



# Far-field optical nanoscopy

## Principles and Applications in the Life Sciences

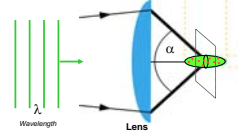


Stefan W. Hell

Max Planck Institute for Biophysical Chemistry  
Department of Nanobiophotonics  
Göttingen



### The diffraction limit



$$\Delta x = \frac{\lambda}{2n \sin \alpha}$$

Wavelength  $\lambda$

Lens

500 nm


200 nm

Vendor (1860)  
Abbe (1873)  
Reinhold (1916)  
Rayleigh (1974)



### The Philosophy:

"... the resolution limiting effect of diffraction can be significantly overcome (...) by fully exploiting the properties of the fluorophores. Combined with modern quantum optical techniques the scanning (confocal) microscope has the potential of dramatically improving the resolution in far-field light microscopy."

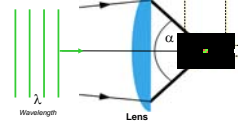


Near-field

Sprague, 1928  
Pohl, Denk, Levins 1984

### "Breaking the diffraction resolution limit by stimulated emission"

Hell & Wichmann, Opt. Lett., 10, 11 (1984)



$$\Delta x = \frac{\lambda}{2n \sin \alpha}$$

Wavelength  $\lambda$

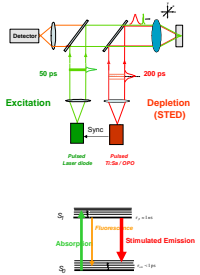
Lens

500 nm

200 nm

Solution: transiently switch off the fluorophores!

### STED microscope



Excitation

Depletion (STED)

50 ps

200 ps

Fluorescence

time [s]

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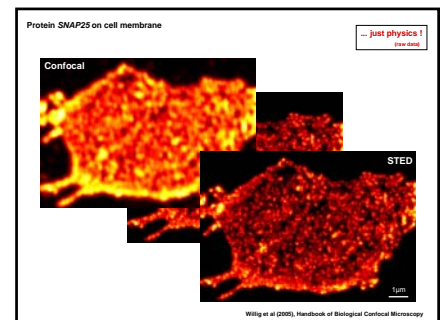
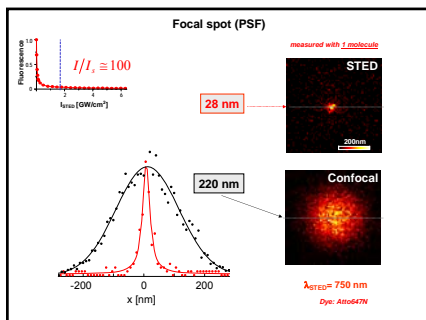
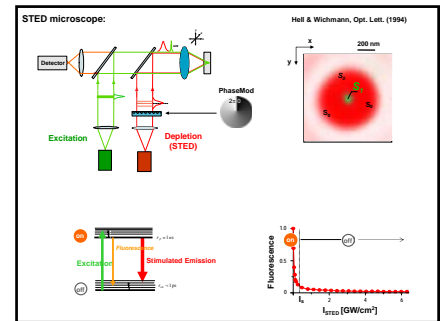
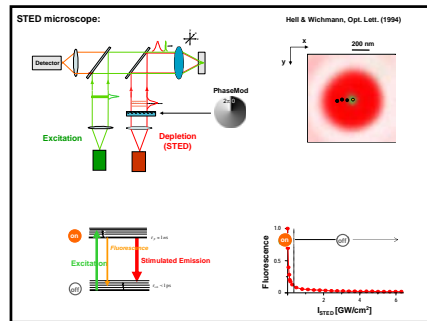
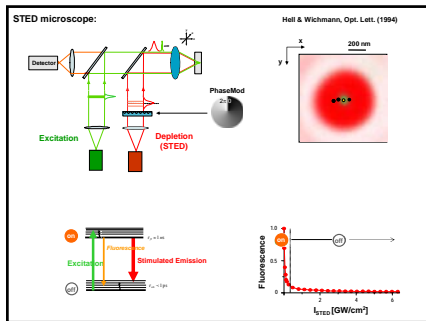
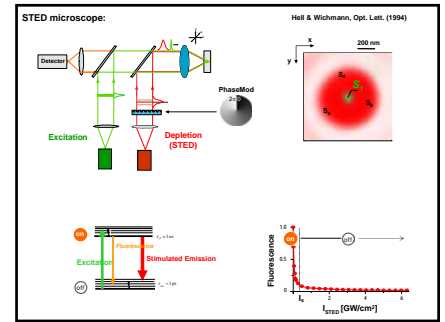
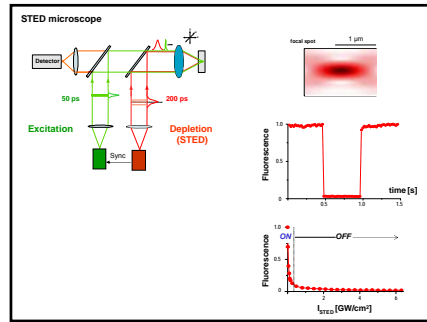
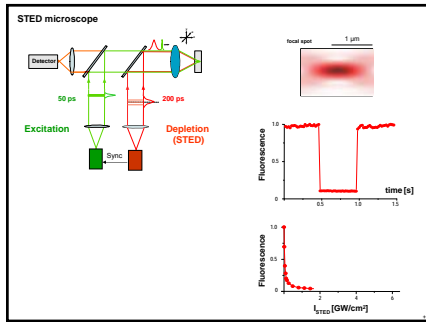
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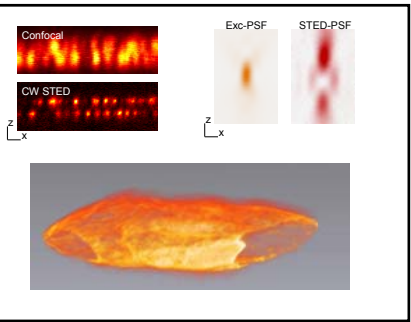
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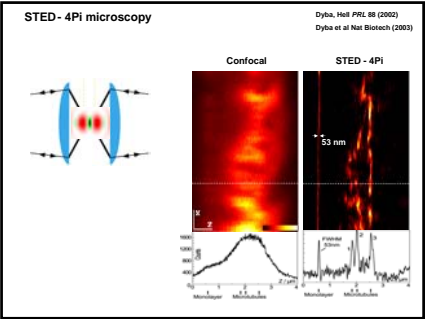
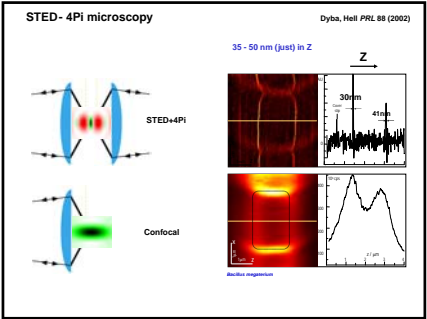
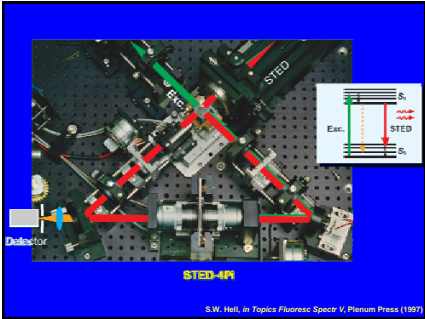




