STED Microscopy

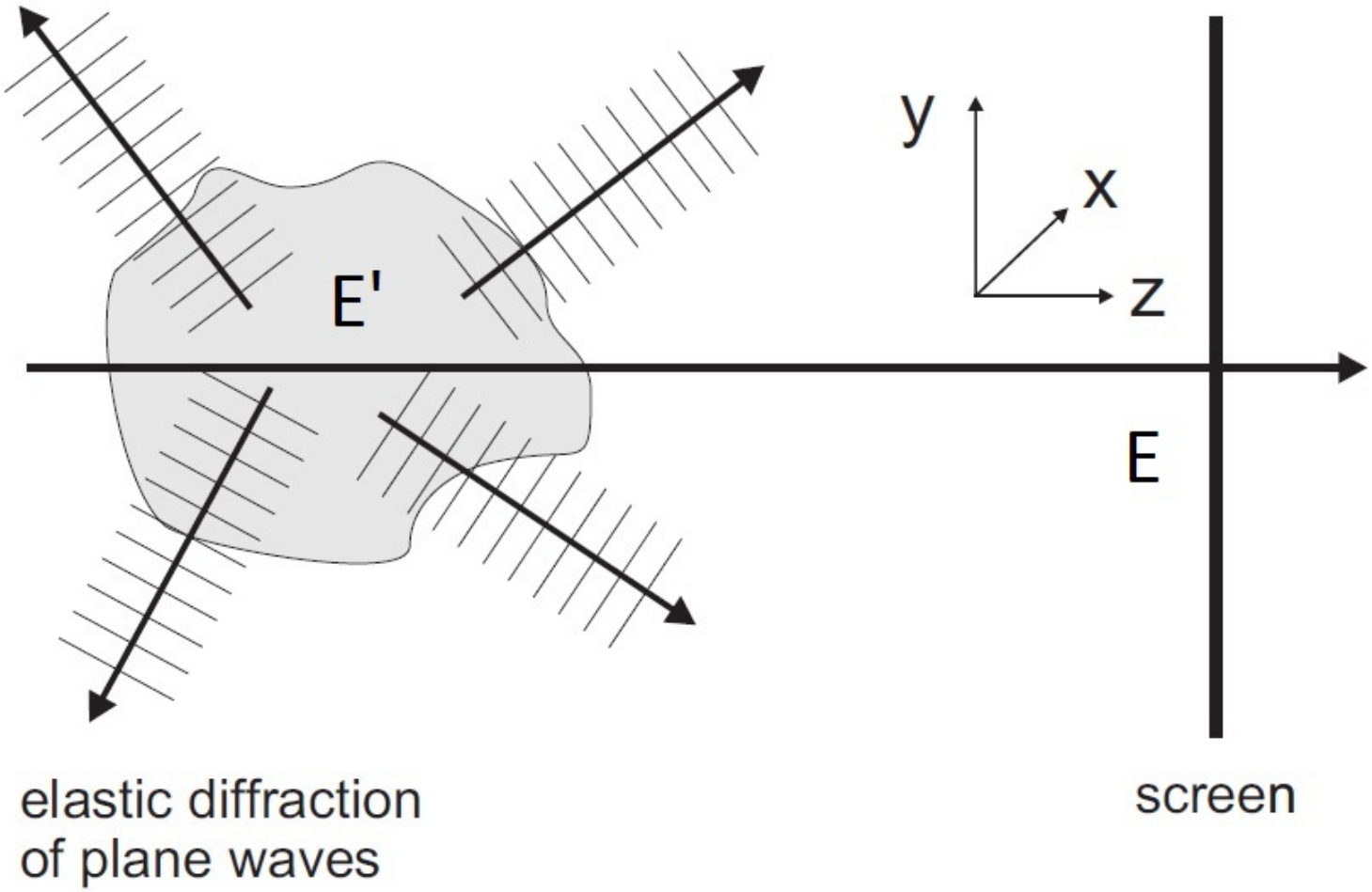
Breaking Abbes-Law

Robert Polster

20. Mai 2010

Inhaltsverzeichnis

Abbes Limit



Abbes Limit

An electromagnetic wave propagates as:

$$\vec{E} = \frac{1}{4\pi^2} \int \int_{-\infty}^{\infty} \vec{E'} \cdot H \; dk_x dk_y dx' dy'$$

with

$$H = e^{i(k_x x + k_y y + k_z z)}$$

k_z can be expressed as:

$$k_z = \sqrt{k^2 - k_x^2 - k_y^2} = 2\pi \sqrt{\frac{1}{\lambda^2} - \rho^2}$$

k_z describes an evanescent wave, if $\lambda < 1/\rho$.

Abbes Limit

A electromagnetic Wave propagates as:

$$\vec{E} = \frac{1}{4\pi^2} \int \int_{-\infty}^{\infty} \vec{E'} \cdot H \; dk_x dk_y dx' dy'$$

with

$$H = e^{i(k_x x + k_y y + k_z z)}$$

k_z can be expressed as:

$$k_z = \sqrt{k^2 - k_x^2 - k_y^2} = 2\pi \sqrt{\frac{1}{\lambda^2} - \rho^2}$$

k_z describes an evanescent wave, if $\lambda < 1/\rho$.

Abbes Limit

Conclusions:
The information of an evanescent wave vanishes in the far field.
Because of the missing information we can not:

- reconstruct the scattering object
- construct infinitely small focus point

Best resolution:

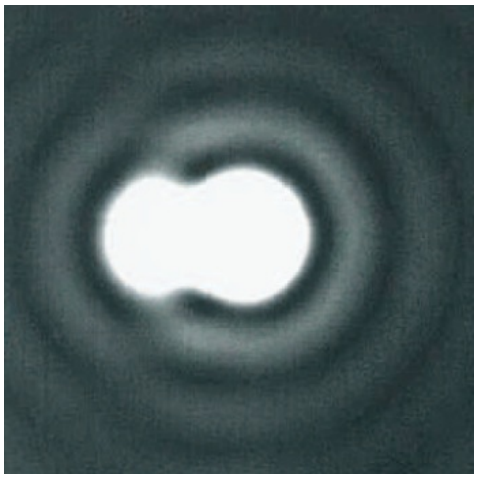
$$\Delta x \approx \frac{\lambda}{2}$$

Optical Aperture

The resolution is $\lambda/2$, if you are able to collect every k-vector. As every microscope has a specific angle of acceptance the resolution is after the Rayleigh-Criterion:

$$\Delta x = 0.61 \frac{\lambda}{NA}$$

$NA = n \sin \alpha$
n = refraction index
α = angle of acceptance



Microscopy methods

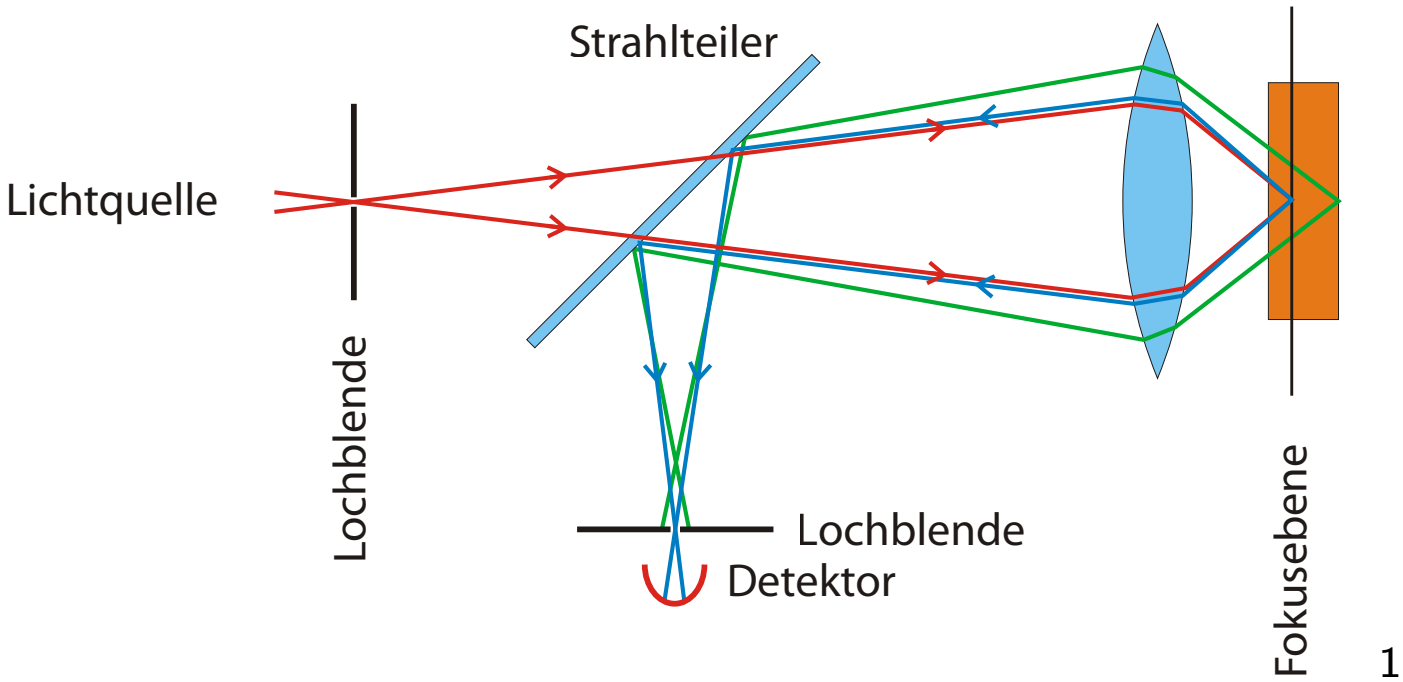
Modern commercial approaches to reach the resolution limit:

