BIO111 Laboratory Course IISER Mohali

(BS-MS First Semester)

- The experiments will be conducted in Biology Teaching Lab 4L2 in Academic Block I
- Get acquainted to your lab and adjoining areas,
- Familiarize with your support staff, tutors, instructors.

General Instructions:

- Labs start at **9 AM sharp** for morning batches and **2 PM sharp** for the afternoon. You should report before time. Late comers might be penalized.
- Instructors will introduce the topics.
- Lab managers will introduce how labs will be conducted.
- You will get a unique opportunity of training in different topics.
- Here everyone is a biologist irrespective of their past education and future interests.
- Grab the opportunity to learn by being a keen observant as every experience will count.
- Attendance in all lab sessions is expected.
- You will be rewarded for your efforts.
- Follow lab etiquettes.
- Wear lab coats.
- Wear proper shoes.
- Mobiles are not allowed. Use of mobile/laptop will be advised for some experiments.
- Use of mobiles with camera may be permitted if useful for the experiment.
- Copying is not allowed.
- Lab journal should be written in your own original words.
- Plagiarism is not allowed.
- Paraphrasing is not allowed.
- Lab journal must be submitted before you leave the lab.
- The class may be allowed to take lab journals if deemed necessary by the instructors.
- Expensive belongings should be avoided.
- Medical leave should be submitted to the Dean Academics office.
- Ensure you are registered in the ERP.
- Mischiefs/ Offenses are taken very seriously.
- Repeat offenders may not be allowed to continue in the lab course.
- Everyone is equal in the lab and should be treated with dignity.
- Try to think out of the box and be curious in nature as curiosity dives us forward. You are future scientists!

Lab Practices to be followed:

1. Do practice fire safety and electrical safety measures.
2. Handle glassware carefully and safely.
3. Keep your bags outside the main lab in the corridor of the building.
4. Don’t eat or drink in the lab.
5. Dispose lab waste properly in waste bins or containers.
6. Know emergency exit routes and the locations of lab safety showers, eyewash stations and fire extinguishers etc.

7. Avoid skin and eye contact with all chemicals and wear gloves during such kind of practical experiments and don't touch the gloves here and there unnecessarily and dispose them off properly after the lab work is done.
8. Assume that all chemicals have some toxicity. Minimize chemical exposures. Consult lab instructors or lab staff if not sure about any chemical substance or solution.
9. While working in the lab be focussed on your experiments and don't create unnecessary chaos.
10. Never mishandle any apparatus/equipment. It is needed by other batches and will be used by your juniors. Even you might need them in future.

Lab Planning of Evaluation:

Continuous evaluation = 50 marks (lab work = 30 marks, lab journal = 20 marks): In principle if a student misses a lab without approved leave, she/he loses 5 marks.

Assignment/Quiz = 20 marks: Two tasks one in November and one in December.

End Sem examination = 30 marks: This will involve performing an experiment.

Today's Exercise

- Open your drawer, note down different materials stored therein.
- Observe different instruments in the lab. Note down their names and broad use.
- Observe laminar flow hood, incubator, shaker, autoclave and note down their use.
- Learn how to use pipettes, balances and centrifuges.

BIO111_MS23_**Weighing, Pipetting and Centrifugation**

Guidelines for Pipetting and Centrifugation

Pipetting:

A pipette (sometimes spelled as pipett) is a laboratory tool commonly used in chemistry, biology and medicine to transport a measured volume of liquid. Pipettes are typically used to pull up and distribute liquid by establishing a vacuum pressure above the container that stores the fluid and deliberately discharging this suction to pull up and distribute the desired volume

1. Use the pipette with the nearest calibration to the volume that you are pipetting, e.g., 2 ml (milliliter) pipettes for 1.8mL -volumes.
2. The pipette should be held upright in a vertical position to prevent taking wrong readings and inaccurate aspiration.
3. In order to obtain higher accuracy and precision it is advised to do slow suction and slow release.
4. Don't strike the pipette tips while assembling. It will cause the pipette parts to loosen due to impact.
5. Release the pipette after use to allow the spring to the prototype.

Centrifugation :

It is a technique used to separate the particles suspended in liquid media under the influence of a centrifugal field. It is one of the most useful and frequently employed techniques in the molecular biology laboratory.

1. Select the appropriate centrifuge tubes or containers, inspect them to make sure there are no cracks or flaws.
2. Make sure that the centrifuge tubes are balanced.
3. Tightly secure the lids on the centrifuge tubes.
4. Balance the tubes within the centrifuge.
5. Make sure the lid of the centrifuge has a tight fit.
6. Set the run speed and run time.
7. Do not leave the centrifuge until it is operating at full speed and the machine seems to be running smoothly. Ensure that there is no abnormal noise or vibration.
8. Once the centrifuge has completed its run, allow the centrifuge to stop its rotation before opening the lid.
9. After the centrifuge has completely stopped moving, you may remove your samples from the centrifuge.

Links to know more –

- Basic centrifugation- https://youtu.be/V-CQP5bLD6A
- Weighing using an analytical balance - https://youtu.be/CDgoJTgIL80
- How to Pipette correctly – EPPENDORF- https://youtu.be/QGX490kuKjg

BIO111_MS23_**Weighing, Pipetting and Centrifugation**

South facing students first perform the exercise in A, followed by the exercise in B, and north facing students perform B followed by A.

