BIO1140

Introduction to Cell Biology

Lab Manual
Winter 2019

Biology Department
University of Ottawa

BIO1140 – Introduction to Cell Biology

Lab manual – Winter 2019

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My Info:

Name: _____ Section: _____

Demonstrators Info:

TA 1: _____ Office hours: _____

email: _____

TA 2: _____ Office hours: _____

email: _____

Questions about **absences**, rescheduling a lab or schedule in general, **please contact the BioLab team at:** biolab1@uottawa.ca

Questions about the lab content, your marks, reports or any other concerns, please **contact the lab coordinator**:

Dr. Fabien Avaron
Email: fabien.avaron@uottawa.ca
Biosciences Complex (BSC) Room 106
Office Hours: Friday 10:30-12:00 (open door the rest of the time).

www.biolab1.uottawa.ca

BIO1140 – Introduction to Cell Biology Winter 2019

Labs take place on the 3rd floor of the Biosciences (BSC) complex between 2:30 and 5:20 pm Monday to Friday. Visit the BIO1140-lab website at **biolab1.uottawa.ca** for more details.

Weekly Schedule:

	Monday (Section & room)	**Tuesday** (Section & room)	**Wednesday** (Section & room)	**Thursday** (Section & room)	**Friday** (Section & room)
Week 1 Sections	A1 BSC312 A6 BSC330 A10 BSC335	A2 BSC312 A7 BSC330 C1 BSC335 C2 BSC310	A3 BSC312 A8 BSC330 C3 BSC310	A4 BSC312 A9 BSC330 A11 BSC335	A5 BSC312 C4 BSC330 C5 BSC335
Week 2 Sections	B1 BSC312 B6 BSC330 B10 BSC335	B2 BSC312 B7 BSC330 C6 BSC335 C7 BSC310	B3 BSC312 B8 BSC330 C8 BSC335	B4 BSC312 B9 BSC330 B11 BSC335	B5 BSC312 C9 BSC330 C10 BSC335

Session Schedule: No labs between Feb. 18 and Feb. 22, 2019 (between the 1st and 2nd week of Lab3).

Mon	14 Jan.	A1, A6, A10	Lab1
Tue	15 Jan.	A2, A7, C1, C2	
Wed	16 Jan.	A3, A8, C3	
Thu	17 Jan.	A4, A9, A11	
Fri	18 Jan.	A5, C4, C5	

Mon	21 Jan.	B1, B6, B10	Lab1
Tue	22 Jan.	B2, B7, C6, C7	
Wed	23 Jan.	B3, B8, C8	
Thu	24 Jan.	B4, B9, B11	
Fri	25 Jan.	B5, C9, C10	

Mon	28 Jan.	A1, A6, A10	Lab2
Tue	29 Jan.	A2, A7, C1, C2	
Wed	30 Jan.	A3, A8, C3	
Thu	31 Jan.	A4, A9, A11	
Fri	1 Feb.	A5, C4, C5	

Mon	4 Feb.	B1, B6, B10	Lab2
Tue	5 Feb.	B2, B7, C6, C7	
Wed	6 Feb.	B3, B8, C8	
Thu	7 Feb.	B4, B9, B11	
Fri	8 Feb.	B5, C9, C10	

Mon	11 Feb.	A1, A6, A10	Lab3
Tue	12 Feb.	A2, A7, C1, C2	
Wed	13 Feb.	A3, A8, C3	
Thu	14 Feb.	A4, A9, A11	
Fri	15 Feb.	A5, C4, C5	

Mon	25 Feb.	B1, B6, B10	Lab3
Tue	26 Feb.	B2, B7, C6, C7	
Wed	27 Feb.	B3, B8, C8	
Thu	28 Feb.	B4, B9, B11	
Fri	1 Mar.	B5, C9 , C10	

Mon	4 Mar.	A1, A6, A10	Lab4
Tue	5 Mar.	A2, A7, C1, C2	
Wed	6 Mar.	A3, A8, C3	
Thu	7 Mar.	A4, A9, A11	
Fri	8 Mar.	A5, C4, C5	

Mon	11 Mar.	B1, B6, B10	Lab4
Tue	12 Mar.	B2, B7, C6, C7	
Wed	13 Mar.	B3, B8, C8	
Thu	14 Mar.	B4, B9, B11	
Fri	15 Mar.	B5, C9, C10	

Mon	18 Mar.	A1, A6, A10	Lab5
Tue	19 Mar.	A2, A7, C1, C2	
Wed	20 Mar.	A3, A8, C3	
Thu	21 Mar.	A4, A9, A11	
Fri	22 Mar.	A5, C4, C5	

Mon	25 Mar.	B1, B6, B10	Lab5
Tue	26 Mar.	B2, B7, C6, C7	
Wed	27 Mar.	B3, B8, C8	
Thu	28 Mar.	B4, B9, B11	
Fri	29 Mar.	B5, C9, C10	

The lab exam will take place on **Saturday March 30th, 2019 at 1PM**. Visit the lab website for later updates including exam rooms.

General Objectives of BIO1140 Laboratories:

The laboratory component of BIO1140 aims to:
1) Allow students, using an experimental approach, to carry out the scientific method in biology,
2) Develop students' ability to effectively analyse and communicate information obtained through experimentation,
3) Reinforce and complement the material presented in the lecture portion,
4) Introduce students to basic microscopic and image acquisition techniques using prokaryotic and eukaryotic cells,
5) Familiarize students with general laboratory skills and safety procedures.

Supplies:

You must bring to EACH lab session:

Lab coat
Safety glasses
Pen and Permanent marker
Lab manual
Combination lock (← very important)

Suggested material you should also bring:

Timer
Lined notebook paper (any format)
Drawing pencil (HB or harder)
Small clear plastic ruler
Eraser

Laboratories web site: www.biolab1.uottawa.ca/BIO1140/

Important documents and instruction files will be posted there. Visit this site regularly, particularly right before your lab session.

Laboratory Sessions:

Lab hours: Monday to Thursday 2:30 to 5:20PM
As labs usually begin with a quiz and a presentation of the day's work, it is necessary to be there on time. **Students arriving late will not be allowed to enter until the pre-lab quiz is completed. Late students will receive a zero for the quiz.** You must read your lab manual **before your lab session**. You may find additional information in relevant chapters of the textbook as well as on the lab website.

Laboratory Rules

Due to safety regulations (Fire Marshal's orders) and space limitations, **no outerwear, briefcases, backpacks, gym bags, etc. will be allowed in the labs**. Outwear and bags must be stored in a locker in the hallway. Do not forget to put a lock on your locker. Locks left on your locker beyond your normal lab period will be removed. **No cell phone should be used** during the lab. **You cannot leave your belongings in a locker outside of your lab time**. Locks will be removed and lockers emptied by staff after regular lab hours.

The Seven Golden Rules:

1. **Lab coats* are obligatory and must be worn at all times in the laboratory.**
2. **Safety glasses or goggles must be brought to each laboratory period and worn during any potentially hazardous procedures.**
3. Cell phones must be used during the lab, nor kept on lab benches.

4. No eating or drinking in the lab, this includes chewing gum.
5. No rowdiness. We work with expensive and sometimes dangerous equipment, as well as poisonous substances.
6. Discussion is encouraged, but general noise is disruptive.
7. Cleanliness: You must keep your own equipment and notes in order. Clean up your equipment and your bench at the end of each lab.

Safety Instructions:

What should you do if:

1. You break some glassware (beakers, pipettes, etc.) or a thermometer.

Notify your demonstrator, who will place the broken glassware in the broken glass container. If a mercury thermometer is broken, a technician (trained in hazardous waste cleanup) will come and deal with the situation.

2. You cut yourself or have any other medical problem.

Notify your demonstrator, who will use the contents of a small First Aid Kit located in the labeled drawer of your lab to deal with your problem (a more complete First Aid Kit is available in BSC 331 and BSC 141). Emergency telephones are available on the podium for medical emergencies (**5411**). In addition, in the hallway outside each lab room you will find emergency eyewash stations, safety showers and a red button on the wall that will trigger an alarm to campus security. If necessary, the student may then be escorted by a demonstrator to Health Services.

3. Your clothes catch fire while in the lab.

Notify your demonstrator, who will bring you to the safety shower located in the hallway to extinguish the flames. The fire extinguishers located beside the doors may also be used for this purpose.

4. You hear the fire alarm.

Do not panic. You should leave your lab, in an orderly manner to the primary fire exit for your room. The primary and secondary fire exits for labs on the 3rd floor of the Biosciences centre are indicated on the evacuation plan posted on the corridor wall and are as follows:

Lab	Primary Fire Exit	Secondary Fire Exit
BSC 312/330	Stairwell A	Stairwell B
BSC 302/335	Stairwell B	Stairwell A
BSC 310	Stairwell B	Stairwell A

If the primary exit is not accessible, proceed to the secondary fire exit. Once you have descended to the main floor, exit the building, moving about 30 meters away from the door and await further instructions.

5. You accidentally spray some toxic liquid into your eyes.

Notify your demonstrator, who will bring you to one of the two emergency eye wash stations in your lab room. Pull down on the water pipe and rinse your eyes in the two water fountains for fifteen minutes. An additional emergency eye wash station is found in the corridor. Thereafter, your demonstrator may take you to Health Services. Please note that it is not recommended to wear contact lenses in the lab.

Attendance to laboratories & Absence:

Lab attendance is mandatory. In case of absence:

- **With Justification:** You must be present to 3 out of 5 labs AND hand in reports for all labs you attend AND write the final exam. If not, the lab component will be worth 12.5% and the remaining 12.5% will be transferred to the lecture part. If you are absent to more than 2 labs AND the final exam you will get a zero for the lab component.
  In case of absence for medical reason, **you must provide a medical note** (give it to your TA or the lab coordinator).
  All **justification notes must be provided within 7 days past your absence** (except in the case of a long lasting absence or medical condition) otherwise you will get a zero for the missed lab.
- **Without justification:** You must attend at least **4 out of 5 labs** AND write 80% of assignments AND write the final exam; otherwise you will get a zero for the lab component. If you missed a lab for a non-medical reason, please contact the lab coordinator ASAP. Any unjustified absence will result in getting a zero for the missed lab. Absence to the lab exam will result in getting a zero for the lab component.

If you know in advance you will not be able to attend a lab, please contact biolab1@uottawa.ca in order to **have your lab rescheduled** (do **NOT** contact your TAs, they cannot do that). You will need a justification note in order to have your lab rescheduled.

Lab reports format:

Format: Text should be printed on **8.5 x 11 inch white paper** (1.5 space, 12 pt), begins with a TITLE PAGE (see page 7), be stapled in the top left hand corner and not be placed in a binder or other plastic cover.
All graphs must be done by hand (=not typed) and on millimetre graph paper. A file containing specific instructions for each report will be posted on the lab website. Make sure you read this file.
Lab reports must meet certain quality standards. For instance, reports written on (or containing) pages **torn from a notebook** will not be accepted and students will receive a zero for it. Ask your TA or the lab coordinator if you have questions.

When is my report due?

Your TA will tell you when to hand in your report. Make sure you write down this information. Due dates will also be indicated on the lab website.
Reports must be handed in before 5:00 pm on the due date or they will be considered late.
Note: The BSC building is locked after 6:00 pm on weekdays and during the whole weekend.

Can I hand in my report late?

Yes, but no more than **2 days** past the due date. A late penalty of 10% (off the maximum mark) will be applied to each of the first two days. After that, you will receive a ZERO (your assignment will be corrected anyway). **Weekend counts as 2 days** = if your assignment is due on Friday, hand it in before 5:00 pm on Monday or you will receive a zero.

Where do I hand in my report?

In the document drop-off boxes located in the first floor lobby of the Biosciences complex (30 Marie Curie entrance, behind the elevator shaft). You will find there a series of mailboxes with a section

number on each. **Hand in your report in the box that corresponds to your section. If you put your report in the wrong drop off box, a 10% penalty will be applied.**
If you got permission to attend the lab with another section, drop off your work in the box that belongs to the section you had your lab with.

ATTENTION: You will spend some time writing your lab report, so **make sure you hand it in the right box otherwise you will get a penalty.**

Plagiarism (make sure you read this part before handing in your first report):

The work must be your own and not a copy of someone else's. If an experiment does not work and there is no time to repeat it, you must notify your TA. Even though you may collaborate or consult with other students, your lab report must be written by you alone.

Attention: If all or part of a report[ab] is identical between two (or more) students, all students involved will receive a ZERO for the corresponding assignment. There will be no exceptions of any sort. If this happens more than once, a formal plagiarism report will be filed and sent to the Faculty of Science.

[a]Including a report from a previous school year. [b]"part of a report" includes graphs, tables, captions, text, etc..

Plagiarism from books, friends' work, etc., is considered academic fraud and will be dealt with severely. Please see http://sass.uottawa.ca/sites/sass.uottawa.ca/files/plagiarism.pdf for what is acceptable and not acceptable citation practice.

Lab skills evaluation:

The laboratory skills mark is granted by your demonstrator based on several criteria: Do you attend and stay for the duration of the laboratory sessions? Do you come prepared and organized to each laboratory session? Do you work well with a partner? Have you mastered all experimental procedures? Do you follow the appropriate safety procedures? Do you return borrowed materials, clean your work area and return your microscope properly to its appropriate location? A lab skills mark will be included in the evaluation of each lab.

Tests and Exam:

Short pre-lab quizzes will be given this term. These will test your preparation for the lab being done that day. Quizzes start right at the beginning of each lab. Be on time or you will miss the quiz and receive a zero.

The final (written) lab exam will be approximately 1.5 hours in duration and will take place the day indicated on the schedule (see page 1). It will include short answer questions concerning all concepts covered in the labs and the theory associated with them. Please contact the coordinator BEFORE the exam if you need any special accommodation.

BIO1140 Lab component evaluation:

You will be evaluated on your technical skills, your preparation to the lab, as well as on lab reports and a final lab written exam.

Lab component evaluation breakdown:	Pre-lab quizzes (labs 3-5):	5%
	Labs 1-5:	11% each
	Final lab exam:	40%

The Lab component is worth 25% of the BIO1140 course, provided you meet the attendance requirements.

Email etiquette:

Always include the course code in the subject field. In the message itself, write down your **name** and **your student number** as a signature. Messages lacking a signature, as well as rude messages will be ignored. Use your uOttawa account if possible to avoid technical difficulties. Professors and TAs will use this email to contact you if necessary. Do **not** send your email to multiple people: this almost guarantees no answer. Contact your TA or the Lab coordinator or biolab1 depending on the nature of your question.

