PRACTICAL 1 MICROSCOPY: PRINCIPLES, USE AND MAINTENANCE

Structure

1.1 INTRODUCTION

We start the Practicals in the Food Microbiology and Safety Course with an orientation to the microscope. This first Practical in the Manual will orient you to the different parts of the microscope, their functions and maintenance. As a student of microbiology, it is very important for us to know about the principle and working of microscopy, different types of microscopes and correct use of microscopes for microbial observation.

Objectives

After studying this practical and undertaking the exercise given herewith, you will be able to:

- identify the different parts of the microscope and recognize their functions,
- discuss the principle behind the working of a microscope,
- recognize different types of microscope, and
- use a microscope correctly in the laboratory.

1.2 THE MICROSCOPE

Microscope is a powerful and crucial basic tool in the field of microbiology. Microbiology, as we have already studied in our theory Course, is the science dealing with the organisms too small to be seen with unaided eye. The existence of microbial life to the world was introduced first by *Antony Van Leeuwenhoek* in 1673, with the help of simple, crude, self-made, single-lens microscope having a magnification of about 300. Over the years, microscopes have evolved to increase the magnification several thousand fold. Modern day microscopes are either light microscopes or electron microscopes.

Light microscopes use either visible light or ultraviolet rays to illuminate specimens. These are generally used to look at intact cells. On the other hand, electron microscopes use electron beams instead of light rays and electromagnets instead of lenses. These are generally used to look at internal structures or details of cell surface.

Let us get to know about these different types of microscope.

1.2.1 Light Microscope

Modern light microscopes are compound microscopes. Here the magnified image formed by the objective lens is further enlarged by one or more additional lenses. A variety of light microscopes are used now-a-days by microbiologists for different purposes. These are enumerated next.

(a) *Bright Field Microscopes* – Figure 1.1 illustrates the compound bright field microscope. This is the most commonly used microscope in biology and microbiology courses. It is called so because it forms a dark image against a brighter background. It contains 2 lens systems for magnification: *ocular lens* in eyepiece and the *objective lens* located in nosepiece, as can be seen in Figure 1.1. The specimen is illuminated by a light focused on it by a sub-stage lens called a *condenser*. With this microscope, specimens are made visible because of the differences in contrast that exist between them and the surrounding medium. Contrast differences arise because cells absorb or scatter light in varying degrees. Live cells, however, are difficult to observe through this microscope due to absence of contrast between specimen and the surrounding medium. It is therefore, used to observe nonviable and stained preparations where contrast is increased and variations in colour between cell structure is evident.

Figure 1.1: Compound bright field microscope

(b) *Dark Field Microscope* – In this microscope, condenser system is modified, so that only reflected and refracted light by specimen forms an

image. Here, the object is brightly illuminated while the field surrounding the object appears black. This microscope can be used to observe live unstained cells and organisms.

(c) *Phase Contrast Microscope* – Unpigmented and unstained living cells can be easily observed by phase contrast microscope. It has a special objective and a condenser that converts slight differences in refractive index and cell density into easily detected variations in light intensity and produce visible image of the structure under study. The image appears dark against a light background. Phase contrast microscopy is used widely to study microbial motility, determining the shape of living cells and detecting microbial structures like inclusion bodies and endospores etc.

(d) *Fluorescent Microscope* – In above said microscopes, image is produced from light that passes through a specimen. In fluorescent microscope, however, specimen image is formed because of the light emitted by the object itself. This microscope is used more frequently to visualize specimens that are tagged with a fluorescent dye. The source of illumination here is an ultraviolet light obtained from a high-pressure mercury vapour arc lamp or hydrogen quartz lamp. Fluorescent dye absorbs the ultraviolet light of desired wavelength and re-emits the energy in visible range. Fluorescent portion of the dye becomes visible against a black background. The fluorescent microscope has become an essential tool in medical microbiology and microbial ecology for identifying the pathogens and other microbes.

The forms of light microscopy just considered, forms two-dimensional images. Besides, certain new forms of light microscopy have been developed which gives three-dimensional images. These are differential interference contrast microscopy, atomic force microscopy and confocal scanning laser microscopy.

Having learnt about the light microscopes, let us get to know about the electron microscopes.

1.2.2 Electron Microscope

Electron microscopes have higher magnification and resolving power than light microscopes. What do we mean by magnification and resolving power? We shall get to know about this later in section 1.4. But for now, it is important to note that in electron microscope, the limit of resolution is 0.2 nm and magnification can be up to more than one million. These microscopes can be used to visualize submicroscopic cellular particles, viruses etc. Electron microscopes are of two types:

(a) *Transmission Electron Microscope* – Here, thin and dehydrated specimen is used. Electrons pass through the specimen and form image on to photographic film. These are used to observe internal cell structure.

