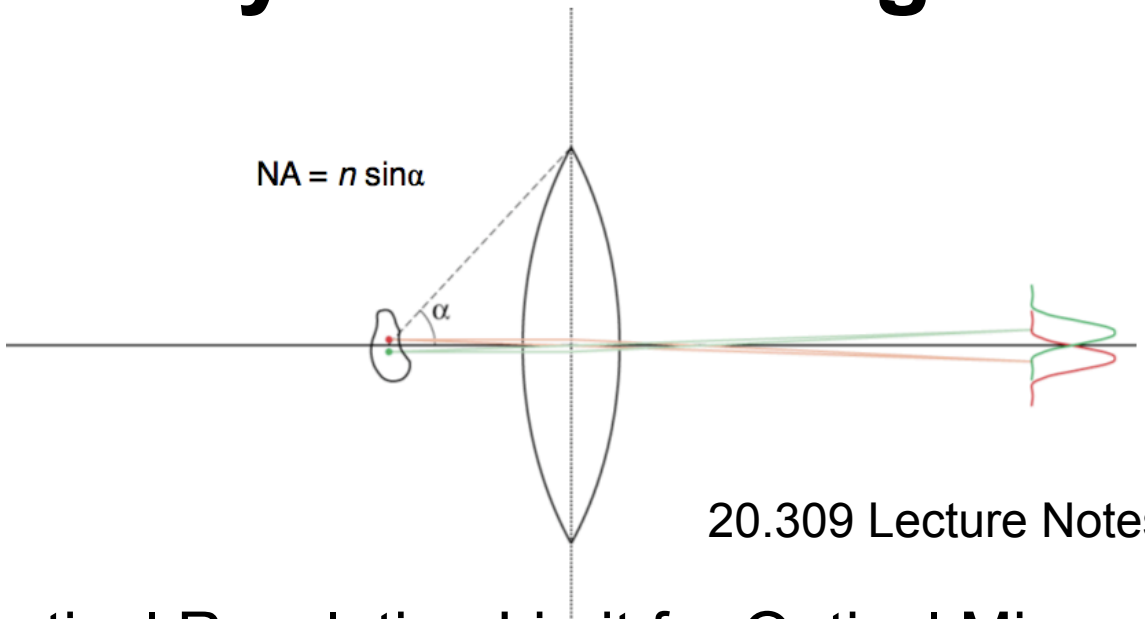
Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)

Michael J Rust, Mark Bates & Xiaowei Zhuang, Nature 2006

Optical Microscopy is Resolution-Limited by the Wavelength of Light

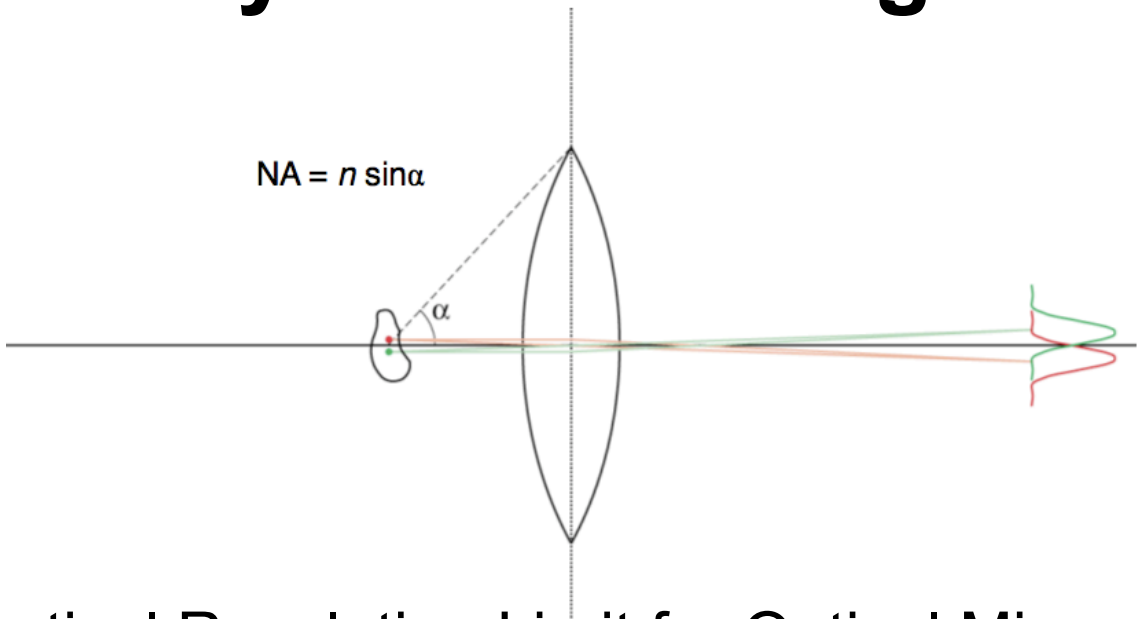


20.309 Lecture Notes: 10

Theoretical Resolution Limit for Optical Microscopy **=**

0.61λ~200nm

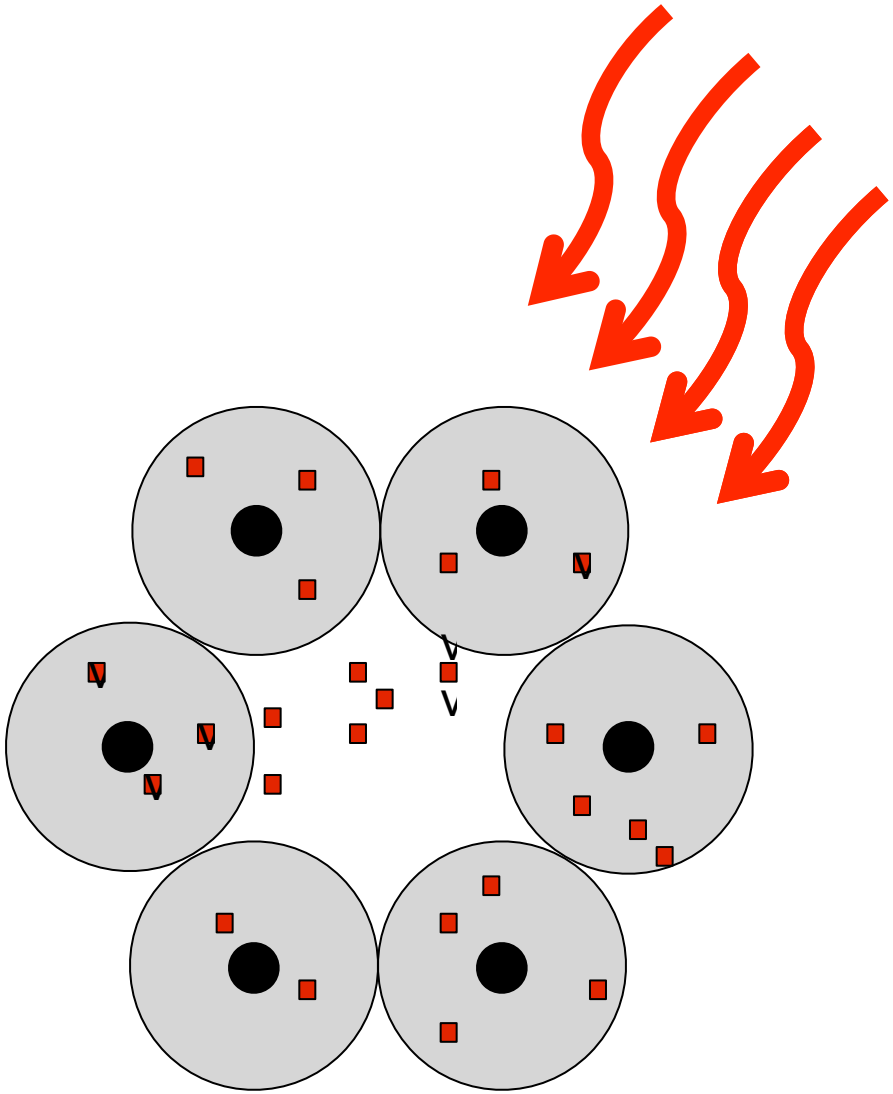
Optical Microscopy is Resolution-Limited by the Wavelength of Light



