

2

Name : _____

Microscope / Cytology

Objectives :

1. Understand the difference between Magnification and Resolution
2. Identify the major microscopic components & demonstrate proper microscope use
3. Define and describe cellular structure
4. Describe structure and function of cellular organelles.

1A. Microscopy :

"The evolution of a science often parallels the invention of instruments that extend human senses to new limits." (Campbell 2002)

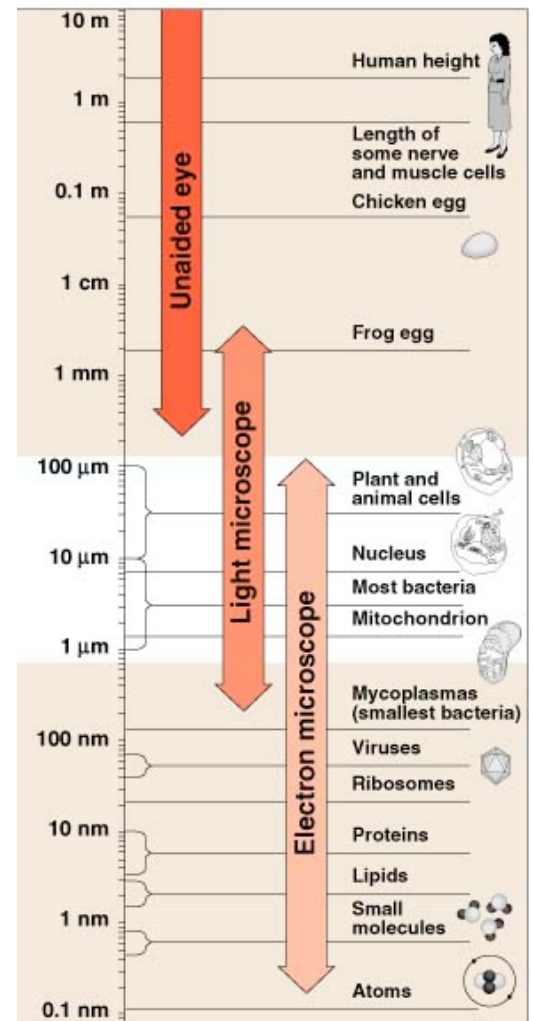
The microscopic study of anatomy is an excellent example supporting this statement. Without the aid of microscopes, our study and acquisition of knowledge about organisms and cells smaller than 0.1 mm (limit of the human eye) would never have been possible. The first microscope constructed and used for the examination of cells was the light microscope in 1665 by Sir Robert Hooke. He utilized the magnifying power of the simple yet powerful light microscope to describe for the first time the general structure of cells, symmetrical repeating patterns of cubes which reminded him of small monastery rooms called cells, hence the name. This began the wondrous journey into the unknown world of cells, launching the field of study known as *Cytology*. Light microscopes function by passing visible light through a specimen & series glass lenses. The lenses function to bend & change the course of the light as it passes toward the eye. The result is the production of a magnified specimen image. Light microscopes are effective in magnifying images up to 1000x. Even today, the light microscope is the most commonly used scientific instrument. It is for this reason that anyone entering into scientific study should become familiar with the use and care of microscopes. Remember, just as the invention of instruments can extend our human senses, the ignorance of their proper use can hinder scientific discovery.

1B. LIGHT MICROSCOPY :

Two important parameters of microscopy are :

1. **Magnification:** The enlargement of a specimen's image size beyond actual size or the ration of an object's image to it's actual size
2. **Resolving Power (Resolution):** A measure of the images clarity; it is the minimum distance that two points can be clearly seen as distinct and separate points.

The limiting factor of most microscopes is the resolving power. For example, light microscopes use light to illuminate images. The smallest wavelengths of visible light are too large to resolve images smaller than 0.25 micrometers (um). This means that when images are enlarged greater than 1,000x,



they will start to appear blurry. Therefore, the greater the magnification, the lower the resolving power. In order to clearly see objects smaller than 0.25 μ m, a media with a much smaller wavelength must be utilized. In the 1950's the development of the electron microscope incorporated the electron technology, focusing a beam of electrons through a specimen or onto its surface. Electron microscopes have the ability to magnify & resolve objects as small as 0.1 nanometers (nm) (Approximately a million times smaller than the period at the end of this sentence) and hundreds of times better than the light microscope.

