Introduction to histology and its methods of study

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1 What is histology

- Definition
- Cell: smalle
- Tissue
- Organ: differ
- System

■Histology is a science that stu m b b a

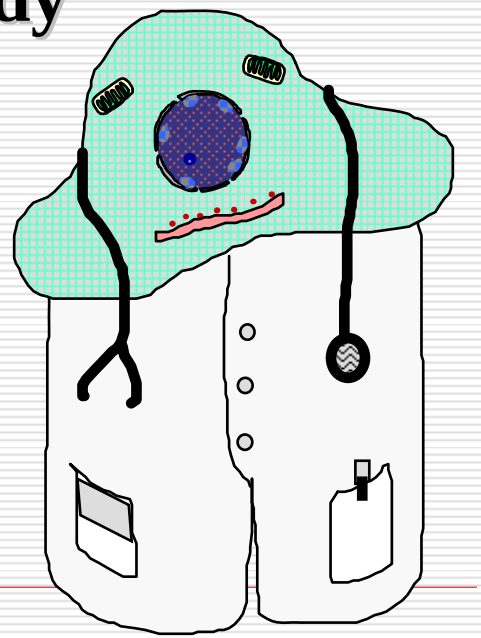
Several organs with related functions

- ■Motor system
- ■Nervous system
- ■Circulatory system
- ■Respiratory system
- ■Digestive system
- ■Urinary system
- ■Reproductive system
- ■Endocrine system

2 Why to study histology

- **Anatomy: macrostructure**
- **Biochemistry: chemical compounds and processes**
- **Pathology: the relation between disease and the structures and functions of the body**

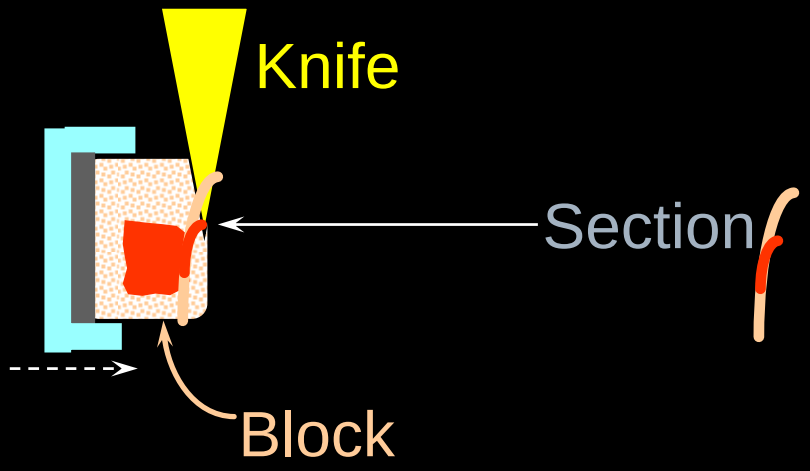
***Although most medical students are not going to become histologists, a thorough knowledge of histology is fundamental for you as future doctors*.**



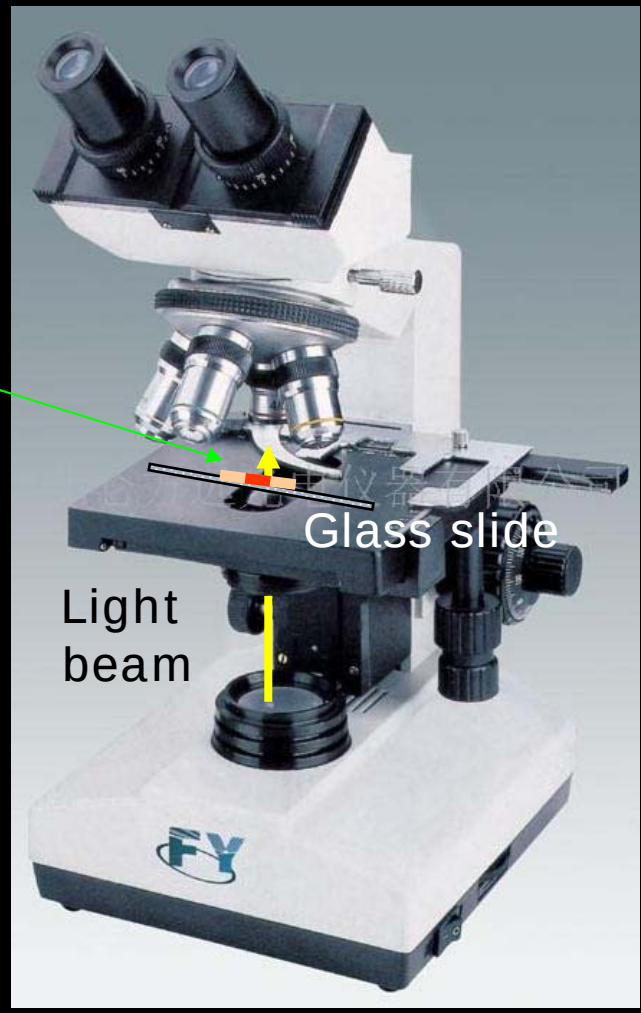
3 How to research on histology

- Preparation of tissue for microscopic examination
  - Paraffin section
  - Frozen section
- Microscopy
- Problems in the interpretation of tissue sections

3 How to research on histology



MICROTOME - a fancy meat-slicer - holds the wax block, & cuts off thin slices, as the block is slowly advanced mechanically

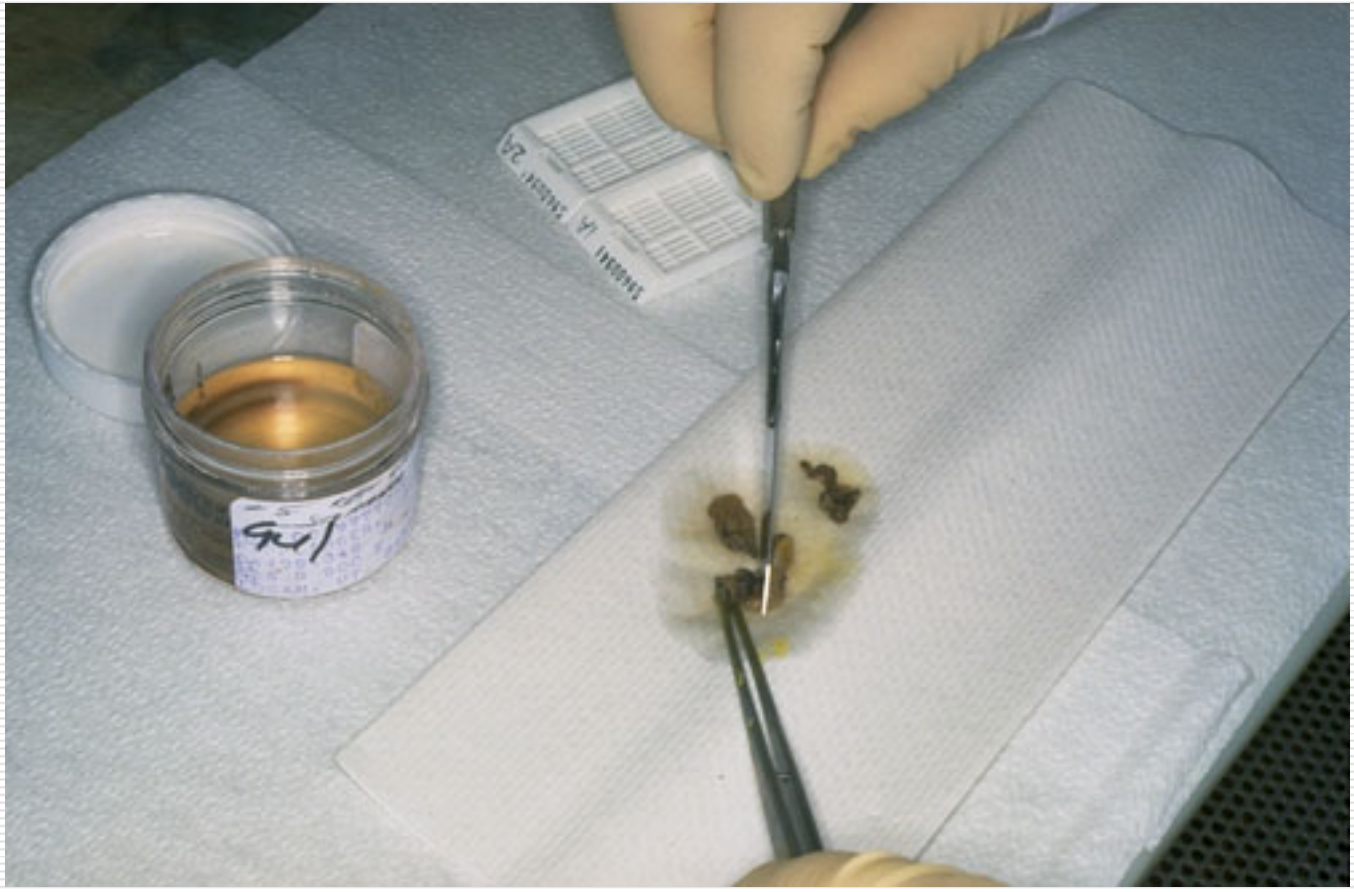


Light microscope

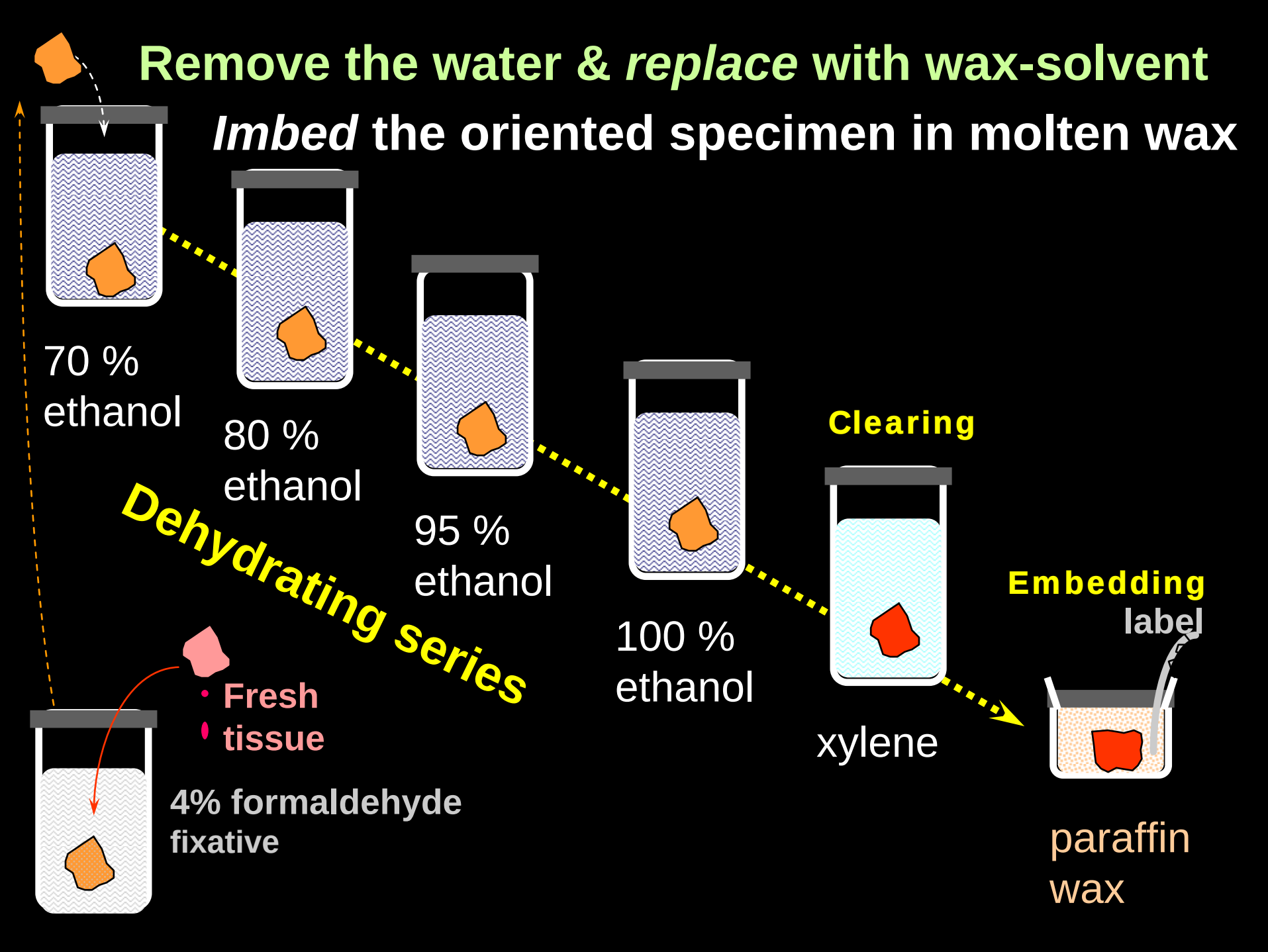
Paraffin section

- Obtaining the specimen
- Fixation
- Dehydration
- Clearing
- Embedding
- Sectioning
- Staining

