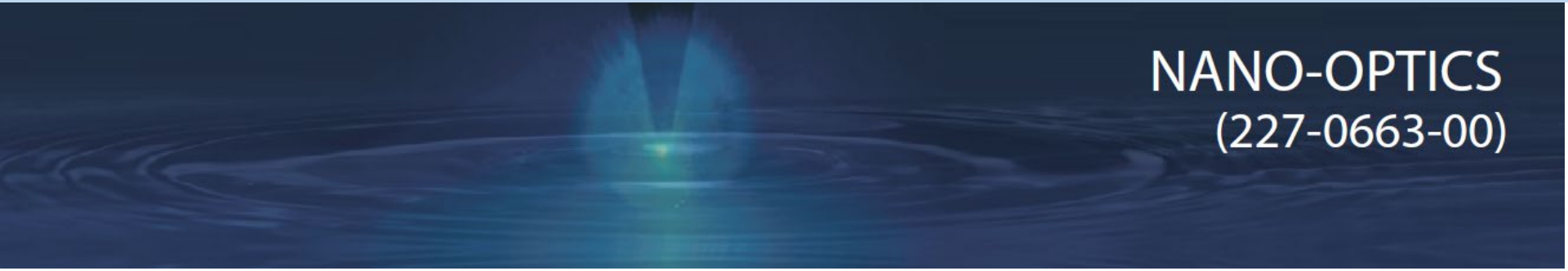
Welcome again!



Martin Frimmer (mfrimmer@ethz.ch)
Photonics Laboratory
HPP M24

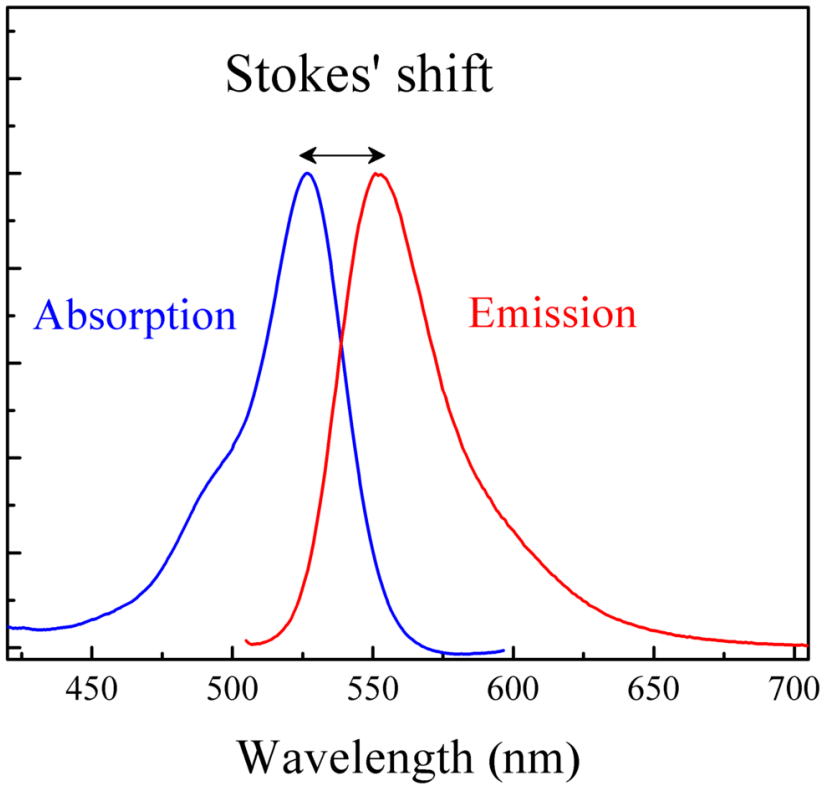
- Start to get into your literature projects!
- You can sign up for a tour of the Photonics Lab on Moodle

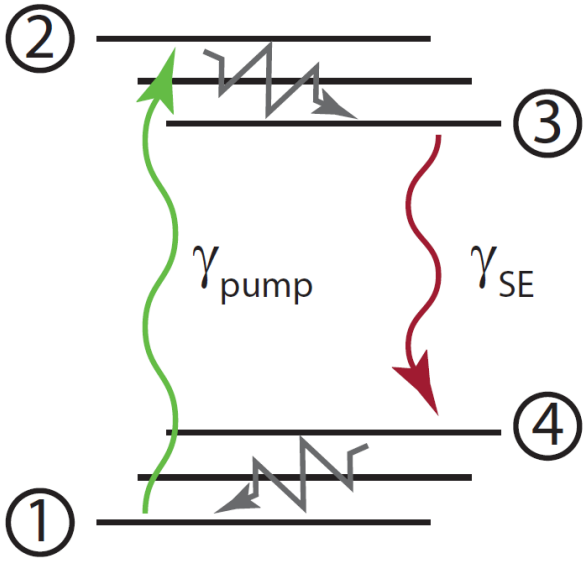
Homework 1

- Very good “electromagnetic fields and waves” knowledge throughout! Excellent job on the content.
  - Our complex quantities have NO time dependence!

- Presentation and form
  - Stick to notation of the course (e.g., vectors vs scalars)!
  - Make it easy for your audience (e.g. files size, order, summary)!
  - Be brief and to the point!

