

Exercise 2: Microscopy and Cells

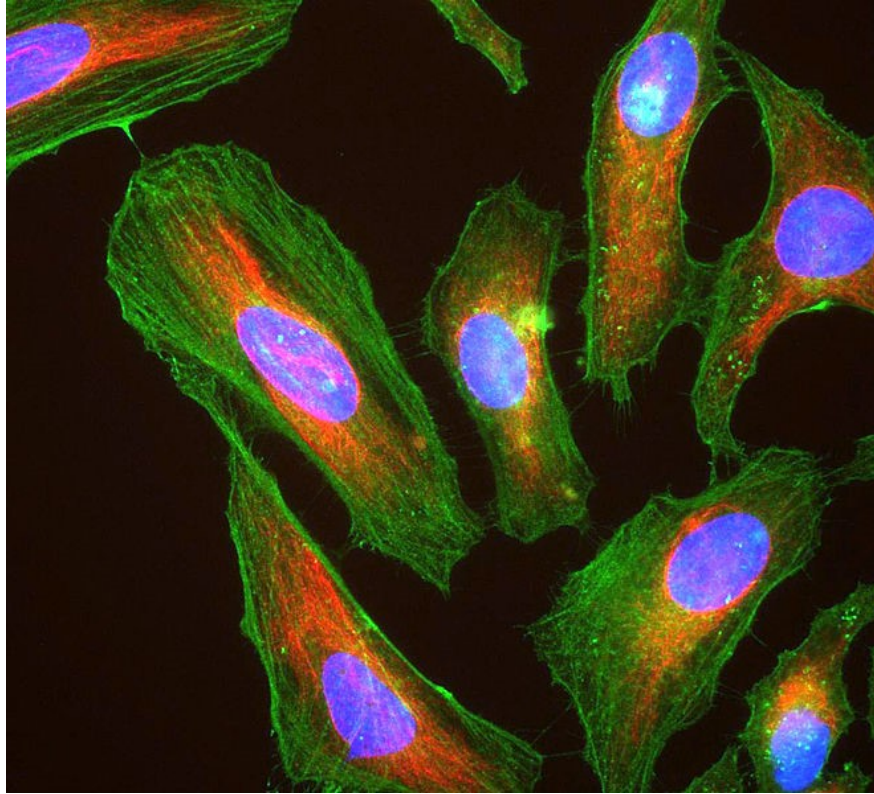


Figure 2.1 HeLa cells are a cell line used for cell culture experimentation from malignant cervical cancer of a woman who lived in the 1950s named Henrietta Lacks. In this image, cells are stained with blue red and green fluorescent dyes. The DNA appears blue, while the actin protein appears green.

Exercise 2 Learning Goals

After completing this lab, you should be able to:

- Define histology or microanatomy.
- Explain why using histology is important in your study of anatomy and physiology.
- Describe the metric conversions provided and where objects of various sizes, from baseball bat to ribosomes and proteins fall on a metric measurement scale.
- Describe at what level of the metric system are light microscopes useful for.
- Identify the parts of the light microscope and explain what they do.
- Describe the four macromolecules, their structure and function and be able to provide examples of each in the human body.
- Identify the parts of the eukaryotic cell.
- Describe the processes of osmosis and diffusion; explain how the process of osmosis can affect cell tonicity (hypertonic, isotonic, hypotonic).
- Describe the steps necessary to use the light microscope to view an object at low resolution and high resolution.
- Be able to estimate the size of objects in the microscope's field of view.
- Describe staining techniques and the color/ appearance of cell parts and protein using these different staining methods.
- Identify the extracellular (interstitial) space; describe the free edges and lumen in histological images.

Pre-Lab Exercise 2

Activity P1: Metric Conversions

Metric Unit	Description of Unit	Representation as a Fraction	Unit as a decimal of the base unit	abbreviation	Example
Meter	Base Unit of Measurement	1/1	1.0 m	m	A baseball bat; a doorway's width; an adult leg; guitar
Decimeter	one-tenth of a meter	1/10	0.1 m	dm	The length of a crayon; the width of your hand
Centimeter	one hundredth of a meter	1/100	0.01 m	cm	Width of a fingernail
Millimeter	one thousandth of a meter	1/1000 m	0.001 m	mm	Thickness of a credit card; paramecium
Micrometer	One millionth of a meter	1/1,000,000 m	0.000001 m	μm	Bacterial cell
Nanometer	One billionth of a meter	1/1,000,000,000 m	0.000000001 m	nm	Amino acids

Exercise P2.1. 1 Complete the following conversions:

1. 10 mm= _____ μm
2. 0.1 mm= _____ dm
3. 1 dm= _____ m
4. 1 m= _____ μm
5. 1 μm = _____ nm

What would be an appropriate unit of measurement for human height? _____

A human kidney? _____

Human red blood cell? _____

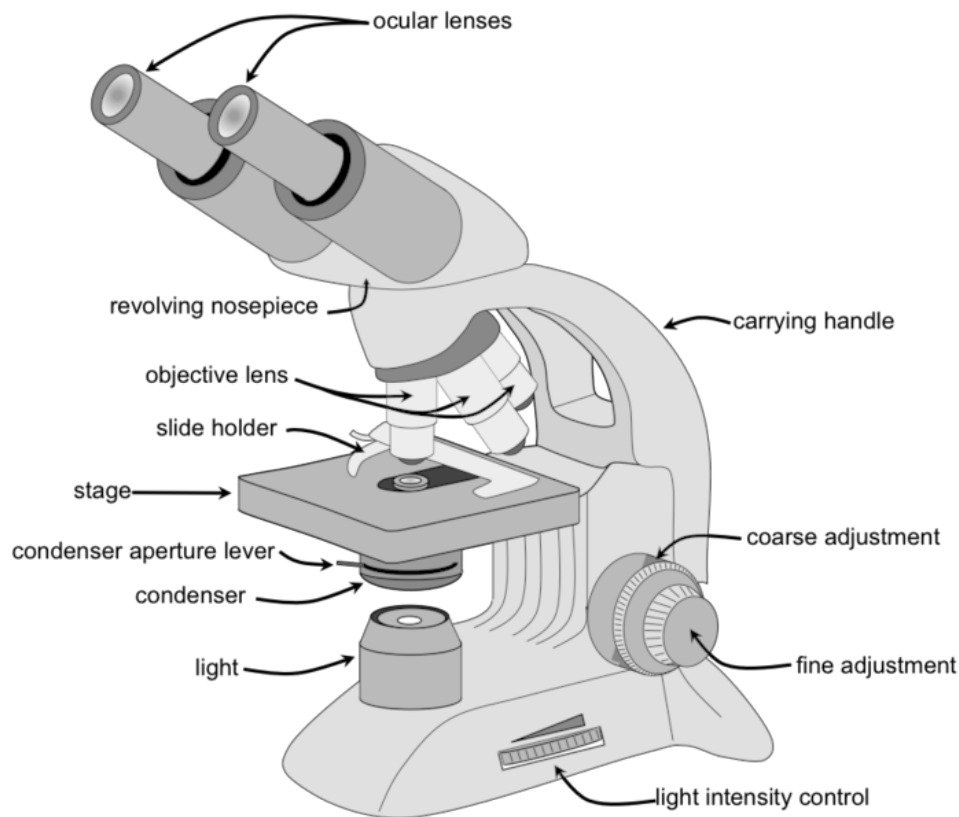
A ribosome? _____

Activity P2: The Light Microscope and Total Magnification

Histology, or microanatomy, is the study of cells and tissues at the microscopic level. There are different types of microscopes that are used for studying cells and tissues; these include scanning electron microscopes, confocal microscopes, and light microscopes. These types differ because they use different mediums to observe objects. The scanning electron microscope, for example, uses a beam of electrons to produce an image of the object. The

confocal microscope uses a laser to produce an image of the object and can give you a three-dimensional view of an object.