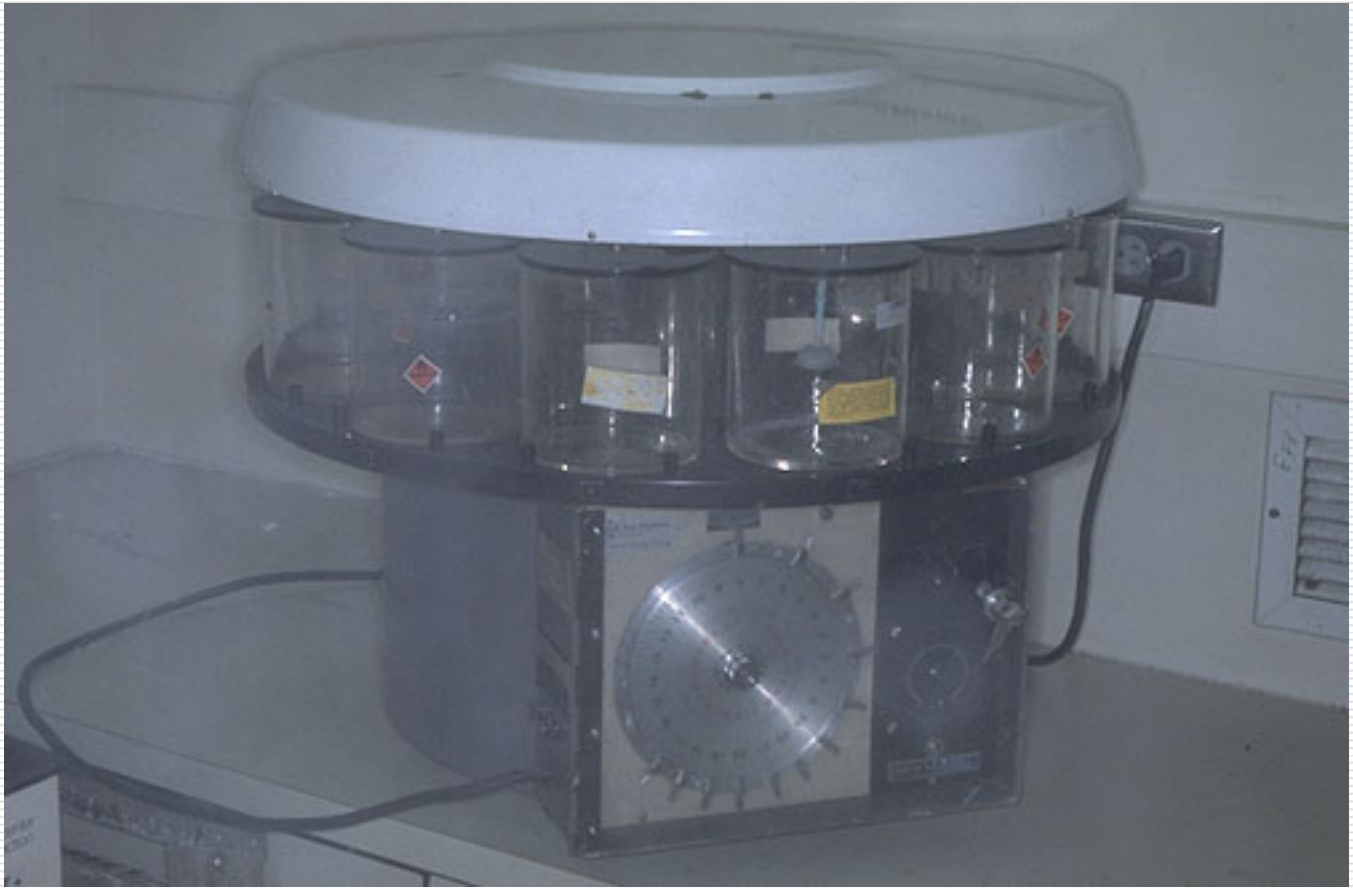
Obtaining the specimen



fresh as possible and small pieces

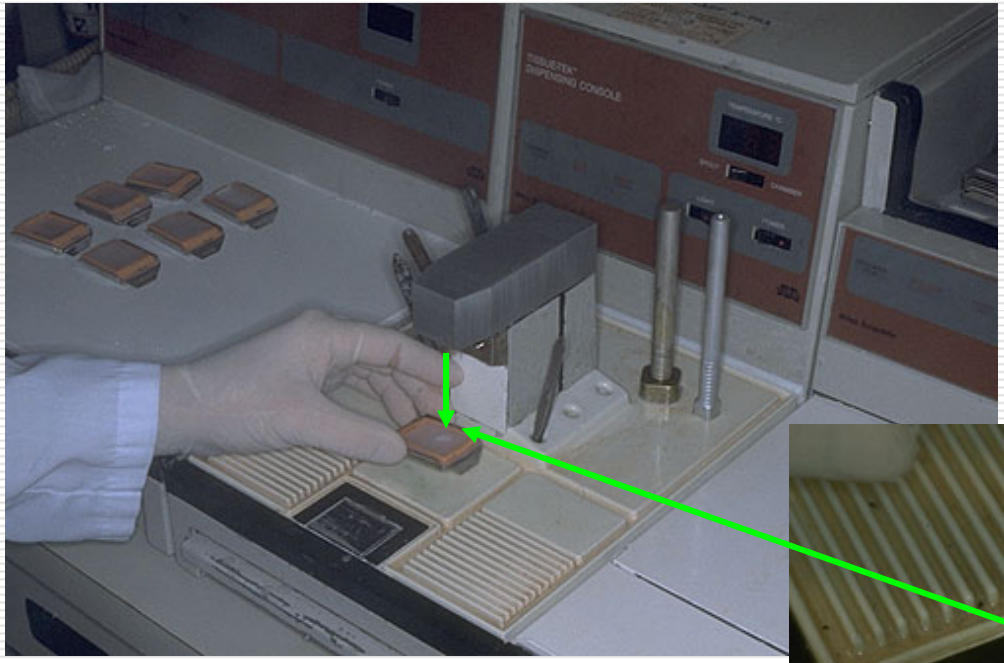

Remove the water & *replace* with wax-solvent
Imbed the oriented specimen in molten wax
70 % ethanol
80 % ethanol
95 % ethanol
100 % ethanol
Clearing
xylene
Embedding label
paraffin wax
Dehydrating series
Fresh tissue
4% formaldehyde fixative

Tissue processor

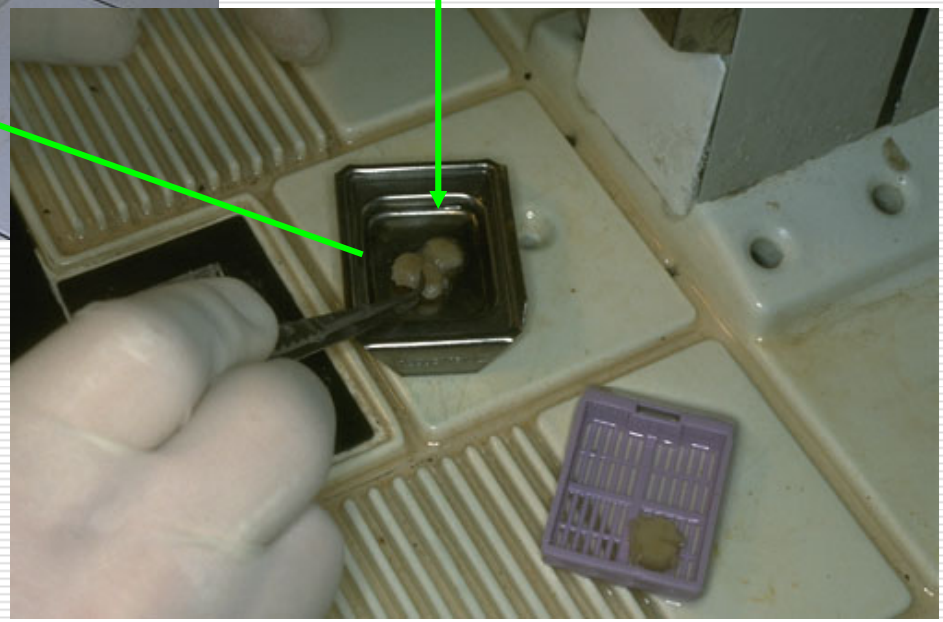


Automatic tissues processor moves the tissues around through the various agents on a preset time scale.

Tissue embedding

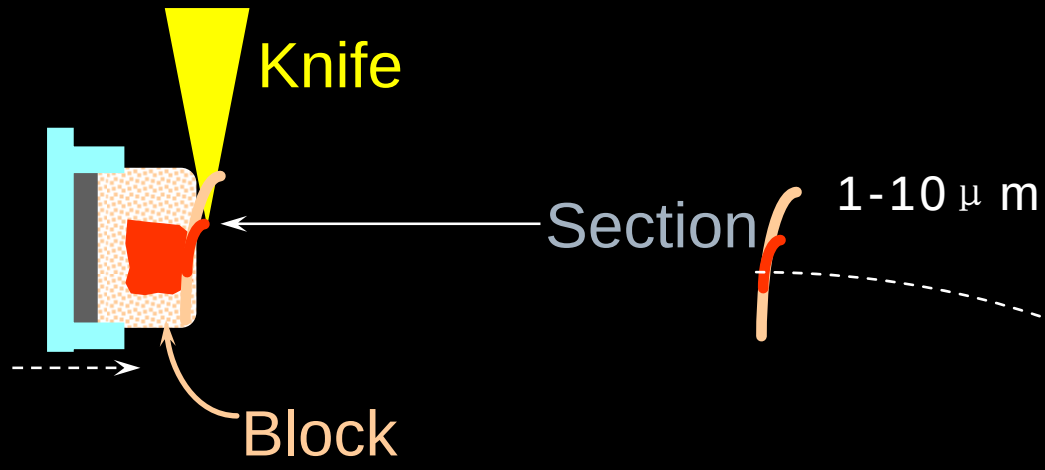


Tissues are infiltrated in molten wax to replace the xyline.



The molten wax drop into a plastic box; then Put the tissues into the box. The molten wax solidify into a block with the tissue inside.

After it is solid, hold the wax block & *cut* slices



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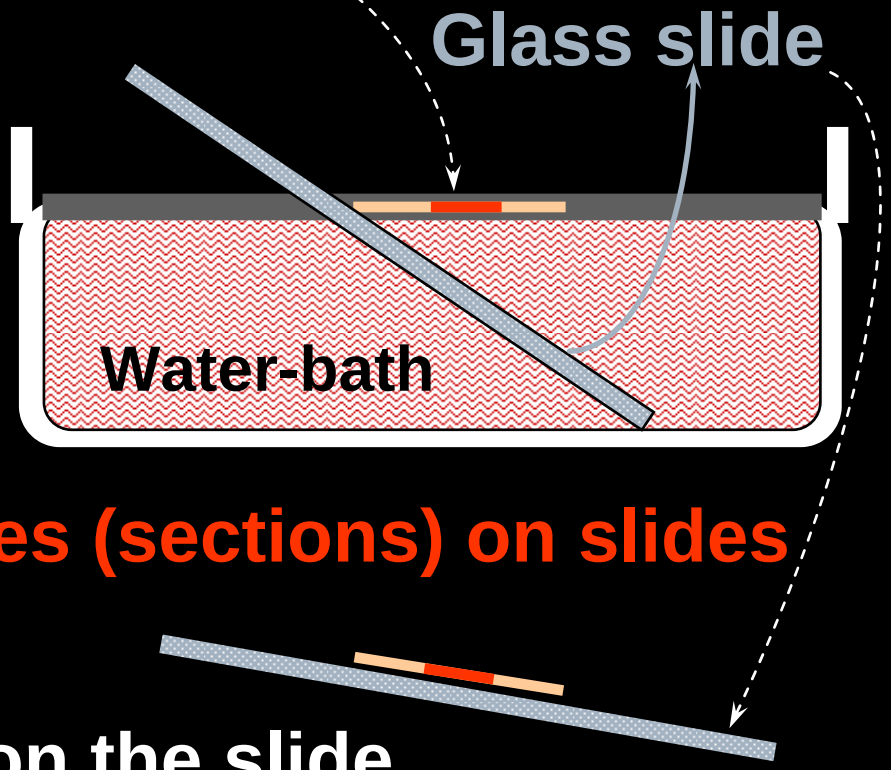


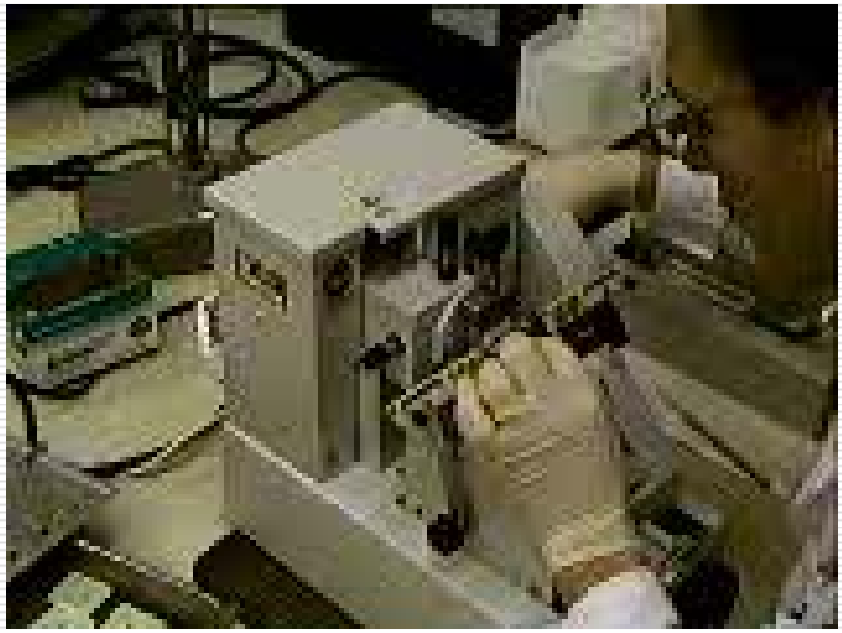
***Mount* the thin slices (sections) on slides**

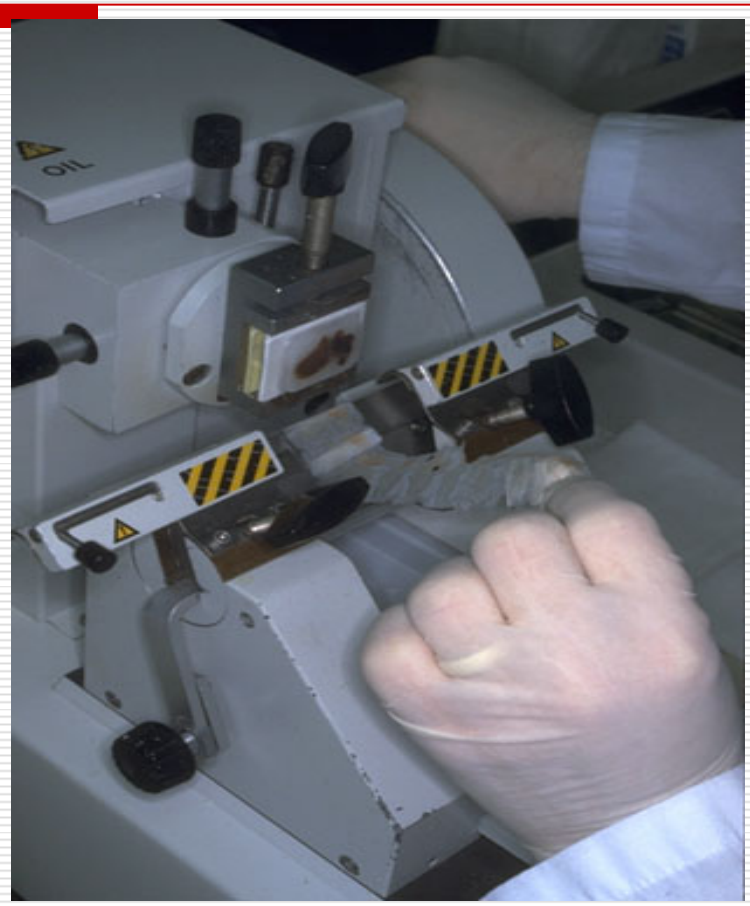
Lift out floating section on the slide

Sectioning with microtome

Rotation of the drive wheel moves the tissue-block holder up and down. Each turn of the drive wheel advances the specimen holder a controlled distance. After each forward move, the tissue block passes over the knife edge, which cuts the sections.

