Correlated Imaging and Analysis of Single Cells: A Biophysical Perspective on Advanced Fluorescence Microscopy

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PIC2 – projeto integrador de 2º ciclo

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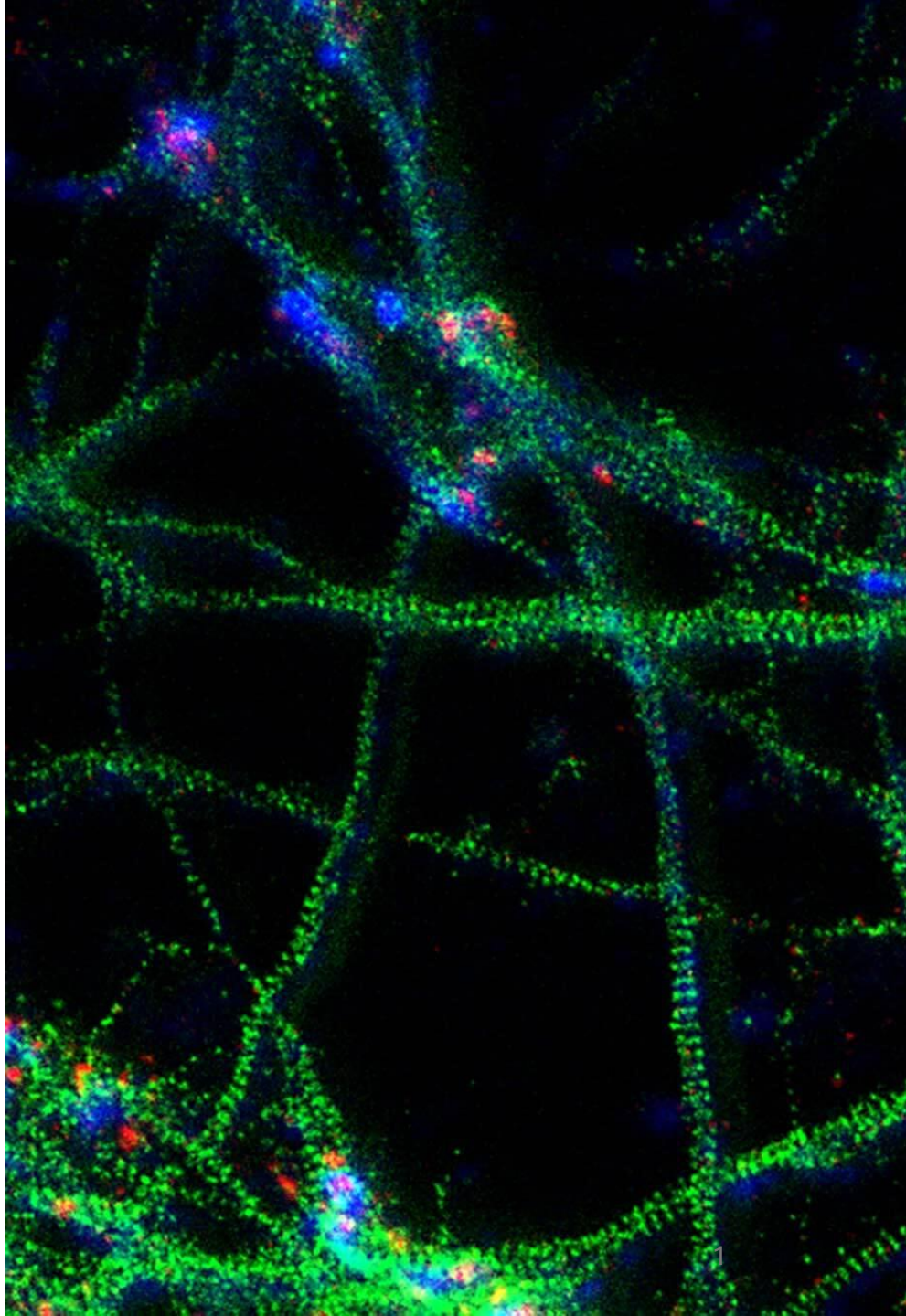
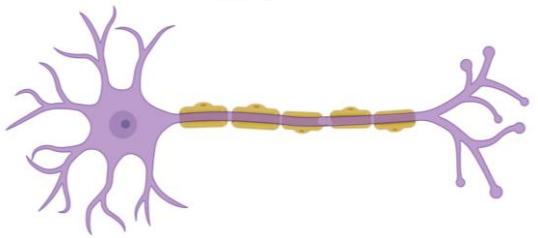


