A guided tour for analyzing and quantifying single-molecule localization microscopy data

Part 1: Analysing raw SMLM data

NEUBIAS@Home webinar

11/06/2020

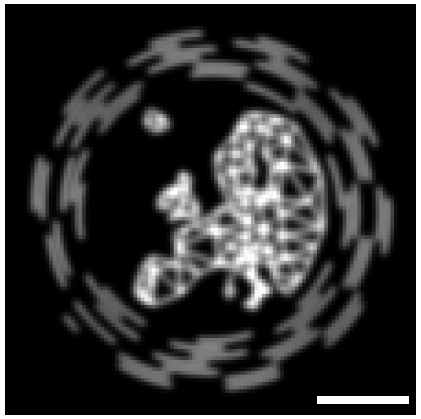
Siân Culley a.k.a. @SuperResoluSian

Overview

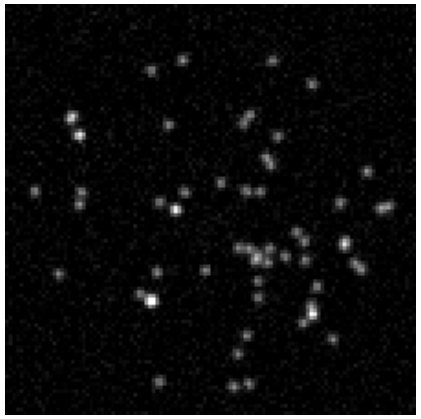
- Basic principles of SMLM analysis
- Analysis for sparse datasets
- Analysis for dense datasets
- Quality control
- 3D localisation

Basic principles of SMLM

Diffraction-limited image



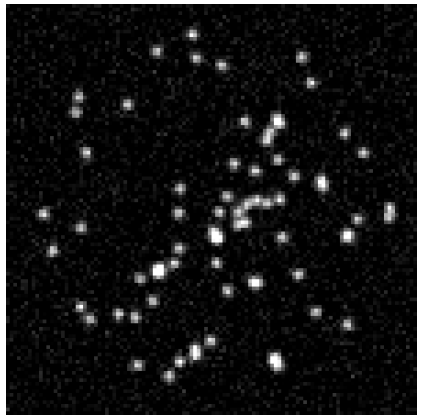
Induce fluorophore 'blinking'



