

# LABORATORY

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## Microscopy and Cell Structure

# 1

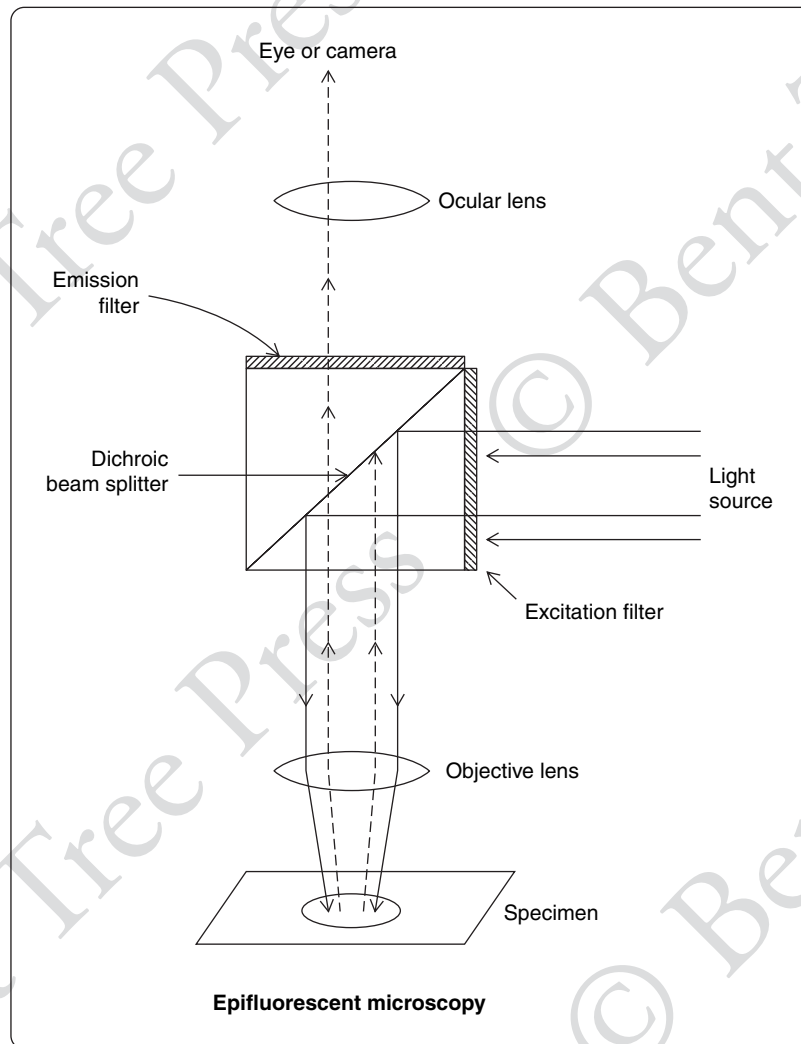
Microscopes are important tools in cell biology for viewing tissues, cells, and some subcellular components. Dramatic images of various cellular details have been generated using the different types of microscopes available, and important functional questions have been investigated. Brightfield and fluorescent microscopes are the focus of this exercise. Using both types of microscope will allow a comparison of the cellular information attainable from each instrument.

Fluorescent microscopy takes advantage of fluorescent molecules (fluors) that will absorb light of a specific short wavelength and emit light of a longer wavelength. The presence or absence of the fluor then determines where fluorescence (light of the longer wavelength) is observed. Fluorescent molecules can also be chemically attached to other molecules with desired binding specificity to direct where the fluor will be localized. This can be used to view details of cellular and subcellular structure, by specific labeling of cellular components. The concentration of ions in cells or parts of cells can also be demonstrated using ion-responsive dyes. These techniques have provided very valuable tools for the study of cellular biology.

There are several types of optics for fluorescent microscopes, with one commonly used type referred to as epifluorescence. This scope is set up with bright illumination for fluorescence at the back of the scope coming through the objective lens toward the specimen. A set of three filters in a cubic holder between the objective and ocular lenses selects and filters both the light that reaches the specimen and emitted light that reaches the eye or camera (see figure below).

This filter cube has an excitation filter to determine what wavelength of light will illuminate the specimen and an emission filter to determine what wavelength of light will be viewed by the eye or camera. These two “bandpass” filters allow light within a certain range (band) to pass through, while blocking wavelengths above and below the band. The appropriate filters are chosen based on the excitation and emission wavelengths of the fluor(s) to be observed. To direct the excitation and emission wavelengths appropriately, the cube also contains, at a 45 degree angle to the others, a dichroic filter or mirror. This dichroic mirror reflects short wavelength (excitation) light toward the specimen, but transmits the longer (emission) wavelengths through the emission filter and on to the eye or camera. A scope typically contains several filter cubes to allow switching from one fluor (color) to another.