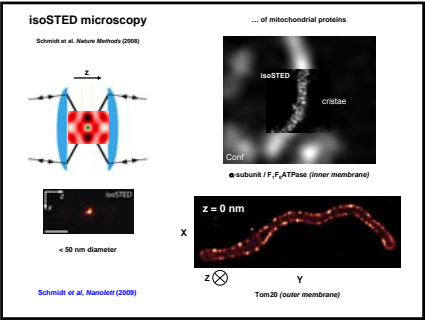
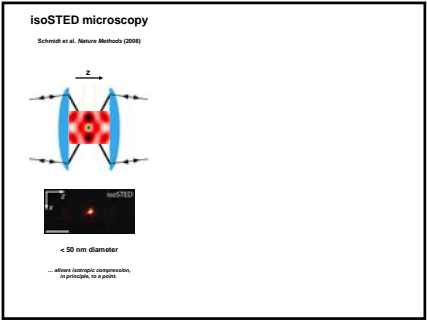
Z- resolution improvement



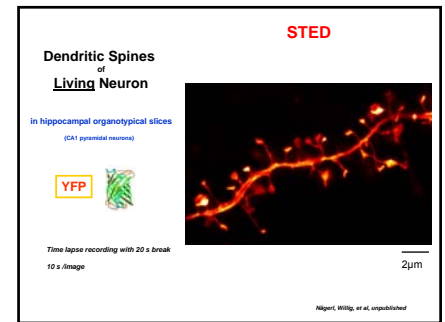
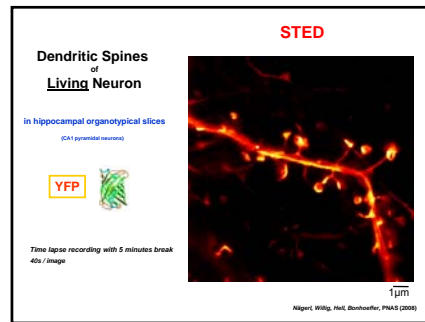
Axial resolution improvement  
by  
STED - 4Pi microscopy



