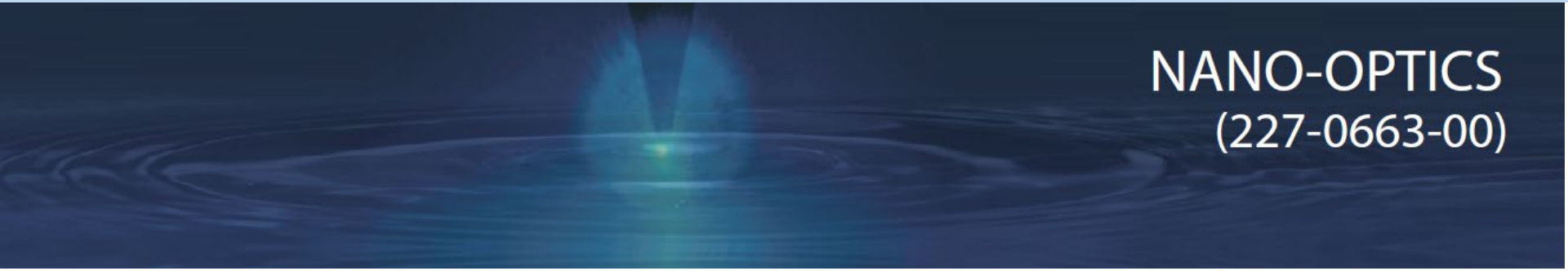
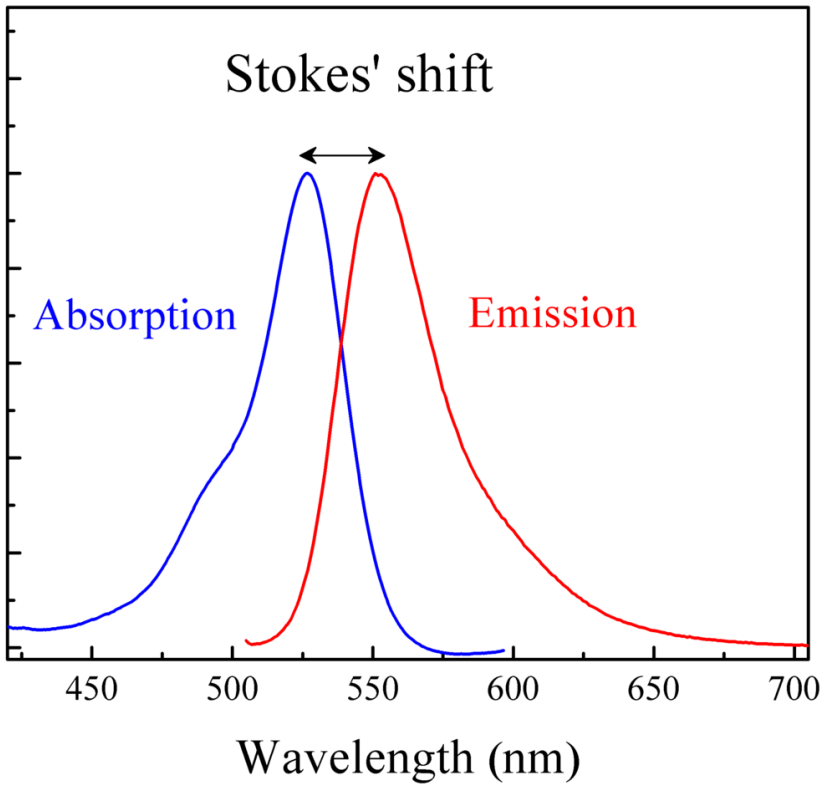
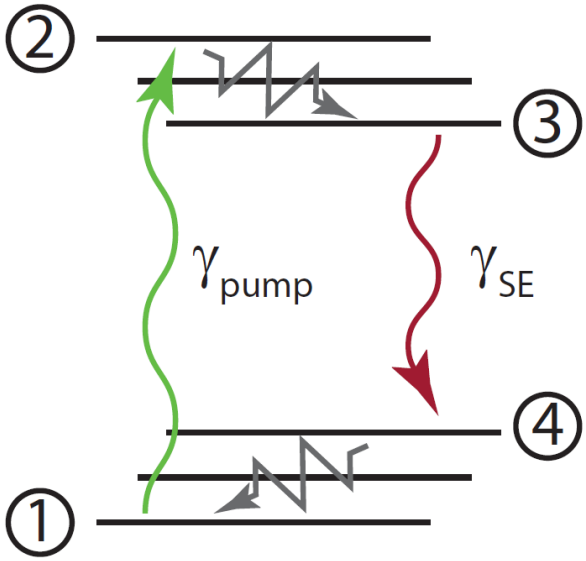
Welcome again!



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

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

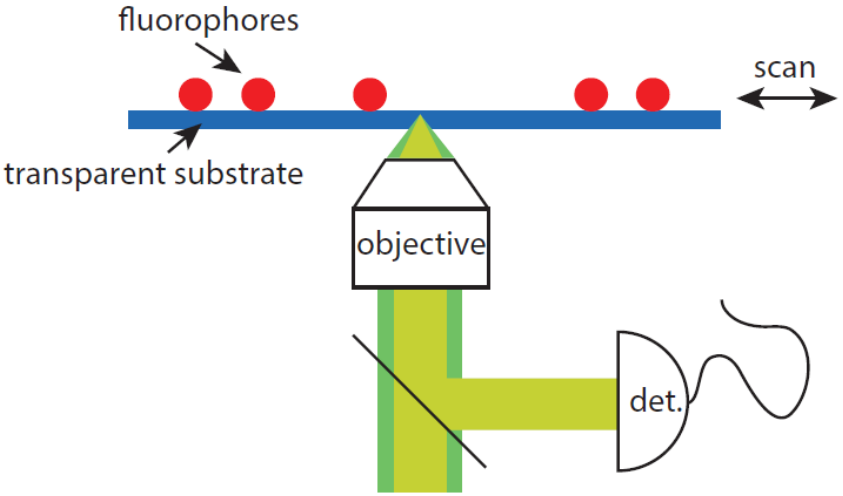
STED – how it works

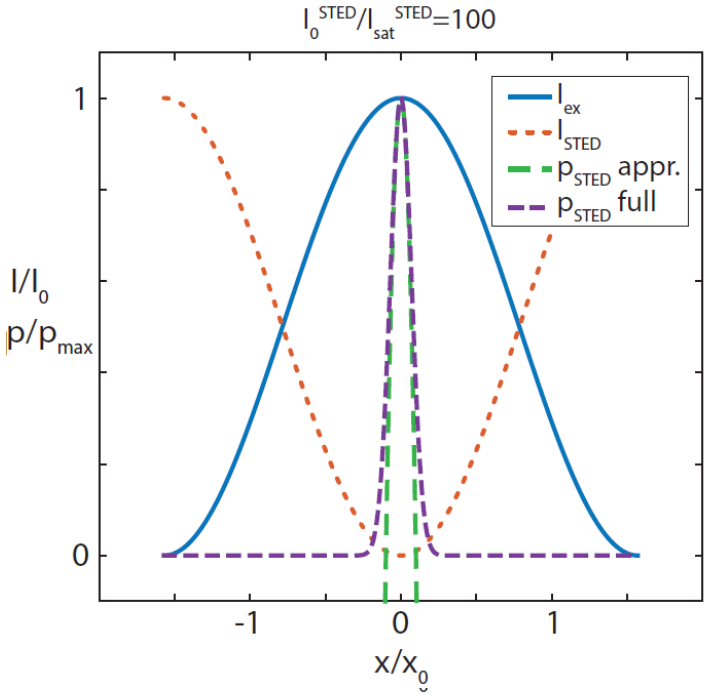
- FWHM of area of remaining pumped fluorophores after STED pulse

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

Characteristic saturation intensity:

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





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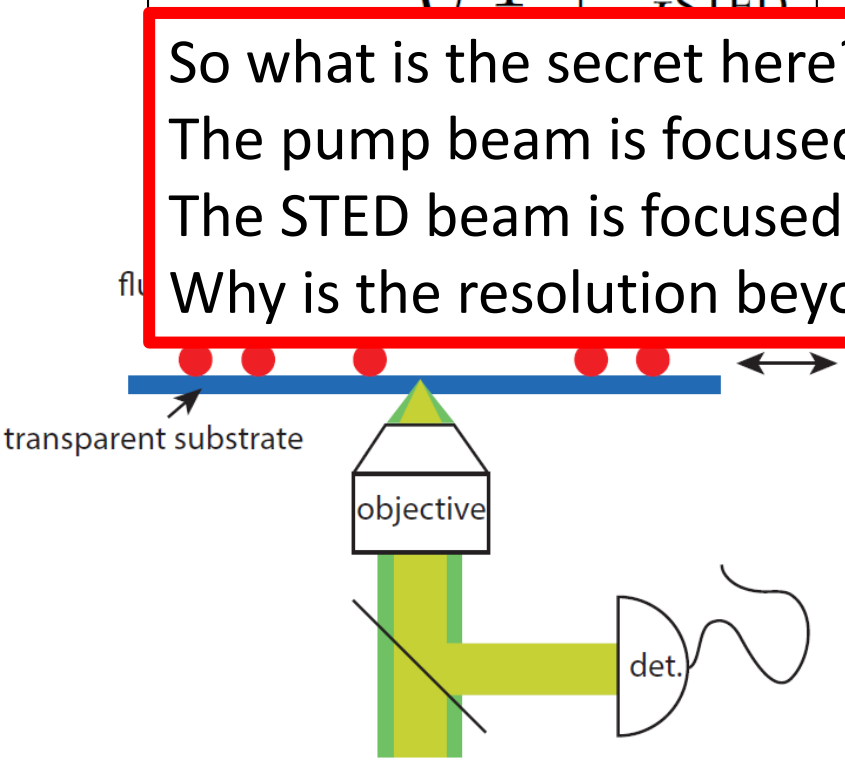
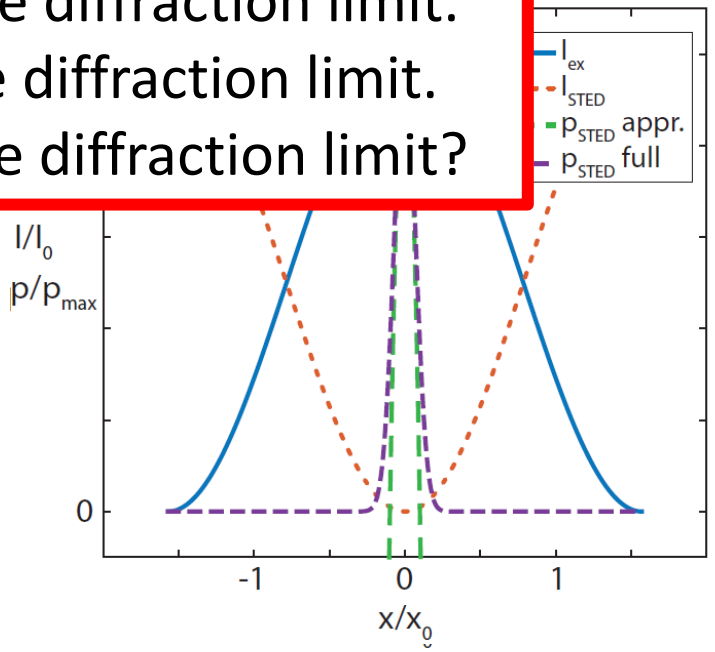
Characteristic saturation intensity:

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

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?





