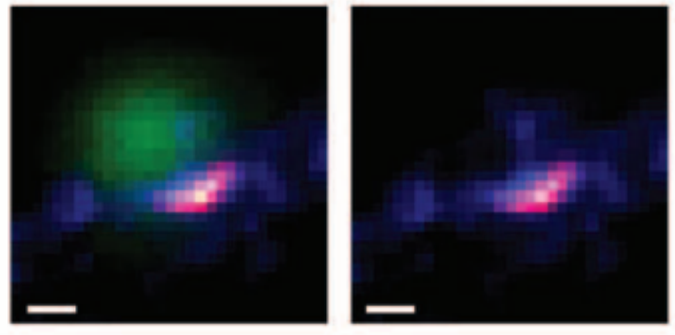
Mature EnvΔCT



immature Env(wt)



immature EnvΔCT



Env clustering observed in mature wt

Env clustering observed in mature and immature ΔCT particle binding

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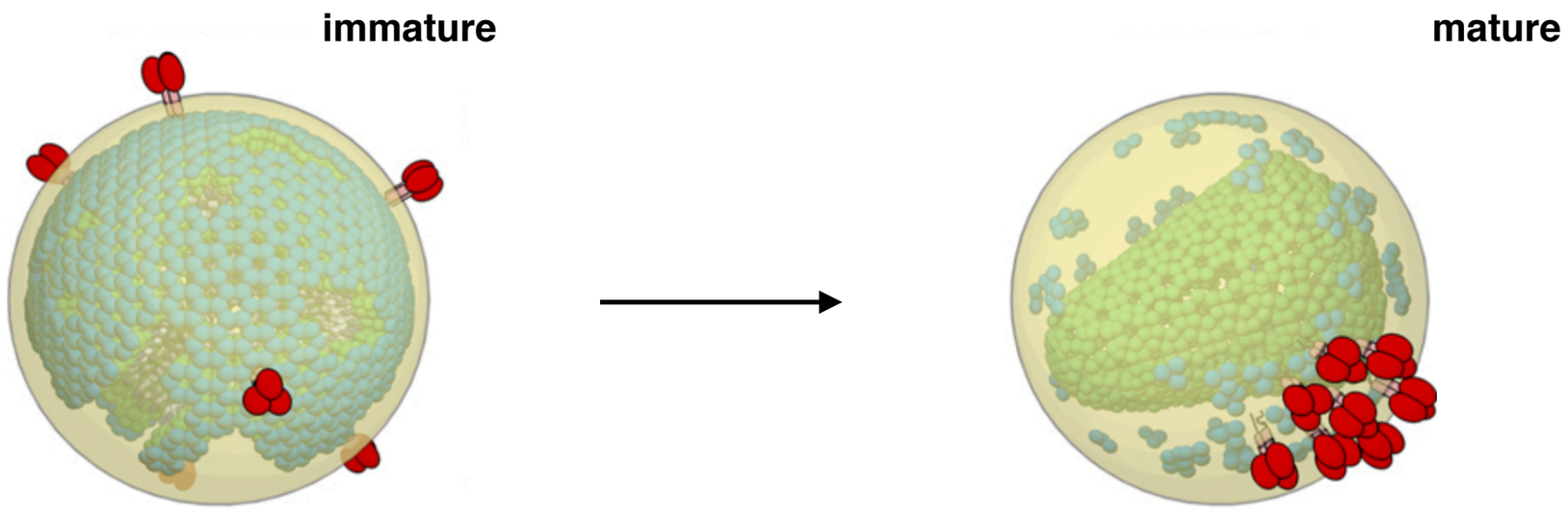
Working Model for Viral Polarization upon Maturation



"inside-out" signaling mechanism

- Gag lattice cleavage allows Env clustering

Only HIV particles with properly formed caspids are capable of viral entry

Chojnacki, J. *et al*. *Science* **2012**, 338, 524

Presentation Overview

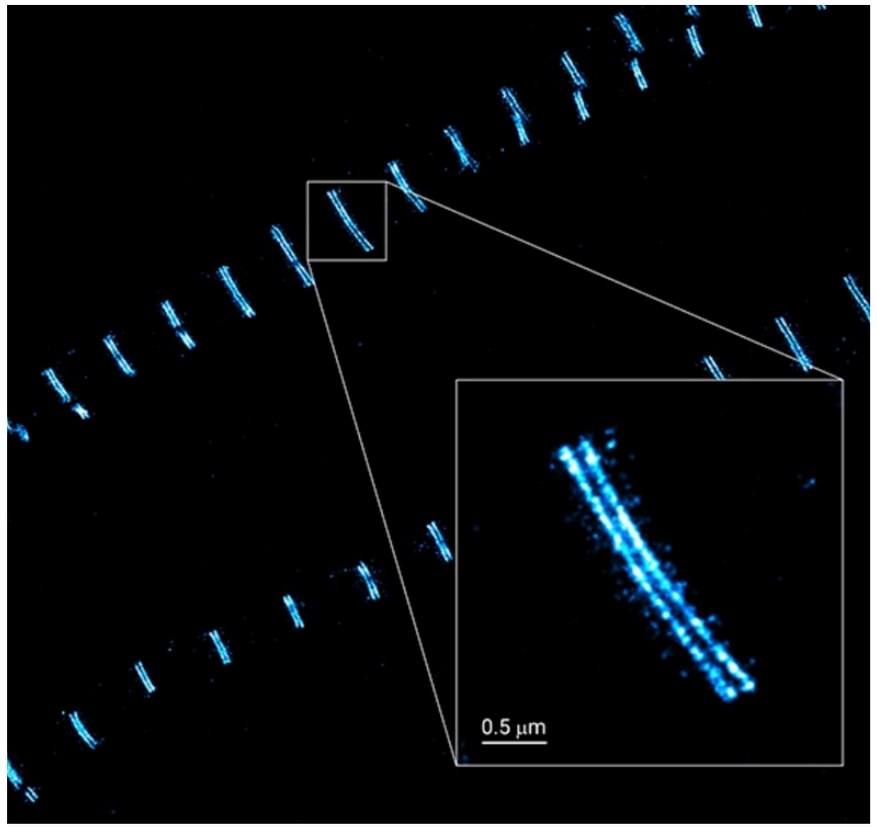
I. Introduction
- Light microscopy in biology
- Scales of biological systems

II. The Diffraction Barrier
- Diffraction
- The Point Spread Function
- The Abbe Equation

III. Stimulated Emission Depletion Microscopy
- STED Theory
- Breaking the Diffraction Barrier
- Selected Publications

IV. Single Molecule Localization Microscopy
- **Super localization**
- **SMLM Theory**
- **PALM**
- **STORM**
- **Selected Publications**

V. Outlook



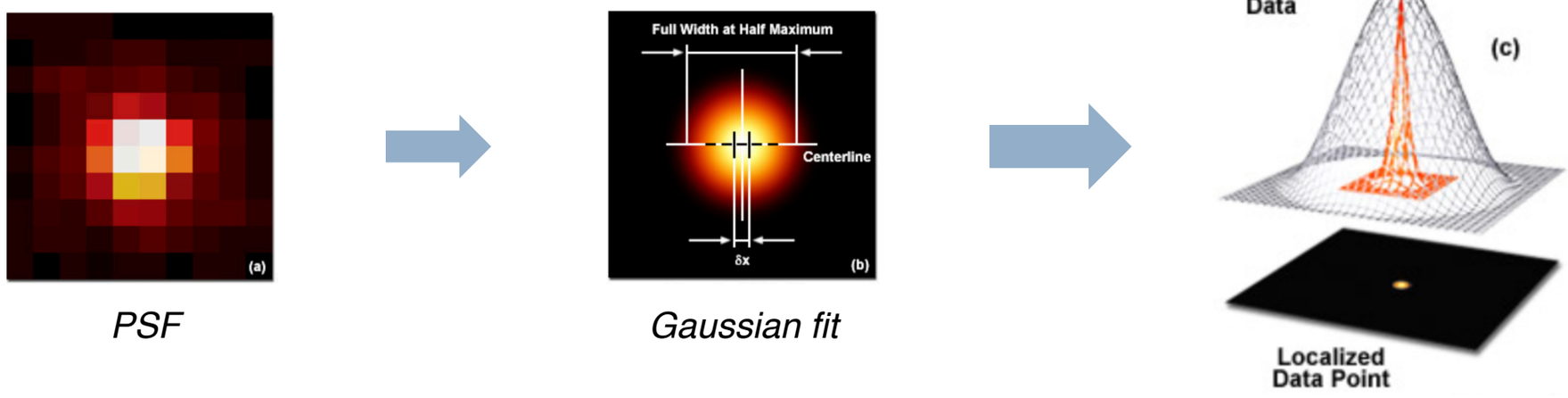
dSTORM image of rabbit psoas myofibrils labeled with the titan antibody T12-AF647

Superlocalization

- ***Center of a diffraction-limited PSF can be determined via curve fitting***

fit Gaussian curve to pixel distribution

localize center

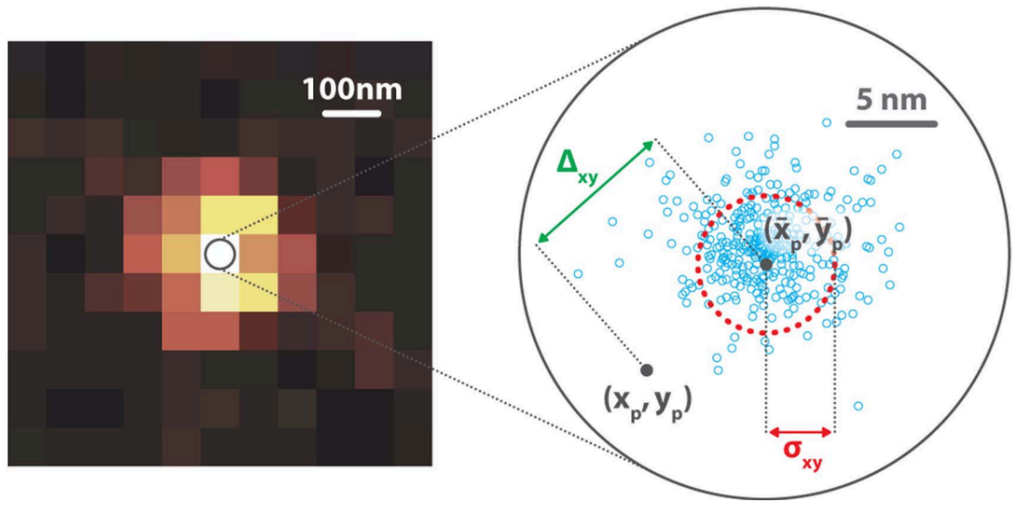


PSF

Gaussian fit

Superlocalization

- ***single emitter coordinates (x_P, y_P) can be estimated from multiple measurements***



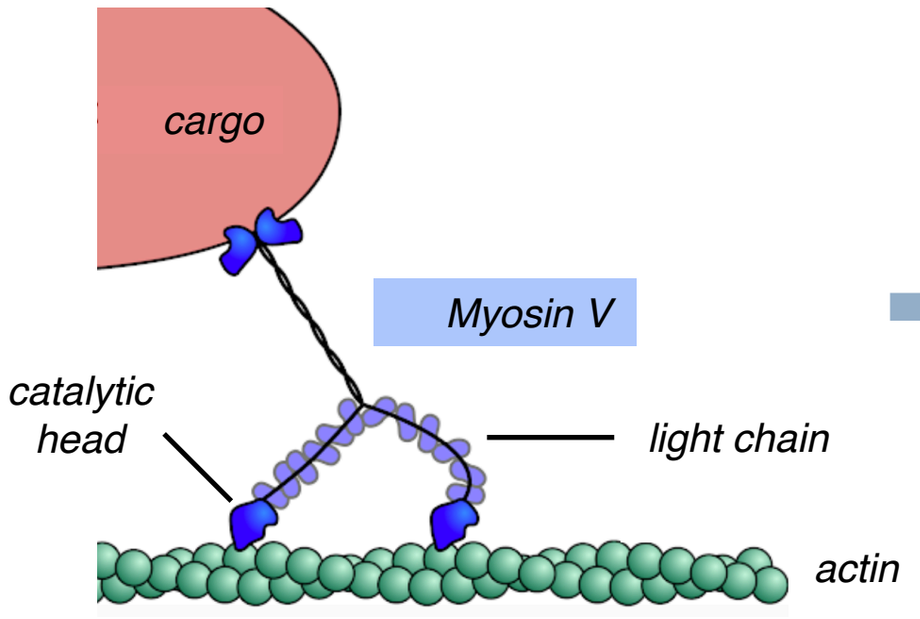
- ***Localization precision depends on number of collected photons***

$$\sigma_{xy}^2 = \left(\frac{s^2}{N_{coll}}\right) + \left(\frac{a^2/12}{N_{coll}}\right) + \left(\frac{8\pi s^4 b^2}{a^2 N_{coll}^2}\right)$$