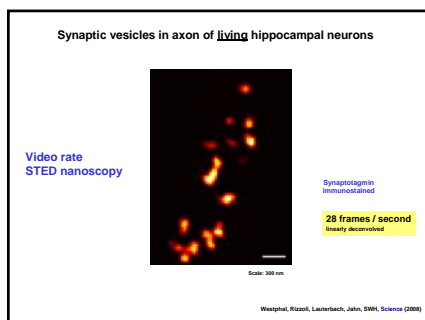
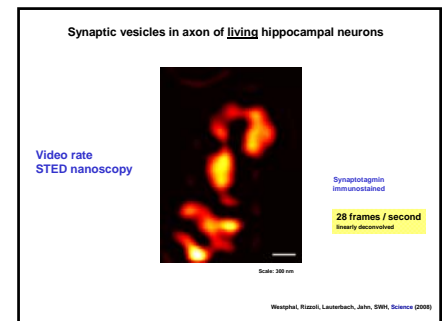
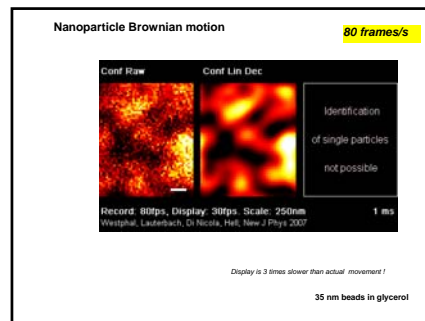
X, Y, and Z



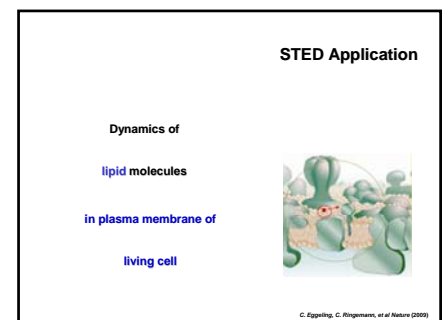
## Recording dynamics

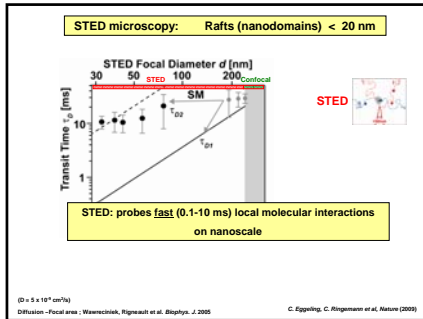
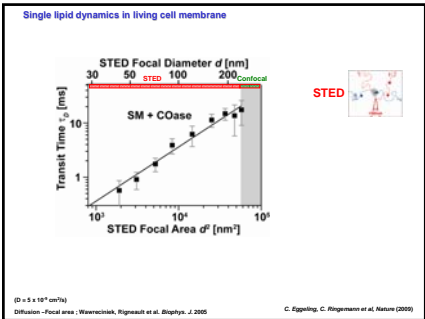
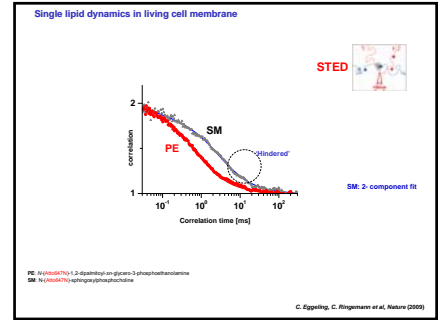
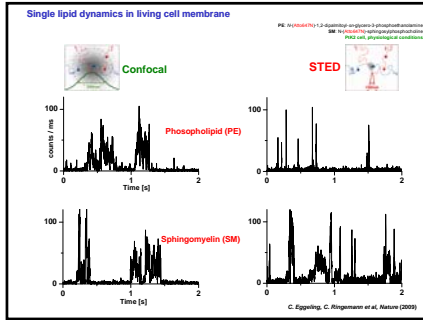
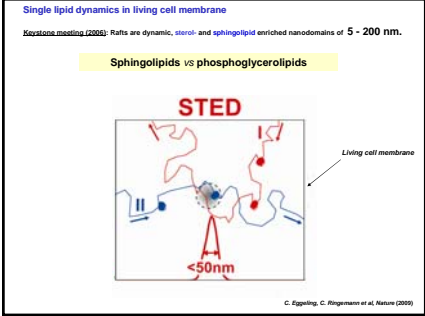