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

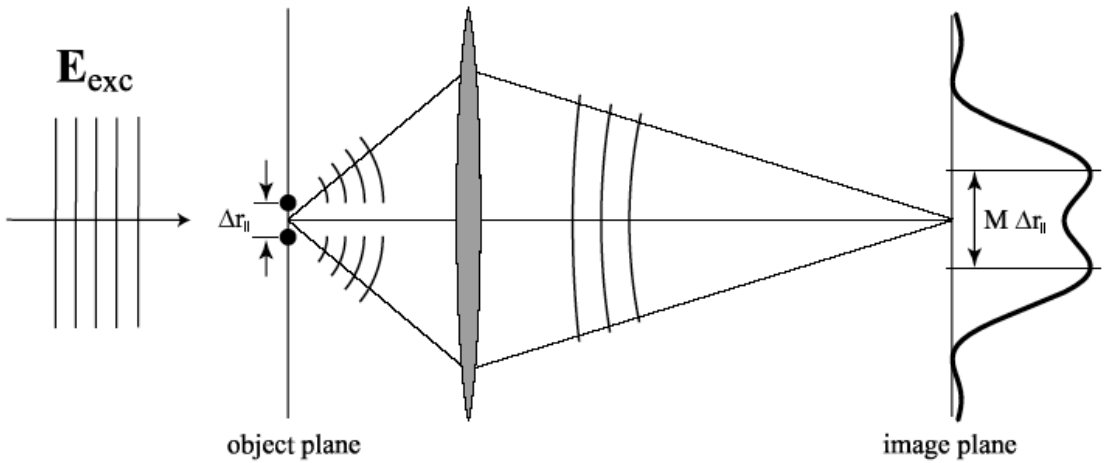
Different names for (in principle) the same technique:

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

STORM – localization microscopy

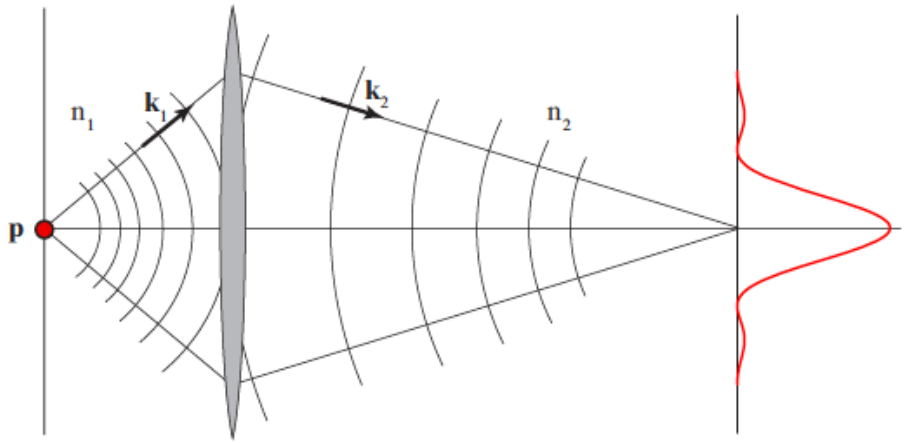
- Abbe tells me how closely spaced two sources can be for them to be discernible

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

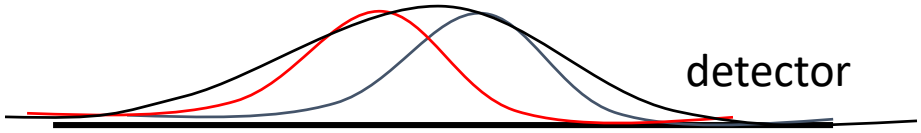


- But how well can I localize a single emitter? (given that I know it is a single one)

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

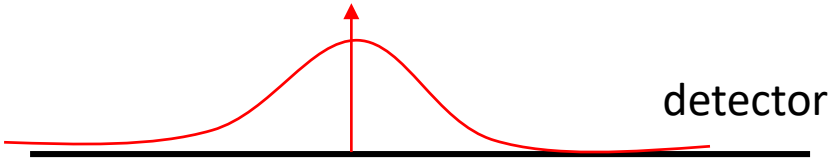


STORM – localization microscopy

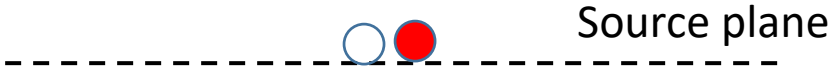


- Let's assume we image 2 emitters spaced at a distance smaller than the diffraction limit

STORM – localization microscopy

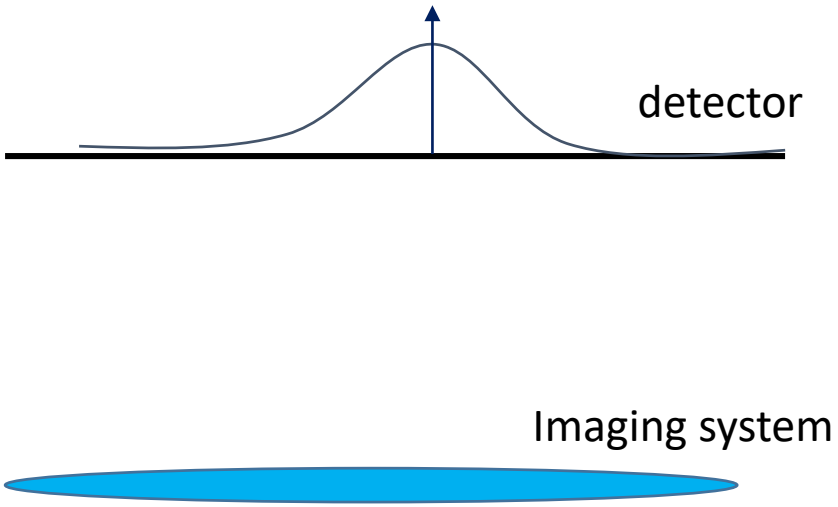


- Emitter 1 on, emitter 2 off
  → localize emitter 1 better than diffraction limit



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

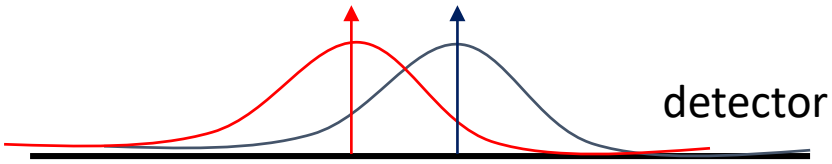
STORM – localization microscopy

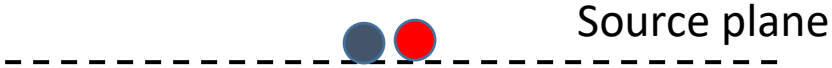


- Let's assume we image 2 emitters spaced at a distance smaller than the diffraction limit
- Emitter 2 on, emitter 1 off → localize emitter 2 better than diffraction limit

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

PALM, STORM – localization microscopy





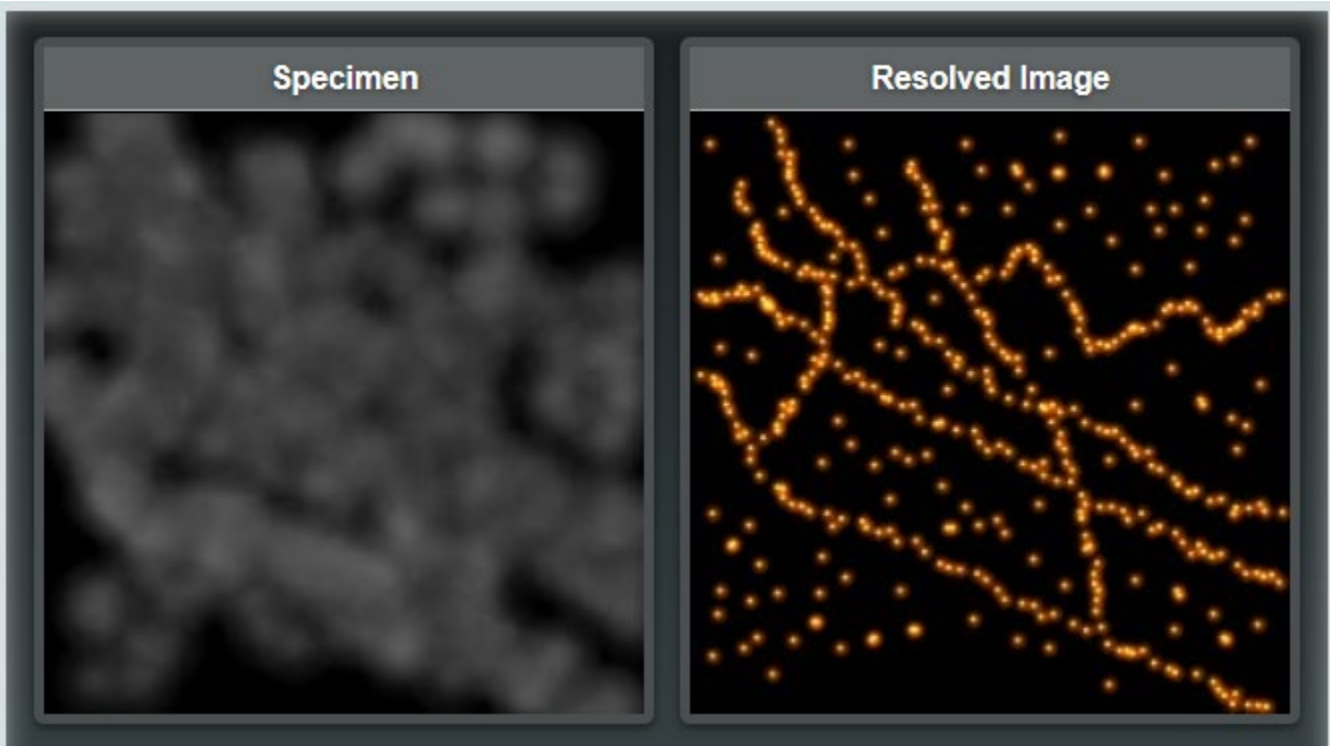
$$\Delta_x = \frac{1}{\sqrt{N}} \frac{\lambda}{2\,\mathrm{NA}}$$

- Emitter 1 on, emitter 2 off → localize emitter 1 better than diffraction limit
- Emitter 2 on, emitter 1 off → localize emitter 2 better than diffraction limit

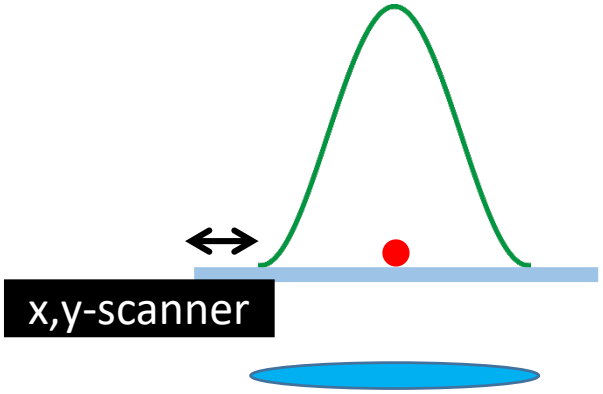
➔For this technique we need fluorophores which can be switched on and off ("photoactivated" or "stochastic")

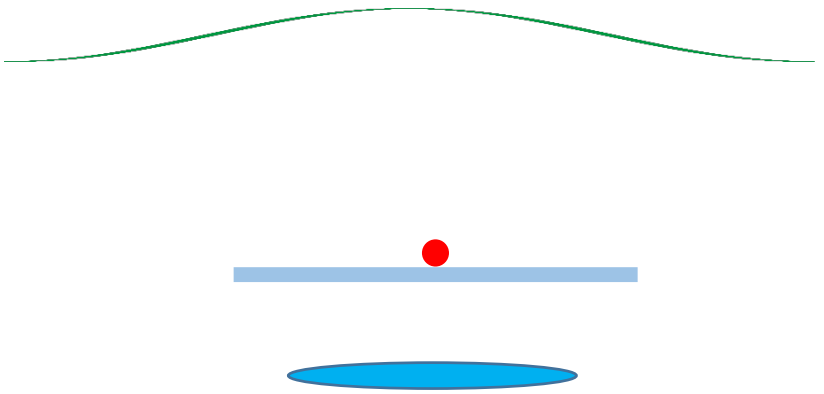
STORM

- https://www.microscopyu.com/tutorials/stochastic-optical-reconstruction-microscopy-storm-imaging



Fluorescence microscopy – scanning vs. wide-field





Scanning technique.

