



ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Benchmarking Spot Localization

Single-Molecule Localization Microscopy

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<http://bigwww.epfl.ch/smlm/>

Benchmarking Spot Localization

Introduction

- ▶ SMLM
- ▶ Benchmarking

Your task

- ▶ Run open software on reference datasets
- ▶ Make the assessment

2D

- ▶ Simulation
- ▶ Datasets
- ▶ Assessment & Metrics
- ▶ Comparison
- ▶ Challenge ISBI 2013

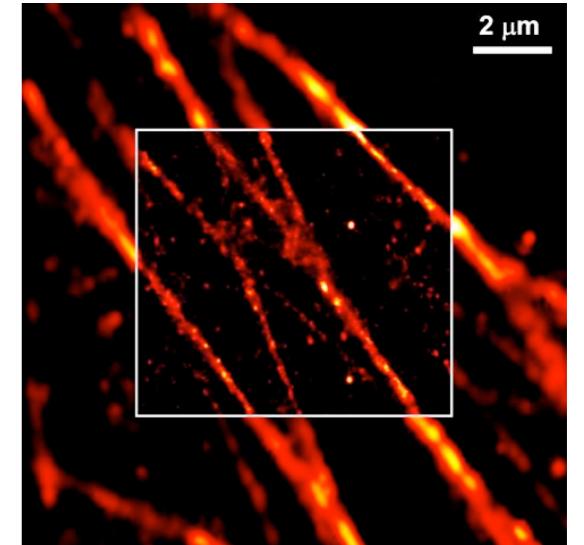
3D

- ▶ Simulation
- ▶ Datasets
- ▶ Assessment & Metrics
- ▶ Comparison
- ▶ Challenge SMLMS 2016

Single-Molecule Localization Microscopy

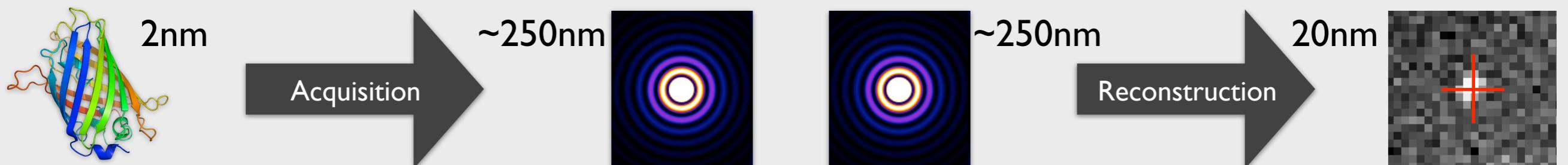
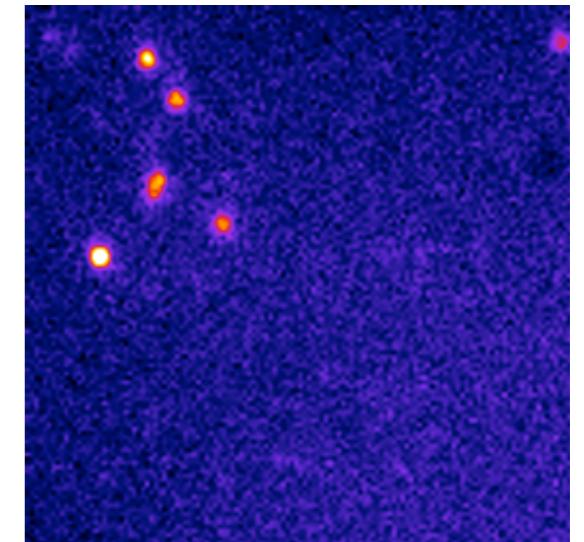
Super-resolution: from microscopy to nanoscopy

- ▶ SMLM: One of the technique to access to nanoscale
- ▶ SMLM: One method ... many acronyms PALM, STORM, FPALM, SMACM, uPAINT, SMLM, SRLM

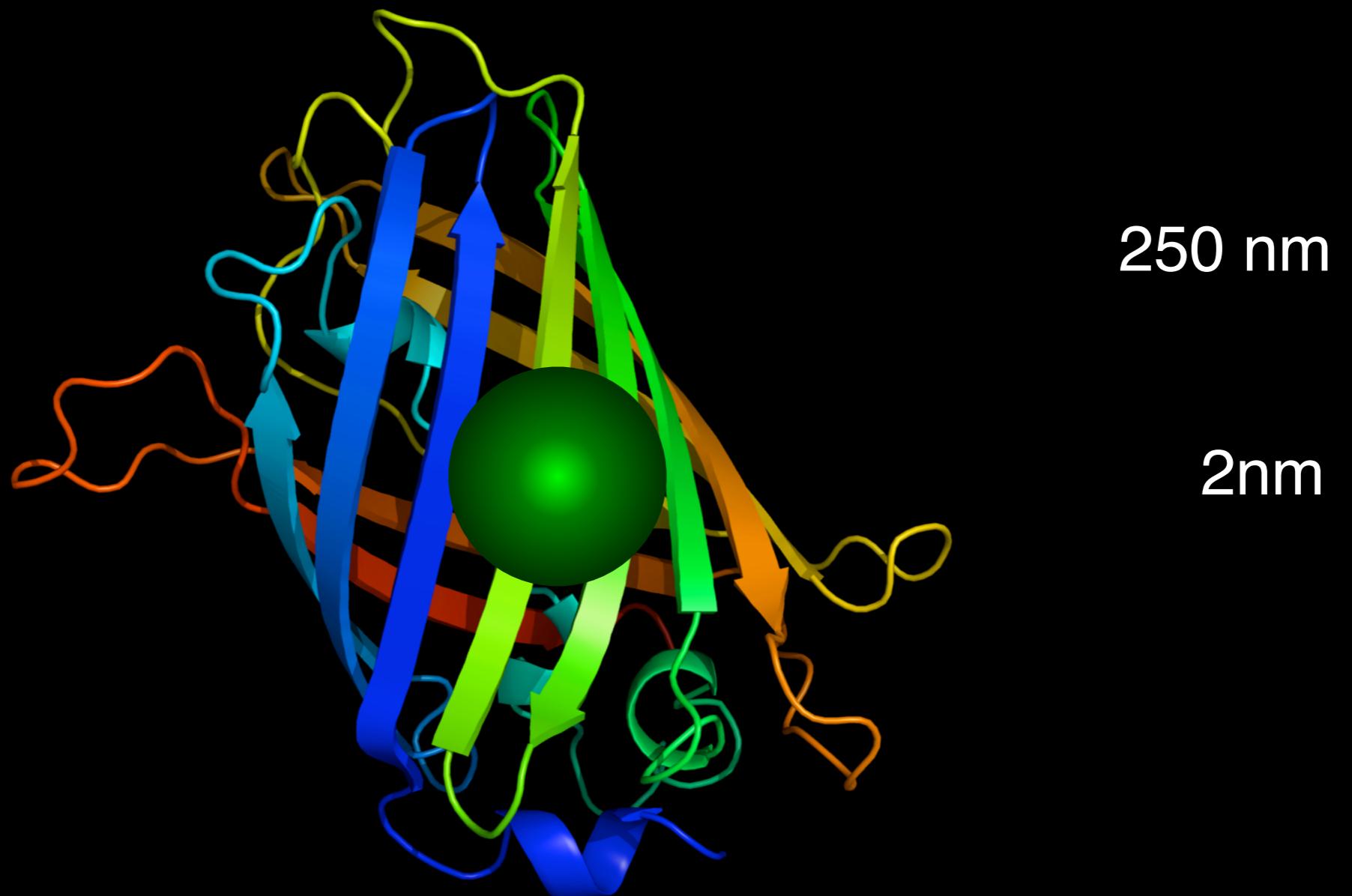


SMLM: Need computational reconstruction

- ▶ Sparse set of activated fluorophores at each time instant
- ▶ Reconstruction from accurate positions of molecules



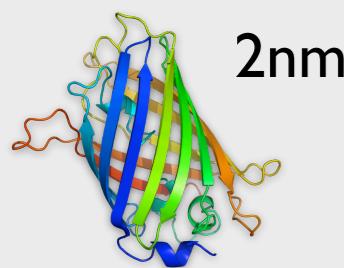
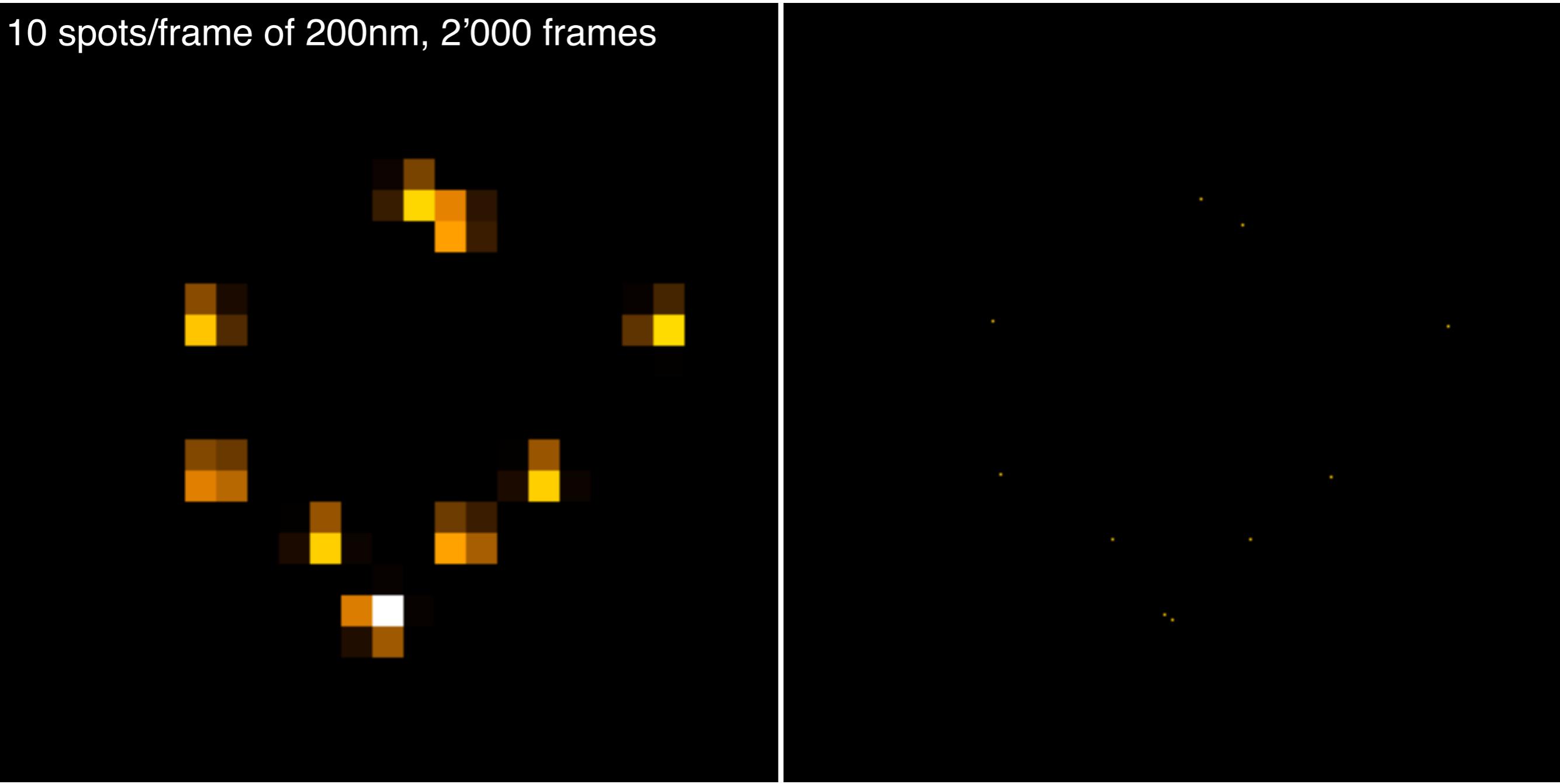
Molecular biology



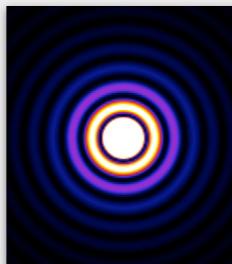
Protein of interest tagged with
fluorescent single-molecule

Single-Molecule Localization Microscopy

10 spots/frame of 200nm, 2'000 frames



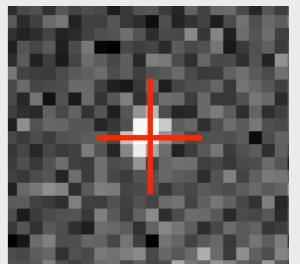
200nm



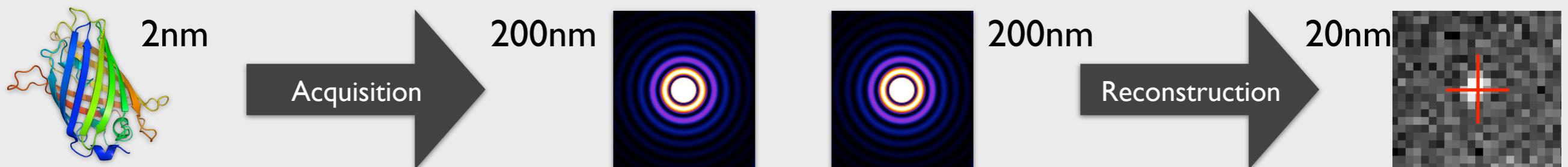
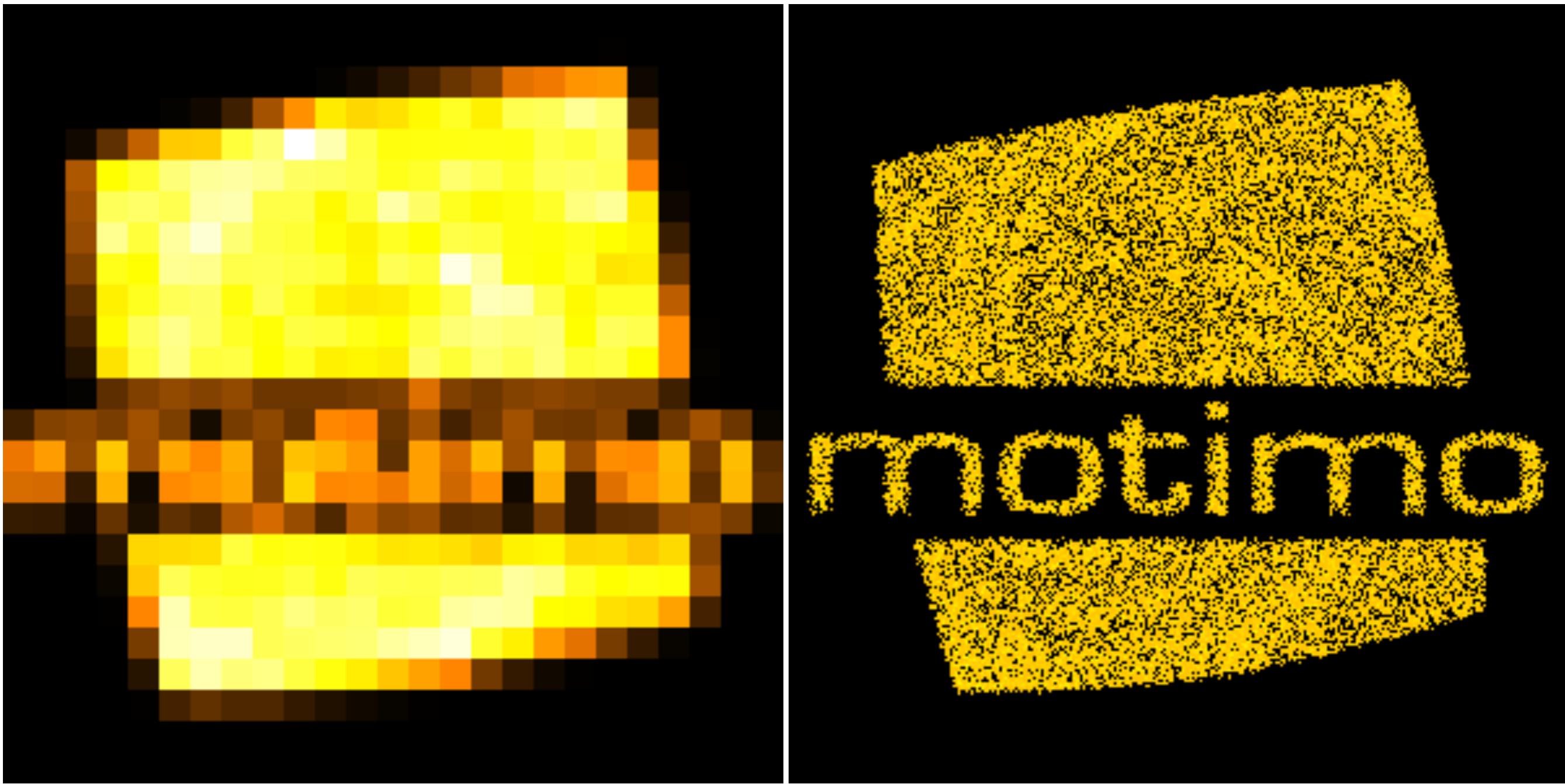
200nm



20nm



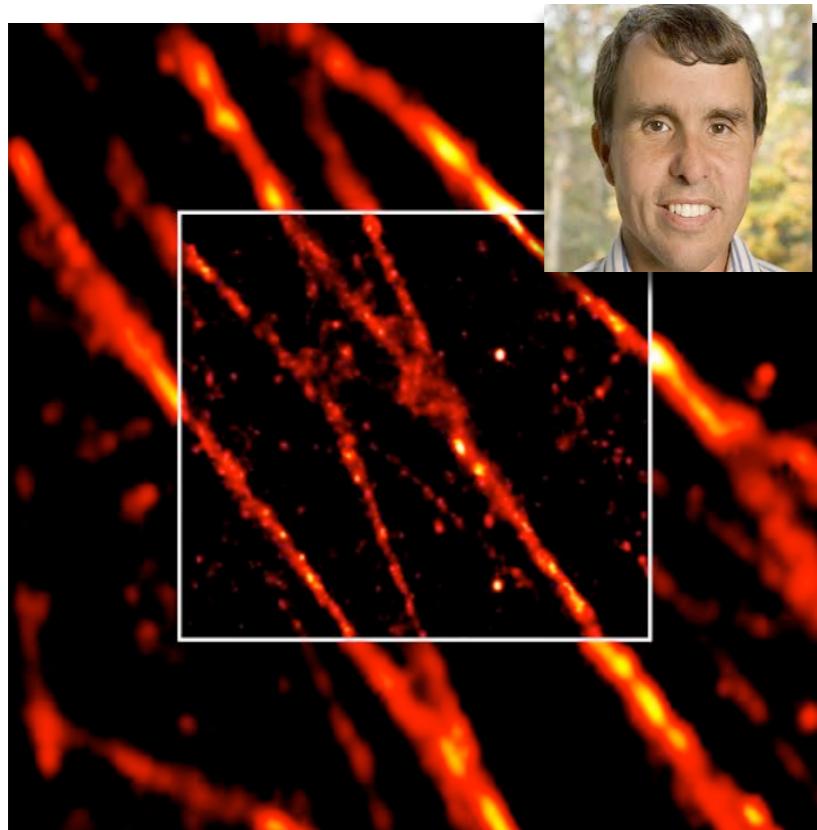
Single-Molecule Localization Microscopy



Single-Molecule Localization Microscopy

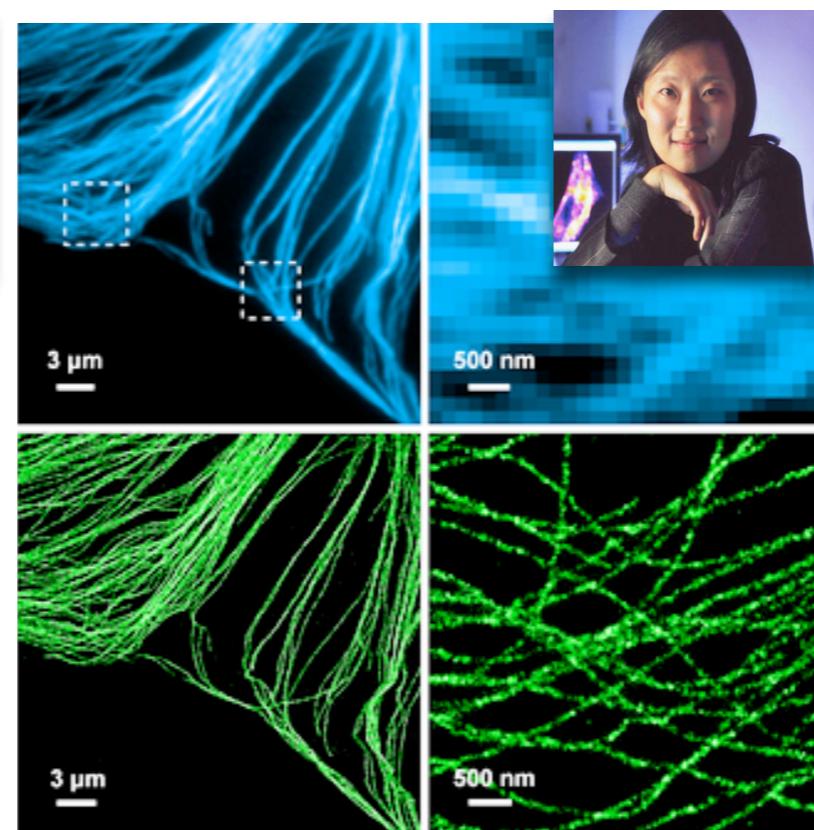
Nobel Prize 2014

Method of the Year 2008



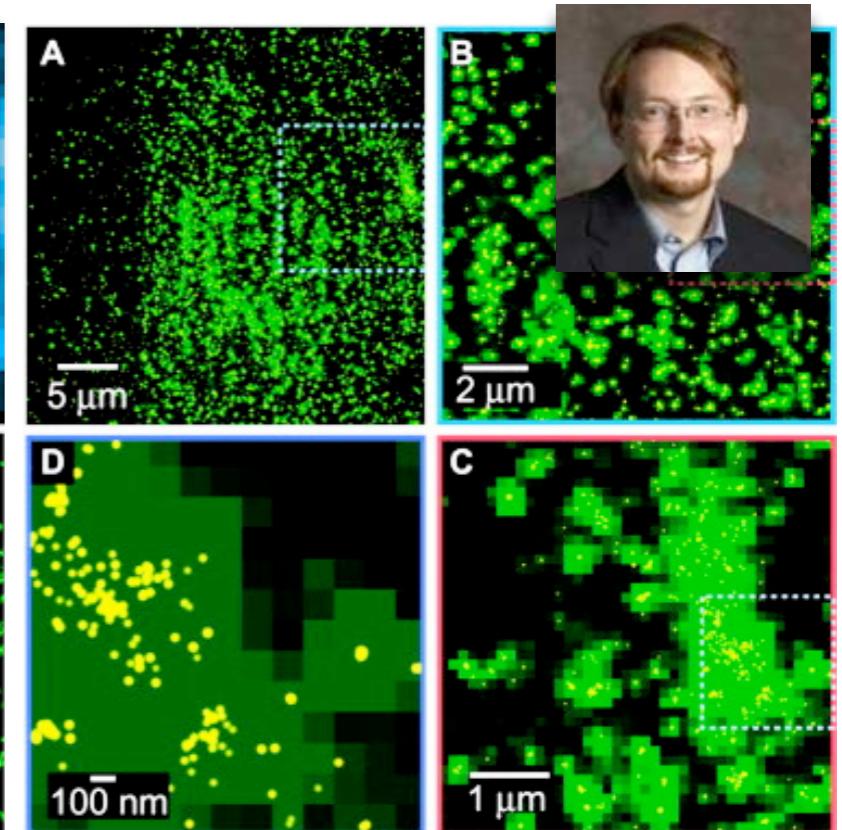
PALM

Photo-Activation Localization
Microscopy
Eric Betzig



STORM

Stochastic Optical Reconstruction
Microscopy
Xiaowei Zhuang



FPALM

Fluorescent Photo-Activation
Localization Microscopy
Sam Hess

Benchmarking in Bioimaging

- ▶ For developers:
 - ▶ Platform to exchange, boost innovation, identification of the community
 - ▶ Good practices: open-source, usability [Carpenter, Nat. Meth. 2012]
- ▶ For end-users:
 - ▶ Comprehensive review of the existing solutions
 - ▶ Large-scale benchmarking as a resource to help to the decision
- ▶ Opportunity to define the field:
 - ▶ Reference common datasets
 - ▶ Metrics to assess the performances
 - ▶ Standardization

Grand-Challenge for Benchmarking

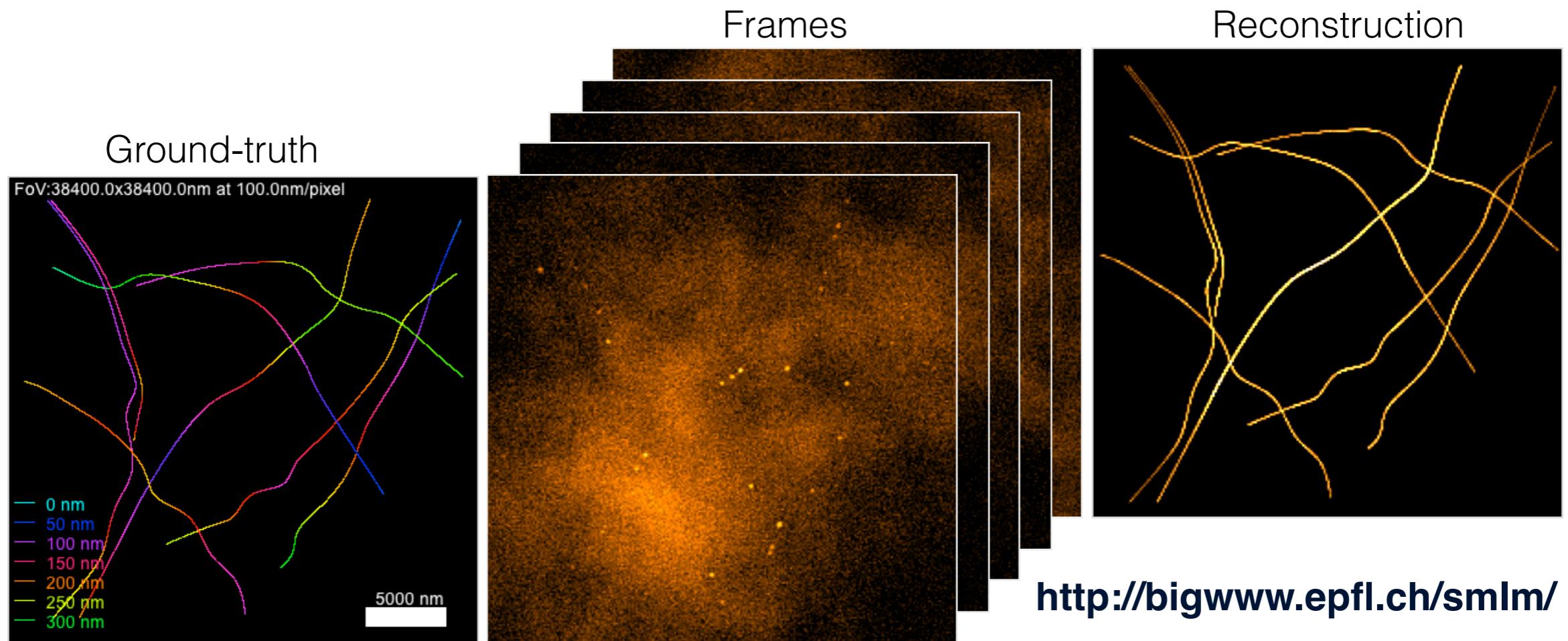
Create the competition
Boost the development
Run by the authors
Inform the final users

“Collaboration through competition”
[Editorial, Nat. Meth. 11, 2014]

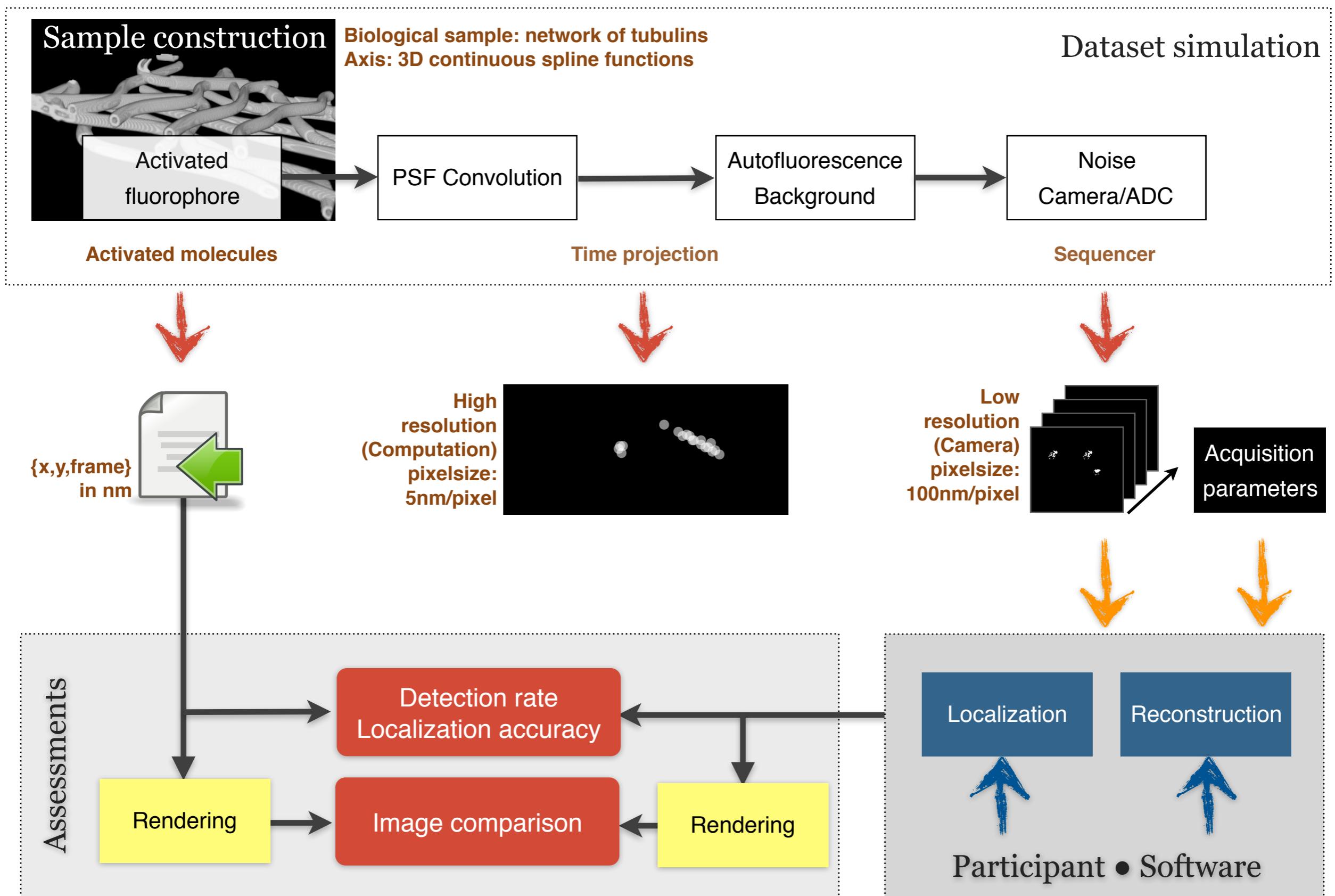
Hide the scientific analysis
Bias by the ranking
Overturning to win

Benchmarking SMLM Software

- ▶ Large offer of software packages: <http://bigwww.epfl.ch/smlm/software/>
- ▶ Missing validation: Robustness to the parameters, validate on specific datasets
- ▶ Well defined input and output in the simulation: localization positions



Validation Protocol



Benchmarking Spot Localization

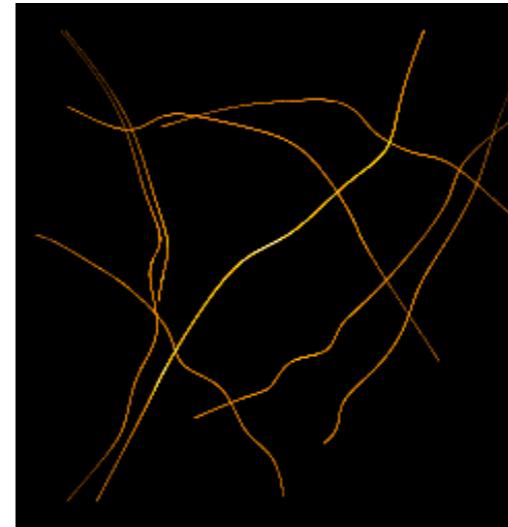
SMLM 2D Simulation

<http://bigwww.epfl.ch/smlm/>

Bio-inspired samples

Biological sample structure

- ▶ 3D geometrical structure, extruded tubes
- ▶ Continuously defined by B-spline basis functions



Example

Network of tubulins
Non uniform illumination



Towards bio-inspired sample, mimics real biological scenario



Highly realistic, projection of 3D structure



Simulation of fluorophore activation, faithful image formation



Discrete representation at any scale

大

Fluorophore Activation

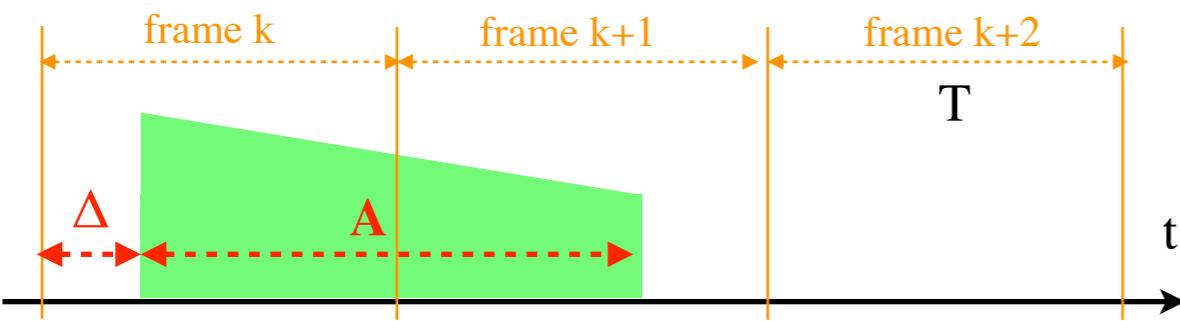
Activation

Density

- Volumetric density: molecules / μm^3
- Uniform spatial distribution
- Uniform temporal distribution

Lifetime model

- Profile: constant, linear, or expo decay
- Delay Δ and activity A are random
- Number of photons F random variable



Emission of photons

Photon flux per dye $F = \Phi \cdot \frac{P}{e} \cdot \sigma$ [photons/s]

- Φ : Quantum yield of the dye
- P : Power laser [W/cm^2]
- $e = h \cdot \frac{c}{\lambda}$: Energy of 1 photon [J]
- $\sigma = 1000 \cdot \frac{\ln(10) \cdot \epsilon}{N_A}$: Absorption cross section [cm^2]
- ϵ : Molar extinction coefficient (EC) or absorptivity [cm^2/mol]

Example

- $\epsilon = 60000 \text{ cm}^2/\text{mol}$
- $\Phi = 0.6$
- $P = 200 \text{ W}/\text{cm}^2$
- $\lambda = 500 \text{ nm}$
- $N_A = 6.022\text{E}-23 \text{ mol}^{-1}$
- $h = 6.626\text{E}-34 \text{ J} \cdot \text{s}$
- $c = 3.0\text{E}8 \text{ m}/\text{s}$
- Absorption cross section: $\sigma = 1000 \cdot \frac{\ln(10) \cdot \epsilon}{N_A} = 2.29\text{E}-16 \text{ cm}^2$
- Energy of 1 photon: $e = h \cdot \frac{c}{\lambda} = 3.97\text{E}-19 \text{ J}$
- Flux of photons per dye per second: $F = \Phi \cdot \sigma \cdot \frac{P}{e} = 68700 \text{ photons/s}$

