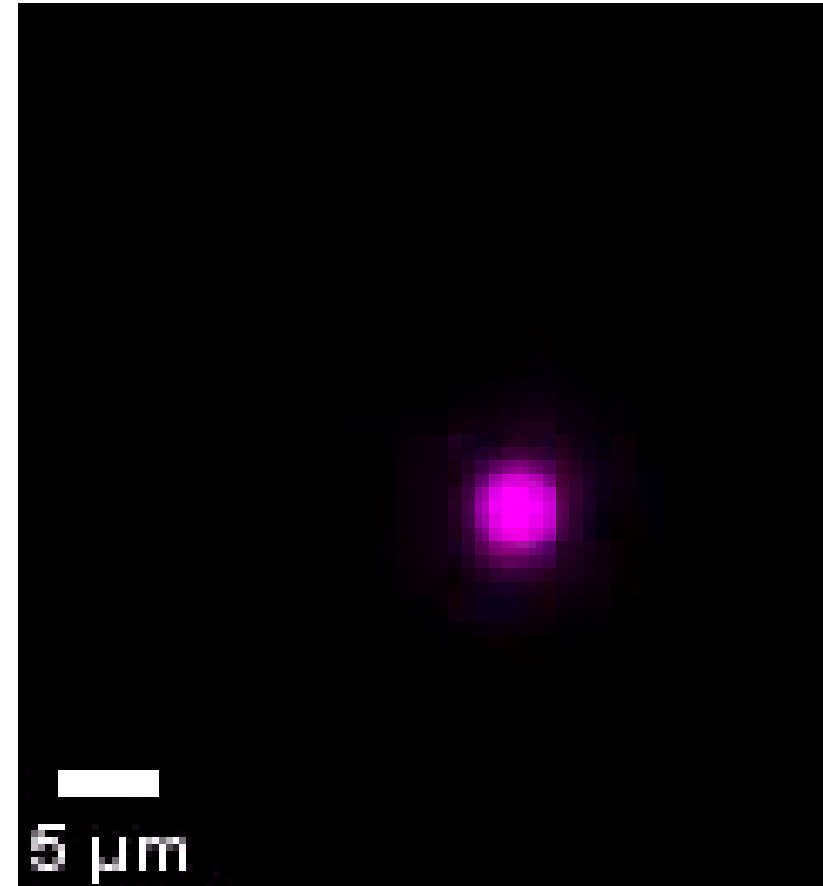
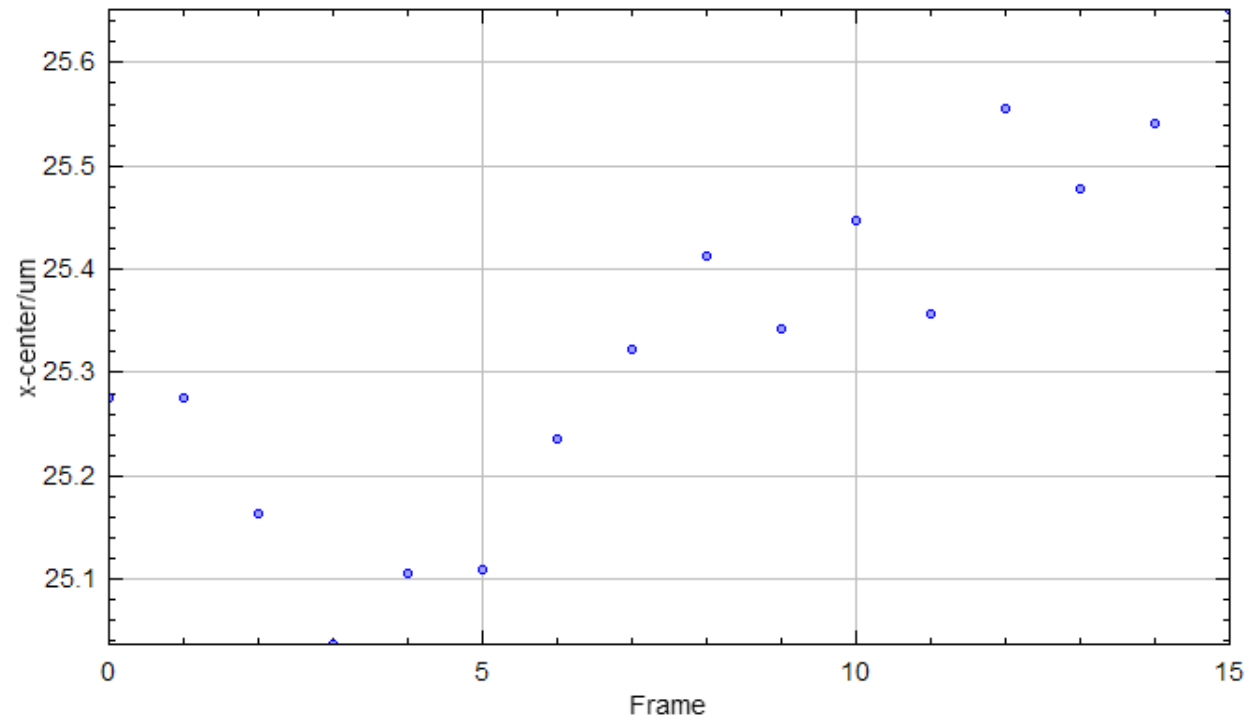


Course

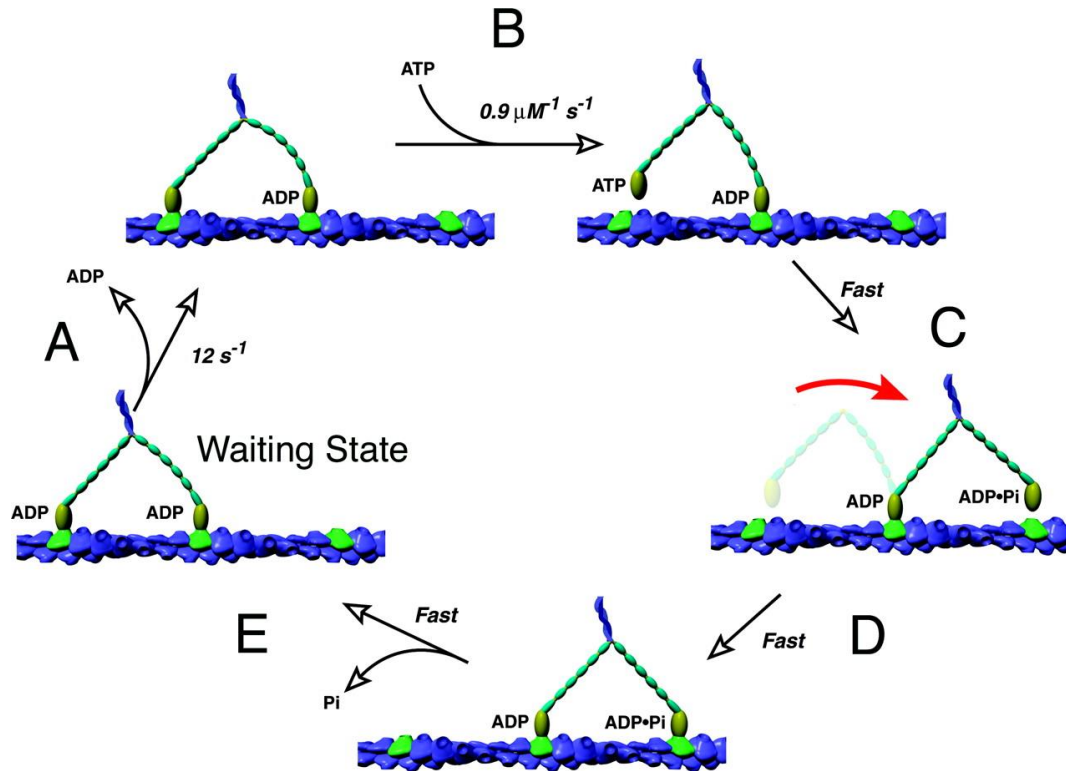
3D Single Molecule Localization Microscopy

BIO-410 □ BIOIMAGE INFORMATICS

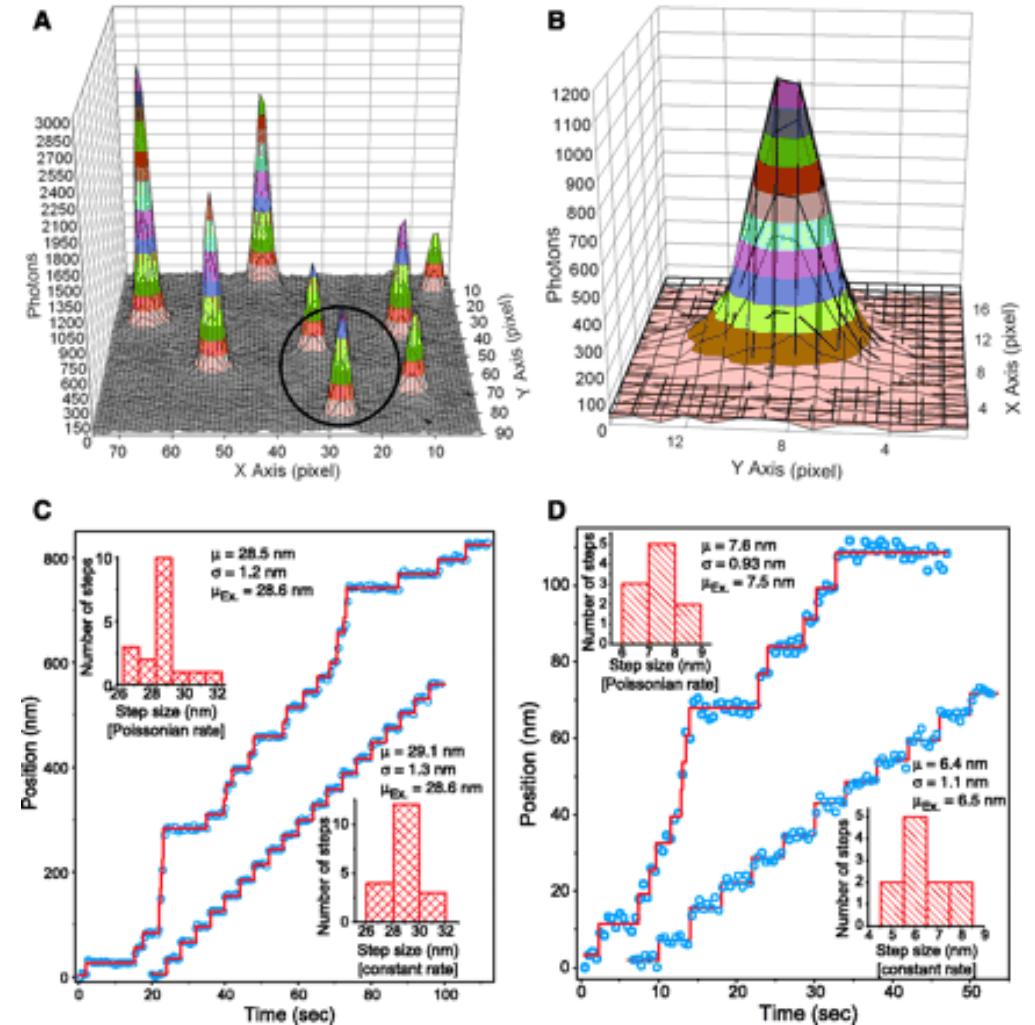
Localisation precision



MyosinV cycle

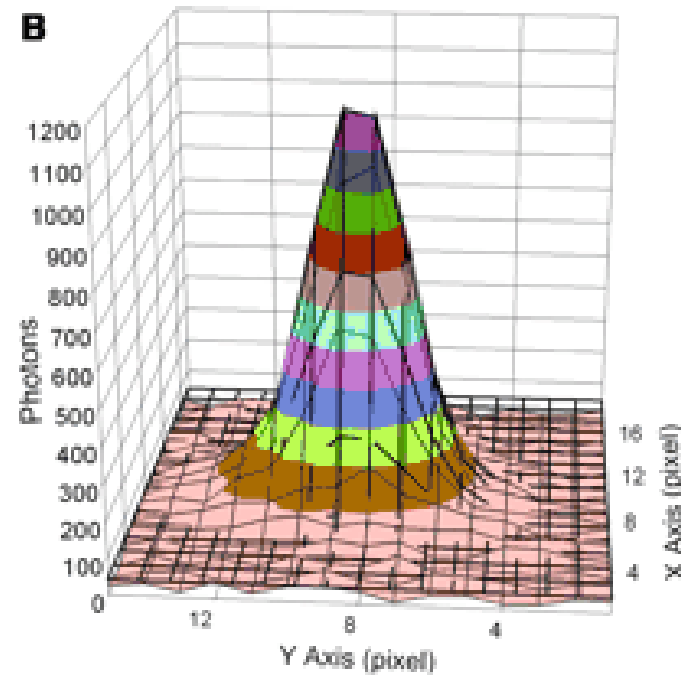
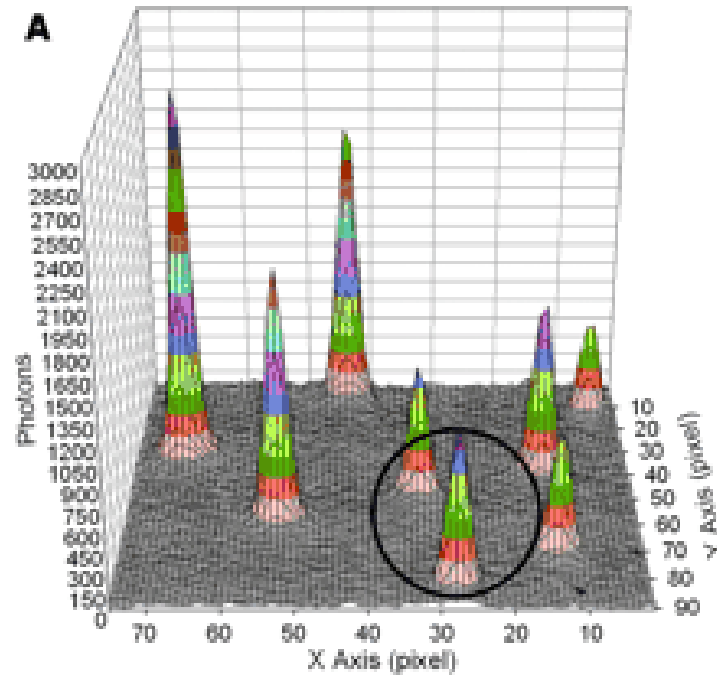


Vale R. D. (2003) Myosin V motor proteins : marching stepwise towards a mechanism *J. of Cell Biology* 163 (3): 445-450.