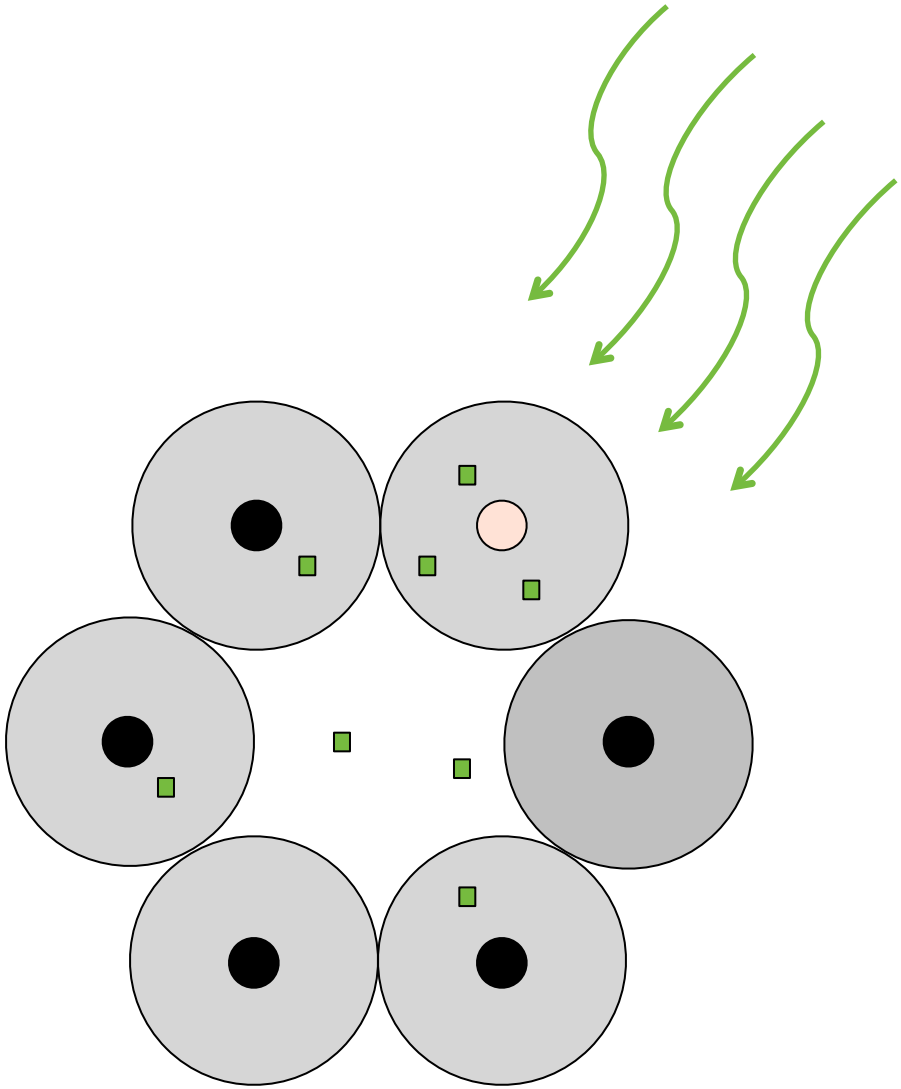
Theoretical Resolution Limit for Optical Microscopy **=**

0.61λ~200nm

However, it is still possible to measure position of a point to much greater accuracy by finding the centroid



Readout



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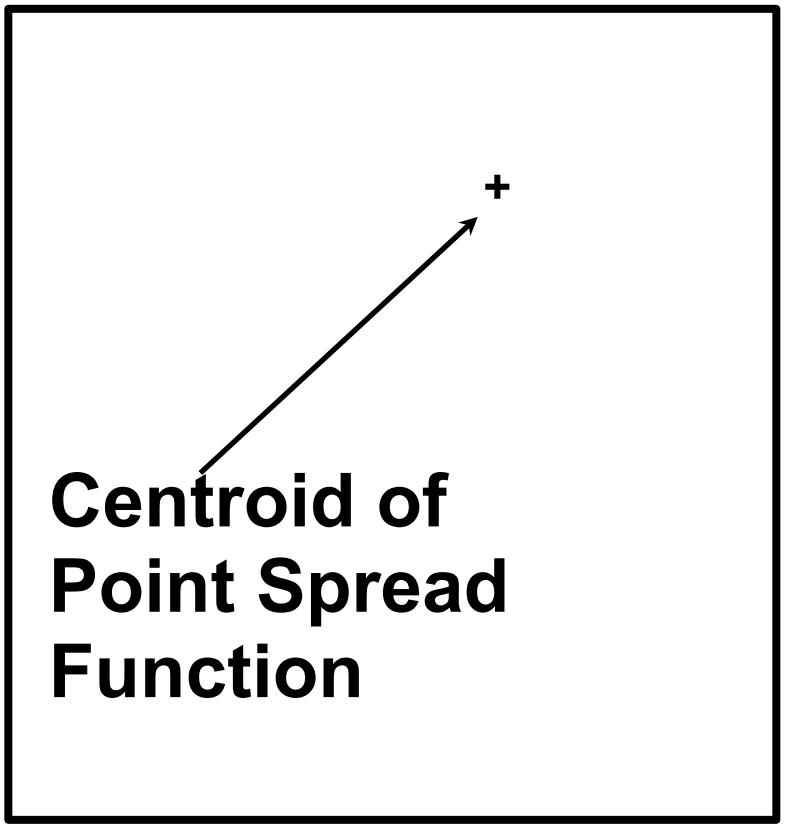