## Faster dynamics ...

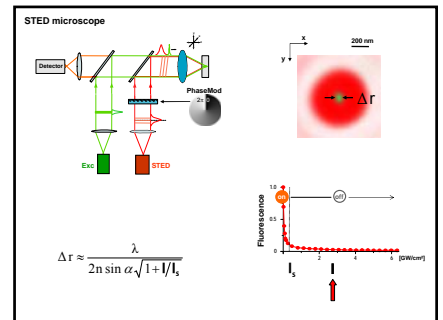
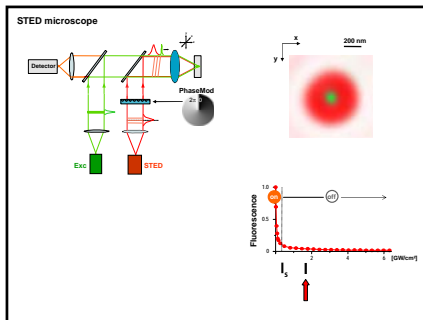
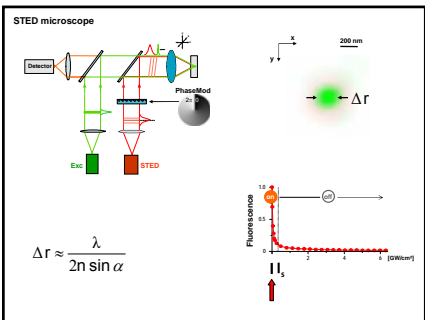


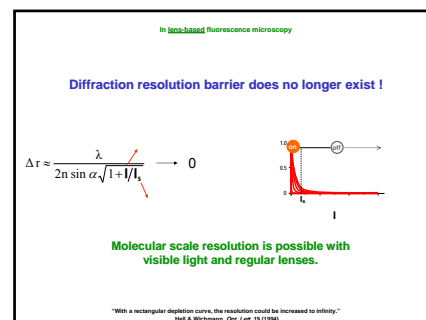
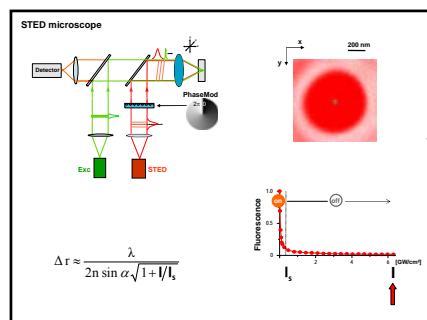
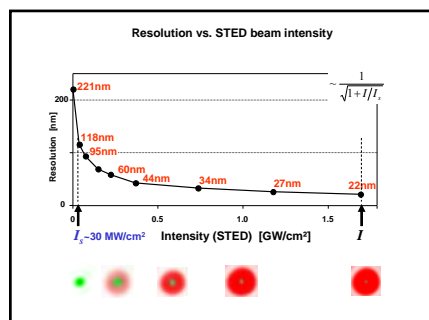
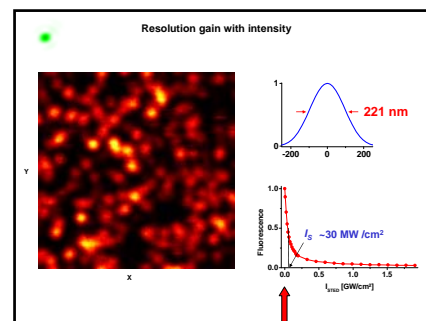
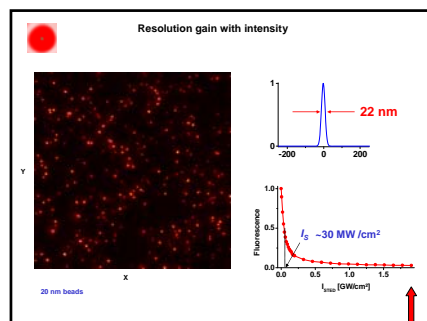
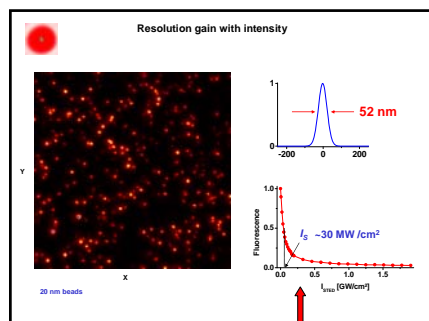
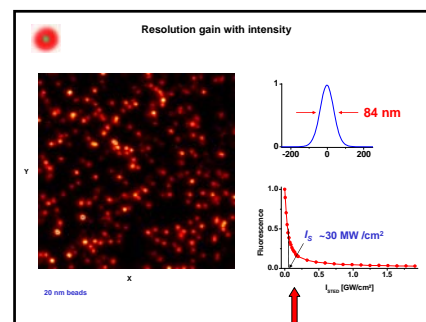
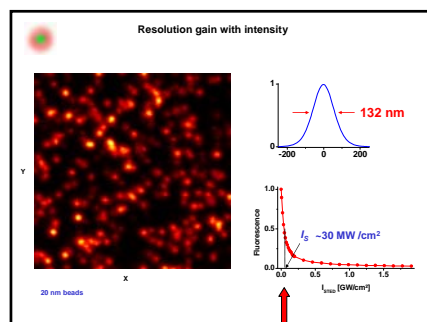
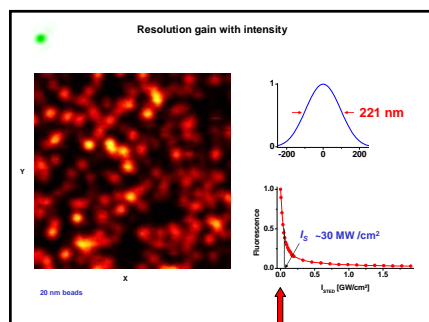
## Even faster (molecular) dynamics ...

