Edvo-Kit # 986

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Analysis of Mammalian Cell Types

Experiment Objective:

This experiment is designed to introduce students to the study of the microscopic structure of cells (histology). At the end of this experiments, students will identify several major cell lines by examining the morphology. This allows students to connect the concepts of cell structure with its function.

See page 3 for storage instructions.

Version 986.240419

SAMPLE LITERATURE
Please refer to included weblink for correct version

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Safety Data Sheets can be found on our website: www.edvotek.com/Safety-Data-Sheets

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Experiment Components

Component	Check (✓)
• Ready-to-Stain multispot slides (4 cell types each)	❑
• Cell fixing agent	❑
• Eosin stain solution	❑
• Methylene Blue stain solution	❑
Supplies	
• Transfer pipets	❑

Storage:
Room Temperature.

This experiment is designed for 6 groups.

Requirements *(NOT included with this experiment)*

- Microscopes (100X total magnification recommended)
- Forceps
- 1.5 mL snap-top microcentrifuge tubes
- Beakers (250 mL or larger recommended)
- Timers
- Distilled water
- Gloves
- Paper Towels or Kimwipes
- Slide coverslips (optional)
- Mounting medium (optional)

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Background Information

CELL MORPHOLOGY

Cell morphology refers to the shape, appearance and structure of a cell. These characteristics are visualized by phase contrast, confocal or electron microscopy.

The morphology of a cell in culture is closely related to the functions of cells within the tissue from which they are derived. The major types of cells that are commonly cultured include fibroblasts, epithelial cells and lymphoblasts.

CELL ATTACHMENT

Cell adhesion plays a critical role in development, wound healing and tumor invasion, as the attachment of cells to the extracellular matrix and to one another is crucial for the maintenance of tissue structure and integrity. Multiprotein complexes called hemidesmosomes are responsible for adhesion of epithelial cells to the underlying basement membrane, a layer of fibrous proteins that separates epithelial cells from the underlying connective tissue. Their importance has become apparent in clinical conditions, in which absence or defects of hemidesmosomal proteins result in devastating blistering diseases of the skin.

The lateral connections between adjacent cells are tight and are characterized by the presence of desmosomes and tight junctions. These lateral connections junctions form an impermeable barrier that prevents bacteria from invading the body. Gap junctions, formed by connexins, allows for cells to communicate with one another through the transport of molecules between cells.

MAMMALIAN CELLS

Mammalian cells are cells that are derived or isolated from tissue of a mammal. In this experiment, students are introduced to four mammalian cell types: fibroblasts, epithelial cells, lymphocytes and macrophages. Lymphocytes are found within the blood. Epidermal cells, fibroblasts, and macrophages are found within the tissues.

FIBROBLASTS

Fibroblasts are present in almost every tissue type (Figure 1). Fibroblasts originate from the embryonic mesoderm, as evidenced by the presence of the intermediate filament protein vimentin. This protein, along with actin and tubulin, forms the structural support of the cell. In vivo, fibroblasts exist as single fusiform cells (wide in the middle and tapered at the ends) embedded within connective tissue.

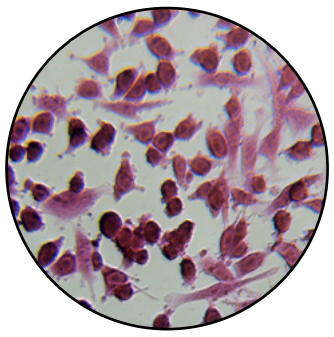

Figure 1: Fibroblasts

Fibroblasts secrete proteins that are important for the formation of the extracellular matrix, including collagen, elastase, fibronectin and laminin. Fibroblasts also play an important role in normal physiological processes such as wound healing by secreting matrix proteins, growth factors and cytokines. Tissue damage stimulates fibroblasts to differentiate into myofibroblasts, which contract to close the wound.

