Single Molecule Localization Microscopy

Marie Olšinová

Superresolution in light microscopy, 13.11.2017

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EURO-BIOIMAGING CZECH-BIOIMAGING

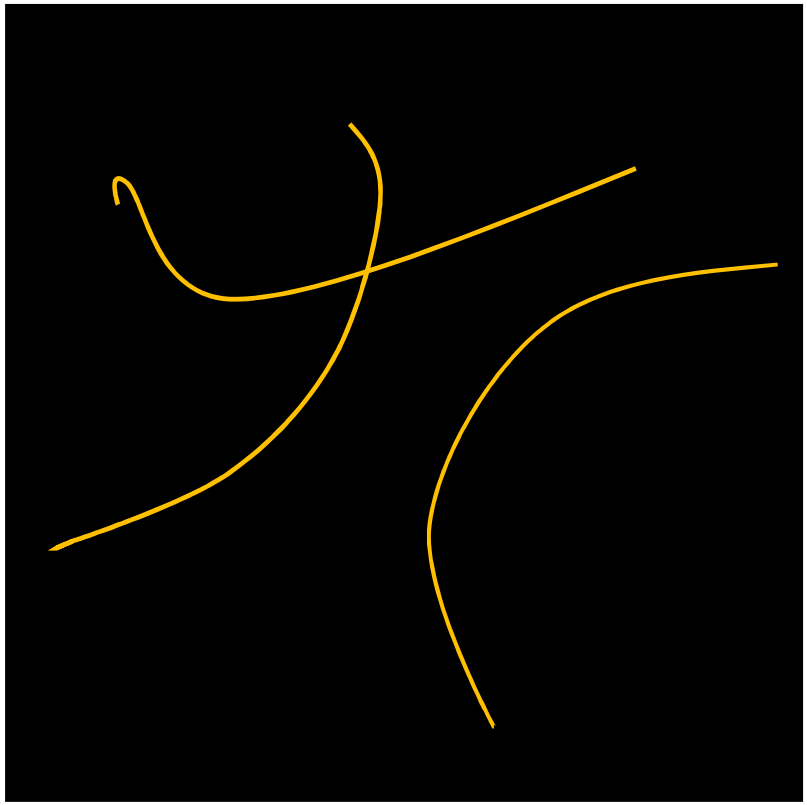
IMG

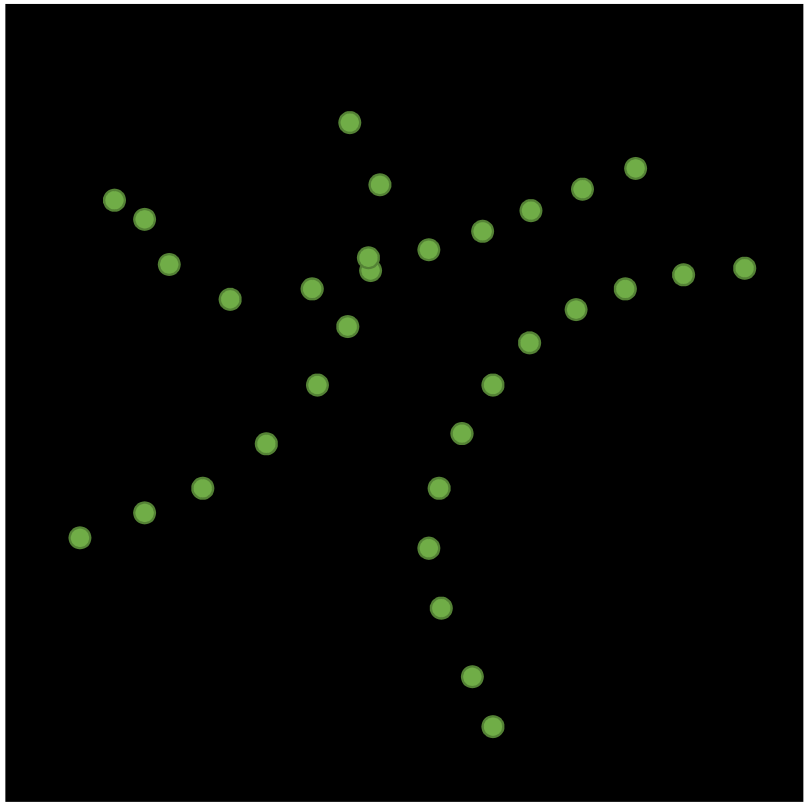
PŘÍRODOVĚDECKÁ FAKULTA Univerzita Karlova

Outline

- Principles of localization microscopy
- Requirements for switching: Fluorophores + Imaging conditions
- Labelling density, temporal resolution
- Multi-color acquisition
- 3D acquisition
- Summary

Localization microscopy: identification of individual molecules





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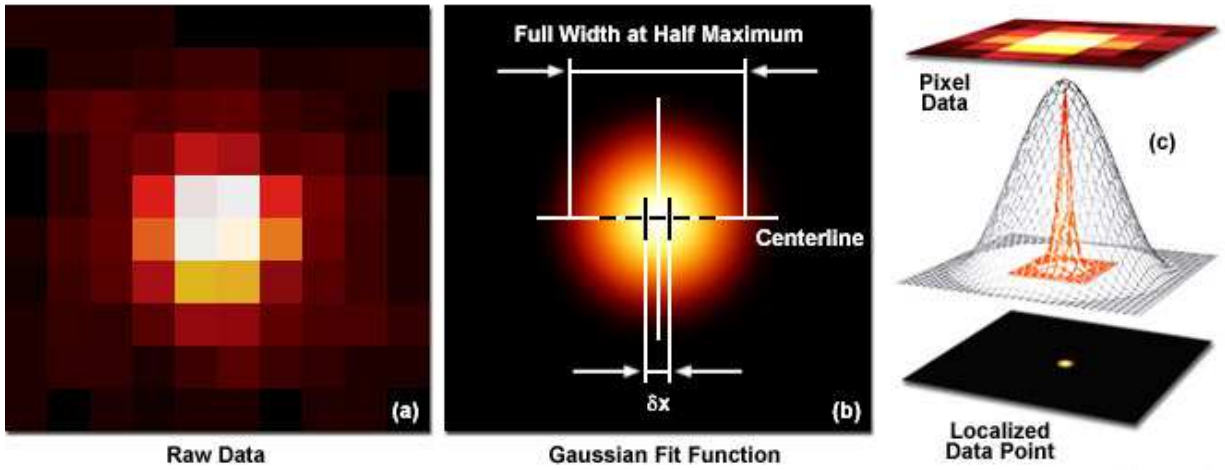
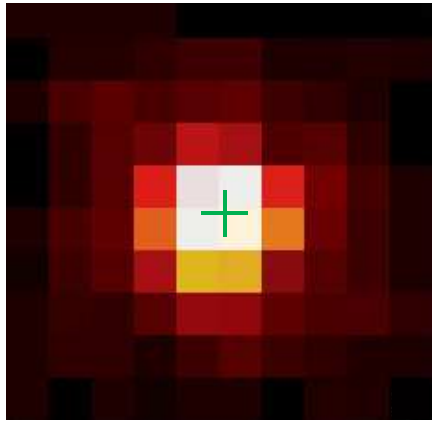
Localization microscopy: identification of individual molecules

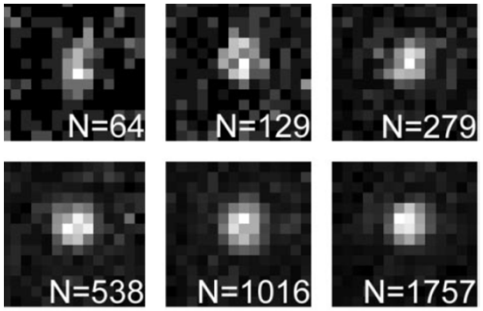




Localisation precision

$$\sigma \approx \sigma_{PSF}/\sqrt{N}$$

Isolated single molecules can be easily localized with precision depending only on signal/noise (~ 10 nm).



