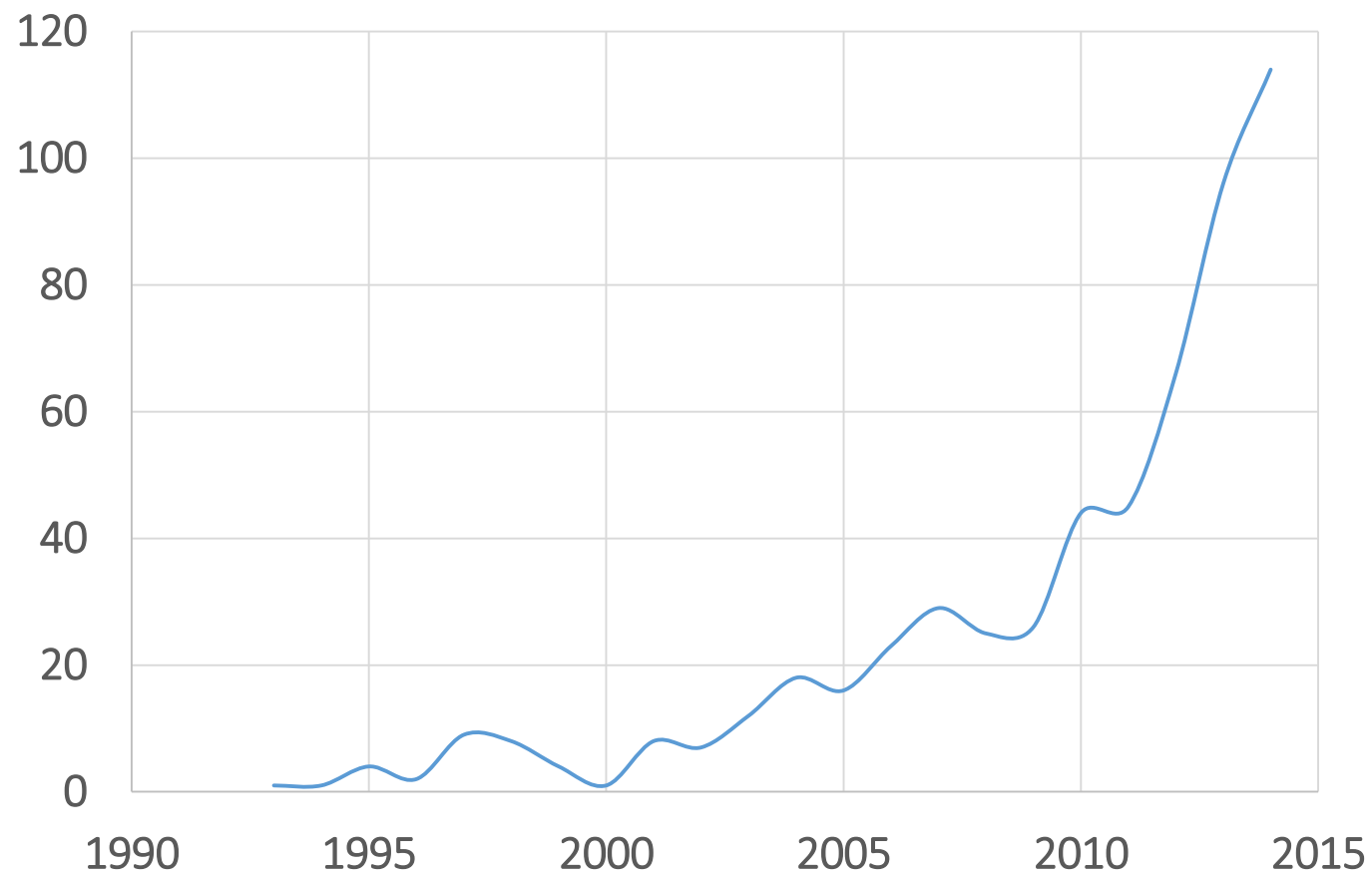


Superrezoluční mikroskopie (nanoskopie)

Jaroslav Zelenka

jaroslav.zelenka@vscht.cz

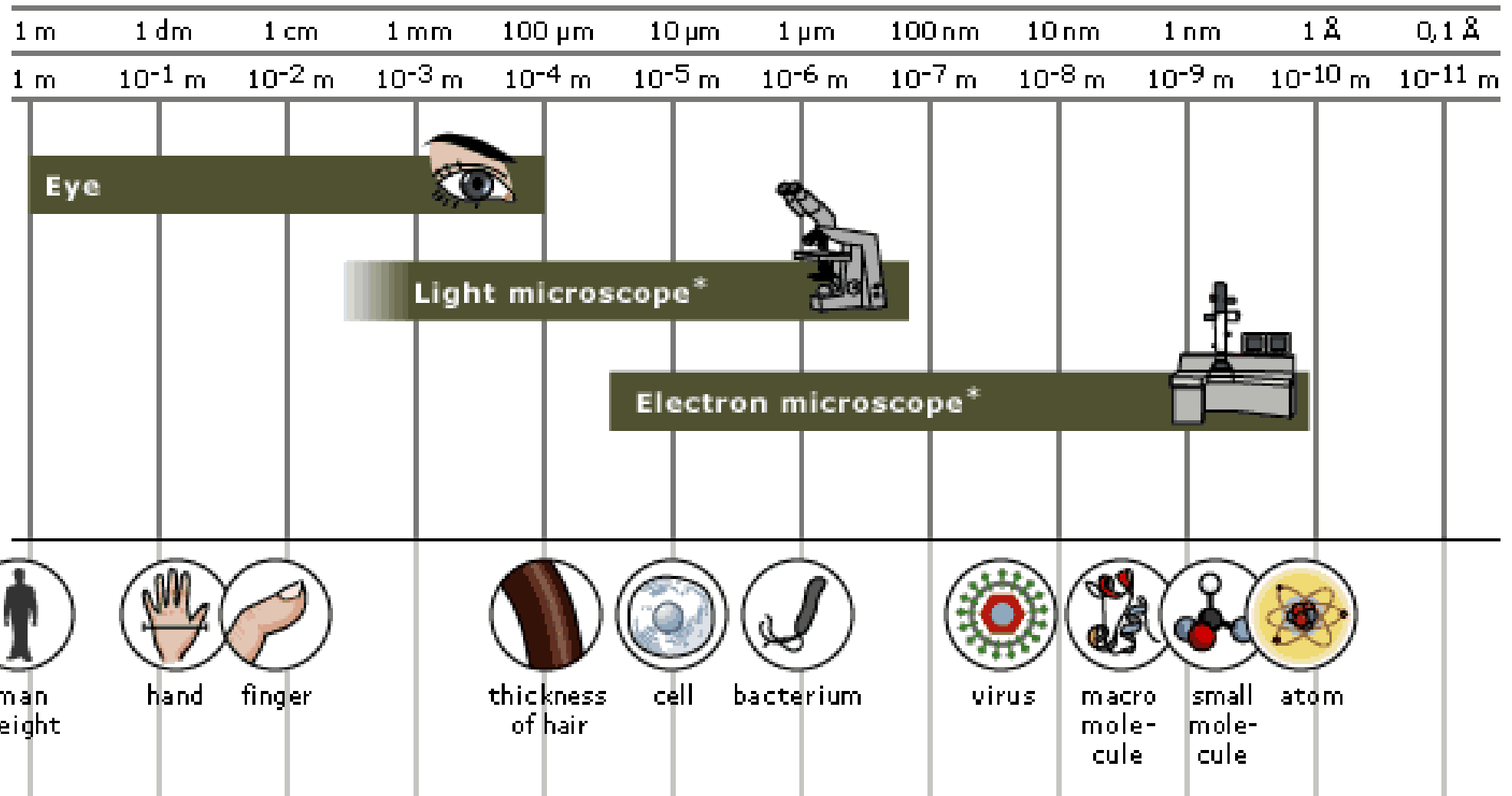
Rychle se rozvíjející novinka



Nobelova cena za chemii 2014



The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.



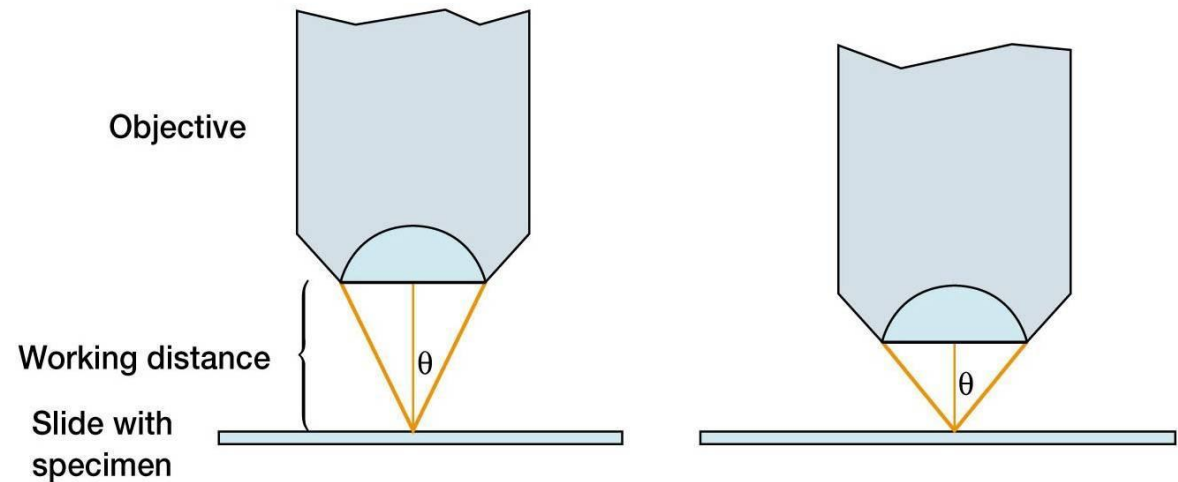
Abbého zákon

Numerická apertura: $NA = n \times \sin\theta$

Rozlišení soustavy:

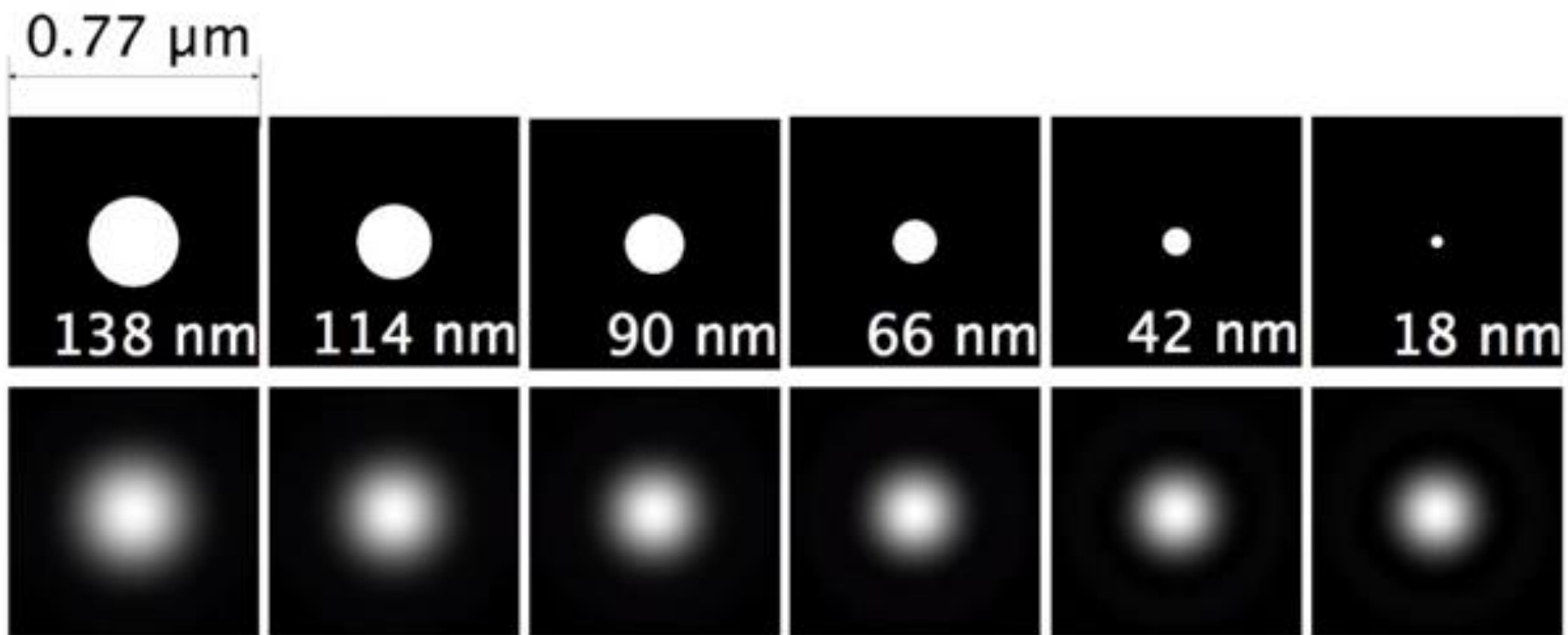
$$d_{x,y} = \frac{\lambda}{2 \times NA} = \frac{500}{2 \times 1.40} = 178 \text{ nm}$$

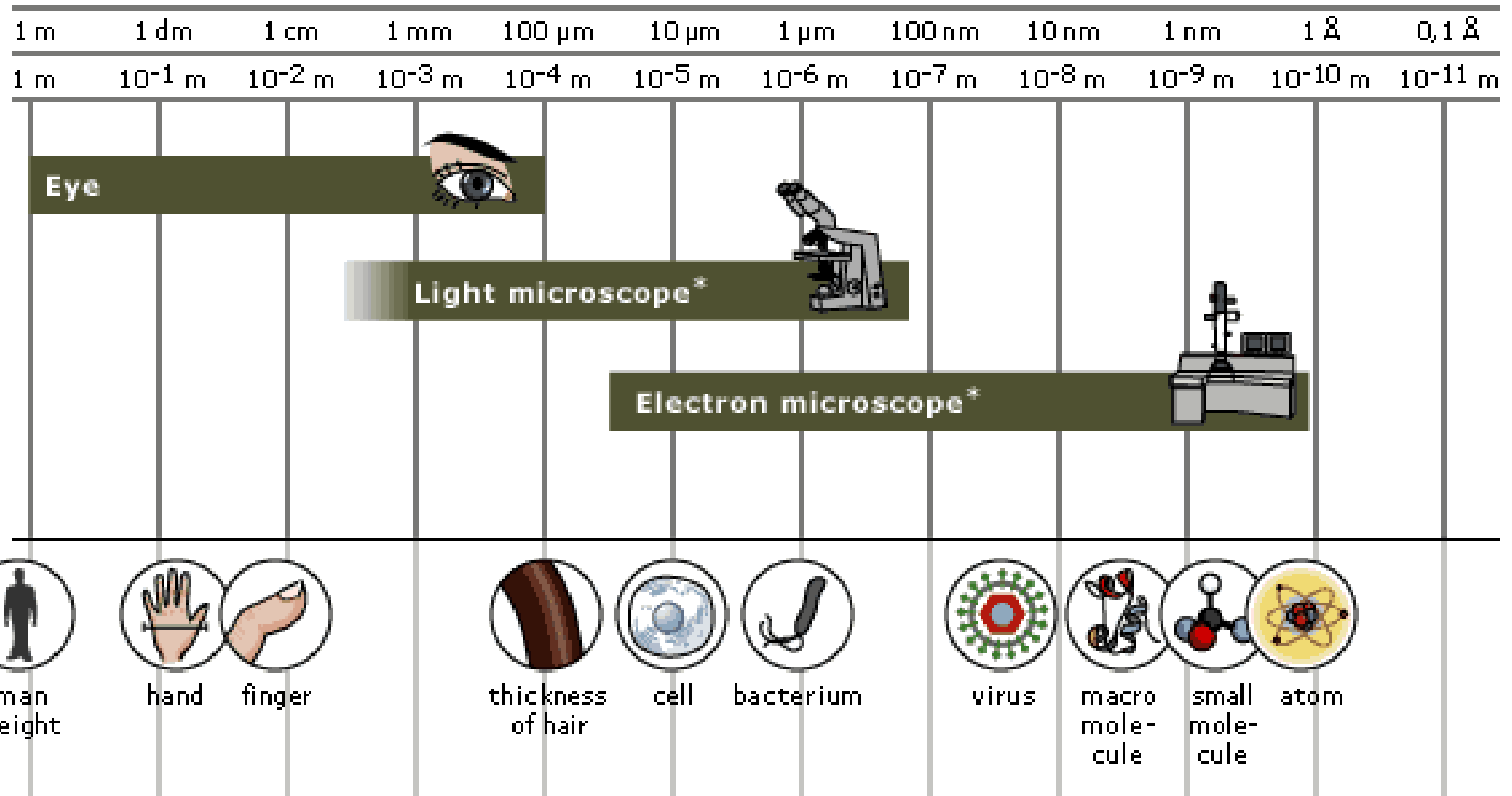
$$d_z = \frac{2 \times \lambda}{NA^2} = \frac{1000}{1.40^2} = 510 \text{ nm}$$



Point spread function (PSF)

Difrakční limit, nikoliv kvantový!



