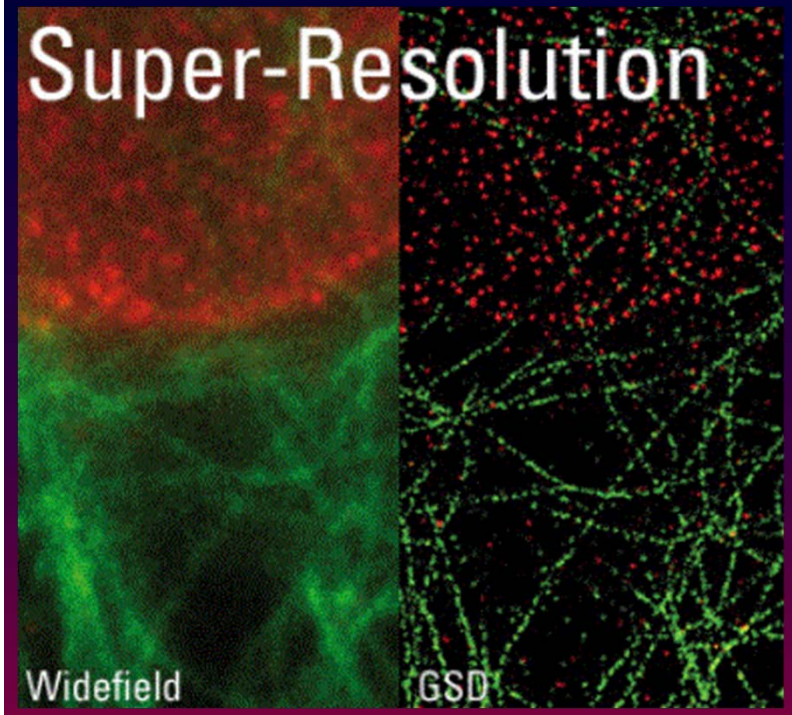
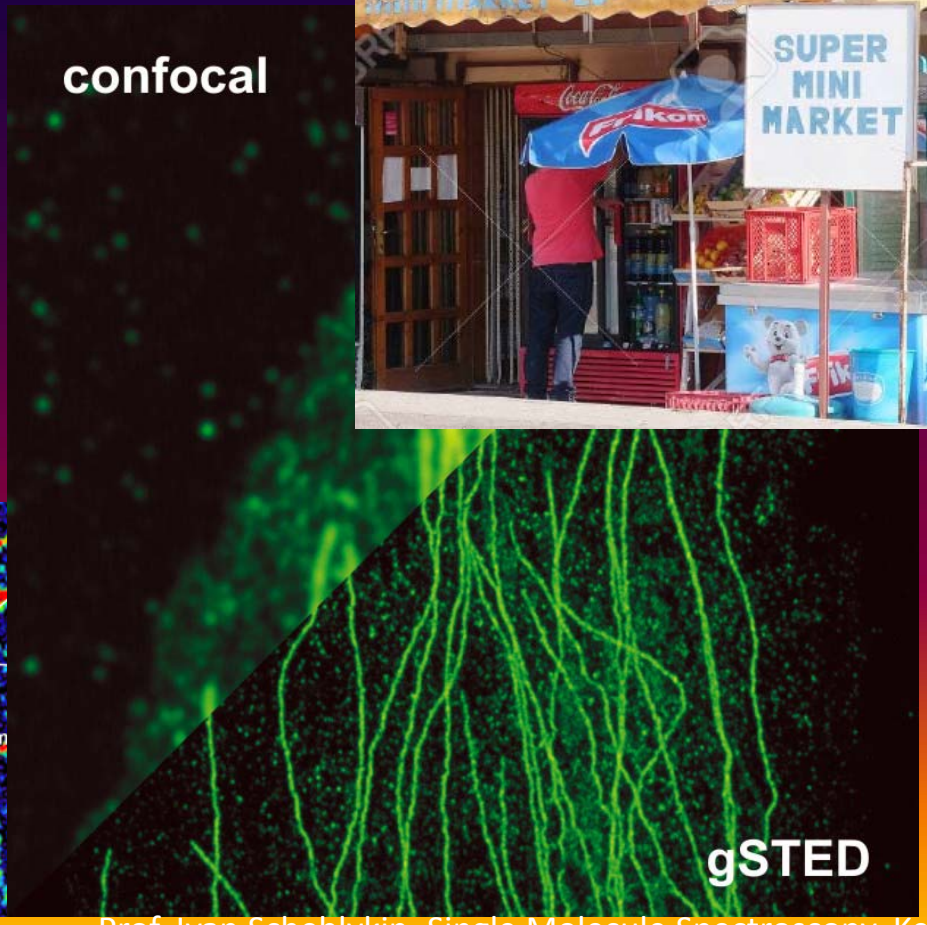
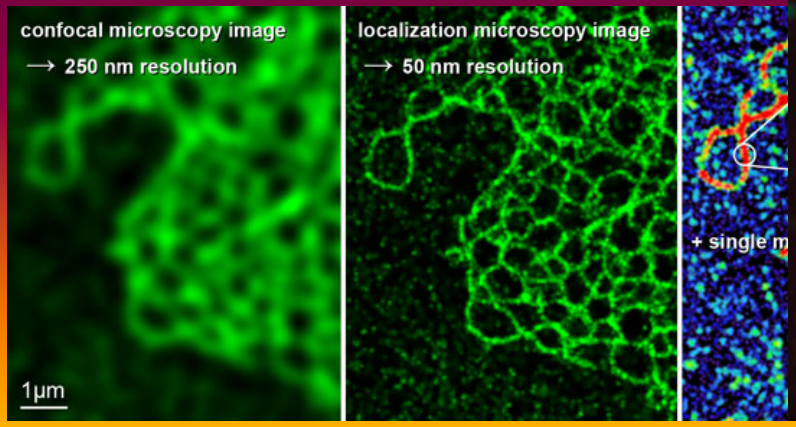
10 min about SUPER-resolution microscopy

Ivan Scheblykin, Kemisk Fysik, LU

Nobel Prize in Chemistry 2014







“Chemistry independent”

Trying to get more information about the object using optical tricks and special analysis

