

Single molecule localization microscopy from concepts to applications

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Sample => Welcome (Full Shot)







Microscopy

" and by the help of Microscopes, there is nothing so small as to escape our inquiry... " Robert Hook

Drawings of the instruments used by Robert Hook. Image reproduced from R.Hook (1665)





Resolution of a Light Microscope

ABBE'S DIFFRACTION LIMIT (0.2 µm)



human eye

light microscope





super-resolution

electron microscope

nobelprize.org

From Concepts to Applications







Point Spread Function (PSF)

- Localization
- Labelling density
- **Fluorophore photo-physics**
- Stochastic readout
- **Applications**

objective (point spread function **PSF**) as



Thompson at al., Biophys. J., 2002; Huang, B.; Bates, M.; Zhuang, X., Annual Review of Biochemistry 2009, 78 (1), 993–1016.

Excitation light



Thompson at al., Biophys. J., 2002; Huang, B.; Bates, M.; Zhuang, X., Annual Review of Biochemistry 2009, 78 (1), 993–1016.

Excitation light

Nobel Prize 2014 in Chemistry

"for the development of super-resolved fluorescence microscopy"





Eric Betzig

Stefan W. Hell







William E. Moerner

Matts Gustafsson (1960-2011)

nobelprize.org

Overcoming the Resolution Limit

- use the properties of fluorophores
- photophysics, photochemistry, ...



targeted readout: molecular density, patterned illumination STED/RESOLFT/SIM...







stochastic readout:

specimen made up of single-molecules **SMLM** (PALM, (d)STORM, PAINT, ...)/S OFI/3B...

Origins of SMLMS-localization use hand

localization precision:

s is the standard deviation of the microscope point spread function (approximated to a Gaussian)

EM-CCD pixel size **a** is defined by the ratio of the physical camera pixel and the system magnification

b² the number of background photons per pixel in the image

Thompson at al., Biophys. J., 2002; Huang, B.; Bates, M.; Zhuang, X., Annual Review of Biochemistry 2009, 78 (1), 993–1016., Annibale et.al. 2012

a







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Localization







Bright fluorescent probes allow more exact PSF fitting and therefore less error in the centroid calculation.



...

 σ 1 nm 2 nm

Thompson at al., Biophys. J., 2002; Huang, B.; Bates, M.; Zhuang, X., Annual Review of Biochemistry 2009, 78 (1), 993–1016., Annibale et.al. 2012



s=1.36 λ /(2 π ObjectiveNA)

20 nm

Fluorophore Stochastic Switching





absorbing scattering spin up . . .

One could imagine breaking the diffraction barrier also in a non-fluorescence far-field optical microscope, provided suitable states and state transitions are identified

S. Hell, nobel lecture; Huang, B.; Bates, M.; Zhuang, X., Annual Review of Biochemistry 2009, 78 (1), 993–1016.



fluorescent

non-fluorescent

non-absorbing non-scattering spin down

...

Eiffel Tower by Night (Youtube)



verage Intensity

STORM

Principle of SMLM –summarized

Principle of SMLM – summarized

Laine at al., Methods and Applications in Fluorescence 2016

From localizations to images

- the other key determinant of resolution is the density of labeled molecules in the specimen.
- The mean distance between neighboring localized molecules **must be at least twice as fine** as the desired resolution. To achieve 10-nanometer lateral resolution, molecules must be spaced a minimum of 5 nanometers apart in each dimension to yield a minimum density of 40,000 molecules per square micrometer.

Microtubule-from localization to images

Optimized localization precision and labeling density

Effect of *suboptimal* localization precision

Deschout et al. Nature Methods 2014

Microtubule-from localization to images

Molecule position

Actual structure

Optimized localization precision and labeling density

Effect of *suboptimal labeling density*

Deschout et al. Nature Methods 2014

Microtubule – from localization to images

Optimized localization precision and labeling density

Effect of *the higher label* displacement

Deschout et al. Nature Methods 2014

Applications

Applications new biology

Spectrin and adducin exhibit quasi-1D, periodic patterns in axons, quantitatively similar to that observed for actin