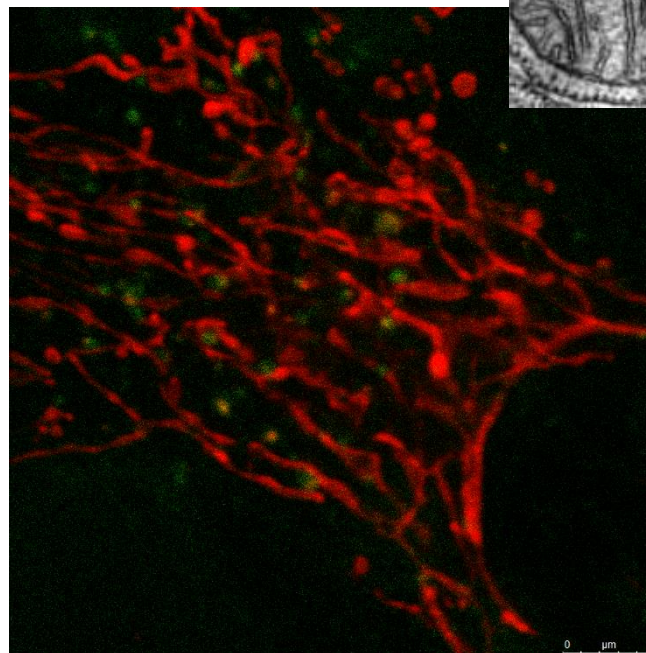


Nevýhody elektronového mikroskopu

Vzorek je vždy fixovaný (nejde live-cell).

Metoda je náchylná k tvorbě artefaktů.

Značení konkrétních molekul je složité.

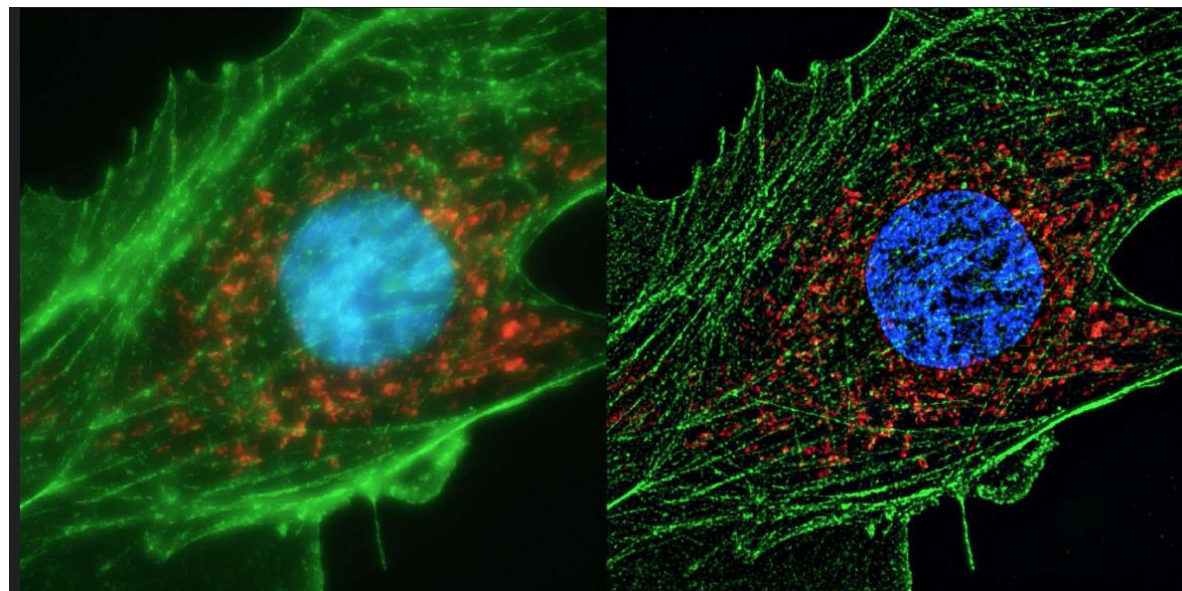


Výhody superrezoluce

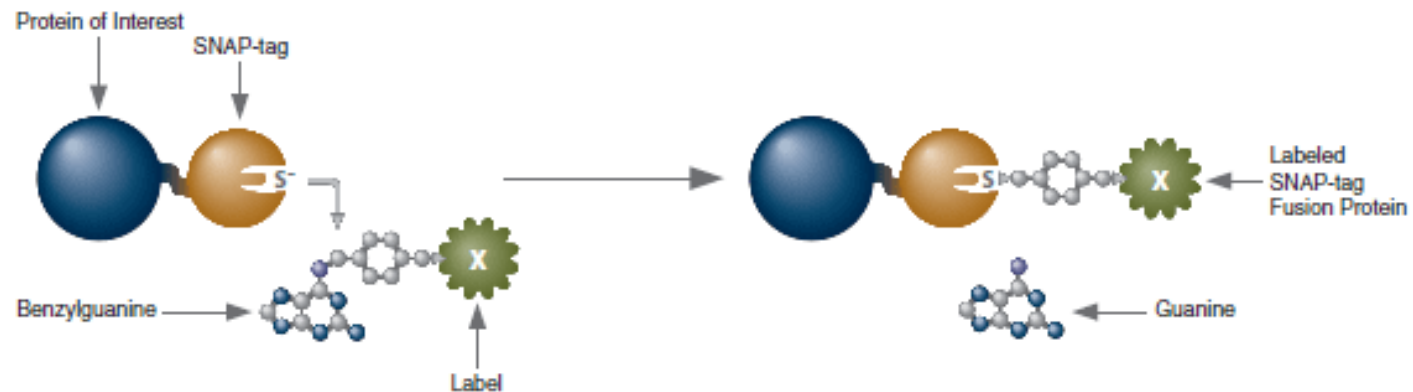
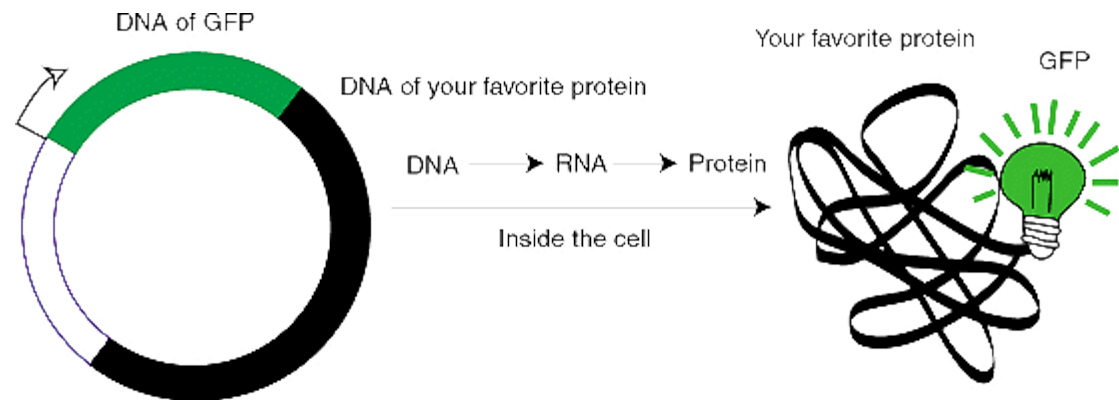
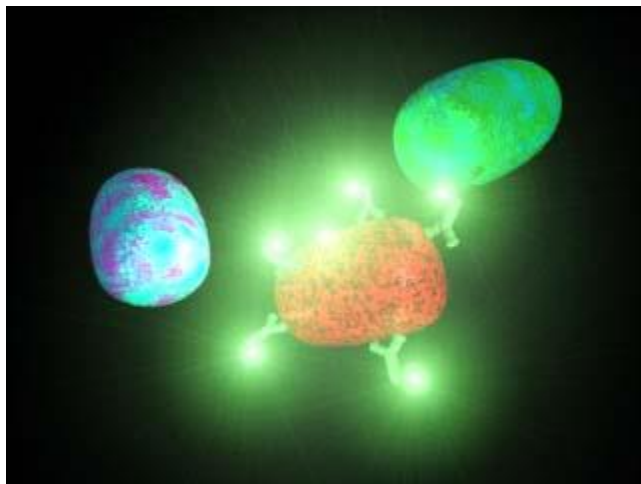
Vzorek může být živý.

Zpracování vzorku je jednoduché.

(Ko)lokalizujeme konkrétní molekuly.



Značení molekul



Druhy superrezoluce

Vylepšená geometrie fluorescenčního mikroskopu (konfo, SIM, 4Pi)

Stimulovaná deplece emise (STED)

Lokalizace jednotlivých molekul (PALM, STORM)

Kombinace: **dobrý nápad + špičková technologie + výpočetní technika**

Structure illumination microscopy (SIM)

Abbého limit snížen na polovinu.

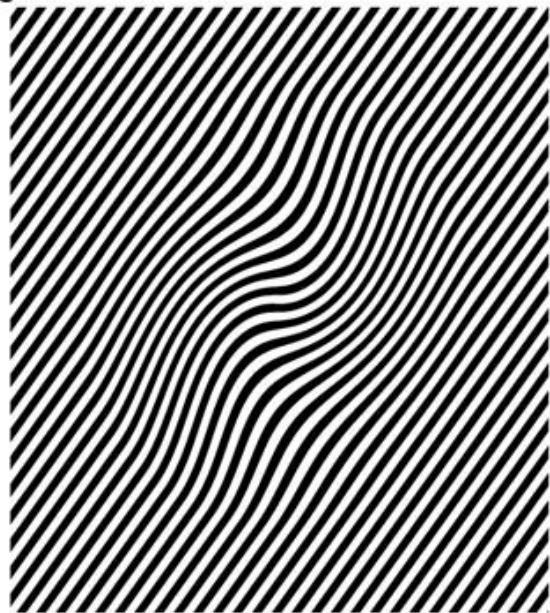
Instalace na VŠCHT, exkurse!

