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"
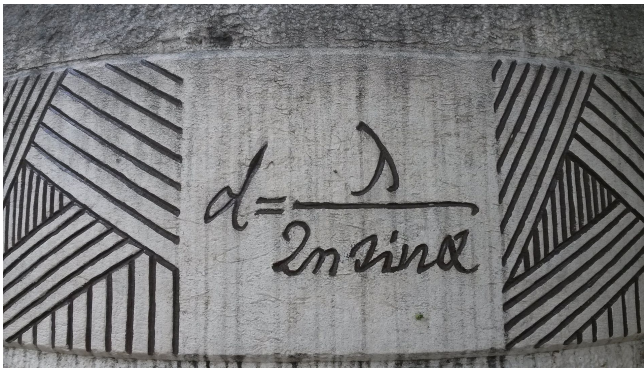 - This means the observed image will be slightly blurred

Resolution Limit Imposed by Wave Nature of Light
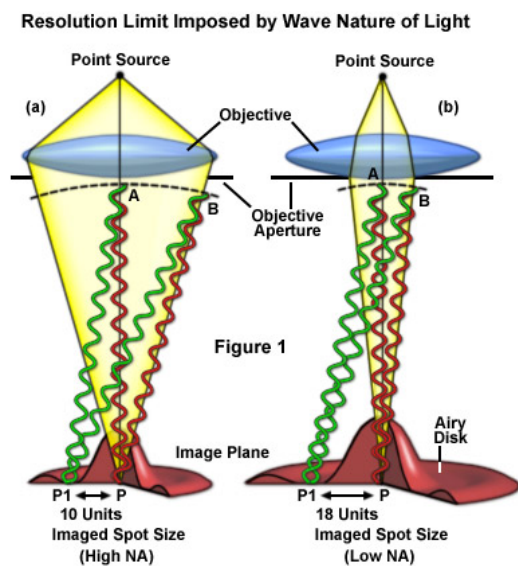
Airy pattern
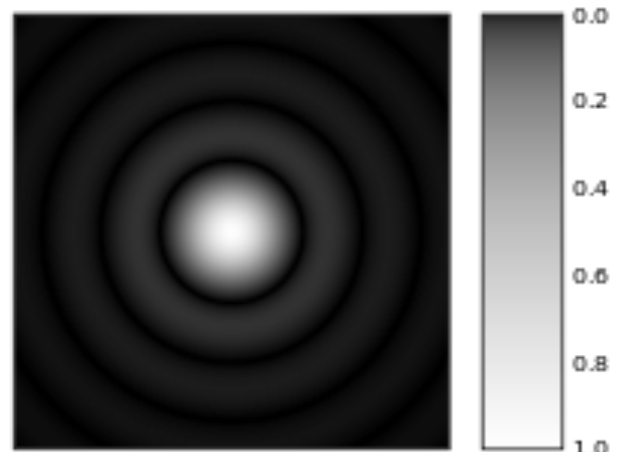
You're not responsible for details of the underlying physics here

The diffraction limit

- 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
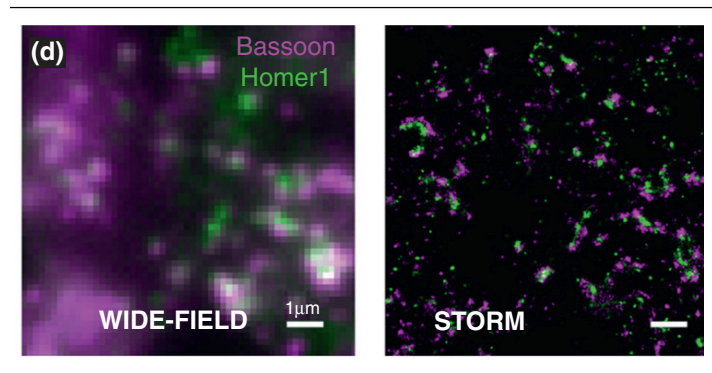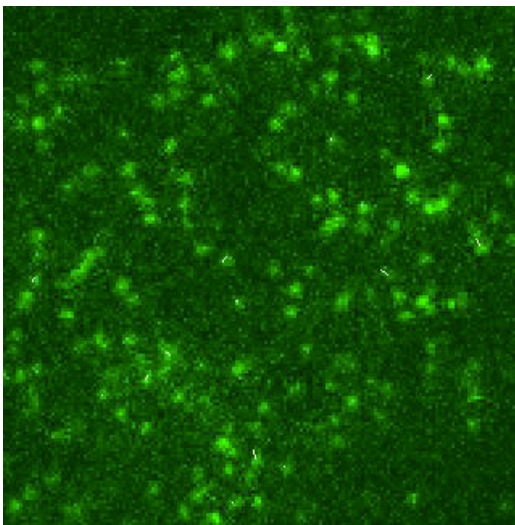

Sigrist & Sabatini, Current Opinion in Neurobiology 22:1-8, 2011

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.
- Tuesday's papers present three methods to achieve better temporal resolution without sacrificing much spatial resolution
  - Improved computational reconstruction method 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
  - Improved computational reconstruction method using low-rank methods
    - Basic idea: leverage the fact that the same "bright spots" (flourophores) tend to appear in consecutive images
  - 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 up a bit of spatial resolution, but substantially increase temporal resolution and substantially decrease damaging light exposure
- To think about: what are the tradeoffs between these approaches?

