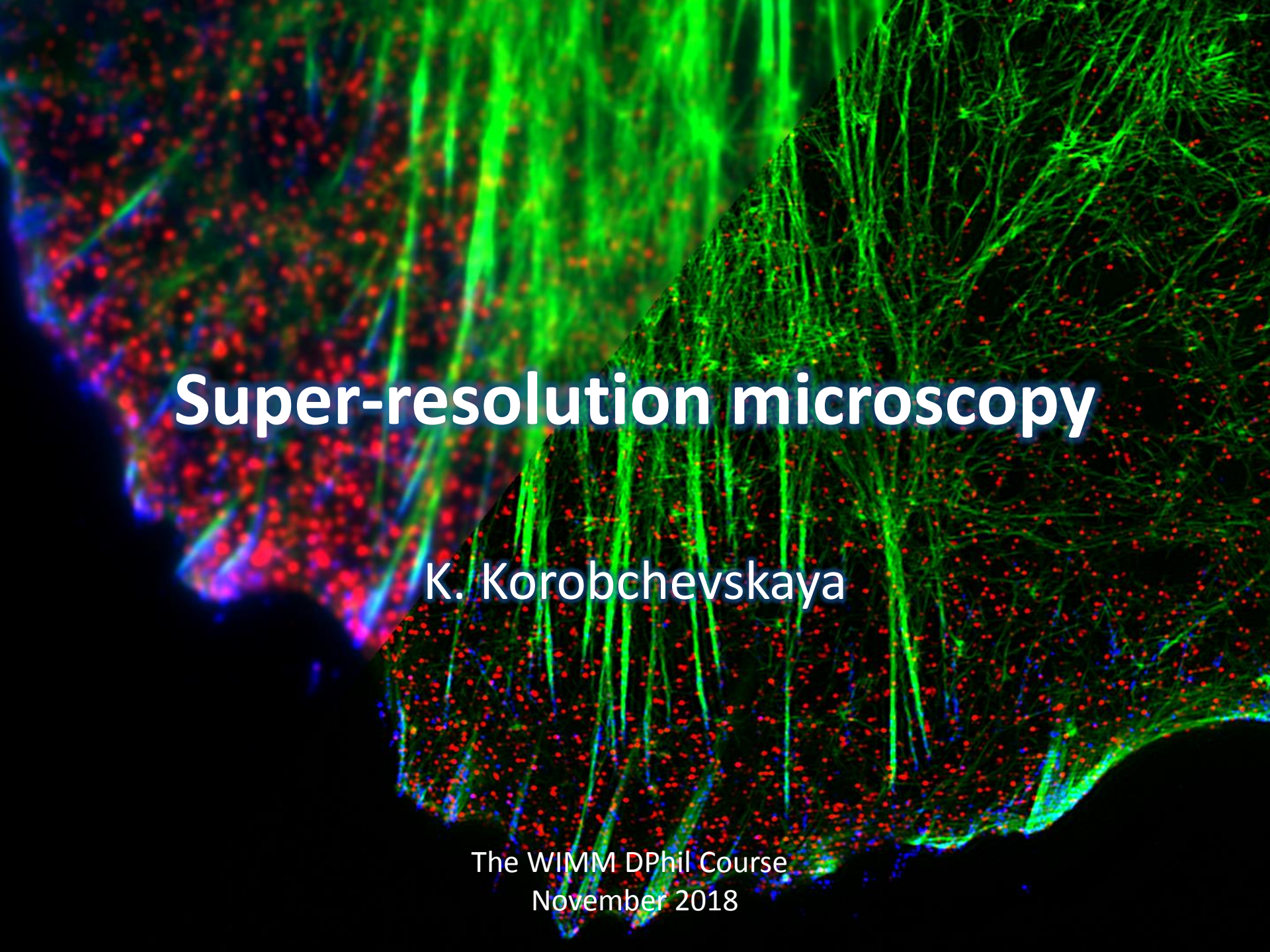


Super-resolution microscopy

K. Korobchevskaya

The WIMM DPhil Course
November 2018

Outline

- Introduction
  - Resolution in optical microscopy
  - PSF and image formation
  - Diffraction limit
- Super-resolution techniques
  - STED concept, examples
  - PALM/STORM concept, examples
  - SIM - concept, examples
- Comparison between SR approaches, applications, pros and cons

Fluorescence microscopy

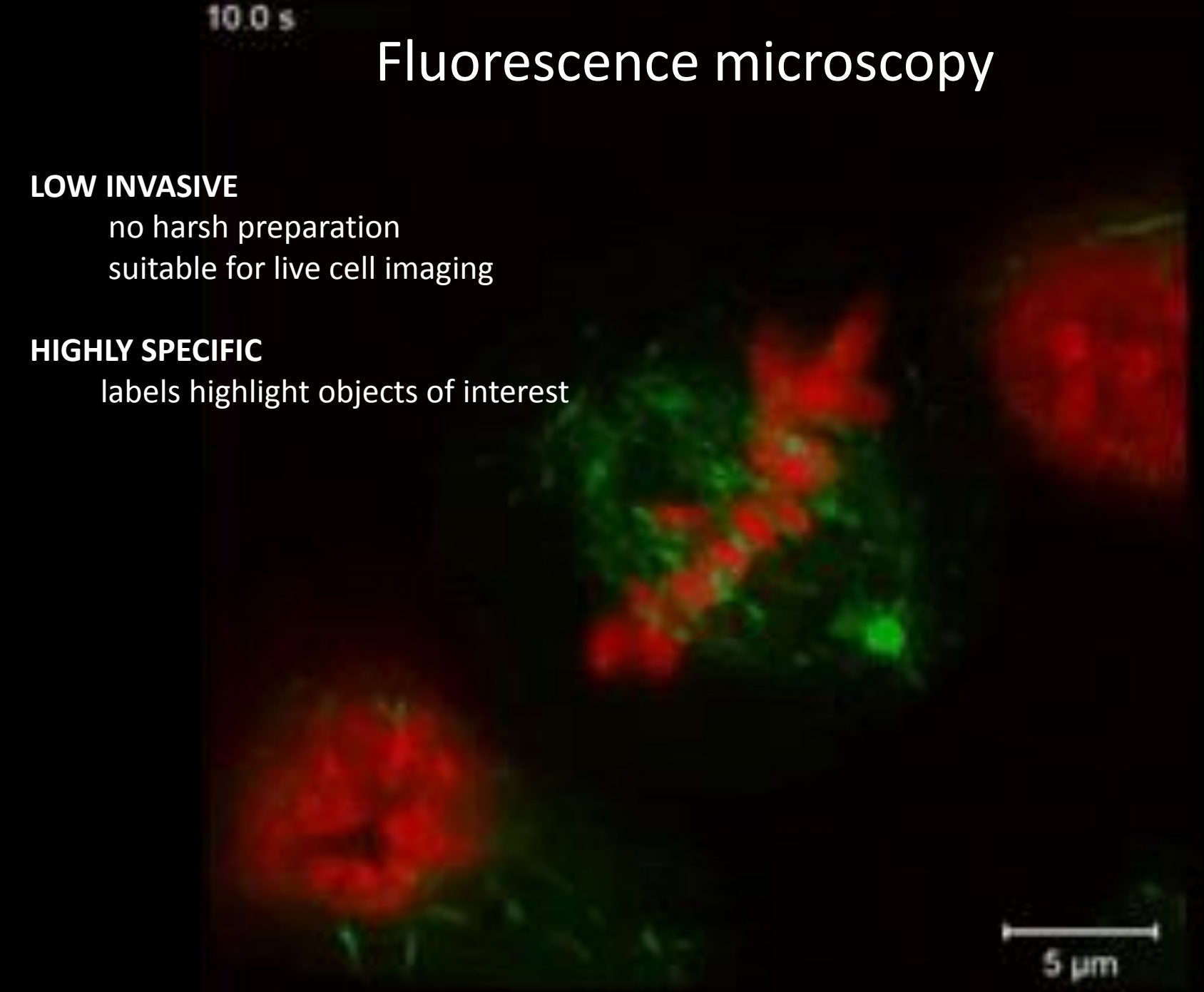
LOW INVASIVE

no harsh preparation

suitable for live cell imaging

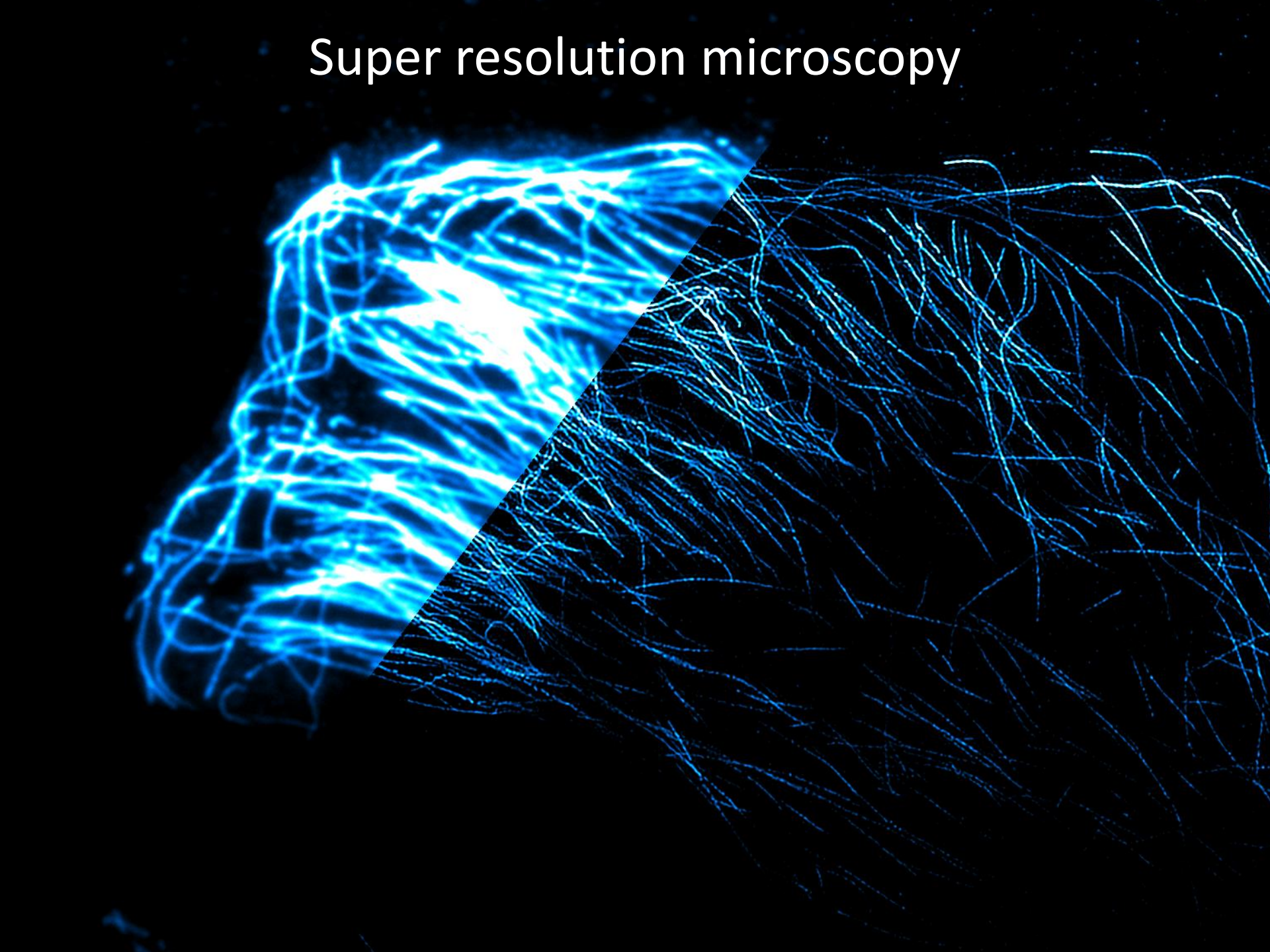
HIGHLY SPECIFIC

labels highlight objects of interest

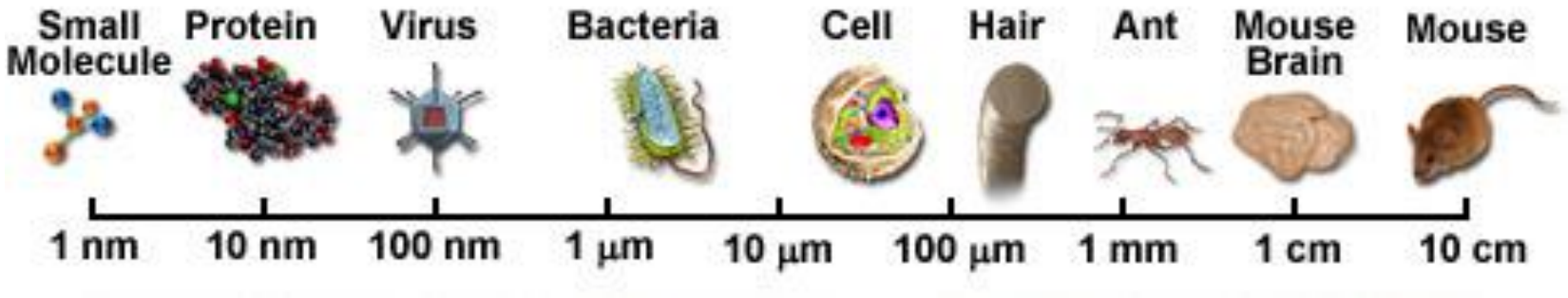


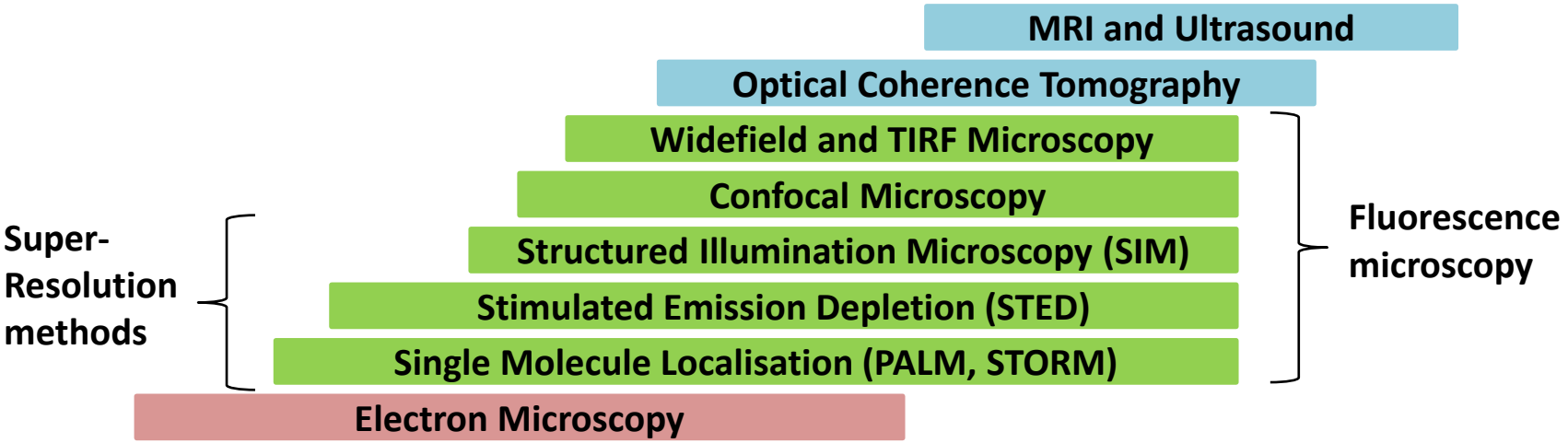
ZEISS LSM 880 with Airyscan: Mitosis in HeLa Kyoto EB3 EGFP Carl, Zeiss

Super resolution microscopy

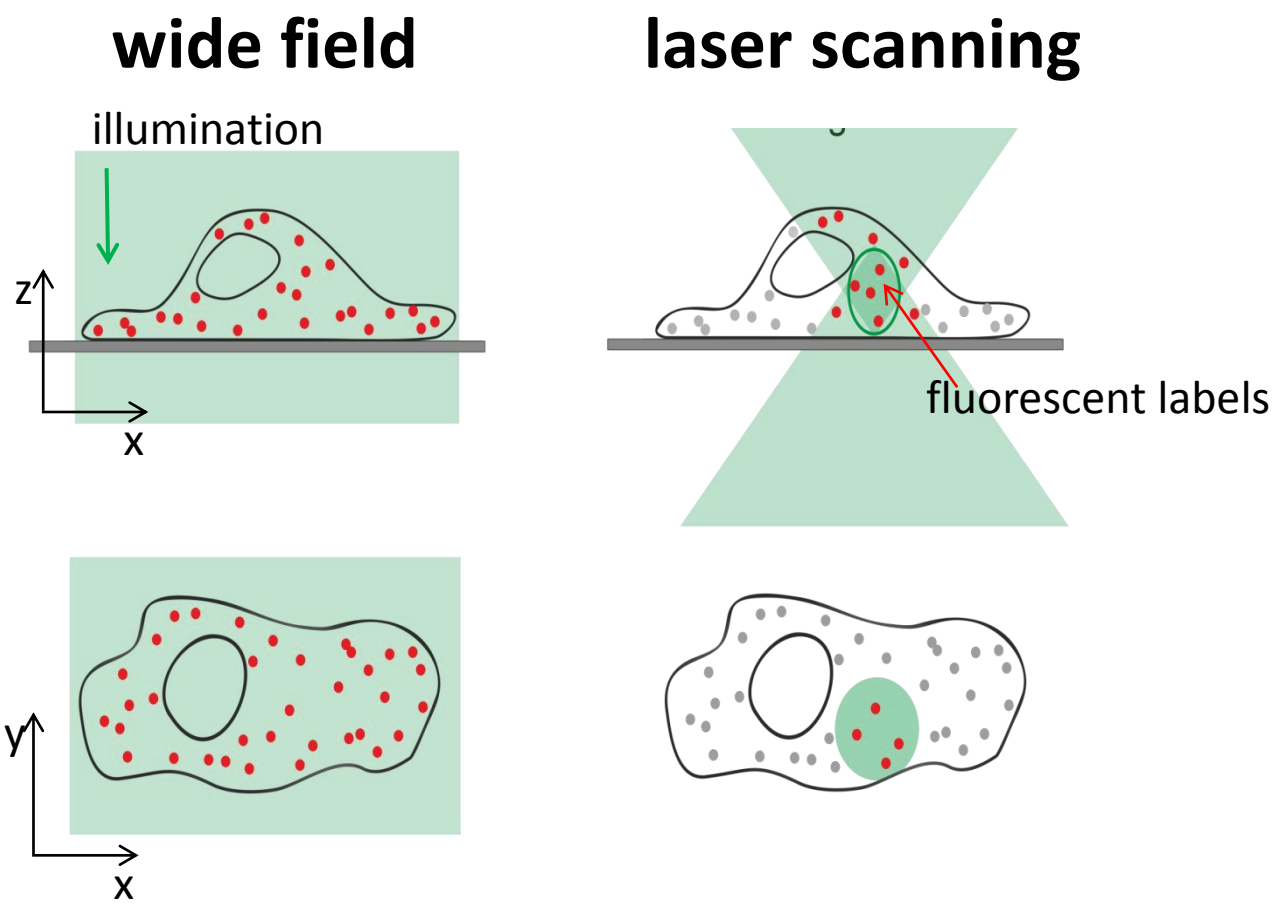


Spatial resolution and Imaging approaches





Excitation and detection approaches in fluorescence microscopy

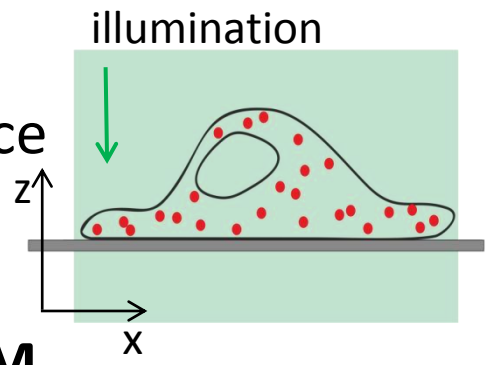


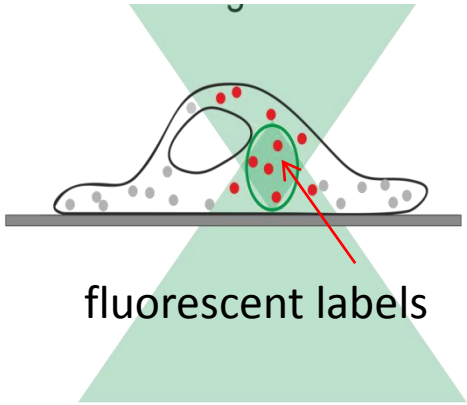
Excitation and detection approaches in fluorescence microscopy

