Challenges in Microscopy Big Data

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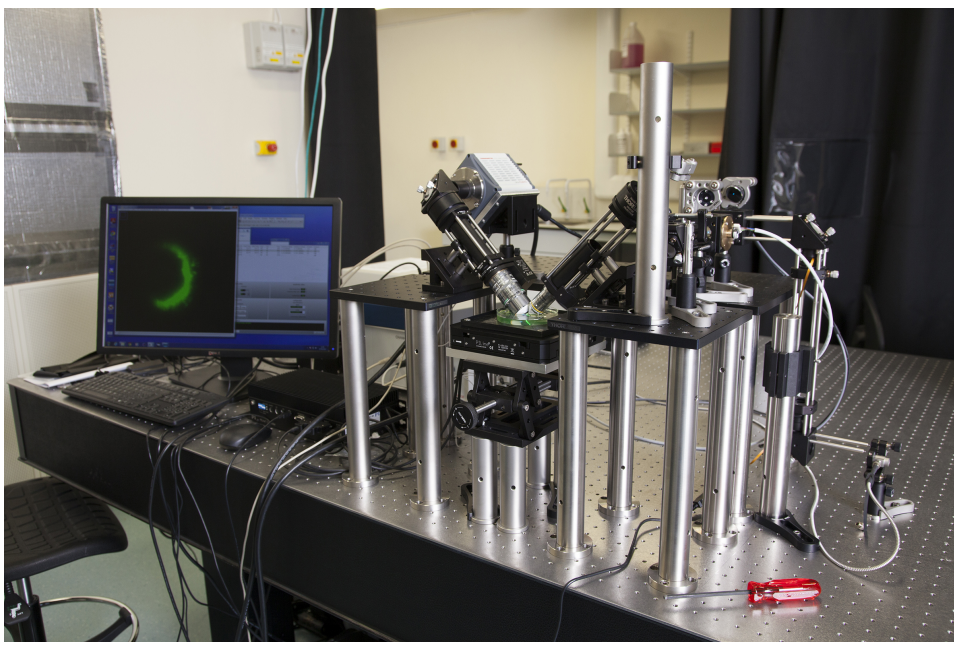
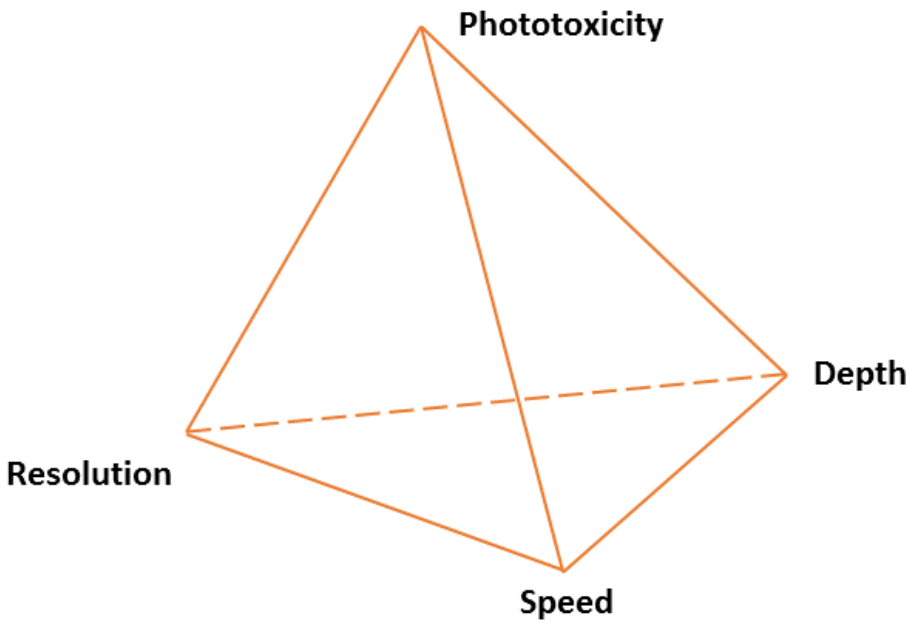
Outline