Fibroblasts exhibit contact inhibition, a growth control mechanism designed to keep fibroblasts growing into monolayers when in contact with neighboring cells. Fibroblasts

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also play an important role in many disease states. Overproduction of connective tissue can change the normal morphology of a tissue, resulting in a condition called fibrosis. Transformed fibroblasts can also give rise to a type of cancer called sarcoma, which comprises tumors from cells of mesenchymal origin (bone, cartilage, fat).

Mouse Fibroblasts (3T3), an extremely well studied cell line, represent fibroblasts in this experiment. In cell culture, these cells exhibit different morphologies depending upon the culture conditions, genotype, and phase of the cell cycle. Most times, fibroblasts maintain their characteristic fusiform morphology. However, in the G2/M phase of the cell cycle, fibroblasts may adopt a rounded shape in preparation for cell division (mitosis and cytokinesis).

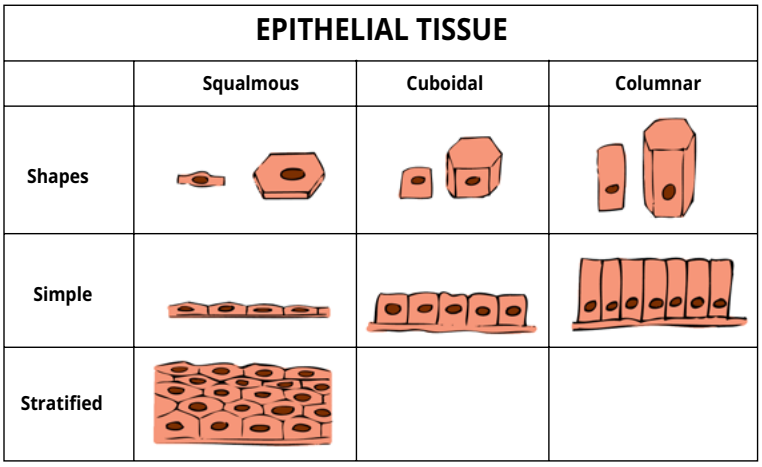

Figure 2: Epithelial Tissue

EPITHELIAL CELLS

Epithelial cells are derived from can be derived from all three germ layers, though true epithelium is considered to be ectodermal (Figure 2). These cells are found as one of three shapes in the body – squamous (flat, scale like cells), cuboidal (height and with are the same) and columnar (cells are taller than they are wide). Epithelial cells develop into sheets or tubes that separate the organism from its environment, allowing for the survival of multicellular organisms. These tissues are found throughout the body, including the epidermis, digestive system, reproductive system, and endocrine system.

Epithelial tissues are classified as simple (stomach, intestine, kidney), stratified (epidermis, esophagus, tongue), or pseudostratified (trachea). Simple and pseudostratified epithelia consist of a single layer of cells that rest on a basement membrane. In contrast, stratified epithelia consist of two or more layers, with only the dividing layer of cells attached to the basement membrane. Communication between epithelial cells allows the tissues to respond in a coordinated fashion to growth, differentiation, and wound healing. In the transformed state, keratinocytes and other epithelial cells give rise to carcinomas.

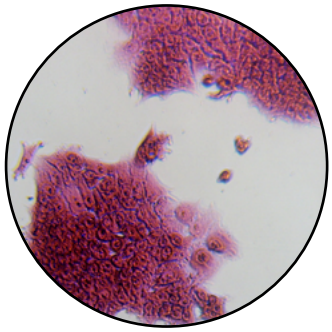

Figure 3: Epithelial Cells

In culture, epithelial cells adhere tightly to the substrate, resulting in flattened cell morphology (Figure 3). The cells will form a contiguous monolayer due to contact inhibition, the process by which monolayers of cells cease to divide and migrate once they come into contact with each other. The intermediate filament protein keratin is also used as a cellular marker for epithelial cells in cell culture.

LYMPHOCYTES

Both red and white blood cells originate from a totipotent cell found in the bone marrow called a hematopoietic stem cell. The program of differentiation from these stem cells appears to depend on both intrinsic and external factors. Red blood cells are responsible for delivering oxygen to the body through the circulatory system. White blood cells, or leukocytes, are responsible for the immune response in mammals.

