Table 1 Staining characteristics	
Characteristic	**Comments**
Staining action	**Substantive**: Stain that acts immediately and directly on the tissue without the intervention of any other substance
	Adjective: Tissue is first treated with an agent, which in turn attaches the stain to the tissue (mordant staining)
	Impregnation: Involves the deposition of sensitive metallic substances over selected cells and tissue structures that are rendered visible by subsequent reduction of the metal
Staining time	**Progressive**: Using the microscope, performed by watching the degree of staining at various points during the process and stopping the process when the desired staining action has been achieved
	Regressive: Used for the best sharpness of differentiation; whole tissue is stained and then differentiated to remove excess dye from the parts that should be relatively unstained
Action on tissue	**General**: Stain that colors all parts of the tissue equally, providing no significant differentiation
	Selective: Stain that differentiates between classes of tissue or between parts of cells
Differentiation	Using regressive staining methods, the process of removing excess stain is called differentiation; this usually provides sharp staining contrasts because the hydronium and hydroxide ions in the solvents used for differentiation diffuse more rapidly than any dye ion; some of the ways in which a tissue section may be differentiated include the use of acidic or basic medium and excess mordant, buffers, or oxidizers
Staining mechanisms	**Capillarity and osmosis**: Account for penetration of the dye into the interior of the tissue
	Absorption or solution: Passage of the dye molecule from the dye bath solution to the solution in the substance being dyed
	Adsorption: Deposition of dye on the surface of the dyed material (physical surface adsorption plus salt linkages of dye to the protein chain)

Chemical factors	**Acid-base**: Based on the fact that certain cell parts are assumed to be acidic and other parts are alkaline
	Ionic strength of dye solution: Dissolved salt, either neutral or buffered, in the dye solution influences the interaction of dye and tissue; increased ionic strength decreases the staining of both acidic and basic dyes; salt ions may compete with color ion for binding site on the protein molecules
	Dye concentration: Greater amounts of dye are bound with increasing concentrations of the dye; amount of dye bound by tissue is limited by the number of available binding sites
	Fixation of tissue: When cells are fixed, the affinity for stains increases; protein molecules are reorganized so that chemical groups are more available to the dye (characteristic shared by all fixatives); greater basic dye uptake after formalin fixation; more acidic dye uptake after fixation with mercuric chloride fixatives
	Temperature: Increase in temperature increases the diffusion rate of dye molecules; increase also causes protein molecules to swell the fibers, rendering them more open to dye penetration
	Staining equilibrium: Staining is a reversible reaction; when the solution environment is changed, the equilibrium concentration in the tissue is altered