

Open-Source Super-Resolution Microscopy and Data-Analysis

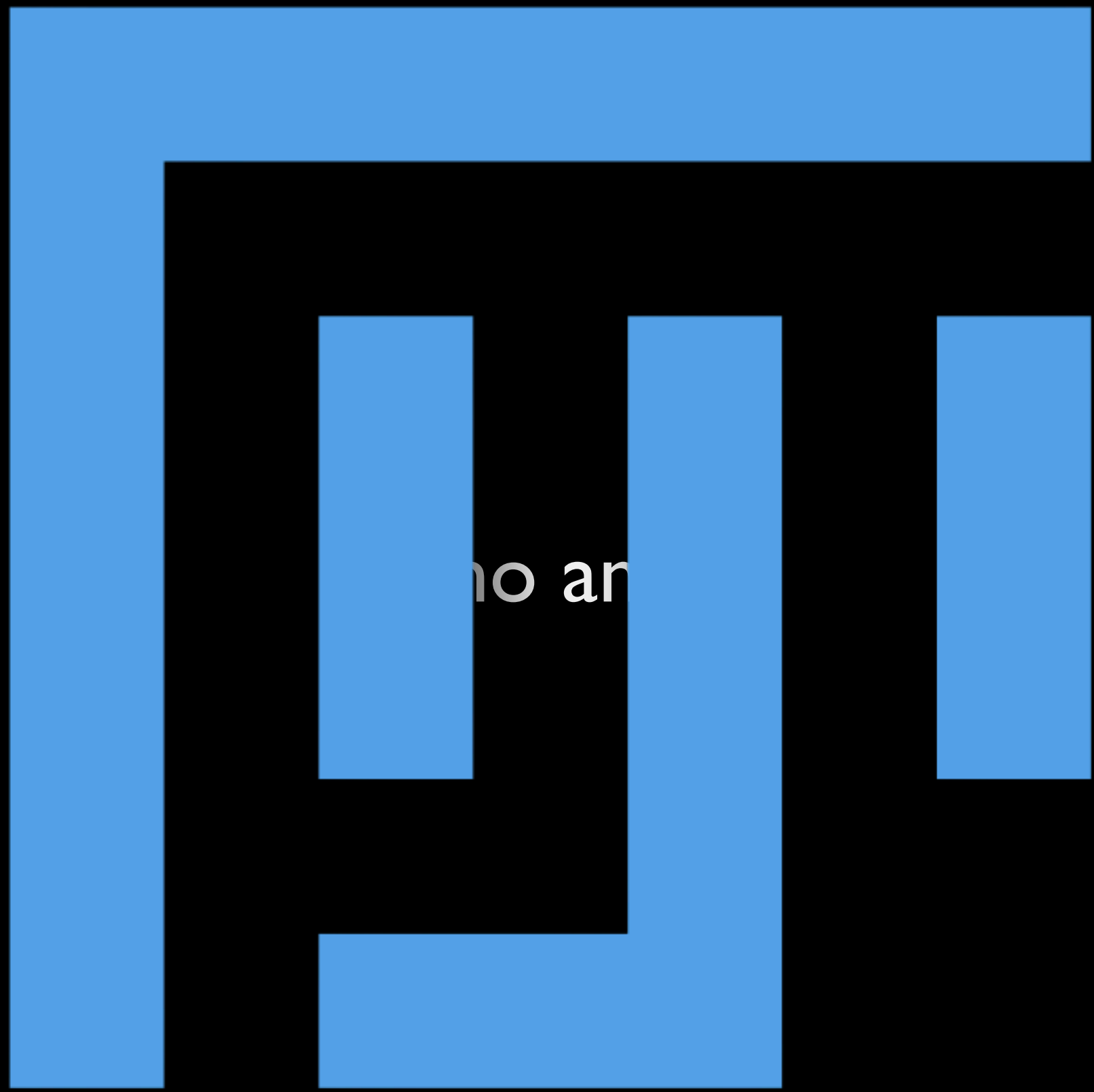
#ESRIC2019

Ricardo Henriques <r.henriques@ucl.ac.uk>
University College London
& The Francis Crick Institute



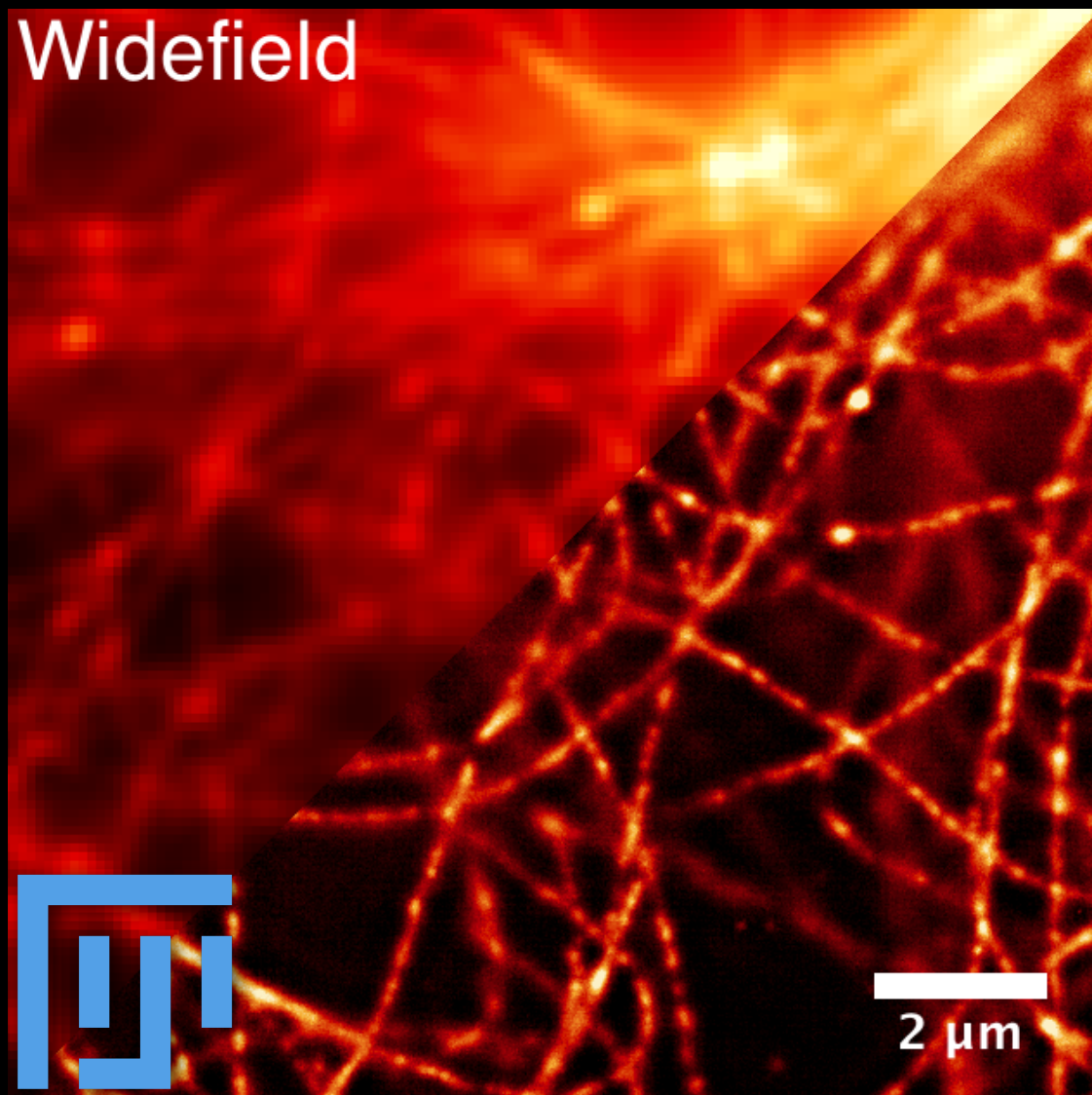
7.00 μm

feel free to tweet, everything is open, in pre-prints or peer-reviewed



no an

Super-Resolution Imaging enabled by ImageJ analytics



- > Open-Source, no hidden features
- > Community review and support
- > For biologists as end users
- > Automated updates

Structured Illumination Microscopy

[Nat Commun.](#) 2016 Mar 21;7:10980. doi: 10.1038/ncomms10980.

Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ.

[Müller M](#)¹, [Mönkemöller V](#)¹, [Hennig S](#)¹, [Hübner W](#)¹, [Huser T](#)^{1,2}.

Single-Mol. Localization (PALM/STORM)

[Bioinformatics.](#) 2014 Aug 15;30(16):2389-90. doi: 10.1093/bioinformatics/btu202. Epub 2014 Apr 25.

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

[Ovesný M](#)¹, [Křížek P](#)¹, [Borkovec J](#)¹, [Svindrych Z](#)¹, [Hagen GM](#)¹.

High-Dens. Localization Mic. (SRRF, ...)

[Nat Commun.](#) 2016 Aug 12;7:12471. doi: 10.1038/ncomms12471.

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

[Gustafsson N](#)^{1,2}, [Culley S](#)¹, [Ashdown G](#)³, [Owen DM](#)³, [Pereira PM](#)¹, [Henriques R](#)¹.

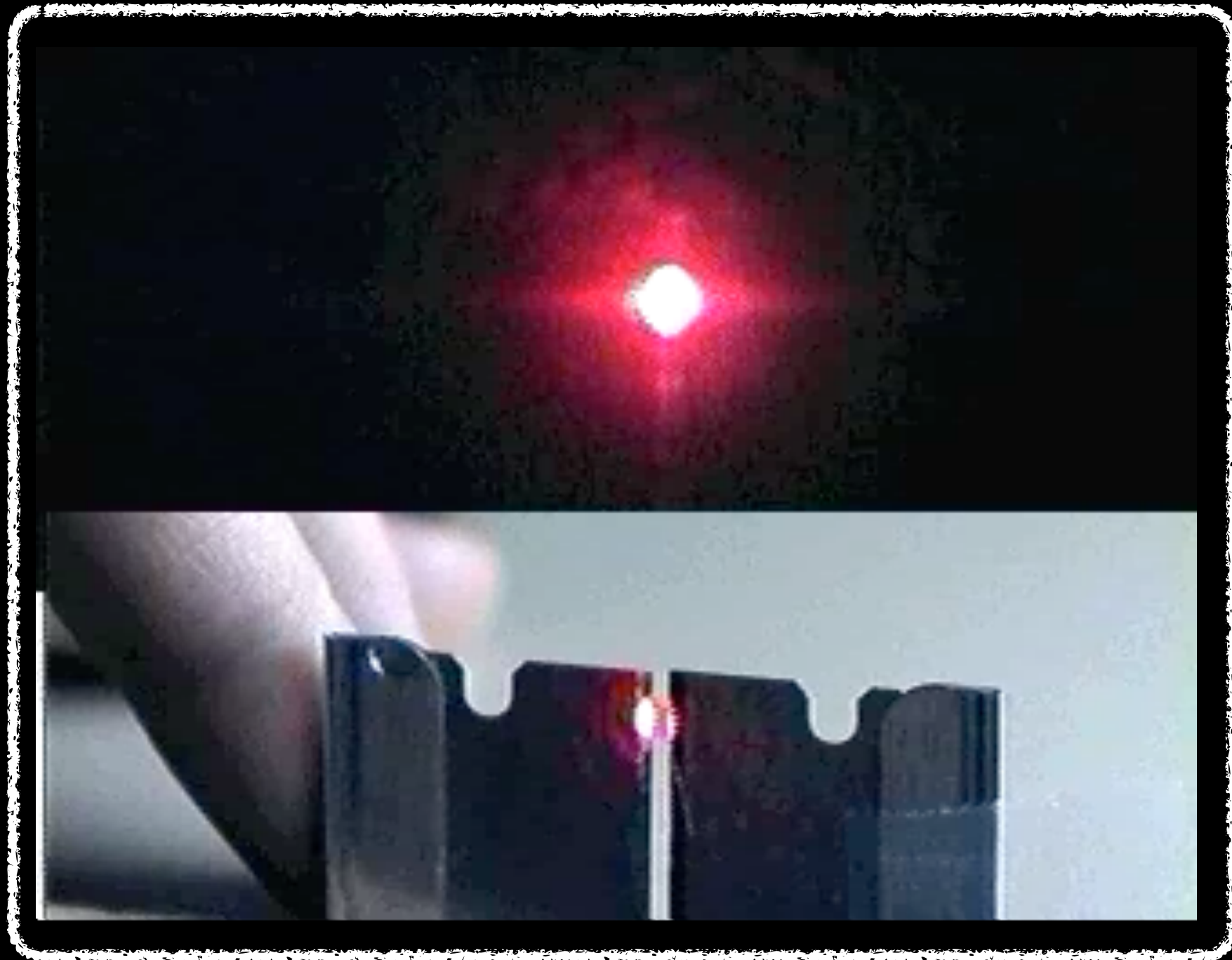
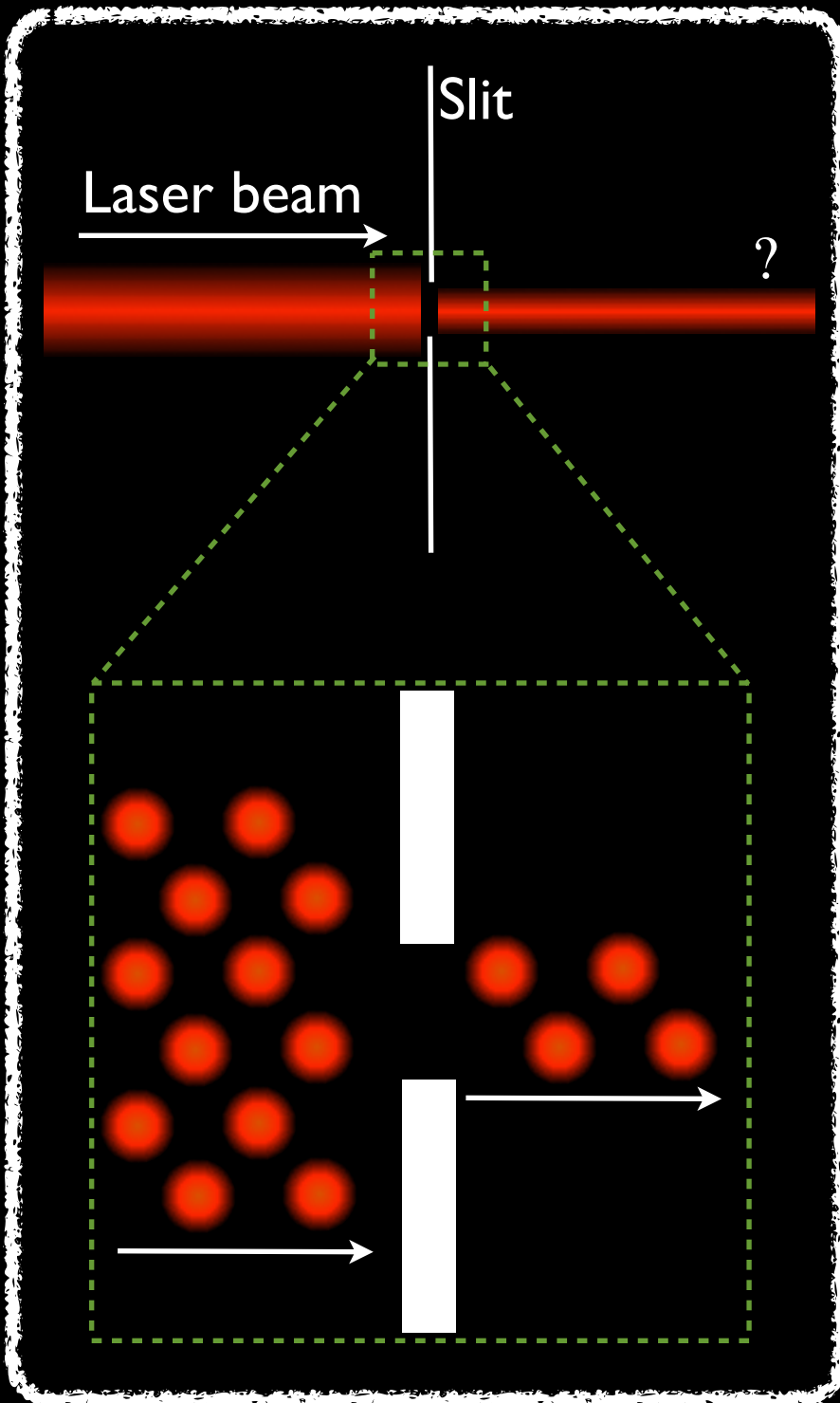
Super-Res. Prediction by Neural-Nets

Content-aware image restoration: pushing the limits of fluorescence microscopy

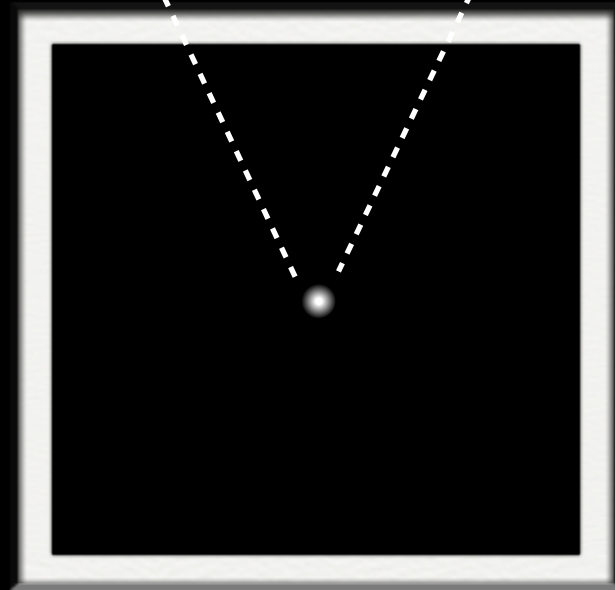
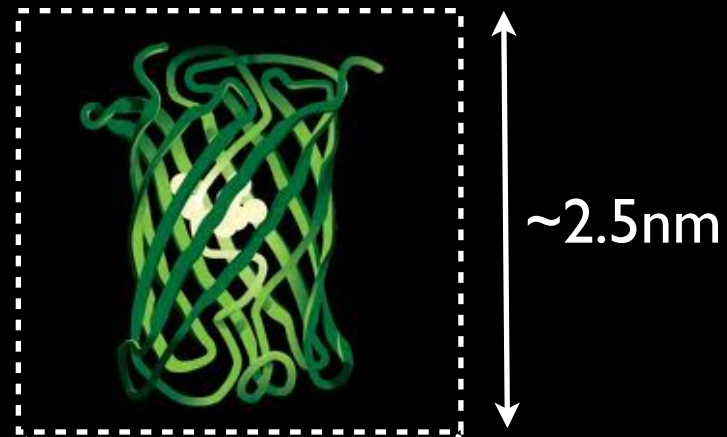
[Martin Weigert](#) ✉, [Uwe Schmidt](#), [Tobias Boothe](#), [Andreas Müller](#), [Alexandr Dibrov](#), [Akanksha Jain](#), [Benjamin Wilhelm](#), [Deborah Schmidt](#), [Coleman Broaddus](#), [Siân Culley](#), [Mauricio Rocha-Martins](#), [Fabián Segovia-Miranda](#), [Caren Norden](#), [Ricardo Henriques](#), [Marino Zerial](#), [Michele Solimena](#), [Jochen Rink](#), [Pavel Tomancak](#), [Loic Royer](#) ✉, [Florian Jug](#) ✉ & [Eugene W. Myers](#)

[Nature Methods](#) **15**, 1090–1097 (2018) | [Download Citation](#) ↓

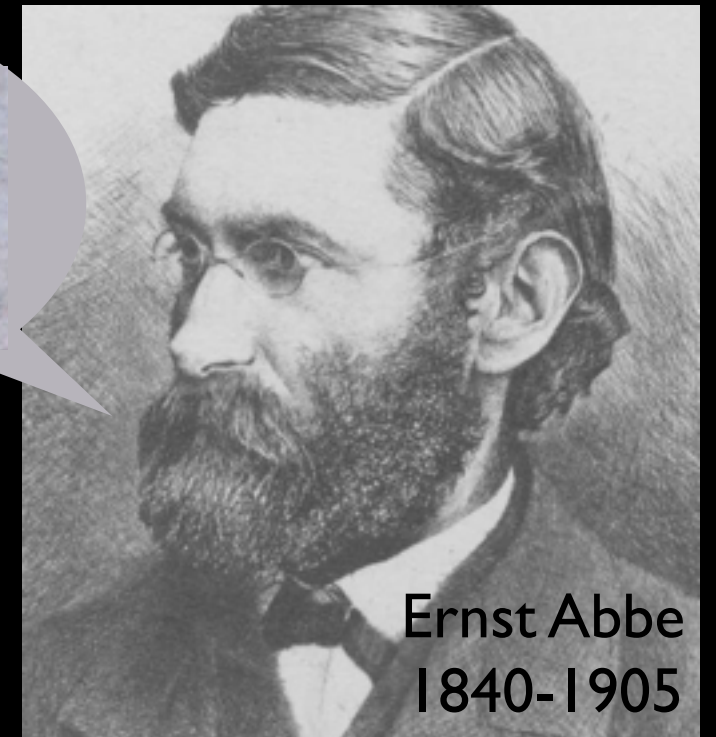
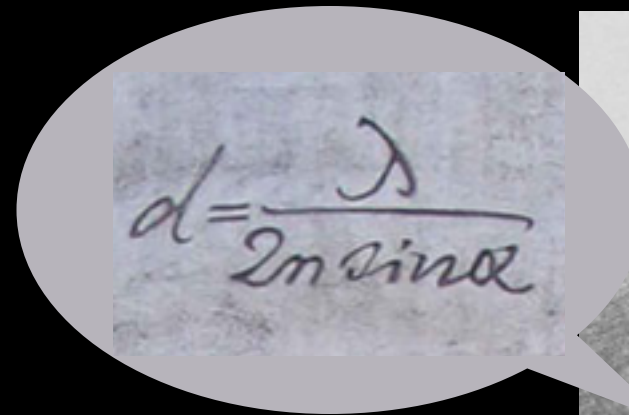
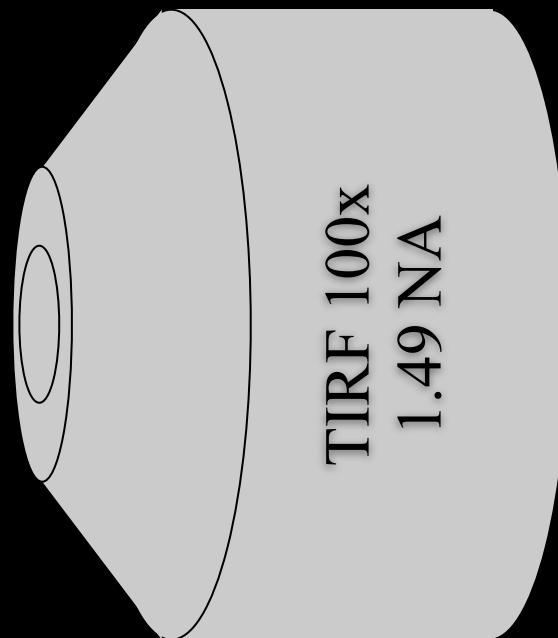
Light through apertures



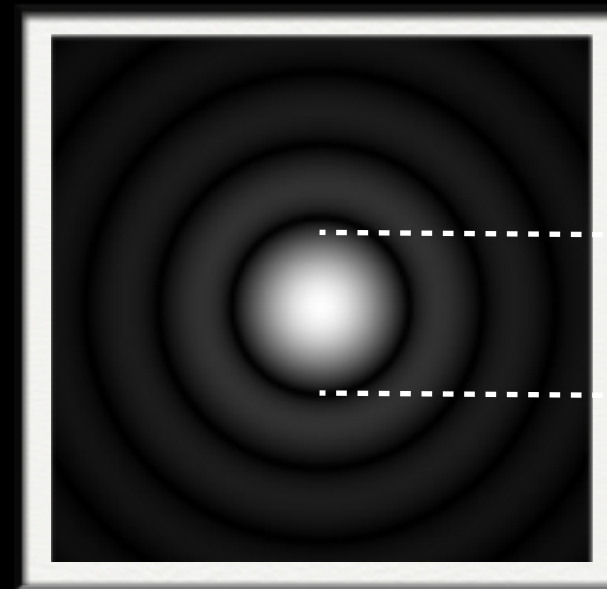
The diffraction limit



Point source of light

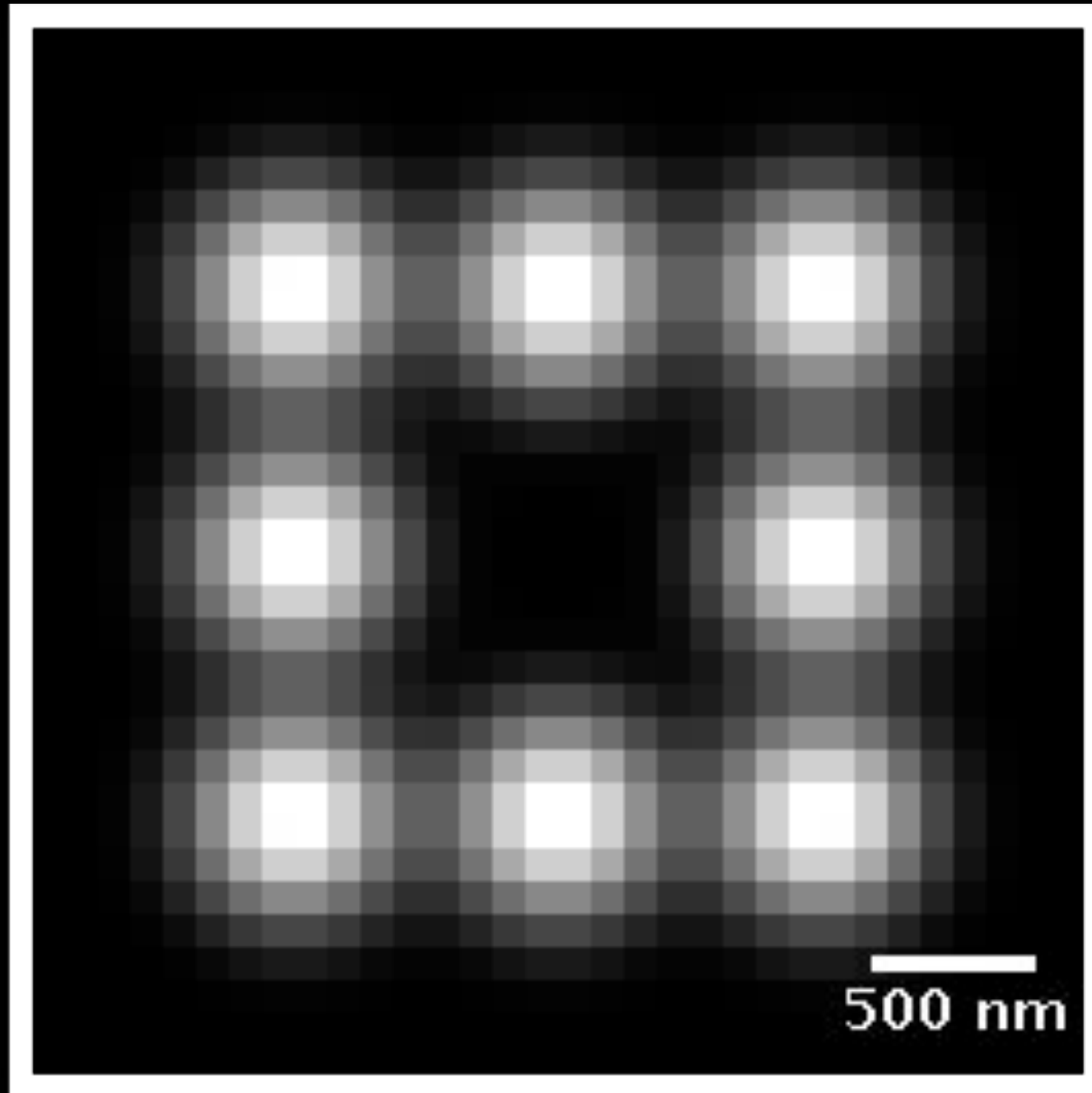


Ernst Abbe
1840-1905



Airy Disk Pattern

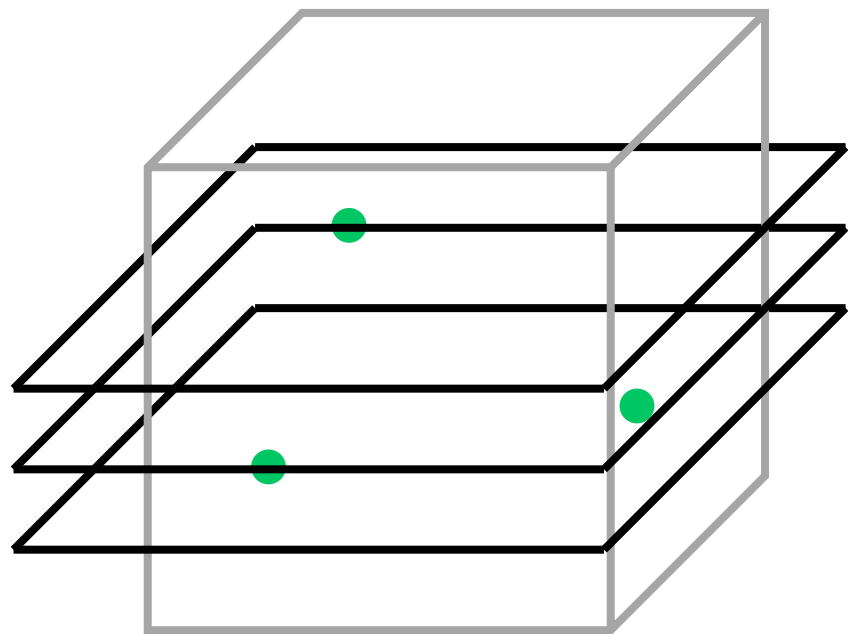
The diffraction limit



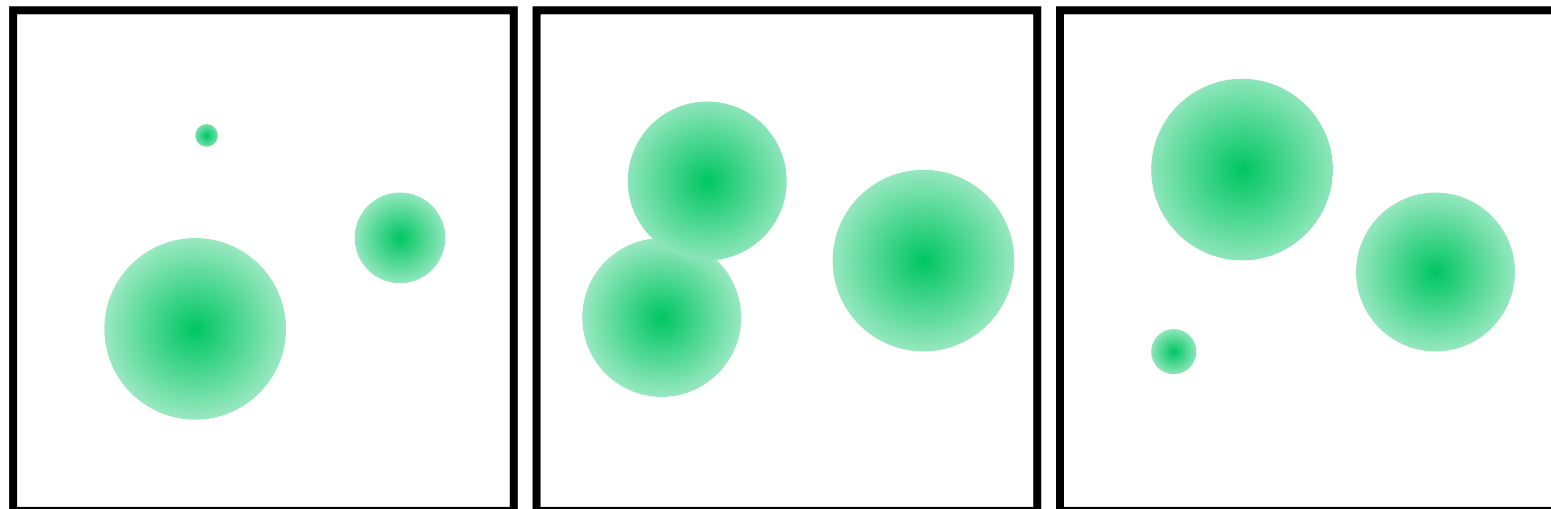
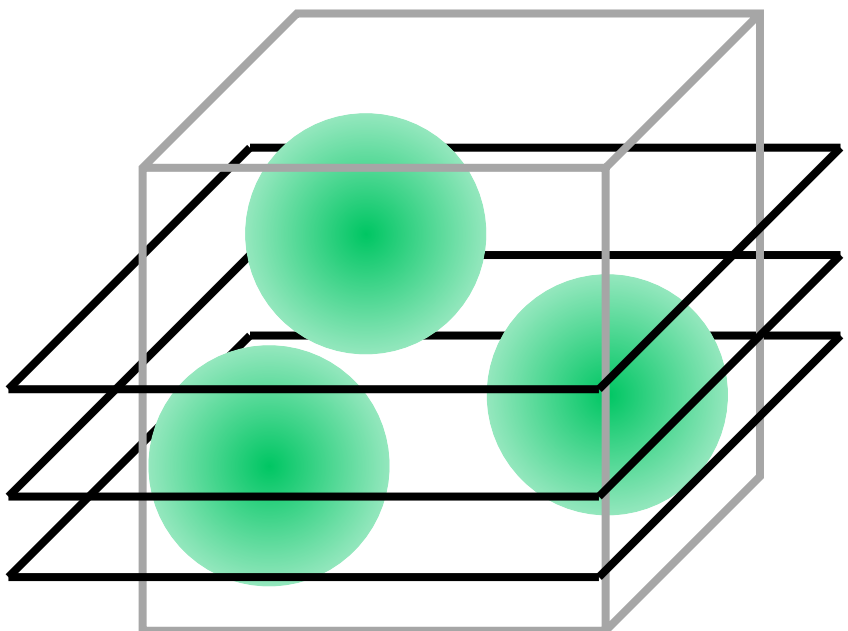
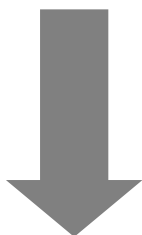
Deconvolution

works on most conventional microscopes

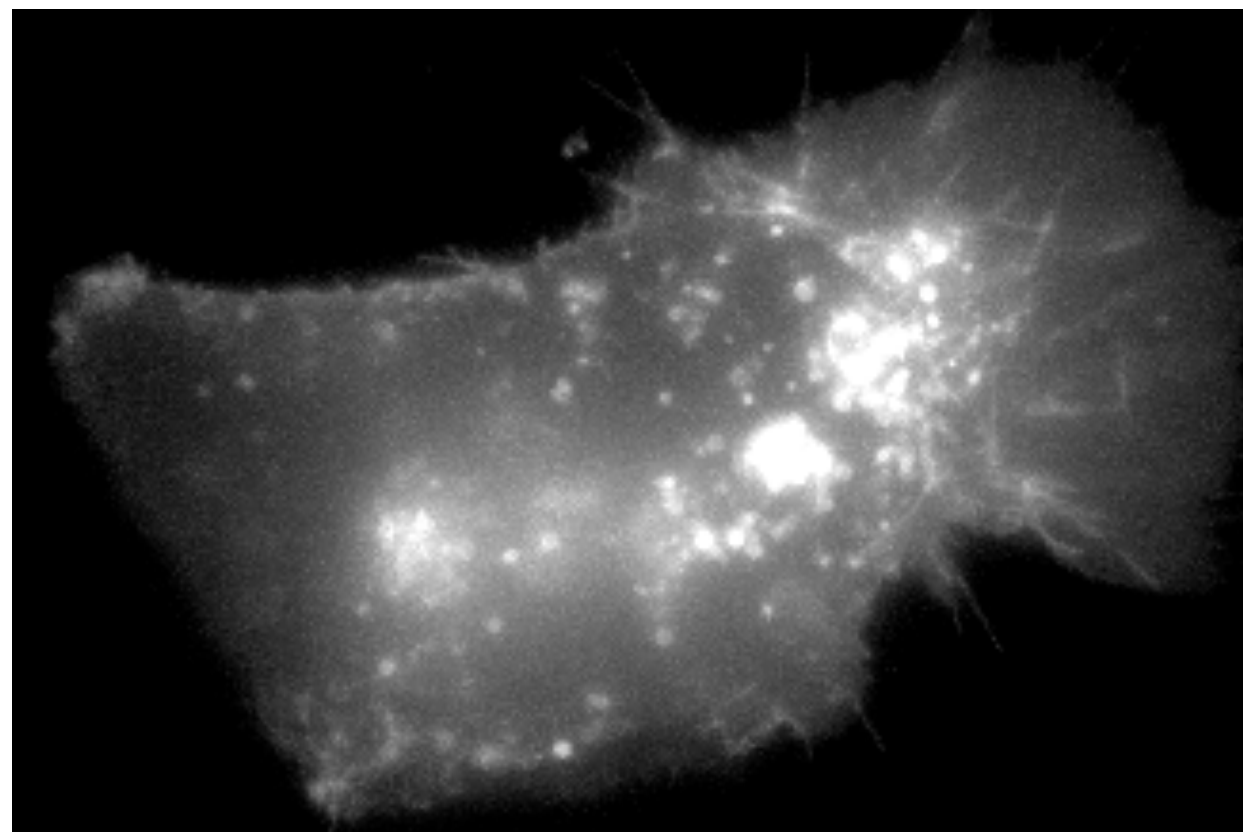
Deconvolution



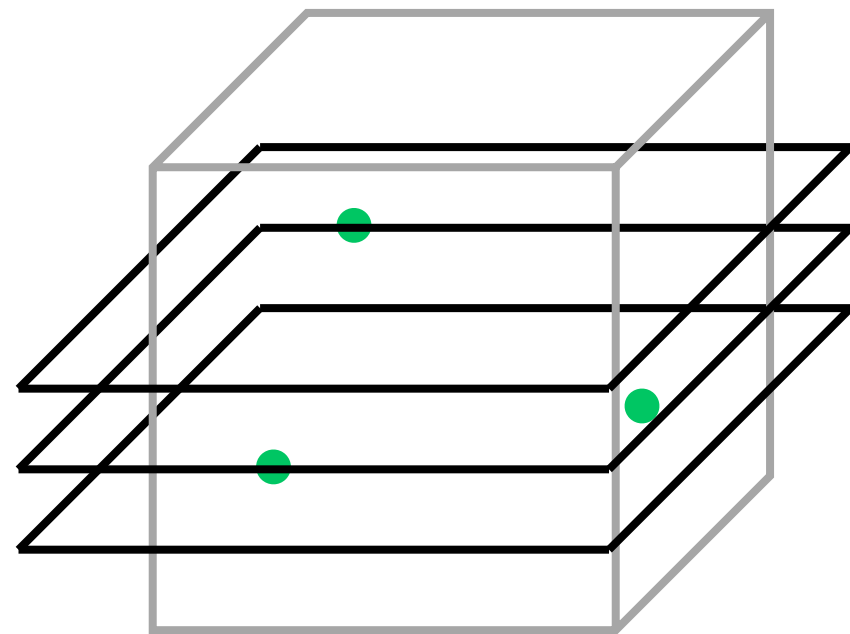
Diffraction



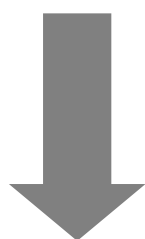
Leterrier et al. 2004



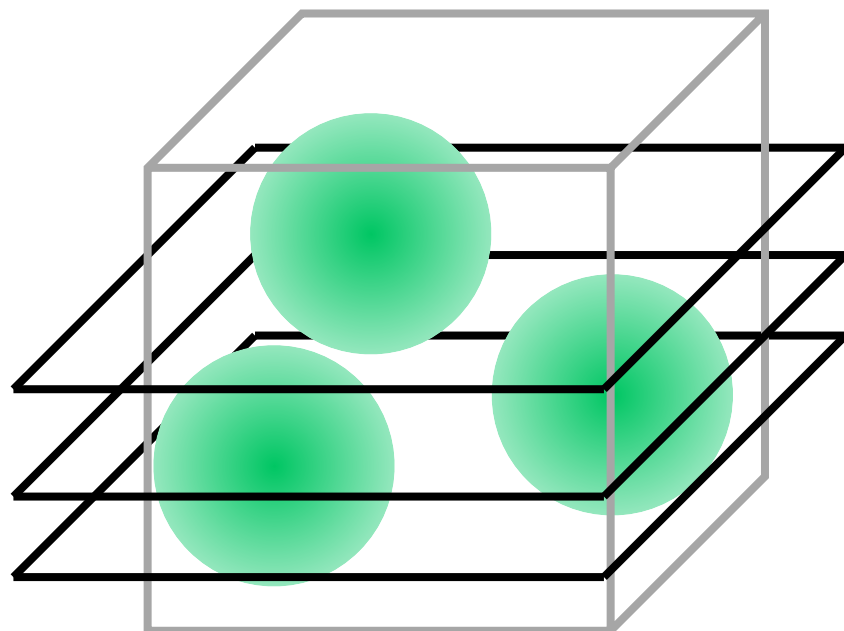
Deconvolution



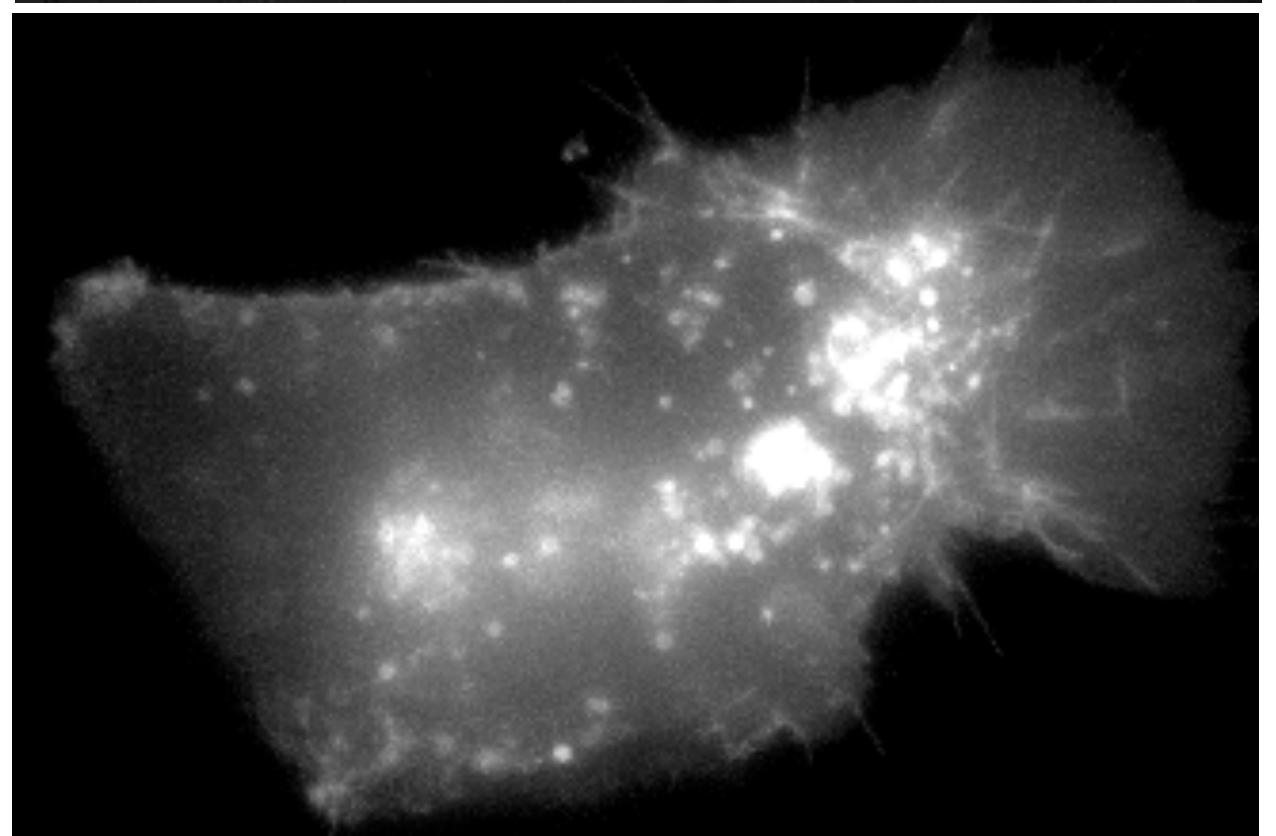
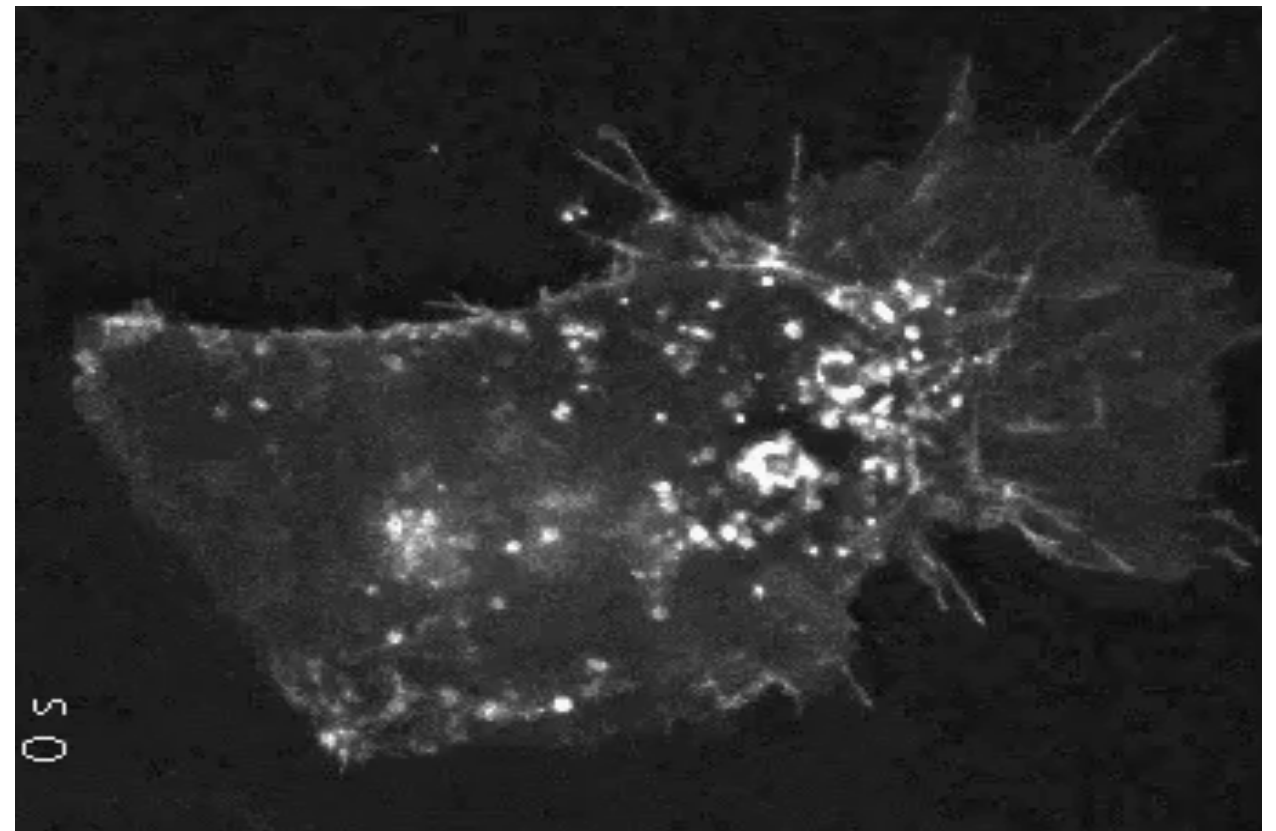
Diffraction