Resolution s

pump spot on sample

(no spatial resolution)

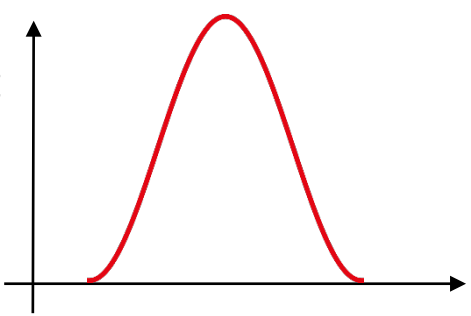
Both limited by diffraction.

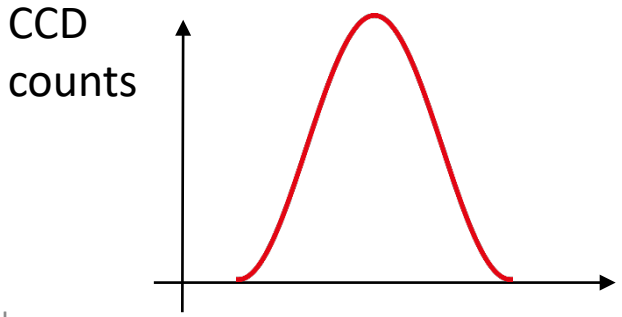
Wide-field imaging.

t by PSF of

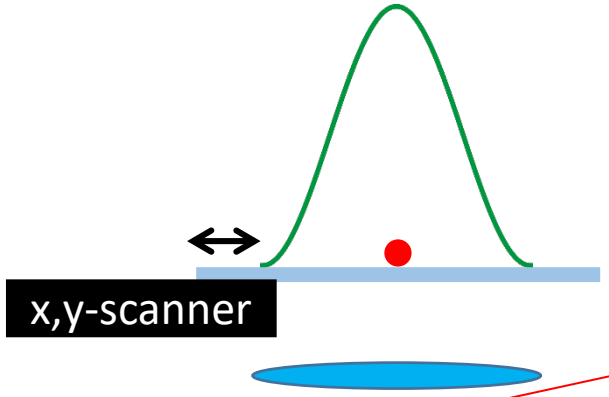
imaging system

CCD camera



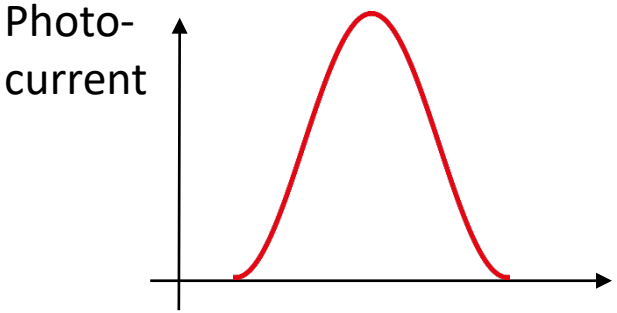


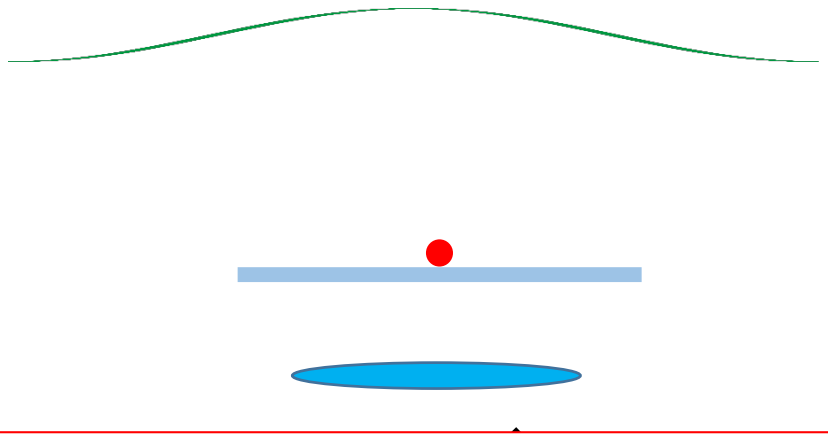
Fluorescence microscopy – scanning vs. wide-field



Should we pump weak or hard?

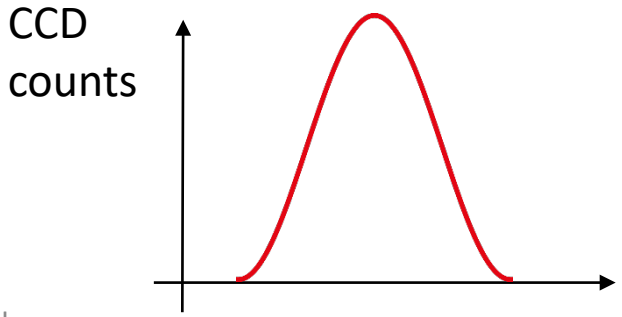
Photodiode
(no spatial resolution)



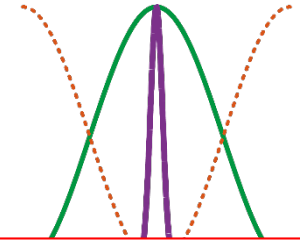


Wide-field imaging.
Resolution set by PSF of imaging system

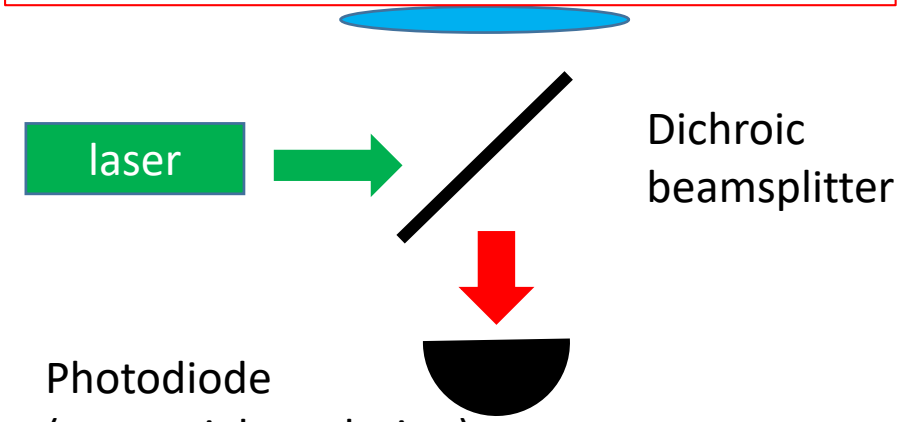
CCD camera



STED vs. STORM microscopy



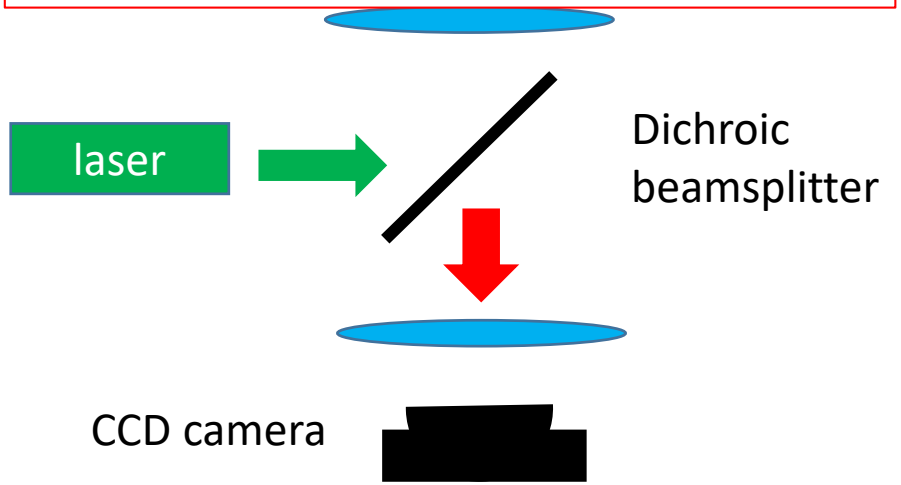
Scanning technique.



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

Stage position

Wide-field imaging.



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

Position on CCD chip

Where do we stand?

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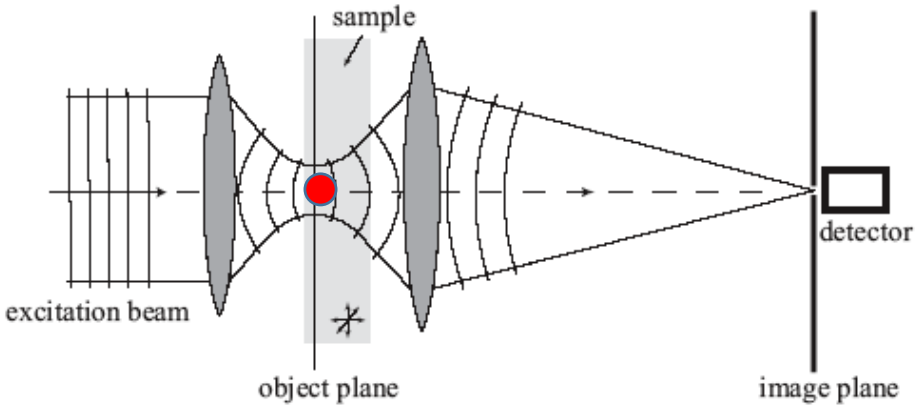
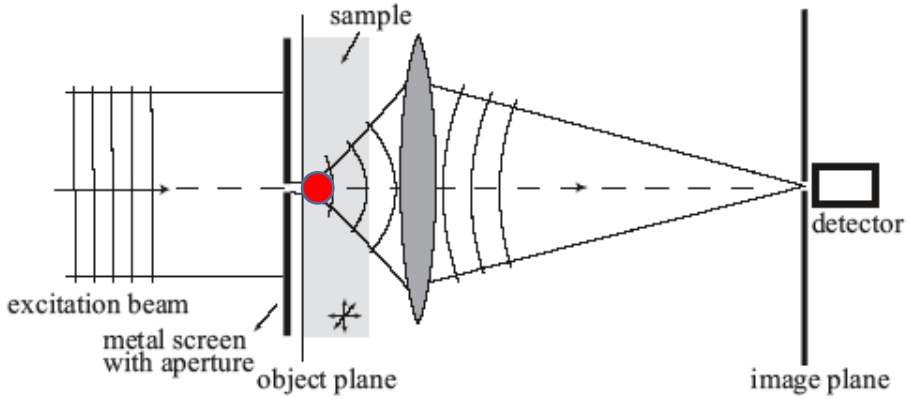
Near-field microscopy

- So far we played some tricks to enhance the resolution of an image in the far-field (what were these tricks?)
- But how can we exploit evanescent (near-)fields?

