Microscopy

CS 279 / BIOE 279 / BIOPHYS 279 / BMDS 245 / CME 279

Oct. 20 and 22, 2026

Ron Dror

Please provide feedback to help us improve the course

- If you haven't already filled out the survey, please do it right after class (by 6 pm today)

- Anonymous survey:

https://forms.gle/oghquk2rLJpc6xMx6

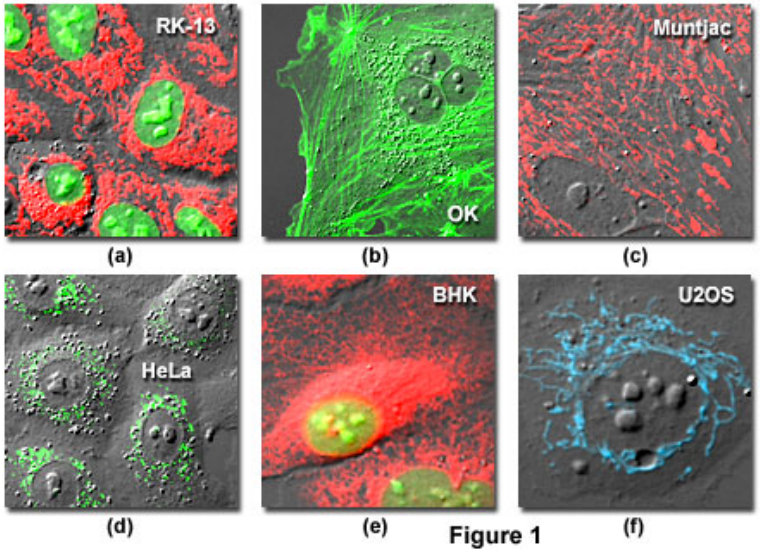
Outline

- Microscopy: the basics
- Fluorescence microscopy
- Resolution limits
  - The diffraction limit
  - Beating the diffraction limit

Microscopy: the basics

Most of what we know about the structure of cells come from imaging

- Light microscopy, including fluorescence microscopy



https://www.microscopyu.com/articles/livecellimaging/livecellmaintenance.html

- Electron microscopy



http://blog.library.gsu.edu/wp-content/uploads/2010/11/mtdna.jpg

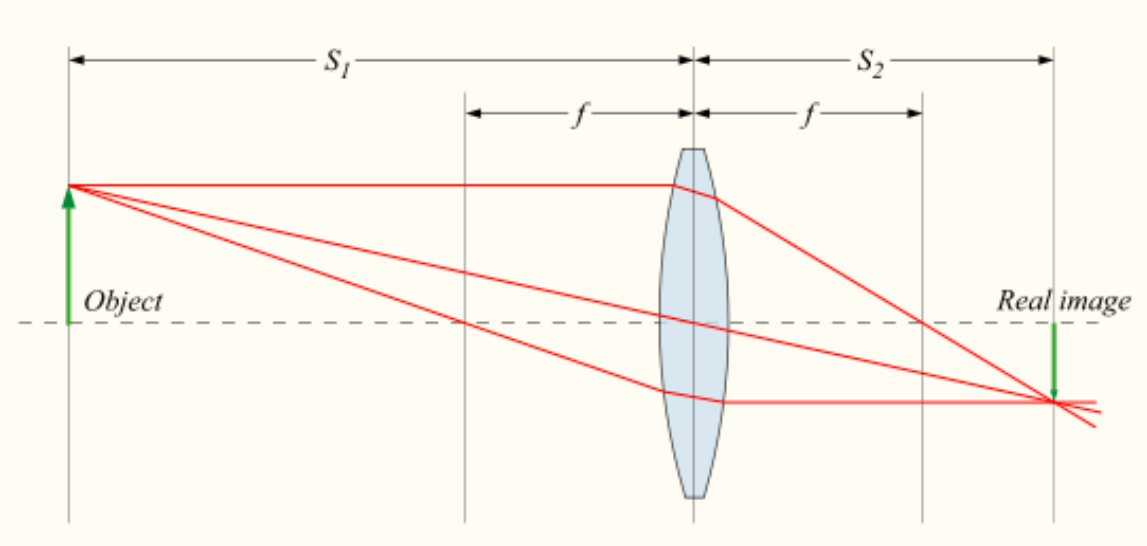
Light microscopy

- Basic idea:
  - Shine light on a biological sample (i.e., one or more cells)
  - Measure the light that is *reflected* or *transmitted*
  - Use lenses
    - Why do we need lenses in a microscope?