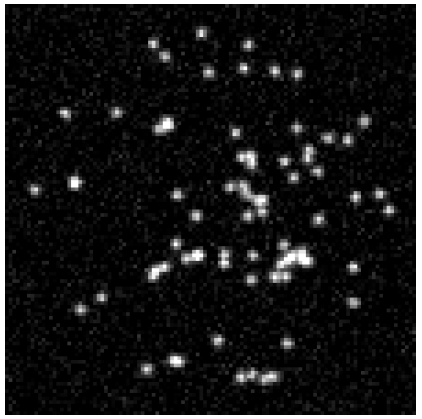
=

Individual frames containing small subset of emitting molecules

Frame 1

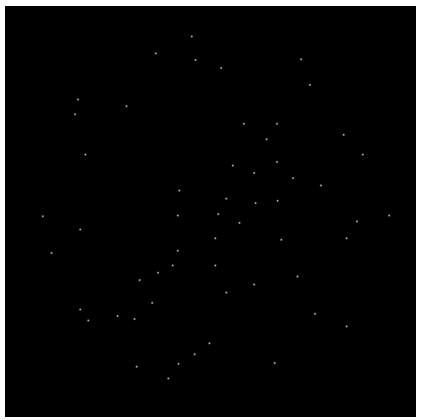


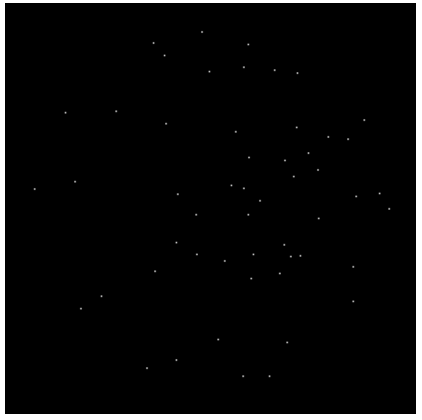
Frame 2



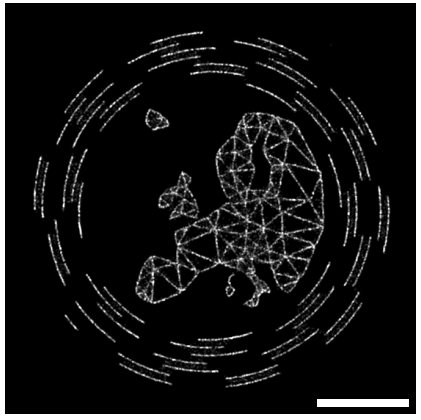
.... Frame n

Detection and localisation



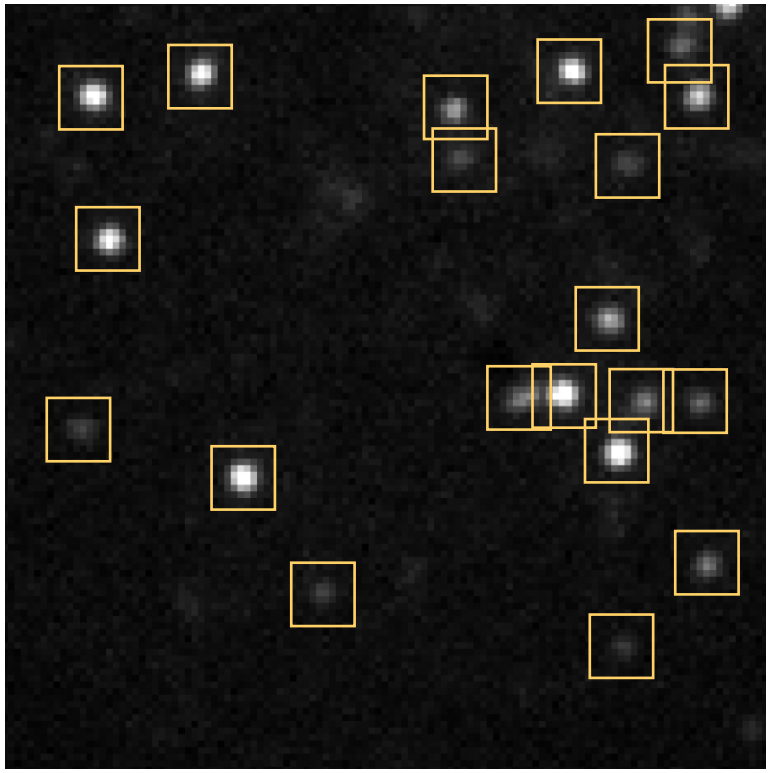


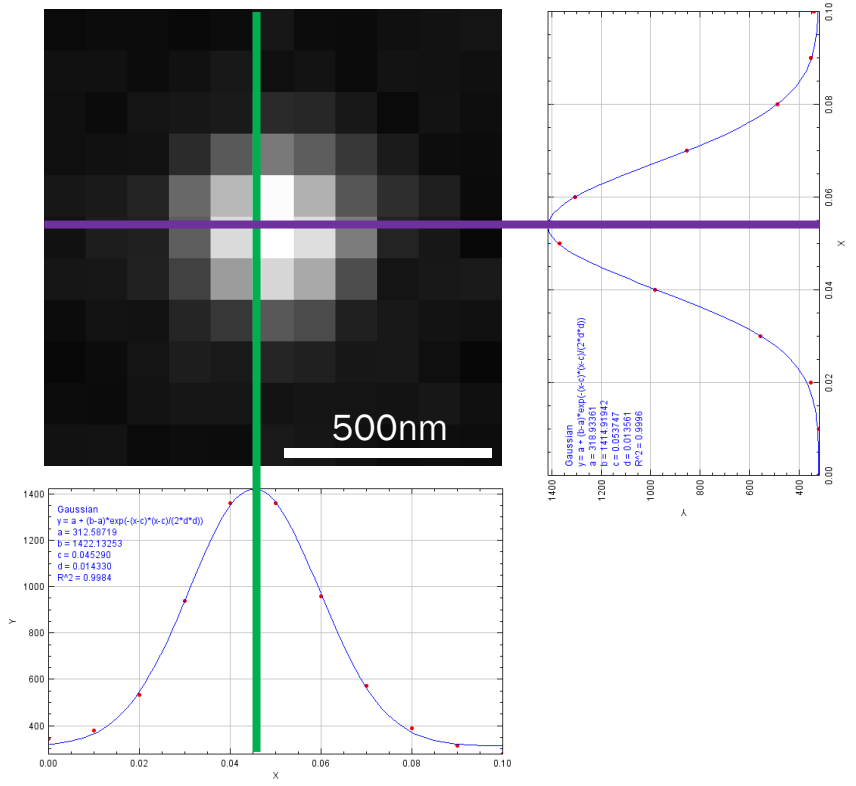
Render super-resolution image



Detection and localization

- Approximate molecule locations = detection
- Accurate determination of molecule centre = localization





(x, y) coordinates of molecule within image

e.g.
(45.29nm, 53.75nm)

ThunderSTORM

- Fiji plugin, all GUI-based
- Can handle 2D and 3D data
- Has options for high density analysis
- Easy to export particles table
- Generates protocol .txt files

BIOINFORMATICS APPLICATIONS NOTE Vol. 30 no. 16 2014, pages 2389–2390
doi:10.1093/bioinformatics/btu202

Bioimage informatics

Advance Access publication April 25, 2014

ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging

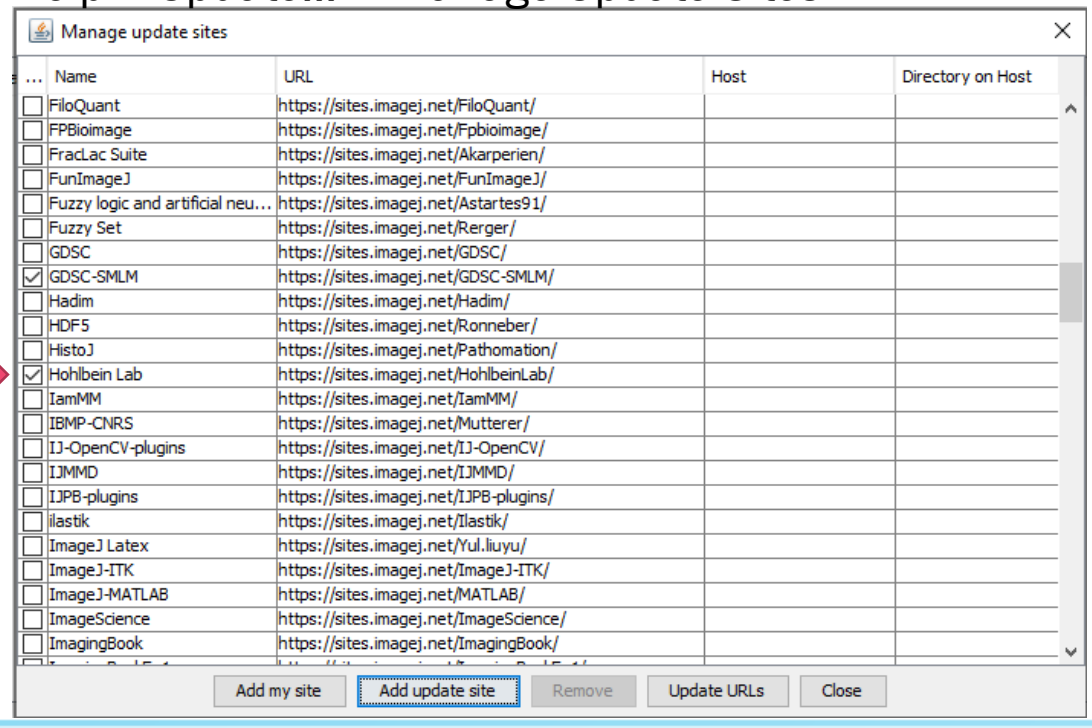
Martin Ovesný, Pavel Křížek, Josef Borkovec, Zdeněk Švindrych and Guy M. Hagen*

Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague, Prague 12800, Czech Republic

Associate Editor: Jonathan Wren

Supplementary data – bonus maths, user manual
https://github.com/zitmen/thunderstorm/wiki

Fiji install:
Help > Update... > Manage Update Sites



Hohlbein Lab update site

nature methods ANALYSIS
https://doi.org/10.1038/s41592-019-0364-4
Corrected: Publisher Correction

Super-resolution fight club: assessment of 2D and 3D single-molecule localization microscopy software

Daniel Sage[1,22*], Thanh-An Pham[1,22], Hazen Babcock[2], Tomas Lukes[3,4], Thomas Pengo[5], Jerry Chao[6,7], Ramraj Velmurugan[7,8], Alex Herbert[9], Anurag Agrawal[10], Silvia Colabrese[1,11], Ann Wheeler[12], Anna Archetti[13], Bernd Rieger[14], Raimund Ober[6,7,15], Guy M. Hagen[16], Jean-Baptiste Sibarita[17,18], Jonas Ries[19], Ricardo Henriques[20], Michael Unser[1] and Seamus Holden[21,22*]

