Module 4 : Laboratory Equipment and Techniques

In this module, the structure and operation of different laboratory equipment are shown, for example, centrifugation, chromatography and spectrophotometry.

Some laboratory techniques, for example, preparation of buffer, gel electrophoresis and use of laboratory stains are also included. You will find yourself gaining better lab techniques after going through this module.

4a. Cell Culture Techniques

Video: Cell Culture Techniques (see website)

4b. Buffer

A buffer is a solution that can resist the change in pH when limited quantities of acid or base are added to it. It consists of a mixture of a weak acid with its conjugate base, or a weak base with its conjugate acid, at equilibrium.

This combination allows the solution to neutralize the added acid/base with the conjugate base/conjugate acid, respectively. However, this characteristic only holds true within the buffer's capacity; exceeding the limit causes significant pH change. A buffer works in balancing the concentrations of acid and base to restore desired equilibrium, a principle which is governed by Le Chatelier's principle.

Buffers are necessary in maintaining pH levels to prevent denaturing of proteins, and degradation of pH sensitive biomolecules that allow for biological reactions to occur consistently. For example, carbonic acid (CH_2CO_3) and bicarbonate (HCO_3^-) are present in blood plasma as a buffer system to maintain a pH level of 7.35-7.45 in the human body for function.

4b(i). Buffer and pH calculation

When calculating pH, we are determining the negative logarithm of the concentration of protons [H^+] in a solution.

- Strong acid/base

HCl - -> $H^+ + Cl^-$

Strong acid/base dissociates completely when reacted with water. The number of moles of H^+ (protons) should be equivalent to the number of moles of H^+ in the acid. Theoretically, water contributes to the amount of H^+ as well, but the amount, in cases dealing with strong acid/base, is insignificantly small. Therefore, pH is calculated using the concentration of H^+ in the acid/base.

pH = - log [H^+]

- Weak acid/base

$HA <- -> H^+ + A^-$

Weak acid/base do not dissociate completely. Instead, they establish equilibrium. The pH level is calculated using the equilibrium constant, Ka.

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

- Buffer

To calculate pH for buffer solutions, an equation derived from equilibrium constant equation of weak acid/base is used. This is the Henderson-Hasselbalch equation.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

4b(ii). Information of different buffers

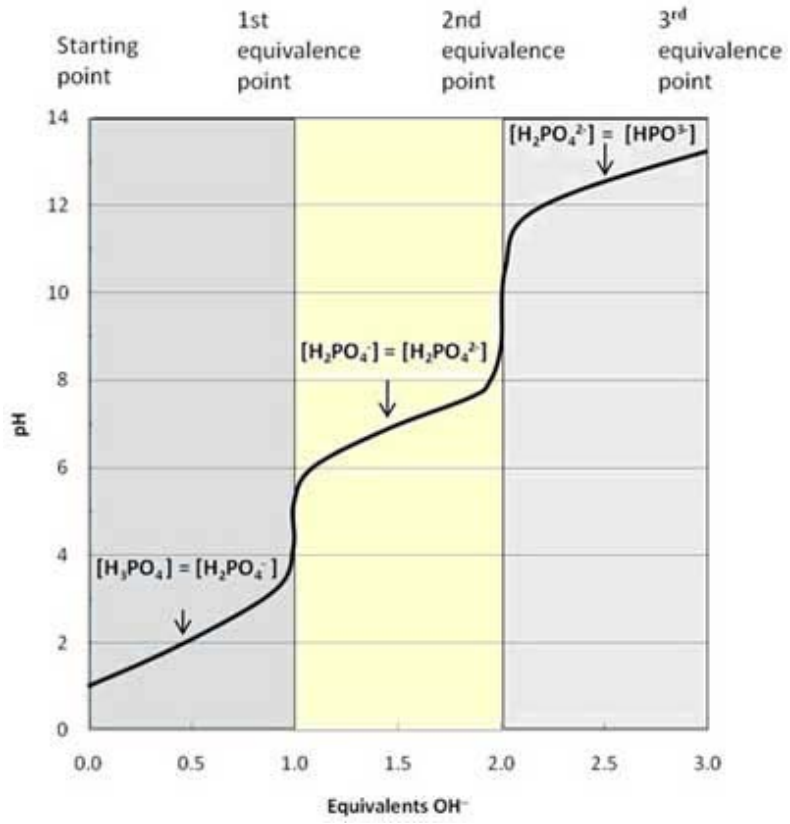
Commonly used buffers			
Buffer	Salts	Use	Remarks
Phosphate buffered solution pH 7.4	-KH_2PO_4 -Na_2HPO_4• 2 H_2O -KCl -NaCl	**Cell culture** To maintain the osmolarity of the cells	Isotonic and non-toxic to cells
Krebs-Ringer bicarbonate buffer pH 7.4	-D-glucose -Magnesium Chloride [Anhydrous] -Potassium Chloride -Sodium Chloride -Sodium Phosphate -Dibasic [Anhydrous] -Sodium Phosphate Monobasic [Anhydrous]	**Tissue culture** To maintain the pH and osmotic balance in the medium and to provide the cells with water and essential inorganic ions	
TAE (Tris-acetate-EDTA) buffer pH 8.0	-Tris base -Acetic acid -EDTA	**Molecular Biology** Used in agarose electrophoresis as running buffer, for the separation of nucleic acids	
TBE(Tris/Borate/EDTA) buffer pH	-Tris base -Boric acid -EDTA	**Molecular Biology** To keep DNA deprotonated and soluble in water	