Lenses in microscopy

- The lenses in a microscope do two things:
  - Magnify the image
  - *Focus* the image, so that much of the light coming from a particular point in the sample ends up focusing on a particular point on either your retina or a sensor (e.g., CCD)
    - You need a lens to form a clear image, even if you have a very high-resolution sensor



https://upload.wikimedia.org/wikipedia/commons/thumb/7/71/Lens3.svg/542px-Lens3.svg.png

Fluorescence microscopy

Fluorescence microscopy: basic idea

- Suppose we want to know where a particular type of protein is located in the cell, or how these proteins move around
- We can't do this by simply looking through a microscope, because:
  - We (usually) don't have sufficient resolution
  - The protein of interest doesn't look different from the ones around it
- If only the protein would glow!
- Can we get a protein (or other molecule of interest) to glow?

Fluorescence microscopy: basic idea

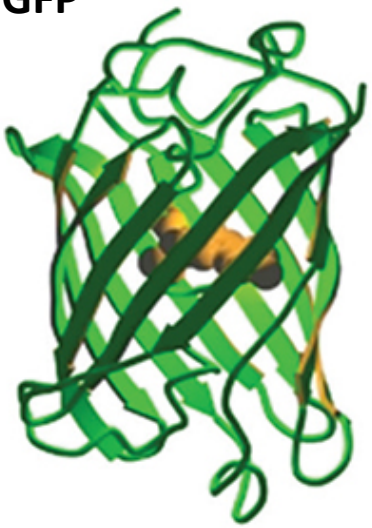
- Make the molecules of interest glow
- Attach a fluorophore (fluorescent molecule) to the molecules of interest
- When you shine light of a particular wavelength on a fluorophore, it emits light of a different wavelength
  - Additional advantage: not only does the molecule glow, the light it emits has a different wavelength than the incident illumination (the light you're shining), making the molecule easier to isolate

Fluorophores

- Fluorophores can themselves be either proteins or small molecules
  - Among the most widely used are:
    - Green Fluorescent Protein (GFP)
    - Alexa fluors (small molecules)

The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien *"for the discovery and development of the green fluorescent protein, GFP"*.

GFP

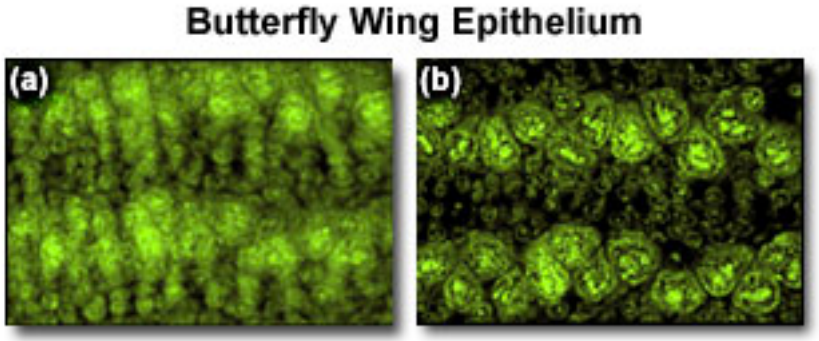






Fluorescence microscopy images

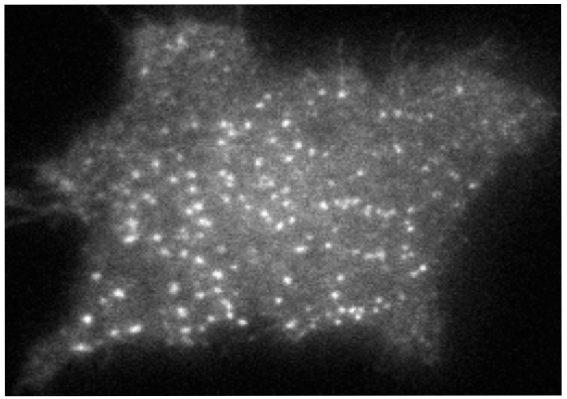
- There are many types of fluorescence microscopy: wide-field, confocal, TIRF (total internal reflectance fluorescence), etc.