Resolution improvement up to 2 times

Super-resolution microscopy

“Chemistry dependent”. Single object emission/excitation

Special labeling + tricks + analysis

Nobel Prize in Chemistry 2014

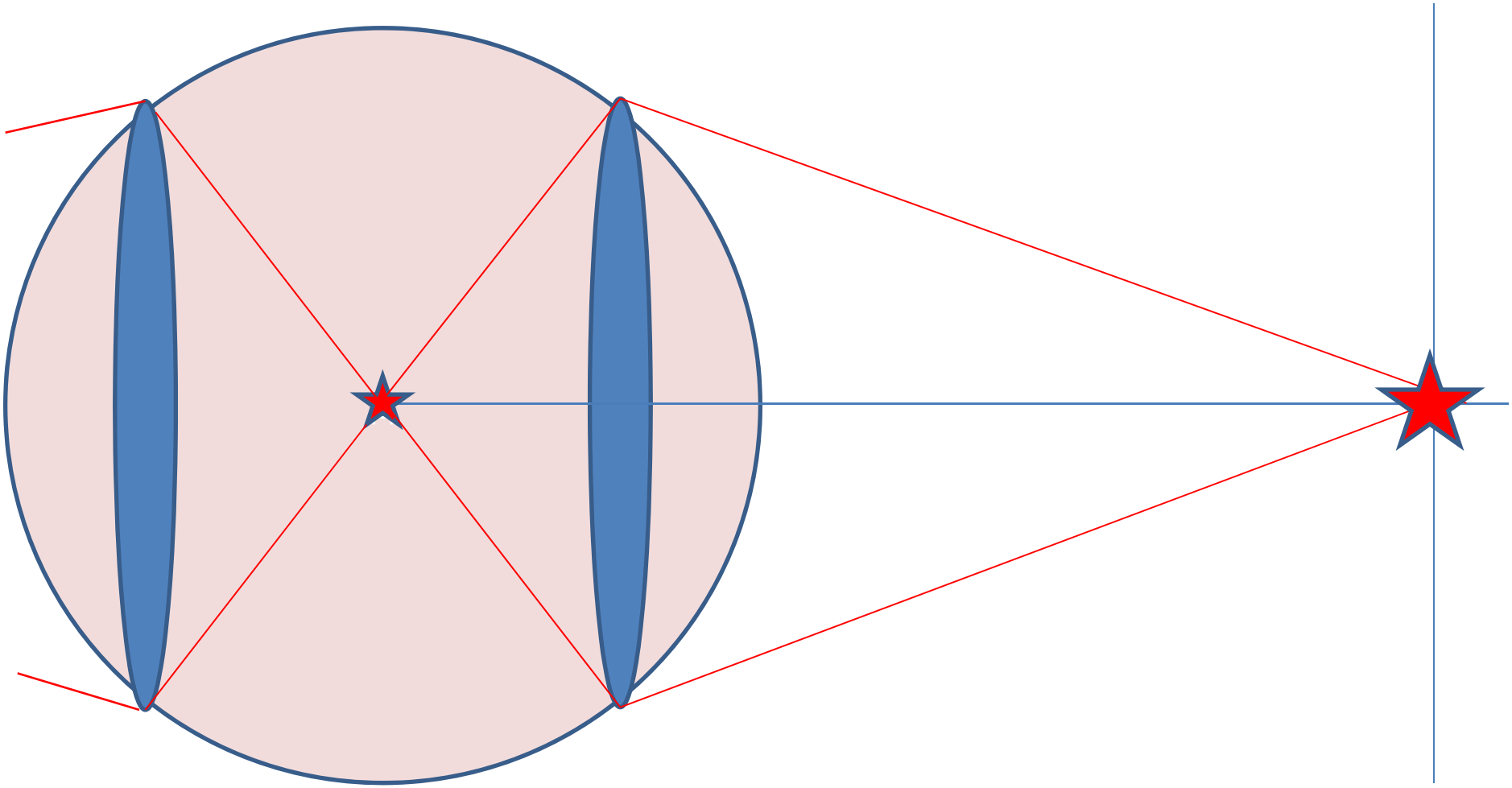
Resolution Improvement – unlimited (in theory), determined by signal to noise ratio

Distribution of the intensity in the image plane

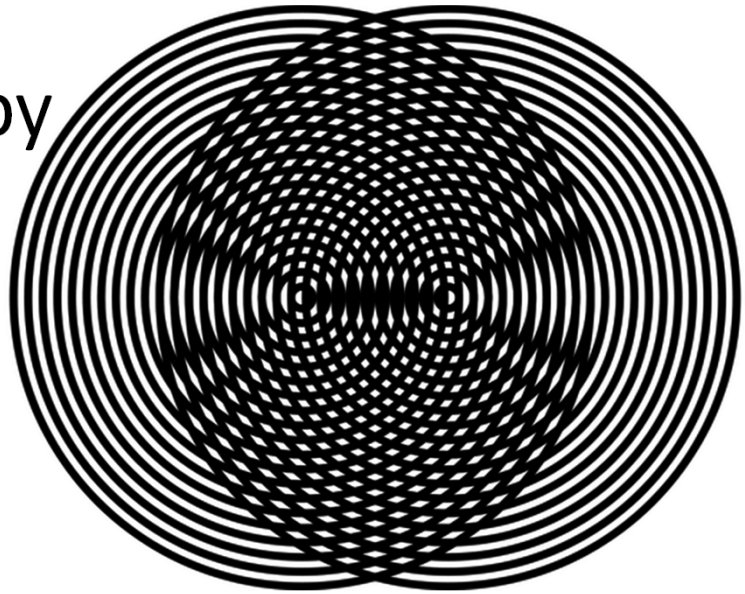
$$\frac{\lambda}{n \sin \varphi}$$

More information
- the smaller, the sharper the image

Maximum collection efficiency - 4π microscopy



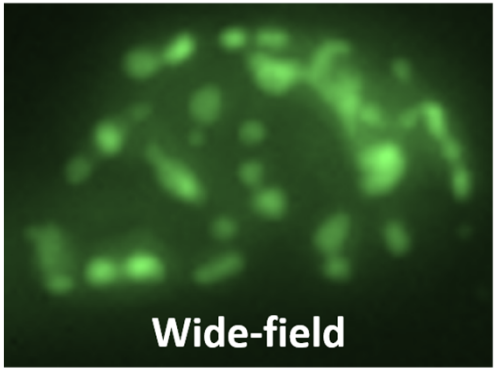
Structural illumination microscopy

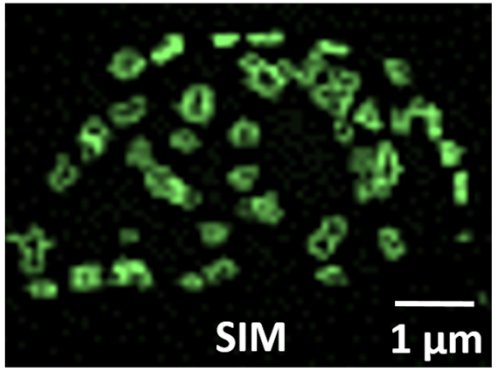


Moiré effect

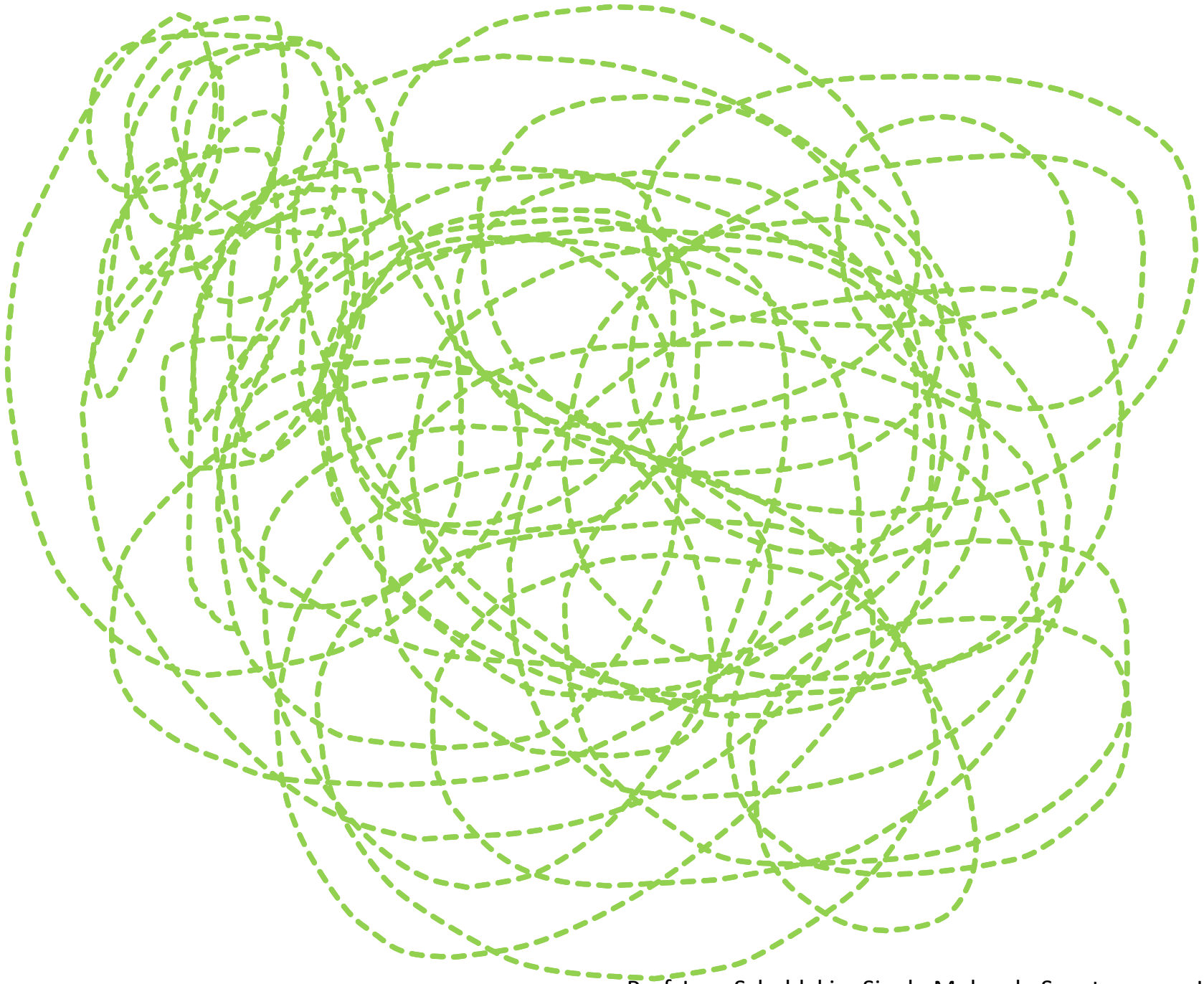
Collection of more information
(higher spatial frequencies)

