AMME1961

- **Lect 1**. Slides on cell structure and function (i.e like prokaryotic, eukaryotic, membranes…)

Lect 2 Microscopes, Light…

Cell culture:

- We can grow both eukaryotic and prokaryotic cells in controlled culture systems if we provide nutrients and remove waste products.
- Requirements can be quite diverse: some cells grow suspended in solutions and some adhere to culture flask surfaces. Growth requirements for mammalian cells are usually quite complex (often including foetal calf serum).
- Cells that grow on surfaces stop dividing when they come into contact with surrounding cells (confluent) resulting in a sheet of cells one cell thick

Purposes for cell culture:

- Making things (e.g beer and wine -> from yeast, therapeutic drugs -> from bacteria or mammalian cells)
- Study of cell function in health and disease
- To generate cells to manufacture human tissues for implantation and to replace mutated cells in patients with genetic diseases

Mammalian cells grown in culture:

- Stem cells cultures -> found in developing embryos. These cells can differentiate into some/all cell types in the body
- Primary cell cultures -> cells isolated from mammal tissues that have a finite ability to divide (after this the cell dies)
- Mammalian cells that have been altered to allow them to continue dividing indefinitely (immortalised) -> this includes cells originally isolated from human cancers

Modern history of the microscope:

- 18th century -> Achromatic microscope objectives made (free of major chromatic aberrations)
- 19th century -> Improvements in lens design due to quality of glass used for microscope optics. Water and oil immersion objectives developed.
- 1873 -> Ernst Abbe provided scientific basis for the production of powerful light microscopes
- 1893 -> Augus Koehler standardised microscope illumination
- 20th century -> Fundamental principles established for fluorescence and single- and two-photon microscopy. Development of Phase contrast (Zernike, 1941), differential interference contrast (Nomarski, 1950s)

Scientific basis for the production of light microscopes:

- Definition of resolution is the minimum distance (in microns) between 2 points where the 2 points can be clearly distinguished from each other.
- The following equation gives the value of the minimum resolved distance, d (this is: The minimum distance (in microns) between two points where the two points can be clearly distinguished from each other). Developed by Ernst Abbe over 140 years ago
  - d = lamda/2(nsin(alpha) -> where nsin(alpha) is the numerical aperture of the lens (Numerical Aperture, NA = is a measure of the microscope's ability to gather light and resolve fine specimen detail at a fixed object distance). lambda is the wavelength of illumination and n is the refractive index of the medium and alpha is half the angular intake of the lens.

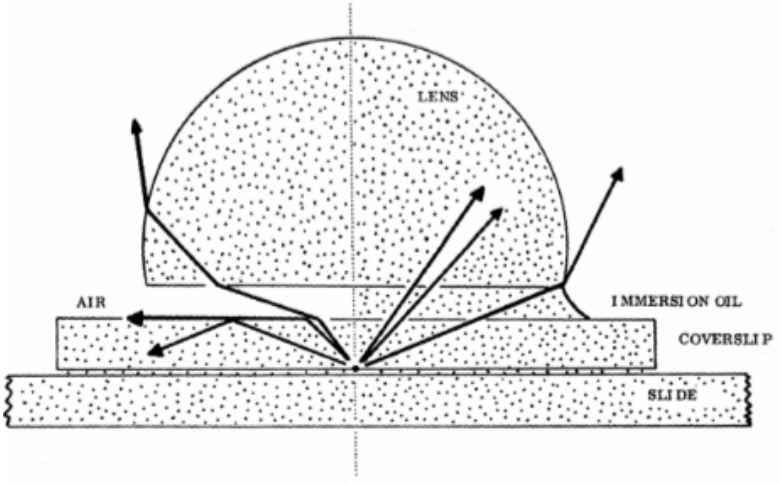
Optical microscope:

- Can be used to view structures of 200nm

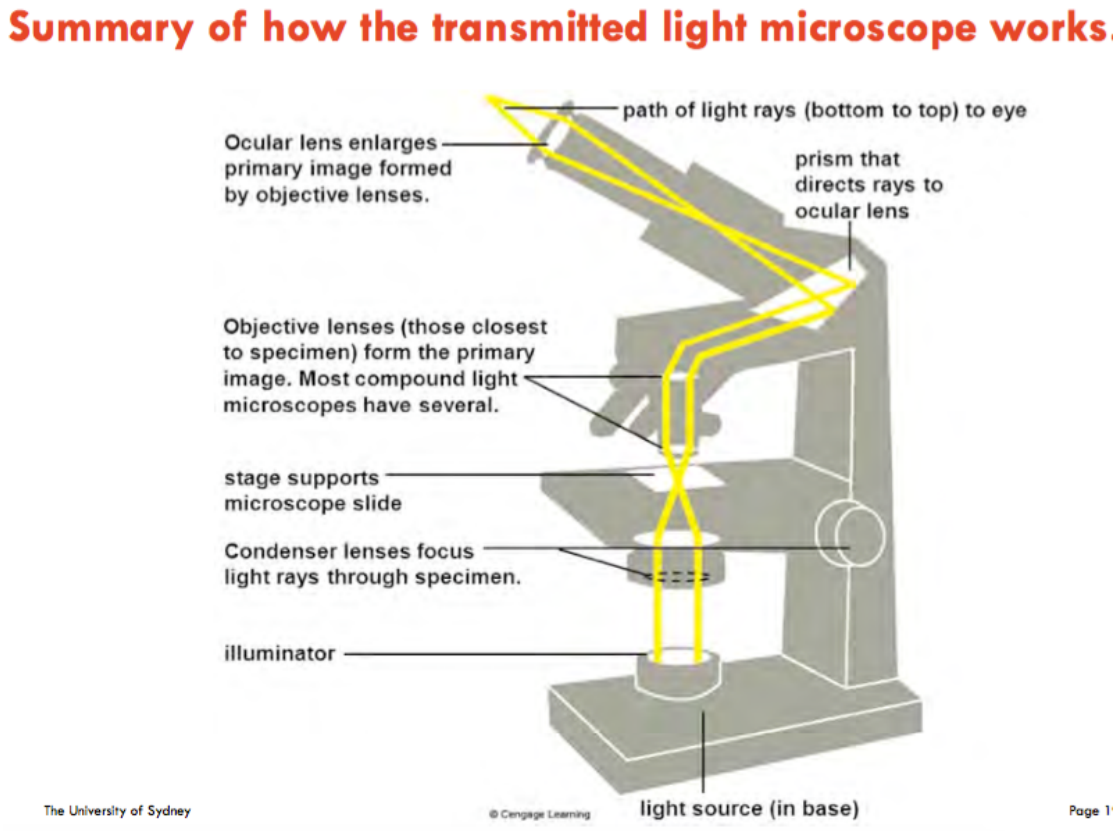
How the light microscope works:

- Main structures: Objective lens, condenser lens, Köhler illumination and Immersion oil.
- OBJECTIVE LENS:
- They are the most important components
- They are responsible for primary image formation
- They are instrumental in determining the magnification of a particular specimen and the resolution under which fine specimen detail can be observed in the microscope.
- Play a central role in determining the quality of images that the microscope is capable of producing
- They are the most difficult component of an optical microscope to design and assemble
- They are the first component that light encounters as it proceeds from the specimen to the image plane
- They are the closest component to the specimen
- CONDENSER LENS:

- Gathers light from the source and concentrates it into a cone of light that illuminates the specimen with uniform intensity over the entire view-field.
- Provides a cone of light and must be properly adjusted
- Two identical NAs are required, the condenser NA exactly matching the objective NA.
- A **condenser of high NA** is chosen that has its NA reduced by an iris diaphragm whenever a lower NA objective is used.
- KOHLER ILLUMINATION:
- A method of illuminating objects in which an image of the source is projected by a collector into the plane of the aperture diaphragm in the front focal plane of the condenser. This latter, in turn, projects an image of an illuminating field diaphragm at the opening of the collector into the object plane.
- OIL IMMERSION:
- NA can also be increased by employing immersion fluids with a high refractive index (*n*)
- Light rays passing through specimen encounter a homogeneous medium between coverslip and immersion oil and are not refracted as enter the lens (shown in diagram below)
- This maximises image contrast



