MICROSCOPY & MICROTECHNIQUE

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OUTLINE

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SUMMARY OF ABBREVIATIONS

ABBREV	Meaning

LEGENDS

Must know	Lecturer	Book	Presentation	Old Trans
❗	👩‍⚕️	📖	🖥️	📝

LEARNING OBJECTIVES

At the end of the lecture, the student should be able to:

- ✔ Define microscope
- ✔ Differentiate the two types of microscope
- ✔ Describe the parts of the microscope
- ✔ Know the different types of light microscopy
- ✔ Know the different types of electron microscopy
- ✔ Describe a simple microtechnique

DEFINITION OF TERMS

- **Microscopy**
  - Science of investigating small objects using the microscope
- **Microscope**
  - An instrument that magnifies an image and allows visualization of greater detail than what is possible with an unaided eye
  - 📖Simplest microscope is a magnifying glass or a pair of reading glasses.
- **Resolving Power**
  - Ability of a microscope lens or optical system to produce separate images of closely positioned objects.
- **Resolution**
  - Smallest distance between two structures at which they can be seen as separate objects.
  - 📖 **Ocular** or **Eyepiece lens**
    - Magnifies the image produced by the objective lens, but cannot increase the resolution.
- **Ocular Piece**
  - Lens at the top where you peep into
- **Draw Tube**
  - Holds the eyepiece in place
- **Body Tube**
  - Connect the eyepiece to the objective lenses
- **Arm**
  - Part where you grasp the microscope
  - 👩‍⚕️ Dito nyo hinahawakan pag binubuhat yung microscope
- **Revolving Nosepiece**
  - Part that holds two or more objective lenses and can be rotated easily to change power
- **Objective Lenses**
  - To gather the light that has passed through the specimen
  - Types:
    - Scanner Objective (4x)
    - Low Power Objective (10x)
    - High Power Objective (40x)
    - Oil Immersion Objective (100x)
  - 👩‍⚕️ HPO and OI are retractable
- **Stage and Stage Clip**
  - Flat platform where you place your slides
- **Aperture**
  - Hole in the middle of the stage that allows light from the illuminator to reach the specimen
- **Rack Stop**
  - An adjustment that determines how close the objective lens can get to the slide
- **Condenser Lens**
  - Aimed to focus the light onto the specimen
- **Diaphragm**
  - Rotating disk under the stage with different sized hoes and is used to vary the intensity and size of the cone of light projected into the slide
- **Coarse Adjustment Knob**
  - Responsible for bringing the objectives down for initial focusing
- **Fine Adjustment Knob**
  - For final focusing and gives a detailed view of the specimen
- **Illuminator**
  - Steady light source used in place of a mirror
- **Base**
  - Bottom of the microscope for support

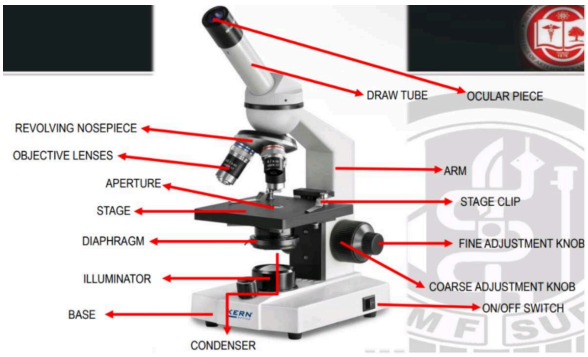

Figure 1. Parts of a Microscope

TYPES OF MICROSCOPE

Light Microscopy

- Bright-field microscope
- Virtual microscope
- Fluorescence microscope
- Phase contrast microscope
- Confocal microscope
- Polarizing microscope

Electron Microscopy

- Transmission Electron Microscope (TEM)
- Scanning Electron Microscope (SEM)

LIGHT MICROSCOPY

- ❗ **Both live and dead** specimens can be seen

Bright-Field Microscopy

- Widely used by students of histology
- Stained preparations are examined by means of **ordinary light** that passes through the specimen
  - 📖 A specimen to be examined with bright-field microscope must be sufficiently thin for light to pass through it
- Consists of:
  - Light source
  - Condenser lens
  - Stage
  - Objective lens
  - Ocular lens