2 times resolution improvement

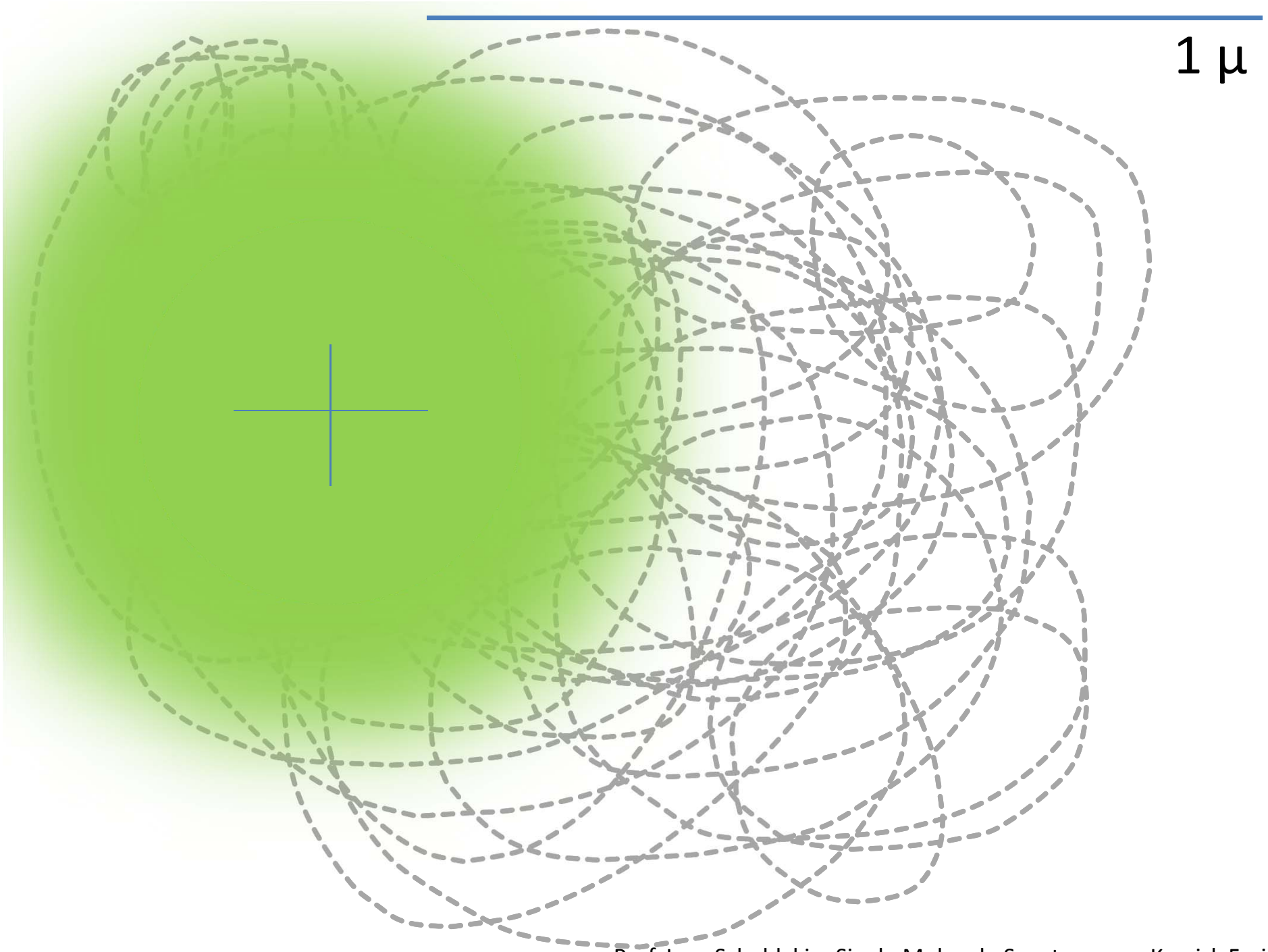


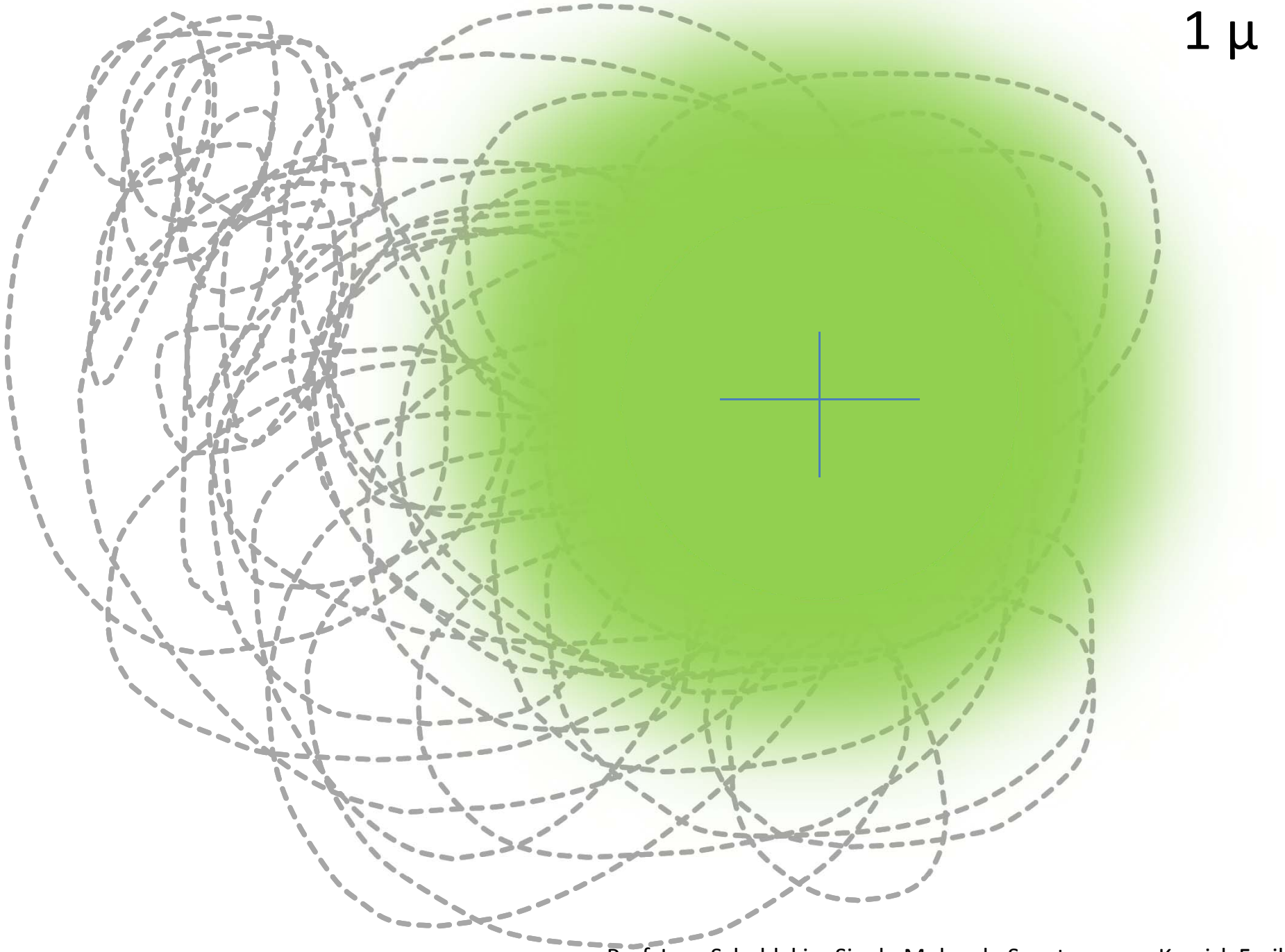


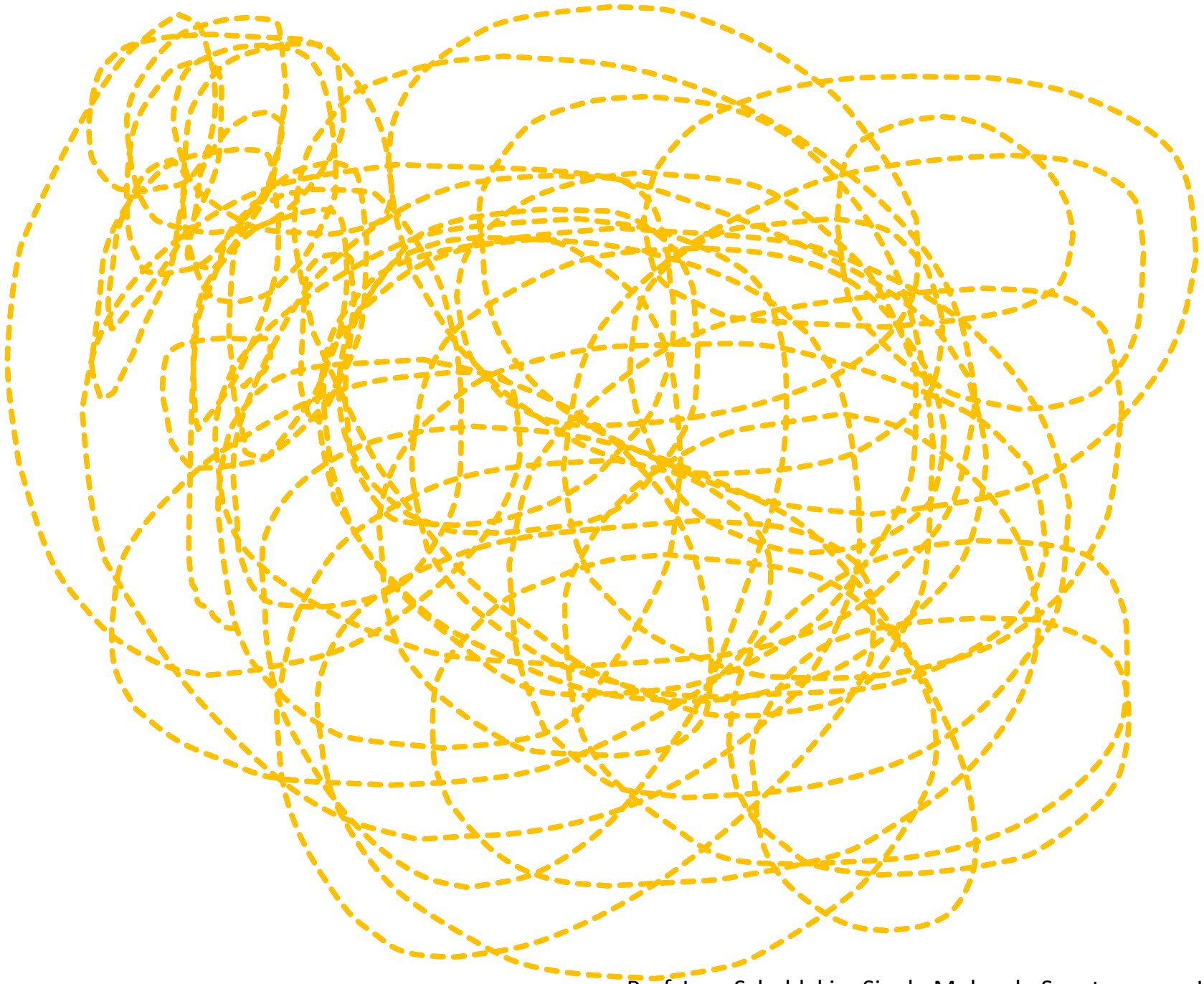
Real SUPER-resolution


1 µ

1 µ

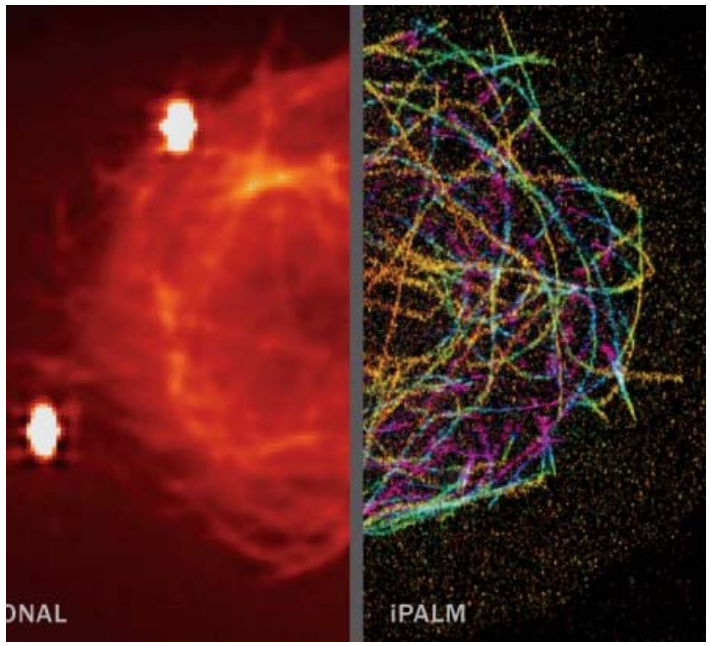

1 µ


