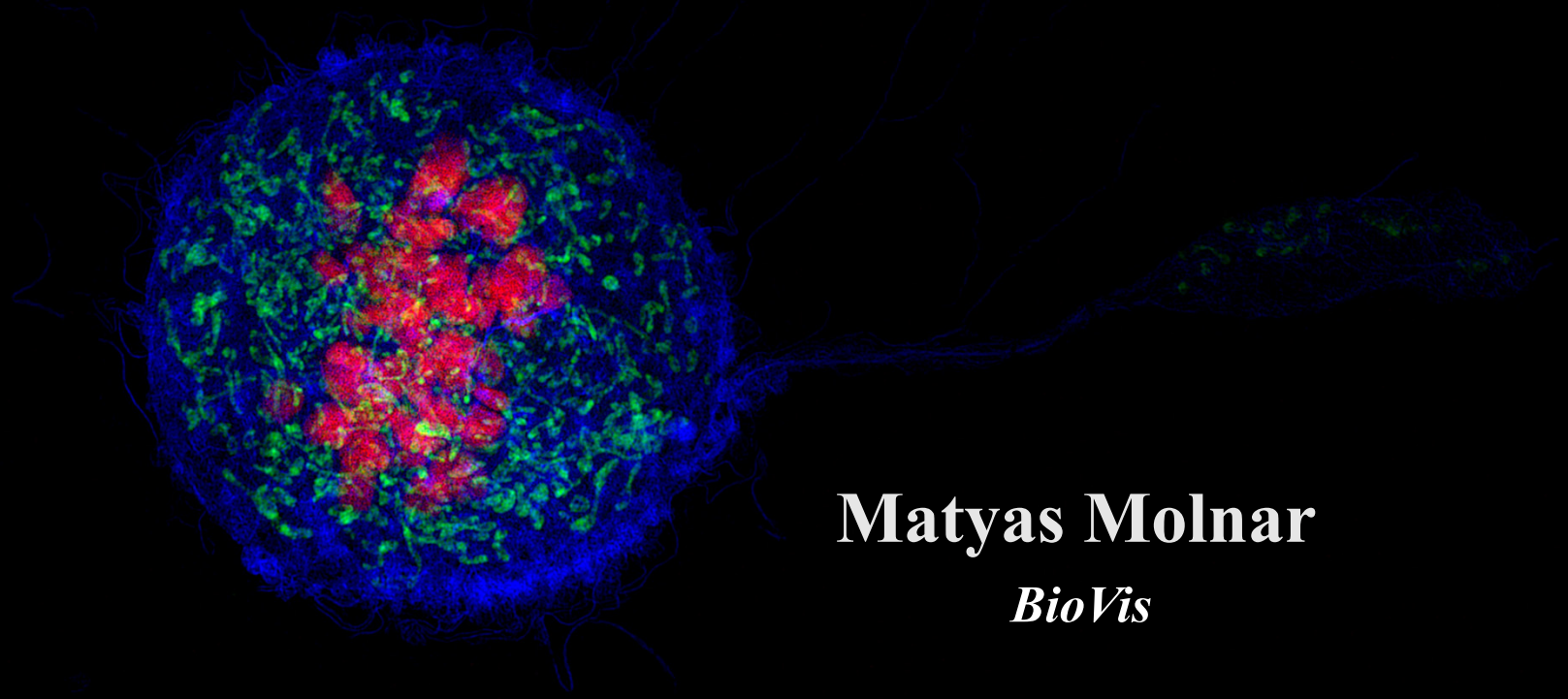
SUPERRESOLUTION MICROSCOPY



Matyas Molnar

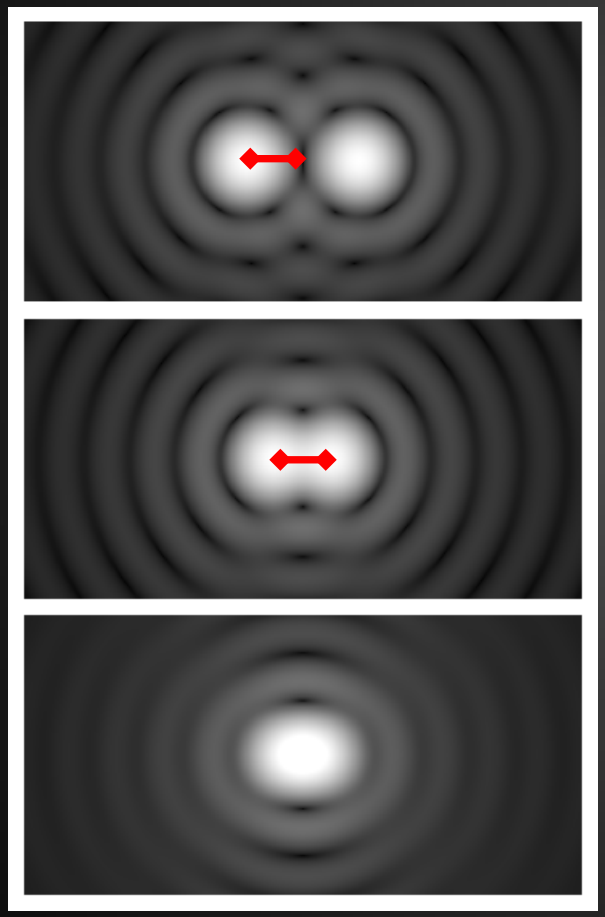
BioVis

. Uppsala University . Platform . Biological Visualization .

BIOVIS

Light & Electron Microscopy : Flow Cytometry : Image Analysis

Resolution of light microscopy



Rayleigh criterion:

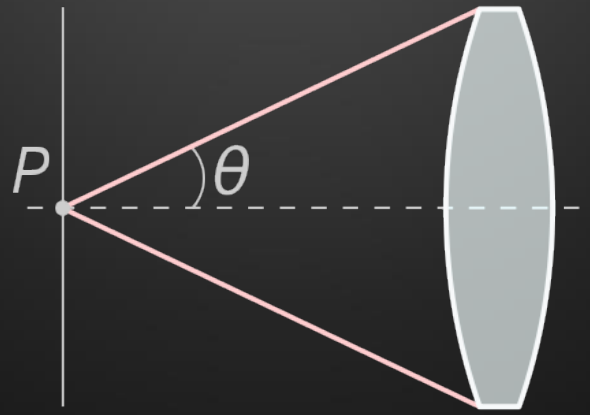
The images of two point sources are resolvable if they are separated by at least the radius of the Airy disk,
i.e. the first minimum of one coincides with the maximum of the other.

Resolution of light microscopy

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

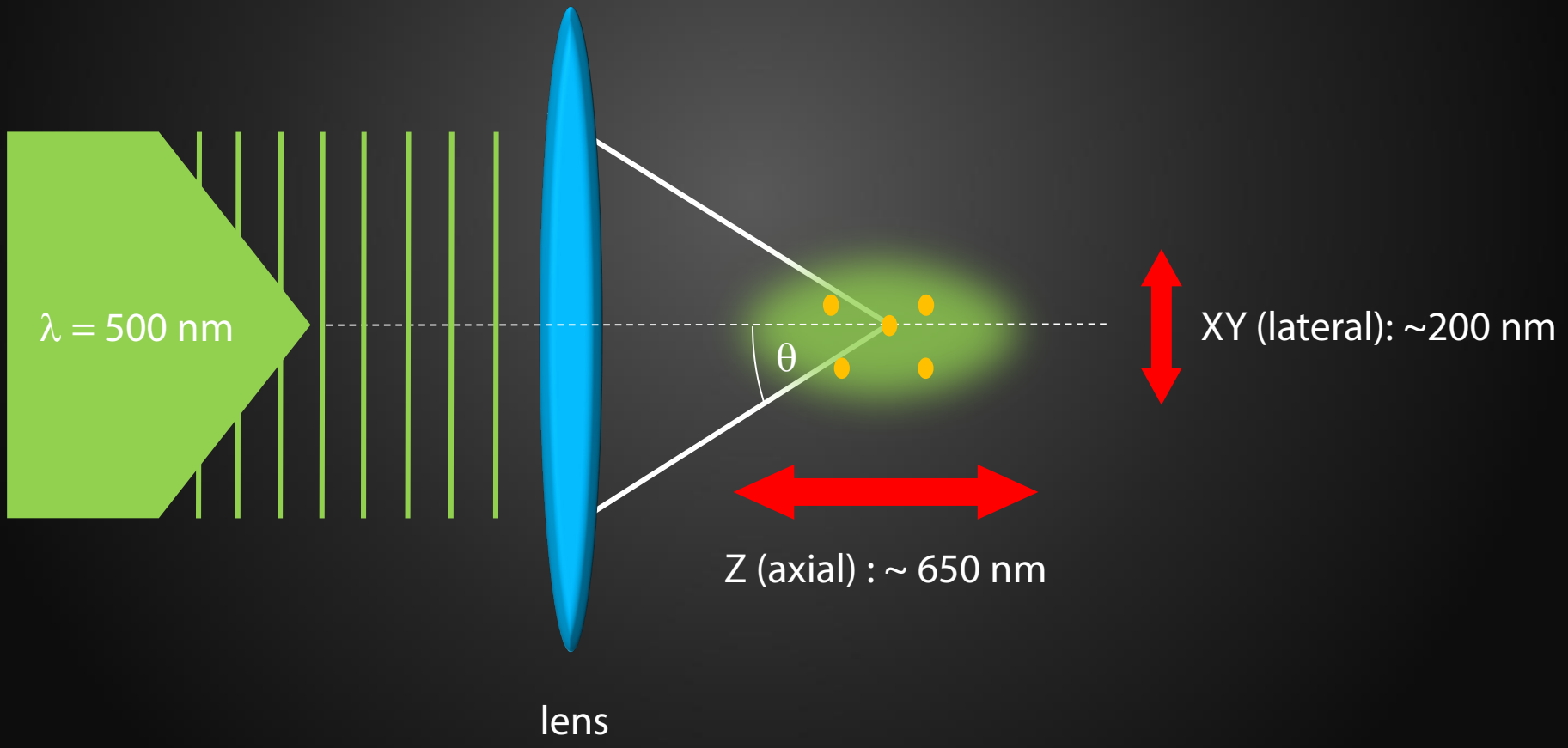
XY limit is around 200 nm for light microscopy

d: point resolution, the shorter the lenght is better for us [nm]
λ: wavelength of light used ([nm], visible light 400-700 nm)
n: refractive index (air: 1, water: 1.3, oil: 1.4-1.5)
ɵ: the maximum cone of light that can enter or exit the lens
nsinɵ: numerical aperture (NA)



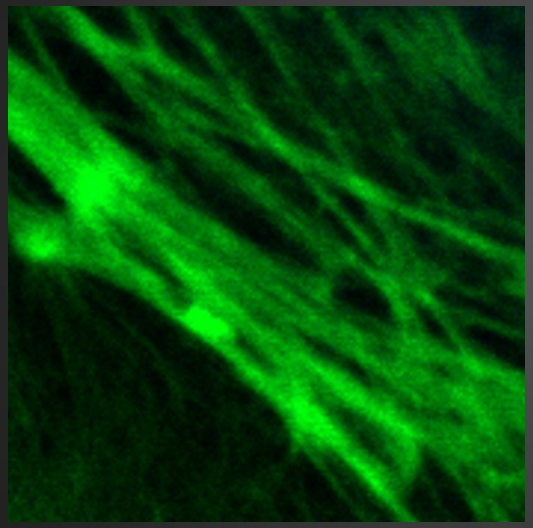
Resolution of light microscopy

Light can only be focused with a lens to a finite illumination spot.
The smallest size of this illumination spot (excitation for microscopy) has a theoretical limit.
All fluorophores within this spot will be excited and their emission will be overlapped.

