Mzuzu University

CENTRE FOR OPEN AND DISTANCE LEARNING

BIOLOGY MODULE

GENERAL BIOLOGY I (PRACTICAL)

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Introduction

Welcome to this practical module on BIOL 1101: General Biology One. Biology learning demands practical orientation and understanding, which essentially complement the theoretical knowledge you gain in the classroom. The module has been designed to enhance understanding of different topics that are covered in general biology, ranging from Laboratory safety, chemistry of living things, cell biology, evolution, and diversity of organisms.

Module objectives

Upon completion of this practical module you should be able to:

1. Identify where exits, eyewash, ventilation hoods, fire extinguisher, first aid kit, and safety shower are located in the laboratory
2. Understand hazard symbols and use microscopes safely
3. Conduct laboratory experiments

Module structure

There are 12 practicals that you are expected to cover in this module, as outlined in the table of contents.

Assessment

The 12 practicals will contribute 20% towards your continuous assessment grade.

Prescribed text books

Solomon, E.P; Berg, L.R and Martin, D.W. (2011). Biology. 9th edition.

Recommended text books

Raven, P.H, Johnson, G.B., Mason et al. (2011). Biology. 10th Edition.

Time Frame

You will require 36 hours to complete this module on site and with more hours of independent learning.

Module assistance

In case you have difficulties while going through this practical module. Kindly contact the Director of CODeL, Mzuzu University, P/BAG 201, LUWINGA, MZUZU 2.

PRACTICAL 1: GENERAL LABORATORY SAFETY

INTRODUCTION

Welcome to the first practical on general laboratory safety. Laboratory safety is the key to reducing injuries and illnesses. This practical module will introduce you to some basic observational measures, laboratory safety practices, hazard symbols and laboratory biosafety levels. There are many exposures in the laboratory that pose a hazard to your health, and you may have never considered them a hazard before. It is important to know, as a laboratory user, the potential dangers that might threaten your health or life.

Learning objectives

By the end of this session, you should be able to:

1. Explain some laboratory safety practices
2. Know where to find and use emergency items
3. Identify some important hazard symbols in the laboratory
4. Describe the laboratory biosafety levels

What is a laboratory- Oxford dictionary defines it as a room or building equipped for scientific experiments, research, or teaching, or for the manufacture of drugs or chemicals.

Know Your Surrounding

Working in a laboratory can be an exciting experience. It can also pose many threats and hazards that a traditional classroom does not. That is why it is important to know your surroundings. Know where the exits to your laboratory are. There might be more than one exit which could be critical in case of an emergency. Your supervisor will go over the emergency action plan including the escape route procedures for your laboratory.

It is also recommended to be aware of the location of fire extinguishers, first aid kit, safety shower and eye wash station in your laboratory. In order to fight a fire one must undergo the proper training. Students are highly encouraged **not** to fight fires. In the event of a fire, and as it is still small, you can use fire extinguisher, fire blanket, moist waster and sand to put it off. If the fire becomes big, the first response is to evacuate the area and notify your instructor. Evacuate calmly without running and assemble at the assembly

point. Do not shout fire, as everyone will get the message at once and this may cause stampede. For purposes of fire incidence only, know the whereabouts of your mate close to you.

Know where the fire alarm is in proximity to your laboratory. If there is a fire, a quick response is the best response. Have your supervisor show you the closest alarm. The fire safety training will detail the safety procedures for your lab.

Hazardous substances in the Laboratory

A **hazardous substance** is defined as a material/substance that poses a physical or health hazard. This includes both chemicals, physical and biological agents.
A **Biohazard** is defined as any organism that is capable of replication and is capable of causing disease in human, animal or plant. There are differences between a physical hazard and a health hazard:

Figure 1: Biohazard symbol

A health hazard has the following characteristics:

- Carcinogenic
- Toxic or highly toxic
- Reproductive Toxins
- Irritant
- Corrosive
- Sensitizers

♦ Hepatotoxin: Chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents.
♦ Nephrotoxin: Toxic agent or substance that inhibits, damages or destroys the cells and/or tissues of the kidneys.
♦ Neurotoxin: Any substance that is capable of causing damage to nerves or nerve tissue. For example, arsenic and lead are *neurotoxins*.

Figure 2: Hazard symbols

A physical hazard has the following characteristics:

♦ Explosive
♦ Flammable
♦ Oxidizer
♦ Pyrophoric- **materials** are often water-reactive as well and will ignite when they contact water or humid air.
♦ Organic peroxide
♦ Compressed gas
♦ Combustible liquid
♦ Unstable (Reactive)
♦ Water-reactive

When physical hazards and health hazards exist, it is very important to know where the eye wash/safety shower is located. Unexpected accidents do occur and knowing where to go at the time of an emergency can reduce injury/illness.

First aid kits have a variety of quick relief items. If your laboratory has a first aid kit, find out where it is. If more than first aid is needed, it is recommended to go to the University Clinic for further treatment.

When there are chemical, biological, or radioactive agents being used, an emergency **Spill kit** (are collections of equipment and absorbents used to safely clean up spills) should be available. If there is a spill kit in your lab, find its location. Further Spill information will be addressed later in this presentation.

Know What Hazards are Present

Each laboratory is faced with different hazards. There could be exposure to biological, physical, chemical, or radioactive material, which may pose a variety of physical and/or health hazards.

A **biological hazard** includes an organism or material of biological origin that could potentially cause harm to humans, animals, or plants.

An **infectious agent** is an organism capable of producing infection or disease in human, animal or plant.

Infectious agents pose a threat because these agents can cause illness or death to both people and animals. Special precautions must be taken to reduce the potential release of these agents. Each laboratory that is using an infectious agent must perform an additional lab specific training. This is to communicate the hazards of the agent that is specific to your laboratory.

The laboratory that works with infectious agents, depending on the Biosafety Level (BSL) will vary in accordance with the safety equipment that is used, the facility (lab) design, the equipment that is used, and the practices that must be followed.

Table 2. **Relation of risk groups to biosafety levels, practices and equipment**

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

Chemicals can pose a significant hazard. They should be limited to the use under a properly working fume hood. Chemicals can release hazardous fumes which not only harm the environment, but they can be a major health threat. They must be handled carefully and disposed of properly.

Laboratory Safety

The following guidelines have been established to minimize the hazards in a laboratory setting. It is important to take responsibility for your actions and to keep in mind that irresponsible acts could have lasting future effects.

Laboratory Attire

You should remember the following:

- No open-toed shoes
- No shorts unless a lab coat is used
- Restrain hair when working with hazardous materials
- Remove protective clothing in public
- Use the proper Personal Protective Equipment for the job

Personal Habits

Personal habits play a large role in minimizing hazards. The following measures must be taken:

- Do not eat, drink, smoke, chew gum or apply cosmetics, or remove/insert contact lenses while in the laboratory
- Do not store food or beverages in the lab or in chemical refrigerator
- Do not mouth pipette
- Wash hands before leaving laboratory or after handling contaminated material

Personal Protective Equipment (PPE)

Watch a video by clicking on the following youtube link on PPE. Good Microbiological Practices and Procedures (GMPP) 1: personal protective equipment (PPE) - YouTube

PPE is short for personal protective equipment. This is the equipment that is necessary to protect yourself from hazardous and bio-hazardous materials. PPE could be gloves, safety glasses, lab coat, shoe covers, respirator or any other item that could protect you from dangerous materials that you may encounter in the laboratory. Knowing *what* to use and *when* to use it is the key to properly protecting yourself. There could be situations that would be more of a risk and require more PPE than others.

Activity 1.1 (self-assessment questions)

Sketch the floor plan of the laboratory. Indicate the locations of exits, eyewash, ventilation hoods, fire extinguisher, first aid kit, and safety shower

Why should you wash your hands before leaving lab?