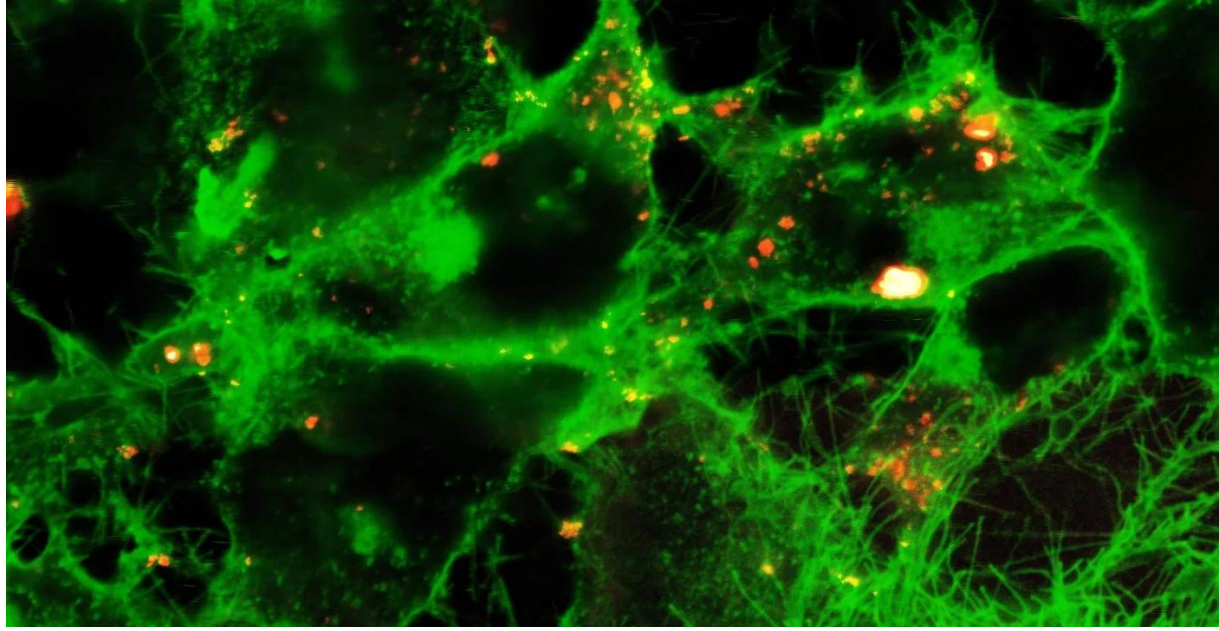
Dynamic cell imaging

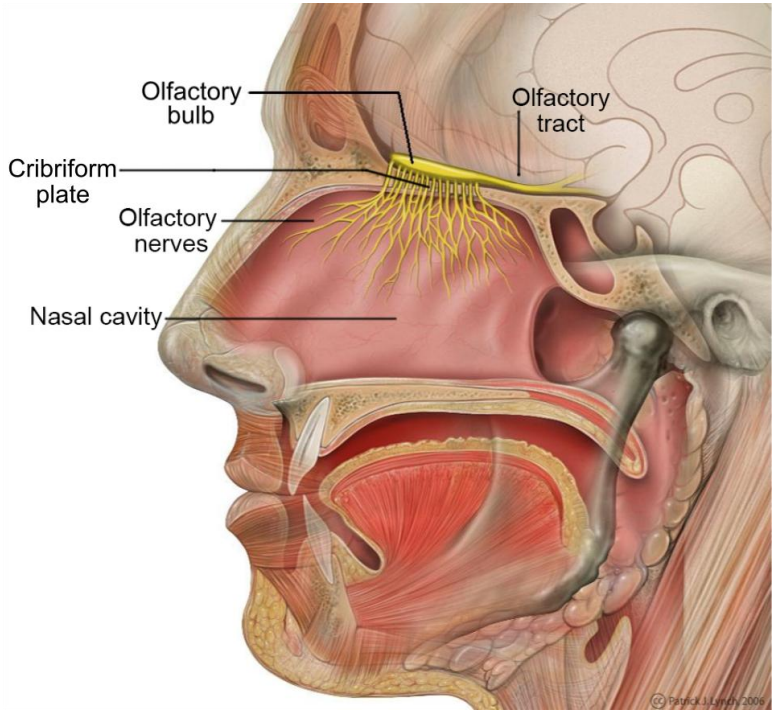
- Biological timescales and speeds:
  - Fruit fly from fertilized egg to segmented embryo – 10 hours
  - E. coli division time – 30 min
  - Bacterium movement – 1 s per its length
  - Axonal and vesicle transport – 1 μm/s
- Imaging speed:
  - 1 pixel – 10 ns
  - 2D: 1000 x 1000 pixels – 10 ms
  - 3D: 10 optical slices – 100 ms or 10 frames per second

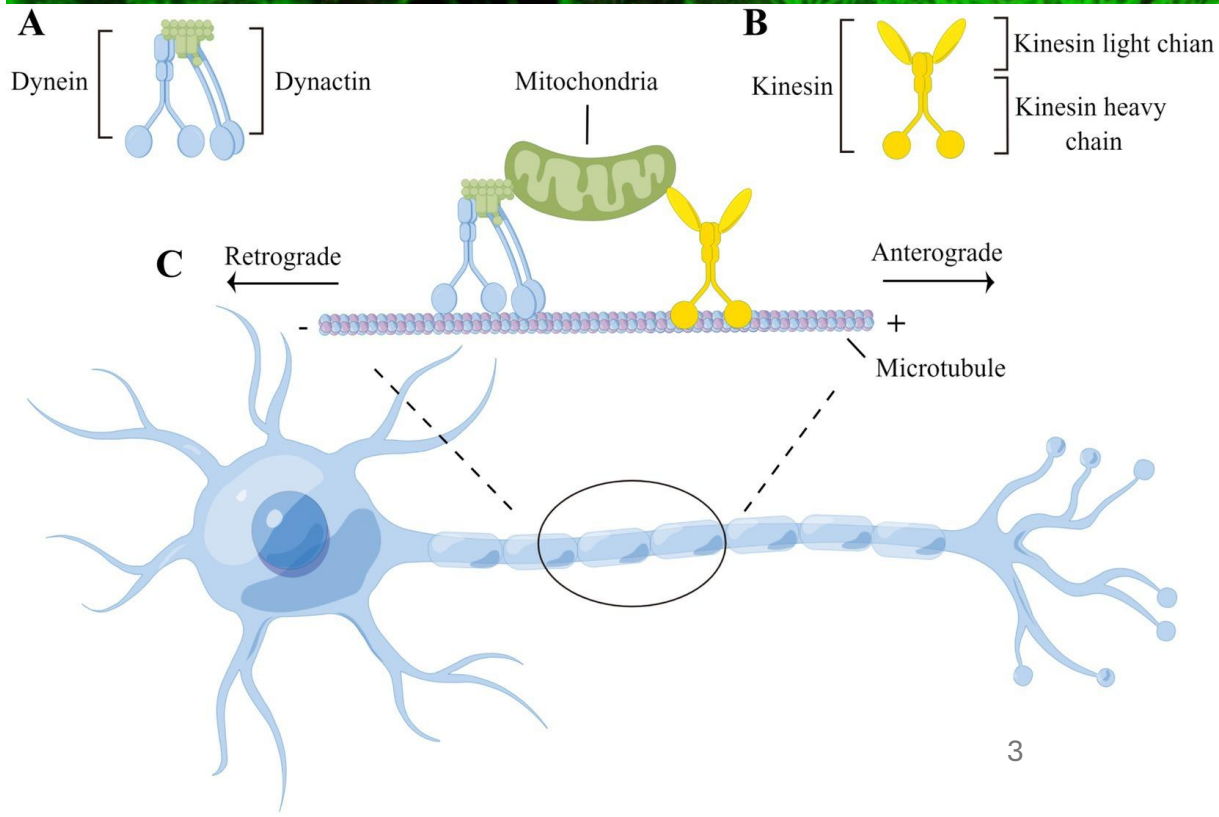


Future work

- Axonal transport
- Nanotoxicology – olfactory nerve
- 2-photon 3D-STED microscope