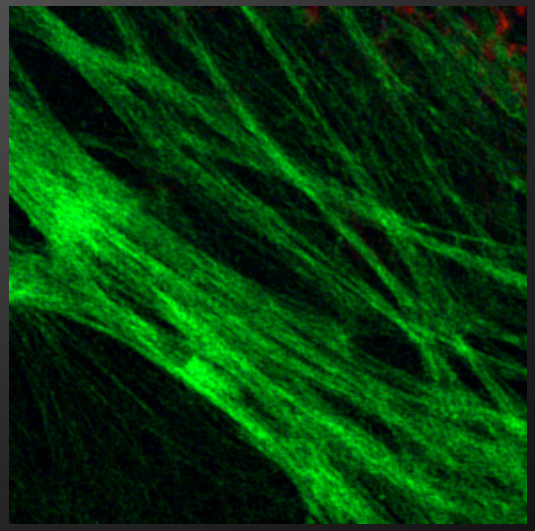


STRUCTURED ILLUMINATION MICROSCOPY

Confocal



SIM



SIM

- SIM resolution is around 90 -100nm
- Mostly for *in vitro* imaging, not for mass imaging
  - Long mathematical post-processing (image reconstruction) is involved
- No special fluorophores needed like with STED, STORM, PALM – normal staining is OK
- No special sample preparation needed, but best as possible sample needed (good staining, immersion oil, coverslip, etc...)
- Drawbacks:
  - artifacts due to the filtering and post processing
  - widefield technique, out of focus signal is visible sometimes

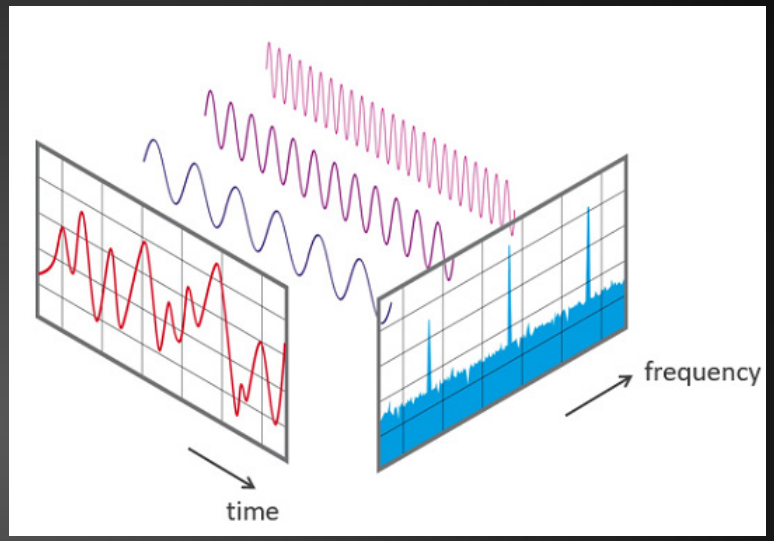
SIM

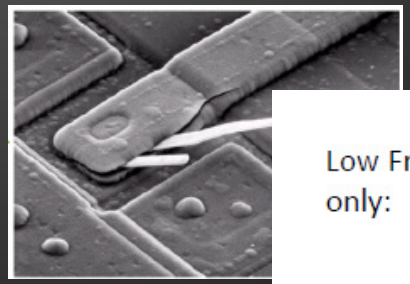
- Any sample, signal or image can be modeled or represented with different frequencies
- With Fourier transform a complex signal can be expressed as the sum of all frequencies
- An image consists of two parts: high frequencies and low frequencies
  - Low frequencies make up the bulk of the information (areas of low variation in intensity)

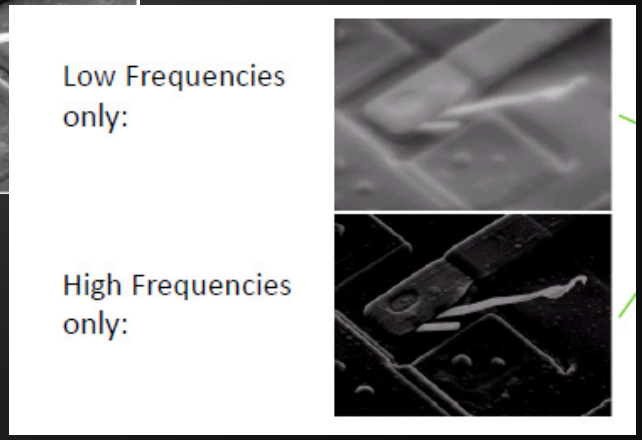
    "Low resolution parts"

  - High frequencies make up the edges and fine detail (areas of high variation in intensity)

    "High resolution parts"





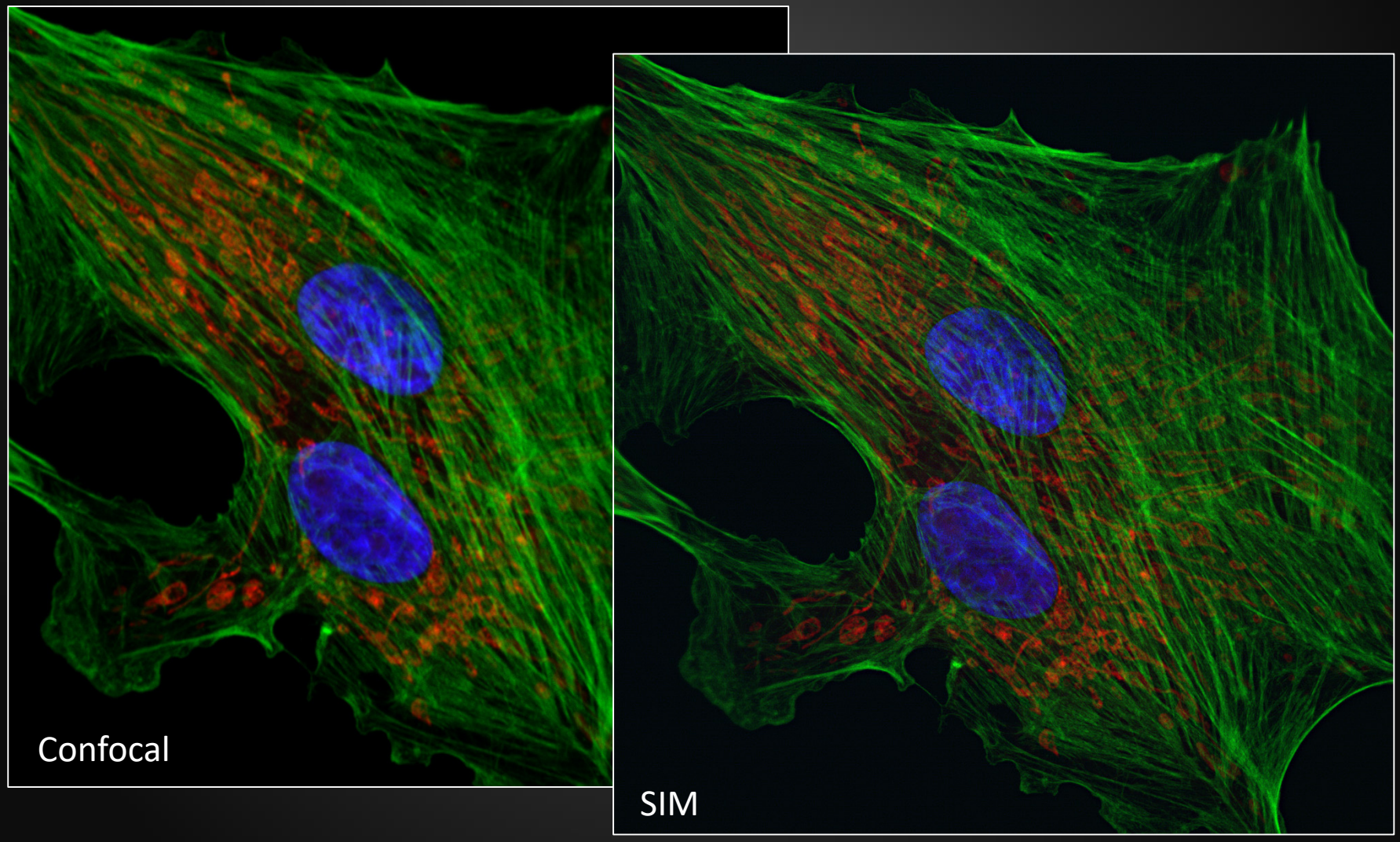


Pics from "The Arc ppt"

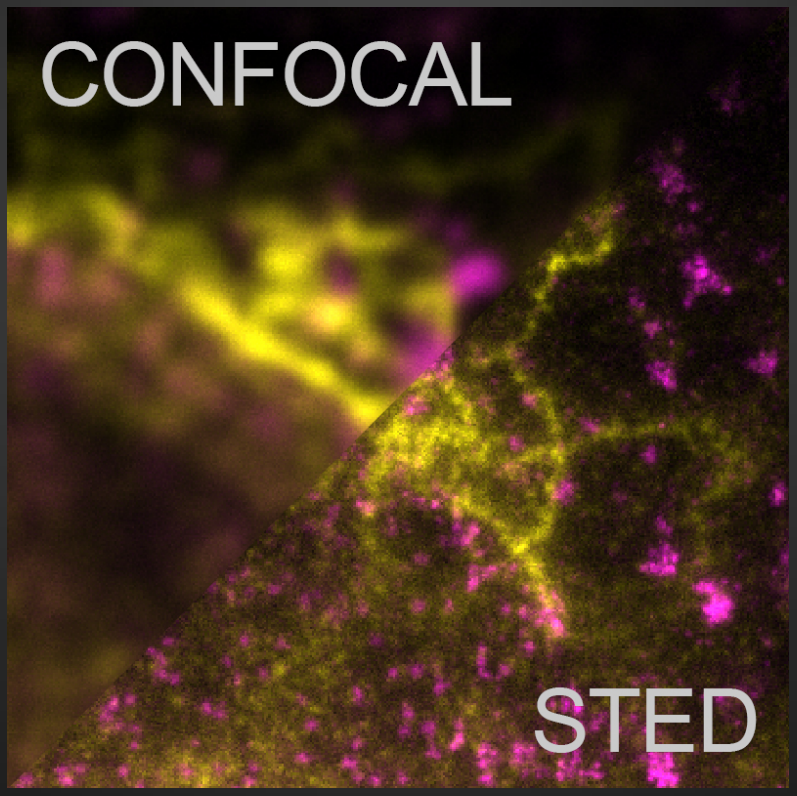
SIM DEMONSTRATION

Moiré demonstration

SIM resolution example



STIMULATED EMISSION DEPLETION MICROSCOPY



Spontaneous and stimulated emission

Spontaneous emission

Stimulated emission

„Normal“ emission is killed,
fluorescence is turned off, depletet.