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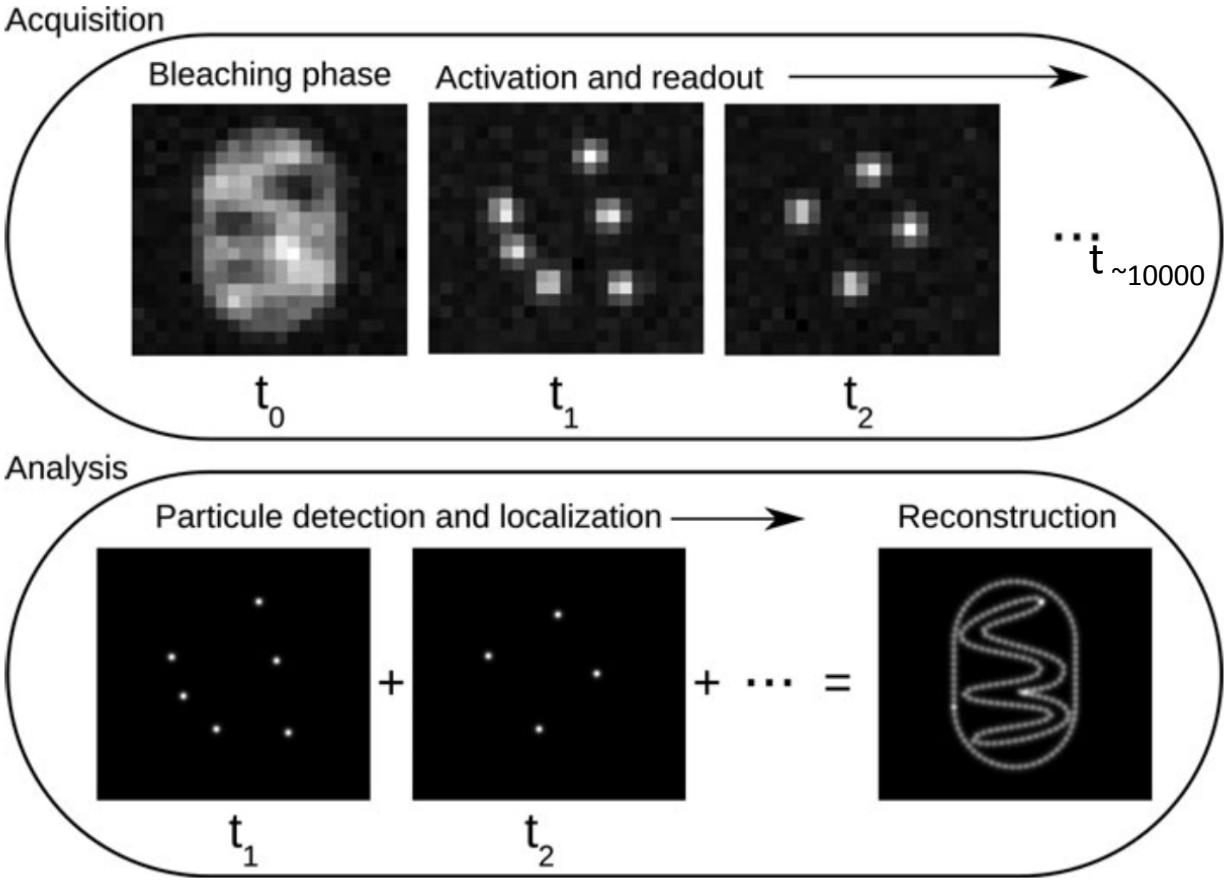
zeiss-campus.magnet.fsu.edu, Thompson et al. 2002 Biophys J

Principle: Localizing of only a small subset of emitters at a time by single molecule switching

Widefield microscopy, high laser powers, sensitive cameras and appropriate sample



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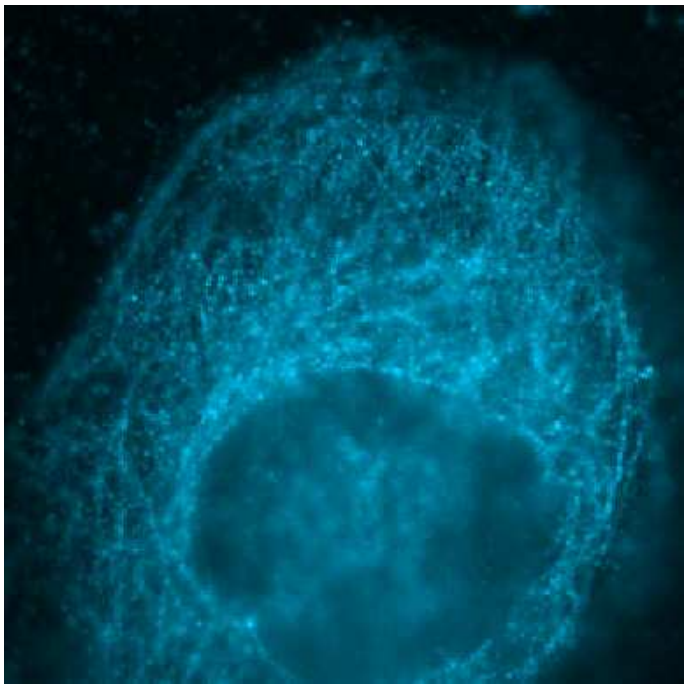
Herbert et al. Microscopy and Microanalysis. 2012

Widefield



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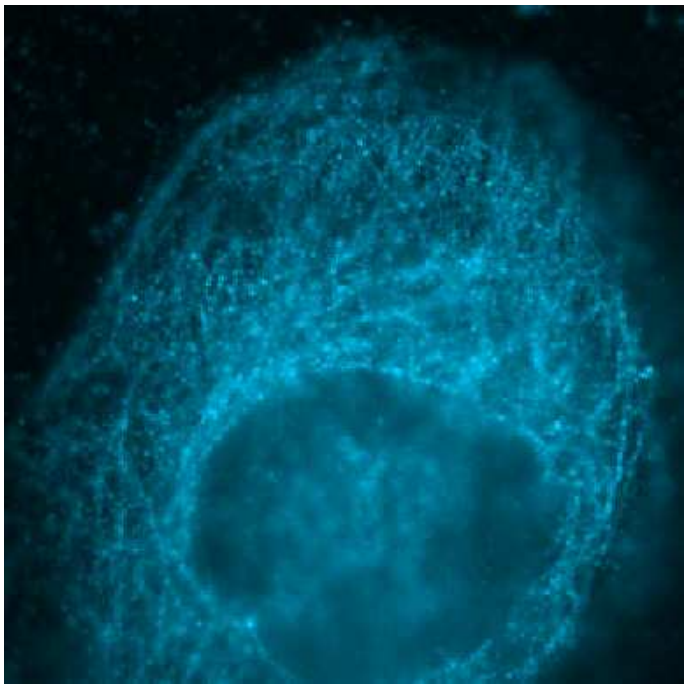
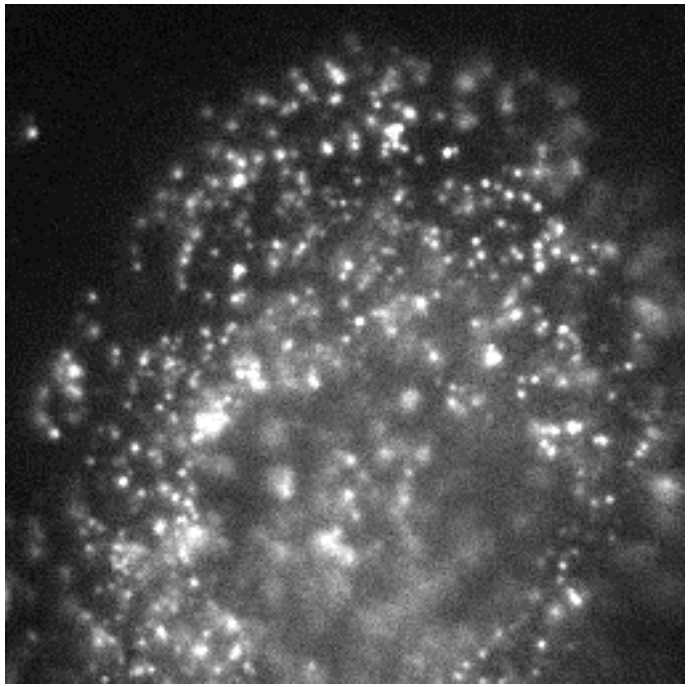
Widefield