Motivation

Main challenge in fluorescence microscopy

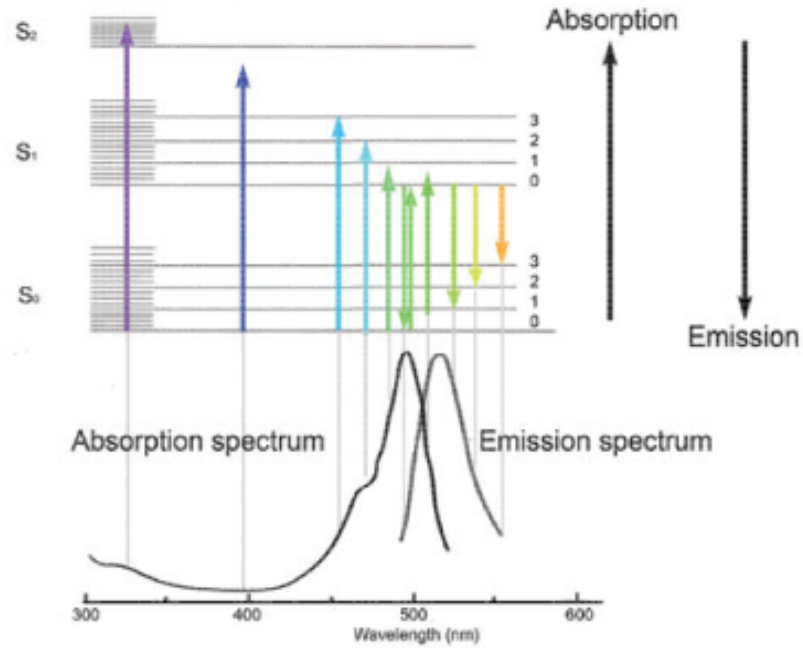


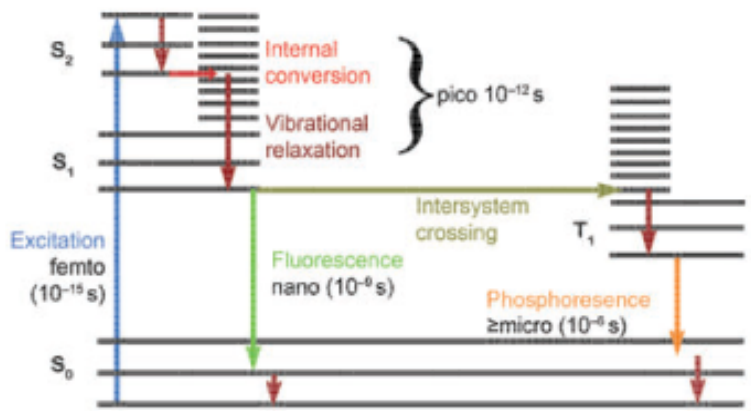


Fluorescence microscopy

Principle

- Fluorescent dyes are added to the sample.
- The dyes are excited by a source of illumination of a given wavelength
- The dyes are emitting at a longer wavelength and their response is registered by a CCD camera
- After several emission cycles, the dyes bleach
- **Phototoxicity** - Excited fluorescent molecules tend to react with oxygen, producing free radicals that can damage the cell





Lichtman J. and Conchello J.-A., Fluorescence Microscopy,

Nature Methods, vol. 2, 2006

Image formation

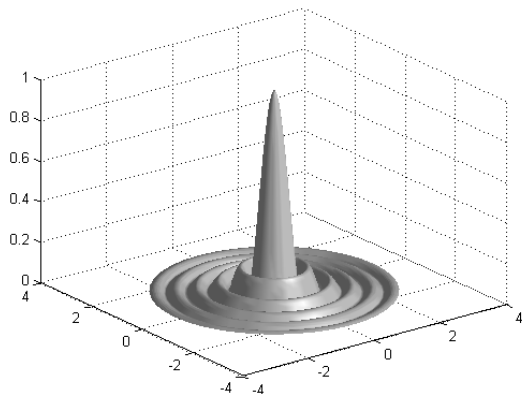
Point spread function

The image of a point source is the point spread function. Fluorophores can be regarded as point sources.

Theoretical models of PSF

- Richards-Wolf model
- Gibson-Lanni model

Approximations of PSF



Airy pattern

"All models are wrong, but some are useful." (G. Box)

- Airy function

$$\mathrm{PSF}(r) = \left(2\frac{J_1(\pi q_c r)}{\pi q_c r}\right)^2,\ q_c = \frac{2NA}{\lambda}$$

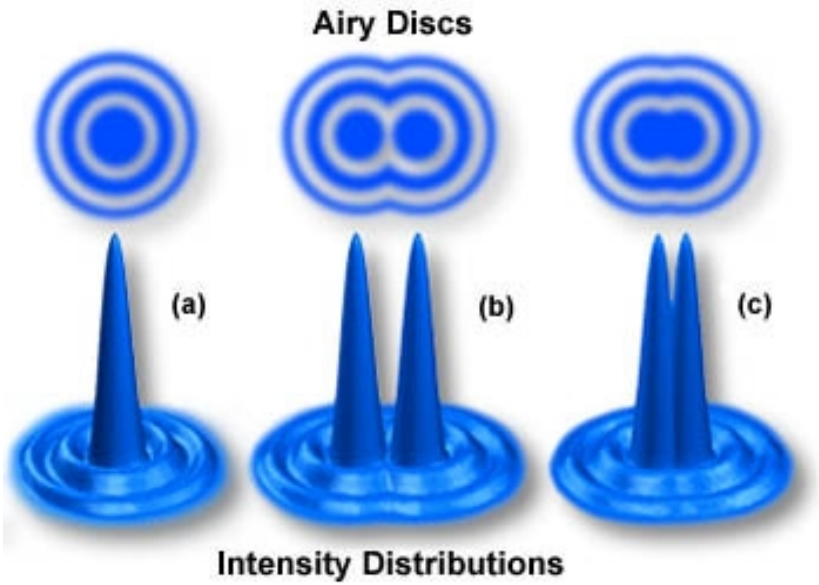
- Gaussian: $G(r) = e^{-\left(\frac{r^2}{2a^2}\right)}$
- modified Lorentzian: $L(r) = \frac{1}{1+\left(\frac{r^2}{a^2}\right)^b}$
- Moffat: $M(r) = \frac{1}{\left(1+\frac{r^2}{a^2}\right)^b}$

Resolution limits

Rayleigh criterion

Two point sources are regarded as just resolved when the principal diffraction maximum of one image coincides with the first minimum of the other.

$$R = \frac{0.61\lambda}{NA}$$



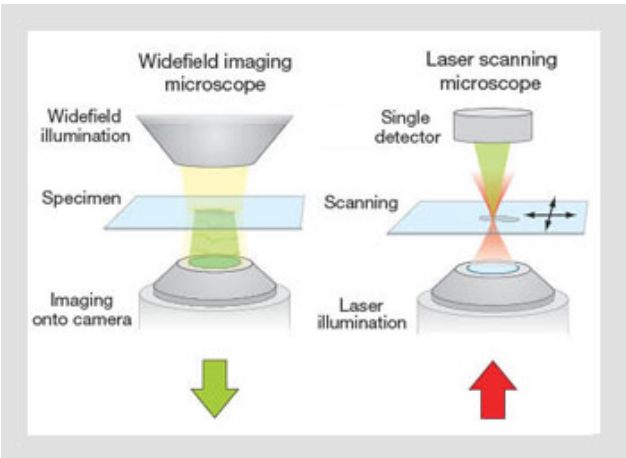
Novel technology

- **Light sheet microscopy** E. Steltzer
  principle: Zsigmondy (1925)
- **Super-resolution fluorescence microscopy**
  E. Betzig, S.W. Hell, W.E. Moerner - Nobel prize for chemistry 2014

These techniques imply huge increase in data and computation needs.