wide field

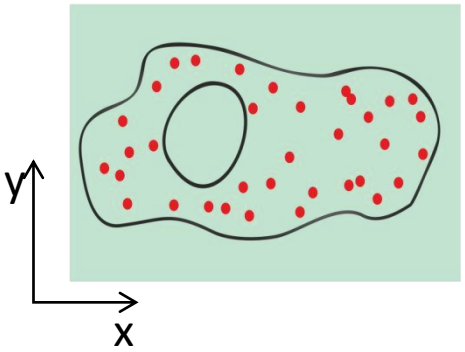
laser scanning

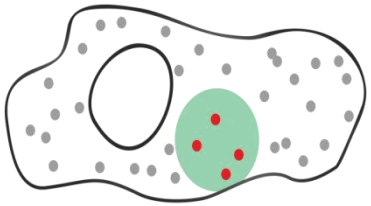
epifluoresence
TIRF
SIM
STORM/PALM





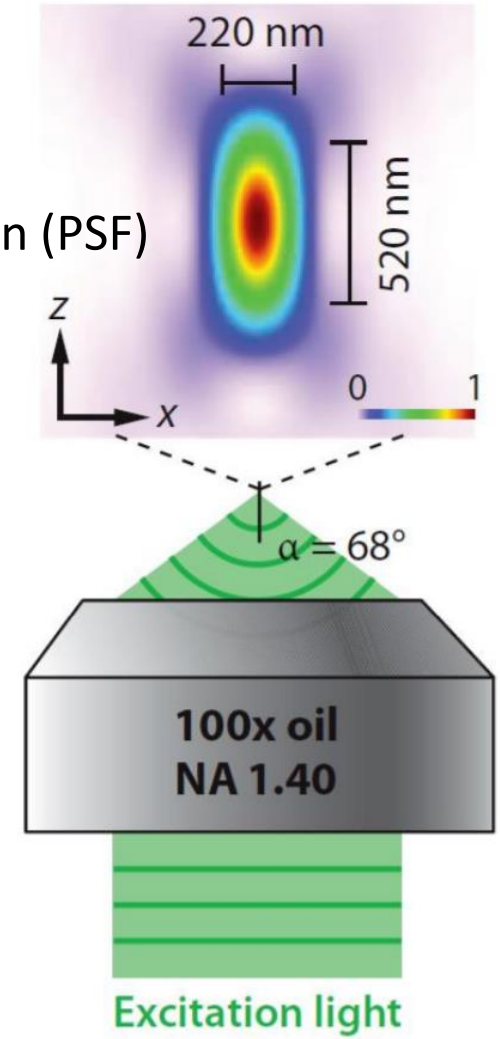
confocal
two-photon
STED





Diffraction limit in optical microscopy

Point spread function (PSF)

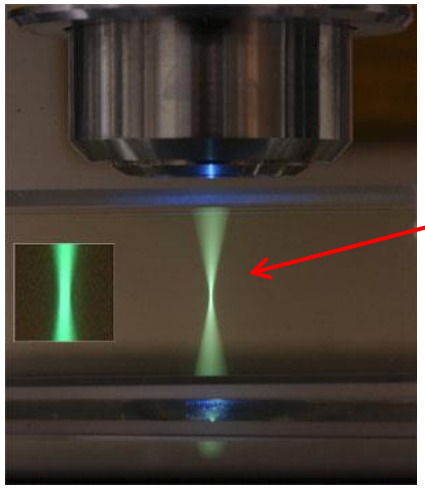


$$d = \frac{\lambda}{2n\sin\alpha}$$

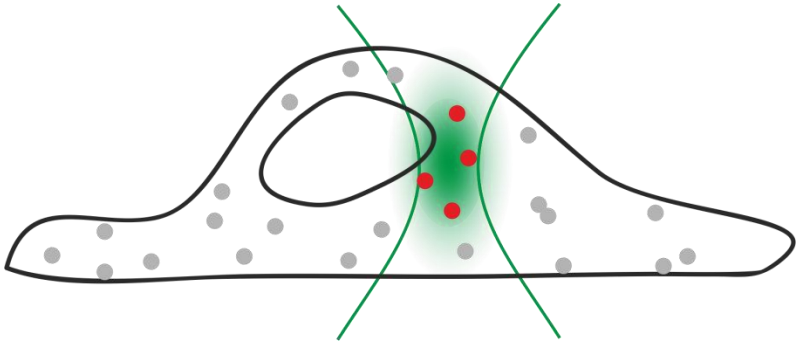
Ernst Abbe
(1840-1905)

Huang B. et al., 2009, Annu. Rev. Biochem. 78:993-016

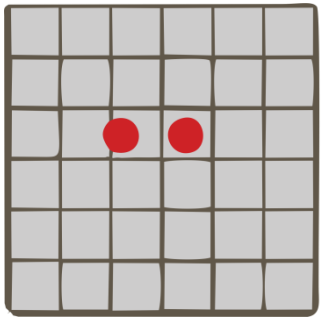
Diffraction limit



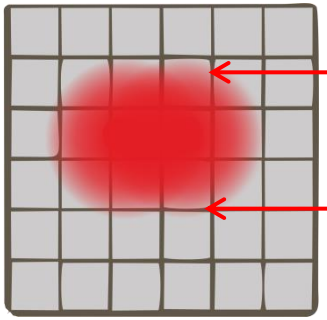
$$d = \frac{\lambda}{2\ NA}$$



Real object



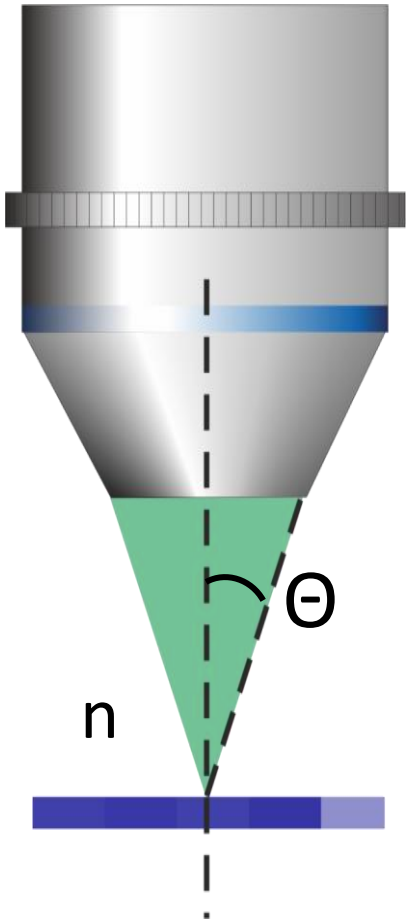
Diffraction limited image



200 – 250 nm

Numerical Aperture (N.A.)

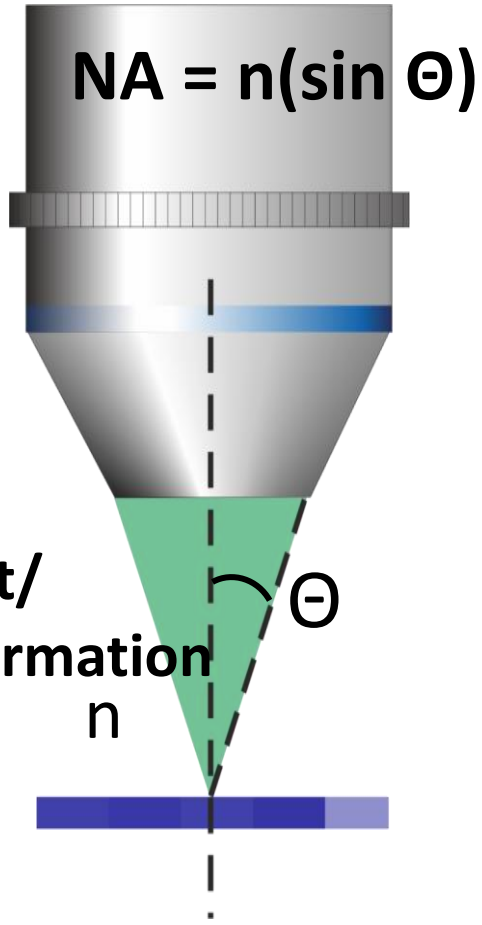
NA = n(sin Θ)



Diffraction limit

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

Numerical Aperture (N.A.)



Higher NA

→

Obtaining more light/

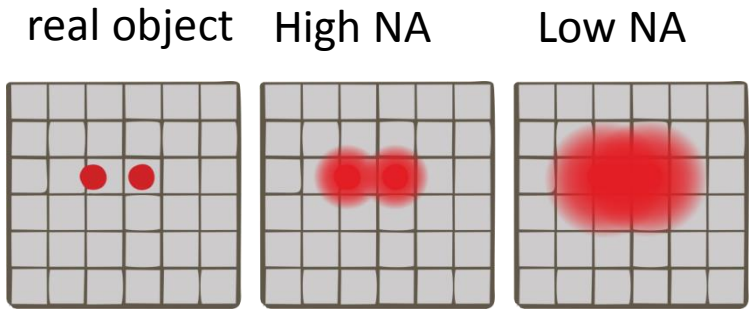
More structural information

→

Higher resolution

Diffraction limit

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



Can we go beyond the diffraction limit?

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

Shorter wavelength

Higher NA

The Nobel Prize in Chemistry 2014

Photo: Matt Staley/HHMI

Eric Betzig

Prize share: 1/3

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

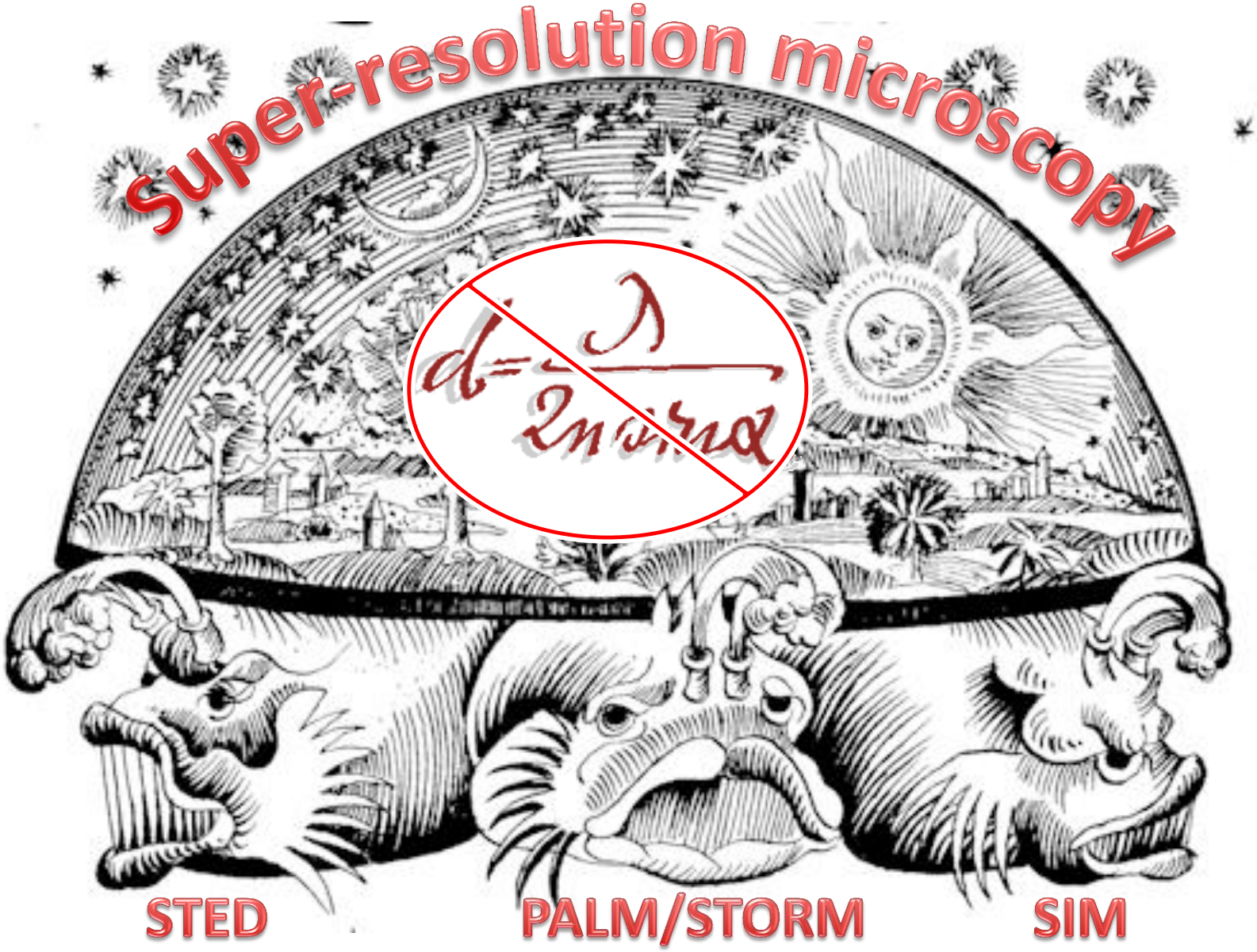
Stefan W. Hell

Prize share: 1/3

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

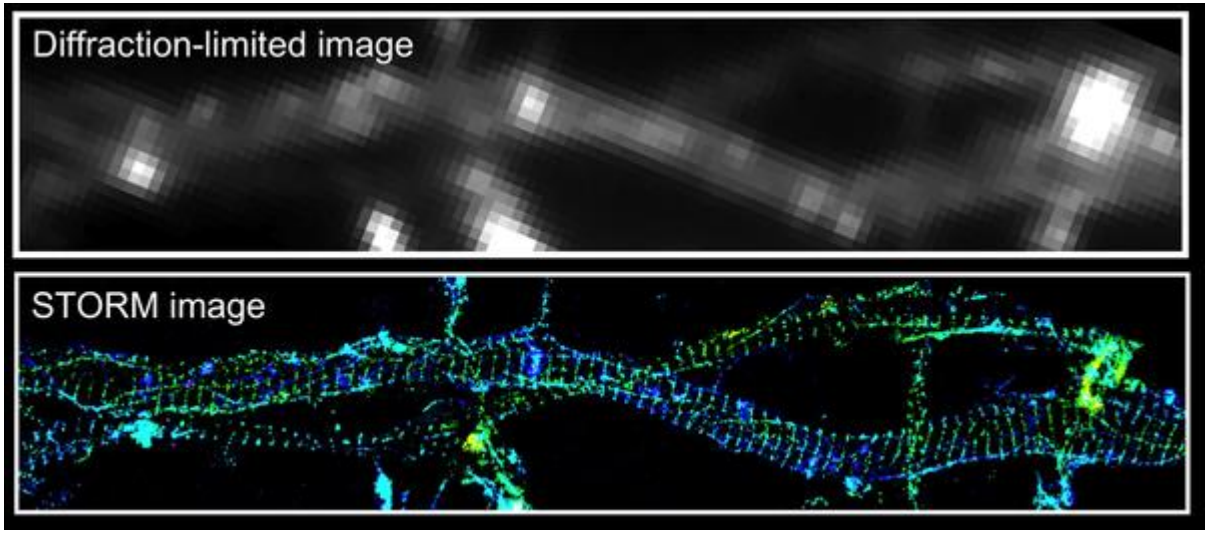
William E. Moerner

Prize share: 1/3


Super-resolution microscopy
$d = \frac{\lambda}{2n\sin\alpha}$
STED
PALM/STORM
SIM

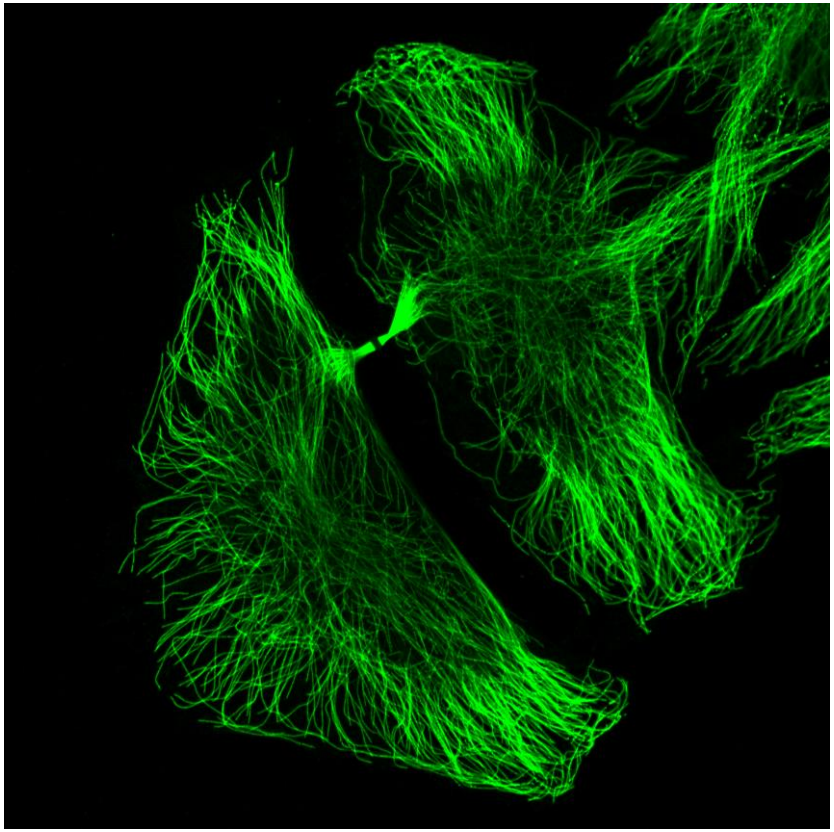
Single molecule localization methods STORM/PALM