Lithium Borate (LB) buffer pH	-Lithium hydroxide monohydrate -Boric acid	**Molecular Biology** Used in agarose gel electrophoresis	Has lower conductivity so that voltage can be increased to speed up electrophoresis
Lysis buffer pH	-Tris-HCl -EDTA -EGTA -SDS -Deoxycholate -TritonX	**Cell Biology** To lyse cells for analyzing the compound of the cells	
		Molecular Biology To release DNA for DNA fingerprinting study	
Glycine-NaOH PH 8.6–10.6	-Glycine -NaOH	**Protein Purification** As an elution buffer at high pH	
GLYCINE–HCL PH 2.2–3.6	-Glycine -HCl	**Protein Purification** As an elution buffer at low pH	

4b(iii). Titration curve

Preparation of phosphate buffer: Titration curve

Phosphate buffer is prepared from NaH_3PO_4 and strong base NaOH. It can be prepared in different pHs, e.g. pH2.15, pH 6.86 and pH 12.32 as the H_3PO_4 is a polyprotic acid. It has multiple dissociation constants. The titration curve of H_3PO_4 is as follow. However, the phosphate buffer is most commonly prepared around pH7. The yellow zone of the titration curve shows the buffer achieves the pH at 7 by NaH_2PO_4 and $Na_2H_2PO_4$. The mid-point of the 1^{st} and the 2^{nd} equivalence point is the point that the ratio of $[H_2PO_4^-]$: $[H_2PO_4^{2-}]$ is 1:1.



Video: Preparation of phosphate (see website)

4c. Centrifugation

Equipment		Speed/G.force	Function
  	Table top	Maximum RPM: 16,000rpm	It collects small amounts of materials that rapidly sediment. For example, small amounts of biological materials such as cells, cell debris or larger cellular organelles are rapidly sedimented.

	Microcentrifuge	Maximum RPM: 14,000rpm	It can rapidly sediment small amounts of biological materials such as cells, cell debris or larger cellular organelles.
	High-speed	Maximum RPM: 30000 rpm	It separates cells, cell debris or larger cellular organelles.
	Ultracentrifuge	Maximum RPM: 120000rpm	(a) Preparative ultracentrifuge Function: It separates of proteins and sub-cellular particles. (b) Analytical ultracentrifuge Function: It studies native molecular mass, assembly models, conformation and shape

Video : Centrifugation (see website)

4d. Chromatography equipment

Chromatography is commonly employed in laboratory for the separation of mixture. Gel filtration (size exclusion) chromatography and ion exchange chromatography are two popular separating techniques students may encounter in their studies.