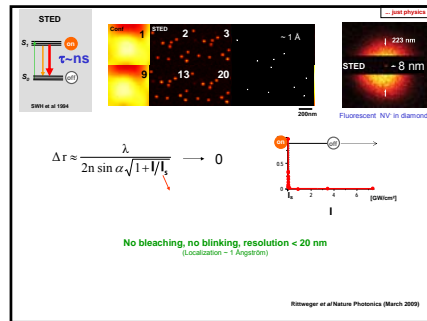
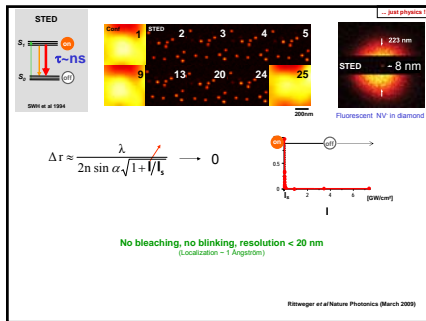
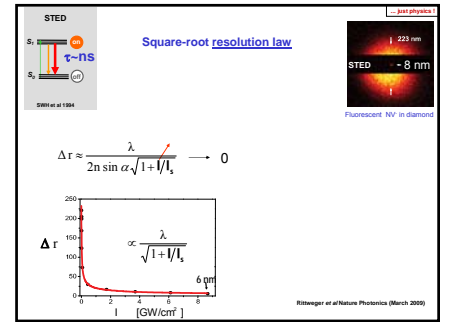
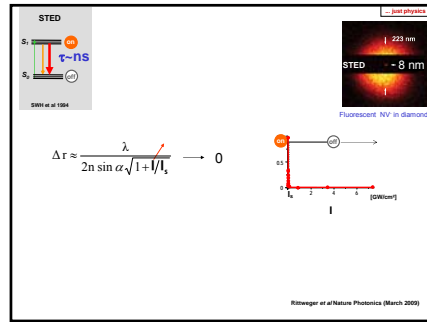
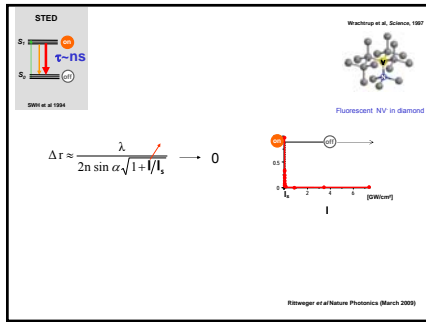




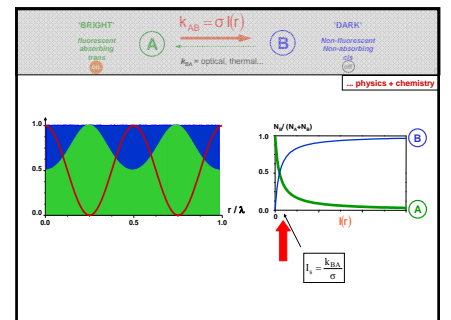
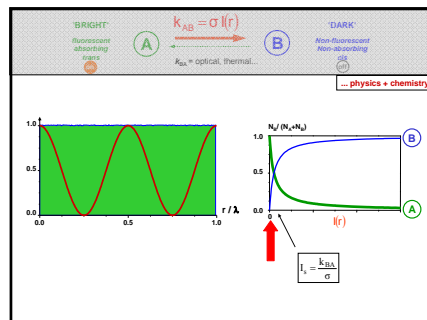
Tuning the resolution



