Resolution in Super-Resolution

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Why do Super-Resolution?

Because ….

- we want/require higher resolution.
- …we want pretty pictures
- …referees ask for them
- …bosses ask for them ….

Expected resolution

Expected resolution				
	XY	Z	volume (fl)	relative volume
Widefield	220	550	0.02662	1
SIM	110	270	0.003267	0.122727273
STED	40	550	0.00088	0.033057851
STORM	20	50	0.00002	0.000751315

Realistic Resolution

Realistic resolution				
	XY	Z	volume (fl)	relative volume
Widefield	220	550	0.02662	1
SIM	110	270	0.003267	0.122727273
STED	80	550	0.00352	0.132231405
STORM	50	100	0.00025	0.009391435

SIM Resolution

- NA and hence stripe width
- Stripe contrast
- Signal to noise

STED Resolution

- Depletion beam power
- Probably gSTED, complicated balance between power and gating
- Signal to noise

Localisation imaging Resolution

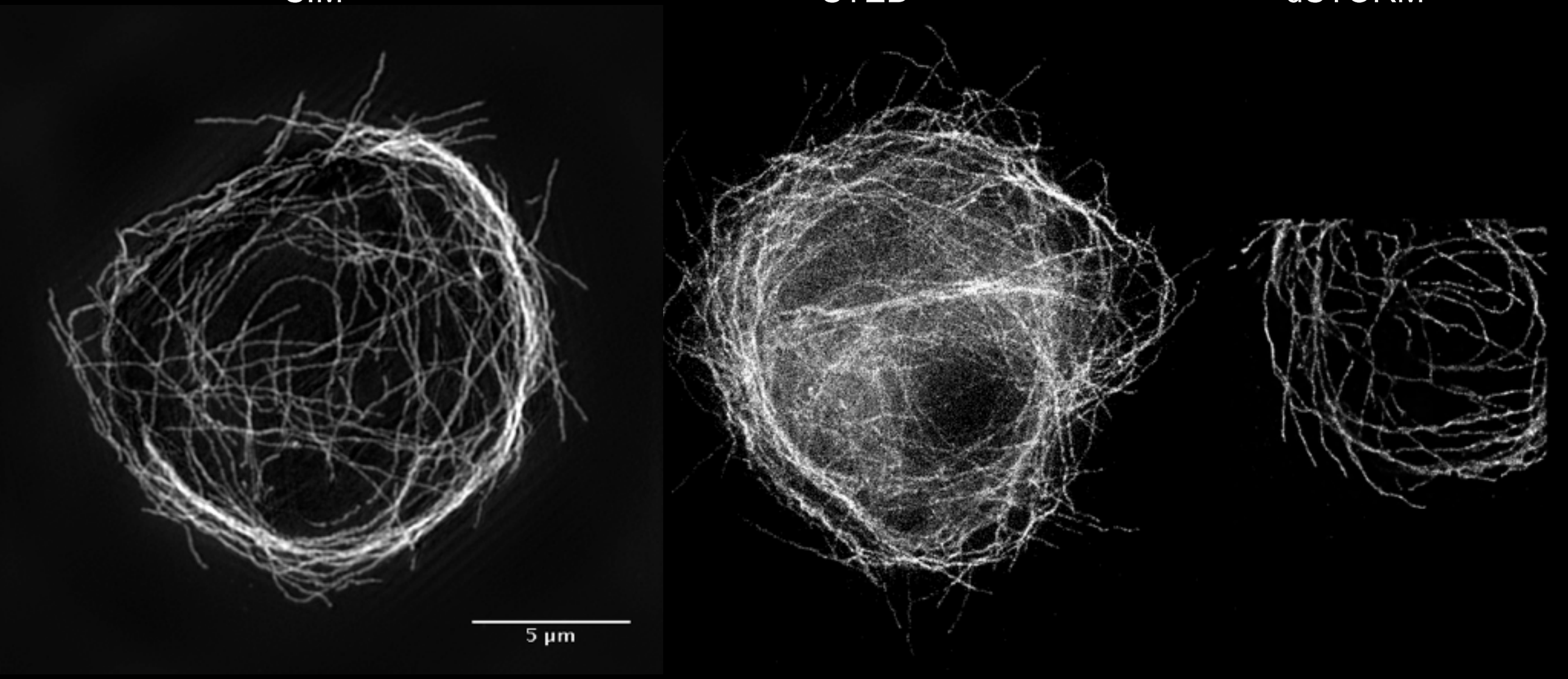
- Photons per localisation
- PSF size
- Labelling density
- Overlapping fitting or rejection.
- Signal to noise