Fluorescent molecules – Jablonski diagram





- Stokes shift of fluorescence allows to spectrally separate (intense) pump light from (weak) fluorescence

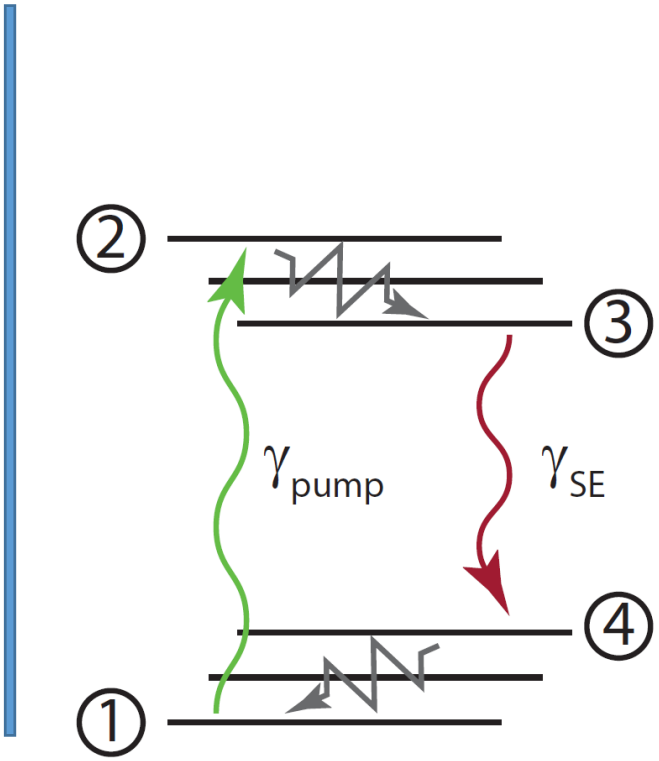
$$\text{Excitation rate} \sim |\boldsymbol{\mu}\cdot\mathbf{E}(x,y;z_o)|^2$$

μ: transition dipole moment

Fluorescent molecules – Jablonski diagram

- In practice, we often quantify the interaction rate between a fluorophore and a light field via a cross section σ

$$\gamma = \frac{|\mathbf{S}|}{\hbar\omega}\sigma$$



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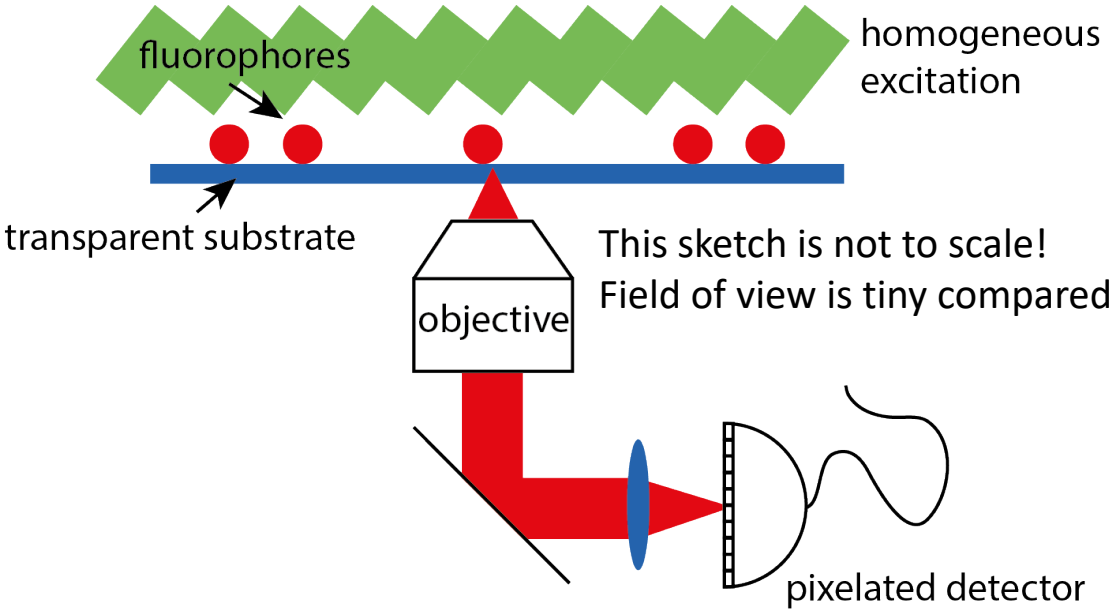
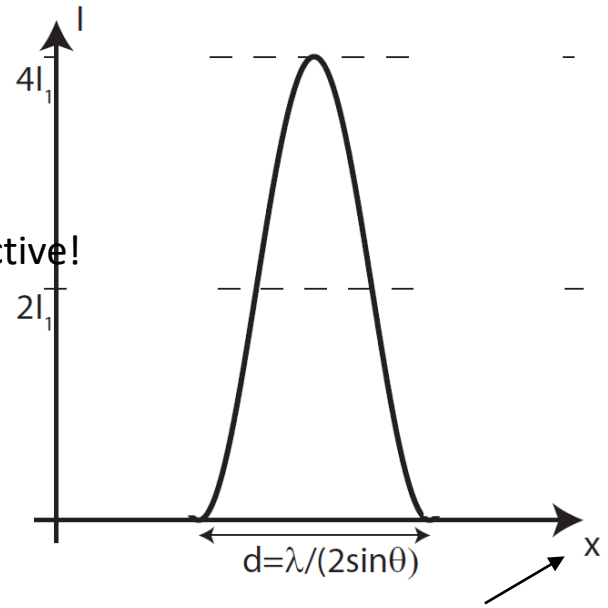
Fluorescence microscopy: Epi-illumination