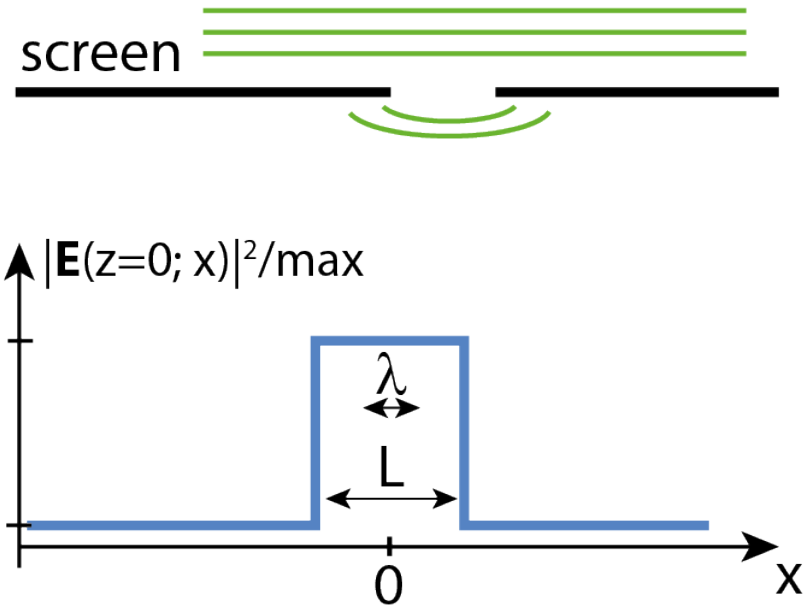
Confocal:



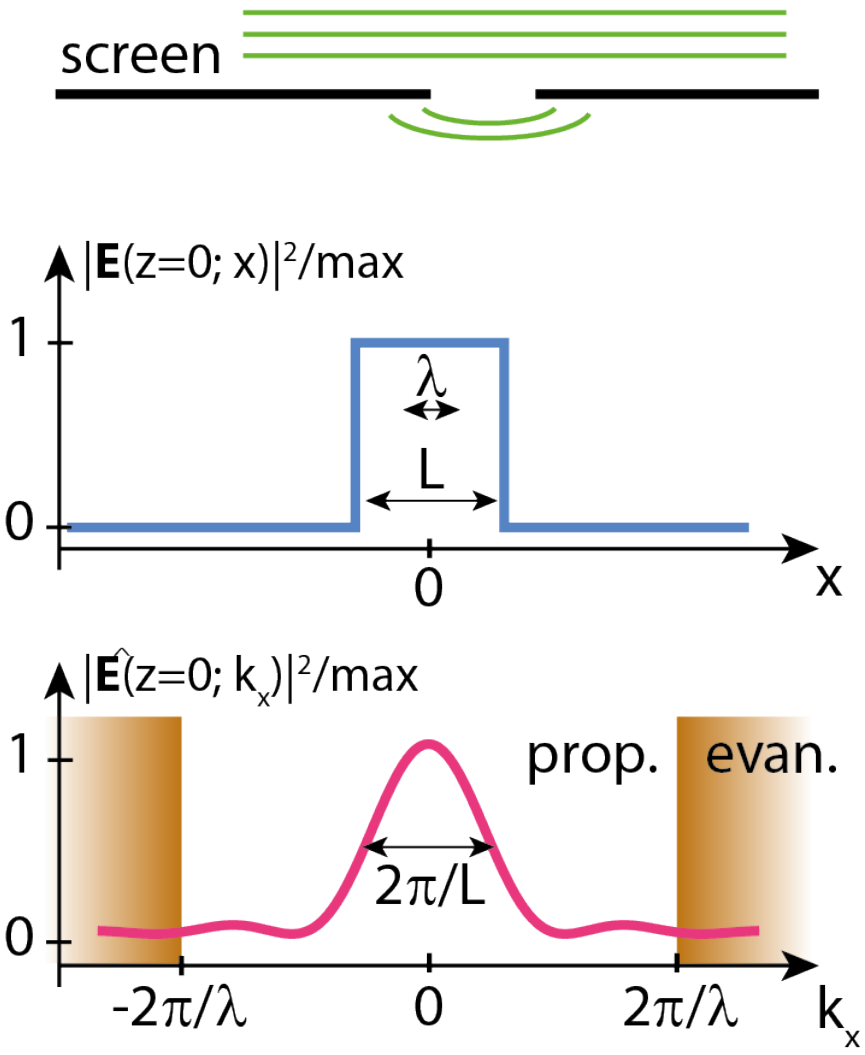
Near-field:



Fields behind an aperture



Fields behind an aperture



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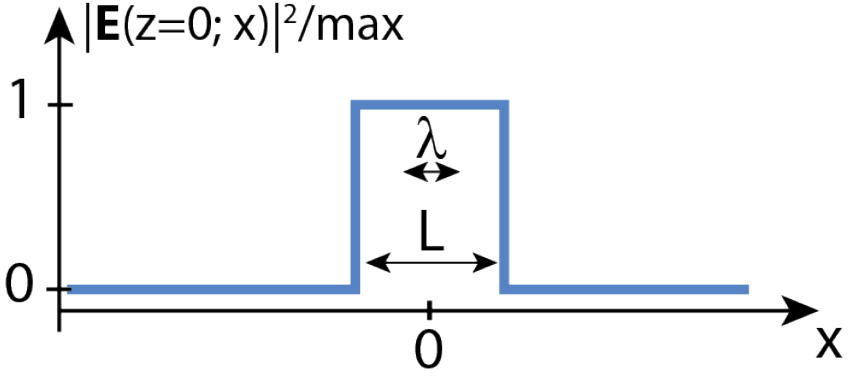

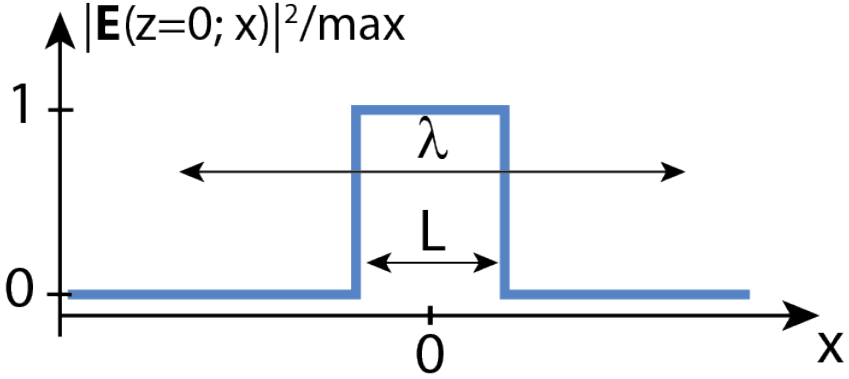

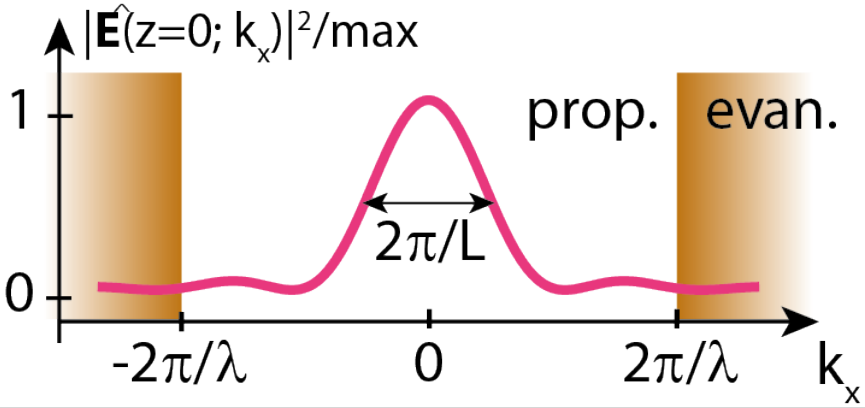

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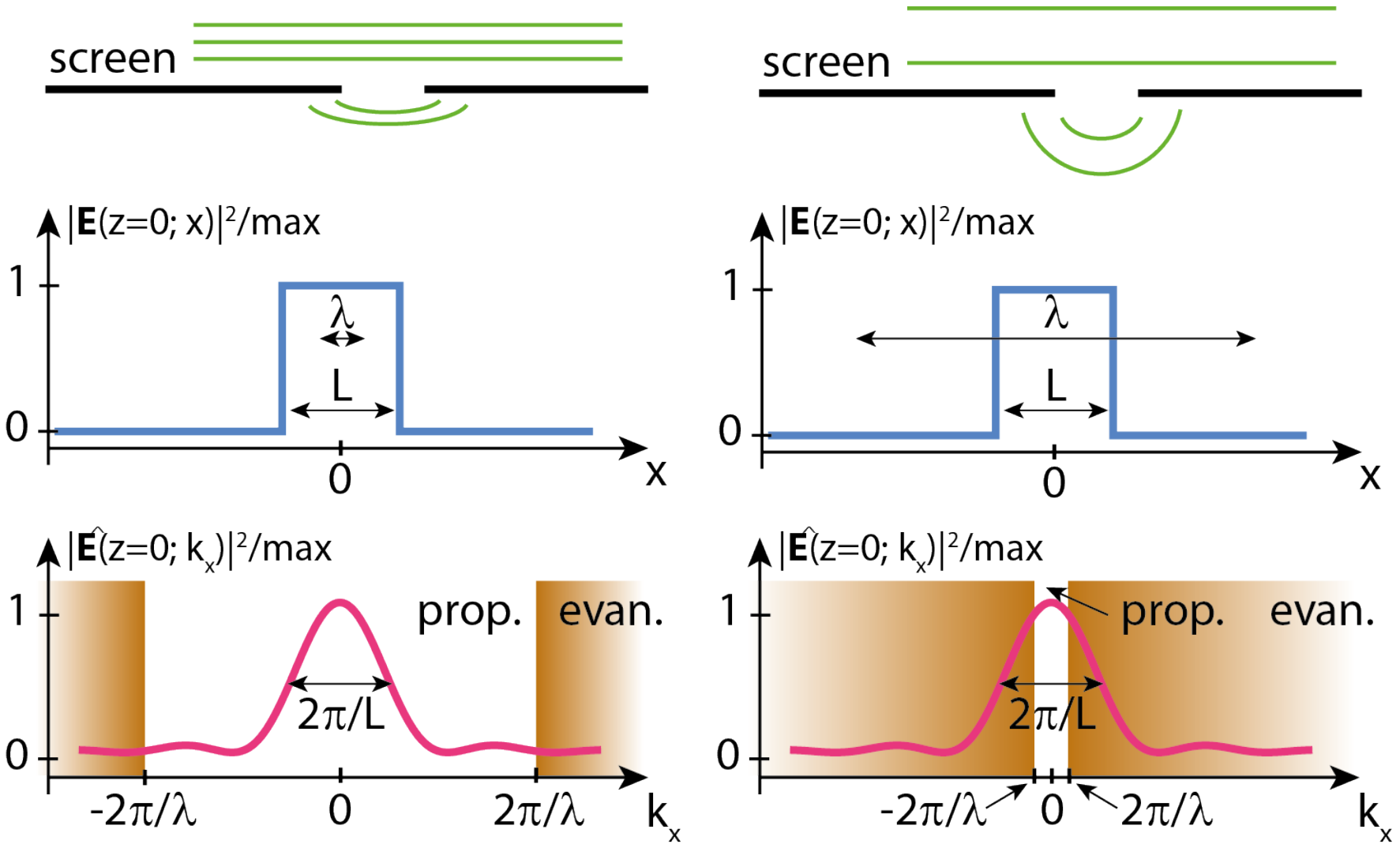

