#### Introduction to the Microscope

#### Microscope History

- **Circa 1000AD** The first vision aid was invented (inventor unknown) called a reading stone. It was a glass sphere that magnified when laid on top of reading materials.
- Circa 1284 Italian, Salvino D'Armate is credited with inventing the first wearable eye glasses.
- **1590** Two Dutch eye glass makers, Zaccharias Janssen and son Hans Janssen experimented with multiple lenses placed in a tube. The Janssens observed that viewed objects in front of the tube appeared greatly enlarged, creating both the forerunner of the compound microscope and the telescope.
- **1665** English physicist, Robert Hooke looked at a sliver of cork through a microscope lens and noticed some "pores" or "cells" in it.
- **1674** Anton van Leeuwenhoek built a simple microscope with only one lens to examine blood, yeast, insects and many other tiny objects. Leeuwenhoek was the first person to describe bacteria, and he invented new methods for grinding and polishing microscope lenses that allowed for curvatures providing magnifications of up to 270 diameters, the best available lenses at that time.
- **18th century** Technical innovations improved microscopes, leading to microscopy becoming popular among scientists. Lenses combining two types of glass reduced the "chromatic effect" the disturbing halos resulting from differences in refraction of light.
- **1830** Joseph Jackson Lister reduces spherical aberration or the "chromatic effect" by showing that several weak lenses used together at certain distances gave good magnification without blurring the image. This was the prototype for the compound microscope.
- **1872** Ernst Abbe, then research director of the Zeiss Optical Works, wrote a mathematical formula called the "Abbe Sine Condition". His formula provided calculations that allowed for the maximum resolution in microscopes possible.
- **1903** Richard Zsigmondy developed the ultramicroscope that could study objects below the wavelength of light. He won the Nobel Prize in Chemistry in 1925.
- **1932** Frits Zernike invented the phase-contrast microscope that allowed for the study of colorless and transparent biological materials for which he won the Nobel Prize in Physics in 1953.
- **1931** Ernst Ruska co-invented the electron microscope for which he won the Nobel Prize in Physics in 1986. An electron microscope depends on electrons rather than light to view an object, electrons are speeded up in a vacuum until their wavelength is extremely short, only one hundred-thousandth that of white light. Electron microscopes make it possible to view objects as small as the diameter of an atom.
- **1981** Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level. Binnig and Rohrer won the Nobel Prize in Physics in 1986. The powerful scanning tunneling microscope is the strongest microscope to date.

### 1. Compound Microscope

Compound microscopes are light illuminated. The image seen with this type of microscope is two dimensional. This microscope is the most commonly used. You can view individual cells, even living ones. It has high magnification. However, it has a low resolution.

#### 2. Dissection Microscope

A dissection microscope is light illuminated. The image that appears is three dimensional. It is used for dissection to get a better look at the larger specimen. You cannot see individual cells because it has a low magnification. (also called stereo microscope)

3. Scanning Electron Microscope

SEM use electron illumination. The image is seen in 3-D. It has high magnification and high resolution. The specimen is coated in gold and the electrons bounce off to give you and exterior view of the specimen. The pictures are in black and white.

4. Transmission Electron Microscope

TEM is electron illuminated. This gives a 2-D view. Thin slices of specimen are obtained. The electron beams pass through this. It has high magnification and high resolution.

### Microscope Care

- Always carry with 2 hands
- Never touch the lenses with your fingers.
- Only use lens paper for cleaning
- Do not force knobs
- Keep objects clear of desk and cords
- When you are finished with your "scope", rotate the nosepiece so that it's on the low power objective, roll the stage down to lowest level, rubber band the cord, then replace the dust cover.

**Ocular lens**: magnifies; where you look through to see the image of your specimen (They are usually 10X or 15X power. Our microscopes have an ocular lens power of 10x.)

Arm: supports the tube and connects it to the base

Stage: the flat platform where you place your slides

Coarse adjustment knob: moves stage (or body tube) up and down

Fine adjustment knob: small, round knob on the side of the microscope used to fine-tune the focus of your specimen after using the coarse adjustment knob

Base: the bottom of the microscope, used for support

Body tube: connects the eyepiece to the objective lenses

**Revolving nosepiece**: the part that holds two or more objective lenses and can be rotated to easily change power

# **Objective lenses**: adds to the magnification

Usually you will find 3 or 4 objective lenses on a

microscope. They almost always consist of 4X, 10X, 40X and 100X powers.

When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X, 400X and 1000X. The shortest lens is the lowest power; the longest one is the lens with the greatest power. Lenses are color coded. The high power objective lenses are retractable (i.e. 40XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide.

**Stage clips**: Stage clips hold the slides in place. If your microscope has a **mechanical stage**, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.

**Diaphragm**: controls the amount of light going through the specimen. Many microscopes have a rotating disk under the stage. This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide. There is no set rule regarding which setting to use for a particular power. Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

Light: makes the specimen easier to see



The proper way to focus a microscope is to start with the lowest power objective lens first and while looking from the side, crank the lens down as close to the specimen as possible without touching it. Now, look through the eyepiece lens and **focus upward only** until the image is sharp. If you can't get it in focus, repeat the process again.

Once the image is sharp with the low power lens, you should be able to simply click in the next power lens and do minor adjustments with the focus knob. If your microscope has a fine focus adjustment, turning it a bit should be all that's necessary. Continue with subsequent objective lenses and fine focus each time.

# Using High Power

**Rotate to 40x objective**, locate desired portion of specimen in the center of the field. Refocus very carefully so that the specimen is focused as sharply as possible. (*Do not alter focus for the Following steps*)

Partially rotate so that 40x and 100x objectives straddle the specimen.

Place a small drop of oil on the slide in the center of the lighted area. (Take care not to dribble on the stage.) Put the small drop of oil directly over the area of the specimen to be examined.

Rotate so that the **100x oil immersion objective touches the oil** and clicks into place.

Focus **only** with fine focus. Hopefully, the specimen will come into focus easily. Do not change focus dramatically.

**Clean up!:** When you have finished for the day, wipe the 100x oil immersion objective carefully with lens paper to remove all oil. Wipe oil from the slide thoroughly with a Kimwipe. Cleanse stage should any oil have spilled on it. Recap the immersion oil container securely, replace in drawer.