Practical 1: Summary

In this practical, you have covered the following main points

1. Definition of a laboratory
2. Safety measures to be followed in the laboratory
3. Hazard symbols in the laboratory
4. Laboratory Biosafety levels

PRACTICAL 2: GENERAL INFORMATION FOR PRACTICAL SESSIONS AND BIOLOGICAL DRAWINGS

Role of Practicals

Biology is a practical science. High quality, appropriate biology experiments and investigations are the key to enhanced learning, and clarification and consolidation of theory. Practical activities are not just motivational and fun: they also enable you to apply and extend your knowledge and understanding of biology in novel investigative situations, which can stimulate interest and aid learning and retention. Crucially, practical work gives understanding of how biological knowledge is generated by experiment and observation.

Requirements for Practical Sessions

The following must be brought with you when coming to the lab for a practical session:

- Practical module/schedule. Read and understand the practical that you are going to undertake.
- Drawing book: A4 plane papers are recommended for biological drawings. A bound booklet is ideal to enable you keep your work.
- Pencil. A sharp pencil is recommended.
- Eraser
- Plastic, transparent, 15cm ruler in mm and cm
- Sharpener

Laboratory Equipment

All laboratory equipment belongs to the laboratory. No single equipment should be taken out of the lab without permission from laboratory staff. Handle all the equipment with greatest care. Follow laboratory procedures correctly to help you handle equipment carefully.

Demonstrations

Demonstration set-ups will be used whenever specimens to be studied are insufficient. Such specimens must be handled with care so that they serve the purpose they were selected for. If microscopes are under demonstration, the slides must not be moved. If specimens are large, they must not be damaged in any way or removed from the demonstration bench. Likewise, labels for the demonstrations must not be interfered with to avoid confusing your colleagues.

General Rules

- All liquid spills, whether on the bench or floor, must be wiped off immediately.
- Leave your working bench tidy after the session.
- All equipment must be handled with care and according to instructions given by your instructor.
- Do not throw wastes on the floor or working bench. Throw the wastes in the bin.
- Other materials not relevant to the practical session should not be brought to the lab. Maintain a good working space for yourself so that you should work comfortably.

BIOLOGICAL DRAWINGS

In biology, you will often be asked to draw what you observe. You do not need to be a great artist in order to make a correct biological drawing. A biological drawing is meant to be an accurate image and a permanent record of the life form, or life process observed. A biological drawing should only be drawn while observing the specimen, not from memory. The specimen should be looked at every few seconds to make sure that the drawing is accurate, precise and a faithful representation of what you have observed. Any mistakes should be erased and corrected.

Rules for Biological Drawings

Technique: Look at the specimen every few seconds as you are drawing. Never draw from memory, but while looking at the specimen. Drawing at a location away from your specimen violates scientific integrity.

Accuracy: Draw what is there, not what you think should be there. Do not idealize the diagram or drawing, it should represent reality. Proportions should be accurate.

Materials: Draw on white, plain paper with a lead pencil. Use a clean eraser for mistakes. Pens are not acceptable because they cannot be erased.

Style: Drawings and/or diagrams should be simple with clean lines. Do not sketch. Do not shade the drawing.

Size: Drawings should be large enough to show all the parts without crowding. The greater the number of parts, the larger the drawing should be. A good rule of thumb is to start at half a page in length for a drawing. You do **not** have to show everything.

Positioning: Start the drawing just to the left of center of the page, reserving the right-hand side for the labels. Do not draw in the corner.

Labels: Leave a good margin for labels. Use the ruler for label lines. Label lines should never cross and should not be ungainly long. Keep the labeling lines horizontal, straight and parallel to each other. Label lines should not have head arrows.

Always include the following:

- Title – Place it at the top of your drawing
- Magnification – it should be below your drawing

Types of Biological Drawings

Anatomical Drawing

Shows as much **details** as possible, especially if the specimen is observed under a microscope. Individual cells, organs or organelles are illustrated.

Map Drawing

Shows only the **outline** of structure or tissues or parts. You do not draw individual cells, but you only show location or position of tissues or organs or structures of interest. For instance, to make a map diagram of a transverse section (T.S) of a root showing xylem and phloem, you only draw an outline around where the xylem and phloem tissues are located – you do not draw individual Xylem and phloem cells. However, the tissue layers on the map should be proportional to that in the section, i.e. structures/organs in the map diagram should be proportional to the specimen from which they were made. See **Figures 1 and 2** below.

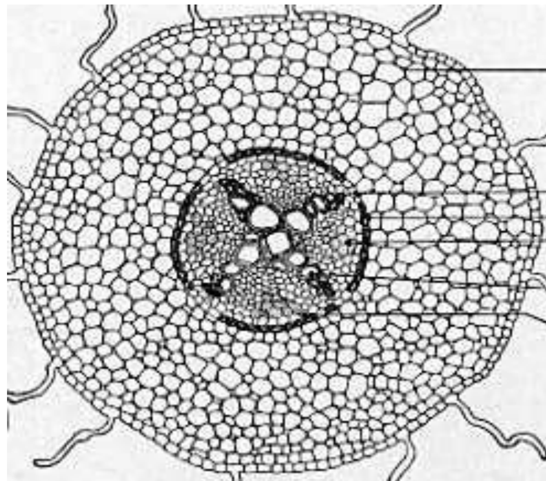

Figure 3: Anatomical drawing showing cross-section of a root

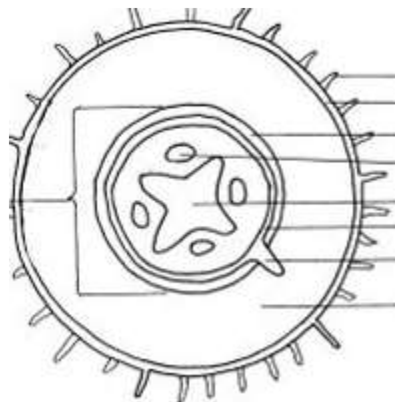

Figure 4: Anatomical drawing showing cross-section of a root

Assignment:

Make a biological drawing of palisade mesophyll cell as seen under a light microscope and label it fully.

Unit Summary

This unit has covered the following points

1. Things to take on board when coming for a practical session
2. Equipment handling in the laboratory
3. Types and rules for biological drawings

………….

PRACTICAL 3: CARE AND USE OF COMPOUND MICROSCOPE

Background

In the study of General Biology I, you will need to view biological structures and organisms too small to be seen by an unaided eye. You will have two types of microscopes to assist you in viewing these specimens. These microscopes are compound microscope and dissecting microscope. The unaided eye has the ability to distinguish between two structures if they are at least 0.1mm apart (resolving power). By using a light microscope we are able to distinguish between two structures that are at least 0.1μm apart.

Objectives

By the end of this session, you should be able to:

1. properly clean and carry a compound and dissecting microscope.
2. identify the parts of the compound and dissecting microscopes and explain the function of each part.
3. adjust the diopters to fit one's own eyes.
4. focus a specimen using all objectives of a compound microscope.
5. focus a specimen using a dissecting microscope.

Activity 1: Care and Cleaning Compound Microscope

Microscopes are delicate precision instruments. They must be well cared for. When carrying a microscope, carry it with both hands. One hand should grab the microscope by its arm and the other hand should be placed under the base of the microscope to ensure the microscope is firmly held. Since the microscope you will be using is also used by several other students, it is important to clean the ocular lens and the objective lens before and after use. To do this take a piece of lens paper and dampen it with the lens cleaning solution found at each table. It is important to only use lens paper and not paper towels, since the latter might scratch the lens.

Questions

1. State two things that you are supposed to use when cleaning ocular and objective lenses.

2. Give a reason why you cannot use other things to clean ocular and objective lenses.
3. Why is it important to clean ocular and objective lenses of a microscope?

Activity 2: Identifying Parts of a Compound Microscope

Now that you have a microscope on your bench and you have cleaned the ocular lens and objective lens, it is time to become familiar with parts of the microscope and their functions. To assist you in doing this you will need to find each part described below on the microscope and then write its name down. We will start at the base of the microscope and work our way up to the oculars.

1. The **light source** is found in the **base** of the microscope (which bears the weight of the microscope). It is activated by turning on the **light switch** at the back of the microscope. The intensity of the light is adjusted by turning the **light intensity control knob** on the base.

