The Microscope

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Objectives

1. To identify the parts of the microscope and list the function of each.
2. To describe and demonstrate the proper techniques for care of the microscope
3. To define total magnification and resolution
4. To demonstrate proper focusing technique
5. To define parfocal, field and depth of field
6. To estimate the size of objects in the field

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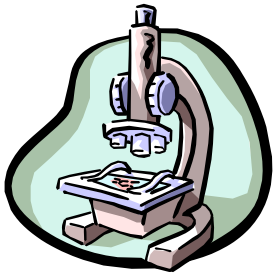
Microscope



Four Types:

1. Scanning Electron – magnifies 20,000x
2. Electron Transmission – magnifies 200,000X
3. Scanning Tunneling – magnifies 100,000,000X, releases electron beams
4. Light – uses visible light, magnifies 100 to 1000x

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Care and Structure of the Compound microscope

- When transporting microscope, hold it in an upright position with one hand on its arm and the other supporting its base. Avoid jarring instrument when setting it down.
- Always begin focusing process with lowest power objective lens in position, changing to the higher power lenses as necessary.
- Use coarse adjustment knob only with lowest power lens
- Always use a cover slip with temporary (wet mount) preparations
- Before putting microscope in storage cabinet, remove slide from stage, rotate the lowest power-objective lens into position,
- Never remove any parts from the microscope

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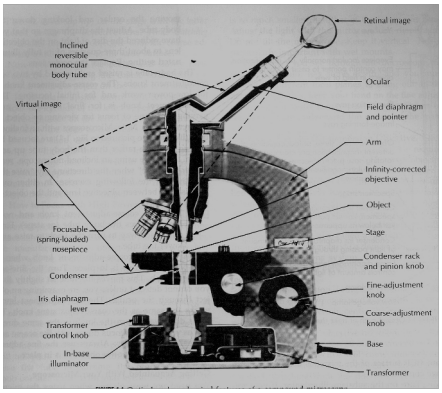
Microscopy



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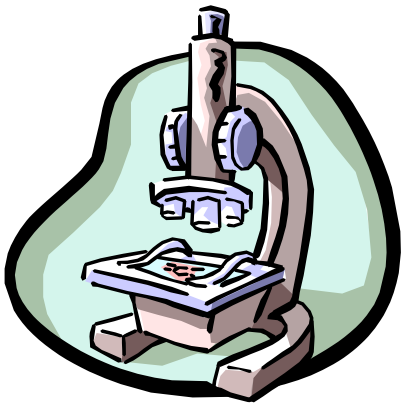

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Mechanical Parts

- Draw tube – hollow cylinder where the eyepiece or ocular is placed.
- Head or Body tube –supports objective lens system mounted on a movable nosepiece and ocular lens or lenses
- Revolving nosepiece – attached to the base of the body tube.
- Dust shield – above the nosepiece which protects the lower lenses.

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Mechanical Parts

- Coarse adjustment knob – consists of large knobs on the side of the tube.
- Fine adjustment knob – used for sharp focus after the object has been brought into view with the coarse adjustment.
- Stage – platform where you put slides for observation
- Stage clips – slide is held in place on stage
  - Spring clips type
  - Clamp type mechanical stage – permits precise movement of specimen

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Mechanical Parts

- Arm – Curved part of the microscope or vertical portion of microscope connecting base and head
- Pillar – short vertical extension on which arm connects with base.
- Base – portion in contact with table.
- Inclination joint – permits tilting of microscope.

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Optical Parts

- Eyepiece – removable cylinder on top of microscope. Contains lenses.
- Lower power objective (LPO) – shorter of the 2 objectives, magnify an objective.
- High power objective (HPO) – give greater magnification.
- Iris diaphragm – found below the stage. Regulates amount of light passing through the stage.

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Parts of Microscope

- Pointer – use to indicate a specific area of the viewed specimen, attached to one ocular and can be positioned by rotating ocular lens.
- Substage illuminator (electric) or reflecting mirror (manual) – reflects light or light from a microscope lamp up into the optical system.
  - Concave surface of mirror is used with natural light or with a separate microscope lamp when there is no condenser on microscope.
  - Plane flat surface of mirror is used when there is a sub stage condenser.

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Focusing with Low Power

- Turn low power objective until it clicks in place over aperture.
- Adjust condenser and diaphragm for optimal illumination
- Obtain a slide containing letter e or any letter.
- Cover slip up, on the stage with letter centered under objective lens.
- While watching from the side, lower objective with coarse adjustment until it is close to the slide surface
- Look through ocular and slowly raise objective.