N_{coll} = *number of collected photons*

Deschout, H.; Zanacchi, F. C.; Mlodzianoski, M.; Diaspro, A.; Bewerdorf, J.; Hess, S.; Braeckmans, K.. *Nat. Methods* **2014**,11,253-266

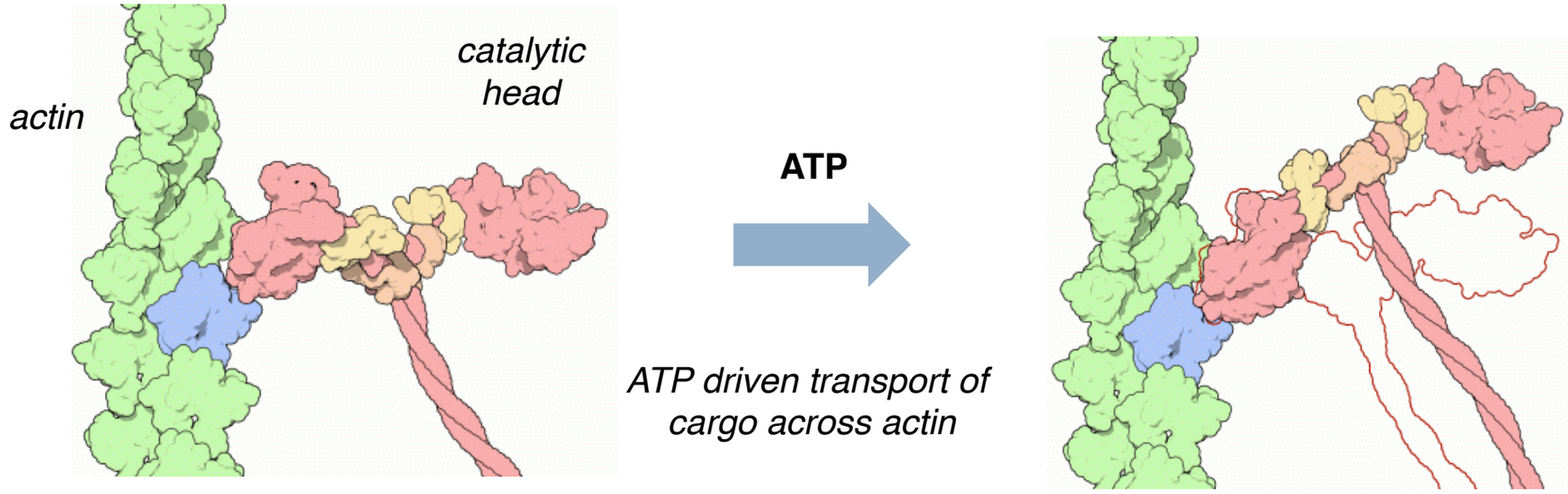
Motility of Myosin V



Mysosin V is a motor protein

■ *Transports organelles, secretory vesicles, mRNA, protein vesicles along the cytoskeleton*

MyoVa transports cargo along cytoskeleton (actin filaments) across the cell



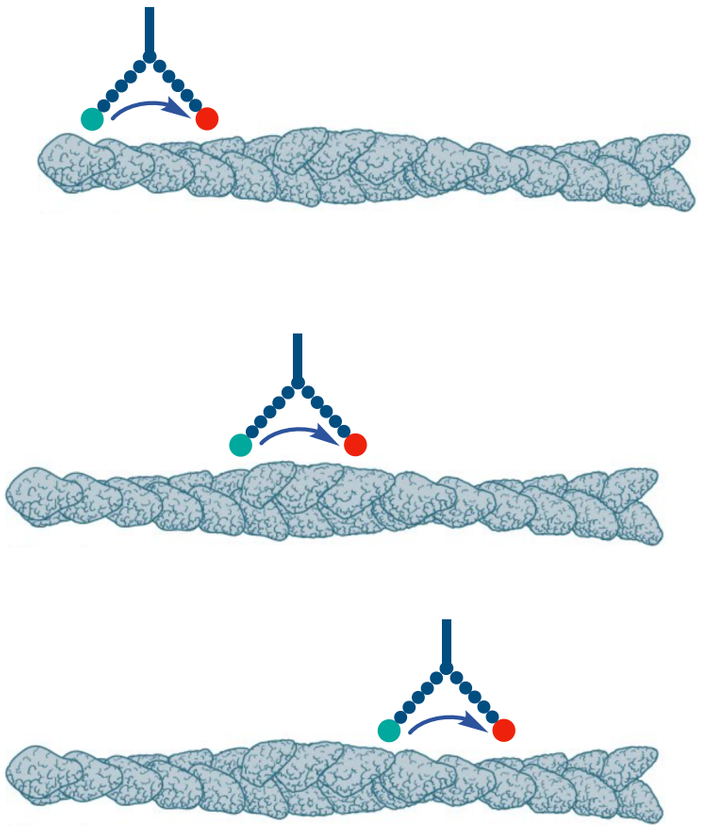
Dominguez, R.; Holmes, K. C. *Annu. Rev. Biophys*. **2011**, 40,169-186

Motility of Myosin V

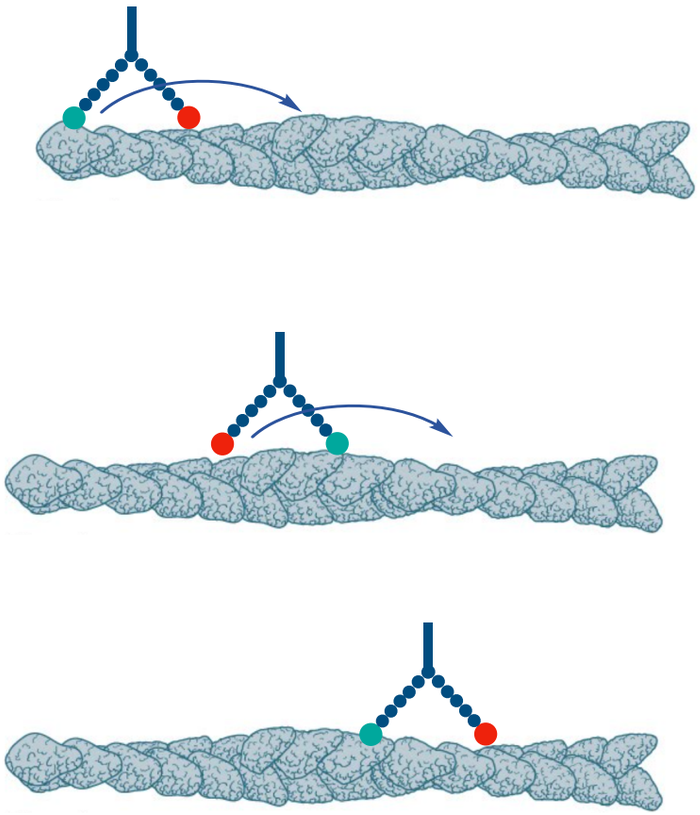
How do the two catalytic heads coordinate to produce steps?

Inchworm vs. Hand-over-hand model

Inchworm model



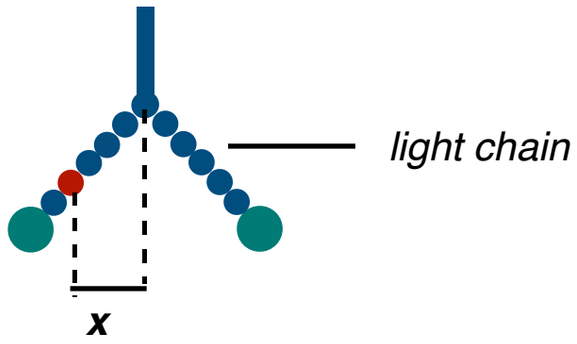
Hand-over-hand model



Dominguez, R.; Holmes, K. C. *Annu. Rev. Biophys*. **2011**, 40,169-186

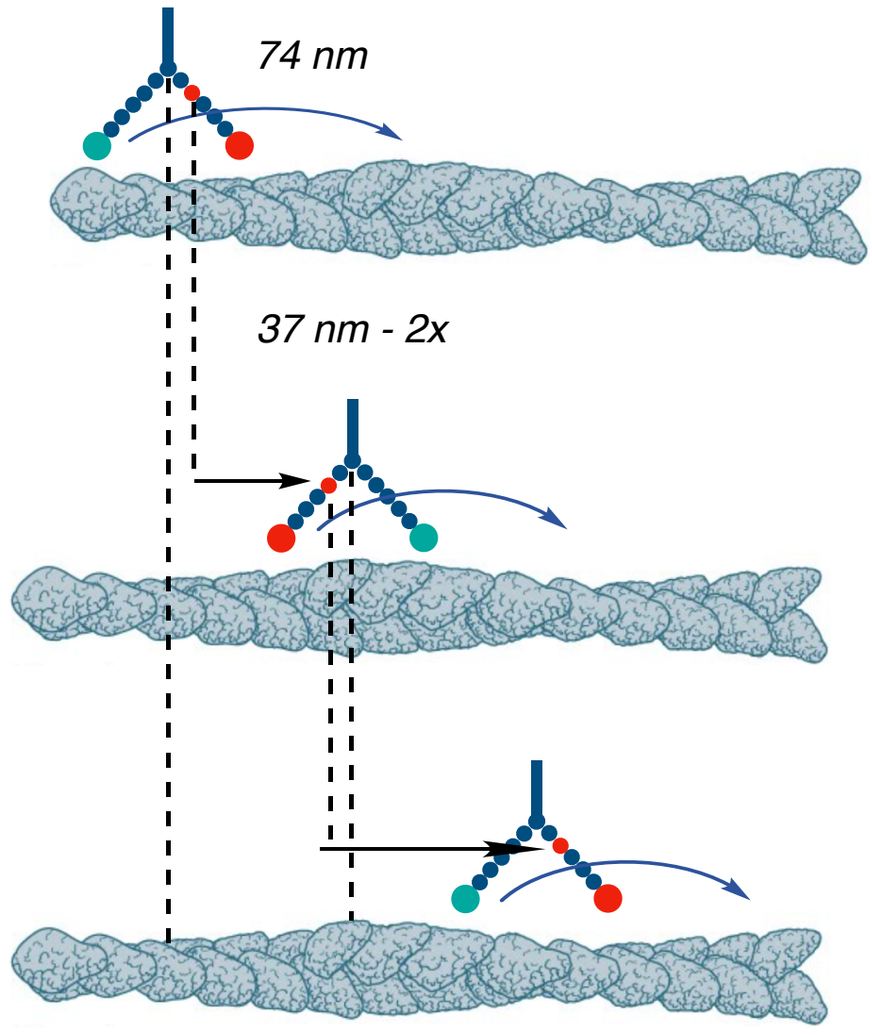
Motility of Myosin V

Hand-over-hand model



- *Geometry of each step moves point on light chain by **37 ± 2x nm***

Geometry of hand-over-hand model



Motility of Myosin V

2003: Single fluorophore imaging of Myosin V to elucidate motility

Fluorophore conjugated to light chain domain

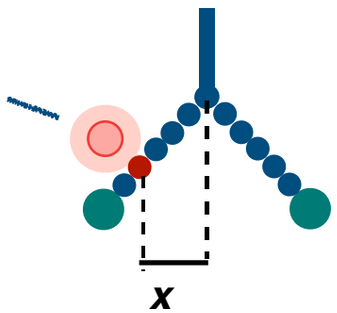
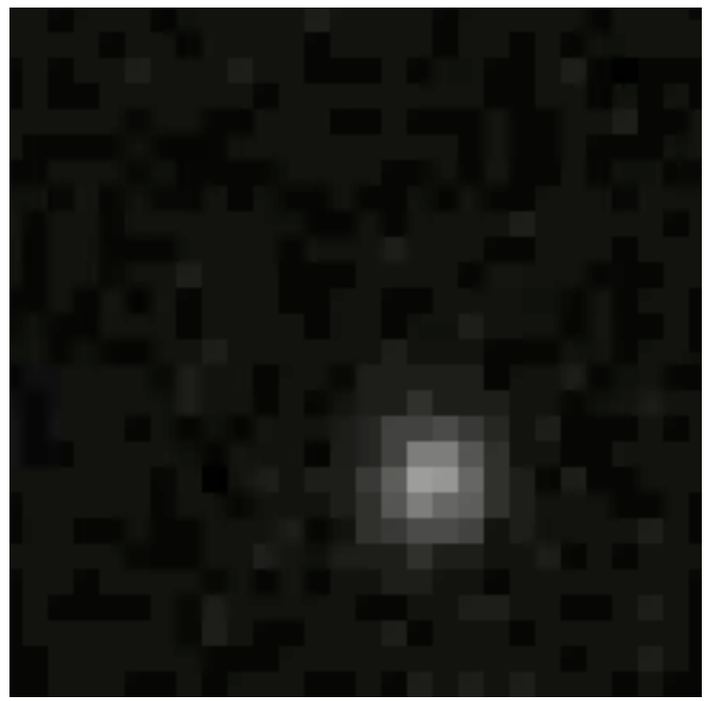