- ordinary Far Field Fluorescence Microscopy
- Scanning Confocal Microscopy

or even to break the resolution limit:

- Near Field Microscopes

Confocal Microscopy



[1]

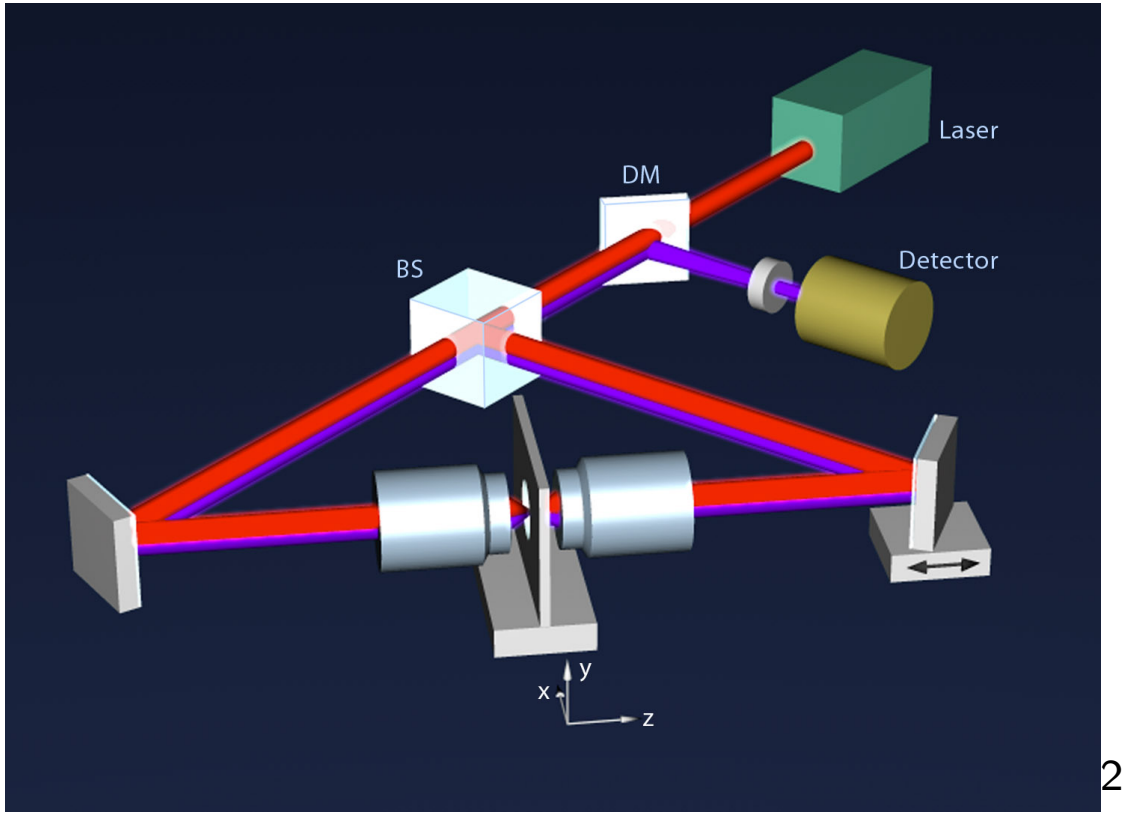
Resolution:

- transversal 200 nm $\propto (PSF)^2$
- longitudinal 500 nm

With a wavelength of:
$\approx 500nm$

[1] source: http://en.wikipedia.org/wiki/Confocal_microscopy

4Pi

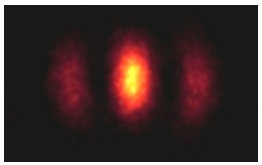


[2]

Resolution:

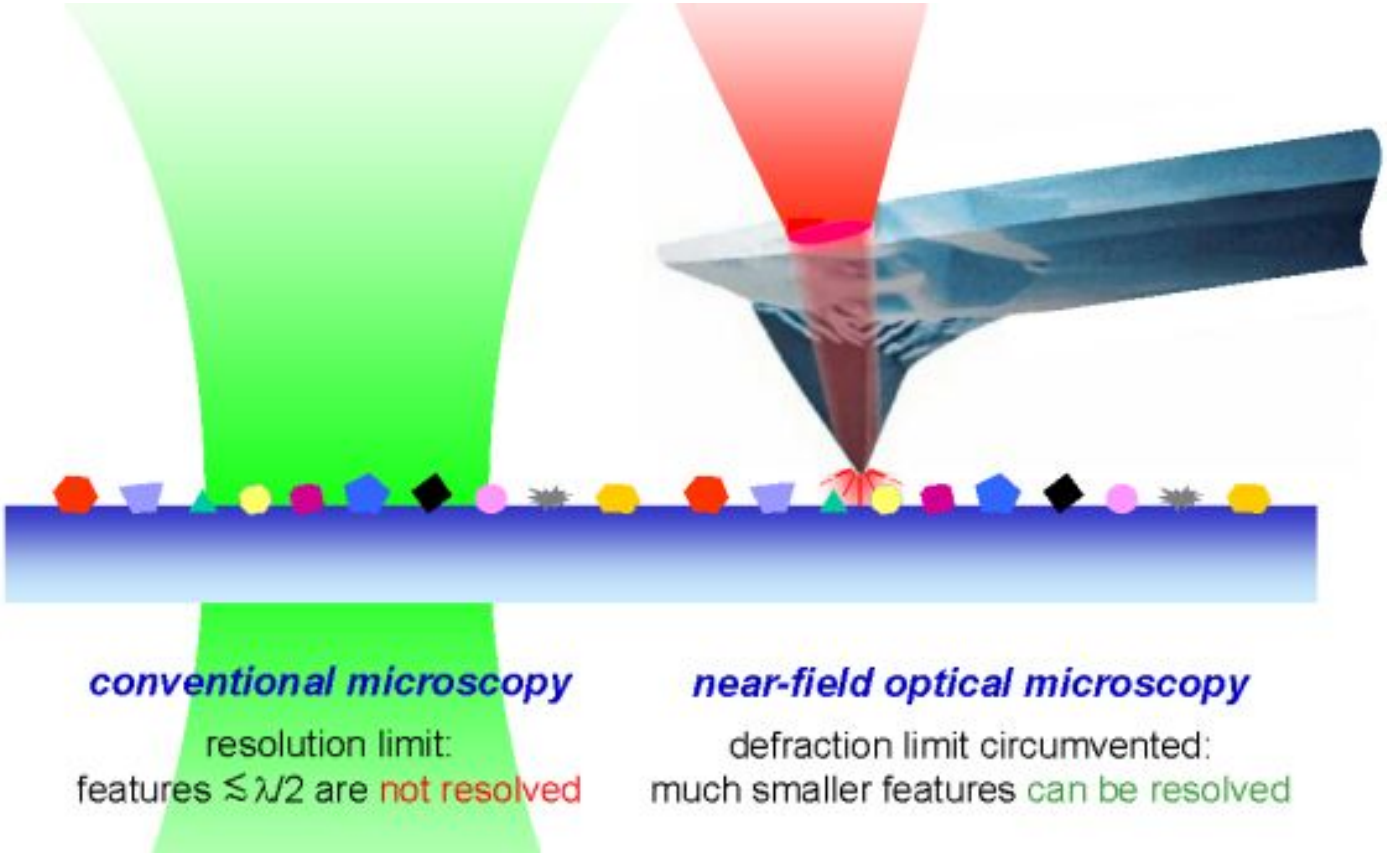
- transversal 200 nm
- longitudinal 80 nm

