Lab protocol

In this lab we will first examine some of cellular structures at the light microscopic level and then go on to look at epithelium, glands and connective tissues. Since most organs are comprised of a mix of epithelium and/or glands and connective tissue, we will be examining several aspects of the slides.

1. **Slide #3** [primate liver, H&E]. Liver cells are a good cell to study due to their involvement in most biochemical processes. Note the general shape of the hepatocyte (the dominant cell type in this slide). Remember that you are looking only at a section of a cell; the most common configuration of an intact, isolated hepatocyte is a 14-sided polyhedron! The cells tend to be lined up in cords, with some spaces in between (that would be filled with blood in life).

   Note the staining pattern of the nuclei and cytoplasm. The nuclei are rather basophilic (blue) with a dot toward the center (the nucleolus) and a rim of blue around the edge of the nucleus (condensed heterochromatin). The remainder of the nucleus is clearer (euchromatin) which is available for transcription. The cytoplasm is pinker (eosinophilic). This is due to the presence of many mitochondria and proteins (that stain with eosin). Darker blue areas (basophilic) in the cytoplasm are areas of ribosomes (and/or rough endoplasmic reticulum). There are regions within the cytoplasm that are much lighter staining. This is because some of the contents of the hepatocyte do not take up stain (such as the Golgi) and because some of the contents (particularly glycogen) dissolve away during processing. Figure 15 of the “EM of organelles” module on virtual histology shows glycogen in the cytoplasm of a hepatocyte. **Slide #10** [liver, PAS] is stained with PAS and shows glycogen as well as glycoproteins, proteoglycans and mucin (carbohydrate rich chemicals). In this slide of the liver most of the purple color is glycogen, demonstrating how ubiquitous it is in liver cells as a store of energy.

   Note that it appears that you can see the plasma membrane between adjacent hepatocytes. This is an illusion resulting from significant interdigitation between adjacent cells. The plasma membrane itself is well below the resolution of the light microscope.

2. **Slide #91** [spinal cord smear, ox; H&E]. This slide will demonstrate the intense basophilia of ribosomes and rough endoplasmic reticulum that is prominent in neurons. Locate the large neurons and examine the deeply basophilic clumps of material within the cytoplasm of these cells, corresponding to the RER. Large stacks of rough endoplasmic reticulum within nerve cells are known as Nissl bodies or the Nissl substance.

3. **Slide #36** [pancreas, monkey; H&E]. The dominant cells on this slide are the secretory cells. These cells manufacture proteins for secretion, and then store them in the cytoplasm. Therefore, the cytoplasm of the basal portion of these cells (the part near the nucleus) is quite blue (basophilic). This is because of the numerous ribosomes associated with rough endoplasmic reticulum. The digestive enzymes produced by the rough ER are packaged in secretory granules. The pink vesicles in the apical part of the cell away from the nucleus contain the digestive enzymes (which are proteins and stain eosinophilic).
These secretory cells are arranged (like pizza slices) in a round circle, with the nucleus away from the center and the enzyme-filled vesicles toward the center of the circle. There is a small lumen at the center into which digestive enzymes are released. This arrangement of secretory cells is termed an alveolus. The secretory product will go from the lumen of this alveolus through a duct to join up with ducts from other alveoli. This particular organ (the pancreas) is comprised of a parenchyma dominated by compound tubuloalveolar glands. There is reticular connective tissue between the alveoli, and blood vessels reside in this connective tissue. There is denser connective tissue between lobules of the gland. Therefore, the glandular parenchyma is supported by a connective tissue stroma.

4. **Slide #93** [sympathetic ganglia; H&E]. Although lysosomes are not seen by usual staining processes, some very long-lived cells (such as neurons and heart muscle cells) contain residual bodies of undigested material. In this slide are neurons (the largest cells found in the section) which contain the brownish-staining lipofuchsin (residual bodies of lysosomes).

5. **Slide #83** [mitral valve, human; H&E]. Lipofuchsin can also be seen within some cardiac muscle cells. It tends to accumulate near nuclei and is quite prominent to the left of the mitral valve attachment toward the bottom of the tissue section on the virtual histology site.

Other inclusions in cells will be seen later in the lab when we examine skin (melanin), the GI tract (mucin) and lipids (fat).