Spontaneous and stimulated emission

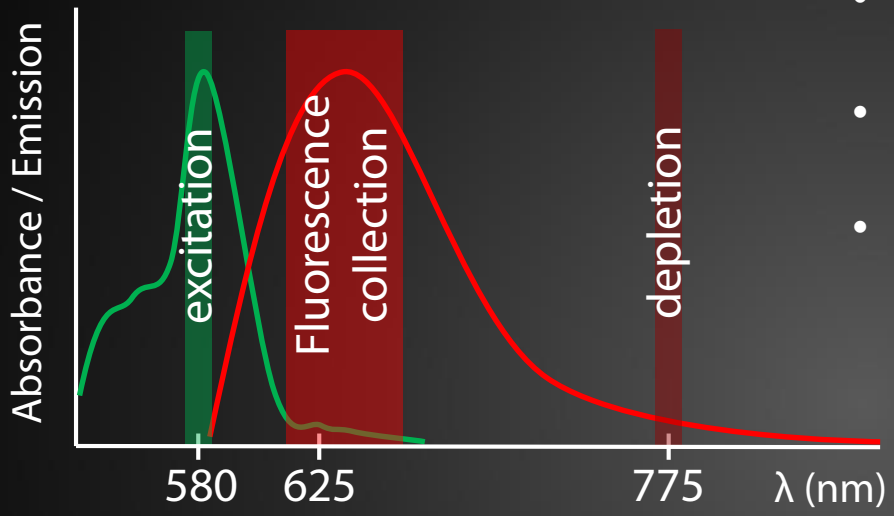
- **Spontaneous emission:** the process is random, hence the gaussian curve (emission spectrum).

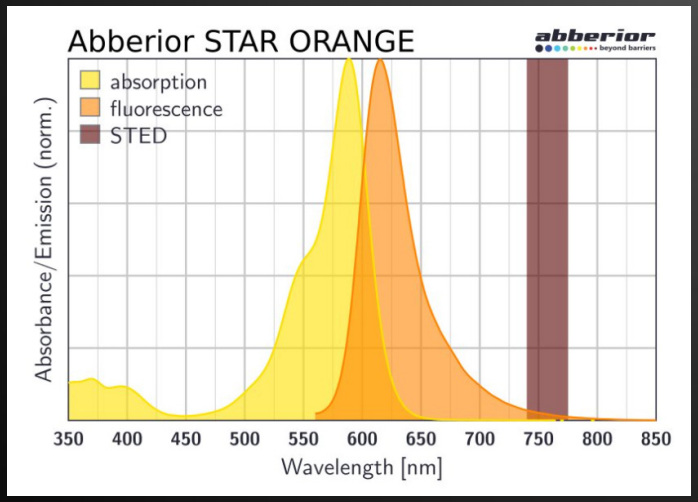
- **Stimulated emission:** a specific frequency of electromagnetic radiation (incident wave, depletion) interacts with an excited atom or molecule causing it to return to a lower state.

  The created photon from the energy transfer is identical to the photons of the incident wave (*i.e* same phase, frequency, polarization, direction).

  The process is identical to atomic absorption (but opposite direction). Lasers are created by stimulated emission, thus they are monochromatic.

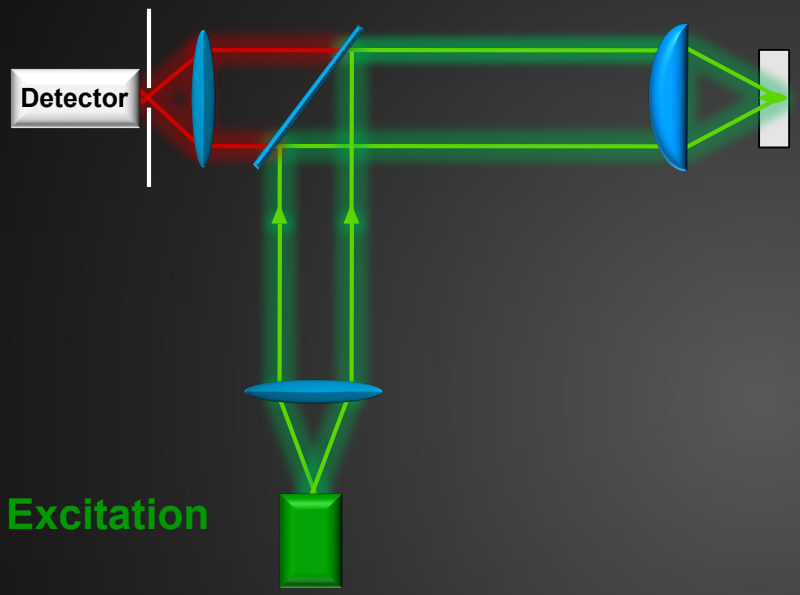
Stimulated emission and targeted depletion

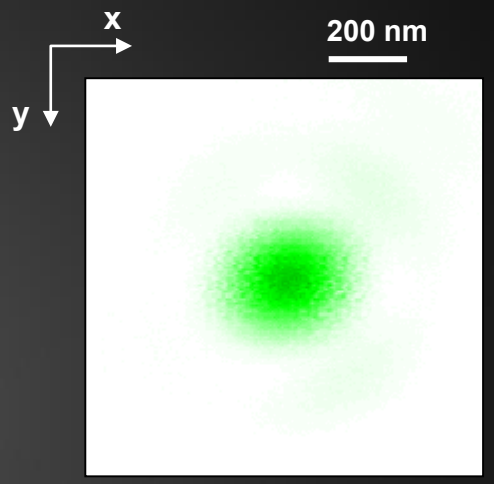


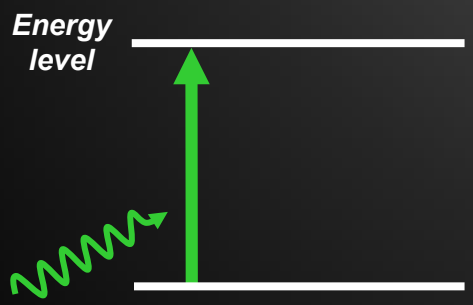


- The depletion must happen in the range of the emission for a fluorophore
- The depletion is usually in the infrared or red region
- For the fluorophore in the pictures:
  - 580 nm laser is used for excitation
  - the emission maximum is at 625 nm
  - depletion for stimulated emission is with a 775 nm laser
  - in this case, if the detection filter is is in the normal range for this dye: BP 610 – 660, no fluorescence can be collected
  - the stimulated emission happens at 775nm, all emitted photons are ignored. The dye is de-exited, effectively turned off.

The STED microscope

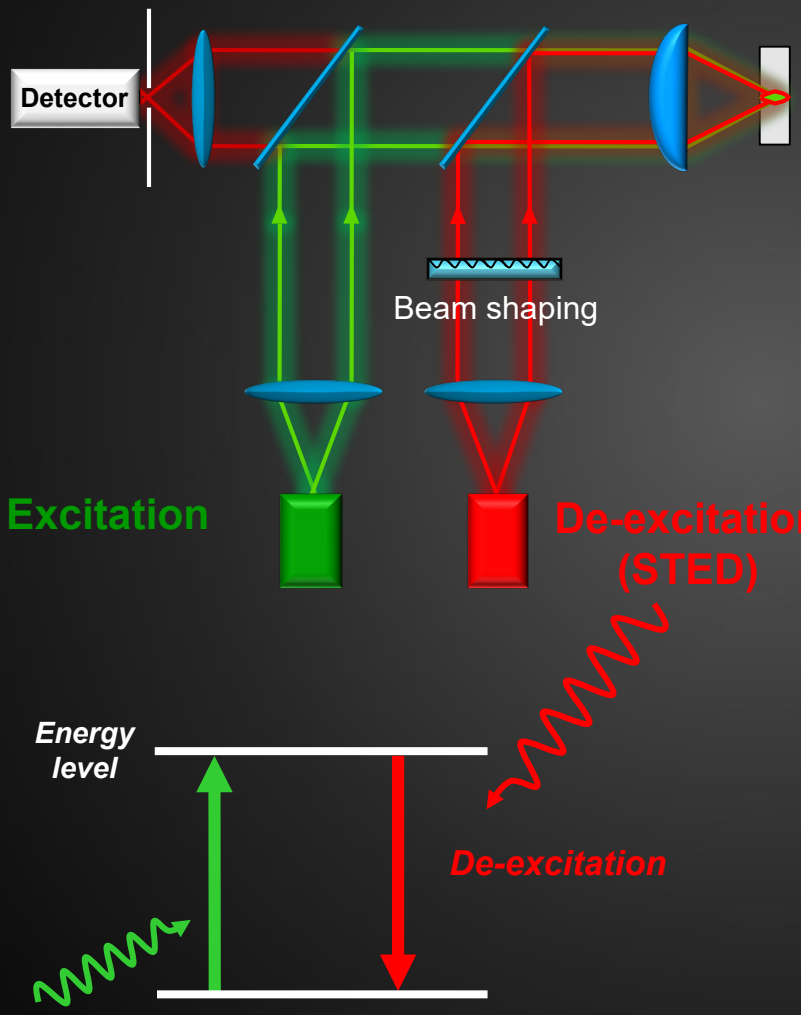


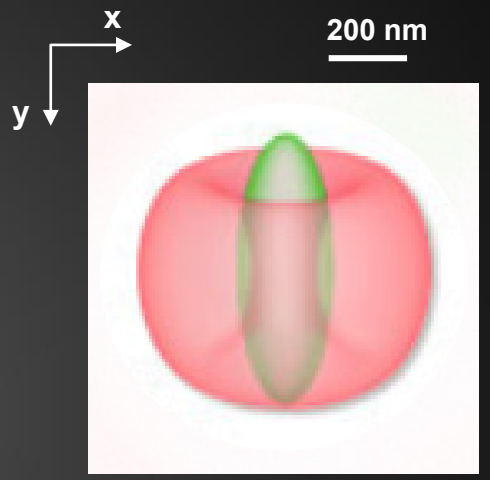




Source: MPI for biophysical chemistry, Göttingen

The STED microscope





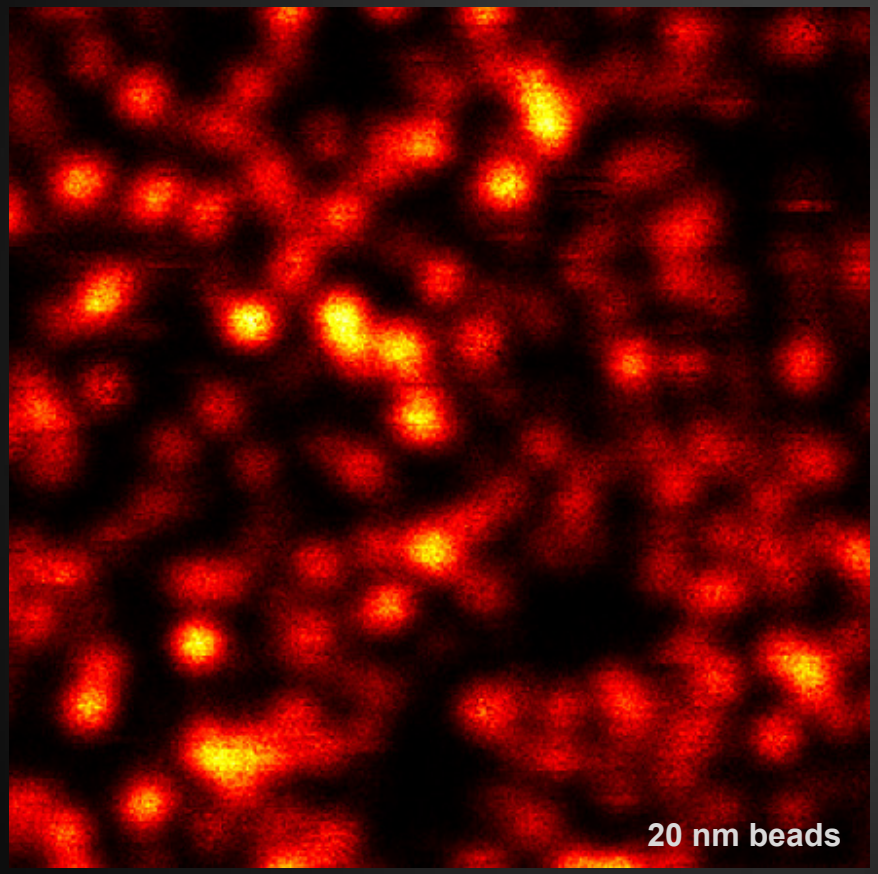
$$d = \frac{\lambda}{2n \sin\theta \sqrt{1 + \frac{I_{max}}{I_S}}}$$