Better resolution because of interference pattern at probe



[2] source: Bewersdorf, A. Egner, S.Hell "4Pi Microscopy". In: Handbook of Biological Confocal Microscopy, pp. 561-570, Ed. J. Pawley, Springer, New York. 2006

Near Field Microscope



3

- tip: local scatterer
- converts near to far field
- transversal resolution: 80 nm

- only surface sensitive
- challenging setup

[3] source: http://nahfeldmikroskopie.de/allgemeines.html

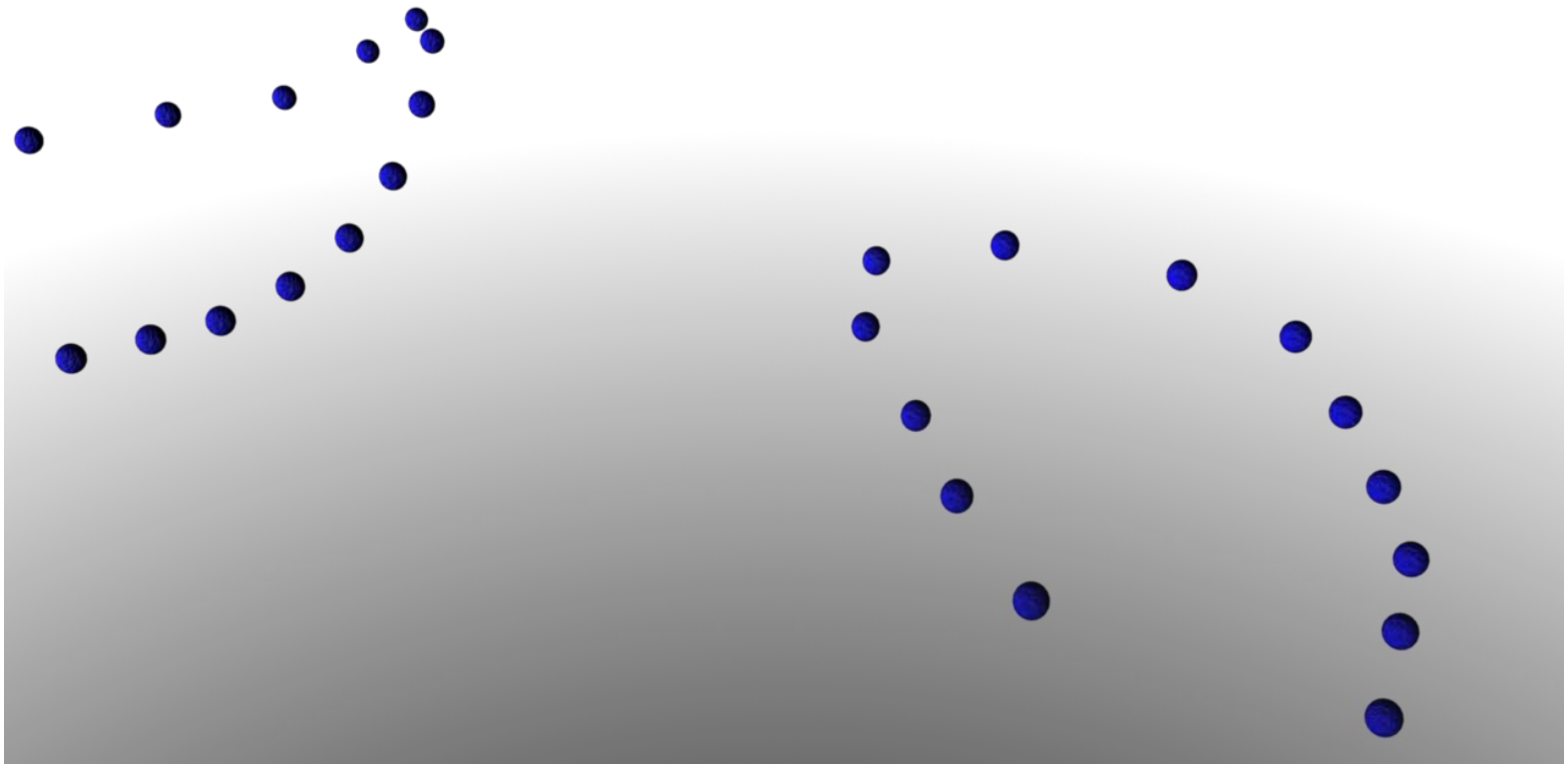
STED Microscopy

Stimulated Emission Depletion Microscopy

Basic Principle Step 0

The goal

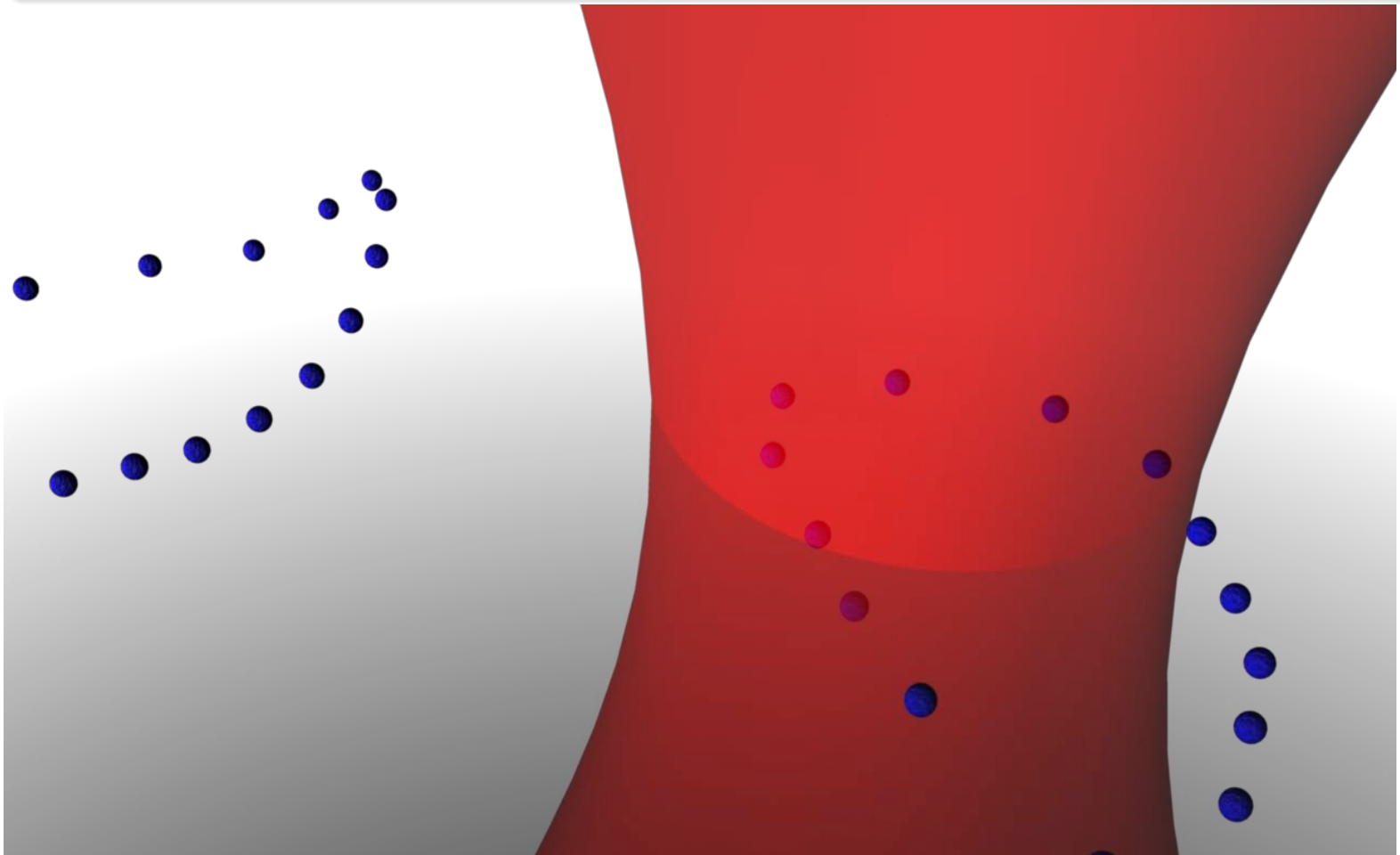
Make an image of a cell with previously inserted dye molecules for fluorescence microscopy.



