



Bioimage Informatics

Daniel Sage & Arne Seitz

Course

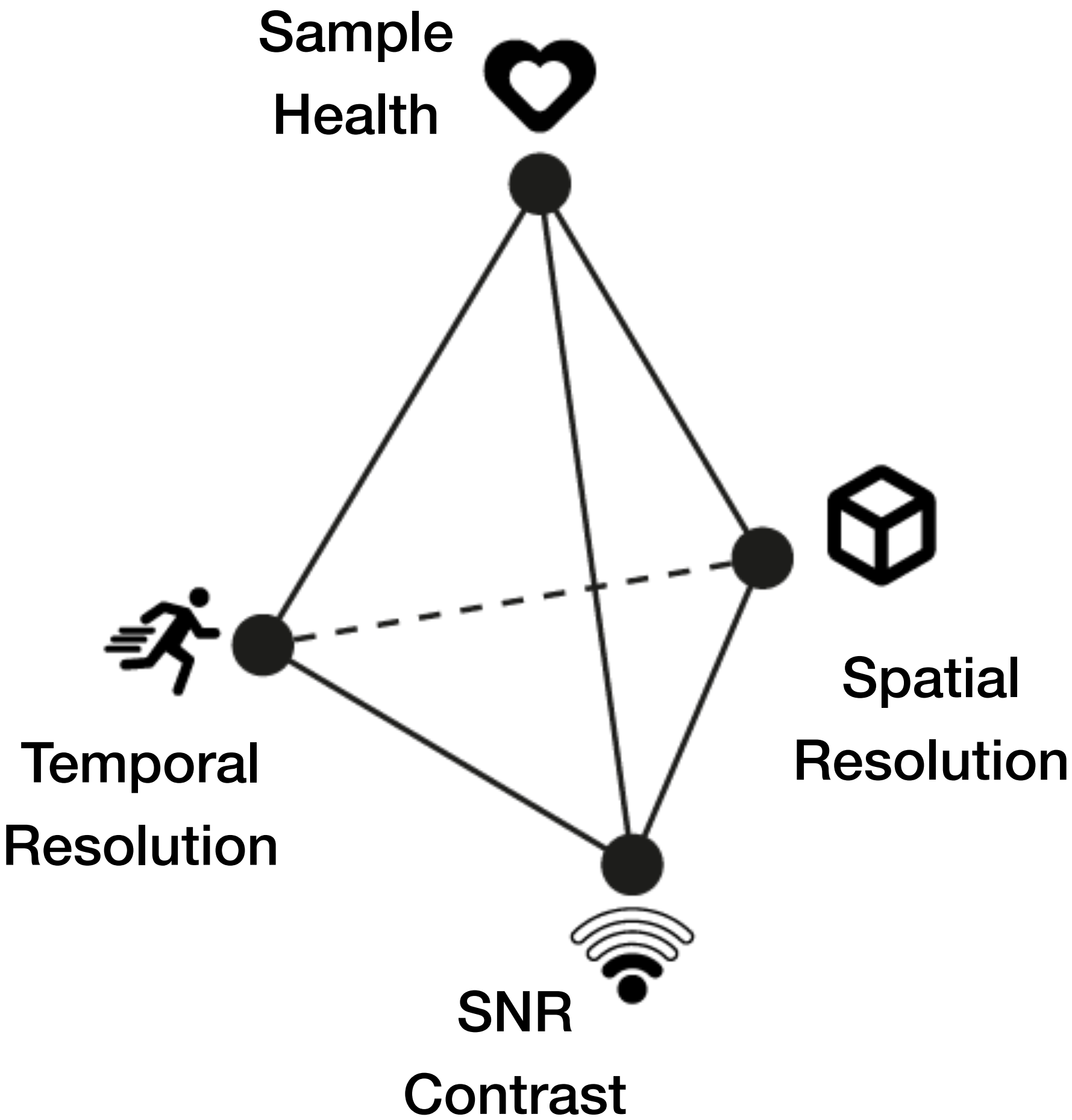
SMLM Data Analysis

The Quest of Higher Resolution

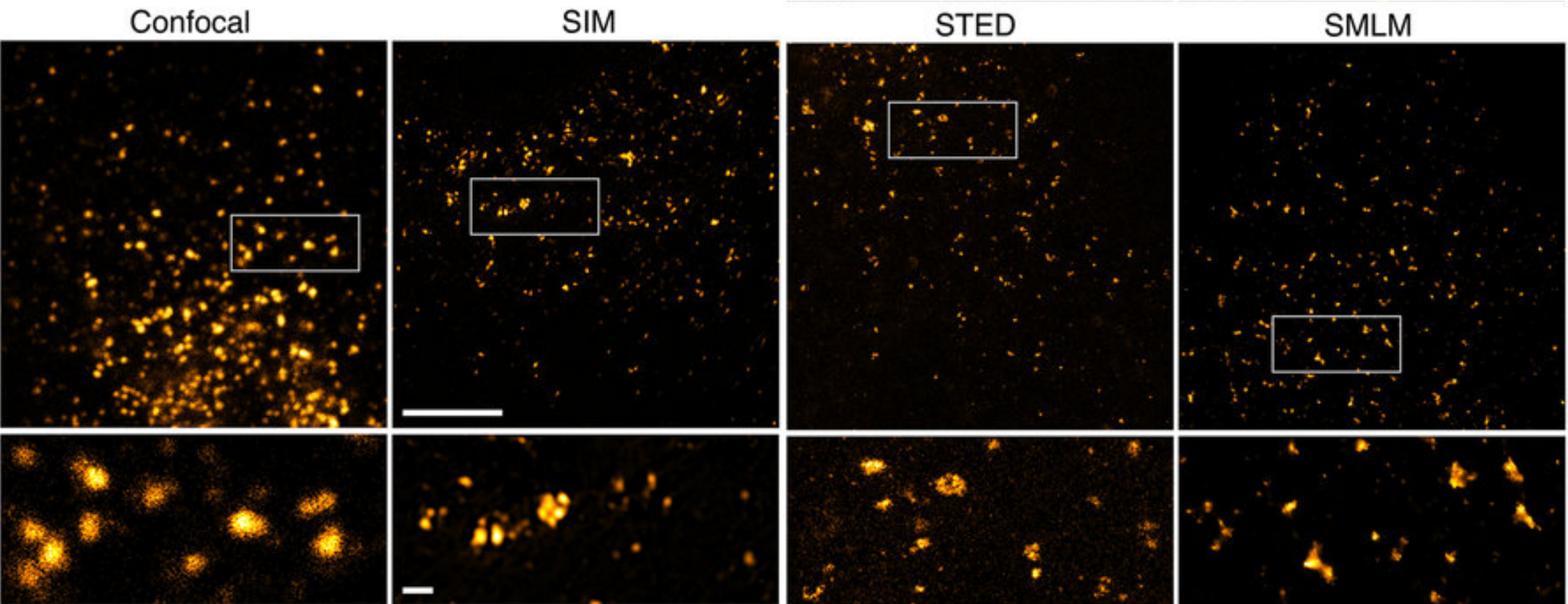


Nobel Prize 2014

PALM/
STORM



Super-resolution	SIM	STED	SMLM
Resolution	x 2	x 5	x 10
Temporal			
Sample prep			
Multi-color			
Cost			



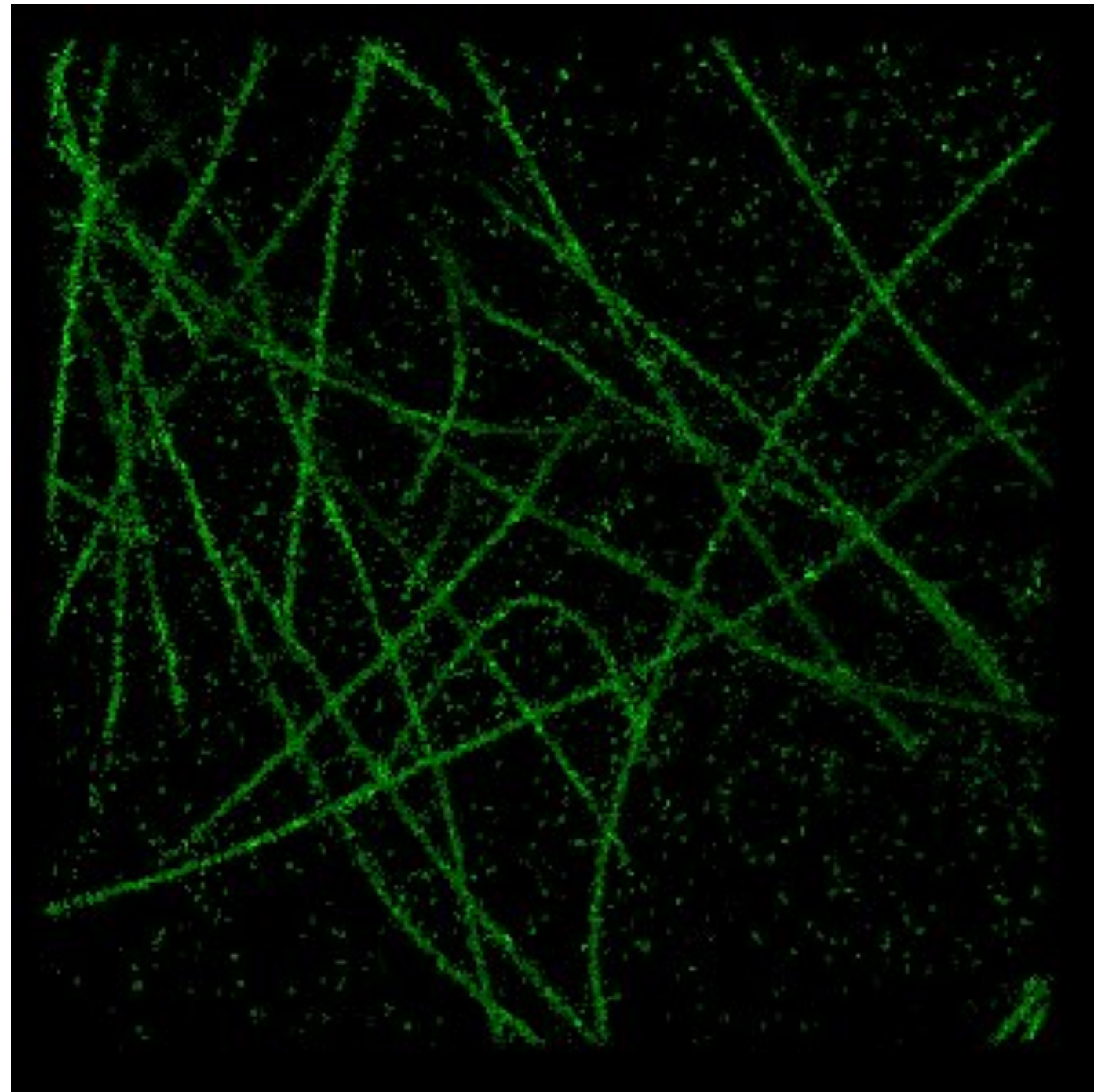


SMLM Image Analysis



1000 frames
 100×100

map of localization 5000×5000 pixels



Sub-Pixel Localization Methods

Direct
Detection

Center of gravity
QuickPALM (FIJI)

