Cells & the Microscope

WALT:

- State the resolution & magnification that can be achieved by a light microscope & electron microscope.
- Explain the difference between magnification & resolution.
- Explain the need for staining samples in microscopy.
- Calculate the magnification of an image.

Make a labelled diagram of a Generalised GCSE animal cell and a plant cell.

- Give yourself 1 point for each labelled part:
  - Nucleus
  - Cell membrane
  - Cytoplasm

  } Animal & plant cells

  - Cell wall
  - Vacuoles
  - Chloroplasts

  } Plant cells only

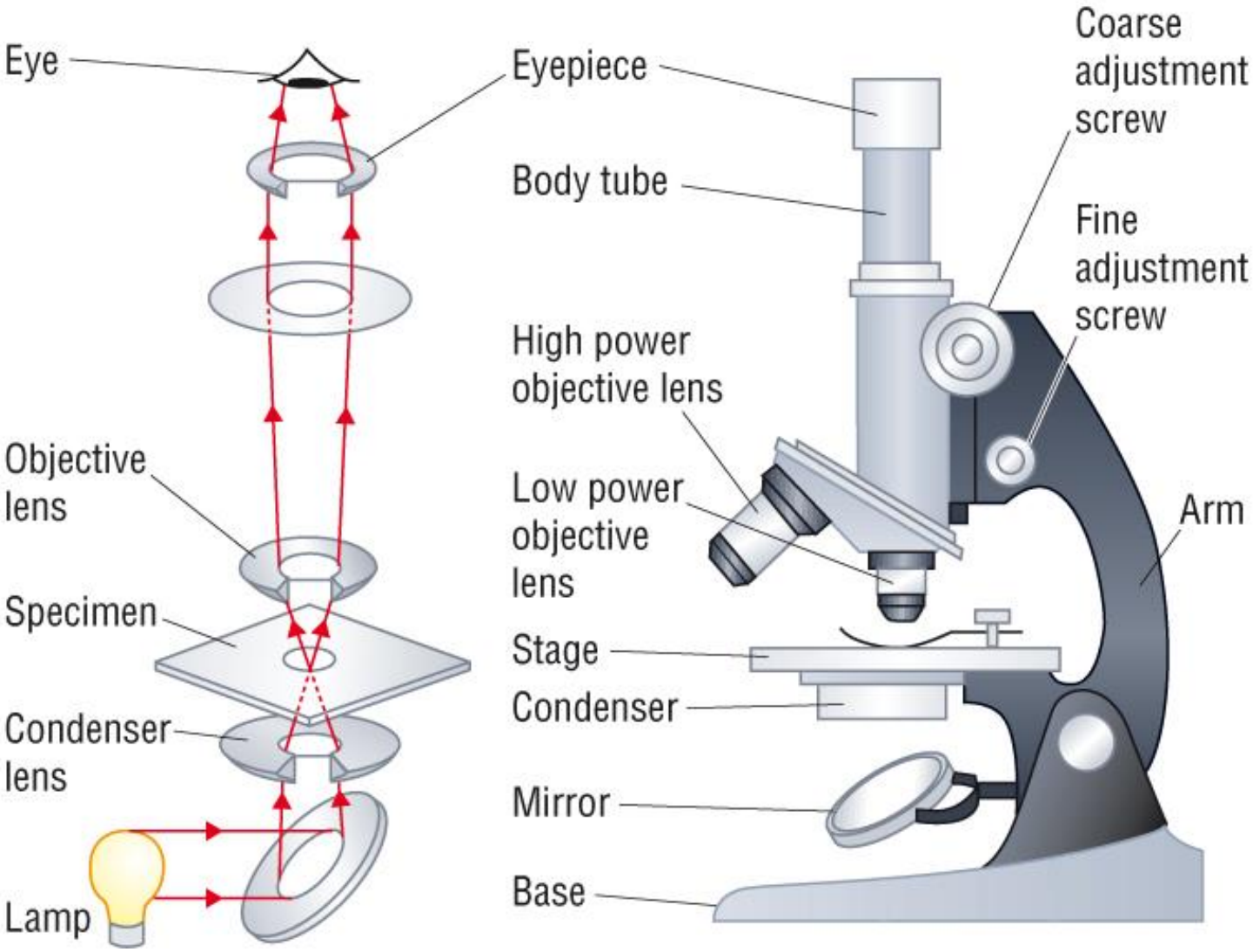
Development of Cell Theory

- Robert Hooke (1660s) developed a microscope using several lenses.
  - He looked at slices of oak bark & saw they were made of tiny chambers.
    - The chambers resembled rooms (cells) that monks lived in.
    - So he called them cells.
- With better microscopes, other scientists saw that all living things are made of cells.

