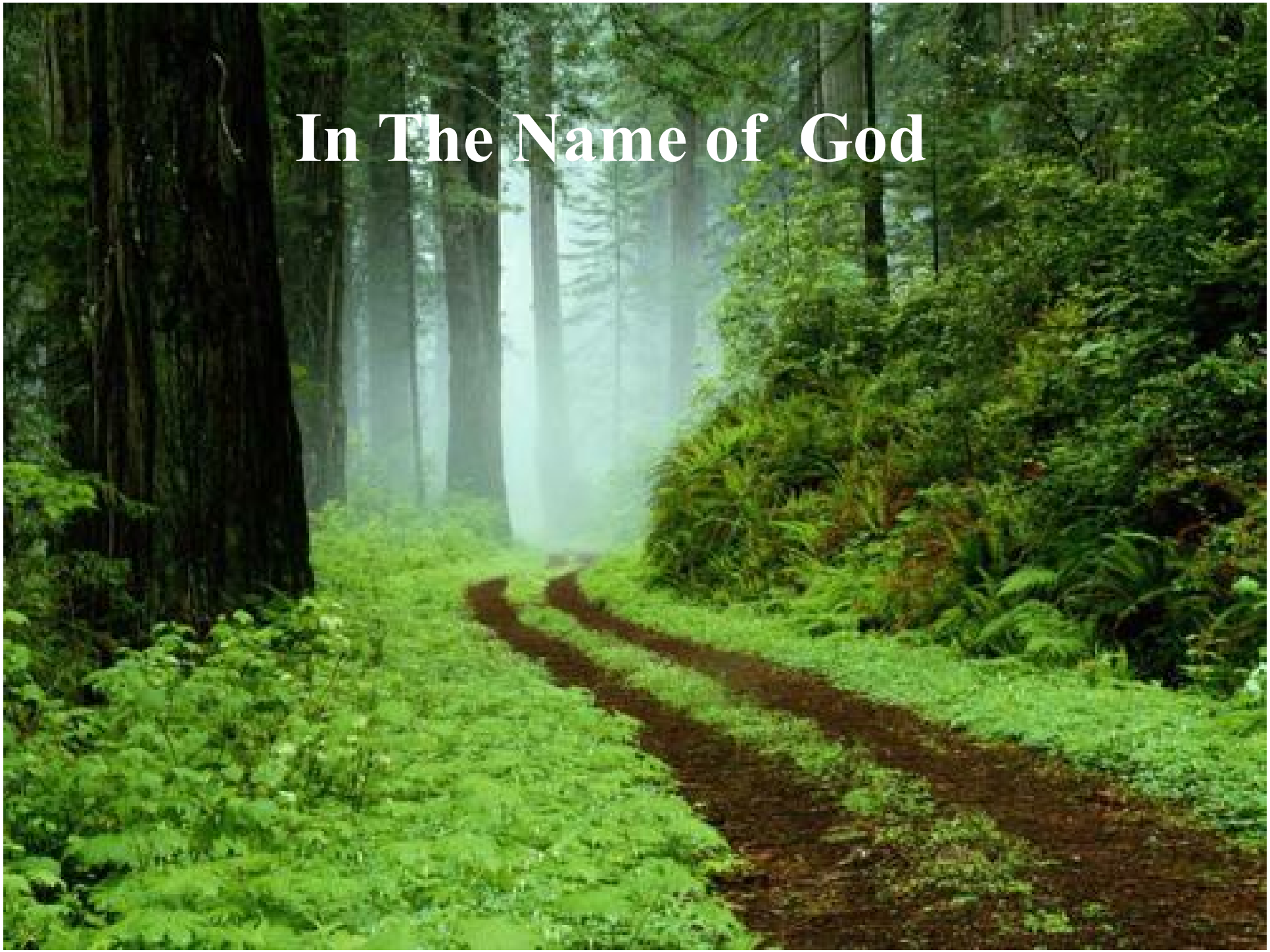

In The Name of God

# Histology



By:
Dr. Borhani

# *Histology*



- Study of tissues of the body & how they are arranged to constitute the organs.
- Histo: tissue or web
- Tissue:

١. Cells

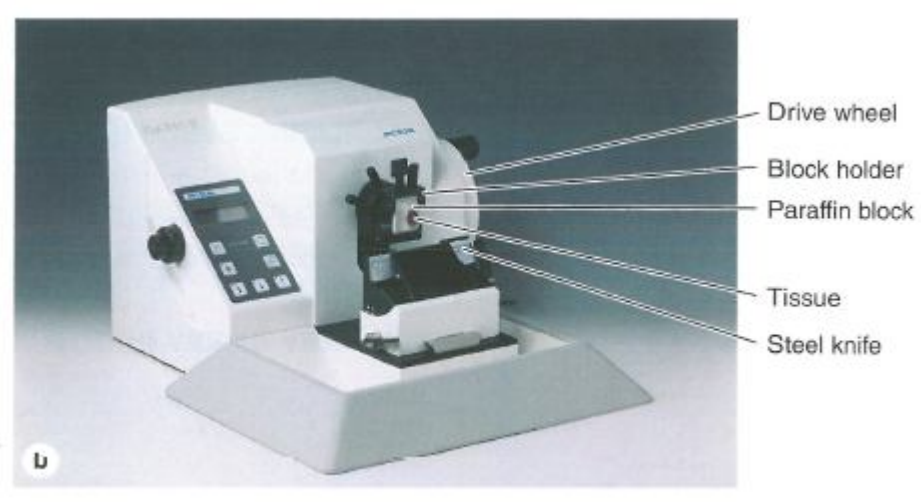
٢. Extra cellular matrix (ECM)