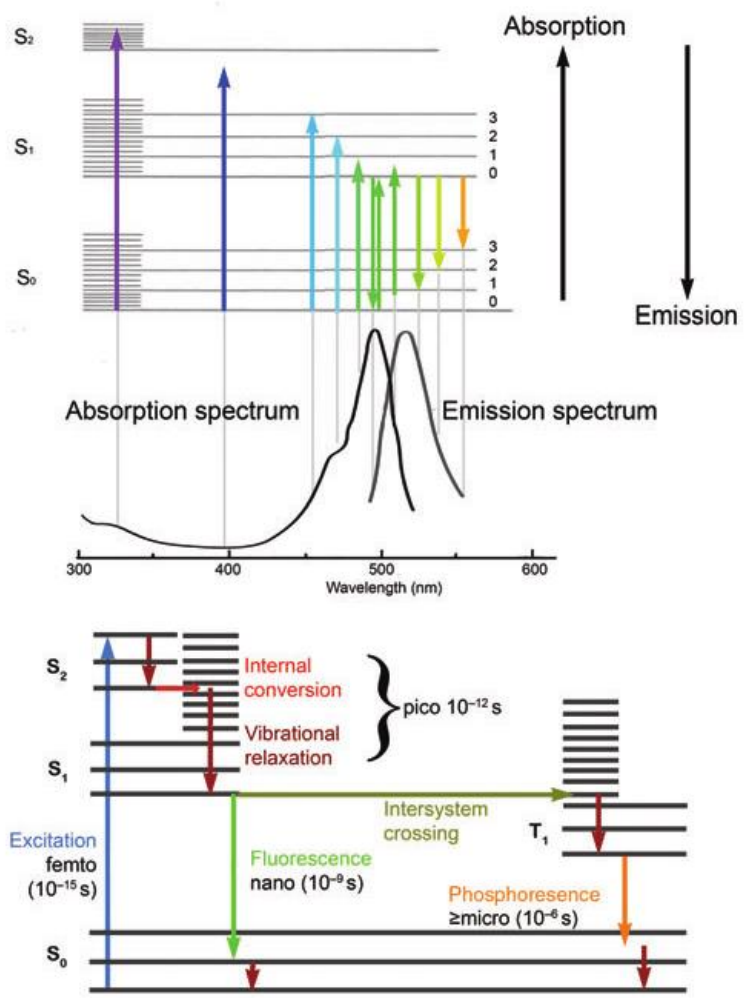
end of pitch

next slides are for answering questions

Introduction

- Fluorescence
- Diffraction limit

$$d = \frac{\lambda}{2n \sin\theta} = \frac{\lambda}{2\mathrm{NA}}$$

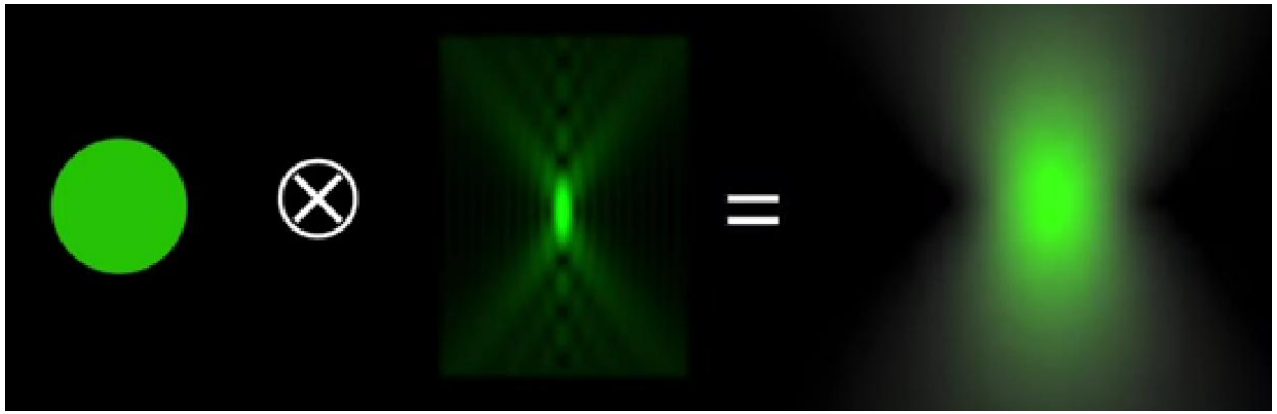


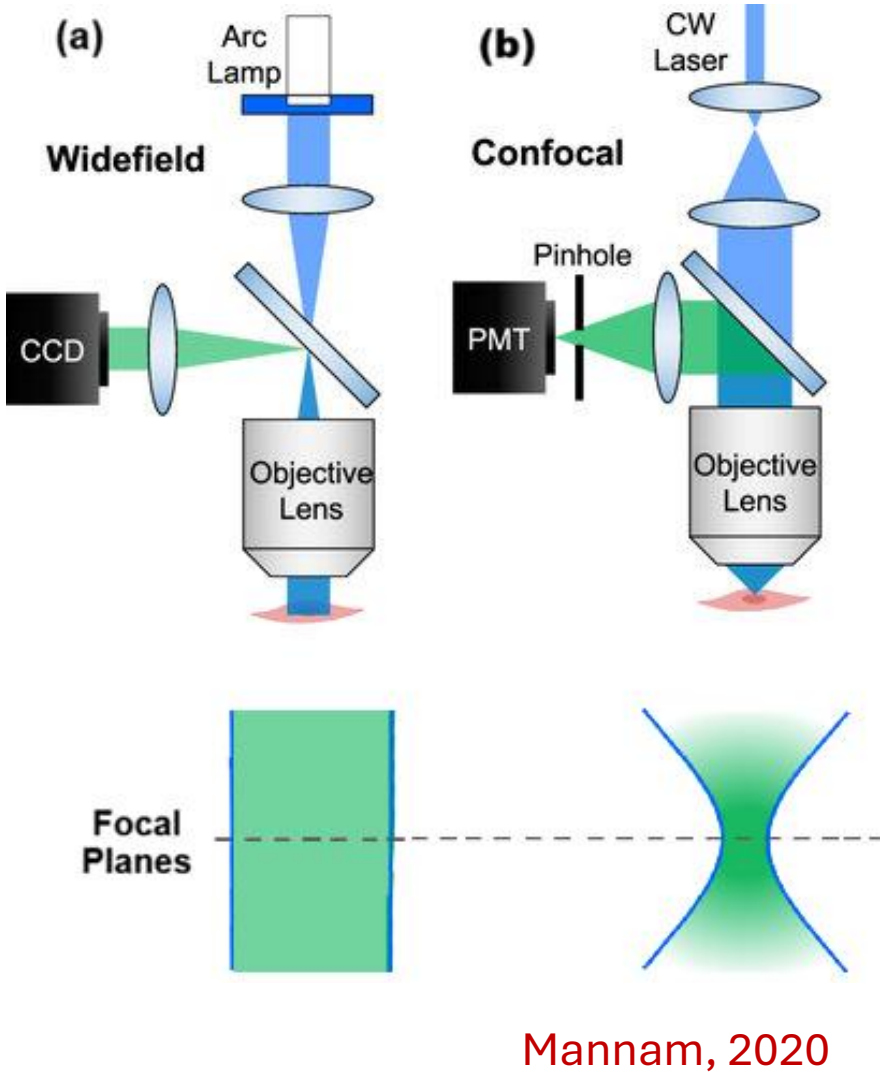
Lichtman, 2005

Confocal microscopy

- Laser + pinhole
- Less out-of-focus fluorescence

- Point Spread Function (PSF)

