Laboratory 1: Microscopes and streaking technique

Microscopes

Light microscope.

The light microscope uses visible light to illuminate specimens. In microscopy, two factors contribute to our ability to see and distinguish structures: MAGNIFICATION and RESOLUTION. Magnification is a specific property of the lenses, while resolution depends on specific properties of light.
Resolution is the ability to distinguish two adjacent objects as distinct and separate. The limits of resolution for a light microscope are 0.2 µm; this means that objects smaller than 0.2 µm cannot be distinguished and that it is not possible to distinguish two objects as separate if the distance between them is smaller than 0.2 µm.

The optical components of a light microscope consist of **three** systems of lenses: the **CONDENSER**, the **OBJECTIVE** and the **EYE PIECE (OR OCULAR LENS)**.

- The condenser collects and focuses light producing a cone of light that illuminates the object to be observed.
- The objective lenses enlarge and project the image in the direction of the eye piece.
- The eye piece (or ocular lens) further magnifies the image of the object and projects it onto the observer's retina.

The total magnification is the product of the magnifications of the objective and ocular lenses (generally the maximum magnification in a light microscope is 1500 times, which is the product of a 10X magnification of the eye piece by a 150X magnification of the objective lens).

Resolution is a function of the light wavelength and of the ability of the objective lens to gather light. The ability of gathering light is indicated on the objective by a number that represents its NUMERICAL APERTURE (NA).

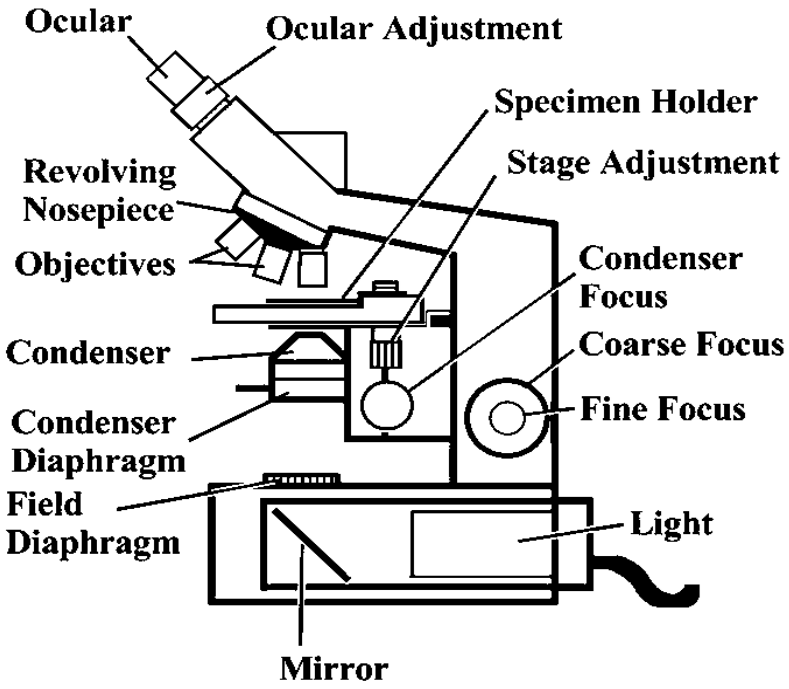
Resolution is expressed by the equation:

$R = 0.5\lambda / NA$

Best resolution is obtained with blue light and an objective with high numerical aperture.

Light microscopes have an OIL IMMERSION OBJECTIVE LENS that increases the ability of the objective to gather light, hence increasing the resolution.
In general there is correspondence between the magnification of a lens and its numerical aperture: the higher the magnification, the higher the NA.

In bright field microscopy the specimens are visualized because of the differences in contrast between them and the surrounding medium. To improve contrast between cells and their medium it is possible to use specific stains that colour the cells and make them more visible.



