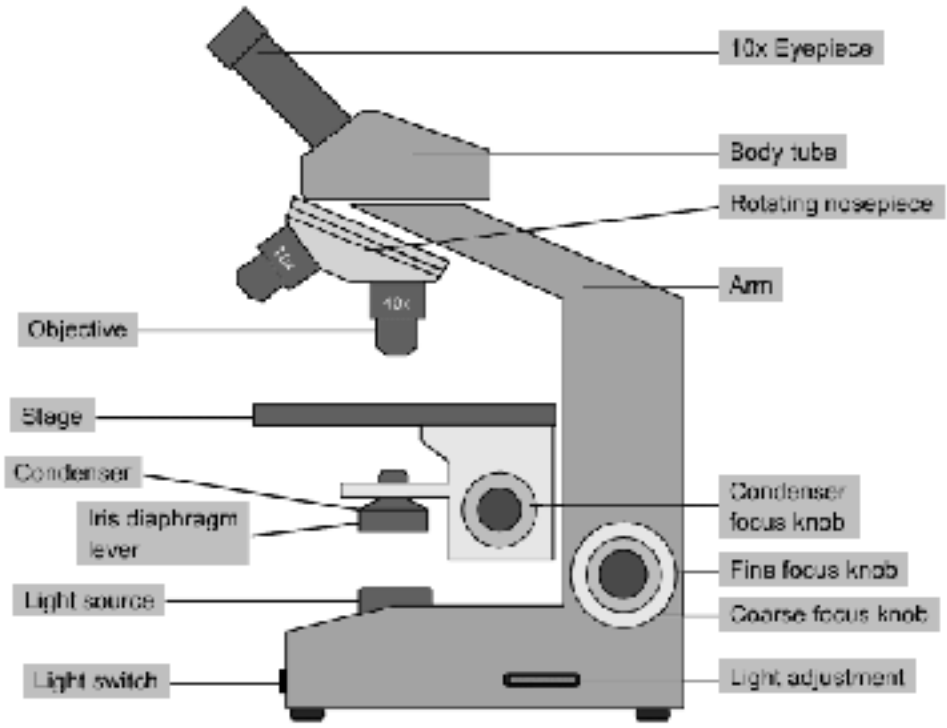
BIOL1903 Notes

THE MICROSCOPE

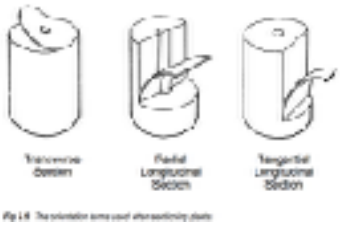
The Compound Light Microscope -

- ***Microscope*** —> optical instrument, needs to be handled with care:
  - When moving microscope, carry in UPRIGHT position with BOTH HANDS (arm with one hand, other hand under base)
  - Place microscope on bench with arm away from you + base approx. 5cm from edge of bench
  - Check before use —> report damage
- Cleaning:
  - To determine if there is dirt on:
    (1) Slide - move slide —> dirt will stay in same place relative to image
    (2) Eye-piece lens - rotate eyepiece —> image of dirt rotates
    (3) Objective lens - change/move objective lens out of position —> dirt disappears
  - To clean lenses: lightly moisten lens-cleaning tissue with lens-cleaning solution (70% ethanol) + gently wipe lens surface



Rules for biological drawing -

- General:
  - Write title
  - Write figure legend (indicates what drawing represents)
  - Label with a ruled line drawn parallel to the bottom of page without arrowheads
  - Labelling lines shouldn't cross
- WITHOUT microscope:
  - Use double lines to indicate hollow tube like structures, *e.g. blood vessels*
  - Orientation:
    - Animals - anterior/dorsal surface towards top of page
    - Plants orientated the way they grow - stem towards top

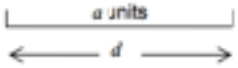


- WITH microscope:
  - Need to:
    - Estimate size of specimens
      - Size of an object viewed on a slide can be determined by estimating the number of times the object fits across the diameter of the field of view
      - *E.g. cheek cell sample viewed at 40x objective; estimate that 1 cheek cell would fit across the field of view 8 times. Using info in table —> field of view diameter is 500 µm —> hence, approximate size of one cheek cell = 500/8 = 62.5 µm*
      - NOTE: 1mm = 1000µm

Objective	Diameter of f.o.v. through ocular (µm)
4x	5000
10x	2000
40x	500

    - Include scale bar
      - ***Scale bar*** - short <u>horizontal</u> line added to bottom corner of biological drawings to represent ratio between actual size of the object and drawn size of object
      - Should represent about a 1/4—>1/3 of the size of the drawn object
      - Units - whole numbers
      - Scale rather than magnification, *e.g. x2*, used as photo-reduction/enlargement could cause distortion
      - How to calculate length of scale bar:

If A = actual size of object
D = drawn size of object
a = actual length represented by scale bar
d = drawn length of scale bar



Therefore

$$\frac{D}{A} = \frac{d}{a}$$

Once we have D, A and a, we can calculate d (the length we have to draw the scale bar).

i.e. $$d = \frac{D \times a}{A}$$

Actual length of pencil (A)	18.0 cm
Length of drawing (D)	7.5 cm
So if the scale bar is to represent, and to be labelled, 5 cm (a), the length of the bar on the drawing should be in actual measurement (d)	$d = \frac{7.5 \times 5}{18.0}$ = 2.1 cm

Table 2.1. Calculation of the scale bar length

    - ***Map diagram*** - map of the relative positions of tissues

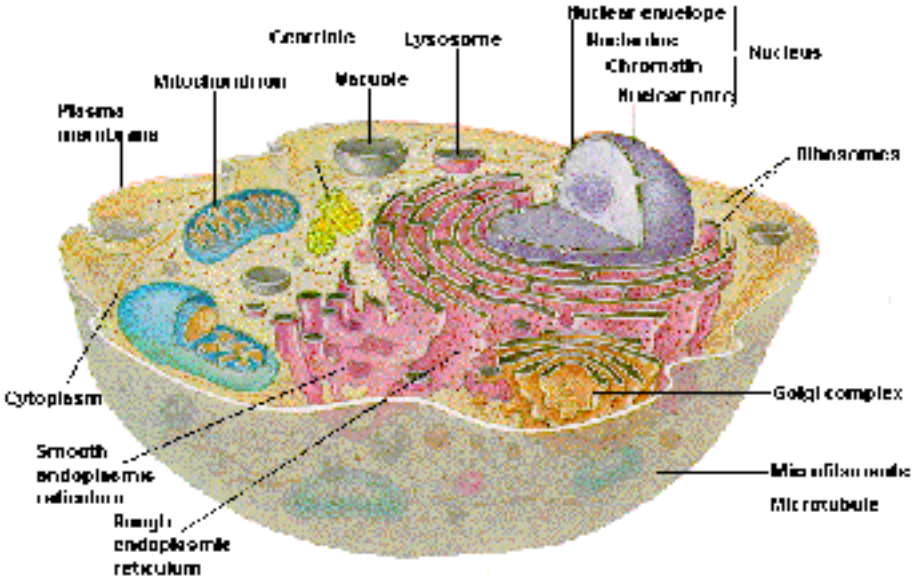
Microscope Theory -

- Light microscope utilises a system of lenses to produce an enlarged image of an object
- ***Magnification*** - enlargement of an image
- ***Minimum resolved distance*** - minimum distance at which 2 objects/images can be identified as distinct from each other
- ***Condenser***:
  - Has 2 parts + functions:
    1. ***System of lenses*** - focus light on slide by moving up + down
       - Objective lenses form INVERTED + MAGNIFIED image of object
       - Ocular (eyepiece) lenses further magnify this image
       - ***Total magnification*** = product of magnification of objective + eyepiece lenses
       - *E.g. x40 objective + x10 ocular lens, total magnification of image seen is x400*
    2. ***Iris diaphragm*** - controls amount of light that passes through specimen + enters objective lens
       - Shutting iris diaphragm decreases angular aperture + amount of light passing through specimen
       - A: angular aperture too wide —> light scattering, glare, contrast too low
       - B: angular aperture too narrow —> field of view too dark, poor resolution, contrast too high
       - C: angular aperture about right
  - A: max resolving power obtained when cone of light just fills objective lens
  - B: condenser too high - cone of light doesn't fill lens of objective —> resolution lost
  - C: condenser too low - some light scattered outside lens of objective —> causes glare, impairs resolution

CELL STRUCTURE

- Atoms > molecules > cells > tissues > organs > organ systems > organism
- 4 fundamental tissue types:
    1. **Epithelial** - covers, lines, forms glands
    2. **Connective** - supports, protects + transports
    3. **Muscular** - contracts, propels, movement
    4. **Nervous** - receives + sends info; controls, coordinates (in all tissues)
- ***Cell theory:***
    - ***Cell*** - basic structural + functional unit of all living organisms
    - All cells come from pre-existing cells
    - Activity (anatomy + physiology) of an organism depends on activity of it's cells
    - Composition of cells (organelles present) determines cell's structure + function
- ***Eukaryotic*** - has membrane bound nucleus or organelles
- ***Prokaryotic*** - no membrane bound nucleus or organelles
- NOTE: ***Cell metabolism*** - sum of all the chemical reactions in the cell

