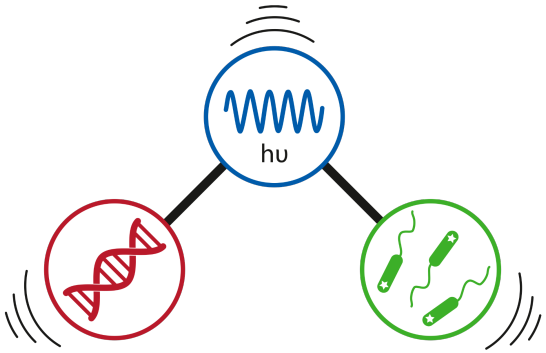
# Fluorescence based super resolution microscopy and single particle tracking: an introduction

Laboratory of Biophysics, Wageningen University & Research

20.11.2017, Johannes Hohlbein




@HohlbeinLab

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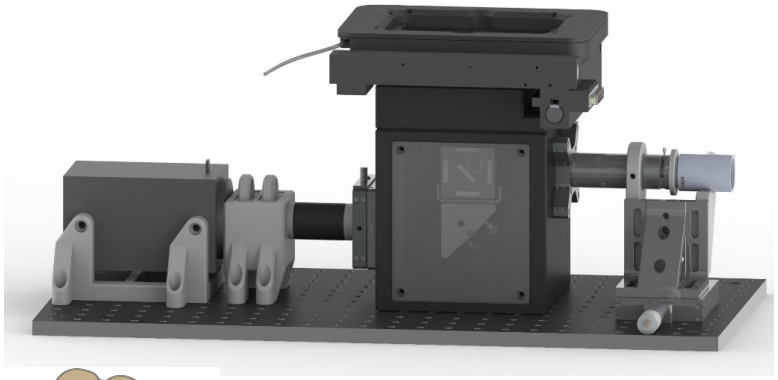
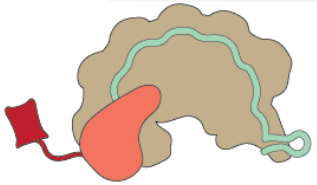
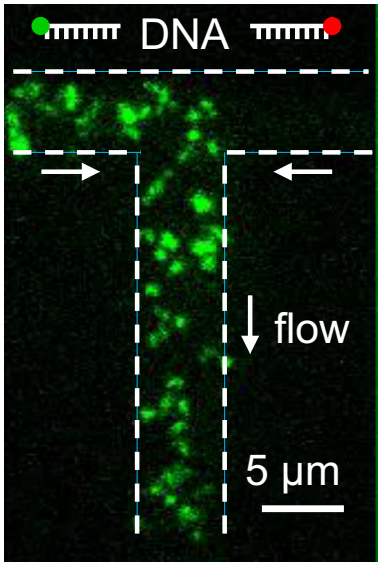
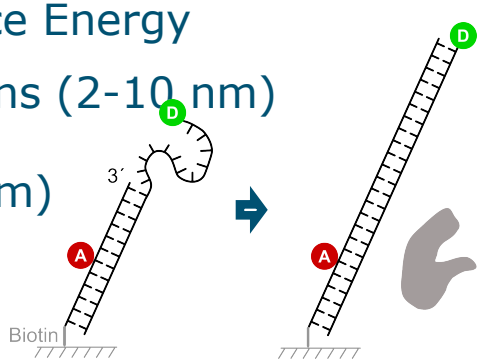
# Outline

- Fluorescence based imaging and the problem of limited resolution
- Super resolution microscopy (20-250 nm)
- Single particle tracking in live bacteria (CRISPR-Cas): temporal information
- Also available @HohlbeinLab:
  - Single-molecule Foerster Resonance Energy Transfer for DNA-protein interactions (2-10 nm)
  - Nanofluidic devices (non-equilibrium)