Light sheet fluorescence microscopy

- Illumination with beams collimated in one and focused in the other direction
- No fluorophores are excited outside the detectors' focal plane, no out-of-focus light (intrinsic optical sectioning) and less photodamage



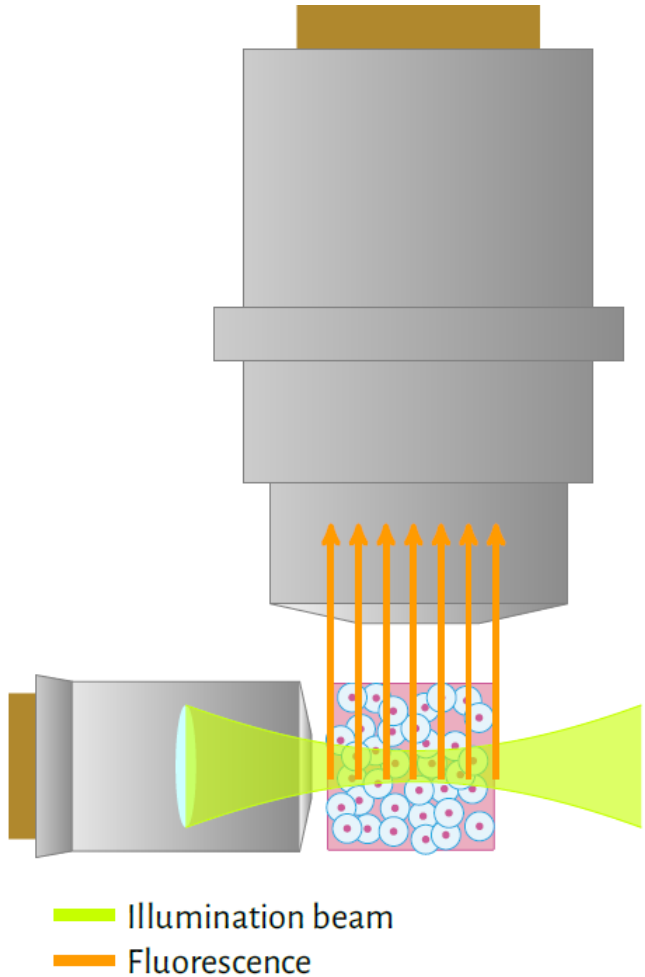
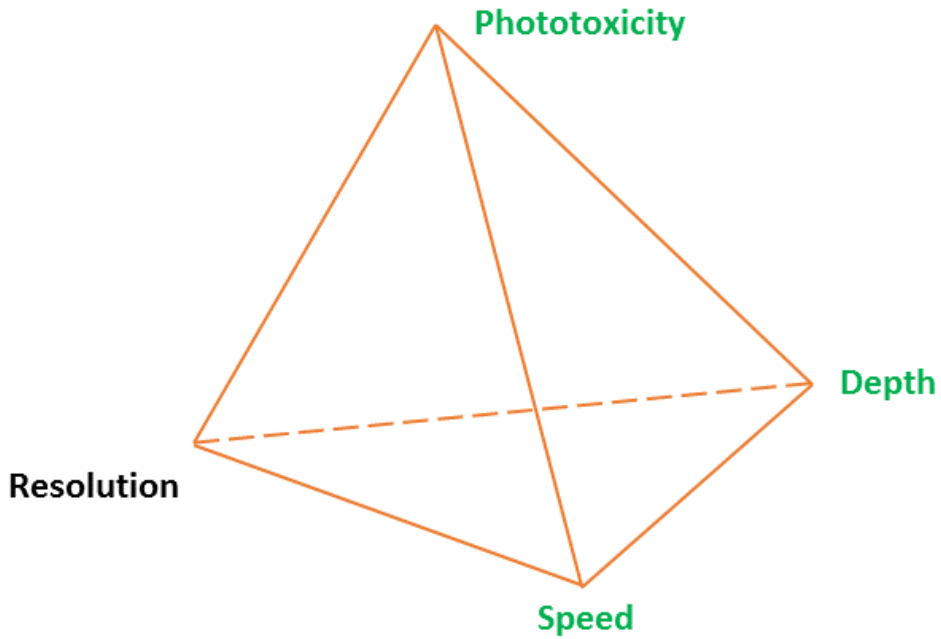


Illustration: Ruth Simms (CAIC)

Light sheet fluorescence microscopy

Advantages

- Speed: ~ 800 MB frame/s , ~ 100 frame/s (limited by the camera). Results in huge amount of data.
- Parsimonious, efficient illumination



