Super-resolution STED microscopy and its application in neuroscience

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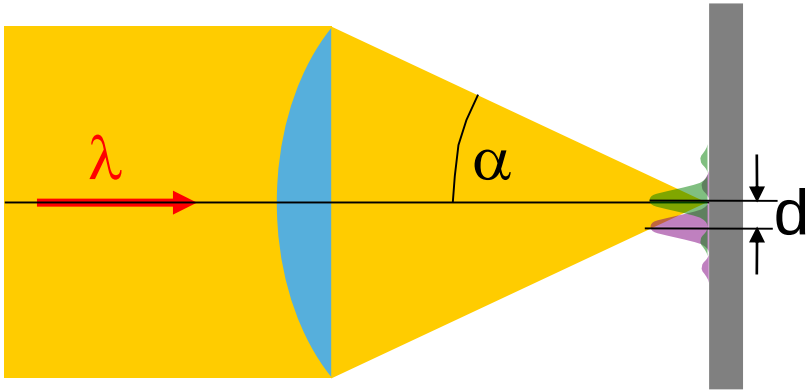
Nanoscale Microscopy and Molecular Physiology of the Brain Cluster of Excellence 171, DFG Research Center 103

MPI of Experimental Medicine

Resolution in far-field light microscopy

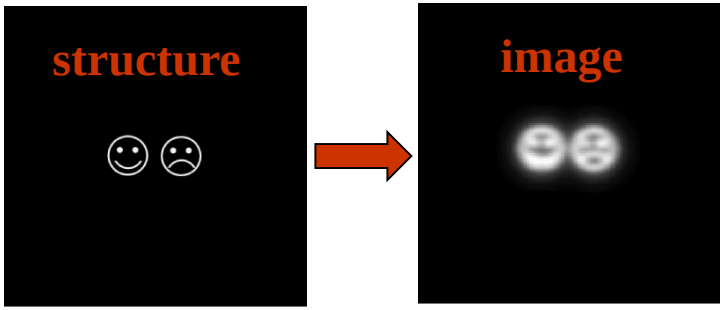


diffraction limit:
minimum resolvable distance

$$d_{\min} \approx \frac{\lambda}{2\, n \sin\alpha}$$

numerical aperture (NA)

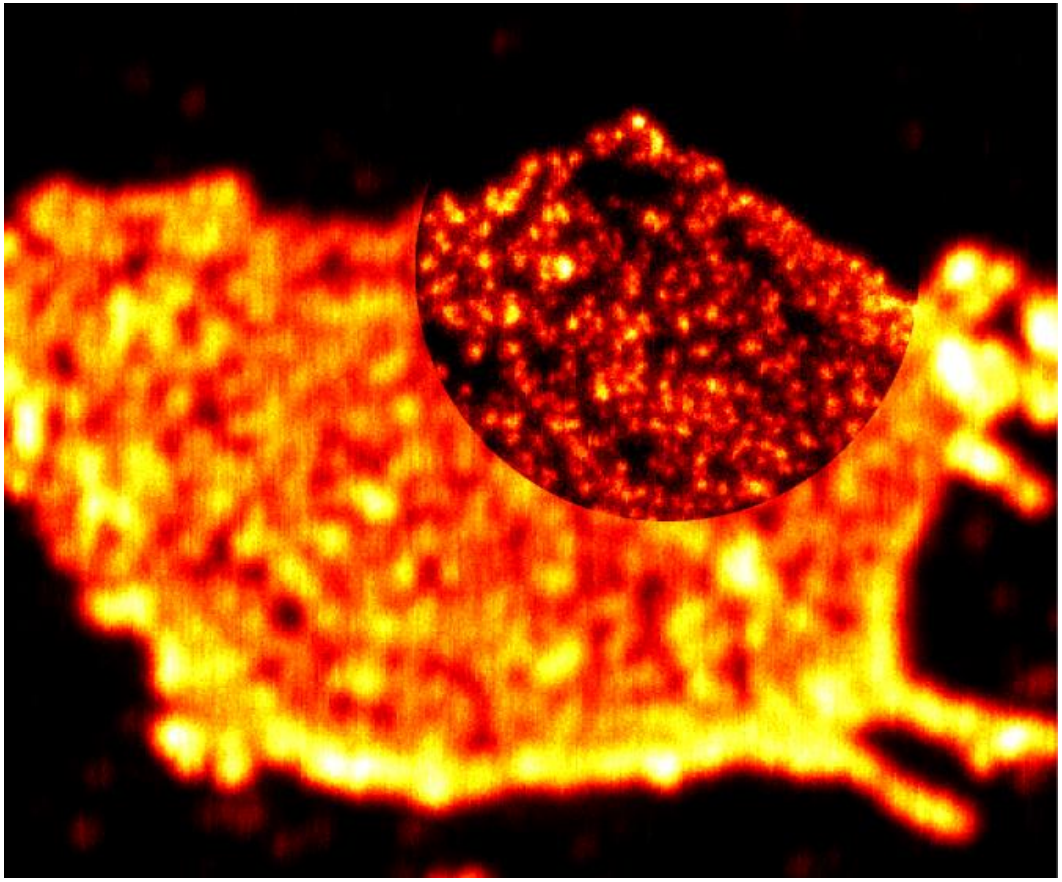
n: refractive index



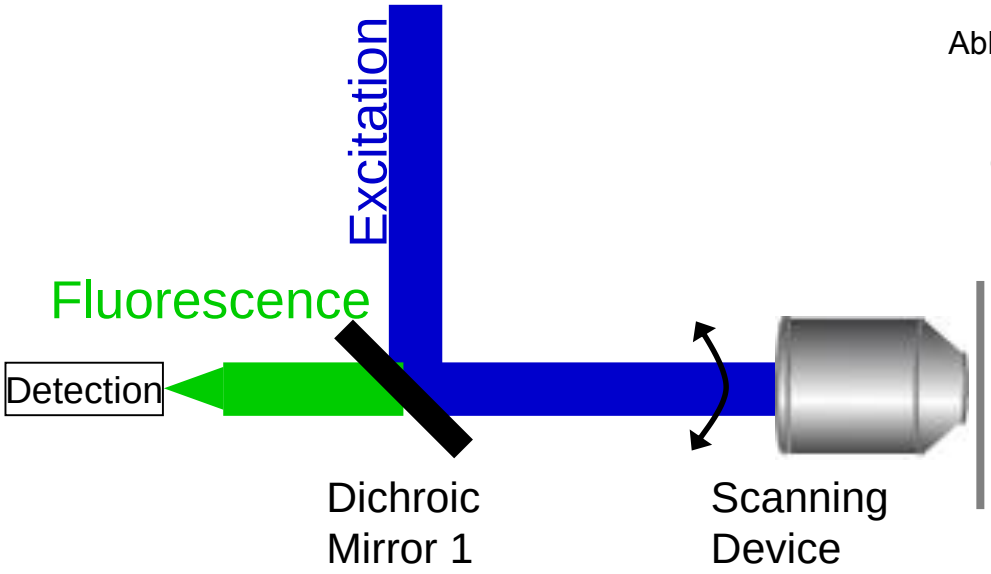
"similar objects closer than about half the wavelength should not be distinguishable in a light microscope"

Ernst Abbe 1873

Standard (confocal) vs. Superresolution (STED)

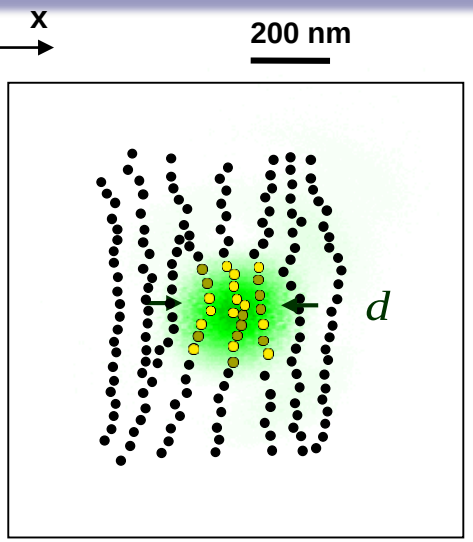


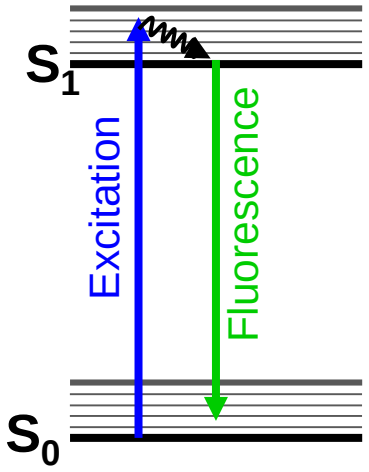
Confocal (fluorescence) microscopy



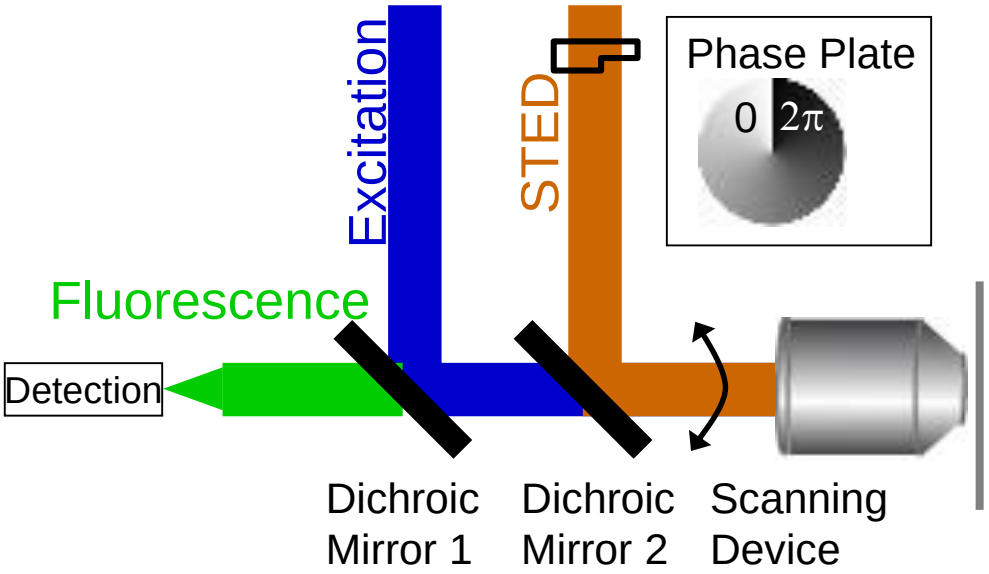
Abbe's equation

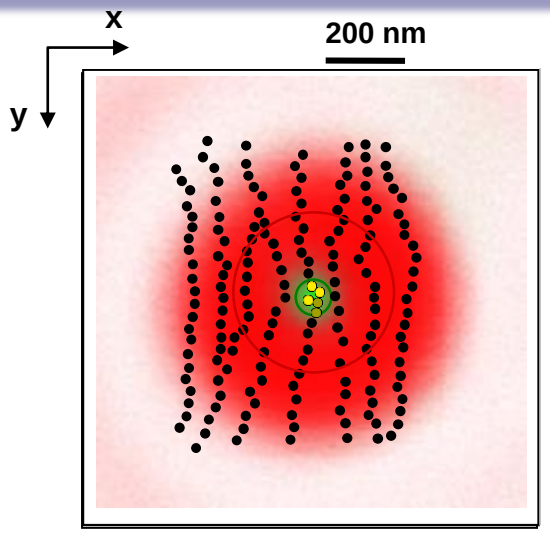
$$d \approx \frac{\lambda}{2n \sin \alpha}$$