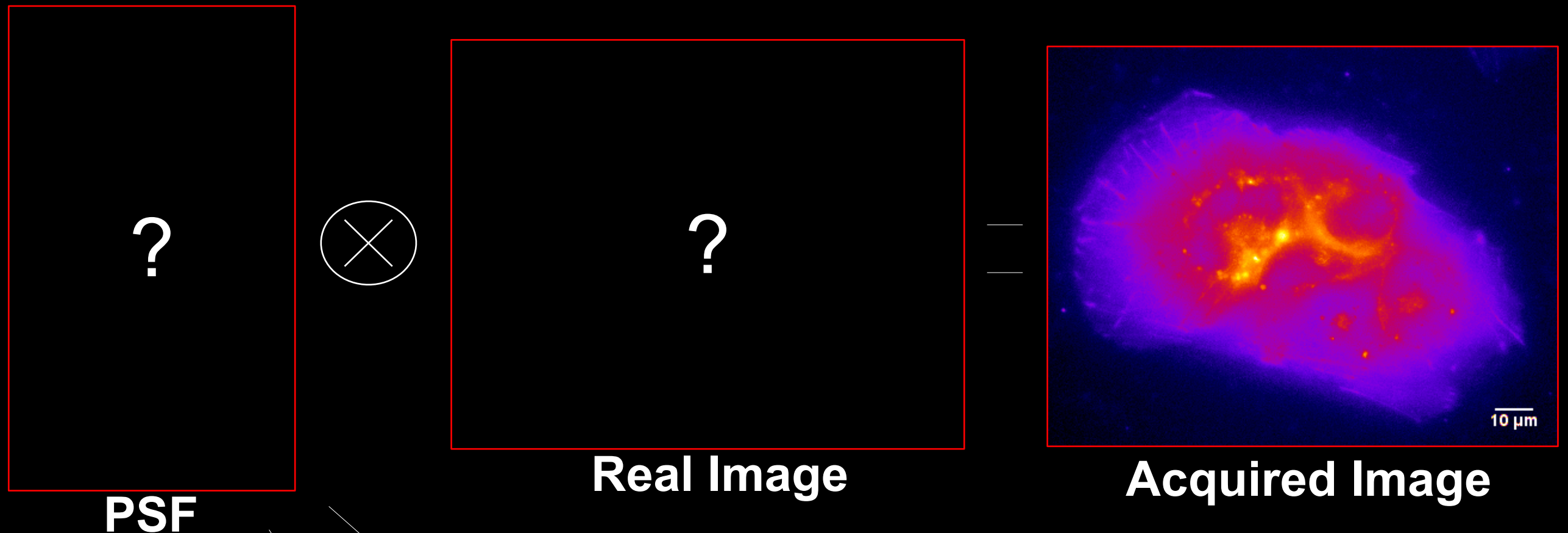
Deconvolution



Leterrier et al. 2004



Deconvolution



DeconvolutionLab2: An open-source software for deconvolution microscopy



Daniel Sage^{a,*}, Laurène Donati^a, Ferréol Soulez^a, Denis Fortun^b, Guillaume Schmit^a, Arne Seitz^c, Romain Guet^c, Cédric Vonesch^b, Michael Unser^a

^a Biomedical Imaging Group, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

^b Center for Biomedical Imaging-Signal Processing Core (CIBM-SP), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

^c BioImaging and Optics Platform, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

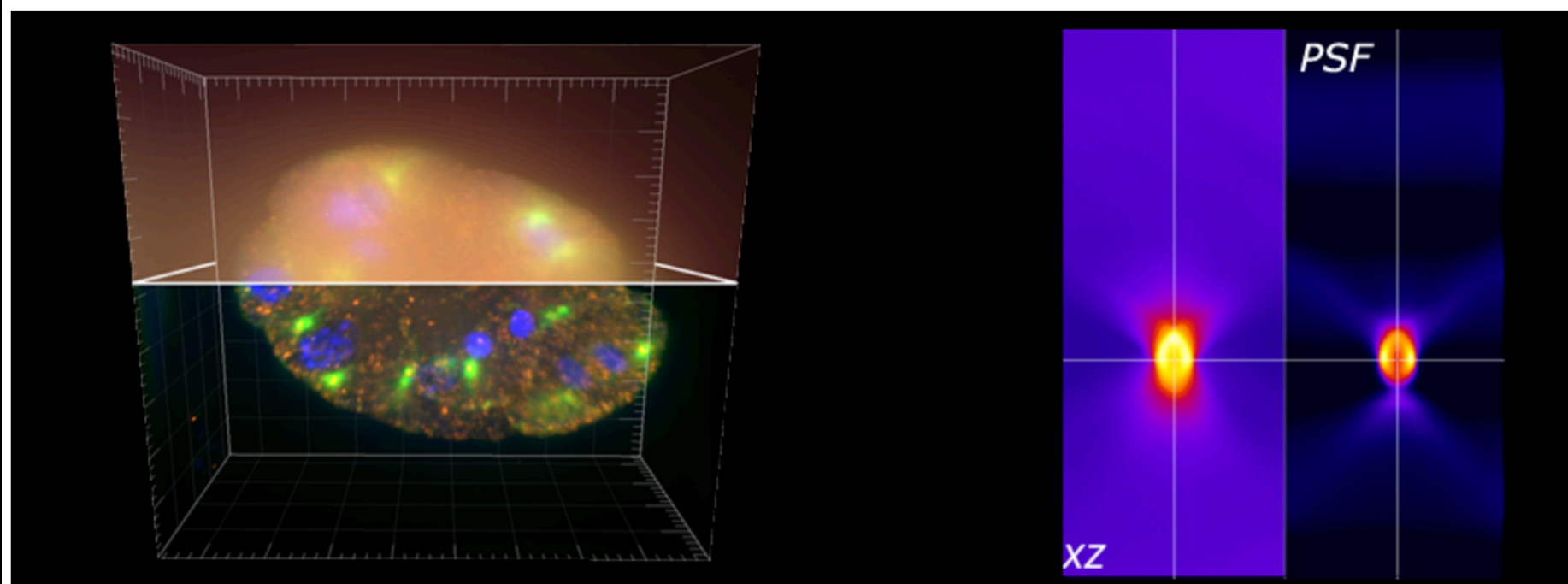
DeconvolutionLab2

The remastered Java deconvolution tool

DeconvolutionLab2 is freely accessible and open-source for 3D deconvolution microscopy; it can be linked to well-known imaging software platforms, **ImageJ**, **Fiji**, **ICY**, **Matlab**, and it runs as a stand-alone application.

The backbone of our software architecture is a library that contains the number-crunching elements of the deconvolution task. It includes the tool for a complete validation pipeline. Inquisitive minds inclined to peruse the code will find it fosters the understanding of deconvolution.

At this stage, DeconvolutionLab2 includes a friendly user interface to run the following algorithms: Regularized Inverse Filter, Tikhonov Inverse Filter, Naive Inverse Filter, Richardson-Lucy, Richardson-Lucy Total Variation, Landweber (Linear Least Squares), Non-negative Least Squares, Bounded-Variable Least Squares, Van Cittert, Tikhonov-Miller, Iterative Constraint Tikhonov-Miller, FISTA, ISTA.



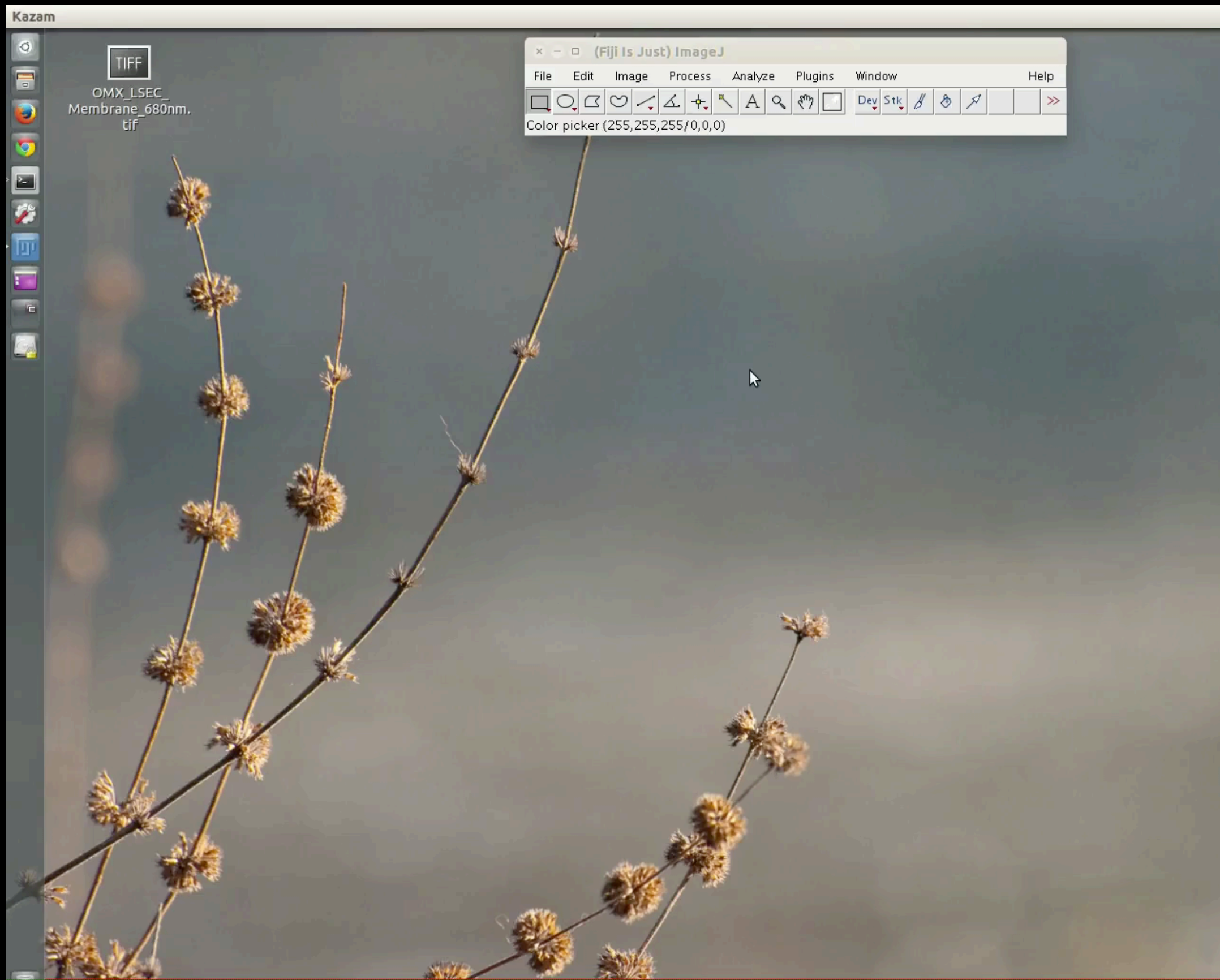
Structured Illumination Microscopy

needs a specialised microscope, but you benefit from
doing your own image processing!

See the talk from Andrew York to know the principles

Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ.

Müller M¹, Mönkemöller V¹, Hennig S¹, Hübner W¹, Huser T^{1,2}.



Single-Molecule Localization Microscopy

generally needs a TIRF or HILO capable microscope

Stochastic Single-molecule localization

switch-OFF



switch-ON
subset



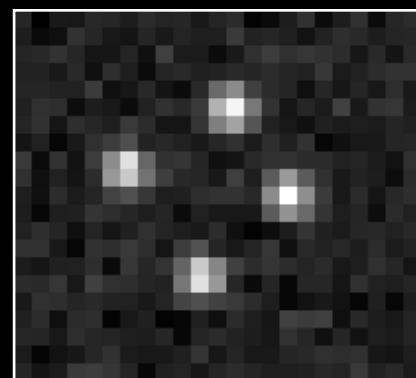
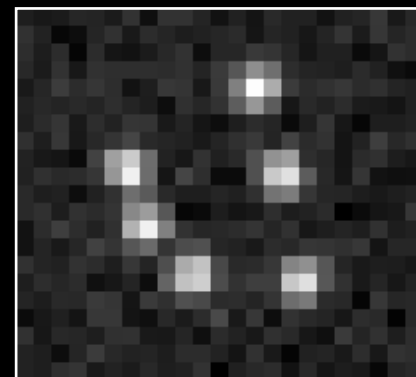
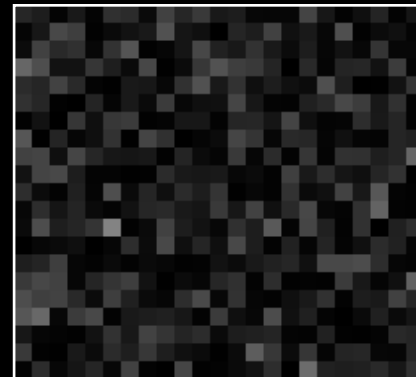
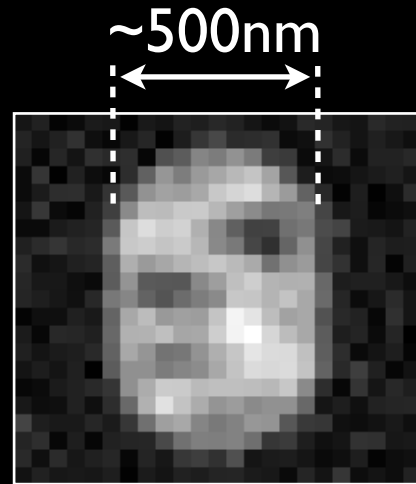
image &
switch-OFF



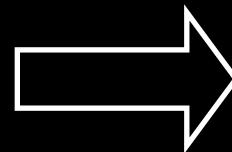
switch-ON
subset



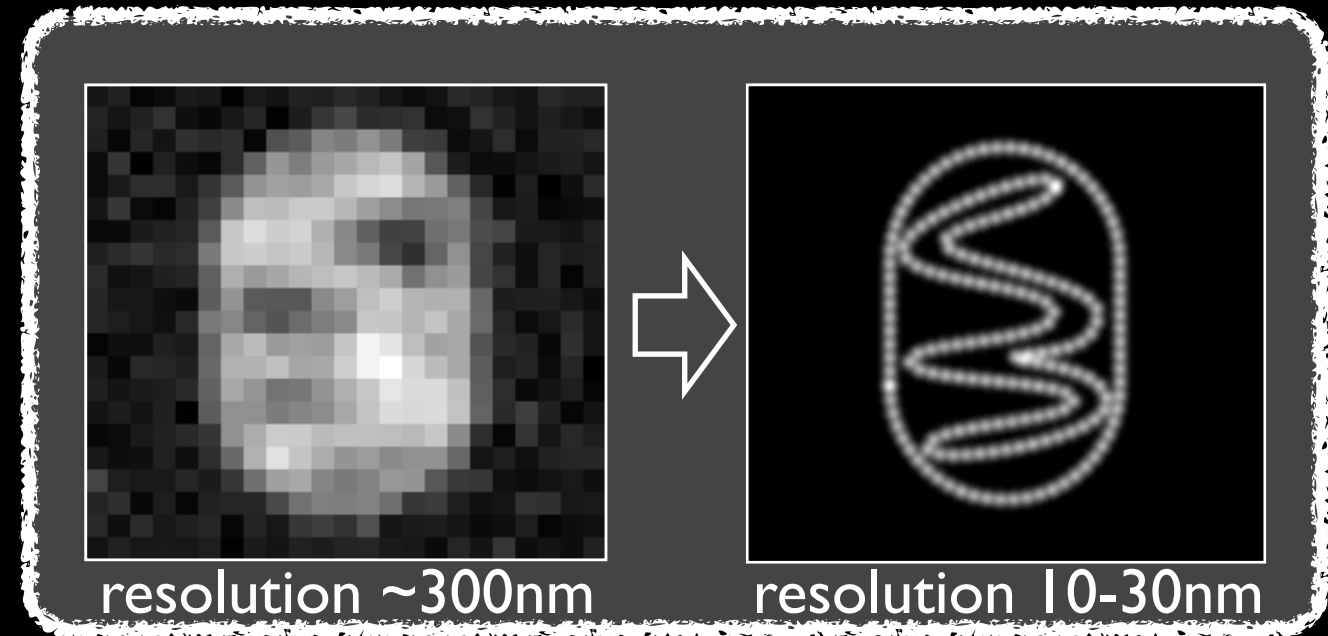
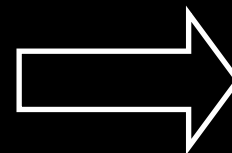
image &
switch-OFF



detect &
localize



detect &
localize

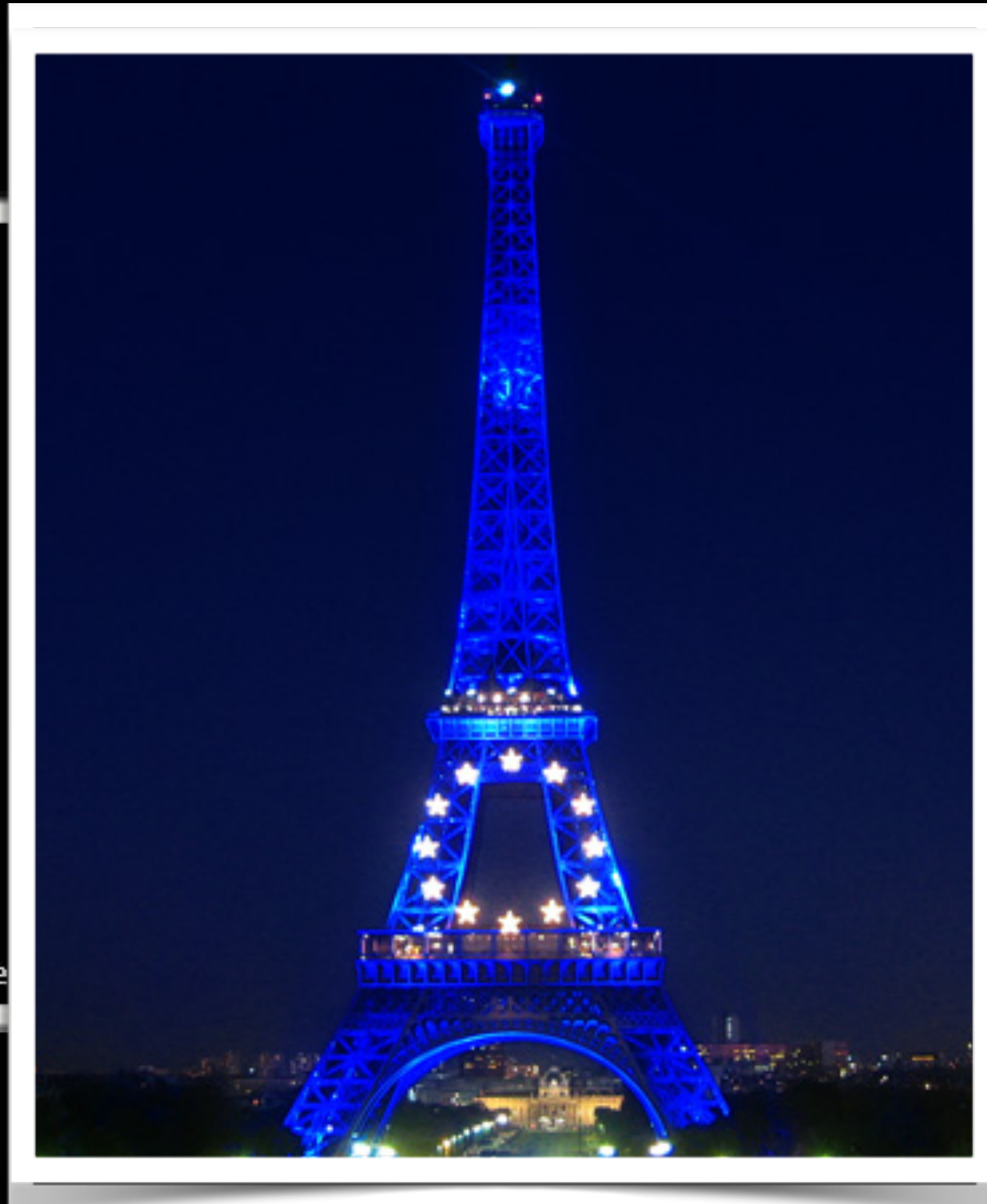


PALM (Betzig et al., '06)
FPALM (Hess et al., '06)
STORM (Rust et al., '06)

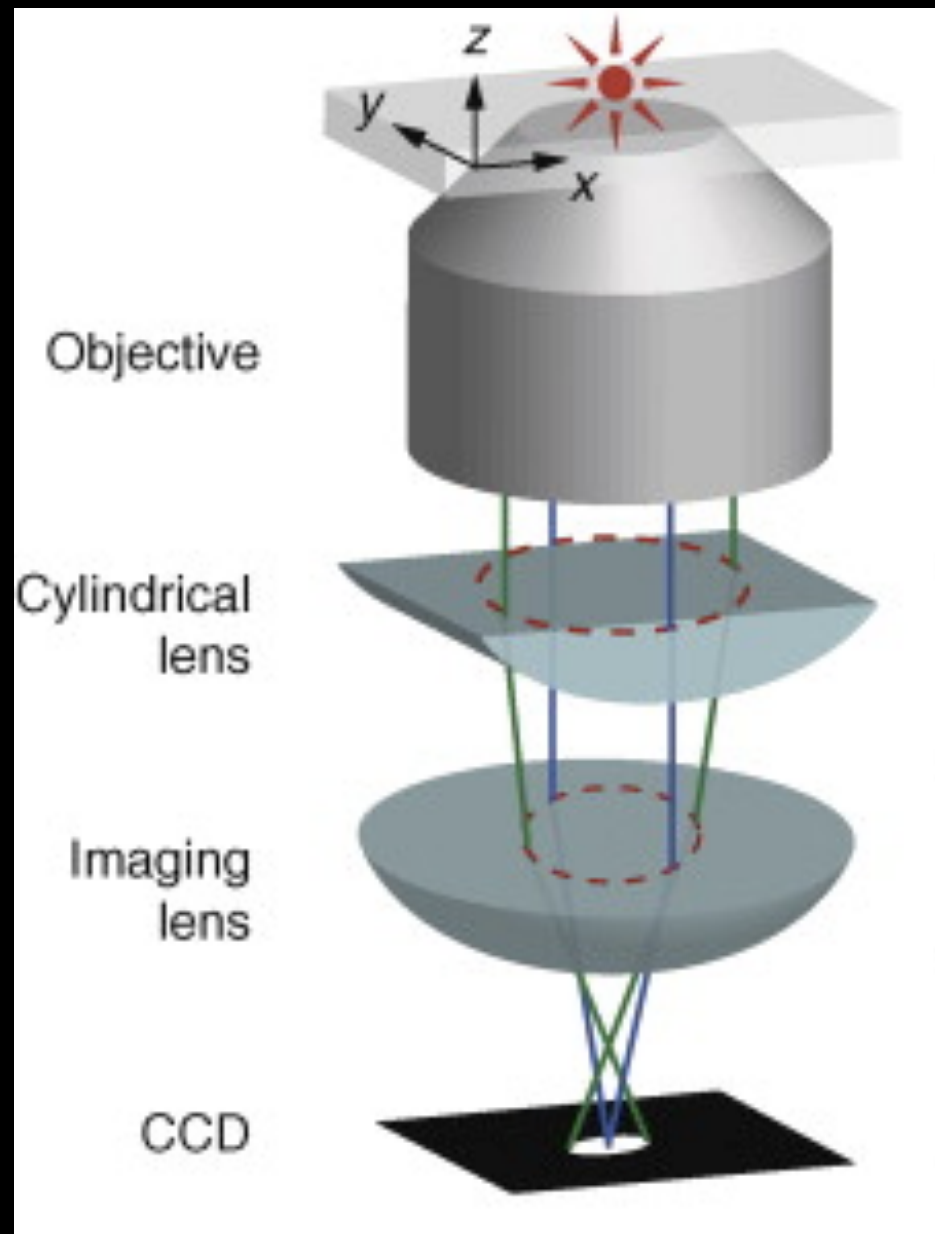
Super-resolving a macro structure



Henriques et al., Nat. Meth., 2010

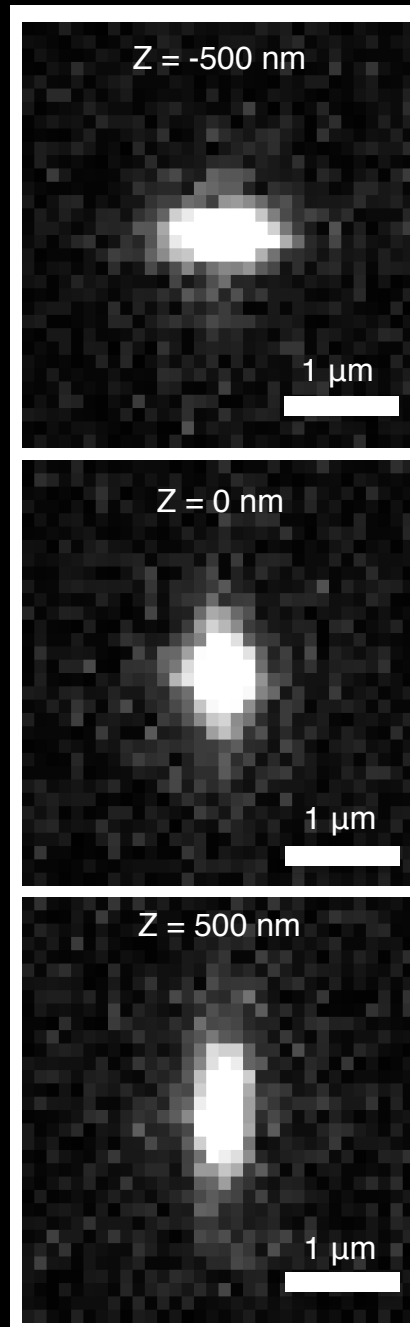


Encoding the z-position into the PSF

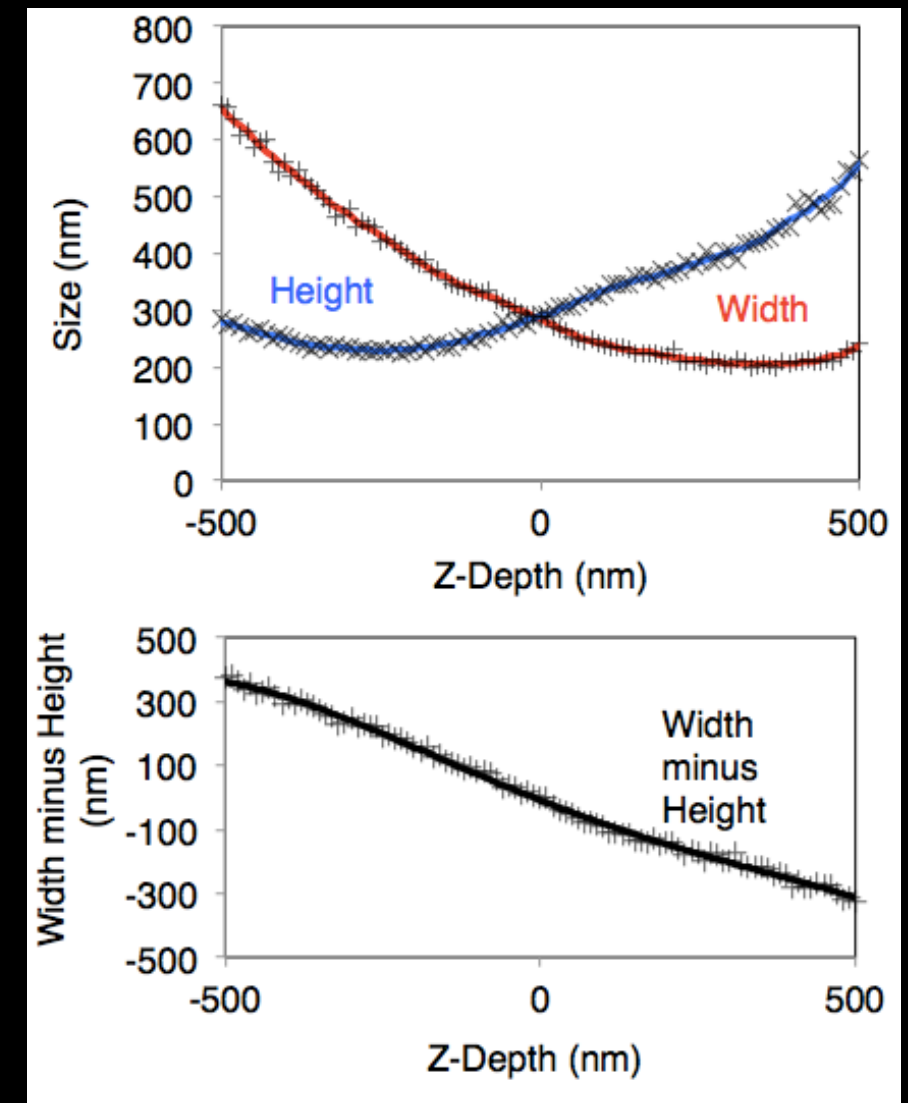


Huang et al., Science, 2008

Astigmatism



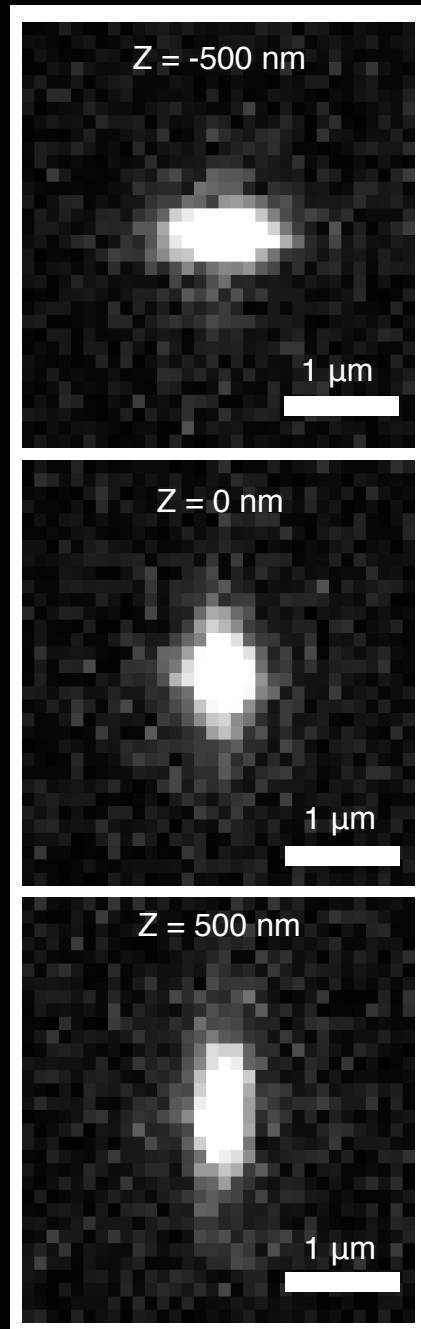
Henriques et al., Nat. Meth., 2010



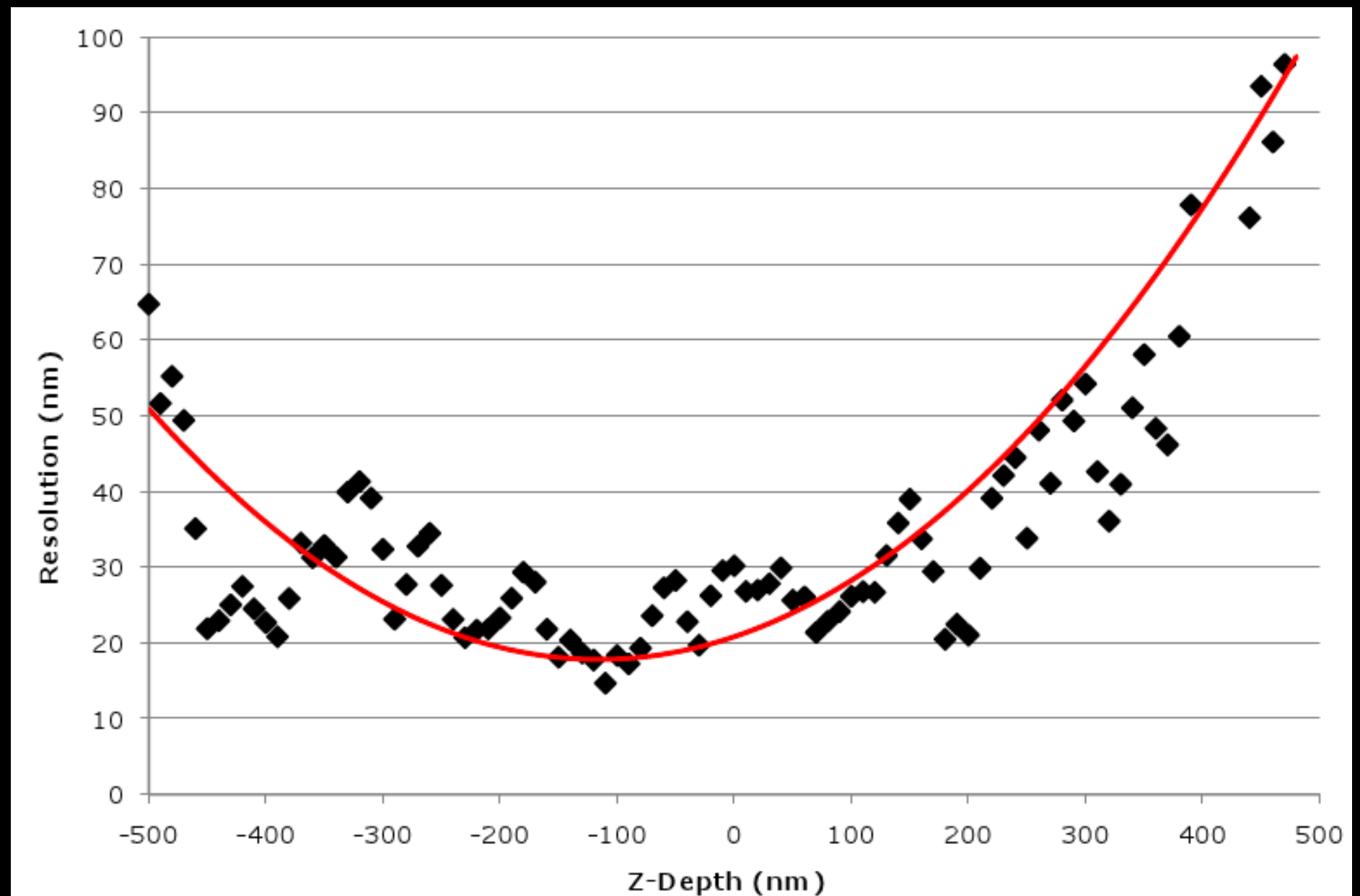
Henriques et al., Nat. Meth., 2010

Encoding the z-position into the PSF

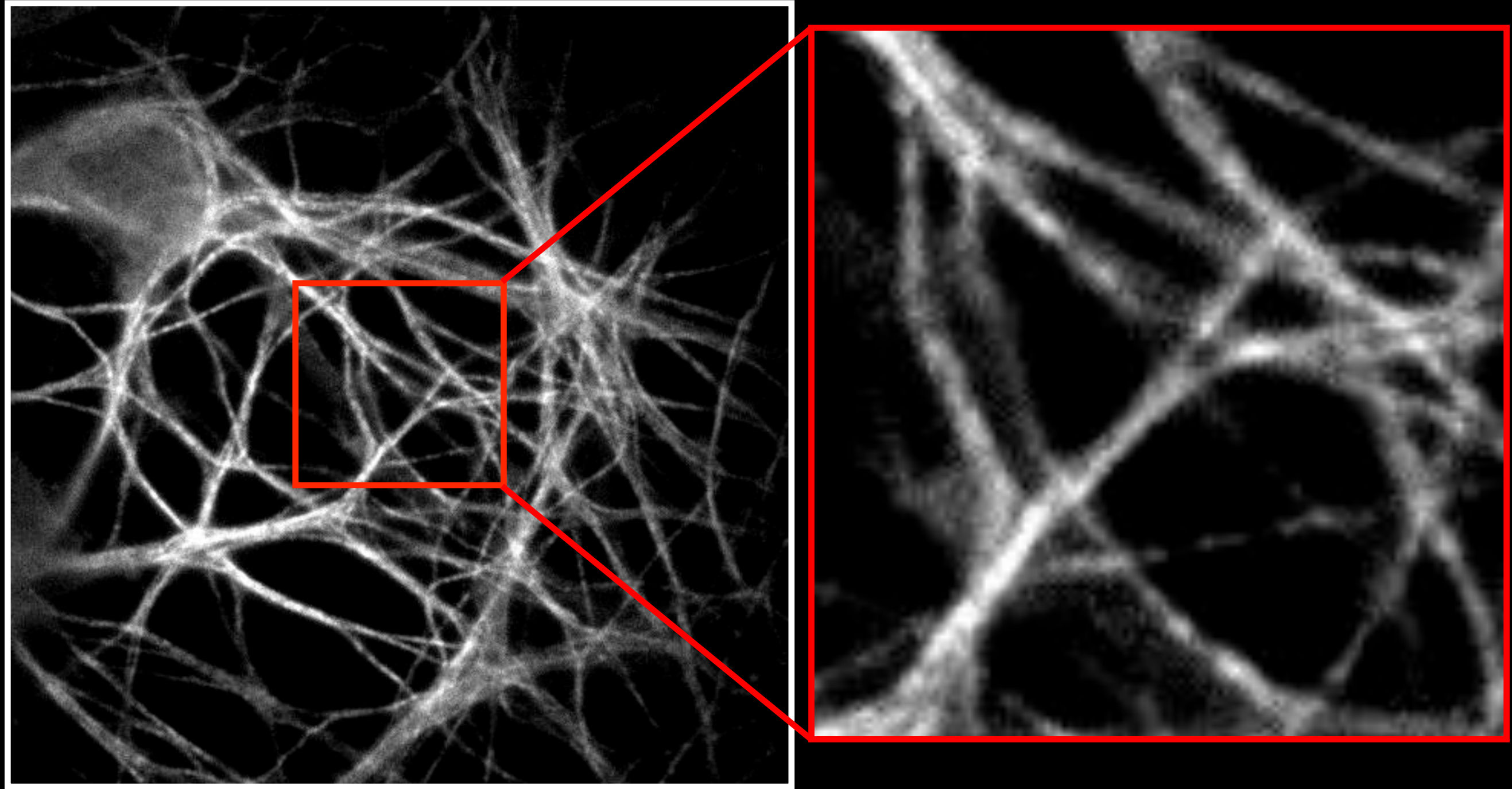
Astigmatism



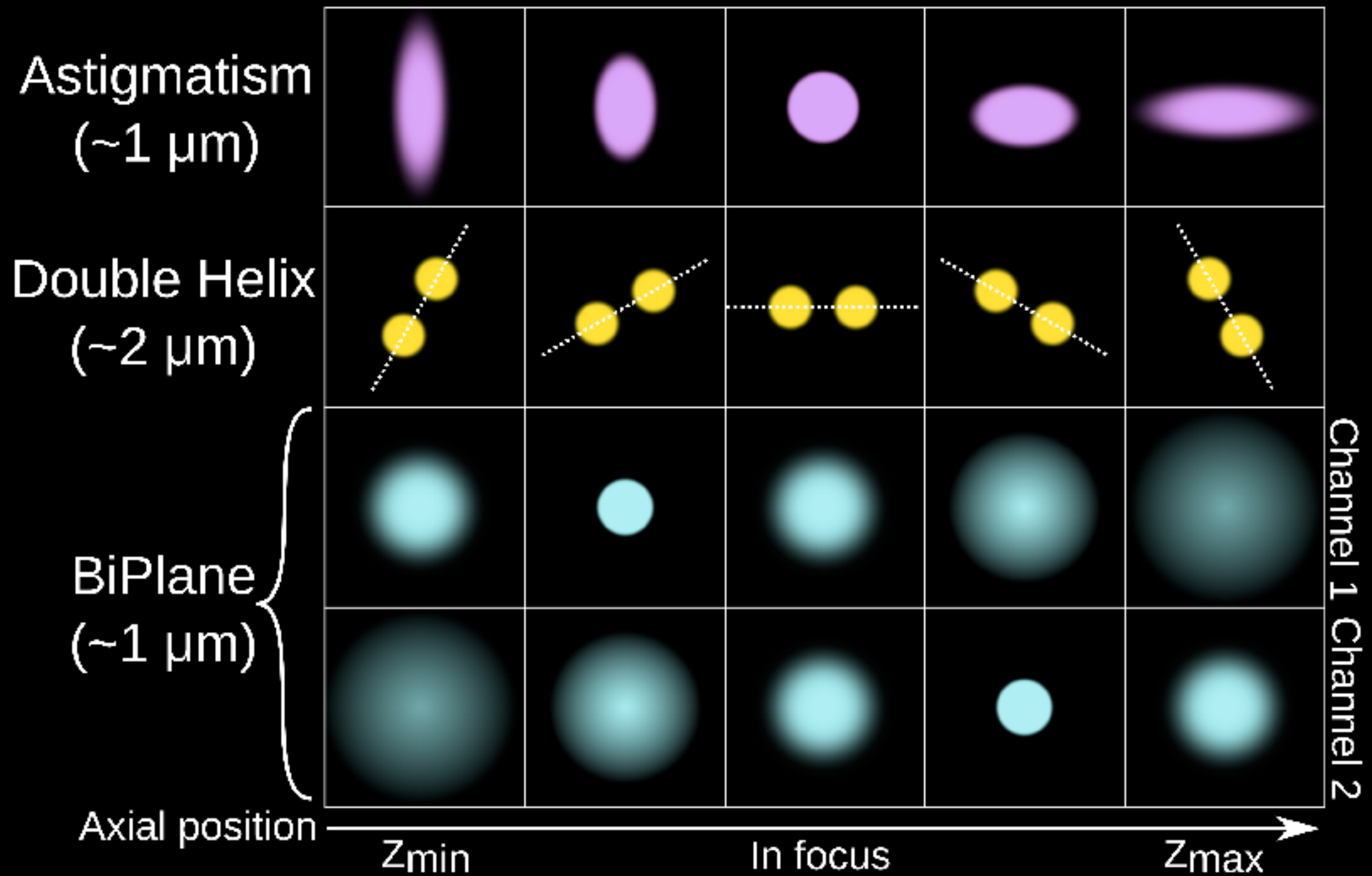
Experimental resolution



Photoswitching under astigmatism



3D particle detection



Herbert, & Henriques, Microsc. and Microanal., in press
 Huang et al., Nat. Meth., 2008
 Rama et al., PNAS, 2009
 Juetten et al., Nat. Meth., 2008

Many different of algorithms for this type of analysis

nature | **methods**

ANALYSIS

<https://doi.org/10.1038/s41592-019-0364-4>

Corrected: Publisher Correction

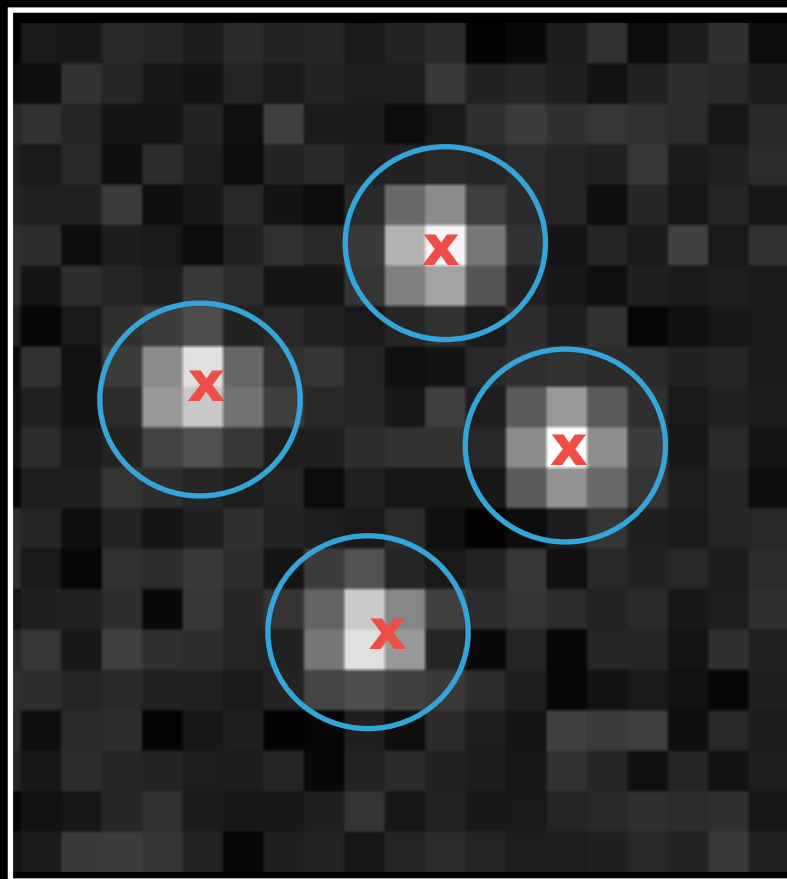
Super-resolution fight club: assessment of 2D and 3D single-molecule localization microscopy software

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 Wheeler¹², Anna Archetti¹³, Bernd Rieger ¹⁴, Raimund Ober^{6,7,15}, Guy M. Hagen ¹⁶,
Jean-Baptiste Sibarita ^{17,18}, Jonas Ries ¹⁹, Ricardo Henriques ²⁰, Michael Unser¹
and Seamus Holden ^{21,22*}

With the widespread uptake of two-dimensional (2D) and three-dimensional (3D) single-molecule localization microscopy (SMLM), a large set of different data analysis packages have been developed to generate super-resolution images. In a large community effort, we designed a competition to extensively characterize and rank the performance of 2D and 3D SMLM software packages. We generated realistic simulated datasets for popular imaging modalities—2D, astigmatic 3D, biplane 3D and double-helix 3D—and evaluated 36 participant packages against these data. This provides the first broad assessment of 3D SMLM software and provides a holistic view of how the latest 2D and 3D SMLM packages perform in realistic conditions. This resource allows researchers to identify optimal analytical software for their experiments, allows 3D SMLM software developers to benchmark new software against the current state of the art, and provides insight into the current limits of the field.