Light microscopes are useful for observing objects in the millimeter-micrometer range. Objects in this size range can still be seen using light waves. This includes the cells and tissues you will be studying in this course. Review the parts of the light microscope in the photo below.



In lab, you will use the light microscope to examine some eukaryotic cells. The eyepiece has a magnification of **10x**. The objective lenses have a magnification of 4x (scanning lens), 10x (low power), 40x (high power), and 100x (oil immersion). To find the total magnification of the object being viewed under the microscope, multiply the **magnification of the eyepiece lens** by the **magnification of the objective lens** being used.

Exercise P2.2.1 Calculating total magnification

Calculate the total magnification when the 4x objective is used:

Eyepiece _____ X 4x= Total Magnification _____

Calculate the total magnification when the 10x objective is used:

Eyepiece _____ X 10x= Total Magnification _____

Calculate the total magnification when the 40x objective is used:

Eyepiece _____ X 40x= Total Magnification _____

Exercise P2.2.2 Match the part of the microscope with the correct description of its function.

- _____ a. stage
- _____ b. carrying handle
- _____ c. light
- _____ d. objective lens
- _____ e. slide holder/ stage clips
- _____ f. condenser
- _____ g. condenser aperture lever/ iris diaphragm

- _____ h. coarse adjustment
- _____ i. fine adjustment knob
- _____ j. ocular lenses
- _____ k. base
- _____ l. stage adjustment knobs (not labeled)
- _____ m. revolving nosepiece

1. Lenses that rotate for use of different magnifications (4x, 10x, 40x, 100x)
2. The broad platform of the microscope that supports it
3. Allows for control of the amount of light that passes through the condenser; is useful for adjusting the contrast of the image
4. Supports the objective lenses
5. Knob that moves stage in the z-direction (up and down) in a coarse (high incremental) fashion
6. Knob that moves the stage in the z-direction (up and down) in a fine (low incremental) fashion, improving clarity/ resolution of image
7. Knob that moves the stage in the xy direction (front and back, left and right)
8. The platform that holds the slide
9. The metal clips/ prongs that hold the slide in place
10. Illuminates the object from below
11. Circular region which concentrates the light on the object
12. Handle for carrying the microscope
13. Lenses found with the eyepiece that give a magnification of 10X

Exercise P2.2.3: Critical Thinking

Why is histology important to the study of anatomy and physiology?

Activity 4: Macromolecules

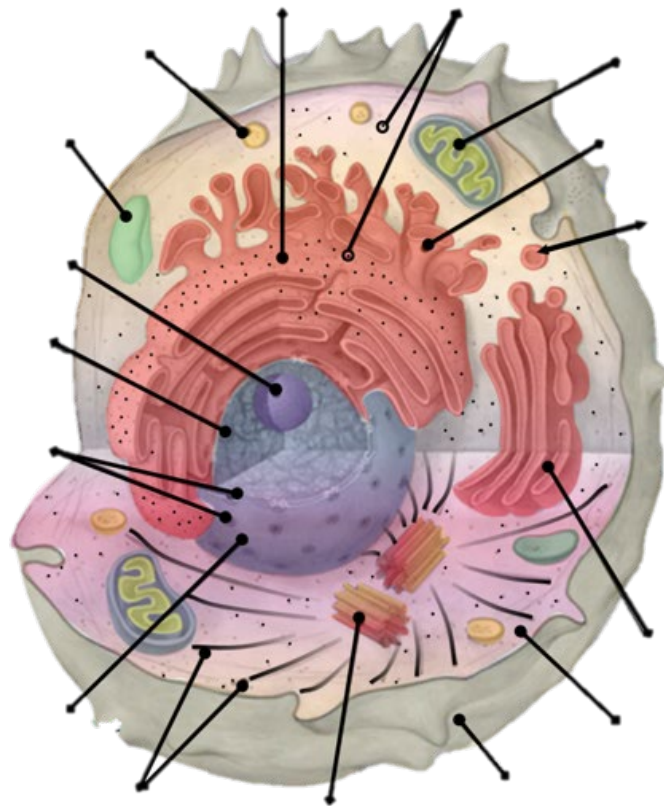
All living things are made up of organic molecules. Organic molecules are those that contain **carbon** and other atoms, such as **nitrogen**, **oxygen**, and **hydrogen**. There are 4 types of organic molecules that are the building blocks of all life. These include carbohydrates, lipids, proteins, and nucleic acids. As you learn about the cell (below), keep in mind that all cell parts are made up of these macromolecules. For example, the plasma membrane of a cell is made up primarily of lipids, the cytoskeleton is made up of different types of proteins, and ribosomes and DNA are made of nucleic acids. It is important to know and understand the structure and function of the macromolecules because all processes in the human body occur around these molecules.

Exercise P2.4.1 Fill out the chart below giving a brief description of the structure and function and examples of such molecules in the human body.

Molecule Type	Structure	Function	Examples
Carbohydrates			
Lipids/ Fats			
Proteins			
Nucleic Acids			

Activity P5: Parts of the eukaryotic cell.

Exercise P2.5.1 Using your textbook, or online resource, label the cell parts on the diagram below.



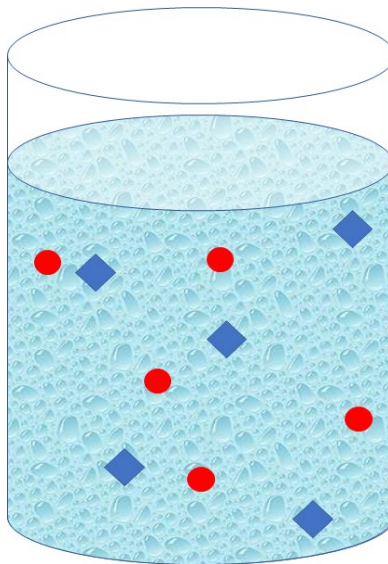
Exercise P2.5.2 Using your textbook or online resource, answer the following questions regarding the parts of the cell.

1. What is the plasma membrane? What is its function?
2. Where do you find the nucleus and the nucleolus? What is found in the nucleus of the eukaryotic cell?

