

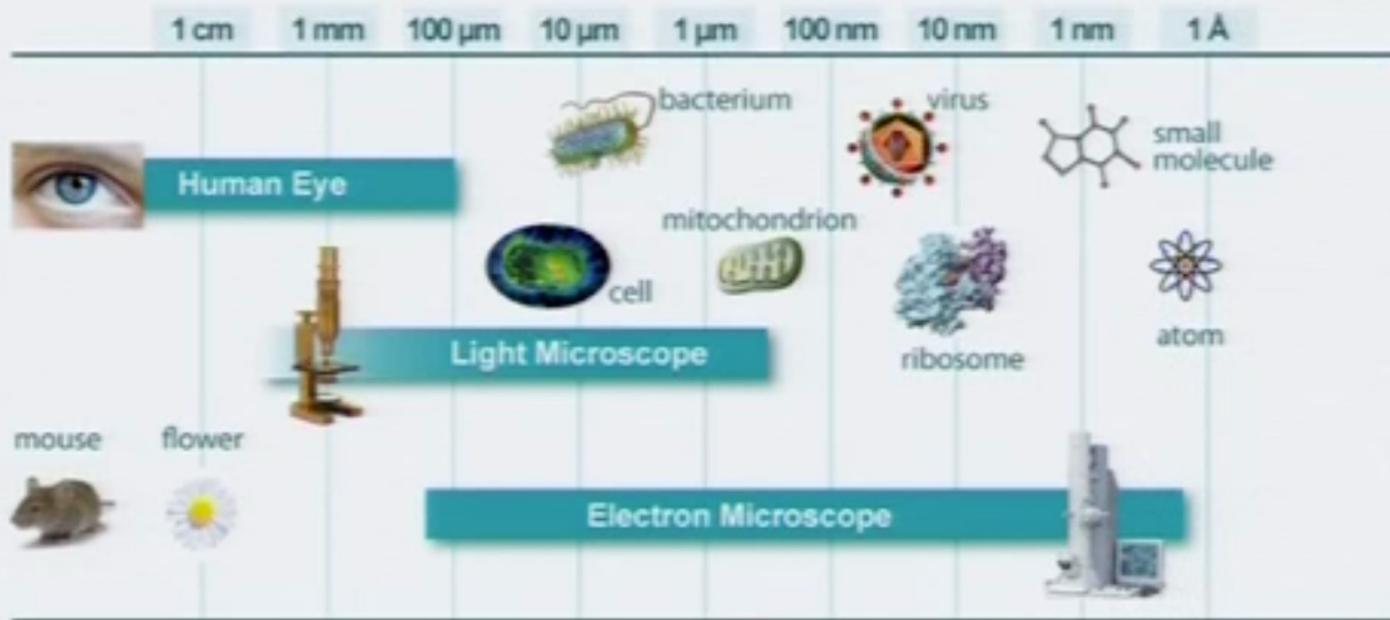
Intelligent Analysis of Biomedical Images

Presenter: Mohammad H. Rohban, Ph.D.

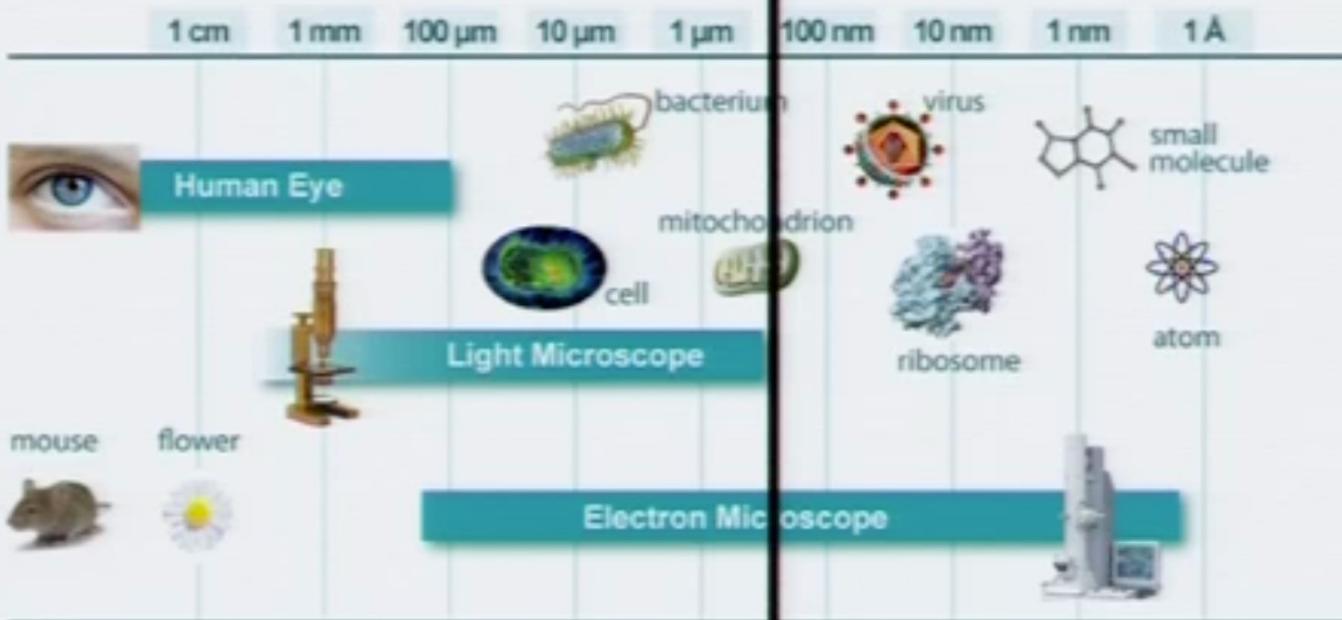
Fall 2023

Courtesy: Some slides are adopted from the videos in iBiology and
Shomu's Biology Channel

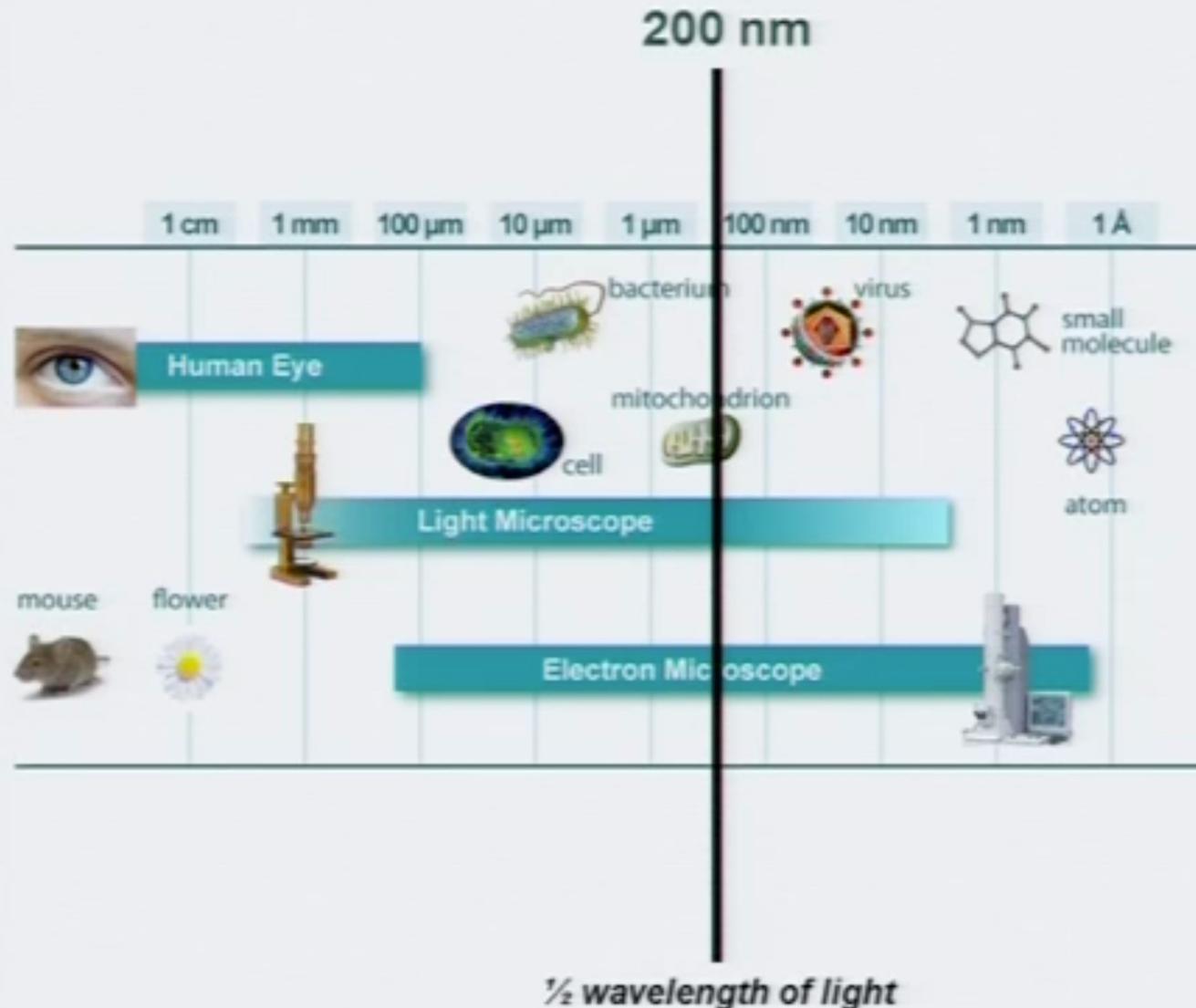
Introduction to Super-Resolution Microscopy



200 nm



$\frac{1}{2}$ wavelength of light





The Diffraction Barrier



Verdet (1869)

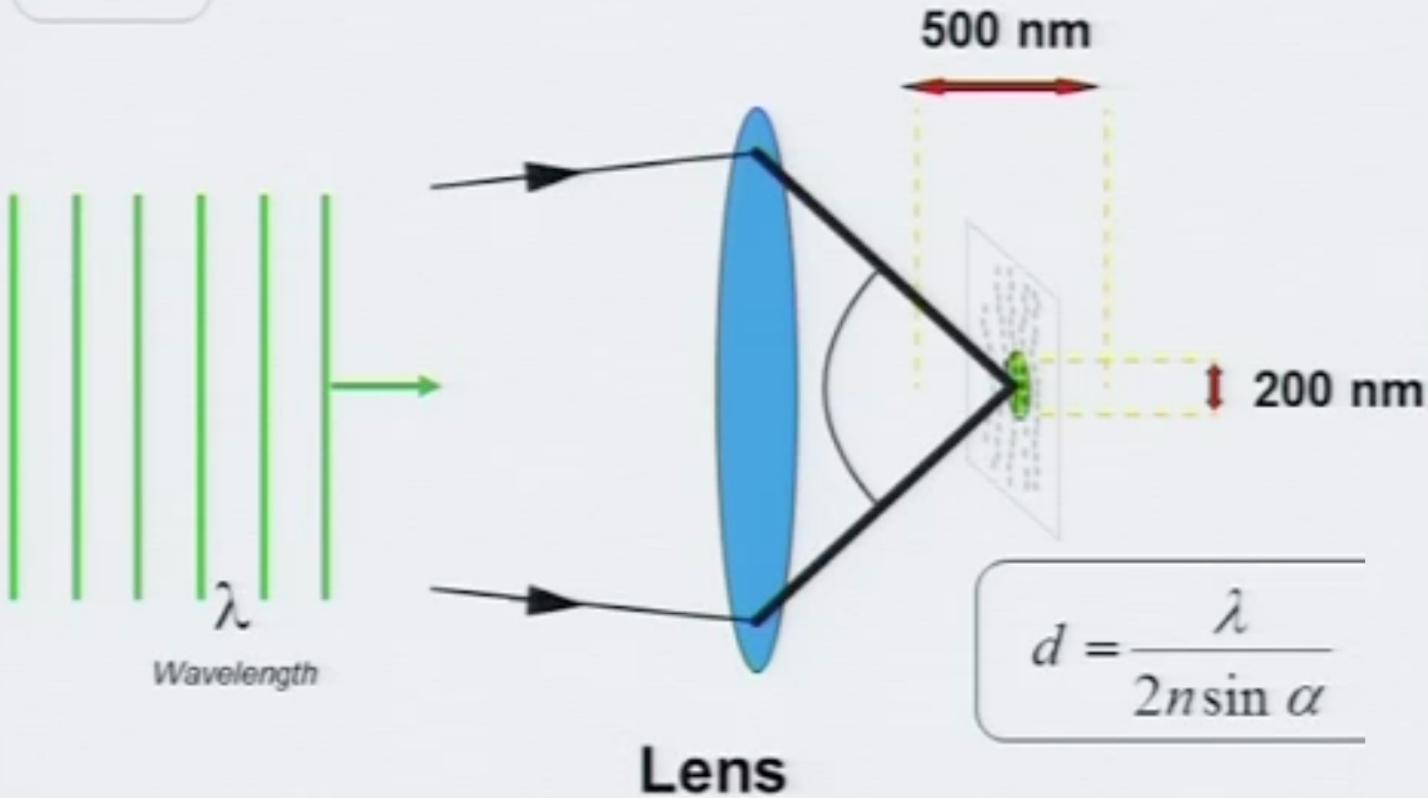
Abbe (1873)

Helmholtz (1874)

Rayleigh (1874)



The Diffraction Barrier



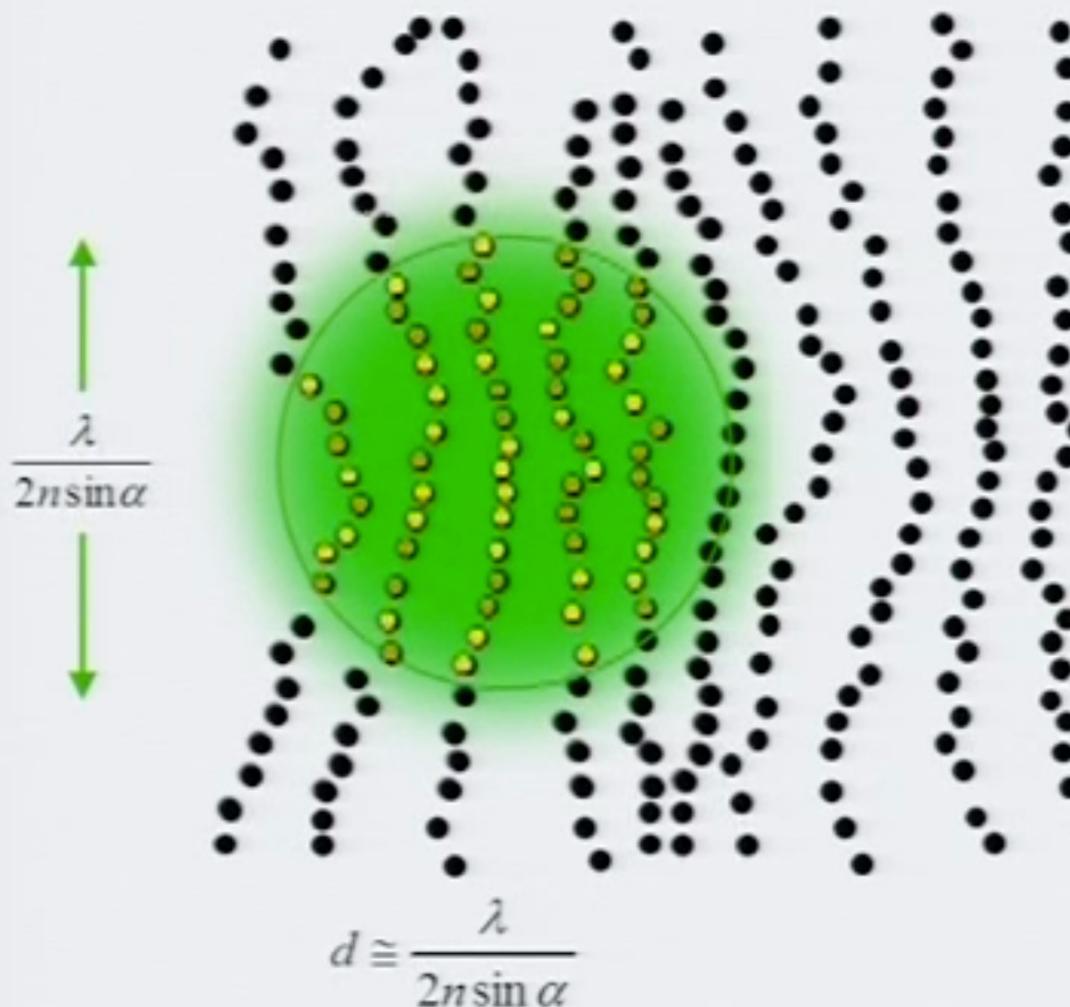
Verdet (1869)

Abbe (1873)

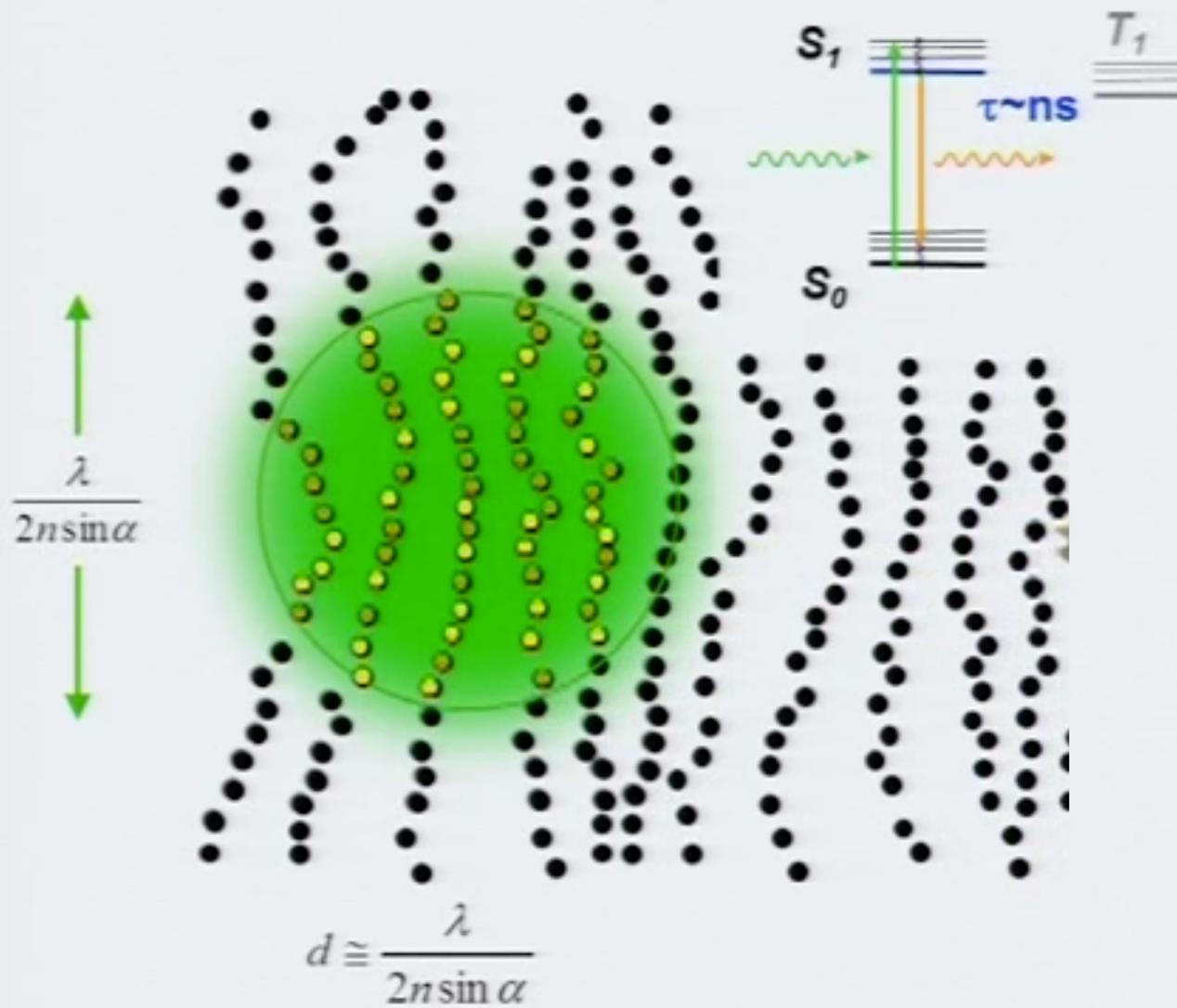
Helmholtz (1874)