(b) *Scanning Electron Microscope* – It can be used to observe intact cells or cell components directly. Then sections are not necessary. It is used for visualizing surface characteristics rather than intracellular structures. A narrow beam of electrons scan back and forth, producing a three-dimensional image as electrons are reflected off the specimen's surface.

Though a variety of optical instruments are available for routine laboratory work, compound bright field microscope is the one commonly found in all biological laboratories. Let us get to know the different parts of this microscope.

1.3 PARTS OF THE MICROSCOPE

Microscope, as you may have noticed in Figure 1.1 or for that matter even seen in a laboratory, is a metal body composed of a base and an arm to which remaining parts are attached. The components of the microscope are:

i) *Light Source* – It is either mirror or electric illuminator present at the base. Identify the mirror in Figure 1.1. Some microscopes have reversible mirror with one side flat and other concave. Concave side of the mirror is used for sunlight and its flat side is used for artificial light, e.g., tungsten lamp. Other microscopes have built in light source.

ii) *Abbe Condenser* – It is present beneath the stage, as shown in Figure 1.1. It collects and focuses a cone of light on the slide. Its position can be adjusted vertically to regulate the light passing on to the specimen. It also has iris diaphragm, a shutter that is also used to adjust the amount of light entering into the lens system.

iii) *Stage* – It is a fixed platform positioned about halfway up the arm and provide surface for keeping the slide. Slide is held in position either by simple slide clip or by a mechanical stage clip, as you can see in the Figure 1.1. Latter helps the viewer in moving the slide around during viewing by use of stage control knobs. Stage has an opening in the center through which light can pass from an illuminating source.

iv) *Body Tube* – Above the stage and attached to the arm is the body tube, to which eye piece(s) or ocular lens and revolving nosepiece is attached. Microscopes having eyepieces for both eyes are called *binocular microscopes*. Nosepiece contains three to five objective lenses of different magnifying power, which can be rotated at will. Microscope should be parfocal, i.e., the image should remain in focus when objectives are changed. The objectives are nearer the specimen and it produces the real magnified image of the specimen at focal plane. This image is further magnified by ocular lens to produce the final image.

From our discussion above, you would have got a clear idea about the parts of a microscope. Next, how does the microscope works? What is the principle behind the working of a microscope? Read the next section and find out.

1.4 PRINCIPLE OF MICROSCOPY

When the light passes from one medium to another, refraction occurs, i.e., the ray is bent at the interface. The direction and the magnitude of bending are determined by the refractive indices of the two media forming the interface. The refractive index is a measure of how greatly a substance slows the velocity of light when light passes from air to glass or vice versa.

When the light rays strikes the lens, a convex lens will focus these rays at specific point called focal point as illustrated in Figure 1.2. The distance between the center of the lens and the focal point is called the focal length. Convex lens act as a magnifier. It provides a clear magnifying image at a much closer range. Lens strength is related to focal length. A lens with a short focal length has a more magnification power than a lens having a longer focal length.

Figure 1.2: Lens function

Magnification means enlargement. In compound microscope, it is carried out by two-lens system – *objective lens* and *ocular lens*. Objective lens produces the real image of the specimen, which is projected up into focal plane and then magnified by the ocular lens to produce the final image, as illustrated through the light pathway of compound bright field microscope in Figure 1.3. Though magnification is important, it has limits. Unlimited enlargement by increasing magnifying power of the lenses is not possible because of the limitations of resolving power.

Microscope should not only produce the enlarged image but also the clear image. Resolution or resolving power of the lens is its ability to separate two close points as separate entities.

Figure 1.3: Light pathway of compound bright field microscope

The minimum distance (d) between two objects that reveals them as separate entities or resolving power of a lens can be calculated by Abbe's equation as:

$$d = \frac{0.5\,\lambda}{\eta sin\phi}$$

Where λ is the wavelength of the light used and $\eta sin\,\phi$ is the numerical aperture (NA) as highlighted in Figure 1.4.

Figure 1.4: Numerical Aperture of a Lens (η sin ϕ)

Numerical aperture is a characteristic of each lens and is printed on the lens. It can be defined as *a function of the diameter of the objective lens in relation to its focal length*. It depends on the refractive index (η) of the medium in which the lens works and also upon the objective itself. Theta (ϕ) is defined as *half of the angle of the cone of light entering an objective*. *Sin* ϕ cannot be more than 1. Therefore, the lens working in air with refractive index 1 can have N.A.1. η will have to be increased for increasing the resolution. This can be done by using the mineral oil. Wavelength is also an important factor in resolution. With shorter wavelength, the resolving power increases. Just like magnification, resolution also has a limit. By decreasing wavelength, resolving power of the lens cannot be increased indefinitely because –

(a) the visible portion of the electromagnetic spectrum is very narrow and borders of very short wavelengths are found in ultraviolet range of spectrum.

