SKOPJE CAMPUS

Laboratory Exercises

in Cell Staining and Microscopy

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Staining techniques and microscopy

Staining is a fundamental technique in biology that helps scientists and students see the tiny details of cells and tissues under a microscope. Many biological samples are naturally transparent, which means that without staining, important structures like the nucleus, cell membrane, or internal organelles might be difficult or impossible to see. By applying **special dyes**, staining enhances the contrast between different parts of a cell, making these components stand out in vivid colors. This process not only improves our ability to study the structure and function of cells but also plays a critical role in identifying and diagnosing diseases in medical laboratories. For example, certain staining methods can help distinguish between different types of bacteria by coloring their cell walls in unique ways. Overall, staining is an essential tool in both research and education, allowing us to explore the intricate world of microscopic life with greater clarity and understanding.

Tools needed for staining

When preparing to stain a biological sample, several key tools are essential. First, **glass slides** and **cover slips** provide a flat, transparent surface on which the **specimen** is placed and observed under the microscope. Fixatives such as **alcohol** or **formaldehyde** help preserve the cell's structure before staining. **Pipettes** and **droppers** are used to accurately apply the **staining dyes**—special chemicals that bind to different parts of the cell to create contrast. Additionally, safety equipment like **gloves** and **lab coats** is important to protect against chemical exposure. These tools work together to ensure that the staining process is effective and that the microscopic details of cells can be clearly observed.

Cheek Cells with Methylene Blue

Objective: Use a basic stain to visualize **nuclei** and **cell membranes** in human cheek cells.

Instructions

1. Collecting Cheek Cells

- Provide students with clean toothpicks.
- **Demonstrate first**: Instruct students to *gently* scrape the inner lining of their cheek (no hard scraping—avoids injury and excess mucus).
- Smear the collected cells onto the center of a clean microscope slide in a circular motion. Allow the smear to air-dry for 30 seconds.

2. Applying Methylene Blue Stain

- Using a dropper, add **1–2 drops of methylene blue** to the dried smear.
- Let the stain sit for **1–2 minutes** (timing matters—too short = faint staining; too long = over-stained).
- **Safety Tip**: Remind students methylene blue stains skin/clothes. Wear gloves!

3. Rinsing and Mounting

- Tilt the slide and *gently* rinse with a slow stream of **distilled water** from a dropper or squeeze bottle. Avoid spraying directly onto the smear.

- Blot excess water with a paper towel by pressing lightly on the edges of the slide.
- Place a **coverslip** over the stained area.

4. Microscope Observation

- Start at **low power (40x)** to locate cells, then switch to **400x** (high power) for detailed observation.
- **Focus on**:
    - ❖ Dark blue **nuclei** (stained by methylene blue's affinity for DNA).
    - ❖ Lighter blue **cytoplasm** and **cell membrane** (semi-transparent).

5. Drawing and Labeling

- Students sketch 2–3 cells, labeling: *cell membrane*, *cytoplasm*, and *nucleus*.
- **Discussion prompt**: "Why don't cheek cells have a cell wall? How does their shape differ from plant cells?"

Troubleshooting Tips for Students

- **No cells visible?** They may have scraped too lightly. Repeat with a slightly firmer scrape.
- **Clumped cells?** Add a drop of saline solution to the toothpick before smearing to spread cells thinly.
- **Over-stained?** Reduce staining time to 45 seconds next time.

Iodine staining for onion cells

Objective:
Use **iodine staining** to visualize the **cell walls** and **nuclei** in onion epidermal cells.

How Does Iodine Work on Onion Cells?

Iodine is used as a **staining agent** in biology. It helps us see cell structures better under a microscope. But how does it actually work?

1. Staining the Cell Structures

- Iodine **binds to starch** molecules and turns them **dark blue or black**.
- Onion cells have **very little starch**, so they don't stain as dark as potato cells.

2. Making Cell Walls More Visible

- Iodine interacts with the **cell wall**, which is made of cellulose.
- This reaction helps outline the **shape of the cells** more clearly under the microscope.

3. Highlighting the Nucleus

- The iodine **partially stains the nucleus**, making it easier to spot.
- This helps in studying the **organization of the cell**.

Why Do We Use Iodine?

Since onion cells are naturally **transparent**, iodine helps us **see their structures more clearly**. It does not change the cells but just makes them easier to observe!