Fitting
Parametric
Function

Single-emitter
RapidSTORM
commercial
software

Multiple-emitter
DAOSTORM
ThunderSTORM

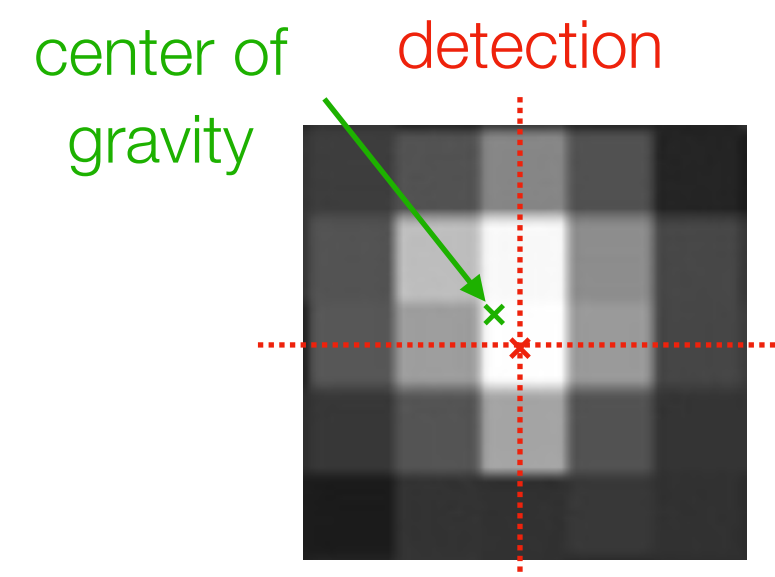
Solving as
a Inverse
Problem

Optimization
ADCG

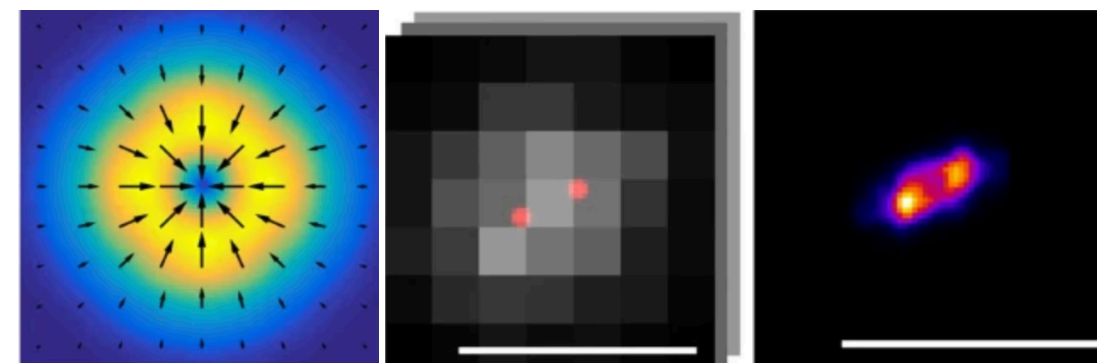
Preprocessing
ANNAPALM

Learning
Approaches

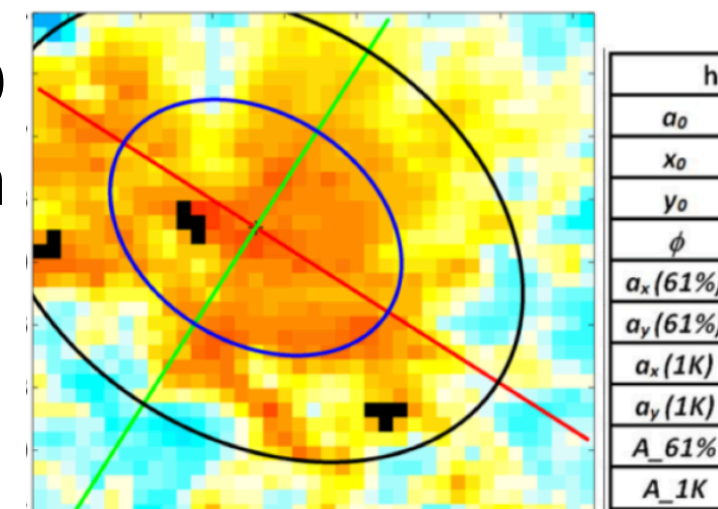
Synthetic datasets
DECODE



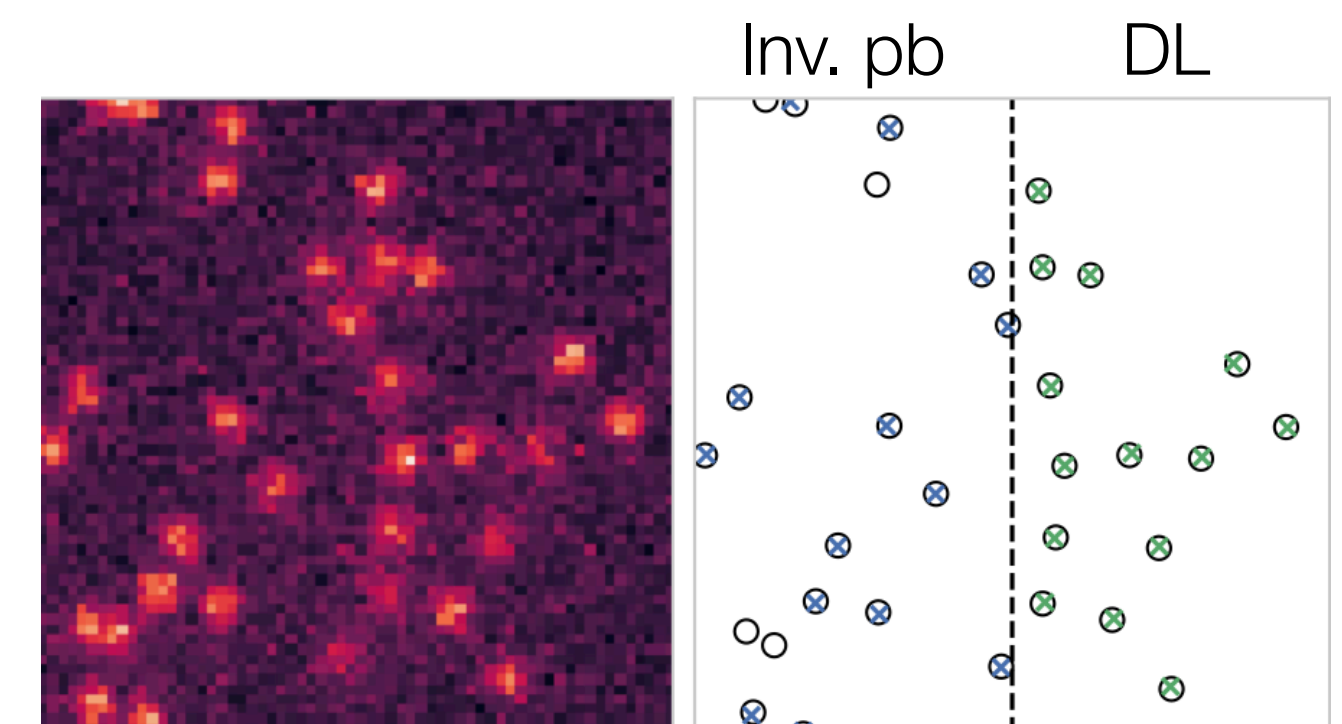
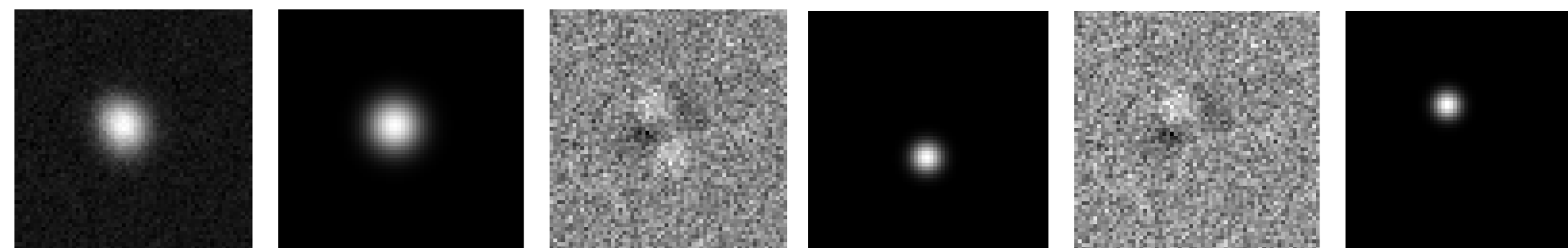
Express
Radiality



Fitting 2D
Gaussian



Fitting on residu



Point Processing and Point Analysis

	A	B	C	D	E	F	G	H	I	J	K	L
1	id	frame	x [nm]	y [nm]	z [nm]	sigma1 [nm]	sigma2 [nm]	intensity [ph	offset [photc	bkgstd [photon]	uncertainty_xy [	uncertainty_z [nm]
2		1	5740.83434	1761.83022	-73.2513	184.16585	233.31909	658.49107	5.95959	5.88896	24.7608	68.89509
3		2	5651.35715	1768.2754	52.98168	214.97157	192.69315	497.43546	8.74365	5.51125	29.31233	79.32229
4		3	5688.11377	1784.34773	80.13173	224.48493	186.72842	776.76044	9.2315	6.25032	21.88203	62.78186
5		4	5749.16986	1777.29827	124.06739	241.43594	179.19385	995.61486	5.54265	8.29197	22.70981	76.66379
6		5	5702.50452	1763.49301	107.69916	234.91776	181.69333	537.04899	8.90223	6.2449	31.20853	98.68351
7		6	5661.68966	1784.76124	97.03721	230.79755	183.51815	409.93424	6.92543	5.7242	36.97788	112.75311
8		7	1767.17136	2364.39846	16.0143	203.44262	202.40569	1579.43826	5.91979	8.46239	14.48774	37.22549
9		8	1763.85639	2371.3338	55.08563	215.67918	192.19542	1227.15187	7.91353	7.11094	15.92052	42.8618
10		9	2602.27882	5117.00944	146.06186	250.52983	176.41348	963.39044	8.50348	6.25944	18.91056	70.10141
11		10	5709.28437	1756.24961	153.70116	253.77066	175.60347	859.14402	7.41058	7.01649	23.20004	90.28823
12		11	5723.28368	1752.92681	25.45325	206.21573	199.75226	842.35646	7.11384	6.57755	20.8658	54.16082
13		12	2563.83532	3871.47058	-103.83586	180.90549	246.30881	1640.95697	5.57923	9.8962	17.07549	51.88794