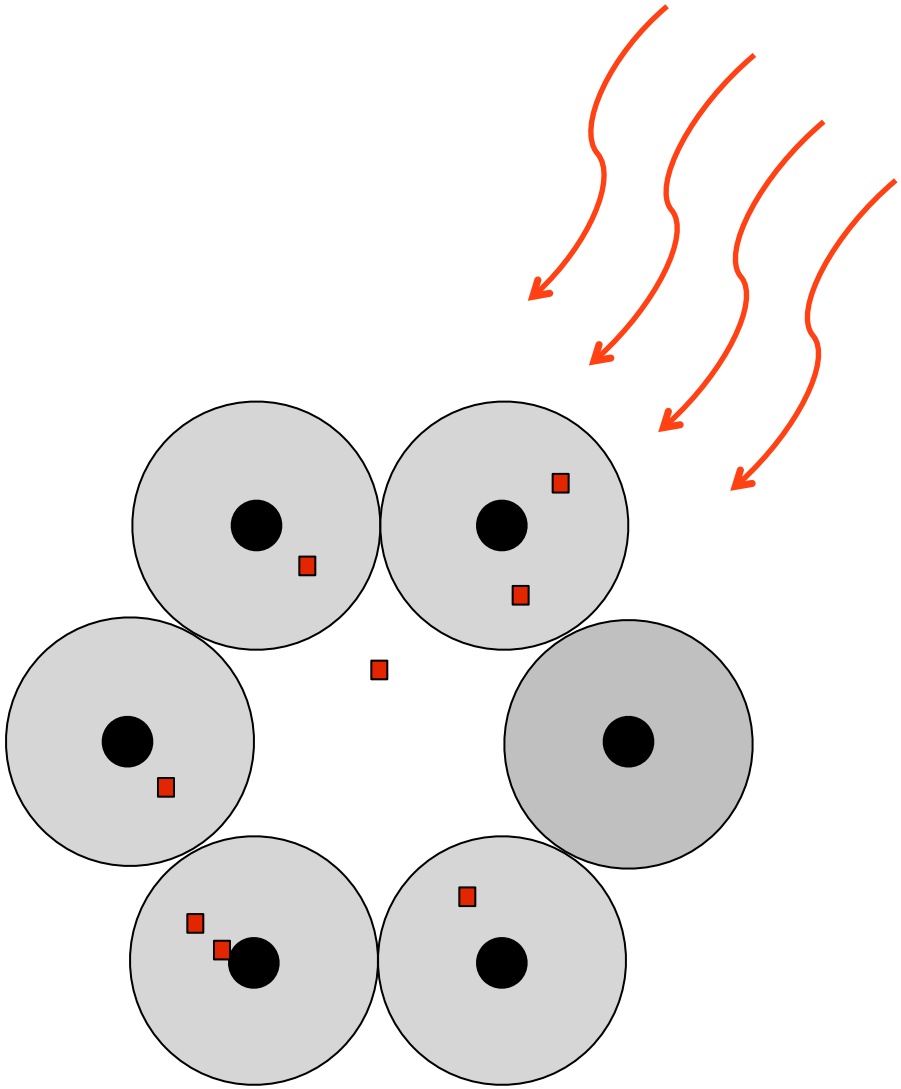

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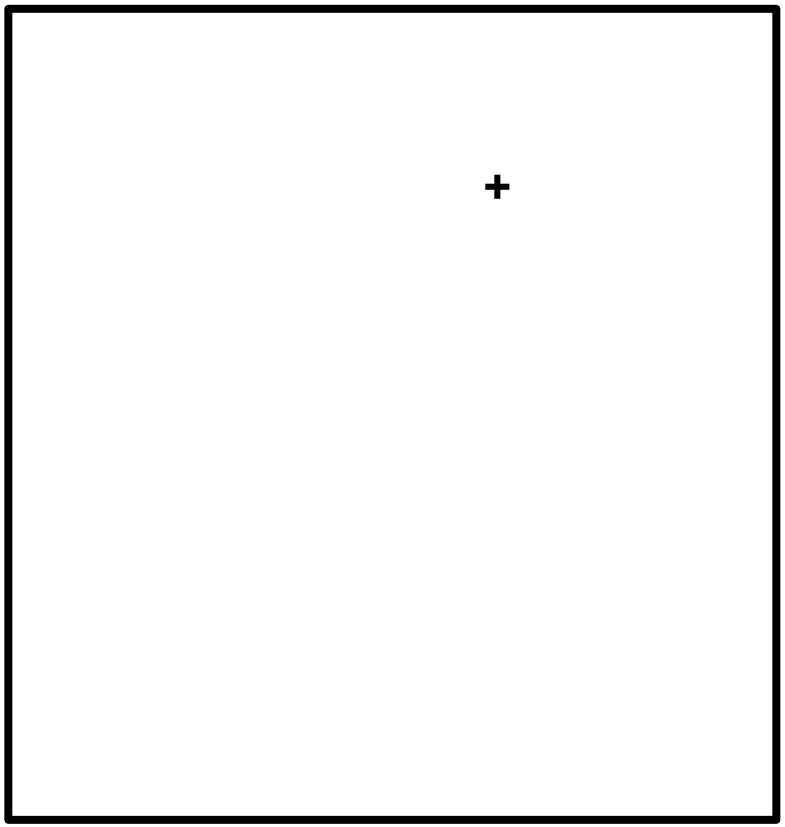