2. The **iris diaphragm** is located just above the light source at the bottom side of the stage. Using the lever attached to it you can increase or decrease the amount of light reaching the specimen.

3. Between the stage and the iris diaphragm is the **condenser**. The condenser further aids in the focusing of the light onto the specimen (it concentrates light). It can be moved up and down by the black knob called the **condenser knob** that is located on the right side of the stage. Take a moment to move the condenser up and down and then position it up close to the stage. For the purpose of this class we do not need to change the position of the condenser. If you have a problem focusing your specimen always check the position of the condenser before calling over the instructor.

4. Above the condenser lies the **stage**. It is mounted at a right angle to the arm and positioned just below the nosepiece. The stage is where you will place your specimen. It is through the movement of the stage up and down that you will bring your specimen into focus.

5. Resting on top of the stage is the mechanical stage. This contains a **spring clip** that will hold the slide in place. To the right of the mechanical stage are two **control**

knobs that allow you to move the slide left and right and backwards and forwards. This will enable you to look at all areas of the specimen.

6. At the back of the stage is the **arm** of the microscope that supports the head of the microscope. It is connected to the base and is a good place for you to grab hold of the microscope when you need to carry it or lift it out of its storage cabinet.

7. Attached to the arm are the **coarse** and **fine adjustment knobs**. These knobs move the stage up and down for the purpose of focusing the specimen. The course adjustment knob moves the stage a large visible distance with a single turn and as such should be used only with 4X and 10X objectives. It should NEVER be used with the 40X and 100X objectives. You run the risk of damaging these objectives and breaking the slide if you do not heed this warning. The fine adjustment knob is used to move the stage up or down only very slightly. Since these microscopes are **parfocal** (all the objective lens focus the image in the same plane), once you have focused your specimen at the 4X or 10X then when you progress to the next objective you will only need to use the fine focus to make the minor adjustment needed for the specimen to be in focus.

8. Above the stage and attached to the **rotating nose piece** are the four objective lenses. They are called **objective lenses** because they are closest to the object or specimen you are looking at. The magnification of the lenses are 4X marked by a red ring, 10X marked by a yellow ring, 40X marked by a blue ring and 100X marked by a white ring. Generally, 4X is referred to as the **scanning lens**, 10X as the low power lens and 40X as the high power lens. The 100X is the oil immersion lens and must be used by placing a drop of immersion oil on the slide before clicking it into place. The 100X lens will NOT be used in this course. Please note that the length of the lens increases as their power of magnification increases.

9. The image magnified by the objective lens in use is passed up through the body tube into the **oculars**. Each ocular contains two lenses for a total magnification of 10X. The total magnification of the microscope is the product of the ocular lens and the magnification of the objective lens in use. This means that if one has the 10X objective lens in place the total magnification that you will see is the product of 10 x 10 or 100X. It is this combined magnifying power that makes this

microscope a compound microscope. What would be the total magnifying power of the microscope if the 40X objective was used?

Questions

1. Why is it discouraged to use coarse adjustment knob with 40X and 100X objectives?
2. Give a reason why 100X objective is called oil immersion objective.
3. The four objectives of a compound microscope are parfocal. What does this mean?
4. What makes a microscope to be a compound microscope?

The Dissecting Microscope

When a biologist needs to look at a specimen too large or thick to be viewed by a compound binocular microscope and too small to be viewed in sufficient detail with the naked eye he/she will use a dissecting microscope or stereoscopic microscope.

1. Use the dissecting microscope on your bench to locate the following parts and write the names down.

2. **Adjustment focus knob** allows you to focus the objective on the specimen.

3. The image magnified by the **objective lens** in use is passed up through the body tube into the **oculars**. Each ocular contains two lenses for a total magnification of 10X.

4. **Light control switches**: There are two switches that control the light on this microscope. One of them turns on either the top light or the bottom light or both. The other knob controls the intensity of the light.

5. **Magnification adjustment knob**: On this microscope instead of individual objectives you can adjust the amount of magnification by turning this knob.
6. **Two light sources**: On this microscope you have a base light source and an upper light source. You may use either one or both depending on the type of specimen you are observing.

Questions

Describe differences between compound microscope and Dissecting microscope.

Activity 3: Viewing Letter "e"

1. Using a piece of lens paper clean the ocular lens, each of the four objectives, the condenser (through the opening in the stage) and the light source.
2. If the 4X objective is not currently in line with the body tube rotate the 4X objective until it clicks into place.
3. Plug in the microscope and turn on the light source.
4. Collect letter "e" from the demonstration bench. Put it at the centre of the glass slides. Add a drop of water. Cover it with a coverslip making sure there are no air bubbles. Open the spring clip and place the slide on the stage so that you can read the "e" as you look at the stage. Using the stage control knobs move the slide until the "e" is positioned over the opening above the condenser lens and is illuminated by the light.
5. Raise the stage up as close as it will go to the objective using the coarse adjustment knob. The microscope has an automatic stop built in to prevent the slide from hitting the 4X (please note this stop will not prevent the slide from hitting the 40X or 100X objectives). As you look through your ocular lens use the coarse adjustment knob to move the stage away from the objective until the object is in focus. If nothing comes into view after several turns of the coarse adjustment knob you will need to check for the following errors: 1) the letter "e" was not positioned over the opening in the stage, 2) you lowered the stage too quickly and missed the letter "e", or 3) you have not lowered the stage far enough to see the letter "e". It may be necessary to repeat steps 4-5 to avoid any of the above three errors.

6. Once you have the letter "e" in focus use the mechanical stage control knobs to move it into the center of your field of view. If the "e" still needs a minor focusing adjustment use the fine focus knob to complete the focusing. If the field of view is too bright you can decrease the light by closing the iris diaphragm.
7. Make observations.
   a. Draw the letter "e" as it appears in your field of view
   b. Draw the letter "e" as it appears when you look at it on the stage
   c. Does the letter appear different when viewed through the microscope?
   d. If so how?

e. Is the letter larger or smaller when viewed through the microscope?
f. While looking through the oculars move the slide away from you. Which way did the letter move in your field of view?
g. While looking through the oculars move the slide to the right. Which way did the letter appear to move in the field of view?
h. Is it possible to bring the entire letter "e" into clear focus with the fine adjustment? Or is the outer edge slightly out of focus when the center is clear?
.

8. Now move the 10X objective in line with the body tube. Since this microscope is parfocal you should only need to make minor adjustments to the focus using the fine focus adjustment knob. You may find that you now need to open the iris diaphragm to let more light in.
    a. Why do you think that is needed?
    b. Is the letter larger or smaller when viewed through the microscope?
    c. While looking through the oculars move the slide away from you. Which way did the letter move in your field of view?
    d. While looking through the oculars move the slide to the right. Which way did the letter appear to move in the field of view?
    e. Is it possible to bring the entire letter "e" into clear focus with the fine adjustment? Or is the outer edge slightly out of focus when the center is clear?
    .
9. Now move the 40X objective in line with the body tube. Since this microscope is parfocal you should only make minor adjustments to the focus using the fine focus adjustment knob. You may find that you now need to open the iris diaphragm to let more light in. Is it possible to bring the entire letter "e" into clear focus with the fine adjustment? Or is the outer edge slightly out of focus when the center is clear? What conclusion do you make?

PRACTICAL 4: TOTAL MAGNIFICATION AND RESOLVING POWER

Objectives

By the end of this laboratory session, you should be able to:

a) define magnification and resolution,
b) work out the total magnification of objectives of a particular microscope,
c) measure Field of View at a particular objective,
d) estimate the size of specimen when viewed through microscope.

Activity 1: Information

The reason for using a microscope is to magnify features to the point where new details can be resolved.

Magnification is the factor by which an image appears to be enlarged. It will be a whole number greater than 1 and is usually followed by a "×", as in 10× magnification. When you look through microscope eyepieces, you are seeing a **virtual image** because in reality, what you are looking at is not as large as it appears through the eyepieces, and because there can be some distortion of the image.