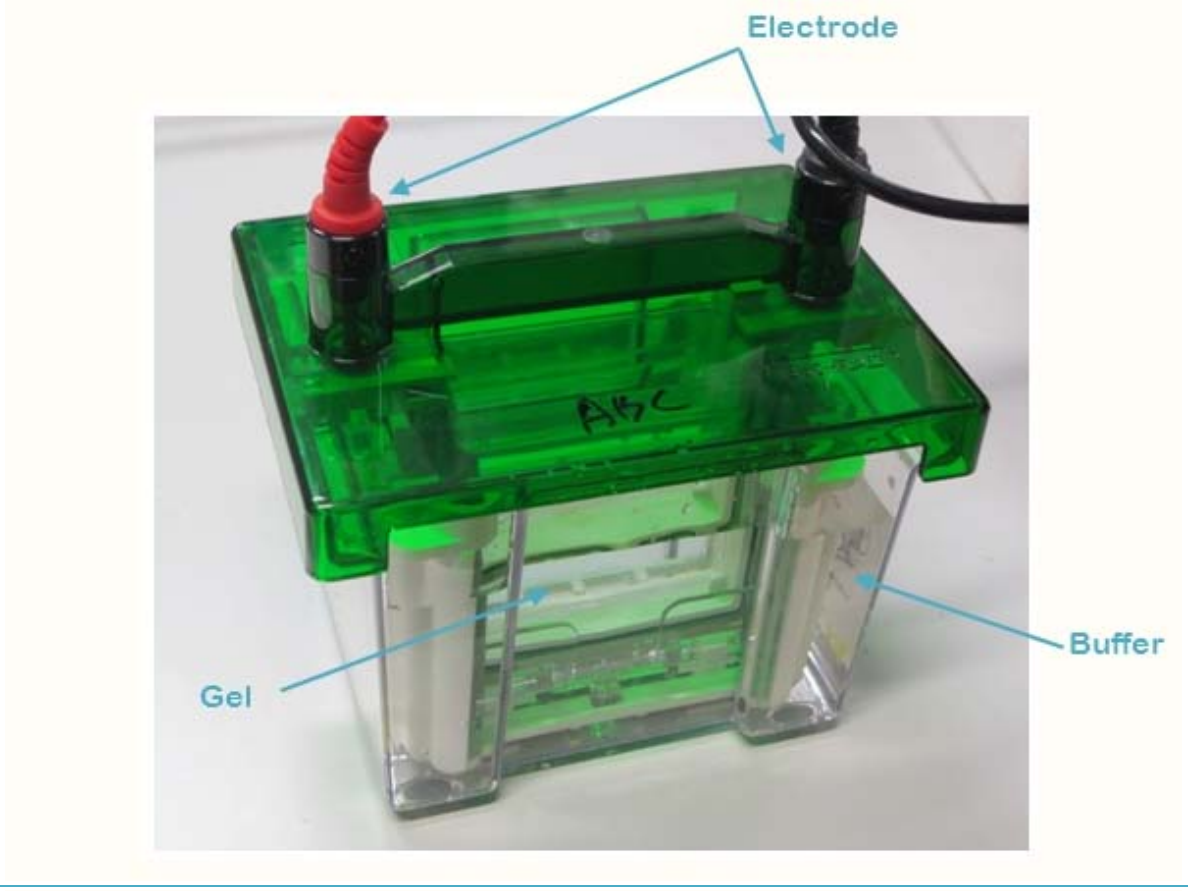
Picture	Equipment	Application
	Paper chromatography	It separates components with different retardation factors in a mixture, like components of chlorophyll.
	Gel filtration Chromatography	Desalting, purification of protein sample, estimation of the molecular weight of sample.
	Affinity chromatography	It purifies proteins bound to tags specifically from a mixture, such as that between antigen and antibody, enzyme and substrate, or receptor and ligand.