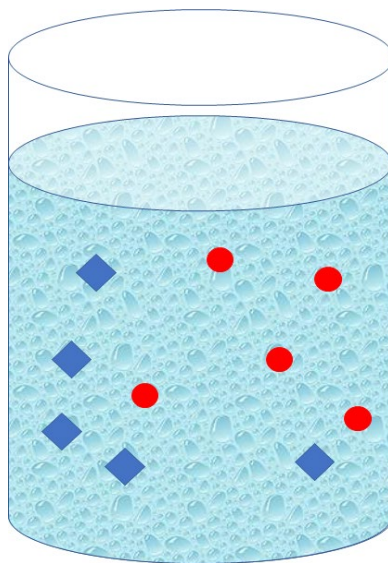
3. Describe the membranes within the cell including the smooth endoplasmic reticulum, the rough endoplasmic reticulum, and the Golgi apparatus. What is the function of each?
4. There are three types of fibers/proteins that help support the cytoplasm of the cell. List all three and give their major function?
5. What is the cell cycle? What is the purpose of mitosis and how does it fit in with the cell cycle? What are the stages of mitosis?
6. What is the purpose of meiosis? What are the stages of meiosis?
7. Define the following terms:
 - a. Diploid:
 - b. Haploid:

Activity 6: Osmosis and Diffusion

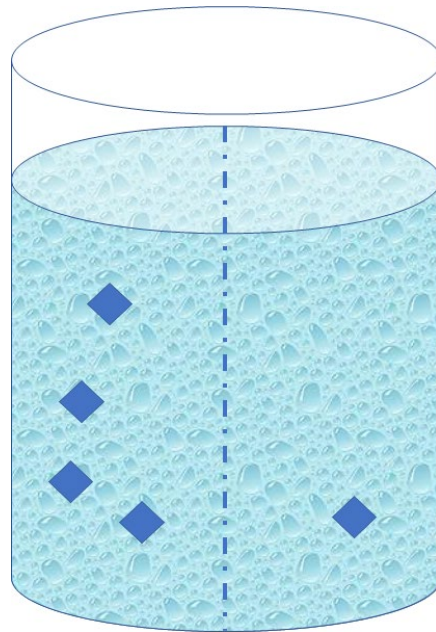
Exercise P2.6.1 Define and label the **solute** and the **solvent**:



Exercise P2.6.2 Define diffusion. Label the direction of movement, using arrows, of the solute from **high** concentration to **low** concentration in the image provided:

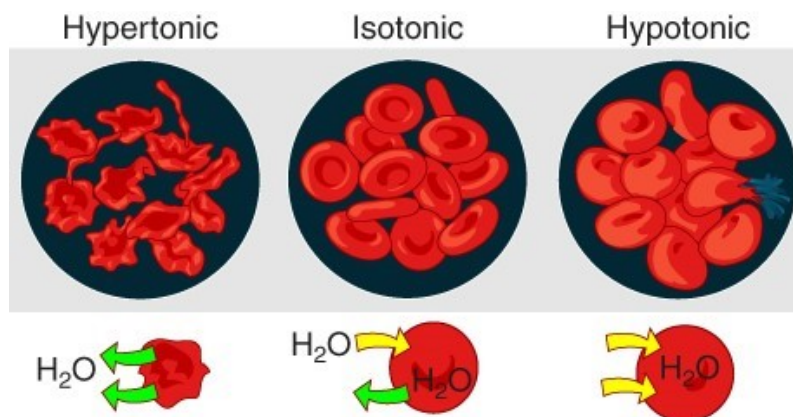


Exercise P2.6.3 Define osmosis. Label the direction of movement, using arrows, of water (solvent) from **low** solute concentration to **high** solute concentration across the semipermeable (permeable to water) membrane:



Activity 7: Tonicity

Any solution is made up of a solute, or solid (such as sodium chloride) and a liquid it is dissolved in, known as a solvent (such as water). Due to the process of osmosis, water will move across a semipermeable membrane (like the plasma membrane of a cell) in order to equalize the concentration of solutes on the inside of the cell with that on the outside of the cell. **Tonicity** describes the ability of the extracellular concentration to move water from the inside or outside of the cell. The states of tonicity, hypertonic, isotonic, and hypotonic describe the concentration of solute in the extracellular fluid (hyper= too much solute; iso=the same amount of solute; hypo=too little solute).



https://commons.wikimedia.org/wiki/File:0346_Concentration_of_Solutions.jpg

Human blood is a connective tissue that is made up of red blood cells, or erythrocytes, plasma (interstitial fluid), white blood cells, and platelets. The concentration of solutes, for example, sodium chloride, in the fluid surrounding red blood cells should be in balance with the concentration of sodium chloride on the inside of the cell. This condition is called _____. When there is *too much* sodium chloride (hypertonic) in the fluid surrounding the cell, water will leave the cell causing it to shrink, or shrivel. When there is *too little* sodium chloride in the fluid surrounding the cell, water will move into the cell, causing the cell to first swell, and then burst. Both the hypertonic and hypotonic conditions are dangerous to the body. It is important for the body to regulate the cell's extracellular environment. The kidneys remove excess water, salt, potassium and waste from the blood plasma. When the kidneys stop working properly, a person must go on **dialysis**, a procedure that filters waste from the blood.



[https://commons.wikimedia.org/wiki/File:Dialysis - arm - 01.jpg](https://commons.wikimedia.org/wiki/File:Dialysis_-_arm_-_01.jpg)

Exercise 2 Activities

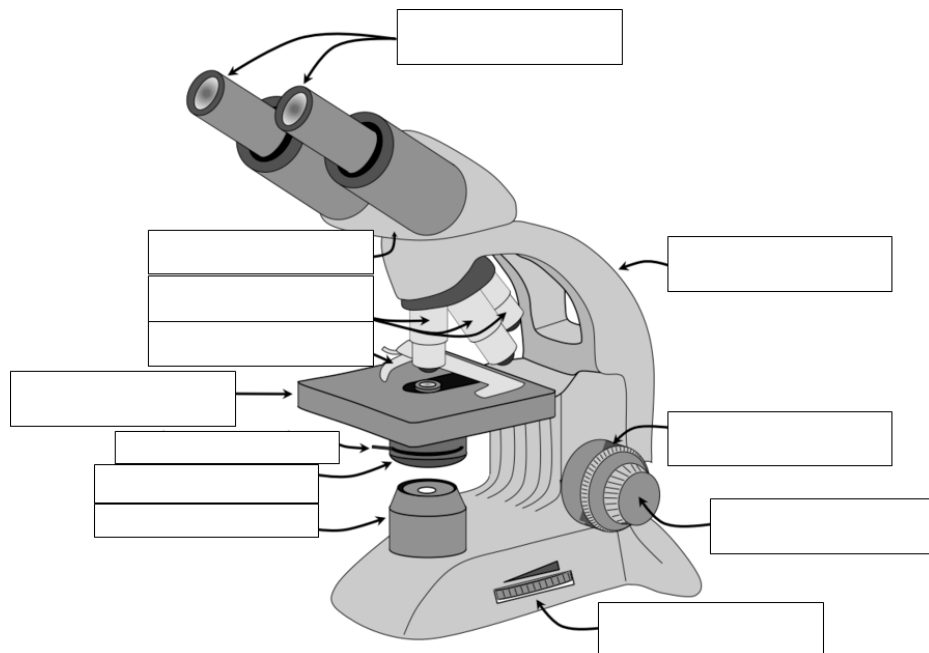
Activity 2.1 Using the Microscope

Using the Microscope

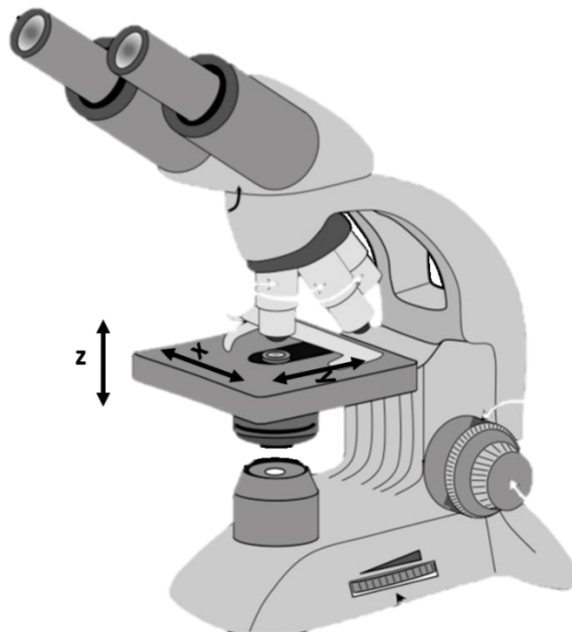
Before beginning, your instructor should review the following basic rules for microscope use and slide care:

1. Always carry the microscope by its arm in an upright position. Hold the free hand under the base of the scope.
2. When storing the microscope, make sure that the slide is removed and the lowest objective (4x) is in position and elevated relative to the stage.
3. Do not allow the lens of the objective to touch the slide. Doing so may break the slide and scratch the lens.
4. Never use the coarse adjustment knob with anything other than the scanning or low power objective.
5. Hold glass slides along their edges.
6. You can clean both slides and objective lenses using lens paper and lens cleaning solution.
7. Always treat the microscope with care and respect!

Exercise 2.1.1 With a light microscope at your table, identify the parts and their function; label the image and fill in the chart, and answer the following questions.



Part	Function
Eyepiece	
Carrying Handle	
Stage and stage clips	
Revolving nosepiece	
Objectives	
Light source	
Condenser	
Fine adjustment knob	
Coarse Adjustment knob	
Stage Adjustment Knob	(not labeled on diagram; locate this on the microscope)



Which knobs moves the stage right and left? How is this movement described in terms of an x, y, z, axis?

Which knobs moves the stage up and down? How is this movement described in terms of an x, y, z, axis?

Exercise 2.1.2 Observe the Slide “e” using the following steps:

1. Make sure the lowest objective lens (4X) is in position and use the coarse knob to move the objective as far up as it will go.
2. Place the slide on the stage, making sure it is stabilized by the stage clips (slide should not move unless the stage clips are moved. Stage clips can work differently: by being positioned on top of the slide, or by spreading apart, so that slide can be wedged between. Check with the instructor if you are not sure that your slide is stable.
3. Position the object on the slide over the light source/ under the objective lens. Then, using the stage adjustment knobs, look through the eyepiece to further position the object on the slide over the light source so that you can see it through the eyepiece. The object will likely be blurry or difficult to see.
4. Once the object is positioned correctly over the light source, use the coarse focus knob to move the stage in the z direction (up and down), until the object becomes a bit clearer.
5. Use the fine focus knob to improve the clarity of the image.
6. Once you have used the 4x to focus the slide, then you may move to the next highest objective (10X).
7. At the next highest objective, it may or may not be necessary to adjust the fine focus knob to improve the clarity/ focus of the image.
8. Once the image is clear, you may switch to the 40X objective and adjust the fine focus knob to gain clarity of the image.

Follow these steps with the slide labeled “e.” What do you notice about the “e” when looking at it through the microscope? What is its orientation? What is the effect of changing the objectives?

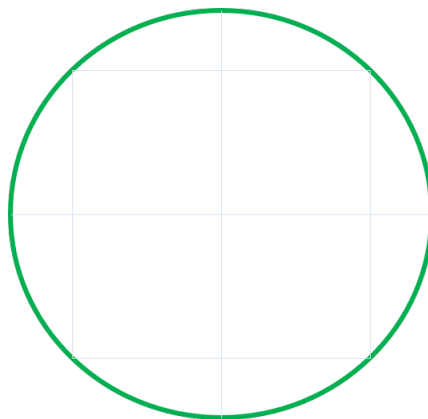
9. Repeat the process with the micrometer slide. Approximate the distance across the field of view for each objective.

Activity 3: Staining and Observing your cells.

1. Use a clean swab/ applicator stick to scrape the internal side of your cheek.
2. Using a clean glass slide, apply a drop of deionized water to the slide using a transfer pipette, and dip the swab in the water. Apply a clean plastic coverslip on top.
3. Use the steps above to observe your cheek cells. What total magnification is useful for looking at your cells? _____
4. Describe what you see (What is their general appearance? How many cells can you see? Are they touching? What is their shape? What type of cells do you think these are? What color are they?)

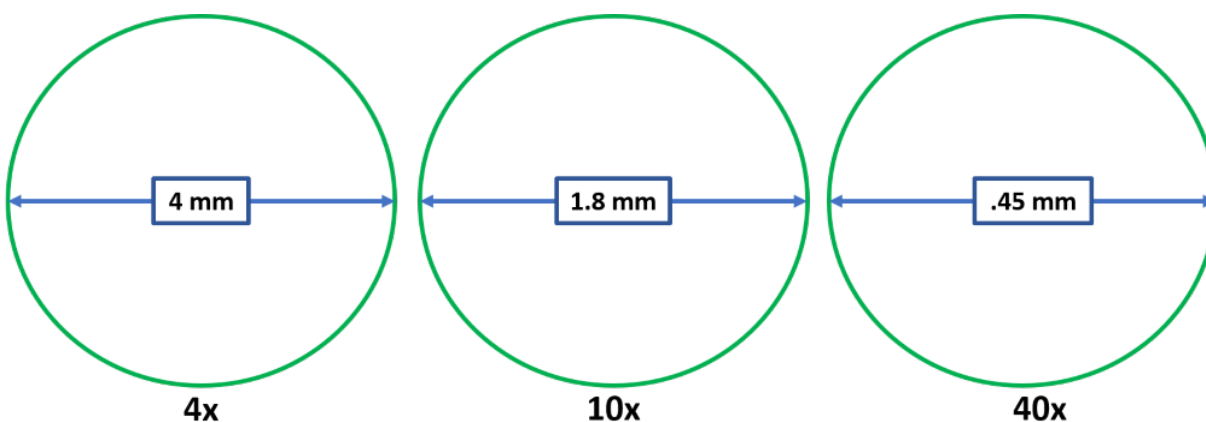
5. Carefully apply a drop of methylene blue stain to the edge of the coverslip.
6. View under the microscope. If you need to, start over from step one above.
7. Describe how the stain has affected your cells. What color is the cytoplasm? What color is the nucleus? Is it easier to see the cells now that they are stained?

8. Make a sketch of your cheek cells in the circle below. Make sure that your sketch is the appropriate size relative to the window of the objective lens. You will use your drawing in the next activity.



Activity 3: Estimating the size of your cheek cells.

Specimens viewed with a light microscope are typically measured using millimeters (mm) or micrometers (μm). The **field of view, or diameter that spans the length of the area visible** using the scanning objective is around 4 mm.



Exercise 2.3.1 Fill in the graph and calculate the size of your cheek cell.

Objective	Total Magnification	Field of View (mm)	Field of View (μm)
4x (scanning)	40x	4	4000
10x (low power)		1.8	
40x (high power)		0.45	

Considering the objective you used, calculate approximately how large your cheek cell is in mm: _____; in μm :_____.