Resolution is the shortest distance between two points that can still be visually distinguished as separate. The resolution of a typical unaided human eye is about 200 µm. Using a microscope decreases the resolution to distances as short as 0.2 µm. Resolution is a property of the eye.

Resolving power is the ability of a lens to show two adjacent objects as discrete. Resolving power is a property of a lens.

Each lens in a microscope has a numerical aperture, or NA, value. This has to do with the angles of light that enter and exit a lens. Its applications are beyond the scope of this lab, but numerical aperture does influence the resolution possible with a particular lens, and so the NA value for the lens is usually printed on each objective and it is a number less than 1.0. The value for numerical aperture measures to what extent the light that passes through a specimen is spread out over and collected by the objective lens. The light that passes through the specimen contains information about what the specimen looks like, that is, about its structure.

Numerical aperture can be defined as:

$NA = n \times \sin \mu$

Where n = refractive index of substance between the specimen and the objective lens (usually air, n = 1.0; quartz, n = 1.5; glass, n= about 1.5; water, n = 1.3)

μ = 1/2 the aperture angle (also called the semi angle). The aperture angle is the angle described by the cone of light that enters the objective lens after passing through the specimen. This angle will depend on the curvature of the lens and also on how close the objective lens is to the specimen when it is in focus.

So, for an objective with an aperture angle of 120° with air between specimen and objective lens,

$NA = 1 \times \sin 60^\circ = \sin 60^\circ = 0.87$

If oil with refractive index of 1.5 is used between the objective lens and the specimen,

$NA = 1.5 \times \sin 60^\circ = 1.5\ (0.87) = 1.31$

Now, numerical aperture is important because it allows us to calculate the resolving power of the objective.

$\text{Resolving Power} = 0.61 \times (\lambda / NA)$

Where λ = wavelength of light (average value for white light ~ 550 nm), NA = numerical aperture

So, for air situation,

$\text{Resolving Power} = 0.61 \times 550\text{nm}/0.87 = 386\text{ nm} = 0.000000386\text{ m} = 0.386\ \mu\text{m}$

For oil immersion,

Resolving Power = 0.61 × 550nm/1.31 = 256 nm = 0.000000256 m = 0.26 μm

Thus, one can see that higher resolution is possible if the substance lying between the specimen and the objective lens has a refractive index as close as possible to that of the lens itself without exceeding the lens' refractive index.

Activity 2: Total Magnification

Each lens in a microscope also has a **magnifying factor**. This is the degree to which that lens magnifies an image. It will be a number larger than 1.0. For instance a 10x objective magnifies the image ten-fold. The magnifying factor for each objective always printed on it, and the magnifying factor for each eyepiece is usually printed on it.

The **total magnification** for any image viewed under a compound microscope is calculated by using the formula:

Total Magnification = Eyepiece Magnifying Factor × Objective Magnifying Factor

There is a compound microscope on the bench. Carry out the activities listed below and answer the questions as you do.

1. Write down the magnification factor for the eyepiece lenses on the microscope in front of you.
2. Using the microscope in front of you, write down all the words and numbers written on each objective on your microscope. There are probably three objectives, but some microscopes might have four. Start with the smallest objective and move through them in order of increasing size.
3. In the above list, for each objective, circle just the magnification factor for that objective. Remember, the magnifying factor is a whole number, and differs for each different objective.
4. Write down the total magnification when using each objective on the microscope in front of you.

Activity 3: Measuring FOV Diameter

Field of view of a microscope is the area you see under the microscope for a particular magnification. The circle you see has a diameter and it is called diameter of field of view

of the microscope. As you increase the magnification, the field of vision is reduced. Depending upon the lens system, this can vary. Note that the extent of the Field of Vision depends on the magnification. Objectives with higher magnifications have smaller Fields of Vision.

Methods of Measuring FOV Diameter

- Measuring using a transparent ruler and calculation using proportion for high and Oil immersion objectives
- Measuring using eyepiece micrometer
- Calculating using information given on the microscope objectives and oculars

Measuring FOV Diameter using Transparent Ruler

The size of the field of view under the microscope decreases proportionately when the magnification is increased. If the diameter of the field of view under the lowest magnification is known, the relative field diameters of other magnifications can be calculated.

Procedure

a) Place a transparent ruler (in mm divisions) on the stage of the microscope (Your ruler is the specimen). Use 4× objective.
b) Focus it and obtain the image of division lines.
c) Align the edge of the ruler along the diameter of the FOV. You should be able see division lines (not full lines)
d) Count the division lines on the ruler that cover the diameter from one end to the other.
e) Measure the diameter of the FOV and record it.
f) Convert the diameter in mm to μm
g) Change to 10× objective. Repeat steps (a) – (f)
h) The division lines of a ruler are too thick to be used to measure diameter of FOV at 40× and 100× objectives. Derive the formula that you should use to calculate diameter at these objectives. Show the formula and conditions under which it can be used. Use the formula to calculate diameter for 40× and 100× objectives.

Measuring FOV Diameter using Eyepiece Micrometer

Procedure

a) Obtain the eyepiece micrometer from the demonstration bench.
b) Calibrate it using a stage micrometer.
c) Align the scale of the eyepiece micrometer along the diameter of the FOV.
d) Count the division lines on the eyepiece micrometer scale that cover the diameter form one end to the other.
e) Measure the diameter of the FOV and record it. Diameter units will be in μm.
f) Change to 10× objective. Repeat steps (a) – (e)
g) Change to 40× objective. Repeat steps (a) – (e)
h) 100× objectives requires oil for it to work. Using 4× objective, calculate diameter at 100× objectives by using proportion.

Calculating FOV Diameter using Information given on the Microscope Objectives and Oculars

To calculate the diameter of field of view of microscope you need to know the field number and objective lens magnification. Once you have this information you can calculate the diameter of field of view of the microscope by dividing the field number by the magnification of the objective lens.

Diameter of Field of View = Field Number (FN) ÷ Objective Magnification
If your microscope only uses an eyepiece you use eyepiece magnification. If your microscope uses both an eyepiece and an objective lens, you use magnifying factor of the objective lens.

For example, consider your eyepiece reads 10X/22, and 40× objective lens.

10× is magnifying factor for the eyepiece
22 is the Field Number
40× is the magnifying factor of the objective lens.

Divide 22 by 40 to get an FOV diameter of 0.55mm. Convert mm to μm. Calculate FOV diameter for the other objectives.

Estimating and Measuring size of Specimen viewed under a microscope

Once the FOV diameter in micrometers is known, it is possible to estimate the size of an object by comparing its size to the FOV diameter. To do this, use prepared slides with algal cells. Focus the cells at scanning objective. Align the cells along the FOV diameter. Count the number of cells that cover the FOV diameter end to end. Use the formula below to work out the size of each cell.

$$\textbf{Actual Cell Size } (\mu\mathbf{m}) = \frac{\textbf{FOV Diameter in micrometers } (\mu\mathbf{m})}{\textbf{Number of Cells in Diameter}}$$

Examine and estimate size for five cells. Find the average size of an algal cell.

To measure the size of an algal cell,

a) Obtain eyepiece micrometer from the demonstration bench and calibrate it using a stage micrometer.
b) Place prepared slide of algal cells on the stage and focus to obtain their image at scanning objective.
c) Choose a cell of your choice. Cover it with eyepiece micrometer scale.
d) Count the number of line divisions of the eyepiece micrometer that cover your cell end to end.
e) Measure the size of cell. Measure five cells and find the average size of the algal cell.
f) Repeat this at low power objective.
g) Draw the algal cell and work out its magnification.
h) Is the estimated size of the algal cell different from the measured one?

PRACTICAL 5: PROPERTIES OF WATER

Activity 1: Cohesion / Surface Tension

Cohesion is the ability of water molecules to stick to themselves (H-bonds). Surface tension is a property of water created by cohesion that enables a drop of water to keep its shape.