Acquisition: blinking



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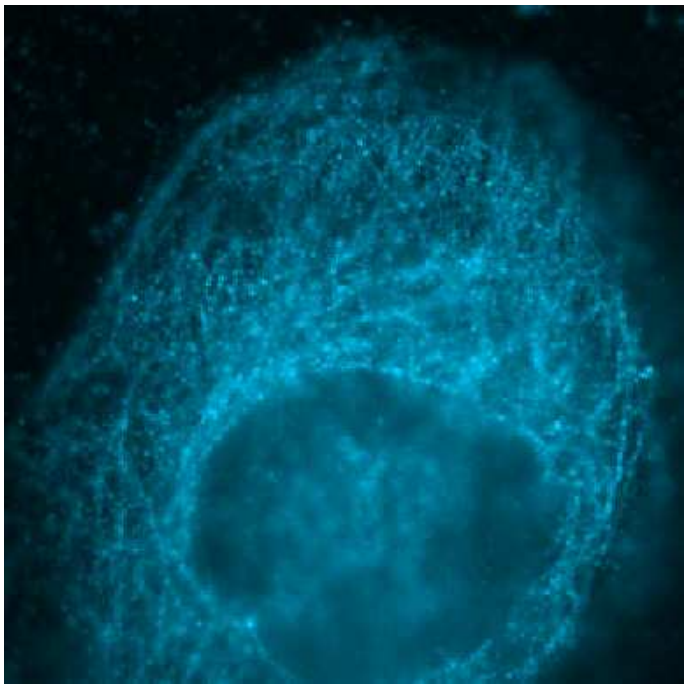
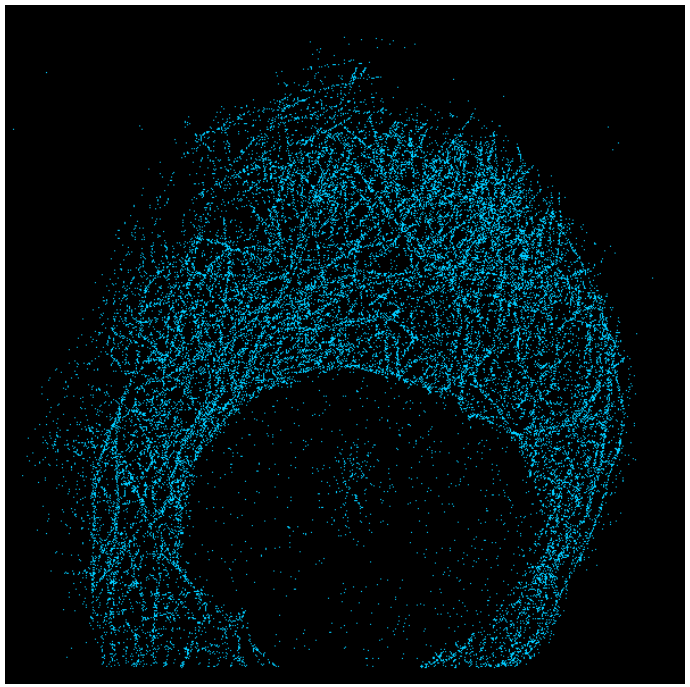
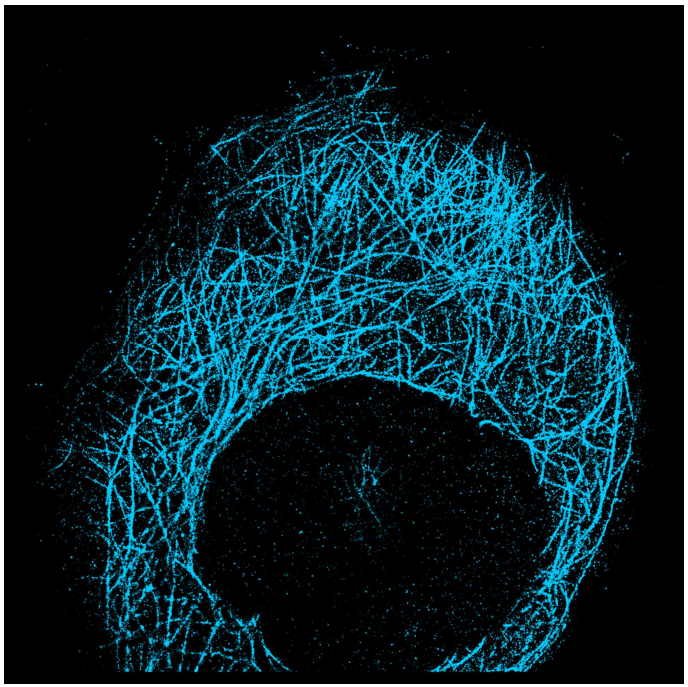
Widefield



Centroid localization



Rendered image



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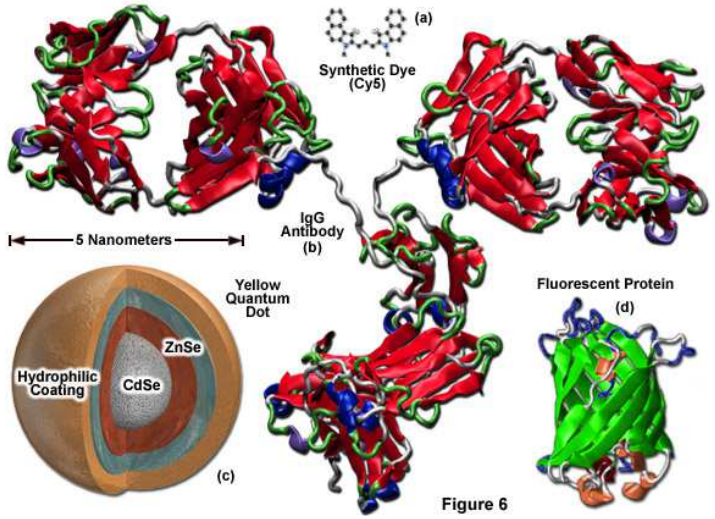
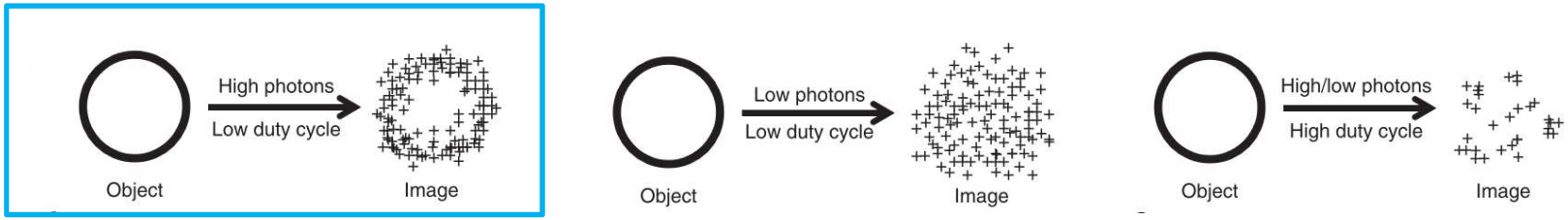
Some parameters to consider for probe selection