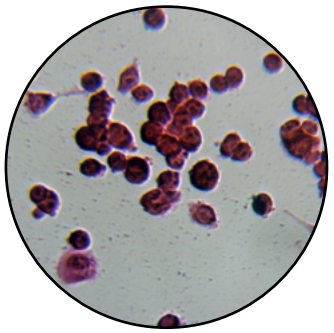

Figure 4: Lymphocytes

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Leukocytes further differentiate into lymphocytes, granulocytes and monocytes. Lymphocytes give rise to T and B cells, which are responsible for cellular and humoral immunity, respectively (Figure 4). T lymphocytes further differentiate into T helper, T suppressor, and cytotoxic T cells. T helper cells stimulate antibody production and release in B cells.

Unstimulated T and B cells are very similar in appearance, even when imaged using high-resolution electron microscopy. Both types of cells are small (only marginally bigger than red blood cells). Very little cytoplasm is observable, as the nucleus occupies most of the cell. Lymphocytes exist as rounded cells in vivo as well as in cell culture. Under normal cell culture conditions, lymphocytes do not adhere to the substrate.

MACROPHAGES

Macrophages do not exhibit contact inhibition and are produced through the differentiation of monocytes, a type of white blood cell in mammals (Figure 5). They are specialized cells with responsibilities in both the innate and adaptive immune system. Macrophages contribute to the innate immune response through phagocytosis of foreign materials (like microbes and fragments of dying cells). In response to pathogens, macrophages secrete cytokines that in turn activate the adaptive immune system. Macrophages are highly motile cells; they move through the body in an amoeboid fashion, which allows them to move to the site of an infection or tissue damage.

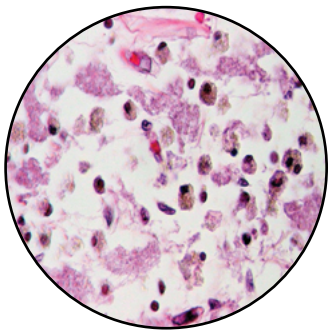

Figure 5: Macrophages

Macrophages are studied in culture for their role in immunity, but also because these cells are specifically targeted by pathogens like tuberculosis and HIV. Both macrophages and monocytes can adhere to the substrate during growth in culture, although not as tightly as epithelial and fibroblasts. This results in a more spherical shaped cell. Like fibroblasts and other mesodermal cell types, monocytes and macrophages can be identified by the presence of vimentin.

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Experiment Overview

EXPERIMENT OBJECTIVE

This experiment is designed to introduce students to the study of the microscopic structure of cells (histology). At the end of this experiments, students will identify several major cell lines by examining the morphology. This allows students to connect the concepts of cell structure with its function.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Wear gloves and goggles while working in the laboratory.
2. Always exercise extreme caution when working in the laboratory.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

