

- 1. Light Microscopy

.Ocular Lens (Eyepiece): Standard magnification is 10x.

.Objective Lenses:

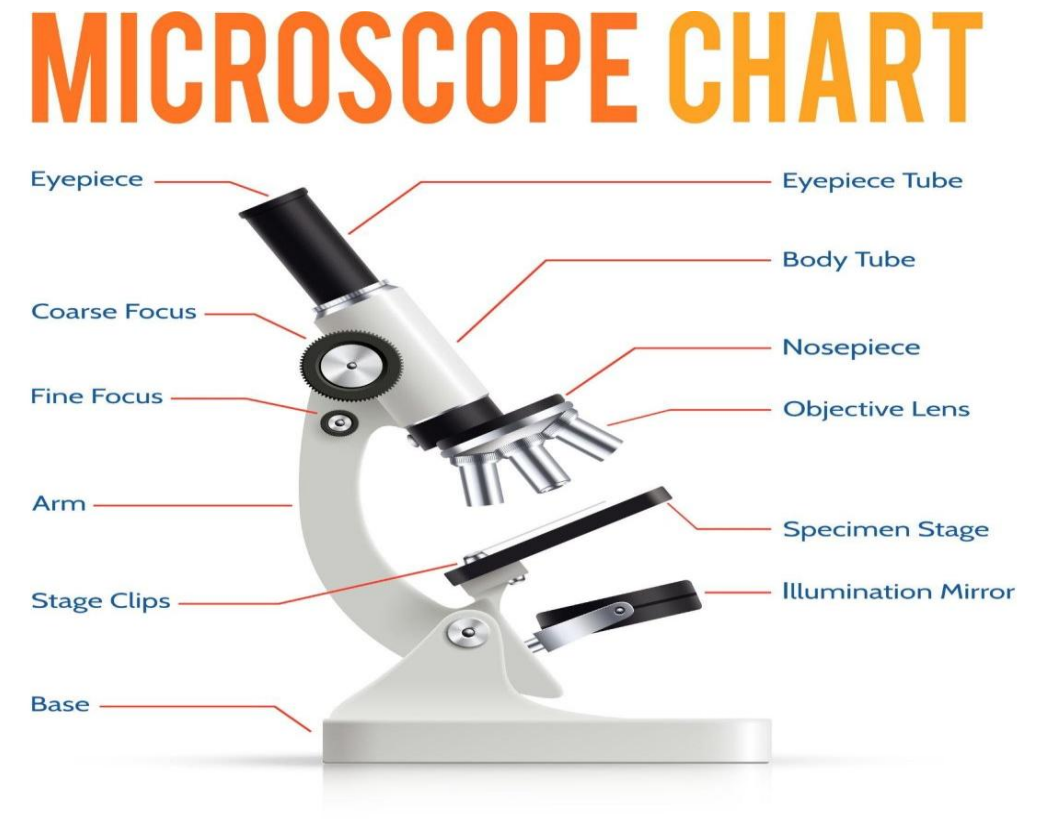
-Scanning power: 4x

-Low power: 10x

-High power (dry): 40x

-Oil immersion: 100x (requires immersion oil to match the refractive index of glass).

.Focusing Control: Use the coarse adjustment knob strictly with low-power lenses (4x, 10x); use the fine adjustment knob for high-power lenses (40x, 100x) to prevent slide breakage