Development of Cell Theory

- By 1880, Cell Theory had been extended to state that:
  - All living things consist of cells.
  - New cells are formed only by the division of pre-existing cells.
  - The cell contains information that acts as the instructions for growth. This information can be passed on to new cells.

The Light Microscope



Using a Light Microscope

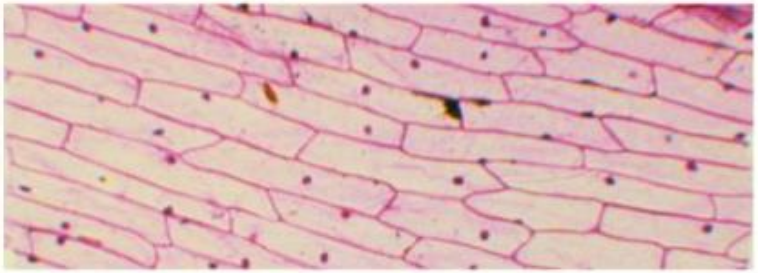
- *Procedure, preparing slides, staining.*

Virtual Microscope

Click here for a Virtual Microscope

Scientific Drawings – the rules:

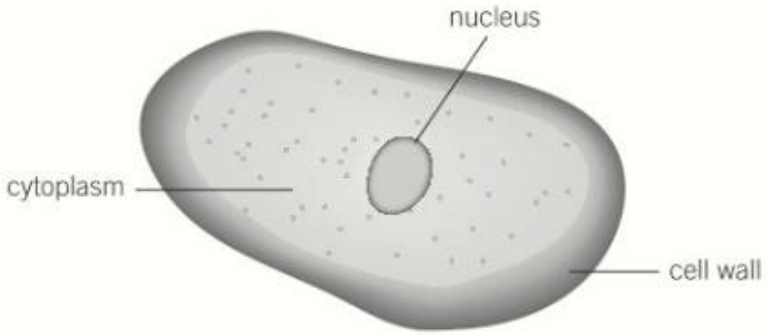
- include a title
- state magnification
- use a sharp pencil for drawings and labels
- use white, unlined paper
- use as much of the paper as possible for the drawing
- draw smooth, continuous lines
- do not shade
- draw clearly defined structures
- ensure proportions are correct
- label lines should not cross and should not have arrow heads
- label lines should be parallel to the top of the page and drawn with a ruler



× 18 magnification

▲ **Figure 7** *Top: Light micrograph of a layer of onion cuticle, showing the bands of large, rectangular cells. The dark spot in the centre of each cell is its nucleus. × 18 magnification. Bottom: A scientific drawing from the micrograph*

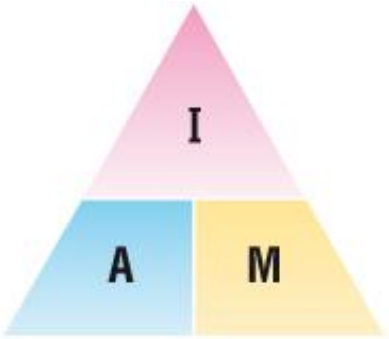
Below is an example of a poor scientific drawings:



1 Describe how this diagram is incorrect as a scientific drawing.

Magnification

- The number of times larger an image appears compared to size of the actual specimen.
  - Magnification = Image size / Actual object size.