Cell & ECM interaction

# Tissue preparation



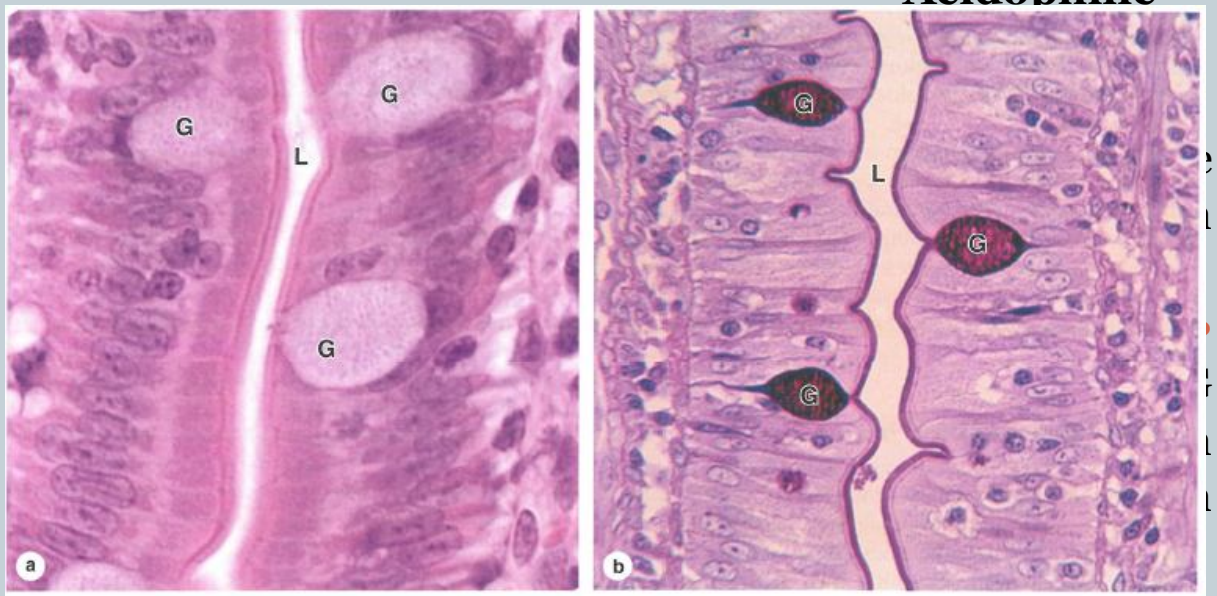
- Fixation
- Embedding
- ectioning
- Staining

# Tissue Staining



- **Acidophilic**

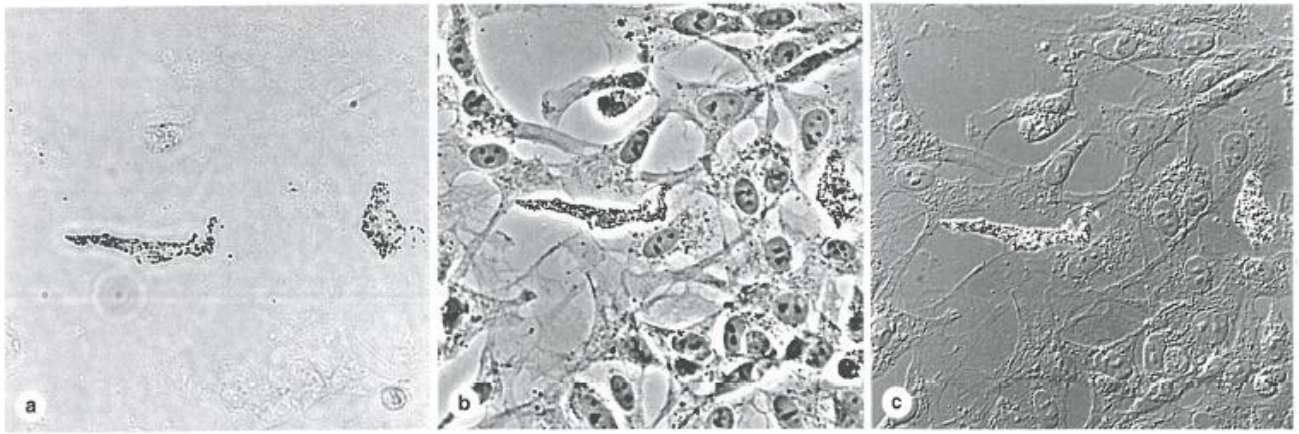



Micrograph of epithelium lining the small intestine, **(a)** stained with H&E, and **(b)** stained with the PAS reaction for glycoproteins. With H&E, basophilic cell nuclei are stained purple while cytoplasm stains pink. Cell regions with abundant oligosaccharides on glycoproteins, such as the ends of the cells at the lumen (**L**) or the scattered mucus-secreting goblet cells (**G**), are poorly stained. With PAS, however, cell staining is most intense at the lumen, where projecting microvilli have a prominent layer of glycoproteins at the lumen (L) and in the mucin-rich secretory granules of goblet cells. Cell surface glycoproteins and mucin are PAS-positive because of their high content of oligosaccharides and polysaccharides respectively. The PAS-stained tissue was counterstained with hematoxylin to show the cell nuclei. Both X300.