Wide-field Confocal

http://www.microscopyu.com/articles/confocal/confocalintrobasics.html

TIRF

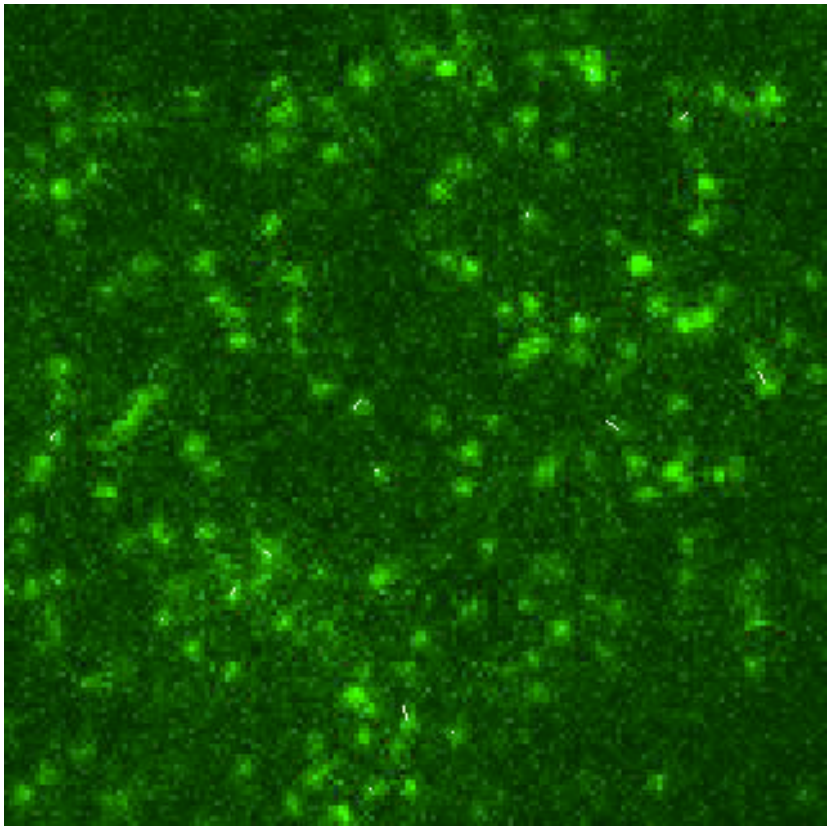


Von Zastrow lab, UCSF

Analyzing this data quantitatively involves the types of image analysis we discussed in previous lectures, and more

Single-molecule tracking

- If the density of fluorescent molecules is sufficiently low, we can track individual molecules
  - Doing this well is a challenging computational problem



