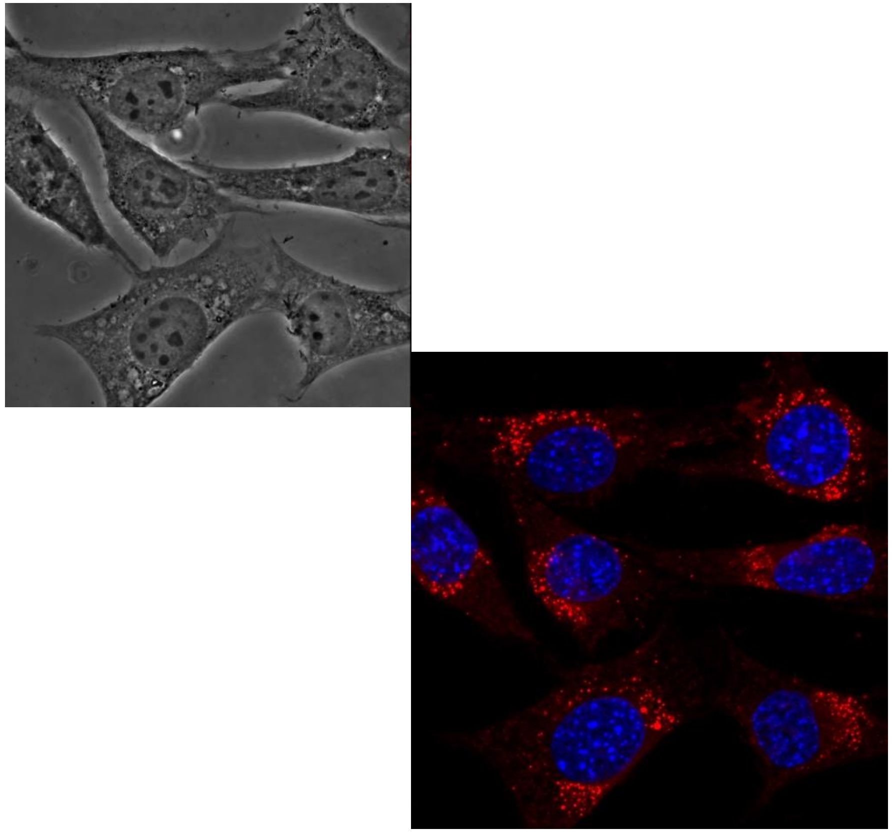
Enhanced axial resolution in **S**ingle **M**olecule (**F**luorescence) **L**ocalisation **M**icroscopy

By Philipp Zelger
Medical University of Innsbruck
Institute of biomedical physics

Supervisor assoz.Prof Alexander Jesacher

MIP Seminar 2019

Fluorescence microscopy



https://www.imaging-git.com/science/light-microscopy/methods-analyze-lipid-bodies-microscopy

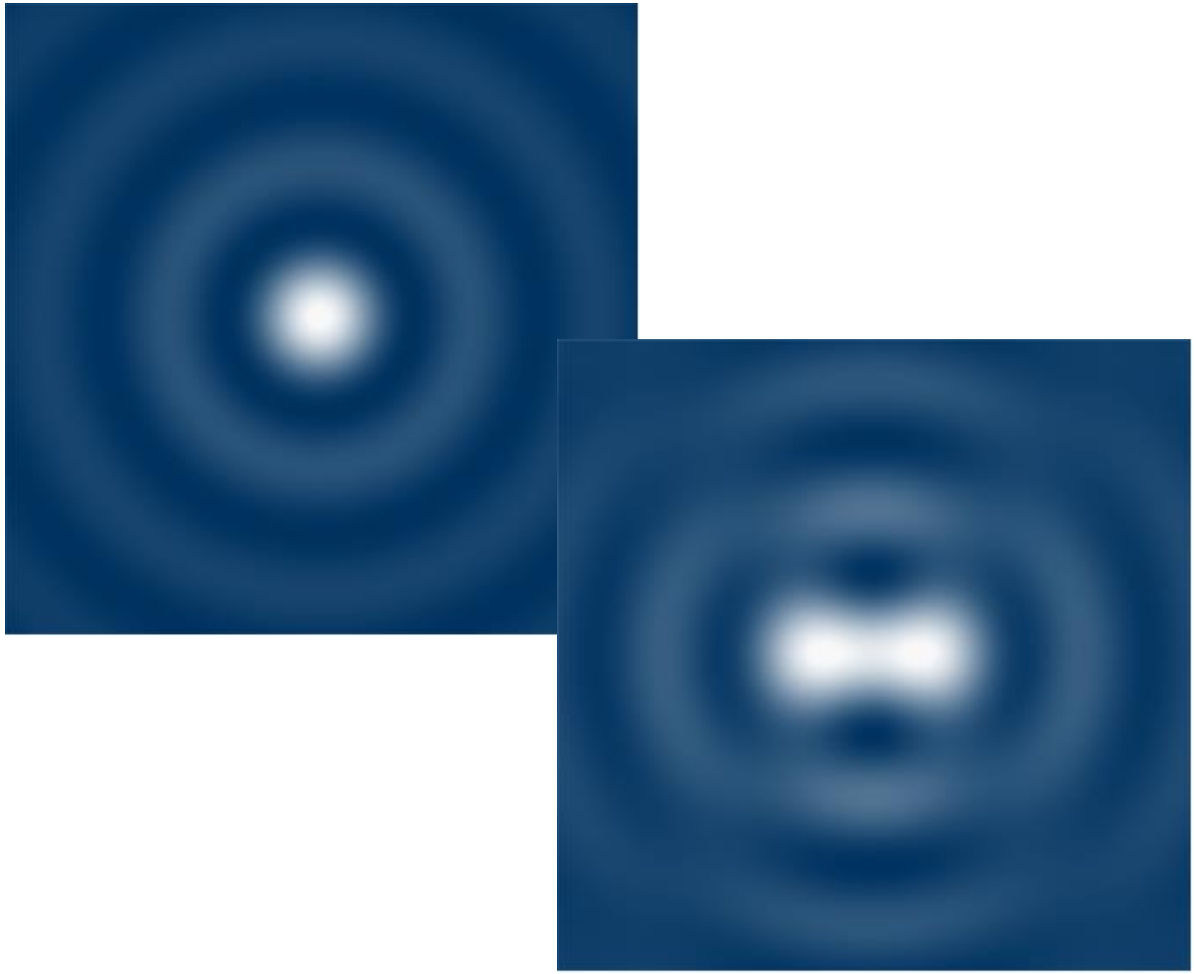
The Problem:

- All of the light goes trough the sample
- No selectivity

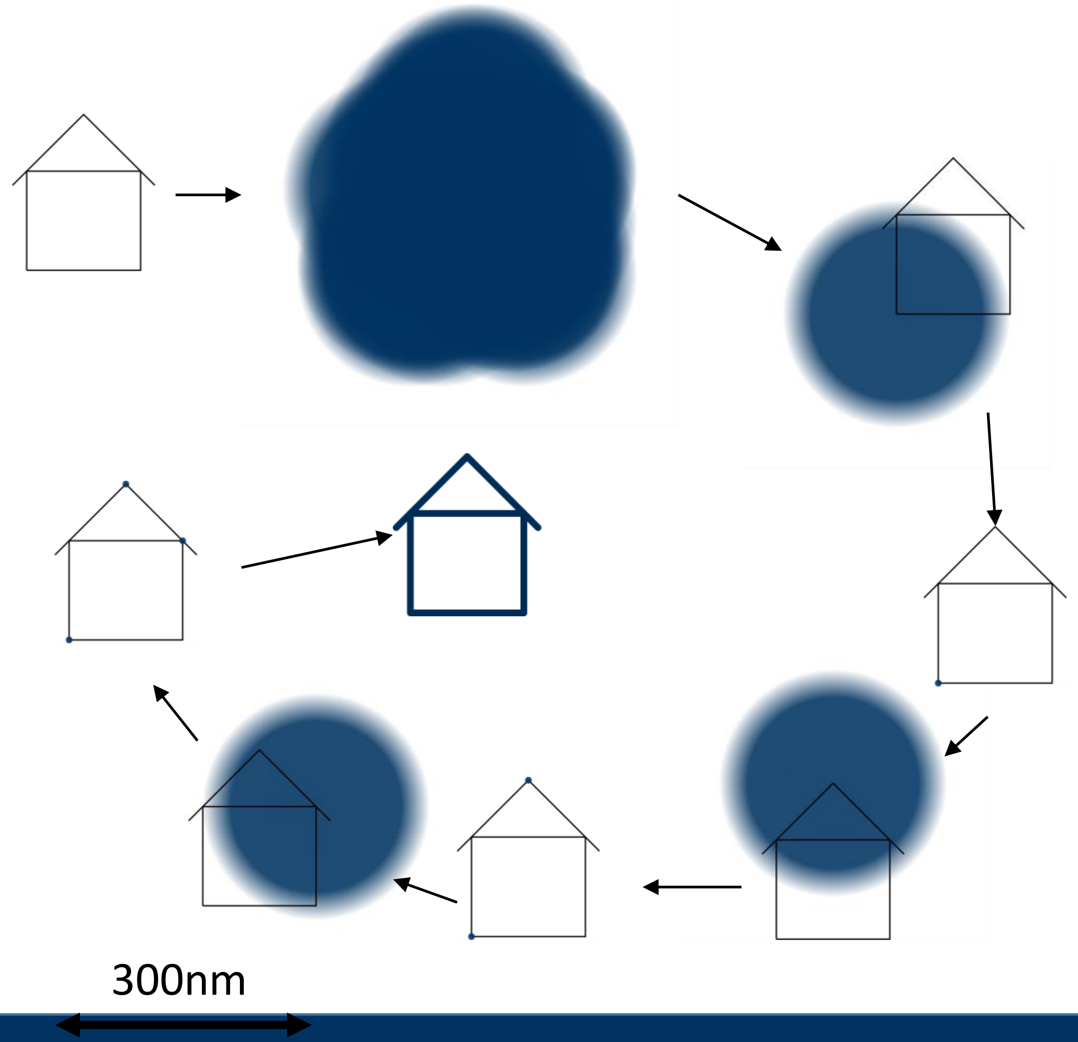
Solution:

- Mark the interesting part with fluorophores
- Fluorescence is the effect of absorbing and reemitting light
- Fluorescent markers bind on selected molecules via antibodies

How far can we go?



- Resolution is defined by the distance of the closest resolvable pair of points
- An imaging system gives the picture of a point
- Image is blurred/spreaded out representation of the point source (**P**oint **S**pread **F**unction)
- Two point are resolvable if they can be distinguished



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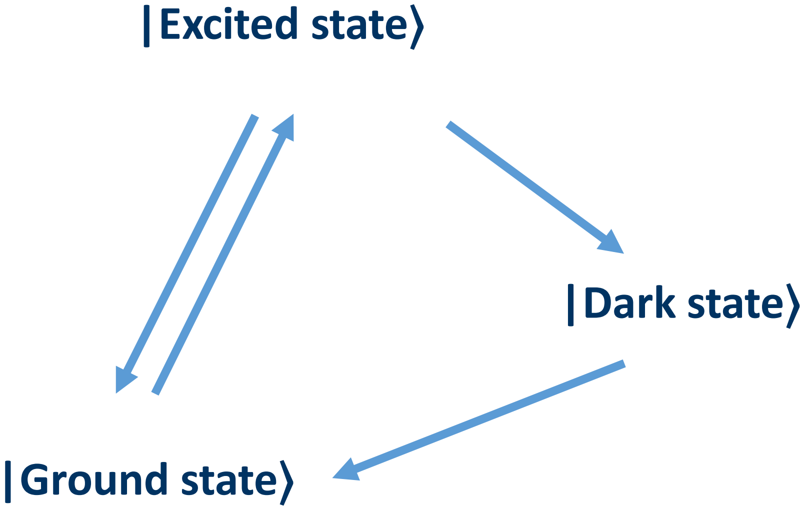
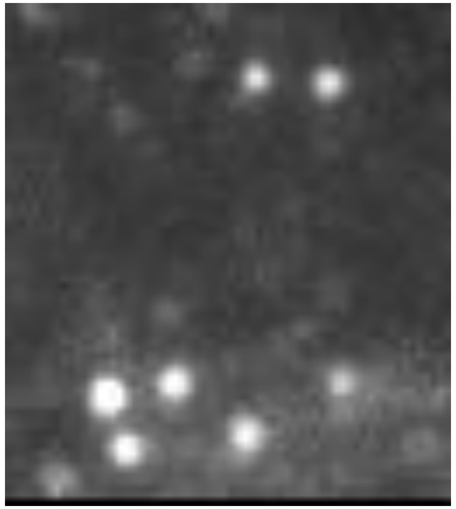
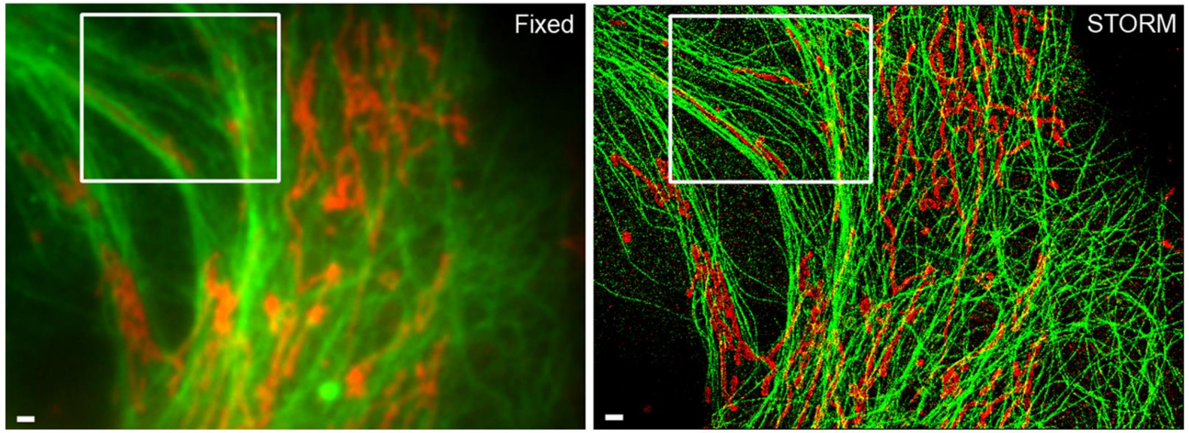
STORM imaging





How can this be done?

