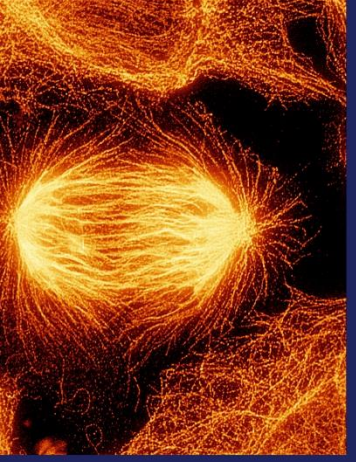
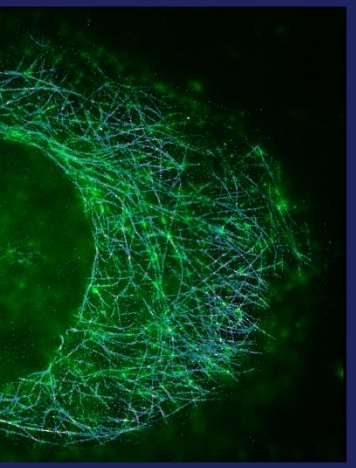
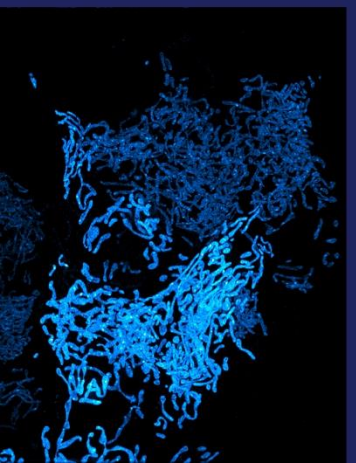
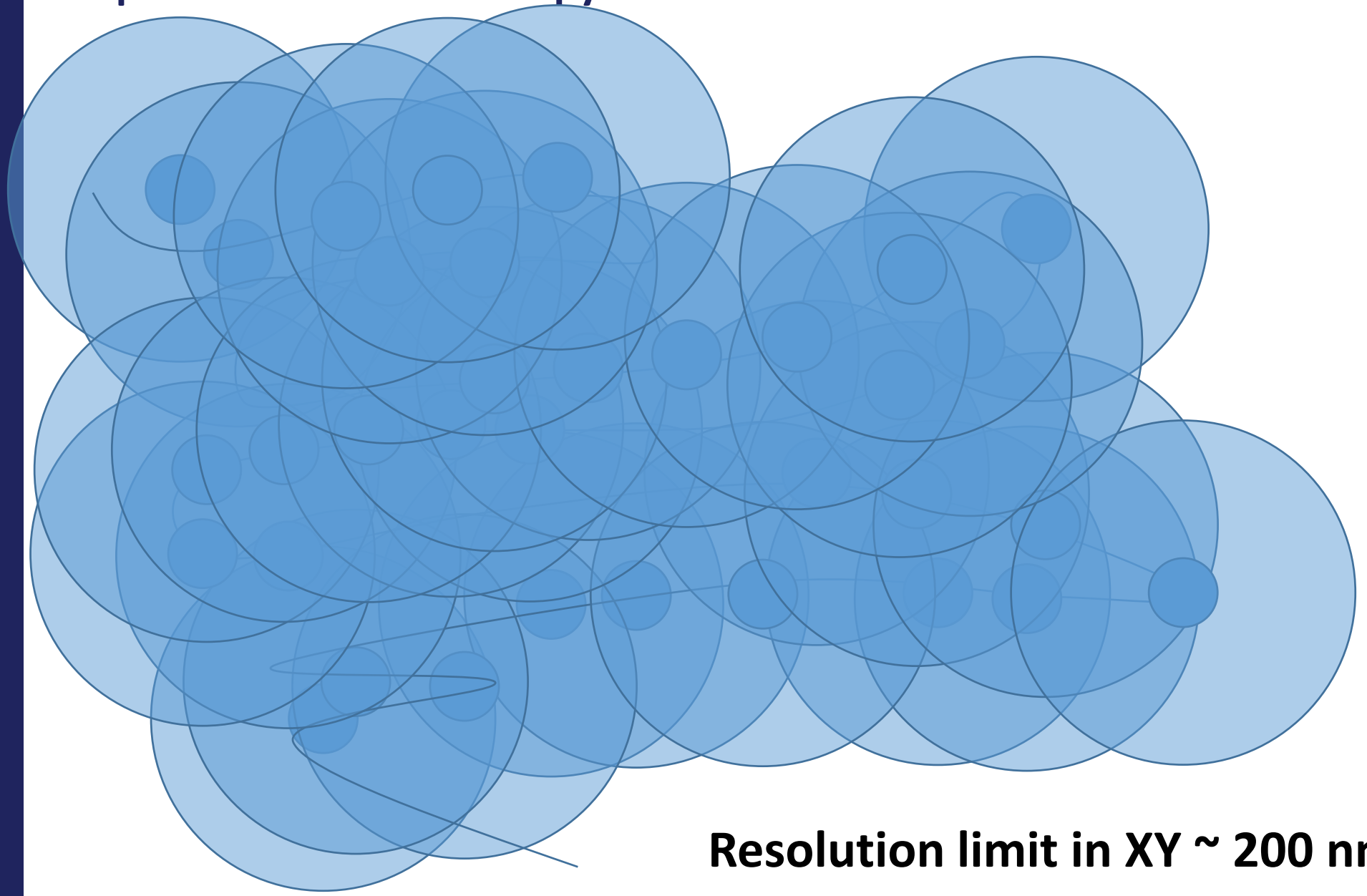






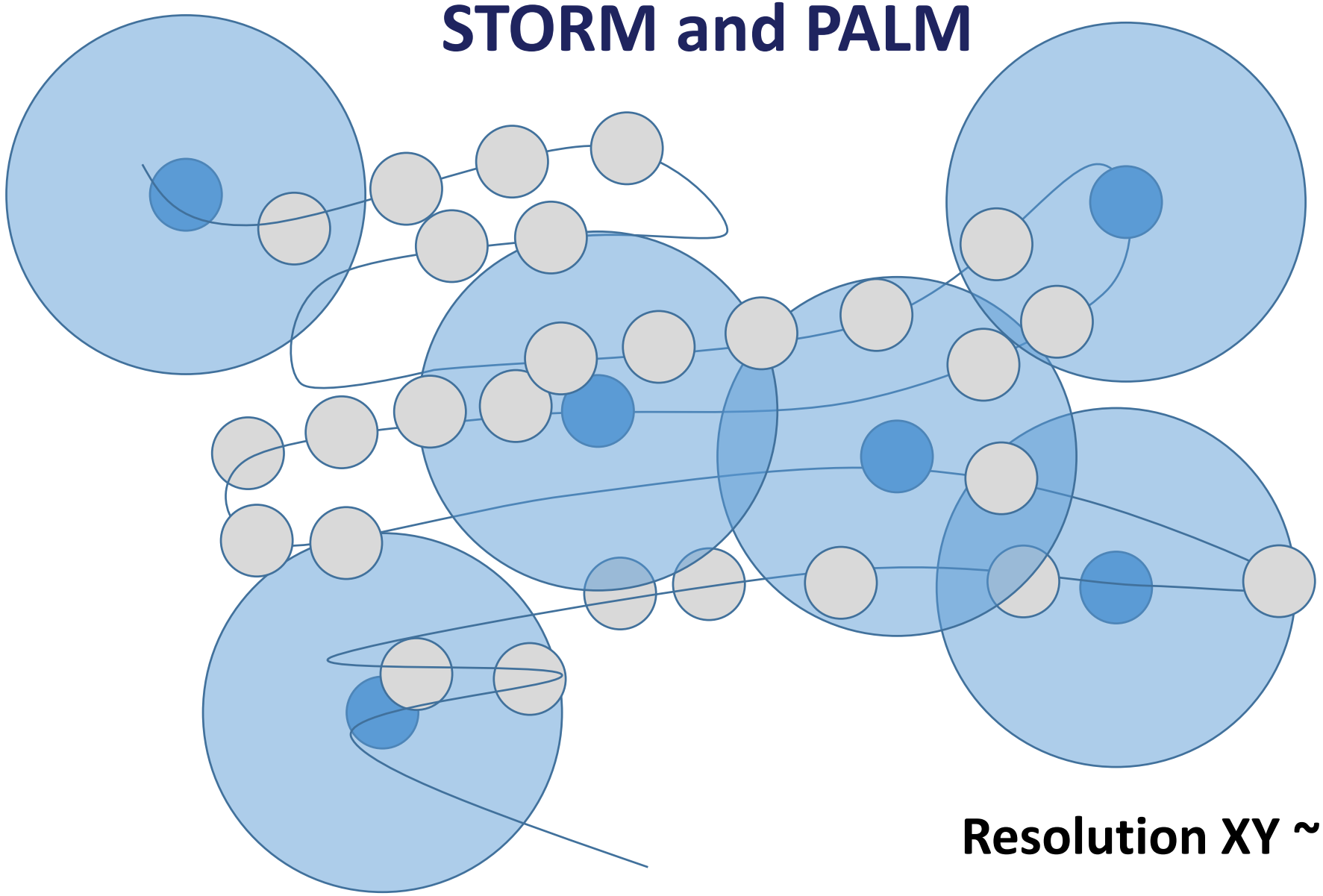
Superresolution microscopy



Resolution limit in XY ~ 200 nm

Superresolution microscopy – single molecule localization microcopy

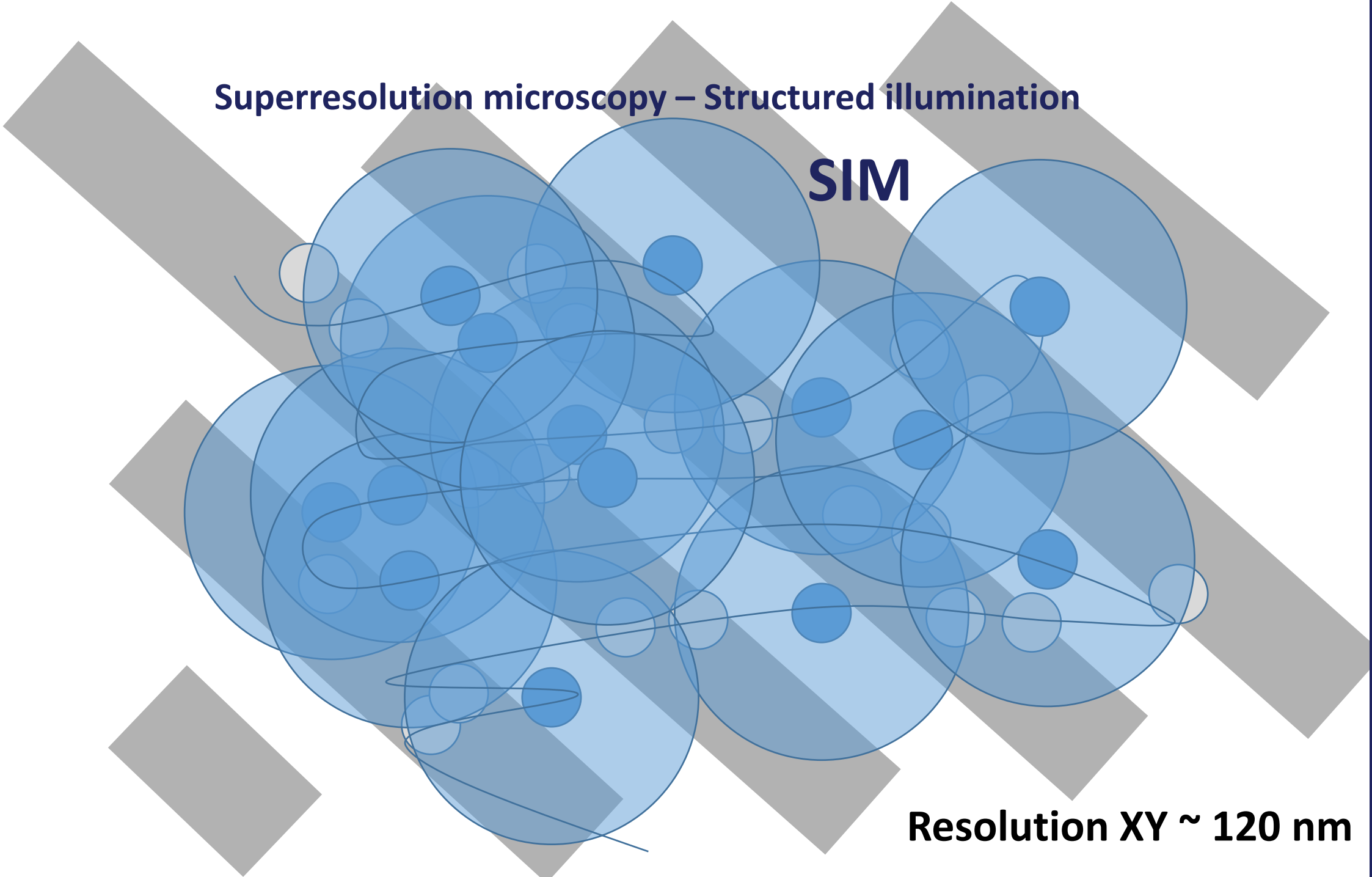
STORM and PALM



Resolution XY ~ 25 nm

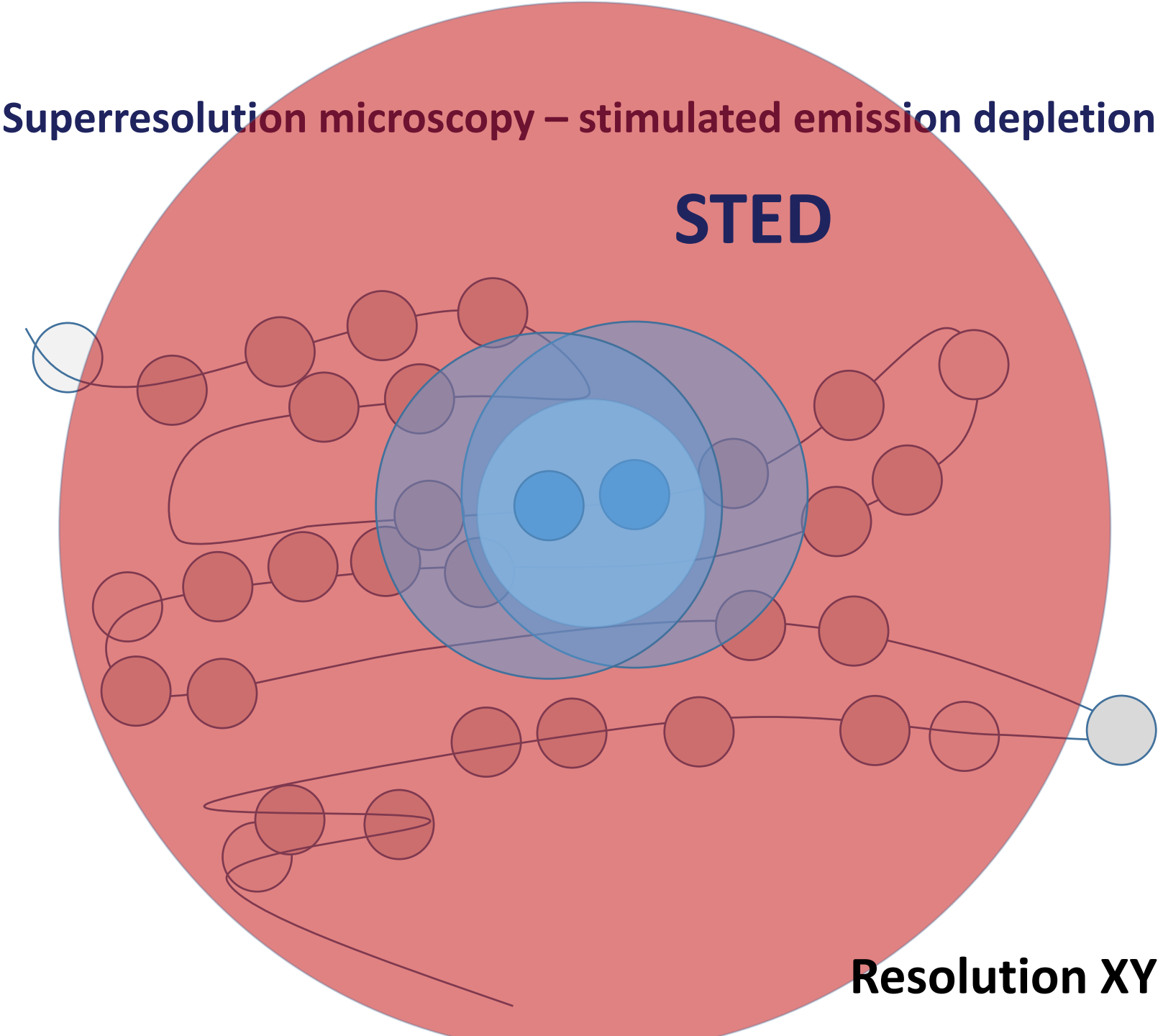
Superresolution microscopy – Structured illumination

SIM



Resolution XY ~ 120 nm

Superresolution microscopy – stimulated emission depletion



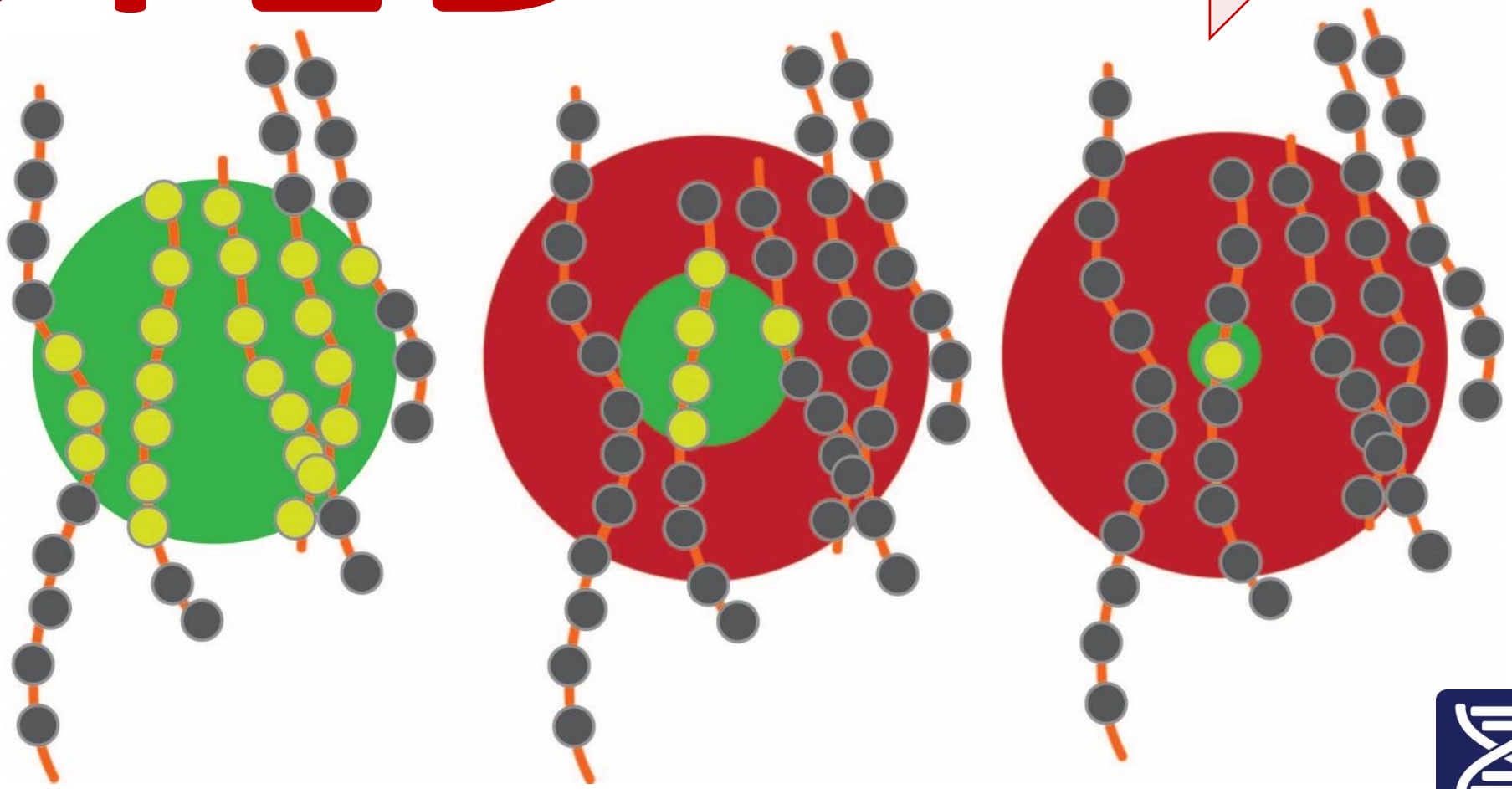
Resolution XY ~ 40 nm

STED

$$d = \frac{\lambda}{2NA}$$

$$d_{STED} = \frac{\lambda}{2NA\sqrt{1+\xi}}$$

break through the resolution barrier



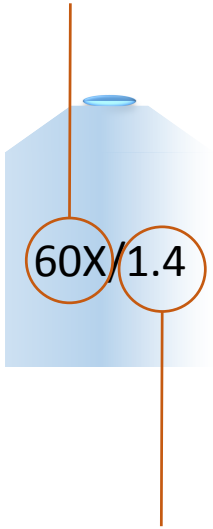
Ivan Novotny, SLM 2017

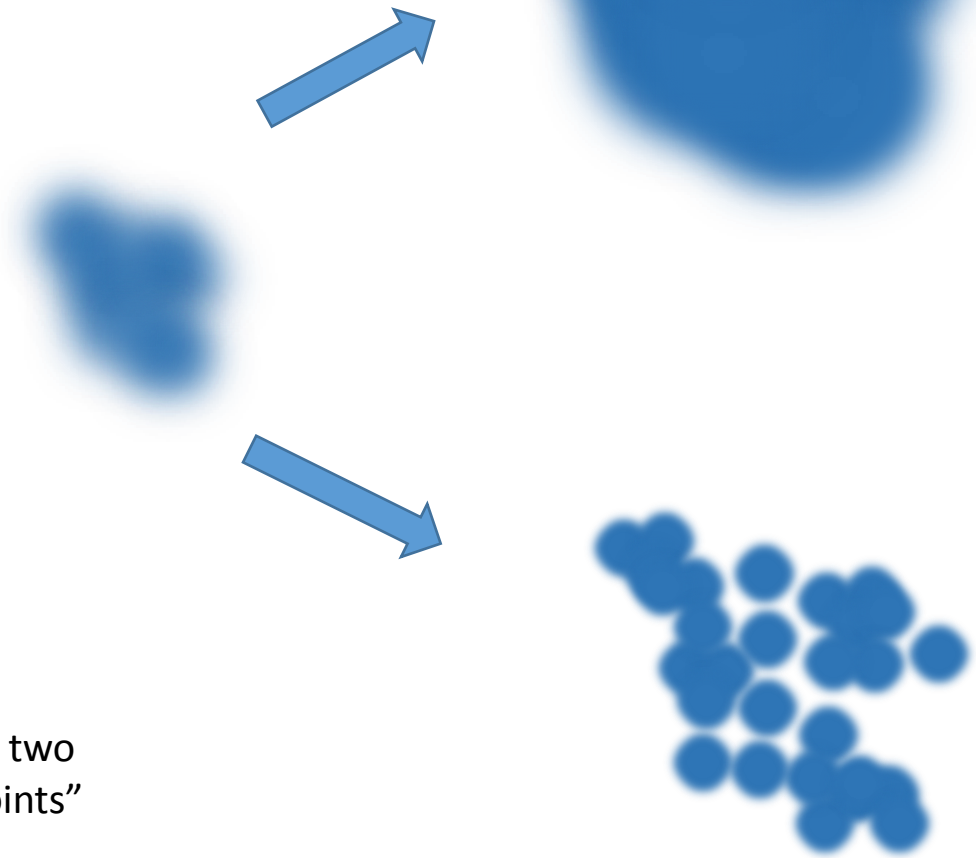
IMG
Light Microscopy Core Facility

Magnification

“How many times the apparent size of the object is increased”

TO SEE THINGS BIGGER





Resolution

“The smallest distance at which two points can be identify as two points”