Data: Bettina van Lengerich, Natalia Jura
Tracking and movie: Robin Jia

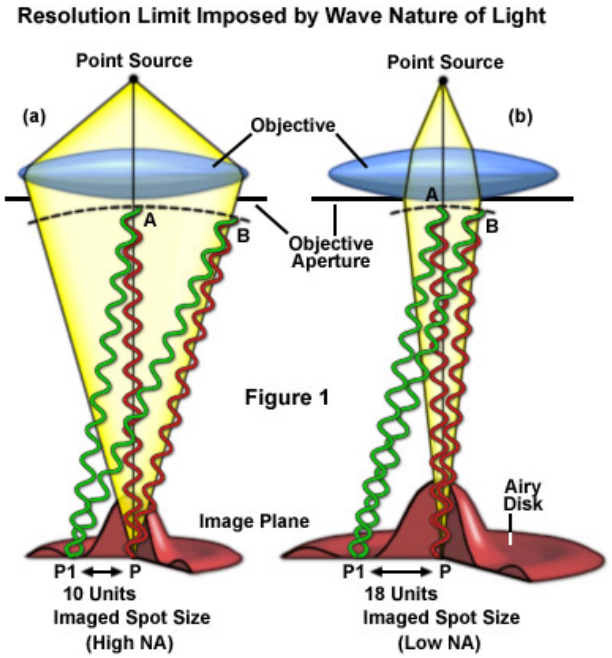
Resolution limits

Resolution limits

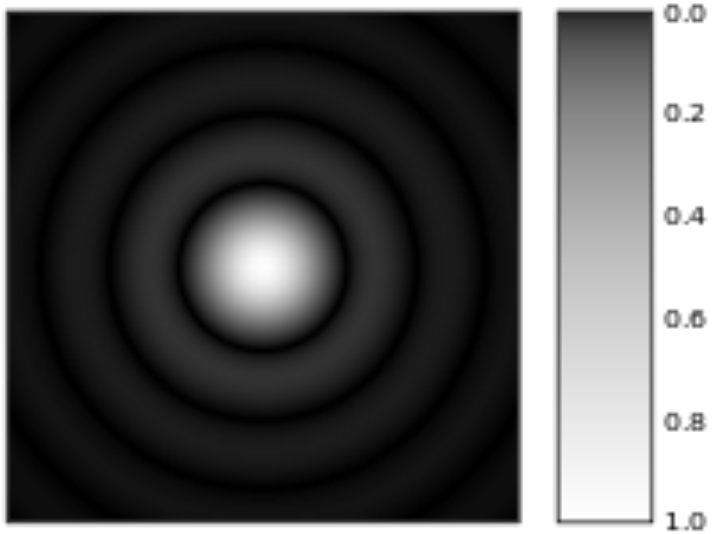
The diffraction limit

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.

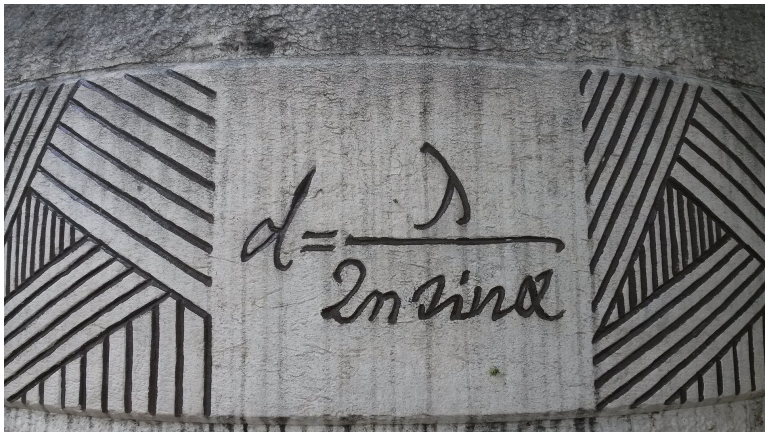


Airy pattern



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 published it in 1873)
- The radius *d* of the Airy disk (the central spot of the Airy pattern) is proportional to the wavelength λ of the light
- It also depends on some other parameters (e.g., lens size) that determine the "numerical aperture" ($n\sin\theta$)
  - The numerical aperture is usually between 0.1 and 1

The bottom line

- Resolution limit of a light microscope:
  - The wavelength of visible light is 400–700 nm
  - A light microscope can't distinguish points that are closer than 200 nm
- Many cellular structures are smaller than this. A protein is just a few nm across.

Question to discuss

How can one achieve resolution better than 200 nm?