Image Formation

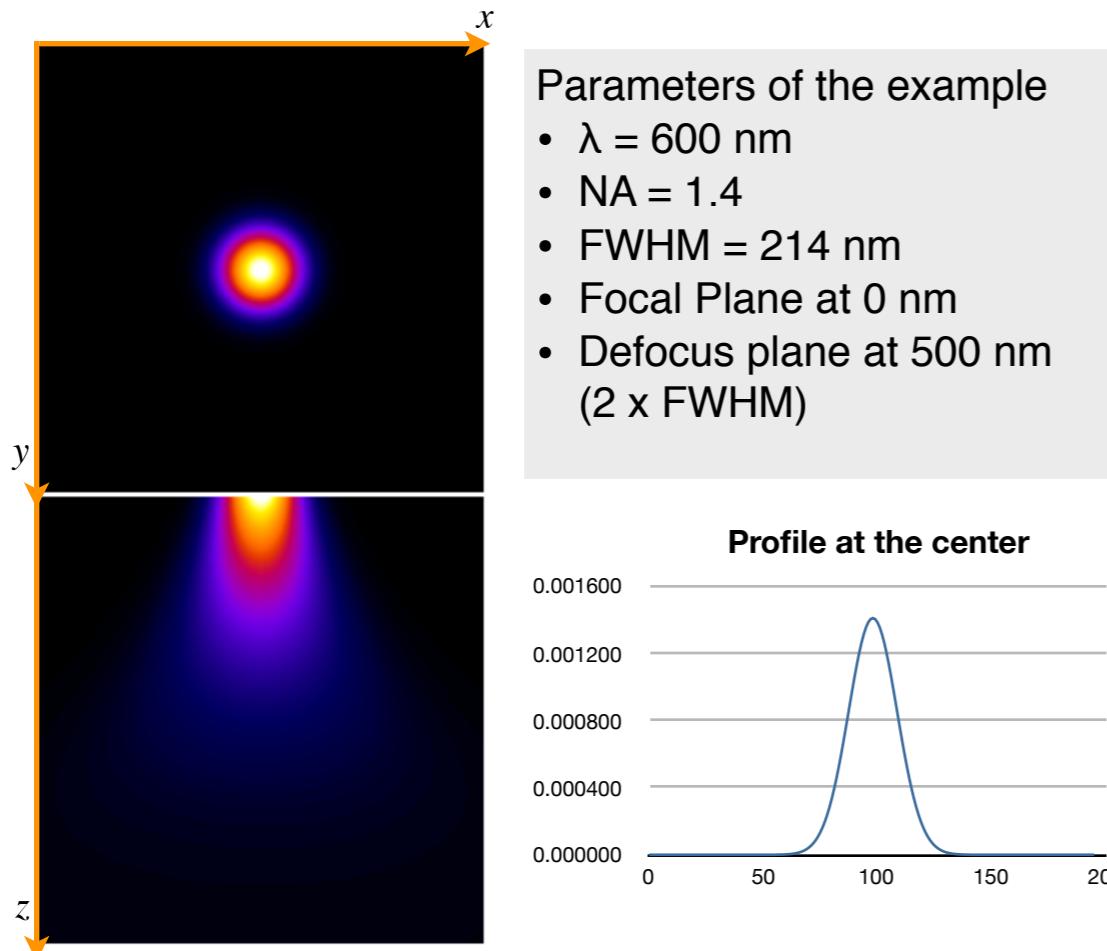
2D defocussed Gaussian PSF

Parameters

- Lateral center: (x_c, y_c) [nm]
- FWHM [nm] at $z_c = \text{diffraction limit} = \frac{\lambda}{2\text{NA}}$ [nm]
- $2\times$ FWHM at : z_d [nm]

Exponential in Z, Gaussian in XY

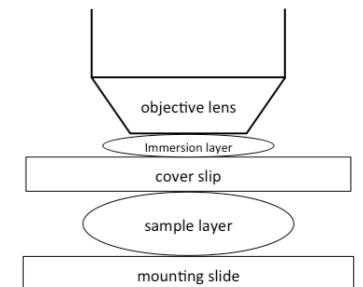
- Exponential in Z: $\Delta_z = e^{-\frac{|z-z_c|\ln(2)}{2z_d}}$
- Gaussian in XY: $f_z(x, y) = e^{-\frac{(x-x_c)^2+(y-y_c)^2}{\sigma^2\Delta_z^2}}$
- Normalization in XY: $\|f_z(x, y)\| = 1$



Gibson & Lanni PSF

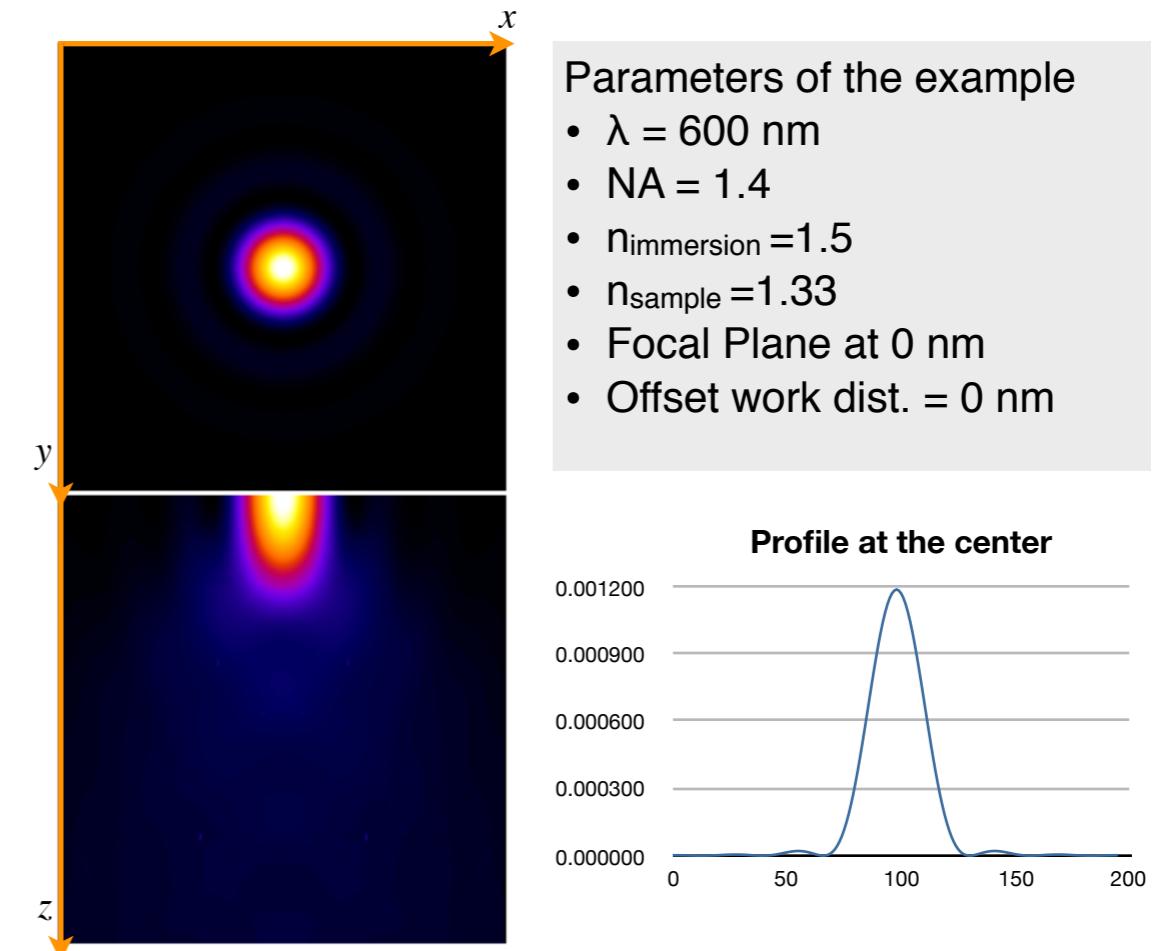
Describe the scalar-based diffraction that occurs in the microscope.

Take into accounts for the immersion, the cover-slip and the sample layers.



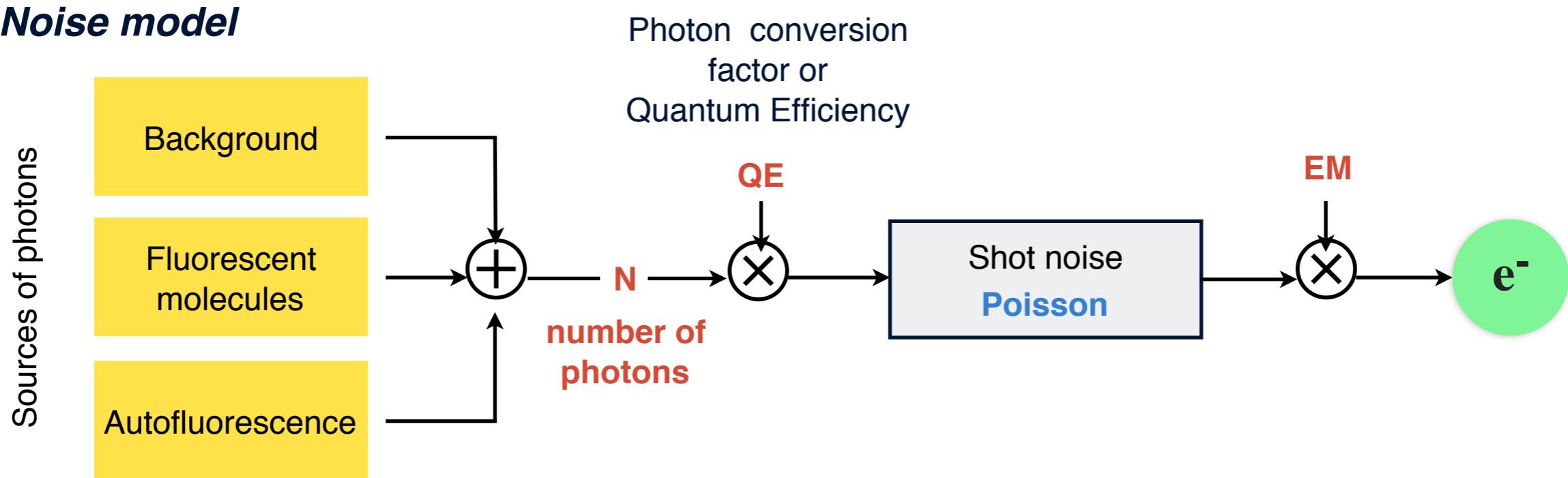
Reference

H. Kirshner and al., [3-D PSF Fitting for Fluorescence Microscopy: Implementation and Localization Applications, J. of Microscopy, 2012.](#)

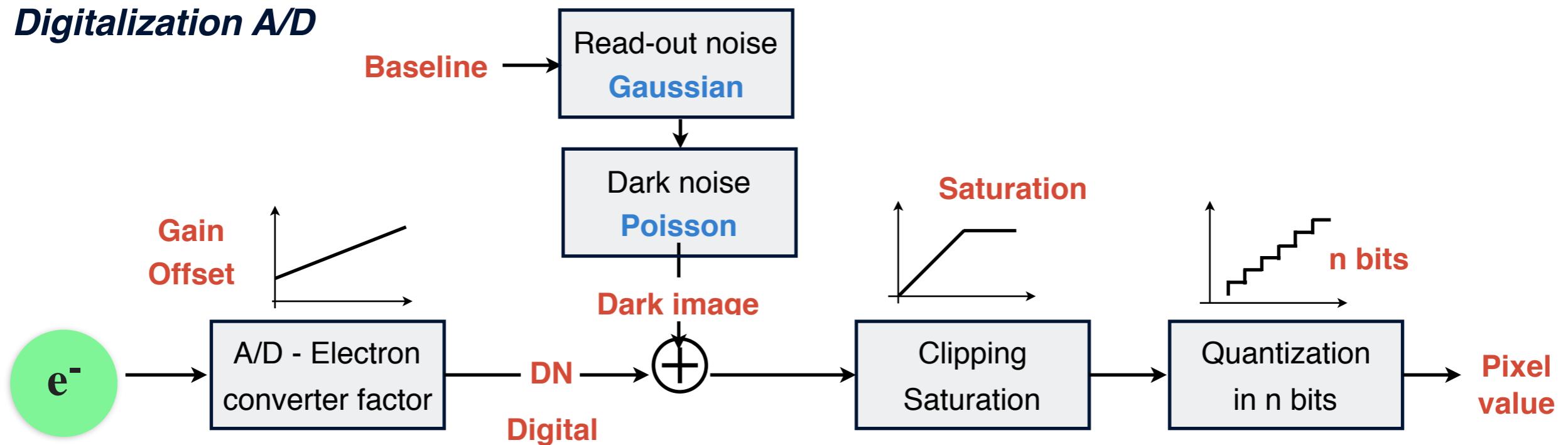


Noise Model

Noise model

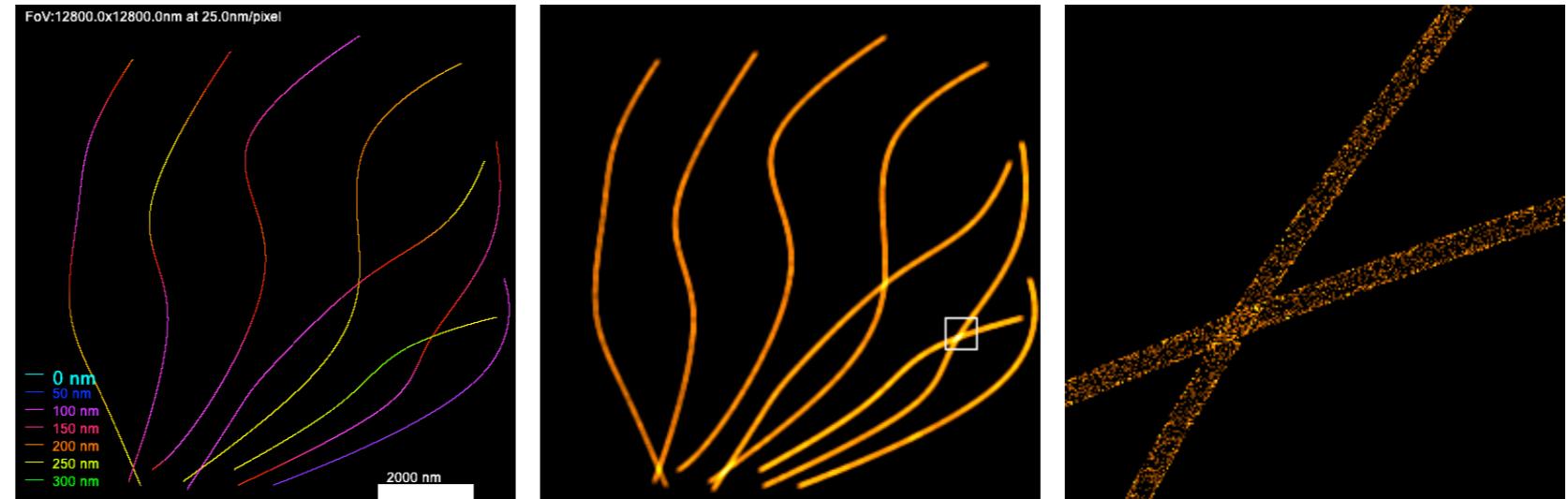


Digitalization A/D



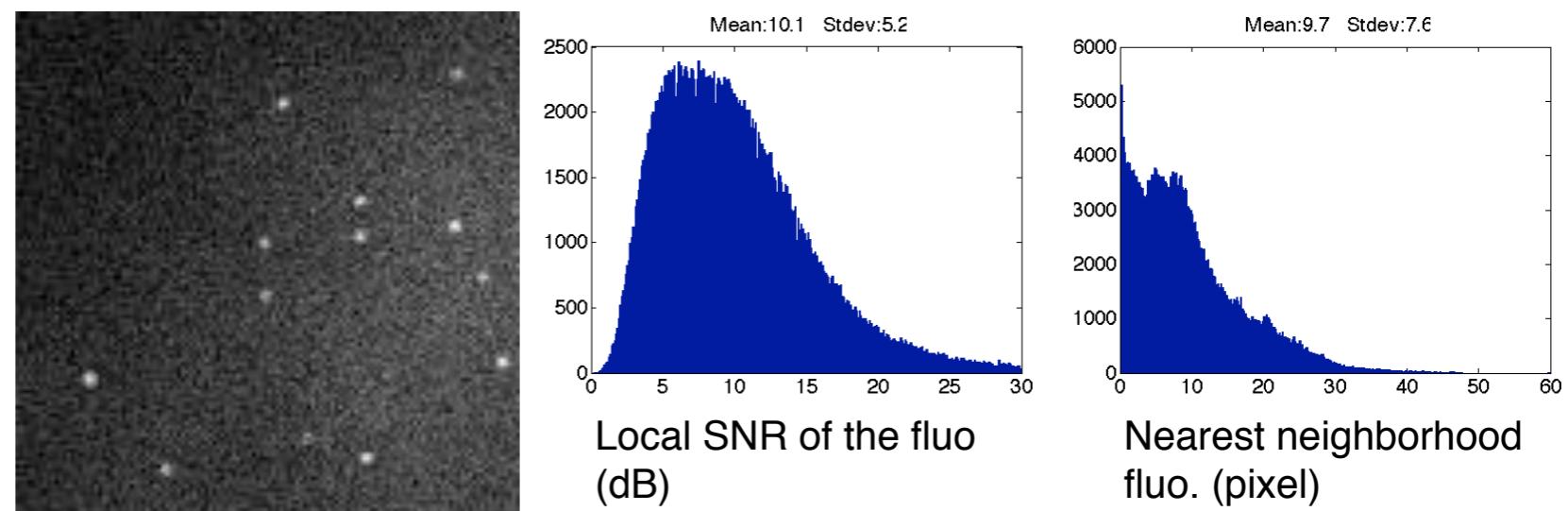
Reference Datasets

Resolution	128 pix. x 100nm
Thickness	53...248 nm
Diffrac. limit	224.32 nm
PSF Model	XY Gauss, Z Expo
# of fluos	289484 fluos



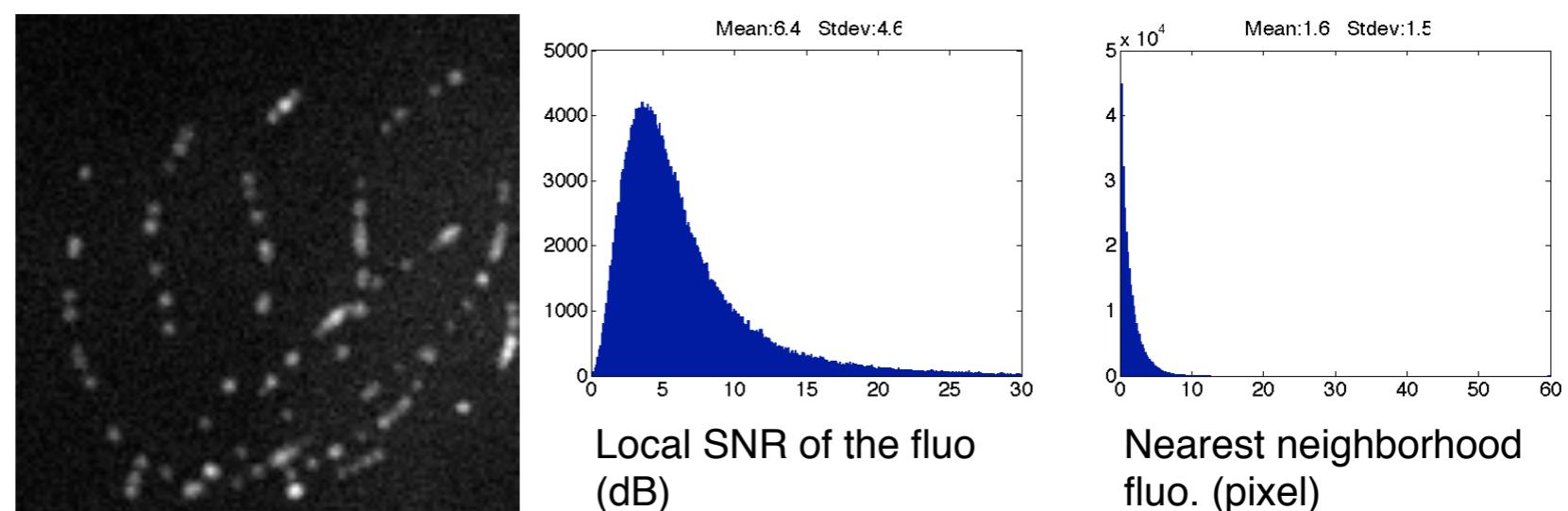
Long-sequence

- ~25 activations / frame
- 10'000 frames



High-density

- ~250 activations / frame
- 1'000 frames

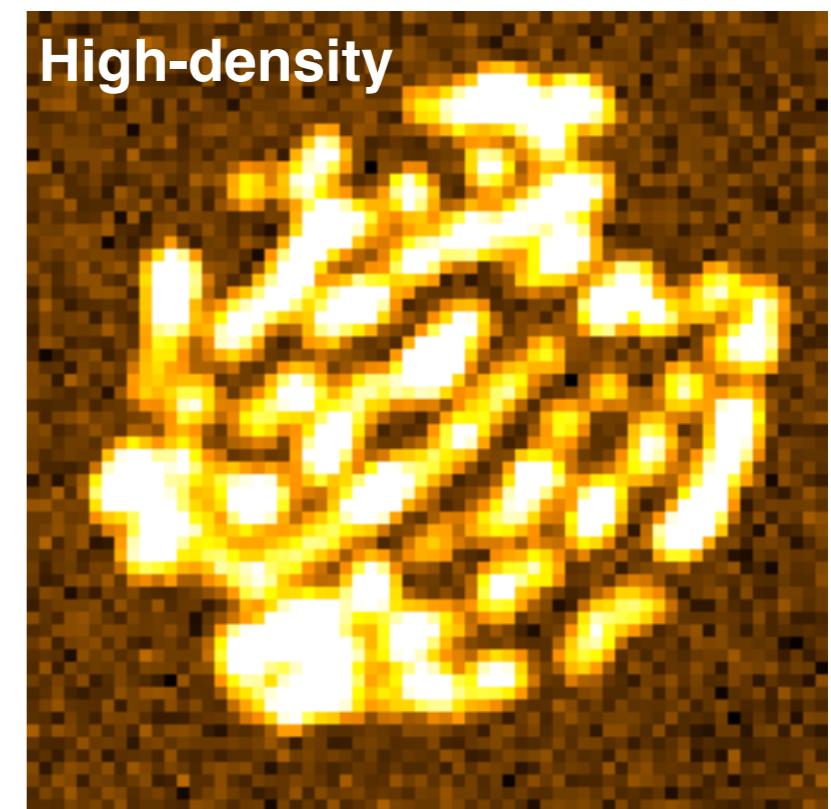
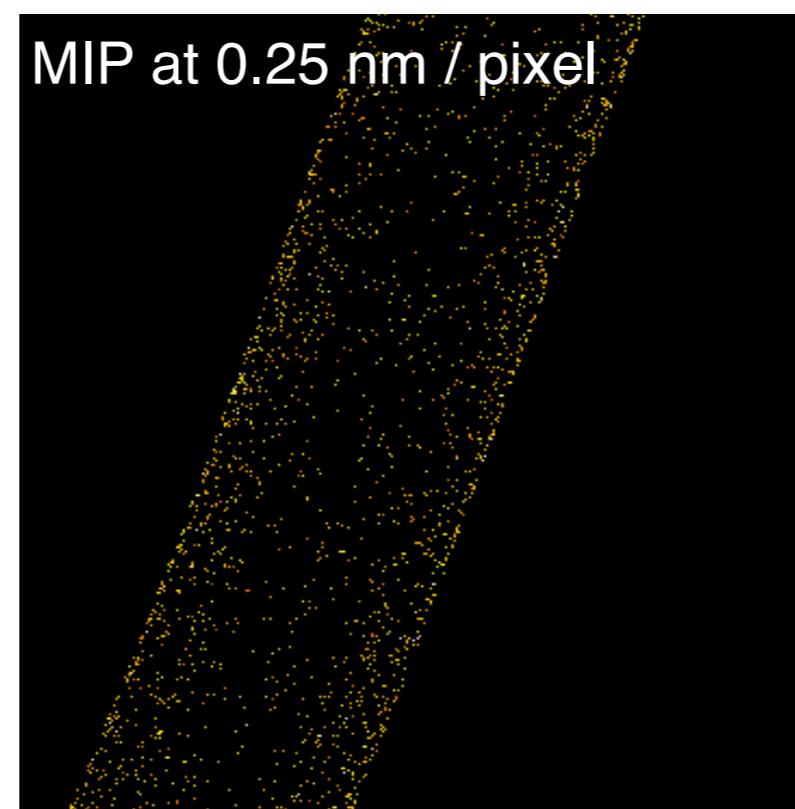
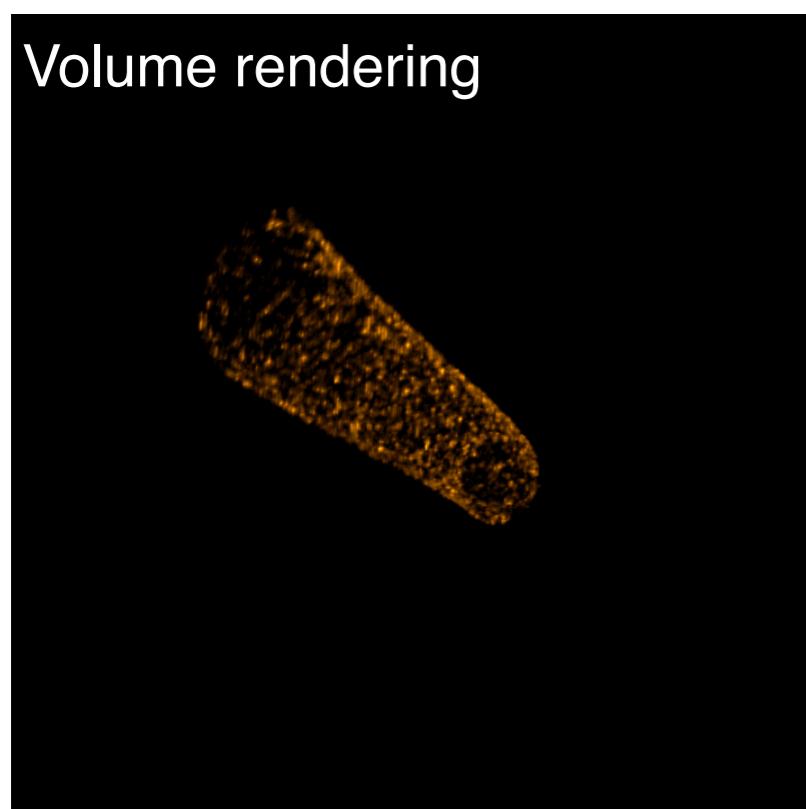
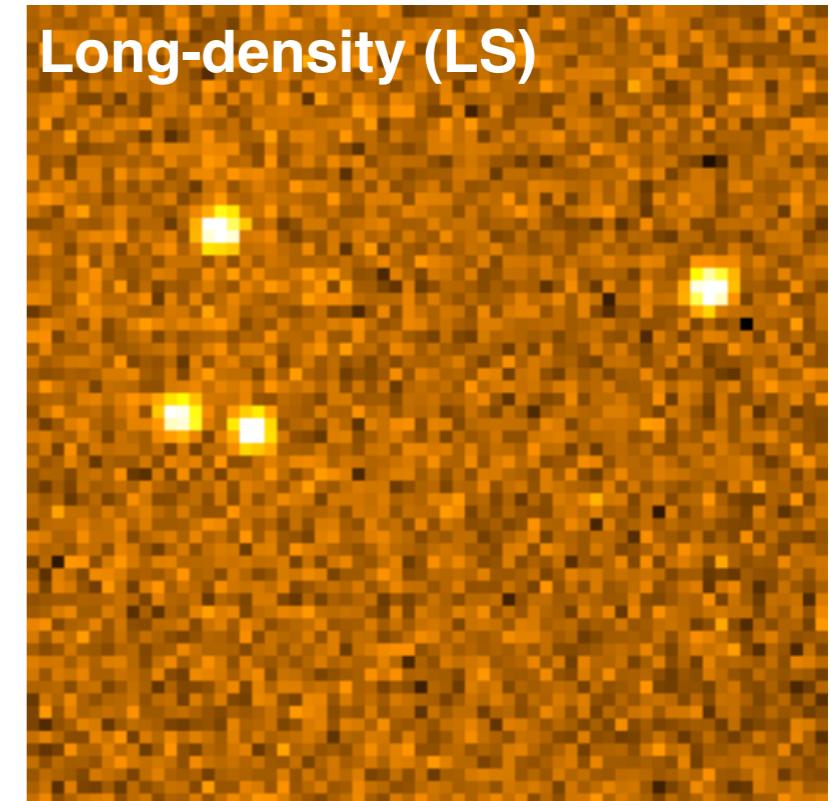
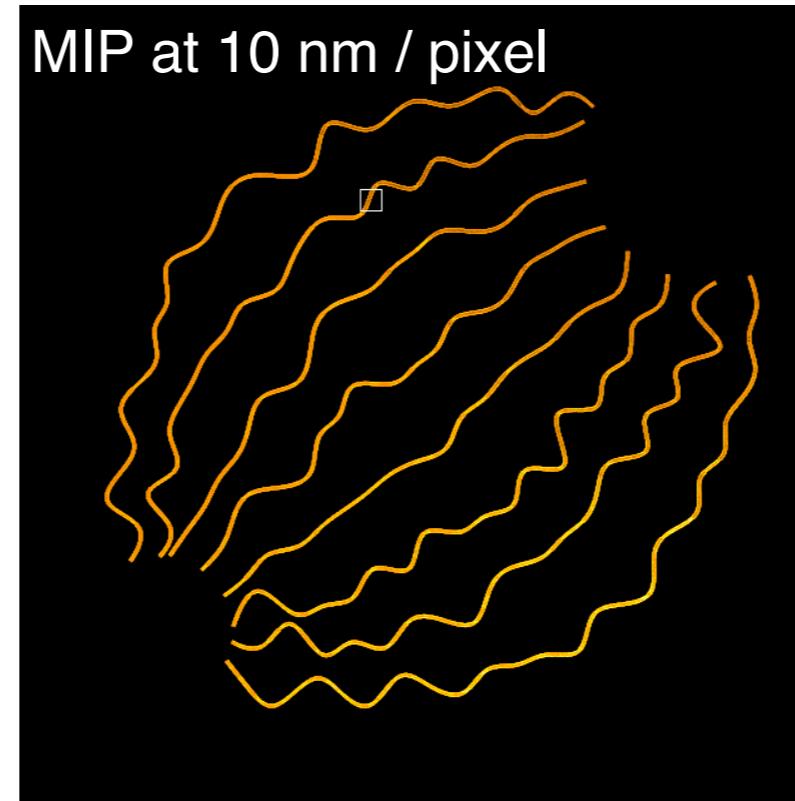
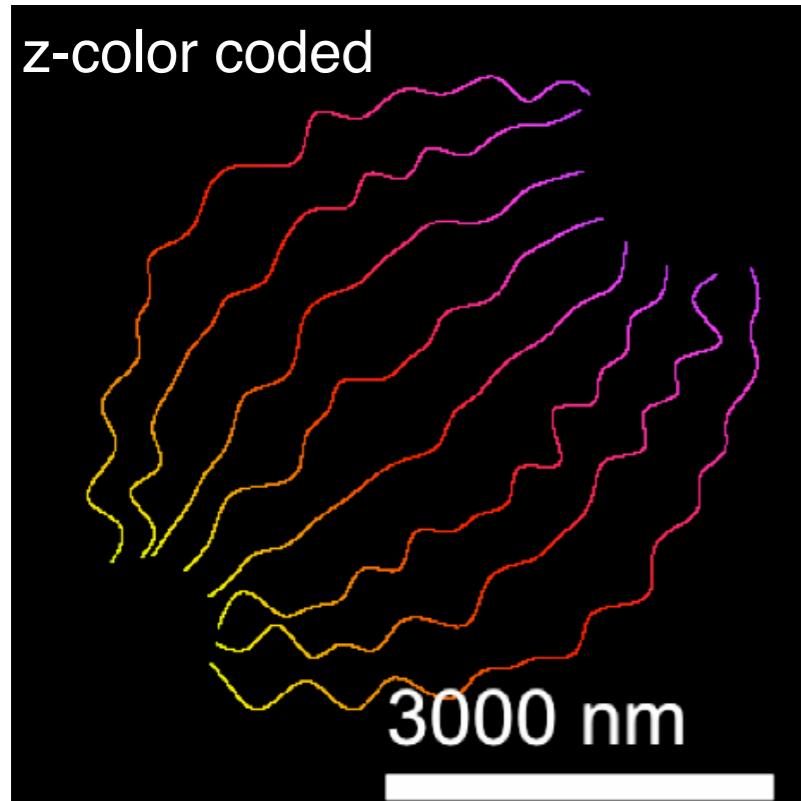


Benchmarking Spot Localization

SMLM 2D Datasets

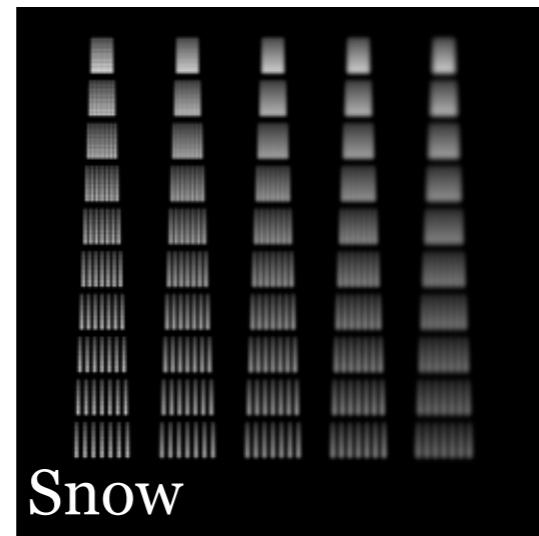
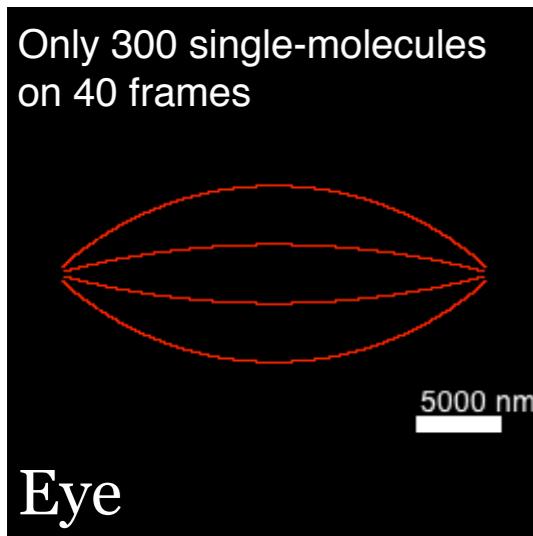
<http://bigwww.epfl.ch/smlm/>

Datasets

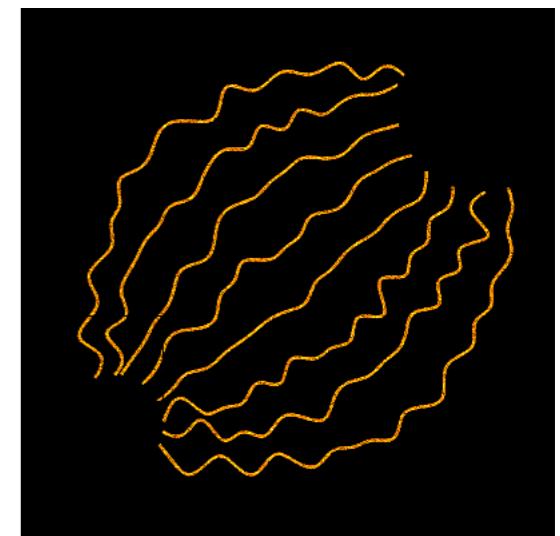
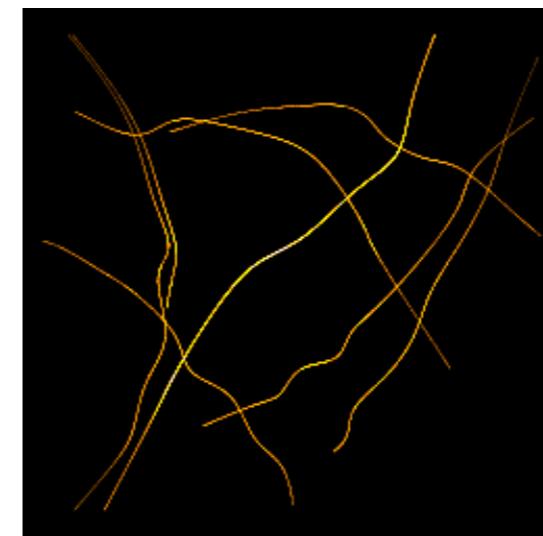


