Histology - Introduction

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(2023)

- ***Histology*** is the study of the various tissues of the body: how these tissues appear, how they interact with each other and how they are arranged to constitute an organ.

- Features of tissues cannot be seen by the un-aided eye. Therefore, their study is done by using a magnifying tool – the *Microscope*.

Components of Tissues

- **Cells**
- **Extracellular Matrix**
  - Fibers
  - Organic and Inorganic molecules
  - Water

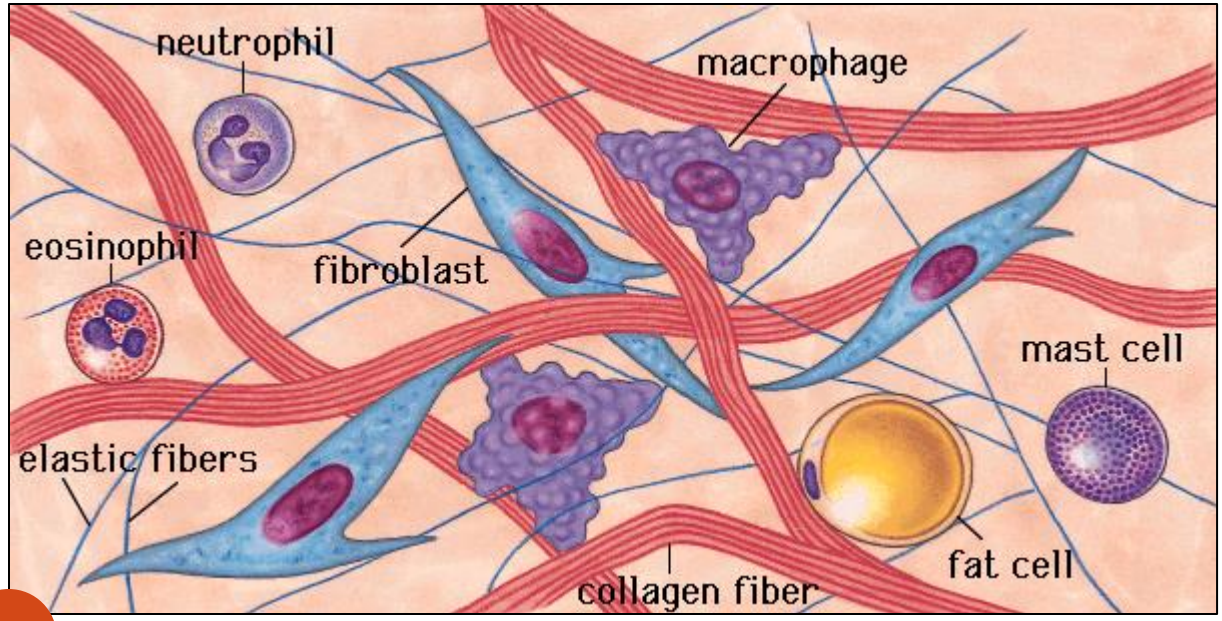
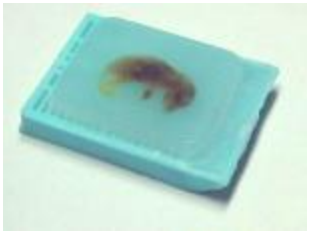


Fig.1: Image showing various components of tissues.

Preparation of tissues for study

1. ***Fixation***: To prevent tissues from being degraded by tissue or bacterial enzymes, a suitable ***fixative*** must be added. These prevent the protein enzymes from functioning. The most famous fixative used is ***Formalin*** (an aqueous solution of formaldehyde) which is used to preserve cadavers in anatomy labs.

After some time



2. ***Embedding***: To facilitate the cutting process, the soft tissues must be first placed into a suitable hard medium (usually ***paraffin wax***).

3. ***Sectioning***: The thick tissues do not allow light to pass through them. Therefore they must be cut into thin slices. This is usually done with a device called the ***microtome***.

Fig.2: Microtome.

4. ***Staining***: Most tissues are colorless. To make them easily visible, they must be stained.