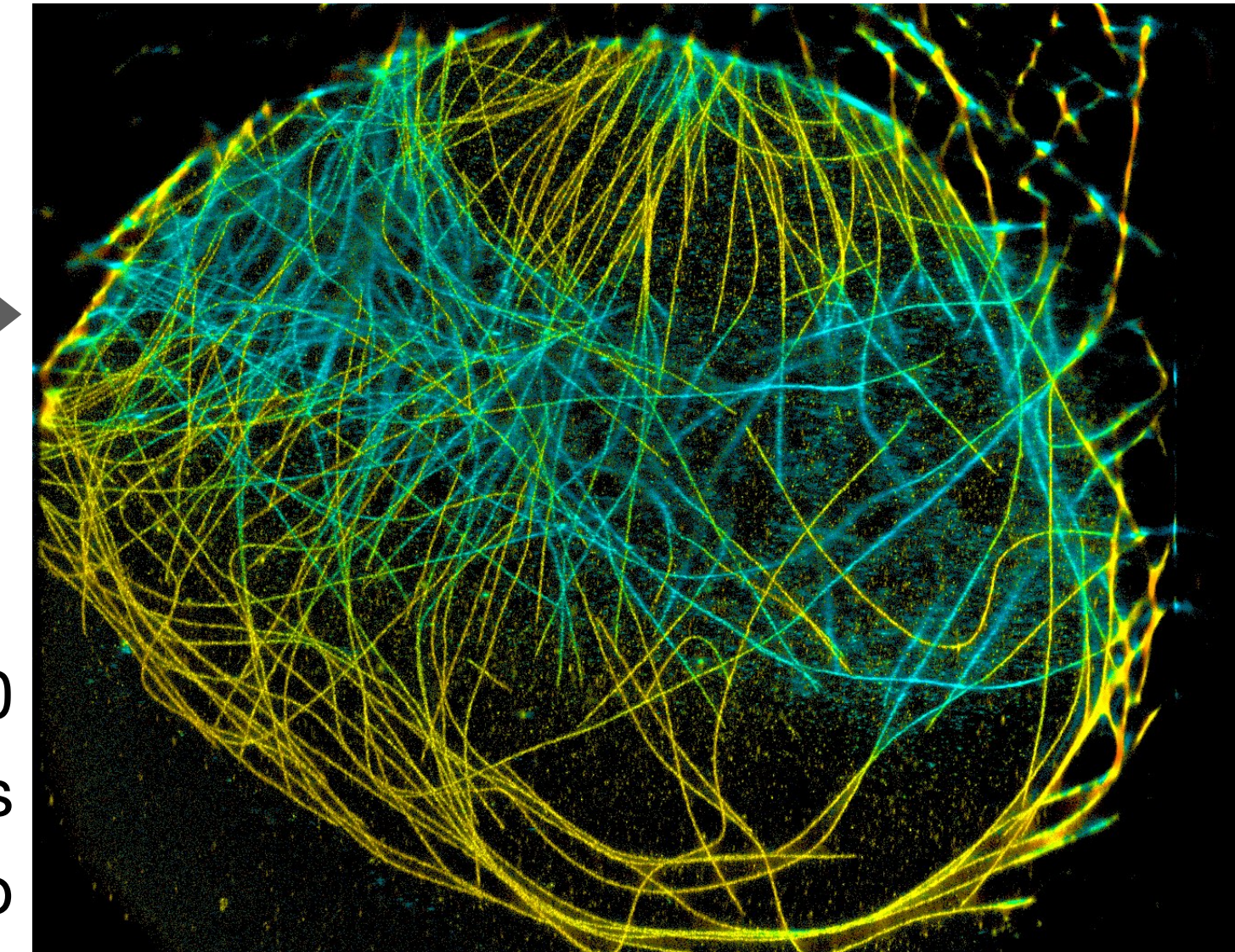
CSV

↑
Frame
XY positions or
XYZ positions

↑
Numbers of photons
Uncertainty

Rendering

2'000'000
localizations
1.17Gb



{x,y,t}

Data preprocessing

- Denoising (Rejection)
- Drift correction
- Temporal grouping

{x,y,t}

{x,y,t}

Data Analysis

- Clustering
- Segmentation
- Tracking

Biological
Measurement
Information



Point Processing

Drift Correction

Fiducial markers-based

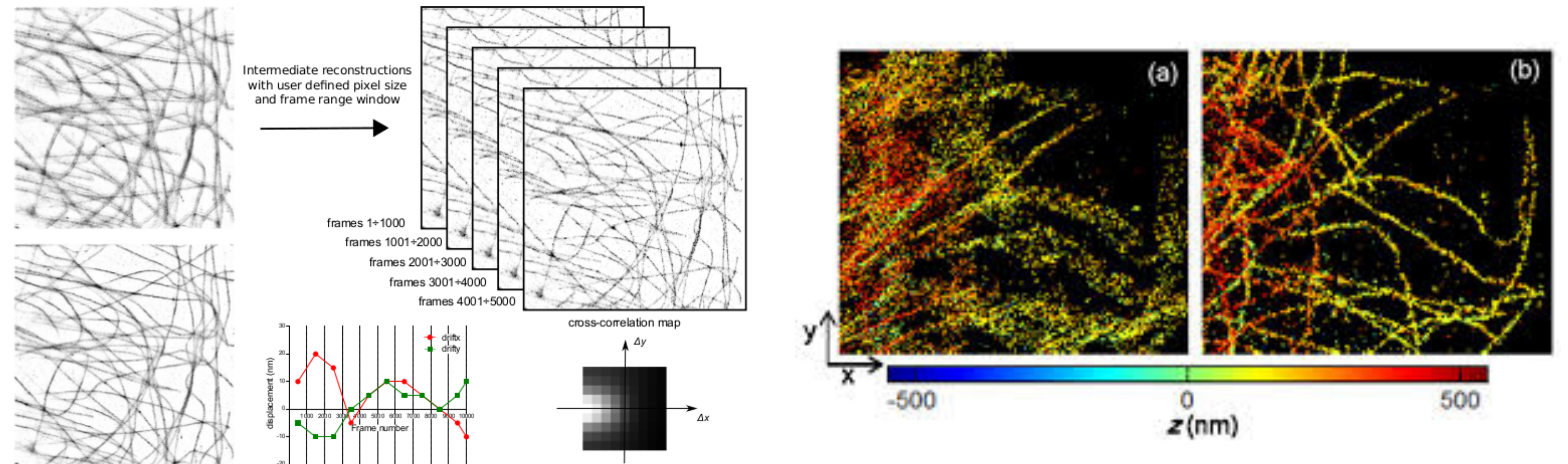
- Tracking the fiducial

Localization-based

- Cross-Correlation of batch of N frames

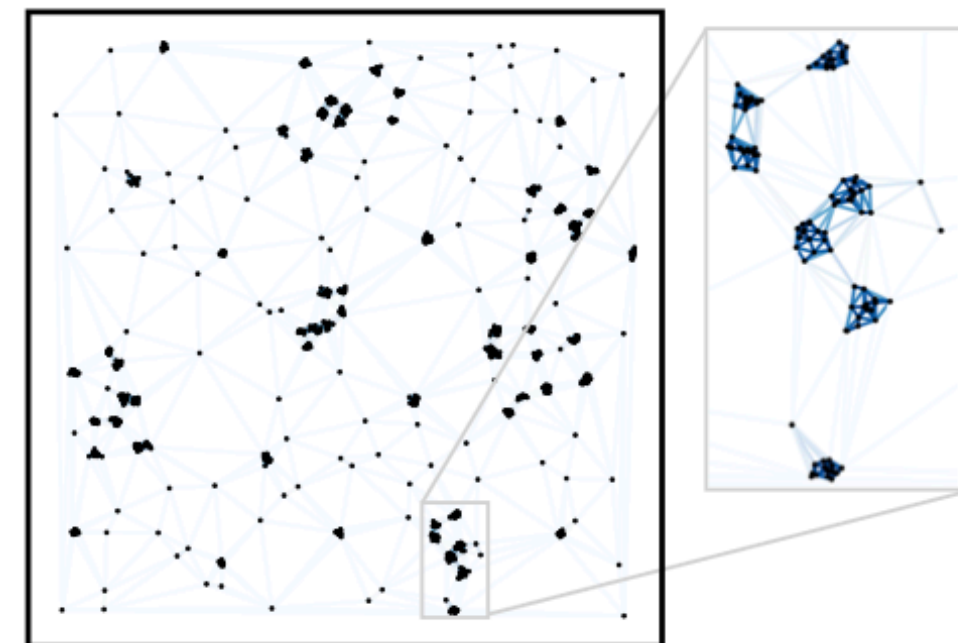
Rendering-based

- Registration

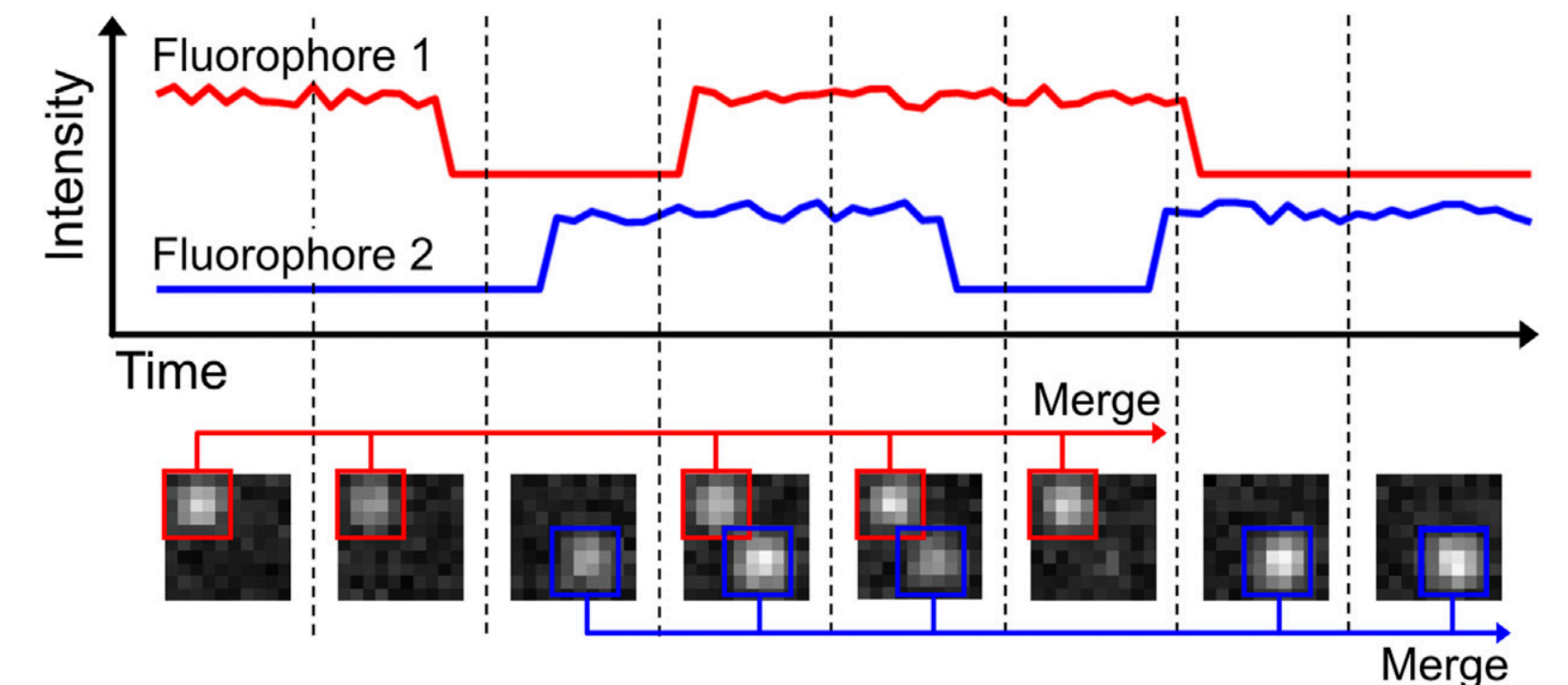


Denoising = rejection of points

- Number of photons
- Uncertainty
- Isolated points
- Low local SNR



Temporal Grouping

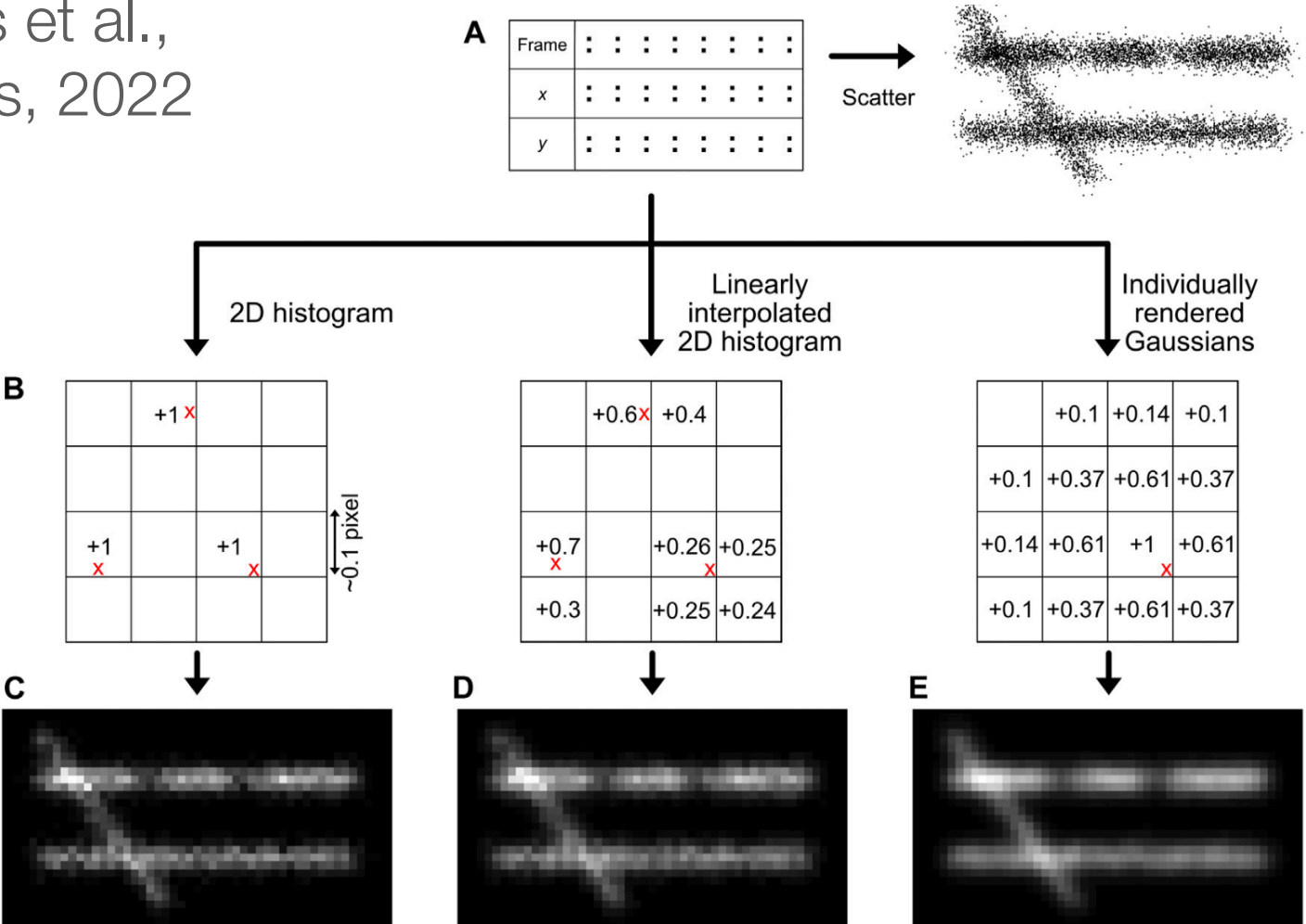