Sectioning with microtome





Rotation of the drive wheel moves the tissue-block holder up and down. Each turn of the drive wheel advances the specimen holder a controlled distance. After each forward move, the tissue block passes over the knife edge, which cuts the sections.

Picking sections up from water bath

sections are floated on a warm water bath that helps remove wrinkles.

Paraffin section

Unstained section on glass slide

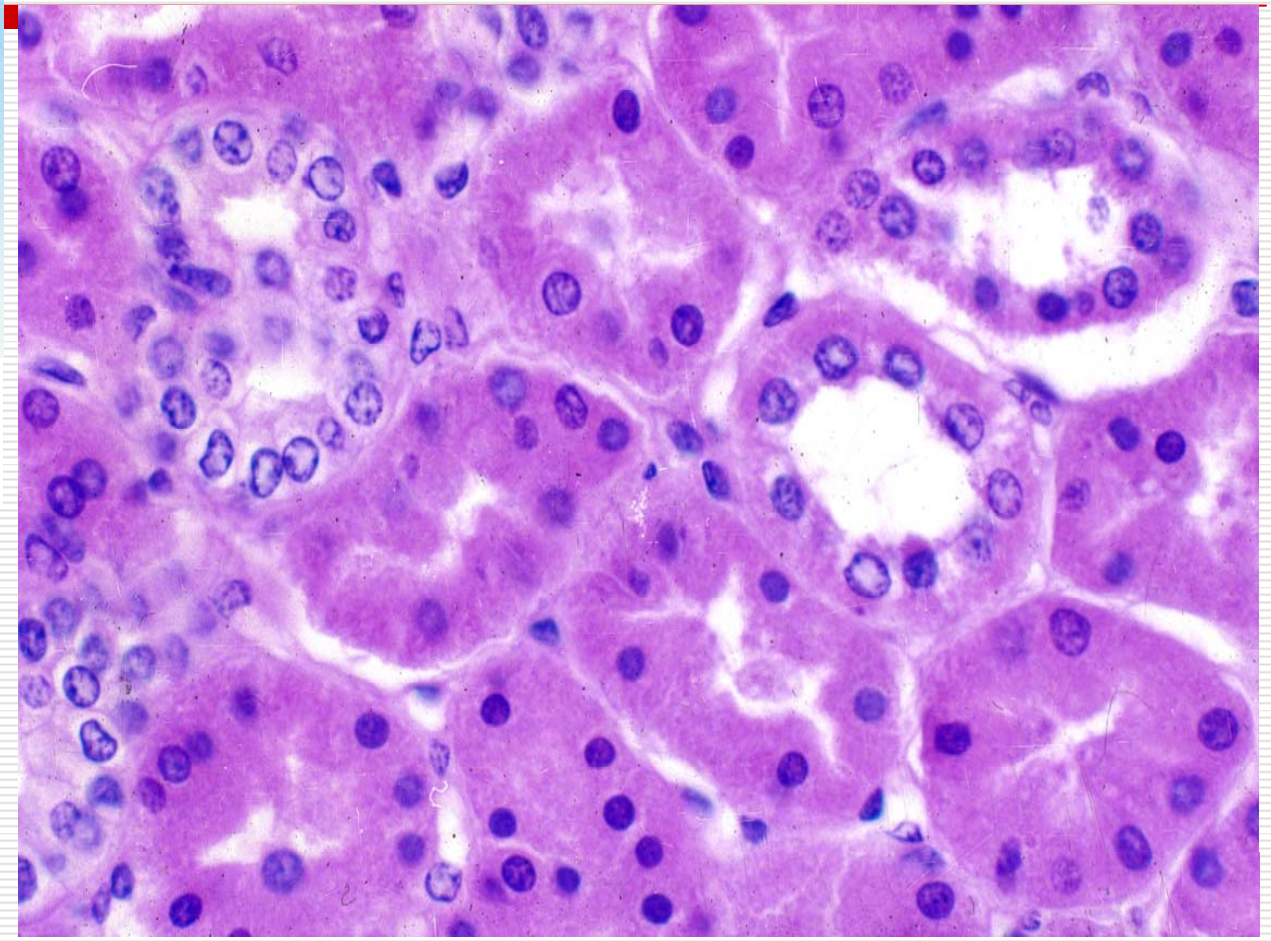
Tray of unstained slides in drying oven

Sections are picked up on a glass slide and placed in a warm oven to help the section adhere to the slide.

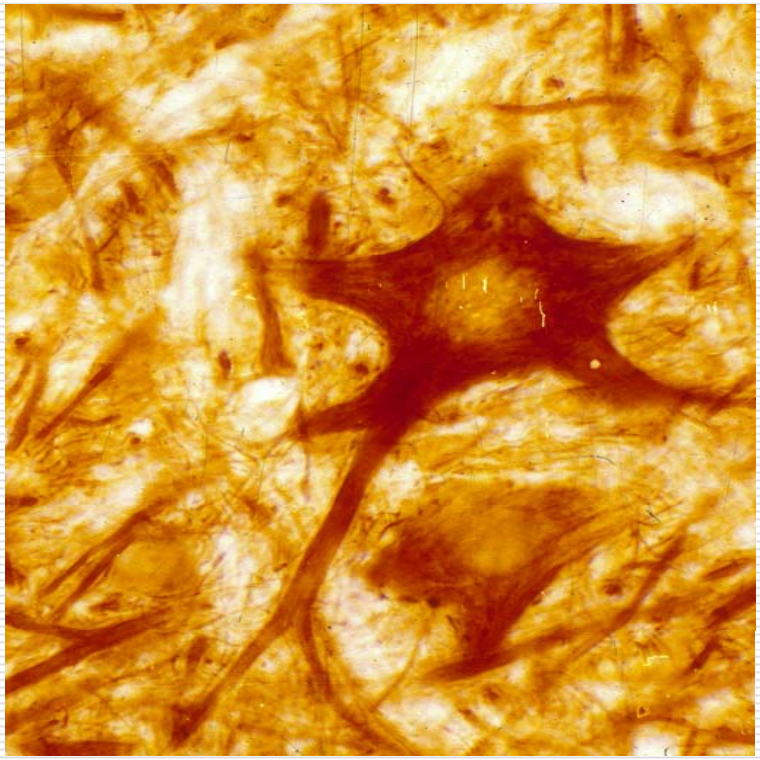
Staining

- Deparaffinized： running through xylene to alcohol to water
- Dye: acidic or basic compounds; electrostatic linkages with tissues
- Hematoxylin & Eosin (H & E) staining
  - Hematoxylin: stains cell nucleus and other acidic structure blue
  - Eosin: stains the cytoplasm and collagen pink
  - Basophilia: affinity for basic dyes
  - Acidophilia: affinity for acid dyes
  - Neutrophilia

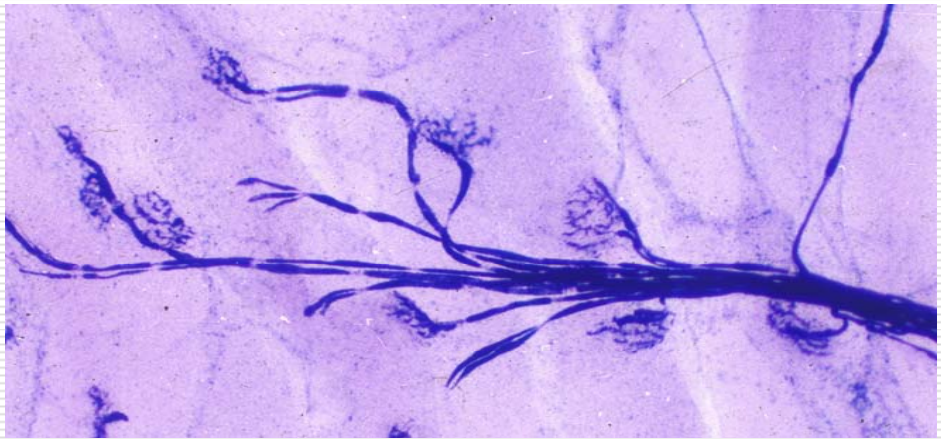
Light Microscope



It is a cross-section of kidney medullar which is made up of lots of tubules. The wall of them is epithelial cells. The cell nucleus is basophilic (blue) and the cytoplasm is acidophilic (pink). HE staining.



Silver staining



Gold staining

Frozen section

- Snap frozen in a cold liquid or cold environment
  Frozen sections are performed with a cryostat.

cryostat

Cutting a frozen section

Frozen section

- It is necessary to get a rapid diagnosis of a pathologic process.
- It is also effective in the histochemical study of very sensitive enzymes or small molecules.

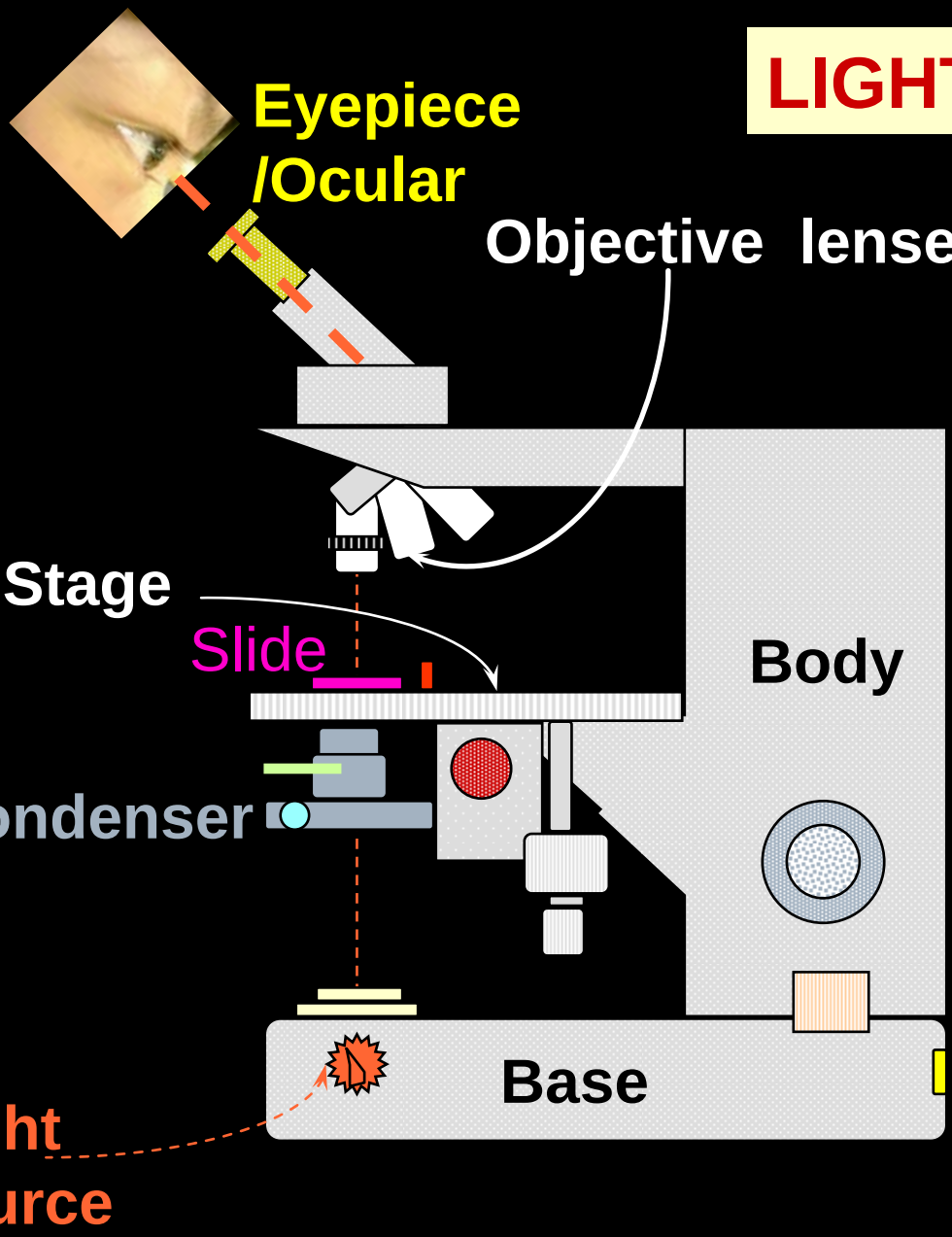
Microscopy

- Light microscopy
  - Conventional light microscopy
  - Phase-contrast microscopy
  - Polarizing microscopy
  - Fluorescence microscopy
  - Confocal microscopy
- Electron microscopy
  - Transmission electron microscopy (TEM)
  - Scanning electron microscopy (SEM)