Extra help

Your own demonstrator will tell you where and when he/she will be available to you for extra help. Contact information of your TAs including email address and office hours schedule will be posted on the **Contact page** of the lab website.

Special needs / concerns about the labs:

Feel free to contact the lab coordinator if you experience difficulties regarding the laboratories. If you need a specific accommodation due to a medical condition or else, please visit the Student Academic Success Service website (access service) at www.sass.uottawa.ca to get additional information, and/or contact the lab coordinator. We will do our best to provide you with the best learning conditions.

I hope you will enjoy this term,

Dr. Fabien Avaron, Lab Coordinator

Example of a typical lab report title page

The rate of transpiration in tomato plants – effects of temperature and air movement

By John P. Smith
1762847

BIO1140 Section A1

Demonstrators:
Anthony Henry and
Penelope Whelan

January 21, 2019

Department of Biology

University of Ottawa

Lab #1 - Introduction to Microscopy & Observation of Prokaryotic and Eukaryotic Cells

Introduction

Many of the cells and organisms that you will be studying are at the lower limits of visibility of light microscopes; therefore, it is extremely important that you attain critical lighting and focussing. It is also important to handle the microscope competently to avoid damaging either the microscope or the preparation you are studying. **Even students who have previously used microscopes should read the instructions below carefully.**

Biolabo

The biolabo website (http://salinella.bio.uottawa.ca/biolabo/) contains many useful ressources relevant to the biology teaching labs. Under **Microscopy** you will find links to pages that describe both types of microscopes you will use this semester, as well as, how to set up and use them. It is strongly recommended that you **visit these pages prior to attending your first lab**.

Infinity Capture

Although you can make all of your observations by watching your specimen directly through the oculars, it can also be done on the computer screen using the digital camera attached to each microscope. For that, you will use the **Infinity Capture** program. Visit the lab website to learn how to use **Capture** (link on the homepage). All observations can be made either on your computer screen or through the oculars. Each method has its advantages and drawbacks; you will have to choose which one is more appropriate (or the one you prefer):

Oculars	Screen
• Greater resolution • Wider field of view	• Can share your observation with others • More comfortable for users • Take pictures while observing

The Compound Microscope

On the Biolabo Guide page, click on the **CX41 Compound Microscope** link then on **Parts and Function**. This will bring up a labelled diagram of your microscope. Familiarize yourself with the various components shown in this figure. Then, click on **Setup and Bright field alignment** in order to know how to use and handle the microscope.

Now, locate your compound microscope in the cupboard below the sink of your workstation. Place it on the counter between the computer and the end of the counter. Be sure that whenever you transport the microscope, it is always kept upright; the ocular lens will fall out if the scope is tilted or swung.
*Even though you don't need the **dissecting microscope** right now, take it out of the cupboard and install it beside the compound microscope.*
Connect **one** USB cable to each of the cameras installed on top of the microscopes.

Parts of the compound microscope

The microscope consists of a system of lenses, a light source, and a geared mechanism for adjusting the distance between the lens system and the object being observed. There are a number of important components and it is essential that you be able to identify them and understand their function before you can proceed. By going through the different modules in Biolabo and using the microscope, you will develop a competency for bright field microscopy.

Identify the following components using Biolabo (Parts and functions figure) and your microscope:

REVOLVING NOSEPIECE: Supports the various objectives - You will only use the 4x, 10x and 40x objectives in the BIO1140 labs (not the 100x).

STAGE: Supports the specimen being observed. A system of knobs on the side of the stage allows you to move the specimen under the objective on the X and Y axes. Try and move the stage.

COARSE FOCUS KNOB: Permits rapid change in distance between the specimen and the objective thereby allowing for rough focussing – Do not use when focusing with the 40x objective.

FINE FOCUS KNOB: Permits small changes in distance between the specimen and the objective and thereby allows for final focussing of the image. It should be used with the 40x objective.

OCULAR OR EYEPIECE: A magnifying element in the microscope, usually 10x. It is through the ocular, or eyepiece, that one looks at the specimen. All our microscopes are parfocal, so that when an object is in focus with one objective, the focus will not be completely lost when changing to the next objective.

OBJECTIVES: The magnifying element that is closest to the specimen. See figure 1 to find out about the engravings on the side of each objective.

CONDENSER: System of lenses that concentrates the light furnished by the illuminator. It does not magnify the object.

CONDENSER HEIGHT ADJUSTMENT KNOB: Allows one to focus the concentrated light onto the specimen.

APERTURE IRIS DIAPHRAGM: Used to reduce glare from unwanted light by adjusting the angle of the cone of light that comes from the condenser.

Production of an Image by a Compound Microscope

The most important part of a microscope is the objective. All the other parts of the instrument are designed to help the objective produce the best possible image. The best image is not the largest; it is the clearest. There is no value to a high magnification. If the resolution is poor you will have no better understanding of the specimen.

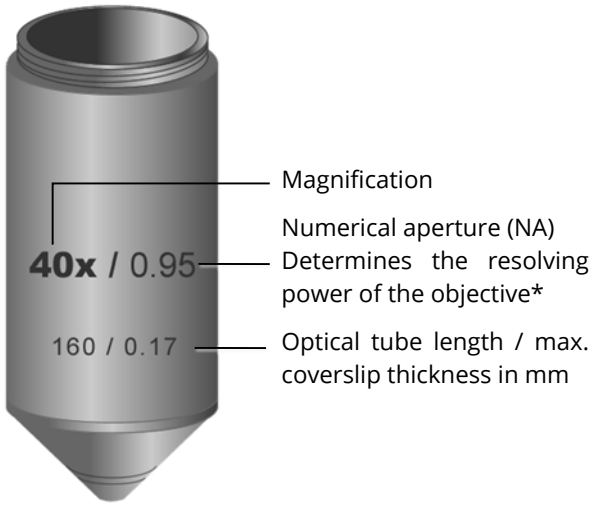


Figure 1: Objectives engravings

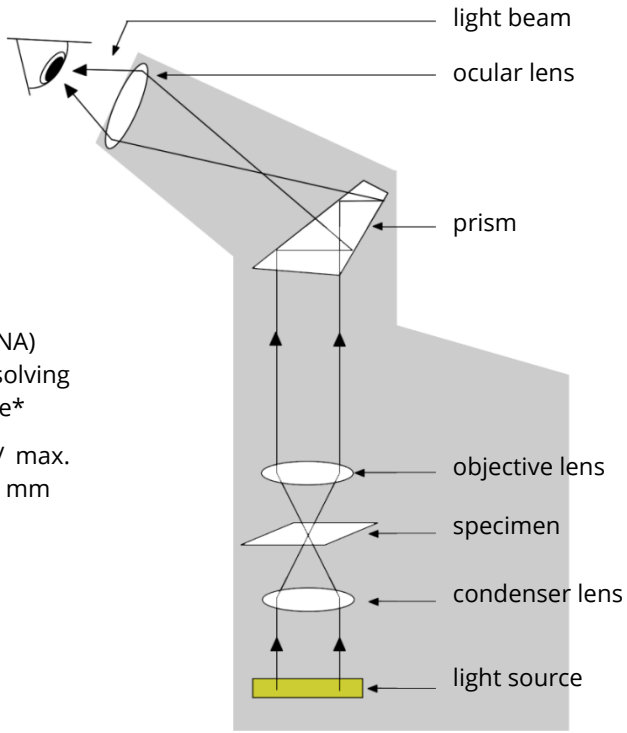


Figure 2: Image production in a compound microscope.

*Resolving power is the ability to see two objects that are very close as two separate objects. The human eye has a resolving power of about 100 µm.

Using the compound microscope

Always handle the microscope **GENTLY**! It is an expensive, delicate and heavy instrument. Carry it with both hands, one hand on the arm, and the other hand under the base. If the ocular or objective is dirty, wipe it clean using ONLY Kimwipes or special lens tissue and cleaning fluid supplied. If you use anything

else you may scratch the lens. Wipe up any cleaning fluid immediately; otherwise it will dissolve the glue which holds the lens in place. REMEMBER, your demonstrator is here to help, so...ASK!

1. Make sure that the power cord is plugged into the back of your microscope and into a power outlet.

2. Using the letter “e” microscope slide provided, follow steps 2 through 13 in the **Setup and Bright field alignment** procedure of Biolabo. Remember, observation can be done on screen or through the oculars.

Orientation and working distance

1. Starting your examination with the 4x objective, position the letter "e" slide on the stage.

2. Draw what you see in the microscope:_____

3. What would a slide with the letter “t” look like under the microscope?

_____

4. Using the knobs located on the side of the stage and looking through the microscope, move the slide slowly to the right, then to the left. Record your observations (Which way does your specimen move?). _____

5. Now, move the slide slowly away from you, then towards you while observing through the microscope. Record your observations

_____

6. Focus on the slide at 10x. Check the distance between the objective lens and your slide (This is called **the working distance**; see also the reference at the end of this chapter). Now switch to the 40x objective and check the working distance. What happens to the working distance as your magnification increases?

Depth of field (depth of focus)

Lenses have a depth of field. It is the number of planes in which an object appears to be in focus. Try this experiment: extend your fist at arm’s length in front of you and hold your thumb up. Focus on your thumb and notice that the objects past your thumb in the background are blurry. Similarly, with a microscope, when it is focussed on one surface, the surfaces located above and below will be out of focus. A high number of surfaces in focus mean that you have a great depth of field (also referred to as deep focus). The depth of field varies with several factors, such as the distance between the object and the lens and the curvature of the lens (which in turn affects the magnification factor of the lens).

1. Position a *Cimex lectularius* (whole mount) prepared slides upon the stage. At low magnification (4x) focus on one of the legs of the specimen.

2. Using the fine focus adjustment, focus up and down slowly and observe the effect on how sharp the image appears.

3. Repeat using different objectives. What can you say about the depth of field at different magnifications? Has it increased or decreased? (i.e., can you see more focal planes at 4x, 10x or 40x)?_____

Magnification

The magnification given by objectives and oculars is engraved on them. The total magnification for any combination of objective and ocular is the product of the magnification of each lens.

Objective magnification	**4x**	**10x**	**40x**
Ocular magnification	10x	10x	10x
Total Magnification	40x	100x	400x
Light intensity	High	Medium	Low
Working distance	22mm	10.5mm	0.56mm

Table 1. Comparison between magnification, working distance and brightness for three different objective magnifications

You also can calculate the magnification of your picture using the following formula:

$$\text{Magnification factor} = \frac{\text{measured size of object}}{\text{actual size of object}} = (\quad X)$$

While the magnification factor may be a useful piece of information, a scale bar is the definitive tool that must be used in order to know the size of things. Therefore, you must always add a scale bar to your biological illustrations (photos or drawing).

Size of the specimen

Before you start this exercise, make sure you have carefully read the website section relevant to the software you will use to take digital pictures (**Infinity Capture**).

The goal of this section is to teach you different techniques that will allow you to determine the size of objects you are observing under the microscope. The general principle is very simple: 2 objects have the same relative size (expressed as a ratio) in the real world and under the microscope.

$$\frac{\text{actual size of object A}}{\text{actual size of object B}} = \frac{\text{on-screen size of object A}}{\text{on-screen size of object B}} \Leftrightarrow \frac{A1}{B1} = \frac{A2}{B2}$$

The following exercises are applications of this formula. Place a slide under the microscope. Choose the right objective and adjust the focus and light level. Then, choose a structure you want to measure and take a picture.

Measuring an object using the field of view (FOV):

There are many methods to determine the size of an on-screen object. The simplest one is to use the known size of the whole field of view (FOV, the whole picture from left to right):

1 - On the computer screen (using a ruler and without writing anything on the screen), measure the object of which you want to determine the size (=A_2)

2 - Then, measure the width of the whole picture on the screen (=B_2).

3 - Refer to table 2 on page 20 to know the actual size of the field of view for the objective you're using (=B_1)

4- Use the following formula:

$$\text{actual size of the object } (A_1) = \frac{\text{actual size of the FOV } (B_1)}{\text{on-screen size of the FOV } (B_2)} \times \text{on-screen size of the object } (A_2)$$

Example: On a snapshot, using the 4x objective, an insect has an on-screen length of 10 cm. The whole picture is 20 cm wide.

What is the actual size of the insect?_____

Do not put the compound microscope back in the cupboard you will need it later this afternoon.

Points to remember concerning microscopes

1. Always work with a clean microscope. Use only the lens paper provided. Don't forget to clean the slide too!

2. Always locate the specimen under low power (4x) and work your way up to the high power objective.

3. Never use the coarse focusing knob when the high power lens is in position. Use only the fine focus knob.

4. Never use the 100x objective in the 1st year labs (we didn't teach you how to)

5. Always readjust illumination whenever you change the objective. Too much light will give you a blurry image that you cannot focus on.

The stereoscopic microscope (dissecting microscope)

The stereoscopic microscope, also called stereoscope or dissecting microscope, is used to view objects that are too large or too thick to observe under the compound microscope.

Stereo microscopes are always equipped with two oculars producing a stereoscopic or three-dimensional image. Unlike the compound microscope, the image is not inverted.

Our stereo microscopes provide magnification in the range of 6.7x – 45x using a zoom-type lens system. By rotating a dial located on the right side of the stereo microscope head, the viewer obtains a continuous change of magnification.

Our stereo microscopes can be used with reflected or transmitted light. Reflected light is directed unto opaque specimens from above and is reflected to the viewer. Transmitted light is used with translucent specimens and passes through the specimen from beneath the stage and into the viewer's eyes.

Use of the stereoscopic microscope

1. On the Biolabo home page, left click on **Stereoscope (Dissecting microscope)** and then on **Stereoscope setup**.

2. Click on Step 1 and read it carefully. Obtain a stereo microscope from the same cupboard as your compound microscope if you haven't yet.

3. Click on and read steps 2 through 7.

4. Place a coin on the stage.

5. Using the focussing knob on either side of the arm, lower or raise the objective until the coin is in focus. Examine it in both reflected and transmitted light.

Which is best for an opaque specimen? Try the various magnifications by turning the zoom knob. The reflected light source is similar to a spotlight and its orientation can be adjusted manually. Try rotating the light upwards and downwards.

6. Examine other materials such as brine shrimp larvae (*Artemia*) in a watch glass using both reflected and transmitted light. Add 1-2 drops of "proto-slow" solution to slow down the larvae. Estimate the actual size of one larva: _____

Prokaryotic and Eukaryotic cells

It has long been recognized that organisms are composed of basic structural and functional units called cells. Cells can be divided into two general types:

prokaryotic and eukaryotic, based on the presence of a nucleus and other membrane bound organelles in the latter.