2D Rendering

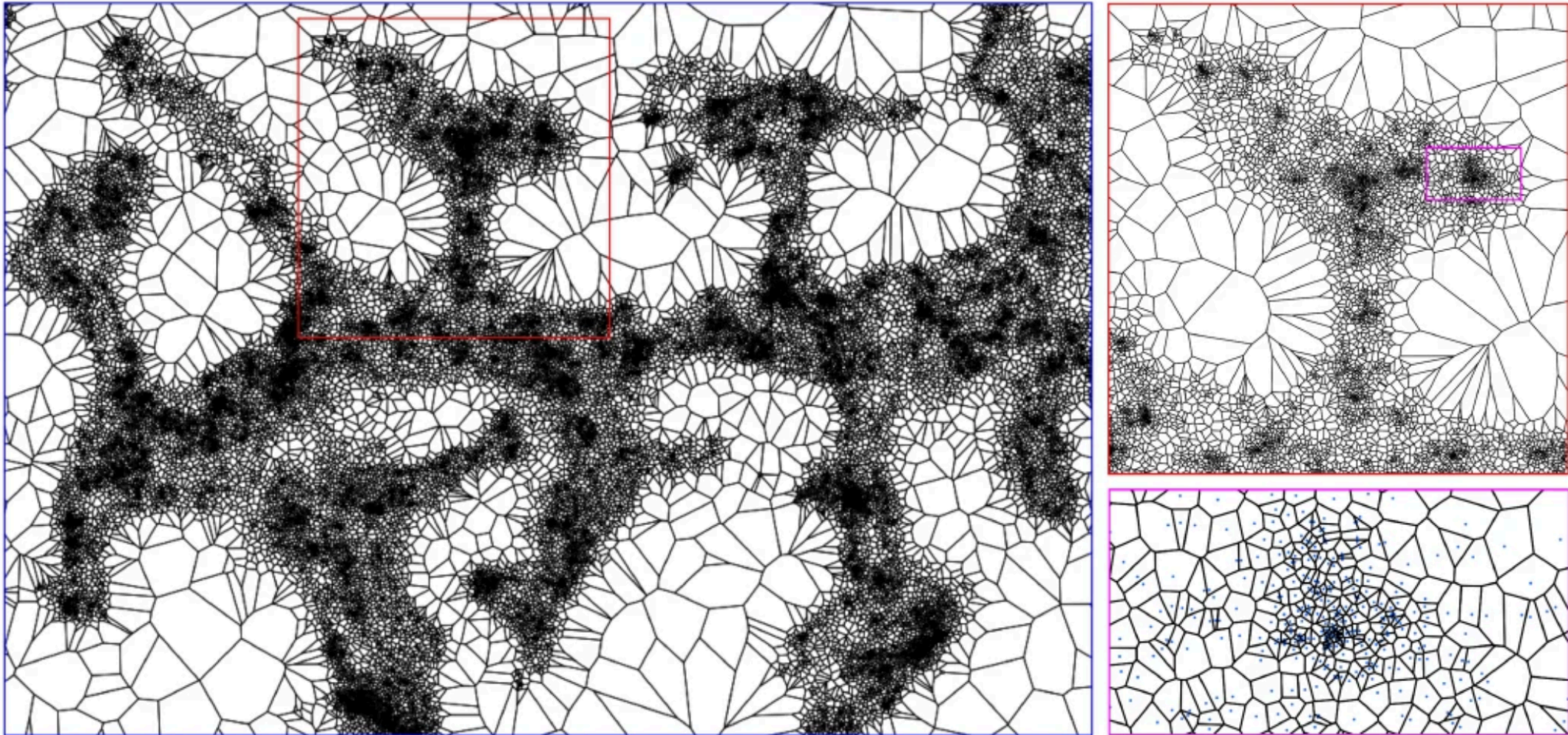
Histogram binning

Martens et al.,
Frontiers, 2022



Colorize Voronoi

Levet et al., SR-Tesseler, 2015



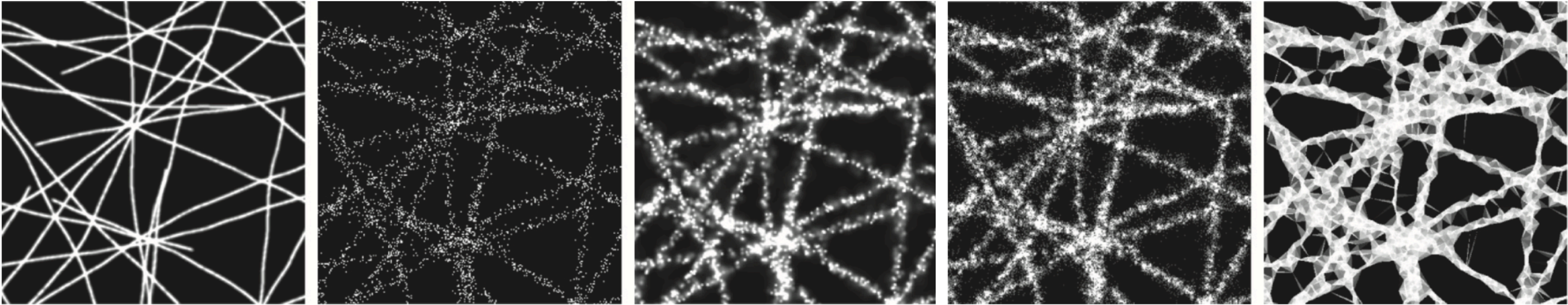
Ground-truth

Histogram binning

Gaussian rendering

Jittering

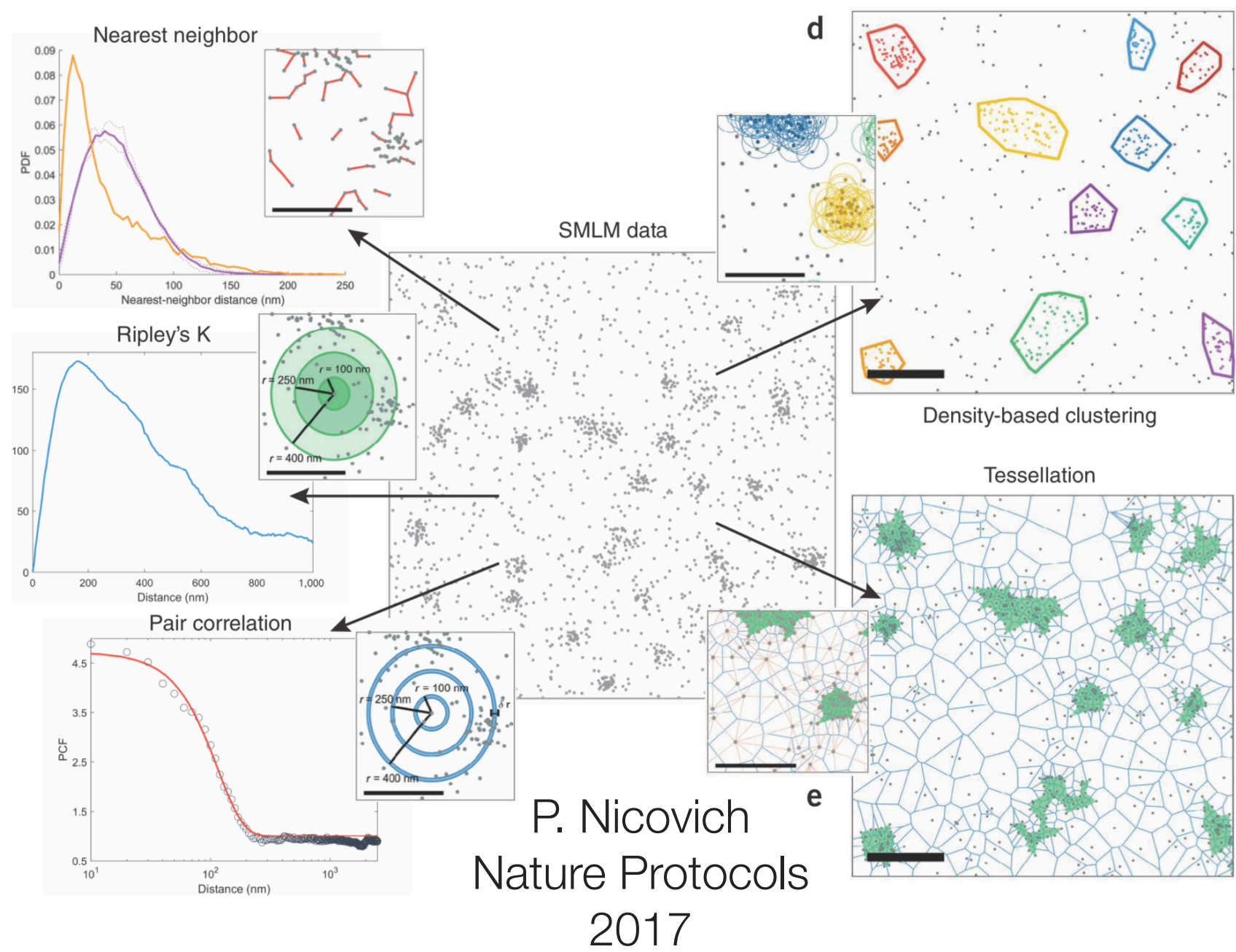
Delaunay



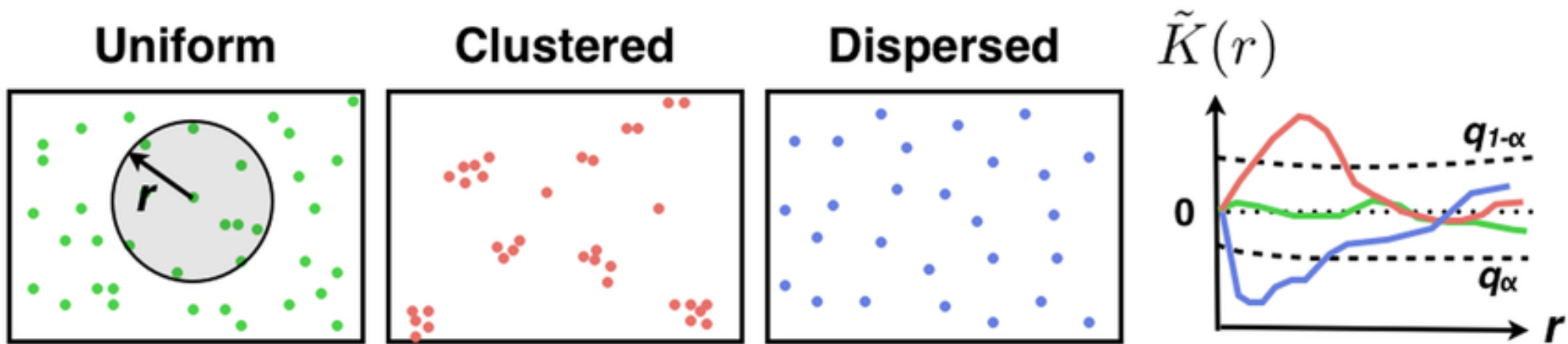
Nieuwenhuizen, 2015



Clustering

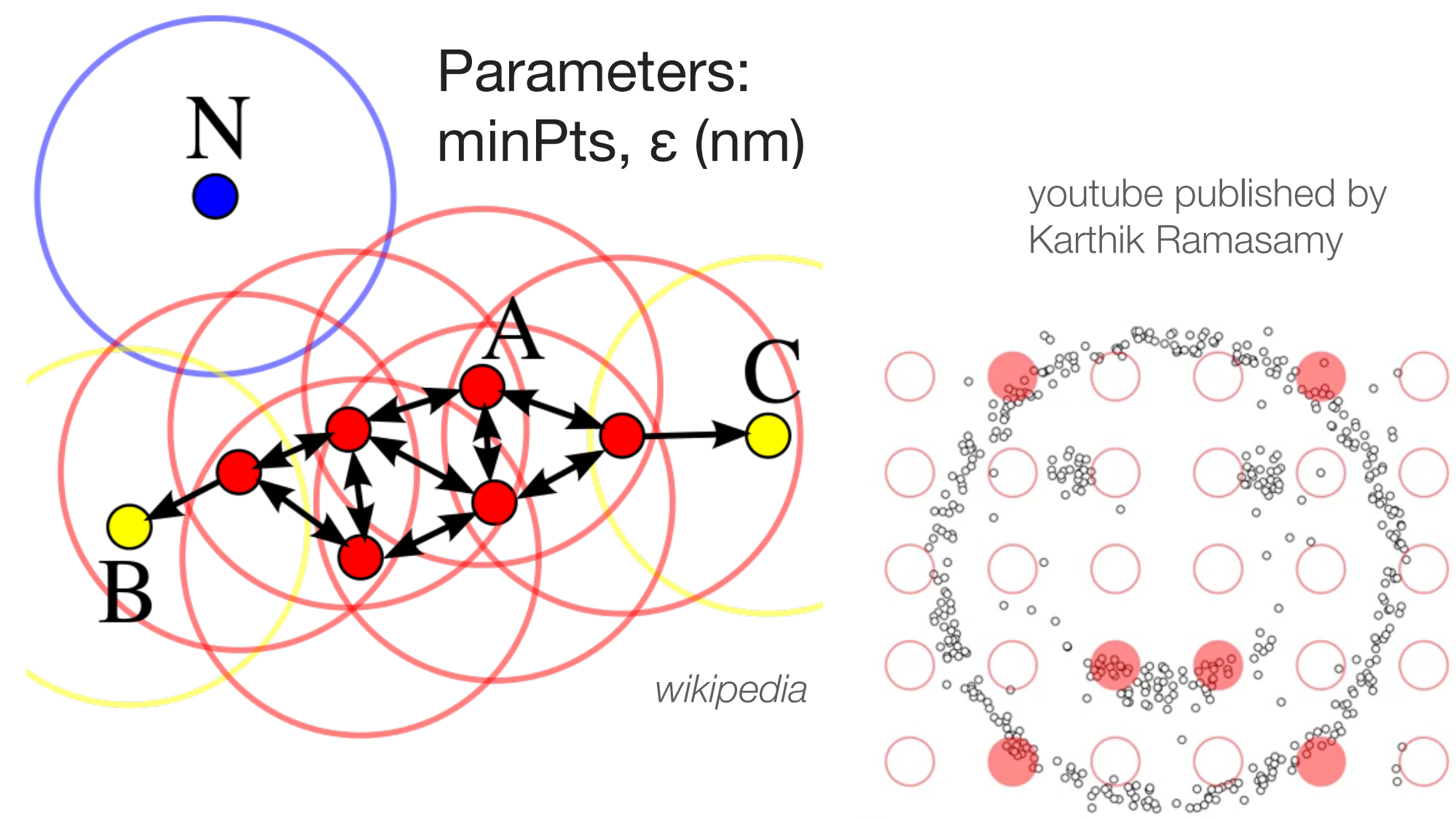


K-Ripley



DBSCAN

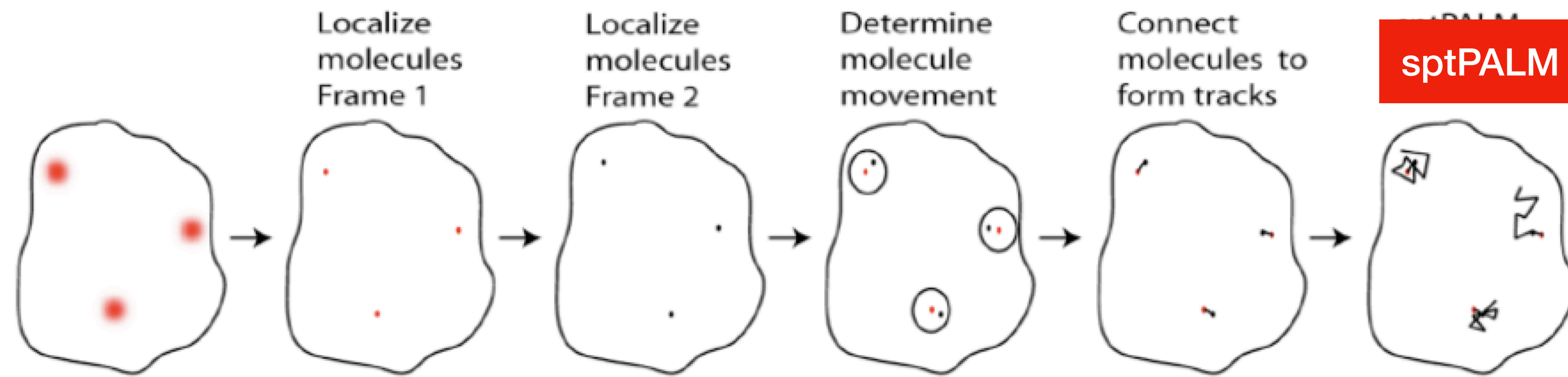
Density-Based Spatial Clustering of Applications with Noise
The most common cluster algorithm
Groups together points that are closely packed together



- Denoise: marks outliers
- Unsupervised clustering: do not required K
- Not deterministic, dependencies to parameters
- Do not work when large differences in densities