- PAS
- Lipid soluble dyes (sudan black)
- Silver salts

# Microscope






Living neural crest cells growing in culture appear differently with various techniques of light microscopy. Here the *same field* of unstained cells, including two differentiating pigment cells, is shown using three different methods (all X200):

**(a) Bright-field microscopy:** Without fixation and staining, only the two pigment cells can be seen.

**(b) Phase-contrast microscopy:** Cell boundaries, nuclei, and cytoplasmic structures with different refractive indices affect in-phase light differently and produce an image of these features in *all* the cells.

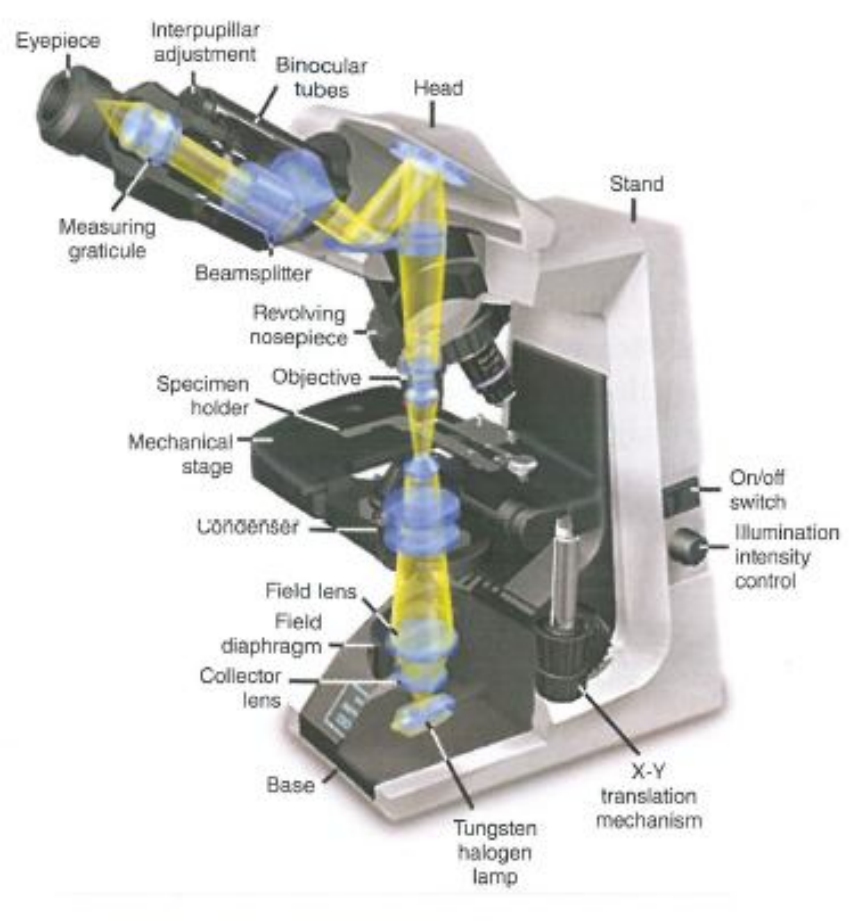
**(c) Differential interference microscopy:** Cellular details are highlighted in a different manner using Nomarski optics. Phase-contrast microscopy, with or without differential interference, is widely used to observe live cells grown in tissue culture.

*(With permission, from Dr Sherry Rogers, Department of Cell Biology and Physiology, University of New Mexico, Albuquerque, NM.)*