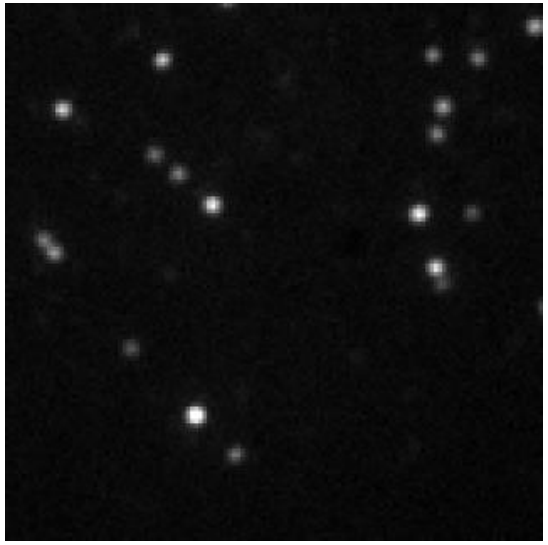
Sage et al, Nature Methods (2019)

Data ‘density’

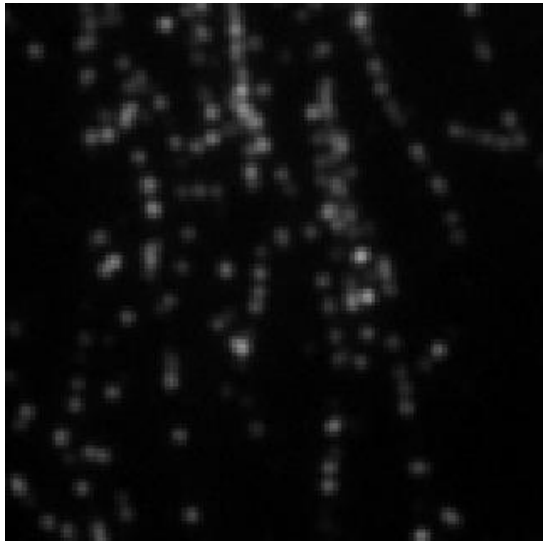
Real experimental datasets from http://bigwww.epfl.ch/smlm/datasets/index.html

Low density (sparse)

- Emitters are all very well separated
- Few emitters per μm^2
- Typically acquired over long period of time (i.e. large number of frames)
- Drift can be severe



High density



- Emitters overlap
- Several emitters per μm^2
- Often fewer frames
- From live cells? Watch out for movement

‘emitter’ = fluorophore in ‘on’ state

Particles tables

ThunderSTORM: results

id	frame	x [nm]	y [nm]	sigma ...	intensity [pho...	offset [photon]	bkgstd [photon]	chi2	uncertainty_...
1	1	417.468	2640.243	223.509	10258.275	44.722	46.445	3323.058	16.741
2	1	481.68	7056.711	159.844	5231.573	80.517	79.321	9647.297	28.435
3	1	506.473	1580.226	153.191	7259.846	80.155	40.213	3105.818	10.029
4	1	669.437	6352.407	128.805	1669.543	78.791	19.009	1276.271	15.073
5	1	624.57	7281.365	168.006	5891.138	59.687	70.991	7192.431	24.982
6	1	792.393	547.101	152.966	4676.928	125.514	67.415	7679.431	24.901
7	1	926.555	5699.312	162.931	3687.128	71.528	28.936	2198.012	15.912
8	1	1033.421	2671.934	289.785	8459.08	35.94	24.049	1469.915	18.011
9	1	1094.673	2843.861	145.309	1351.337	119.062	44.481	3936.051	51.264
10	1	1372.559	3299.007	170.209	7062.607	70.419	15.525	523.907	5.881
11	1	1348.05	11233.328	155.389	2743.277	58.665	27.535	1996.595	18.464
12	1	1600.821	1641.058	150.626	2556.421	67.228	17.356	820.734	12.487
13	1	1656.894	1201.632	172.935	5218.995	68.678	13.517	483.853	7.076
14	1	1622.658	8669.813	133.462	2179.423	77.529	35.173	2802.113	21.755
15	1	1742.882	2685.776	139.385	2338.193	82.256	10.663	337.574	8.315
16	1	1723.45	5215.905	143.719	1401.231	63.303	7.832	277.202	10.909
17	1	1945.564	6888.527	178.277	3650.194	61.406	37.683	3457.906	24.342
18	1	1846.094	12430.179	147.388	2036.822	61.811	22.148	1310.547	18.259
19	1	1923.11	9138.857	133.476	5498.35	71.501	19.218	924.297	5.621
20	1	2189.383	7182.671	172.179	4087.851	53.205	37.227	3116.562	20.171
21	1	2343.263	3617.819	119.901	709.139	68.663	5.785	154.815	11.86
22	1	2443.59	313.595	143.091	4378.591	95.276	38.468	3472.473	13.741
23	1	2531.572	2171.223	198.974	3405.234	60.527	29.085	2358.366	25.157
24	1	2633.573	2235.262	142.937	1772.464	86.638	36.36	3707.549	31.268
25	1	2627.909	4278.172	136.752	3577.781	70.637	17.783	982.555	7.992
26	1	2813.703	10091.223	141.82	4869.598	58.596	20.503	1092.351	7.221
27	1	2745.725	12256.168	122.87	1646.154	68.07	10.394	475.463	8.928
28	1	2788.333	4687.385	175.656	2821.404	49.672	31.47	2321.055	25.637
29	1	2894.192	11634.63	137.295	5426.208	63.545	24.844	1432.882	7.189
30	1	2920.342	2761.895	126.548	4446.844	74.274	15.003	727.497	5.248
31	1	2962.616	691.525	153.818	2965.512	55.643	19.615	976.249	12.52
32	1	3009.069	8109.174	117.923	1277.683	82.249	21.674	1522.845	18.522
33	1	3028.664	8574.056	123.54	2865.356	80.048	17.519	978.756	8.083
34	1	3175.319	7603.575	157.209	2051.041	65.701	22.3	1772.093	20.562

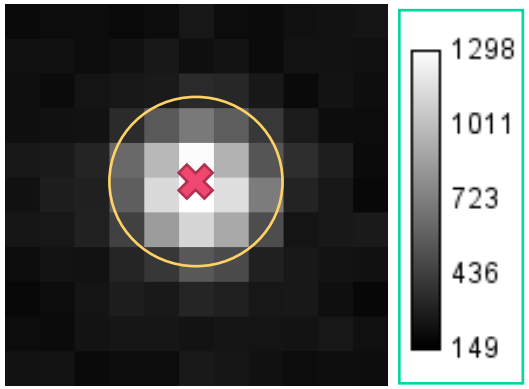
Filter | Density filter | Remove duplicates | Merging | Drift correction | Z-stage offset