After putting the slide on the stage of the microscope, the light is turned on and the specimen must be observed and put into focus with the objective that has the smaller magnification. This is done by using first the coarse focus knob and only when the image is roughly focused the fine focus knob. Once the specimen is in focus we can change objective to one with higher magnification. **Once the next objective is in place we will need to use only the fine focus knob to visualize the specimen.**

TO MOVE INTO OIL IMMERSION, 100X MAGNIFICATION:

1. Do NOT MOVE the focus knobs or the stage knobs. Swing the **40X objective (high dry)** out of the way. Place a single drop of immersion oil on the slide right over where the light is coming through the stage, and rotate the **100X objective (oil immersion)** into place. The lens will actually GO INTO THE OIL DROP.
2. Now look through the oculars, increasing your light with your iris diaphragm lever. Your object should still be in the field of vision, probably out of focus. Use the fine adjustment knob to focus clearly.
3. Once you have gone into oil immersion, do NOT GO BACK TO THE 40X OBJECTIVE. The objective will get oil on it, and you will have to really clean it to get the oil off. The 10X can be returned to, since the lens should not touch the slide anyway.
4. **Once through with the microscope, *use the lens paper to wipe the oil* from the 100X objective lens.**

See Appendix 1 for the description of other types of microscopes.

LABORATORY EXERCISE:

Learn how to focus and obtain a clear image on the microscope.

Prepared slides (Letter e)

Wet mount from mouth microbiota

Observe how the picture changes with changes in magnification.

Table 1.2 Troubleshooting the Microscope

Problem	*Possible Corrections*
Insufficient light passing through ocular	Make certain the power cord is out of the way
	Raise the condenser
	Open the iris diaphragm
	Check the objective: is it locked in place?
Particles of dust or lint interfering with view of visual field	Wipe the ocular and the objective (*gently*) with clean lens paper
Moving particles in hazy visual field	Caused by bubbles in immersion oil; check the objective
	Make certain that the oil-immersion lens is in use, not the high-dry objective with oil on the slide
	Make certain the oil-immersion lens is in full contact with the oil.

Streaking

In natural environments, microorganisms usually exist as mixed populations. However, if we are to study, characterize, and identify microorganisms, we must have the organisms in the form of a pure culture. A **pure culture** is one in which all organisms are descendants of the same organism. In working with microorganisms, we must also have a sterile nutrient-containing-medium in which to grow the organisms. Anything in or on which we grow a microorganism is termed a **medium**. A **sterile** medium is one which is free of all life forms. It is usually sterilized by heating it to a temperature at which all contaminating microorganisms are destroyed.

There are several ways to grow and stock microorganisms, and which method we choose depends on various factors.
When cultures need to be kept for a not too long time (days or weeks) the best method is to grow them on a solid medium. Solid media contain **AGAR**, a complex polysaccharide that is used as a thickener in foods such as jelly or ice cream. Agar has some very important properties that make it valuable for microbiology:

1. Few microorganisms can degrade agar so the medium is an inert substance and remains solid.
2. Agar liquefies at about 100ºC (the boiling point of water) and at sea level remains liquid until the temperature drops to about 40ºC. Once the agar has solidified it can be incubated at temperatures up to 100ºC before it liquefies again.

The liquefied agar can be poured in Petri dishes or in test tubes. Petri dishes are shallow dishes with a lid that nests over the bottom to prevent contamination. Once the agar is solid it is possible to grow microorganisms on its surface.

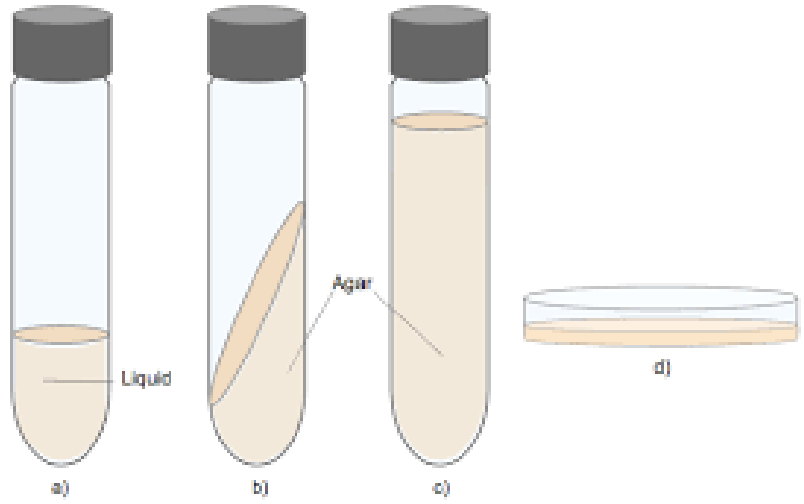
Slant tubes are tubes containing a nutrient medium plus agar. The medium has been allowed to solidify at an angle in order to get a flat inoculating surface.