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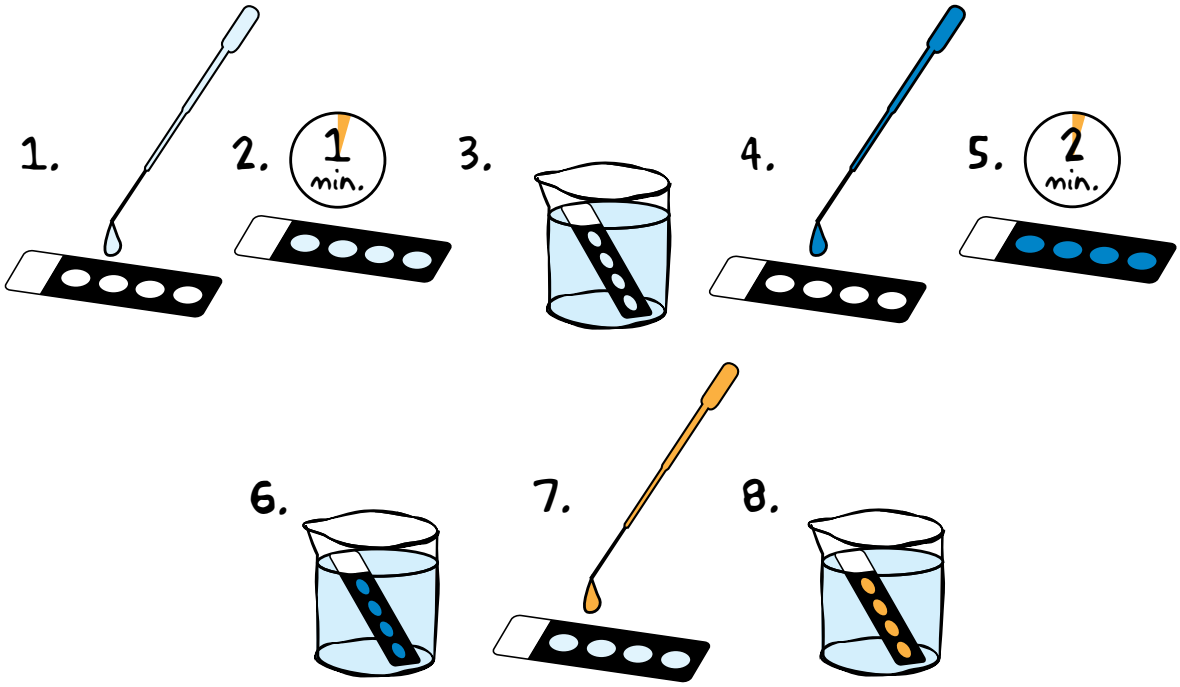
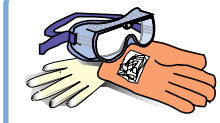
Module I: Staining the Pre-fixed Cells



NOTE: Before beginning the experiment, ensure that the slide is facing upright.


Wear gloves and safety goggles

1. Using a transfer pipet, **ADD** 1 or 2 drops of cell fixing agent to **COVER** each well.
2. **INCUBATE** the slide for 1 minute at room temperature.
3. **RINSE** the slide briefly by submerging in the beaker of distilled water. Gently tap the slide on a paper towel to remove excess water.
4. Using a fresh transfer pipet, **ADD** 1 or 2 drops of methylene blue stain to **COVER** each well.
5. **INCUBATE** the slide for 2 minutes at room temperature.
6. **RINSE** the slide briefly by submerging in the beaker of distilled water. Gently tap the slide on a paper towel to remove excess water.
7. Using a fresh transfer pipet, **ADD** 1 or 2 drops of eosin stain to **COVER** each well.
8. Immediately **RINSE** the slide by submerging in the beaker of distilled water. If residual stain remains, change water and repeat until the water no longer turns orange. Gently tap the slide on a paper towel to remove excess water. **PROCEED** to Module II: Microscopic Observation.

OPTIONAL STOPPING POINT:
At this point, the stained slides can be stored at room temperature. If a coverslip is required by your microscope, one can be added by following the instructions in Appendix A.

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Module II: Microscopic Observation

1. **LOCATE** cells in well #1 (Figure 6, below) using the lowest magnification objective. Adjust the slide to find a random field of nicely stained cells that contains at least 15-20 cells.
2. **DESCRIBE** the overall morphology of the cells in the table on the following page. Features you might record include the number of cells, overall cell morphology, shape and size of the nucleus, and the intensity or color of staining.
3. Using the space provided, **DRAW** an image of the field of cells that you observe.
4. **MOVE** the slide and observe a second field of cells. Are your observations from the first area consistent with the second cell type.
5. **SWITCH** to a higher magnification and record your observations as in steps 2-3.
6. **CHANGE** the microscope back to the lower magnification objective and repeat steps 1-5 for the cells in well #2, #3, and #4.
7. Based on your observations, **CLASSIFY** each cell type as lymphocytes, macrophages, fibroblasts, or epithelial cells.