1. Predict how many drops of water will fit on the surface of a K10 coin before it spills over.
2. Put drops of water (one at a time) on the top of the coin until the water spills over the edge. Record your data in the table. Include the data of your class then calculate the average number of drops.
3. Adding surfactant to the surface will reduce the surface tension of water causing it to lose its shape and spread out. Predict how many drops of water will fit on the surface of a dry coin after it has been smeared with a surfactant.
4. Place one drop of soap on your finger then spread it on the surface of a dry coin.
5. Put drops of water (one at a time) on the top of the penny until the water spills over the edge. Record your data in the table. Include the data of your class calculate the average number of drops.

Activity 2: Water as a Good Solvent

A solvent is something a substance is dissolved into. Complete the following test to see if water really does dissolve substances better than any other liquid.

1. Measure 50 mL of each of the following: water, rubbing alcohol and oil into two clean beakers for each of the three substances.
2. Place 1 spatula full of salt, sugar and sodium chloride in each of the beakers at the same time.
3. Stir the contents in the beakers using stirring rod.
4. Observe for 1 minute then record the data below using the key shown below.
5. Discuss why water is referred to as a universal solvent.

Key: ++ *100% soluble, + partially soluble, - Did not dissolve*

Activity 3: pH

Common house hold items have a variety of different pH levels. Test the following unknowns to determine which are acidic, basic, or neutral.

1. Dip the small strip of pH paper into each of the 5 unknown substances. Be sure to use a different strip of paper for each substance.
2. Using the pH scale on the package, immediately read the pH of each level.
3. Record your data for each substance in the table. Also indicate if the substance was acidic, basic, or neutral.

Activity 4: Adhesion (capillarity)

Adhesion is the attractive force that occurs between two different substances as in glass and water. Demonstrate this force by completing the procedure below.

1. Place the capillary tube vertically into the cup of colored water.
2. Observe, describe and sketch what happens. Be sure to include in your description which property of water allows this action to occur.

Activity 5: Water is Less Dense as a Solid

1. Observe the ice floating on the water and the frozen water bottle.
2. What normally happens to materials when they get colder? (Expand or shrink?)
3. Think about why the bottle has been "pushed outward".
4. Explain why ice floats on water. (What makes it less dense?)
5. How is this important to life on Earth? Give an example.

Activity 6: High Heat of Vaporization

1. Spray the back of your hand with the water. Think about how that feels on your skin.
2. When you boil water it seems to take forever for the water to heat up. Why do you think that is?
3. What is meant by the term "high heat of vaporization"?
4. Explain why this property of water is important to life on Earth.

PRACTICAL 6: INDICATIVE TESTS FOR MACROMOLECULES

Introduction

One characteristic of life is that living things are made up of molecules containing carbon. These are called **organic molecules**. In class you have been referring to them as macromolecules since they are necessary for life. The most common organic compounds found in living organisms are **lipids, carbohydrates, proteins**, and **nucleic acids**. Common foods, which often consist of plant materials or substances derived from animals, are also combinations of these organic compounds. Simple chemical tests with substances called indicators can be conducted to determine the presence of organic compounds.

Objective

To carry out indicative test for presence of lipids, carbohydrates and proteins in various food stuffs.

Activity 1: Tests for Lipids

Spot test

1) Draw 5 small squares, approximately 3 cm each on a brown paper, and label each with the name of one of the foods (Cooking oil, A, B, C, D and distilled water)
2) Put 1 drop of each of the foods in the corresponding boxes on the brown paper.
3) Allow the brown paper to dry.
4) Look for a grease stain or transparency in the box for presence of lipids.
5) Record your observations in the data table.

Sudan IV Test

1) Put 1 dropper full of each foods (Cooking oil, A, B, C, D and water).
2) Add 2 cm^3 of water to each of the test tubes.
3) Add a few drops of Sudan IV and shake.
4) Note the change in colour
5) Oils are stained red with Sudan III. Since they are less dense than water, they separate out as a red layer on the water surface

Activity 2: Tests for Carbohydrates

Iodine Test

1) Put 1 dropper full of each food (Starch, A, B, C, D and distilled water) in 6 different test tubes.
2) Add 10 drops of Iodine Solution to each test tube.
3) Check for any color change and record in data table.

Benedict's Test

1) Put 1 dropper full of each food (Glucose, A, B, C, D and distilled water) in 6 different test tubes.
2) Add 10 drops of Benedict's Solution to each test tube and place them all **carefully** into the hot water bath for 3-5 minutes.
3) Remove test tubes from hot water bath using designated tongs and place them into test tube holders. Note the color change and record into the data table. Colour change should progress in the colors of blue (with no glucose present) to green, yellow, orange, red, and then brick red or brown

   NB: A sample of food which tests negative may contain other non-reducing sugars.

Activity 3: Tests for Proteins

Biuret Test

1) Put 1 dropper full of each food (Egg albumin, A, B, C, D and distilled water) in 6 different test tubes.
2) Add 10 drops of Biuret's Solution to each test tube.
3) Check for any color change and record in data table.

Ninhydrin Test

1) Put 1 dropper full of each food (Egg albumin, A, B, C, D and distilled water) in 6 different test tubes.
2) Add 2 ml of Ninhydrin solution.
3) Boil the contents and check for any colour change.

Questions

1. What name is given to the process in which monomers join together to form larger molecules?
2. Glucose and fructose have the same formula. What is the main difference between them?
3. Briefly describe the underlying principle for each of the following:
    a. Iodine test.
    b. Benedict's test.
    c. Biuret test.
4. In your view, does Malawi have a wide source of protein? Discuss with examples. (Not more than 10 sentences)

PRACTICAL 7: CELL STRUCTURE AND FUNCTION

Introduction

The cell is the basic unit of structure and function of all living things. All organisms are made up of at least one cell. Large organisms, such as humans, are made up of trillion of cells. Understanding of the structure and function of the cell is essential in understanding the process of life. There are two types of cells: the prokaryotic cell and eukaryotic cell. A prokaryotic cell has no nuclear membrane and their genetic material (the DNA) is said to be "naked." Internal membranous subcellular structures called organelles are absent in the prokaryotic cells, while these are present in the eukaryotic cell.

In this practical, you are going to examine under the microscope two different kinds of cells: plant cells and human epithelial cells.

Objectives

a. Explain the difference between prokaryotic and eukaryotic cells and be able to distinguish each type under the microscope.
b. Compare and contrast animal and plant cells and be able to distinguish each type under the microscope.
c. Identify the following parts of the cell and explain the functions of each: plasma membrane, cytoplasm, nucleus, and cell wall.
d. Examine the diversity in cell size and shape.
e. Properly prepare and view wet mount slides under the microscope.

Plant Cells: Epidermal cells of an onion *(Allium cepa)*

1. Obtain a clean slide and a cover slip.
2. Cut the onion bulbs in quarters.
3. Using forceps, break off a small portion of a leaf and gently pull up a portion of the epidermis (It should be very thin and mostly transparent).
4. Place the peeled epidermis on the slide and add one drop of iodine on the surface.
5. Holding the cover slip at an angle on the edge of the drop of iodine, slowly lower the cover slip down on top of the epidermis. There should be minimal air bubbles if done correctly.
6. Gently wipe the bottom of the slide before loading it on the microscope if any iodine has escaped the cover slip.

7. Examine the epidermis at scanning, low and high power. Note the shape of the cells.
8. Draw one cell as seen at HP objective and label the following parts: nucleus, cytoplasm, cell membrane and cell wall. What is the function of each of these parts?
9. Estimate the size of the cell you have drawn. Use the estimated size to work out the magnification of your drawing.