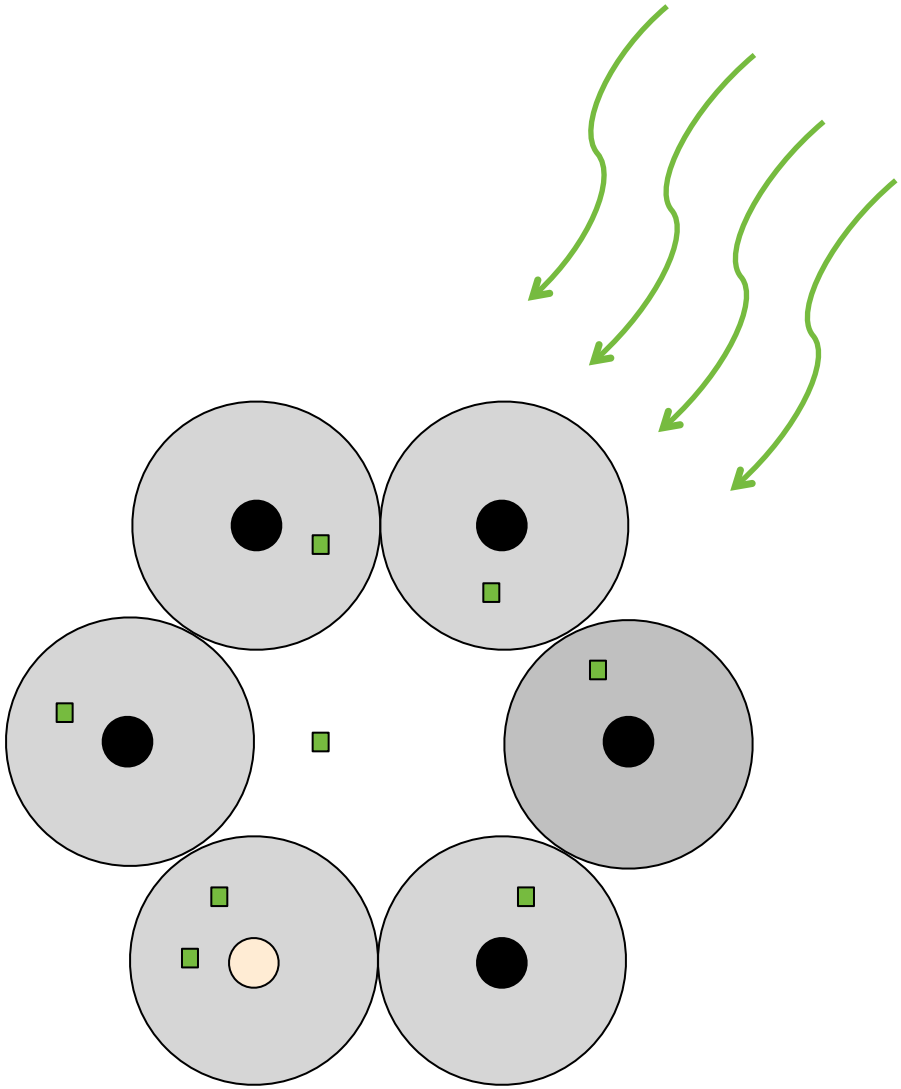

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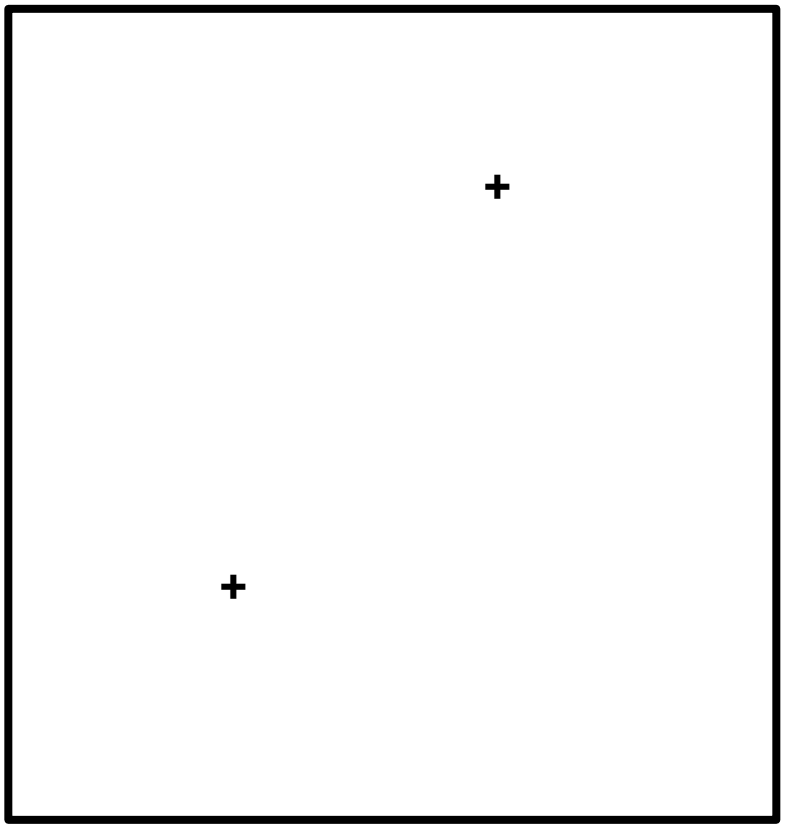
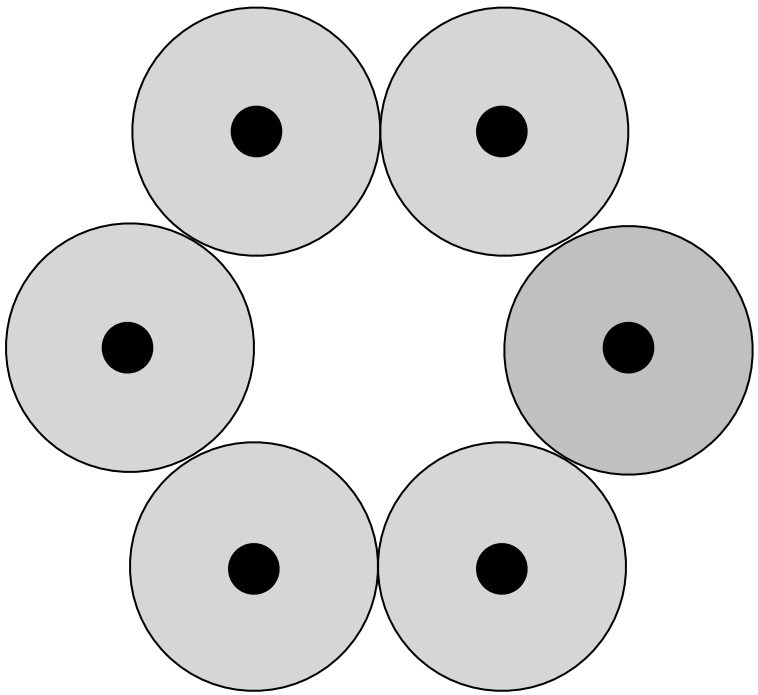


Resolution is Now Limited by Shot Noise (i.e. by number of photons), not by Wavelength of Light