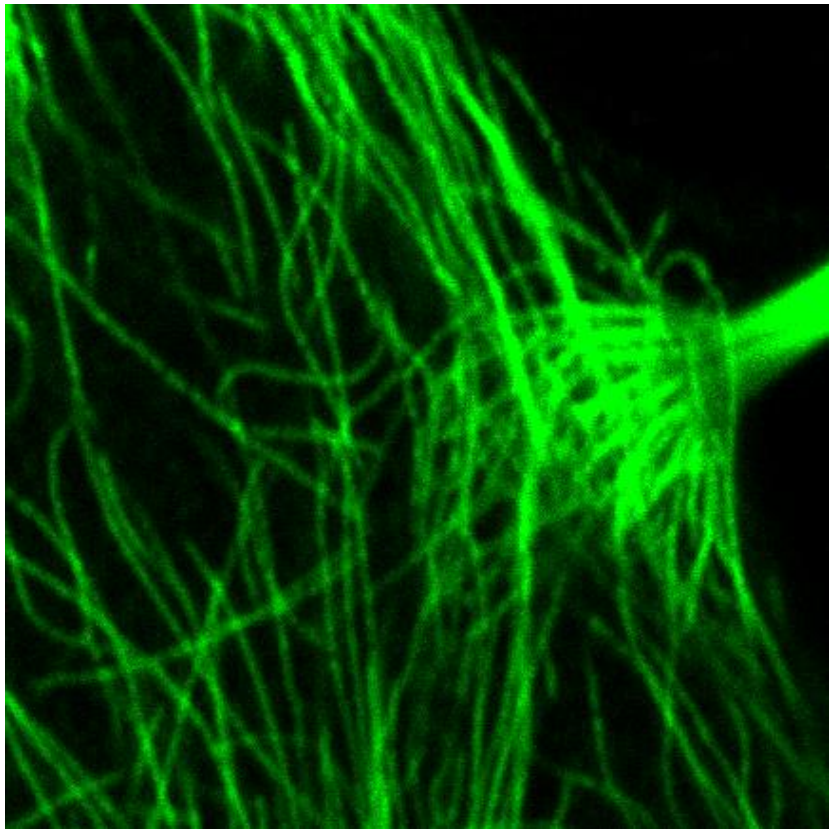
STORM/PALM microscopy overview

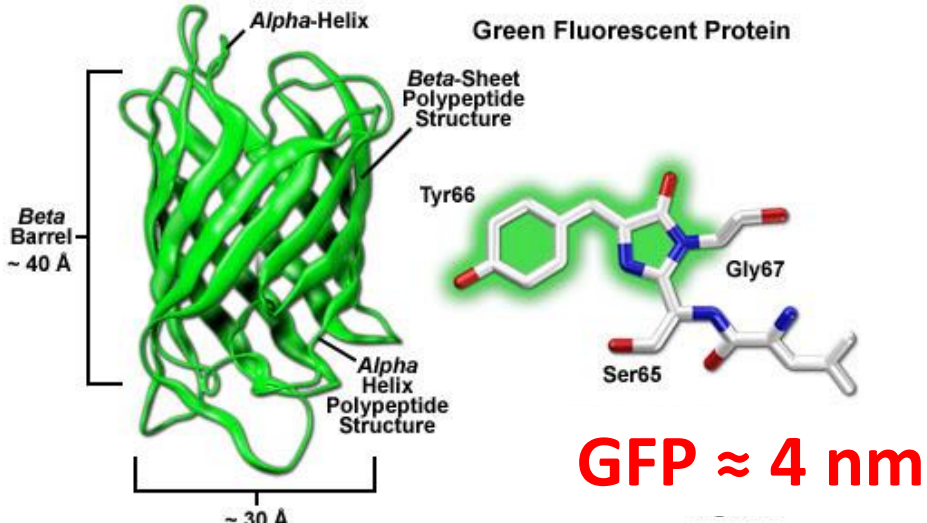


Xu at al., Actin, spectrin and associated proteins form a periodic cytoskeleton structure in axons, Science 339 452-456 (2013)

- Wide field technique (camera acquisition)
- Resolution is only limited by labelling quality
- Require careful image reconstruction, especially for multicolour co-localization

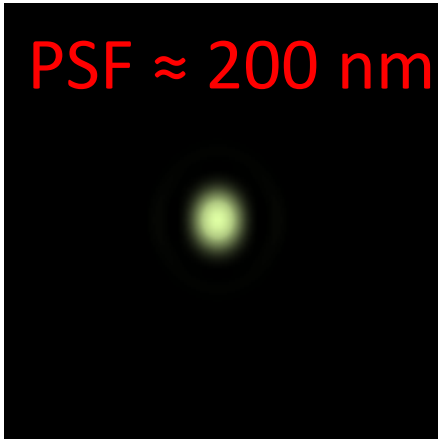




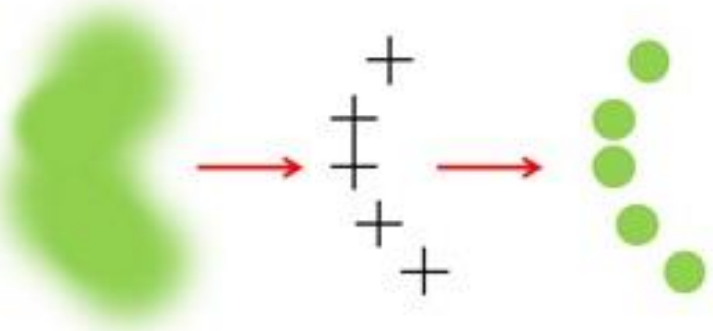

Alpha-Helix
Green Fluorescent Protein
Beta-Sheet Polypeptide Structure
Beta Barrel ~ 40 Å
Tyr66
Gly67
Ser65
Alpha Helix Polypeptide Structure
~ 30 Å

GFP ≈ 4 nm

<<


PSF ≈ 200 nm

Single molecule localization



Conventional fluorescence microscopy: fluorophores too close to resolve

Centroid localisation

Computer rendered super-resolution image

$$\sigma = \sigma_{PSF}/N^{1/2}$$

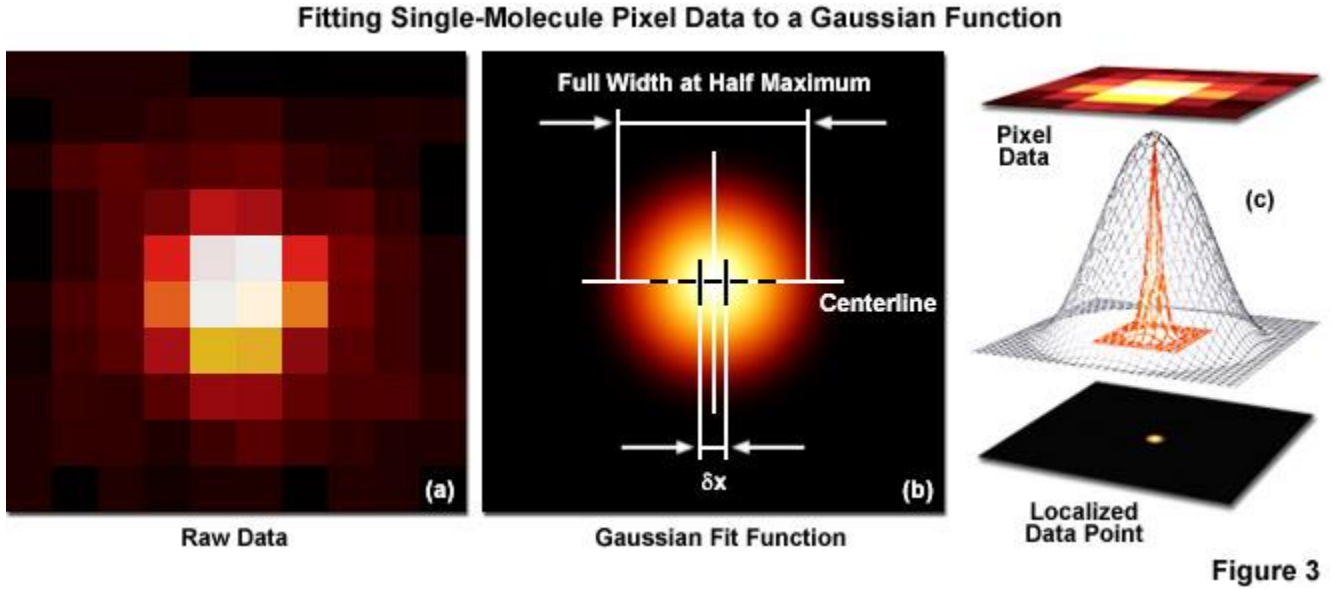
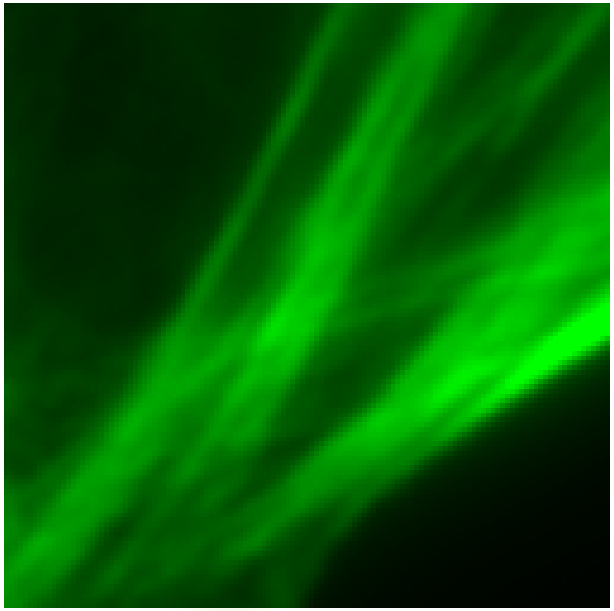
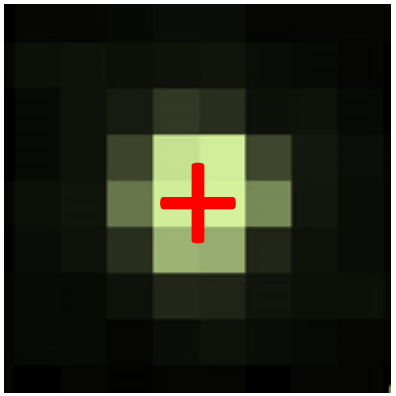


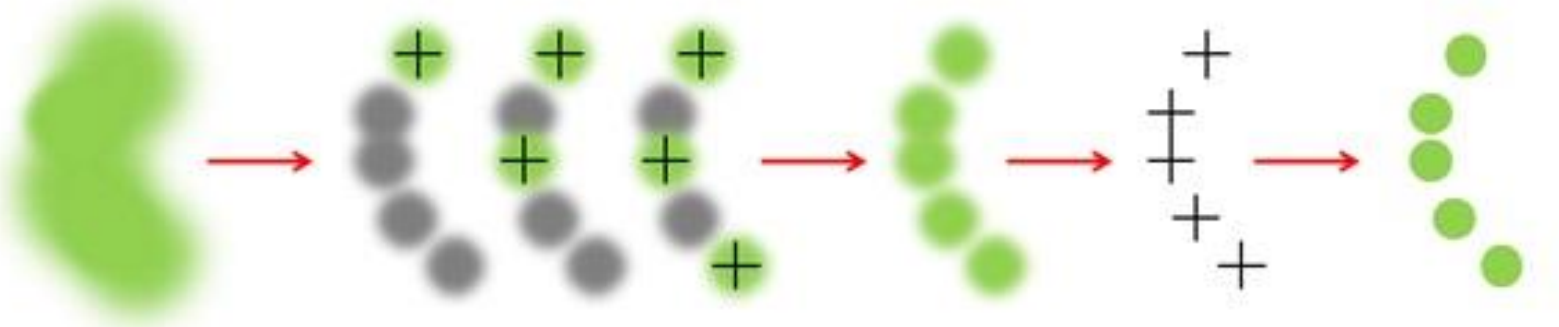
Figure 3

http://zeiss-campus.magnet.fsu.edu/articles/superresolution/palm/practicalaspects.html

Single molecule localization

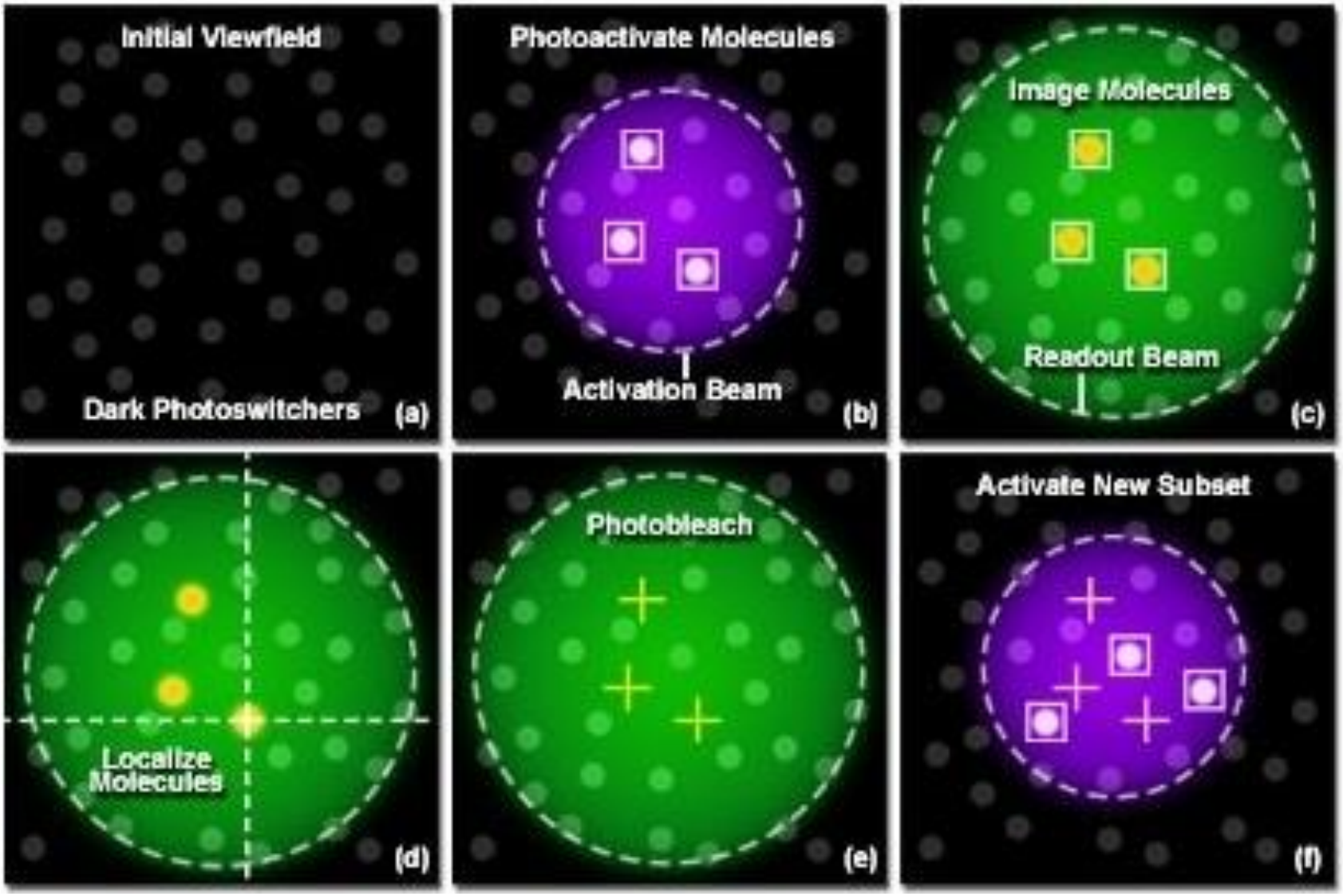




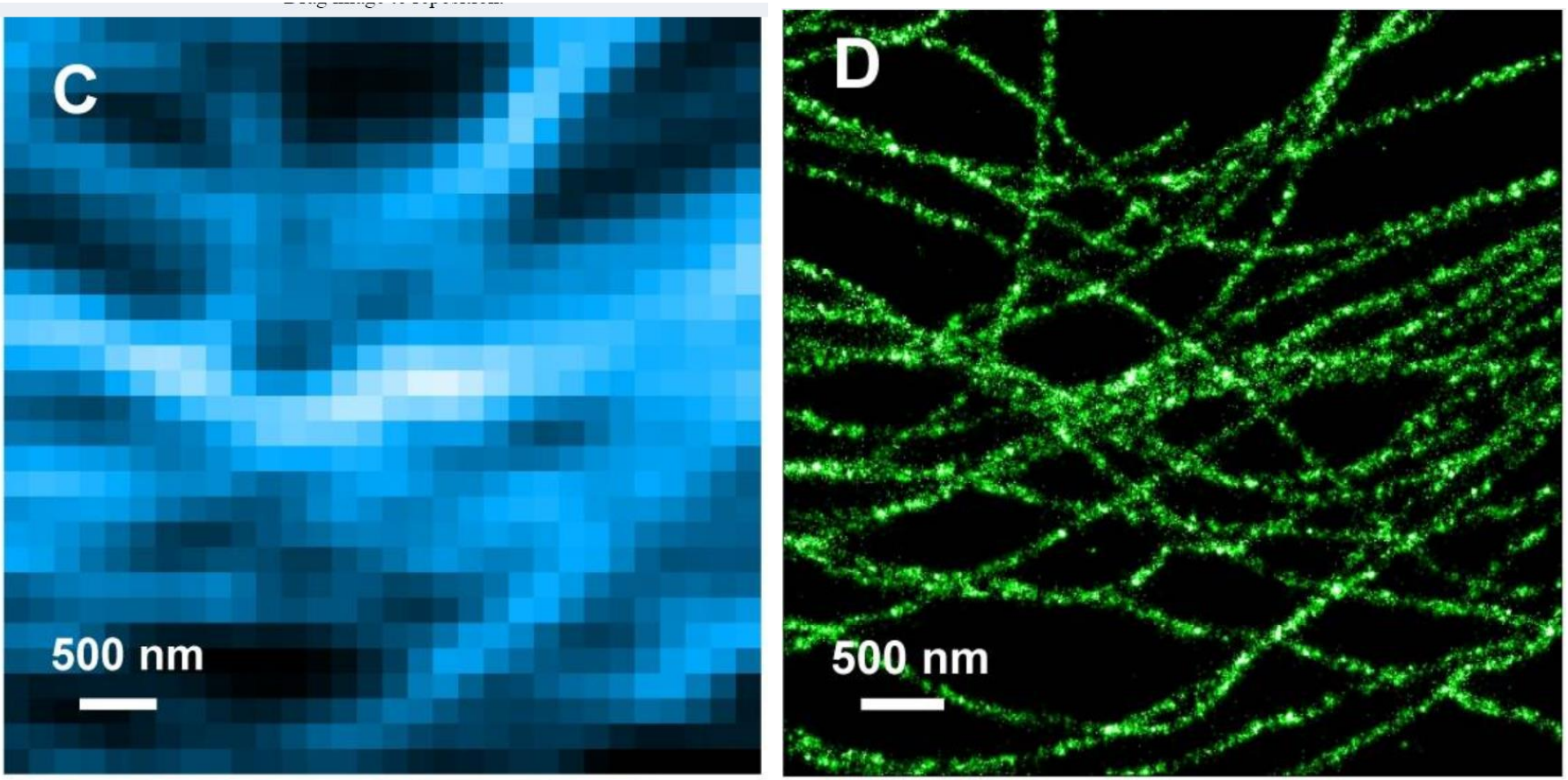


https://cam.facilities.northwestern.edu/588-2/single-molecule-localization-microscopy/

STochastic **O**ptical **R**econstruction **M**icroscopy (**STORM**)/ **P**hoto-**A**ctivated **L**ocalization **M**icroscopy (**PALM**)