Image and localize emission signal via FIONA (fluorescence imaging with one nm accuracy)

Motion of single Cy3-labeled myosin V molecule



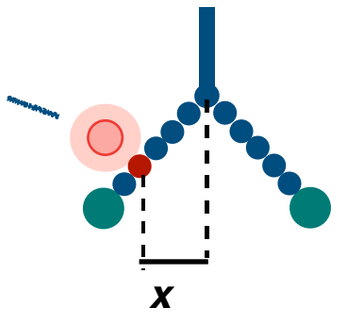
- *'Steps' of 74 nm observed within ± 3 nm precision*

Yildiz, a.; Forkey, J. N.; McKinney, S. A.; Ha, T.; Goldman, Y. E.; Selvin P. R. *Science* **2003**, 300, 2061-2065

Motility of Myosin V

2003: Single fluorophore imaging of Myosin V to elucidate motility

Fluorophore conjugated to light chain domain



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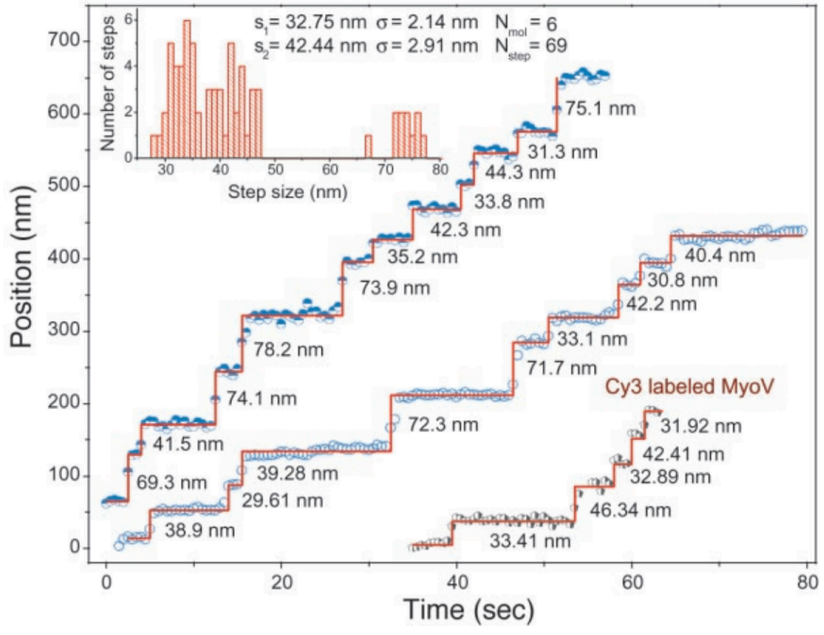


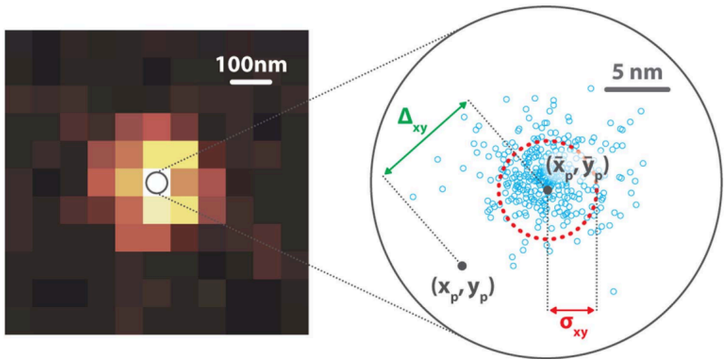
Motion traces for three individual Myosin V molecules

- *Emitters localized with up to 1.5 nm precision*
- *Alternating steps of 42 and 33 nm observed*

- ***37 ± 2x nm geometry observed, confirming hand-over-hand model for Myosin V***

Yildiz, a.; Forkey, J. N.; McKinney, S. A.; Ha, T.; Goldman, Y. E.; Selvin P. R. *Science* **2003**, 300, 2061-2065

Single Molecule Localization Microscopy



Super-localzation to determine fluorophore coordinates

Cannot create super resolution image if points cannot be resolved

Eric Betzig (1994): Utilize super localization to break the diffraction barrier

Eric Betzig
UC Berkely

Proposed method for molecular optical imaging

E. Betzig

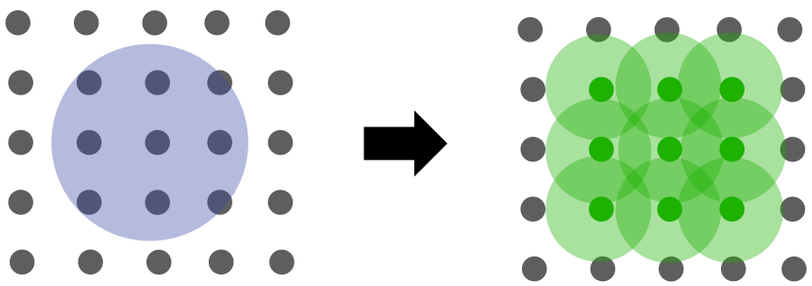
NSOM Enterprises, 17 Webster Drive, Berkeley Heights, New Jersey 07922

Received September 20, 1994

We can resolve multiple discrete features within a focal region of m spatial dimensions by first isolating each on the basis of $n \geq 1$ unique optical characteristics and then measuring their relative spatial coordinates. The minimum acceptable separation between features depends on the point-spread function in the $(m + n)$-dimensional space formed by the spatial coordinates and the optical parameters, whereas the absolute spatial resolution is determined by the accuracy to which the coordinates can be measured. Estimates of each suggest that near-field fluorescence excitation microscopy/spectroscopy with molecular sensitivity and spatial resolution is possible.

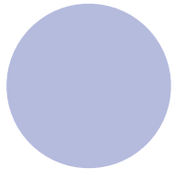
Betzig, E. *Opt. Lett.* **1994**, 20, 237

Single Molecule Localization Microscopy



Multiple fluorophores activated

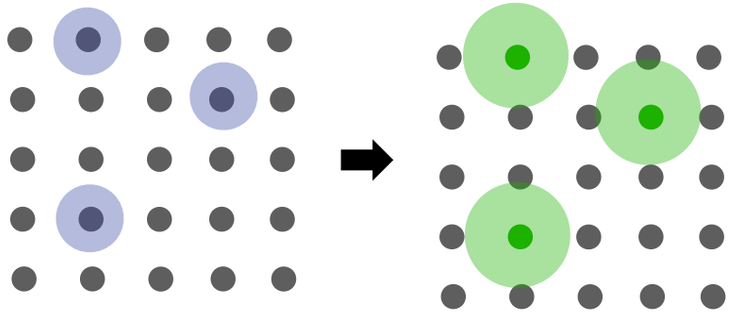
Diffraction-limited spots overlap



excitation beam

Single Molecule Localization Microscopy

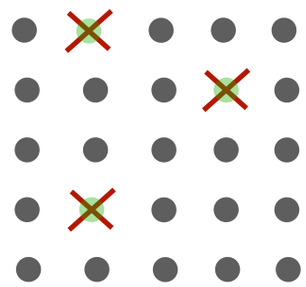
Spatially separated activation and localization of fluorophores



