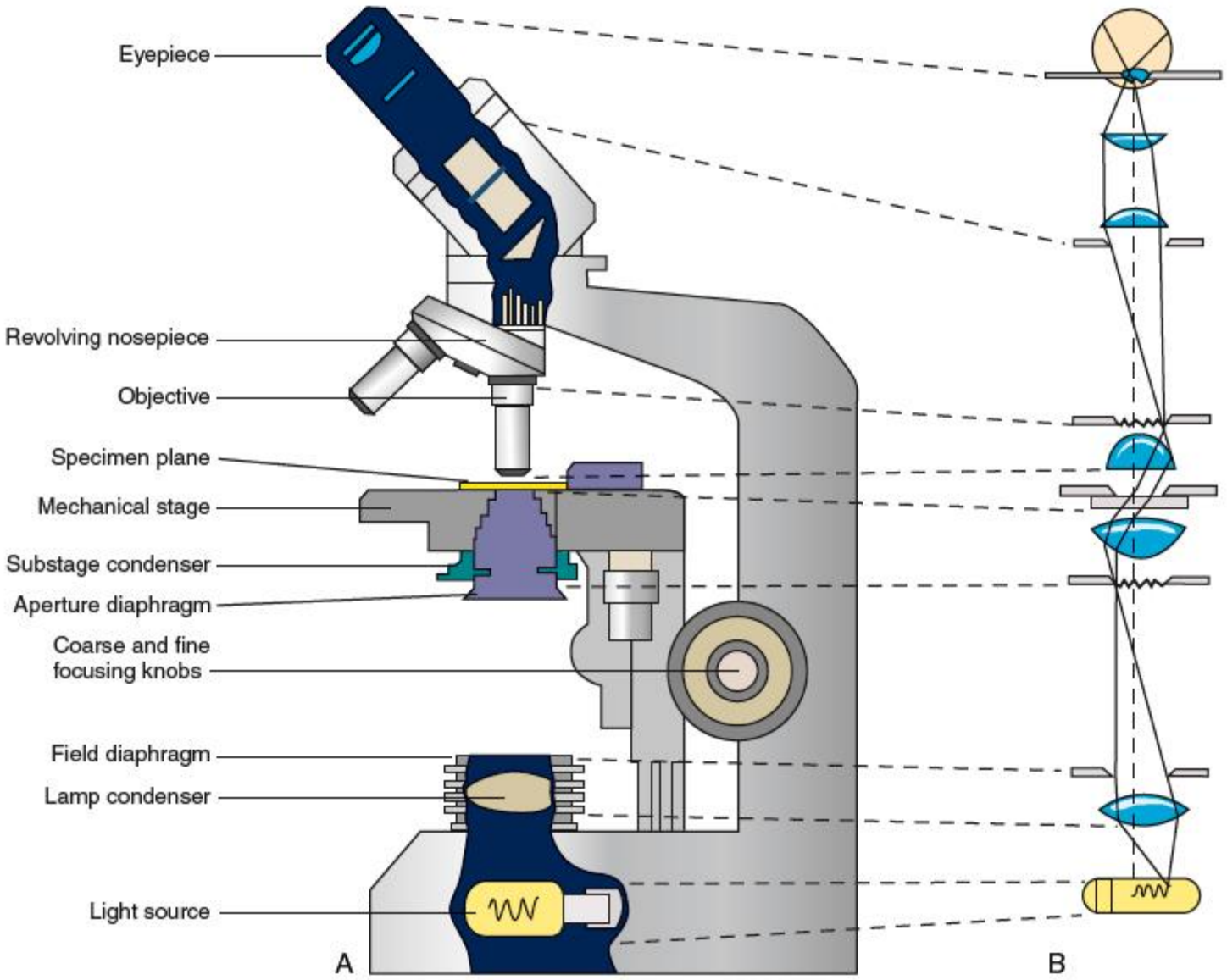
Chapter 18

MICROSCOPY

Parts of Microscope

Microscope body includes four basic components:

- Optical tube with lenses (eyepieces and objectives)
- Stage on which specimen is placed to be viewed
- Condenser to focus light onto the specimen
- Illumination source



Field of View (FOV)

Field of view (diameter determined by eyepiece and field number assigned to eyepiece)

FOV determined by formula:

$$\text{FOV} = \frac{\text{Field number}}{\text{M}}$$

- Example: Eyepiece with field number of 18 has a diameter of 18 mm when using a 1x objective, 1.8 when using a 10x objective, and 0.18 when using a 100x objective

If using more than 1 microscope for urinary sediment examination, both should have same FOV to allow consistent reporting of formed elements per FOV

- Use eyepieces with the same field number

Resolution

Also known as *resolving power*

Ability of lens system to reveal fine detail

The smallest distance in microns between two points or lines at which they are distinguished as two separate entities

Depends on wavelength of light used (λ) and the numerical aperture (NA) of the objective lens

$$R = \frac{0.612 \times \lambda}{NA}$$

Resolution (Cont.)