Filter: Apply | Restrict to ROI

Post-processing history: - Reset

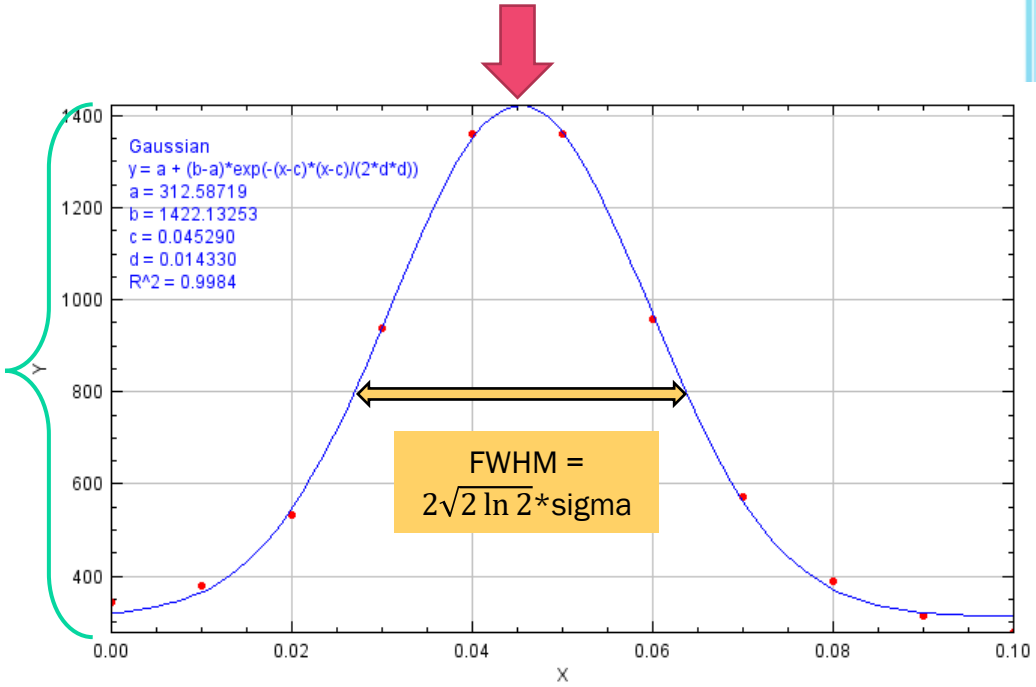
Preview | Defaults | Plot histogram | Visualization | Import | Export

central coordinate



width of Gaussian

amplitude and offset of Gaussian



χ^2 goodness-of-fit

uncertainty

$$\langle(\Delta x)^2\rangle = \frac{2\sigma^2 + a^2/12}{N} + \frac{8\pi\sigma^4 b^2}{a^2 N^2}$$

Analysis woes for high-density data…

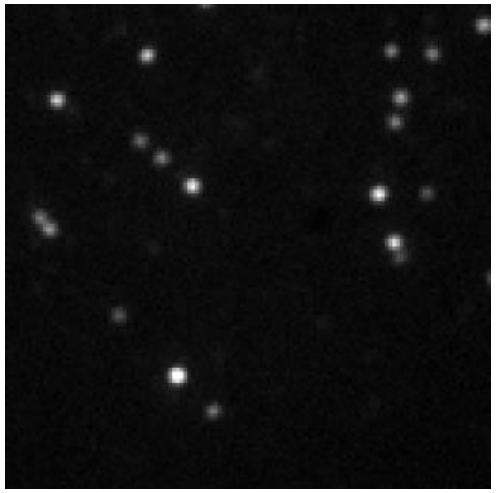
Raw data

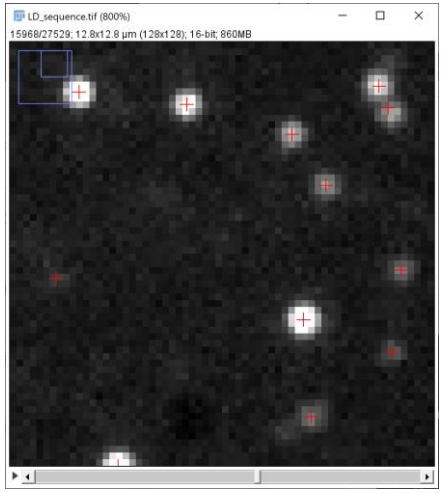
Detections

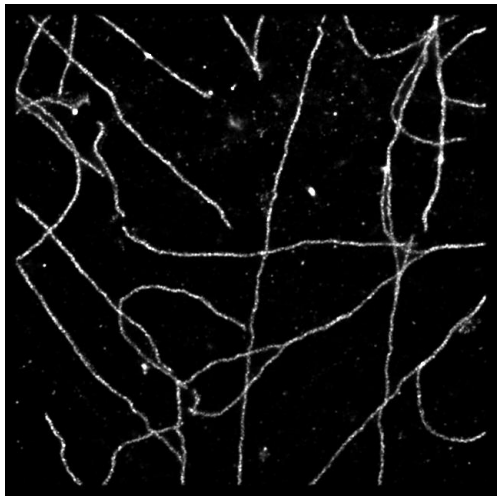
Rendered image

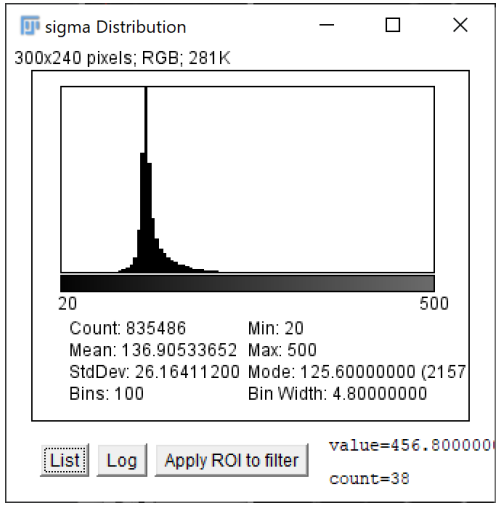
Widths of fitted Gaussians
(‘Plot Histogram’ button)