1C. Components of the Microscope

The microscope is the most basic and widely used instrument in both clinical and research laboratories. In order to make the microscope an effective and useful scientific instrument; you should become familiar with the names and functions of the following microscope parts listed below and diagramed in figure 1:

1. **Ocular (Eyepiece)** : The last lens through which light passes before entering your eye.
The ocular lens has the ability to magnify images **10x**. Most of our microscopes have only one ocular lens (Monocular Scope) versus the traditional two ocular lenses microscopes (Binocular Scopes). The binocular microscopes are generally preferred to the monocular microscopes because they provide a greater perception of the depth of field
2. **Arm** : Supports the body and associated lenses.
3. **Base** : Supports the arm & provides stability to the microscope.
4. **Nosepiece** : Mounts the objective lenses to the body. By rotating the nosepiece, each objective lens can be brought into the path of light & used for magnification.
5. **Objective Lenses** : Function to differentially magnify images by specified degrees (10x, 43x, 98x).
 - a. Scanning (4x) - present on only some microscopes used in this class
 - b. Low power (10x) – used during initial focusing; used for searching and gaining an overview of the a slide or specimen, often low power is sufficient for most observations
 - c. High power or high dry (43x) - used when viewing greater detail is desired
 - d. Oil immersion (98x) - present on only some microscopes used in this class; increases the resolving power of the 98x objective by decreasing the refraction of light at the glass air interface of the slide & lens.
6. **Stage** : Flat surface below objectives upon which slides are placed for viewing
7. **Mechanical Stage** :
 - a. Mechanical Retainer Clamps (Stage Apparatus or Slide Holder) - clamps which hold the slide on the stage
 - b. Adjustment Knobs - two knobs which control the movement of the clamps thus providing a means of precise movement the slide over the stage
8. **Course & Fine Adjustments** : Focusing Knobs - move stage closer or farther from the objective lenses to bring specimen into focus. Microscopes contain parfocal lenses. This means that once an image is brought into focus through one objective, it will be in focus in all other objectives with just minimal fine focusing.
 - a. Fine Adjustment (focus) Knob (smaller inner knob on either side of arm) - moves stage in small increments
 - b. Course Adjustment (focus) Knob (larger outer knob, on either side of arm) - moves stage in large increments
9. **Condenser** : Adjustable sub-stage lens which functions to focus (or "Condense") light onto the specimen