Activation of a subset of fluorophores

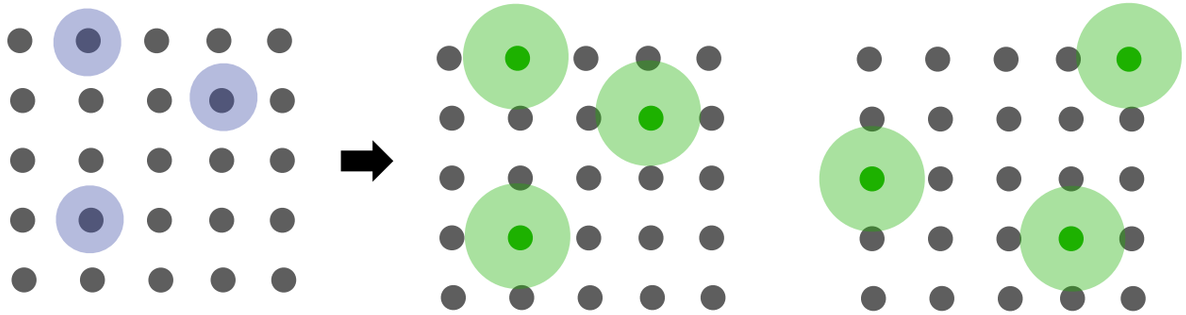
$$d > \frac{\lambda}{2n \sin\alpha}$$

fit and localize



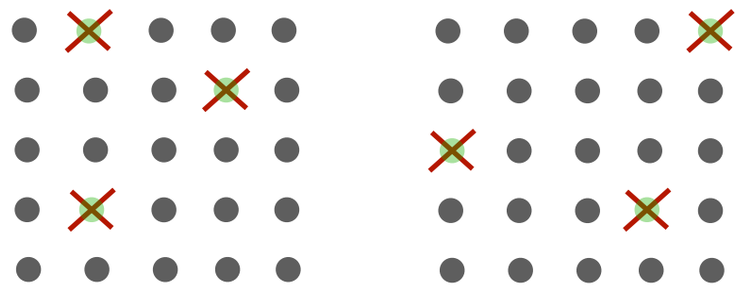
Single Molecule Localization Microscopy

Spatially separated activation and localization of fluorophores



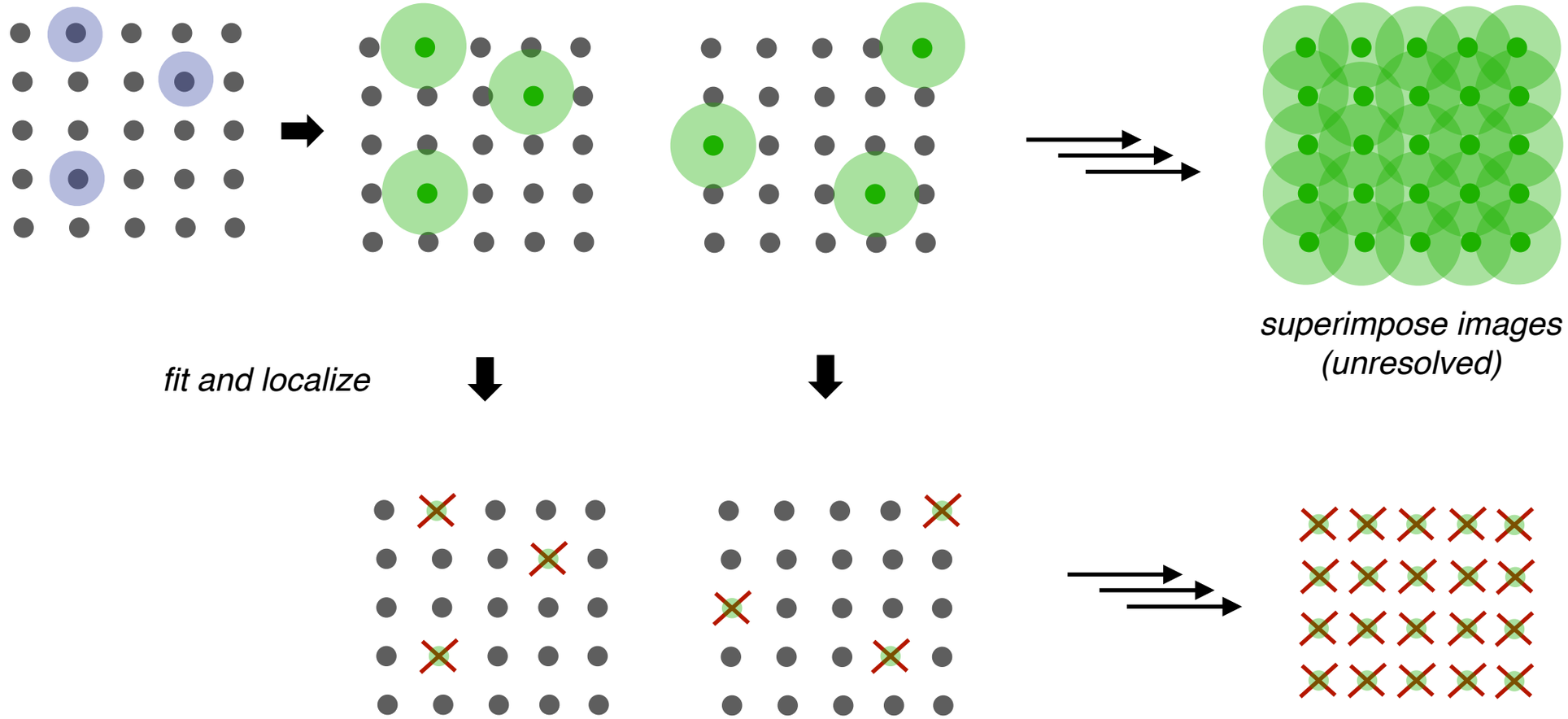
Repeat subset activation

fit and localize



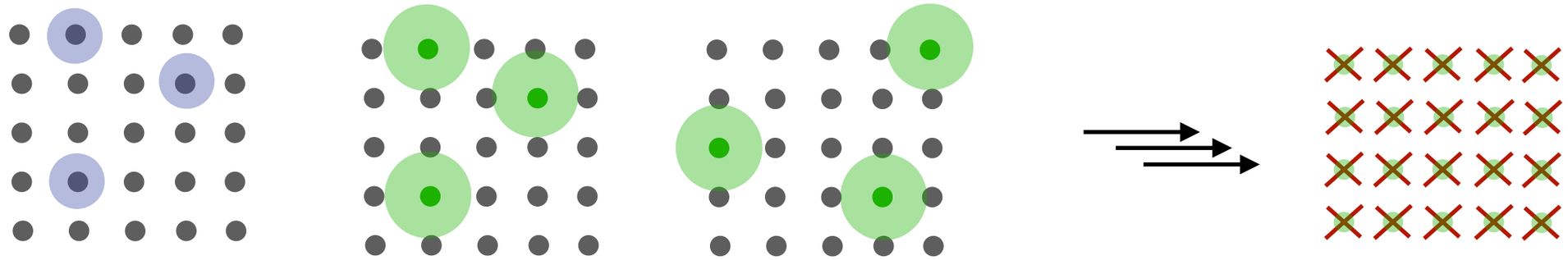
Single Molecule Localization Microscopy

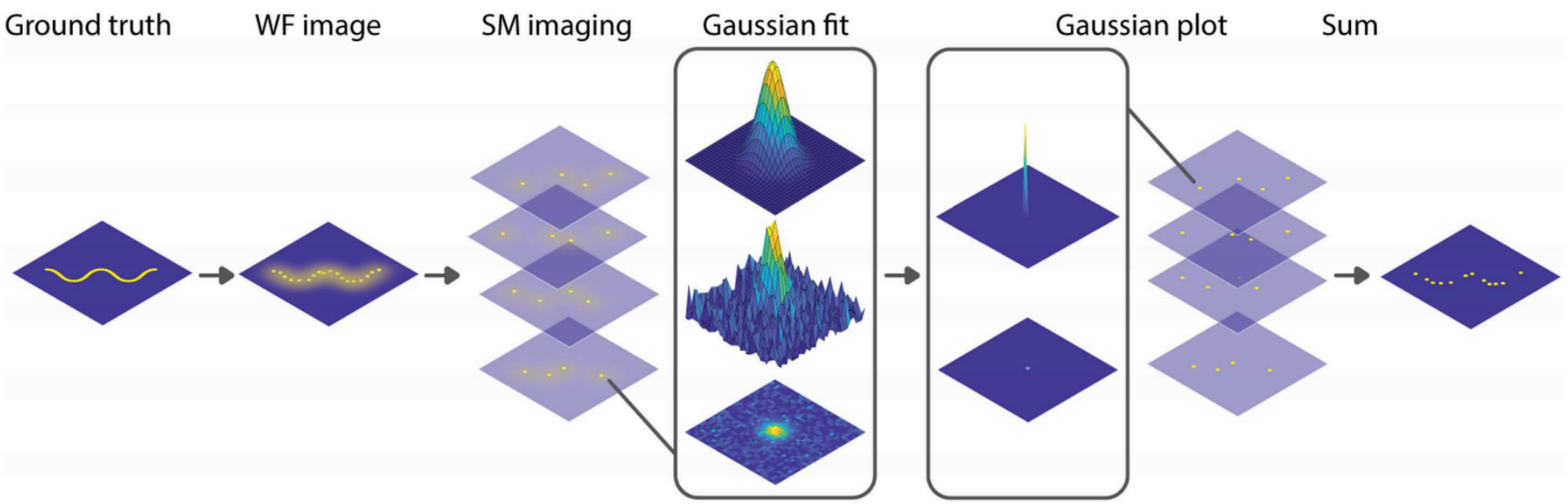
Spatially separated activation and localization of fluorophores



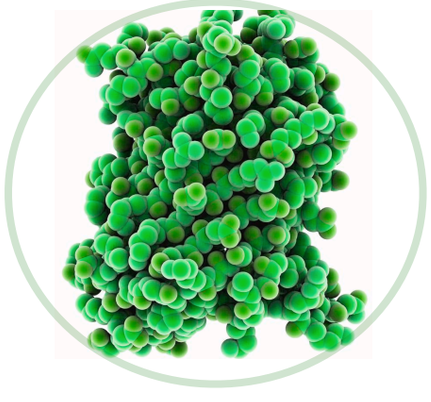
Single Molecule Localization Microscopy

Spatially separated activation and localization of fluorophores





Photoactivation of Green Fluorescent Protein

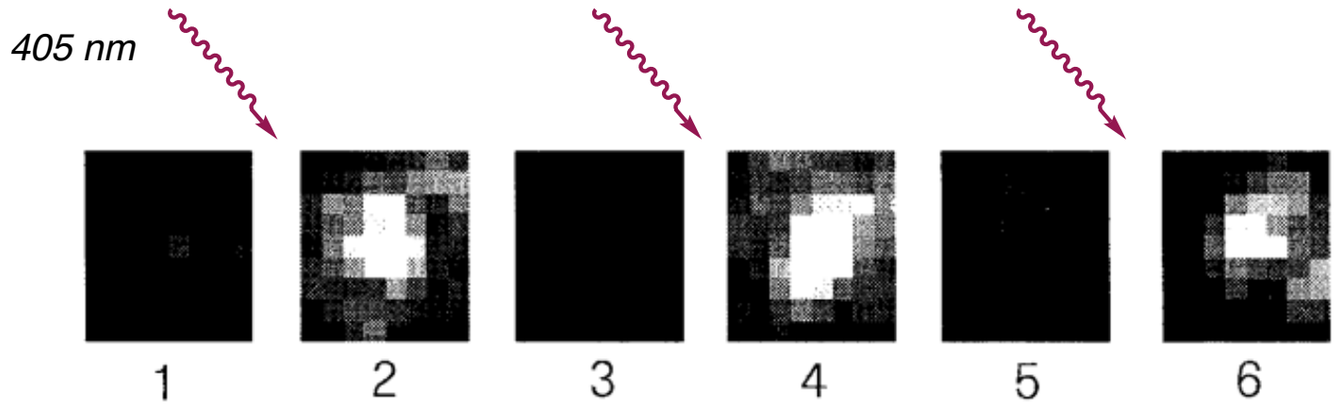


Green fluorescent protein (GFP)

- *highly biocompatible fluorophore*
- *2008 Nobel prize in chemistry*

William E. Moerner
Stanford University

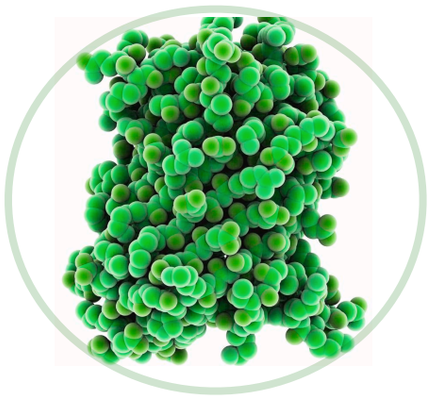
Photoactivation of GFP observed at the single molecule level



- *Fluorescence recovery from dark state with UV light*

Dickson, R. M.; Cubitt, A. B.; Tsien, R. Y.; Moerner, W. E. *Nature* ***1997***, 388,355-358

Photoactivation of Green Fluorescent Protein



Green fluorescent protein (GFP)

- *highly biocompatible fluorophore*
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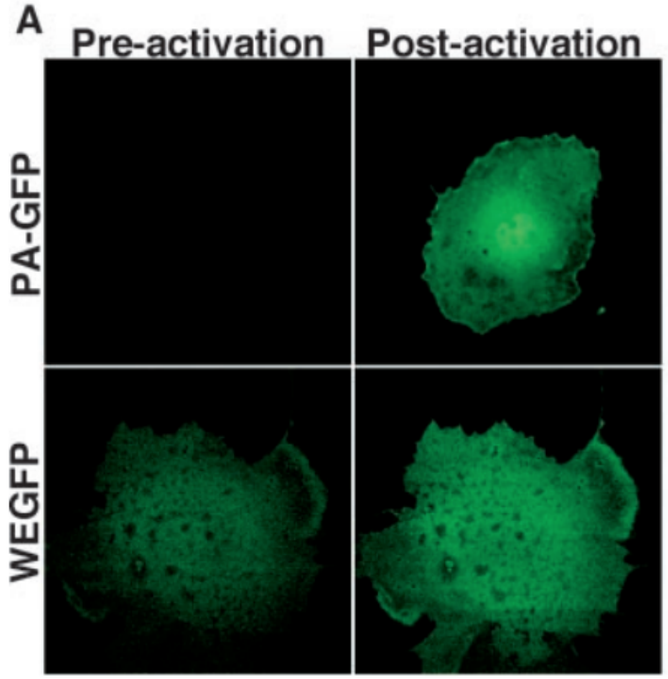
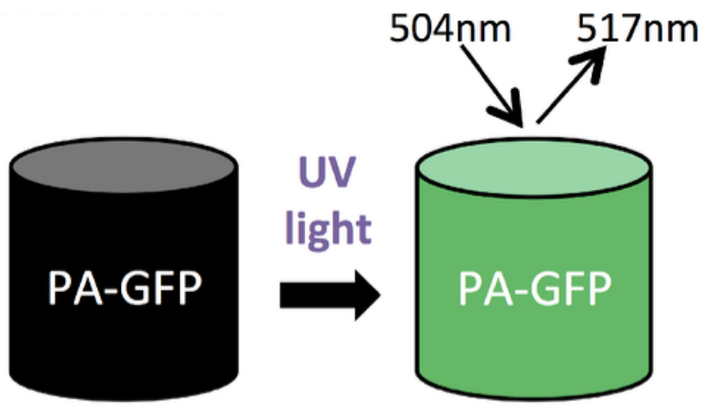


(2002) Development of photoactivatable GFP (PA-GFP)



dark state *fluorescent state*

Patterson, G. H.; Lippincott-Schwartz, J. *Science* ***2002***, 297,1873-1877

Photoactived Localization Microscopy (PALM)

Photoactivated Localization Microscopy (PALM)

Development of first PALM microscope (La Jolla labs, 2006)

