Introduction to Histology

The name "**Histology**" is derived from the Greek word for a tissue "*Histos*", and "*-logos*" = “the study of”

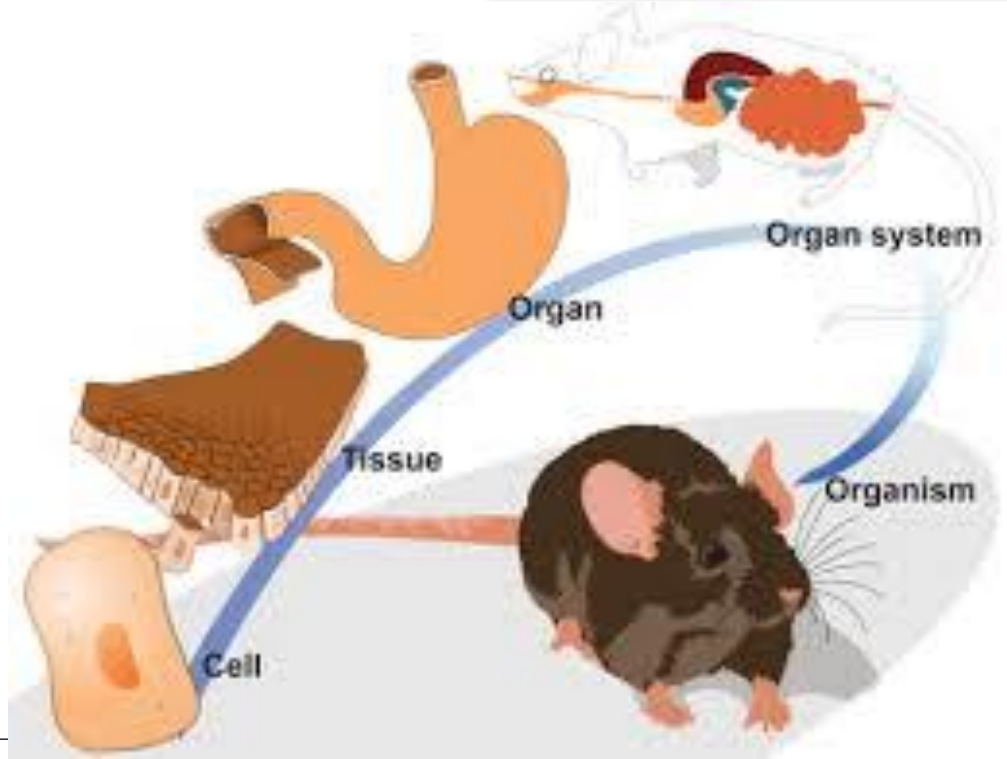
It is tightly bounded to molecular biology, genetics, immunology and other basic sciences

Cells are the basic unit of structure and function in living things

Organs made up of tissues that work together to perform a specific activity

Tissues made up of cells that are similar in structure and function and which work together to perform a specific activity

Systems are groups of two or more organs that work together to perform a specific function for the organism



Theory	**Practical**
Introduction to histology	----------
Cell overview	Microscopes and Microtechniques
Epithelium 1	Cell overview
Epithelium 2	Epithelium-1
Connective tissue 1	Epithelium-2
Connective tissue 2	Revision- Quiz
Cartilage	Connective tissue-1
Midterm exam	
Bone 1	Connective tissue-2
Bone 2	Cartilage
Muscular tissue	Bone-1
Nervous tissue	Bone-2
Skin 1	Muscular tissue
Skin 2	Skin

Suggested Histology Reference

Junqueira's Basic Histology

Text and Atlas

13th edition

By Anthony L. Mescher

History

- **Bichat** is the first anatomist who defined the term "TISSUE" without the use of microscope

Units used in microscopy

- One millimter = 1000 micrometer (μm)
- One micrometer = 1000 nanometer (nm)

Microtechniques

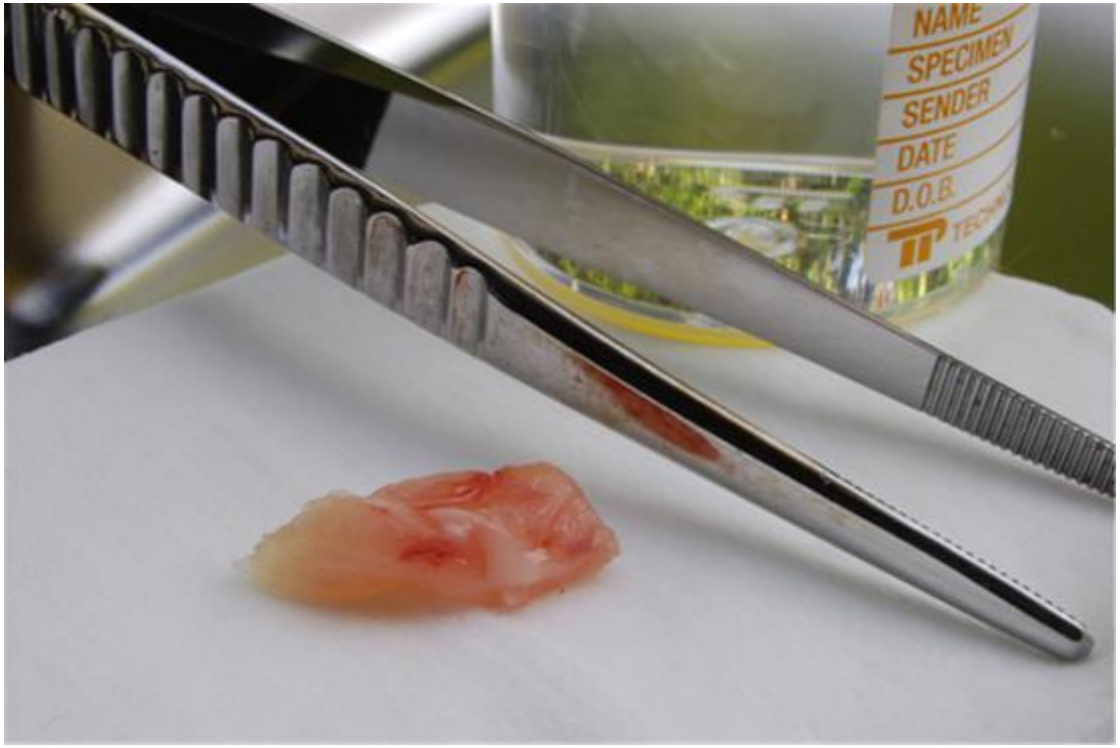
- Tissue preparation for microscopic examination
- There are different methods but the basic principles are similar

Hardening and sectioning of the tissue

❖ Examples : paraffin and freezing techniques

Microtechniques

1. Fixation
2. Dehydration
3. Clearing
4. Impregnation (infiltration)
5. Embedding
6. Section cutting
7. Staining
8. Mounting



Fixation: Exposing the tissue to chemical agents called fixatives i.e paraformaldehyde

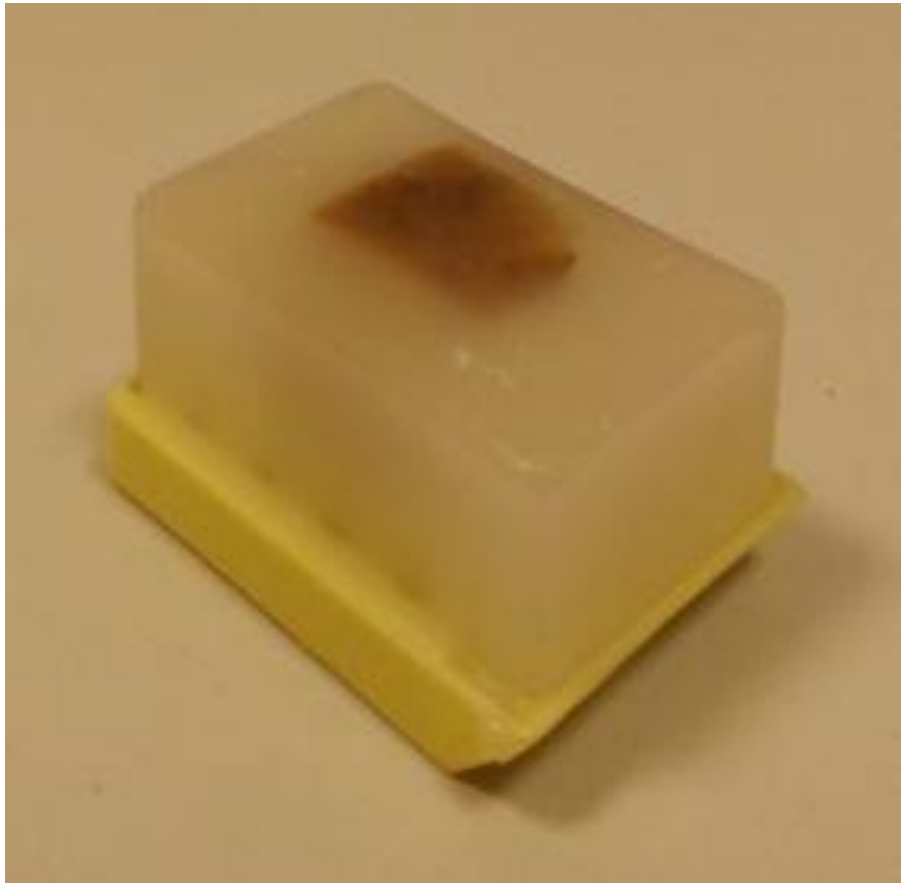
Dehydration

The process to remove the water by using a graded series of alcohol
Then the tissue can be filled with the paraffin or other embedding agent