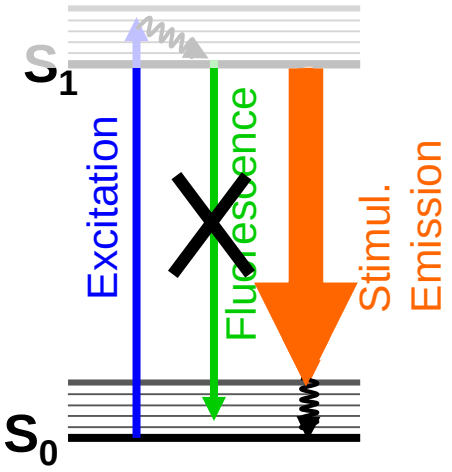


STED (STimulated Emission Depletion) microscopy



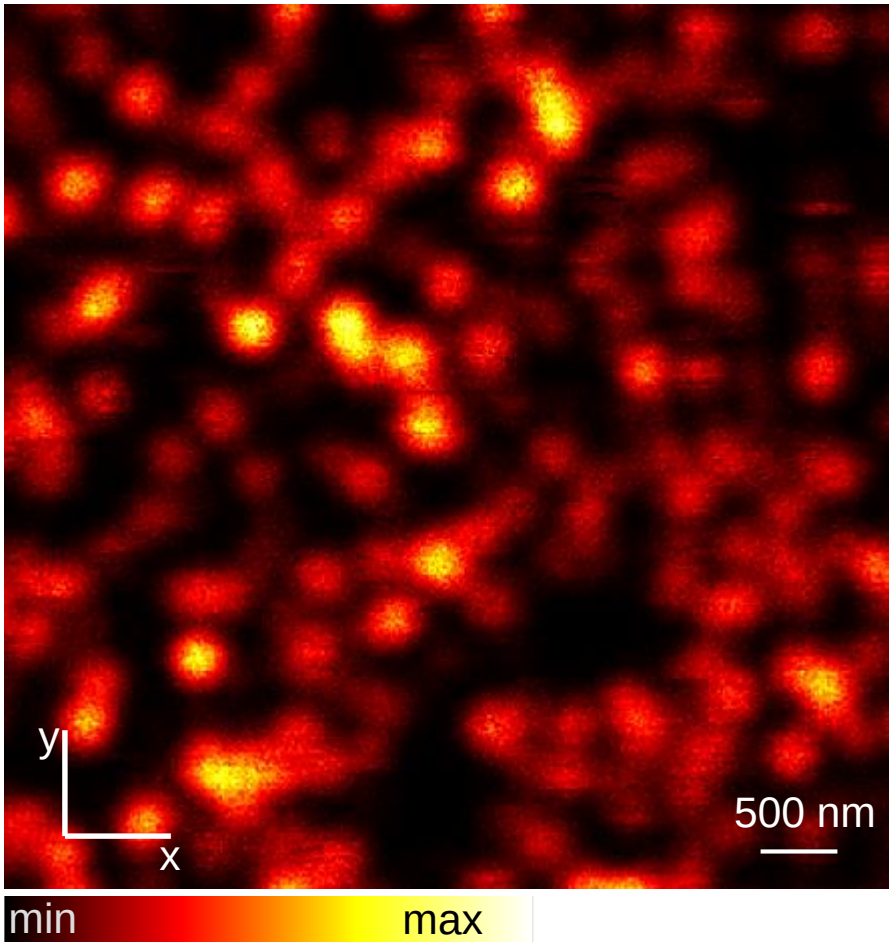


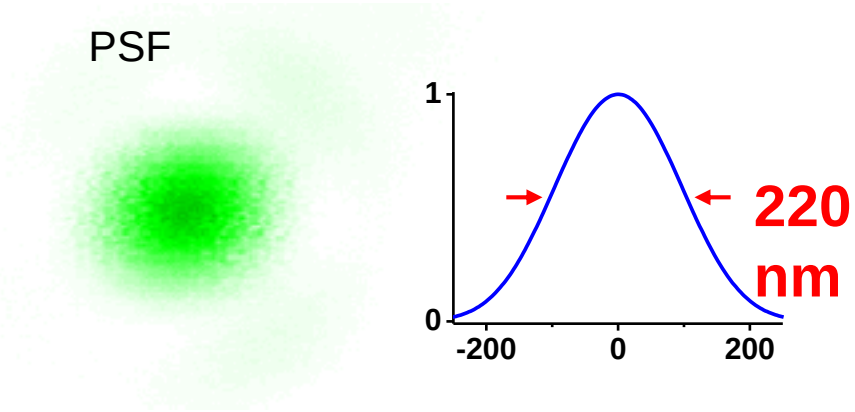
Nobel Prize in Chemistry 2014 to Betzig, Hell & Moerner
"for the development of super-resolved fluorescence microscopy."

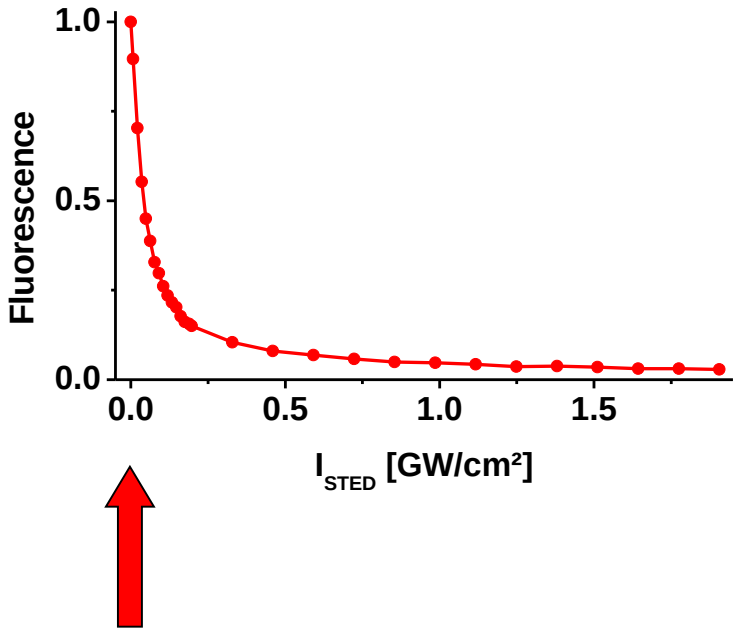


STED beam: keeps molecules non-fluorescent

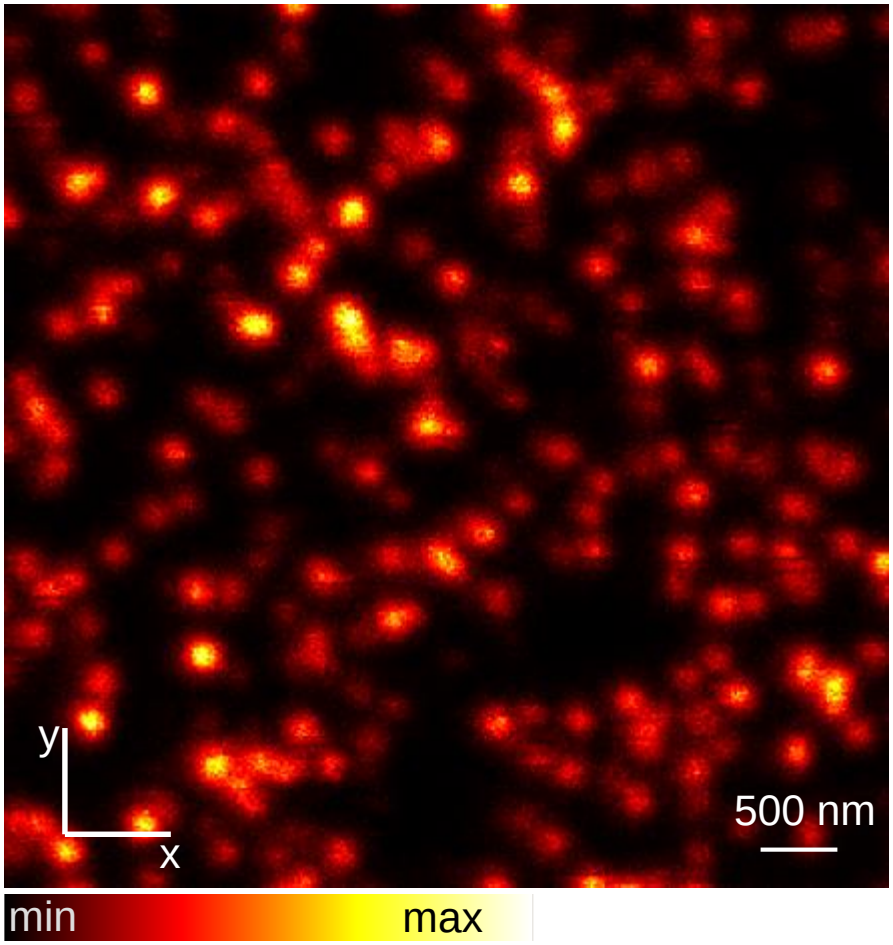
Diffraction limited resolution



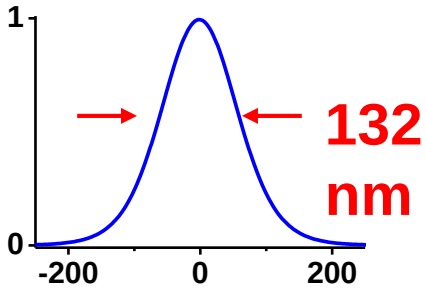


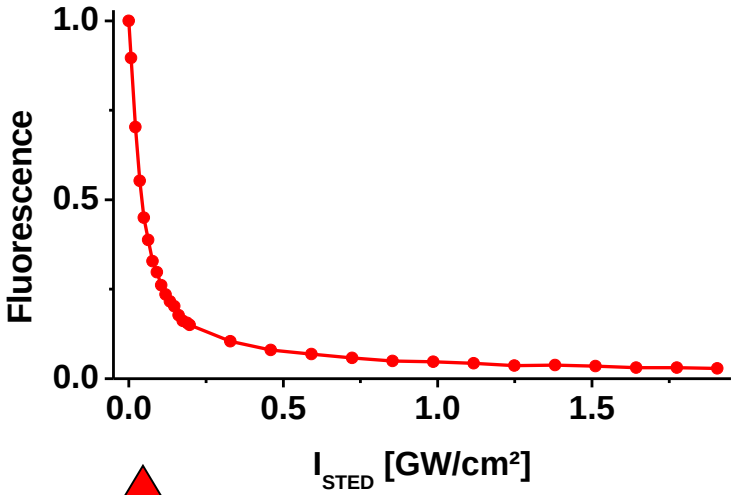


Subdiffraction resolution



Depletion distribution





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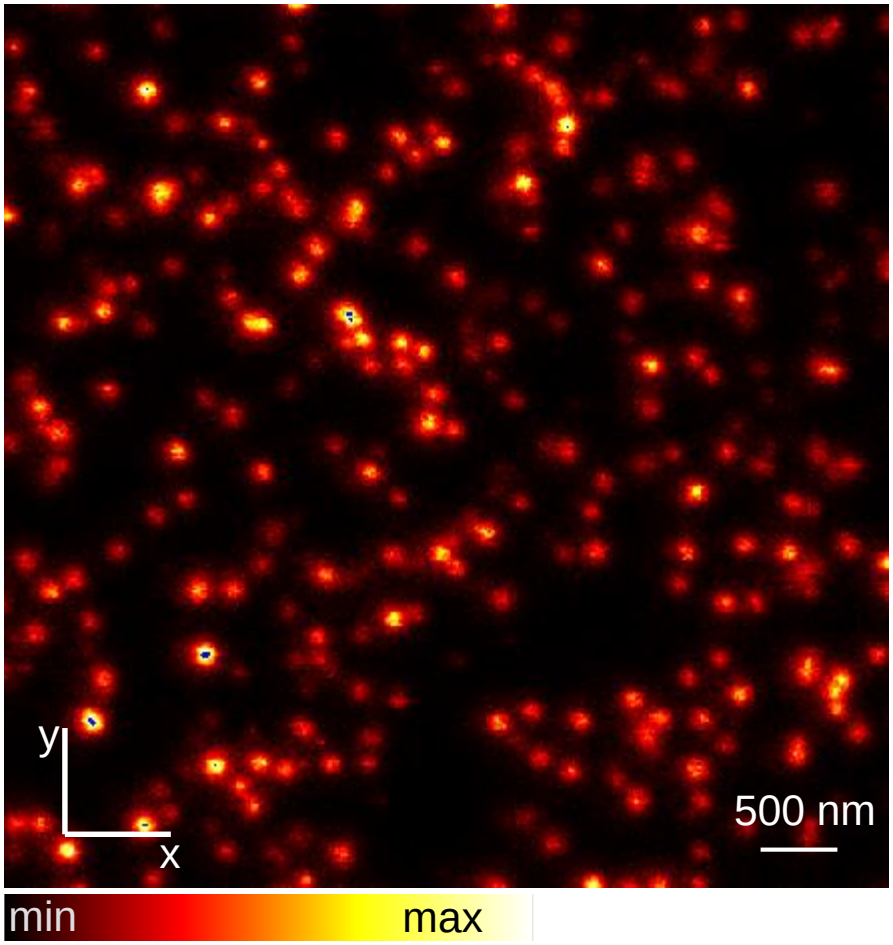
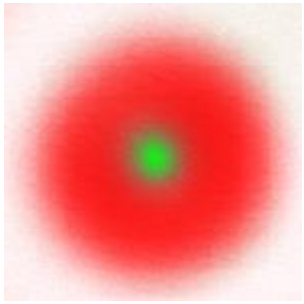
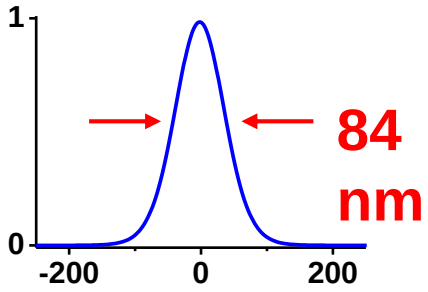
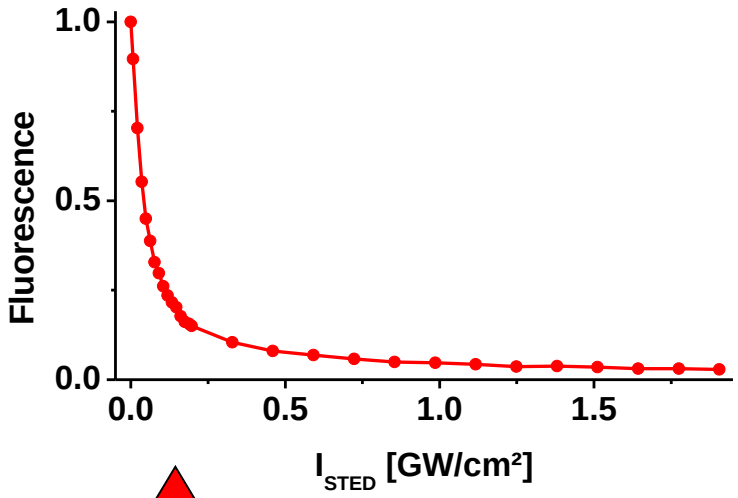