Datasets

Control datasets

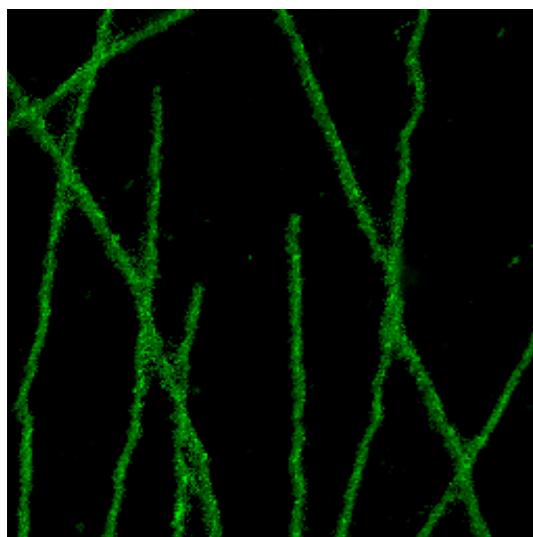


Training datasets

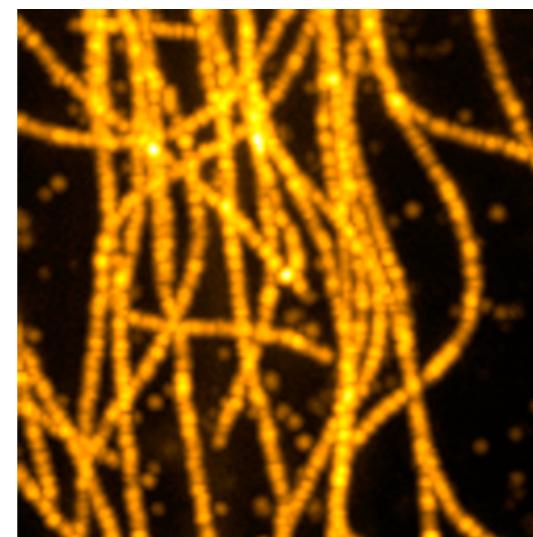


Real datasets

Long sequence
15'000 frames, 64x64 pixels

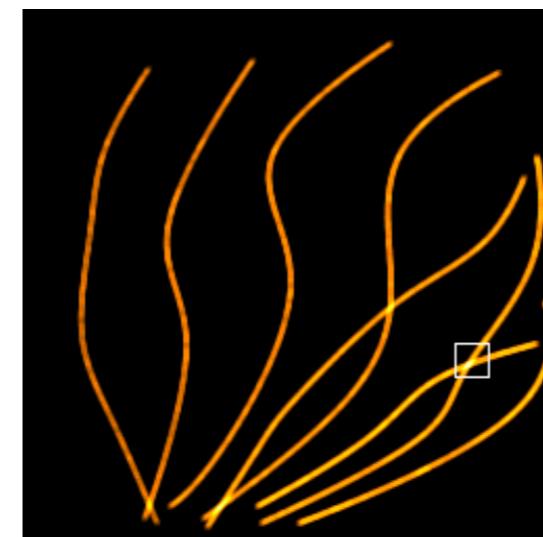


High density
500 frames, 64x64 pixels

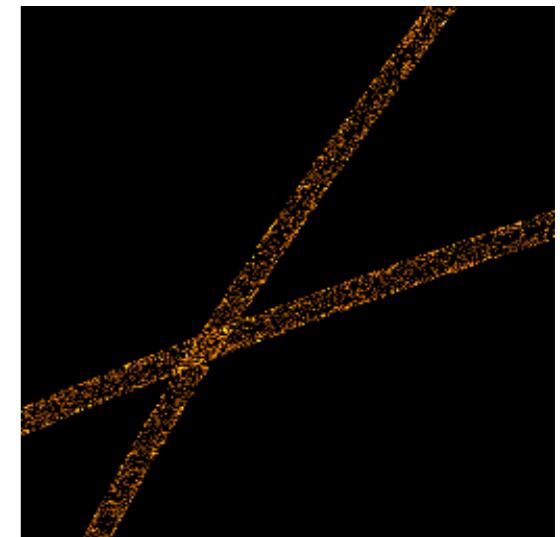


Contest datasets

2 versions: low level of noise
and high level of noise



2 versions: long sequence
and high density

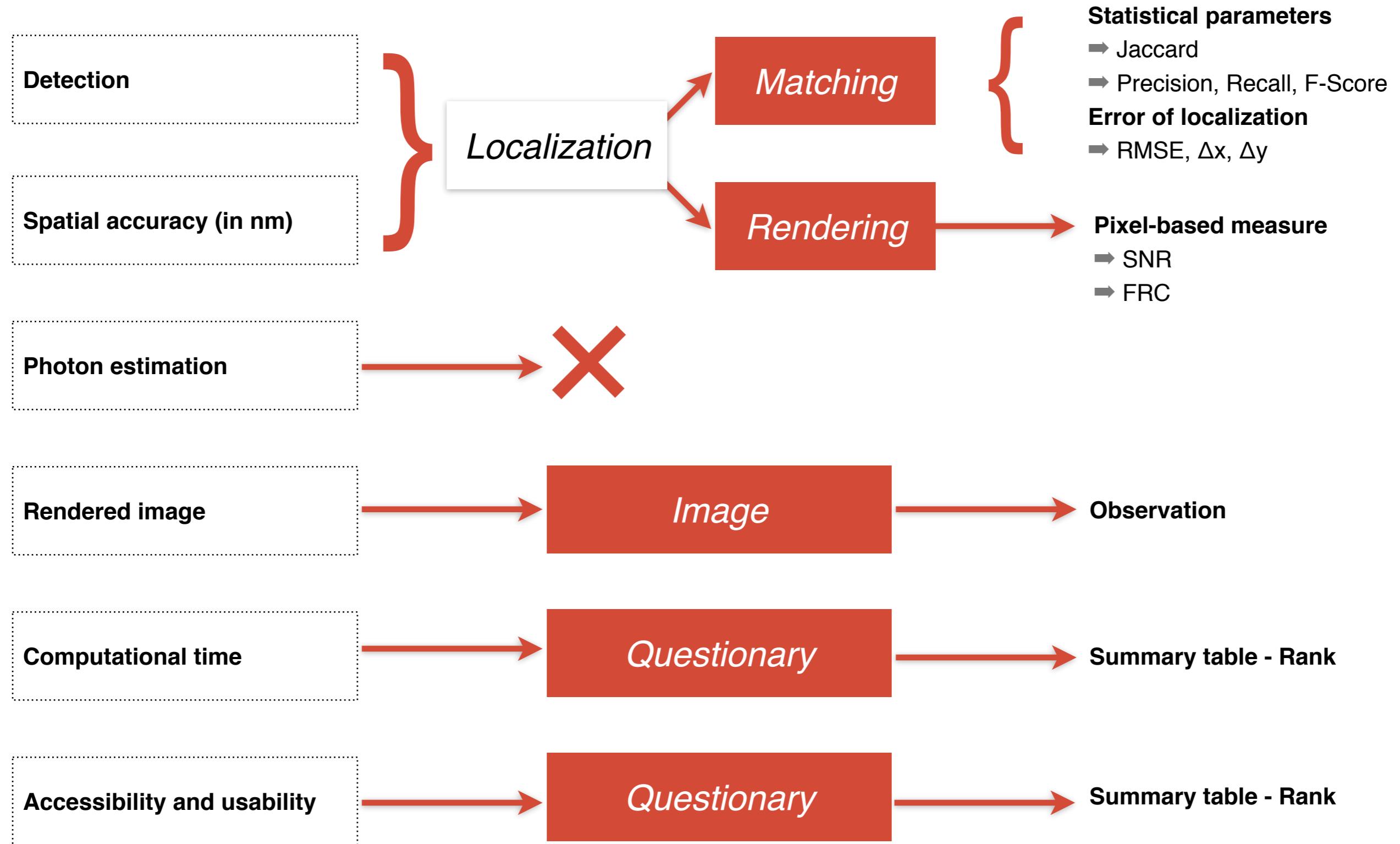


Benchmarking Spot Localization

SMLM 2D Assessment & Metrics

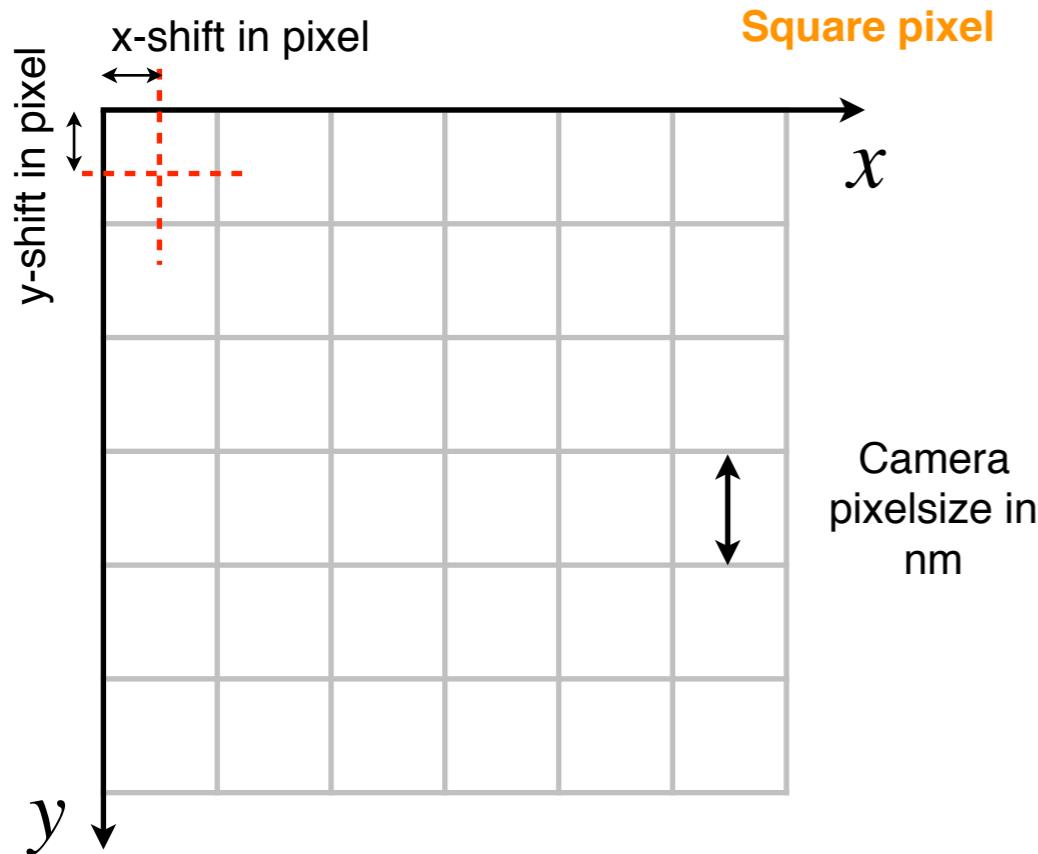
<http://bigwww.epfl.ch/smlm/>

Qualitative vs Quantitative Assessment



Standardization of the Localizations

Coordinate system



File format

position x and y in nm

frame number

Sheets

Charts

SmartArt Graphics

WordArt

Particles Table.xls

A	B	C	D	E	F	G	H	I	J	K	L	M	
1	Intensity	X (px)	Y (px)	X (nm)	Y (nm)	Z (nm)	Left-Width(px)	Right-Width(px)	Up-Height (px)	Down-Height (px)	X Symmetry	Y Symmetry	
2	1	1003	110.883475	141.220837	16632.5212	21183.1256	0	1.03984958	0.46597121	0.52720521	0.91707428	0.61889332	0.73005982
3	2	900	53.63154	120.22394	8044.731	18033.576	0	0.85596836	0.67161754	0.51899267	0.91354023	0.87931885	0.72458045
4	3	995	145.853233	113.848964	21877.9849	17077.3446	0	1.00425512	0.48335731	0.99923089	0.48729769	0.64984306	0.65561833
5	4	987	130.996454	113.460808	19649.4682	17019.1212	0	1.17282677	0.36261968	0.69252316	0.75964238	0.47233127	0.95377992
6	5	908	87.176413	139.609494	13076.462	20941.424	0	0.49039567	0.96936188	0.83510569	0.69029183	0.67188647	0.90506484
7	6	888	167.76567	139.632428	25164.8505	20944.8643	0	0.90119354	0.53134337	0.85609815	0.67678548	0.74340049	0.88302264
8	7	1094	227.010177	122.284969	34051.5266	18342.7453	0	0.37494471	1.16502114	0.52290148	0.84159186	0.48695198	0.7664405
9	8	926	214.848163	131.994003	32227.2248	19799.1005	0	0.99829024	0.51374319	1.16994195	0.41520202	0.67953946	0.52386663
10	9	996	164.781233	115.147516	24717.1851	17272.1274	0	0.91951279	0.53204285	0.48895729	0.00337331	0.73306573	0.65529353
11	10	986	31.7567191	119.146171	4763.50786	17871.9257	0	0.89065834	0.54889596	0.48818651	1.00495626	0.76267748	0.65390467
12	11	969	166.264372	139.785254	24939.6558	20967.7881	0	0.56883915	0.86583385	0.92424388	0.54177207	0.79298788	0.73910802
13	12	896	71.6398116	137.77826	10745.9717	20666.739	0	0.86657262	0.66901005	0.91601177	0.52180593	0.87134358	0.72583044
14	13	958	178.285342	116.76252	26742.8013	17514.3779	0	0.58804281	0.84115284	0.89748552	0.5571581	0.82290037	0.76604069
15	14	881	137.253496	113.597707	20588.0244	17039.656	0	0.54109741	0.87863487	0.8270909	0.70669893	0.76225274	0.92150687
16	15	935	215.098026	123.070223	32264.7041	18460.5335	0	0.48228842	1.06162155	0.46140039	1.09434708	0.62476236	0.59315589
17	16	1075	134.059113	169.660202	20108.8669	25449.0303	0	0.41442498	1.10742442	0.77705765	0.58615475	0.54463337	0.84298618
18	17	998	59.014774	153.511456	8852.2161	23026.7184	0	0.36773284	1.15961099	0.73385809	0.71302528	0.48153249	0.9856016
19	18	981	220.95465	130.544713	33143.1975	19581.707	0	1.12362268	0.38698496	0.76498912	0.69080836	0.51235669	0.9490459
20	19	977	76.2686949	116.784737	11440.3042	17517.7106	0	0.5743811	0.86074613	0.92363582	0.54111241	0.80046018	0.73884699
21	20	1014	57.8291241	102.132684	8674.36861	15319.9027	0	0.97587902	0.49646485	0.47620307	1.02083036	0.67438709	0.63619564
22	21	1004	65.9081633	156.791812	9886.22449	23518.7718	0	1.06890816	0.44737533	0.93196254	0.52197863	0.59009457	0.71801891
23	22	930	109.977786	113.870775	16496.6679	17080.6163	0	1.15085382	0.43116868	1.02490231	0.50287177	0.54508539	0.56830646

Description file XML

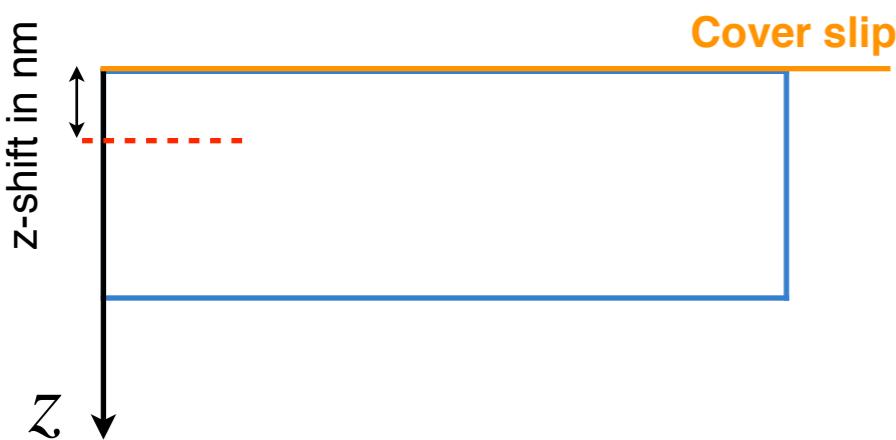
Generic reader to parse localization text file

Separator of columns	tab
Row of the first localization	2
Column of the frame	1
Column for the X position in nm	1
Column for the Y position in nm	2
Column for the Z position in nm	3
Column for the intensity	
Column for the confidence (%)	
x-shift	0
y-shift	0
z-shift	0

```

<xml>
<firstrow>2</firstrow>
<frame>1</frame>
<xnano>1</xnano>
<ynano>2</ynano>
<znano>3</znano>
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<yshift>0</yshift>
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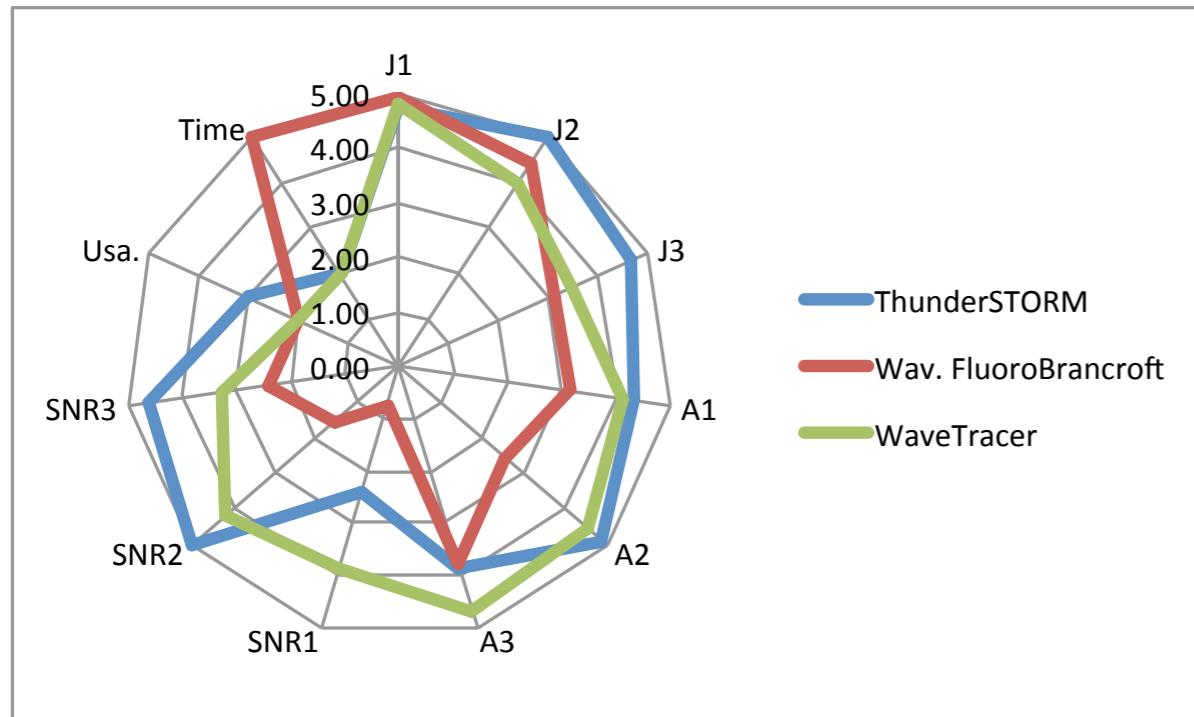
```



Metrics

5 criteria

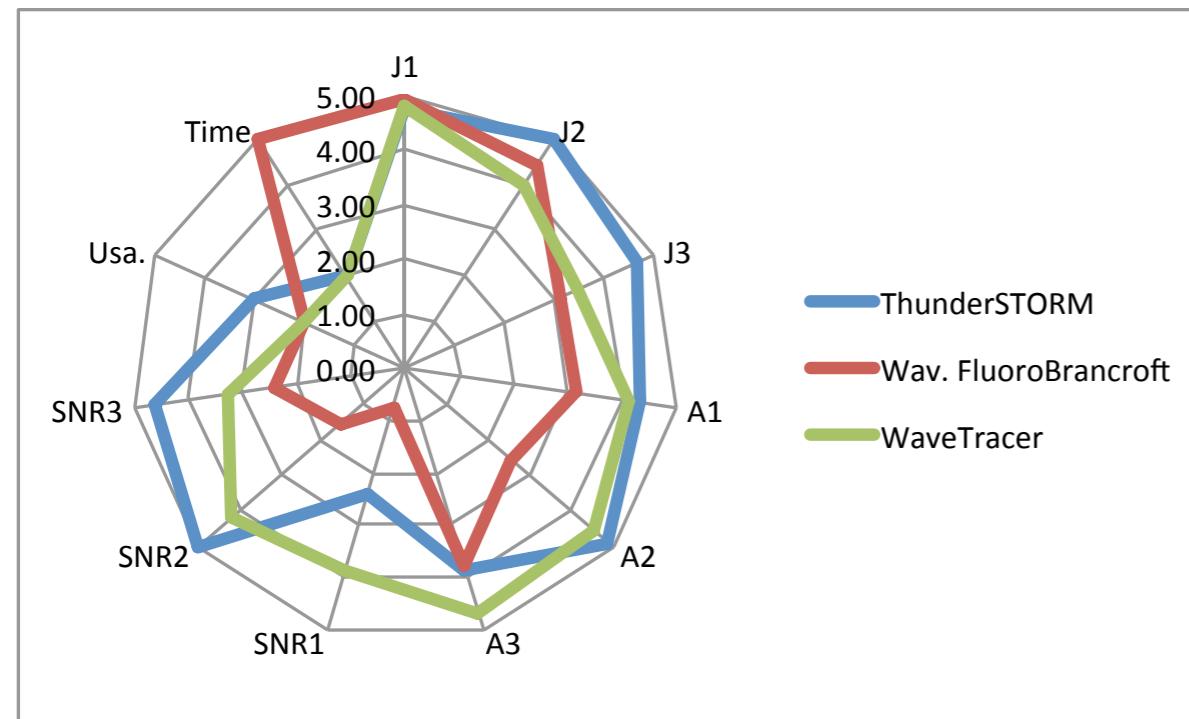
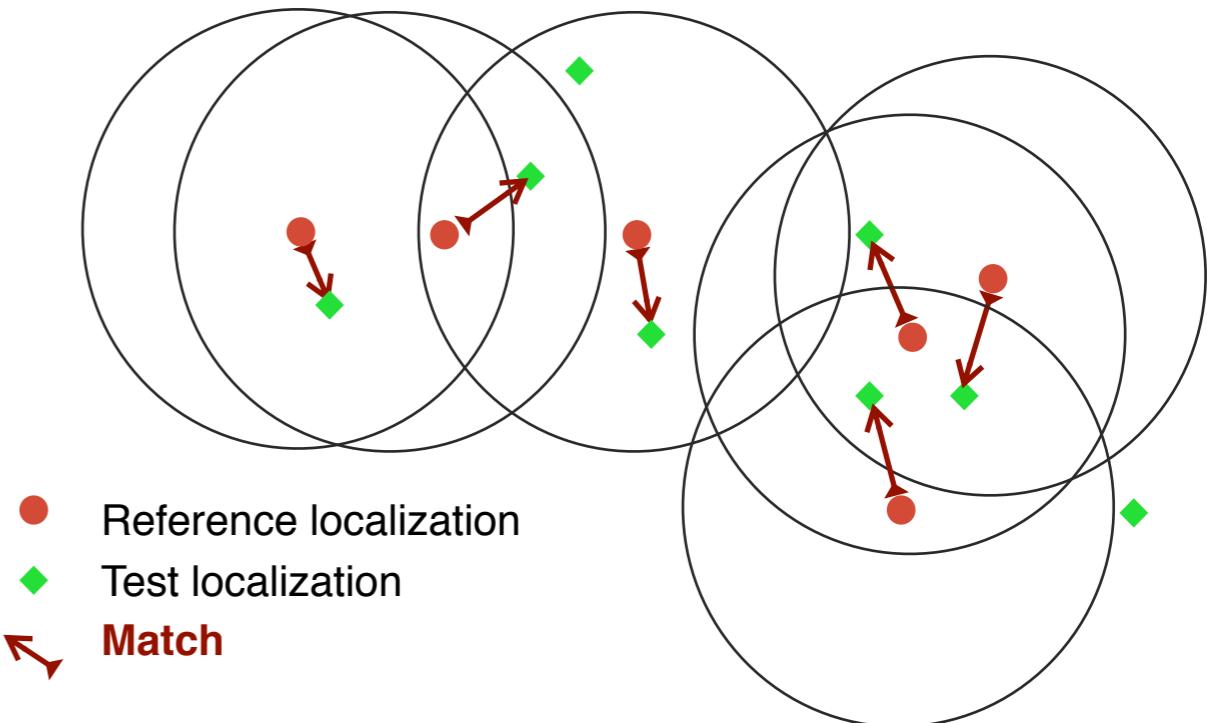
- ▶ Jaccard index (%)
- ▶ Accuracy (nm)
- ▶ SNR (dB)
- ▶ Usability (grade)
- ▶ Computation time (grade)



Metrics

6 criteria

- ▶ Jaccard index (%)
- ▶ Accuracy (nm)
- ▶ SNR (dB), FRC (nm)
- ▶ Usability (grade)
- ▶ Computation time (grade)



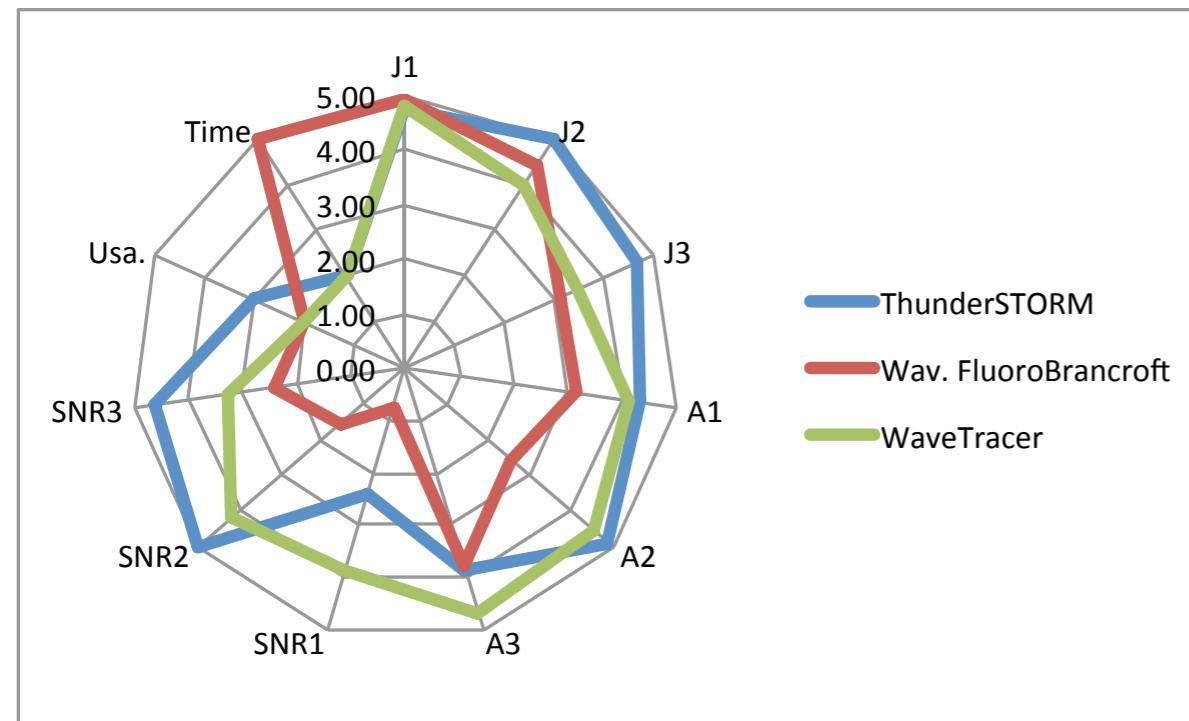
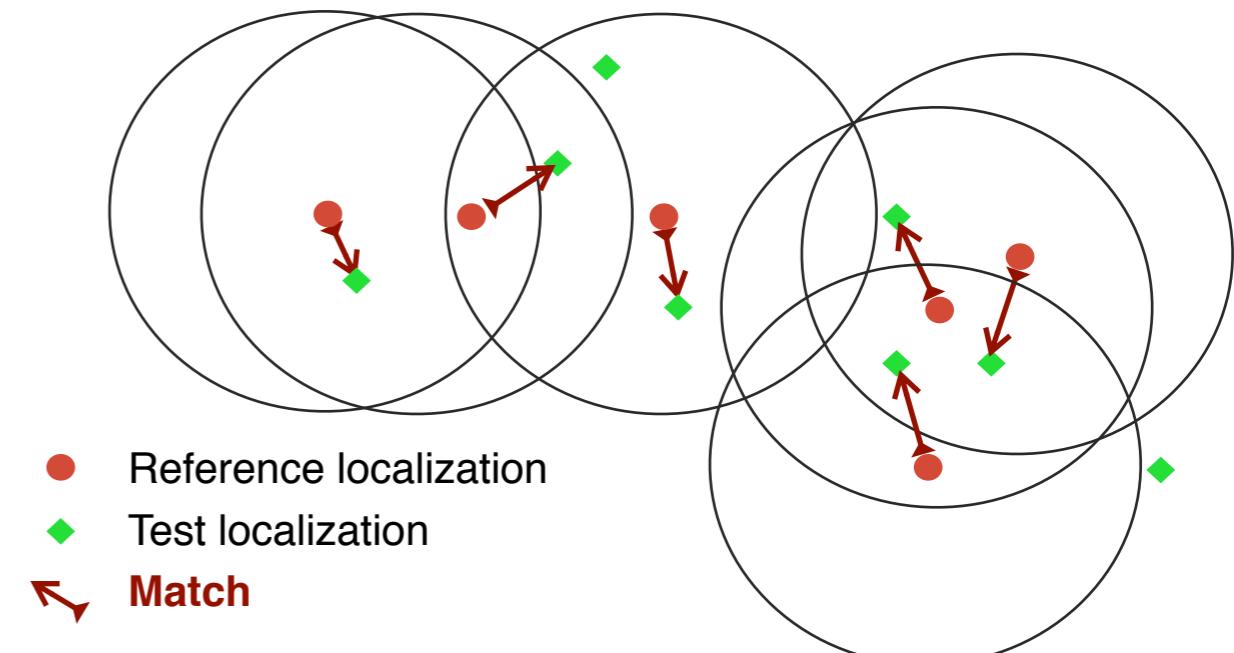
Statistical parameters

- Recall: $r = \frac{TP}{TP+FN}$
- Precision: $p = \frac{TP}{TP+FP}$
- F-Score: $f = \frac{2 \times p \times r}{p+r}$
- Jaccard : $j = \frac{|R \cap T|}{|R \cup T|}$

Metrics

6 criteria

- ▶ Jaccard index (%)
- ▶ **Accuracy (nm)**
- ▶ SNR (dB), FRC (nm)
- ▶ Usability (grade)
- ▶ Computation time (grade)



Error of localization

RMSE $d^2(R, T) = \frac{1}{|R \cap T|} \sum_{i=1}^{|R \cap T|} (p_r(x, y) - p_t(x, y))^2$

Shift $\Delta_x(R, T) = \frac{1}{|R \cap T|} \sum_{i=1}^{|R \cap T|} (p_{rx} - p_{tx})$

$$\Delta_y(R, T) = \frac{1}{|R \cap T|} \sum_{i=1}^{|R \cap T|} (p_{ry} - p_{yx})$$

Only use to detect errors in the origin of the coordinates systems

Metrics

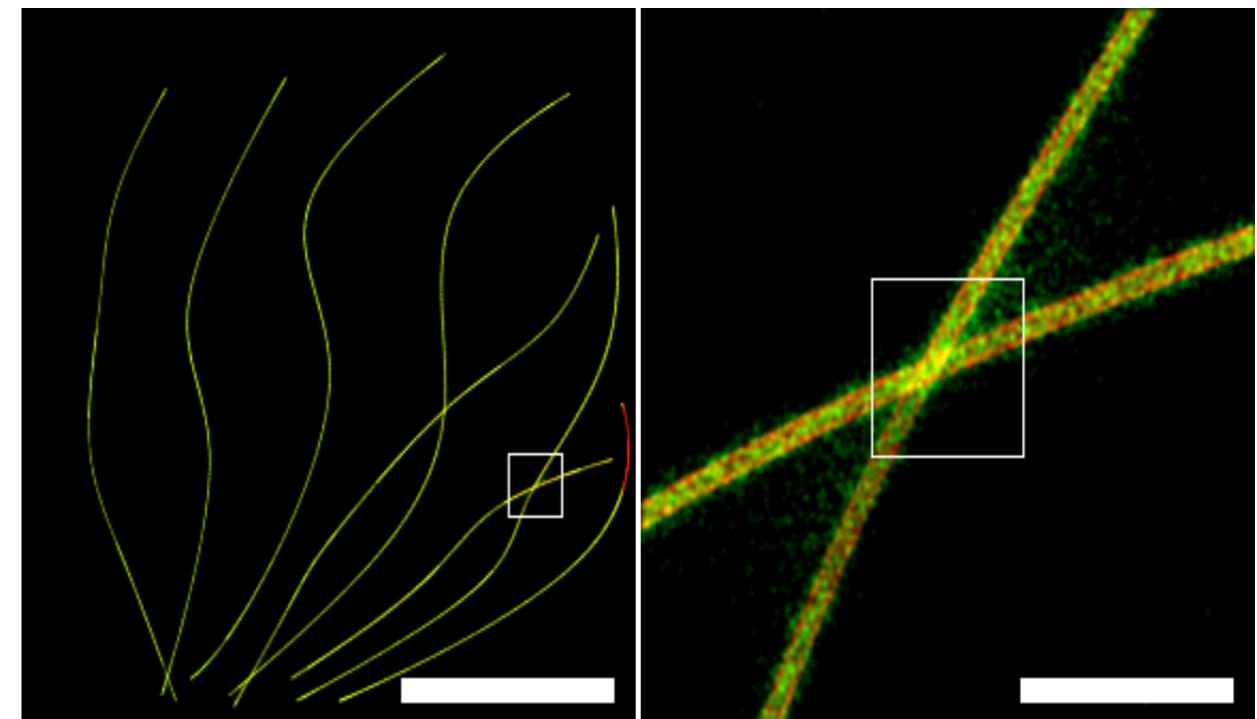
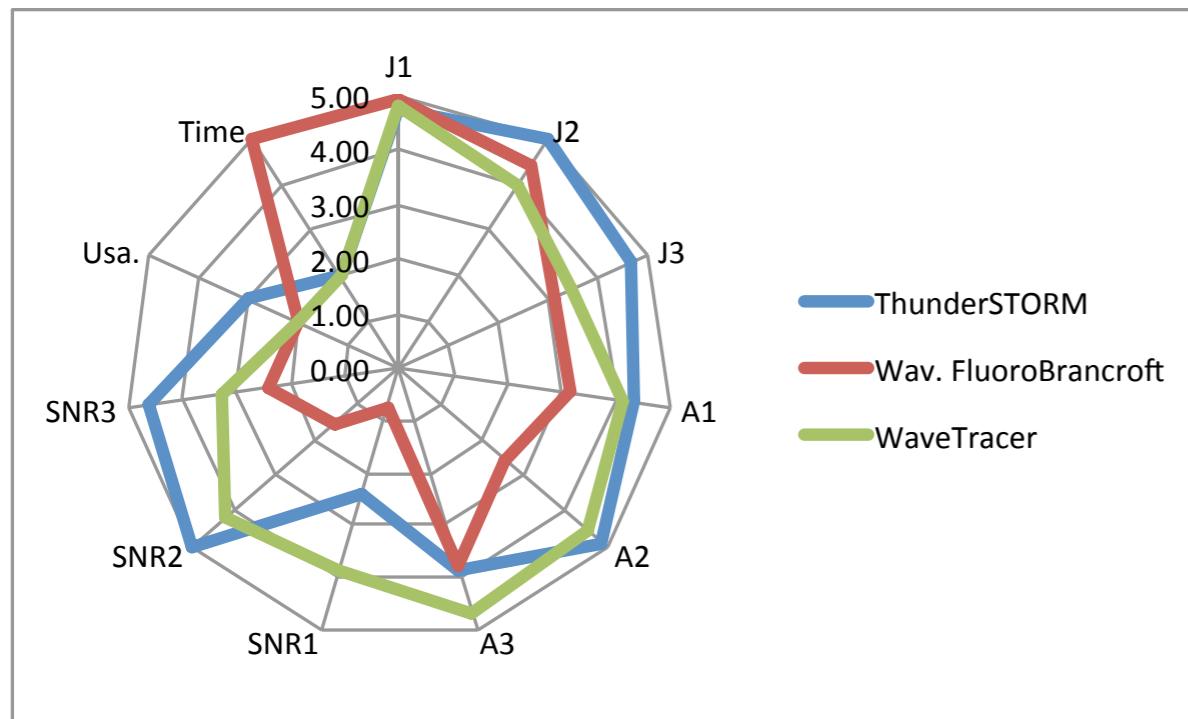
5 criteria

- ▶ Jaccard index (%)
- ▶ Accuracy (nm)
- ▶ **SNR (dB), FRC (nm)**
- ▶ Usability (grade)
- ▶ Computation time (grade)