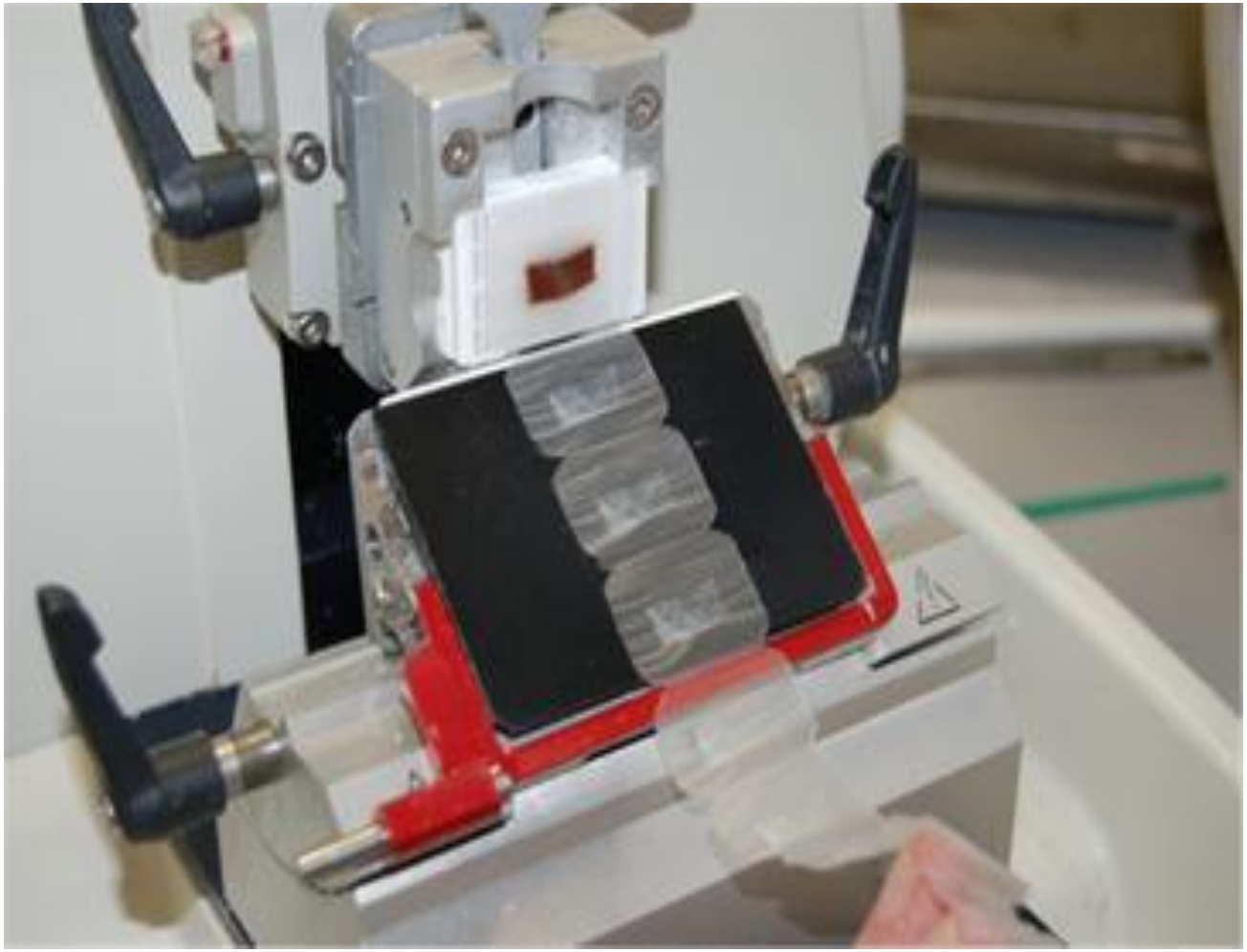
Clearing

- Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium. i.e Xylene

Embedding

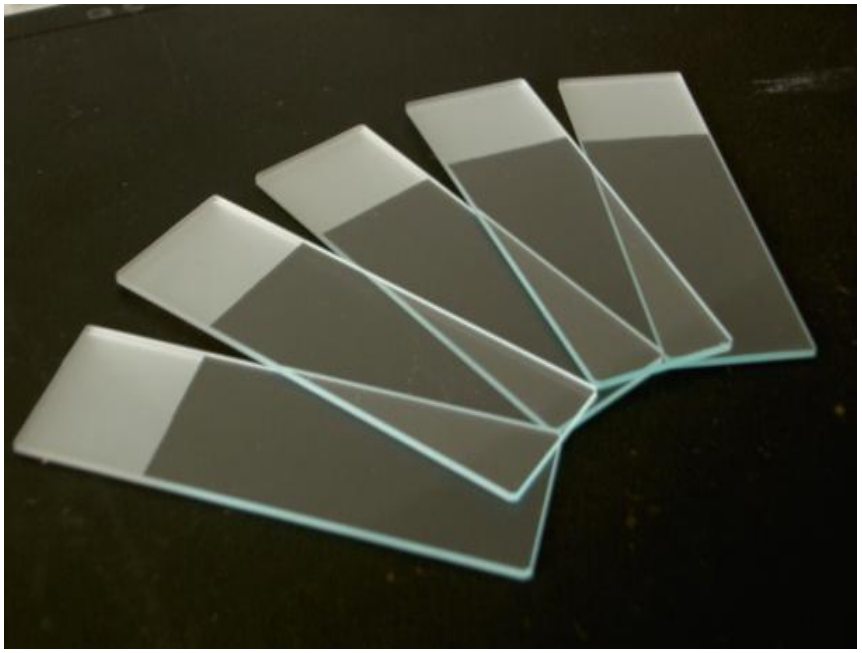


Sectioning



Mounting

- The process to place (mount) the tissue sections on the adhesive coated glass slides

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SLIDES

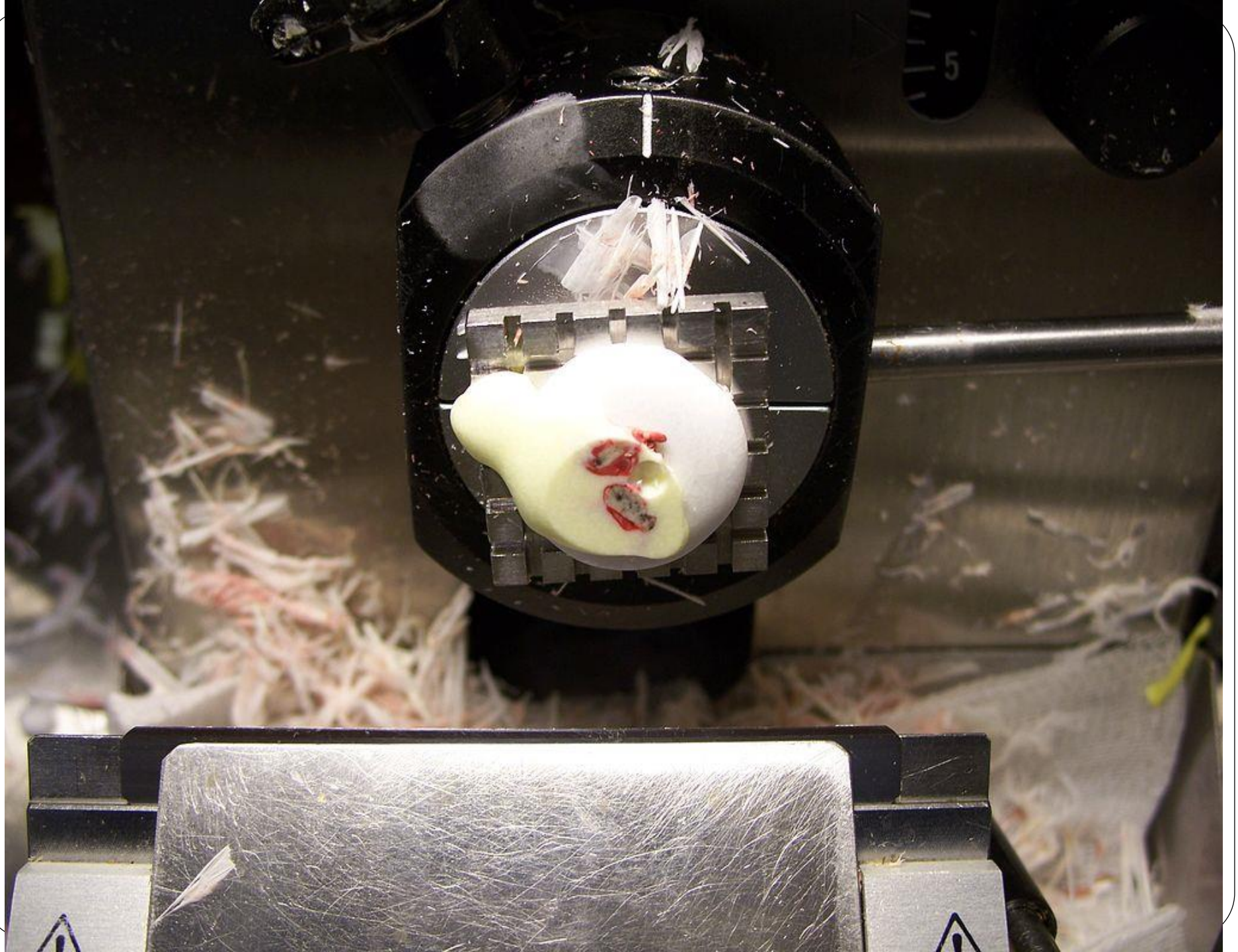

Geoff

Freezing technique

- Tissues are frozen using liquid nitrogen
- Frozen tissues are sectioned by cryostat
- It is faster and preserve tissue components