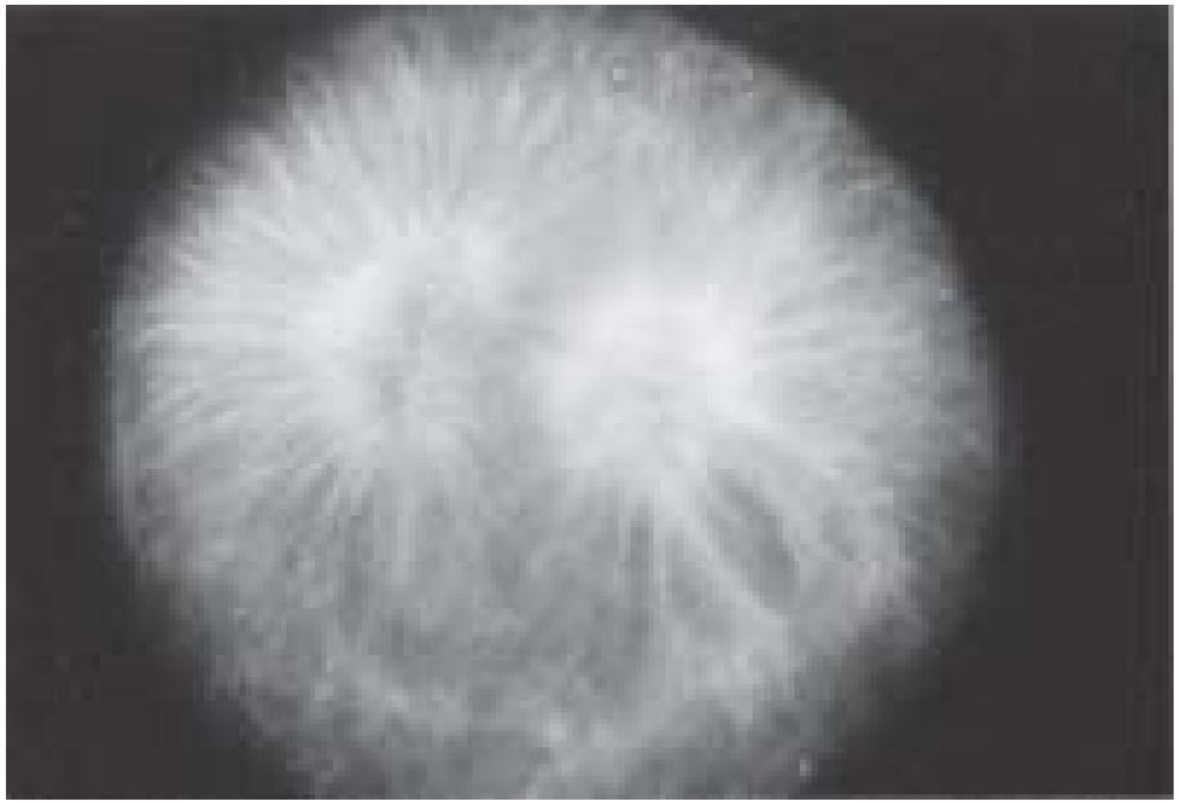


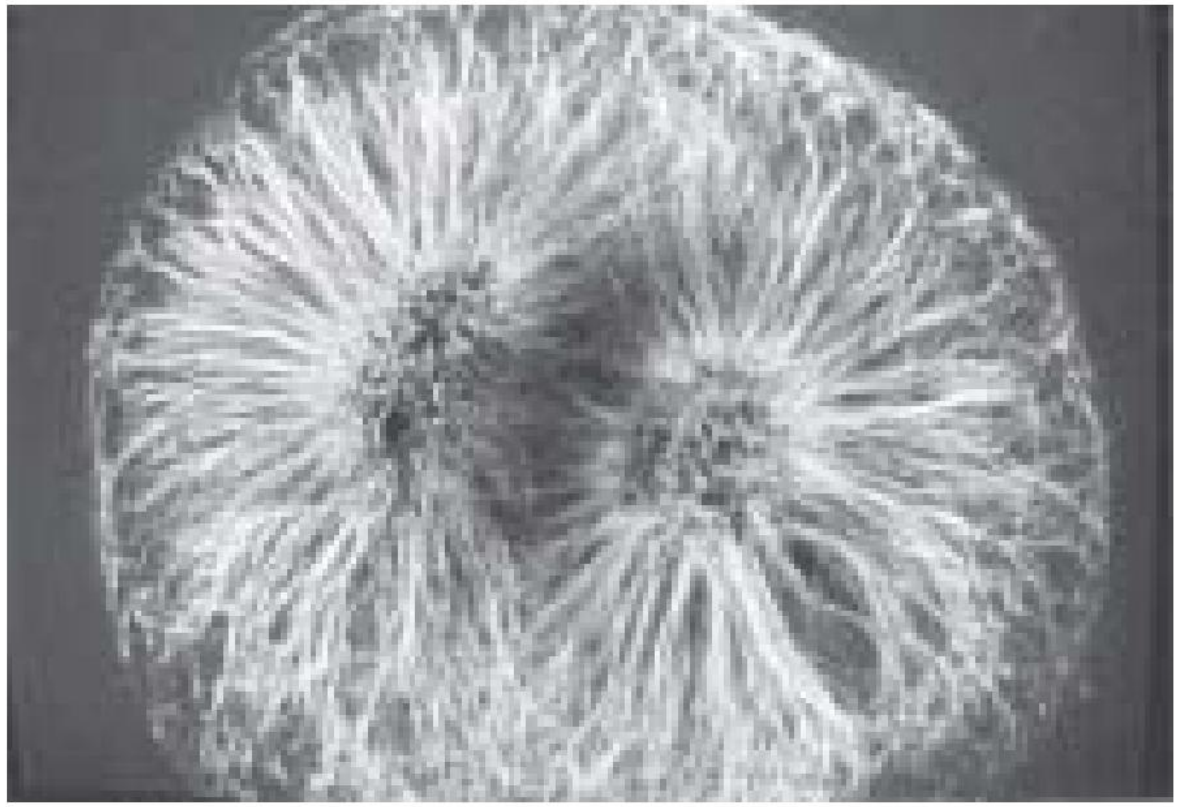


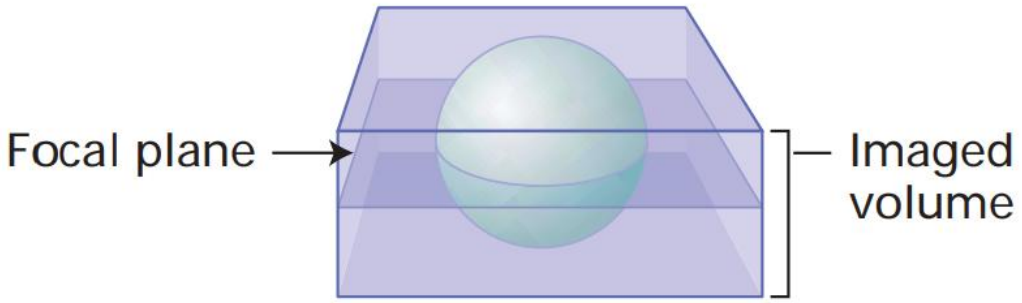
Mannam, 2020

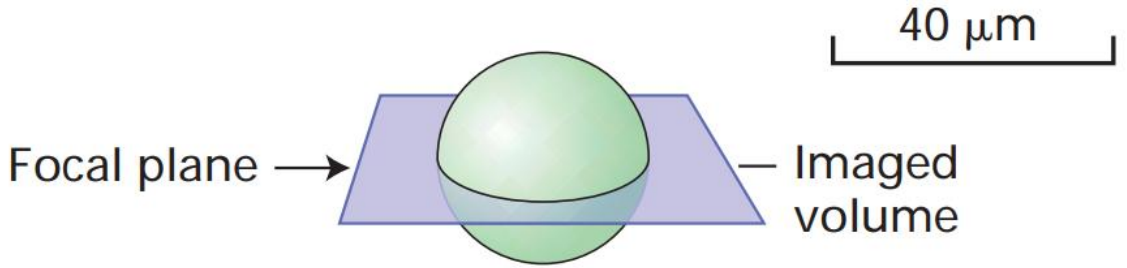
(a) Conventional fluorescence microscopy

