

(A) Cell Structure

Microscopy

AICE Biology

History

- Robert Hooke: 1665 – started with cork cells
 - Discovered and described- *cells are the fundamental unit of all living things*
- Schleiden: 1838 – all plants are made up from cells
- Schwann: 1839 – all animals are made of cells
- Virchow’s Cell Theory of 1855:
 - All cells arise from pre-existing cells

Cell Theory

- All living things are composed of cells
- Cells are the basic units of structure and function in living things
- New cells are produced from existing cells

Cell biology - cytology

- Fluid mosaic membrane
- Partially permeable membrane
- Active and passive transport of materials inside and outside of the cell
- Simple acts of diffusion through the membrane
- Considered biotic or living
- Groups of similar cells make tissues

Units of Measurement in cell studies

Fraction of Meter (metre)	Unit	Symbol
One thousandth = 0.001 = 1/1000 = 10^{-3}	Millimeter (re)	mm
One millionth = 0.000 001 = 1/1 000 000 = 10^{-6}	Micrometer (re)	μm
One thousandth millionth = 0.000 000 001 = 1/1 000 000 000 = 10^{-9}	Nanometer (re)	nm

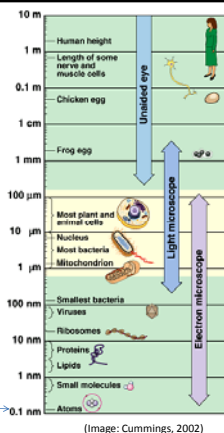
μ is the Greek letter mu
1 micrometer is a thousandth of a millimeter
1 nanometer is a thousandth of a micrometer

Therefore, to change mm into μm , multiply by 1000

Textbook pg. 5

Biological Structures

- Minimum resolution of a light microscope is about 2 microns
 - size of a small bacterium
- Light microscopes can magnify effectively to about 1,000 times the size of the actual specimen
 - at higher magnifications, the image blurs

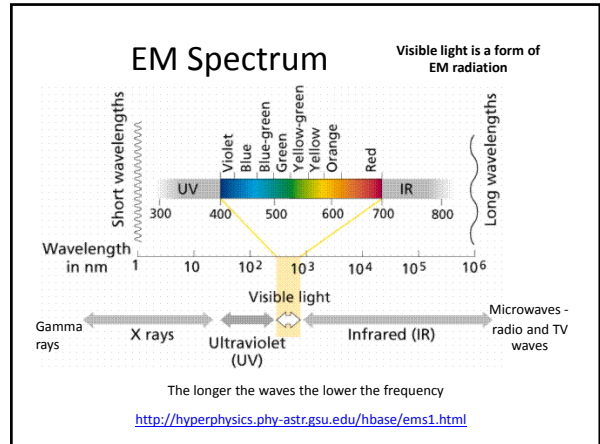


(Image: Cummings, 2002)

Magnification and resolution

- **Magnification**= size of image/size of specimen. Drawing or picture in mm converted to μm /measured with graticule in μm
- **Resolution**= the ability to distinguish between two separate points. If you cannot resolve between two points, then it is only one point.
 - Maximum resolution is 200 nm, if two points are closer than 200 nm, they cannot be distinguished as separate.
 - The limit of resolution is only half the wavelength of the radiation used to view the specimen

$\text{magnification} = \frac{\text{size of image}}{\text{actual size of object}}$ $\text{actual size of object} = \frac{\text{size of image}}{\text{magnification}}$



Measuring Cells & Calibration

Field of View Intro > Field of View > Calibrate > Estimate

Great practice Link on daily assignment page

When you look into a microscope, the "field of view" is the visible circular area. See how the object looks longer when the magnification of the objective (Obj) changes from 4x to 10x to 40x. By knowing the size of the field of view (diameter), you can measure the size of objects in the microscope.

Because the size of objects in the field of view is different at each magnification, you have to calculate the diameters of the fields of view at each magnification. This process is called "calibrating your microscope".

Every microscope is different; even two identical-looking microscopes in the same classroom can differ significantly in how big objects in the microscope appear. For this reason, each microscope needs to be individually calibrated.

The easiest process of calibration involves determining how wide the field of view appears.

If you record that information, you can use it to estimate the size of any object in your microscope's view!