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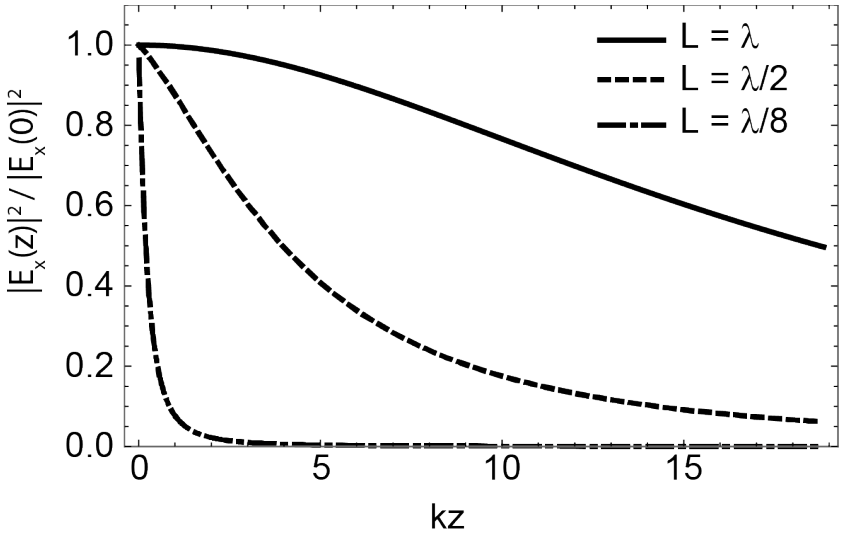
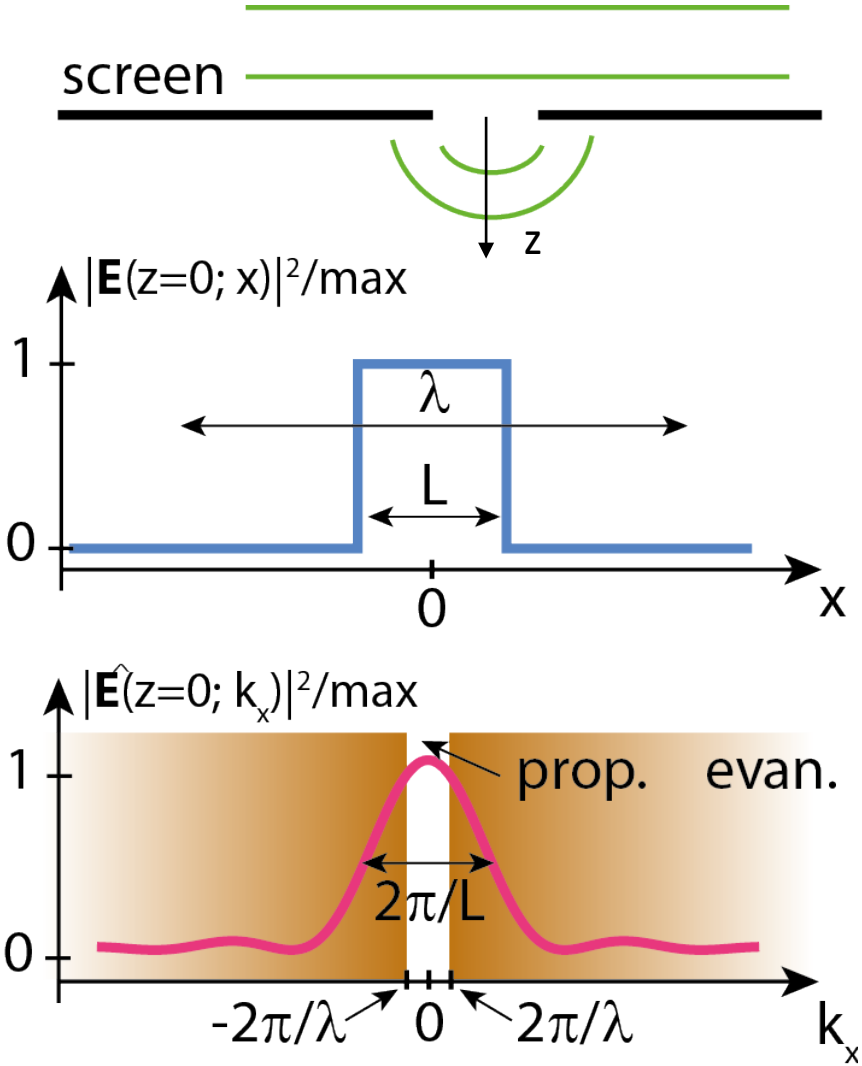
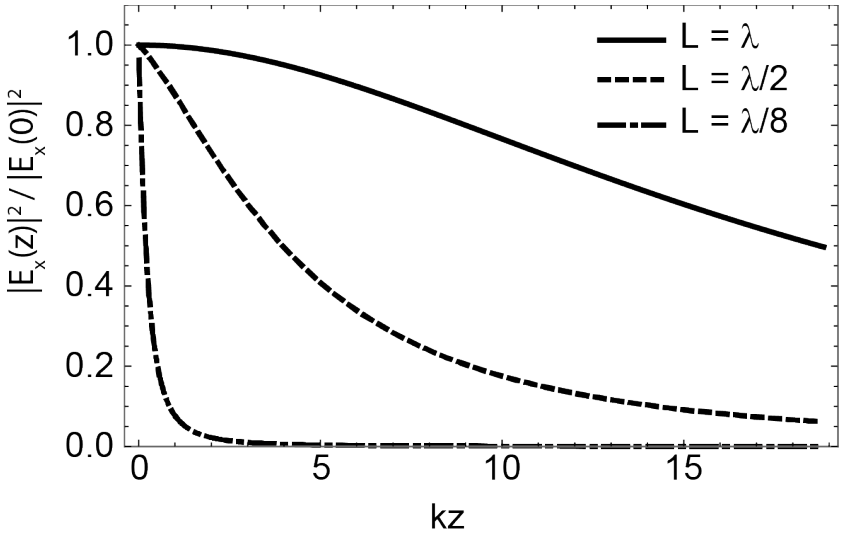
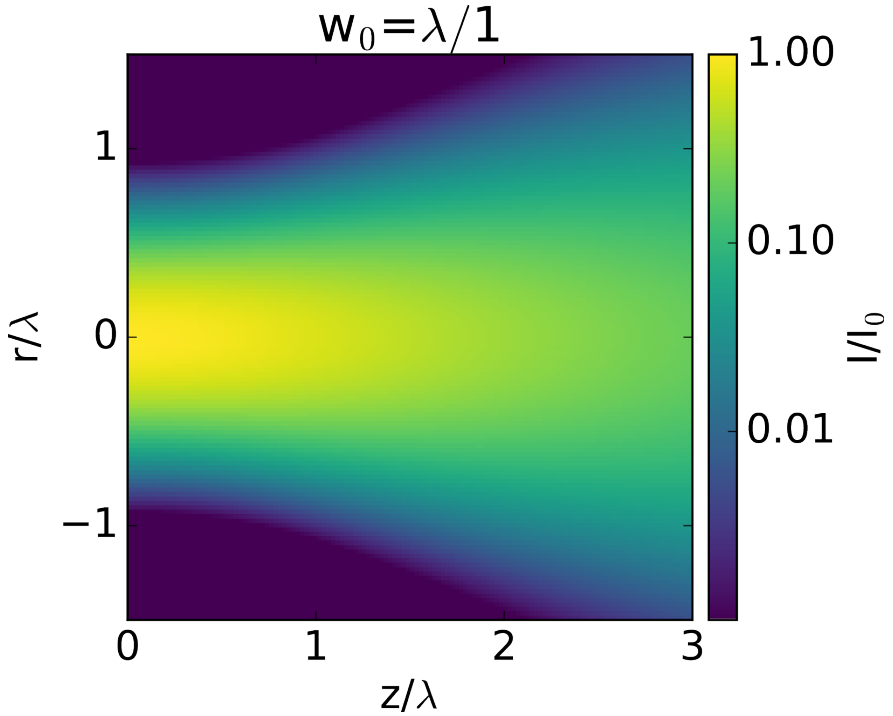