Activate subset of GFP

↓

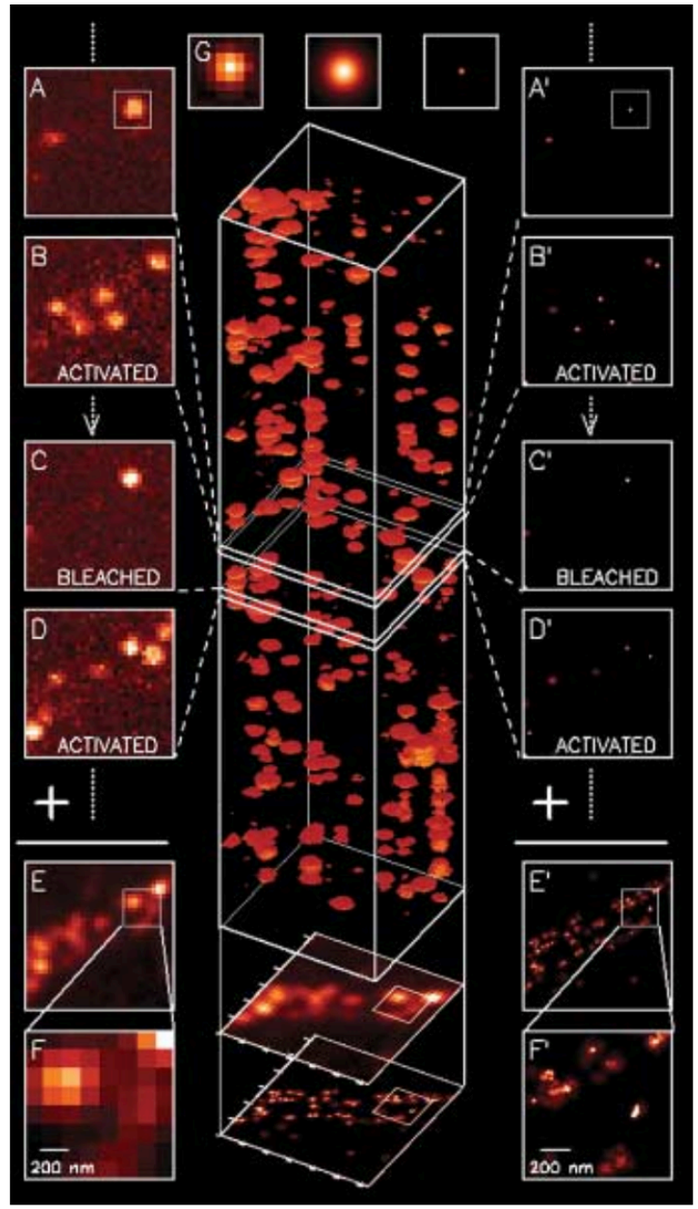
Image till photobleach

↓

Localize signals

Diffraction limited

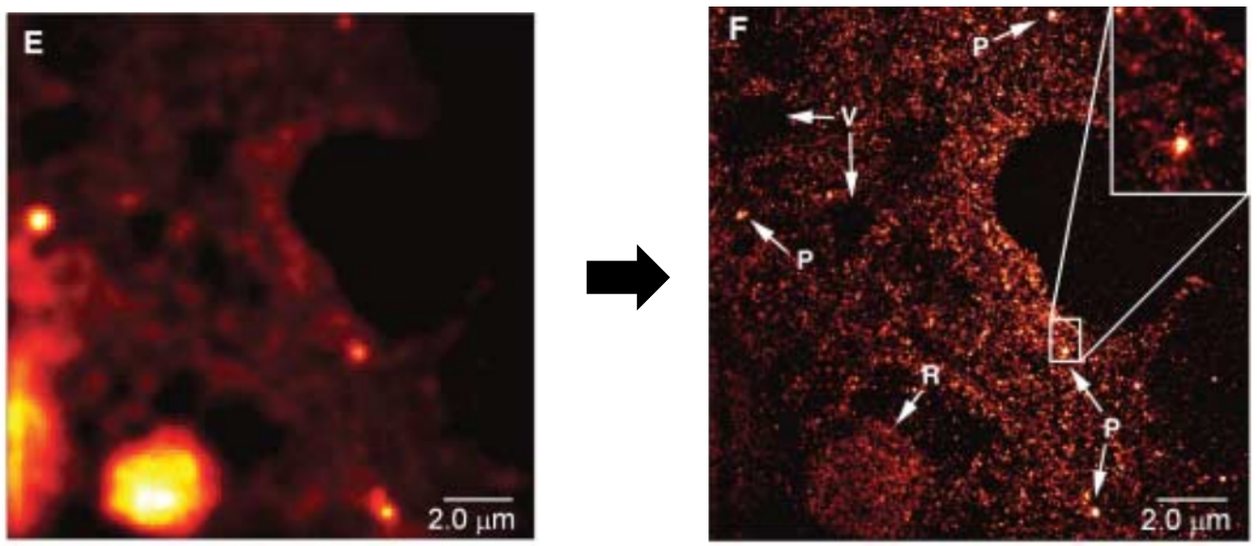
Super-localized



Betzig, E.; Patterson *et al*. *Science* **2006**, 313, 1642

Photoactived Localization Microscopy (PALM)

TIRF (DL) ***PALM***

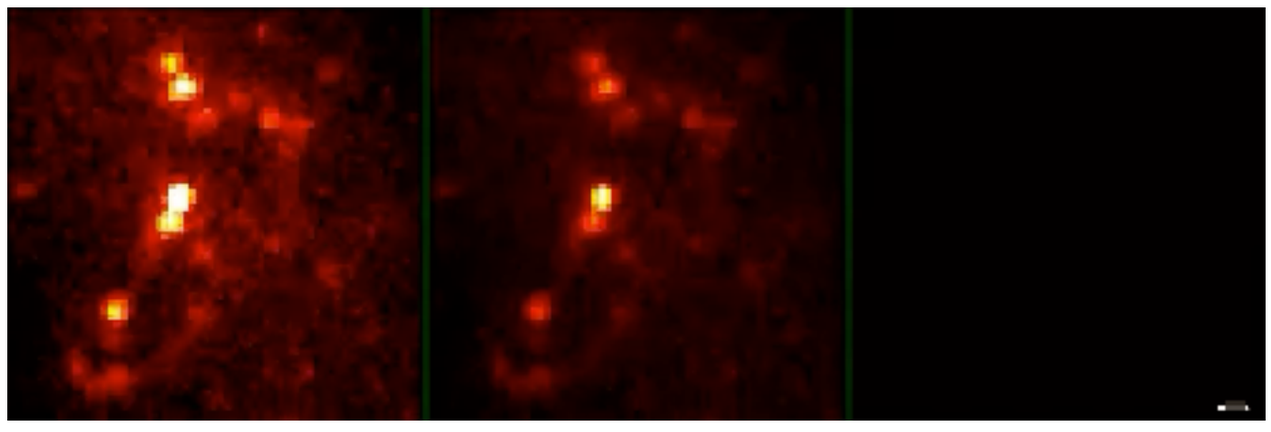


2006: Single molecule localization allows super-resolution imaging

- *20 nm resolution in the image plane achieved*

COS-7 cell expressing the retroviral protein Gag tagged with dEos

Image frames *DL image* *PALM image*



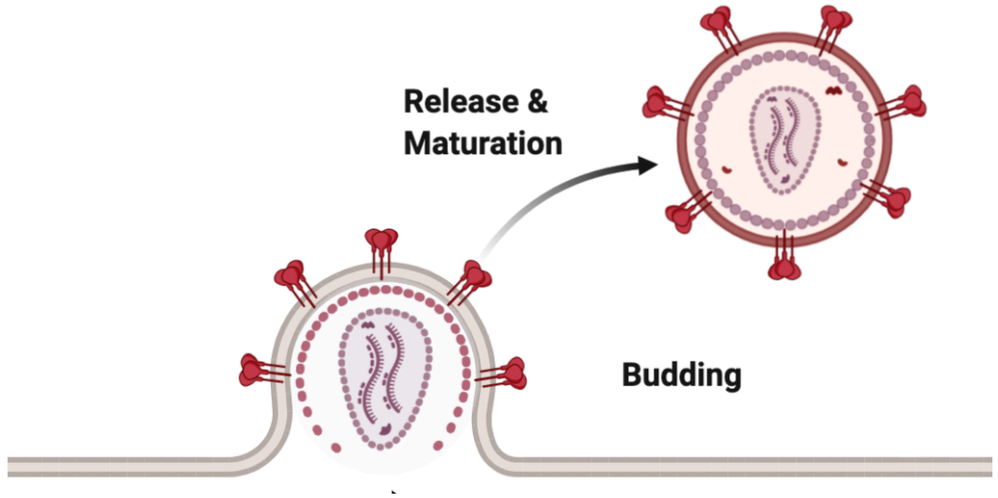
COS-7 cell expressing lysosomal CD63 tagged with PA-FP Kaede

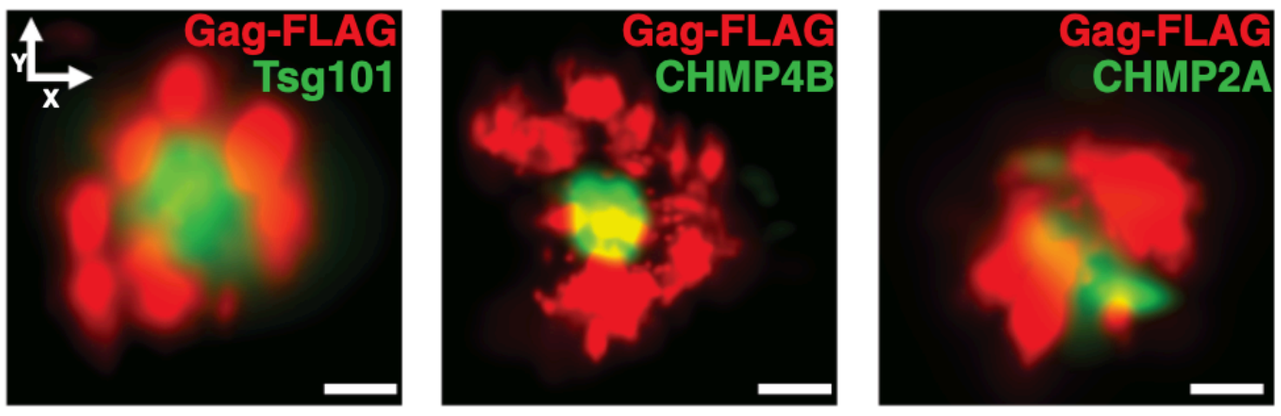
Betzig, E.; Patterson *et al*. *Science* **2006**, 313, 1642

Applications of PALM

How does viral budding and release occur?

- *Virus hijaks host ESCRT proteins*
- *ESCRT protein mediates fission of budding viral particle*





iPALM images of budding viral particles

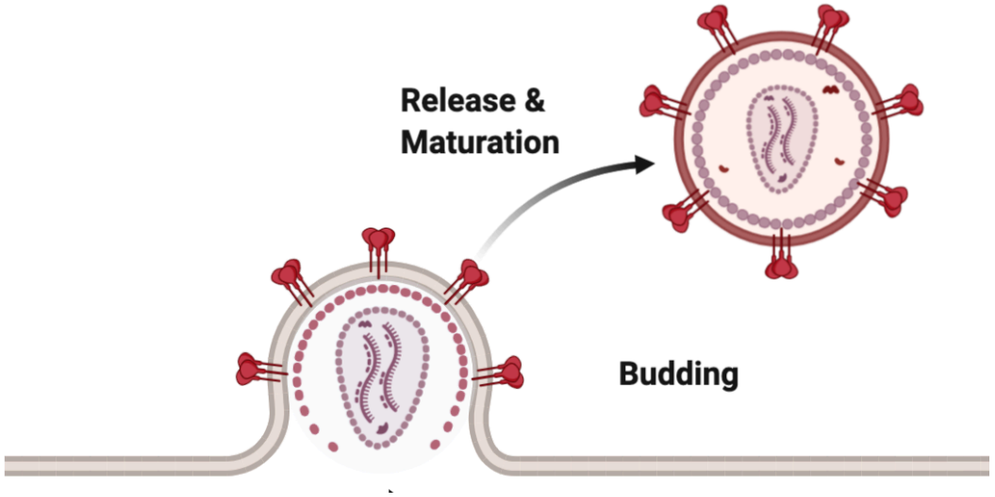
- ***ESCRT subunits localized inside of assembling particles***

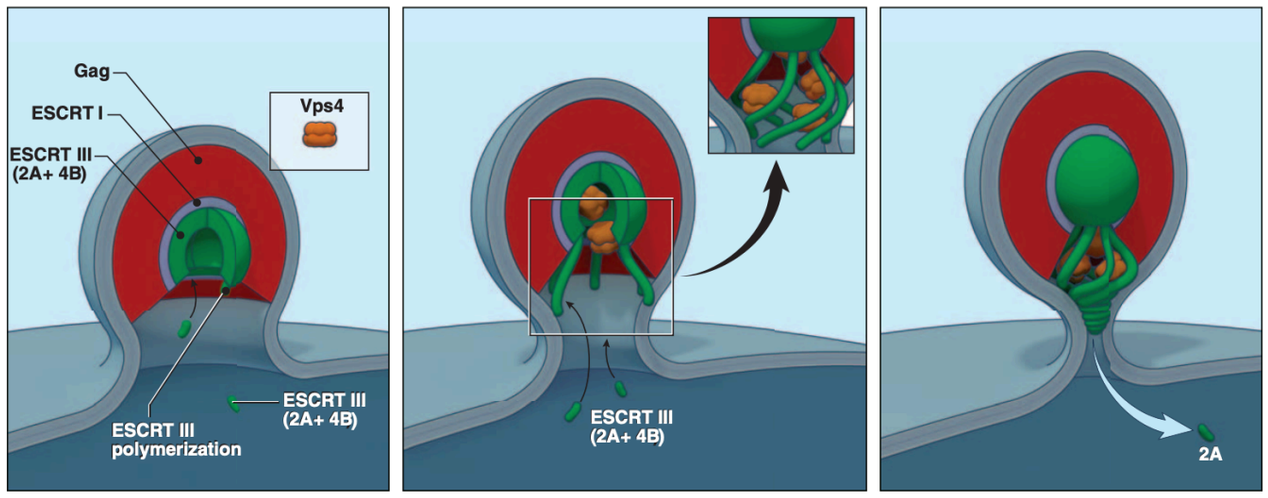
Van Engelenburg, S. B. *et al*. *Science* **2014**, 343, 653-656

Applications of PALM

How does viral budding and release occur?