- A fluorophore can be described as a three state system
- When the fluorophores is illuminated it transfers in to the bright state
- From there the can go back to the ground state, go in to a dark state or even be destroyed
- A buffer medium prevents the destruction of the molecules and keeps (most) them in the dark state
- Some of them still make it in to the ground/exited state and start to blink randomly



https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0115512.g003

Problem:

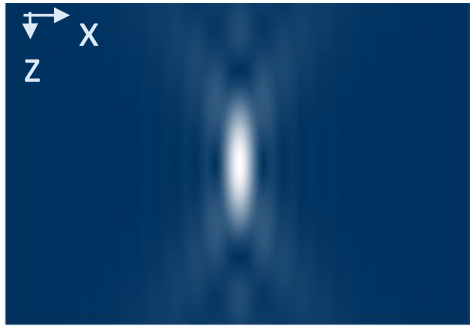
- It is difficult to resolve images in different distances to the camera plane
- The three dimensional PSF is elongated in z-direction
- We just see a cross section of the 3D PSF on the camera plane

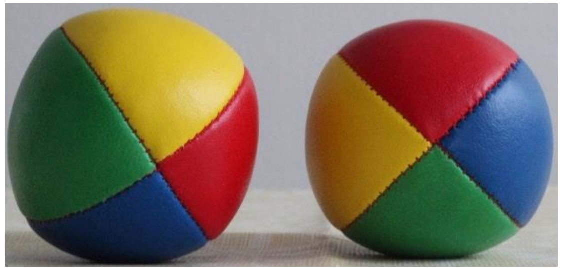
Solution:

- We need a property which changes with z position
- With that we can influence the shape of the PSF
- Supercritical angular fluorescence (SAF) light

What are we interested in?

- Cell membranes in close vicinity to the glass surface
- How close is close?
- Between 0 and 250 nm

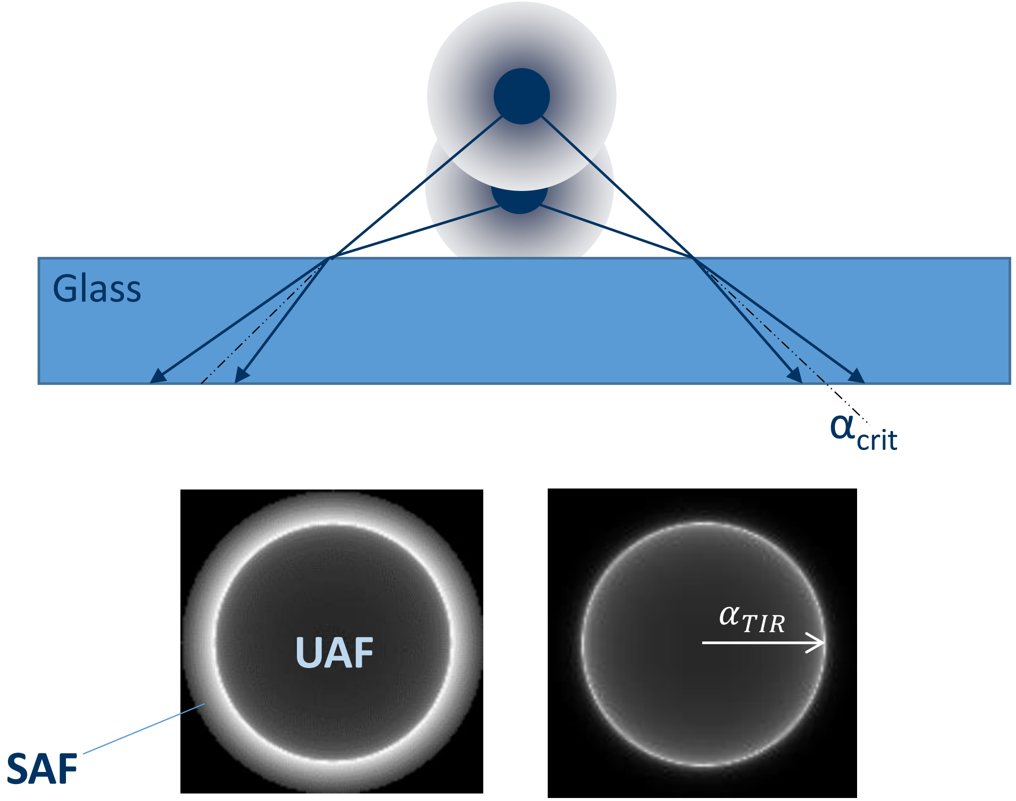




Supercritical angle fluorescence

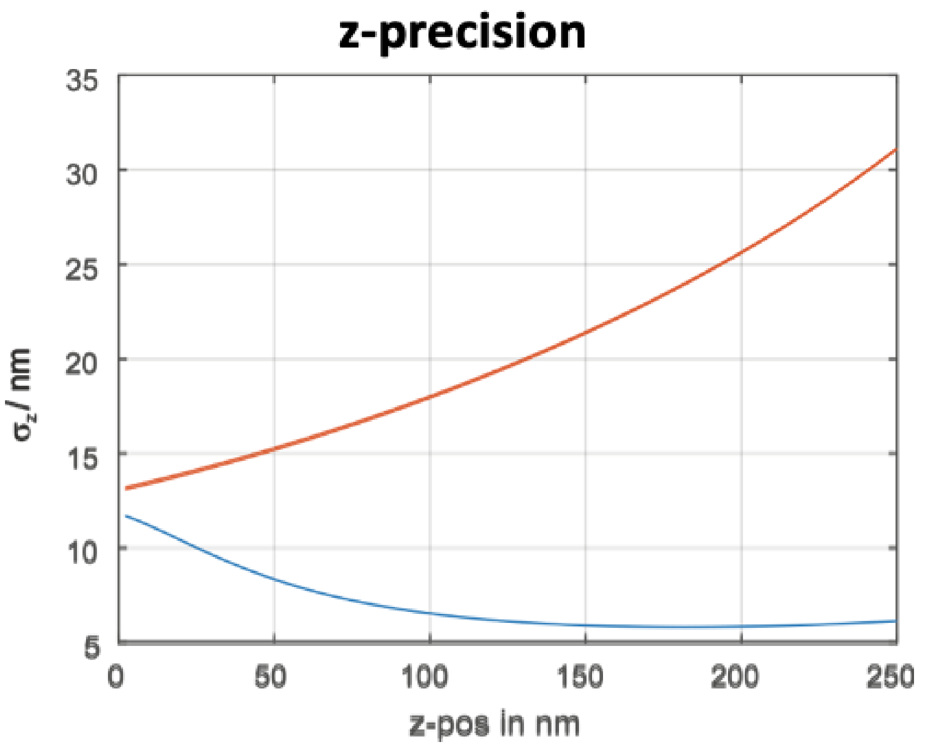
<u>Fluorescent molecules can be described as dipoles:</u>

- Tiny Antenna with oscillating charges
- The far field propagates -> Light is emitted
- The near field does not propagate under normal conditions
- But if the dipole gets close to a dielectric material (glass) it starts to propagate into the material
- The amount of SAF-light can be used to determine the z-position



Information content

- The best possible resolution can be calculate by the Cramér Rao lower bound (CRLB)
- The CRLB expresses a lower bound on the variance of unbiased estimator of a deterministic parameter
- Signal or photon number ratio contains only a part of the information
- Blue curve: taking into account the PSF shape as well