Conventional light microscopy

- Mechanical parts
- Optical parts
  - Condenser collects and focuses light to illuminate the object
  - Objective enlarges and projects the image of the object in the direction of the eyepieces.
  - Eyepieces magnify this image and project it onto the viewer's retina

LIGHT MICROSCOPE



Max MAGNIFICATION

Eyepiece (10X)

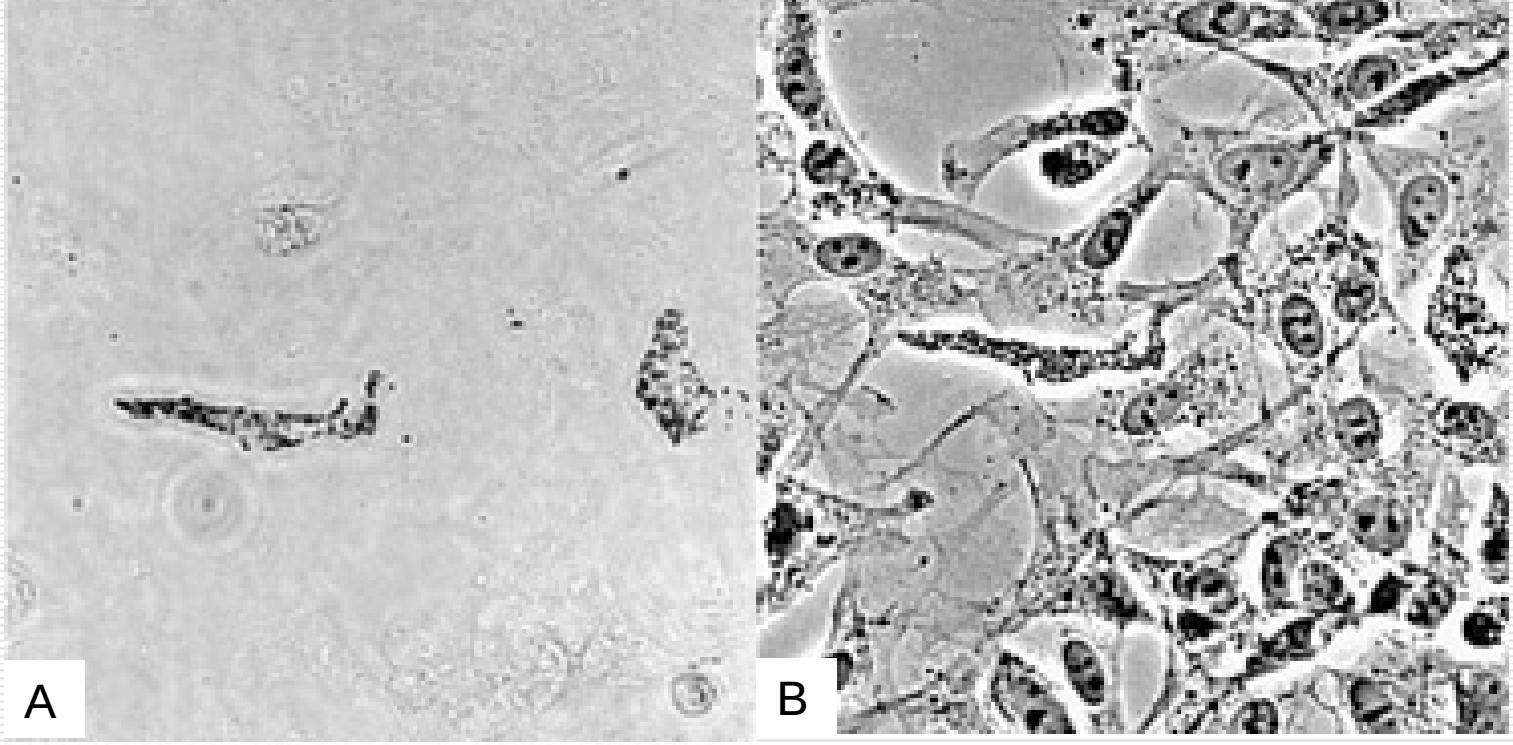
Objective (40X)

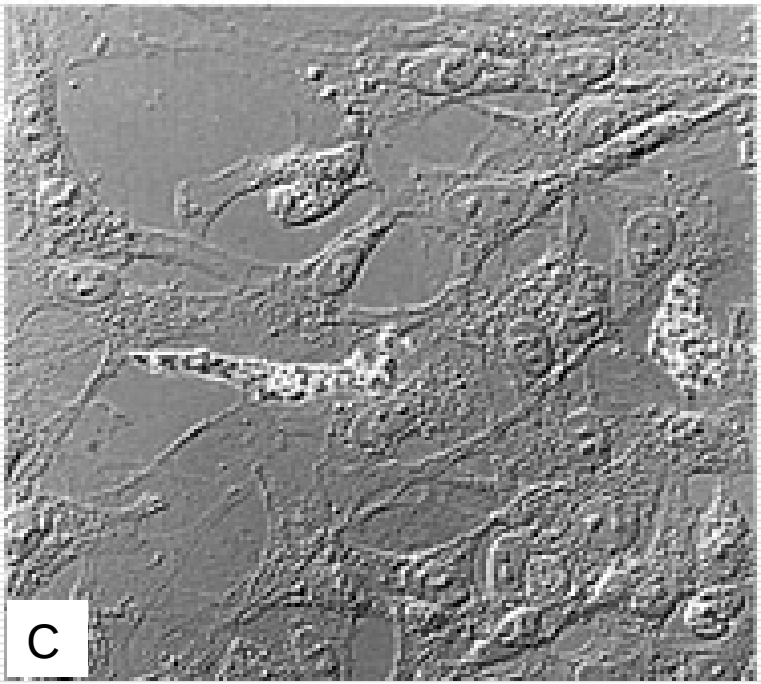
= 400X

Schematic diagram of light microscope

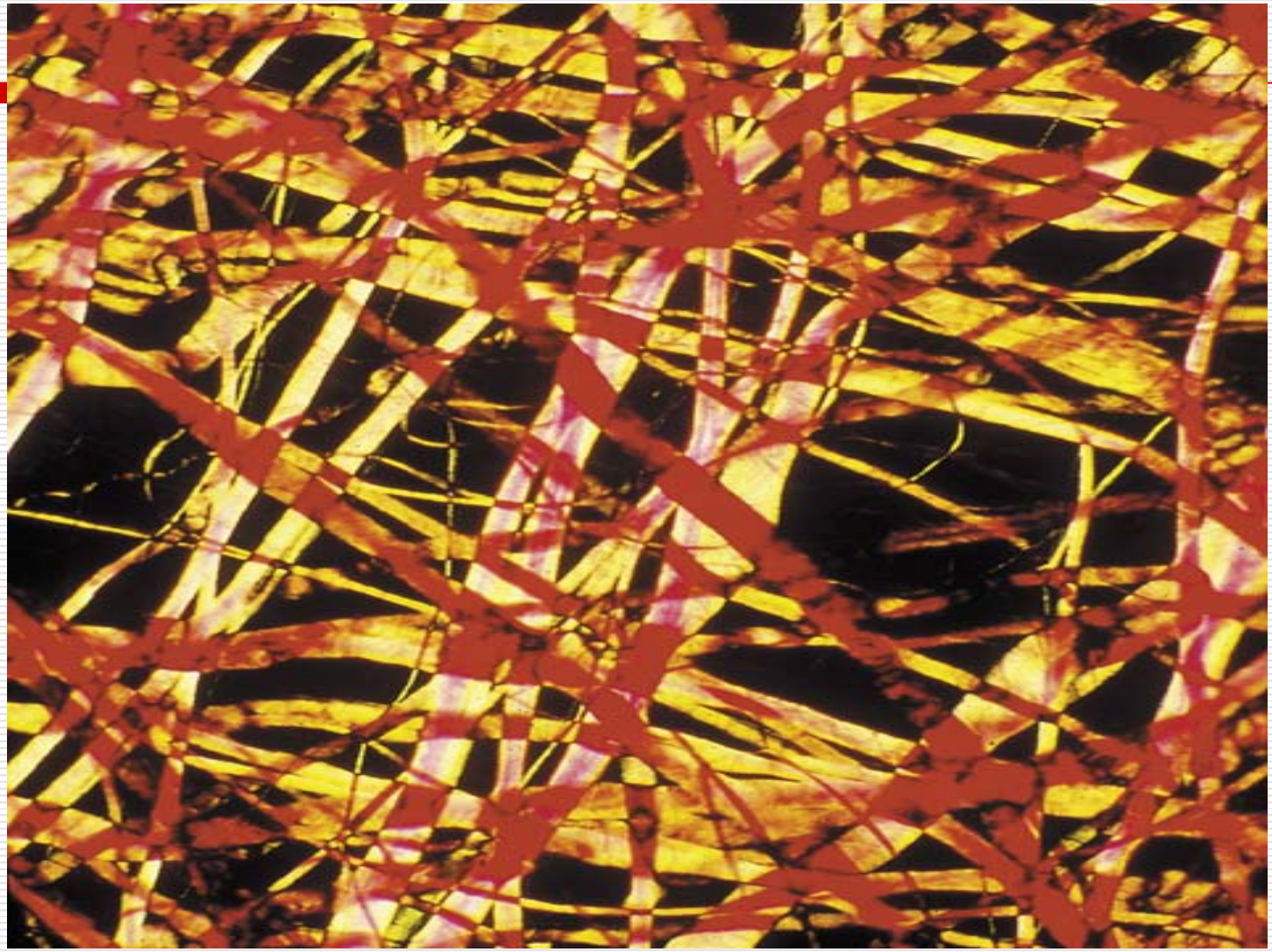
Phase-contrast microscopy & differential interference microscopy

- Phase-contrast microscopy
  - light changes speed when passing through cellular and extracellular structures with different refractive indices.
- Differential interference microscopy
  - produces an three-dimesional image
- Two types of microscopy are used to observed living cells.





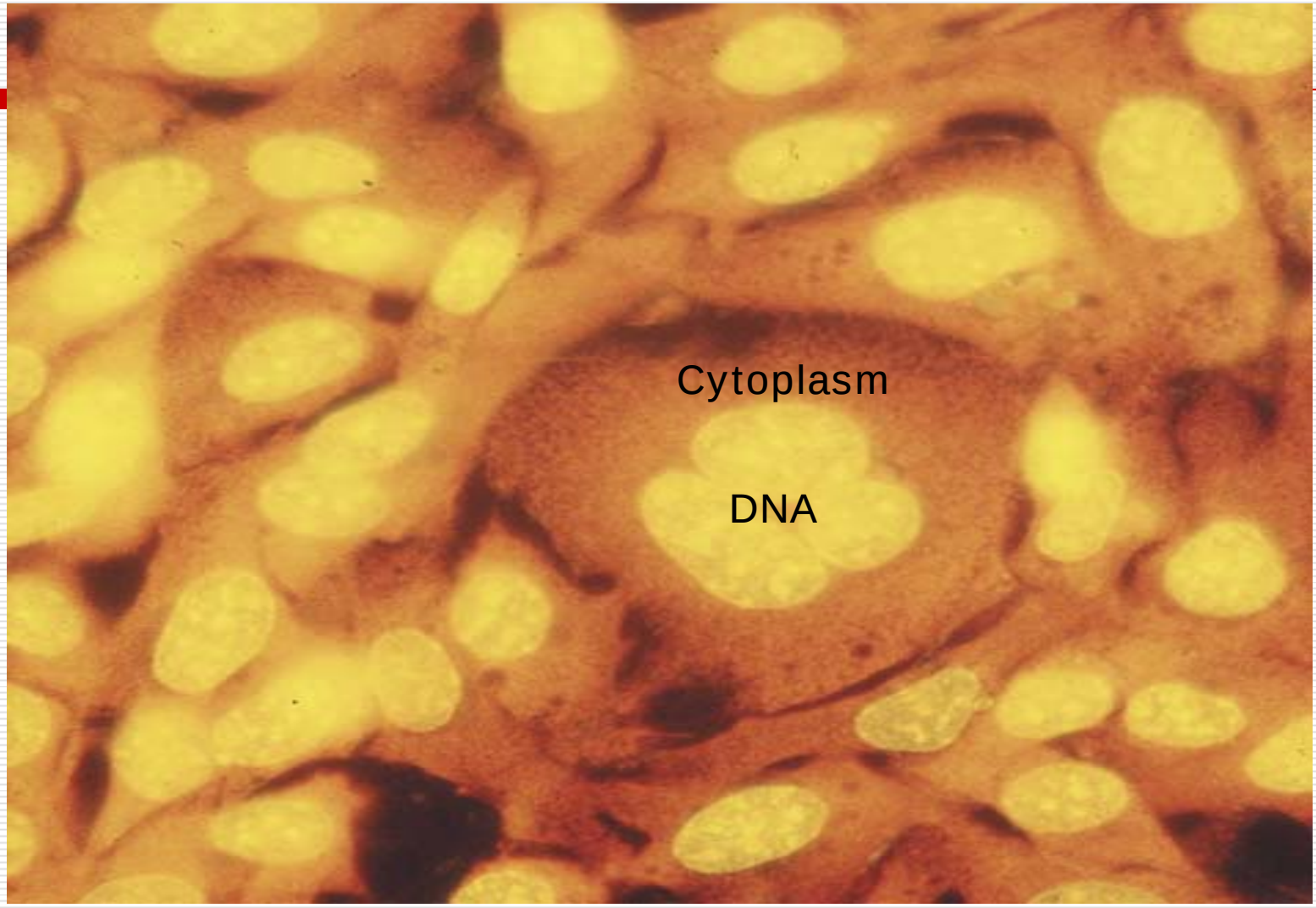
Cultured neural crest cells seen with different optical techniques.
A: Conventional light microscopy.
B: Phase contrast microscopy.
C: Nomarski differential interference microscopy.



Under polarized light microscopy, collagen fibers appear brilliant or yellow.