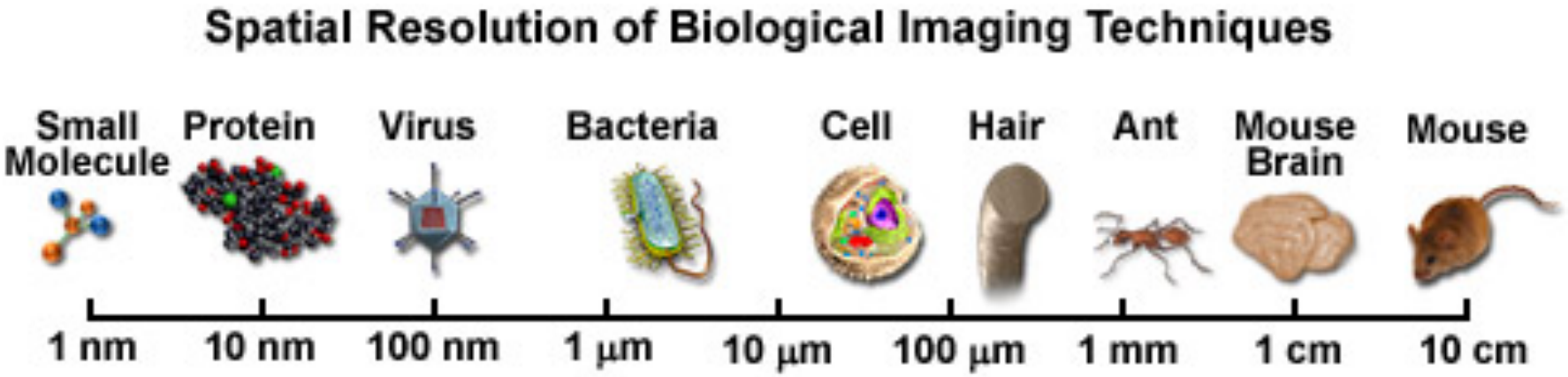






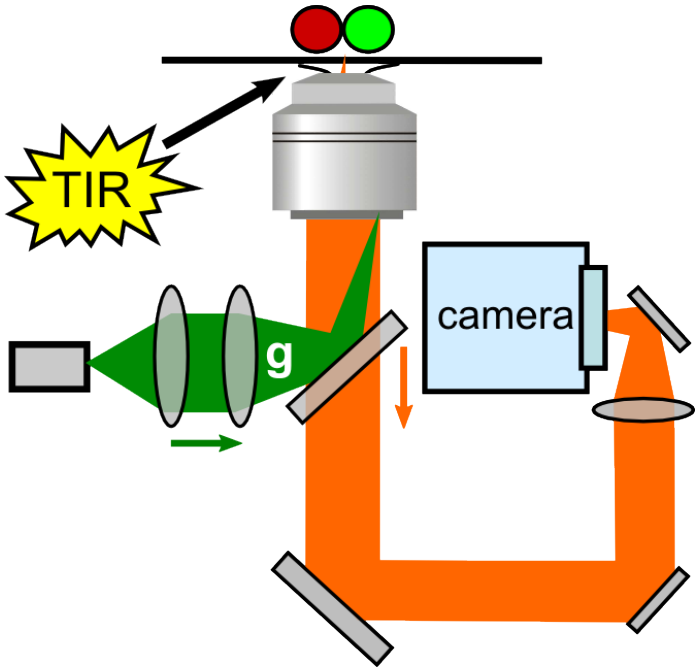



# Length scales in Life Sciences...

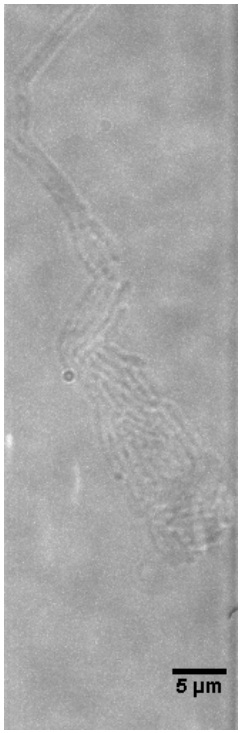



http://zeiss-campus.magnet.fsu.edu/print/superresolution/introduction-print.html

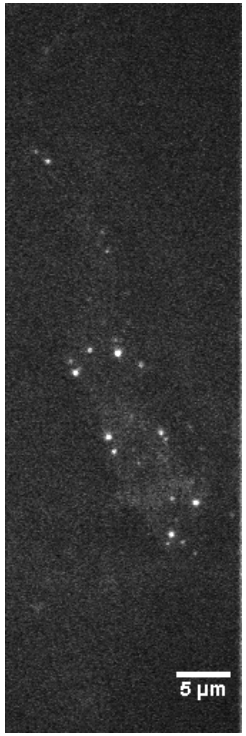
# Fluorescence imaging: Widefield microscopy




Brightfield

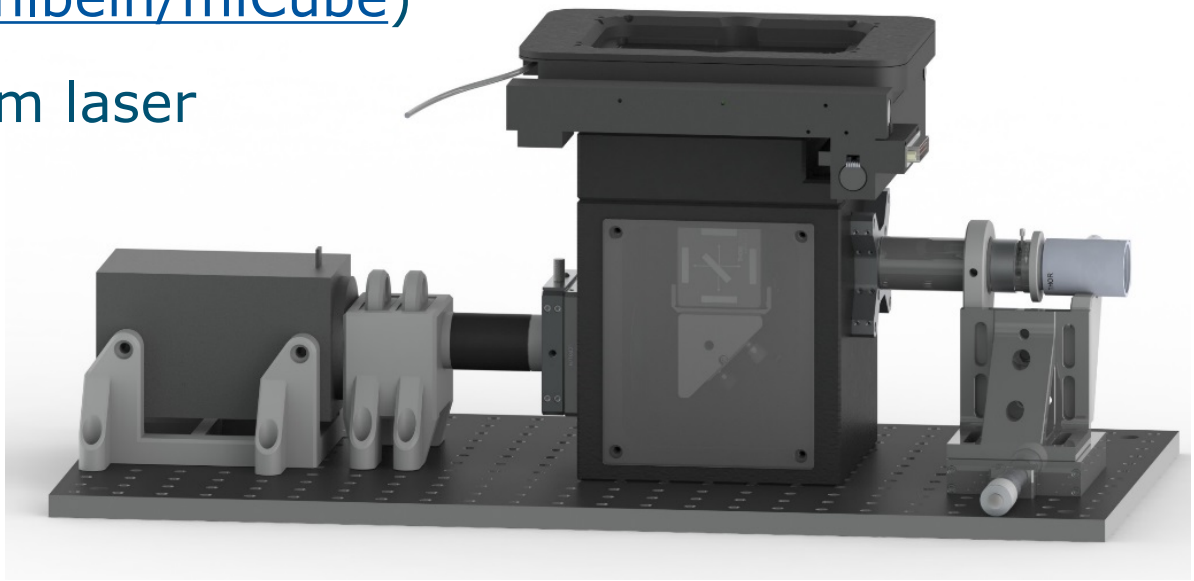



Fluorescence



# miCube: modular fluorescence microscopy

- Concept published under a Creative Common licence (www.jhohlbein/miCube)
- **405**, **488**, **561** and **638** nm laser excitation
- Single-molecule sensitivity
- Scanning in *x,y,z*
- Easy to operate!

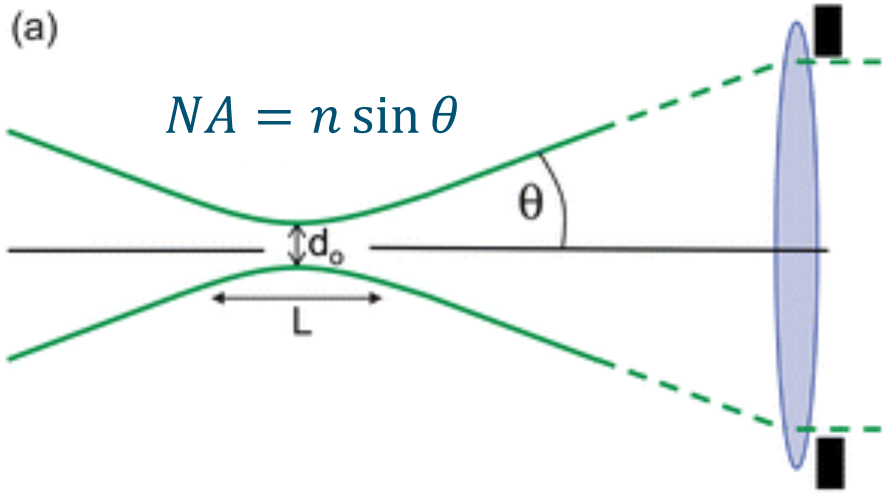


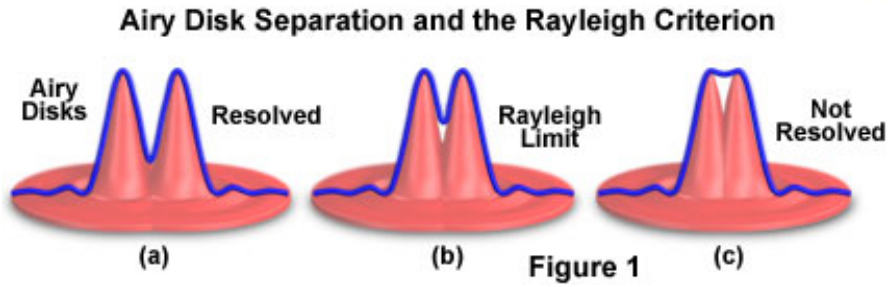
# Problem: Diffraction limit and spot size