Instructions

1. **Preparing the Onion Membrane**
   - **Collecting the Sample:** Provide each student with an onion and a pair of tweezers or forceps.
   - **Peeling:** Demonstrate how to gently peel a thin, transparent layer (the epidermis) from the inner surface of the onion without tearing it.
   - **Slide Preparation:** Place the peeled membrane flat in the center of a clean microscope slide.

2. **Applying Iodine Stain**
   - **Stain Application:** Using a dropper, add 1–2 drops of iodine solution directly onto the onion membrane.
   - **Staining Duration:** Let the iodine sit on the sample for 1 minute (timing is crucial—too little time might result in faint staining; too long could lead to over-staining).
   - **Safety Tip:** Iodine stains skin and clothing, so remind students to wear gloves and handle the solution carefully.

3. **Rinsing and Mounting**
   - **Rinsing:** Tilt the slide and gently rinse with a slow stream of distilled water from a dropper, taking care not to wash away the sample.
   - **Blotting:** Lightly blot the slide with a paper towel to remove excess water, ensuring the membrane stays in place.
   - **(Optional) Coverslip:** If desired, carefully place a coverslip over the stained area to protect the sample during observation.

4. **Microscope Observation**
   - **Initial Scan:** Begin at low power (e.g., 40x or 100x) to locate the stained cells on the slide.
   - **Detailed Examination:** Switch to high power (400x) for a closer

look.

• **Observation Focus:**

- ❖ **Cell Walls:** Notice the iodine's affinity for cellulose, highlighting the cell walls.
- ❖ **Nuclei:** Observe any nuclear staining and contrast it with the rest of the cell structure.

• **Comparison:** Compare these results with an unstained onion membrane (prepared earlier) to appreciate the contrast provided by the iodine stain.

5. **Drawing and Labeling**

• **Sketching:** Have students draw 2–3 cells from their observation, clearly labeling the cell wall and nucleus.

• **Discussion Prompt:** "How does iodine staining improve our ability to distinguish the components of onion cells compared to unstained samples?"

Troubleshooting Guidelines for Students

- **No Visible Cells:** Verify that the onion epidermis is sufficiently thin. A thicker peel may prevent clear observation of the cells.
- **Over-Staining:** If the cells appear excessively dark or if details are obscured, consider reducing the iodine exposure time in future experiments.
- **Uneven Staining:** Ensure that the iodine is evenly distributed across the membrane and rinse the sample gently to avoid dislodging any parts.

Blood Smear Preparation and Examination

Objective:

Students will learn how to prepare, stain, and examine a blood smear under a microscope to identify different blood cells and understand their functions.

Materials Needed:

- Glass slides
- Sterile lancets
- Alcohol swabs
- Capillary tubes
- Microscope
- Staining reagents (Wright's stain or Giemsa stain)
- Distilled water
- Dropper
- Gloves and lab coats
- Waste disposal container

Safety Precautions:

- Use gloves and lab coats to prevent contamination.
- Dispose of lancets in a sharps container.
- Follow proper hand hygiene before and after the procedure.
- Ensure that blood samples are handled safely and ethically.

Procedure:

1. **Blood Collection:**
    - Clean the fingertip with an alcohol swab.
    - Use a sterile lancet to prick the fingertip and wipe away the first drop.
    - Collect a small drop of blood using a capillary tube or directly onto the slide.
2. **Smear Preparation:**
    - Place a drop of blood near one end of a clean slide.
    - Use another slide at a 30-45 degree angle to spread the drop across the slide in a thin film.
    - Allow the smear to air-dry completely.
3. **Staining Process:**
    - Place the dried smear on a staining tray.
    - Flood the smear with **Wright's** or **Giemsa stain** and wait for 1-3 minutes.
    - Add an equal amount of distilled water and mix gently.
    - Allow the mixture to sit for 5-10 minutes.
    - Rinse the slide with distilled water and let it air-dry.
4. **Microscopic Examination:**
    - Place the stained slide under the microscope.
    - Start with low magnification (10x) to locate the smear.
    - Switch to higher magnification (40x or 100x with oil immersion) for detailed examination.
    - Identify red blood cells (RBCs), white blood cells (WBCs), and platelets.

Observations & Discussion:

- Compare the size, shape, and staining properties of different blood cells.
- Identify abnormalities in cell morphology if present.
- Discuss the significance of blood smears in diagnosing diseases like anemia, infections, and leukemia.

Conclusion:

Students will develop microscopy skills and gain an understanding of blood cell morphology and its importance in medical diagnostics.