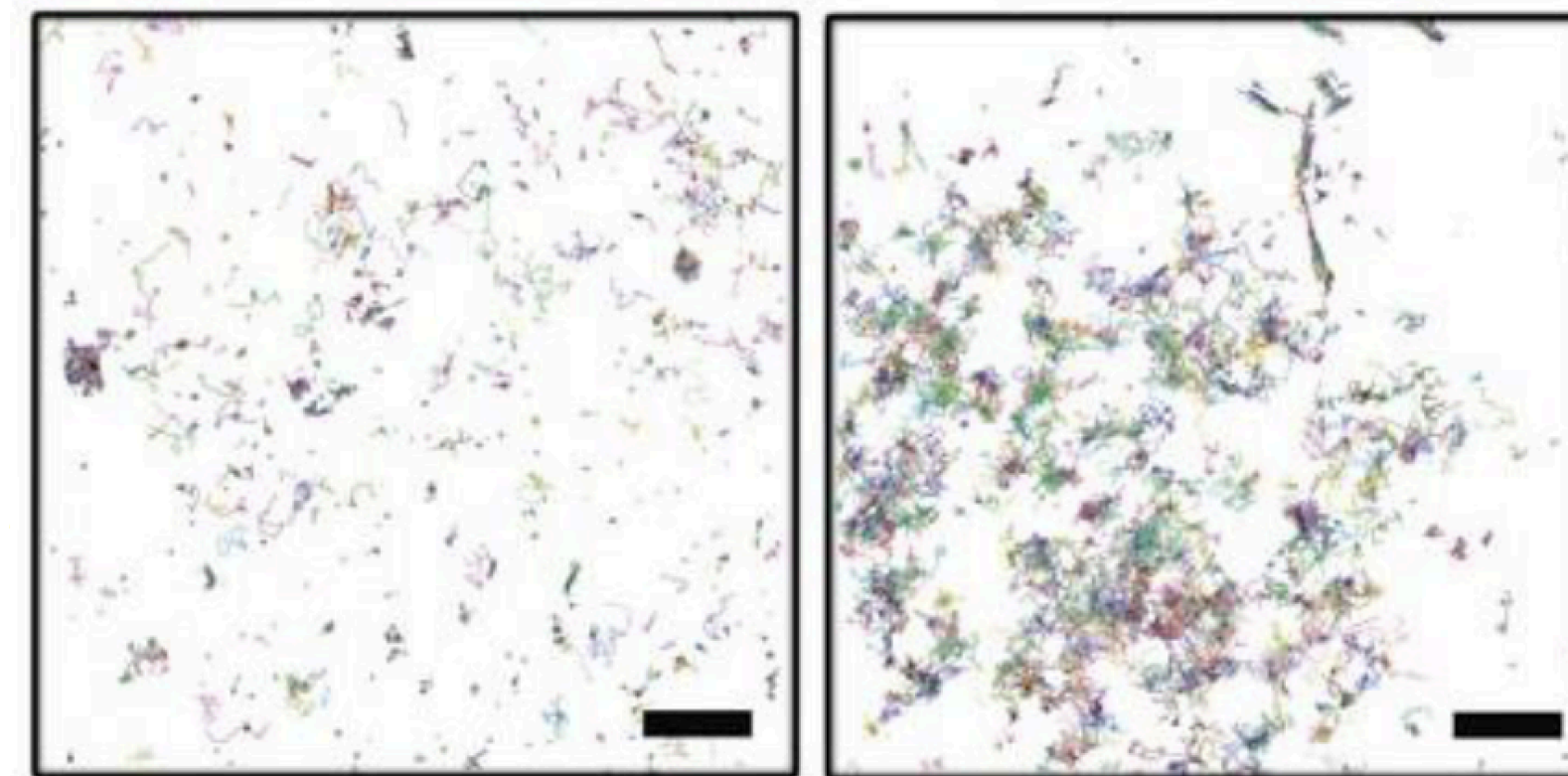
Tracking

Single Particle Tracking

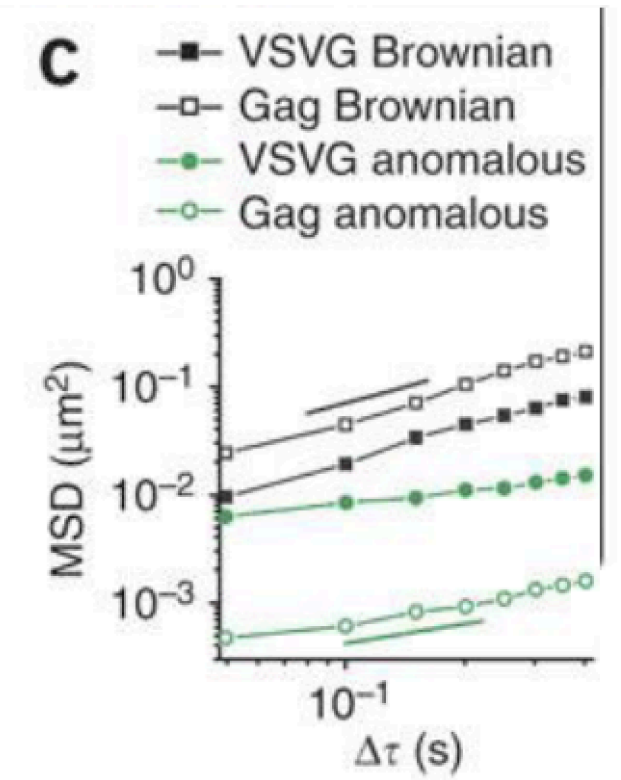
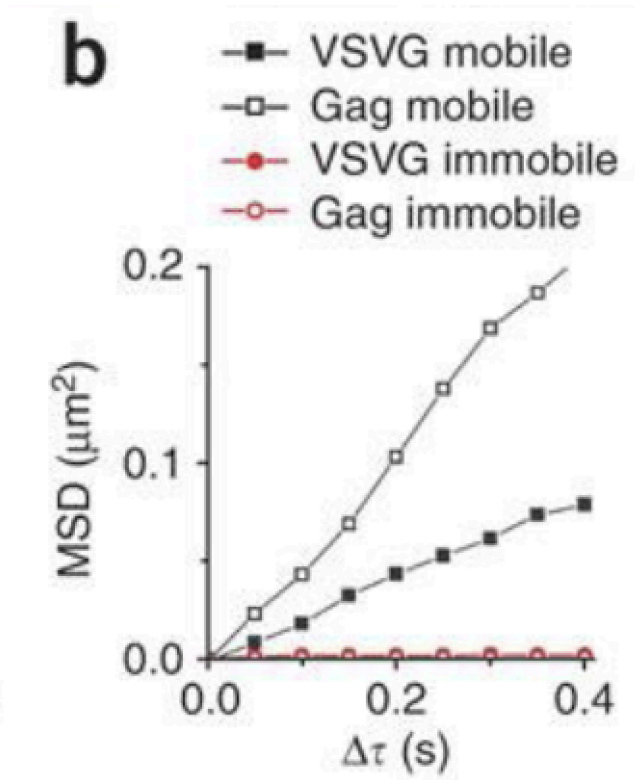
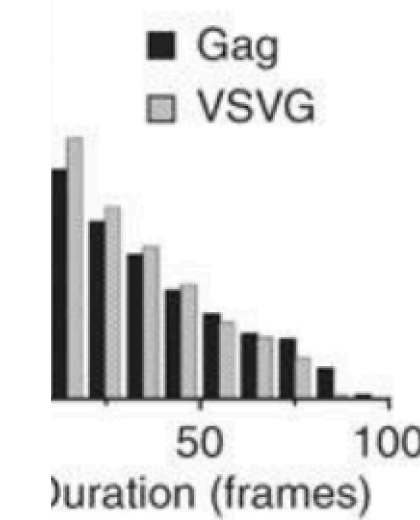


Gag -Eos

VSVG -Eos



Manley, Nat.
Methods, 2008





PSF Shaping

Interferometry	iPALM	Shtengel et al. PNAS 2009
	SEFI	Bon et. al. Nat Methods 2018
Engineered PSF	Astigmatism cylindric lens	Huang et al. Science 2008
	Astigmatism adaptive optics	Izzedin et al. Optics Express 2011
	Double-helix	Pavani et al. PNAS 2009
	Saddle	Shechtman et al. Phys Rev Let 2014
	Tetrapod	Aristov et al. Nat. Comm. 2018
Multiplane	Biplane	Juette et al. Nat. Methods 2008
	Multiplane, MUM Biplane	Ram et al. Biophysical Journal 2008
	Multifocus, MFM	Abrahamsson et al. Nat. Methods 2013
	Beam Splitter	Geissbuelher et al. Nat. Comm. 2018

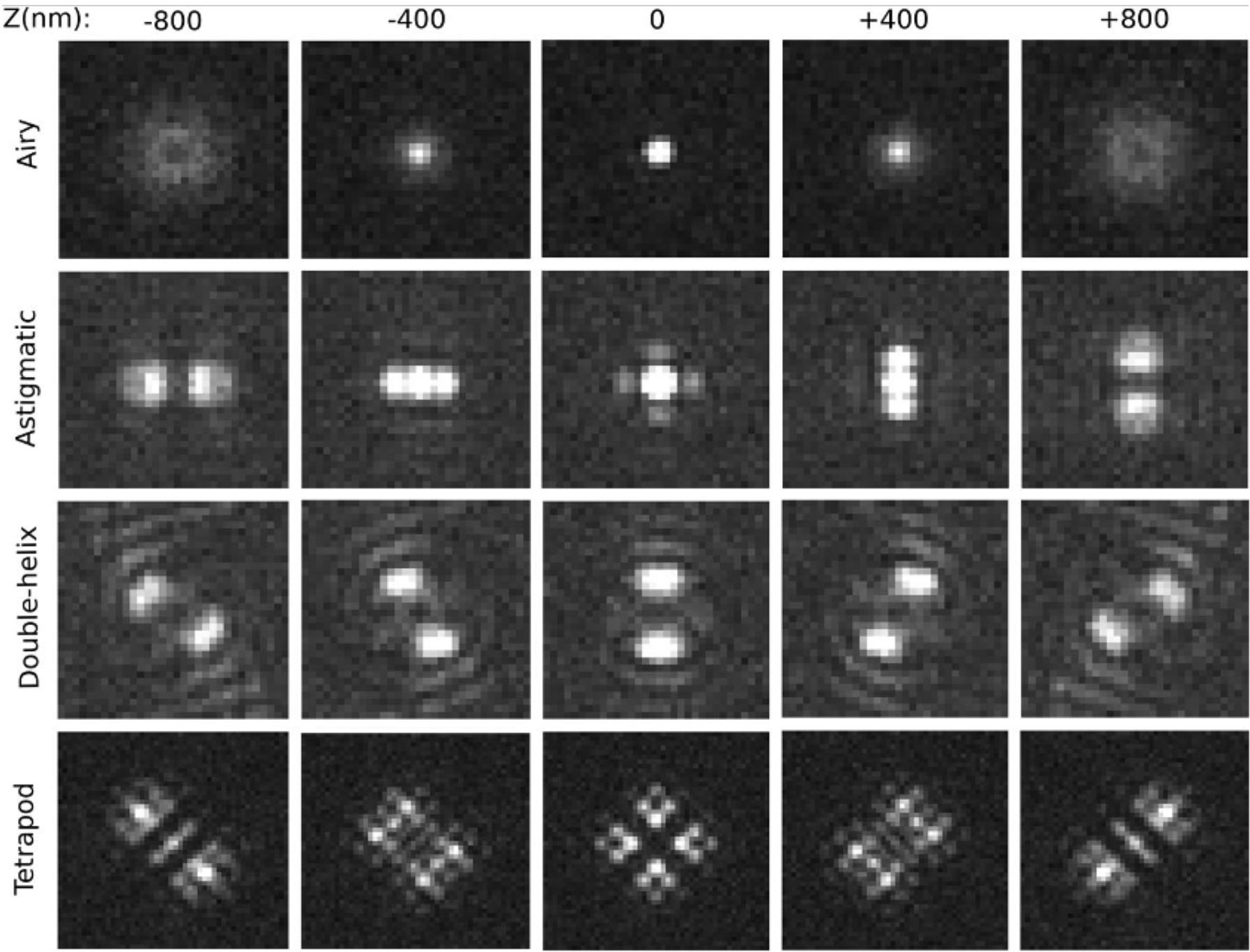
Astrid Magenau 2014 and Bassam Hajj 2018

Principle

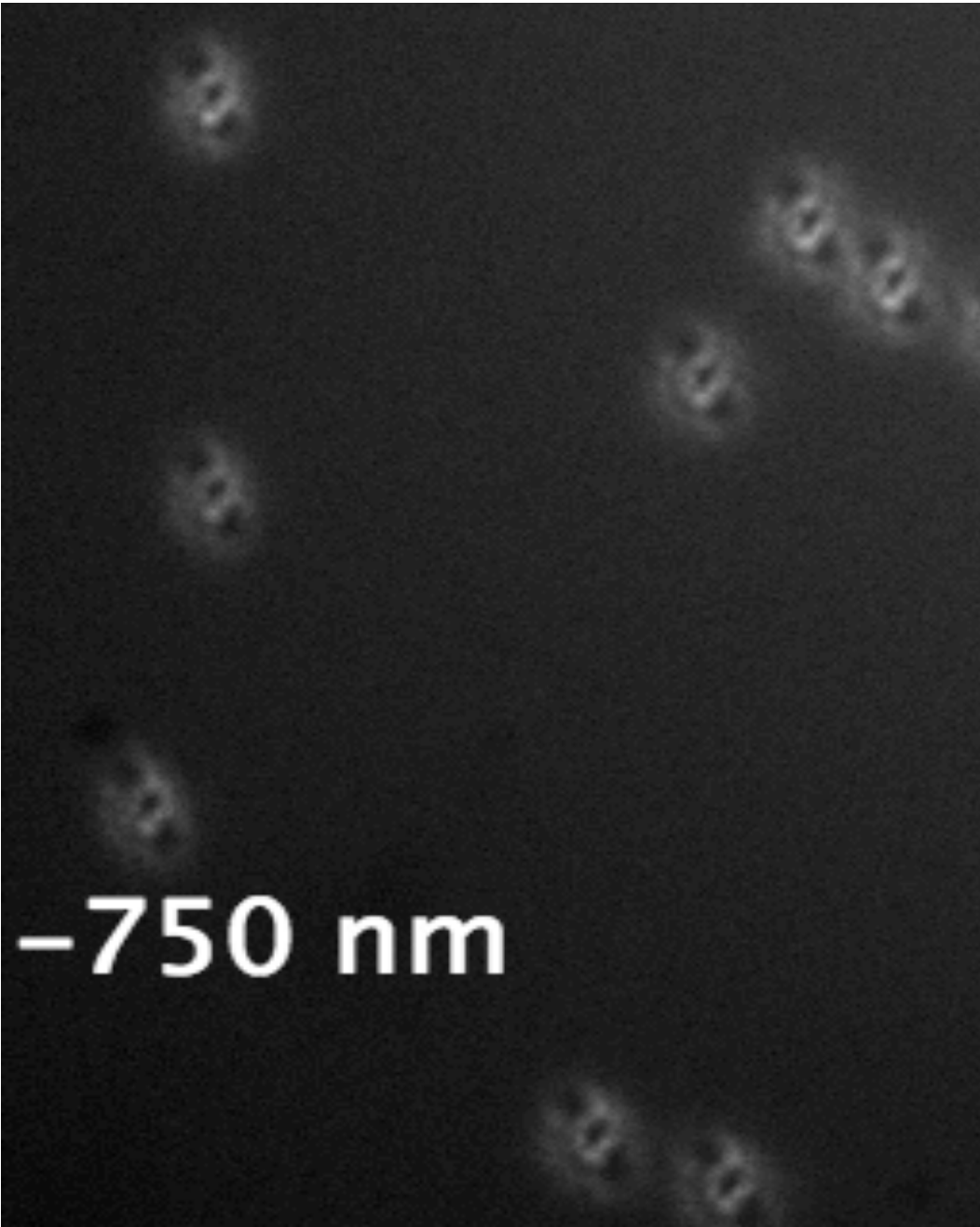
Acquisition in 2D

3D PSF: Stacking all the 2D-PSF

Calibration: Find relation $z = f(\text{shape_parameters_2D})$



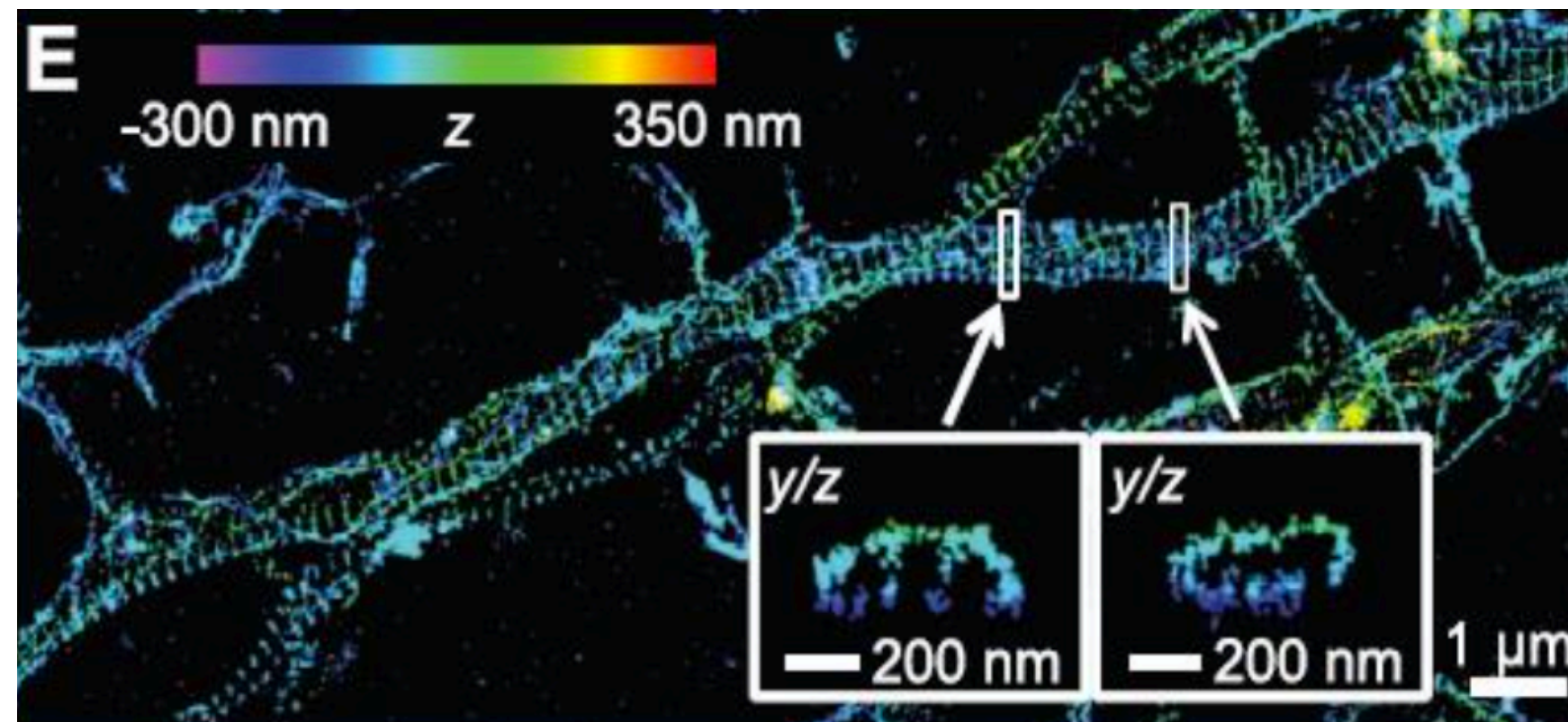
Fazel et al. Biophysics and Bioengineering, 2022