1 µ

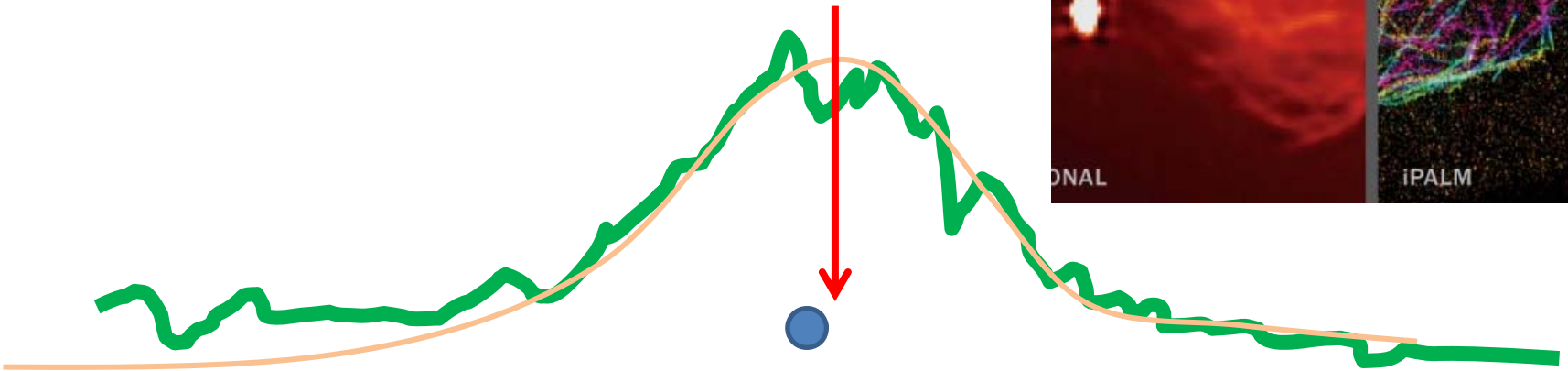

1 µ

So, the idea is to see one chromophopre at a time

- single molecule detection



Accuracy – all depends on the signal/noise ratio

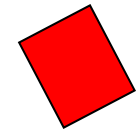