# Illustration of compressed sensing: the single-pixel camera

- To record a 1000-by-1000 image, a traditional camera makes one million measurements (one per pixel)
- The "single-pixel camera" can very closely approximate this same image with just a few thousand measurements.

https://cnx.org/contents/
_S5wmtzY@4/Single-pixel-camera
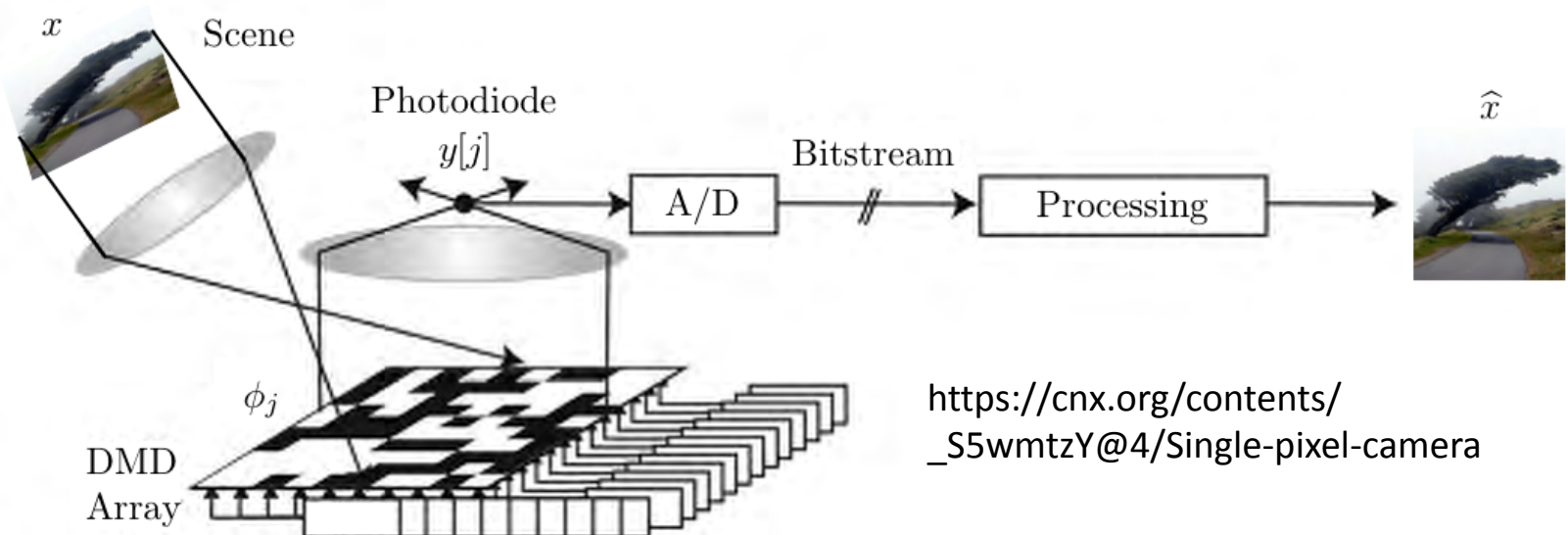
How is this possible?

- The single-pixel camera simply records the mean brightness of different selections of pixels in the image, then computationally reconstructs the original image
- One can do this using relatively few measurements because real photographic images represent a tiny fraction of all possible images (i.e., all possible combinations of pixel values)
  - If represented in the appropriate way (e.g., using wavelets), real photographs are mostly zeros, with just a few non-zero values. In other words, they are "sparse."
- Computational techniques to do the reconstruction effectively are relatively new.
- These techniques are known as "compressed sensing"

randomly chosen
pixel values
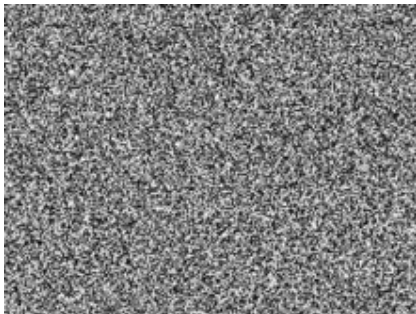
real
photograph

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://users.isr.ist.utl.pt/~aguiar/CS_notes.pdf
  - This article is intended to be introductory, but it assumes some knowledge of signal processing/information theory. It's optional!