- *Virus hijaks host ESCRT proteins*
- *ESCRT protein mediates fission of budding viral particle*



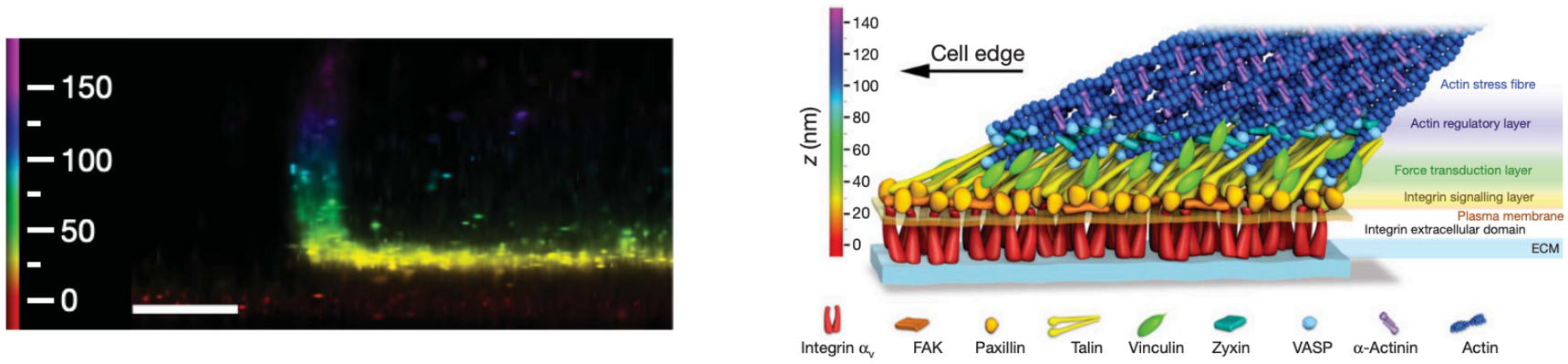


- *ESCRT remodeling within viral particles leads to scission*

Van Engelenburg, S. B. *et al*. *Science* **2014**, 343, 653-656

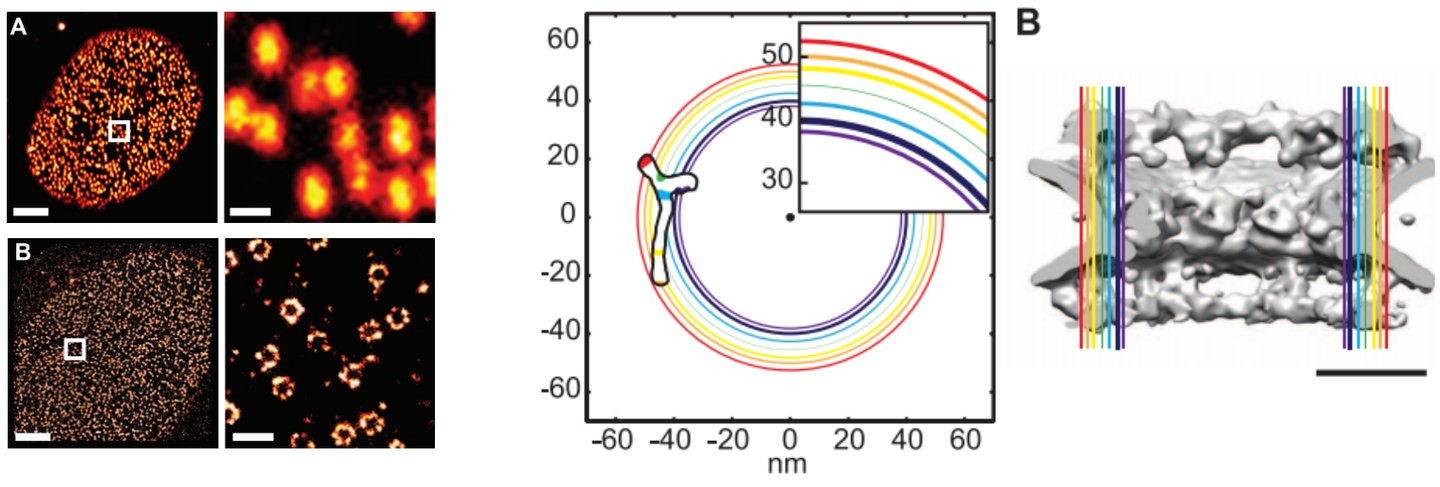
Applications of PALM

Mapping of nanoscale protein organization in focal adhesions



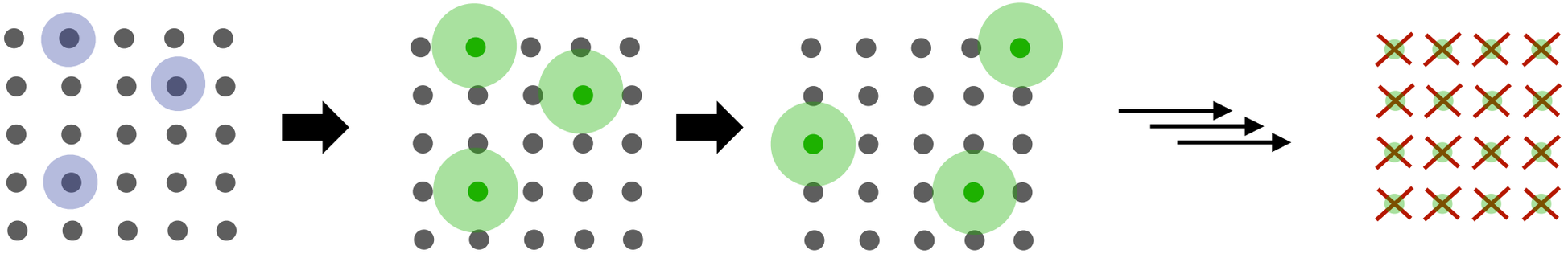
Kanchanawong, P. e*t al. Nature* **2010**, 468, 580-584

Structural elucidation of the nuclear pore scaffold structure



Symborska, A. *et al*. *Science* **2013**, 341, 655-658

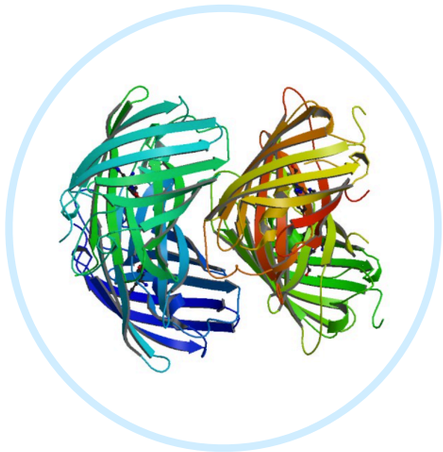
Stochastic Optical Reconstruction Microscopy (STORM)

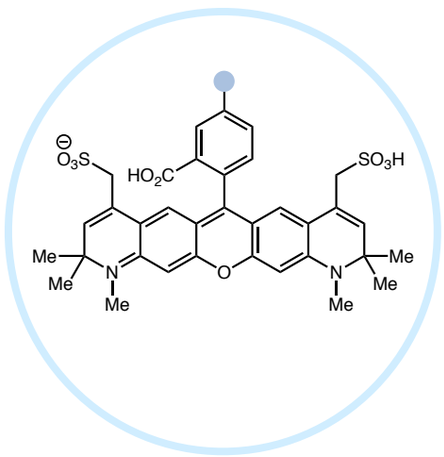


Photoactivated Localization Miroscopy (PALM)

Photoactivateable proteins

(PA-GFP, Kaede, eDos)



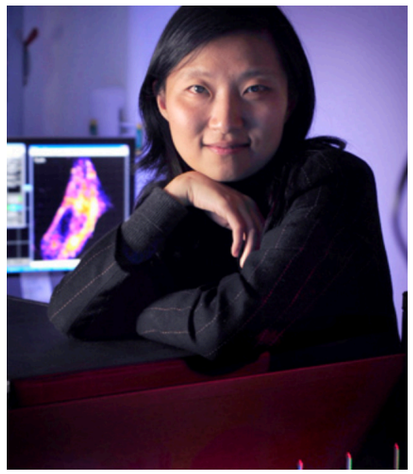


Photoaswitcheable dyes

(Cy3, Alexa Fluor 594, 647)

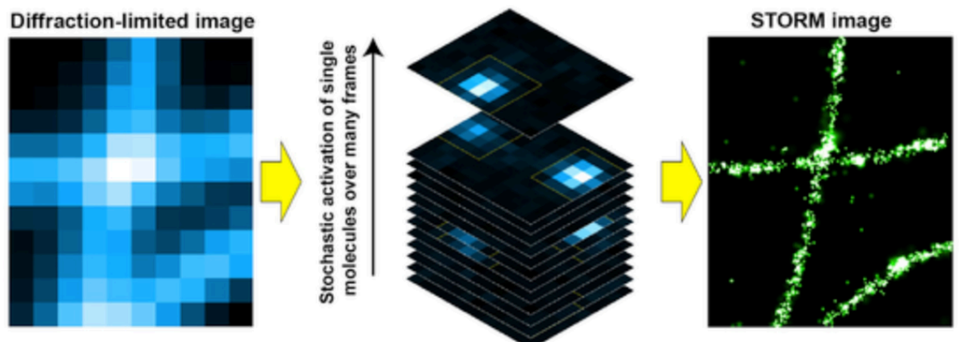
Stochastic Optical Reconstruction Microscopy (STORM)

Stochastic Optical Reconstruction Microscopy (STORM)

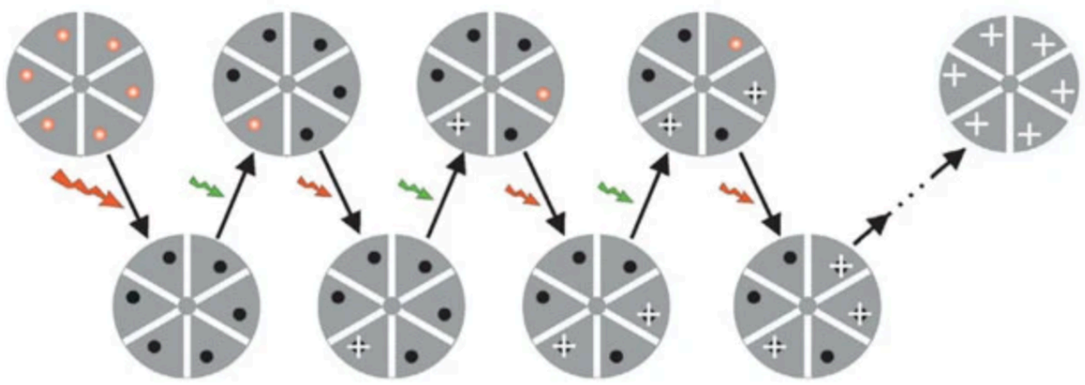


Xiaowei Zhuang
Harvard University, HHMI

Stochastic Optical Reconstruction Microscopy (STORM)



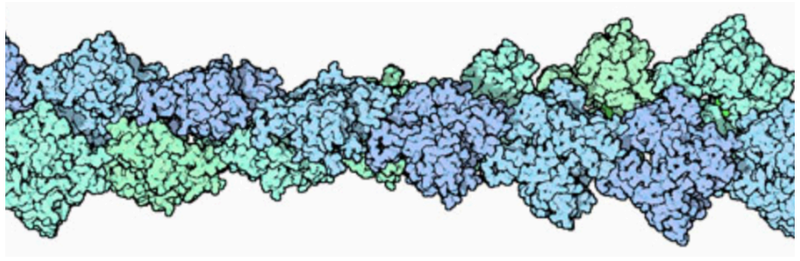
Photoswitching of dyes to achieve super-resolution imaging



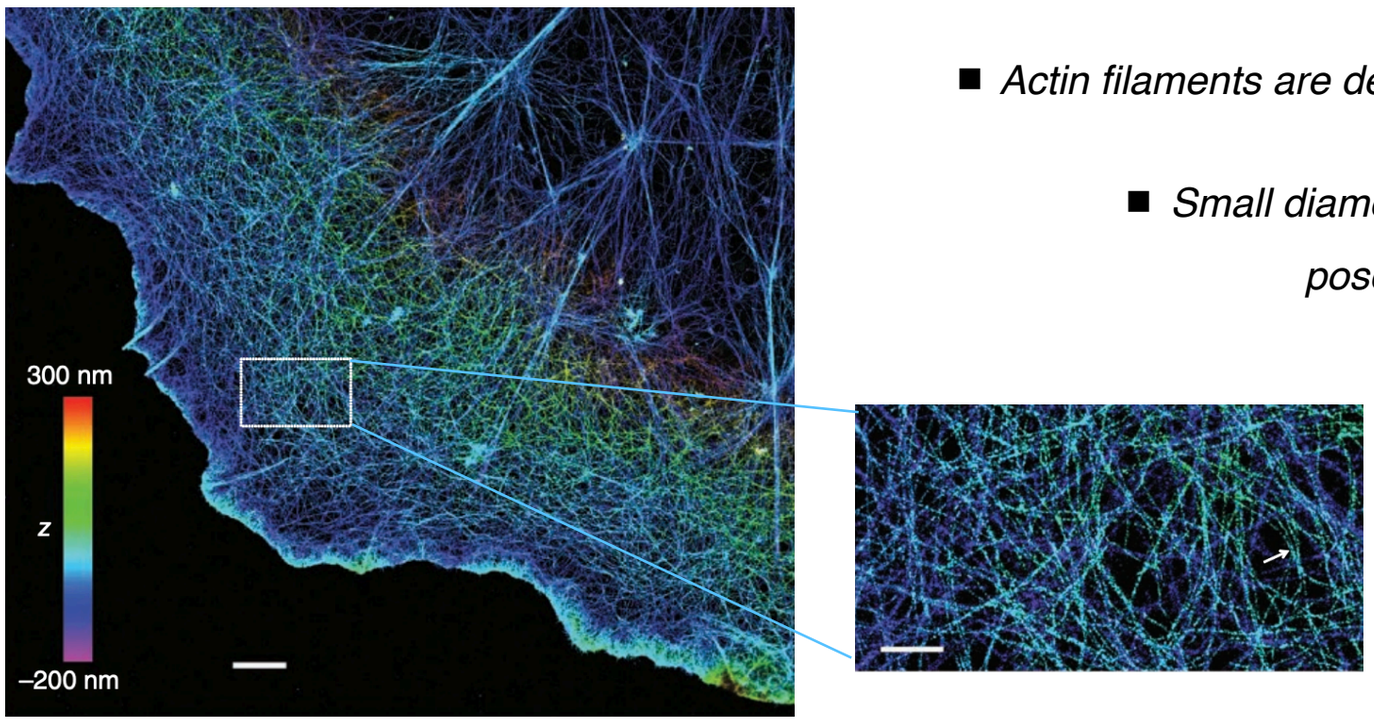
Rust, M. J.; Bates, M.; Zhuang, X. *Nat. Methods,* **2006**, 3, 793-795