Animal Cells: Human Epidermal Cells (Cheek Cells)

The tissue that lines your cheeks contains multiple layers of flattened cells that are constantly sloughing off as you eat and drink. The layered nature of these cells serves to protect the underlying tissue against this abrasion. New cells are constantly being produced in the lower layers to replace those that are lost. Prepare a cheek smear slide of your own cells using the following method:

1. Obtain a clean slide and a cover slip.
2. Gently rub the inside of your cheek with a toothpick and smear the collected fluid onto the slide.
3. Stir/tease the scraping in a drop of water at the centre of the glass slide. Stirring or teasing dislodge individual cells or makes the scraped material thin.
4. Discard the used toothpick in the bleach solution at the instructor bench.
5. Add a drop of dilute methylene blue stain to the slide and cover with the cover slip.
6. View the slide at scanning, low and high power objectives. Note the cell shape.
7. Draw one cell and label the following parts: plasma membrane, cytoplasm, and nucleus.
8. Estimate the size of the cell you have drawn. Work out the magnification of your drawing.

***Note**: When you are finished with the cheek slide, place it in the container of bleach at the instructor bench.*

Questions

1. Give two examples of each of the following: (a) Prokaryotes (b) Eukaryotes
2. Discuss differences between a prokaryotic cell and eukaryotic cell.
3. Compare and contrast plant and animal cells.

PRACTICAL 8: RATE OF OSMOSIS AND DIALYSIS

Activity 1: Rate of Osmosis

In this experiment you will examine the effect of a concentration gradient on the speed of water movement across a semipermeable membrane (dialysis tubing). You will compare the rate of osmosis for 3 different combinations of solutions:

Materials

Dialysis bags soaking in water
3 beakers
1 funnel
Rubber bands
Solutions: 10% sucrose, 20% sucrose, 1% sucrose
Paper towels
Watch

Bag Setup **BAG**	**INSIDE BAG**	**IN BEAKER**
1	tap water	20% sucrose
2	1% sucrose	tap water
3	10% sucrose	tap water

Procedure

1. Take one dialysis bag out of the beaker and tie off one end (instructor will demonstrate how to tie off the bags to prevent leaks). Fill the bag with 20 ml of tap water, using the funnel. Squeeze any air out of the bag, being careful **not** to use your fingertips (the oil on the skin of your fingertips can damage the dialysis membrane). Tie off the opposite end of the bag.
2. Dry the bag thoroughly with paper towels, especially the knotted ends. Weigh the bag on the balance.

3. Put the bag in a labeled 400 ml beaker, and fill the beaker with 20% sucrose to just cover the bag. Note time.
4. Fill the second dialysis bag with 1% sucrose, tie it off, dry it, weigh it, put it in a separate, labeled, 400 ml beaker with enough tap water to cover the bag, and again note the time
5. Fill the third dialysis bag with 10% sucrose, tie it off, dry it, weigh it, put it in a separate, labeled 400 ml beaker with enough tap water to cover the bag, and once more note time.
6. Weigh each bag every 15 minutes for one hour - make sure you dry the bag thoroughly before each weighing. Also, make sure the bags stay submerged in the liquid - if necessary, weight them down with a pen or pencil.
7. Use the table below to keep track of your weighing times and the weights of the dialysis bags.

	Weight at T = 0 min	Weight at T = 15 min	Weight at T = 30 min	Weight at T = 45 min	Weight at T = 60 min
Bag 1					
Bag 2					
Bag 3					

8. Plot a graph of weight change of each bag against time for each experiment.
9. Calculate the initial rates of osmosis for bags 1, 2 and 3. Initial rate of osmosis = weight at 15 min - weight at 0 min / 15 min.
10. Given the formula for the initial rate of osmosis, write the formula for the final rate of osmosis and calculate the final rates of osmosis for bags 1, 2 and 3.

Questions

1. Why did some of the dialysis bags gain weight while other bags lost weight?
2. What produced the difference in the rate of weight change among the 3 bags?
3. Do you think there will be a difference in the initial and final rates of osmosis for any of the bags? Why or why not?
4. What molecule was moving across the dialysis membrane to produce the weight changes observed in the dialysis bags?

Activity 2: Dialysis

The ability of a molecule to diffuse through a semipermeable membrane depends on its size and shape. The process of dialysis takes advantage of a molecule's ability to diffuse across a semipermeable membrane in order to separate large and small molecules. In this experiment you will compare the ability of glucose and starch molecules to cross dialysis tubing, a semipermeable membrane.

Materials

1 piece of dialysis tubing, soaking in water
Beaker
Funnel
4 test tubes
Test tube holder
Colored tape and marking pen
Iodine solution and Benedict's solution
Starch (10%) and glucose (5%) solution
Rubber bands

Procedure:

1. Tie off one end of the dialysis tubing with rubber bands.
2. Using a funnel, fill the bag with 20 ml of the starch/glucose solution. Make sure all the air is out of the bag, and tie off the other end with twine.
3. Immerse the bag in a beaker of tap water, and make sure the bag stays under the surface of the water.
4. Let the bag sit in the beaker of water for 15 minutes.
5. Label 4 test tubes:
   - IN - starch
   - OUT - starch
   - IN - glucose
   - OUT – glucose
6. At the end of 15 minutes, cut one end off the dialysis bag and pour a few ml into the "IN" test tubes. Pour a few ml of the beaker water into the "OUT" test tubes.

7. Add 10 drops of iodine solution to the tubes labeled IN - starch & OUT – starch. A dark blue color indicates the presence of starch. Record your results in the table below.
8. Add 10 drops of Benedict's solution to the tubes labeled: IN - glucose & OUT – glucose. Put the test tubes containing the Benedict's solution in a boiling water bath (on the side bench) for 1-2 minutes. The blue color will change to green, orange or yellow in the presence of glucose. Record your results in the table below.

Questions

Based on what you know about the relative size of glucose and starch molecules, identify which molecule(s) will diffuse out of the bag and which molecule(s) will stay inside the bag.

PRACTICAL 9: OBSERVING MITOSIS IN ONION ROOT TIPS

Objectives:

By the end of this practical, students should be able to:

a) Understand the process and stages of mitosis.
b) Prepare your own specimens of onion root tip in which you can visualize all of the stages of mitosis.
c) Apply an analytical technique by which the relative length of each stage of mitosis can be estimated.

Procedure

1. Take the onion plant with newly sprouted roots and cut two root tips using scissors and transfer them into a vial tube.
2. Fill 1/2 of the tube with 1N HCl using a dropper.
3. Place the tube in a 60°C water bath and incubate the tube for 12- 15 minutes.
4. Remove the tube from the water bath after the incubation.
5. Discard the HCl from the tube using a Pasture pipette to the running tap water.
6. Add some drops of distilled water into the tube and rinse the root. Then remove the water from the vial tube using the Pasture pipette. (Rinse the roots at least three times).
7. After the washing step add 2-3 drops of aceto-carmine stain into the tube with root tips and incubate the roots for 12-15 minutes. (During the incubation, the very tip of the root will begin to turn red as the DNA stains the numerous small actively dividing cells at the time).
8. After the incubation remove the stain using a Pasture pipette.
9. Again rinse the root tips with distilled water. (Rinse the roots at least three times).
10. Transfer a root from the tube to the centre of the microscopic slide and add a drop of water over it.
11. Take a razor blade and cut most of the unstained part of the root.
12. Cover the root tip with a cover slip and then carefully push down on the cover slide with the wooden end of a dissecting probe. (Push hard, but do not twist or push the cover slide sideways). The root tip should spread out to a diameter of about 0.5 - 1cm.
13. Observe it under a compound microscope in 10x objective. Scan and narrow down to a region containing dividing cells and switch to 40x for a better view. Examples are shown below.

14. Draw cells showing each stage of mitosis.

Estimating the relative length of each stage of mitosis

For this procedure, we will use permanent slides of onion root tip. Where possible use slides prepared above.

Procedure

1. Starting at the 10x objective, find the region of active cell division.
2. Switch to the 40x objective and begin observations at the lower end of this region.
3. Take turns as observer and recorder. The observer should call out the stage of mitosis of each cell to be tallied by the recorder in the results table. Roles should be switched for the second slide. Since prophase and prometaphase are difficult to distinguish, classify these cells as prophase. Only count as prophase cells that contain distinctly visible chromosomes.
4. Systematically scan the root tip moving upward and downward through a column of cells.
5. Tally each cell in a stage of mitosis that you observe, being careful not to record the same cell twice.