- Number of photons per switching event (organic dyes ~6000 photons proteins ~500 photons)
- Fraction of time a fluorophore spends in the on state (duty cycle)
- Number of switching cycles
- Size of probe





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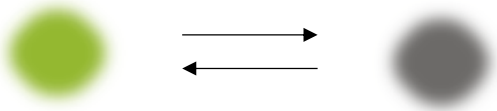
zeiss-campus.magnet.fsu.edu, Dempsey et al. Nature Methods. 2011

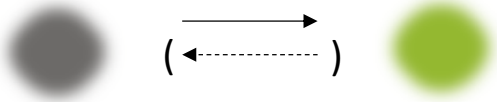
How to make molecule blinking?

Organic dyes under specific imaging conditions („blinking buffer“ + high laser power)



Bright state Dark state

Photoactivable/photocovertible/photoswitchable proteins



Dark state/one color Bright state/red-shifted color

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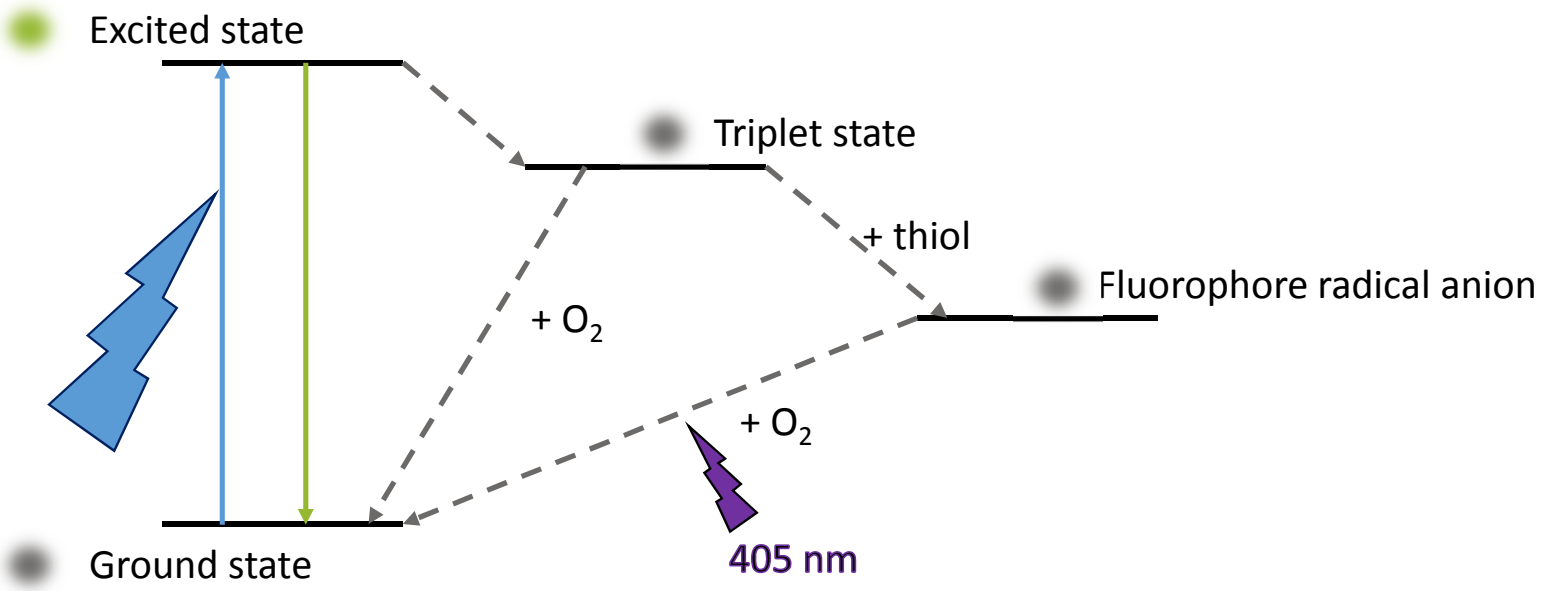
Reversible photoswitching of fluorescent dyes



+ O_2 Enzymatic oxygen scavenger system

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SUPER-RESOLUTION MICROSCOPY

Protocol for d-STORM Imaging Buffer

Materials

- Cysteamine (MEA) #30070-10G (Sigma)- store at 4 °C
- Glucose oxidase type seven from Aspergillius #G2133-50KU (Sigma)-store at -20 °C
- Catalase from Bovine liver C40-100 mg (Sigma)-store at -20 °C
- 1M Tris pH 8.0 # 22638 500 ML (Affymetrix / USB)-store at room temperature
- NaCl
- Glucose

Stocks

- Buffer A: 50 mM Tris-HCl (pH 8.0) + 10 mM NaCl)-store at room temperature
- Buffer B: 50 mM Tris-HCl (pH 8.0) + 10 mM NaCl + 10% (w/v) glucose- store at 4 °C
- 1 M MEA: 77 mg MEA dissolved in 1 mL Buffer A- store at 4 °C
- Gloxy: glucose oxidase (Gluox)+catalase mixture dissolved in buffer A- store at 4 °C

- When making Gloxy stock, calculate the amount to add based on the active units (AU) since not all the protein in the bottle is active. Both the Catalase and Glucose from Sigma should have the information written on the tube. For 10x stock of Gloxy mix 1,688 AU Gluox + 14,040 AU Catalase into 1mL of 50 mM Tris+10 mM NaCl (pH 8.0) and vortex.

Imaging buffer –Make the mixture on ice or at 4 °C fresh before imaging

Typically 50 mM MEA + 1x Gloxy in buffer B

Mix 50 uL of 1 M MEA + 100 uL of 10x Gloxy+ 850 uL of buffer B in a 1.5 ml centrifuge tube and vortex.

Buffer can be used at room temperature imaging for approximately 2 hours.