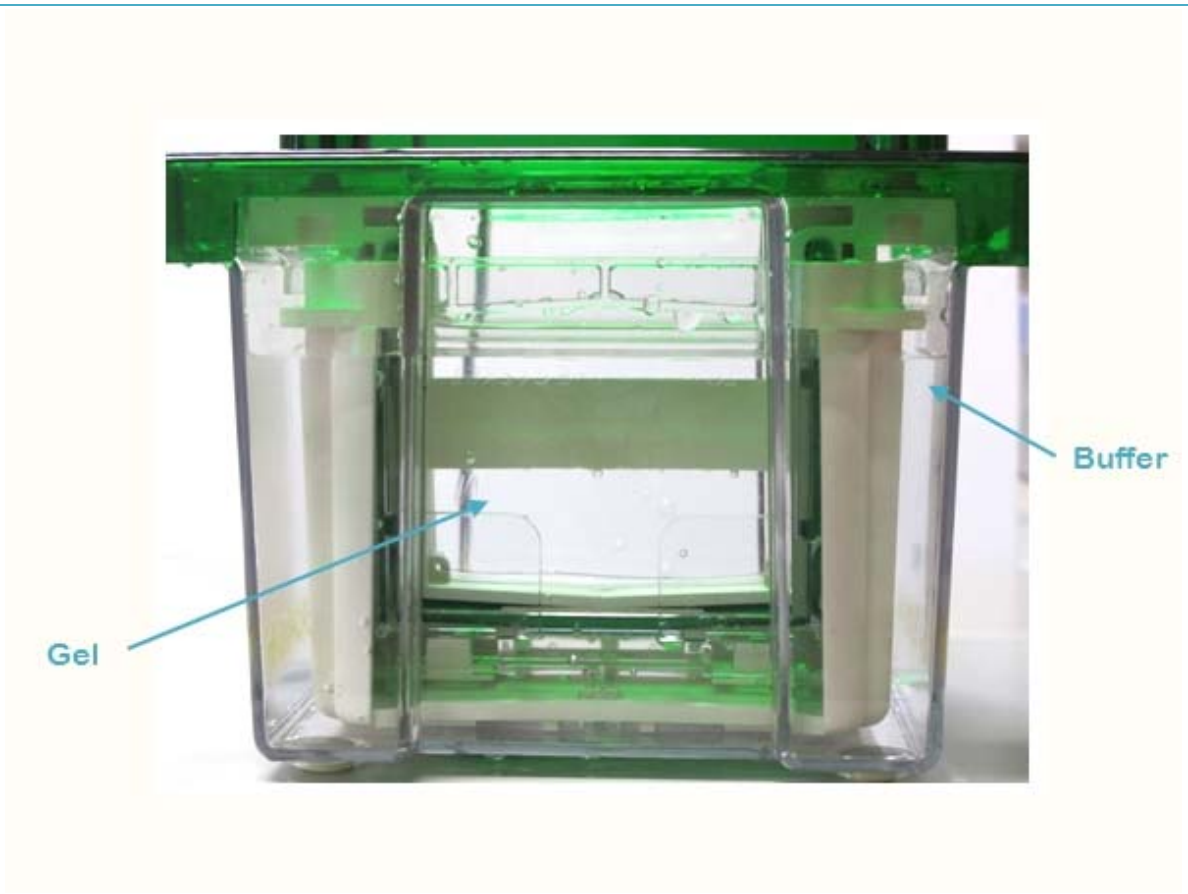
4e. Electrophoresis equipment

The equipment for gel electrophoresis allows an electric field to be maintained across the gel matrix for macromolecule separation. There are horizontal and vertical gel systems.

In our laboratory, "mini" gel apparatus is commonly used. It is a vertical system made to a smaller scale. Advantages of this apparatus is the ease in preparation and the shortened run time. In this case, the cassette (glass-gel-glass combination) is mounted on a u-shaped frame, and fully submerged in buffer for optimal cooling.

