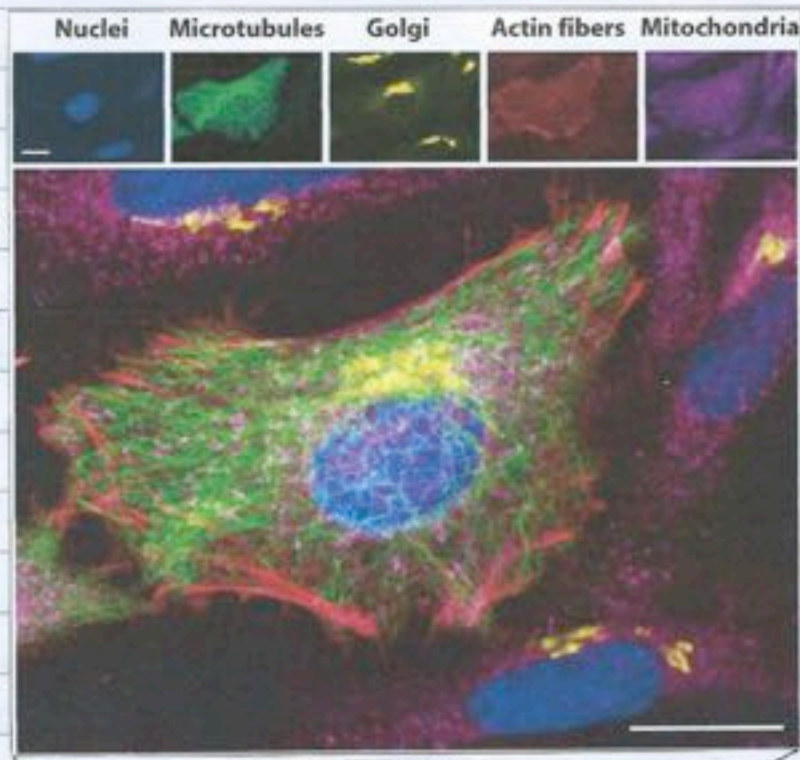
(tb: 397 - 436)
chapter 9

Week 2 - Characterization I



- The picture shows staining on different parts of the cell with various stains (which are coloured differently) → combined together to show the cell and its components

Nuke blue → used to stain double-stranded DNA (to show the nucleus)
↳ it doesn't fluoresce unless it's stuk to DNA (same for cyber green)
→ It's similar to cyber green but it won't work with L6 cells

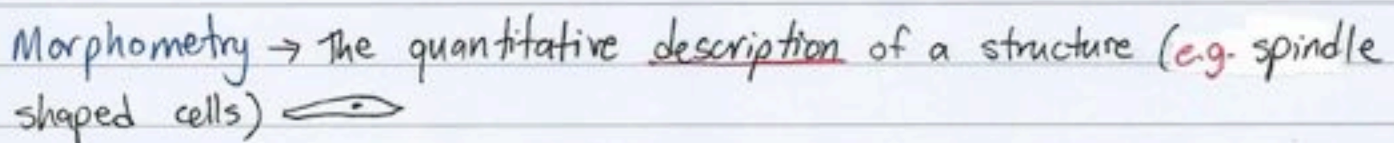
- When there's a high concentration of them they just start to fluoresce

Organelle lights → stains that are specific to organelles

- the big image shows that it's excited by the different wavelengths of light which shows all the different colours
(a scale bar must always be included in reports)

Microscopy is: Characterization

Morphometry → the quantitative description of a structure (e.g. spindle shaped cells)



Stereology → the extraction/interpretation of 3D data from 2D data
(e.g. sections of objects)

Image analysis → information of a data image (e.g. area, perimeter, length, etc).