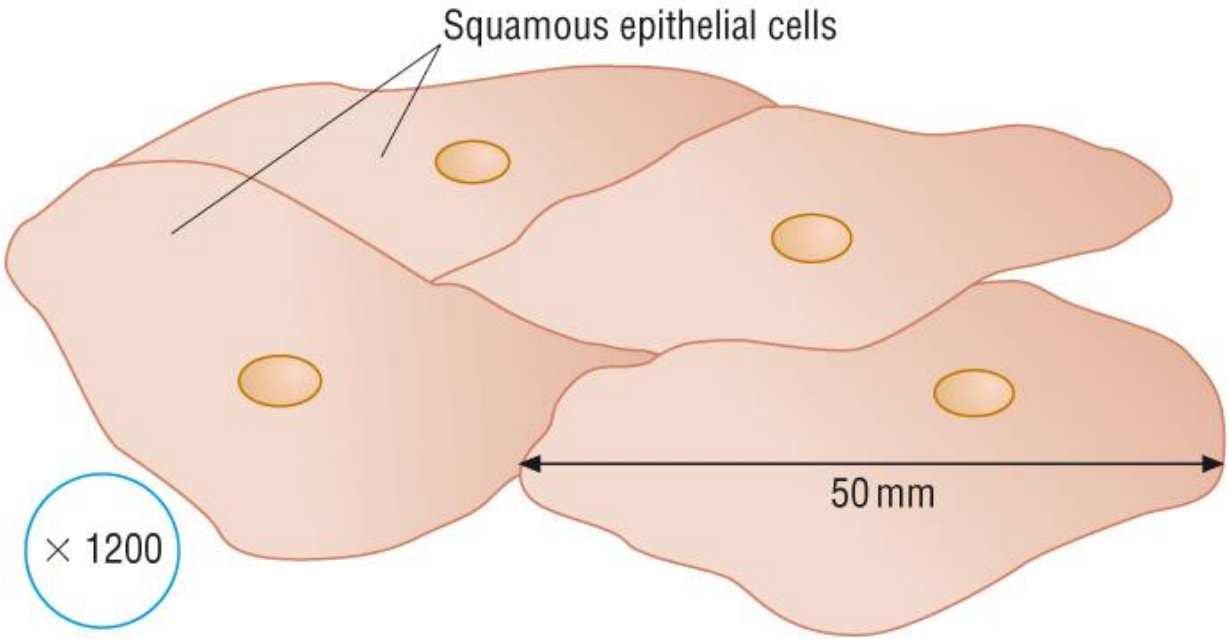


$$\text{Actual size} = \frac{\text{Image size}}{\text{Magnification}}$$

$$\text{Magnification} = \frac{\text{Image size}}{\text{Actual size}}$$

Magnification Examples

1) Calculate the actual length of the marked cell below.



(I) Image size = 50 mm
(M) Magnification = 1200

2) If a 5µm nucleus is magnified with a x400 microscope, what will be the size of the image produced?

5 x 400 = 2000 µm = 2mm

Measurements & Units

- Complete the table below to show different measurements in various units

Rewrite these measurements	5mm	26μm	300nm
In metres (m)			
In millimetres (mm)			
In micrometres (μm)			
In nanometres (nm)			

Resolution

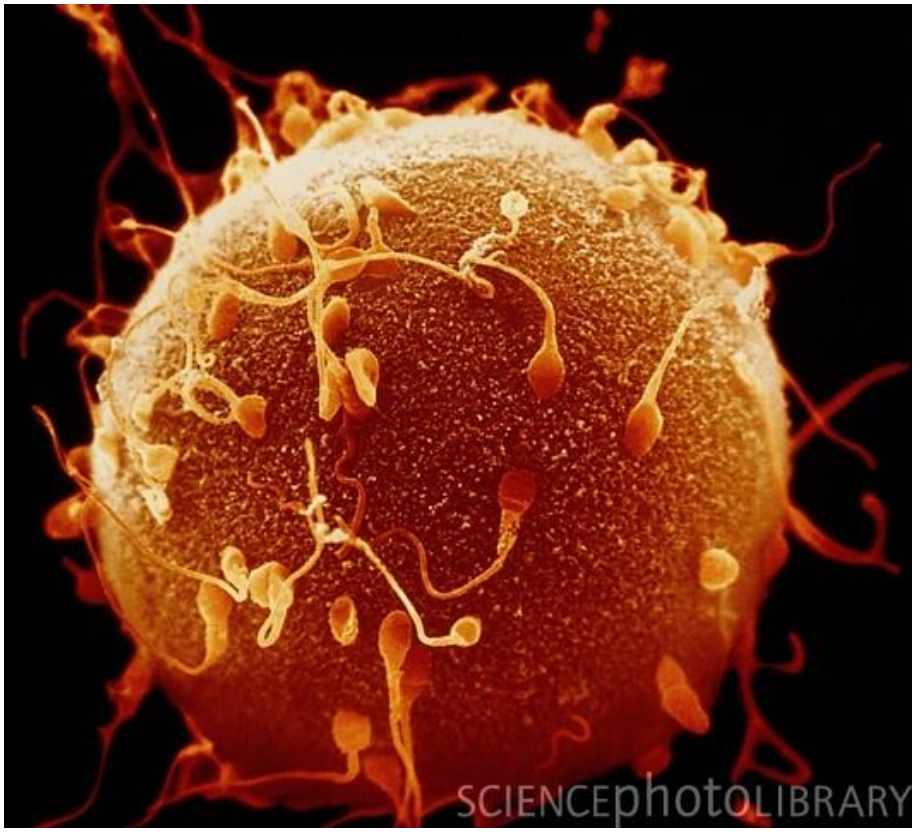
- The degree to which you can distinguish between two objects that are close together.