Microtubules in *Drosophila* macrophages

SIM

STED

dSTORM



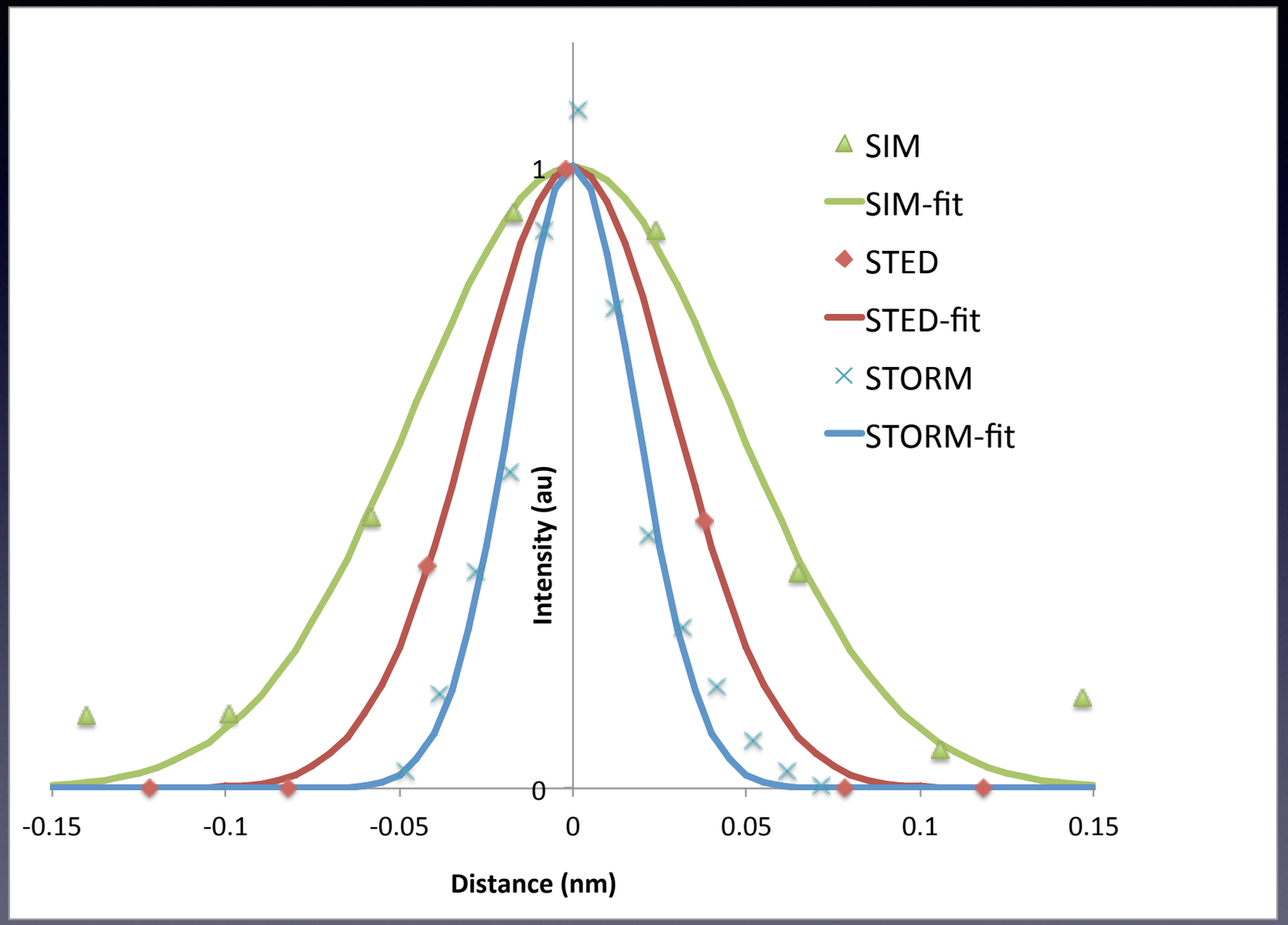
SIM: maximum intensity projection
of two 125 nm z-sections

Primary antibody anti α tubulin, secondary antibody coupled to Alexa FIuor 488

Measuring Resolution

- Line widths of sub resolution objects (eg Microtubules)
- Fall off in intensity with frequency in Fourier Transforms

Microtubule widths



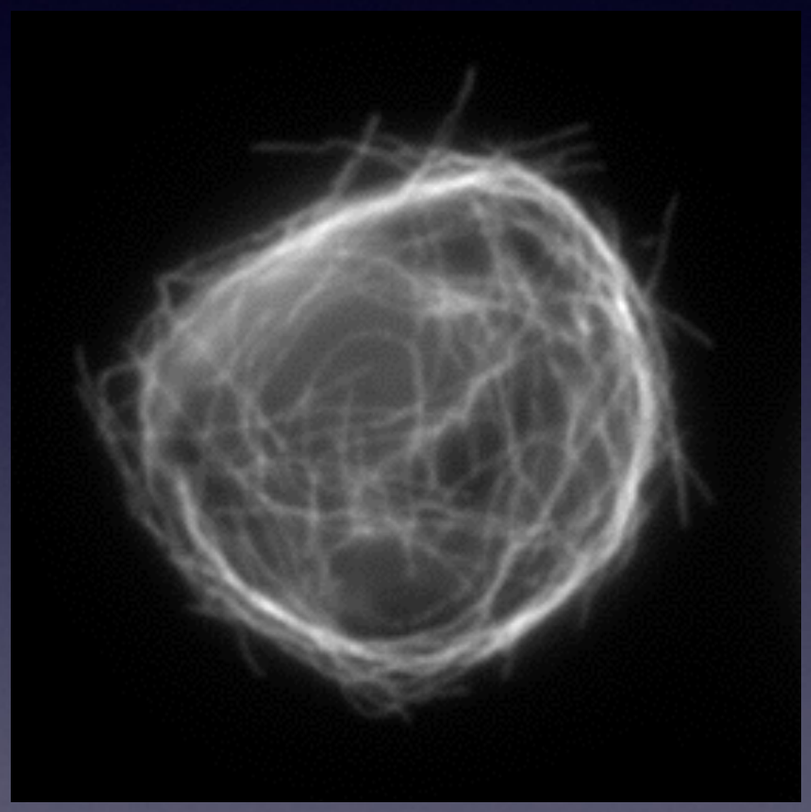
FWHM

SIM	108±5 nm
STED	63±20 nm
dSTORM	42±4 nm

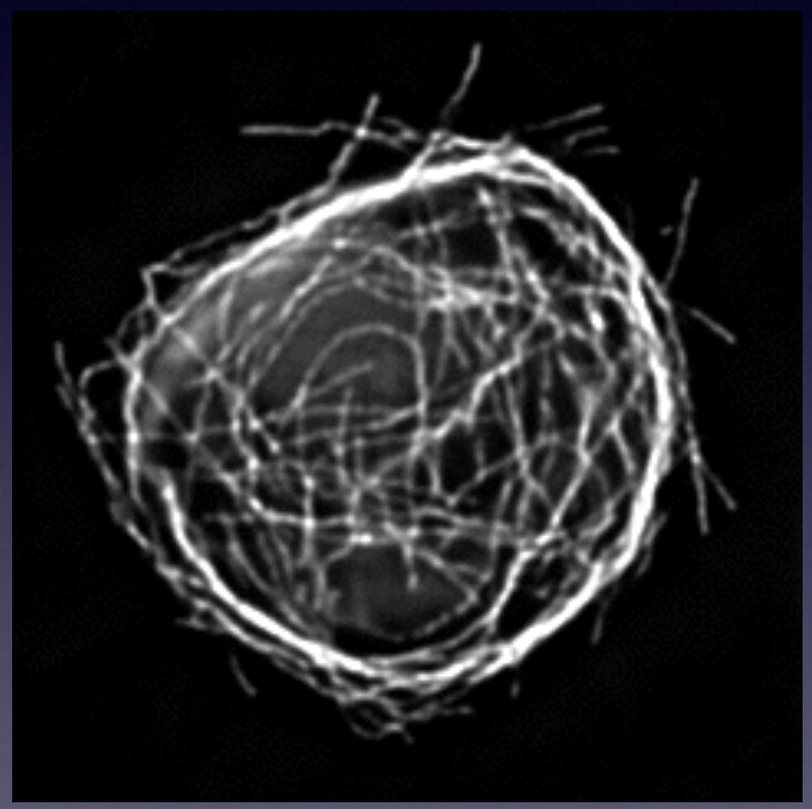
(n >10)