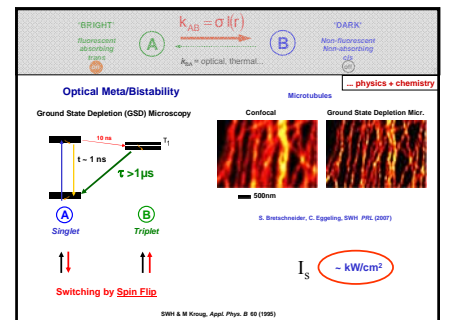
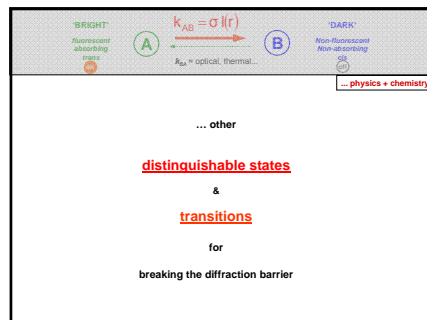
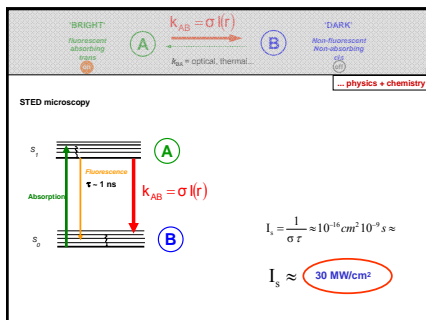
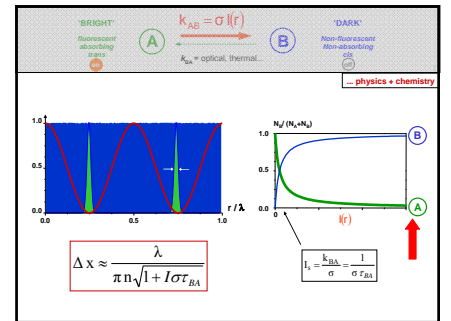
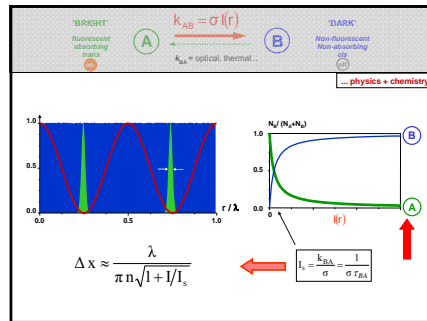
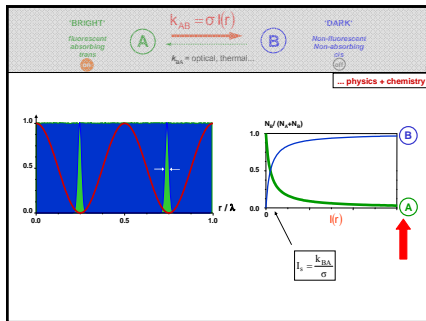
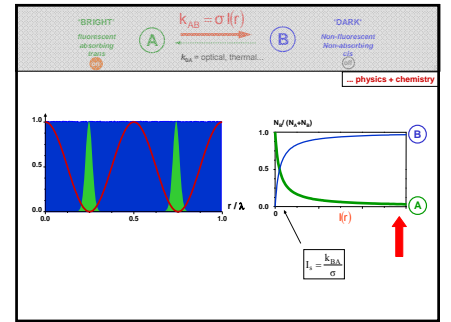
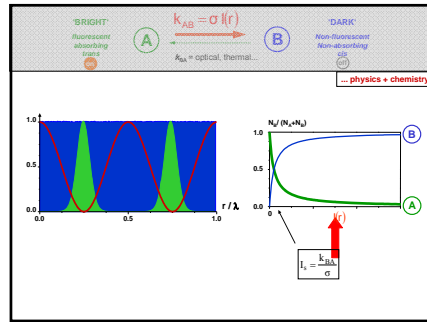
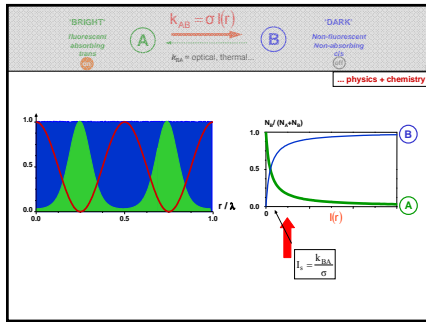