Basic Principle Step 1

Excite the dyes

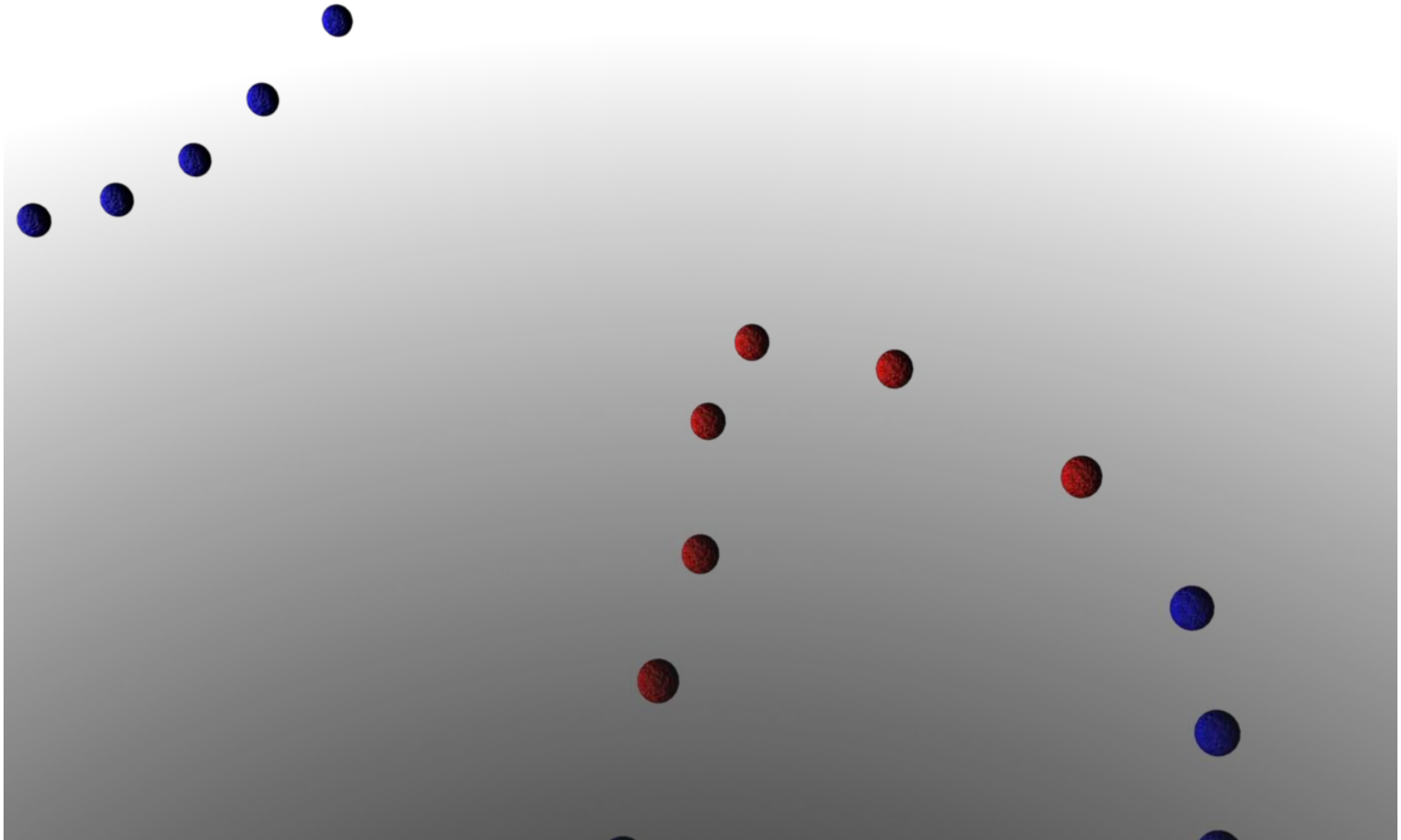
As in every fluorescence microscopy the dye molecules are excited by a Laser.



Basic Principle Step 2

At the edge of breaking the limit

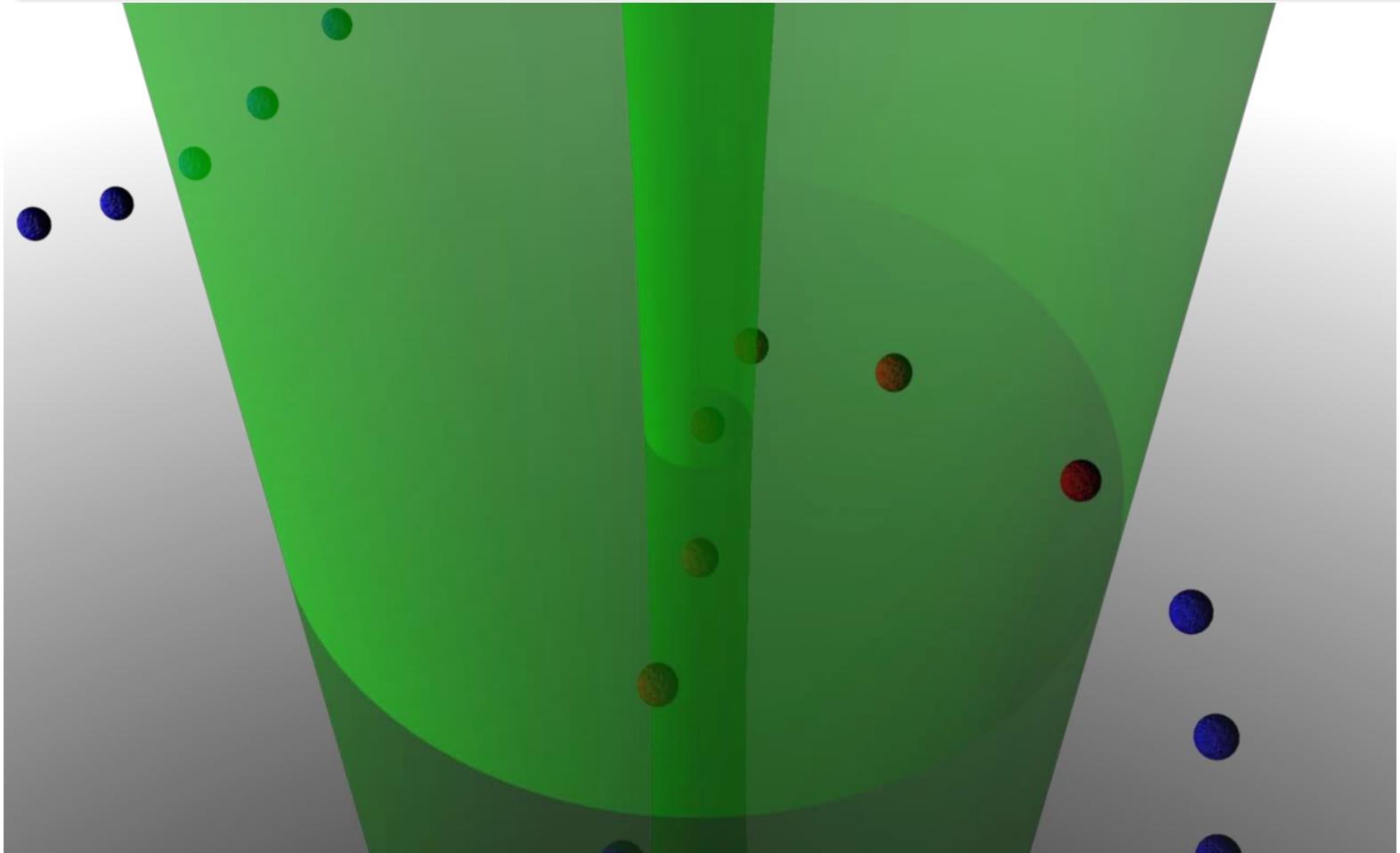
The Laser excites too much dye molecules for a good resolution. As we could not distinguish the light of the different dye molecules.



