Lab # 1

Depth of field (or Focus) – how many optical planes you can focus on simultaneously

- when mag increases, the area of image in focus decreases.
- high # of surfaces in focus = great depth of field.

* Connect camera to microscope before starting infinity capture.

White balance — Live histogram — Options → Focus meter

the bar should use 70 – 80% of window.

Kimwipes to clean ocular or objective

Oculars	Screen
• wider field of view • greater resolution.	• shareable. • more comfortable • take pictures.

Working distance: d b/w objective lens & slide.

a system of knobs on the side to move specimen in x & y axes.

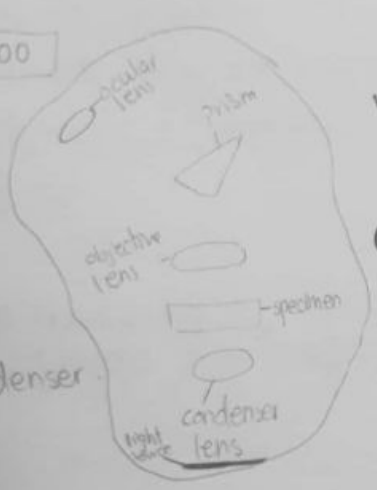
Coarse focus knob = permits rapid change in d b/w specimen & objective.

Fine focus knob = small changes in distance

Ocular / eyepiece = a magnifying element (10×)

Parfocal = when object is in focus in one objective, focus will not be completely lost when moving to other.

×4, ×10, ×40, ×100



Visual mag. = power of eyepiece × objective lens. (doesn't apply to pictures)

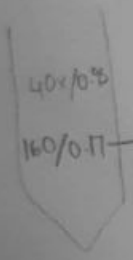
Condenser = system of lenses that concentrates light furnished by illuminator. No mag.

Light

illuminator → condenser

Condenser height adjustment knob: focus concentrated light onto specimen.

Aperture Iris Diaphragm: reduce glare from unwanted light by adjusting angle of cone of light that comes from the condenser.



optical tube length / max coverslip thickness in mm

numerical aperture is on objective.

→ determines resolving power of objective (ability to see 2 objects that are very close as 2. (human = 100 μm)

Insect - Cimex lectularius

Eukaryote - Elodea (plant) — convex side of leaf on top.

Prokaryote - Oscillatoria (cyanobacterium)

Brine shrimp - Artemia (used protoslow to slow it down).

Cytoplasmic Streaming - directed flow of cytosol & organelles around plant cells. (or cyclosis)

Cell - cell wall = protoplasm.

Prokaryote	Eukaryote
• small	• ~~a~~ large
• circular chromosome	• linear chromosomes
• more abundant	• less
• no membrane bound organelles (nucleus, mitoch. chloroplast)	• larger genome.
	• uni or multicellular.
• ~~s~~plasmids.	

Stereoscope mixes mag. 6.7x — 45x

Calculations

Magnification = measured size of object / actual size of object.

actual size of object A / actual size of object B = on screen size of A / on screen of B

Actual size of object = actual size of FOV (table) / on-screen size of FOV × on-screen size of object.

Aperture Iris diaphragm

closed → less light, more contrast

open → more light & colours, less contrast

Lab #2 – Hemolysis

Q9 what

Why erythrocytes?
- easily obtain large # of homogenous cells.
- kept for long time in isolated state
  (3-4 days in isotonic NaCl or week in 4°C serum)
- contain large quantities of pigment

Isotonic = isosmotic

Electrolyte – compound that dissociates into ions in ~~water~~ solution.
Osmolarity – concen. of all slts present in a solution.
Tonicity – ability of a slt to cause a cell to absorb or reject water.
(ability to create movement of water) (depends on how well solutes can cross membrane & their concen.)

Water movements are driven by tonicity adjustments

A hypertonic slt will cause the cell to lose water & the membrane will start folding.

Solutes can diffuse through a membrane at various speeds depending on the functional groups they possess.

How hemolysis can be reached:
- placing cells in isosmotic slt of a penetrating subs. (ethylene glycol)
  Osmotic pressure may be equal out & in at first but when it enters the cell, tonicity & osmotic pressure change. Osmotic pressure inside cell increases.

Since some cells are more resistant than others, when 75% of population has undergone hemolysis, the suspension of RBCs becomes transparent suddenly.

