INTRODUCTORY QUIZ & TECHNIQUES FOR STUDYING CELLS. 3313
See Chapter 1 - Ross & Rommel
Chapter 9 - “Visualizing cells” in Alberts 'Molecular Biology of the Cell' (4th edition). [Note: is Chapter 4 “How cells are studied” in some earlier editions].

Introductory Histology Quiz

What do you remember from second year?

What can you deduce by careful examination and discussion?

Working in groups.
- Examine and discuss the micrographs (1-12) set out for this session.
- Make a record of your conclusions for a class forum at the end of the session.

For each micrograph reach agreement about:-
- **What type of microscope has been used to prepare it?**
- **Which cell features are clearly discernible?**
- **What type of stain has been used?**
- **What tissue types are present?**
- **Try to identify the organ from which this photo has been taken.**
- **Identify any specifically labelled features.**
- **Make an estimation of the magnification of the photograph.** Either measure the scale bar with your ruler and then calculate the magnification e.g if a scale bar of 2um measures 20mm (20,000um) the magnification is calculated by dividing 20000 by 2 = 10,000. Alternatively, can measure the diameter of a nucleus (~5um) with a ruler and then calculate e.g if the nucleus (~5um) measures 2mm (2000um), divide 2000 by 5 = 400 times magnification.
- **Try and DRAW the tissue to show WHERE the transverse sections have been cut for #1&2 (similar pictures) and also #4. Try and visualise the whole tissue**

Cell dimensions and the need for light and electron microscopy
- The diameter of a typical animal cell is 10-20um and the nucleus is about 5um. A small dot made by a pencil on paper measures about 0.05mm (= 50um) and this is about 4 times bigger than a cell. Thus cells cannot be studied by the naked eye.
- 1um (micron or micrometre) = 10^-6 metres (m): therefore 1um = 10^-3 millimetres (mm), i.e 1000um (10^3 um ) = 1mm.
- 1nm (nanometer ) = 10^-9 m. 1um = 1000nm (10^3 nm), i.e 1nm = 0.001um.
- 1 Angstrom = 10^-10 m
- The study of cells is only possible with the help of microscopes. Because cells and tissues are soft and composed largely of water it is necessary to freeze or fix them in order to observe their organisation. Because cells are colourless and translucent, stains are needed to make the various structures visible.

The resolution of light microscopy is limited by properties of the wave length of visible light and the smallest objects that can be resolved are bacteria and mitochondria. (about 0.5um wide). Can magnify as much as you like e.g. by projecting onto a screen, but cannot get finer resolution (where 2 objects can still be seen as distinct) than about 0.2um with the light microscope.

The electron microscope uses electrons and can achieve far higher resolution. The practical resolution for a transmission electron microscope is around 2nm (= 0.002um or 20Angstroms) which is about 100 times better than the light microscope (0.2um).
Comments on the QUIZ

#1, #12, #13
ILEUM (453-464; p488 Ross, Romell & Kaye [R&R]; p218 Gartner & Hiatt). Cross sections of villi of the ileum.

#1
Light microscope. These are finger like projections of the mucosa that extend into the lumen giving it a velvety appearance - on the surface of the villi are microvilli (increase the area x600) and all are designed to maximise the surface area for absorption of digested material (from the lumen) in the end of the lower intestine. The CORE is an extension of the lamina propria with capillaries and lacteal (blunt ended lymphatics) beneath the basement membrane that separates it from the epithelium. The COLUMNAR EPITHELIUM is simple and tall and has microvilli on the lumen surface and these cells (enterocytes) absorb fat and other digested food from the lumen and transport it to the circulatory system. Among the epithelium are goblet cells that secrete mucus onto the surface of the lumen (they also have some microvilli). Lymphocytes move across these cells in both directions from the lamina propria to the lumen.

- Imagine the shape when cut in the other plane i.e. Longitudinally (LS) and compare the TS and LS views in your mind to reconstruct to 3D image of the tissue. Try to draw the 3D image.
- Where would the fluids moving though the gut and bathing these cells be located in the image?
- What is the benefit of the huge surface area provided by the microvilli?

#2
Similar view of ileum but stained with toluidine blue. Is a plastic resin section. 
**Magnification** Think of a nucleus as about 5 µm (0.005mm) in diameter. In the photo they are about 2mm (2000µm). Therefore divide 2000 by 5 = 400. i.e is magnified about 400 times.

A= brush border/ microvilli, B=core of lamina propria with vessel which could be a lacteal or a blood vessel, C=goblet cell, D=lymphocyte.

COMMENT ON FIXATION compare the histological detail with #1

#3
Electron micrograph of brush border. The apical (luminal) ends of 2 epithelial cells are shown with their microvilli on the surface, smooth endoplasmic reticulum in the cytoplasm and a desmosome junctional complex connecting the 2 cells. **Magnification** is about 50 x more than #12 (i.e width of brush border is about 50mm cf 1mm) therefore magnification is about 20,000.

Note: really high power EM view (x 85,000) of microvilli on p457, R&R.

WHERE LOCATED IN #1 & 2?