- In order to investigate cells and their component parts, we need both a high magnification and resolution.

What is this?

Click to **magnify** only

What is this?



Click to increase **magnification** & **resolution**

Limitations of a Light Microscope

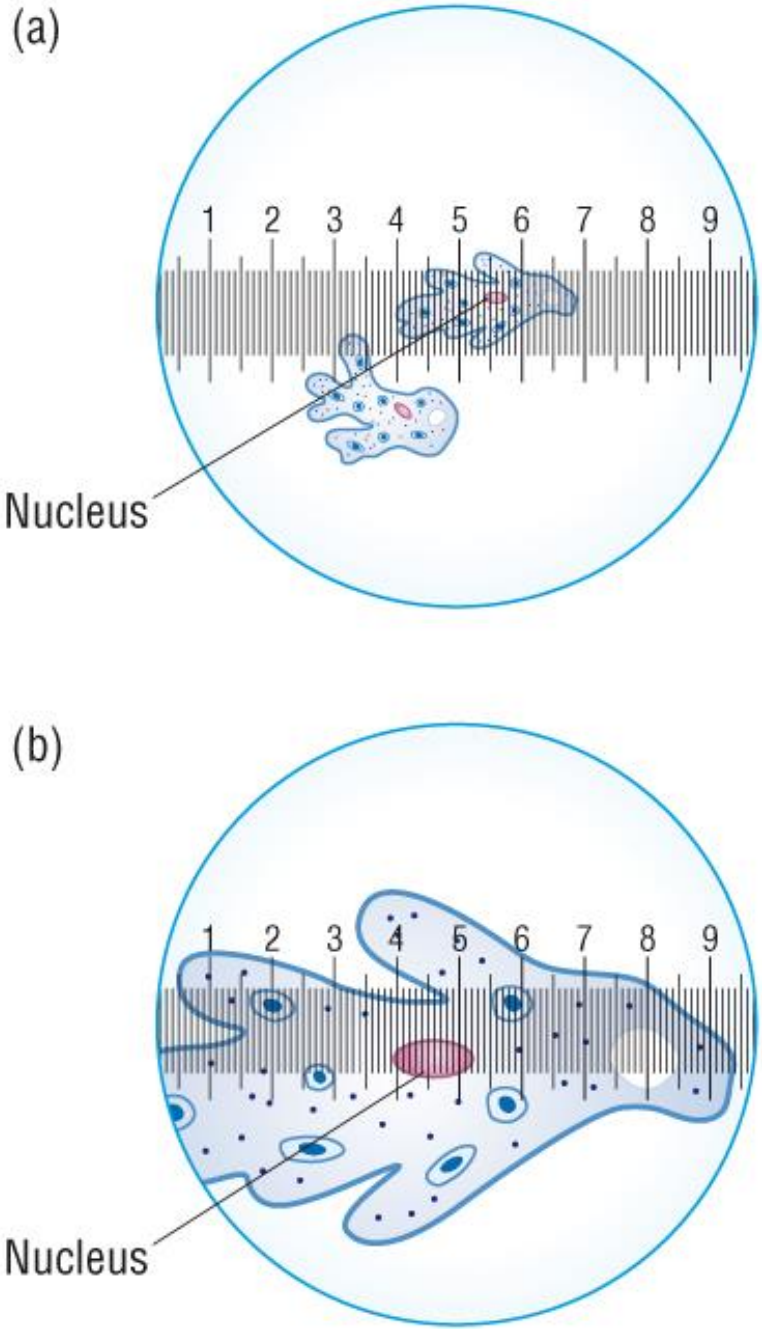
- Magnification:
  - Most light microscopes are only capable magnification of up to x1500.
- Resolution:
  - Light microscopes are only capable of resolving 200nm.