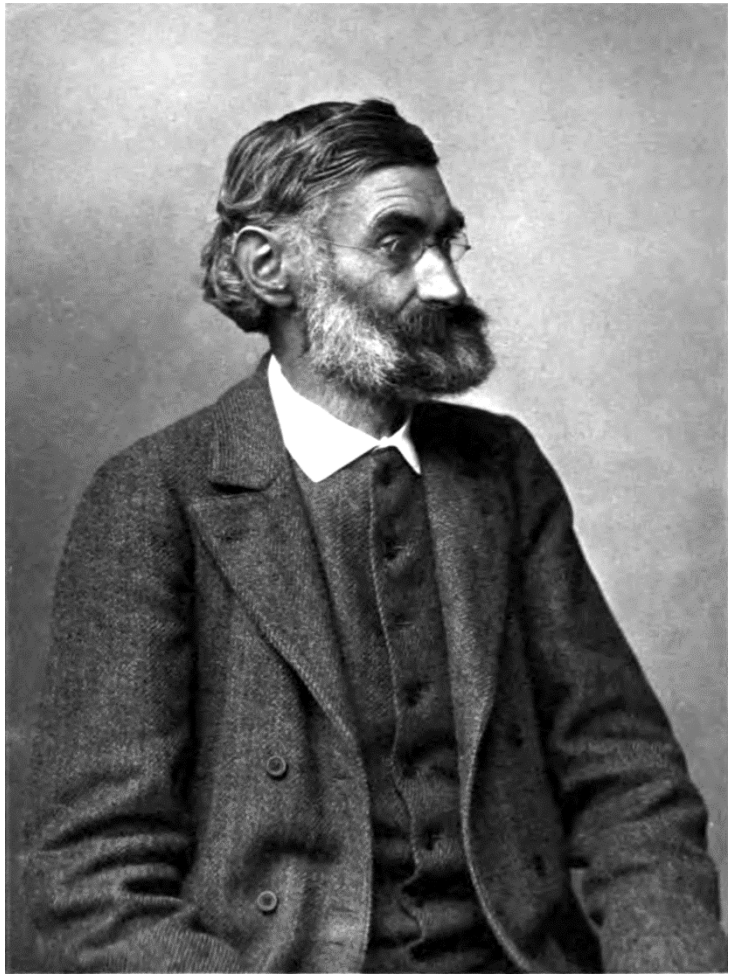
TO SEE MORE DETAILS

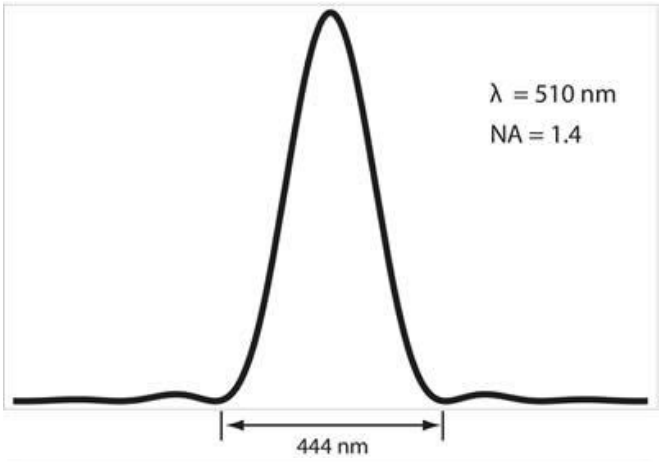
resolution

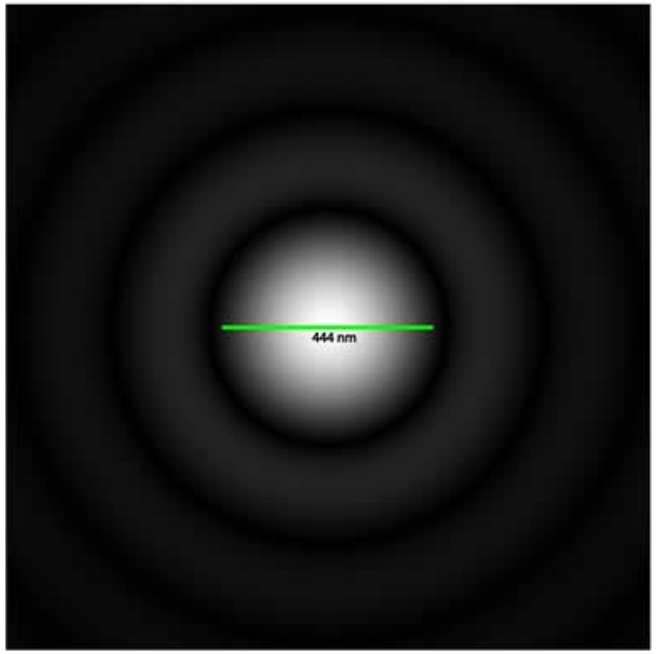
Ernst Abbe

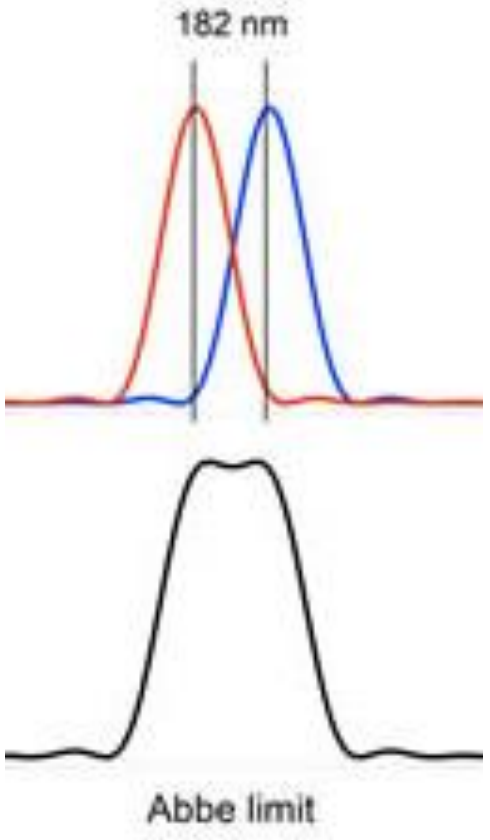
Resolution barrier

$$d = \frac{\lambda}{2NA}$$



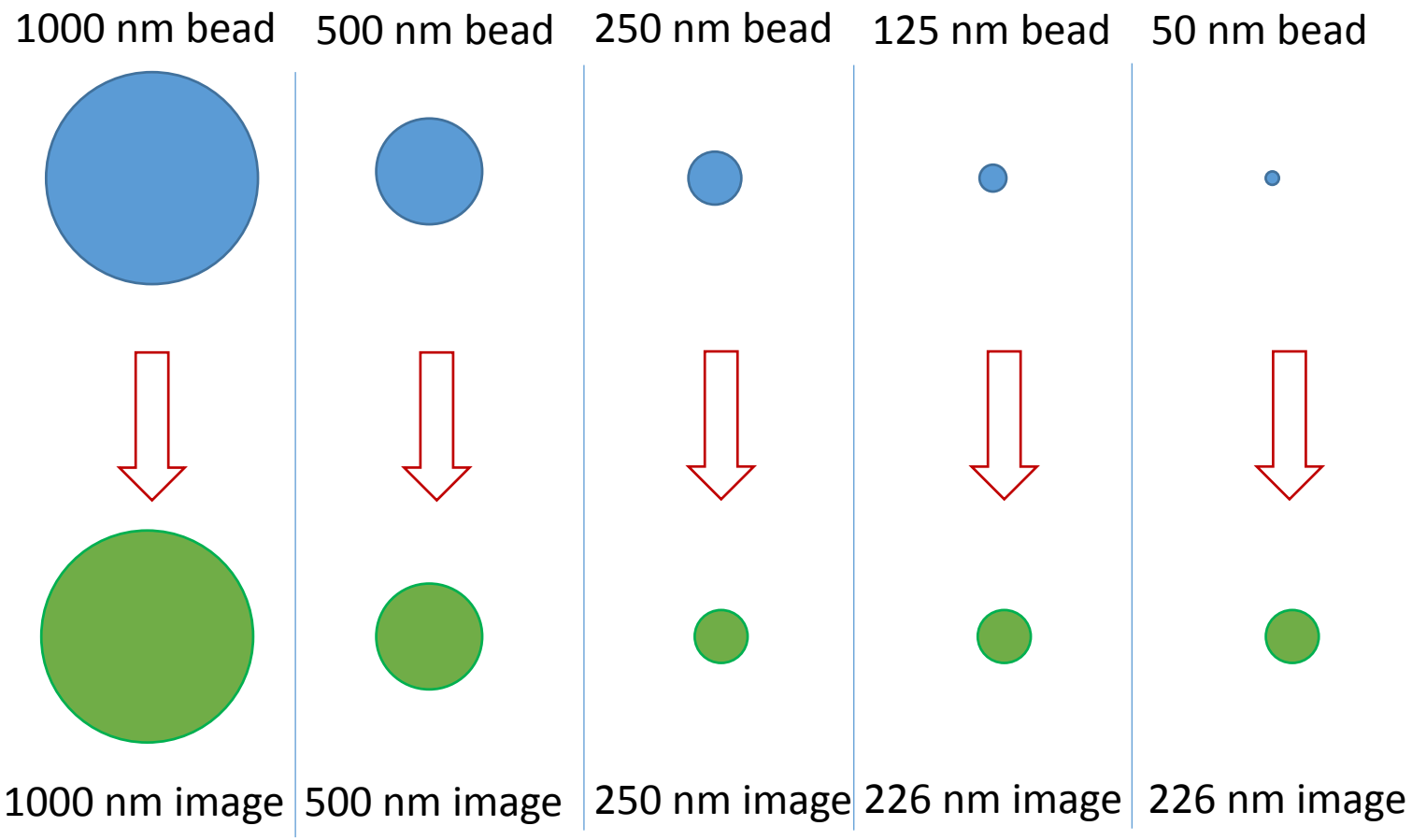


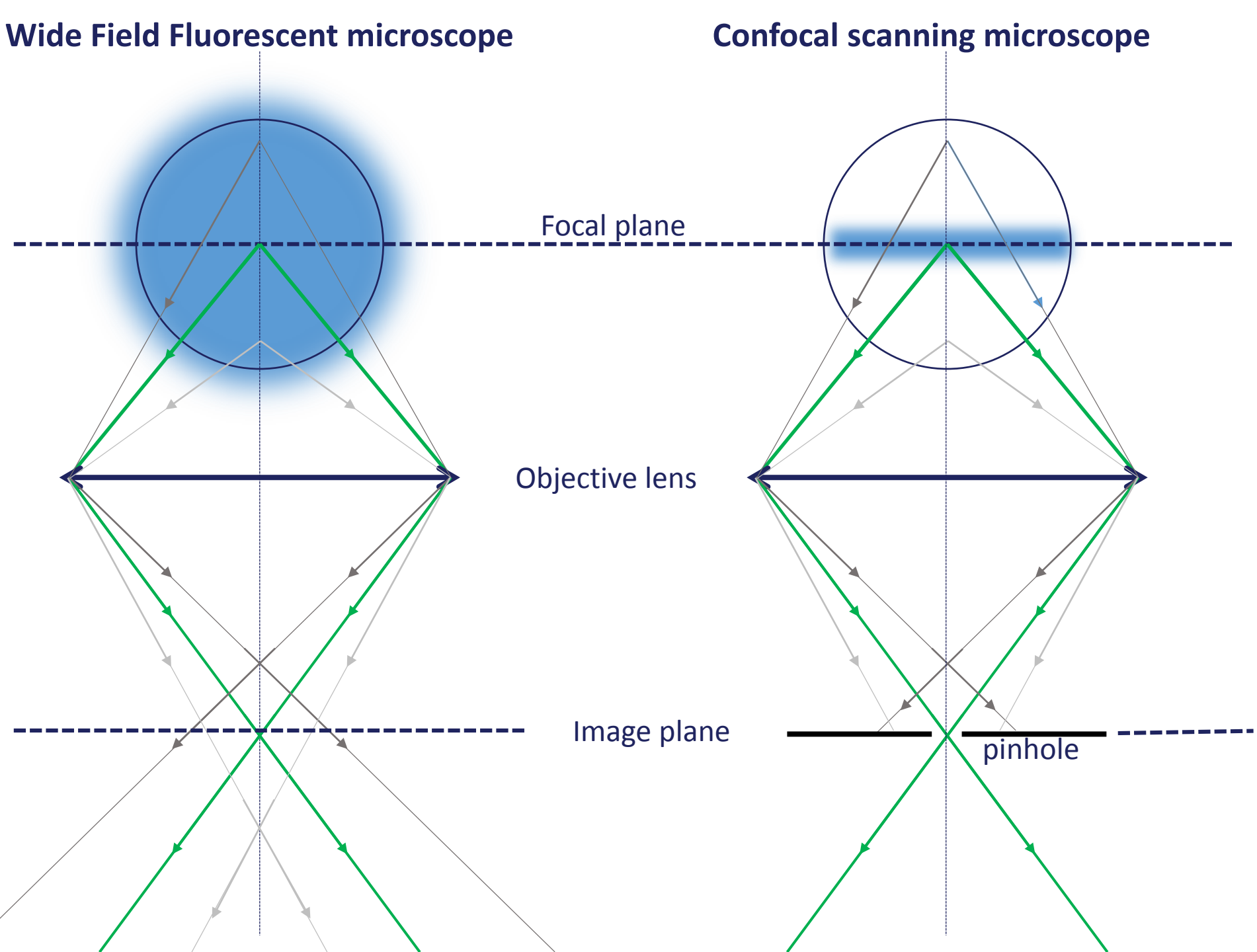




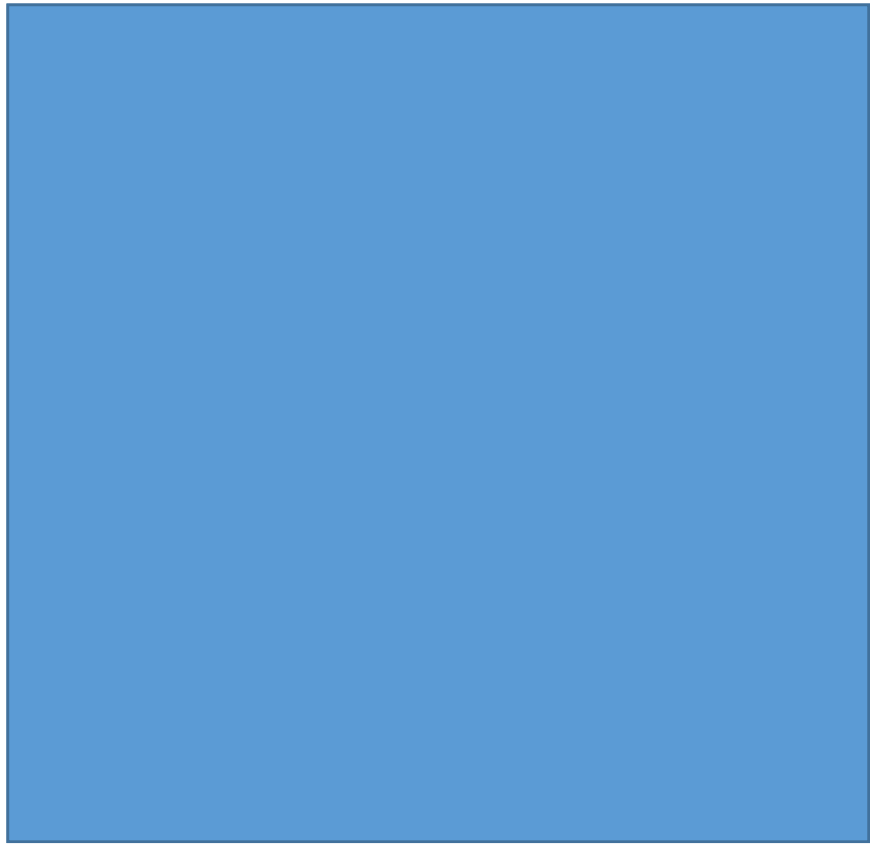
$$\boldsymbol{d = \frac{\lambda}{2NA}}$$

e.g., for λ = 520 and NA = 1.4, the resolution is 226.6 nm:

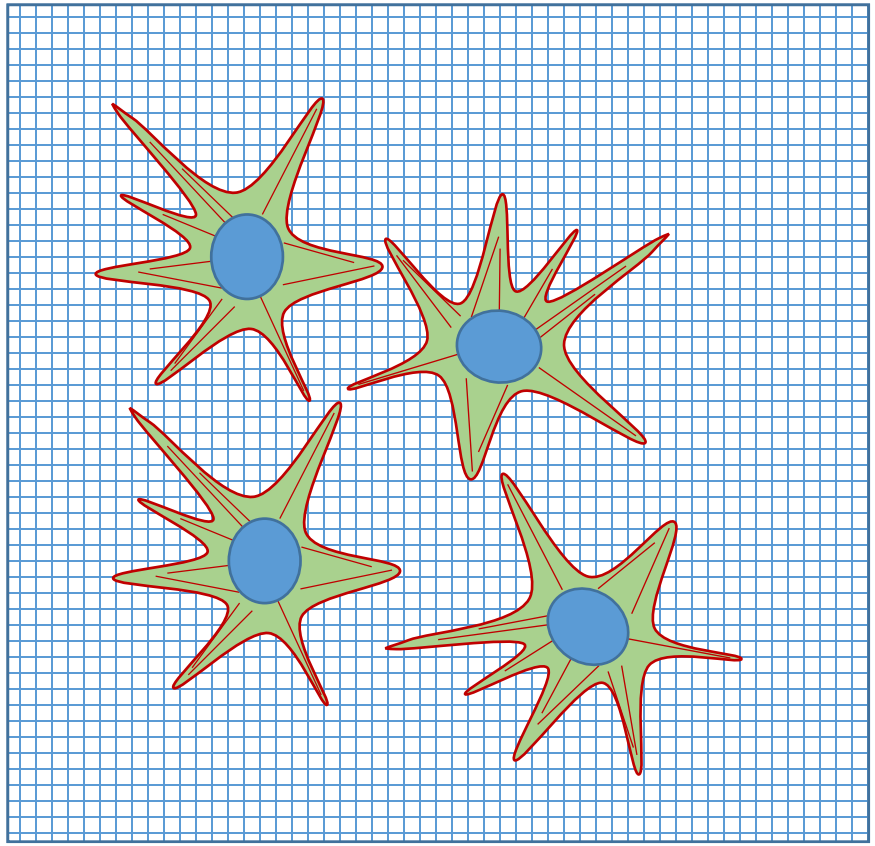



Wide Field Fluorescent microscope
Confocal scanning microscope
Focal plane
Objective lens
Image plane
pinhole

