أسس مختبرية ت 104

Laboratory bases

Essentials of practical work — Week 1

All knowledge and theory in science have originated from practical observation and experimentation. Practical work is an important part of most courses and often accounts for a significant proportion of the assessment marks.

In order to develop a practical skills there are some important aspects we must have and consider:

1. Designing experiments
2. Observing and measuring
3. Recording data
4. Analyzing and interpreting data
5. Reporting / presenting the data

Ethical and legal aspects

When you are specialized in biological work you will need to consider the ethical and legal implications included in such work, this includes:

1. Safe working which means following a code of safe practice and avoid causing harm to yourself and others

2. Any laboratory work that involves working with humans or animals must be carefully considered.
3. Fieldwork have a legal aspect so you must consider seeking approvals or making sure there is no legislation against your work before started.

Bioethics

Bioethics is the philosophical study of ethical issues arising from recent advances in biology and medicine, it aims to provide a framework for making decisions based on specific morals principles that take into account the reason underlying different choices and the consequences of specific decisions. Bioethics can be divided into three main areas:

1. Environmental ethics: for example, the use of genetically modified organisms (GMOs) and the hunting and killing of endangered species.
2. Animal ethics: for example, factory farming, the use of transgenic animals and xenotransplantation.
3. Human ethics: dealing with medical and social issues, such as human cloning, embryo and stem cell research, genetic testing and the storage of DNA profiles on databases.

Research involving human subjects

If the work involve human participants an ethical approval must be obtained this usually require you to submit a request for ethical approval of your proposed project to your institute. There are three main aspects that should be considered when working with human participants irrespective of whether the research is **invasive** (collecting samples from mouth swabs from participants to study a particular DNA sequence) or **non- invasive** (e.g. questioning participant about their a particular social habit or study patterns of exam performance). They are:

1. **Providing information**

   All participants should be informed in writing and through oral explanation about the main aspects of the research study, typically through the use of **Participant Information Sheet** that should include the following aspects:

   I. An invitation to participate in the study.
   II. A succinct outline of the purpose of the study.
   III. A summary of what the participant will be expected to do.
   IV. A statement of any possible benefits or risks involved in participating in the study.
   V. An assurance of confidentially and anonymity in use of data from the study.
   VI. Information about the funding source.
   VII. Names and contact details of researchers/ supervisors.

2. Seeking informed consent

After reading the **Participant Information Sheet** subjects are then requested to complete and sign an **Informed Consent Form.**

3. Assuring confidentiality

All participant must be assured that their personal details will be protected through processes that ensure anonymity. This means that all data recording and storage must be de-identified (using codes for individuals rather than names).

Health and safety

Terms to understand

Hazard : the intrinsic ability of a substance or biological agent to cause harm.

Risk : the likelihood that a substance or biological agent might be harmful under specific circumstances.

e.g. one of the hazards associated with water is drowning. However, the risk of drowning in few drops of water is negligible.

There are many routes of entry of harmful substances into the body including (inhalation, ingestion, inoculation or absorption and absorption from spillage) as illustrated in figure 1.1.

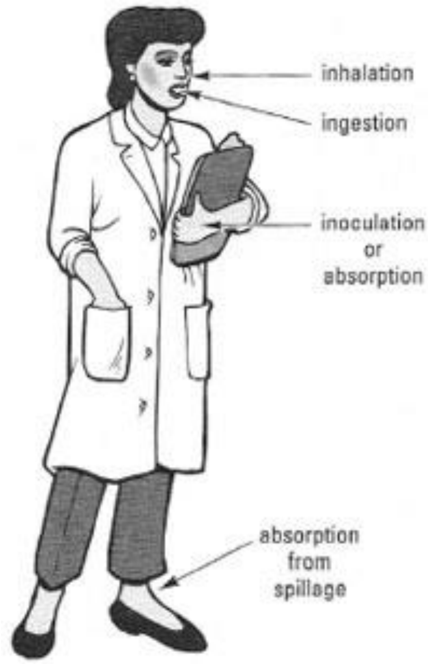

Figure 1.1. Routes of entry of harmful substances into the body

Warning labels that appears on the laboratory, the chemical containers and on tape used to label working vessels such as figure 1.2.