The quality of the section is poor with more artifacts
While paraffin technique produces intact tissue with less artifacts


LEICA 3050 S



Staining techniques

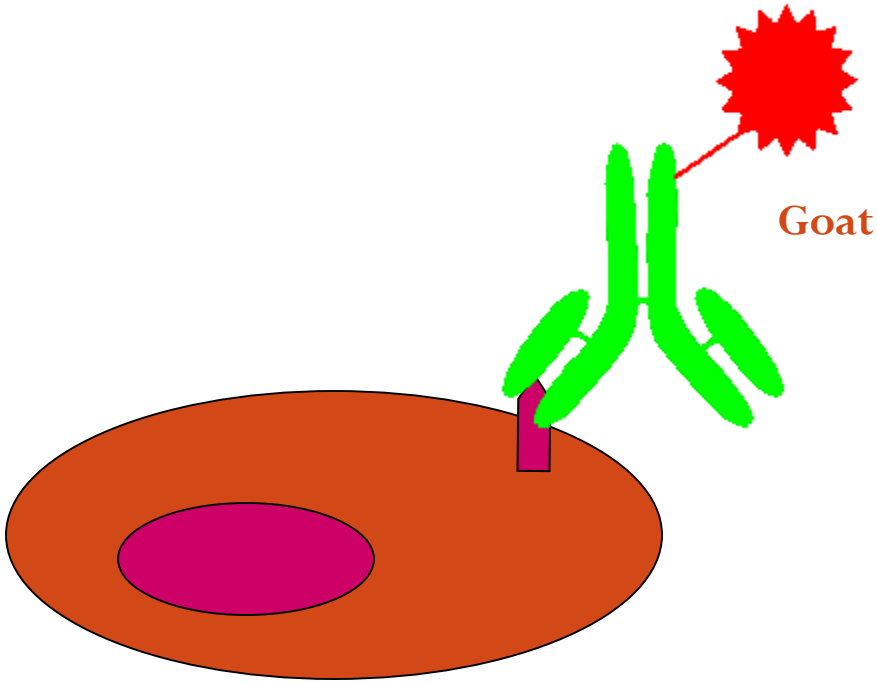
- The stain is a chemical substance which reacts with certain tissue components producing a color

1. Ordinary stains
2. Immunohistochemistry and Immunocytochemistry
3. Hybridization techniques

Immunohistochemistry

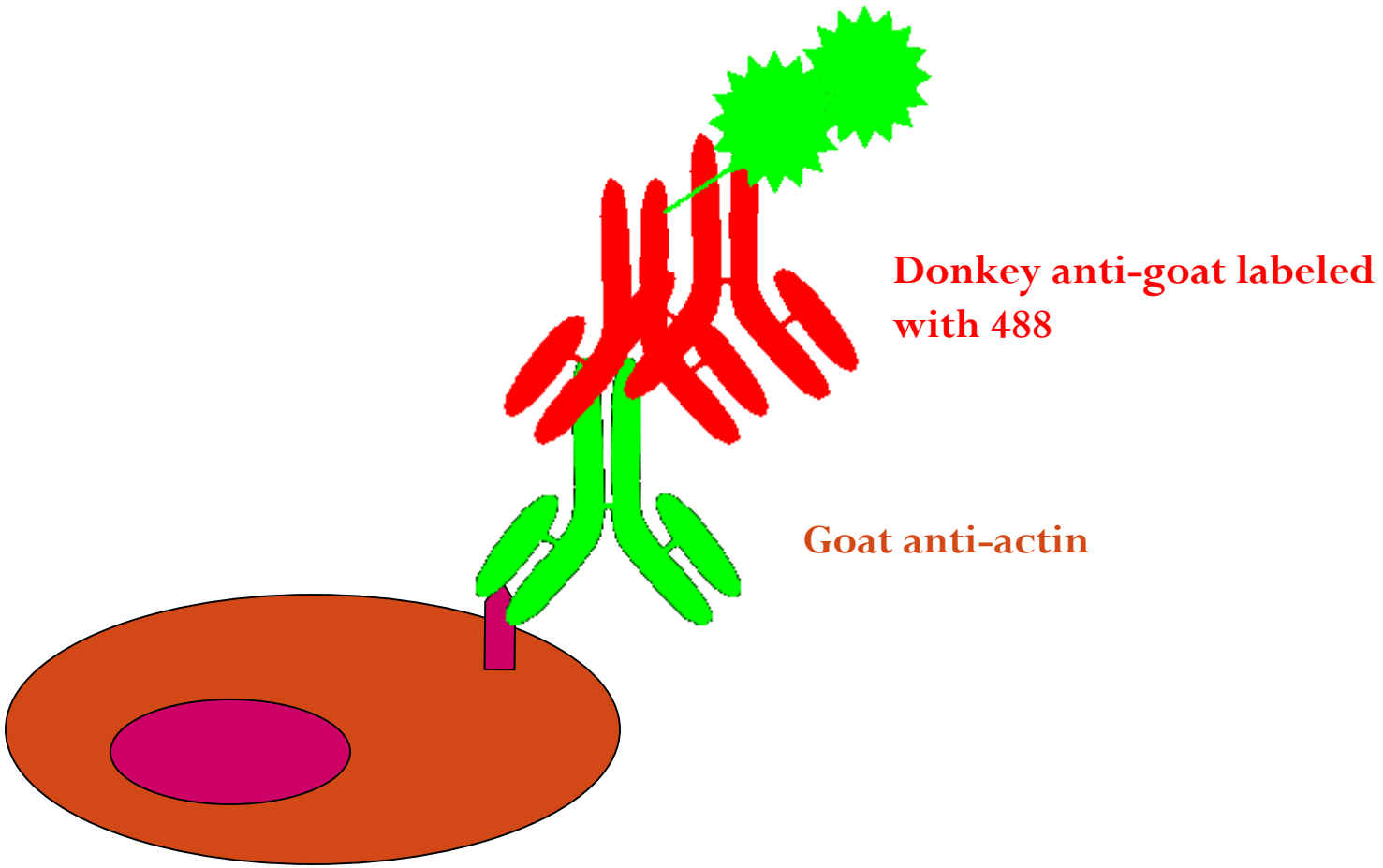
- Rely on the use of antibody directed against molecule of interest, usually protein
- The antibody is usually labeled with a colored substance

Direct method-
primary antibody only



Goat anti-actin labeled with 594

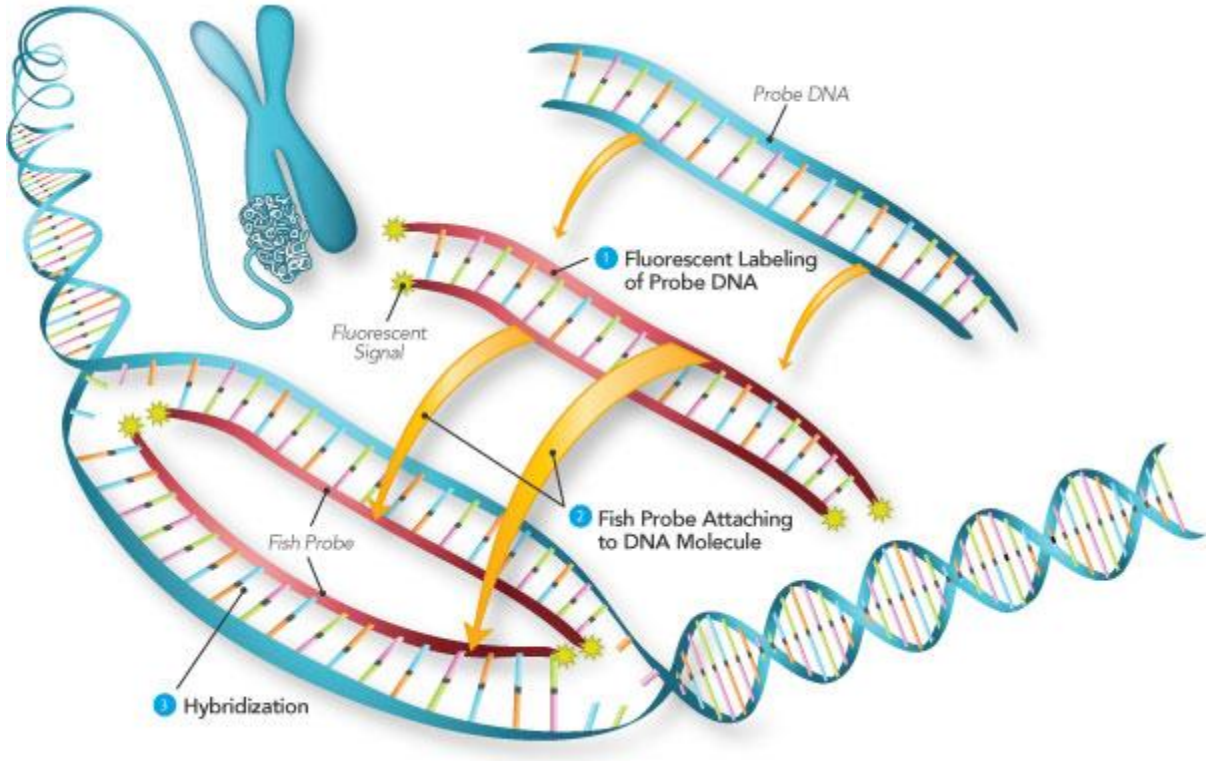
Indirect method – primary and secondary antibodies