Depletion distribution







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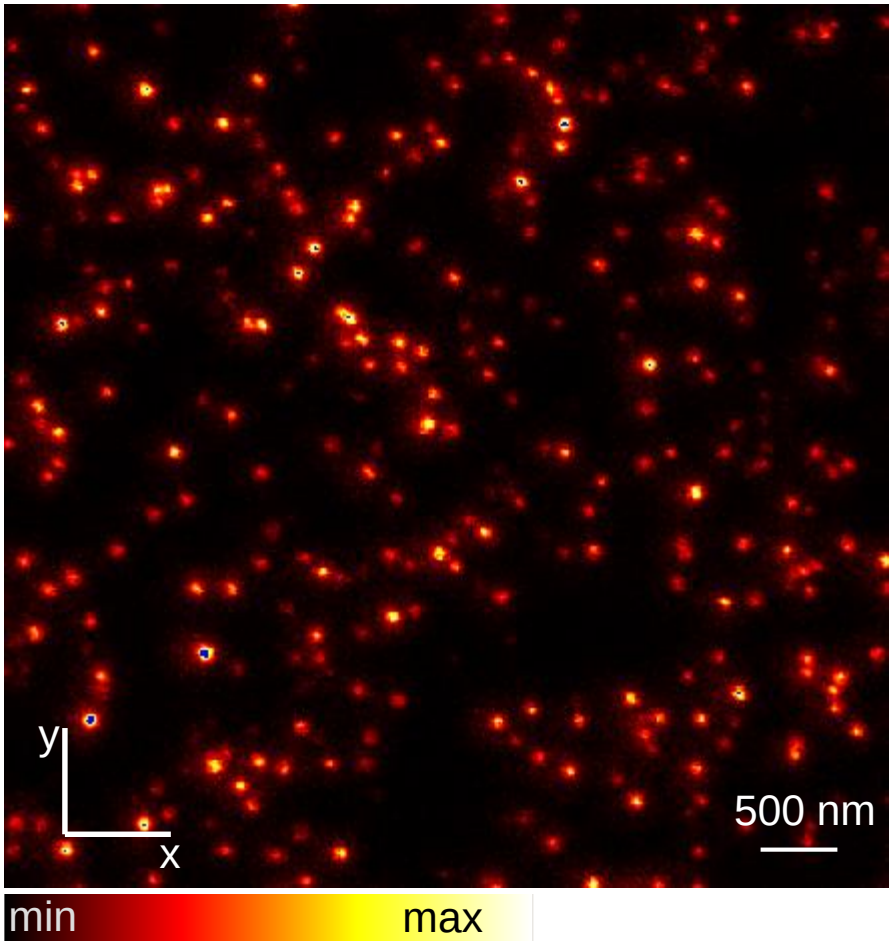
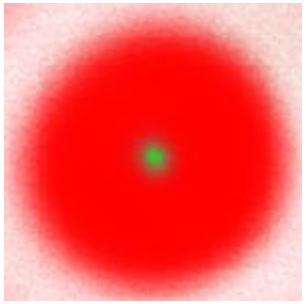
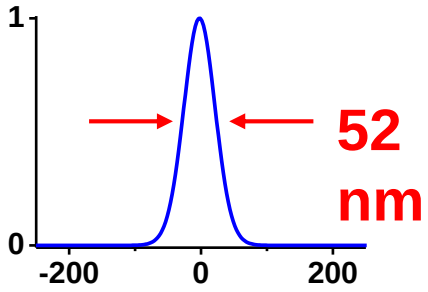
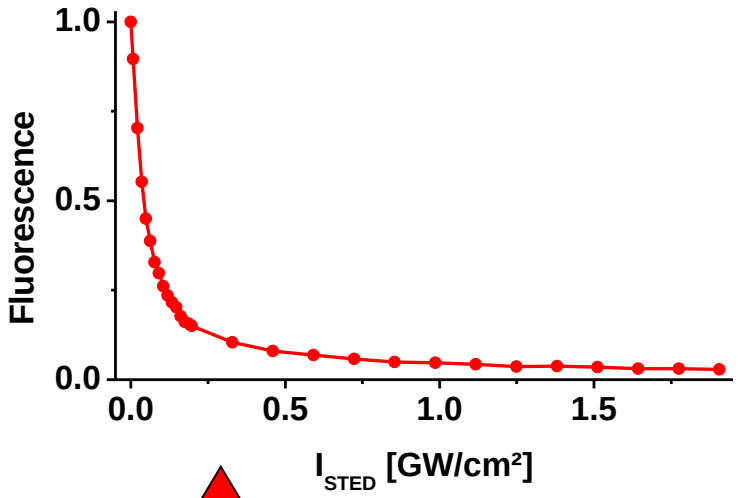