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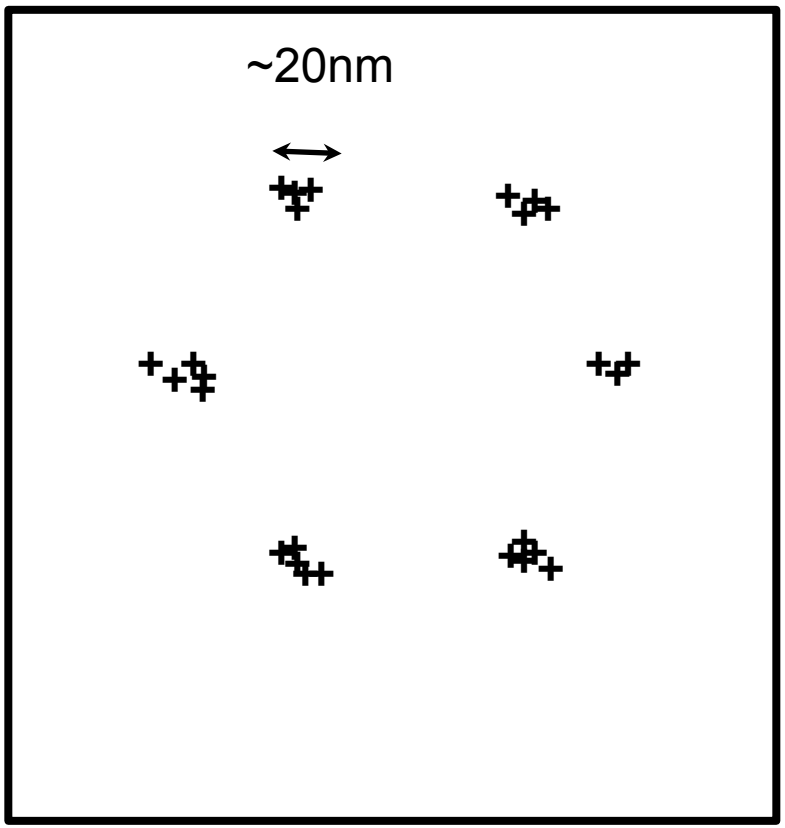
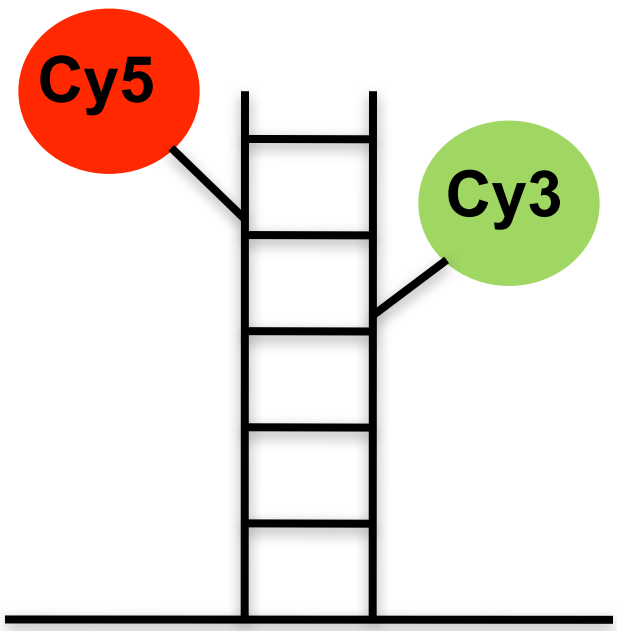
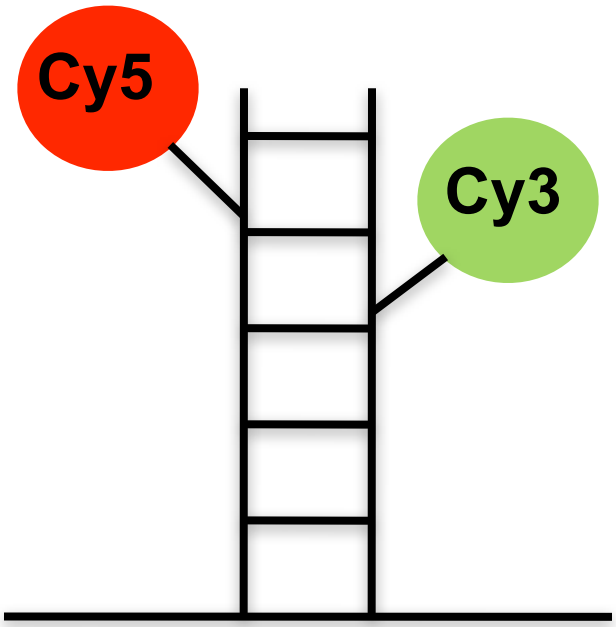


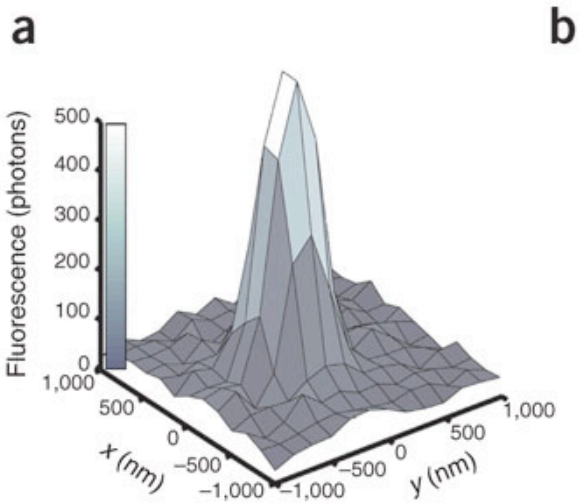
STORM uses a cyanine switch

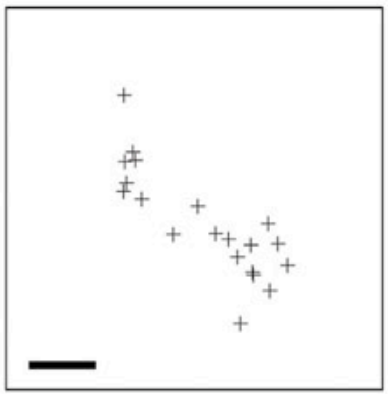


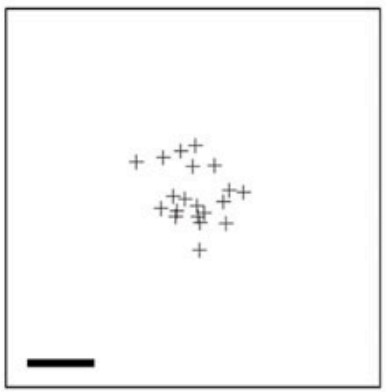
- **Red light** causes **fluorescence followed by a stable dark state**
- **Green light** causes a **return to fluorescent state**
- Recovery rate depends on proximity to Cy3
- Can be used hundreds of times before photobleaching occurs
- Approx 3000 photons/s -theoretical position accuracy 4nm (8nm measured)