Can increase NA of a lens by changing refractive index of optical medium or by increasing aperture angle

Using immersion oil achieves both; increases NA and thus increases resolution

Maximum resolution is only achieved if microscope condenser NA is equal to or slightly greater than objective lens NA (necessary to ensure adequate illumination to the objective lens)

Objectives

Most important optical components because they produce primary image magnification

Each objective has a different “working distance”

- Distance between objective and coverslip on slide

Care required when changing or focusing objectives to avoid damage because working distance decreases as object magnification increases

Engravings on Objective

Objective Corrections

Chromatic aberrations

- Occur because different wavelengths of light bend at different angles after passing through a lens, resulting in specimen with undesired color fringes
- Achromatic lenses bring red and blue into same focus
- Apochromatic lenses bring red, blue, and green into same focus

Spherical aberrations

- Occur when light rays pass through different parts of lens and are not in same focus, so specimen appears blurred
- Objectives corrected to bring all light entering lens into same focus

Other Objective Characteristics

Parcentered

- Relates to ability of objectives to retain same central field of view when objectives are switched
- Keeps object in center of field of view when objectives are switched to higher magnification

Parfocal

- Refers to ability of objectives to remain in focus regardless of objective used
- Allows initial focusing at low power, and only fine adjustments needed when objectives are changed

BOX 18.2 Microscope Dos and Don'ts

Dos

- Always use lens paper on optical surfaces.
- Always use a commercial lens cleaner to clean optical surfaces.
- Protect the microscope from dust when not in use. Avoid temperature extremes and direct sunlight.
- Document all cleaning and maintenance; have microscope professionally serviced annually.

Don'ts

- Never use gauze, facial tissue, or lint-free tissue on lens surfaces.
- Never touch optical surfaces with fingers or hands.
- Never wipe off dust or particulate matter; remove using suitable brush or air syringe.
- Never wear mascara while performing microscopy.
- Never clean the back lens of the objectives.
- Never use grease or oil on mechanical parts.
- Never disassemble the microscope for repair; call a service representative.
- Never leave microscope tubes without eyepieces; insert dust plugs as necessary.

Brightfield Microscopy

Oldest and most common type of illumination

Refers to dark appearance of specimen against brighter background

Kohler illumination focuses light on condenser diaphragm allowing for bright even illumination

Principal type of microscopy used in urinalysis

Phase-Contrast Microscopy

Used to view components that are difficult to view due to low refractive index (hyaline casts, mucus threads, trichomonads)

Variations in refractive index are converted into variations in light intensity or contrast

Components retard light to different degrees

Best contrast achieved when light retardation is one-quarter of a wavelength

Areas of the objects appear light and dark with haloes of various intensity

Polarized Microscopy

Polarized microscopy requires two filters:

- Polarizing—below condenser
- Analyzer—between objective and eyepiece

Polarized light splits into two beams when passing through optically active substance:

- Original light path
- Rotated at 90 degrees

Can identify birefringent substances such as crystals, fibers, bones, or minerals

Birefringent refers to ability of a substance to refract light in two directions (at 90 degrees)

Interference Contrast Microscopy

Converts differences in optical path through sample to intensify differences in the image

Achieves specimen images of high contrast and resolution without haloing

Three-dimensional appearance revealing contour detail

Excellent for viewing details of unstained images

Two types:

- Modulation contrast (Hoffman)
- Differential interference contrast microscopy (Nomarski)

Darkfield Microscopy

Produces bright image against dark background

Used on unstained specimens and for identifying spirochetes

Special condenser directs illumination source light through the specimen plane only from oblique angles

Resulting shining image on dark background is enhanced visually by increased contrast

Helps to clarify edges and boundaries of objects

Fluorescence Microscopy

Allows visualization of fluorescent substances

Occurs when light is absorbed at one wavelength and emitted at a different, longer wavelength

Uses two filters:

- Excitation filter—selects wavelength to present
- Barrier or emission filter—selects emitted wavelength to be measured

Most applications of this technique require staining sample with fluorescent dyes called *fluorophores*

TABLE 18.2 Comparisons of Microscopic Capabilities

	MICROSCOPY TYPES						
Features	**Brightfield**	**Phase Contrast**	**Modulation Contrast**	**Differential Interference Contrast**	**Polarizing**	**Darkfield**	**Fluorescence**
Unstained Specimens							
Resolution	Poor	Limited by contrast	Excellent	Excellent	Limited by contrast	Fair	NA*
Contrast	Poor	Optimal with thin, flat structures†	Excellent for most specimens; adjustable	Excellent for most specimens; adjustable	Maximum for birefringent specimens	Good for most specimens	—
Three-dimensional image	No	No	Yes	Yes	No	No	No
Halo	No	Yes, can be excessive	No	No	No	No	No
Optical sectioning	No	Limited by halo	Yes	Yes	No	No	No
Stained Specimens							
Resolution	Optimal	Often reduced	Optimal	Optimal	NA‡	NA‡	Adequate§
Image enhancement (compared with brightfield)	—	Only for faintly stained objects	At boundaries and gradients	At boundaries and gradients	—	—	Maximal, not visible otherwise
General Considerations							
Detailed imaging of birefringent specimens	Yes	Yes	Yes	Limited	Limited	Yes	No
Technical training required¶	Minimal	Moderate	Moderate	Moderate	Moderate	Minimal	Minimal
Comparative cost	Low	Medium	Medium	Medium	Medium	Low	High (owing to cost of source)

Modified from Hoffman Modulation Contrast System, Modulation Optics, Inc., Greenvale, NY.