Yildiz A., Forkey J. N., McKinney S. A., Ha T., Yale E Goldman Y. E., Paul R Selvin P.R. (2003) Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging With 1.5-nm Localization *Science* (5628):2061-5

Localization precision



$$\sigma_{\mu_i} = \sqrt{\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}}$$

N = number of collected photons

a = pixel size (detector)

b = standard dev. background

s_i = width of the distribution

$$\sigma_{\mu} = 1.24 \text{ nm}$$

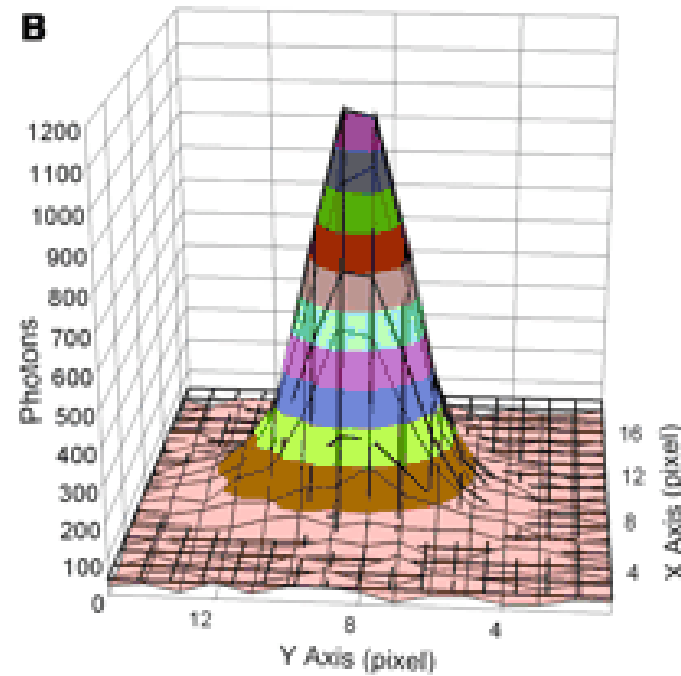
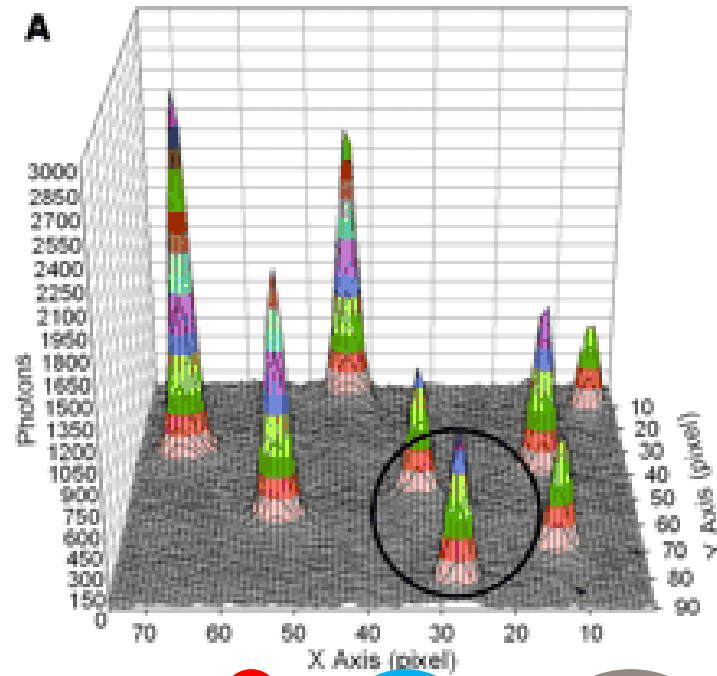
$$N = 14200$$

$$a = 86 \text{ nm}$$

$$b = 10$$

$$s_x = 125 \text{ nm}$$

$$s_y = 122 \text{ nm}$$



$$\sigma_{\mu_i} = \sqrt{\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}}$$

Shot noise
finite pixel size
Back-ground

$$\sigma_{\mu} = 1.24 \text{ nm}$$

$$N = 14200$$

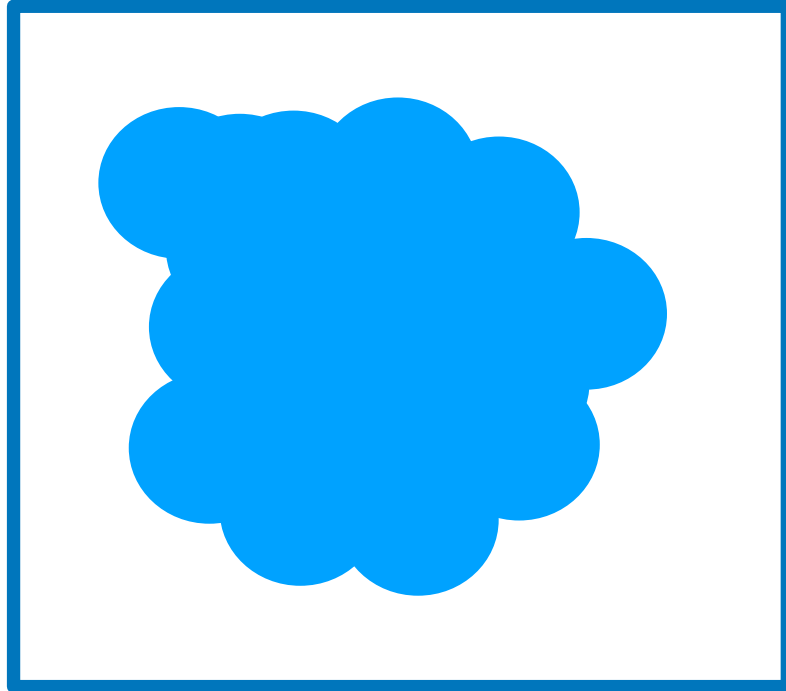
$$a = 86 \text{ nm}$$

$$b = 10$$

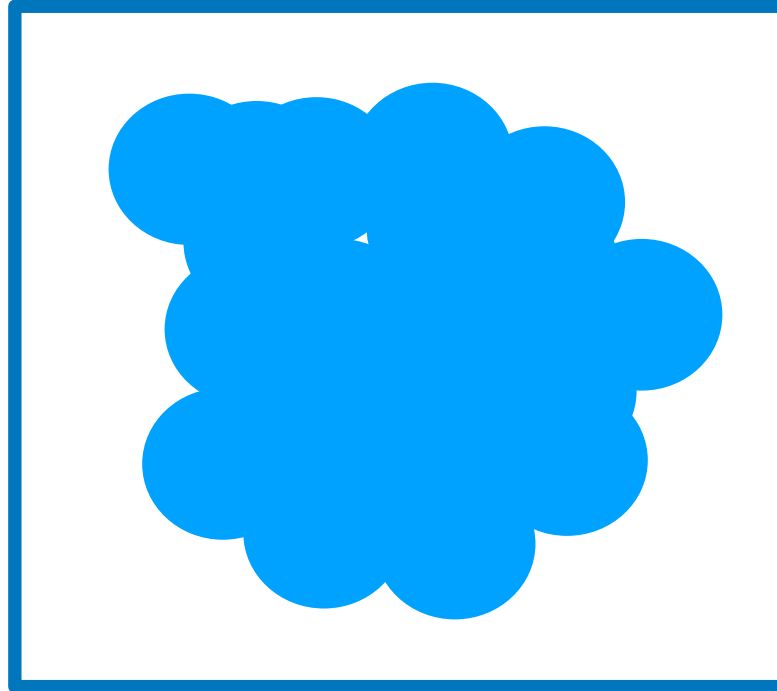
$$s_x = 125 \text{ nm}$$

$$s_y = 122 \text{ nm}$$

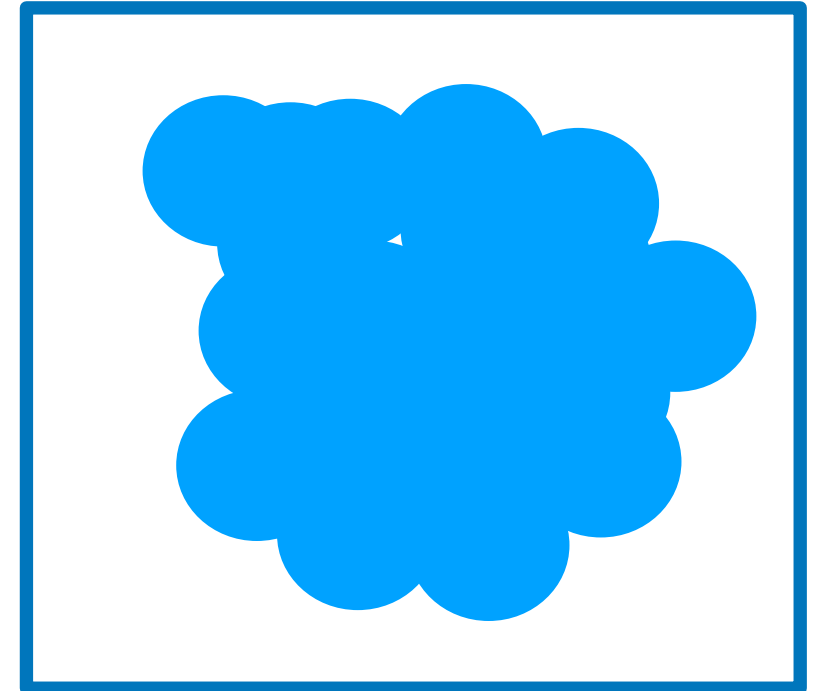
EPFL Localization Microscopy Principle



spatially overlapping
temporally overlapping

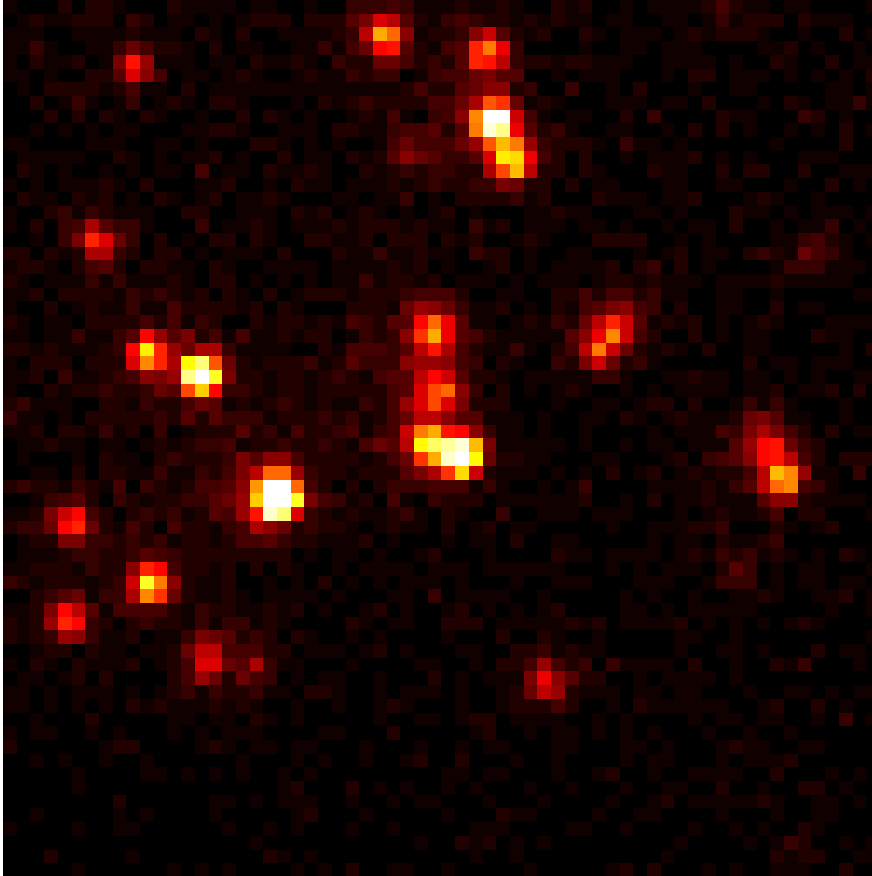


spatially overlapping
temporally separated

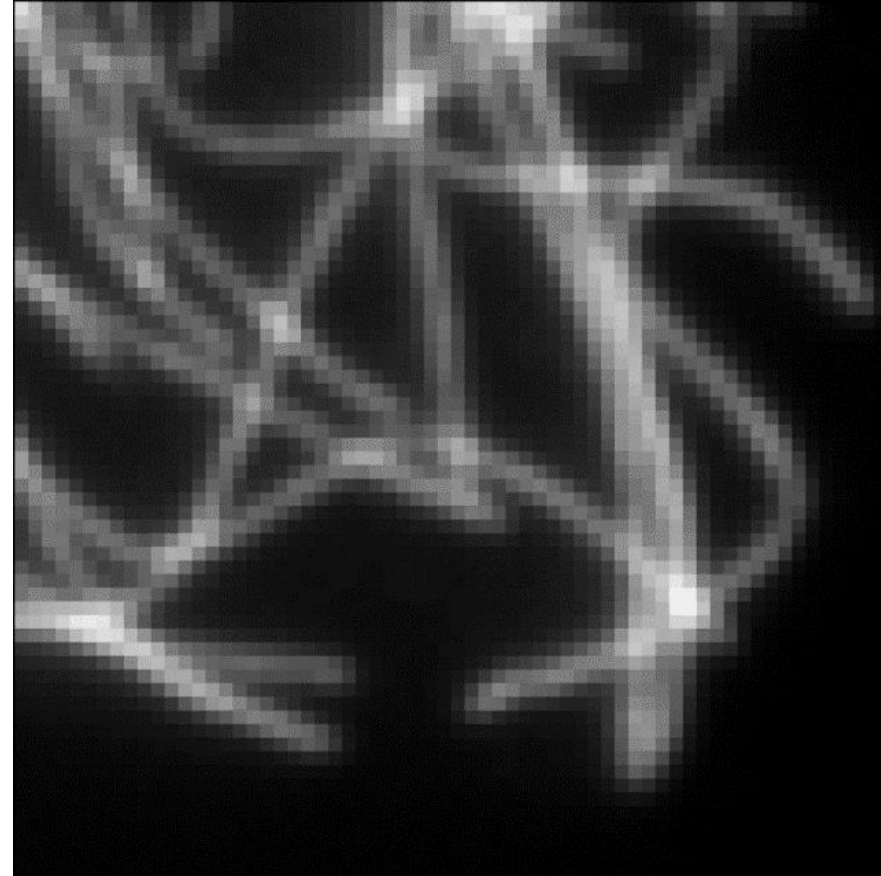


spatially overlapping
temporally partially
separated

Localization Microscopy Principle

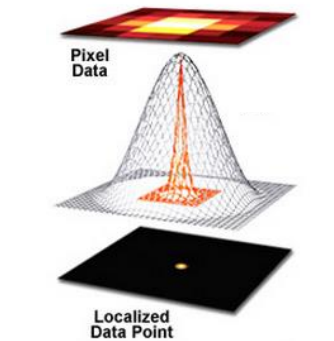
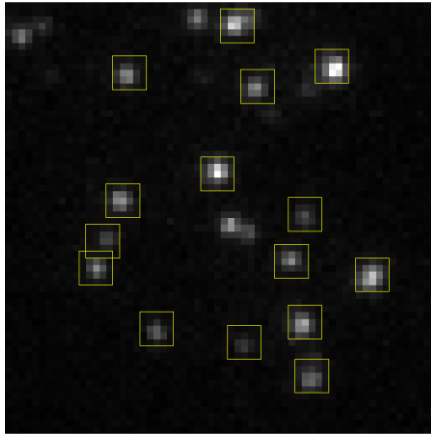