- Limited resolution in optical microscopy

$$d_0 = \frac{\lambda}{2NA}$$

~200 nm

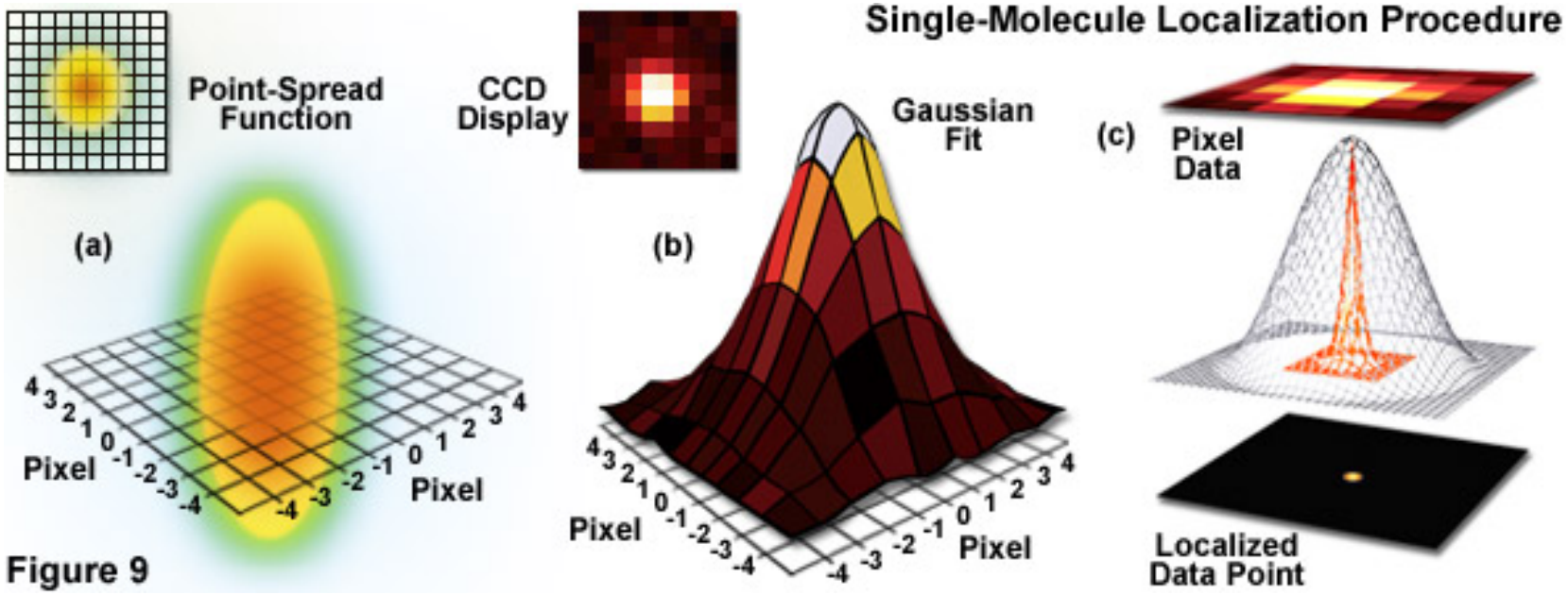



Numerical aperture (NA)

# Localisation of single emitters

- Localise a single emitter with high precision



# Super resolution microscopy

- How do resolve features λ/2 (~250nm)?


→ Separate emitters in time
to separate them in space

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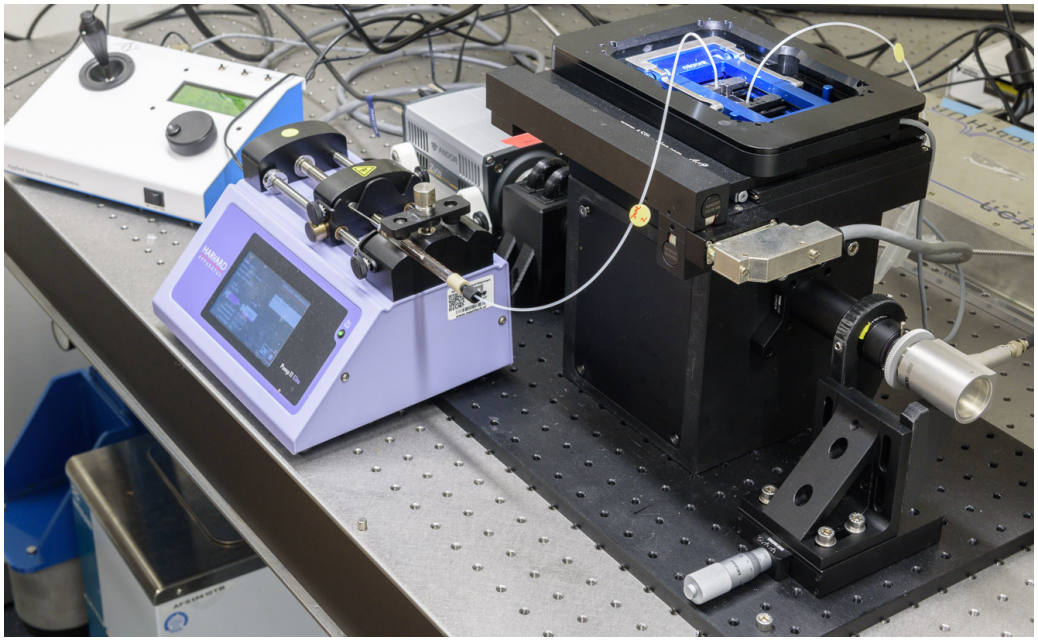
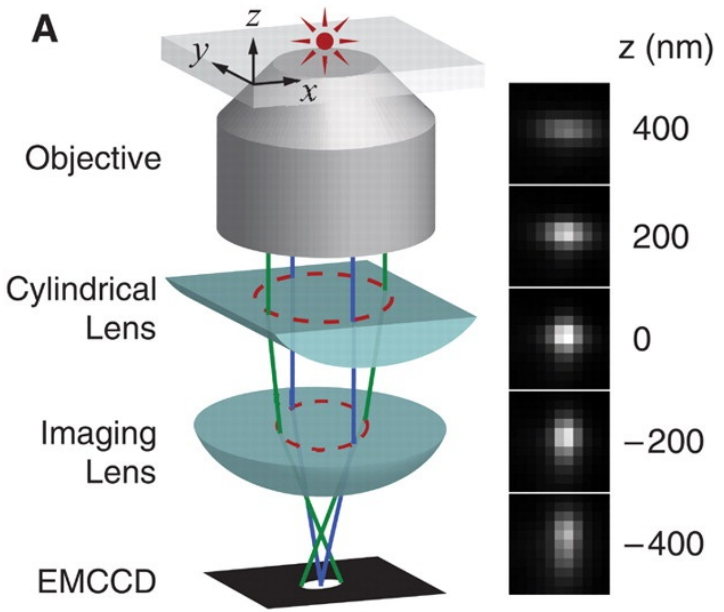
# miCube: modular fluorescence microscopy

- www.jhohlbein/miCube



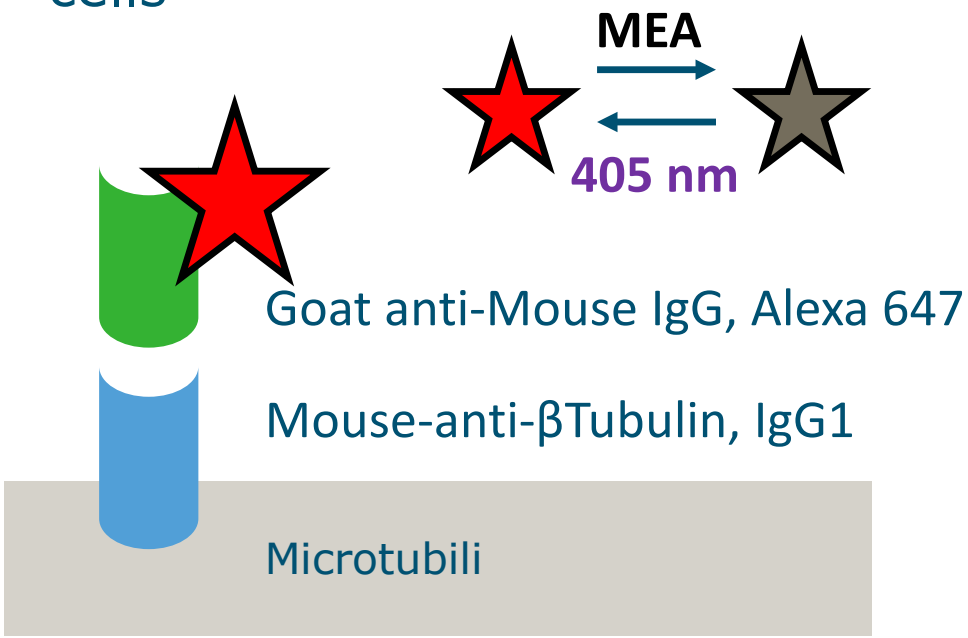
- 3D SMLM (astigmatism)