Covered testtubes with parafilm (all concen. were 0.3 M)
Time started (t=0) after tube is inverted once.
Suspension = turbid ⟶ transparent

* Inversion is to homogenize.

Y = measurement
n = sample size

$$\text{mean} = \bar{Y} = \frac{\Sigma Y}{n} \qquad s = S.D = \sqrt{\frac{\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}}{n-1}}$$

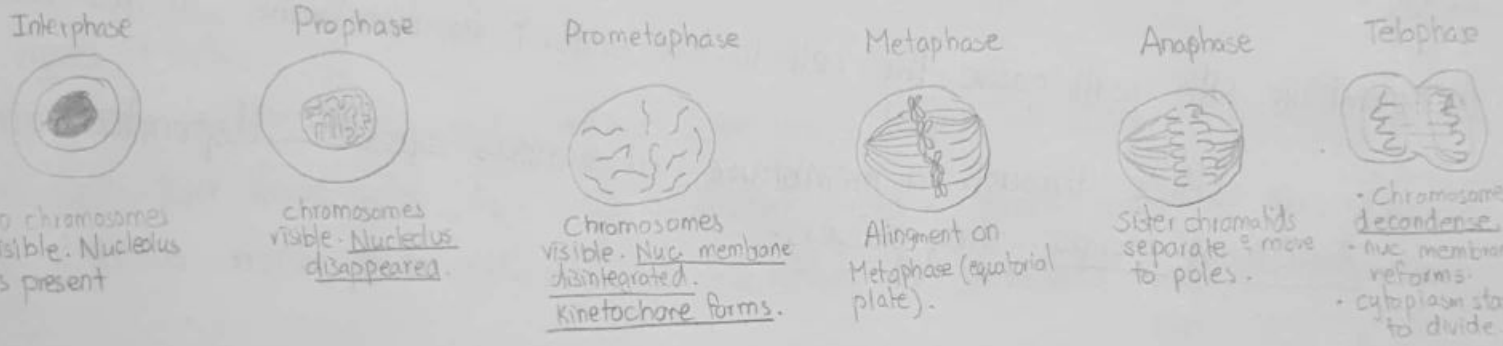
$$S.E = \frac{s}{\sqrt{n}}$$

Lab #4- Mitosis

Keep root tips in water so they don't dry out.

1) label microtube
2) discard fixative (Carnoy-lebrun) — stops processes & preserves tissues.
3) rinse tips once with 95% alcohol EtOH.
4) discard EtOH, add preheated 1N HCl (60°C) — removes purine bases.
5) incubate at 60°C for 10 minutes.
6) Discard & add ice-cold dH_2O.
7) Discard & add Felgen stain (30-40 mins). Rinse with water - 2 to 3 times.
8) Add drop of acetic 50% acid to slide, and cut the last 2mm of root tip — to soften tissue.
9) to squash, add cover slip & press down vertically until its 1 cm wide.

Vicia faba (broad bean)



Broad bean (squash): Vicia faba (squash)
Onion (root tip): Allium cepa (longitudinal section)
Whitefish (blastula): Coregonus clupeaformis. (cross section).

Prokaryotes & unicellular eukaryotes rely on cell division to produce new individuals.
In multicellular organisms, cell division allows development of embryo, growth of structure & replacement of cells to aging/damage.

Cell cycle: M phase (mitosis + cytokinesis) (or cell division; nuclear division; cytoplasmic division) + Interphase (G_0, G_1, S, G_2)

... Lab #4 continued

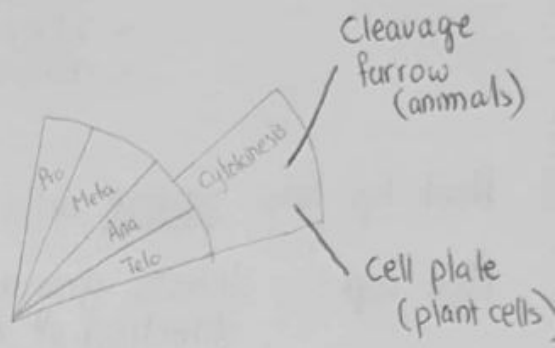
G_1 = • growth
• synthesis of macromolecules (RNA & proteins)
• organelles (mitoch. & ribosomes) are duplicated. & centrosome

G_2 = • protein synthesis
• production of structures for mitosis (spindle fibres)

S = • DNA replication
• synthesis of DNA associated protens (histones or microtubule associated proteins like centrioles).

joined by cohesin protein

G_0 = cell cycle arrest.