Moiré efekt

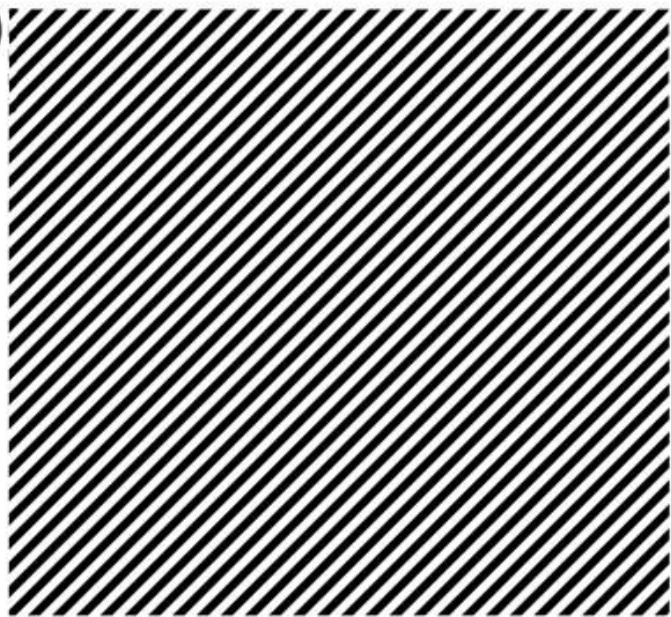


Moiré efekt

a



b



c



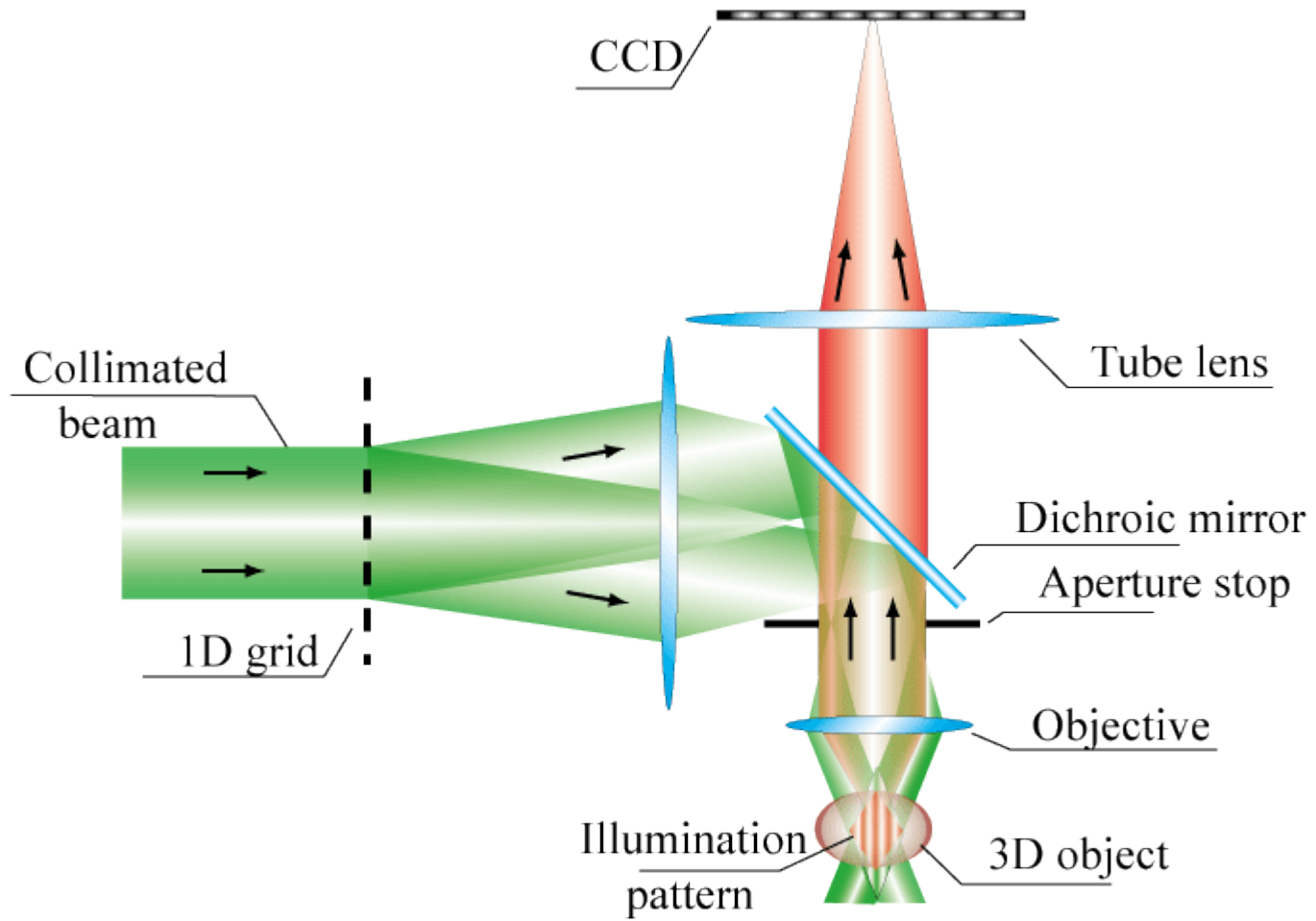
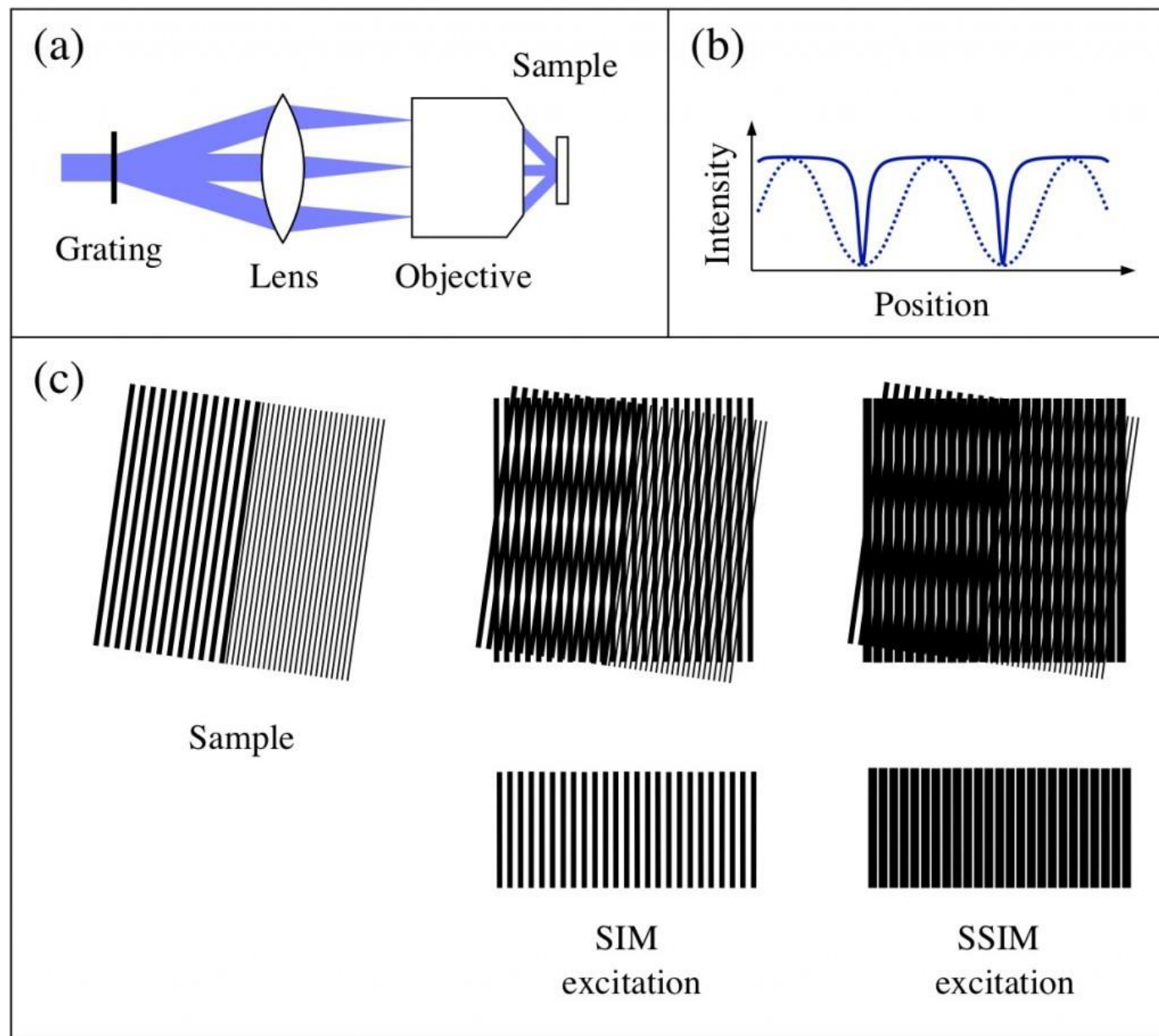
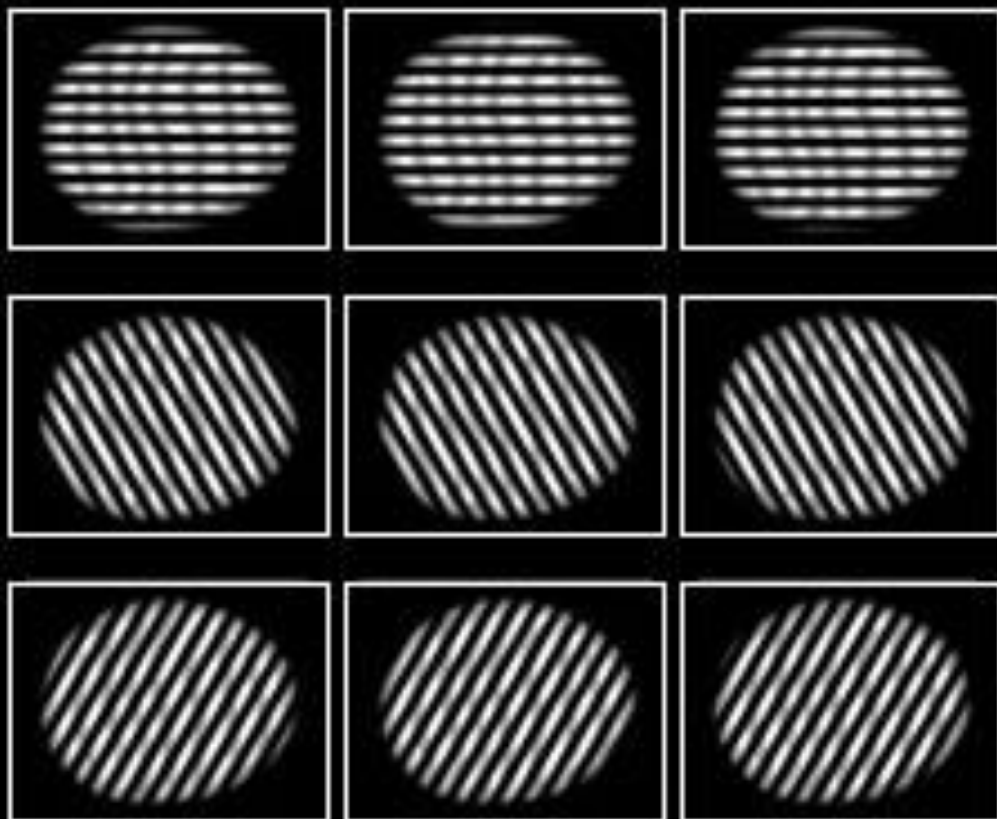


Fig.4 Schematic of conventional SI microscope.

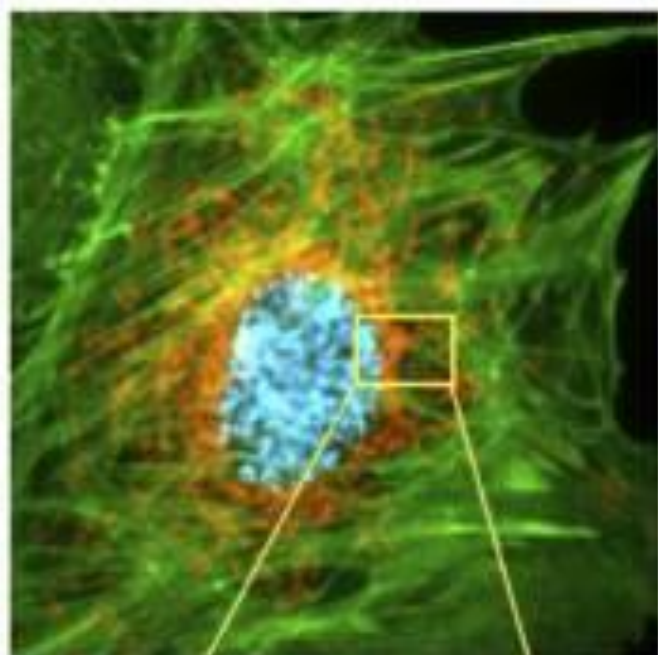
SIM



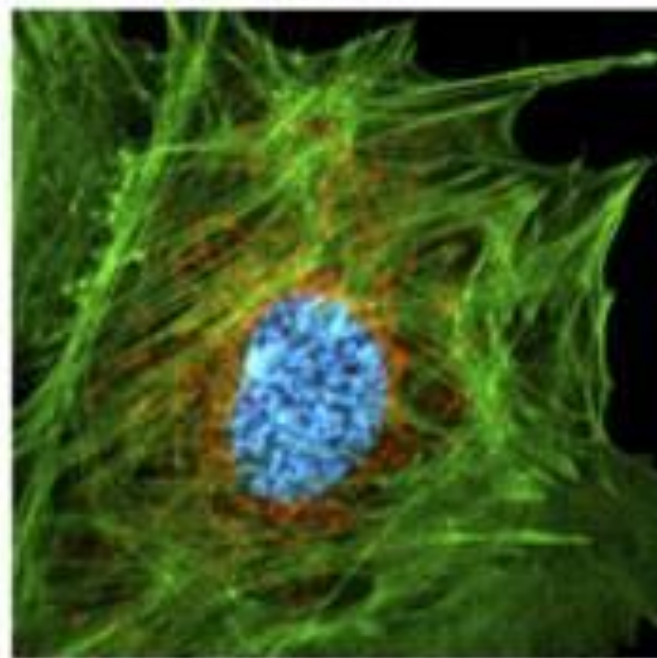


A super-resolution image is constructed through an image restoring process which uses multiple images captured in different phases and moving directions of the striped patterns.

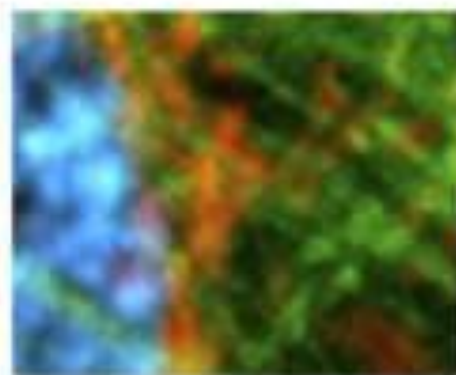
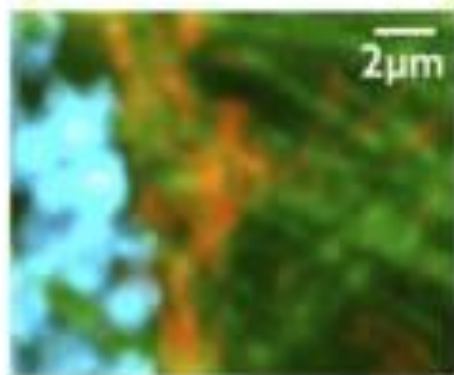
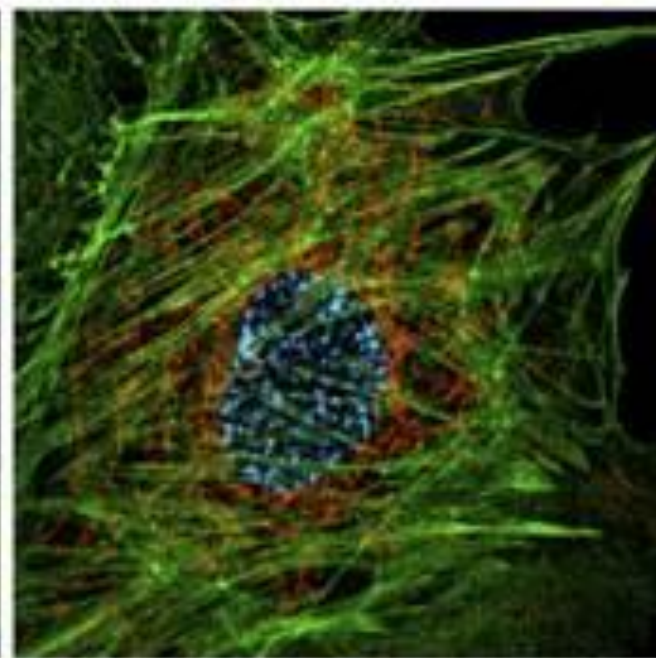
Epifluorescence

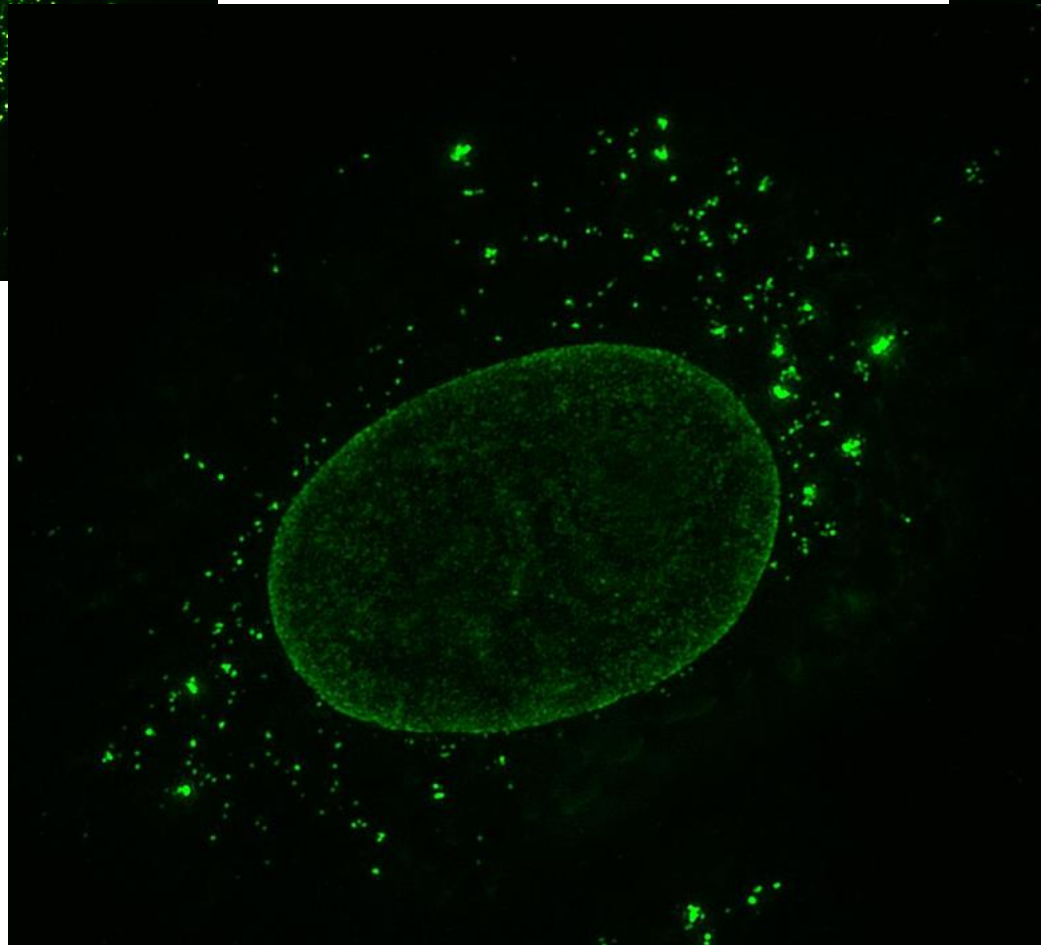
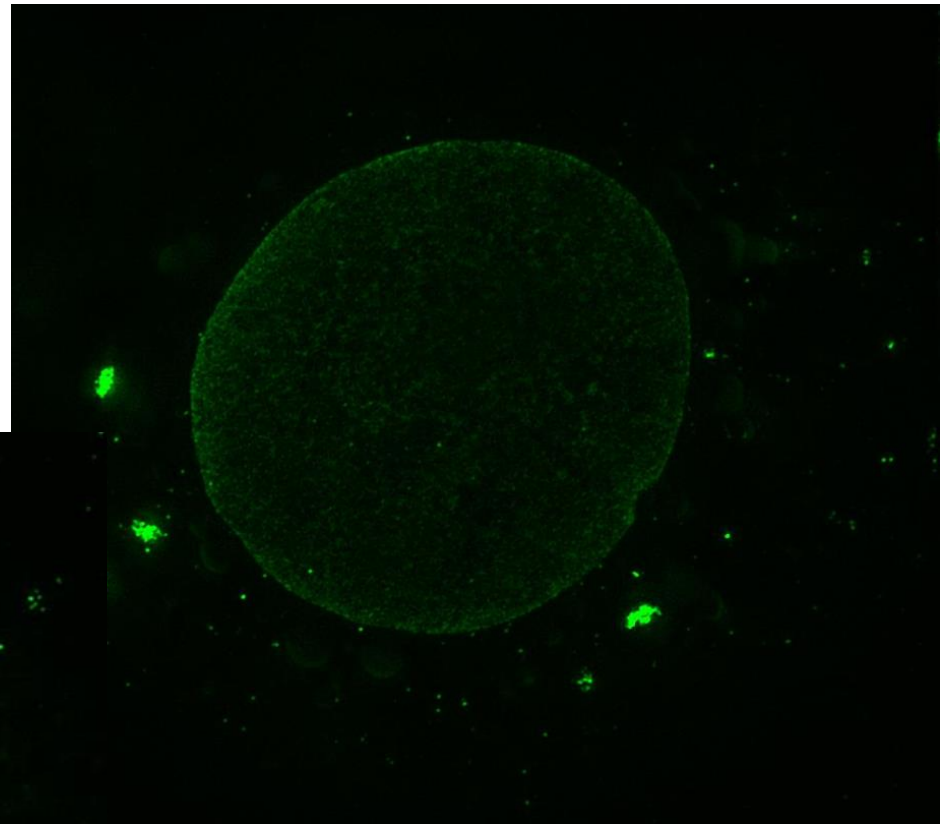
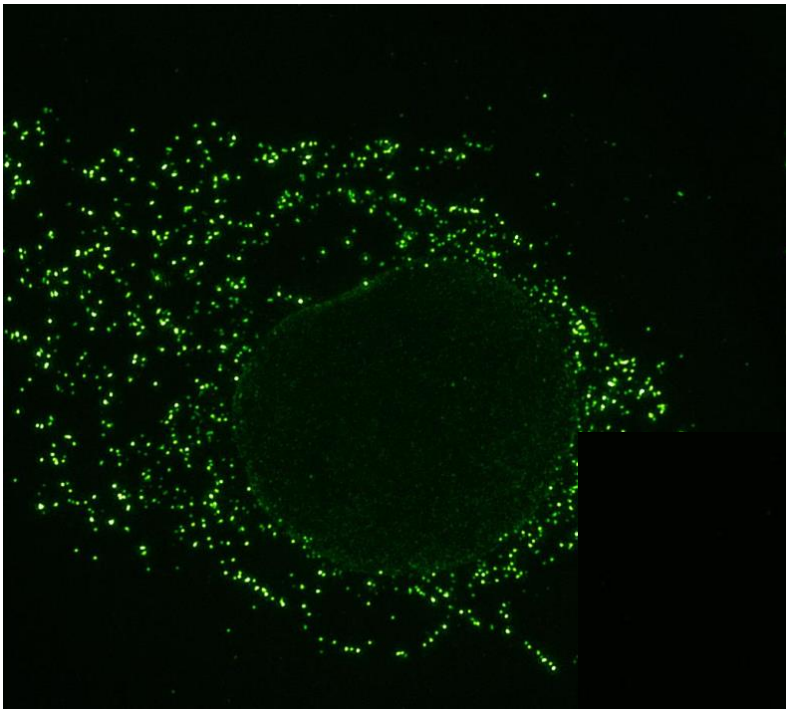