Signal = 2k photons
Bg-level 100 photons/pix

Maximum Likelihood Estimation (MLE)

‘Comparing’ the measurements I to a numerical model S:

– Least square fitting

$$argmin_{\theta}\left[\sum_k |S_k - I_k(\theta)|^2\right]$$

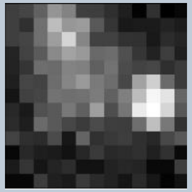
with $\sum_k$ the parameters θ = (x,y,z,Int,BG) and the pixel index k

- Maximum Likelihood for Poisson noise

$$argmin_{\theta}\left[\sum_k |S_k - I_k(\theta)\ log(S_k)|^2\right]$$

Problems:

- For each molecule you need to find the global minimum in a 5-dim. space!
- Therefore it is really slow (~30s per molecule image)
- Fails at high molecule densities



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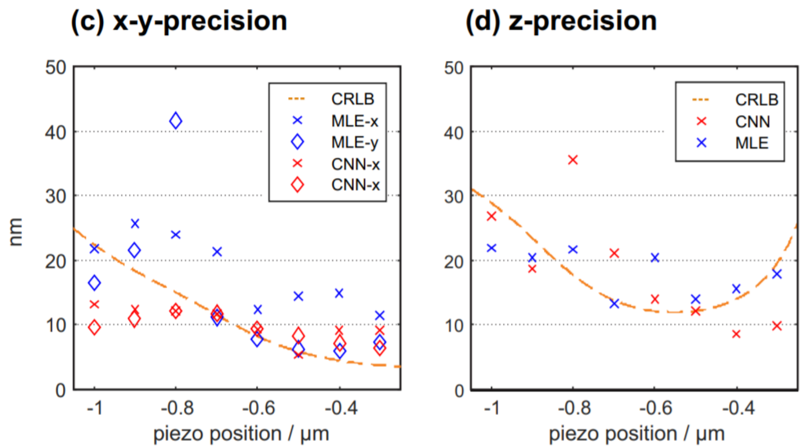
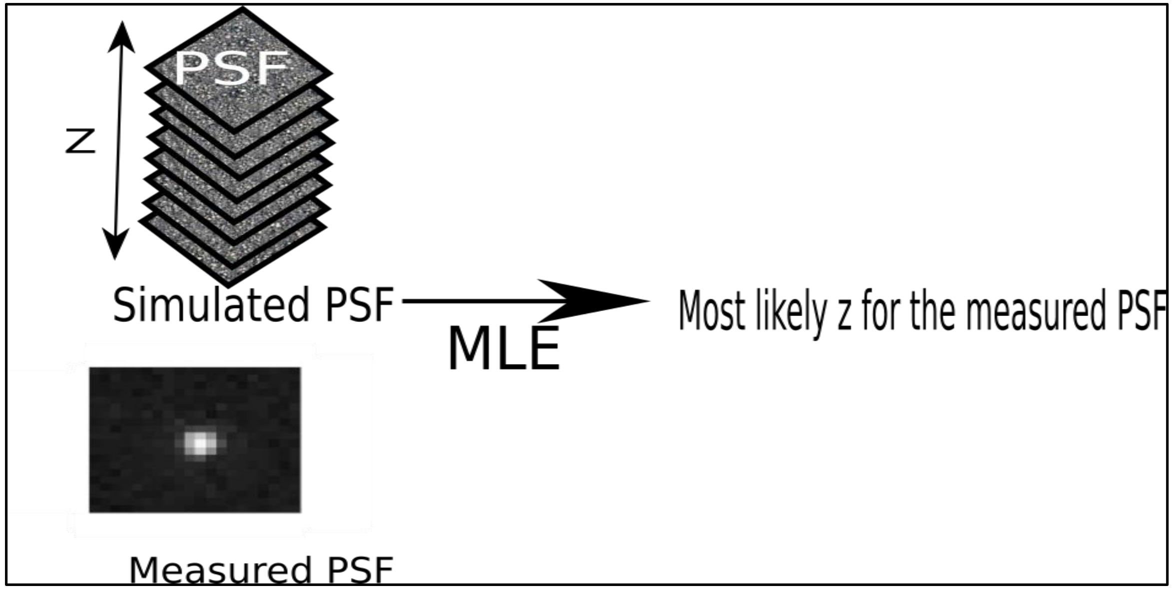
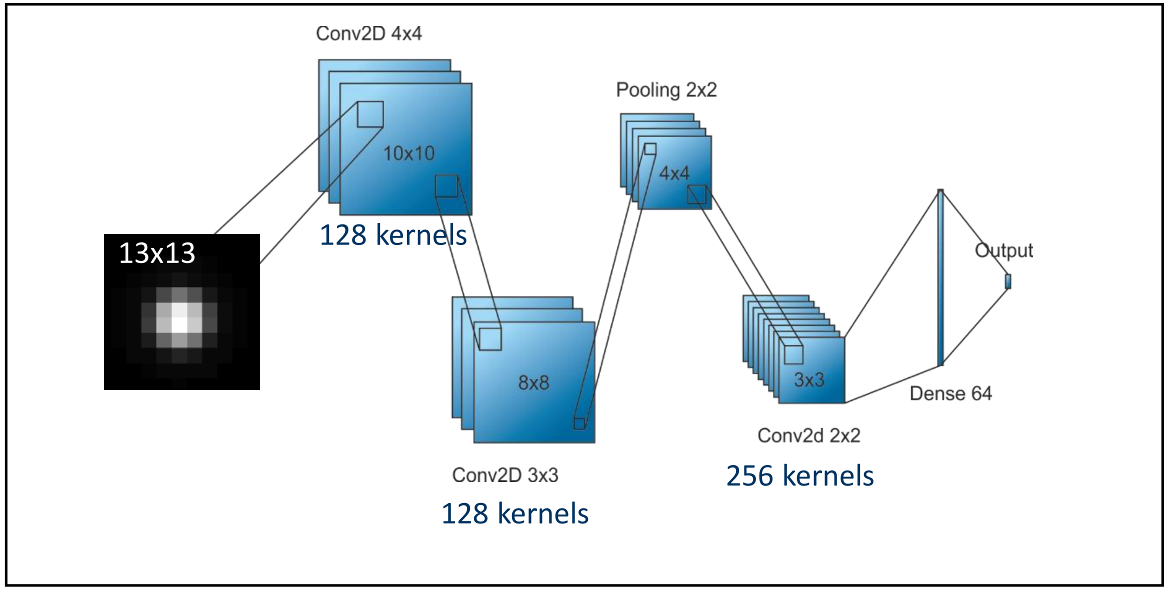
MLE vs CNN