Prokaryotic cells belong to 2 big groups: archaea and eubacteria. They are usually smaller than eukaryotic cells (typically 1-5 µm). These unicellular organisms may be small, but they are the most abundant organisms on the planet, representing about half the biomass (Biology, Brooker *et al.* 2010, McGraw-Hill&Ryerson). They are devoid of membrane bound organelles such as the nucleus, mitochondria or chloroplasts. Their genetic material is usually composed of one circular chromosome plus other extra chromosomal elements called plasmids.

Eukaryotic cells are usually much larger. They possess a membrane bound nucleus, their organelles are more complex and numerous, and their genome is larger than prokaryotes. Eukaryotic organisms can be uni- or multicellular. You will have a chance to observe many eukaryotic cells during this semester: *Amoeba*, Lilly, Whitefish....

In today's exercise you will take a first look at the similarities and differences between prokaryotic and eukaryotic cells, as well as the diversity within these groups.

You should familiarize yourselves with a whole array of cellular structures and organelles you will probably encounter during the course of this exercise. Before your scheduled lab session, write down the definition and function for each of the following terms: plasma (cell) membrane, cell wall, protoplast, cytoplasm, vacuoles, nucleus, nucleolus and chloroplasts.

Eukaryotic Cells: *Elodea* (plant)

1 - Get a young green *Elodea* leaf from the jar. Mount it in a drop of water on a clean microscope slide with the convex side of the leaf uppermost. Cover the preparation with a coverslip.

2 - Observe the preparation at 4x, then at 10x. If you see brownish oval structures on the leaf surface, ignore then. These are probably epiphytic diatoms. Concentrate your attention on the cells near the central rib at the base of the leaf and on the marginal cells at the edge of the leaf.

- Can you distinguish several layers making up the leaf?____
- What is the average length _____ and width _____ of the cells in micrometres?

3 - Focussing at 40x, locate the **cell wall**, the **vacuole**, the **cytoplasm** and the numerous green **chloroplasts**.

- What important biological process takes place in the chloroplasts? _____
- What pigment is responsible for their green colouration? _____

- What is the shape of chloroplasts?

  _____

- Are the chloroplasts moving? What sort of movement?

  _____

- The phenomenon you are observing is called **cytoplasmic streaming** or **cyclosis**. What do you think the function of such a process could be?

  _____

4 - You have probably realised that the **plasma membrane** cannot be seen in plant cells. It is too thin to be resolved with the compound microscope. In order to see the true limiting boundary of the cytoplasm, it is necessary to treat the cells in such a manner that the plasma membrane becomes withdrawn away from the rigid cell wall. This can be done by placing the cell in a strong salt solution. This will cause water to diffuse out of the cell by osmosis, thereby decreasing the cell volume. The unaffected cell wall remains in its original state. What can then be seen is a space between the cell wall and the limiting boundary of the **protoplast** (the cell minus the cell wall) which thereby becomes visible.

- Remove your *Elodea* slide from the microscope stage. Delicately remove the coverslip, add one drop of 5% NaCl solution, then put back the coverslip on your preparation.
- Refocus at 40x (don't forget: you must first focus at 4x, then 10x and finally at 40x).
- Are the cells plasmolyzed? (If not, wait a while longer). How do they look like now?_____
- Has the cell wall been affected?_____
- What became of the large central vacuole during plasmolysis? _____
- Take a picture of a plasmolyzed *Elodea* cell. How does it compare to the previous picture?

Prokaryotic Cells: *Oscillatoria* (eubacteria: cyanobacteria)

1. Take a close look at the sample in the jar. Which colour would best describe its appearance?_____
2. Prepare a wet mount of fresh *Oscillatoria* by following the procedure below:
   - Using a plastic pipette put a very small amount of green matter on a clean slide
   - Carefully place a coverslip over it. Make sure it lies flat on the preparation. Don't worry if there are just a few air bubbles. With practice, your skills will

improve. However, if too many air bubbles are present, your preparation risks drying out very quickly during the viewing, thereby compromising your observations.

3. Starting with the **4x** objective, focus on your preparation.
   - Can you see numerous green filaments?_____
   - Are the filaments moving?_____
4. Switch to the **10x** then the **40x** objective and focus using the fine focus knob only:
   - Do you see the individual cells making up each filament?_____
   - Estimate the width of one filament in micrometres:_____
   - What's the filament width in **millimetres (mm)**? _____
   - REMEMBER: You are working with living cells. Work quickly and keep your specimen wet at all times. Dead, dry or damaged biological preparations are useless.

Returning the microscopes after use

After completing all observations, turn and click the low power objective (4x) on the compound microscope into position.

Remove the slide from the stage and return it to its correct box.

Wipe the stages with a clean paper towel.

Carefully disconnect the USB cable from the camera.

Make sure you turned off the light on each microscope, then unplug the power cord and make a loose coil of it around the eyepieces.

Return the microscope to the cupboard.

The TAs will check that you properly returned the microscopes to the cupboard with the cord properly attached and no slide present on the stage. You will lose marks for this lab (and other labs) if you don't do so.

Evaluation

A short quiz on microscope components, specimen observations and measurement of objects will take place at the beginning of Lab 2.

Be on time, the quiz will start at **2:30 pm SHARP.**

Reference:

1 - Size of fields of view (fov) for the different cameras:

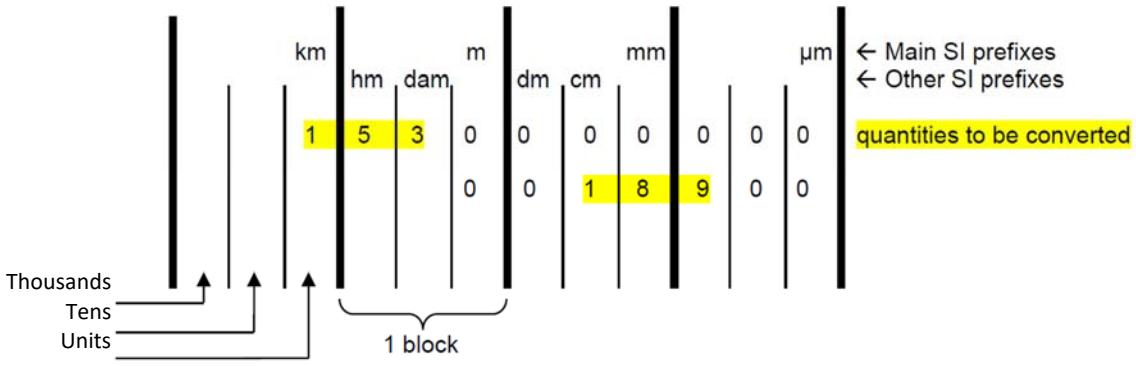
Table 2: Fields of View: Olympus CX41 Compound Microscope

Objective	Camera field of view (width in mm)
4x	1.80
10x	0.72
40x	0.180
100x	0.072

Table 3: Fields of View – Olympus SZ61TR Dissecting Microscope

Zoom Setting	Camera field of view (width in mm)
0.67x	24.5
1x	15
2x	6.6
4.5x	3.1

2- A simple system for converting SI units



How to use: This table consists of repeated **blocks** each containing 3 columns (the left and right borders of each block are thicker to make it easier to read). Each column represents one prefix of a given SI unit (in the example above, prefixes of the metre).

In the right column of each block (units), write down the main SI prefixes (i.e. kilo-, *base*, milli-, micro-, nano- etc....) of the unit you're working with

Optional: enter secondary prefixes (hector: h, deka: da, deci:d and centi: c) in the appropriate columns if needed. Read the SI Units appendix if needed.

In order to convert a quantity, write down its value in the appropriate column (see example below). You may then add as many zeros on the left and right sides as you need.

Ex: Measured quantity: 1.53 km.

1. First, enter the quantity in the table: put 1 in the km column, then 5 then 3 in the columns immediately at the right. You don't have to write the dot separating the decimals.
2. You're done! You now can easily read directly in the table that 1.53 km = 153 dam, 1 530 m, 153 000 cm and 1530 000 000 µm.

   Similarly: 18.9 mm = 0.0189 m = 0.189 dm = 18 900 µm

No calculation required, just read the table. Add as many zeros at the left and the right as you need. You can easily reproduce this table during an exam if need, it only consists of a series of 3 vertical lines.

This table will not work for non SI units, (e.g. time units such as years, month, days, hours and minutes), except for the **litre**, that is often used to measure volumes (1 l=1 dm^3).

The table will not work with imperial units (inch, feet gallons etc...). These do not belong in a science lab anyway.

You can also use similar tables to convert area and volume units:

For square metres (m^2, measuring areas): double the number of columns within one block (so that there will be 6 column per block): 1 m^2 = 1000000 or 10^6 mm^2

For cubic metres (m^3, measuring volumes): triple the number of columns with one block (9 columns per block): 1m^3 = 1000000000 or 10^9 mm^3.

3 - Metric system units (see also appendix II at the end of lab manual):

1 centimetre cm = 10^{-2} metres (m)

1 millimetre mm = 10^{-3} metres

1 micrometre µm = 10^{-6} metres

1 nanometre nm = 10^{-9} metres

Lab #2 - Permeability of the Red Blood Cell

Introduction

During lab 1, we investigated tonicity changes upon *Elodea* cells. Today, we will study spontaneous movements of molecules (water and solutes) through the membrane of animal cells: Erythrocytes (or red blood cells). These cells offer numerous advantages for the study of cell membrane permeability:

1. One can easily obtain large quantities of homogenous cells.

2. Cells can be kept for a long time in an isolated state (three or four days in isotonic NaCl or one week in serum at 4°C).

3. Erythrocytes contain large quantities of the pigment hemoglobin. When the volume of a cell exceeds a critical volume, the cell ruptures and pigment is released to the external environment. This phenomenon is referred to as **hemolysis**.

Osmosis

Osmosis is defined as the spontaneous movement of **water molecules** across a semi-permeable membrane. Water can freely cross the plasma membrane, mostly thanks to specialized proteins called aquaporins that form canals through the membrane. Water movements are mostly driven by tonicity adjustment: water will move from the compartment with a lower solute concentration to the compartment with a higher solute concentration.

Tonicity is defined as the ability of a solution to create movement of water. Therefore, the volume of a cell placed in a hypotonic solution will increase due to massive entry of water inside the cell, in order to decrease the concentration of solutes in the intracellular environment. If the solution is very hypotonic, the increase in volume will tear the plasma membrane. This will cause the death of the cell and the release of the intracellular content into the outside environment. In red blood cells, this phenomenon is called hemolysis.

A hypertonic solution, on the other hand, will cause water to flow from the inside of the cell to the outside. The volume of the cell will decrease and the plasma membrane will start folding (like a deflated balloon).

Diffusion

Molecules in a solution can also spontaneously cross the membrane following their own concentration gradient. This phenomenon is called diffusion.

In today's experiment, we will study the diffusion of solutes through the plasma membrane of red blood cells, as well as the movement of water caused by diffusion. Solutes can diffuse through a membrane at various speeds depending on the functional groups they possess. Some molecules cross the membranes easily, whereas the membrane is almost impossible to cross for others. Visit the lab 2 webpage for more examples and explanations about diffusion.

Hemolysis

This process describes the destruction of red blood cells that occurs when too much water flows inside the cell and causes the rupture of the plasma membrane.

When erythrocytes are placed in a hypotonic environment, water enters the cells and their volume increases. Cells eventually reach a critical volume and undergo hemolysis.

Hemolysis can also be reached by placing the cells into an isosmotic solution of a penetrating substance (e.g. ethylene glycol). A solution may be called isosmotic if it has the same concentration of solutes (penetrating and non-penetrating) as the cellular fluid. Therefore, even though the osmotic pressure may be equal at the onset, as the penetrating substance enters the red blood cell, the tonicity and osmotic pressure change. The osmotic pressure inside the cell increases, while the external environment becomes hypotonic. Water enters the cell via osmosis in an attempt to maintain equilibrium. As water enters, the cell swells and eventually undergoes hemolysis.

The time it takes a red blood cell to undergo hemolysis in a solution of penetrating substances is a practical criterion that allows us to estimate the permeability of the membrane to those substances.

In all populations of erythrocytes, certain cells are more resistant than others to hemolysis. However, when 75 % of the cell population has undergone hemolysis, the suspension of red blood cells becomes suddenly transparent. This event will be used as your criterion to compare the permeability of a given red blood cell to different penetrating substances and to compare the permeability of different types of mammalian erythrocytes.

Experimental Procedures

A - Permeability Experiments

Overview

You will measure the time it takes 5 solutions to cause hemolysis in sheep red blood cells. Each measurement will be performed 3 times in order to calculate the mean and standard error of the mean of the hemolysis time.

Material provided

- One index card with a horizontal line across its center attached the faucet at the center of your lab bench.

- 5 graduated plastic tubes (used to measure volumes)

- 15 parafilm squares

- 15 glass test tubes

- One of these two sets of solutions in 20 mL flasks:

Set A :	Distilled water	Set B :	Distilled water
	Glycerol 0.3 M		Thiourea 0.3M
	Ethylene glycol 0.3 M		Triton X-100 2% (v/v)
	Sucrose 0.3 M		D-Glucose (Dextrose) 0.3 M
	Urea 0.3M		Ethanol 0.3 M

Procedure:

1. Preparation of tubes: use one of the graduated tubes to measure 5 mL from the flask containing solution 1. Then pour the liquid into a test tube. Repeat two times to get 3 test tubes filled with 5 mL of solution 1. Label tubes 1-1, 1-2 and 1-3.

Repeat the procedure with all 5 solutions to test (label tubes 2-1, 2-2 etc....)

Once all 15 tubes are filled, get prepared for the measurements, starting with solution 1:

2. Take a test tube and tip it slightly. Then, using the plastic pipette put 1 small drop of blood on the side of the tube.

*do not press the pipette too much or you may waste some blood and will not have enough for the whole experiment.

3. Quickly cover the top of the tube with a piece of parafilm and invert once. Start your timer now: this is time equals zero. Hold the test tube up to the index card so the horizontal line is behind it (about 10 cm away from the card). The suspension should initially be turbid. After a moment, the suspension will suddenly turn transparent and the line will be clearly visible. When this occurs, stop your timer; you have reached hemolysis, the endpoint of this experiment. Record your time to hemolysis.

Hint: hold the hemolysis tube no closer than 10 cm from the index card; otherwise, you may not be able to determine precisely when hemolysis is complete.

4. Repeat steps 2 + 3 with the 2 other tubes for the current solution

5. Repeat steps 3 and 4 for the other four solutions

It is important that the experimental conditions are identical from one determination to another. In this way, the results are highly reproducible.