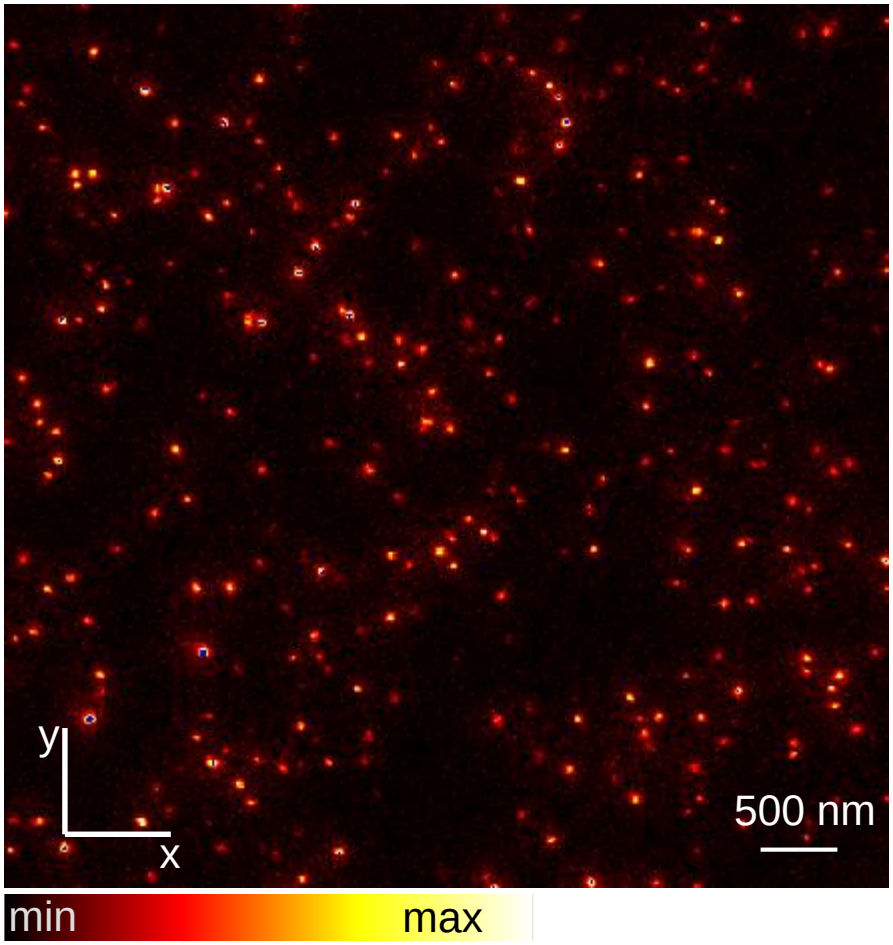
Depletion distribution



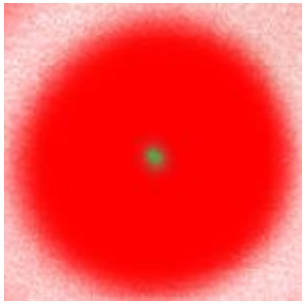


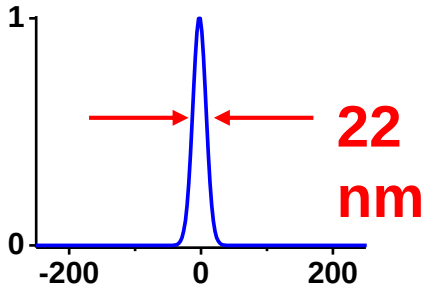


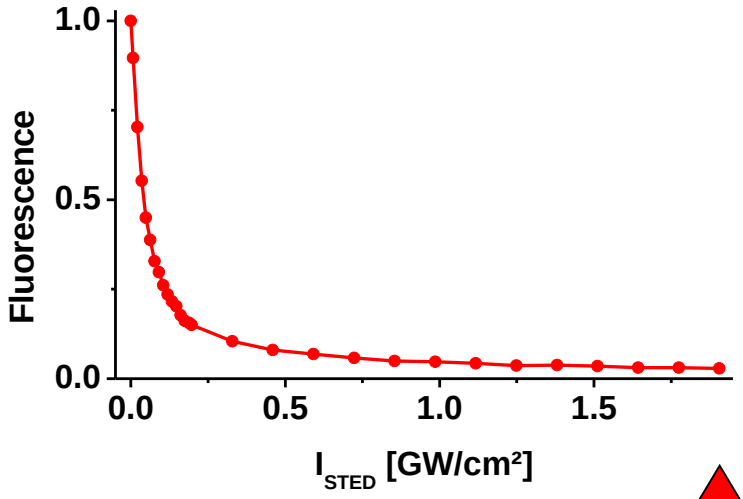
Subdiffraction resolution



Depletion distribution







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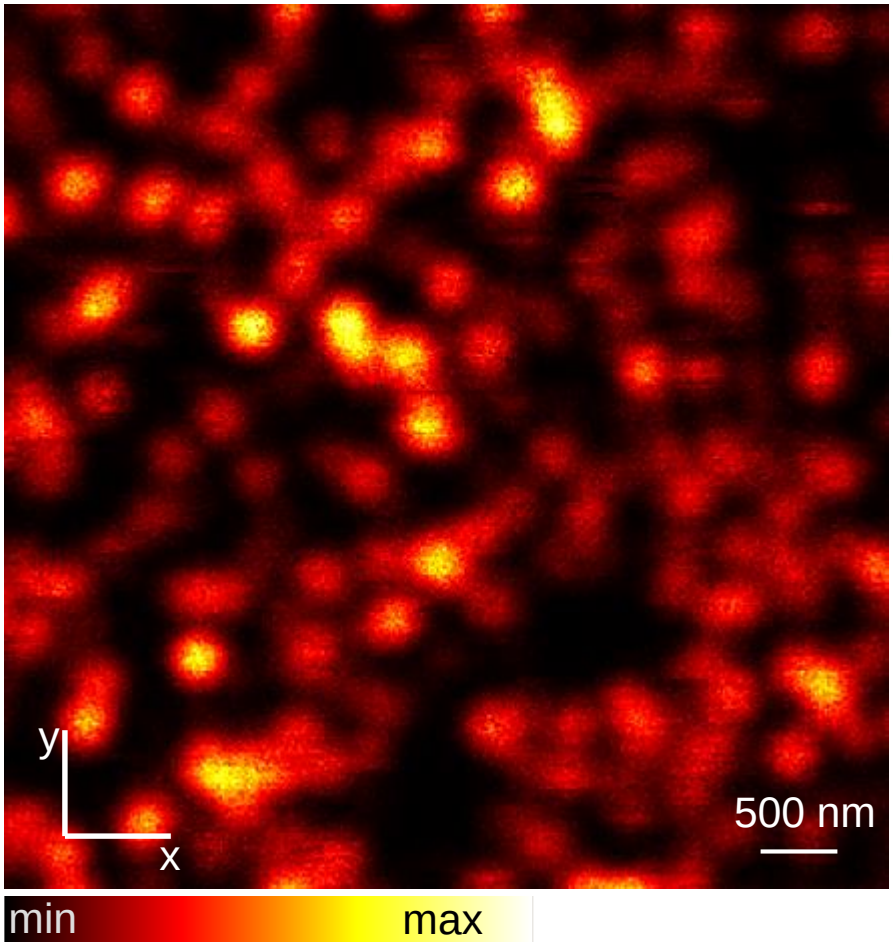
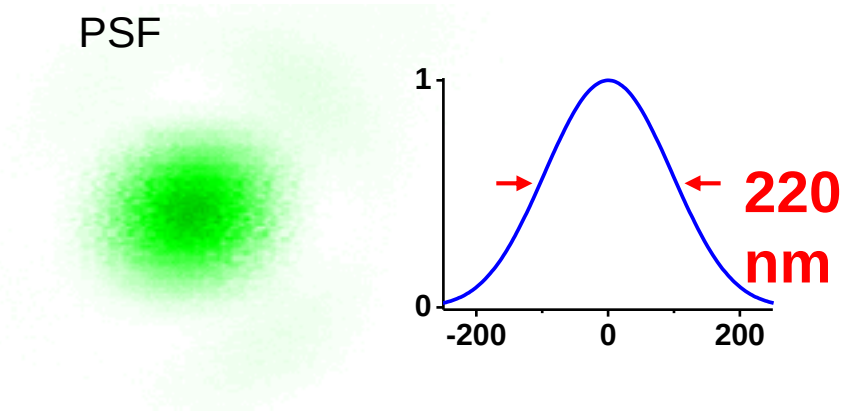
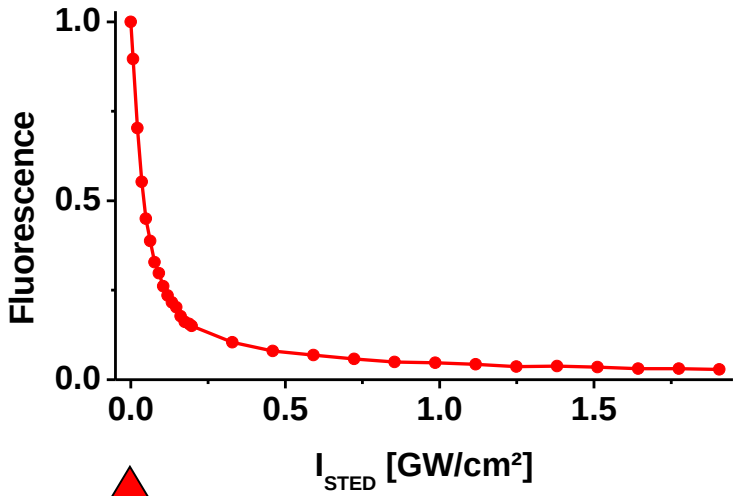




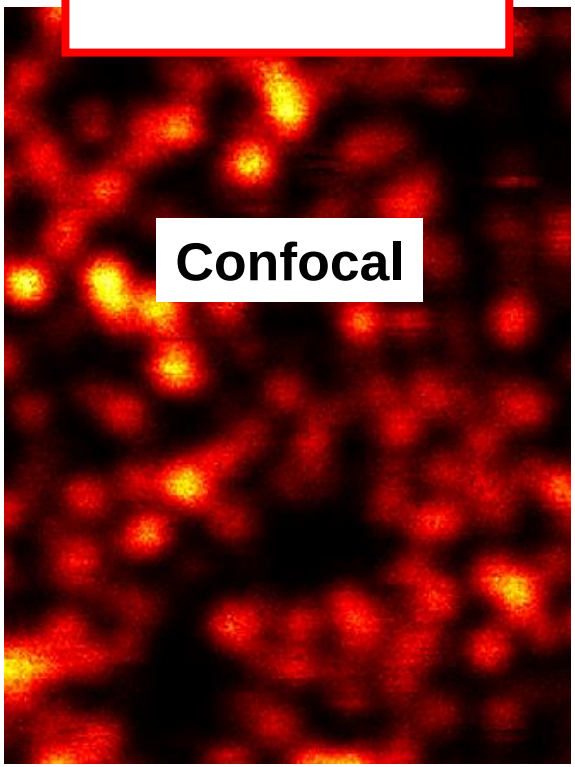


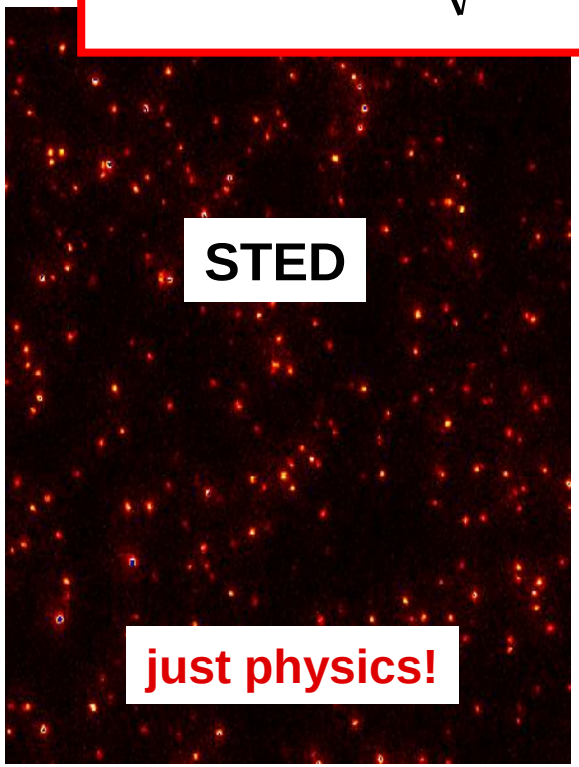
Subdiffraction resolution

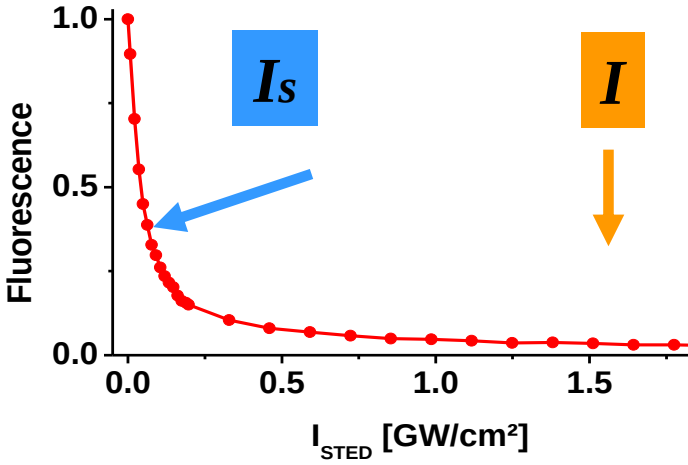
$$\Delta r \approx \frac{\lambda}{2n\sin\alpha}$$

$$\Delta r \approx \frac{\lambda}{2n\sin\alpha\sqrt{1+I/I_s}}$$

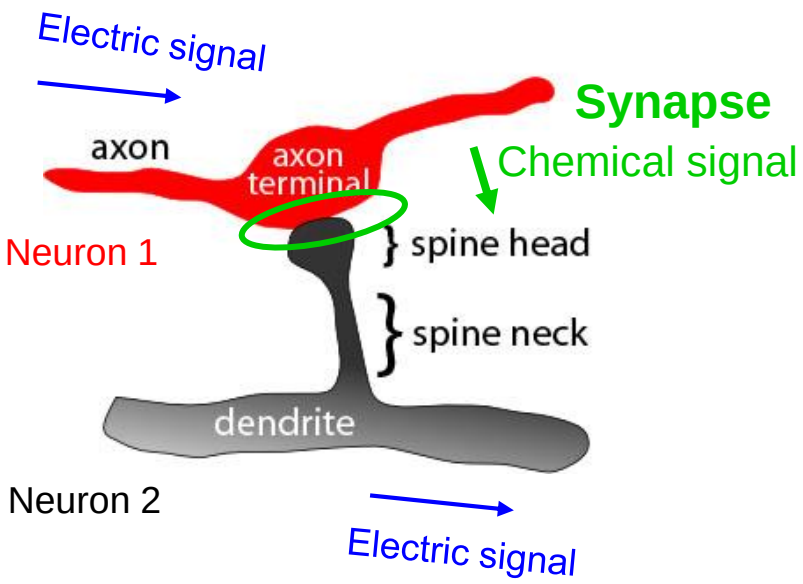
Modified Abbe equation



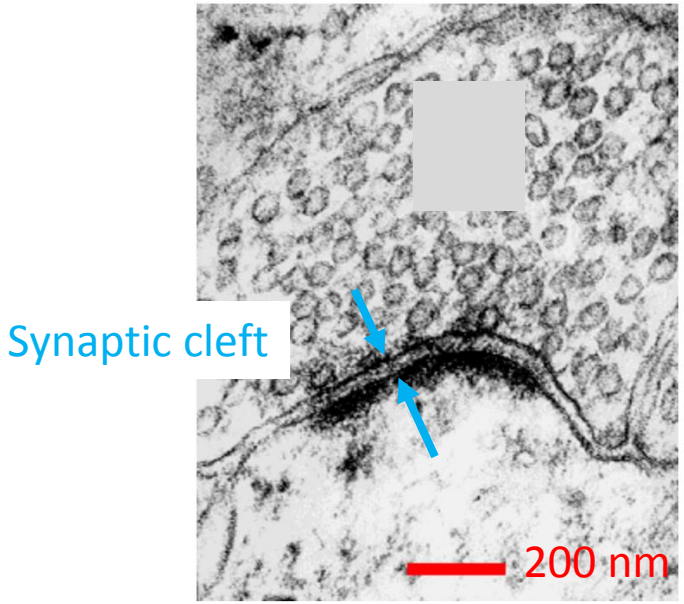




Synapse: connecting neurons

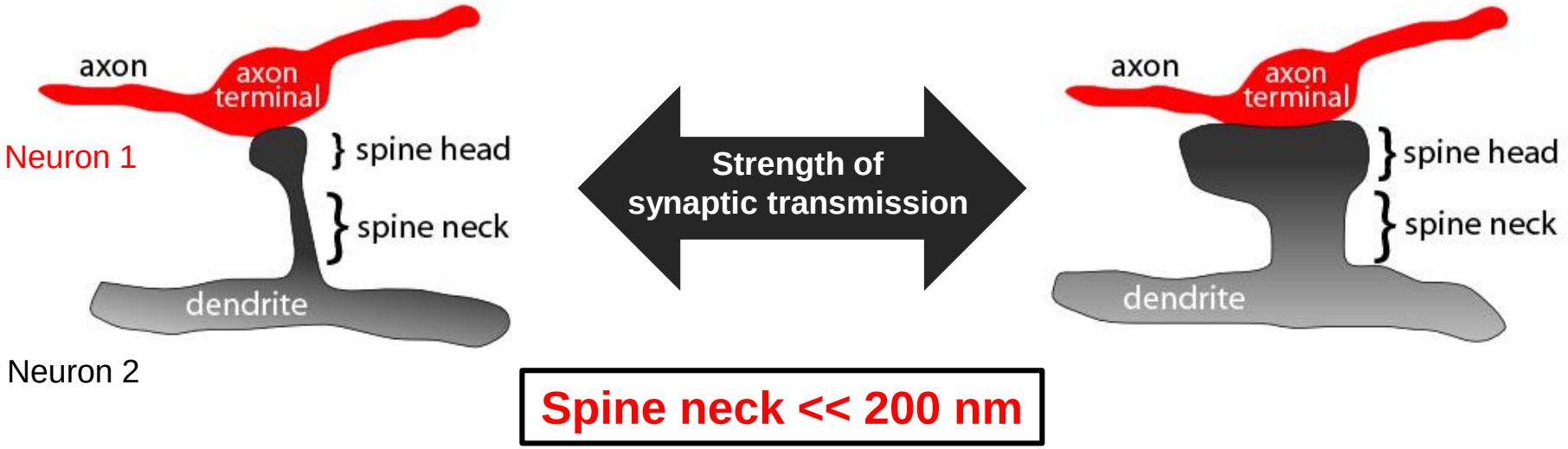


Electron microscopy:



Kristen M. Harris lab
https://synapseweb.clm.utexas.edu/16-chemical-synapses-11

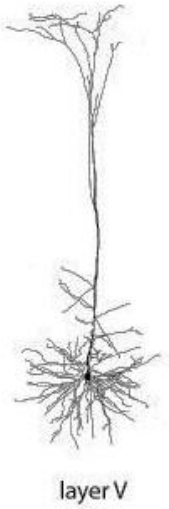
Spine/synapse plasticity requires nanoscale resolution



Spine neck << 200 nm

- Synapses are key functional information processing units of neural circuits.
- Spines are morphological correlates of synaptic strength
- Synaptopathies: Defects in synaptic proteins cause > 100 brain diseases (Autism, Schizophrenia…)

Why imaging spines *in vivo*?