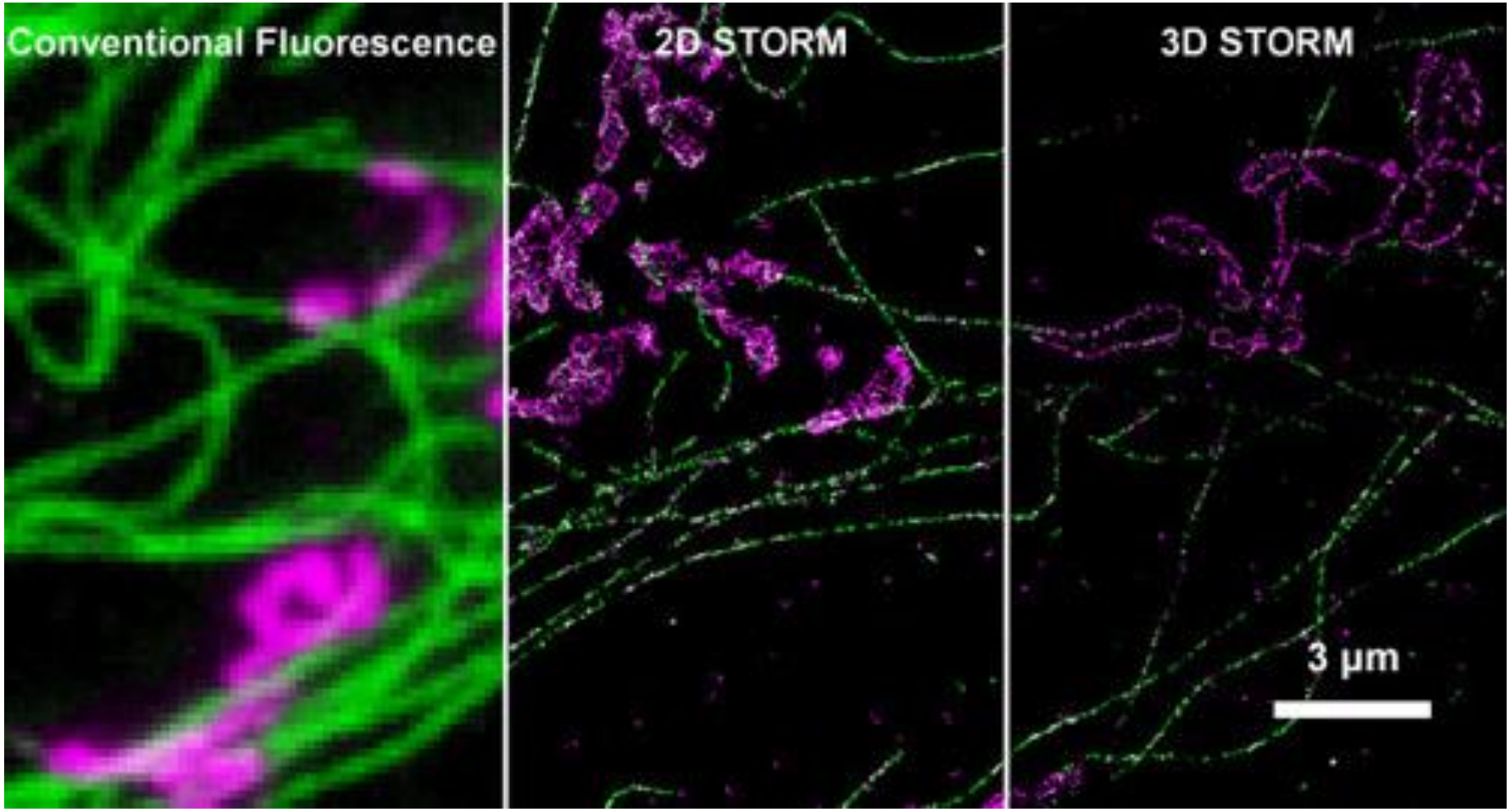


http://zeiss-campus.magnet.fsu.edu/articles/superresolution/palm/practicalaspects.html



Bates at al, Science, 2007

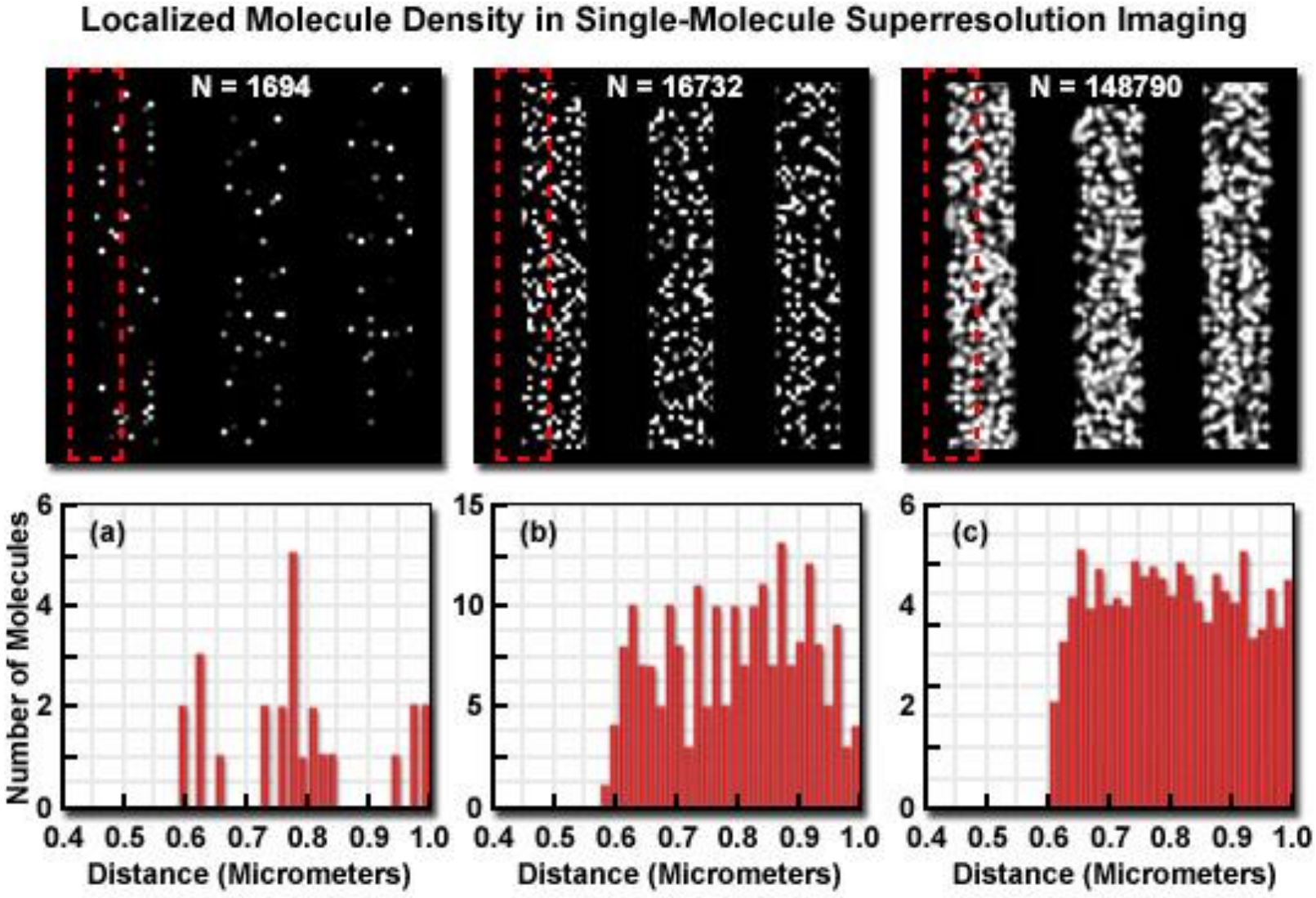
STochastic **O**ptical **R**econstruction **M**icroscopy (**STORM**)/ **P**hoto-**A**ctivated **L**ocalization **M**icroscopy (**PALM**)



Resolution depends on **LOCALIZATION PRECISION** and **MOLECULAR DENSITY** of fluorescent probes in the specimen.

http://huanglab.ucsf.edu/STORM.html

Importance of molecular density for resolution for PALM/STORM



http://zeiss-campus.magnet.fsu.edu/articles/superresolution/palm/practicalaspects.html

Single Molecule Localisation Microscopy summary

PROS:

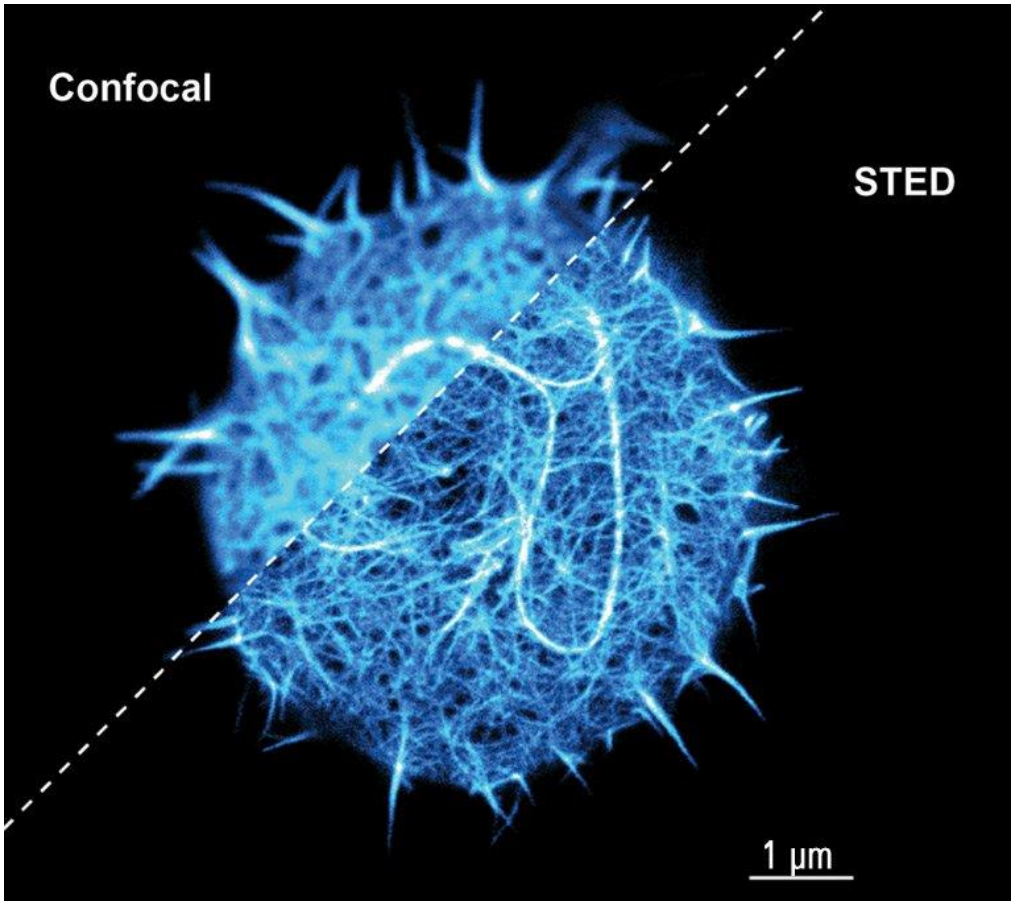
- **Outstanding resolution performance – up to 10 nm**
- **Ideal for single molecule co-localisation**
- **Quantitative data**

CONS:

- **Long acquisition times**
- **Fitting algorithms**
- **Need of specific dye**
- **Labelling density is crucial for high resolution**

STED

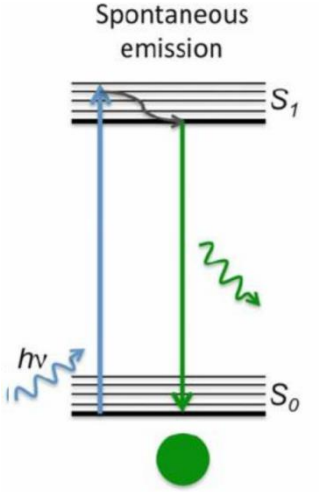
STED microscopy overview

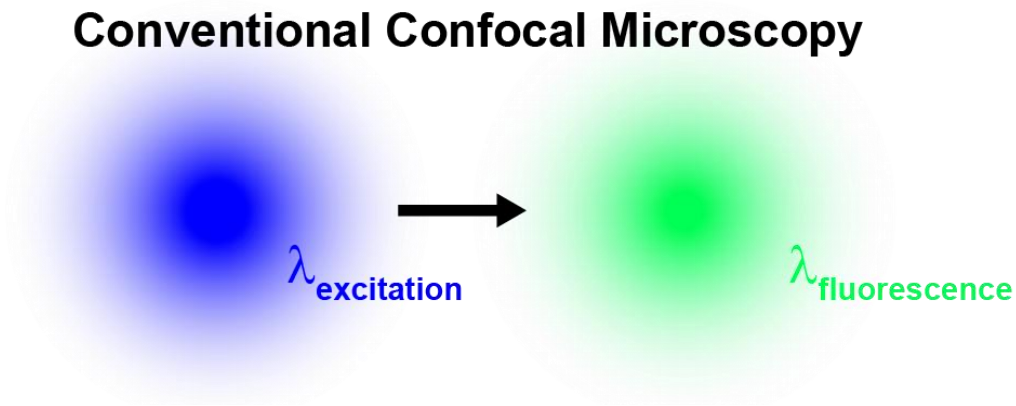


Actin filaments in resting T-cell taken on Leica STED system at WIMM
image courtesy M.Fritzsche

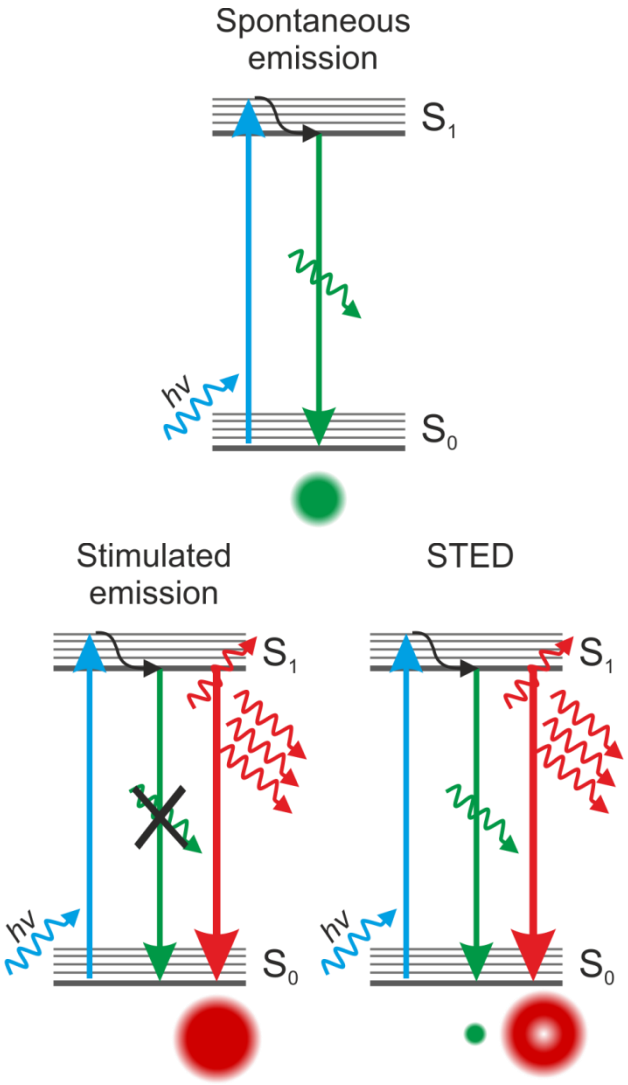
- Confocal microscopy based super-resolution technique (laser scanning)
- Reduces the effective PSF dimensions, so resolution is only limited by sample degradation
- Does not require subsequent image reconstruction

STimulated **E**mission **D**epletion (STED) Microscopy principle

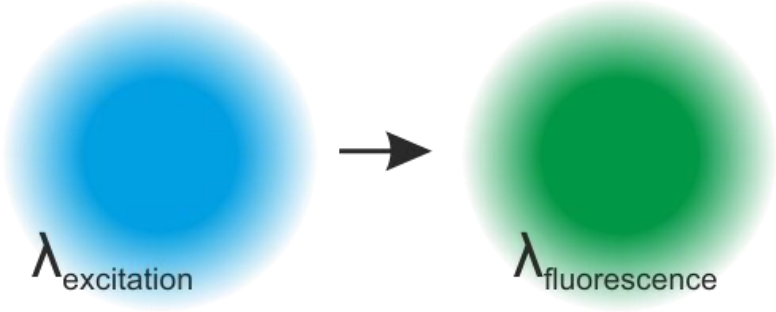




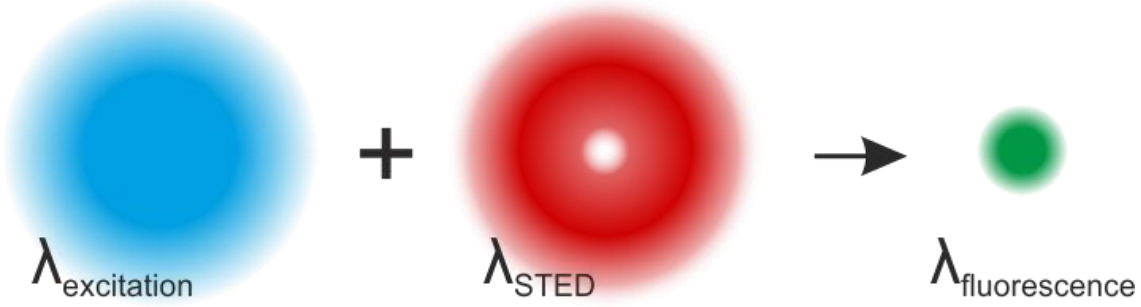
STimulated **E**mission **D**epletion (STED) Microscopy principle



