The Abdus Salam
International Centre
for Theoretical Physics

Principles of Microscopy II: Super-resolution

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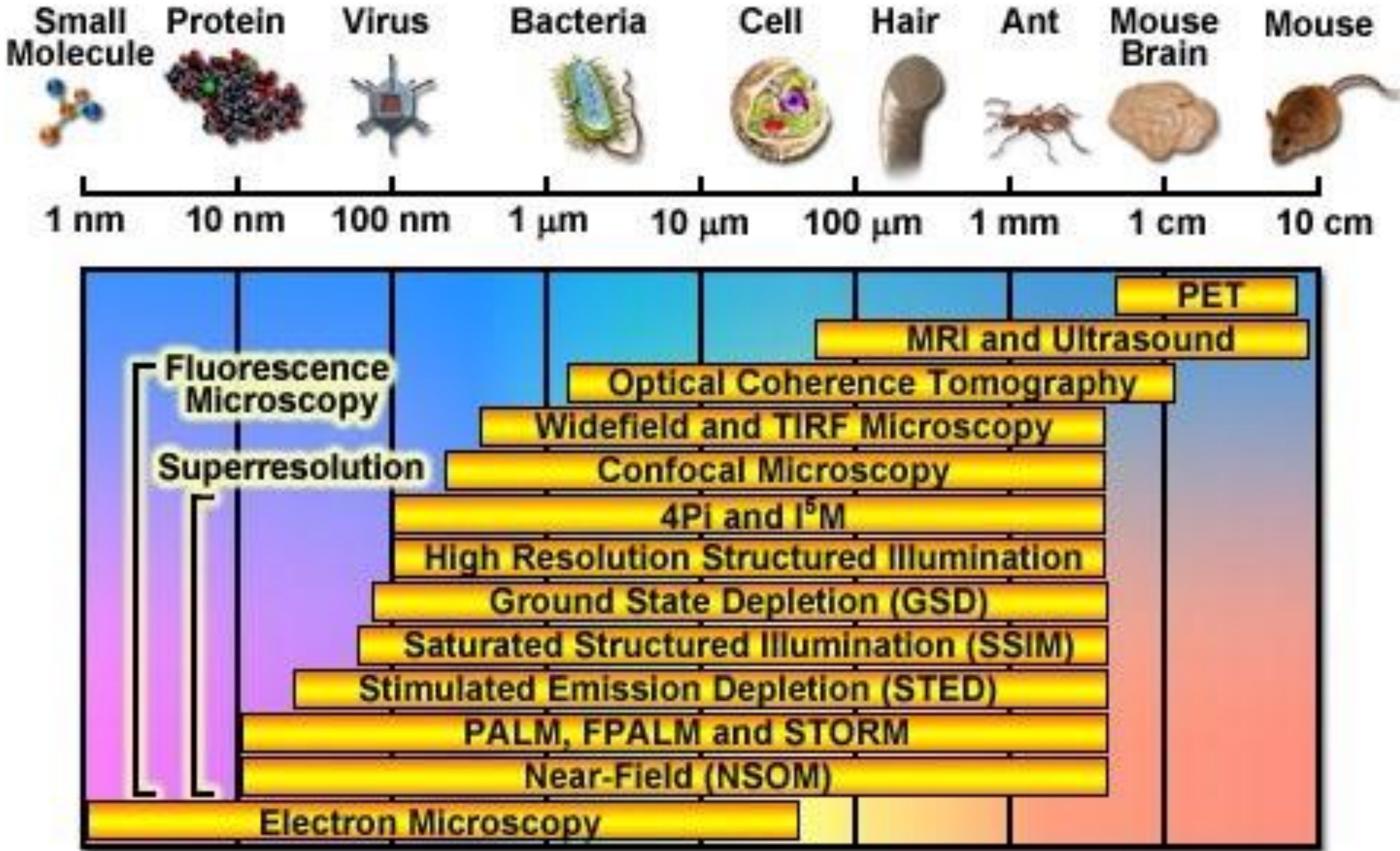
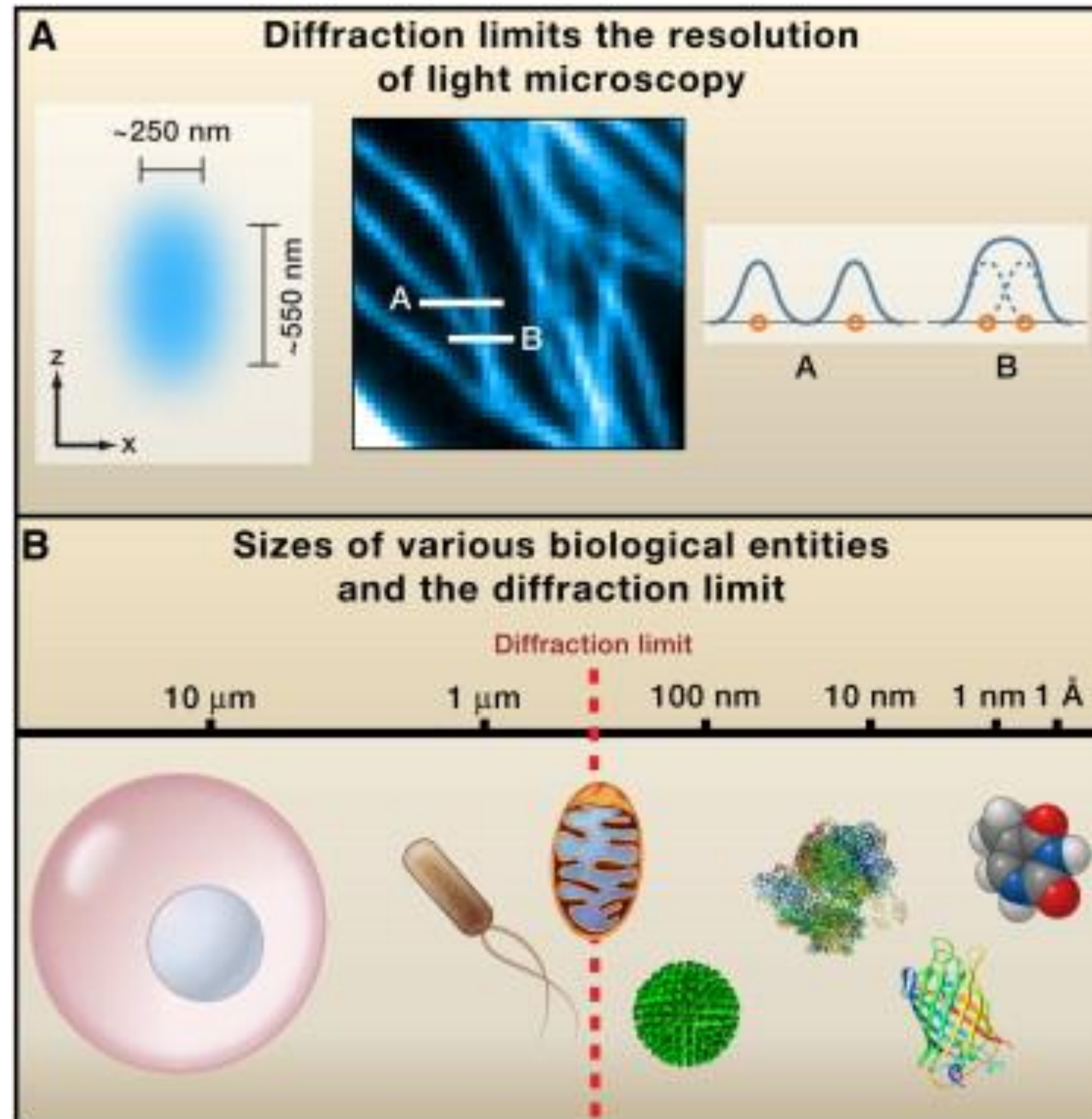
Preparatory School to the Winter College on Optics: Advanced Optical Techniques for Bio-imaging

