ISTA 352

Lecture 26

Images in Biosciences

Administrivia

- Grade replacement with a project option
  - Up to 2 bad grades (e.g., one quiz and one assignment)
  - Group projects allowed
  - Projects graded based on presentation and write up
  - Project presentations will be during our scheduled final exam time
  - Interested people need to get me a proposal by November 05

Quiz next time

- Material through first half of last lecture
  - Visualization of data will be tested next time

- Focus is on material from lecture 17 ("retrospective") onwards

Main quiz topics (not necessarily everything)

- Some review questions similar to, and on the same topics as, the last quiz
- One mark for each guest lecture, perhaps choose two from three as I did with Mary's lecture
- 17) Similar triangles and previous quiz
- 18) Homography transformation
- 20) Conformal mapping, geodesics, latitude, longitude, great circles, meridians
- 20) Three kinds of point projections. Perhaps prepare by sketching them.
- 21) Rhumb lines, key properties of the Mercator, universal transverse mercator
- 22) Level sets (e.g., elevation contour lines)
- 23) Perspective, shading, and shadows in painting
- 24) Lambertian reflection, dielectric light interaction model, specular reflection, and mirrors. Interpreting shading.
- 25) Shadows (umbra, penumbra). Concept of local structure versus global consistency.

Biomedical imaging

- Three (!) guest lectures in biomedical imaging next week

- Today we will discuss some optical issues and types of microscopes

Important optical systems characteristics

- Do you capture enough light?
  - Some things you want to see in the night sky are bigger than you think, but too dim to see
  - The bigger the telescope, the more light you get
  - The longer the exposure the more light you get

- Can you distinguish two points that are close together?
  - The ability to do so is called resolving power
  - This is fundamentally limited (next slide)
    - The bigger the telescope, the better the resolution
    - Being "diffraction" limited is hard---usually other effects (e.g., atmospheric) dominate
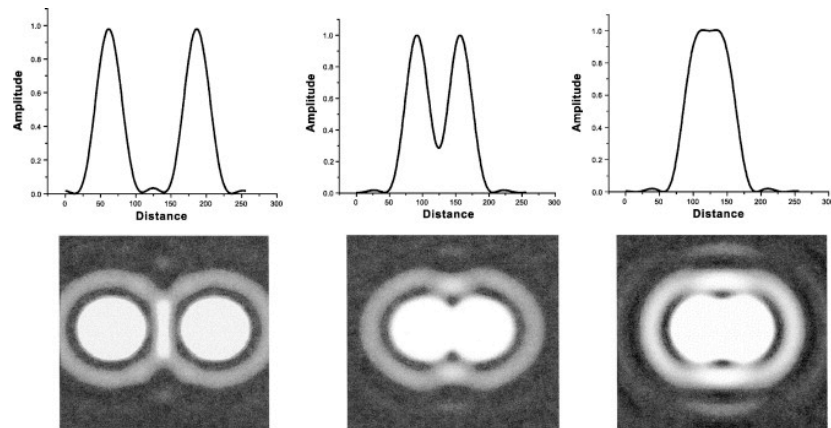
What is the image of a point source?

- Ideally the image of a point source is a point

- Unfortunately, even with a perfect optical system, this is not the case due to the wave nature of light.
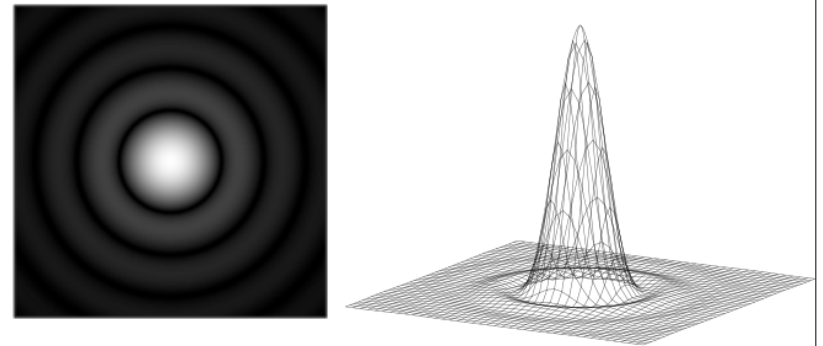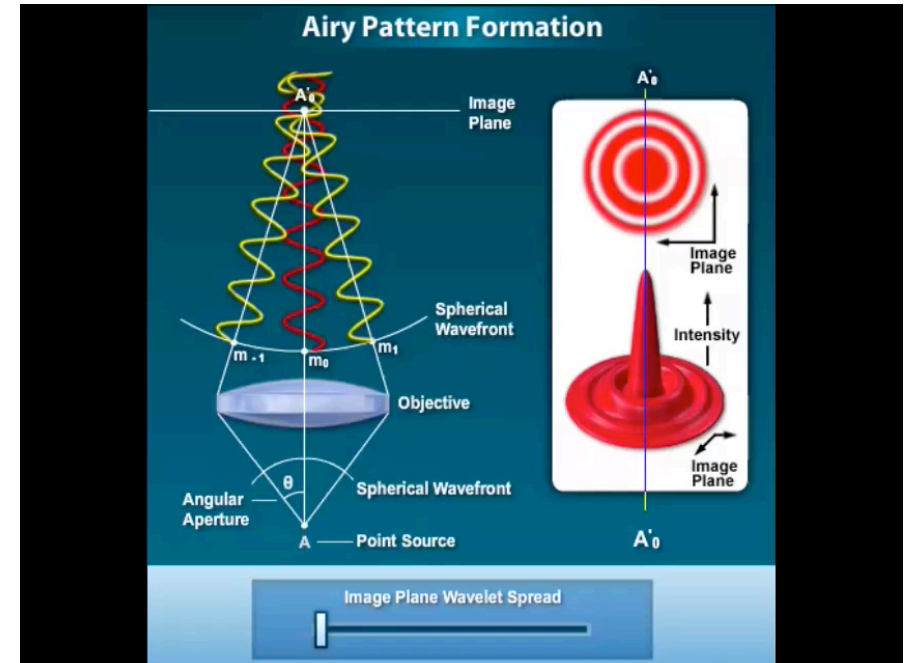