Representative single microtubules with Gaussian fits

Fourier Transforms to Assess Resolution

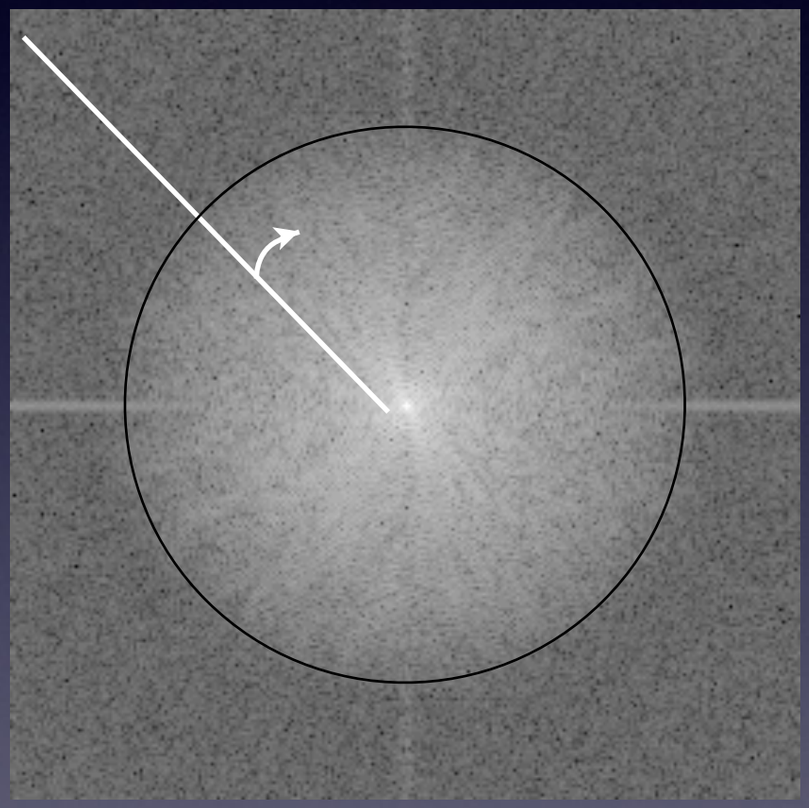


Widefield

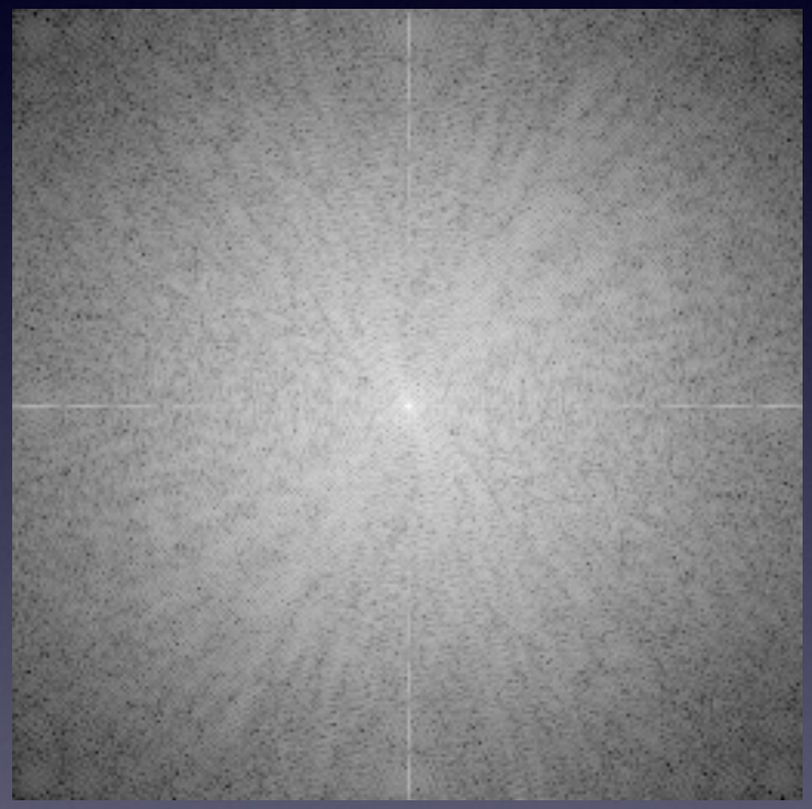


Deconvolved Widefield