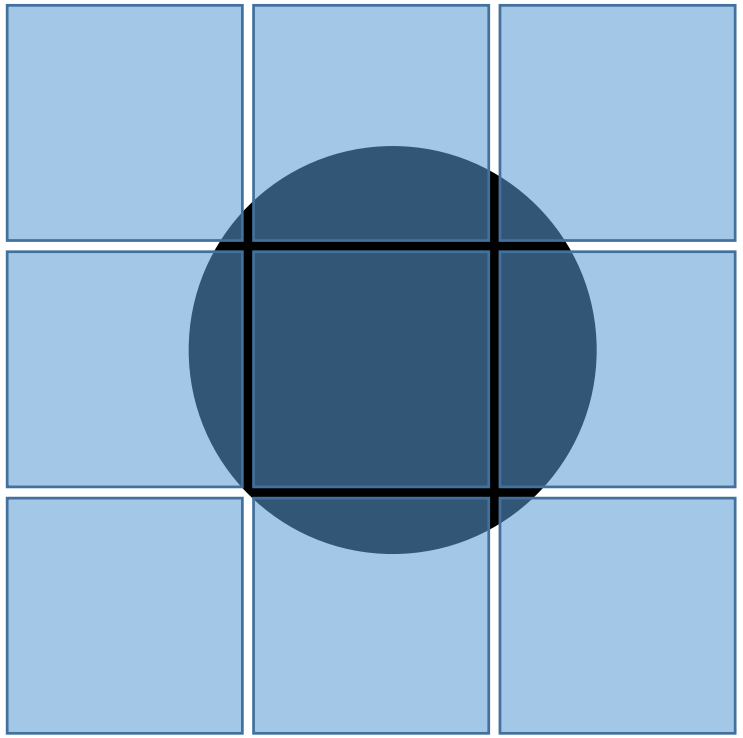
Wide field microscope

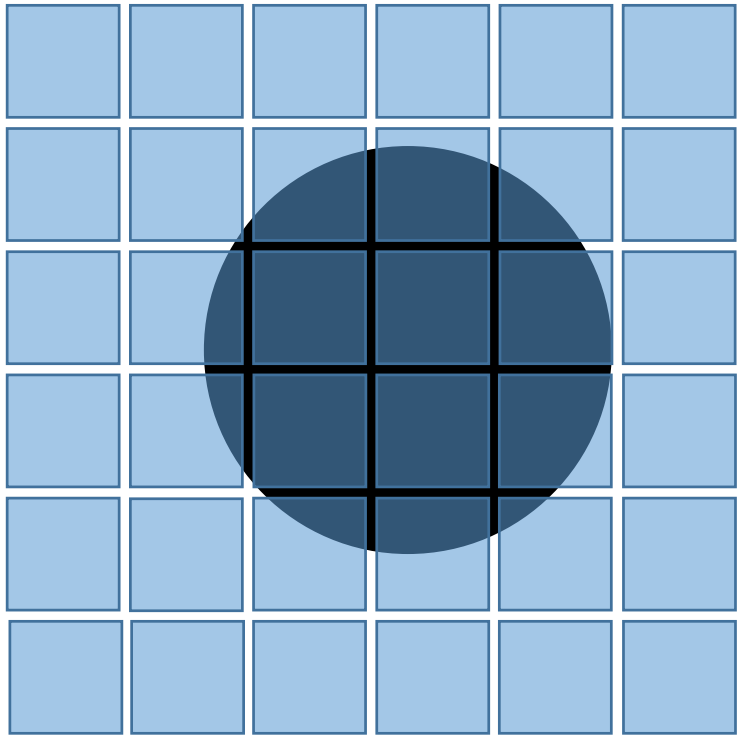


Confocal scanning microscope

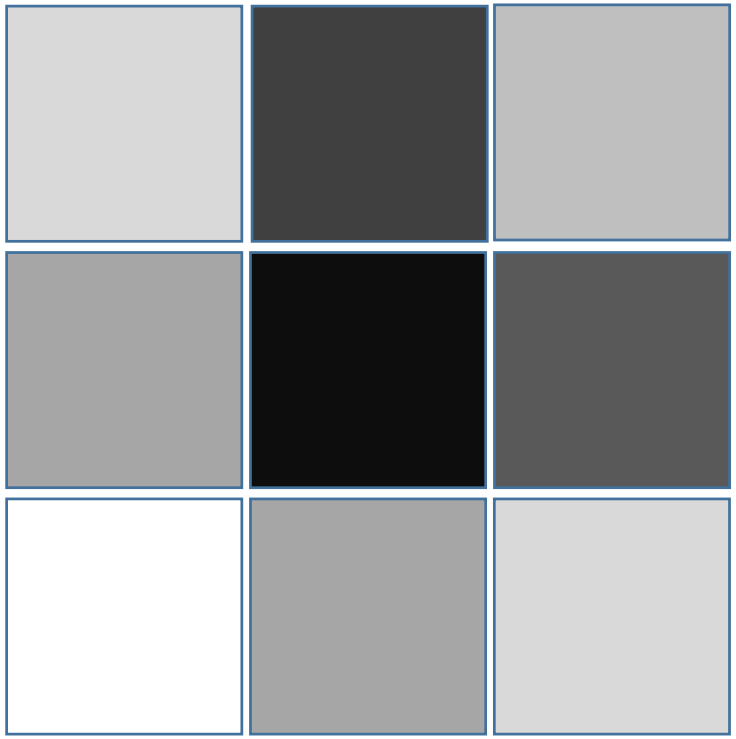


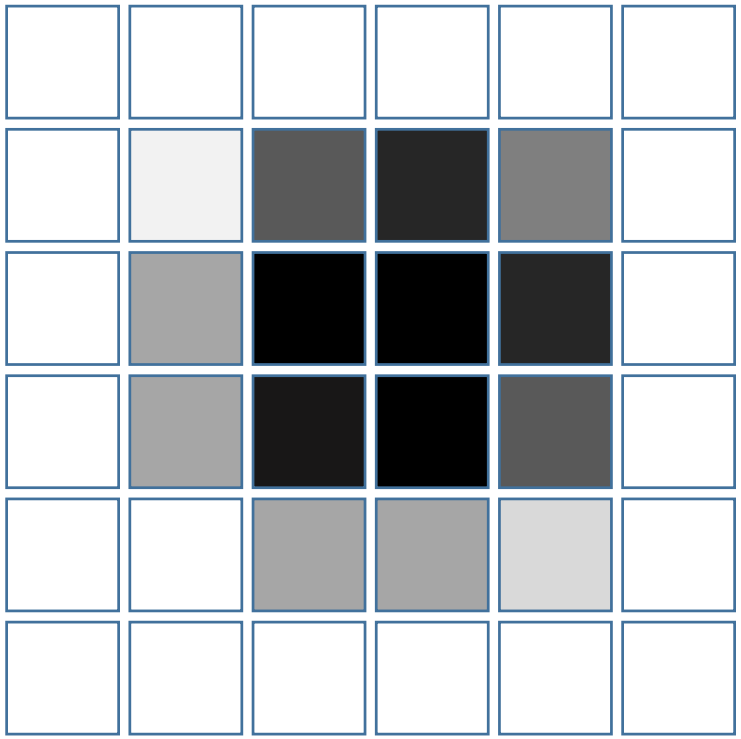
Confocal scanning microscope

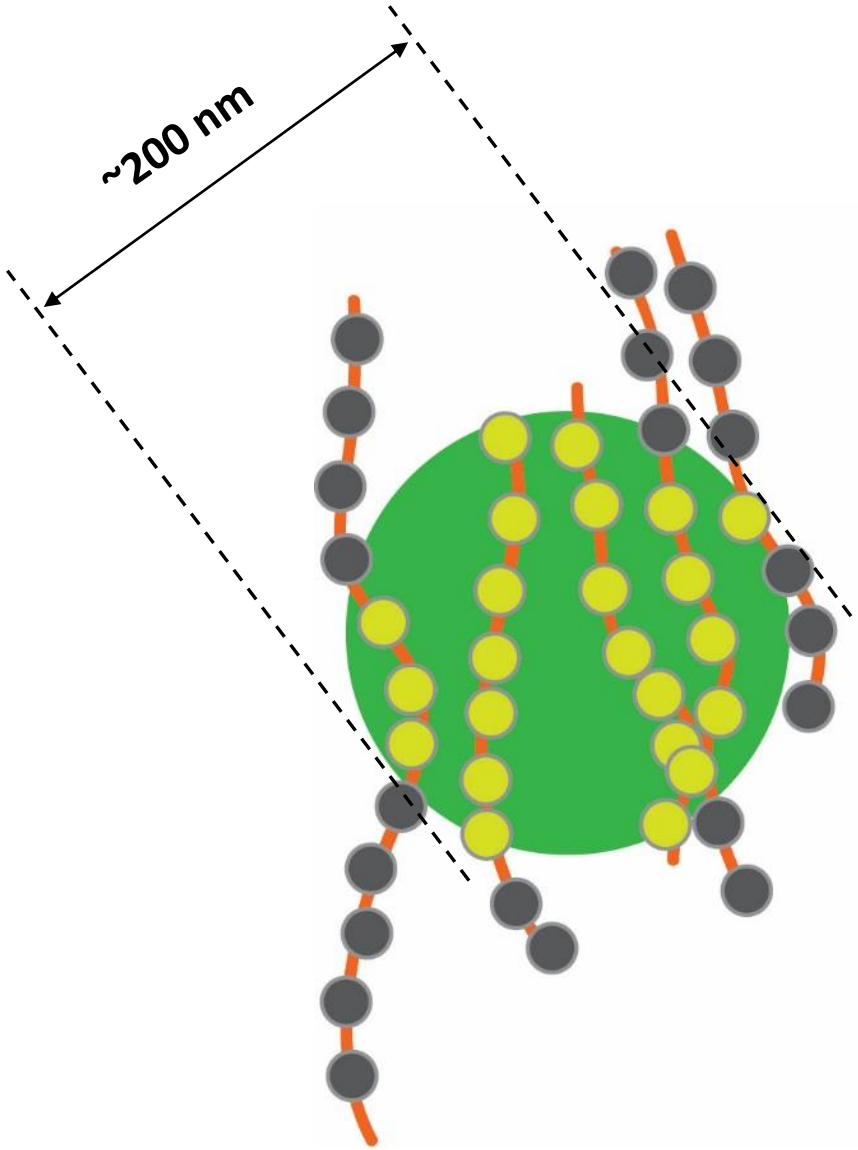




Confocal scanning microscope



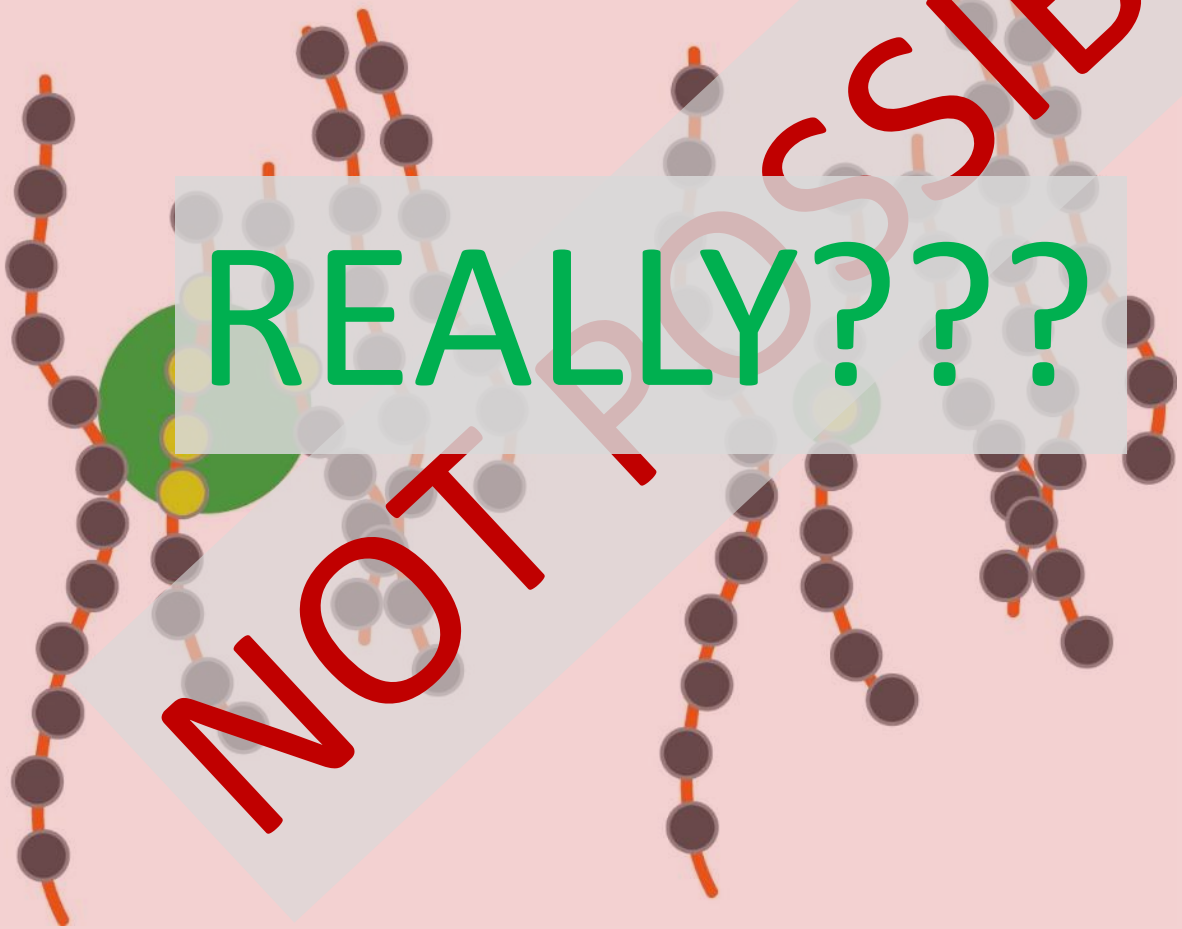




Ernst Abbe

Resolution barrier

$$d = \frac{\lambda}{2NA}$$



REALLY???

NOT POSSIBLE

Stefan W. Hell

Born: 23 December 1962, Arad, Romania

Affiliation at the time of the award: Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, German Cancer Research Center, Heidelberg, Germany

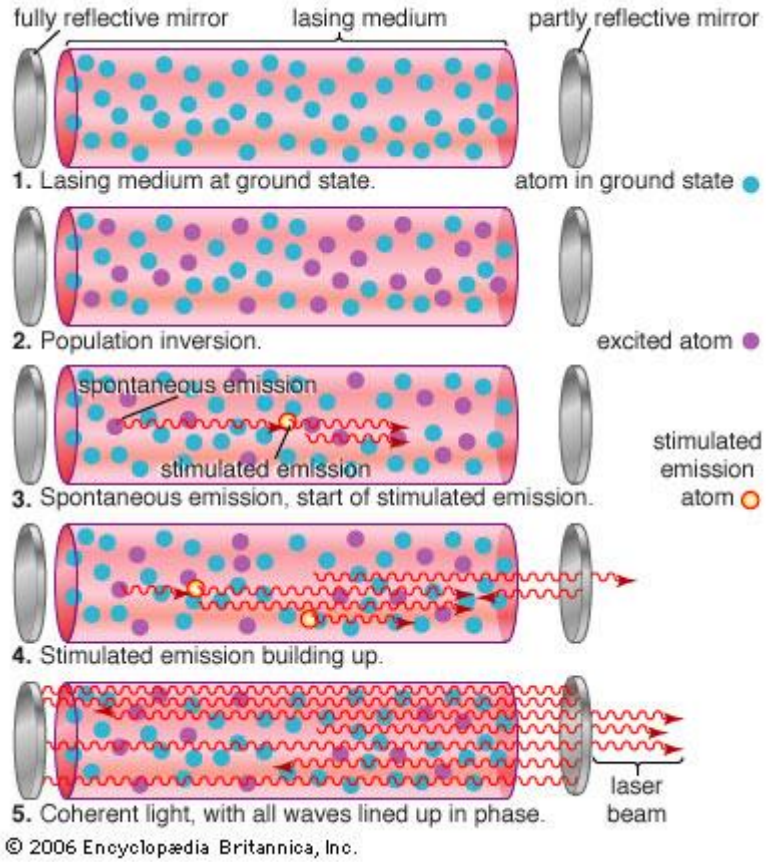
Prize motivation: "for the development of super-resolved fluorescence microscopy"

Field: physical chemistry

Prize share: 1/3

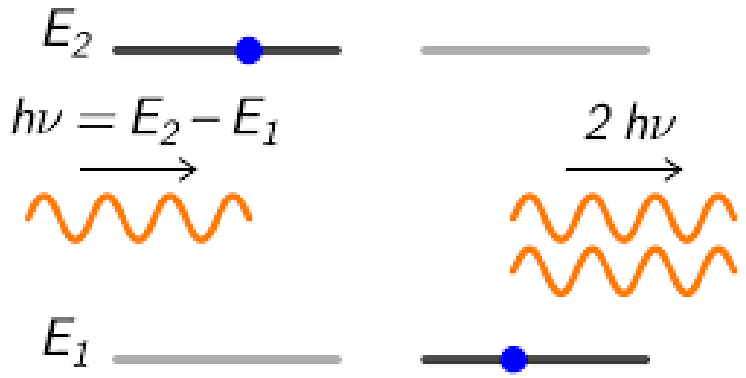
https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/hell-facts.html

Principle of the LASER – stimulated emission

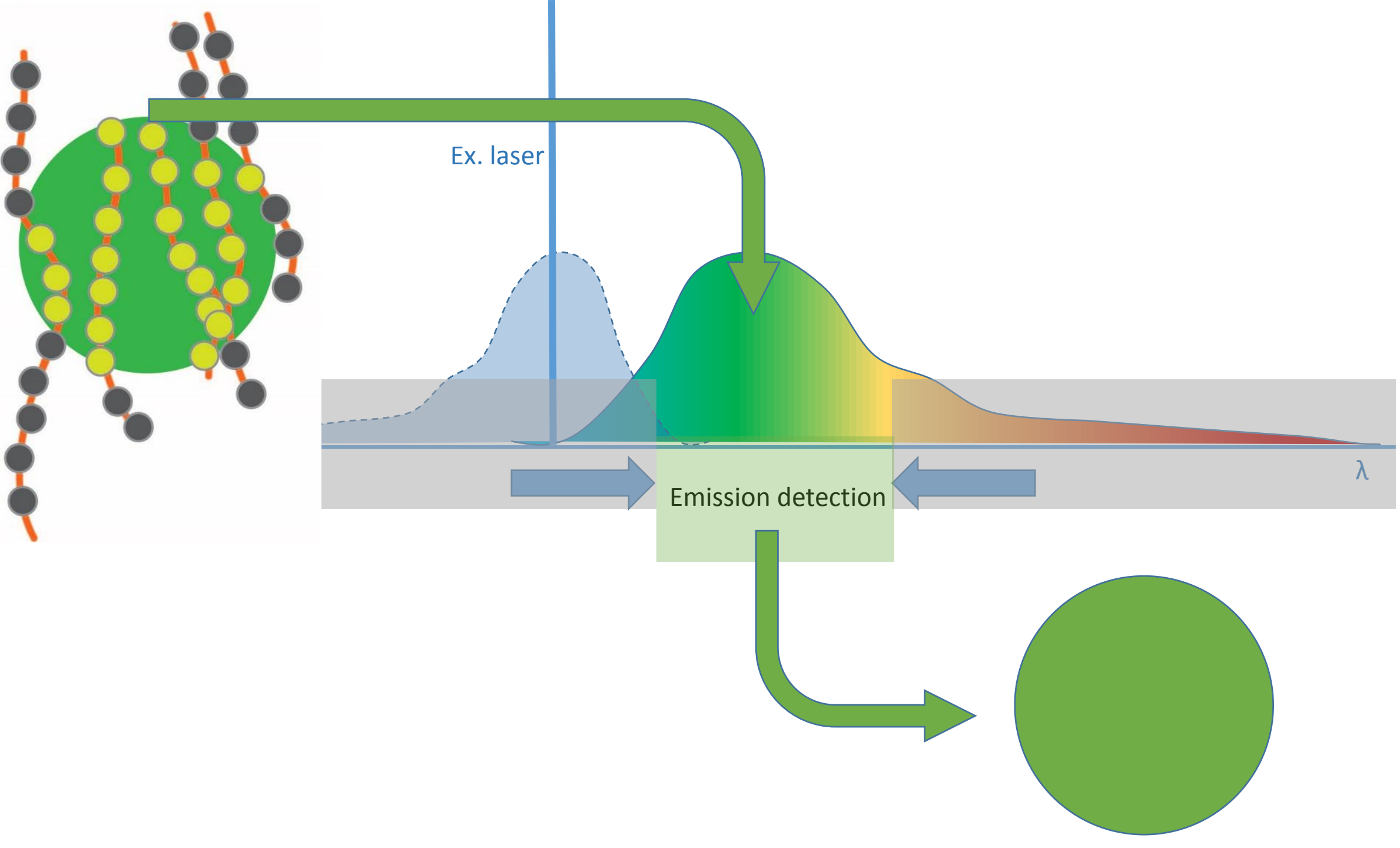


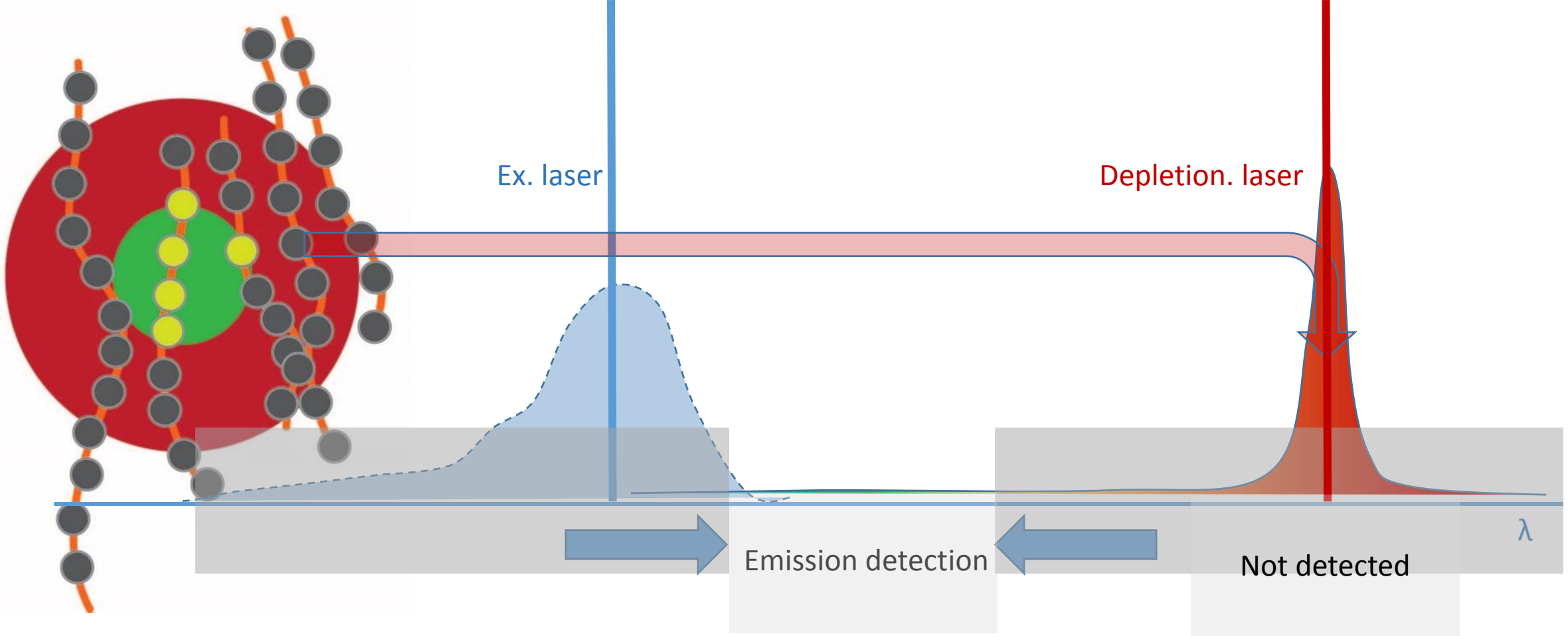
https://www.britannica.com/technology/stimulated-emission

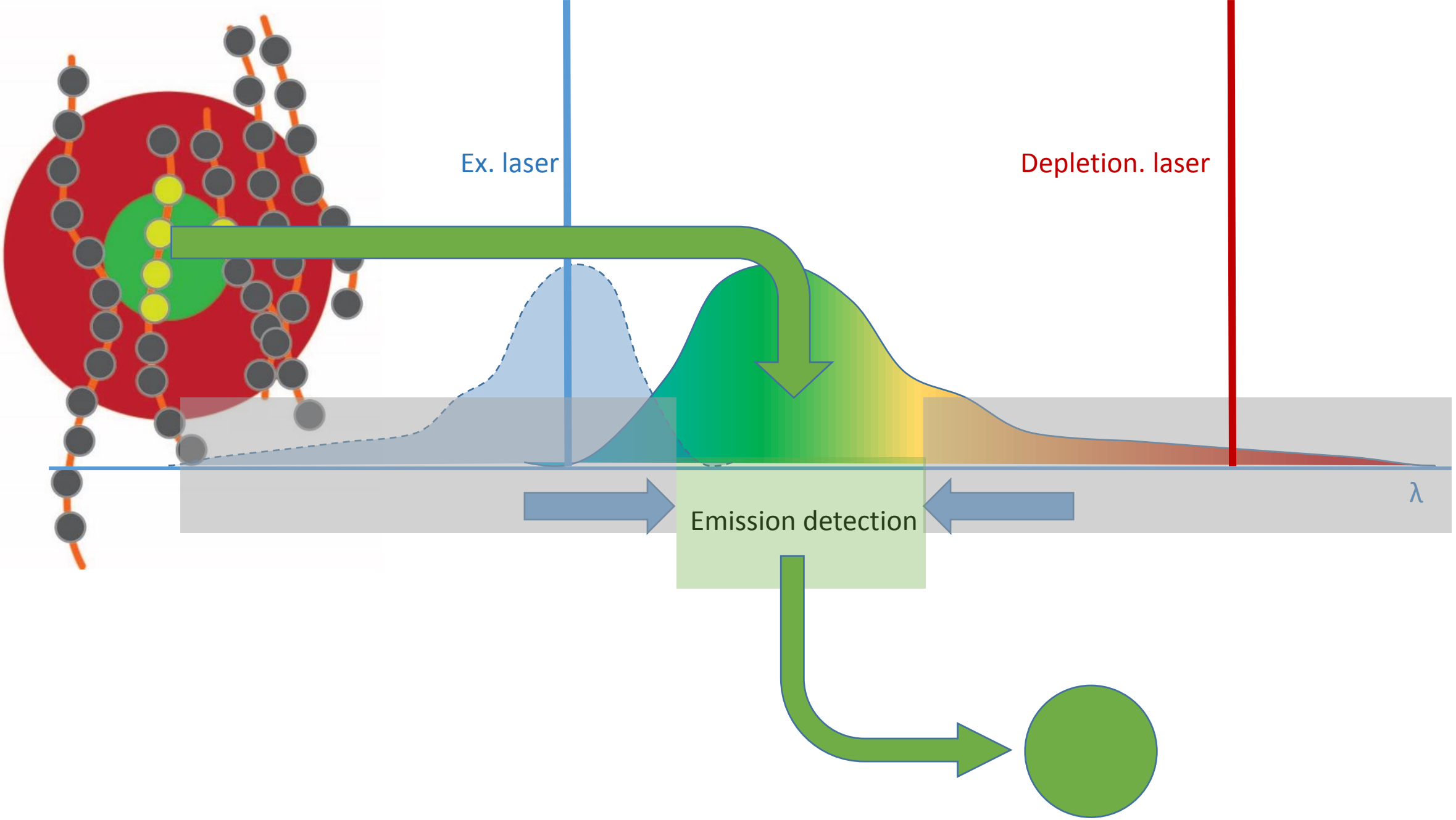
LASER = light amplification by stimulated emission of radiation.