confocal



SIM





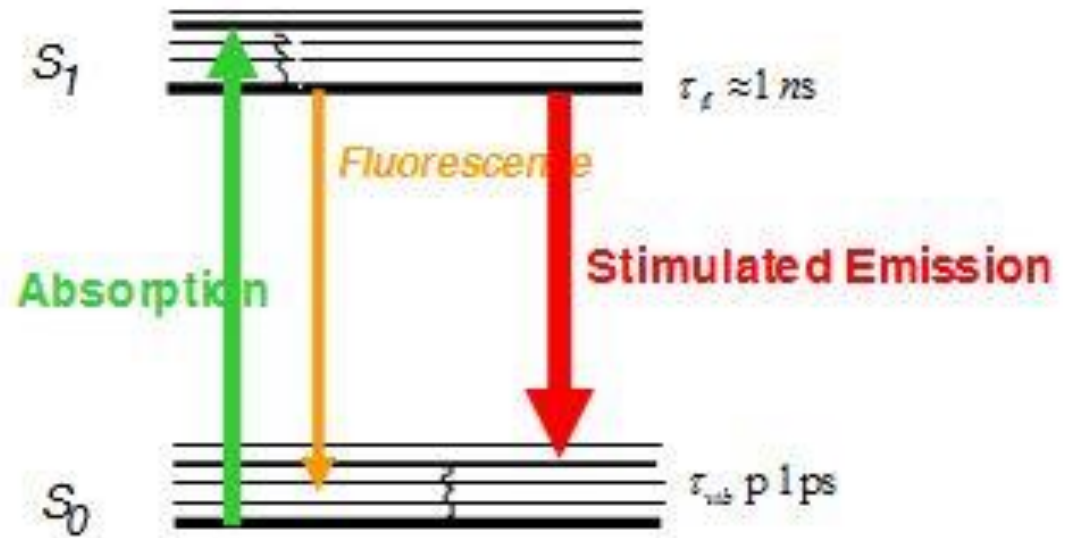
+video SIM

Stimulated emission depletion (STED)

Vysoké rozlišení

Poměrně drahý

Vysoká intenzita záření



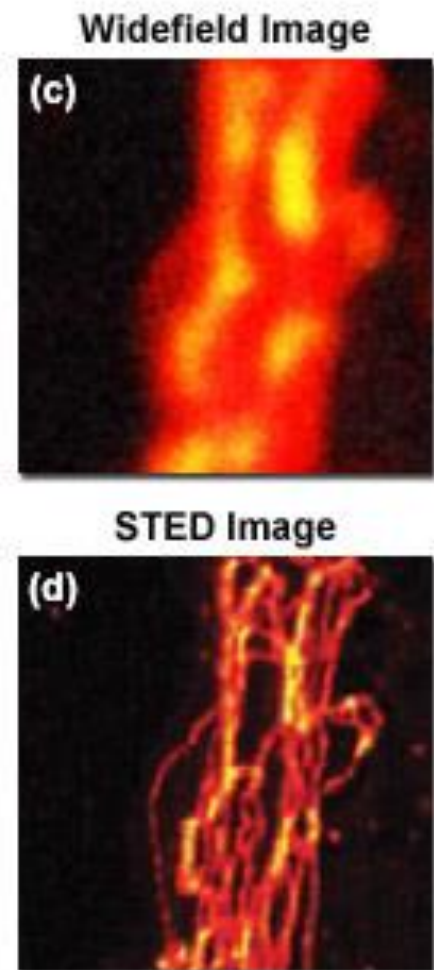
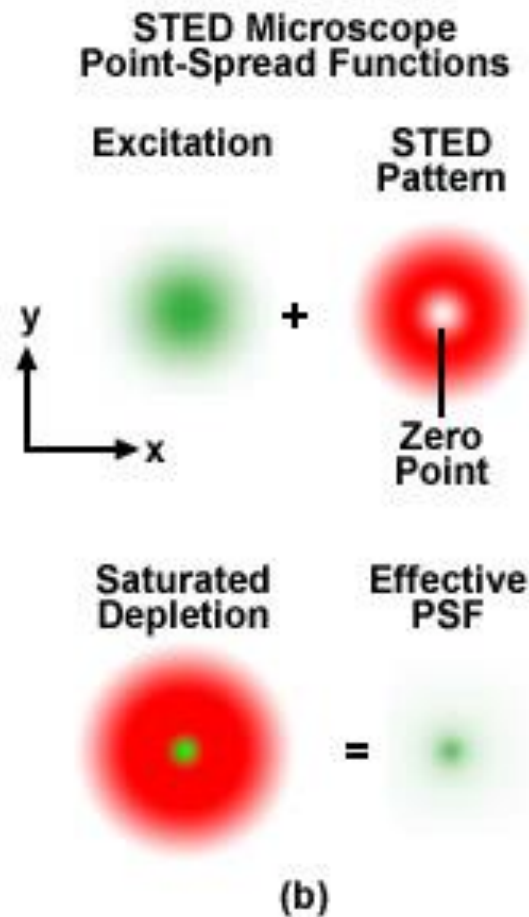
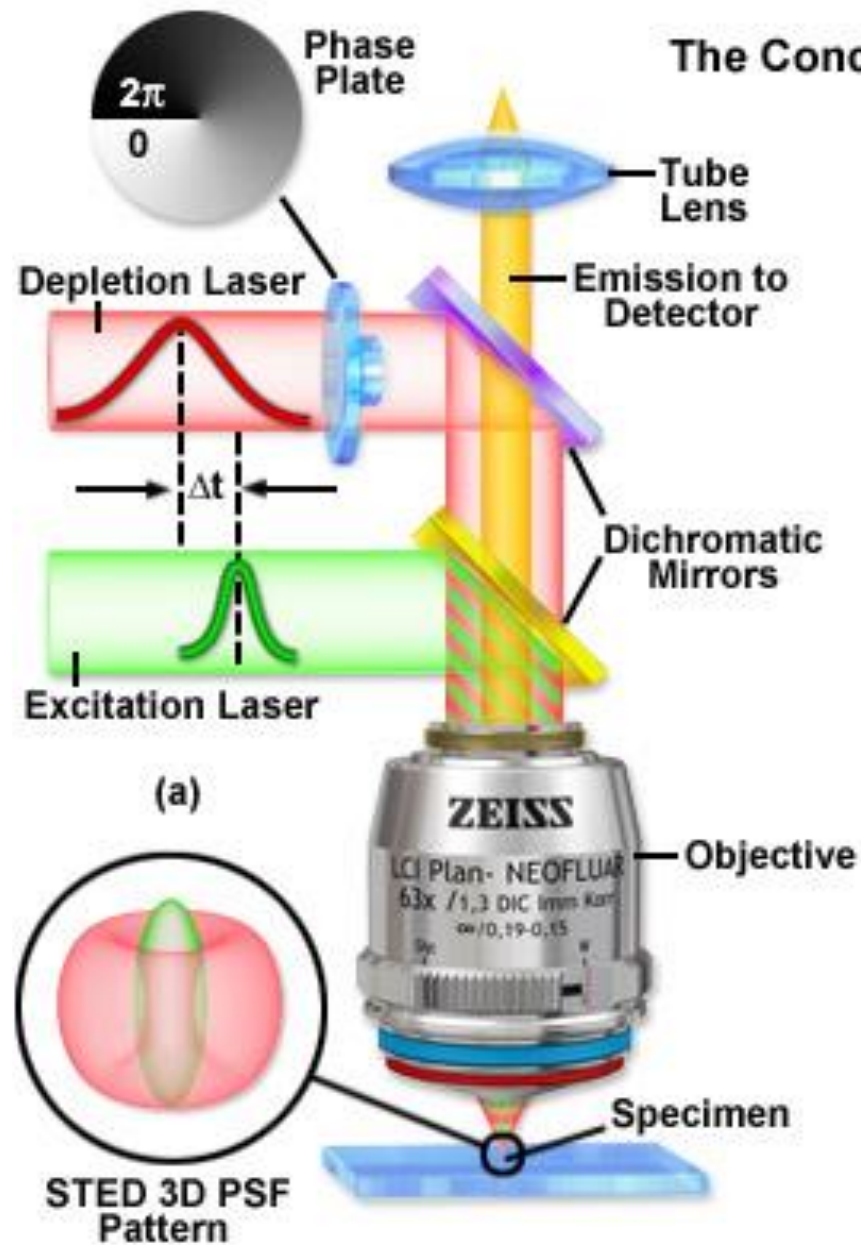
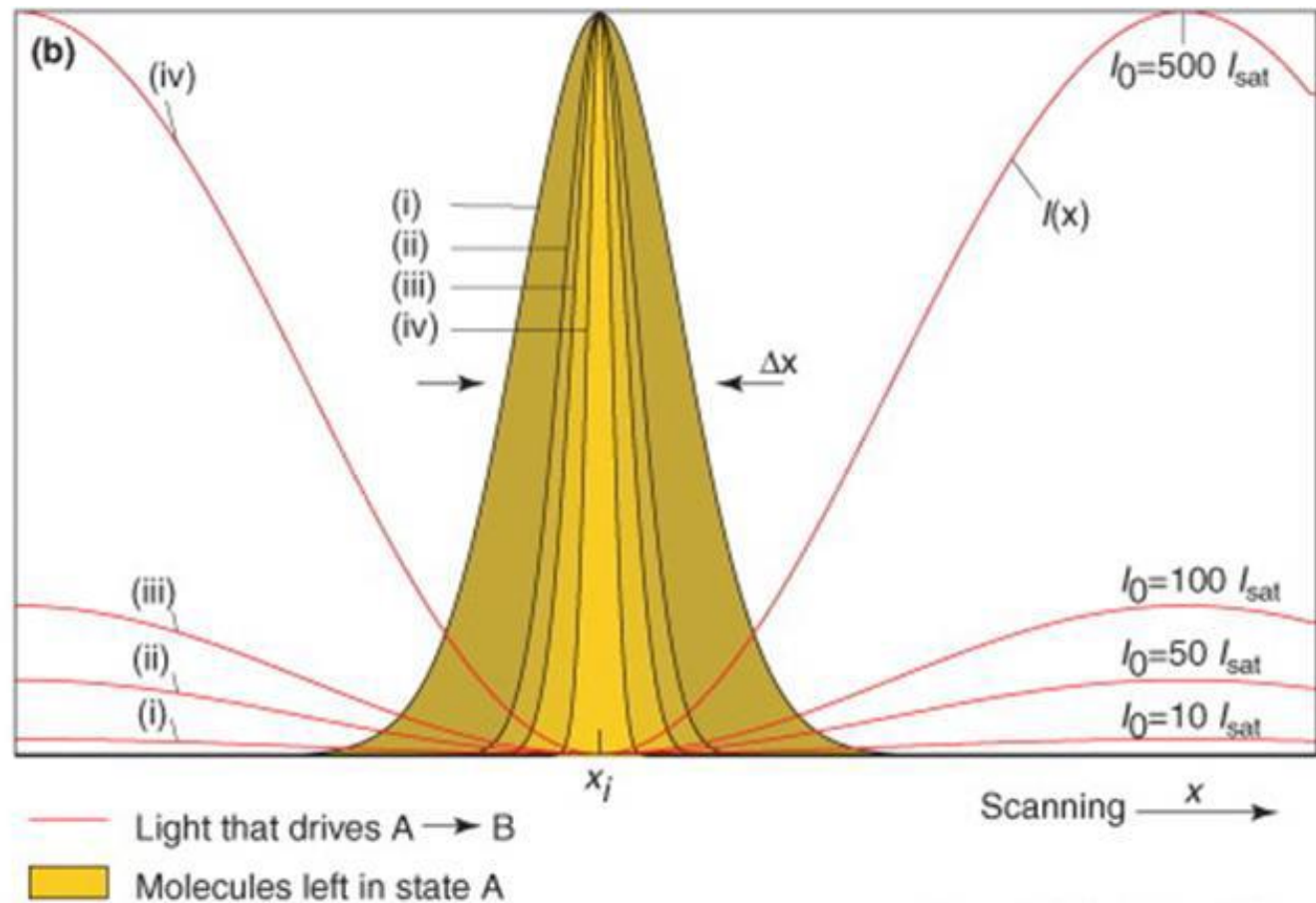
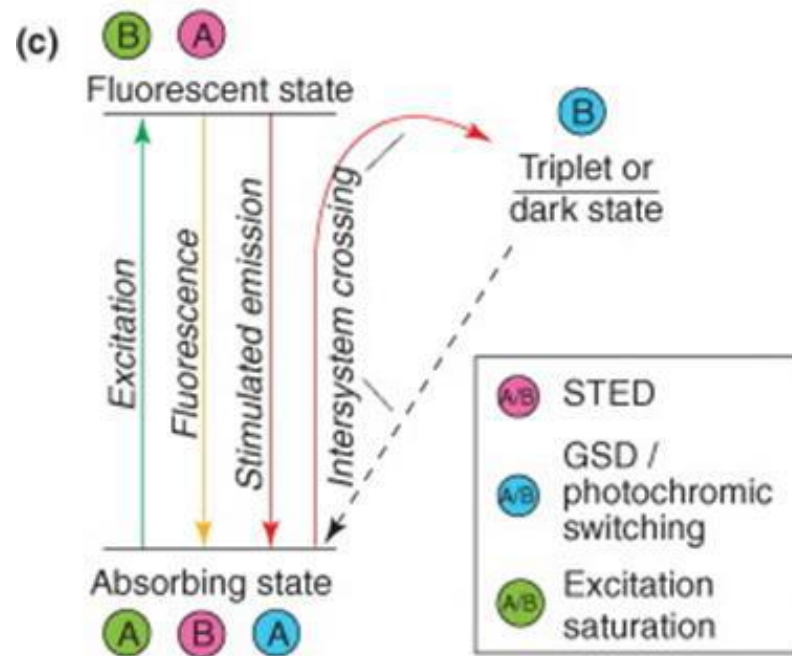
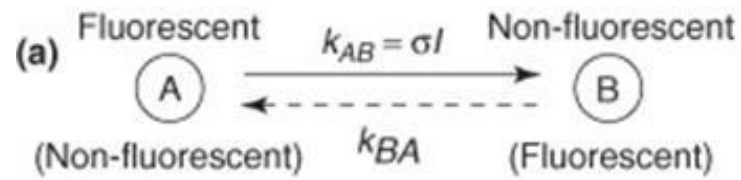