Rayleigh (1874)

Problem: Features (molecules) within < 200 nm not discernible

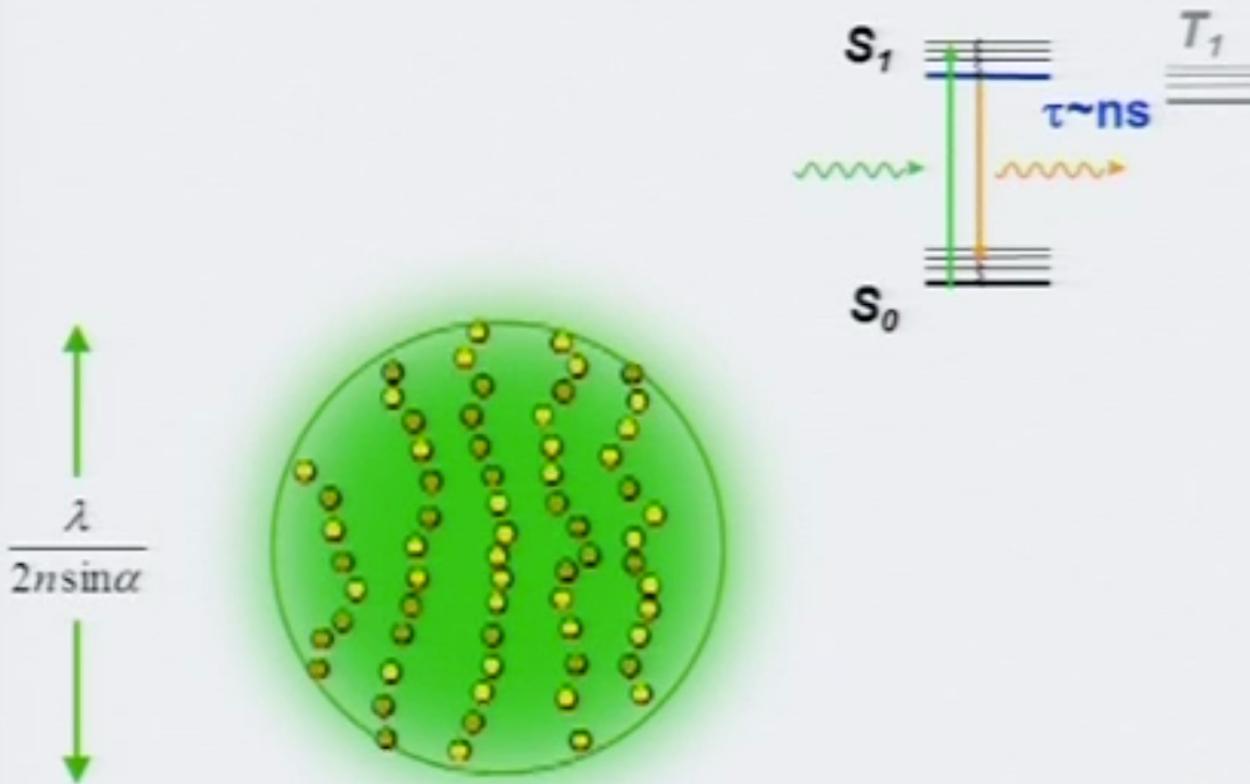


Problem: Features (molecules) within < 200 nm not discernible



Problem: Features (molecules) within < 200 nm not discernible

Solution: Keep some features (molecules) dark.

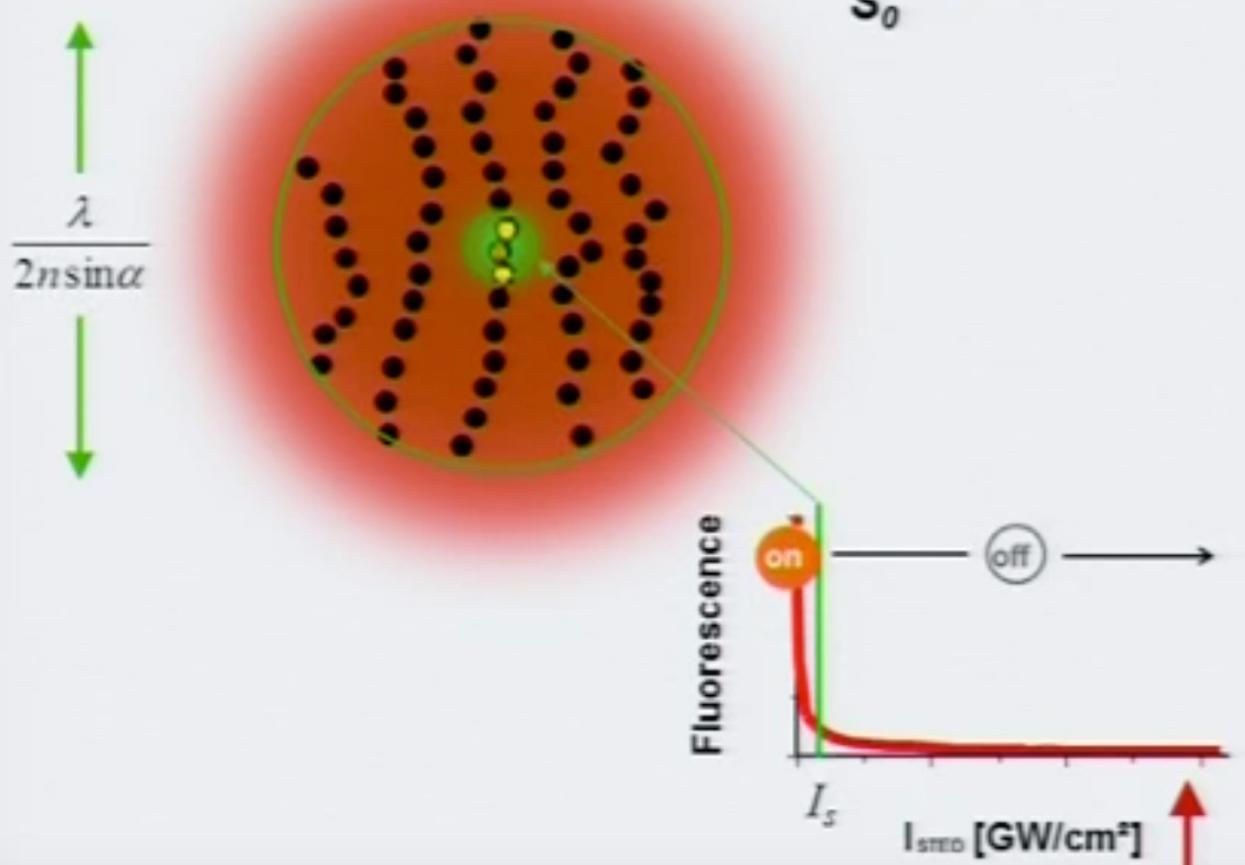


Problem: Features (molecules) within < 200 nm not discernible

Solution: Keep some features (molecules) dark.

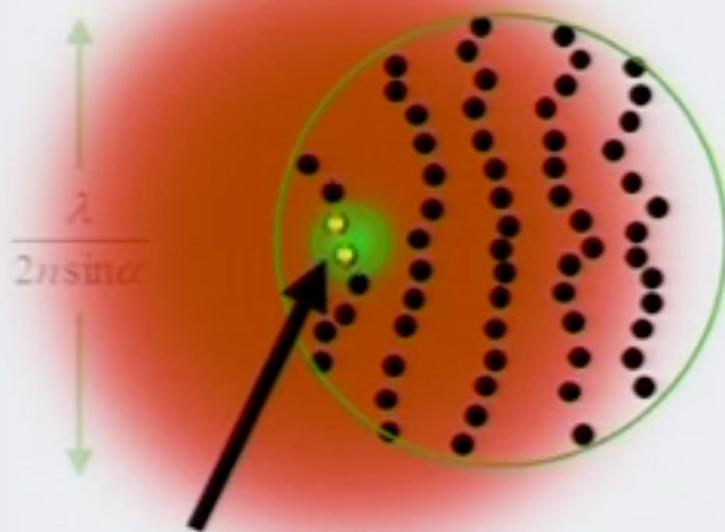
STED

SW Hell, J Wichmann Opt Lett (1994)

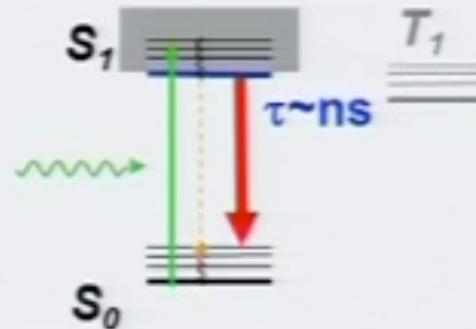


Keep some fluorophores dark. Play ON / OFF

STED

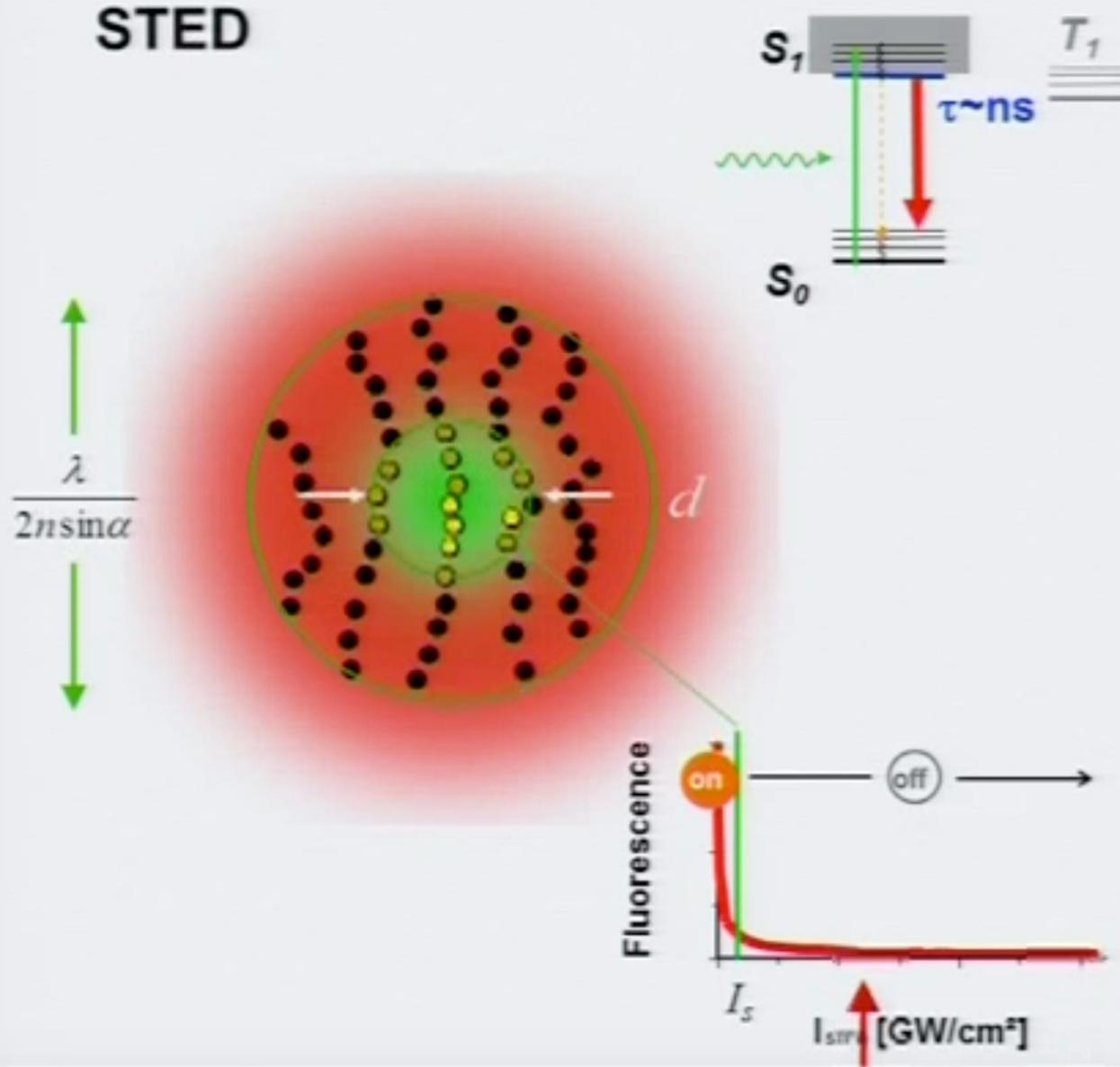


Coordinates preset : nm precision



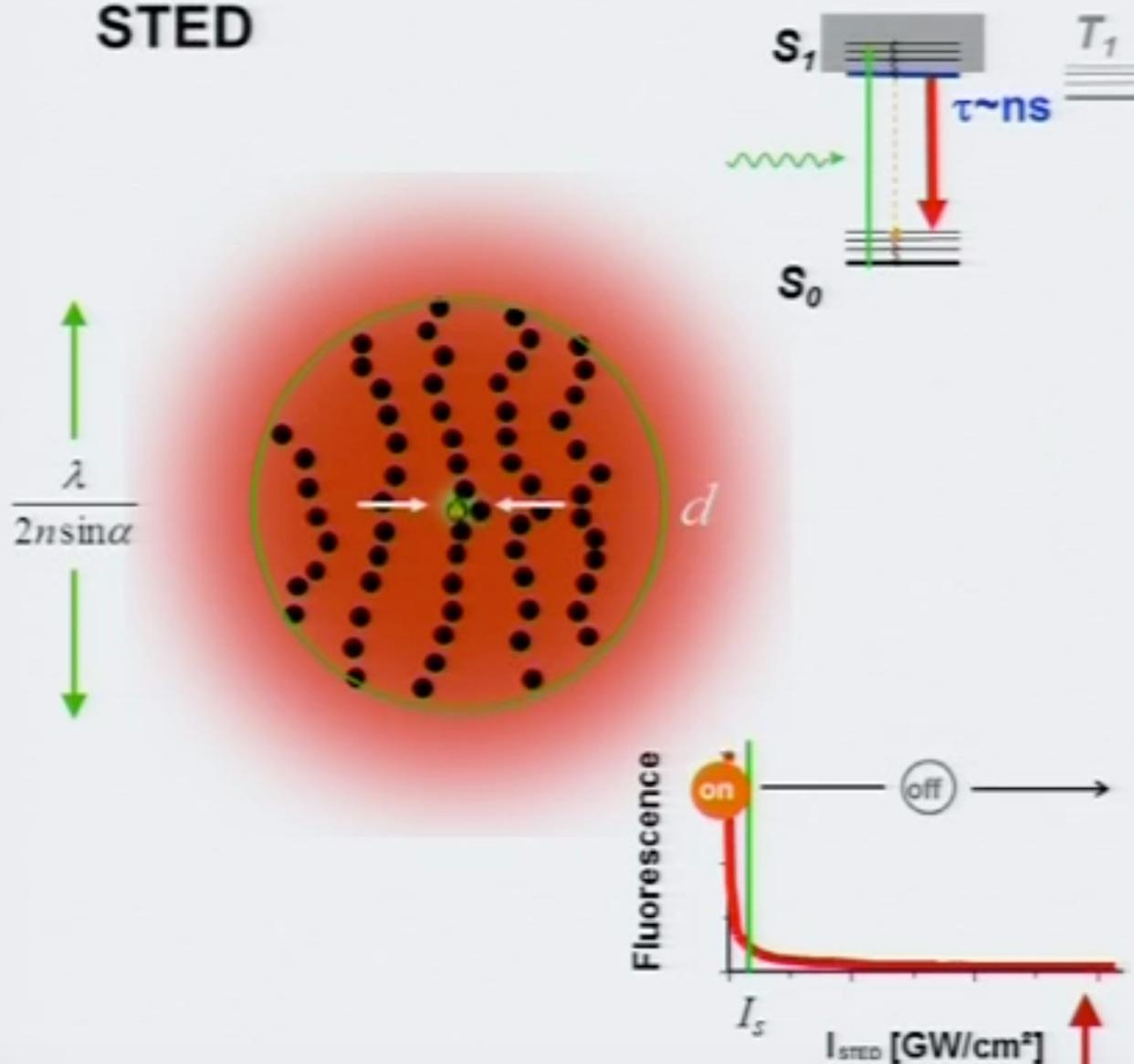
Keep some fluorophores dark. Play ON / OFF

STED



Keep some fluorophores dark. Play ON / OFF

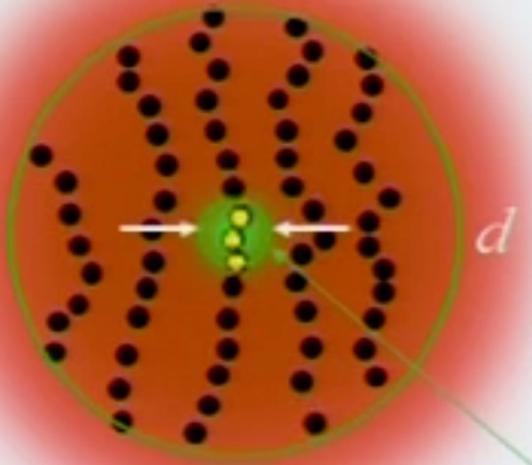
STED



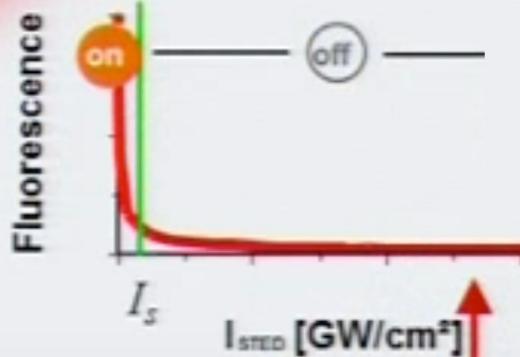
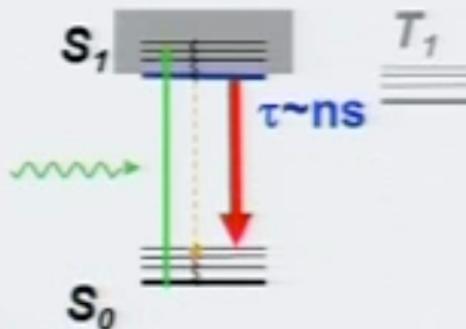
Keep some fluorophores dark. Play ON / OFF

