

Lab instruments ...Lecture-1

The light microscope

جامعة المعقل
الكلية التقنية الصحية
قسم تقنيات التحليلات المرضية

Microscope

- An optical instrument that uses a lens or a combination of lenses to produce magnified images of small objects, especially of objects too small to be seen by the unaided eye.

- A device that transmits light through several lenses to produce an enlarged image of a microscopic specimen.
- Modern compound light microscopes, under optimal conditions, can magnify an object from 1000X to 2000X (times) the specimens original diameter.

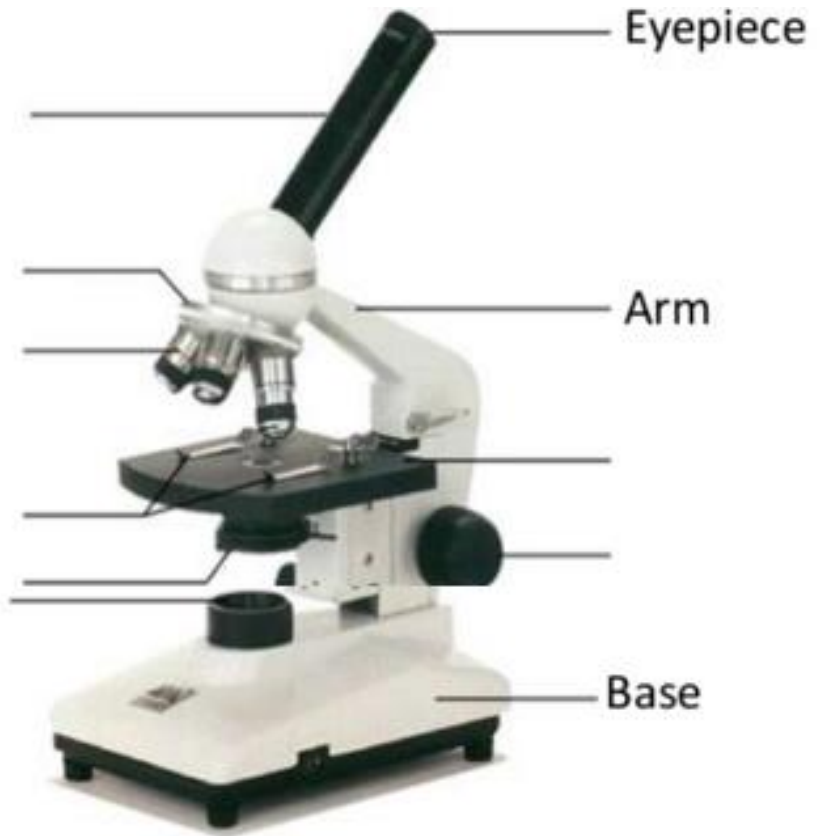


Microscope Parts and Functions

- Arm- Supports the tube and connects it to the base
- Base- The bottom of the microscope, used for support
- Eyepiece- Where you look to see the image of your specimen.