Cells & connections intact,
Natural environment

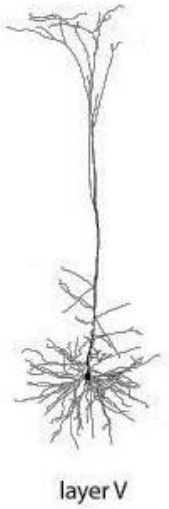
State-of-the-art imaging:
Two-photon microscopy

Zuo et al., 2005, **Neuron, 46, 181**

Information processing

e.g. visual stimulation, learning tasks

STED is ideal to superresolve structures *in vivo*



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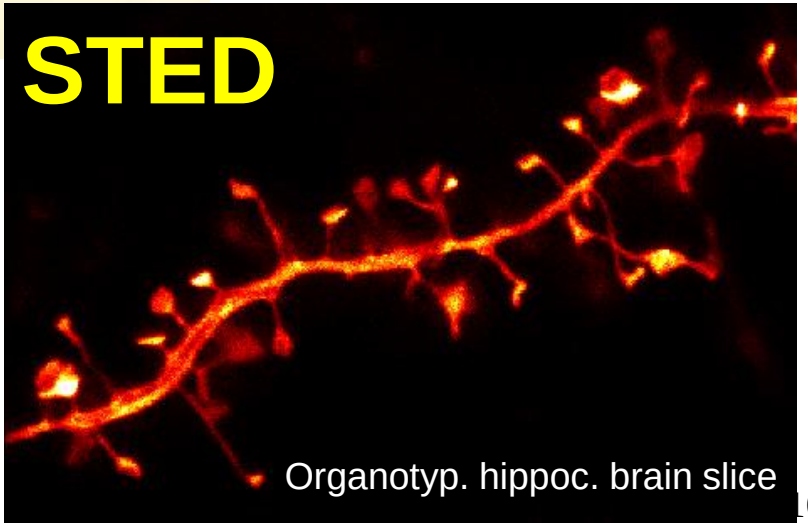
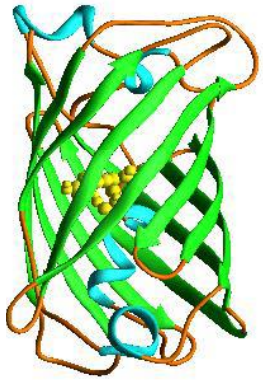
Super-resolution

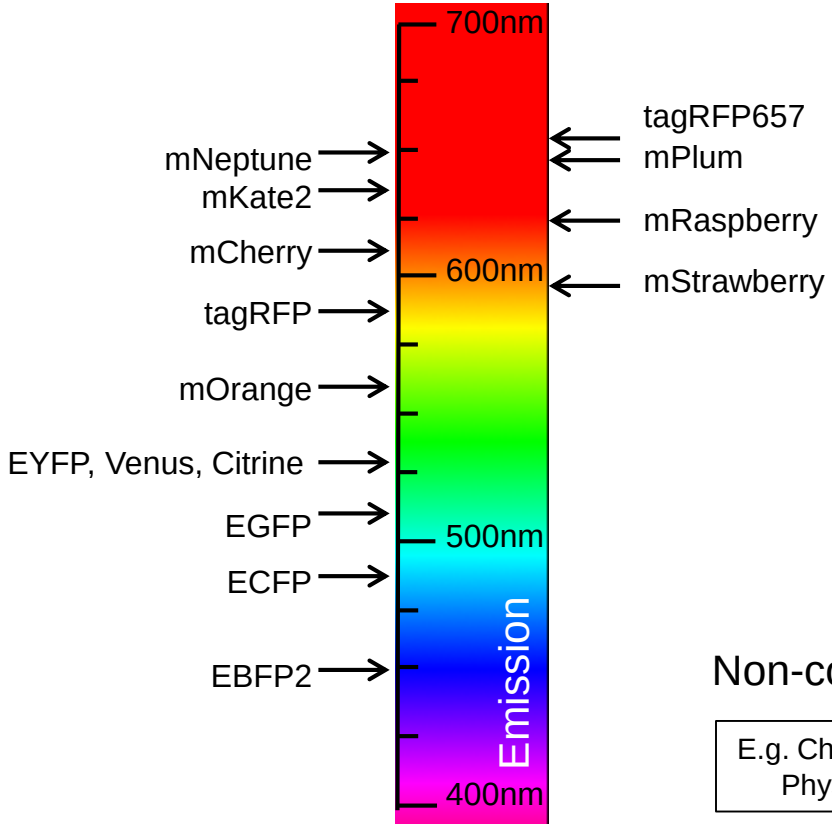


Fluorescent proteins for live-cell STED microscopy

First discovered:
Green Fluorescent Protein (GFP);
(Shimomura 1961)

Aequorea victoria





Non-comprehensive

E.g. Chudakov et al. (2010)
Physiol Rev 90: 1103

Genetically encoded fusion-proteins
for live-cell imaging

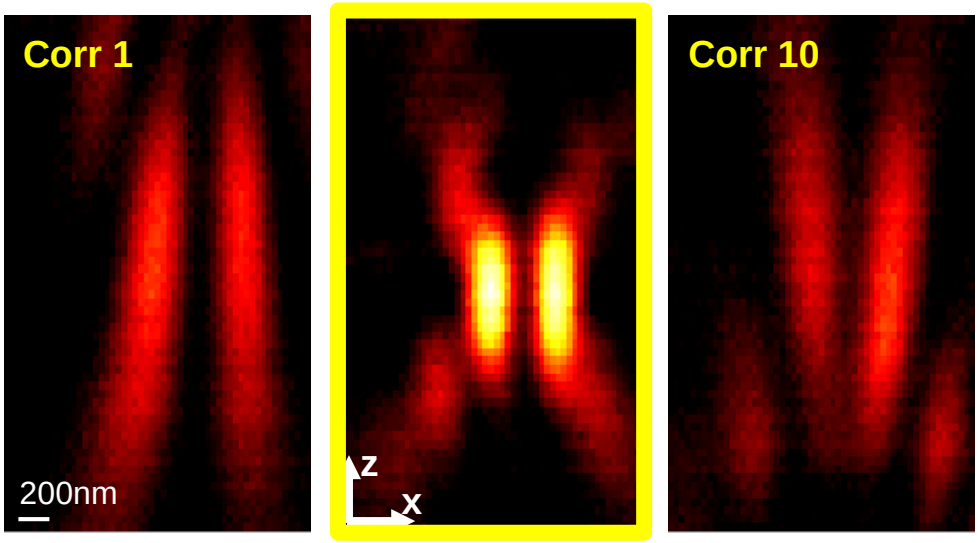
Challenge of tissue imaging: Penetration depth

Problem: refractive index mismatch!

➔ spherical aberrations

Glycerine immersion objective

n = 1,45
NA = 1,3
63x



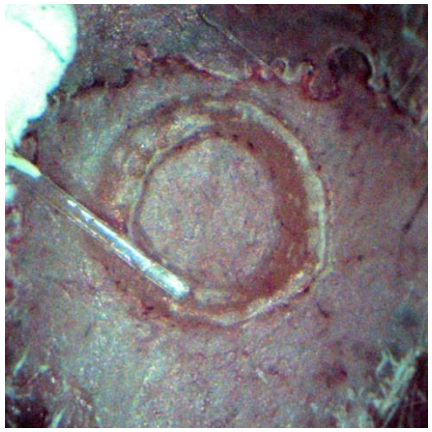
Correction collar

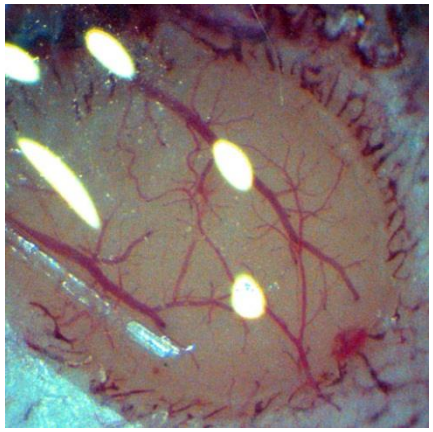
... Compensates spherical aberrations

Exploring the brain *in vivo*

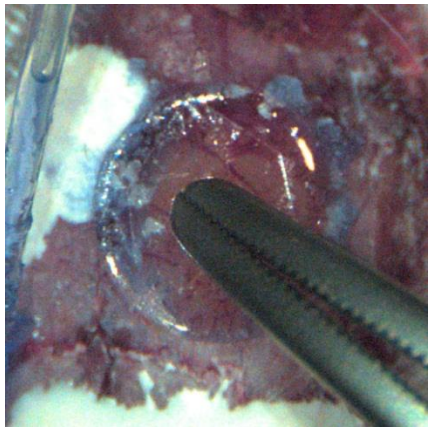
Cranial window for *in vivo* nanoscopy

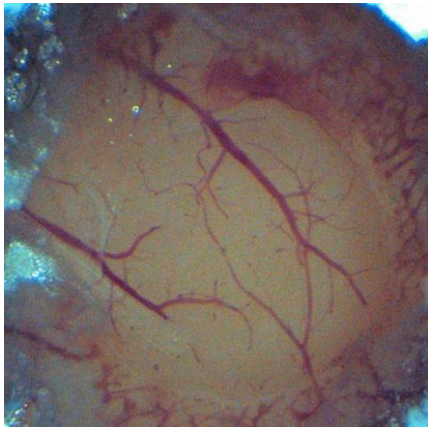
Craniotomie
(open-skull)



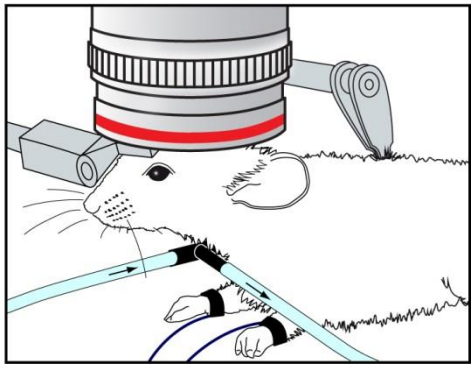


Glass window



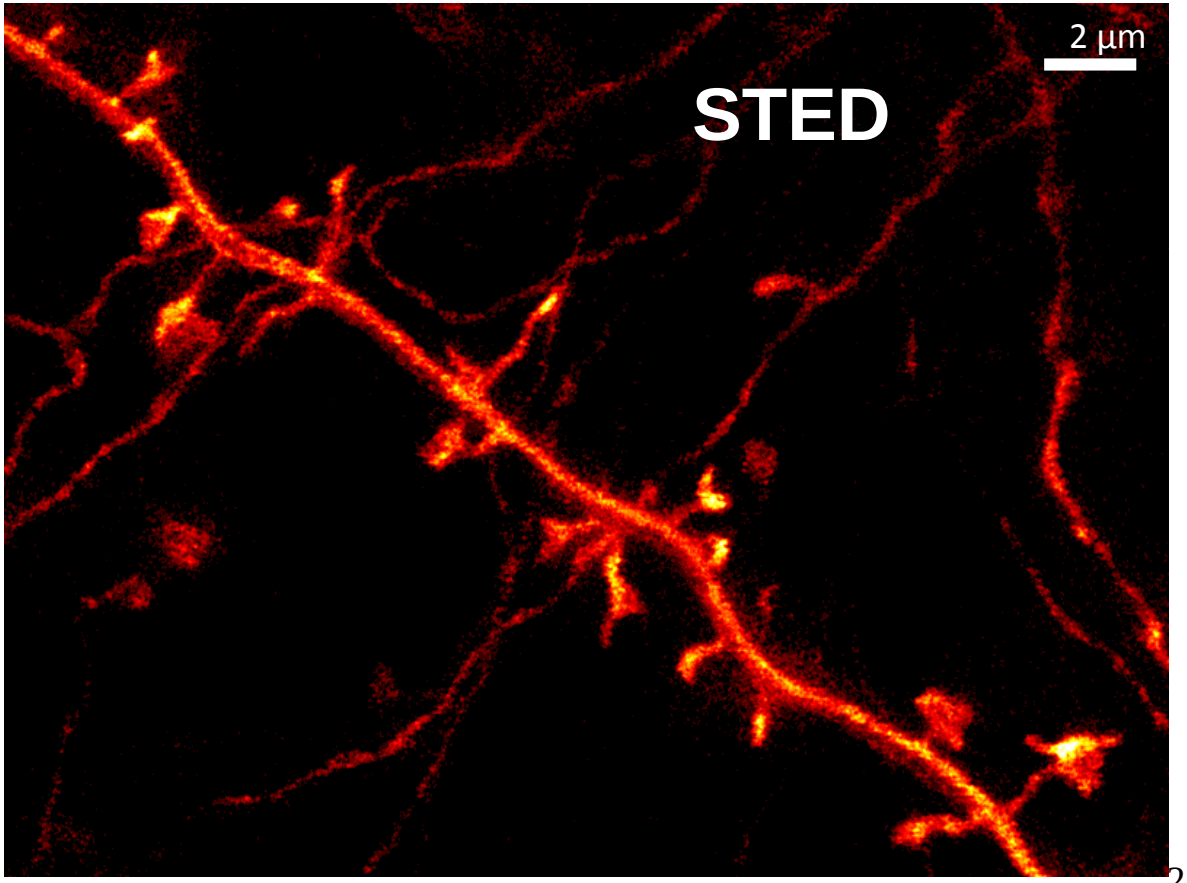


In vivo STED microscopy



Neurons expressing cytoplasmic EYFP

Visual cortex of living mouse



Berning, Willig, Steffens, Dibaj, Hell (2012) Science

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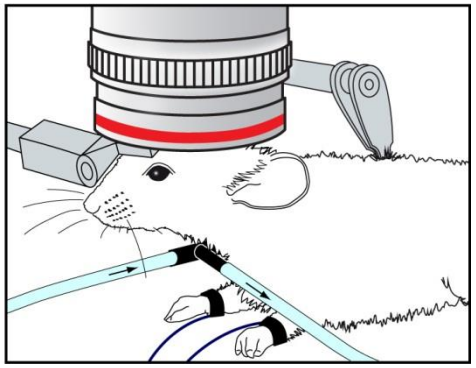