Light Microscope:



How to check the resolution of your light microscope:

- Set up the microscope with 40x/0.65 objective using Kohler Illumination
- Examine the siliceous outer wall of diatom Pleurosigma angulatum
- If you can see the diatom patter you are using the microscope correctly
- If you cannot see it this may be due: to the condenser too low, the aperture iris closed, auxiliary lenses in the illumination chosen incorrectly, or using a red filter

Phase Contrast Microscopy (Zernike, 1938)

- This form of microscopy centres around the idea that when light travels through glass the imperfections of the glass (e.g thickness) would cause the light to not be in "phase" anymore. I.e the waves -> peaks and troughs do not line up. Thus a phase contrast microscope is used to transform these phase differences seen when light passes through unstained live cells, for example, into intensity differences thereby increasing contrast.
- This has revolutionised the light microscope.
- Advantages: do not need to fix or stain cells, high contrast, quantitative phase imaging, affordable
- Disadvantages: not ideal for thick specimens, halo effect (results in one area influence that in other areas) can obscure details.

Electromagnetic Spectrum:

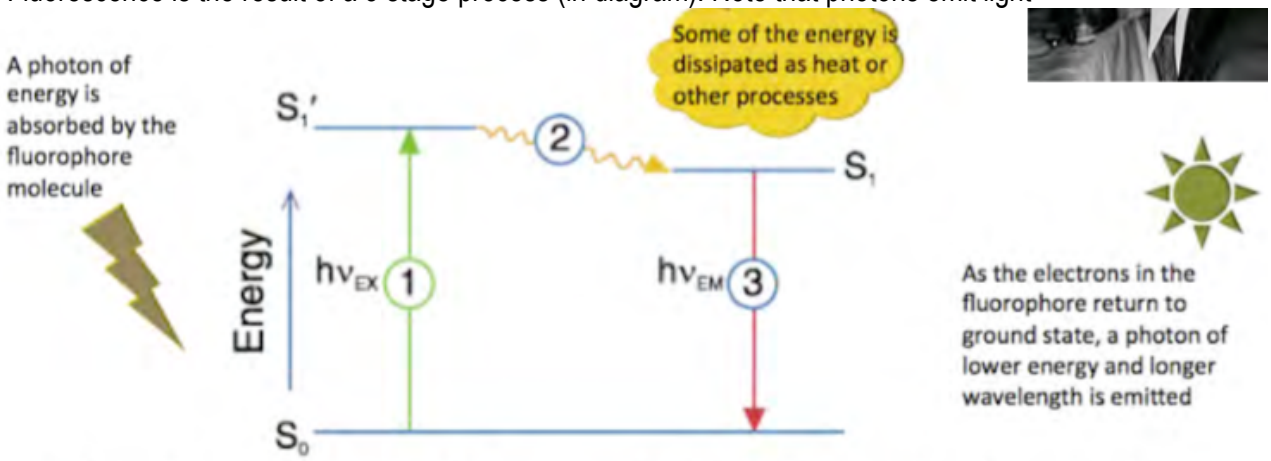
- Can be broken into different regions with different wavelengths. Infra-red (700nm), Visible red-orange-yellow-green-blue-indigo-violet (700-390nm), Ultraviolet & X-rays & gamma rays (380-10nm),
- Longer wavelength = low frequency = less energy
- Shorter wavelength = high frequency = more energy

Absorption of Light:

- Highly dependent on the wavelength
- Absorption in the microwave is due to molecular rotations
- Absorption in the infrared is due to molecular vibrations
- Absorption of UV/visible light is due to electrons in the molecule being promoted to higher states

Fluorescence:

- Fluorescence is the result of a 3 stage process (in diagram). Note that photons emit light



Jablonski diagram illustrating the processes involved in the creation of an excited electronic singlet state by light absorption and subsequent emission of fluorescence.