Record your results in your notes, then calculate the mean and the standard error of the mean of the hemolysis times you measured.

Calculation of mean and standard error of the mean

Y= measurement; n=sample size

Arithmetic Mean : $\overline{Y} = \frac{\sum Y}{n}$ Standard deviation: $s = \sqrt{\frac{\sum Y^2 - \frac{\left(\sum Y\right)^2}{n}}{n-1}}$

Standard Error : $SE = \frac{s}{\sqrt{n}}$

Your TAs will demonstrate how to calculate the standard error using MS Excel.

B - Red Blood Cells subjected to various osmotic conditions.

Put a very small quantity* of blood on a microscope slide then add one drop of 0.145 M NaCl. Cover the preparation with a coverslip. Observe the cells using a compound microscope. How would you describe the shape of these red blood cells? Sketch them and measure their diameters on a digital picture. Repeat this procedure with blood + 0.350 M NaCl and then blood + 0.065 M NaCl. Do not forget to place the coverslips in the broken glass container. Please wash your hands after this manipulation.

*Do not add too much blood (a 1-2mm drop is enough). Too much blood will cause your preparation to be too dense and you will not be able to see individual RBCs or to focus properly at 40x.

Read instructions for the lab report on the BIO1140 lab website

Lab #3 - Cellular Processes in *Amoeba proteus*

General objectives of the lab

- Observe the amoeboid movement under the microscope
- Locate and identify various organelles and cell structures of *Amoeba proteus*
- Follow the contractile vacuole (CV) under the microscope and take pictures of it
- Determine the volume of the CV through its cycle and represent the variation of volume versus time on a graph
- Observe endocytosis after adding an inducing agent, as well as phagocytosis

Introduction

Amoeba proteus is a protist that belongs to the Amoebozoa group. It is a **single-celled eukaryotic organism** common to the bottoms of freshwater ponds and lakes. It continuously changes shape and is very mobile. It extends its pseudopodia as a way to move, and to catch food (including preys) that will then be digested in food vacuoles. In terms of size, it may obtain a length of 600 micrometers (µm). It is easy to culture in the lab and is a good model for demonstrating many cellular processes.

Amoeboid Movement

It is in the Amoebozoa group where amoeboid movement is the most typical and the most spectacular.

The first activity of this lab consists of observing the amoeboid movement under the microscope. In order to conduct prolonged observations and prevent the amoebas from being crushed we will use small chambers made of parafilm melted onto a microscope slide. Amoebas are very sensitive to light of short wavelengths and to heat. We should therefore turn off the light of your microscope between observations, and work at a relatively low light intensity. Also, make sure the coverslip on top your amoeba wet mount covers the whole chamber otherwise the water will evaporate quickly and the amoeba will die.

As mentioned earlier, *Amoeba proteus* uses membrane protrusions called pseudopodia ("fake feet"), to achieve movement. After one or several **pseudopodia** form the rest of cell seems to crawl in that direction. The formation of pseudopodia is relatively well described and mostly relies on a process shared by all moving cells: extension of long polymers of **actin**. This protein is present in the cytoskeleton (microfilaments) of eukaryotic cell and is involved in muscle contractions in animals. During the formation of a pseudopodium, molecules of free actin (G-actin) bound to each other to create a series of long polymer fibers (called F-actin in this form). As these fibers grow they push the plasma membrane in the direction of the extending pseudopodium (fig.1). Adhesive molecules will attach the newly formed pseudopodium to the substratum, whereas the same adhesive molecules will detach at the "back"* of the cell (called the uroid). Then, a series of contractions occur at the uroïd (one can see the membrane

folds in the uroïd region where these contractions occur) helping the cell content to be pushed towards the front*of the cell.

*The amoeba cell doesn't have an actual "front" or a "back" *per se*, but it is fine to use these terms relative to the direction the movement.

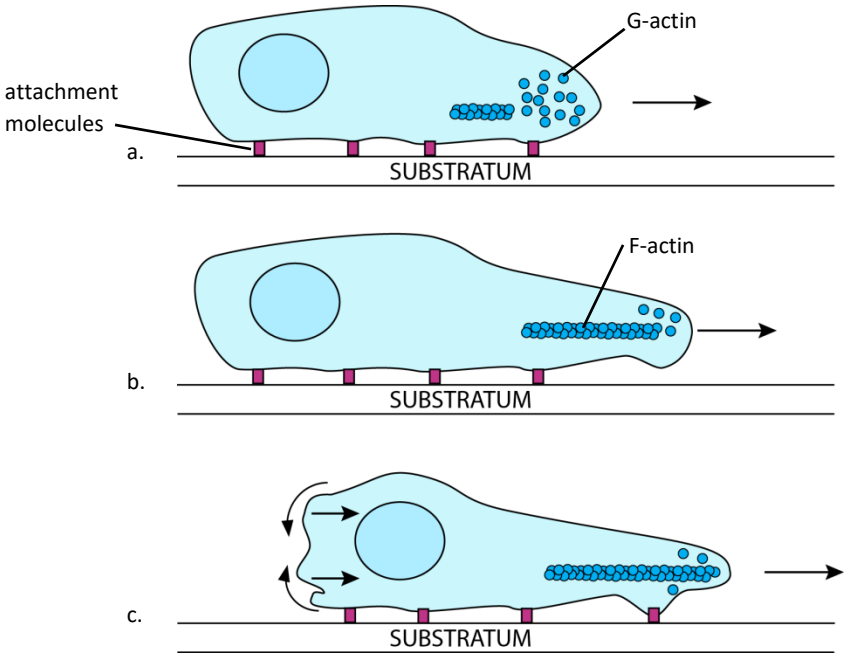


Fig. 1: Schematic representation of amoeboid movement (Modified form ref.1).

Microscopic observations

1. Using the 4x objective, scan through the slide and find a living amoeba. Then, switch to the 10x objective and observe the changes in shape and direction of movement. Estimate the speed at which pseudopodia form and disappear.

2. At a higher magnification, you should be able to distinguish the principal regions of this cell: hyalin ectoplasm, granular endoplasm (fluid or gelatinous), uroid and the hyalin cap (cytoplasm).

3. Locate and identify these organelles: nucleus, contractile vacuole, food or digestive vacuoles, crystals (different forms), lysosomes (surrounding food vacuoles).

Contractile Vacuole

Like all organisms that live in water, especially single-celled ones, amoeba must constantly fight excess water entry by osmosis (the extracellular environment is hypoosmotic compared to amoeba's cytosol).

Amoebas, as well as many other protists, release the excess water thanks to a contractile vacuole. This organelle goes through a cycle during which it fills up by taking in water in the cytosol, and then releases this water into the extracellular environment. The size and number of contractile vacuole(s) varies depending on species. *Amoeba proteus* only has one contractile vacuole. The contractile vacuole cycle is depicted in fig.1.

Your task will be to track the CV under the microscope during two full cycles and take a picture every 30 seconds using the 40x objective.

Time zero corresponds to the time when the amoeba empties the content of its contractile vacuole to the outside (=**systole**). Start measuring time as soon as the vacuole from the previous cycle disappears and take a picture every 30 seconds.

You may not be able to observe the vacuole up to 2 minutes past the systole, in this case record 0 as the diameter.

Note: A typical contractile vacuole cycle lasts for 4-6 minutes but it sometimes takes more time. Stop measuring the diameter after 480 seconds, since the cycle may halt for a long time, for example during phagocytosis.

Once you took pictures over two full cycles, use the snapshots to measure the CV diameter (in µm) and enter your results in the excel spreadsheets on the designated computers.

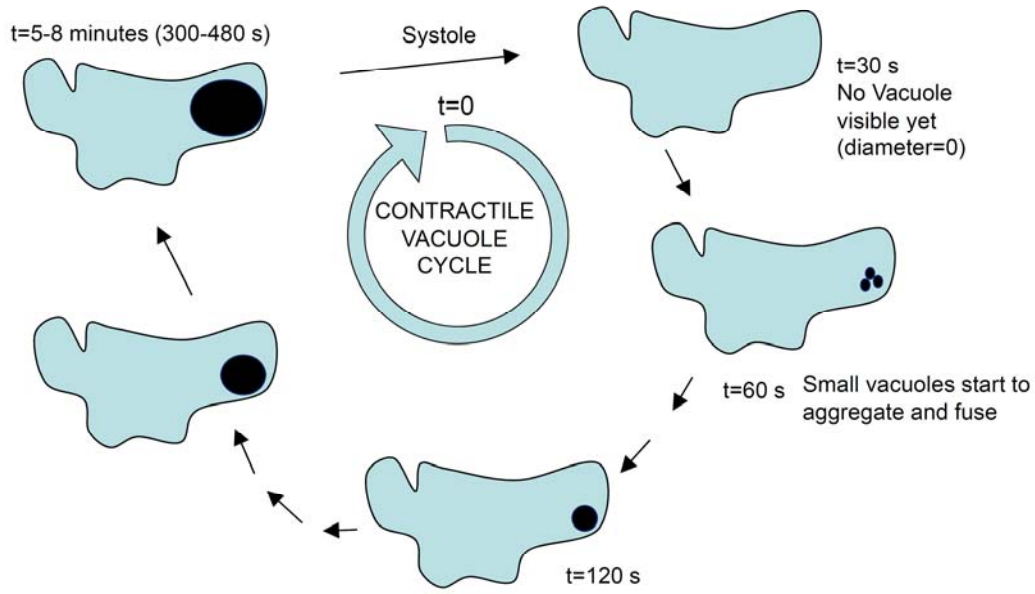


Figure 2: Schematic representation of the contractile vacuole (CV) cycle. Durations are approximate.

Endocytosis

(Bulk-phase endocytosis or pinocytosis)

It was in the *Amoeba* where endocytosis was first described. Since then it has been induced in a great variety of cells but the amoeba remains the organism of choice for its demonstration. Bulk-phase (or nonspecific) endocytosis may be induced by a large variety of substances: proteins, amino acids, salts (particularly cations), basic stains, etc... We will use a solution of 0.05% Alcian blue at pH 4.4, containing 1% bovine plasma albumin to induce pinocytosis.

Place some amoebas in a watch glass with minimum liquid, then add one drop of inducing agent. After 30 seconds prepare a wet mount using the slide in which you observed the amoeboid movement. Do not add too much water and make sure that the coverslip covers the whole preparation.

Once the amoeba is in contact with the inducing agent, the cell will stop moving and its shape will become "rounder". The endoplasm flow will also stop and endoplasm will become restricted to the central region of the cell. Small protrusions (like mini pseudopodia) will appear. Endocytosis canals can be observed more easily in these structures. Observe the formation of canals and accumulation of alcian blue dye in the endocytic vesicles.

Phagocytosis

You may observe phagocytosis in your amoeba depending how hungry it is at the time of the lab. Observe the various steps of phagocytosis and the evolution of the food vacuole. Let your TAs and the other students know so that they can observe this interesting process as well.

Read instructions for the Lab report on the Lab website

Lab #4 – Mitosis

Introduction

Cell division

The cell is the basic functional structure that defines living creatures. All cells have in common the fact that they will have to, at one point during their life, reproduce. This is necessary for the continuity of life, and allows organisms to reproduce. The process that permits one cell to divide into two is called cellular division. Prokaryotes and unicellular eukaryotes rely on cell division to produce new individuals. In multicellular organisms cell division allows the development of an embryo (from one cell to a complete organism), the growth of structures, and replacement of cells due to aging or damage.

In eukaryotes, there are two types of cell division:

1- Cell division that will give rise to two daughter cells identical to the original mother cell. Mitosis belongs to this type of division and it designates the process where the genetic material is evenly separated in two.
2- Cell division that will produce gametes (meiosis). Cells produced by meiosis are different from the original cell and contain a different genetic content. These cells will be used for sexual reproduction. We'll cover meiosis in our next chapter (Lab 5).

The cell cycle

During its life span, a **eukaryotic** cell goes through a series of events called the **cell cycle** (Fig. 1). The cycle may last for a few minutes or hours in actively dividing cells (as in plant meristems or in animal embryos) or years (as in adult bone cells). The cell cycle can be broken down into different phases:

Cell Cycle = **M Phase** **+** **Interphase**

Mitosis + Cytokinesis G_1 ($\pm G_0$) + S + G_2

Cell division (or **M phase**) as seen in this equation is composed of both nuclear (**mitosis**) and cytoplasmic (**cytokinesis**) divisions. **Interphase** is the phase separating two cell divisions, in which cells enter a period of growth and potentially preparation to the next **M phase**. Figure 1 shows that a cell spends most of its time in interphase and it is during this time that most cellular processes are carried out. Interphase may be further divided into sub-stages: the **G_1** (first gap) stage is a period of growth and active synthesis of all groups of macromolecules (including RNAs and proteins). It is also during G_1 that cytoplasmic organelles (like mitochondria, ribosomes, etc…) are duplicated. The length of this stage is quite variable and dependent on the physiological condition of the cell, cell type and species. It may last from as little as 0.5 hours to months or even years. The **S** (synthesis) **phase** follows with precise replication

of all DNA and synthesis of DNA-associated proteins (such as histones in eukaryotic cells) or microtubule-associated proteins (such as the centrioles in animal cells). At the end of the S phase, each duplicated chromosome consists of two **chromatids** joined together by protein complexes called cohesins. The connections between chromatids are tighter in the centromere region where the duplicated chromosome looks narrower. Once S stops, the subsequent stages of the cycle usually follow without delay. The **G_2** (gap 2) stage involves protein synthesis and production of structures needed for mitosis, like spindle fibres.

Some cells stop dividing completely once their growth and differentiation are finished.