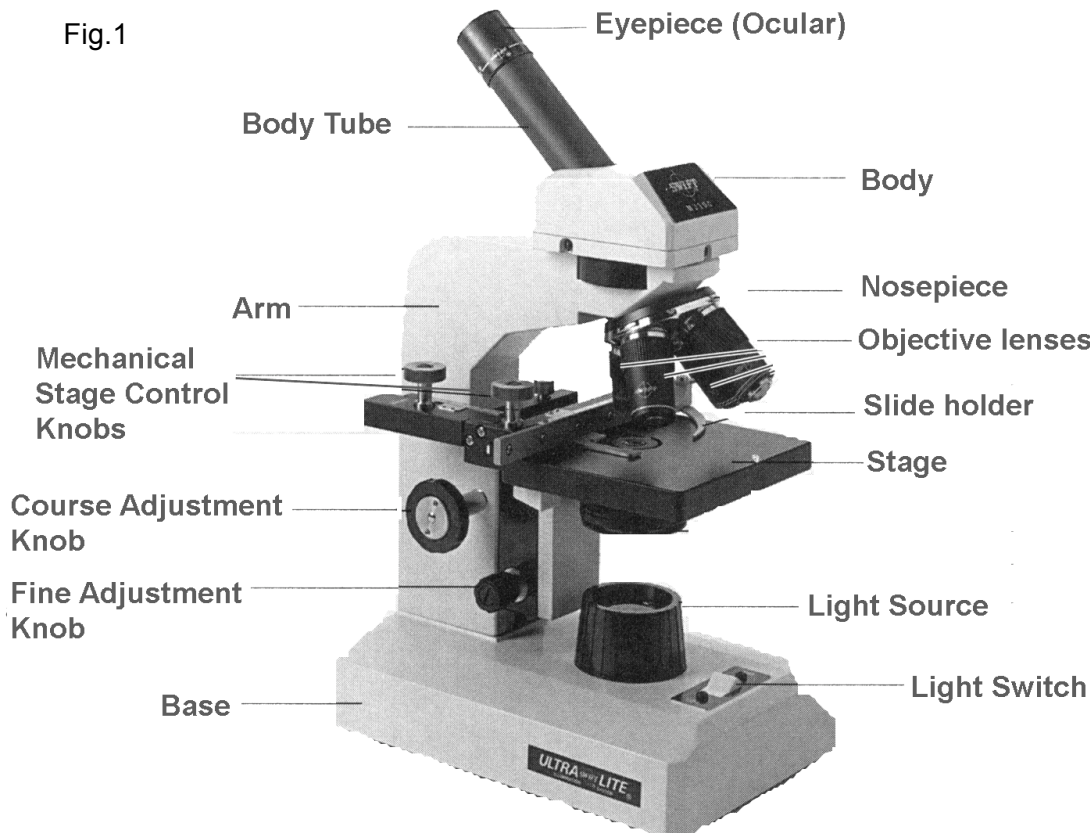
a. Condenser (Substance) Adjustment : Moves the condenser up or down, thereby focusing light.

The condenser is in relatively good focus when the condenser is positioned just slightly below the stage.

10. Condenser or Iris Diaphragm : Adjustable sub-stage opening which functions to control the amount of light illuminating the specimen. By adjusting the condenser diaphragm an images' *contrast* can be increased or decreased. Decreasing the condenser diaphragm is essential when viewing specimens which are not highly pigmented and have little contrast.

Listed on the next page are the main microscope parts. You should be able to locate and describe the function of all microscope parts listed.

Fig.1



1D. Handling and Care :

The microscopes in this laboratory are stored in numbered cubby holes corresponding to the microscope's numbers and in the cabinets at some of the student desks. Microscopes must be returned to their assigned cubby upon return. The following is a check list detailing the manner in which the microscopes should be handled and returned.

1. Always carry the microscope with two hands close to your body
2. ONLY *clean* microscope lenses (objective lenses) with LENS paper (stored in lab table drawer)
3. When putting away the microscopes :
 - a. Remove all slides from the stage
 - b. Move lowest power objective into viewing position
 - c. Move stage down (away from the objective lenses) with the course adjustment
 - d. Center the stage
 - e. Coil electrical cord around *ocular lens* and *course focus adjustment knob*.
 - f. Return microscope to correct cubby

★ *Each student is responsible for his/her own microscope.*

1E. Initial Focusing and Correct Use of the Microscope :

In order to effectively use the microscope it is important that the user be able to find and focus on images with relative ease. The following is a list of steps which should be followed EVERYTIME you put a slide on the stage. Using these simple steps will eliminate most of the frustration which generally accompanies microscopy.

1. Plug microscope into closest outlet, position cord so that it does not hang over the edge of the table.
2. Turn on microscope by rolling the “on switch” backwards (or push on button).
3. Make sure the lowest power objective is in the viewing position.

(ALWAYS START WITH THE *LOWEST* POWER OBJECTIVE)

4. Secure the slide to be viewed in mechanical retainer clamps (stage apparatus). Be sure that the slide's inferior surface is not wet, this will inhibit the slides movement.
5. While looking from the side, center the slide over (or the specimen to be viewed) the stage aperture (opening).
6. Using the course adjustment knob, move the stage upward toward the objective lenses (if using low power objective the slide will NOT hit the objective lens)
7. In order to focus, Look through the ocular lens and use the COURSE adjustment to move the stage DOWNWARD (away from the objectives) continue moving the course adjustment until the image comes into focus, then use the fine adjustment knob to make any minor adjustments in clarity. If you do not see the image come into focus, you have probably moved past the focal point. Reposition the stage to its initial position (moved all the way up) and start over.