Airy patterns (after George Biddel Airy, who analyzed them in 1835). The part inside the first zero is the "Airy disc"

Resolution is limited by the Airy disc size.

Airy Pattern Formation

What is the image of a point source?

- The image of a point source is a disc that is nominally the radius of the first zero in the Airy pattern

- The radius of this disk is approximately

$$x = (1.22)\frac{\lambda f}{d}$$ where λ is wavelength, f is focal length, and d is aperature

For angular resolution

$$\theta \cong (1.22)\frac{\lambda}{d}$$ $(\theta \cong \frac{x}{f}$ for small angles$)$

Airy disk take home message

$$x = (1.22)\frac{\lambda f}{d}$$ where λ is wavelength, f is focal length, and d is aperature

- To get more resolving power you need a bigger telescope

- To see extremely small things, you want to use a smaller wavelength
  - A key motivation for the electron microscope

- There is not much point having sensors (e.g., CCD wells) which are substantively closer together than the Airy disk radius.
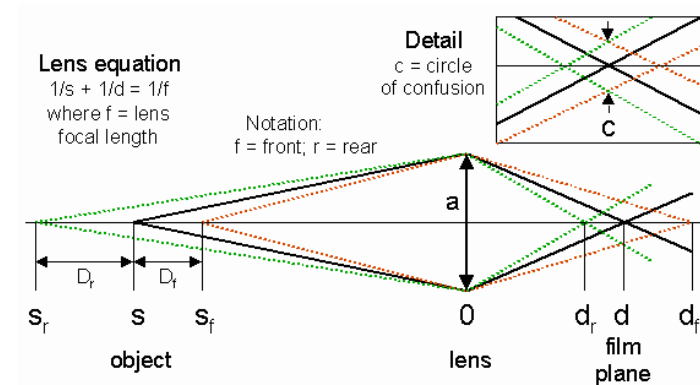
Depth of field

- Objects at a given distance are in focus only in one imaging zone
  - Not an issue in astronomy because all objects are far enough away to be considered "at infinity"
  - Important issue in photography and microscopy

Image from WikiMedia commons

Depth of field

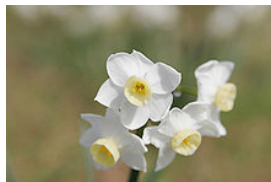Details will not appear on quizzes

Depth of field (DOF)

- Ignoring diffraction (i.e., interference of waves), depth of field is related to "f-stop" (or "f-number")

$$F^{\#} = \frac{f}{D}$$ where f is focal length and D is aperature

- As f-number increases, DOF decreases

f/32   f/5.6   f/2.8

Careful with the fancy reciprocal like notation from photography. Large f-number is on the left.

Depth of field (DOF)

- Ignoring diffraction (i.e., interference of waves), depth of field is *inversely* related to "f-stop" (or "f-number")

$$F^{\#} = \frac{f}{D}$$ where f is focal length and D is aperature

- As aperture decreases, DOF increases
  - A pinhole camera has a very large DOF
    - Limited only by diffraction

Types of Microscopes

- Too many kinds to do in one lecture!!!

- A few important ones
  - Basic optical microscope
  - Confocal microscope
  - Electron microscope
    - Transmitted electron microscope
    - Scanning electron microscope
  - Scanning probe microscope

Basic Optical Microscope

- Brightfield microscope passes light through the sample
  - Staining is often needed to make features visible
  - Not good for seeing surfaces

Basic Optical Microscope

- Fluorescent tagging
  - Add a genetic tag to the organism that glows when light at a specific wavelength stimulates the sample
  - Often used in conjunction with a confocal microscope


Alternaria Data (1)