Temporal separation



Time projection

Point localization and reconstruction



Fitting of individual spots

Position list

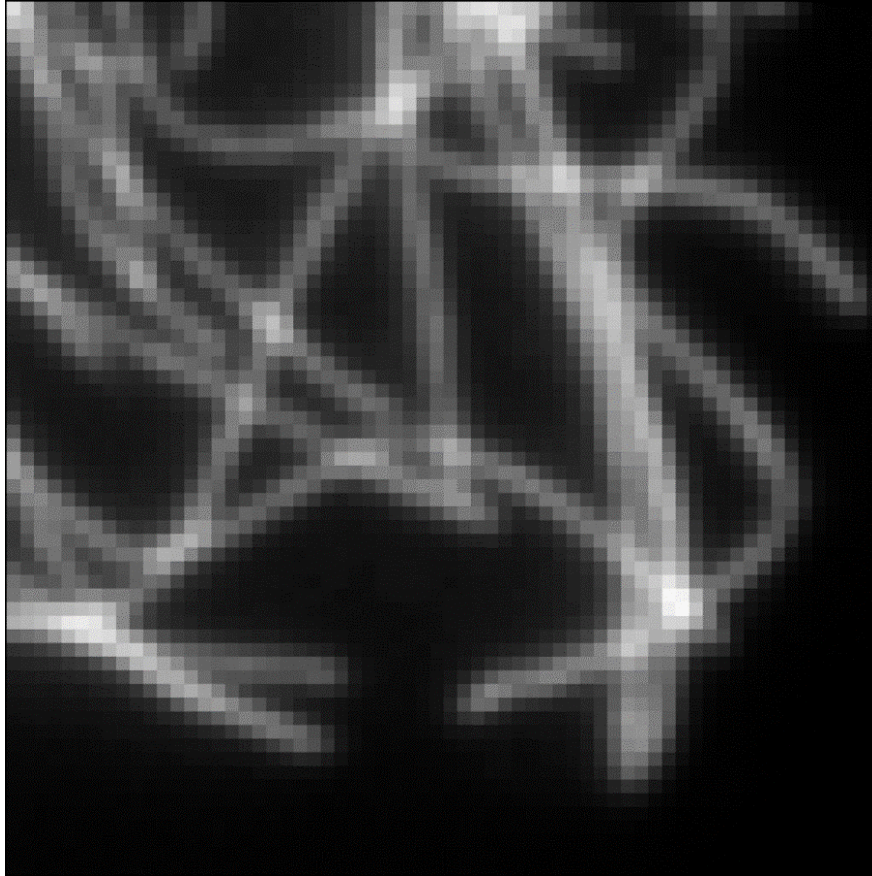
File Edit Font Results														-	□
	Intensity	X (px)	Y (px)	X (nm)	Y (nm)	Z (nm)	Left-Width (px)	Right-Width (px)	Up-Height (px)	Down-Height (px)	X Symmetry (%)	Y Symmetry (%)	Width minus Height (px)	Frame Number	
21	36	8.524	55.986	681.931	4478.897	0	0.734	0.694	1.161	0.362	0.972	0.476	-0.095	2	
22	36	23.396	15.196	1871.663	1215.663	0	1.612	1.463	1.384	1.525	0.952	0.952	0.166	2	
23	33	33.731	49.764	2698.450	3281.319	0	0.860	0.552	0.899	0.526	0.762	0.738	-0.012	2	
24	36	25.135	41.016	2010.769	3281.319	0	1.577	1.322	1.785	1.464	0.912	0.901	-0.350	11	
25	36	33.155	51.887	2652.371	4150.928	0	0.475	0.995	1.044	0.445	0.646	0.598	-0.019	11	
26	40	8.021	36.333	641.702	2906.667	0	0.380	1.152	0.548	0.785	0.496	0.823	0.199	11	
27	36	53.750	41.463	4300.000	3317.008	0	1.355	1.094	1.708	1.749	0.894	0.988	-1.007	11	
28	34	32.157	22.075	2572.567	1765.977	0	1.668	1.774	1.253	1.804	0.969	0.820	0.384	11	
29	36	6.811	56.268	544.880	4501.443	0	0.955	0.507	0.555	0.962	0.694	0.784	0.046	11	
30	38	42.160	47.978	3372.764	3838.219	0	1.261	1.800	1.941	0.741	0.824	0.553	0.379	11	
31	39	46.365	11.898	3709.197	951.825	0	0.574	0.747	1.057	0.436	0.869	0.584	-0.171	11	
32	38	5.622	21.900	449.778	1752.000	0	0.732	0.577	1.059	0.438	0.881	0.595	-0.188	11	
33	36	62.058	54.524	4164.658	4361.918	0	0.390	1.108	0.733	0.694	0.521	0.973	0.072	11	
34	36	10.479	6.938	838.345	555.034	0	0.699	0.729	1.104	0.392	0.979	0.524	-0.068	11	
35	35	21.525	57.183	1721.972	4574.648	0	0.728	0.688	0.468	0.961	0.972	0.655	-0.013	11	
36	33	8.520	15.218	681.624	1217.417	0	0.719	0.683	0.489	0.921	0.974	0.694	-0.007	11	
37	33	30.687	29.321	2454.925	2345.672	0	0.808	0.589	0.583	0.799	0.943	0.943	0.015	11	
38	34	17.332	16.432	1386.556	1314.523	0	0.577	0.786	0.611	0.669	0.846	0.954	0.084	11	
39	36	39.731	13.003	3178.512	1040.220	0	1.607	1.496	0.937	2.046	0.964	0.628	0.121	5	

Position list

Row	Result	Est. FWHM Results													-	+	X
		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 (%)	Width minus Height (px)			
21	36	8.524	55.986	681.931	4478.897	0	0.734	0.694	1.161	0.362	0.972	0.476	-0.095	2			
22	36	23.396	15.196	1871.663	1215.663	0	1.612	1.463	1.384	1.525	0.952	0.952	0.166	2			
23	33	33.731	49.764	2698.450	3281.319	0	0.860	0.552	0.899	0.526	0.762	0.738	-0.012	2			
24	36	25.135	41.016	2010.769	3281.319	0	1.577	1.322	1.785	1.464	0.912	0.901	-0.350	11			
25	36	33.155	51.887	2652.371	4150.928	0	0.475	0.995	1.044	0.445	0.646	0.598	-0.019	11			
26	40	8.021	36.333	641.702	2906.667	0	0.380	1.152	0.548	0.785	0.496	0.823	0.199	11			
27	36	53.750	41.463	4300.000	3317.008	0	1.355	1.094	1.708	1.749	0.894	0.988	-1.007	11			
28	34	32.157	22.075	2572.567	1765.977	0	1.668	1.774	1.253	1.804	0.969	0.820	0.384	11			
29	36	6.811	56.268	544.880	4501.443	0	0.955	0.507	0.555	0.962	0.694	0.784	0.046	11			
30	38	42.160	47.978	3372.764	3838.219	0	1.261	1.800	1.941	0.741	0.824	0.553	0.379	11			
31	39	46.365	11.898	3709.197	951.825	0	0.574	0.747	1.057	0.436	0.869	0.584	-0.171	11			
32	38	5.622	21.900	449.778	1752.000	0	0.732	0.577	1.059	0.438	0.881	0.595	-0.188	11			
33	36	62.058	54.524	4164.658	4361.918	0	0.390	1.108	0.733	0.694	0.521	0.973	0.072	11			
34	36	10.479	6.938	838.345	555.034	0	0.699	0.729	1.104	0.392	0.979	0.524	-0.068	11			
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36	33	8.520	15.218	681.624	1217.417	0	0.719	0.683	0.489	0.921	0.974	0.694	-0.007	11			
37	33	30.687	29.321	2454.925	2345.672	0	0.808	0.589	0.583	0.799	0.943	0.943	0.015	11			
38	34	17.332	16.432	1386.556	1314.523	0	0.577	0.786	0.611	0.669	0.846	0.954	0.084	11			
39	36	39.731	13.003	3178.512	1040.220	0	1.607	1.496	0.937	2.046	0.964	0.628	0.121	5			