Making Measurements Using a Microscope

- How can we judge the size of an image seen?
  - The eyepiece can be fitted with a graticule.
    - This is a tiny ruler etched onto the eyepiece lens.
  - The graticule is superimposed onto the image so the specimen can be measured.

The Eyepiece Graticule

- The graticule scale is arbitrary.
  - It represents different lengths for different magnifications.
- The scale needs to be calibrated.

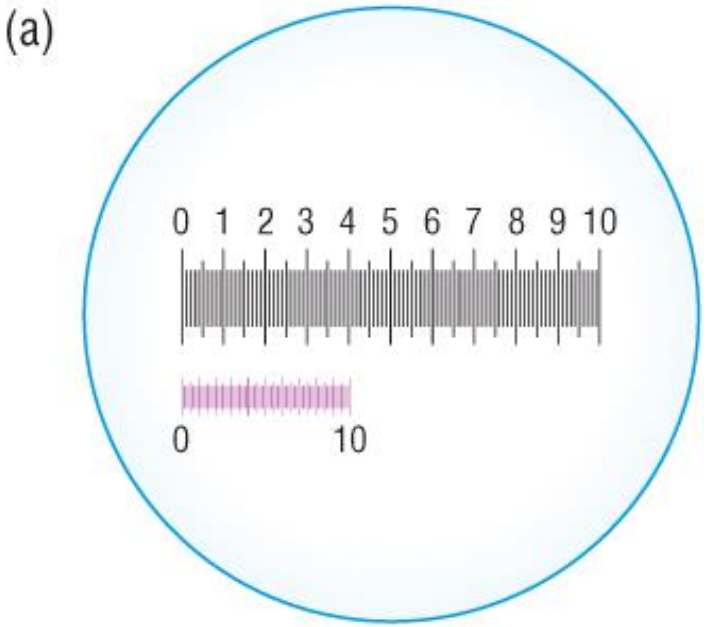


Calibrating the Eyepiece Graticule

- A stage micrometer is placed onto the microscope stage.
  - This is a tiny 1mm ruler divided into 100 10µm divisions that is etched onto a microscope slide.
- By focussing on the stage micrometer we can compare the eyepiece graticule with the stage micrometer.

Calibrating the Eyepiece Graticule

- At x40 magnification (a), 40epu = 1mm = 1000μm.
  - So 1epu = 1000/40 = 25μm.



Using the Calibrated Graticule

Using the previous examples at x100

Objective lens used	x10
Eyepiece magnification	x10
Total magnification used	x100
Length of amoeba nucleus (epu)	3.2 epu
Value of one epu (μm)	10 μm
Actual length of amoeba nucleus (μm)	32 μm

Web animation

Practical Work

- Prepare some onion epidermal cells on a slide stained with:
  - Iodine
- Calibrate the eyepiece graticule for 2 different magnifications.
- Measure the diameter of a cell nucleus.
- Make labelled sketches (include the magnification).

The Electron Microscope

- Why use electrons?
  - Visible light has a wavelength of 400-750nm.
  - A beam of electrons has a wavelength of 0.004nm.
  - So a much greater resolution can be achieved using electrons.
  - A magnification of about x900 000 can also be achieved.

But we can't see electrons & they are not diffracted by lenses

- The image produced by an EM is projected onto a screen or photographic paper.
  - These images can be printed & are called electron micrographs.

- Glass lenses are ineffective at focussing electrons.
  - Magnets are used instead.

Types of Electron Microscope

- Transmission Electron Microscope (TEM)
  - Electrons pass through a thin section of the sample.
  - The image is 2D.