**Mitosis is an important cellular function. This can be studied to some degree with the light microscope.**

6. **Slide #19** [White fish blastula; iron hematoxylin-orange G stain]. The mitotic stages are more clearly visible in these cells. First, scan the slide at low power before selecting areas to view at higher power. Note that sections of six different blastulae are mounted on this slide. Nearly all of the cells in the blastulae are in mitosis so you probably will not see any interphase stages. (The cells are dividing so rapidly that they proceed from telophase into the next prophase almost without pause.) The cytoplasm of the cells stains a granulated orange; the chromosomes are stained a dense black. (The round, black objects in the cytoplasm are fat droplets.) Examine a number of mitotic cells, trying to find examples of each of the major stages of mitosis. Can you discern the spindle apparatus? What is the diploid number of chromosomes in these cells?

7. **Slide #21** [human cell culture; H&E]. These excellent slides show fibroblasts (connective tissue cells) isolated from an aborted fetus. This is not a section of tissue. Rather, the cells were cultured directly on the glass slide and subsequently fixed and stained with H&E. At low power select areas for more detailed study where cells are well separated. Cells in mitosis are much smaller, more rounded and basophilic than interphase cells. First, select several interphase cells for study using progressively higher power. Note the staining characteristics of the nuclei and cytoplasm. What in a cell
stains with a basophilic dye such as hematoxylin? Are the nuclei of some cells larger than average (~2X)? What might be the explanation for this? Note the fusiform or stellate appearance of the interphase fibroblasts. In the living state, these cells are quite motile, producing broad cytoplasmic processes (pseudopodia).

The kidney is an excellent organ in which to view simple squamous and cuboidal epithelial cells lining the ducts.

8. **Slide #26 [kidney, rabbit; PTS]**. Concentrating on the apex of this wedge-shaped tissue section, there are many examples of squamous and cuboidal epithelia. These two types of simple epithelia will be seen lining the tubules comprising the renal medulla. Most of the tubules are cut in cross-section, so that the cells are arranged around a central lumen. Because the slide has been stained using PAS and hematoxylin, the basement membranes underlying the epithelium (and outlining the individual tubules) can be readily discerned (it is purple). Identify the apical and basal surfaces of the epithelial cells based on the location of the lumen and basement membrane.

Some types of epithelia have specific names based on their location. The simple squamous cells that line blood and lymphatic vessels are called endothelium. Small blood vessels lined with endothelium (also simple squamous in structure) are found amongst the kidney tubules. However these are difficult to distinguish from the tubules unless you can see red blood cells in their lumen. Endothelium will be studied in more detail when the cardiovascular system is discussed.

The gastrointestinal tract is a classic place to view pillar-like, columnar epithelial cells, which cover the finger-like projections (villi) of the intestinal surface.

9. **Slide #28 [jejunum, monkey H&E]**. Observe the variation in appearance of the epithelial layer, which is caused by different angles of cutting. Identify the location of the basement membrane underlying the bases of the cells (since this slide is not stained with PAS, you can’t actually see it). Note the proximity of connective tissue and blood capillaries to the epithelium. The luminal surface of the cells appears “fuzzy” due to the presence of many tiny projections of the luminal plasma membrane. Also observe microvilli in figs. 1 & 6 of the “EM of epithelium” module of virtual histology. Look for simple tubular glands (intestinal glands) at the base of the villi (which are finger-like projections). Note that some of these will be cut longitudinally, while others will be cut in cross-section or obliquely. Look for mitotic figures in the epithelial cells lining the deep tubular glands (crypts). In this preparation mitotic figures are seen as deeply basophilic bodies (clumped chromosomes) in the central region of the cytoplasm.

The connective tissue in the core of the villi is comprised of loose areolar connective tissue. There are many cells within the connective tissue that have round nuclei and prominent chromatin. These are mostly plasma cells. The classic description of plasma cells describes them as having a “clock face” chromatin pattern in the nucleus with a basophilic cytoplasm and a clear area (Golgi) near the nucleus. See if you can find a
classic example of a plasma cell in this or the next slide. Plasma cells synthesize antibodies. Why would you want to manufacture antibodies in this location?