How are the images created?

- Many different algorithms exist (see Sage 2015, Sage 2018)



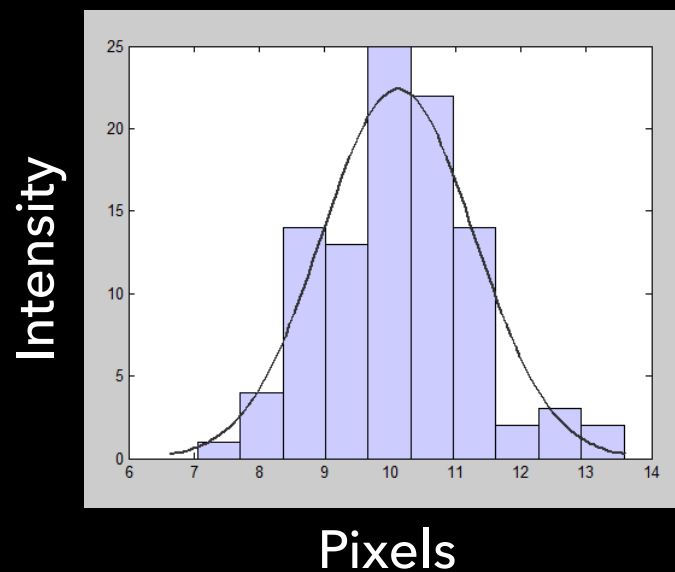
STEP 1: DETECTION - 'DOES THIS LOOK LIKE A FLUOROPHORE?'

Is the intensity what you'd expect?

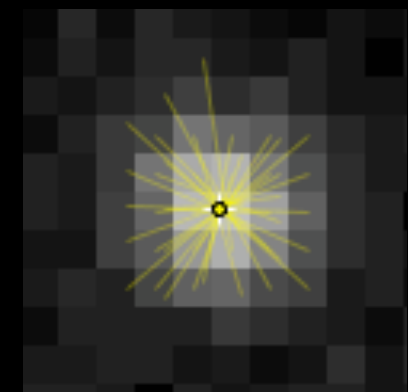
Are the size and shape what you'd expect?

STEP 2: LOCALISATION - WHERE ARE THE CENTRES OF THE FLUOROPHORES?

Gaussian fitting



Symmetry measurements



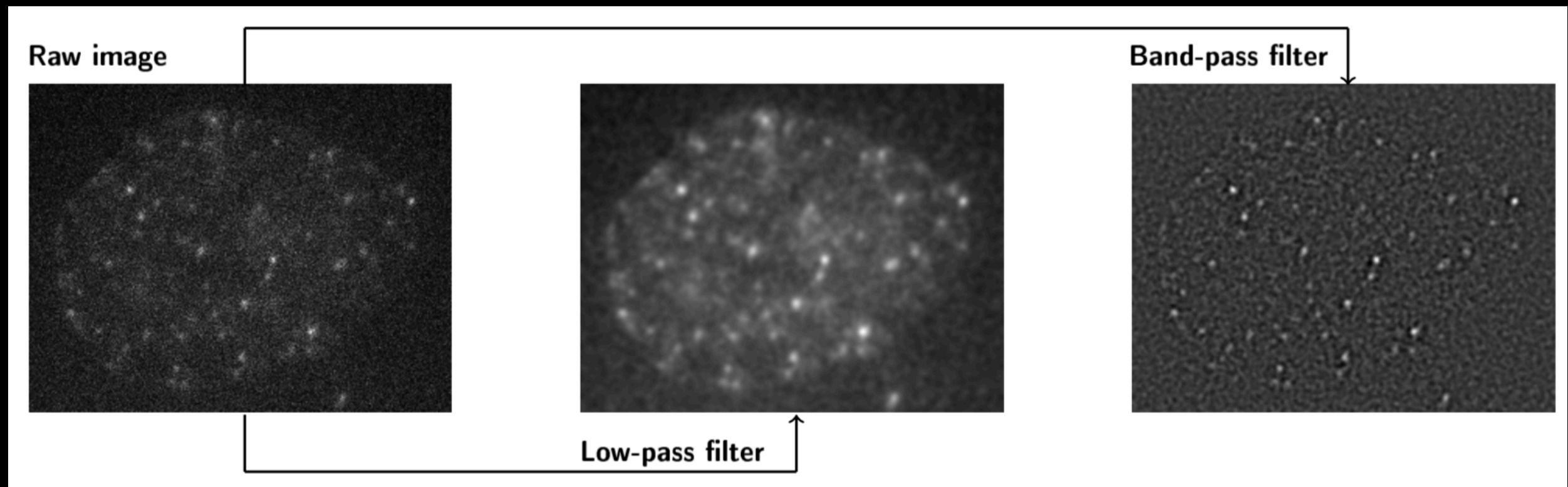
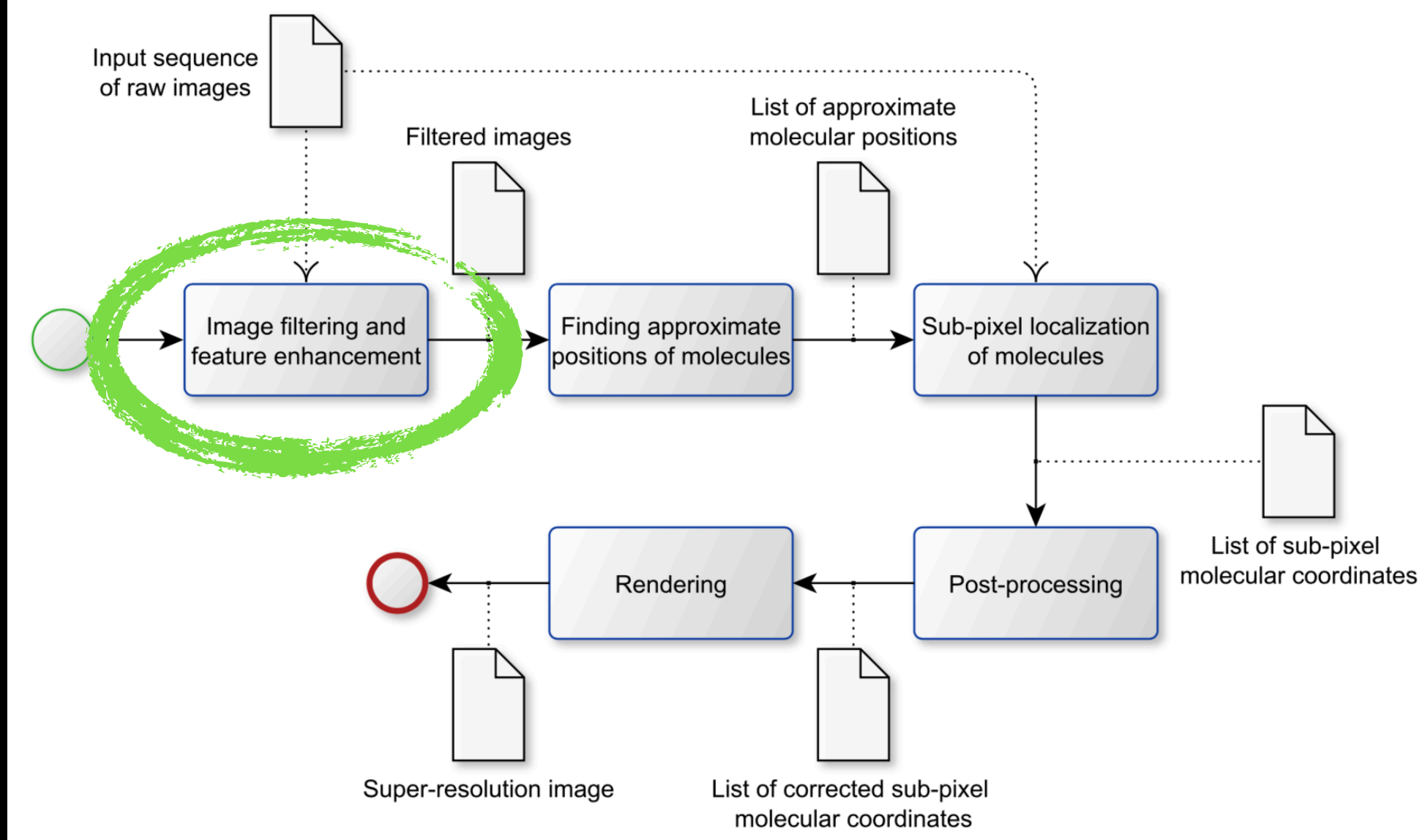
Going from a sequence of frames with
blinking molecules to a super-resolution
reconstruction...

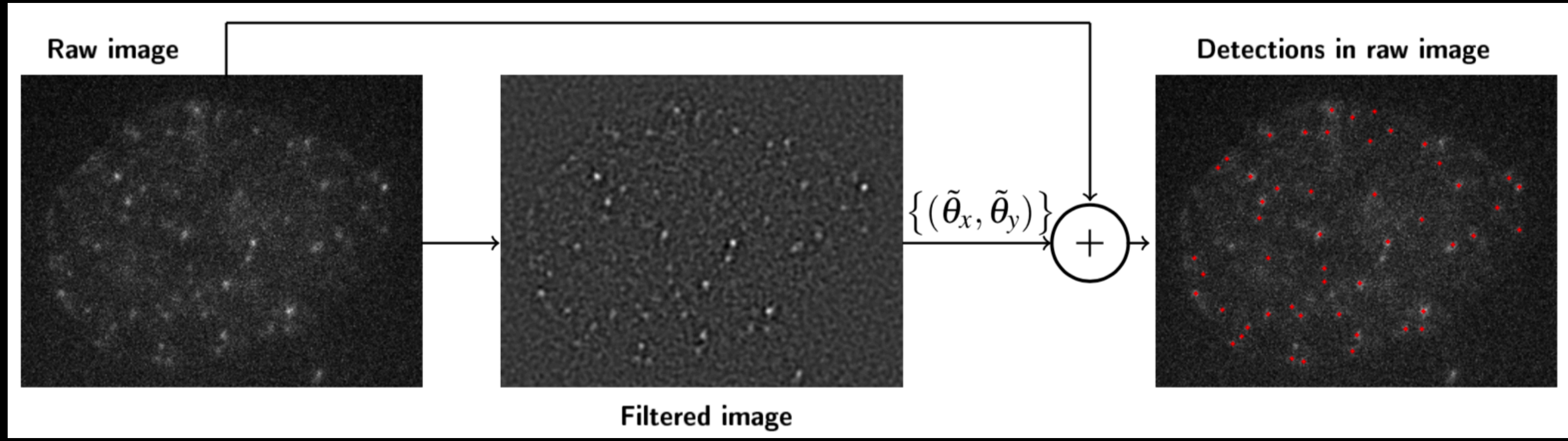
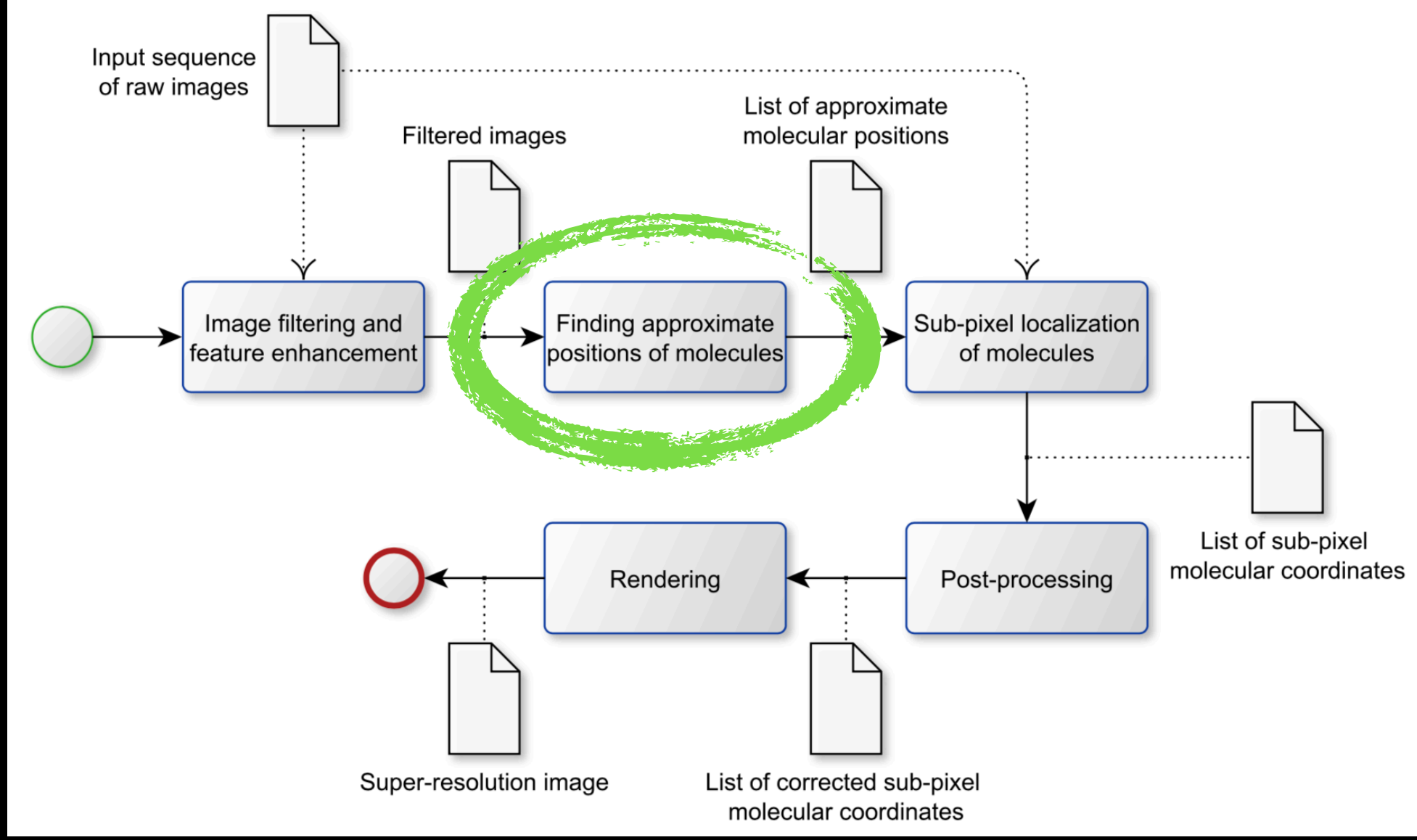
*See thesis of Martin Ovesný, who wrote
ThunderSTORM (it's awesome)*

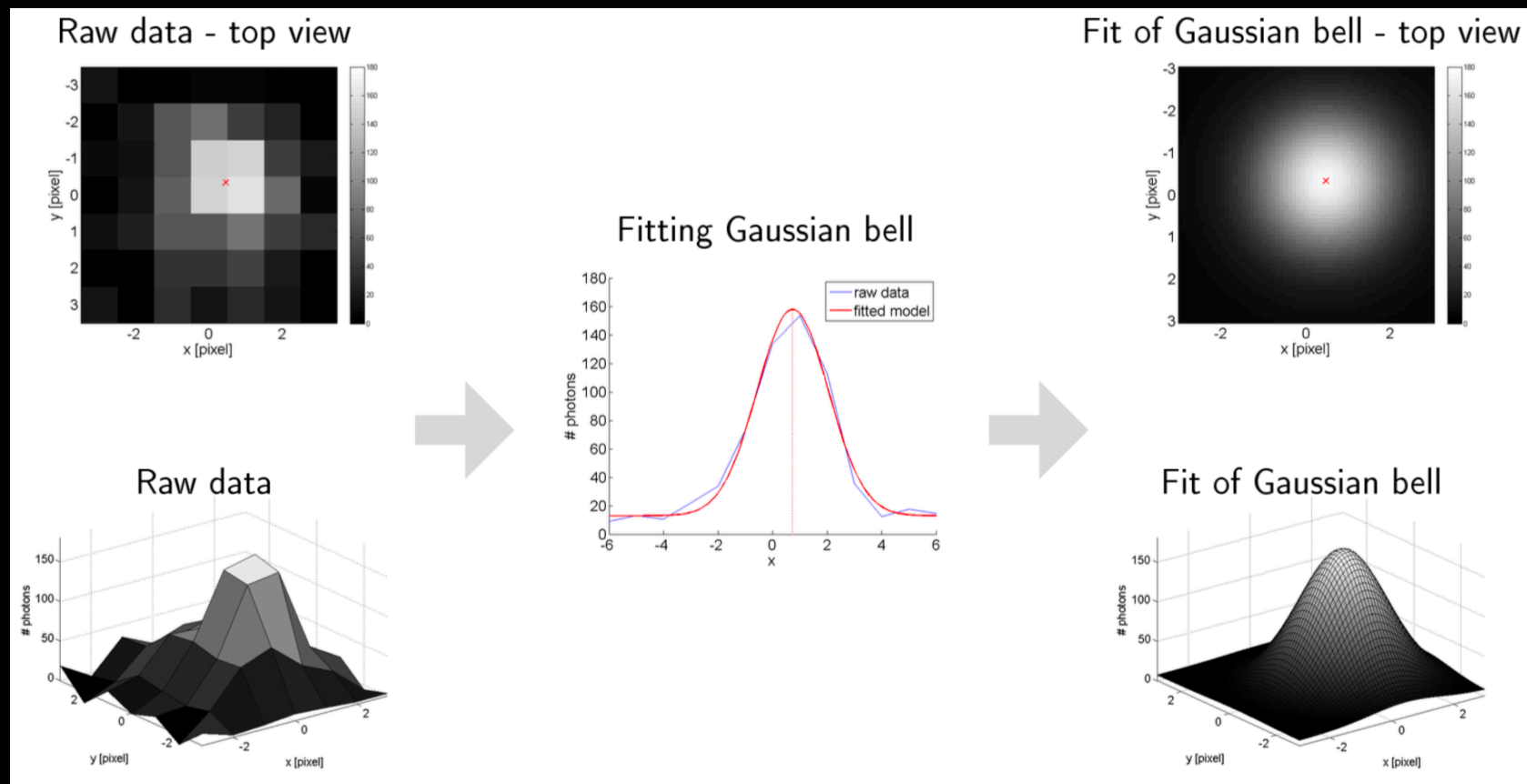
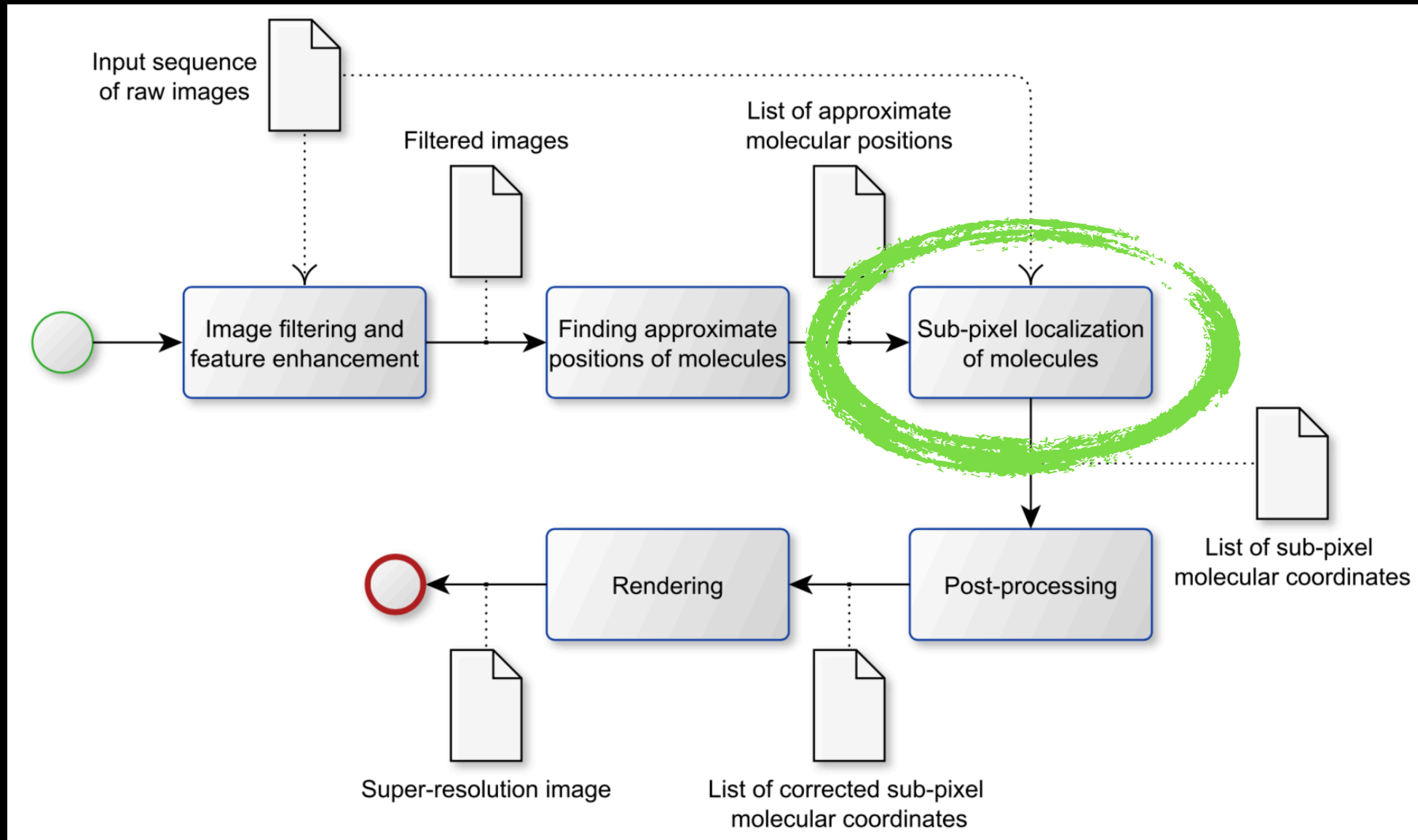
[Bioinformatics](#). 2014 Aug 15;30(16):2389-90. doi: 10.1093/bioinformatics/btu202. Epub 2014 Apr 25.

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

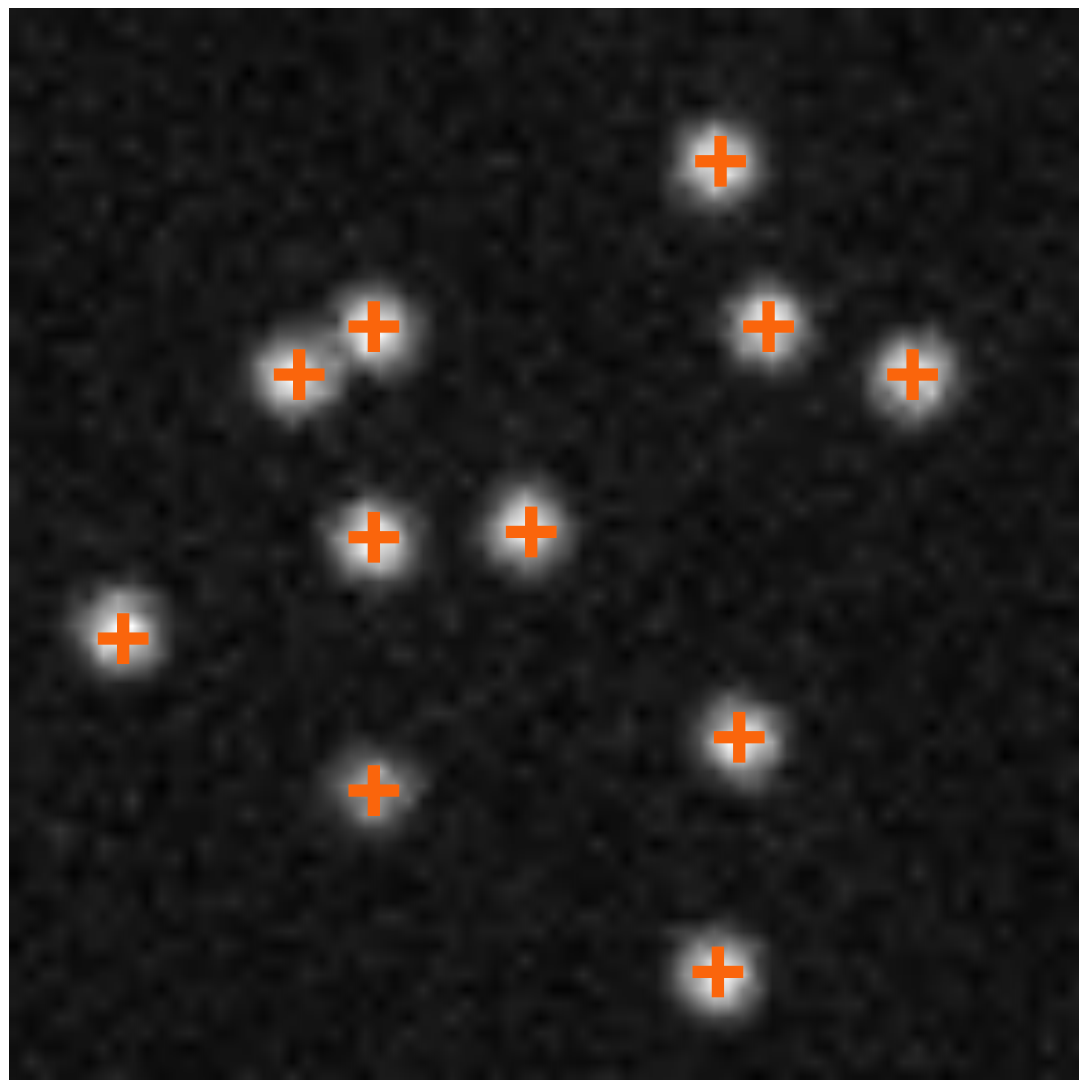
[Ovesný M](#)¹, [Křížek P](#)¹, [Borkovec J](#)¹, [Svindrych Z](#)¹, [Hagen GM](#)¹.



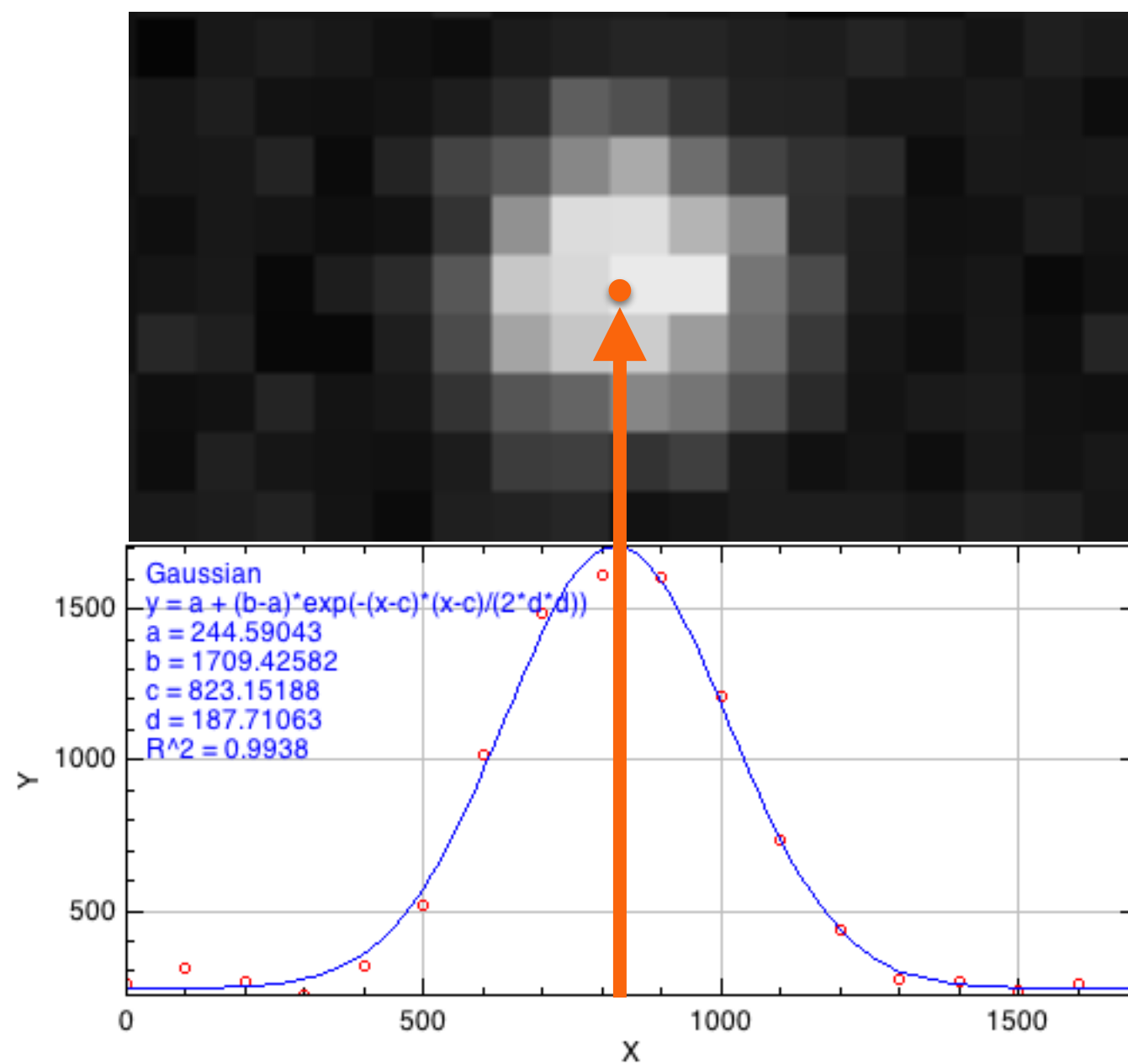


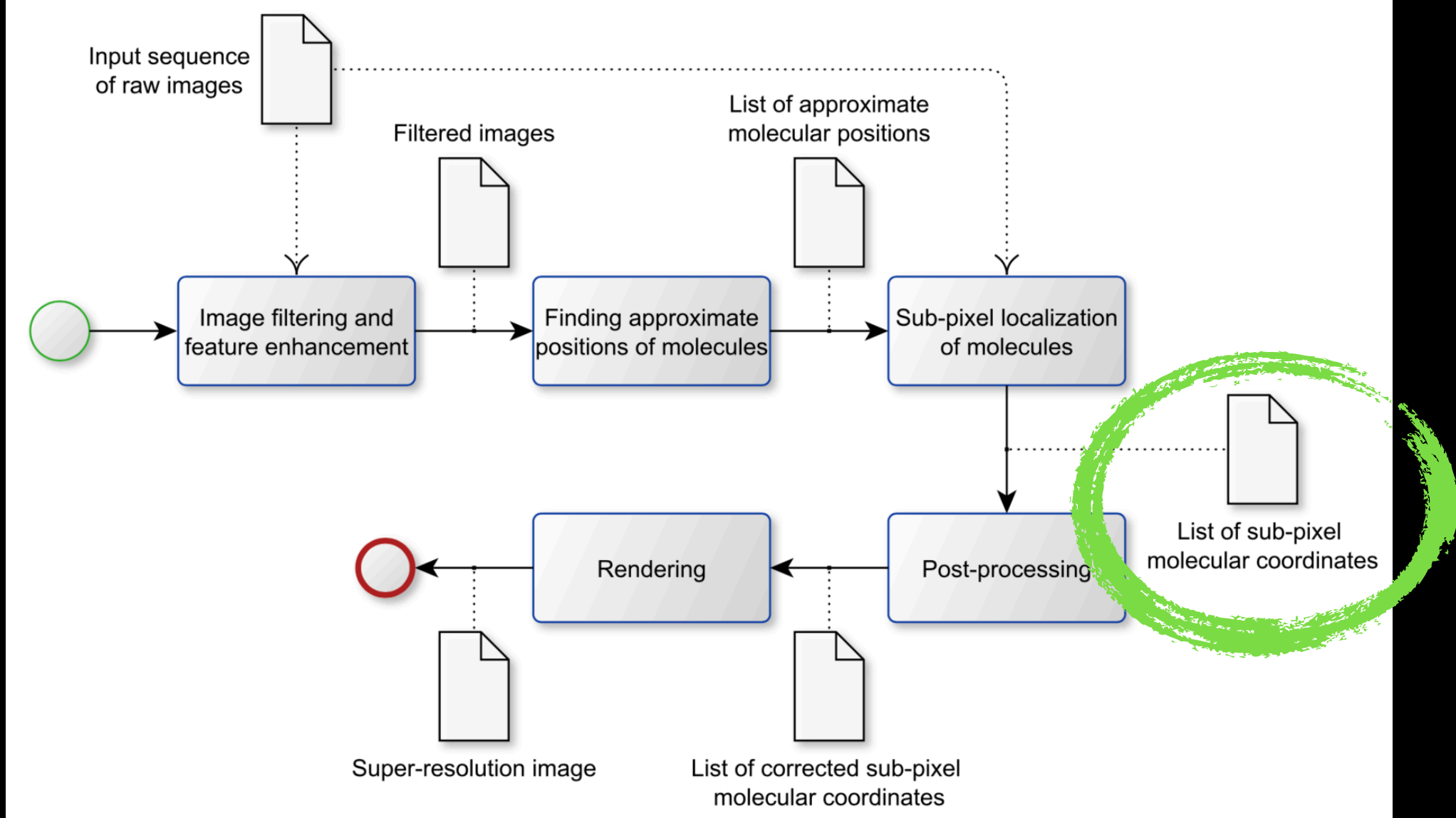


Detection

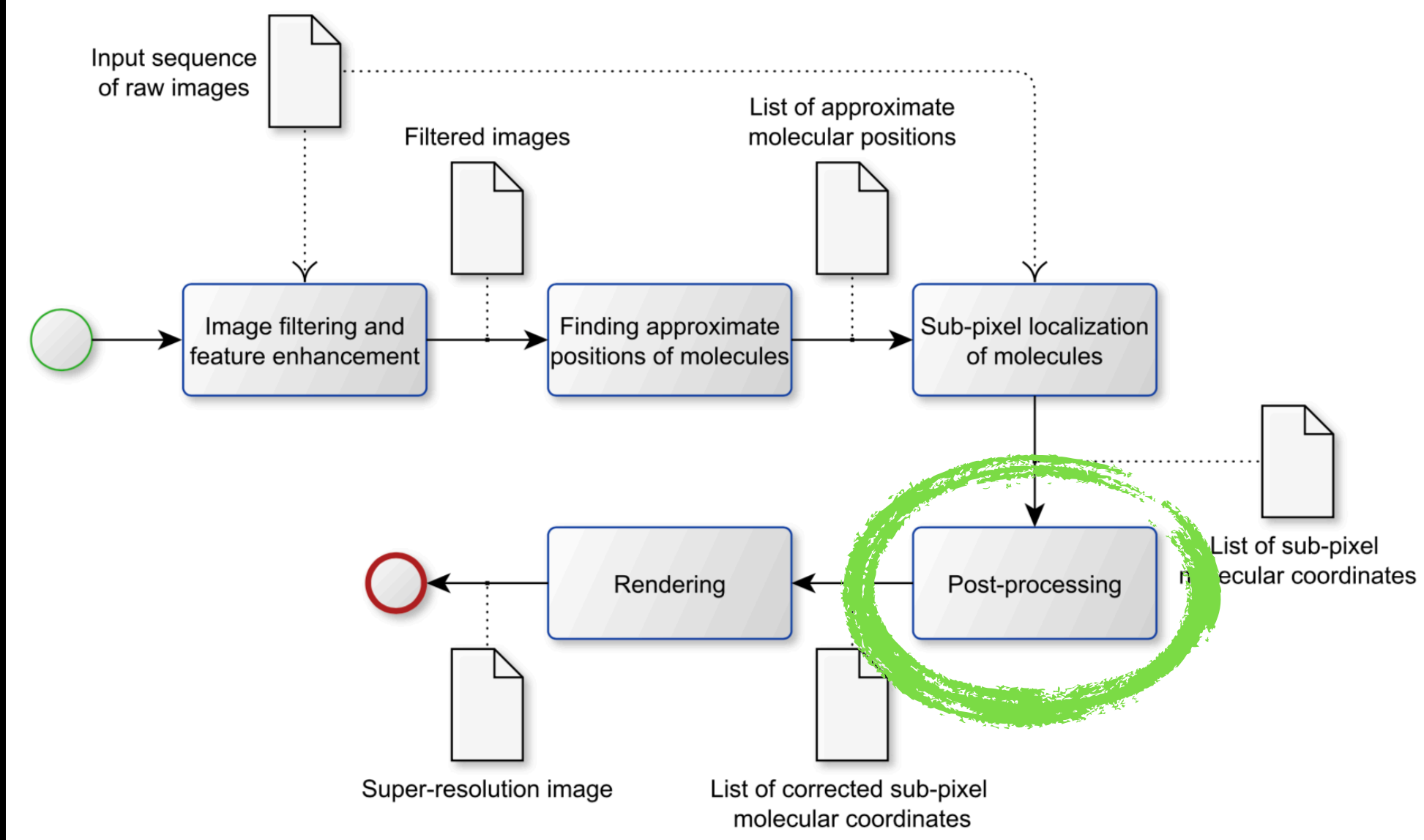


Localisation



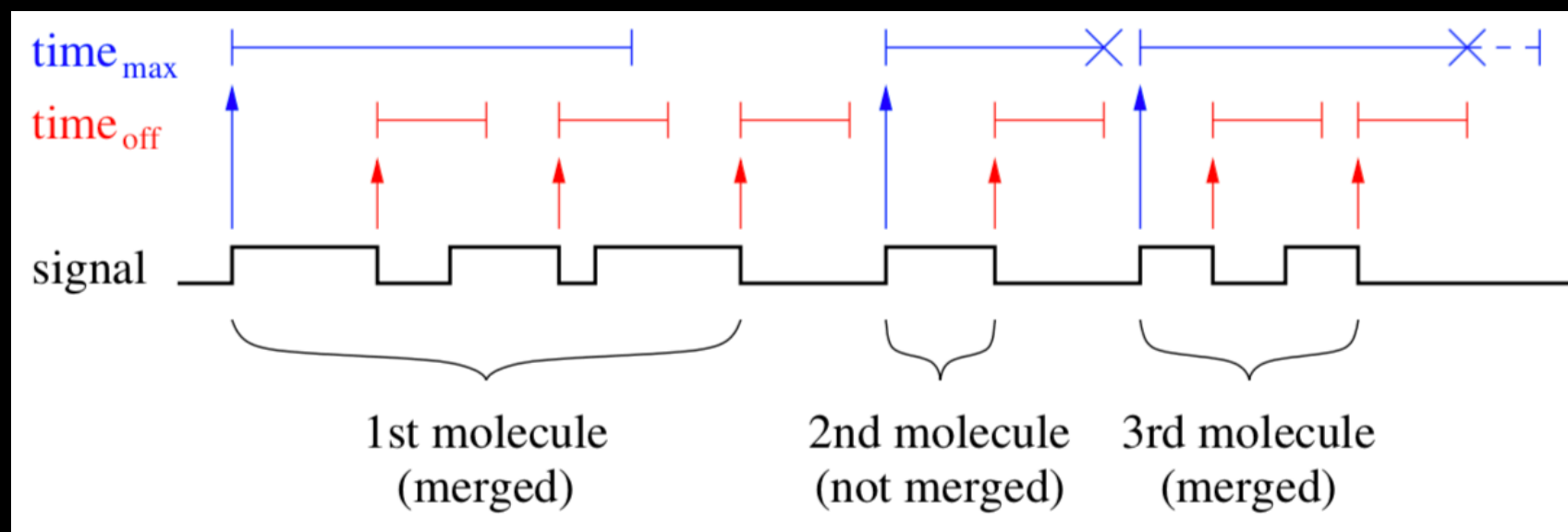


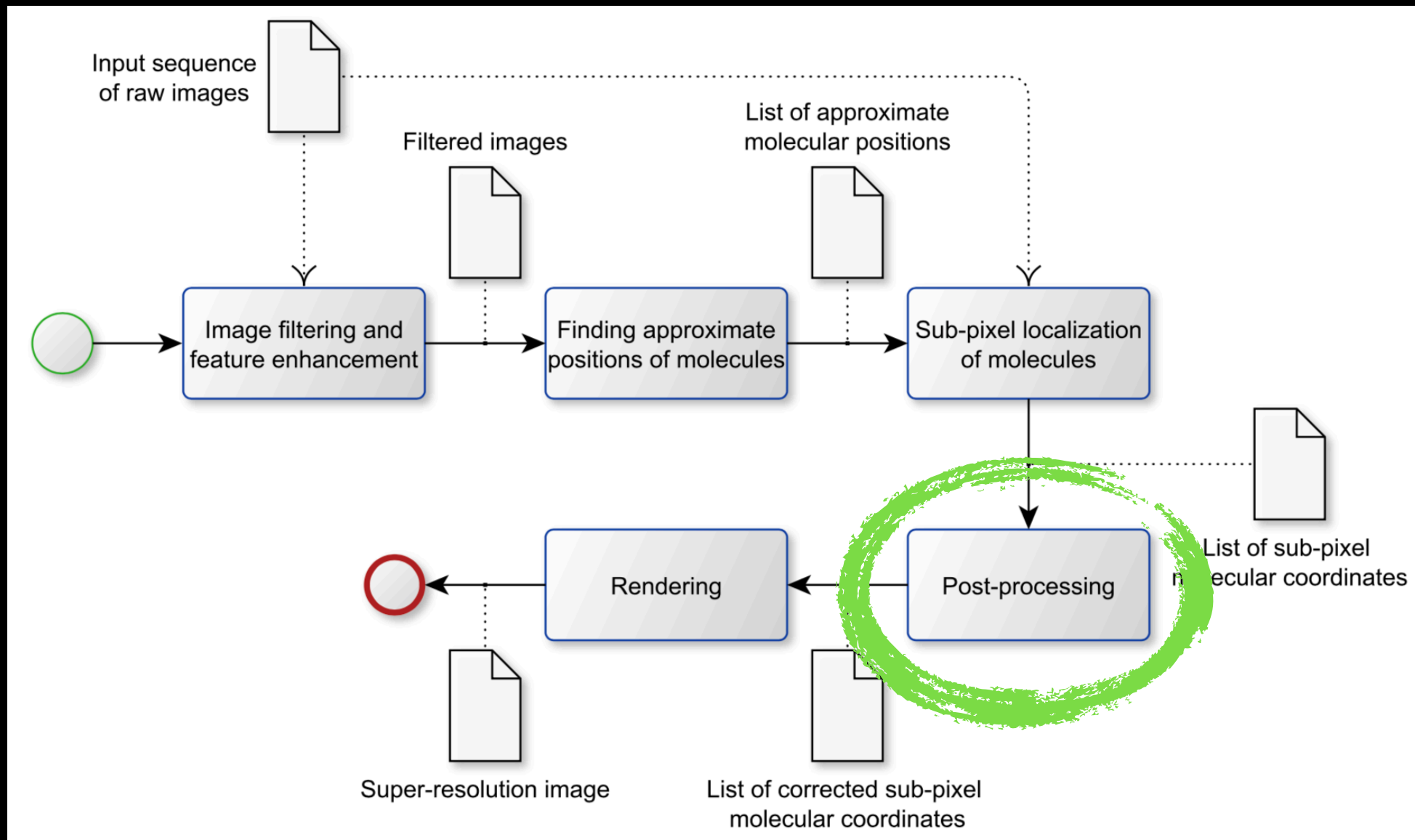
id	fra...	x [nm]	y [nm]	sigma [nm]	intensity [photon]	offset [photon]	bkgstd [photon]	uncertainty_xy [nm]
32	1	2942.434	4255.307	100.92	14915.506	1052.982	343.014	12.752
33	1	3055.493	8625.645	119.206	52862.355	743.964	300.399	4.342
34	1	3096.546	976.925	154.281	44270.007	634.941	166.758	4.753
35	1	3029.857	1758.821	324.997	282845.394	0	526.804	9.972
36	1	2952.374	1685.813	223.189	75638.283	2062.452	696.986	23.421
37	1	3269.731	2401.032	141.983	49091.545	1591.74	390.903	8.423
38	1	3209.651	3253.642	354.319	402335.319	0	784.701	12.378
39	1	3314.96	3525.231	251.578	342911.395	0	494.867	4.674
40	1	3500.277	7511.153	197.371	55806.365	706.021	506.44	18.135
41	1	3426.383	4409.018	132.057	37899.705	718.012	158.518	3.937
42	1	3406.202	5603.179	112.778	20181.755	606.216	82.872	2.985
43	1	3482.003	6281.227	166.165	27199.399	518.336	233.58	12.318
44	1	3509.645	5055.962	133.456	31613.686	597.874	87.873	2.782
45	1	3510.991	6610.576	172.262	27118.525	521.79	225.917	12.816
46	1	3612.288	2222.58	205.252	37028.525	614.626	206.115	8.241



Input: A list of coordinates with sub-pixel positions of the molecules.