Hybridization techniques

- To detect and localize the presence or absence of specific DNA sequences on chromosomes
- to detect and localize specific RNA targets (ex. mRNA) in cells
- a small oligonucleotide which is complementary to the target DNA/RNA sequence is used (ex. fluorescent probes)
- Can be applied to tissue sections, smears or chromosomes

Hybridization techniques



Microscopy

- Light Microscopy
  - ✓ Phase contrast
  - ✓ Interference
  - ✓ Fluorescence
  - ✓ Polarizing

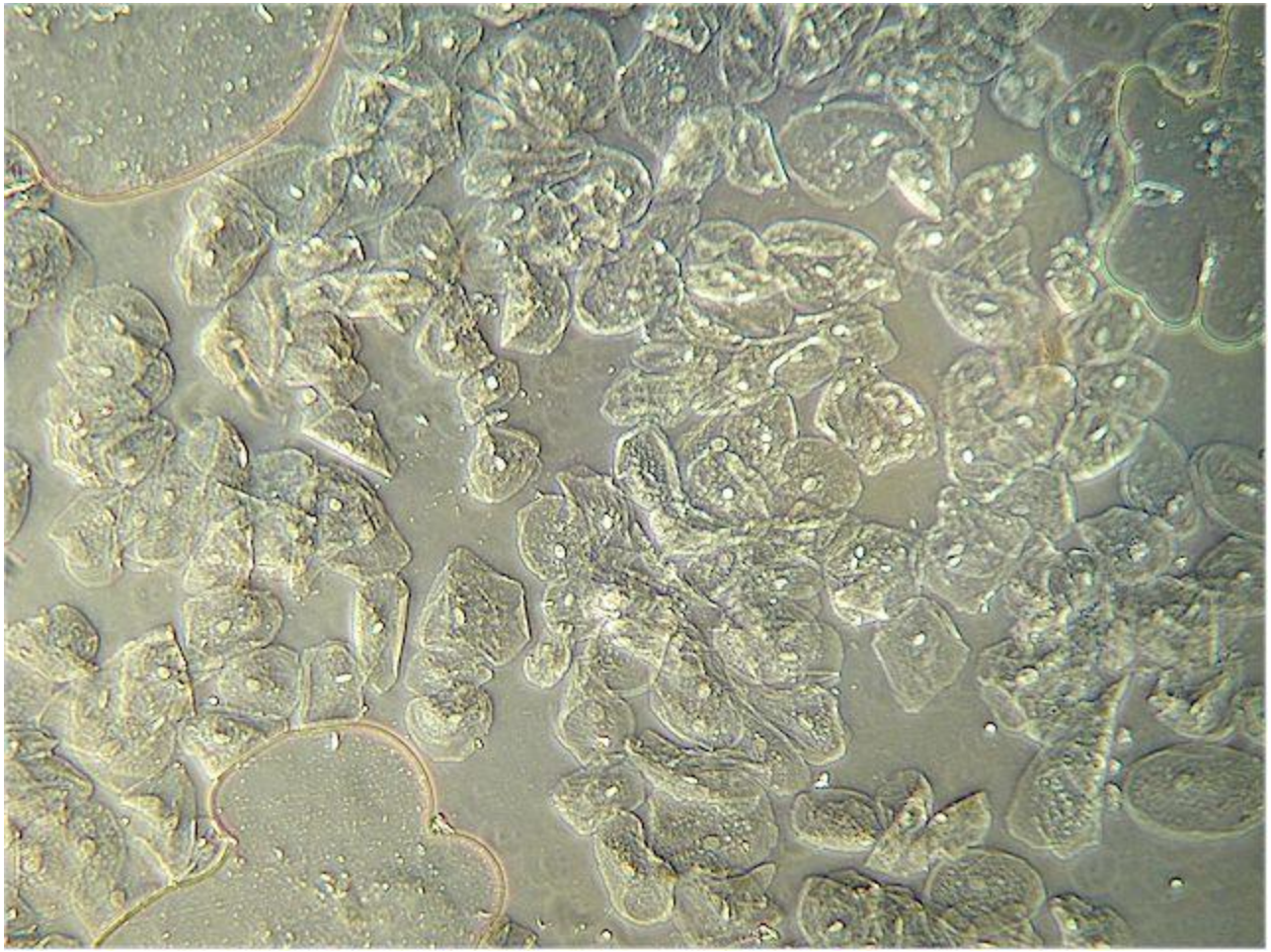
- Electron Microscopy
  - ✓ Transmission EM
  - ✓ Scanning EM

Light microscopy

- The basic functional unit consists of a tube; having an objective lens at one end and an ocular lens at the other
- The objective lens enlarges the image of the object in the direction of the ocular lens
- The ocular lens further magnifies this image toward the observer's eye
- The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses

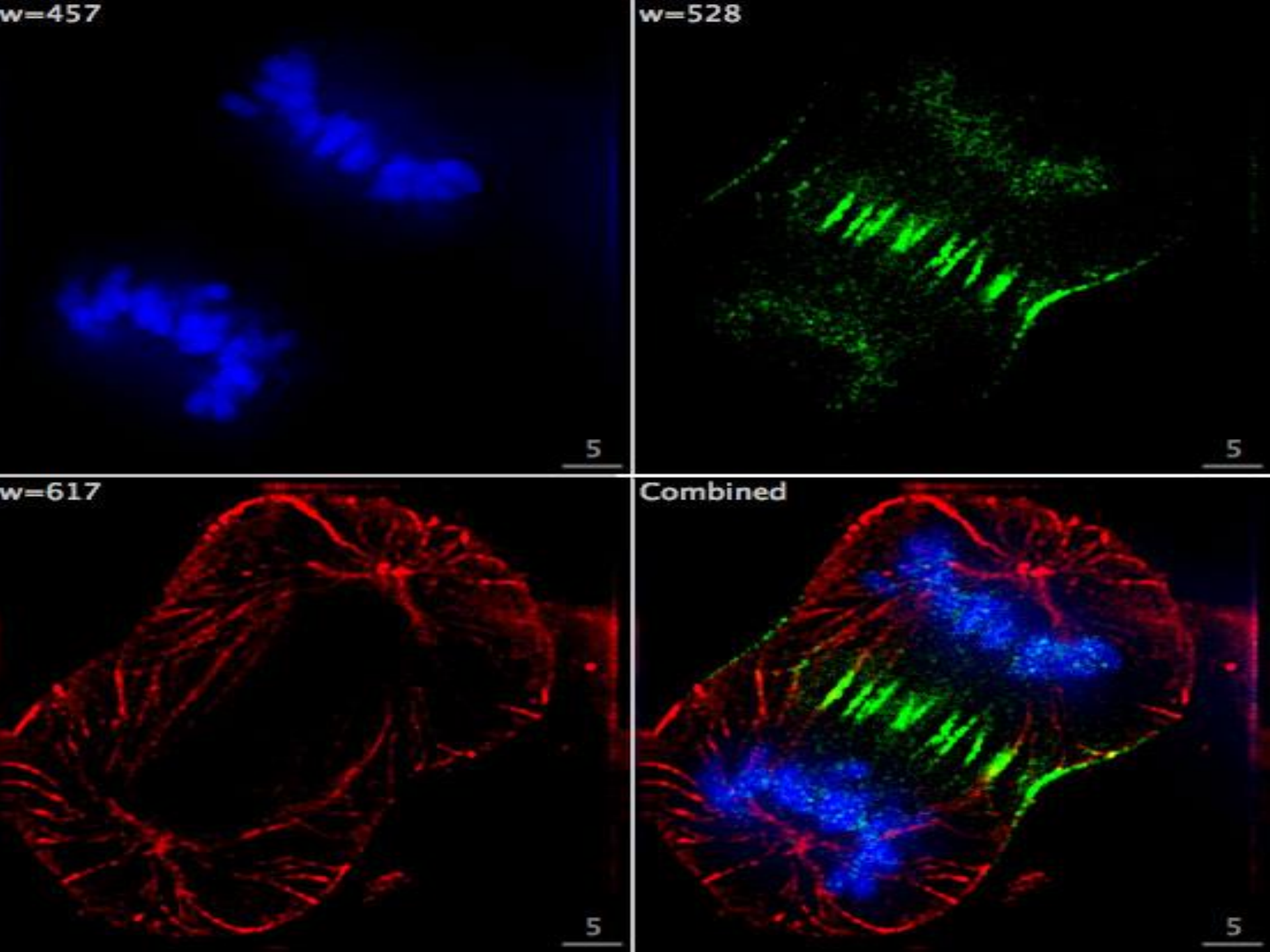
Phase Contrast Microscopy

- It uses a lens system that produces visible images from transparent objects
- The structures appear lighter or darker relative to each other
- The light changes its speed and direction when passing in different media
- Useful in tissue culture