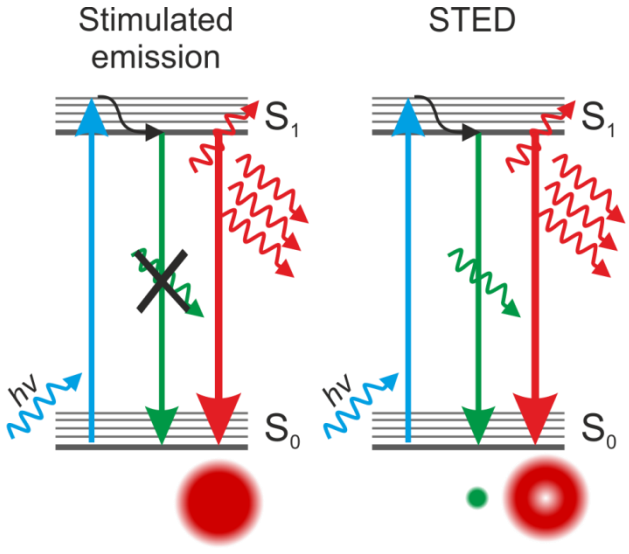
Conventional fluorescence microscopy

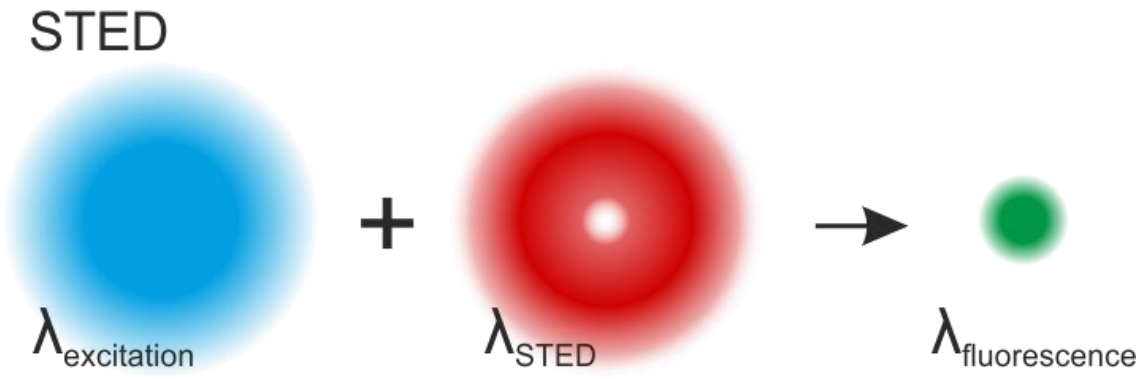


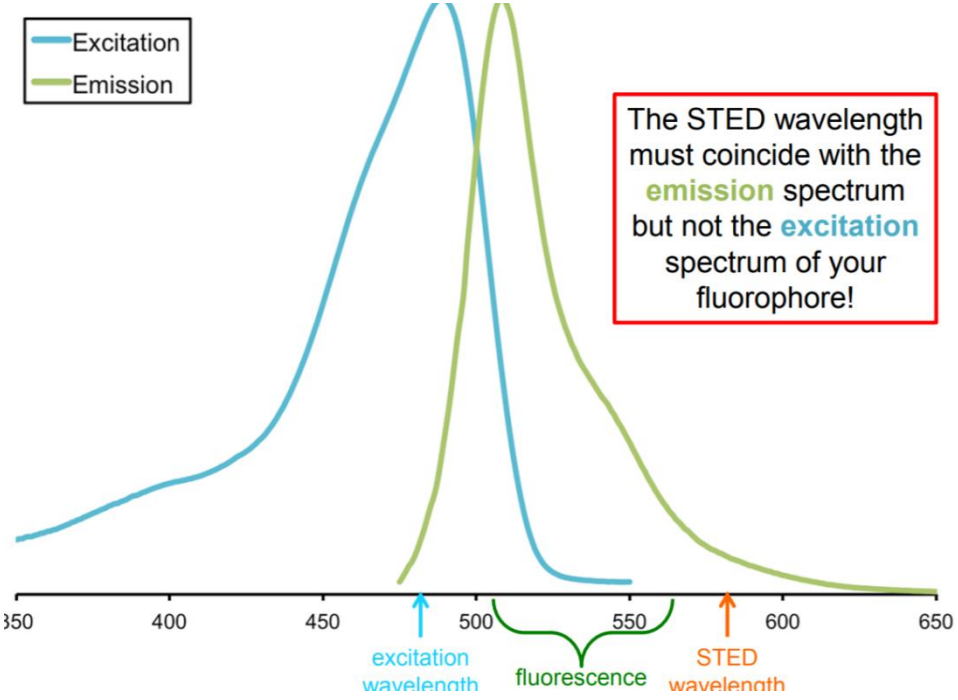
STED



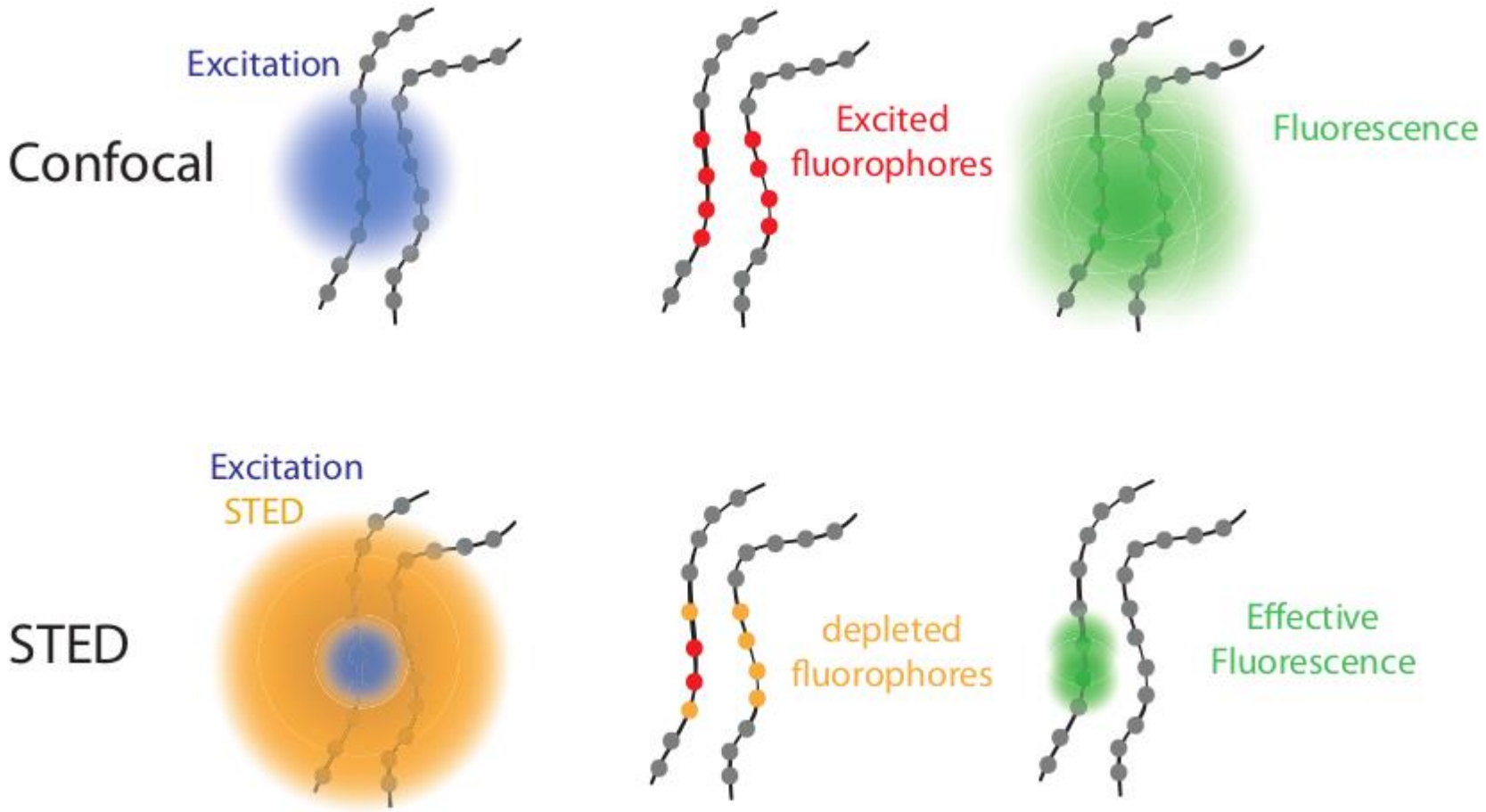
STimulated **E**mission **D**epletion (STED) Microscopy principle





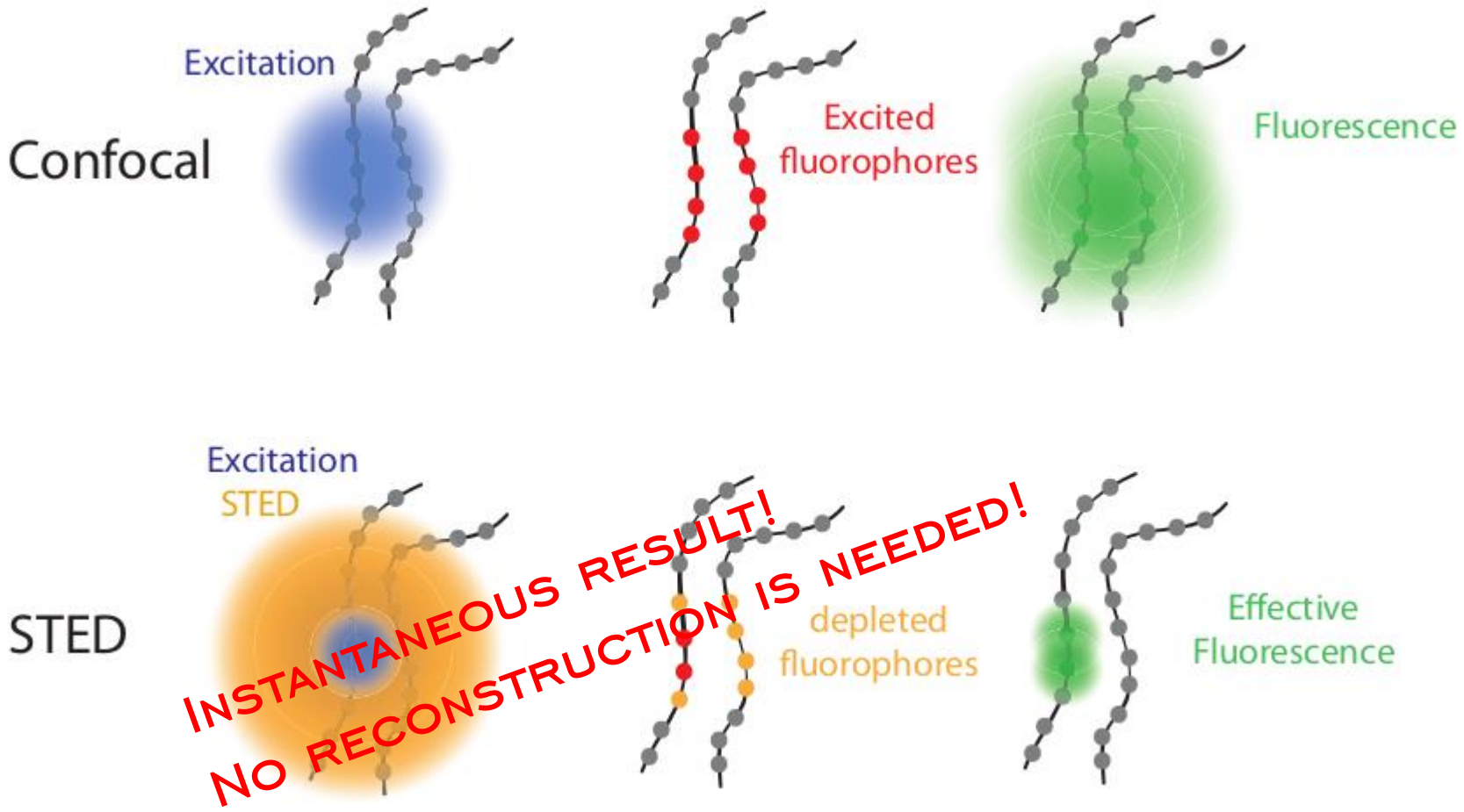


STimulated **E**mission **D**epletion (STED) Microscopy principle



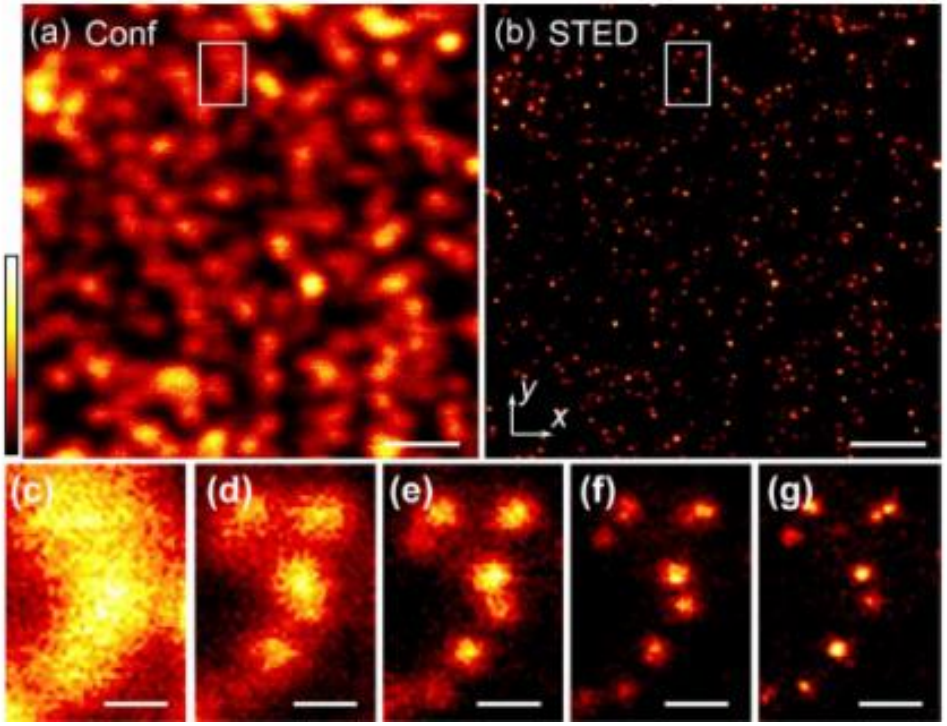
https://svi.nl/STEDMicroscopy

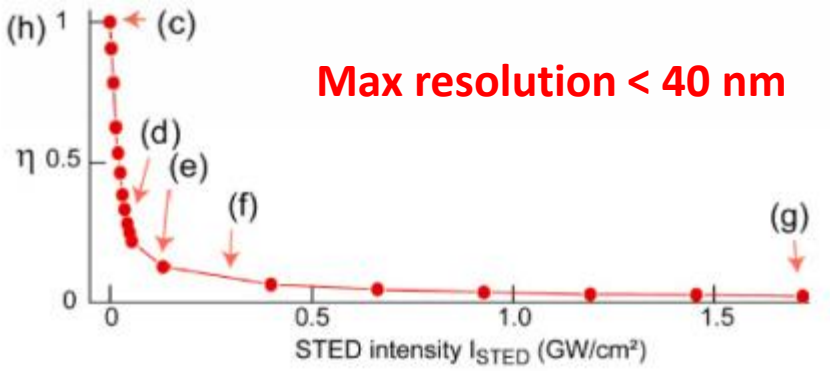
STimulated **E**mission **D**epletion (STED) Microscopy principle



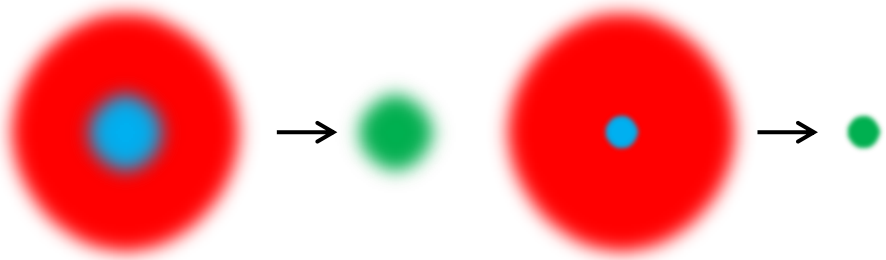
https://svi.nl/STEDMicroscopy

Resolution in STED microscopy





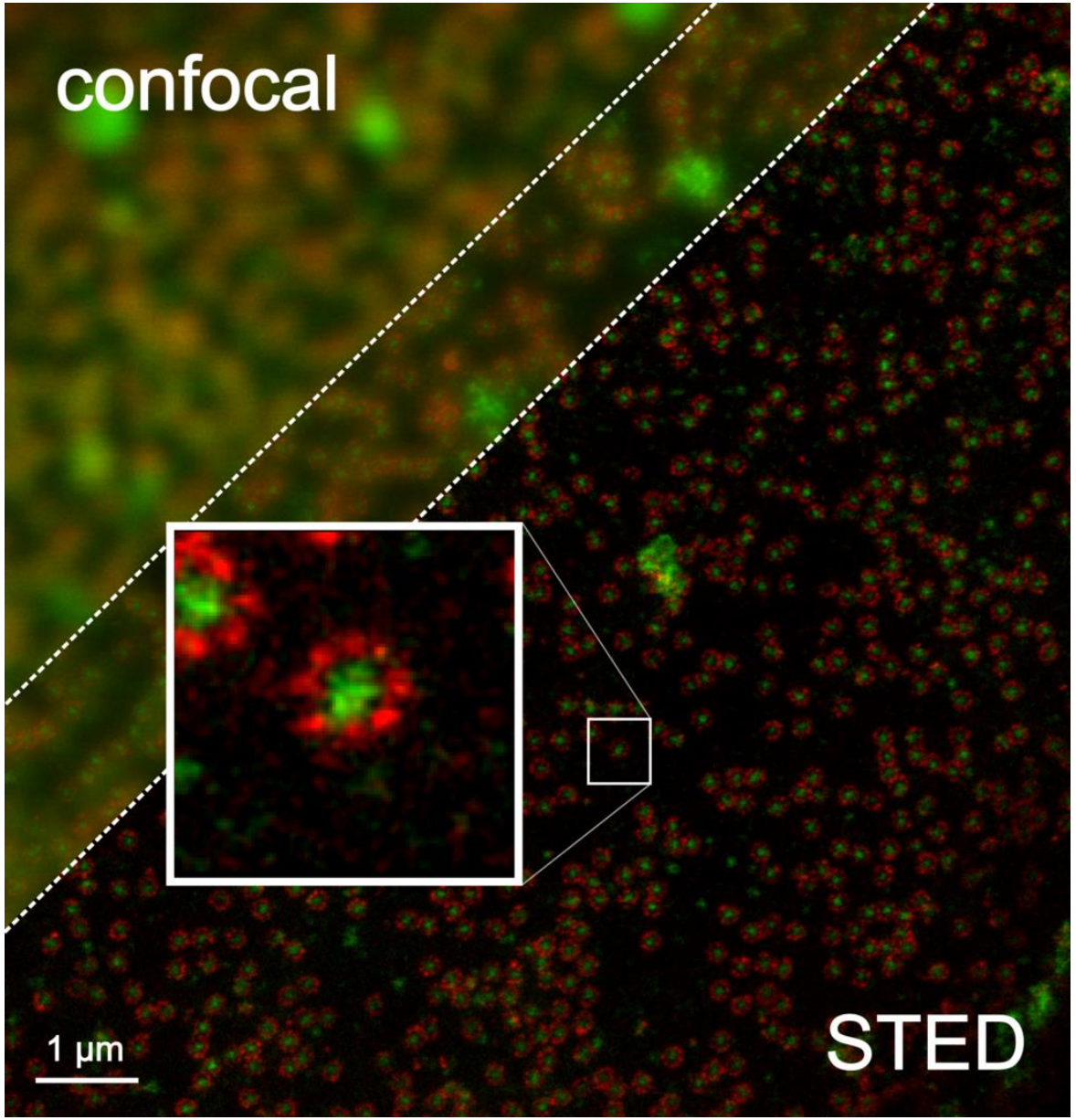
Max resolution < 40 nm



Resolution is defined by the STED beam intensity!