Stab tubes (deeps) are tubes of hardened agar medium which are inoculated by "stabbing" the inoculum into the agar.

Agar plates are sterile Petri dishes that are aseptically filled with a melted sterile agar medium and allowed to solidify. Plates are much less confining than slants and stabs and are commonly used in the culturing, separating, and counting of microorganisms. Single colonies of microorganisms on agar plates can be described according to their shape, consistency, colour etc.

When cultures are going to be used within a short period of time it is possible to grow them and keep them in a liquid medium that is poured either in a test tube or a flask. Often microorganisms grow faster into liquid media and can reach a considerable density in a relatively short time. **Broth tubes** are tubes containing a liquid medium. A typical nutrient containing broth medium contains substrates for microbial growth, sodium chloride, and water. After incubation, **growth** (development of many cells from a few cells) may be observed as one or a combination of three forms:

a. **Pellicle**: A mass of organisms floating on top of the broth.
b. **Turbidity**: The organisms appear as a general cloudiness throughout the broth.
c. **Sediment**: A mass of organisms appears as a deposit at the bottom of the tube.



Petri dishes and agar tubes are the methods of choice for stocking microorganisms. The main advantage of using them is that they can be easily stacked and take up much less space compared to liquid cultures. Also they are less prone to contamination in comparison to liquid cultures.

Whichever is the method that we choose for our cultures, after the microorganisms are allowed to grow and reach the desired density we need to stop the growth and keep them under conditions that do not kill them. The most common way to do this is by transferring the cultures in a refrigerator and keep them at a temperature of 4ºC which slows the growth down to almost zero.

For very long term keeping (years) microorganisms can be transferred to small test tubes (1.5 ml Eppendorf tubes) in a medium that contains glycerol and stored at -20ºC.

In working with microorganisms, we must have a method of transferring growing organisms (called the **inoculum**) from a pure culture to a sterile medium without introducing any unwanted outside contaminants. This method of preventing unwanted microorganisms from gaining access is termed **aseptic technique** and the instrument most commonly used is the inoculating loop.

ASEPTIC TECHNIQUE

To carry out this technique you need to follow a specific procedure:

1. Sterilize the inoculating loop.
The inoculating loop is sterilized by passing it at an angle through the flame of a gas burner until the entire length of the wire becomes orange from the heat. In this way all contaminants on the wire are incinerated. Never lay the loop down once it is sterilized or it may again become contaminated. Allow the loop to cool a few seconds to avoid killing the inoculum.

2. Remove the inoculum.

a. Removing inoculum from a broth culture (organisms growing in a liquid medium):

- Hold the culture tube in one hand and, in your other hand; hold the sterilized inoculating loop as if it were a pencil.
- Remove the cap of the pure culture tube with the little finger of your loop hand. **Never lay the cap down or it may become contaminated.**
- Very briefly flame the lip of the culture tube. This creates a convection current which forces air out of the tube and prevents airborne contaminants from entering the tube. The heat of the gas burner also causes the air around your work area to rise, and this also reduces the chance of airborne microorganisms contaminating your cultures.
- Keeping the culture tube at an angle, insert the inoculating loop and remove a loopful of inoculum.
- Again flame the lip of the culture tube.
- Replace the cap.

b. Removing inoculum from a plate culture (organisms growing on an agar surface in a Petri plate):

- Sterilize the inoculating loop in the flame of a gas burner.
- Lift the bottom of the culture plate slightly and stab the loop into the agar away from any growth to cool the loop.
- Scrape off a small amount of the organisms and close the lid.