Updated September 27, 2012

vutara

Thiols:

cysteamine, β-mercaptoethanol

Oxygen scavenging system:

1 β-D-Glucose + 1 O_2 → 1 D-glucono-1,5-lactone + H_2O_2 (Glucose Oxidase)

2 H_2O_2 → 2 H_2O + O_2 (Catalase)

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Recommended organic dyes:

640 nm — Alexa Fluor 647 | Cy5 | Dyomics654

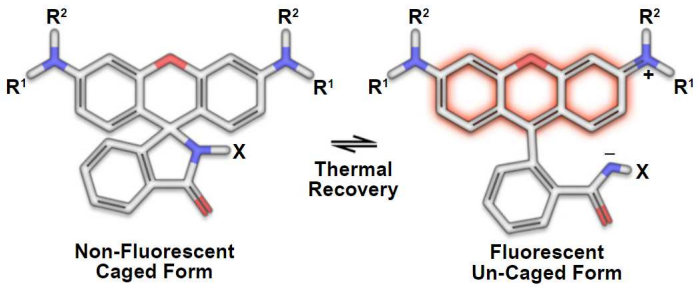
561 nm — Cy3B | Alexa Fluor 568

488 nm — Atto 488 | Alexa Fluor 488

Dempsey et al. *Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging.* Nature Methods. 2011

CF dyes
https://biotium.com/technology/cf-dyes/

caged rhodamine spiroamides (RSAs)



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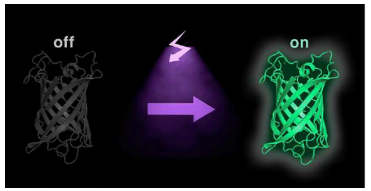
www.microscopyu.com



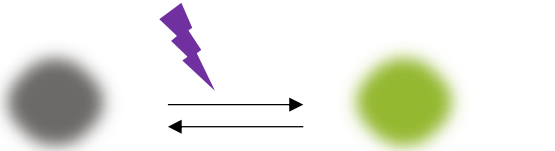
Photoactivable proteins

PA-GFP, PA-mCherry, PS-CFP2, …

Photoconvertible proteins

Kaede, mEos2, Dendra2, …

Photoswitchable proteins



Dronpa, rsFastLime, rsEGFP, rsCherryRev,…

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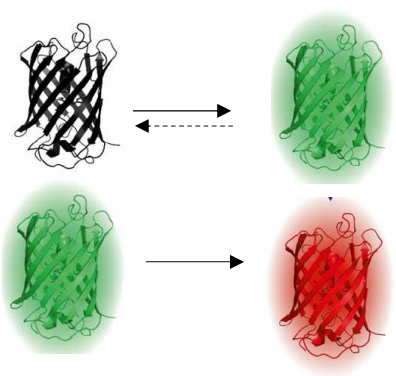
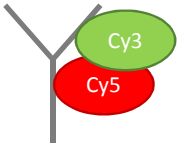
Localization methods

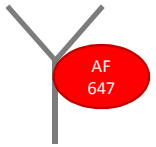
PALM	Photoactivated Localization Microscopy	Betzig et al. (2006)
FPALM	Fluorescence Photoactivation Localization Microscopy	Hess et al. (2006)



STORM	Stochastic Optical Reconstruction Microscopy	Rust et al. (2006)



dSTORM	Direct STORM	Heilemann et al. (2008)
GSDIM	Ground State Depletion Microscopy	Fölling et al. (2008)



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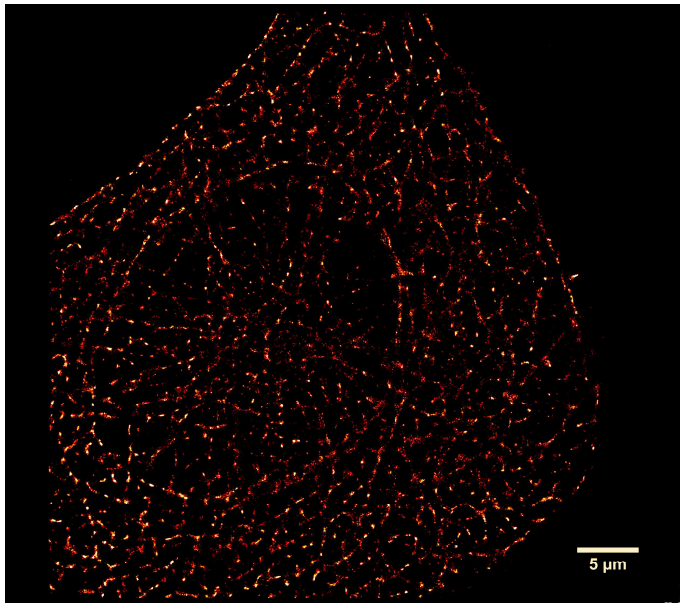
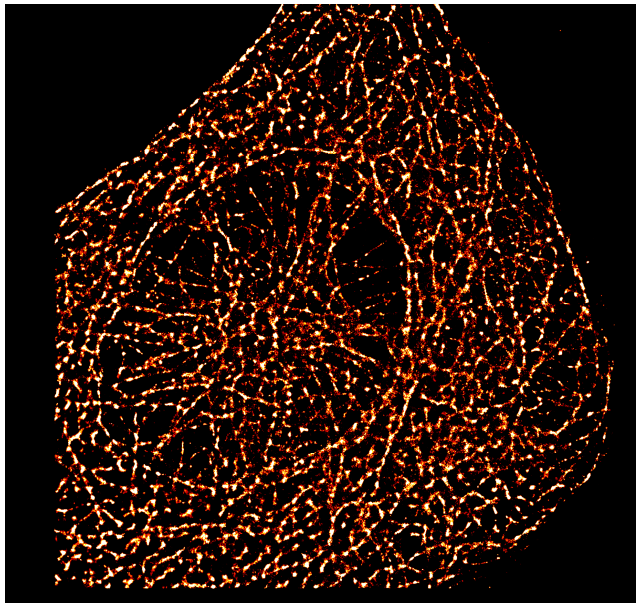
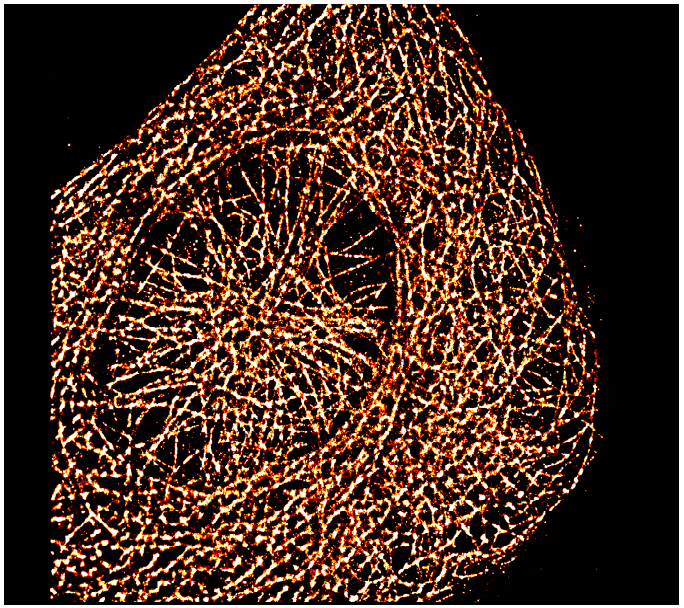
Frames: 1-2000
#Localizations: ~200 000

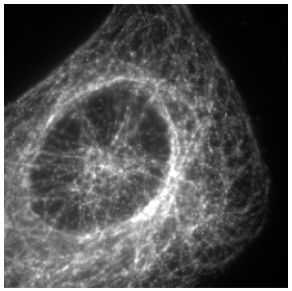


Frames: 1-4000
#Localizations: ~600 000



Frames: 1-10000
#Localizations: ~1 200 000





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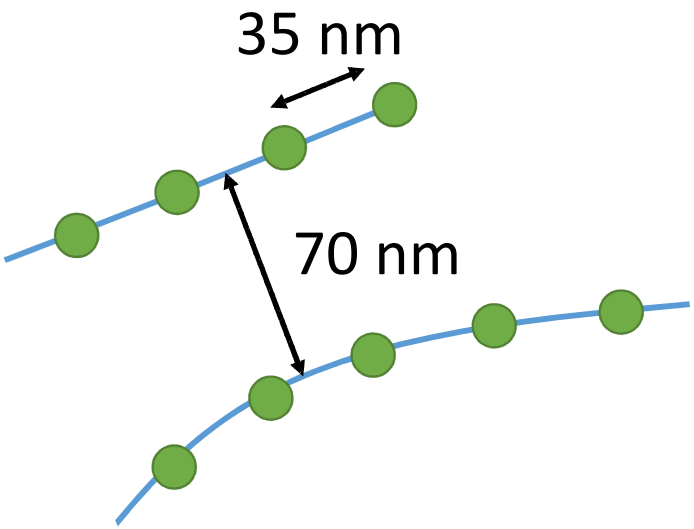
Labelling density: Nyquist sampling theorem