Low density

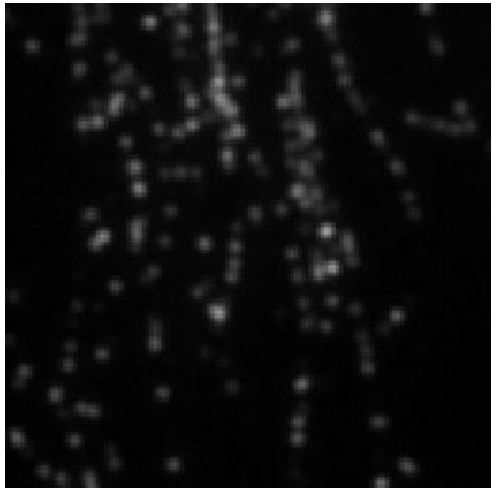


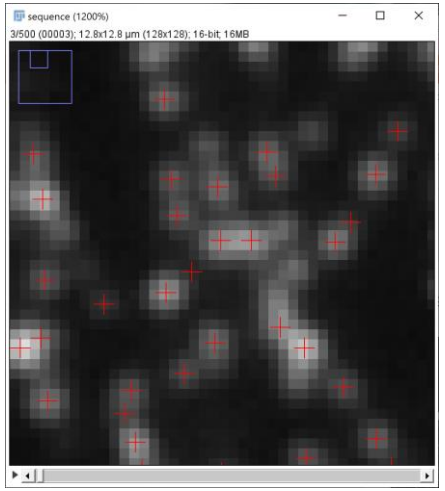


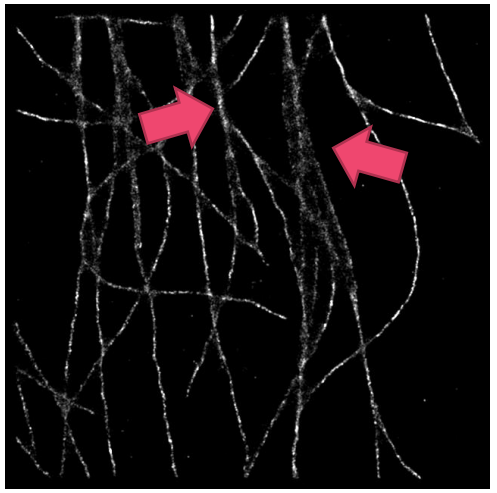


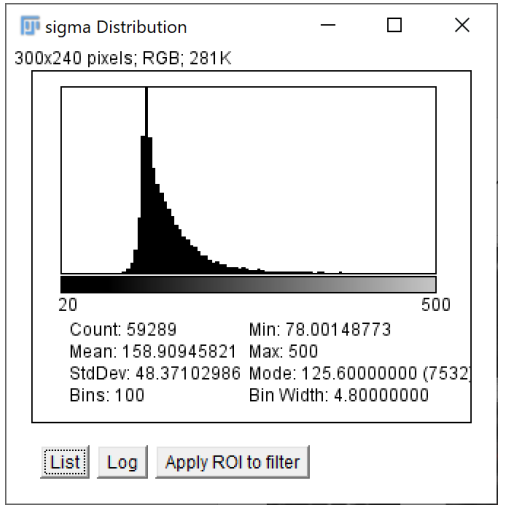


High density



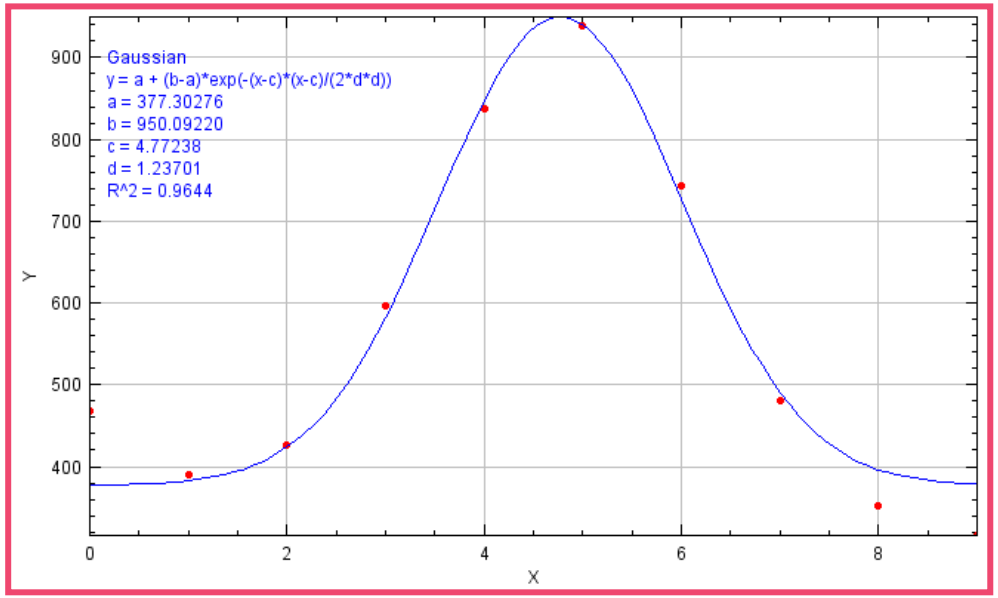


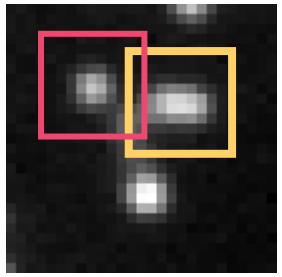


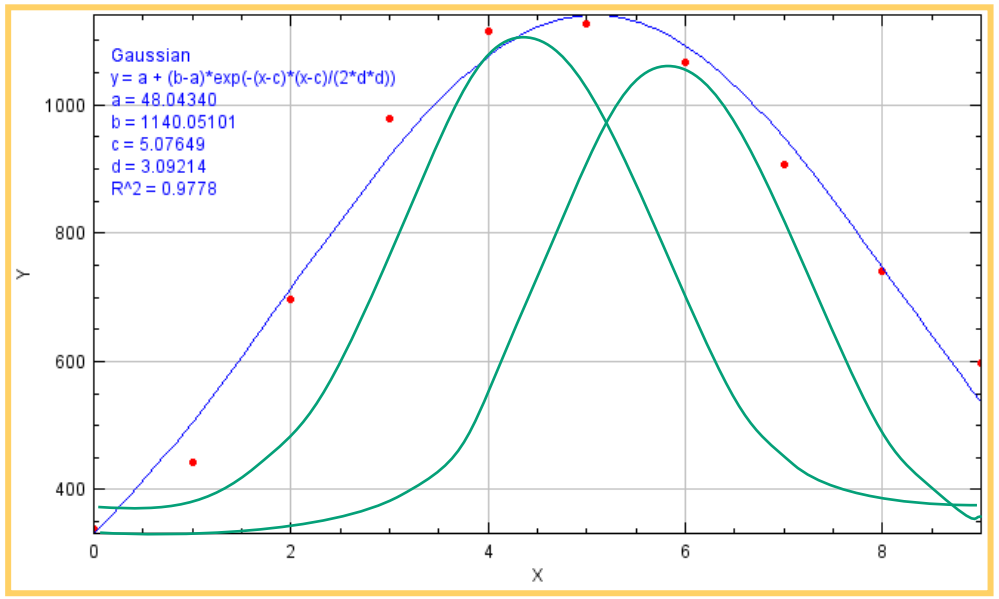


High density data in ThunderSTORM

- Just filtering out ‘bad’ detections?
- ThunderSTORM: multi-emitter fitting

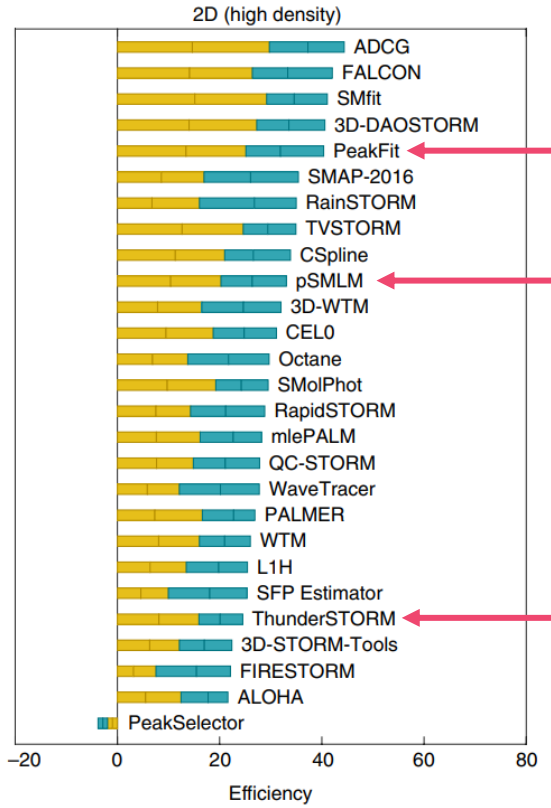






ThunderSTORM alternatives…

- HD algorithms…



Fiji

Localization method available in ThunderSTORM (Hohlbein Lab update)

Sage et al
Nature Methods 2019

Pre-processing high density data

- HAWK wavelet filtering
- Makes datasets more ‘sparse’

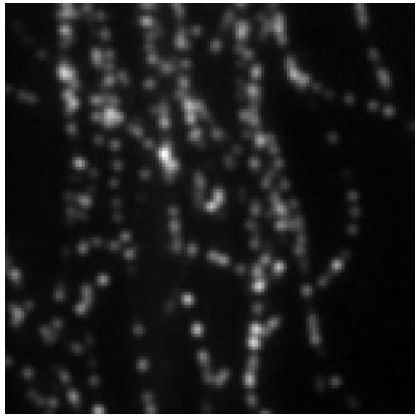
nature methods
BRIEF COMMUNICATION
https://doi.org/10.1038/s41592-018-0072-5

Artifact-free high-density localization microscopy analysis

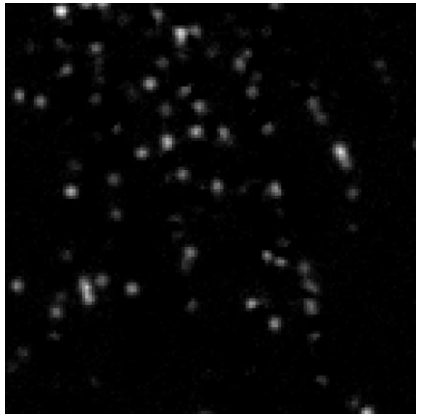
Richard J. Marsh, Karin Pfisterer, Pauline Bennett, Liisa M. Hirvonen, Mathias Gautel, Gareth E. Jones and Susan Cox*

Download from: www.coxphysics.com