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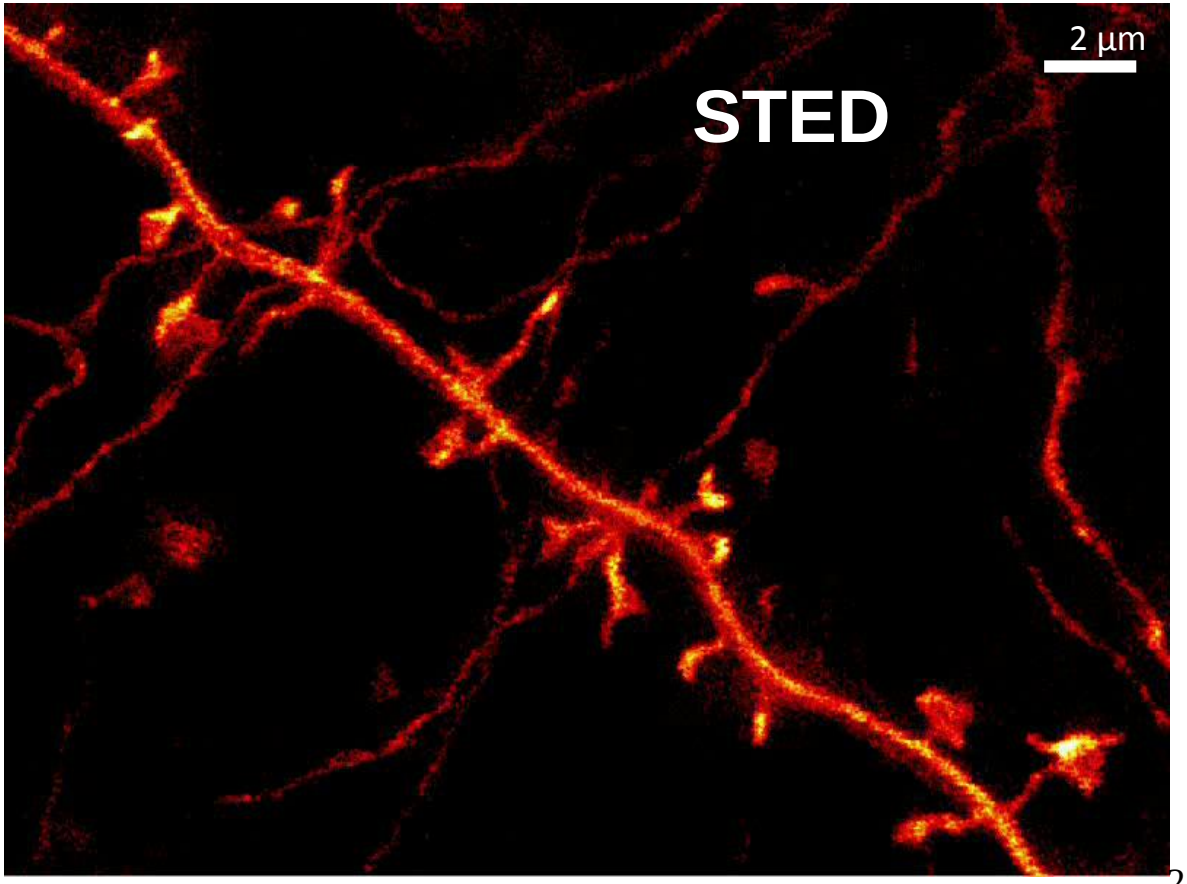
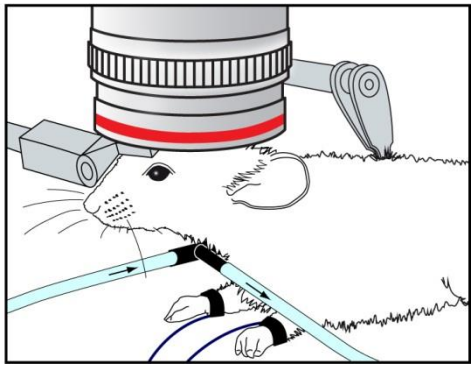


23 x 18 x 3 µm,
10µs / px, 800 x 600 x 5 px, interval 5 min

Berning, Willig, Steffens, Dibaj, Hell (2012) Science

In vivo STED microscopy



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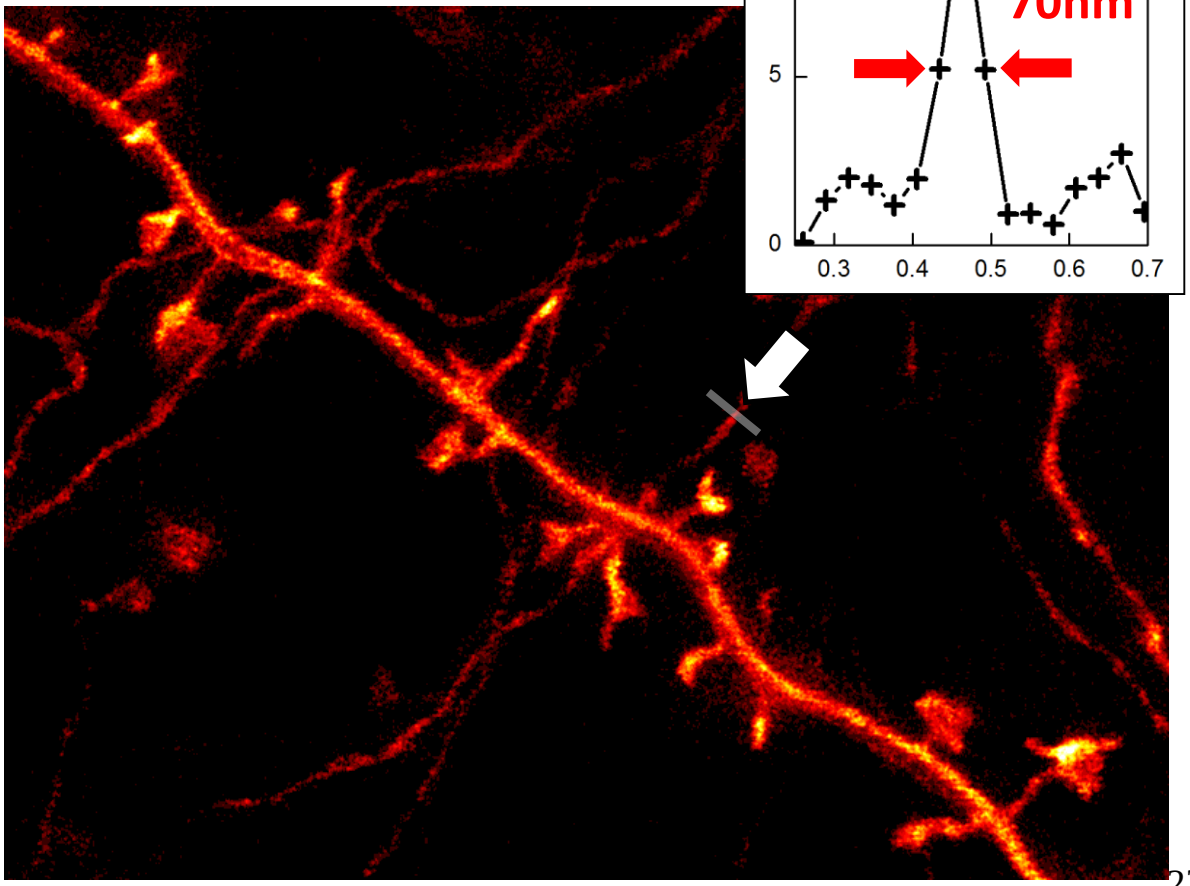

Berning, Willig, Steffens, Dibaj, Hell (2012) Science

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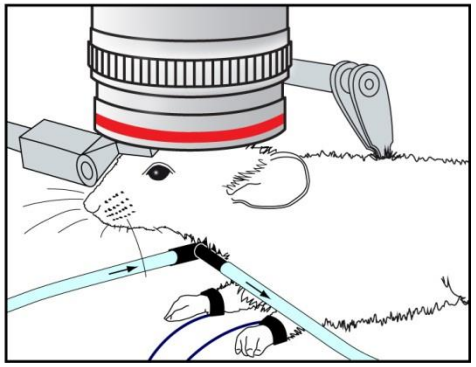

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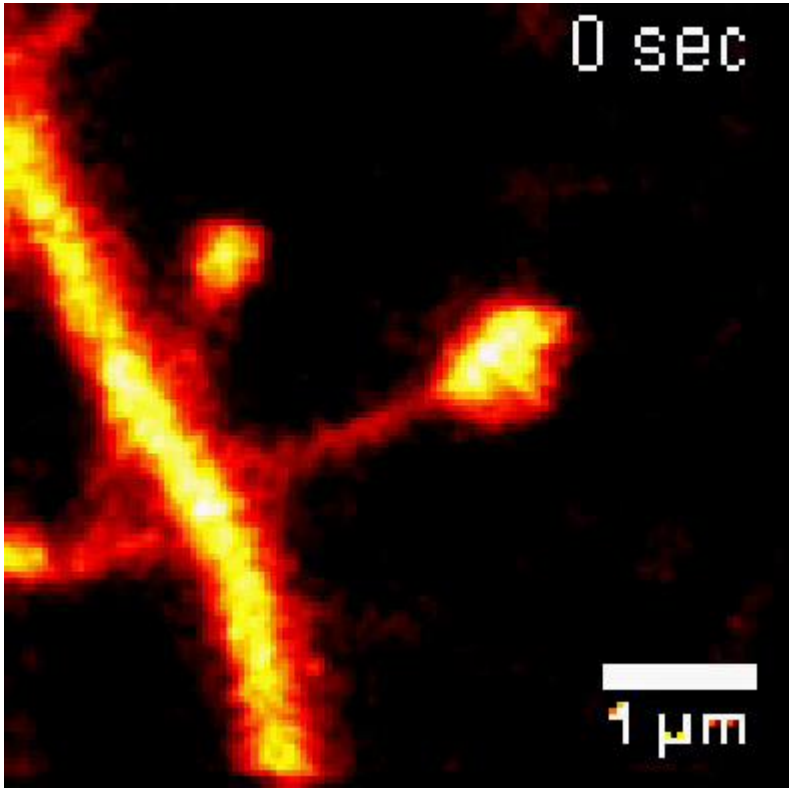
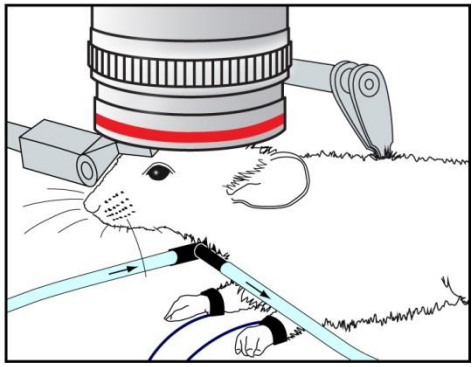