(b) Confocal fluorescence microscopy



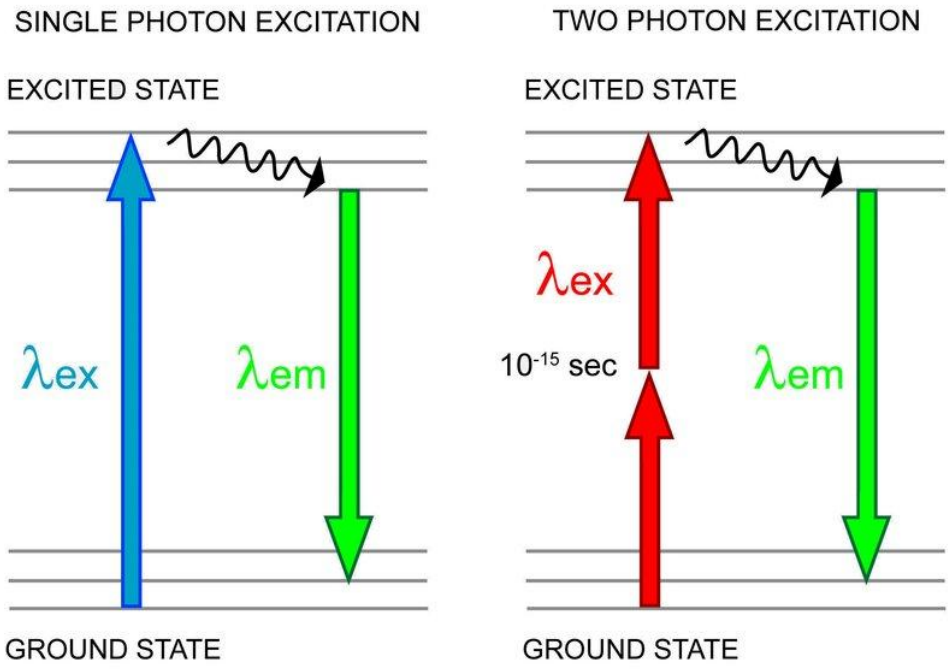




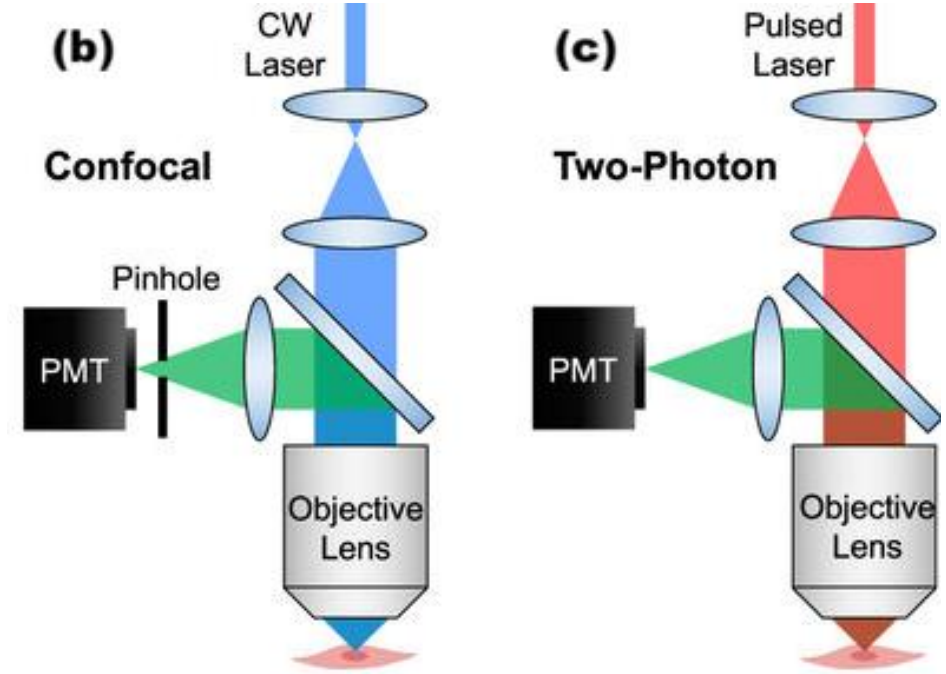


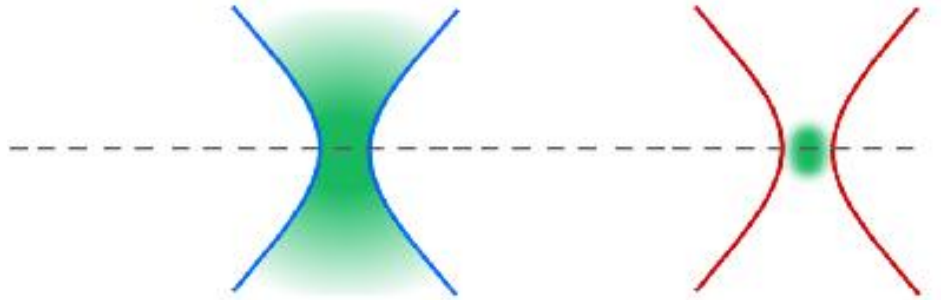
Lodish, 2003

2-photon Microscopy



Castelli, 2019





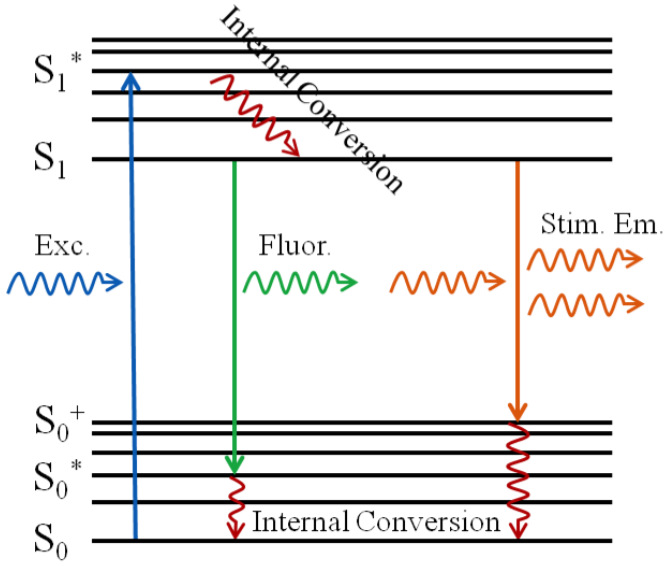
Mannam, 2020

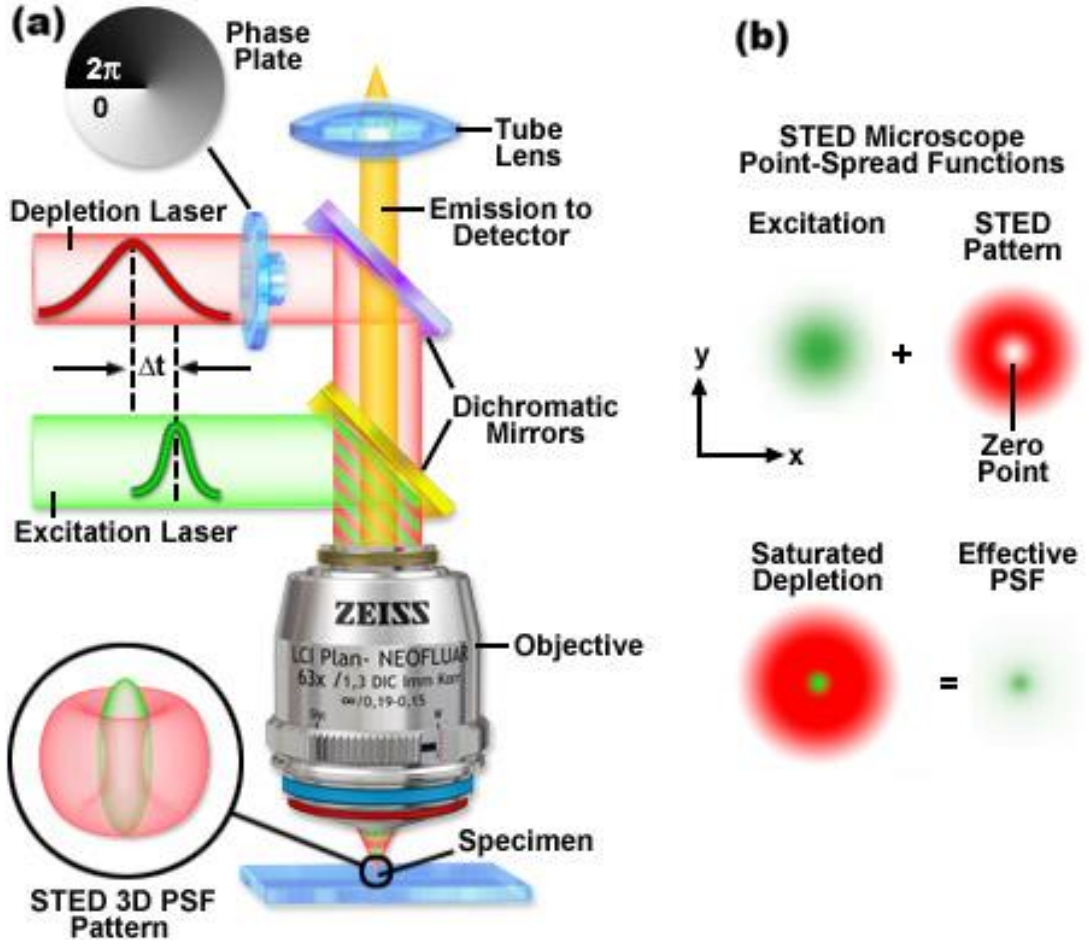
$$R \propto I^2$$

Stimulated Emission Depletion (STED)