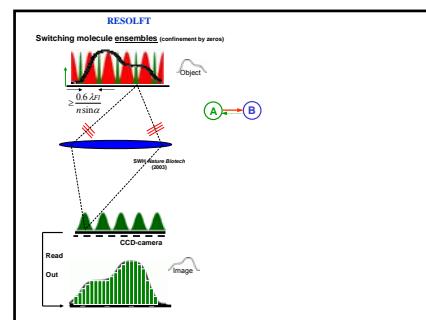
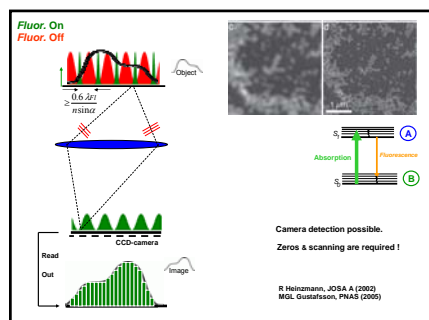
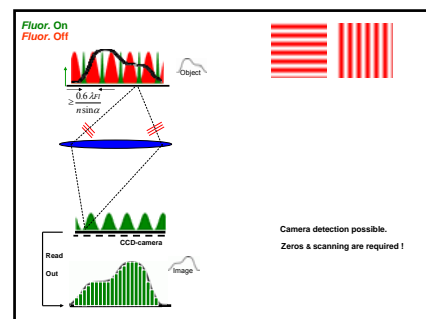
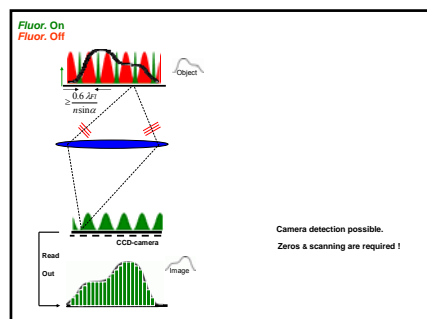
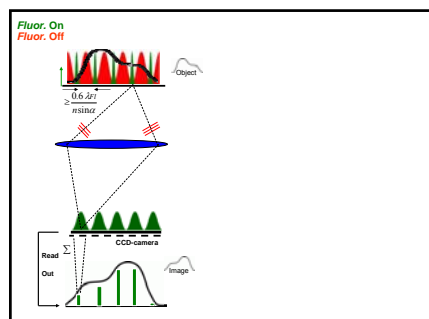
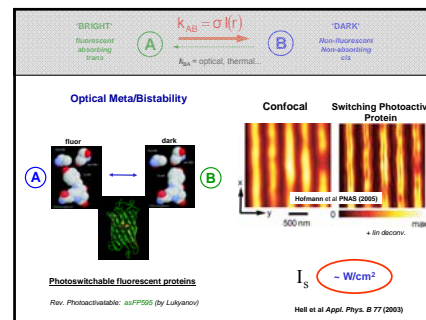
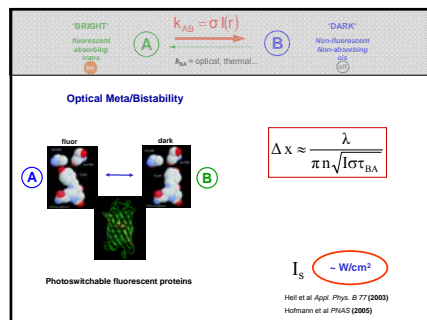
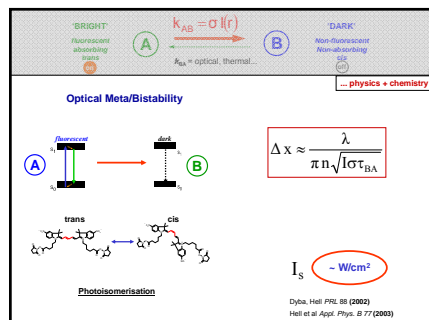