Fourier Transforms to Assess Resolution



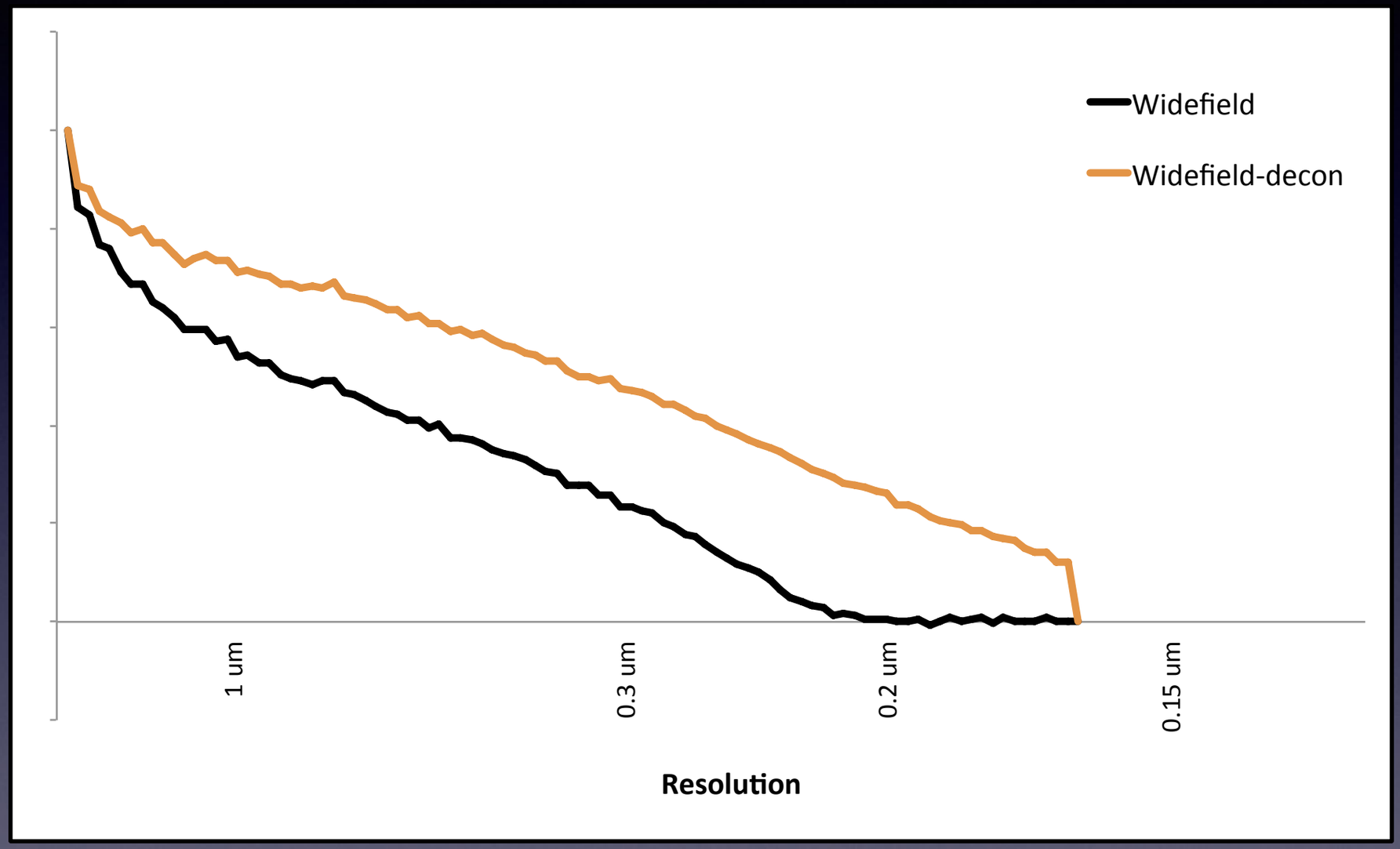
FFT of Widefield



FFT of deconvolved Widefield

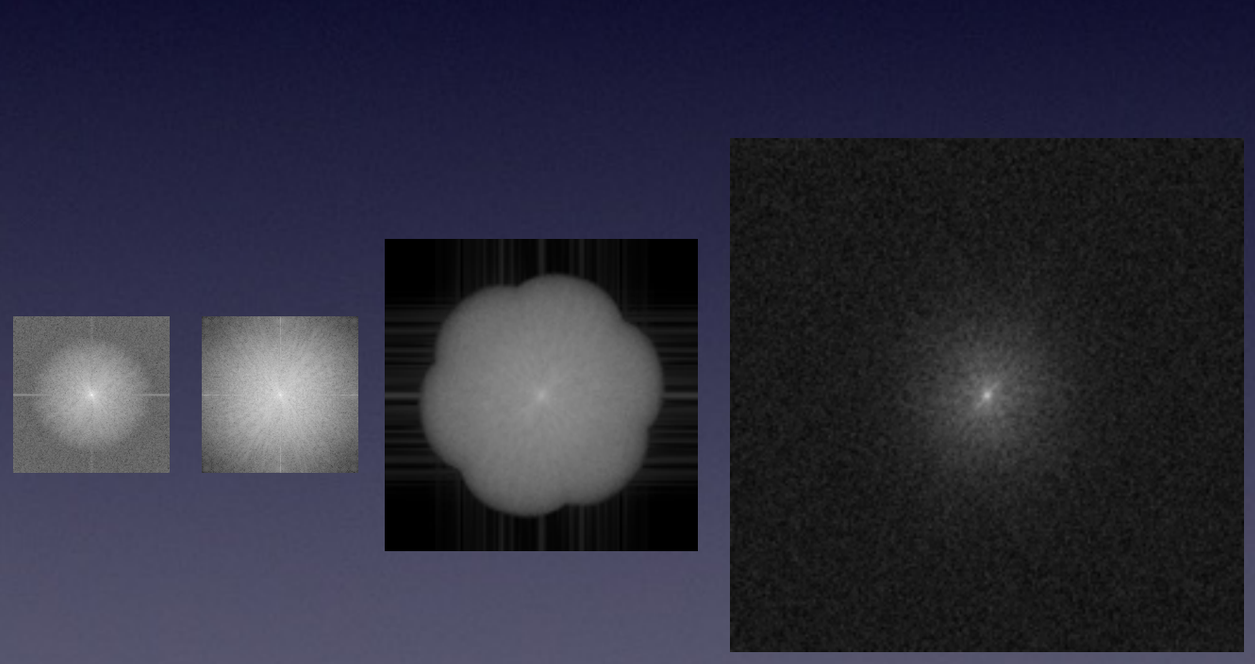
FTs of Microtubule images at equivalent scale