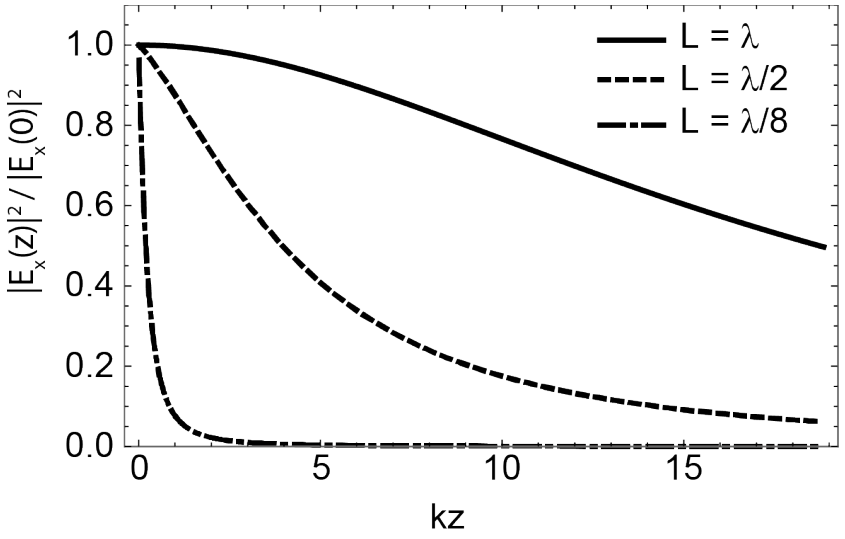


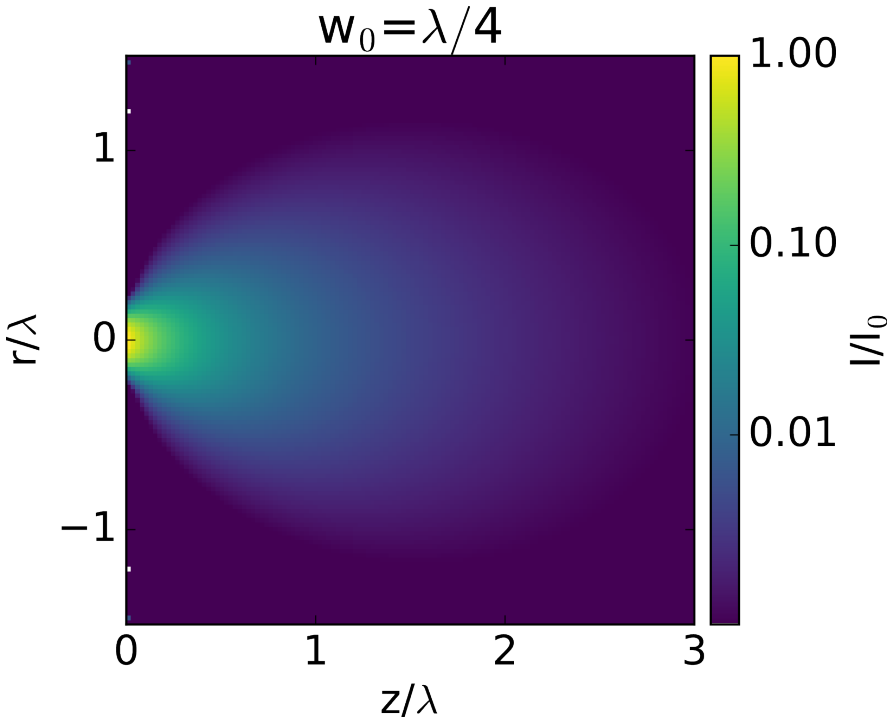
Fields behind a (Gaussian) aperture



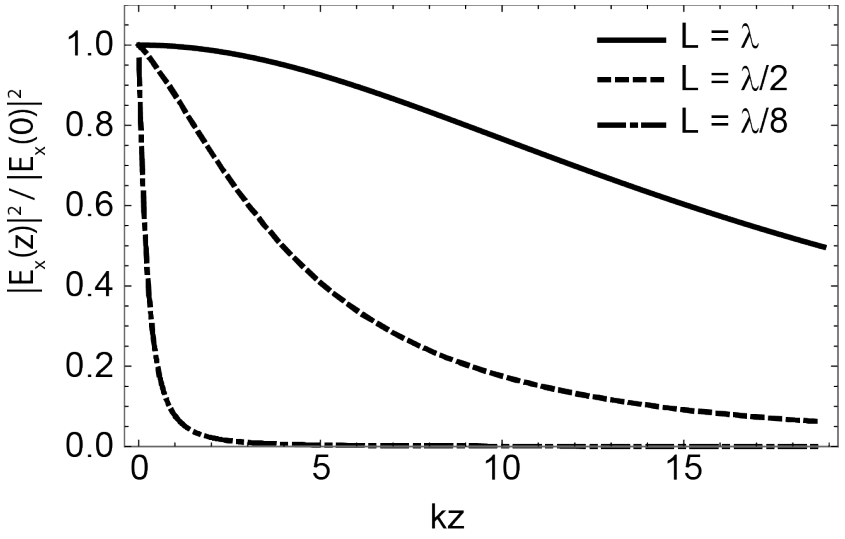


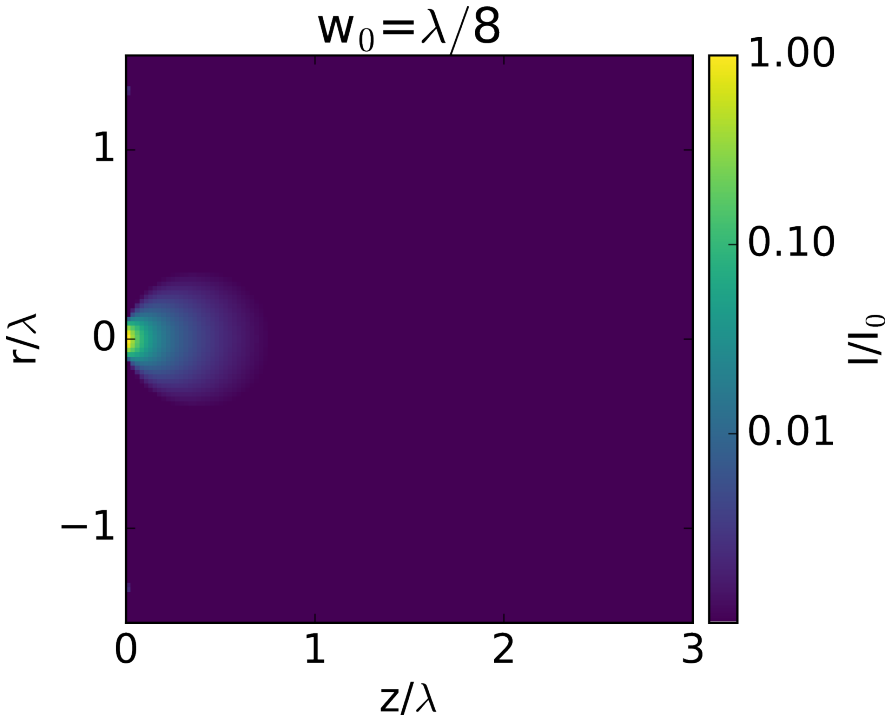
Fields behind a (Gaussian) aperture



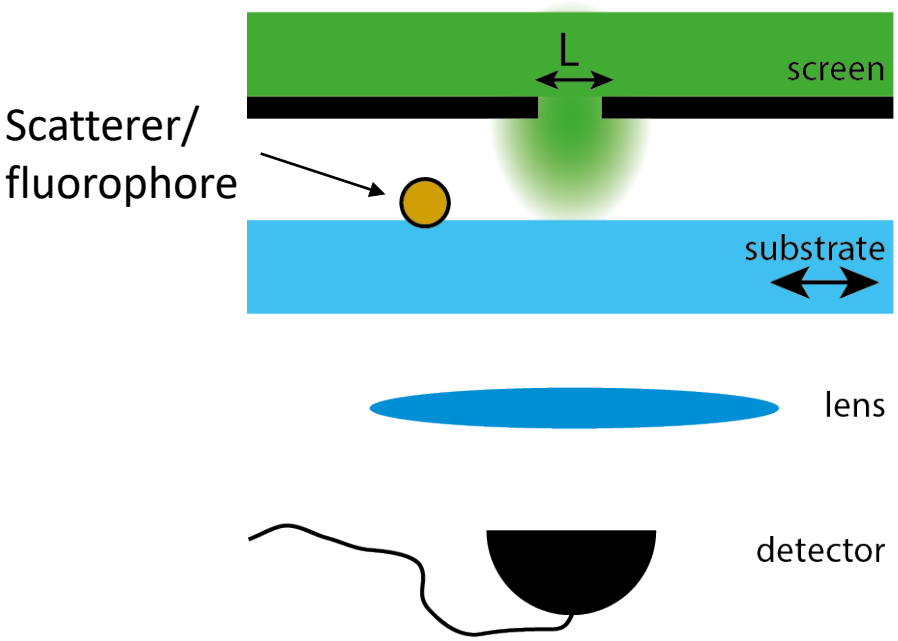


Fields behind a (Gaussian) aperture

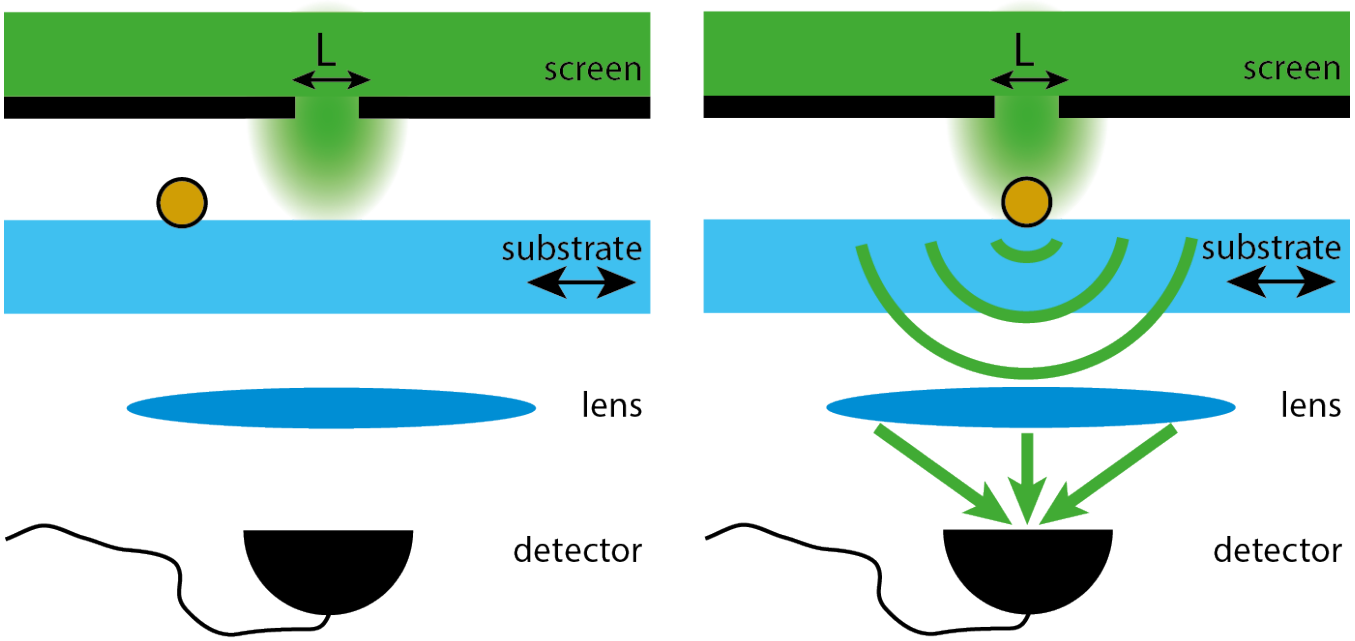




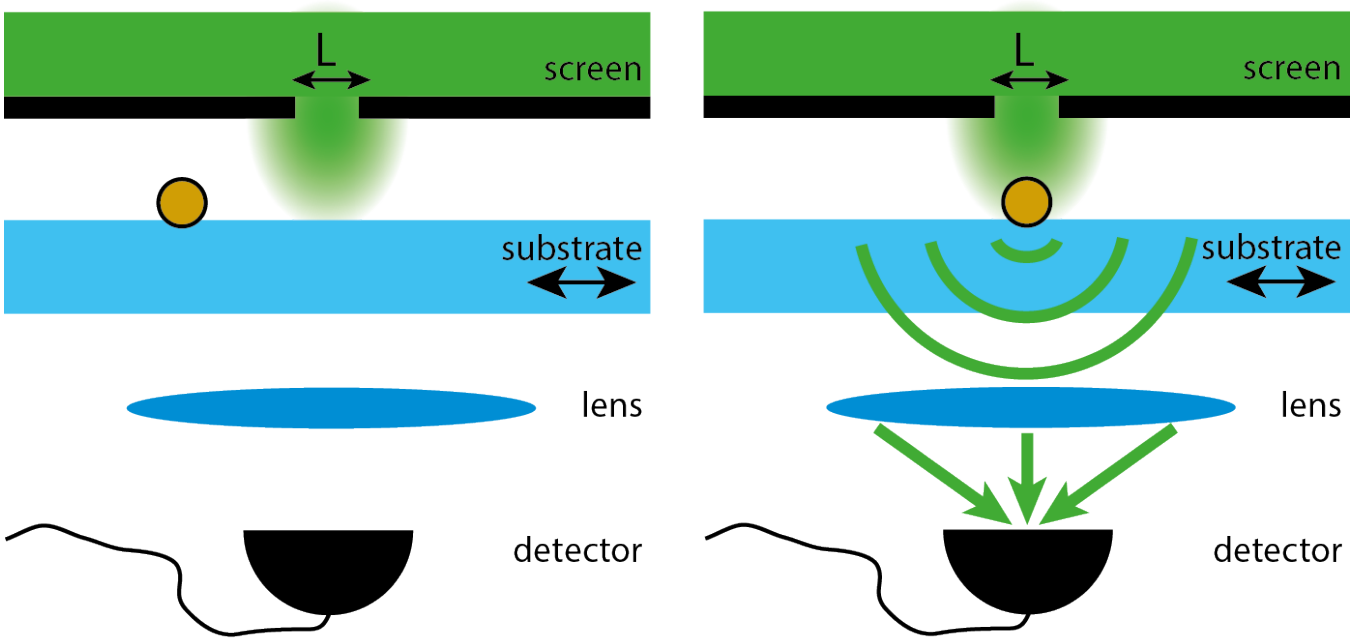
The principle of NSOM

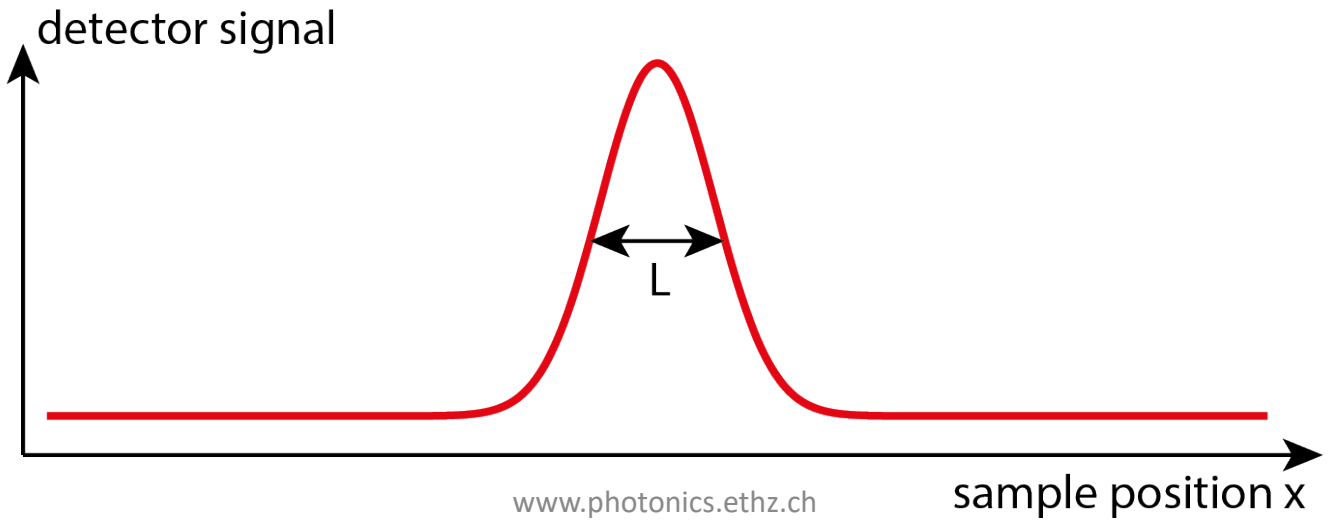


The principle of NSOM



The principle of NSOM

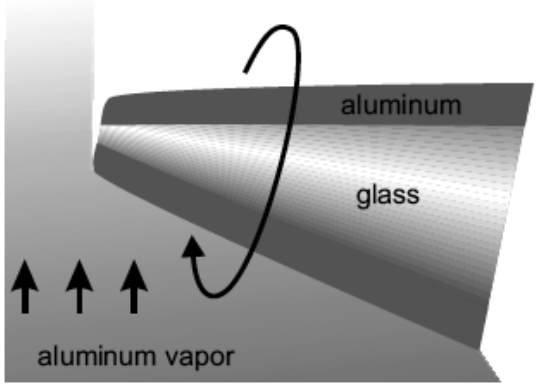


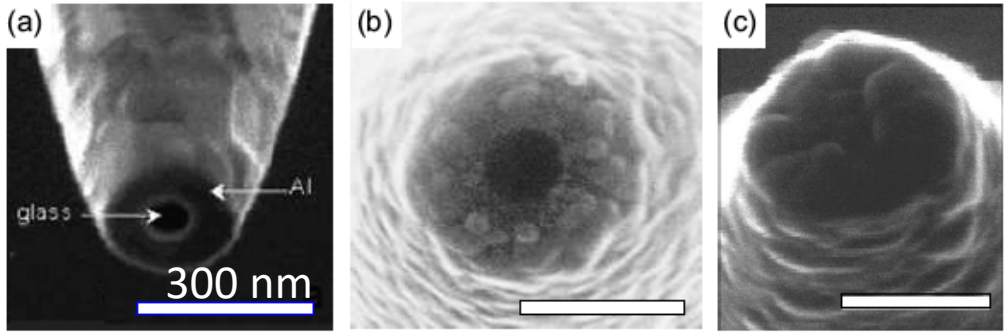


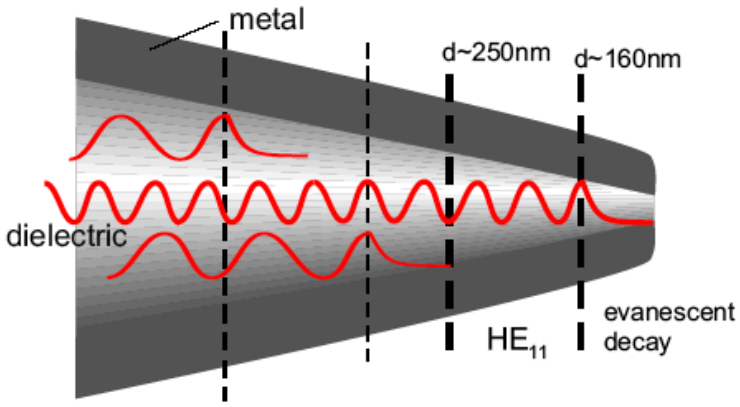
NSOM – how it's really done

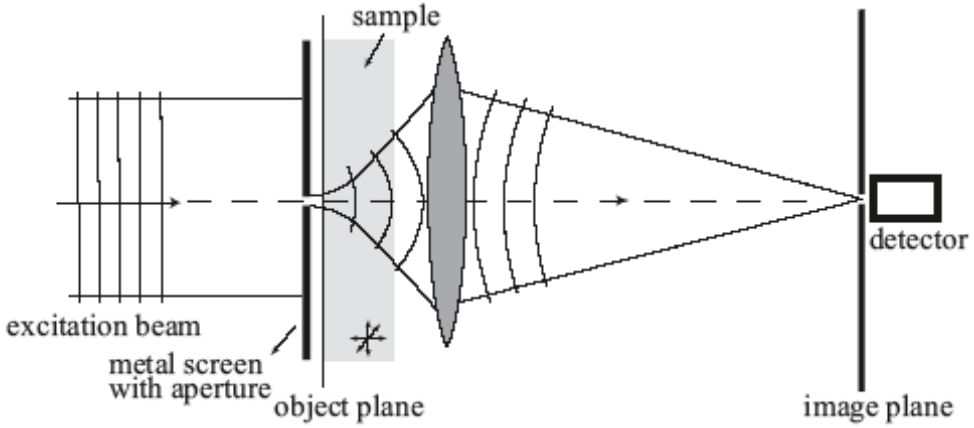
- Metal coated fiber tip

Hecht et al., J Chem. Phys. 112, 7761