10. **Slide #37 [small intestine, hamster; P.A.S. + H]**. The lining cells of this piece of intestine are simple columnar. Examining the epithelial cells closely, the brush borders of these cells are distinctly “PAS positive” (stain purple/red). Why? Note the presence of many goblet cells in the epithelium (single cell glands secreting mucus). Again, look for the intestinal glands (simple tubular) as well as the loose connective tissue (with plasma cells) under the glands and in the core of the villi. With 40X magnification, observe the abluminal (non-villous) surface and note a delicate, thin layer of **simple squamous epithelial cells**. This may be artifically missing in some places in the section. Generally only the flattened nuclei of this layer will be seen; the cytoplasm is too attenuated to be seen in cross sections of these cells. This layer of simple squamous epithelium is part of the **visceral peritoneum (mesothelium)** that covers the surface of many of the organs within the abdominal cavity. The next two views will be of mesentery where two layers of mesothelium encase a small amount of loose areolar connective tissue.

**Mesenteries** are connections between the GI tract and the body wall. They are membranes that resemble a sandwich. Two layers of simple squamous epithelium are the bread, with loose areolar connective tissue as the filling of the sandwich (see the figure below). These are often viewed in a “whole mount”, where the mesentery is placed between the slide and the cover slip, and then stained in place. Therefore, you are looking across a rather thick specimen (top to bottom of the sandwich). Although you can see most things on the virtual histology site, in these thick sections it is useful to be able to focus up and down, thereby giving a sense of depth to the structures.

11. **Slide #30 [mesentery, rat; whole mount, silver stain]**. With careful focusing, the irregular outlines of the plate-like mesothelial cells (simple squamous) covering the surface of the mesentery can be discerned. These squamous cells are like fried eggs, with a nucleus in the center (yolk) and the edges (outlined in black by the silver stain) abutting one another.

12. **Slide #53 [mesentery, whole mount, resorcin-fuchsin]**. This is another view of mesentery, which is stained more for the connective tissue elements. It is covered on both sides by a layer of **mesothelial cells** (simple squamous epithelial cells), which may be seen if you focus up and down carefully. However, these cannot be easily seen on the virtual site where there is no ability to focus up and down. The slide is like looking at a translucent sandwich, with two layers of bread (the layers of simple squamous cells) and the filling (the loose areolar connective tissue). The largest nuclei that can be seen belong to the squamous mesothelial cells. Between the mesothelial layers on both sides of the piece of tissue is loose, irregularly arranged (areolar) connective tissue (see diagram below). Many **fibroblasts** and perhaps a few macrophages are present in the connective tissue. Fibroblasts have oval nuclei that are larger and have finer chromatin material than do the nuclei of macrophages. Often surrounding the dense irregular nuclei of the macrophages are dark particles, which the macrophages have phagocytosed into their
cytoplasm. Do not spend an inordinate amount of time trying to distinguish macrophages in this preparation, as you will see much better examples in later slides. A number of mast cells are also present. Their cytoplasm is usually so filled with metachromatic (purplish) granules that their rather small, round nuclei are often not visible. A small number of other cell types (e.g., white blood cells) not pertinent to this study are also present in this connective tissue.

In addition to cells, the areolar connective tissue contains fibers. Type I collagen fibers are visible as thick, eosinophilic/acidophilic (pinkish-purple) strands extending throughout the tissue. Elastic fibers are the much thinner, dark purple-stained, branching strands. (Blood capillaries, venules or arterioles also course through the connective tissue in most of the slides… we will study these in a later lab)

13. **Slide #80 [uterus, human, H&E]**. The uterus is lined with simple columnar epithelium that has to be regenerated after each menstrual cycle. Look for simple branched tubular glands in the endometrial lining of the uterus. Beneath the epithelial cells is a loose connective tissue containing many fibroblasts and white blood cells.

14. **Slide #39 [oviduct, human; H&E]**. This organ is lined with simple columnar epithelium, even though, in some tangential sections, it may appear pseudostratified. The presence of occasional, basally-located lymphocytes may give this appearance. These epithelial cells are different than in the intestine since they have cilia. What function does ciliated epithelium serve here? Also review the internal structure of cilia in figs 3 & 4 of the “EM of epithelium” module of virtual histology).

15. **Slide #29 [trachea, monkey; x-section, PTS]**. The lumen of much of the respiratory tract (including the trachea) is lined by pseudostratified columnar epithelium (sometimes just called respiratory epithelium). Note that all the surface cells have cilia at their free (apical) surface. What function do these cilia serve in the trachea? Mucus-secreting unicellular gland cells (“goblet cells”) are interspersed among the ciliated cells (these cells appear clear or “foamy” due to the fact that the cytoplasm is full of mucin, which does not
stain except with special stains. The epithelium is sitting on a basement membrane (not visible) that is supported by loose connective tissue.