Activity 4: Staining Techniques

Why are cells and tissues stained? As you saw in the previous activity, cells and their parts appear translucent/ clear and colorless under the light microscope. Staining cells and tissues helps to make cells, cell parts, and tissues more visible. Different types of staining include basophilic and acidophilic, H&E (**Haematoxylin and Eosin**), PAS (**Periodic Acid-Schiff Reaction**), **Masson's Trichome**, **Alcian Blue**, van Geison, Giemsa, Toulidine Blue, Silver Stain, Nissle and Methylene Blue, and various types of fluorescent dies can be used as well.

In the following images, label the cell parts and structures that are visible below. Note the differences in color that are based on the different types of stains that are use.

H&E staining

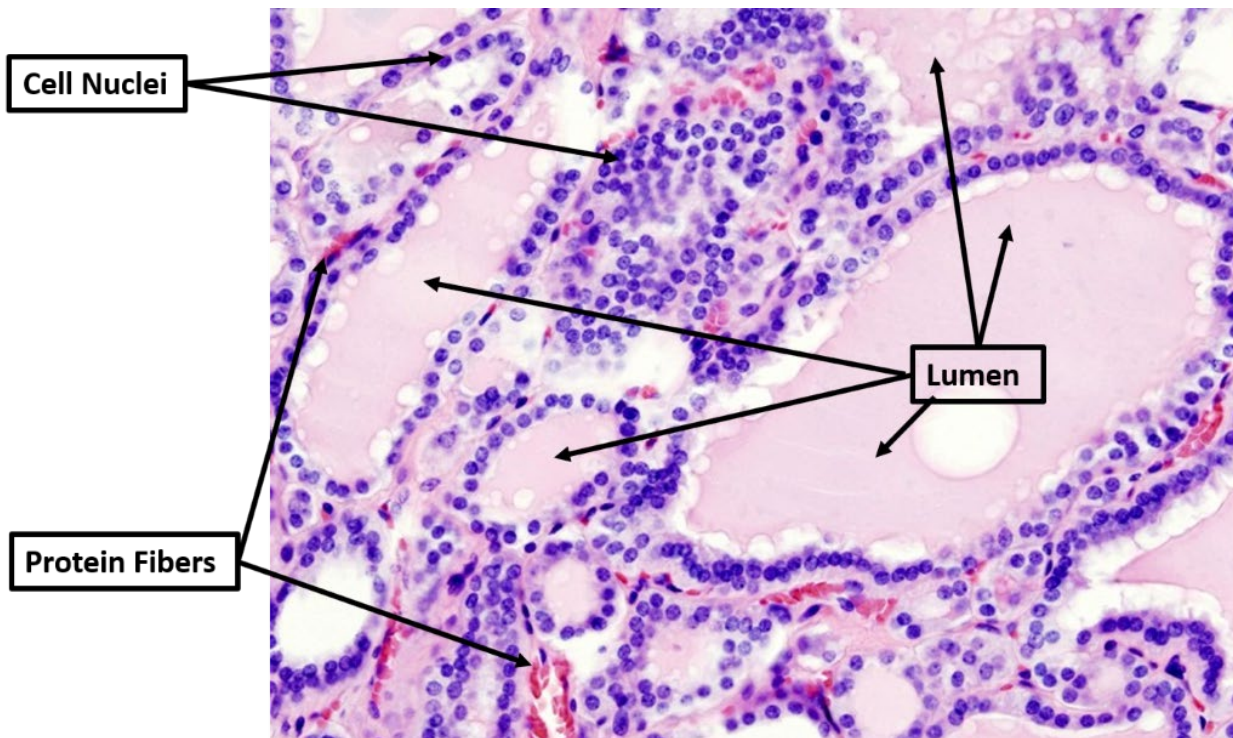


Figure 5 In this image, tissue from the thyroid gland has been stained using H&E staining. In an H&E stain, the nucleic acids within the cell are stained purple/ blue by Haemotoxylin, giving the nuclei a blue/purple color. The Eosin stains proteins pink. Spaces, or lumen, appear white. This image shows hyperplasia of the thyroid gland, indicating hyperthyroidism

[https://commons.wikimedia.org/wiki/File:Hyperthyroidism_\(1\).jpg](https://commons.wikimedia.org/wiki/File:Hyperthyroidism_(1).jpg)

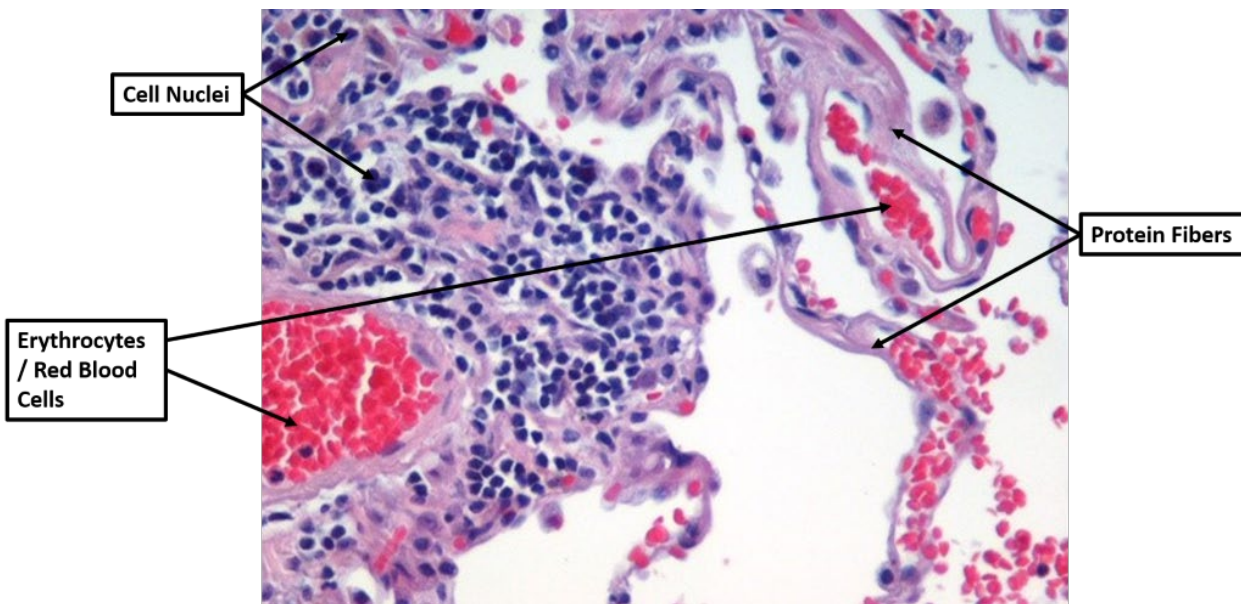


Figure 6 Another example of an H&E stain. This is lung tissue from a patient with Emphysema. In this image you can see red blood cells which appear bright pink. **Why do these cells appear pink and not purple?** The purple nuclei are from cells that line the bronchioles. These types of

cells, as you will learn about in the next lab, are simple squamous epithelial cells, good at absorption and diffusion for gaseous exchange. The light pink lines are protein fibers. Lung tissue must have a lot of elasticity in order to expand with each breath and return to its original shape; wavy pink lines indicate elastic fibers.

https://commons.wikimedia.org/wiki/File:Emphysema_H_and_E.jpg

In an H&E stain, nuclei appear _____ in color, while proteins appear _____ in color.