# Bright field microscope

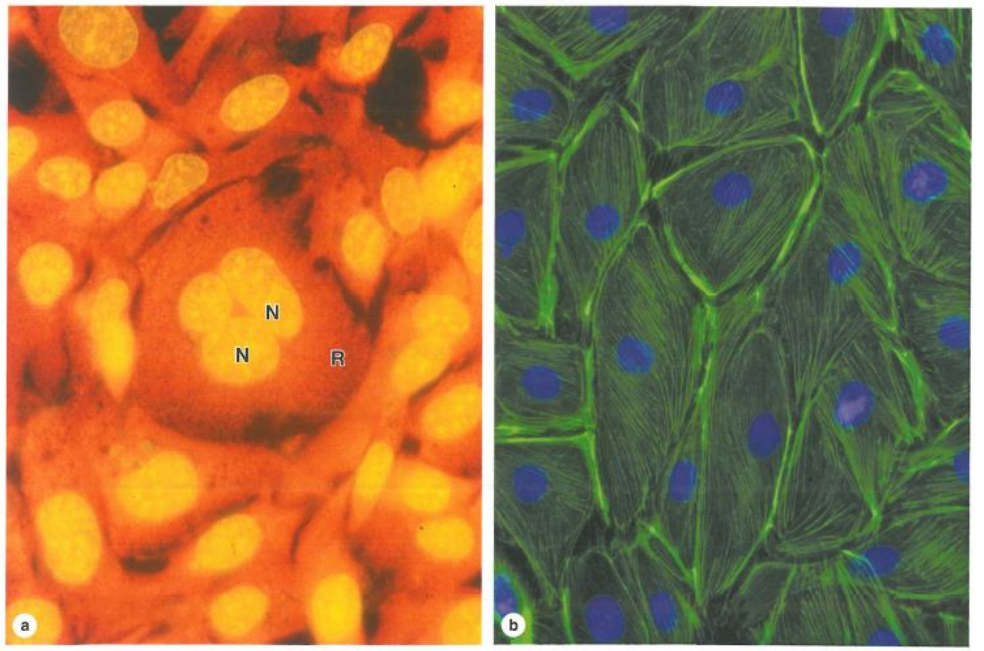


- Condensor



# Microscope





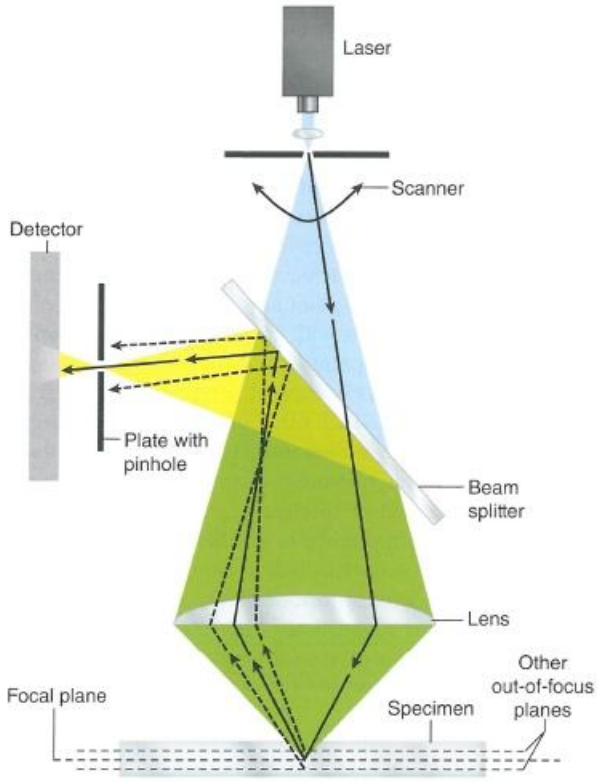

Components of cells are often stained with compounds visible by fluorescence microscopy.

**(a)** Acridine orange binds nucleic acids and causes DNA in cell nuclei (**N**) to emit yellow light and the RNA-rich cytoplasm (**R**) to appear orange in these cells of a kidney tubule.

**(b)** Cultured cells stained with DAPI (4′,6-diamino-2-phenylindole) that binds DNA and with fluorescein-phalloidin that binds actin filaments show nuclei with blue fluorescence and actin filaments stained green. Important information such as the greater density of microfilaments at the cell periphery is readily apparent. Both X500.

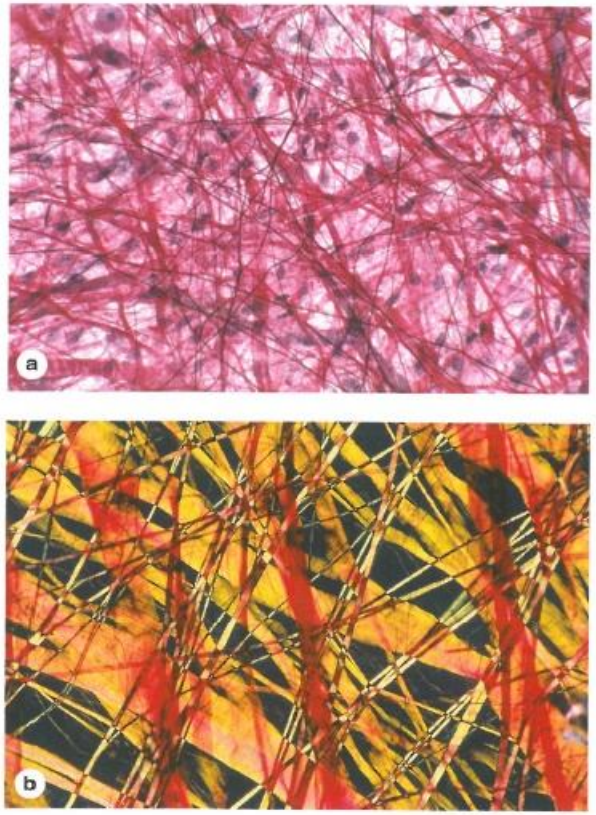
*(Figure 1–4b, contributed with permission, from Drs Claire E. Walczak and Rania Risk, Indiana University School of Medicine, Bloomington.)*

**FIGURE 1–6 Principle of confocal microscopy.**




Although a very small spot of light originating from one plane of the section crosses the pinhole and reaches the detector, rays originating from other planes are blocked by the blind. Thus, only one very thin plane of the specimen is focused at a time. The diagram shows the practical arrangement of a confocal microscope. Light from a laser source hits the specimen and is reflected. A beam splitter directs the reflected light to a pinhole and a detector. Light from components of the specimen that are above or below the focused plane is blocked by the blind. The laser scans the specimen so that a larger area of the specimen can be observed.