Basic Principle Step 3

De-excitation of the dye molecules

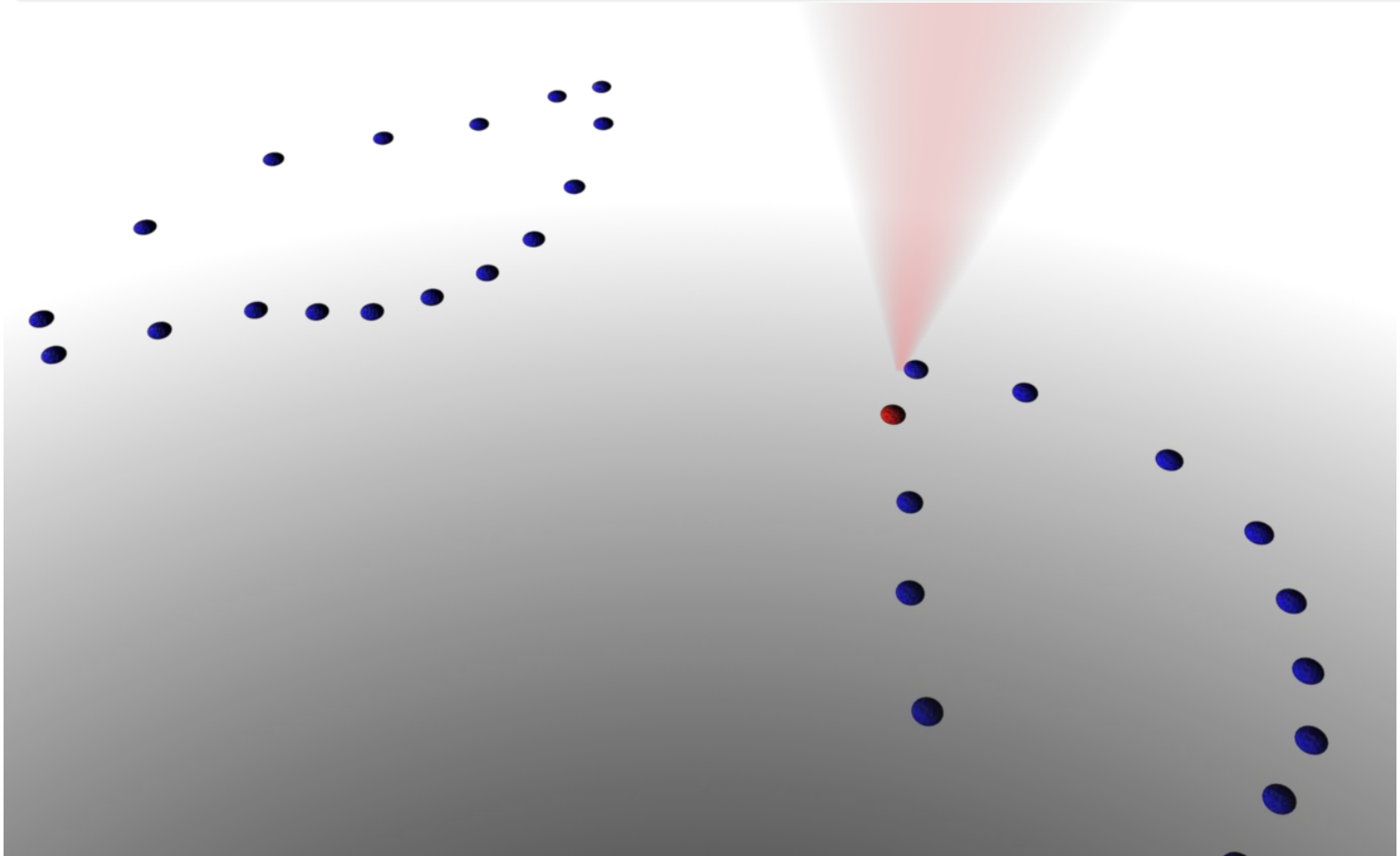
Another Laser with a donut shaped focus deexcite the molecules by stimulated emission.



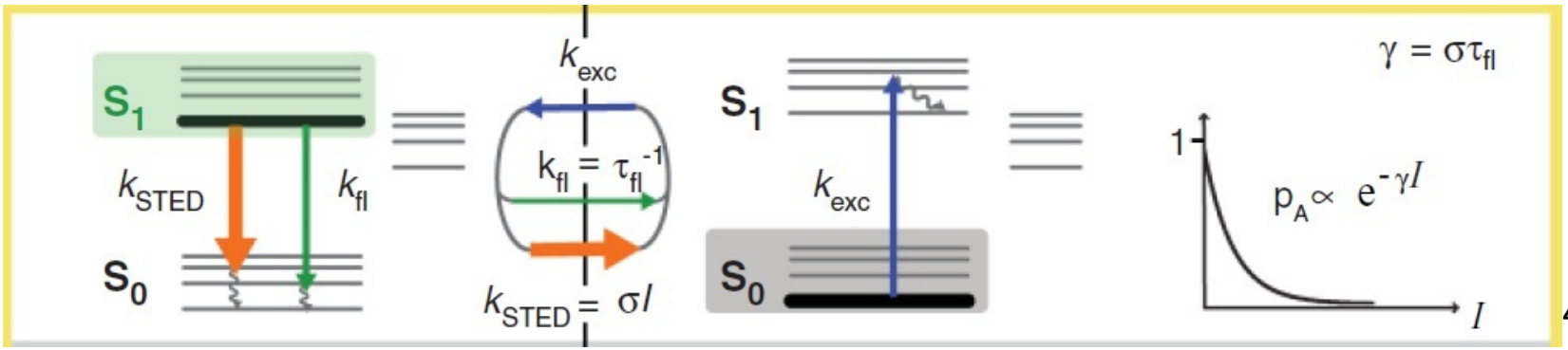
Basic Principle Step 4

Finish

Only selected molecules are still excited and can emit. Hence Abbes-Law is broken!



The Dye



4

Important attributes of the dye:

- 2 level system
- the lower level has sub levels
- decay time of excited level(k_{Fl}^{-1}) >> laser pulses
- decay rate of sub levels(k_{vib}) >> re excitation rate

4 source: S.W. Hell, Far-Field Optical Nanoscopy, www.sciencemag.org, 25/05/07

STED-Beam Intensity

Depletion of the fluorescence level:

$$\frac{dN_1}{dt} = -N_1 \sigma I_{STED}/\hbar\omega + N_0 \sigma I_{STED}/\hbar\omega - N_1 k_{Fl}$$

STED-Beam Intensity

Depletion of the fluorescence level:

$$\frac{dN_1}{dt} = -N_1\sigma I_{STED}/\hbar\omega + N_0\sigma I_{STED}/\hbar\omega - N_1 k_{Fl}$$

$$\frac{dN_0}{dt} = N_1\sigma I_{STED}/\hbar\omega - N_0\sigma I_{STED}/\hbar\omega - N_0 k_{vib}$$

STED-Beam Intensity

Depletion of the fluorescence level:

$$\frac{dN_1}{dt} = -N_1\sigma I_{STED}/\hbar\omega + N_0\sigma I_{STED}/\hbar\omega - N_1 k_{Fl}$$

$$\frac{dN_0}{dt} = N_1\sigma I_{STED}/\hbar\omega - N_0\sigma I_{STED}/\hbar\omega - N_0 k_{vib}$$

$$k_{vib} >> \sigma I_{STED}/\hbar\omega >> k_{FL} \rightarrow N_0 \approx 0$$

STED-Beam Intensity

Depletion of the fluorescence level:

$$\frac{dN_1}{dt} = -N_1\sigma I_{STED}/\hbar\omega + N_0\sigma I_{STED}/\hbar\omega - N_1 k_{Fl}$$

$$\frac{dN_0}{dt} = N_1\sigma I_{STED}/\hbar\omega - N_0\sigma I_{STED}/\hbar\omega - N_0 k_{vib}$$

$$k_{vib} >> \sigma I_{STED}/\hbar\omega >> k_{FL} \rightarrow N_0 \approx 0$$

$$\Rightarrow N_1 \propto e^{-\tau\sigma I_{STED}/\hbar\omega}$$

STED-Beam Intensity

Depletion of the fluorescence level:

$$\frac{dN_1}{dt} = -N_1\sigma I_{STED}/\hbar\omega + N_0\sigma I_{STED}/\hbar\omega - N_1 k_{Fl}$$

$$\frac{dN_0}{dt} = N_1\sigma I_{STED}/\hbar\omega - N_0\sigma I_{STED}/\hbar\omega - N_0 k_{vib}$$