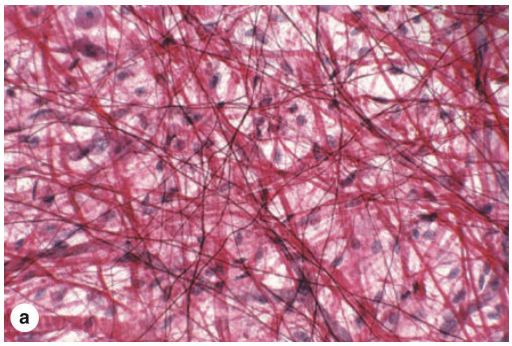

Figure 2. Light Microscopy

Virtual Microscopy

- 📖 **Digital procedure** alternative to the examination of glass slides using a light microscope
- **Virtual Slide**
  - 📖 Digital representation of a glass slide, which can be viewed remotely without a light microscope

Figure N. Virtual Microscopy

Fluorescence Microscopy

- Irradiated by **fluorescence**
- 📖 Light with a longer wavelength
- Tissue sections are usually irradiated with UV light
- Fluorescent substances **appear brilliant on a dark background**
- Fluorescent compounds with affinity for specific cell macromolecules may be used as fluorescent stains
- 📖 Fluorescence microscope is used to display naturally occurring fluorescent (autofluorescent) molecules such as **vitamin A** and some **neurotransmitters**

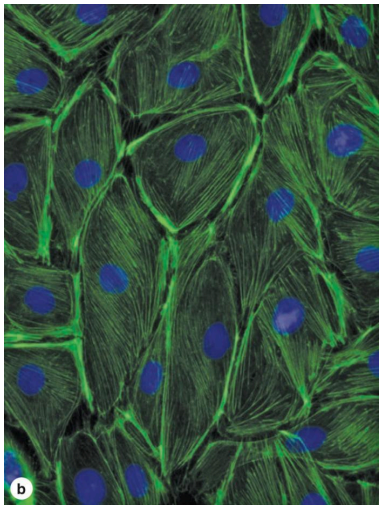

Figure N. Fluorescence Microscopy

Phase-Contrast Microscopy

- Uses a lens system that produces visible images from **transparent** and **colorless specimens**
- Light changes its cellular and extracellular structure with different reactive indices
- Interference microscope
- Differential interference microscope

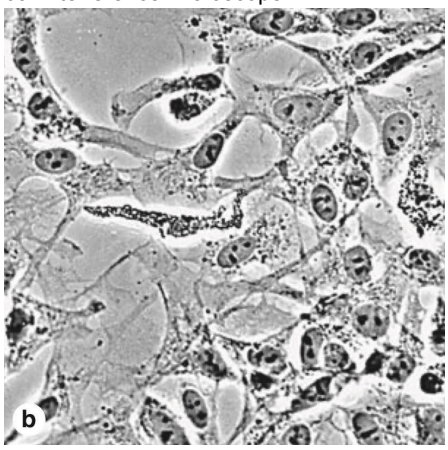

Figure N. Phase-contrast Microscope

Confocal Microscopy

- Small point of high intensity light (**laser**)
- Plate with a pinhole aperture in front of the image detector
- Allows only "in-focus" light to pass into a photomultiplied (detector) device, whereas the "out-of-focus" light is blocked from entering the detector
- 📖 **Confocal scanning microscope**
  - Allows visualization of a biologic specimen in ***three dimensions***

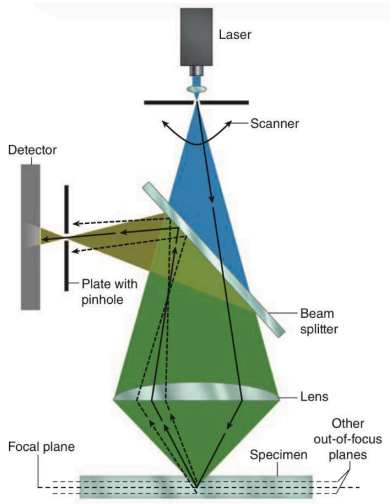

Figure N. Confocal Microscopy

Polarizing Microscopy

- Allows the recognition of stained or unstained structures of highly organized subunits
- **Polarizing Filter**
  - Between the light source and the specimen
- **Analyzer**
  - Between the objective lens and viewer
- This allows the recognition of stained or unstained structures made of highly organized subunits
- Birefringence
- 📖 Simple modification of the light microscope in which a polarizing filter (polarizer) is located between the light source and the specimen, and a second polarizer (analyzer) is located between the objective lens and viewer