Compartments of spectrophotometer





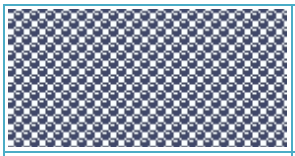
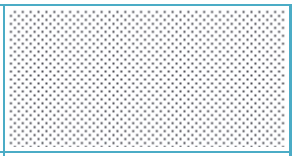
4e(i). Agarose gel electrophoresis

Video: Agarose gel electrophoresis (see website)

4e(ii). Factors affecting the rate of DNA migration in agarose gels

1. Concentration

   Agarose is usually used in concentrations of 0.5 to 2.0%. The increase of agarose concentration increases the stiffness of the gel, and decreases pore size.

	
1% agarose	2% agarose

2. Voltage The choice of power supply affects the range of control over the voltage gradient which affects the resolution and the migration rate of DNA.

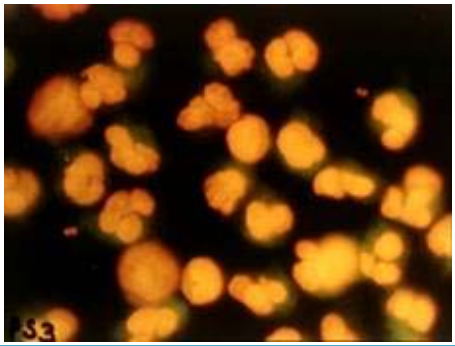
4f. Staining Techniques

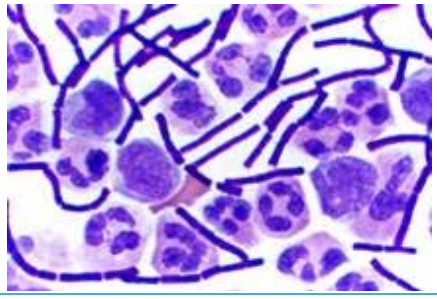
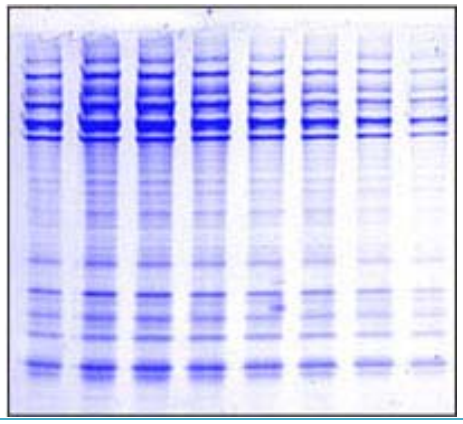
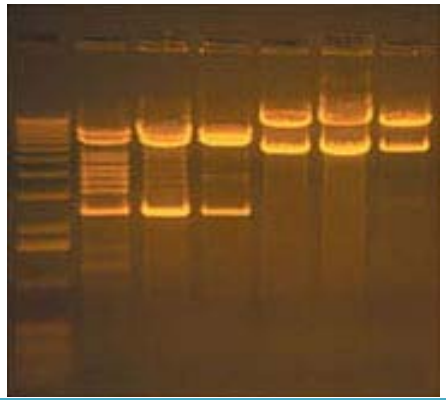
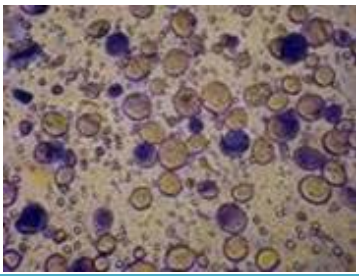
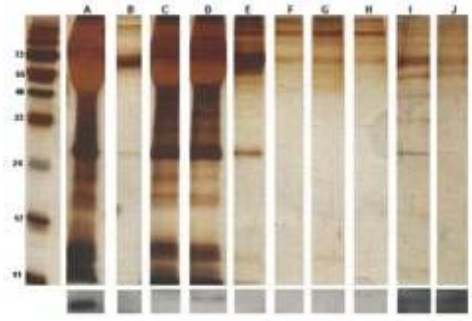
Stains and dyes are often used to highlight biological structures for examination. The staining technique enhances contrast in microscopy. In biochemistry, the use of dyes is to visualize the presence and quantity of interest molecules/cells. They are also used for tracking purposes (e.g. visualizing DNA/protein migration during gel electrophoresis).