👁 3D PSF Calibration - Astigmatism

Astigmatism: the affordable 3D solution

- Simple set-up, calibration on beads, low-cost
- Commercial microscope, open-source software
- Mid-range performances



Discovery of the periodic cytoskeletal structure in axons [Xu, Science 2012]

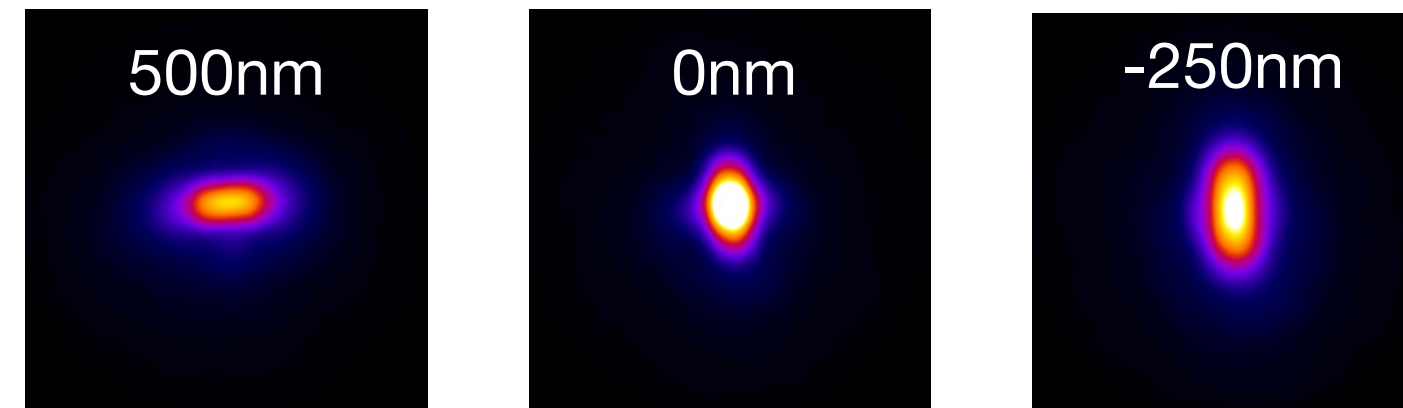
Z-Stack of beads

- Small beads of very high SNR
- Small z-step

Calibration find the axial curve

$$z = f(\alpha_1, \alpha_2, \dots) \quad z \in [z_{\min}, z_{\max}]$$

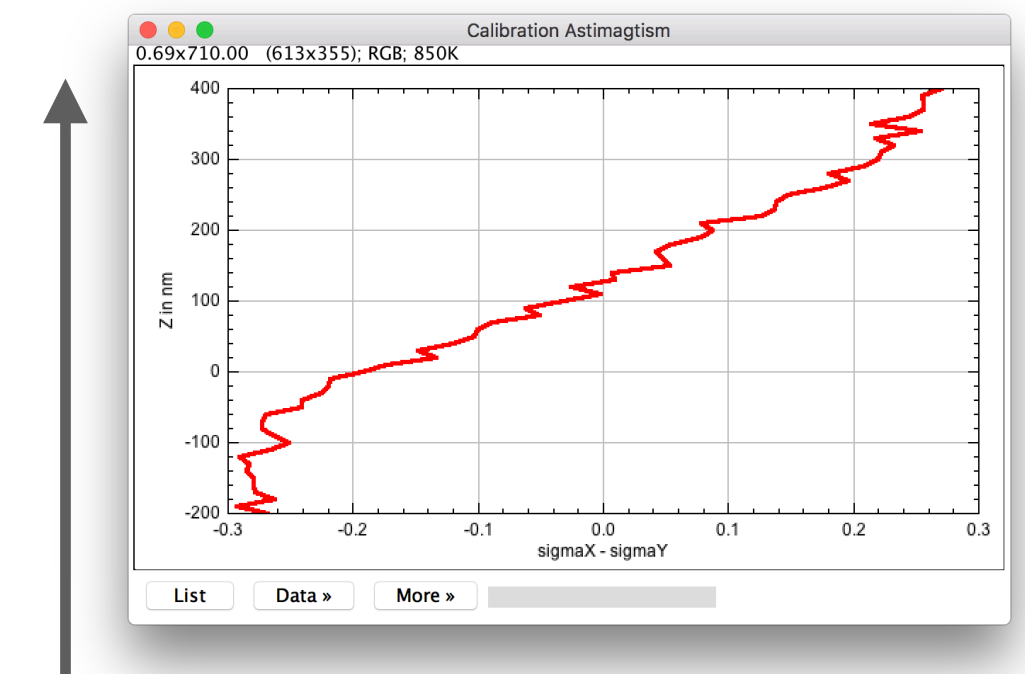
Gaussian-based model in astigmatism



$$\text{PSF}(x, y) =$$

$$\frac{N}{2\pi\sigma_1\sigma_2} \exp\left(-\frac{x^2}{2\sigma_1^2} - \frac{y^2}{2\sigma_2^2}\right) + b$$

QuickPALM

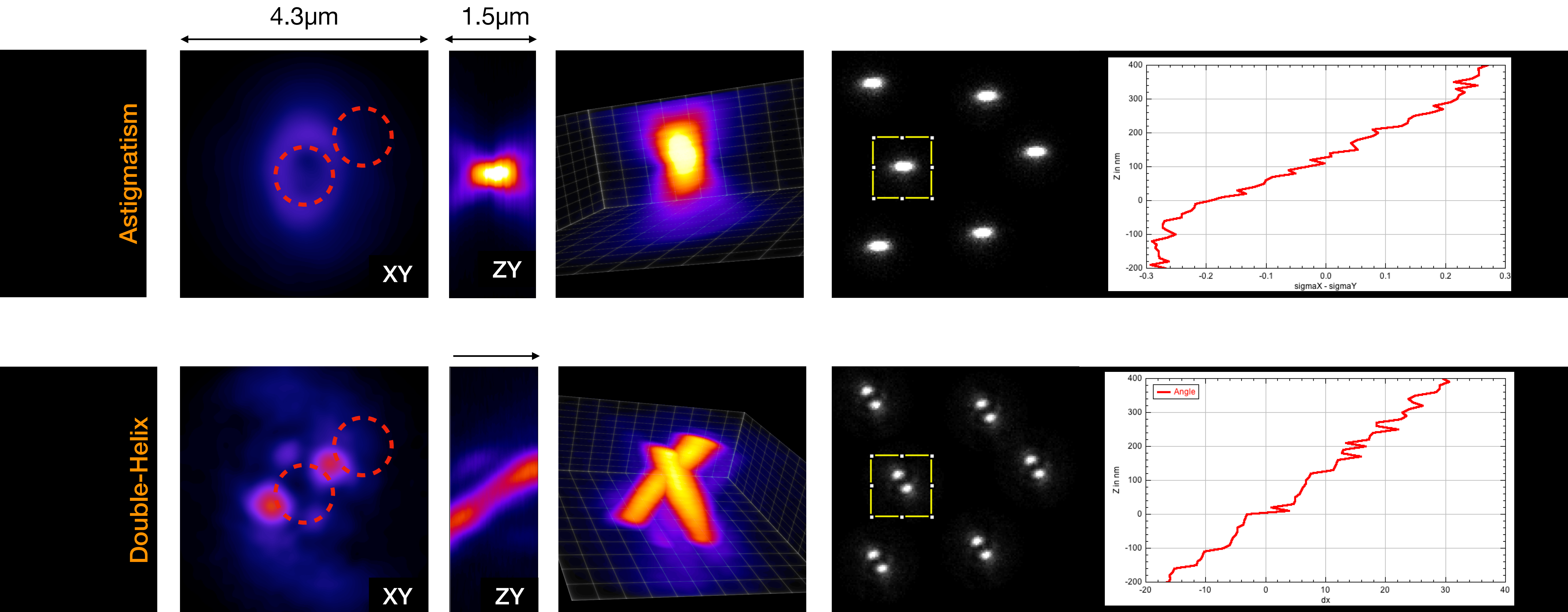


$$z = f(\sigma_x - \sigma_y)$$

PSF model

- Gaussian PSF
- Zernike coefficients
- Continuous model (derivable)

👁 3D SMLM High-Density

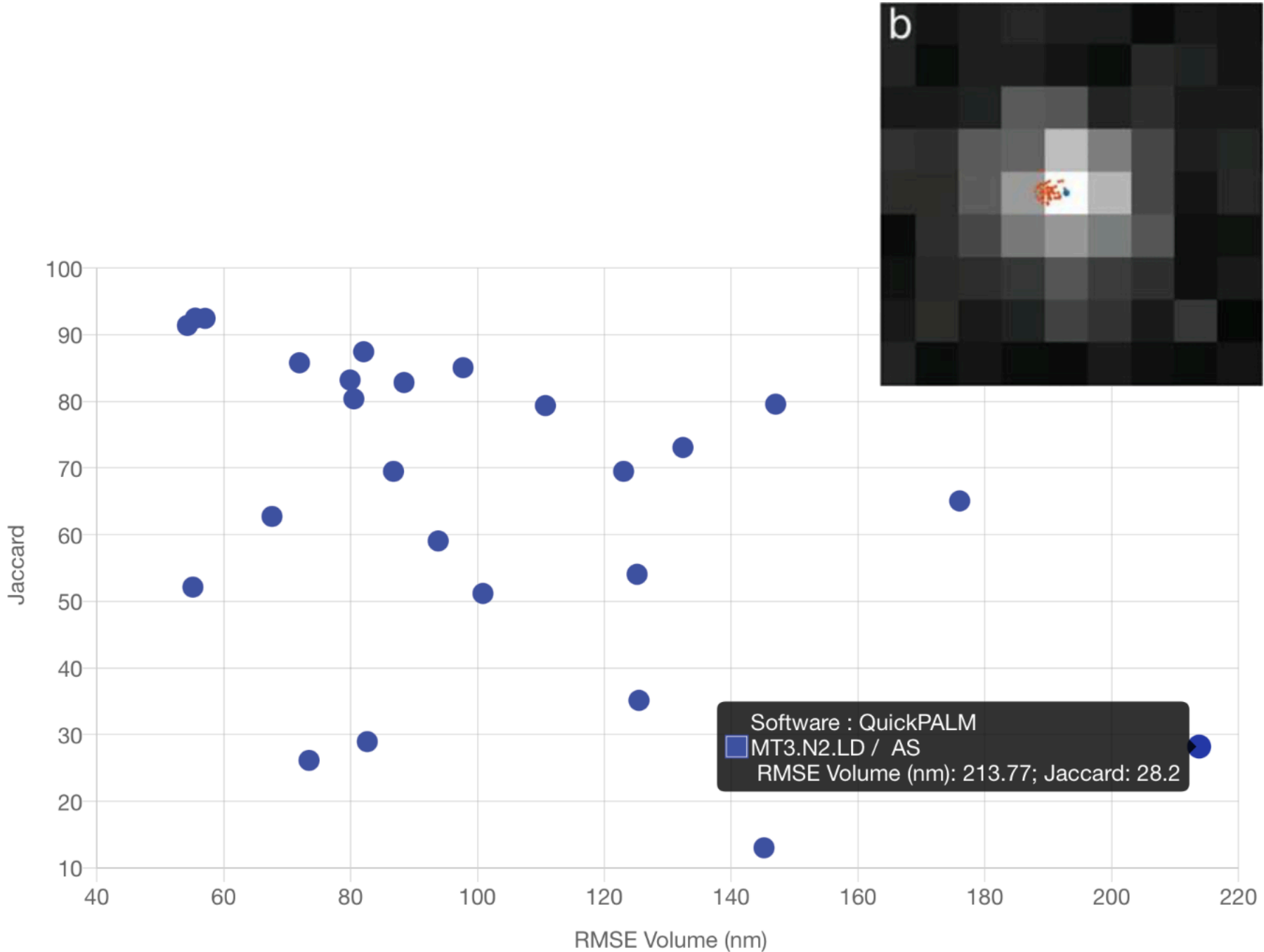


How to detect and localize when overlapping? 2 spots at $\sim 1\mu\text{m}$

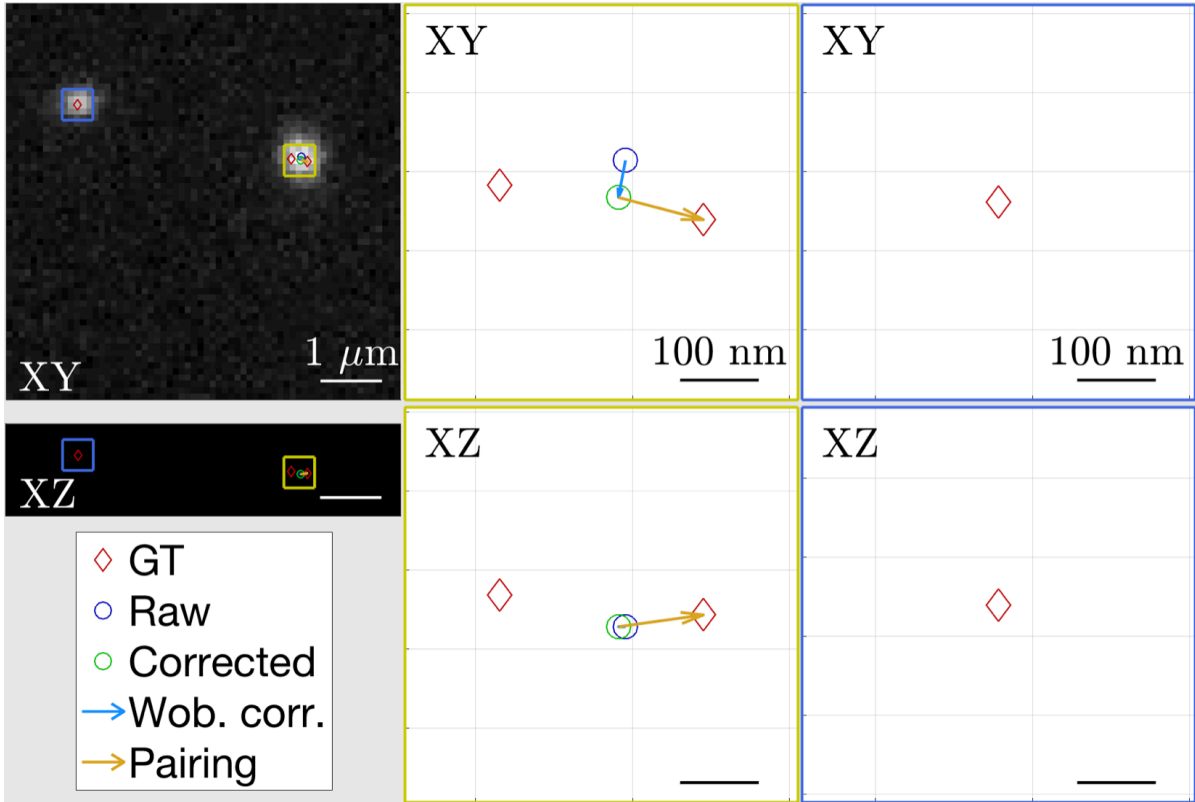


Software	PSF	Platform	Contact
3D-DAOSTORM	Gaussian	Python / C	Harvard University, MA, USA
3D-STORM	Gaussian	Qt framework	University of Upper Austria Linz, Austria
3D-WTM	Gaussian	Stand-alone	Hamamatsu Photonics K.K., Japan
Cspline	Learnt	Python / C	Harvard University, MA, USA
DECODE	Encode	Python / Theano	Janelia Farm, USA
Localizer	Gaussian	Igor	University of Leuven, Belgium
MIATool	Bessel	Java	Texas A&M University, Texas, USA
mlePALM	Gaussian	Matlab	Laboratory of Nanoscale Biology EPFL, Swiss
pSMLM-3D	Gaussian	ImageJ	Wageningen University, NL
QC-STORM	Gaussian	ImageJ	Wuhan National Laboratory, China
QuickPALM	Gaussian	ImageJ	Institut Pasteur Paris, France
RainSTORM	Gaussian	Matlab	University of Szeged, Hungary
RapidSTORM	Gaussian	Stand-alone	University of Wurzburg, Germany
SMAP	Learnt	Matlab	EMBL Heidelberg, Germany
SMolPhot	Gaussian	Python / C	University of Tartu Institute of Physics, Estonia
ThunderSTORM	Gaussian	ImageJ	Charles University Prague, Czech
TVSTORM	Gaussian	Matlab	The Ohio State University Columbus OH, USA
WaveTracer	Gaussian	Metamorph	University of Bordeaux, France
ZOLA-3D	Zernike	ImageJ	Pasteur Institute