You would use various equipment for your experiments in Biology. To use them you need to be trained well. Also, to get reproducible data, you should ensure their performance by checking them (or getting them checked) from time to time. In this exercise you will learn how to use micro-pipettes, weighing balances and centrifuges, and check the performance of your pipettes and weighing balance.

A. Weighing and Pipetting

These are basic exercises for every researcher in Biology. Micro-pipettes and micro-centrifuge tubes are used often for experiments in molecular biology, cell biology, biochemistry, evolution etc. Weighing is needed in experiments involving chemicals, cells, buffers etc.

You work in a group of 5. Every student performs their own experiment.

Materials: Water, micro-pipettes, microfuge tubes, balance

Method:

1. Each one of you weigh an empty 1.5 ml tube. Note down the weight.
2. Pipette one of the following volumes of water: 63µl, 109µl, 213µl, 355µl, 481µl, 591µl, 658µl, 777µl, 801µl, 924µl, 1.07ml, 1.19ml, 1.25ml, 1.36ml, 1.42ml.
3. You can use up to 3 pipettes, but minimize the number of pipetting steps.
4. Plan the pipette to be used and the pipetting steps to be performed for the given volume.
5. Weigh the tube with water after **every pipetting step**. Note down the total weight of the tube with water.
6. Calculate the weight of water by subtracting the weight of the tube after **every pipetting step**. See if the pipetted volume of the water correlates with its weight*.
7. Make a plot with yours and your group's data with volume on X-axis and weight on Y axis. What should be the ideal slope of the graph? Will the graph have any intercept on X or Y axes?
8. Calculate percentage error in your measurement from the expected value.
9. Errors can also come from the pipettes and the weighing balance. Discuss the performance of pipettes and weighing balance.
10. In case of errors, how would you know if the source of the error was the pipettes or weighing balance? Discuss in your lab journal.

*Density of water is ~1.0, therefore, pipetted volume of water should be equal to the weight.

BIO111_MS23_**Weighing, Pipetting and Centrifugation**

B. Pipetting and Centrifugation: Washing and harvesting of bacteria

In this exercise you will learn about different parts, features and operation of a centrifuge. You will also learn how to harvest cells.

Various organelles and macromolecules (such as DNA, RNA, protein, lipids) and metabolites can be isolated from cells and tissues. For this, cells need to be grown in specific media until desired growth phase before harvesting. You will be harvesting of *Escherichia coli* grown in LB media.

You work in a group of 5. Every student performs their own experiment.

Materials: *E. coli* culture, micro-pipettes, microfuge tubes, balance, centrifuge

Method:

1. Each one of you weigh an empty 1.5 ml tube. Note down the weight.
2. Pipette 1.00 ml the given bacterial culture in a 1.5 ml microfuge tube*. Note down appearance of the cell suspension.
3. Place the tube in centrifuge rotor and balance the opposite side of the rotor with a tube with equal weight (from your group member).
4. Note down orientation (angle) of your tube in the centrifuge.
5. Centrifuge the culture at 5000 rpm for 3 min.
6. Note down change in appearance of the media after centrifugation.
7. Observe the cell pellet compare its orientation (angle) with respect to the orientation during centrifugation. Draw your tube with the cell pellet in your lab journal.
8. Carefully remove the supernatant (media) using a pipette without disturbing the cell pellet.
9. Add 1 ml sterile water to the pellet. Resuspend the cell pellet by mixing using a vortexer.
10. Note down the appearance of cell suspension in water.
11. Centrifuge the culture at 5000 rpm for 2 min.
12. Observe the cell pellet compare its orientation (angle) with respect to the orientation during centrifugation. Draw your tube with the cell pellet in your lab journal.
13. Carefully remove the supernatant (water) using a pipette without disturbing the cell pellet.
14. Weight the tube with pellet. Calculate the weight of the cell pellet.
15. How much cell biomass can be obtained from 1 liter of cell suspension?

With further processing steps, the cell pellet can be used for isolating proteins, nucleic acids, metabolites and even organelles (in case of eukaryotic cells).

*You could use 2 ml tubes too, however, slanted bottoms of 1.5 ml tubes hold various pellets better.

BIO111_MS23_Lab-3a: Growth media and Sterilization

Perform BIO111_MS23_Lab-3a first and BIO111_MS23_Lab-3b in the next class

Preparation of growth media and sterilization. Testing the need of sterilization by keeping un-autoclaved media in parallel

*Living organisms are formed from a single cell (unicellular organism) or many cells (multi-cellular). Constituents of the cell usually represent the whole organism from which it is derived. Thus, pure cultures of cells are used for experiments in Biology to understand the organism. Since cell growth and division requires nutrients, cell/organism-specific media is used to culture them. For example, the bacterium Escherichia coli is grown in lysogeny broth (**LB**) liquid media. It can also be grown on LB Agar plates (with solidifying agent agar) in the form of colonies where each colony originates from a single bacterium.*

*In this exercise, you will prepare liquid and solid LB media for E. coli. To obtain pure culture, contaminating organisms must be avoided. Sterilization by **autoclaving** using steam at high temperature (121°C) and pressure (15 psi) for 15 mins is a common method to kill all such contaminants before the media can be used for growing the desired bacteria.*

You work in the group of five students on your workbench. The work should be shared by every member of the group. Each one of you is responsible for the outcome of the group's exercise. Discuss each step with your group members.