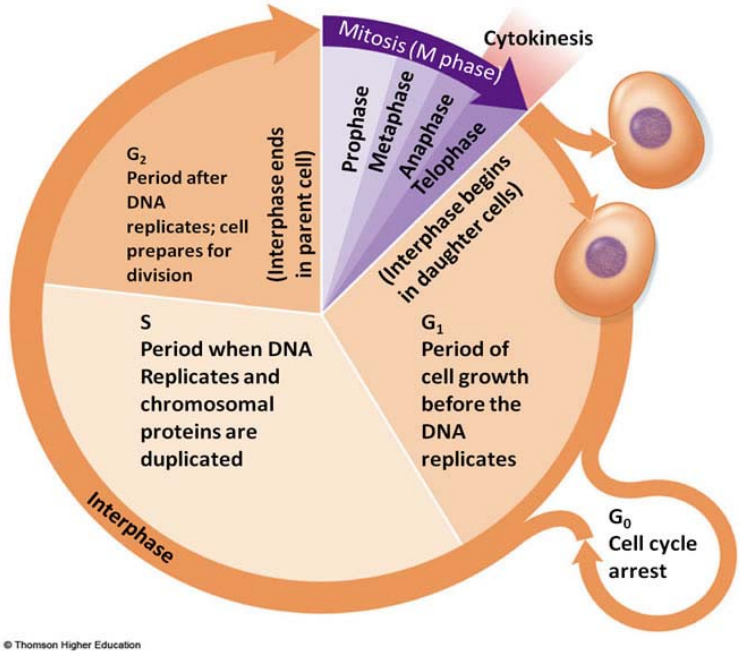


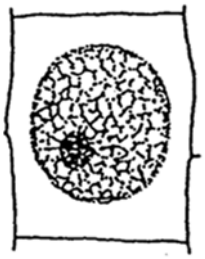
Figure 1: Schematic representation of a typical eukaryotic cell cycle (source: Biology – Russel et al. 1st Canadian edition – Nelson Publishing)

Mitosis

Mitosis is the part of cell division which refers specifically to the process of nuclear division where the chromosomes are equally distributed between two daughter nuclei. After mitosis and cytokinesis, both daughter cells are identical to each other and to the parental cell. In addition, after these two processes, three principal cell types may be found: 1) cells that divide continually (e.g. cells of the gut epithelium or germinal layers of the skin), 2) cells that leave the cell cycle (G_0) and do not divide again during the life of the organism (e.g. nerve cells) and 3) cells that enter a resting state (G_0 , G_1 or G_2) but after some stimulus return to the cell cycle and divide (e.g. liver cells after partial removal of the liver or blood lymphocytes after being stimulated by an antigen). Mitosis involves a dynamic series of events with often rapid physiological and morphological changes. For convenience, it is usually divided into five main stages:

prophase, prometaphase, metaphase, anaphase and telophase. In reality, it is a continuous series of changes with no sharp breaks in between. In fact, it is often necessary to use the terms "early" or "late" to describe a dividing cell in between stages. Closely associated (but not always) with nuclear division is the division of the cytoplasm, known as **cytokinesis**. It begins in late anaphase and continues through telophase. It is seen as a **cleavage furrow** which forms in the middle of animal cells or a **cell plate**, which is laid down at the equator of plant cells.

Summary of cell cycle phases in plants



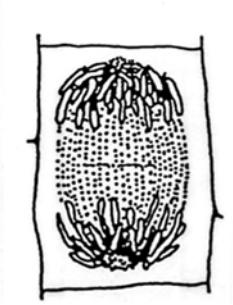
Interphase: This is the stage that the cell is in between mitotic divisions. Growth, synthesis of macromolecules and assembly of organelles, as well as DNA replication occur during this phase. Features of this phase are a clear-cut **nucleus**, darkly staining **nucleoli** within the nucleus and **heterochromatin** in the nucleus. Why will most of the cells you see appear to be in this stage?

Prophase: Chromosomes shorten and thicken and each one can now be seen to consist of two **chromatids** attached at the **centromere** region. Each chromatid of a chromosome contains identical information and comprises the DNA duplicated during interphase (S Phase). During prophase the **microtubules** of the cytoskeleton disassemble into tubulin subunits that begin to reassemble, forming the mitotic spindle. The **nucleoli** gradually disappear. The **centrosome** (including its two **centrioles**) duplicates just before the S phase of interphase. During prophase in animal cells, the two centrosomes separate and migrate towards the opposite poles of the cell. Centrosomes are also the site where microtubules of the mitotic spindle are produced.

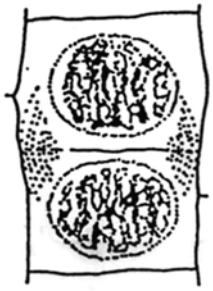
Prometaphase: The fragmentation or breakdown of the nuclear membrane marks the beginning of prometaphase. The centrosomes will eventually be found at opposite poles of the cell (**spindle poles**). A protein complex forms on the centromere of each chromatid: **the kinetochore.** The kinetochore of one sister chromatid attaches to microtubules from one pole (**polar microtubules**) while the kinetochore of the other sister chromatid attaches to polar microtubules of the opposite pole. As chromosomes are being pulled toward opposite poles simultaneously, they slowly migrate toward the **metaphase (equatorial) plate**, an imaginary plane located midway between each pole of the cell.

Metaphase: Mitotic spindle microtubules are fully formed between the poles. The centromeres of all chromosomes are located at the metaphase

plate. Cohesin complexes are cleaved by an enzyme called separase, which releases the junctions between sister chromatids. Then, centromeres start to separate.



Anaphase: Each chromatid (now called a **daughter chromosome**) moves to opposite poles of the cell. The movement of chromatids towards the pole is driven by the shortening of microtubules, which occurs by depolymerisation at the level of kinetochores.



Telophase: Chromosomes now located at the poles decondense and become longer and thinner. Nuclear membrane reappears and the nucleolus reforms. Spindle disappears. Cytokinesis usually occurs during this phase. In plant cells, the **phragmoplast** guides the formation of the new cell wall across the centre of the cell, while in animal cells a **cleavage furrow** pinches the cell in two.

Microscopic observation of mitosis

Mitosis is the method of cell division in almost all animals and plants. It is generally more clearly seen in plants than in animal tissues as chromosomes stand out more clearly. In addition, plants have growth confined to certain areas and mitotic cells are usually abundant there. These growth centres occur principally in root tips and in stem tips (**apical meristems**). We will study growth and cell division in root tips and also in whitefish (*Coregonus clupeaformis*) embryo cells for comparison.

Though sections are often used, the simplest way of making slides for chromosome study is the "squash preparation". In this method, the tissue is first fixed and stained (hard tissue is also softened by acid treatment) and then squashed beneath a coverslip. This gives a preparation of a single layer of flattened cells, with the chromosomes well spread for microscopic observation. A number of stains may be used for chromosomes, such as the **Feulgen stain,** which give a strong and specific staining.

Each student team will prepare a Feulgen squash of a broad bean (*Vicia faba*) root tip. At the root tip the cells are dividing actively, and a large percentage will be seen in mitosis.

A - Mitosis in plants

1 - Feulgen Stain

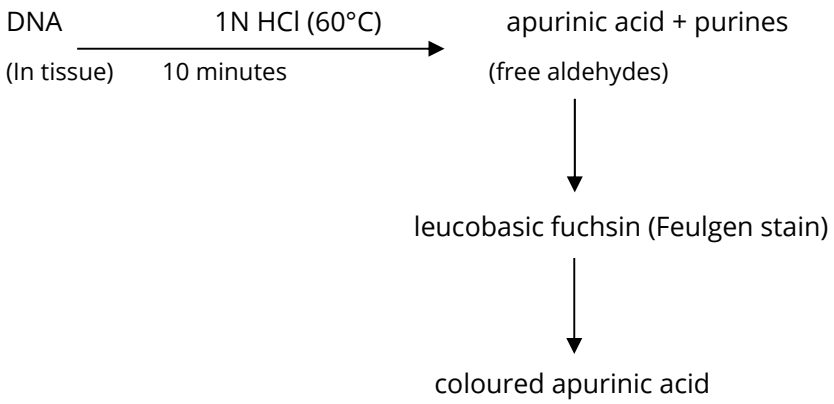
The Feulgen stain (named after one of its originators) specifically colours DNA in magenta red. Chemically, this substance is a "Schiff base" which will react specifically with aldehyde groups to form a coloured reaction product.

Intact DNA does not contain free aldehyde groups and will not react directly with Feulgen stain. However, DNA does contain **deoxyribose** sugar molecules chemically linked through aldehyde groups to purine and pyrimidine bases. To form free aldehyde groups in DNA so that it will react with the Feulgen stain, the DNA is first hydrolysed with hot (60°C) 1N HCl which removes the purine bases and frees the aldehyde groups of the deoxyribose sugar. The resulting product is called apurinic acid, i.e. DNA lacking purines, and this material reacts specifically with Feulgen stain.

The acid hydrolysis may be carried out on whole tissue pieces or on mounted sections. It has the added value that it removes all RNA from the tissue so that it cannot react, and it softens the tissue, which is of value in making squash preparations. Timing of the acid hydrolysis (**10 min**.) is important: hydrolysis of short duration will not free all aldehyde groups while over hydrolysis will cause destruction of the apurinic acid. Both lead to reduced staining.

The hydrolysed tissue containing apurinic acid is placed directly in Feulgen stain where the aldehyde reaction occurs. Colour formation is essentially complete in 1-2 hrs.

Reactions involved in Feulgen staining may be summarized as follows:



CAUTION: Feulgen stain looks like an inoffensive chemical but it can stain clothes and
skin in a vivid magenta colour. Wear old clothes or a lab coat.

2 - Preparation of a root tip squash.

General recommendations: Use the plastic pipette to transfer all liquids. Try to keep root tips inside the microtube except for washes, only add enough liquid to cover the

root tips (do not fill the microtube). Discard all liquid in waste beaker located on each bench. Keep the stained roots in water so that they won't dry.

Keep your fingers away from Feulgen stain especially if bright pink is not your favourite colour. You must wear safety goggles during all steps of the preparation of root tip squash.

Protocol:

1. Write your name on the microtube containing the broad bean root tips in Carnoy Lebrun Fixative.

2. Using the plastic pipette, remove the fixative and wash the root tips once in 95% alcohol. Then take out the alcohol.

3. Add hot 1N HCl then place microtube in tube rack in waterbath at 60°C for 10 min. This time is important.

4. Quickly take out the HCl (using the plastic pipette) and add cold distilled water to stop the hydrolysis.

5. Discard the water and add the Feulgen stain (just enough to submerge the sample). Leave roots in stain for about ½ hour, until the tips are deep red. If not deep red, leave them in longer (up to 1 hour). Note that the deep colour is in the root tip, where the cells are small and actively dividing. The zone of elongation has much less colour.

6. Discard the stain in the chemical waste bucket.

7. Rinse in water twice.

8. Place one drop of 50% acetic acid on a slide and place one root tip in it. Using a razor blade, cut off the last 2 mm of the deeply stained tip and discard the rest.

9. Put on a coverslip and squash by pressing with the eraser end of a pencil. Squash until the circle of tissue is about 1 cm across. Be careful not to break the coverslip.

10. Show your slide to your demonstrator for technical evaluation.

11. Using the squashes you have prepared, find and take a picture of all stages you can observe.

3 - How is root growth accomplished?

Growth can be accomplished by increasing the number of cells by cell division or by increasing the size of individual cells. What is happening in root growth?

We know from our work so far that: Many cells in the root tip are undergoing mitosis. Look at a prepared slide of a longitudinal section of a root and work out what is happening. Note the **root tip** with its square cells many of which are undergoing mitosis.

Around the end of the root tip is a mass of irregular dead cells with thicker walls. These are the **root cap** which protects the apical meristem of the root tip as it is pushed into the soil. As the root penetrates deeper, the sloughed off cells form a covering around

the root end to lubricate its passage through the soil. The root cap is also believed to be a site that detects gravity and controls the direction of root growth.

Immediately above the root cap, there is the **region of the apical meristem**. It is a zone of cell division or the site of apical growth of the root. At the base of the apical meristem is the **quiescent centre**. It is a relatively inactive region where the cells are arrested in G_1 of interphase.

Above the region of cell division, but not sharply delimited from it, is the **region of elongation**. The elongation of cells in this region results in most of the increase in length of the root.

Following the region of elongation is the **region of maturation** in which most of the cells of the primary tissues mature. Root hairs are also produced in this region. Near the centre of the root you may be able to see very narrow thick walled cells which are differentiating into vascular bundles. The **xylem cells** of these bundles transport water and salts from the soil to the rest of the plant. The **phloem cells** transport carbohydrates from the photosynthetic portions of the plant to the roots. The roots do not have **chloroplasts** and cannot make their own food.

1. Label the line diagram of the main regions of the root. (Figure 2)

2. Study the prepared slide of the longitudinal section of the onion *(Allium cepa)* root tip and find all the regions described above.

3. Answer the questions 4 to 6.

B - Animal Mitosis

Mitosis in animal cells is essentially the same as that in plant cells, but as there is no cell wall around the cell membrane, and no cell plate is formed at telophase. At the end of nuclear division the membrane simply pinches together in the middle dividing the two new cells. In animal cells there is an **aster**, a semicircle of fibrils around each end of the spindle. There are no asters in plant cells.

Many animal cells such as nerve, muscle and red blood cells do not divide; they lose their ability to divide during their differentiation and development. Embryo cells, however, divide rapidly and many can be seen in mitosis.

Study the prepared slides of whitefish embryos (**blastula**). These slides are very pale and the cells small. Set them up on the microscope carefully, focusing with care with the low objective first. Turn to the medium power and focus again. Turn to high power and focus with the fine focus only. This is a real test of your ability to use the microscope. Broken slides are caused by careless focusing.

C - Timing of the cell cycle

Objective: Determine the relative time spent by cells in each phase of the cell cycle.

Procedure: Working with your partner, count how many cells of each phase you can observe in three fields of view (or at least 150 cells in total) for each of the 3 samples: broad bean squash (made by you), prepared slides of the onion root (longitudinal

section) and the whitefish blastula embryo (cross section). The duration of each phase of the cell cycle is proportional to the number of cells you can observe in any given phase (ex. if 10% of the cells are in prophase then 10% of the cycle time must be taken up by prophase).

Enter your observations in the table on the next page while counting cells, and then enter your results on the designated computer (follow instructions given by your TAs). Percentages will be automatically calculated in the excel sheet, but you can do it manually by dividing the **number of cells** in each phase by the **total** number of cells observed.