Calculations

1. Pool your data with that of the class, and then record the class totals in the table.
2. Calculate the percentage of cells in each stage.
3. Calculate the relative time span of each stage. (Hint: Percentage of cells in each stage is also the percentage of time span for mitosis for that particular organism).

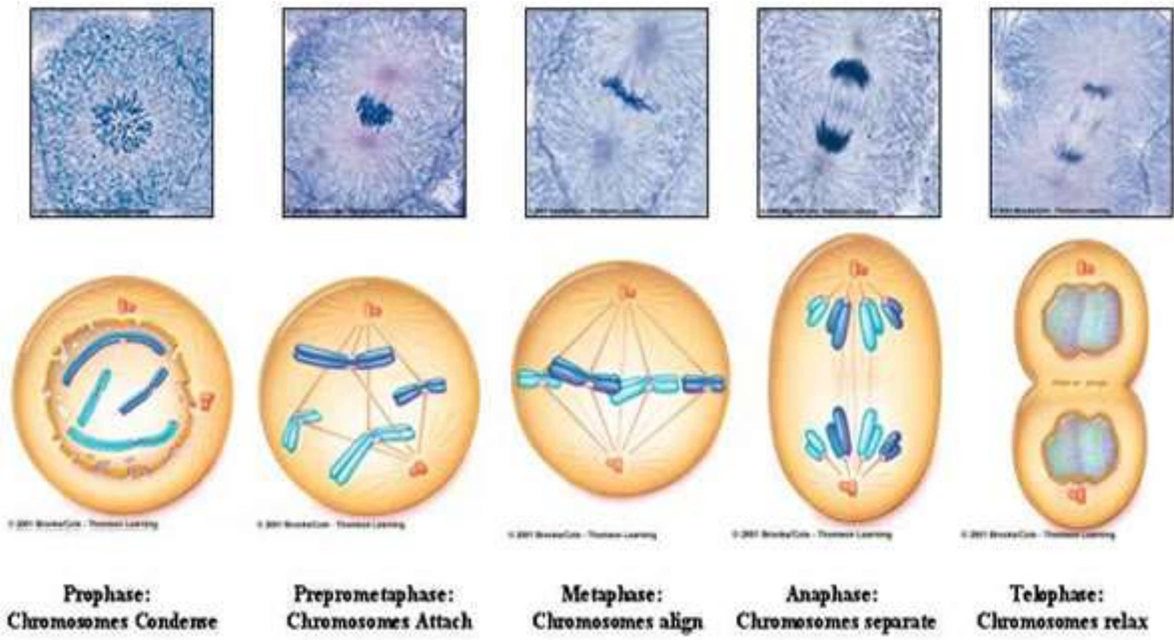

Figure 5: Stages of mitosis

PRACTICAL 10: DIVERSITY OF ORGANISMS: BACTERIA, FUNGI AND PROTOZOA

Activity 1: Bacteria

Bacteria are microorganisms that grow everywhere. We can collect and grow them in specially prepared petri dishes. Blood agar is an excellent medium for supplying bacteria with nutrients and an environment in which we can see them grow.

1. Obtain prepared petri dishes from your instructor.
2. Using a dropping pipette, add 3 drops of pond water on the surface of agar plate. Spread the water using a sterile swab. Cover the agar plates. Allow them to stand for 5 minutes.
3. Tape close, and label each dish.
4. Incubate the petri dishes at room temperature with covers down.
5. You should see growth within a couple of days. The dishes will start to smell which means the bacteria are growing.
6. Make observations and keep records of what you see growing in each dish.
7. In what form are you able to see bacteria? Are you able to see bacterial cells with unaided eyes?
8. Count the number of different types of colonies in each plate.
9. Examine permanent slides for shapes of bacteria. Make a drawing of each shape of bacteria.

Activity 2: Fungal Enumeration

1. Obtain prepared petri dishes from your instructor.
2. Suspend 10g of soil in 100 mm of sterile water. Let the suspension stand for 10 minutes.
3. Using a dropping pipette, add 3 drops of soil suspension on the surface of agar plate. Spread the suspension using a sterile swab. Cover the agar plates. Allow them to stand for 5 minutes.
4. Tape close, and label each dish.
5. Incubate the petri dishes at room temperature with covers down.
6. You should see growth within a couple of days. The growth will be in form of colonies.

7. Make observations and keep records of what you see growing in each dish.
8. Describe colonies of fungi. How do they differ from those of bacteria?
9. Count the number of different types of colonies found on each plate.
10. Pick three different mycelia colonies, one at a time, and place at the centre of glass slide.
11. Place a drop of lacto-phenol blue on the mycelia and tease them. Place a cover slip over the preparation. Observe each fungus under the microscope and draw the structures of fungi you are able to see.
12. Study the fungi displayed on the demonstration bench. How do they compare with those that you have studied above?

Activity 3: Protists

1. You are provided with a culture of protists.
2. Using a dropper, put one drop of the liquid culture on the centre of a clean glass slide.
3. Cover the drop with a cover slip, do it gently to minimize air bubbles.
4. Observe various protists under a microscope, starting with scanning objective, then progress to low power or high power objectives.
5. Examine live *Amoeba proteus* and prepared slides of *Amoeba proteus*. Draw and label the following parts: plasma membrane, contractile vacuole, nucleus, food vacuoles, endoplasm, ectoplasm and pseudopodium.
6. Examine both live and whole mount slides of Euglena. Draw and show the following parts: nucleus, contractile vacuole, chloroplast, flagellum and eyespot. Observe the movement of a live Euglena.
7. Examine Paramecium, both live and on prepared slides. Draw and label the following parts: cilia, macronucleus, micronucleus, food vacuole and oral groove.
8. Identify and list down other protists that you are able to see.

Activity 4: Algae

1. Obtain a filament of green algae for the demonstration bench.
2. Put it at the centre of glass slide and examine it under a microscope.
3. Draw each algal cell and label it fully as seen under a microscope.
4. Discuss whether algae is a plant or not.

PRACTICAL 11: PLANT DIVERSITY AND EVOLUTION: NON-TRACHEOPHYTES AND NON-SEED TRACHEOPHYTES

Note: Study your lecture notes well in advance before coming for this practical to make your work easier.

In this practical, you will be introduced to the diversity of land plants. These include the non-tracheophytes (liverworts and mosses) and the non-seed tracheophytes (ferns, *Lycopodium sp, Selaginella sp* and horsetails).

You will use live specimens for most of these groups and also some prepared slides to help you locate the innovations that are important to the invasion of land by plants. Around the lab, there are stations for each of the major groups of land plants. This schedule will guide you through the laboratory stations and ask you questions that highlight the various structures and characters of certain groups. Take notes, make drawings, refer to your text, and talk to your instructor and your classmates as you work through the practical. Enjoy these organisms as some of you have never ever seen them before.

Activity 1: Non-tracheophytes (Mosses and Liverworts)

1. Place a small sample of sporulating moss in a Petri dish and study it under a dissecting microscope. Draw the moss plant showing gametophyte and the sporophyte. Note their relative positions. Indicate the ploidy levels for each on your drawing. Find and label the following structures: seta, capsule, calyptra, operculum, peristome teeth, leaves, stem and rhizoids. In which structure does meiosis occur?
2. Tug gently on the sporophyte and note that it is attached to the gametophyte. What generation is dominant in mosses? Give reasons for your answer.
3. Non-tracheophytes (mosses and liverworts) do not have an advanced vascular system (e.g., tracheids or vessel elements), but they do have either water absorbing cells (liverworts) or filaments made up of many cells (mosses). Find these structures on your moss specimen. What are they called? Speculate (offer at least two reasons) on how these structures may have been important to the invasion of land by plants.

4. Look at the products of meiosis under a compound microscope. What are these called, and are they haploid or diploid? What will they develop into? With regard to the transition to land, what is the selective advantage of producing these structures elevated on a stalk? Note the peristome teeth. What might these teeth do?
5. Examine the slide of the moss antheridia and archegonia; where are they located on the moss & how can you tell them apart? What are produced in these structures? How many of each type? From which of these will the sporophyte develop?