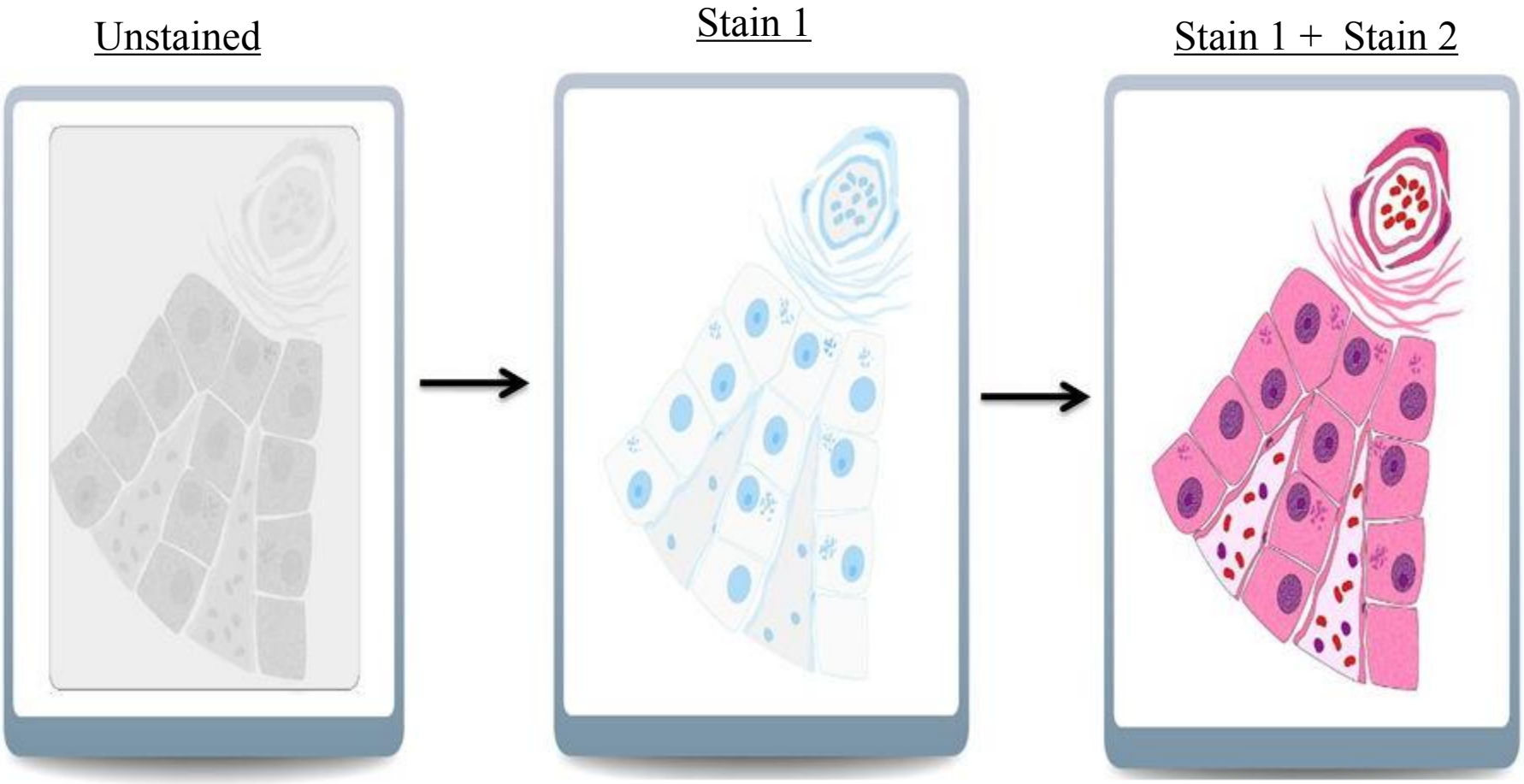


Fig.3: Benefit of staining.