		Broad bean				**Onion root**				**Whitefish blastula**			
	Field of view #	**1**	**2**	**3**	**Total**	**1**	**2**	**3**	**Total**	**1**	**2**	**3**	**Total**
Number of cells observed	**Interphase**												
	Prophase												
	Metaphase												
	Anaphase												
	Telophase												
		Grand total=				**Grand total=**				**Grand total=**			

Questions

1. In which stage did you find the most cells?_____

The fewest cells? _____

2. Which stage, therefore, would you conclude is the longest in duration?

The shortest? _____

3. In which part of root tip section would you expect to find the youngest

cells? _____

4. How do young cells differ from old cells with respect to:

a) shape: _____

b) size:_____

5. Do all cells appear to have a nucleus? (If not, explain why) _____

_____

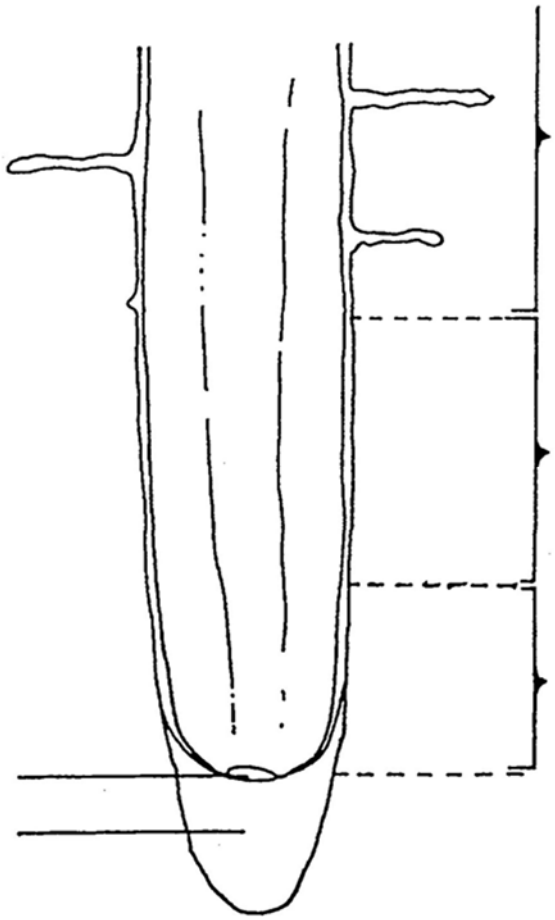


Figure 2: Longitudinal section of an onion (*Allium cepa*) root tip (add labels to drawing)

Assignment: Carefully read the instruction file located in the Lab 4 section of the lab website.

Lab #5 – Meiosis

Introduction

Meiosis (from the Greek meaning "to make smaller") is a process of nuclear reduction division. In mitosis, there is a duplication of DNA in interphase followed by a single nuclear division. In meiosis, there is also DNA duplication, but it is followed by 2 divisions. The net result of meiosis is 4, not 2 cells, and because of events that occur during meiosis, the 4 daughter cells are not identical to each other nor to the mother cell. In addition, each daughter cell contains only n chromosomes, half the number in the mother cell. Upon fertilization, the 2n number of chromosomes is restored. Meiosis therefore assures: 1) that the chromosome number will be stable from generation to generation, 2) that each offspring resulting from sexual reproduction will receive two entire sets of genetic instructions and 3) that genetic diversity is promoted among the product (through differential parental inheritance and homologous recombination).

As we stated in lab 4, meiosis only occurs in sexually reproducing organisms. The life cycles of plants are characterised by an alternation of generations between diploid and haploid stages. The diploid (2n) generation or **sporophyte** undergoes meiosis to form haploid **spores**. These spores divide mitotically to become multicellular haploid individuals or **gametophytes**. They eventually produce **gametes** which fuse to form diploid **zygotes**. Zygotes divide mitotically, developing and differentiating into a multicellular diploid organism, the sporophyte. This type of meiosis is called **sporic meiosis**.

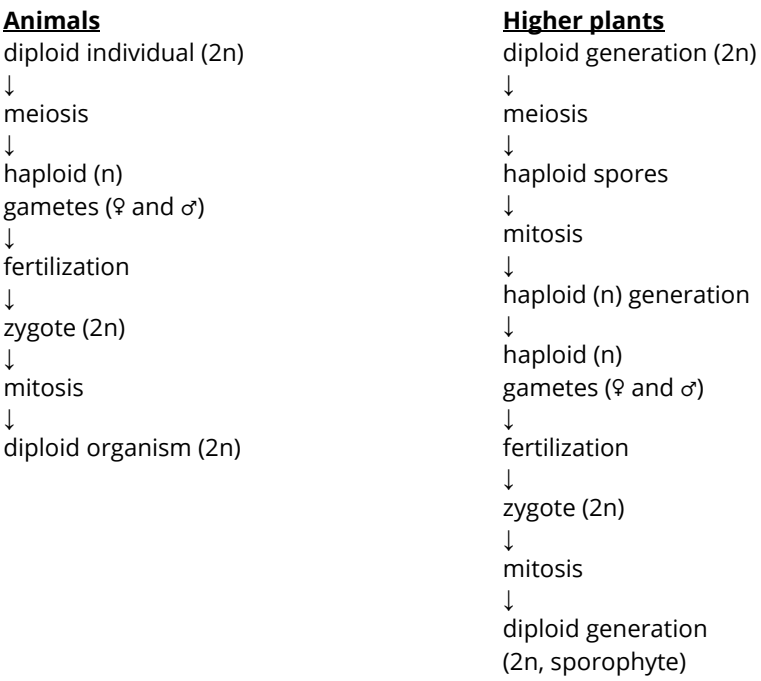
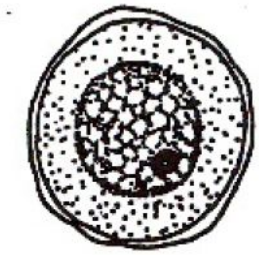


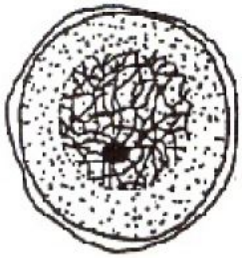
Fig 5.1: Comparison between meiosis in animals and plants

In most animals, an alternation of **ploidy level** also occurs. The diploid individual produces haploid gametes by meiosis (**gametogenesis**). Male and female gametes fuse to form a diploid zygote. The zygote then divides mitotically to become a multicellular diploid organism. This type of meiosis is called **gametic meiosis**.

Stages of Meiosis I



Premeiotic Interphase: This phase precedes meiosis 1 and is composed of substages similar to mitotic interphase (i.e. G_1, S and G_2). However, premeiotic S phase may be up to 20 times longer than premitotic S in the same species. Chromosome replication (DNA and proteins) occurs during this stage. The result is two identical sister chromatids attached at the centromere. In animal cells, the centriole pairs also replicate.

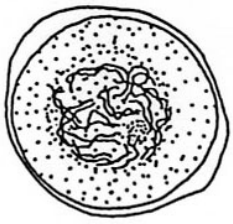


Prophase I: In this stage, homologous chromosomes pair, recombination occurs and large amounts of RNA and proteins are synthesized. This is one of the longest stages in meiosis lasting anywhere from weeks to years, depending on the species. Prophase I can be divided into 5 sub-stages:

1) Leptotene: Initial condensation of chromosomes occurs so they appear as fine single threads (**sister chromatids** are indistinguishable). Tips of the chromosomes (**telomeres**) are attached to the nuclear membrane.

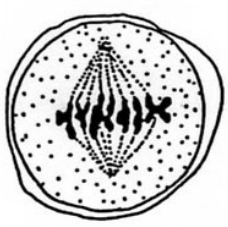
2) Zygotene: The maternally and paternally derived copies of each homologous pair of chromosomes (=**homologues**) line up along their length in a process called **synapsis**. As the chromatids between homologous chromosomes intertwine, a **synaptonemal complex** is formed. This stage may last from several hours to three days.

3) Pachytene: Recombination occurs as adjacent chromatids break and join in a process called **crossing over**. Fully paired homologues are called **tetrads** (four chromatids). This stage may last from days to weeks. Genetic material is exchanged between homologous chromosomes. This is a key event in creating genetic diversity.



4) Diplotene: The separation between homologues increases so that 4 chromatids are visible**. Chiasmata** (region of crossing over) becomes visible. The synaptonemal complex disappears and chromosomes detach from the nuclear membrane. Extensive RNA transcription occurs in some species. The length of this stage is quite variable (up to 12 years).

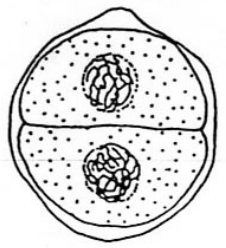
5) Diakinesis: Condensation of chromosomes finishes and final modifications occur, ensuring that the chromosomes are ready for division**.** RNA transcription stops and the nucleoli disappear**.**



Metaphase I: At the beginning of metaphase I, the nuclear membrane breaks down. The paired homologues line up on the equator of the divisional spindle (**metaphase** or **equatorial plate**). The ordering of

maternal and paternal homologues occurs at random. Two sister chromatids of one homologue attach their **kinetochores** via microtubules to the same spindle pole while sister chromatids of the other homologue use microtubules to attach to the other pole.

Anaphase I : The centromeres of the doubled chromosomes do not split as in mitosis, but the complete homologues are pulled away from each other toward opposite poles. This is a key difference between meiosis and mitosis that contributes to creating genetic diversity.



Telophase I: Chromosomes decondense**,** the spindle breaks down and the nuclear membrane reforms**.** The length of this stage and degree to which these events occur vary widely among different species. Cytokinesis continues, resulting in two daughter cells, each with half the number of chromosomes, but with each chromosome containing two chromatids.

Interkinesis: During this interphase-like stage between meiosis I and II, no DNA replication occurs. Centrioles and asters, if present, divide. Centrioles however, do not duplicate, such that a single centriole is found at each pole of the spindle in each daughter cell of prophase II.

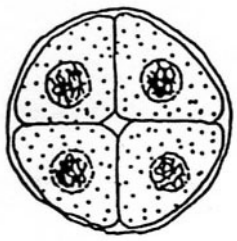
Stages of Meiosis II

The second meiotic division is very similar to mitotic division.

Prophase II: The nuclear envelope breaks down and chromosomes recondense during this stage. The spindle forms with kinetochores attached to spindle microtubules in mitotic fashion. Chromosomes begin to move to equator.

Metaphase II: The chromosomes, each consisting of two chromatids, line up at the metaphase plate (equator of the cell).

Anaphase II: Similar to mitotic anaphase, sister chromatids separate and move to opposite spindle poles. Cytokinesis begins late in this stage.



Telophase II: Chromosomes decondense, nuclear membranes form around each product, nucleoli reform and cytokinesis finishes. After cytokinesis, 4 haploid cells are present. There are four potentially genetically different gametes that may contain all maternal, all partenal or a mix of maternal and paternal chromosomes

Meiosis in plants

In angiosperms (flowering plants), meiosis is involved in the formation of both male and female spores (**microspores** and **megaspores,** respectively).

Male gametophyte formation in *Lilium*, for instance, occurs in structures called **anthers**. Microspores are produced by a reduction division within the four **microsporangia** or **pollen sacs** of the anther.

Early in differentiation of the anther, four fertile groups of cells (**sporogenous cells**) may be observed. They are surrounded by several layers of sterile cells: the innermost layers (the **tapetum**) will provide nutrition to the developing microspores, while the outermost ones will form the wall of the **microsporangium**. The sporogenous cells develop into **microsporocytes** (microspore mother cells). These diploid cells divide by meiosis to produce four haploid, single-celled **microspores**.

After meiosis, the microspores divide by mitosis to form a **tube cell** and a **generative cell,** the **microgametophyte**. These two cells, together with the spore wall, constitute a **pollen grain**.

Examine prepared slides of microsporogenesis in the lily (*Lilium*). Try to find as many meiotic stages as you can and draw them or take a picture. Is pairing evident in prophase I? Can you see any crossing over? How can you distinguish the stages of meiosis II from those of meiosis I?

Meiosis in Animals

In the formation of male gametes (**spermatogenesis**), four viable sperm are produced from each pair of meiotic divisions. In the female (**oogenesis**), the whole cytoplasm and stored food must be retained in the egg for the use of the developing embryo. Here, one of the products of the first division of meiosis is pushed out of the surface of the other as simply a nucleus without its cytoplasm. The large cell is the egg; the small one is called a **polar body**. In the second division, the same thing happens. This leaves one large egg and two small polar bodies, which do not survive any further.

Observation of meiosis in *Ascaris*

One of the earliest studies of meiosis was made by Van Beneden (1883) on egg formation in the parasitic roundworm *Ascaris*. The diploid number of chromosomes in this species is four and thus the process is comparatively easy to follow.

In *Ascaris*, as in all animals, the spermatozoa complete meiosis in the testis of the male. The mature sperm then leaves the testis and travels to the seminal vesicle. In the female, the eggs are shed from the ovary in prophase of the first division of meiosis. They travel down the oviduct in this stage without going into metaphase I.

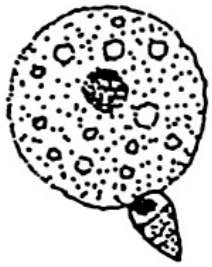
After copulation, as the spermatozoa reach the eggs in the oviduct, one penetrates each egg. It moves into the centre of the egg and loses its membrane, leaving a dark mass of chromatin. Sperm entrance stimulates the egg to resume meiosis (and enter metaphase I): The egg nuclear membrane breaks down and a small spindle forms at

the periphery of the egg, perpendicular to the plasma membrane. Homologous chromosomes are paired (as tetrads) and aligned in between each spindle pole. During anaphase I, the homologous chromosomes are separated and pulled away in opposite direction by the spindle. One set (that migrated towards the periphery of the egg) is pinched off and expelled from the cell as the first polar body. Both the egg and the polar body are now haploid.

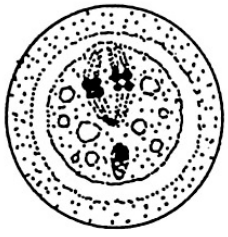
A second meiotic spindle forms after that in preparation for the second division. This time, sister chromatids separate and one set is expelled as the second polar body, whereas the other half remains in the egg. The first polar body sometimes divides to give a total of three polar bodies.

The haploid egg and sperm nuclei remain in interphase for a while. At this time, they are called the male and female **pronuclei**. Subsequently, the first mitotic spindle forms. Several mitotic divisions follow and result in the formation of an embryo. Each embryo is surrounded by a chitinous shell secreted by the uterus to protect it from drying. The embryos are then released by the female.

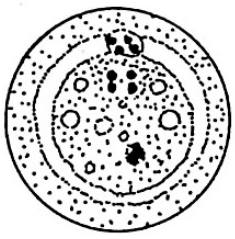
Histological preparations and a *powerpoint* presentation showing a series of *Ascaris* eggs during meiosis will be provided. Each slide and picture represents a section of the **oviduct**. The oviduct walls are shown on the top and bottom. Between these walls are numerous eggs in meiosis. Since it is a section, all the eggs will not be cut through the middle. Several representative stages were selected. Observe the presented material and identify the specific features allowing you to later recognize each meiotic stage.



1. **Sperm entrance**: The blue acorns seen among the ovocytes are the spermatozoa. Note that each lacks a flagellum. The black dot at the rounded end of the sperm cell is the haploid nucleus. The ovocyte cytoplasm is vacuolated and its nucleus in prophase I is barely visible. The ovocytes **do not have shells yet,** and many have lost water by osmosis during preservation and appear shrunken. In life, they would be spherical. As the sperm cell enters the primary oocyte, the egg nucleus resumes meiosis. The sperm cell loses its outline and becomes a black amorphous mass of chromatin in the egg.