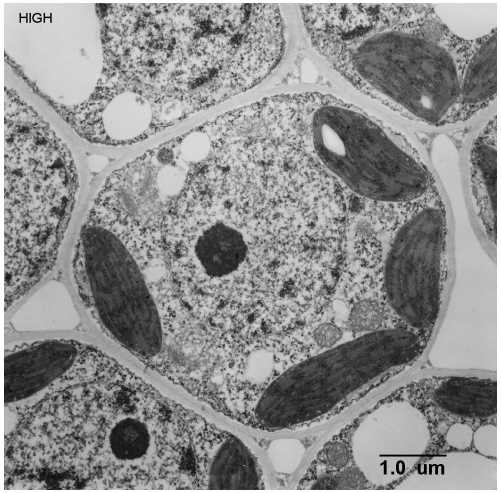
Resolution limits

Beating the diffraction limit

Option 1: Decrease the wavelength

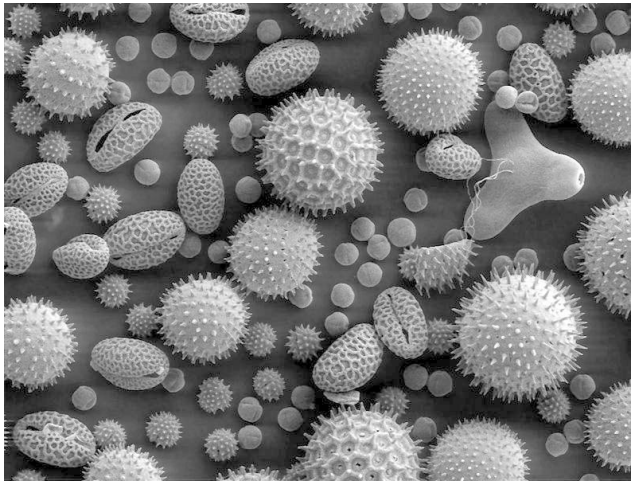
- Higher-frequency radiation (e.g., x-rays) has shorter wavelengths and thus allows higher resolution
  - It also damages the sample more
- It's possible to image with electrons, which have a *much* shorter wavelength (~.002 nm)
  - Electron microscopy can thus achieve much higher resolution
  - Disadvantages: can't use living cells, and molecules of interest won't glow

Transmission electron microscopy



http://www.cas.miamioh.edu/~meicenrd/ANATOMY/Ch2_Ultrastructure/Tempcell.htm

Scanning electron microsopy



http://www.newscientist.com/data/images/ns/cms/dn14136/dn14136-1_788.jpg

Option 2: super-resolution fluorescence microscopy

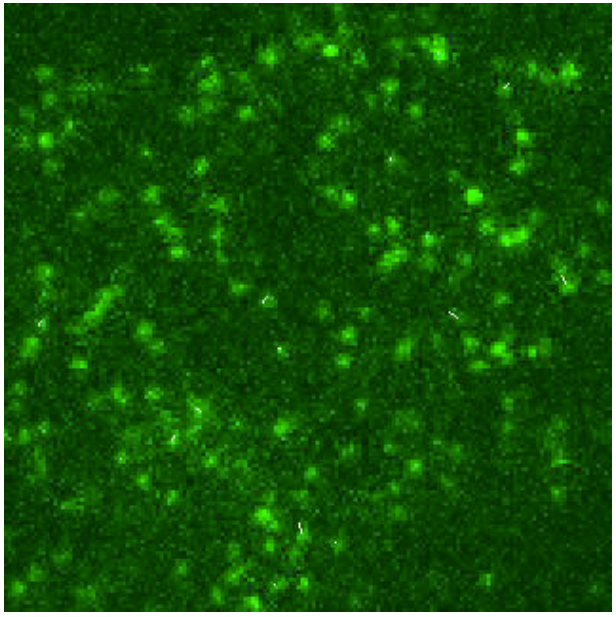
- A number of recently developed techniques achieve resolution well beyond the diffraction limit
  - This requires violating some of the assumptions of that limit
- Major approaches:
  - Single-molecule localization (e.g., STORM & PALM)
  - Structured illumination (e.g., STED)
  - Combination of the two (e.g., MINFLUX)
- All of these involve collecting many images of the sample and then processing them computationally to obtain a composite image at higher resolution

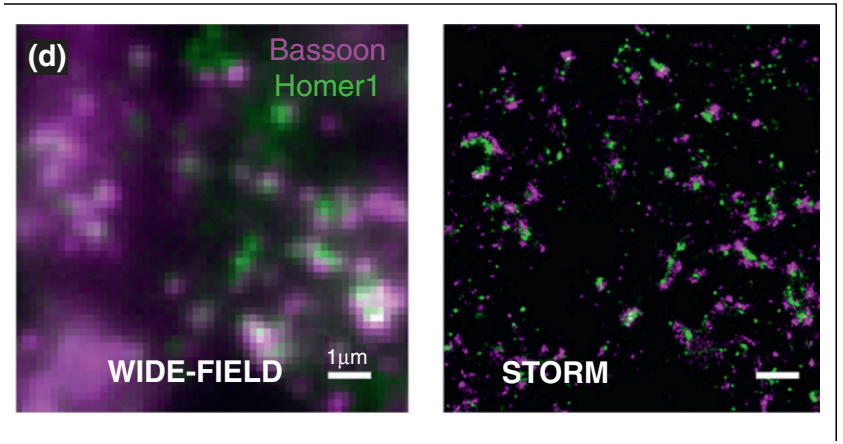
The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.