6. Look at the gametophyte of the liverwort (Marchantia). How does the morphology of Marchantia differ from the mosses? Given the external differences in morphology, speculate on which group is more dependent upon water. What is the evidence to support your answer?
7. Notice the gemmae cups (singular: gemma) on the surface of the liverwort. Are the gemmae a means of sexual or asexual reproduction?
8. Using a dissecting microscope, look closely at the surface of the liverwort gametophyte (Marchantia). Can you find pores? How are pores and stomata different? What is the function of pores?
9. Look at the live specimen of Marchantia. Note the large, flat-topped stalked structures attached to the thallus; these are reddish (especially in the center). What are they and what is their ploidy level? What is dispersed from them? What is the ploidy level of the items that are dispersed?

Activity 2: Non-Seed Tracheophytes (Ferns, *Lycopodium sp, Selaginella sp* and *Equisetum sp*)

1. We have several examples of adult ferns in the lab. Examine their morphology and find the following: fronds, fiddleheads, rhizome and sori. You will likely need to look at more than one fern to find all of these structures. What generation are you looking at? Is it haploid or diploid? Draw the fern and label it fully.
2. What is the function of each of the following: fronds? Rhizomes? Sori?
3. Look at the "rabbit's foot" fern. What are the furry "rabbit's feet" in botanical terms? How can these function in reproduction? Is this an asexual or sexual process? What would the ploidy level be of offspring from such reproduction?

4. Use the dissecting microscope to magnify the sori (singular: sorus) on a fresh fern frond. There is also a prepared slide of indusia (singular: indusium) with sporangia at this station. Indusia are plate-like structures that protect the sporangia. Indusia are thought to have a protective function for the sporangia; how do you think they might also aid in spore dispersal?
5. Are the spores of a fern produced by mitosis or meiosis?
6. Find the cuticle enclosing the leaf; what is its function?
7. Finally, note the green staining groups of cells arranged throughout the leaf section. These are vascular bundles that contain xylem and phloem specialized tissues that transport water and nutrients to the leaves.
8. Unlike the liverworts and mosses, ferns are tracheophytes and have vascular tissue composed of xylem (water-conducting cells) and phloem (sap-conducting cells). Examine the cross section of a fern rhizome. A rhizome superficially looks like a root, but it is actually an underground stem. Note the bundles of vascular tissue. Typically, xylem cells stain red and are located towards the inside of the bundle, whereas phloem cells stain green and are located towards the outside of the bundle. Find both of these tissues. Which of these cell types have thicker cell walls and are dead at maturity? What might this suggest about another function of this cell type and how does this help plants get big?
9. How have the derived tissues of xylem and phloem present in ferns (and other tracheophytes) enabled the growth form of the fern sporophyte to appear different than that of the mosses and liverworts? Note differences in size, structure, and color.
10. Examine the heart-shaped fern prothallus under the dissecting microscope. Find the rhizoids at the base of the prothallus.
11. What is the ploidy level of the prothallus? Which generation are you looking at?
12. Compare the size of the fern prothallus to the equivalent generation in the mosses.
13. Have a quick look at a prepared slide of a prothallus stained to show antheridia. Consider how these structures compare with those that are present in mosses. What is produced inside the antheridia?
14. What evidence suggests that at least early in its development, the embryo (or young sporophyte) of a fern receives nutrients from the prothallus? What happens to the prothallus after the sporophyte begins to produce its own food? Which generation is dominant in ferns? Why?

15. Study the collection of *Lycopodium sp* and *Selaginella sp*. Make brief notes on *Lycopodium sp* and *Selaginella sp.* Make a drawing of short length of Selaginella sp showing the arrangement of microphylls and label it fully.

16. Make brief notes on *Equisetum sp* (horsetails) with reference to sporophyte, leaf arrangement and location of strobili. Summarize in a table form differences among *Lycopodium sp, Selaginella sp* and *Equisetum sp*.

PRACTICAL 12: PLANT DIVERSITY AND EVOLUTION: SEED PLANTS

In this practical, you will be introduced to seed plants (gymnosperms and angiosperms).

Activity 1: Gymnosperms

1. Examine a pine branch and notice the arrangement of leaves. At the tip of the branch new leaves are being produced. Which generation are you looking at? Is it haploid or diploid?
2. Notice the small staminate (male) cones at the tips of the branch. Male cones contain microsporangia. What important process occurs in these sporangia? What is produced?
3. Using the dissecting microscope, examine the prepared slide of a long-section through a staminate cone. Notice that the cones are comprised of many scales that contain within them microspore mother cells. These microspore mother cells undergo meiosis to produce microspores which then divide by mitosis to produce pollen grains (see life cycle). What is the male gametophyte?
4. The pollen grains in pines have a distinctive structure. Use the compound microscope to examine and draw the pine pollen grain in the prepared slide. How does their structure relate to the function of pollen grains? Likewise, why might it be advantageous for a plant to produce male cones on the tips of their branches?
5. Fresh female cones are available here. Within species, female cones are consistently larger than male cones. Note the position of the pine seeds within the cones; often the seeds leave telltale scars where they were positioned on the scale. What is the significance of the shape of pine seeds? Is a seed a sporophyte or a gametophyte?
6. Examine a cross section of a pine leaf. Find the vascular tissue (xylem & phloem). Recall that xylem stains red and has thick cell walls, whereas phloem stains green and has thinner cell walls, and that these two tissue types are associated together in bundles. Note also the stomata and the leaf cuticle. Recall that the development of the cuticle was important in the evolution of land plants. List two reasons why.
7. One of the major trends in the evolution of land plants is the reduction in size of the gametophytes. Describe the male and female gametophytes of pines in terms of size, location, and fate (what happens to them). Refer to the pine life cycle chart and diagrams.

Activity 2: Angiosperms

1. Primary growth refers to the growth of plants at their apical meristems. In contrast, in some plants, secondary growth arises from lateral meristems and results in the thickening of the stem. Refer to the handout at this station. Look at the cross section of wood, can you find the growth rings? How old is this wood?
2. Examine the prepared slides of stomata from angiosperms. The Sedum leaf shows "typical" stomata, looking down on the surface of the leaf. The Nerium slide is a transverse section of an Coleander leaf. This particular species is drought-adapted, being able to withstand hot and dry conditions. To this end, the stomata are sunken into "stomatal crypts" and long hair like trichomes extend into the crypts. Speculate on how the trichomes might help prevent water loss. Note also the thick cuticle.
3. Dissect a lily flower. Draw and label as many of the following structures as are present: sepals, petals, filaments, anthers, stigma, style and ovary. Can you distinguish sepals from petals? Can you find ovules within the ovaries? Where are the gametophytes produced?
4. Examine the sunflower. Can you find all the same parts that you did in the lily? Draw and label the parts.
5. Examine the slide of germinating pollen grains from multiple species. Following successful pollination (i.e., landing on a conspecific stigma), a pollen grain germinates to produce a pollen tube. The pollen tube grows down the style until it reaches the opening to the embryo sac. During this growth, one of the nuclei in the pollen grain divides into two sperm nuclei. What are the fates of these two sperm (i.e., what do they fuse with in the embryo sac & what do they ultimately form)? What is the significance of this double fertilization event?
6. Describe the relationships among the following: ovules, ovaries, seeds, and fruits.
7. Look at the collection of seeds. Identify the method of dispersal for each seed.
8. Look at the collection of flesh fruits. Classify each of the fruits on the collection.
9. There are four organs that make up the angiosperm plant body: roots, stems, leaves, & flowers. In most plants, roots are specialized for water/nutrient uptake and anchoring the plant; stems for stature, support, and conduction, leaves for photosynthesis (food production), and flowers for reproduction.
10. Humans eat all of these organs. On this table are examples of food items of each organ. List two examples for each category. Feel free to discuss with your classmates on food items not present here. Roots; Stems; Leaves and Flowers.

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