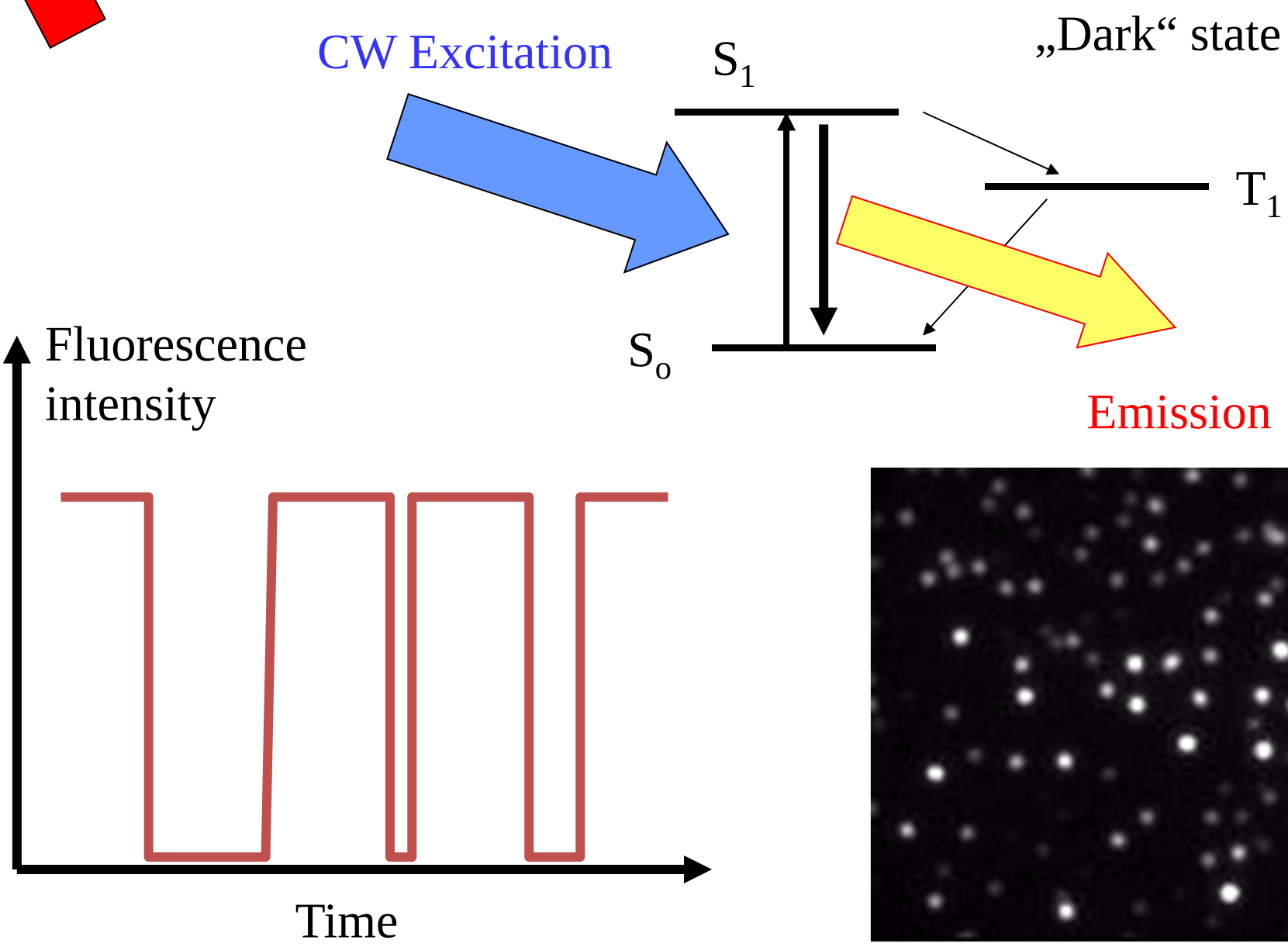
In practice **special dye lables** need to be used to reach the required blinking (switching) pattern !!!

Ivan Scheblykin

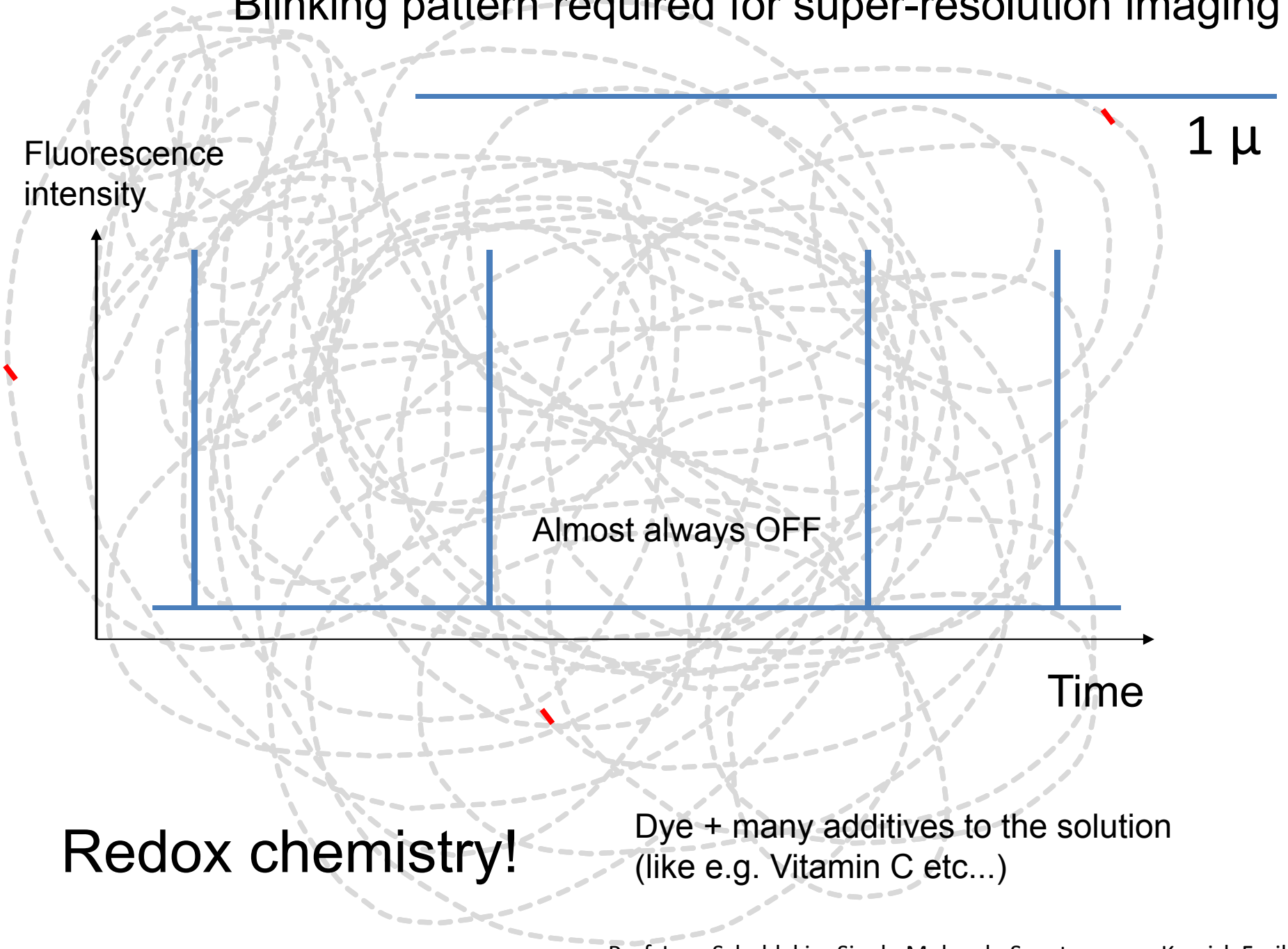
Single Molecule

Fluorescence “blinking”