Diffraction limit of imaging techniques



The diffraction limit

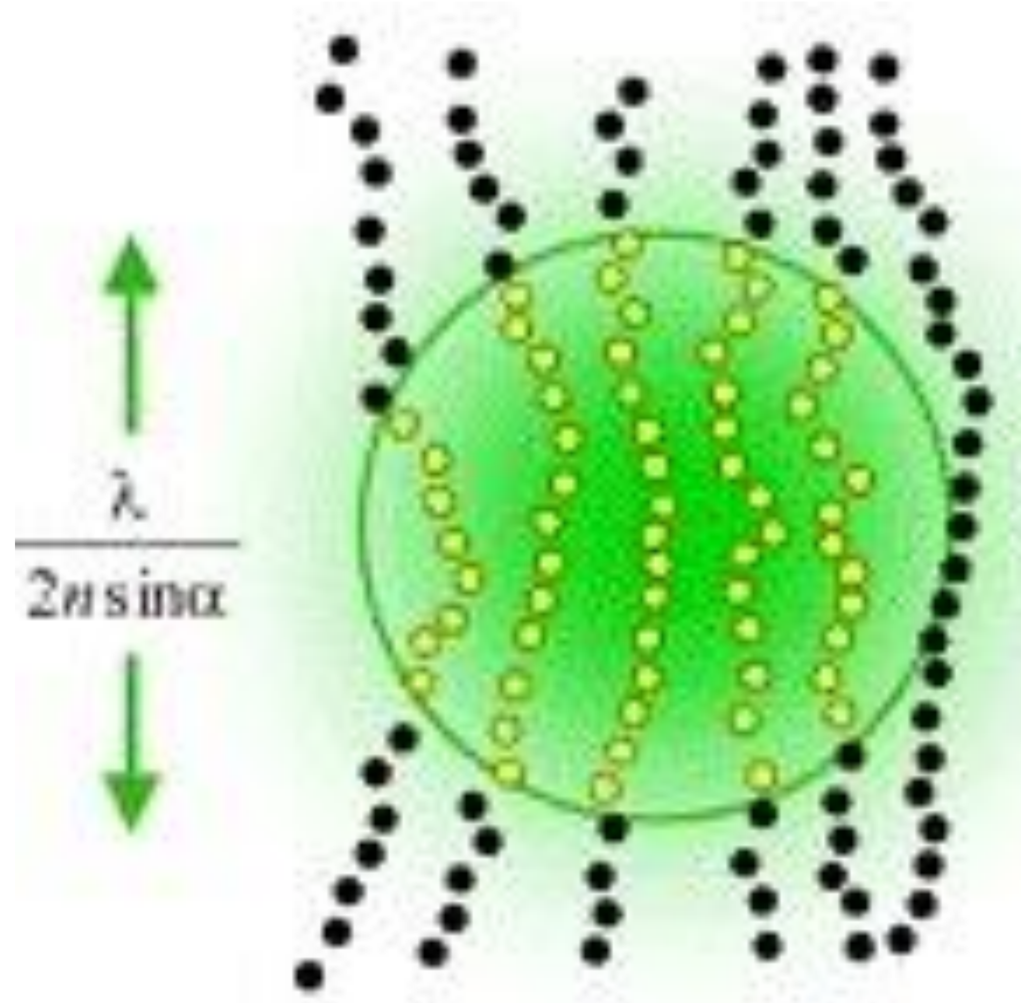


The diffraction limit of light

Ernst Abbe
(1840-1905)