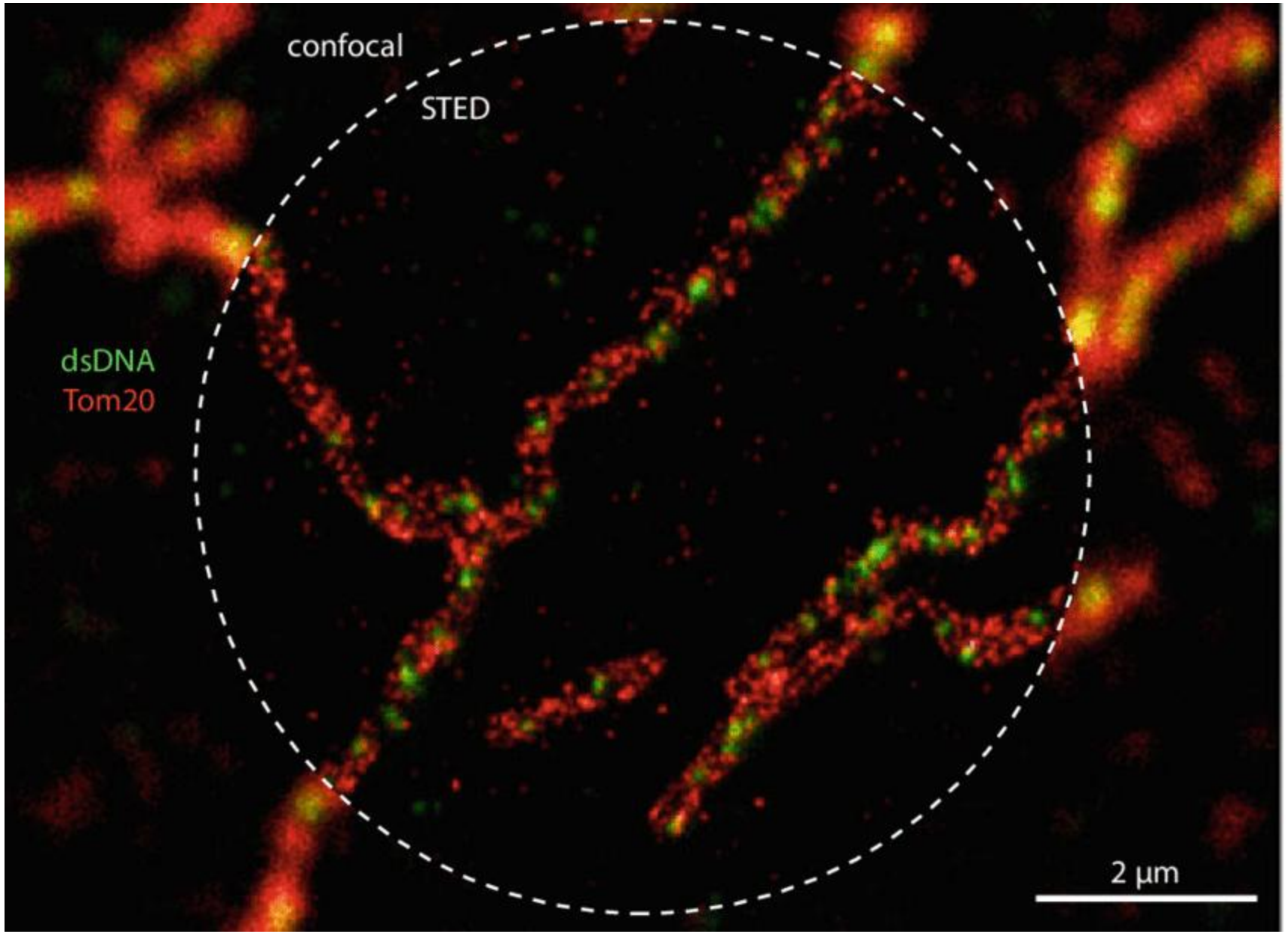
- Breaking the diffraction limit

$$d_{STED} \approx \frac{\lambda}{2NA\sqrt{1 + I/I_{sat}}}$$



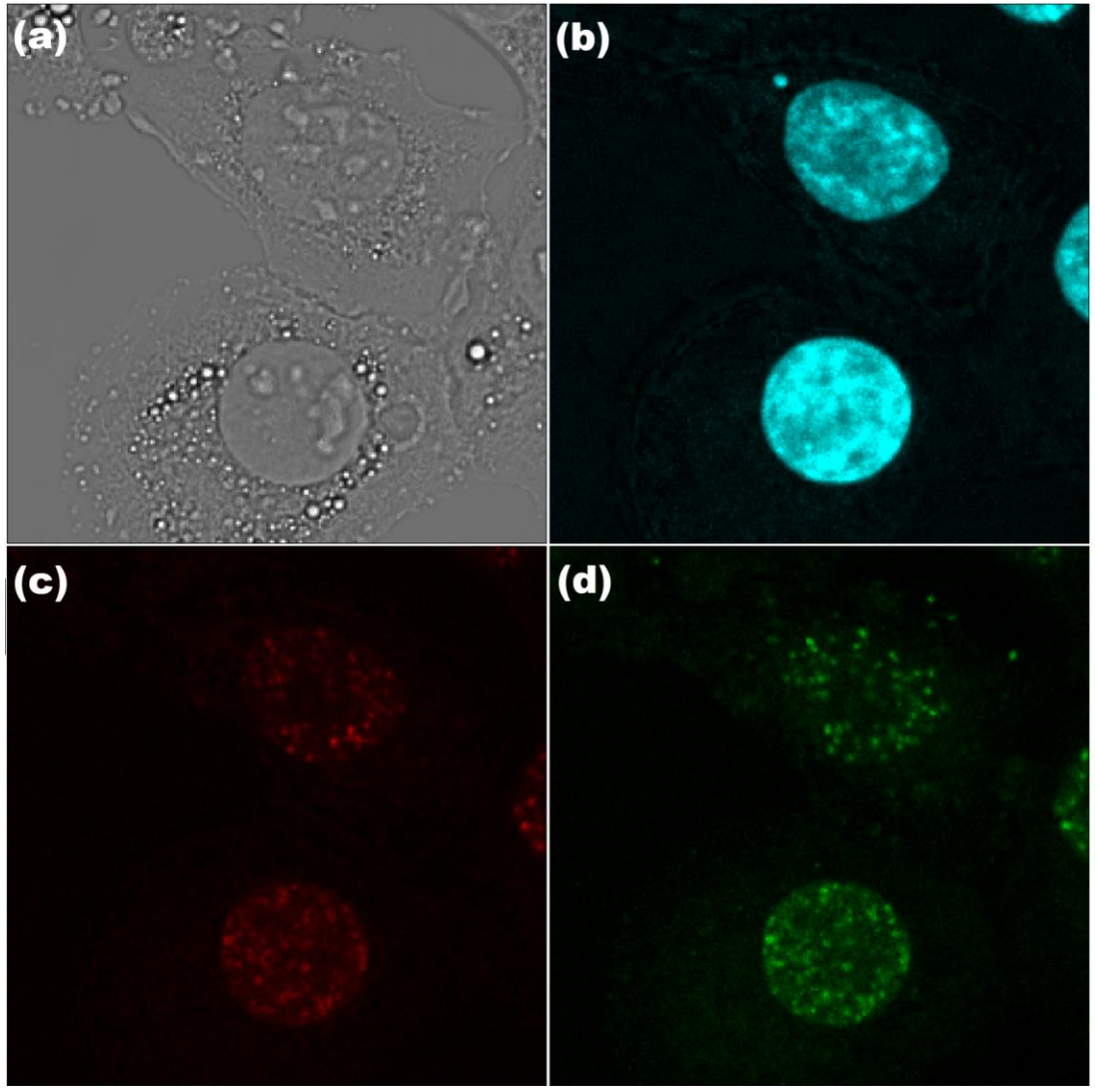


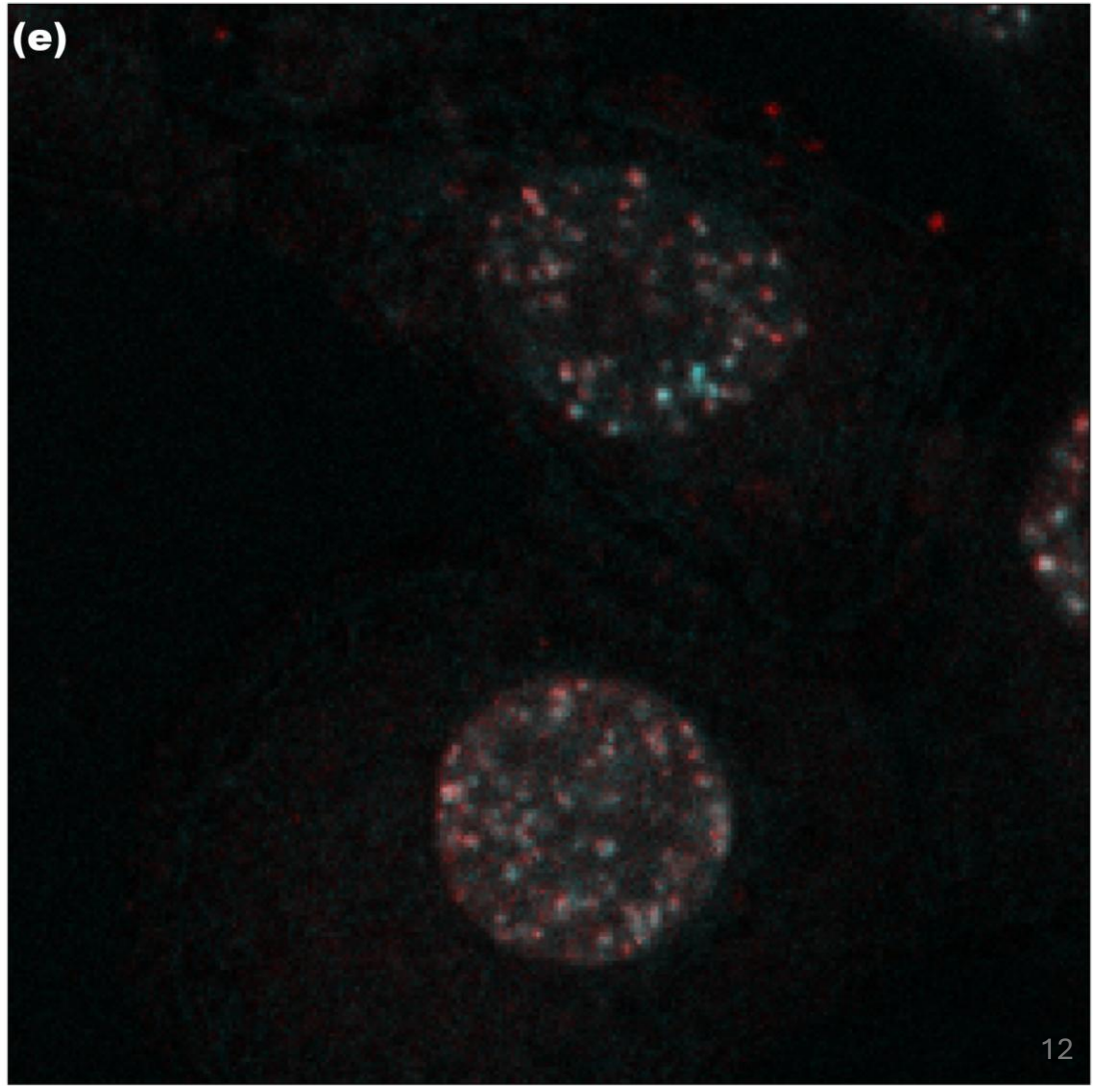
Zeiss Microscopy



Enger, 2020