(ALWAYS FOCUS AWAY FROM THE OBJECTIVES)

8. ONLY when a specimen is in focus on low power should you then switch to a higher magnification. Use the FINE adjustment ONLY to sharpen the image.
9. ONLY use the *FINE adjustment* whenever you are using a *HIGH power objective* or oil immersion lens.
10. ONLY use lens paper on objective lenses.

★ *Each student must be familiar with the steps used in initially focusing*

1F. Important Terminology :

Like any instrumentation, the light microscope has specialized features and properties which must be defined. The following is a list of features and properties which are important for the understanding and correct use of a microscope.

1. **Magnification** : When viewing images through a microscope, it is important to be able to convey how much larger the image is than the actual specimen size. By convention the magnification is always expressed as the “Total magnification” of the scope (not the magnifying power of the lens being used). The total magnification of the microscope can be easily determined by multiplying the magnifying power of the ocular lens (10x) by the magnifying power of the objective lens being used (4x, 10x, 43x, 98x).
i.e. : The total magnification of the microscope (image) when using the 43x objective is 430x.
2. **Field (Field of view)** : The area of the slide which is visible when viewing through the microscope. The size of the field of view decreases with increasing magnification.
3. **Depth of Field** : The clearly visible distance *into* the slide (remember a slide is 3 dimensional). The depth of field decreases with increasing magnification.
4. **Parfocal Microscope** : Microscope designed to allow changing of the objectives without significant changes in focus.

5. Working Distance : The distance between the specimen and the objective lens.
6. Wet mount slide : A temporary slide preparation where the specimen is suspended in a liquid (often under a cover-slip)
7. Prepared Slide : A permanent slide preparation where the specimen is fixed and bonded under a cover slip

1G. Image Orientation

When using a microscope it is important to be aware of the relationship between specimen and the image's orientations. Because light microscopes pass the image of the viewed specimen through several lenses the image of the specimen and the actual orientation of the specimen are different. Light microscopes function to invert the image into a position which is ultimately *upside down and backwards* relative to the actual specimen. You will be asked to confirm this relationship between image and specimen orientation.

1H. Estimating Field Diameter & Depth of Field & their Relevance to Magnification

Microscopy is a crucial component of the study of anatomy. Much of the evaluation of anatomical structure begins at a cellular level where the relationship between the size of structures is an important observation. In order to be able to effectively use the microscope as an observational and evaluative tool you must be able to approximate the size of the specimens being examined. This can be easily accomplished with the approximation of the field of view diameter. Once the field of view diameter is known, specimen size can also be estimated. This activity will be a part of the laboratory exercises you will be asked to complete.

In addition, it is important to understand how the magnification alters both the field diameter and depth of field of the images viewed. As already discussed, as the magnification of the microscope increases both the field diameter and the depth of field will decrease. In essence you will be able to see less of a specimen but what you see will be larger. Because the field of view will be much reduced when using the higher power objectives, it is important to estimate the field diameter under the low and high power objectives. This too will be an exercise you will be asked to complete.

2A. Introduction to Cell Structure:

Note: This introduction to cell structure is a review. You should already be familiar with this information. If the following information is new, then you should review the general cell structure in your anatomy text.

Cells were first described in 1665 by the British scientist Robert Hooke. Hooke observed the small compartments comprising the cork specimen he was viewing and called them *cells*. Since then scientists have been racing to understand and describe these smallest units of life. We now understand that the cell is in fact the *basic structural & functional unit* of life. From the unicellular bacteria which can invade our tissues to the estimated 50 - 100 trillion cells which comprise our bodies, cells comprise all living things. Multi-cellular organisms, like ourselves, are composed of over 200 different cell types. These different cell types are then organized into cooperative groups of similar cells in order to perform specific functions. The cooperative groups of cells are called *tissues*, of which there are *four primary tissue types*: 1. Epithelial, 2. Connective, 3. Muscular, & 4. Nervous. From these four tissues all organs in our bodies are constructed. Although the body is comprised of many specialized cells, every cell has the same basic needs and therefore has the same basic or generalized structure. A generalized or composite cell will be used in describing cell structure.