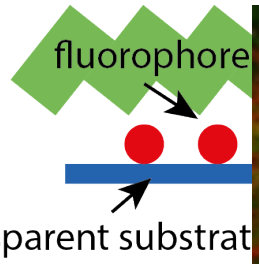


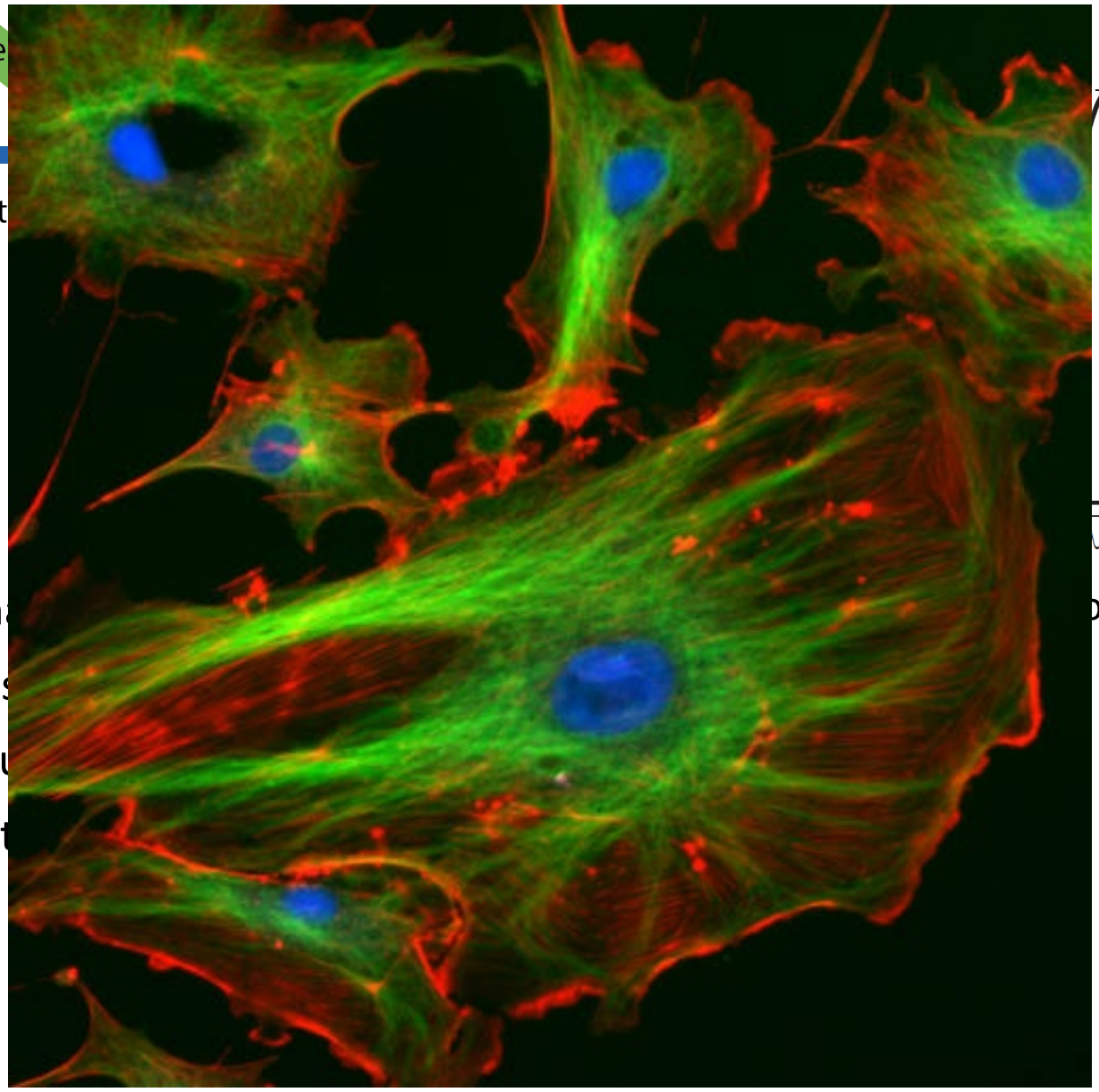


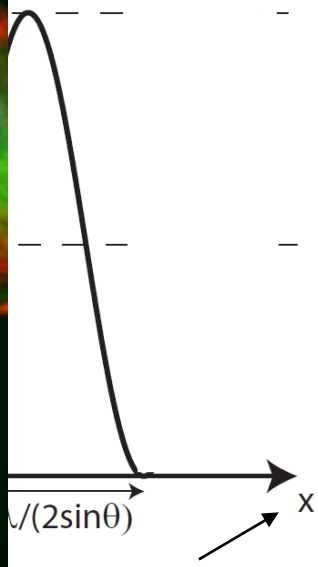
- Illuminate entire sample homogeneously
- Image sample plane onto pixelated detector
- Each fluorophore generates a signal according to the PSF
- Resolution is