- Scanning Electron Microscope (SEM)
  - Electrons don’t pass through the specimen but bounce off it.
  - The image is 3D.

Advantages of the EM

- A resolution of 0.1nm (2000x more than LM)
  - Detailed images of cells & organelles can be produced.
- 3D images can be produced of the surface of a sample (SEM).

Limitations of the EM

- Electrons are deflected by air molecules so the sample needs to be placed in a vacuum.
  - Living tissue would not survive.
- Electron microscopes are expensive.
- Using an EM requires highly skilled & trained personnel.

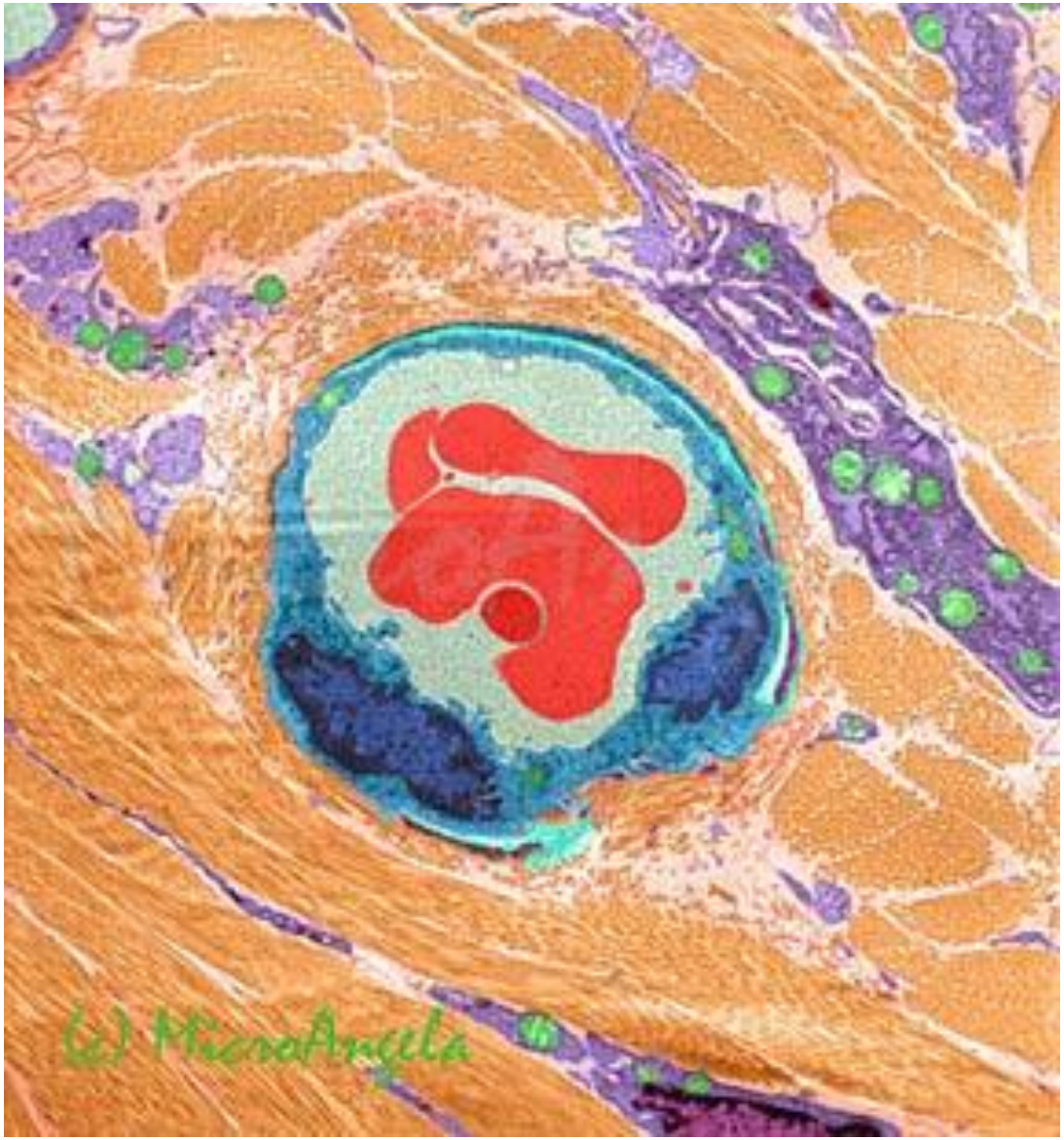
Using a TEM

- Preparing specimens:
  - Fix the sample in glutaraldehyde to make it firm.
  - Dehydrate it by replacing water with alcohol.
  - Embed the dehydrated tissue in a resin.
  - Cut very thin slices (100nm) using an ultramicrotome.
  - Stain the sections using lead salts.
  - Mount sections onto a small copper grid.
  - Place the grid into the vacuum specimen chamber.