- Note: many approaches were developed more recently (including, MINFLUX, which gives resolution down to about 2 nm)

Single-molecule localization microscopy

- 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 (computationally), and combining that information across many images, we can build a composite image of very high resolution
  - STORM (stochastic optical reconstruction microscopy) and PALM (photoactivation localization microscopy) are two different experimental strategies for making only a subset of fluorophores glow at a time





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

Structured illumination (e.g., STED)

- Shine many different light patterns on the sample, then combine the resulting images computationally to obtain a composite image at higher resolution
  - Early approaches used sinuisoidal illumination patterns
  - Stimulated emission depletion (STED) uses a combination of two lasers that are scanned across the sample

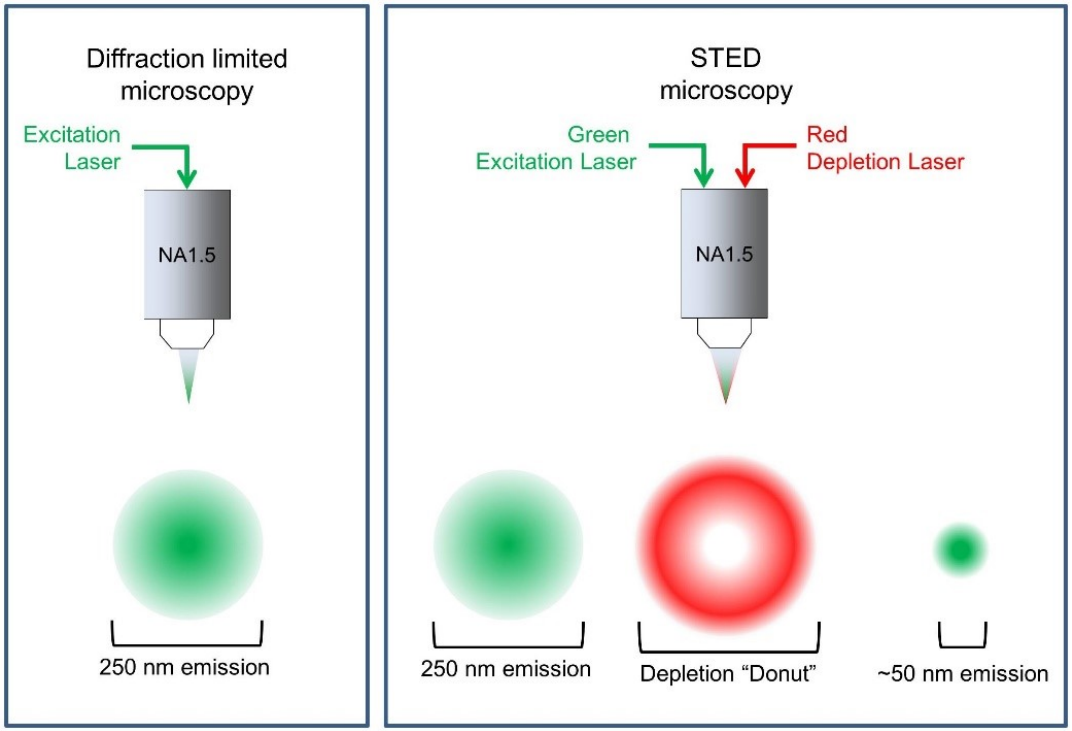


Image from https://www.technologynetworks.com/neuroscience/articles/what-is-super-resolution-microscopy-sted-sim-and-storm-explained-328572

Major area of current research: improving these methods via deep learning/AI

- Examples:
  - Increase accuracy of the reconstructed image by learning to recognize the “point spread function”—i.e., the appearance of individual fluorophores under the microscope
  - Speed up imaging by allowing analysis of images with a higher density of active fluorophores, thus requiring fewer individual images (e.g., DEEP-STORM)
- See optional reading on course website