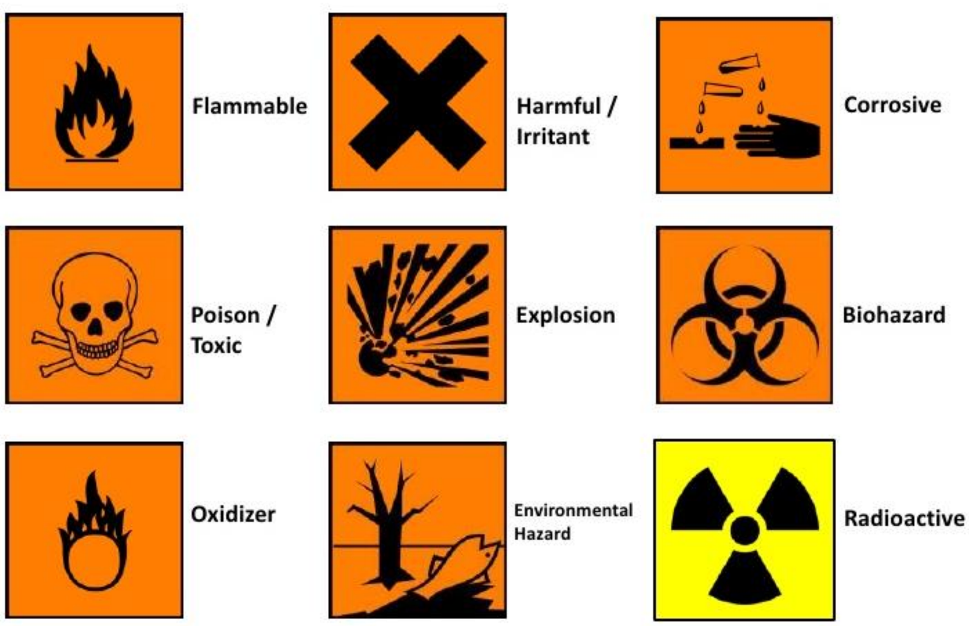

Figure 1.2. Warning labels

Types of hazards in a medical or clinical laboratory

1. Physical hazards

Physical hazards may be present in what thought of as ordinary equipment or surroundings such as:

A. Electrical equipment: all electrical supplies must be disconnected even when a minor repair is undertaken (replacing a bulb in a microscope).

B. Fire: every one works in the laboratory should know the location and the use of fire extinguisher and the fire escape route. They must pay attention to loose clothes or long hair when using Bunsen burners.

C. All equipment must be used as instructed by the manufacturer e.g. the centrifuged lid must be opened only if the centrifuged is completely stoped.

2. Chemical hazards

Chemicals may cause burns such as strong acids and bases, others may be poisonous or carcinogenic. In any case of contact with theses chemical the area must be washed immediately to prevent and damage.

3. Biological hazards

Biological specimens and reagents present a special problem because they may contain agents which are potentially harmful but they are not obvious, e.g. commercial plasmas, sera and other reagents derived from blood may be capable of transmitting hepatitis. There are many important rules to prevent biological hazards:

A. The laboratory work area always should be disinfected before and after use.

B. No eating, drinking or smoking is not allowed inside the laboratory.

C. Mouth pipetting of biological samples is not allowed.

D. Plastic gloves should be worn.

E. A laboratory coat should be worn to prevent contamination of the clothes by microorganisms.

F. Biological specimens and any contaminated articles such as lancets and tubes should be placed in a special biohazard bags or containers.

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Laboratory bases

Introduction to microscopy **Week 2**

Many features of interest in biological system are too small to be seen by the naked (unaided) eye and can only observed with microscope.

Microscope is an optical instrument that uses a lens or a combination of lenses to produce magnified images of small objects, especially of objects too small to be seen by the naked (unaided) eye. A light source is used (either by mirrors or lamps) to make it easier to see the subject matter.The word microscope is derived from the Greek mikros (small) and skopeo (look at).

Microscope versus microscopy

The microscope is the instrument that is used to magnify objects while microscopy is the use of microscope for investigation.