Fluorescence microscopy

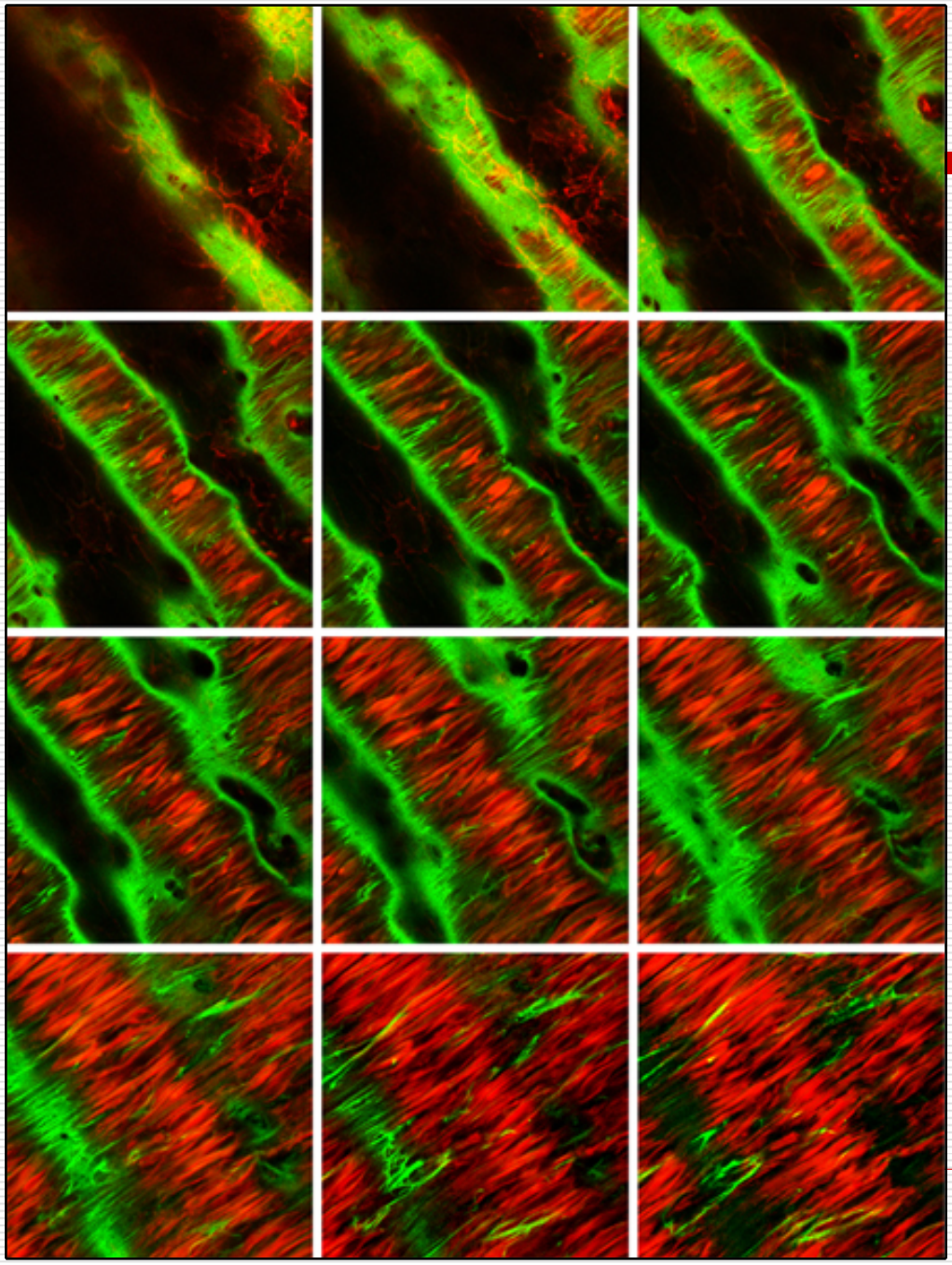
- Fluorescence: substances irradiated by certain light emit light with a longer wavelength.
- Fluorescence microscopy
  - tissue sections are irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum.
  - The fluorescent substances appear brilliant or colored on a dark background.

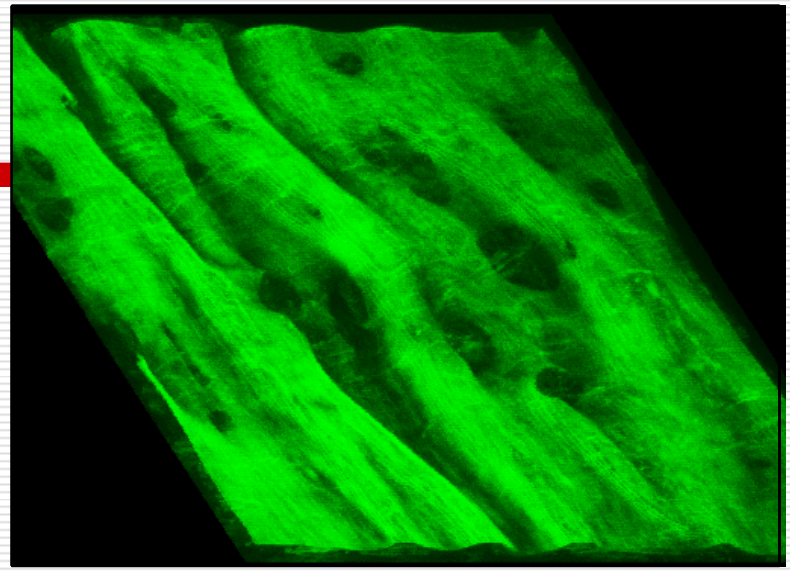


Photomicrograph of kidney cells stained with acridine orange. DNA (within the nuclei) emits yellow light, and the RNA-rich cytoplasm appears reddish or orange.

Confocal microscopy

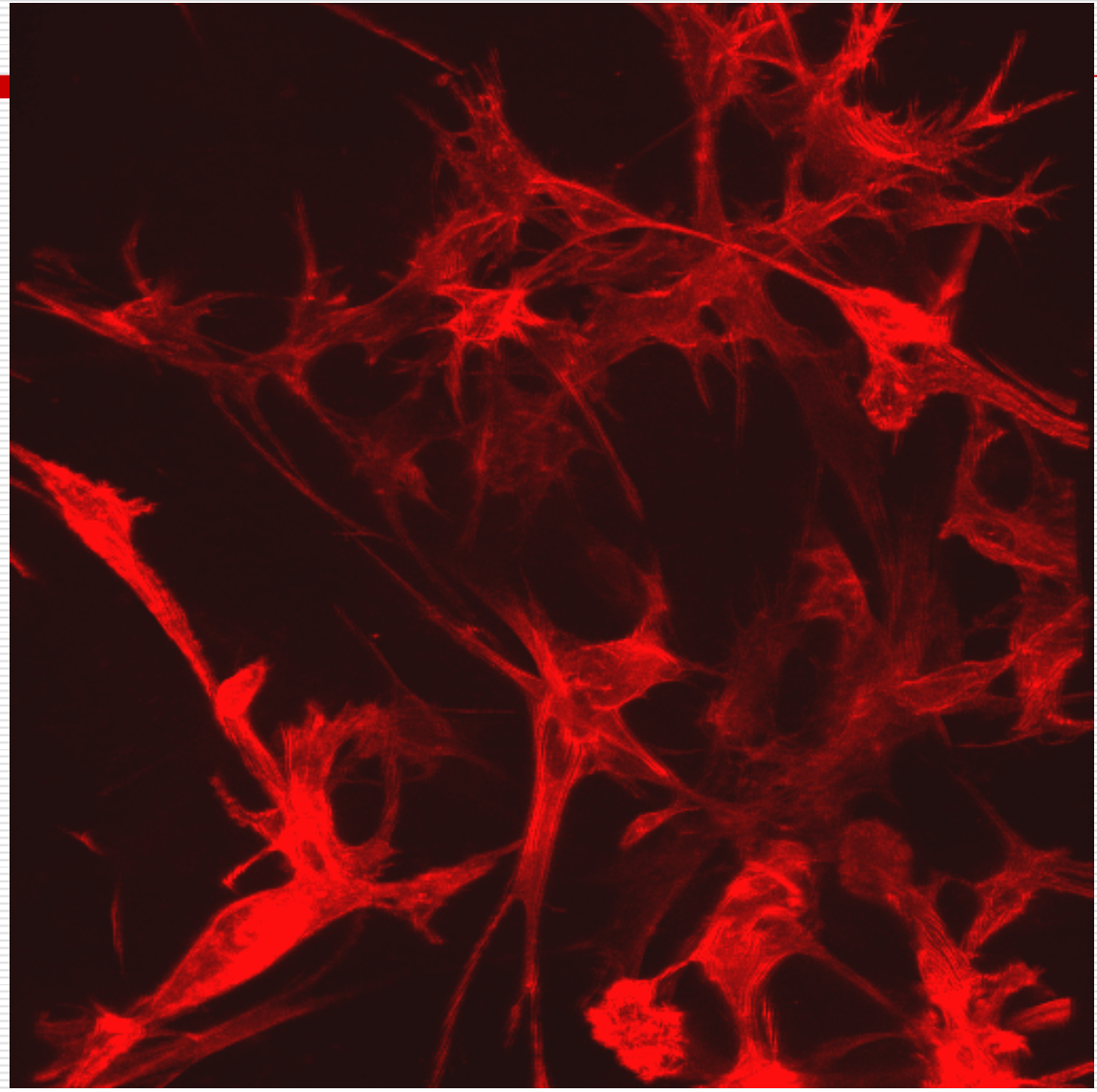
- A laser source
- Different layers of the specimen are seen in different focus simultaneously.
- Merged image of a three-dimension
- Clearer image



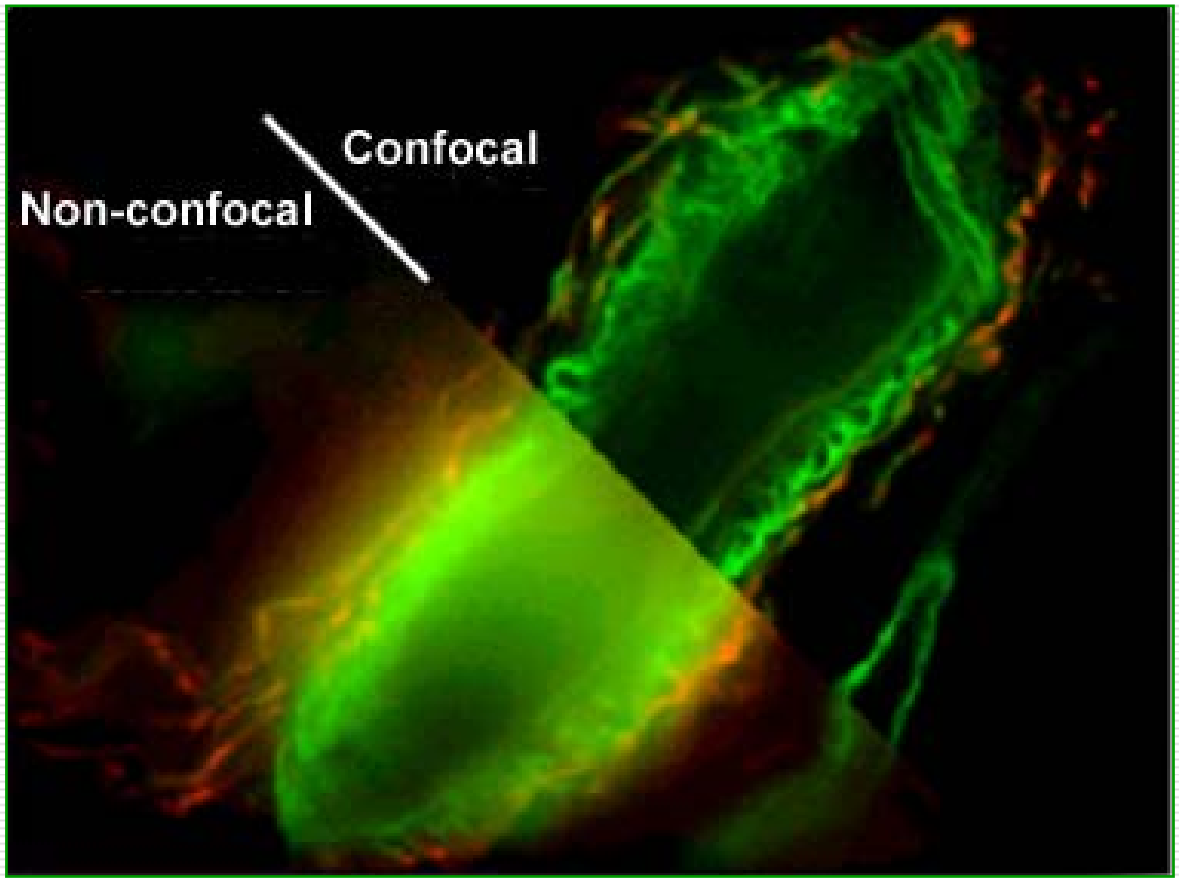


Different layers of the specimen are seen in different focus simultaneously.

A merged image of a three-dimensional object could be got.