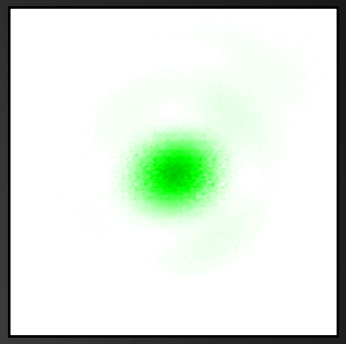
I: depletion laser's intensity

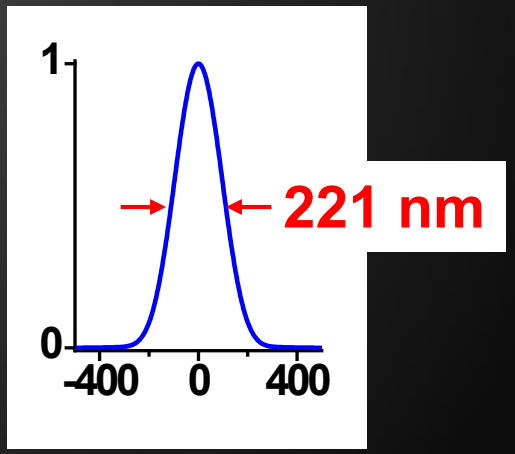
I_s: saturation intensity of the off-transition