16. **Slide #40 [epididymis, human, H&E]**. Notice that this organ also has pseudostratified epithelium, with projections into the lumen from the apical surface. However, in the case of the epididymis, these projections are **stereocilia**. What is the structure of stereocilia? See if you can make out the “terminal bars” located apically between contiguous epithelial cells. Remember, these are the specializations that hold individual epithelial cells in proper relation to one-another.

The most important stratified epithelium is “stratified squamous”. Remember, the epithelium is named by the shape of the most superficial cells not by the basal cells which appear cuboidal.

17. **Slide #32 [esophagus, rat; x-section, H&E]**. Give particular attention to changes in the character of the cells as you study the various strata proceeding from the **basal layer** toward the surface. The most superficial cells still contain visible, albeit, shrunken **pyknotic nuclei** and may retain some viability prior to their sloughing off. The human vagina is also lined by this type of epithelium. The epithelium rests on a basement membrane that is supported by a loose connective tissue containing many fibroblasts and wandering tissue cells, as well as blood vessels. There is also smooth muscle which we will study later.

18. **Slide #33 [thick skin, primate; H&E]**. Stratified squamous epithelium (keratinized) covers the dry surface of the skin. Note the pyknotic changes in the nucleus as one progressed toward the surface from the basal layers of the skin. Eventually, the nucleus disappears altogether. Keratinization is complete in the superficial cells, i.e., cells are devoid of nuclei, are filled with keratin and keratin-matrix proteins yielding dead, scale-like remnants of the epithelial cells. The thickness of this keratinized (cornified) layer of cells varies with the skin on different parts of the body. Where might you expect it to be thickest? Note that there are brownish granules in the cytoplasm of the cells of the deeper layers of the skin. This is yet another type of cell inclusion, termed melanin, which is designed to protect the nucleus from damage by UV radiation and also gives the skin its color.

Dense irregular connective tissue of the dermis is located beneath the stratified squamous epithelium (epidermis) of the skin. Both collagen and elastic fibers are present in abundance, but only the former can be seen in routine H&E preparations (elastic fibers don’t stain with H&E). Identify fibroblasts, recognizable by their dense-staining, oval to spindle shaped nuclei between collagen fibers. Deep to the dermis is the **superficial fascia** layer consisting of adipose (fat) tissue and less dense fibrous connective tissue. There is no sharp line separating dermis and superficial fascia.

Next, look in the deeper connective tissue (amongst the fat in the superficial fascia). Find **simple coiled tubular glands** (e.g., sweat glands of skin). Since these glands are coiled, you will see many cross-sectional (circular/oval) profiles. You cannot tell that these are a
single, coiled tube from studying a section like this, but their location tells you that they are sweat glands. You may see sweat ducts extending through the dermis and even the epidermis but will not be able to follow one all the way from the gland to the surface.

19. **Slide #58 [scalp, human, H&E] on the VIRTUAL HISTOLOGY SITE.** Examine the stratified squamous epithelium and the hair follicles. Adjacent to many of the hair follicles are sebaceous glands that empty their secretion into the follicles. These are holocrine glands, meaning that the cells themselves disintegrate and make up the oily secretion. Again look for the dense irregular connective tissue (between hair follicles) and the adipose tissue in the deeper layers of the skin. There is another dense connective tissue layer deep to the fatty layer.

20. **Slide #42 [bladder, monkey; H&E] contains two sections of bladder, one fixed in the non-distended state and the other in the distended state. Which is which? The epithelium here is “transitional”, meaning that it can appear squamous when stretched and cuboidal to round when not stretched. This is found in the urinary system, where it is often called “urothelium”.

21. **Slide #41 [submandibular gland, human; H&E] with your 10X objective.** The salivary glands are an example of a compound tubuloalveolar gland. This type of gland is much like a bunch of grapes, with a branching duct leading to a circular cluster of cells (an acinus) with a lumen. The cells secrete into the lumen and the duct carries the product to the surface. The submandibular gland contains serous acini, mucous acini and mixed seromucous acini (i.e., mucous gland cells with “serous demilunes”). Most of the acini in the specimen are serous (dark blue cells). The mucus acini have foamy appearing cytoplasm. The ducts are lined by cuboidal to columnar epithelium, and there are many blood vessels. See if you can find stratified columnar epithelium lining the lumen of the larger ducts. This is a rare type of epithelium. Note how denser connective tissue separates the gland into lobules and even sends delicate extensions in between acini, to provide support.