$$x_0 = \frac{\lambda}{2\,\mathrm{NA}}$$

Fluorescence microscopy: Epi-illumination



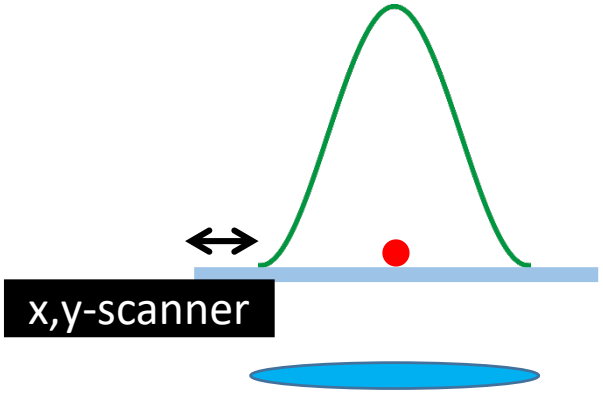


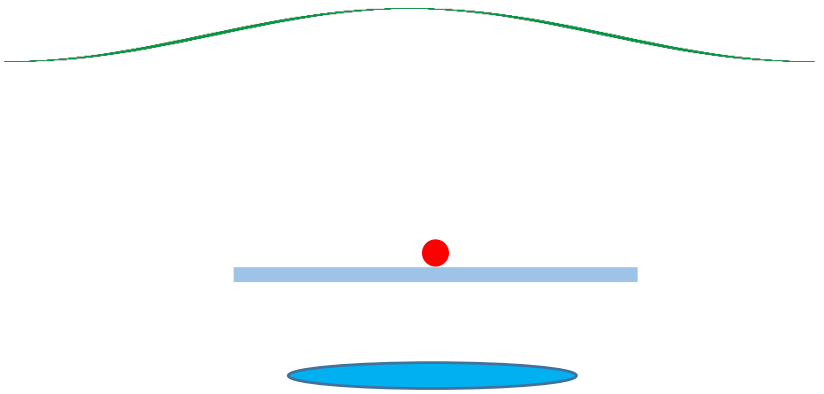


- Illumin
- Image s
- Each flu
- Resolut

on on detector

Fluorescence microscopy – scanning vs. wide-field





Scanning technique.

Resolution s

pump spot on sample

(no spatial resolution)

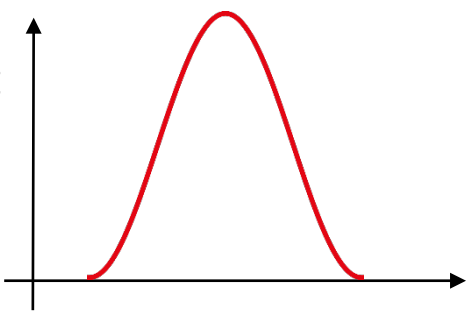
Both limited by diffraction.

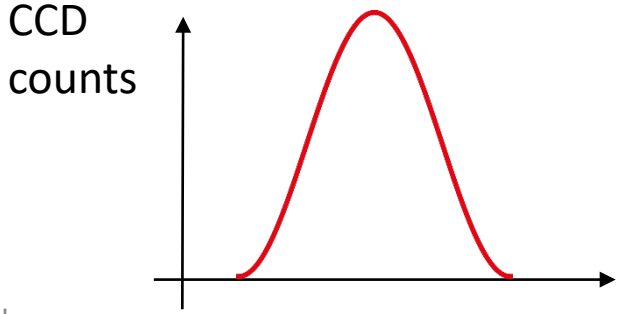
Wide-field imaging.

t by PSF of

imaging system

CCD camera





STED microscopy

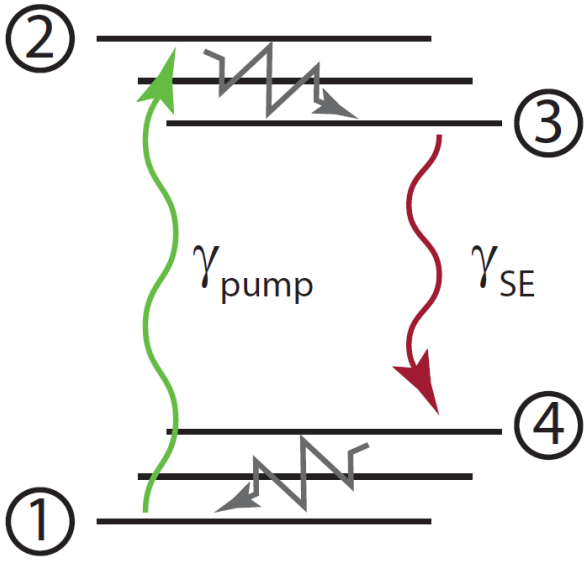
Westphal and Hell, Phys. Rev. Lett. 94, 143903 (2005)

- STED = stimulated emission depletion
- Allows fluorescence microscopy beyond the diffraction limit
- Ingredients:
  - (at least) 4-level system
  - Pump laser
  - Depletion laser

- We need to understand
  - The diffraction limit
  - A four-level system in the presence of light fields