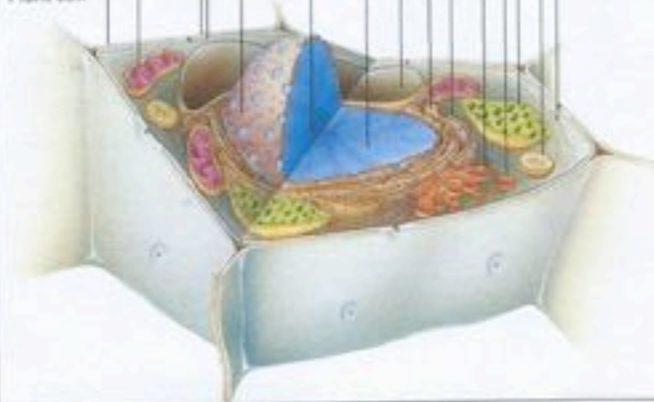
Temporal → time dependent aquision and analysis of objects in 2D/3D, referring to the overall time (e.g. 4D cell responses to drugs)

Image processing → computer enhancement of a digitized image (e.g. using various filters to remove noise, improve contrast and pseudocolouring) → not real colours

spectroscopic → the quantitative and behavioural analysis of chemical species (e.g. 'Lifetime': free and bound NADH)

(use zeiss software, look for black or blue → put in jpeg/tiff image & gives info)

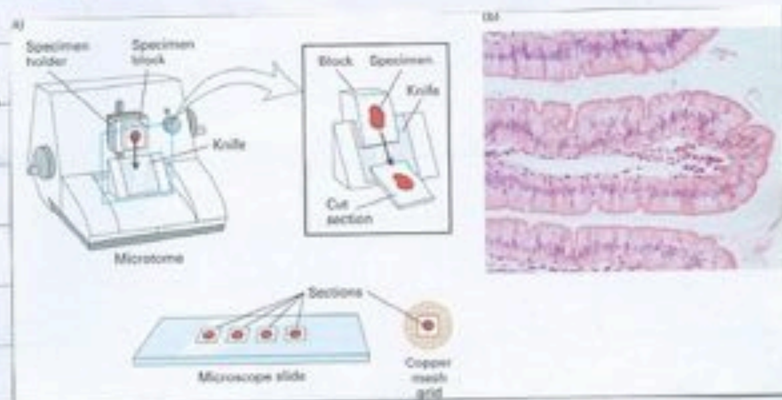
Cell Organelles



1. Plasma membrane controls movement of molecules in and out of the cell and functions in cell-cell signaling and cell adhesion.
2. Mitochondria, which are surrounded by a double membrane, generate ATP by oxidation of glucose and fatty acids.
3. Lysosomes, which have an acidic lumen, degrade material internalized by the cell and worn-out cellular membranes and organelles.
4. Nuclear envelope, a double membrane, encloses the contents of the nucleus; the outer nuclear membrane is continuous with the rough ER.
5. Nucleolus is a nuclear subcompartment where most of the cell's rRNA is synthesized.
6. Nucleus is filled with chromatin composed of DNA and proteins; site of mRNA and tRNA synthesis.
7. Smooth endoplasmic reticulum (ER) synthesizes lipids and detoxifies certain hydrophobic compounds.
8. Rough endoplasmic reticulum (ER) functions in the synthesis, processing, and sorting of secreted proteins, lysosomal proteins, and certain membrane proteins.
9. Golgi complex processes and sorts secreted proteins, lysosomal proteins, and membrane proteins synthesized on the rough ER.
10. Secretory vesicles store secreted proteins and fuse with the plasma membrane to release their contents.
11. Peroxisomes detoxify various molecules and also break down fatty acids to produce acetyl groups for biosynthesis.
12. Cytoskeletal fibers form networks and bundles that support cellular membranes, help organize organelles, and participate in cell movement.
13. Microvilli increase surface area for absorption of nutrients from surrounding medium.
14. Cell wall, composed largely of cellulose, helps maintain the cell's shape and provides protection against mechanical stress.
15. Vacuole stores water, ions, and nutrients, degrades macromolecules, and functions in cell elongation during growth.
16. Chloroplasts, which carry out photosynthesis, are surrounded by a double membrane and contain a network of internal membrane-bounded sacs.