Example raw high-density frame



Example high-density frame after HAWK



Non-particles table approaches

- SOFI, SRRF…

RESEARCH ARTICLE

Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI)

T. Dertinger, R. Colyer, G. Iyer, S. Weiss, and J. Enderlein

PNAS December 29, 2009 106 (52) 22287-22292; https://doi.org/10.1073/pnas.0907866106

Edited by John W. Sedat, University of California, San Francisco, CA, and approved October 29, 2009 (received for review July 15, 2009)

Open Access | Published: 12 August 2016

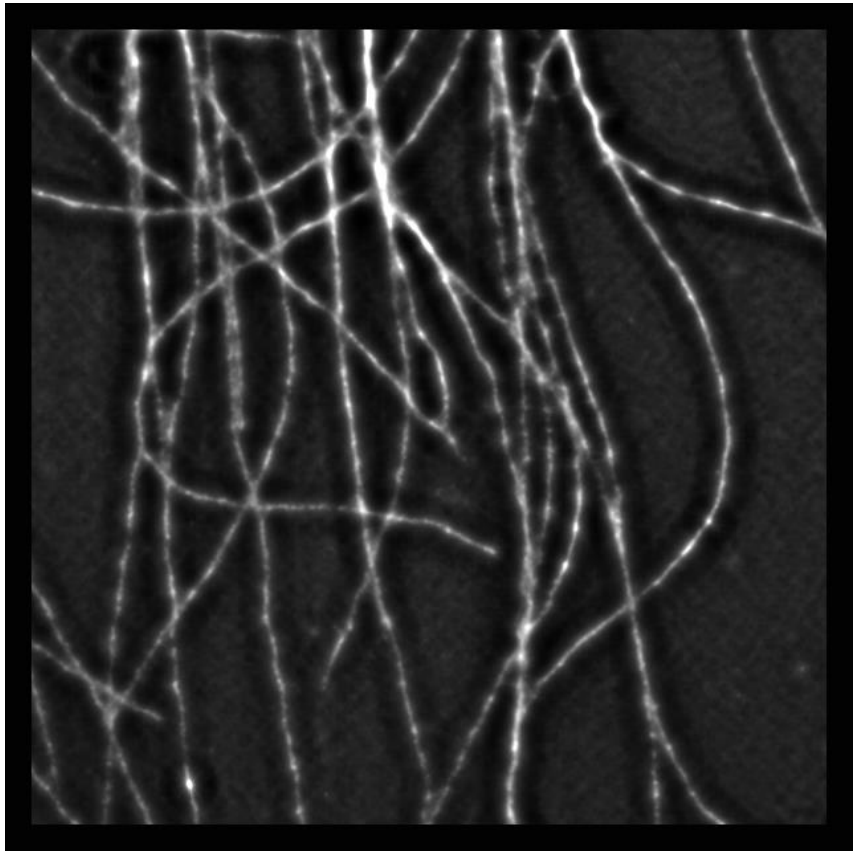
Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations

Nils Gustafsson, Siân Culley, George Ashdown, Dylan M. Owen, Pedro Matos Pereira & Ricardo Henriques

Nature Communications **7**, Article number: 12471 (2016) | Cite this article

10k Accesses | **141** Citations | **95** Altmetric | Metrics

SRRF analysis of high density data

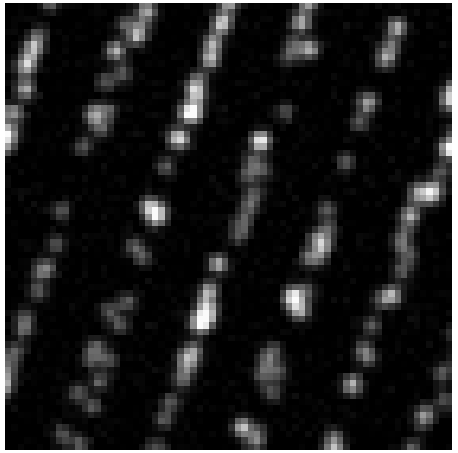


- SRRF is available as a Fiji plugin
- Used for very high density data
- Image ≈ probability map, not intensity or molecule coordinates