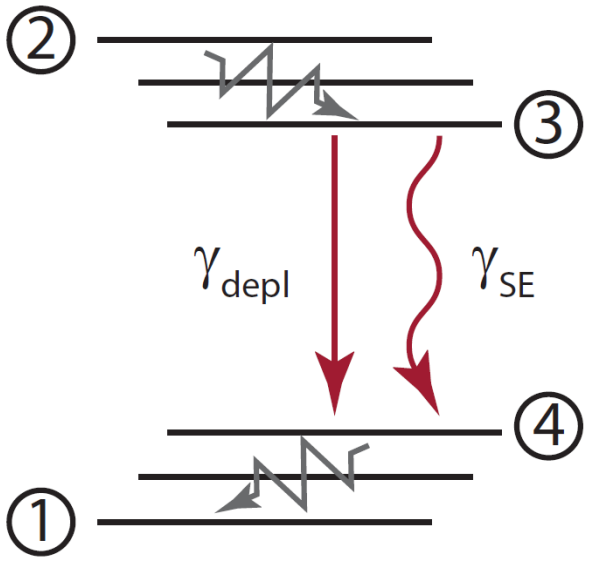
Population of excited state in absence of STED beam

- 4-level system created by two electronic states (of a fluorophore) and vibrational excitation
- Vibrational relaxation infinitely fast
- Start in ground state, turn on pump



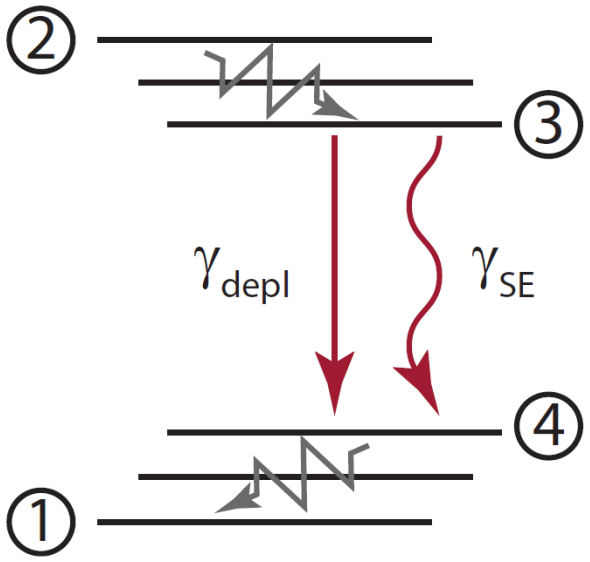
Population of excited state in presence of STED beam

- Start in excited state (with certain probability), turn on depletion laser



Population of excited state in presence of STED beam

- Start in excited state (with certain probability), turn on depletion laser
- Exponential decrease of population as function of time
- Depletion field “helps” spontaneous emission



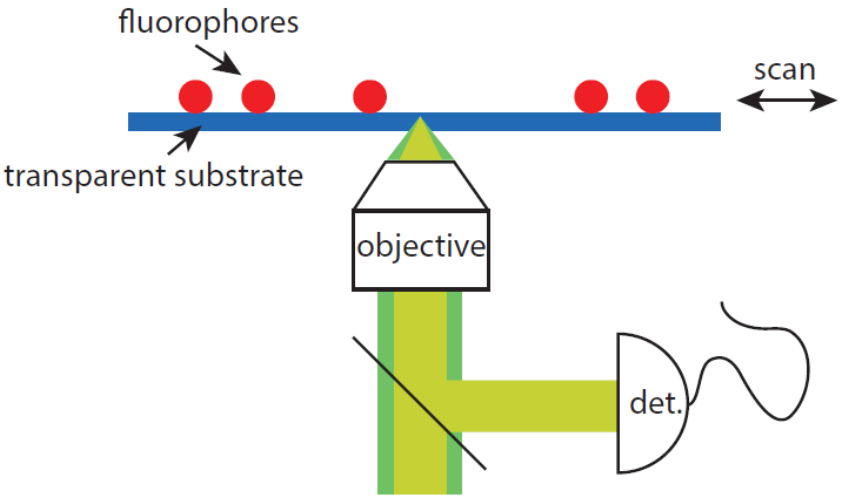
STED – how it works

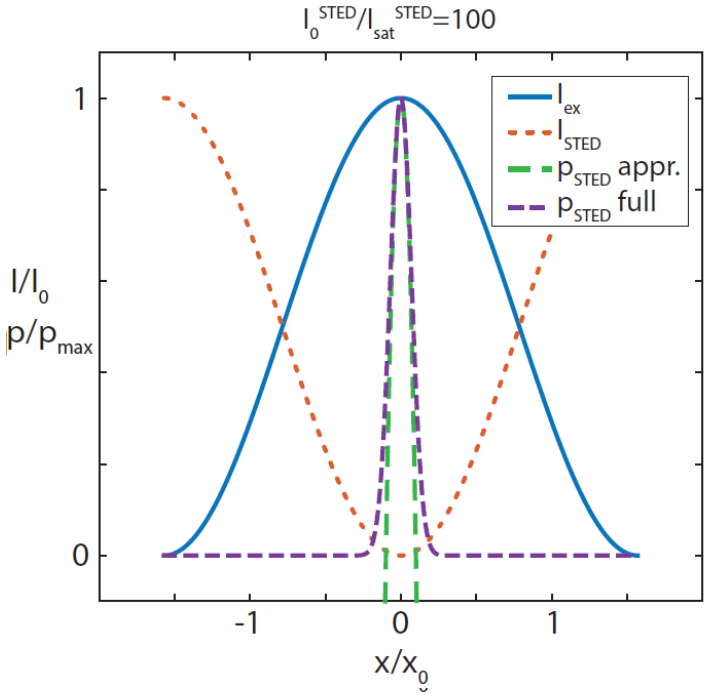
- FWHM of area of remaining pumped fluorophores after STED pulse

