Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY
LABORATORY

EXPERIMENT # 1

AIM

To learn the principles of the microscope

LEARNING OBJECTIVES

a) Learn the names of the major parts of the light microscope and how they function.
b) Understand the importance of resolution and learn what affects the resolving power of a microscope.
c) Learn how to check your microscope before you use it and before you put it away.
d) Draw the microscope, label all of the parts. Briefly note various functions

What should you know by the end of this class?

1. Learn the principles of optical Microscopy and be able to identify each part of an optical microscope along with its function.
2. Understand the basic idea behind other types of microscopy (Dark field, DIC, Fluorescence, Confocal, Electron)
3. How to use a microscope (focusing, aperture adjustment etc.)
4. Precautions and instrument care

Using the Microscope

a. **Lower** the Slide platform and place the slide in position using the **slide clips.**
b. Use the **stage adjustment knobs** to position the slide such that the edge of a stain (where the dye seems to be brightest) is just above the **condenser** opening.
c. Switch on the lamp and place it at an intermediate brightness. Also use the condenser slider to **full open position**.
d. Position the 4X lens at the sampling position and lower it as much as possible **without** touching the slide, then while looking through the eyepiece, slowly raise the lens using the **coarse adjustment knob**. When you see something, use the fine adjustment knob to bring it into clear focus. The fine adjustment knob only works over a small range, so bring it as near as possible to focus using the coarse adjustment knob, then us the fine adjustment knob.
e. Now that you have brought the right layer into focus, you can use the **stage adjustment knobs** to move the slide slowly and identify a region of good density of cells (At this stage, you cannot identify anything, but you can get identify the cells from the colour of the dye). If everything looks fine, then move on to the next step.
f. **Without moving the slide (don't touch the stage adjustment knobs), use the coarse adjustment knob to lower the stage**. Put the 10X in the sampling position, and repeat the above procedure for focusing. Don't move the stage using the stage adjustment knobs until you have brought the slide into focus. After you have obtained the focus, then you can move the slide to find the best spot (most number of cells/best looking ones etc).
g. Do the same with the 40X magnification.
h. If required, then cover the slide with a cover slip and place a droplet or 2 of **oil** on the cover slip. Lower the 100X such that it is in contact with the oil and the **oil forms a layer between the objective lens and the coverslip**. This increases the resolution of the microscope allowing you to use the 100X. The same procedure for focusing may be followed as above, here the lower most position is when the lens just touches the cover slip.

Precautions

1. To move the microscope, hold it by the arm, don't ever hold it using the eyepiece or lens holder or the stage.
2. Switch off the lamp when not in use, since the lamps have a short lifetime.
3. Don't move the coarse adjustment knobs too fast, the lens might crash into the slide!!!
4. While changing the slide, always lower the stage.
5. Don't ever touch the lens bottom directly