In activity assays, artificial substrates are converted into colored products by catalytic cycles to mark the presence of specific proteins. This principle is applied as a staining technique to visualize active proteins in a gel.

Different stains or dyes used concentrates of different cell/tissue structures. This provides an advantage in specific revelation depending on the choice of stains.

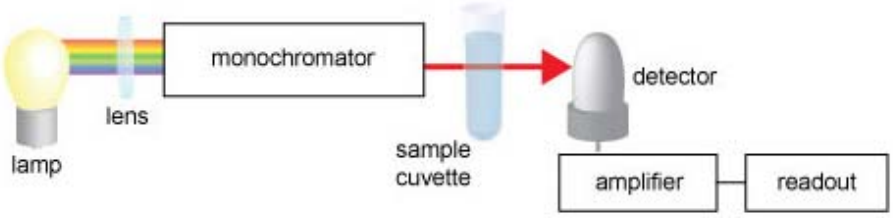
4f(i). Information of different kind of stains

Acridine Orange	
	• a cell permeable dye that is attracted to DNA/RNA • used for cell cycle determination • often combined with Ethidium Bromide to differentiate between live and apoptotic cells

Crystal Violet	
	• commonly used to stain cell wall purple • can also bind to DNA, which makes it useful for cell viability assays; however, it causes replication errors in live cells
Coomassie Blue	
	• stains proteins blue • generally applied in gel electrophoresis to track migration
Ethidium Bromide	
	• intercalates with DNA, resulting in a red-orange fluorescence • utilized to visualize gel electrophoresis results • marks cells during apoptosis since membranes are easily penetrated at this stage, and is used in conjunction with Acridine Orange
Iodine	
	• an indicator of the presence of starch • forms a starch/iodine complex that possesses an intense dark blue colour
Silver	
	• highly sensitive dye used to detect proteins • can be applied to gel electrophoresis result visualization

4g. Spectrophotometry

A spectrophotometer is an instrument used to measure absorbance, the amount of light absorbed by a sample. It utilizes a monochromator to isolate a specific wavelength of light from a light source to be directed through the sample (incident light) by a series of lens and mirrors. The light that passes through the sample (transmitted light) is measured by a detector, and an absorbance reading is made.



There are a variety of spectrophotometer models that differ in outer appearance, controls, and wavelength ranges. The common spectrophotometer used in most labs is the UV-Vis spectrophotometer, which measures absorbance in the ultra violet and visible light range.

4g(i) Introduction of Beer-Lambert Law

When the intensity of light before passing through a sample is greater than the intensity after passing through the sample, it means that the sample absorbed light. The ratio of intensity before and after the sample is referred to as *transmittance*.

$T = I / I_0$ where,

T = transmittance

I = transmitted light intensity (after sample)

I_0 = incident light Intensity (before sample)

As the transmitted light intensity (after passing through the sample) decrease, it means that the amount of light absorbed by the sample increases. The relationship between transmittance and absorbance is inverse logarithmic relationship.

$A = \log (1/T)$ where,

A = absorbance

Absorbance is dependent on the concentration of the sample, and the path length that the light travels. Both factors increase the amount of molecular interaction which affects absorbance.

The ***Beer-Lambert law*** is a combination of the equations that show the relationship between absorbance, transmittance, and concentration.

