Lecture 2 August 28th 9:30am: Studying Cells

1. Microscopes aid in studying cells
    a. 17th century development of lenses that allow magnification
    b. Put two lenses together and created the compound microscope
    c. 0.2mm is the minimum resolvable by unaided eye
    d. 200nm is the minimum resolvable by light microscope – can only go so far with a light microscope because the wavelength of light is .2nm, so it can't see through more than that.
    e. 0.2 μm is the minimum resolvable by the electron miscroscope
2. Light Microscopy
    a. Can view living or fixed specimens
    b. Live stains: GFP (Green fluorescent Protein)
    c. Fixed stains: Fluorescent probes
    d. Different light sources
        i. Regular light
        ii. Phase contrast – changes the light direction by using filters of liquid. This makes the areas that are denser, darker.
        iii. Differential interference contrast – use polarized light. Light is shined on a polarized filter with a grid that only allows light that shines in a certain direction to go through the filter. This shifts the light and gives it a 3D effect.
        iv. Dark field – moves the light. The light hits the area in a different direction and creates a dark background with bright features. Ex. Holding a flashlight under your face.
3. Fluorescent Microscopy
    a. Colors travel at different wavelengths, so we use filters to absorb all the colors/wavelengths except the one we want showing.
    b. As atoms absorb the lights energy they get excited, and atoms don't want to be in this state. So, they go back to original state and release that energy in a color lower in wavelength. Ex. Green to Red, or Blue to Orange.
    c. Chemical fluorophores can be used in live or fixed specimens
        i. Chemical fluorophores are small molecules that absorb and emit light at different wavelengths
        ii. Examples: rhodamine, DAPI ALEXA, cy
        iii. Can be covalently attached to other molecules (protein inhibitors, antibodies, etc.)
        iv. Different structures inside the cell can be different colors
        v. And they can also change colors under different conditions i.e. binding and releasing substrates.
    d. Antibodies
        i. Antibodies are generated for a specific protein. (actin is for DNA)
        ii. They are shaped like a Y with the antigen binding sites on the two tips of the Y. The top of the Y is the light chain and the bottom is the heavy chain

iii. Fluorescent dyes attach to the heavy chain and glow under the microscope to help us identify where certain structures are. Since the antibodies only bind to a certain protein we can find these proteins under the microscope this way.

e. Confocal Microscopy
   i. Specialized type of fluorescent microscopy using a laser scan specimen
   ii. Focus light and capture one specific focal plane instead of lots of focal planes like in regular fluorescent microscopy.

f. Deconvolution Microscopy
   i. Take spheres less than 2 μm and capture the image on a computer
   ii. The computer is then trained to correct the size of the spheres
   iii. And the image that results is a defined image

4. Electron Microscopes
   a. Two types: Scanning and Transmitter electron microscope
      i. In TEM electrons go through the specimen and are detected beneath them
         1. Give you images that look like light sections
         2. Put a gold particle attached to antibody to determine which proteins are which in the cell. Just like in fluorescent microscopy but with gold instead.
      ii. In SEM Specimen is covered in scanning oils so electrons bounce off and are detected off to the side at an angle. The image that results is a 3D image
5. Cell breakage
   a. Many ways to break down the cell
   b. Force it through a small hole with a great force
   c. Shear the cells in a blender
   d. Break them with a high frequency
   e. Or Centrifugation
      i. Differential centrifugation: Use different speeds to separate different size parts of the cell.
      ii. Velocity Centrifugation: Centrifuge and drain fast(bigger) sedimenting components
6. Great Experimental Organisms
   a. E. Coli (Prokaryote) – grow and reproduce very quickly. Most of the basic processes that occur in E. Coli also occur in our own cells
   b. Saccharomyces cerevisiae (eukaryote fungus) – rapid growing yeast cell. Single celled fungus that carries out the basic tasks that eukaryotes perform, but divides almost as quickly as bacteria.
   c. Arabidopsis (eukaryote plant) – can be grown indoors and in large numbers. Studying it gives us insight to the crops that we need to live.
   d. Drosophila melanogaster (eukaryote multicellular animal) – help us study chromosomes and heredity because they reproduce so quickly.