Output: A corrected list of coordinates with sub-pixel positions of the molecules.

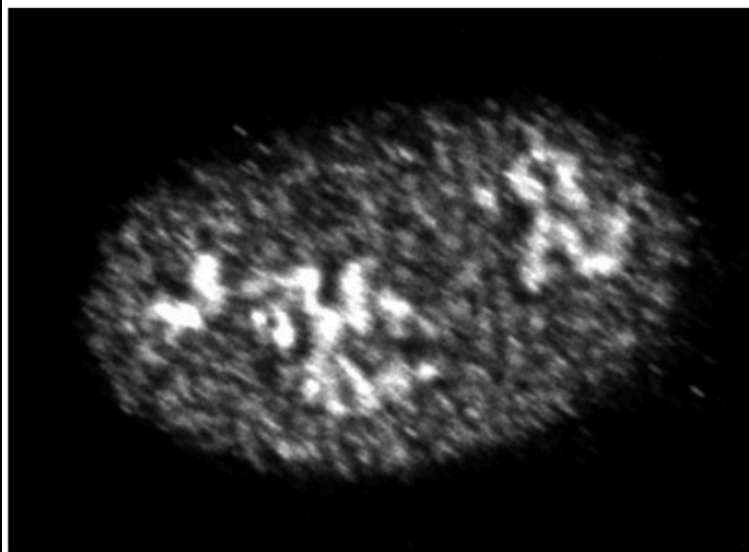




Input: A list of coordinates with sub-pixel positions of the molecules.

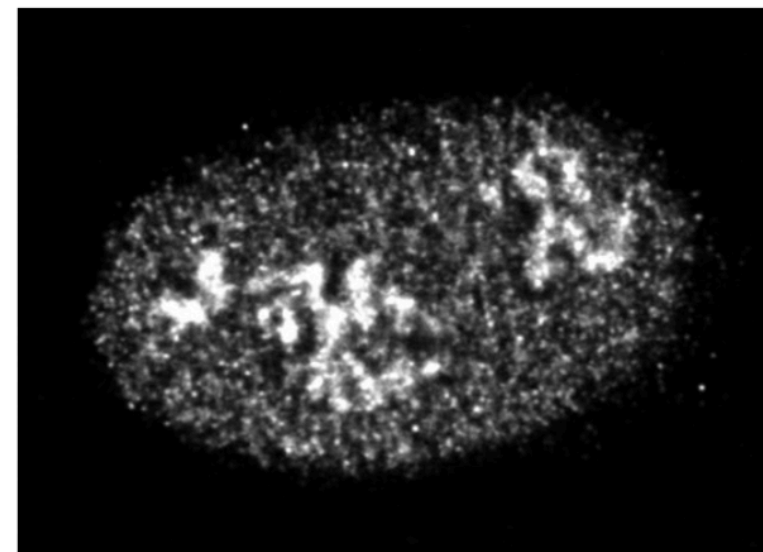
Output: A corrected list of coordinates with sub-pixel positions of the molecules.

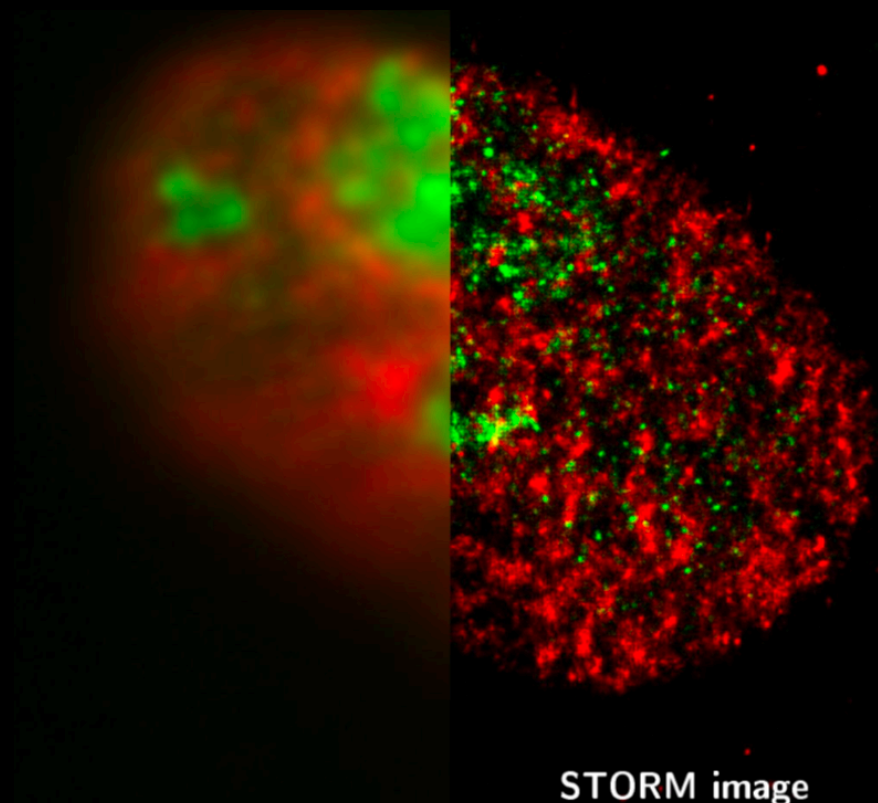
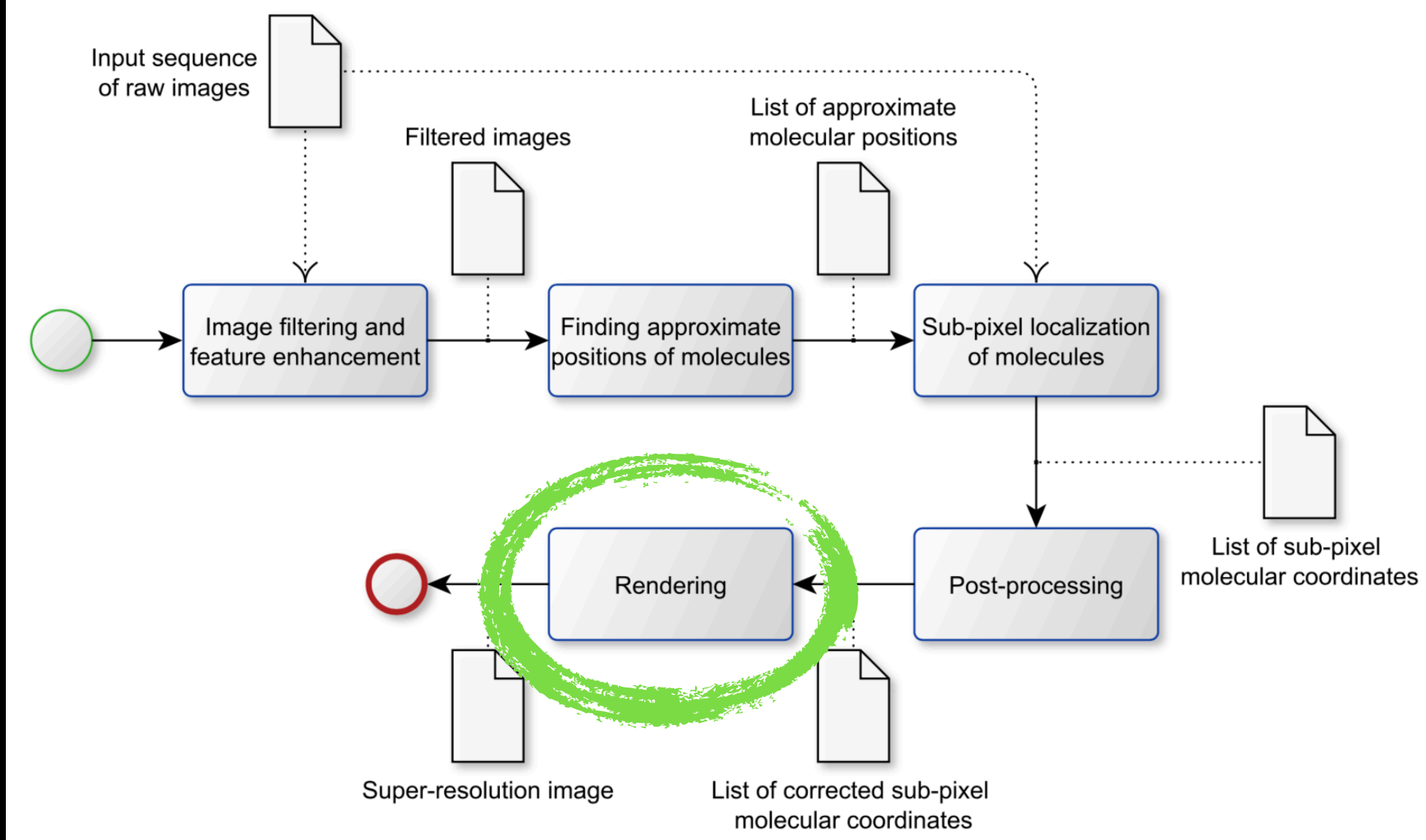
Result of image processing



Drift correction →

Drift corrected image





STORM image

Give a try to ThunderSTORM

Bioinformatics. 2014 Aug 15;30(16):2389-90. doi: 10.1093/bioinformatics/btu202. Epub 2014 Apr 25.

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

Ovesný M¹, Křížek P¹, Borkovec J¹, Svindrych Z¹, Hagen GM¹.

Run analysis

Camera
Camera setup

Image filtering
Filter: Wavelet filter (B-Spline)
B-Spline order: 3
B-Spline scale: 2.0

Approximate localization of molecules
Method: Local maximum
Peak intensity threshold: std(Wave.F1)
Connectivity: ☒ 8-neighbourhood
☐ 4-neighbourhood

Sub-pixel localization of molecules
Method: PSF: Integrated Gaussian
Fitting radius [px]: 3
Fitting method: Weighted Least squares
Initial sigma [px]: 1.6
Multi-emitter fitting analysis: ☐ enable
Maximum of molecules per fitting region: 5
Model selection threshold (p-value): 1.0E-6
☐ Same intensity for all molecules
☒ Limit intensity range [photons]: 500:2500

Visualisation of the results
Method: Normalized Gaussian
Magnification: 5.0
Update frequency [frames]: 100
3D: ☐
Colorize Z: ☐
Lookup Table: KENNETH1
Z range (from:step:to) [nm]: -700:100:700
Lateral uncertainty [nm]: 20.0 ☐ Force
Axial uncertainty [nm]: 100.0 ☐ Force

Defaults Preview Ok Cancel

GitHub, Inc. [US] | <https://github.com/zitmen/thunderstorm/wiki/Tutorials>

Apps Cristina Ricardo

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Tutorials

Martin Ovesny edited this page on 5 May 2015 · 4 revisions

Here are some of the examples of how to work with ThunderSTORM:

- Processing 2D data
- Processing 3D data with astigmatism
- Post-processing modules
- Guidelines for the choice of parameters
- Analyzing super-resolution images with ImageJ
- Specifying a threshold for approximate detection of molecules
- Simulation engine
- Batch processing

Variants worth knowing...

Bioinformatics. 2014 Aug 15;30(16):2389-90. doi: 10.1093/bioinformatics/btu202. Epub 2014 Apr 25.

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

Ovesný M¹, Křížek P¹, Borkovec J¹, Svindrych Z¹, Hagen GM¹.

THE JOURNAL OF CHEMICAL PHYSICS **148**, 123311 (2018)



Phasor based single-molecule localization microscopy in 3D (pSMLM-3D): An algorithm for MHz localization rates using standard CPUs

Koen J. A. Martens,^{1,2} Arjen N. Bader,^{1,3} Sander Baas,¹ Bernd Rieger,⁴ and Johannes Hohlbein^{1,3,a)}

¹Laboratory of Biophysics, Wageningen University and Research, Stippeneng 4, 6708 WE Wageningen, The Netherlands

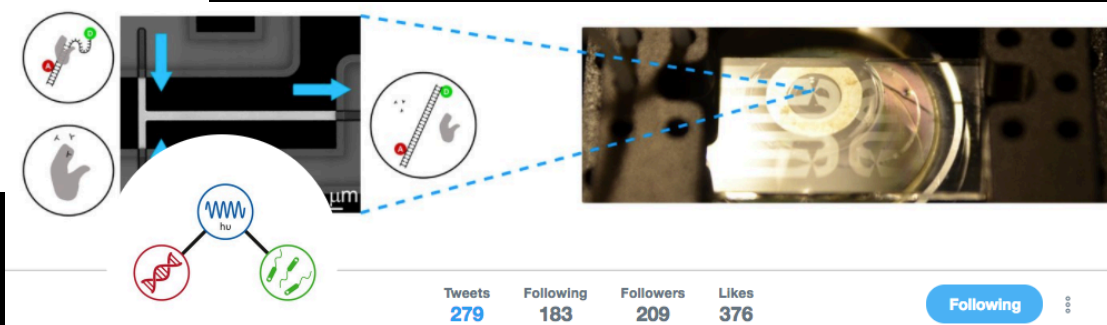
²Laboratory of Bionanotechnology, Wageningen University and Research, Bornse Weiland 9, 6708 WG Wageningen, The Netherlands

³Microspectroscopy Research Facility, Wageningen University and Research, Stippeneng 4, 6708 WE Wageningen, The Netherlands

⁴Faculty of Applied Sciences, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands

(Received 20 September 2017; accepted 6 December 2017; published online 22 December 2017)

We present a fast and model-free 2D and 3D single-molecule localization algorithm that allows more than 3×10^6 localizations per second to be calculated on a standard multi-core central processing unit with localization accuracies in line with the most accurate algorithms currently available. Our algorithm converts the region of interest around a point spread function to two phase vectors (phasors) by calculating the first Fourier coefficients in both the x- and y-direction. The angles of these phasors are used to localize the center of the single fluorescent emitter, and the ratio of the magnitudes of the two phasors is a measure for astigmatism, which can be used to obtain depth information (z-direction). Our approach can be used both as a stand-alone algorithm for maximizing localization speed and as a first estimator for more time consuming iterative algorithms. © 2017 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>). <https://doi.org/10.1063/1.5005899>



Variants worth knowing...

Bioinformatics. 2014 Aug 15;30(16):2389-90. doi: 10.1093/bioinformatics/btu202. Epub 2014 Apr 25.

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²Laboratory of Bionanotechnology, Wageningen University, 6708 WG Wageningen, The Netherlands

³Microspectroscopy Research Facility, Wageningen University, 6708 WE Wageningen, The Netherlands

⁴Faculty of Applied Sciences, Delft University of Technology, 2628 CD Delft, The Netherlands

(Received 20 September 2017; accepted 20 September 2017)

We present a fast and model-free 2D algorithm for single-molecule localization microscopy (SMLM) with localization accuracies in line with the theoretical limit. The algorithm converts the region of interest into a phasor space by calculating the first Fourier coefficients of the intensity profiles. These are used to localize the center of the single-molecule spot. The phasor space two phasors is a measure for astigmatism. Our approach can be used both as a standalone algorithm or as a first estimator for more time consuming algorithms. *except where otherwise noted, is licensed under a Creative Commons Attribution 4.0 International License* (<http://creativecommons.org/licenses/by/4.0/>)

GitHub, Inc. [US] | <https://github.com/kjamartens/thunderstorm/tree/phasor-intensity-1/Compiled%20plugin>

Cristina Ricardo

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kjamartens / thunderstorm
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Code Pull requests 1 Projects 0 Wiki Insights

Branch: phasor-intensi... thunderstorm / Compiled plugin /

Create new file Upload

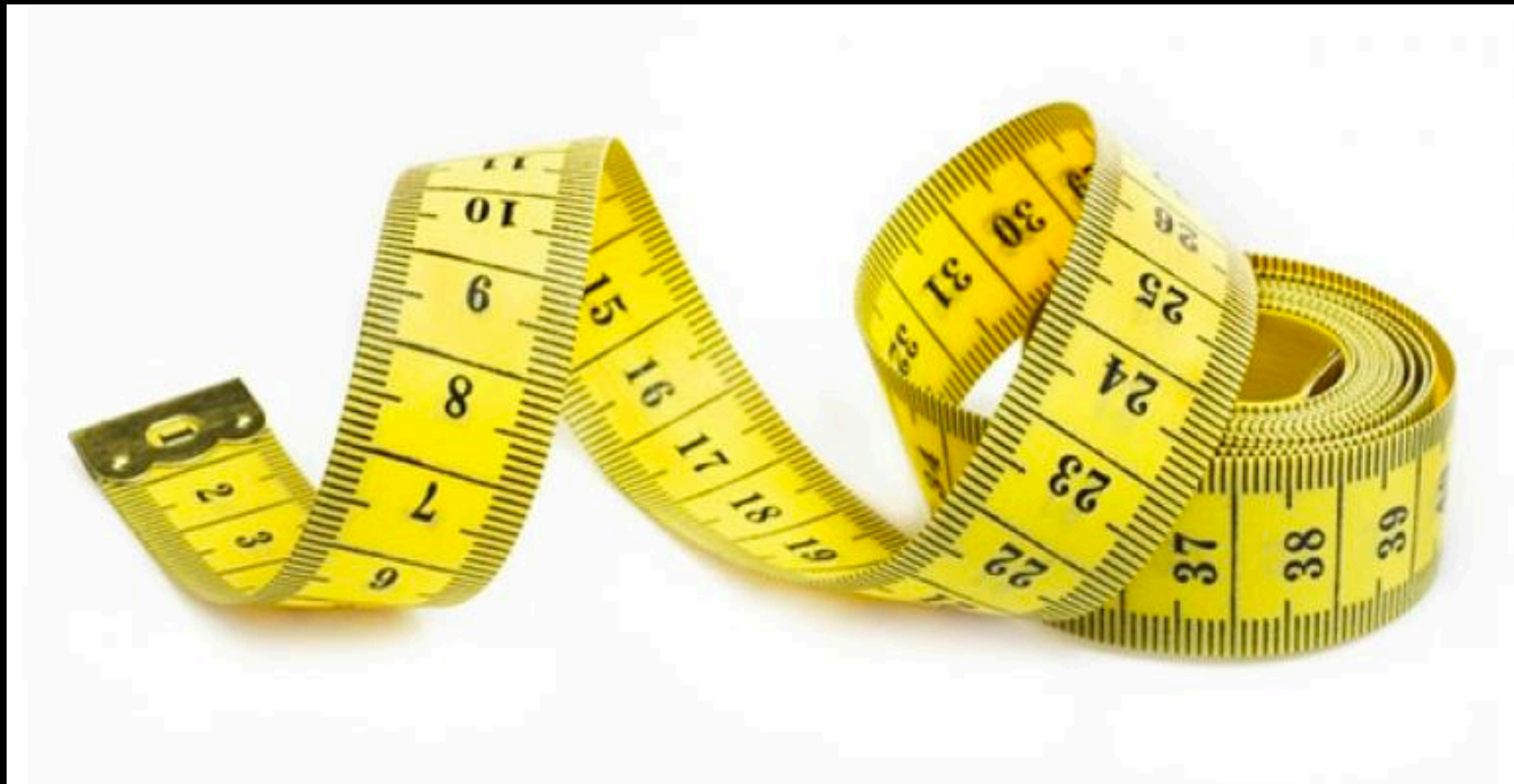
This branch is 23 commits ahead, 3 commits behind zitmen:master.

kjamartens Update 19-03-2018 - fixing banding issue Latest commit

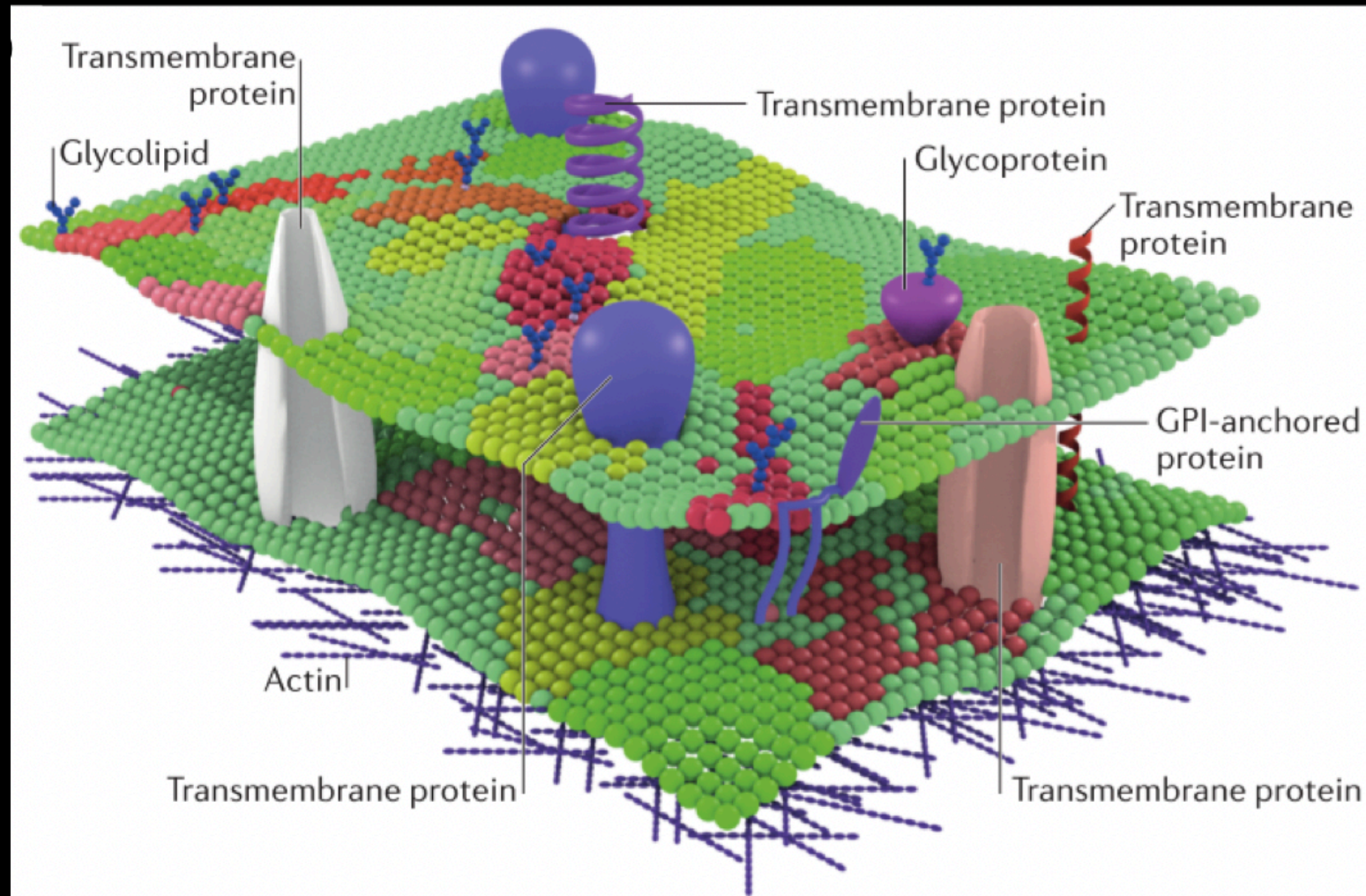
..

Deprecated versions	Update 19-03-2018 - fixing banding issue
Thunder_STORM-FIJI_20180319.jar	Update 19-03-2018 - fixing banding issue
Thunder_STORM-ImageJ_20180319.jar	Update 19-03-2018 - fixing banding issue

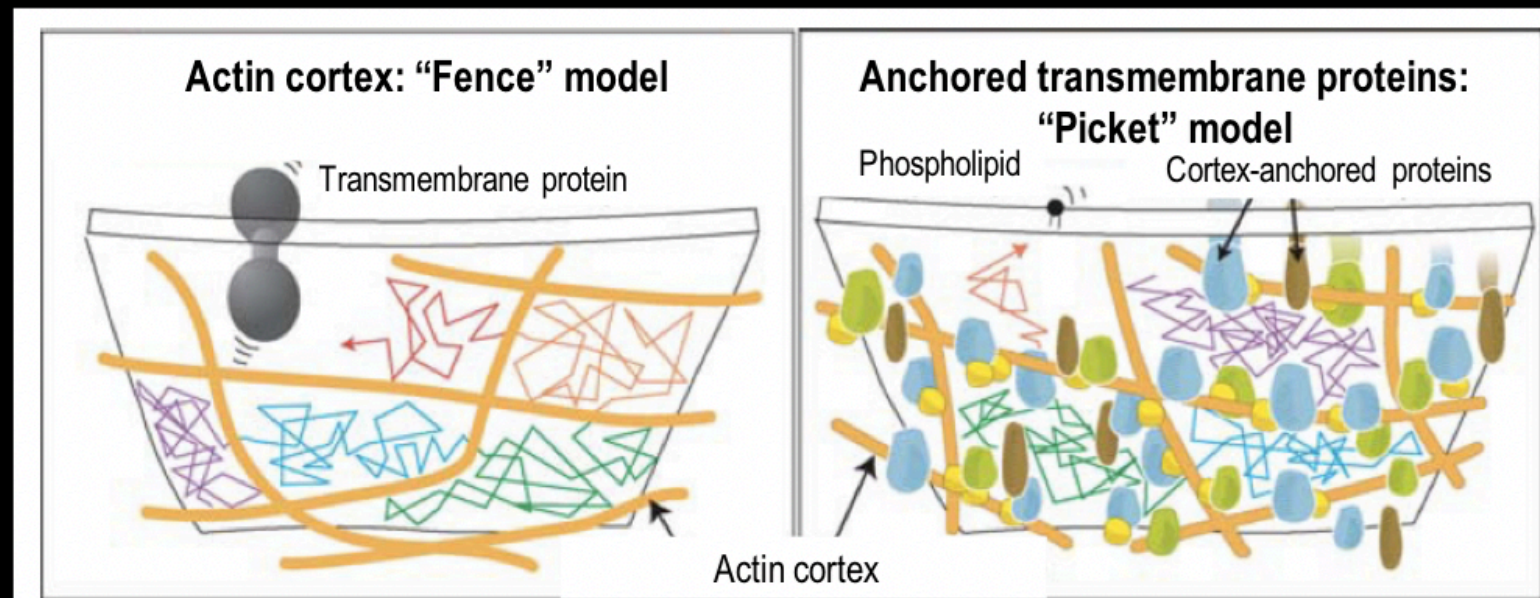
We have our data,
what about now?



Study protein clustering....

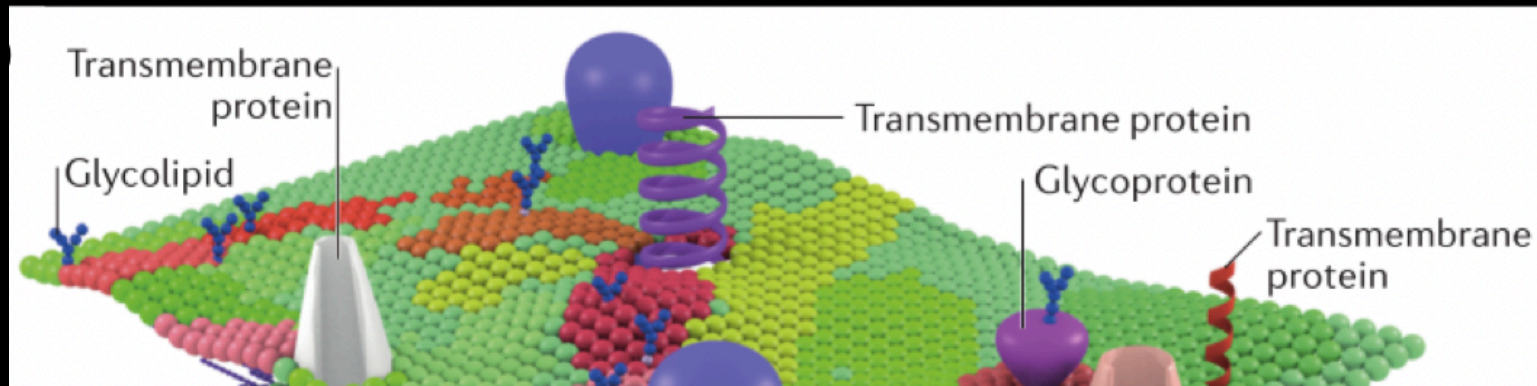


Sezgin et al. 2017



Kusumi et al. 2005

Study protein clustering...

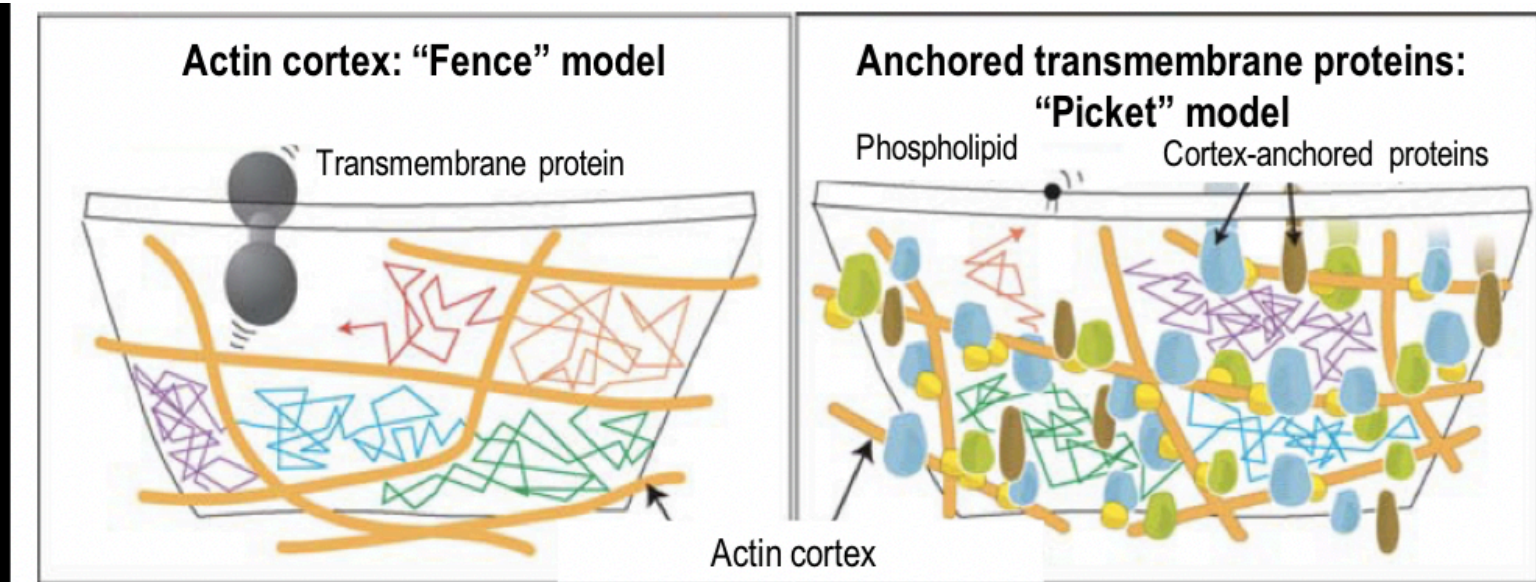


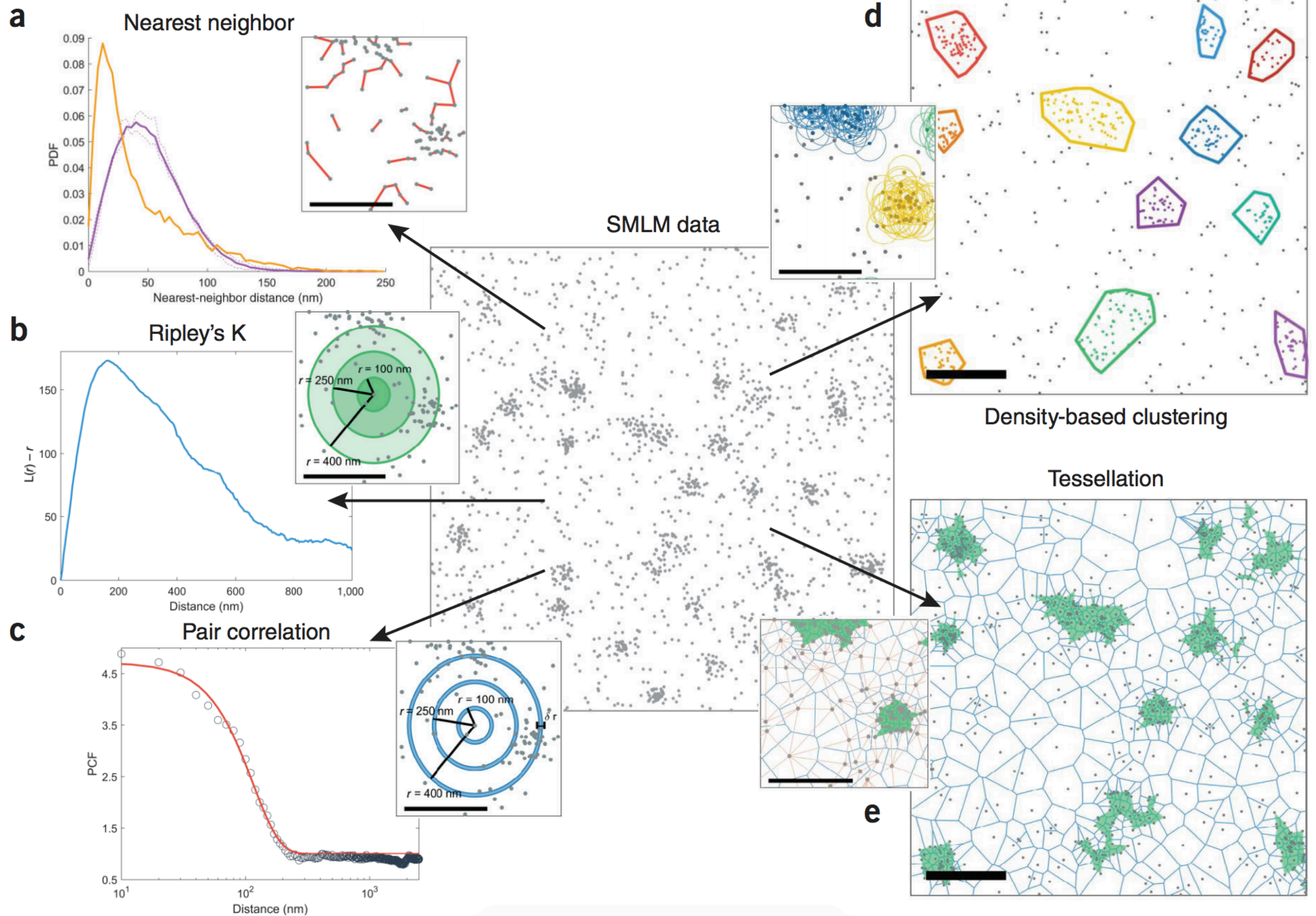
Turning single-molecule localization microscopy into a quantitative bioanalytical tool

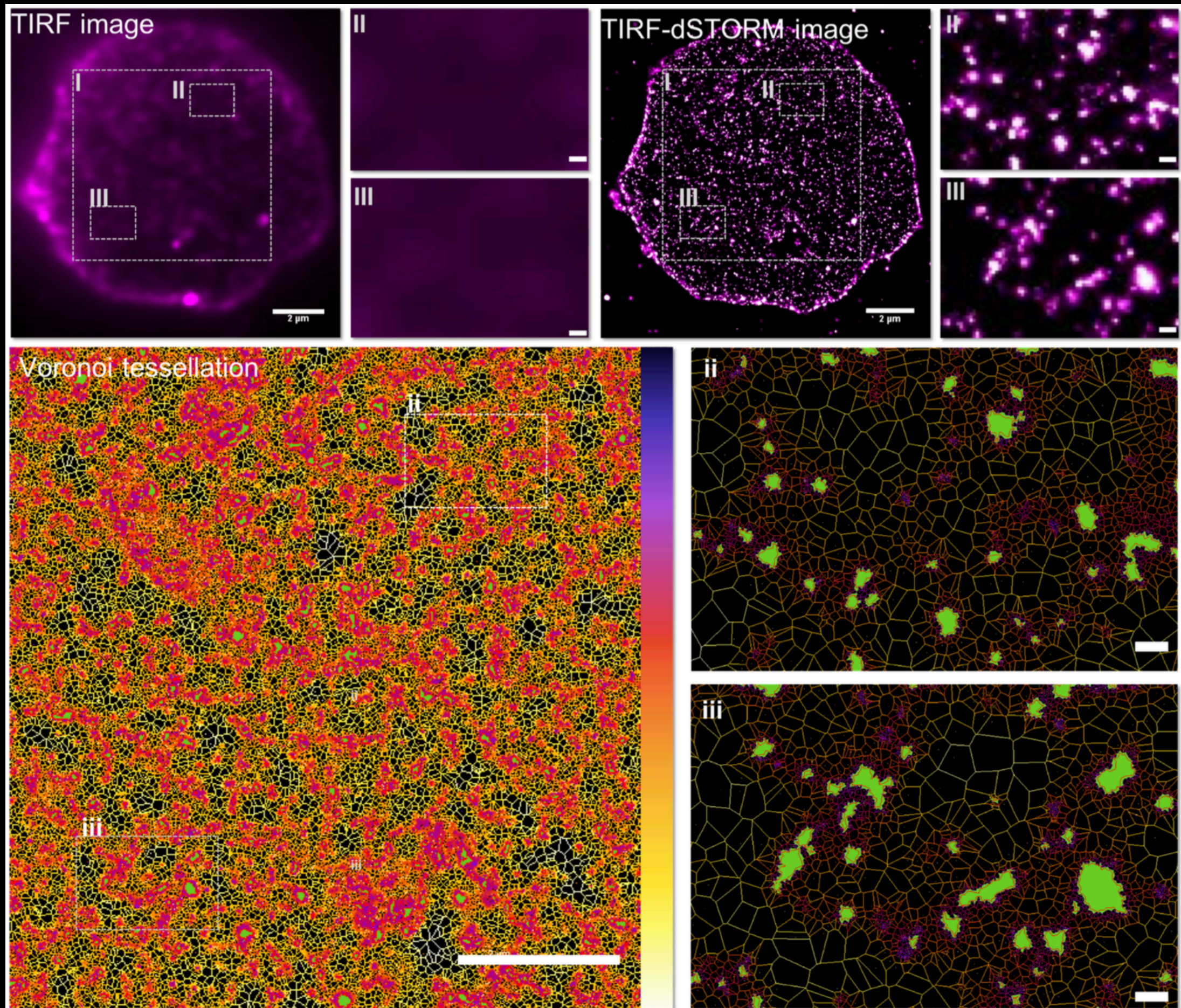
[PR Nicovich](#), [DM Owen](#), K Gaus - Nature protocols, 2017 - [nature.com](#)