Bright Field Microscopy

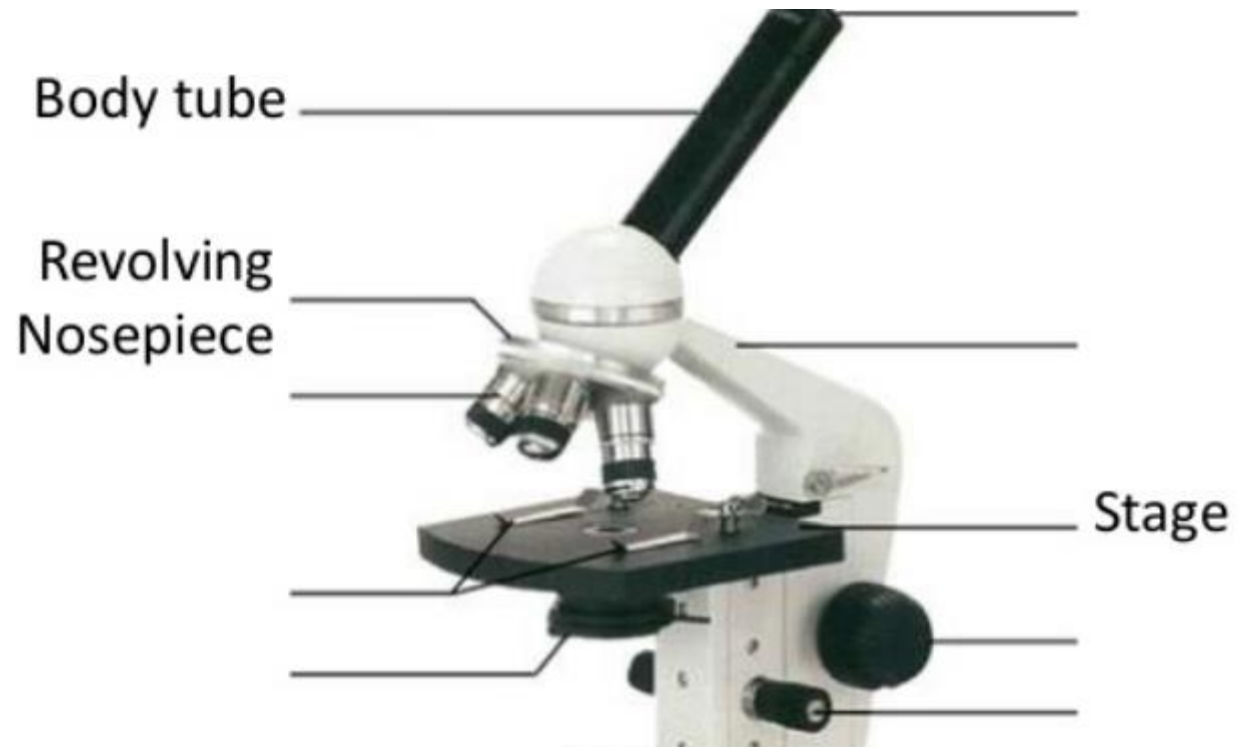
- Simplest optical microscopy illumination technique
- Uses visible light as source of illumination
- *> the shorter the wavelength, the greater the resolution (blue is the best)*
- Contrast comes from absorbance of light in the sample, or from staining.
- When the diaphragm is wide open the image is brighter and contrast is low.



Microscope Parts and Functions

- Body tube- Connects the eyepiece to the objective lenses.
- Revolving Nosepiece- This is the part that holds two or more objective lenses and can be rotated to easily change power.
- Stage- The flat platform where you place your slides.