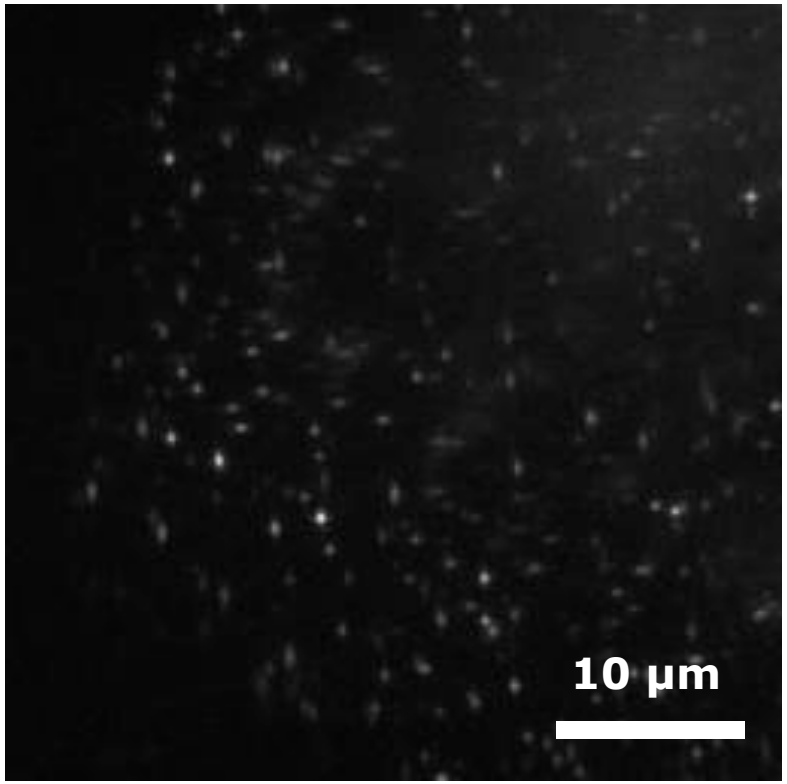



Huang et al, Science, 2008

# Example: dSTORM microscopy

- Use of antibodies and blinking fluorophores to label target in cells




Data courtesy of Christophe Letterier, CNRS/Aix-Marseille University

# pSMLM-3D

- Ultrafast localisation algorithm:

New Results

**Phasor based single-molecule localization microscopy in 3D (pSMLM-3D): an algorithm for MHz localization rates using standard CPUs**

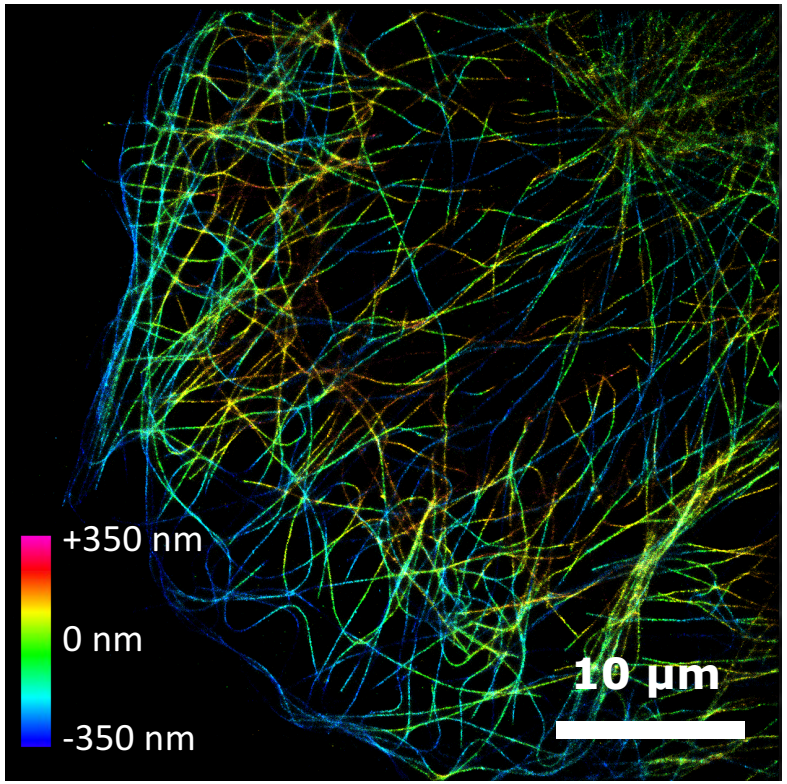
Koen Martens, Arjen N. Bader, Sander Baas, Bernd Rieger, Johannes Hohlbein

**doi:** https://doi.org/10.1101/191957



https://www.biorxiv.org/content/early/2017/09/21/191957

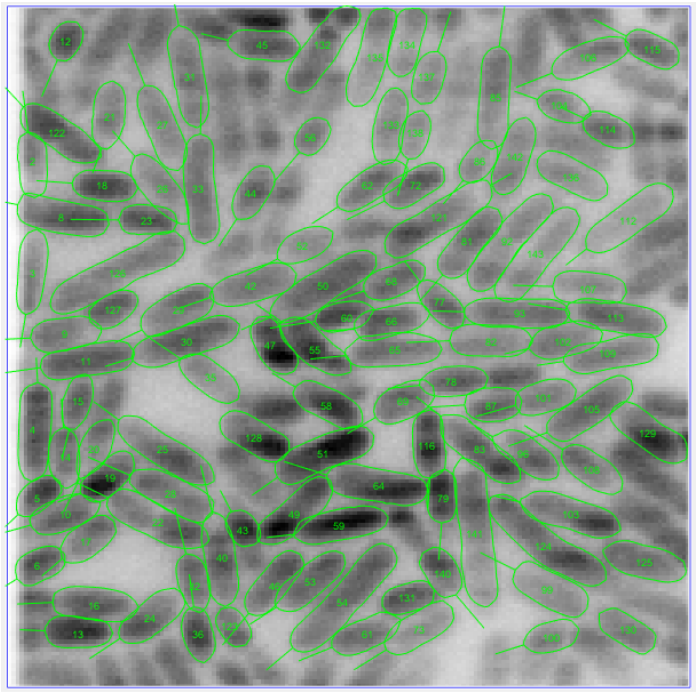
- Implementation in ThunderSTORM (imageJ)