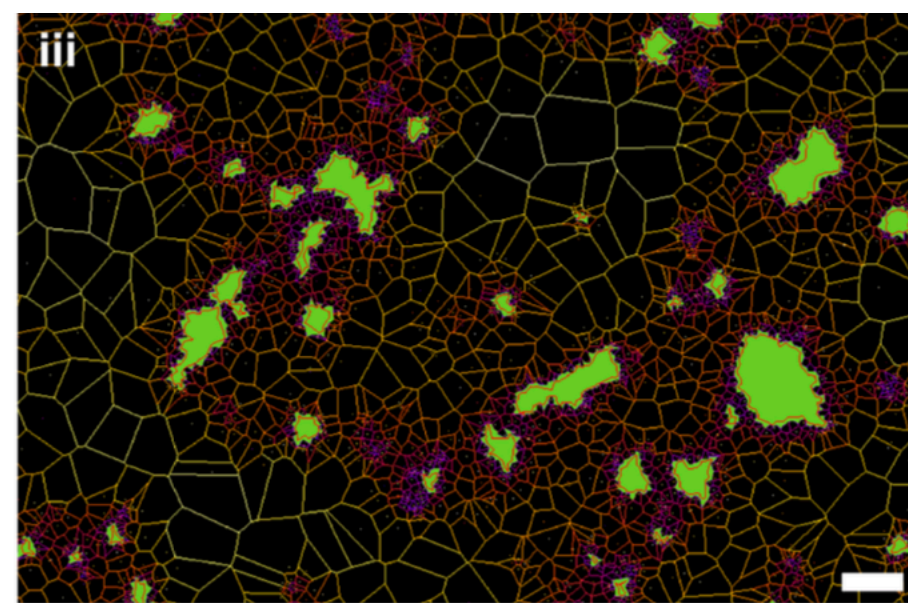
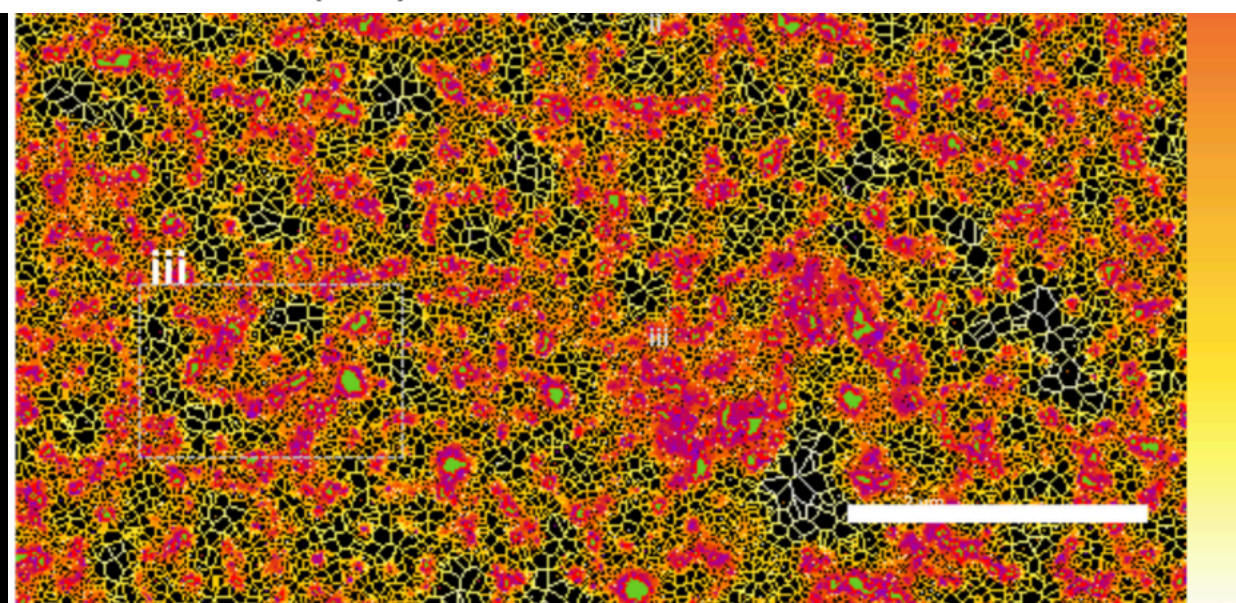
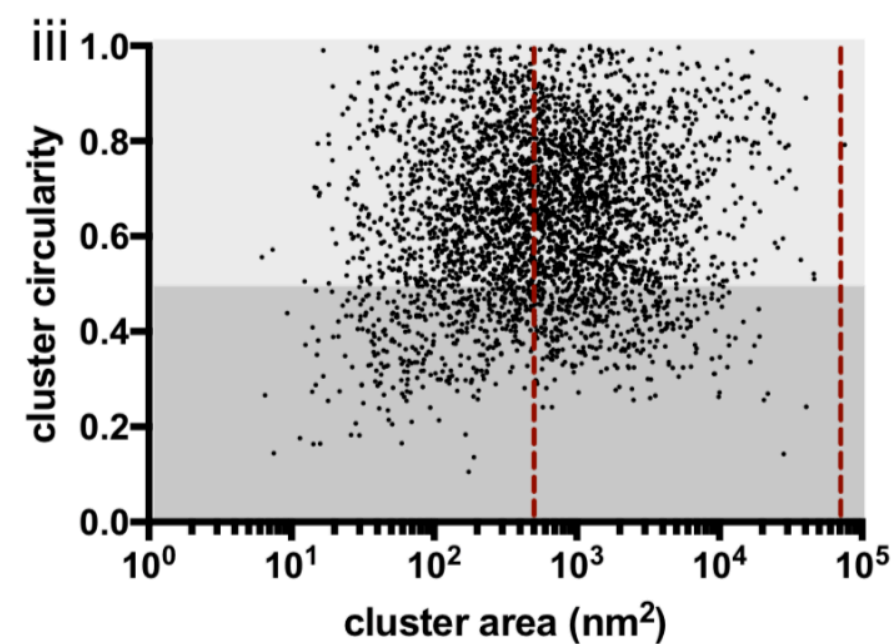
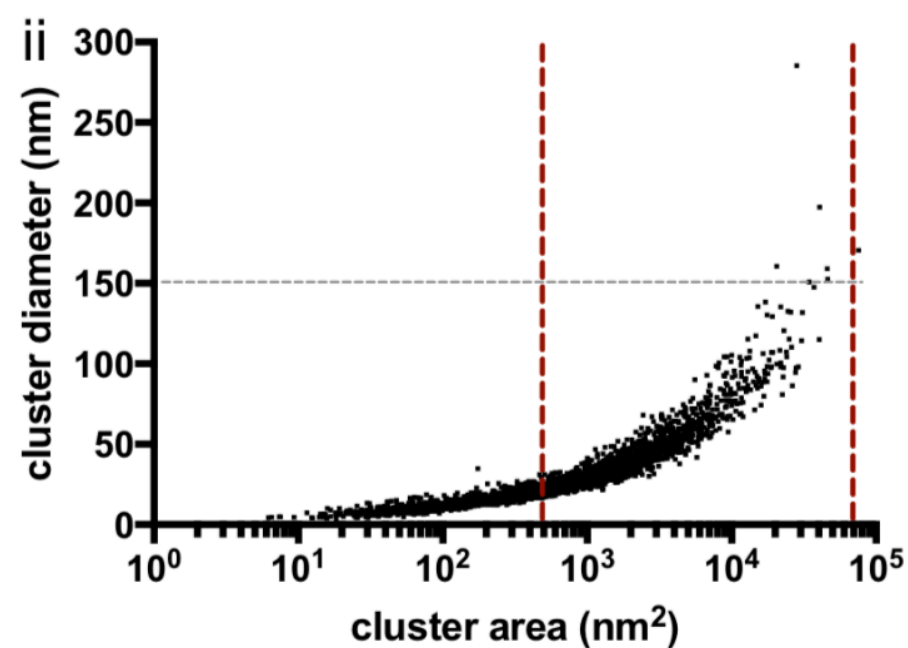
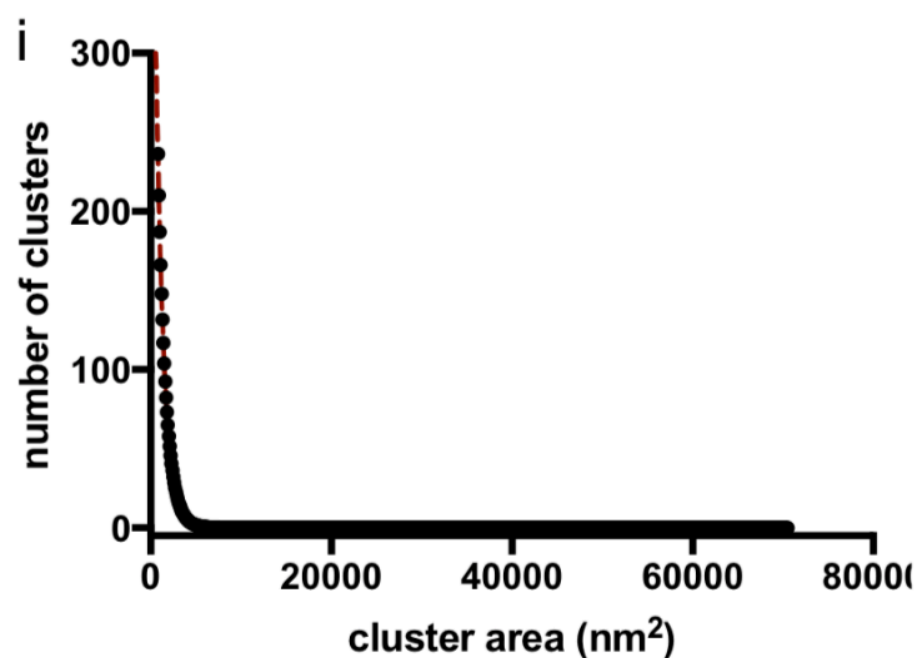
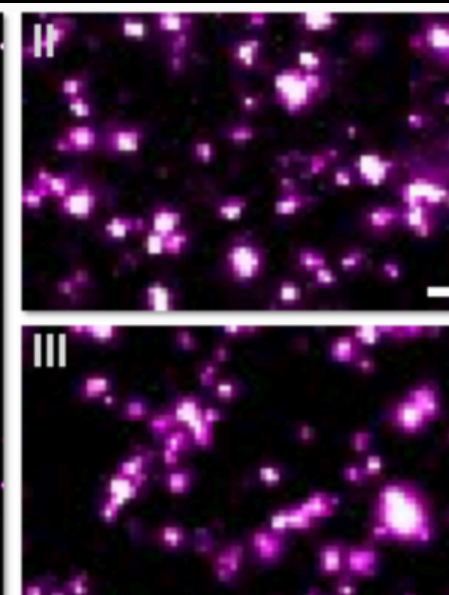
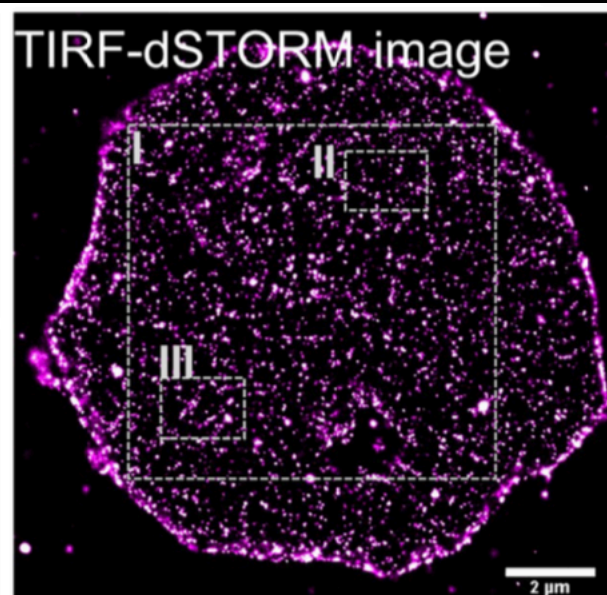
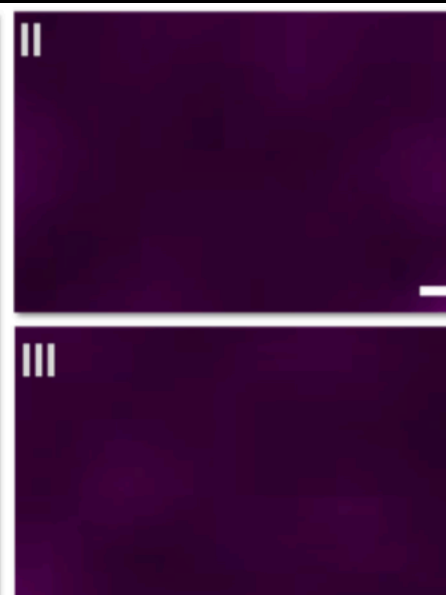
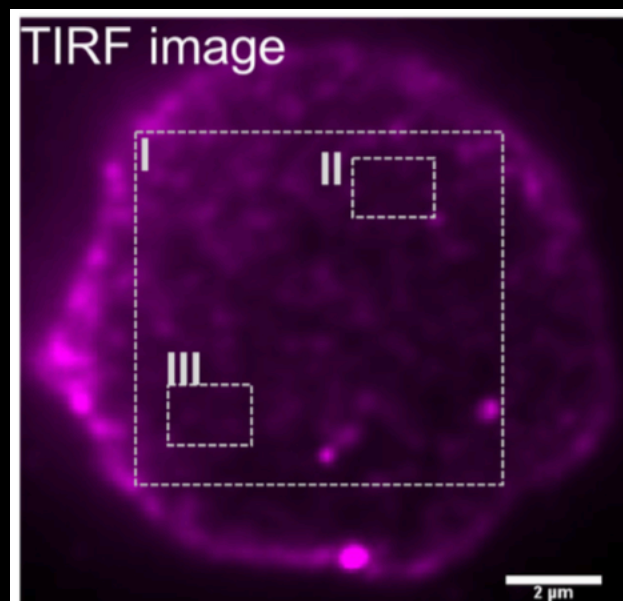
Single-molecule localization microscopy (SMLM) generates super-resolution images by serially detecting individual fluorescent molecules. The power of SMLM, however, goes beyond images: biologically relevant information can be extracted from the mathematical relationships between the positions of the fluorophores in space and time. Here we review the history of SMLM and how recent progress in methods for spatial point analysis has enabled quantitative measurement of SMLM data, providing insights into biomolecule ...

☆ 𐀀 Cited by 22 Related articles All 6 versions Web of Science: 12 𐀀

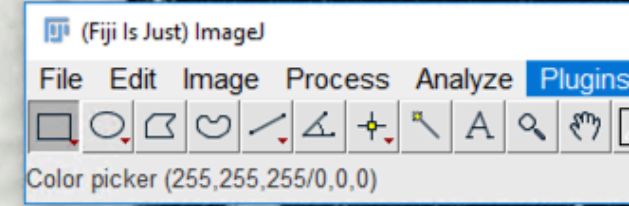
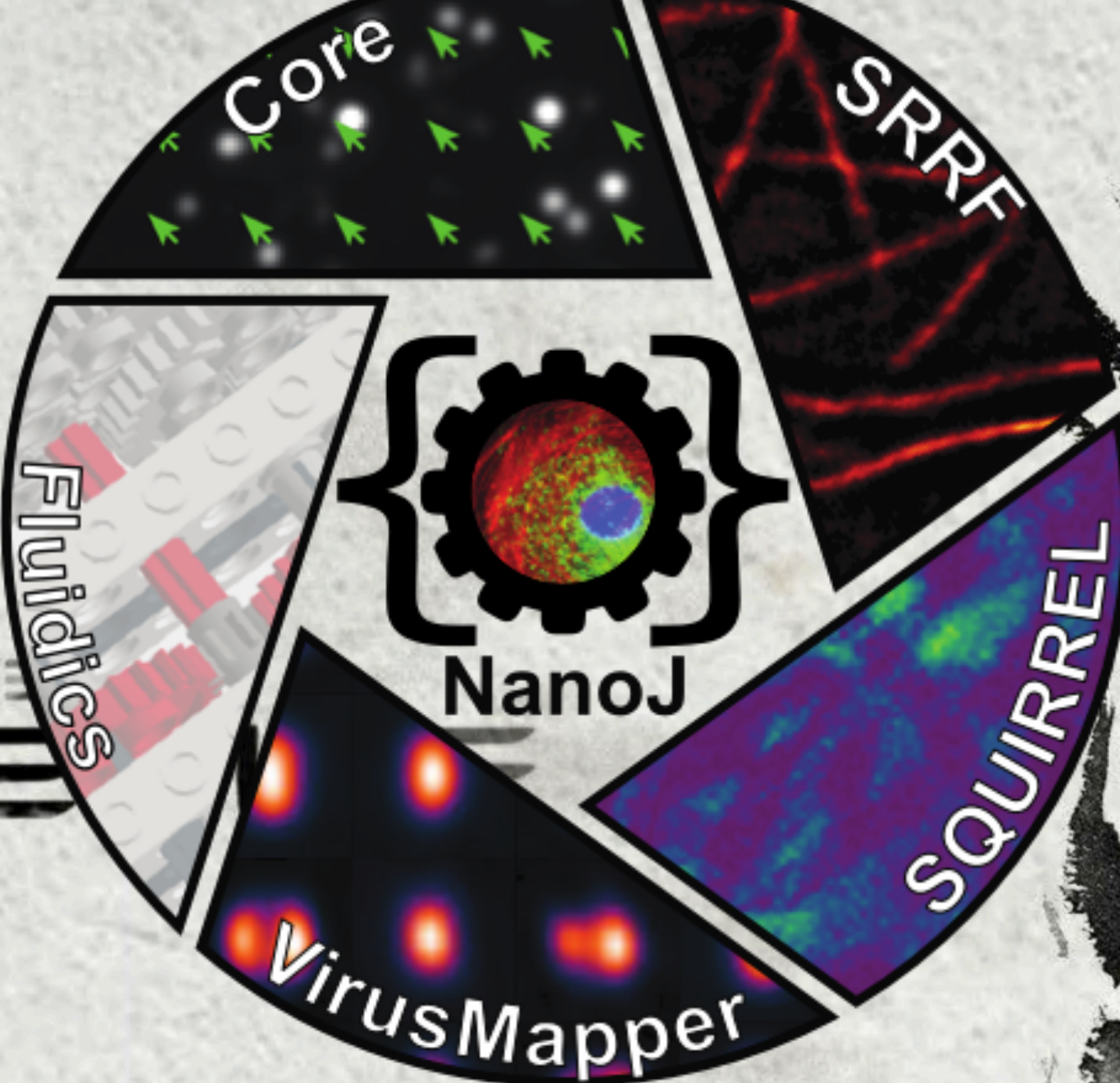








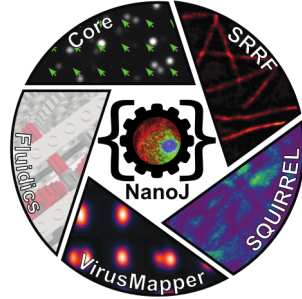
NanoJ - A little bit
about us!



- Drift Correction
- OpenCL
- Peak Localisation
- Registration
- Open NanoJ Image (NJI)...
- Save Image as NJI...
- Convert image sequence files into NJIs...
- Open NanoJ Table (NJT)...
- Save Results-Table as NJT...
- Convert Results-Table files (.xls) into NJTs...
- Set Compression (NJI & NJT)...
- Open image files in folder as image sequence...
- Extract ROIs and save as NJIs...
- Stop NanoJ execution...
- Reset Preferences...
- Show MTA
- NanoJ-Core Version
- NanoJ-Core What's New
- NanoJ-Core User Manual and Paper

- Feature Extraction
- HDF5
- Image5D
- Integral Image Filters
- Janelia H265 Reader
- LOCI
- LSM Toolbox
- Landmarks
- Multiview Reconstruction
- NanoJ-Core**
- NanoJ-Fluidics
- NanoJ-LiveSRRF
- NanoJ-SQUIRREL
- NanoJ-SRRF
- NanoJ-TheSims
- NanoJ-VirusMapper
- OptiJ
- Optic Flow
- Process
- Radial Profile
- Registration
- SPIM Registration
- Segmentation
- Skeleton
- Stacks
- Stitching
- ThunderSTORM
- Time Lapse
- Tracking
- Transform
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- Volume Viewer
- Debug
- Sandbox
- AutoRun
- Kymograph
- Musical
- Scripting
- SulfoscopeAnalysisGUILiveSRRF

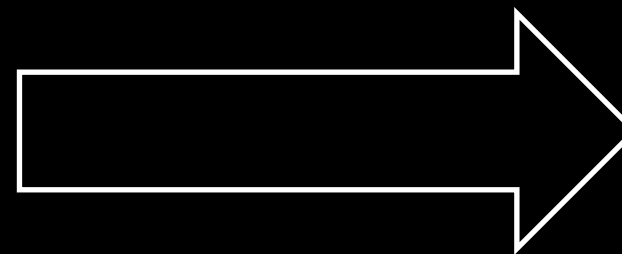
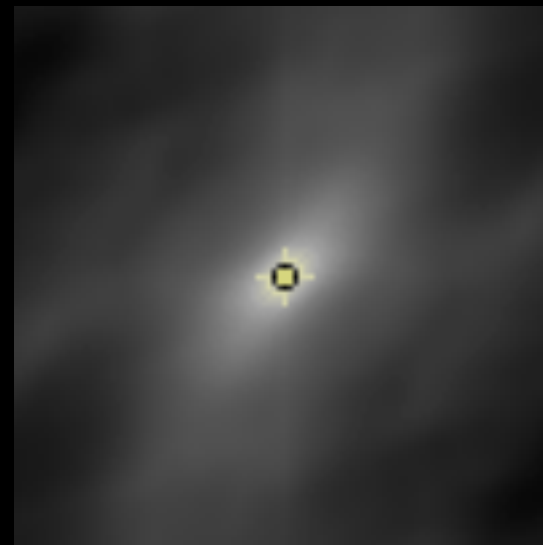
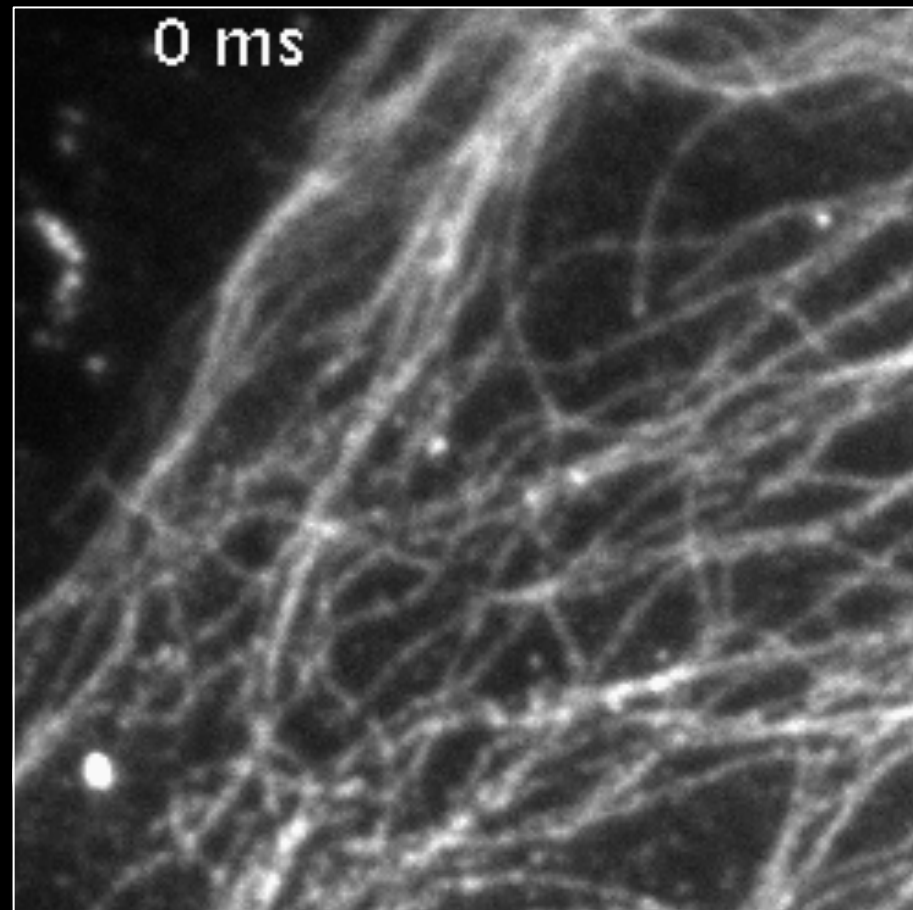
Laine R.F., Tosheva K., *et al.*, 'NanoJ: a high-performance open-source super-resolution microscopy toolbox'. J.Phys.D (2019)



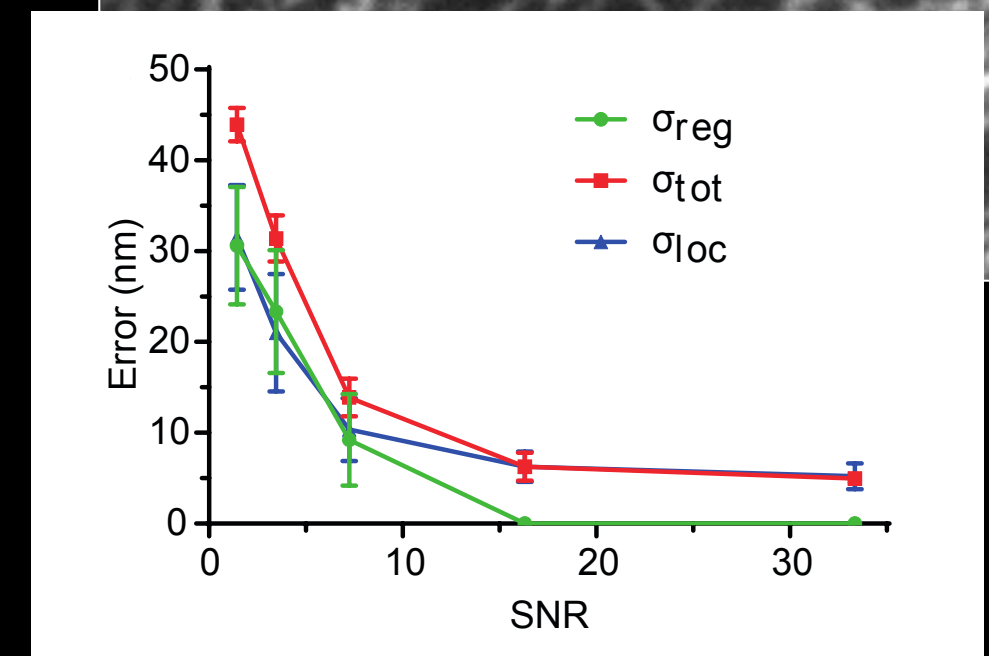
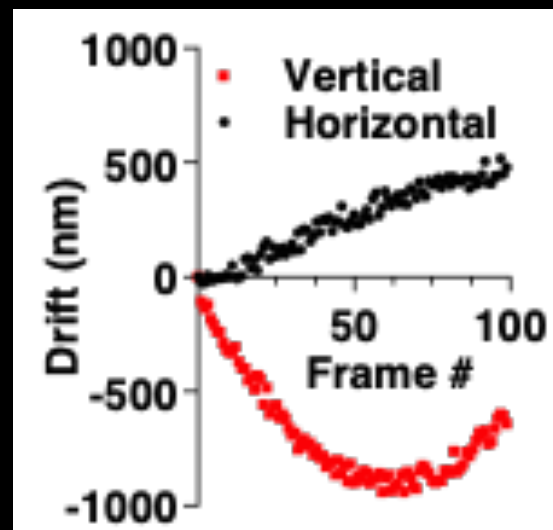
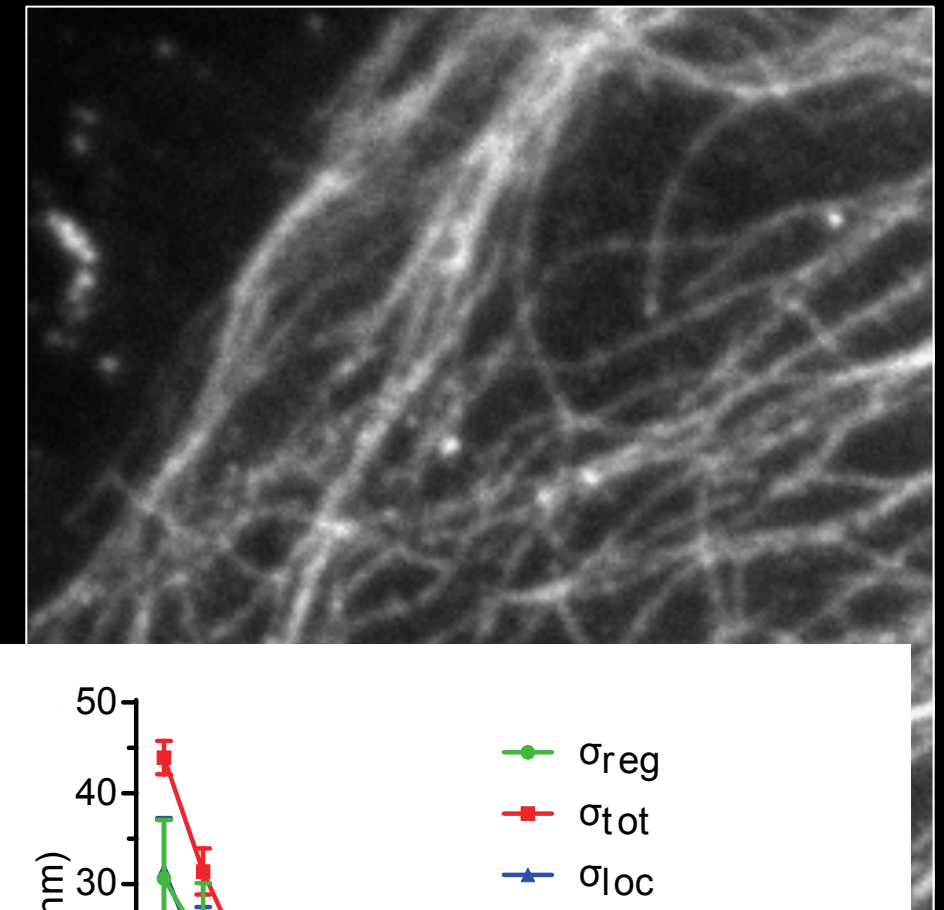
Pre-processing steps

CCM

Prior to drift correction



After drift correction



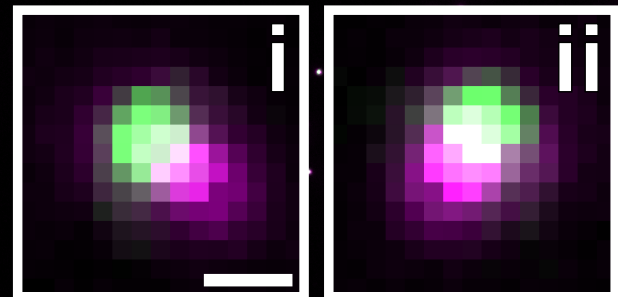
Channel alignment

NanoJ-Core

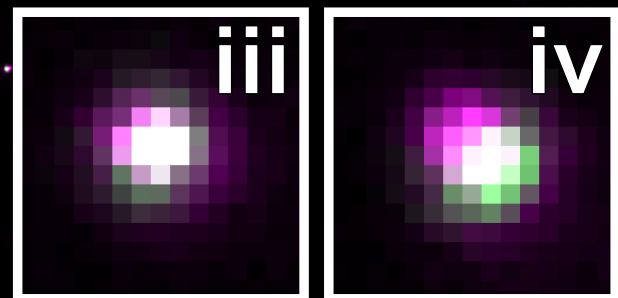
Before registration

i

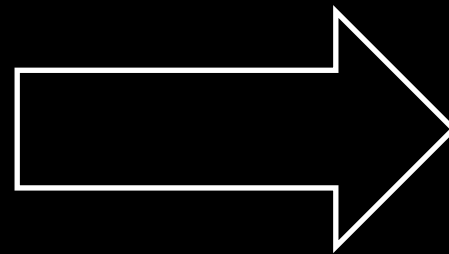
ii



iii



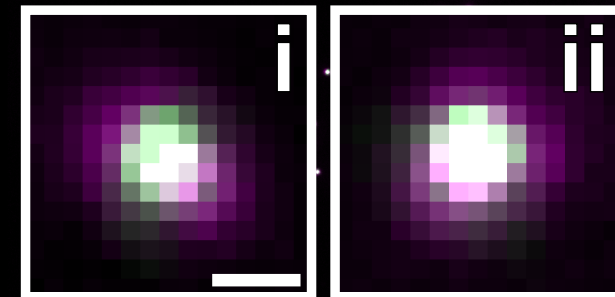
iv



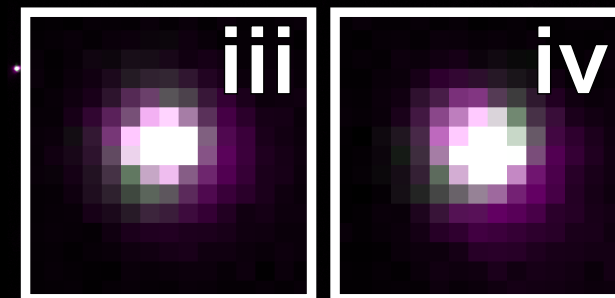
After registration

i

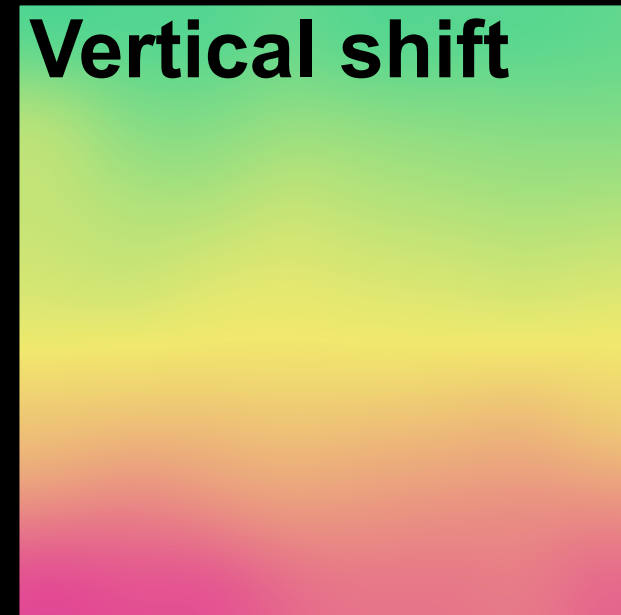
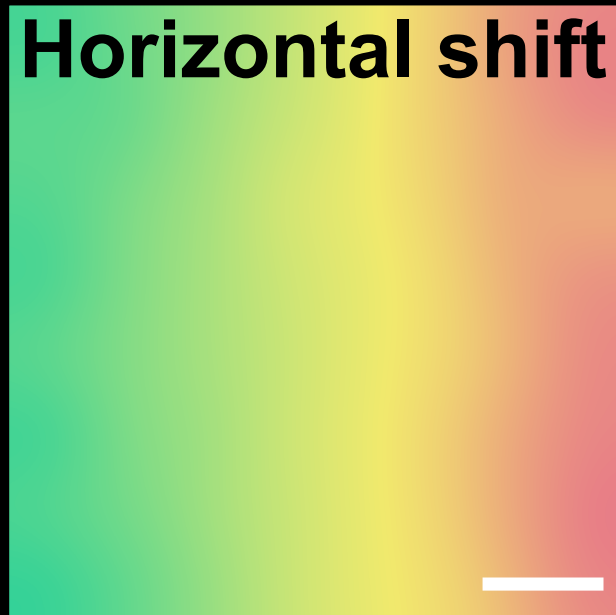
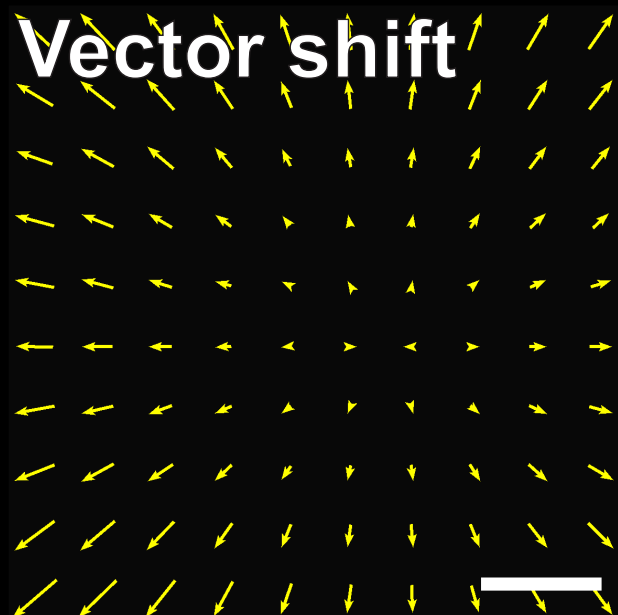
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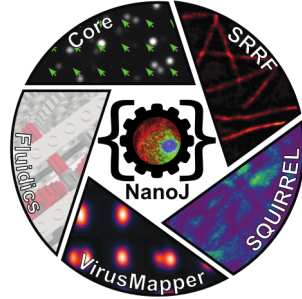


iii



iv





Super-Resolution image reconstruction

small organisms,
nano-histology of
tissues

plant cell

animal cell

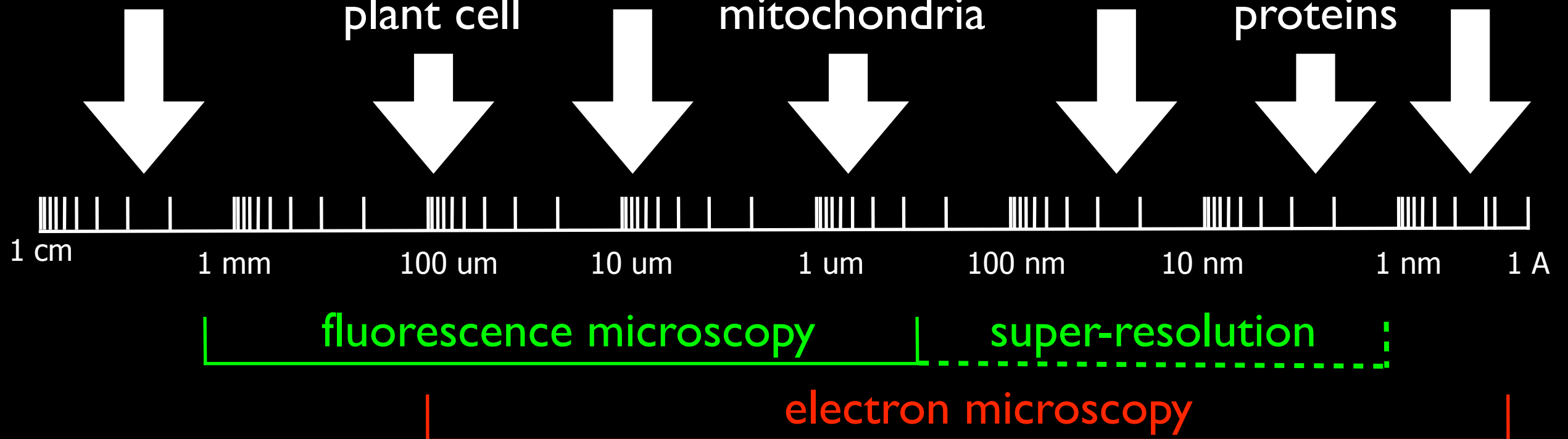
mitochondria

bacteria,

viruses,
vesicles

proteins

small
molecules

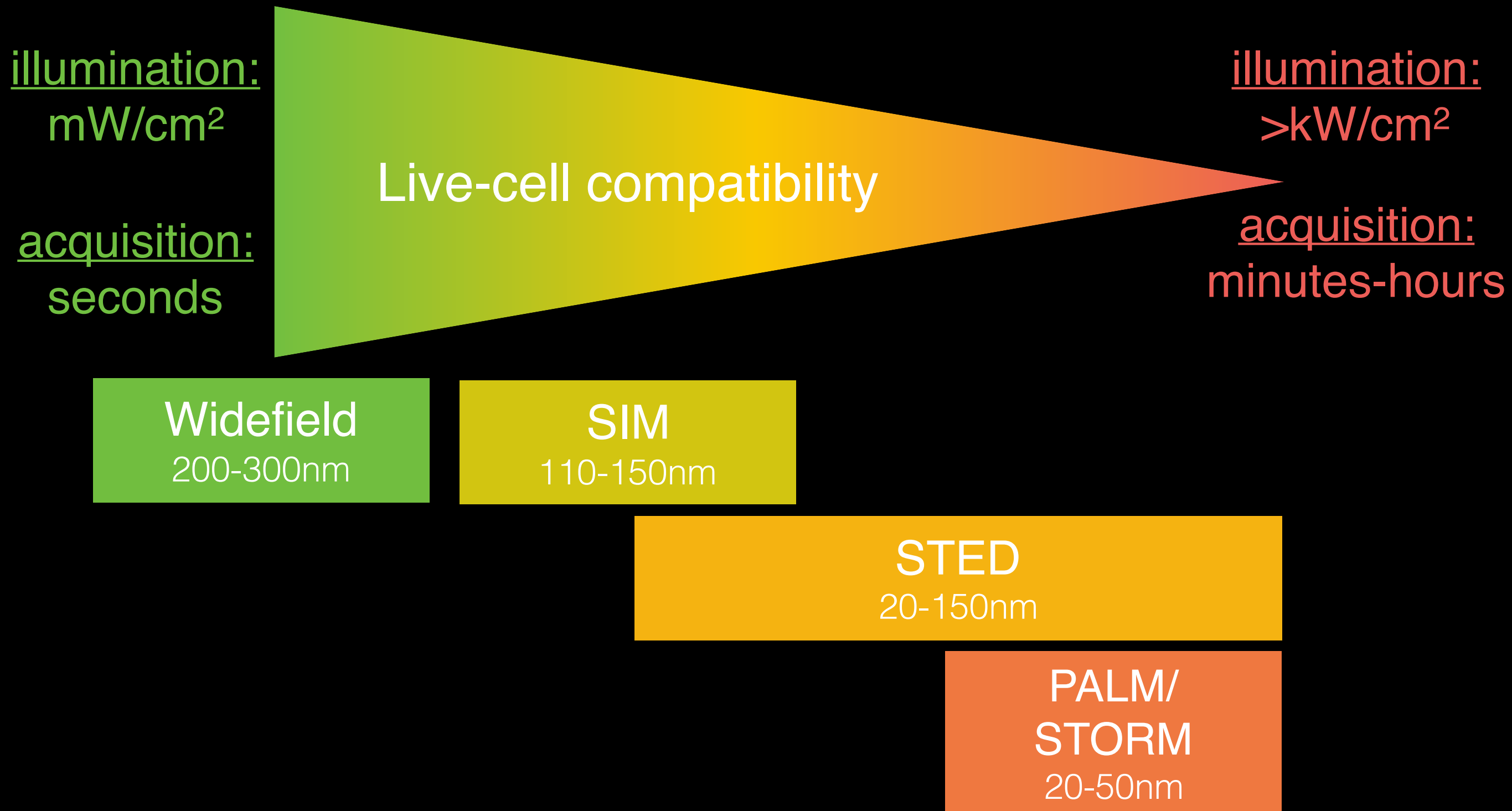


Reviewers:

HFSP: “this would be a great project for Cryo-EM”

MRC: “super-resolution in general should be seen at face value as a sophisticated way of analyzing blobs that can only be complementary to high-resolution EM”