Figure 6

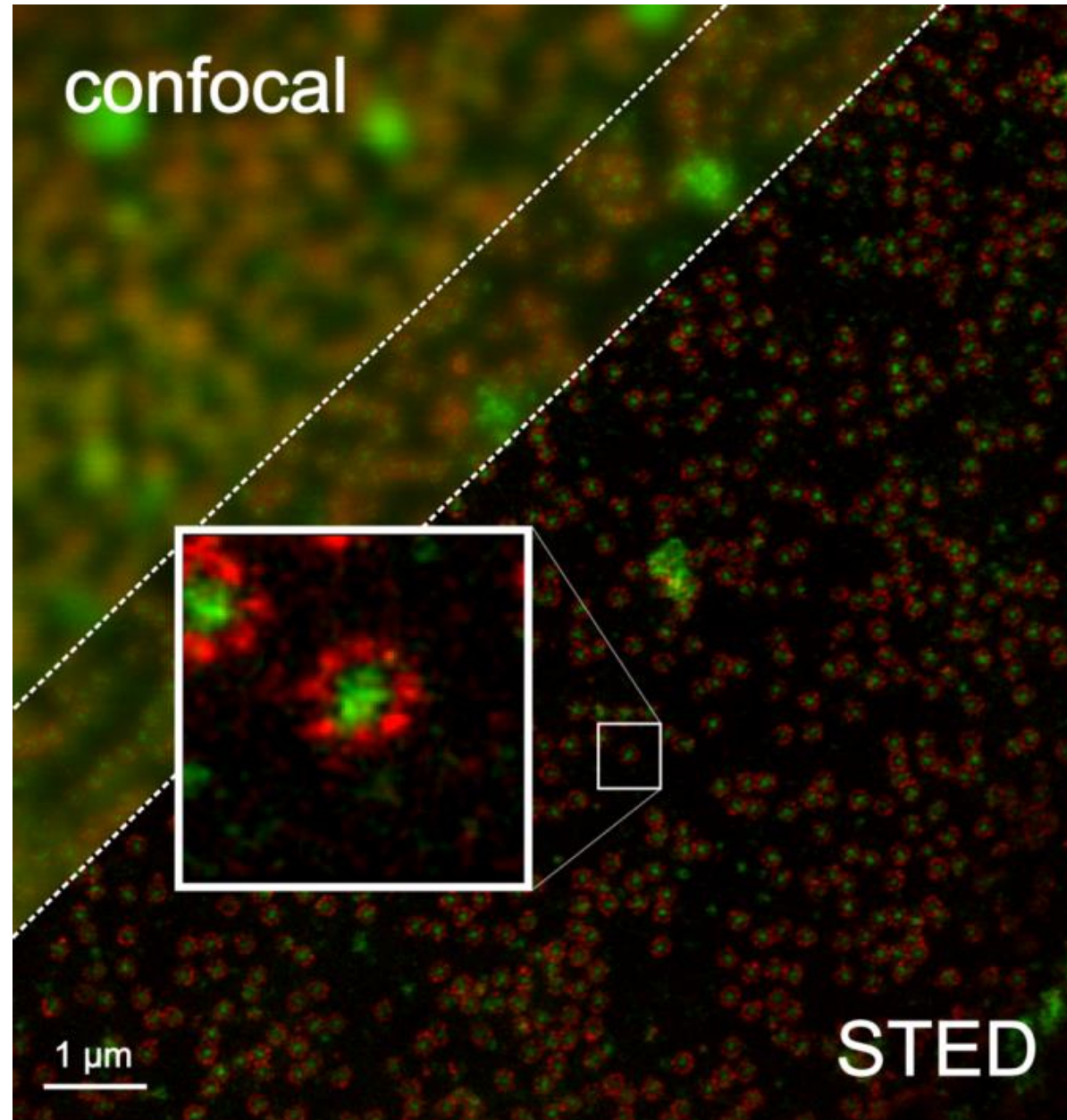
+ video STED



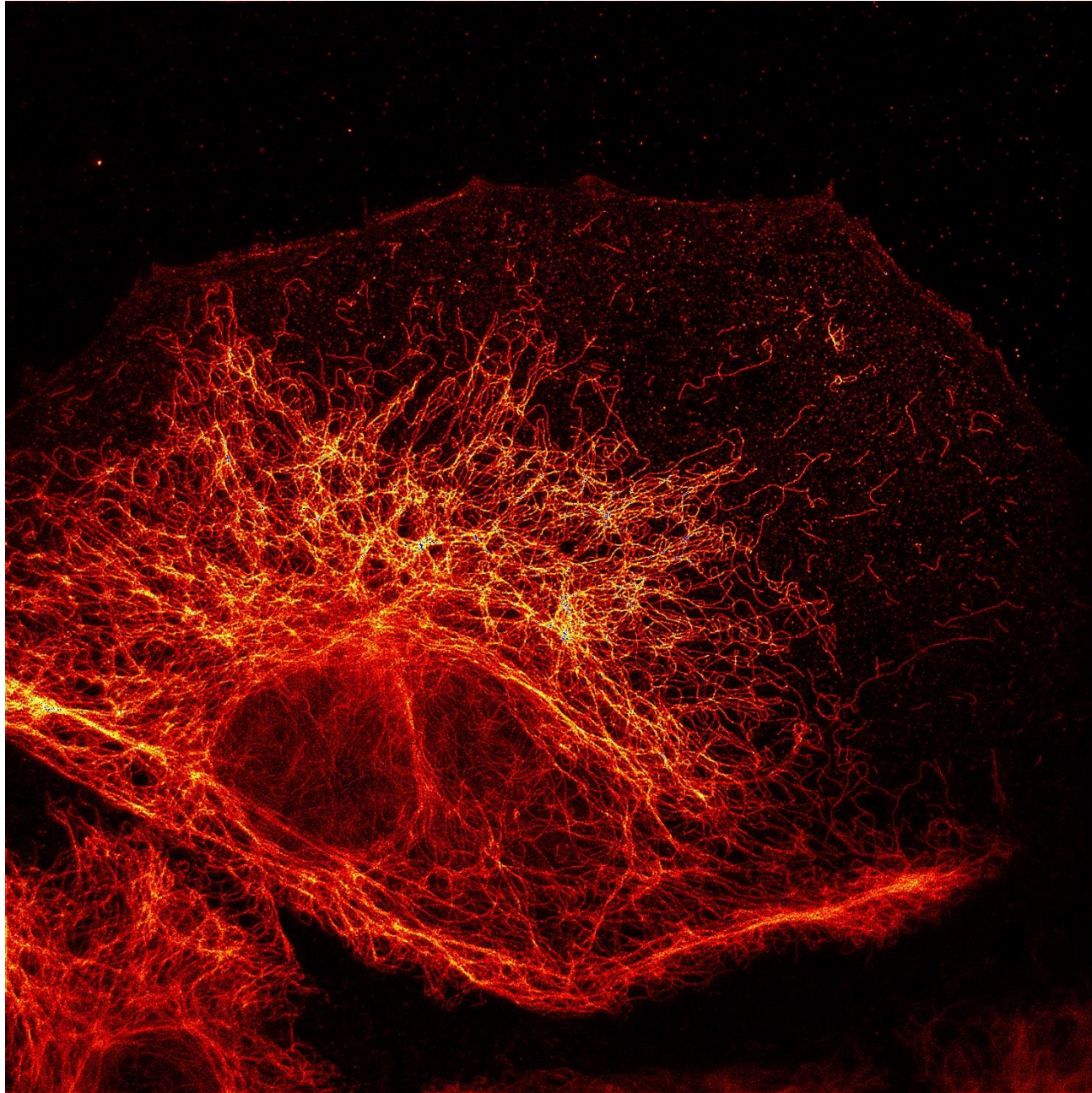
Current Opinion in Neurobiology

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

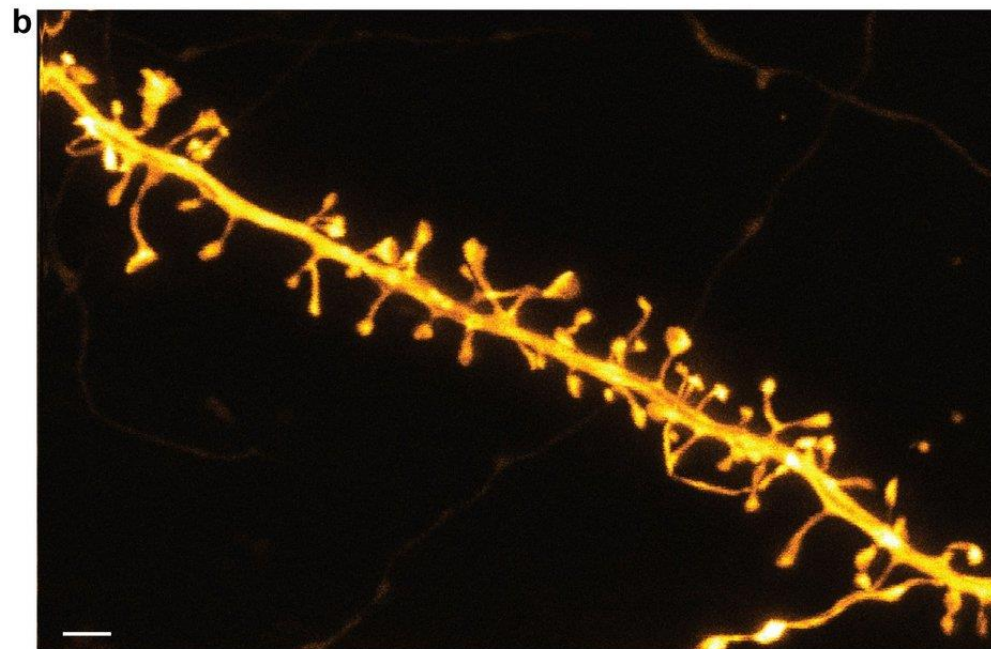
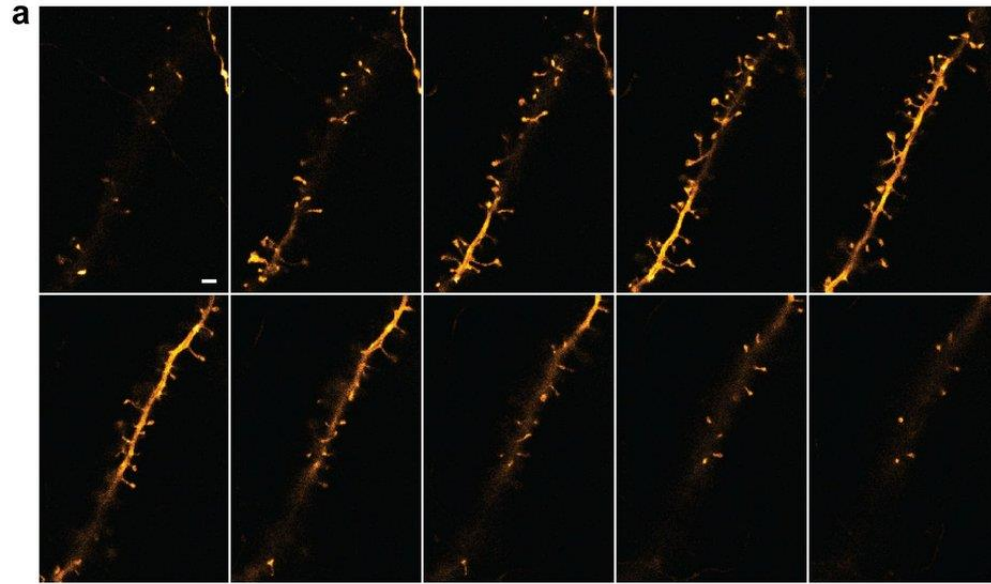
STED



STED

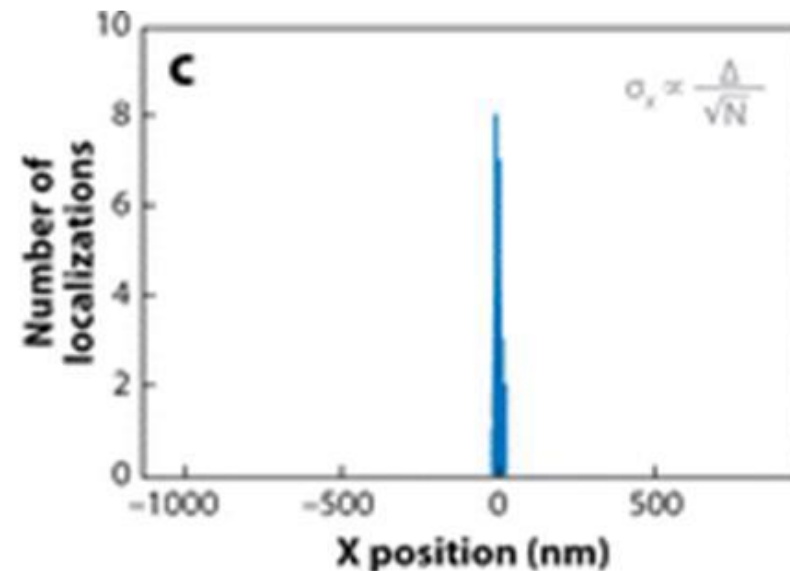
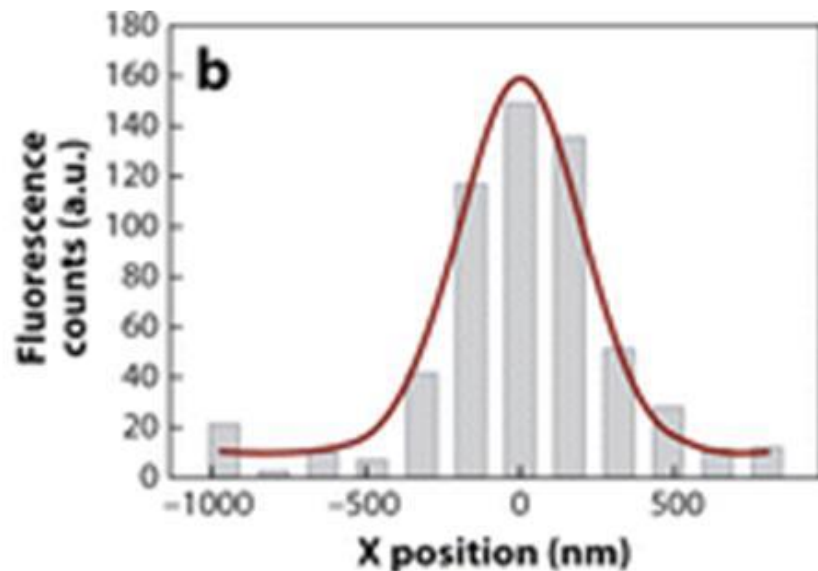
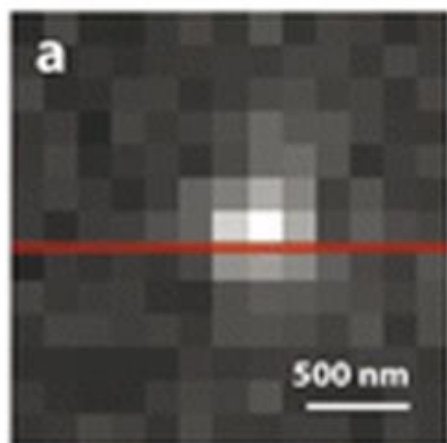