Imaging a single switch attached to DNA









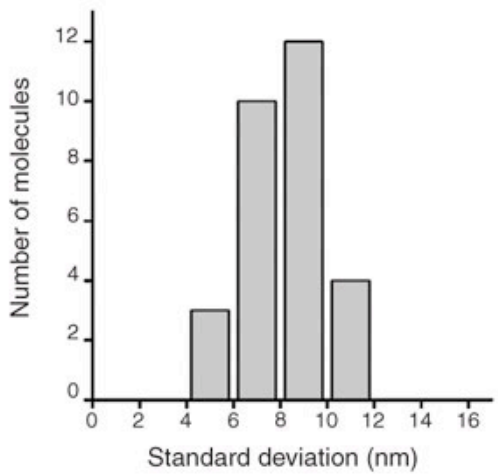
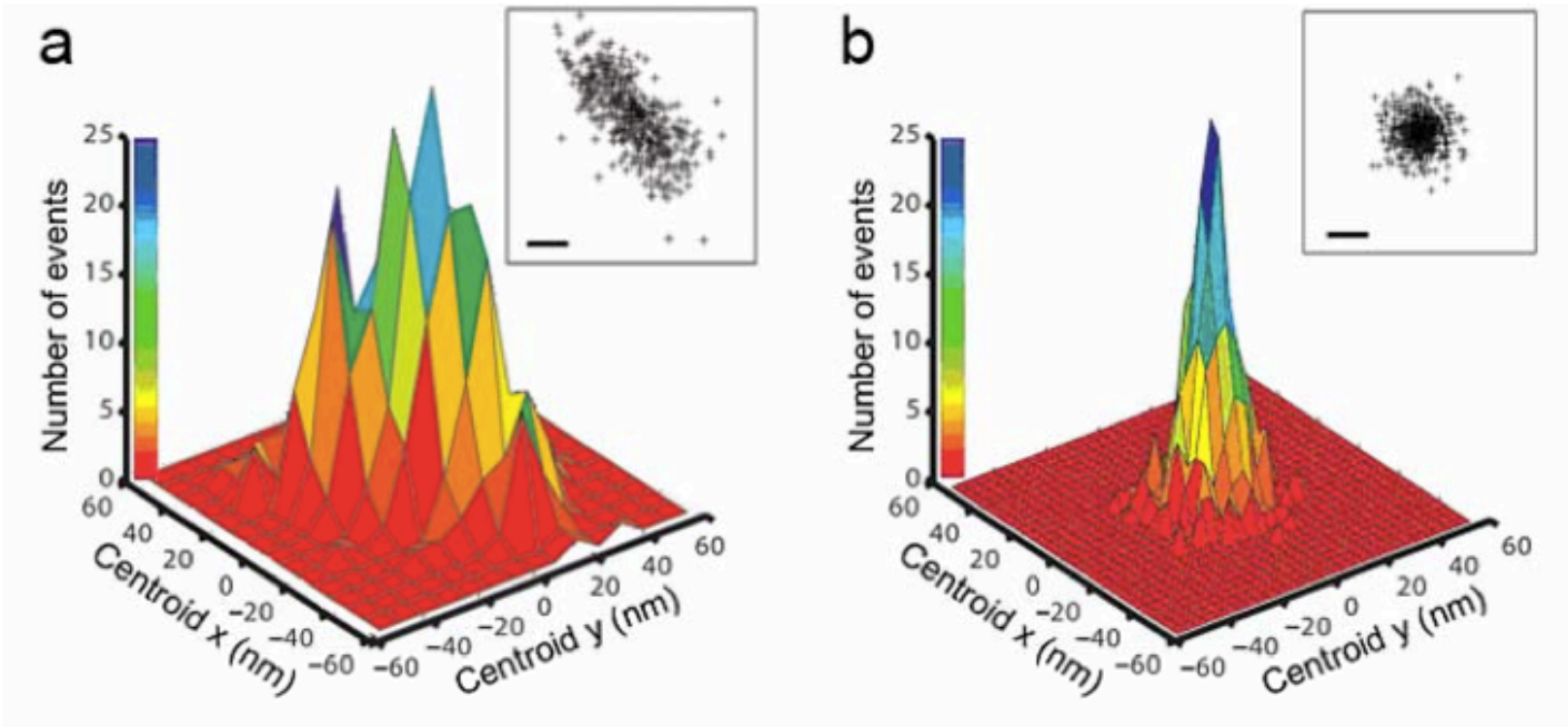


Image processing reveals a resolution limit of 18nm.