Giesma Stains are often used to stain blood smears from bone marrow.

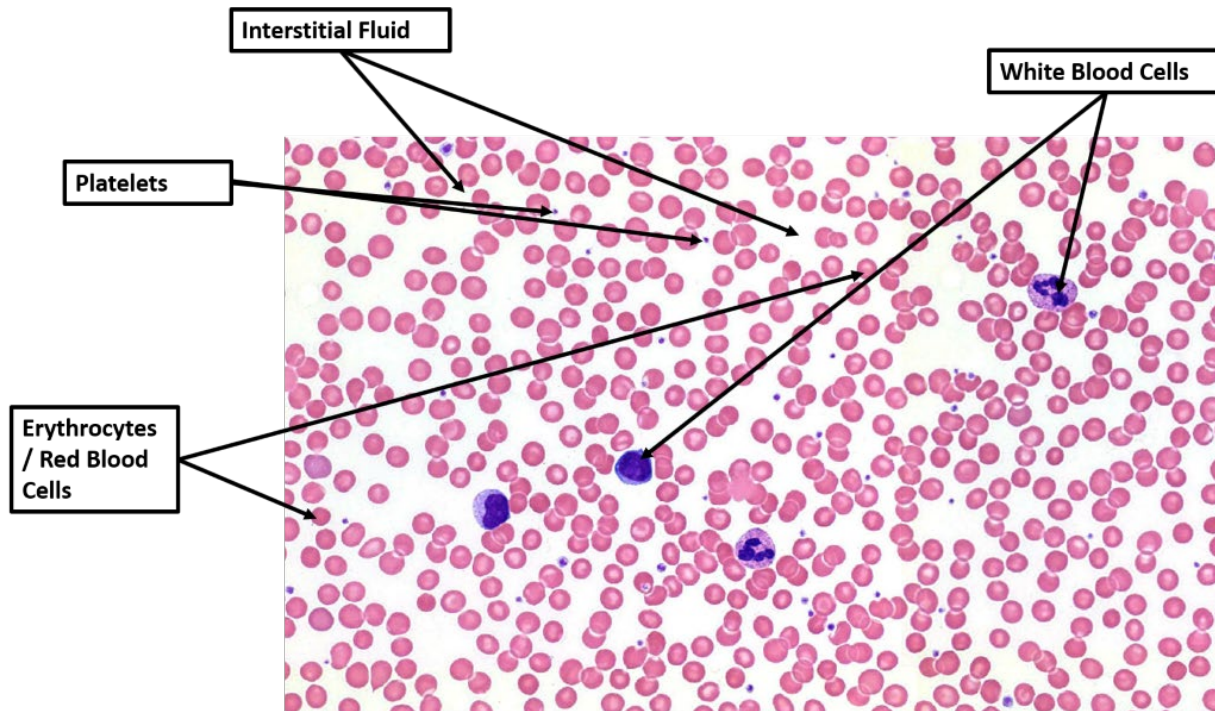


Figure 6 shows a normal blood smear with a Giesma stain. Red blood cells (erythrocytes) are prolific throughout this type of connective tissue and appear pink/red. White blood cells and platelets stain purple because they have a nucleus. White blood cells are large and contain a large dark purple mass (monocyte) or smaller dark purple globules within (leukocytes). Platelets are smaller than erythrocytes and appear purple also. Micrograph provided by the Regents of University of Michigan Medical School © 2012

<http://virtualslides.med.umich.edu/histology/Cardiovascular%20System/Hematology%20Lab%20Normal1%2063X.svs/view.apml?X=0&Y=0&zoom=4.77038310412574>

Sum it up:

In an Giesma stain, cells containing DNA appear _____ in color, while cells without DNA (red blood cells/ erythrocytes) and proteins appear _____ in color.

Silver Stain

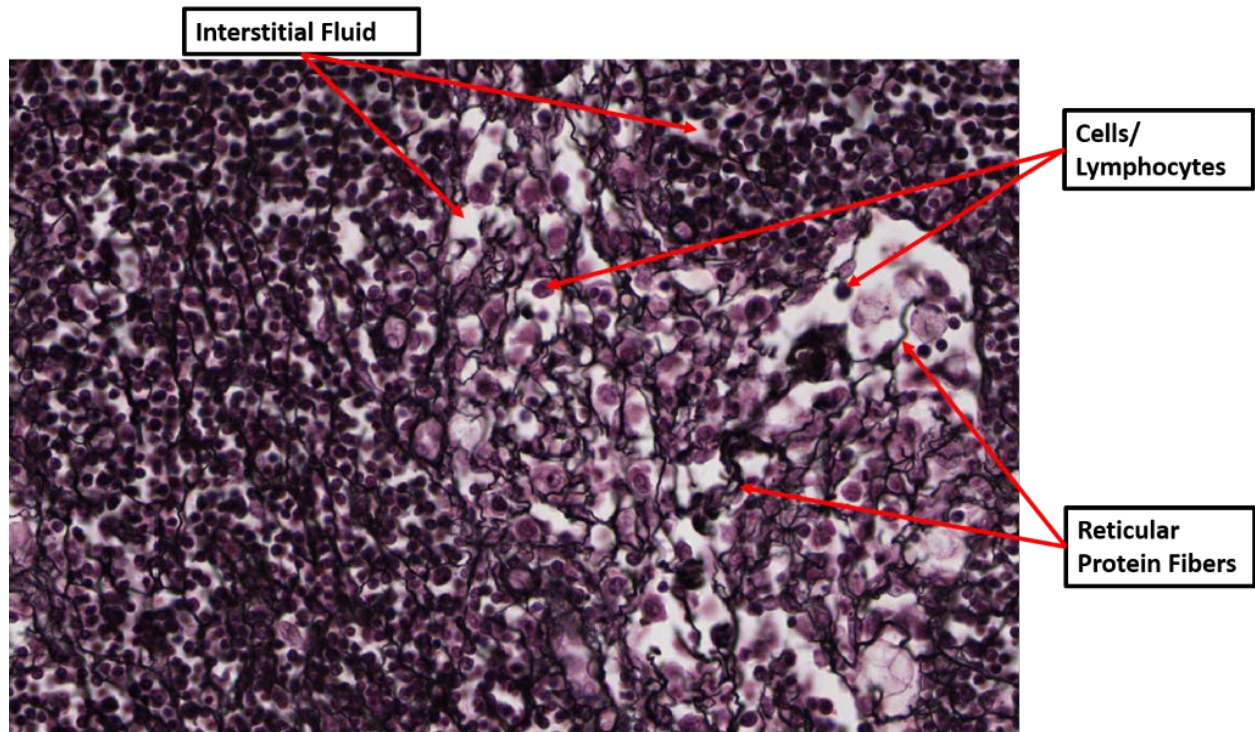
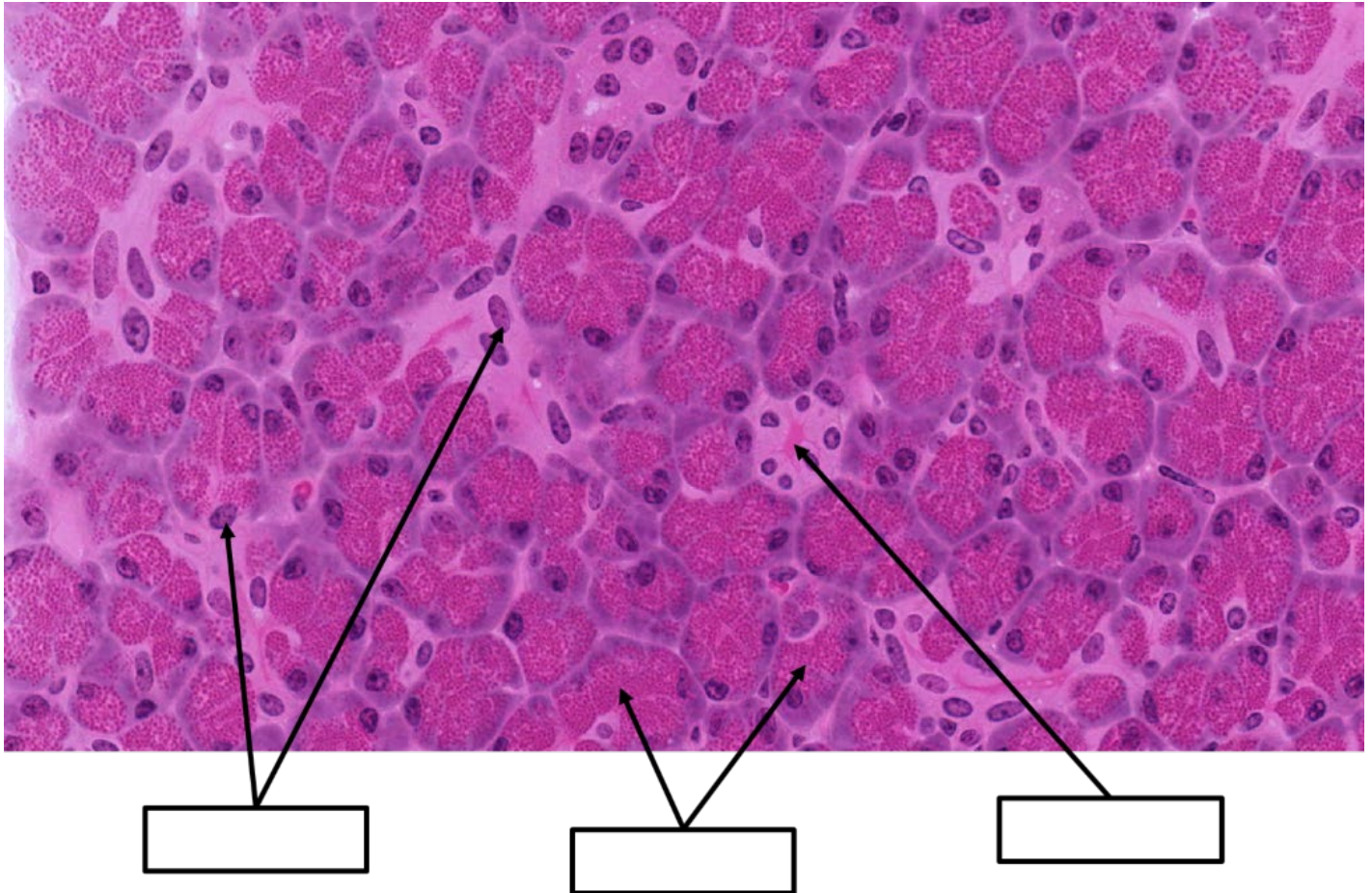


