**Light Microscopy**

Light microscopy is based on the interaction of light and tissue components and can be used to study tissue features.

# Upon completion of this lecture, the student should be able to:

## Explain the basic principles of each type of light microscopes.

## Explain the additional materials required for the examination with each microscope.

## Define: total magnification, resolving power, quality of the image, optical section.

## Choose the appropriate microscope type according to the specimen type and aim of the examination.

**Bright-Field Microscopy**

With the **bright-field microscope** (e.g. Compound Microscope), widely used by students of histology, stained preparations are examined by means of ordinary light that passes through the specimen. The microscope is composed of **optical** and parts **mechanical** (Figure 1–3). The optical components consist of three systems of lenses. 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 illuminated image of the object in the direction of the eyepiece. The **eyepiece** or ocular lens further magnifies this image and projects it onto the viewer's retina, photographic film, or (to obtain a digital image) a detector such as a charge-coupled device (CCD) camera. The **total magnification** is obtained by multiplying the magnifying power of the objective and ocular lenses.

The critical factor in obtaining a detailed image with a light microscope is its **resolving power**, defined as the smallest distance between two particles at which they can be seen as separate objects. The maximal resolving power of the light microscope is approximately 0.2um; this power permits good images magnified 1000–1500 times.

Objects smaller or thinner than 0.2um (such as a ribosome, a membrane, or a filament of actin) cannot be distinguished with this instrument. Likewise, two objects such as mitochondria will be seen as only one object if they are separated by less than 0.2 um. {*The resolving power of a microscope depends mainly on the quality of its objective lens*}. The **quality** of the image (its clarity and richness of detail) depends on the microscope's resolving power. {*The magnification is of value only when accompanied by high resolution*}*.* The eyepiece lens enlarges only the image obtained by the objective; it does not improve resolution. For this reason, when comparing objectives of different magnifications, *those that provide higher magnification also have higher resolving power.*

Video cameras highly sensitive to light enhance the power of the bright-field and other light microscopes and allow the capture of digitized images suitable for computerized image analysis and printing. The frontiers of light microscopy have been redefined by the use of such cameras. With digital cameras and image-enhancement programs (to enhance contrast, for example), objects that may not be visible when viewed directly through the ocular may be made visible in the video screen. These video systems are also useful for studying living cells for long periods of time, because they use low-intensity light and thus avoid the cellular damage from heat that can result from intense illumination. Moreover, software developed for image analysis *allows rapid measurements and quantitative study of microscopic structures*.

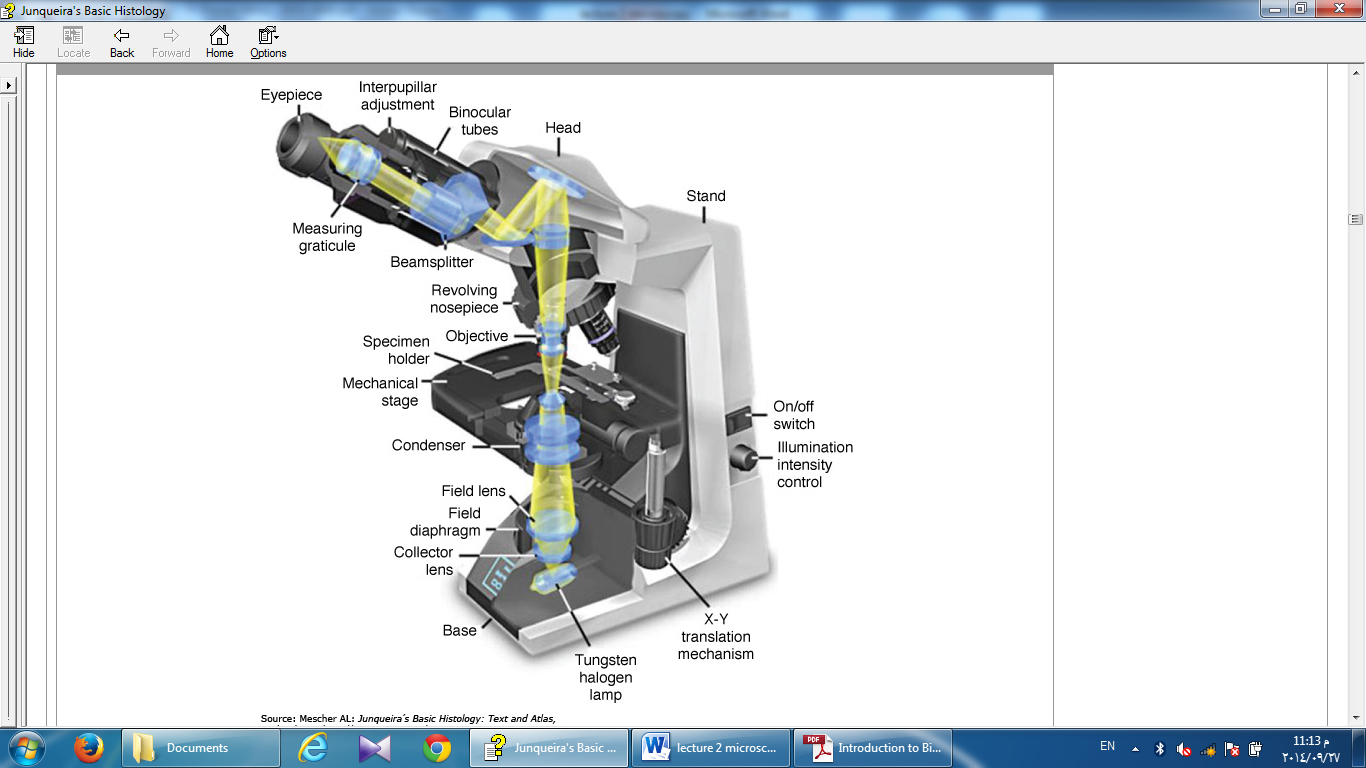


Figure 1: Bright field microscope