Material required: LB broth, agar, spatula, weighing balance, 2x 125 ml conical flask, 1x 250ml conical flask, cotton

A. Liquid growth media (LB broth) for *E. coli*:

1. Prepare 100 ml LB broth (10g/L peptone + 5g/L Yeast Extract + 5g/L NaCl) for your group. Calculate how much of each component you would need.
2. You will get pre-mixed LB broth purchased from a company of which 2% solution is prepared.
3. Weigh desired amounts of the above component and transfer to a beaker.
4. Add ~90 ml MilliQ water. Mix well.
5. Pour the mixture in a measuring cylinder. Adjust the volume to 100 ml with MilliQ water.
6. Distribute 50 ml of the broth into two 250 ml flasks.
7. Learn how to prepare cotton plugs.
8. Put cotton plugs to avoid entry of aerial microorganisms.
9. Discuss use of **autoclaving** and **cotton plugs** with tutors and write in your lab journal.
10. Autoclave the media in one of the flasks for **sterilization**. Put a piece of autoclave tape as an indicator of successful sterilization.
11. Keep the second flask without autoclaving. This flask will serve as a **control** for demonstrating the need of autoclaving / sterilization.
12. After autoclaving is done, store both flasks in your table cabinet. Observe growth of organisms, if any, in the next class.
13. You will use your flask with the sterile LB media for bacterial growth experiments.

B. Solid growth media (LB agar plates) for *E. coli:*

1. Prepare 150 ml LB agar (10g/L peptone + 5g/L Yeast Extract + 5g/L NaCl + 15g/L Agar) for your group. Calculate how much of each component you would need.
2. Observe appearance of the mixture.
3. Learn how to prepare cotton plugs.
4. Put a cotton plug to avoid entry of aerial microorganisms.
5. Put a piece of autoclave tape as an indicator of successful sterilization.
6. S**terilize the media by** autoclaving.
7. After autoclaving is done, observe change in appearance of the mixture from muddy in the beginning to clear after autoclaving. Discuss reasons for this change in appearance with your tutors.
8. **Each one of you** label a 9 cm diameter plate with your roll no. inside laminar flow hood under sterile conditions. Do not open the plate. Frequent opening of the plate might get contaminants even inside a laminar hood.
9. Pour ~25 ml of warm LB Agar media in your plate in a laminar hood.
10. Observe appearance of the media in the plate before and after solidifying. Discuss reasons for this change in appearance with your tutors.
11. You will use your plate for plating/spreading bacteria and cell counting per ml in your next practical class.

BIO111_MS23_Lab-3b: Serial dilution and optical density for cell counting; serial dilution and plating for cell counting (to be performed by students who prepared LB broth and agar in the last class)

Observe your autoclaved and non-autoclaved LB media from the previous week. Record your observation for the need/use of sterilization.

Sterile LB media was inoculated with E. coli. Cells were grown at 37℃ in a shaker-incubator. How would you estimate number of cells per unit volume of a culture?

OD_{600} in a spectrometer *is often used to measure density of bacterial and yeast cultures. Lights of 600nm wavelength falling on a cell tend to scatter. The extent of scattering by a culture is proportional to the cell density. Therefore, this measure reflects cell numbers per unit volume. However, the OD_{600} measurement is accurate only when cell density and the extent of scattering is within a linear range of the spectrophotometer. For microorganisms, the linear range is usually within 0.1 – 1.0 OD_{600}.*

Nevertheless, culture density does not differentiate between living and dead cells. Therefore, ***viable cell count*** *must be performed. The best method for E. coli is* ***plating*** *diluted culture on a solid media such as LB Agar.*

Materials: *E. coli* culture, spectrophotometer (OD_{600}), cuvettes, 2 ml microfuge tubes, pipettes, LB agar plates,

1:2 serial dilution of *E. coli* culture cell counting by spectrophotometer

1. Pipette 1.0ml H_2O in 5x 2.0 ml microfuge tubes. Label the tubes 1-5.
2. Add 1.0ml *E. coli* culture in tube #1. Note down the dilution factor (DF) of the original culture. It will be 1/2.
3. Transfer 1.0ml of the diluted culture from tube #1 to #2. Note down DF of the original culture. It will be 1/4.
4. Transfer 1.0ml of the diluted culture from tube #2 to #3. Note down DF of the original culture.
5. Transfer 1.0ml of the diluted culture from tube #3 to #4. Note down DF of the original culture.
6. Transfer 1.0ml of the diluted culture from tube #4 to #5. Note down DF of the original culture.
7. Measure OD_{600} of tube #1-5. Take a blank with water. Mix the diluted culture before pipetting in the cuvettes for the measurements. See where the drop in OD_{600} is proportionate to the dilution factor.
8. Calculate OD_{600} per ml of the original culture.

1:100 serial dilution of *E. coli* culture and cell counting by plating

The complete exercise should ideally be performed under aseptic condition a laminal flow hood to avoid contaminations. However, due to a limited availability of time, steps #1-6 will be performed on the bench. Step #7 must be performed under a laminar flow.