Data courtesy of Christophe Letterier, CNRS/Aix-Marseille University

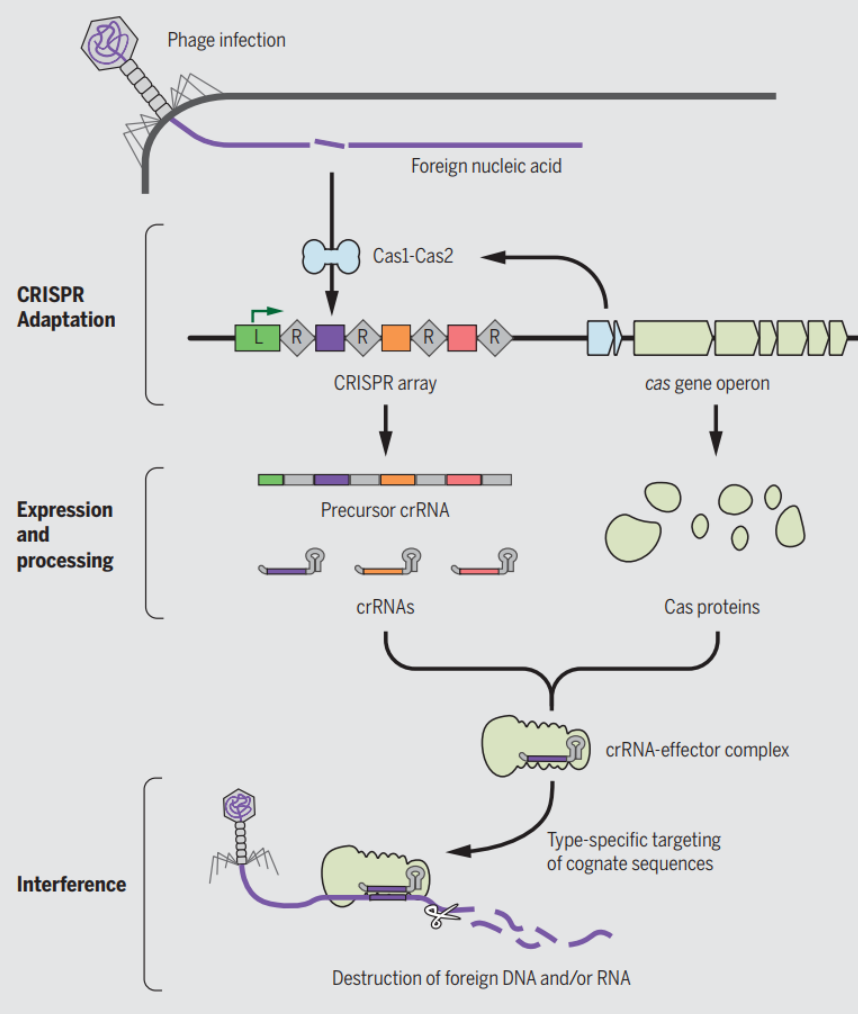
# Insights into cellular target search of Cascade

- CRISPR-Cas *in vivo*
  - Jochem Vink (TUD)
  - Stan Brouns (TUD)
  - Koen Martens (BIP @ WUR)
  - Stan van de Wall (BIP @ WUR)

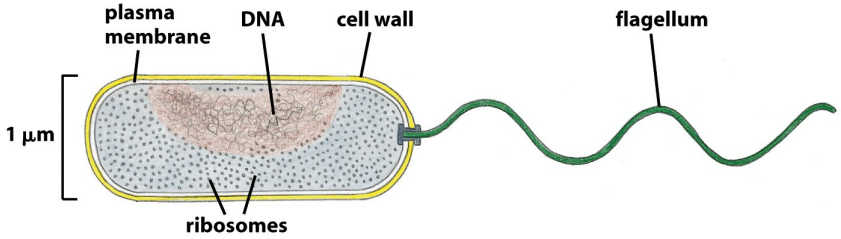


*E. coli* cell segmentation

# The CRISPR model...




## Target finding is a number game...




*Molecular Biology of the Cell*, 5th, Garland Science 2008

- Life cycle virus: ~20 minutes
- E.coli genome: 4-5 Mio. bp
- Viral genome: 20-100 kbp
- Copy number viruses: 1-10000

Jackson et al., Science, 2017

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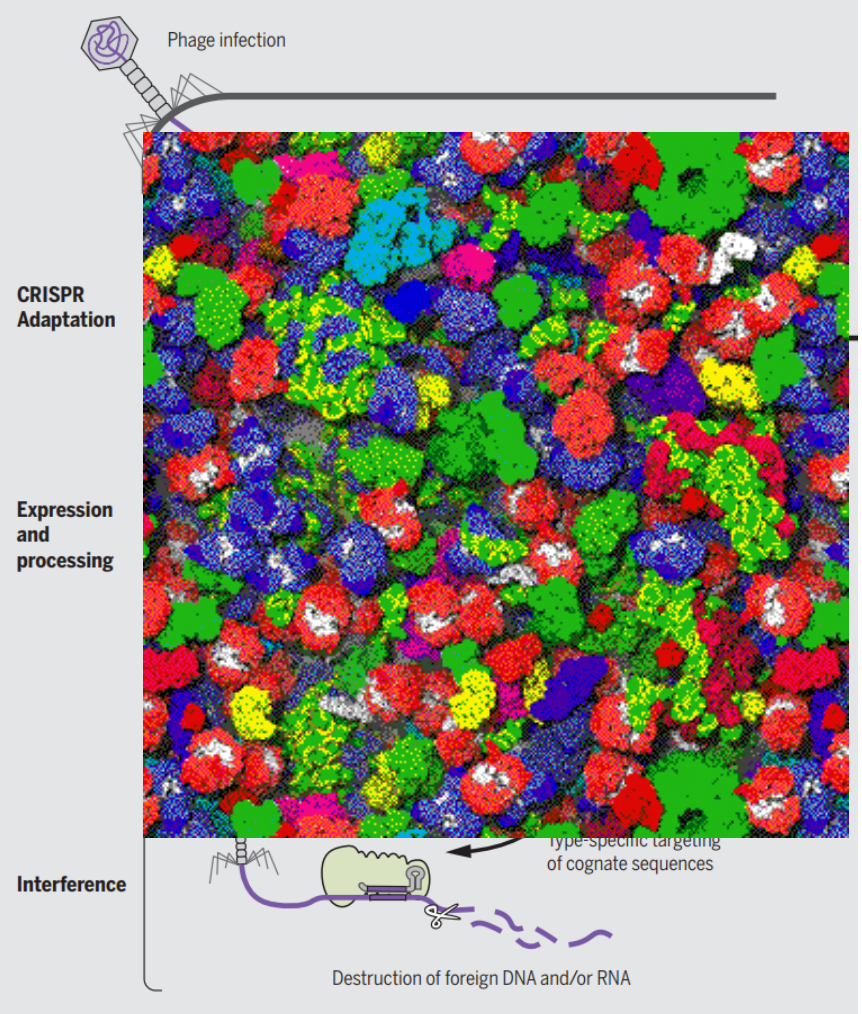


