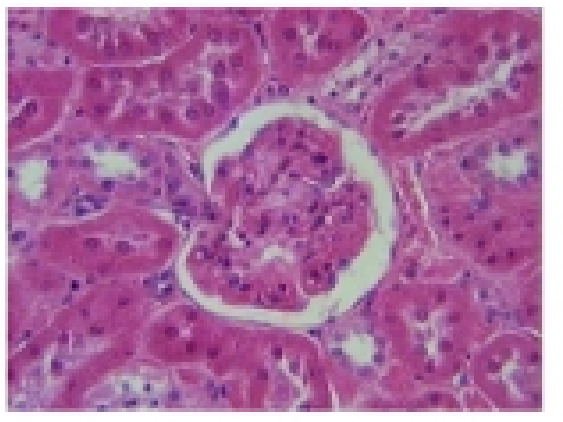
Cell Structure & Function 3096
Lecture 4 – August 31, 2015

Today's Outline:

Development of technologies – thin sectioning
Contrast
Staining
Various Types and different tissues
Fluorescence
Phase Contrast

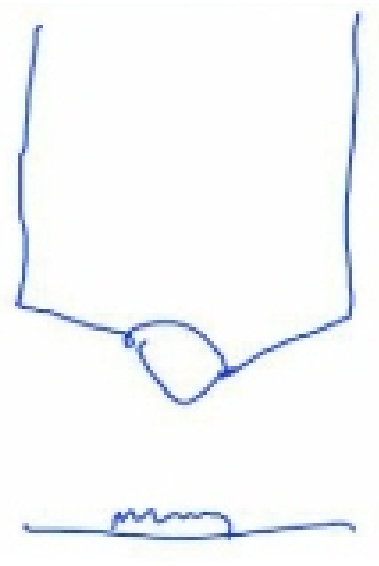
- Tissue Preparation
  - In order to be able to look at things through a microscope, the sample have to be thin
  - How do you get things thin enough to look at and preserve as much tissue as you can? We will look at things from bottom up
    - Embedding the sample in a hard matrix – For Light microscopy, you will use wax, Ice (freeze the sample), Plastic (TEM – transitional electron microscope)
      - Ice usually destroys the tissue, but sometimes is used
      - For electron microscope, wax cannot be used, so plastic is used
    - It's hard to get the wax to replace the water in the sample
      - You don't just surround the tissue but you also need to penetrate the whole tissue
        - Hard to do
      - So you have to use a series of intermediate solutions – Xylene dissolves wax
        - But it's still not soluble in water
        - So you must find something that xylene is soluble in but also soluble in water
      - In this case, you can use Ethanol or Acetone (can be dissolved in water and in xylene)
      - You start to replace the water with alcohol or acetone then you replace alcohol or acetone with xylene then you replace the xylene with wax
      - You do this in steps and let it happen gradually
      - As you pass them through these solvents, you are extracting a lot of components of the cells, so to preserve much of the cell, you need to pretreat the tissues with "fixatives" which includes Formaldehyde or Glutaraldehyde
        - They cross link the proteins in the tissue and hold the tissue stable

        - These hold the tissues together while going through all the chemicals
- Then you cut the tissue with a microtome
    - Thin section (full of wax), placed on a glass slide
        - But because it's full of wax, its hard to see the material so you must remove the wax
        - You do the same process, backwards
- Removing the wax – xylene – alcohol – water
- How do you know that this whole process isn't creating an artifact, then you see the tissue shrink and then see distortion?
    - You believe that what you see is happening with everything you work with
    - What other technique can you use (frozen vs. wax)
        - Once you have the tissue, you then stain with dyes that seem to react to different parts of the tissue
            - Hematoxylin – blue, stain nuclei
            - Eosin – pink, stains mucus, polysaccharides, cytoplasm
- Picture of the noodle
    - Depending on how you cut it, you get different shapes
        - Rectangles, ellipse, circle
- PICTURE OF KIDNEY
    - 
    - You can see both the Hematoxylin and the Eosin
    - One slice of tissue (pink and blue)
    - What kinds of structures are shown?
        - Vesicle? Tubule?
    - The dimensions of what you see may change as you cut the wax, it compresses, stretches, etc.
- Fluorescence
    - Fluorescence – family of stains
    - When a sample is illuminated with light of a given wavelength, in certain cases, it then emits light of a different (lower energy) wavelength
        - Ex. If you illuminate it with red light, you might get blue fluorescence
    - Error in Karp…
        - All wavelengths are visible light and all wavelength (infrared, etc) can be used to stimulate fluorescence.
    - Chlorophyll – fluorescent themselves

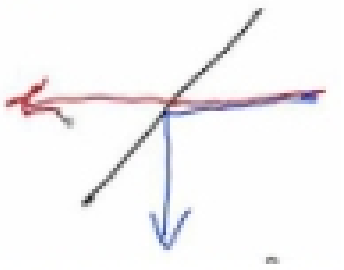
- Fluorescent proteins (found in jellyfish) – green fluorescent protein (GFP) people can clone these proteins and make them fluorescent

- Dyes that bind to specific components – DAPI binds to DNA
- Dye molecules that can be coupled chemically to specific proteins – Coupled to Antibodies
  - You can label the antibodies and follow where they go in the tissue
- GFP – protein

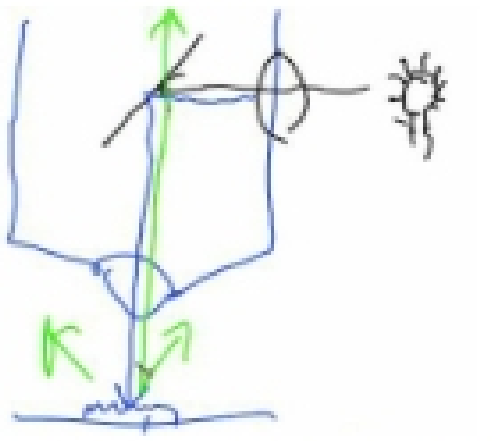
- Microscope Design



- Piece of glass
  - Dichroic Filter – reflect some wavelength, and pass others
  - Ex. Shine red light + blue light at the filter (blue light will be reflected down and red light will go through)



  - When the filter is in the microscope, along with a source of light coming from a lens.



- Blue light will reflect and shine on the sample