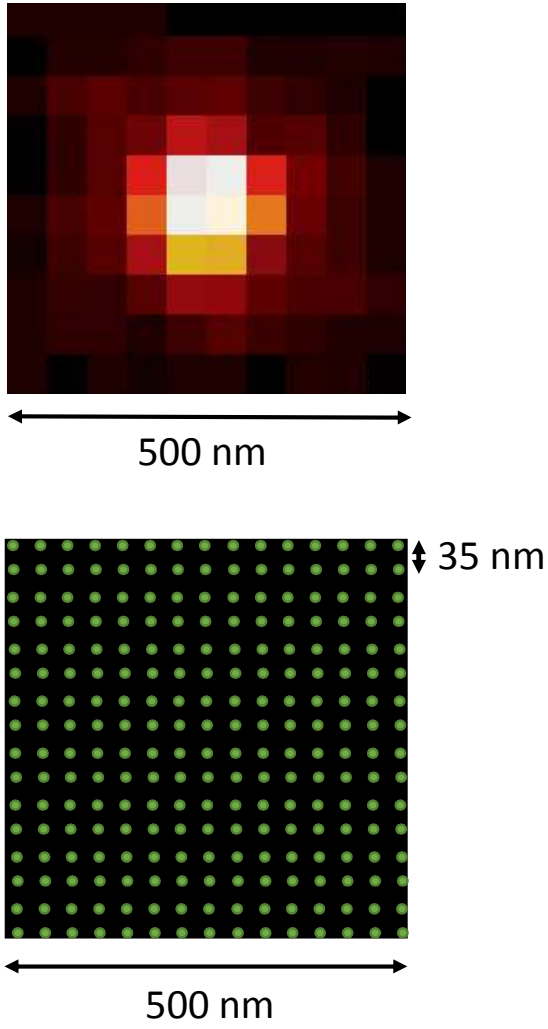
Mean distance between neighboring localized fluorophores (the sampling interval) must be at least twice as fine as the desired resolution.

How many fluorophores?

Assuming: Single molecule can be well localized from single frame into 500 nm x 500 nm

Nyquist theorem: for resolution 70 nm in 2 dimensions, there needs to be a fluorophore on average every 35 nm.

=> $(500/35)^2$ ~ 200 fluorophores/0.25 μm^2



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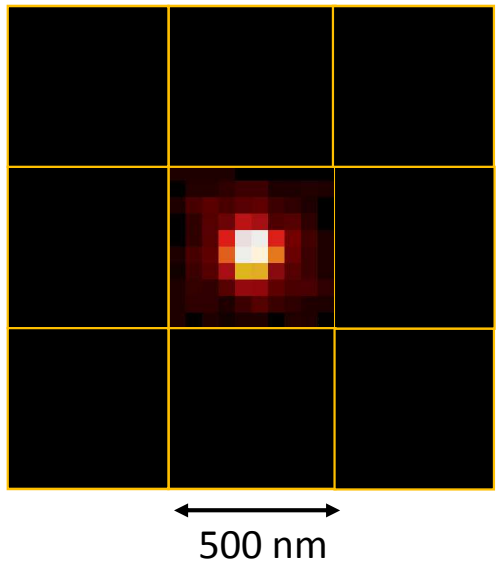
How many frames?

For correct molecule center fitting, no emission can take place in neighboring 500 nm x 500 nm squares

$\Rightarrow$ Average density of emitting molecules 0.11 / 0.25 µm^2

~ 200 fluorophores/0.25 µm^2

=> we require 2000 frames for 70 nm resolution



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We require 2000 frames for 70 nm resolution.

At 100 fps, the time resolution is 20 s.

If we want to have resolution of 20 nm, we need approximately 25000 frames, at 100 fps it will take 250 s.

Live cell imaging is difficult…

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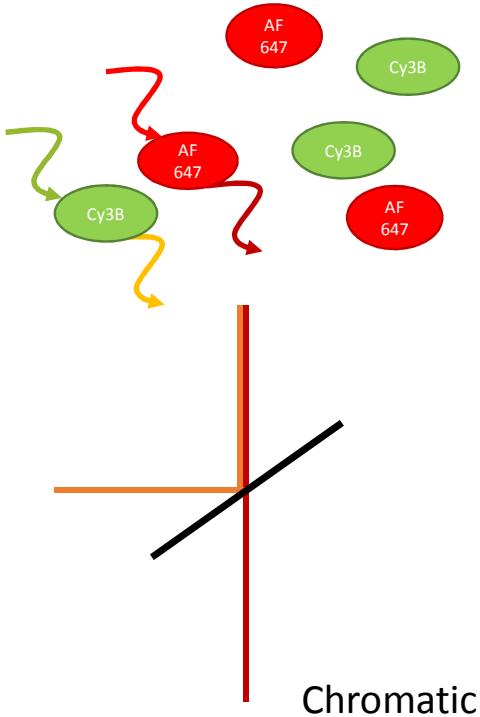
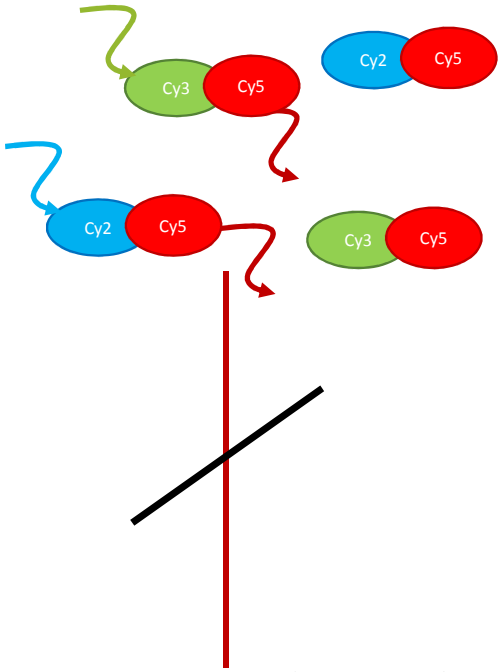
Multi-color imaging

Spectral separation



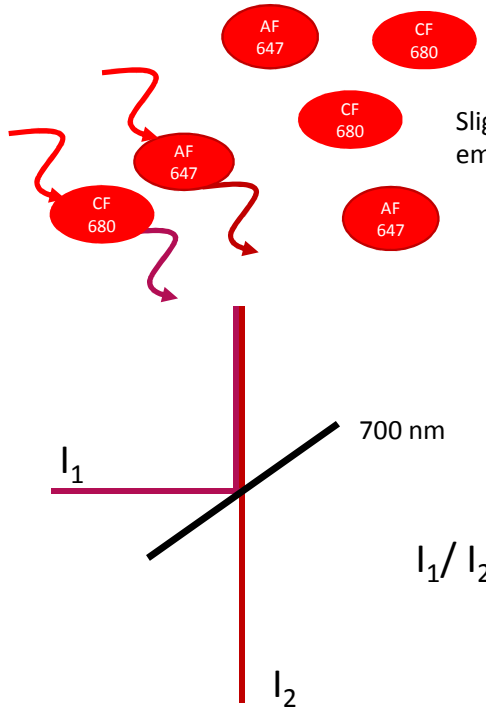
Chromatic shift
(Fiducial markers help)

Several activator/reporter systems



Cross-activation

Ratiometric separation



Slightly shifted emission spectra.