STED

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

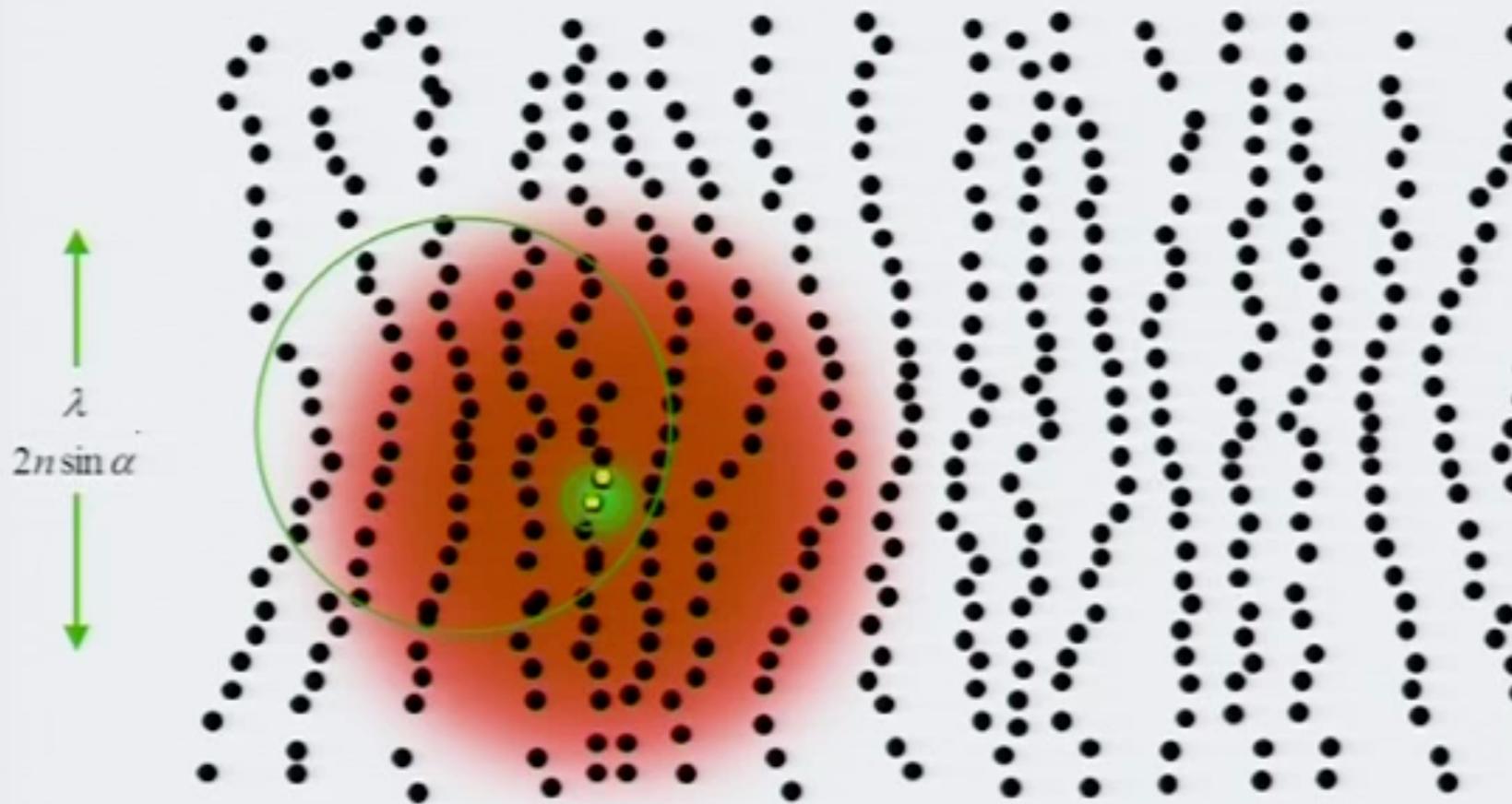


$$d \approx \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_c}}$$



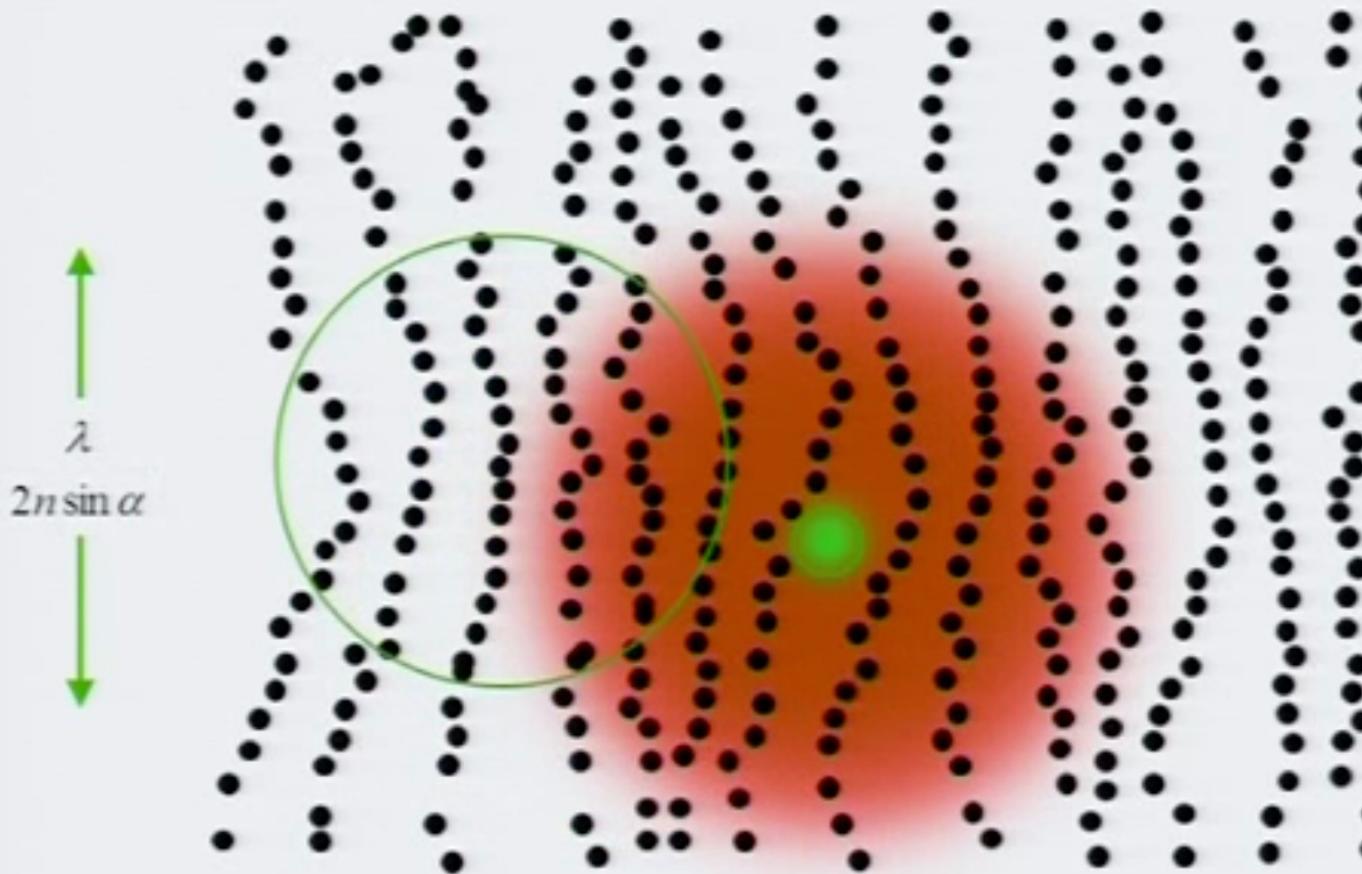
Keep some fluorophores dark. Play ON / OFF

STED

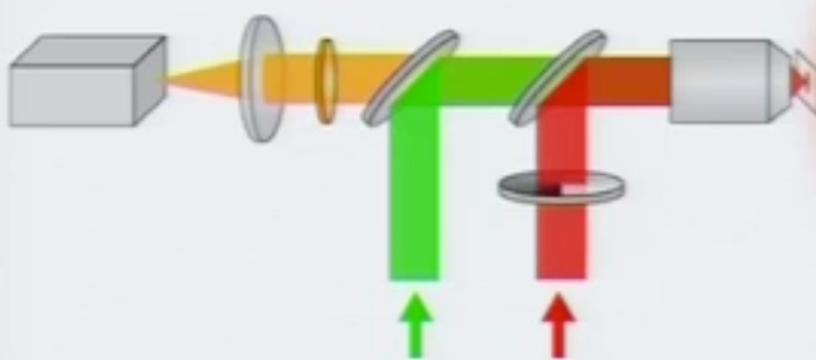


Keep some fluorophores dark. Play ON / OFF

STED



STED Microscope



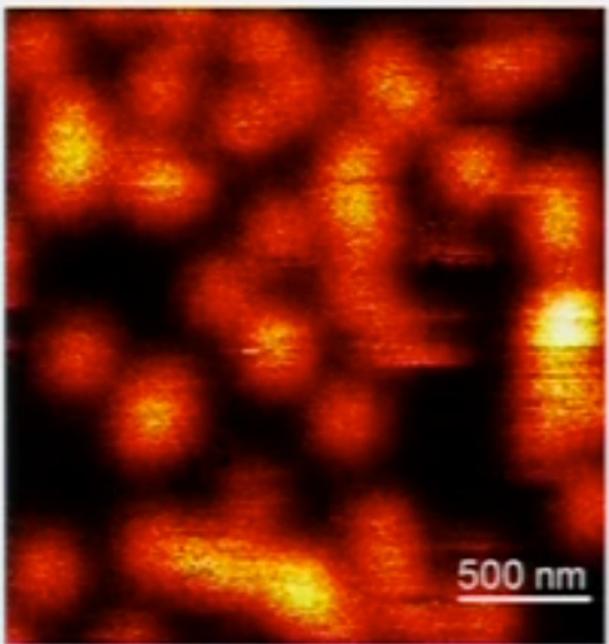
excitation de-excitation
(STED)

$$d \cong \frac{\lambda}{2n \sin \alpha \sqrt{1 + I/I_z}}$$



Single molecules

Confocal

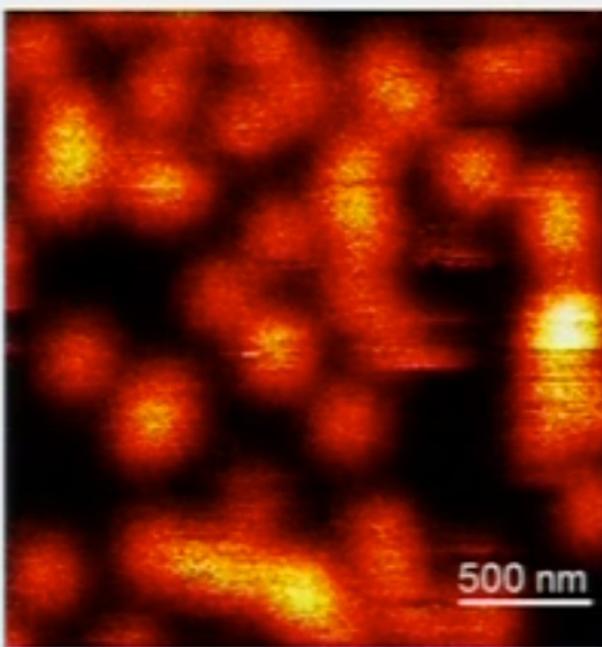


STED

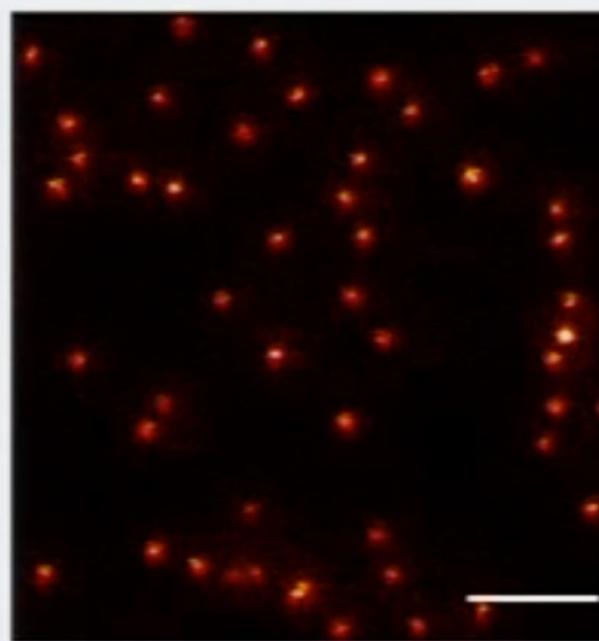
$\lambda_{\text{STED}} = 750 \text{ nm}$

Single molecules

Confocal



STED

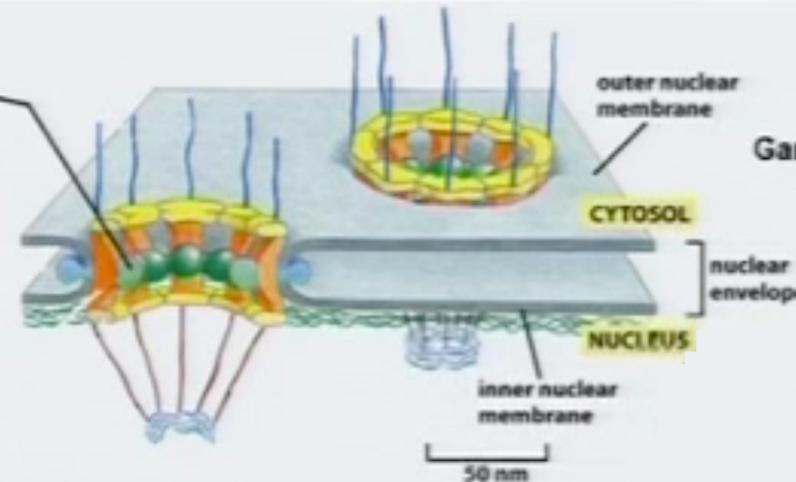


$$\lambda_{\text{STED}} = 750 \text{ nm}$$

Nuclear pore complex

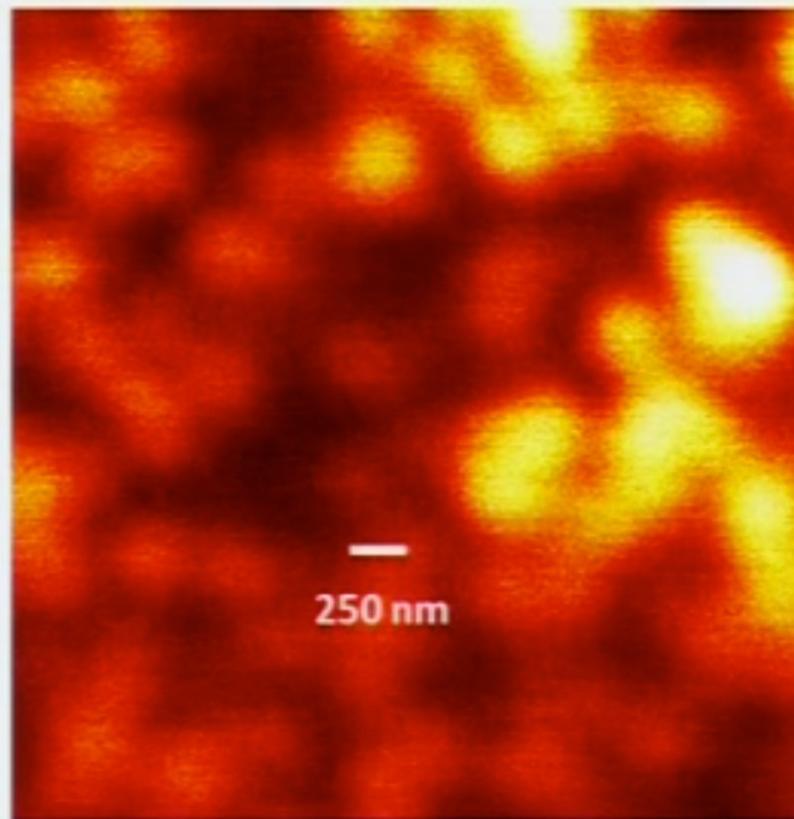
gp210

Xenopus laevis / epithelial cell line



Garland, Science 2008

Confocal

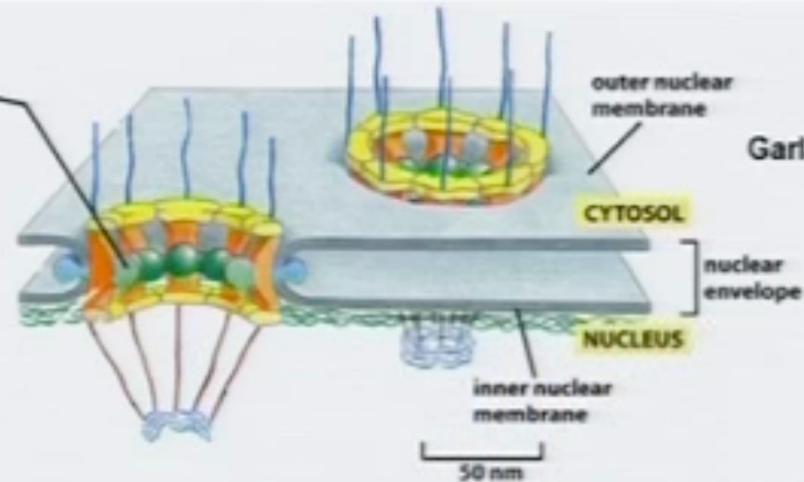


Göttfert et al (2012)

Nuclear pore complex

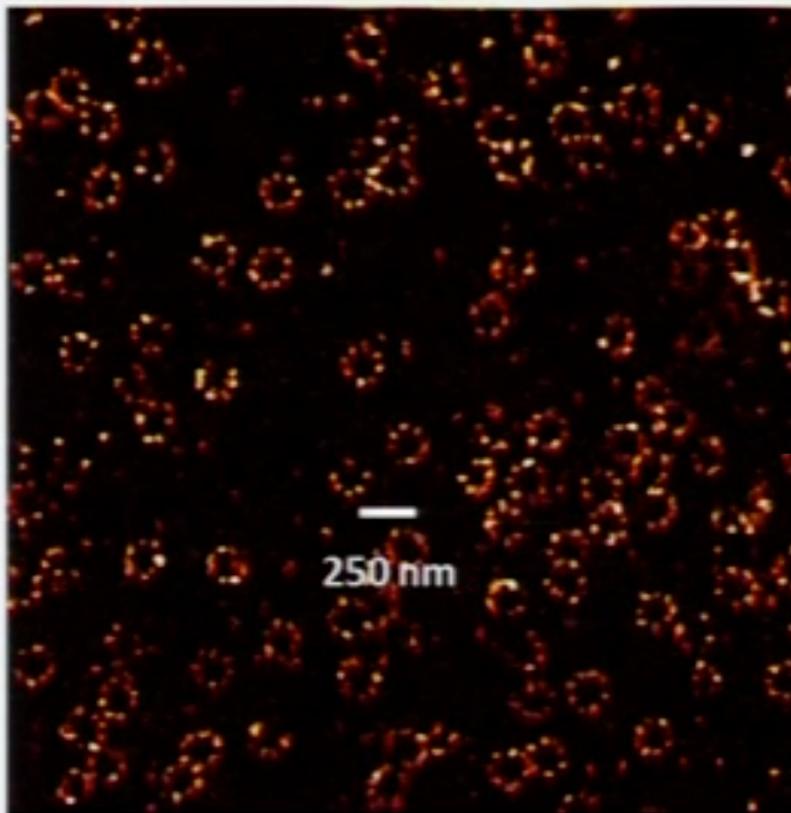
gp210

Xenopus laevis /epithelial cell line



Garland, Science 2008

STED

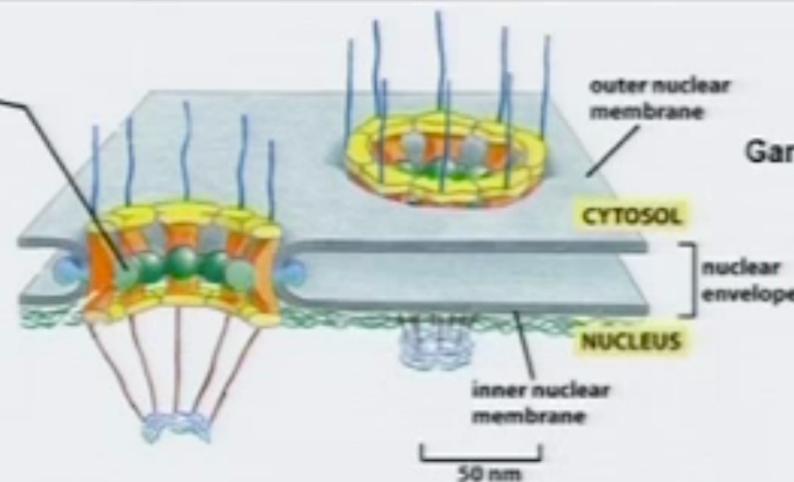


Göttfert et al (2012)