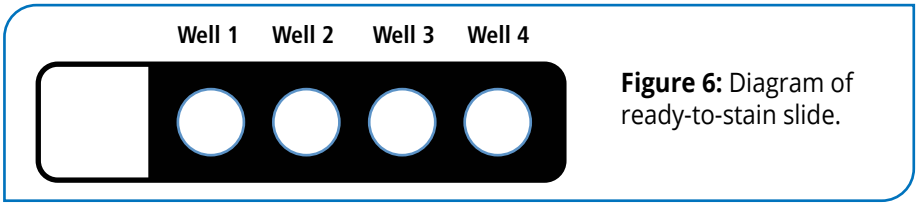

Figure 6: Diagram of ready-to-stain slide.

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Module II: Microscopic Observation, continued

Well	Magnification	Observations	Drawing

Classification:

Well #1:_____ Well #2:_____

Well #3:_____ Well #4:_____

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Study Questions

1. How does the morphology of each cell type relate to its function?
2. Give an example of external stratified epithelium, and internal stratified epithelium.
3. Which cell types are found within the blood? Which cell types are found within the tissues?
4. Which cell types are the largest? The smallest? What would be the advantage of a smaller cell?
5. Which cells are the most adherent (flattest)? The least adherent? What is the advantage of each?

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Instructor's Guide

This lab is designed for six groups of 2-4 students.

This lab explores the differences in morphology between four different cell types. Students will work in groups to stain the pre-fixed cells with methylene blue and eosin. The samples are examined using light microscopy, allowing students to identify the distinguishing characteristics of each cell type through exploration.

It is important to remind students that natural variations can occur in cultured cells, both within a single well and when comparing the same condition across multiple slides. In addition, differences in staining technique or timing can alter the intensity of staining from group to group. When examining the slides, students should comment on general trends in each cell type.

PRE-LAB PREPARATION

Pre-lab preparation should take **approximately 30 minutes** and can be performed any time before the lab period.

1. Label eighteen (18) 1.5 mL snap-top microcentrifuge tubes as follows:
   - 6 – Cell fixing agent
   - 6 – Methylene blue stain
   - 6 – Eosin Stain

2. Use a separate transfer pipet for dispensing each component into the appropriately labeled tube.
   - Add approximately 0.3 mL of each solution to the tube.
   - Cap the tubes and store at room temperature.

3. Prepare beakers and distilled water for washing slides. The water level must be high enough to fully submerge the slides. If beakers are not available, slides can be gently washed under running water.

4. Distribute the following to each student group, or set up a workstation for students to share materials:
   - 1 Ready-to-Stain slide
   - Cell fixing agent
   - Methylene blue stain
   - Eosin stain
   - 3 Transfer pipets
   - 1 Pair of forceps (optional)
   - Beaker of distilled water
   - Paper towels or Kimwipes
   - Microscope

NOTE: Rarely, microscopes will require a coverslip/cover glass for best results. Coverslips and mounting medium are not included in this experiment - please refer to the manual included with your microscope for specific information on the required coverslips.

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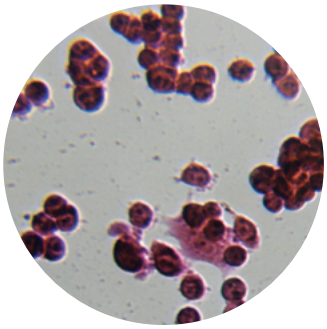
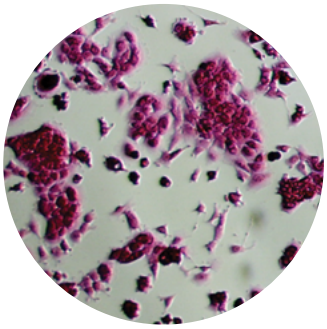
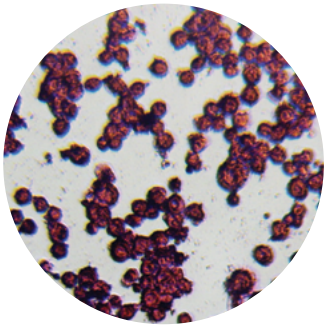
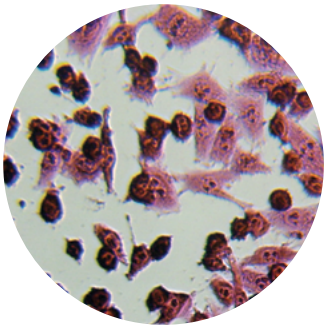
Experiment Results and Analysis