Rendering of the localizations

- ▶ Resolution: 10 nm
- ▶ 2D Gaussian function accumulator
- ▶ Compute the ISNR between the test software and the ground-truth

$$SNR = 20 \log \frac{|I_{Oracle}|^2}{|I_{Test} - I_{Oracle}|^2}$$

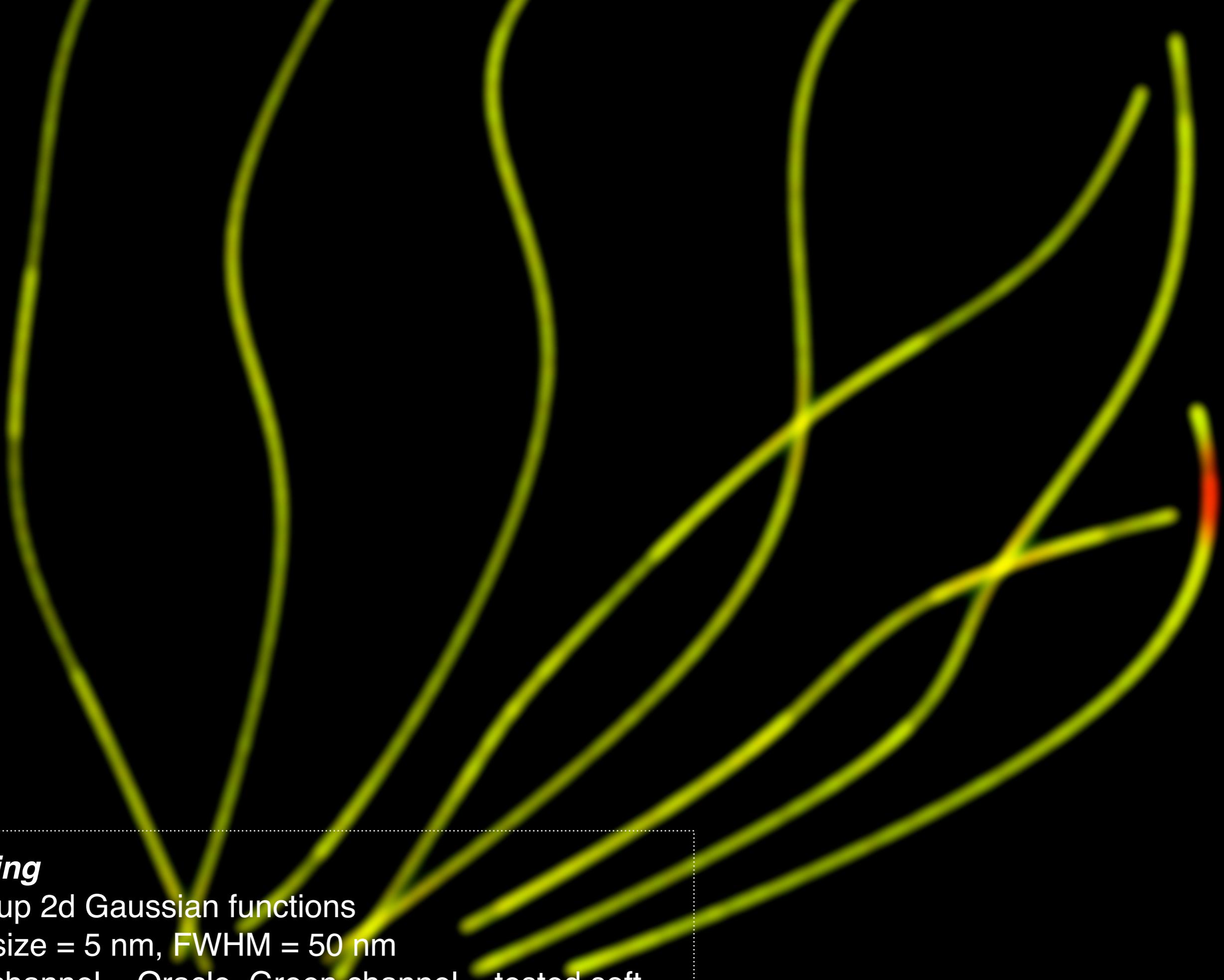


Benchmarking Spot Localization

SMLM 2D Comparison

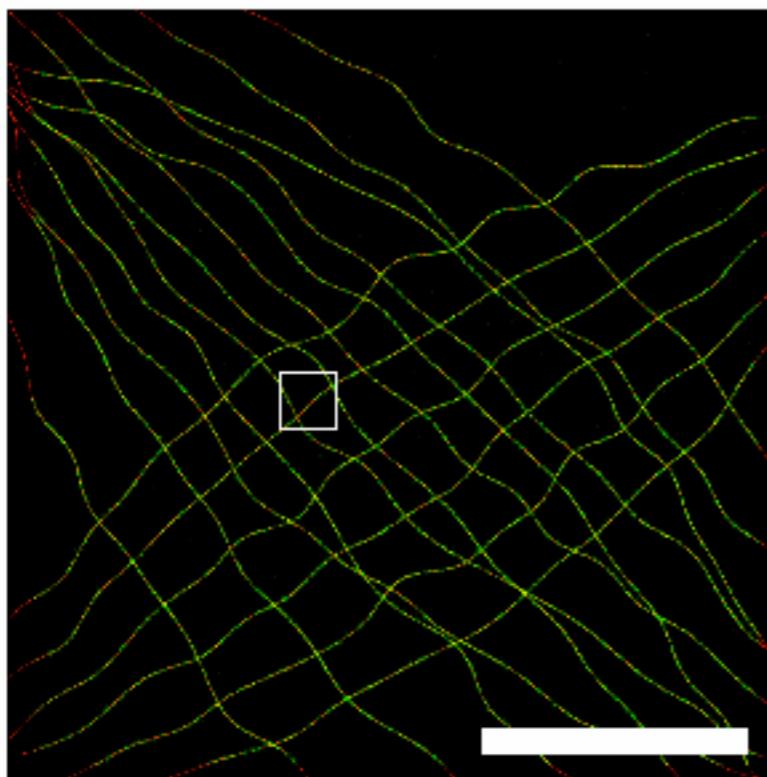
<http://bigwww.epfl.ch/smlm/>

1	B-recs	Run by participants		Stand-alone	Open source	nature methods
2	ClearPALM	Run by organizers		MATLAB		
3	CSSTORM	Run by organizers		MATLAB	Open access	nature methods
4	DAOSTORM	Run by participants		python	Open access	nature methods
5	FacePALM	Run by participants		python	Under dev.	
6	Falcon	Run by participants		MATLAB	Under dev.	IEEE
7	Fast-ML-HD	Run by participants		MATLAB	Under dev.	
8	FPGA Estimator	Run by participants		Stand-alone	Open access	Conf. FPGA
9	Gauss2dcirc	Run by organizers		MATLAB	Open access	Langmuir
10	GPUgaussMLE	Run by participants		MATLAB	Open access	nature methods
11	GraspJ	Run by participants		ImageJ	Open access	Optical Nanoscopy a SpringerOpen Journal
12	Insight3	Run by participants		Stand-alone		
13	a-livePALM	Run by participants		MATLAB		nature methods
14	M2LE	Run by participants		ImageJ	Open access	Optics Letters
15	μManager LM	Run by participants		μ-Manager	Open access	
16	Octane	Run by organizers		ImageJ	Open access	Biophysical Journal
17	PeakFit	Run by participants		ImageJ	Under dev.	
18	PeakSelector	Run by organizers		IDL 5.1		Science
19	PYME	Run by participants		python	Open access	Nano Research May 2011
20	QuickPALM	Run by participants		ImageJ	Open access	nature methods
21	RadialSymmetry	Run by participants		MATLAB	Open access	nature methods
22	rapidSTORM	Run by participants		Stand-alone	Open access	nature methods
23	SimplePALM	Run by participants		Stand-alone	Under dev.	IEEE
24	simpleSTORM	Run by participants		Stand-alone	Open access	
25	SNSMIL	Run by participants		Stand-alone	Under dev.	
26	SOSplugin	Run by participants		ImageJ	Under dev.	
27	ThunderSTORM	Run by participants		ImageJ	Under dev.	Optics EXPRESS
28	Wavelet FluoroBancroft	Run by participants		MATLAB	Open access	Optics EXPRESS
29	WaveTracer	Run by participants		MetaMorph	Commercial	Optics EXPRESS
30	CenterOfGravity	Control			Open access	



Rendering

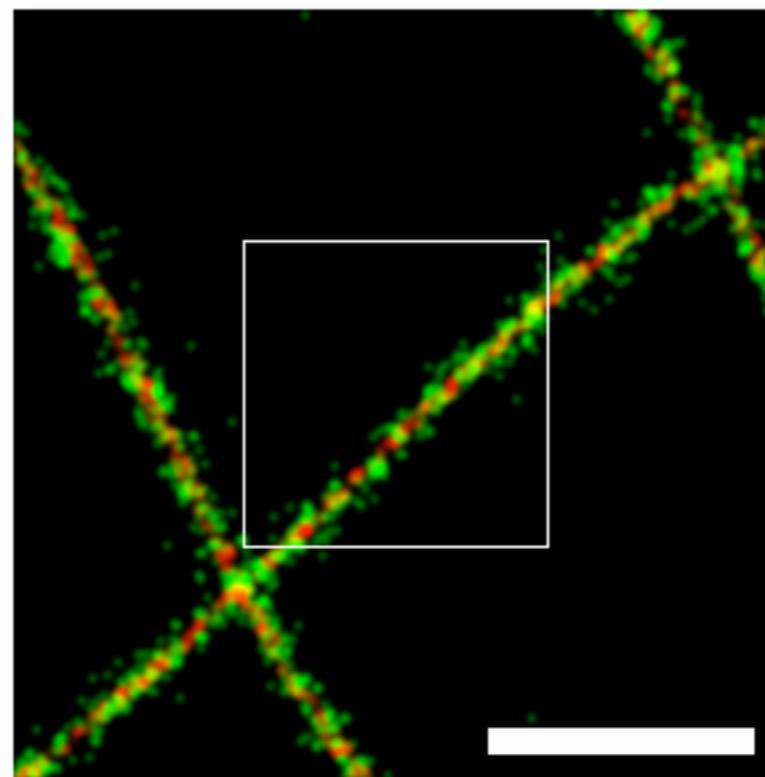
- ▶ Sum up 2d Gaussian functions
- ▶ Pixelsize = 5 nm, FWHM = 50 nm
- ▶ Red channel = Oracle, Green channel = tested soft.



Scale bar: 6600 nm

Rendering pixelsize: 66 nm/pixel

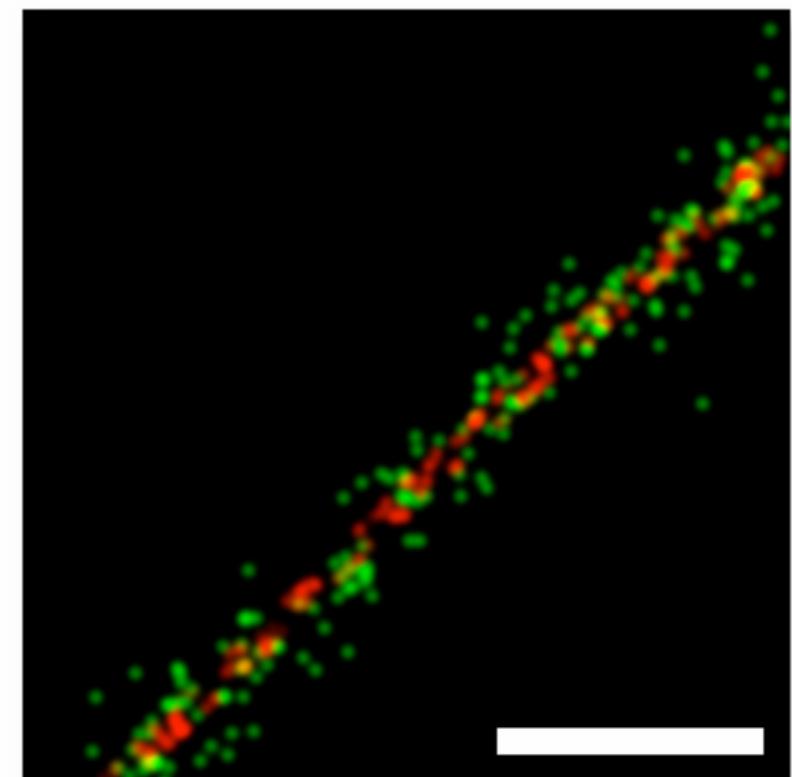
FWHM: 10.0 nm



Scale bar: 500 nm

Rendering pixelsize: 5 nm/pixel

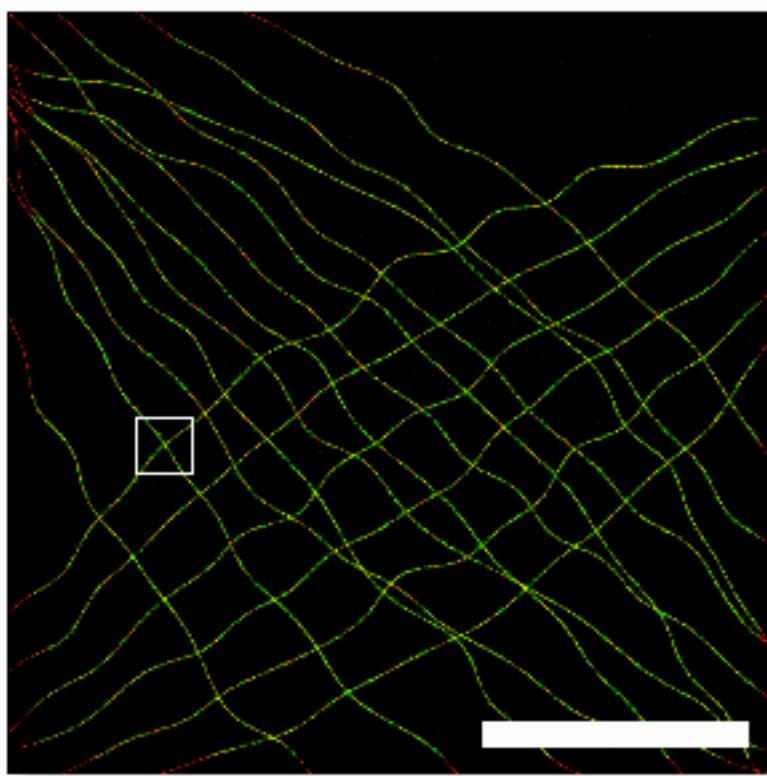
FWHM: 8.0 nm



Scale bar: 200 nm

Rendering pixelsize: 2 nm/pixel

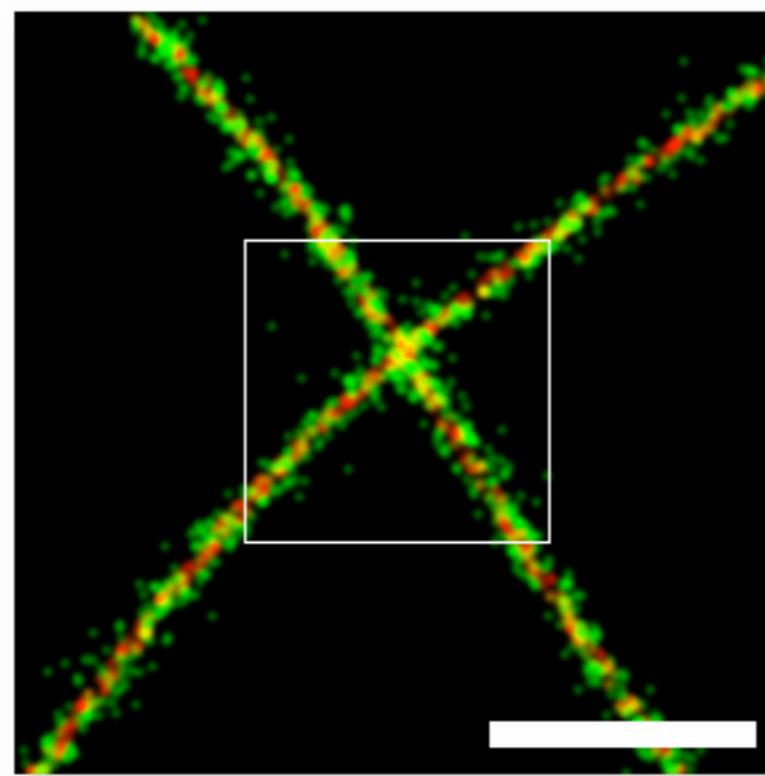
FWHM: 4.0 nm



Scale bar: 6600 nm

Rendering pixelsize: 66 nm/pixel

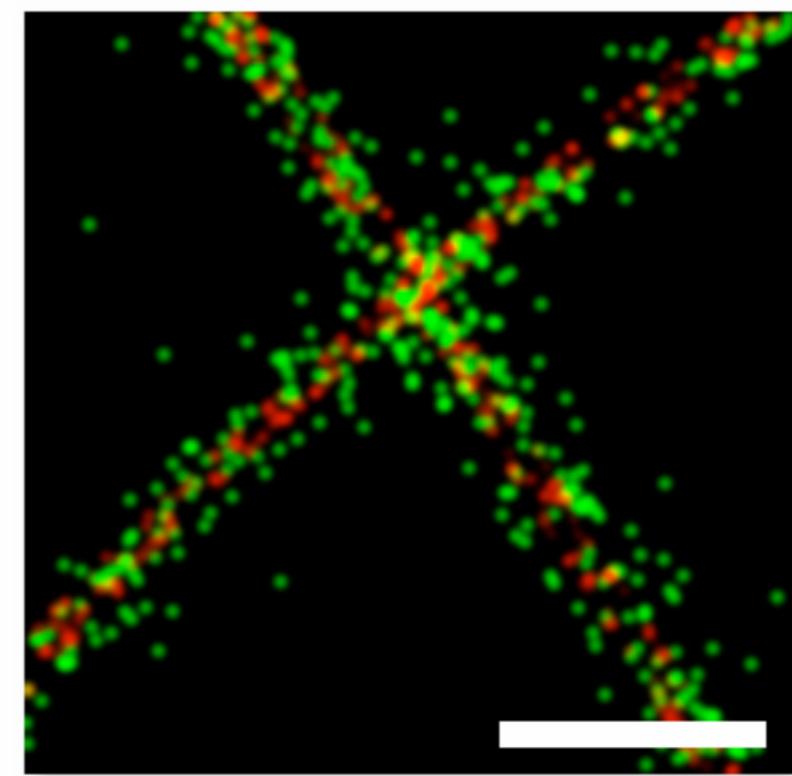
FWHM: 10.0 nm



Scale bar: 500 nm

Rendering pixelsize: 5 nm/pixel

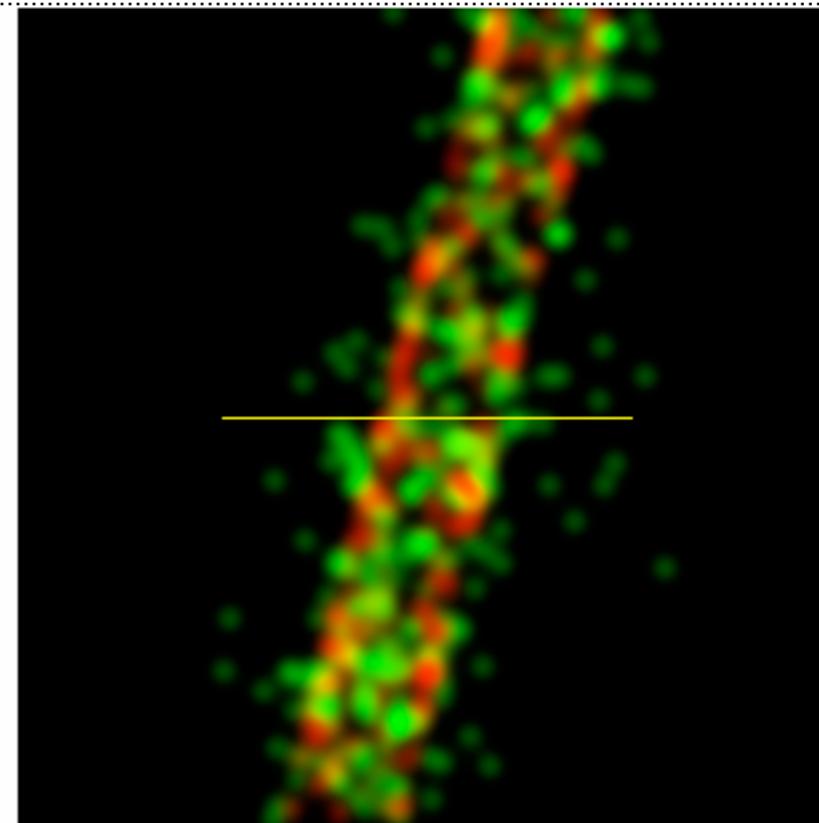
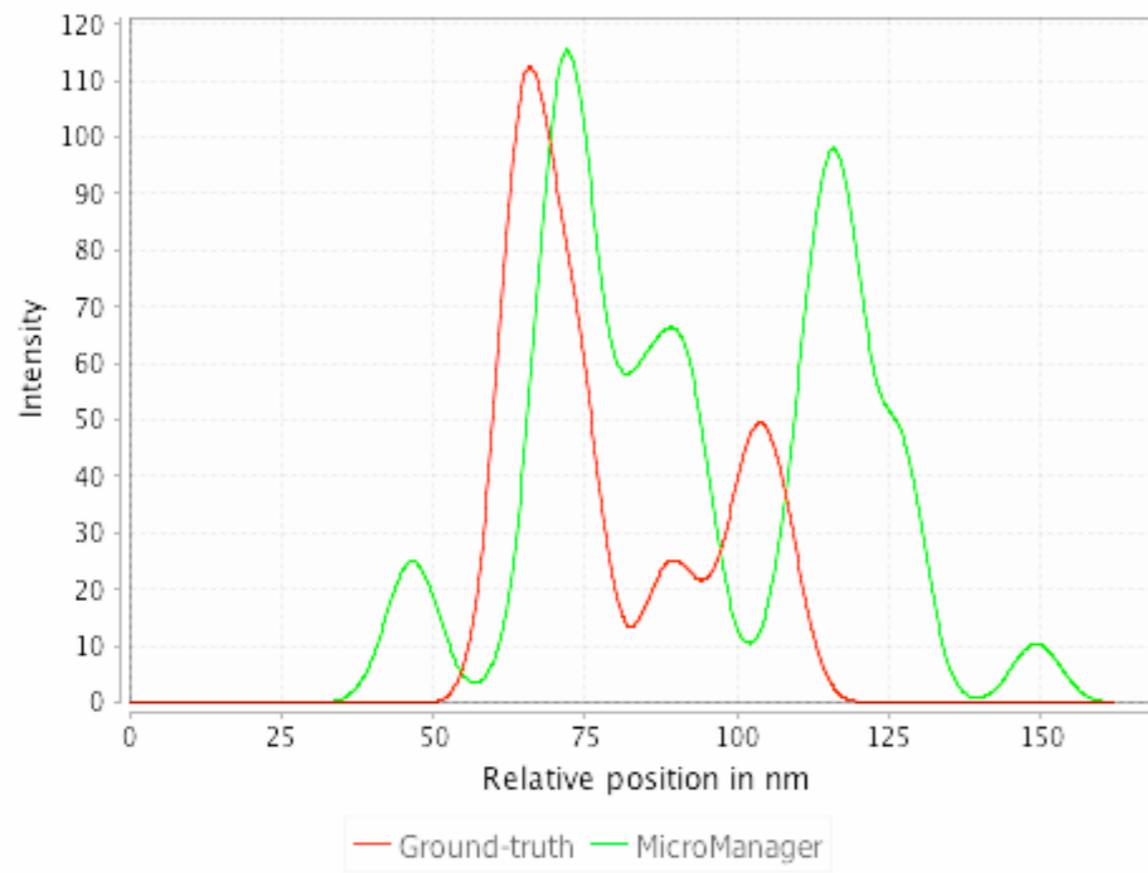
FWHM: 8.0 nm



Scale bar: 200 nm

Rendering pixelsize: 2 nm/pixel

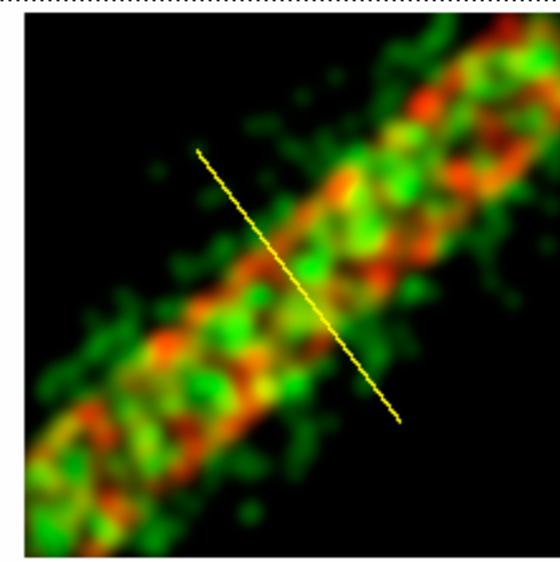
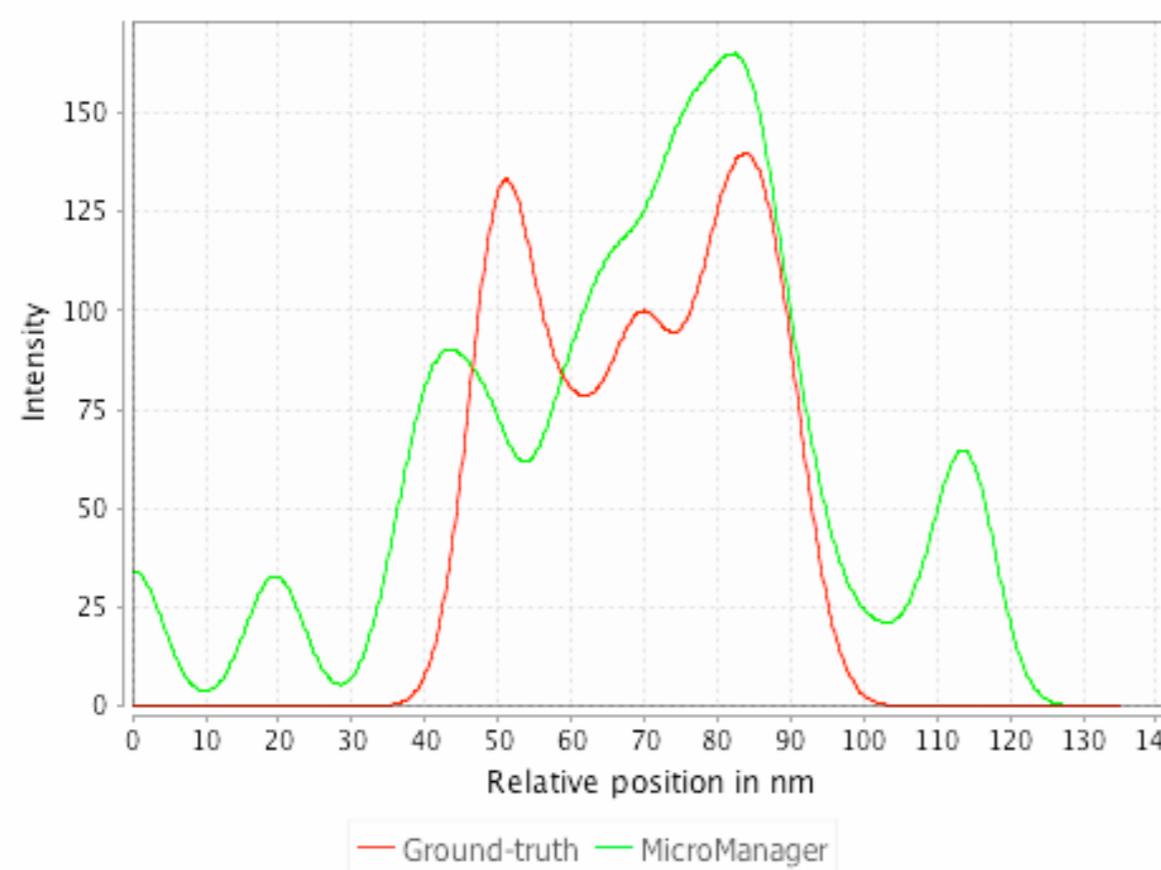
FWHM: 4.0 nm



AA Cross-section on a rendering image

Position of the section in nm: (1790.00,3700.00) to (1880.00,3700.00)

Rendering Pixelsize: 0.56 nm/pixel, FWHM of the 2D Gaussian function : 2.2 nm



AA Cross-section on a rendering image

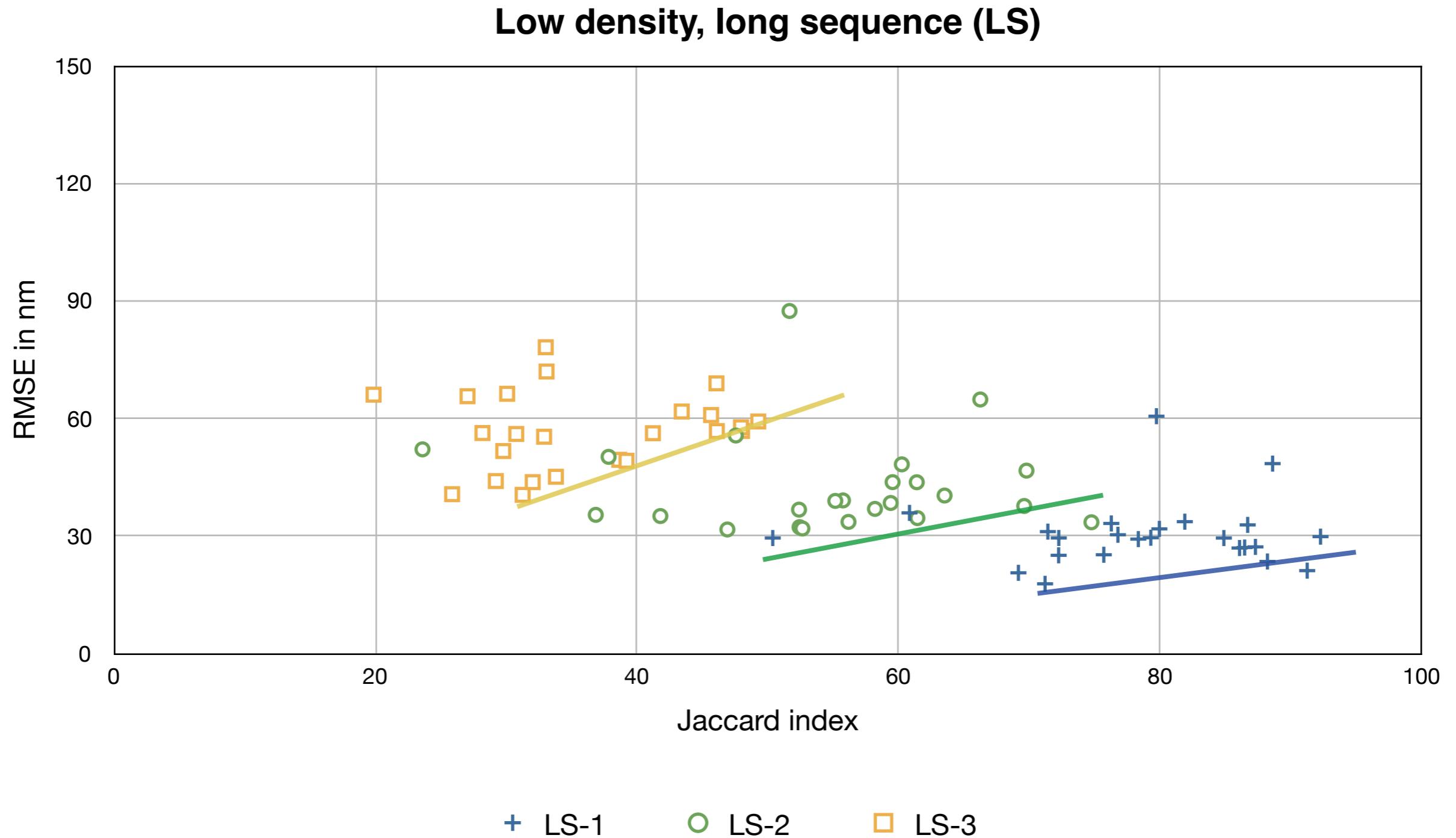
Position of the section in nm: (9675.00,10050.00) to (9720.00,10110.00)