- RNA is made in the nucleus
- Nuke blue is excluded from the nucleus when it's busy making RNA

Biological stains of living/fixed cells



- various fixatives are added which makes the cells in the tissue fixed for light microscopy or electron microscopy

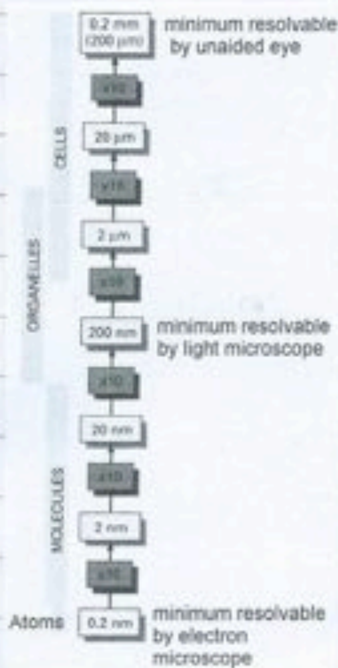
- fixed cells/tissue are usually embedded in paraffin or various resins
  - this allows sections to be made:
    Paraffin: 10-20 μm
    Resin: 1-2 μm (thin), 0.5 μm (semi-thin), 0.06-0.1 μm (ultra thin)

- unfixed cells/tissues, the tissues are frozen then sliced up with a cryomicrotome
  - allows the antigenicity of a molecule to be preserved
    - vaccines are antigens (8-24 AAs), our immune system makes antibodies for it, so it's not affected when infected by the actual infection
  - we cut the cells while it's cold → so they don't change their state

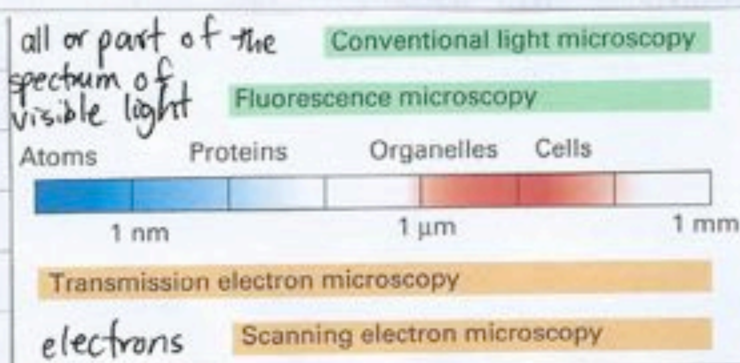
Two basic types of microscopes

Resolving power is the ability to distinguish between 2 pixels/lights

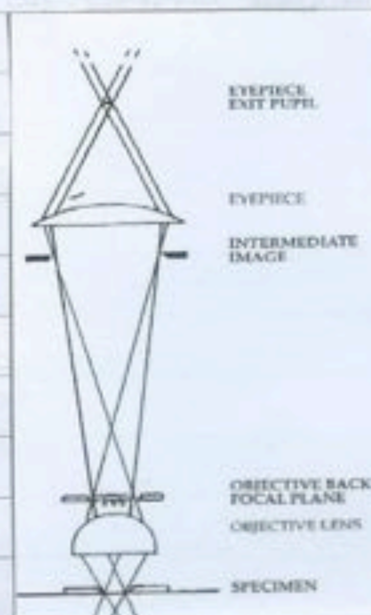
- light microscopy uses light to discriminate between 2 points



- fluorescent microscopy is now able to deal with sub-nanometer and picometer → looking at the light we emit