$$\Delta x = \frac{x_0}{\sqrt{1 + \frac{I_0^{\mathrm{STED}}}{I_{\mathrm{sat}}^{\mathrm{STED}}}}}$$

Characteristic saturation intensity:

$$I_{\mathrm{sat}}^{\mathrm{STED}} = \hbar\omega_{\mathrm{STED}} / (\sigma_{\mathrm{STED}} \tau_{\mathrm{STED}})$$





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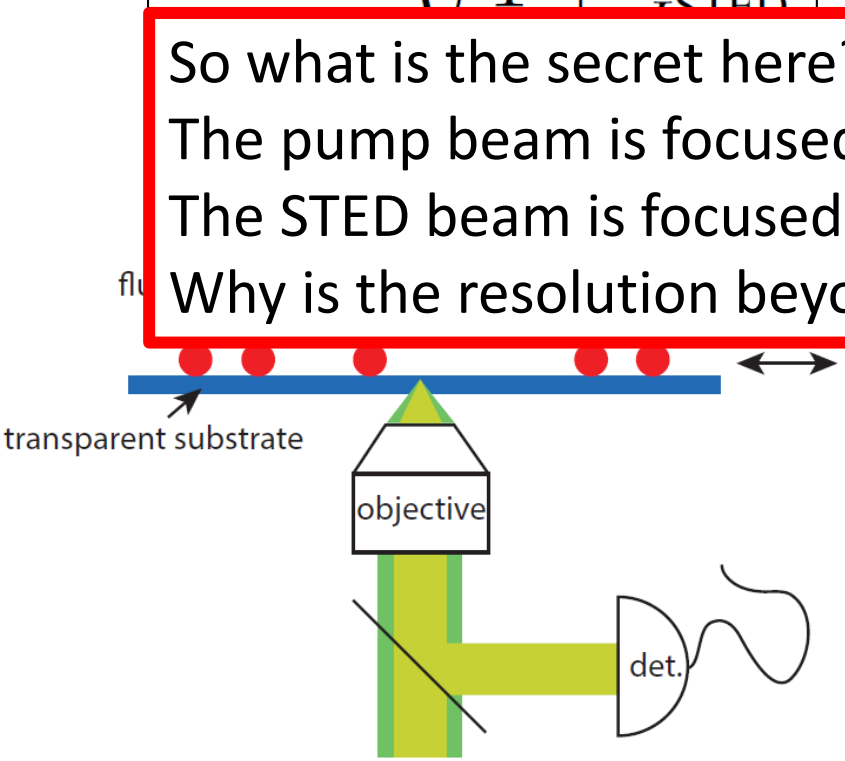
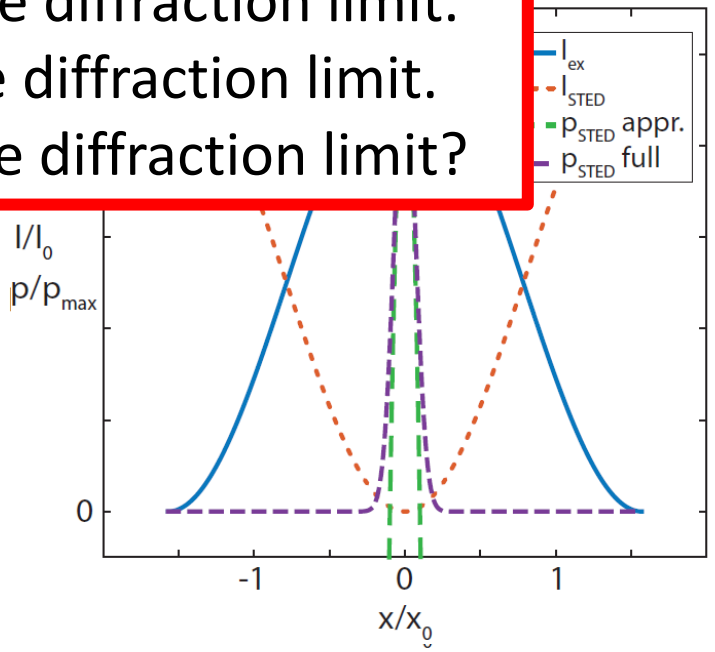
So what is the secret here?
The pump beam is focused to the diffraction limit.
The STED beam is focused to the diffraction limit.
Why is the resolution beyond the diffraction limit?