Who discover the microscope ?

In 1670s, A Dutchman, Anton van Leeuwenhoek, is considered the father of microscopes because of the advances he made in microscope design and use. He worked as an apprentice in a dry goods store where magnifying lenses were used to count the threads in cloth. Anton was inspired by these glasses and he taught himself new methods for grinding and polishing small lenses which magnified up to 270x.

Lens system of a microscope

The microscope consist of two lens systems :

1. The ocular lenses or eyepiece : located nearest to the eye

2. The objective lenses : located close to the object being viewed.

Forms of microscopes

1. Monocular microscope which has one ocular or eyepiece, this type is cheaper so it can be used for education purposes in high schools.
2. Binocular microscopes which is used frequently in most laboratories and has two eyepieces which reduces eyetrain. (figure 2.1)

Monocular microscope

Binocular microscope

Figure 2.1 Forms of microscopes

Types of microscopes

1. Light microscope
2. Electron microscope

1. Light microscope

Light microscope is the most common type and the principle of its operation can be illustrated in figure 2.2. as the light is emitted from a light source through a condenser to direct it towards the specimen and then through the objective lens to a prism that direct the light to the ocular lens and finally reaches the eye.

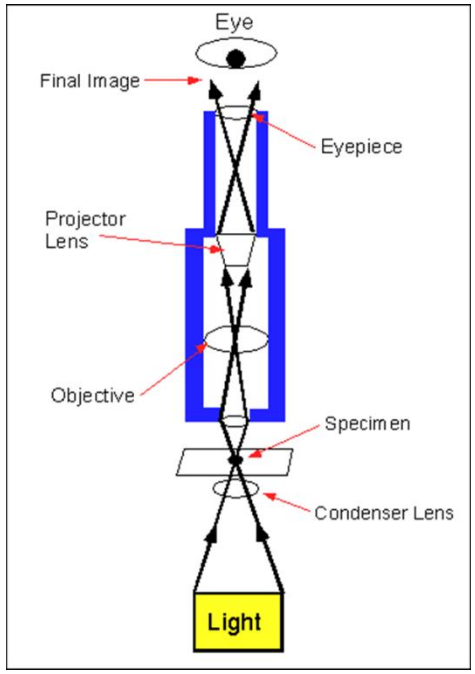

Figure 2.2 Principle of light microscope

The light microscope has many subtypes that serves different purposes in pathological analyses and in scientific research, as follow:

1. Compound light microscope (illustrated later in the lecture)
2. Dissecting microscope (illustrated later in the lecture)
3. Dark field illumination: involves a special condenser which causes reflected and diffracted light from the specimen to be seen against a dark background, this is useful to near transparent specimens and for delicate structures like flagella.
4. Ultraviolate microscopy : uses short-wavelength UV light to increase resolution and contrast of the specimens, special light sources, lenses and mountants are required in addition, filters must be used to prevent damage to users eyes.
5. Phase contrast microscopy : useful to increase contrast when viewing transparent specimens, better image than the dark field illumination microscope.

6. Nomarski or differential interference contrast (DIC) microscopy : used to optain a three-dimensional images.
7. Polarised light microscopy : used to detect the presence and orientation of optically active compounds within the specimen such as starch grains and cellulose fibers as it show them brightly against dark background.
8. Confocal microscopy : used to obtain a three dimensional view of cells.

2. Electron microscope

A microscope that uses a beam of electrons to create an image of the specimen. It is capable of much higher magnifications and has a greater resolving power than a light microscope, allowing it to see much smaller objects in finer detail.

Types of electron microscope

1. **Transmission electron microscopy (TEM or conventional transmission electron microscopy** or **CTEM**)

A microscopy technique in which a beam of electron is transmitted through a specimen to form an image. The specimen are either Ultra thin section less than 100 nm thick or a suspension on a grid. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a charge-coupled device.

2. **Scanning electron microscope** (**SEM**)

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. Figure 2.3 show the differences between TEM and SEM.