A computer-controlled camera is linked to the fluorescent scope for the capture of images. In a typical setup with multiple staining of the specimen, three different images are taken (e.g., red, green, and blue), and then a composite image with all three colors can be generated by image processing on the computer. This allows one to view the separate fluors or the combined image of the staining of several cellular components.



## MATERIALS

Microscope slides and coverslips  
 Solutions X, Y, and Z  
 Ocular and stage micrometers  
 Molecular Probes Fluocells #1 prepared slide from Invitrogen  
 Plant cells for autofluorescence study  
 (e.g., African violet, carnation or coleus leaves, carrot or beet roots)  
 Razor blades  
 Brightfield and Fluorescence microscopes

Brightfield Microscope Tissues:

**Red Onion:** Obtain a very thin slice or peeling of the red portion of the onion and prepare a wet mount on a microscope slide.

**Elodea:** Obtain a leaf from the growing tip and prepare a wet mount of a small piece or slice on a microscope slide.

## PROCEDURE 1: MEMBRANES AND OSMOSIS

- a. Prepare 3 wet mounts of your tissue sample, one in each of solutions X, Y, and Z. Some pairs will do Onion, and some *Elodea*.
- b. Observe cellular structure, cell wall, and pigmentation. Where is the pigmentation located in the cell?
- c. Compare what you see for cells in each of the 3 solutions. Observe any differences in cell structure.
- d. Can it be determined which solution is hypertonic, isotonic, or hypotonic? What cellular processes are causing observed changes in cellular structure?
- e. Observe the other cell type examined by another student group. How do the observations vary between groups?

## PROCEDURE 2: SUBCELLULAR ORGANELLES AND MOVEMENT

- a. In a wet mount of tissue, observe subcellular components and draw a sample.
- b. Can you identify cell organelles (e.g., vacuole, cytoplasm, chloroplasts, etc.)?
- c. Note whether there is any cytoplasmic streaming of organelles within the cells. Describe what the function(s) of cytoplasmic streaming in a cell might be.
- d. Compare to observations of the other cell type, prepared by another group.

## PROCEDURE 3: CELL MEASUREMENT

- a. Choose a cell type and take a cell measurement in the microscope.
- b. This is done by obtaining a slide micrometer (slide with micrometer etched in it) and an ocular micrometer (eyepiece with an arbitrary scale marked in it). Use the two micrometers together to calibrate the size scale of the ocular micrometer markings when using the 40X objective. Then use the ocular micrometer alone, with your cell slide, to measure the cell.
- c. Use your calibration numbers to determine the actual size of the cell.
- d. Include a labeled drawing of the measured cell and indicate the dimension that was measured.

## PROCEDURE 4: FLUORESCENCE MICROSCOPY

Use of the microscope will be demonstrated and supervised by the instructor.

- a. Students will observe prepared slides of cultured mammalian cells that have been stained to image several subcellular components. Molecular Probes Fluocells prepared slide #1 from Invitrogen contains bovine pulmonary artery endothelial cells stained with DAPI, Alexafluor phalloidin, and Mitotracker Red, for viewing of labeled nucleus, actin cytoskeleton, and mitochondria.
- b. Obtain slide and focus on cells under phase contrast or brightfield optics.
- c. Switch to fluorescent optics, view cells, and choose interesting field of view. Critically focus fluorescent image.
- d. Take photos of blue, green, and red fluorescence, without any adjustment to focus or movement of slide between switching of filter sets. Be sure computer camera settings are correct for each separate filter image.

- e. Use computer to merge the three images to provide a composite multicolor image. Adjust brightness of colors in composite as necessary.

## PROCEDURE 5: AUTOFLUORESCENCE

Plant tissues from diverse sources may demonstrate autofluorescence. This is a result of specific molecules present in the tissues, which have a fluorescent property. Striking images can be obtained of this autofluorescence using a confocal microscope. See <http://www.olympusfluoview.com/gallery/plants/grassleaf.html> for example images. Students will attempt to observe autofluorescence in plant tissue.

- a. Make a wet mount of thin slices of the chosen plant tissue.
- b. Observe slide in the fluorescent microscope for the presence of autofluorescence with different filter sets.
- c. Take photos of any observed autofluorescence. Generate composite multicolor images when appropriate.

## QUESTIONS TO CONSIDER BEFORE LAB

1. What is meant by the terms hypertonic, hypotonic, and isotonic? Explain the expected results when a plant cell is put into each solution type. Would this differ for an animal or bacterial cell? Why?
2. What details of cellular structure are not readily observable in the brightfield microscope? How might one effectively view subcellular components?
3. What are the major advantages and disadvantages of using the fluorescence microscope?
4. How is a multicolored fluorescent image gathered/generated by the microscope?
5. What is a confocal fluorescent microscope and how are images obtained with this instrument? What are the advantages of this type of scope?

**Reporting:** Clearly labeled drawings and written descriptions of what is observed should be included in the results. Digital images from the scope will also be part of the results. Discussion is to include interpretation of these observations.