1. Pipette 0.99 ml sterile H_2O* in 5x 1.5 ml microfuge tubes. Label the tubes 1-5.
2. Add 10µl *E. coli* culture in tube #1. Note down DF from the original culture. It will be 1/100 or 10^{-2}.
3. Transfer 10µl of the diluted culture from tube #1 to #2. Note down DF of the original culture. It will be 1/10000 or 10^{-4}.
4. Transfer 10µl of the diluted culture from tube #2 to #3. Note down DF of the original culture.
5. Transfer 10µl of the diluted culture from tube #3 to #4. Note down DF of the original culture.
6. Transfer 10µl of the diluted culture from tube #4 to #5. Note down DF of the original culture.
7. Take the diluted cultures to a laminar hood. Label LB agar plate with your roll number and dilution. Mix the cells in the tube by turning the tube upside down. Put 100 µl of the diluted cultures on a LB agar plate. **Spread** the cells on the plate. Incubate the plates at 37°C for 16 hr.
8. **Next morning:** Observe bacterial growth in the form of colonies on the plates. On some plate the growth will appear as lawn of colonies. Count the number of colonies on the plates where ever it is countable.
9. Calculate the total number of colonies (which reflects number of living cells) per ml of the original culture.

Compare OD_{600} and live cell count per ml of the *E. coli* culture to find out how many cells are present per OD_{600} of the bacterial culture. Thus, in principle, you can measure OD_{600} of a culture to estimate the cell count without the need of plating and colony counting every time. You should also be able to calculate proportion of cells that are unable to divide and form colonies and maybe dead.

*In the exercise we are using water for serial dilution of the culture, but ideally culture media should be used.

BIO111_MS23_Lab-4a: Acid, Base, pH and Serial Dilution

Perform BIO111_MS23_Lab-4a first and BIO111_MS23_Lab-3b in the next class.

Acids and bases are commonly used to prepare buffers. ***Serial dilution*** *is performed to obtain precise concentration of* ***working solutions*** *from their concentrated* ***stocks****. You will be given an acid and a base. You will also learn to make 1:100-fold serial dilutions of them. Measure pH of the diluted solutions using the pH meter. You will be calculating pH of all solutions. Compare observed pH with calculated values. Recall acid, base and pH from your earlier classes. Discuss with your group members.*

You work in the group of five students on your workbench. The work should be shared by every member of the group. Each one of you is responsible for the outcome of the group's exercise. Discuss each step with your group members.

Materials: 1 M HCl, 1M NaOH, pH meter, Pipettes, 4x 100 ml Beakers, measuring cylinder, Pure water, magnetic stirrer, bead and bead retriever.

Theory:

Ionization of water: $H_2O \leftrightarrow H^+ + OH^-$ (very small amount of H_2O stays in its ionic forms H^+ and OH^-)

Ionization constant of water: $Kw = [H^+] \times [OH^-] = 10^{-14}$ (take negative log)

$-\log[Kw] = \{-\log[H^+]\} + \{-\log[OH^-]\} = -\log[10^{-14}]$

$\therefore pKw = pH + pOH = 14$

$\therefore pH = 14 - pOH$

$Kw = [H^+] \times [OH^-] = 10^{-14}$

For H_2O at standard conditions: $[H^+] = [OH^-]$

$\therefore [H^+] = 10^{-7}$ and $[OH^-] = 10^{-7}$

$\therefore -\log[H^+] = -\log[OH^-] = -\log[10^{-7}] = 7$

$\therefore pH = pOH = 7$

Considering the following:

$-\log[Kw] = pK$

$-\log[H^+] = pH$

$-\log[OH^-] = pOH$

$-\log[10^{-14}] = 14 \log 10 = 14$

Acid: Serial Dilution and pH of HCl

1. Ionization of a strong acid: $HCl \leftrightarrow H^+ + Cl^-$ (since strong acids are fully ionized in water, HCl stay in its ionic forms H^+ and Cl^-). $\therefore [HCl] = [H^+]$.
2. You are given 1M HCl solution. *Calculate its pH by applying the above formula.*
3. Take **4 beakers**. Label them #1-4. Add 99 ml water in each beaker. Measure pH of water in one of the beakers.
4. Mix 1 ml of 1M HCl acid with water in beaker #1. *This will dilute the acid by 1:100. The concentration will now become 0.01M. Calculate pH.* Label the concentration and pH on the beaker.
5. Transfer 1 ml of 0.01M HCl from beaker #1 to beaker #2. Mix. *This will dilute the acid again by 1:100. The concentration will now become 0.0001M. Calculate pH.* Label the concentration and pH.
6. Transfer 1 ml of 0.0001M HCl from beaker #2 to beaker #3. Mix. *This will dilute the acid again by 1:100. The concentration will now become 0.000001M. Calculate pH.* Label the concentration and pH.
7. Transfer 1 ml of 0.000001M HCl from beaker #3 to beaker #4. Mix. *This will dilute the acid again by 1:100. The concentration will now become 0.00000001M. Calculate pH.* Label the concentration and pH.
8. Measure pH of each solution #3-6 4-7 using a pH meter. Compare the pH measured by the pH meter with the calculated pH obtained after dilutions of 1M HCl.
9. Learn about pH meter, electrode and its maintenance from tutors. Note down in you journal.