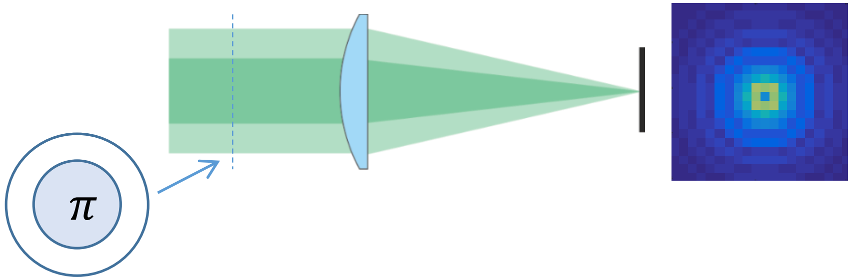


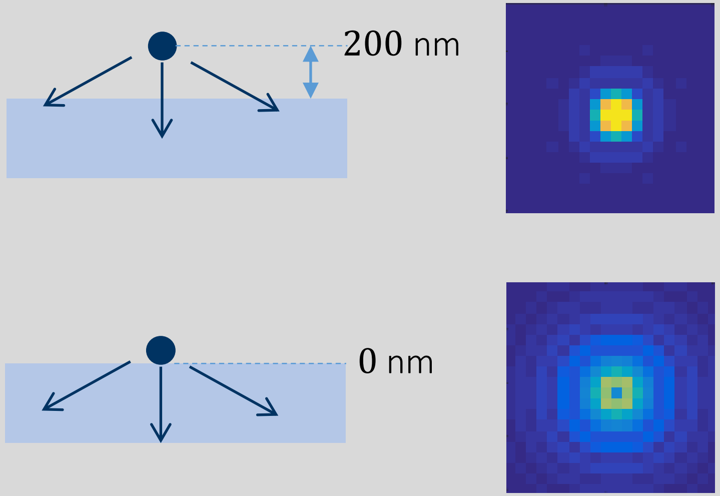




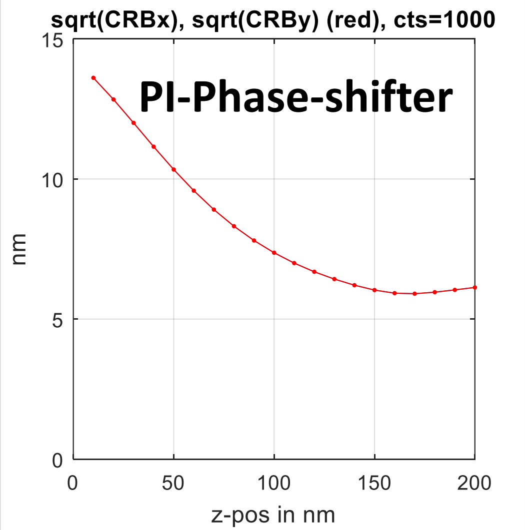
P. Zelger, K. Kaser, B. Rossboth, L. Velas, G. J. Schütz, and A. Jesacher, Opt. Express 26, 33166-33179 (2018)

First Idea: use a π phase shifter for optimal interference

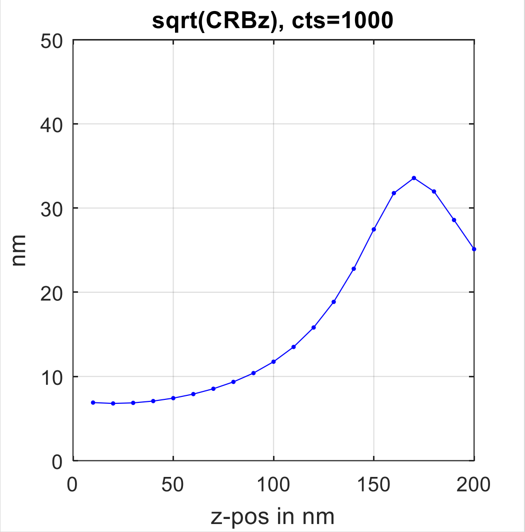




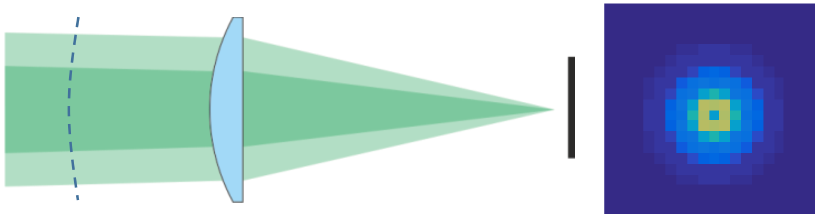
CRLB x/y



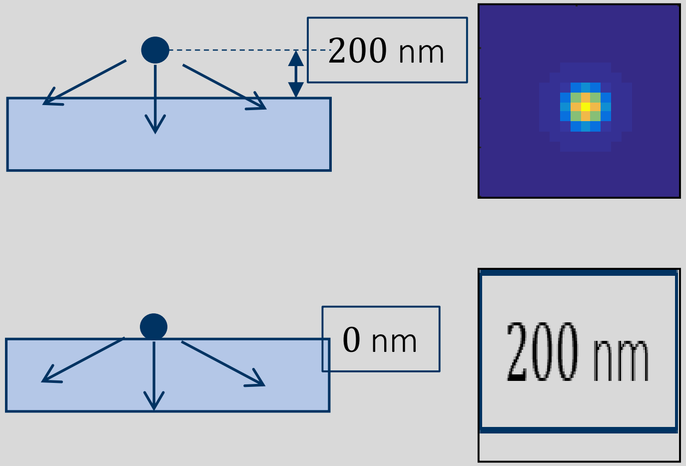
CRLB z



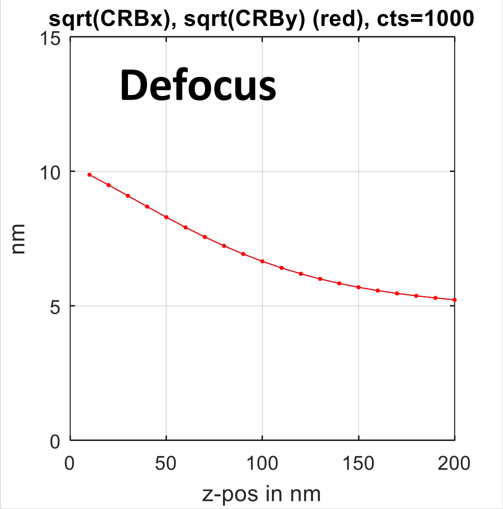
Even simpler „engineering“: defocusing the image