Source: MPI for biophysical chemistry, Göttingen

Depletion intensity and resolution

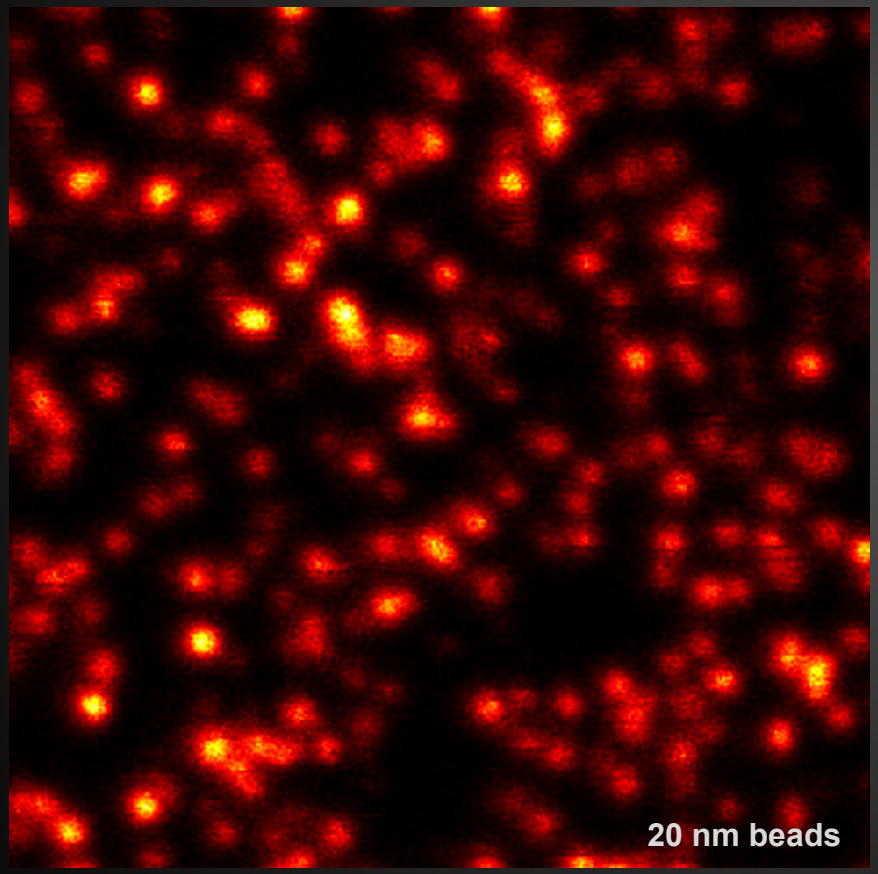


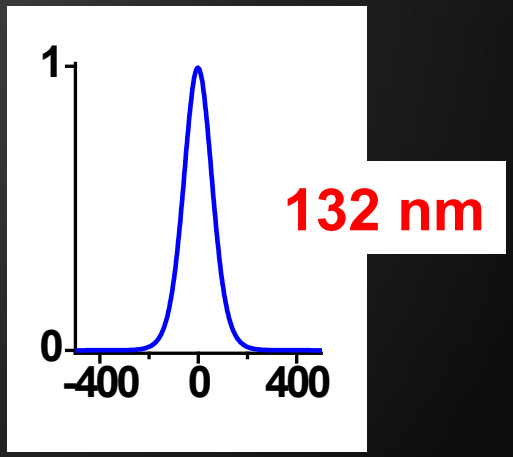




Source: MPI for biophysical chemistry, Göttingen

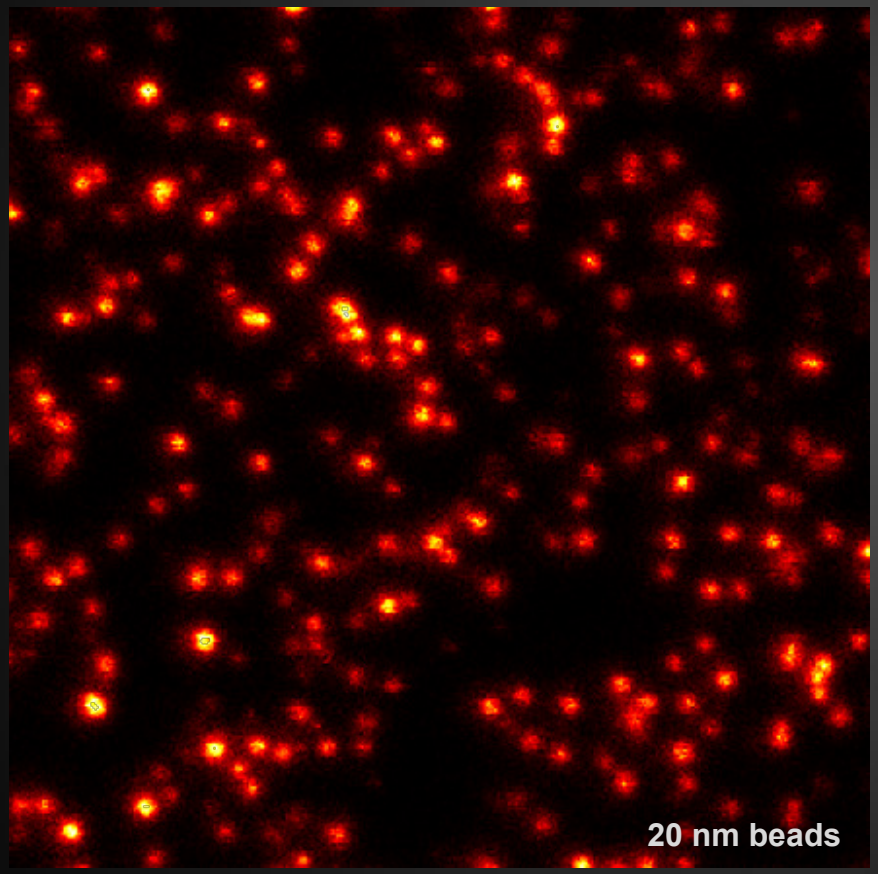
Depletion intensity and resolution

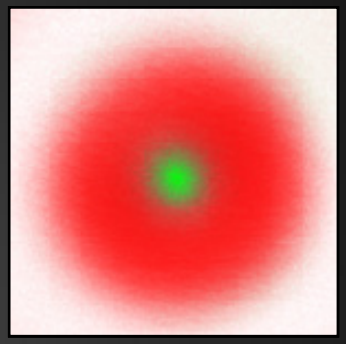


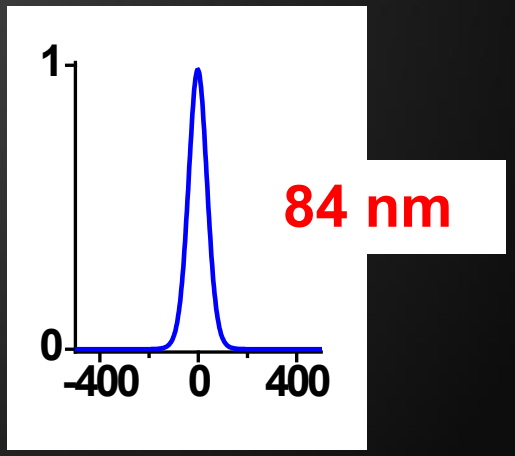


Source: MPI for biophysical chemistry, Göttingen

Depletion intensity and resolution

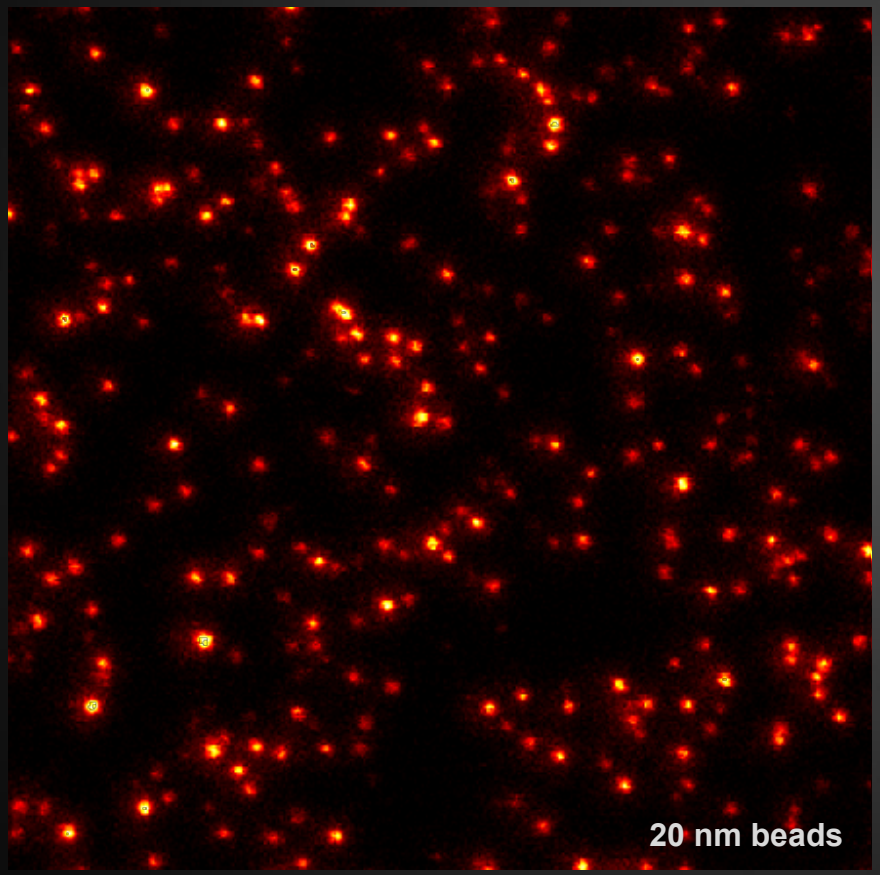


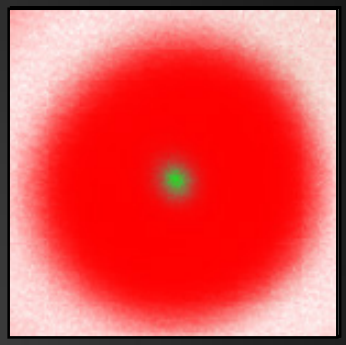


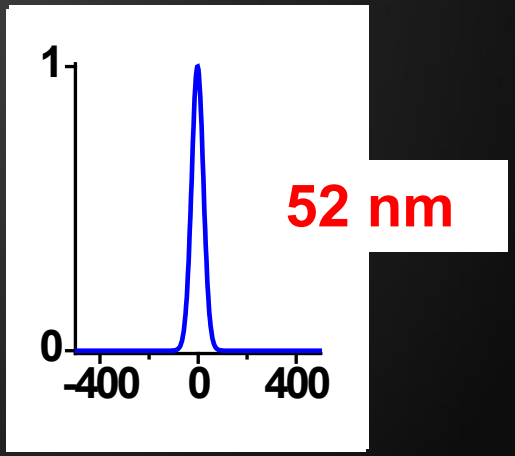