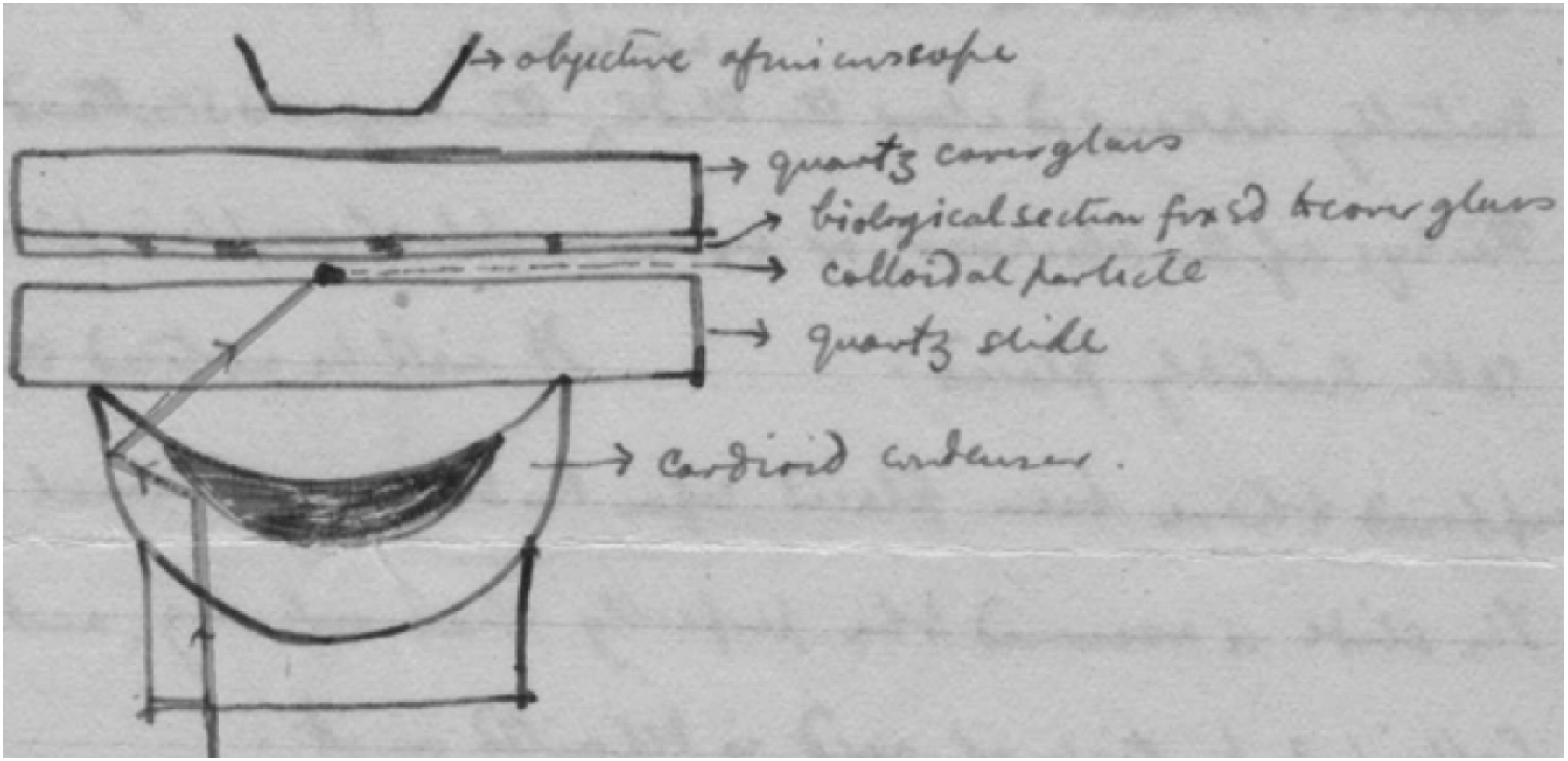






The idea of NSOM is not new…

Sketch sent by Synge to Einstein in 1928



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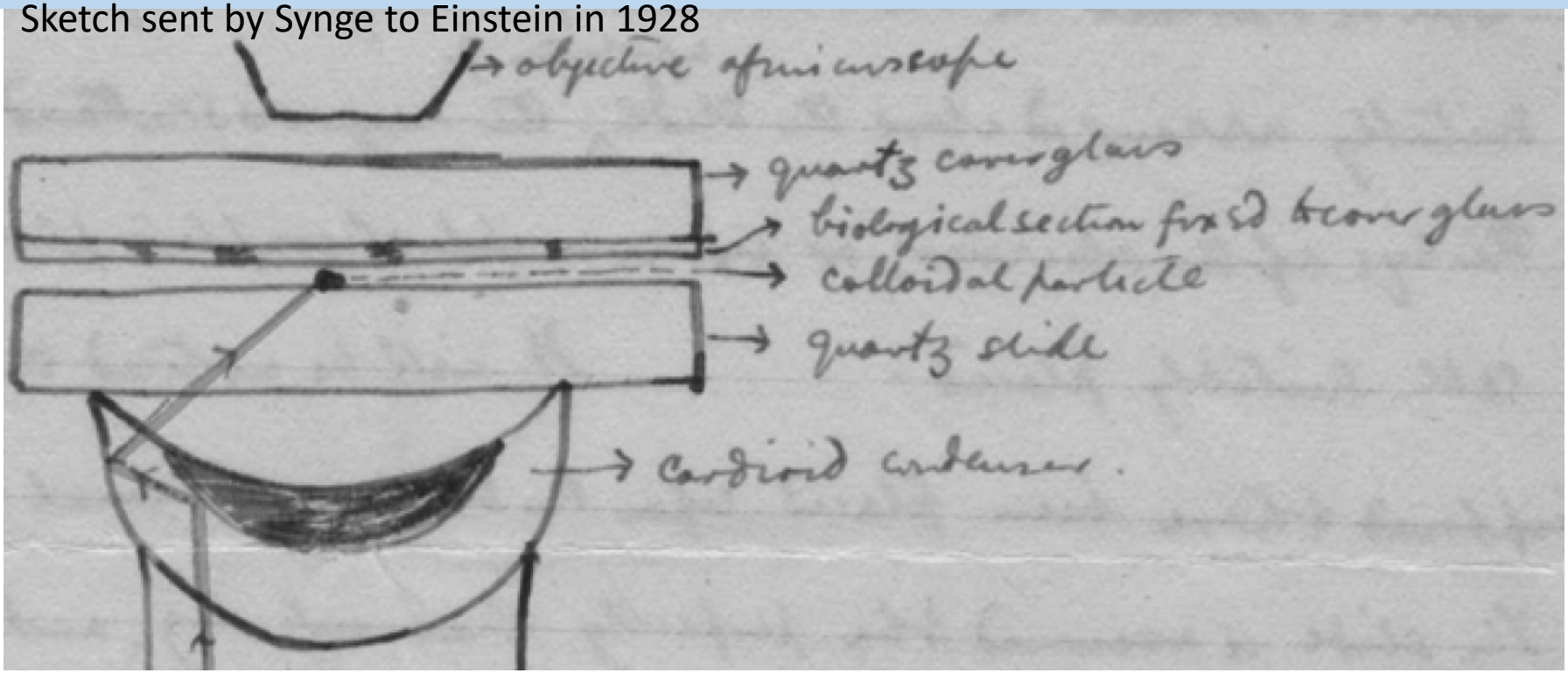

If a small colloidal particle, e.g. of gold, be deposited upon a quartz slide placed above a Zeiss cardioid condenser of NA 1.05, then, all rays of light from the condenser which reach the surface of the slide will be totally reflected by the surface, except those which strike the surface at the base of the particle. These will be scattered in all directions and if the objective of a microscope is suitably arranged above the slide, a proportion of the rays so scattered will come to a focus in the eye of an observer, or upon a photographic plate, or a photo-electrical cell suitably placed.