Figure 7 In the histological image above, a silver stain is used to show the structure of reticular tissue (a type of connective tissue). This tissue type is found in bone marrow, lymphatic organs, around individual smooth muscle cells, and beneath most epithelia (skin, for example). This image is from a lymph node. The lymphocytes (cells found in lymphatic tissue) appear light purple. Staining reticular tissue with a silver stain allows you to see the reticular fibers, which are the dark purple/ black lines. Reticular fibers are a type of protein that help give structure and support to the tissues. Micrograph provided by the Regents of University of Michigan Medical School © 2012

http://141.214.65.171/Histology/Basic%20Tissues/Epithelium%20and%20CT/028-2_HISTO_40X.svs/view.apml?

Exercise 5.1.1 For the following histological images, identify the cell, the protein, and the type of stain used. On each image, label one or more of the following: lumen, interstitial region, cell, nucleus, and protein fiber.

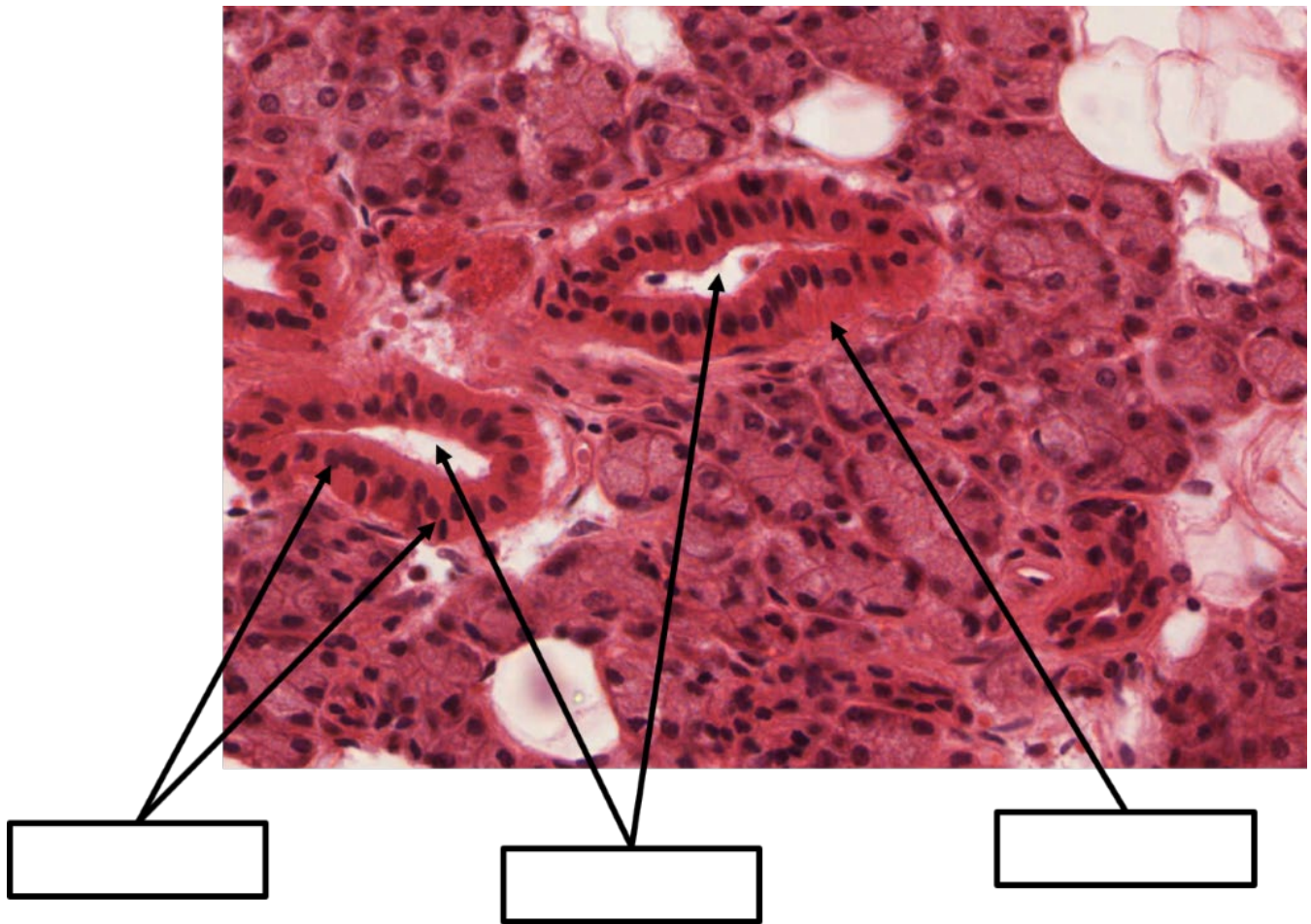
Image 1: Pancreas (Digestive/ Endocrine System)