#4
PERIPHERAL NERVE: Cross section of myelinated and unmyelinated nerves and support Schwann cells (p264 -270, R&R; p112 G&H). Electron microscope (x50,000). A= axon with E = thick myelin sheath and C = peripheral cytoplasm of Schwann cell with
basal lamina separating it from the supporting connective tissue (P267 R&R). B = smaller axons of unmyelinated nerves lying within grooves but enclosed by the Schwann cell cytoplasm.

- What cell type makes the myelin sheath?

#5
LUNG: electron micrograph of type II alveolar cell with alveolar spaces and red blood cells. (p542-546 R&R). **Magnification** Estimation: nucleus measures about 30mm (=30,000 µm) therefore magnification is 6,000. Scale bar of 20mm represent 2µm, therefore magnification actually is 10,000. The type II alveolar cell (pneumocyte) has very distinctive multilamella bodies that make granules of phospholipid surfactant that are secreted into the alveolar air space, the cell has a dome shaped apical surface with some short microvilli. The surfactant covers the alveolar epithelium and reduces the surface tension and prevents the alveoli collapsing on exhalation. The alveolar epithelium is composed mainly of type I (95%) and type II (5%) alveolar cells. Connective tissue cells and several thin walled capillaries with RBC are present.

#6

#7
THYROID FOLLICLE Electron microscope view of colloid and cuboidal follicular cells (p603-606, p612, 630 R&R; p157 G&H). The follicular cells have lots of vesicles and ER. The colloid consists of thyroglobulin which is secreted by the follicular cells and is the inactive storage form of the hormones T4 and T3. Only after further cellular processing are the thyroid hormones liberated from the colloid and passed out to the capillaries. Apical pseudopods within the colloid show that the follicular cells are removing the colloid. The thyroid is unique amongst endocrine glands because it stores its secretory product extracellularly. **Magnification** Estimate gives about 8,000, scale bar gives 35,000 divided by 5 = 7,000.

#8
BLOOD CELLS Light microscope of blood smears stained with Wright’s stain (p188-207, p210-213 R&R; p71-73 G&H). A = Neutrophil (polymorphonuclear leukocyte - lobulated nuclei), B = Eosinophil (pink granules and sausage-shaped nucleus), C = RBC (erythrocyte - many of these, no nucleus, clear area is thinnest area of biconcave disk, long diameter is 7-8), D = Monocyte (large cell, acentric kidney shaped nucleus and lack of granules), E = Platelet (tiny bits of cytoplasm 2µm in diameter, no nucleus). x 1,000.

#9, #10
CARTILAGE. Longitudinal section through the trachea showing hyaline cartilage (blue) and the dense connective tissue of the perichondrium (pink) surrounded by epithelium (brown) (p132-138, p140-149, 177, R&R; 53, 61 G&H). #8 Low power (x 100) stained with Alcian blue, light microscope. Shows chondrocytes and matrix proteins. Note the pronounced lacunae around the chondrocytes due to the processing. In contrast in #10 in the EM (x 10,000) the lacuna is less pronounced and the chondrocytes are closely associated with the matrix.
#11
BONE Undecalcified ground compact bone, paraffin cross section treated with Indian ink to show the features x 100. (p150-169, 171 R&R; p56-59 G&H). Osteocytes in lacunae with canaliculi, the lamelli of deposited bone matrix and Haversian canals are shown

#12
COLLAGEN FIBRILS. Very high power EM showing the typical 64nm banding of collagen. (p96-104, p98, R&R) Magnification 100nm = 0.1µm measures 20,000µm (20 mm), therefore = 200,000.
#12,#13 see #1

#13
GUT Low power view through the large intestine showing the 4 layers that make up the wall of the colon. The mucosa (B), the submucosa, the muscularis externa (A & D) and the serosa. (p464-469, p491 R&R; p221 G&H) A = inner circular smooth muscle, B = the mucosa with tubular glands (crypts of Lieberkuhn) that extend to the muscularis mucosae, C = lymphoid tissue, D = outer circular smooth muscle (tenia coli)

#14
CILIATED EPITHELIUM on microvilli. There are no cilia on the epithelium in the gut but there are on the vas deferens and the fallopian tube and in the respiratory tracts (see p537 R&R). Scanning electron micrograph (x 400). Note the long cilia on some cells (all waving in the same direction at the time of fixation) whereas only short microvilli are present on other cells.

#15
JUNCTION OF STRATIFIED SQUAMOUS EPITHELIUM AND COLUMNAR EPITHELIUM.
Light microscope stained with Alcian blue and haematoxylin (x 400). Beneath the epithelium is loose connective tissue and glands. No cilia. Probably the cervix (Fig 4 p727 R&R) at the junction of the vagina (squamous) and the cervical end of the uterus (columnar -p723, R&R) (p267 - 271, G&H)

#16
EM OF MYOTUBE. Multinucleated young muscle fibres (myotubes) are formed by the fusion together of mononucleated muscle precursor cells (myoblasts). This process of myogenesis occurs during embryogenesis and also in adult animals regenerating after muscle damage.

Such fusion is an unusual event in biology. NAME 2 OTHER CELL TYPES WHERE FUSION OCCURS (trophoblasts, multinucleated phagocytes giant cells)