Blinking pattern required for super-resolution imaging

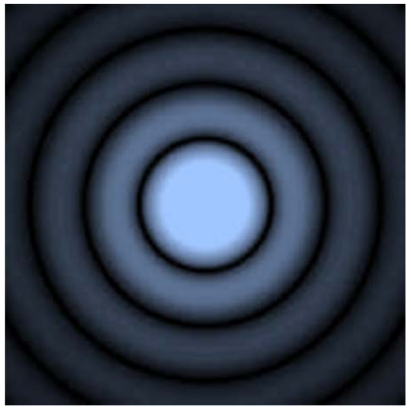


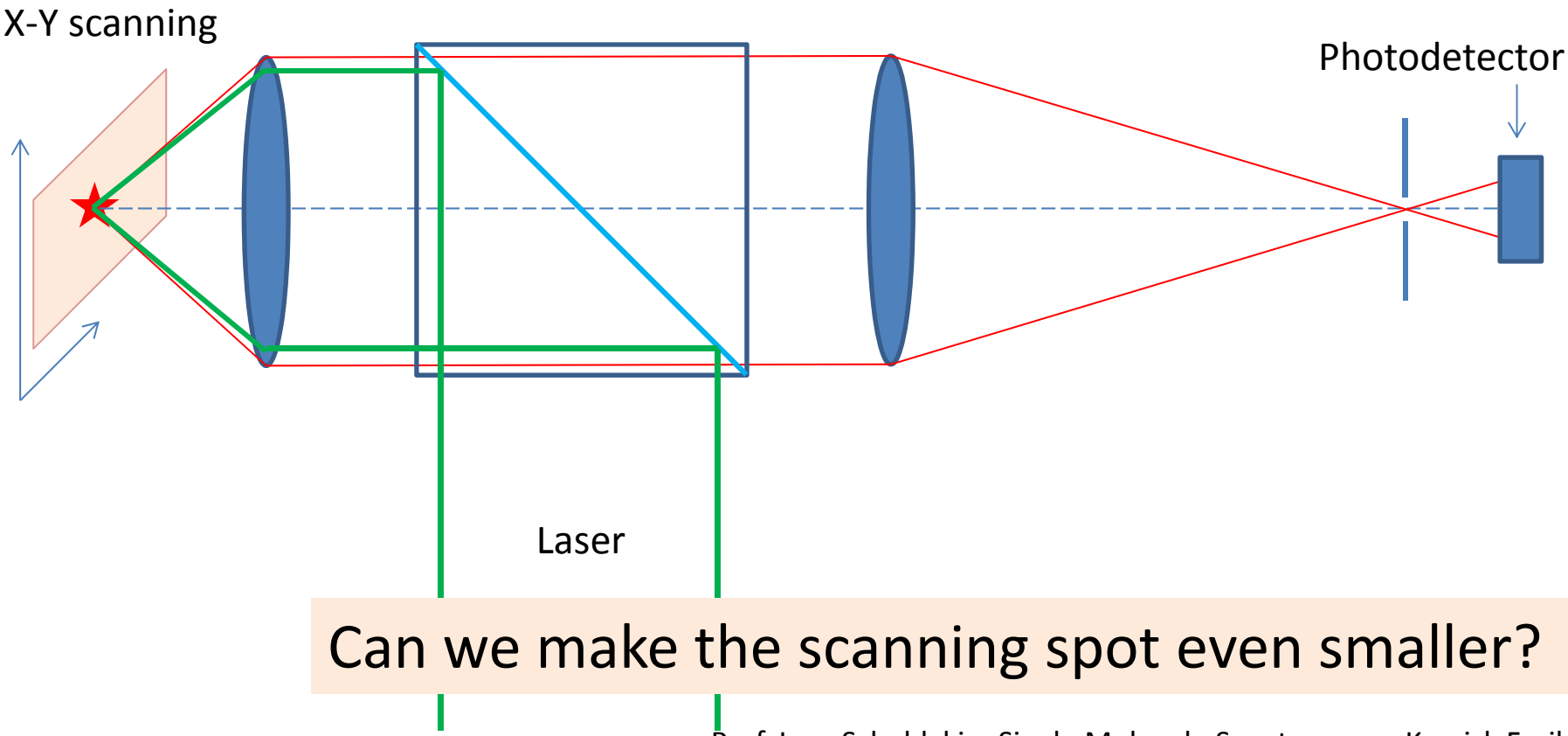
Redox chemistry!

Dye + many additives to the solution
(like e.g. Vitamin C etc...)

Scanning confocal microscopy

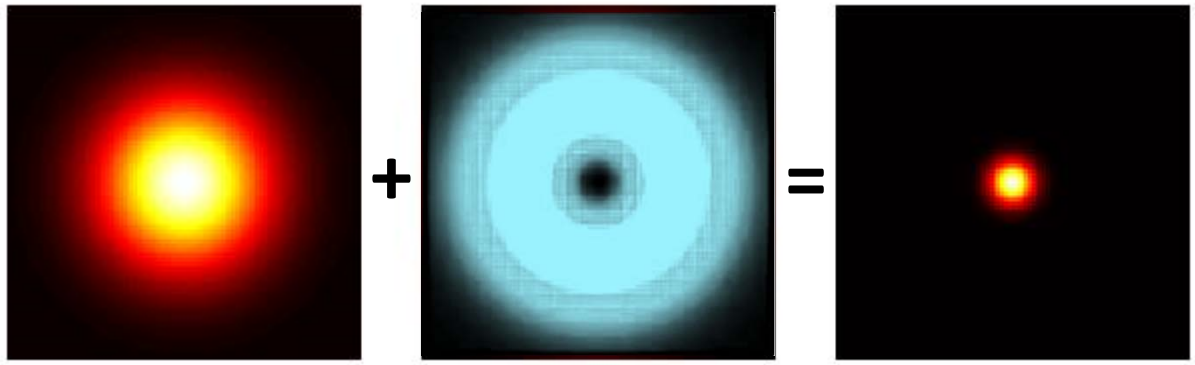
Laser is focused to the smallest spot possible - diffraction limited





Can we make the scanning spot even smaller?

STED - Stimulated emission depletion microscopy



The Nobel Prize in Chemistry 2014

Photo: Matt Staley/HHMI

Eric Betzig

Prize share: 1/3

Photo: Wikimedia Commons, CC-BY-SA-3.0

Stefan W. Hell

Prize share: 1/3

Photo: K. Lowder via Wikimedia Commons, CC-BY-SA-3.0

William E. Moerner

Prize share: 1/3

Hell, S. W.; Wichmann, J. (1994).