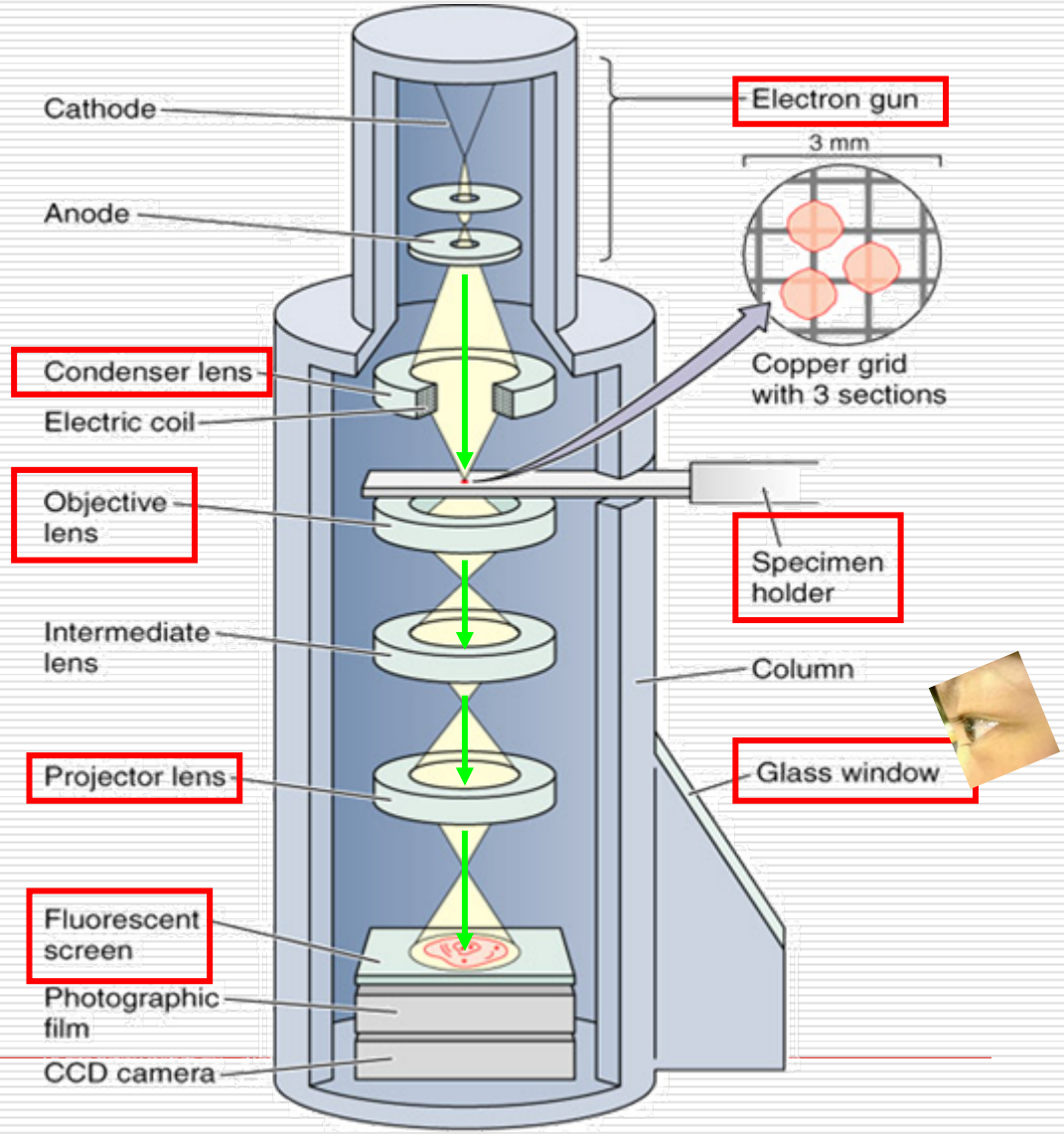
a 3-D image of cultured cells

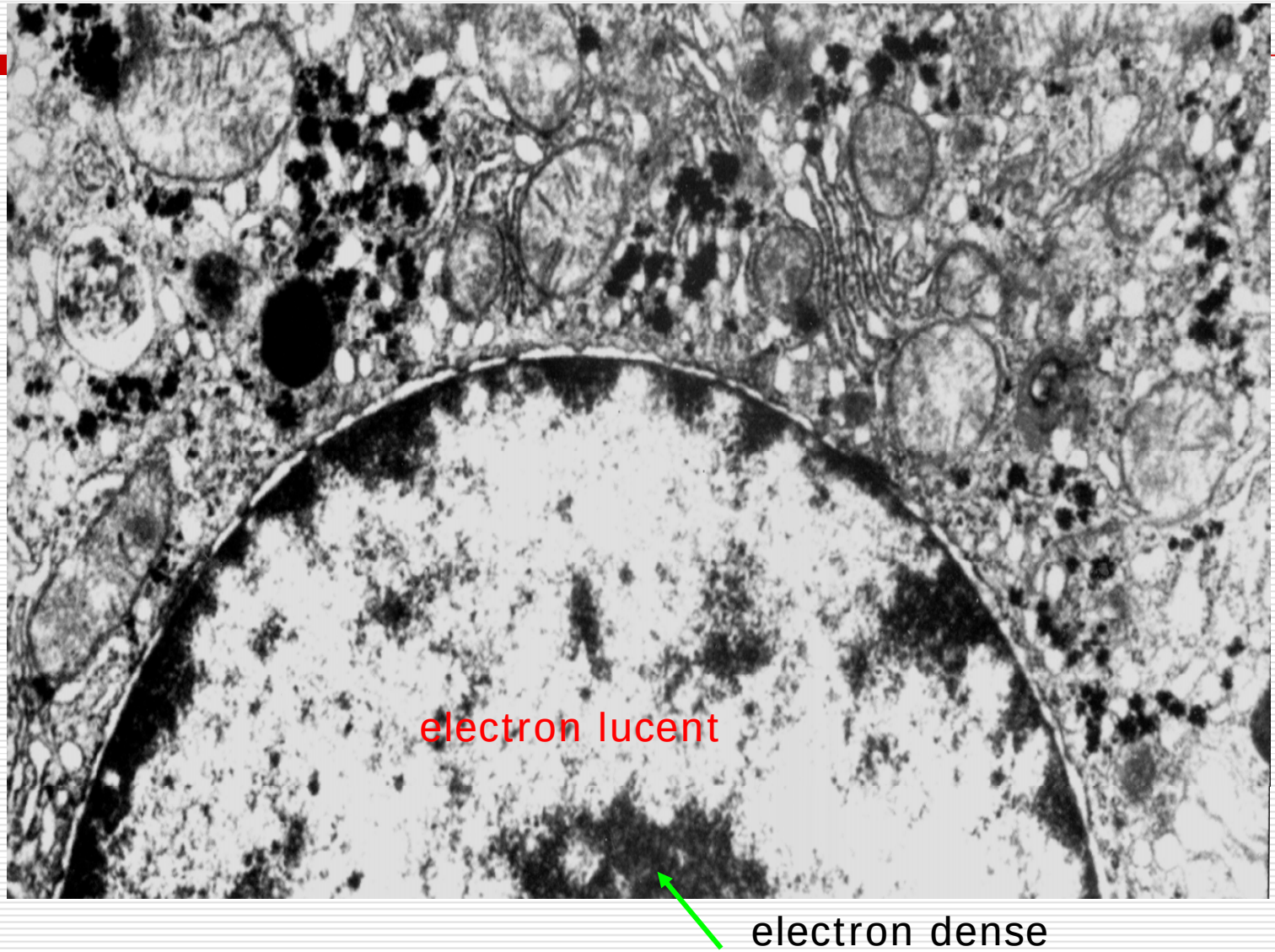


The image of specimen is clearer than in common fluorescence microscope.

Transmission electron microscope

high resolution (0.1nm)