Fluorescence Microscopy

- Uses ultraviolet light
- When certain fluorescent substances are irradiated with ultra violet light, it emits light
- They appear as shiny particles on a dark background
- Placed in dark room


w=457
5
w=528
5
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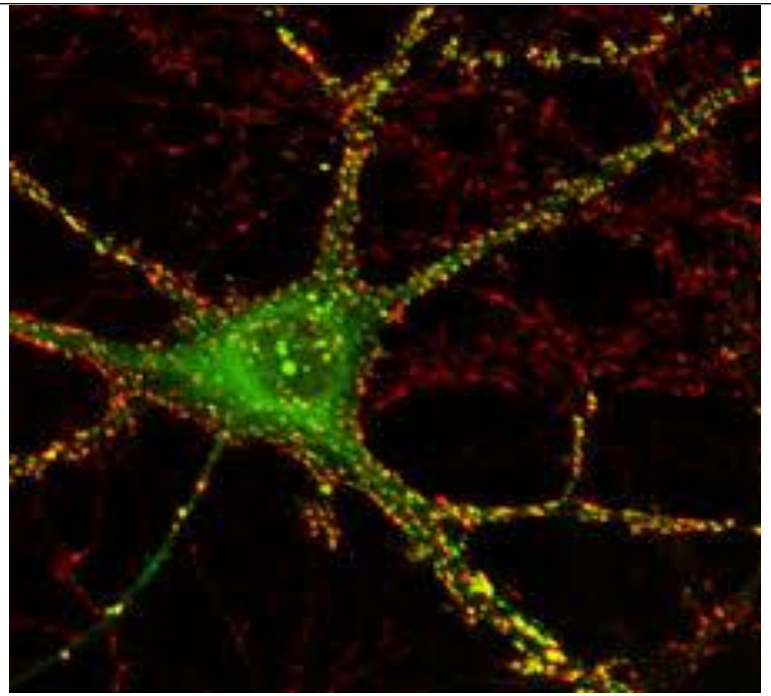
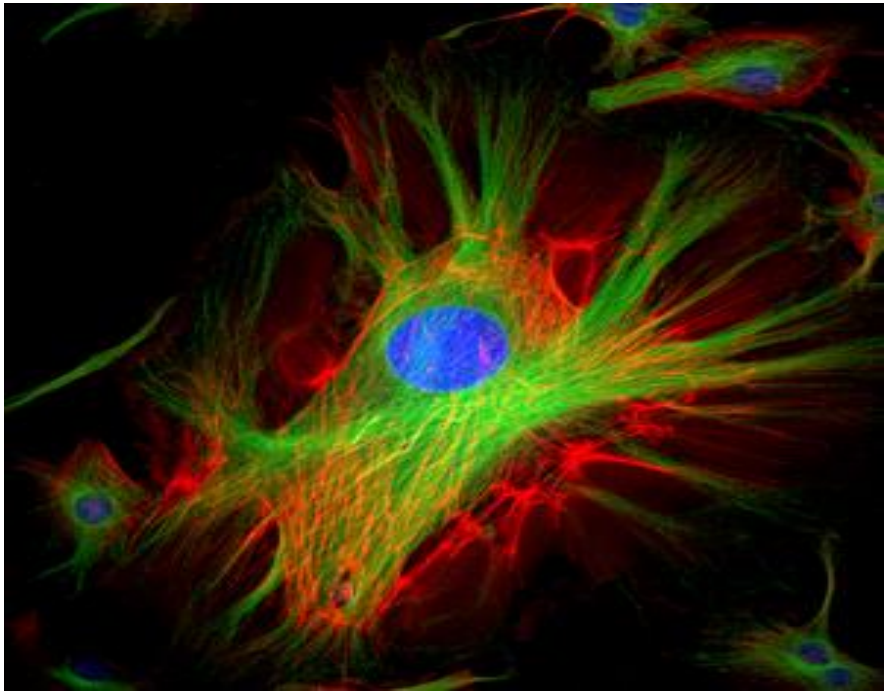
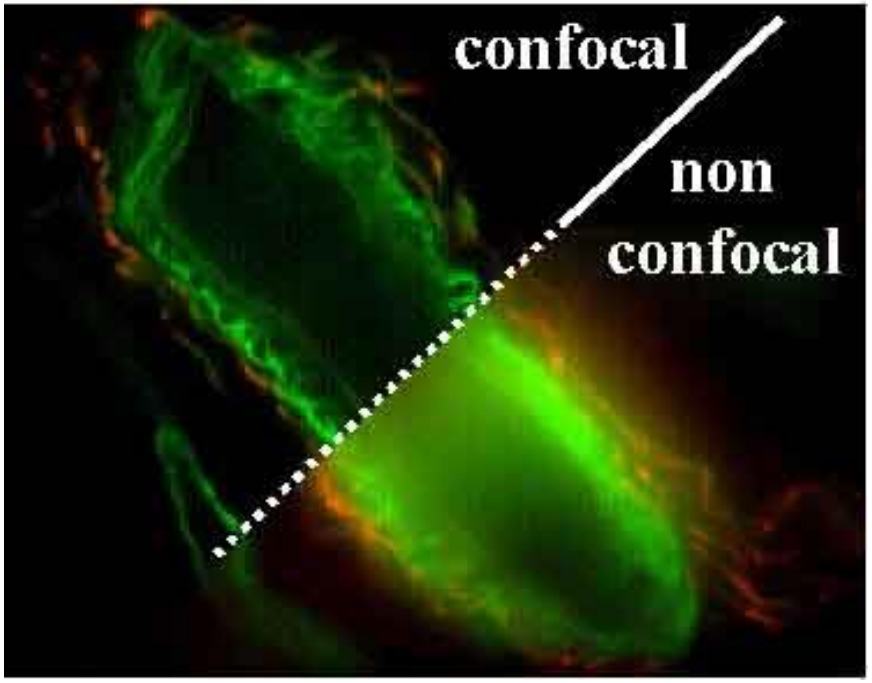
5
Combined
5

Confocal Microscopy

- Uses laser beams
- the laser can be moved (scanned) across the specimen as well as down into the specimen, it can produce 3D images
- Can be used in living and cultured cells and tissue sections






confocal
non
confocal

Electron Microscopy

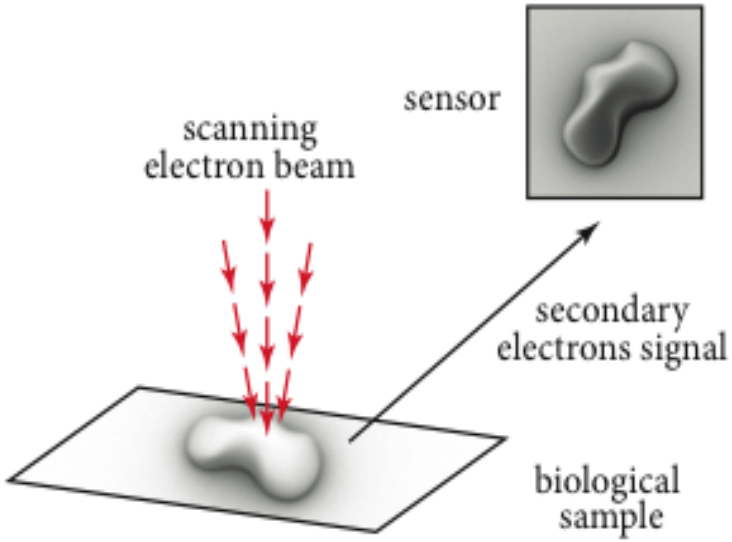
- Uses electron beams instead of light
- Provides the highest resolution of subcellular structures
- Electromagnets to focus the electrons (versus glass lenses to focus the light)
- Detect by fluorescent screen or photographic emulsion
- Requires ultrathin sections (0.02-0.1 μm)
- Uses hard epoxy resin for embedding
- Ultrathin sections are produced by ultramicrotome

  (Diamond or Glass knives)

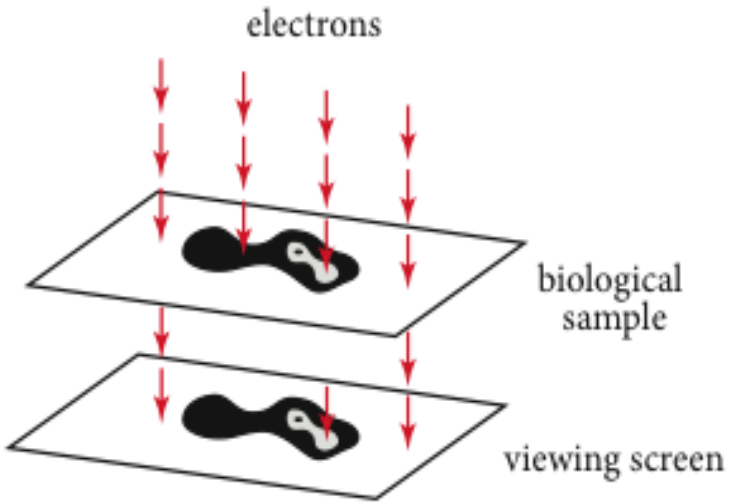
Types

- Transmission EM
  - Views the ultrastructural details in shades of gray
  - The bright areas of the images are unstained (the electrons passed through the sample) and the darker regions are areas which have taken up stain and either absorbed or scattered the electrons