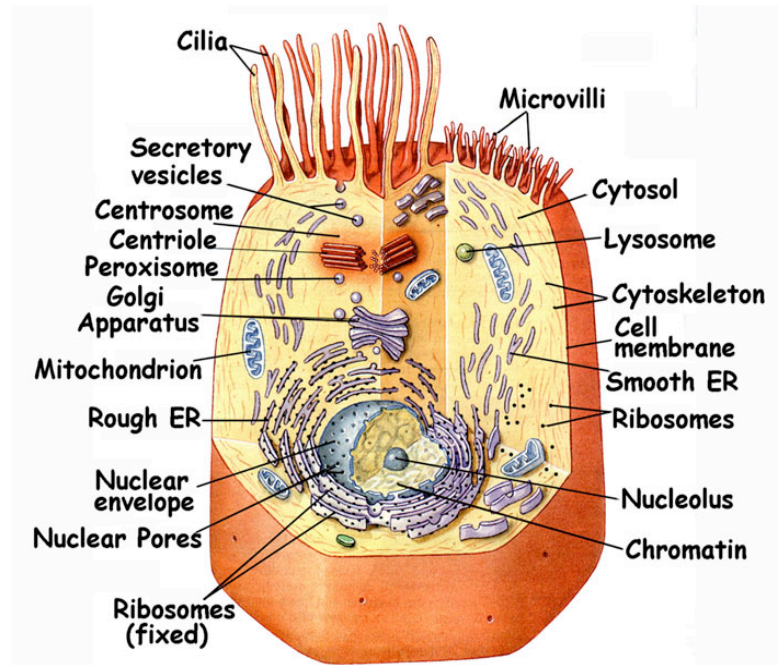
You will begin to study the basic structure and components of single cells : Cytology, and later how these cells are organized into tissues : Histology.

2B. Cytology : *cyto* = “cell” ; *logos* = “study of”

Although mature cells perform extremely specialized functions, each has basic physiological needs which translate into a similar anatomical structure or “structural plan”. As you observe the cells which comprise the four different tissue types, look at the different cellular morphologies as these will prove useful in understanding their function. When using a light microscope, you will only be able to discern the cell membrane (plasma membrane) and the darkly staining nucleus. You will be unable to resolve the sub-cellular structures present within the cell’s membrane.

Hint : In order to discern cellular structure it is often helpful to use the *nuclear shape* as an indicator for the entire *cell shape*. For example example : Squamous (flattened) cells not only have a flattened cell shape, but also a flattened nucleus. Cuboidal (square) cells have a rather round nucleus, similar to the cell shape.

Although with the use of a light microscope you will not be able to see the sub-cellular organelles which perform all of the cell’s functions you should be familiar with their structure and functions. Diagramed are all relevant structures followed by a list of the major major organelles & their generalized structure & structure & functions.



Organelle	Composition	Function
1. Cell Membrane	Lipid bilayer, containing phospholipids, steroids, and proteins	Isolation, protection, sensitivity support, control of entrance/exit of materials
2. Cytosol	Fluid component of cytoplasm	Distributes materials by diffusion
3. Cytoskeleton	Proteins organized in filaments Microtubules & Microfilaments	Strength and support, movement of cellular structures & materials
4. Microvilli	Non-mobile membrane extensions containing microfilaments	Increase surface area to facilitate absorption of extra-cellular materials
5. Centrioles	Microtubule subunits	Essential for the movement of chromosomes during division (form the spindle apparatus), organize cytoskeleton
6. Cilia	Mobile membrane extensions containing microtubules	Movement of materials over cell surface
7. Ribosomes	RNA and protein structures (attached to endoplasmic reticulum & free in cytoplasm)	Protein synthesis