Reconstruction



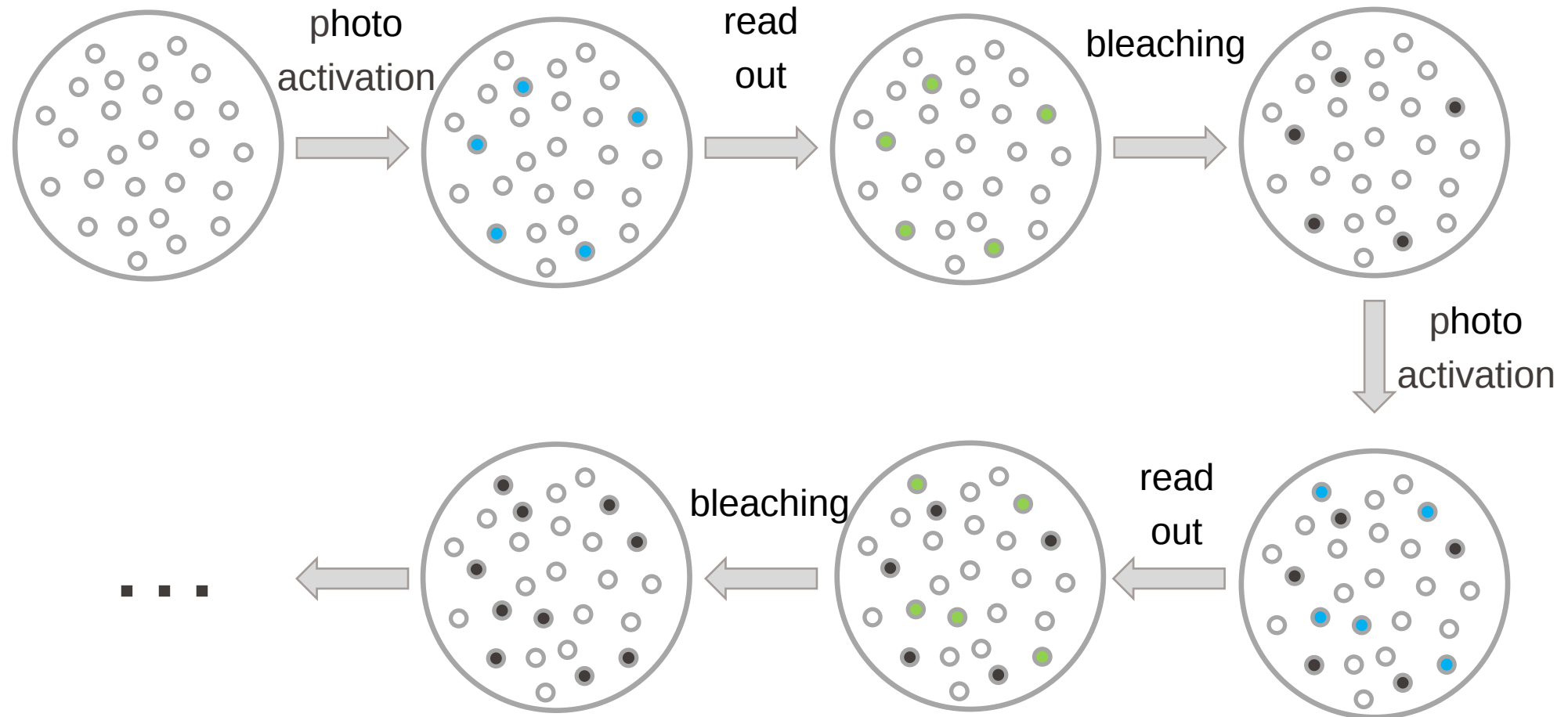


Diffraction limited

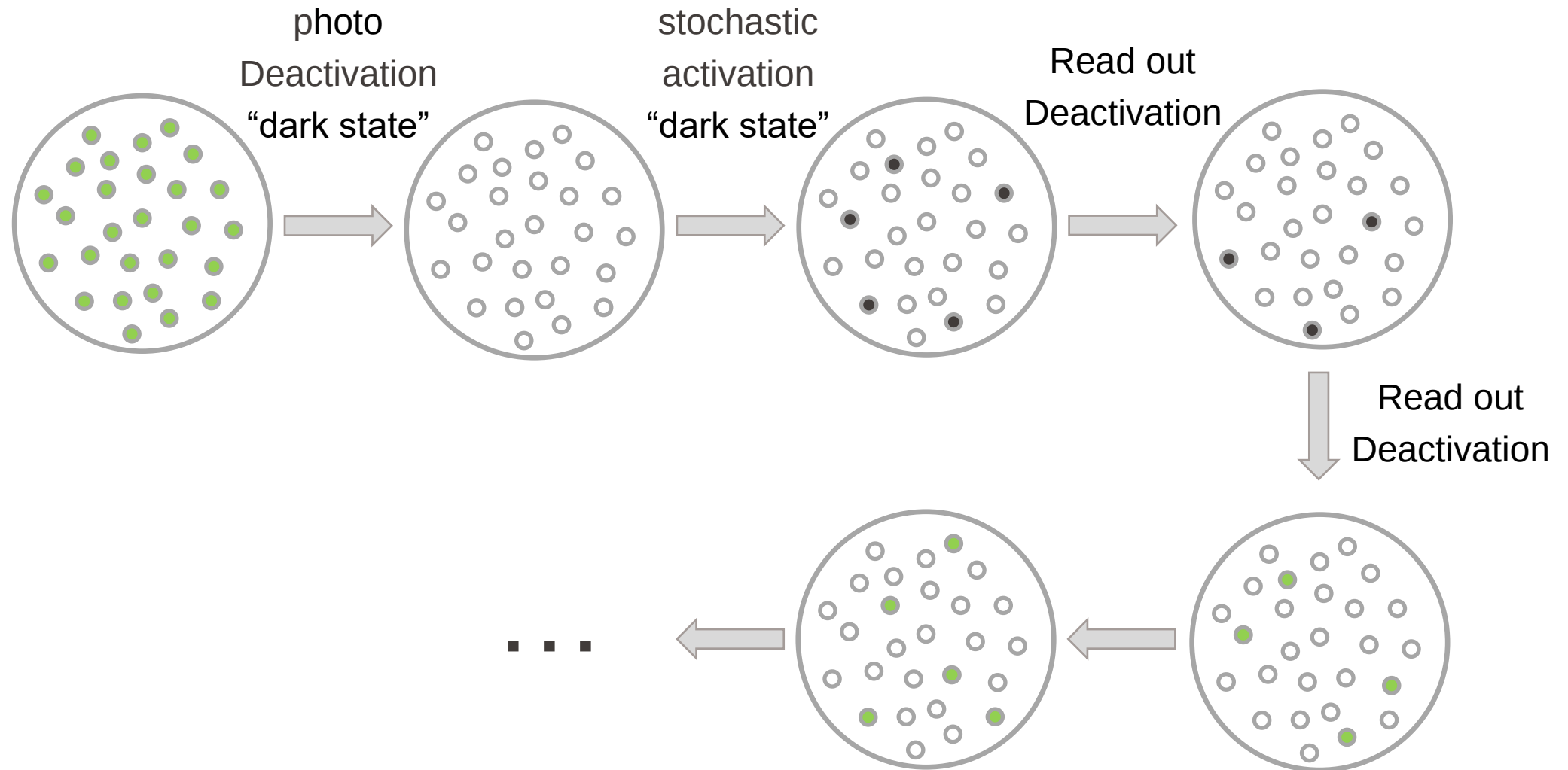


Super resolution

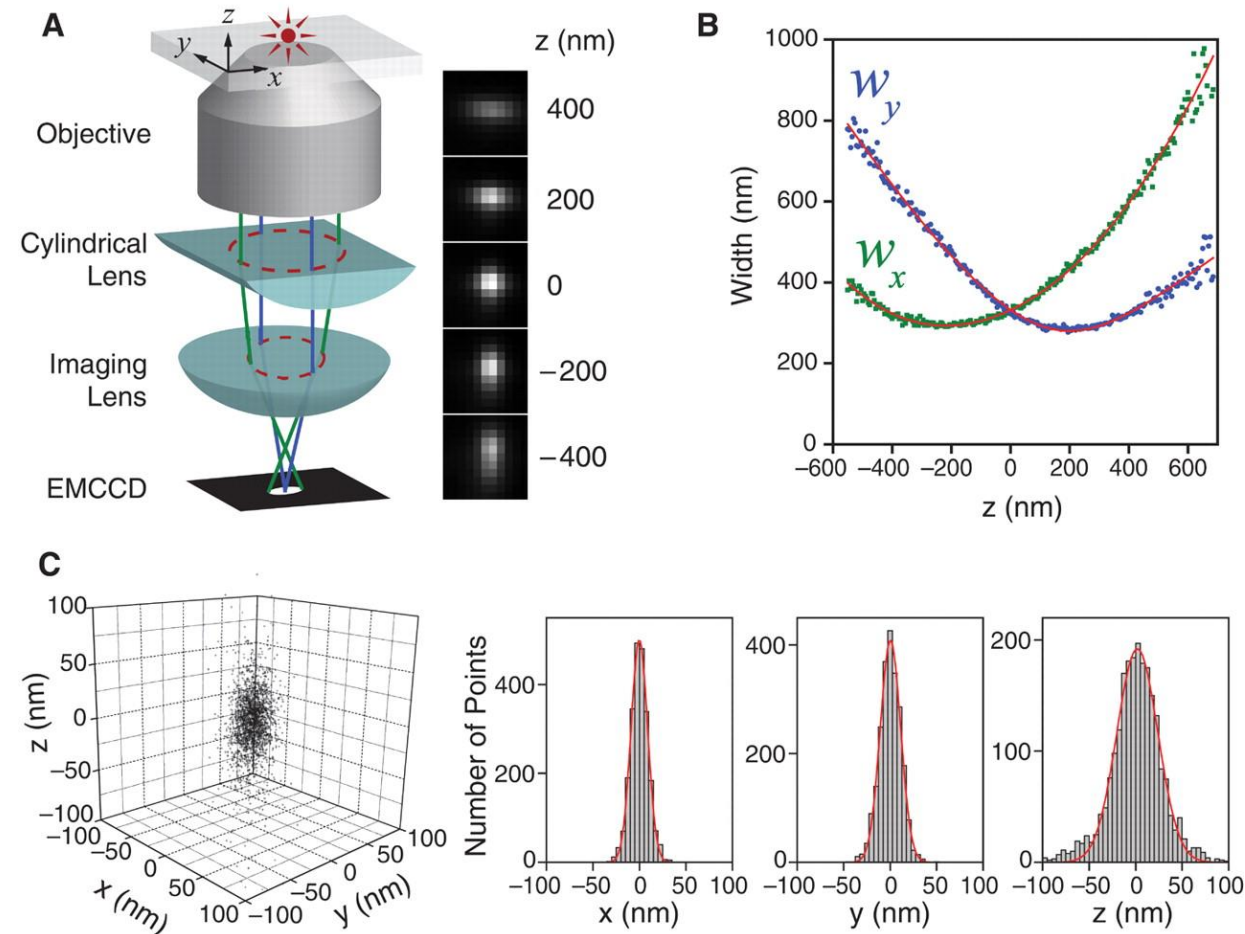
Photoactivated Localization Microscopy (PALM)



Stochastic Optical Reconstruction Microscopy (STORM)



3D Localization

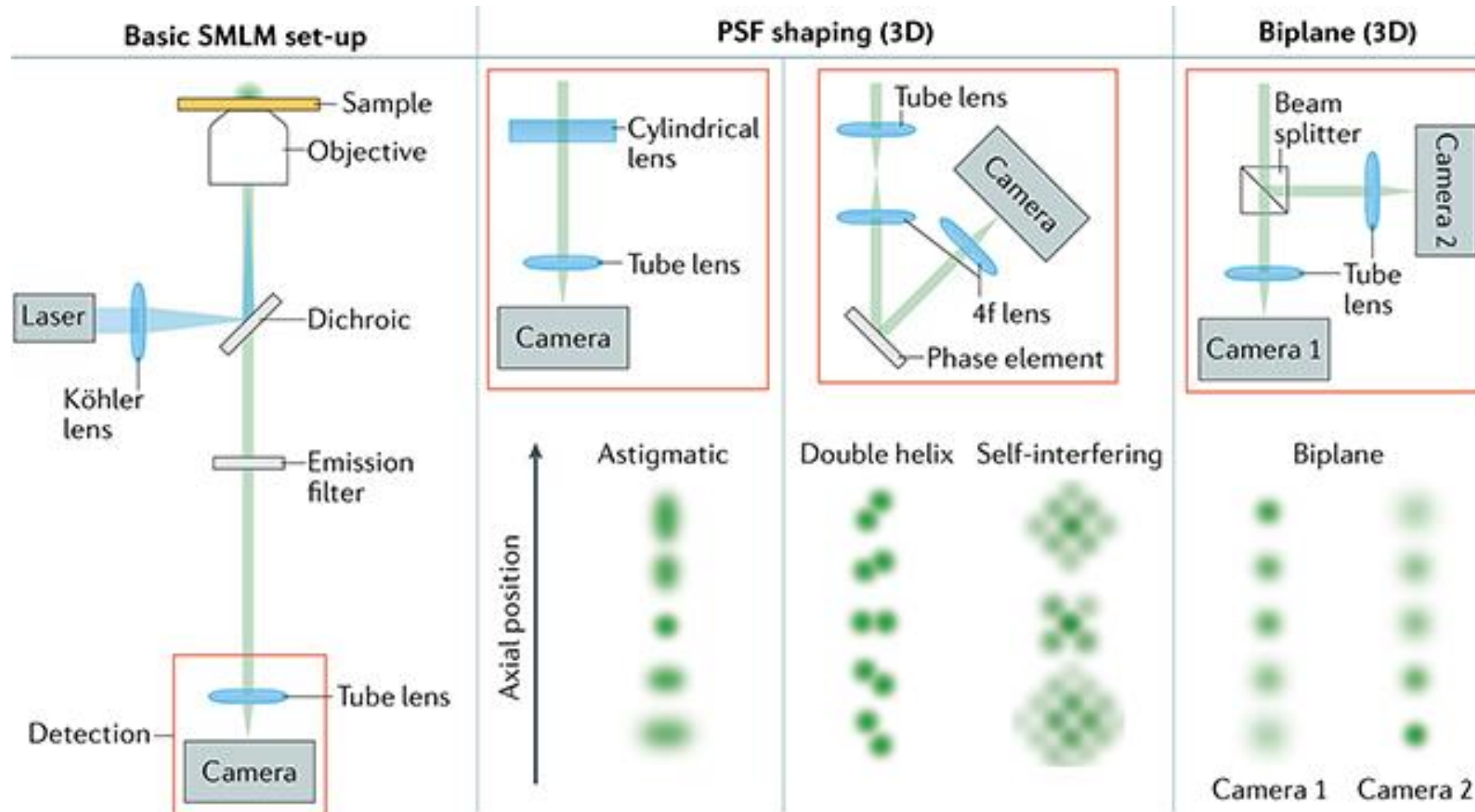


Bo Huang et al.

Three-Dimensional Super-Resolution Imaging by Stochastic Optical Reconstruction Microscopy