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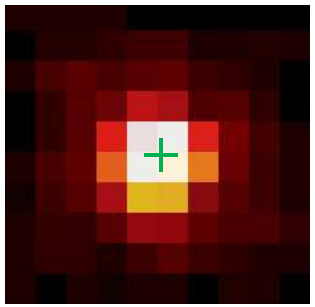
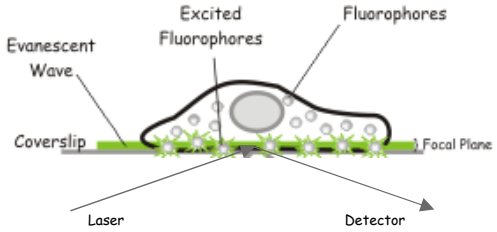
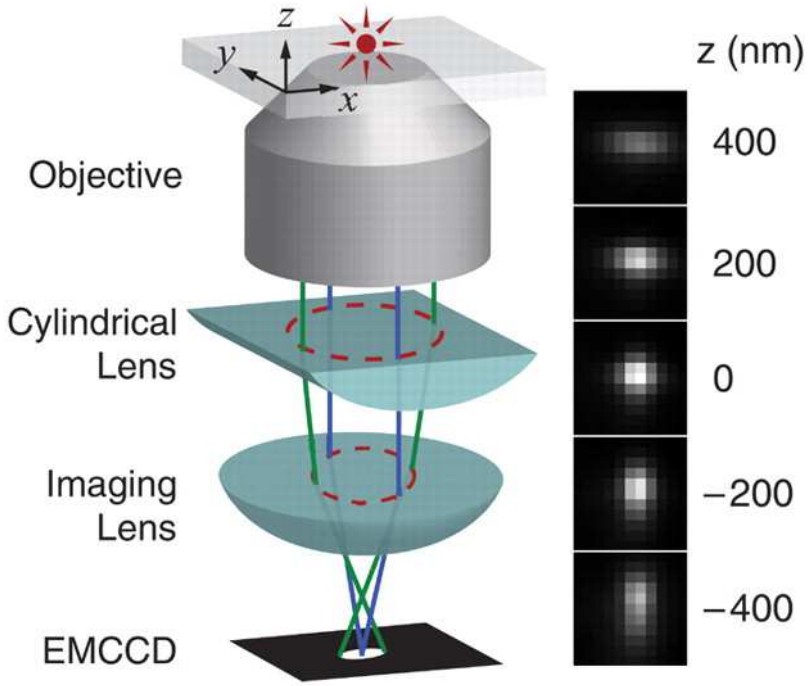
3D imaging



[x, y]

TIRF ~100nm





Cylindrical lens causing astigmatism
Achievable resolution:
x,y - 25 nm
z - 70 nm

Huang Bo et al. Science (2008)

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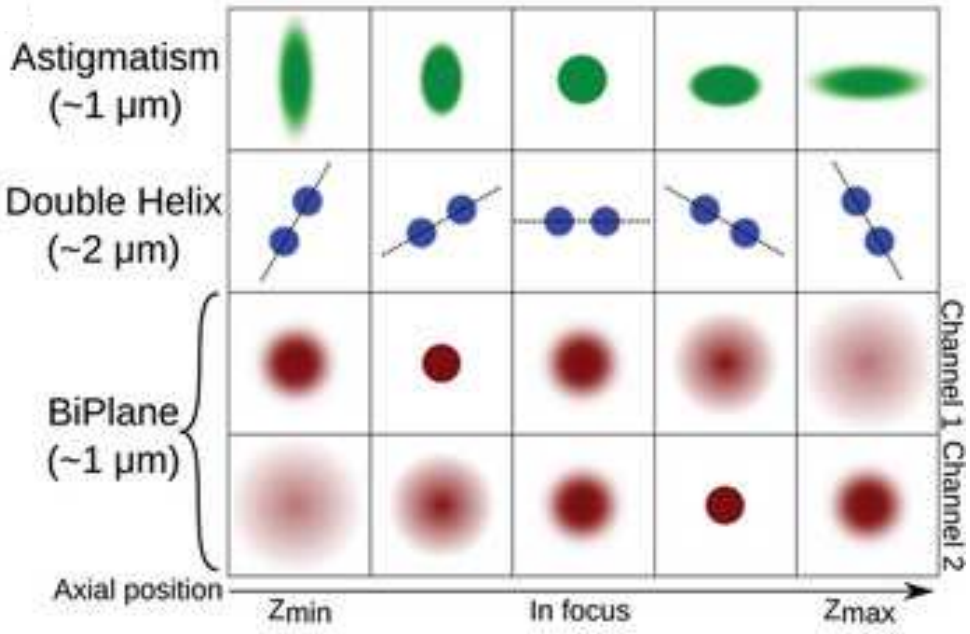
3D imaging



Biocev (N-STORM)

Faculty of Science, Viničná 7 (Zeiss Elyra SP1)

Institute of Physiology CAS, group of prof. Ježek (Biplane FPALM)

Dual-objective interferometry (iPALM)

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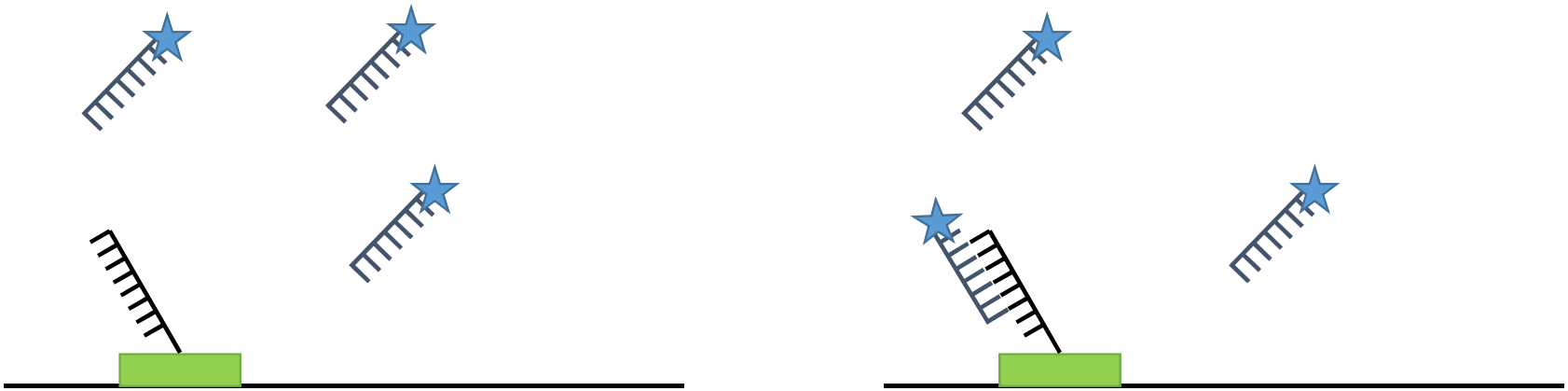
Herbert et al. Microscopy and Microanalysis. 2012