Radial Integrals of FTs



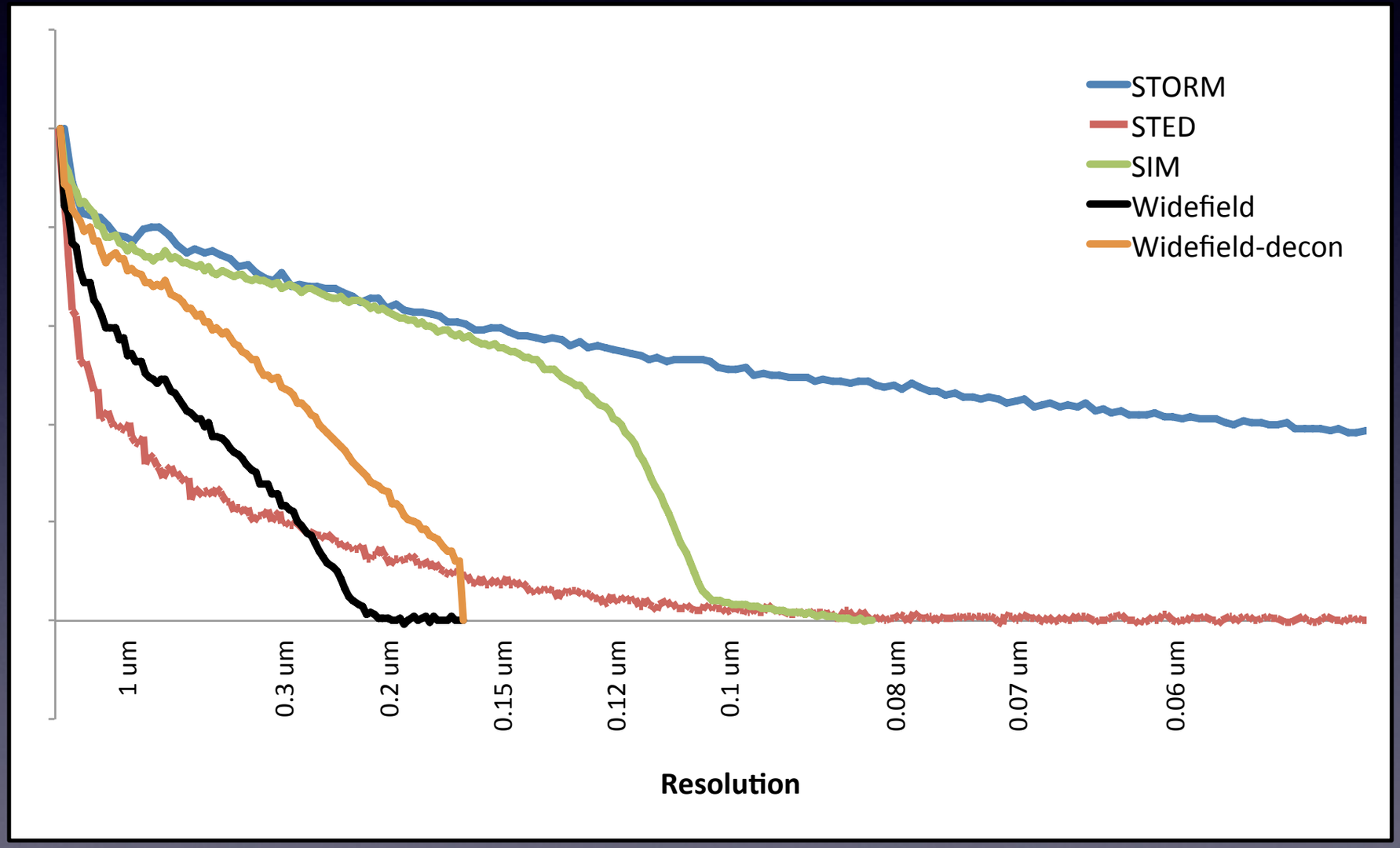
Fourier Transforms to Assess Resolution

WF SIM STED dSTORM

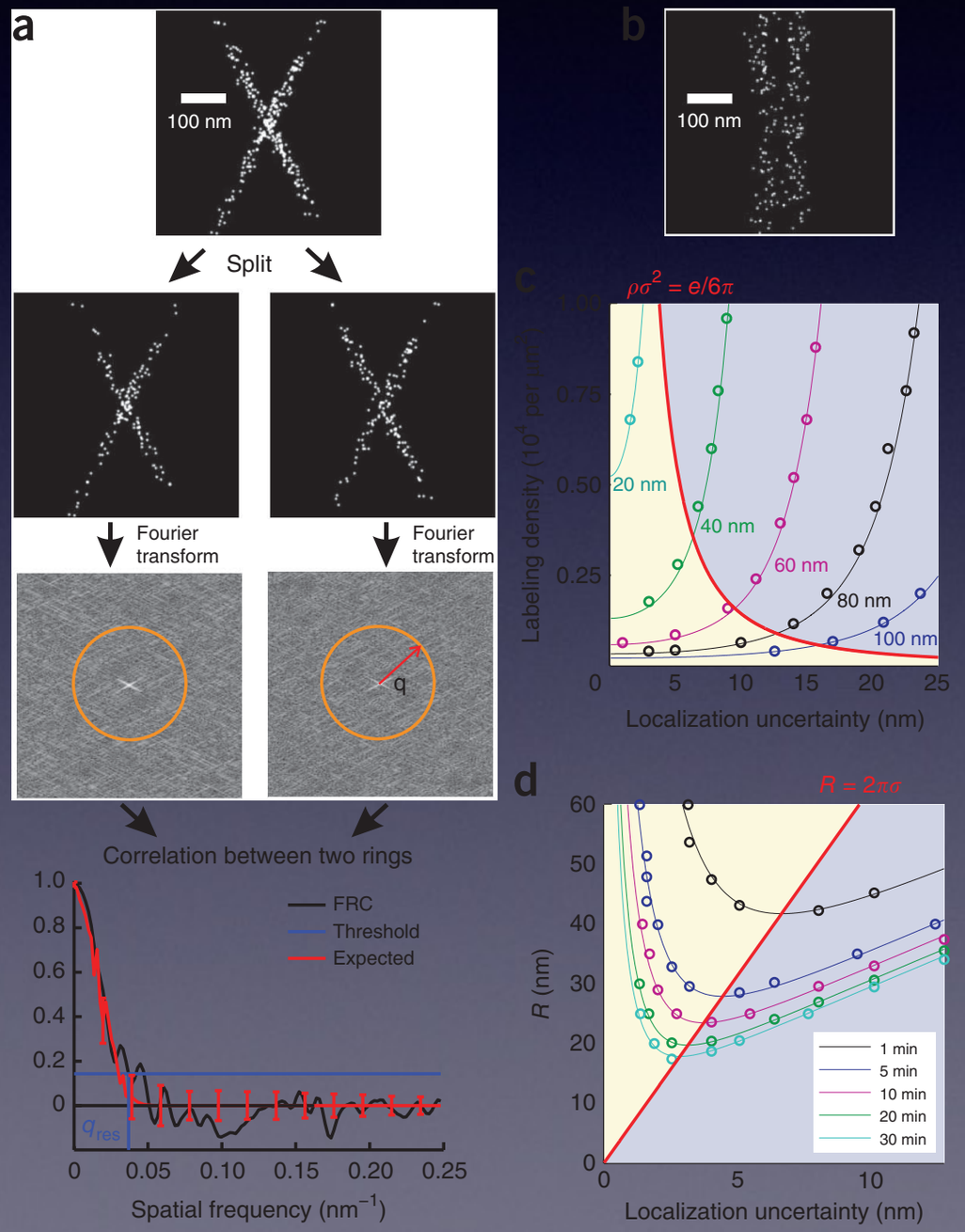