Rendering Pixelsize: 0.56 nm/pixel, FWHM of the 2D Gaussian function : 2.2 nm

Presentation of Results

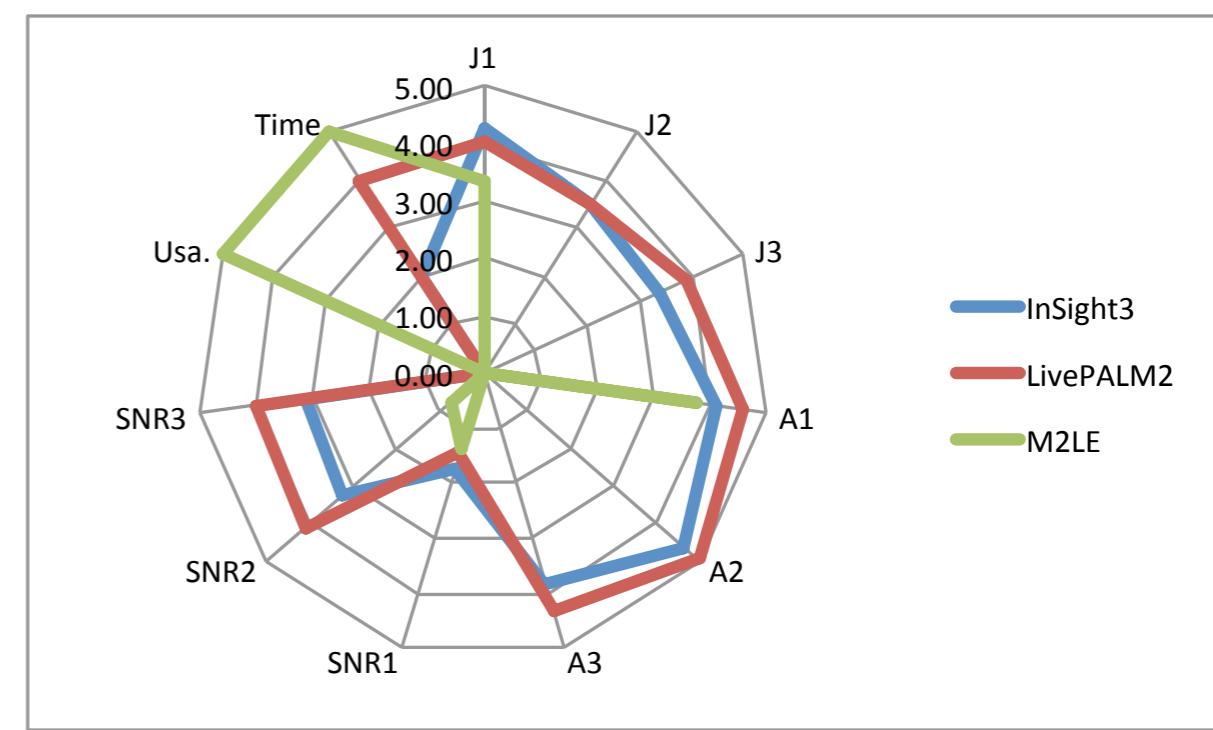
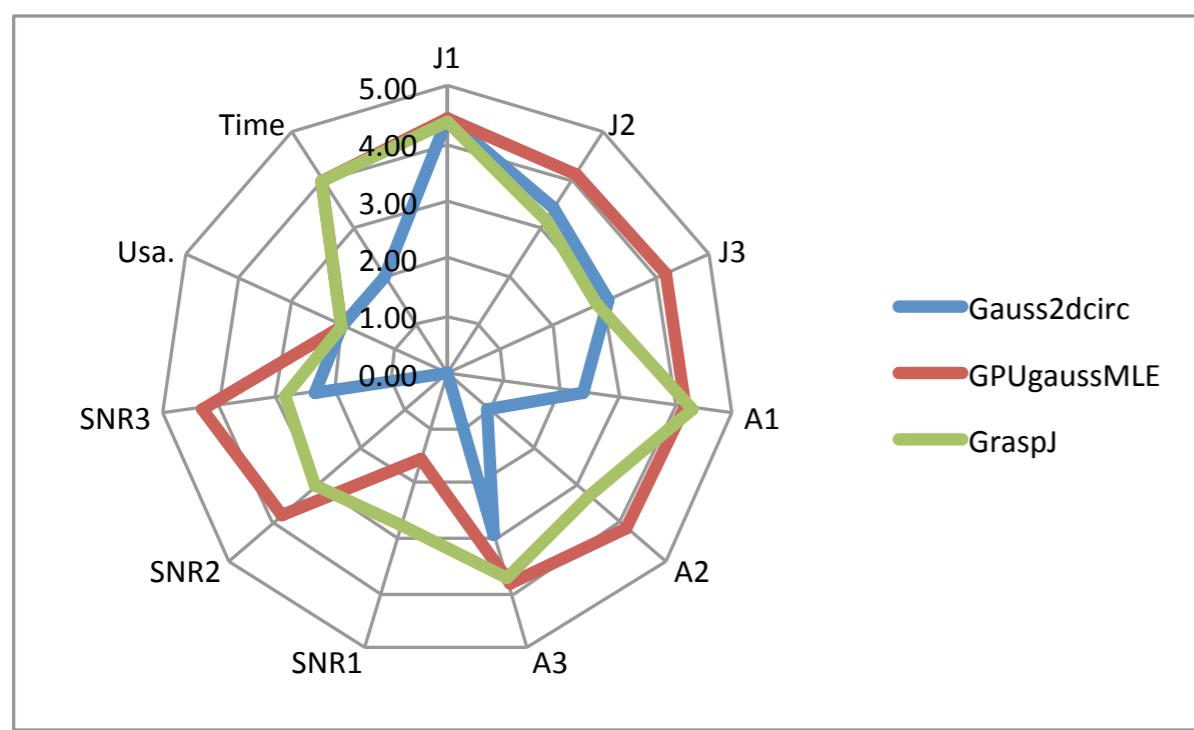
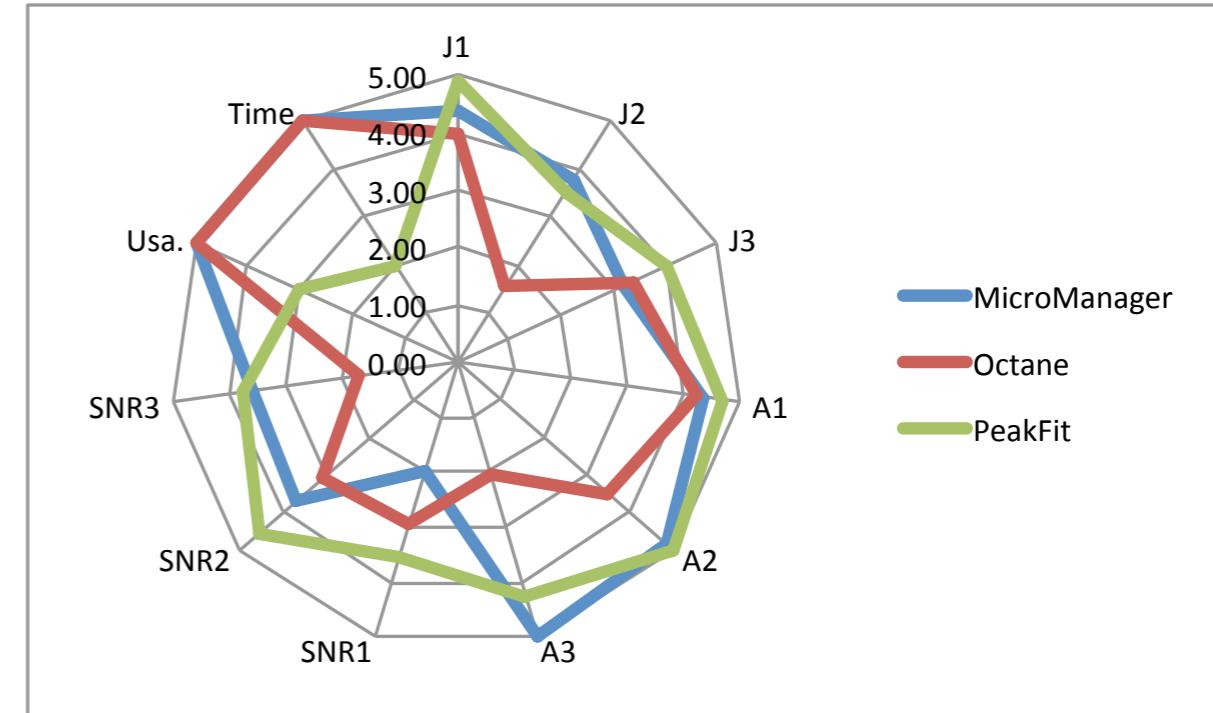
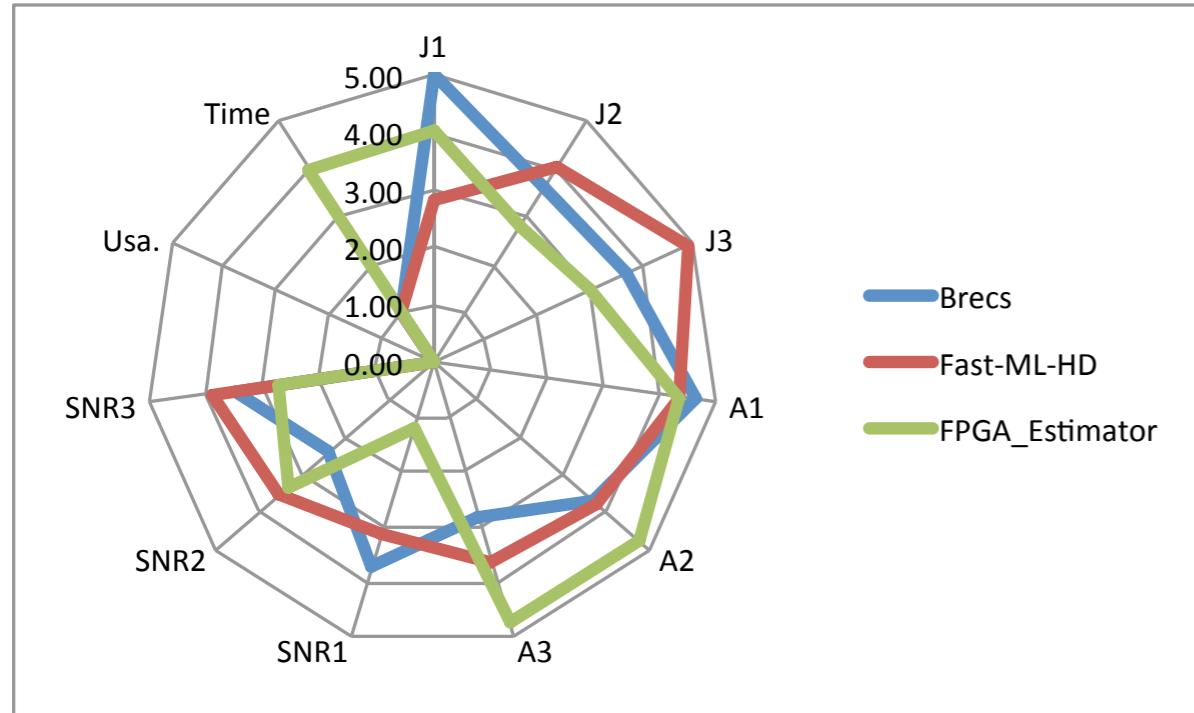
Long-sequence
Dataset 1

Comparison



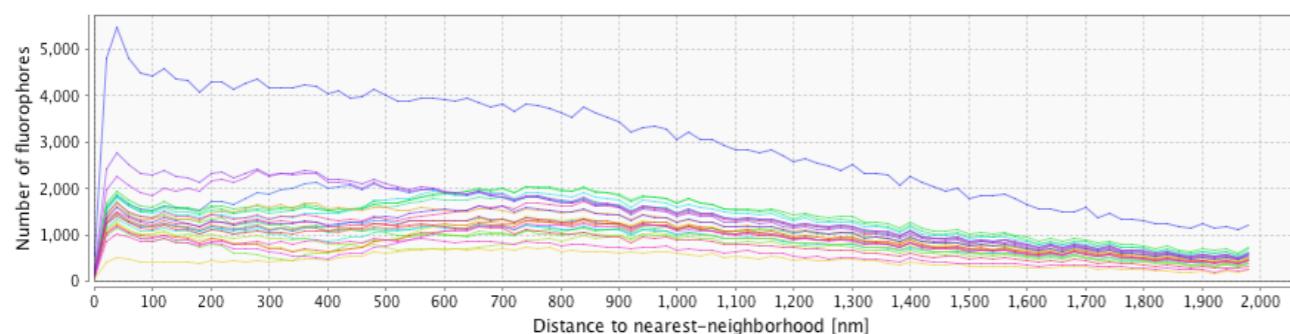
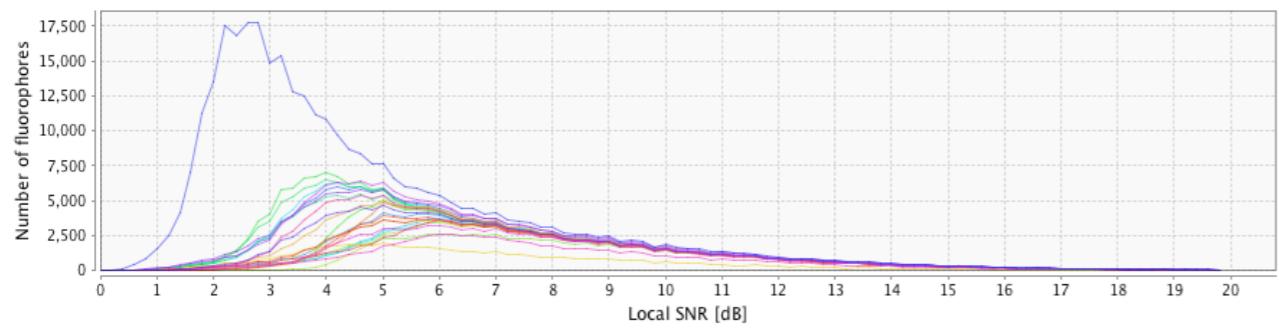
Comparison

Results on long-sequence datasets (1/2)



J1, J2, J3: Jaccard grade for 3 datasets. A1, A1, A3: Accuracy grade. SNR: SNR grade. Usa.: Usability grade. Time: Computational time.

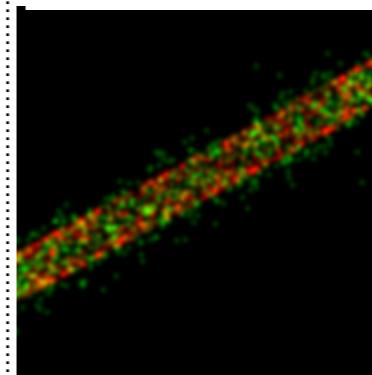
Analysis of Results



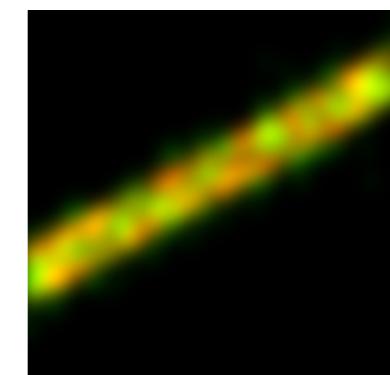
Common rendering image from localization

- Sum-up 2D Gaussian functions
- 1 localization = 1 normalized Gaussian
- Parameters:
 - Pixelsize
 - Size of the Gaussian
 - Number of frames

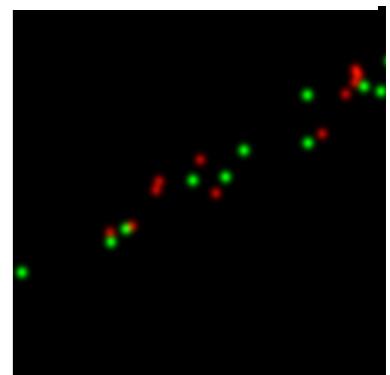
Reference in the red channel, test in the green channel



Detail at 1nm/pixel
FWHM=2nm
10'000 frames



Detail at 1nm/pixel
FWHM=9nm
10'000 frames



Detail at 1nm/pixel
FWHM=2nm
1'000 frames

Benchmarking Spot Localization

SMLM 2D Challenge

<http://bigwww.epfl.ch/smlm/>

Challenge ISBI 2013



Results

- ▶ Reference datasets
- ▶ Protocol for comparison: performance, usability, computational time
- ▶ Identification the main actors of the field
- ▶ First discussion around the new directions for the localization software

B-recs		Stand-alone	
ClearPALM	USA	MATLAB	nature methods
CSSTORM	USA	MATLAB	nature methods
DAOSTORM	CH	python	nature methods
FacePALM	FR	python	FOM 2013
Falcon	KR	MATLAB	IEEE
Fast-ML-HD	KR	MATLAB	IEEE
FPGA Estimator	DE	Stand-alone	Conf. FPGA
Gauss2dcirc	USA	MATLAB	Langmuir
GPUgaussMLE	FR	MATLAB	nature methods
GraspJ	ES	ImageJ	Optical Nanoscopy a SpringerOpen Journal
Insight3	USA	Stand-alone	
a-livePALM	DE	MATLAB	nature methods
M2LE	USA	ImageJ	Optics Letters
μManager LM	USA	ImageJ	Biophysical Journal
Octane	USA	ImageJ	
PeakFit	UK	ImageJ	Science
PeakSelector	USA	IDL 5.1	Nano Research
PYME	UK	python	
QuickPALM	FR	ImageJ	nature methods
RadialSymmetry	USA	MATLAB	nature methods
rapidSTORM	DE	Stand-alone	nature methods
SimplePALM	FR	Stand-alone	
simpleSTORM	DE	Stand-alone	
SNSMIL	CN	Stand-alone	
SO\$plugin	PL	ImageJ	Optics EXPRESS
ThunderSTORM	CZ	ImageJ	Optics EXPRESS
Wavelet FluoroBrancroft	USA	MATLAB	PLOS ONE
WaveTracer	FR	ImageJ	FOM 2013
Wedge Template	CH	Stand-alone	

Challenge ISBI 2013

Ranking: multiple criteria decision

- ▶ Jaccard index = 1
- ▶ Accuracy = 1
- ▶ SNR = 1
- ▶ Usability = 0.1
- ▶ Computation time = 0.1

Low-Density

1. SimplePALM, CNRS Gif/Yvette
2. SimpleSTORM, Heidelberg Univ.
3. ThunderSTORM, Prague Univ.

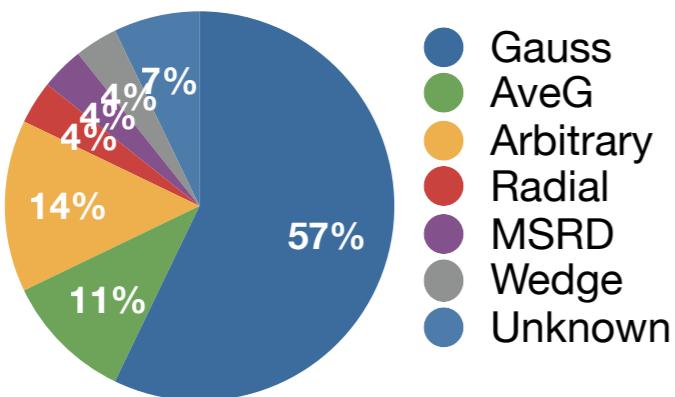
High-Density

1. B-recs, Janelia Farm
2. WTM, Hamamatsu
3. Octane, Univ. of Connecticut HC

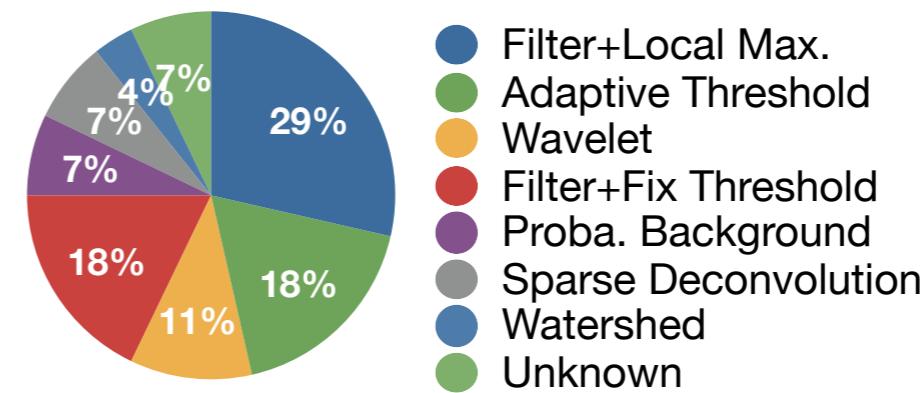
Online permanent challenge: <http://bigwww.epfl.ch/smlm/>

Analysis of Results

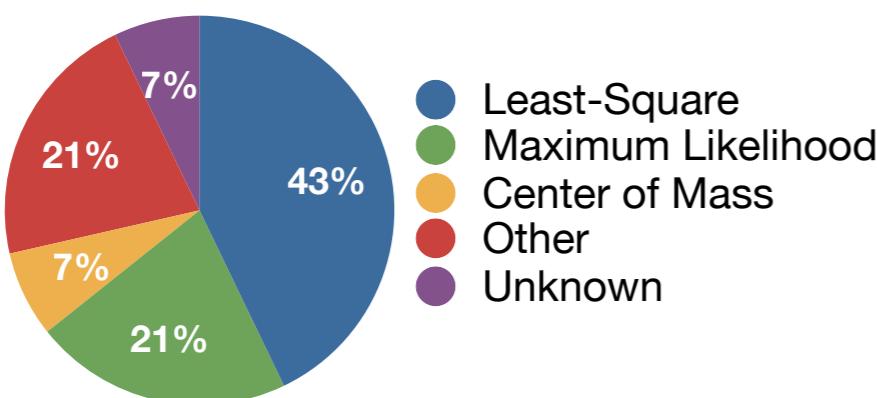
PSF Model



Detection Method



Fit Method



Analysis of the results

- ▶ Rate of detection: 30% to 80%
- ▶ Accuracy: 20 nm to 60 nm
- ▶ Average time: 5 minutes

Benchmarking Spot Localization

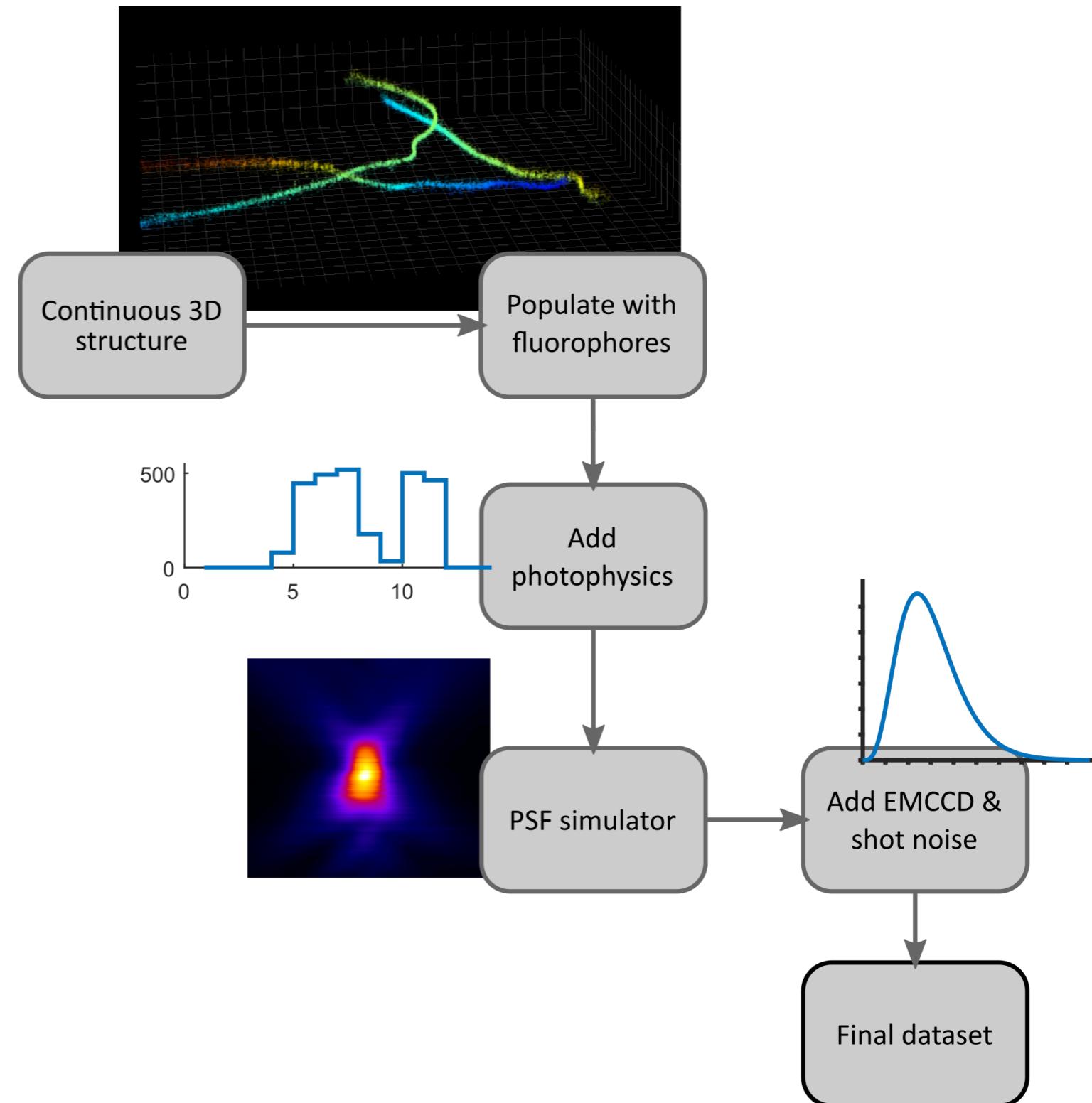
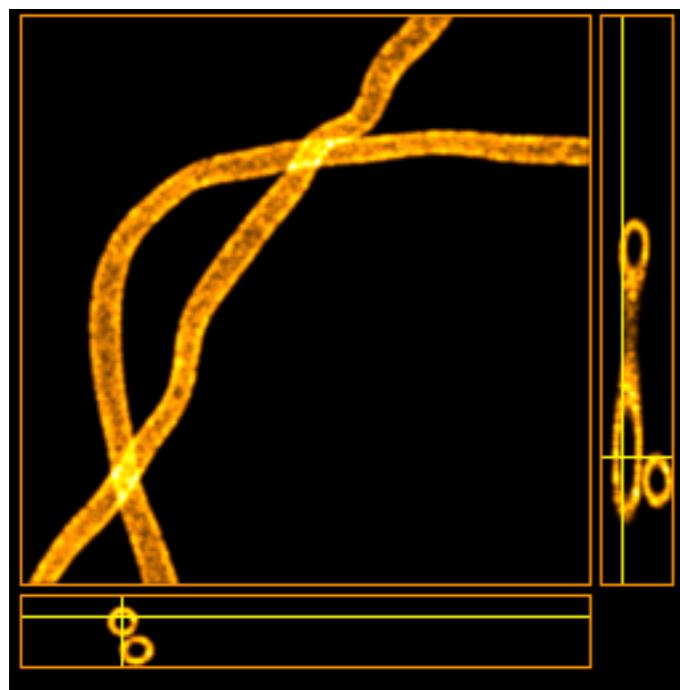
SMLM 3D Simulation

<http://bigwww.epfl.ch/smlm/>

Simulation

Highly realistic datasets

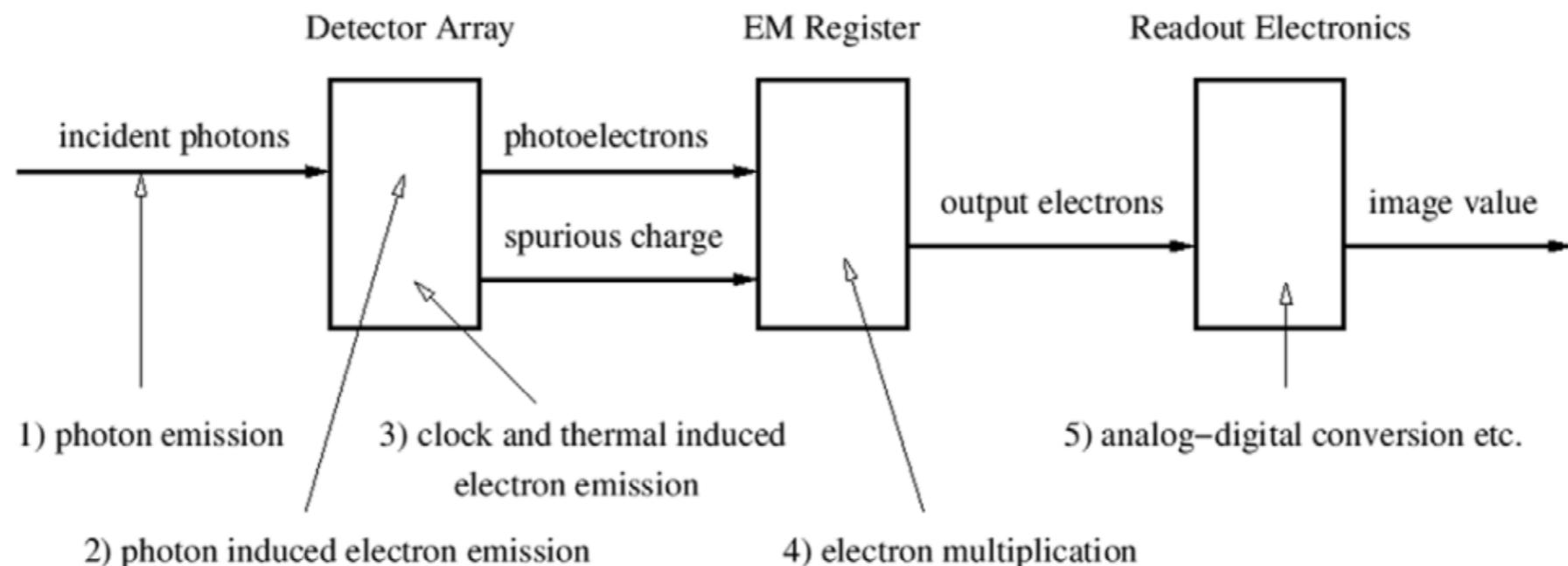
- ▶ Simulates photo physic activation
- ▶ Simulates EMCCD noise
- ▶ Experimental PSF



Camera Noise Model

Noise Model

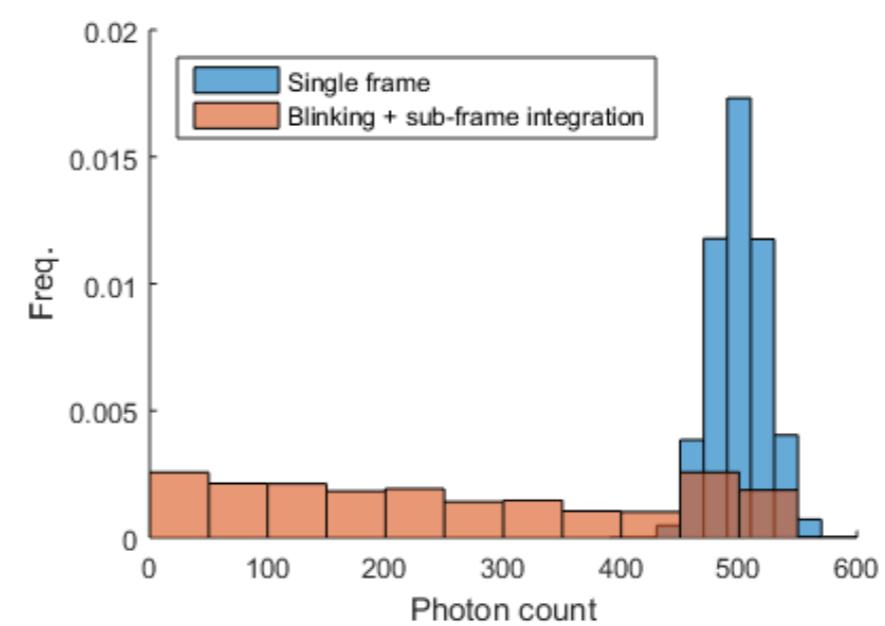
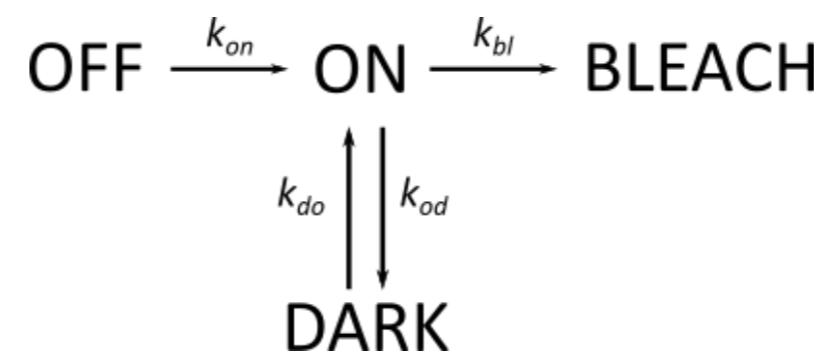
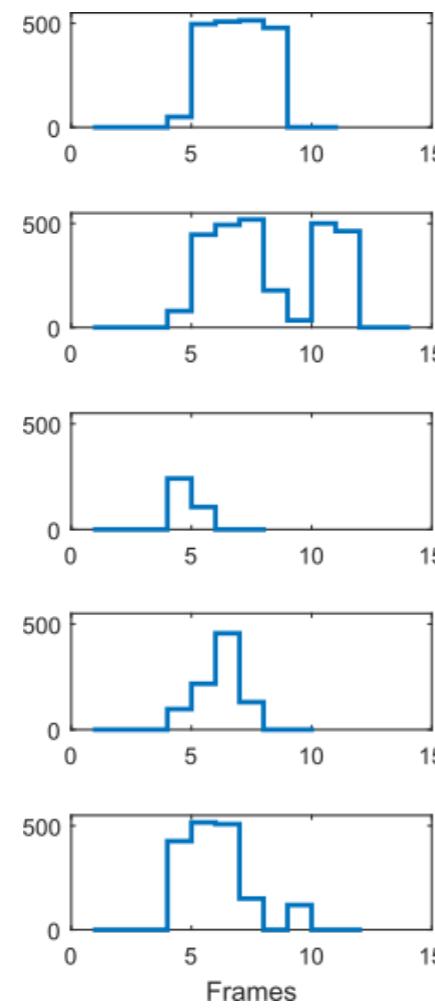
- ▶ Accurate shot noise
- ▶ EMCCD noise simulation



Photophysics Activation

Experimental PSF

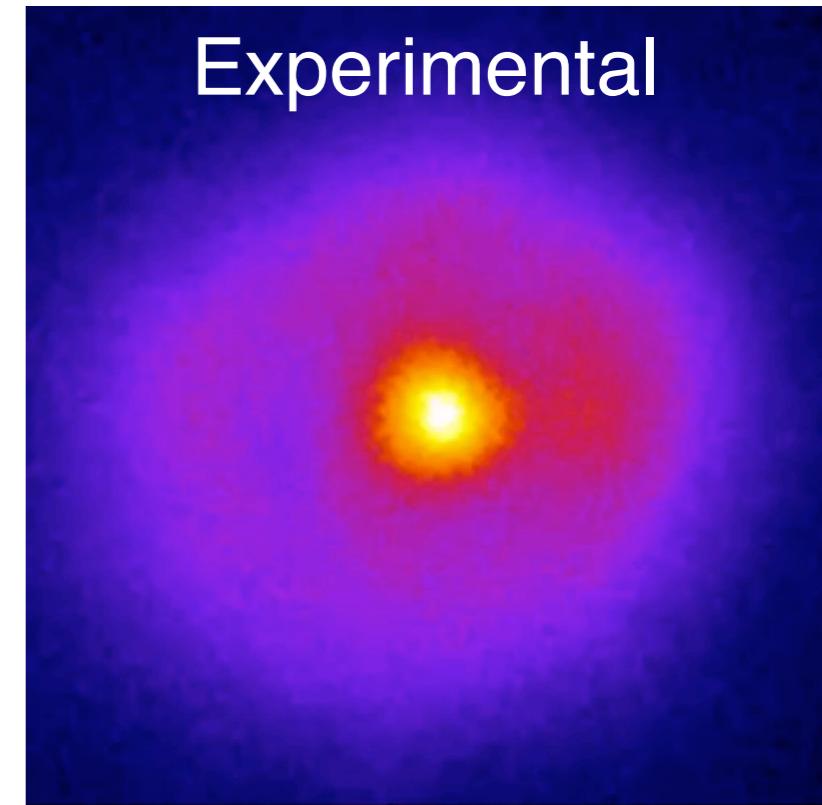
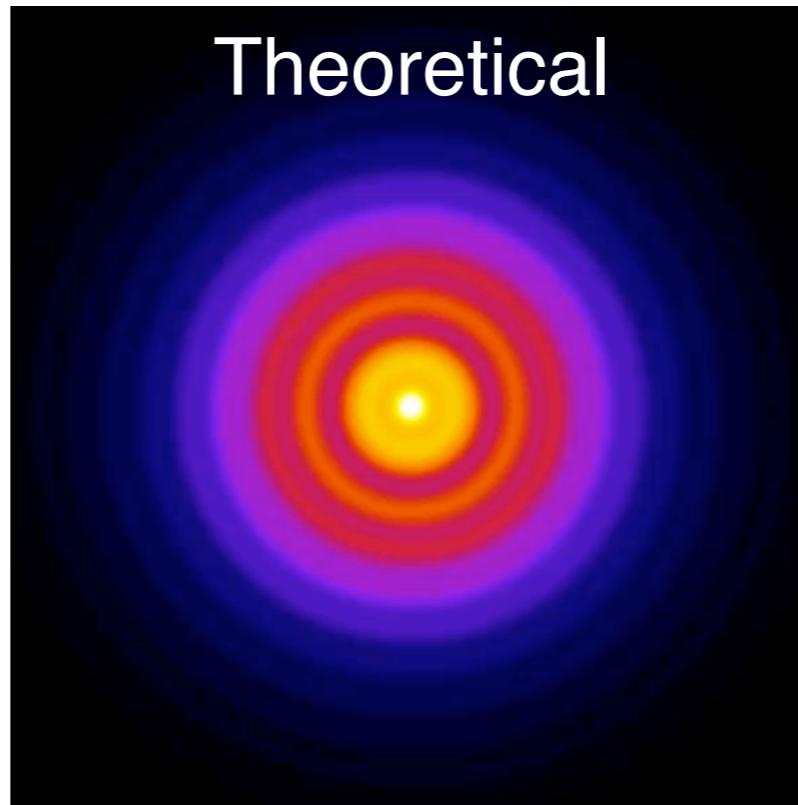
- ▶ Real molecules blink
- ▶ 4 states model



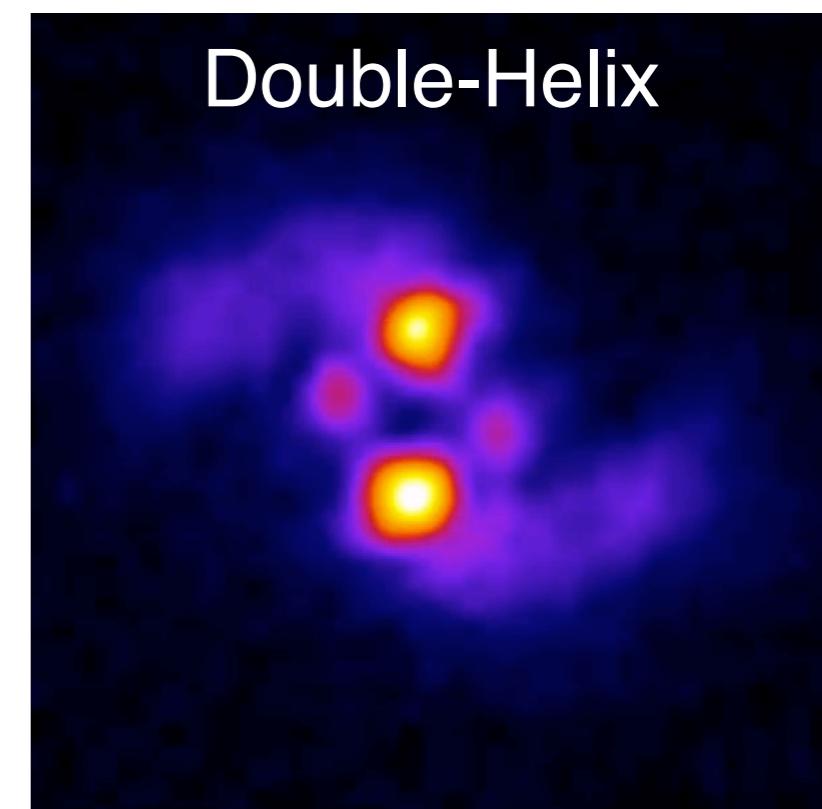
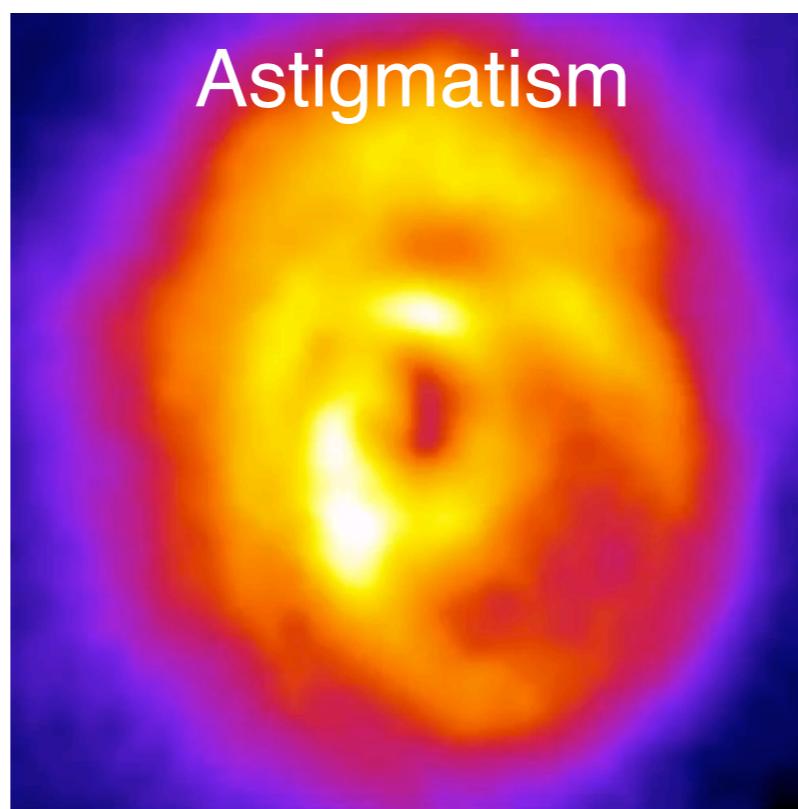
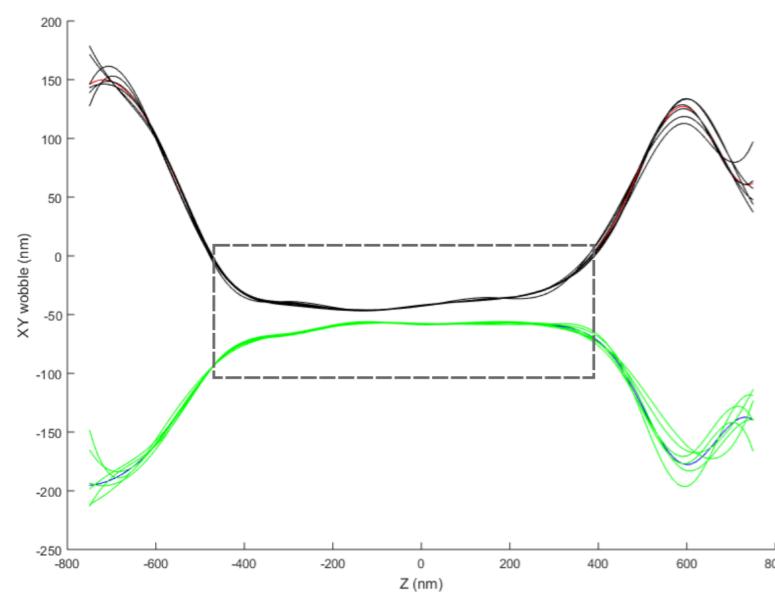
3D Point-Spread Function