STED

128 z-stacks, 5 slices
interval 10 sec

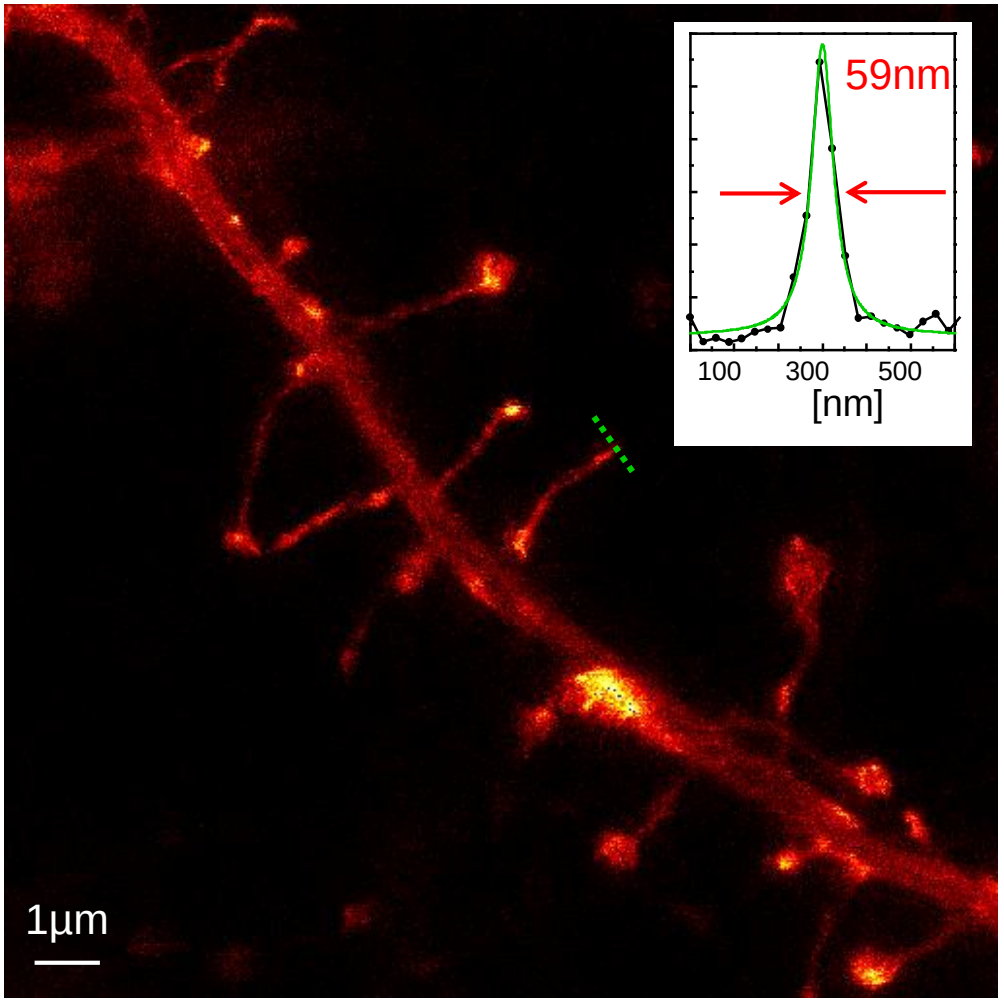
Berning, Willig, Steffens, Dibaj, Hell (2012) Science

In vivo STED microscopy of actin



Actin

Lifeact-YFP

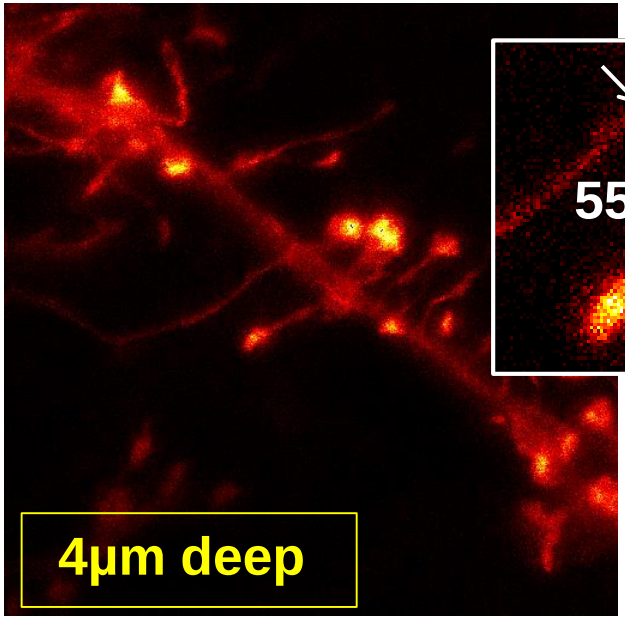


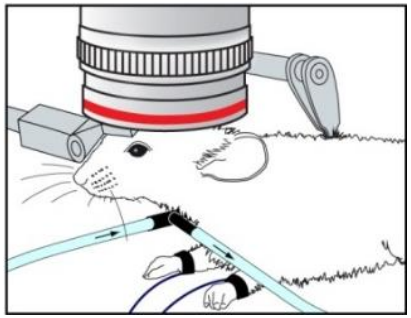
Maximum intensity projection

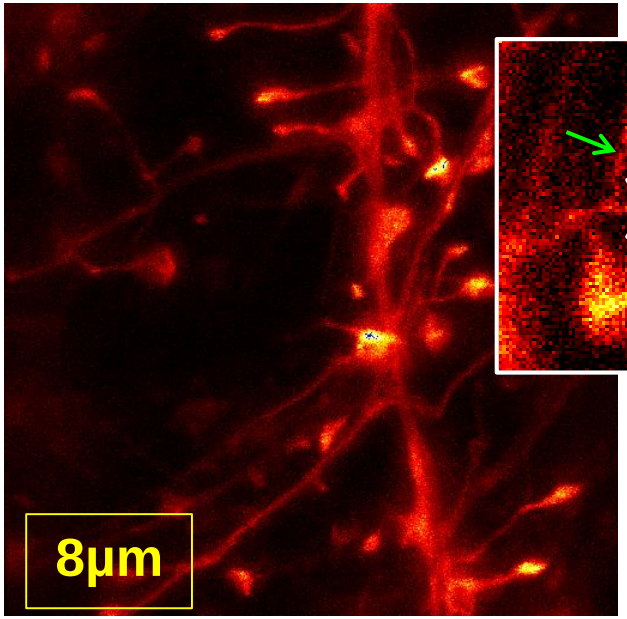
Raw data

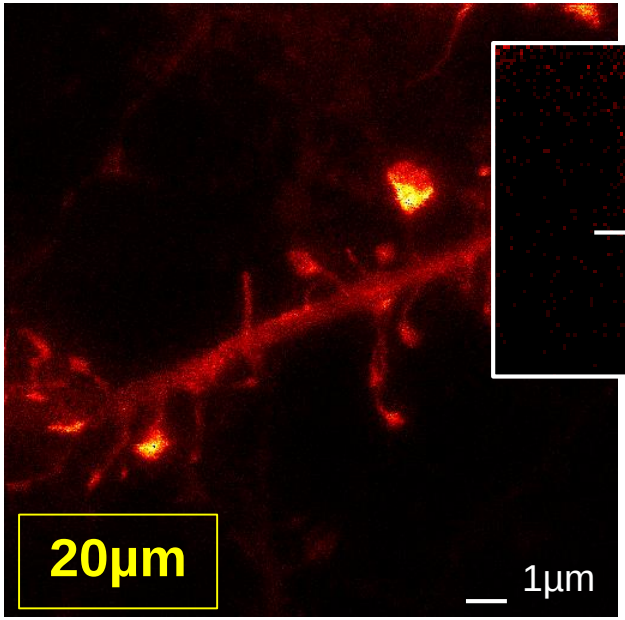
Willig et al. (2014)
Biophys. J. 106

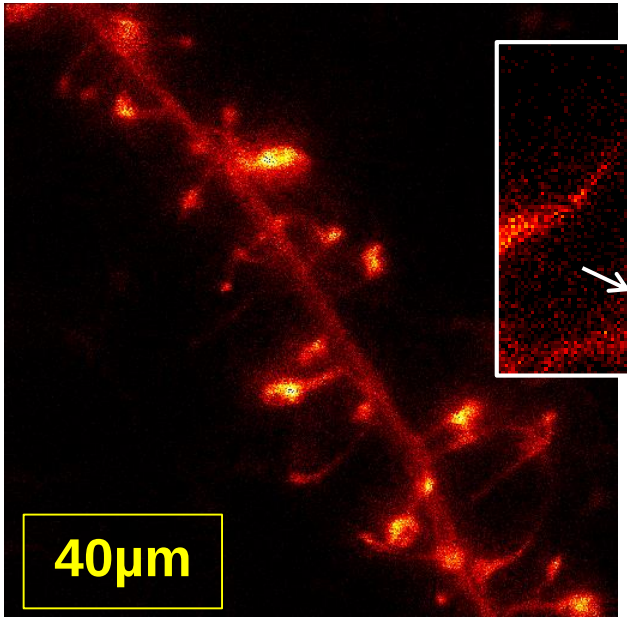
Actin





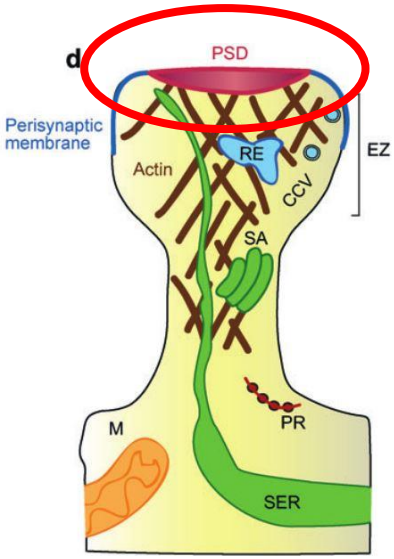


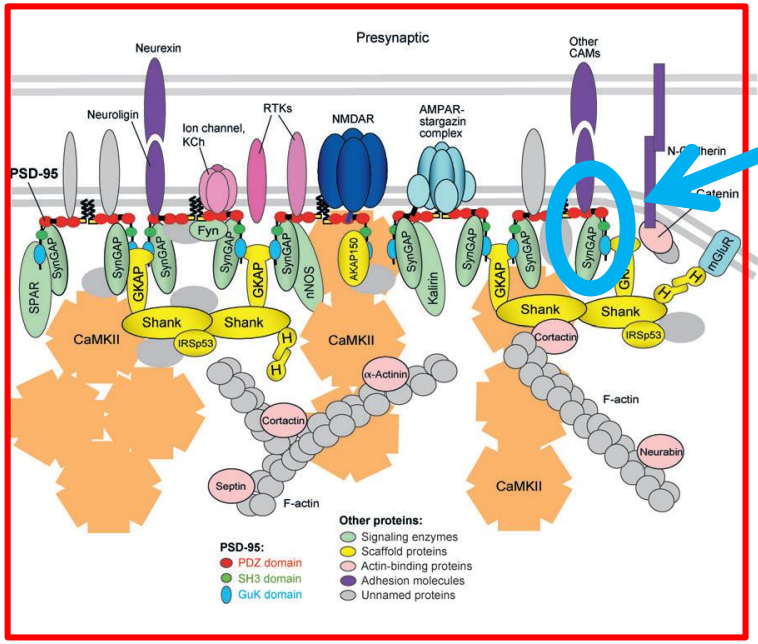




Willig et al. (2014)
Biophys. J. 106

In vivo STED microscopy of the post-synaptic density





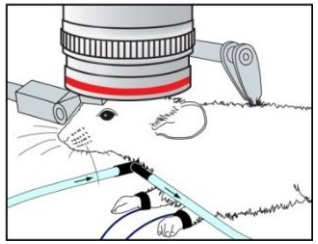
PSD95
Scaffolding protein & signalling platform

Sheng & Hoogenraad *Annu. Rev. Biochem.* 2007 76:823

- **High complexity:** PSD comprises ~1500 proteins
- **PSD** (post-synaptic density) size correlates with synaptic strength