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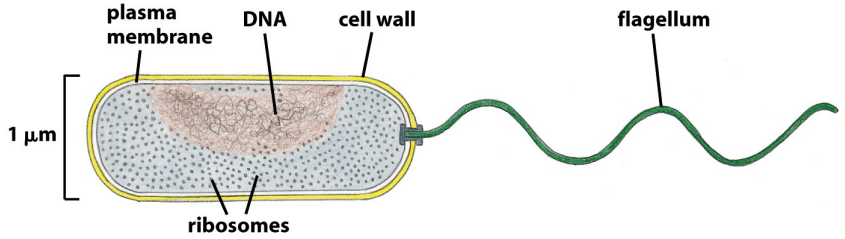


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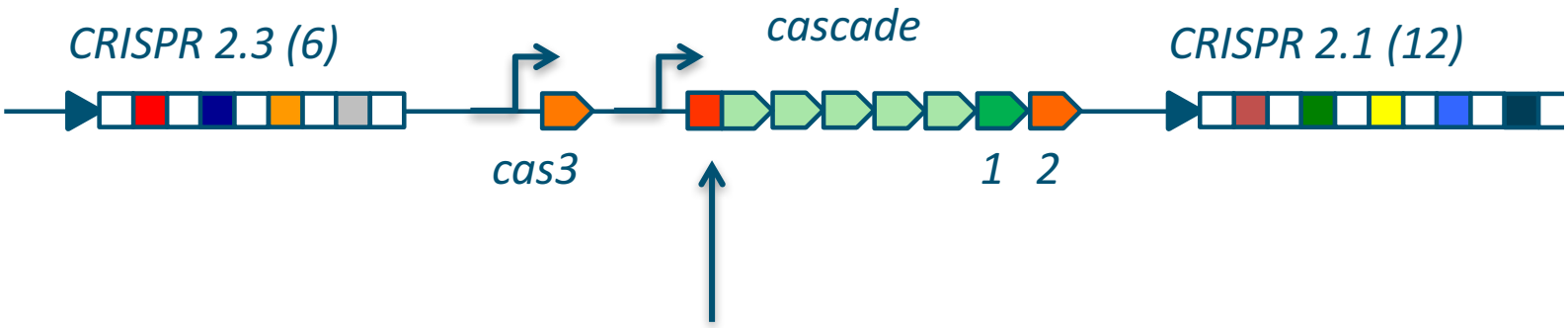
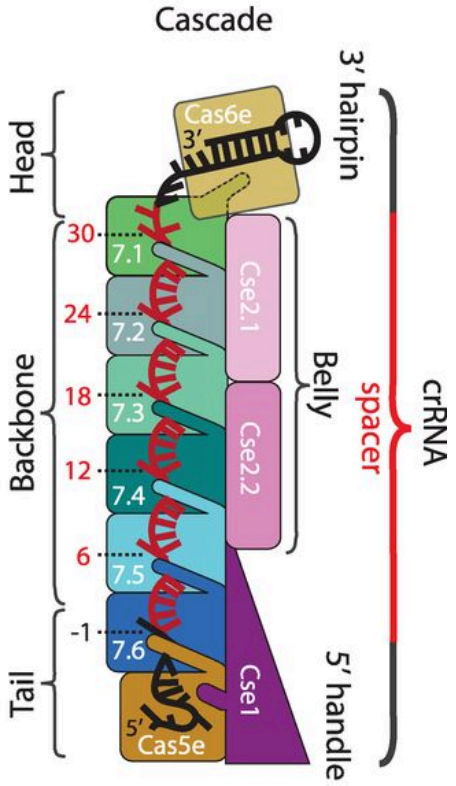
Jackson et al., Science, 2017

McGuffee & Elcock, PLOS com.biol., 2010

# The biological setup: type I-E, *E. coli* K12

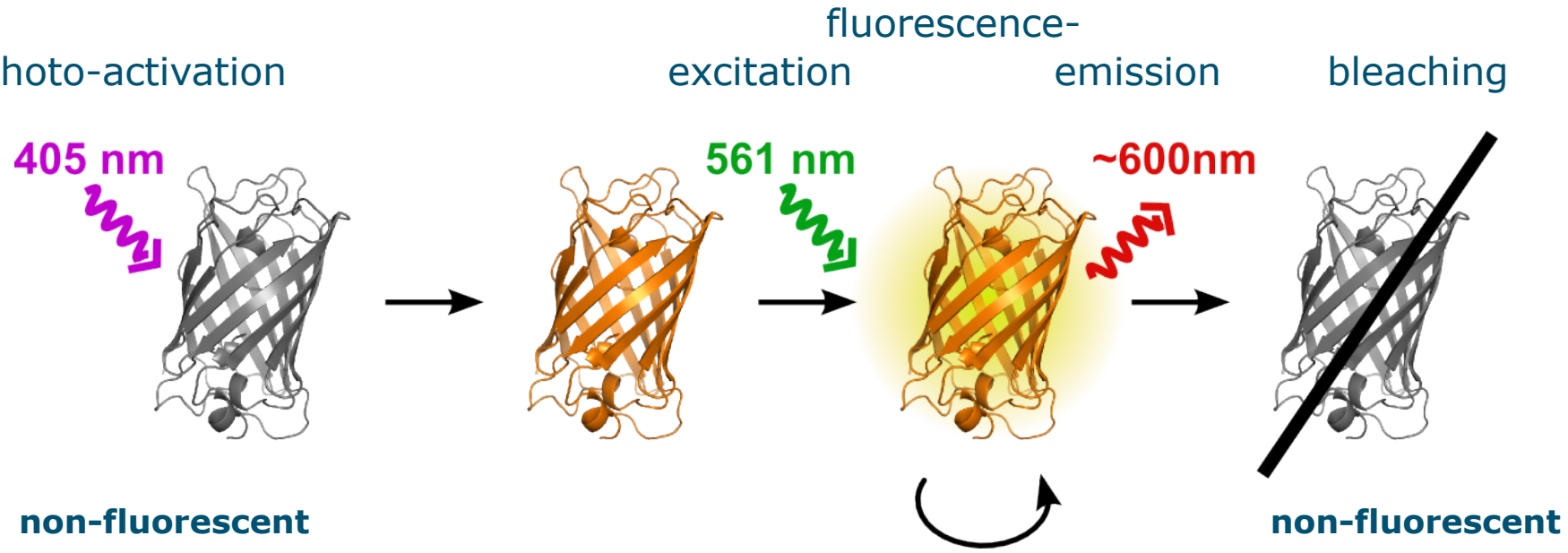



Jackson et al., Science, 2014

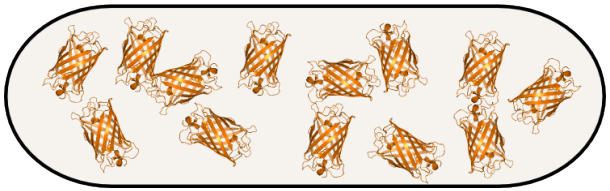
# Photoactivated localization microscopy (PALM)