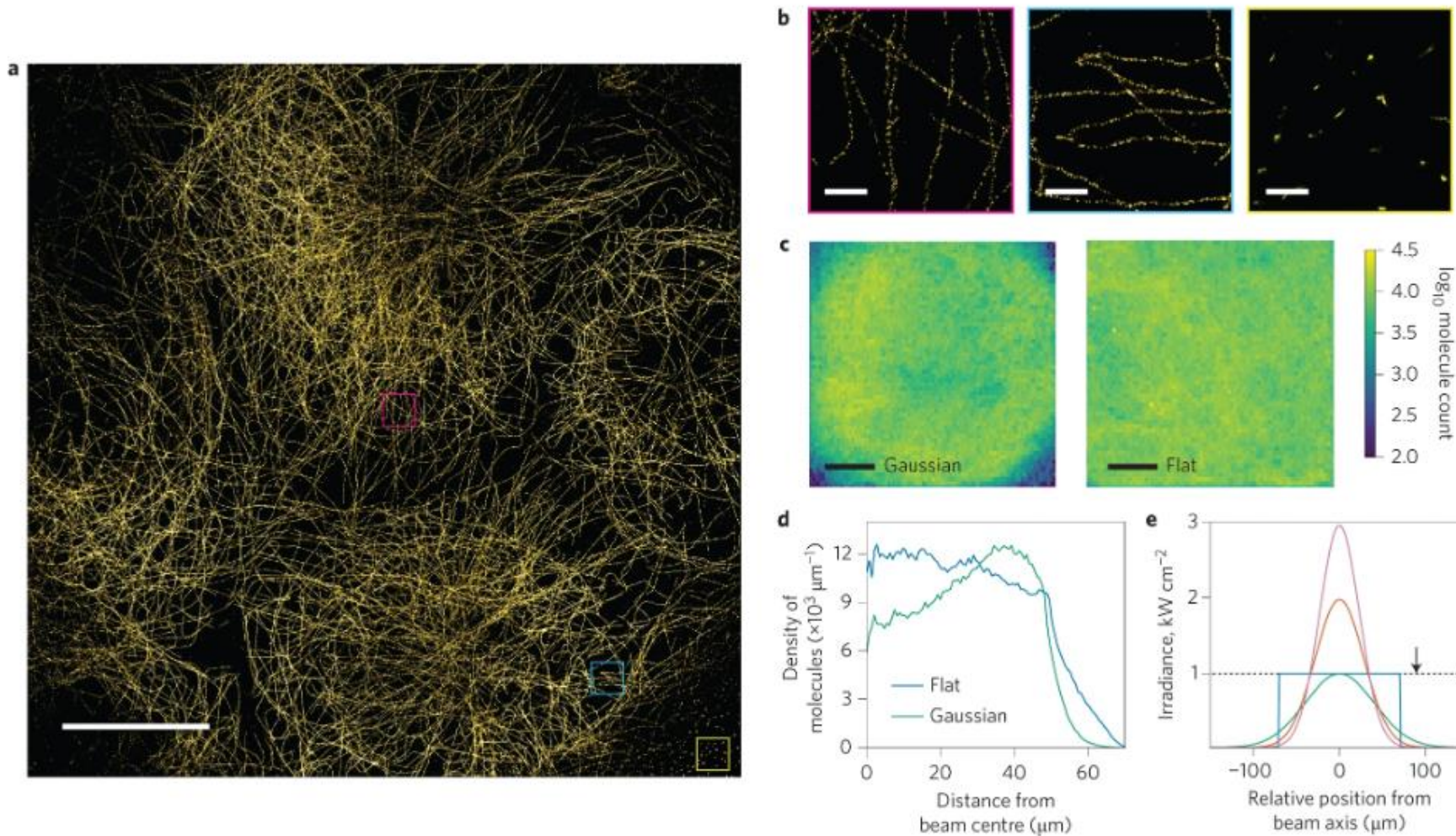
DOI: 10.1126/science.1153529

3D Localization



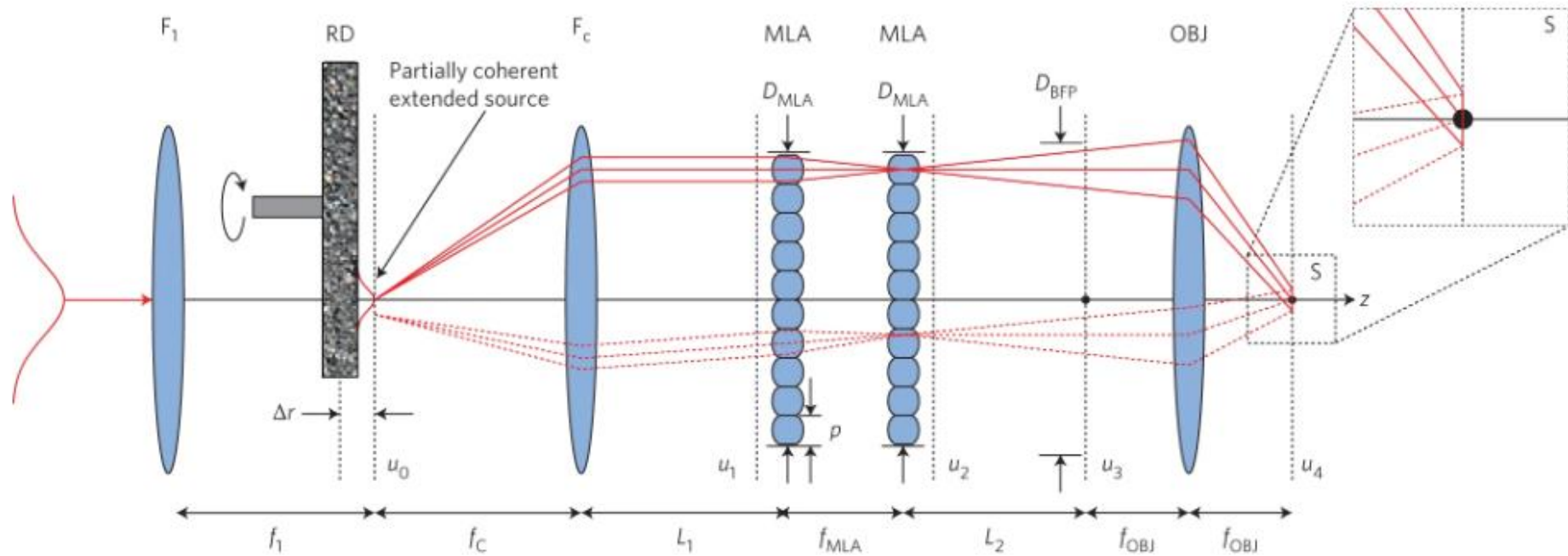
Lelek et al.
 Single-molecule localization microscopy
 doi: [10.1038/s43586-021-00038-x](https://doi.org/10.1038/s43586-021-00038-x)

Experimental Challenges



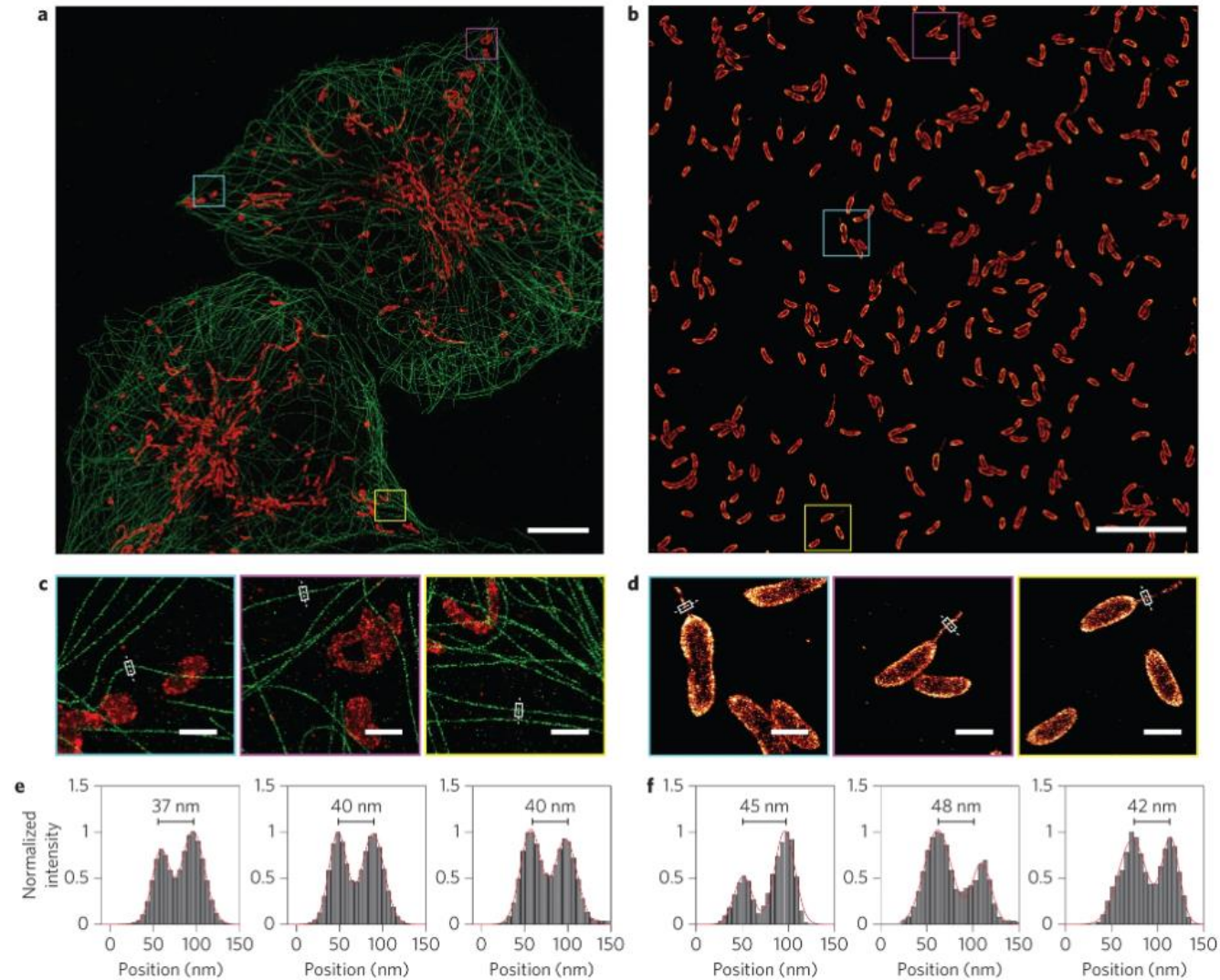
Douglass, K., Sieben, C., Archetti, A. *et al.* Super-resolution imaging of multiple cells by optimized flat-field epi-illumination. *Nature Photon* **10**, 705–708 (2016). <https://doi.org/10.1038/nphoton.2016.200>

Experimental Challenges



Nature Photon **10**, 705–708 (2016). <https://doi.org/10.1038/nphoton.2016.200>

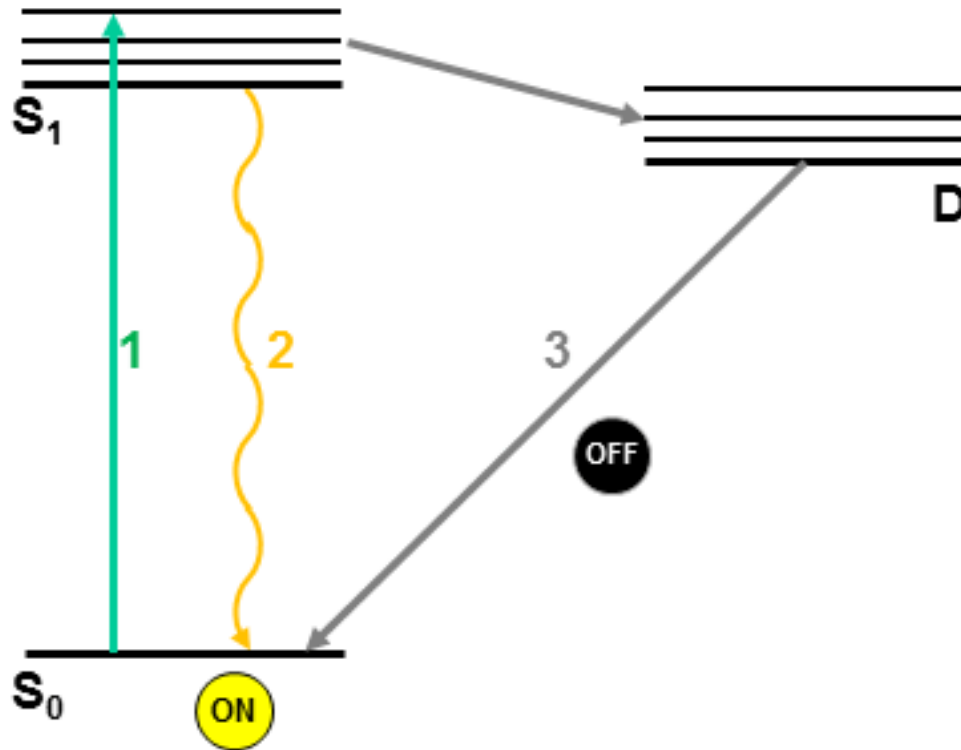
Experimental Challenges



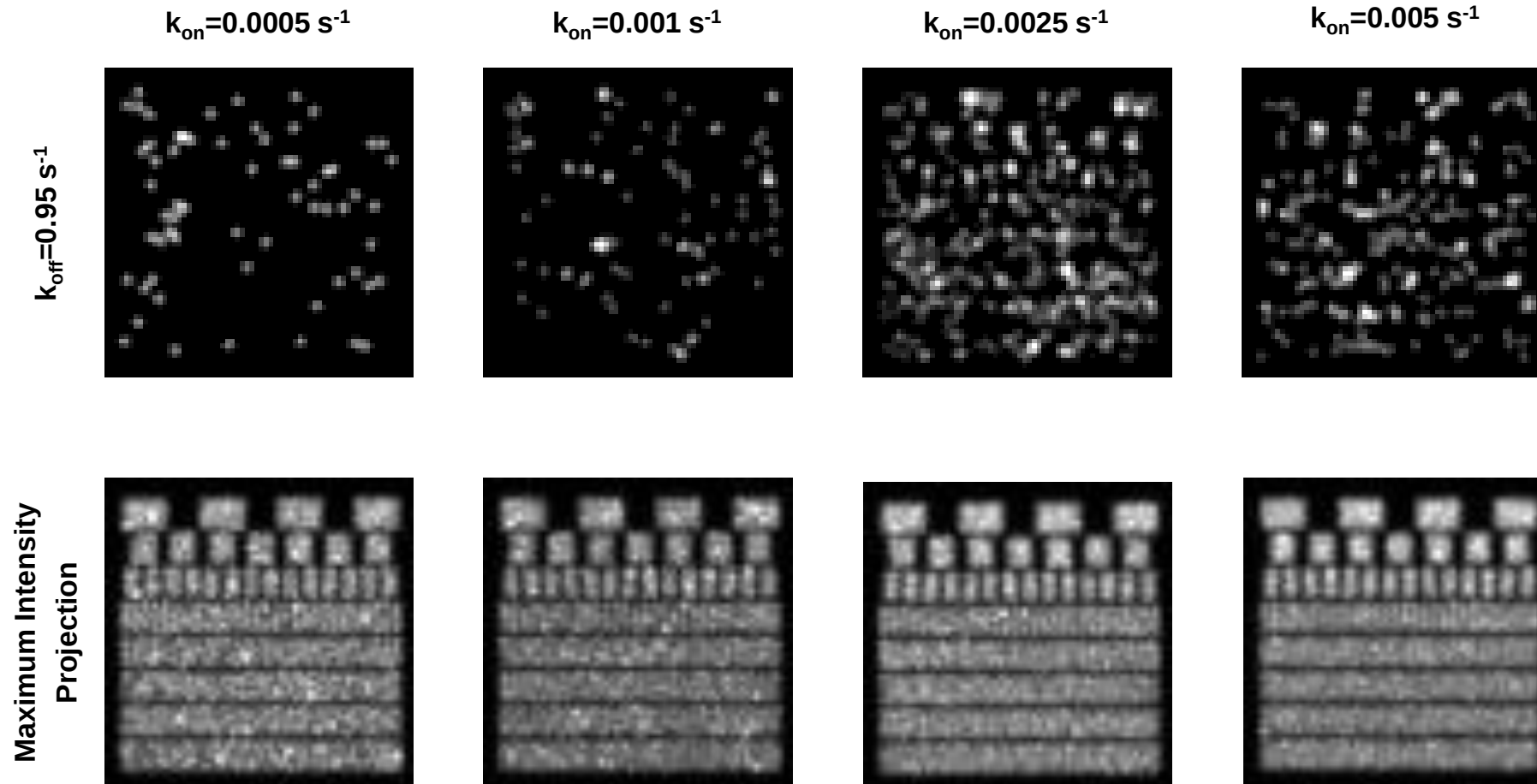
Nature Photon **10**, 705–708 (2016). <https://doi.org/10.1038/nphoton.2016.200>