Nuclear pore complex

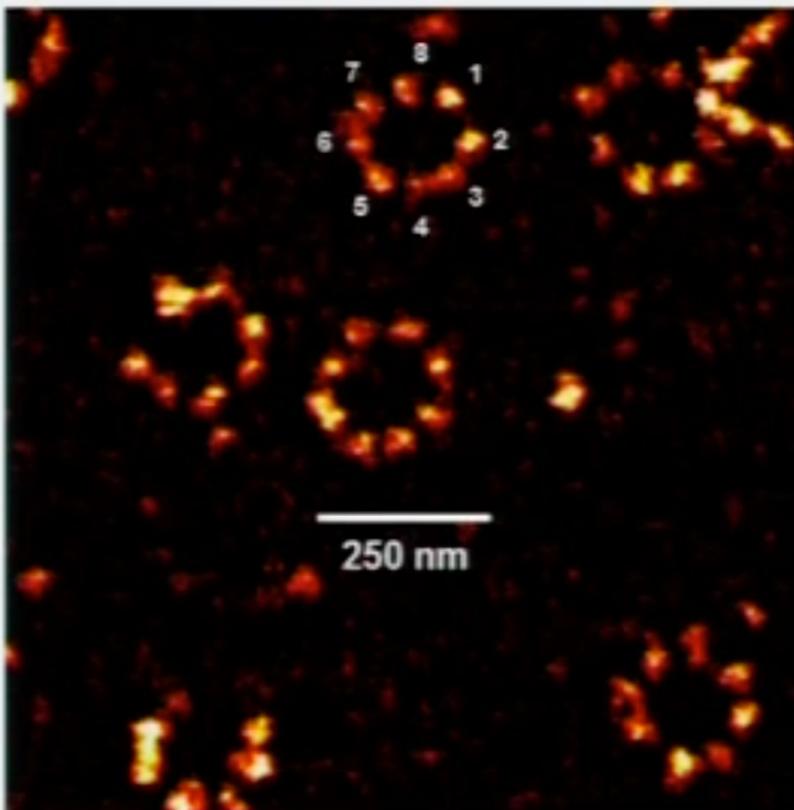
gp210

Xenopus laevis /epithelial cell line



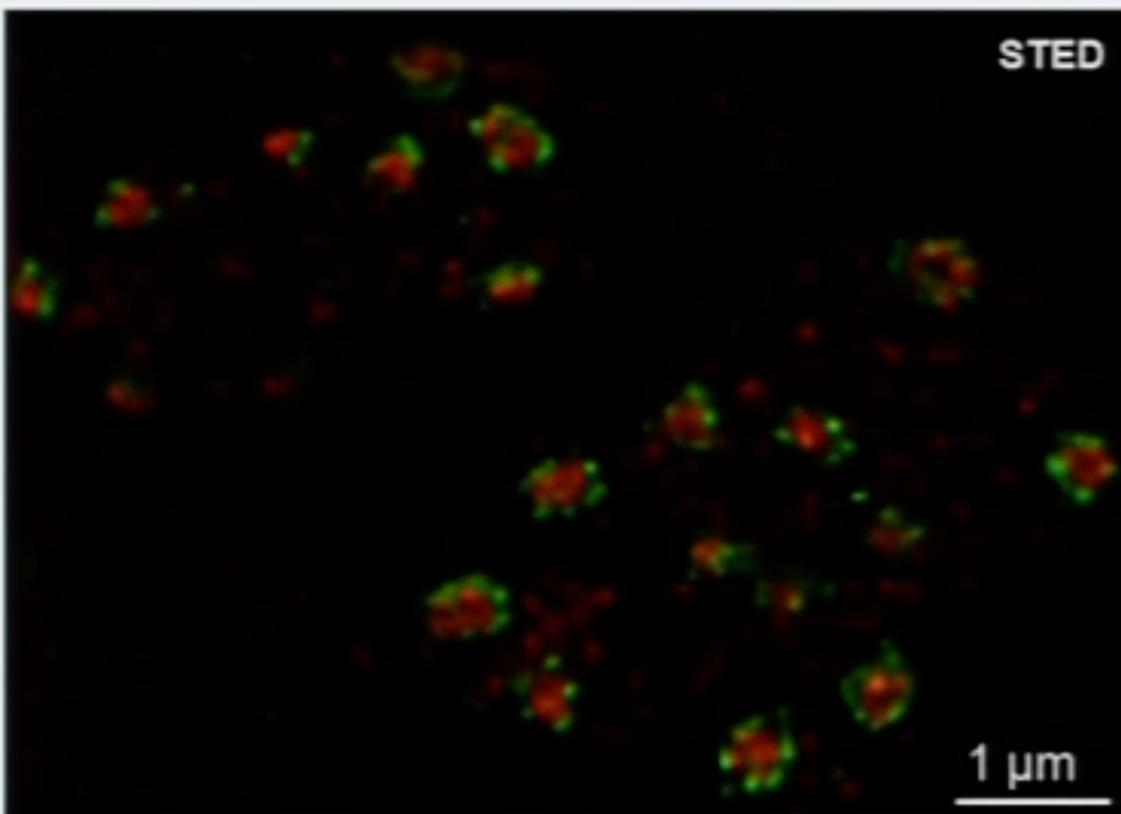
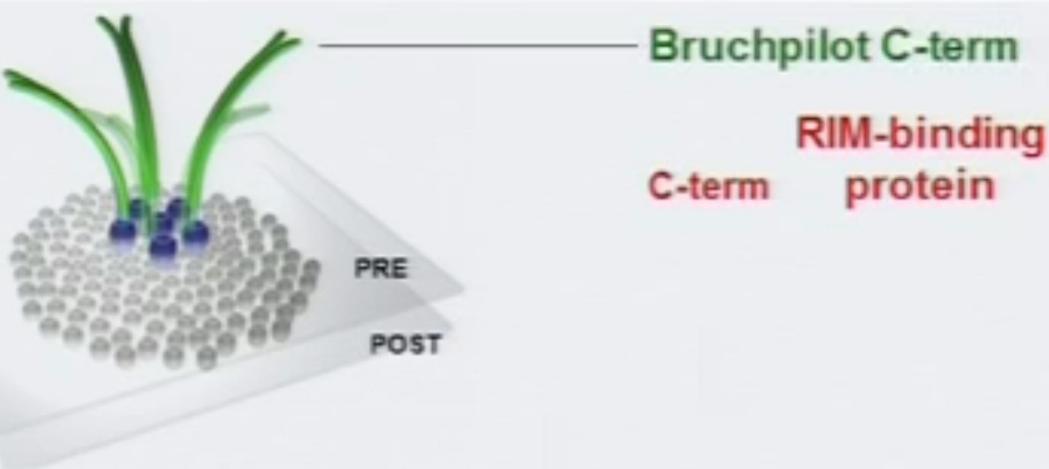
Garland, Science 2008

STED



Neuromuscular junction

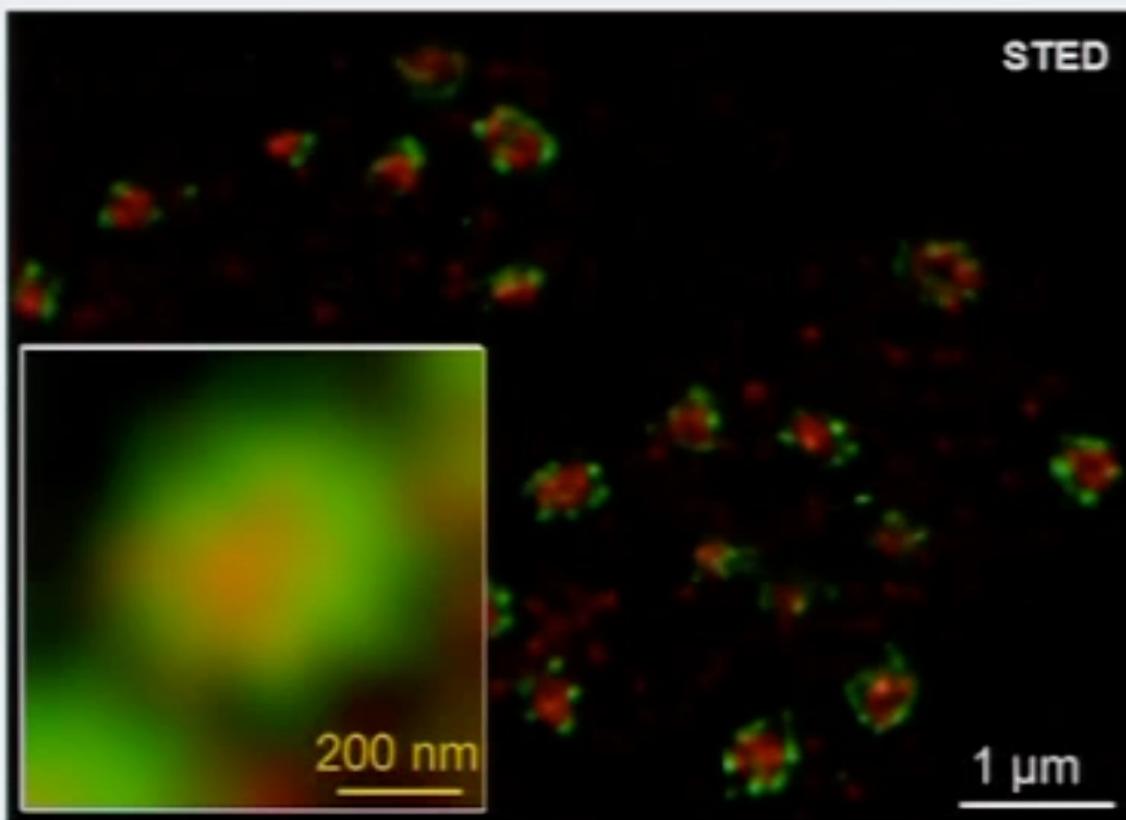
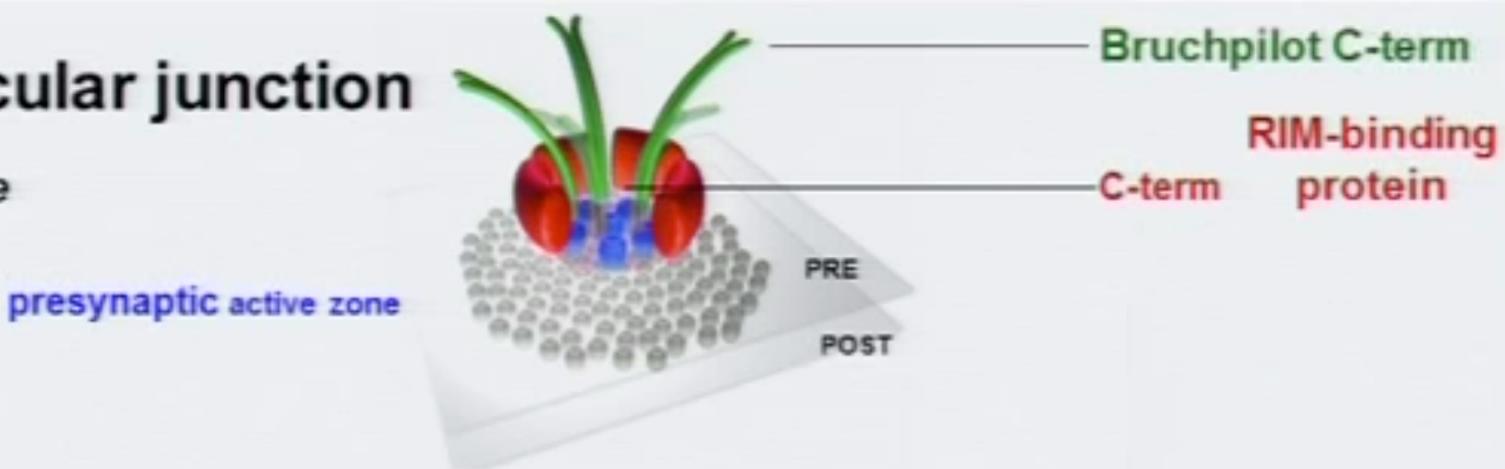
Drosophila larvae



Liu...Bückers, Hell...Sigrist, *Science* (2011)

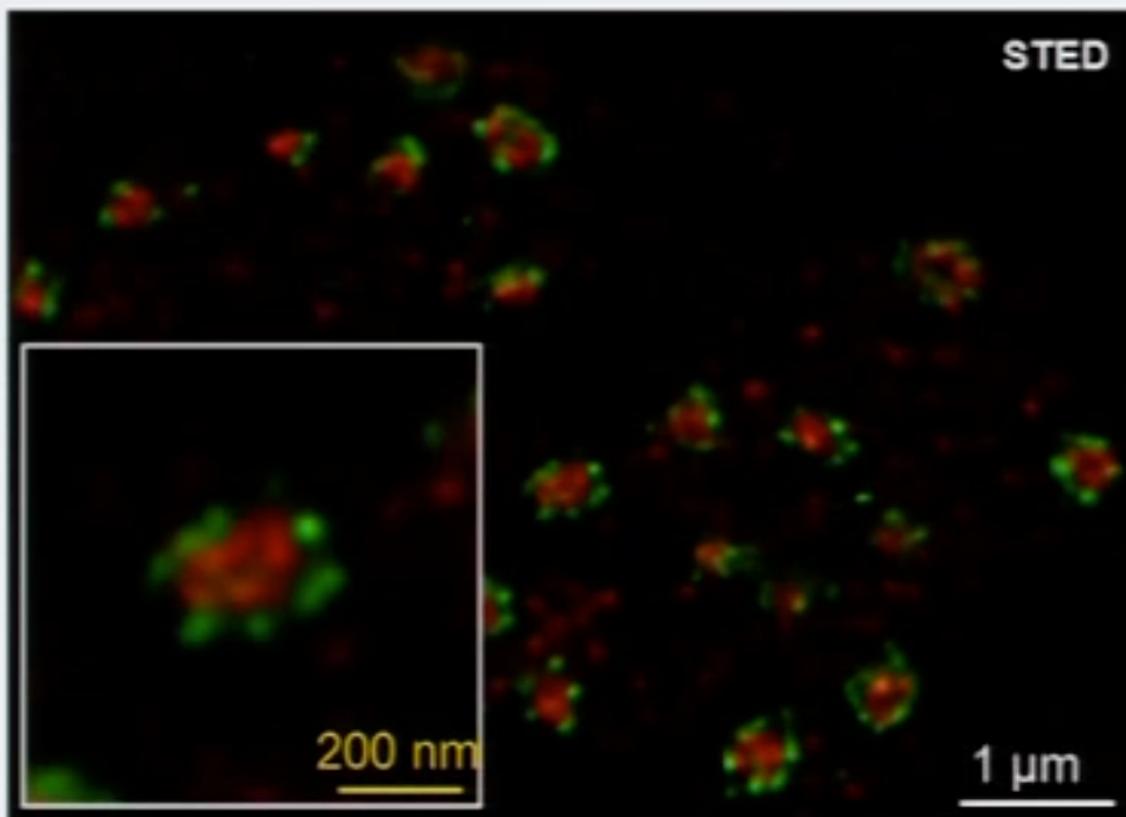
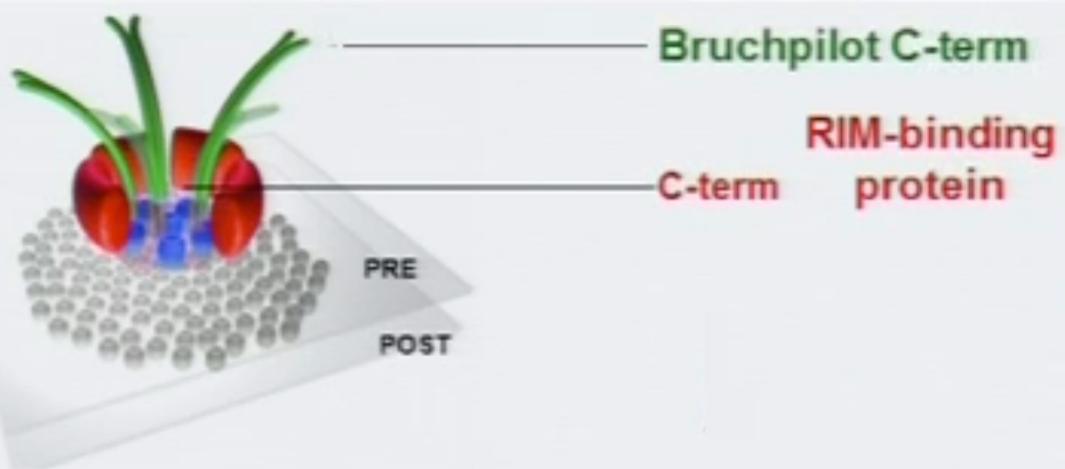
Neuromuscular junction

Drosophila larvae



Neuromuscular junction

Drosophila larvae



Neurophysiology

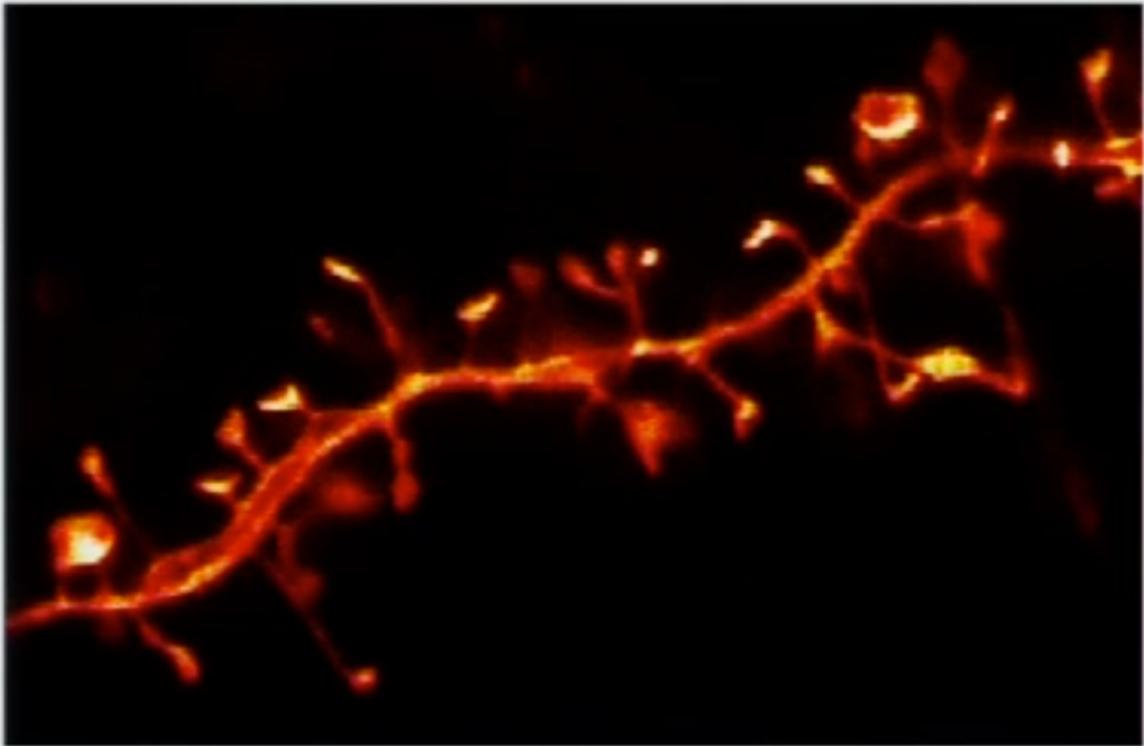
STED



Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



Neurophysiology

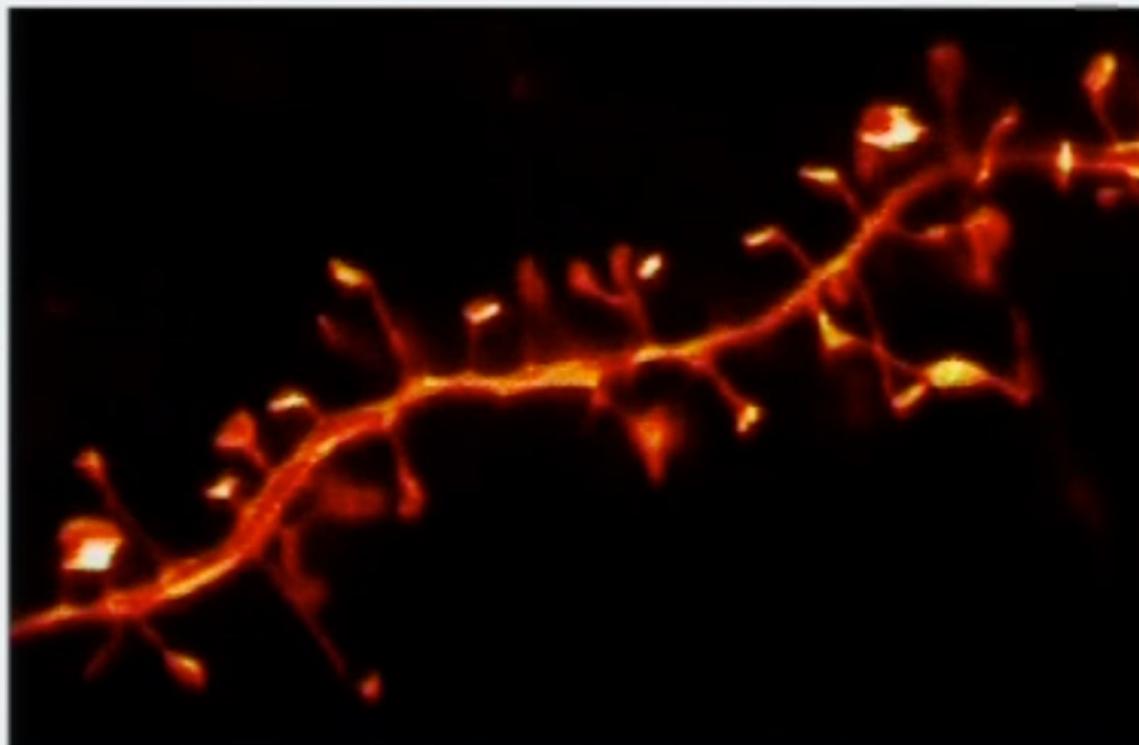
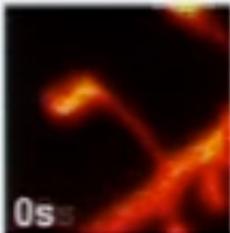
STED



Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



2μm

Neurophysiology

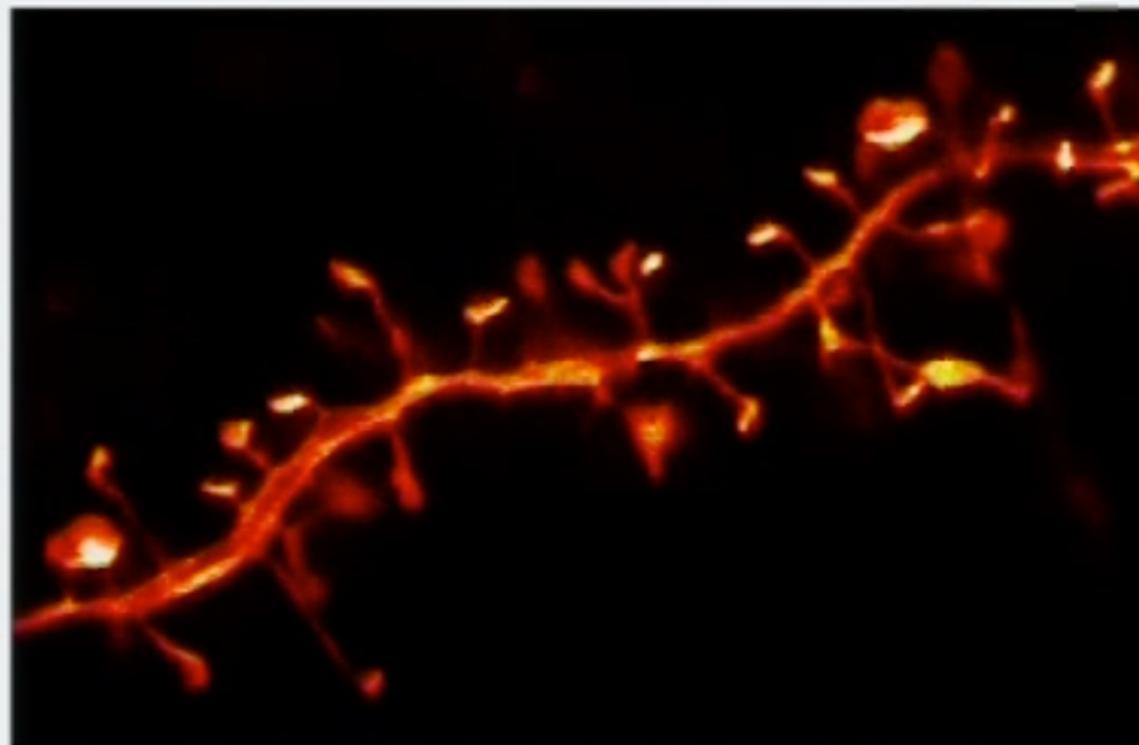
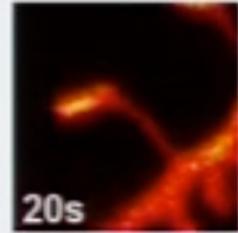
Dendritic Spines Living Neuron

STED



Hippocampal organotyp slices

CA1 pyramidal neurons



2μm

Neurophysiology

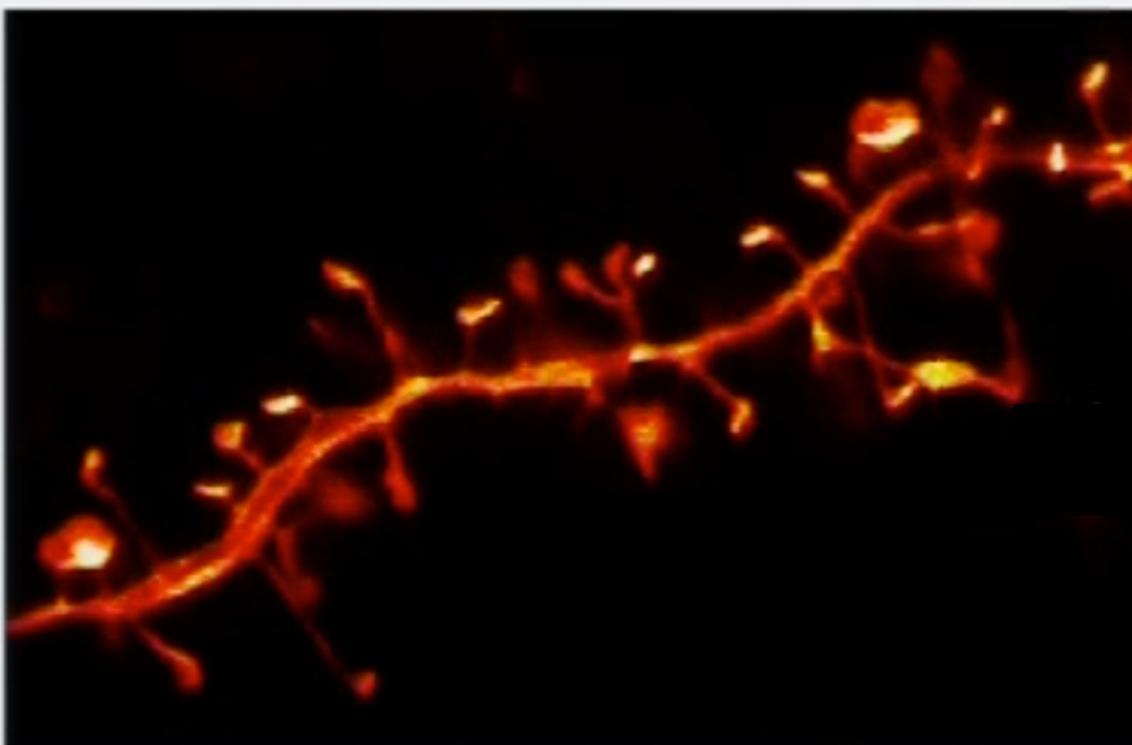
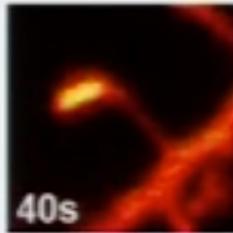
STED



Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



2μm

Neurophysiology

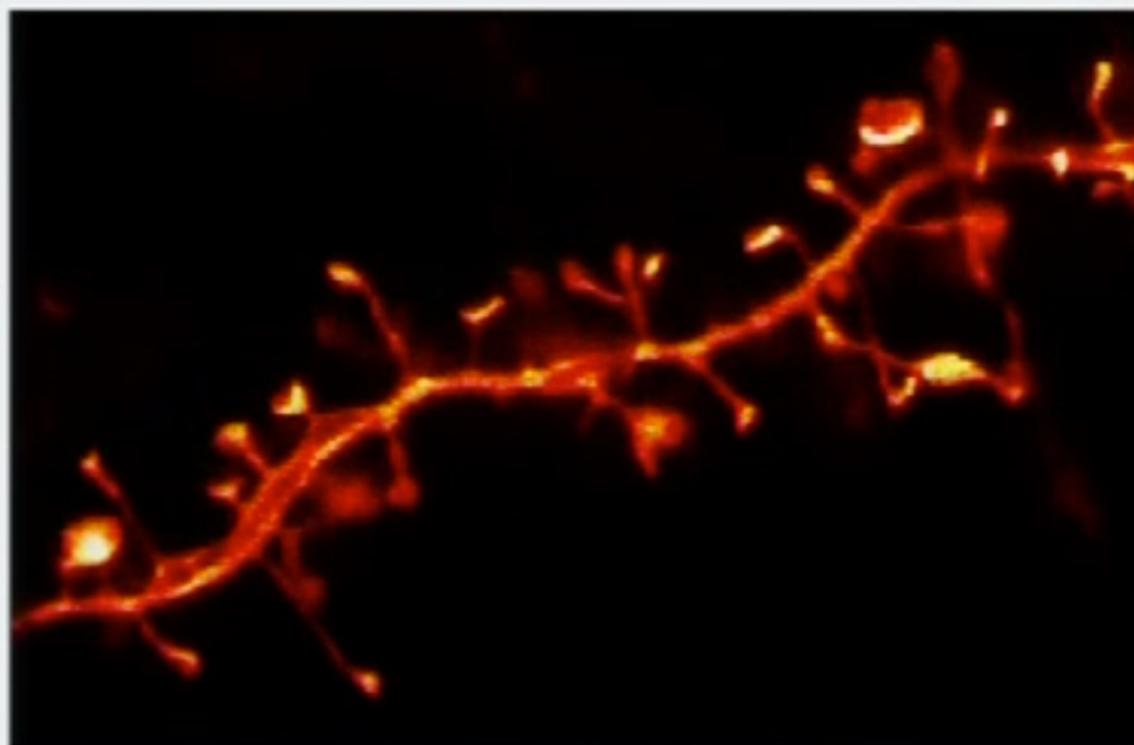
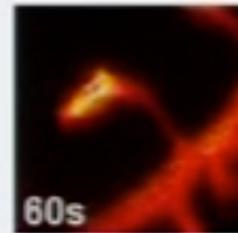
STED



Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



2μm

Neurophysiology

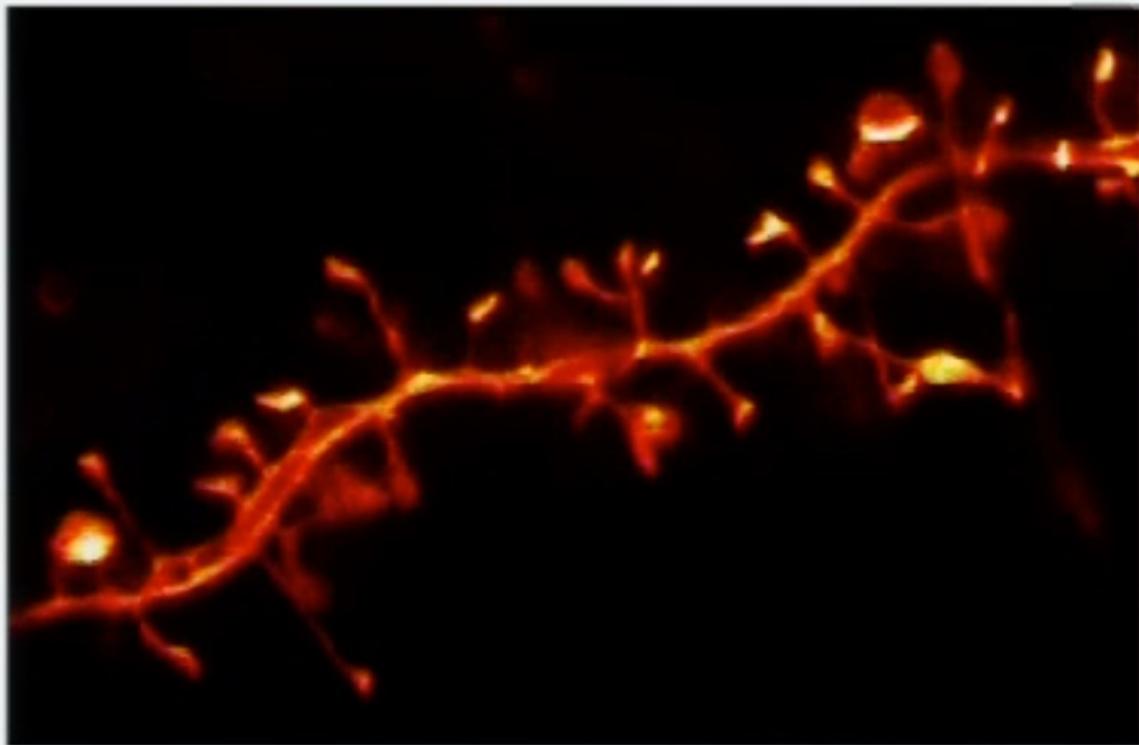
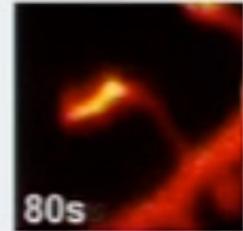
Dendritic Spines Living Neuron

STED



Hippocampal organotyp slices

CA1 pyramidal neurons



2 μ m

Neurophysiology

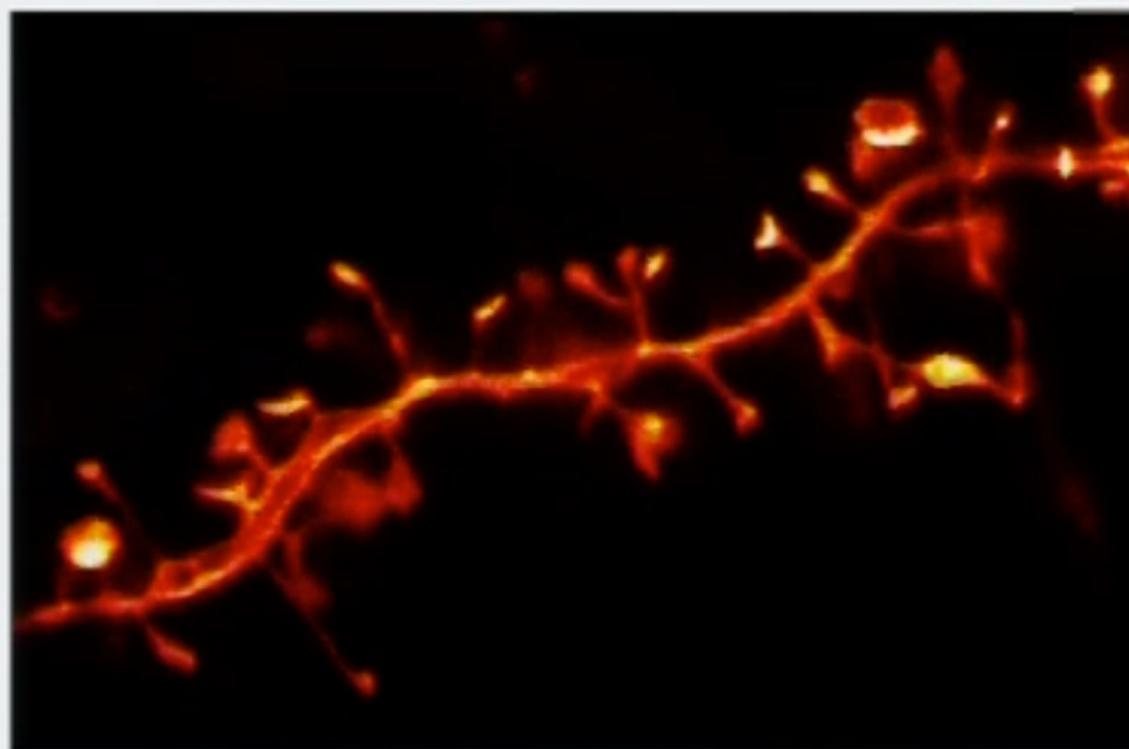
Dendritic Spines Living Neuron

STED



Hippocampal organotypic slices

CA1 pyramidal neurons



2μm

Neurophysiology

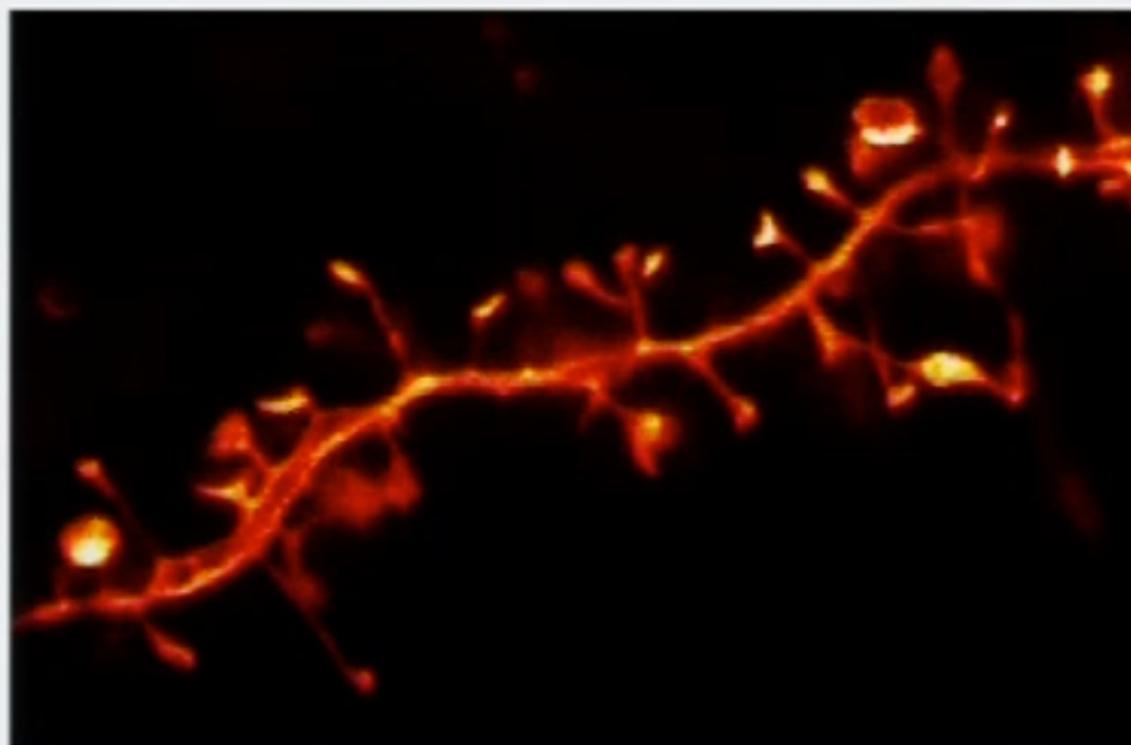
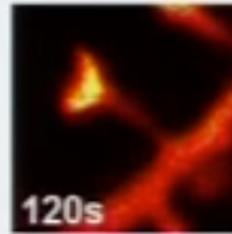
Dendritic Spines Living Neuron