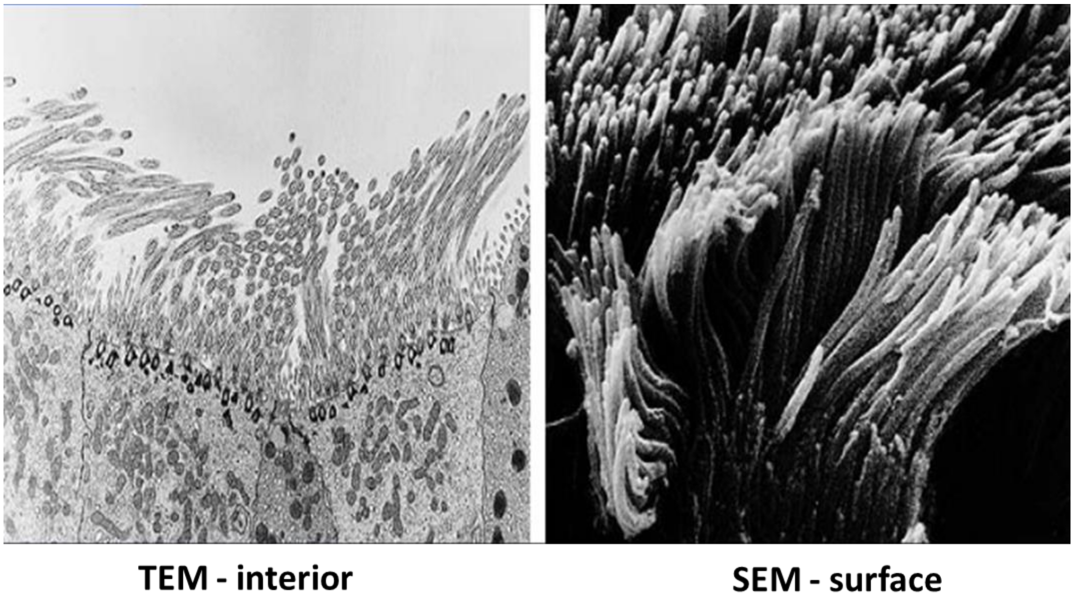

Figure 2.3 Differences in images between TEM and SEM

Parts of the light compound microscope

Magnification

The ocular or eyepieces located at the top of the microscope they are attached to a barrel or tube that connected to the microscope arm. The ocular lenses have a magnification power of 10X and sometimes they are available at 15X or 20X (figure 2.4)

Figure 2.4 The ocular lenses of the microscope

The objectives lenses are attached to a revolving nosepiece that is attached to microscope arm. Most microscopes have three objectives

- Low power : 4X or 10X
- High power : 40X , 43X or 45X
- Oil immersion objective : 95X, 97X or 100X

Each objective is marked with color coded bands as showed in figure 2.5 and each one have its own parameters as shown in figure 2.6

Figure 2.5 the color coding bands of microscope objectives

Figure 2.6 the parameters of microscope objectives

Light

The arm of the microscope connects the objectives and eyepieces to the base which supports the microscope. The base also contains the light or mirror which supplies light to the object viewed the light or mirror has a movable condenser and iris diaphragm located above it. The condenser focuses or directs the light into the object while the iris diaphragm regulates the amount of light which strikes the objects it is adjusted by a movable lever.

Focusing

The two focusing knobs located just above the base, there are two focusing knobs:

1. Coarse adjustment : used only to focus with the low power objective and it has a bigger working distance.
2. Fine adjustment : used to give a sharper image after the object is brought into view with coarse adjustment (used with the high power objective) and it has a shorter working distance.

Working distance is the space between the specimen (the slide) and the objectives (figure 2.7)

Low power , bigger working distance

High power , shorter working distance

Figure 2.7 Working distance of the microscope

Stage

The stage of the microscope is supported by the arm and is located between the nosepiece and the light source, it supports the object being viewed (slide) and has a clip to keep the slide stationary (figure 2.8). Some stages are movable by a knobs located below it. The knobs moves the stage left and right or backward and forward. Other stages are immovable (fixed) and in this case the slide must be moved manually to view different areas.