Organelle	Structure/Function
Nucleus	Directs cell's life processes; contains DNA + nucleoli
Nuclear membrane	Separates nucleus from cytoplasm - controls exchange between them
Nucleolus	Where ribosomal RNA (rRNA) synthesised + combined with proteins
Cytoplasm (cytosol)	Semi-fluid material in which organelles are found
Cell membrane (plasma membrane)	- Regulates movement of substances into cell (intracellular) + out of cell (intercellular) - ***Phospholipid bilayer*** with attached/imbedded proteins - Phospholipids have: Internal facing <u>hydrophobic</u> tail (<u>lipid</u>) Outward facing <u>hydrophilic</u> head (<u>phosphate</u>) NOTE: protein channels: Passive - allow movement across conc. gradient from high —> low Active - requires energy to move ions across membrane
Mitochondrion	- Cellular respiration (change glucose to ATP) - ATP breaks down into ADP + energy NOTE: have own DNA; theorised that eukaryotic cell ingested/internalised a prokaryotic cell (mitochondria) + they now have a <u>symbiotic</u> relation
Rough endoplasmic reticulum	- Site of protein synthesis + transport - Has ribosomes attached to surface
Smooth endoplasmic reticulum	- Site of lipid + carb synthesis - Detoxification of drugs/alcohol - Stores Ca NOTE: only in muscle cells
Golgi body/apparatus	Stores, modifies, packs + secretes substances manufactured within the cell NOTE: secretion = ***exocytosis***
Lysosome	Contains hydrolytic enzymes —> cellular digestion NOTE: involved in cell defence (macrophages) —> ***endocytosis*** of bacteria in vesicle which fuses with lysosome + is digested
Ribosome	Protein synthesis (translation) NOTE: only non-membrane bound organelle in this list
Cytoskeleton	Microtubules, intermediate filaments, microfilaments



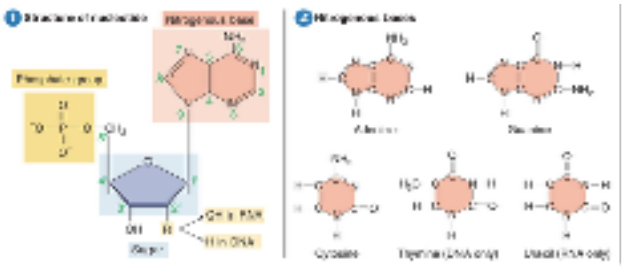
Surface area to volume ratios -

- SA:V ratio + rate of diffusion in/out of cell increases as cell size decreases because there is more SA per unit of V across which to exchange substances
- This determines max size to which cell can grow
- NOTE: cell can increase max size to which it can grow by:
  - Developing an intracellular transport system (membrane systems, *e.g. E.R.*)
  - Compartmentalising chemical processes, i.e. organelles (so chemical reactions don't interfere)

Molecular basis of inheritance -

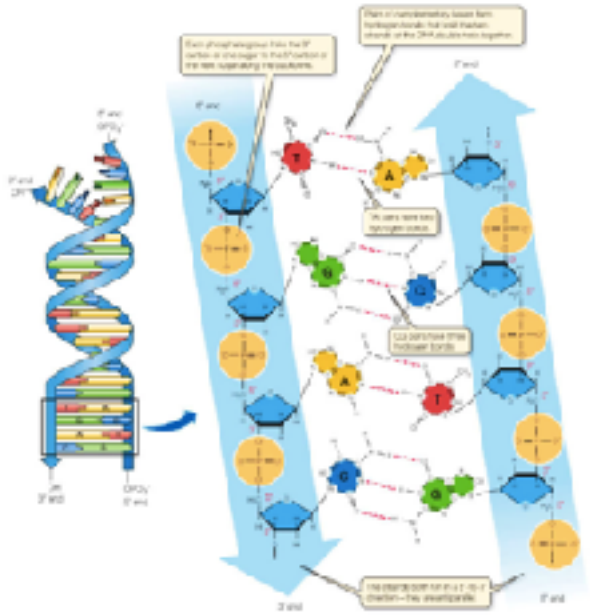
- What was known about genes in 1940-50:
  - ***Genes*** = hereditary 'factors' carried on chromosomes that cause certain traits
  - DNA consists of 4 nucleotides
  - Physical structure unknown
- ***Linus Pauling*** - amino acids = carriers of hereditary material ('DNA too simple - only 4 nucleotides')
- Experiments showing DNA was hereditary material:
  - ***Griffith***
    - Live S strain - mouse dead
    - Live R strain - no effect
    - Heat-killed S strain - no effect
    - Live R + heat-killed S strain - mouse dead (transformation - bacteria take genetic info from environment —> live S strain recovered)
  - ***Avery***
    - Continuation of Griffith's experiment
    - Selectively destroyed chemicals in S strain, i.e. lipids, RNA, proteins etc. before injecting into mouse with live R strain
    - Only after removing DNA did mouse survive (no live S strain recovered)
    - Proved DNA carries genetic info
- Linus Pauling 2 - began to accept DNA as hereditary material —> triple helix model —> inherently flawed as DNA ceased to be an acid