Independent Work

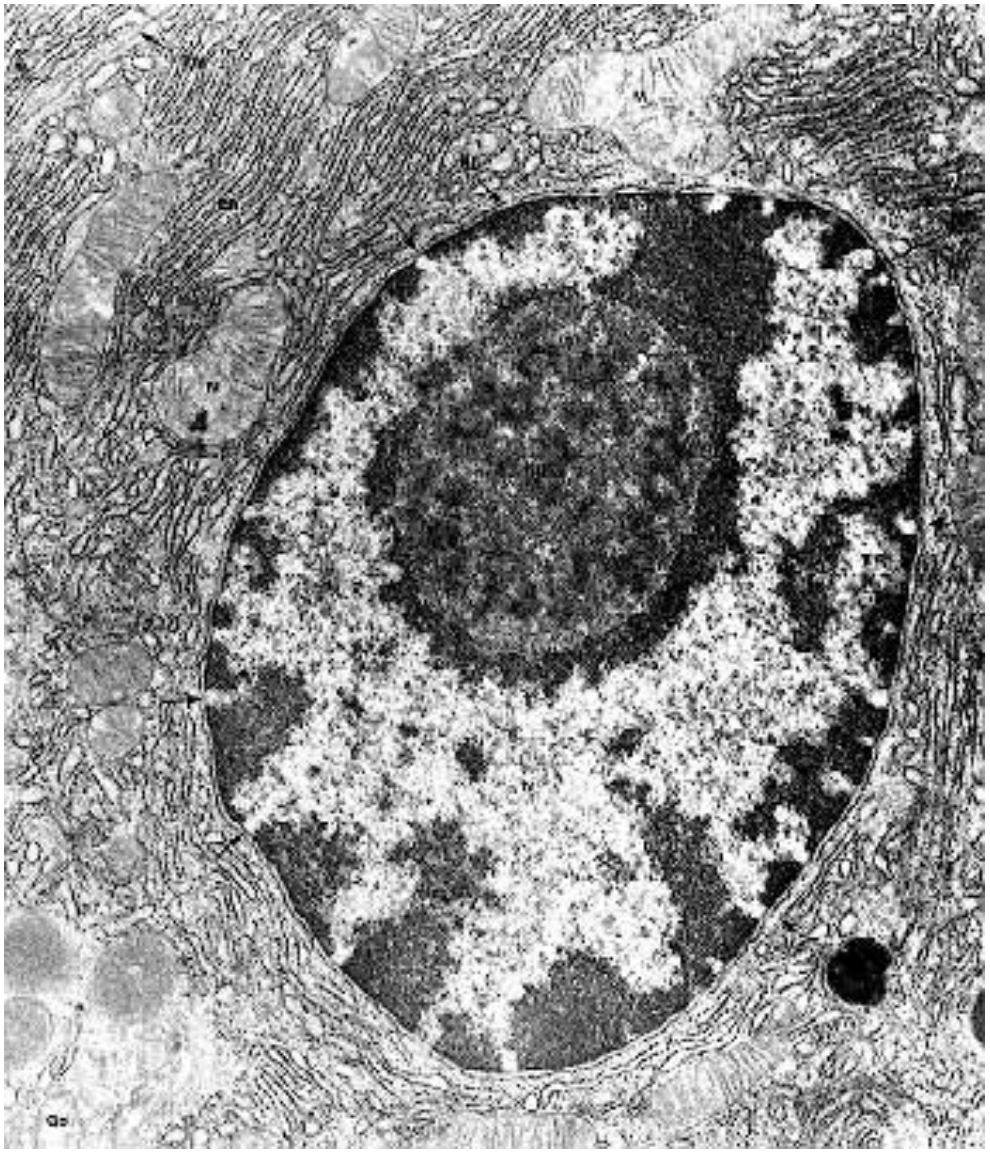
- Produce a venn diagram to show the similarities & differences between light & electron microscopes.
- Produce a summary page to describe each of the following alternative microscopy techniques:
  - Atomic force microscopy
  - Laser scanning confocal microscopy
  - Super-resolved fluorescence microscopy