3. Transfer the Inoculum to the Sterile Medium.

a. Transferring the inoculum into a broth tube:

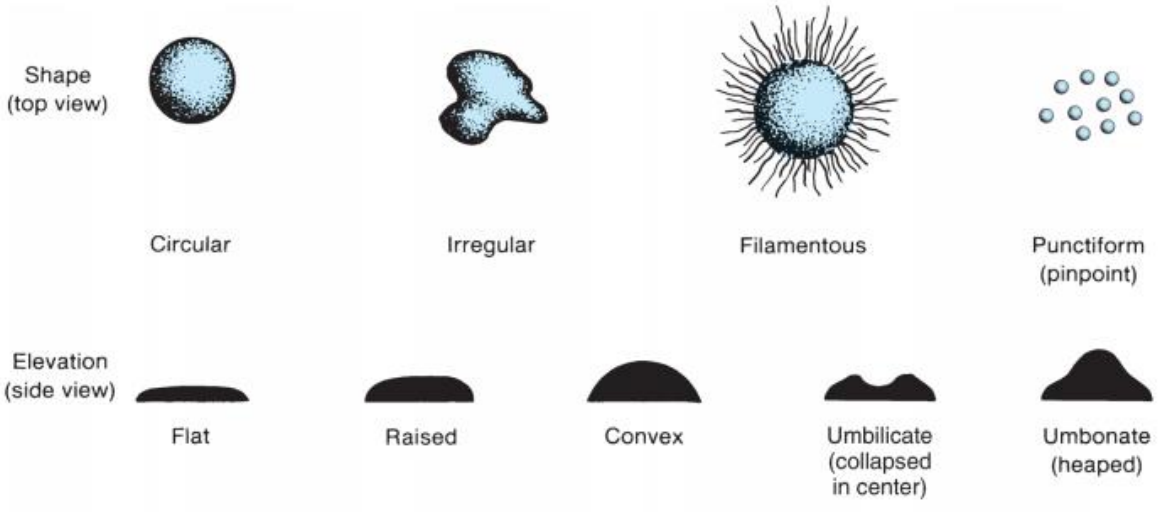
- Pick up the sterile broth tube and remove the cap with the little finger of your loop hand. Do not set the cap down.
- Briefly flame the lip of the broth tube.

- Place the loopful of inoculum into the broth, and withdraw the loop. **Do not lay the loop down!**
- Again flame the lip of the tube.
- Replace the cap.
- Re-sterilize the loop by placing it in the flame until it is orange. Now you may lay the loop down until it is needed again.

b. Transferring the inoculum into a Petri plate:

- Lift the edge of the lid just enough to insert the loop.
- Streak the loop across the surface of the agar medium using either of the patterns shown in the next page. These streaking patterns allow you to obtain single isolated bacterial colonies originating from a single bacterium. In order to avoid digging into the agar as you streak the loop over the top of the agar you must keep the loop parallel to the agar surface. Always start streaking at the "12:00 position" of the plate and streak side-to-side as you pull the loop toward you. As you follow either pattern, each time you flame or cool the loop between sectors, rotate the plate counter clockwise so you are always working in the "12:00 position" of the plate.
- Remove the loop and close the lid.
- Resterilize the inoculating loop.

Colonies growing on agar plates can show different shapes and forms.



Laboratory Exercise:

Materials:

a. **LB agar plates**
b. **Inoculating loops, wire loops**
c. **Bunsen burner**
d. **Stock culture of bacteria on plates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis***
e. **LB agar slant and straight tubes**

f. Lens paper
g. Fat solvent
h. Pipettes, pipette aid
i. Matches

Procedure:

1. Prepare streak plates from liquid cultures of: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis*. Incubate the plates upside down for 24 hours at 37ºC, then remove from the incubator and place in the refrigerator.
2. Prepare liquid cultures from plates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis*. Incubate for 24 hours at 37ºC, then remove from the incubator and place in the refrigerator.
3. Inoculate slant tubes and agar tubes with *E. coli* and *Micrococcus luteus* using both the inoculating loop and wire.
4. Record your findings.