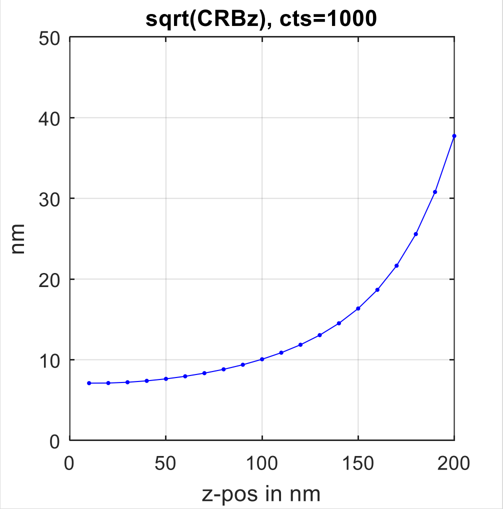
Defocus $\approx 400 - 500$ nm



CRLB x/y



CRLB



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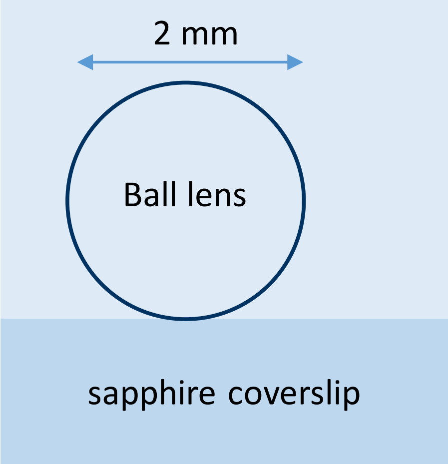
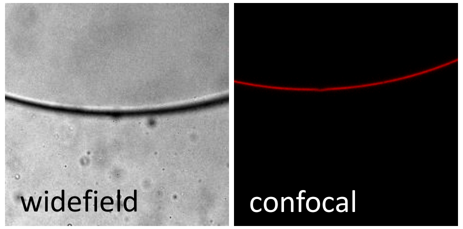
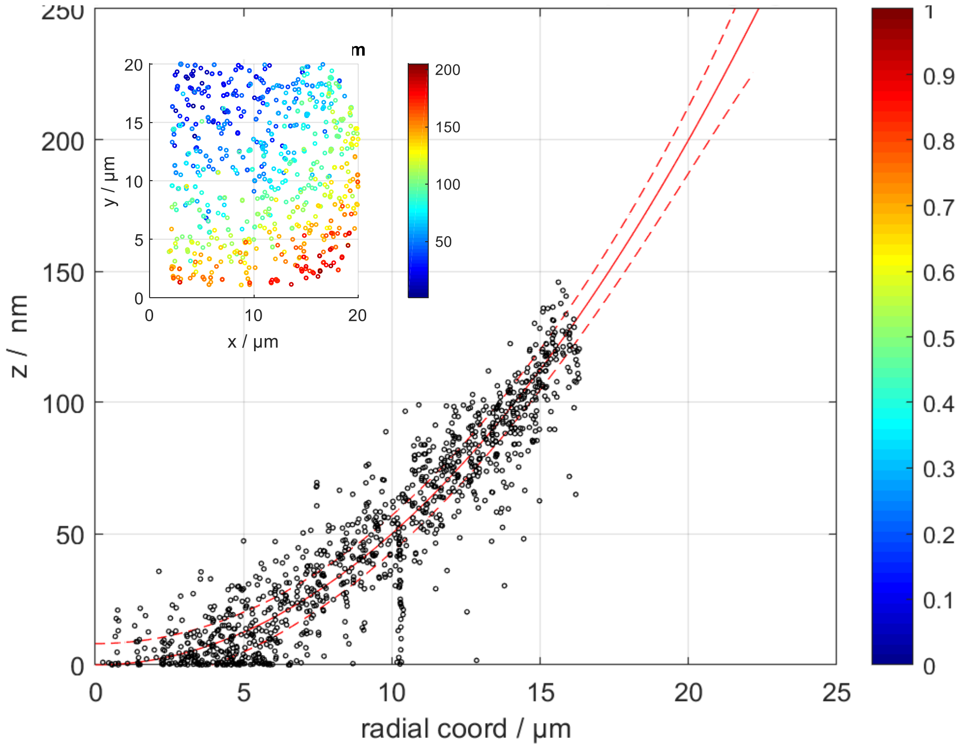
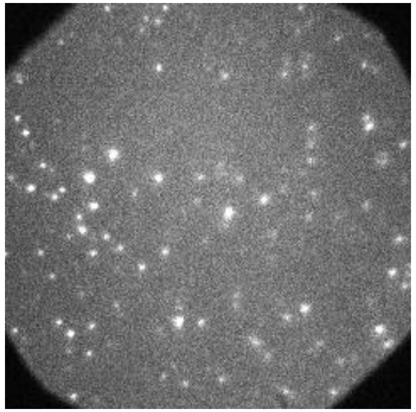
Test Sample



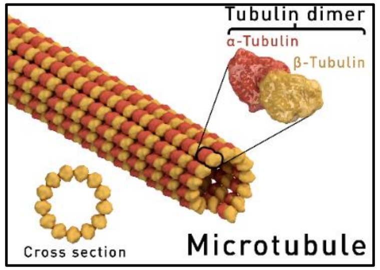


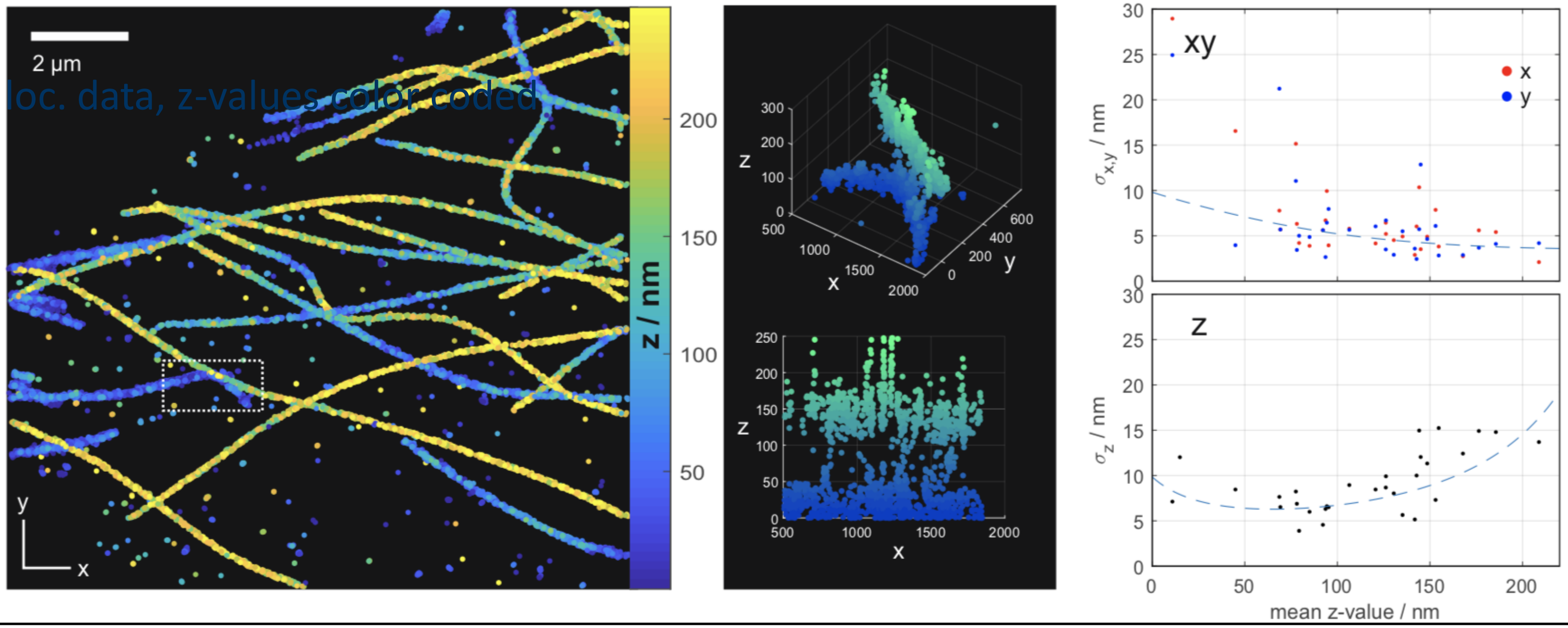




Results

Experimental results from dSTORM measurement on stained microtubules in COS7 cells





Defocused imaging exploits supercritical-angle fluorescence emission for precise axial single molecule localization microscopy. 2019 Biooptics Express