Fluorophore properties

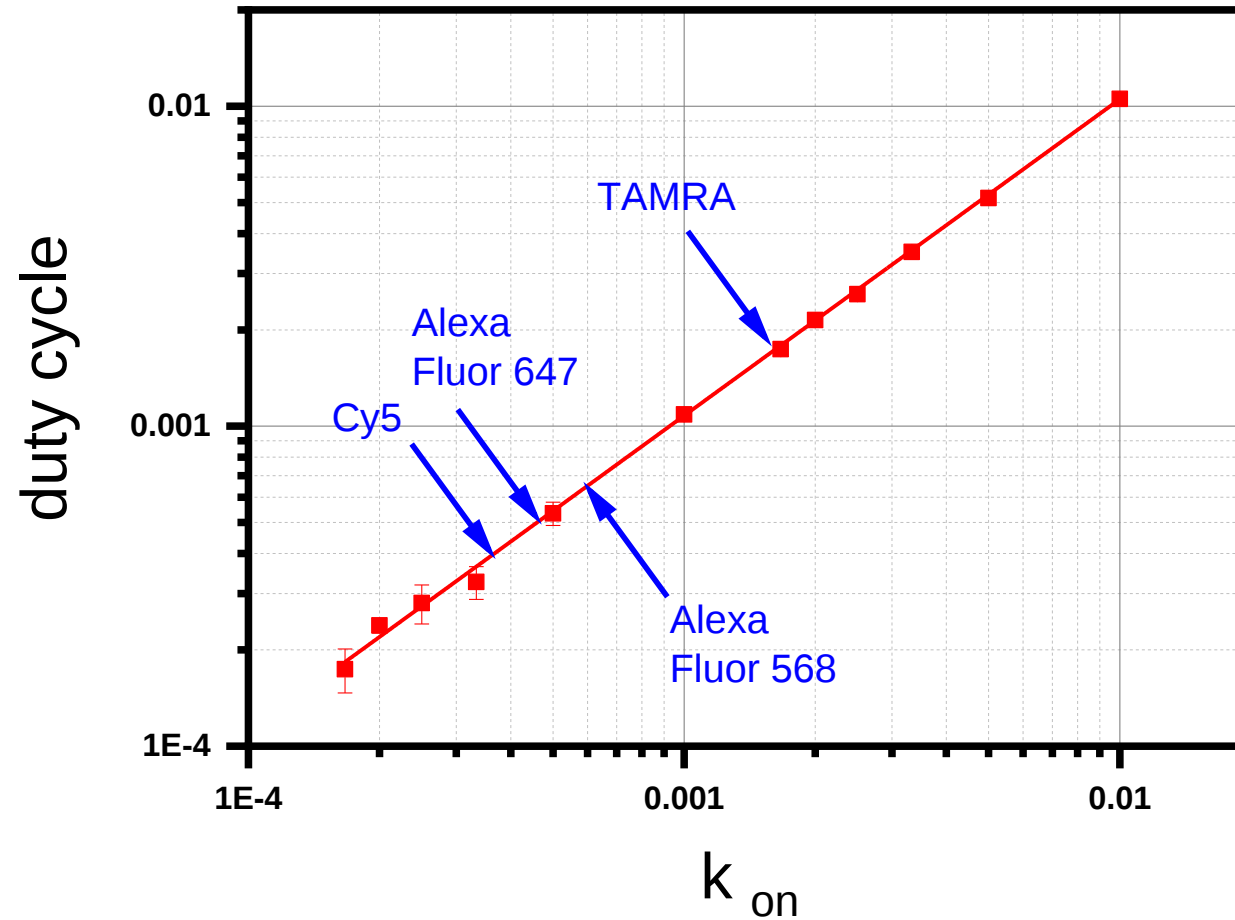
Molecular blinking mechanism



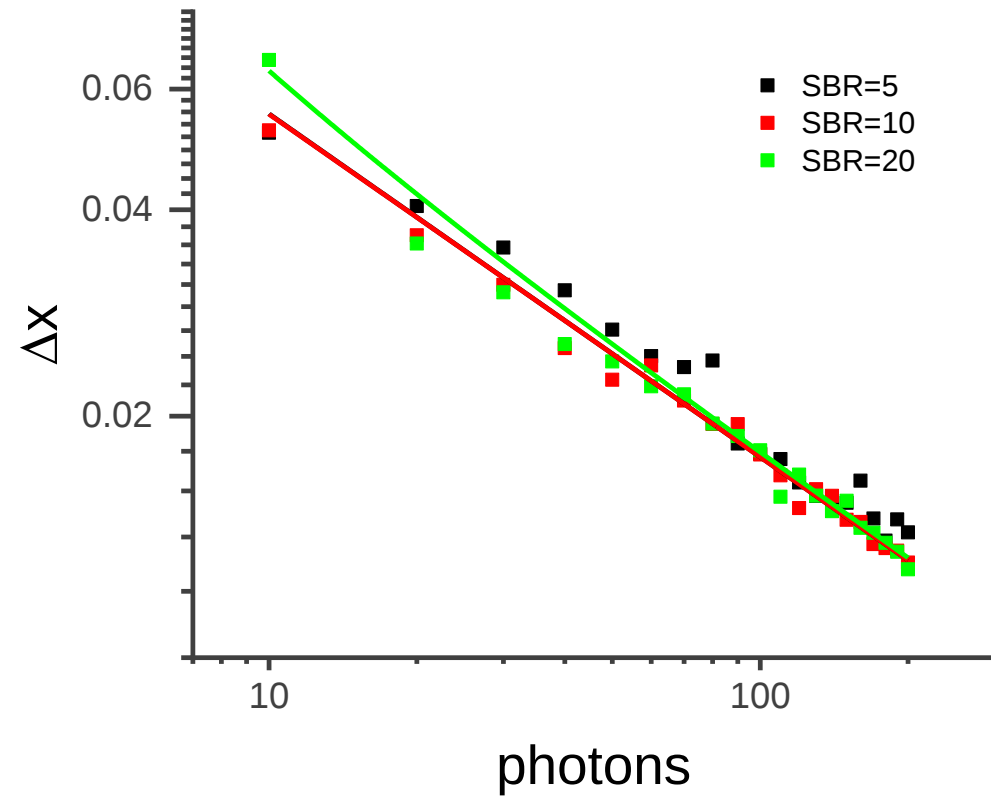
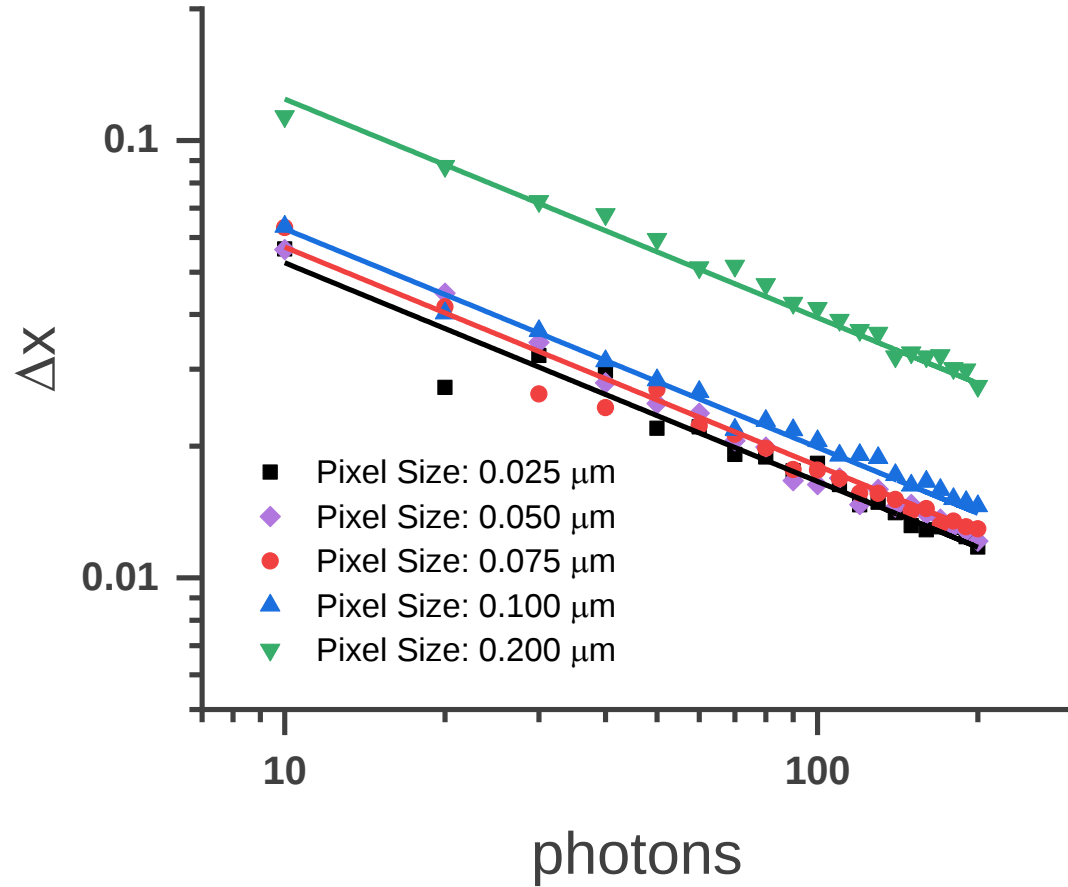
Duty Cycle



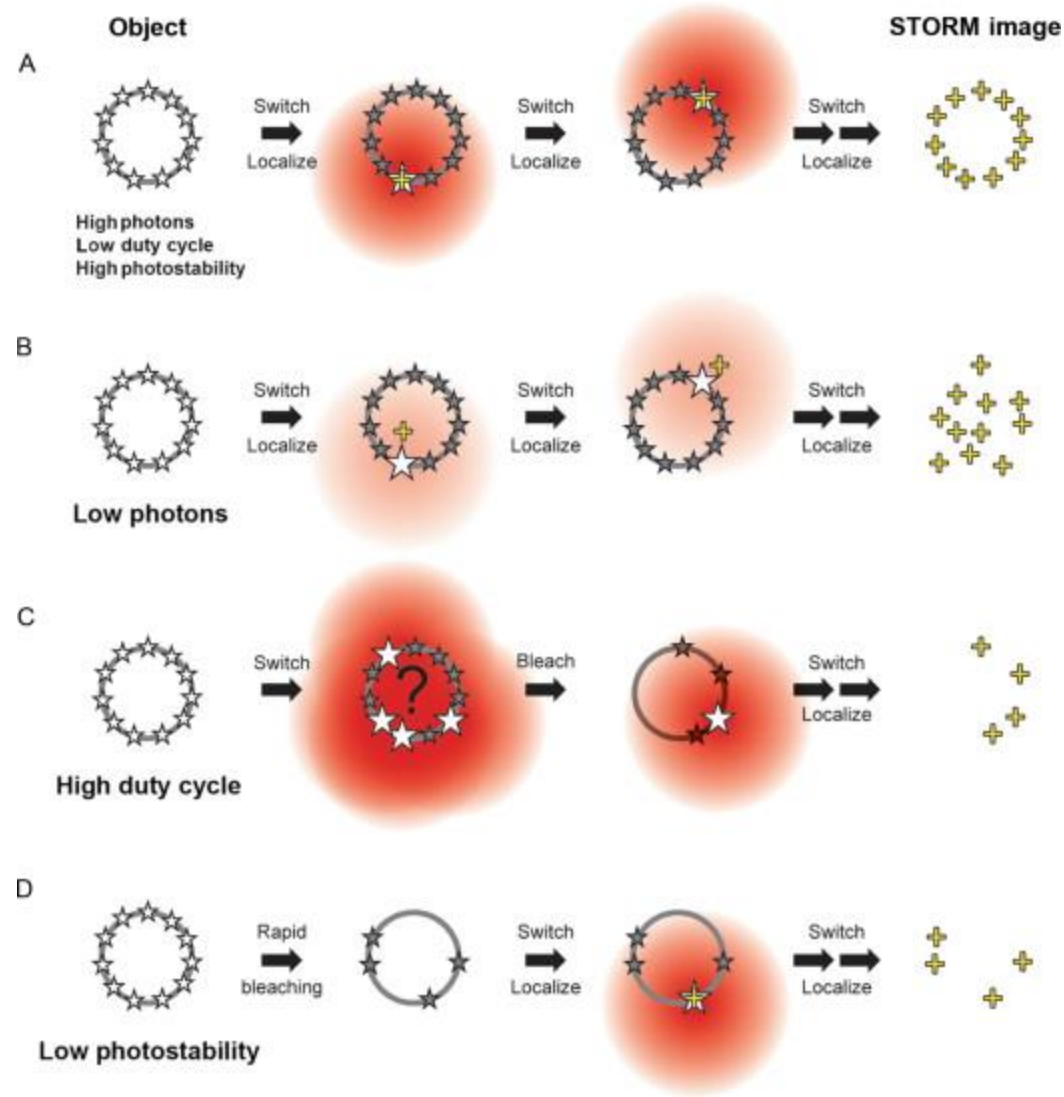
Duty Cycle



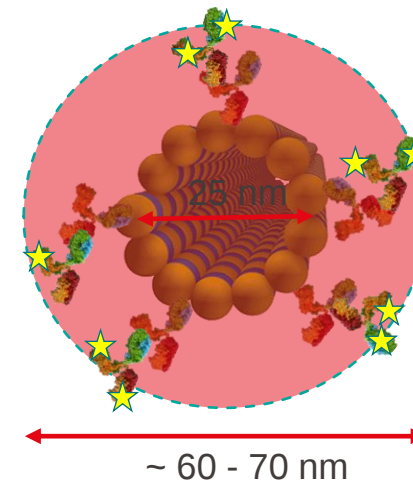
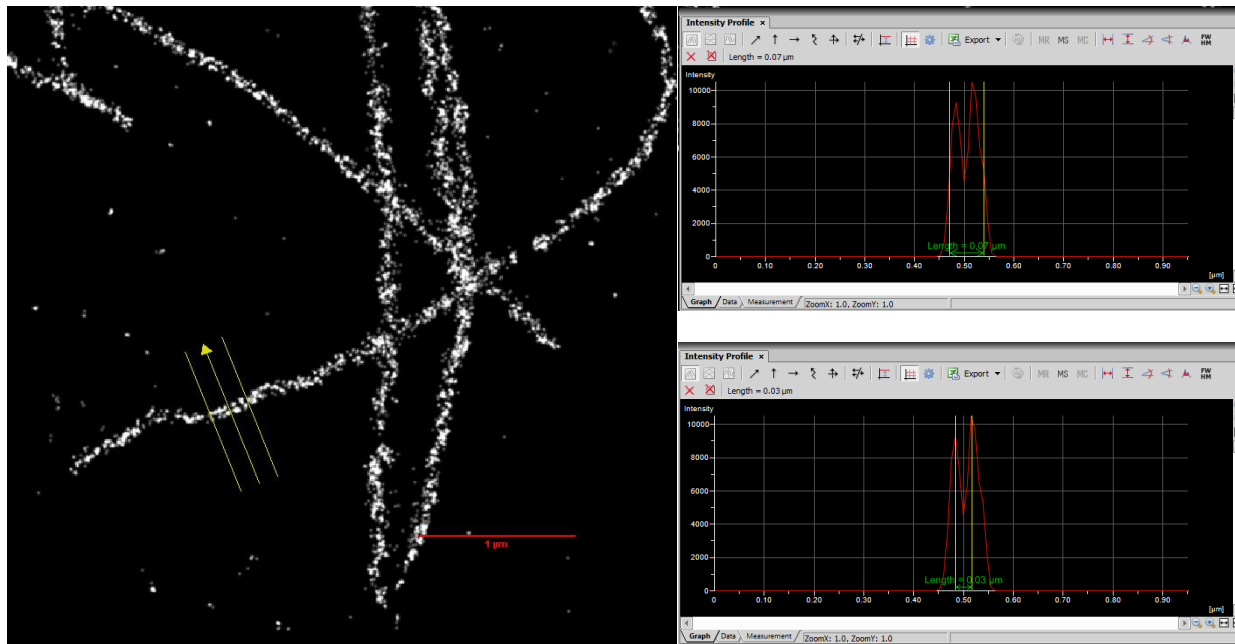
Photon output