Main Principle of Staining:

- Components of cells/tissues with a ***net negative charge*** react with ***basic dyes*** (which are positively charged and usually blue). These components are, thus, called ***Basophilic***. Examples: ***DNA and RNA, Glycosaminoglycans***, and others.

$$Component^{-} + \mathbf{Dye}^{+}Cl^{-} \rightarrow \mathbf{Dye}Component + Cl^{-}$$

- Components of cells/tissue with a ***net positive charge*** react with ***acidic dyes*** (which are negatively charged and usually red). These components are, therefore, called ***Acidophilic***. Examples: ***proteins (as in collagen fibers and mitochondria)*** and others.

$$Component^{+} + Na^{+}\,\mathbf{Dye}^{-} \rightarrow Component\mathbf{Dye} + Na^{+}$$

Microscopes and Microscopy

- Several types of microscopes are used in histology.

- They can be generally divided into 2 types:
  - ***Light microscopes***: which use the ordinary beam of light

  - ***Electron microscopes***: which use a narrow beam of electrons

The Light Microscope

1) Bright-Field Microscope

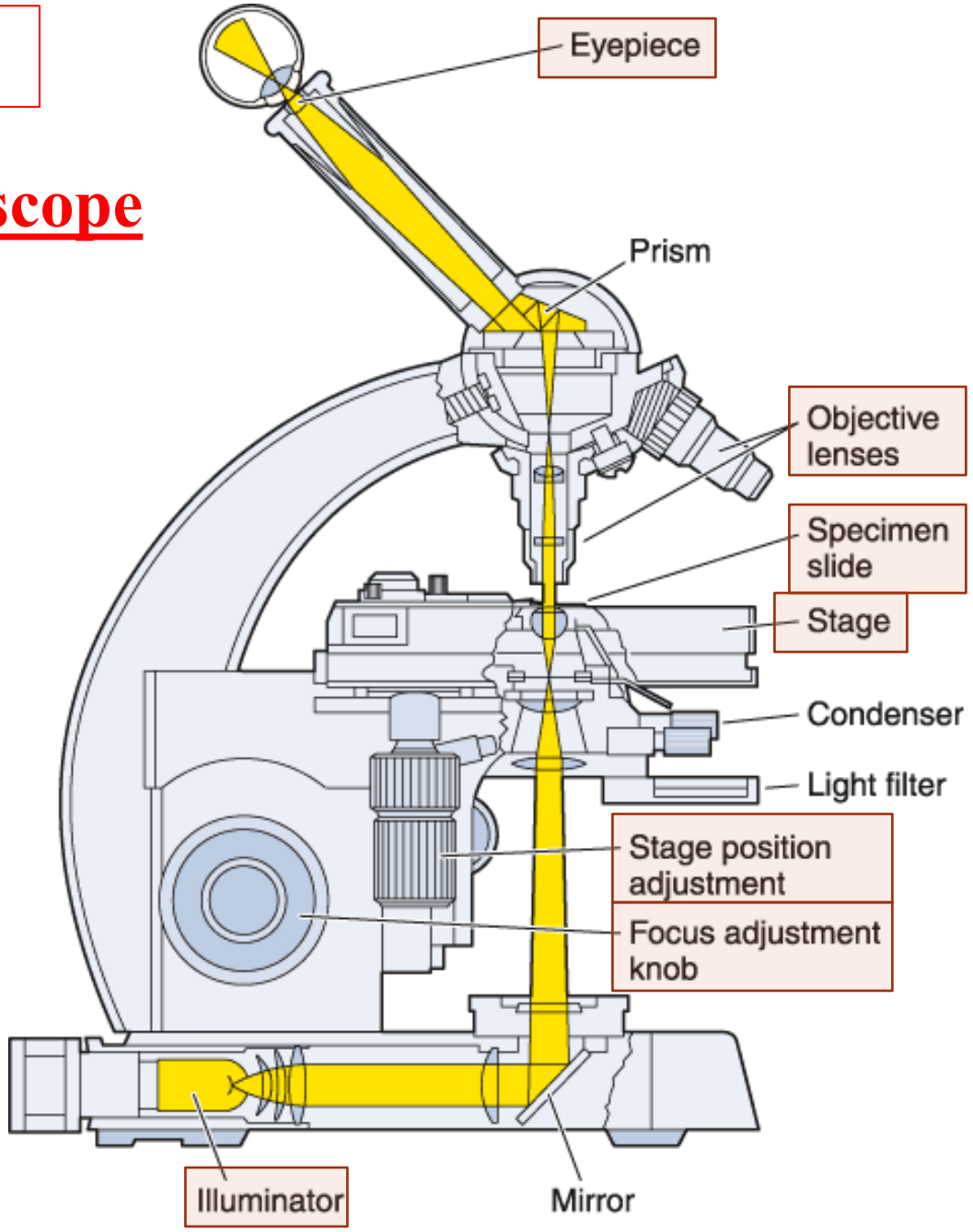


Fig.4: Image showing various parts of a Bright-field light microscope.

- The Resolving power of the light microscope is about 0.2μm.
- ***Resolving power***: the minimum distance between two points that enable the device to recognize them as two points*.

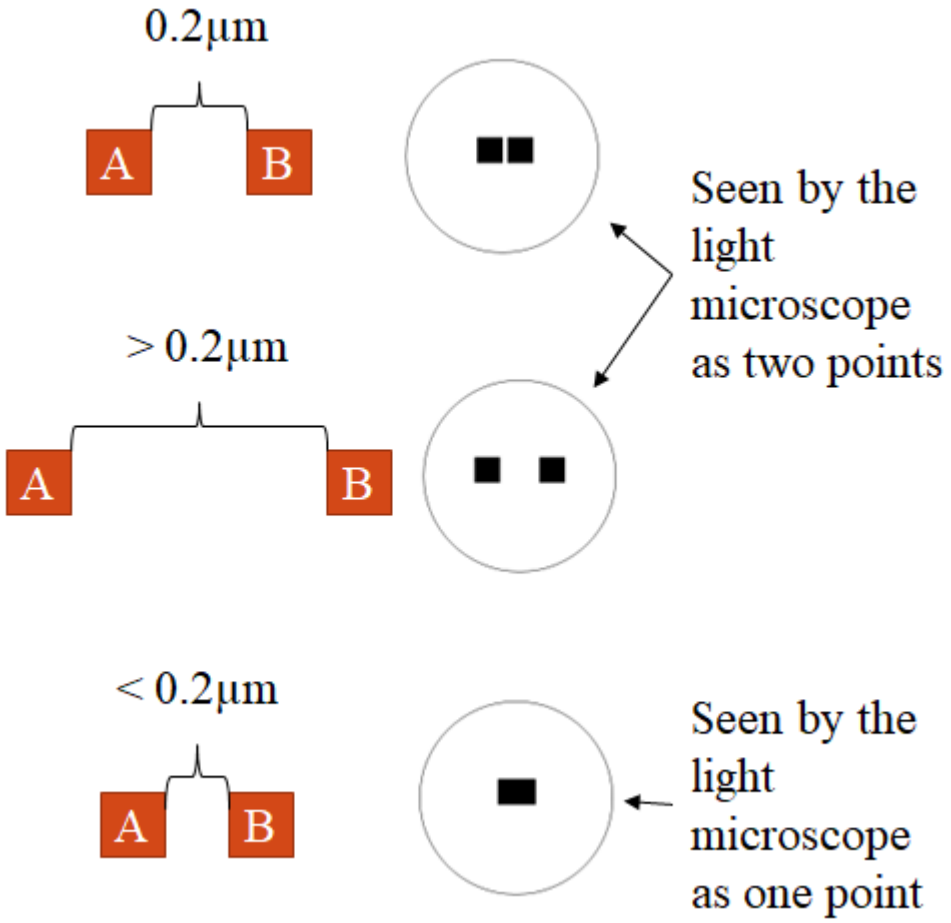


Fig.5: How distance between two points affects their appearance under the microscope.

* This same definition of resolving power can be used for cameras, television sets, computer monitors, and the human eye.