Experimental PSF

- ▶ 3D Astigmatism
- ▶ Double-Helix
- ▶ Biplane



Wobble



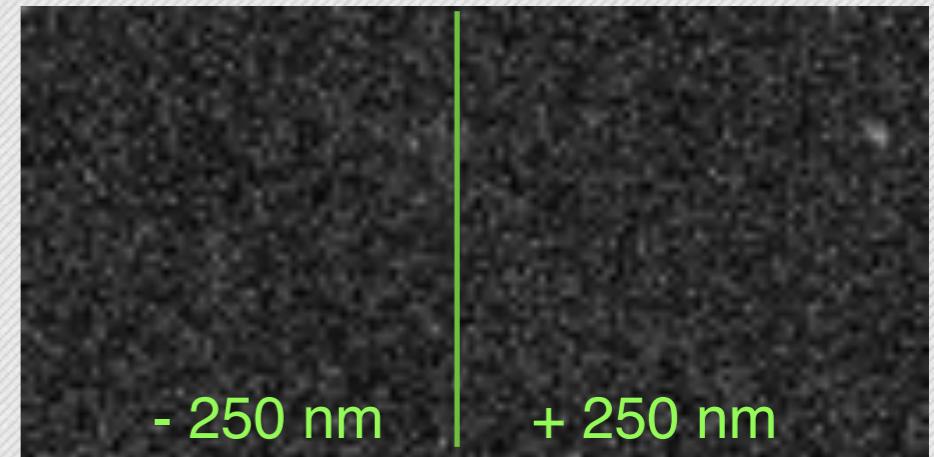
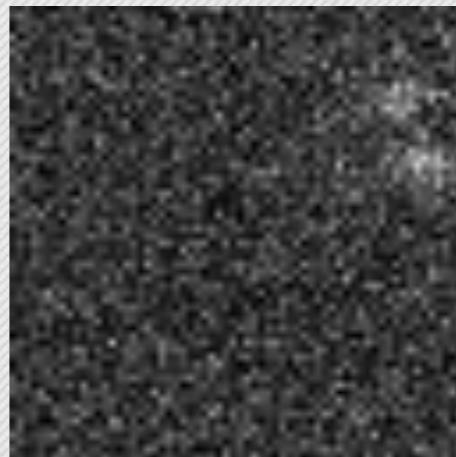
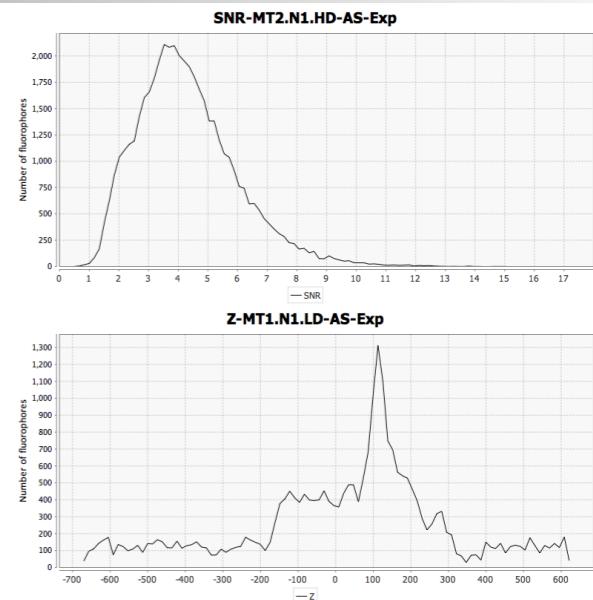
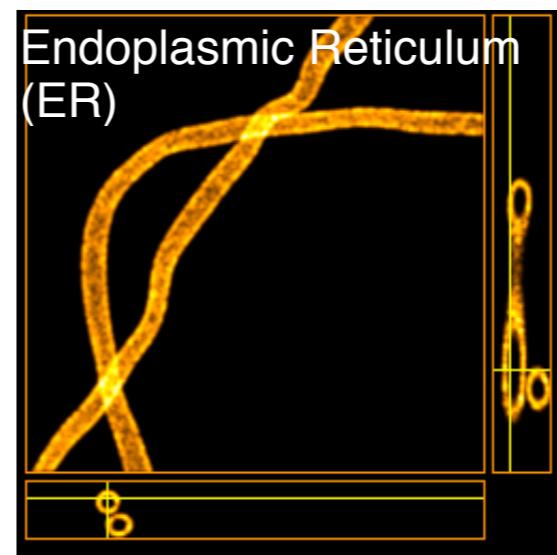
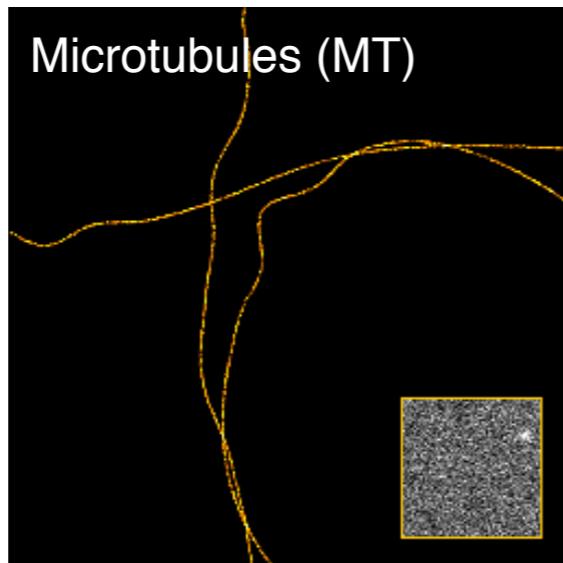
Benchmarking Spot Localization

SMLM 3D Datasets

<http://bigwww.epfl.ch/smlm/>

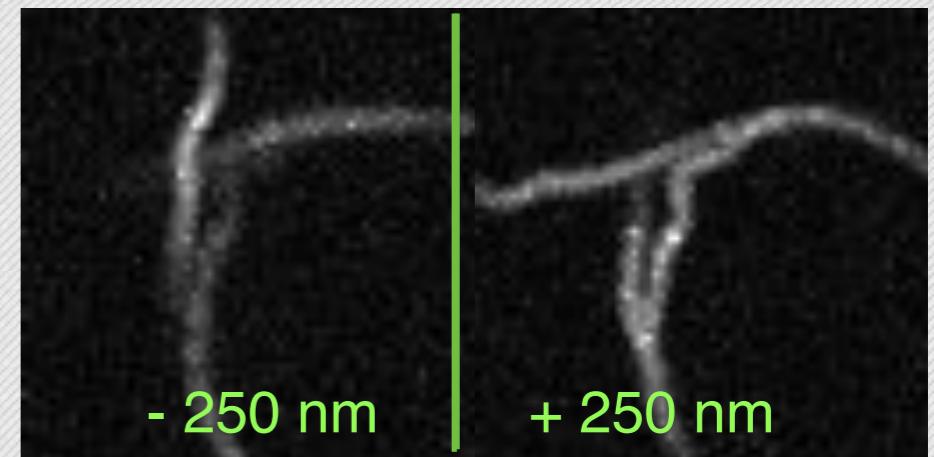
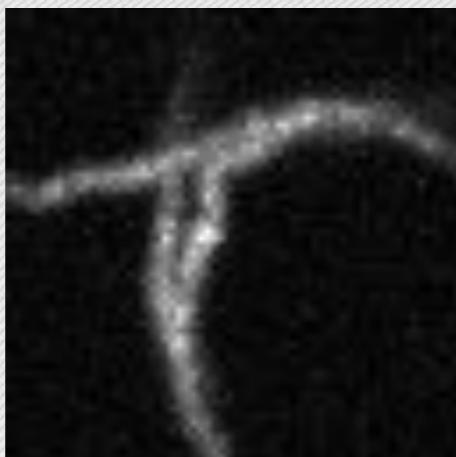
Datasets

Ultra-realistic simulation



- 250 nm

+ 250 nm



- 250 nm

+ 250 nm

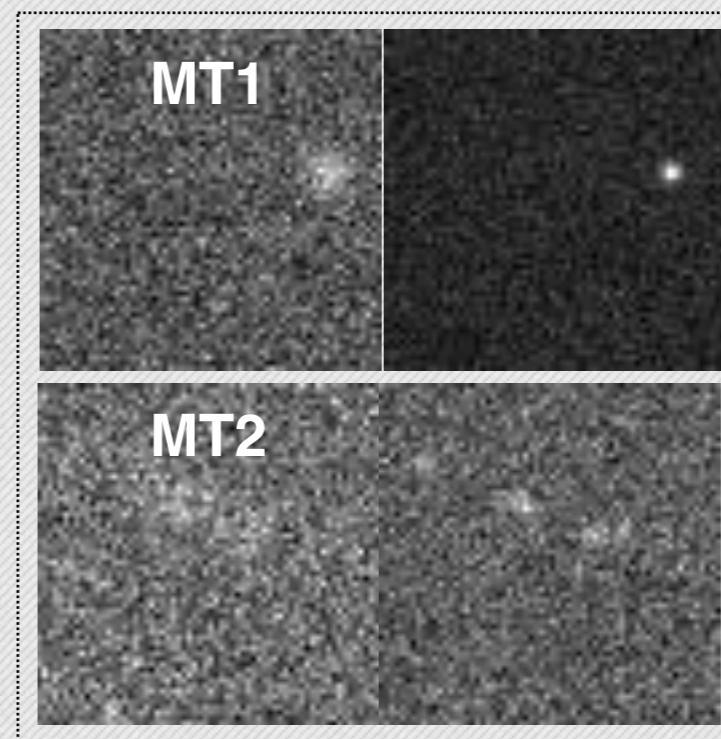
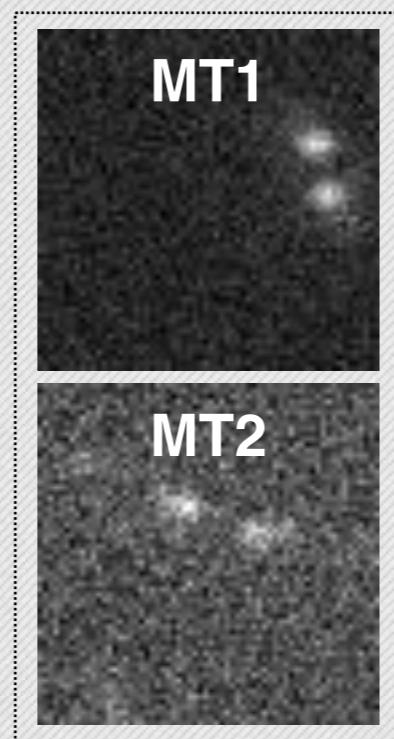
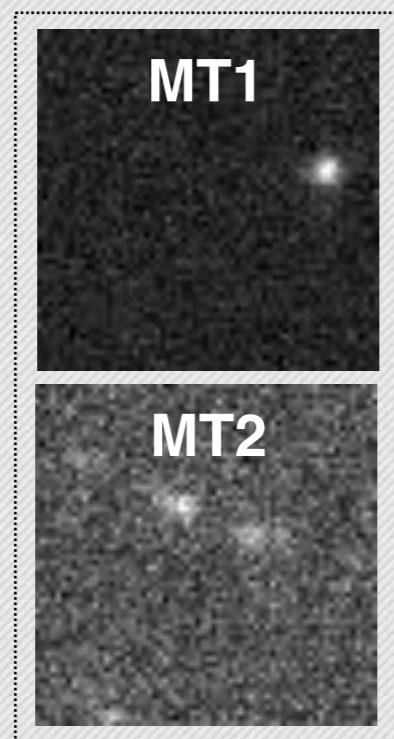
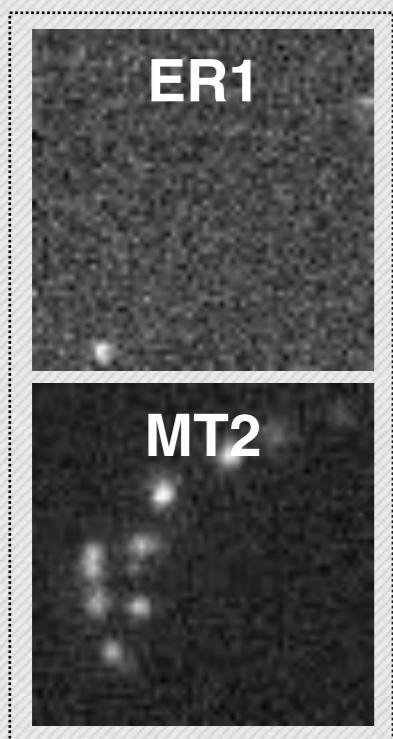
2D

Astigmatism

Double-Helix

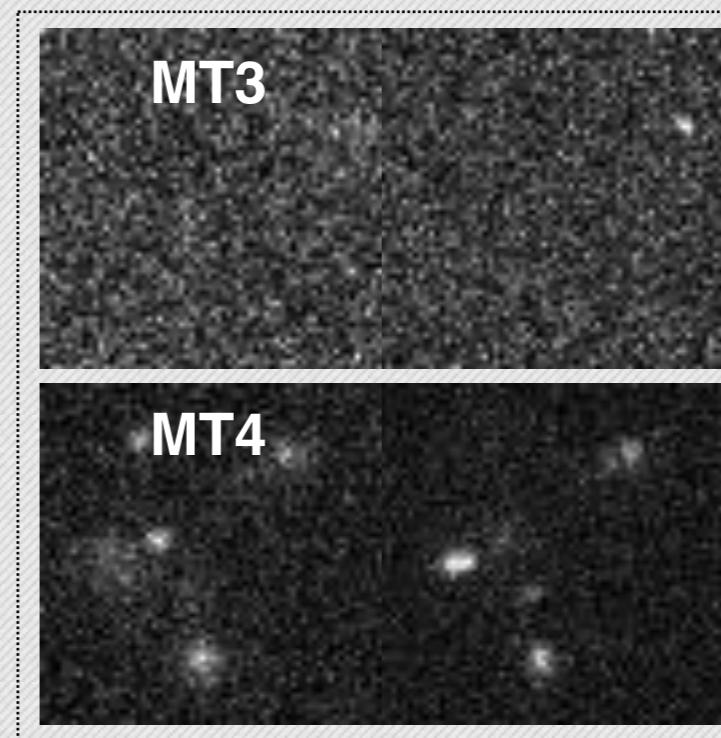
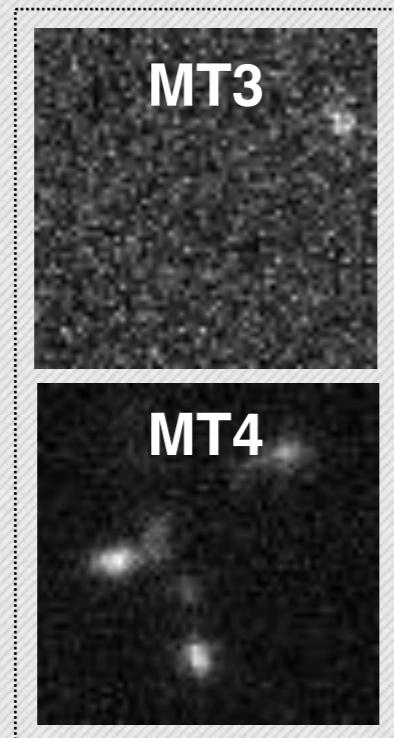
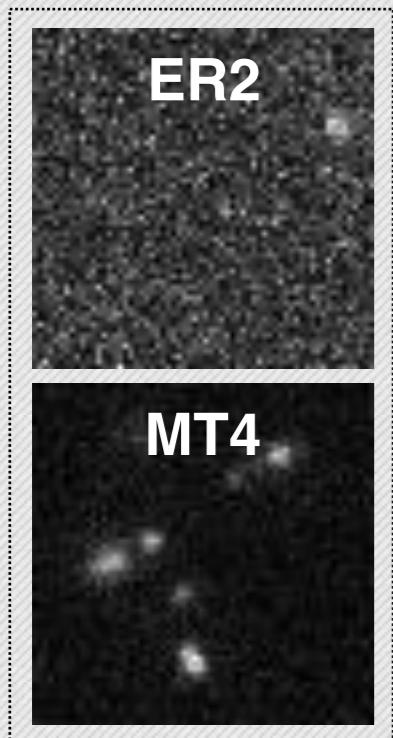
Biplane

Max. Intensity 500 first frames

2D**Astigmatism****Double-Helix****Biplane****Low Density (LD)** ~20'000 frames, ~1.5 fluos / frame

~5000 ph/f/m
autofluo ~10

~500 ph/f/m
autofluo ~100

High Density (LD) ~3'000 frames, ~15 fluos / frame

~5000 ph/f/m
autofluo ~10

~500 ph/f/m
autofluo ~100

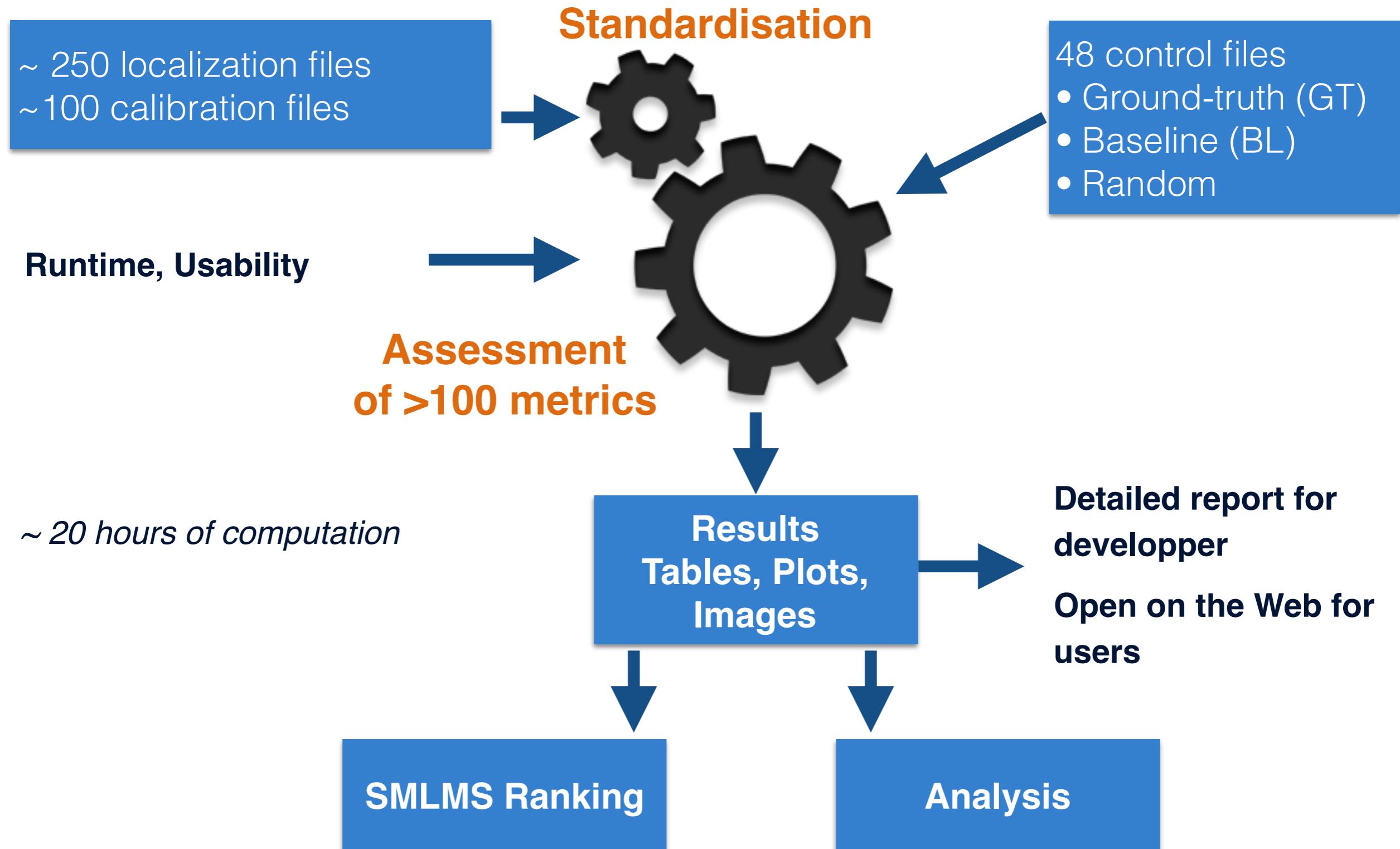
2D**AS****DH****BP**

Benchmarking Spot Localization

SMLM 3D Assessment & Metrics

<http://bigwww.epfl.ch/smlm/>

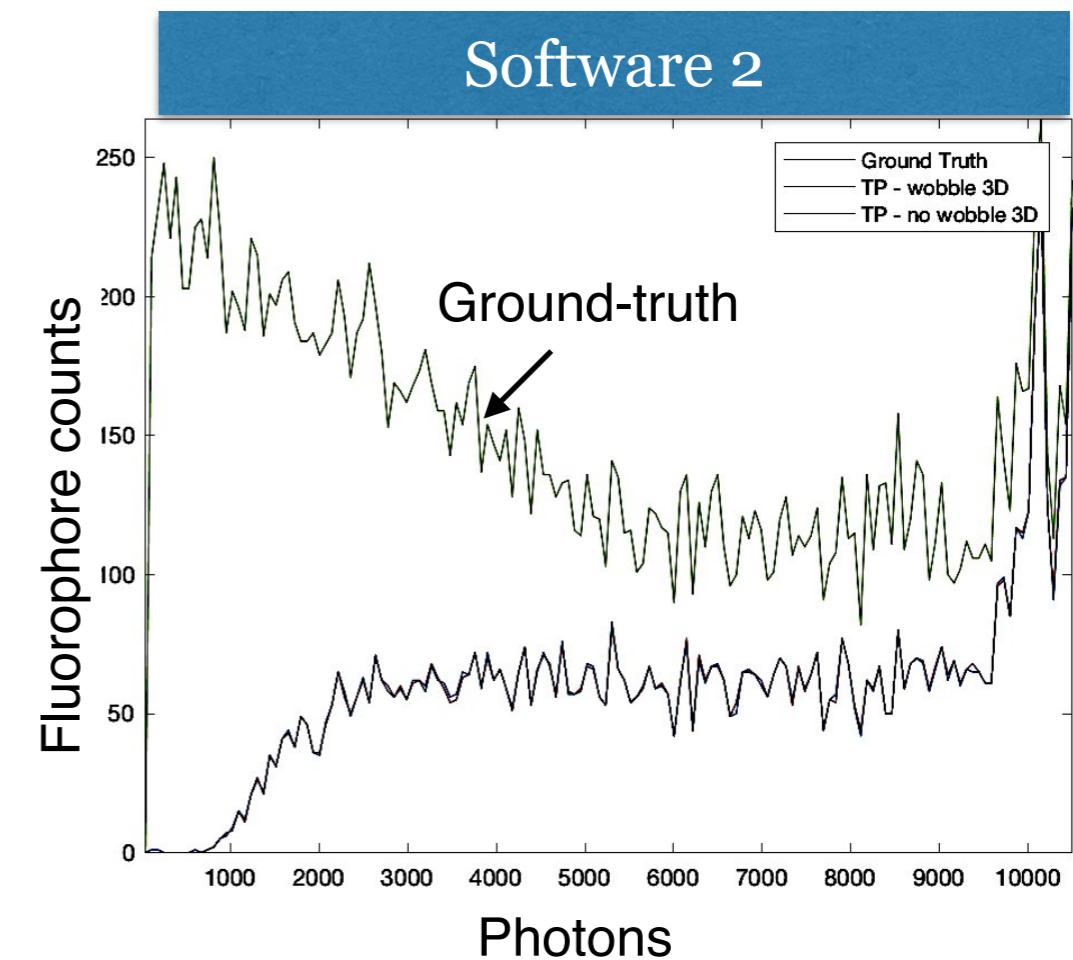
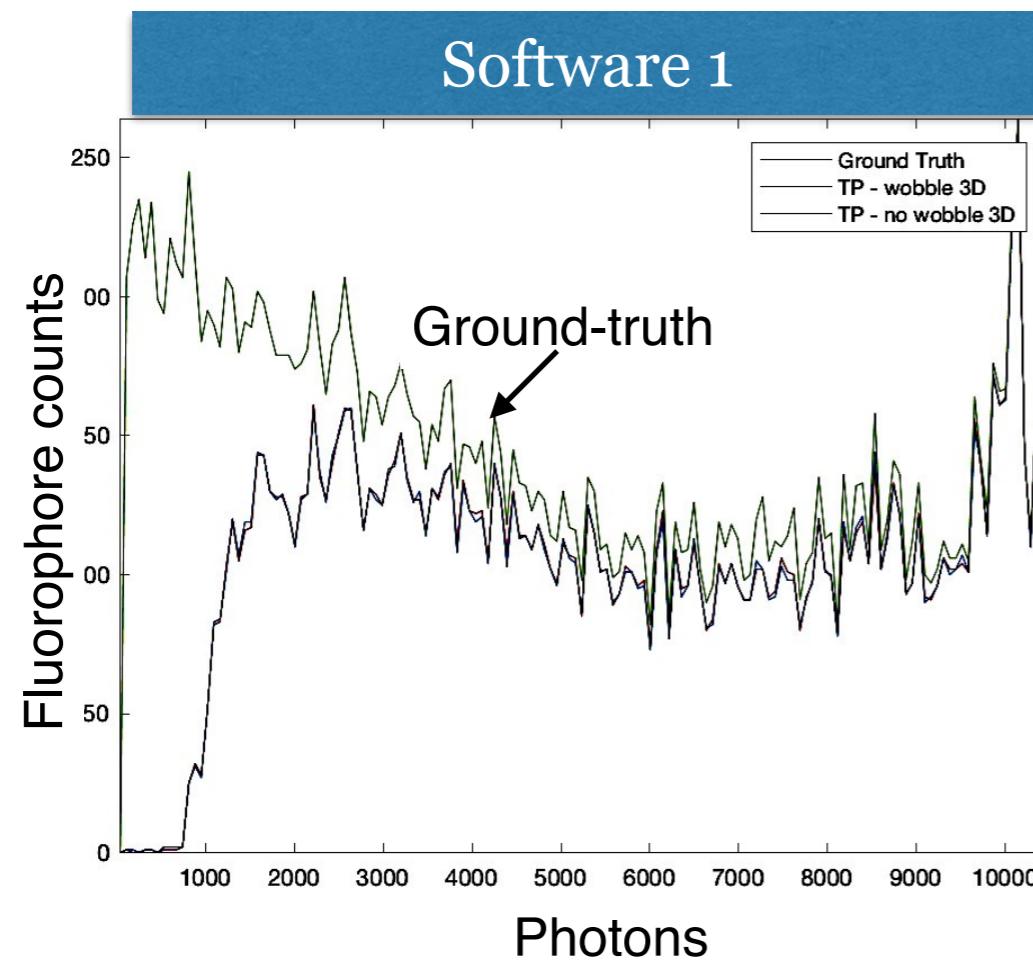
Assessment



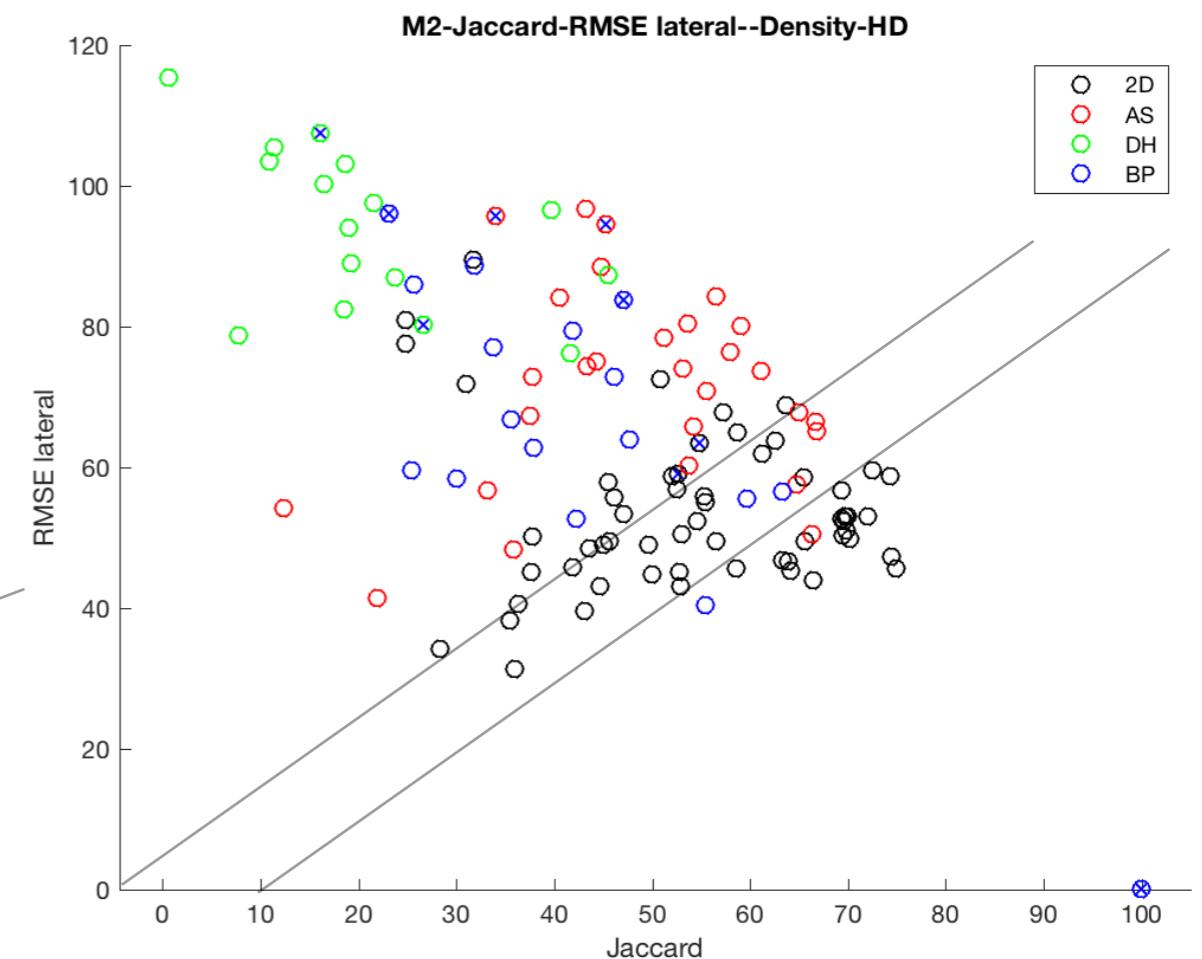
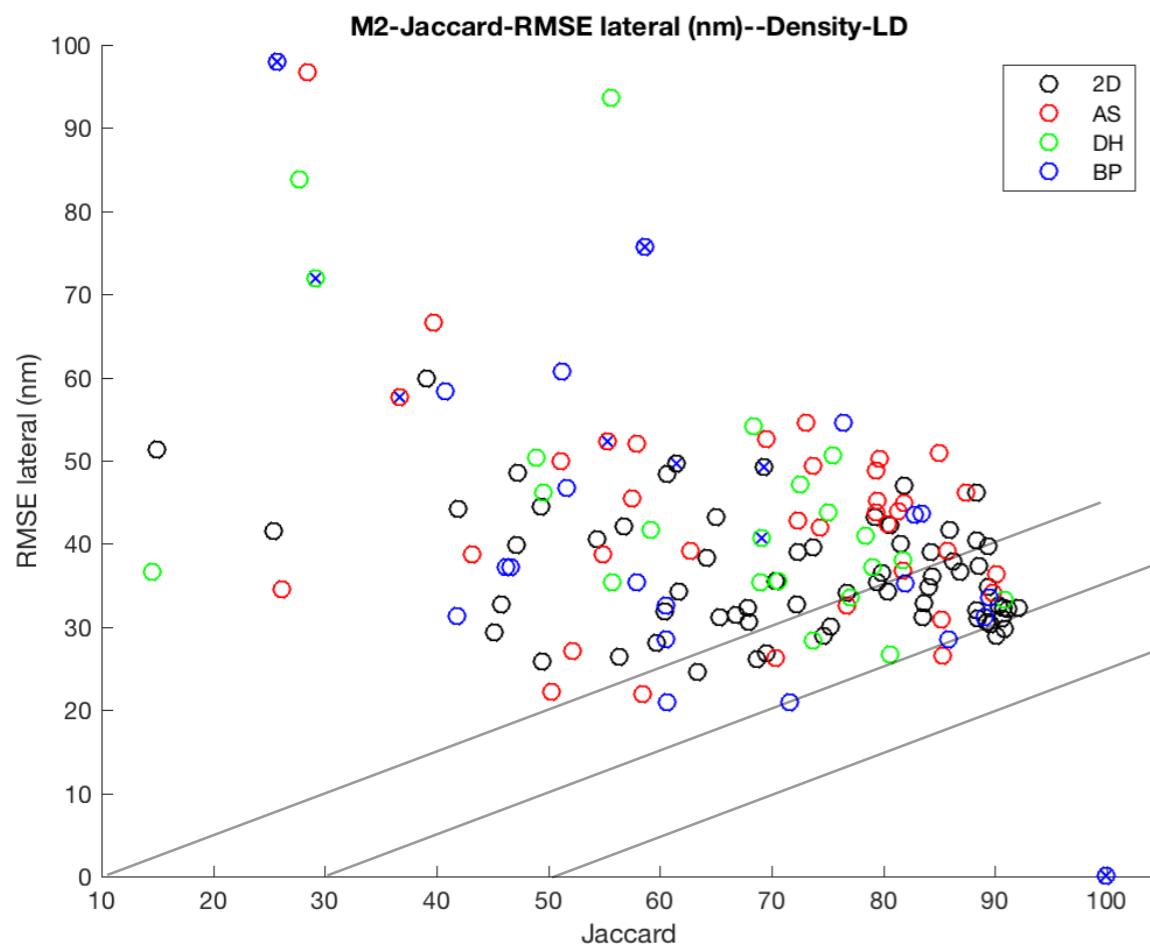
Detailed Assessment