**FIGURE 1–7 Tissue appearance with bright-field and polarizing microscopy.**

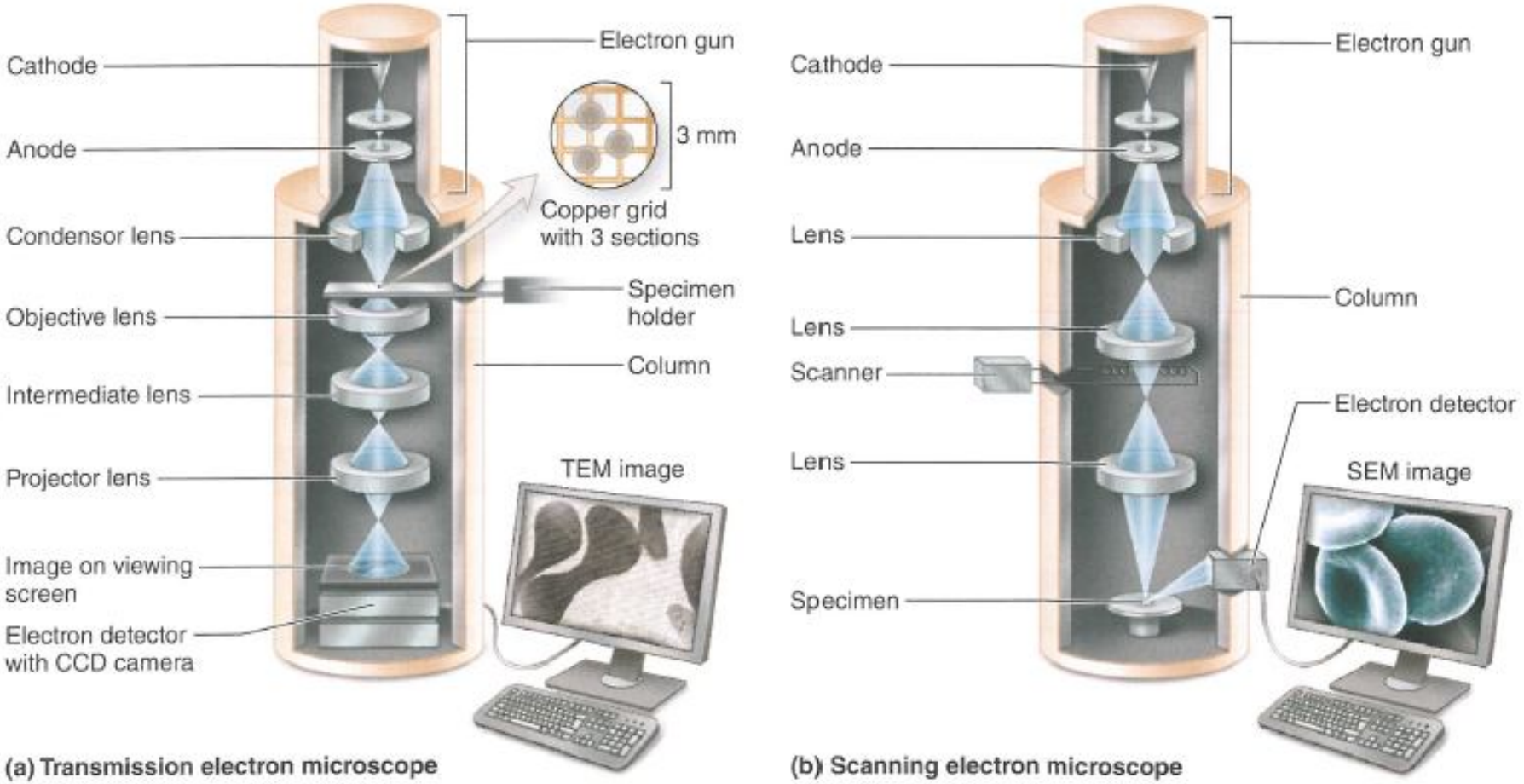



Polarizing light microscopy produces an image only of material having repetitive, periodic macromolecular structure; features without such structure are not seen. Pieces of thin, unsectioned mesentery were stained with red picrosirius, orcein, and hematoxylin, placed on slides and observed by bright-field **(a)** and polarizing **(b)** microscopy.

**(a)** With bright-field microscopy collagen fibers appear red, with thin elastic fibers and cell nuclei darker.

**(b)** With polarizing microscopy, only the collagen fibers are visible and these exhibit intense yellow or orange birefringence (a: X40; b: X100).