Resolution

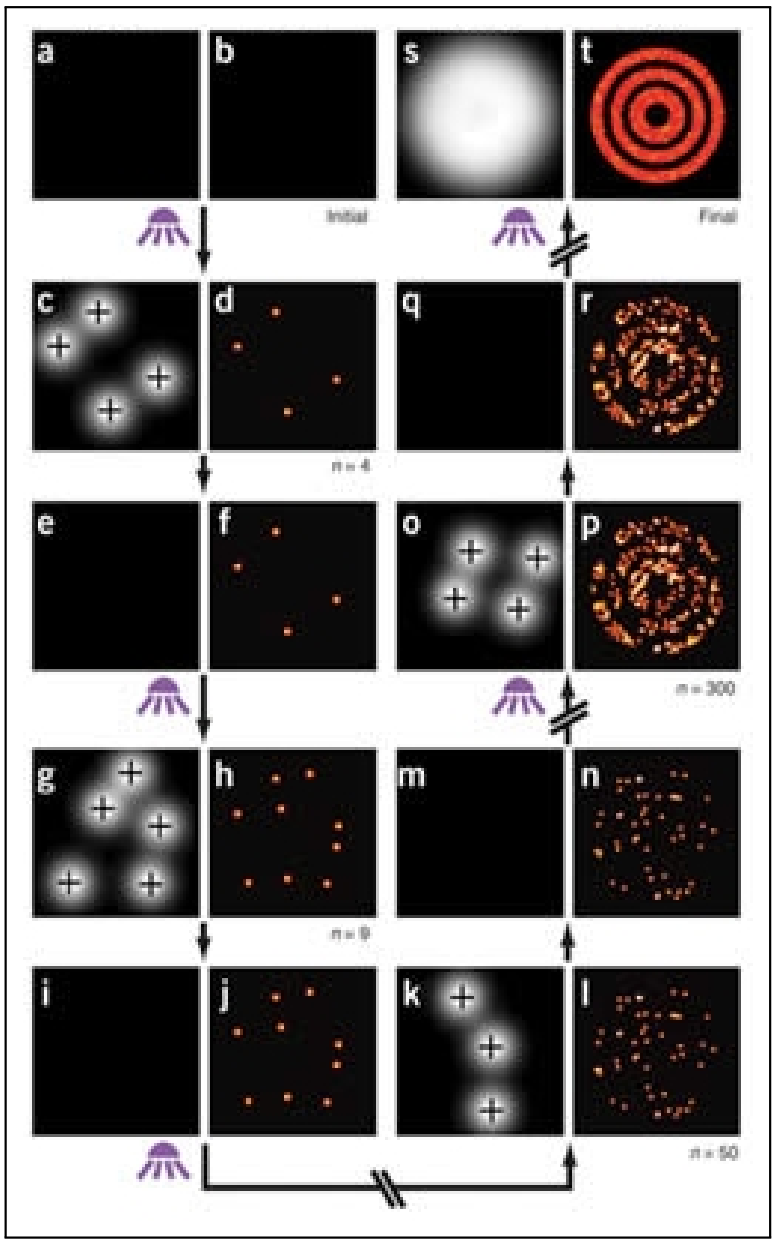
Conventional confocal microscope

- Lateral $(x-y)$ resolution limit: $\lambda/(2NA)$ (~ 200 nm)
- Axial (z) resolution limit: $2n\lambda/NA^2$ (~ 500 nm)

Light sheet microscope microscope

- The axial resolution is dominated by the thickness of the light sheet and is less affected by the PSF defined by the NA of the detection lens.
- The isotropy of the PSF is much better than other microscopes for low NA systems

What is "nanoscopy"?



Super-resolution light microscopy

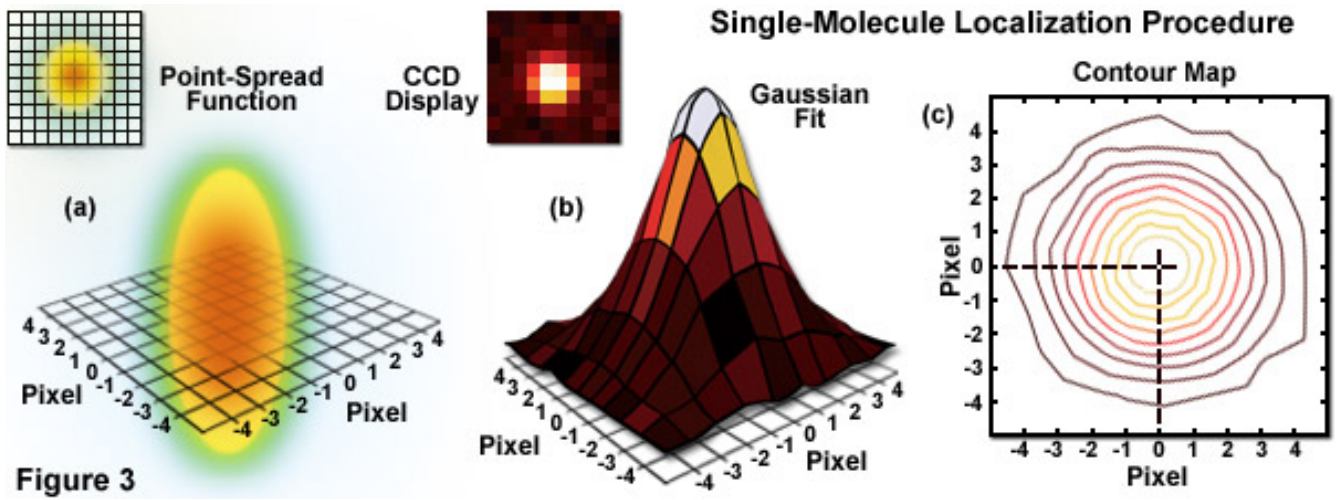
- Photo-activated localization microscopy (PALM)
- Stochastic optical reconstruction microscopy (STORM)
- Other (STED, SIM)

Characteristics (STORM/PALM)

- Fluorophores are stochastically alternating between active and dark state
- Single, well-separated fluorophores are imaged
- Algorithms localize with high precision individual fluorophores in images