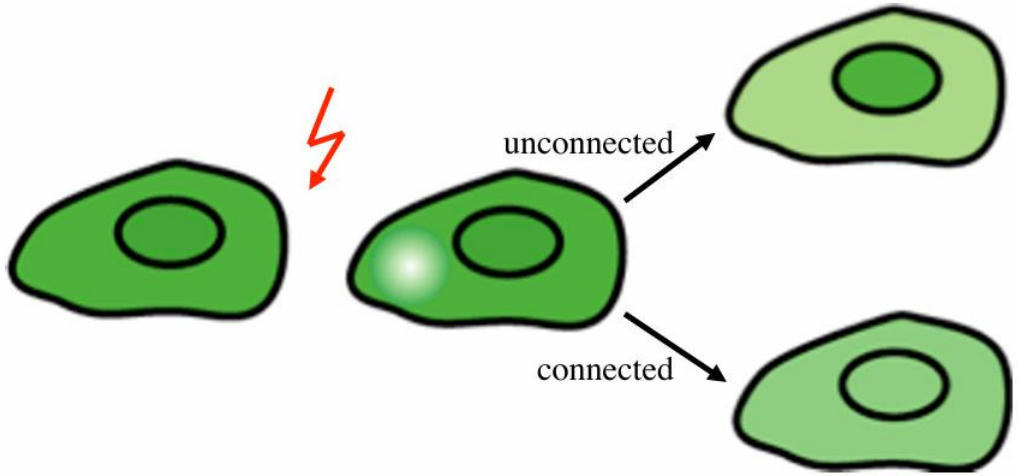
Correlated Imaging

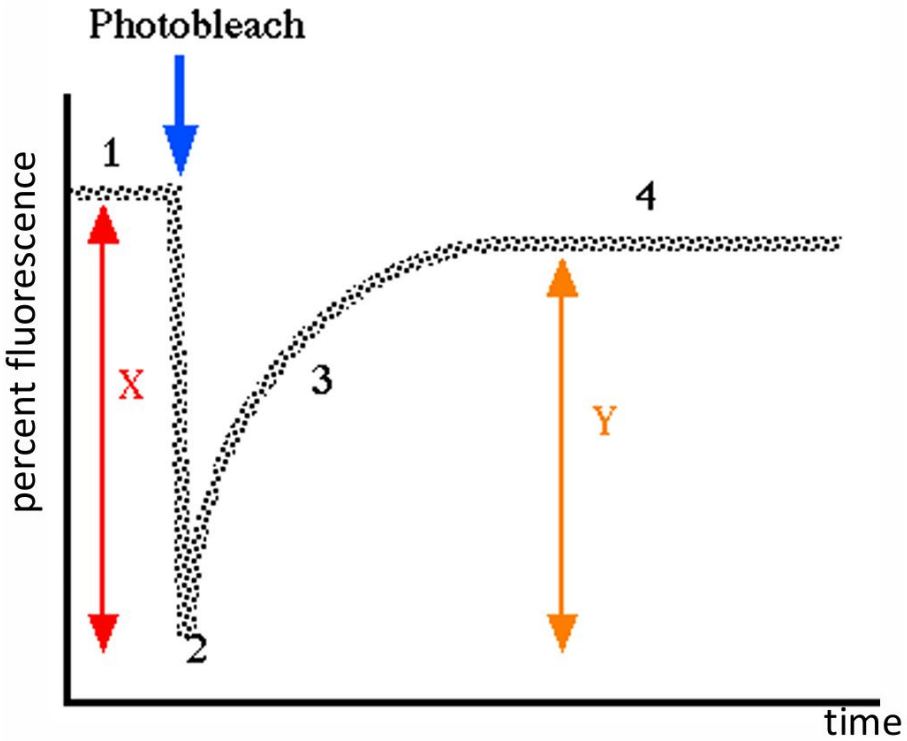


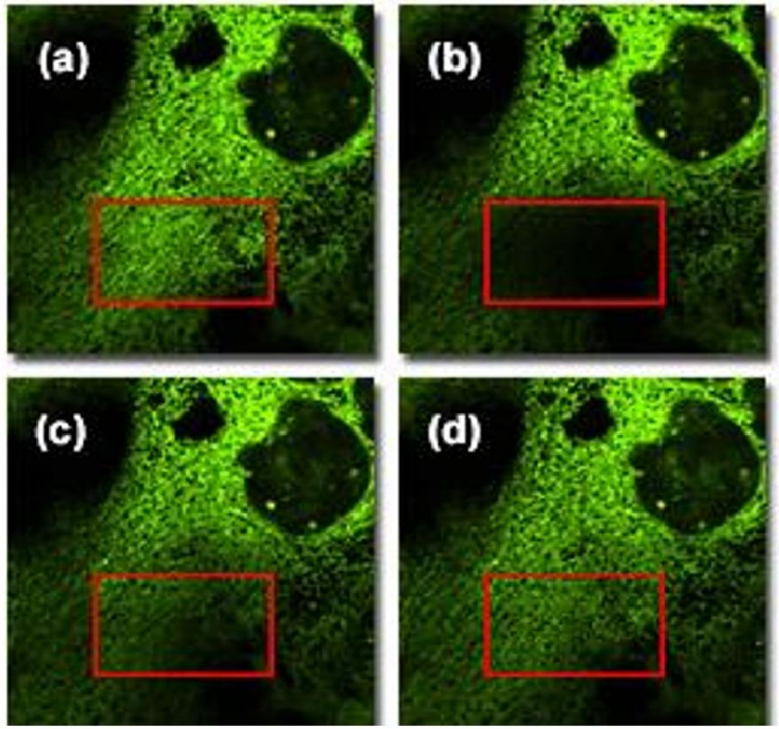


Photobleaching methods

- Diffusion, active transport (FRAP)
- Connectivity of compartments (FLIP)