- Photo-activation turns a non-fluorescent protein into a fluorescent one

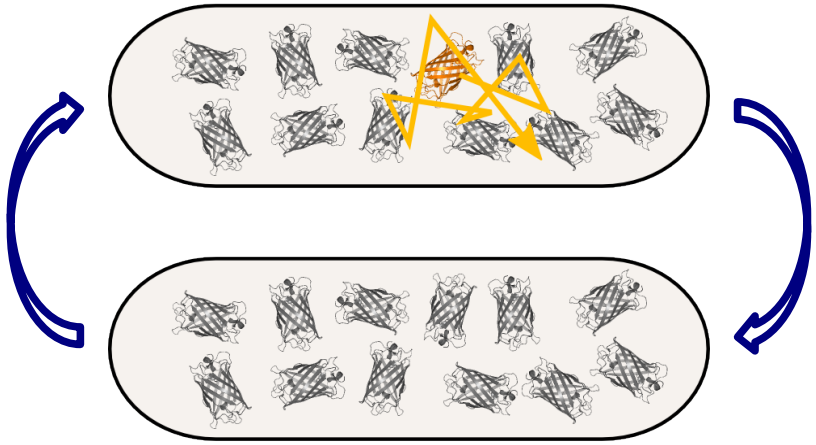


# Photoactivated localization microscopy (PALM)

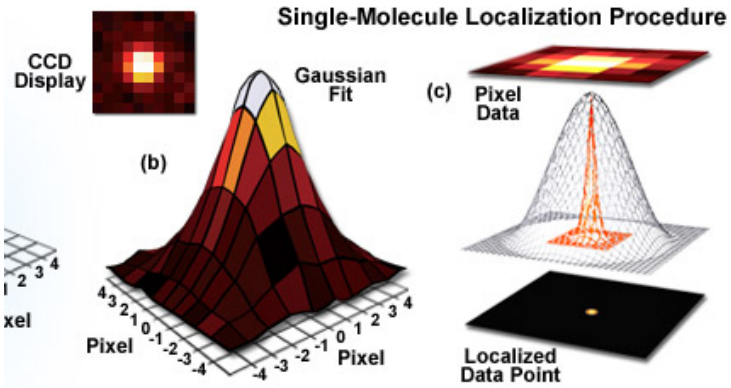
- From many active emitters per cell to a single one



Manley et al., Nat. Met., 2008; English et al., PNAS, 2011

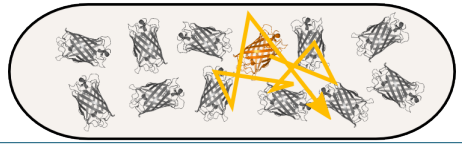


- Localisation of emitters with high precision



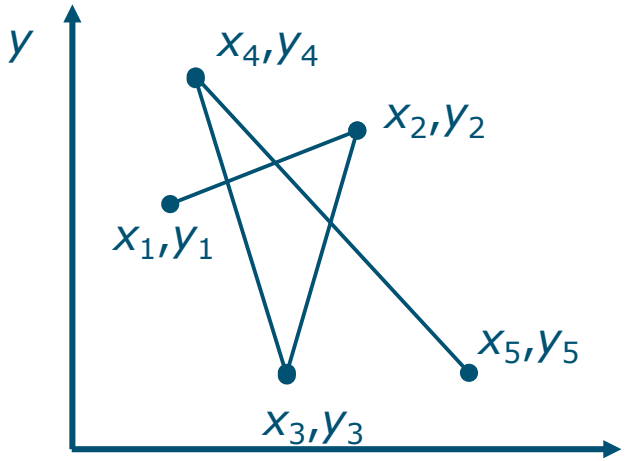

http://zeiss-campus.magnet.fsu.edu/print/superresolution/introduction-print.html

# Quantifying movements...



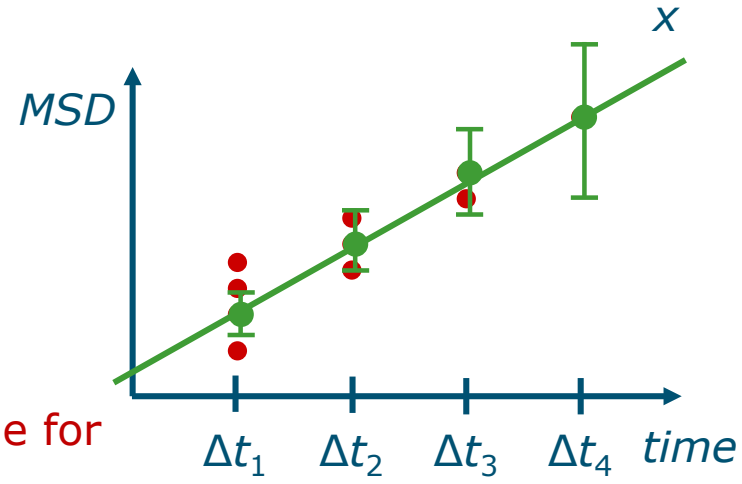
- ...using mean squared displacement

$$\overline{MSD}_n = \frac{1}{N-n}\sum_{i=1}^{N-n}(\vec{r}_{i+n} - \vec{r}_i)^2, n = 1, \ldots, N-1$$