Base: Serial Dilution and pH of NaOH

1. Ionization of a strong base: $NaOH \leftrightarrow Na^+ + OH^-$ (strong bases are fully ionized in water, NaOH stays in its ionic forms Na^+ and OH^-). $\therefore [NaOH] = [OH^-]$.
2. You are given 1M NaOH solution. *Calculate its pH by applying the above formula.*
3. Take **4 beakers**. Label them #1-4. Add 99 ml water in each beaker. Measure pH of water in one of the beakers.
4. Mix 1ml of 1M NaOH acid with water in beaker #1. Mix. *This will dilute the acid by 1:100. Thus, the concentration will now become 0.01M. Calculate pH.* Label the concentration and pH on the beaker.
5. Transfer 1ml of 0.01M NaOH from beaker #1 to beaker #2. Mix. *This will dilute the acid again by 1:100. The concentration will now become 0.0001M. Calculate pH.* Label the concentration and pH.
6. Transfer 1ml of 0.0001M NaOH from beaker #2 to beaker #3. Mix. *This will dilute the acid again by 1:100. The concentration will now become 0.000001M. Calculate pH.* Label the concentration and pH.
7. Transfer 1ml of 0.000001M NaOH from beaker #3 to beaker #4. Mix. *This will dilute the acid again by 1:100. The concentration will now become 0.00000001M. Calculate pH.* Label the concentration and pH.
8. Measure pH of each solution #3-6 4-7 using a pH meter. Compare the pH measured by the pH meter with the calculated pH obtained after dilutions of 1M NaOH.

BIO111_MS23_Lab-4b: Choosing and preparing buffers. Investigate if amino acids could work as buffer in the cell (to be performed by students who did pH in the last class)

Optimal intracellular pH is essential for survival of the organisms. Enzymatic activities are optimal only at a small pH range. For example, cells in our body must maintain a narrow pH range around 7.0. Even small deviation results in acidosis or alkalosis. Prolonged deviations can be life threatening.

How do cells resist change in pH? Could cellular components such as metabolites, amino acids, proteins etc work as buffering agents in the cell? Recall buffers as solutions that resist change in pH upon addition of an acid or base.

Exercise 1: Identify good buffering agents from NaCl/Na_2HPO_4/NaH_2PO_4/glycine and their buffering pH range.

Exercise 2: Prepare 100ml buffer of pH 6.8.

Materials: 1M NaCl, 1M Na_2HPO_4, 1M NaH_2PO_4, 1M Glycine, 1M Hydrochloric acid, 1M Sodium Hydroxide, pH Meter, 6x 100 ml Beaker and Magnetic stirrer

Methods:

1. You are given stock solutions at 1M concentration. Calculate how much stock solution you would need to prepare 50ml of 0.1M NaCl/Na_2HPO_4/NaH_2PO_4/glycine solution. Prepare the solutions into 100ml beakers.
2. Put one of the solution on a magnetic stirrer. Add a magnetic bead stir **gently**. Stop the stirrer.
3. Wash thoroughly the pH electrode in distilled water. Carefully assemble the pH meter on the magnetic stirrer. Make sure the magnet bar is not hitting the pH electrode.
4. Start the stirrer. Make sure the magnet bar is not hitting the pH electrode.
5. Note down starting pH of the solution.

Titrate with HCl

6. Add 50µl of 1M HCl and note the pH after each addition.
7. Continue adding the acid until the pH goes below 2.
8. Make a table of HCl volume added and the respective pH obtained.

Table

Sr No	Volume of HCl (µl)	pH
1	0	?
2	50	?
3	100	?
4		?

Titrate with NaOH

9. Add 50µl of 1M NaOH and note the pH after each addition.
10. Continue adding the base until the pH goes above 12.
11. Make a table similar to the above of volume of NaOH added and the respective pH obtained.

12. Each one of you must titrate at least one solution.
13. Plot a graph of the volume of HCl/NaOH on X-axis and pH on Y axis.
14. Compare data obtained by your team. Study the graph carefully.
15. Estimate if the above solutions can serve as a buffering agent and within which pH range?
16. Discuss why only some of the above solutions work better as buffer.
17. Find their buffering range. Discuss why do they have different buffering pH ranges?
18. Why do other solution may not work as buffer?
19. Discuss if Glycine could be used as a buffering agent.
20. Choose a right combination of the salt solutions to prepare a buffer of pH 6.8. Discuss your plan with your tutors. Record the rationale of the choice of salts in your lab journal. Prepare 100ml of a suitable buffer of pH 6.8.