Size 200-800nm
50nm thin

In vivo nano-organization of PSD95

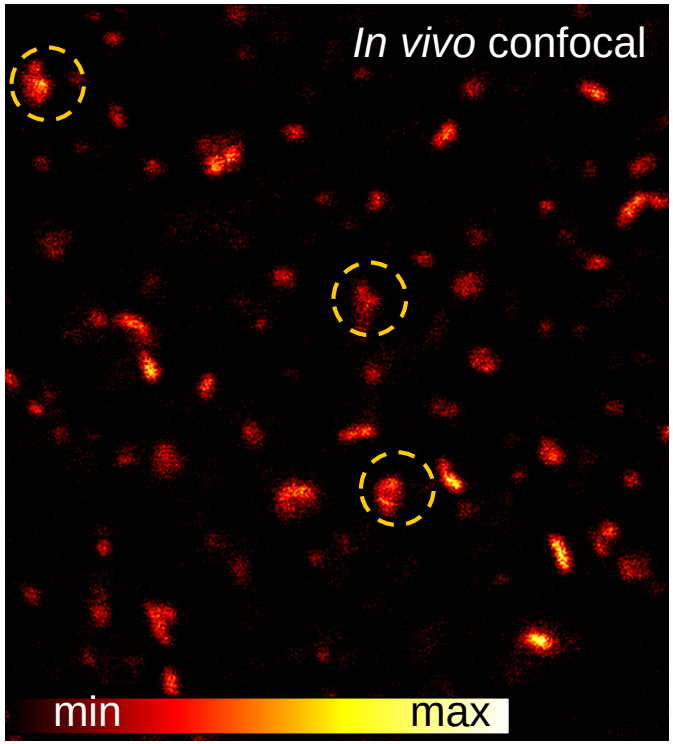


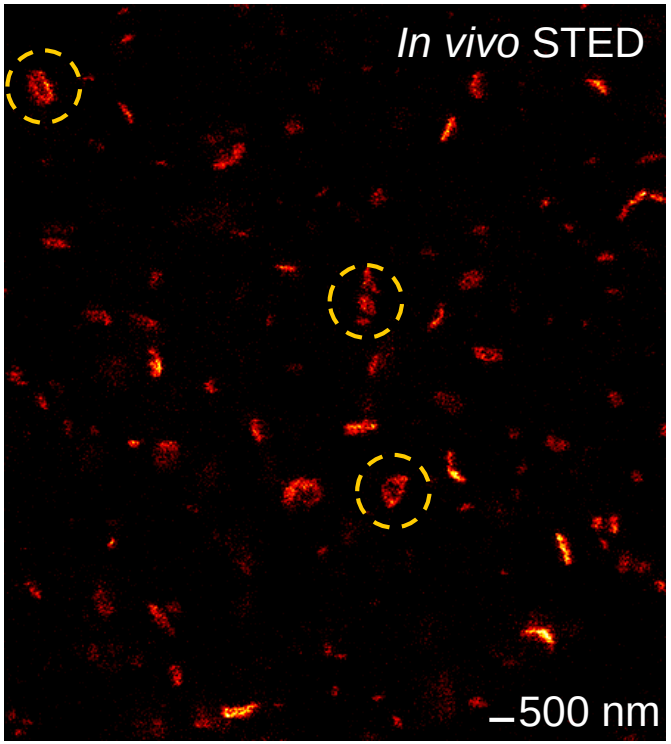
Knock-in mouse
PSD95-GFP
Visual cortex

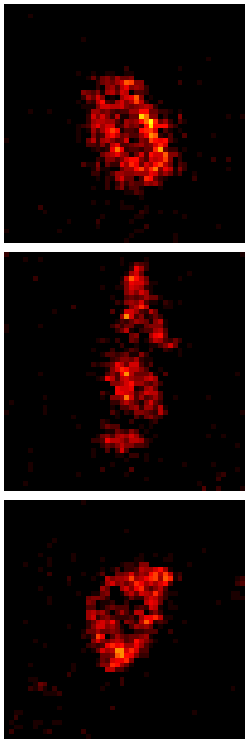
Side-view:

Top-view:

PSD95-GFP







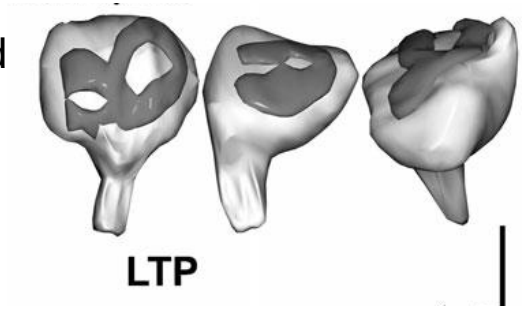
Wegner, Mott, Grant, Steffens, **Willig** (2018)
Sci Rep. 8, 219-219

Nano-organization of PSD95: EM vs. *in vivo* STED

EM

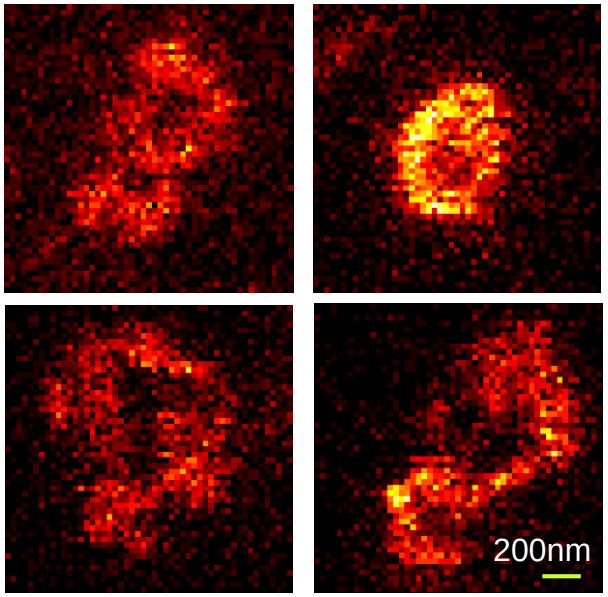
Mushroom spines

Perforated PSD



fixed

STED



Perforated PSD95

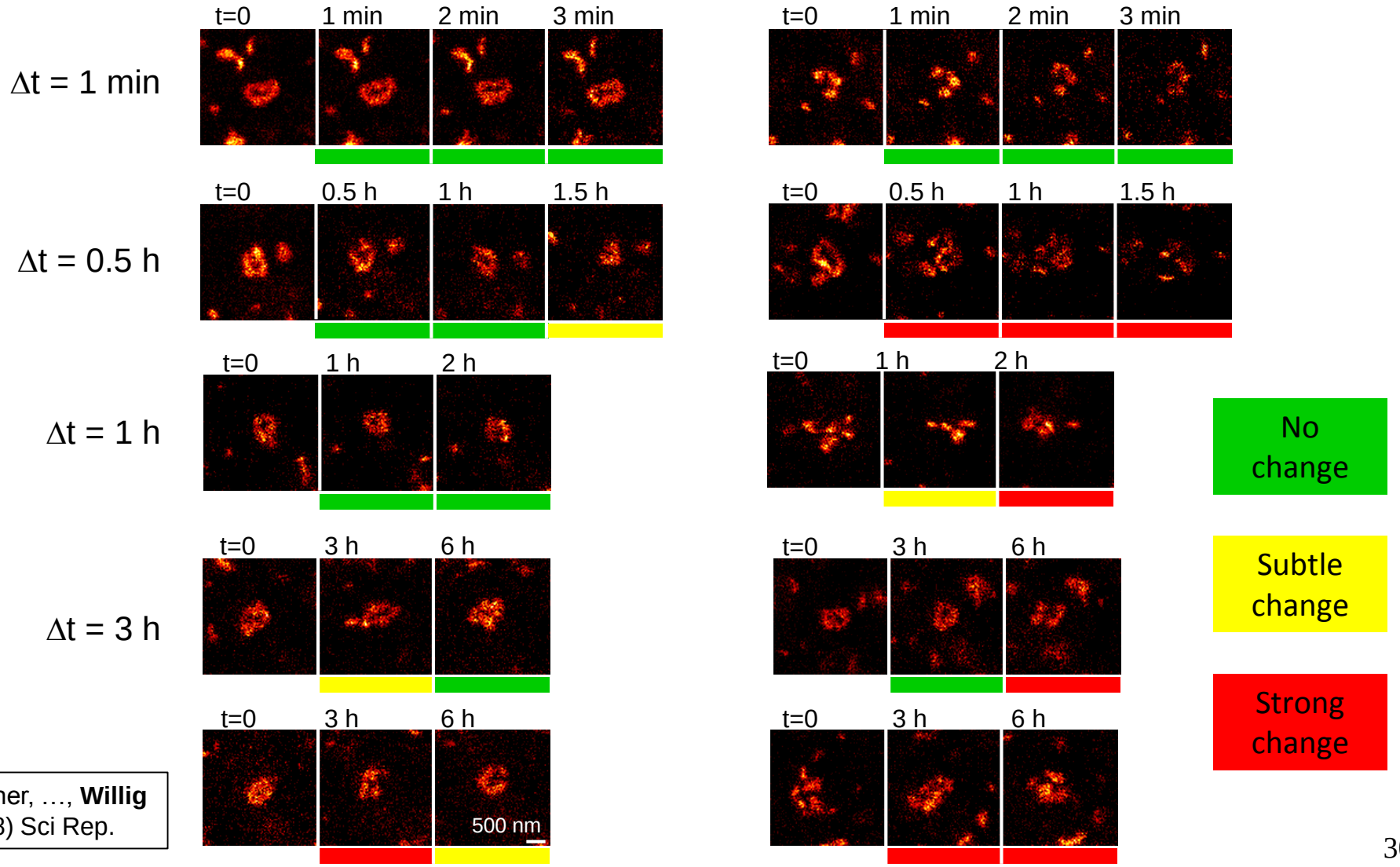
In vivo

Stewart et al. (2005). European Journal of Neuroscience, 21(12), 3368–3378.

Morphological changes of large PSD95 assemblies *in vivo*

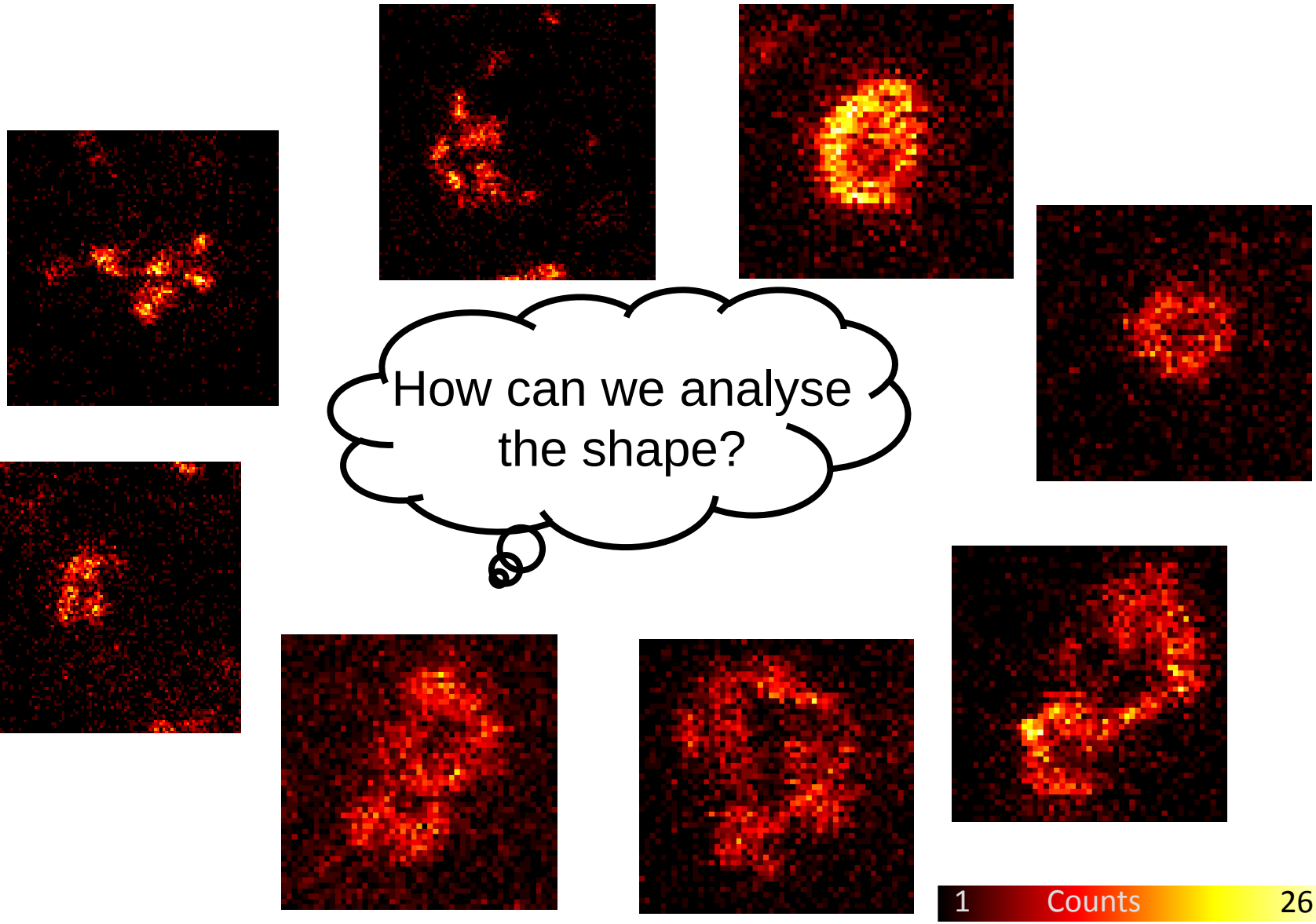
Time intervall

***In vivo* STED**



Wegner, …, **Willig** (2018) Sci Rep.

Dissecting the PSD *in vivo*



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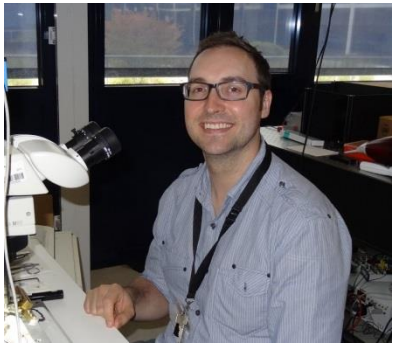

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Nanoscale Microscopy and Molecular Physiology of the Brain Cluster of Excellence 171, DFG Research Center 103

MPI for Experimental Medicine