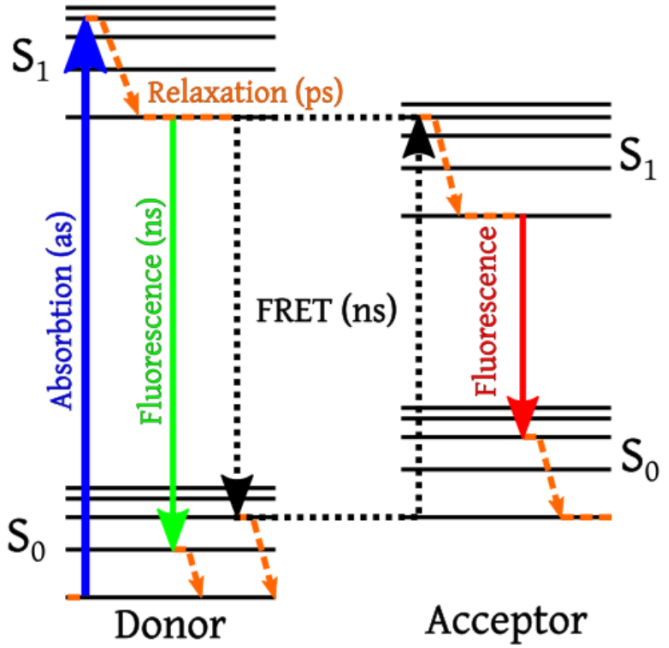


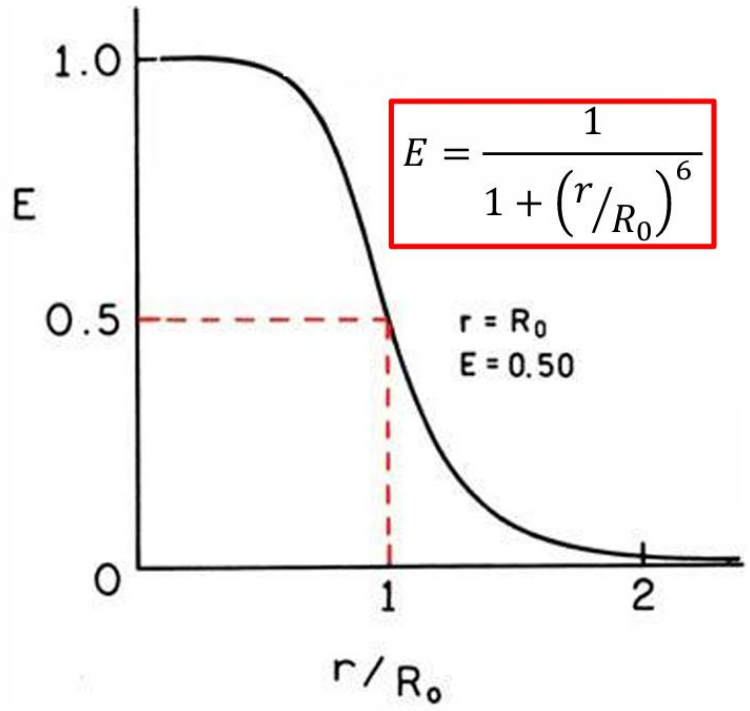


Göhler, 2014
(lecture notes)

Fluorescence Resonance Energy Transfer

- Two types of fluorophores
- Dipole-dipole interaction
- A molecular ruler





R_0 = 0.5 – 10 nm