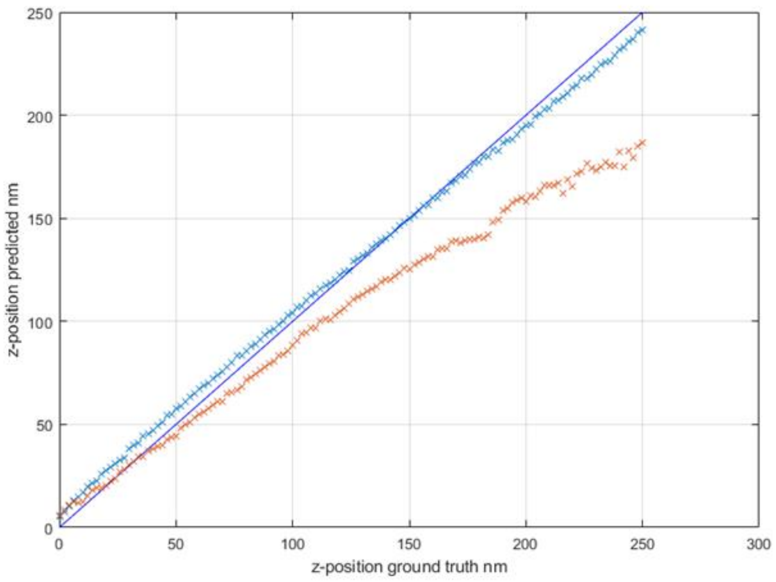
Philipp Zelger; Alexander Jesacher et. All (peer review)

What I did not show:

- The setup
- Bio experiments

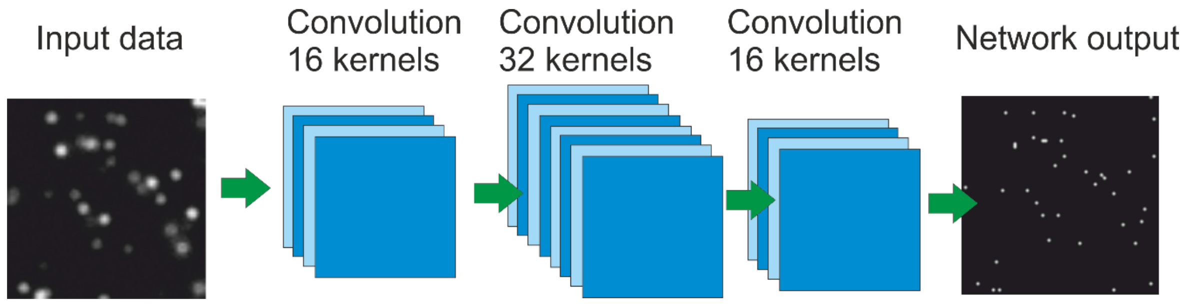
What comes next:

- Biplane imaging
- New CNN to estimate aberrations

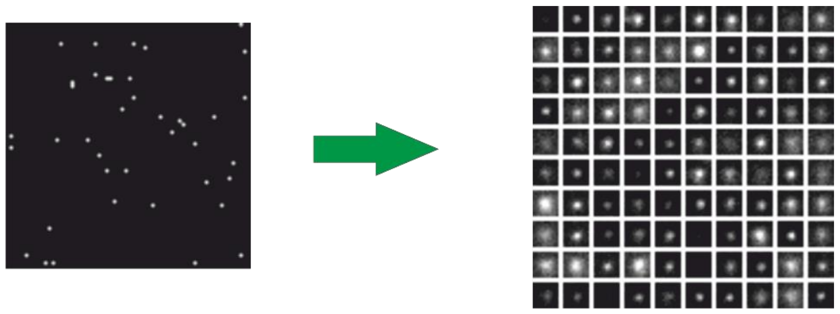


Additional information

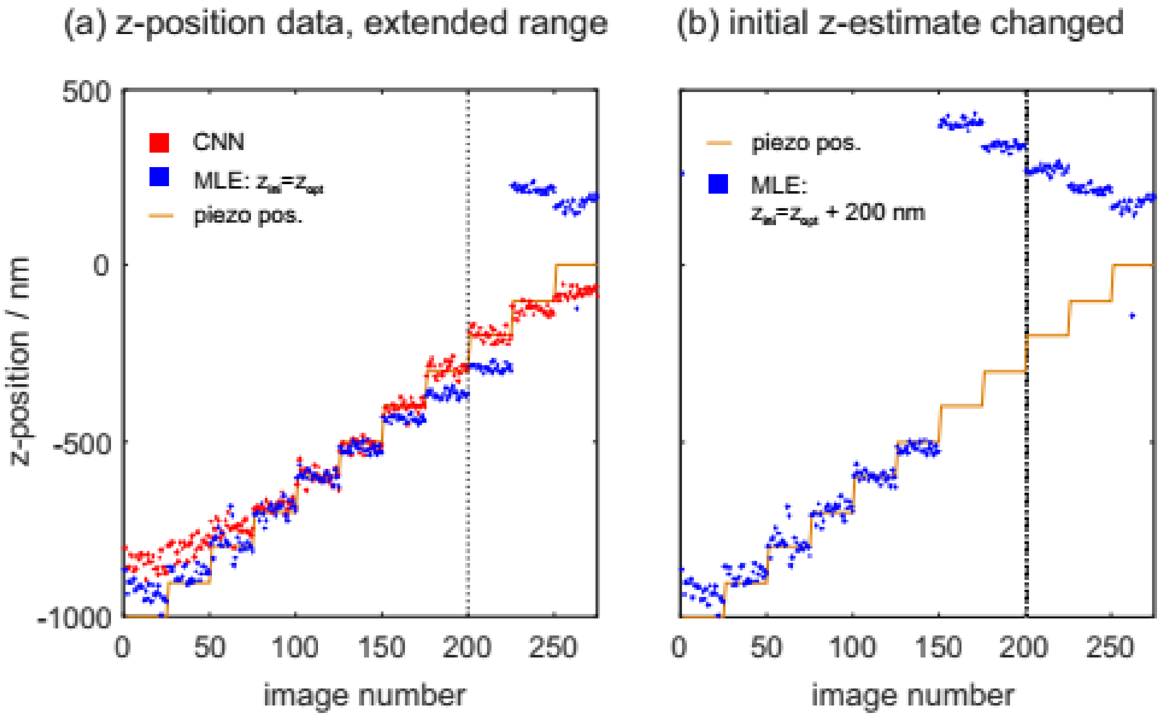
- Autoencoder network deconvolves raw images from camera