Always a part of paper 3

Measuring Cells

- You should be able to work out the real size of an object if you are told how much it has been magnified

$\text{actual size of object} = \frac{\text{size of image}}{\text{magnification}}$

- **Ex:** the mitochondrion has been magnified 100 000 times
 - Use your ruler to measure its length in mm.
 - Let's say it is 50 mm long
 - As it is a very small object, convert this to μm by multiplying by 1000
 - Substitute into the equation:
 - actual size of object = size of image/magnification
 - = 50 000/100 000
 - = 0.5 μm

Magnification calculation using a scale bar

- Measure the length of the scale bar.
- Calculate its magnification using the formula

$$\text{magnification} = \frac{\text{size of image}}{\text{actual size of object}}$$

$$= \frac{\text{length of scale bar}}{\text{length the scale bar represents}}$$

$$= \frac{200000}{2}$$

$$= 100000$$
- Measure the length of the image of the chloroplast in mm, and convert to μm . You should find that it is 80000 μm long.
- Calculate its real length using the formula

$$\text{actual size of object} = \frac{\text{size of image}}{\text{magnification}}$$

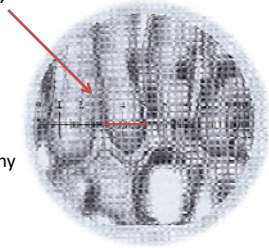
$$= \frac{80000}{10000}$$

$$= 8 \mu\text{m}$$

(Jones, 2010)

Measuring cells using a graticule

- Scale bar in your light microscope
- Marked off in “**graticule units**”
- Width of one cell, **23 units**
- Convert graticule units to real units (mm or μm)
 - Calibration
- Done by using a **stage micrometer** marked off in a tiny scale
 - Smallest markings are 0.01 mm apart (10 μm)



Graticule, continued

- Next, take the specimen off the stage of the microscope and place the stage micrometer on the stage using the same magnification. Focus.
- Line up the micrometer scale and the eyepiece graticule scale
 - Notice the 50 mark on the stage micrometer is lined up with the 1.0 mark on the eyepiece graticule
 - Work along until you see another two lines that line up exactly
 - 68 on the stage micrometer and 9.0 on the graticule align well

Therefore, 80 small eyepiece graticule markings = 18 stage micrometer markings

$$= 18 \times 0.01 \text{ mm} = 0.18 \text{ mm}$$

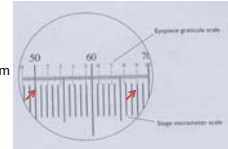
$$= 180 \mu\text{m}$$

$$1 \text{ small eyepiece graticule marking} = 180/80 = 2.25 \mu\text{m}$$

Now calculate the real width of the plant cell ...

$$\text{Which is } 23 \times 2.25 = 51.75 \mu\text{m}$$

Ta Da!



- If you change the objective lens then you will need to calibrate the eyepiece graticule units all over again using the new lens setting



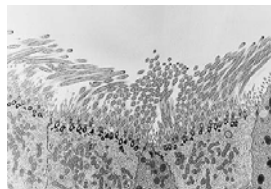
- Once you've calibrated for the different lenses (4x, 10x, 40x) you can save those measurements while using the same scope and same graticule

Electron microscope

- Using excited electrons with a very short wave length
- Negatively charged and therefore can be focused easily using electromagnets. *Page 11*
- Must be in a vacuum, dehydrated and dead material only
 - Transmission electron microscope TEM:
 - Through the specimen
 - Scanning electron microscope SEM:
 - Surface of specimen

Transmission Electron Microscope (TEMs)

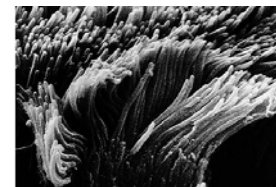
- Very thin sample/specimen, in a vacuum
- used mainly to study the internal ultra-structure of cells
- aims an electron beam through a thin section of the specimen.
- Image is focused and magnified by electromagnets
- To enhance contrast the thin sections are stained with atoms of heavy metals



(Cummins, 2002)

Scanning Electron Microscope (SEMs)

- useful for studying surface structures.
 - The sample surface is covered with a thin film of gold.
 - The beam excites electrons on the surface.
 - These secondary electrons are collected and focused on a screen.
- great depth of field resulting in an image that seems three-dimensional



(Cummins, 2002)

Resources

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