STED



Hippocampal organotypic slices

CA1 pyramidal neurons



2μm

Neurophysiology

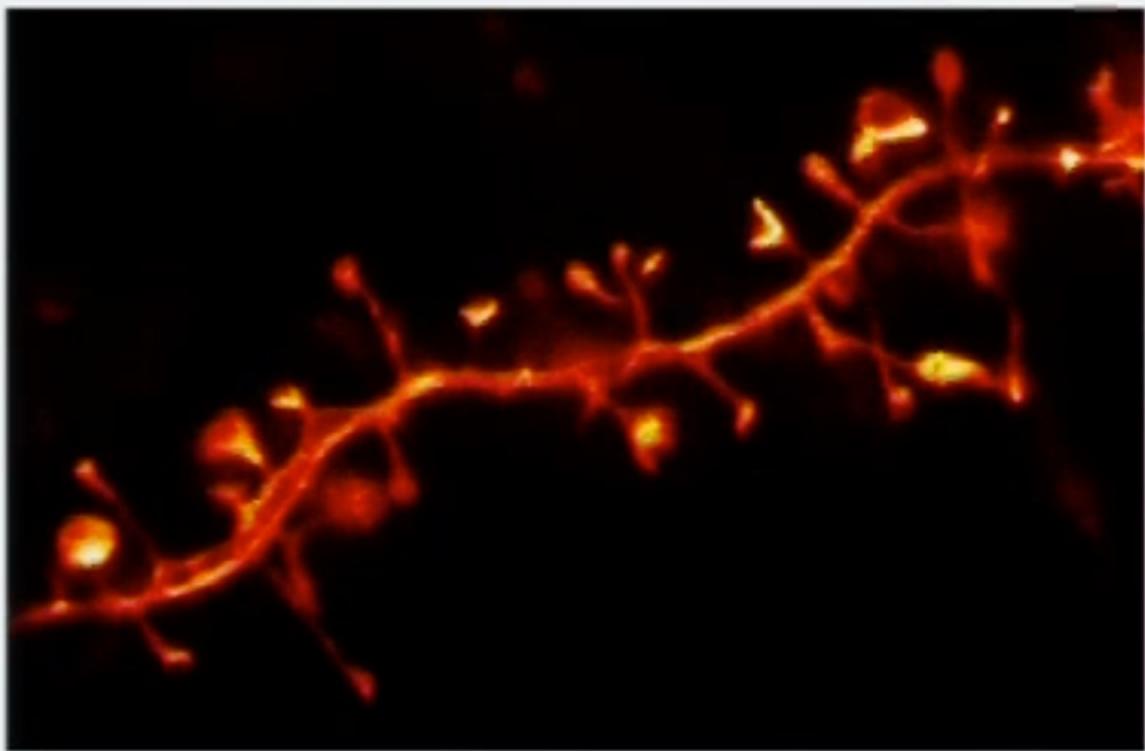
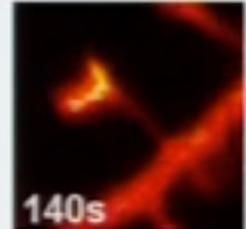
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Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



Neurophysiology

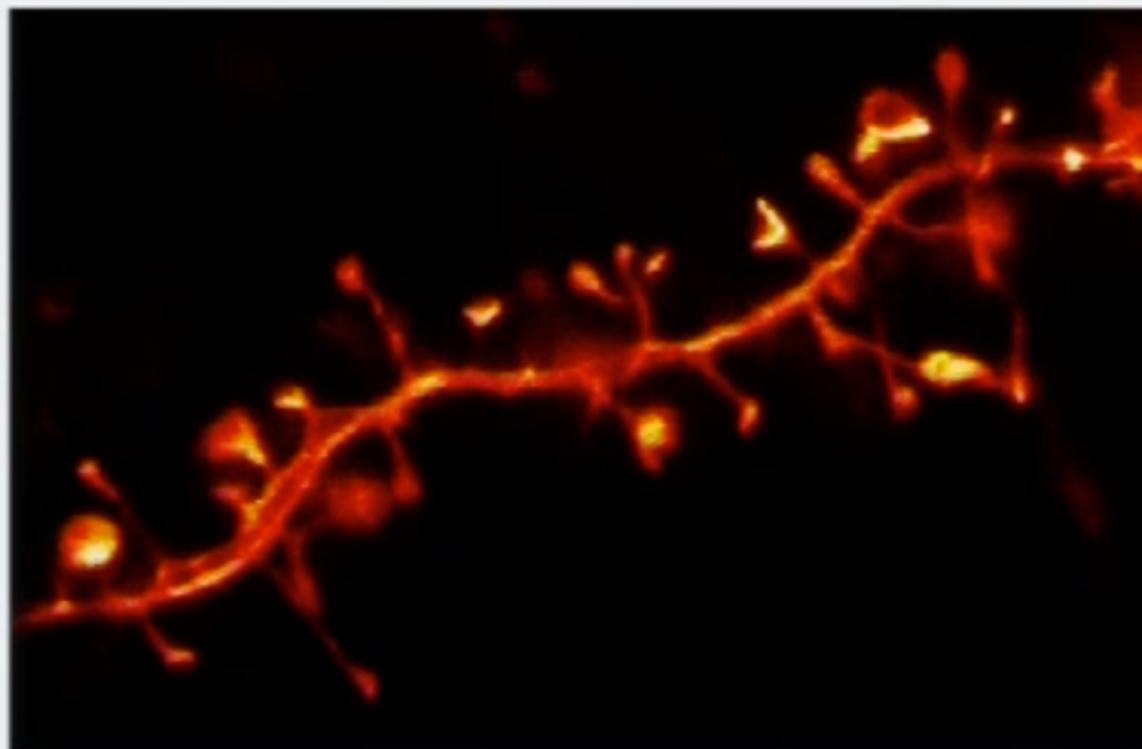
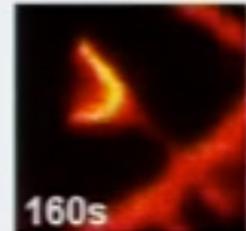
Dendritic Spines Living Neuron

STED



Hippocampal organotyp slices

CA1 pyramidal neurons



2μm

Neurophysiology

STED



Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



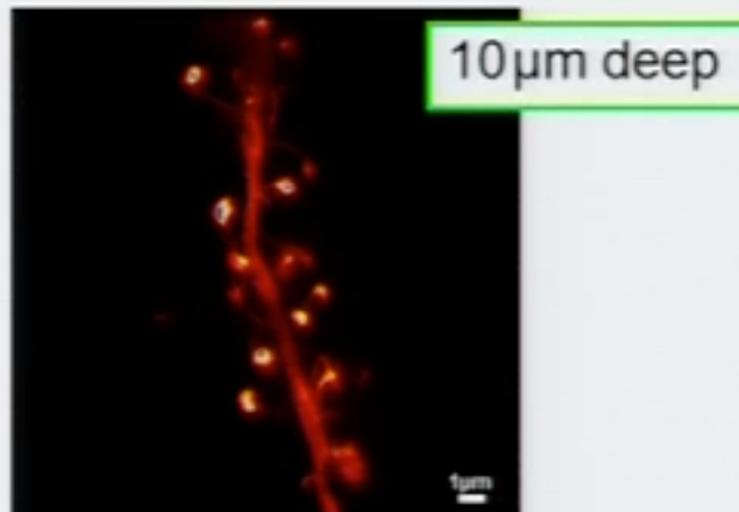
2μm

Neurophysiology

Dendritic Spines Living Neuron

STED

Lifeact-YFP

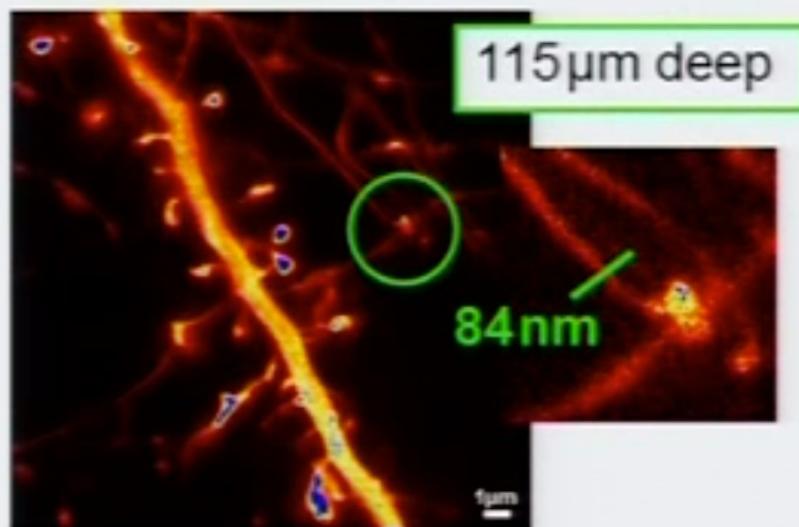
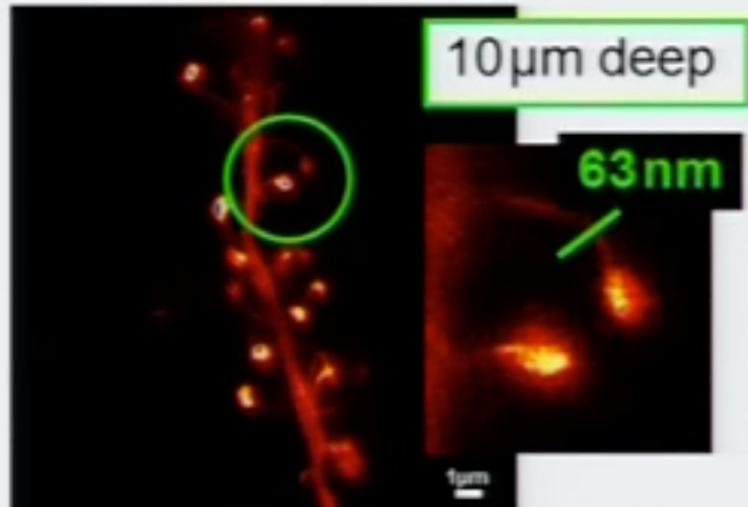


Neurophysiology

Dendritic Spines Living Neuron

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Lifeact-YFP



Neurophysiology *in living mouse brain*

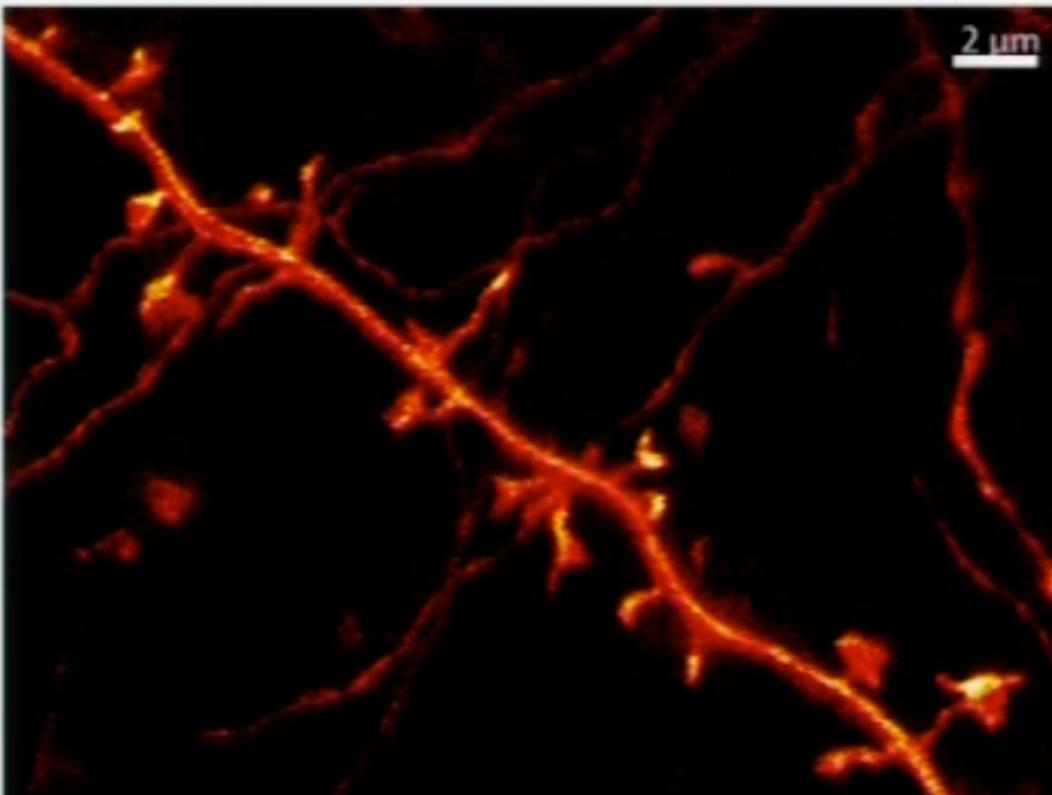


Cortical neurons expressing
cytoplasmic EYFP

STED



$\sim 20 \mu\text{m}$ deep

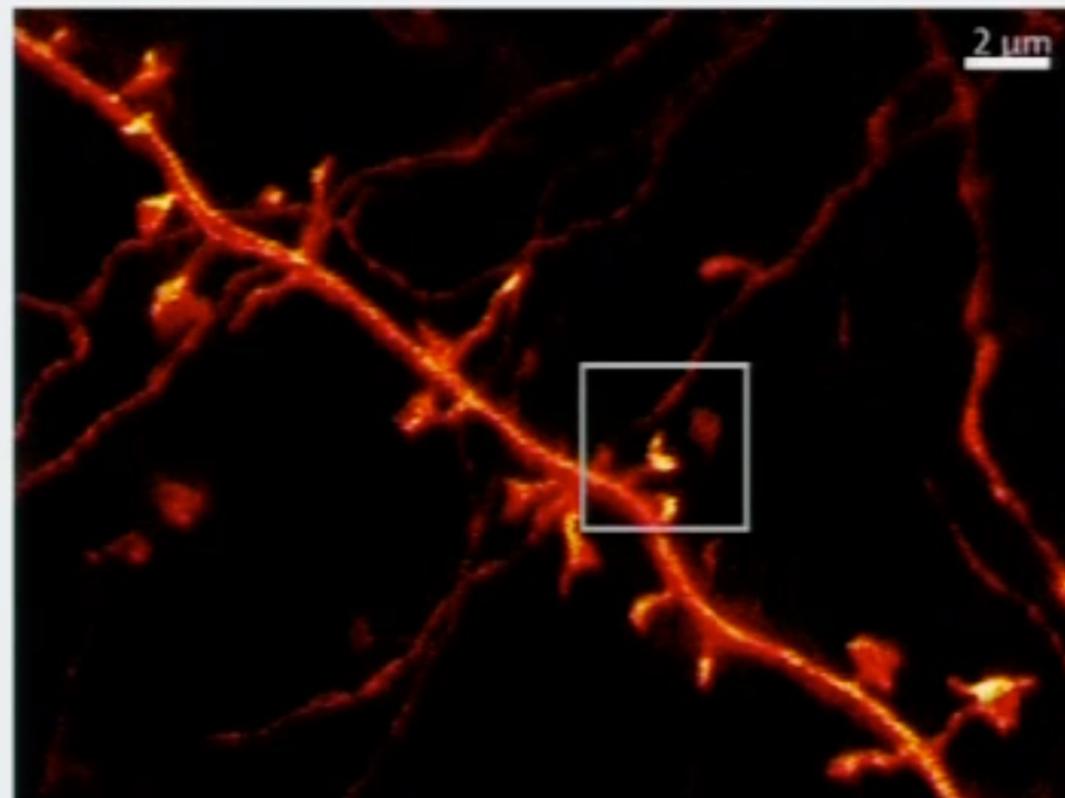


Neurophysiology *in living mouse brain*



Cortical neurons expressing
cytoplasmic EYFP

STED

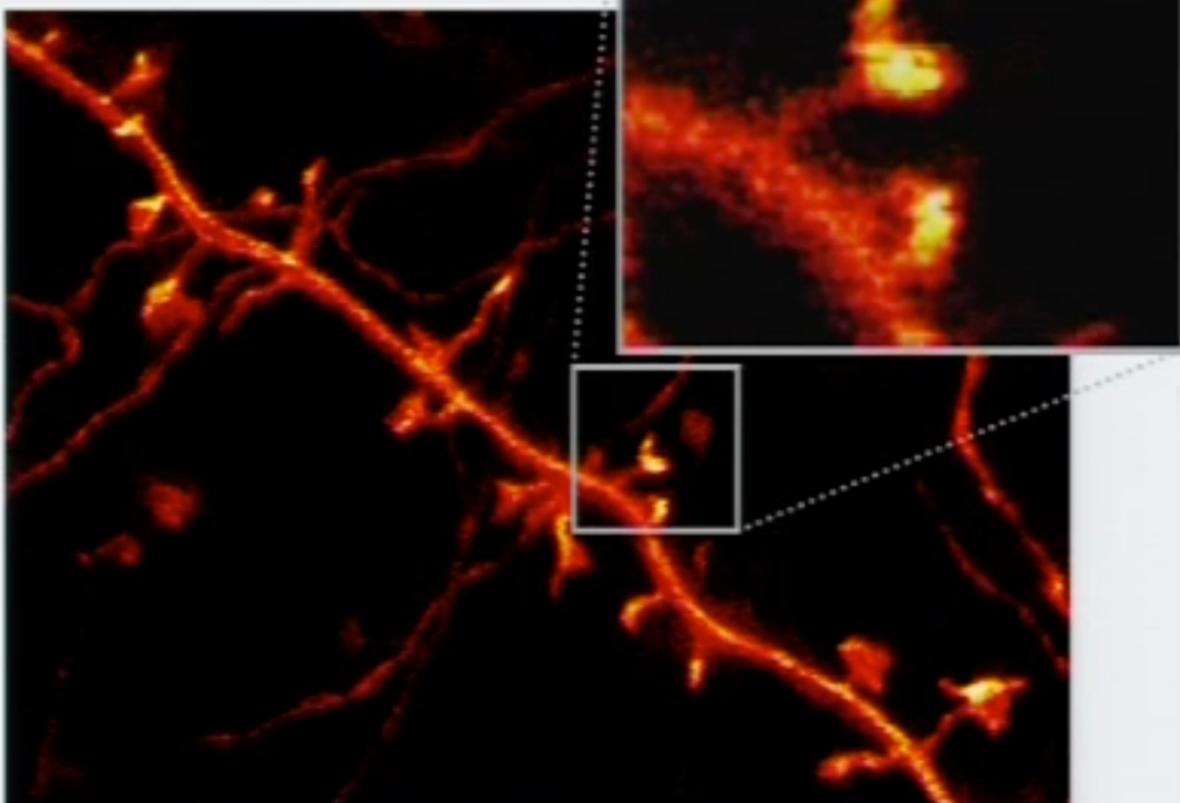


Neurophysiology *in living mouse brain*



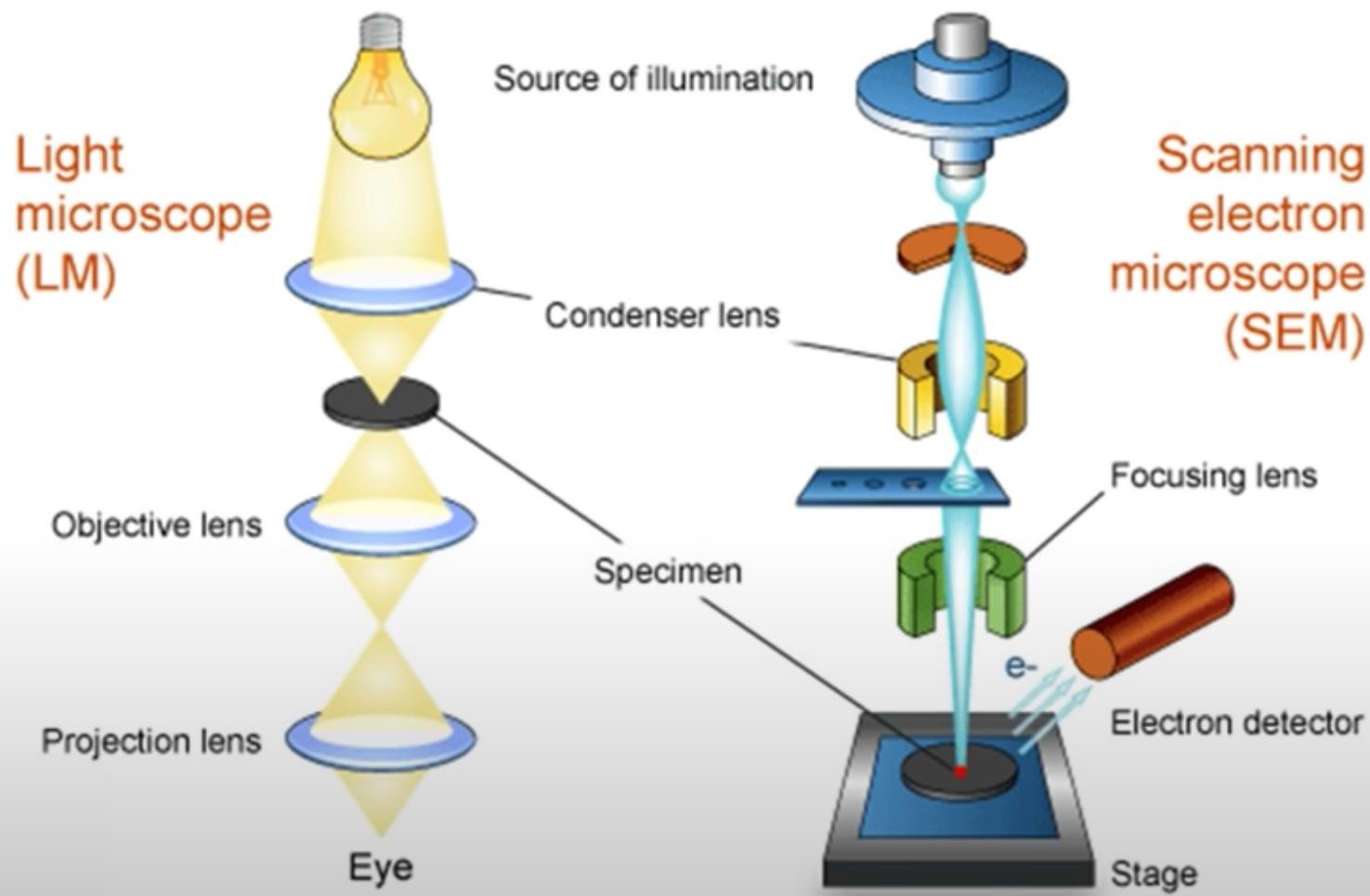
Cortical neurons expressing
cytoplasmic EYFP