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Observations?

- Is the image upside down? Is it reversed?
- Does the left side of the letter appear on the right and right side on the left?

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Focus with high power

- Focus object first with low power, slowly rotate high power objective in position.
  - Parfocal – if the object in focus with low power will be nearly in focus under high power. Lenses are said to be parfocal with respect to each other.
  - Not parfocal – focus first with low power and then raise the tube by turning coarse adjustment knob one-half turn, carefully swing high power into place and lower it slowly to about 1 mm from cover glass. Look through the ocular, use fine adjustment to focus object.

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Using Oil Immersion Objective

- Focus specimen with LPO and then with HPO.
- Carefully center point of interest in field of view, rotate nosepiece to move HPO off to one side.
- Place a single drop of immersion oil on cover slip at point where objective will come into position.
- Move the OIO carefully into position. Watch from side to be certain that the lens clears the cover slip.
- Note: Oil should bridge between lens and cover slip.
- Carefully adjust fine focus to bring specimen to focus. Adjust diaphragm or substage condenser or mirror to increase light as required.
- When finished, clean with xylol and lens tissue.

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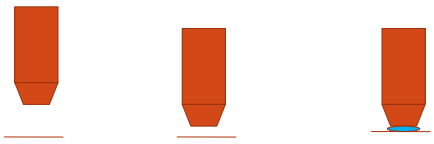
Relative Working Distances of Objectives



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Magnification and Resolution

- Magnification – achieved through interplay of 2 lenses, ocular lens and objective lens.
- Objective lens magnifies specimen to produce a real image projected to the ocular.
- Real image is magnified by ocular lens to produce virtual image seen by your eye.

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Magnification Power

Ocular lens X Objective = Magnification

Example:

5X 10X 50 x

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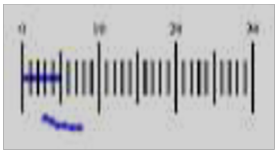
Calibrating the Microscope

Ocular Micrometer



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Calibrating the Microscope

Stage Micrometer

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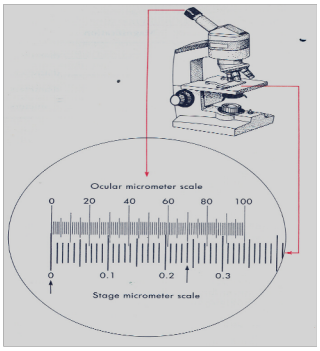
Calibrating the Microscope



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Calibration Formula

$$\frac{\text{Stage}}{\text{Ocular}} \times 0.01 \text{ mm}$$

Why 0.01 mm?

Smallest space on stage micrometer

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Determining length or width of specimen

$$z = s \times c$$

Z = measurement
S = number of divisions of ocular micrometer covered by specimen
C = calibration constant

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Getting measurement in microns

$$z \times 1000$$

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Determining Size of Microscope Field

- Measure diameter field of view by estimating proportion of field occupied by the objects.
  - Scanning objective in position, place a transparent ruler on stage and focus on its edge so you can see the scale, move ruler right or left so one of vertical mm lines is visible at edge of circular field of view.
  - Count no. of mm lines spanning the field.
  - Diameter in mm convert to microns.

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Microscope Field

- Diameters of fields of view can't be measured directly with transparent ruler because of high magnification. To calculate field diameter of LPO and HPO.

$$\text{Diam of LPO} = \frac{\text{Magnification of scanning objective}}{\text{Magnification of LPO}} \times \text{Diameter of Scanning objective field}$$

$$\text{Diam of HPO} = \frac{\text{Magnification of LPO}}{\text{Magnification of HPO}} \times \text{Diam. Of LPO field}$$

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Microscope Field

- Diameter of LPO Field
  - Ranges between 1.5 and 1.6 mm (1500 and 1600 µm)
- Diameter of HPO Field
  - Ranges between 0.36 and 0.42 mm (360 and 420 µm)

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Stereoscopic Dissecting Microscope

- Objects too large or too thick
- Furnishes a 3-dimensional view of objects at very low power
- Image not inverted
- Ample space for manipulation and dissection under the lens.
- Microscope stage can be illuminated either by transmitted light (light passing through object below) or reflected light (light illuminating object from above and being reflected by object into microscope)

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Using stereoscopic dissecting microscope

- Place object on stage and illuminate with reflected light.
- Look through oculars, adjust to fit distance between eyes.
- Adjust focus using the focus knob.

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