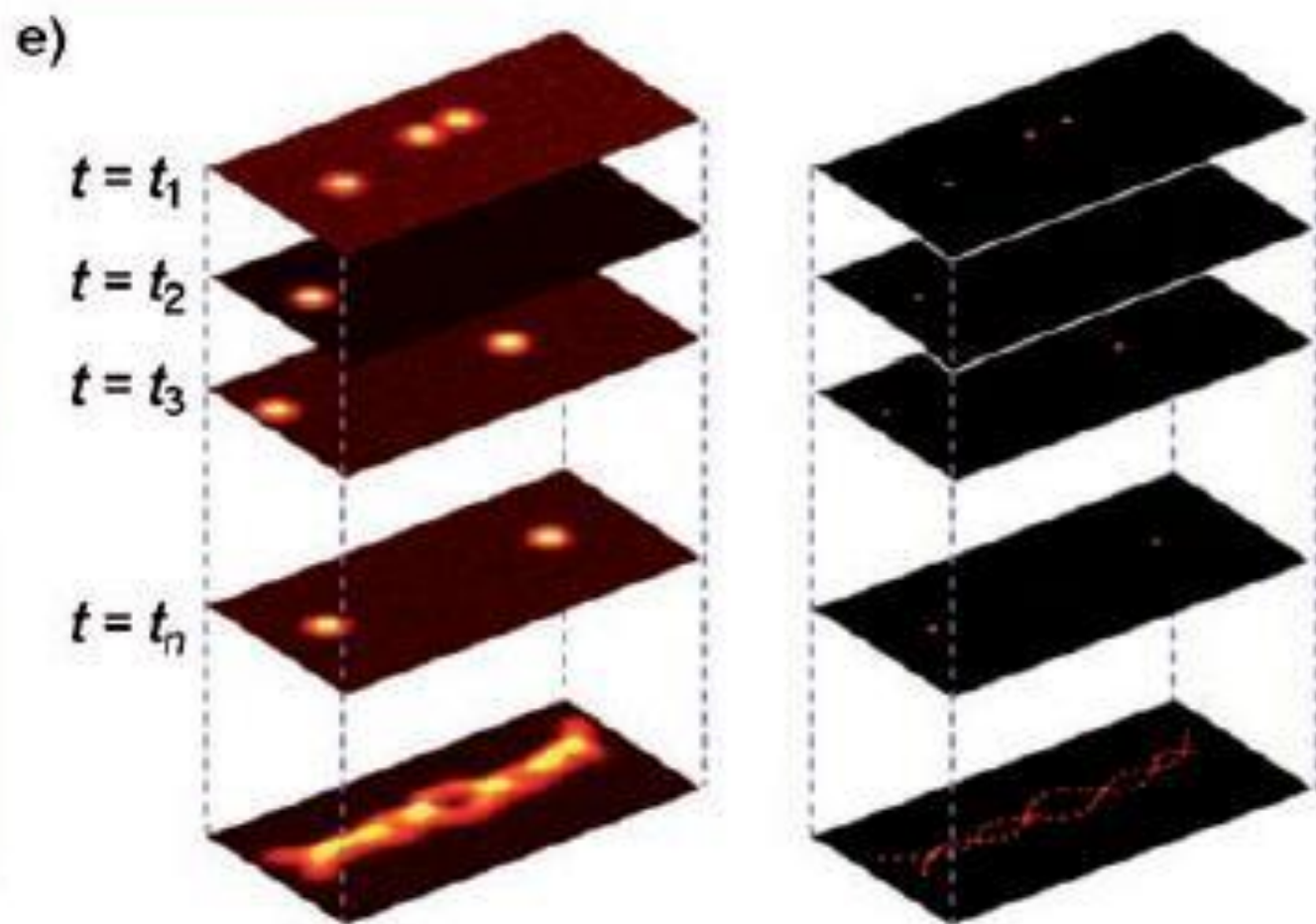
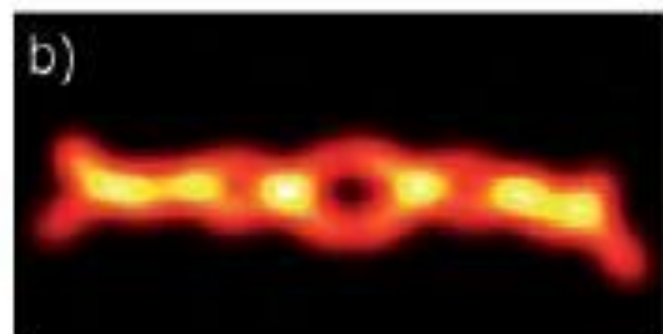
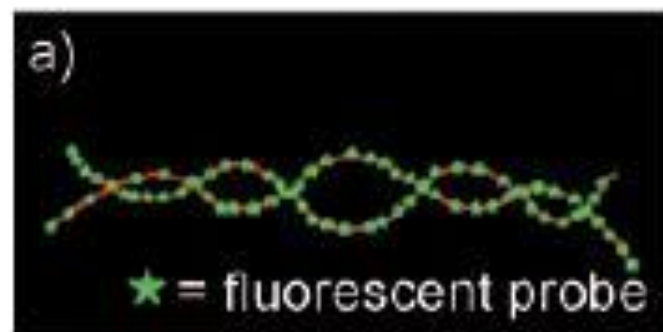
STED



Lokalizační mikroskopie (PALM, STORM)

Lokalizace jednotlivých molekul vzdálených $>$ Abbého limit





+ video Eiffel

Photoactivated localization microscopy (PALM)

Fotoaktivované proteiny

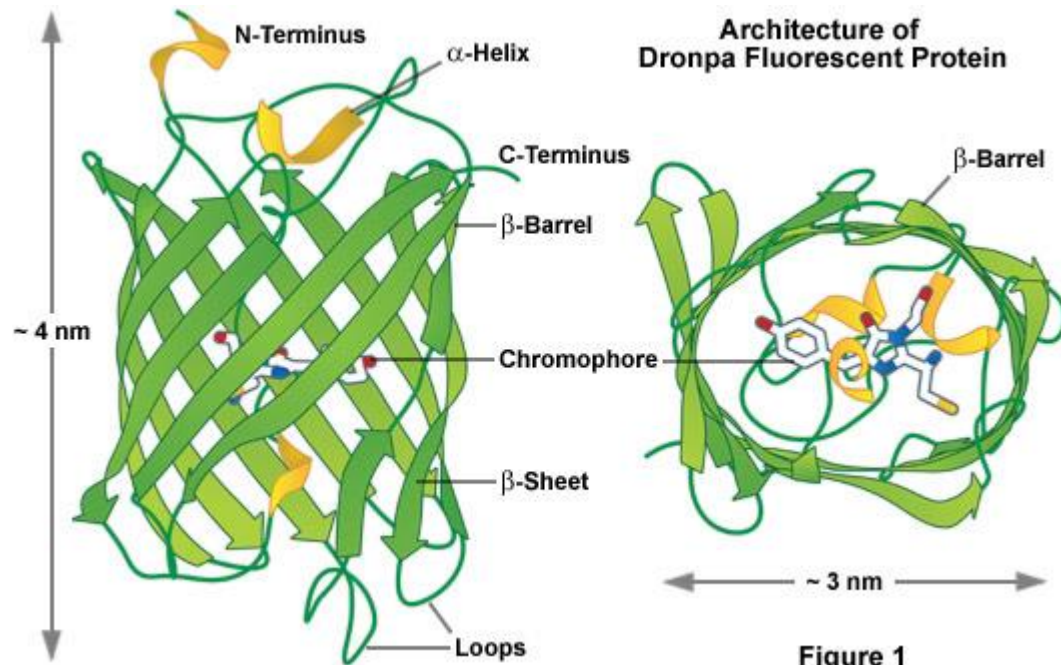
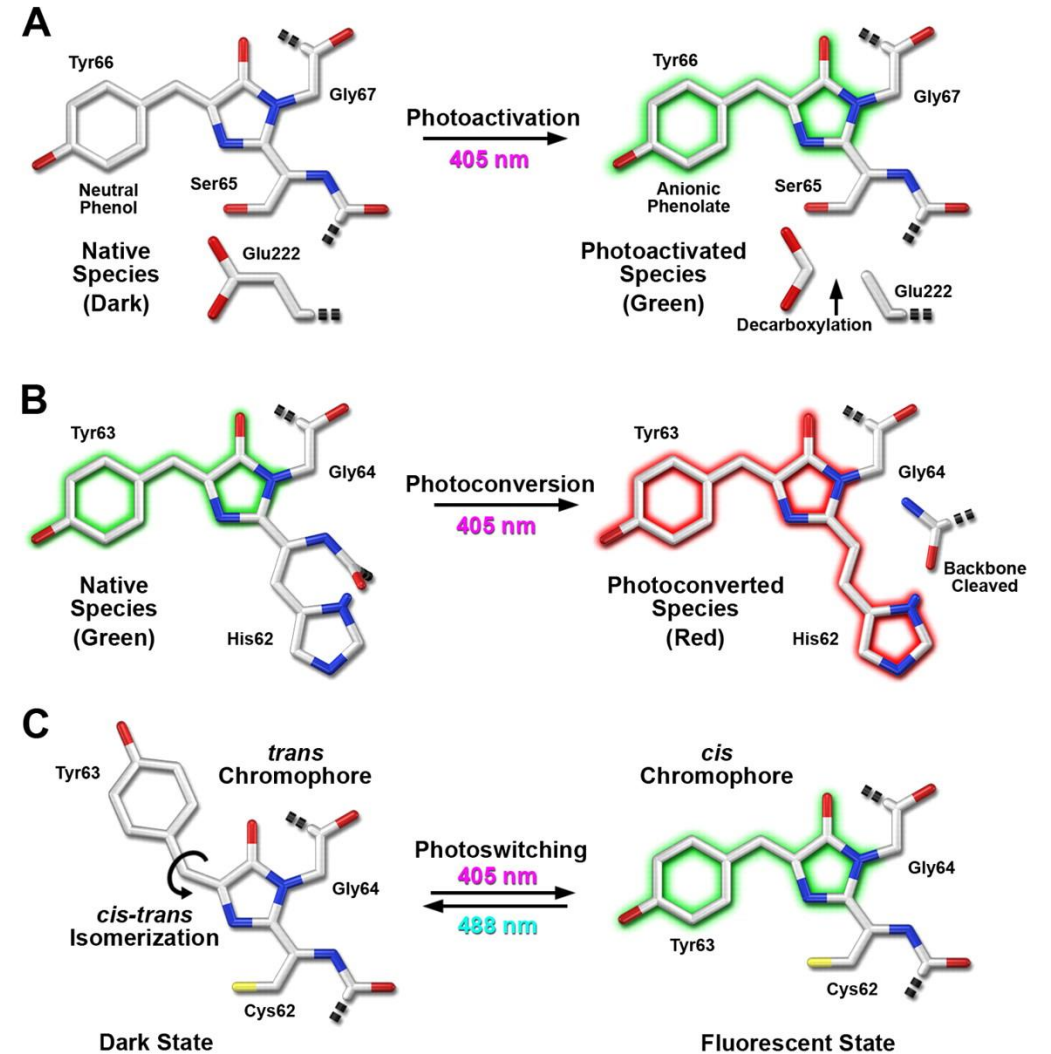


Figure 1



Single-Molecule Superresolution Basic Microscope Configuration

Activation
Laser
(405 nm)

Readout
Laser
(561 nm)