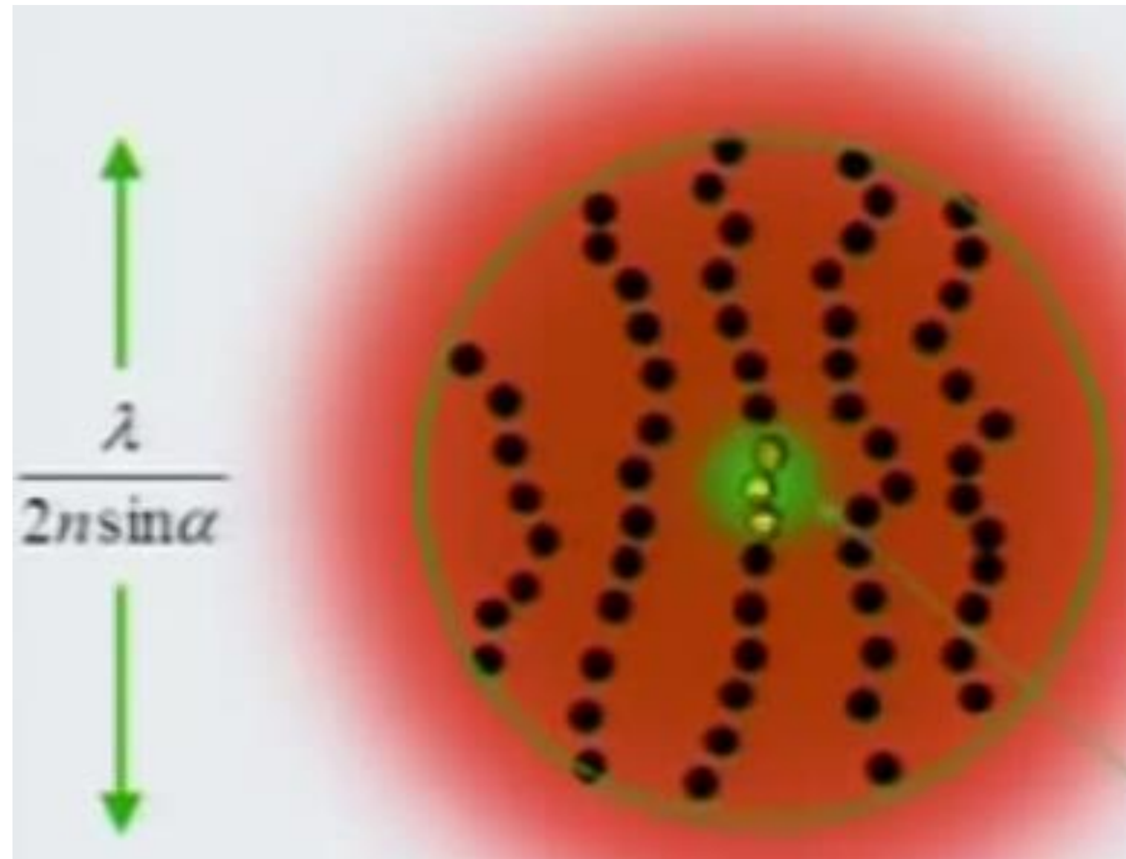
$$d = \frac{\lambda}{2n \sin \alpha}$$

The diffraction limit

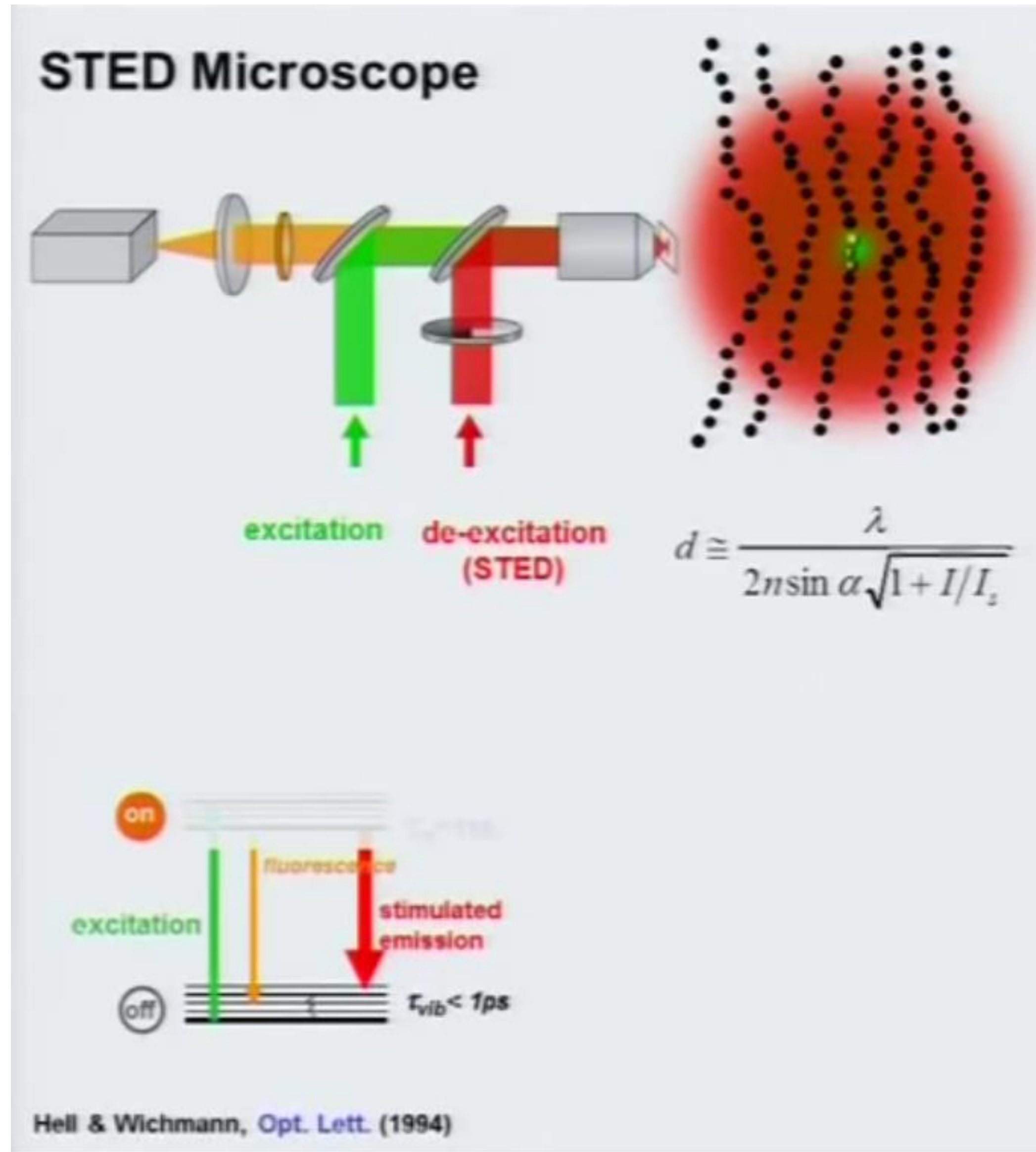


Molecules within the región < 200 nm are not discernible

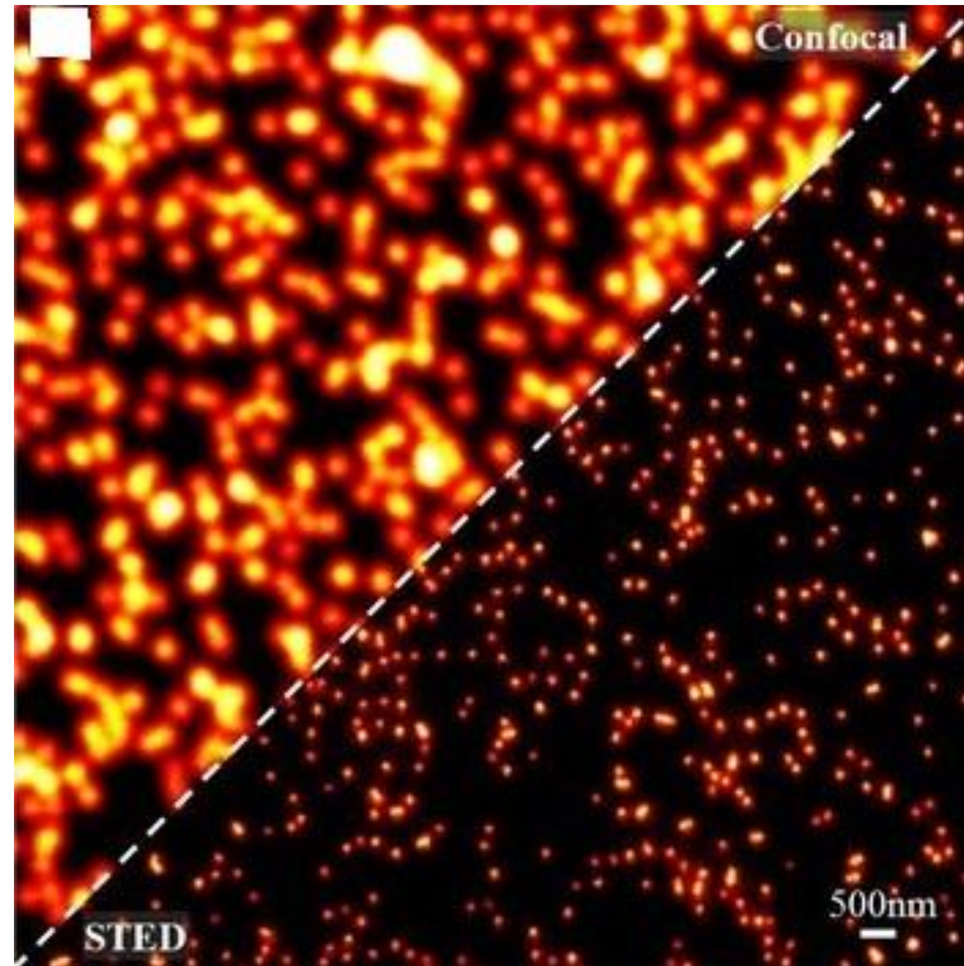
STED microscopy



Experimental setup



Confocal versus STED microscopy