Source: MPI for biophysical chemistry, Göttingen

Depletion intensity and resolution

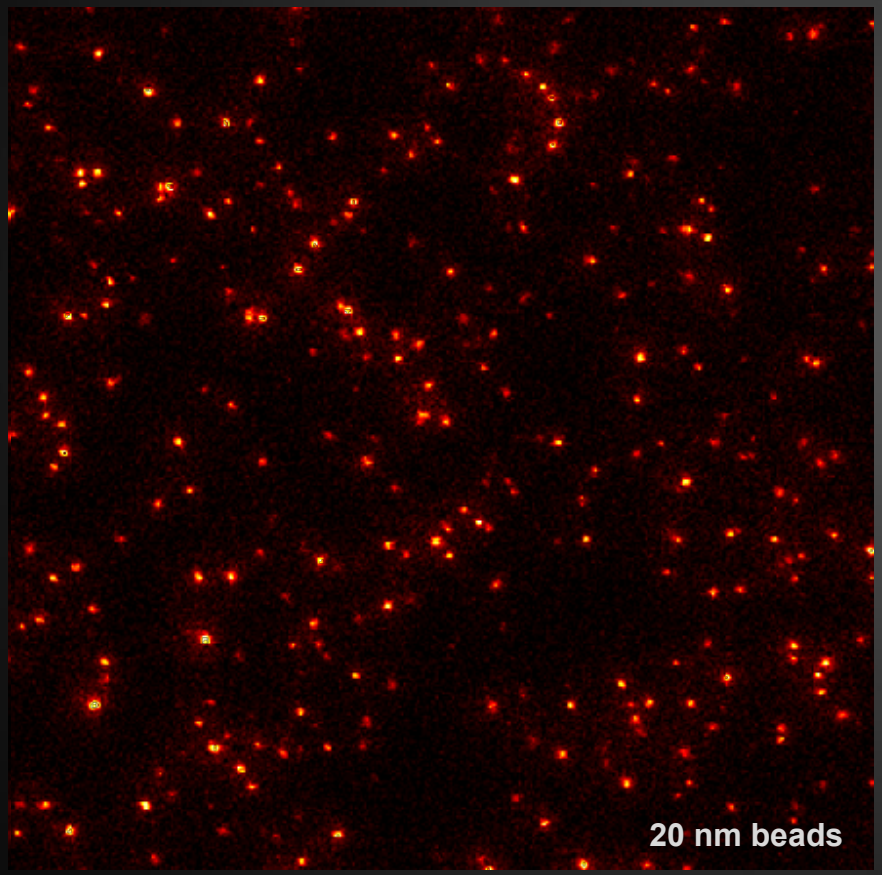


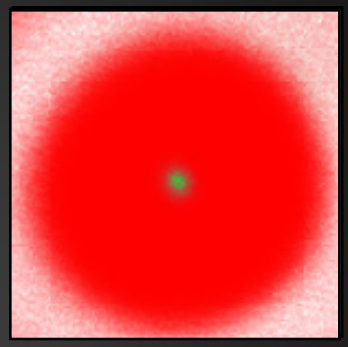


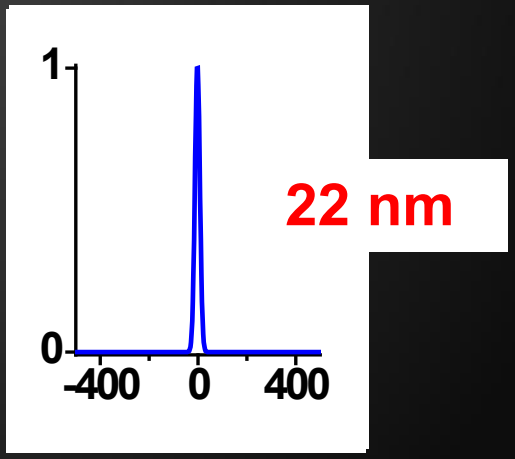


Source: MPI for biophysical chemistry, Göttingen

Depletion intensity and resolution

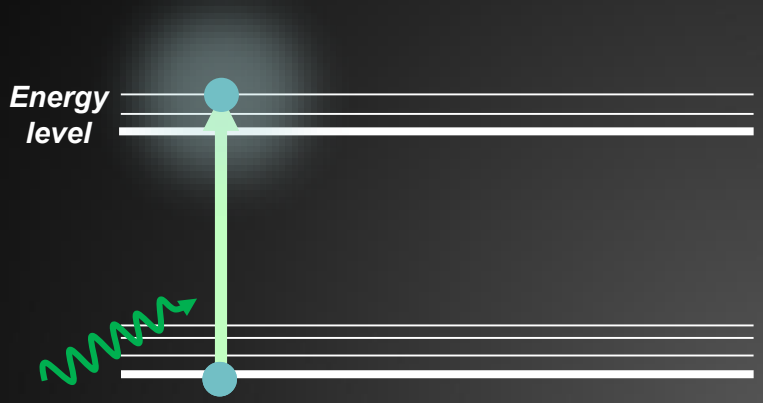


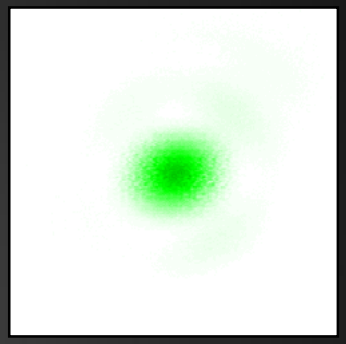


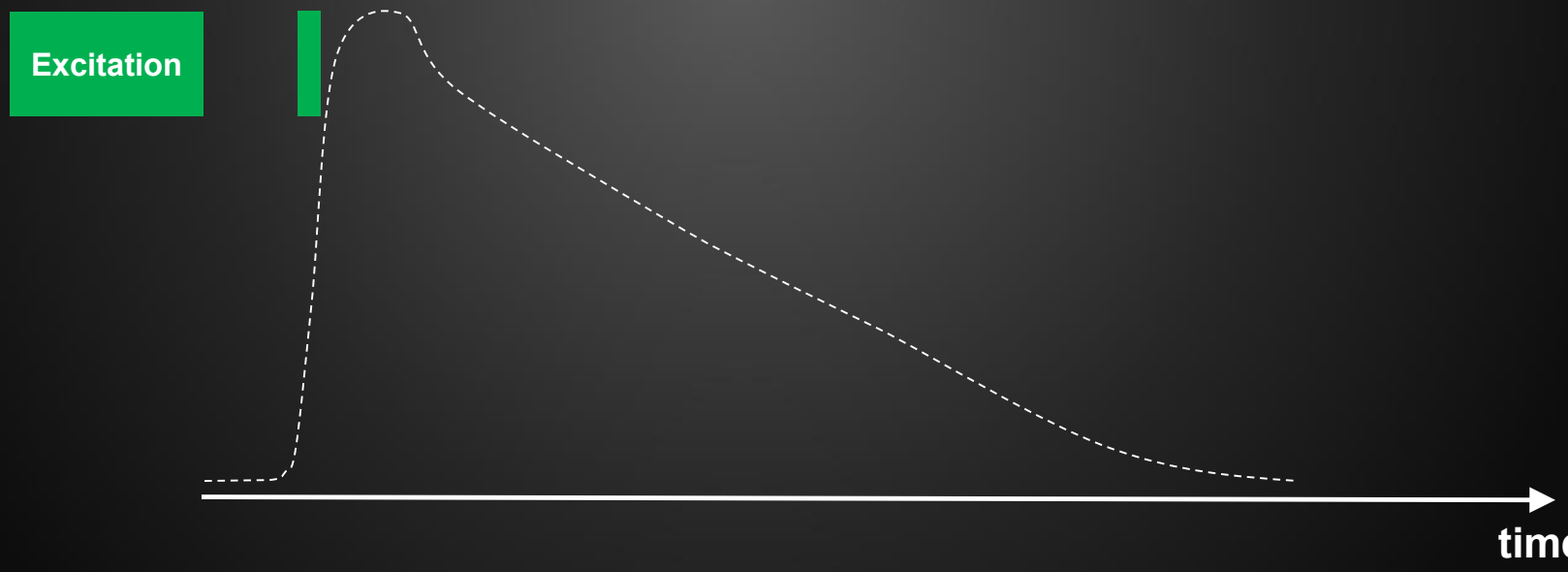


Source: MPI for biophysical chemistry, Göttingen

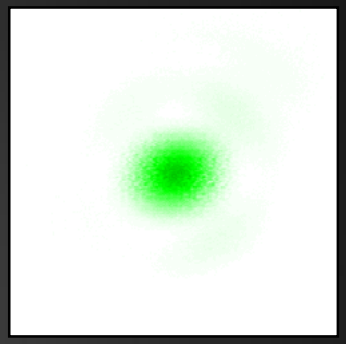
Timeline of STED

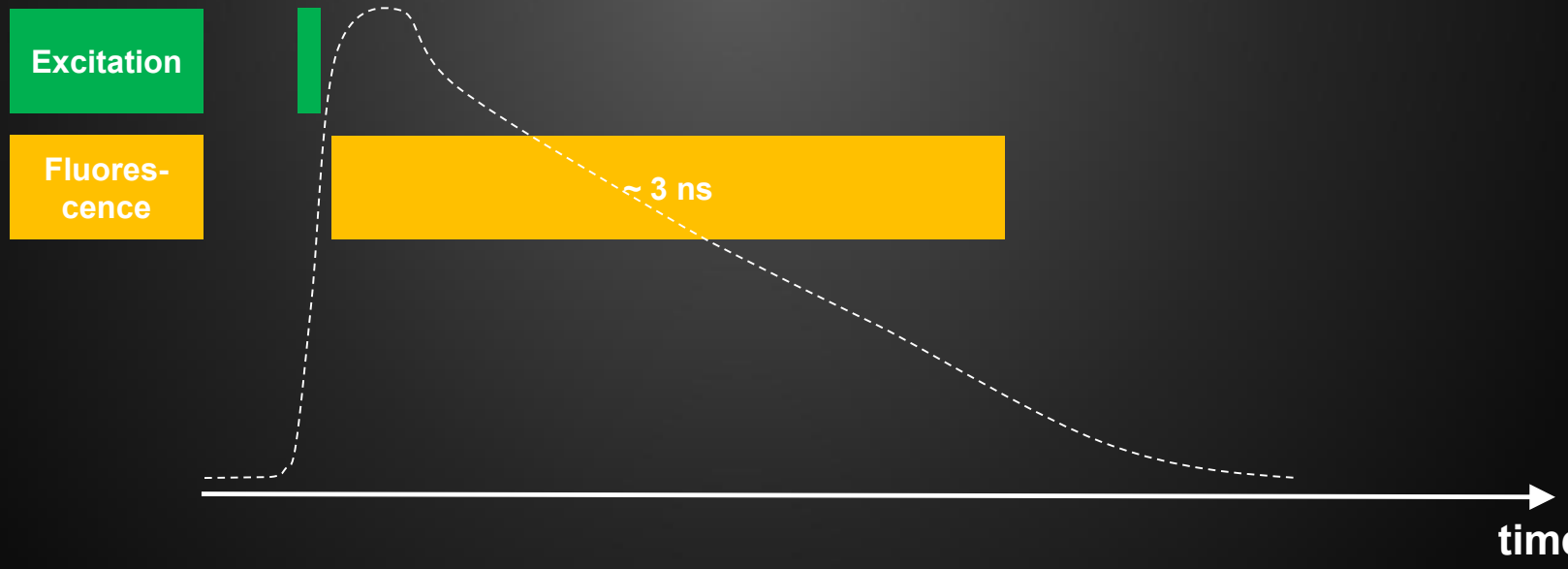




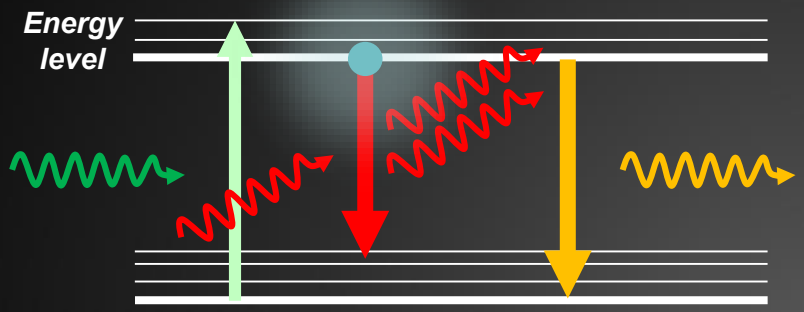