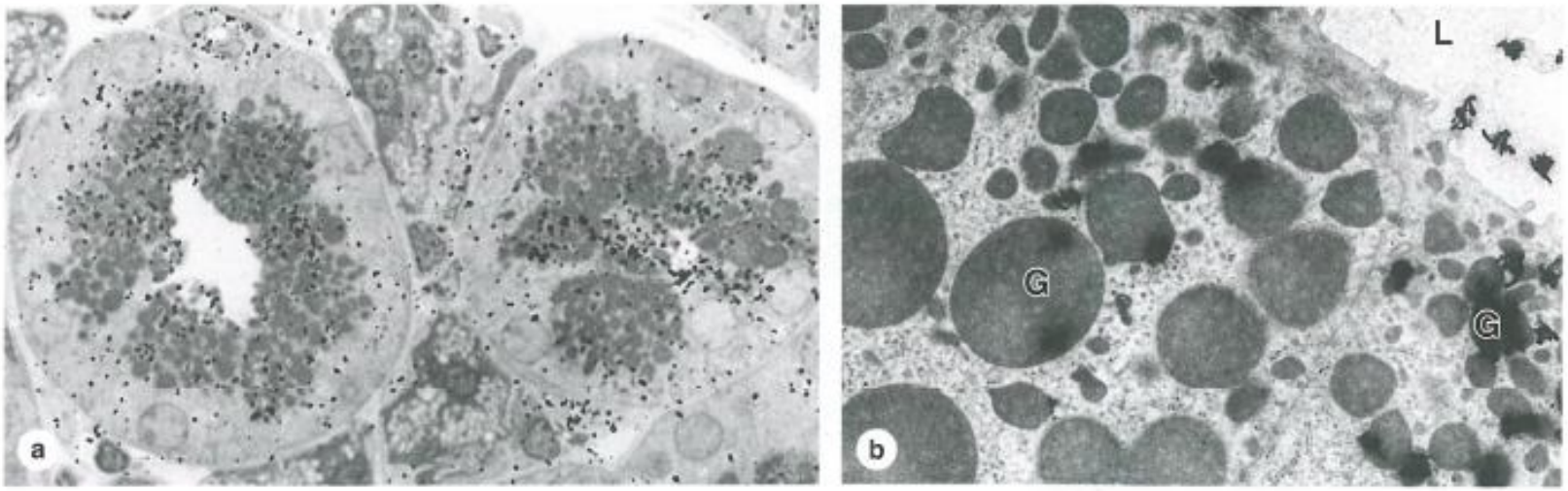
FIGURE **1–8** **Electron microscopes.**




**(a) Transmission electron microscope**

**(b) Scanning electron microscope**

## FIGURE 1–9 Microscopic autoradiography.




Autoradiographs are tissue preparations in which particles called **silver grains** indicate the cells or regions of cells in which specific macromolecules were synthesized just prior to fixation. Shown here are autoradiographs from the salivary gland of a mouse injected with $^{3}$H-fucose 8 hours before tissue fixation. Fucose was incorporated into oligosaccharides, and the free $^{3}$H-fucose was removed during fixation and sectioning of the gland. Autoradiographic processing and microscopy reveal locations of newly synthesized glycoproteins containing that sugar.

**(a)** Black grains of silver from the light-sensitive material coating the specimen are visible over cell regions with secretory granules and the duct indicating glycoprotein locations. X1500.

**(b)** The same tissue prepared for TEM autoradiography shows silver grains with a coiled or amorphous appearance again localized mainly over the granules (**G**) and in the gland lumen (**L**). X7500.

*(Figure 1–9b, with permission, from Drs Ticiano G. Lima and A. Antonio Haddad, School of Medicine, Ribeirão Preto, Brazil.)*

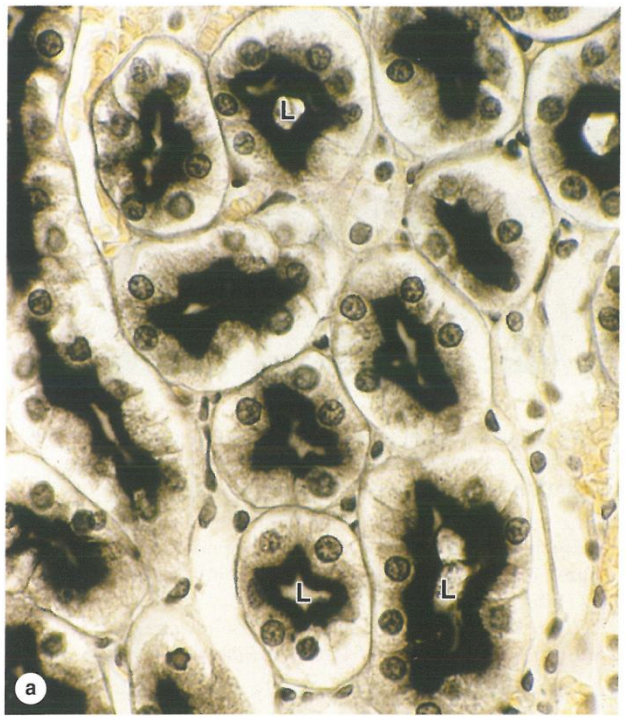
# Cell & Tissue Culture

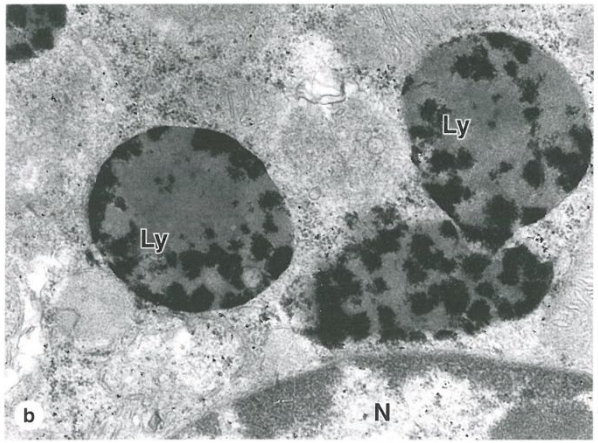
# Enzyme histochemistry



- Phosphatases
- Dehydrogenases

FIGURE **1–10** Enzyme histochemistry.