22. **Slide #77 [tongue, rabbit; H&E].** The tongue is covered with stratified squamous (nonkeratinized) epithelium with some unusual cells (taste buds) intervening (don’t worry about taste buds at this point). There is a loose connective tissue supporting the folds of the tongue (papillae). At deeper levels of the tongue, embedded among the muscles of this organ, are serous and mucous glands. (NOTE: some slides of this number have no mucous glandular units).

23. **Slide #51 [fetal pig head, H&E].** Primitive mesenchyme is a form of connective tissue that is most prevalent in embryonic life. In this slide it is best seen in the area between the skin and the developing cranium (see diagram below). Note the fusiform (spindle) or stellate (star) shape of these undifferentiated cells and the apparent high ratio of nuclear to cytoplasmic volume. The intercellular material that surrounds these cells consists of sparse delicate collagen fibrils and a viscous amorphous ground substance, neither of which can be seen well in this routine type of preparation.
24. **Slide #52 [Human, umbilical cord, H&E]**. The connective tissue consists of a specialized extracellular matrix whose ground substance is called Wharton’s Jelly. This contains **Type I collagen** and a mucoid ground substance containing much chondroitin sulfate and hyaluronic acid (it appears vaguely eosinophilic and darker near the fibroblasts). There are two umbilical arteries and an umbilical vein inside, with the cord surrounded by amnion with a simple squamous to very low cuboidal epithelium (see below).

25. **Slide #54 [ectocervix, Masson’s trichrome]**. This section has a stratified squamous (nonkeratinized) lining on one part that blends with a simple columnar epithelium (not easy to see with this stain). There are some branched tubular glands on the side with the columnar epithelium. These glands have high-cuboidal to low columnar epithelium. The bulk of the slide is **dense, irregular fibrous connective tissue**. The section was stained by the Masson’s trichrome technique (one of the so-called "connective tissue stains"). Epithelium and muscle stain varying shades of red to purple; collagen is green. Study the collagen bundles at 40X and identify the nuclei of interspersed fibroblasts. Note: there are also many blood vessels, which are more densely staining and the red-staining cells mixed in which the connective tissue fibers are smooth muscle cells.

26. **Slide #55 [tendon; H&E]**. This section of a tendon shows dense regular connective tissue. Ligaments and aponeuroses (a sheet or band of fibrous connective tissue
separating or binding together muscles) have a similar appearance. Note the rows of blue-stained fibroblast nuclei in between the longitudinally-oriented bundles of acidophilic collagen fibers (which have a slightly wavy appearance). Note the paucity of blood vessels in this type of connective tissue.

27. **Slide #56 [external ear, iodinated iron-hematoxylin - Verhoeff’s elastic stain]**. Find coarse, dark purple elastic fibers in the sub-epidermal connective tissue (dermis) and in the walls of large arteries. This slide also contains elastic cartilage, which will be studied in the next lab.

28. **Slide #57 [lymph node, silver stain]**. This is an example of the arrangement of reticular connective tissue. The reticular fibers are the rather delicate black-staining strands supporting the parenchyma and contributing to the perivascular connective tissue.

28. **Slide #90 [rodent spinal cord in situ; H&E]**. Brown fat can be seen in this slide near the spine. The fat cells in brown fat are multiloculated (i.e., have multiple fat droplets in them) and the cytoplasm is more eosinophilic due to the presence of a high concentration of mitochondria.

29. **Slide #60 [rabbit liver, H&E, injected with carbon particles]**. This slide demonstrates the fixed macrophages (Kupffer cells) lining the blood sinusoids of the liver. To make this preparation a rabbit received an intravenous injection of India ink (carbon) particles 24 hours before sacrifice. Sections of the liver from this animal display many Kupffer cells literally engorged with phagocytosed carbon particles. While this preparation is useful for demonstrating the abundance, distribution and obvious phagocytic capacity of these cells, the morphology of the macrophages is obscured by the carbon particles.

30. **Slide #61 [liver, human H&E]**. This is another section of liver (not injected with carbon). It is not as well fixed as the prior slide, but it shows many macrophages (Kupffer cells). They are found in the liver sinusoids between the simple cuboidal epithelial cells that make up the liver parenchyma. They are easily identified by the greenish-brown particles (most likely remnants of phagocytosed red blood cells) found in their cytoplasm.