https://web2.ph.utexas.edu/~coker2/index.files/xrayslasers.htm


Ex. laser
λ
Emission detection


Ex. laser
Depletion. laser
λ
Emission detection
Not detected

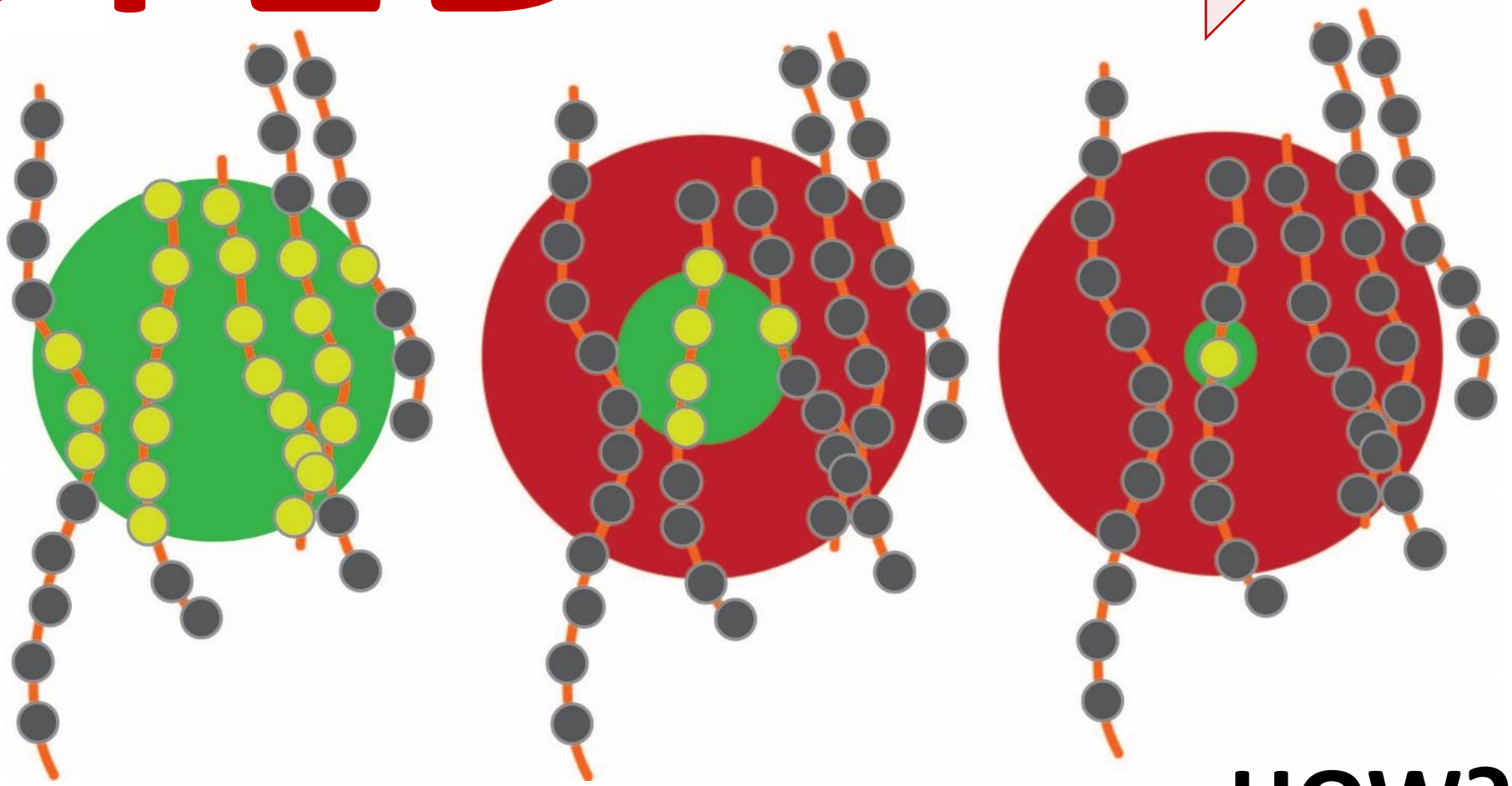

Ex. laser
Depletion. laser
λ
Emission detection

$$d = \frac{\lambda}{2NA}$$

$$d_{STED} = \frac{\lambda}{2NA\sqrt{1+\xi}}$$

STED

break through the resolution barrier

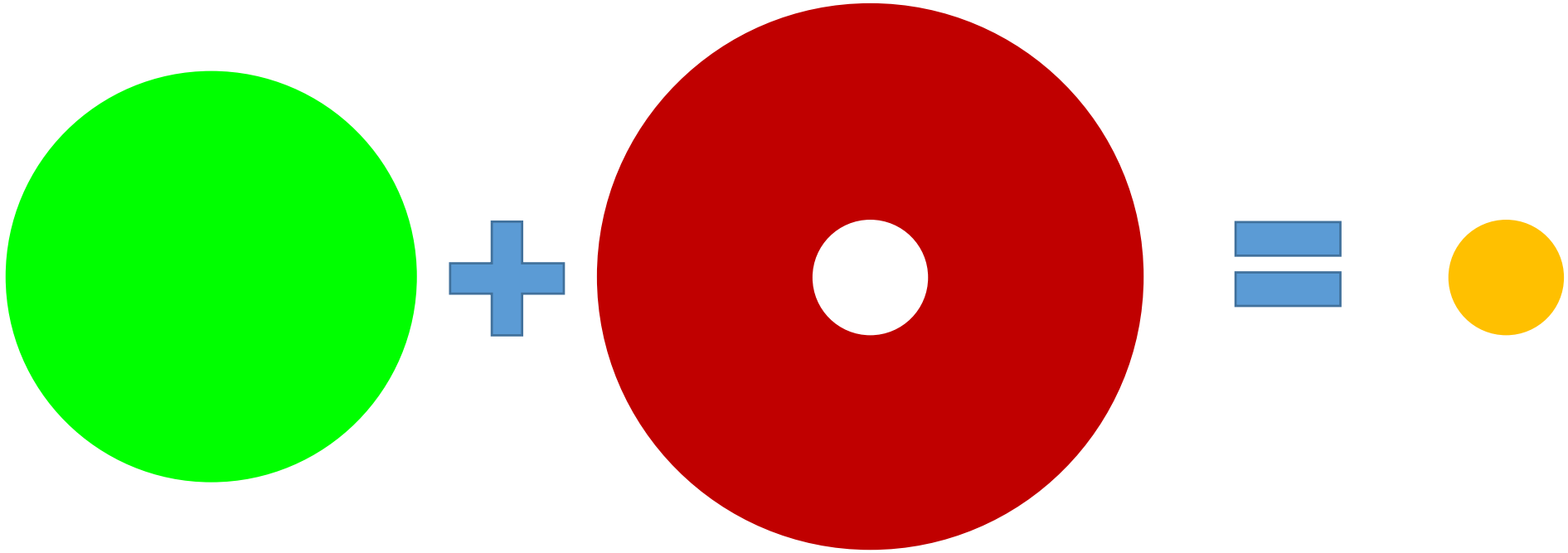


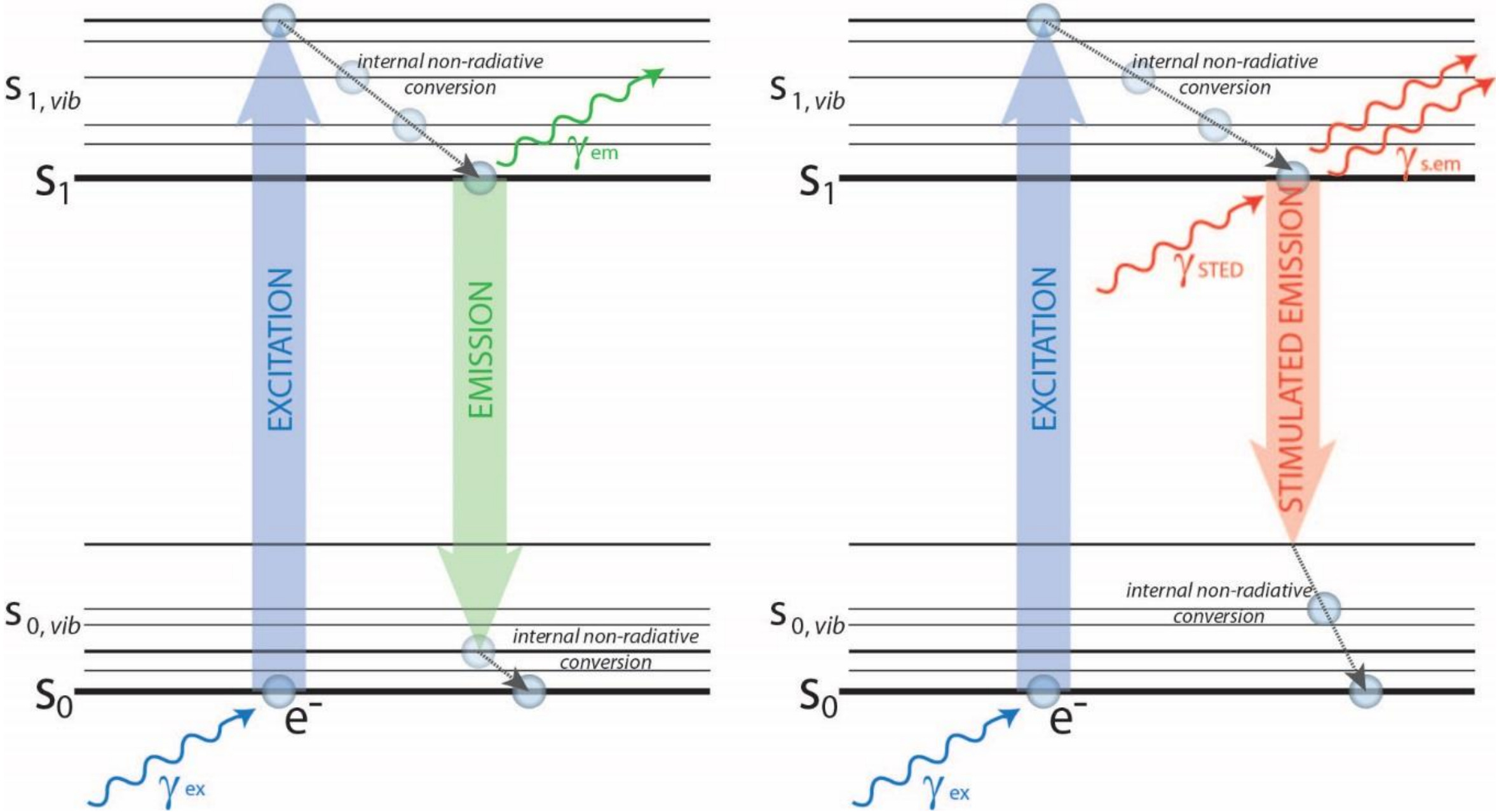
HOW?

Excitation LASER spot

Depletion laser donut

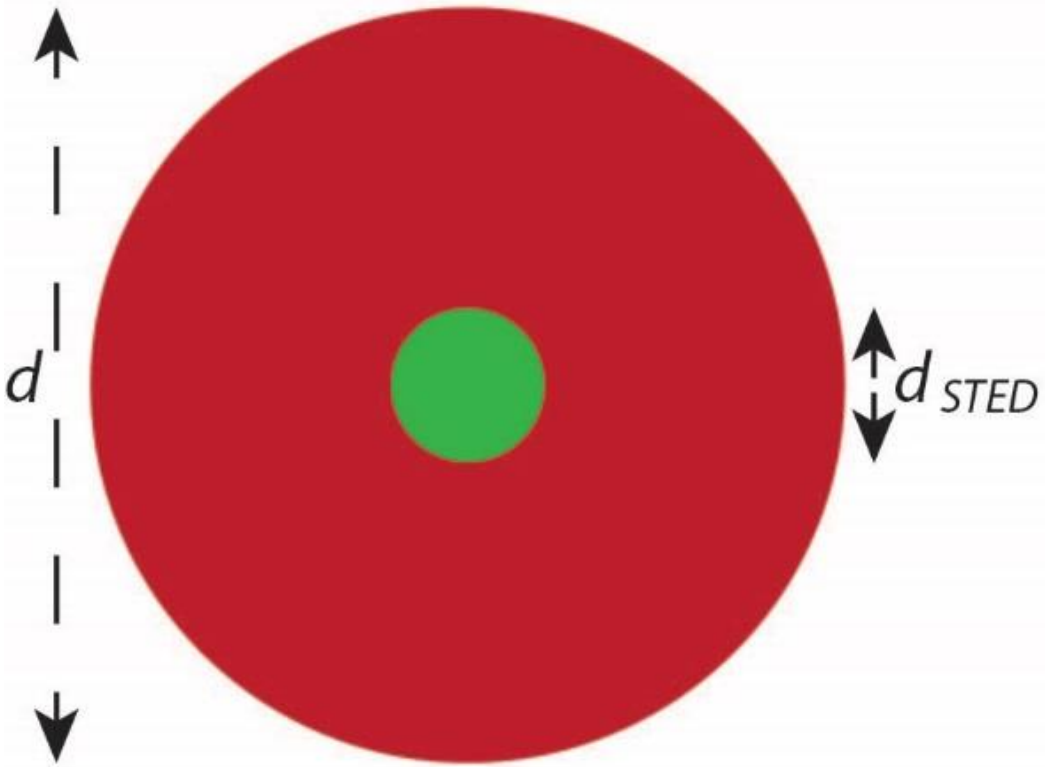
Acquired emission

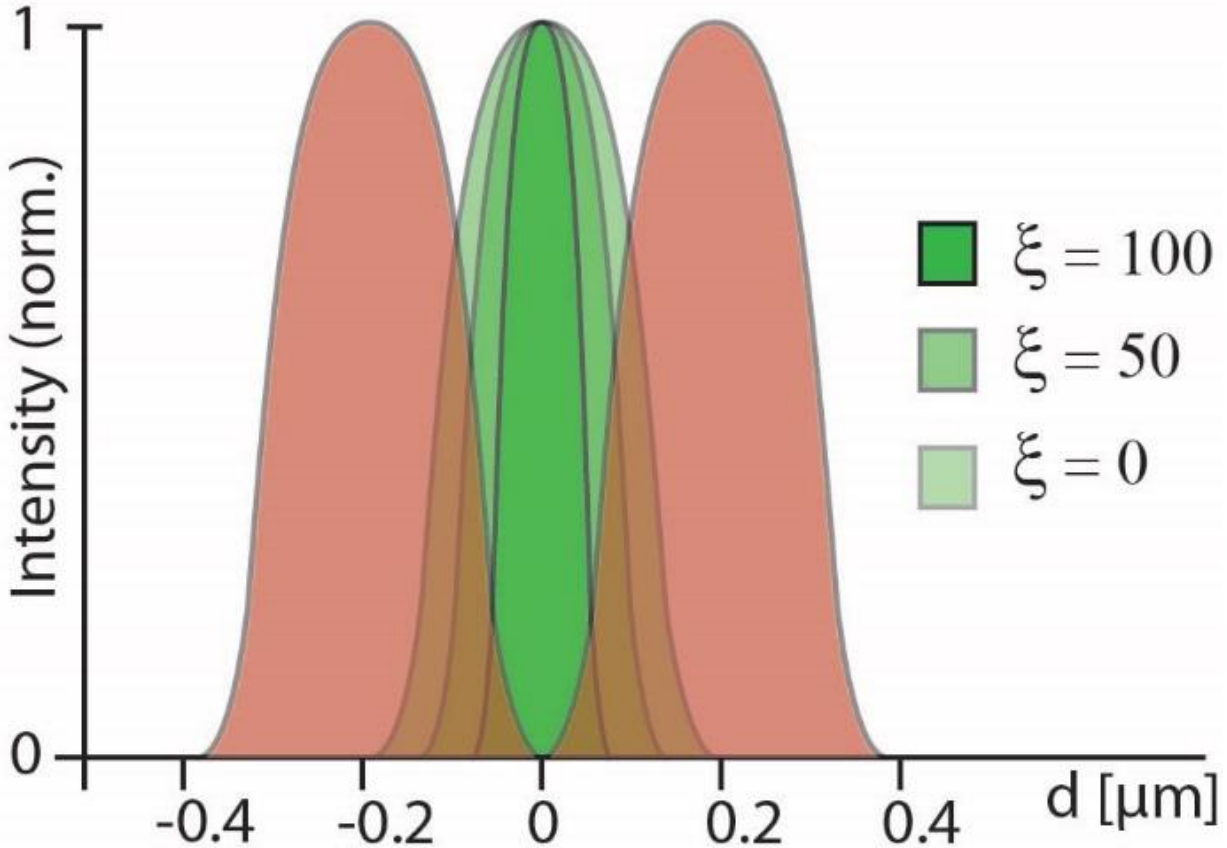



S 1, vib
S1
internal non-radiative conversion
γ em
EXCITATION
EMISSION
S 0, vib
S0
internal non-radiative conversion
e-
γ ex
S 1, vib
S1
internal non-radiative conversion
γ s.em
γ STED
EXCITATION
STIMULATED EMISSION
S 0, vib
S0
internal non-radiative conversion
e-
γ ex

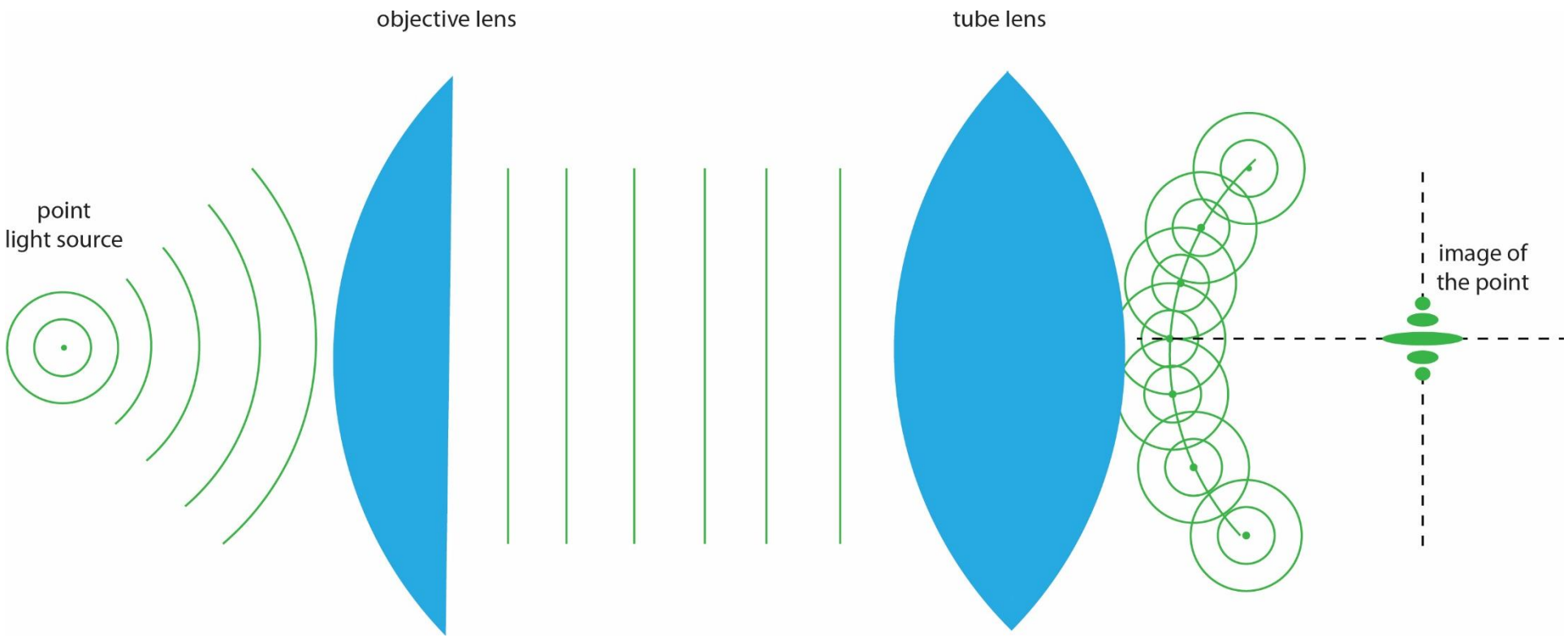
$$d_{STED} = \frac{\lambda}{2NA\sqrt{1+\xi}}$$

$$\xi = \frac{I_{STED}}{I_S}$$

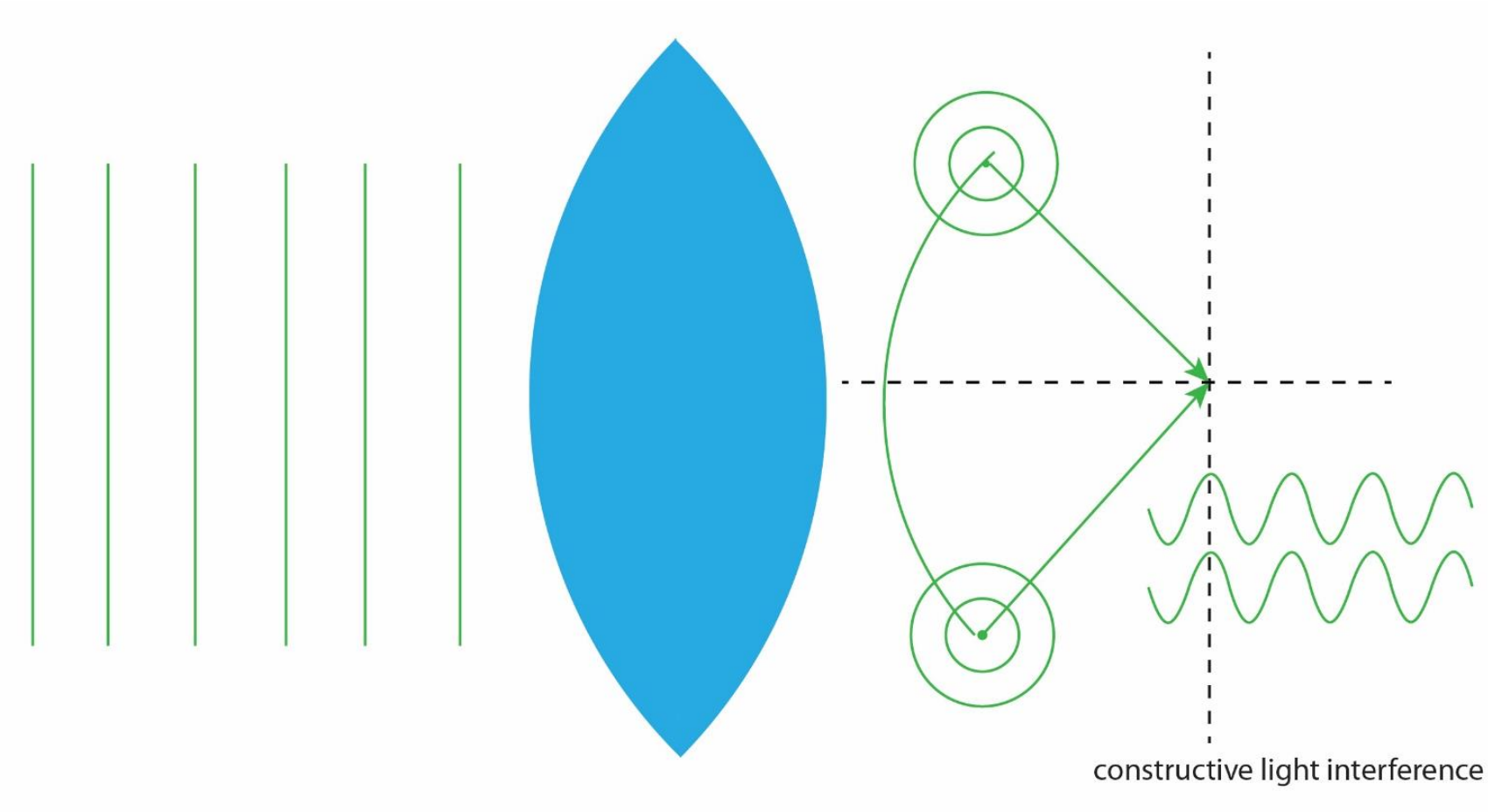




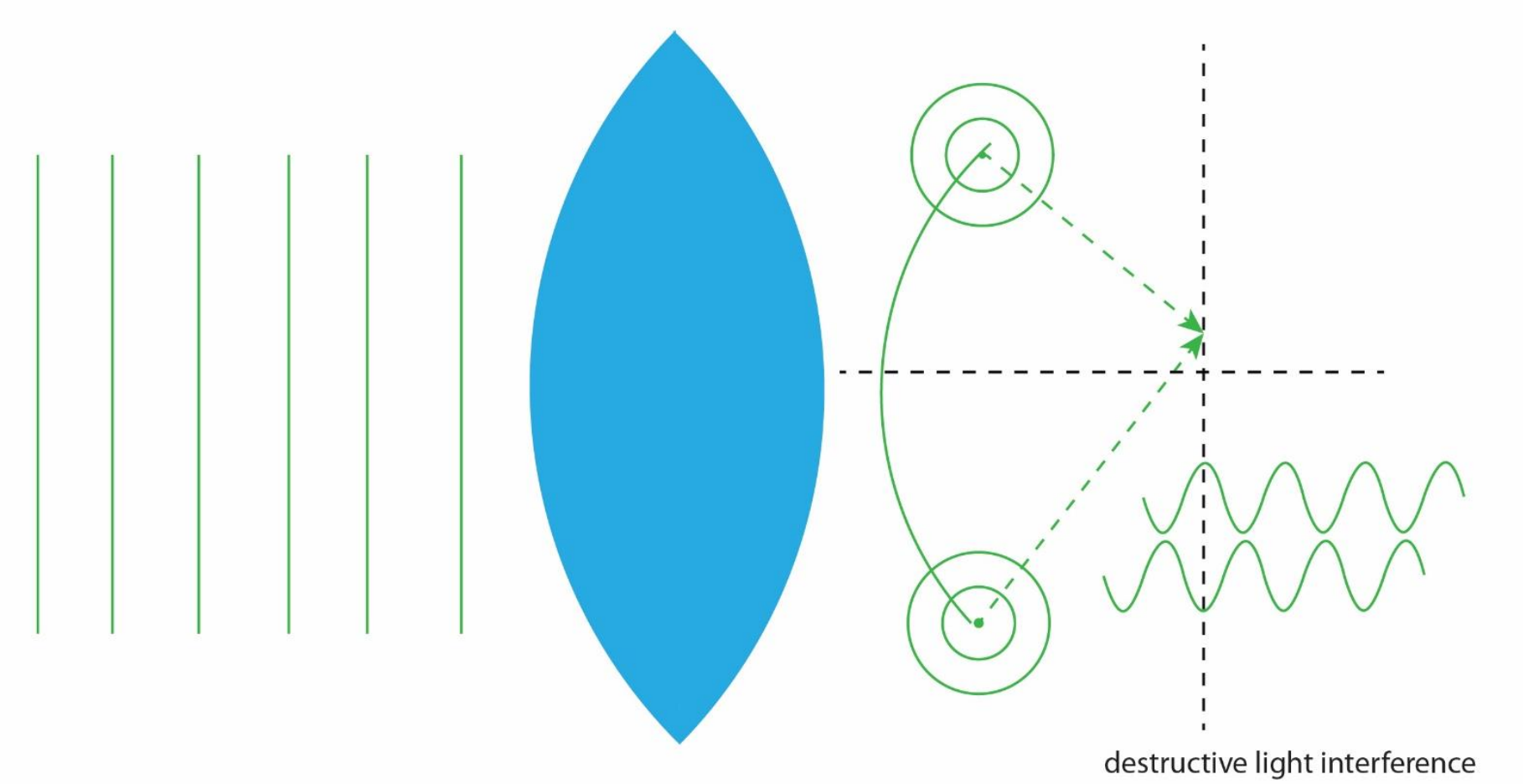
How the donut is created?