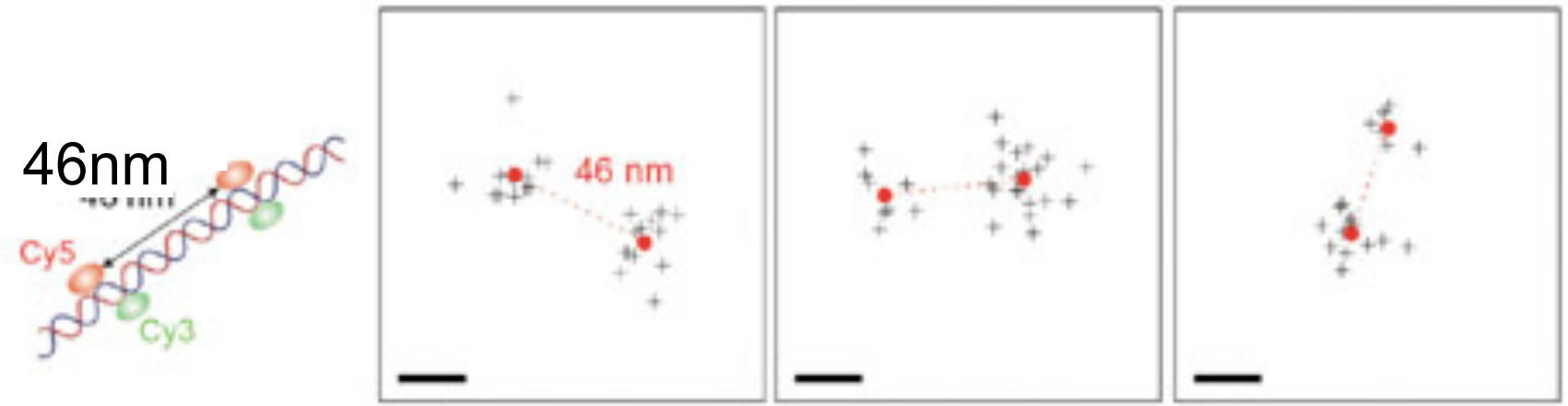


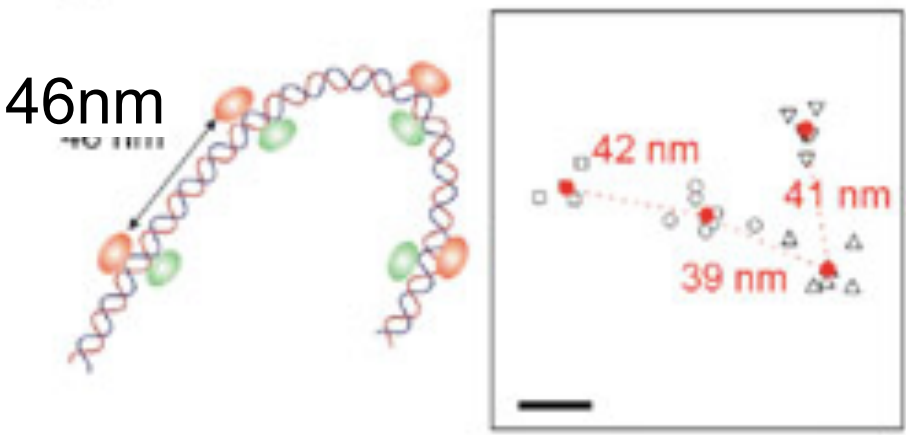
Before Drift Correction

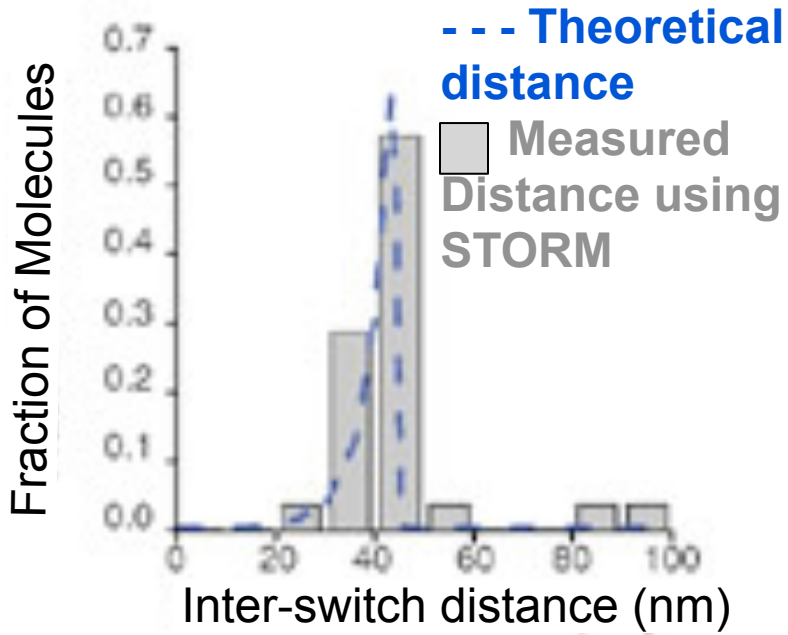
After Drift Correction

FWHM = 18nm

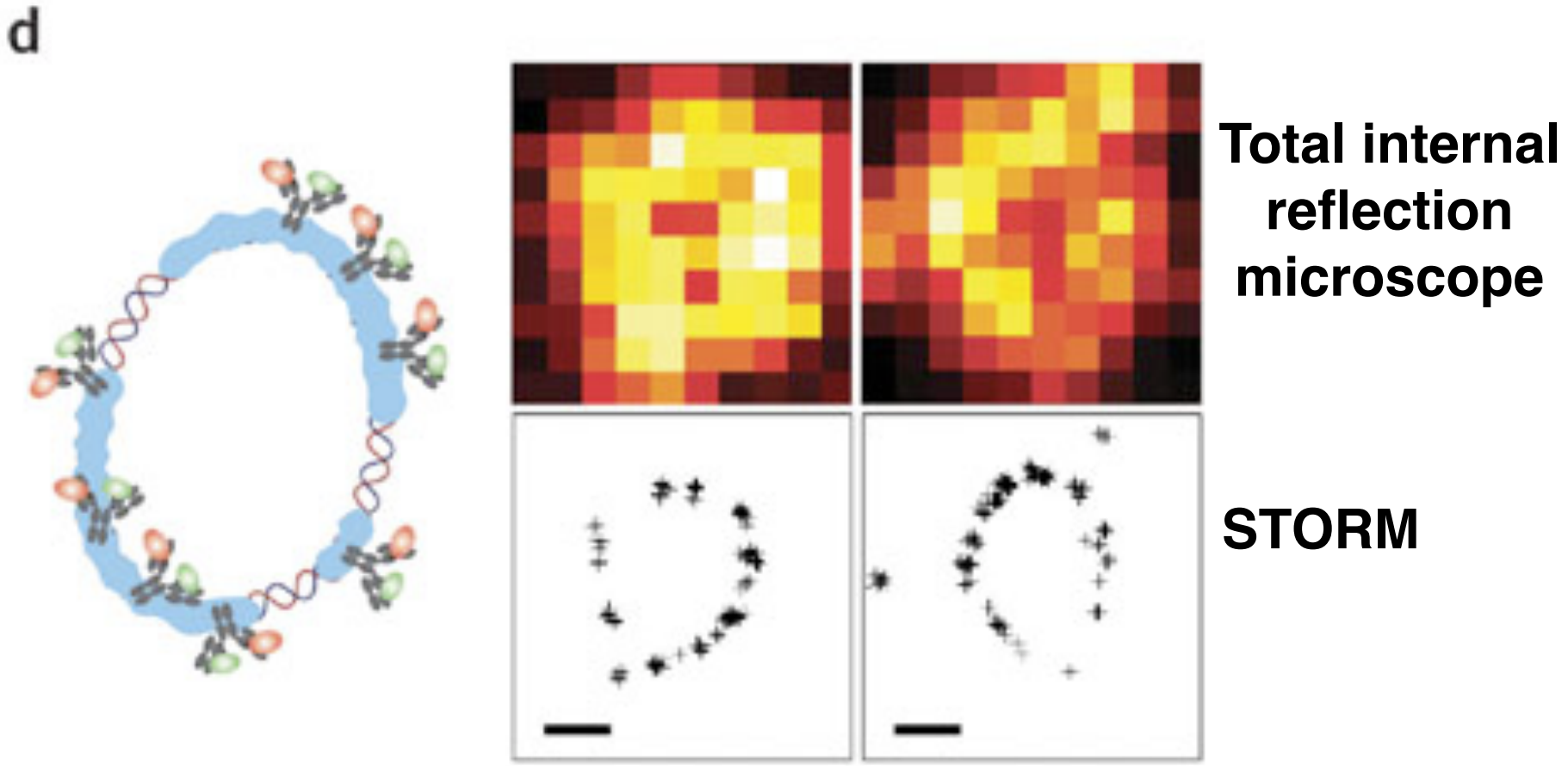
STORM visualizes switches on a plasmid.