The order of the cells on the slide from frosted end: Lymphocytes, Epithelial, Macrophages, Fibroblasts.

Cell Type	Lower Power Observations	Higher Power Observations	Classification	Photo
1	Cluster of circular cells	Nearly colorless, uniform nuclei	Lymphocytes	
2	Uniform, compact cells. Monolayer or tight clusters of cells.	Uniform nuclei, tight cell-cell junctions.	Epithelial	
3	Cluster of round cells	Large in size, monocuclear cells	Macrophages	
4	Polygonal and irregularly shaped cells	Flat, elongated cells, disorganized arrangement	Fibroblasts	

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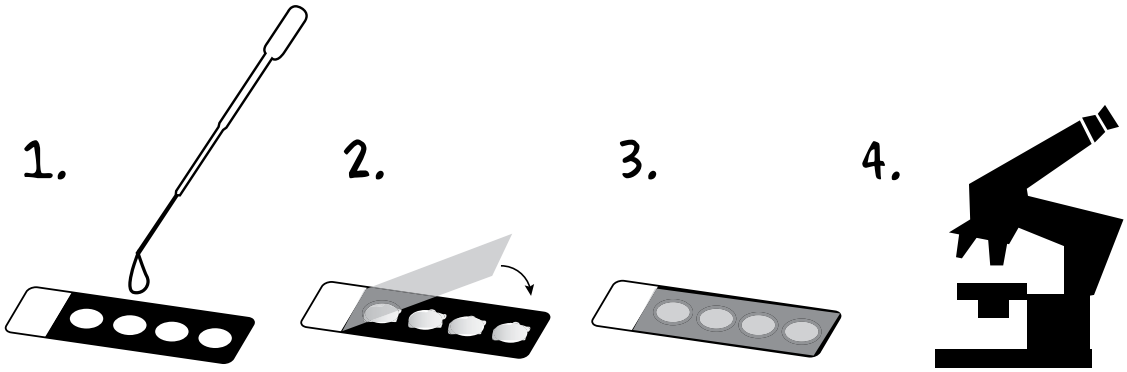
Please refer to the kit insert for the Answers to Study Questions

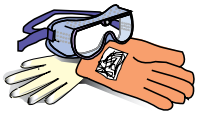
Appendix A

Mounting Glass Coverslips (Optional)

NOTE: Mounting medium and slide coverslips are not included with this kit.



Glass coverslips may be required for some microscope objectives and can help to increase the visibility of nuclei and organelles on these microscopes. Occasionally, mounting media can cause lightly stained cells to fade. Because of this we recommend observing slides without a coverslip unless necessary. If time allows students can visualize slides before and after adding mounting media and coverslips.



Wear gloves and safety goggles

ADDING A COVERSLIP

1. Using a fresh transfer pipet, **ADD** 1 small drop of mounting medium to each well.
2. Carefully **PLACE** a coverslip on top of the mounting medium to cover all four wells. **HINT:** Avoid bubbles by placing the cover slip and at 45° angle to the slides and slowly lowering. If bubbles are seen, gently press on the coverslip to displace.
3. Gently **ADJUST** the coverslip so that it is centered on the slide and fully covers all four wells.
4. **PROCEED** to Module II: Microscopic Observation.

OPTIONAL STOPPING POINT:
Once the coverslip has been placed, the stained slides can be stored at 4° C for up to 24 hours before moving to Module II.

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