Harke et al 2008, Optics Express

STED image example



https://en.wikipedia.org/wiki/STED_microscopy

STimulated **E**mission **D**epletion (STED) summary

PROS:

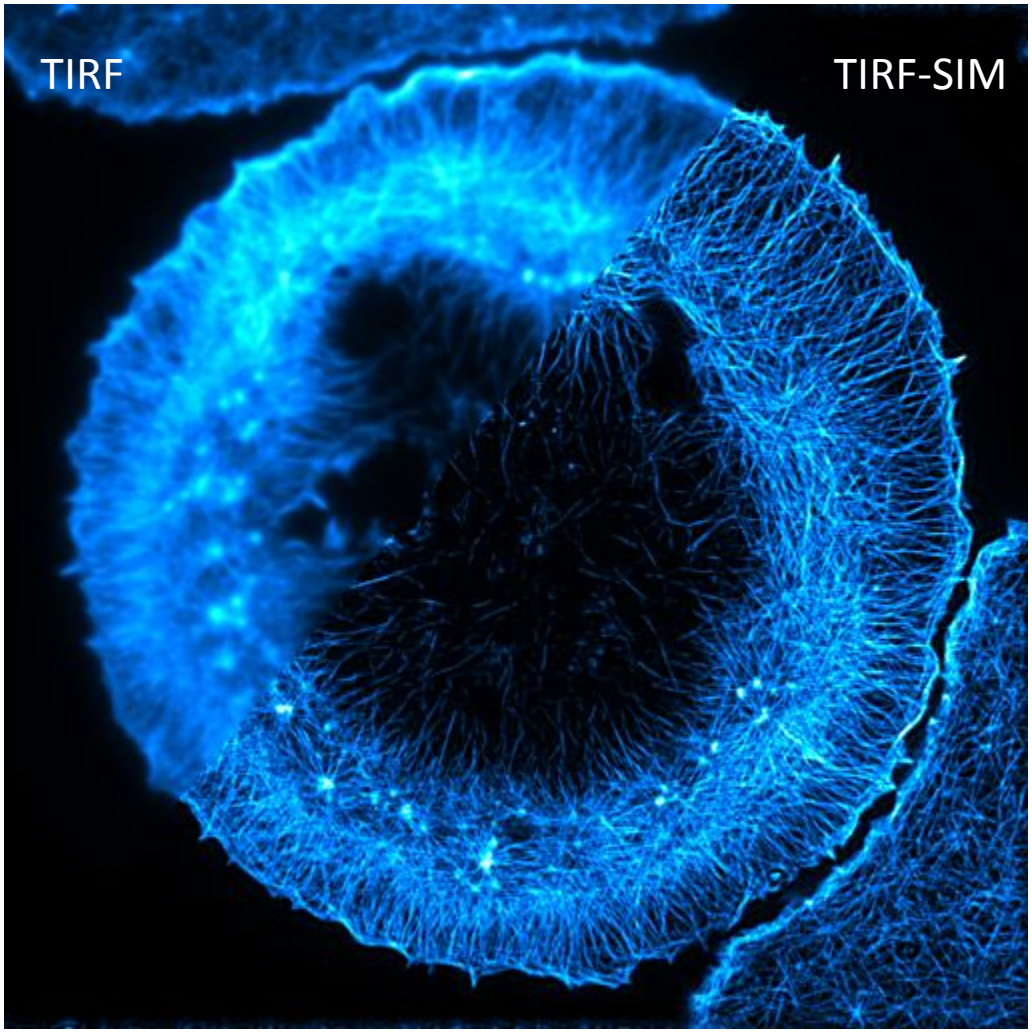
- **Confocal based – good penetration depth, 3D imaging**
- **Resolution can be tuned by STED beam power**
- **No image post processing is needed**

CONS:

- **High resolution require high laser intensity – not suitable for prolonged imaging**
- **Small improvement in z-resoluton compare with confocal**
- **Beams alignment is critical**

Structured Illumination microscopy

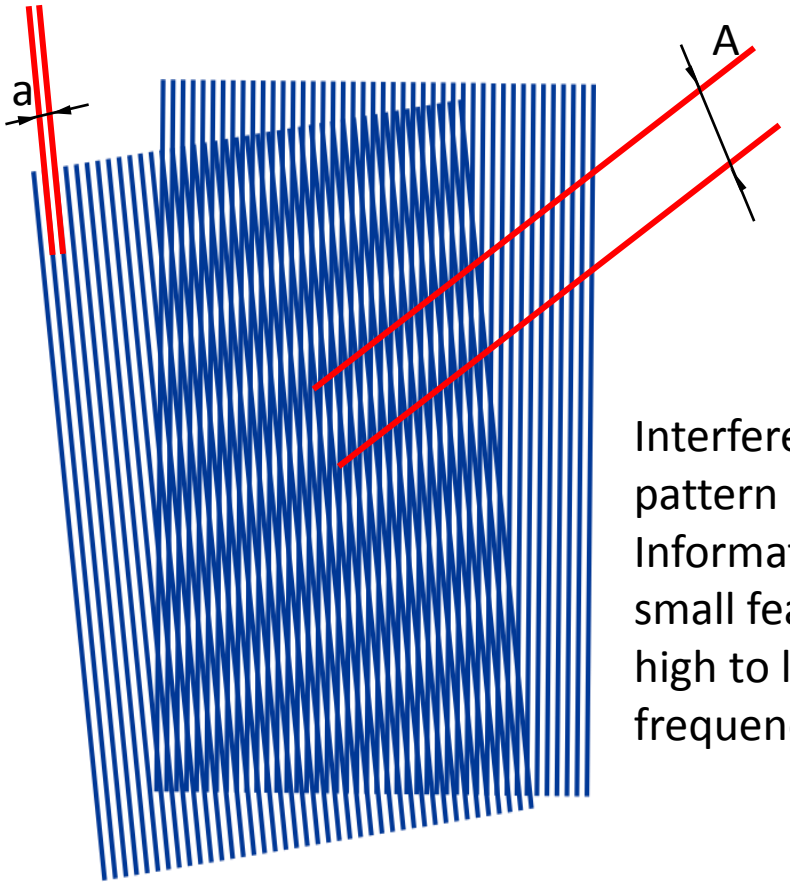
SIM microscopy overview



Actin filaments in activation RBL taken on custom built TIRF-SIM system at KIR

- Wide field technique (camera acquisition)
- Resolution is limited by the ¼ of imaging wavelength
- Requires careful image reconstruction

Moiré Interference

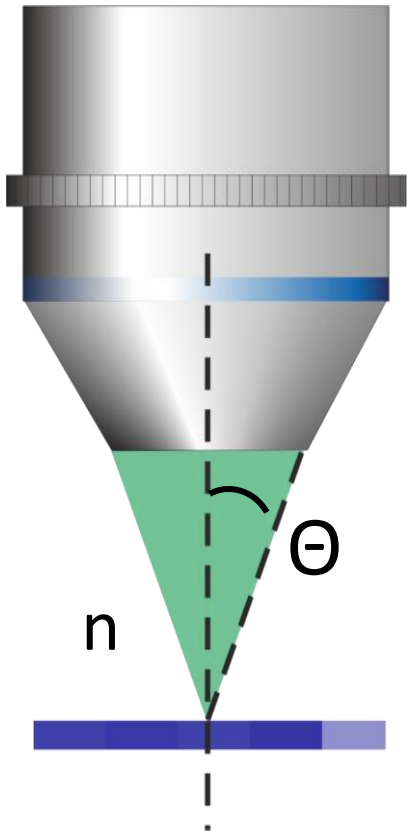


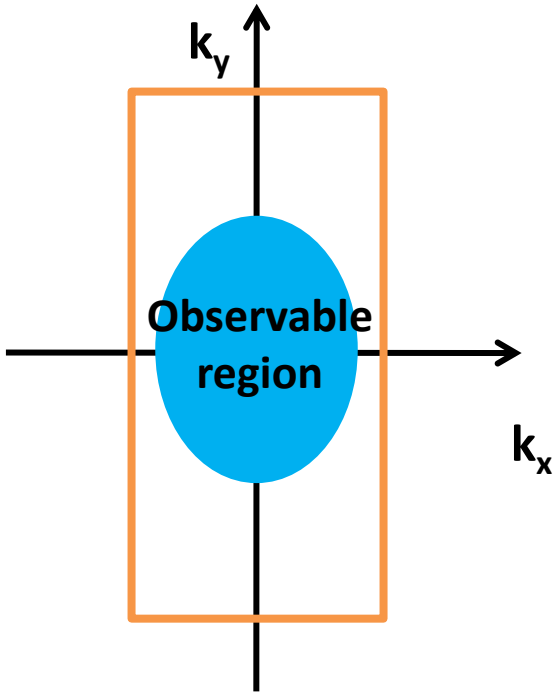
Interference pattern brings Information about small features from high to low frequency domain

a << A

Numerical Aperture (NA) of objective and resolution

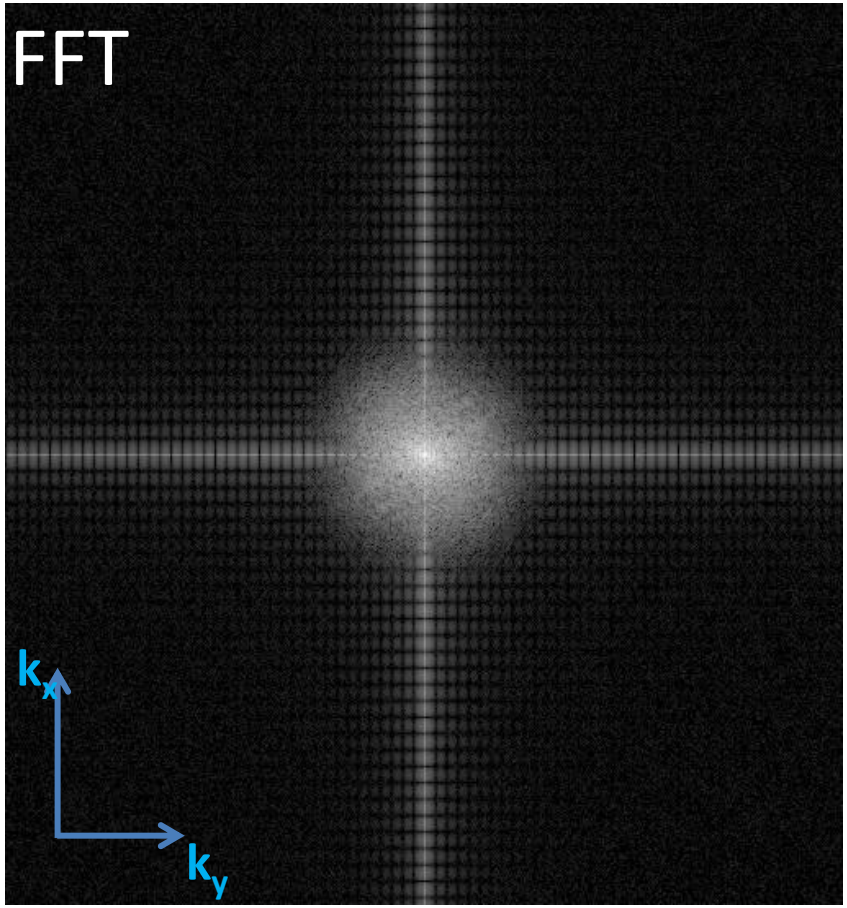
$$d = \frac{\lambda}{2n\sin\theta} = \frac{\lambda}{2\mathrm{NA}}$$





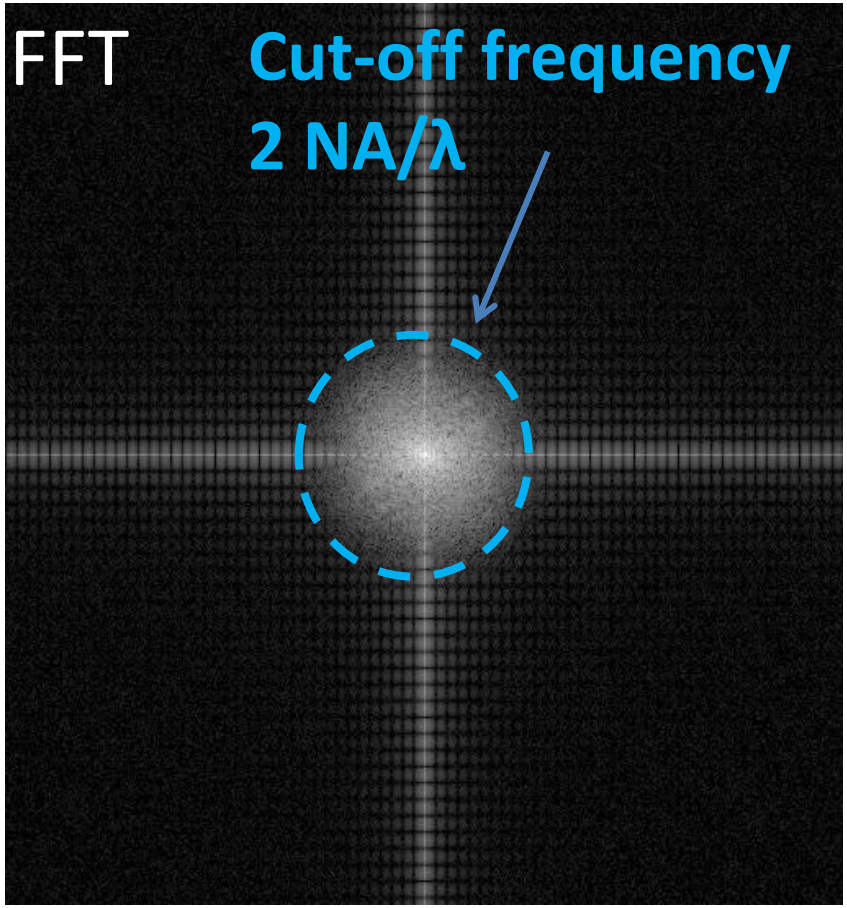
Real space (x,y) $\xrightarrow{\text{FFT}}$ Frequency space (k_x,k_y)

Low NA



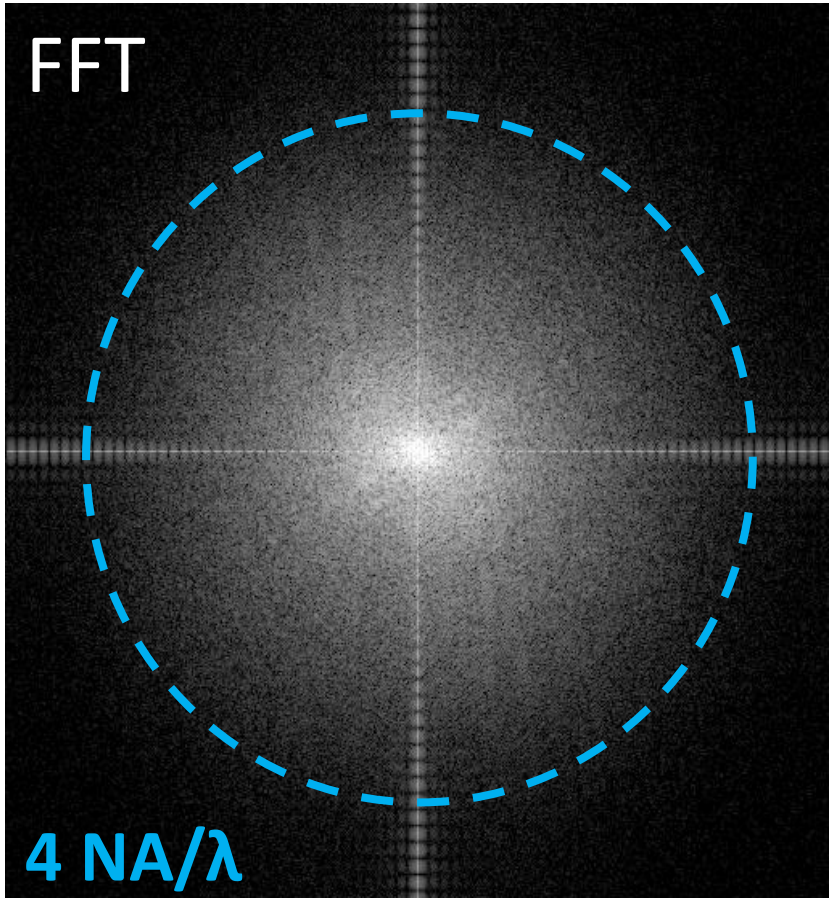
Real space (x,y) $\xrightarrow{\text{FFT}}$ Frequency space (k_x,k_y)

Low NA

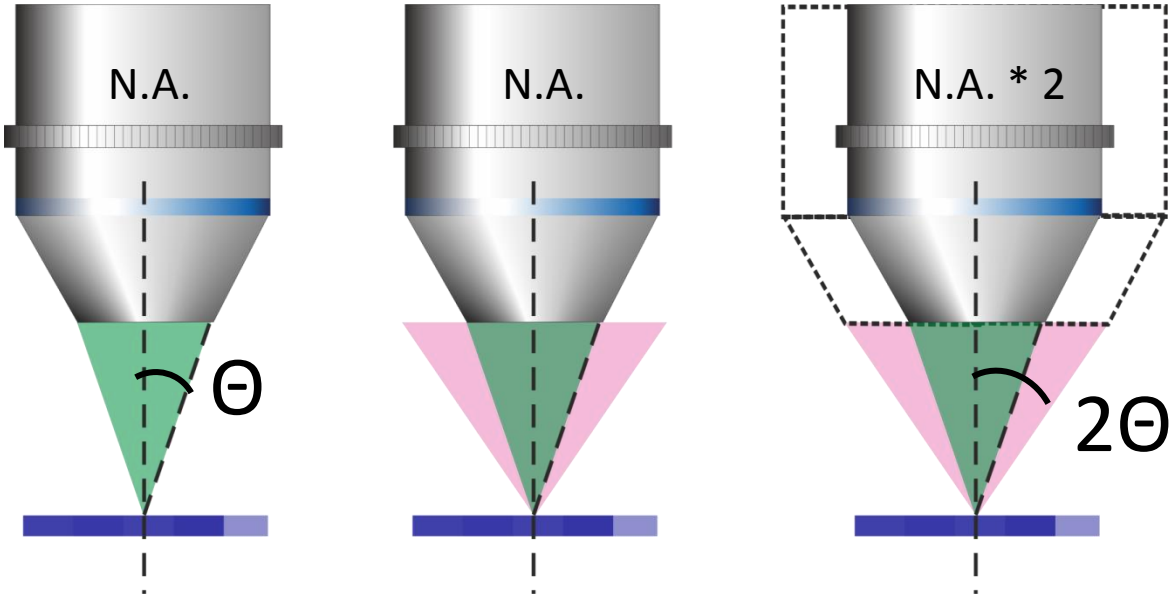


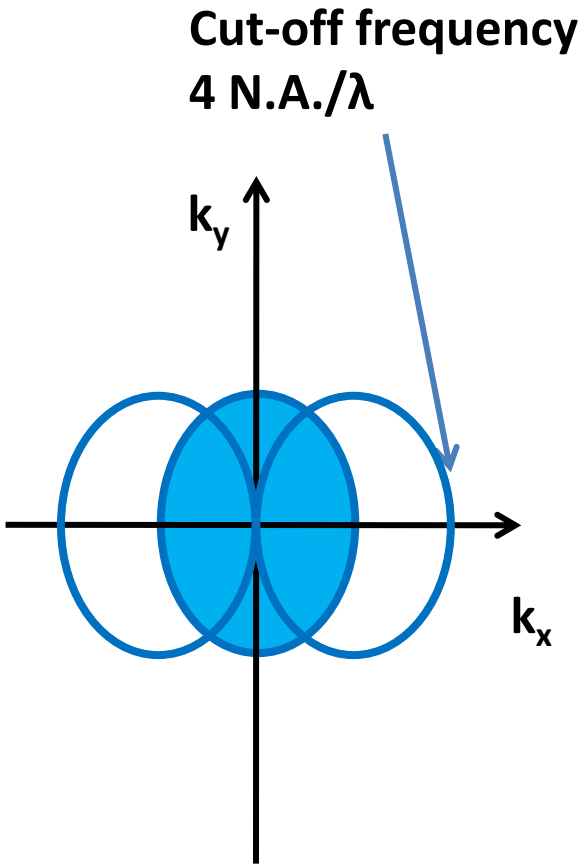
Real space (x,y) $\xrightarrow{\text{FFT}}$ Frequency space (k_x,k_y)