8. Endoplasmic Reticulum (Rough)	Network of membranous channels with attached ribosomes	Modification and packaging of newly synthesized polypeptides
9. Endoplasmic Reticulum (smooth)	Network of membranous channels (cisternae)	Lipid and carbohydrate synthesis without ribosomes attached
10. Golgi Apparatus	Stacks of flattened membranes (sacculles)	Storage, alteration, and packaging of newly synthesized proteins
11. Lysosomes	Vesicles containing digestive enzymes	Intracellular removal of damaged organelles &/or pathogens
12. Peroxisomes	Vesicles containing degradative enzymes	Catabolism of fats and other organic compounds
13. Mitochondria	Double membraned structure filled with metabolic enzymes	Converts 95% of organic energy into cellular energy (ATP)
14. Nucleus	Double membraned structure containing cellular DNA organized into chromosomes	Storage and processing of genetic of genetic material

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Name : _____

Microscope / Cytology

Laboratory Activities :

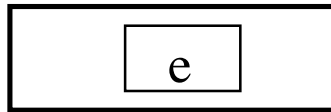
1C. Components of the Microscope

The microscope is the most basic and widely used instrument in the life science laboratory. In order to make the microscope an effective scientific instrument; you should become familiar with the names and functions of the microscope parts listed in the lab manual and diagramed in figure 1.

1. Obtain a microscope from the microscope cabinet. Be sure to carry the microscope with 2 hands, one under the base and one on the arm.
2. Obtain a prepared letter "e" slide.
3. Turn on the microscope and secure the electrical cord so that it does not dangle from the desk top
4. Locate all microscope component identified on page 2 and in figure 1.
5. Be able to describe the function of each component.

1E. Initial Focusing and Correct Use of the Microscope :

1. Correctly place the slide on the stage in the mechanical retainer clamps. Place the "e" on the stage in the correct readable position (as viewed from the side rather than through the microscope)



2. Swing the low power (10x) objective (green band) into place. Place the letter "e" slide right side up at the center of the stage over the stage opening.
3. Use the coarse adjusting knob to bring the stage as close to the objective lens as possible. Then, while looking through the eyepiece very slowly turn the coarse adjusting knob away from you until the letter comes into view.
4. Open the iris diaphragm and then close it down until you find a level of light intensity that is comfortable for you.
5. Without changing the focus, change to the high power (43x) objective (yellow band). Then clarify your view with the *fine focus* adjusting knob. Adjust the light intensity as needed.

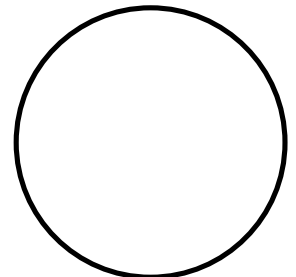
Questions :

- a. Do you need more or less light when using the high power objective lens?
- b. Which focusing knob do you NEVER use under HIGH power? Explain.
- c. Under what conditions would you use low power?
- d. Under what conditions would you use high power?

1G. Image Orientation :

1. Observe the orientation of the "e" as viewed through the microscope relative to actual position of the "e" on the slide on the stage.

Sketch the appearance of the "e" as viewed *through* the microscope :



a. Total microscope magnification : _____

b. Describe the orientation : _____

2. While moving the mechanical stage (slide) to the right, observe the movement of the “e’s” image.

Describe the direction of the images movement when the slide is moved to the right : _____

Describe the relationship between the actual movement of the slide and the image’s movement : _____

1H. Estimating Field Diameter & Depth of Field & their Relevance to Magnification

Field of View : Estimation

1. Obtain a small plastic ruler from your instructor (front desk).
2. Using the 10x objective, place the ruler over the stage aperture (hole) with the millimeter marks in the field of view.
3. Focus on the millimeter marks. Roughly estimate the diameter of the field of view using the millimeter marks.