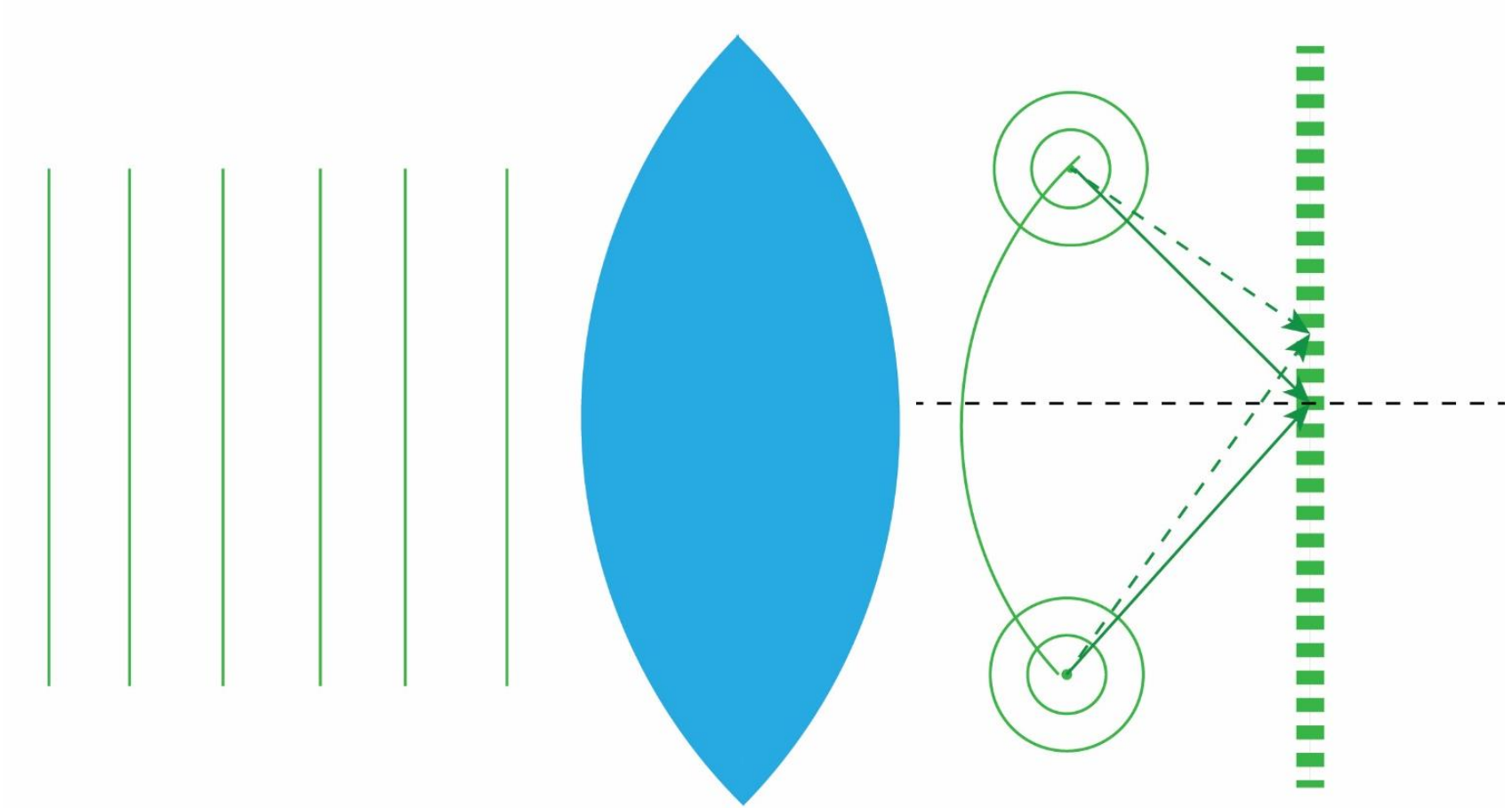
How the donut is created?



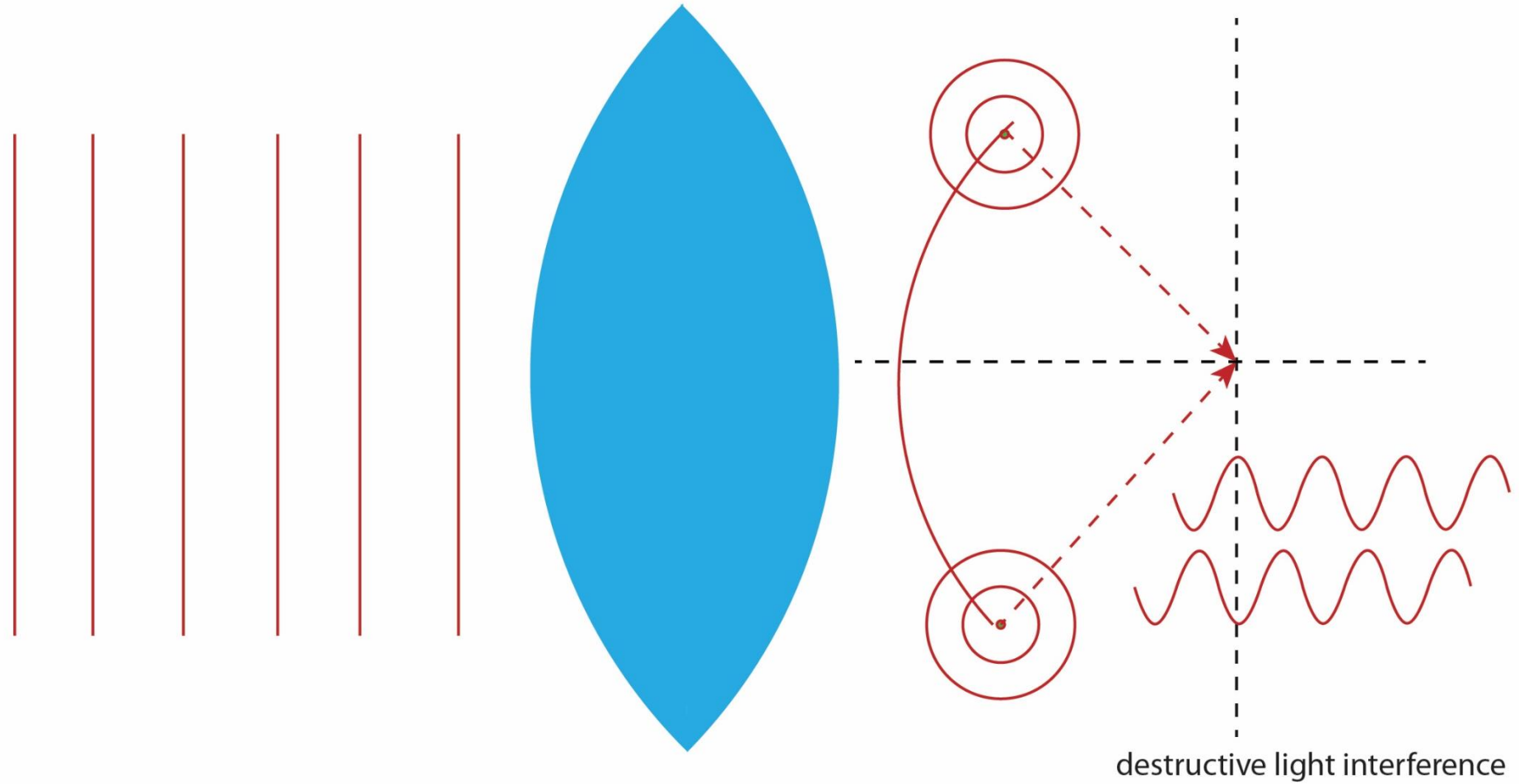
How the donut is created?



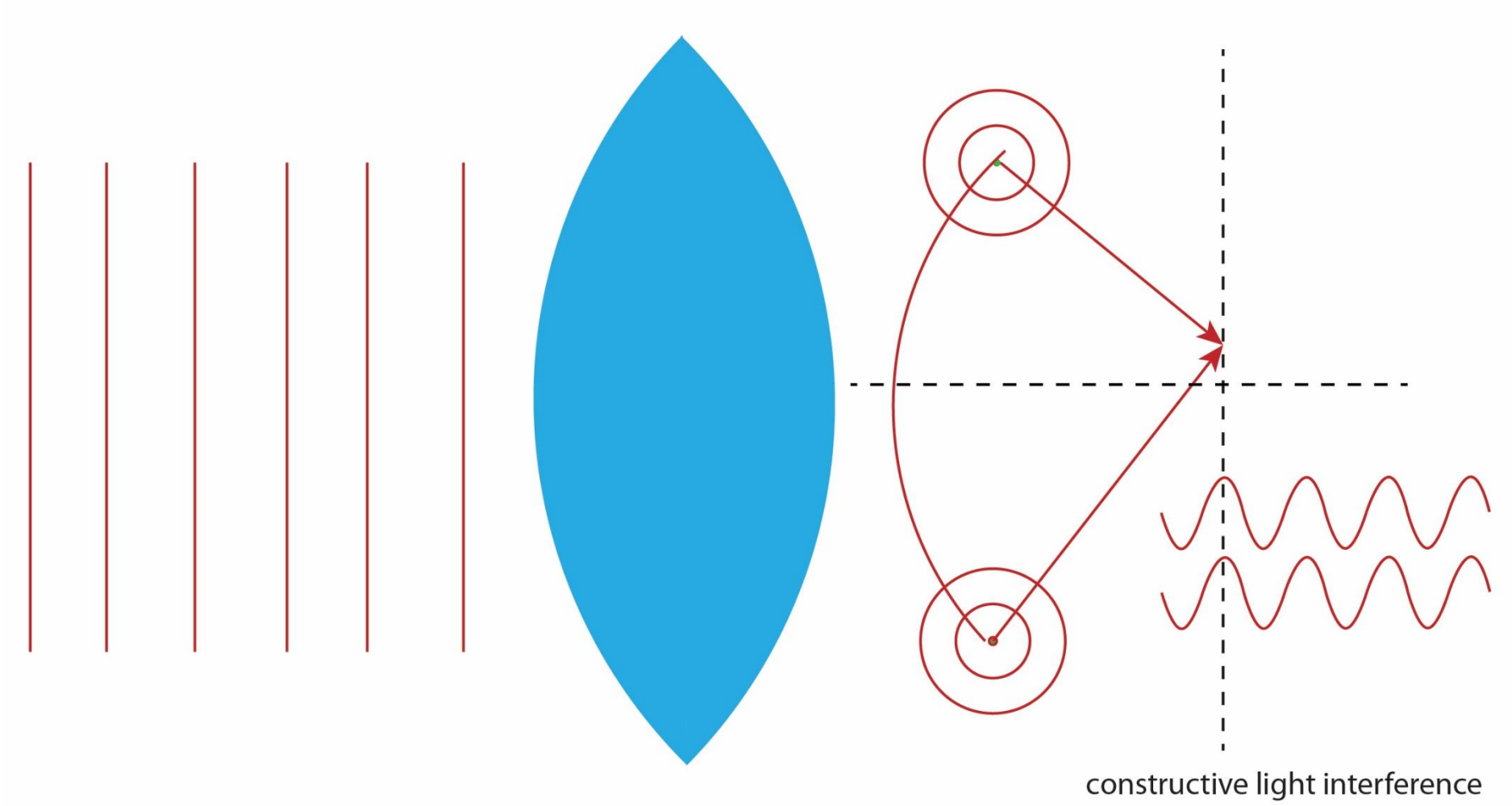
How the donut is created?



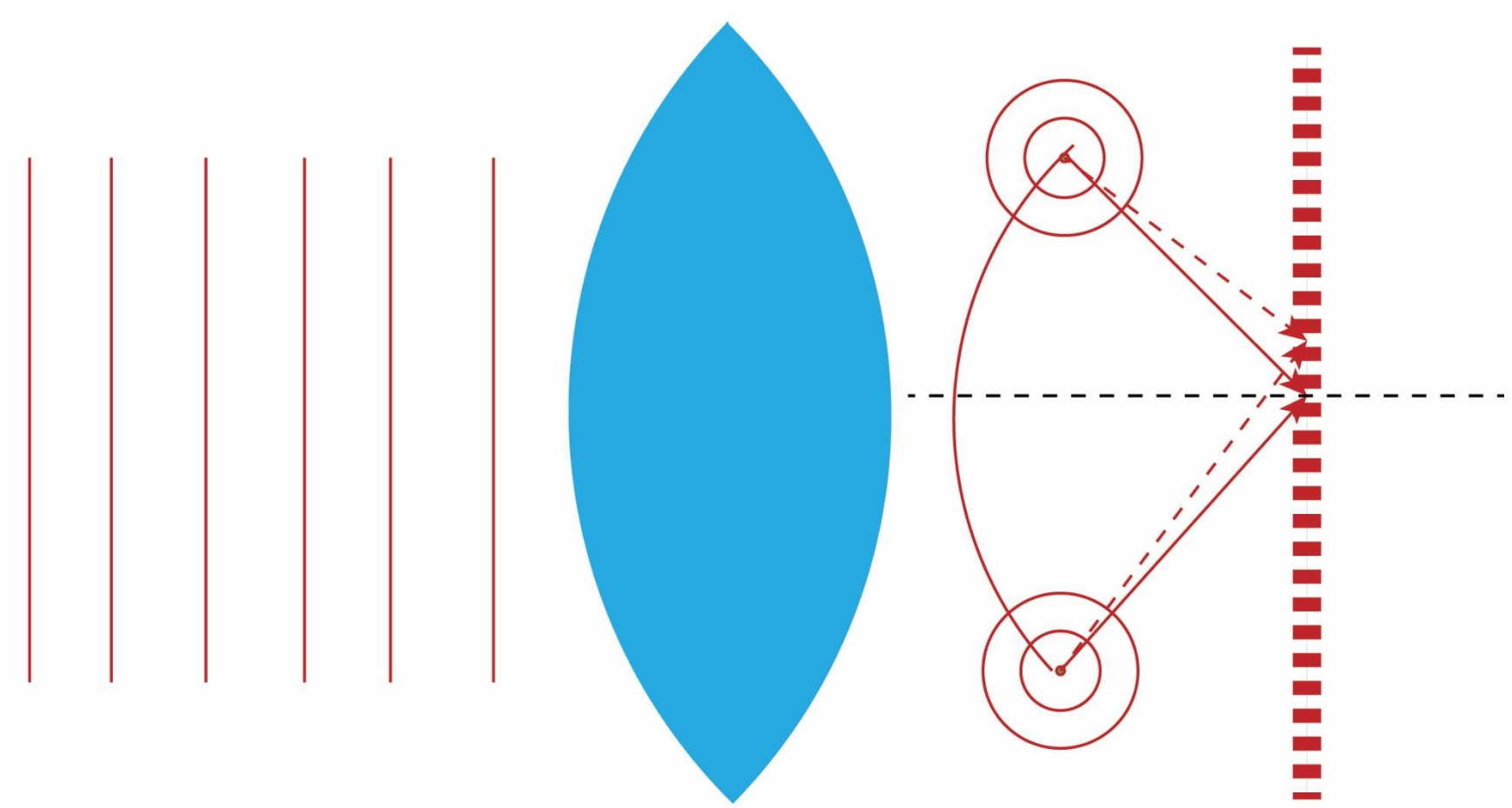
How the donut is created?



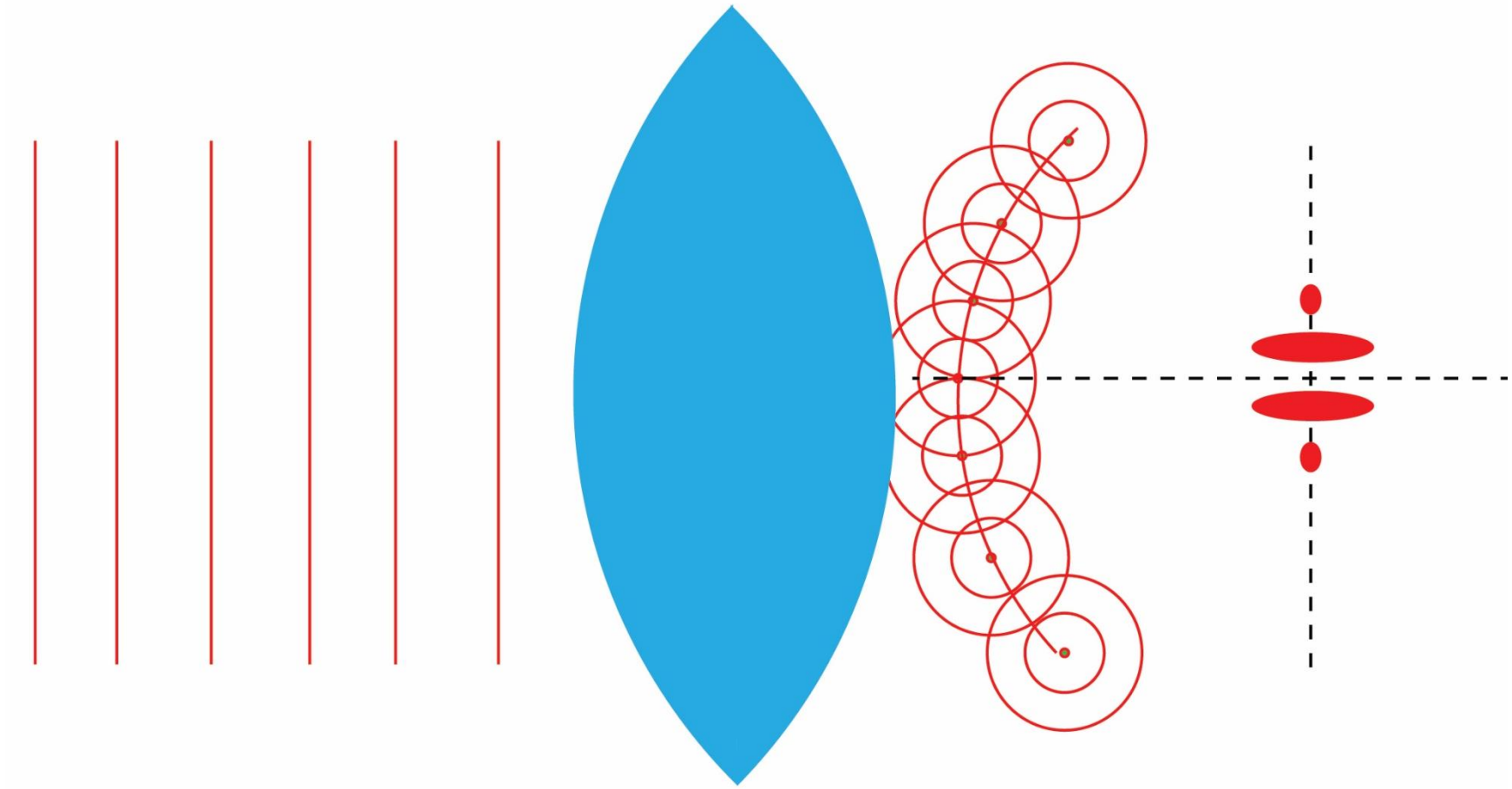
How the donut is created?



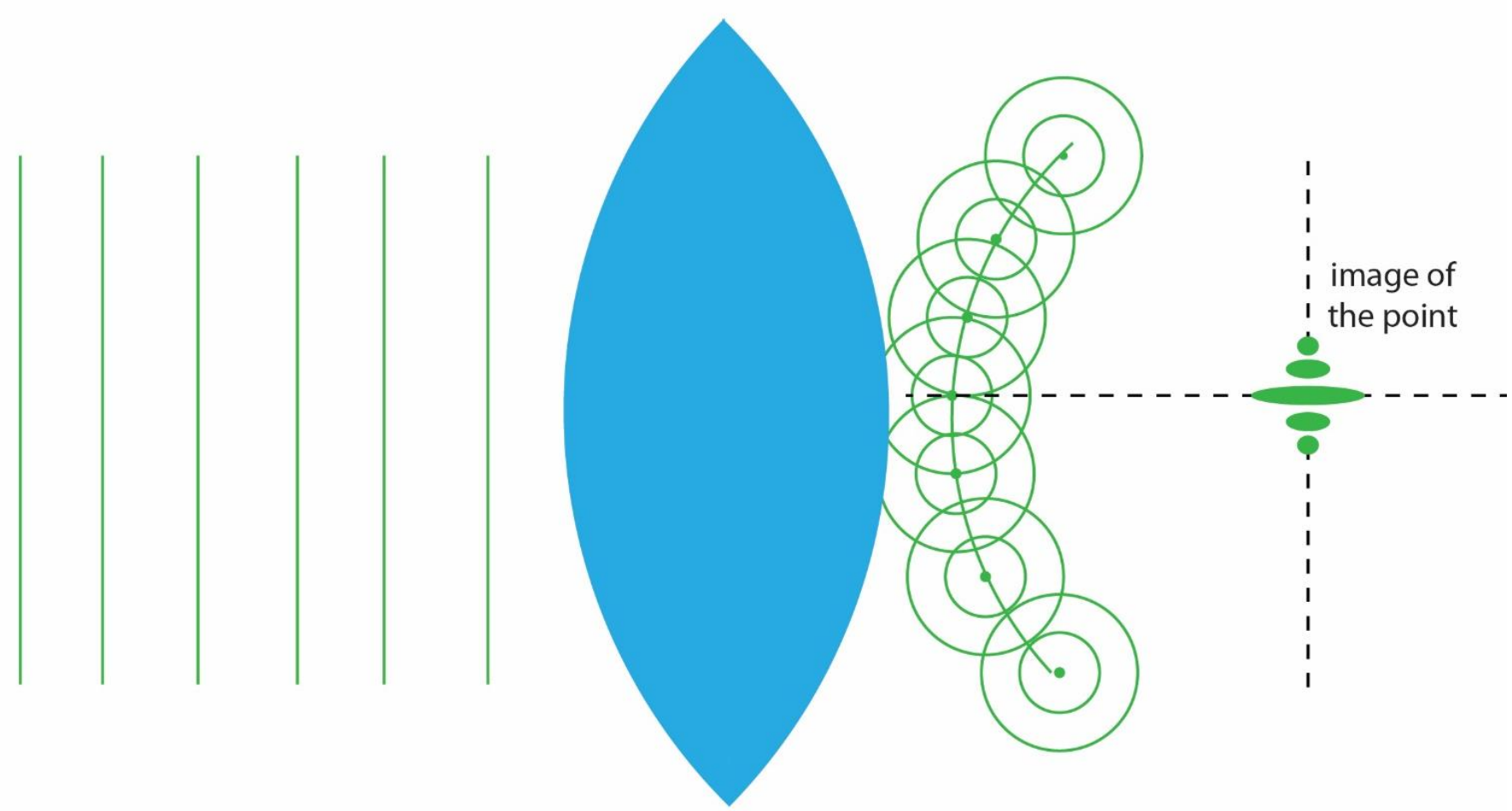
How the donut is created?



How the donut is created?

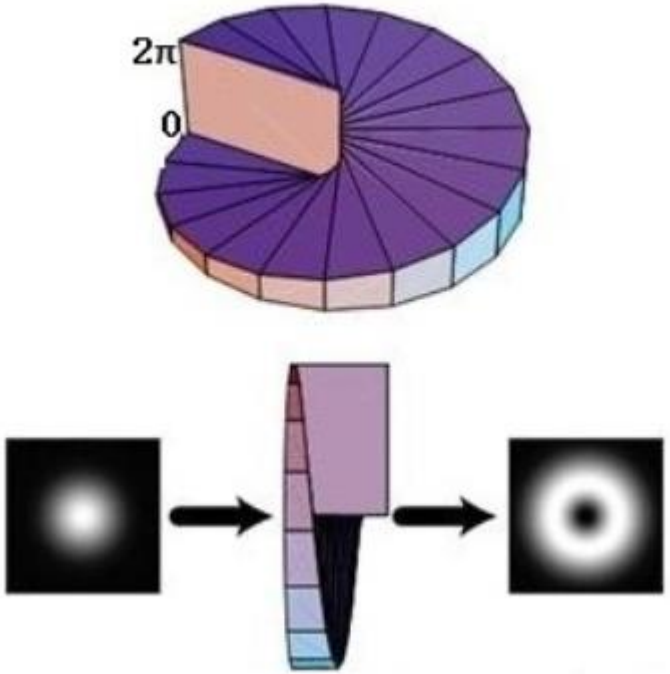


How the donut is created?