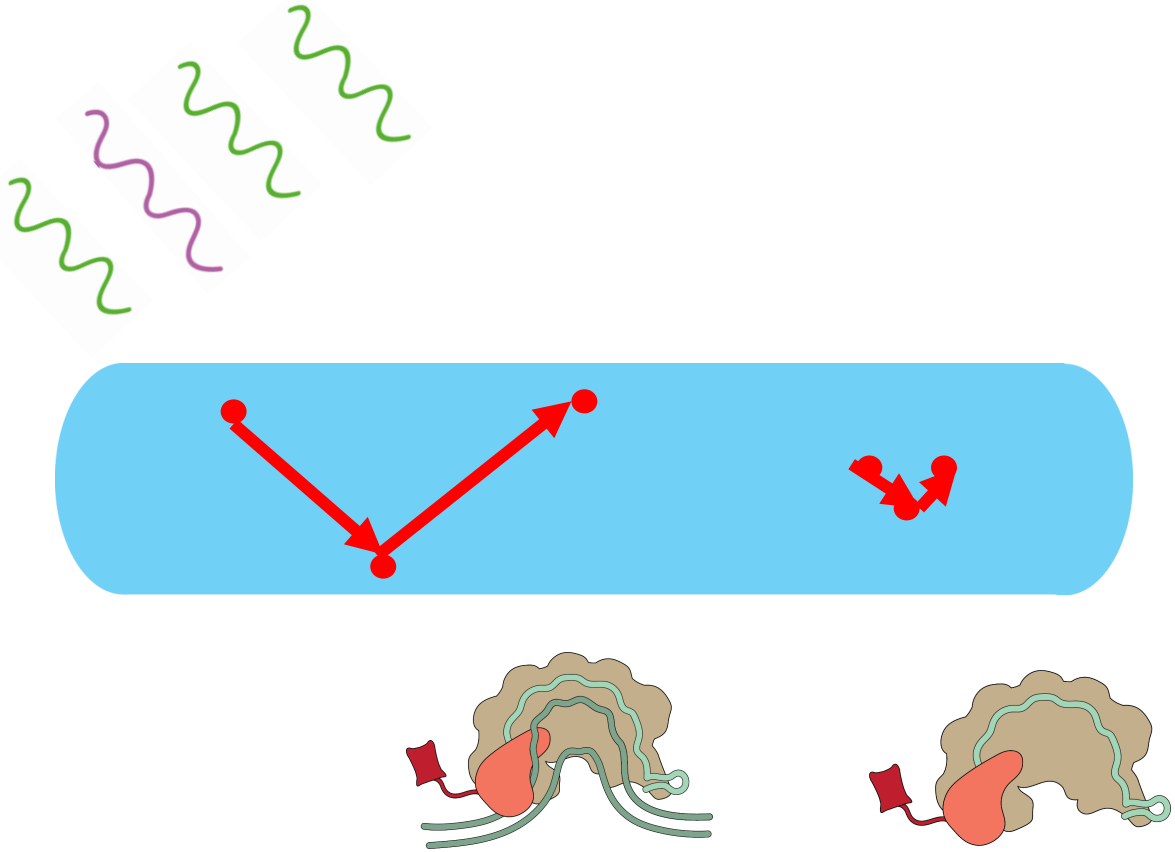

- Diffusion coefficient *D** describes mobility of the molecule

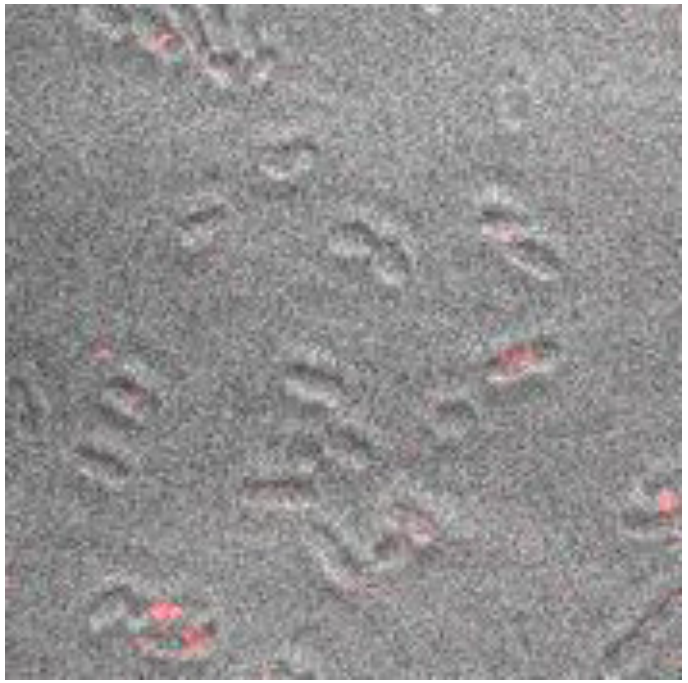
$$MSD = 4D^*\Delta t$$



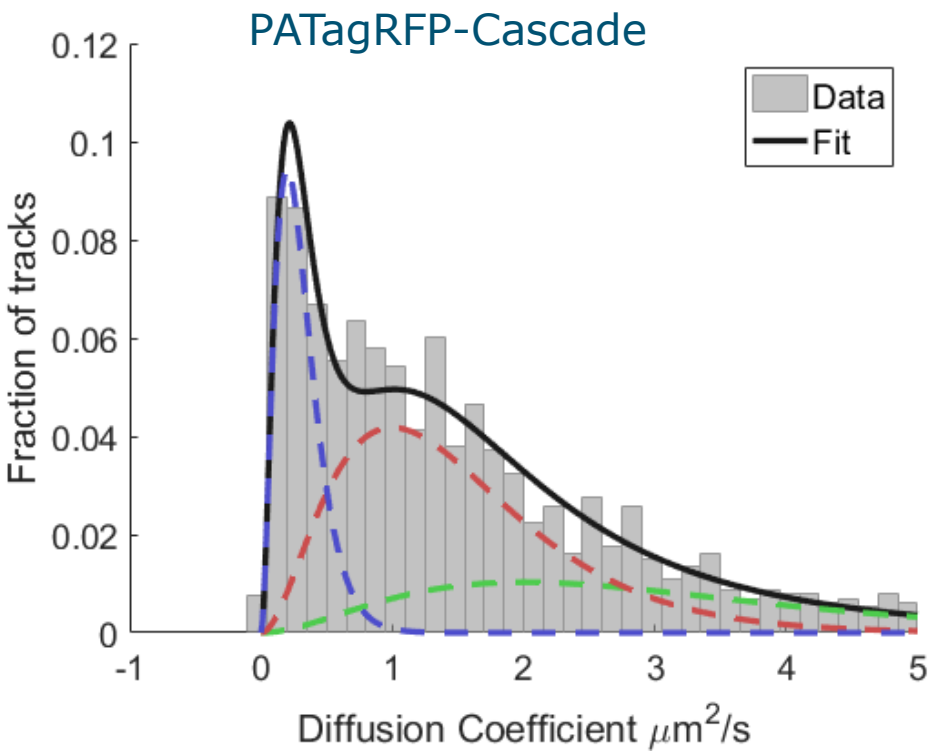

→ Every short track leads to a value for an apparent diffusion coefficient

# Tracking in the cell...

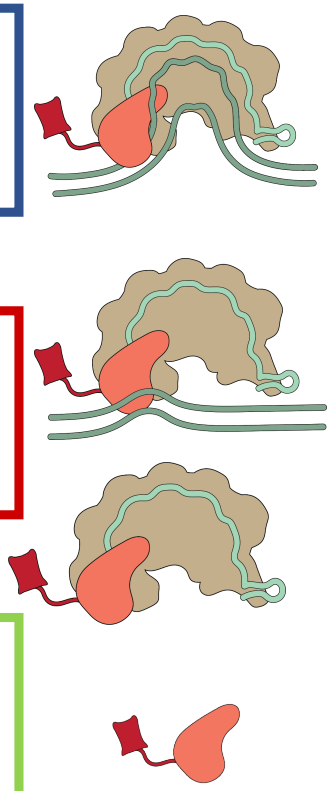




# Diffusional states



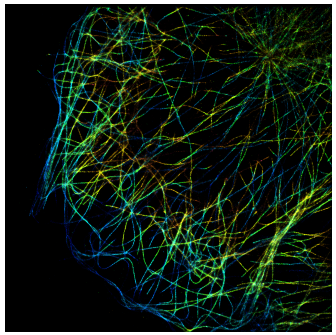

| | |
|---|---|
| Immobile | 0.3 $\mu m^2/s$<br>25% |
| Slow moving | 1.5 $\mu m^2/s$<br>51% |
| Fast moving | 3.0 $\mu m^2/s$<br>24% |

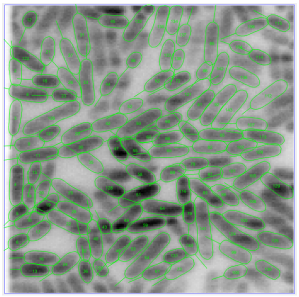


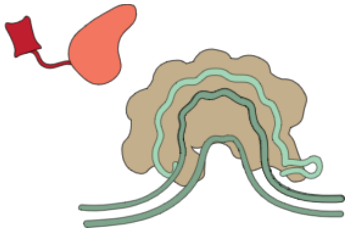
Fitting model: Stracy et al., PNAS, 2015

# Summary

- Advanced fluorescence imaging techniques available @ WUR allow resolutions down to 10 nm
- Broad spectrum of potential applications (DNA paint, dSTORM, PALM, SPT,..)
- *In vivo* studies reveal target search mechanisms of Cascade complexes









Funding: EPS and VLAG Graduate schools