Introduction: Superresolution fluorescence microscopy

CS/CME/Biophys/BMI 371

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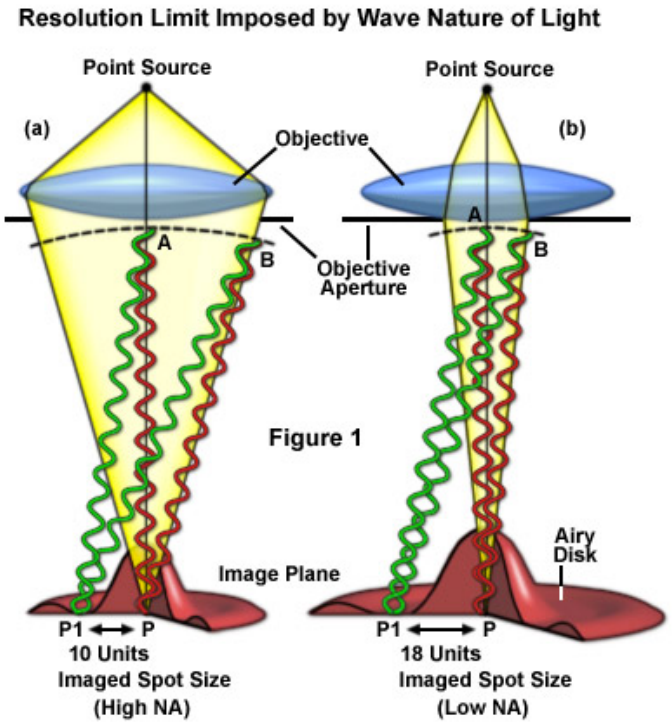
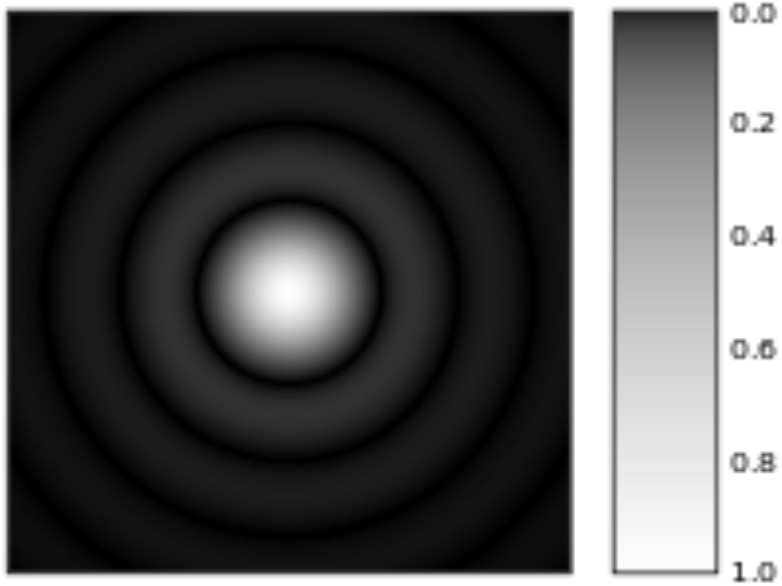
A limit on focusing light

- The physics of light—in particular, the fact that it is a wave—imposes a fundamental limit on how well a lens can focus it
- The light from a single point in space will not focus to a single point
- Instead, it will focus to a disk-like pattern called an “Airy pattern”
  - This means the observed image will be slightly blurred
  - In fact, we can think of the observed image as the true image convolved with the Airy pattern. This constitutes a low-pass filter.

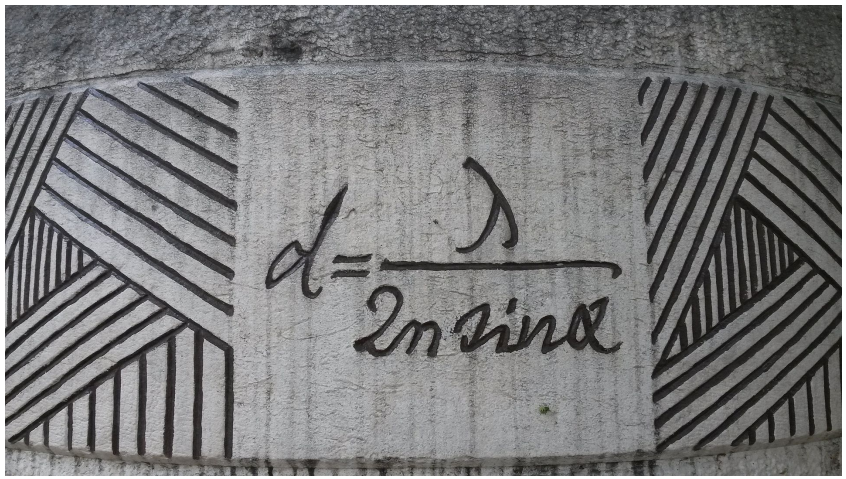


Airy pattern



You’re not responsible for details of the underlying physics here

The diffraction limit



http://en.wikipedia.org/wiki/STED_microscopy#mediaviewer/File:Ernst-Abbe-Denkmal_Jena_F%C3%BCrstengraben_-_20140802_125708.jpg