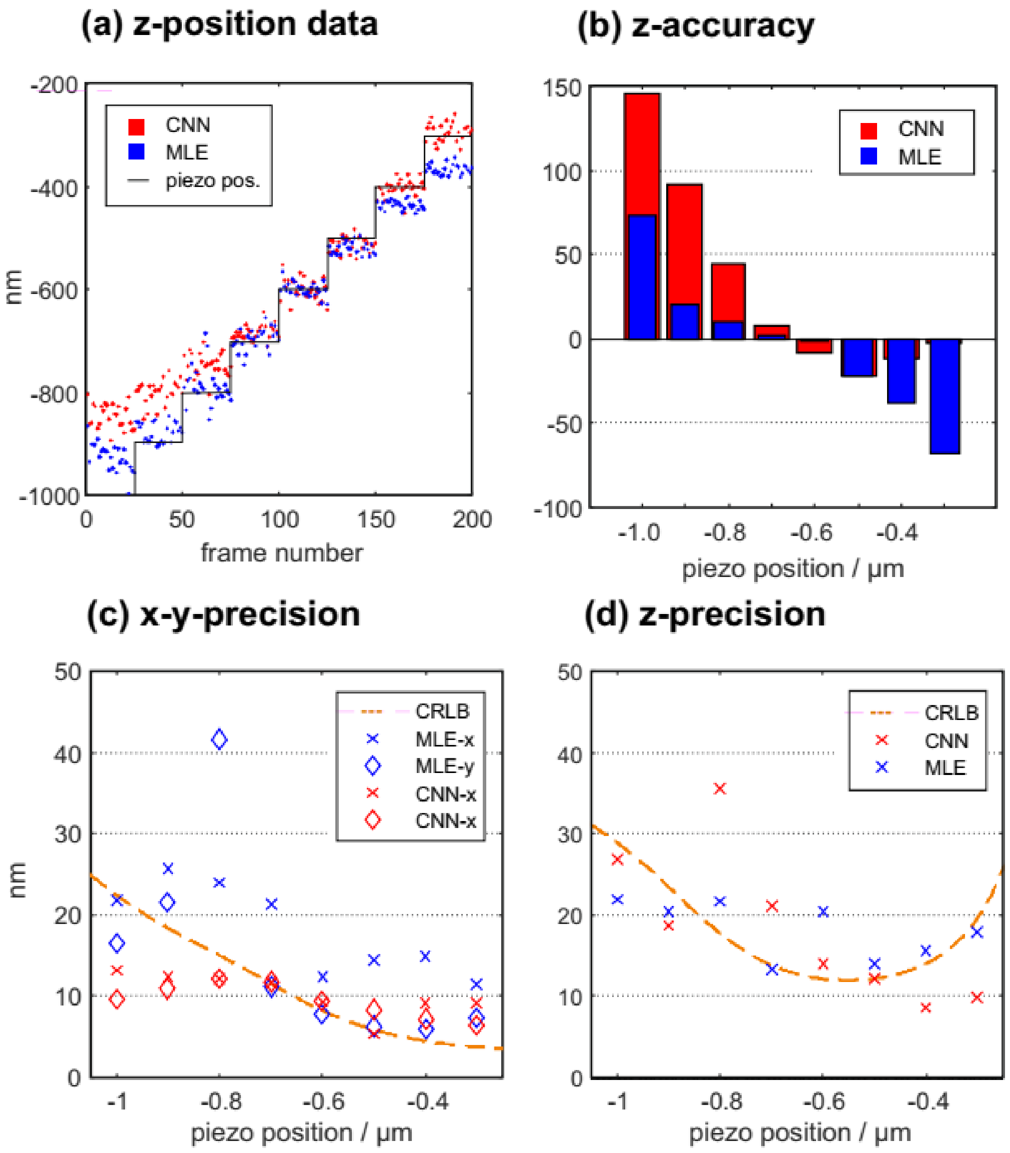


- Cropping of individual molecules is facilitated



Comparison between MLE & CNN





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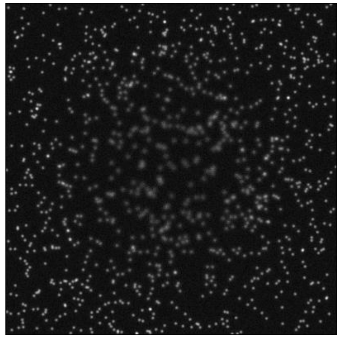
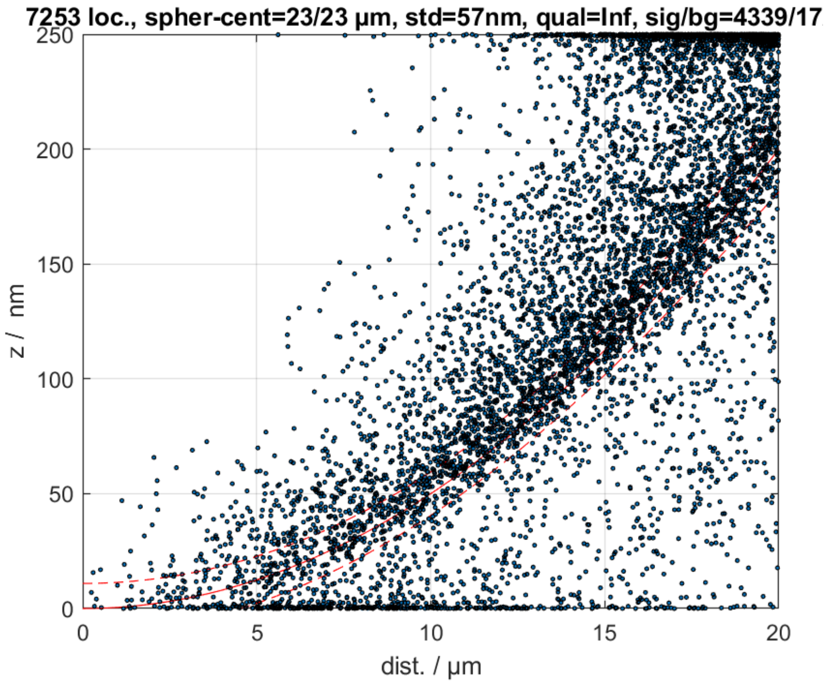
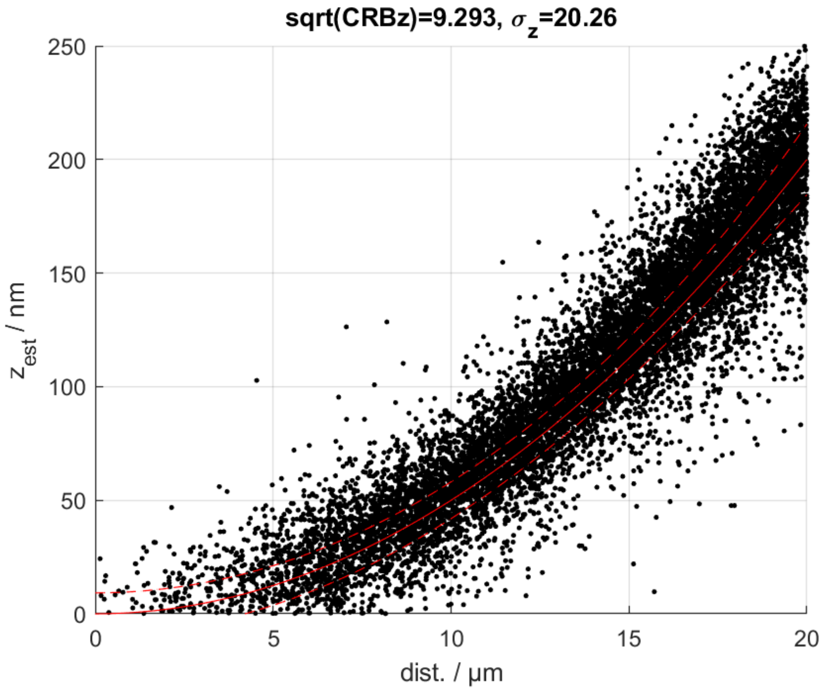
Additional information

Simulation: dense sample 0.5 mol./µm²







Additional information

Experiment: unfiltered data