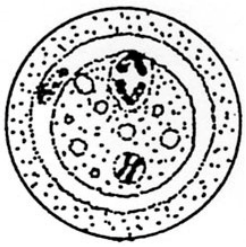


2. **Metaphase I and Anaphase I**: In metaphase I, there are two tetrads, the paired homologous chromosomes are lined up at the equator of the spindle at the edge of the **primary oocyte**. It is sometimes difficult to distinguish the homologues and to see that each homologue is double. During metaphase I the oocyte secretes its protective shell. In anaphase I, the homologous chromosomes separate and move toward the opposite poles of the spindle. The male pronucleus is visible as a dark spot in the lower part of the oocyte.

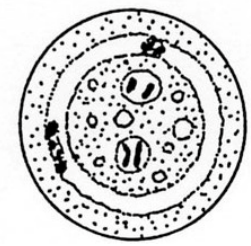


3. **Telophase I**: The first polar body is extruded from the newly formed secondary oocyte carrying with it two double chromosomes (four

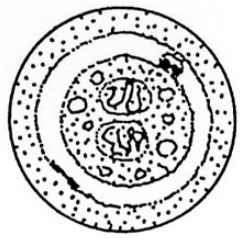
chromatids). It is NOT a tetrad being expelled here. What would be the consequence if that was the case?



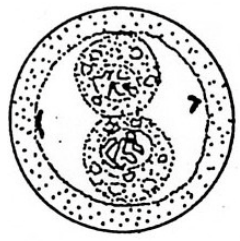
4. **Metaphase II and Anaphase II:** Note the spherical shape of the secondary oocytes surrounded by a thick shell. The oocyte floats in a fluid filled space called perivitelline space. The sperm nucleus is near the centre of the secondary oocyte (in the illustration beside, it is in the lower part of the oocyte). The first expelled polar body is visible in the space between the oocyte and the shell. The two remaining chromosomes (each formed of two chromatids) line up at the equator in metaphase II. In anaphase II, the centromeres split and the chromatids separate.



5. **Telophase II:** The second polar body is expelled (black spot at the top), carrying with it two chromatids. The two chromosomes remaining in the mature ovum will be single and be of each of the two types. Now the haploid ovum has two single chromosomes.



6. **Interphase:** The haploid male and female **pronuclei** remain in interphase for a while and then fuse: this is called fertilization. The resulting zygote is now diploid and has two pairs of homologous chromosomes (i.e. four chromosomes).



7. **Cleavages:** The zygote divides by mitosis. First, the embryo is formed of two cells, then four, then eight, etc... The cells don't necessarily divide synchronously. In *Ascaris*, the cells at the eight celled stage are not equal in size.

Gametogenesis in mammals

In this exercise you will examine the process of gamete formation in mammalian gonads (rat and rabbit).

Spermatogenesis

Since spermatogenesis is a continuous process, all its stages can be observed within the cross section of one tubule. You will notice that it occurs in an orderly fashion from the outside of the tubule to the lumen (see figure 5.2).

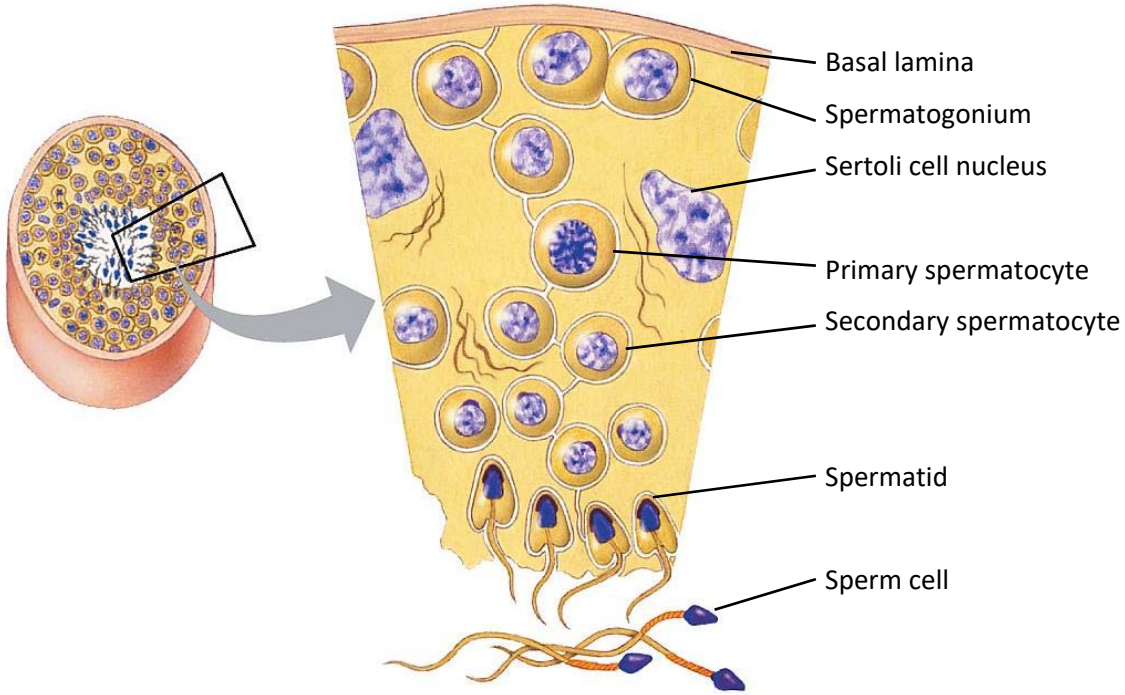


Figure 5.2: Overall view (left) and cross section (right) diagram of a mammalian testis showing spermatogenesis in progress within a seminiferous tubule (lumen at the bottom of the figure). Modified from Campbell Biology 4th Edition (2010).

Obtain a slide of a cross section of a mammalian testis. Scan the slide at low power to see numerous cross sections of seminiferous tubules. Switch to medium power and choose one tubule not too darkly stained with a defined clear area (the lumen) at its centre. Now turn to high power and study the following cell types:

1. **Spermatogonia** (2n). These are the large darkly stained outer cells with well-defined nuclei. They divide by mitosis to produce more spermatogonia. About half of the spermatogonia produced undergo meiosis to become sperm cells while the other half divide again by mitosis to replenish the spermatogonia population.

2. **Primary spermatocytes** (2n). Located just below the spermatogonia and not as darkly stained, the primary spermatocytes are larger cells undergoing the first meiotic division.

3. **Secondary spermatocytes** (n). These slightly smaller cells are further toward the lumen of the tubule. They are the product of the first meiotic division. The secondary

spermatocytes are difficult to observe because they rapidly undergo meiosis II to produce spermatids.

4. **Spermatids** (n). These very small circular cells will differentiate into functional spermatozoa.

5. **Spermatozoa** (n). Upon maturity these are easily recognizable by their very long thin flagellum bordering the lumen.

6. **Nurse cells** (or Sertoli cells). The large nurse cells are also found in the walls of the **seminiferous tubules**. These cells feed and regulate the differentiation of spermatids into mature spermatozoa.

Oogenesis

Unlike tubular ovaries as seen previously in *Ascaris*, mammalian ovaries are solid structures in which egg-forming cells grow in small cell-lined cavities known as follicles (see figure 5.3).

Ovaries of a newborn mammalian female already contain all the **ovocytes** she will ever need during her reproductive years. Each **ovocyte** is then surrounded by a layer of **follicular cells** and together they constitute a **primary follicle**. At the onset of meiosis I, the ovocyte enlarges to become a primary oocyte. The follicular cells surrounding it proliferate and together with the primary oocyte form a **growing follicle**. Cytokinesis of the primary oocyte at the end of the first meiotic division produces a large secondary oocyte and a small first **polar body**. Meiosis II of the secondary oocyte is initiated, goes as far as metaphase II and then stops. The growing follicle containing a secondary oocyte then awaits the stimulation of female hormones to develop further. In response to hormonal influences the growing follicle will enlarge and form a **mature follicle** (or **Graafian follicle**). At that stage, the secondary oocyte (still stopped at metaphase II) is called a **mature oocyte**. After a short while, the mature follicle ruptures at the surface of the ovary by a process called ovulation and releases the mature oocyte. The mature oocyte will only resume and finish meiosis II if it is penetrated by a **spermatozoon**.

Obtain a slide of a cross section of a mammalian ovary and observe the following structures at the appropriate magnification:

1. **Primary follicles**. These are the numerous small round structures at the periphery of the ovary. Each primary follicle contains one ovocyte (2n) filling most of the space within one layer of follicular cells.

2. **Growing follicles**. These are larger follicles with a few layers of follicular cells. Each growing follicle contains a primary oocyte (2n) or a newly formed secondary oocyte (n) in its small fluid filled cavity.

3. **Graafian follicles**. Most of the space of these very large follicles is occupied by a large clear fluid-filled cavity. If you are lucky, your slide will show a mature oocyte (actually a secondary oocyte (n) stopped at metaphase II) nestled in a bulge of follicular cells at the centre of the follicle.

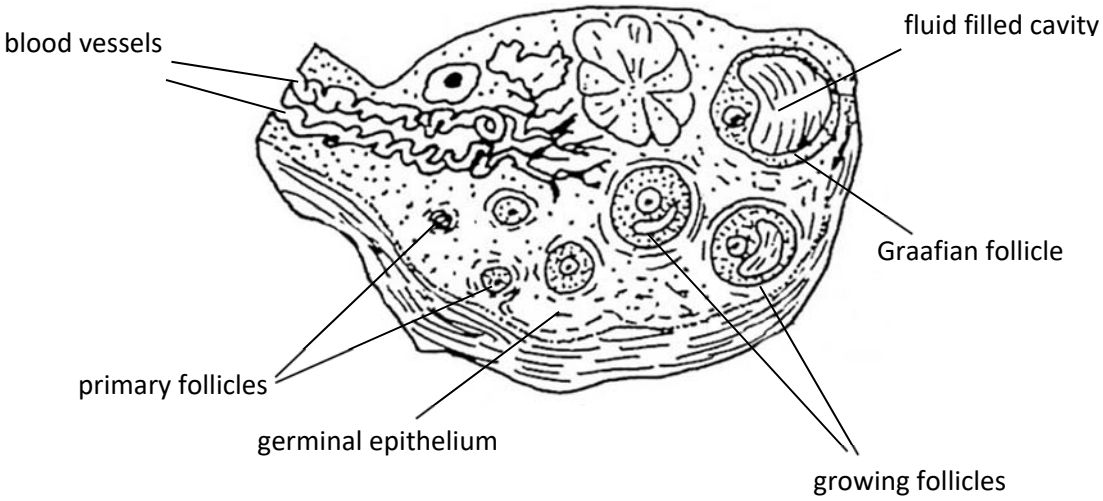


Figure 5.3: Cross section of a mammalian ovary showing follicular development.

Table 1: Characteristics of Mitosis versus Meiosis

Mitosis	Meiosis
(1) Sister chromatids separate at anaphase.	(1) The first stage is a reductive division which separates homologous chromosomes at first anaphase; sister chromatids separate in an equational division during the second anaphase.
(2) One division per cycle	(2) Two divisions per cycle
(3) Chromosomes fail to synapse; no chiasma forms; genetic exchange between homologous chromosomes does not occur	(3) Chromosomes synapse; chiasma forms; Genetic exchange occurs between homologues
(4) Two products (daughter cells) produced per cycle	(4) Four cellular products (gametes or spores) produced per cycle.
(5) Genetic content of mitotic products is identical	(5) Genetic content of products is different; chromosomes may be replicas of either maternal or paternal chromosomes or various combinations of both.
(6) Chromosome number of daughter cells is the same as that of the mother cell.	(6) Chromosome number of meiotic products is half that of the mother cell
(7) Mitotic products are usually capable of undergoing additional mitotic divisions	(7) Meiotic products cannot undergo another meiotic division although they may undergo a mitotic division
(8) Normally occurs in most somatic cells.	(8) Occurs only in specialized cells of the germ line

Instructions for the lab report can be found on the lab website

Lab exam covers all specimens observed in lab5 (as well as those observed in the other labs).

APPENDIX

Graphical representation of quantitative data

Use a graph when you wish to:

- see overall trends, patterns or relationships in the data
- compare two or more factors in a general or quantitative fashion,
- present large data sets in a comprehensible way and analyse data

General Principles of Graph Construction

A- Plot type

Here are some examples of different graph or plot types:

Bar graphs (horizontal or vertical)

These graphs consist of proportional bars of equal width and variable length. Quantitative variables are placed **along one axis only** (the x-axis for horizontal bar graphs and the y-axis for vertical bar graphs). Bars can represent discrete values (for example locations, categories, time periods…) and are separated by empty spaces. Bar graphs should not be confused with **histograms,** which have quantitative scale along both axes.

Histograms

Histograms are similar to bar graphs in that they consist of a number of proportional bars of equal width and variable length. They differ in that **histograms are used to analyse and study distributions**. When building a histogram, the data range must first be divided into a number of intervals and the number of observations falling into each interval recorded. The percent of observations in each interval can be then calculated and plotted on the y-axis.

Two panel bar graph or histogram (vertical layout):

Les règles générales des histogrammes ou graphiques à barres s'appliquent, plus les suivantes :
General rules regarding histograms or bar graphs apply, plus

- The Y axis spreads over the 2 panels.
- The scale of the Y axis on both panels is the same (even though the range of the data may be different).
- Label for the X panel is located below the lower panel
- Tick marks are placed on both X axes
- Only one symbol key is used for both panels.

Pie charts

A pie chart shows the relationship or proportion of parts to a whole. It is useful if one element makes up a significant portion of the whole. Since generally no scale is provided one must judge the sizes of the angles to infer percentages or proportions represented by a given slice. This type of chart is poor for determining exact values **should be avoided**.

Straight-line graphs

They are generally used when many data points (n>30) are available at constant intervals, in order to see trends or changes in a variable through time. Points are connected by straight lines to indicate

the fluctuation in values through time.

Scatterplots

Scatterplots are used to investigate the relationship between two different sets of data. In these graphs variable quantities are scaled along both axes. The independent is always presented on the x-axis, whereas the dependant variable (the one whose value depends on the independent variable) is presented on the y-axis. With these graphs it is possible to quantitatively evaluate the relation between two variables.