(b) This relationship of Resolving Power with λ is valid only when light rays are parallel. As such 'Resolving Power' is also dependent on another factor, i.e., *refractive index*. When the light passes from air to glass slide, and from glass slide to air, there is a loss of light due to bending of rays as can be seen in Figure 1.5. This reduces the numerical aperture and thus the resolving power of the lens. This loss in light can be compensated by using mineral oil in between glass slide and objective lens. Mineral oil is a colourless liquid having same refractive index as glass. This does decrease the bending of ray, as shown in Figure 1.5, so that more light rays enter the objective lens thus increasing the resolving power. Proper specimen illumination is also important in determining resolution.

Figure 1.5: Immersion lens operating in air and immersion oil

Resolving Power of a microscope depends upon the numerical aperture of both condenser and objective, i.e.,

$$d_{\text{microscope}} = \frac{\lambda}{NA_{\text{Objective}} + NA_{\text{Condenser}}}$$

It was found that limit of resolving power of a microscope at best be about 0.2 μm and limit of magnification is about 1000 times the numerical aperture of the objective lens.

With an understanding about the principle of the microscope, we are now ready to use the microscope.

To ensure good results and observations, it is important that we use and take good care of our microscope. This aspect is highlighted in the next section.

1.5 USE AND CARE OF MICROSCOPE

Proper care and maintenance of microscope is needed. Following points should be kept in mind while handling the microscope:

(i) Instrument should be kept in special cabinets while not in use.

(ii) Microscope should be held firmly by holding the arm with right hand and base with left arm.

(iii) All the lens systems should be cleaned with lens tissue to remove dust, oil, etc., which may decrease the efficiency of the microscope. Blotting paper, cloth or towel should not be used for cleaning.

(iv) If the lens is sticky or oily, the lens can be cleaned with xylol followed by 95% alcohol. The lens is wiped dry with lens paper. This should be performed only if necessary as consistent use of xylol may loosen the lens.

Now answer the questions given in the next section and judge your understanding on this topic.

1.6 REVIEW QUESTIONS

1. Name different types of microscopes used in microbiology.

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2. Define the following terms:

   Magnification .....

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   Resolution .....

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   Numerical aperture .....

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   Parfocal .....

3. What is the use of immersion oil? How it increases the magnification?

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4. Label the diagram of compound light microscope.

5. What is the function of following parts of microscope?

   - Iris diaphragm .....
   - Condenser .....
   - Coarse Adjustment Knob .....
   - Fine Adjustment Knob .....
   - Stage Clip .....
   - Mirror .....
   - Objective Lens .....
   - Ocular Lens .....

6. Why the body tube of the microscope should not be lowered while looking through the ocular lens?

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7. What precautions should be taken while working with light microscope?

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Now conduct the following exercise.

EXERCISE 1

USE OF LIGHT MICROSCOPE

Date : .....

Aim : To study different parts of light microscope.

Requirement : Compound light microscope

Slide of *Bacillus* sp. and *Aspergillus* sp. or any other bacterial and fungal specimen.

Before you begin this exercise, study different parts of the microscope, as described in section 1.4.

Procedure:

Now carry out the exercise following the steps enumerated herewith.

1. Place the microscopic slide with any specimen on the stage with the help of stage clips. Move the slide to keep the specimen above the opening in the stage.

2. Adjust the light with the help of mirror and by adjusting the height of the condenser with the condenser knob. Iris diaphragm can be opened or closed to regulate the amount of light entering the condenser.

3. Rotate the scanning or low power lens into position. Lower the body tube to its lowest position and then raised slowly to bring specimen into focus with the coarse adjustment knob. Sharp focusing can be done with fine-adjustment knob.

4. Rotate the nosepiece to bring high power or oil immersion lens into position. For viewing fungal slide (i.e., *Aspergillus*), use high power lens (40x) and for bacteria (i.e., *Bacillus*) use oil immersion lens (100x). Because of parfocal nature of microscopes, minor focus adjustment can be done by using fine-adjustment knob. As the magnification of lens increases, the distance between the objective lens and slide, called working distance decreases. For oil immersion lens, a drop of oil is placed over the specimen before being used.

5. Scan the slide without the application of additional immersion oil.

6. After using microscope, clear all lenses with dry lens paper and use xylol to remove oil from stage.

7. Lower the body tube completely and place the low power objective in position.

8. Put the microscope back in cabinet.

Precautions:

1. Clean the lens system properly.

2. Use xylol only when necessary.

3. Never lower the body tube while looking through the ocular lens.

4. Use only a drop of immersion oil for oil immersion lens.

5. Focus the slide first at low-power lens using coarse adjustment knob. Then change to other lens in position and focusing should be done only with fine adjustment knob.

6. High power objective lens should not touch the oil drop on slide.

Observations and Results:

Record your observations in the space provided herewith. Comment on the process or adjustments (if any) you had to make in observing the slide. Give description of what you see in the slide.

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Submit your responses for evaluation.

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Counsellor Signature

LIST OF EXERCISES

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