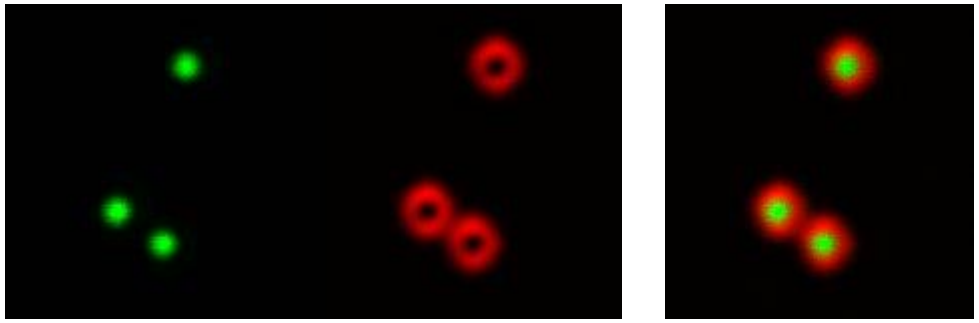


The donut is created via optical vortex

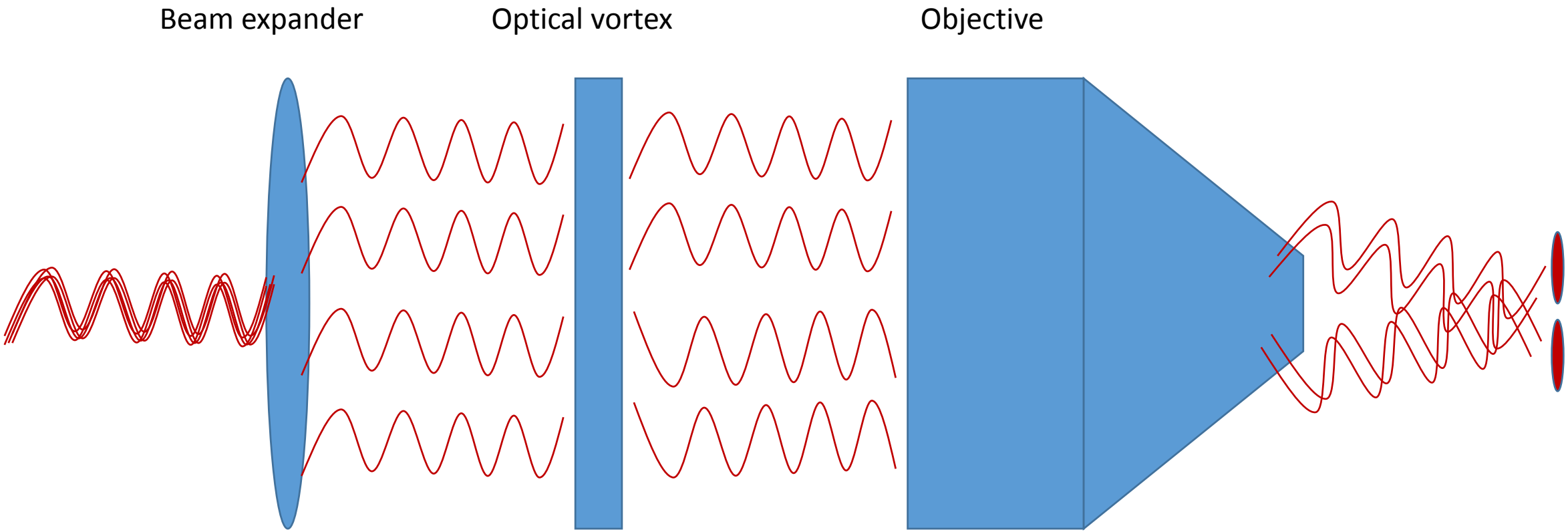
Courtesy of Courtial and O'Holleran, 2007



Excitation beam Depletion beam Overlay



80 nm gold beads, 550 nm excitation, 660 nm depletion

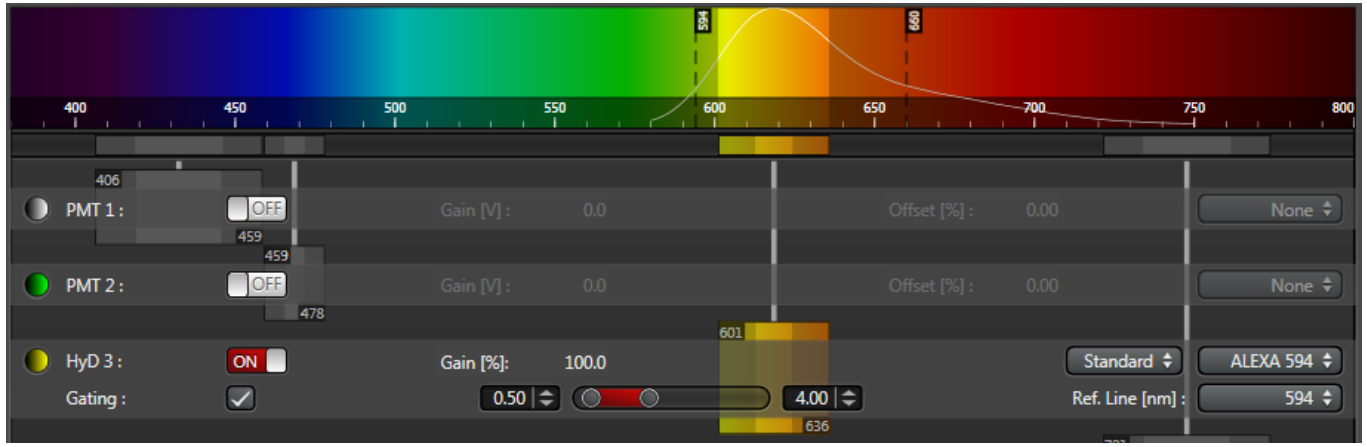

Beam expander
Optical vortex
Objective

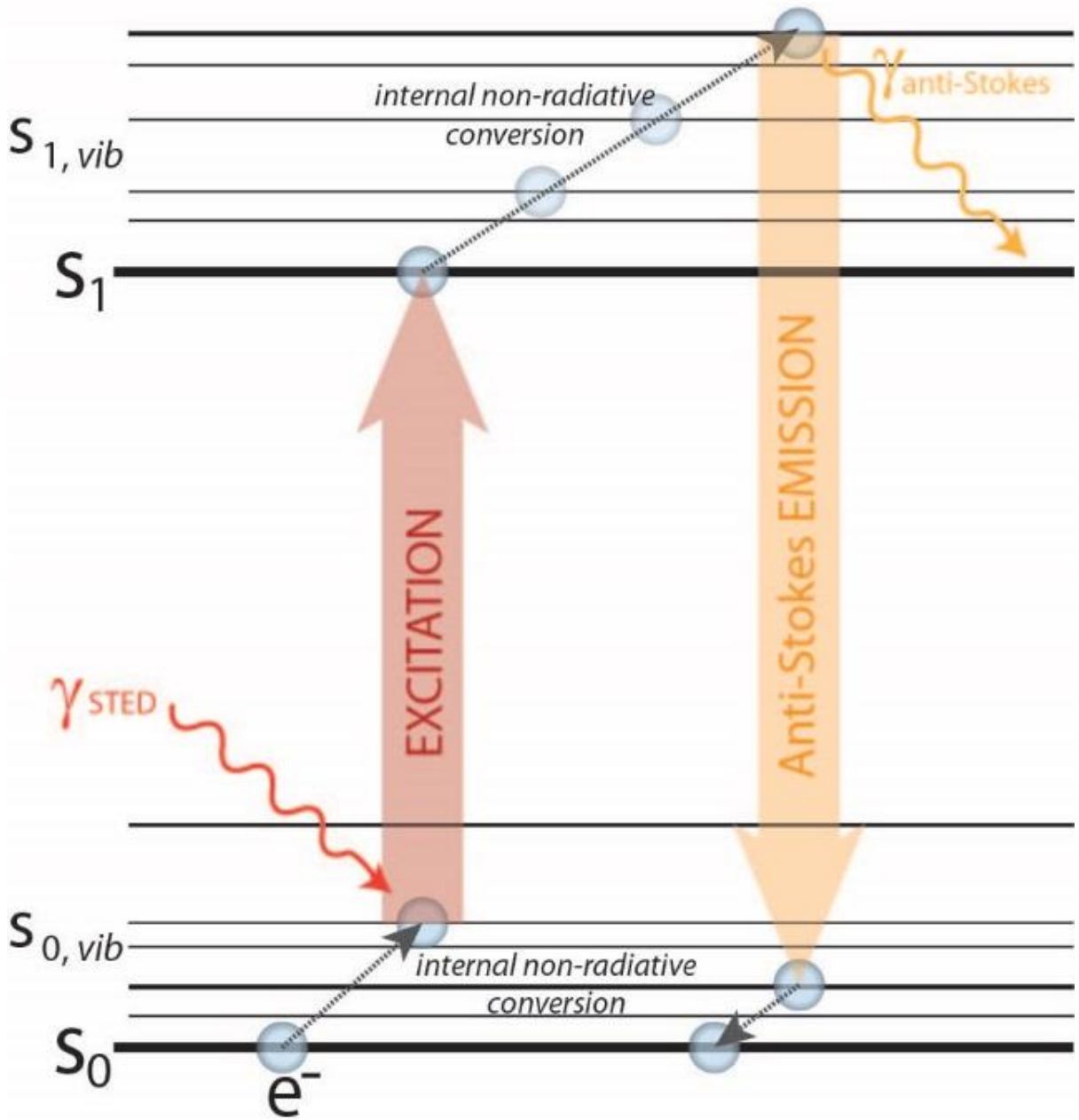
AF488

Depletion laser 660 nm

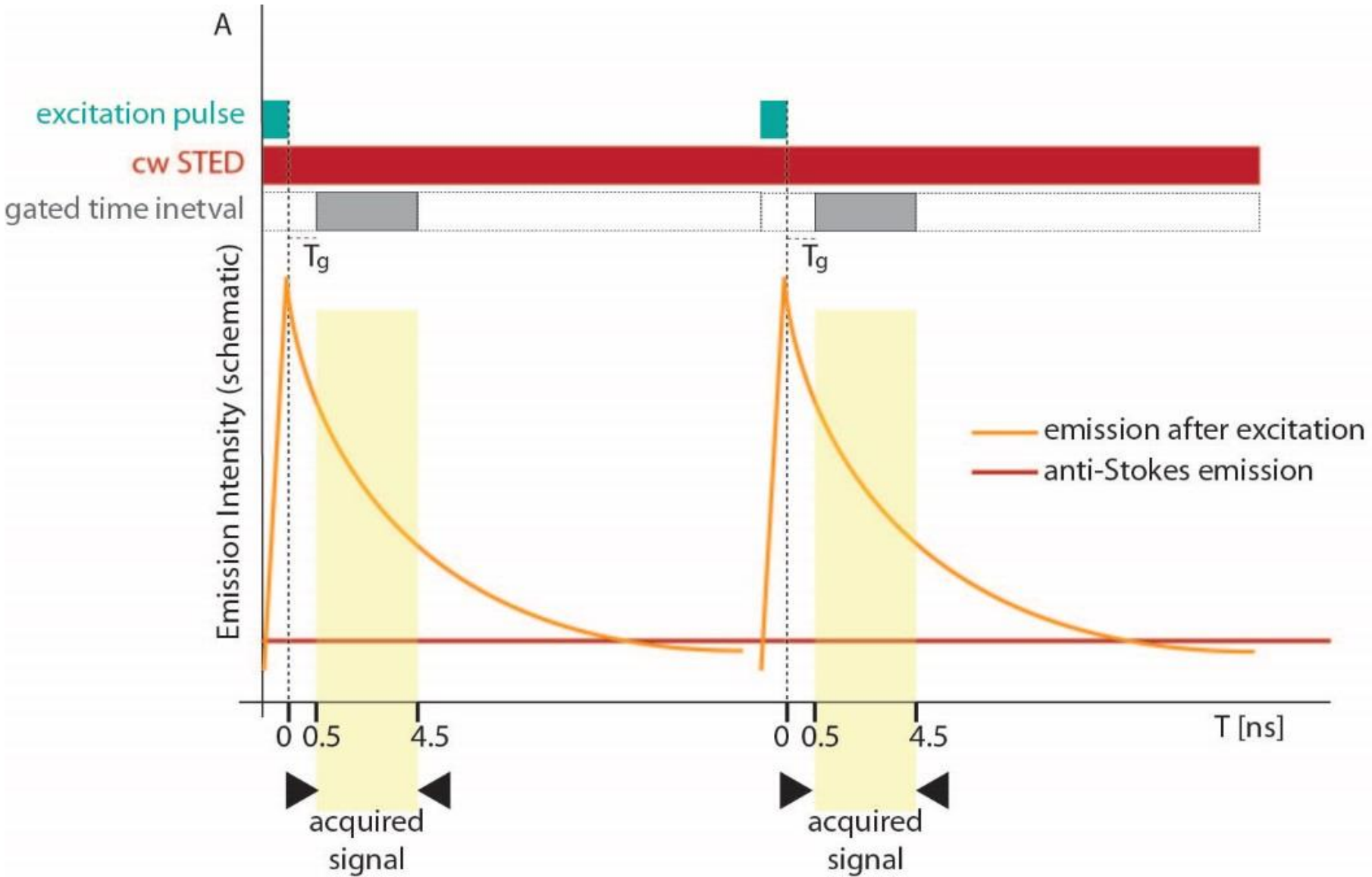
AF555

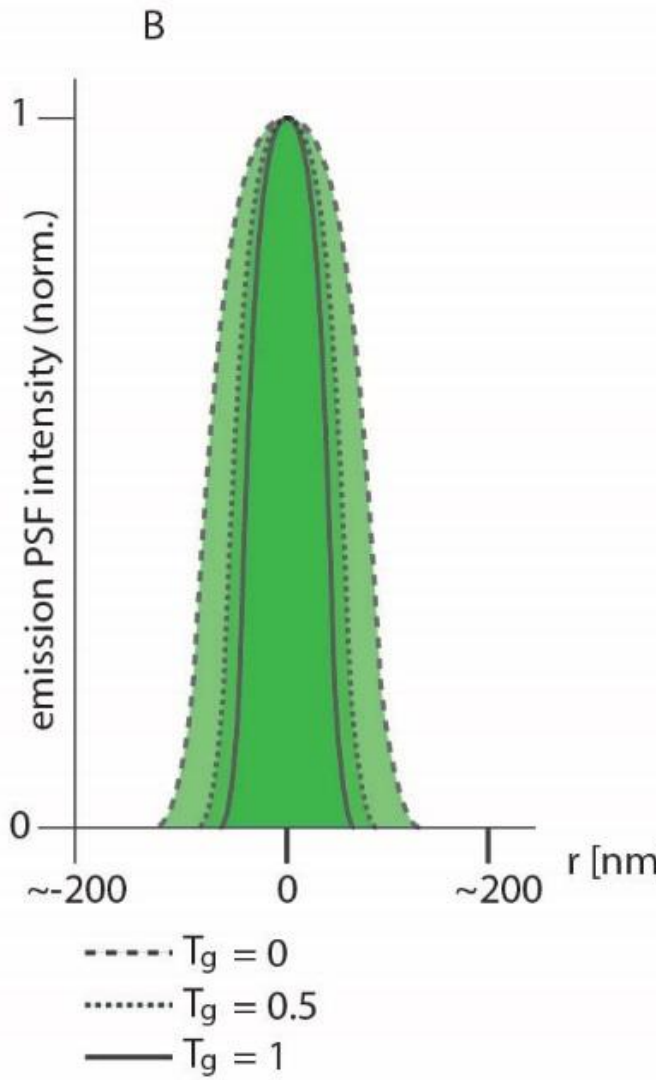
AF594



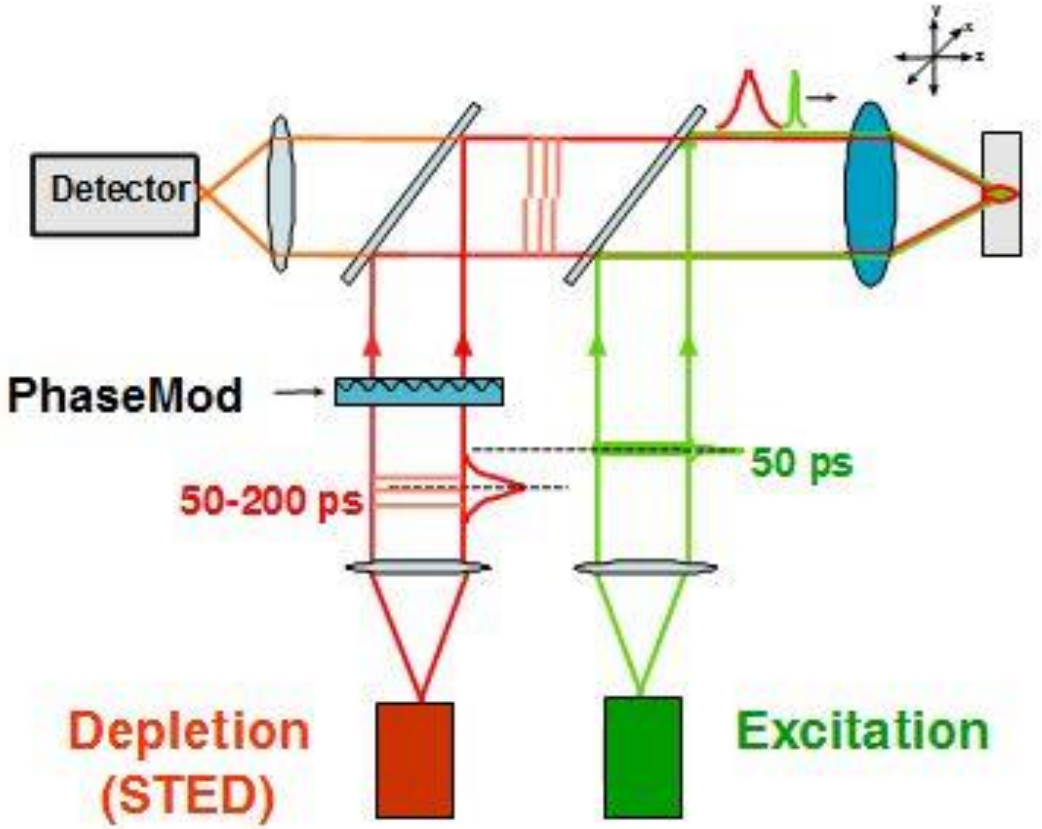

S 1, vib
S1
internal non-radiative
conversion
γ anti-Stokes
EXCITATION
Anti-Stokes EMISSION
γ STED
S 0, vib
internal non-radiative
conversion
S0
e-

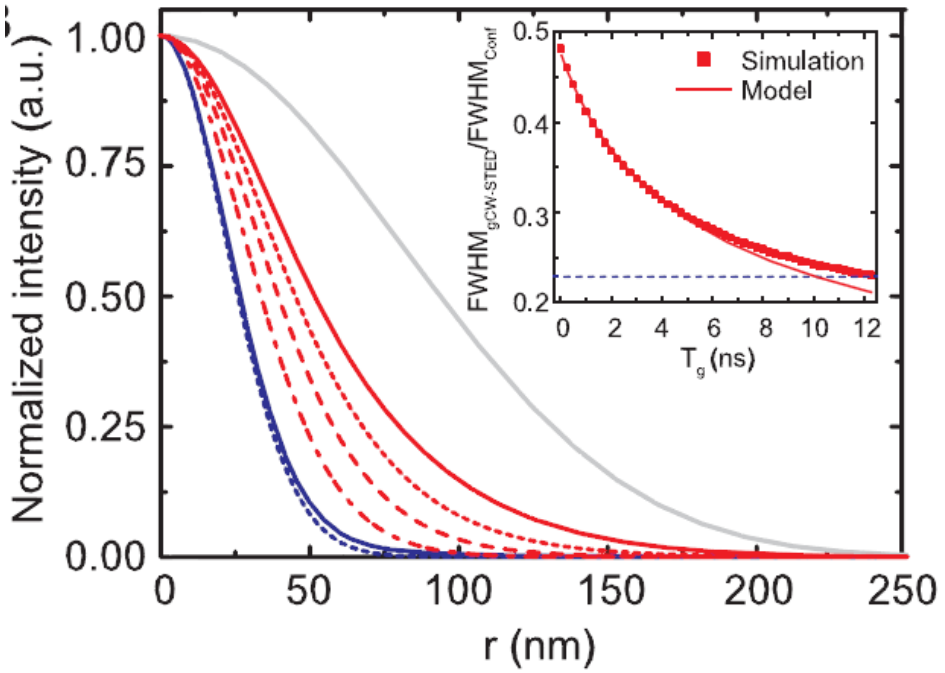
Time Gating in continuous wave STED laser