FTs of Microtubule images at equivalent scale

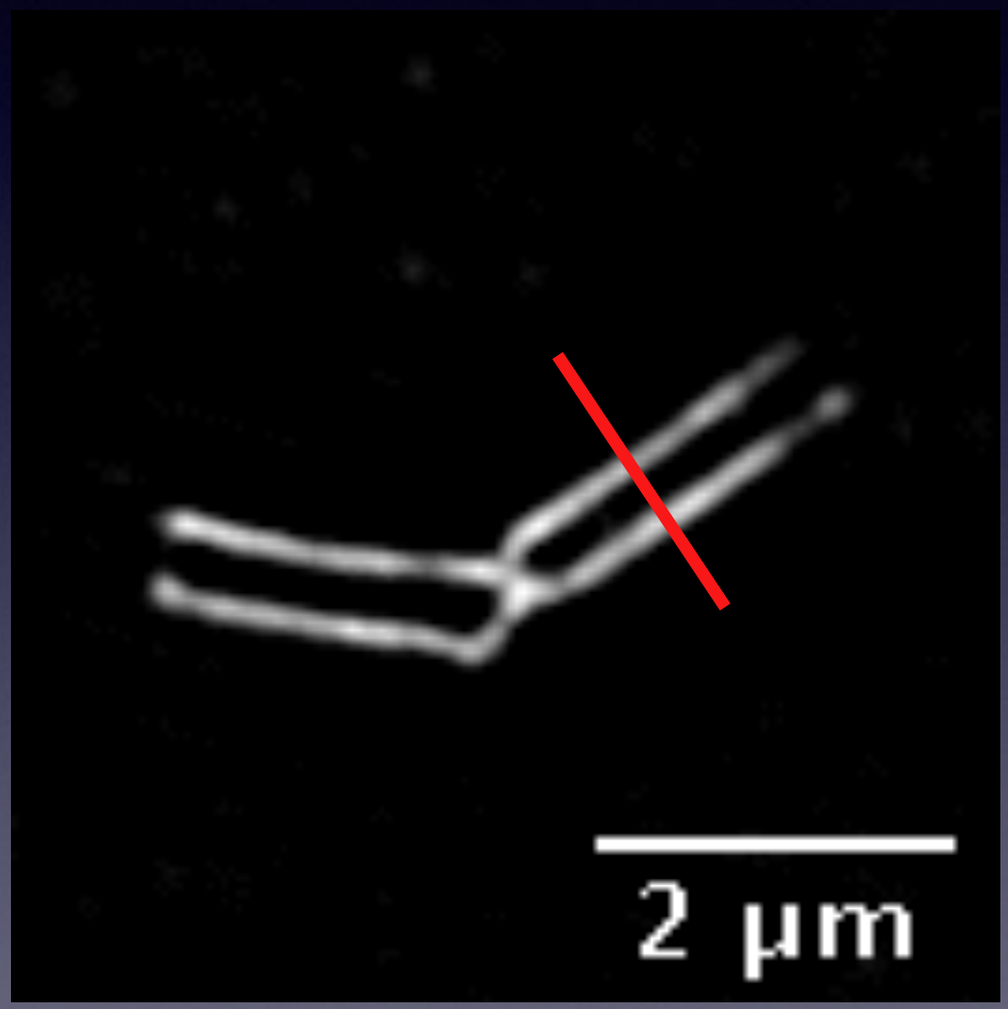
Radial Integrals of FTs



Localisation precision by Fourier ring correlation

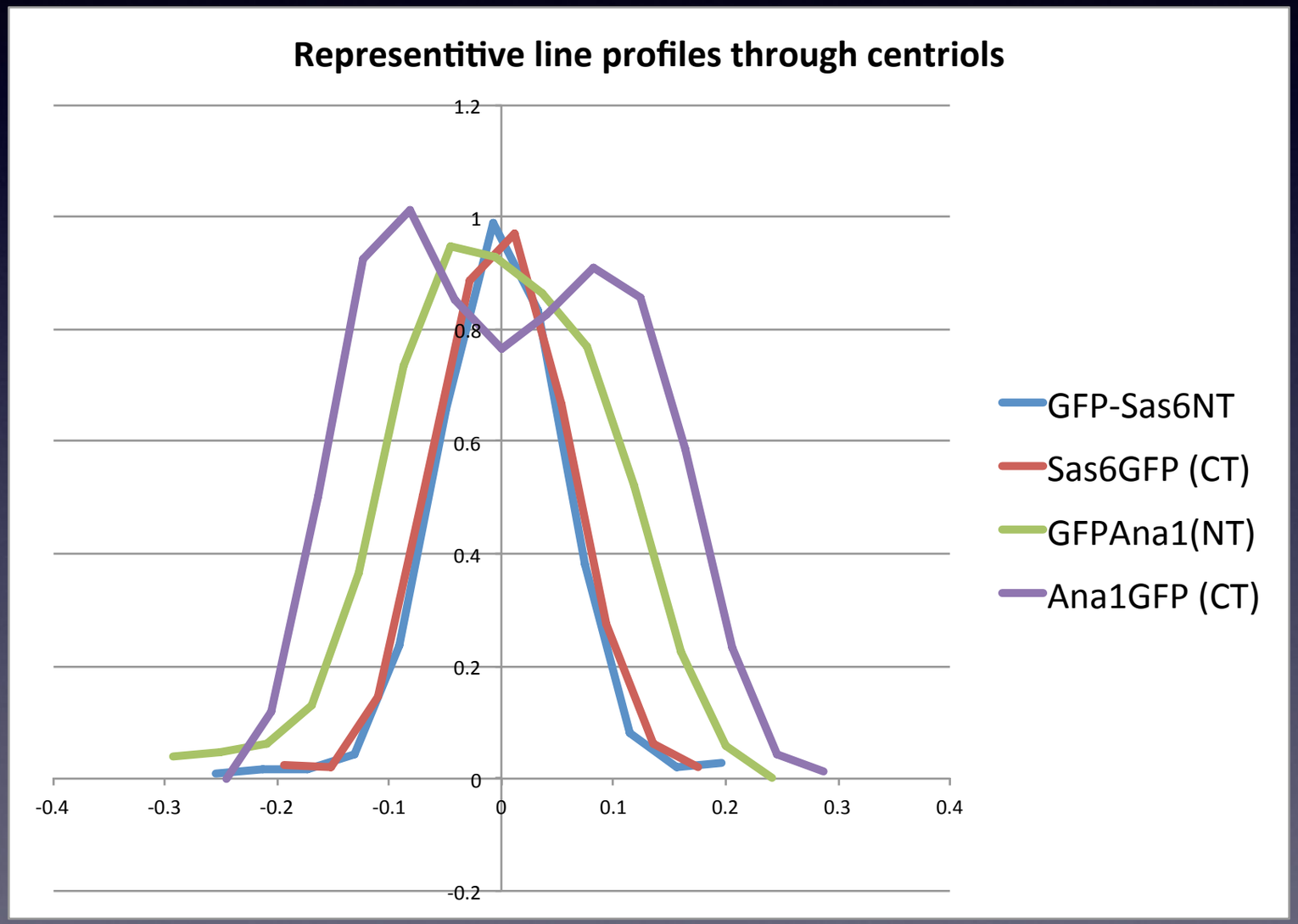