Figure 2.8 Movable stage of the microscope

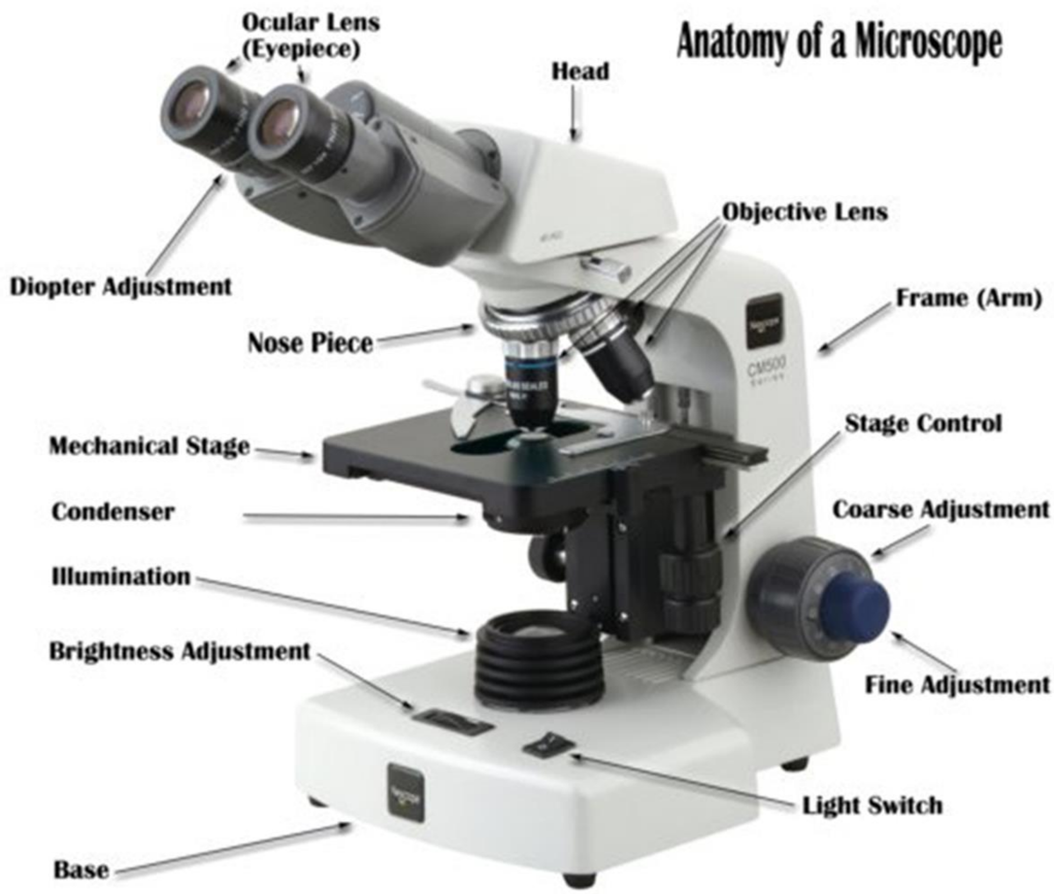

Figure 2.9 represent all parts of the light compound microscope

Adjustment of ocular for binocular microscopes

Glossary

Specimen or slide: The specimen is the object being examined. Most specimens are mounted on slides, flat rectangles of thin glass.

The specimen is placed on the glass and a cover slip is placed over the specimen. This allows the slide to be easily inserted or removed from the microscope. It also allows the specimen to be labeled, transported, and stored without damage.

Stage: The flat platform where the slide is placed.

Stage clips: Metal clips that hold the slide in place.

Stage height adjustment (Stage Control): These knobs move the stage left and right or up and down.

Aperture: The hole in the middle of the stage that allows light from the illuminator to reach the specimen.

On/off switch: This switch on the base of the microscope turns the illuminator off and on.

Illumination: The light source for a microscope. Older microscopes used mirrors to reflect light from an external source up through the bottom of the stage; however, most microscopes now use a low-voltage bulb.

Iris diaphragm: Adjusts the amount of light that reaches the specimen.

Condenser: Gathers and focuses light from the illuminator onto the specimen being viewed.

Base: The base supports the microscope and it’s where illuminator is located.