A_1

102 images

# Confocal Microscope

- Recall the depth of field formula

$$F^{\#} = \frac{f}{D}$$ where f is focal length and D is aperature

- A confocal microscope uses optical trickery to arrange a small aperture
  - This blocks out of focus light
  - Provides sharp images for a point at a specific focal plane
  - A lot of light is blocked, so we scan, point by point, to make an image
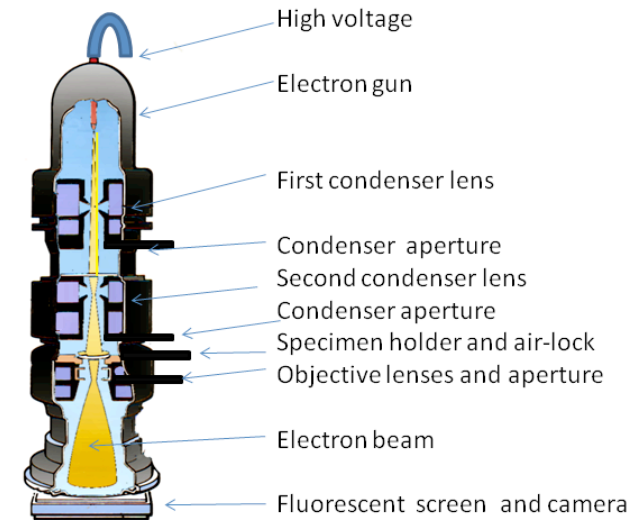

Confocal microscopy

WikiCommons
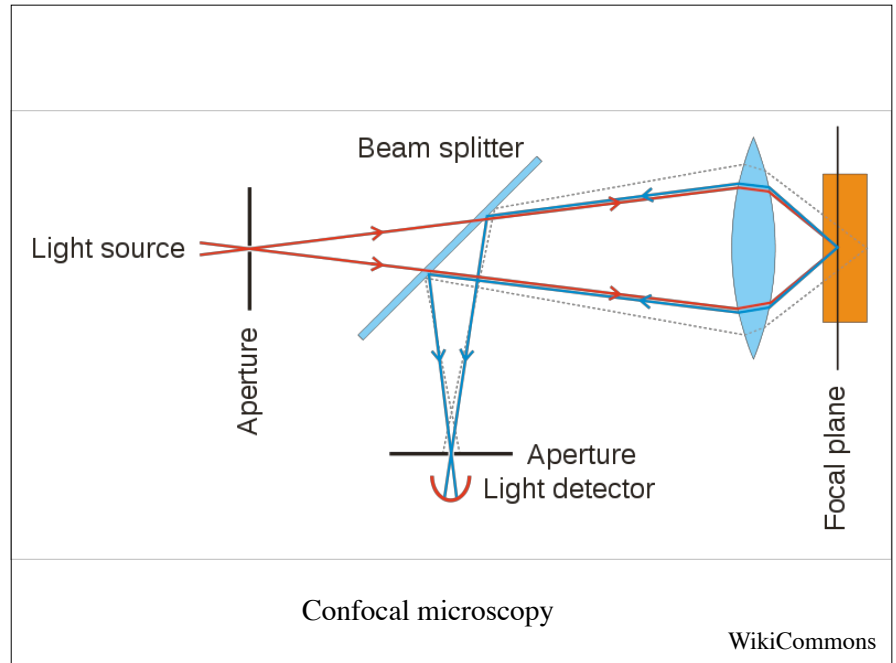
Electron Microscope

- Recall the Airy disc
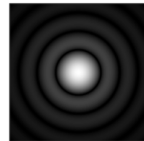
$$x = (1.22)\frac{\lambda f}{d}$$ where λ is wavelength, f is focal length, and d is aperature

- Smaller wavelength means higher resolution

- Electrons at energies usable for imaging have much smaller wavelength than visible light (even x-rays).

- Electron beams are focused with magnets.

Transmission Electron Microscope
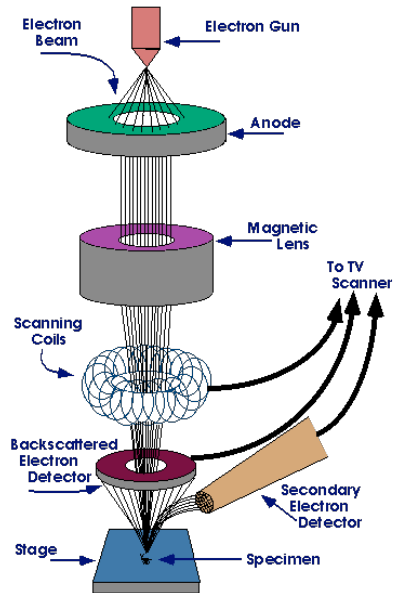
Scanning electron microscope (SEM). Note that electrons are reflected from the surface, rather than going through the sample (as in the transmission case)

Caption for picture on the next slide (taken with a scanning electron microscope)

Pollen from a variety of common plants: sunflower (*Helianthus annuus*), morning glory *Ipomoea purpurea*, hollyhock (*Sildalcea malviflora*), lily (*Lilium auratum*), primrose (*Oenothera fruticosa*) and castor bean (*Ricinus communis*). The image is magnified some x500, so the bean shaped grain in the bottom left corner is about 50 μm long.