Going beyond the image resolution

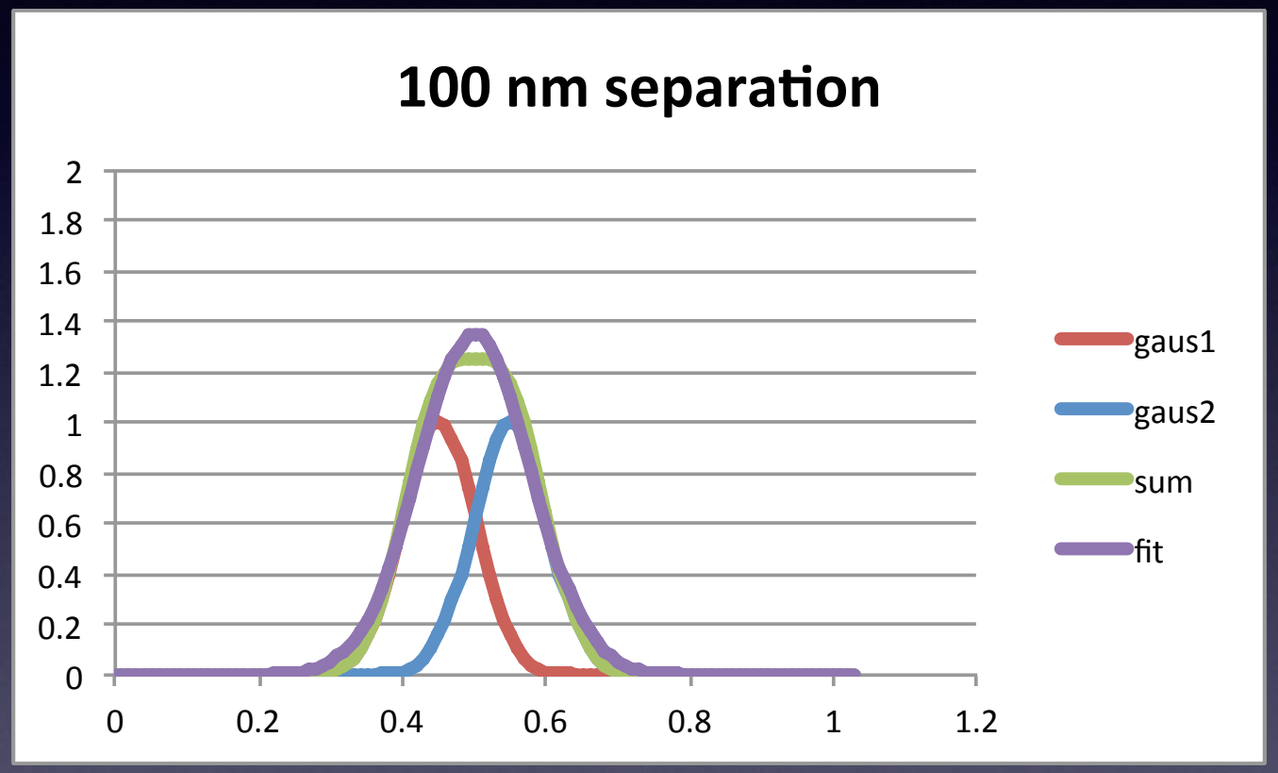


Centriol, imaging

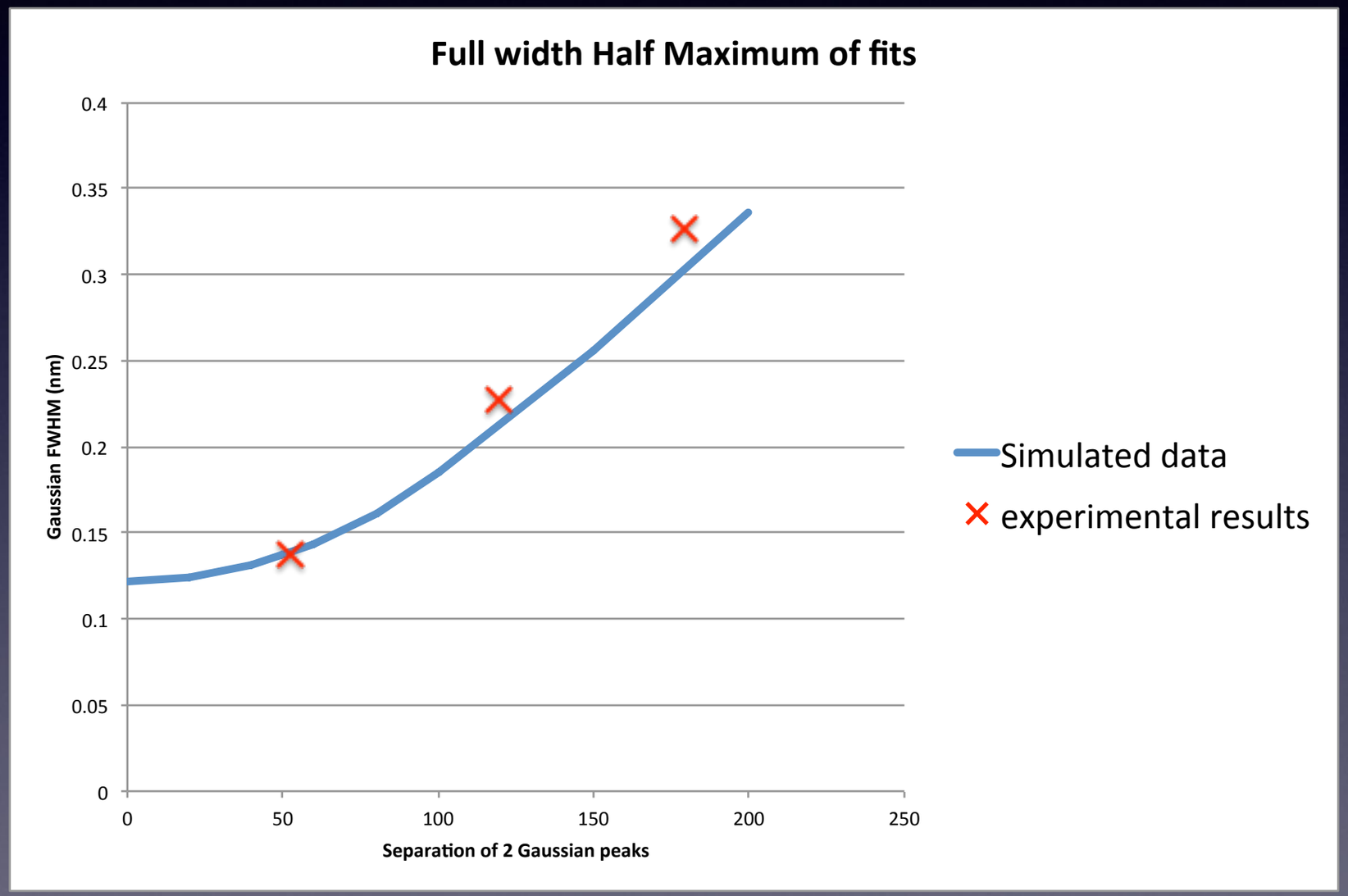
Line profiles from different proteins