STED

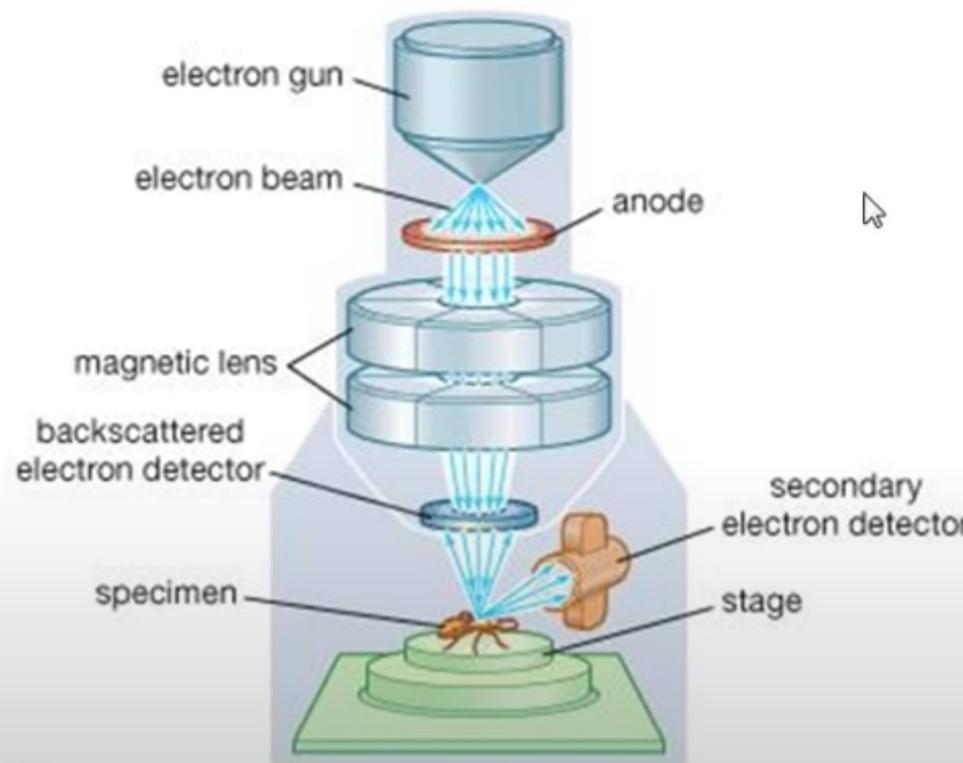


Introduction to Scanning and Transmission Electron Microscopes

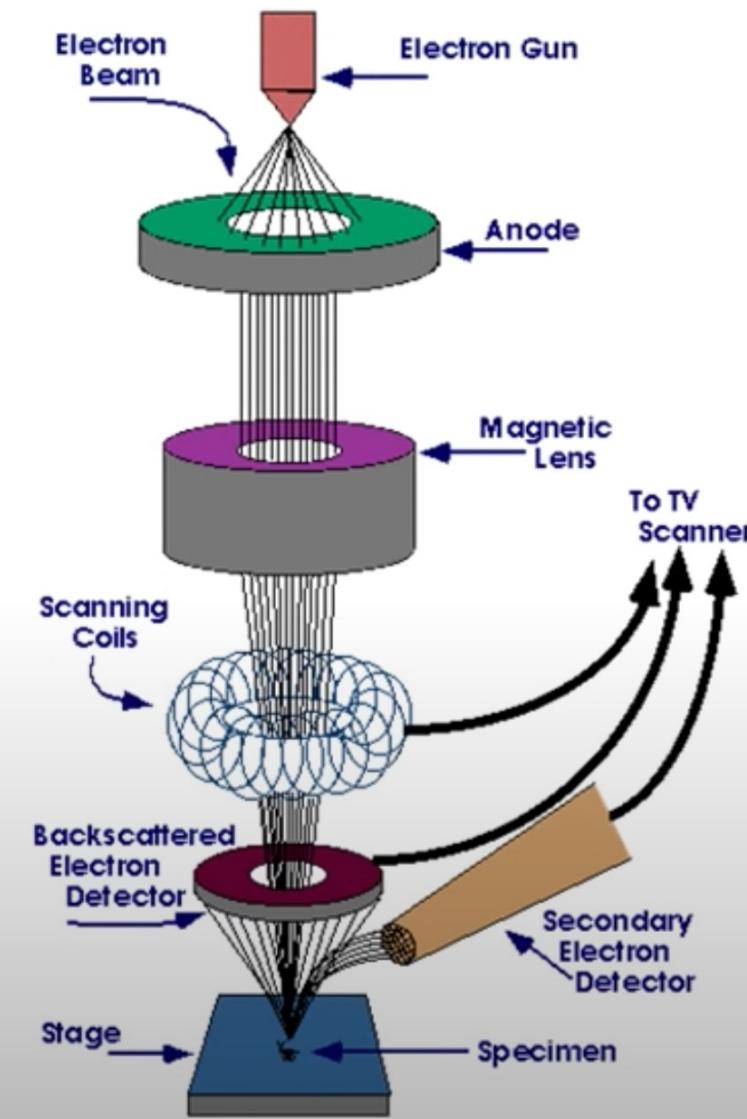
Light vs electron Microscopy

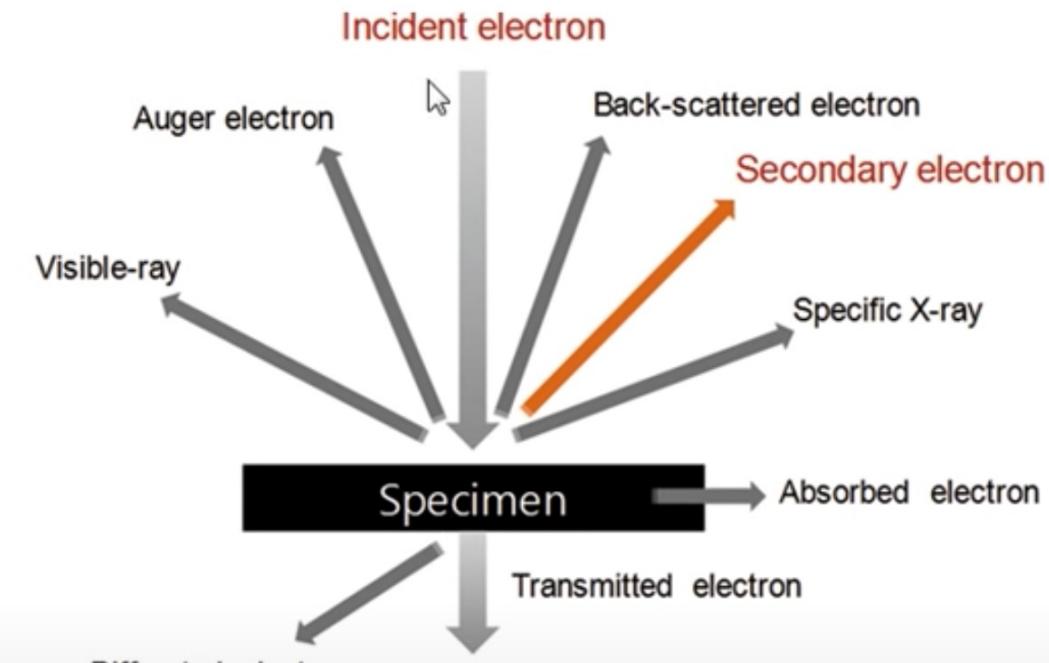
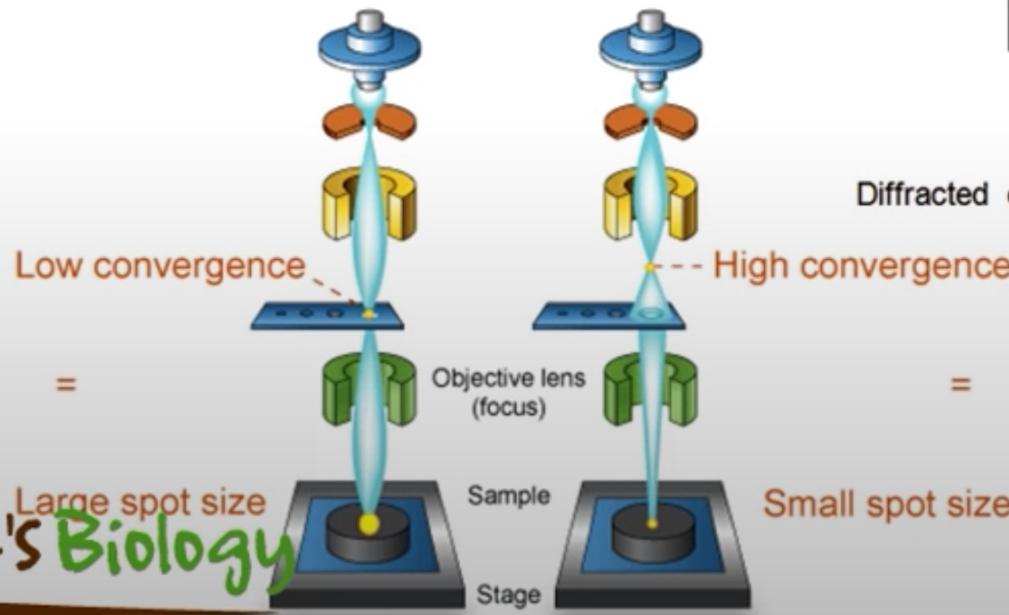
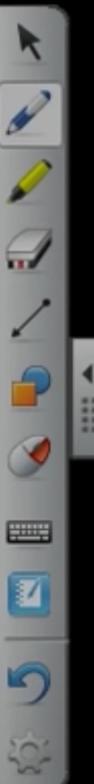


Principle

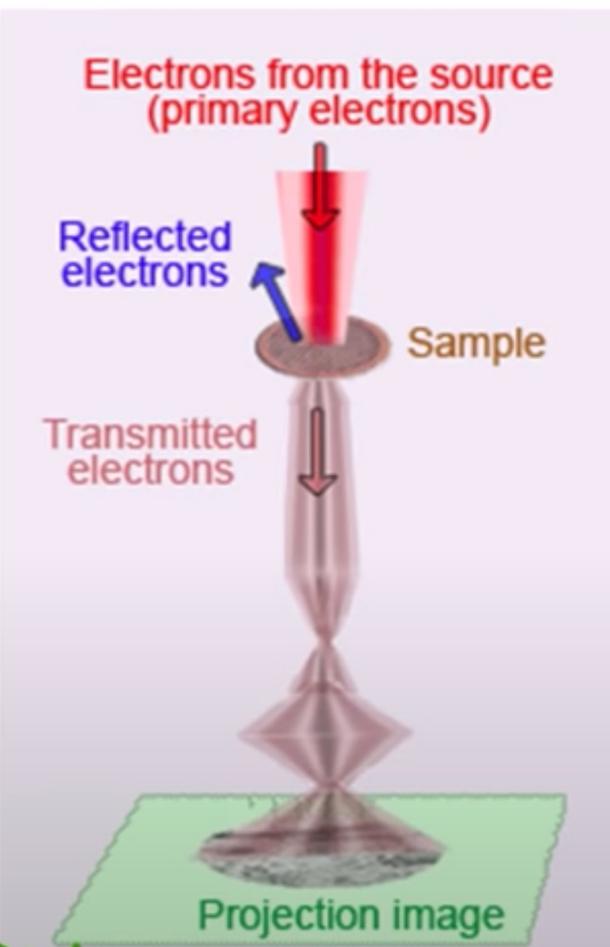


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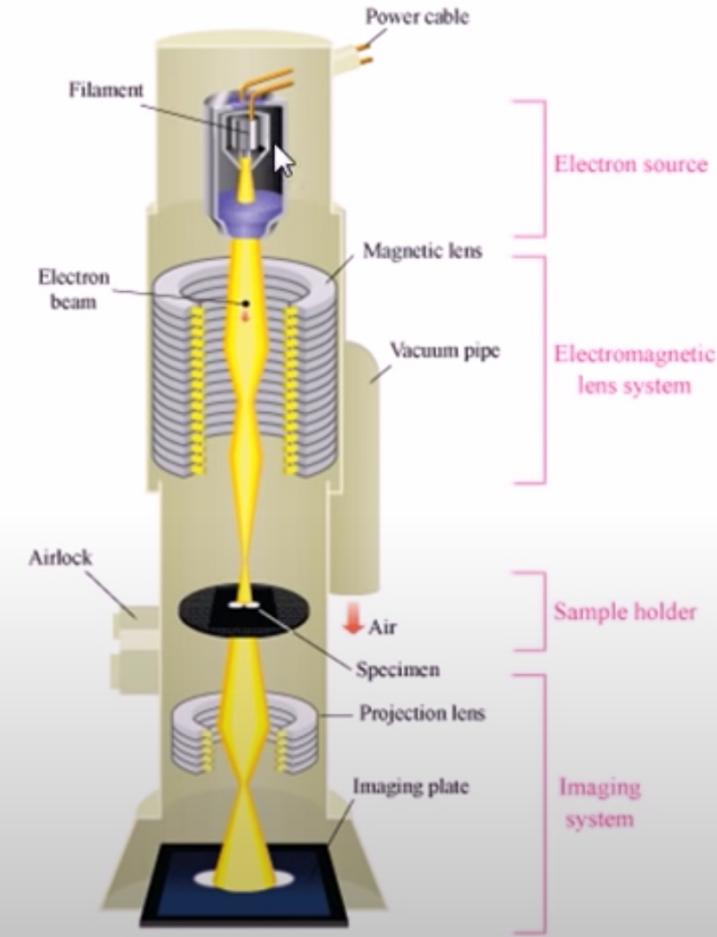
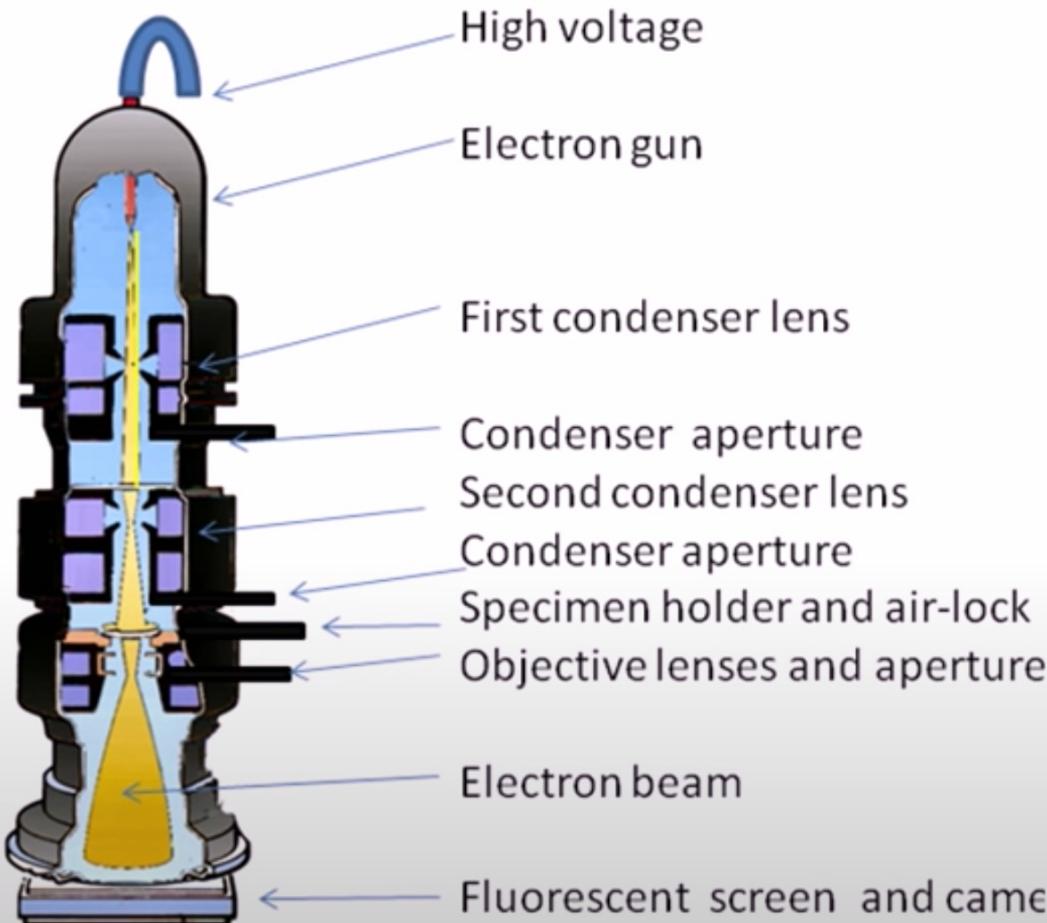




Principle



Working mechanism

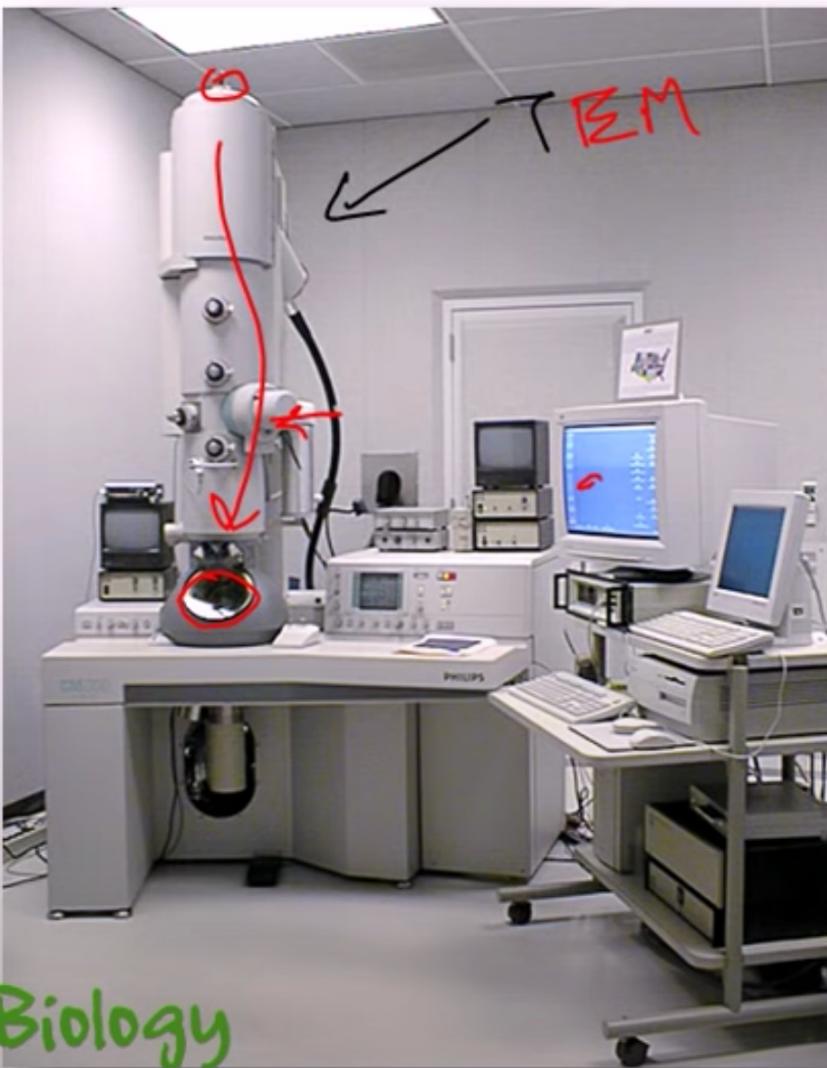


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In action



In action



OPEN

AxonDeepSeg: automatic axon and myelin segmentation from microscopy data using convolutional neural networks