Systematic assessment of the performances

- ▶ Comparison to others and to ground-truth
- ▶ Useful source of information



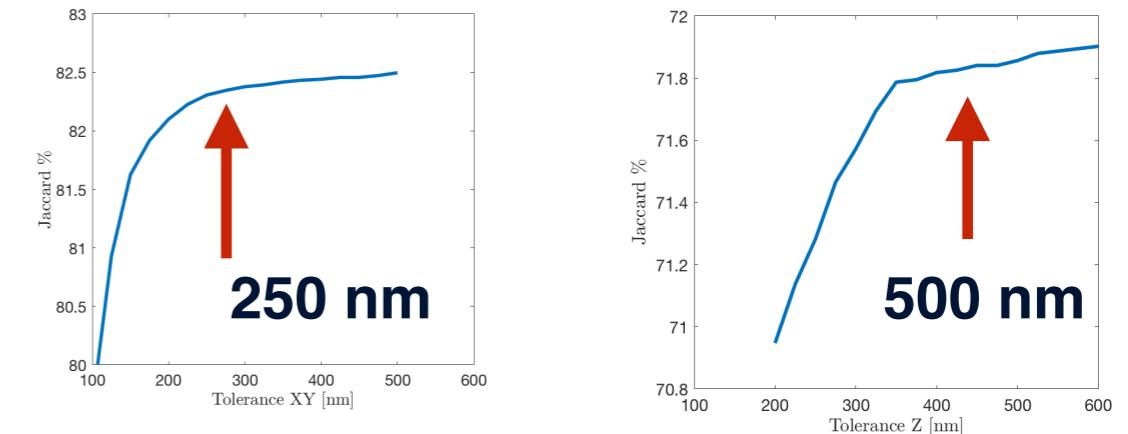
Wobble Correction for 3D



Metrics

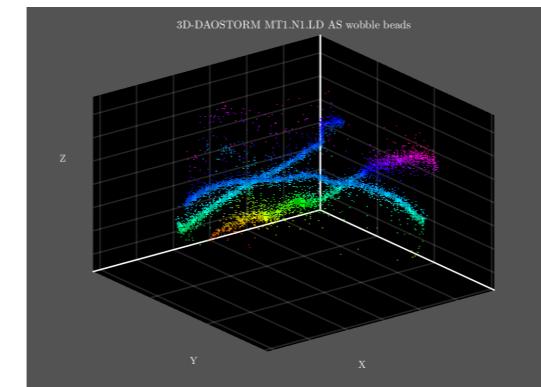
Efficiency [Sage, Nat. Meth. 2015]

- ▶ RMSE Lateral vs Jaccard ($T_{xy} = 250\text{nm}$)
- ▶ RMSE Axial vs Jaccard ($T_z = 500\text{nm}$)



Resolution

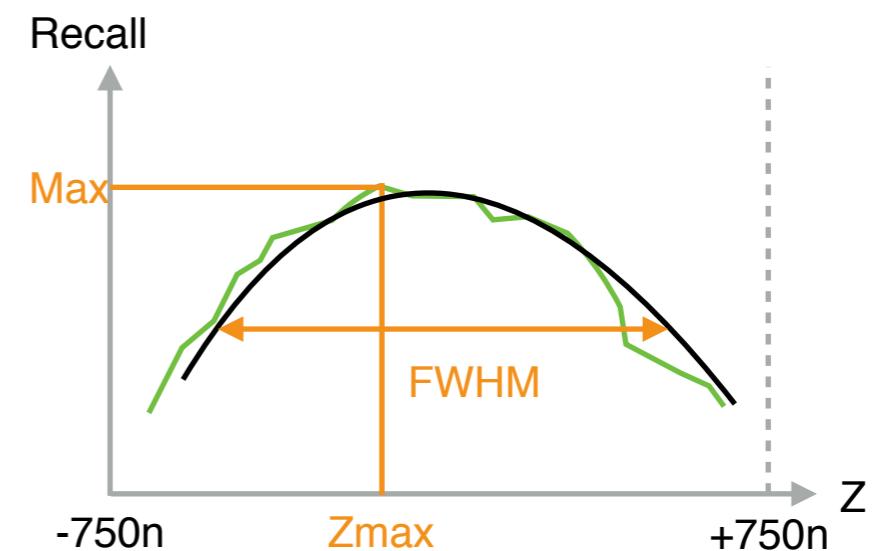
- ▶ Fourier Ring Correlation (FRC)
- ▶ Fourier Shell Correlation (FSC)



[Nieuwenhuizen,
Nat. Meth. 2013]

Consolidated Z Range

- ▶ Max Recall $0 \leq m \leq 1$
- ▶ Consolidated Range = $m \times \text{FWHM}$



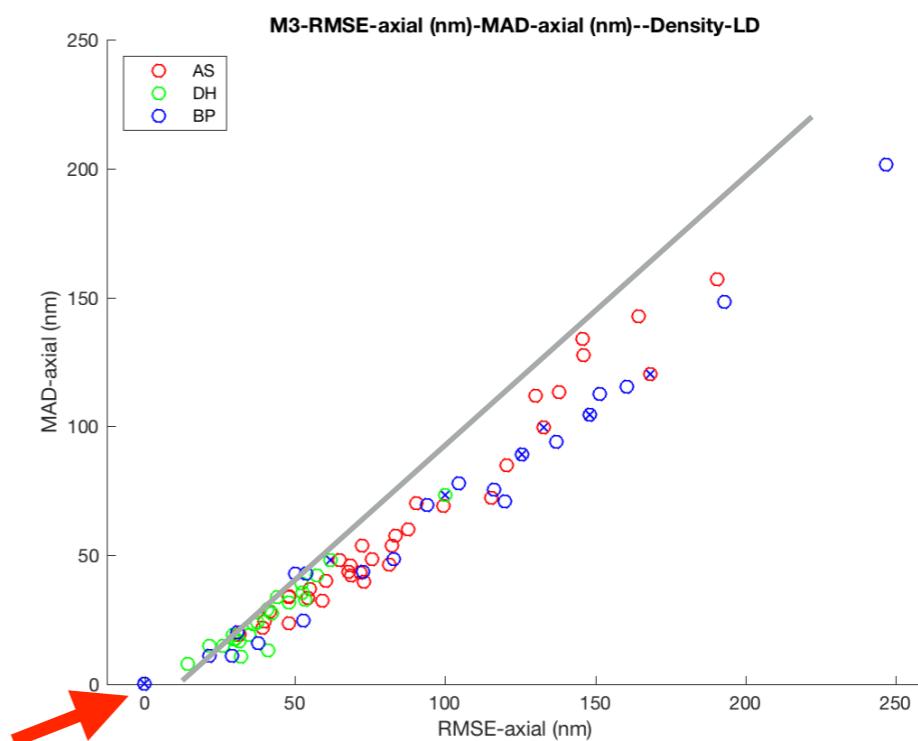
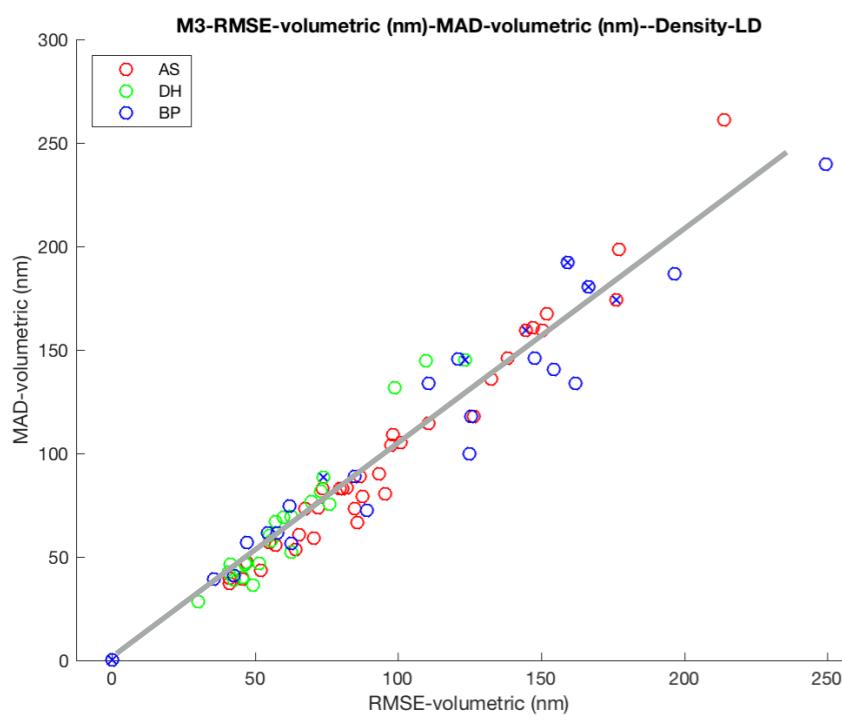
Metrics: Precision Measurement

Root Mean Square Error

- $RMSE_{vol} = \left[\frac{1}{TP} \sum_{i=1}^{TP} d(\mathbf{x}_i^r - \mathbf{x}_i^t)^2 \right]^{\frac{1}{2}}$
- $RMSE_{lat} = \left[\frac{1}{TP} \sum_{i=1}^{TP} (x_i^r - x_i^t)^2 + (y_i^r - y_i^t)^2 \right]^{\frac{1}{2}}$
- $RMSE_{axial} = \left[\frac{1}{TP} \sum_{i=1}^{TP} (z_i^r - z_i^t)^2 \right]^{\frac{1}{2}}$

Mean Absolute Deviation

- $MAD_{vol} = \frac{1}{TP} \sum_{i=1}^{TP} |\mathbf{x}_i^r - \mathbf{x}_i^t|$
- $MAD_{lat} = \frac{1}{TP} \sum_{i=1}^{TP} |x_i^r - x_i^t| + |y_i^r - y_i^t|$
- $MAD_{axial} = \frac{1}{TP} \sum_{i=1}^{TP} |z_i^r - z_i^t|$



Ground-truth

Metrics: Photons and Molecules

Number of Photons

- ▶ Coefficient of correlation (Pearson)

- $$\text{corr}(R, T) = \frac{\text{cov}(R, T)}{\sigma_R \sigma_T}$$

Molecule-wise matching

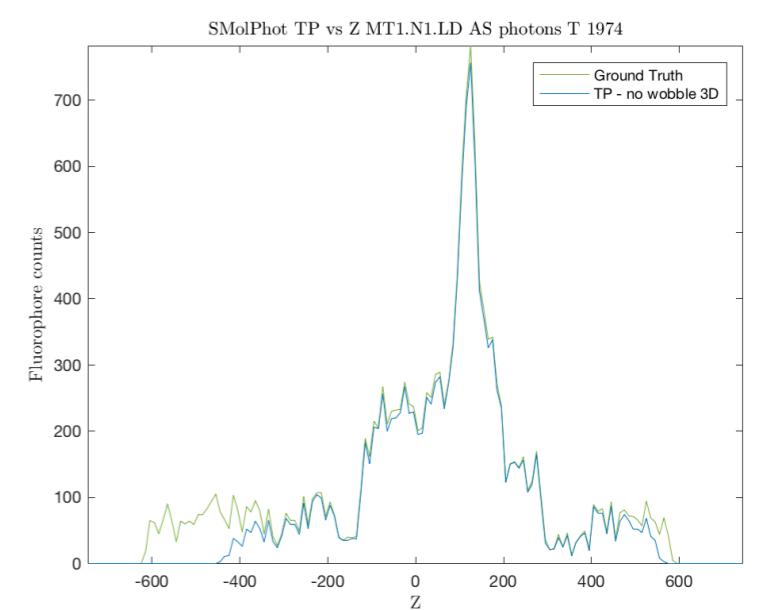
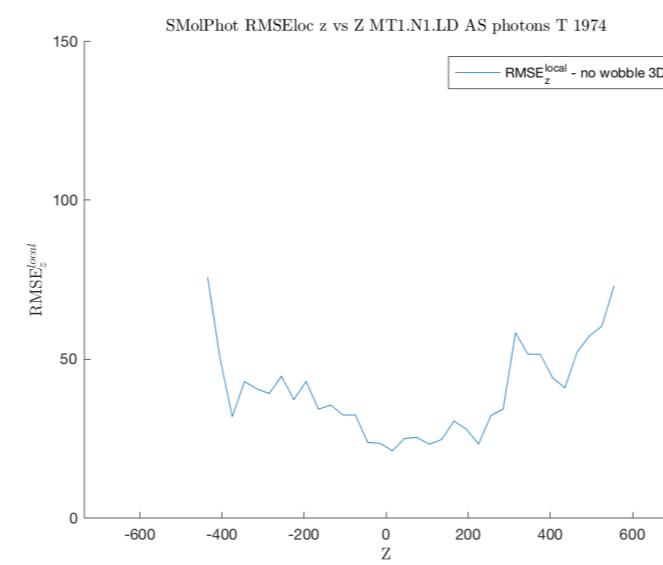
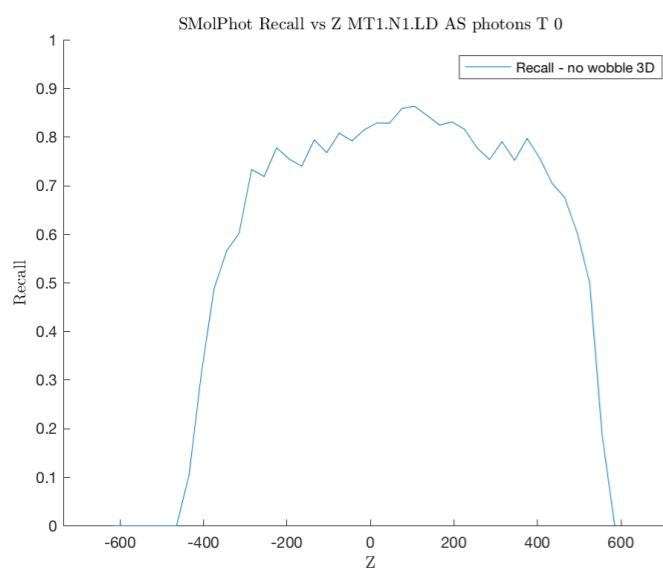
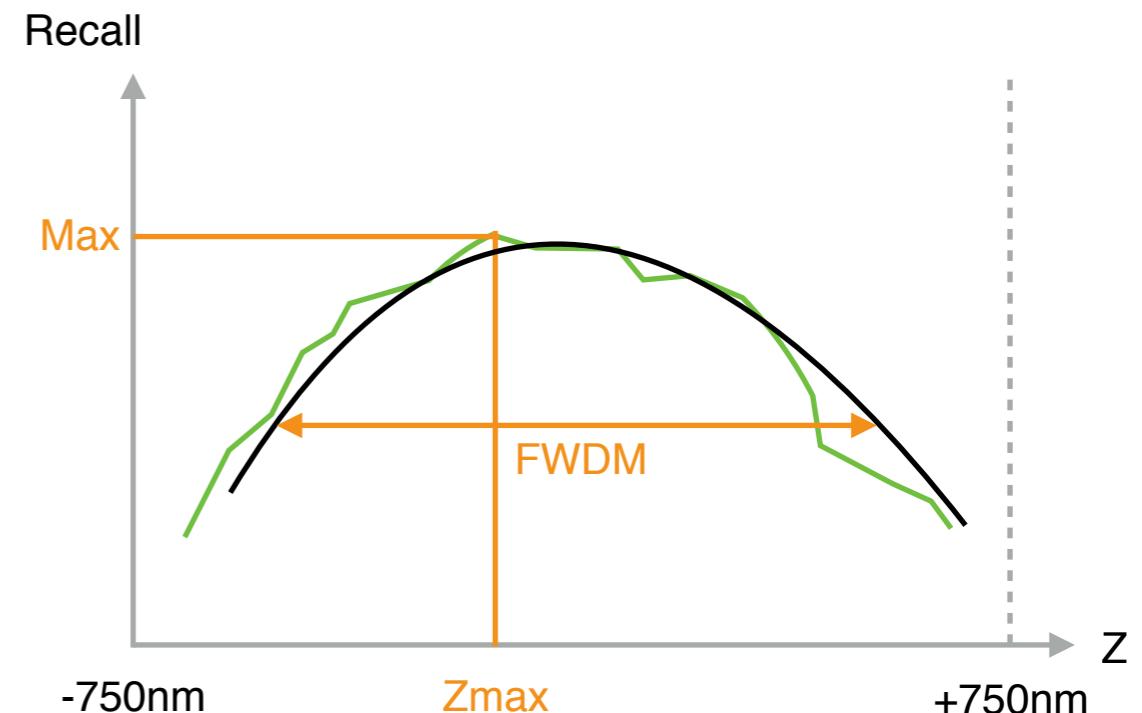
- Number of molecule in ground-truth: N_{mol}
- True Positive (molecule-wise): TP_{mol}
- $$\text{Recall}_{mol} = \frac{TP_{mol}}{N_{mol}}$$
- $$\text{Ratio}_{mol} = \frac{N_{Frames\ TP}}{N_{Frames\ mol}}$$



Metrics: Axial Assessment

Consolidated Z Range

- ▶ Max Recall $0 \leq m \leq 1$
- ▶ FWHM
- ▶ Consolidated Range = $m \times \text{FWHM}$

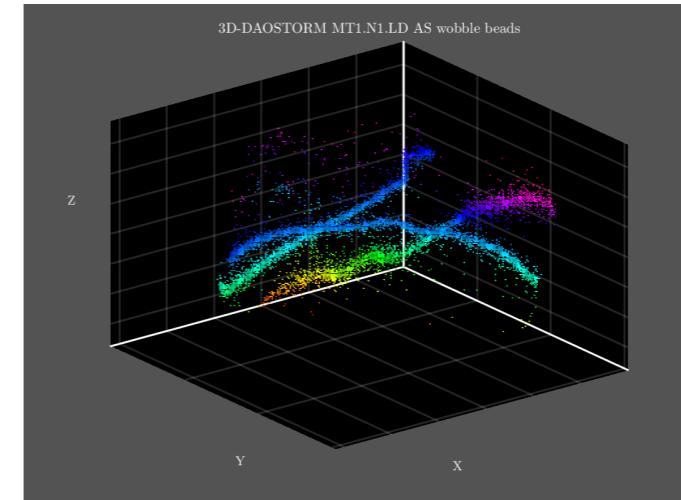


Metrics: Image-Based Criteria

Image quality by Signal-to-noise ratio

- ▶ SNR Volumetric (XYZ) in 3D
- ▶ SNR YZ
- ▶ SNR XZ
- ▶ SNR XY in 2D

Resolution: 10 x 10 x 10 nm
Gaussian rendering FWHM = 10nm



Resolution by Fourier Ring Correlation

- ▶ FSC (Fourier Shell Correlation) in 3D
- ▶ FRC YZ
- ▶ FRC XZ
- ▶ FRC XY in 2D

R. Nieuwenhuizen et al.,
Measuring image resolution in optical nanoscopy,
Nature Methods 10, 2013.

Benchmarking Spot Localization

SMLM 3D Comparison

<http://bigwww.epfl.ch/smlm/>



A A

www.google.ch/?gfe_rd=cr&ei=_YHAV_izFMbw8Aehqa7YAw



RCC ▾ leaderboard graphs datasets report Icy biii.info LM ▾ Maps ▾ Web ▾ Savoir ▾ Voyages ▾ Guide ▾ Education ▾ TM Nad et Jess >> +

Gmail Images



Sign in



s|

sbb**sbb** – Swiss Federal Railways, Railway company**sbb** – Serbia Broadband, Cable television company**skyscanner****swiss air****swiss air** – Swiss International Air Lines, Airline company**swiss air** – Swissair, Airline company**spotify****sncf****skype**