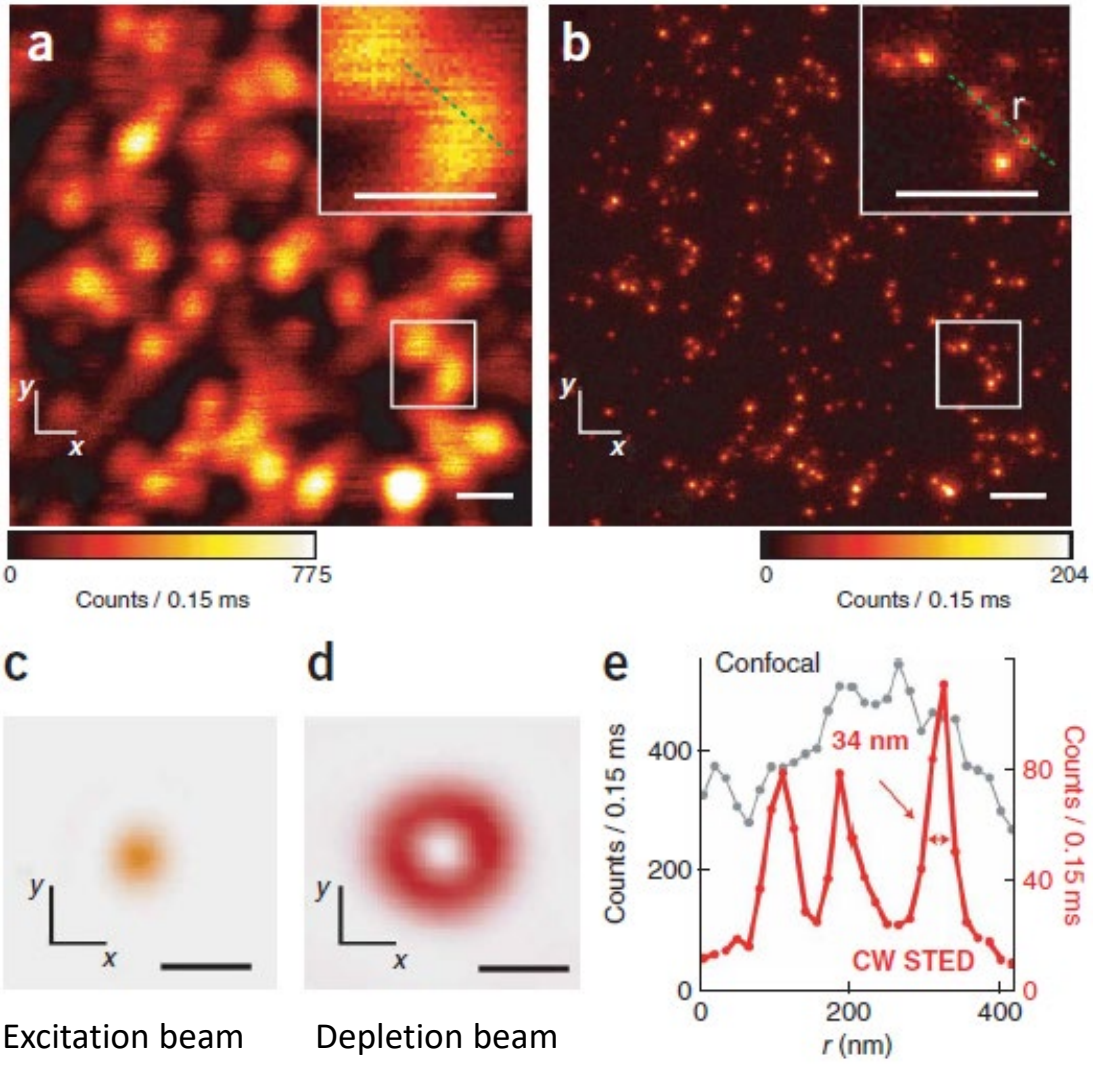




STED – how it really works

Willig et al., Nat. Meth. 4, 915(2007)

Figure 2 | Nanoscale imaging with CW STED. (**a**,**b**) Raw data of confocal (**a**) and corresponding CW-STED (**b**) image of fluorescent 20-nm-diameter beads. The images were recorded simultaneously with an excitation power of 11 μW (at 635 nm) at the sample and by turning the STED laser (825 mW, 730 nm) on and off line by line. Insets, magnification of the boxed area. Scale bars, 500 nm. (**c**,**d**) The measured focal spot of the excitation light (**c**) along with the measured focal STED doughnut exhibiting a minimum of 250 nm (FWHM; **d**). (**e**) The profile along the dashed line in **a** and **b** exhibits a spot size of 34 nm, indicating an effective resolution of ~29 nm.