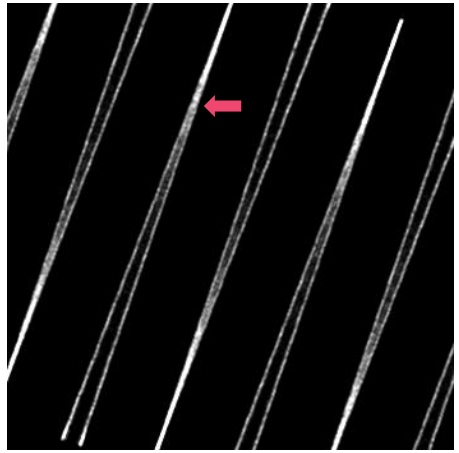
Artefacts in SMLM reconstructions

- Merging of closely-separated structures
- Incomplete representation of structures
- Intensity non-linearities

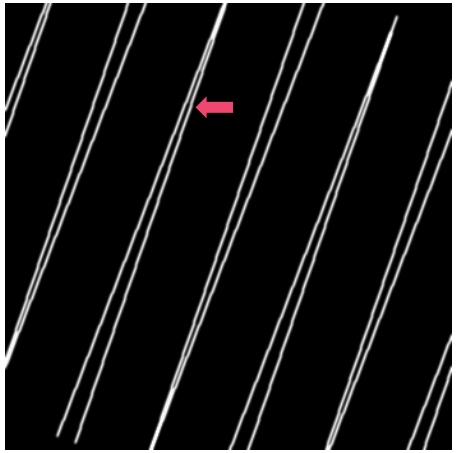
Simulated raw data



SMLM reconstruction

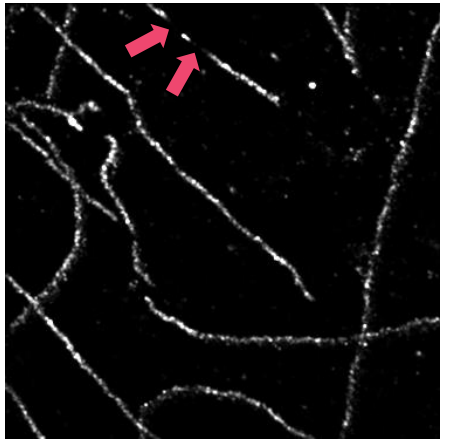


Real structure



2µm

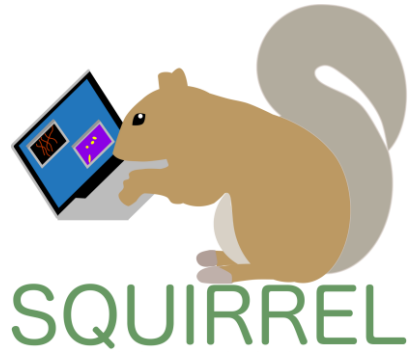
Low density data – filtering around fiducial marker



2µm

Using SQUIRREL to assess image quality

- Can compare different algorithm results
- Can map resolution across image
- Can test how many frames you need to represent an image



Published: 19 February 2018

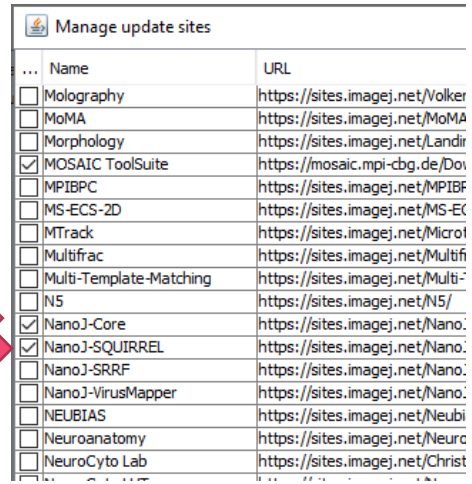
Quantitative mapping and minimization of super-resolution optical imaging artifacts

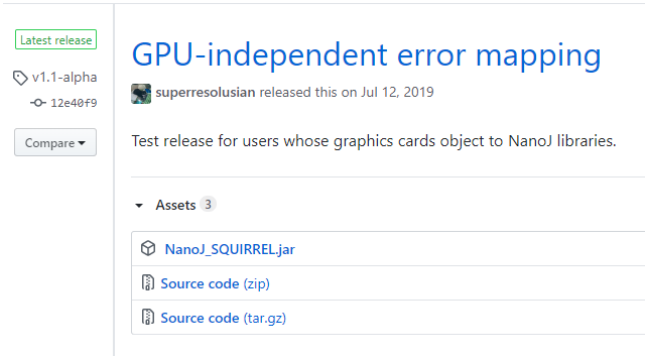
Siân Culley, David Albrecht, Caron Jacobs, Pedro Matos Pereira, Christophe Leterrier, Jason Mercer & Ricardo Henriques