Cytoskeletal Structure of Actin in Neurons

Actin filaments underly cell morphology, motility, and functions



- *Cell division*
- *Intracellular transport*
- *Sensing environmental forces*



- *Actin filaments are densely packed in the cellular environment*
- *Small diameter (6 nm) and packing density pose imaging challenges*

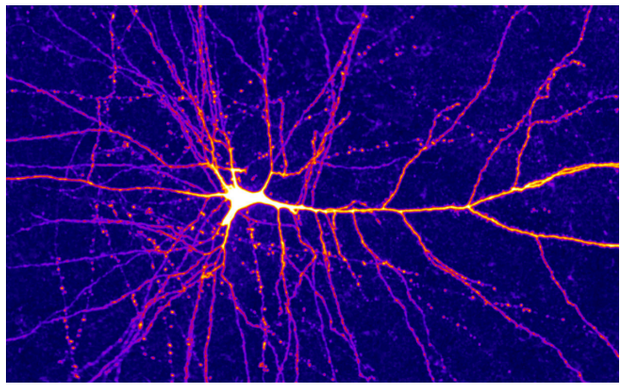
STORM image of actin labeled with Alexa Fluor 647 in a COS-7 cell

Xu, K.; Babcock, H. P.; Zhuang, X. *Nat. Methods,* **2012**, 9, 185-188

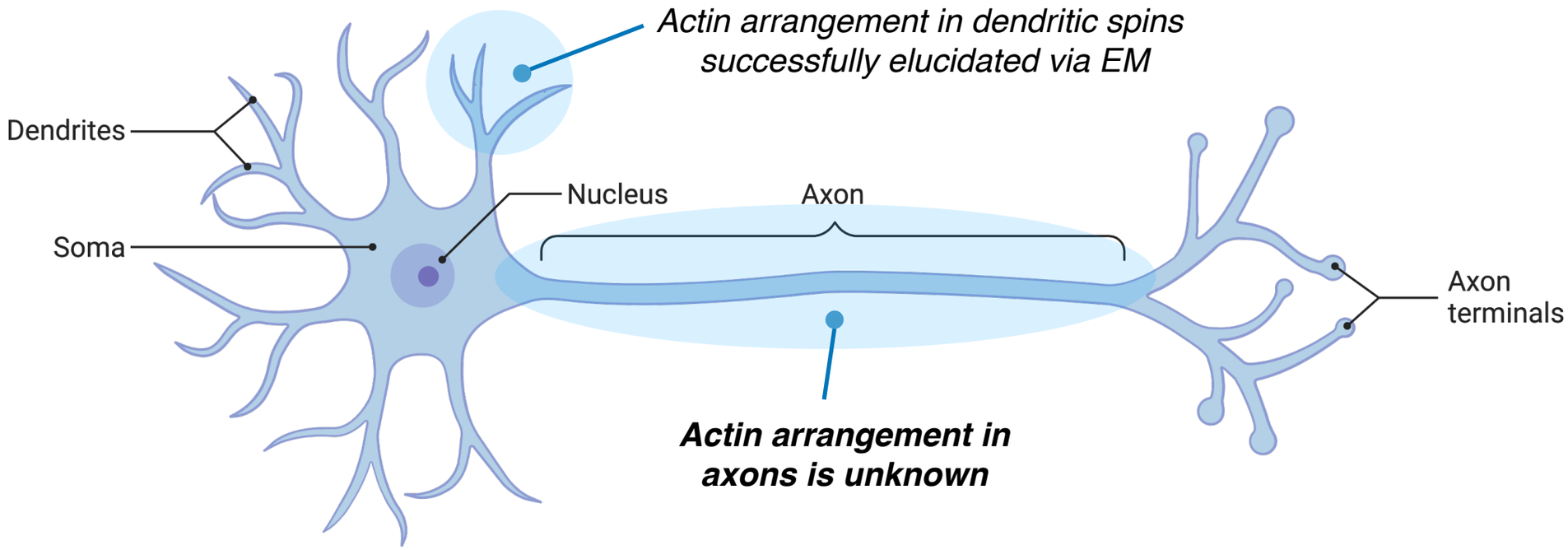
Cytoskeletal Structure of Actin in Neurons

Actin organization plays a critical role in neurons

- *cargo transport*
- *neurite growth*
- *structural stabilization*

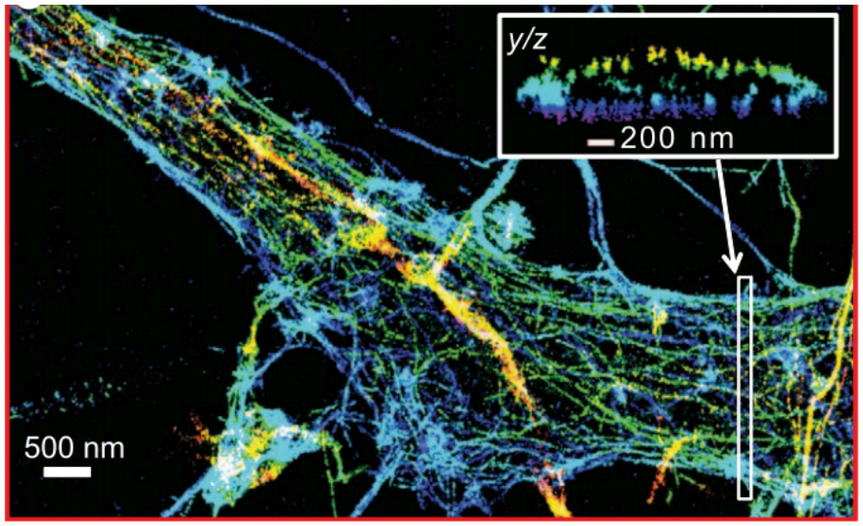


How is actin organized in neurons?

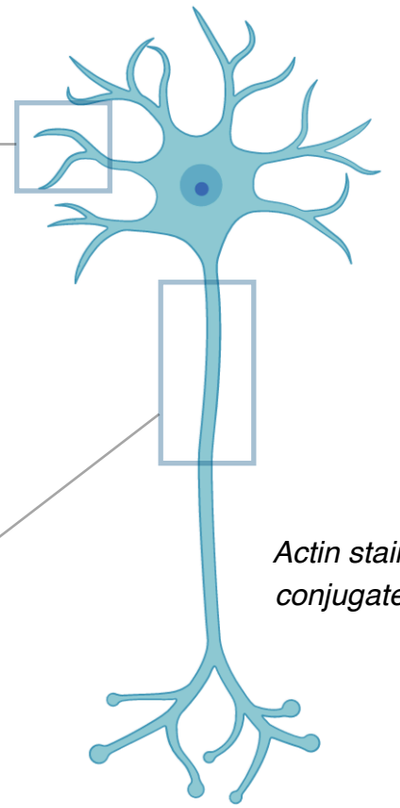


Cytoskeletal Structure of Actin in Neurons

STORM image of dendrites stained with Alexa Fluor 647

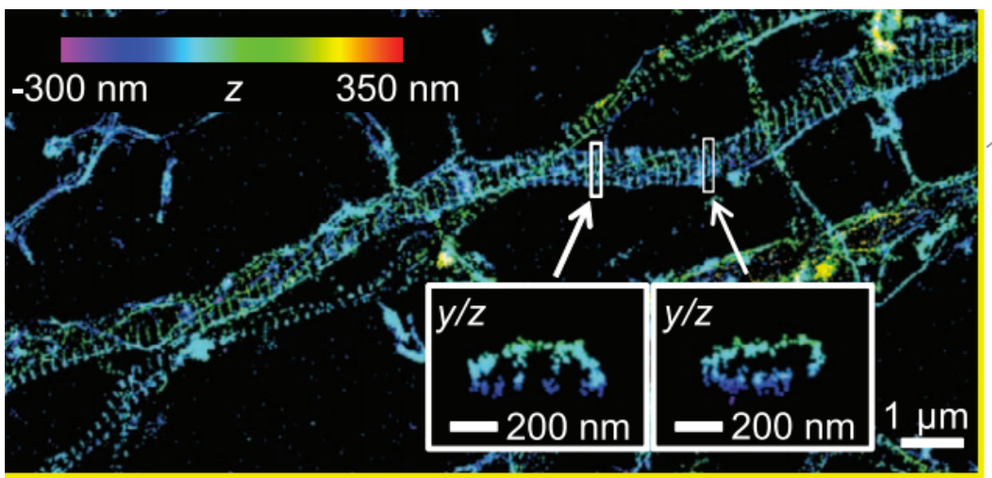


- *Actin filaments run along axis of dendrites*



Actin stained with phalloidin conjugated Alexa Fluor 647

STORM image of axons stained with Alexa Fluor 647

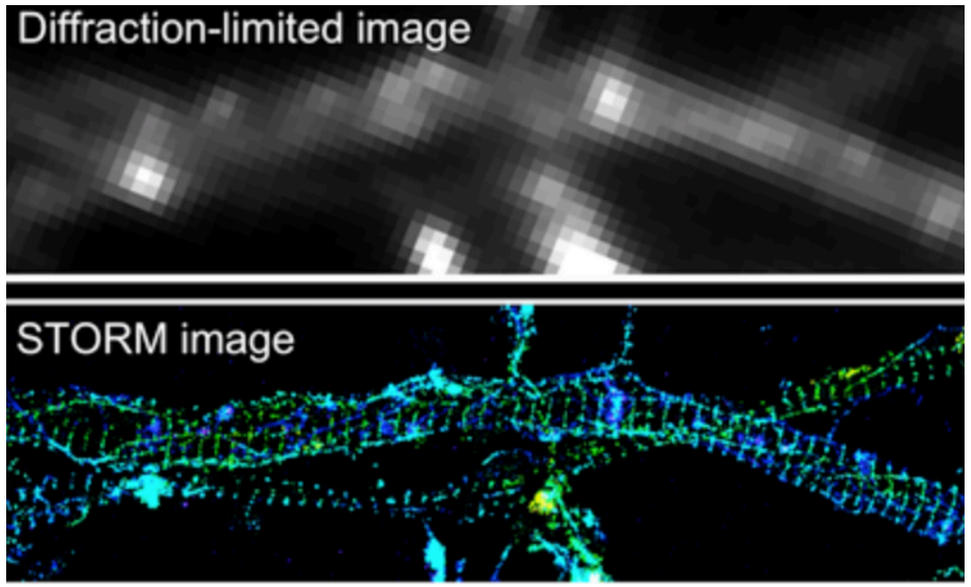


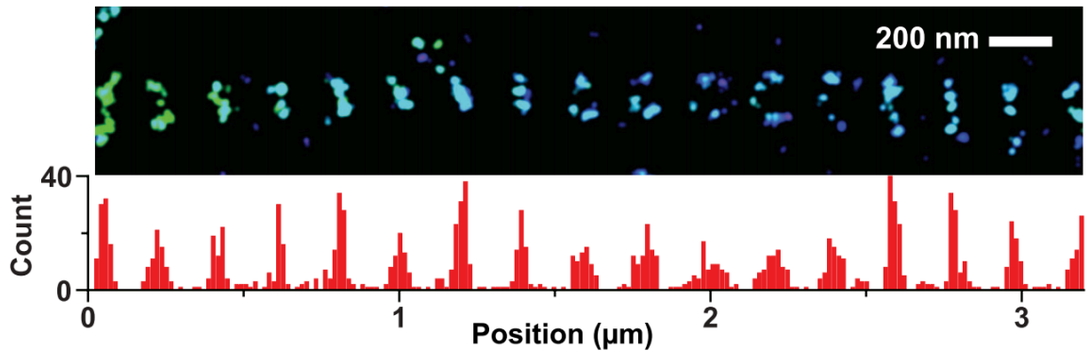
Unexpected arrangement of actin observed in axonal region