Field Diameter (10x) = _____ mm

3. Once in focus on the 10x objective, Estimate the field diameter using the 43x objective.

Field Diameter (43x) = _____ mm

4. Describe the relationship between magnification and field of view : _____

Depth of Field : Relationship with Magnification: Most biological specimens you will sample are several cell layers thick. You will need to be able to discern and focus on each layer separately. Your ability to do this will rely on your understanding of the microscope’s depth of field (focus). The characteristic of the microscope that allows one to examine of each separate layer is called the depth of field (sometimes called the depth of focus) or how much of the specimen is in focus at one time.

1. Obtain a “silk fiber” slide from the instructor’s desk.
2. Using the 10x objective, focus on the silk threads. (use the condenser diaphragm to increase the contrast of the threads)
3. Try to determine which thread is on top, middle and bottom in the slide. Remember at lower magnifications more of the slide will be in focus at one time (ie greater depth of field)

Top : _____

Middle : _____

Bottom : _____

4. Use the 43x objective and re-observe the orientation of the silk fibers. Remember the higher the magnification the less of the depth of field.

Top : _____

Middle : _____

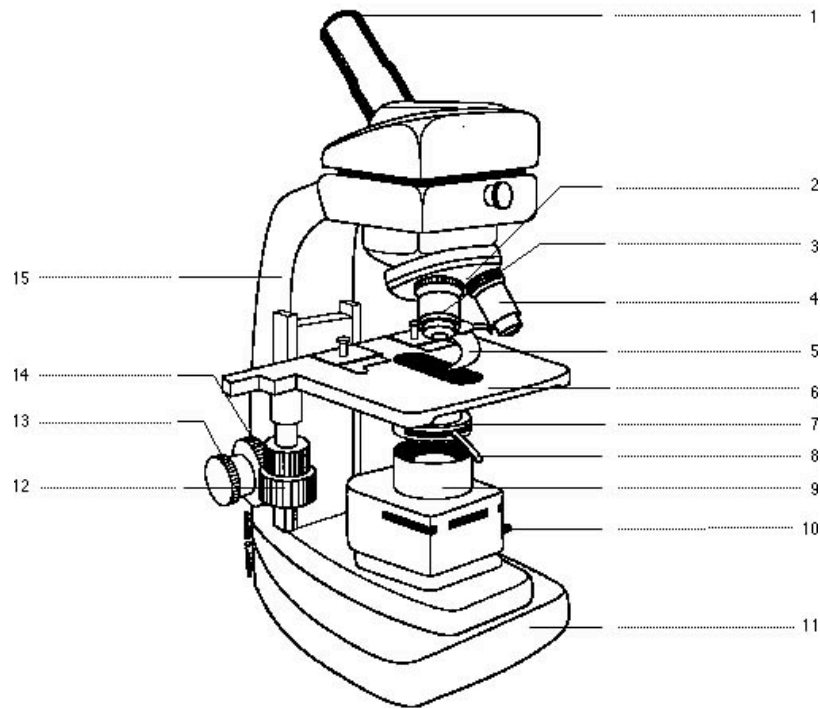
Bottom : _____

2A. Introduction to Cell Structure :