High
NA



Structured Illumination doubles the resolution





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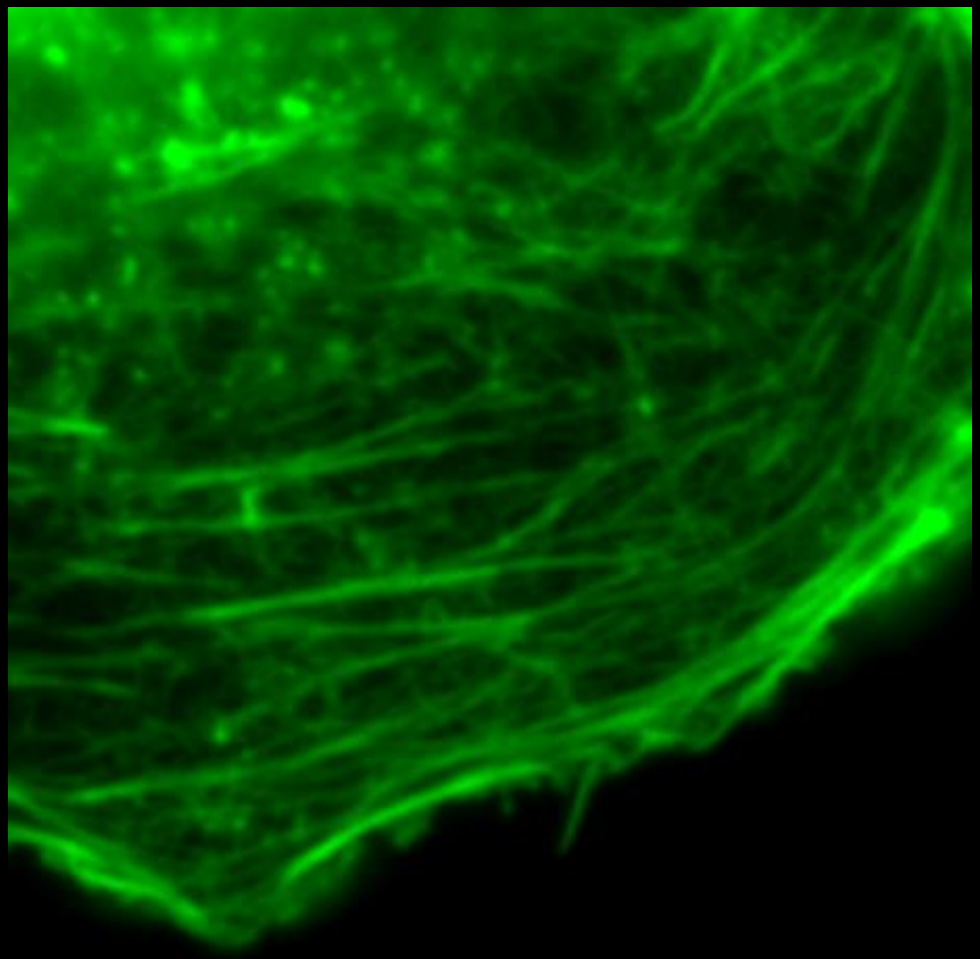
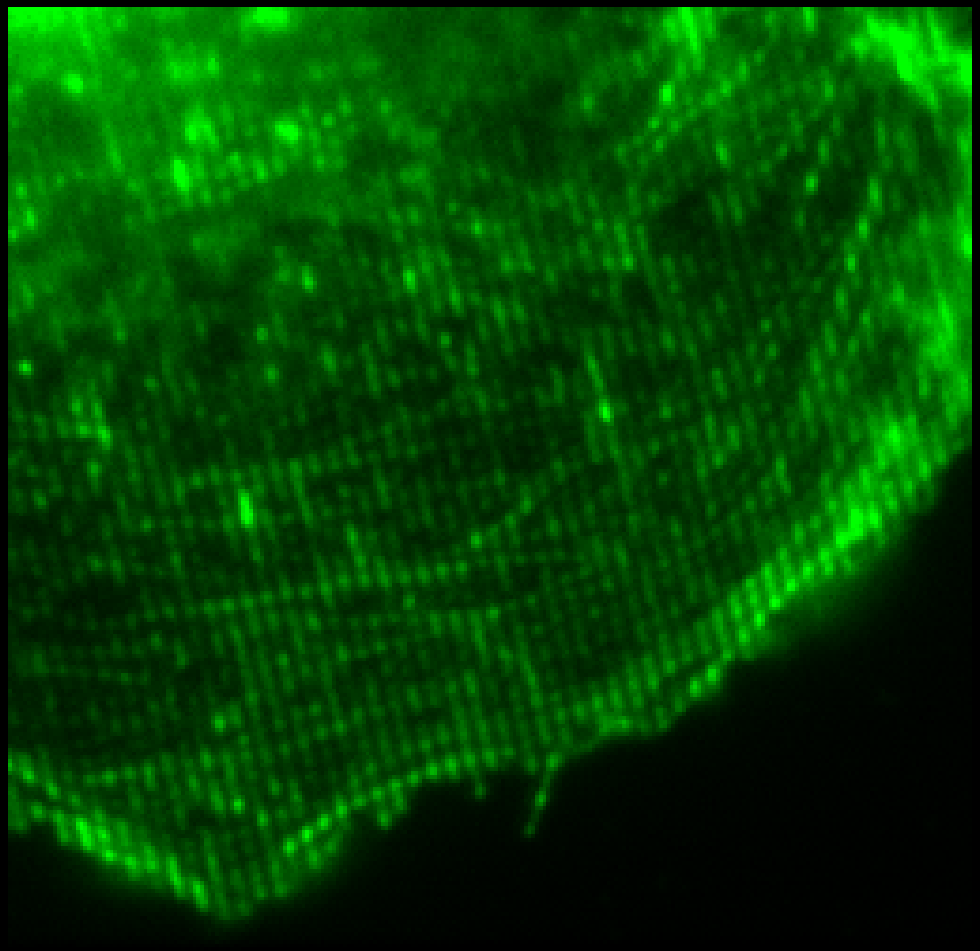
Widefield