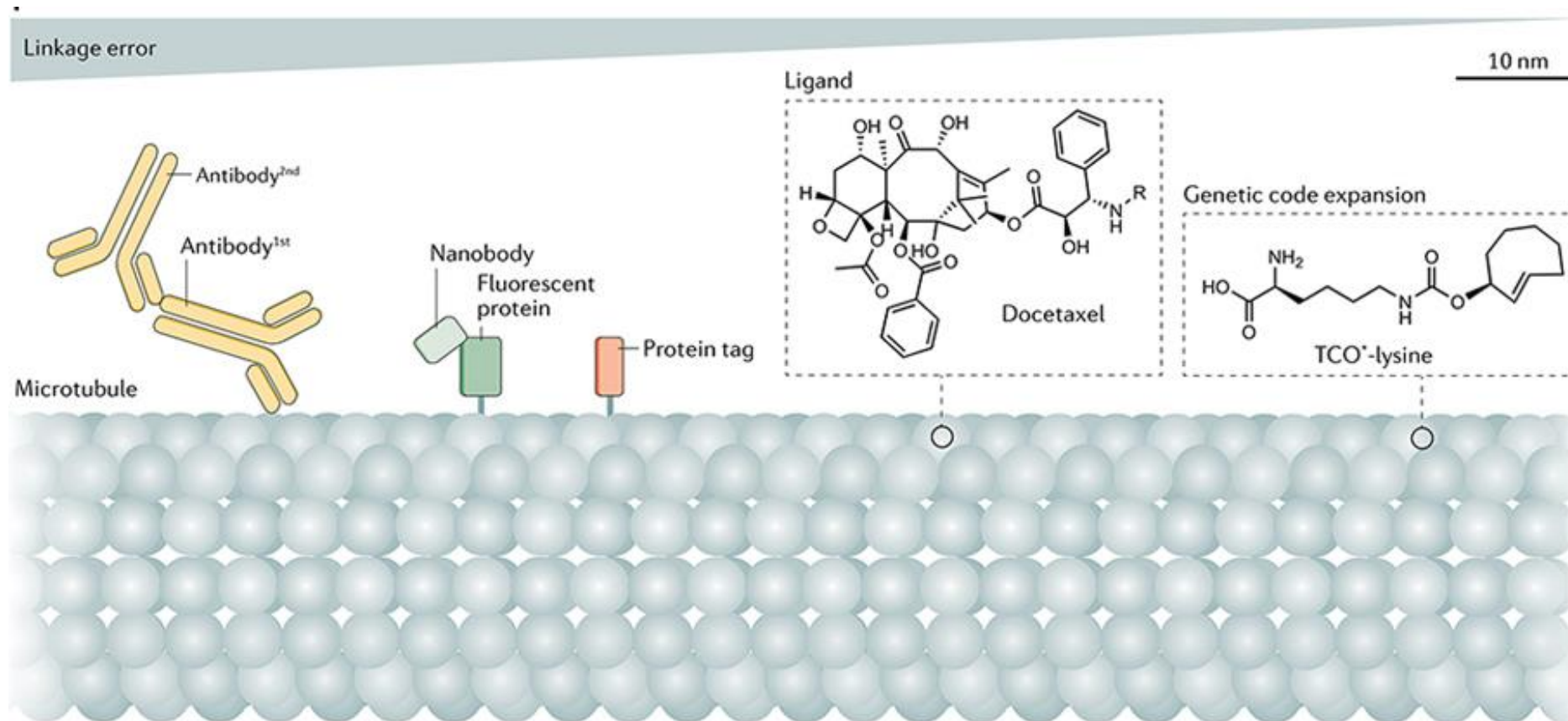
Summary Fluorophore properties



Performance of Localization Microscopy

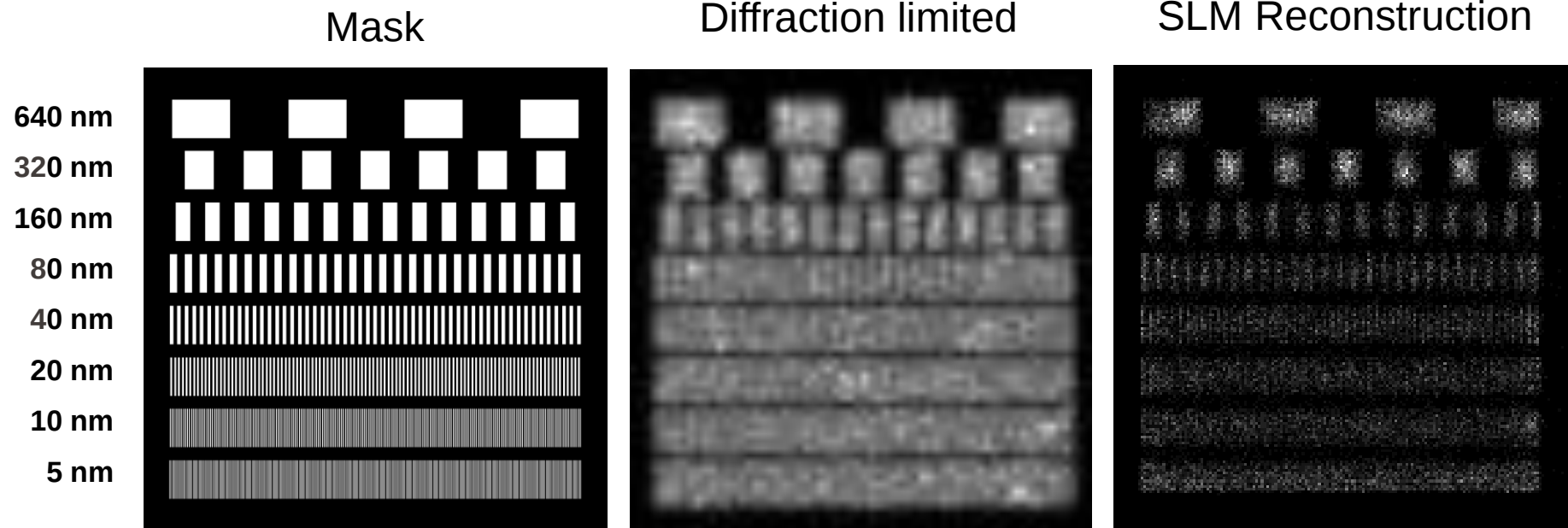


Performance of Localization Microscopy

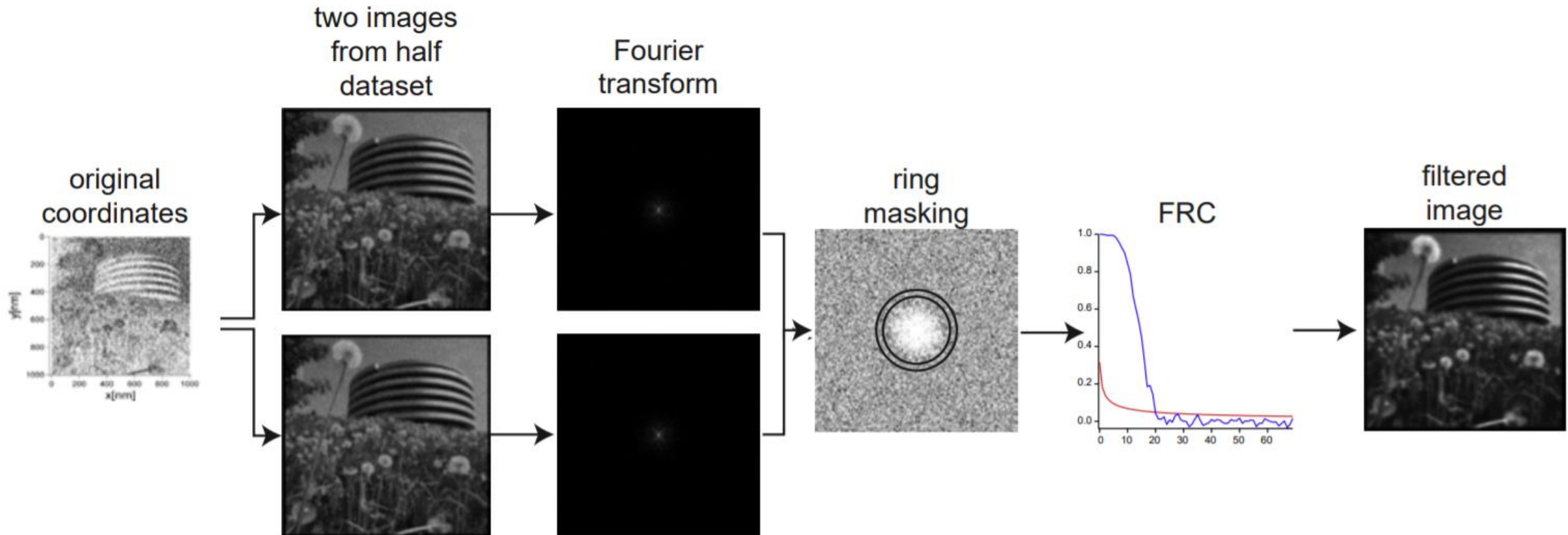


Lelek et al.
Single-molecule localization microscopy
doi: [10.1038/s43586-021-00038-x](https://doi.org/10.1038/s43586-021-00038-x)

Measuring resolution

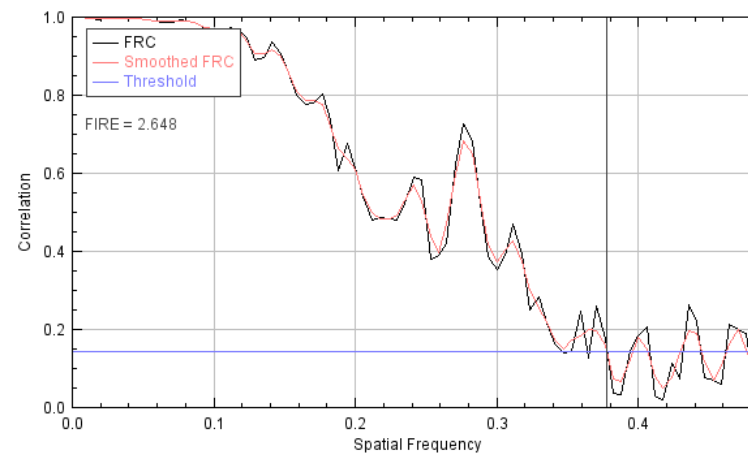
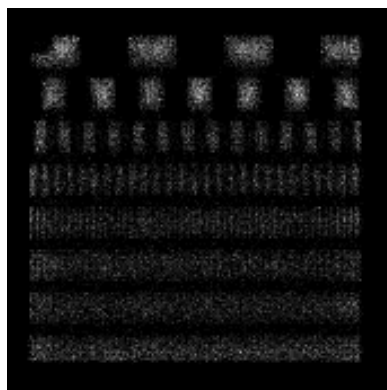
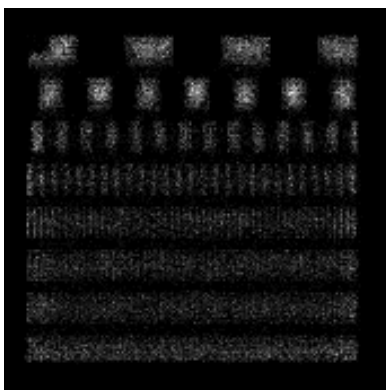
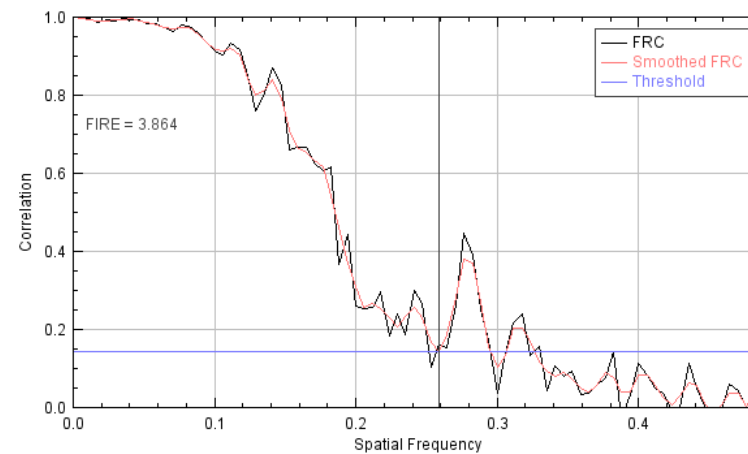
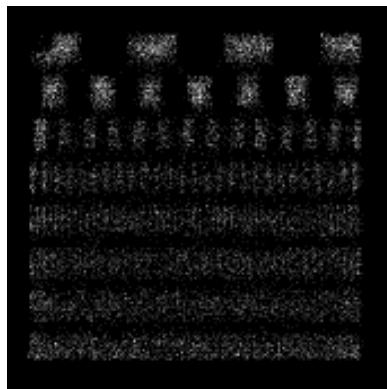
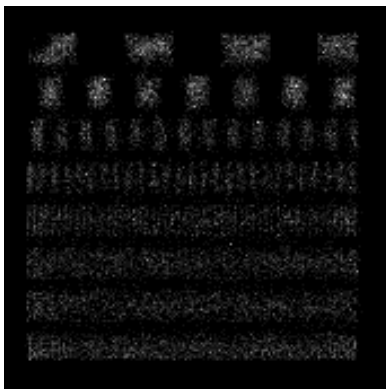