Live-cell super-resolution

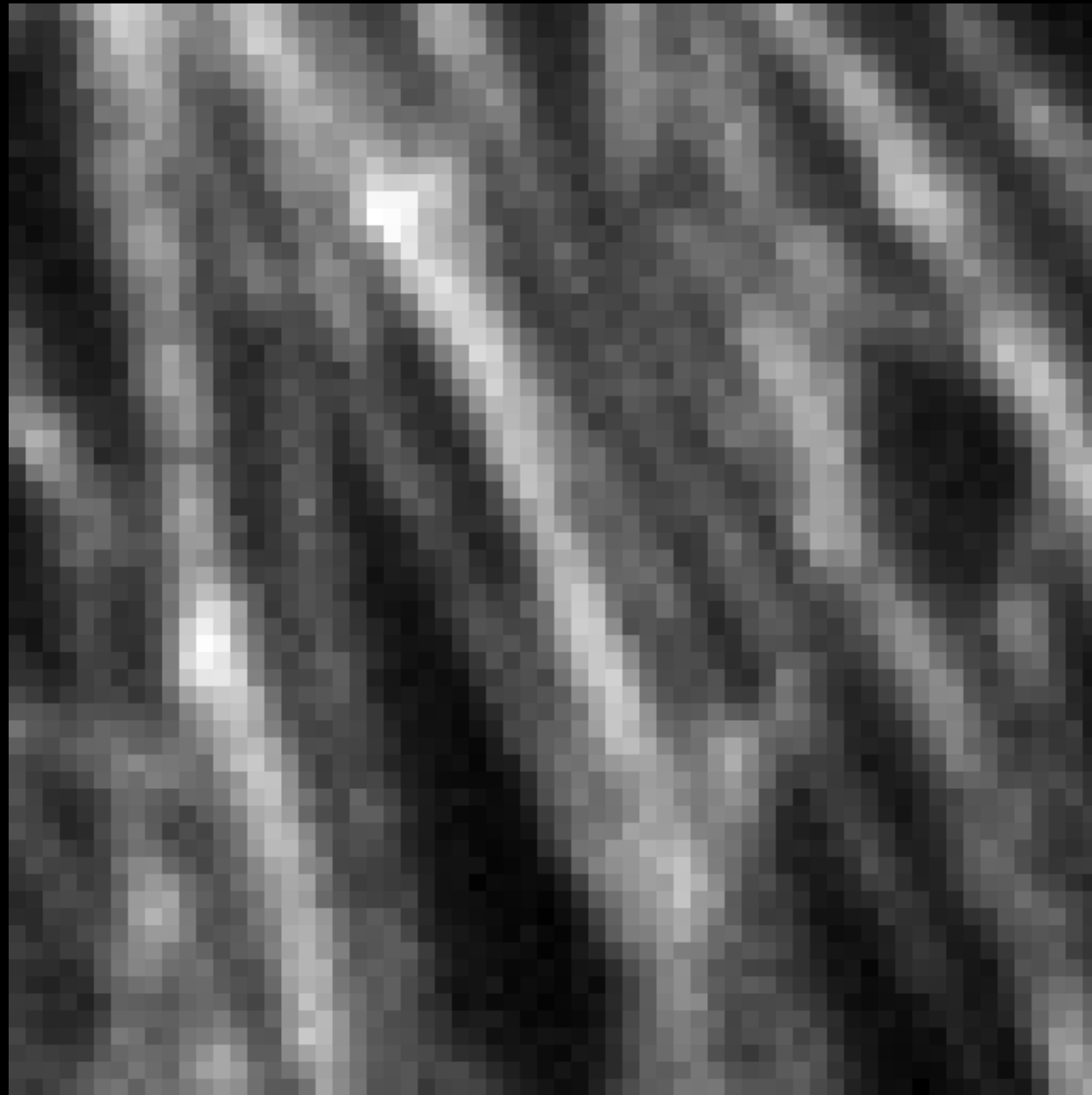


Super-Resolution Radial Fluctuations (SRRF, reads as *surf*)

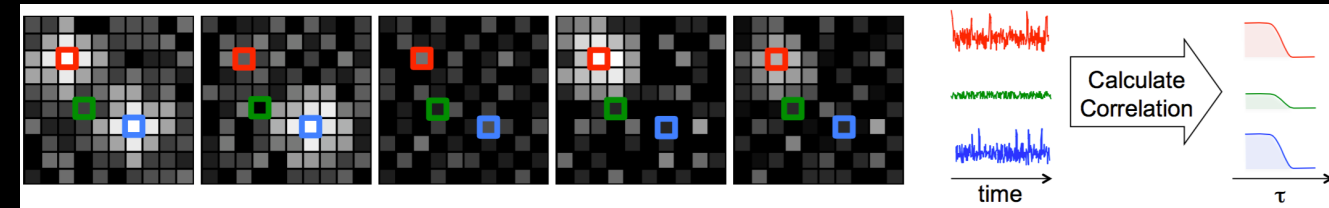
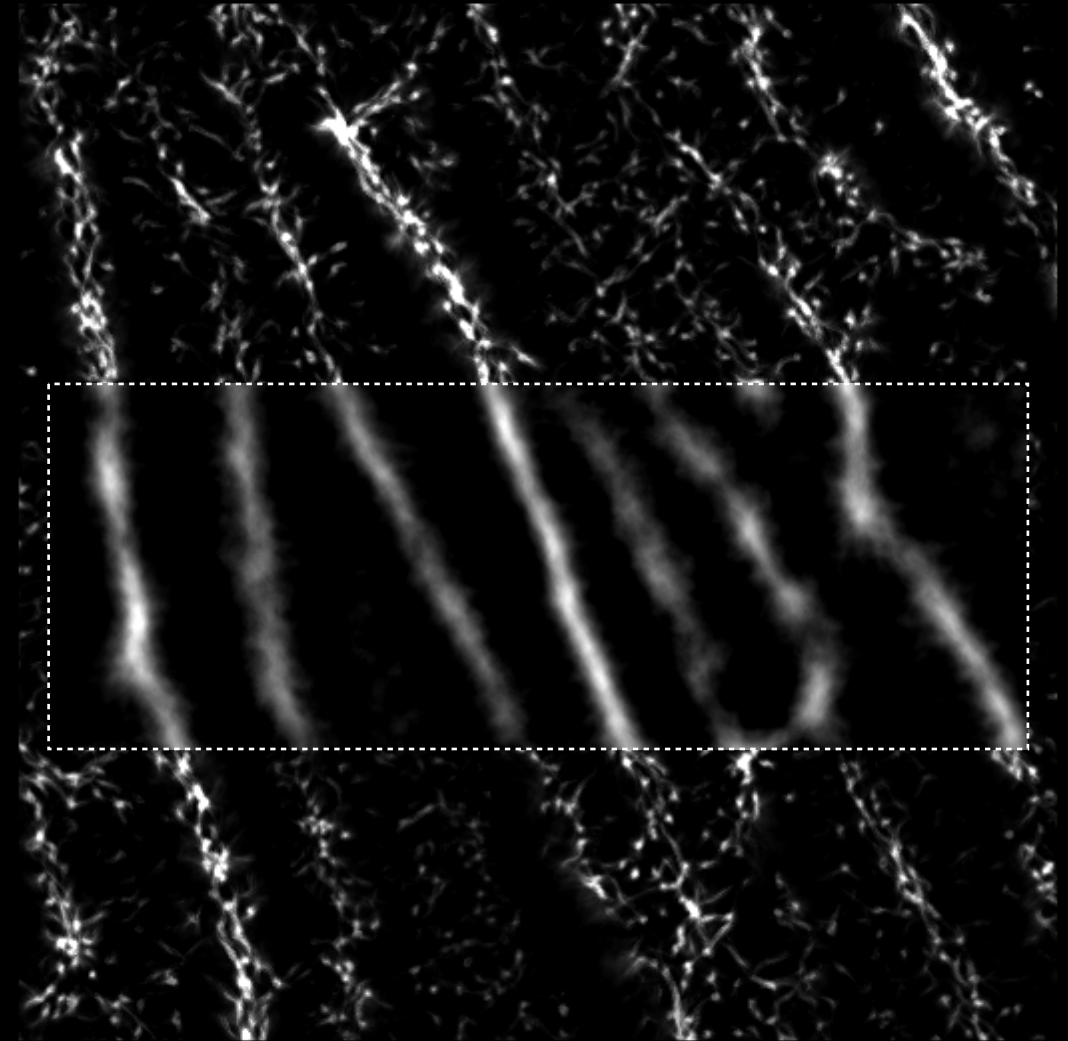


100 frames at 40mW/cm²

Super-Resolution Radial Fluctuations (SRRF, reads as *surf*)



100 frames at 40mW/cm²



bottom line... for a very dead and fixed cell,
SRRF will be similar to PALM & STORM

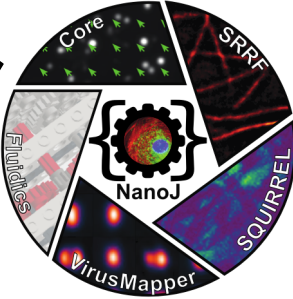
... but for a live-cell, labeled with GFP,
using 10.000-fold less illumination



Siân Culley

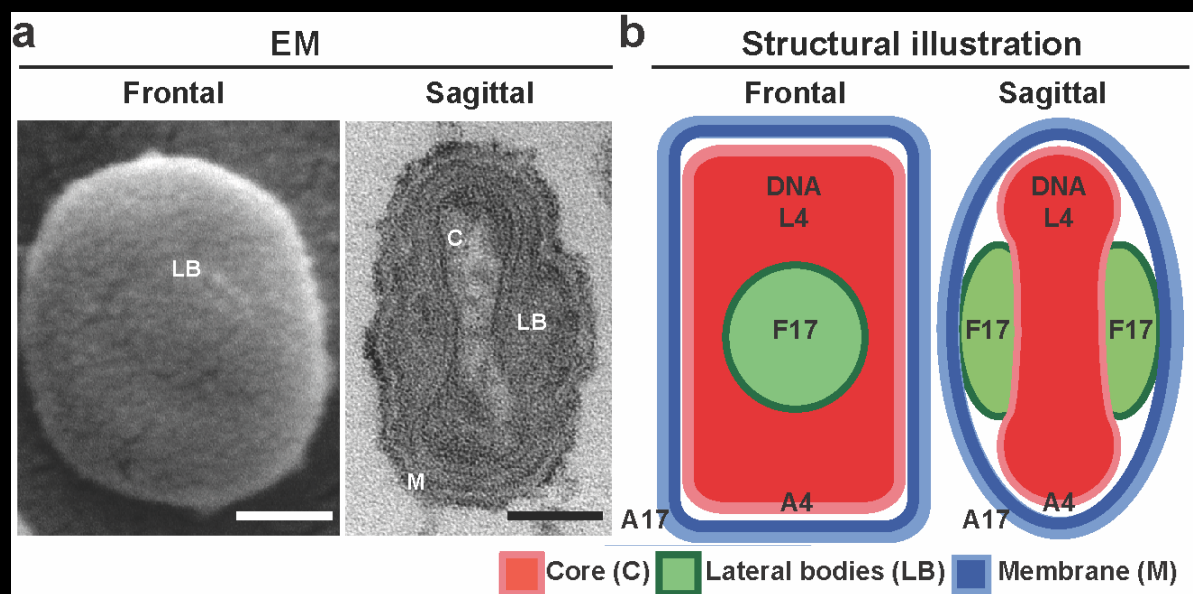
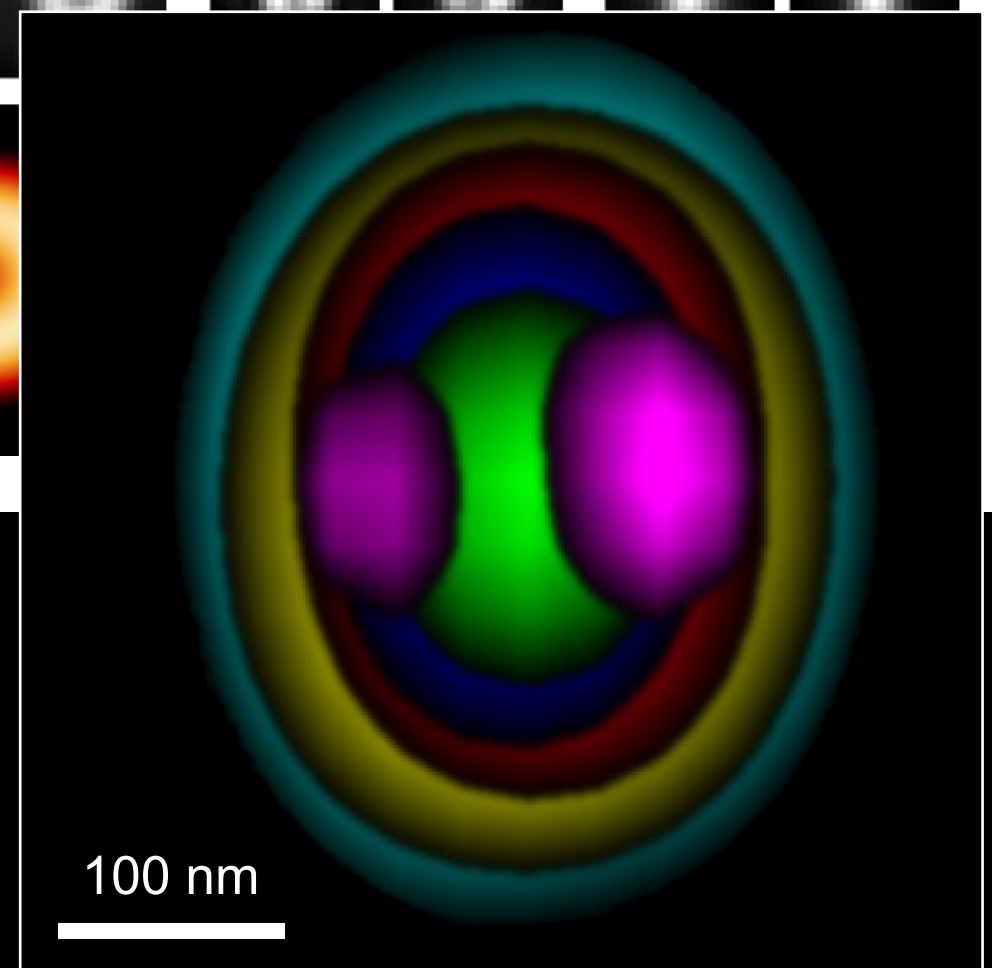
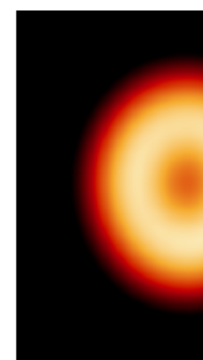
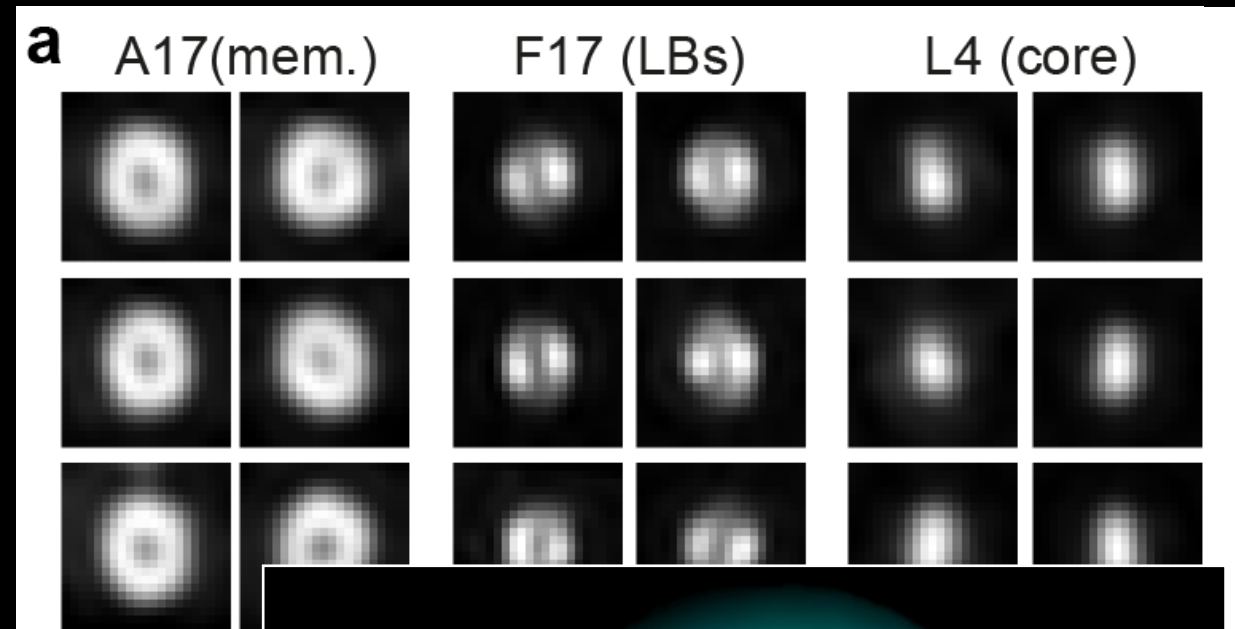
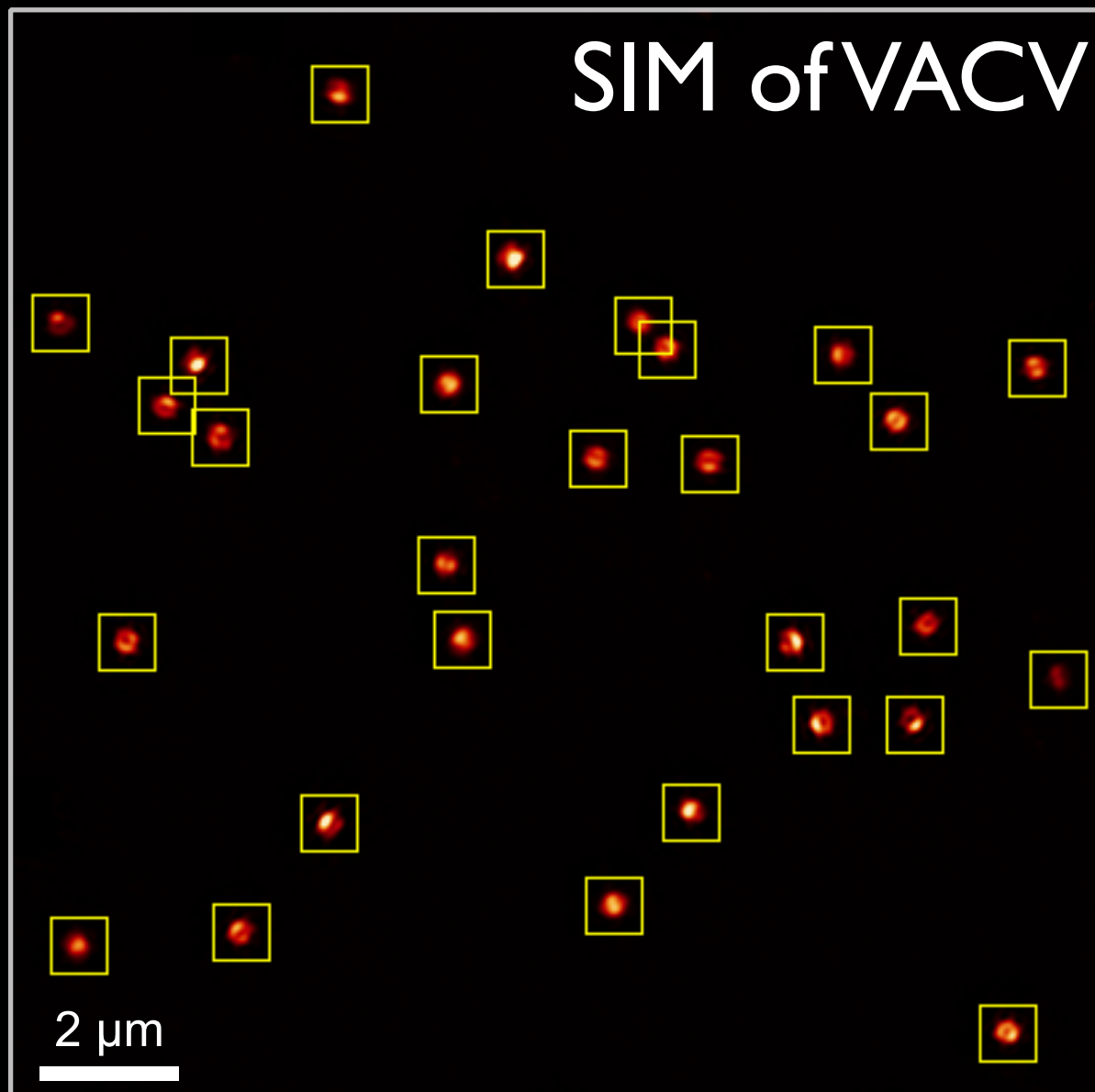


Kalina Tosheva



Single-particle averaging

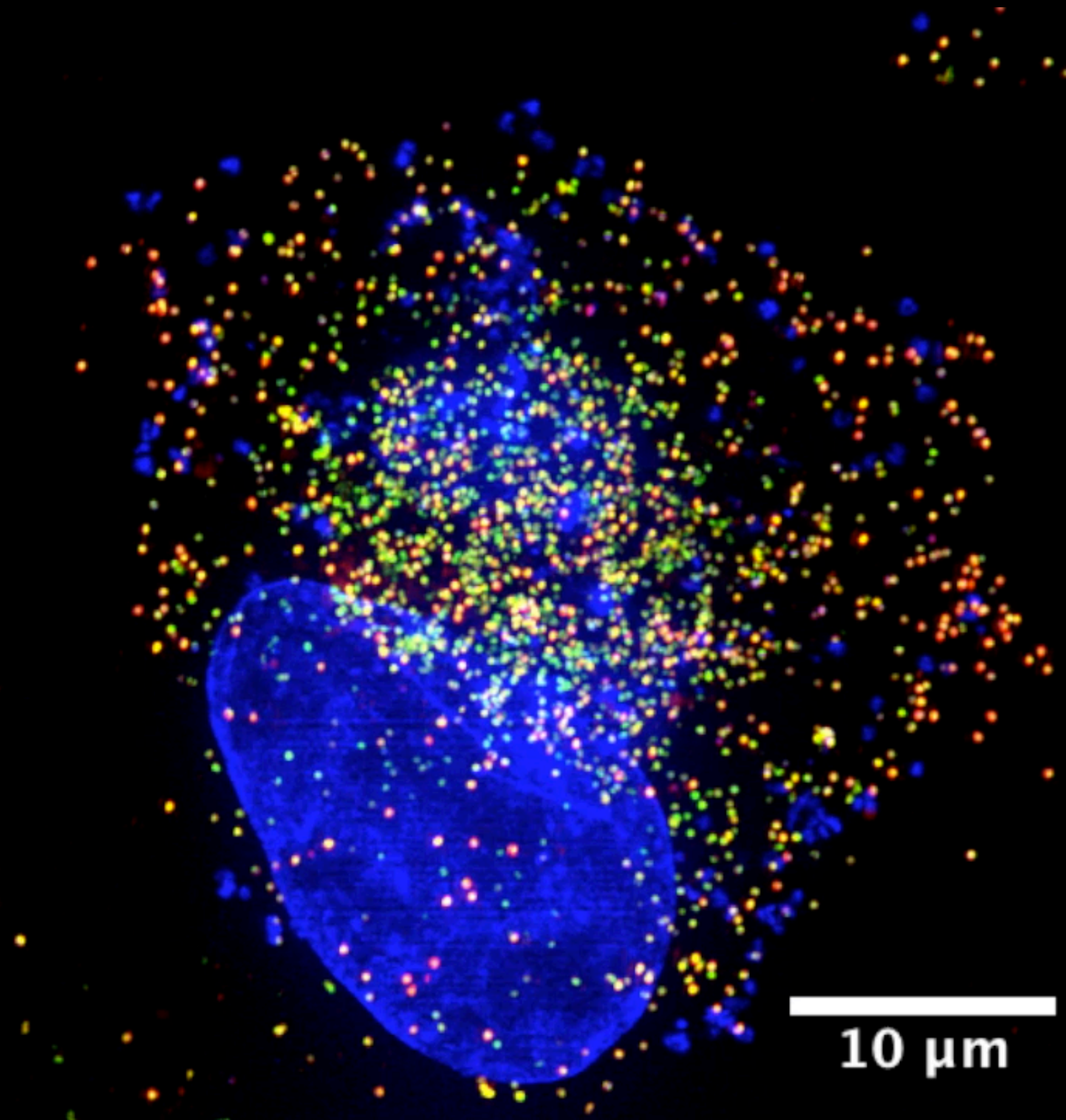
Single-particle averaging (VirusMapper)

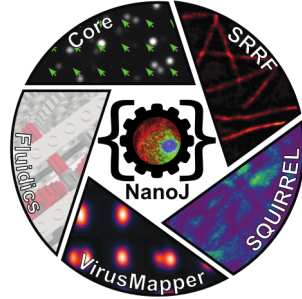


Gray R., *et al.* 'VirusMapper: open-source nanoscale mapping of viral architecture through super-resolution microscopy' *Sci. Rep.* (2016)

Single-particle averaging (VirusMapper)

NanoJ-VirusMapper



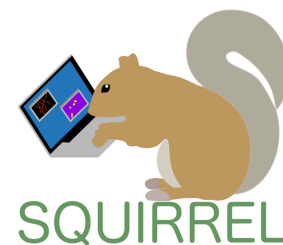


Quality control in super-resolution microscopy



Siân Culley

NanoJ-SQUIRREL - is
your image nuts?



Quality control (SQUIRREL)

NanoJ-SQUIRREL

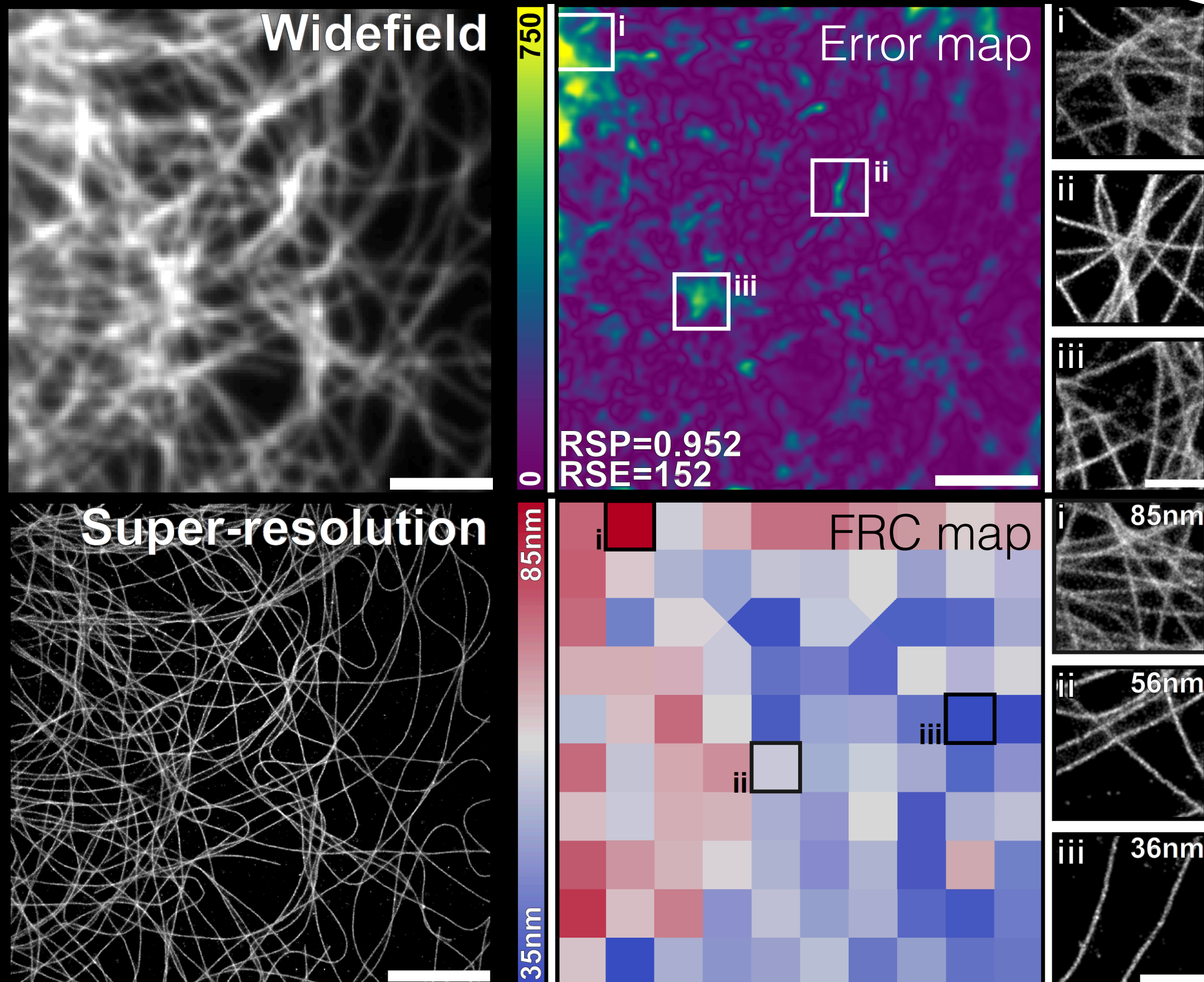
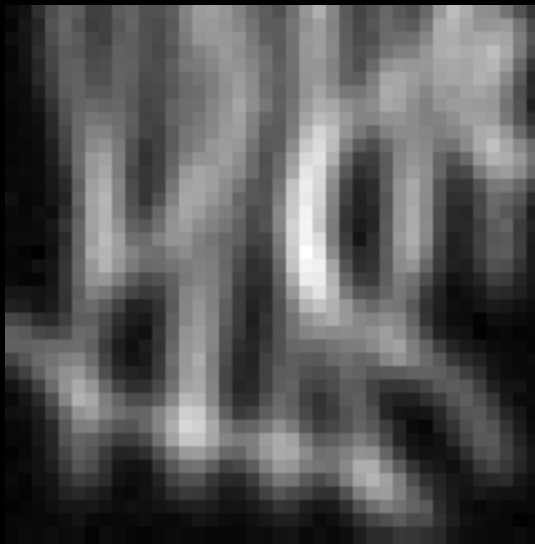