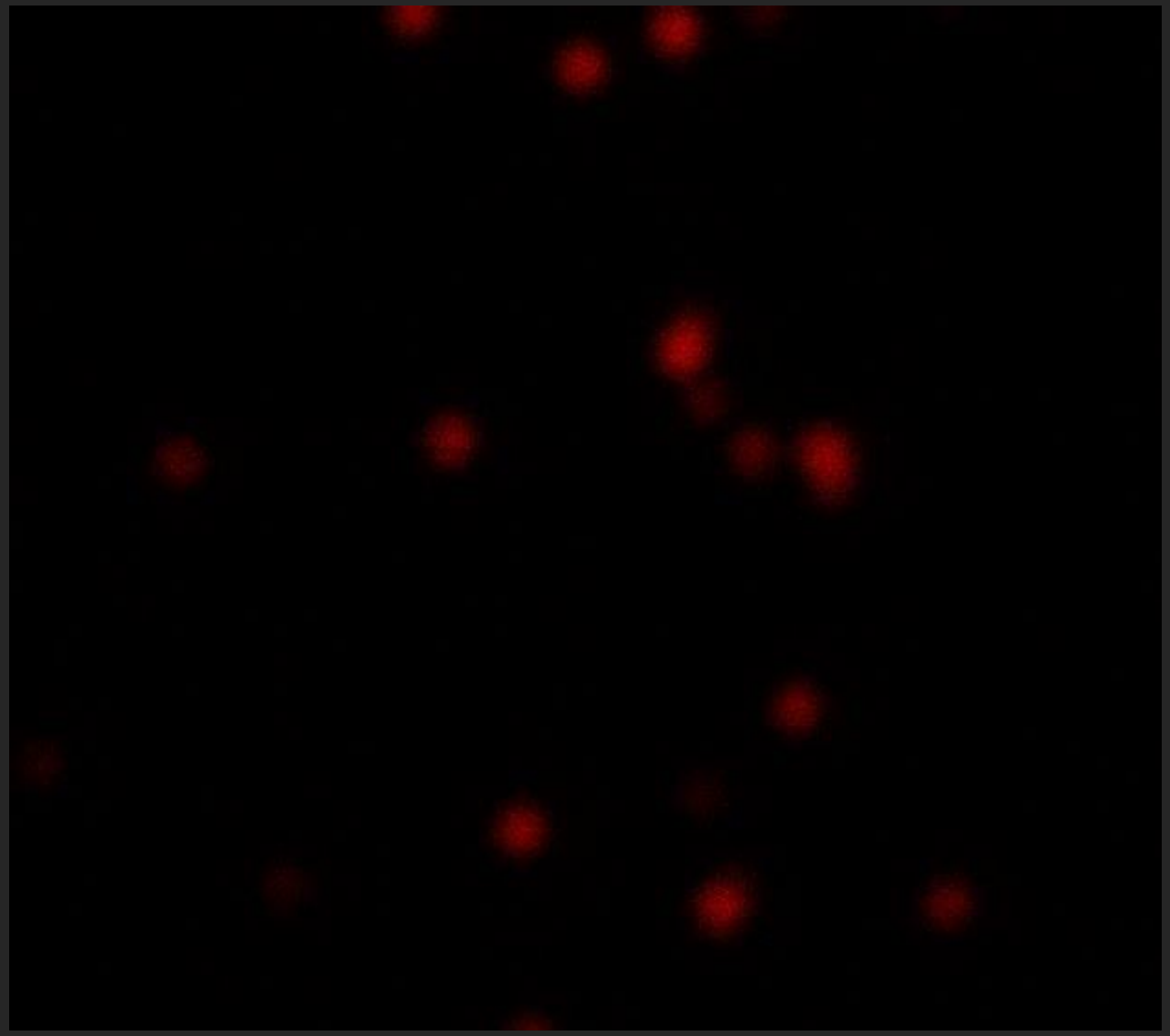


Pulsed-STED and the time-gating in compare to CW-STED time-gating

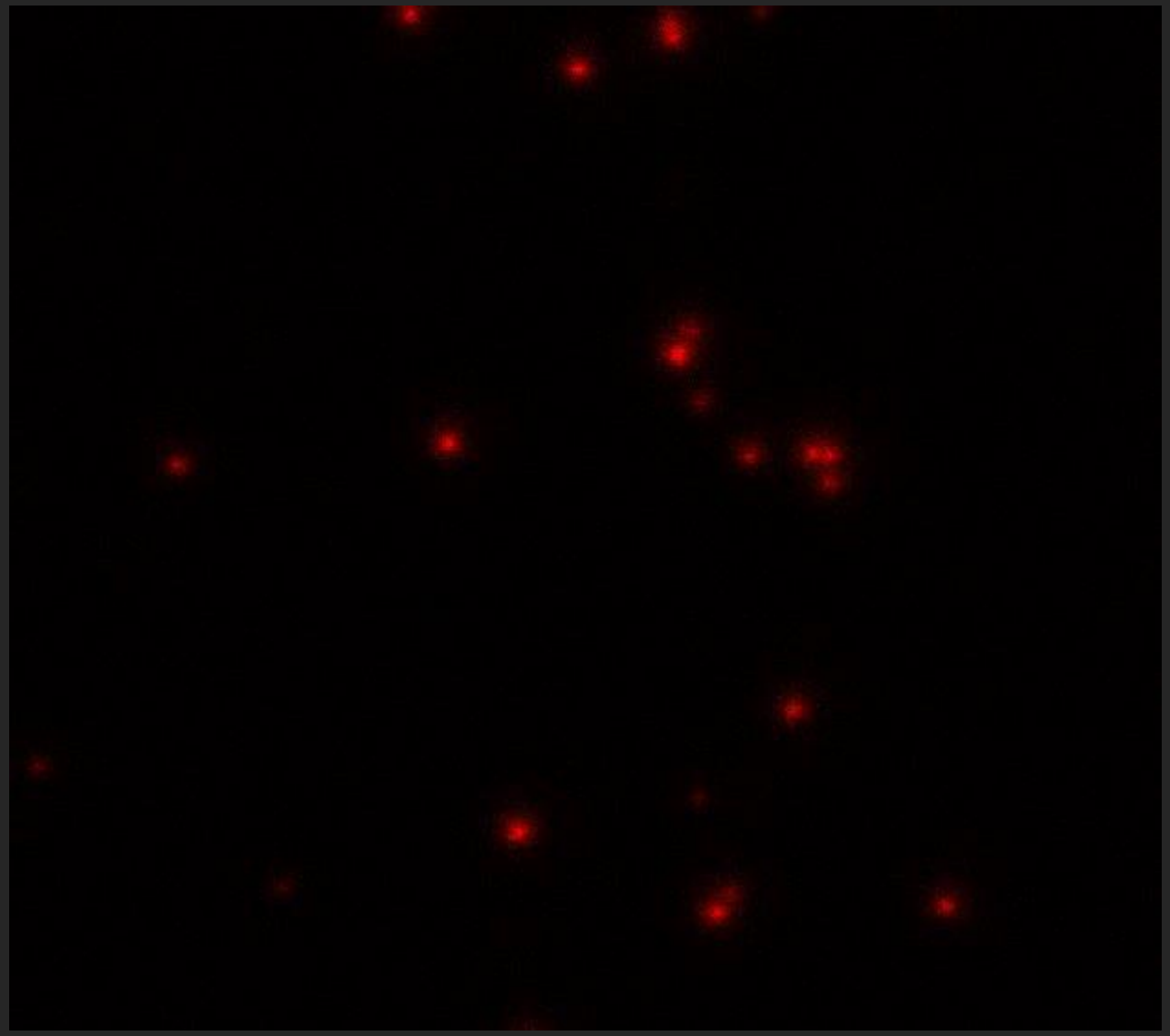




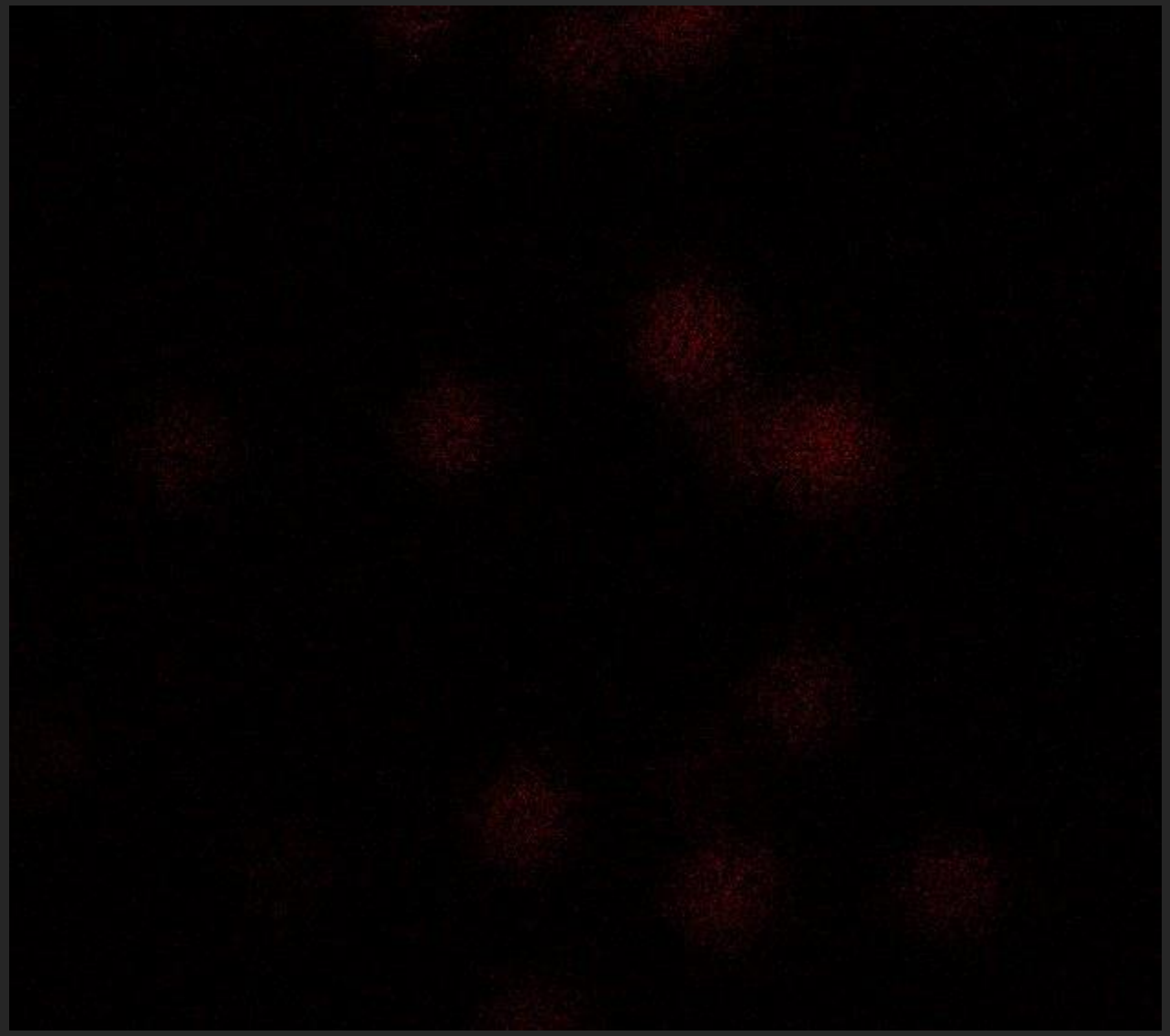
48 nm beads; confocal



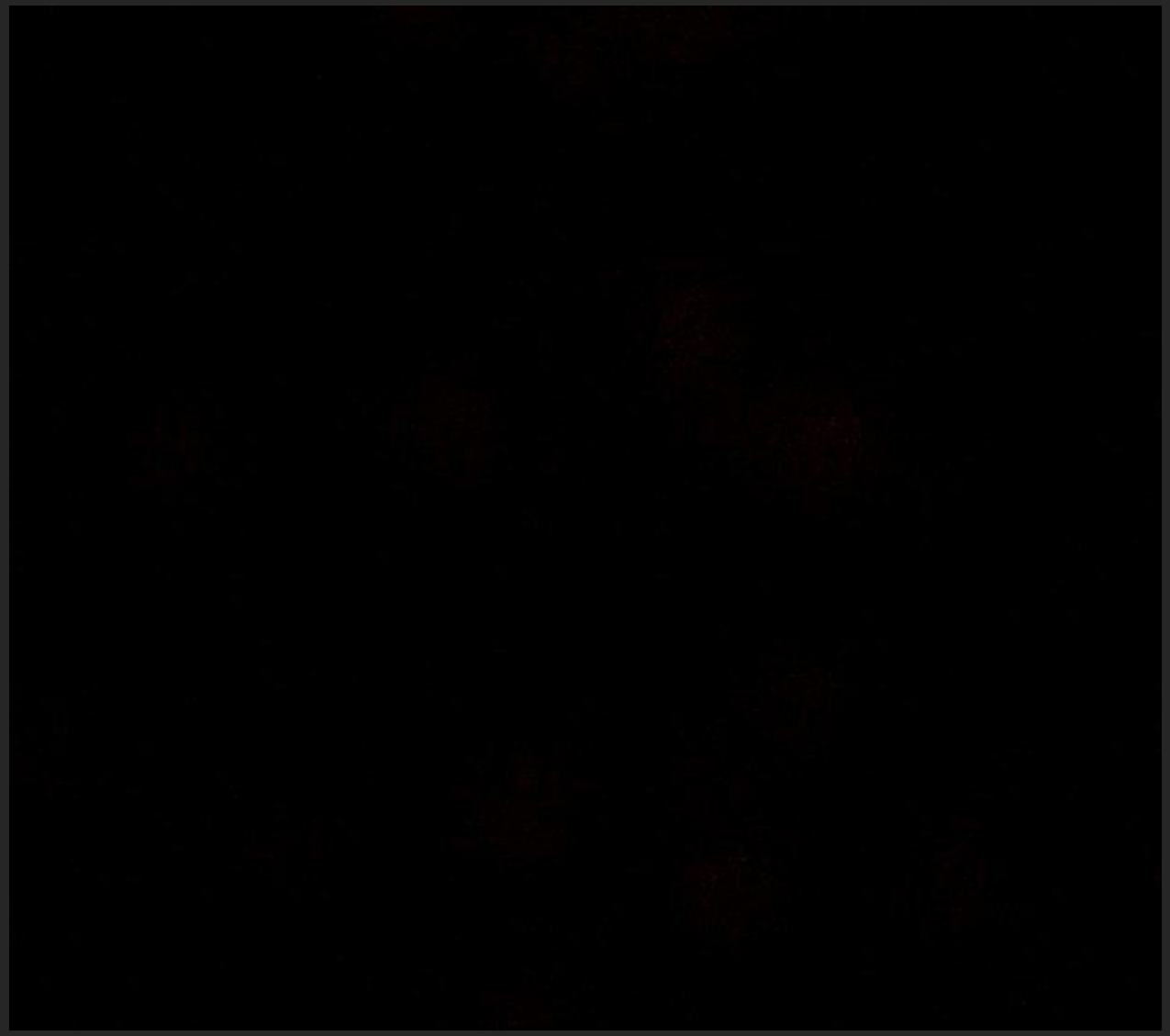
48 nm beads; non-gated cw-STED (660nm)



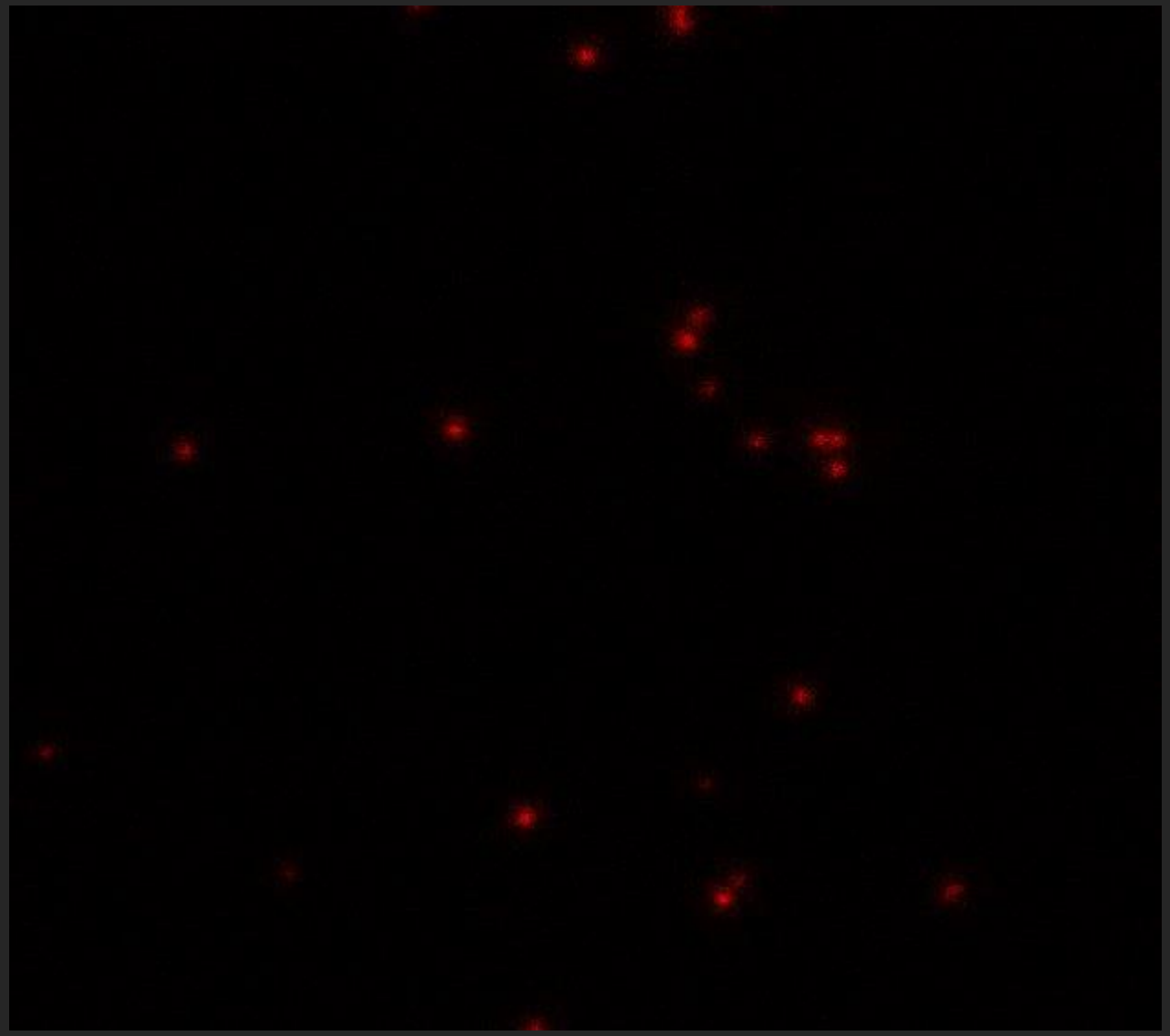
48 nm beads – anti-stokes signal, non-gated cw-STED (660nm)



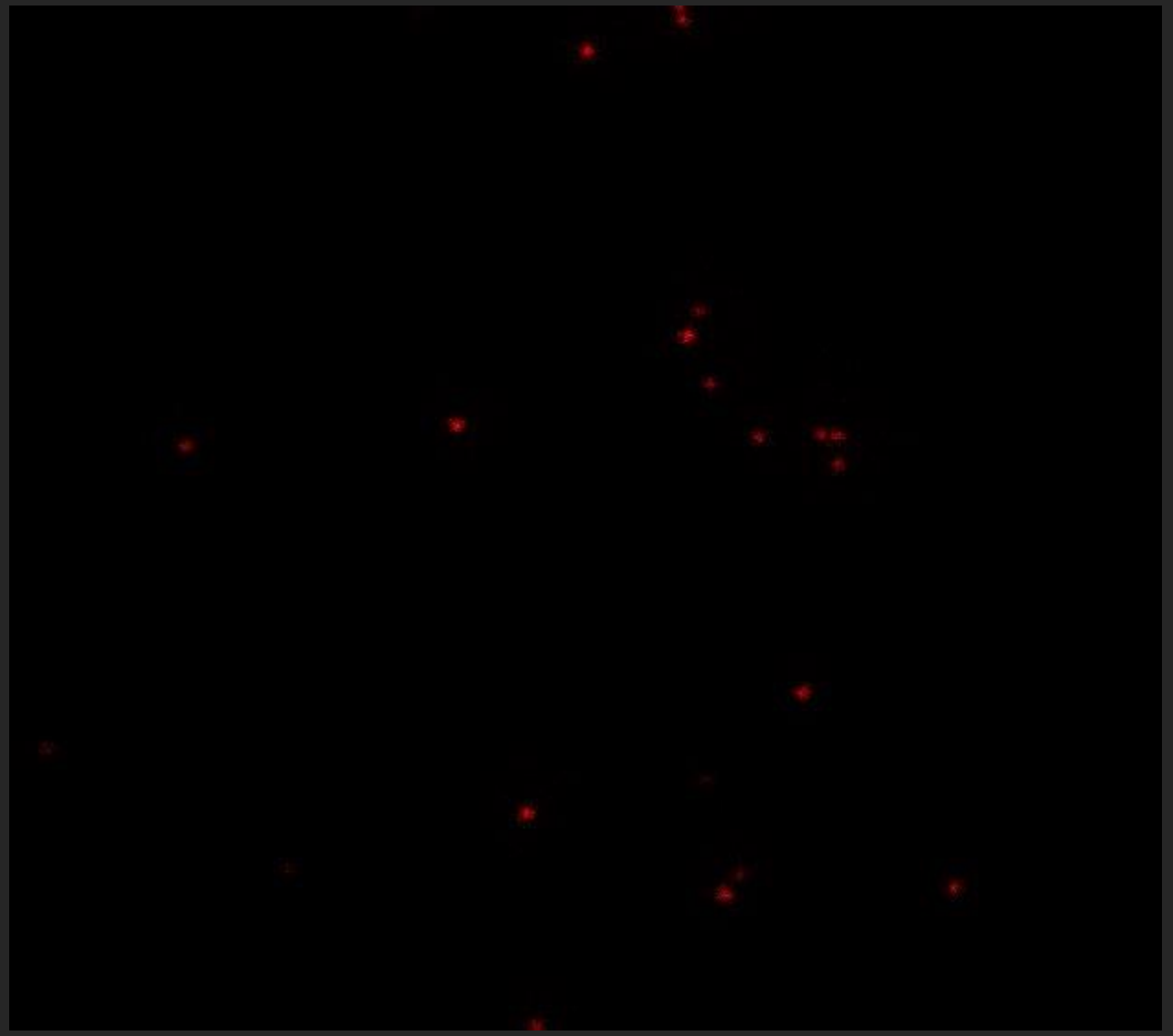
48 nm beads – anti-stokes signal, partially gated cw-STED (660nm)



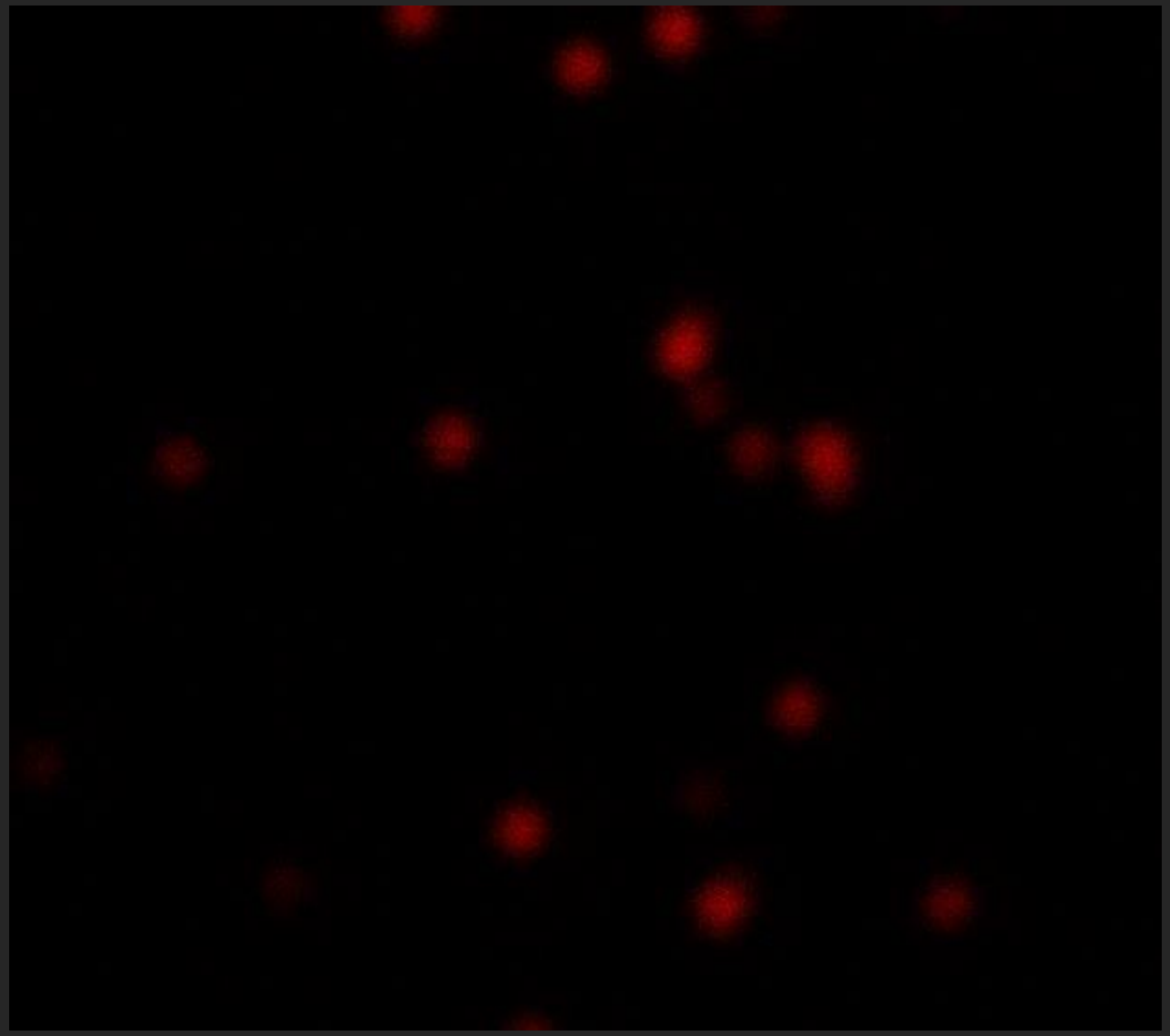
48 nm beads, partially gated cw-STED (660nm)



48 nm beads, gated cw-STED (660nm)



48 nm beads; confocal



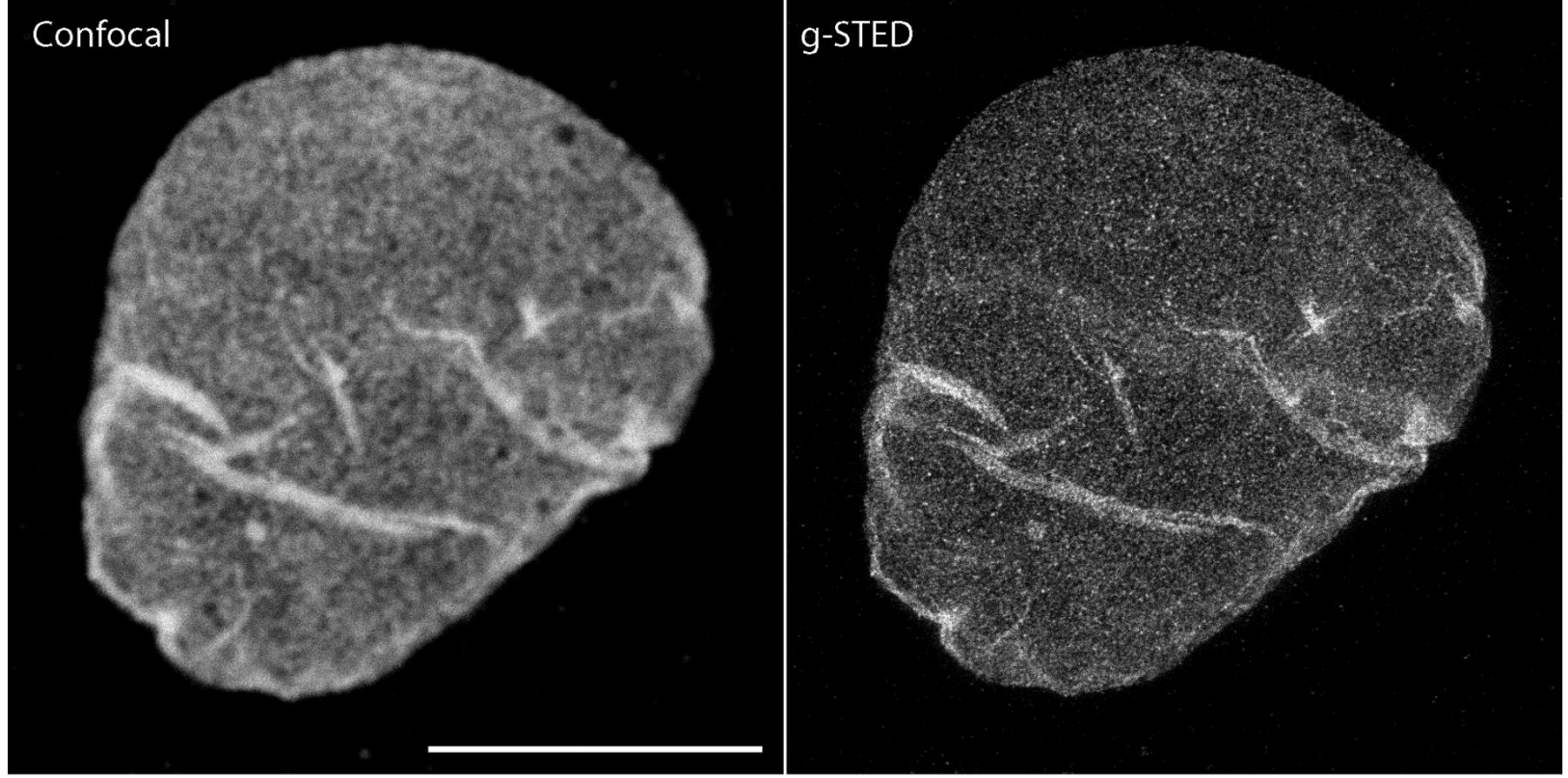
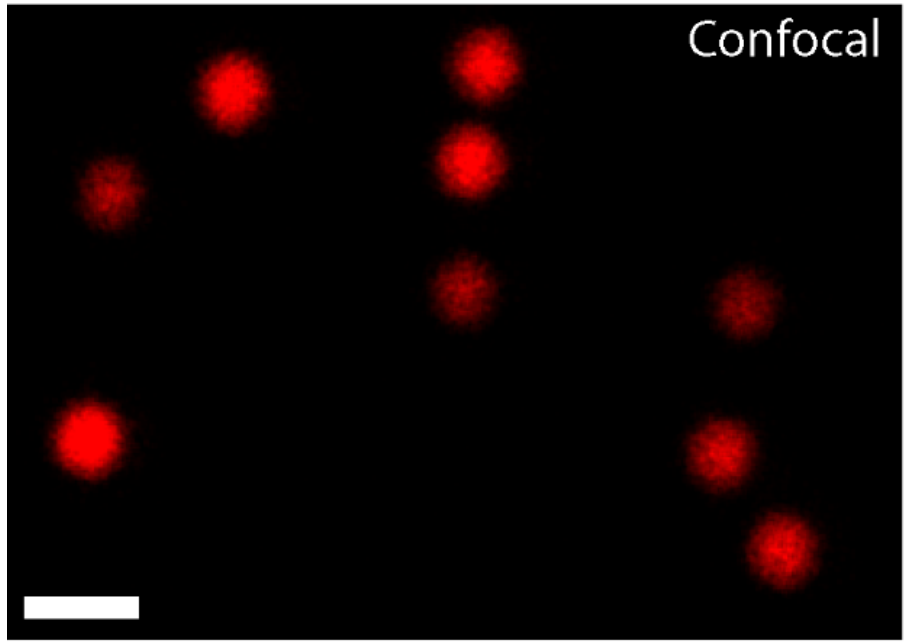
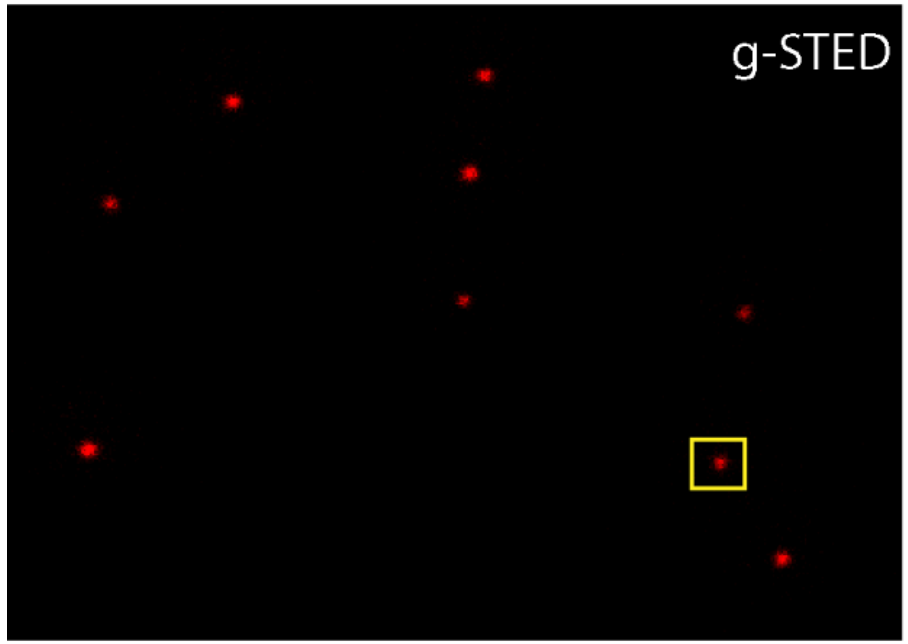
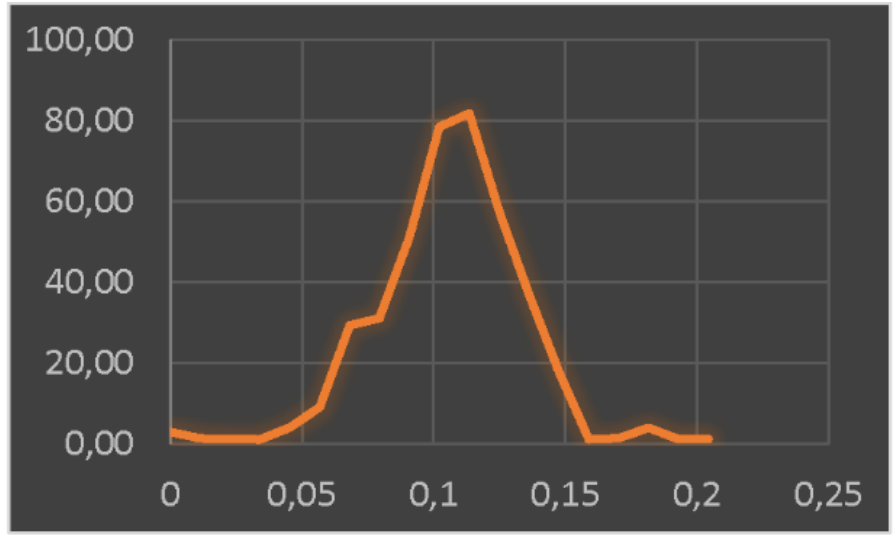