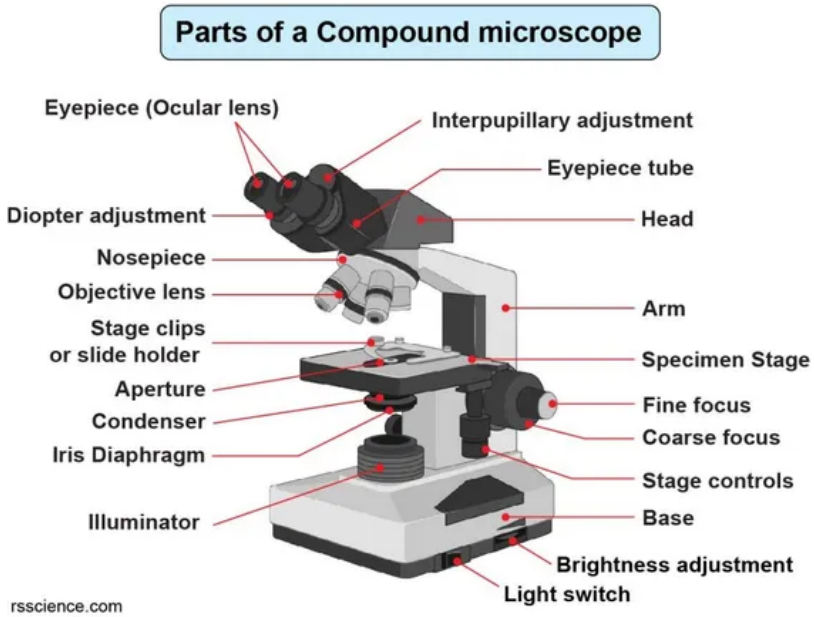
BIO111_MS23_Lab-5a_Microscopy and Model Organisms

Microscopy are commonly used for research. The technique is not only used to identify an organism but also to visualize a cell and its intracellular contents.

Model organisms are commonly used for research in Biology. Their use not only simplifies complex investigations but also make the research ethically feasible and economically viable. For example, ethical clearances is mandatory while planning any research but certain kind of studies involving humans are not permitted. Similarly, a study involving a large number of complex animals and plants may not be economically viable. Therefore, discovering as much information from model organisms greatly helps in research. In fact, a majority of fundamental discoveries have been made by explorations in model organisms.

You are given a microscope. You are also give cell suspensions of a few commonly used model organisms, including the bacteria *Escherichia coli*, the budding yeast *Saccharomyces cerevisiae*, and the fission yeast *Schizosaccharomyces pombe*. Learn how to use the microscope. Identify different model organisms and study their applications.

***A. Microscope and function of its different parts*:** Study your microscope carefully. Locate its different parts from the image shown below. Note down function of its different parts. Each one of you are expected to learn to use the microscope by focusing and identifying a model organism under the microscope. Practice handling the microscope with care. For example, how to switch on light, move specimen stage horizontally and vertically.



B. Study microorganisms using the microscope: Stain *E. coli*, *S. cerevisiae* and *S. pombe* with Safranin and observe them under different objectives. *Study their appearances and estimate their size (diameter).*

1. Prepare a slide of any cell suspension given to you.
2. Pipette 50 µl of the culture in a microfuge tube. Add a tiny drop of safranin. Mix gently for 30 seconds. Put 10 µl of the mix on a slide. Put a coverslip. Gently remove the excess fluid by using a tissue paper.
3. Focus the sample with the objectives in increasing order of magnification (i.e. start with 10x – 40x). See you can locate your cells.
4. Put a small drop of oil on top of the coverslip in the centre. Try focusing your cells with 100x objective.
5. Note down your observation on handling the microscope, focusing trick of cells and appearance of the cell.
6. Compare appearance of your cells with slides prepared by your group members. If necessary, re-prepare the slides.
7. Feel free to use your mobiles to click pictures. Paste representative pictures in your lab journal.
8. Identify different model organisms. Study some of their key applications and note down in your lab journal.
9. Estimate the size (length/diameter) of each cell types*.
10. Make a table of different types of cells that you have observed in the course and their size estimates.

Clue: Cell size ≈ Apparent size of image from the microscope / Total magnification (you can click pictures using your mobile for estimating cell sizes seen by your eyes in the microscope)

Total magnification = magnification of the eyepiece x magnification of the objective

*More advanced microscope are fitted with camera for this and similar tasks.

BIO111_MS23_Lab-5b_Study of microorganisms in homemade curd and a commercial probiotic

You are given curd, yoghurt and commercial prebiotics to study their microorganisms. Curd is formed from fermentation of milk by the bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus,* and yeast cells. The given probiotic contains *Lactobacillus casei* strain *Shirota.*

Aim: Study the presence of above microorganisms in curd and the probiotic by Gram staining.

Principle: The method was devised by a Danish bacteriologist, Hans Christian Joachim Gram (1884) as a method of staining bacteria. Gram stain is used to differentiate between Gram-positive and Gram-negative bacteria. The two types of bacteria differ in thickness of their peptidoglycan layers; Gram-positive bacteria has a thicker layer.

The reaction is dependent on the permeability of the bacterial cell wall and membrane to the dye–iodine complex. In Gram-positive bacteria, the crystal violet dye iodine complex combines to form larger aggregates which precipitates within the cell. The alcohol/acetone mixture which acts as a decolorizing agent causes dehydration of the multi-layered peptidoglycan of the cell wall. This reduces porosity of the peptidoglycan causing the cell wall to trap the crystal violet iodine complex within the cell. The Gram-positive bacteria do not get decolorized of the primary crystal violet dye and remain violet.

In the case of Gram-negative bacteria, the alcohol, being a lipid solvent, dissolves the outer lipopolysaccharide membrane of the cell wall. It also damages the cytoplasmic membrane to which the peptidoglycan attaches. As a result, the dye-iodine complex is not retained within the cell and diffuses out of the bacterium during decolonization. Hence, they take up the counter stain Safranin and appear pink.

Lactobacillus is a Gram-positive bacillus. *Streptococcus thermophilus* is a Gram-positive coccus. *E. coli* is a Gram-negative smaller bacillus. Yeast cells appear Gram-positive.