Image formation

Diffraction Limited

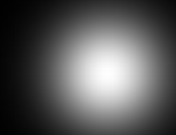


$\approx$

$\approx$



$\otimes$



$=$

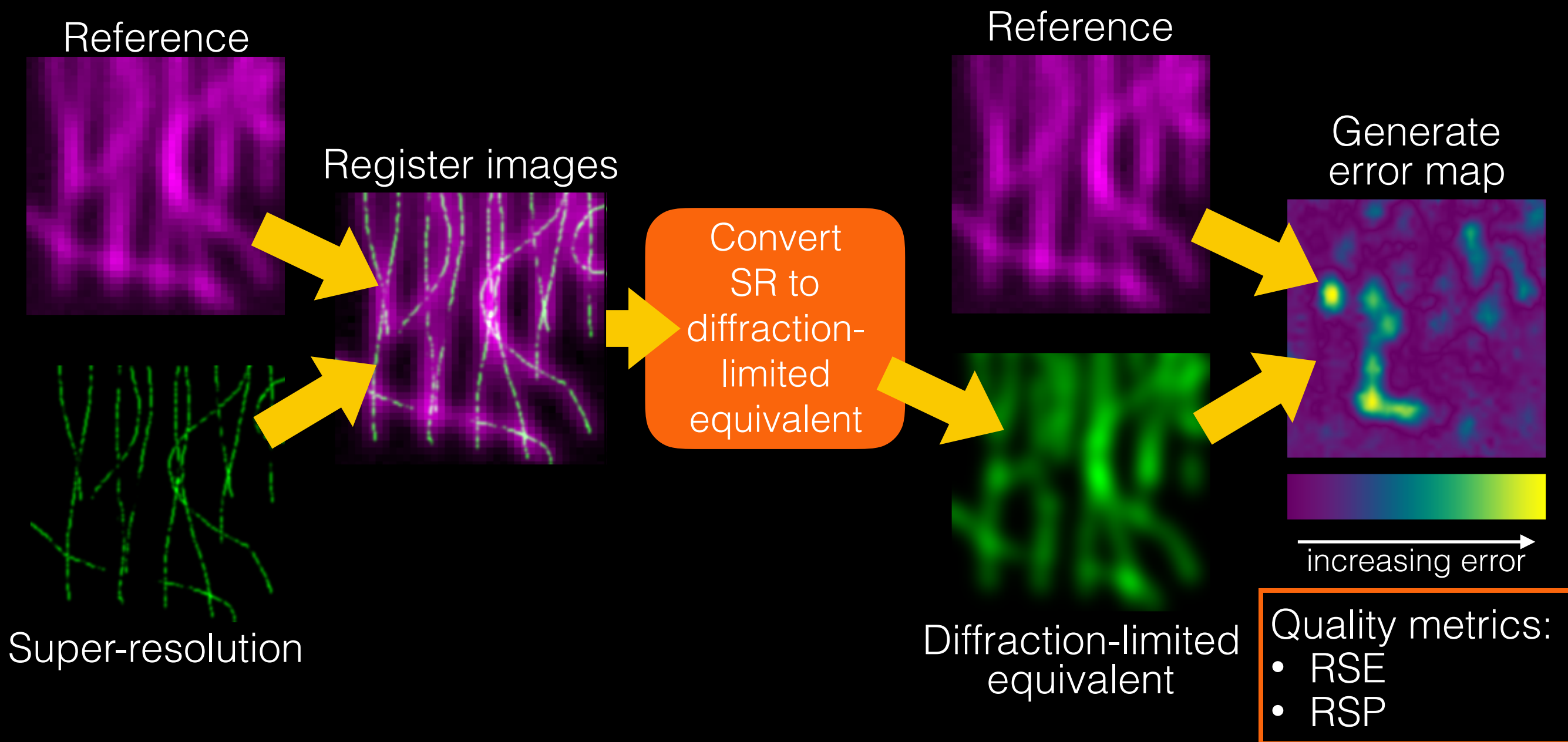


$\approx$

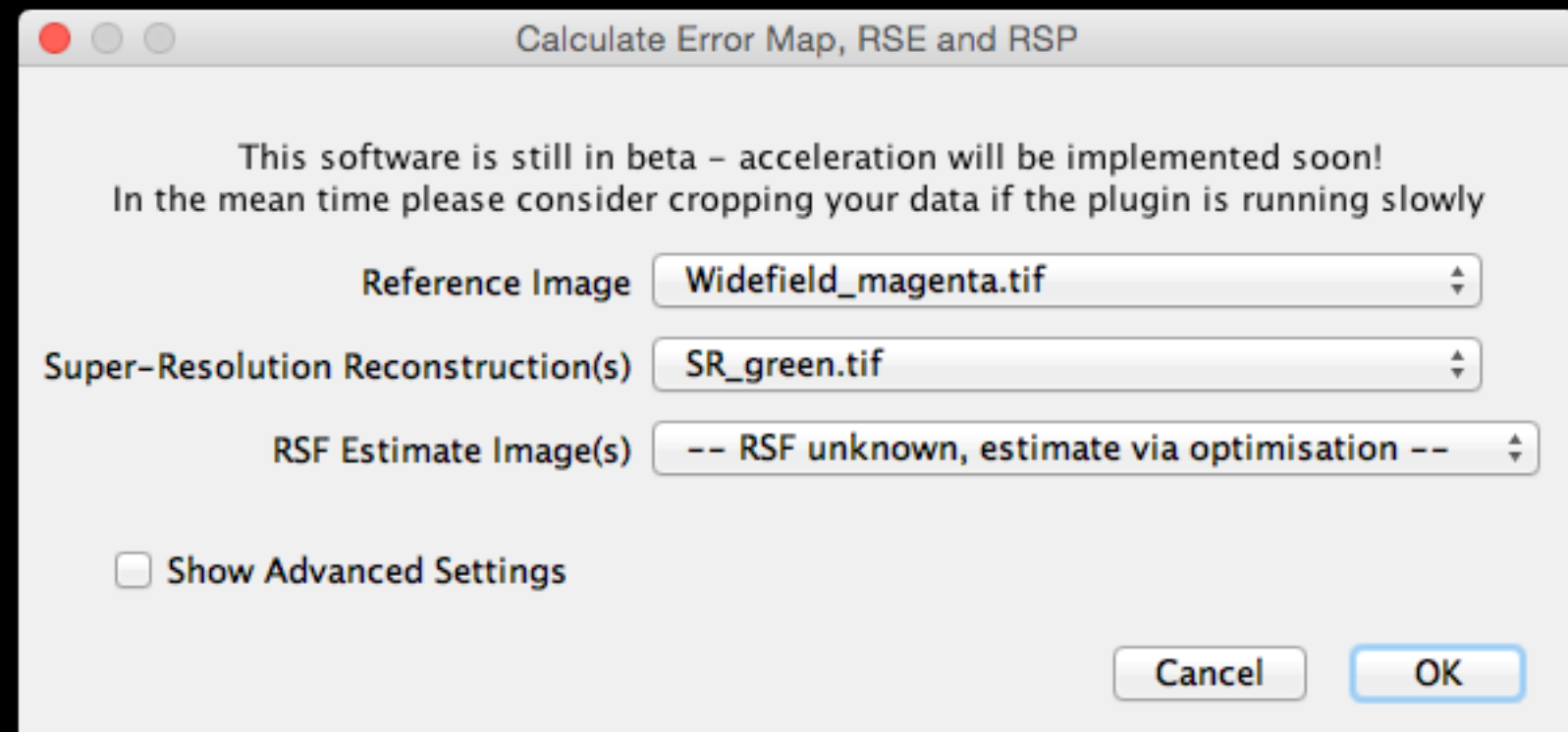
Artefact-**full** SR

Diffraction Limited

SQUIRREL error-mapping pipeline

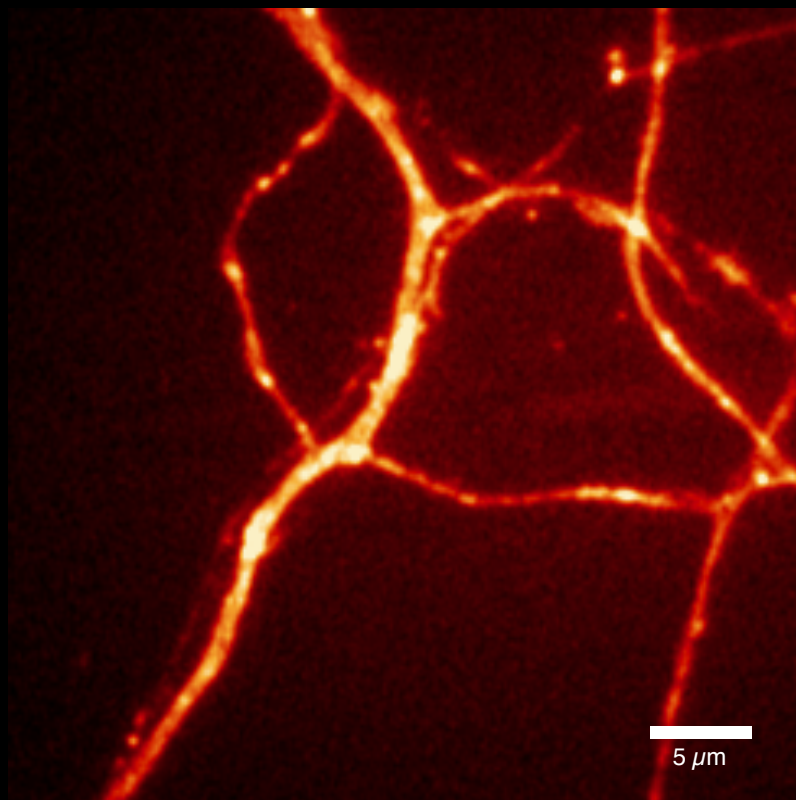


Er, this sounds complicated

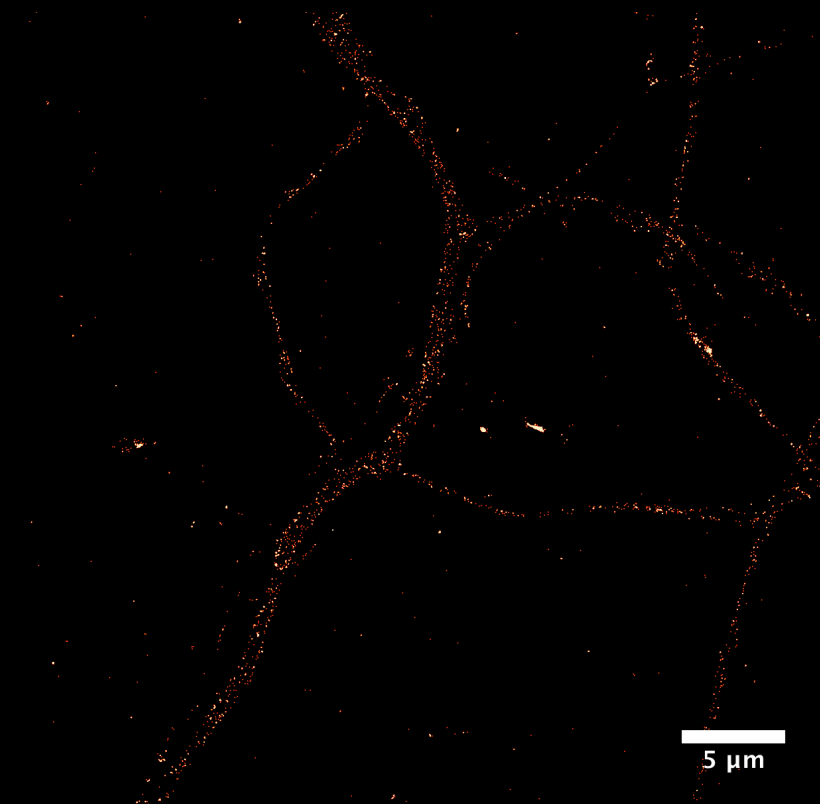


Improving acquisitions

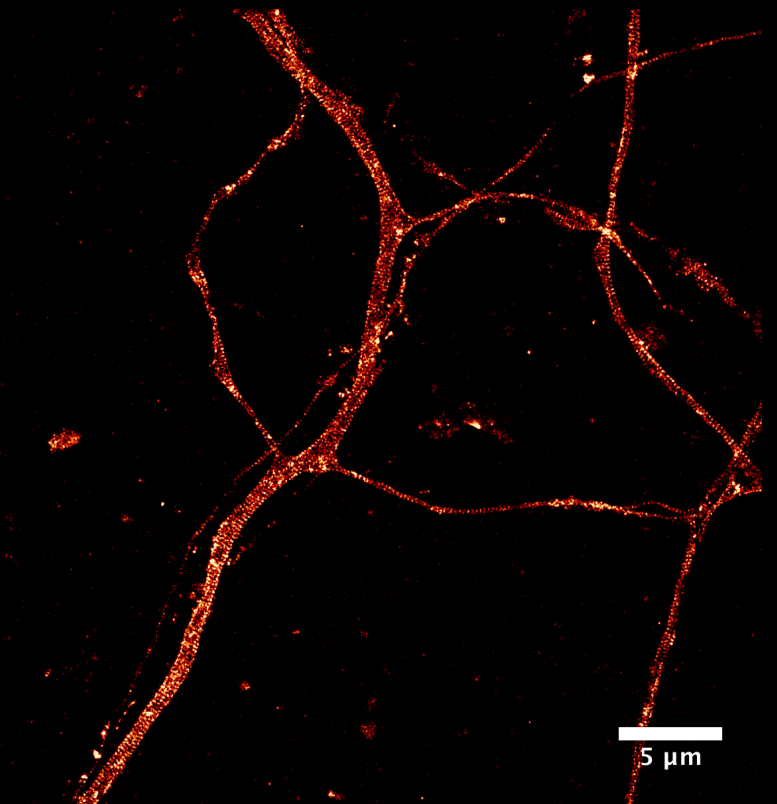
Reference



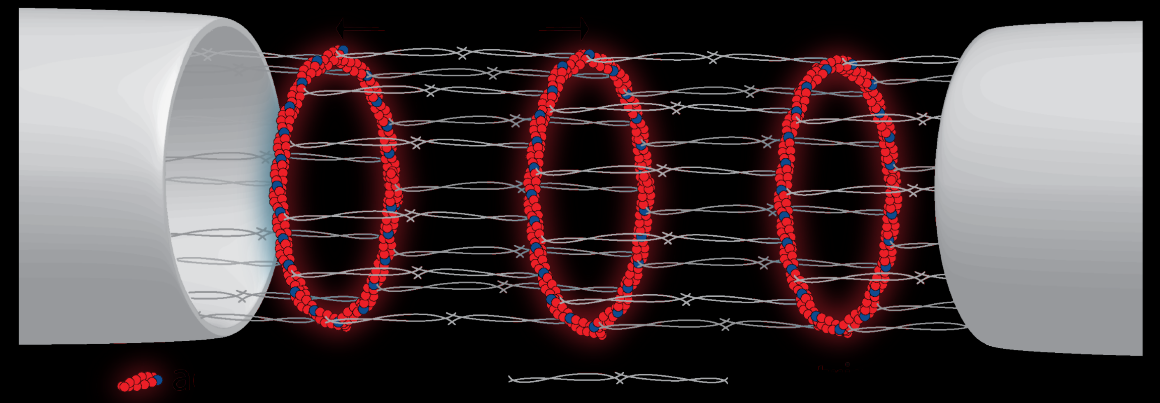
500 frames



60000 frames

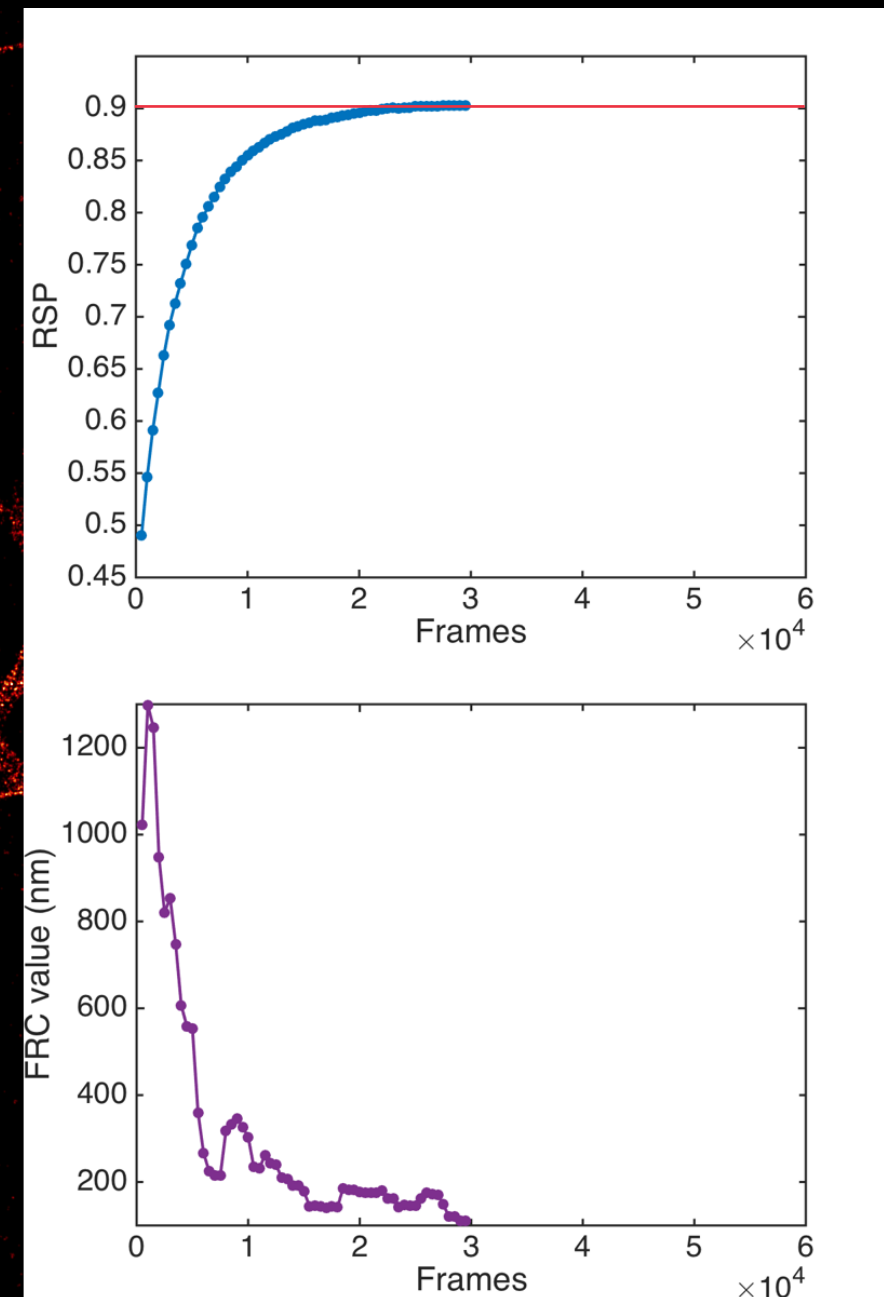
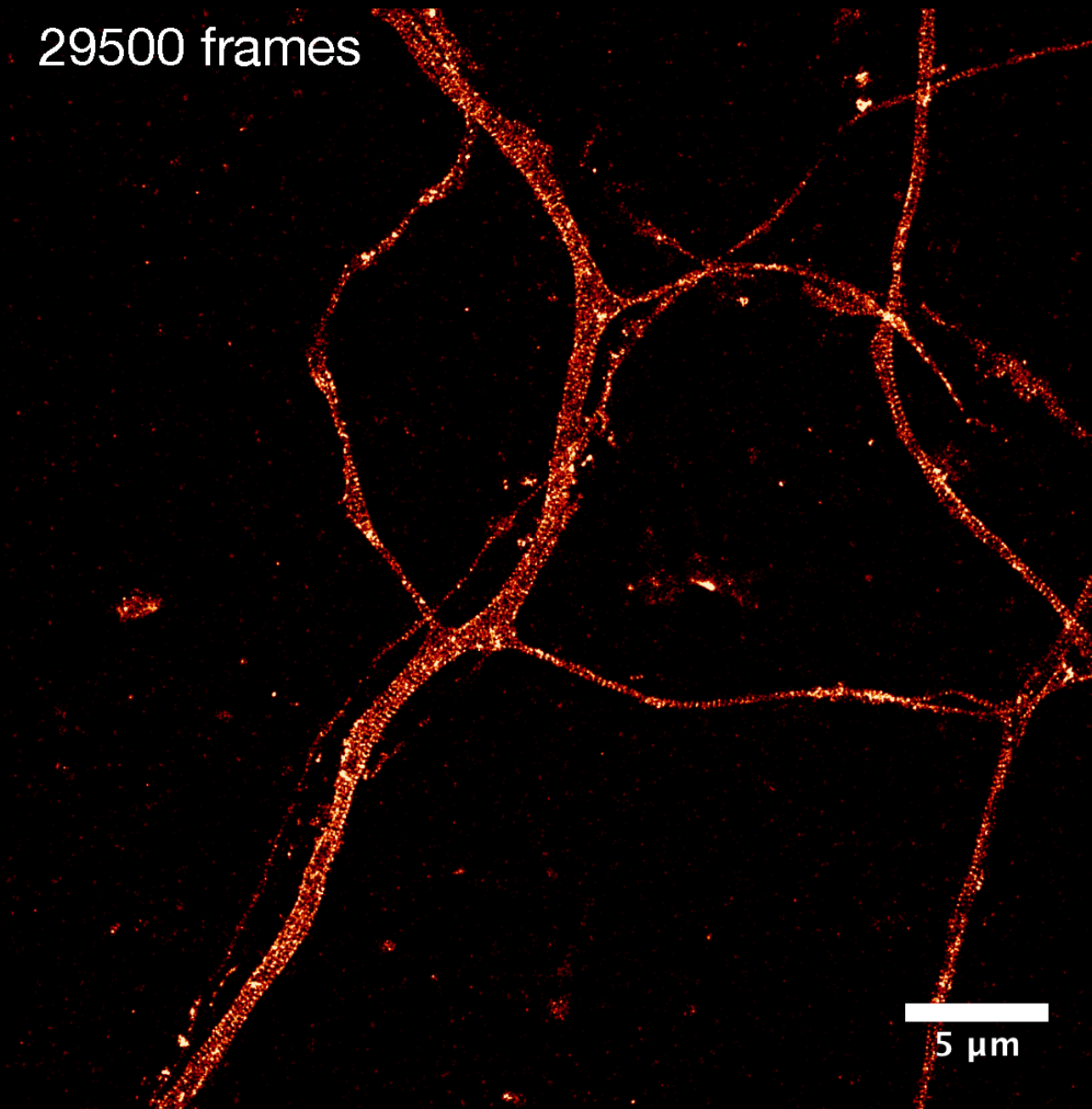


Alexa 647-phalloidin
60000 frames dSTORM



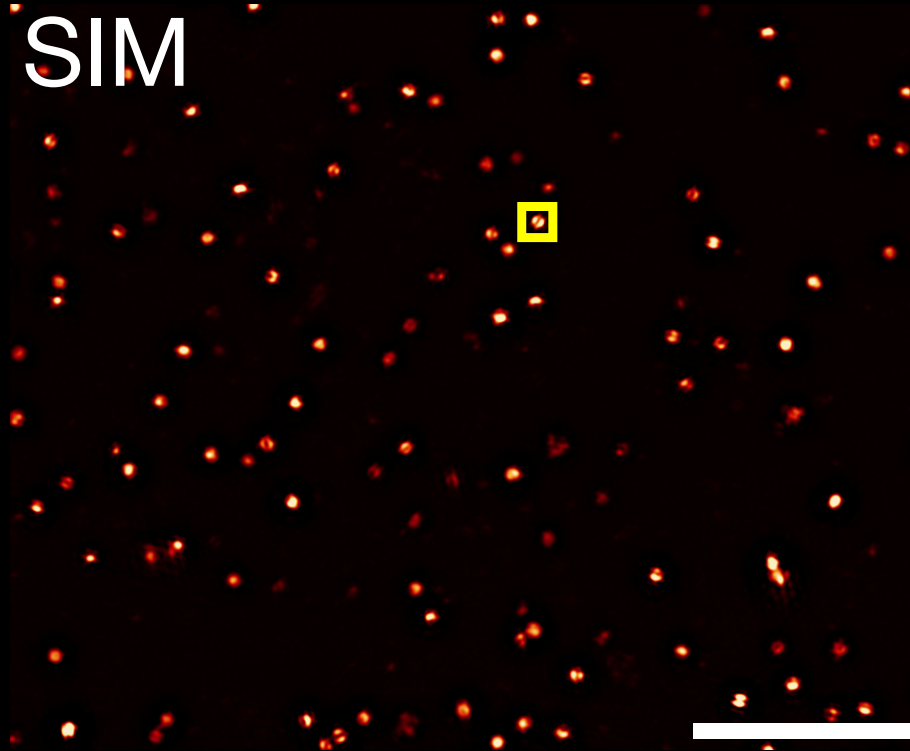
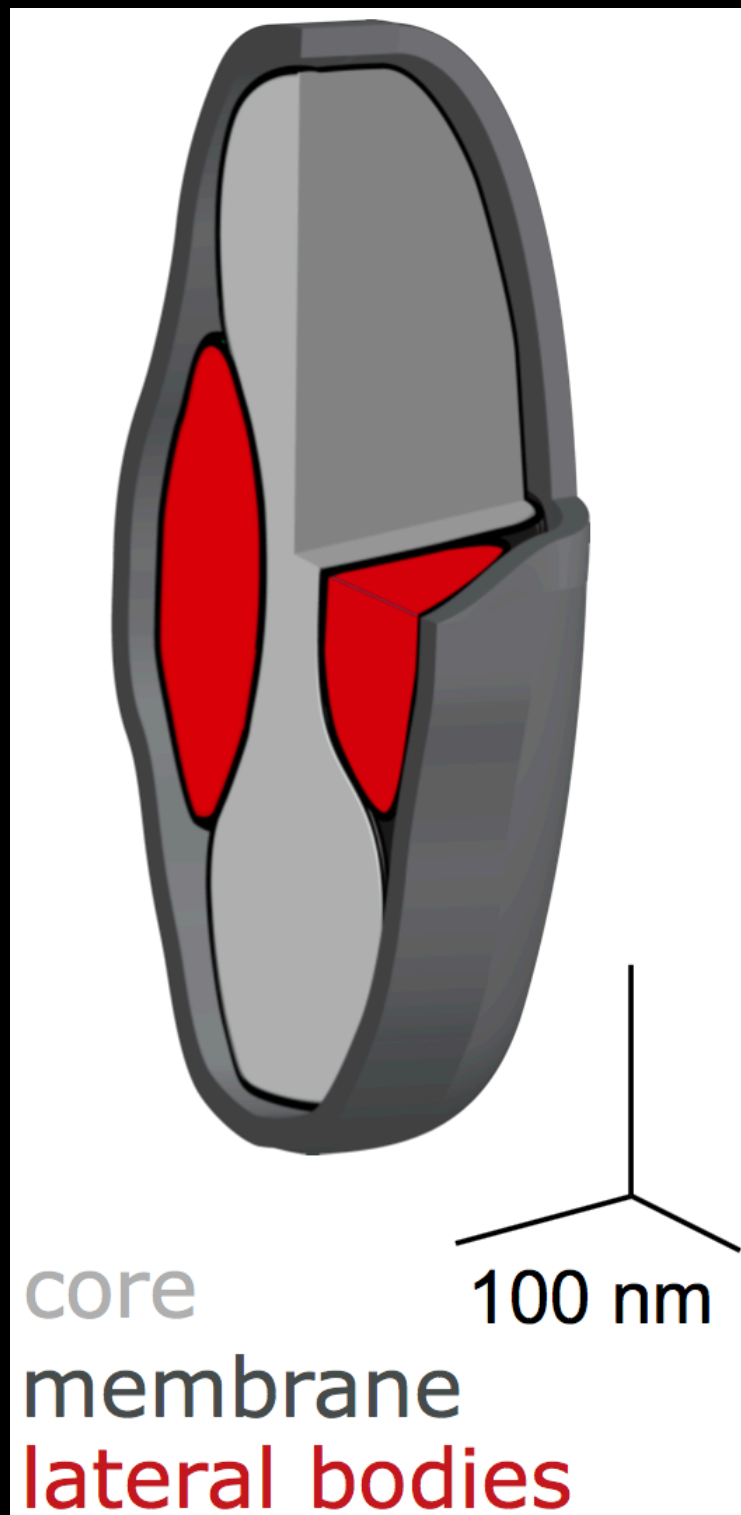
Improving acquisitions

29500 frames

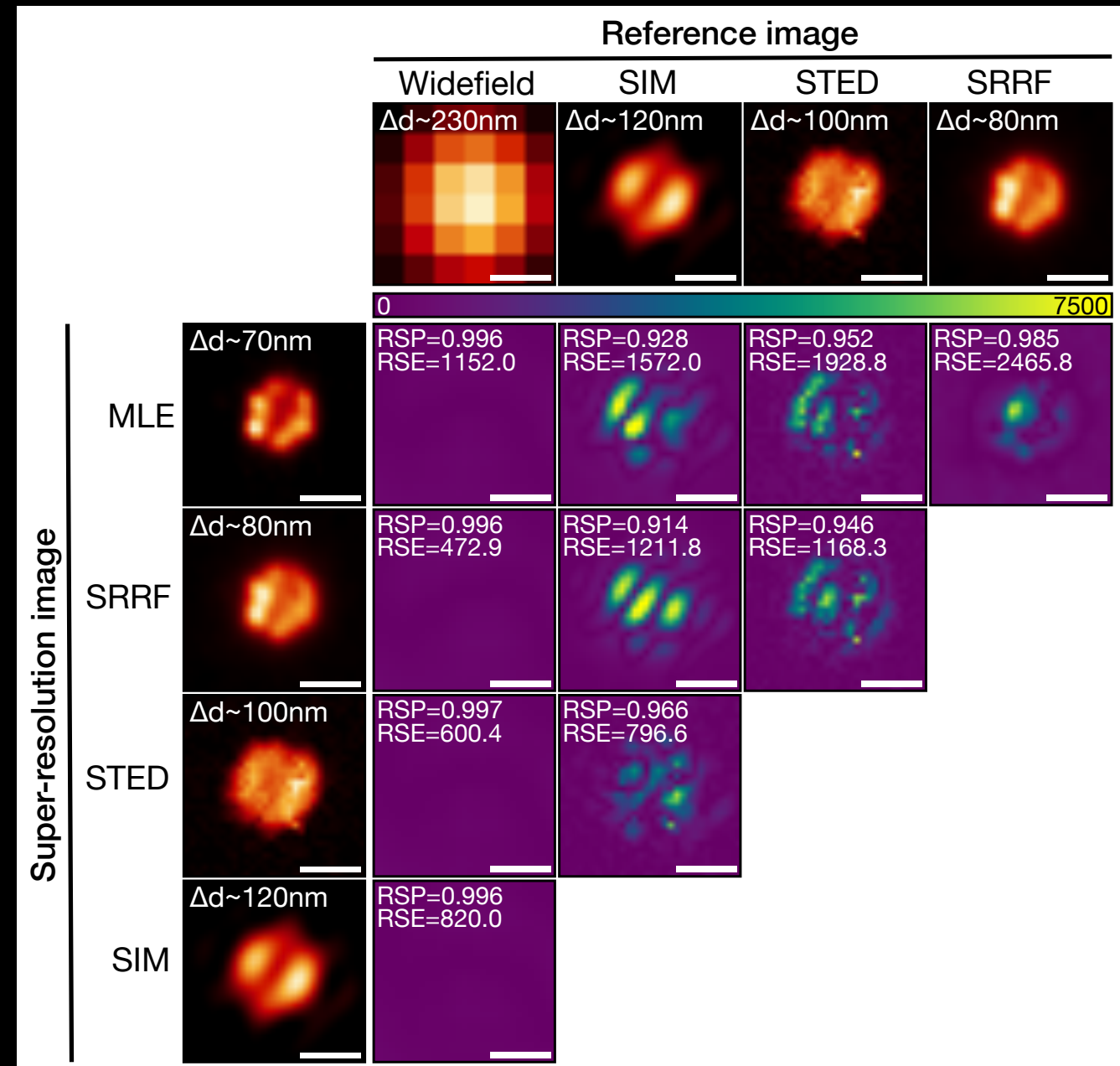
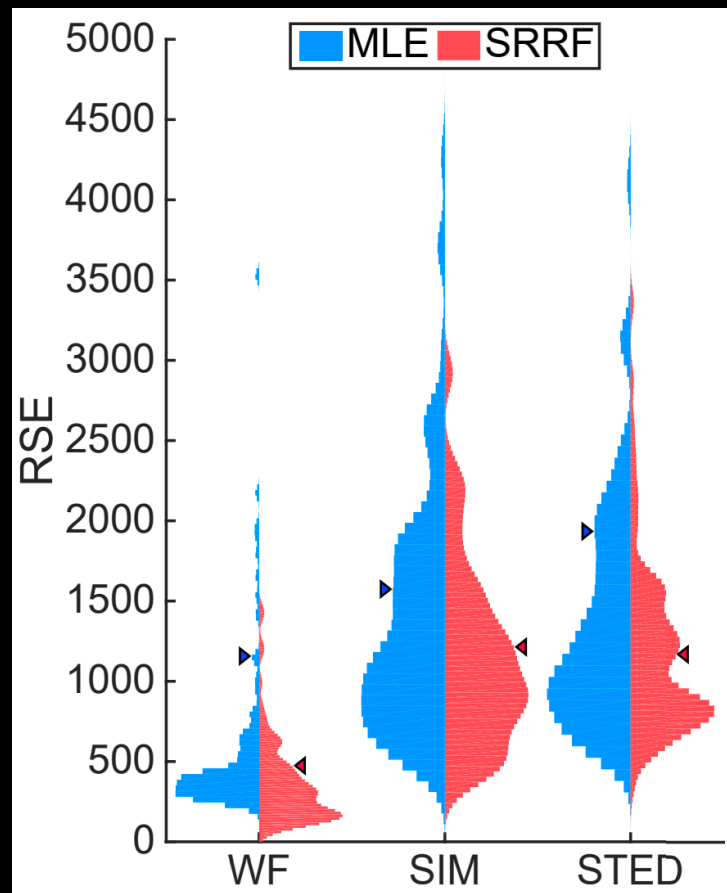
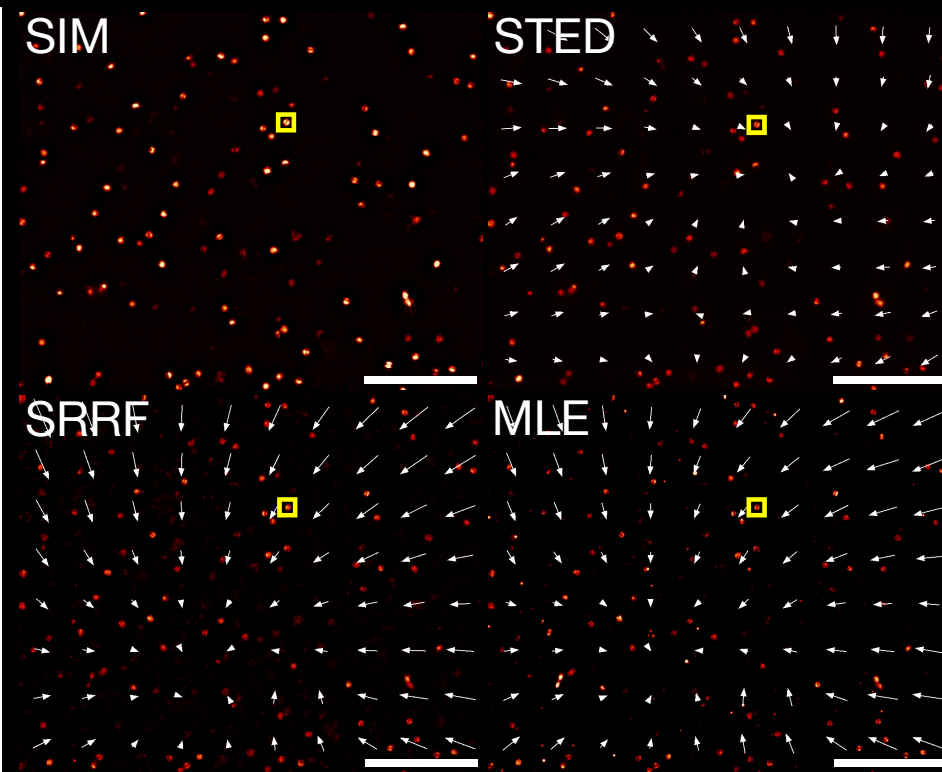
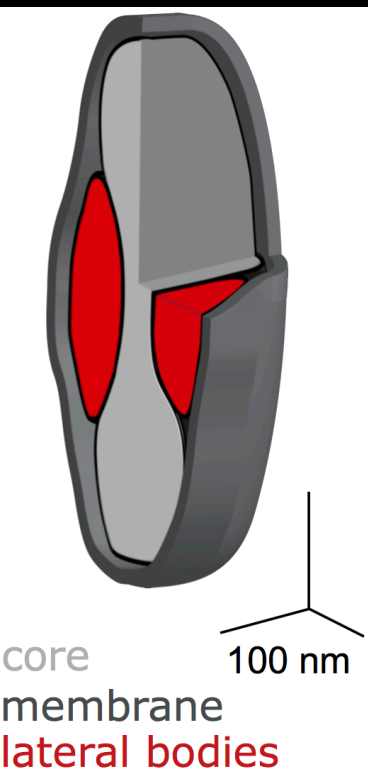


Over half of the acquired data is disruptive

Can we do better?



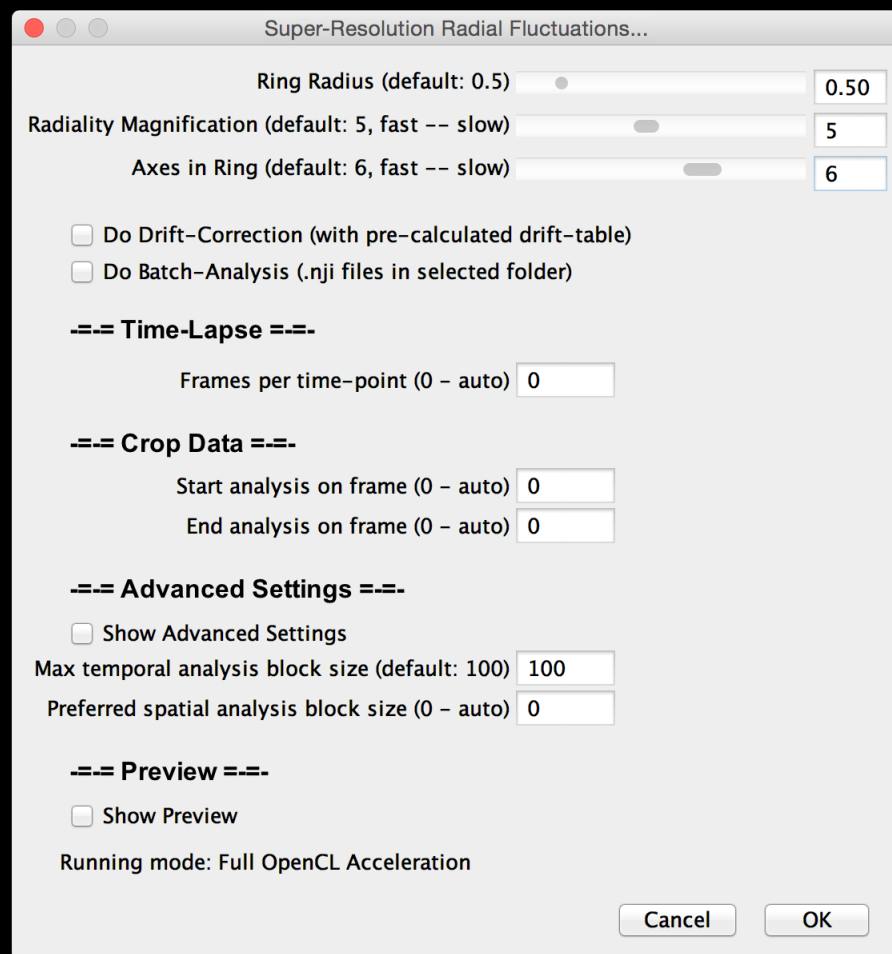
Can we do better?



SRRFing SQUIRRELS

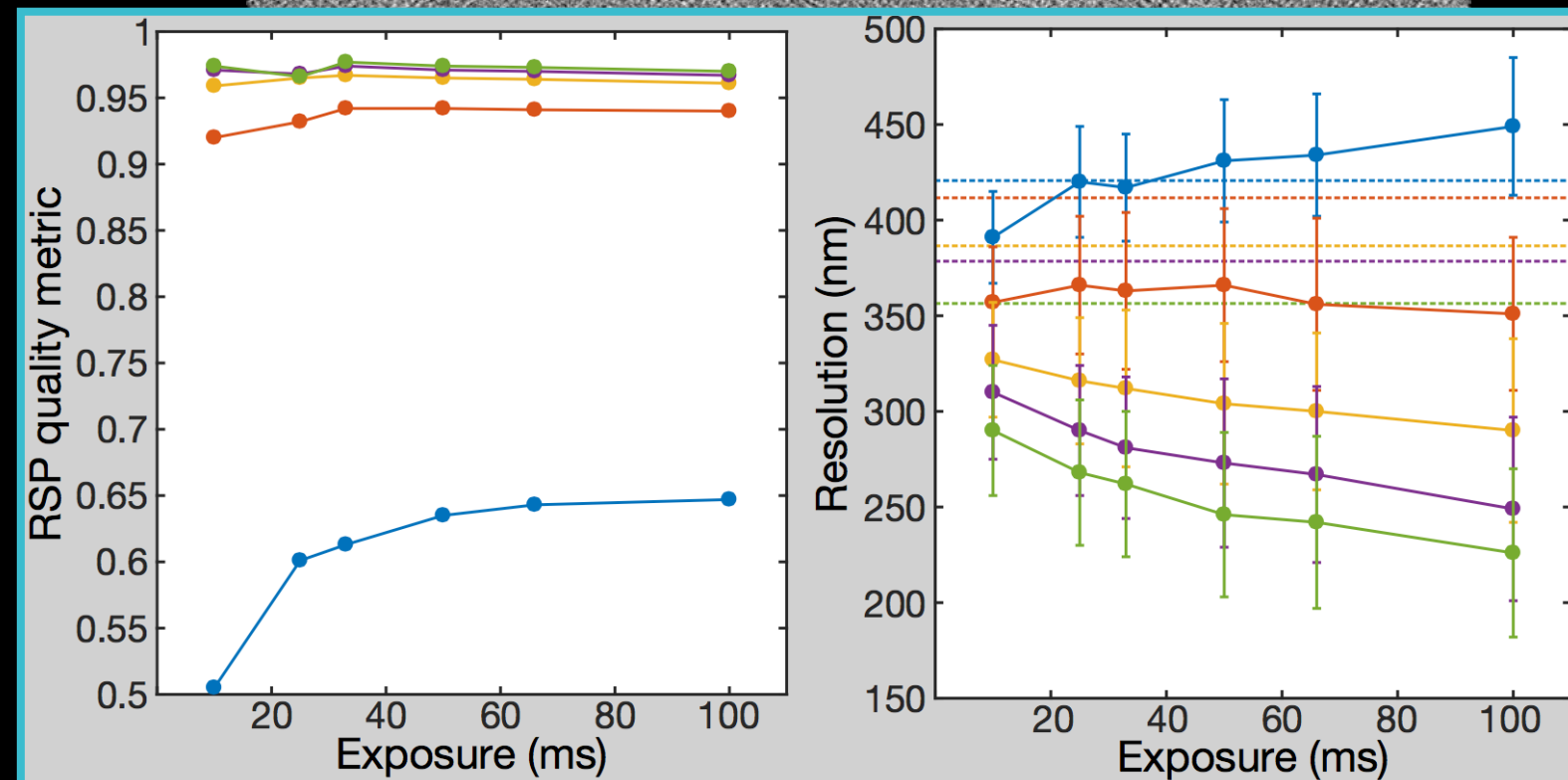


MitoTracker Red - fixed sample
1 second acquisition



1% 560nm LED

100 x 10ms



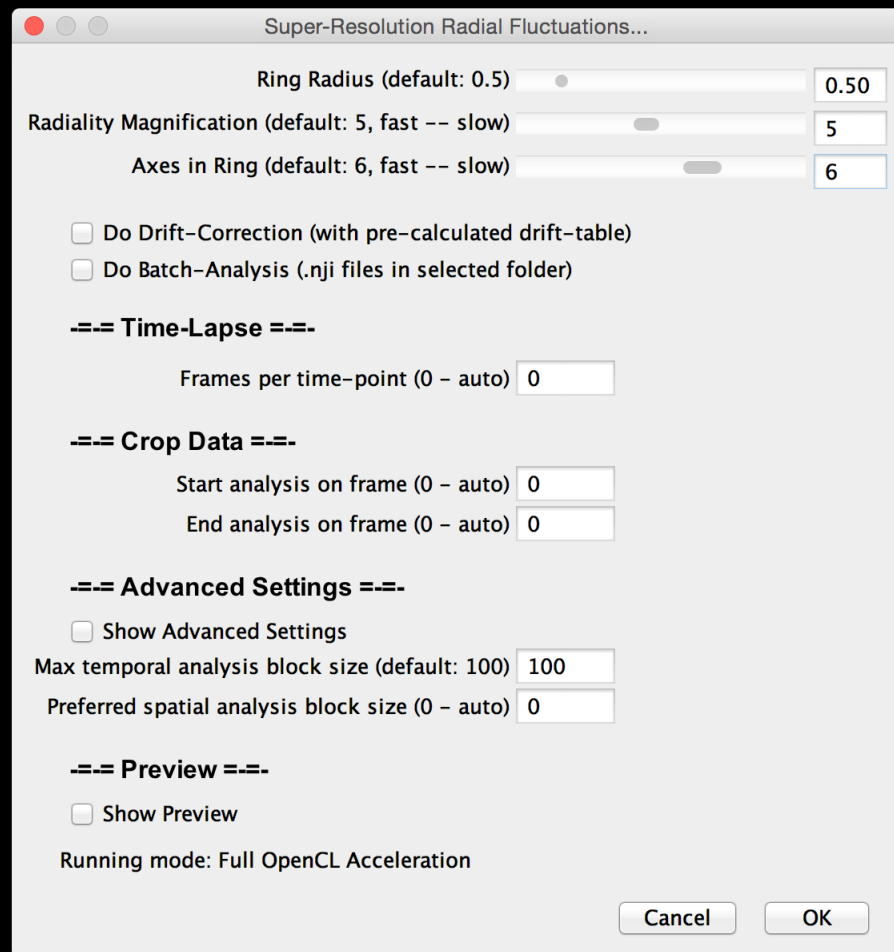
Gustafsson et al, Nature Communications 2016

What is the best
combination of number of
frames, LED power and
exposure time?

SRRFing SQUIRRELS

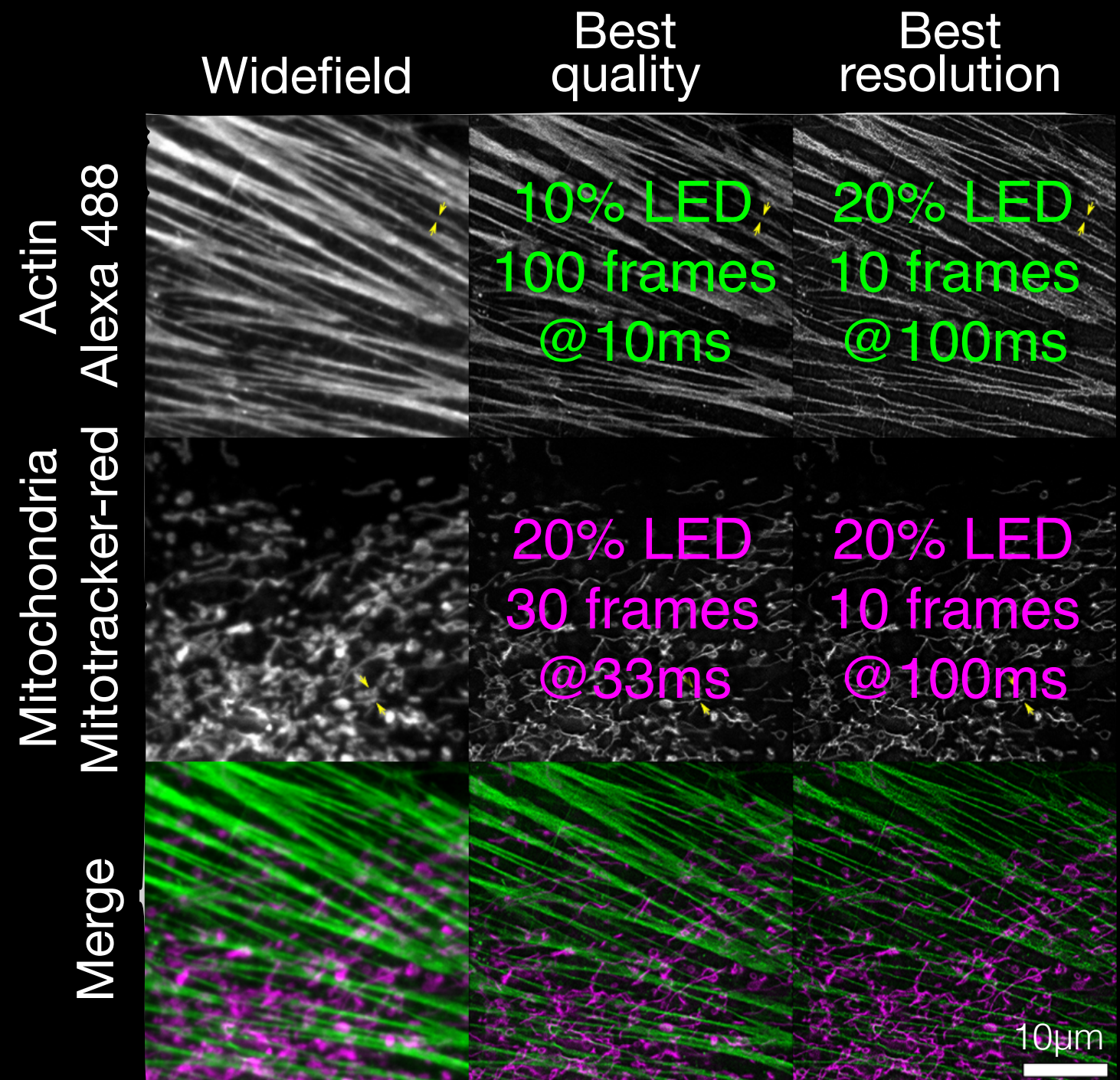


Siân Culley

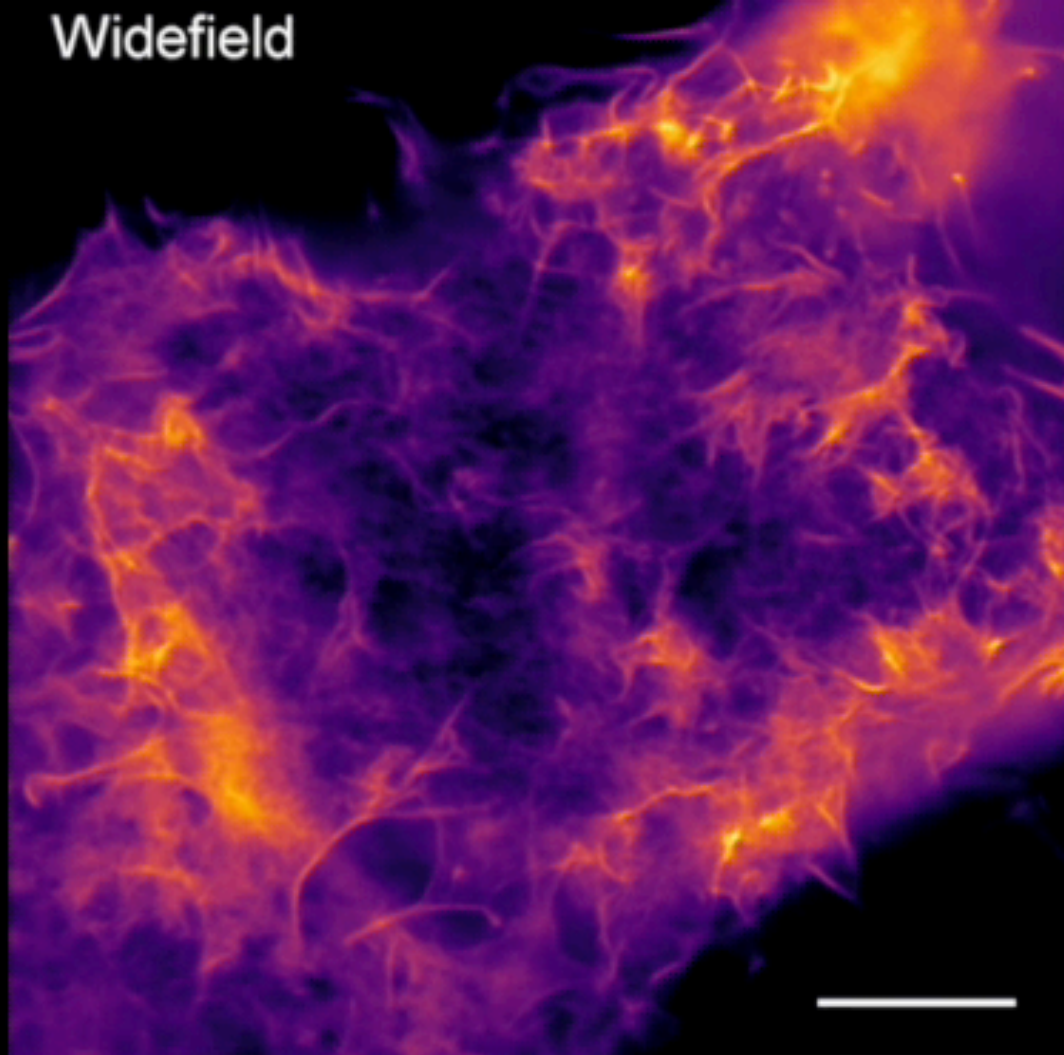


Gustafsson et al, Nature Communications 2016

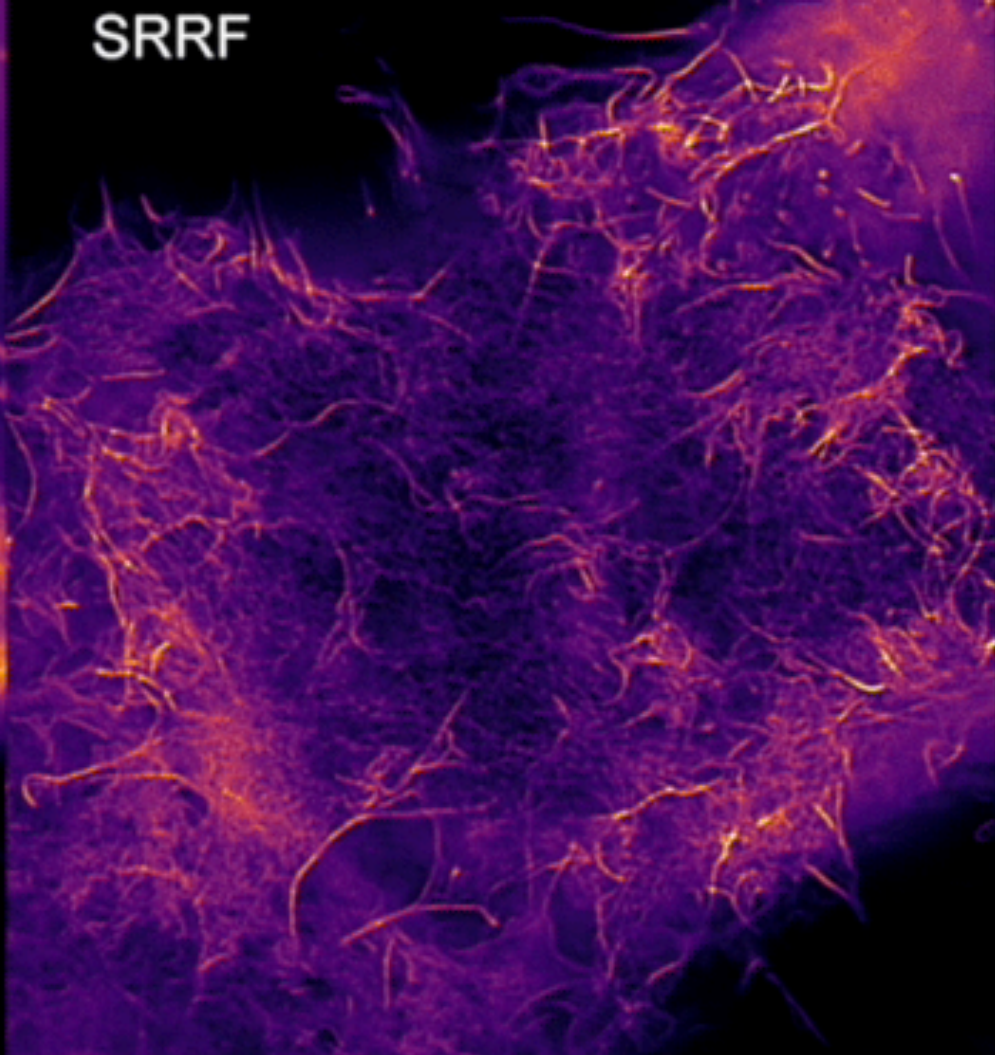
What is the best combination of number of frames, LED power and exposure time?