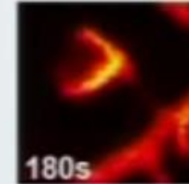
Applications of STED microscopy

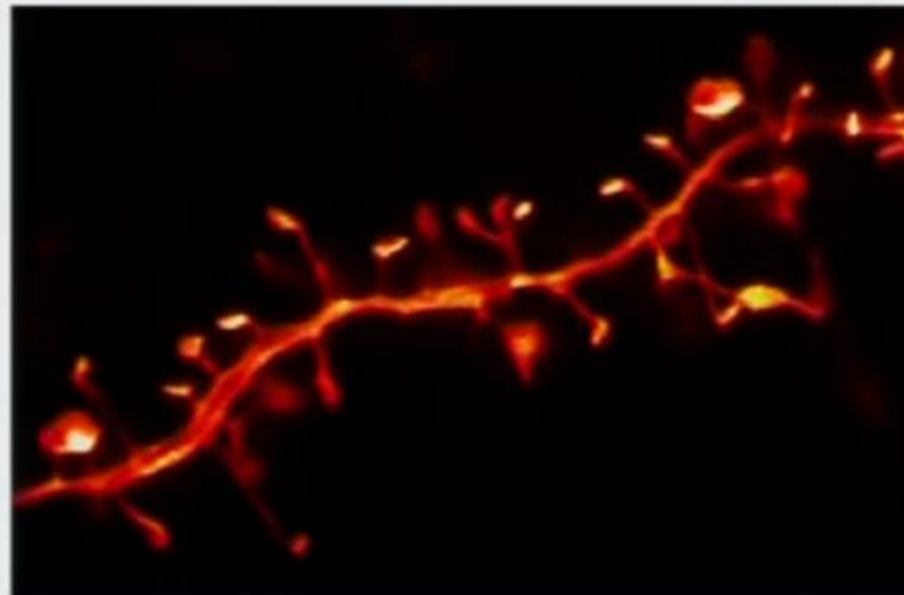
Neurophysiology

Dendritic Spines Living Neuron

Hippocampal organotyp slices

CA1 pyramidal neurons



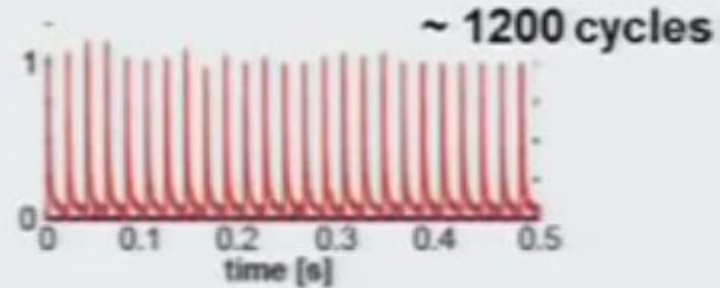


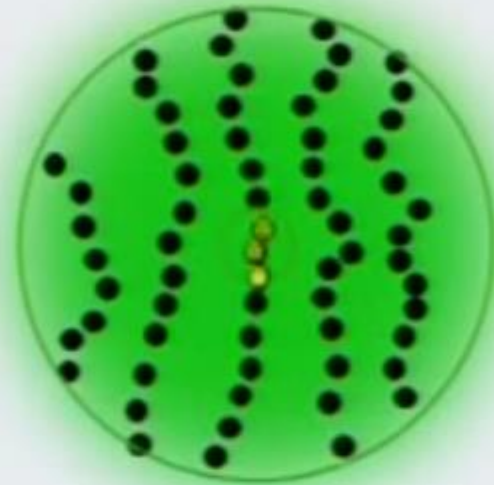
2µm

Urban, Willig, Hell, Nägerl, *Biophys J* (2011)