In 1986, the super-resolution optical microscope based on stimulated emission was patented by **Viktor Okhonin**.[15]

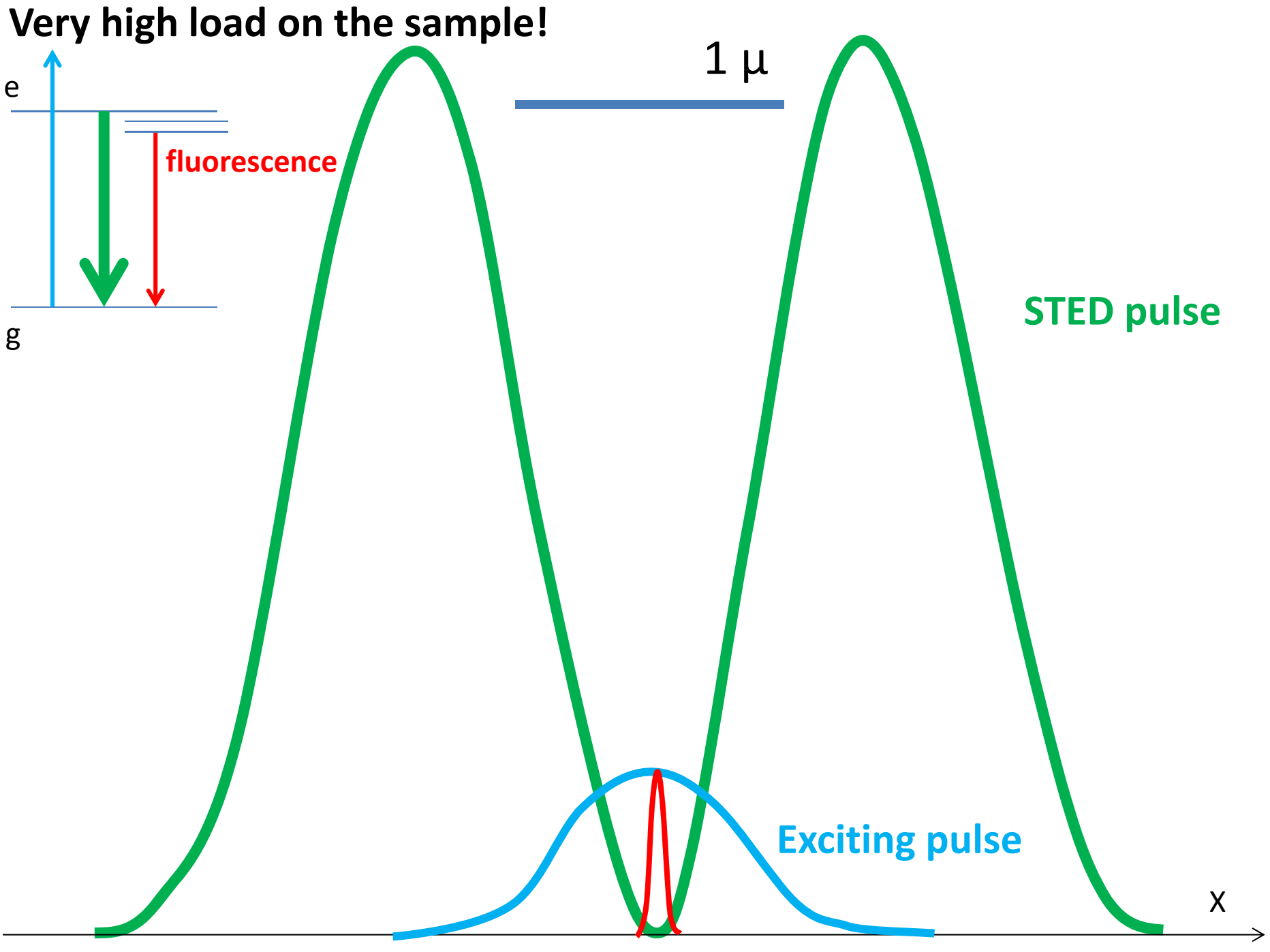
Institute of Biophysics,

USSR Academy of Sciences,

Siberian Branch, Krasnoyarsk

Last 20 years

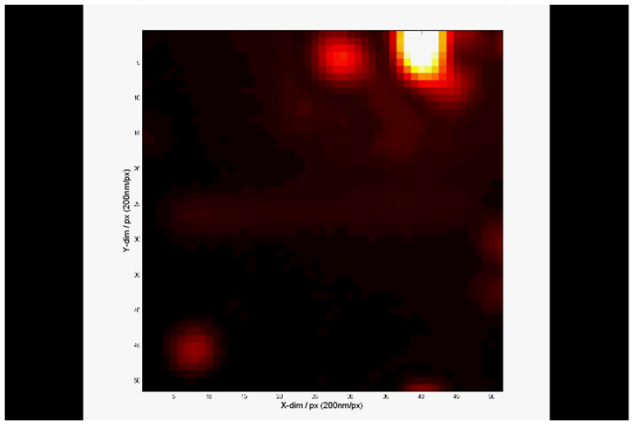
- IT specialist in Canada

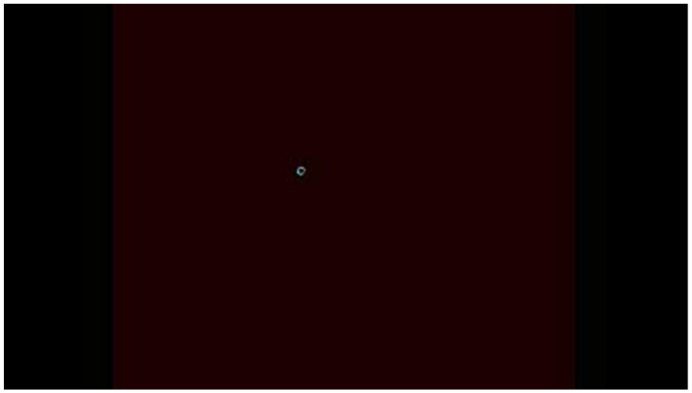

Very high load on the sample!
e
g
fluorescence
1 µ
STED pulse
Exciting pulse
X

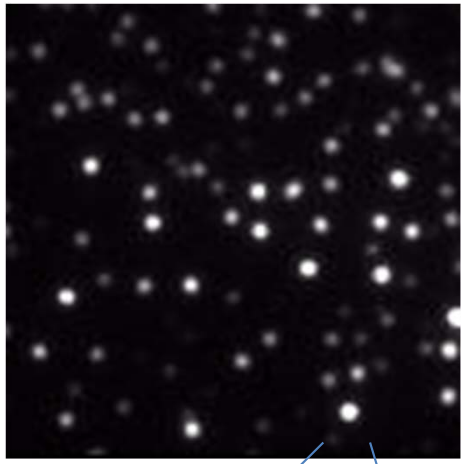
Blinking of nano and micro-crystals of organo-metal halide perovskites (solar cell material)

10 µ

It is the long-range energy or charge carrier migration which makes possible blinking of micrometer-sized object (semiconductors)



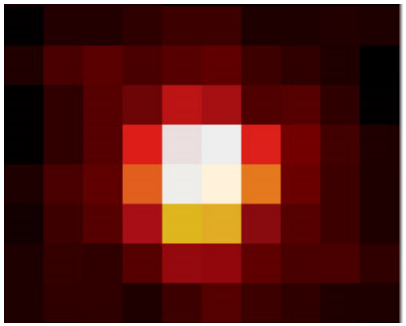




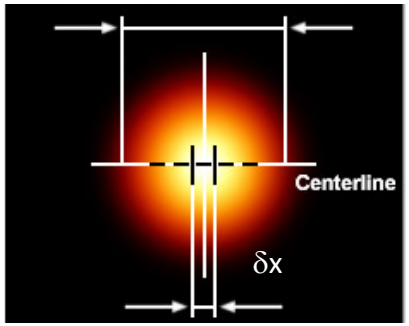
Where the emission is coming from ?

Optical super-resolution

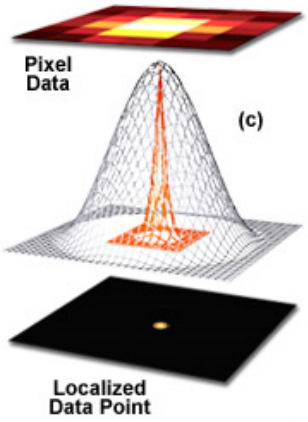
Adapted from zeiss-campus.magnet.fsu.edu/ Full width ~ λ/2

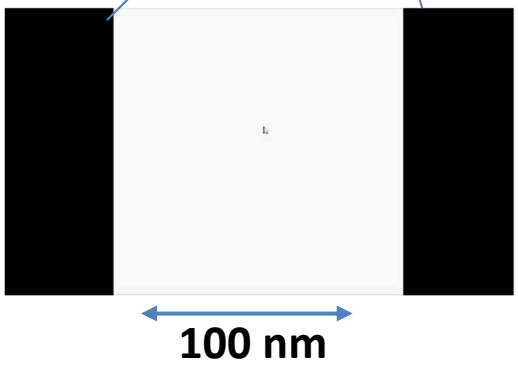


Raw image



Gaussian Fit Function





Uncertainty δx of the emitter localization is much smaller than λ/2

Fitting the image of an object by a 2D Gaussian
Determination the position of the center