- ***Watson + Crick***
  - Proposed double helix structure
  - Info available to them:
    - Chemical structure of DNA, i.e.
      - Nucleotides composed of:
        - Sugar
        - Phosphate
        - Nitrogenous base
      - Sugar has 5 carbons, aka primes
        - 1st prime - connected to nitrogenous base
        - 2nd prime - determines if DNA or RNA (R = H or OH)
        - 5th prime - connected to phosphate
      - A + G have 2 carbon rings (***purines***)
      - T + C have 1 carbon ring (***pyrimidines***)
    - Chargaff's rules of base composition:
      Amount A = amount T; Amount G = amount C
    - Franklin's work - X-ray diffraction pattern (controversial)



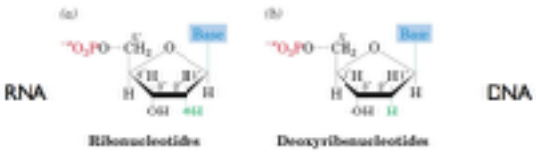
DNA structure and replication -

- DNA described as a ladder:
  - 'Sides of ladder' (backbone) = alternating sugar + phosphate groups
    - 2 backbones twisted to create double helix
    - Double helix not exactly facing each other - slightly shifted to have ***major*** + ***minor groove***
  - 'Rungs of ladder' = nitrogenous bases
    - Each phosphate group links 3' carbon of 1 sugar to 5' carbon of next sugar
    - Complimentary base pairs form hydrogen bonds that hold 2 strands of DNA in double helix together
- Chains run in opposite directions - ***antiparallel***
- Features of DNA:
  - Stable - can self repair
  - Encodes lots of info
  - Can replicate
- ***DNA replication***
  - ***Semi-conservative***, i.e. daughter DNA contains 1 parent strand + 1 replicate strand
  - Addition of new unit always happens in 5 —> 3 direction
  - Enzymes can ONLY add nucleotides to 3' carbon (5' already used)
  - During replication, 1 strand made continuously in 5' —> 3' direction (***leading strand***)
  - Other strand made discontinuously (***lagging strand***) - it's broken into ***Okazaki fragments*** aka short DNA fragments (DNA synthesis begins at fork + continues in direction opposite to unwinding, + so it soon runs out of template)
  - NOTE: ligase enzymes join fragments
- Genetic code almost universal of all phyla —> suggests common origin of life



Protein Synthesis -

- Info contained within DNA molecules directs production of proteins within cells
- Proteins used by cell as:
    A. Structural components
    B. Enzymes
- Protein synthesis requires 3 types of RNA:
    1. messenger RNA (mRNA)
    2. transfer RNA (tRNA)
    3. ribosomal RNA (rRNA)
- RNA VS DNA
    - Single stranded
    - U instead of T
    - Sugar backbone made of ribonucleotides NOT deoxyribonucleotides - on 2nd carbon: DNA has H, RNA has OH (hydroxyl)



- Central dogma of gene expression - protein synthesis involves:
    - ***Transcription*** - making a copy of information in a gene
    - ***Translation*** - converting copied information into a protein
- Process:
    - Promoter attaches to DNA
    - RNA polymerase binds to promoter + starts to unwind DNA
    - RNA polymerase reads template strand from 3' —> 5' + produces transcript by adding nucleotides to 3' end —> cannot add to 5' end
    - Introns cut out + exons spliced together to form mRNA
    - mRNA —> cytoplasm where it attaches to ribosome (smaller ribosome subunit attaches to mRNA, larger ribosome subunit attaches to complex)
    - NOTE: 5' = cap; 3' = poly-A-tail
    - tRNA brings amino acid —> A (acceptor) site on ribosome; joins anticodon to codon on mRNA
    - tRNA follows into P site where peptide bonds form between amino acids
    - tRNA exits ribosome at E (exit) site
    - Polypeptide chain grows until complete
    - NOTE: mRNA has 3 reading frames —> often, 2/3 end abruptly + produce non-functional polypeptides
    - NOTE: open reading frame (ORF) - makes sensible transcript - has start + stop codon