Materials: Compound light microscope, bunsen flame, clean grease-free slides, Crystal violet (basic dye) Gram's iodine, 95% ethanol (decolorizing agent), 1% safranin, curd, prebiotic

Methods:

1. Take clean and grease-free slides for making smears of curd and prebiotic.
2. Take 20 µl of the samples and place them on the slide. Bend the pipette tip and using the bent tip spread the samples into a thin area on the slide.
3. Allow the smears to air dry.
4. Holding the slide at one end. Heat fix the smears by quickly passing the smear over the flame of the Bunsen burner two to three times.
5. Cover the smear with one drop of crystal violet stain by tilting the slides at different angles. Wait for one minute.
6. Dip the slides 2-3 times into a beaker containing tap water. Let the water evaporate from the slides.
7. Cover the smear with one drop of Gram's iodine for one minute.
8. Dip the slides 2-3 times into the beaker with water. Let the water evaporate from the slides.
9. Add 50 µl 95% alcohol to the smears.
10. Dip the slides 2-3 times into the beaker with water. Let the water evaporate from the slides.
11. Cover the smear with safranin for one minute.
12. Dip the slides 2-3 times into the beaker with water. Put a coverslip.
13. Observe the smears under the oil immersion (100x) objective. Feel free to use your mobiles to click pictures.

Observation: Note down the presence of different microorganisms in curd and the prebiotic. Gram-positive bacteria will appear purple or violet colour. Gram-negative bacteria will appear pink or red in colour. Cocci will appear round in shape and bacilli will appear rod in shape.

You will be performing both 6a and 6b

BIO111_MS23_Lab 6a_Onion peel observation under the microscope

In this exercise, you will learn how plant cells appear in the microscope (6a). You will also observe different stages of cell division. Importantly, you will also observe cells in different stages of mitosis (6b).

Aim: To prepare a specimen slide of a plant cell (onion peel) for observation under the microscope

Background: Onion is a multicellular plant. Like other plant cells, the cell of onion peel consists of a cell wall, cell membrane, cytoplasm, a large vacuole and a nucleus. The nucleus lies at the periphery of cytoplasm and vacuole is located in the centre. Presence of large vacuoles and cell wall confirms that cells of onion peel are plant cells.

Materials: Raw onion, glass slide, coverslip, watch glass, forceps, safranin stain, tissue paper, glycerine and compound microscope.

Procedure:

1. Take a thin transparent peel from the inner surface of a small piece of onion using a forcep.
2. Keep this thin transparent onion peel (onion epidermal peel) on to a watch glass.
3. Add 2-3 drops of saffranin stain in the watch glass to stain the onion peel and incubate for 2 minutes
4. Wash the onion peel with distilled water to remove extra stain.
5. Add 2-3 drops of aceto orcein stain and again incubate for 2 minutes.
6. Wash the onion peel with distilled water to remove extra stain.
7. Take a clean glass slide and put a small drop of glycerine in the centre of the glass slide.
8. With the help of a forceps, transfer the onion peel on to the glass slide. Glycerine prevents the onion peel from drying up.
9. Carefully cover it with the coverslip and avoid the formation of any air bubble.
10. Remove any excessive glycerine with filter paper.
11. Observe the prepared mount of the onion peel under 10x and 40X magnification of a microscope. You should be able to see nucleus.
12. Take a picture for calculating size of the cell.

BIO111_MS23_Lab-6b_Cell Cycle – Different stages of mitosis

Aim: Temporary mount of onion root tip to study different stages of mitosis

Background: *All organisms are made up of cells. For an organism to grow, mature and maintain tissue, new cells must be made. All cells are produced by division of pre-existing cells. Continuity of life depends on cell division. Mitosis is very important to life because it provides new cells for growth and replaces dead cells. Mitosis is the process in which a eukaryotic cell nucleus splits in two, followed by division of the parent cell into two daughter cells.*

Onions have larger chromosomes than most plants and stain dark. The chromosomes are easily observed through a compound light microscope.

Phases of plant cells division:

1. **Prophase**: During prophase the nuclear envelope starts to break down and all the chromosomes start to coil up in the center of the cell.

2. **Metaphase** is the middle stage at which point all the chromosome line up in the center at metaphase plate of the cell along spindle fibers that pull to either side of the cell.

3. **Anaphase:** The spindle fibers become shorter and pull each chromatid apart to the opposite ends of the cell.

4. **Telophase:** The nuclear envelope is reformed. A new cell wall is created down the center and two daughter cells are formed.