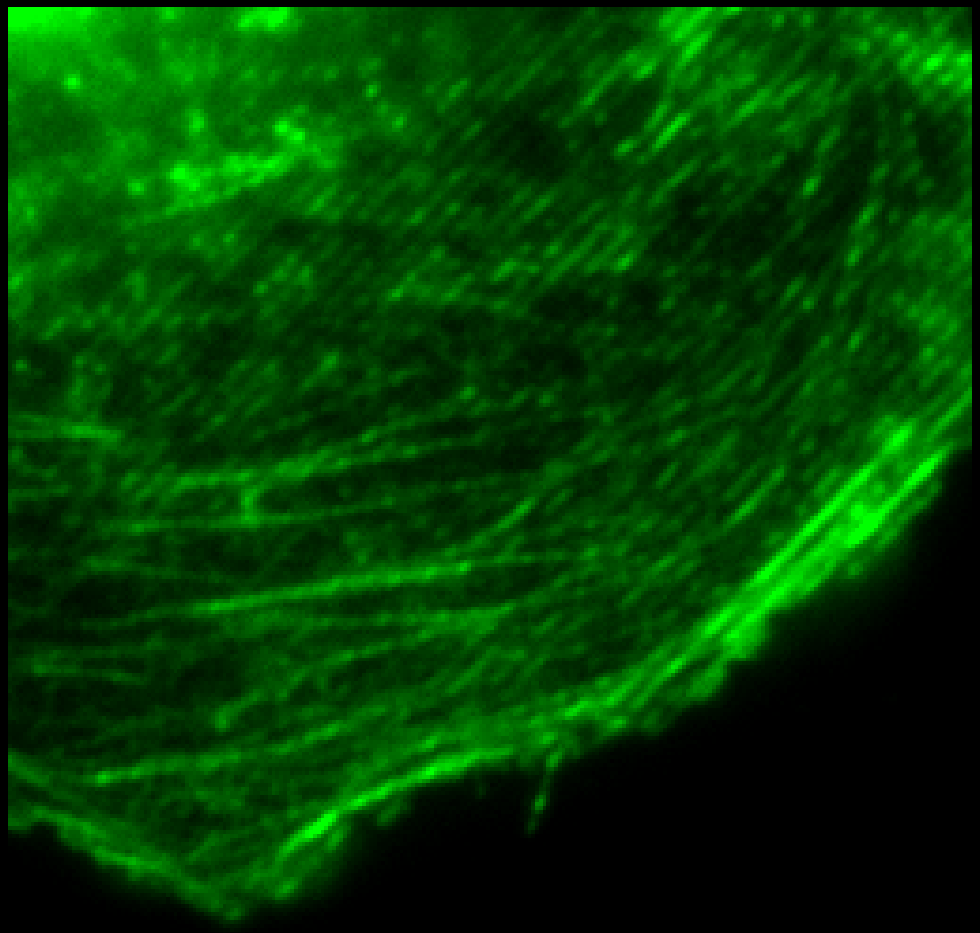


Sample image from https://fairsim.github.io/

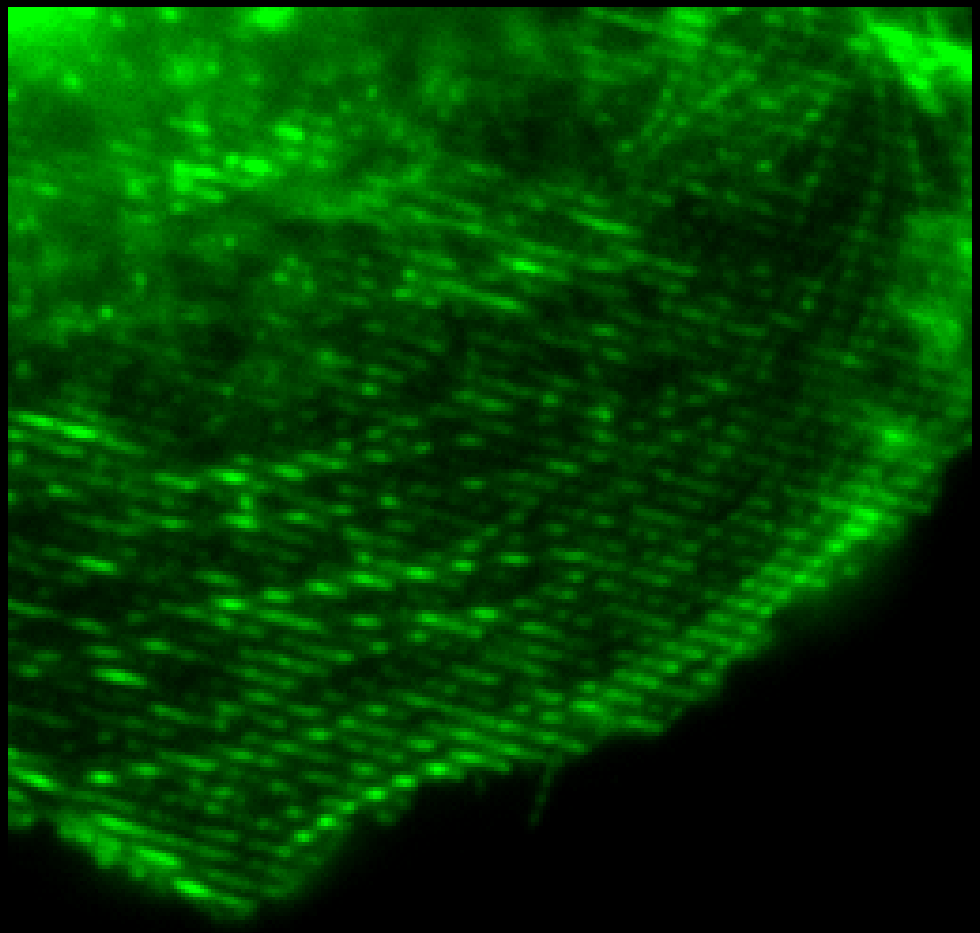
SIM
angle 1



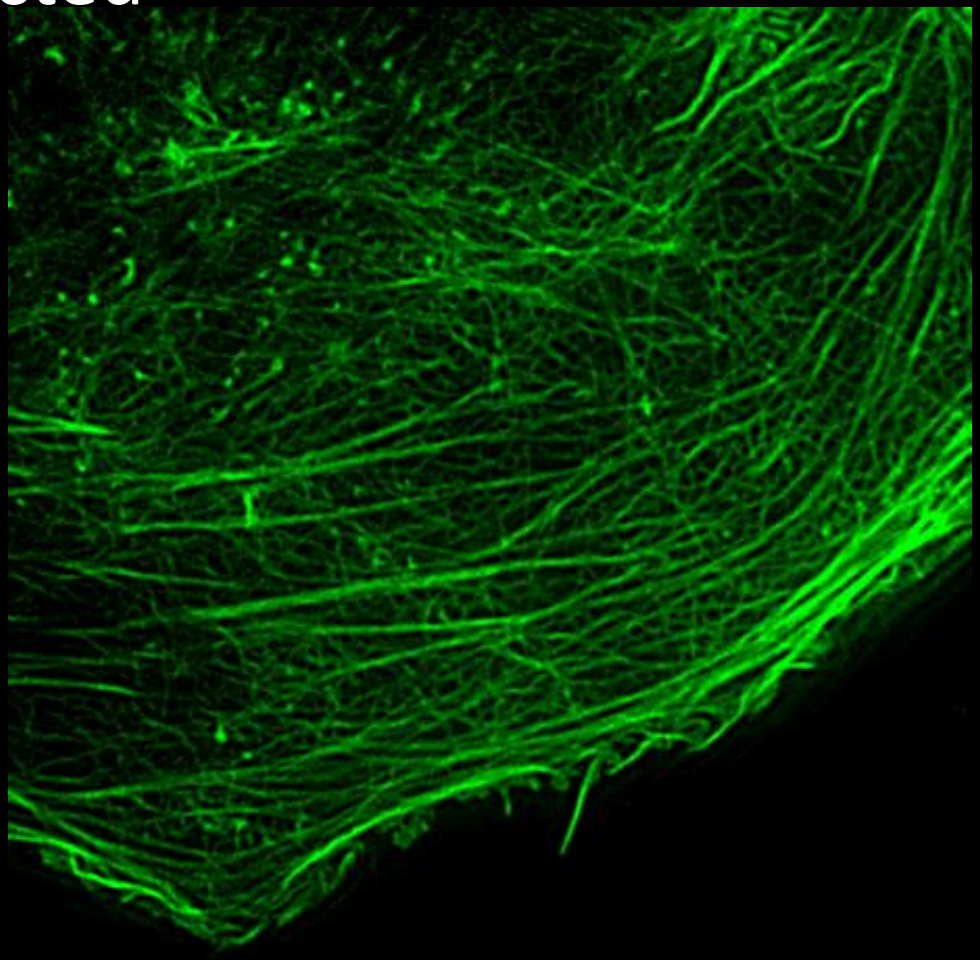
SIM
angle 2

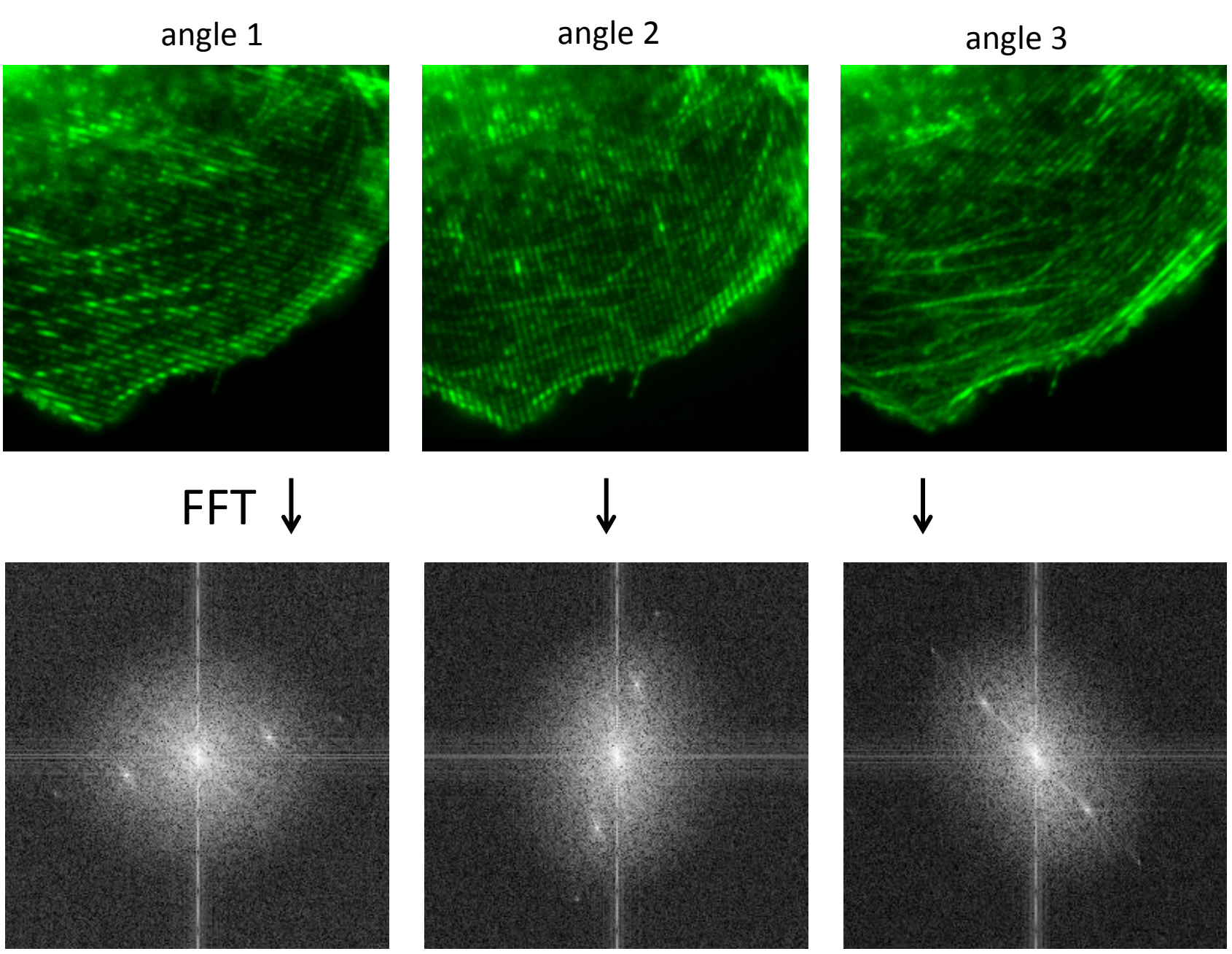


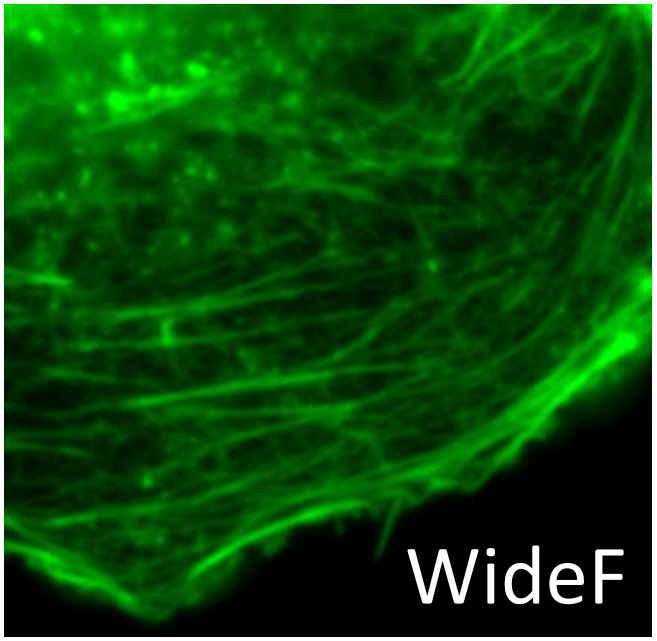
SIM
angle 3

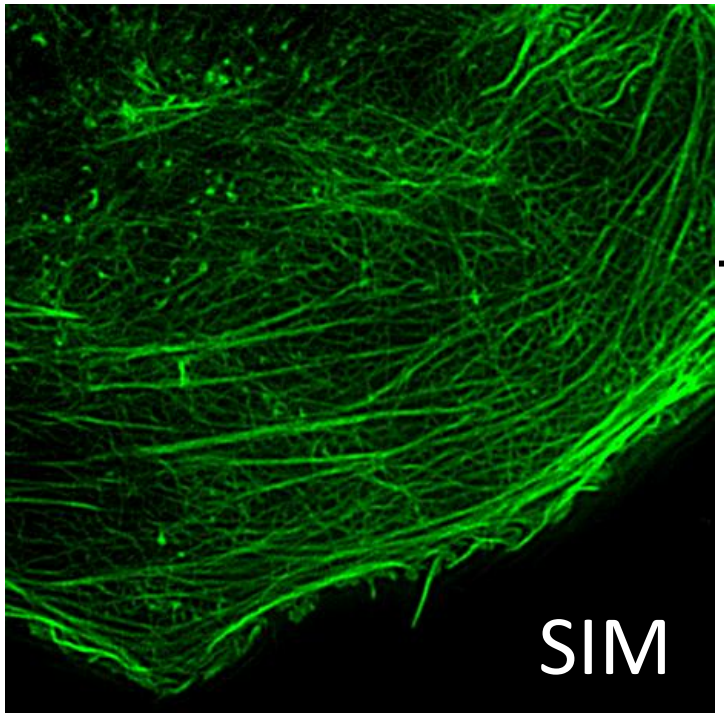


SIM
reconstructed



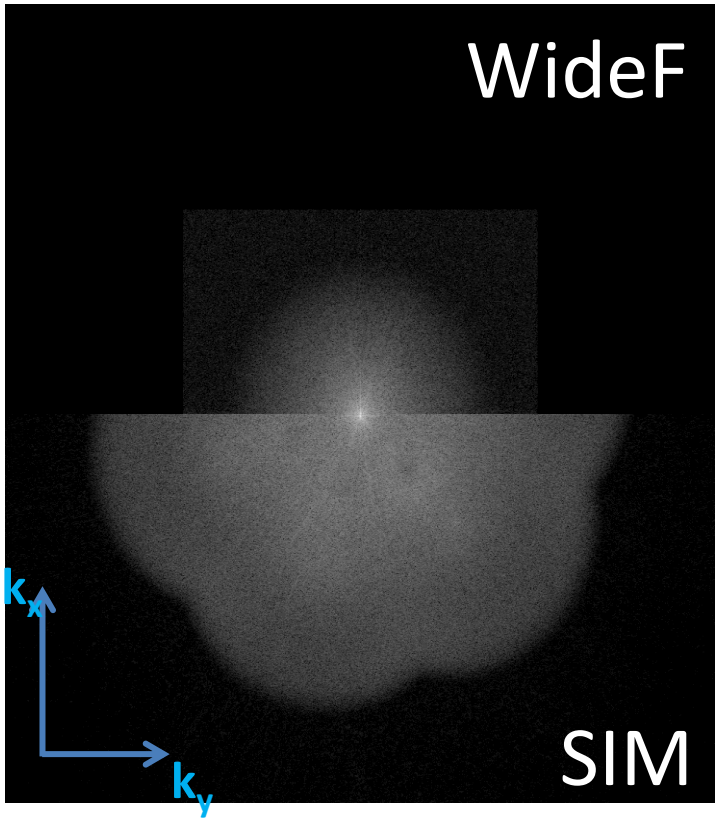

angle 1
angle 2
angle 3
FFT



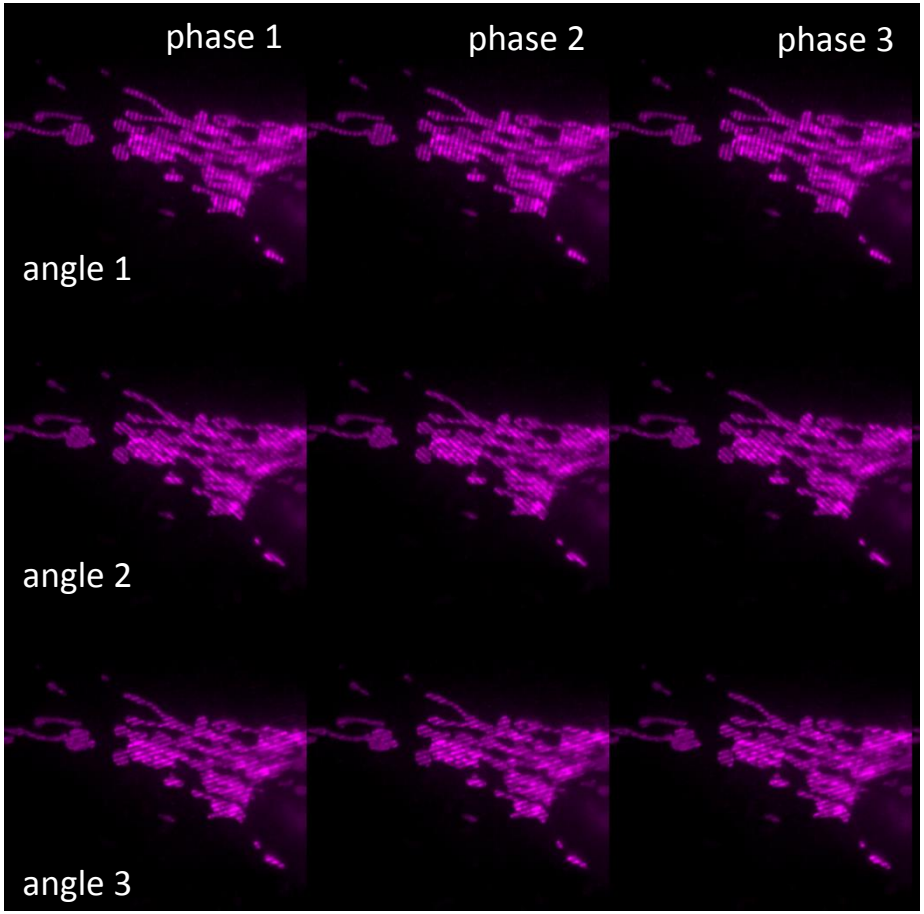


FFT

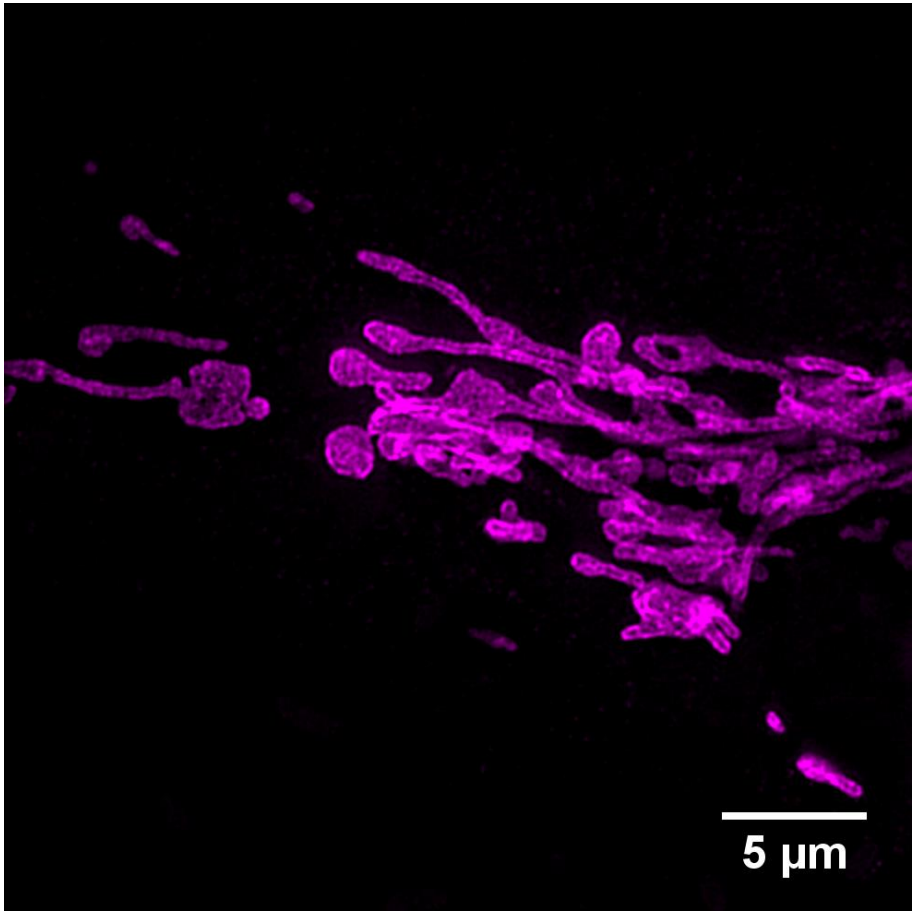
Cut-off frequency



SIM raw data



SIM reconstructed



Structured Illumination Microscopy summary

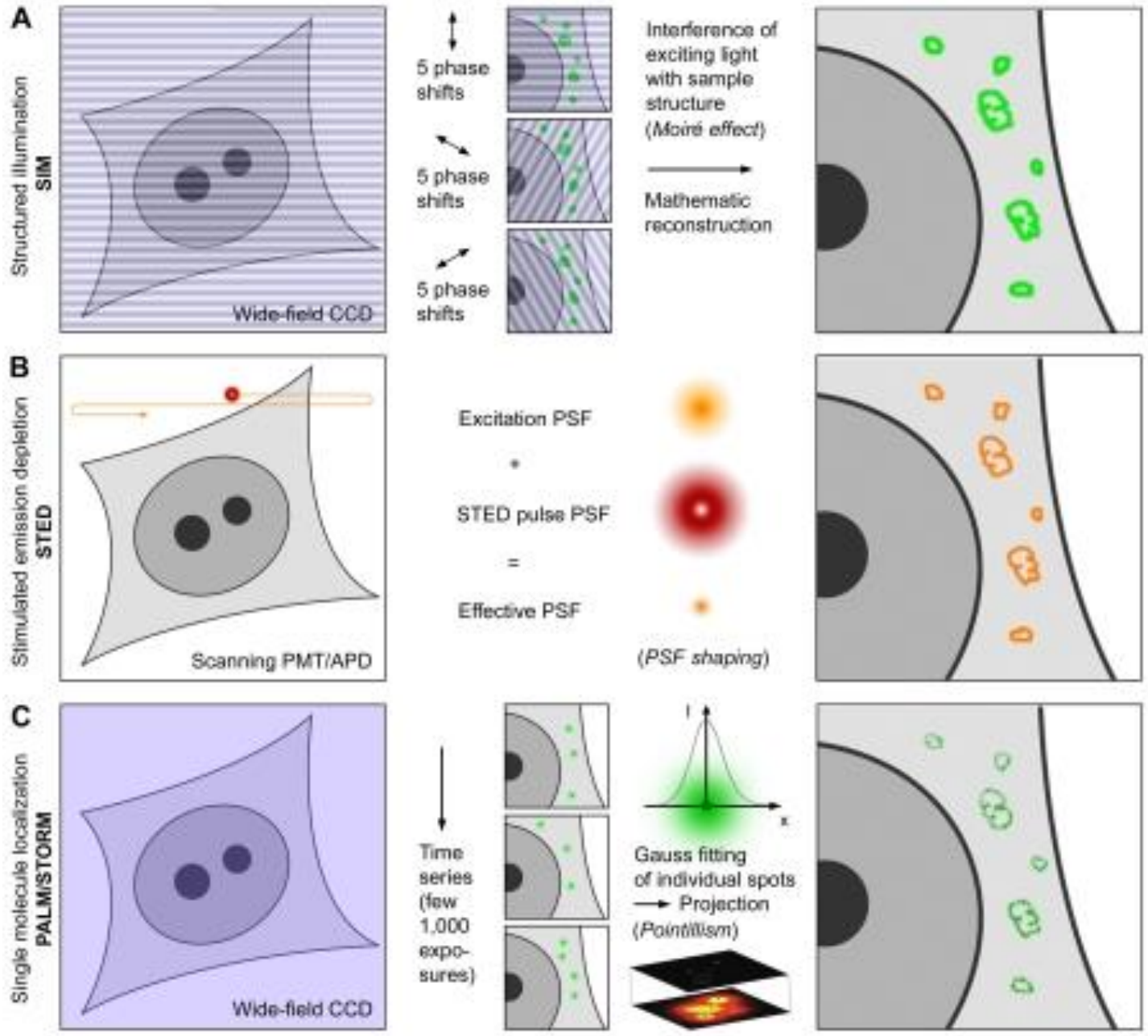
PROS:

- **Wide field based approach – very fast acquisition times, ideal for life cell imaging**
- **Low illumination powers**
- **No need for specific dye**

CONS:

- **Post processing is necessary**
- **Only moderate resolution improvement up to 90nm**

Summary



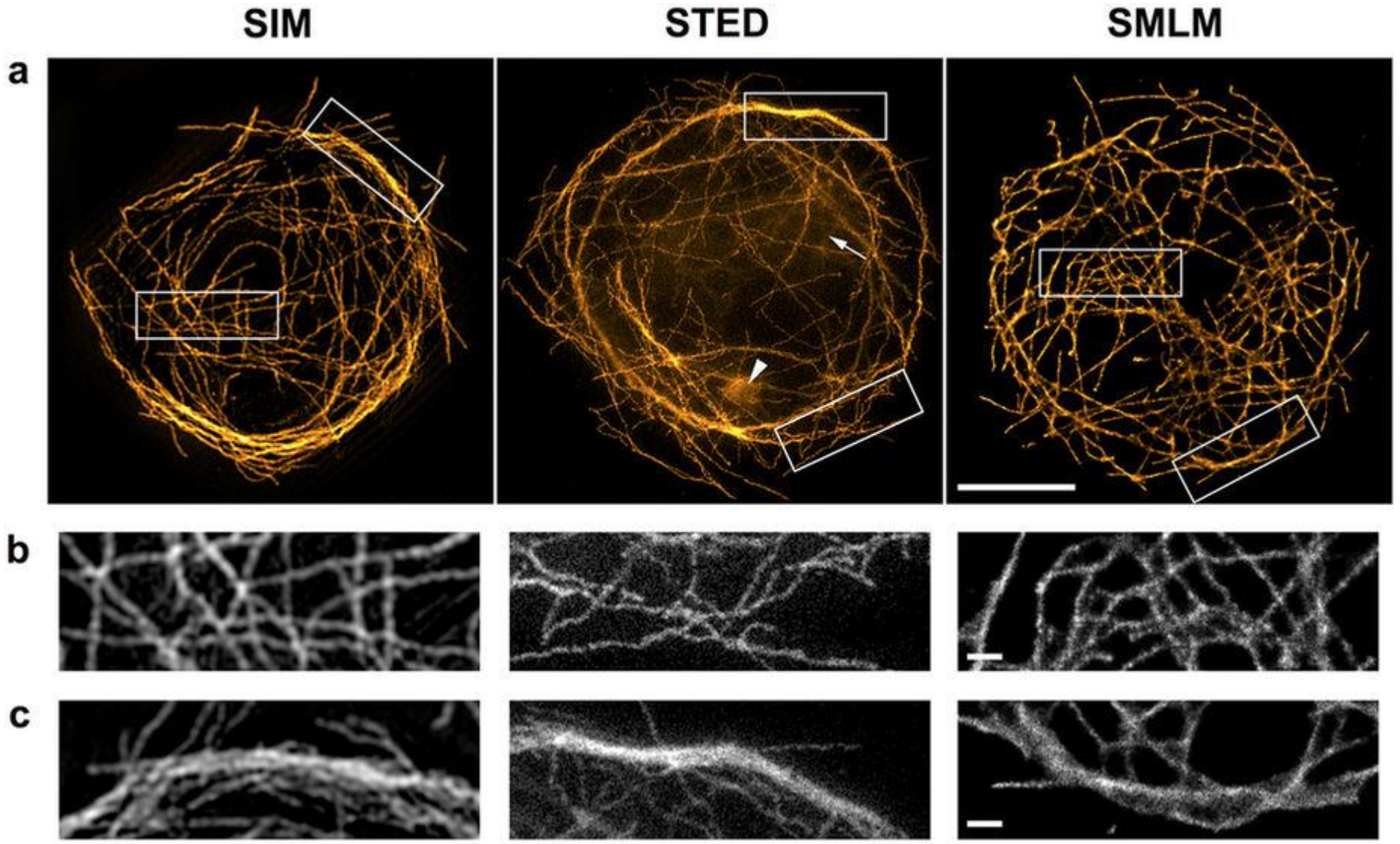
resolution
<100nm
Speed
<1-2s
Power
W/cm²

resolution
<10nm
speed
~ min
Power
MW/cm²

resolution
<10nm
speed
~ min
Power
kW/cm²

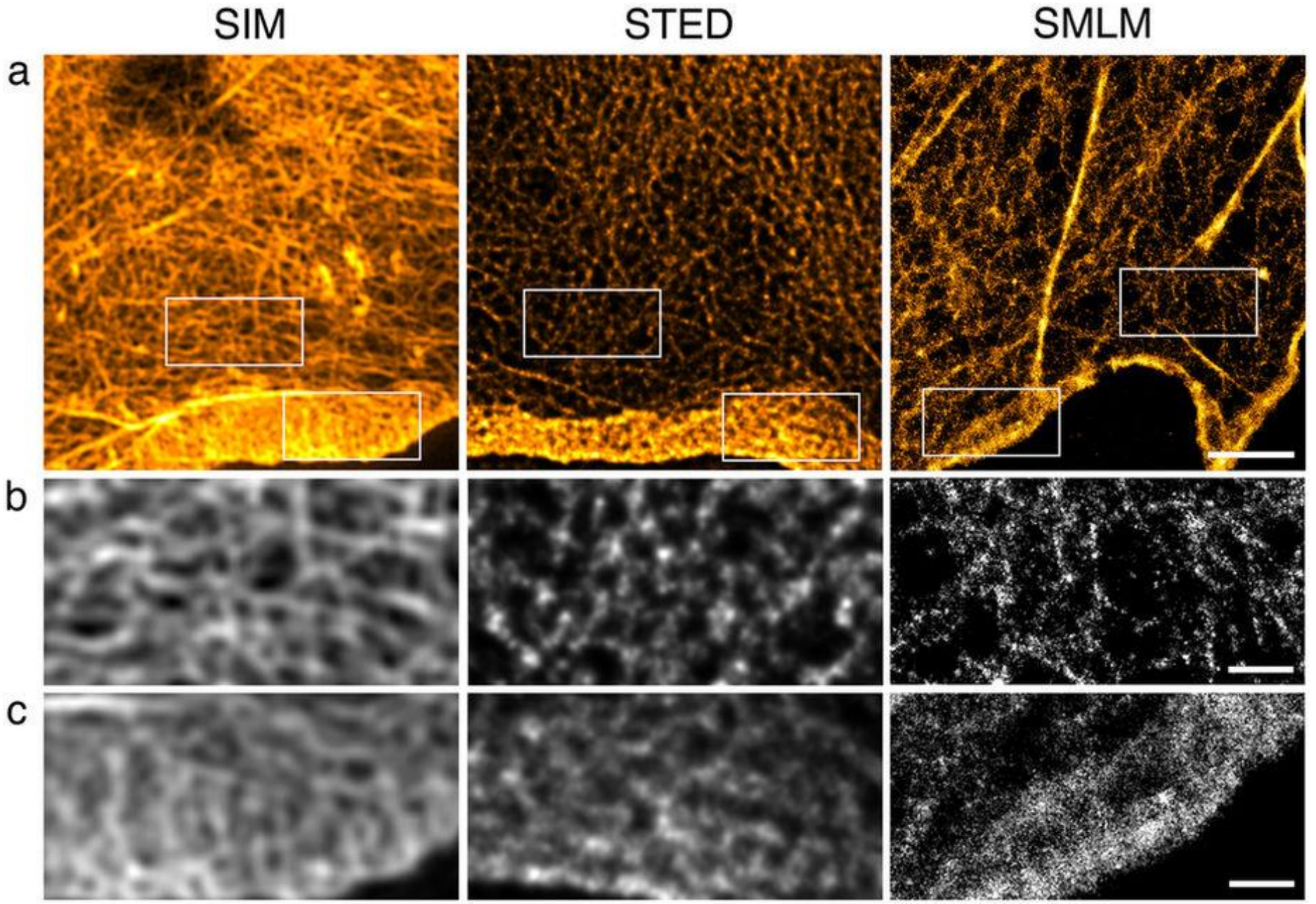
Schermelleh at al. A guide to super-resolution fluorescence microscopy. J Cell Biol. 2010 Jul 26;190(2):165-75

Microtubules in Drosophila macrophages



Wegel et. al., Scientific Reports **6**, : 27290 (2016)

Actin in COS7 cells



Wegel et. al., Scientific Reports **6**, : 27290 (2016)

Comparison of the three super-resolution techniques

	SIM	STED	SMLM
Resolution	🔴	🟡	🟢
Separated structures	🟢	🟢	🟢
Densely packed 3D structures	🟢	🟡	🔴 ?
Unknown structures	🟡	🟡	🟡
Image reconstruction issues	🔴	🟢*	🟡
Sample preparation	🟢	🟡	🟡
Ease of multi-colour imaging	🟢	🔴	🟡
Cost of purchase and system complexity	🟡	🔴	🟢

Green, good; yellow, medium; red, problematic. *The resolution gain in STED is achieved by optical methods so no reconstruction is strictly needed, however use of image contrast enhancement techniques such as deconvolution can introduce artefacts

Wegel et. al., Scientific Reports **6**, : 27290 (2016)