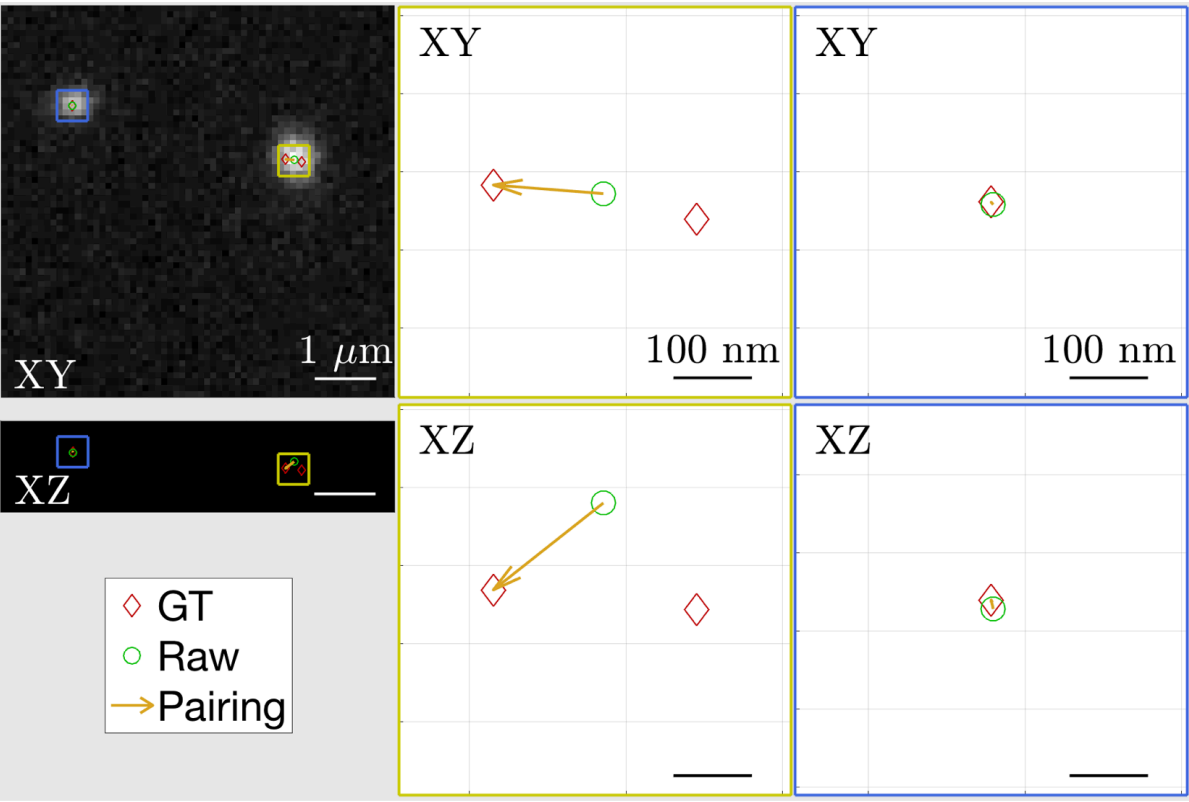
Criteria
Runtime
Detection Rate
Accuracy
Parameters
User interface
PSF model
Learning?
Accessible
Robust to HD



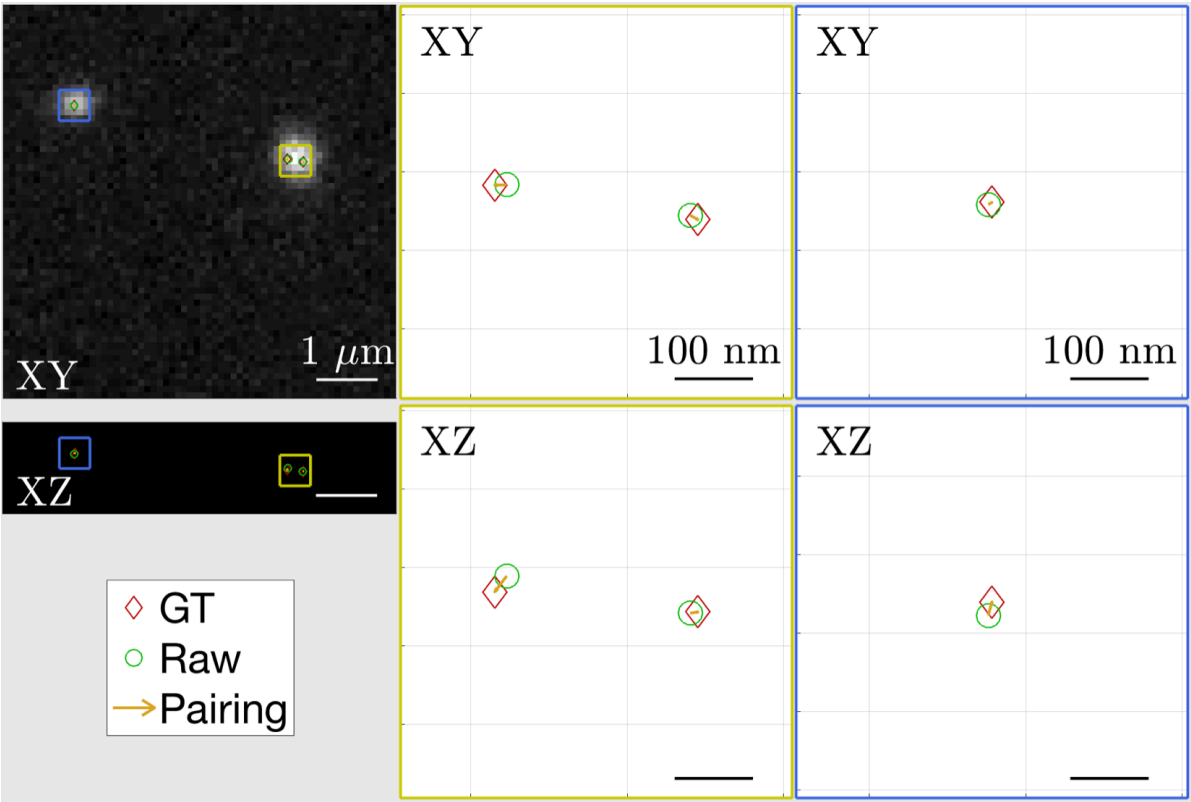
QuickPALM (center of gravity)



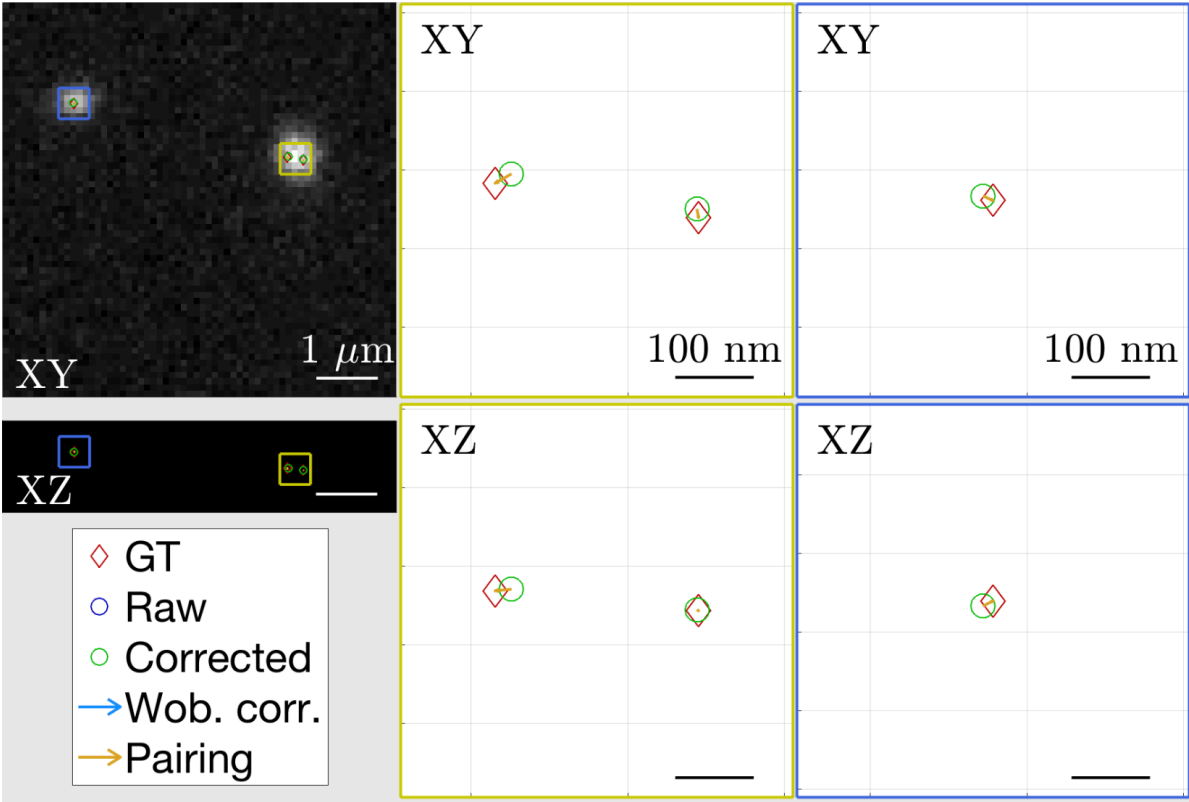
MIATools (single-emitter fit)



Spline (multi-emitter fit PSF)



DECODE (Learning)

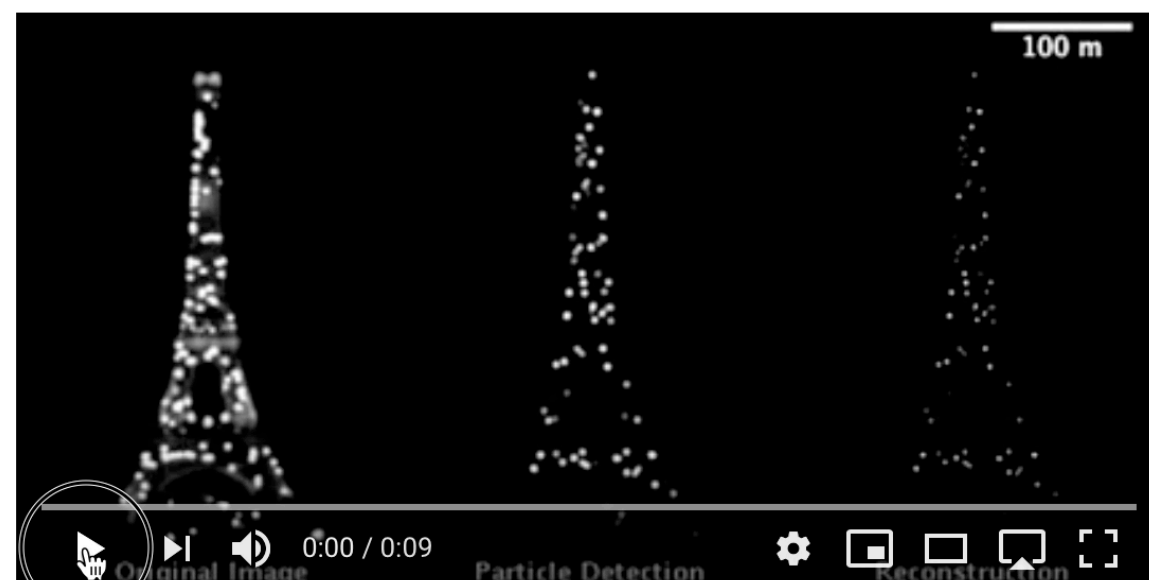


QuickPALM

Ricardo Henriques, Nat. Methods, 2010

The historical tool for a fast reconstruction

- Fast (without GPU)
- Method: centroid
- Popular: embedded in FIJI
- 2D and 3D (AS)
- On-line reconstruction

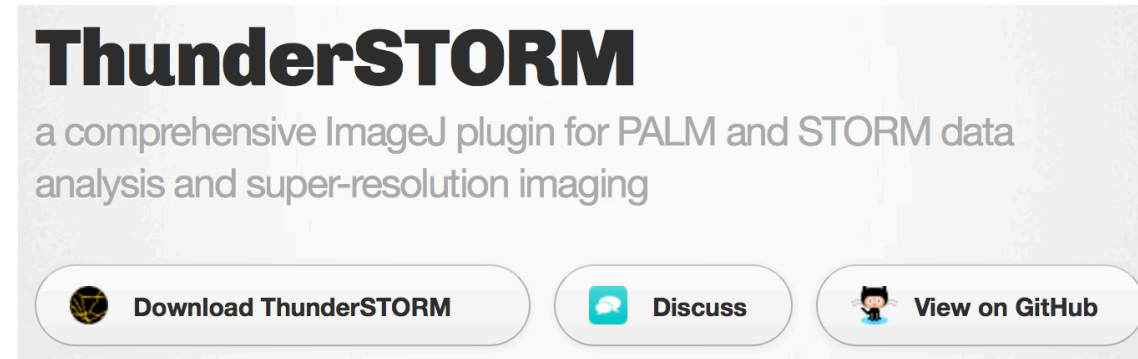


ThunderSTORM

Martin Ovesný, Bioinformatics 30, 2014

A comprehensive ImageJ plugin for PALM and STORM data

- Easy-to-use on Fiji
- Extensive collection of methods
- 2D and 3D (AS, BP)
- Post-processing: drift, grouping