T. J. Gould, V. V. Verkhusha, S.T. Hess, Nature Protocols 4, 291-308 (2009).

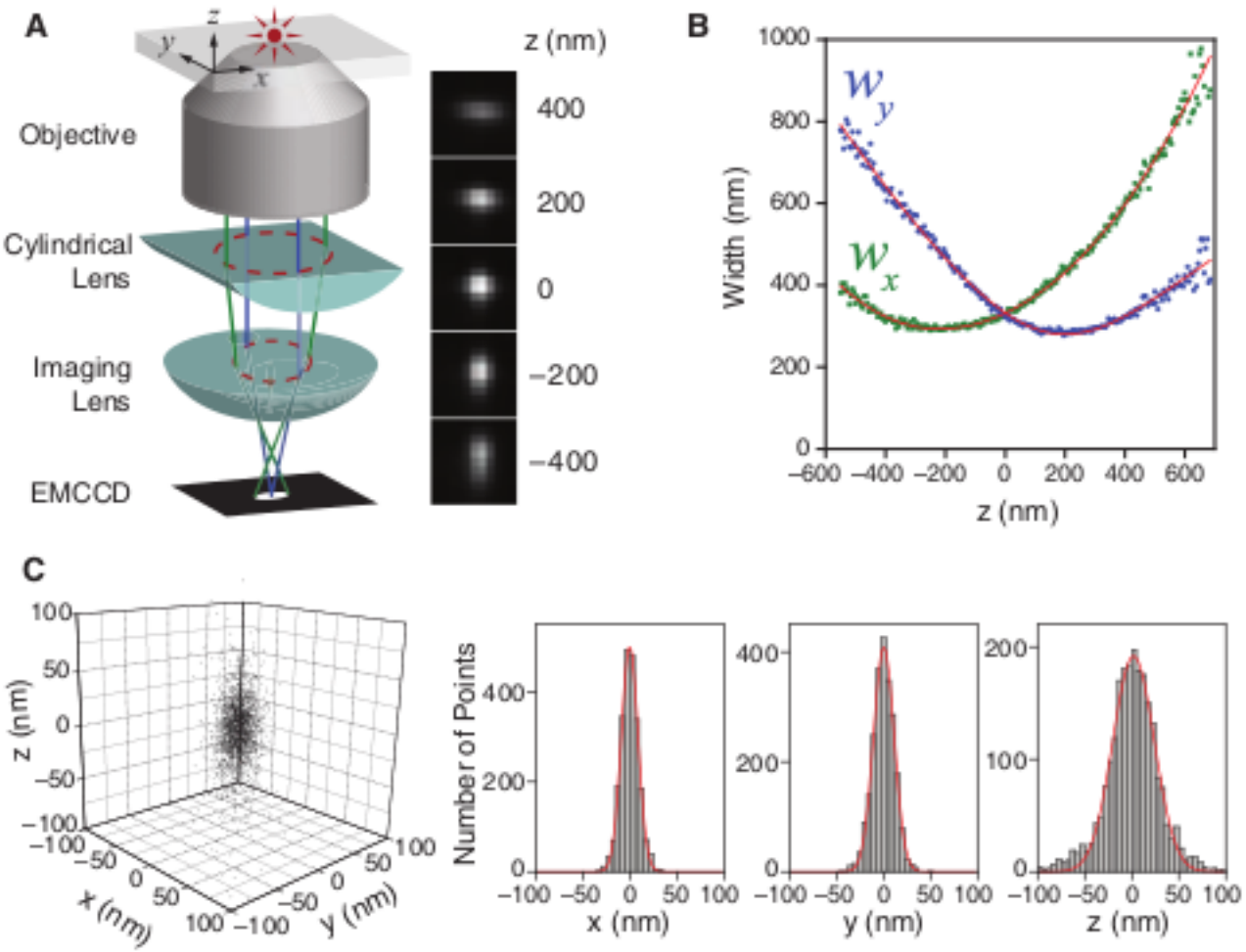
2d and 3d superresolution



Zeiss-campus

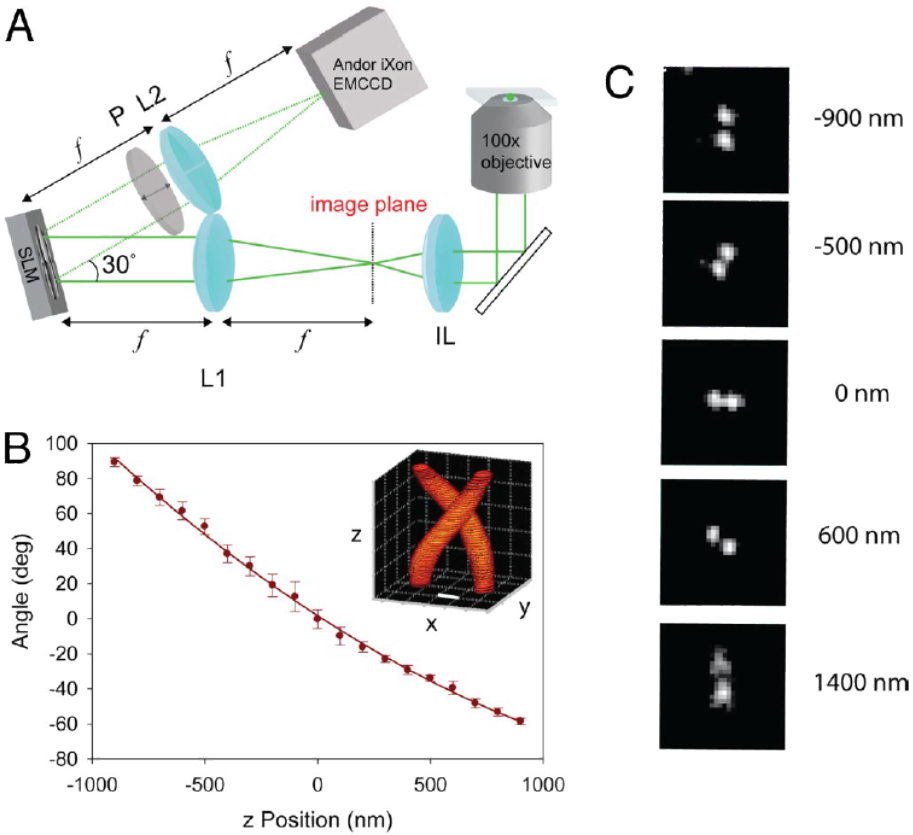
The image of the single molecule is the same at a distance d below and above the focal plane.
To gain information on z-position from the PSF shape: the symmetry has to be broken!

3d super-resolution via a cylindrical lens



B. Huang, W. Wang, M. Bates, X. Zhuang, "Three-dimensional Super-resolution Imaging by Stochastic Optical Reconstruction Microscopy", Science 319, 810-813 (2008)

Engineered point spread function



Double helix PSF

- spatially rotating point-spread functions (depth from diffracted rotation)
- inspired from depth from defocus techniques (circular aperture)

A SM near focus in a DH microscope appears as two spots on a detector.
Lateral (x, y) position of the SM - the midpoint between spots
Axial(z) position - the angle of the line connecting the two spots and a fixed orientation (calibration measurement).

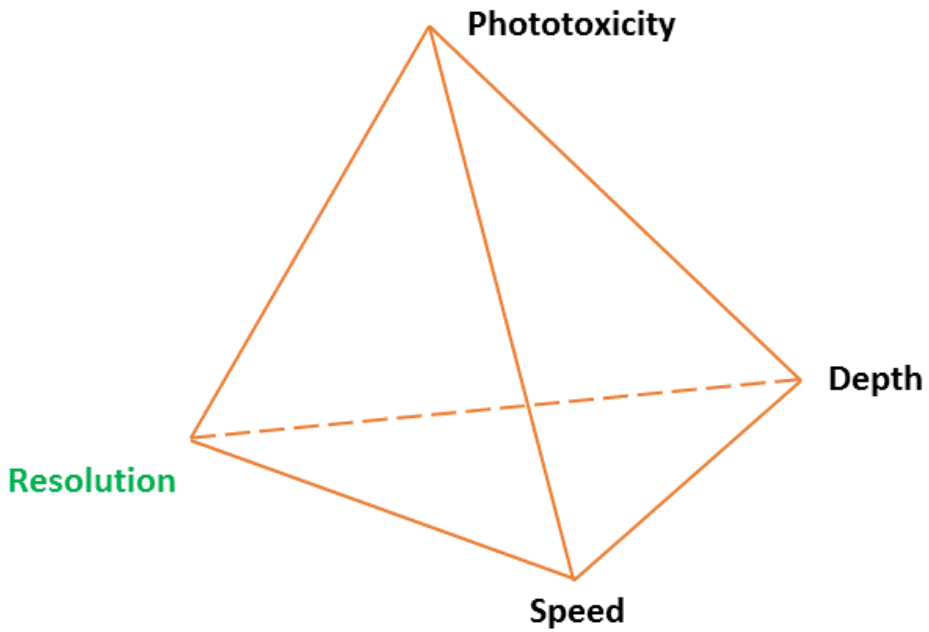
S.R. Prasanna Pavani, M.A. Thompson, et al. - Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function PNAS, vol.106 , no.9, pp. 2995-2999, 2009