STED, RESOLFT microscopy

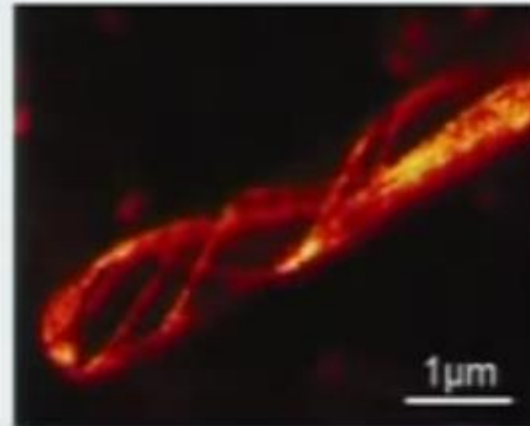
Hell, Jakobs, Kastrup, *Appl Phys A (2003)*
Hell, *Nat Biotech (2003)*
Hofmann et al, *PNAS (2005)*





~ 10^5 times lower
intensity than in STED

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



Grotjohann, Testa et al, *Nature (2011)*
Brakemann *et al*, *Nat Biotech (2011)*

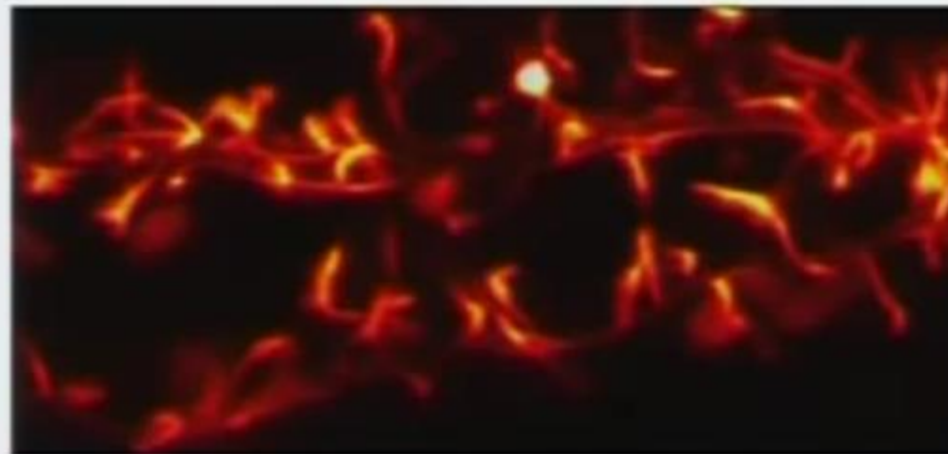
$I_s = 1/(\sigma\tau) \sim$ W/cm^2

STED, RESOLFT microscopy

Hell, Jakobs, Kastrup, *Appl Phys A (2003)*
Hell, *Nat Biotech (2003)*
Hofmann et al, *PNAS (2005)*

2 hours continual scanning, no visible photodamage.

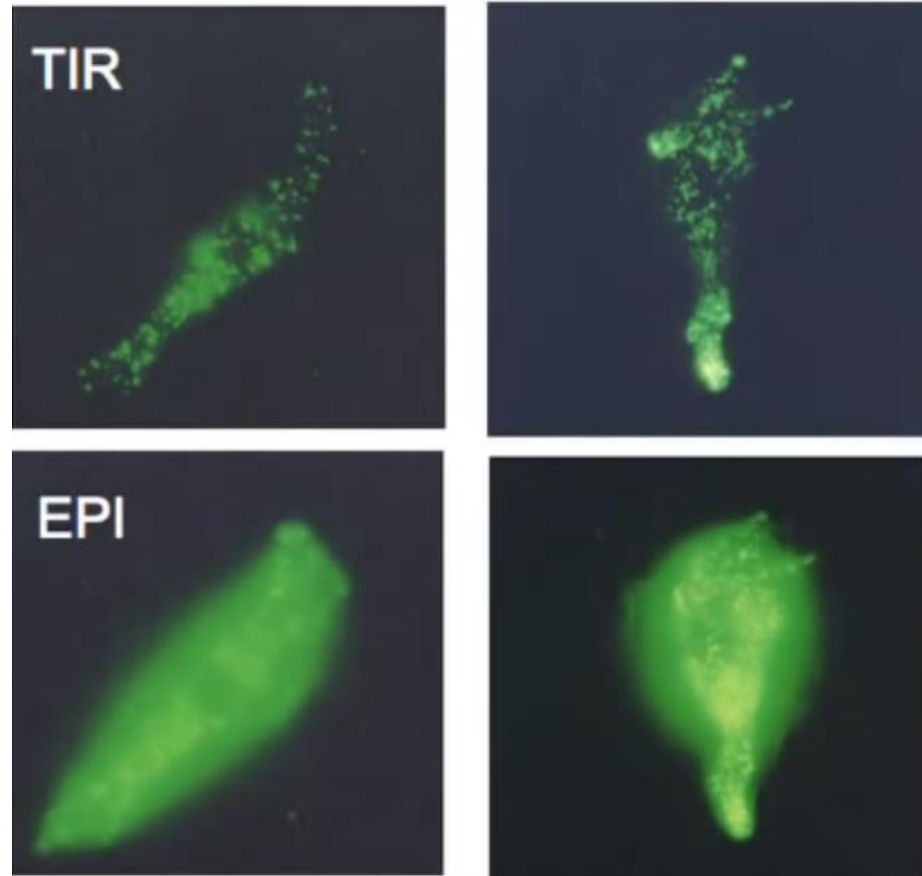
Living neuron, hippocampal organotyp slice, F-Actin



1 µm

Testa, Urban *et al*, Neuron (2012)