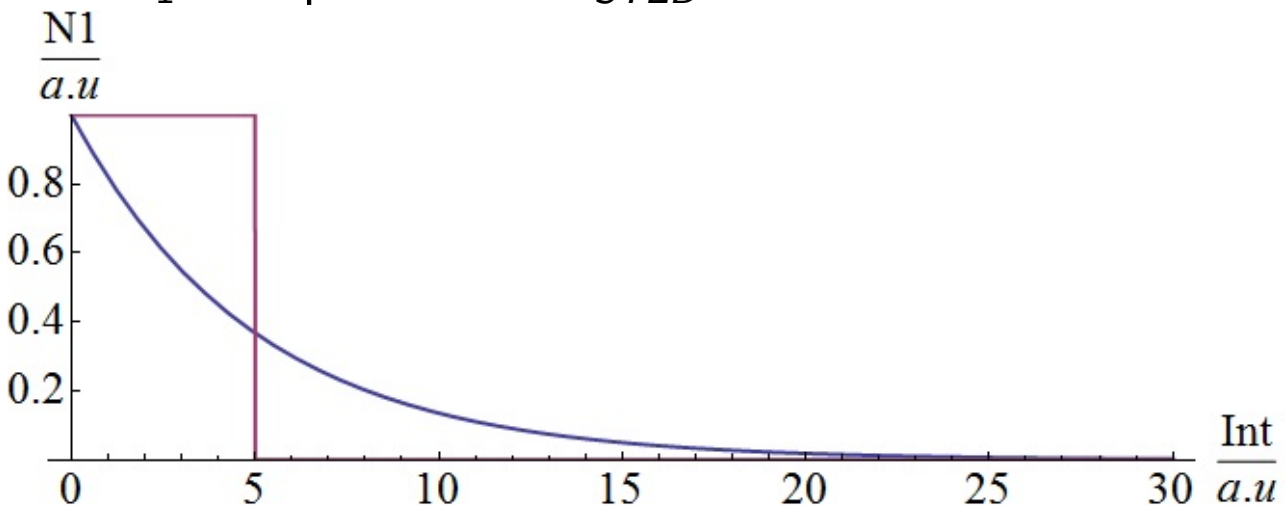
$$k_{vib} >> \sigma I_{STED}/\hbar\omega >> k_{FL} \rightarrow N_0 \approx 0$$

$$\Rightarrow N_1 \propto e^{-\tau\sigma I_{STED}/\hbar\omega}$$

We can estimate $N_1(I_{STED})$ as a step function which becomes 0 at $I_{STED} = \frac{\hbar\omega}{\tau\sigma}$

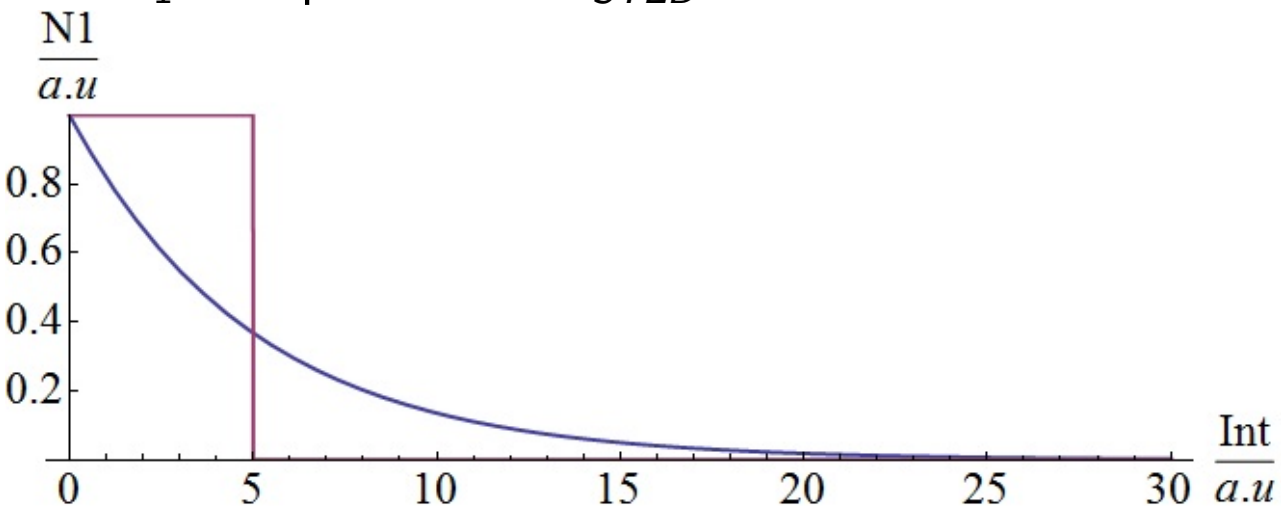
Area of Excited Molecules

The N_1 occupation over I_{STED}:

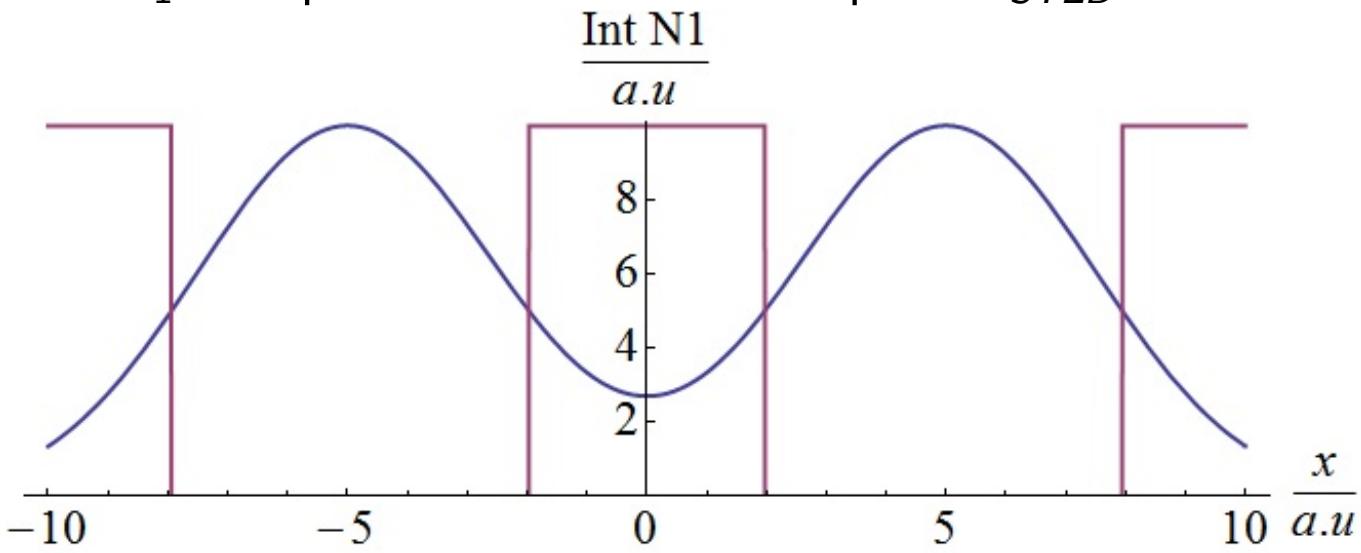


Area of Excited Molecules

The N_1 occupation over I_{STED}:



The N_1 occupation folded with the spatial I_{STED} distribution:



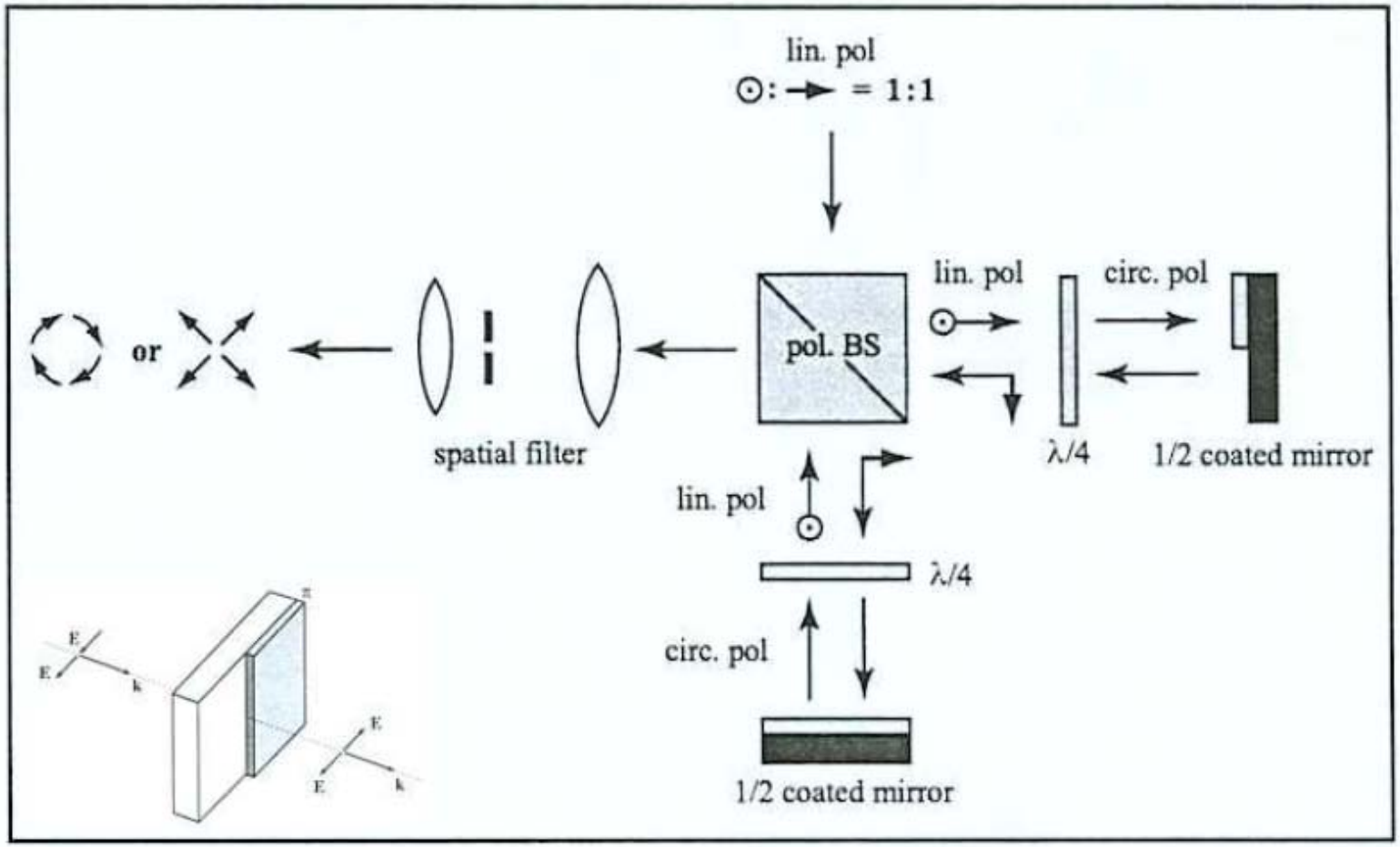
New Resolution

Resolution of a STED microscope

$$\Delta x = \frac{\lambda}{2 \cdot NA \cdot \sqrt{1 + \frac{I_{STED}}{I_{Sat}}}}$$

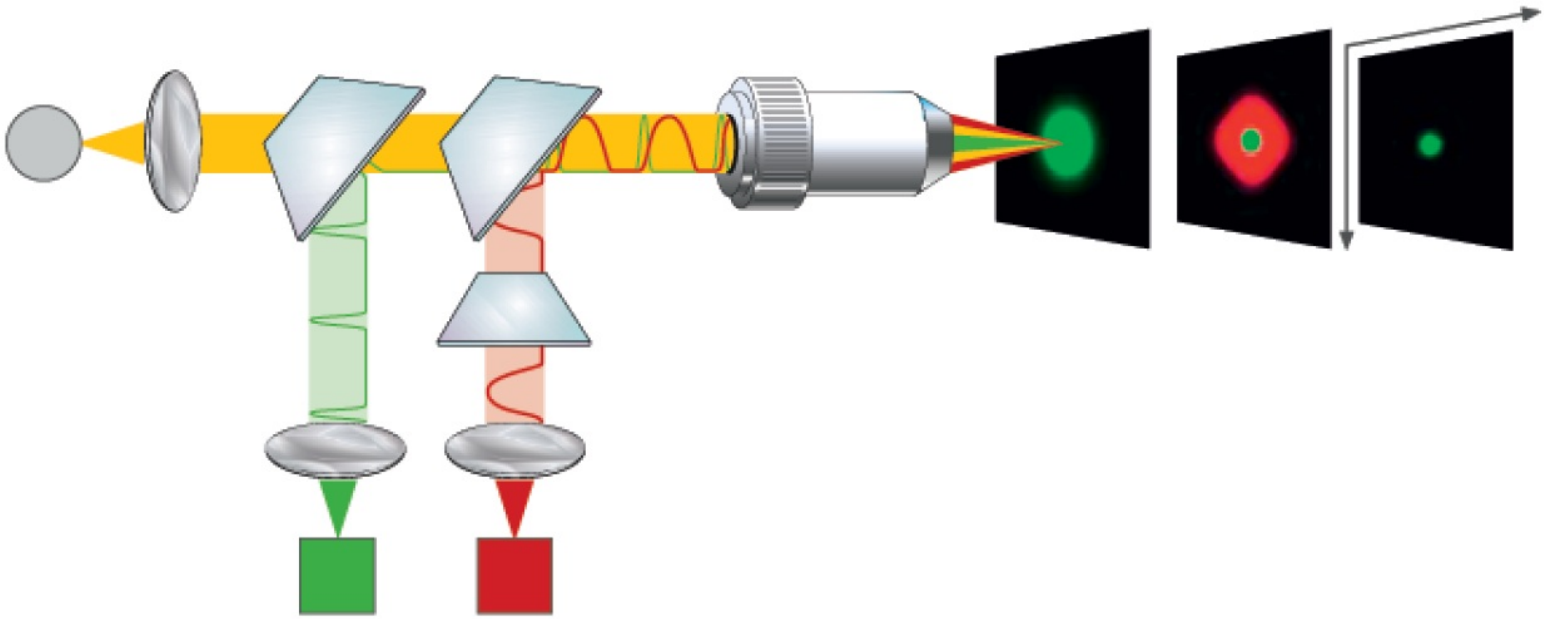
As the improvement is due to the limited area of light sources I_{STED} must be the crucial parameter.

The Donut-Mode



The minimum in the center of the STED Focus is made by giving the half of the inner laying beams an phase shift of π

The STED Setup

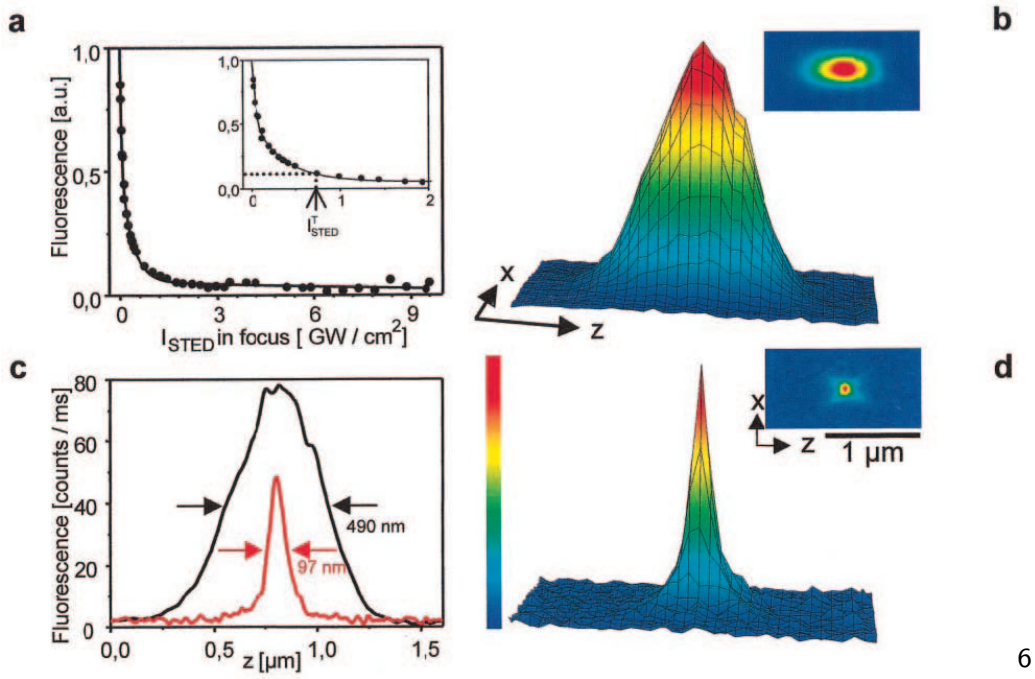


5

The STED setup is similar to the setup of a confocal or 4Pi microscope. With the difference that one more beam has to be coupled into the optical axes.