Super-resolution microscopy

Resolution: Double helix PSF

In thick samples, superlocalization of single fluorescent molecules with precisions as low as 10 nm laterally and 20 nm axially over axial ranges $\sim 2\mu m$

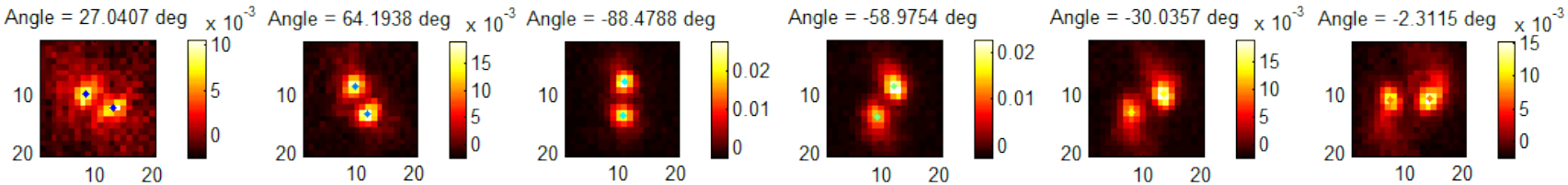
(Moerner, PNAS, 2009)



Goal: Combining the two techniques (light sheet and super resolution)
Need for fast and accurate image analysis algorithms!

Outline

Existing analysis: EasyDHPSF algorithm



Step 1: Detection

- Each template is phase correlated with the image
- The correlations are combined
- Peaks in the combined correlated image are validated to filter out extraneous matches.

Step 2: Fitting

Least-squares fitting of a double-Gaussian function:
robust to low signal, high background, optical aberrations, sample drift, or any combination of experimental non-idealities

M.D. Lew, A.R.S. von Diezmann, W.E. Moerner - Easy-DHPSF open-source software for three-dimensional localization of single molecules with precision beyond the optical diffraction limit, Protocol Exchange, 2013

Existing analysis: EasyDHPSF algorithm

User-friendly, good documentation!

Performance:

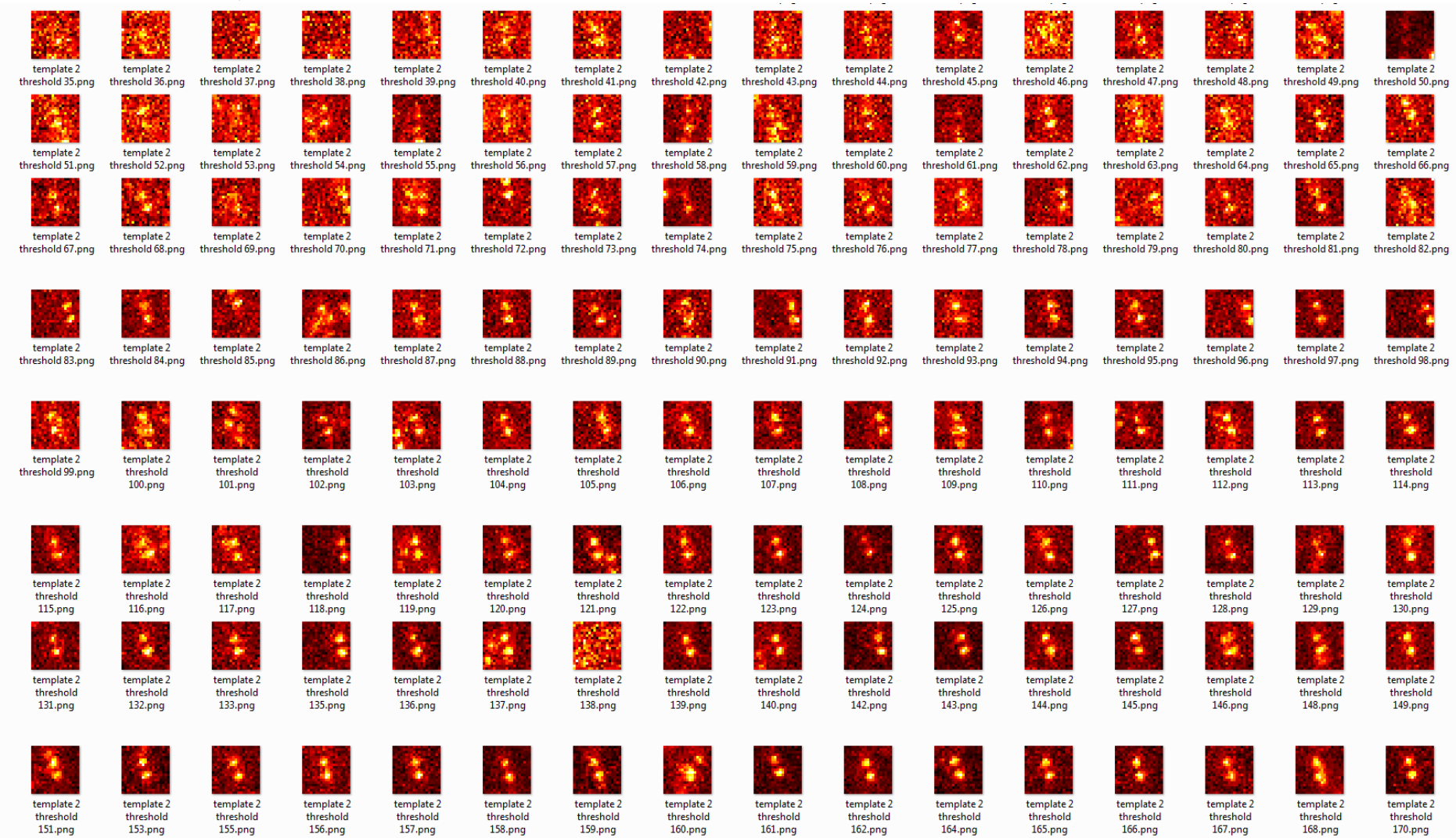
- $15 - 30$ molecules per second on a 3 GHz Intel Core 2 Duo workstation
- 3D super-resolution reconstruction of 100,000 molecules over a $20 \times 20 \times 2\mu m$ field of view (processing 128×128 pixels $\times 20000$ frames) in 75 min.