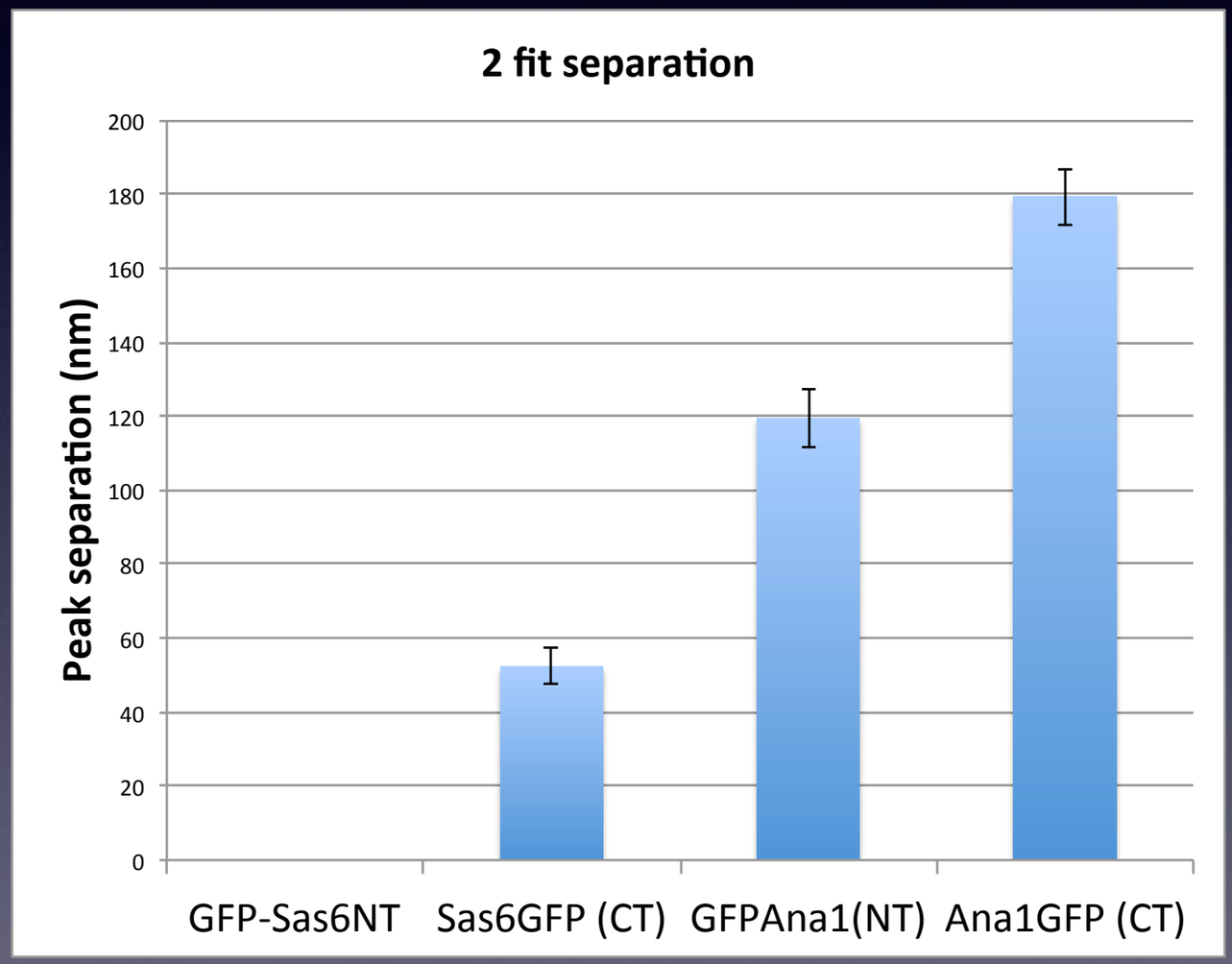
Simulations of 2 Gaussian peaks



Simulated and experimental results



Results of 2-peak fits



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