Resolving Power



Abbe Equation: R = 0.5 ·

R → minimum resolving distance

I → the wavelength of illumination

NA → the numerical apeture of the objective

- for light microscopes the best Minimum Resolving Distance (MRD) comes from using:

green = 550 nm or blue = 500 nm

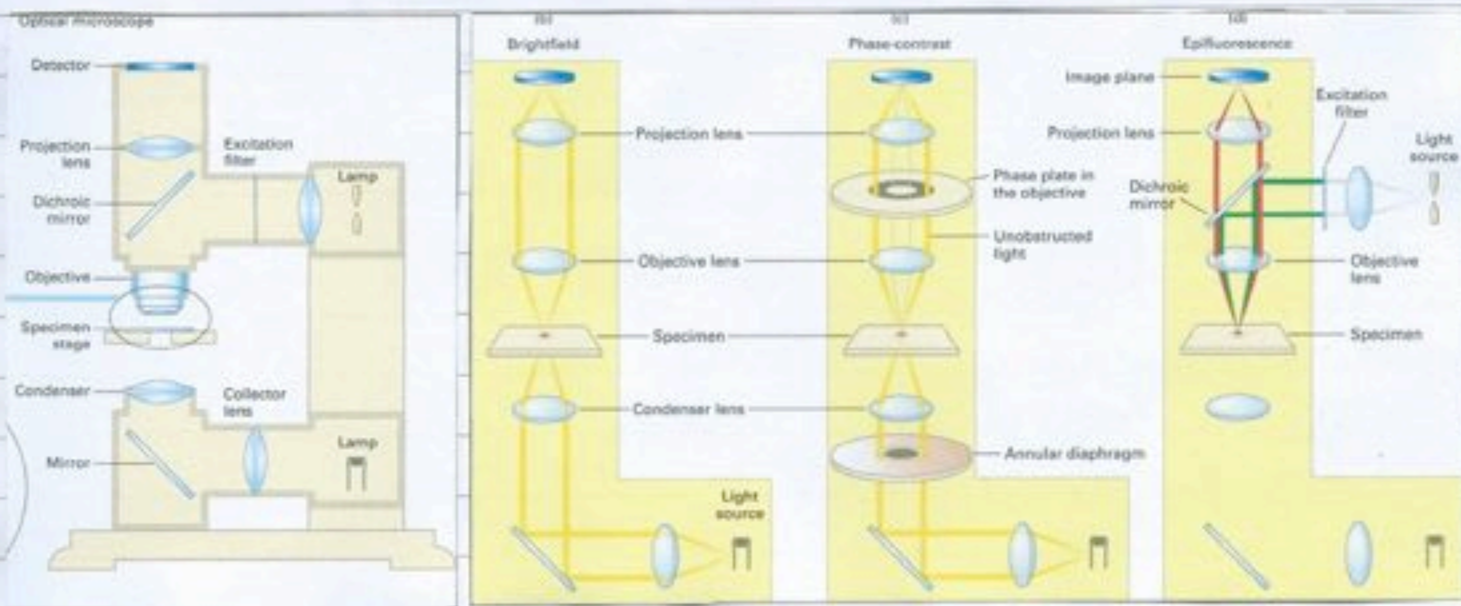
- Resolution → the ability of a system to distinguish separate points under a particular condition of usage

- usually air is the medium but in confocal it'll be in water
  - ↳ we use 63x zoom with water

Refractive Index:
- RI of mountain media range from 1.400 to 1.700
- RI of air = 1.0000
- RI of water is 1.333
- RI of immersion oil & embedded plastic is 1.515

- these lenses have a very short working distance: 40x & 100x objectives
- for the best resolution → oil condenser + oil immersion objectives + Köhler illumination is used

Microscope Configuration



- magnification is ascertained by multiplying the objective and eyepieces values
- what we used was an upright field (counting cells)
- In phase contrast we'll change the phase of light
- In fluorescence light comes in and hits a mirror → bounces off the mirror (green light) → specimen reaches a higher energy state → when it comes back down to a lower energy state it emits light everywhere, which goes back (now in the red wavelength) to pass through the mirror & to our eyes.