Nature Methods **15**, 263–266(2018) | Cite this article

3013 Accesses | **64** Citations | **160** Altmetric | Metrics

NanoJ-Core,
NanoJ-SQUIRREL
update sites

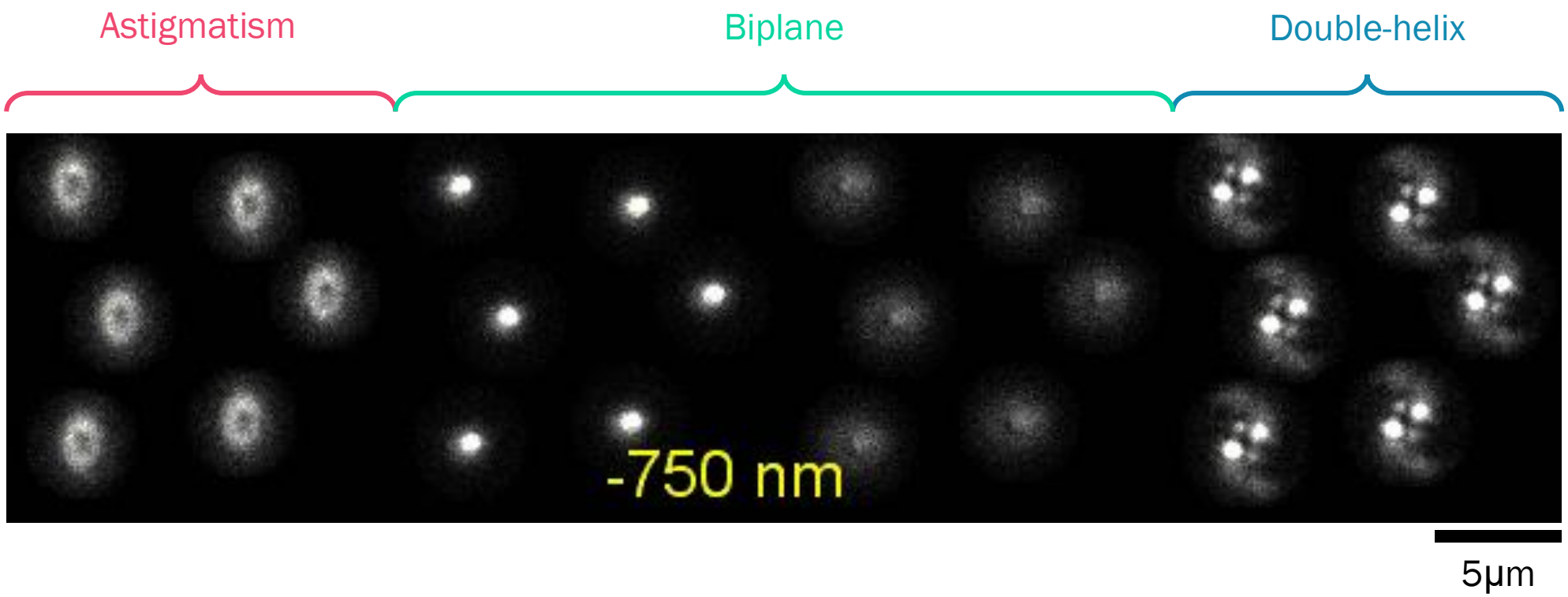




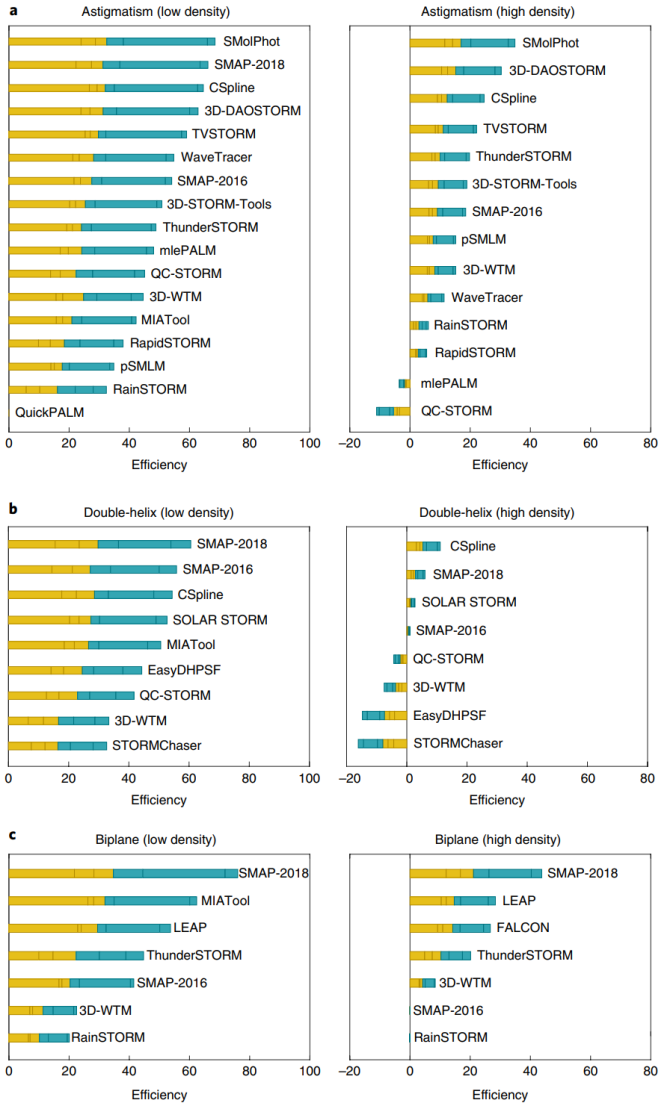
github.com/superresolusian/NanoJ-SQUIRREL/releases

3D data

- You can’t get axial localizations unless you used specific optics to encode axial information
- You need to have calibration data for your microscope



Algorithms with 3D data



- ThunderSTORM is good for astigmatism
- SMAP can do all 3D modalities (very high performance for biplane)

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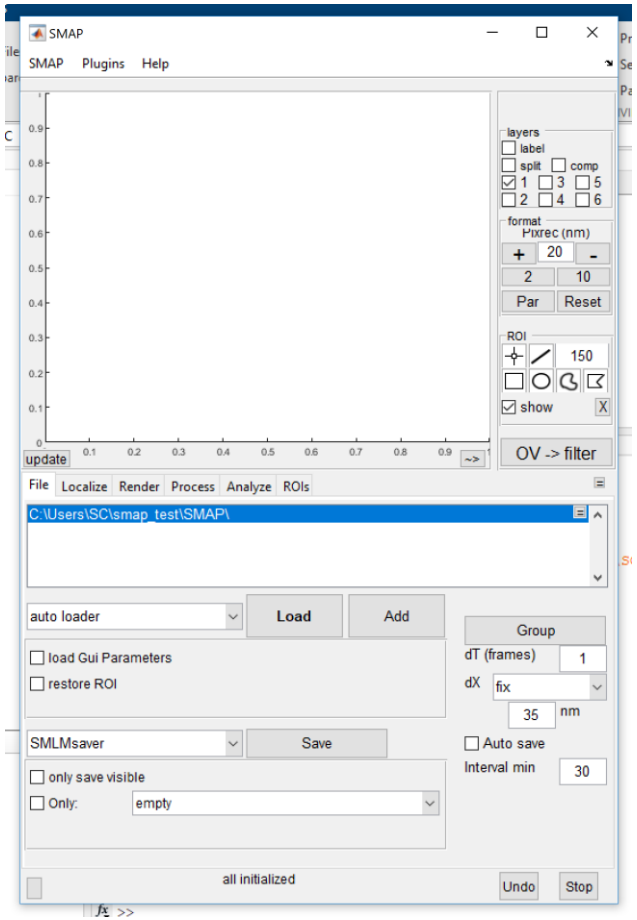
Real-time 3D single-molecule localization using experimental point spread functions

Yiming Li, Markus Mund, Philipp Hoess, Joran Deschamps, Ulf Matti, Bianca Nijmeijer, Vilma Jimenez Sabinina, Jan Ellenberg, Ingmar Schoen & Jonas Ries

Nature Methods **15**, 367–369(2018) | Cite this article

3400 Accesses | **36** Citations | **55** Altmetric | Metrics



Sage et al
Nature Methods 2019

General advice for SMLM analysis

- Walk before you run
- Test analysis on small crops of data before running on whole datasets
- Get familiar with one piece of software

Report what you did!

- What algorithm did you use?
- What parameters did you use?
- How are your images rendered?

- Did you test any other algorithms/parameters – how did you decide which was best?