Types of cells:

1) Divide continually - gut epithelium, germinal layers of skin
2) Cells that leave cycle (G_0) & do not divide again - nerve cells
3) enter a resting state (G_0, G_1, G_2) but returns after stimulus - liver cells after removal blood lymphocytes after stimulation of antigen.

Interphase

✓ nuclear membrane
✓ nucleolus
✓ heterochromatin in nucleus.

Prophase

✓ microtubules of cytoskeleton disasseble & reassmble forming mitotic spindle.
✓ nucleoli disappear
✓ centrioles travel to poles.

Prometaphase

✓ break down of nuclear membrane.
✓ protein (kinetochore) forms on centromere.
✓ polar microtubules attach to kinetochore.

Metaphase

✓ lined at equatorial plate
✓ separase breaks down cohesin.
✓ centromeres start to separate.

Anaphase

✓ each chromatid → daughter chromosome
✓ microtubules shorten by: depolymerization at kinetochores.

Telophase

✓ chromosomes at poles decondense & become long & thin.
✓ nuclear mem. & nucleolus reform.
✓ In plants, phragmoplast guides formation of cell wall. In animals, cleavage furrow.

Plants have growth confined to specific areas = apical meristems.

Feulgen stain: magenta red, Schiff base (reacts with aldehyde groups to give colour)

Root tip has square cells

Root cap: detects gravity & controls direction of root growth.

The elongation of cells in region of elongation results in most of the increase in root length.

Region of maturation: root hairs are produced here.

Near centre, you may see very narrow, thick walled cells which are differentiating into vascular bundles.

The roots do not have chloroplasts.

* In animal cells there is an aster (semicircle of fibrils) around each end of the spindle. - not present in plant cells.

Duration of each stage is proportional to # of cells of each stage

Lab # 5 - Meiosis

Micros progeneris in Lilium
Meiosis in ascaris
Spermatogenesis in rat testis
Oogenesis in rabbit ovary

Ascaris (worm)

Primary oocyte (primordial) – • arrested in prophase I
• no shell.
• penetration of spermatozoid causes it to continue meiosis

Metaphase I – • shell is present.
• chromosomes lined up.
• male pronucleus visible in centre.

Anaphase I – • homologues separate.

Metaphase II – polar body can be seen in perivitelline space.

Anaphase II – • centromeres split
• chromatids separate.

Interphase – • following telophase II, pronuclei remain in interphase before fertilization.
• 2nd polar body is expelled.
• a zygote results from fertilization.

Mitotic cleavage of embryo.

* Gametogenesis in mammals

Rat testis (Rattus norvegicus) = spermatogenesis. (cross-section)

- occurs within walls of seminiferous tubules
- mature stages near the lumen.

basal lamina ⟶ sertoli cells (nursing cells) in walls of seminiferous tubule. ⟶ spermatogia (2n) ⟶ primary spermatocyte (2n) ⟶ secondary spermatocyte (n) → spermatids (n) → sperm (n)

sertoli cells: feed & regulate differentiation of spermatids ⟶ mature spermatozoa.

> A secondary oocyte remains in metaphase II until its penetrated by spermatozoon.

Rabbit Ovary (Sylvilagus floridanus) = oogenesis

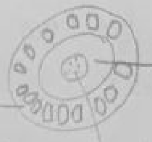
Maturation stages depend on :
- position in ovary
- no. of layers of follicular cells.
- presence of fluid filled cavity

epithelium ⟶ primordial follicle ⟶ primary unilaminar follicle ⟶ Primary multilaminar / Growing follicle ⟶ Mature or Graafian follicle.

Meiosis I produces growing follicle (secon. oocyte) + polar body

Primordial follicle: (surrounded by 1 layer of squamous follicular cells)

Primary unilaminar follicle:
- contains primary oocyte.
- 1 layer of cuboidal follicular cells.