What type of staining is used here? _____

Micrograph provided by the Regents of University of Michigan Medical School © 2012
<http://virtualslides.med.umich.edu/WesternUniv/98-9652.svs/view.apml>

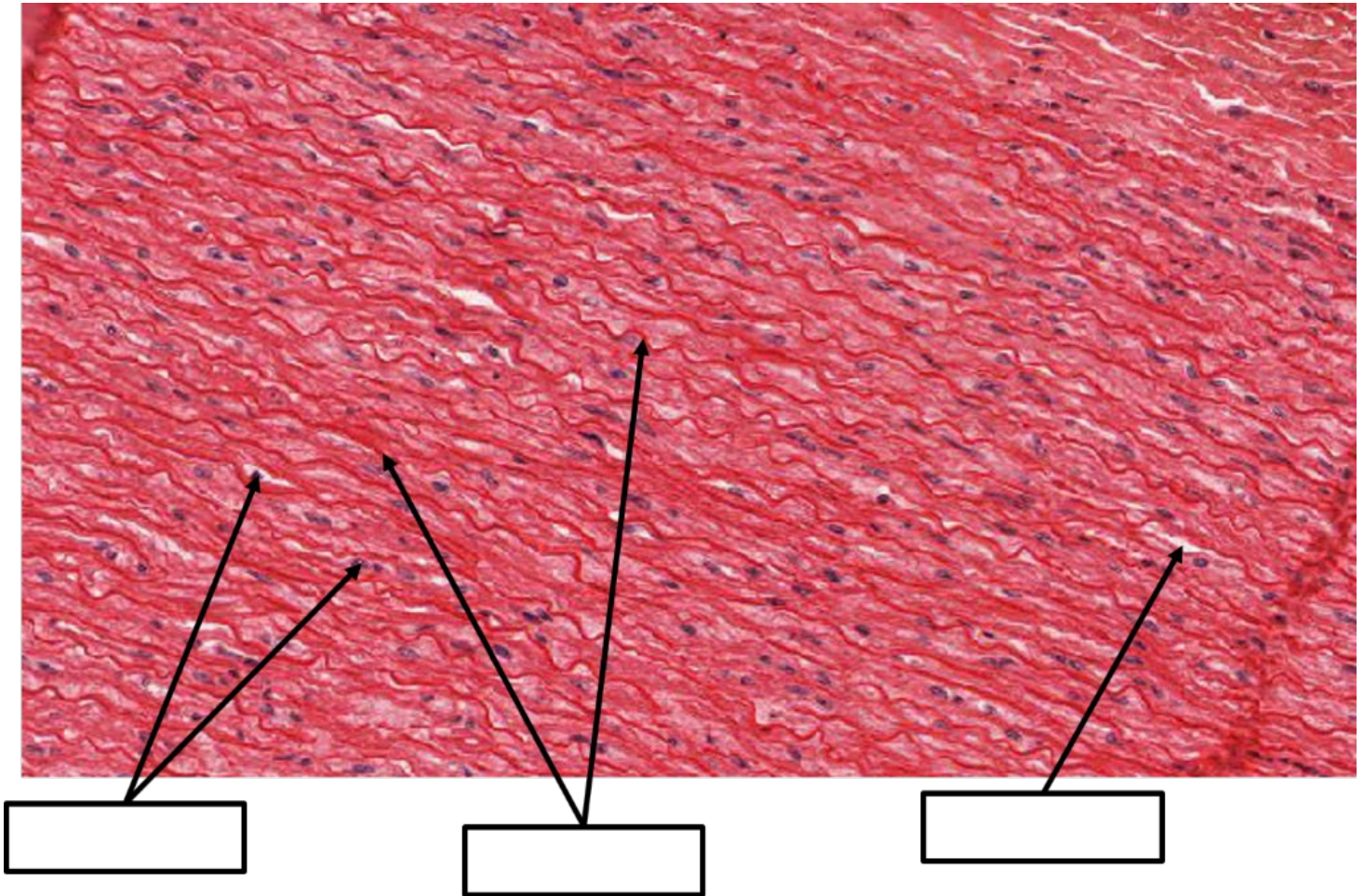
Image 2: Parotid Gland (Digestion)



What type of staining is used here? _____

Micrograph provided by the Regents of University of Michigan Medical School © 2012
http://141.214.65.171/Histology/Digestive%20System/Oral%20Region/180-1_HISTO_40X.svs/view.apml

Image 3: Elastic Connective Tissue from the Aorta (Cardiovascular)



What type of staining is used here? _____

Micrograph provided by the Regents of University of Michigan Medical School © 2012
http://141.214.65.171/Histology/Cardiovascular%20System/088_HISTO_20X.svs/view.apml?width=953&height=1025

Post-Lab 2 Review Questions

Answer the following questions:

1. What is the total magnification when the **scanning objective** lens is being used?
 - a. 10x
 - b. 40X
 - c. 100x
 - d. 4x
2. Which of the following is total magnification of the **high-power objective**?
 - a. 4x
 - b. 10x
 - c. 40x
 - d. 100x
3. What is the approximate size of a human cheek cell?
 - a. 6 μm
 - b. 60 μm
 - c. 6,000 μm
 - d. 6 mm
4. What part of the microscope helps to move the stage in the x-y (back and forth) direction?
 - a. The condenser
 - b. The coarse/ fine adjustment knobs
 - c. The scanning objective lens
 - d. The stage adjustment knob
5. Which macromolecule must have both an amino group (NH_2) and a carboxyl group (COOH) present on its monomer?
 - a. Carbohydrate
 - b. Lipid
 - c. Nucleic acid
 - d. Protein
6. Osmosis is the process by which water will move through a semipermeable membrane towards _____.
 - a. High solute concentration
 - b. Low solute concentration
 - c. High solvent concentration
 - d. Balanced solvent concentration

7. When a red blood cell swells, you can conclude that it is in a(n) _____ environment.
- a. Hypertonic
 - b. Hypotonic
 - c. Isotonic
 - d. Lysotonic
8. Which type of staining allows you to see reticular fibers?
- a. H&E
 - b. Silver
 - c. Giesma
 - d. Methylene blue
9. In an H&E stain, nuclei appear _____.
- a. Pink
 - b. Purple
 - c. Blue
 - d. Red
10. Which organ in the body regulates the concentration of solute in the blood, helping to expel waste when solvent level is too high?
- a. Pancreas
 - b. Thalamus
 - c. Stomach
 - d. Kidney