PSF fitting

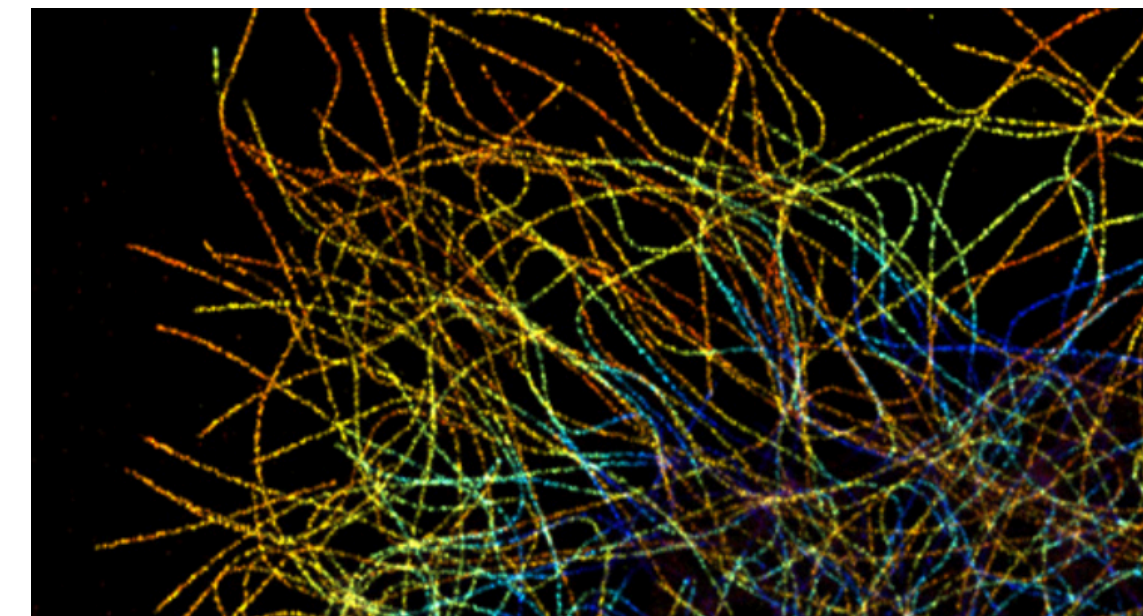
- Single emitter fitting: detection-localisation-rejection
- Multiple emitter fitting: optimal determination of the number of molecules (**high-density**)

SMAP

Jonas Ries, Nature Methods 2018

Super-Resolution **M**icroscopy **A**nalysis **P**latform

- Easy-to-use, Matlab based tools
- Extensive collection of methods
- 2D and 3D (AS, DH, BP)
- Post-processing: drift, grouping



PSF model

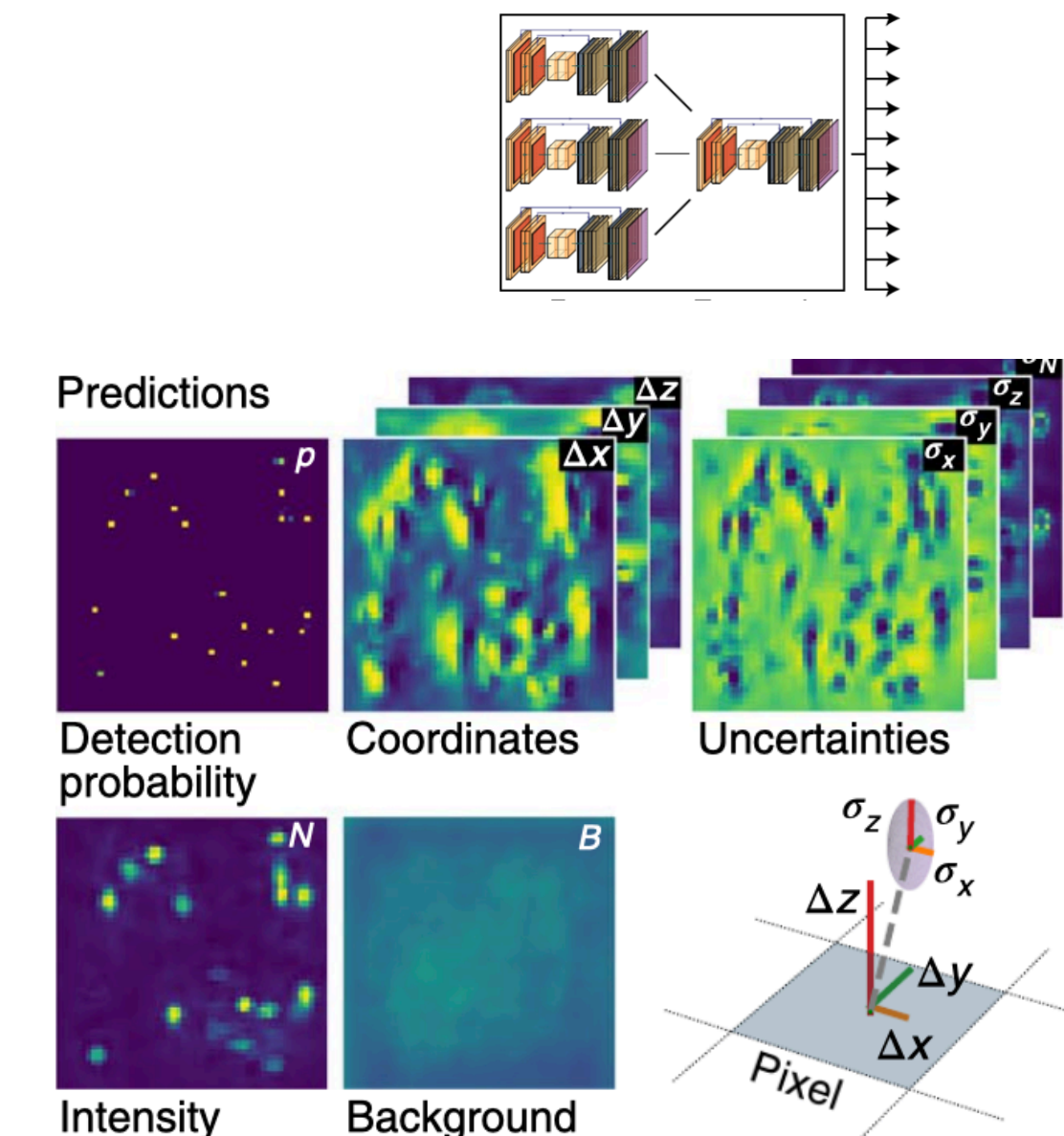
- Include the 3D fit of PSF
fit3Dspline

DECODE

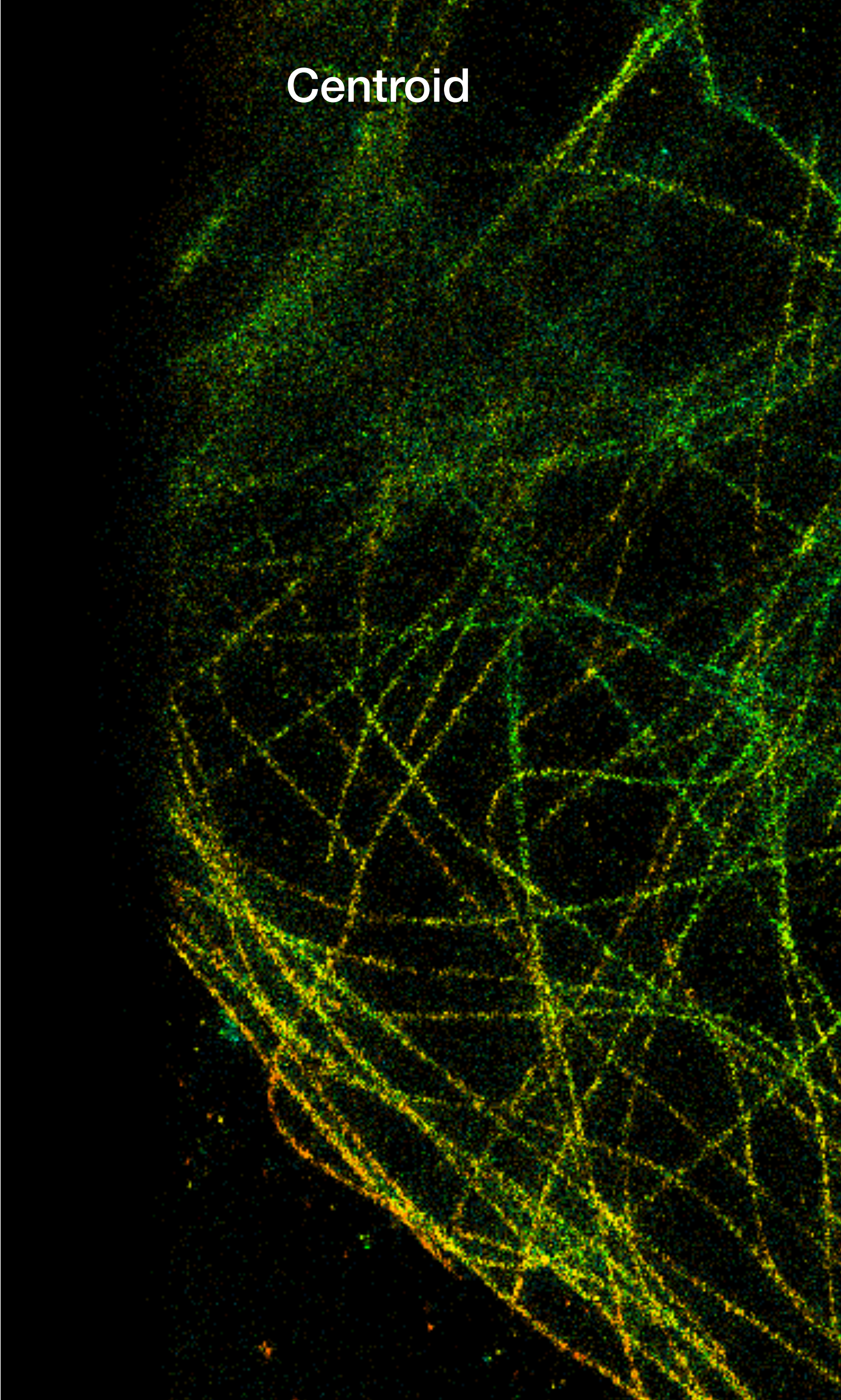
Arthur Spieser, Nature Methods 2021

Deep Learning
Prediction 3D localizations

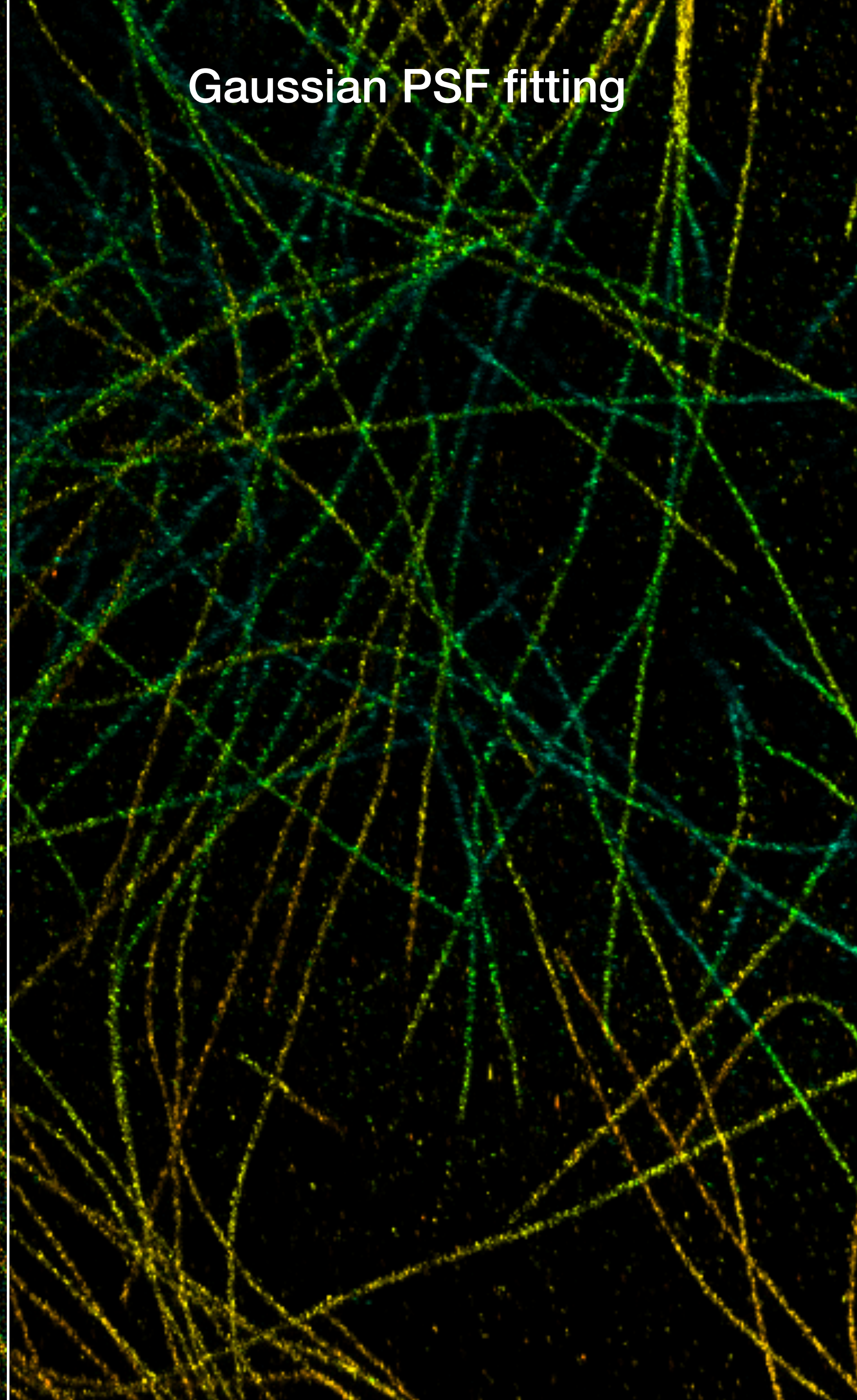
- 2D and 3D. Python-based
- Prediction: detection, intensity, **coordinates**, uncertainties
- Frame-to-frame and temporal analysis



Centroid



Gaussian PSF fitting



Cubic-spline PSF fitting