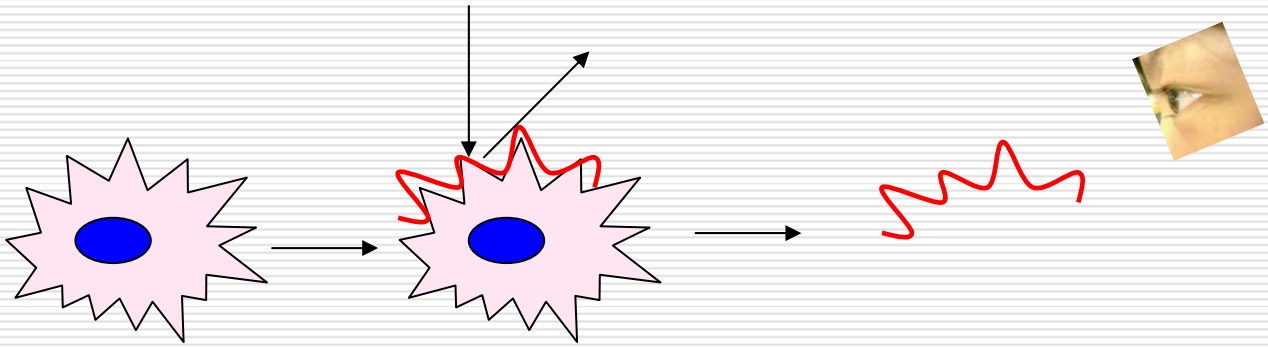


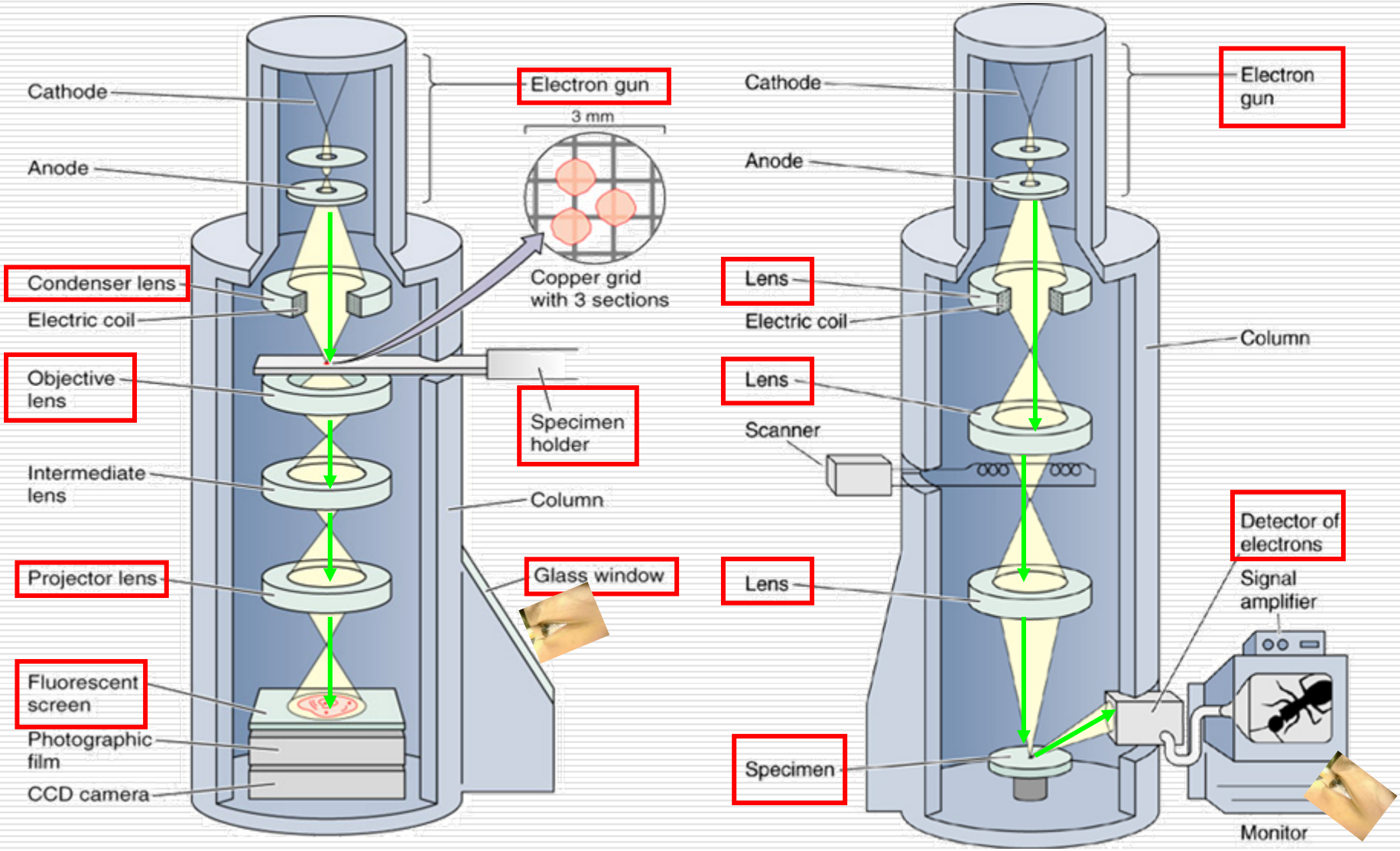


TEM micrograph of hepatocyte

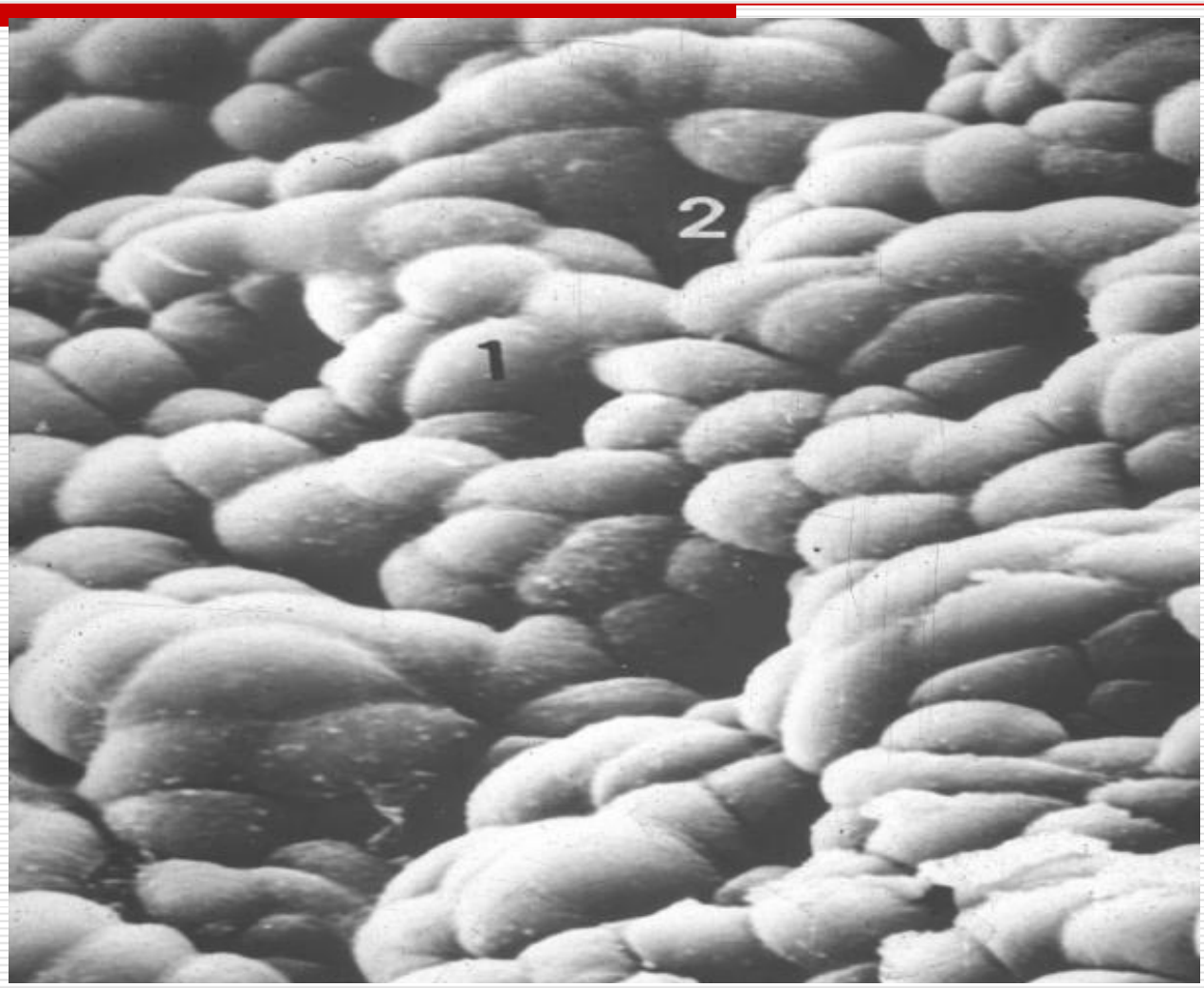
Scanning electron microscopy

- pseudo-three-dimensional views of the surfaces
- A very thin metal coating
- The electron beam interacts with this metal coating and produces reflected or emitted electrons.





Schematic view of a transmission and scanning electron microscope



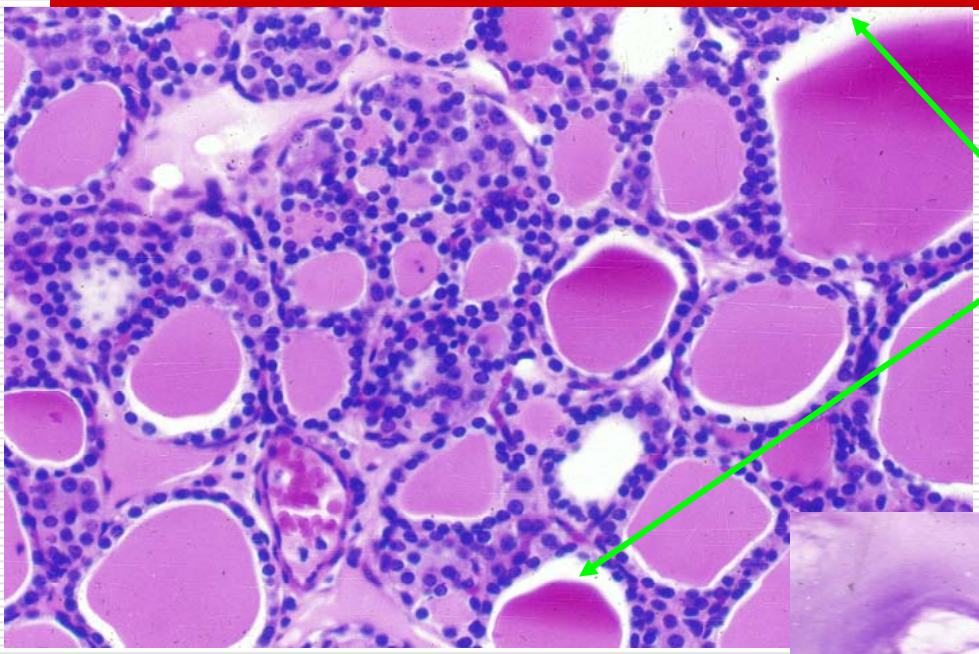
SEM micrograph of the epithelium of stomach

Problems in the interpretation of tissue sections

- Distortions & artifacts caused by tissue processing
  - shrinkage
  - Artificial spaces

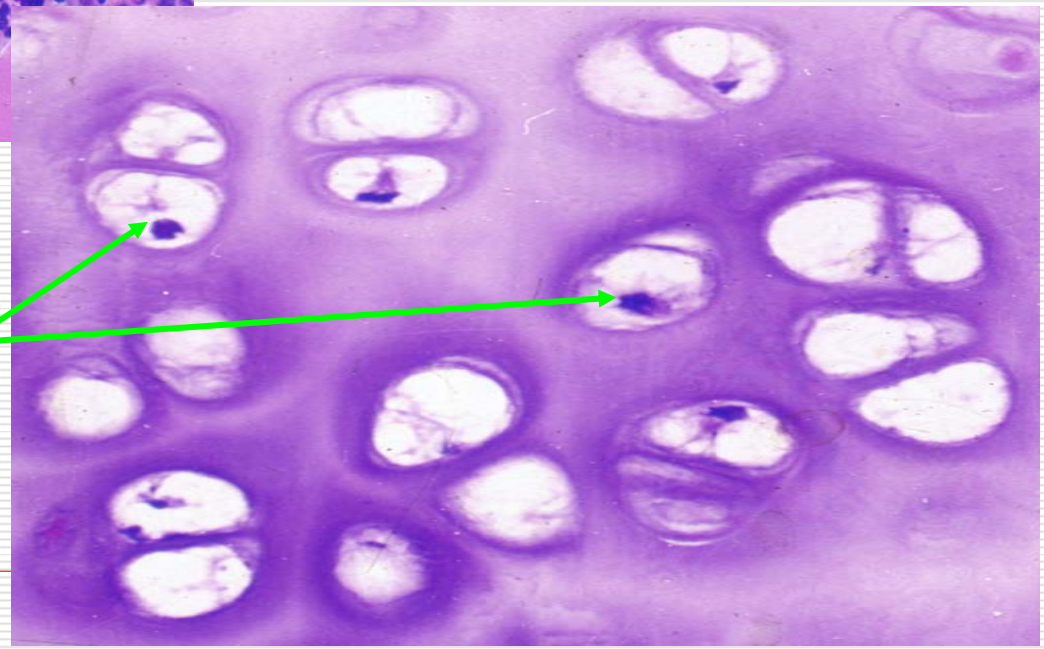
  } artifact
  - Wrinkles of the section
  - precipitate of stain
- Totality of the tissue
- Two dimensions & three dimensions

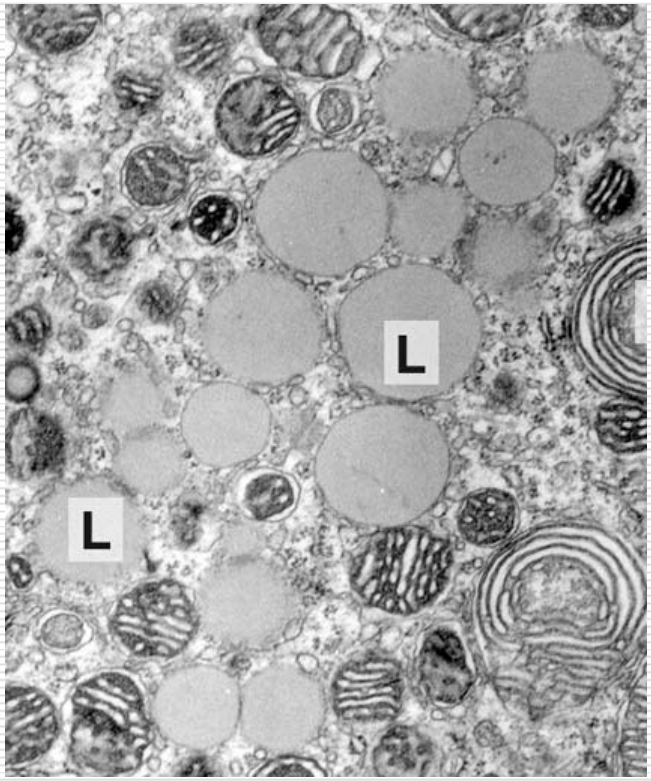
Shrinkage caused by tissue processing

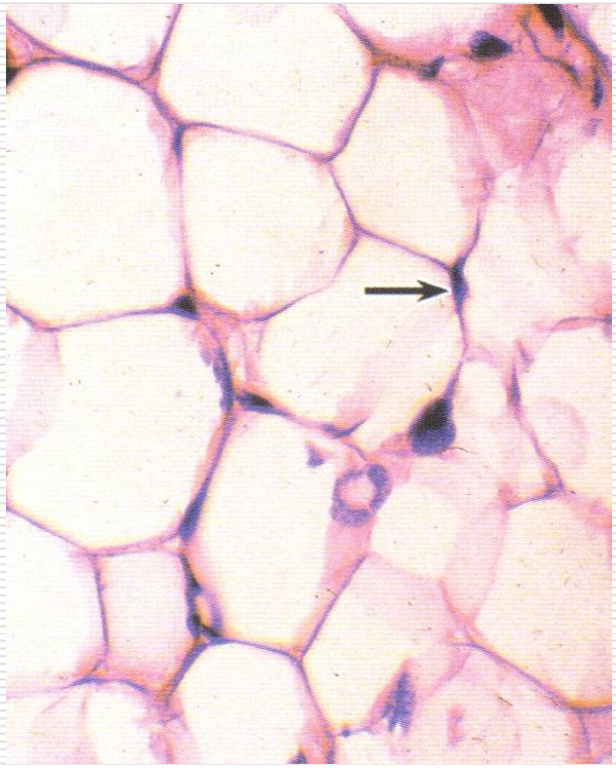


artificial spaces between the colloid and the follicular wall in the section of thyroid gland.

Shrinkage of cells in hyaline cartilage

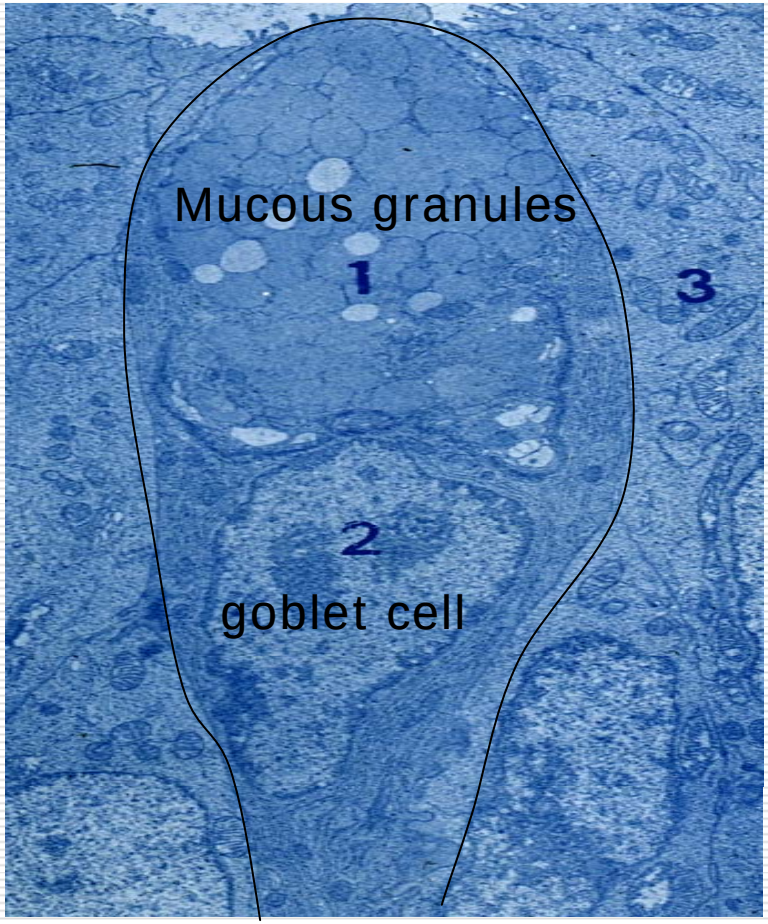


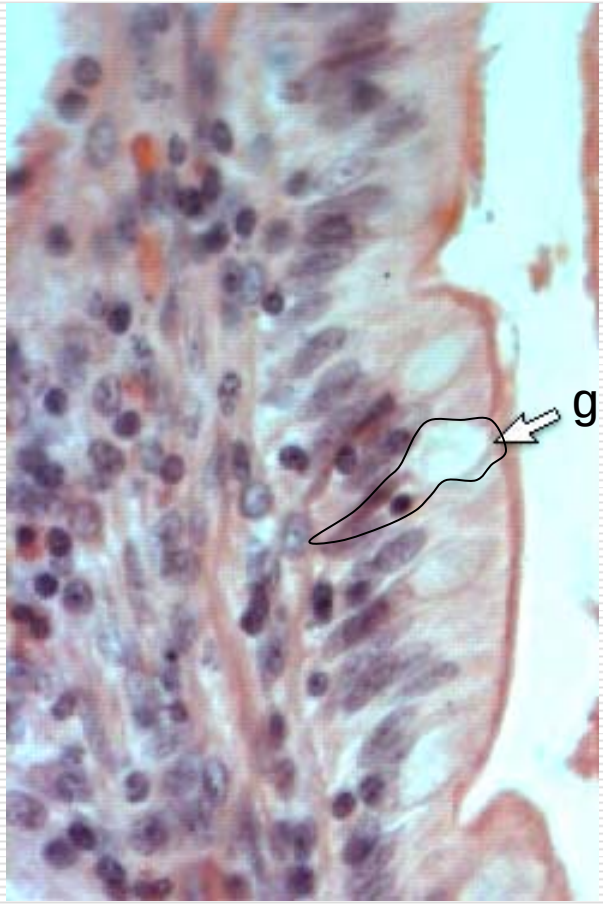




Lipid droplets infat cells are lost during tissue preparation.