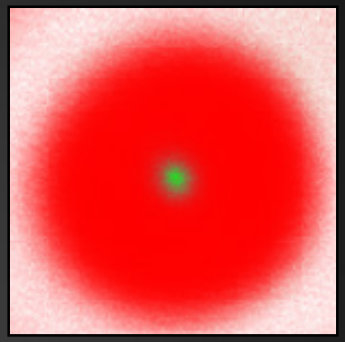
Timeline of STED

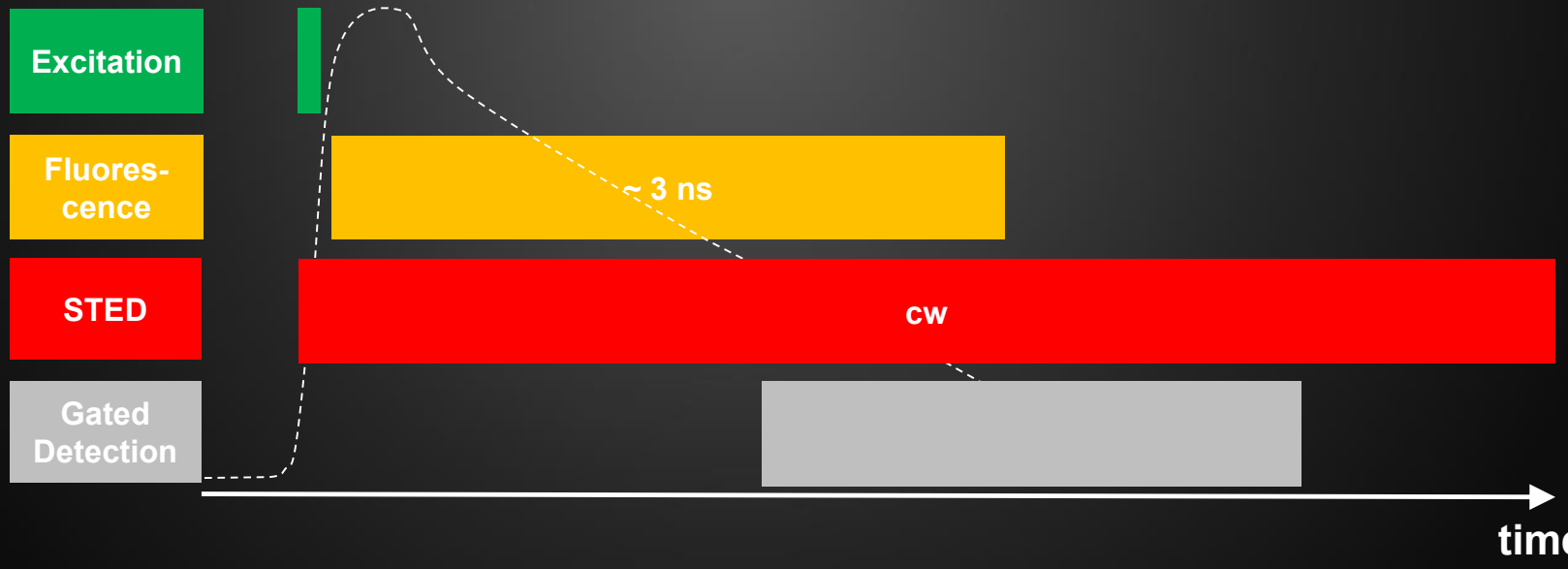




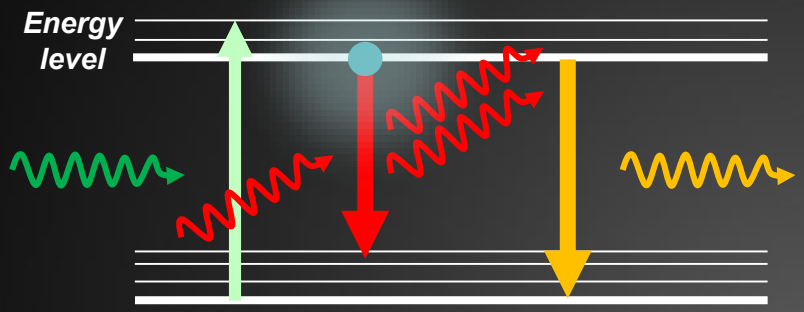
Timeline of STED – CW STED

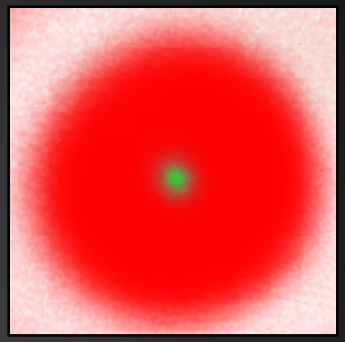


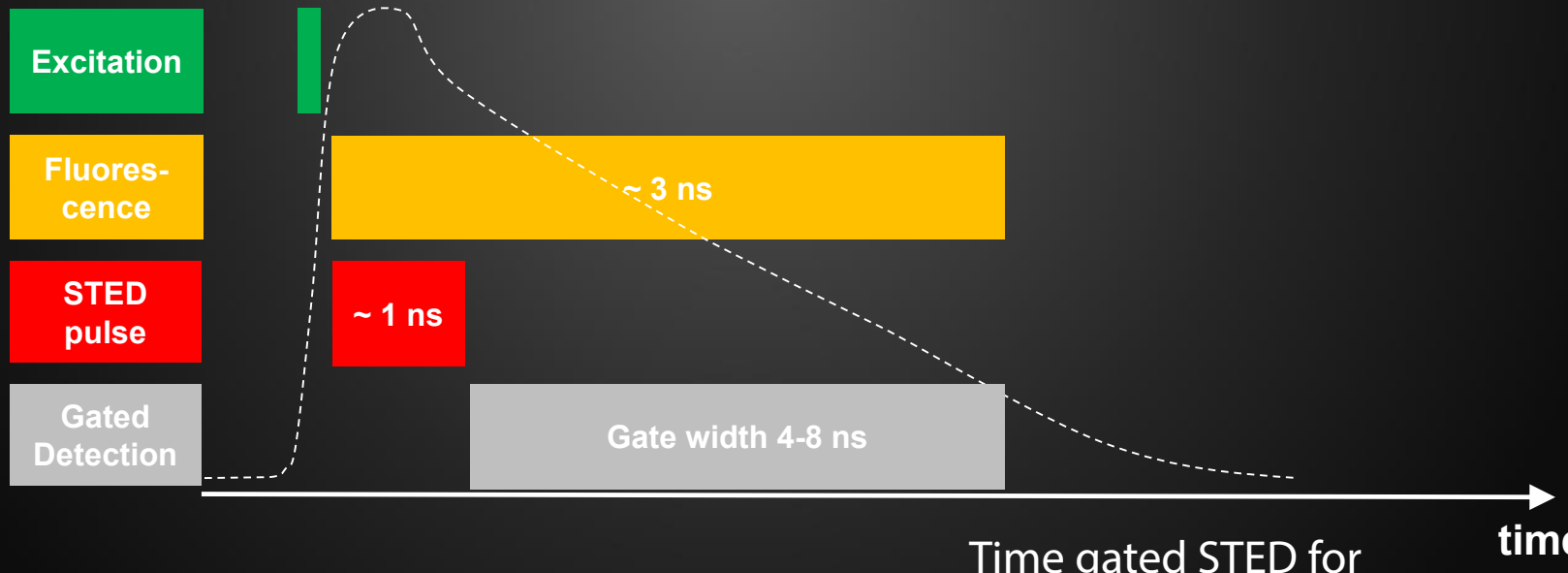




Timeline of STED – Pulsed STED

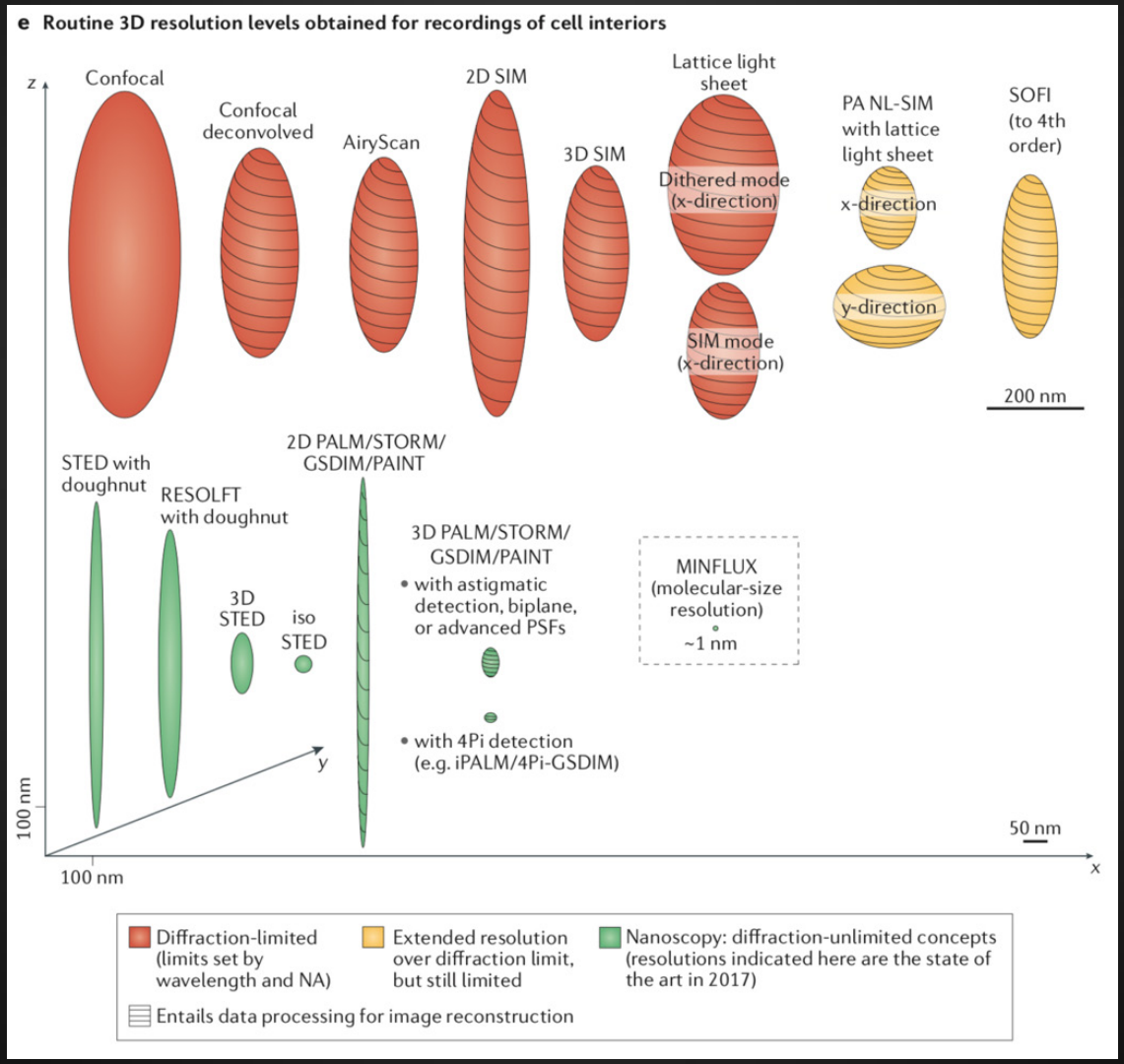






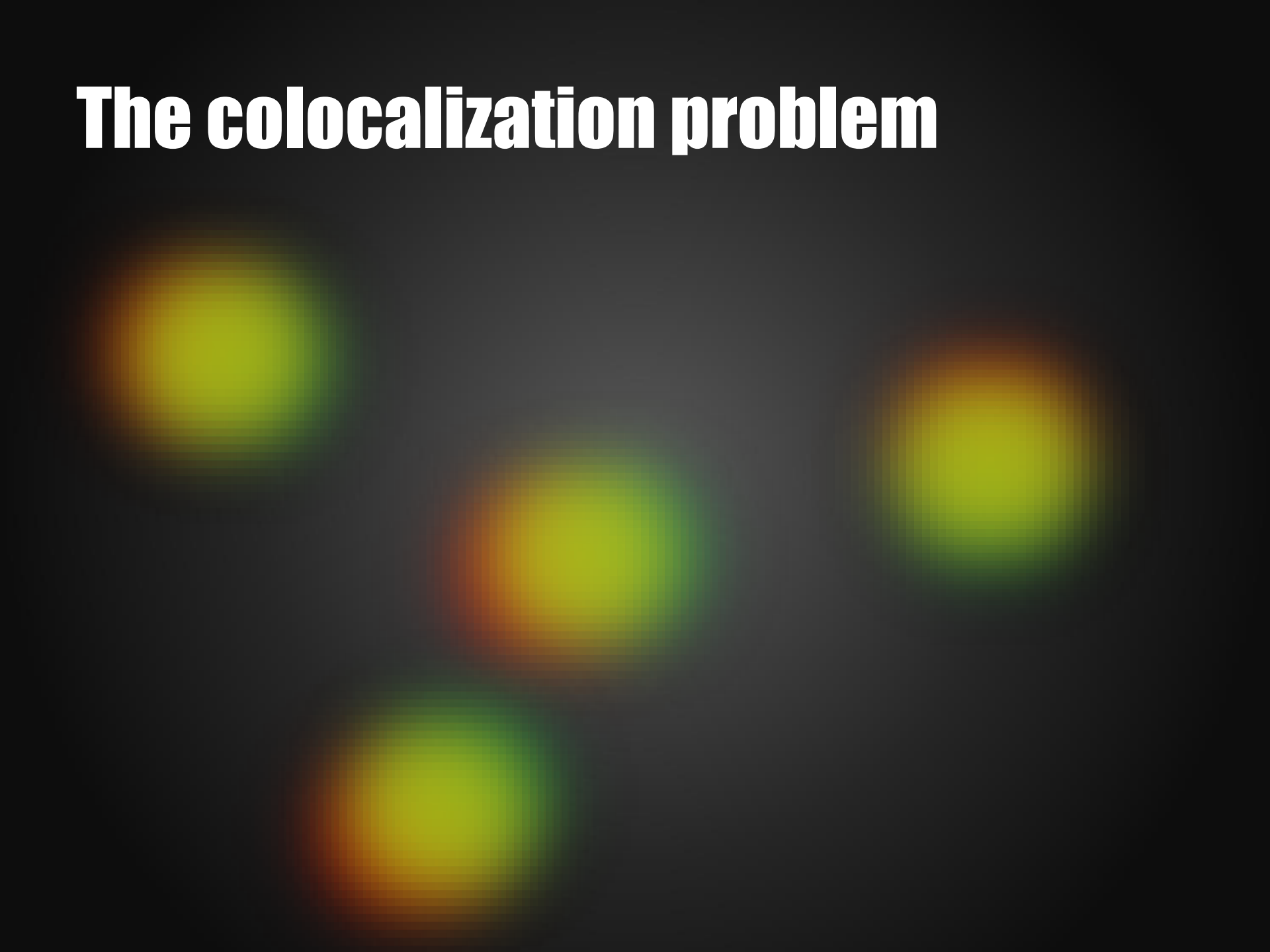
Time gated STED for
enhanced resolution

Superresolution techniques



The colocalization problem

The colocalization problem



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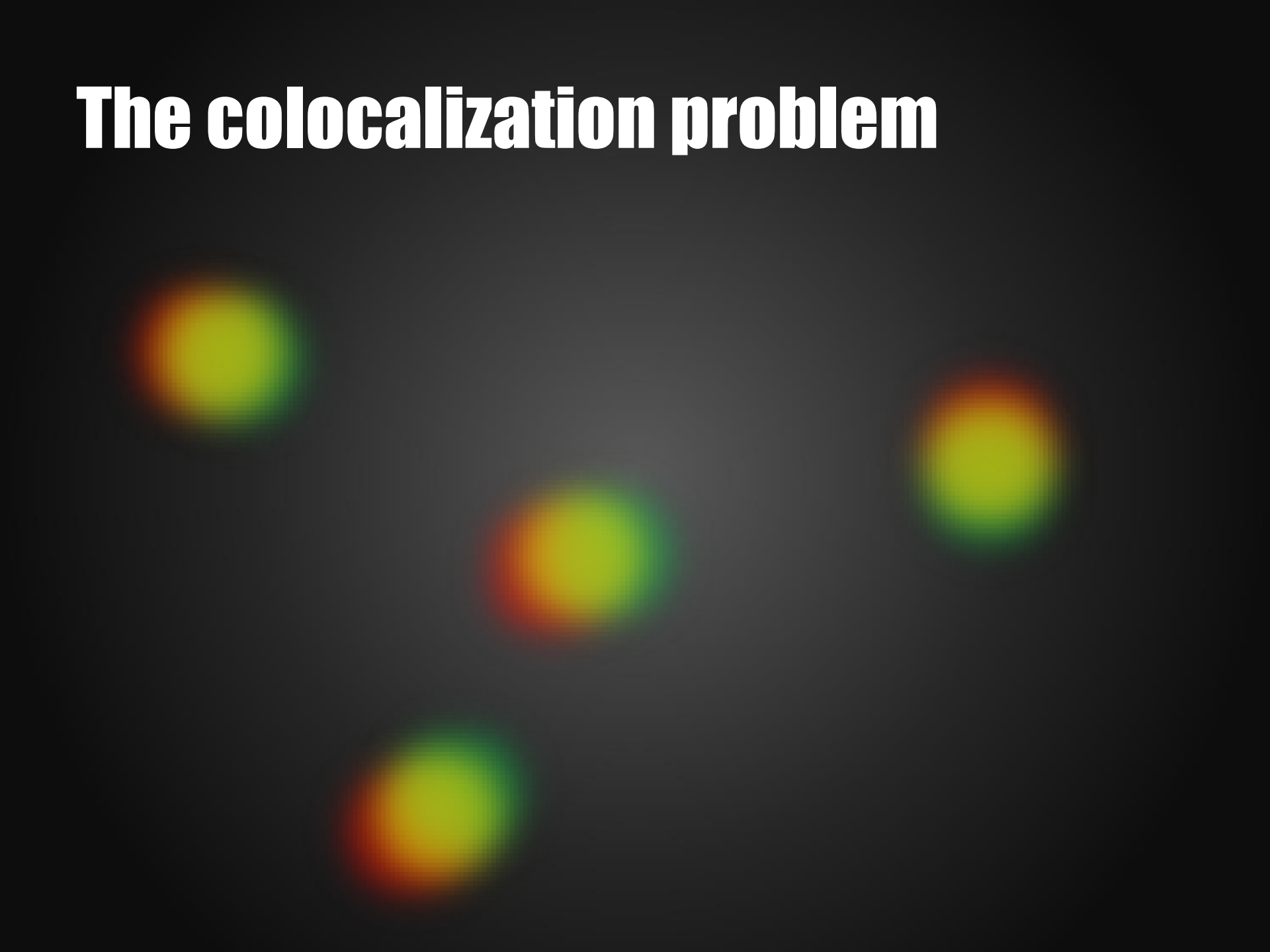