Parameters:

- Six thresholds have to be selected by the user (one for each template)

Existing analysis: EasyDHPSF algorithm

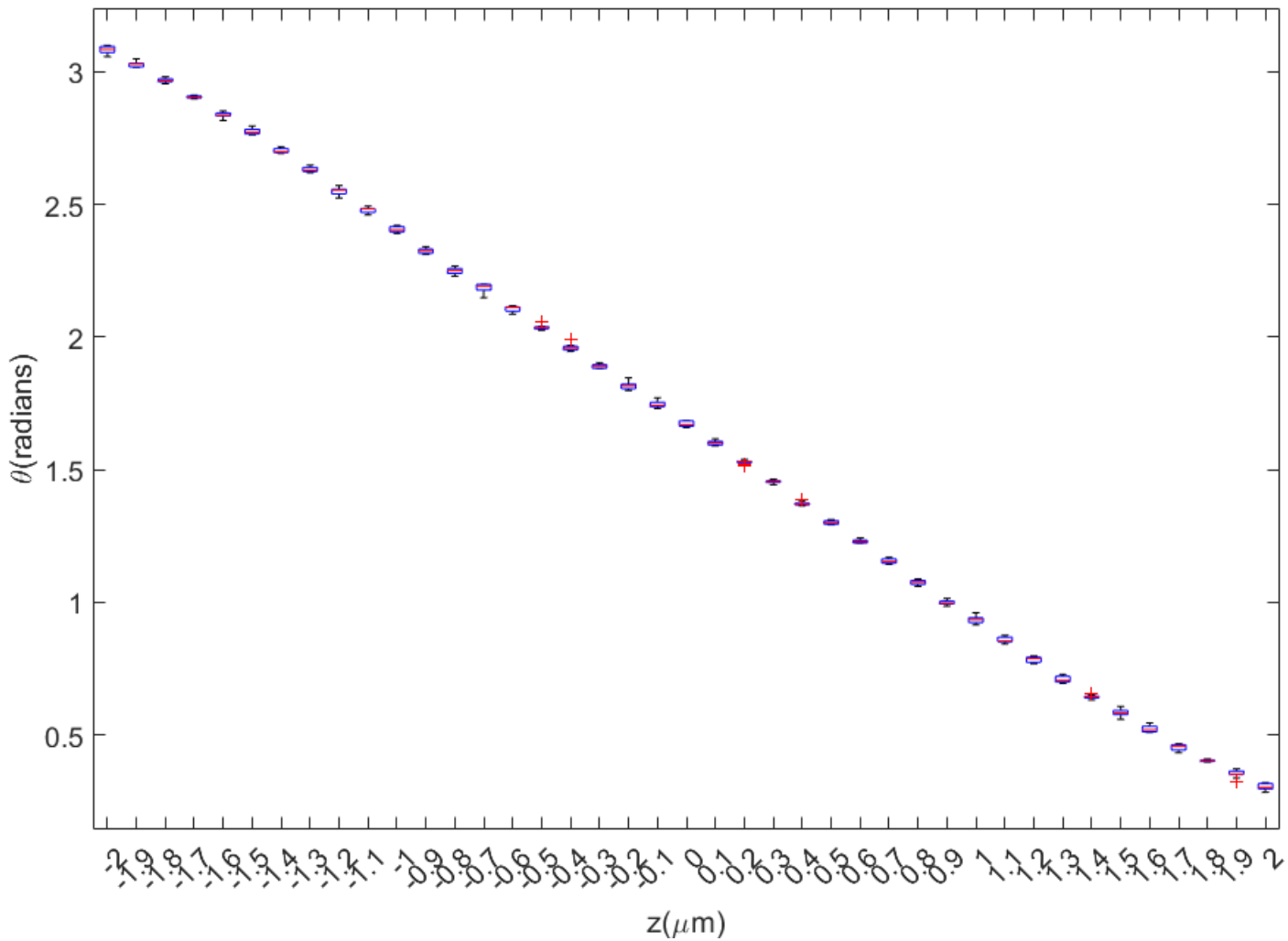
Parameter adjustment



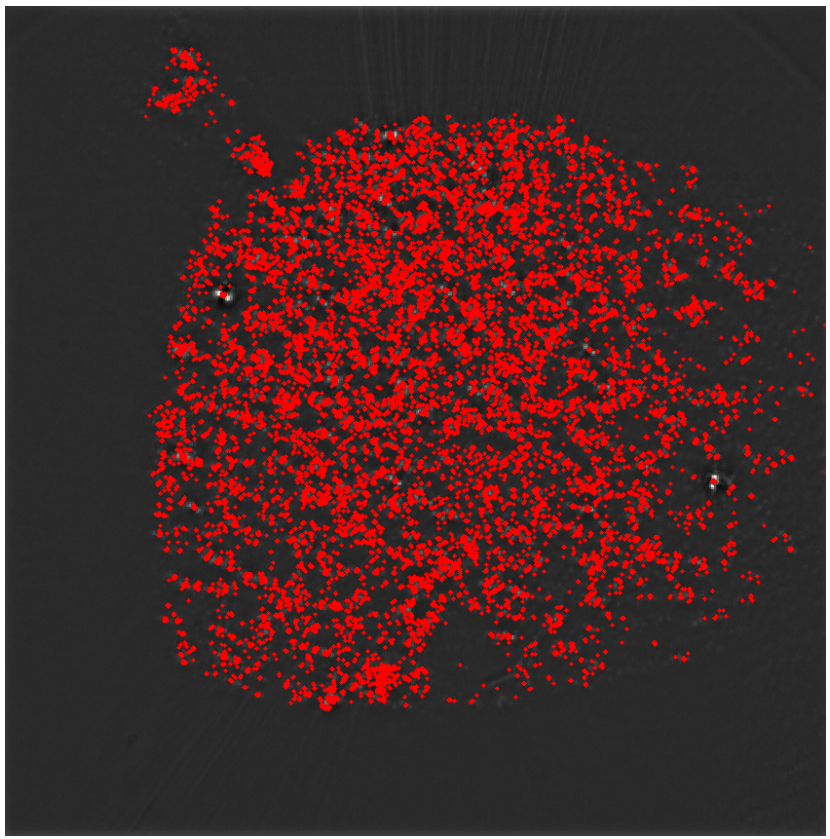
Outline

Axial calibration from orientation information

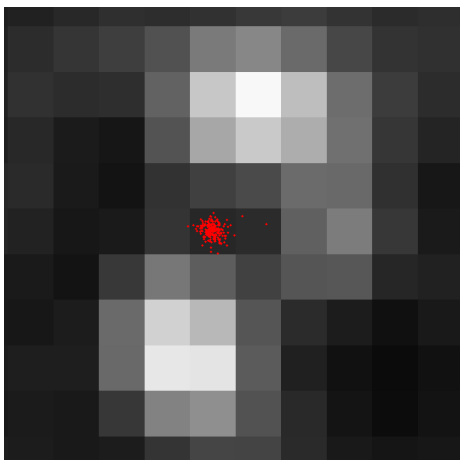
Calibration on beads: 41 z-steps at 0.1 μm, 10 acquisitions per step