Received: 7 November 2017

Accepted: 6 February 2018

Published online: 28 February 2018

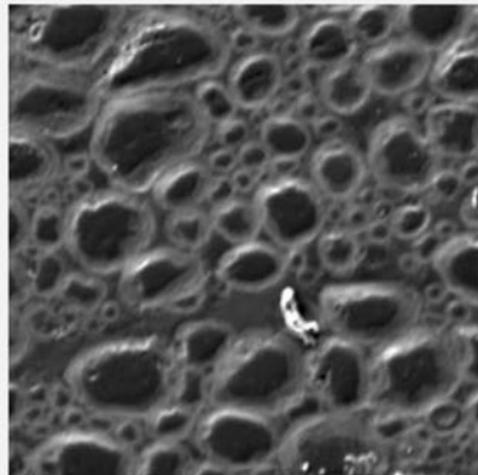
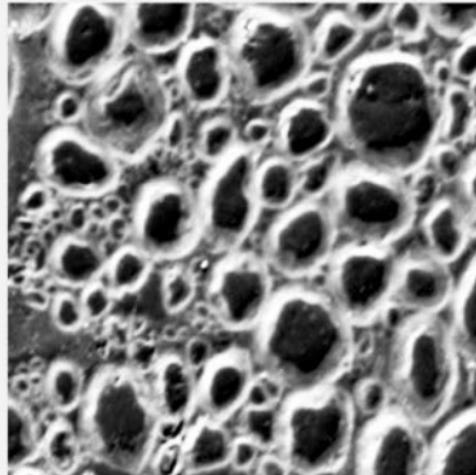
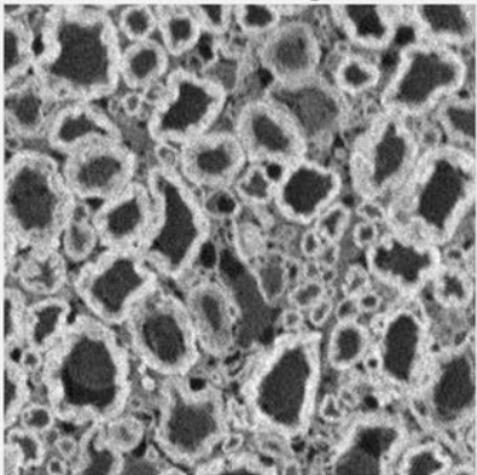
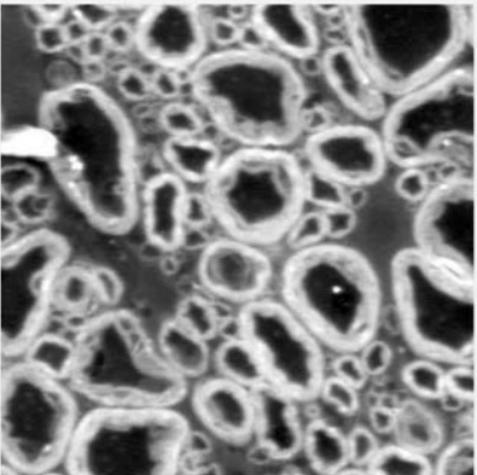
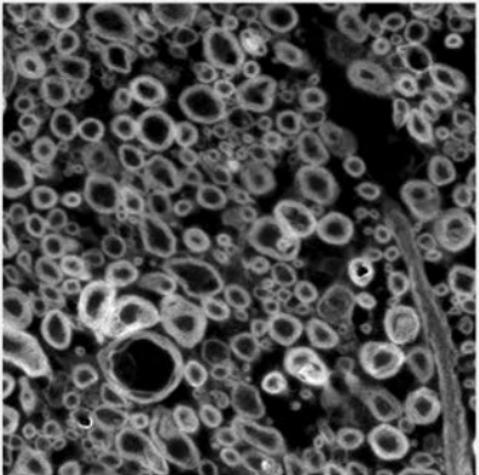
Aldo Zaimi¹, Maxime Wabbartha^{1,2}, Victor Herman^{1,2}, Pierre-Louis Antonsanti^{1,3}, Christian S. Perone¹ & Julien Cohen-Adad^{1,4}

increase the transmission speed^{1,2}. Pathologies such as neurodegenerative diseases (e.g., multiple sclerosis) or trauma are associated with myelin degeneration, which can ultimately lead to sensory and motor deficits (e.g., paraplegia)^{3,4}. Being able to image axons and myelin sheaths at high resolution would help researchers understand the origins of demyelination and test therapeutic drugs^{5,6} and could also be used to validate novel magnetic

resonance imaging biomarkers of myelin⁷. High resolution histology is typically done using electron microscopy following osmium staining to obtain myelin contrast. Then, axons and myelin can be analysed on the images to derive metrics such as axon density or myelin thickness. However, given that 1 mm² of white matter can contain

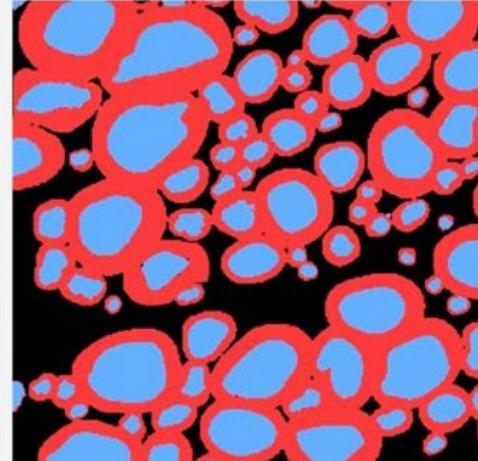
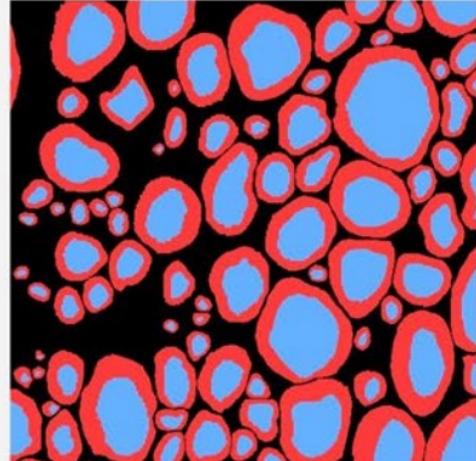
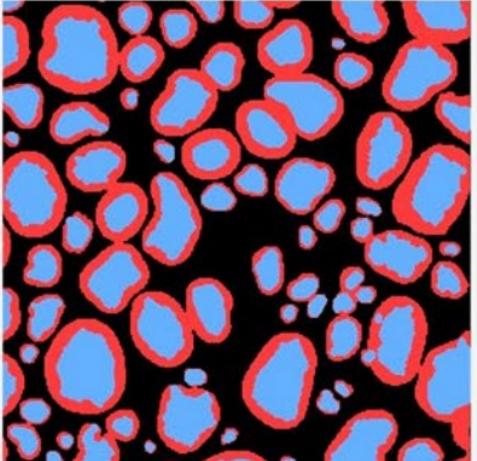
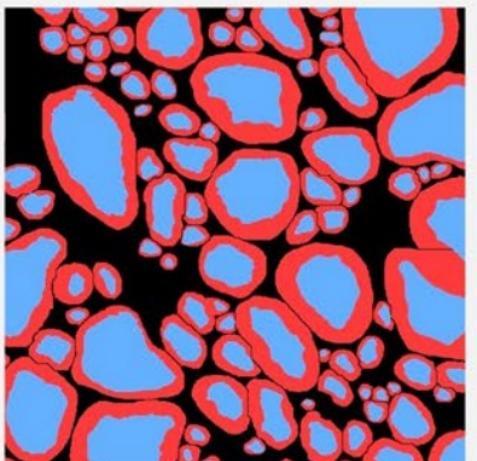
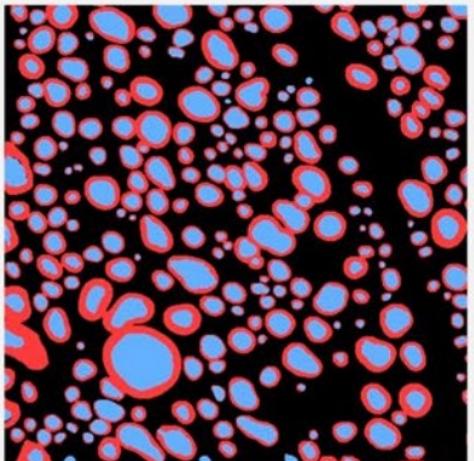
a) SEM dataset

Example image patches:



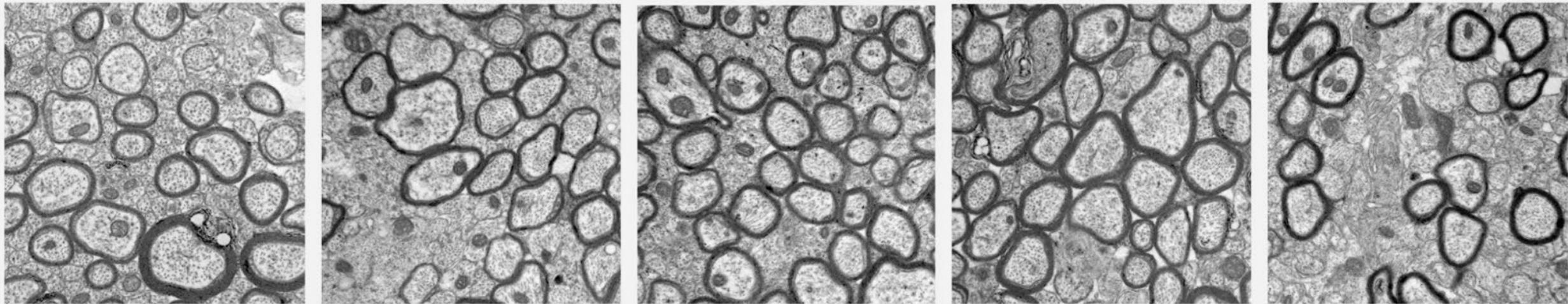
10 μm

Corresponding labels:



b) TEM dataset

Example image patches:



1 μm

Corresponding labels:

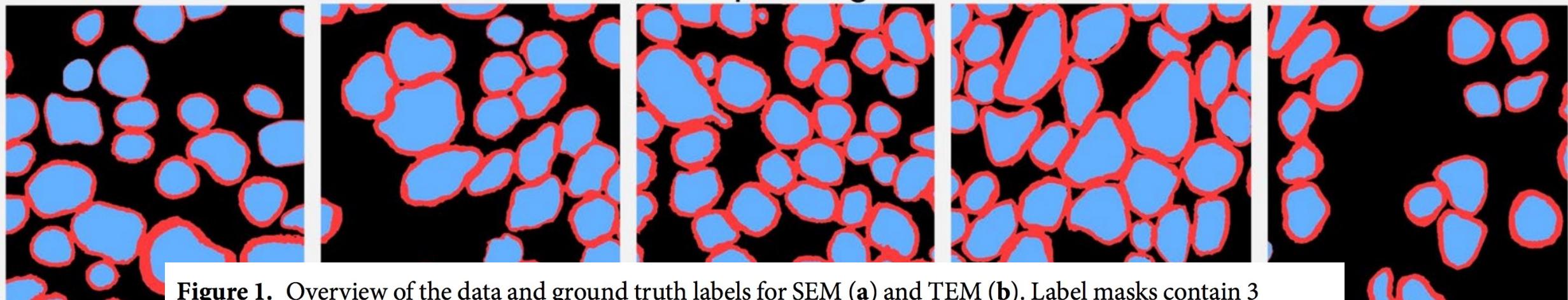


Figure 1. Overview of the data and ground truth labels for SEM (a) and TEM (b). Label masks contain 3 classes: axon (in blue in the figure), myelin (red) and background (black). All SEM and TEM samples shown here are cropped to 512×512 pixels. SEM patches have a pixel size of $0.1 \mu\text{m}$, while TEM patches have a pixel size of $0.01 \mu\text{m}$ (see section “Pipeline overview”).

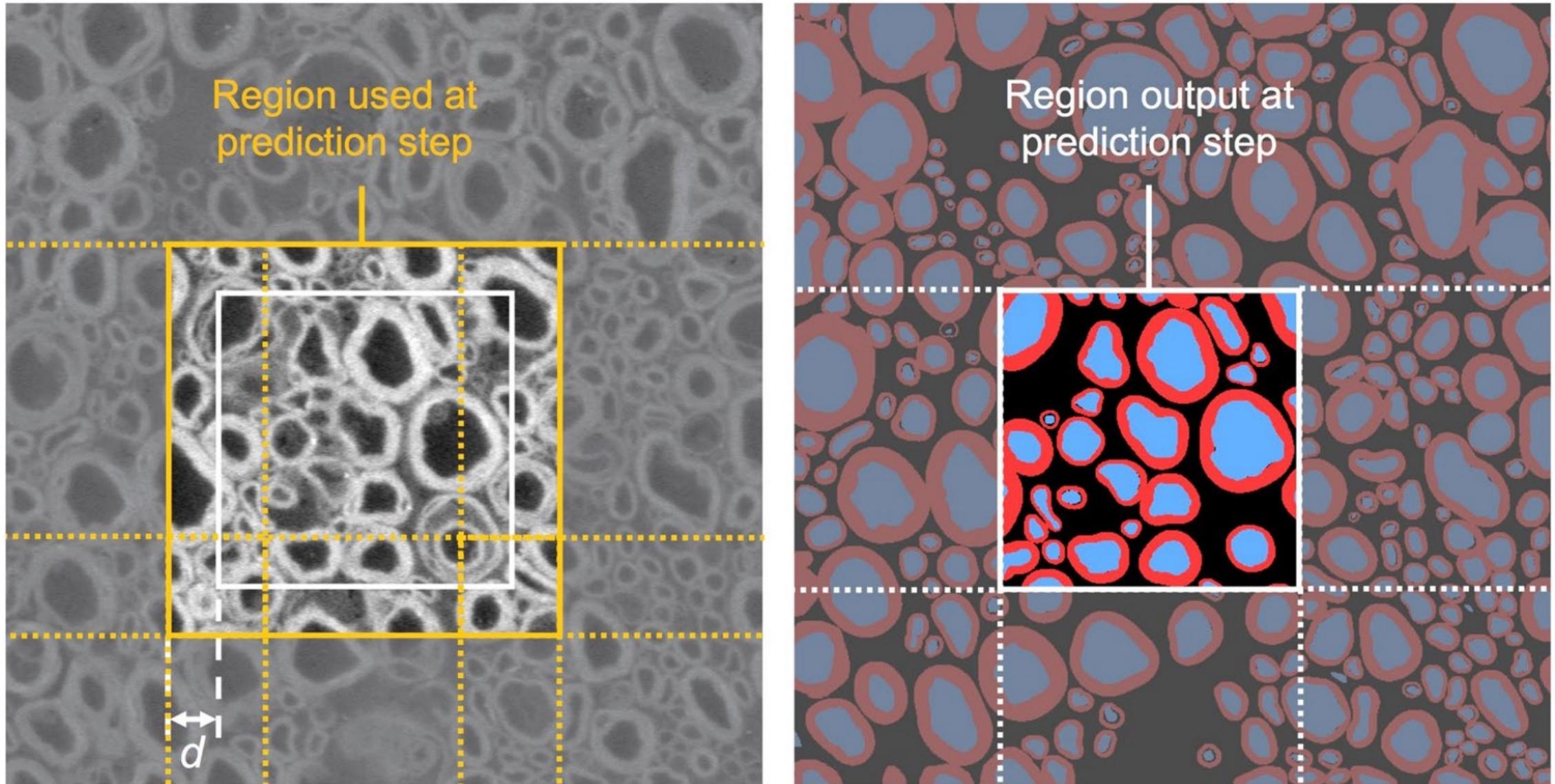


Figure 4. Overlapping procedure during inference. To avoid border effects during prediction, inference is run on the orange square, but only the white square is output. The algorithm iterates by shifting the inference window by the size of the white square. The overlap default value d was set to 25.

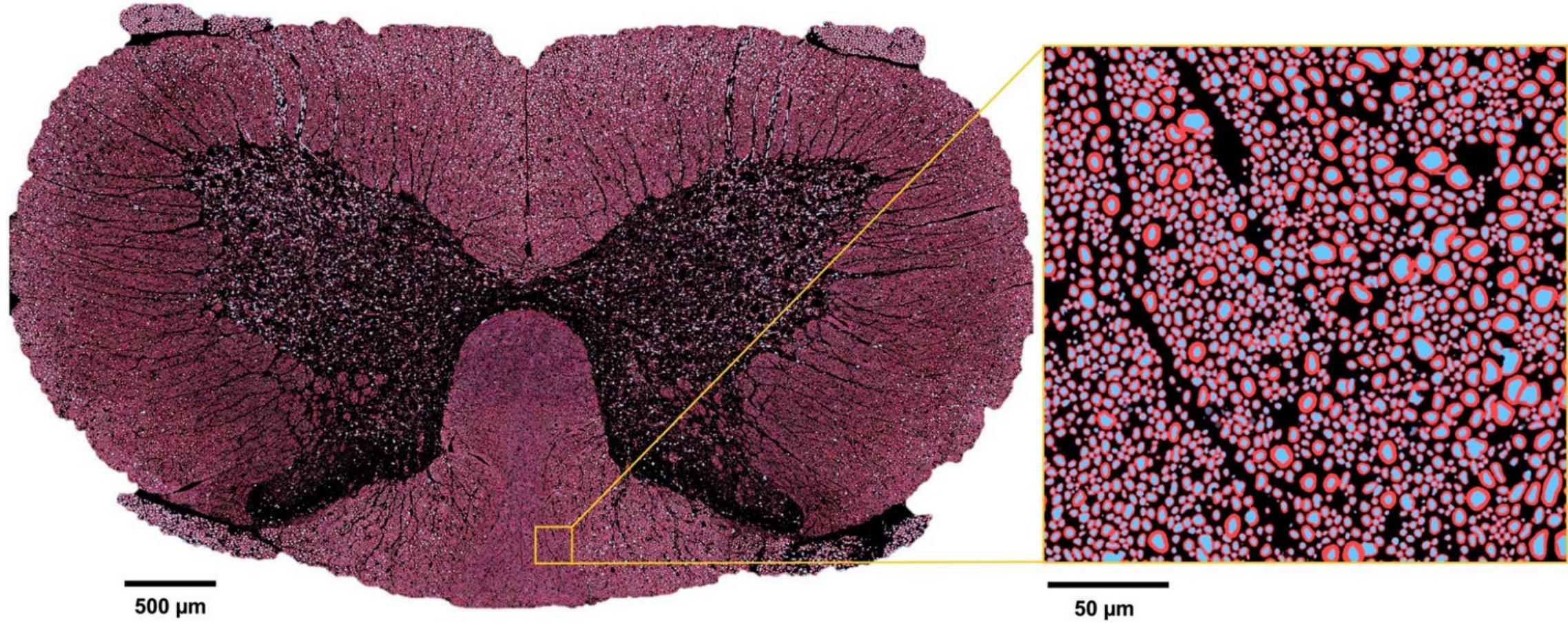


Figure 6. Full slice of rat spinal cord showing segmented axons (blue) and myelin sheaths (red). The zoomed panel illustrates the segmentation performance and sensitivity to fiber size: the left half of the panel contains smaller axons (mean diameter around $1.75 \mu\text{m}$) while the right half contains larger axons (mean diameter around $2.5 \mu\text{m}$).

- Axon diameter mean and standard deviation: arithmetic mean and standard deviation of the distribution of equivalent axon diameters (computed for each axon object as $\sqrt{(4 * \text{Area}/\pi)}$);
- Axon density: number of axons per mm²;
- Axon volume fraction (AVF): ratio between area of axons and total area of the region;
- Myelin volume fraction (MVF): ratio between area of myelin and total area of the region;
- G-ratio: ratio between axon diameter and myelinated fiber (axon + myelin) diameter, which can be estimated with the following formula⁷: $\sqrt{(1/(1 + \text{MVF}/\text{AVF}))}$.