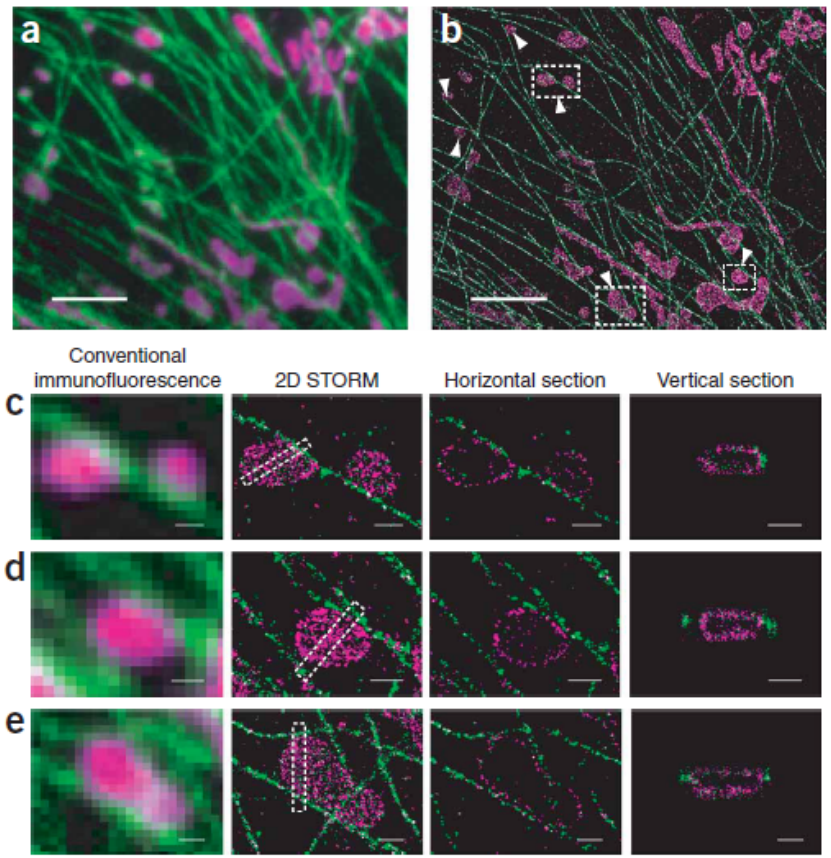


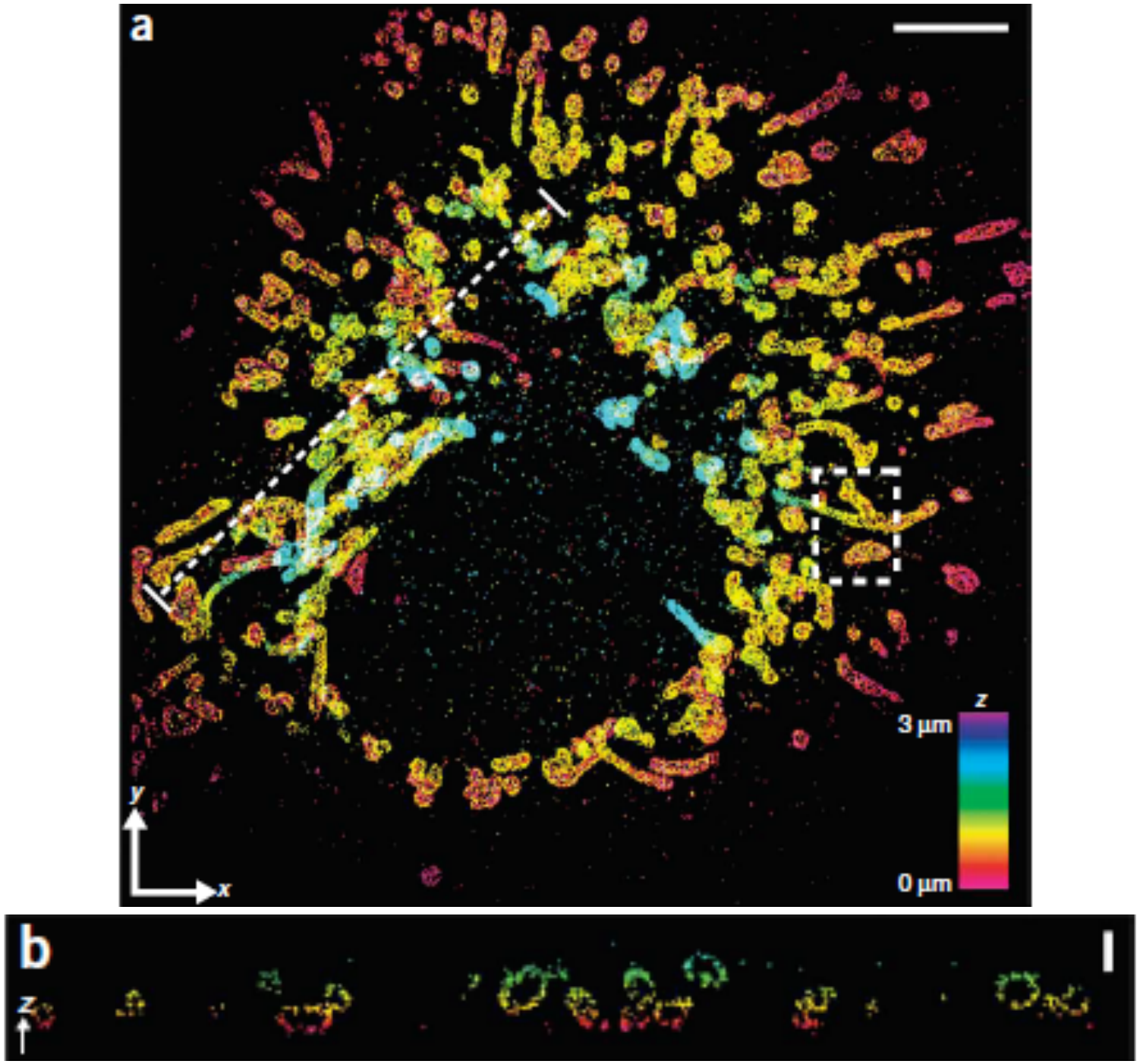
Storm Imaging Compared to TIRM



Whole-cell 3D STORM reveals interactions between cellular structures with nanometer-scale resolution

Bo Huang, Sara A Jones, Boerries Brandenburg & Xiaowei Zhuang (2008)




a
z
3 µm
0 µm
y
x
b
z

Possible Future Directions

- Imaging speed can be improved by increasing switching rate
- High-resolution fluorescence in situ hybridization and immunofluorescence imaging
- Potentially allows for high-resolution, live-cell imaging

Questions?

Acknowledgements

Michael J Rust	Harvard University
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Switching of Cy5-Cy3-labeled antibody.