Expected result: linear dependence



Quantum dots

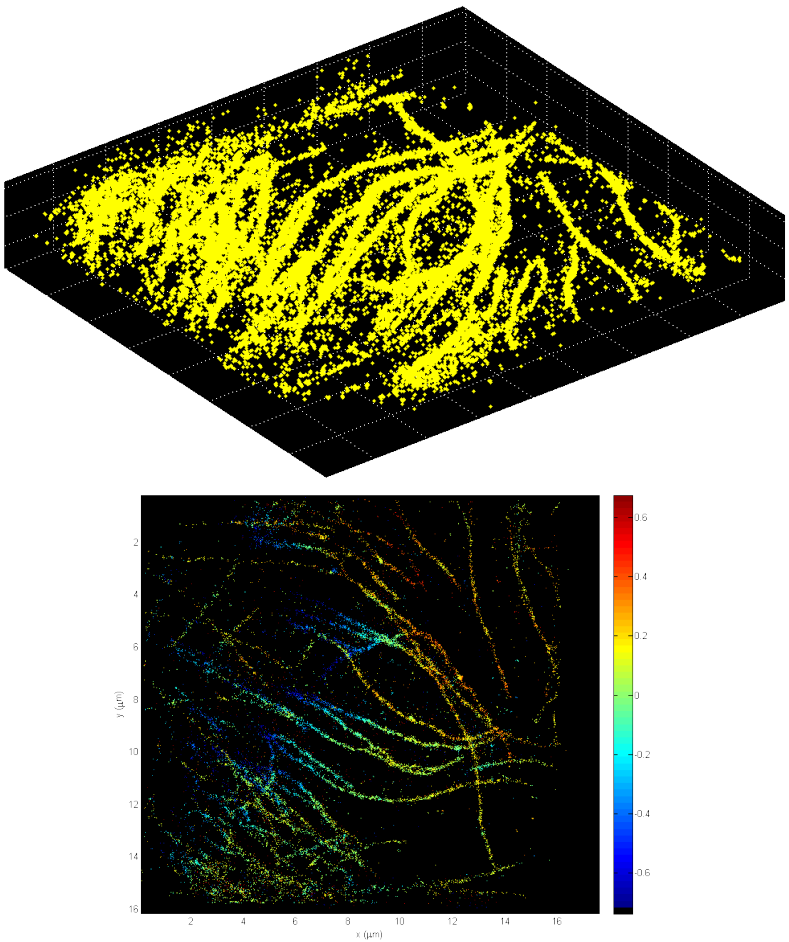


Average of 200 images of quantum dots and the respective fitted positions

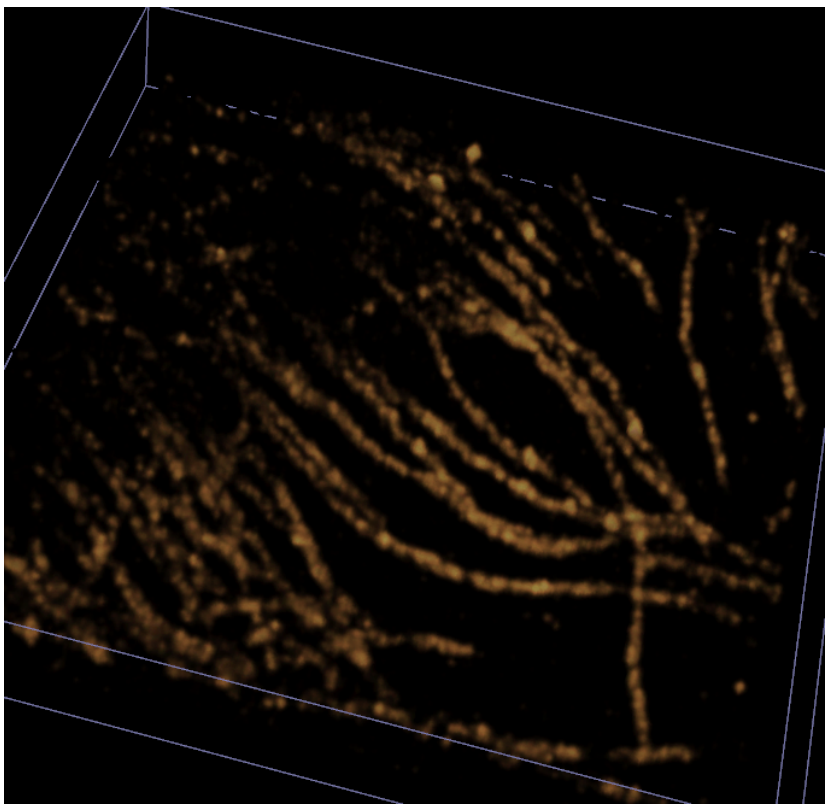


Comparison of reconstructions

EasyDHPSF



Our algorithm.



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Thank you!