Electronmicrographs



Capillary TEM

Electronmicrographs

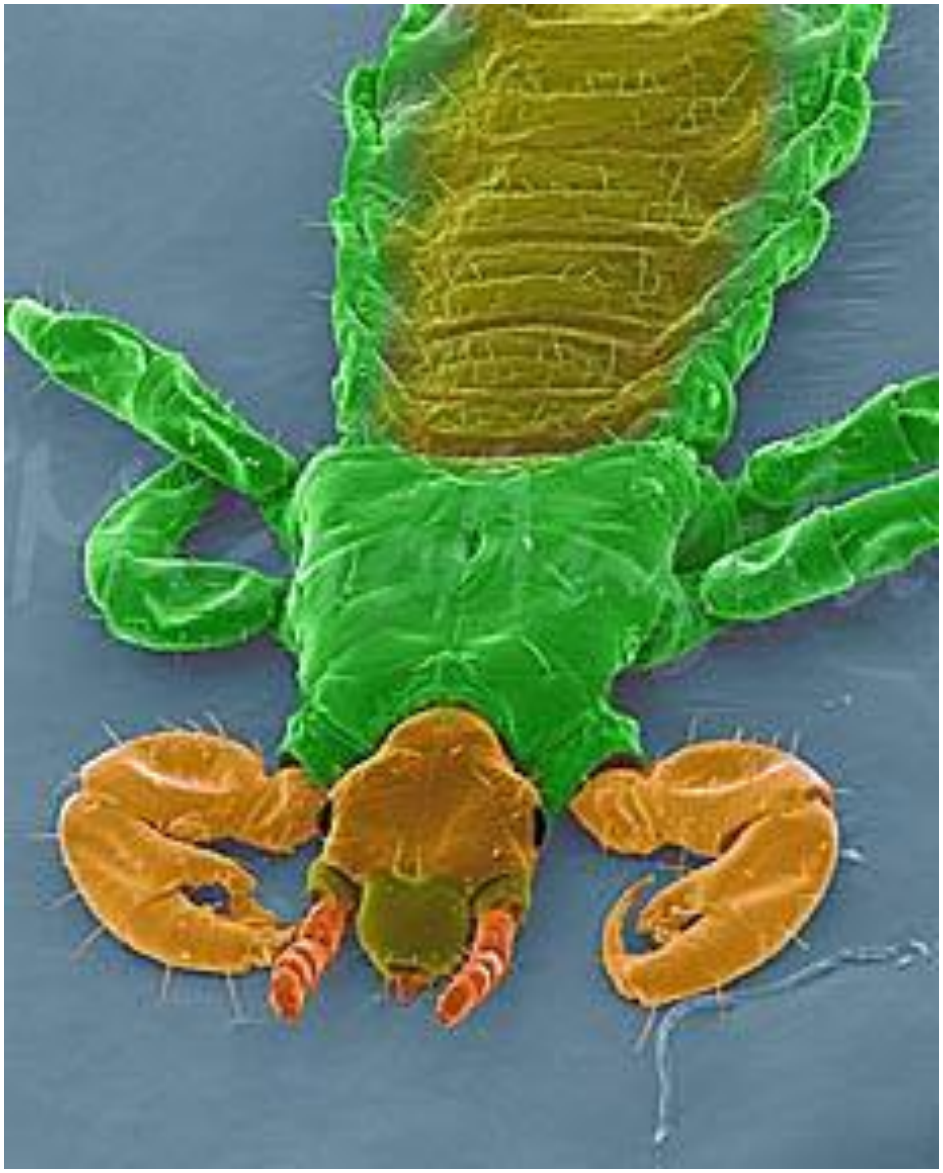


Nucleus TEM

Electronmicrographs

Bedbug
SEM

Electronmicrographs



Headlouse SEM