Specimen

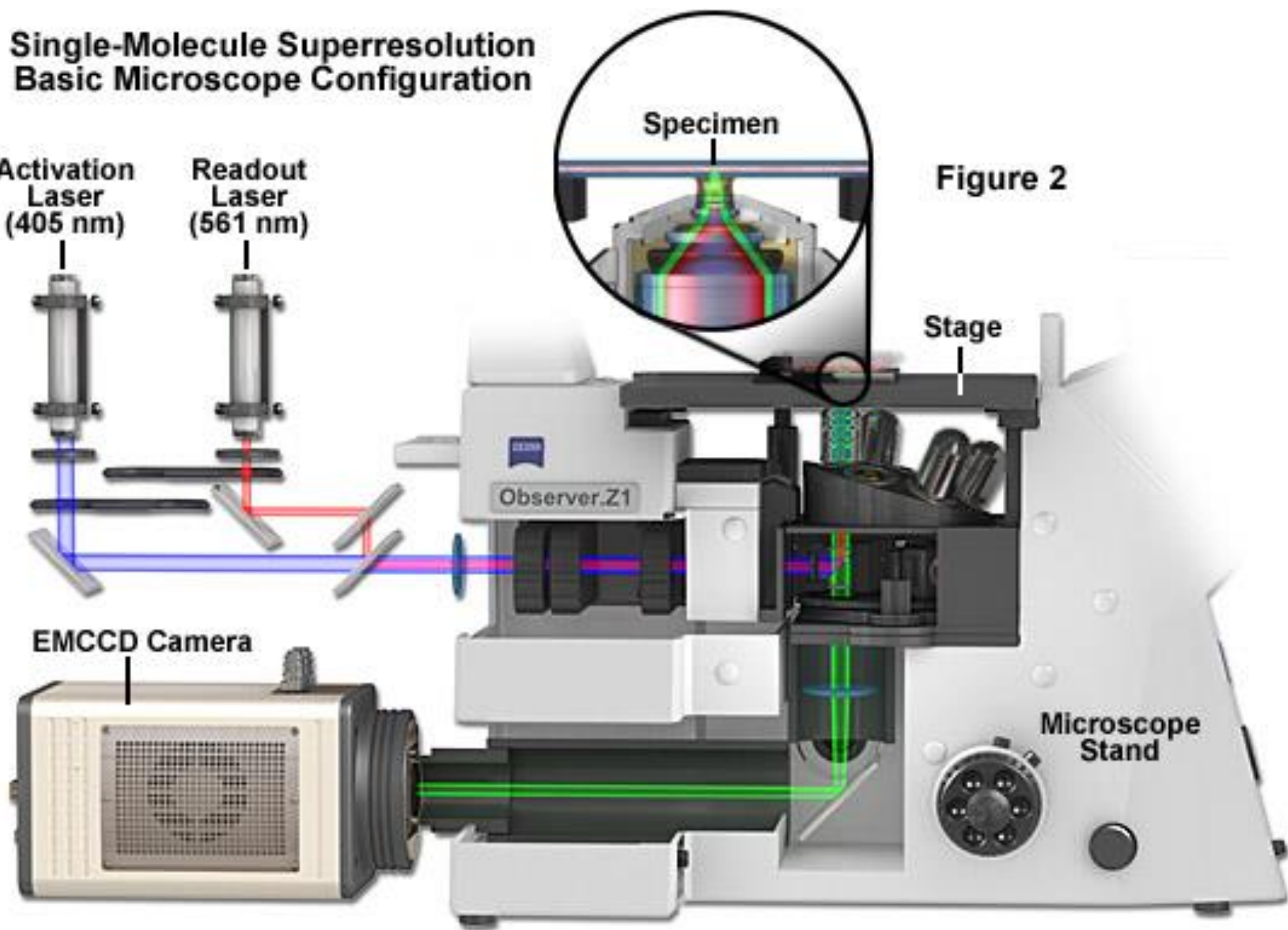
Figure 2

Stage

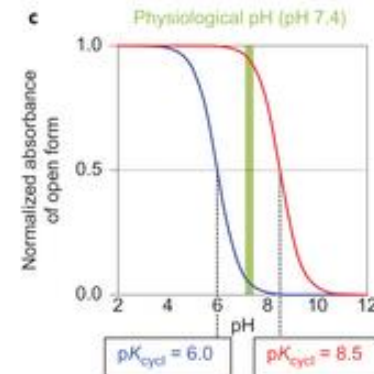
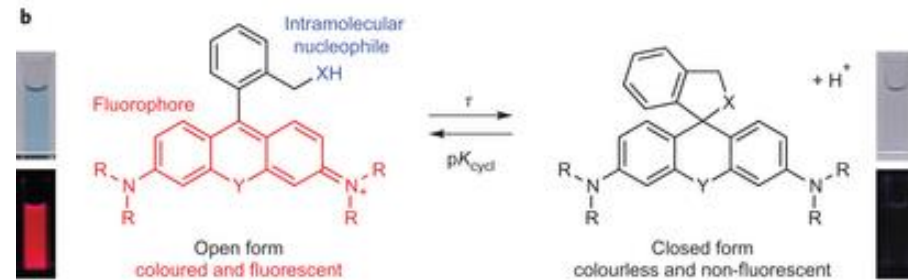
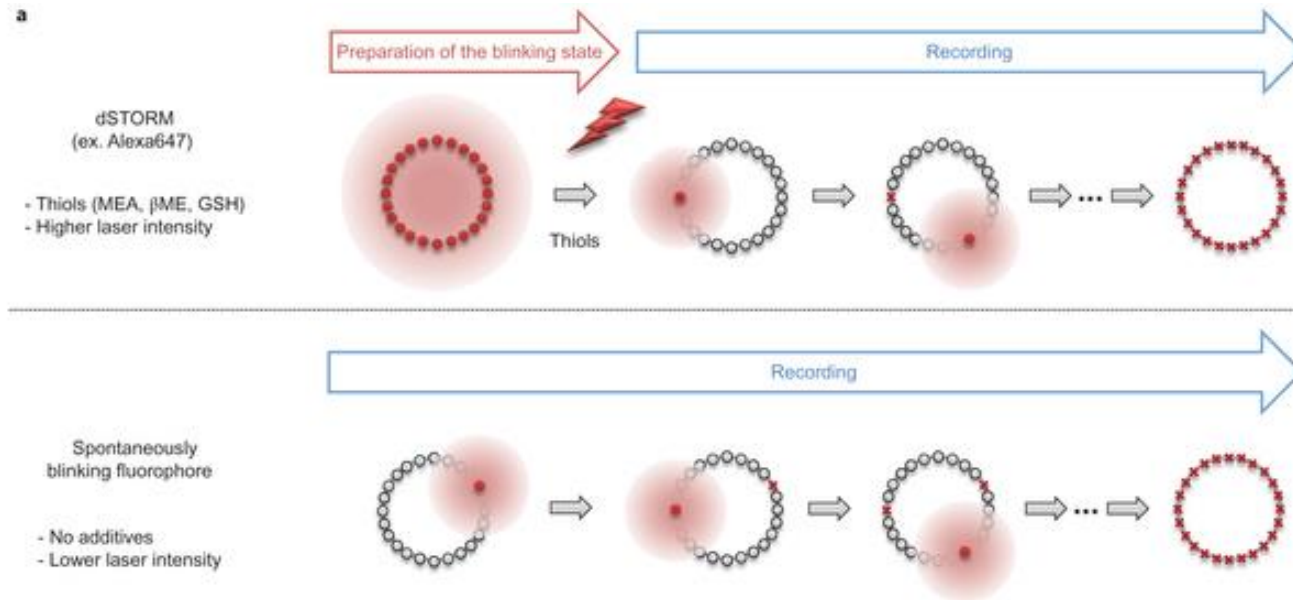
Observer.Z1

EMCCD Camera

Microscope
Stand



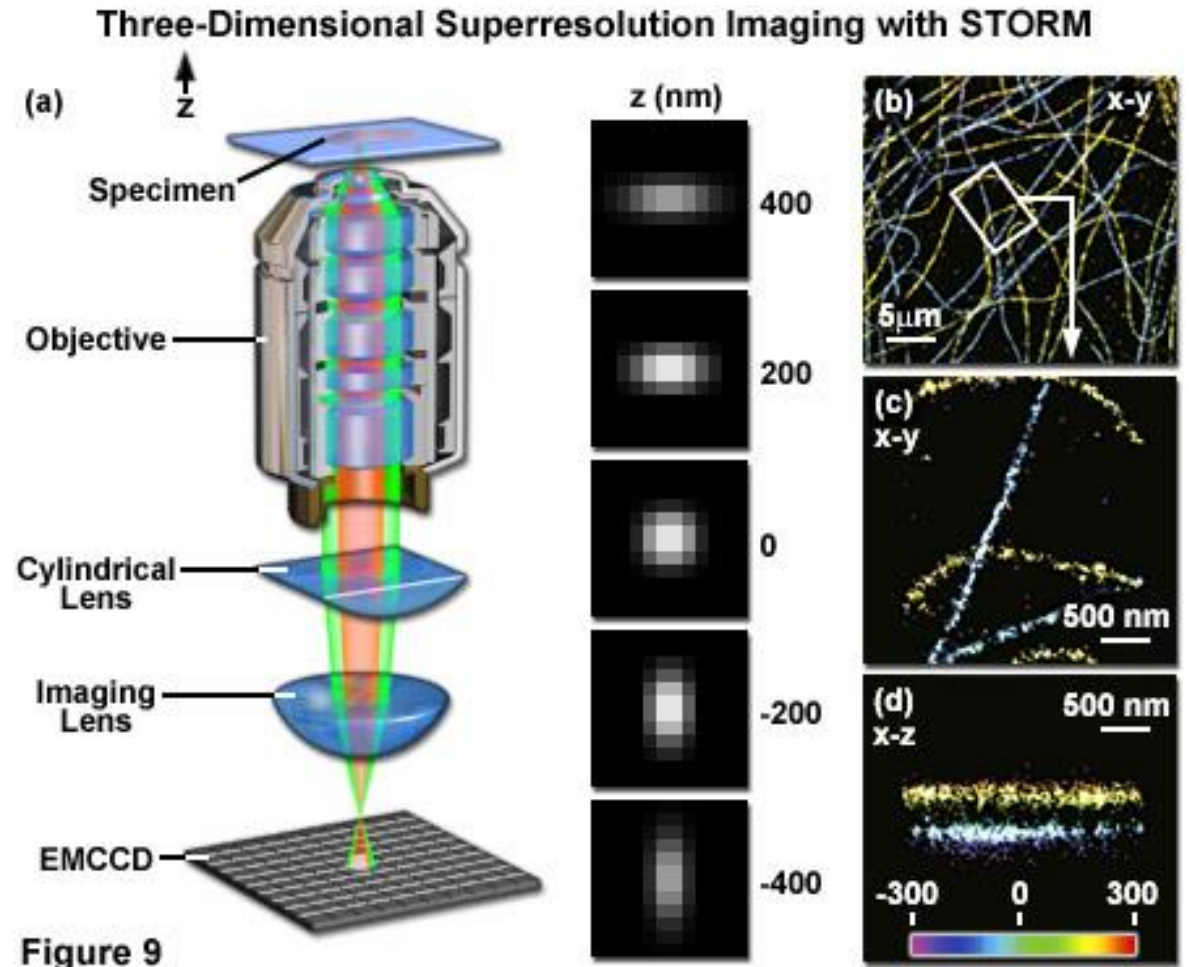
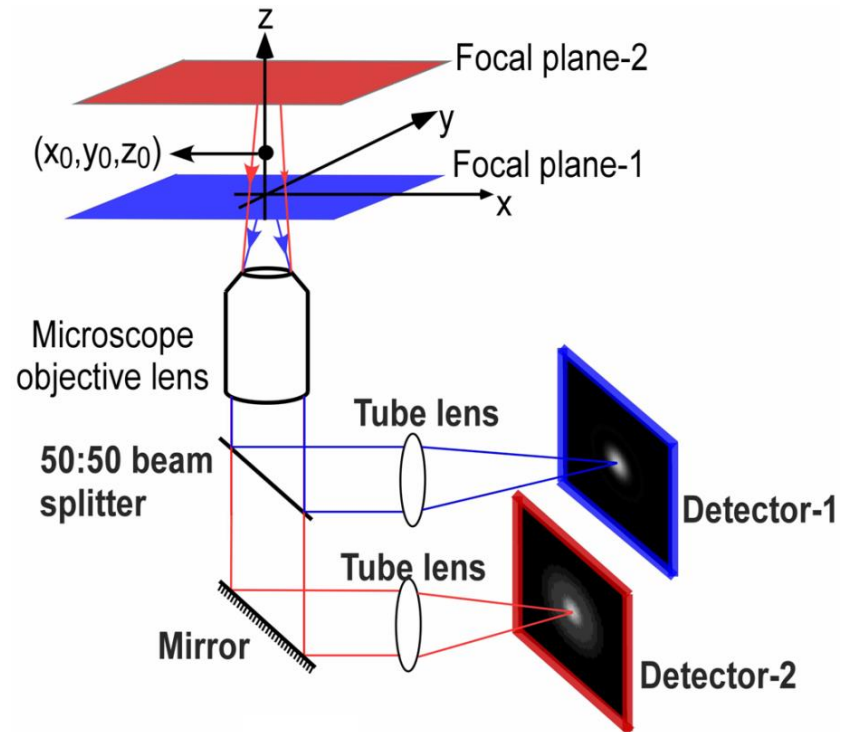
Stochastic optical reconstruction microscopy (STORM)