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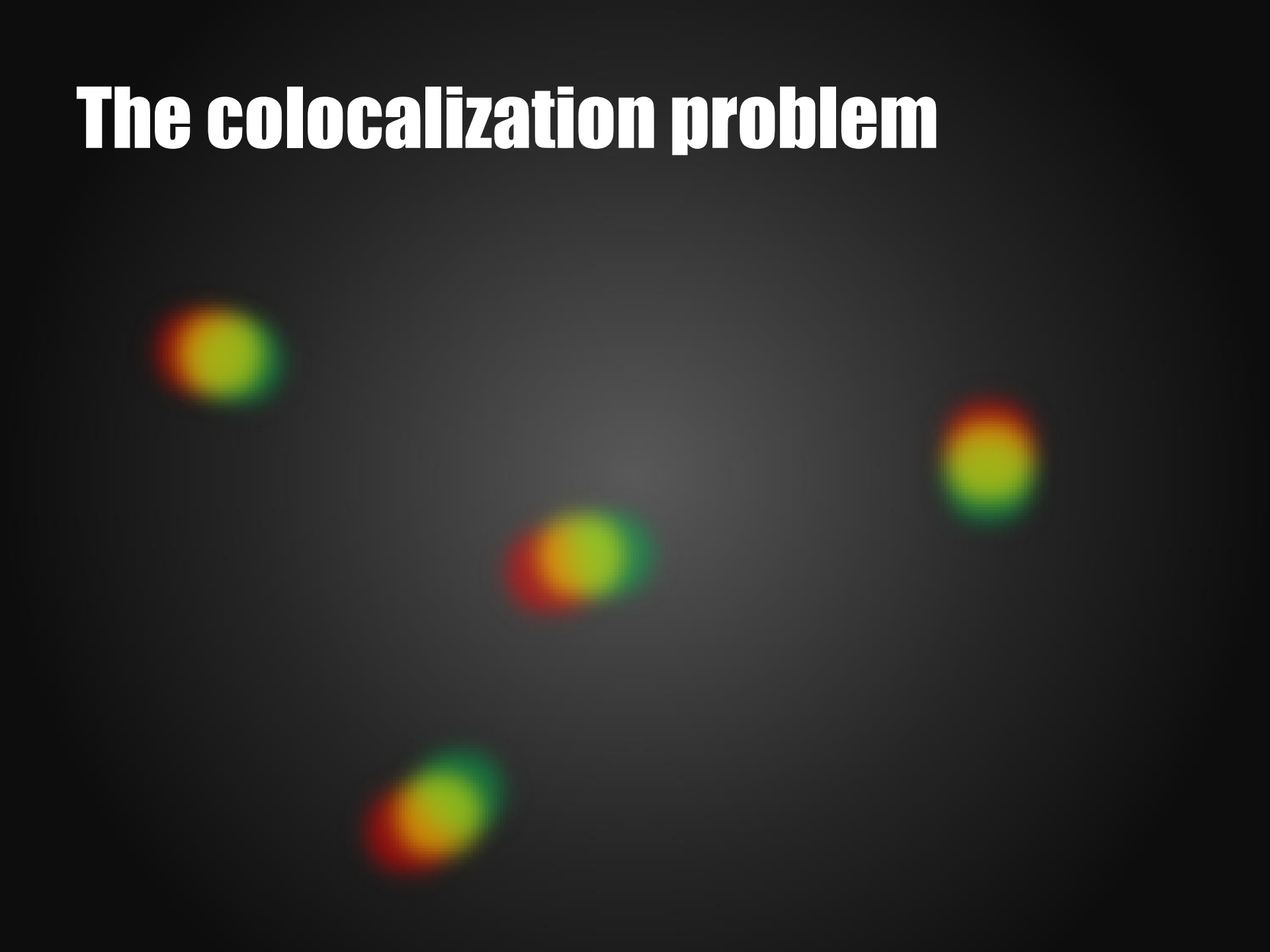

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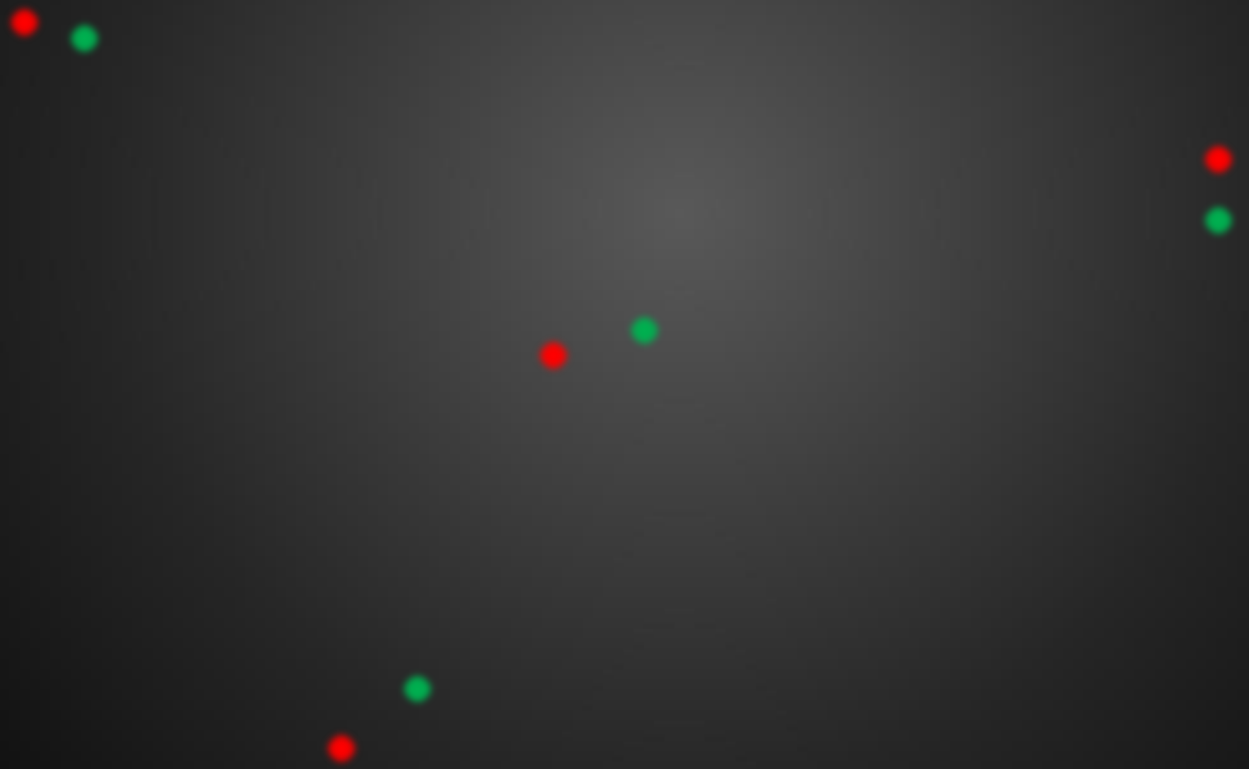
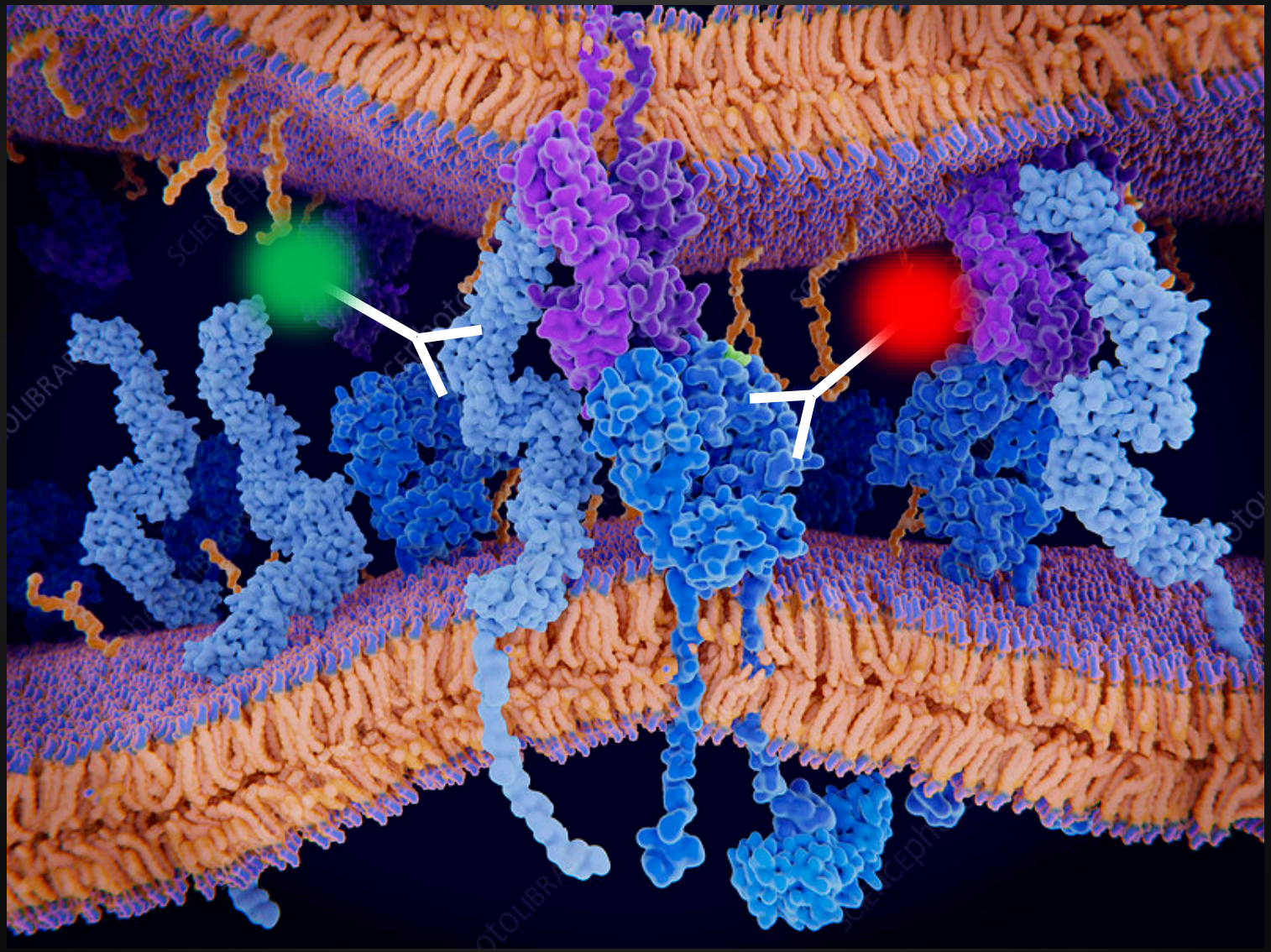


The colocalization problem



THANKS FOR YOUR ATTENTION!