Figure N. Polarizing Microscope

ELECTRON MICROSCOPY

- ❗ Only **dead and dried specimen** can be seen

Transmission Electron Microscope (TEM)

- An **electron source** (cathode, electron gun) such as a heated tungsten filament, emits electrons
- The electrons are attracted toward an anode
- An electrical difference between the candid cover and the anode imparts an accelerating voltage of between 20,000 and 200,000 volts to the electrons, creating the electron beam
- The beam then passes through a series of electromagnetic lenses that serve the same function as the glass lenses of a light microscope
- 📖 The optics of TEM are, in principle, similar to those of the light microscope, except that **TEM uses a beam of electrons rather than beam of light**

Scanning Electron Microscope (SEM)

- Provides a high-resolution view of the surfaces of cell, tissues, and organs
- Produces and focuses a very narrow beam of electrons
- The surface of the specimen is first dried and spray coated with a very thin layer of heavy metal-like gold
- The refracted electrons are captured by a detector, producing signals that produce a black-and-white image
- 📖 The electron beam does not pass through the specimen but is scanned across its surface

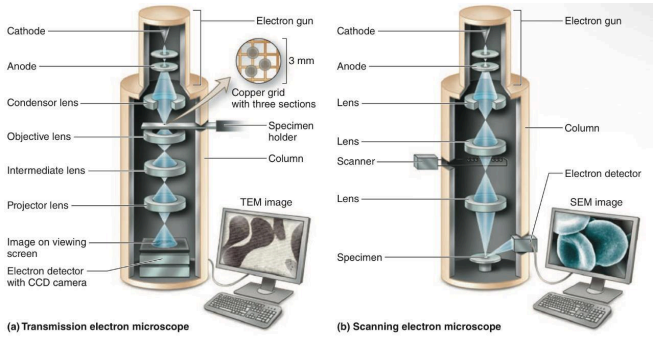

Figure N. TEM and SEM

MICROTECHNIQUES

ELECTRON MICROSCOPY

- Set of procedures in the preparation of tissue specimens for histological examination
- 👩‍⚕️ The first step for tissue preparation should be **procurement** or **collection of tissue specimen**

Fixation

- Used to:
  - Terminate cell metabolism
  - Prevent enzymatic degradation of cells and tissues
    - Referred to as **autolysis** - self digestion
  - Kill pathogenic microorganisms such as bacteria, fungi, and viruses
  - Harden the tissue as a result of either cross-linking or denaturating protein molecules
- 📖 **Formalin**
  - Widely used fixative for light microscopy
  - Buffered isotonic solution of 37% formaldehyde
  - Preserves the general structure of the cell and extracellular components by reacting with the amino groups of proteins (most often cross-linked lysine residues)

Dehydration

- The tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100% which removes all water
- 📖 **Ethanol** is commonly used for dehydration

Clearing

- Alcohol is removed in toluene or other agents in which both alcohol and paraffin are miscible
- 📖 **Xylol or tuluol** are used to remove the alcohol before infiltration

Infiltration

- The tissue is then placed in melted paraffin until it becomes completely infiltrated with the substance
- Tissue is placed in melted paraffin in an oven at 52°- 60°C

Embedding

- Paraffin-infiltrated tissue is placed in a small mold with melted paraffin at room temperature and is allowed to harden

Sectioning

- Using a microtome machine to about 5-6 µm in thickness

Mounting

- Mounting the cut sections on clean slides
- Dissolving or melting the embedding medium

Rehydration

- Immersing the tissue in decreasing concentrations of alcohol

Staining

- H & E stain is most commonly used
- Hematoxylin is a basic (blue) stain
  - 📖 Stains DNA in the:
    - Cell nucleus
    - RNA-rich portions of the cytoplasm
    - Matrix of cartilage
- Eosin is an acid (red) stain
  - 📖 Used as a counterstain to distinguish additional features of a tissue
- Placing a cover slip with Canada balsam to secure it in place

Labelling

- Writing a small information about the specimen

APPENDIX

1. Powerpoint: Desiderio, J. (2026). Microscopy and Microtechnique.
2. Mescher, A. L. (2016). Basic Histology: Text & Atlas (14th ed.). McGraw-Hill Education.
3. Previous Trans: 2024