Fourier Ring Correlation (FRC)



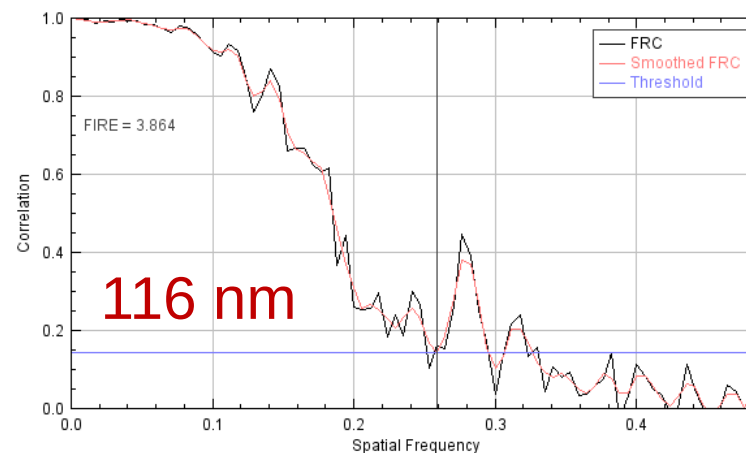
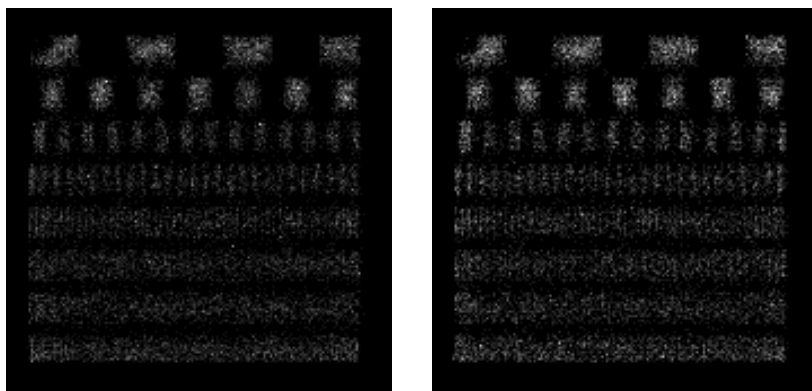
[J Struct Biol.](#) 2013 Sep;183(3):363-367.

Resolution FRC

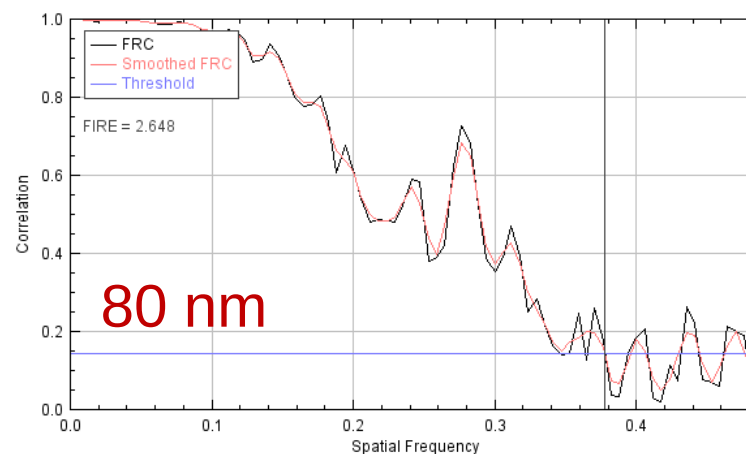
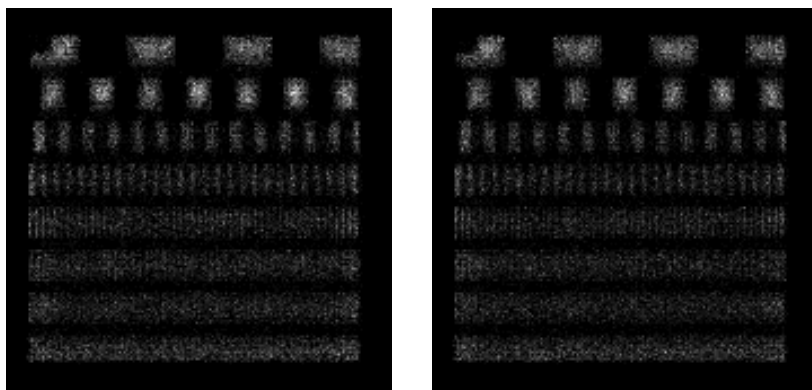


Resolution FRC

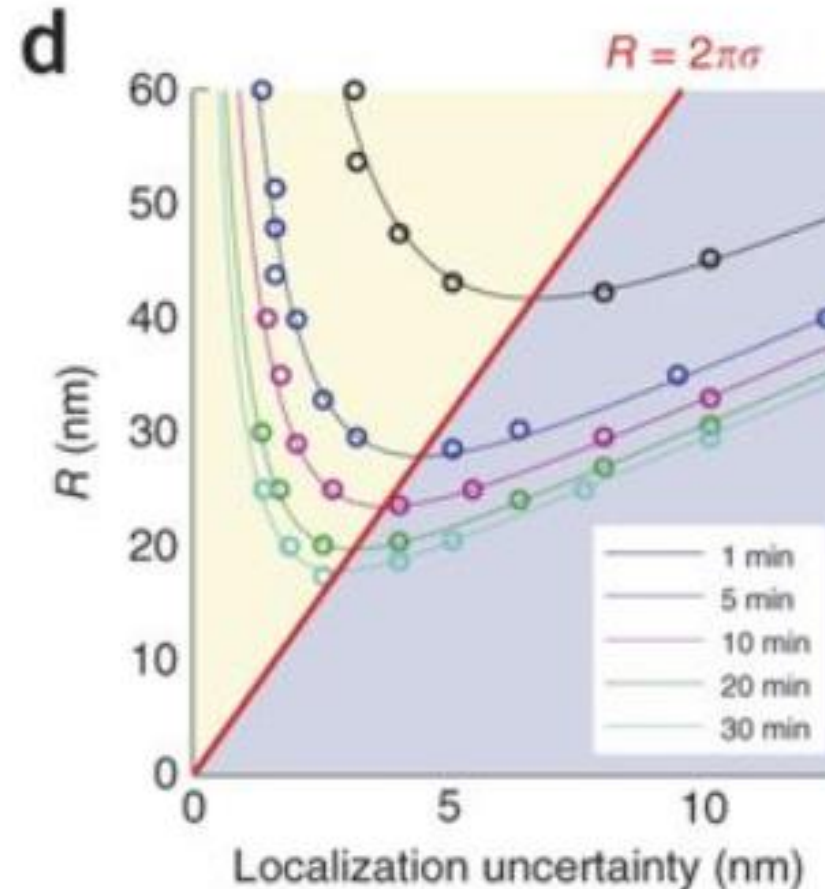
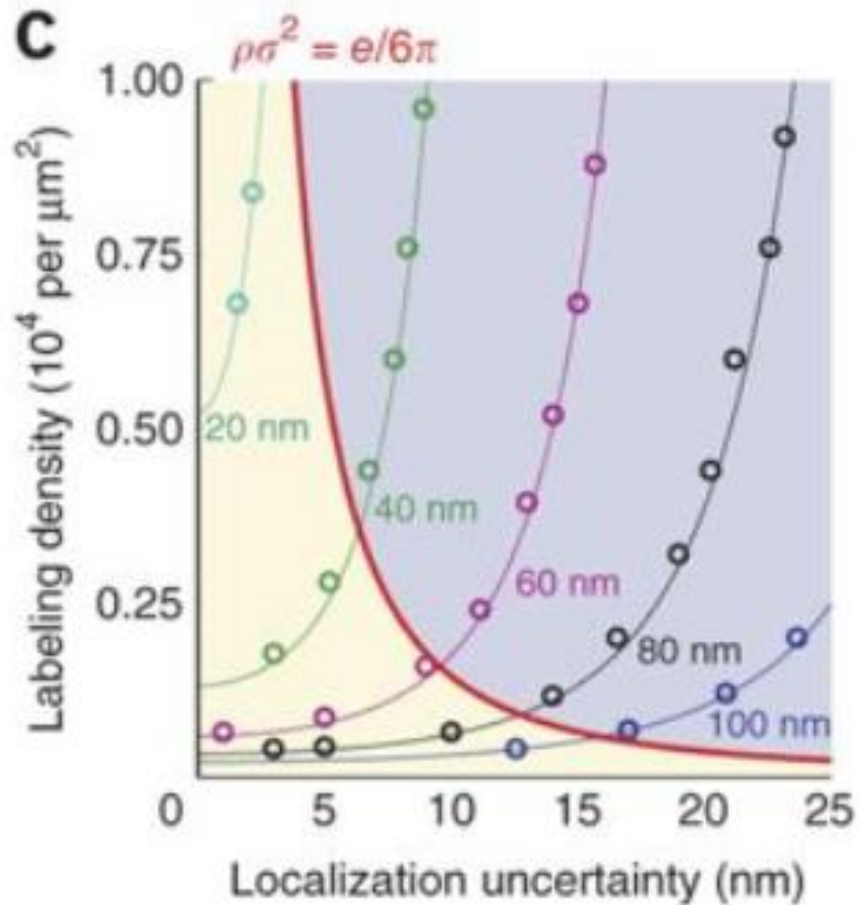
2000 frames, ~ 20 k localizations



5000 frames, ~ 40 k localizations

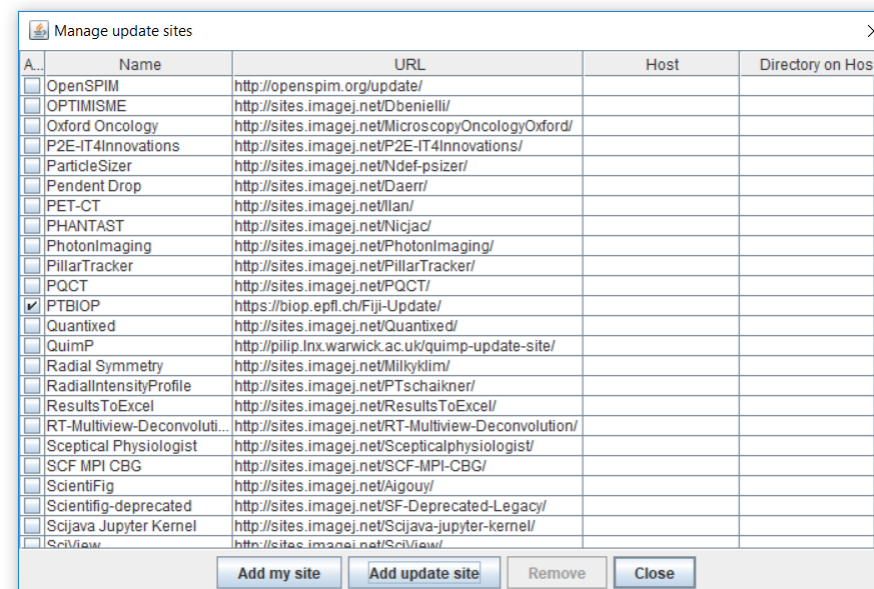
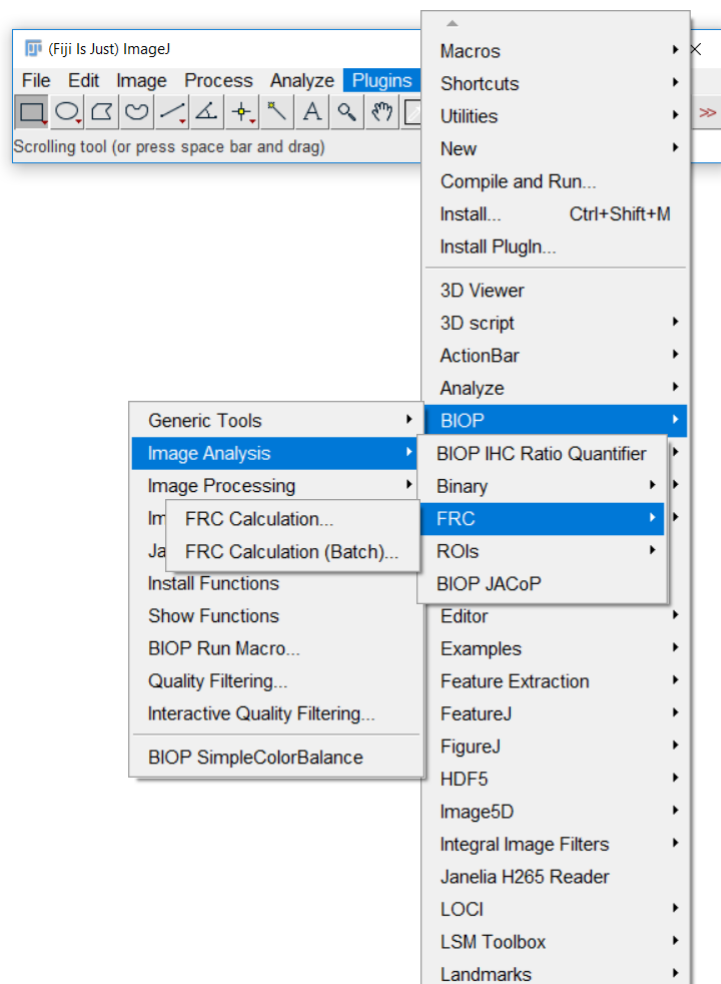


Fourier Ring Correlation (FRC)



Nature Methods **volume10**, pages557–562 (2013)

FRC in FIJI



SMLM Data Acquisition Summary

- Fluorophores can be localized with nm precision
 - Number of photons
 - Independent of wavelength
- Resolution of reconstructed image
 - Density of localized fluorophores
 - Properties of fluorophores
- Data acquisition
 - Low temporal resolution