$A = \log (I_0 / I) = \varepsilon\, l\, c$ where,

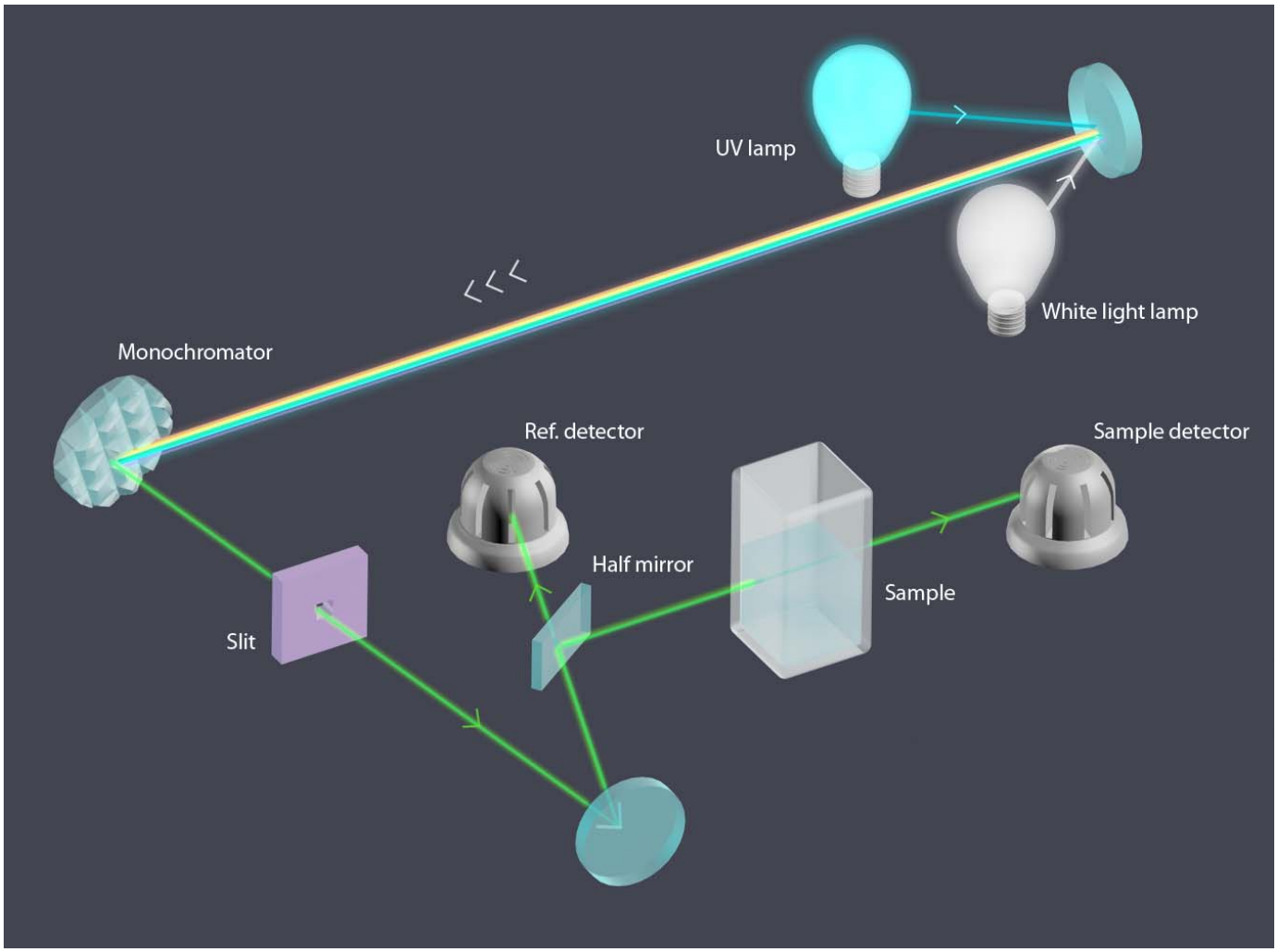
l = path length (1cm for standard cuvette)

c = concentration of sample

ε = molar absorptivity

Beer-Lambert's equation is used to determine quantities by using absorbance measurements. It is most commonly used to determine concentrations of interest substances in a sample.

4g(ii). Compartments of spectrophotometer



4g(iii). How to use spectrophotometer

Video (see website)