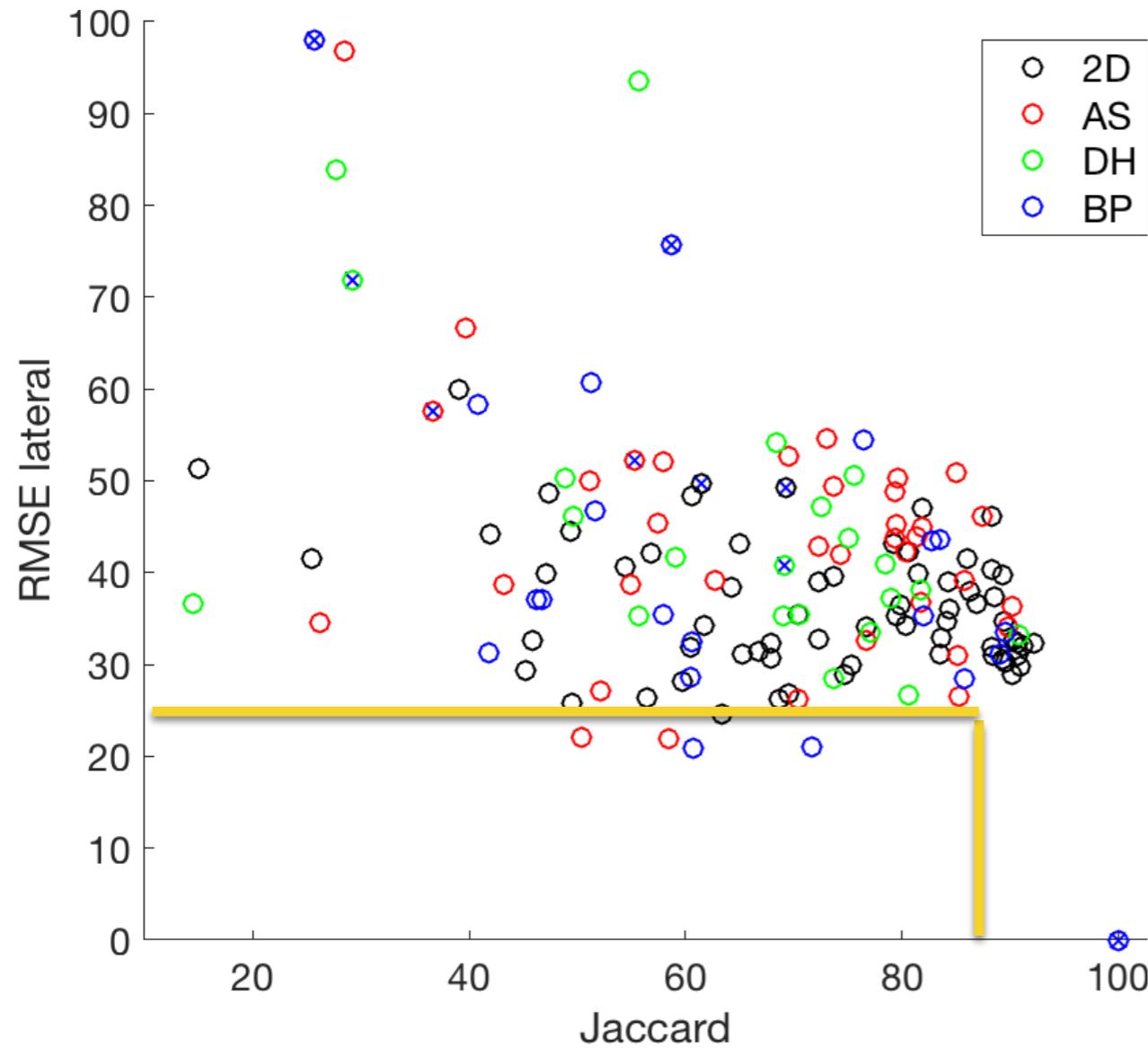
Google Search

I'm Feeling Lucky

Learn more

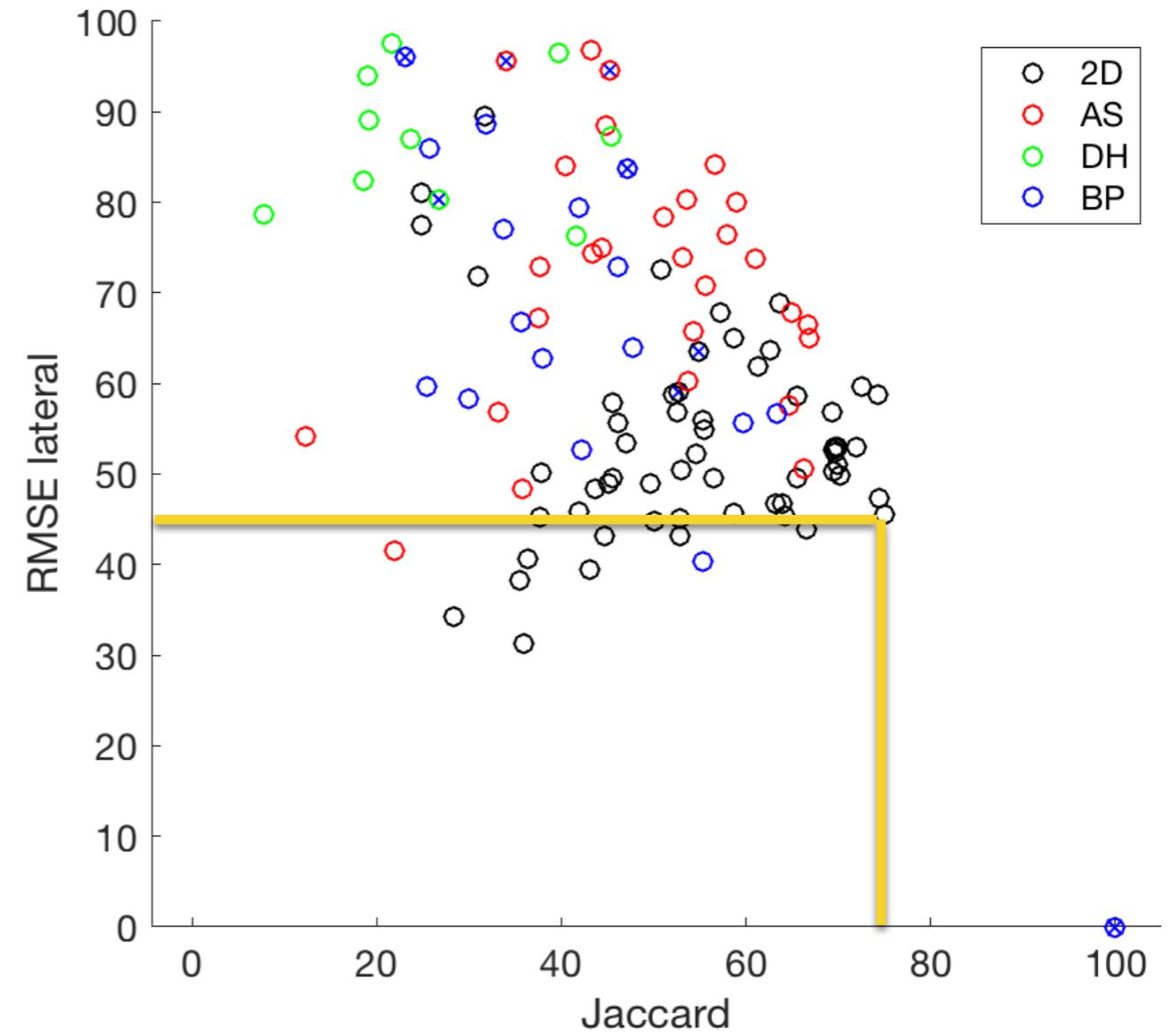
Lateral Efficiency

Low-Density



~ 85% ~ 25 nm

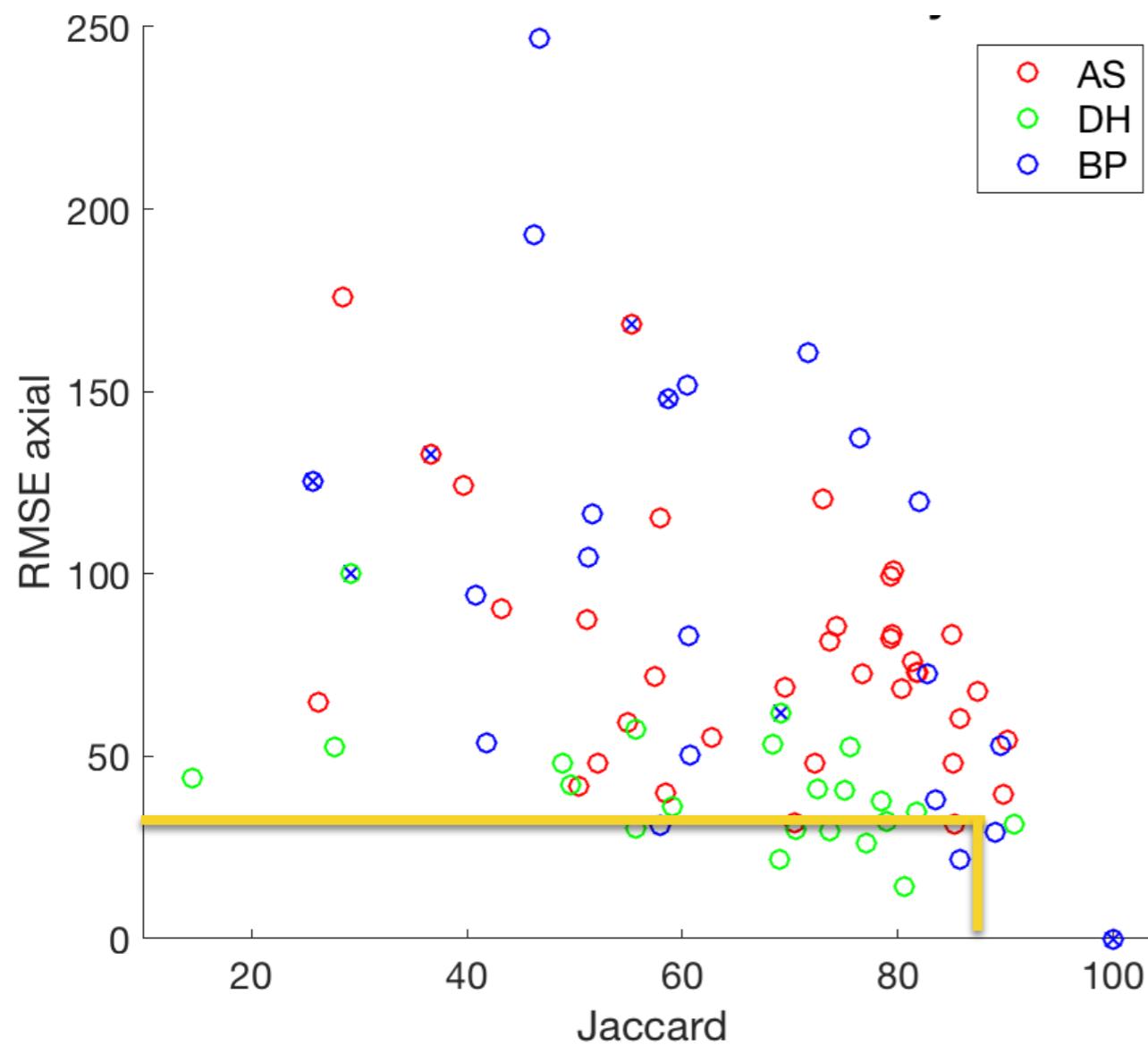
High-Density



~ 75% ~ 45 nm

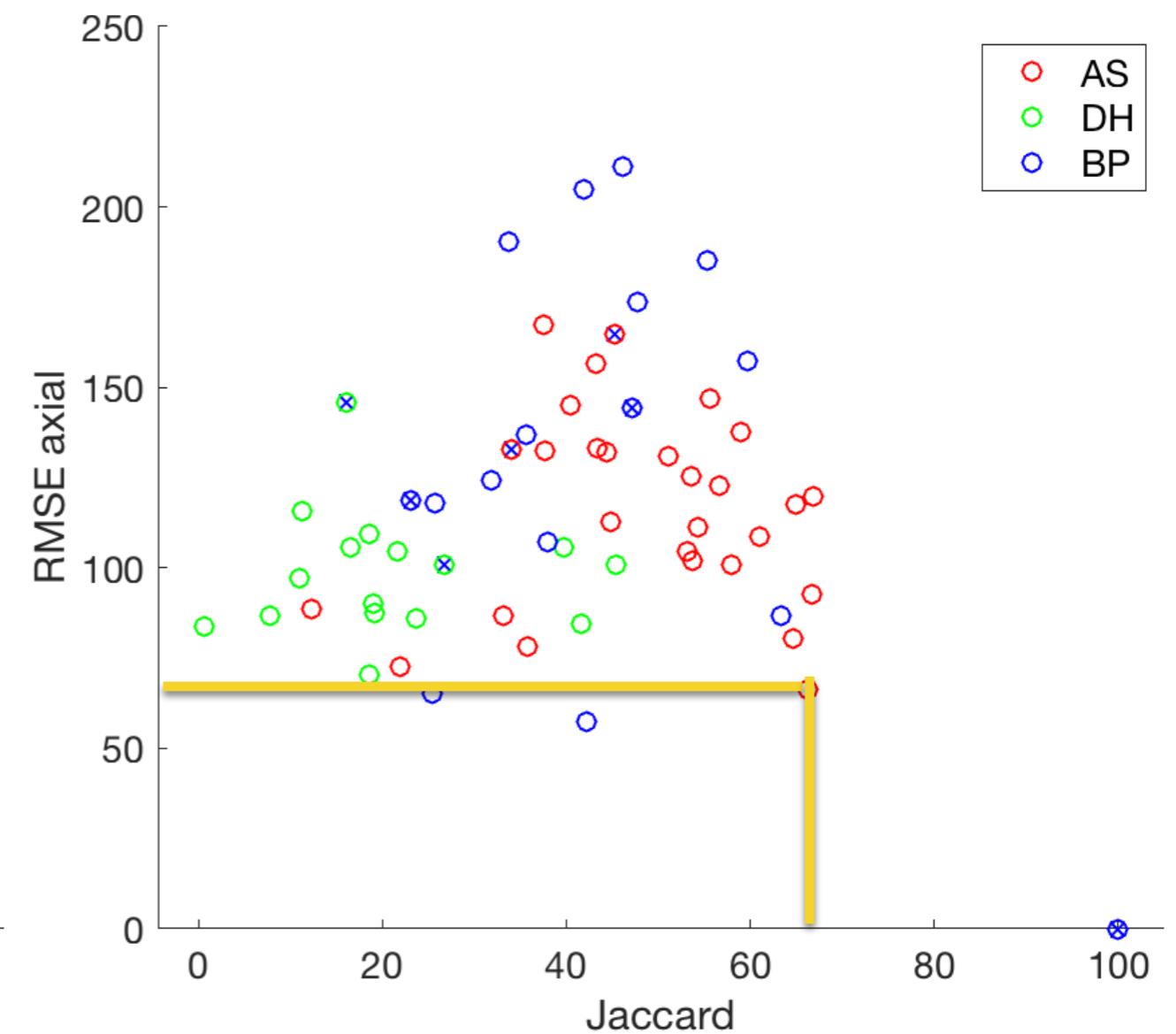
Axial Efficiency

Low-Density

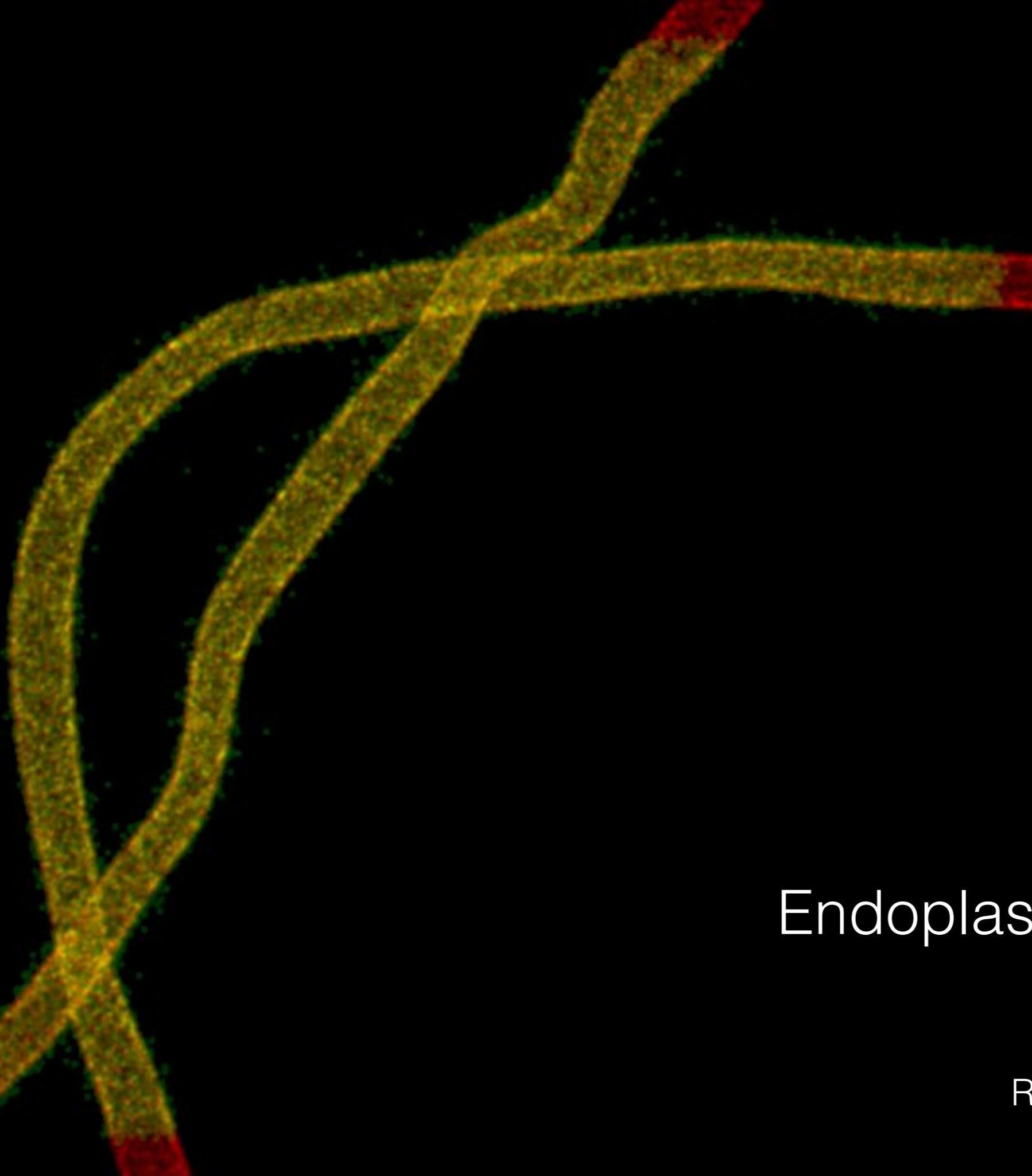


~ 85% ~ 30 nm

High-Density



~ 65% ~ 60 nm



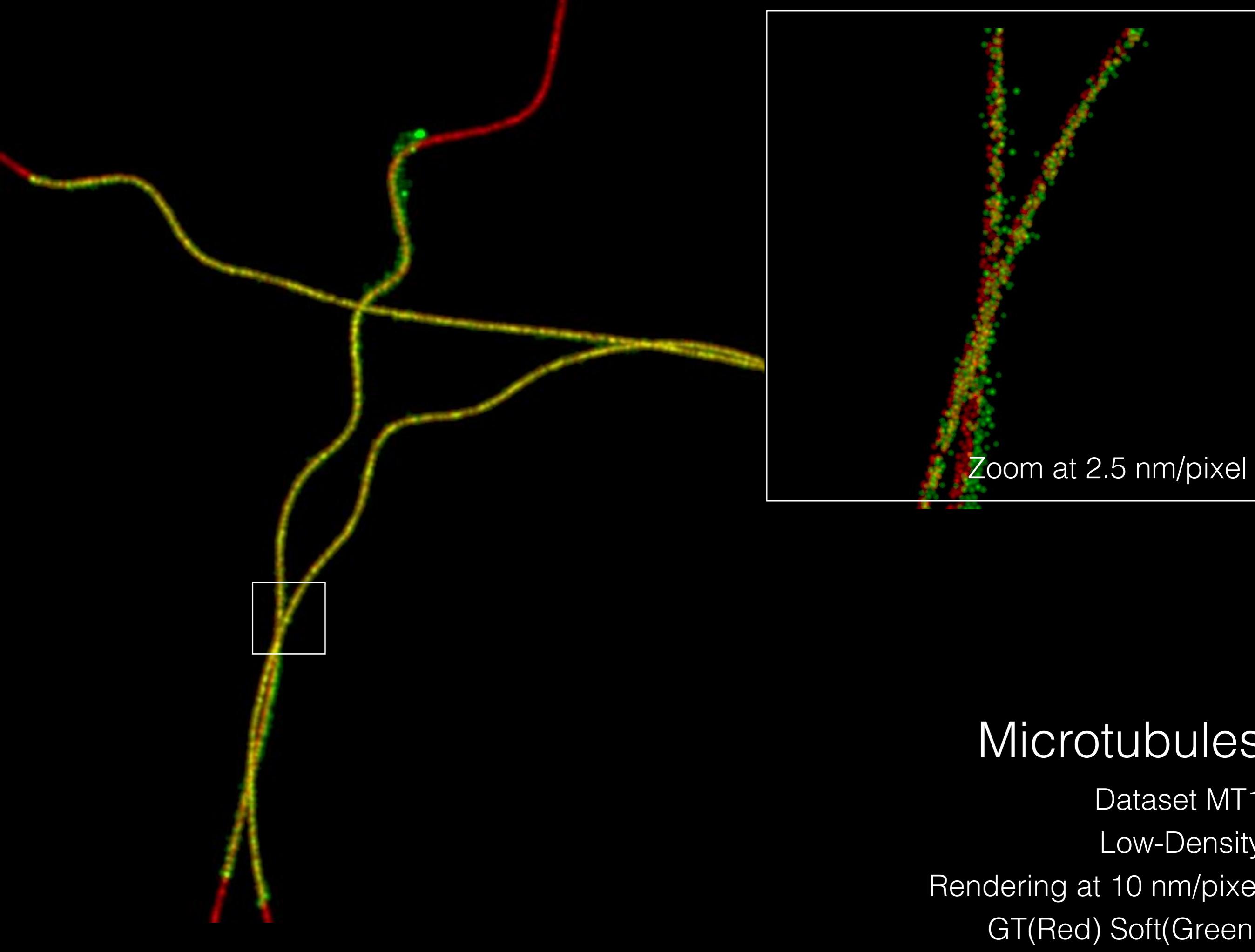
Endoplasmic Reticulum

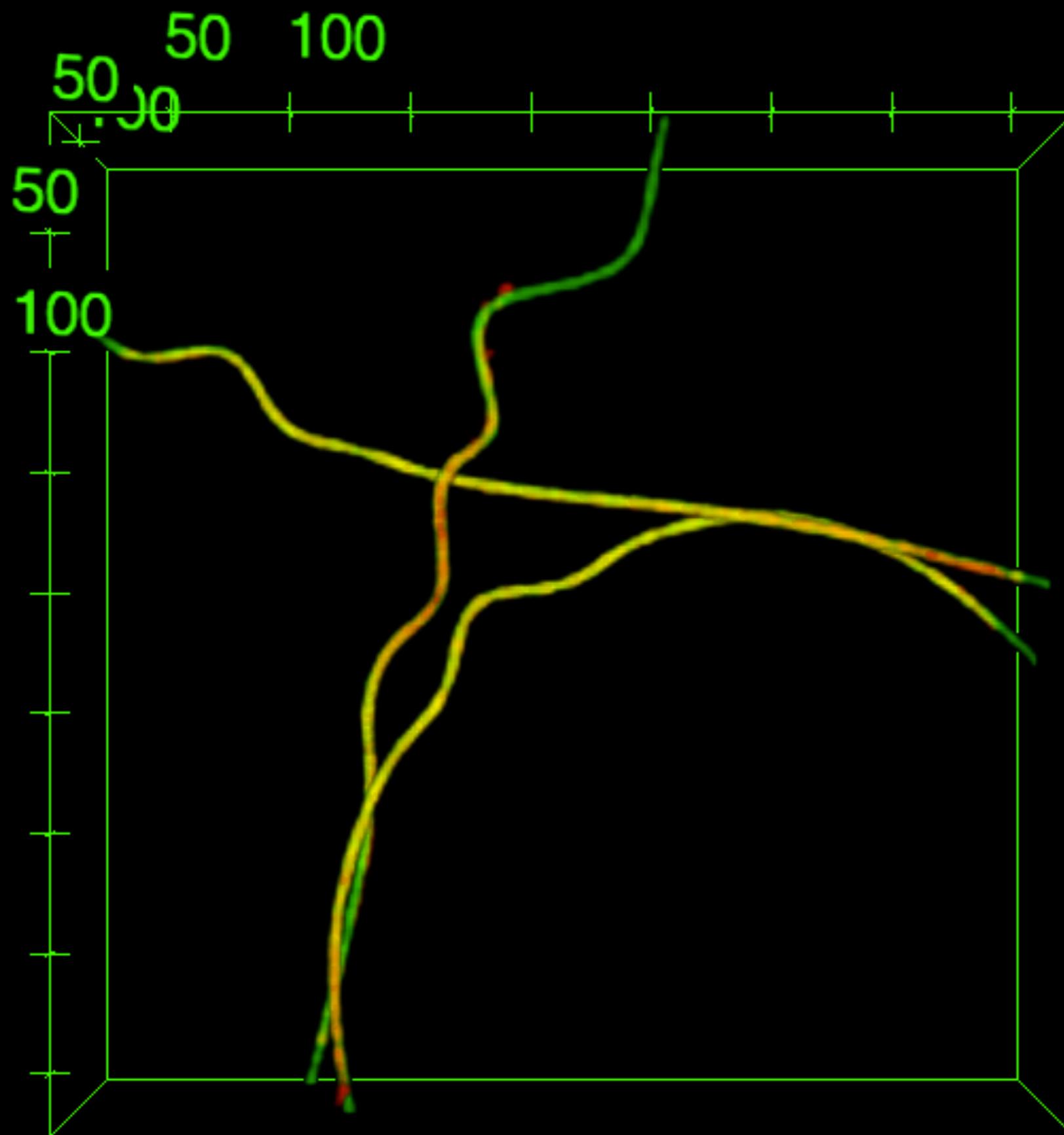
Dataset ER1

Low-Density

Rendering at 10 nm/pixel

GT(Red) Soft(Green)

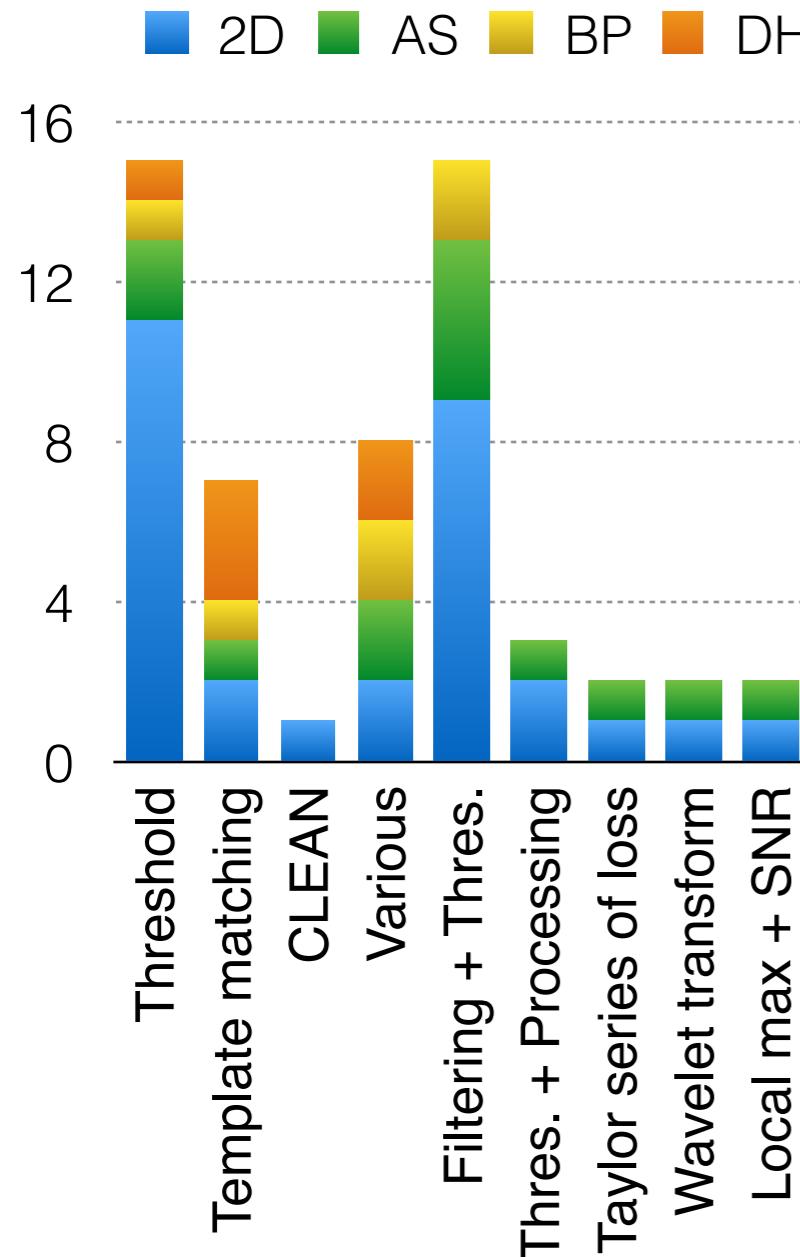




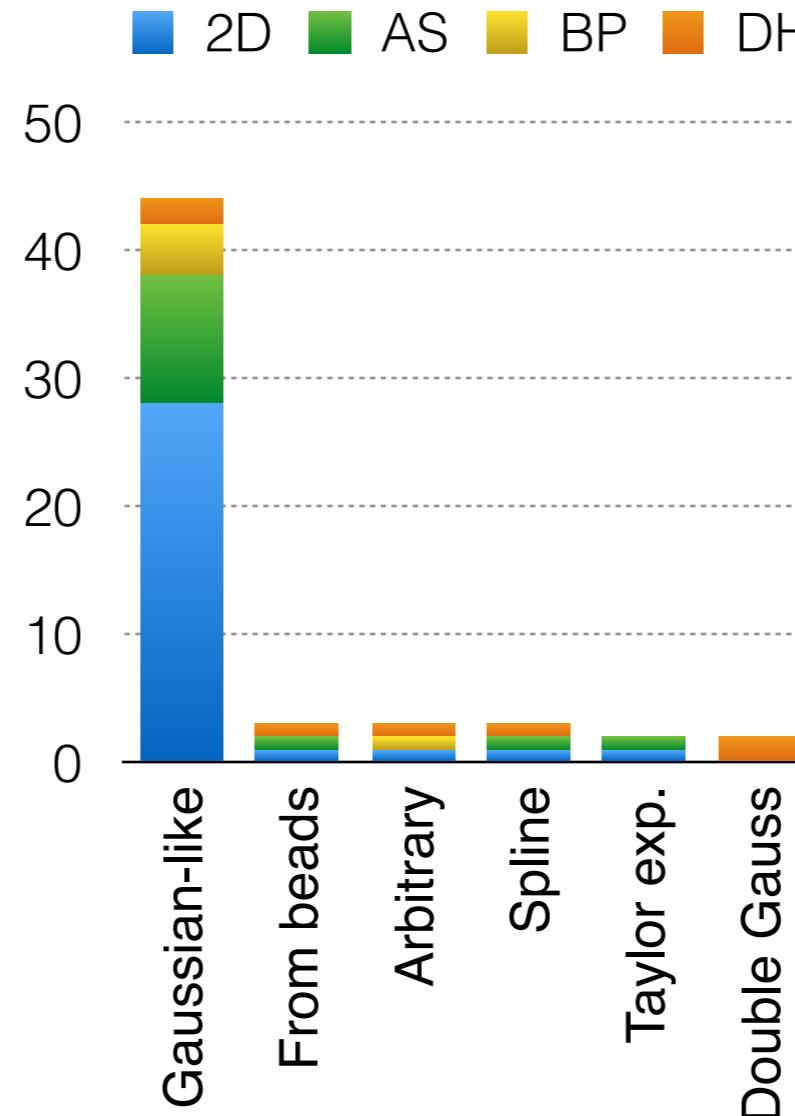
Microtubules
Dataset MT1
Astigmatism Low-Density
Rendering at $10 \times 10 \times 10$ nm
GT(Green) Soft(Red)

Algorithms

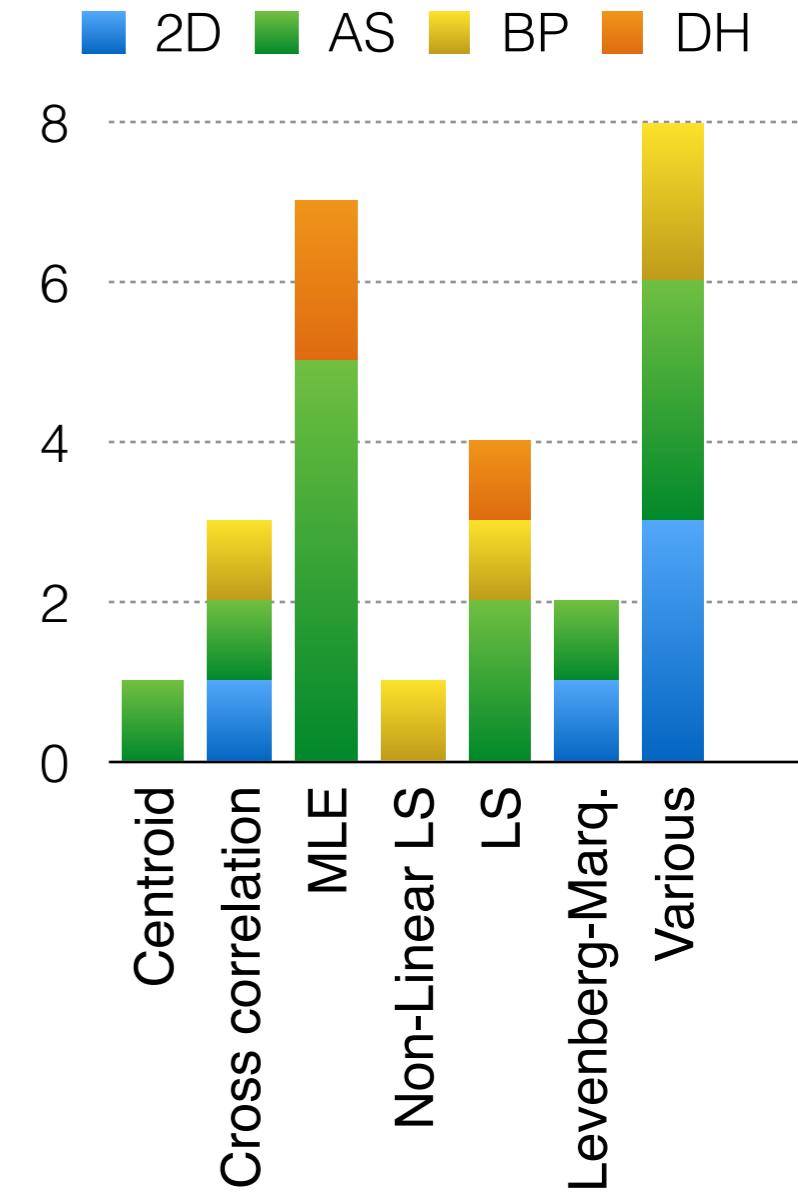
Detection Algorithm



PSF Model



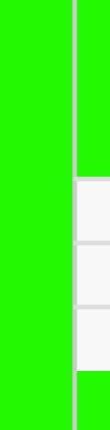
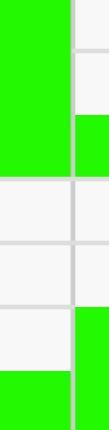
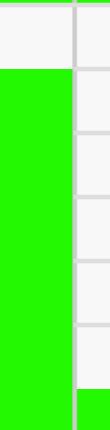
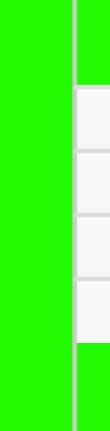
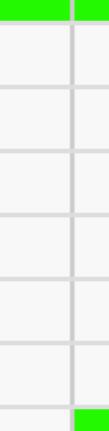
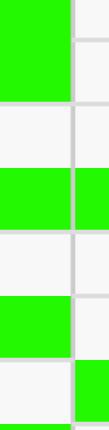
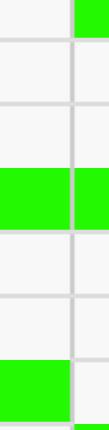
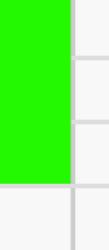
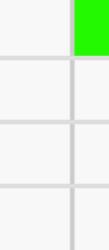
Localization Algorithms



Benchmarking Spot Localization

SMLM 3D Challenge

<http://bigwww.epfl.ch/smlm/>

Software	2D	AS	DH	BP	Run by	Contact	Affiliation	Country	Reference
3D STORM Tools					Author	Fabian Hauser	University of Austria, Linz	Austria	
3D-DAOSTORM					Author	Hazen Babcock	Harvard	USA	Opt. Nanoscopy 2012
3D-WTM					Author	Shigeo Watanabe	Hamamatsu Photonics	Japan	
ADCG					Author	Nicholas Boyd and al.	Berkeley	USA	ArXiv 2015
ALOHA					Author	Junhong Min, Kyong Jin	KAIST	Korea	ArXiv 2015
BreCs					Author	Hervé Rouault	Janelia Farm	USA	
CSpline					Author	Hazen Babcock	Harvard	USA	
Easy-DHPSF					Author	Alex Diezmann	Stanford University	USA	Proto. Exchange 2013
FALCON					Author	Junhong Min	KAIST	Korea	Sci Report 2015
FIRESTORM					Author	Thomas Von Hof	Ulm University	Germany	
L1H					Author	Hazen Babcock	Harvard	USA	Optics Express 2013
Localizer					Expert	Ann Wheeler	University of Edinburgh	UK	J. Biomed. Optics 2013
MaLiang					Author	Wang Yujie	Huazhong University	China	Optics Express 2010
MIATool					Author	Ram Velmurugan	Texas A&M University	USA	IEEE TITB 2010
mlePALM					Author	Hendrik Deschout	EPFL	Switzerland	
Octane					Expert	Thomas Pengo	University of Minnesota	USA	Biophysical Journal 2012
PALMER					Author	Wang Yujie	Huazhong University	China	Optics Express 2012
PeakFit					Author	Alex Herbert	University of Sussex	UK	
PeakSelector					Expert	Thomas Pengo	University of Minnesota	USA	PNAS 2009
QuickPALM					Author	Ricardo Henriques	UCL	UK	Nature Methods 2010
RainSTORM					Author	G. Tamas, J. Sinkó	University of Szeged	Hungary	Optics Express 2013
RapidSTORM					Expert	Seamus Holden	Newcastle University	UK	Nature Methods 2012
SFP Estimator					Author	Manfred Kirchgessner	University of Heidelberg	Germany	
SMAP					Author	Jonas Ries	EMBL	Germany	
SMfit					Author	Hayato Ikoma	Stanford University	USA	
SMolPhot					Author	Martti Pärs and al.	University of Tartu	Estonia	
STORMChaser					Author	Anna Arletti	EPFL	Switzerland	
ThunderSTORM					Author	Martin Ovesní	Charles University	Czech Rep.	Bioinformatics 2014
TVSTORM					Author	Jiaqing Huang	Ohio State University	USA	ISBI IEEE 2016
WaveTracer					Author	Adel Kechka	Université de Bordeaux	France	Plos One 2013
WTM					Author	Shigeo Watanabe	Hamamatsu Photonics	Japan	FOM 2013

Participation

Participants

- ▶ Attract the attention of many groups: 31 software
- ▶ 87% run by the author
- ▶ 61% run at least one 3D modality
- ▶ 65% are new competitors



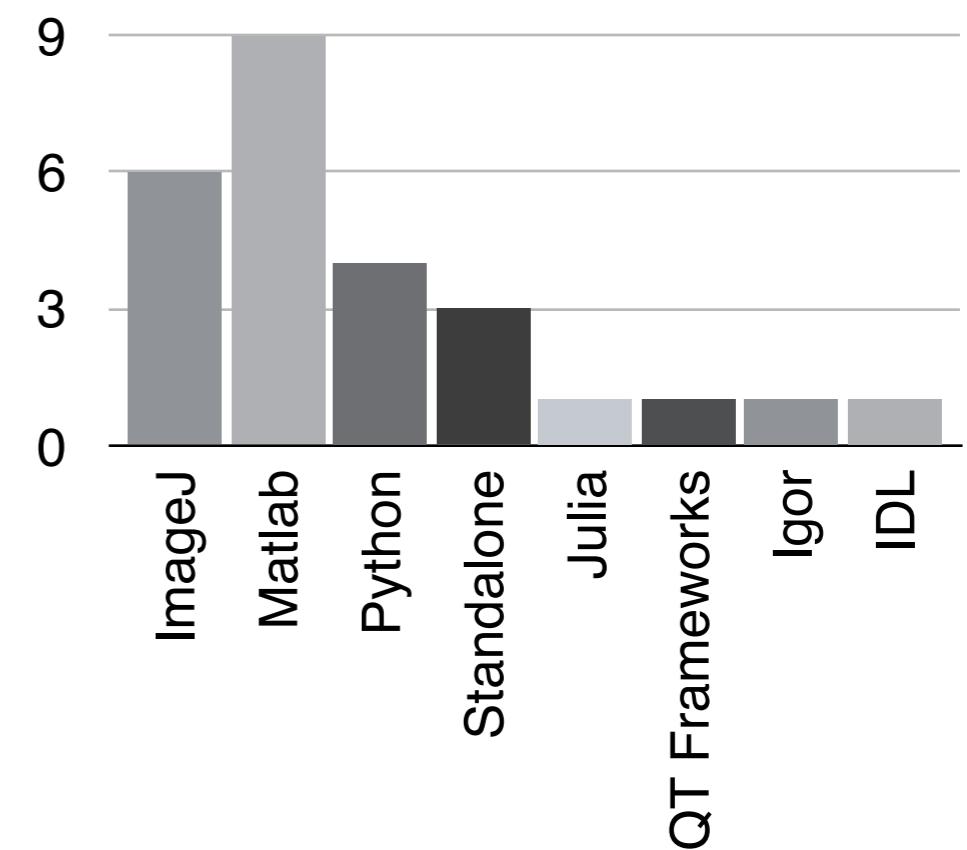
3D-WTM

MIATool

SMAP

By-products of the challenge

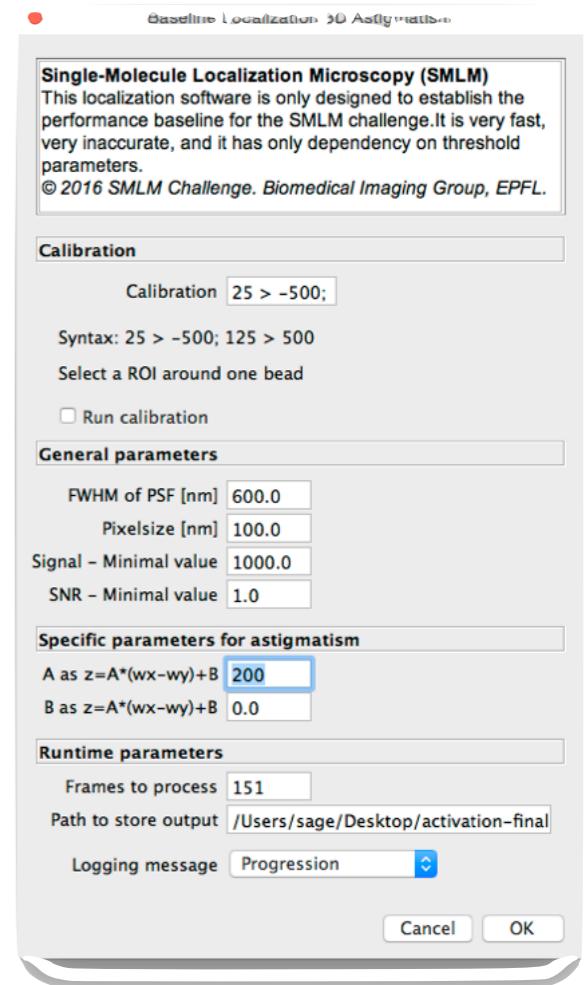
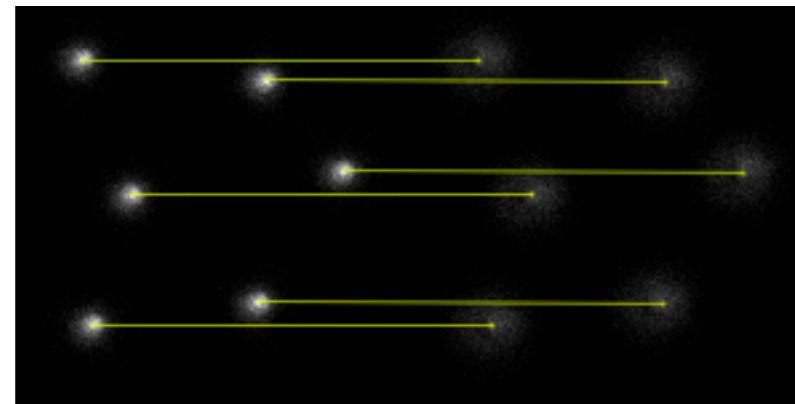
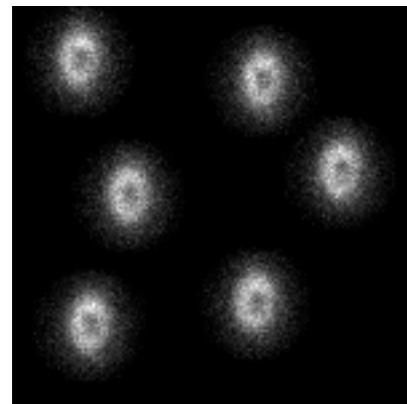
- ▶ Encourage multi-modalities (3D)
- ▶ Promote the open-source
- ▶ Foster usability



Baseline

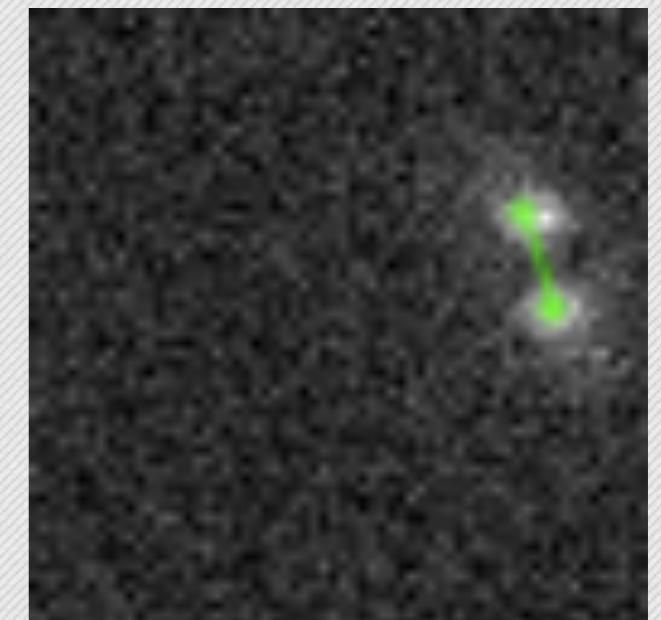
Make a code as simple as possible

- ▶ Make it available (ImageJ)
- ▶ Multiple modalities: 2D, AS, BP, DH
- ▶ Calibration on beads for 3D



Double-Helix

- ▶ Detect by Band-pass filter, local max., threshold
- ▶ Localize by center of gravity
- ▶ Match the closest ones
- ▶ Convert the angle to Z



Grading

Assignment of the grades

- ▶ Linear mapping: RND=0, BL=1, GT = 5
- ▶ Normalized on each feature, every datasets
- ▶ Rescale to have the maximum fro GT

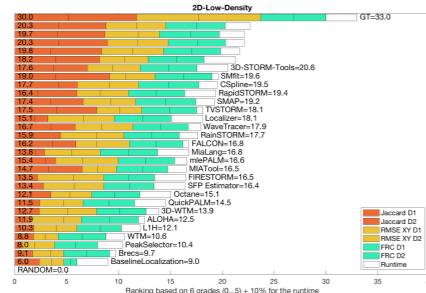
Score_Lateral	Jaccard1 + Jaccard2 + RMSELat1 + RMSELat2 + FRC1 + FRC2	30 pts
Score_Axial	ZRange1 + ZRange2 + RMSEAxial1 + RMSEAxial2 + FSC1 + FSC2	30 pts

2D	Score_Lateral + 3 points for runtime	33 pts
AS, BP, DH	Score_Lateral + ScoreAxial + 6 points for rutime	66 pts

Categories of the Challenge

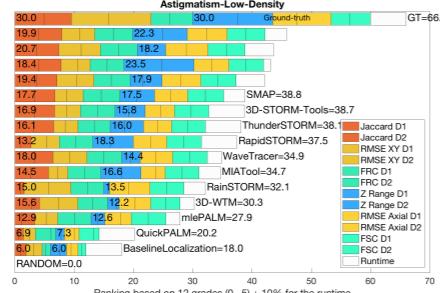
Low-Density

2D



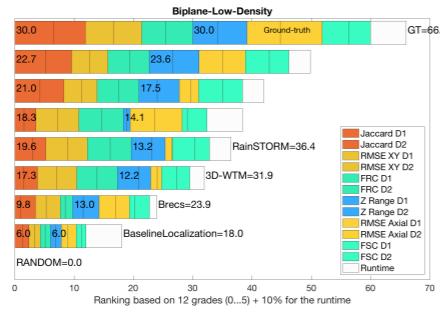
31 participants

AS



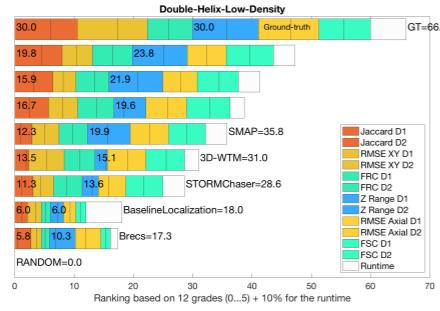
17 participants

BP



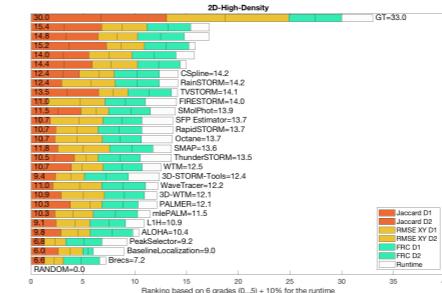
9 participants

DH

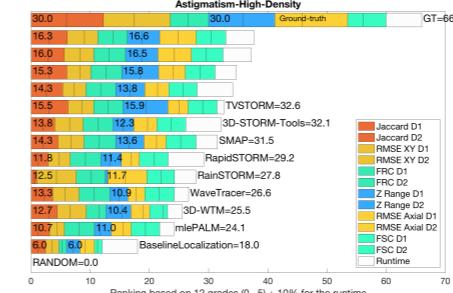


10 participants

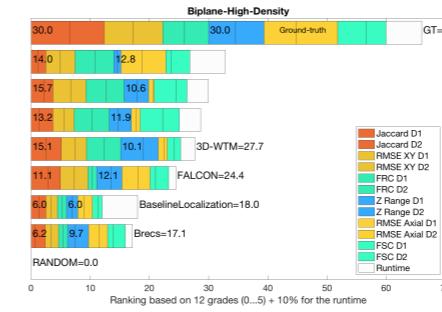
High-Density



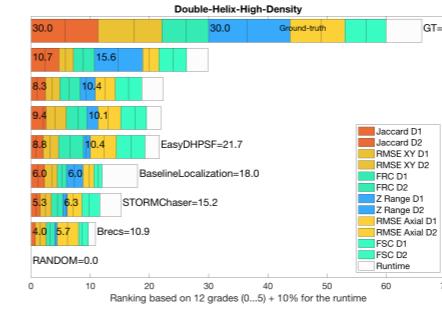
28 participants



15 participants



9 participants



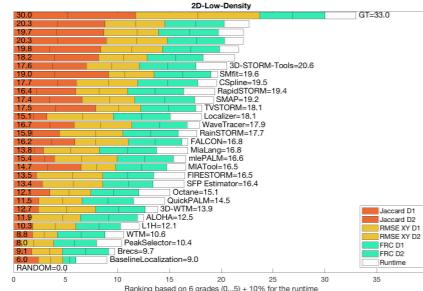
10 participants

Assess several instances: rank only only the main instance of a software
Access to ground-truth localization: Daniel Sage and Thanh-An Pham

Winners in SMLMS 2016

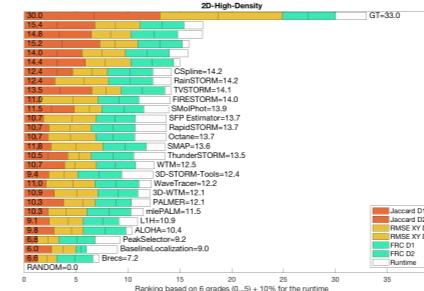
2D

Low-Density



3D-DAOSTORM

High-Density

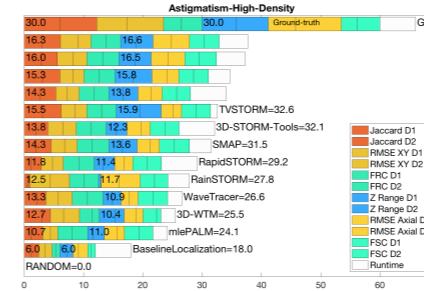
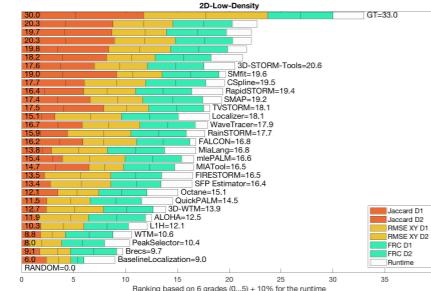


ADCG

AS

CSpline

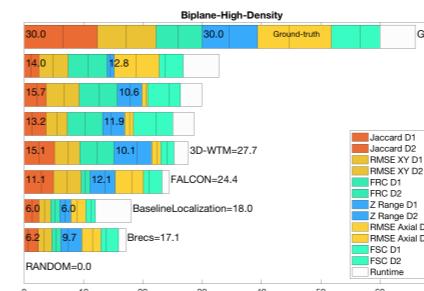
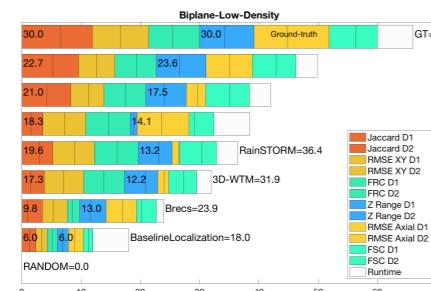
SMolPhot



BP

MIATool

ThunderSTORM



DH

CSpline

CSpline