Total internal reflection fluorescence (TIRF) microscopy



Principles of TIRF microscopy

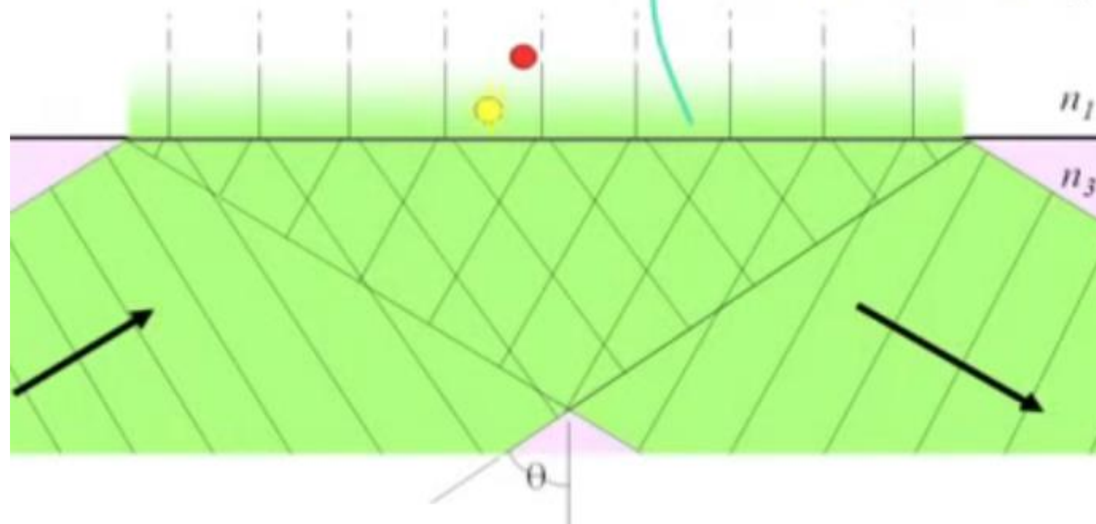
Evanescent field produced by TIR

$$I(z) = I(0)\, e^{-z/d}$$

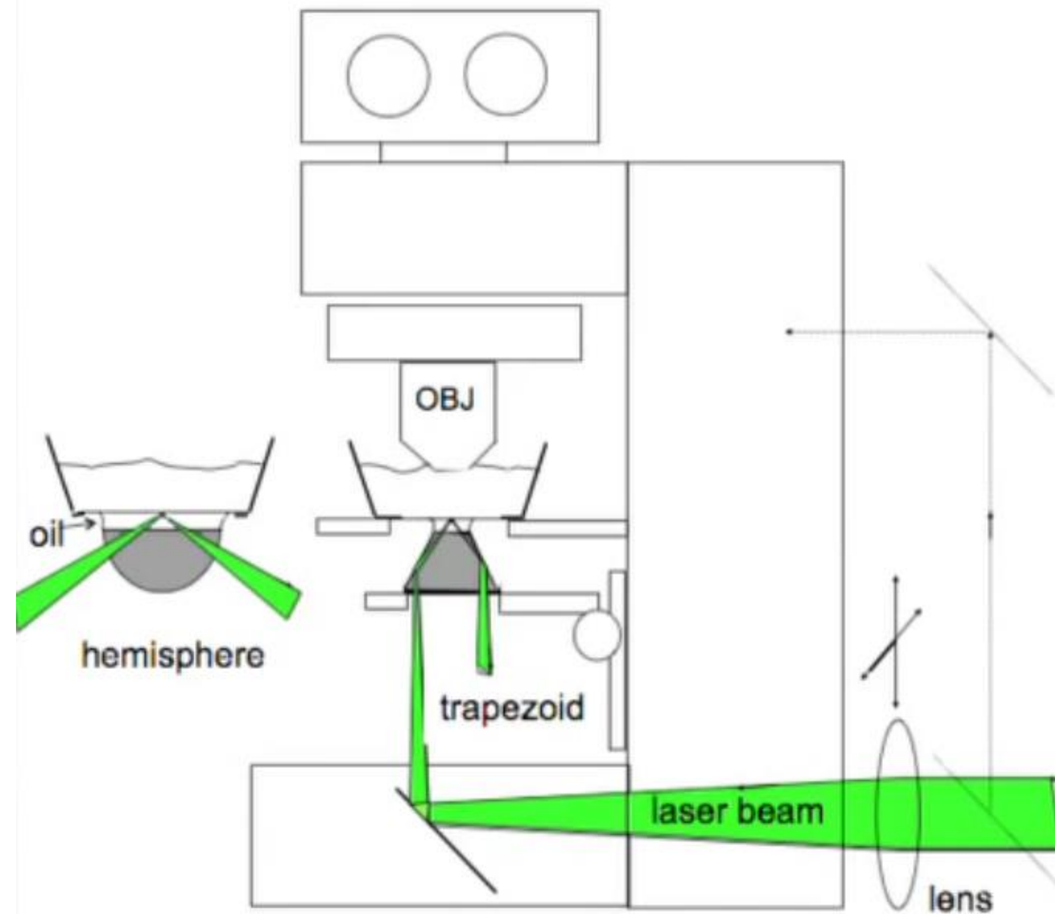
$$d = \frac{\lambda_o}{4\pi}\left(n_3^2 \sin^2\theta - n_1^2\right)^{-1/2}$$