- This limit on how well one can focus light is known as "the diffraction limit"
  - It's literally "written in stone" in Jena, Germany (on a memorial to Ernst Abbe, who derived it)
- The radius *d* of the Airy disk (the central spot of the Airy pattern) is proportional to the wavelength λ of the light
- Don't worry about the other parameters ($n \sin\theta$, the "numerical aperture," is usually between 0.1 and 1)

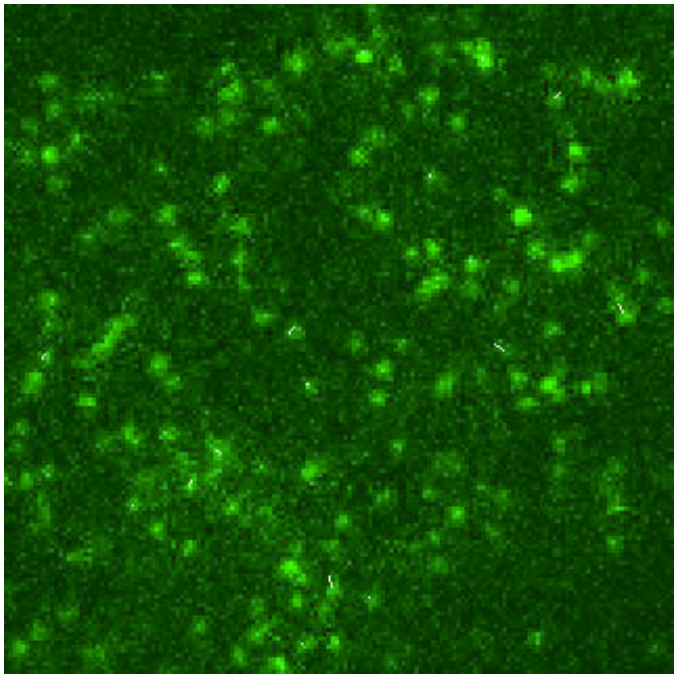
Super-resolution fluorescence microscopy

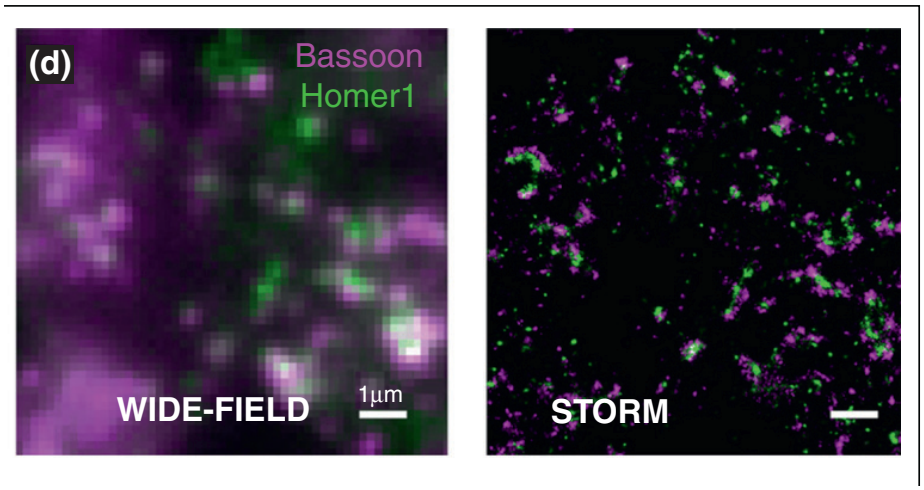
- A number of recently developed techniques achieve resolution well beyond the diffraction limit
  - This requires violating some of the assumptions of that limit—e.g., assuming some prior knowledge of the true image
- The most popular of these techniques is known as either STORM (stochastic optical reconstruction microscopy) or PALM (photoactivation localization microscopy)

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.

STORM/PALM

- If we have only a few fluorophores in an image, we can localize them very accurately
- Thus by getting only a few fluorophores to turn on at a time, identifying their locations in each image, and combining that information (computationally) across many images, we can build a composite image of very high resolution





Sigrist & Sabatini, Current Opinion in Neurobiology 22:1-8, 2011

Tradeoff in spatial vs. temporal resolution

- STORM/PALM requires collection of many raw images for a single reconstructed image. This limits its temporal resolution, and also increases total light exposure, which damages cells.
- Monday's papers present two methods to achieve better temporal resolution without sacrificing much spatial resolution
  - Improved computational reconstruction techniques using compressed sensing (originally due to Emmanuel Candes, Stanford)
    - Basic idea: leverage the fact that the reconstructed image (in PALM/ STORM) is sparse, meaning there are few non-zero pixels
  - Lattice light sheet microscopy: a new super-resolution imaging method, from the inventor of PALM.
    - Basic idea: use advanced optics to illuminate only a very thin plane. Give us a bit of spatial resolution, but substantially increase temporal resolution and substantially decrease damaging light exposure

Background information

- Review on super-resolution microscopy ("Optical super-resolution microscopy in neurobiology")
  - http://www.sciencedirect.com/science/article/pii/S0959438811001802
- My introduction to fluorescence microscopy from CS/CME/Biophys/BMI 279:
  - http://web.stanford.edu/class/cs279/lectures/lecture11.pdf
- If you'd like a more thorough coverage of compressed sensing (also called compressive sensing), see:
  - http://dsp.rice.edu/sites/dsp.rice.edu/files/cs/CSintro.pdf
  - This article is intended to be introductory, but it assumes some knowledge of signal processing/information theory. It's optional!