**(a)** Micrograph of cross sections of kidney tubules treated histochemically to demonstrate alkaline phosphatases shows strong activity of this enzyme at the apical surfaces of the cells at the lumens (**L**) of the tubules. X200.

**(b)** TEM image of a kidney cell in which acid phosphatase has been localized histochemically in three lysosomes (**Ly**) near the nucleus (**N**). The dark material within these structures is lead phosphate that precipitated in places with acid phosphatase activity. X25,000.

(*Figure 1–10b, with permission, from Dr Eduardo Katchburian, Department of Morphology, Federal University of São Paulo, Brazil.*)

# Visualizing specific molecules



- Phalloidin (actin)
- Protein A (FC of ntibody)
- Lectins (carbohydrat)

- **Immunohistochemistry**

Direct

Indirect

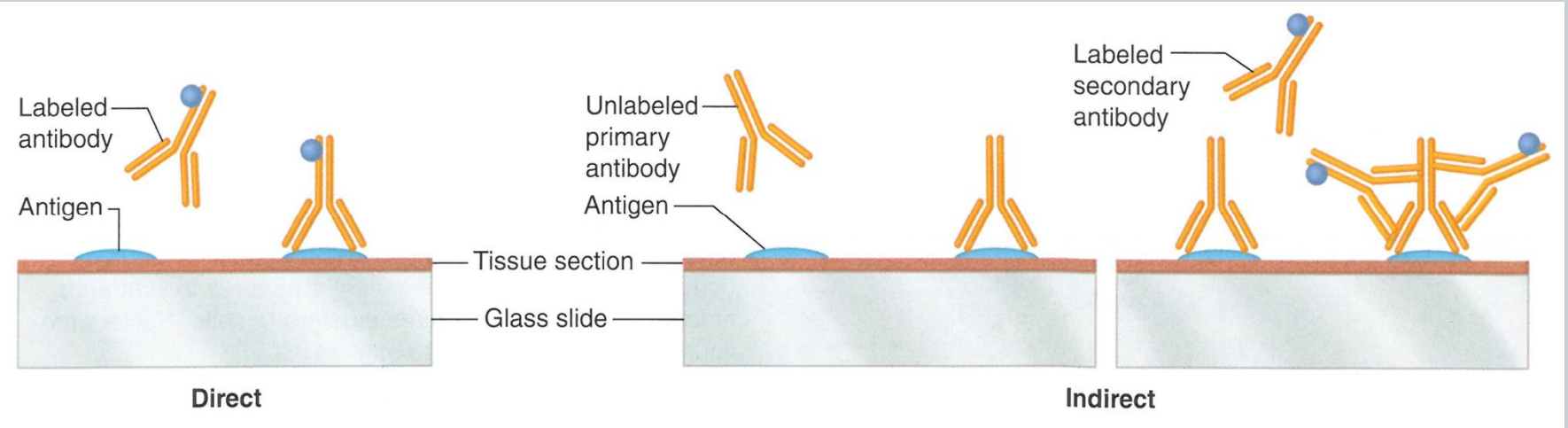
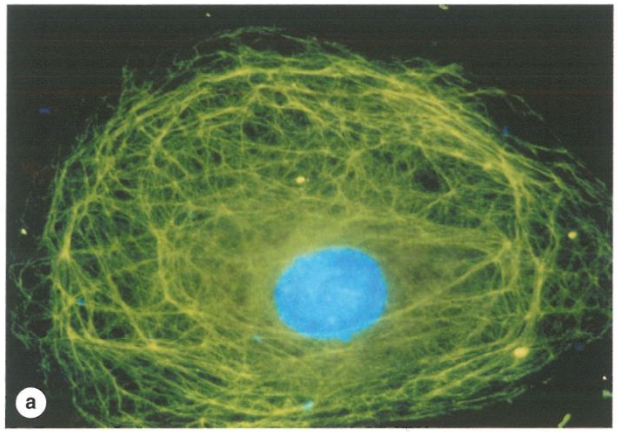
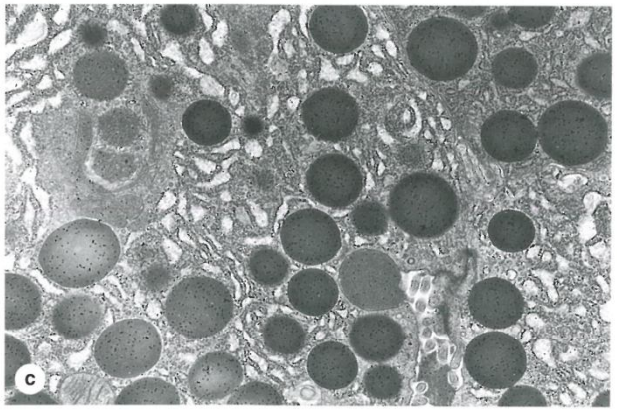
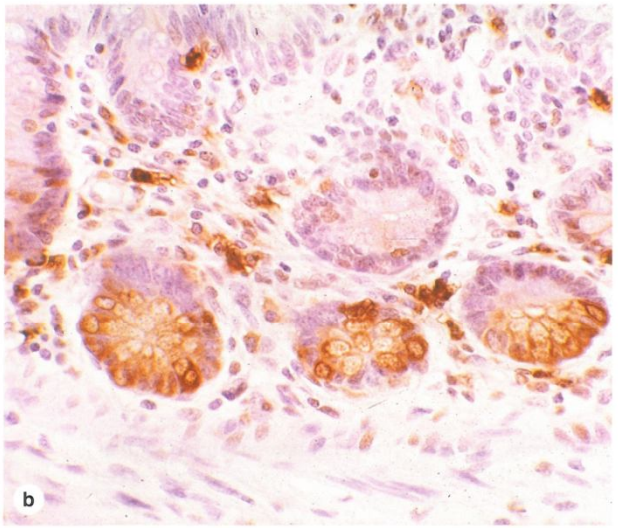