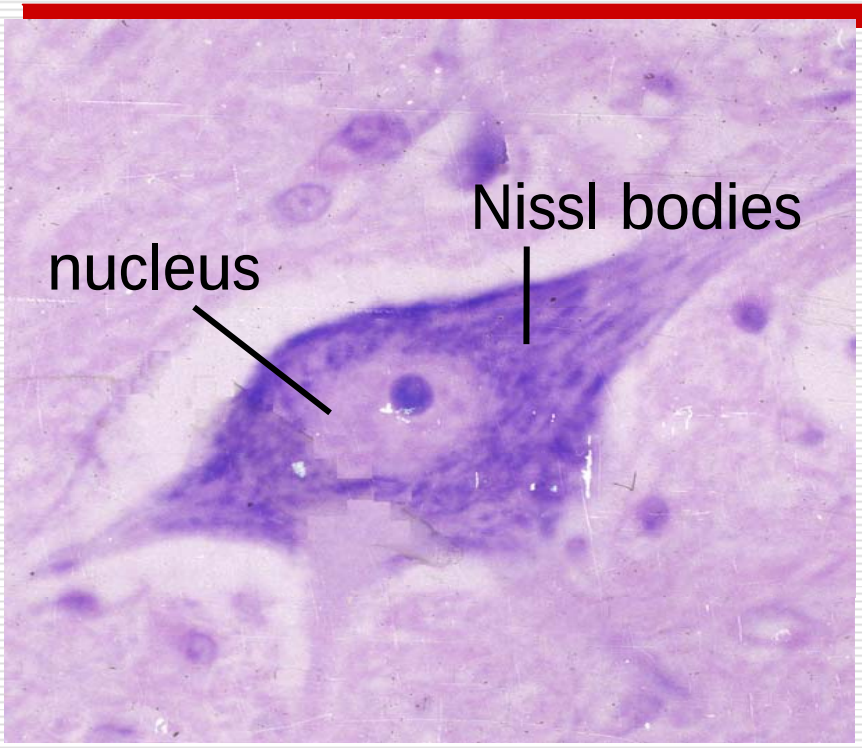
Artifacts caused by tissue processing





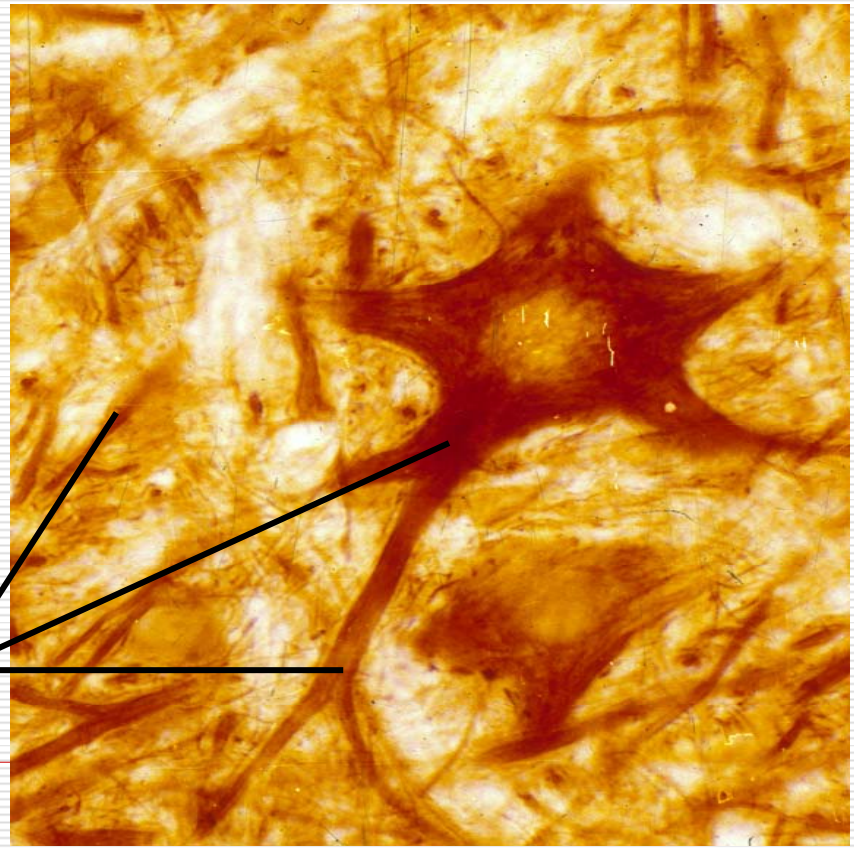
Mucous granules containing glycoprotein in the cytoplasm of goblet cells are lost during tissue preparation.

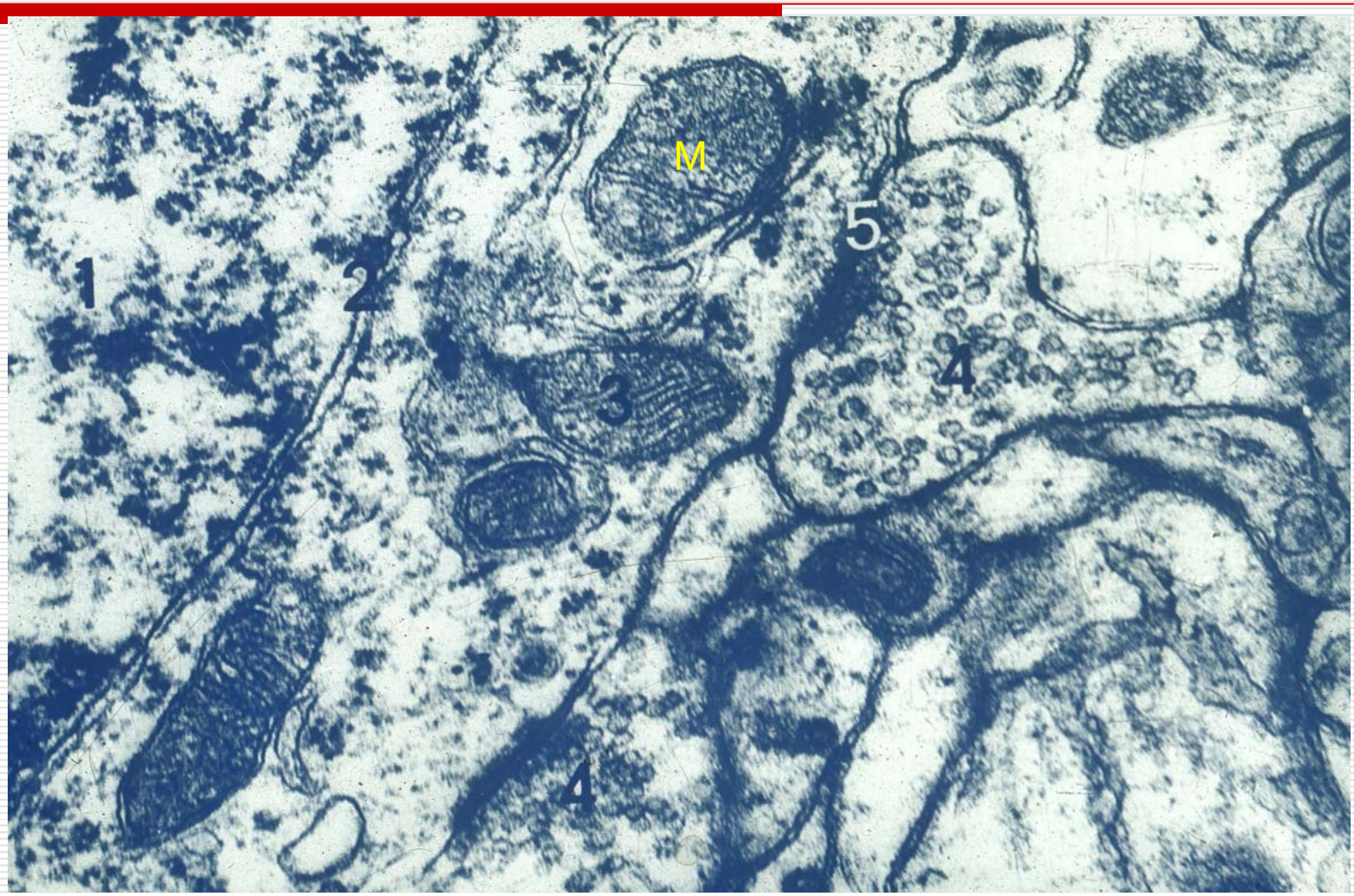
Totality of the tissue



H&E staining

Sliver staining

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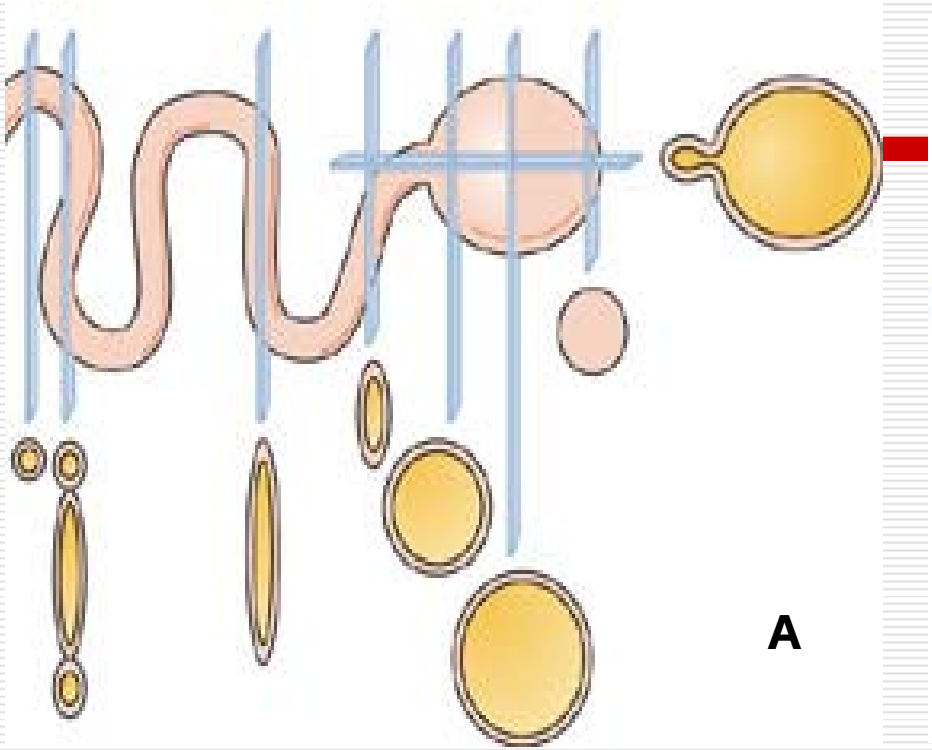

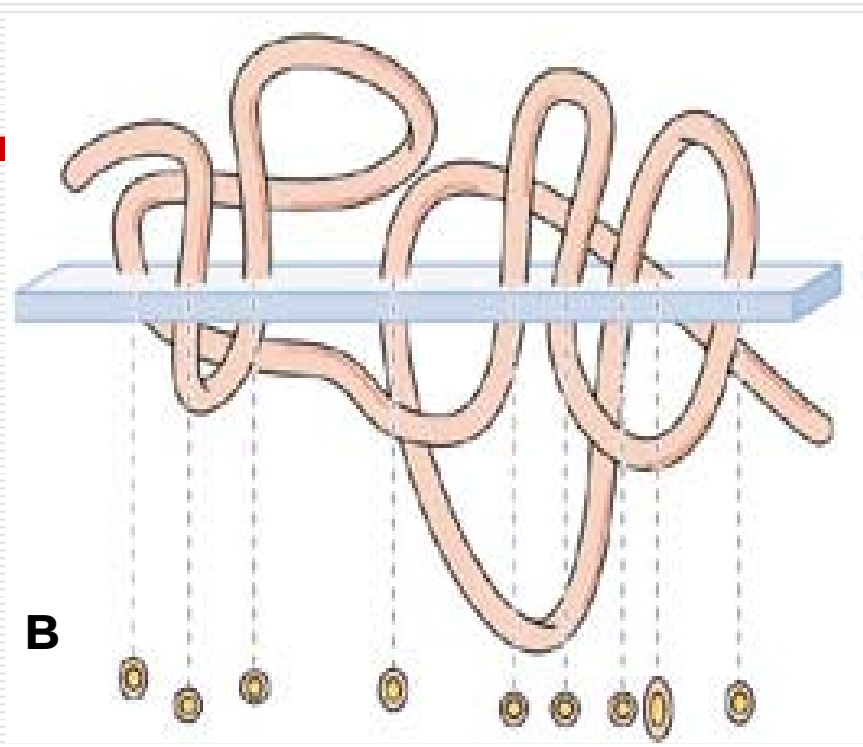

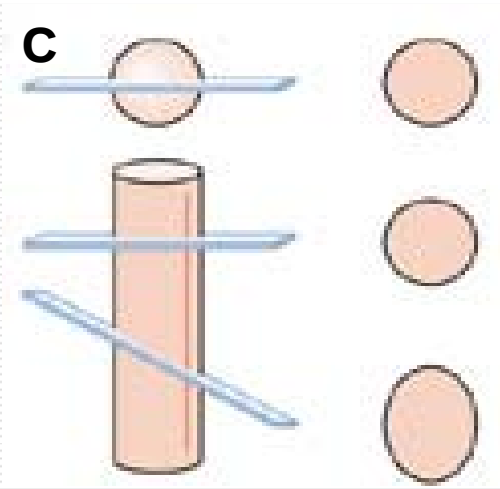

How different 3-dimensional structures may appear when thin-sectioned.
A: Different sections through a hollow ball and a hollow tube.
B: A section through a single coiled tube may appear as sections of many separate tubes.
C: Sections through a solid ball (above) and sections through a solid cylinder (below).

Important questions

- Hematoxylin & Eosin (H & E) staining
- basophilic
- acidophilic