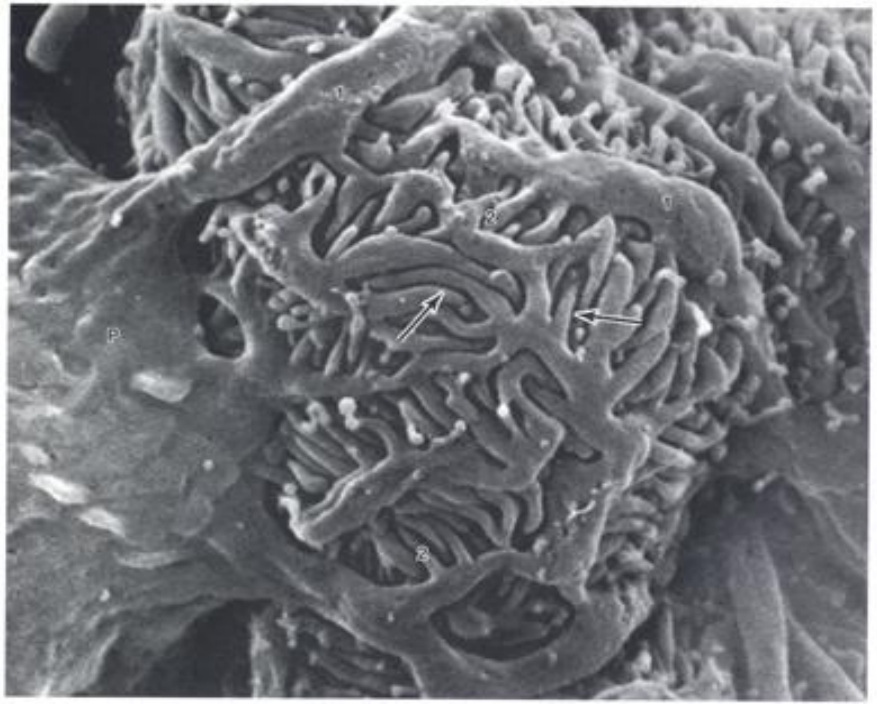
- Scanning EM
  - Provides information about the surface of a specimen
  - Samples are coated with a gold-carbon film. The electron beam is then scanned across the specimen surface and the electrons that are reflected off of the surface are captured by the detector
  - Views only the structure as a 3D image