Localization of emitting sites in complex materials

Aboma Merdasa's Ph.D. Thesis, 2017

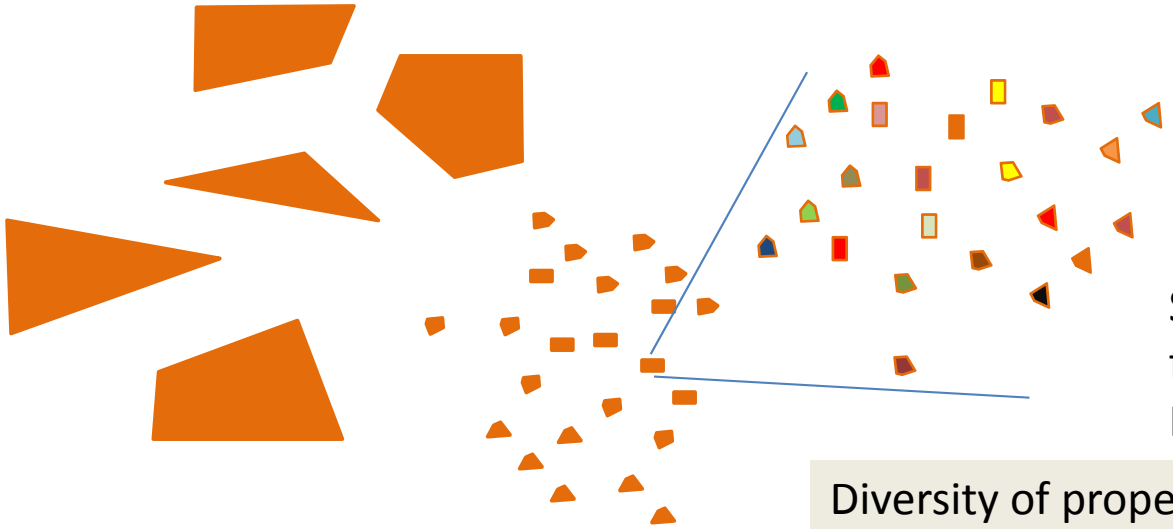
Single molecule spectroscopy in service of Material Science

Prof. Ivan Scheblykin, Chemical Physics

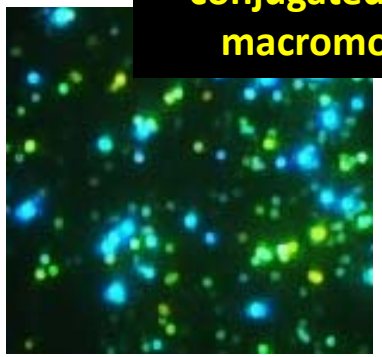
Samples – whatever luminescent material/particles/crystals etc

Bulk materials
- Ensemble averaged properties

"Cutting down" the material to nano-size



Different colours of conjugated polymer macromolecules



Size 1 – 1000 nm
from a molecule to a particle
Properties become INDIVIDUAL

Diversity of properties – Effect of the environment, molecular conformation, presence of defects etc

<u>Dynamics</u> at milliseconds and slower scale

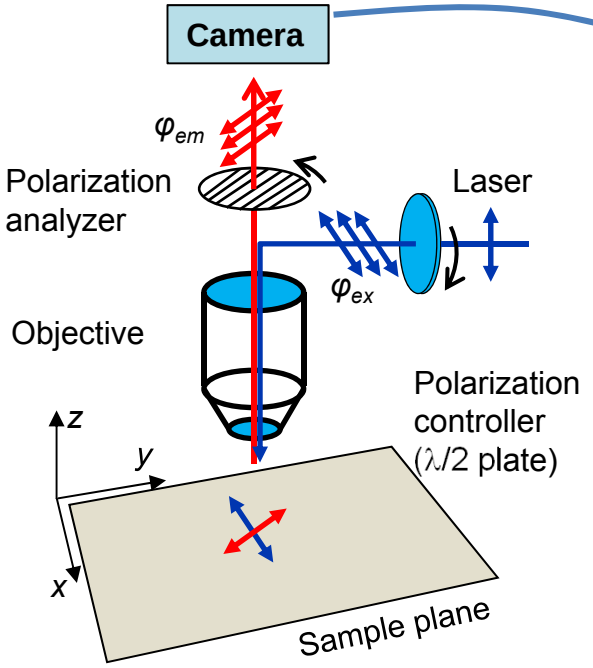
Fluorescence blinking
Spectral fluctuations

Chemical reactions (e.g. redox reactions)
Conformational fluctuations

Just one quenching site/redox site/trap controls luminescence of the whole object

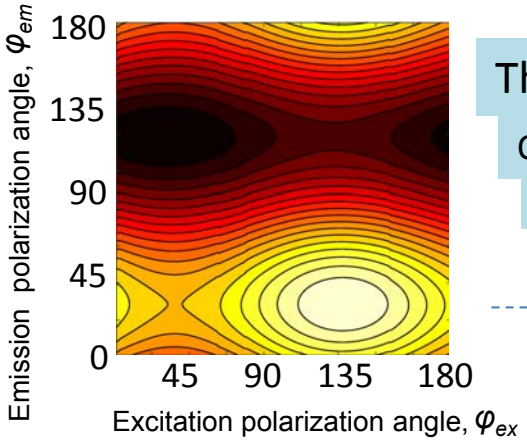
Polarization-sensitive fluorescence microscope
2D POLIM – 2D POLarization IMaging

Prof. Ivan Scheblykin, Chemical Physics



”Polarization portrait”

Fluorescence (φ_{ex}, φ_{em})



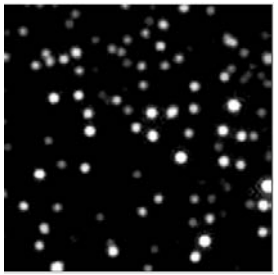
New imaging contrasts:

-Polarization modulation depth
- Polarization phase of excitation
- Polarization phase of emission
- Energy funneling efficiency

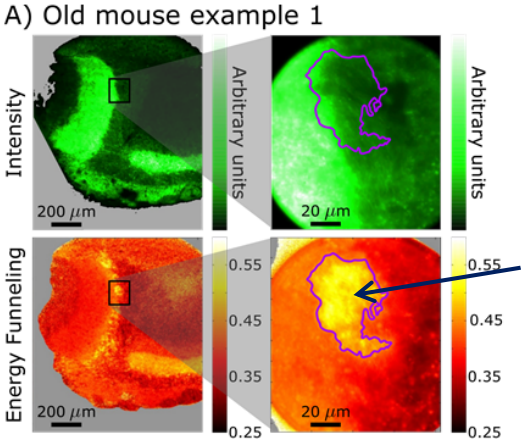
They tell us about:

Orientation of chromophores which absorb light
Orientation of chromophores which emit light
Energy transfer between them

Single molecule spectroscopy

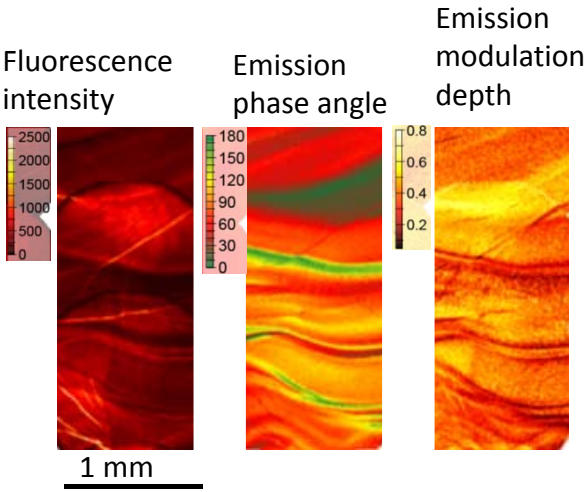


Biology:



Visualization of protein aggregation in mouse brain

Material science:



Conjugated polymer film with oriented domains