[5] source: Imaging with the Leica TCS STED a Practical Guide

Proof of Concept



6

Experiment details:

- dye: LDS 751
- $I_{STED} = 2.8 GW/cm^2$
- $\lambda_{exc} = 540nm$
- $\lambda_{STED} = 700nm$

Results:

a) predicted dependence N_1 from I_{STED}

b) measured lateral FID[7] without the STED beam

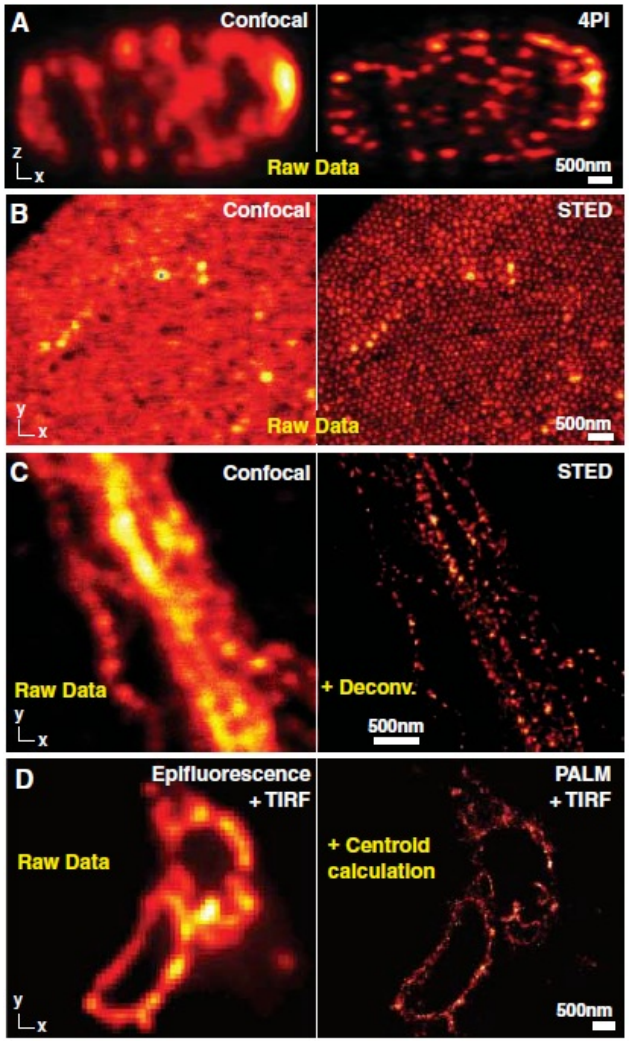
c) reduced axial FID with the STED beam

d) reduced lateral FID with the STED beam

6 source: Hell et al., Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission

7 FID stands for fluorescence light intensity distribution

STED Applications: Biophysics



Resolution:

- transversal 16 nm
- longitudinal 32 nm

Probes:

a) microtubules in a neuron

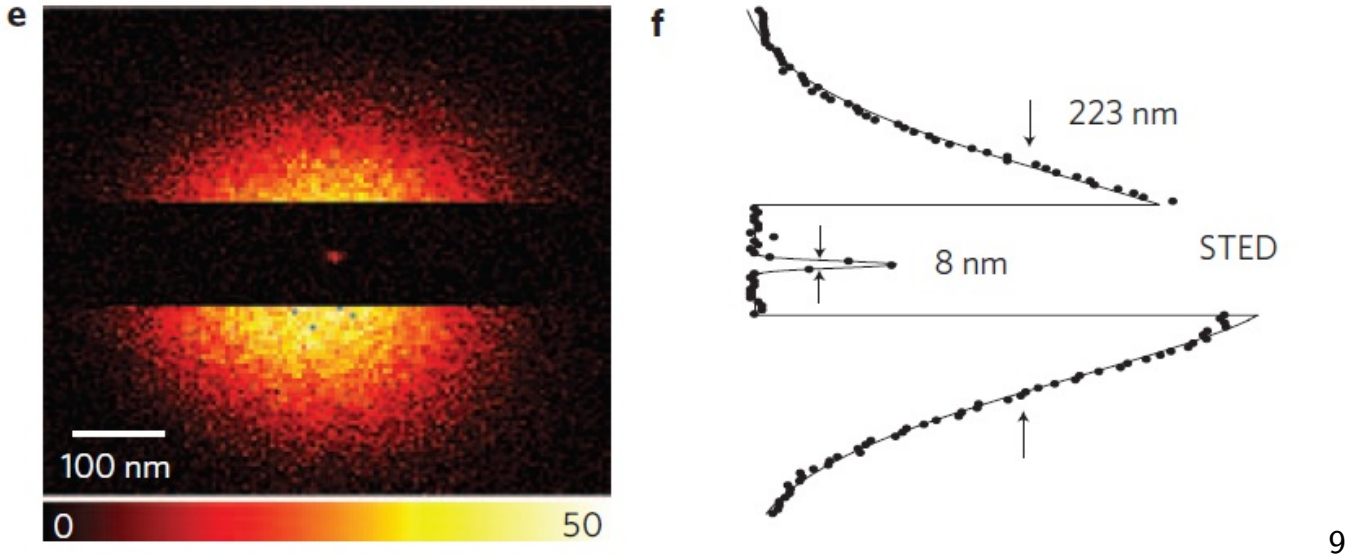
b) silica nanobeads

c) neurofilamente

d) cell membrane

[8] source: S.W. Hell, Far-Field Optical Nanoscopy, www.sciencemag.org, 25/05/07

Record of Resolution



[9]

Experiment details:

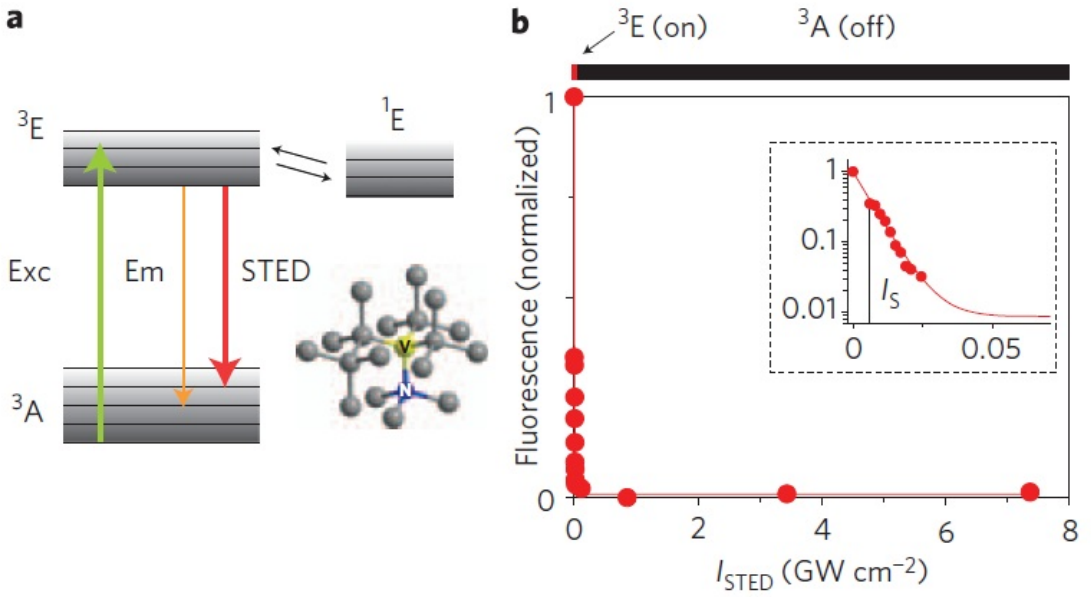
- dye: nitrogen vacancies in diamond
- $I_{STED} = 3.7 GW/cm^2$
- $\lambda_{exc} = 532nm$
- $\lambda_{STED} = 775nm$

Results:

- resolution goes up to 8 nm
- with $I_{STED} = 8.6 GW/cm^2$ even 6 nm

[9] source: see next page

Diamond Colour Centers

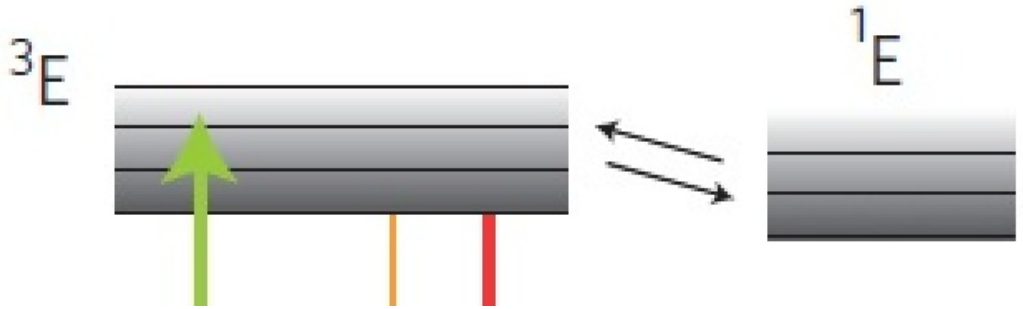


10

- luminescent transitions arising from nitrogen vacancies in the diamond structure

10 Hell et al, STED microscopy reveals crystal colour centers with nanometric resolution, nature photonics , 22/02/09

Diamond Colour Centers



[11]

- Centers with $m_s = 0$ emit more strongly, because the $m_s = \pm 1$ centers convert often to the metastable state ^1E
- optical measurement of spins
- usable as magnetic field sensor or data storage

[11] Hell et al, STED microscopy reveals crystal colour centers with nanometric resolution, nature photonics , 22/02/09

Conclusion

- Theoretically no more resolution limits for far field microscopy
- Practically bounded by the destruction threshold or cross section of the medium