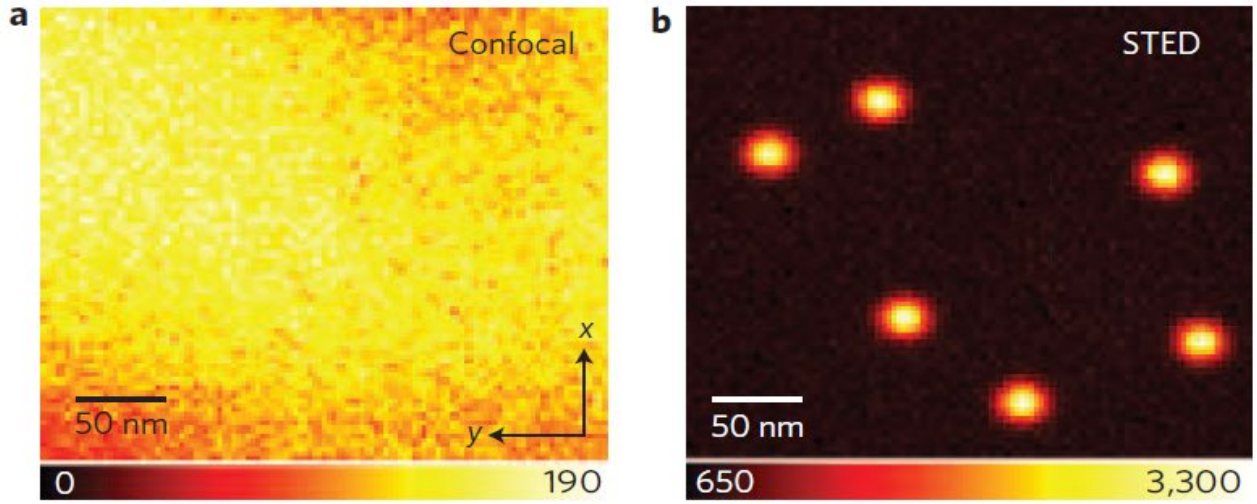
Excitation beam profile

Depletion beam profile

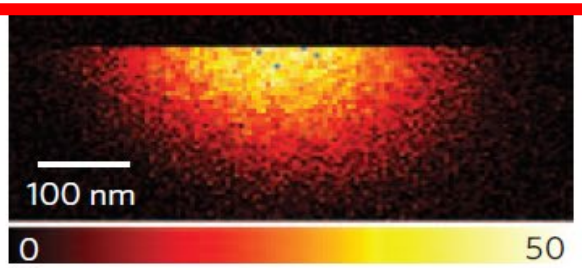
STED microscopy - example

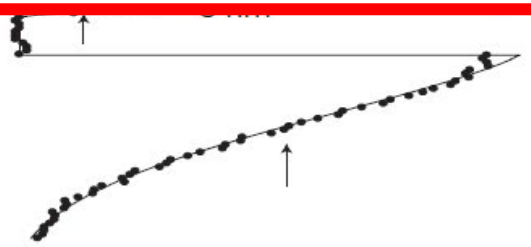
Rittweger et al., Nat. Photonics 3, 144 - 147 (2009)

- Imaging color centers in diamond



- Why do I need laser pulses?
- Could I also do this with CW lasers?
- If yes, how?





Where do we stand?

- Optical imaging:
  - Focusing by a lens
    - Angular spectrum
    - Paraxial approximation
    - Gaussian beams
    - Method of stationary phase
  - The diffraction limit: How well can we focus light?
  - Optical microscopy
    - Optical imaging systems
    - Real-world (dipolar) sources: Fluorophores and scatterers
    - Example: Fluorescence microscopy
    - Example: STED microscopy
    - Example: Localization microscopy
    - Example: Scanning probe microscopy

STORM/PALM – localization microscopy

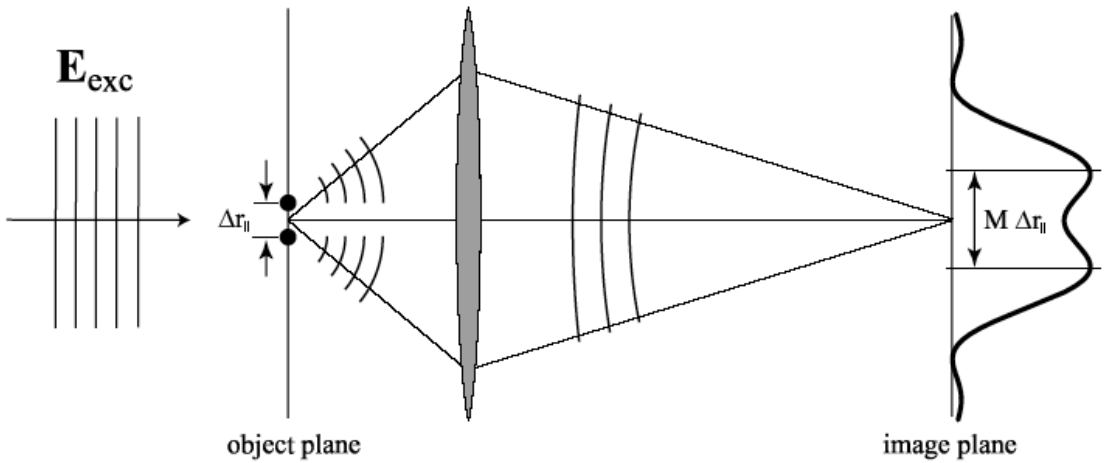
Different names for (in principle) the same technique:

- Photoactivated localization microscopy (PALM)
- Stochastic optical reconstruction microcopy (STORM)

STORM – localization microscopy

- Abbe tells me how closely spaced two sources can be for them to be discernible

$$\mathrm{Min}\,[\Delta r_{||}] = 0.6098\,\frac{\lambda}{NA}$$



- But how well can I localize a single emitter? (given that I know it is a single one)

$$\Delta_x = \frac{1}{\sqrt{N}}\,\frac{\lambda}{2\,\mathrm{NA}}$$