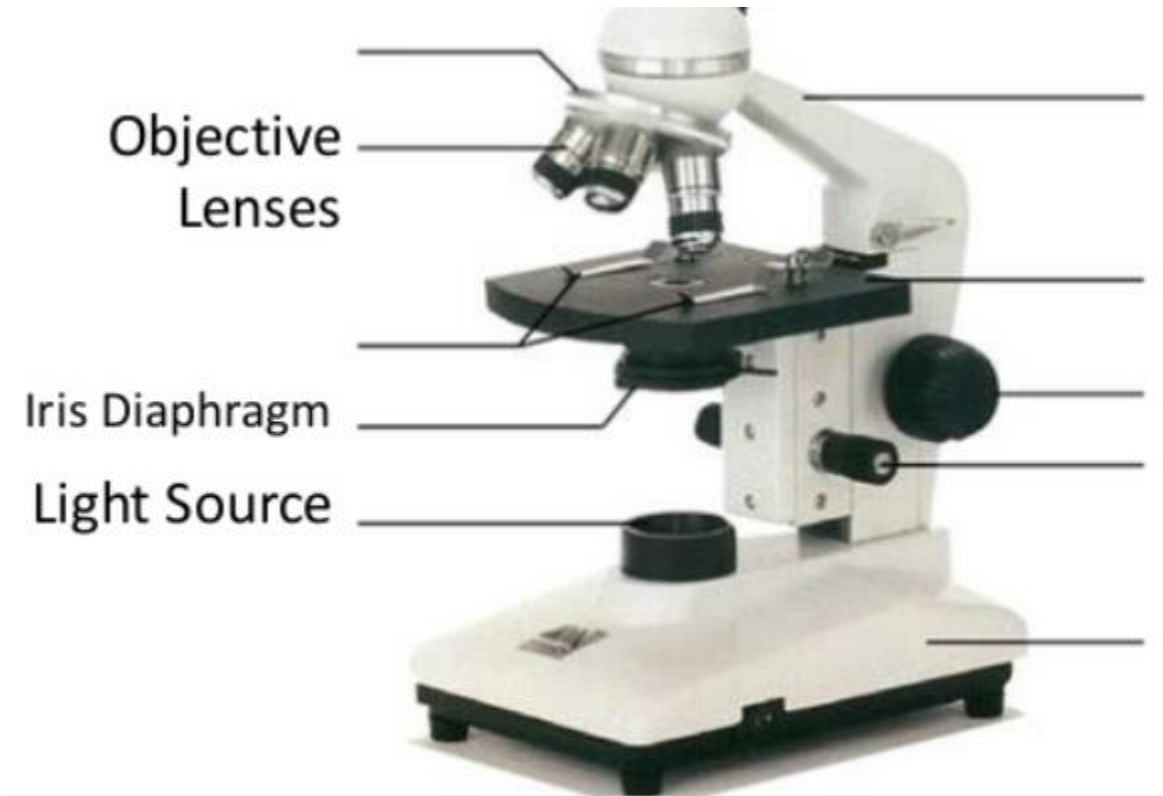
Microscope Parts and Functions

- Fine Adjustment Knob-small, round knob on the side of the microscope used to fine-tune the focus of your specimen
- Coarse Adjustment Knob--large, round knob on the side of the microscope used for focusing the specimen.
- Stage Clips-hold the slide in place



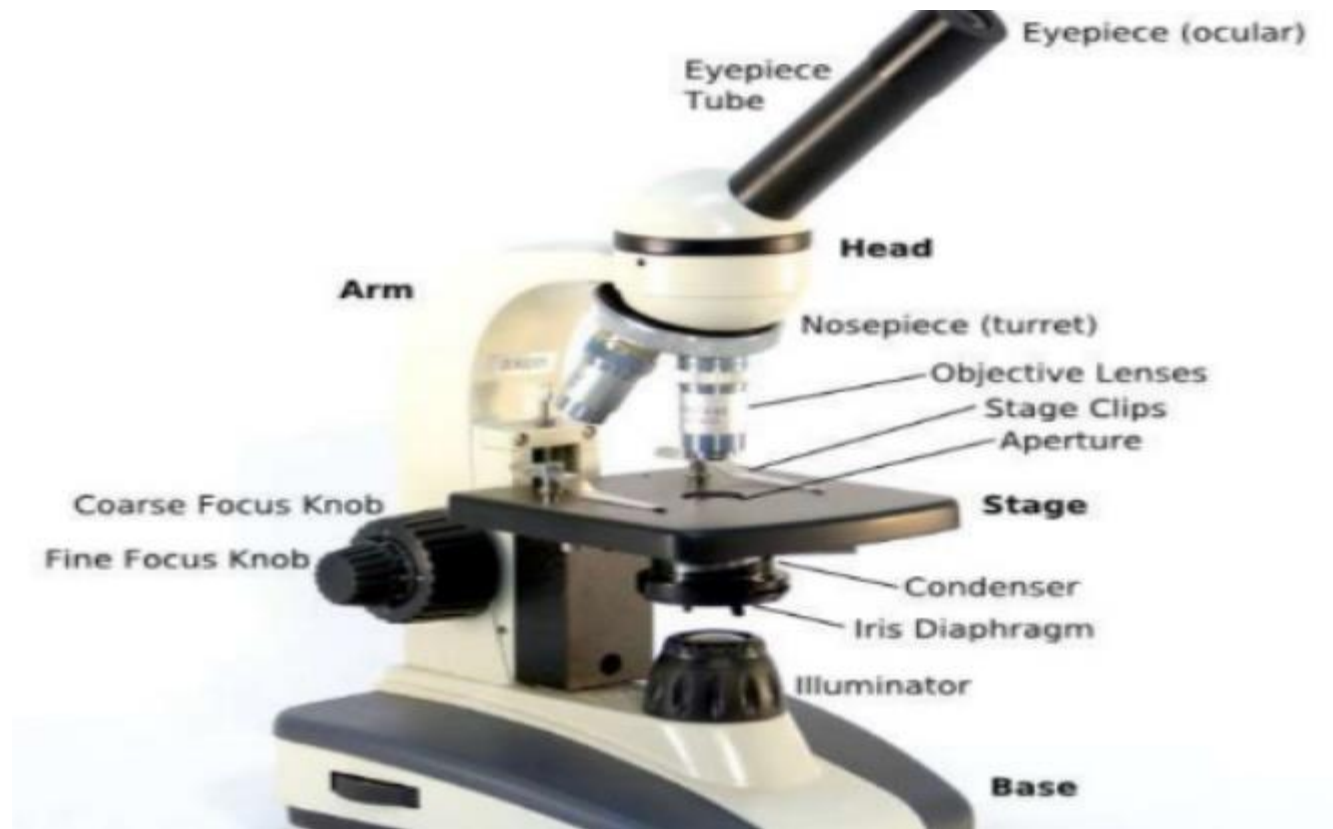
Microscope Parts and Functions

- Iris Diaphragm- controls the light going thorough the aperture.
- Mirror/Light source-used to reflect light to the specimen/source of light.
- Objective lenses-may have (scanner, Low, high Oil immersion objectives) and is used use to increase the magnification of the specimen.