$$d \leq \sim 100\ nm$$

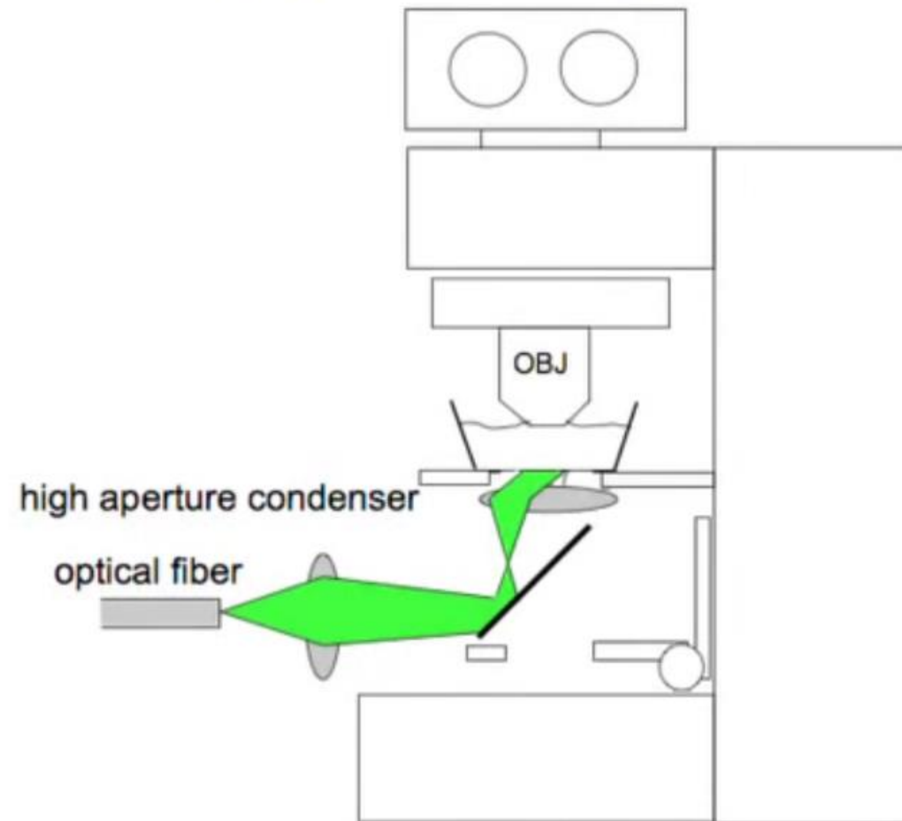
$I(0)$ similar to incident intensity



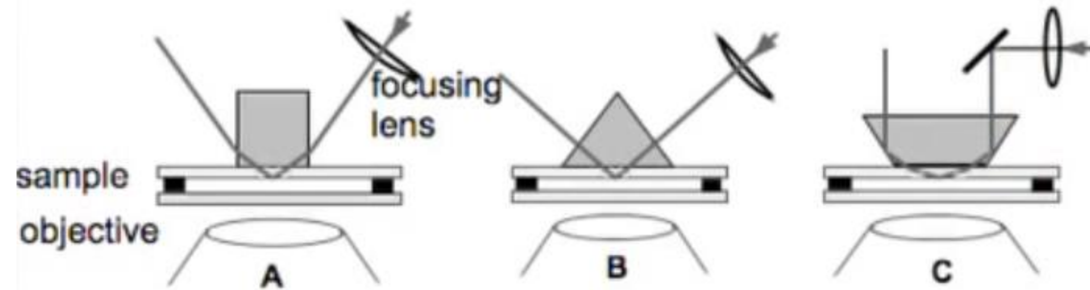
Experimental setup

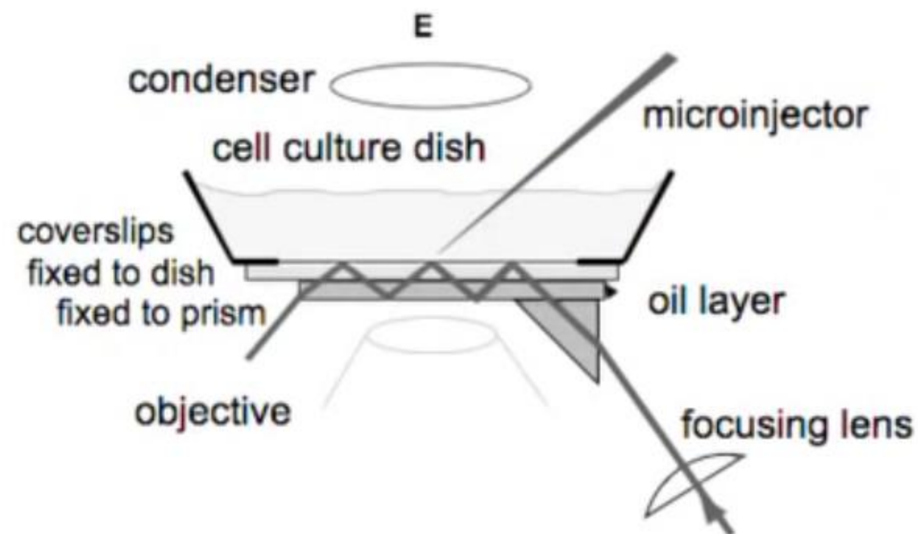


Experimental setup

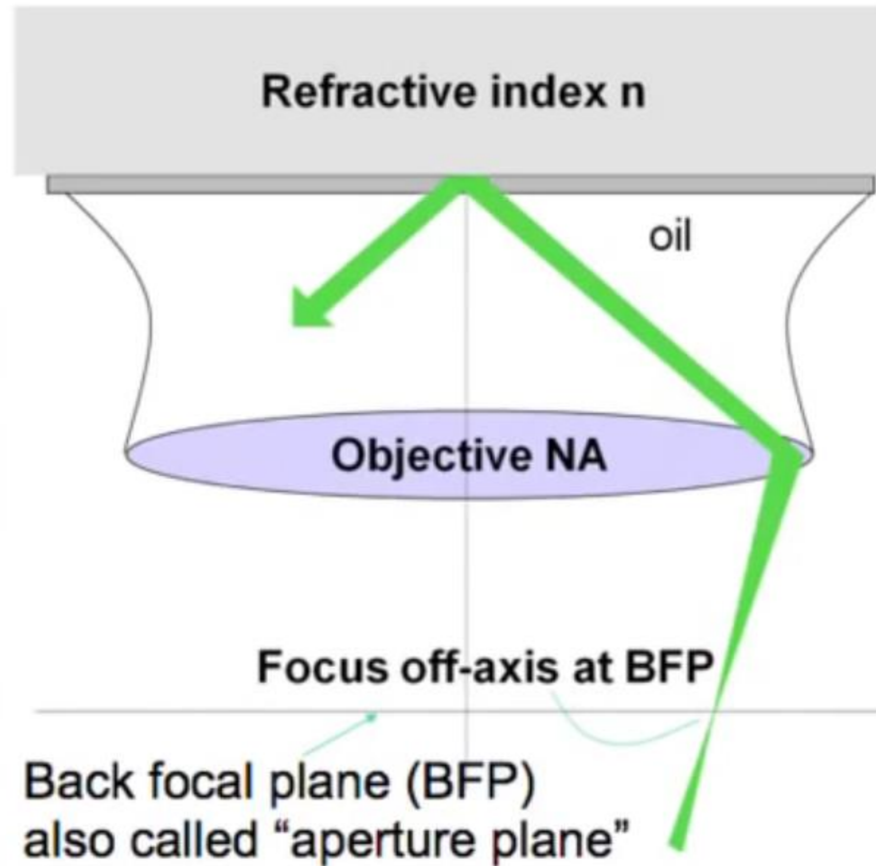


Experimental setup

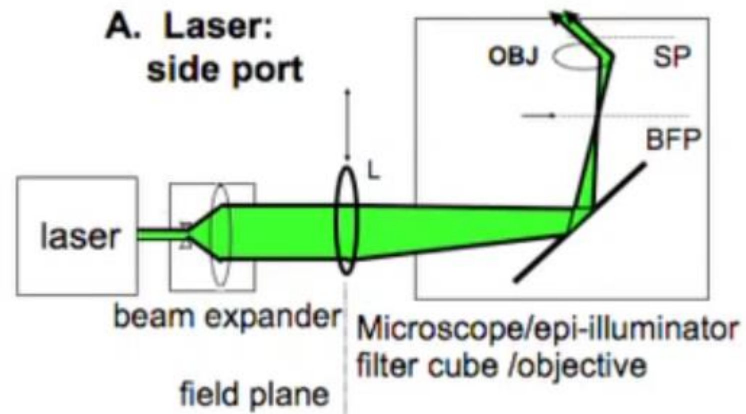


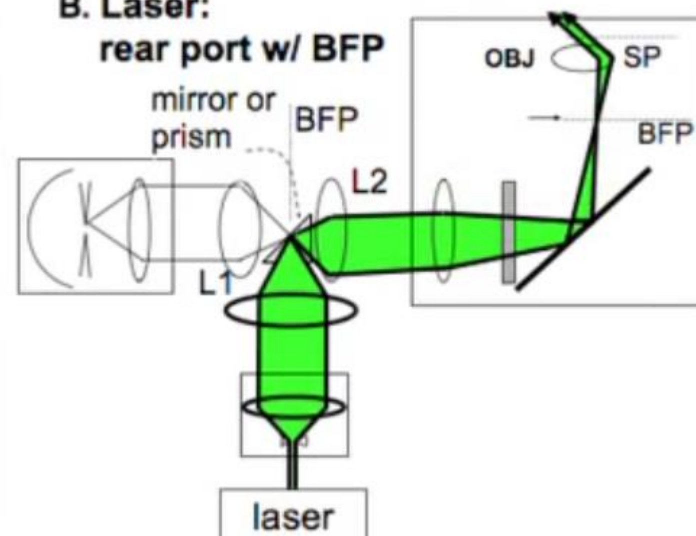


Experimental setup

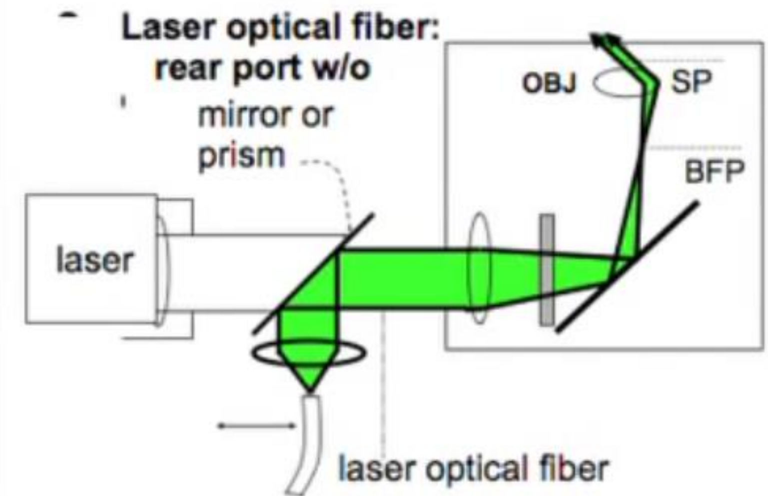


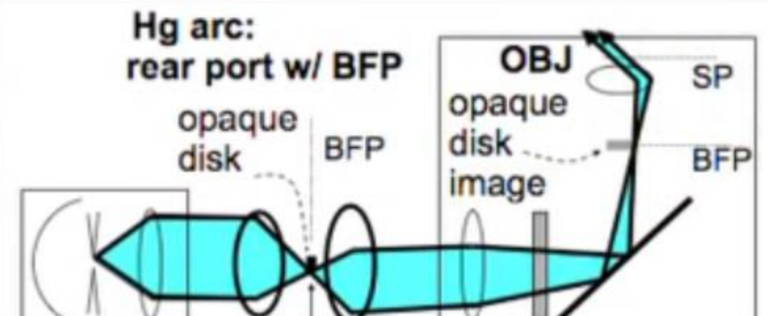
Experimental setup





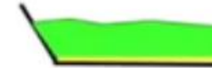
Experimental setup

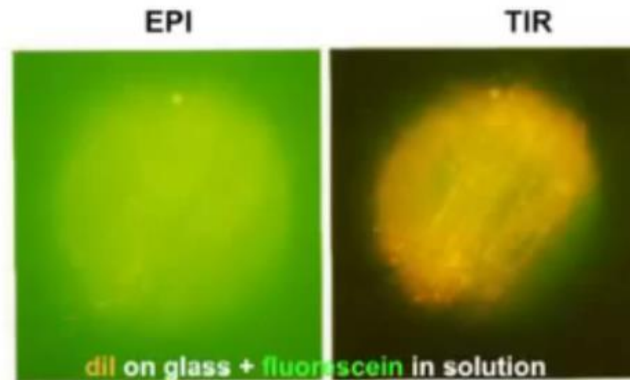