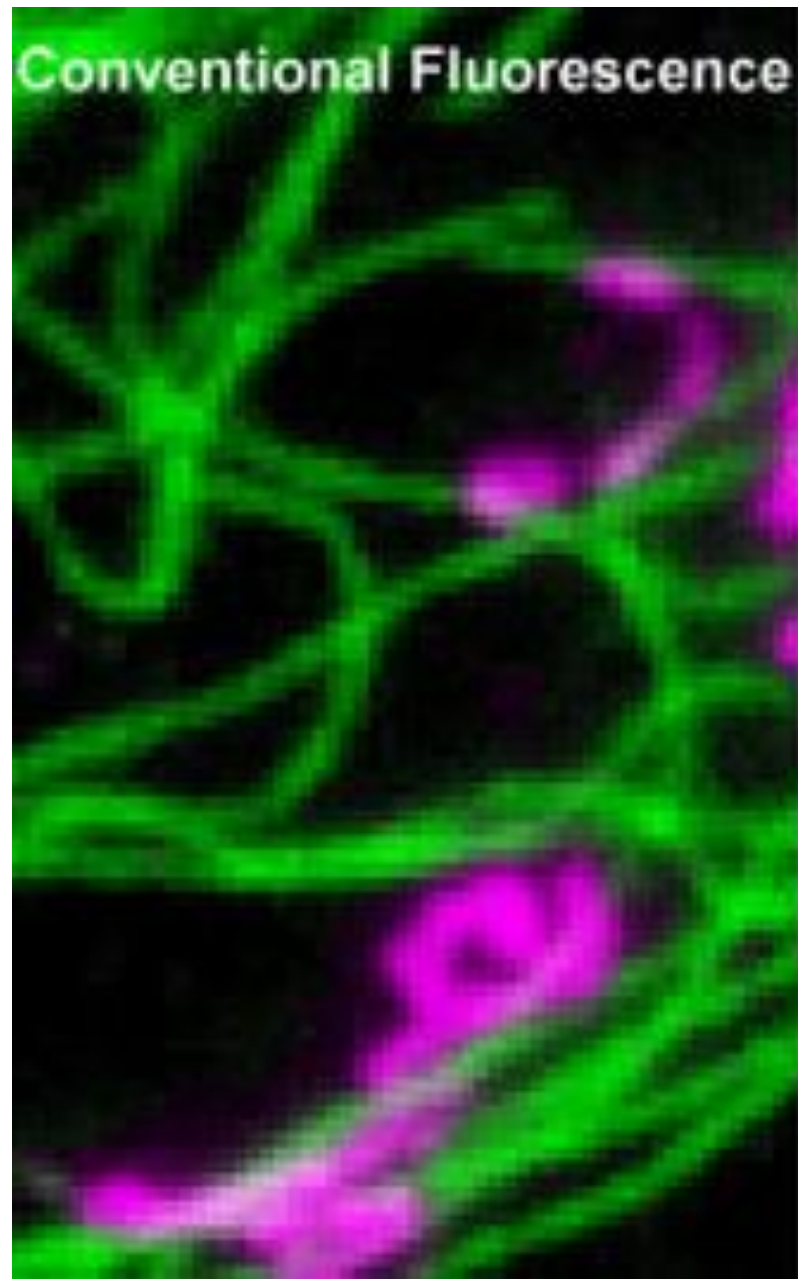
PALM + STORM

$$d_{x,y} = \frac{\lambda}{\sqrt{n} \times 2 \times NA}$$

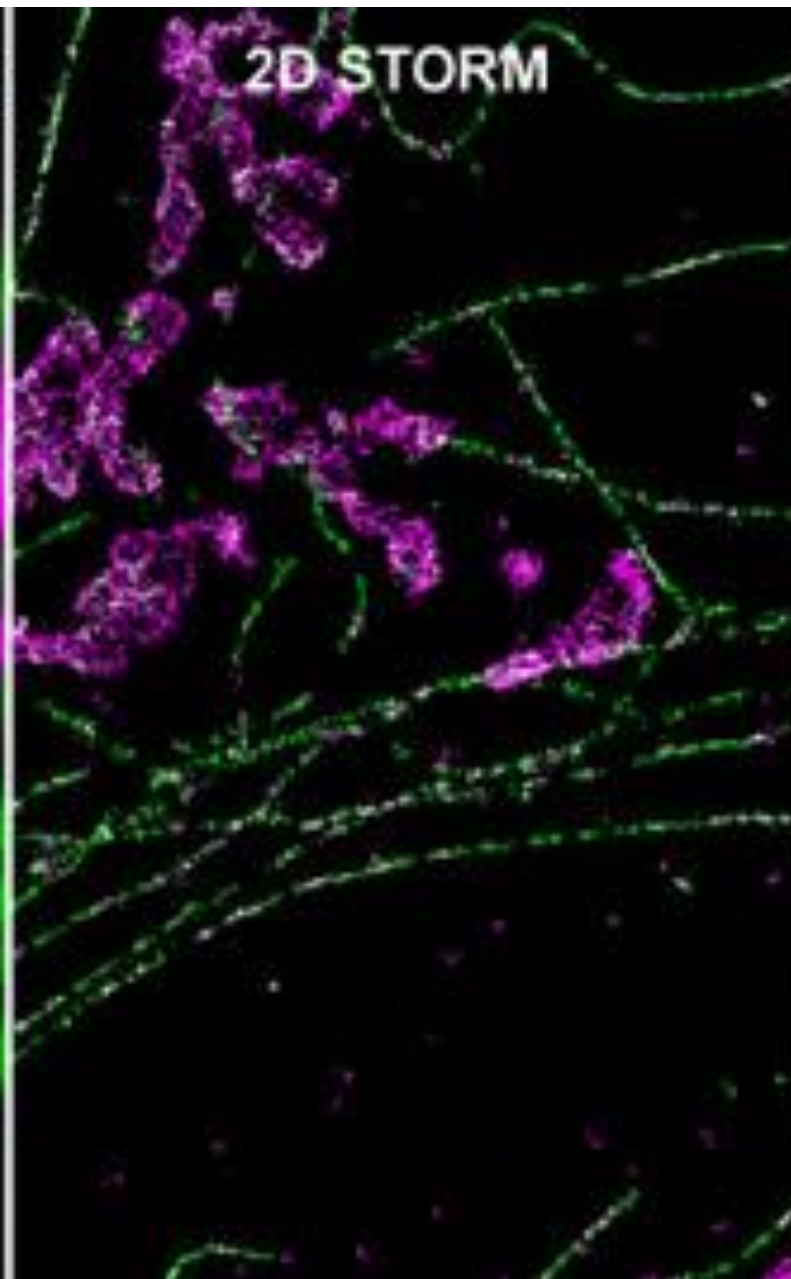
Bi-plane konfigurace



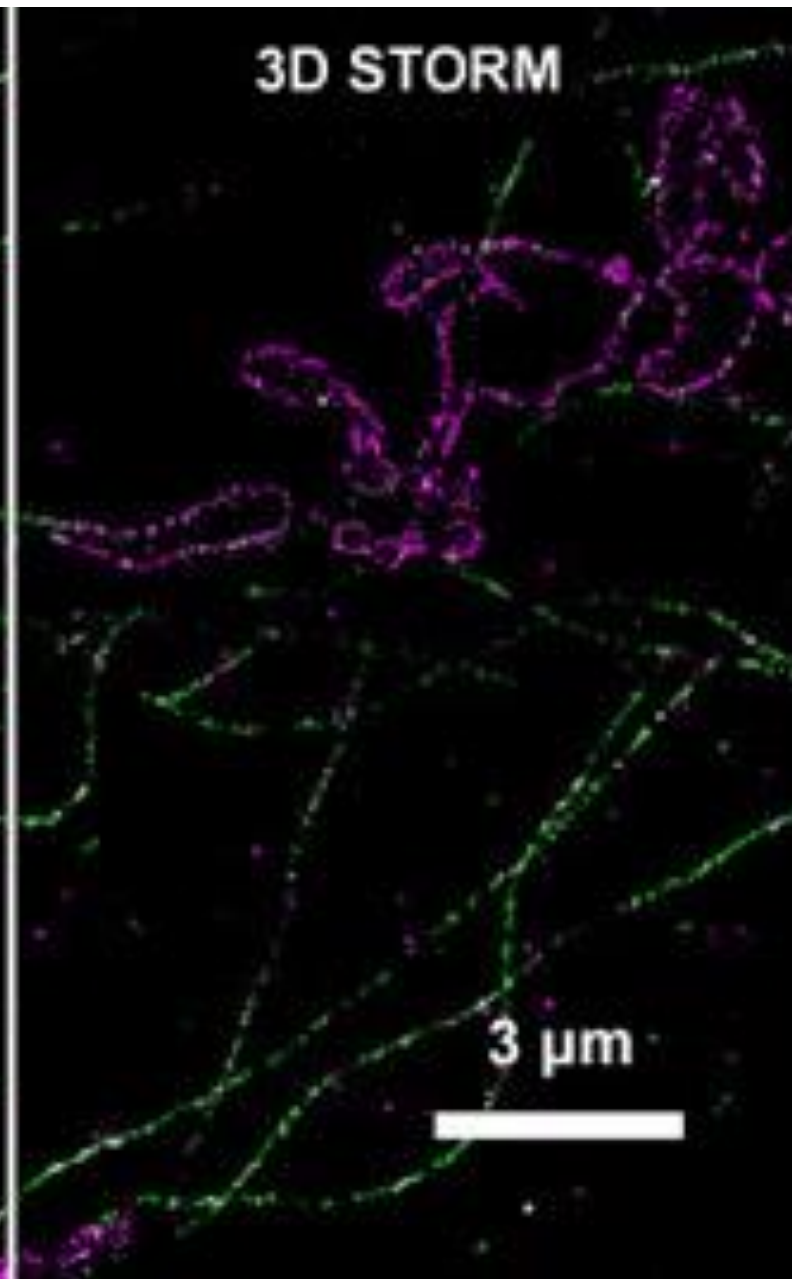
Conventional Fluorescence

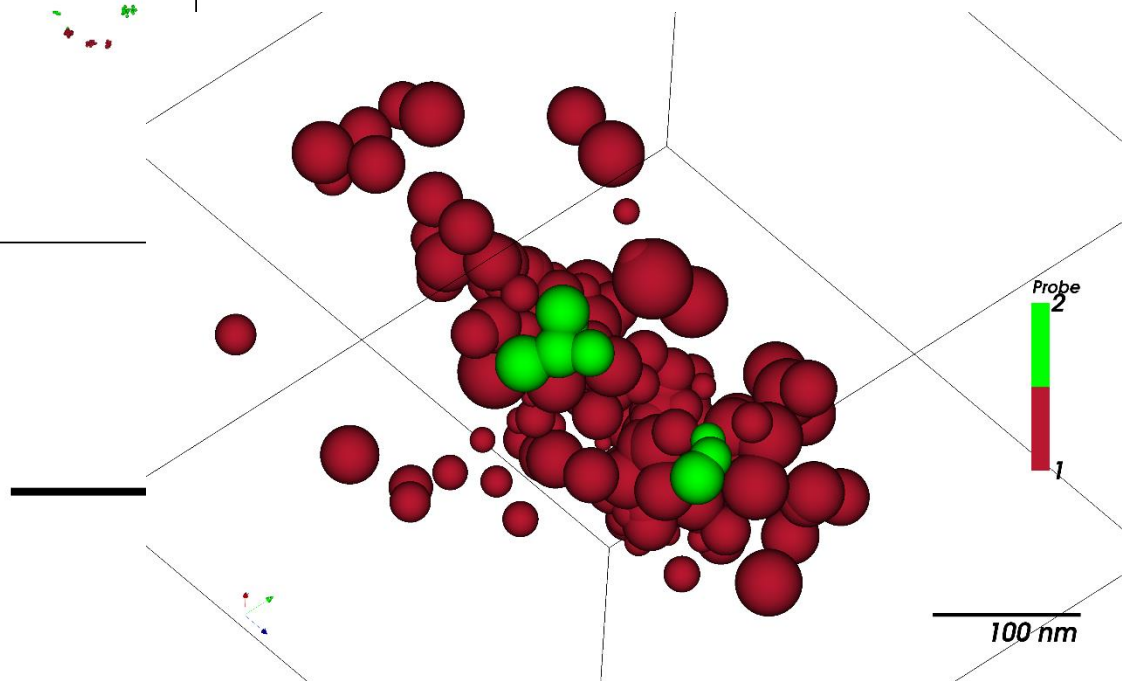
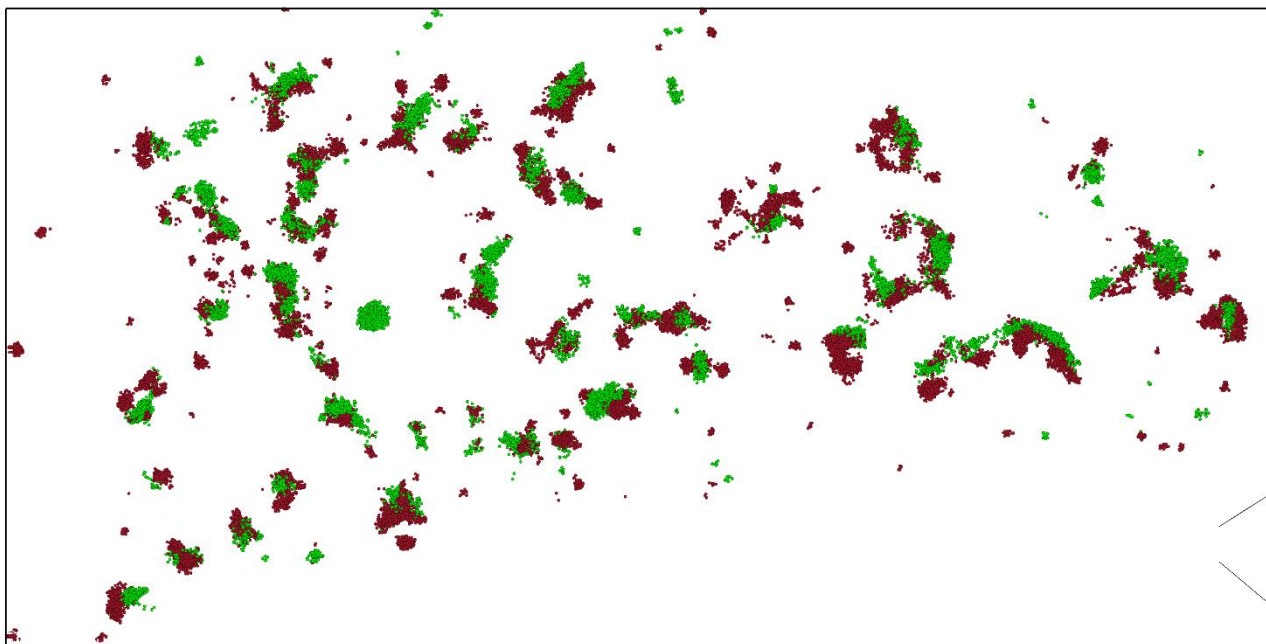


2D STORM



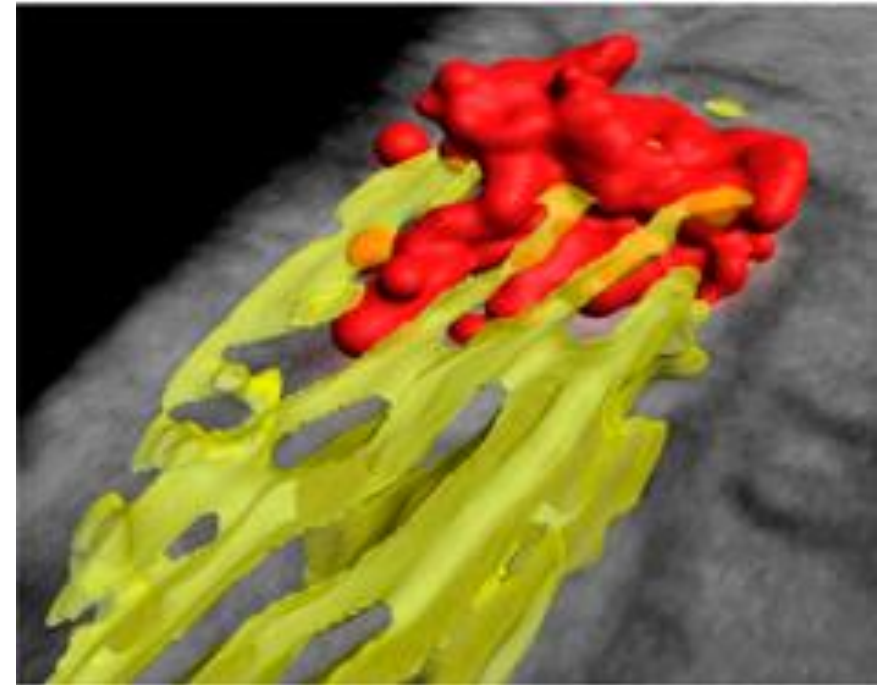
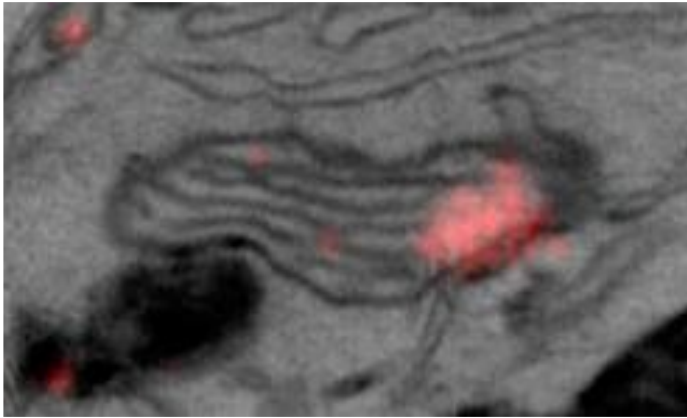
3D STORM





x

Correlative 3D superresolution and electron microscopy



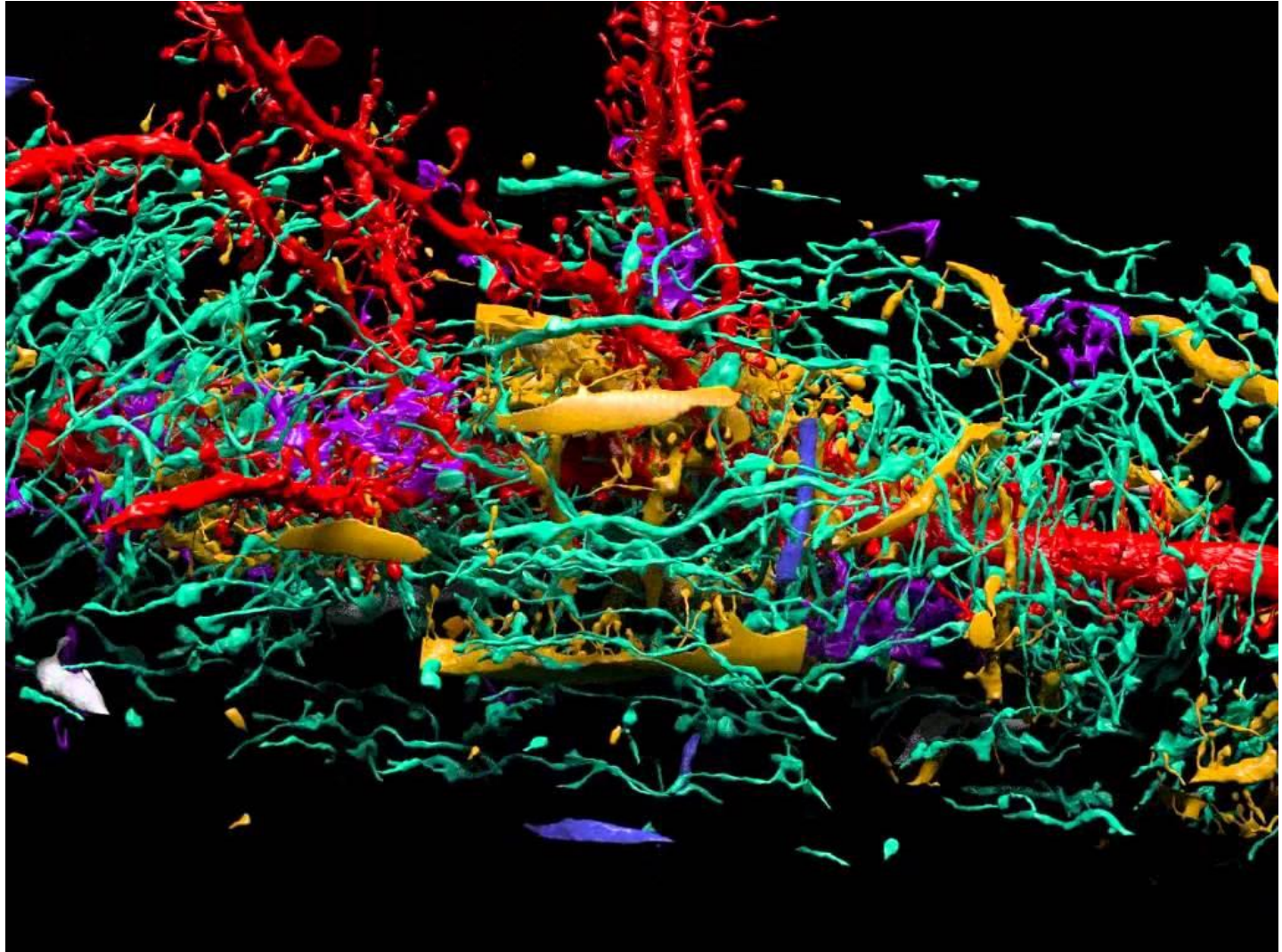
[Correlative 3D superresolution fluorescence and electron microscopy reveal the relationship of mitochondrial nucleoids to membranes.](#) **Kopek** BG, Shtengel G, Xu CS, Clayton DA, Hess HF. *Proc Natl Acad Sci U S A.* 2012 Apr 17;109(16):6136-41. doi: 10.1073/pnas.1121558109.

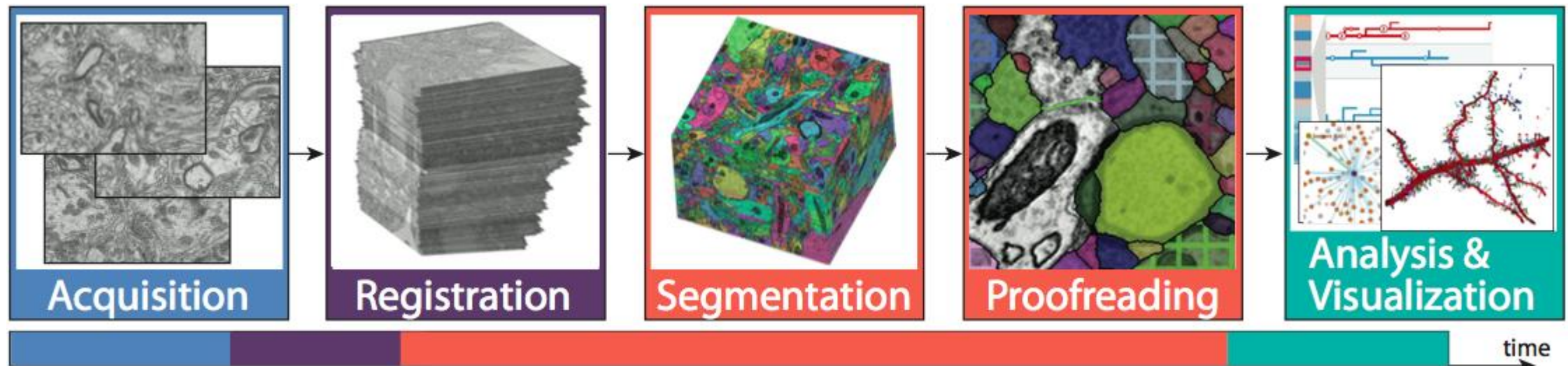
Konektomika – nový příslib



Human brain architecture project

Ještě větší výzva než HUGO





Děkuji za pozornost

Exkurze: konfokální mikroskop + SIM