FIGURE **1–12** **Cells and tissues stained by immunohistochemistry.**




Immunocytochemical methods to localize specific proteins can be applied to either light microscopic or TEM preparations using a variety of labels.

**(a)** A single cultured uterine cell stained fluorescently to reveal a meshwork of intermediate filaments (green) throughout the cytoplasm. Primary antibodies against the filament protein desmin and fluorescein isothiocyanate (FITC)–labeled secondary antibodies were used in the indirect staining technique, with the nucleus counterstained blue with DAPI. X650.

**(b)** A section of small intestine treated with an antibody against the enzyme lysozyme. The secondary antibody labeled with peroxidase was then applied and the localized brown color produced histochemically with the peroxidase substrate 3,3′-diamino-azobenzidine (DAB). The method demonstrates lysozyme-containing structures in scattered macrophages and in the large clusters of cells. Nuclei were counterstained with hematoxylin. X100.

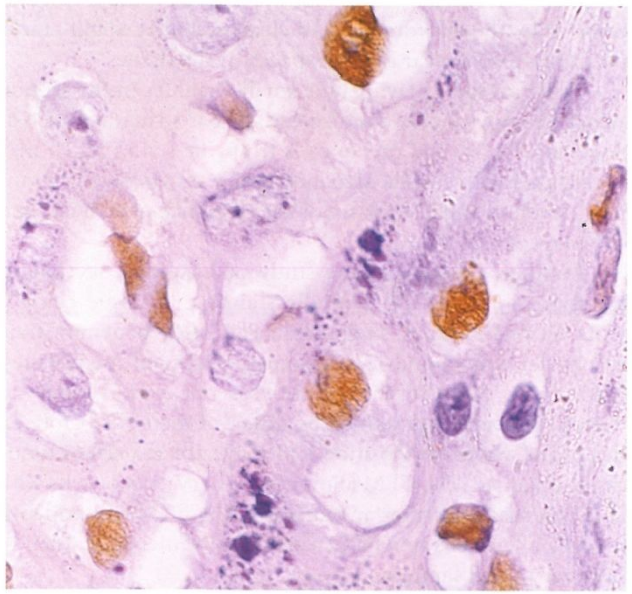
**(c)** A section of pancreatic cells in a TEM preparation incubated with an antibody against the enzyme amylase and then with protein A coupled with gold particles. Protein A has high affinity toward antibody molecules and the resulting image reveals the presence of amylase with the gold particles localized as very small black dots over dense secretory granules and developing granules (left). With specificity for immunoglobulin molecules, labeled protein A can be used to localize any primary antibody. X5000.

(*Figure 1–12c, with permission, from Dr Moise Bendayan, Departments of Pathology and Cell Biology, University of Montreal, Montreal, Canada.*)

**TABLE 1–1** Examples of specific antigens with diagnostic importance.

| Antigens | Diagnosis |
|---|---|
| Specific cytokeratins | Tumors of epithelial origin |
| Protein and polypeptide hormones | Certain endocrine tumors |
| Carcinoembryonic antigen (CEA) | Glandular tumors, mainly of the digestive tract and breast |
| Steroid hormone receptors | Breast duct cell tumors |
| Antigens produced by viruses | Specific virus infections |

# Hybridization techniques



ISH of this tissue section reveals that many cells contain the human papilloma virus (HPV). The section was incubated with a solution containing a digoxigenin-labeled complementary DNA (cDNA) probe for the HPV DNA. The probe was then visualized by direct immunohistochemistry using peroxidase-labeled antibodies against digoxigenin. This procedure stains brown only those cells containing HPV. X400. H&E.

*(With permission, from Dr Jose E. Levi, Virology Lab, Institute of Tropical Medicine, University of São Paulo, Brazil.)*

# Interpretation of structures in tissue sections



## Artifact

In thin sections 3D structures appear to have only two dimensions. Such images must be interpreted correctly to understand the actual structure of tissue and organ components. For example, blood vessels and other tubular structures appear in sections as round or oval shapes whose size and shape depend on the transverse or oblique angle of the cut. A highly coiled tube will appear as several round and oval structures. In TEM sections of cells, round structures may represent spherical organelles or transverse cuts through tubular organelles such mitochondria. It is important to develop such interpretive skill to understand tissue and cell morphology in microscopic preparations.