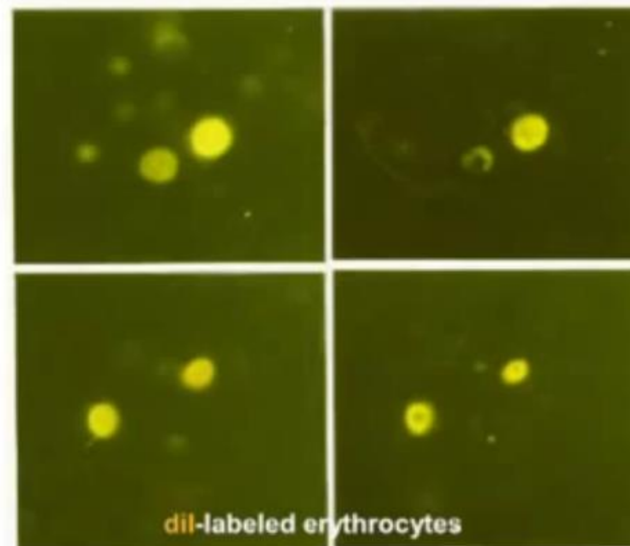


Applications

TIRF with an ARC LAMP source







Summary

TIRFM is useful to study the **cellular dynamics** of:

1. Small random motions of organelles toward or away from the membrane.
2. Submembrane events: exocytosis, cytoskeletal dynamics
3. Submicroscopic membrane folding and indentations
4. Kinetic rates of association/dissociation at the membrane, even in continuous presence of fluorophore in bath.

TIRFM is also useful in **surface biochemistry**:

1. Single molecule fluorescence (low background)
2. Chemical kinetics and diffusion at surfaces

Thanks