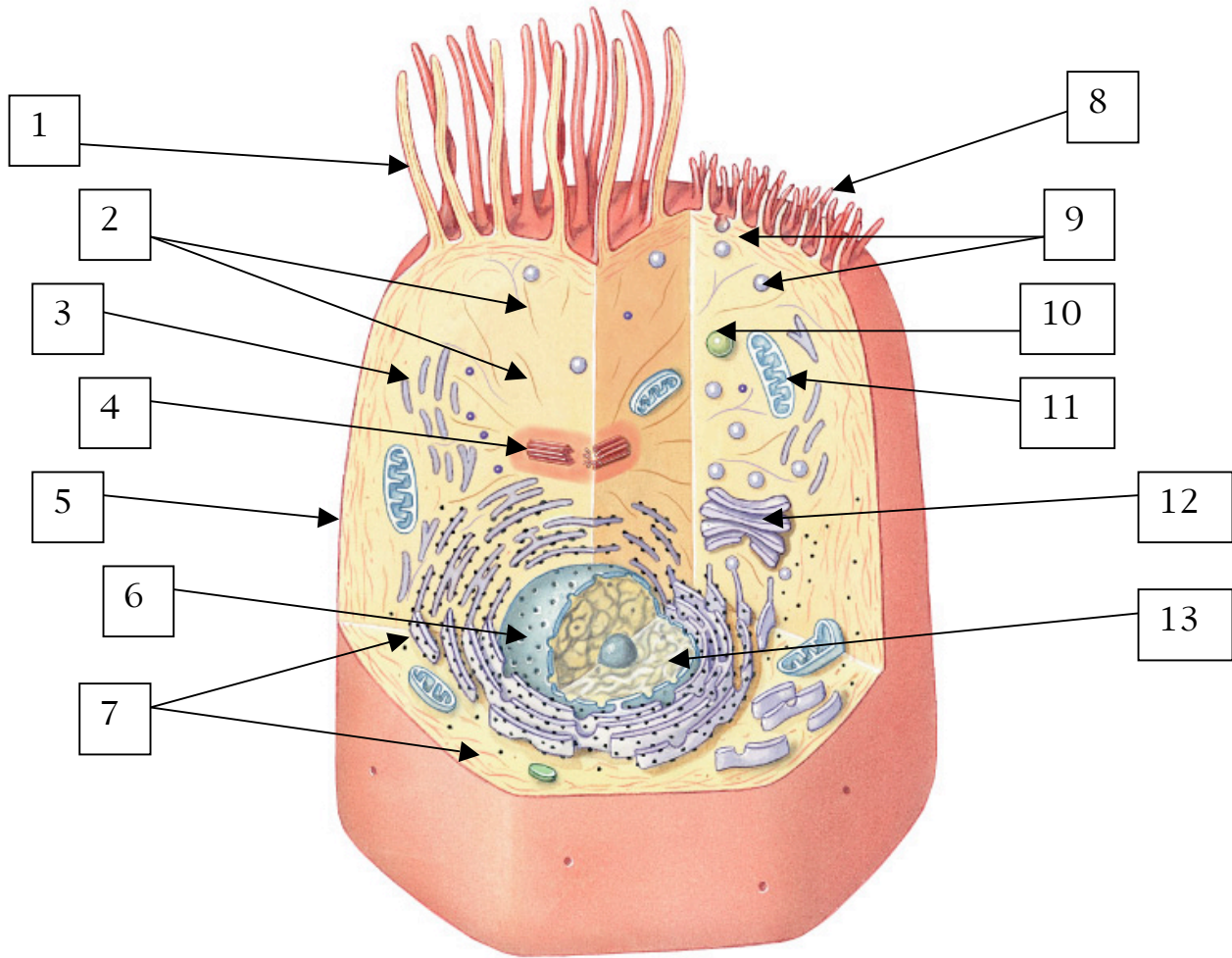
Matching 2.1 :

- | | |
|--------------------------------|--|
| 1. Ribosome | ___ a. Vesicles containing digestive enzymes |
| 2. Peroxisomes | ___ b. Modification & packaging of newly synthesized polypeptides |
| 3. Mitochondria | ___ c. Membrane extensions containing microfilaments, increase cell surface area |
| 4. Microvilli | ___ d. Converts 95% of cellular energy into ATP |
| 5. Centrioles | ___ e. Protein synthesis |
| 6. Rough Endoplasmic Reticulum | ___ f. Strength and support; movement of cellular structures & materials |
| 7. Cytoskeleton | ___ g. Catabolism of fats & other organic molecules |
| 8. Golgi Apparatus | ___ h. Stacks of flattened membranes (sacculs) |
| 9. Lysosome | ___ i. Movement of materials over the cell surface |
| 10. Cilia | ___ j. Essential during cell division, organization of microtubules |

Labeling : 2.2 : Label the following diagrams :



- | | |
|----------|-----------|
| 1. _____ | 9. _____ |
| 2. _____ | 10. _____ |
| 3. _____ | 11. _____ |
| 4. _____ | 12. _____ |
| 5. _____ | 13. _____ |
| 6. _____ | 14. _____ |
| 7. _____ | 15. _____ |
| 8. _____ | |



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