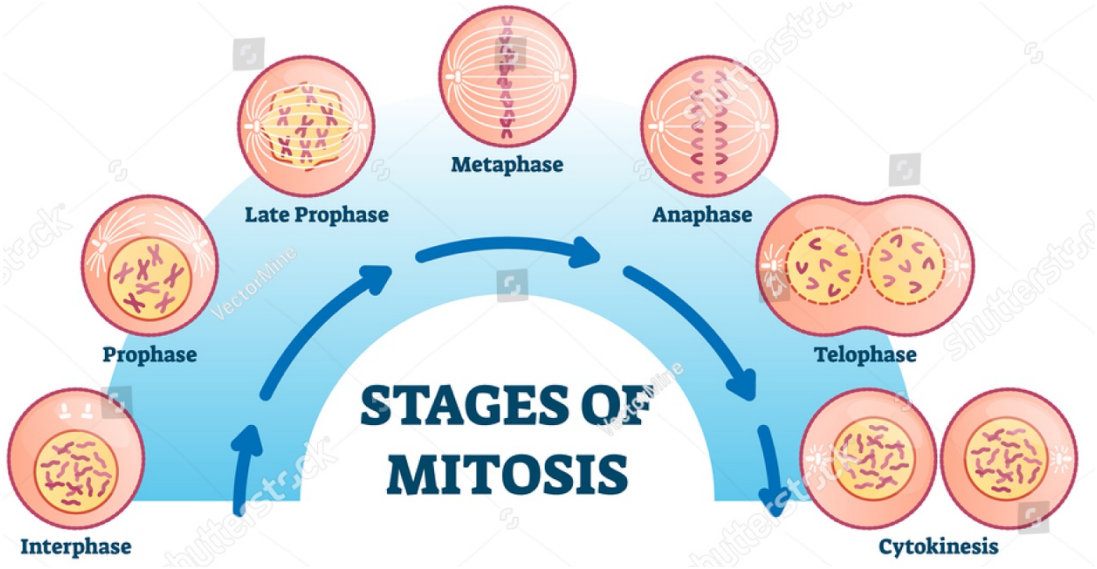
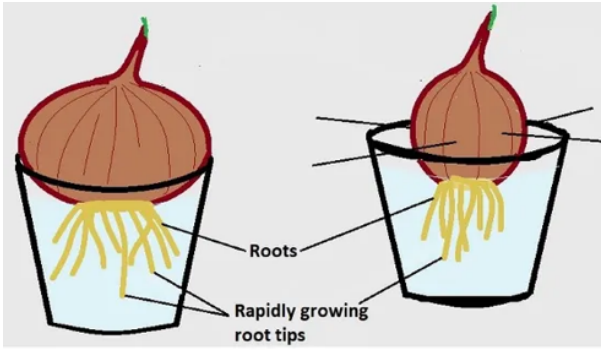


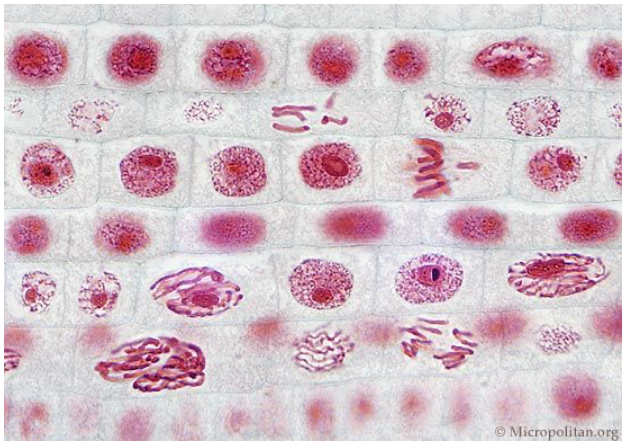
Fig 1: Different stages of the mitosis in a cell

Principle: Somatic growth in plants and animals takes place by the increase in the number of cells. In plants, cell divides mitotically to form two daughter cells wherein the number of chromosomes remains the same as in the mother cell. In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where the stages of mitosis can be easily observed.

Requirements: Onion bulbs with growing roots, 1N HCl, acetoorcein stain, safranin stain, decolorizer, scalpel blade, 1.5 ml microfuge tubes, forceps, microscopic slides and cover slips, water bath, light microscope.

Fig 2: A) Onion root tips grown and provided. B) different stages of the cell division seen under microscope





Procedure:

1. Take a newly sprouted onion root tip (given to you) and transfer it into a 1.5ml micro centrifuge tube.
2. Fill the tube with 1N HCl using a dropper.
3. Place the tube at 60°C in a water bath and incubate the tube for 10 minutes.
4. Remove the tube from the water bath after the incubation.
5. Discard the HCl from the tube using a pasture pipette.
6. Add some drops of distilled water into the tube and rinse the root. Then remove the water from the micro centrifuge tube using the pasture pipette. (Rinse the roots at least three times).
7. After the washing step transfer the root on to glass slide with the help of forceps.
8. Add 2-3 drops of aceto-orcein stain onto the root tip and incubate for 5 minutes. (During the incubation, the very tip of the root will begin to turn red as the DNA get the stained in the cells).
9. After the incubation rinse the root tips with distilled water to remove the extra stain using a pasture pipette.
10. Add 2-3 drops of safranin stain onto the root tip and incubate for 1 minute.
11. Again rinse the root tips with distilled water.
12. Add few drops of decolorizer (ethanol) for 2-3 seconds to remove the excess stain and rinse it with water.
13. Take a scalpel blade and cut most of the unstained part of the root and keep the dark stained tip portion.
14. Cover the root tip with a cover slip and then carefully press on the coverslip with a tissue paper (**apply sufficient pressure so that the root tip placed between slide and coverslip spreads horizontally**, but *do not let the coverslip slide sideways*).
15. Observe it under a compound microscope in 10x objective. Scan and narrow down to a region containing dividing cells and switch to 40x objective for a better view.
16. Count number of cells in interphase and in different stages of mitosis.