[Synge then proposes to place a very thin stained biological section onto a quartz cover glass and to raster scan it in close distance over the irradiated particle. He argues that the amount of light received from the particle and collected by the objective will depend upon the relative opacity of the different parts of the section.]

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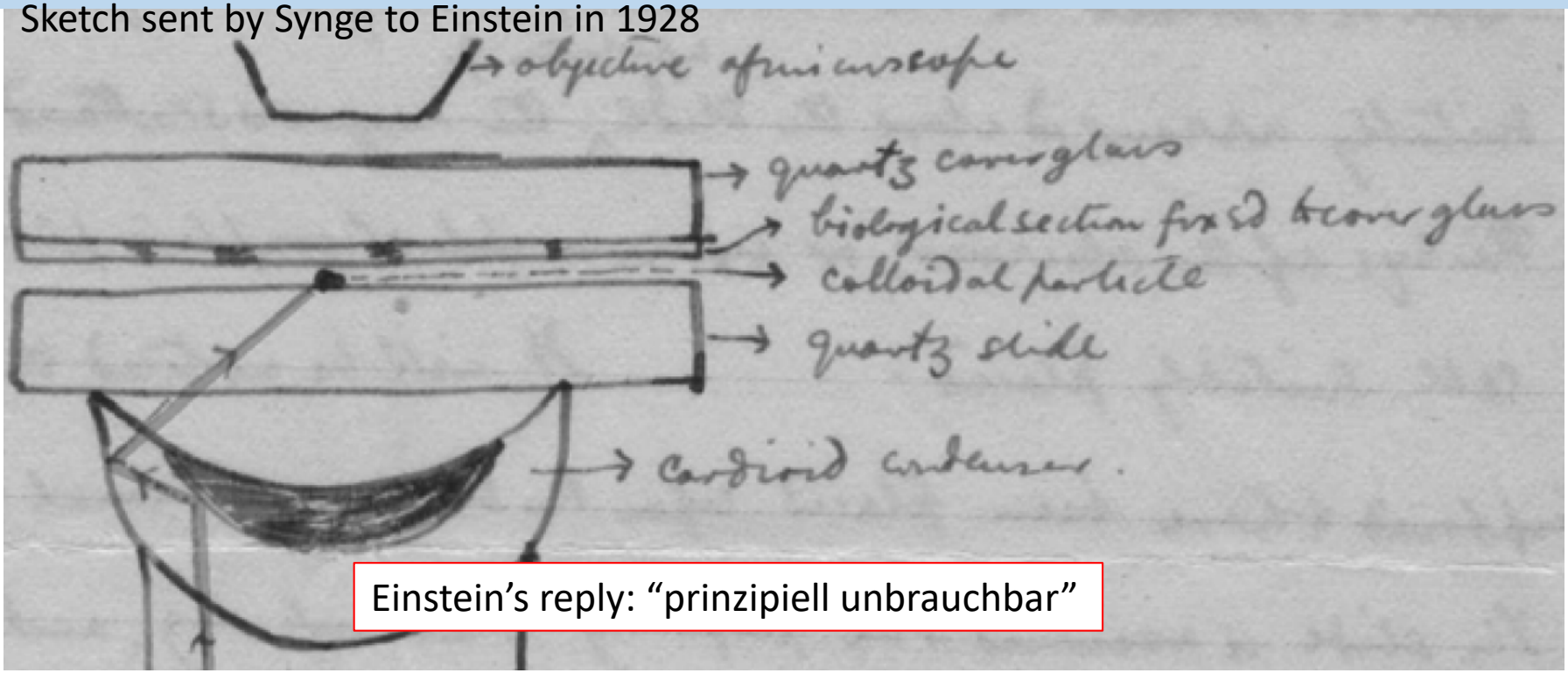

Einstein's reply: "prinzipiell unbrauchbar"

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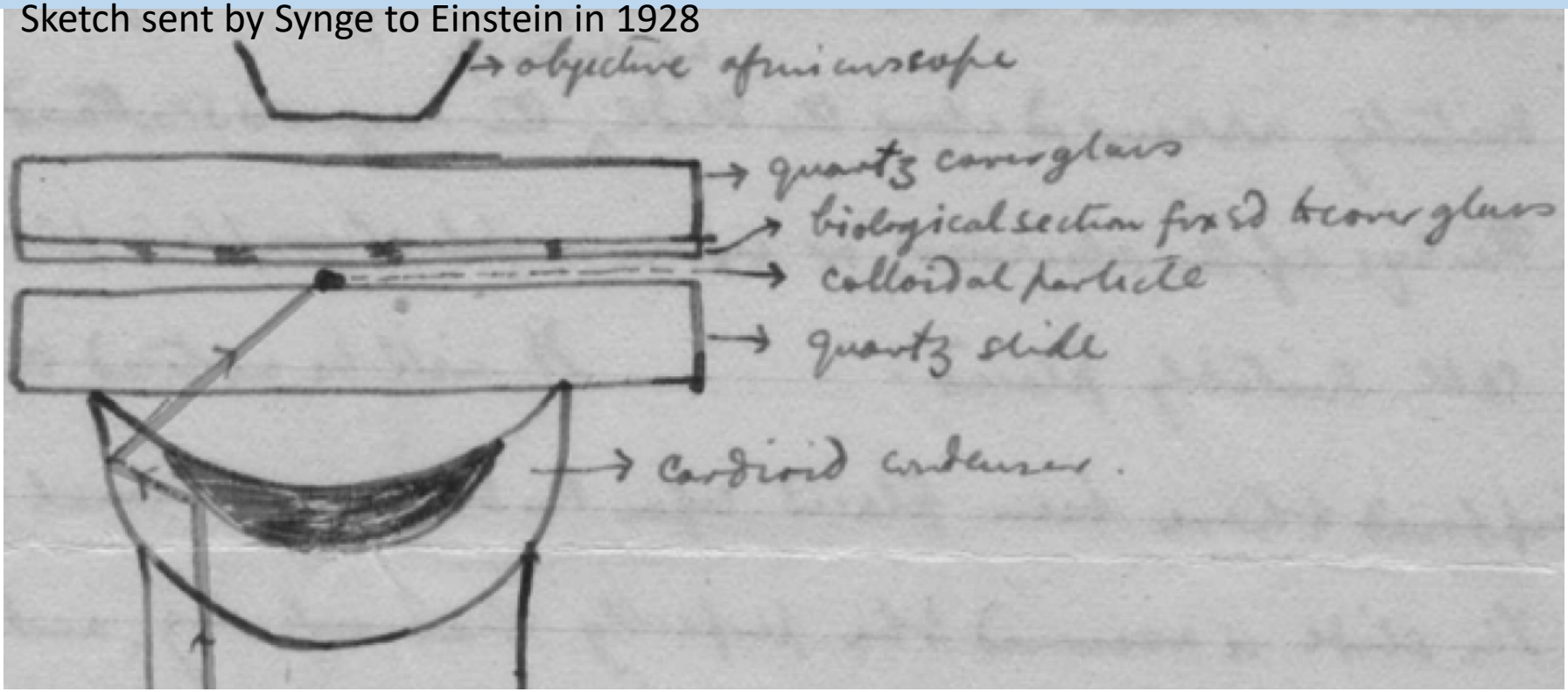

Synge: "It was my original idea to have a very small hole in an opaque plate, as you suggest, and it was in this form that I had mentioned it to several people… A better way could be, if one could construct a little cone or pyramid of quartz glass having its point P brought to a sharpness of order 10−6 cm. One could then coat the sides and point with some suitable metal (e.g. in a vacuum tube) and then remove the metal from the point, until P was just exposed. I do not think such a thing would be beyond the capacities of a clever experimentalist.

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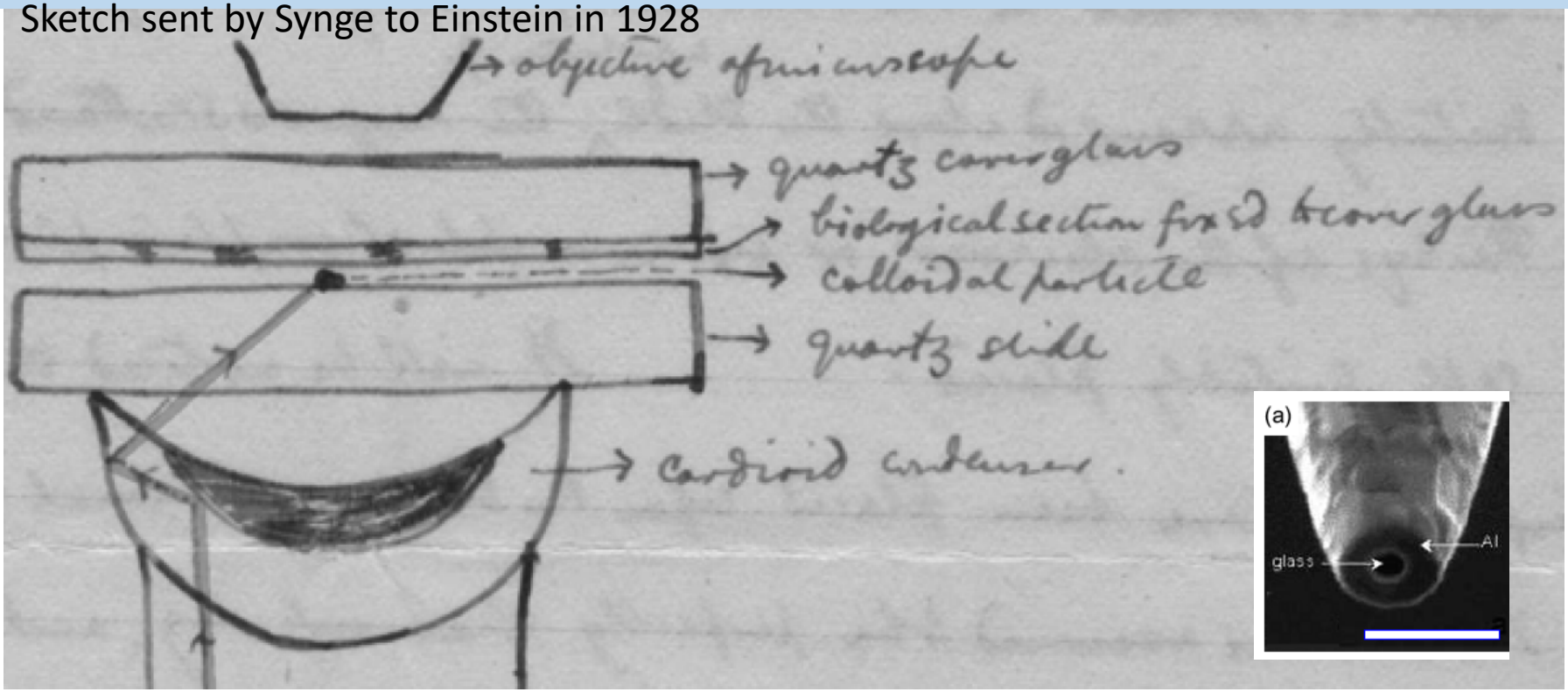

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