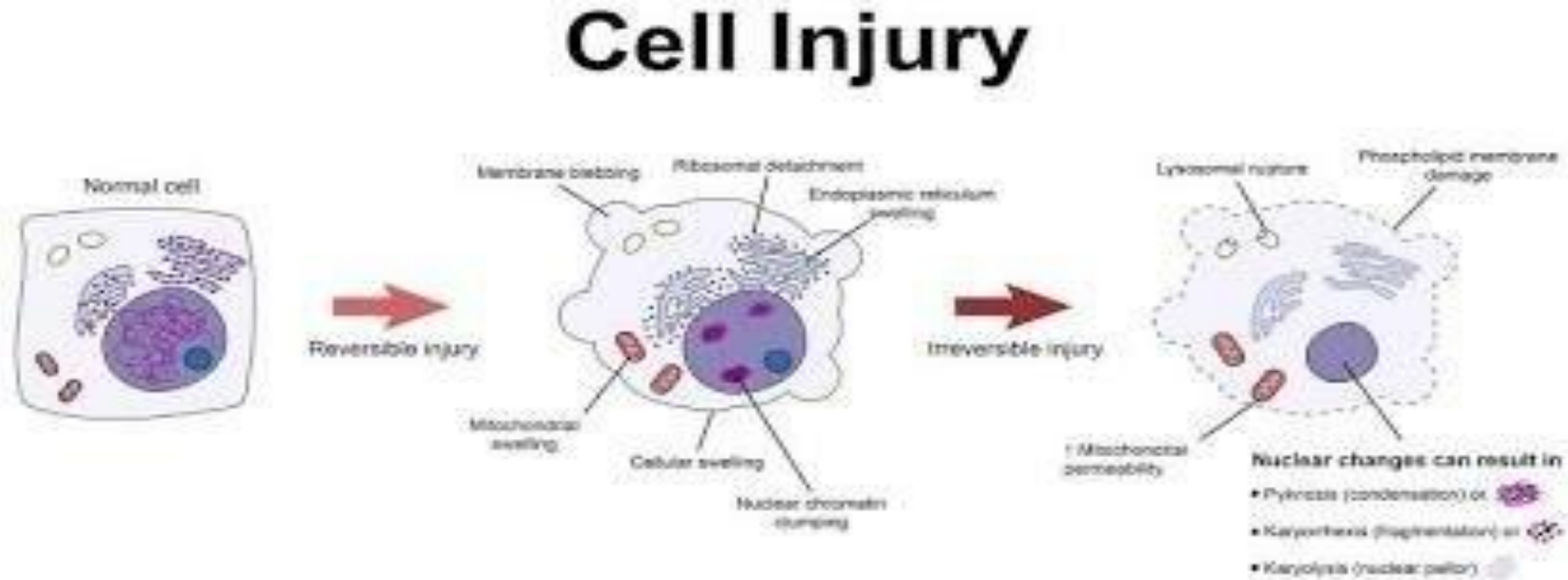


• 2. Tissue Handling & Processing

- **Fixation:** Halts tissue autolysis and putrefaction. The universal fixative is 10% Neutral Buffered Formalin (NBF).
- **Tissue Processing Steps:**
 - **Dehydration:** Graded ascending series of ethanol (70% to 100%) to remove free and bound water.
 - **Clearing:** Hydrocarbon intermediate (typically Xylene) miscible with both alcohol and paraffin.
 - **Infiltration & Embedding:** Penetration with molten paraffin wax, followed by casting into a solid block.
 - **Sectioning:** Slicing the block at 3–5 μm thickness using a microtome.
 - **Staining:** Routine Hematoxylin & Eosin (H&E) stain:
 - **Hematoxylin (Basic):** Stains nuclei deep blue/purple (basophilic structures).
 - **Eosin (Acidic):** Stains cytoplasm and extracellular matrix pink/red (eosinophilic structures).

- **Part 2: Cell Injury and Cell Death**

- Cell injury occurs when environmental stresses exceed the cell's baseline physiological adaptation mechanisms.
- Reversible vs. Irreversible Cell Injury



feature	Irreversible Cell Injury (Cell Death)	Reversible cell injury
definition	Point of no return crossed; the cell inevitably progresses to necrosis or apoptosis.	Functional and structural changes resolve if the injurious stimulus is removed
Plasma membranemito	Severe structural breakdown; loss of membrane integrity and leakage of cellular contents.	Intact structure .blebbing .blunting . Or loss of microvilli
mitochondria	Severe persistent swelling; formation of large amorphous calcium-rich densities.	Mild. Reversible swelling
Nuclear changes	Irreversible breakdown: shrinkage, fragmentation, dissolution.	Chromatin clumping (reversible)
Histological hallmarks	Cytoplasmic hypereosinophilia, membrane rupture, and surrounding inflammatory response (in necrosis).	Cellular swelling (hydropic change) and fatty chang (steatosis)
Cell fate	Progression to necrosis or apoptosis	Returns to baseline homeostasis

- **Patterns of Cell Death: Necrosis vs. Apoptosis**

- **Necrosis:**

- Unregulated, pathological cell death resulting from severe ischemia, hypoxia, or acute toxins.
- Cellular swelling, rupture of membranes, and release of intracellular contents.
- Always triggers a significant host inflammatory response.

- **Apoptosis:**

- Highly regulated, energy-dependent (ATP-requiring) programmed cell death.
- Seen in both physiological settings (embryogenesis, hormonal involution) and pathological states (DNA damage, viral infection).
- Cell shrinks, forms apoptotic bodies, and is rapidly phagocytosed without eliciting an inflammatory response.