Microscope Parts and Functions

- **Aperture** is the hole in the stage through which the base (transmitted) light reaches the stage.
- **Condenser** is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.



Proper Way of Focusing the Microscope

- Always observe the specimen or object using the **LOWEST POWER** object first.
- Focus using the **COARSE ADJUSTMENT KNOB** to bring the object into focus. Bring the object into sharp focus by using the fine adjustment knob.

Proper Way of Focusing the Microscope

- Focus, and then move to a higher power objective, if needed.
- Use only the FINE ADJUSTMENT KNOB when using the HIGHEST (longest) POWER OBJECTIVE.

Proper Way of Focusing the Microscope

- Keep both eyes open to reduce eyestrain. Keep eye slightly above the eyepiece to reduce eyelash interference.
- To find out the total magnification of the object, multiply the power of the eyepiece lens (10X) by the power of the objective.

Handling the Microscope

- Always use two hands to move the microscope. Place one hand around the arm, lift the scope, and then put your other hand under the base of the scope for support.
- Be gentle.





Storing the Microscope

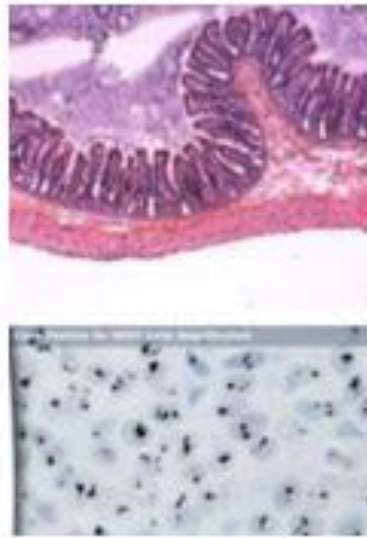
- Dust is an enemy to microscope lenses; always keep the microscope covered when not in use.

Types of microscopes

Compound Light Microscope



Bright Field Microscopy



Dark Field Microscopy



Pros & cons of dark field microscope

Advantages

- Used to view unstained specimens more clearly.
- Can be used to study various live bacteria, protists, algae, fungi, and many other cultures.
- Can examine the external of the specimen with detail

Disadvantages

- Can be inaccurate compared to other methods.
- Special care if more needed for this type of microscopy to prevent aberrations.
- Needs intense amount of light which can hurt the eyes and cause glare.
- Air bubbles in the slide can cause problems.

Phase contrast microscope

- Type of light microscope
- Enhances contrast in micrographs by converting phase shifts of light waves into brightness
 - Offers more contrast than brightfield microscopy
- Does not require the use of staining procedures which usually kill cells
 - Especially useful for examining living, unpigmented cells

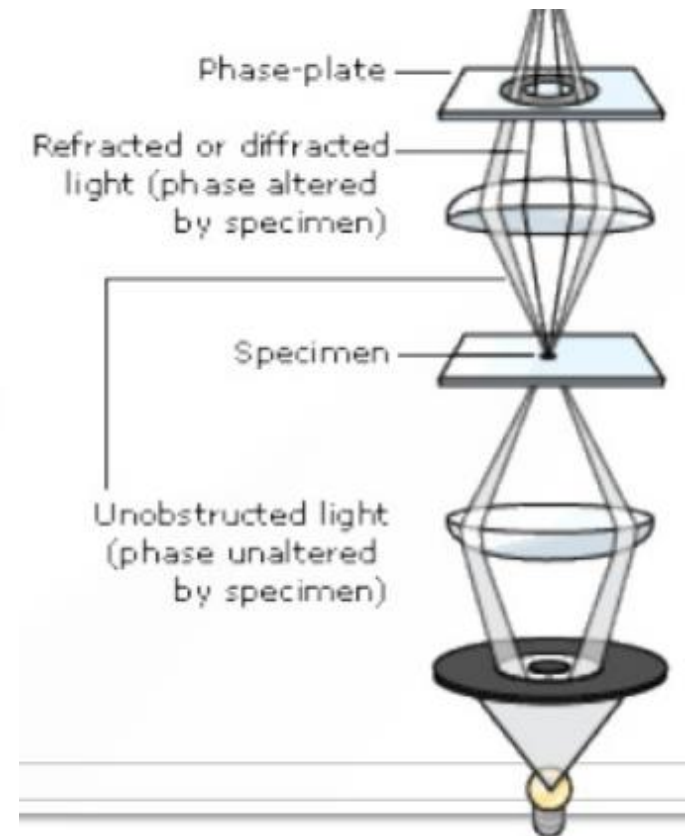
Phase contrast objective lenses (Nikon) come in 4x, 10x, 20x, and 40x powers, so the total magnification for phase contrast microscopes range from **40x to 400x**