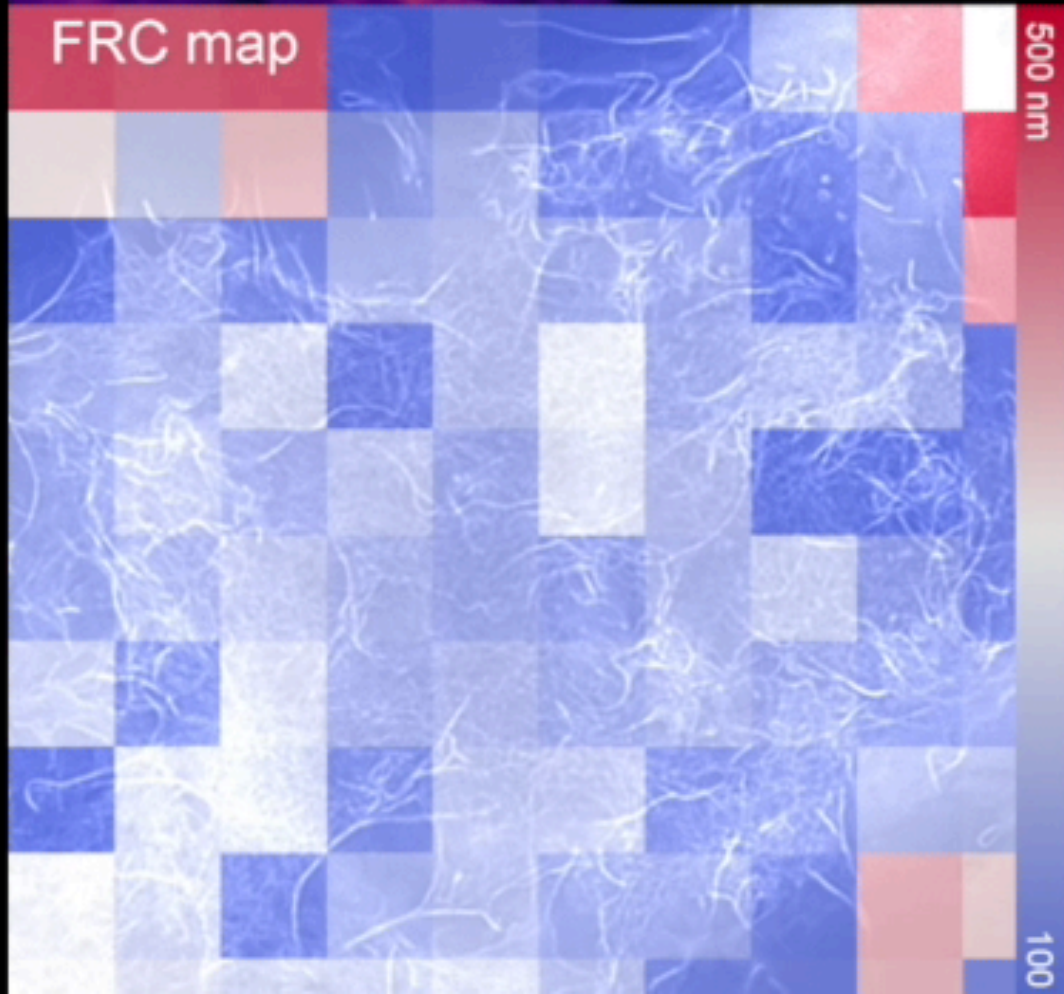
Widefield



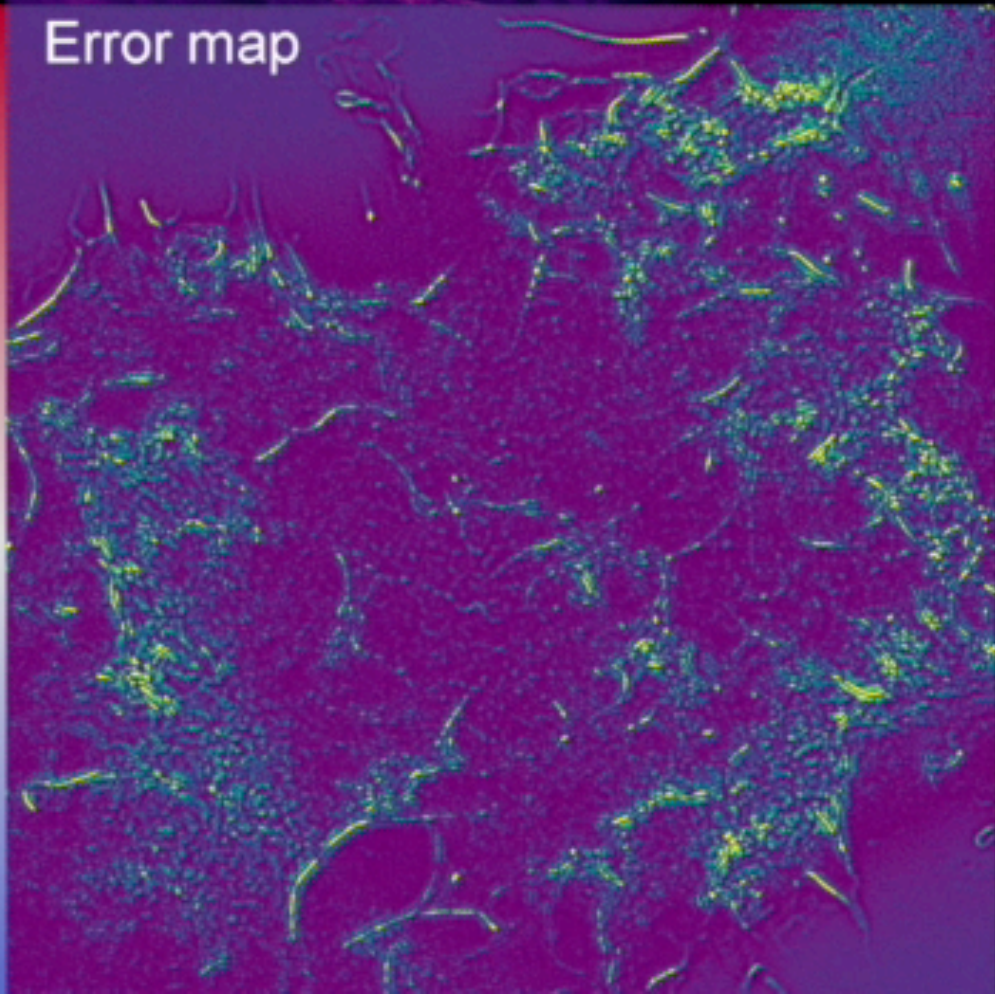
SRRF

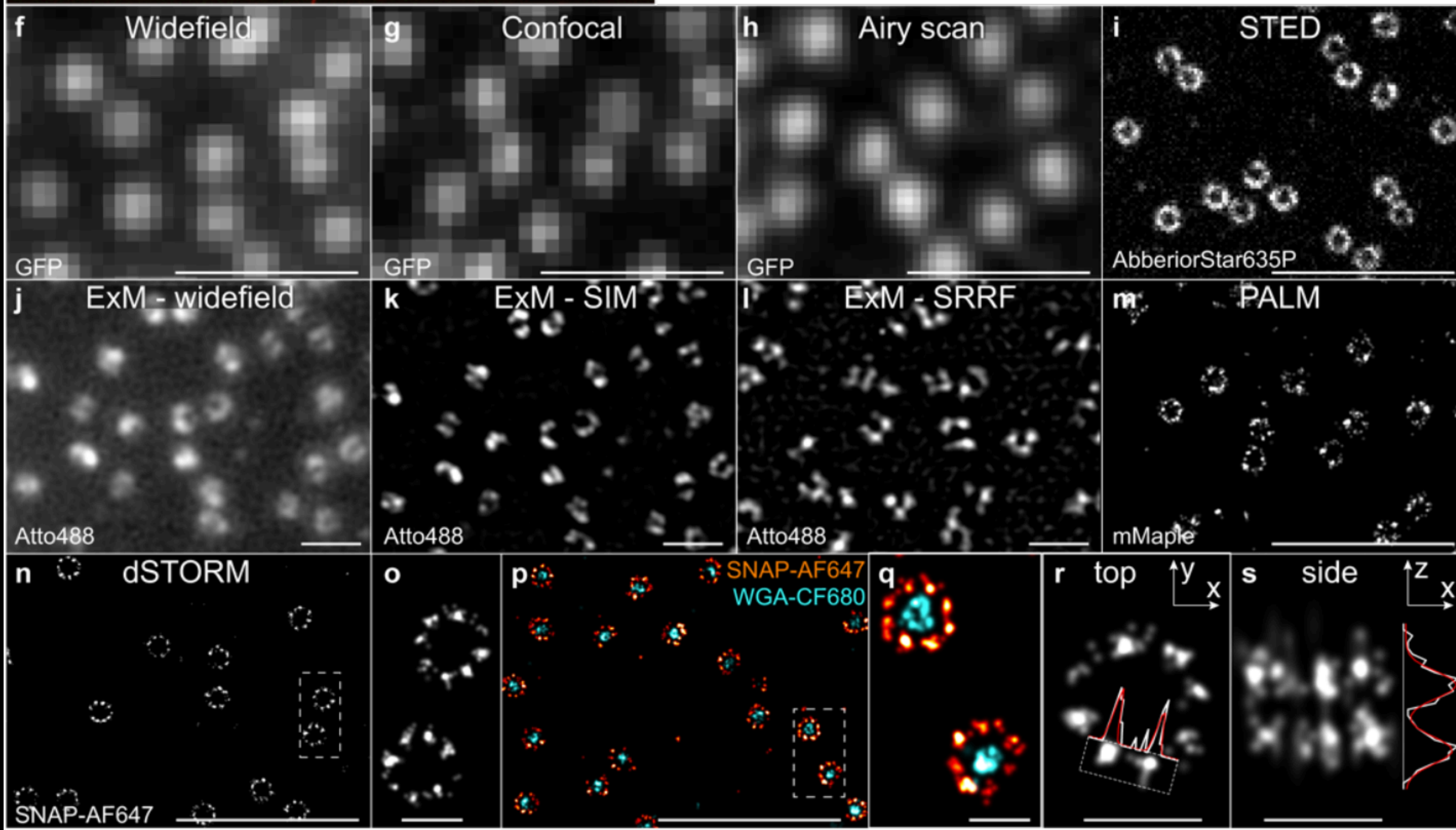
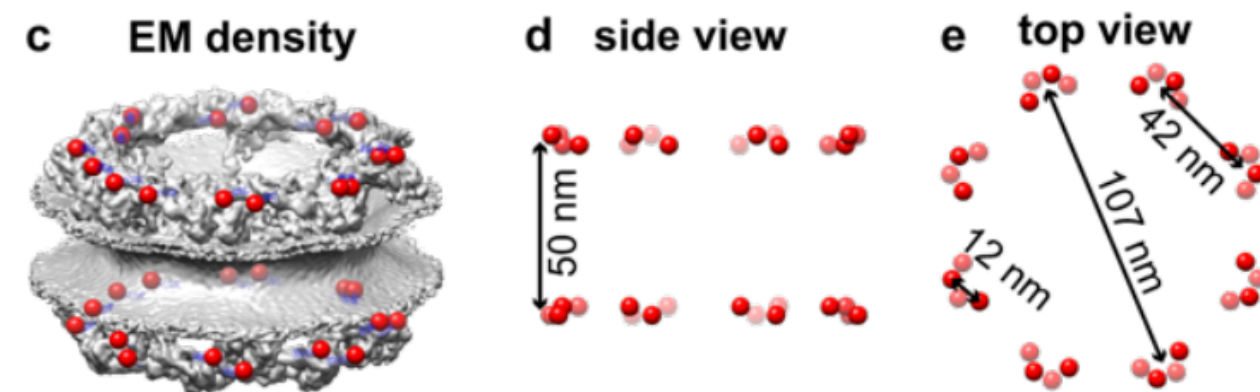
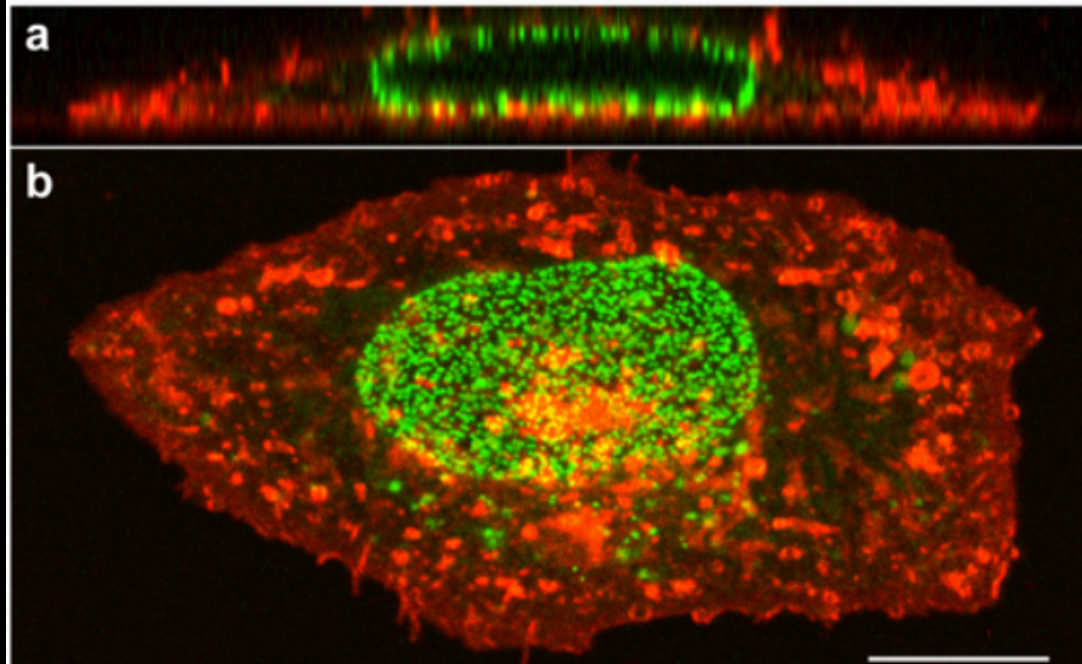


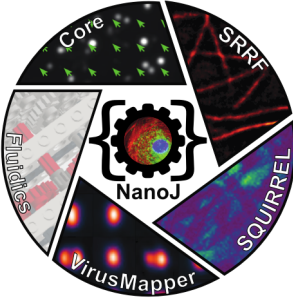
FRC map



Error map

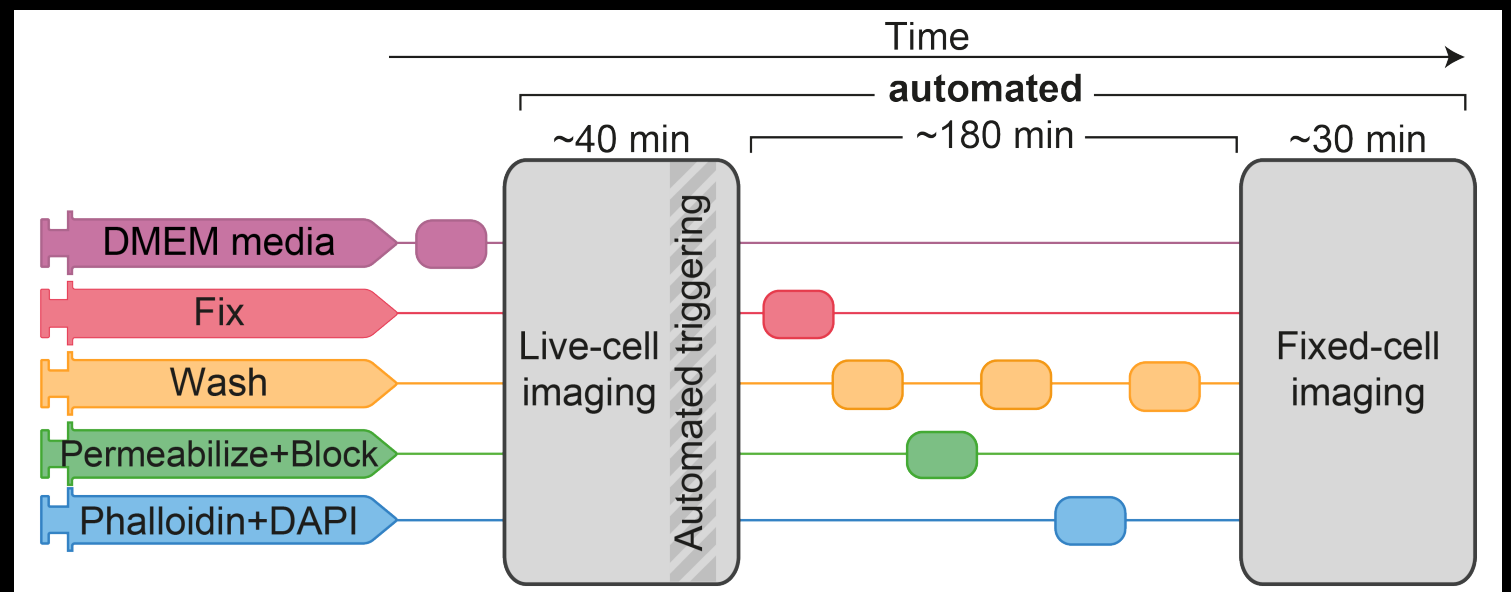
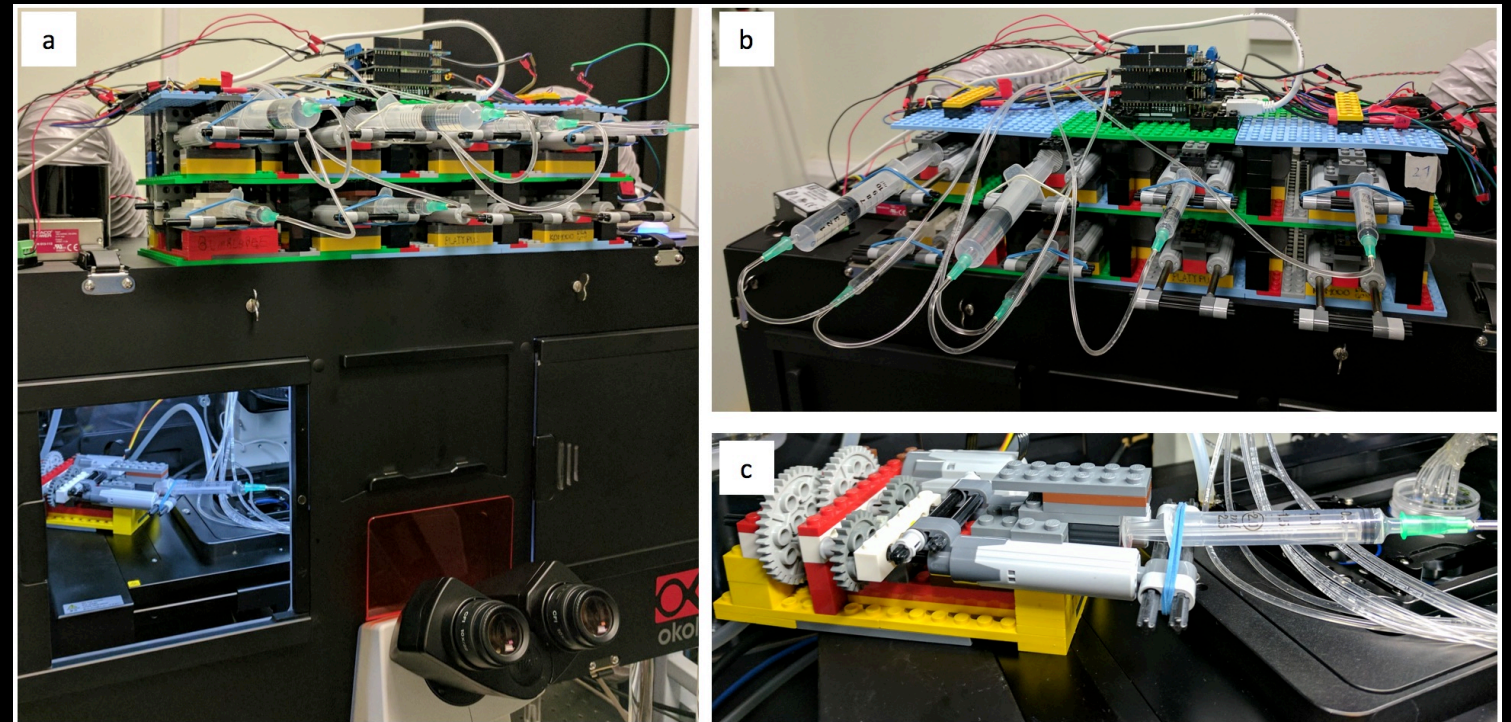
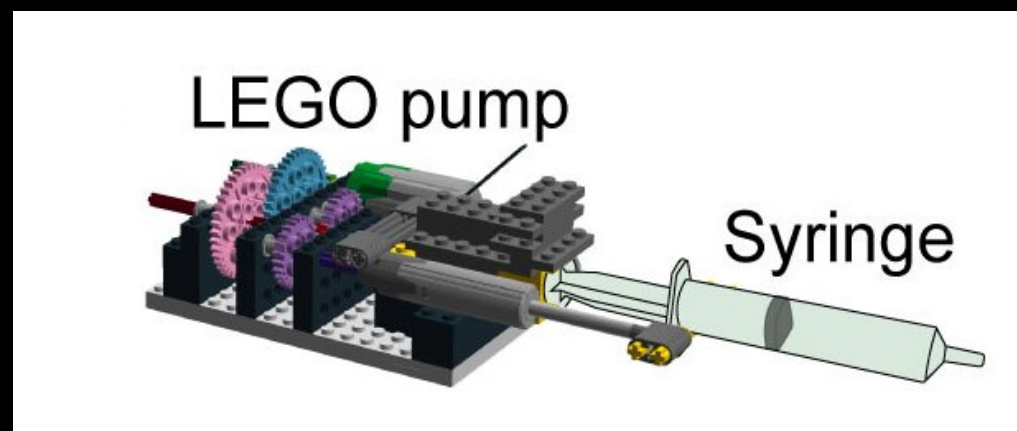
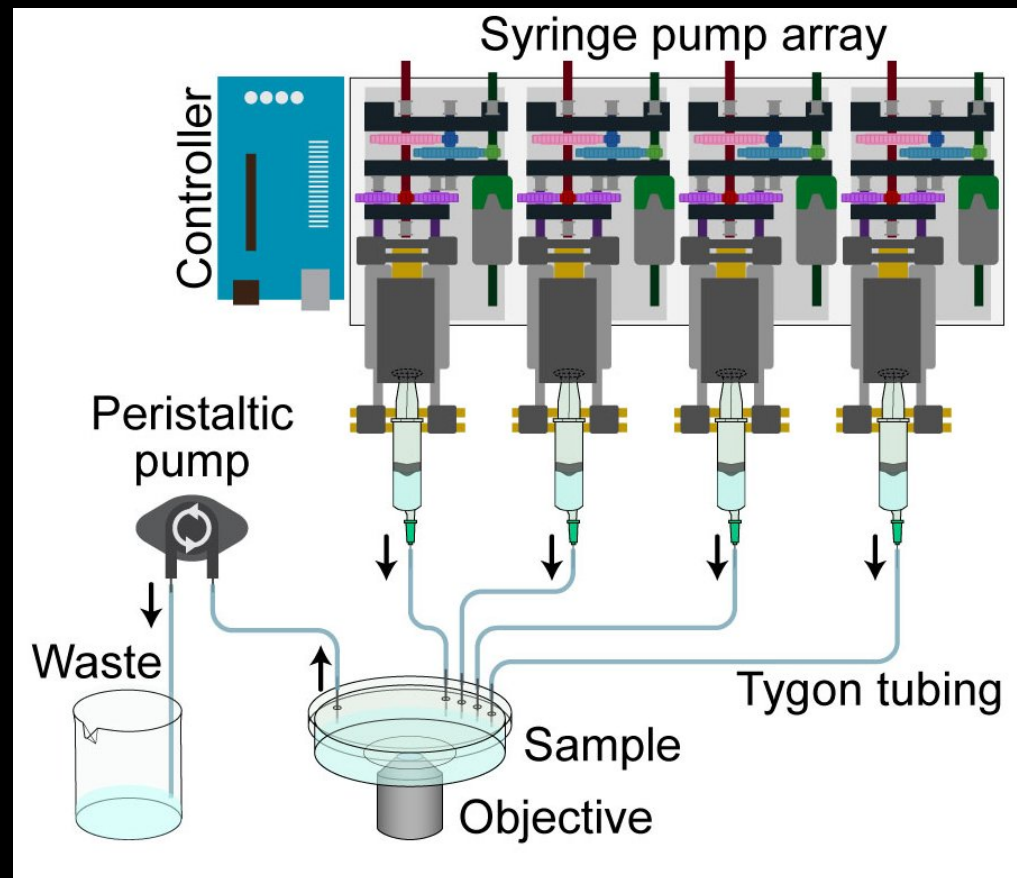






Multimodal imaging

Fluidics exchange (Pumpy)

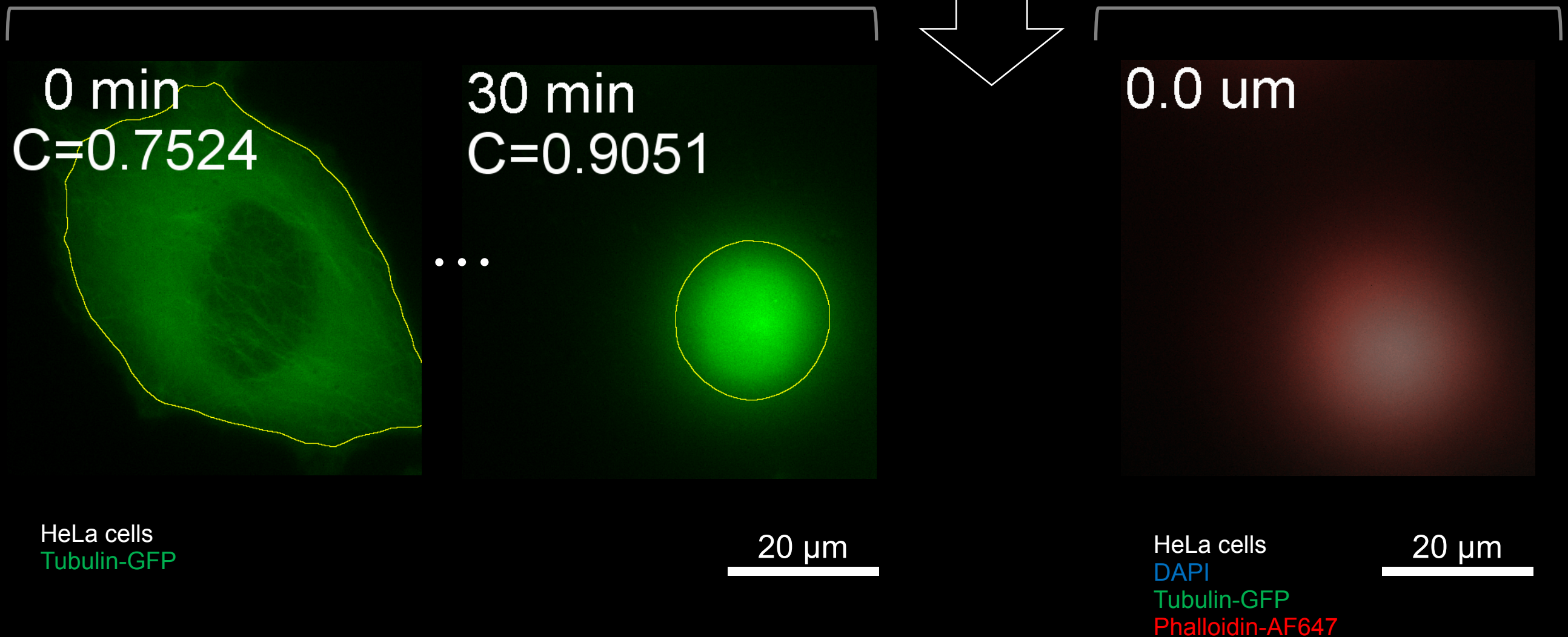


Fluidics exchange (Pumpy)

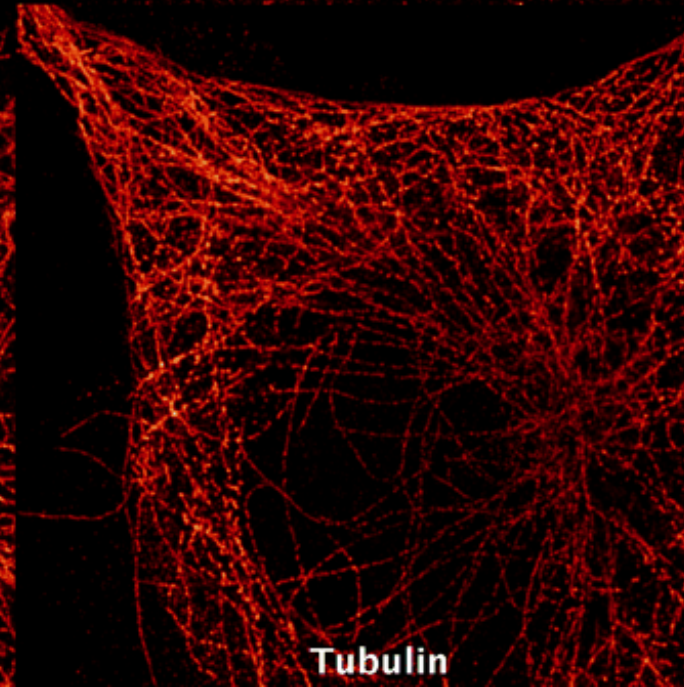
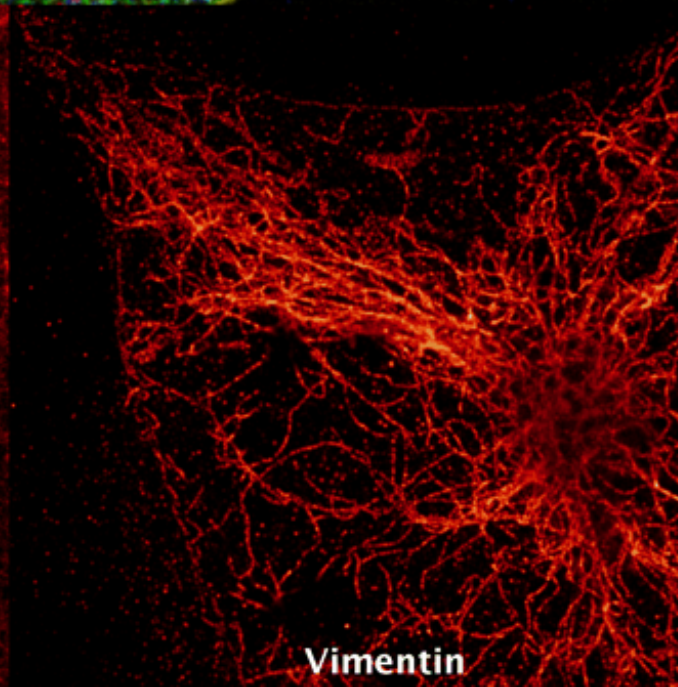
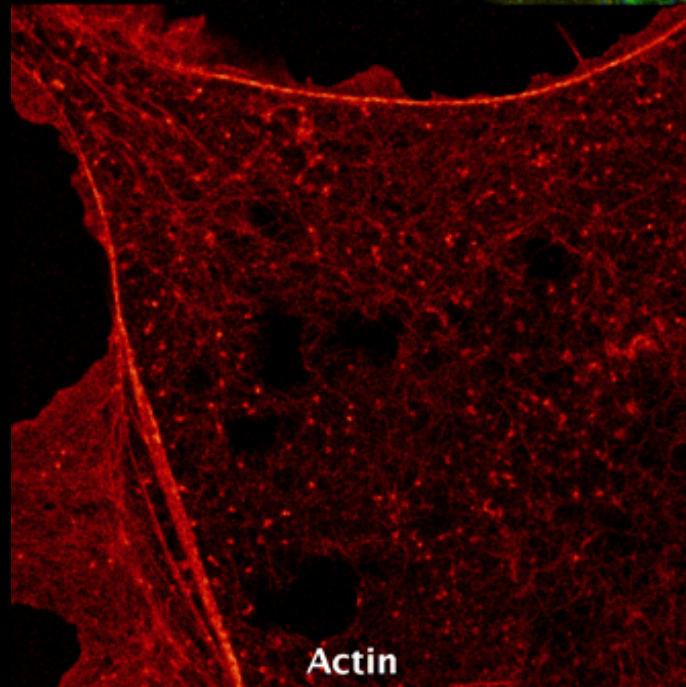
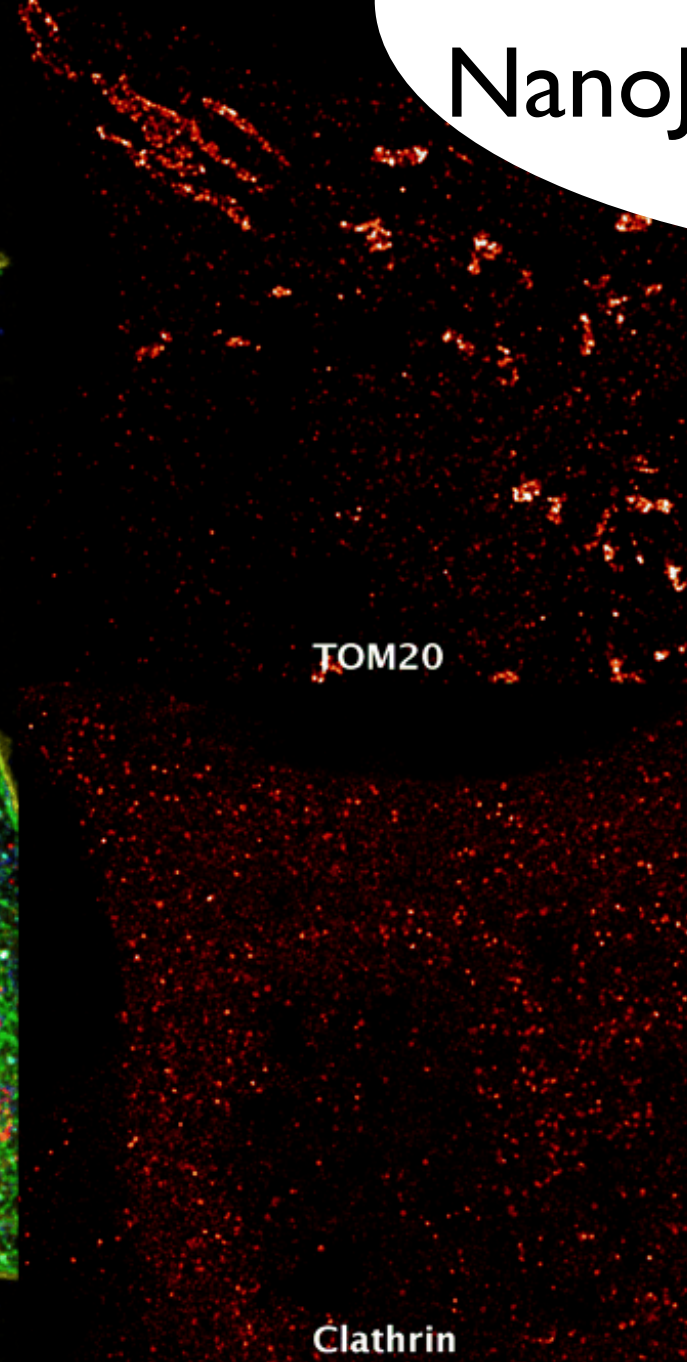
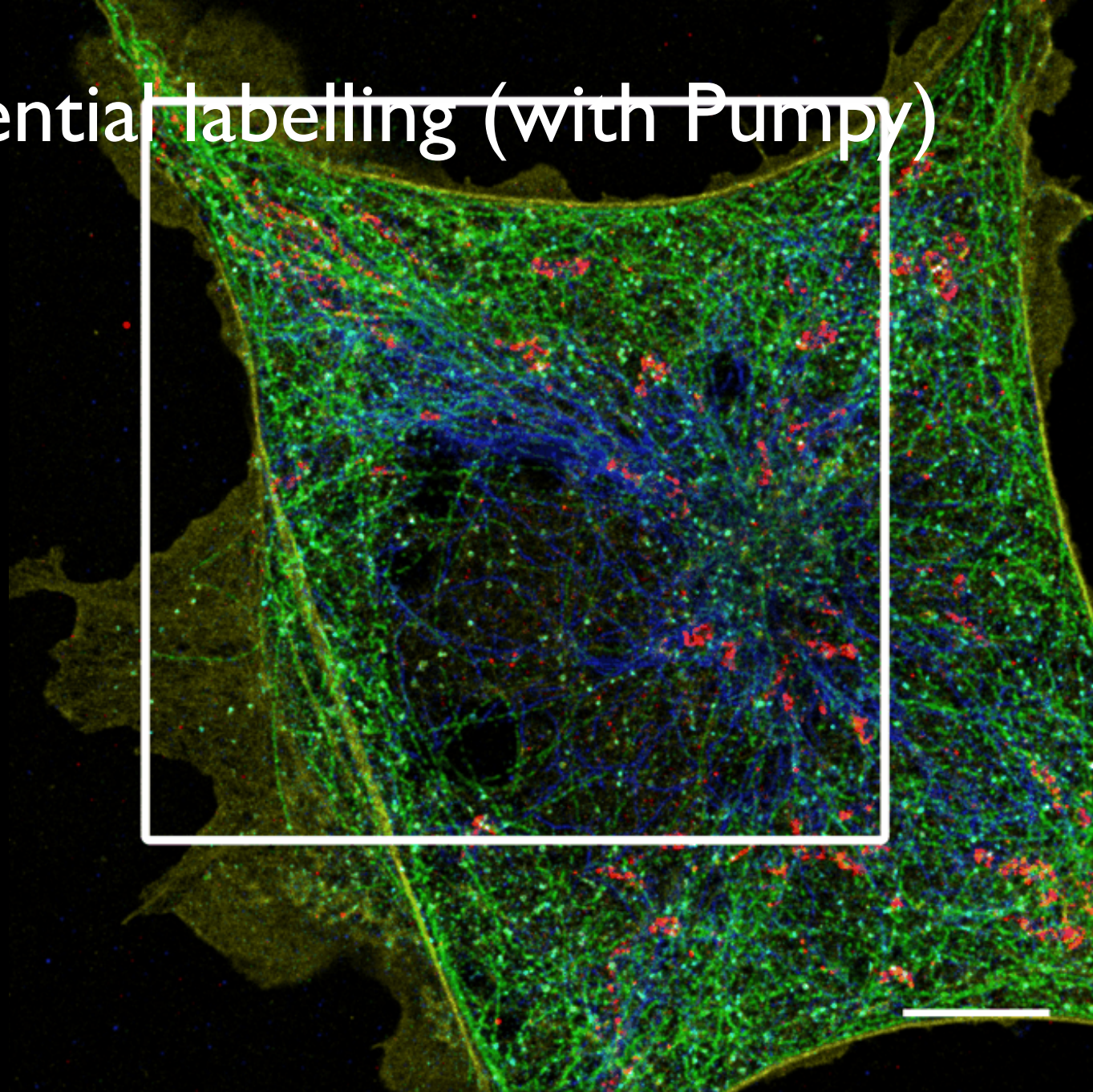
Live-cell imaging

Automated fixation
and labelling

3D imaging



Sequential labelling (with Pumpy)



Actin

Vimentin

Tubulin



Matthew Meyer

Replied to Henrique's post

Fully LEGO



IMBmicros

Hmmm. ST



Ed Phelps

@UFCPET

and Arduino

using open

lab #pump



Alexandre

Replied to Henrique's post

No problem

😊 Thank y



Friedrich F

The advant

set-up in o

providing p

things etc.!



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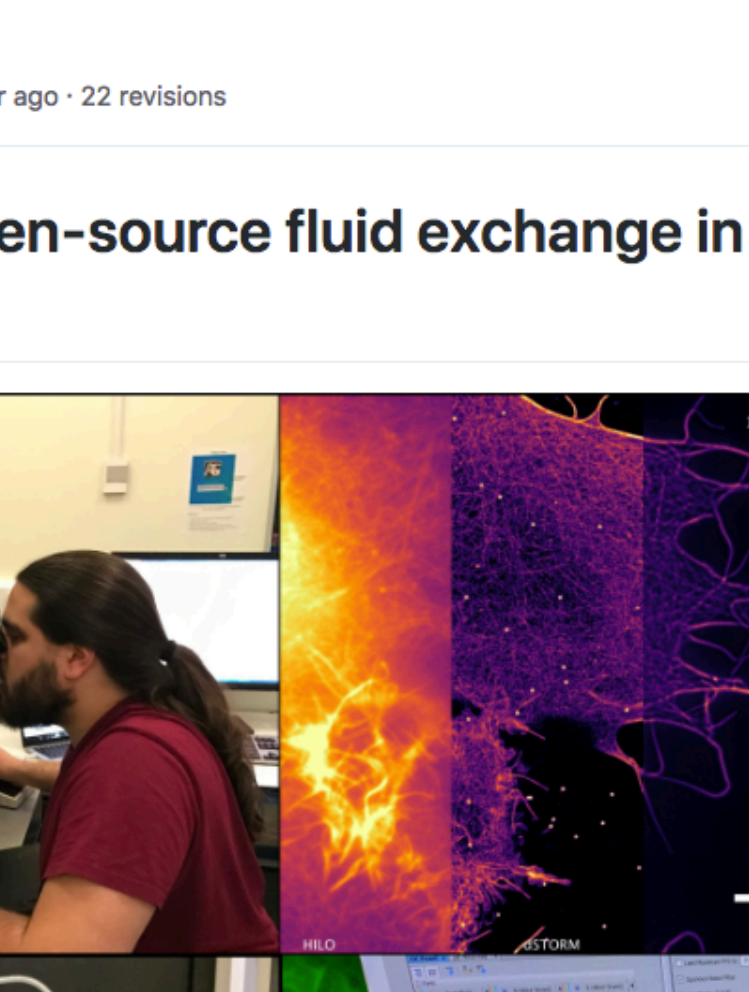
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Ricardo Henriques edited this page an hour ago · 22 revisions

NanoJ-Fluidics: open-source fluid exchange in microscopy



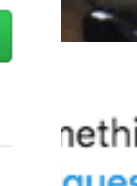
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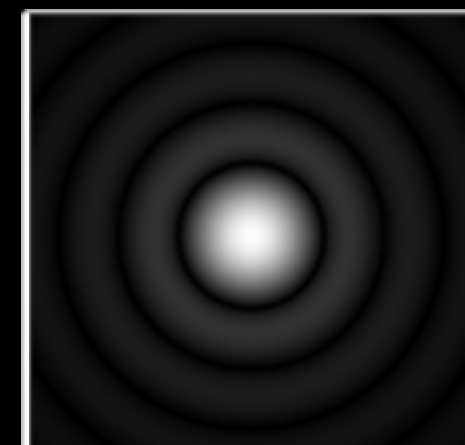
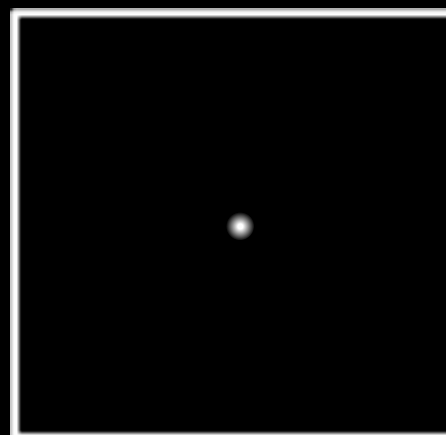
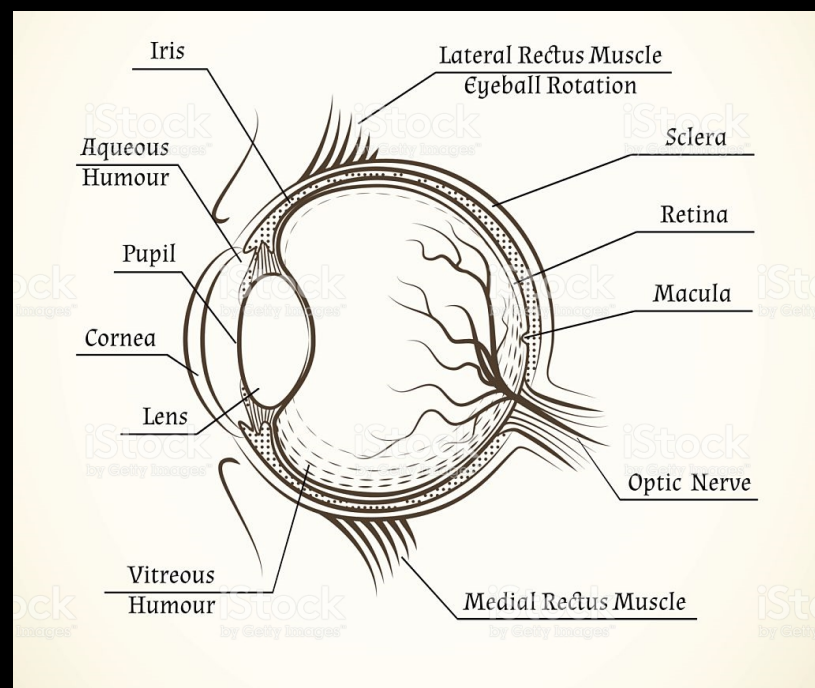
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<https://github.com/HenriquesLab/NanoJ-Fluidics>

NanoJ-Fluidics is an open-source device, composed of easily accessible LEGO-parts, electronics and labware. It is designed to automate and simplify fluid exchange experiments in microscopy. Check the paper in Nature Communications: [Automating multimodal microscopy with NanoJ-Fluidics](#).





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Thank you for listening....



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Ralf Jungmann (MPI Biochemistry, Germany)