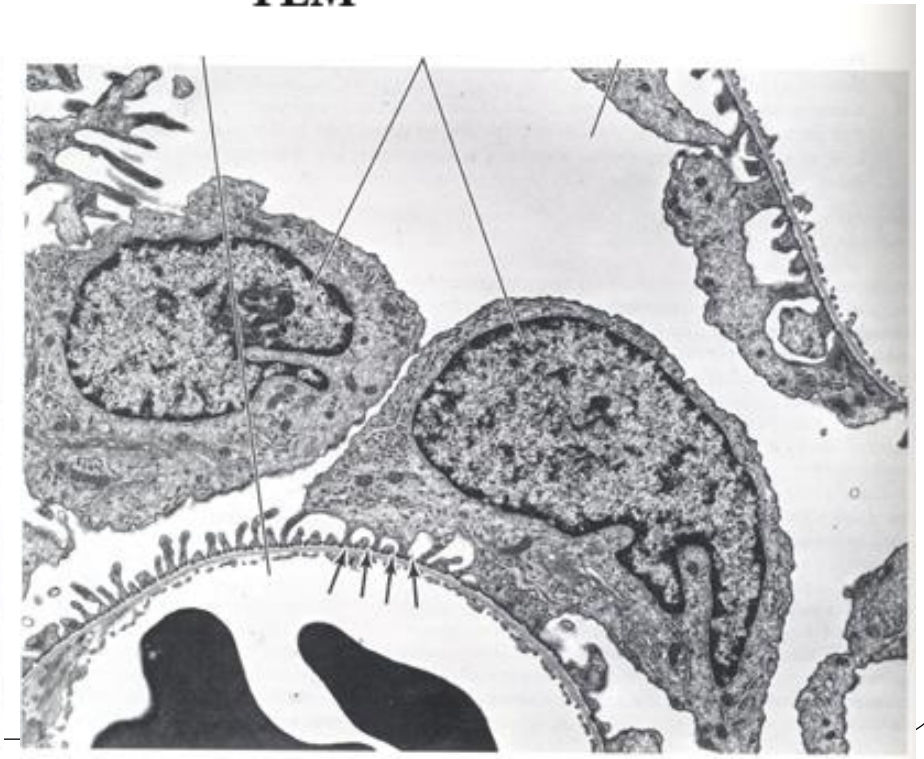

sensor
scanning
electron beam
secondary
electrons signal
biological
sample

SEM


electrons
biological
sample
viewing screen

TEM

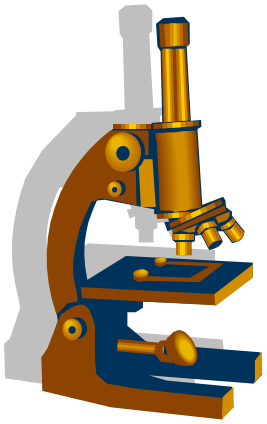


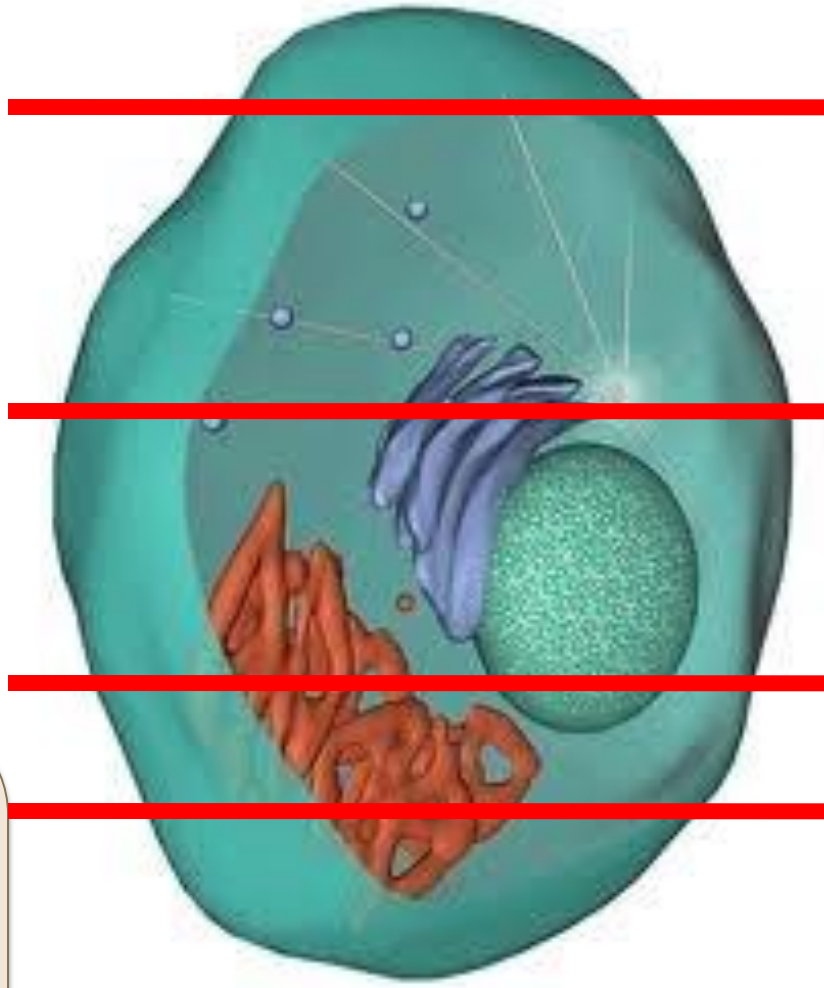



PHILIPS


JEOL
JEOL JSM-6610LV

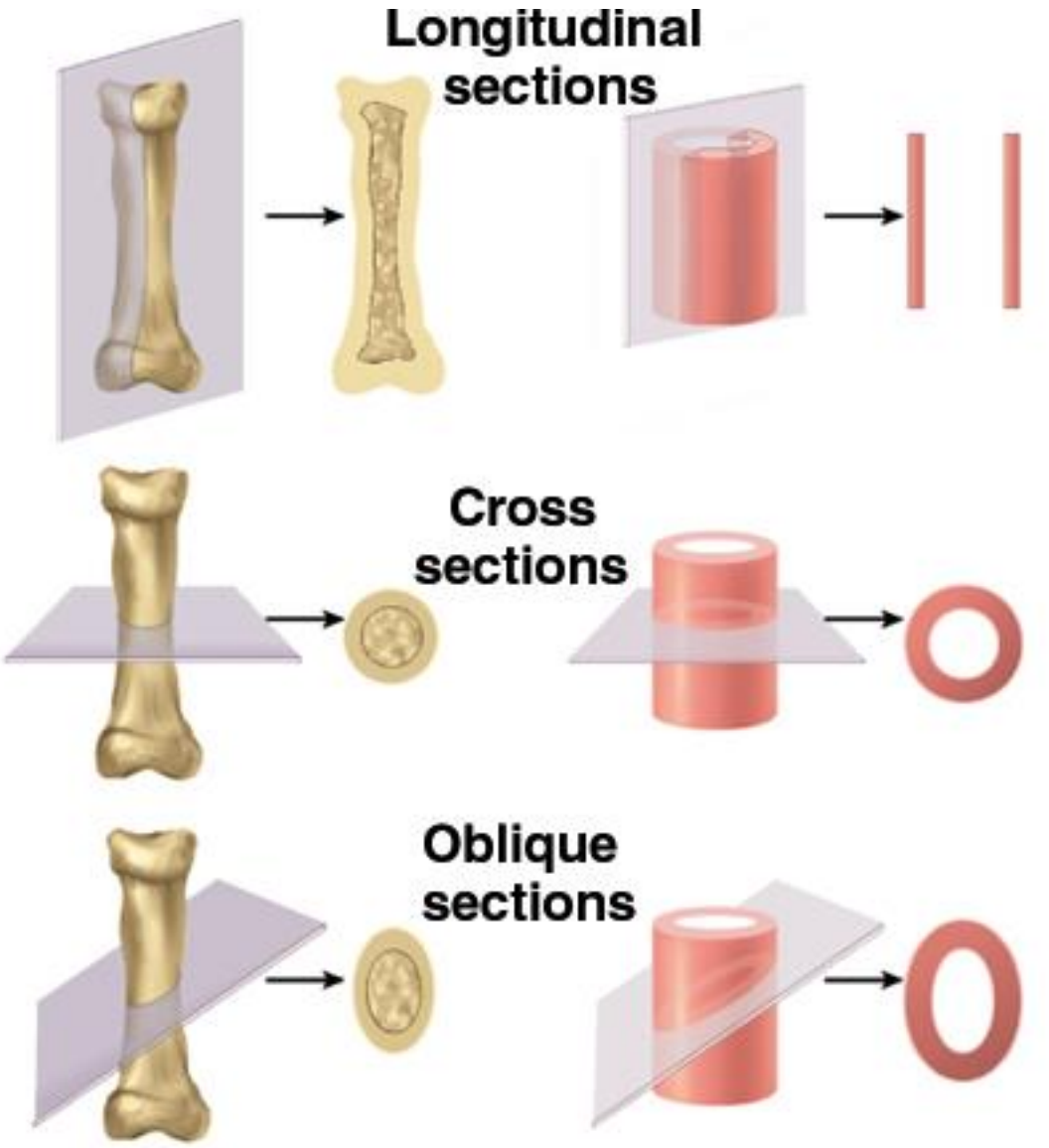
	Light microscope	Electron microscope
Image	Color images	black and white images
Images produced by	Visible light rays	Electron beam
Magnification	up to 1500x but a wider field of view and easier orientation	Up to 2,000 000x
Resolution	Resolving power to 0.25µm	Resolving power to 0.1nm
Time	Frozen sections can yield an image within 20 minutes	One day at least
Section thickness	Ranges from 1-30 µm	Ranges from 0.02-0.1 µm
Specimen placed on	Glass slide	Copper mesh





Histology is a two dimensional study of a three dimensional reality.

§ Types of Tissue Sections (1)



Longitudinal section

- tissue cut along the longest direction of an organ

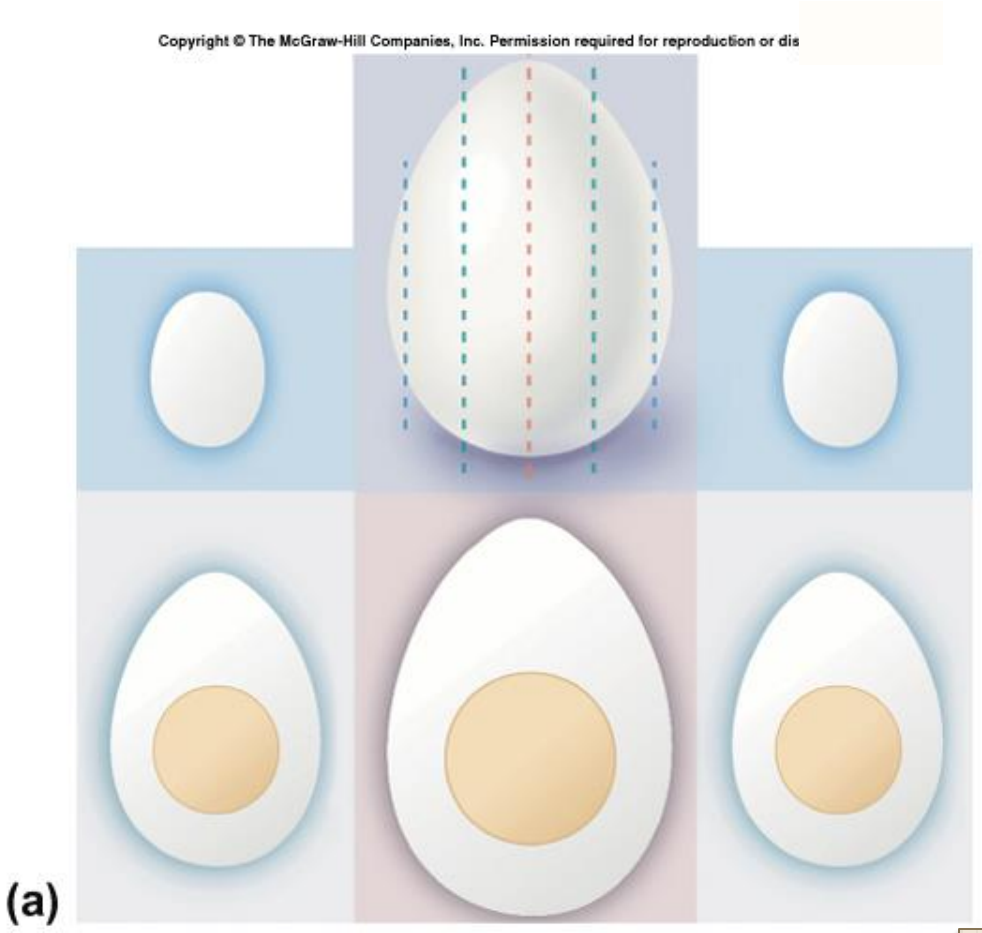
Cross section

- tissue cut perpendicular to the length of an organ

Oblique section

- tissue cut at an angle between a cross & longitudinal section

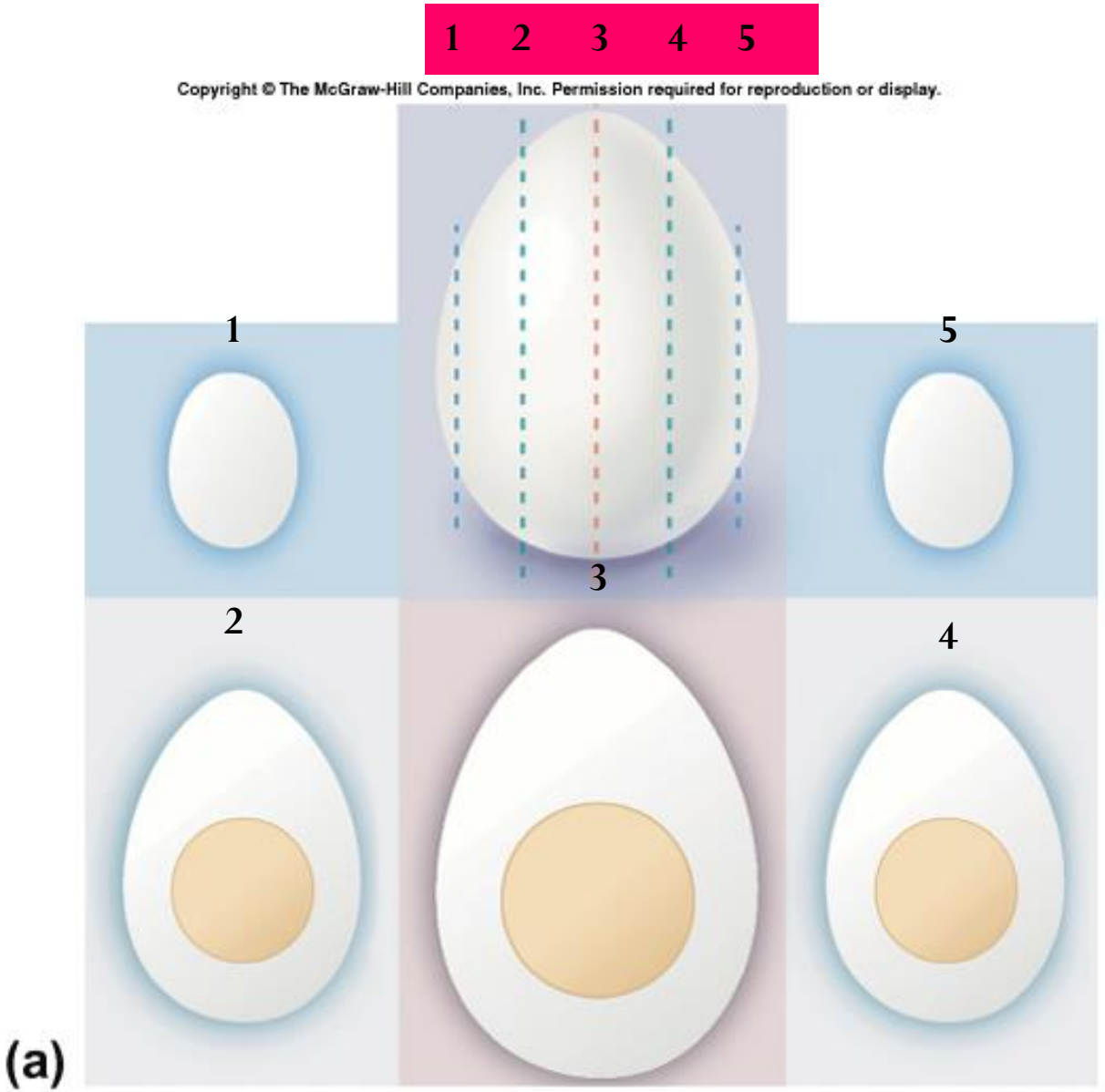
Types of Tissue Sections (2)



- Would you classify the egg sections as longitudinal, cross, or oblique sections?
- How would the egg look if sectioned in the other two planes?