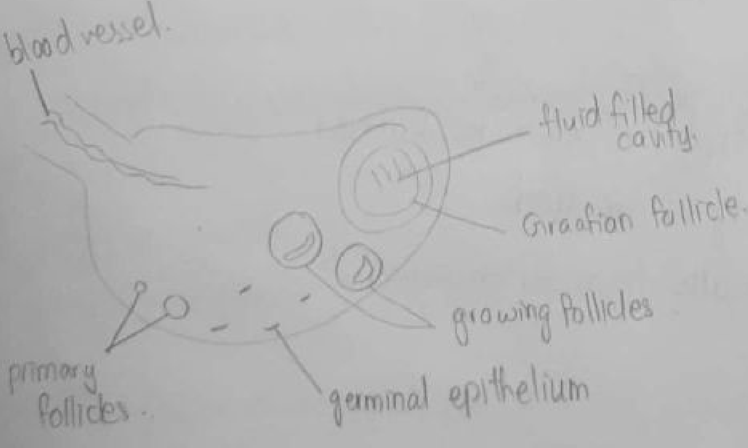


Primary multilaminar:
- several layers of follicular cells.
- contains primary oocyte.
- prim. oocyte in fluid filled space.
- contains secon. oocyte stopped at metaphase II

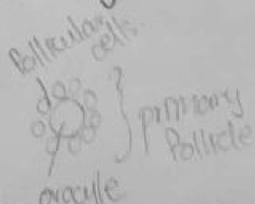
hormonal stim. causes it to grow.

Mature or Graafian follicle:
- largest
- many layers of follicular cells.
- one big or several fluid filled cavities
- contains sec. oocyte stopped in metaphase II.
- comes near to ovarian epithelium prior to ovulation

Surrounding follicles are : interstitial cells.



Ovaries of a newborn mamal female contain all the ovocytes she will need.



Ovocyte ↓ on onset of meiosis I. primary oocyte

Appendix

Bar graphs
- Bars represent discrete values & are separated by empty spaces.
- Quantitave variables on 1 axis only.
- bars of equal width but variable length

Do not connect data on each side of scale break.

Histograms
- quantitative ~~&~~ scale on both axes
- analyze & study distributions
- % of observations in each interval recorded y-axis.

If you plot logarithsm of a variable, axis label should correct to tick mark label.

Two-panel
- y-axis spreads over 2 panels
- scale on y-axis is same, range may be different.
- only one symbol key for both panels.

- 95% confidence interval.
- sample S.D
- S.E of the mean

Pie chart
- proportion of parts to whole.
- useful if one element makes up a significant portion.
- no scale provided.

Do not describe trends in caption.

Straight-line
- many data points ($n > 30$) are available at constant intervals to see trends & changes in variable through time.

Scatterplots
- investigate relationship b/w two sets of data
- allow to quantitatively evaluate relationship b/w two variables.

Linnaeus

2/3 of page - graph
1/3 of page - caption
(in portrait orientation)

No. in superscript to indicate how many points overlap.

Use visually prominent plotting symbols. More prominent than line connecting them.

°C = kelvin − 273.15

C = K − 273.15

candela (cd)

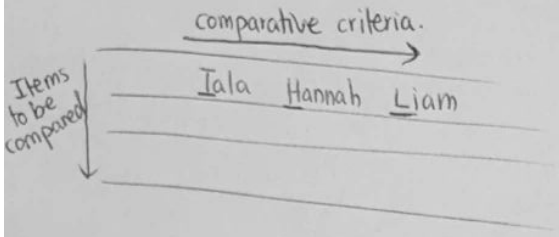
pico (p) = 10^{-12}

nano = 10^{-9}

μm = 10^{-6}

centi = 10^{-2}

deci = 10^{-1}



Caption is above for tables only.

Identify non-standard symbols in caption or footnotes at bottom of table.

Drawing Biological Specimens

- Drawing centre or slightly to left.
- half the page.
- no frame
- clean, crisp, continuous lines.
- deep or hidden structures shown by dotten lines.
- lines of equal density/thickness/darkness.
- label size (9-10 pts = 3mm height if my hand).
- all labels on drawing, not to side
- use pointers if structure is small or not enough space.
- brackets used to distinguish regions of specimen.
- one scale bar per panel on bottom right corner with approp. conversion.
- type of preparation should be indicated in caption (c.s., whole mount, prepared slide)

Scale bar

$$\frac{\text{measured width (cm)}}{\text{actual width (table) (μm)}} = \frac{x \text{ (scale length)}}{50 \text{ (μm)}}$$

$$\frac{x \text{ cm}}{50 \text{ μm}}$$