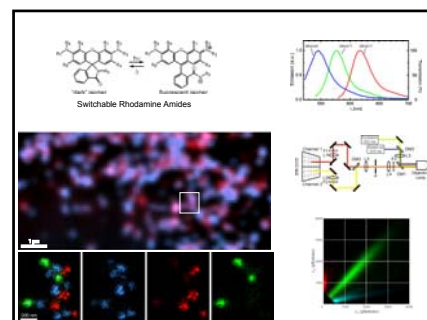
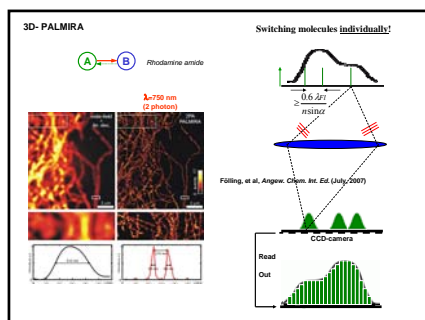
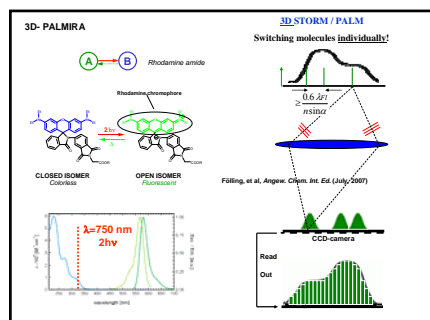
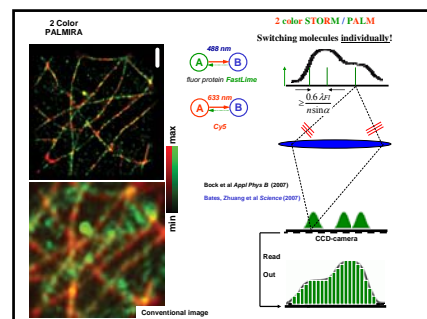
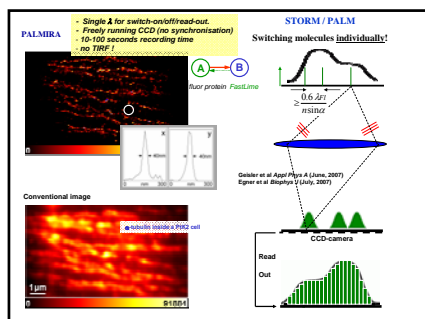
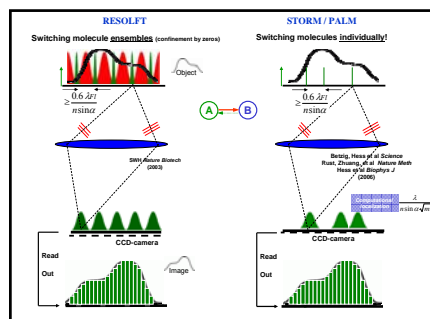
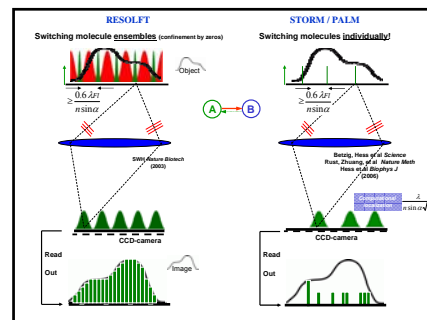
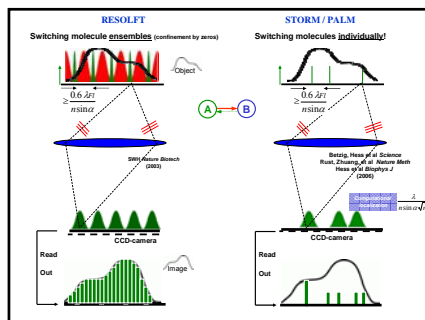
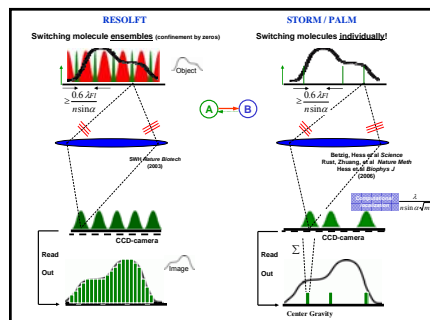
Basic idea behind  
STED etc. ....



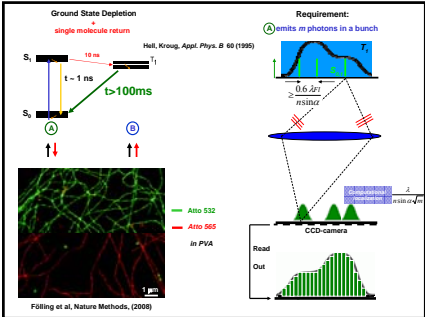
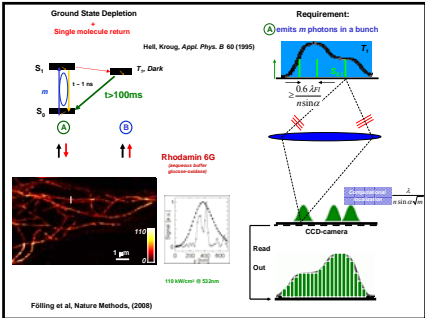
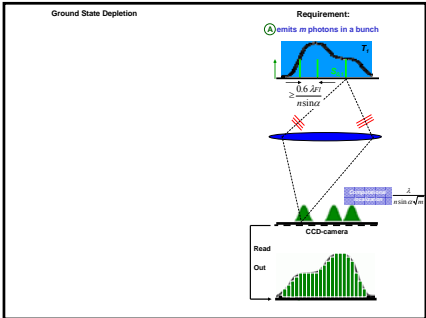








Is photoactivation (= active switching on) really required  
as the name PALM suggests ?



What's THE enabling element  
in  
current nanoscopy schemes?