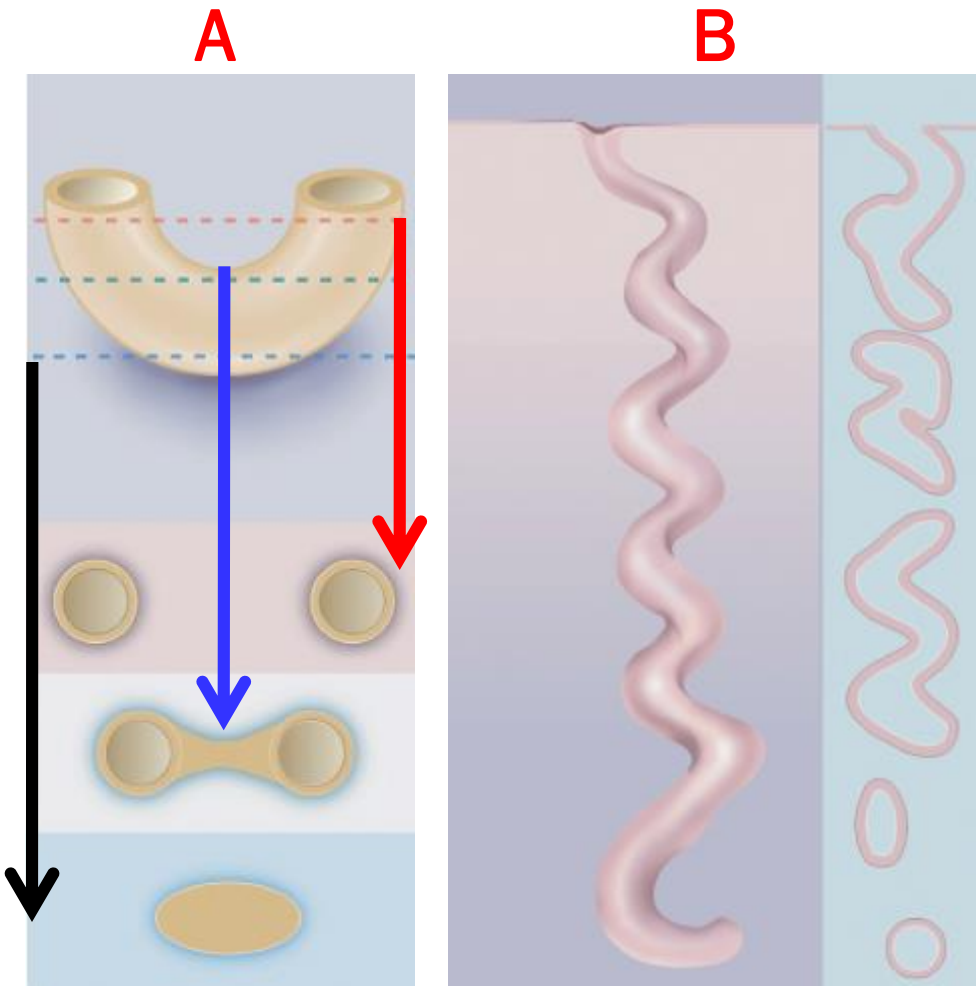
Practice at home.

Tissue Sectioning (2)



- **Slices 1 & 5** miss the yolk / cell nucleus
- Cell nucleus is smaller in **sections 2 & 4**

Tissue Sectioning (3)



- **Image A** is a cross section of elbow macaroni, resembling a blood vessel, piece of gut, or other tubular organ.
- **Image B** is a longitudinal section of a sweat gland. Notice what a single slice could look like

Stains .. examples

Standard stain (dye). H & E (Hematoylin & Eosin); Specialized stains include PAS, Ag, Aldehyde fuchsin, Orcein

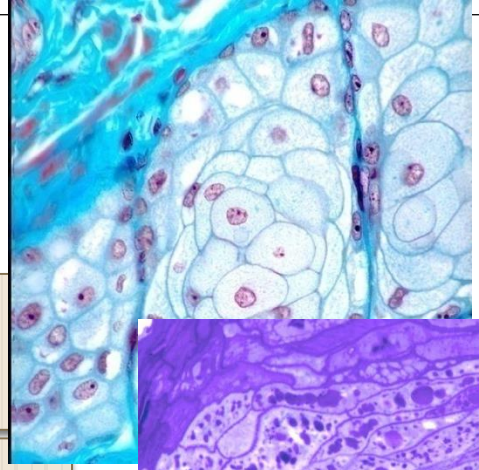
PAS- detects glycogen, glycoproteins, glycolipids and mucins in tissues

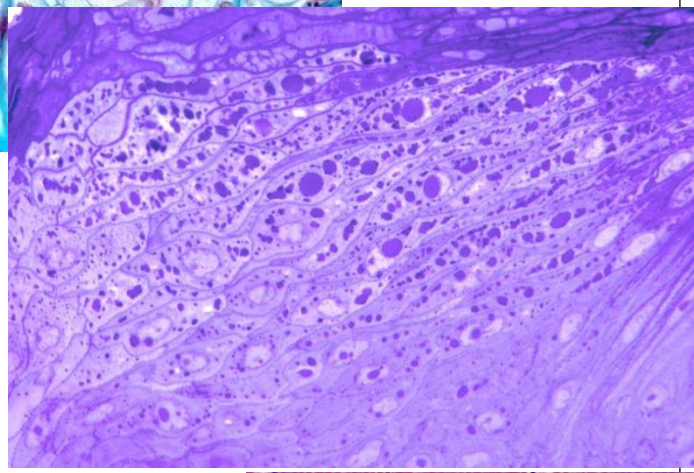
2 steps; CHO are oxidized with periodic acid to aldehyde groups. The Schiff reagent reacts with aldehyde groups to form a deep red-reaction (magenta) product

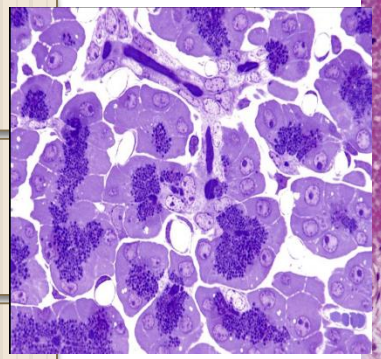
Aldehyde fuchsin stains elastic fibres & ß-cells islets of pancreas

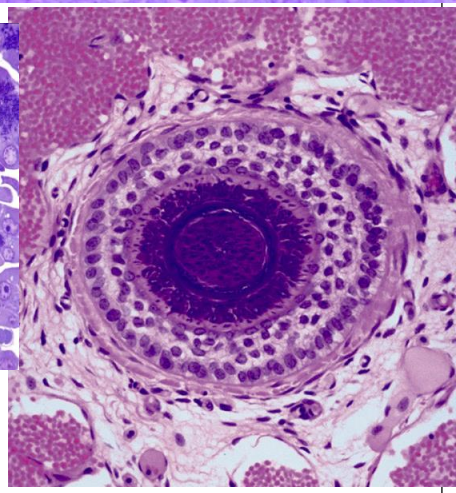
Orcein stains elastic fibres dark brown

Silver stain stains reticular fibres (type III collagen)







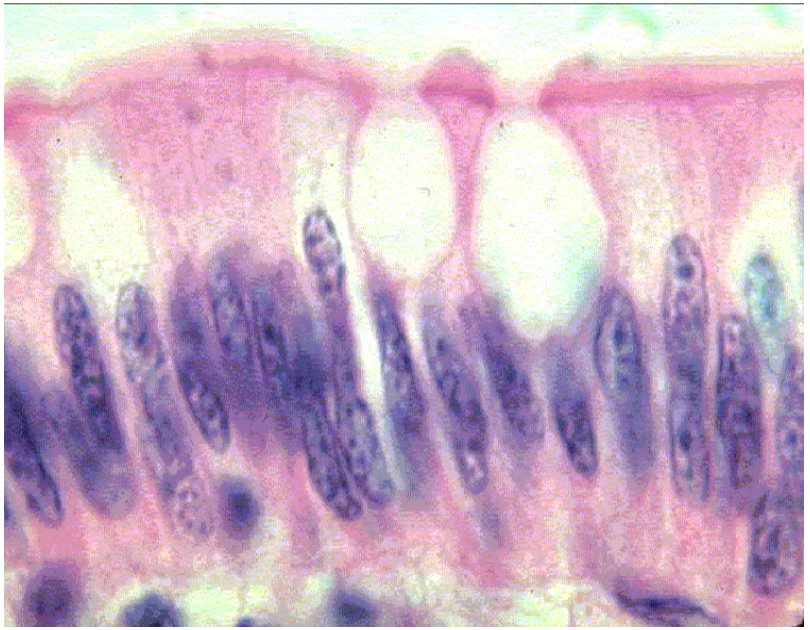


Basophilia

Basophilic structures are stained by basic dyes:

- Basic dyes are **positive**
- Basophilic structures are **negative** (ex. DNA, RNA, ribosomes, RER)

Basophilic = **B**lue



Acidophilia

Acidophilic structures are stained by acid dyes:

- Acid *dyes* are **negative**
- Acidophilic structures are **positive** (ex. Proteins, collagen, cytoplasm)

Eosinophilic = Pink