Image of U2OS cell nucleus; nuclear lamina visualized using indirect immunofluorescence, secondary antibody conjugated with AlexaFluor 555 (samples prepared in collaboration with *Jindriska Fiserova, Laboratory of Epigenetics of the Cell Nucleus, IMG CAS, Prague*)
Images were acquired in Confocal and g-STED mode.

Leica TCS SP8 STED 3X
660 nm depletion LASER
Bar: 5 μm



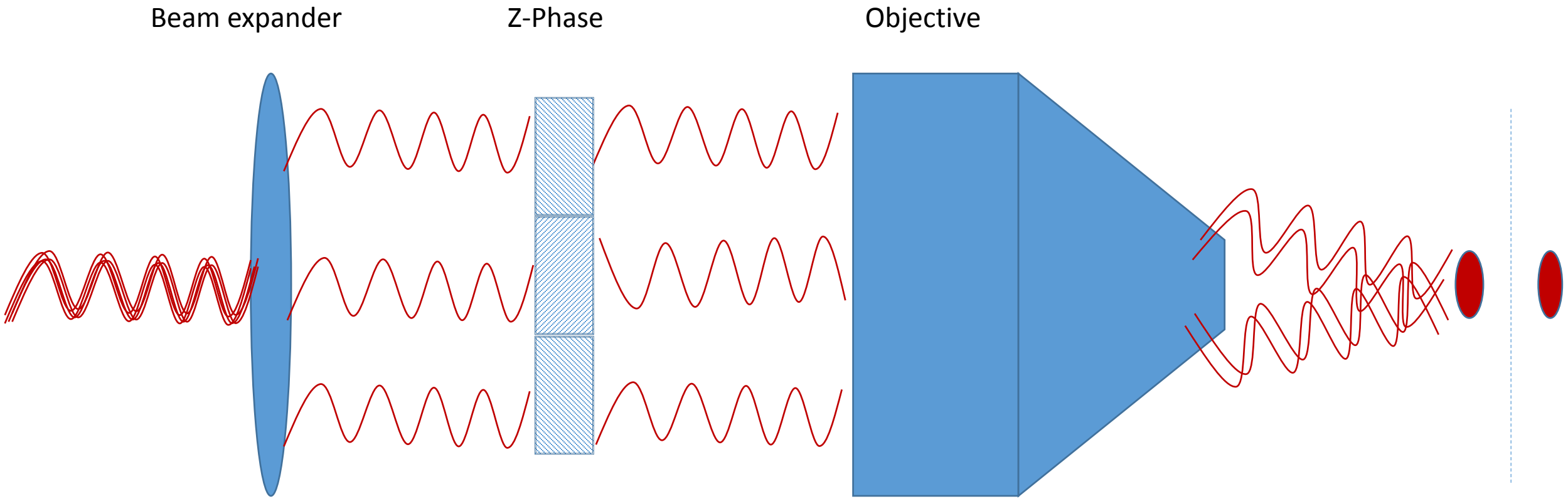


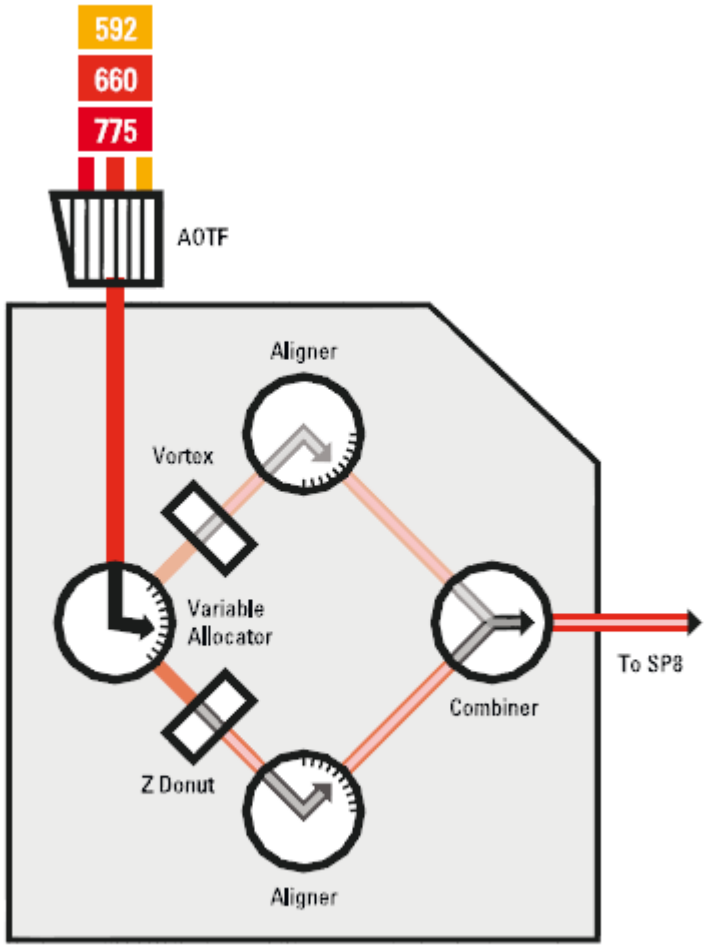


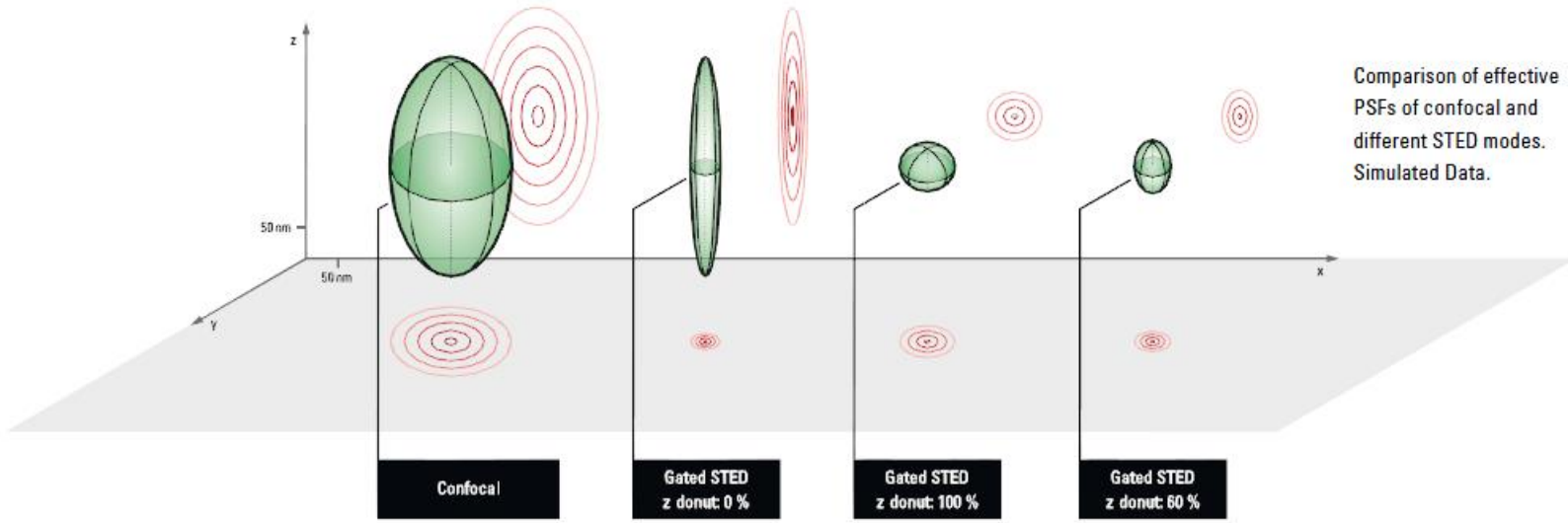
Fluorescence bead sample optimized for STED, acquired in Confocal and g-STED mode (left top and bottom).
Plot profile (above) shows 50 nm FWHM.
(X: microns, Y: intensities; 8bit depth)

Leica TCS SP8 STED 3X
660 nm depletion LASER
Bar: 300 nm

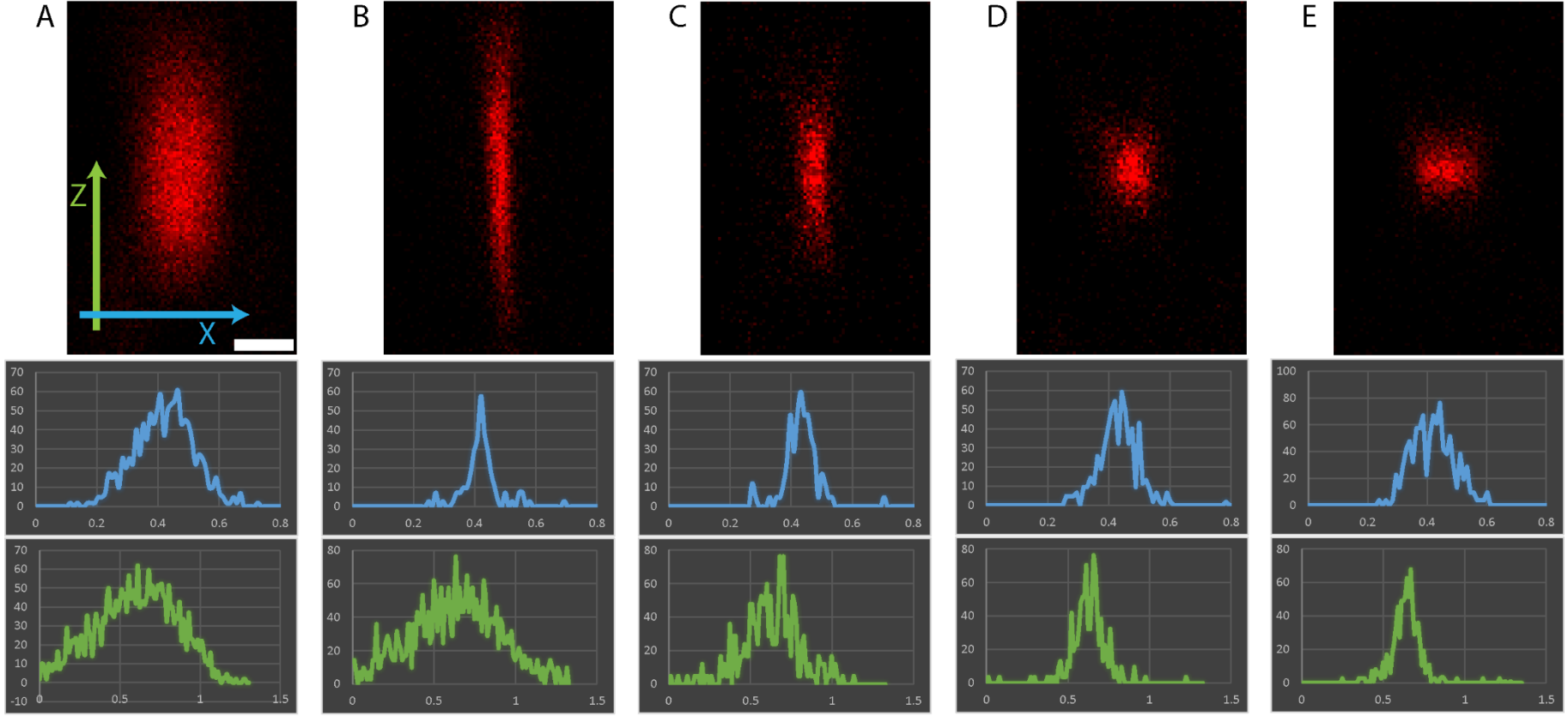
3D STED







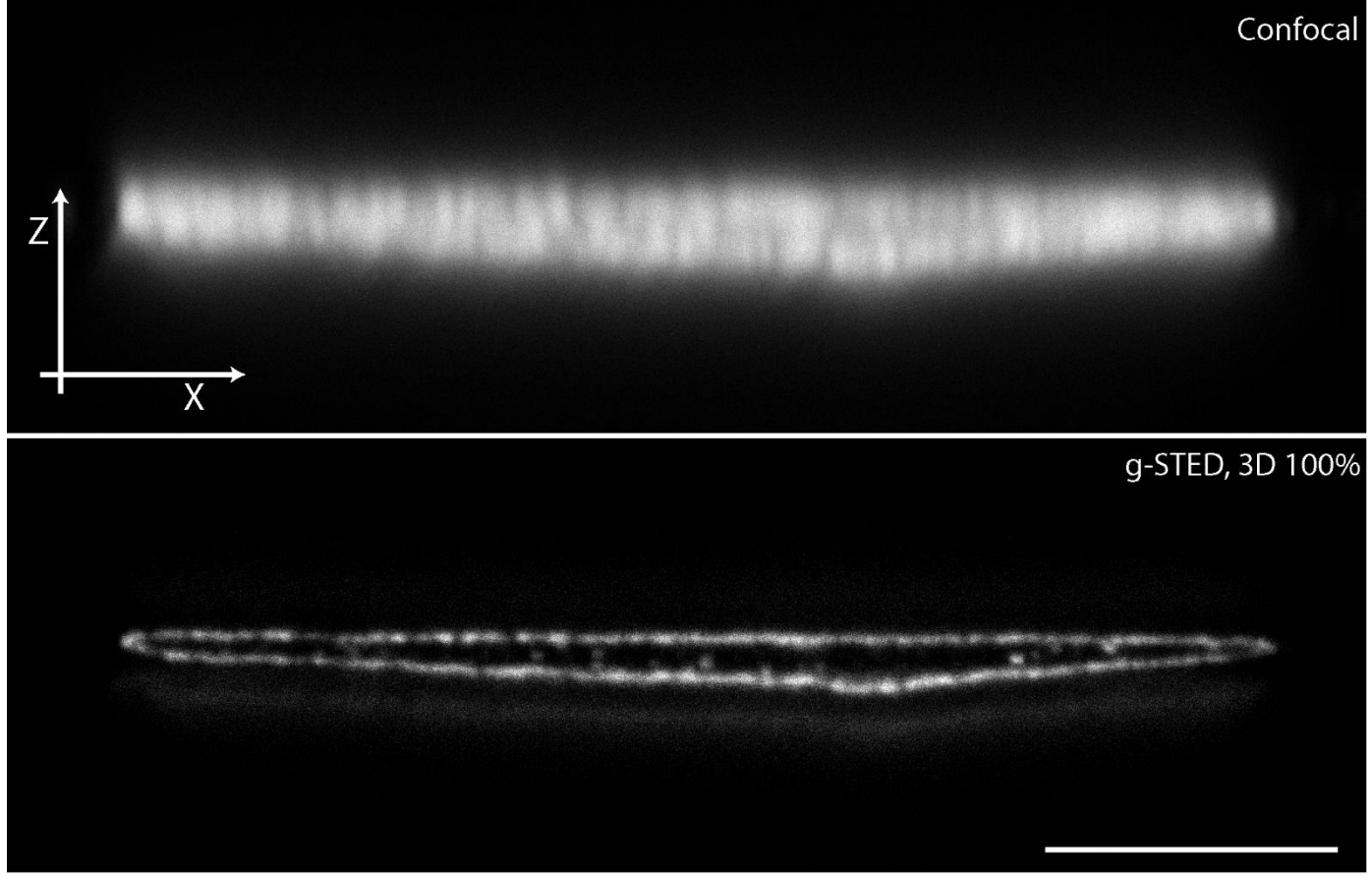
Comparison of effective PSFs of confocal and different STED modes. Simulated Data.



PSF of fluorescence bead optimized for STED, image acquired using XZ scan.
Plotes above demonstrate the intensity profile of each PSF in X (blue) and Z (green) axis. The beads were imaged in the manner:
A: confocal
B: g-STED, 3D 0%
C: g-STED, 3D 20%
D: g-STED, 3D 70%
E: g-STED, 3D 100%

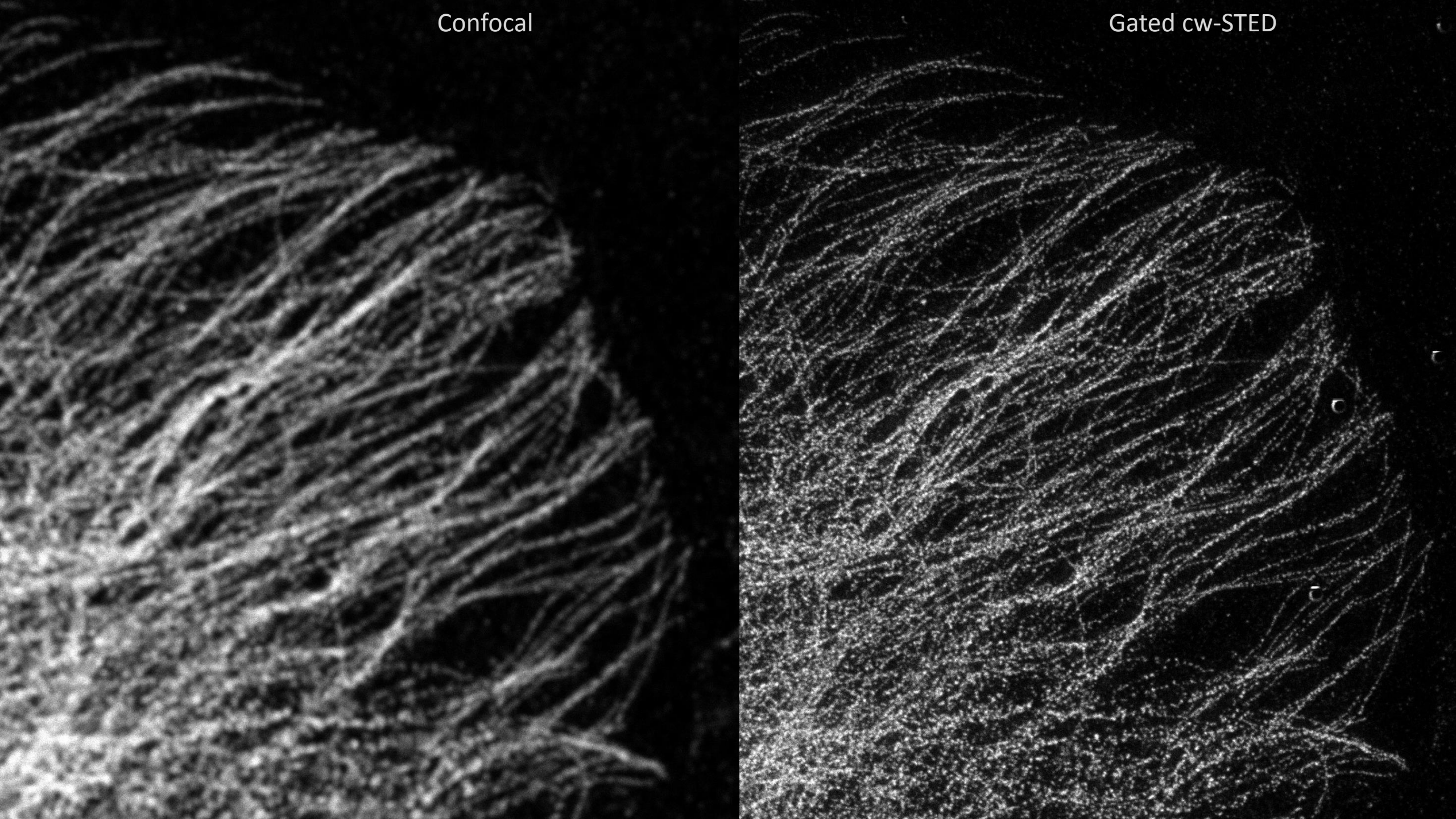
Leica TCS SP8 STED 3X
660 nm depletion LASER

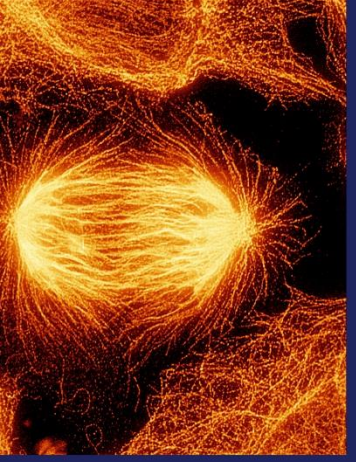
Bar: 300 nm

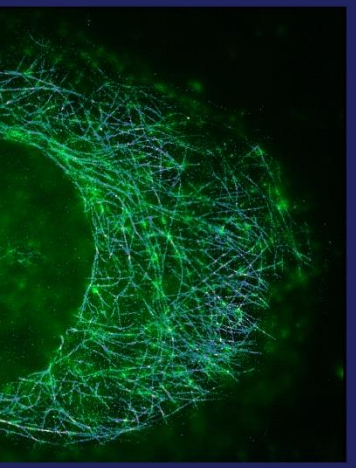


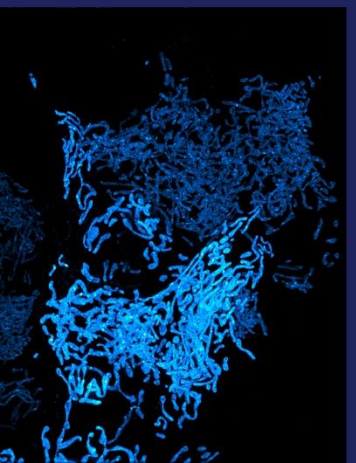
XZ profile of U2OS cell nucleus; nuclear lamina visualized using indirect immunofluorescence, secondary antibody conjugated with AlexaFluor 555 (samples prepared in collaboration with *Jindriska Fiserova, Laboratory of Epigenetics of the Cell Nucleus, IMG CAS, Prague*)
Images were acquired using XZ scan in Confocal and g-STED mode.

Leica TCS SP8 STED 3X
660 nm depletion LASER
Bar: 5 μm


Confocal
Gated cw-STED







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IMG
Light Microscopy Core Facility

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