B- Presentation

I -Page layout

Layout should consider the size, placement and orientation of the graph. Typically the graph uses the top 2/3 of the page (in portrait orientation), the last 1/3 being used for the caption

II- Data to ink ratio

The data to ink ratio tries to emphasise the importance of the data itself relative to the other elements of the graph. Maximise the data to ink ratio by reducing the amount of non-data ink (grids, borders and unnecessary text…).

III- Data

1. Symbols

- Use visually prominent plotting symbols to show the data. The size and appearance of the symbols should be considered. Open or filled circles, squares and triangles are, for example, appropriate. Data symbols should be more prominent than any line linking them or drawn through them.
- Lessen the visual impact of data labels so they do not interfere with the data or disorder the graph.
- Axis labels must be concise and precise.
- Symbols that overlap must be distinguishable. Use distinct symbols (e.g. different shapes or numbers in superscript) to indicate how many data points overlap.
- If data sets superpose, they should be visually separable.
- If more than one series of data is plotted and /or you use several symbols, a key for the symbols should be used. Place the symbol key within the plot area of the graph in a space devoid of data. If this is not possible, place it either immediately above or to the right of the x-y axis area. The use of colours should be avoided.

2. Error Bars

- If means are plotted, error bars must be traced. Error bars may be either the sample standard deviation, the standard error of the mean or the 95% confidence interval (indicate what error bars represent in the caption).
- The error bars should be slightly less prominent than the data points.
- If a bar graph is used, only present the means + the error bars.

3. Plotting area

- This is an ***imaginary*** rectangle which encompasses **all the data points and error bars**. A larger plotting area means a clearer and more easily readable graph (see also page layout).

IV- Axes

1. Axes scale

- Choose a scale for the axis so that the data uses most of the available space (see also page layout)
- Choose an interval that comprises your whole data set (including error bars, if present)
- Only when necessary use a scale break (a break in the x- or y-axis). Do not connect the data points on each side of the break.
- Add 3 to 5 tick marks on quantitative axis. Tick marks should be evenly spaced and all plotted data should be contained between the lower tick mark and the higher tick mark.
- Tick marks number is not limited on non-quantitative axes. Put one tick mark for each category.
- Tick marks should point outwards so they do not obscure data points (they do *not* cross the axes)
- Each tick mark must be labelled with the value or the category it represents.

2. Axis identification (labels)

- Axes labels must be appropriate for the variables plotted. Units between brackets must be included after the text of the label.
- Appropriate SI abbreviations for units must be used (see appendix, next)
- The label text should be brief and precise.
- If you plot logarithms of a variable, the axis label should correspond to the tick mark labels.

V. Caption

A graph must have a caption, located right **below** the graph

1. What should be included in the caption?

- The first sentence in the caption should be a specific and informative title. For instance, indicate what data is presented on each axis, as well as the complete name of the studied organism (see binominal nomenclature below), if applies.
- The body of the caption should be enough to explain briefly the mechanism by which the data were collected and analysed.
- If only two symbols are used in the graph, they may be explained in the caption as an alternative to a symbol key. For more than 2 symbol types use a key (see Symbols).
- If several observations were combined or if a mean average was calculated, indicate the number of observations (= sample size). The abbreviation for sample size is 'n' (e.g. n=20).
- Do NOT describe trends in your data. The graph is right above the caption and speaks by itself.

2 Binomial nomenclature of Organisms

In addition to common name(s) given to organisms, each of them possesses a unique name based on phylogenetic relationships it shares with all other organisms. The most common way to name an organism is to use the binomial nomenclature initiated by the 18th century biologist Linnaeus. This nomenclature indicates the **genus** and **species**, the most specific characteristics in the hierarchy of designations. Specific typography rules apply: **only the first letter of the genus is capitalized (not the species name)** and **both genus and species are printed in italics** (or underlined when hand written). Ex: dog (common name) is *Canis domesticus* or Canis domesticus. Binomial nomenclature must be used in captions.

3 Description of the type of data presented on the graph

- If means are presented, this should be stated, as well as the sample size.
- If data were transformed (if you did the calculation), the type of transformation should be indicated.

- Briefly explain the error bars. For instance: mean average ± standard deviation, mean average ± standard error, mean average ± 95% confidence interval, etc…

C- Example of a graph:

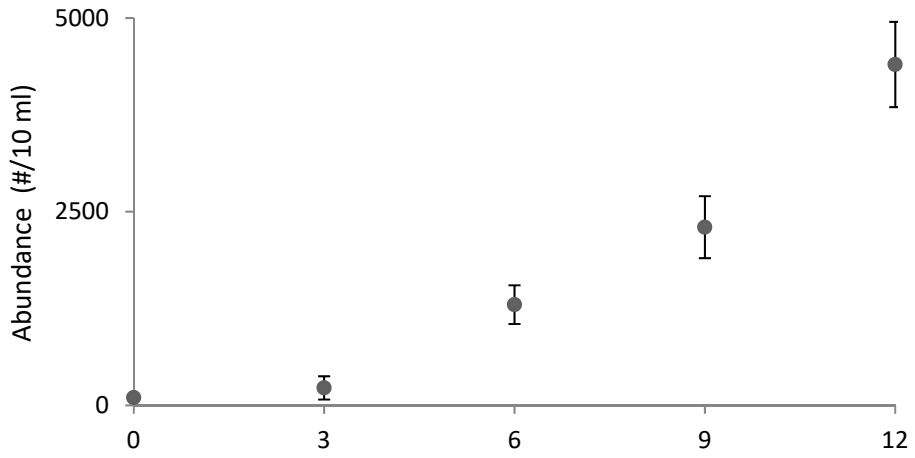


Figure 1: Population growth of *Paramecium aurelia* at 20°C in a sterile solution of 1% (w/v) peptone versus time. Means ± standard error (n=20 counts) are presented.

D- Evaluation Criteria

Evaluation criteria for graphs (not inclusive):
Presentation: Graph type, data: ink ratio, layout, clarity and tidiness.
Data: Symbols, error bars, axes units and labels
Caption: Description, nomenclature, sample size…
All graphs must be done **by hand** and on **millimetre paper.**

References:

Cleveland, W.S. (1994). The elements of graphing data - 2nd Edition Hobart Press, Summit, Nj
Davis, P. (1974). Science in geography 3, data description and presentation - Oxford University Press, London, R-U.
Reynolds, L. and Simmonds, D. (1981). Presentation of data in science - Martinus Nijhoff Publishers, Boston, Ma.

SI (système international) units and prefixes

SI Units:

Base units:

Length: metre (m)

Mass: kilogram (kg)

Time: second (s) [lower case]

Electric current: ampere (A)

Thermodynamic temperature: kelvin (K)

Amount of substance: mole (mol)

Luminous intensity: candela (cd)

Non-SI units sometimes used:

Litre (L or l) as a special name for dm^3, used to measure volume.
Angstrom (Å) as special name for 10^{-10}m (100pm) (should not be used).
Minute (min), hour (h), day (d) for the quantity of time.
Degree Celsius (°C) for thermodynamic temperature: temperature (°C) = temperature (K)-273.15

SI prefixes:

SI prefix	SI symbol	Decimal value	10^x (scientific) value
pico-	p	0.000000000001	10^{-12}
nano-	n	0.000000001	10^{-9}
micro-	μ	0.000001	10^{-6}
milli-	m	0.001	10^{-3}
centi-	c	0.01	10^{-2}
deci-	d	0.1	10^{-1}
(no prefix)		1	10^{0}
deca-	da	10	10^{1}
hecto-	h	100	10^{2}
kilo-	k	1,000	10^{3}
mega-	M	1,000,000	10^{6}
giga-	G	1,000,000,000	10^{9}
tera-	T	1,000,000,000,000	10^{12}

Adapted from Let's get ready for biology – Pearson Benjamin Cummings ©2005-2007

External references:

Prefixes: http://www.bipm.org/en/si/si_brochure/chapter3/prefixes.html
Base units: http://www.bipm.org/en/si/base_units/

Preparation of Tables

Use tables when you wish to:

-show exact numerical values,
-present a small data set
- make many localised comparisons
-summarise characteristics of lists of different components

Principles of table construction

1. Tables should convey the greatest amount of coherent information with the least amount of wasted space and redundancy.
2. Tables should stand alone and be comprehensible without reference to the text of the report.
3. Before constructing a table you ask yourself, "What information does the reader have and what information is he/she seeking?"
4. Column and row headings should relate to the information the reader has and lead him/her to the body of the table.
5. Strive for simplicity and clarity.
6. Present the information in a sequence similar to one used by the reader, i.e. left to right and top to bottom.
7. Items that need to be compared should be listed vertically and the comparative criteria horizontally.
8. In numerical tables, the information should be given in full, i.e. the reader should not have to add two values to obtain a third not shown in the table.
9. The table is composed of a caption, column and row headings and the body that contains the data.
10. Spaces and/or horizontal lines may be used to separate each of the table components.
11. Items that are close together are viewed as being more closely related than those further apart. Therefore, the use of space and/or lines to relate and separate information should be considered carefully in designing a table.

I - Caption:

1. The caption is located above the table body.
2. The caption should begin with a table number, followed by a meaningful and specific title.
3. Identify and/or explain all non-standard symbols or abbreviations in the caption or in footnotes at the bottom of the table.

II - Column and row headings:

1. Capitalise the first word, proper nouns and appropriate symbols.
2. Use spaces and/or horizontal lines to separate headings. Do not use vertical lines.
3. They should be brief and carefully placed so it is clear to which data they refer.
4. They should be clear enough so that the data are understandable without reference to the text.
5. Abbreviations are acceptable but must be defined either in the caption or by the use of footnotes.

III - Body of table:

1. Spaces may be used to separate or group rows of data (horizontal lines are rarely used).
2. Vertical lines should never be used.
3. Only significant digits should be presented.

IV - Footnotes:

1. May be used to explain an abbreviation, symbol or term in the caption, heading or body of the table.
2. Follow the item by a superscripted letter (start with [a] and follow alphabetically for other items).
3. The superscripted letter is then repeated below the body of the table, followed by a brief explanation. Only provide enough information so that the meaning of the item is clear.

Sample table:

Table 1. Mean observed fork lengths (LF)[a] for yellow perch (*Perca flavescens*) sampled in Lake Charlotte. Sample sizes (*N*) are in parentheses and age is in years.

	LF (mm)	
Age	Mean (N)	Range
0	31 (35)	25-40
1	42 (123)	27-72
2	65 (197)	52-76
3	75 (74)	64-86
4	94 (31)	83-104
5	113 (10)	101-123
6	205 (13)	180-216
7[b]	264 (24)	234-278
8	275 (19)	257-297

[a] Measured distance from the tip of the snout to the tip of the middle caudal ray.

[b] At this age they begin feeding solely on other fish.

Drawing biological specimens

Biology students are frequently asked to draw their dissections and preparations as seen through the microscope. There are three reasons for this. First, and most important, drawing is a very important aid to study. It is all too easy to look at the specimen and assume that you have seen all there is to see. It is, however, only after examining every curve and boundary line and every connection between structures, as you must do when drawing, that you have a good grasp of the subject. Second, you have a record in your notes for later study and comparison. Third, your demonstrator can now check to see if you understand and give help when necessary.

Scientific drawing has more in common with mechanical drawing than with art, and it is a skill that must be acquired.
Study the material carefully before beginning to draw and frequently while making the drawing. Draw from the specimen; draw only what you see. Do not copy from books or from the work of other students, because copying (or plagiarism) defeats the purpose of the laboratory course - actual observation by you. Your instruction sheets and reference books should be used as guides to what to look for, but your specimen ought to be the chief source of information. Previous drawings may be inaccurate, but the specimen is always right! As you draw and label each part, make sure you know what it is, what its functions are, and how it is connected or related to adjacent parts, both anatomically and functionally.

You should purchase:
1. Good quality typing paper (very thick) that will erase easily.
2. A hard lead pencil, HB or harder, well sharpened.
3. A good eraser.

Instructions and Evaluation Criteria

Layout

Position: Your drawing should be centred or slightly to the left of the centre of the page. Use only one side of the sheet for your drawing.

Size: It should be about one-half of a page in size.

Frame: No frame should be present around your drawing.

The layout of your biological drawing should be similar to that of the printout using the template file (see lab 5 instructions)

Drawing:

Lines: Clean, crisp, continuous lines should be drawn in pencil. Make sure line connections are perfect and not overlapping. Deep or hidden structures may be shown by dotted lines. Line width should be appropriate (not too thin or thick). No stippling

Line thickness: Lines should be of equal density (darkness) throughout the drawing and all lines should join seamlessly.

Detail and content: Only draw one side of paired structures (unless details of paired members are different). No stippling, shading, cross-hatching, ink or colours should be used.

Shading: No shading or pattern should be used on the biological drawing

Proper proportions: Proportional size of structures should reflect differences seen in the specimen.

Labelling:

Label format: You can label in sequence A, B, C etc. or take the labels' letters from the structure: O (Ovary), T (testis) using one or more letters in the label: Ov (Ovary), Te (Testis). Avoid writing the whole name of the structure you label. Labels must be easily readable - choose the colour and style so that it will stand out from the background drawing.

Label size: 9-10 pts = 3mm height (approximately if done by hand)

Label position and pointers: As much as possible, put all labels directly on the drawing, not off to the side, unless they would hide important structures or would not be easily readable. Locate labels directly on the structure is possible (without hiding the structure). If needed, when there is not enough space or when the designated structure is very small, use a pointer. Pointers should be a straight line (or an arrow). The pointer should not hide any part of the illustration.

Accuracy and Completeness: The structures you label should reflect what is clearly visible. All structures must be labelled. Brackets may be used to distinguish regions of a specimen.

Scale: An indication of size or scale must be included in your drawing. A scale bar should be presented in the bottom right corner of the diagram, with its appropriate conversion (e.g. _____).
You need to put one scale bar per panel. 10 µm

Caption

Location: The caption should be located immediately below the drawing and begin with a figure number.

Content: It must be concise, descriptive and complete (scientific name as well as sex of the organism, if applicable). The aspect (e.g. dorsal or ventral view) and type of preparation should be indicated (i.e. whole mount, cross-section, prepared slide, etc.). The first sentence is in effect the title. **Do not write "A drawing of...".**

Abbreviations used on the drawing must be listed at the end of the caption alphabetically.

Sample Caption: Figure 1. Transverse section of an elderberry (*Sambuca canadensis*) stem at the end of the first year's growth. Note the xylem rays passing through vascular cambium and secondary xylem. e: epidermis, ca: cambium, ph: phloem, xy: xylem.

For additional instructions about labelling and caption, read the "Illustration essentials" pages on the Biolabo guide (http://salinella.bio.uottawa.ca/BIOlabo/)