Differences in **density** (Campbell et. al., 1999) or **refractive index** (Tortora et. al., 2007) within the specimen or cell causes light waves to be diffracted at different degrees

Diffraction of light waves implies a **change in the phase** of their wavelength

A unique part of the phase-contrast microscope, called the **phase-plate**, amplifies this change in phase to one-half wavelength

When both the direct (undiffracted) and reflected (diffracted) types of light waves converge at the ocular lens, **constructive and destructive interference** occurs

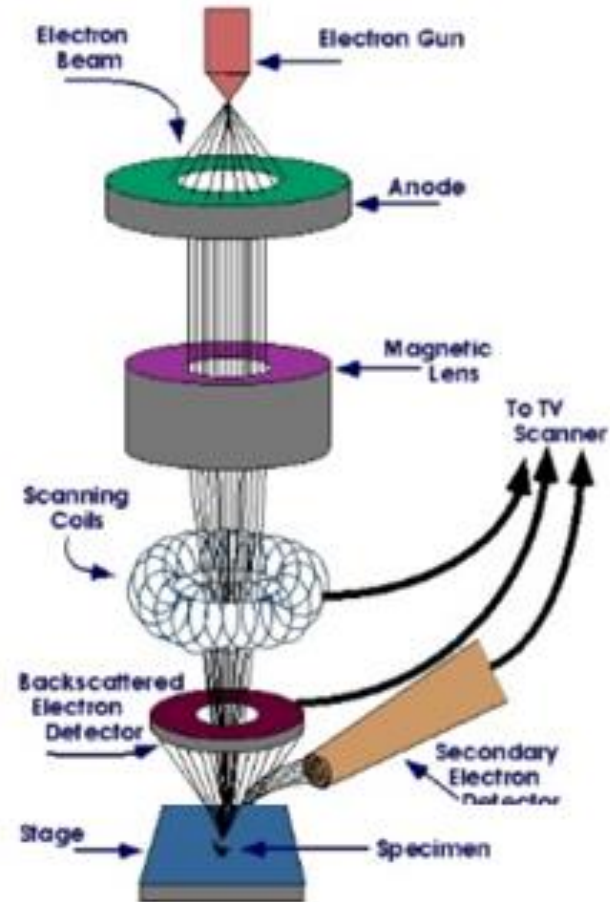


Electron Microscopy

- Illumination: electrons
- Magnification: $\sim 100,000\times$
- How it works: Detect electrons back-scattered by the sample.
- Image: Monotone (but may be color enhanced), 3-D surface of specimen

Electron Microscopy

- Magnetic lens—focuses electron beam
- Scanning coils—for systematic scanning (left to right, then down)
- Backscattered Electron Detector—detects electrons that bounced off the film
- Secondary Electron Detector—detects electrons emitted by



Source: <http://www.purdue.edu/rem/rs/graphics/sem2.gif>

Pros

- High magnification
- High resolution
- Shows the surface of specimen

Cons

- Needs specimen to be in vacuum
- Needs living cells and tissues and whole, soft-bodied organisms to be treated, usu. coated w/ gold film
- No color
- Cannot examine live specimen
- Really. Big. And Expensive. Equipment.

References

- <http://www.thefreedictionary.com/microscope>
- http://www.saskschools.ca/curr_content/biology20/unit1/UNIT1MODULE2LESSON1c.htm
- <http://www.microscope-microscope.org/basic/microscope-parts.htm>