Other approaches to…

… fluorophores blinking

Transient binding/unbinding events



PAINT, uPAINT	(Universal) Points Accumulation for Imaging in Nanoscale Topography Sharonov at al. PNAS 2006, Giannone et al. Biophys J 2010
BALM	Binding-Activated Localization Microscopy Schoen et al. Nano Letters 2011

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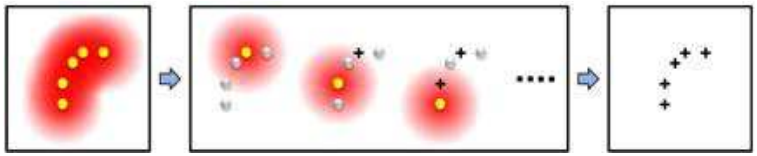
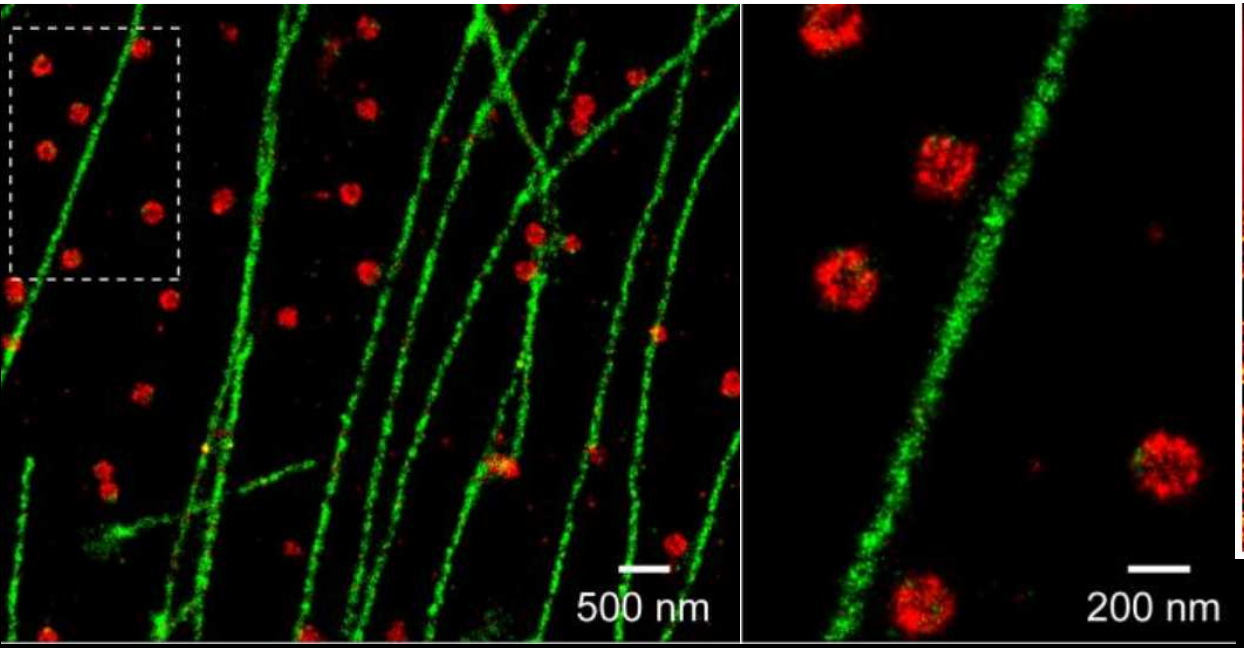
Summary



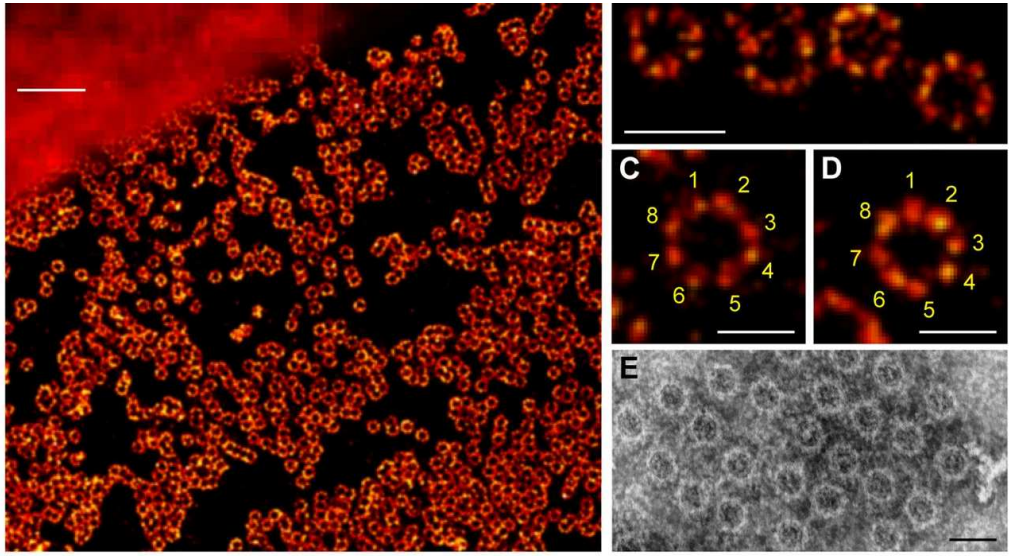
- ✓ High localization precision (tens of nm)
- ✓ High lateral resolution (20-30 nm)
- ✓ Axial resolution 100-200 nm in TIRF mode, 50-100 nm with specialized setup
- ✓ Excellent for small, dim, punctate, or filamentous objects
- ✓ Simple instrumentation
- ✓ Quantification of single molecules (cluster analysis, single particle tracking)

- ▪ Long imaging times
- ▪ Typically one plane only (no 3D sectioning)
- ▪ Special fluorophores and imaging media
- ▪ Fixed samples

huanglab.ucsf.ed, www.micron.ox.ac.uk

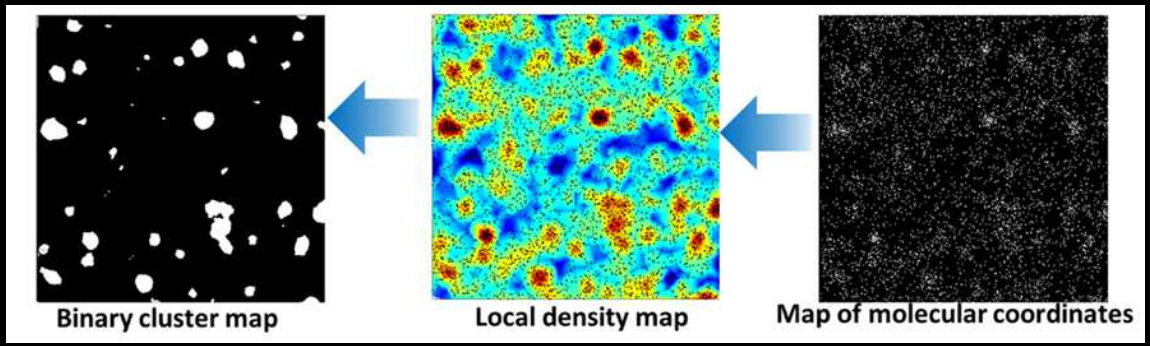


Microtubules and clathrin coated pits. Bates at al. Science 2007



Nuclear pores. Löschberger at al. J Cell Science 2011

Thank you for your attention.



Protein clustering at plasma membrane.
Owen&Gauss. Front Plant Sci 2013