- *Actin arranged in rings around axonal region*

Xu, K.; Zhong, G.; Zhuang, X. *Science,* **2013**, 339, 452

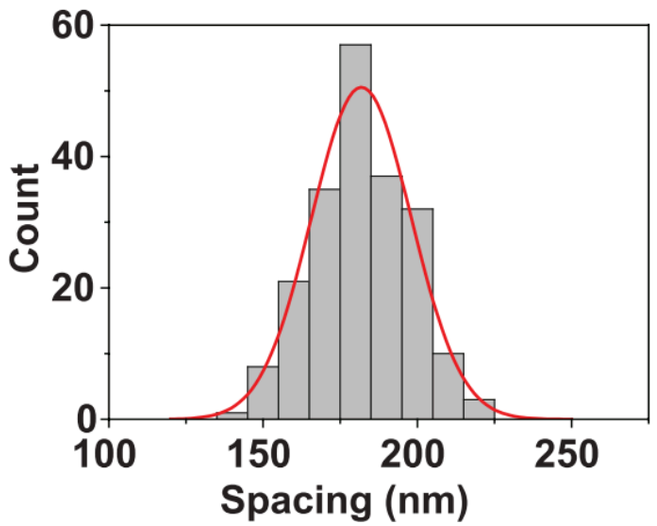
Cytoskeletal Structure of Actin in Neurons





- *Highly periodic spacing of actin rings observed*

Spacing between actin rings along axon

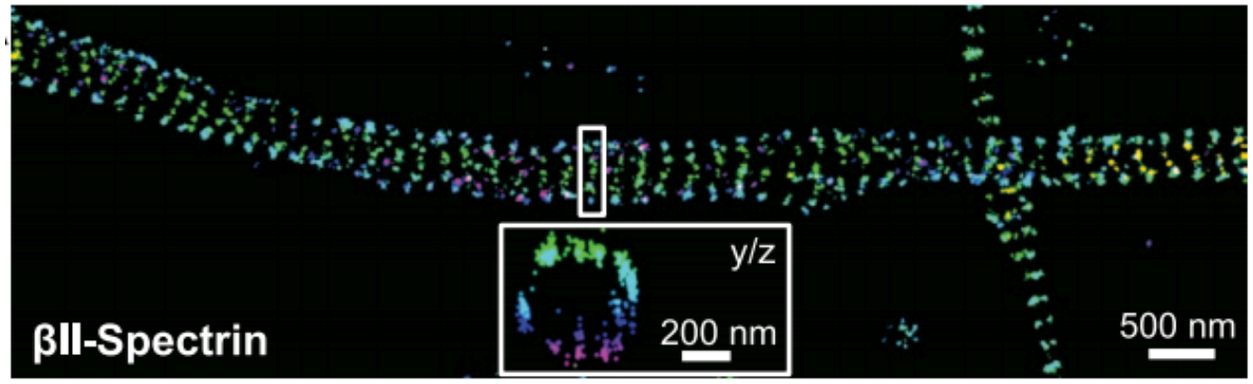


- ***Actin ring periodicity of ~182 nm (stdv = 16 nm)***

Does spectrin, another cytoskeletal protein, underlie this structure?

Xu, K.; Zhong, G.; Zhuang, X. *Science,* **2013**, 339, 452

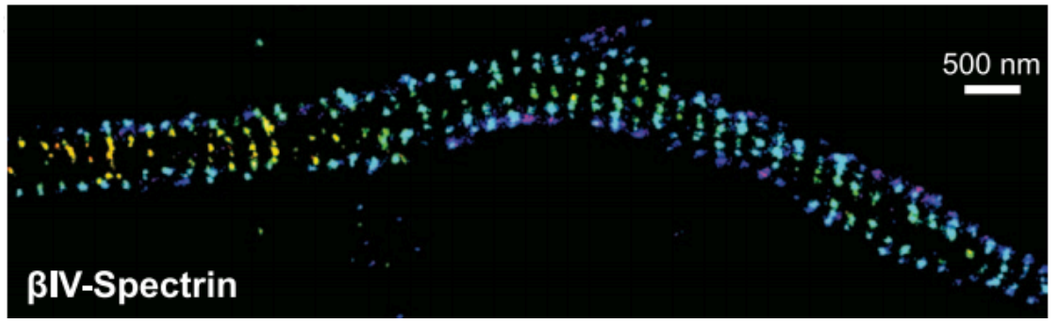
Cytoskeletal Structure of Actin in Neurons



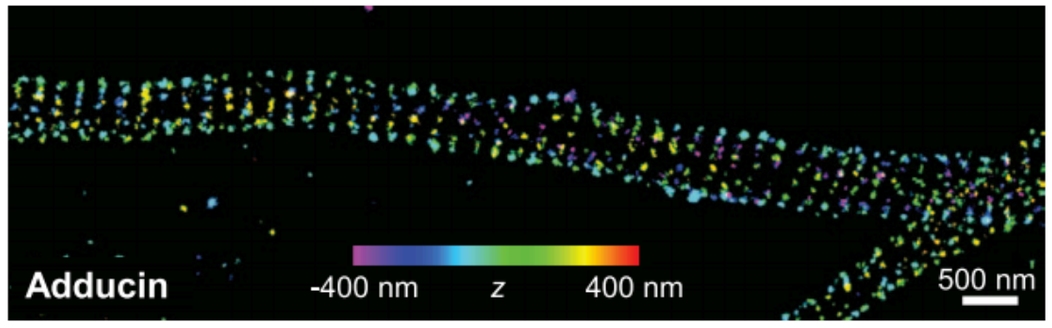
Highly periodic arrangement of Spectrin observed

- *spectrin periodicity identical to actin (~182 nm)*

- *periodic arrangement of Spectrin observed in initial axon segments (BIV-Spectrin)*



- *periodic arrangement of Adductin observed (actin capping protein)*



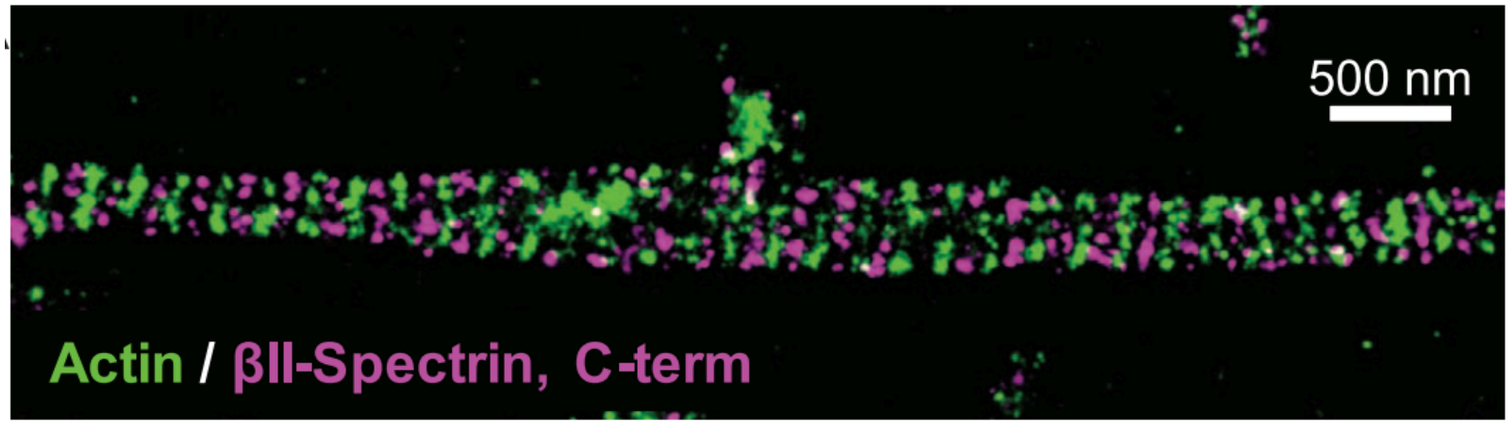
Xu, K.; Zhong, G.; Zhuang, X. *Science,* **2013**, 339, 452

Cytoskeletal Structure of Actin in Neurons

Two color STORM to determine actin/spectrin axonal oragnization

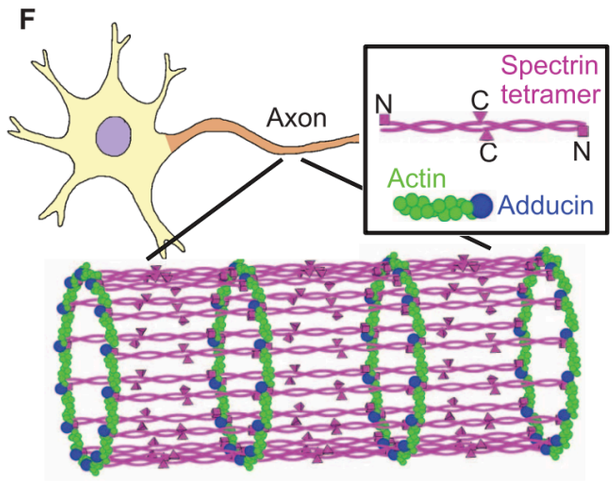
Spectrin

Actin

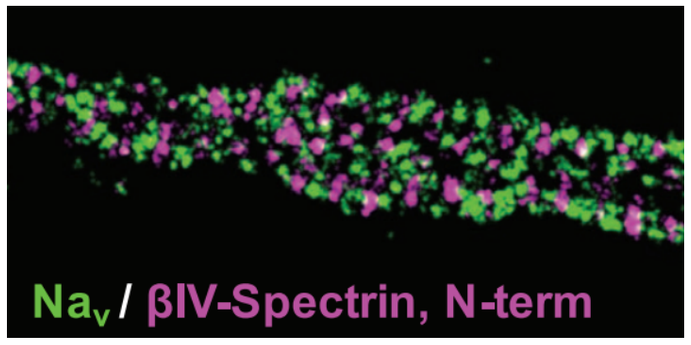


- *Highly regular alternating pattern of actin/spectrin observed*

Actin and spectral are interconnected



Cytoskeletal arrangement influences membrane organization



- *Periodic organization of Nav on initial axon segments*

Xu, K.; Zhong, G.; Zhuang, X. *Science,* **2013**, 339, 452

2014 Nobel Prize in Chemistry

Over 1 order of magnitude improvement over Abbe's diffraction limit with super-resolution microscopy

$$d = \frac{\lambda}{2n\sin\alpha}$$

200 nm ⟶ ***~20 nm***

- ***2014 Nobel prize in chemistry***

Eric Betzig
UC Berkeley

William E. Moerner
Stanford

Stefan W. Hell
Max Planck

"for the development of super-resolved fluorescent microscopy"

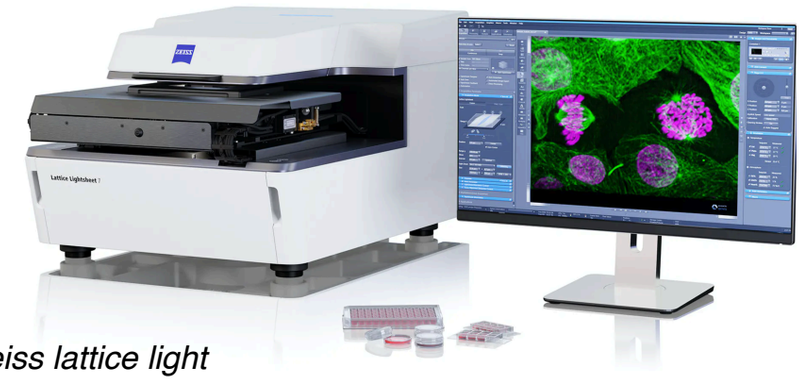
Outlook

■ ***Comparison of super-resolution platforms***

	SIM	STED	PALM/STORM
xy Resolution	100–130 nm	20–70 nm	10–30 nm
Temporal resolution	Milliseconds to seconds	Milliseconds to seconds	Seconds to minutes
Photodamage	Low to moderate	Moderate to high	Moderate
Post-image processing required?	Yes	No	Yes
Maximum number of simultaneous colors	4	3	PALM: 2 STORM: 3
Considerations	Straightforward multicolor experiments and sample preparation. Reconstruction algorithm may cause artifacts	Best temporal resolution at the highest spatial resolution; however maximal in-plane can be at the expense of axial resolution	Highest spatial resolution; however sensitive to labeling density. Crosstalk between fluorophores maybe an issue

■ ***Sought-after improvements for super-resolution microscopy***

- bio-compatibility
- Multi-color imaging
- spatial resolution
- Temporal resolution



Zeiss lattice light sheet 7

Questions?