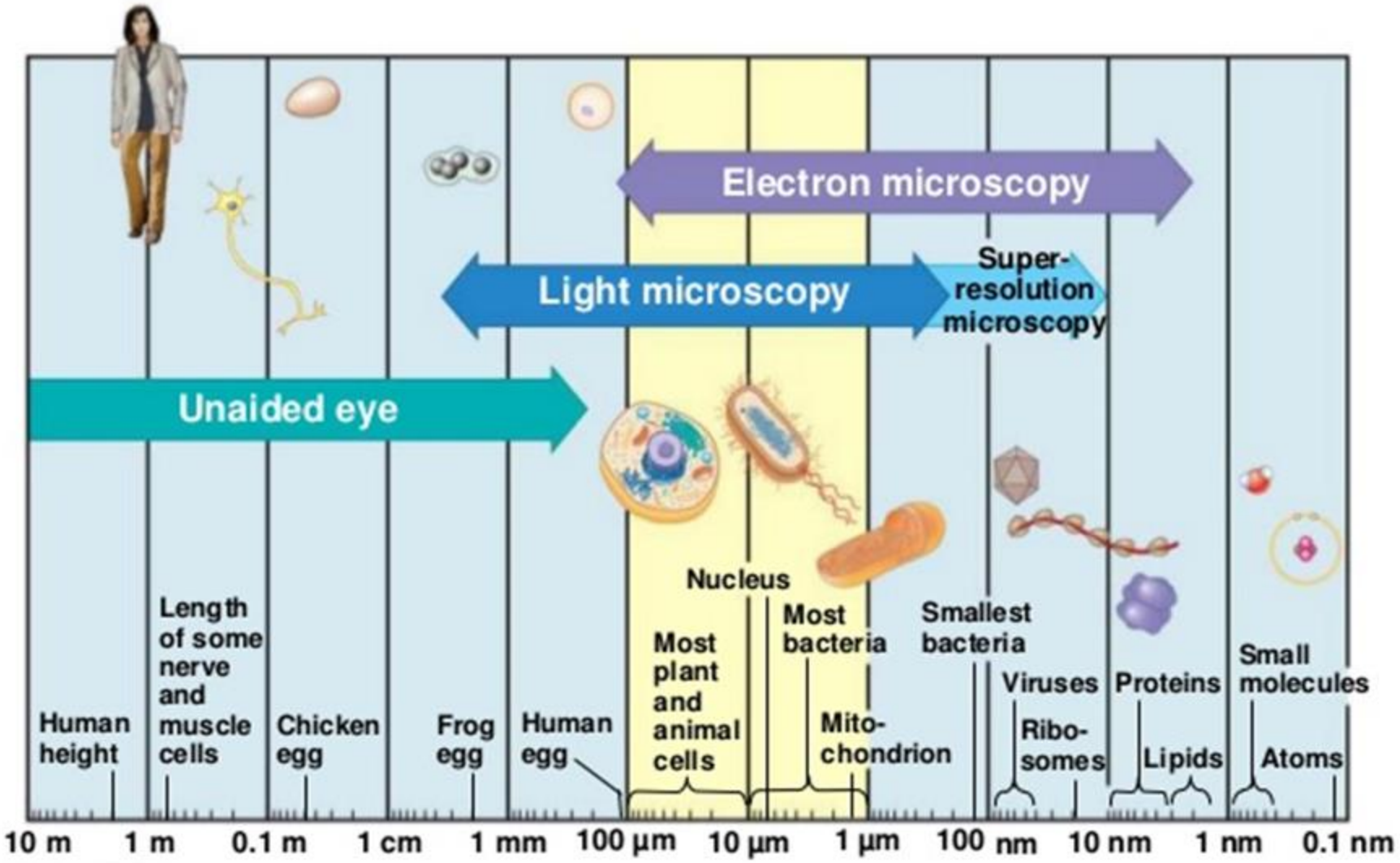


Fig.6: Resolving power of various optical devices.

2) Fluorescence Microscopy

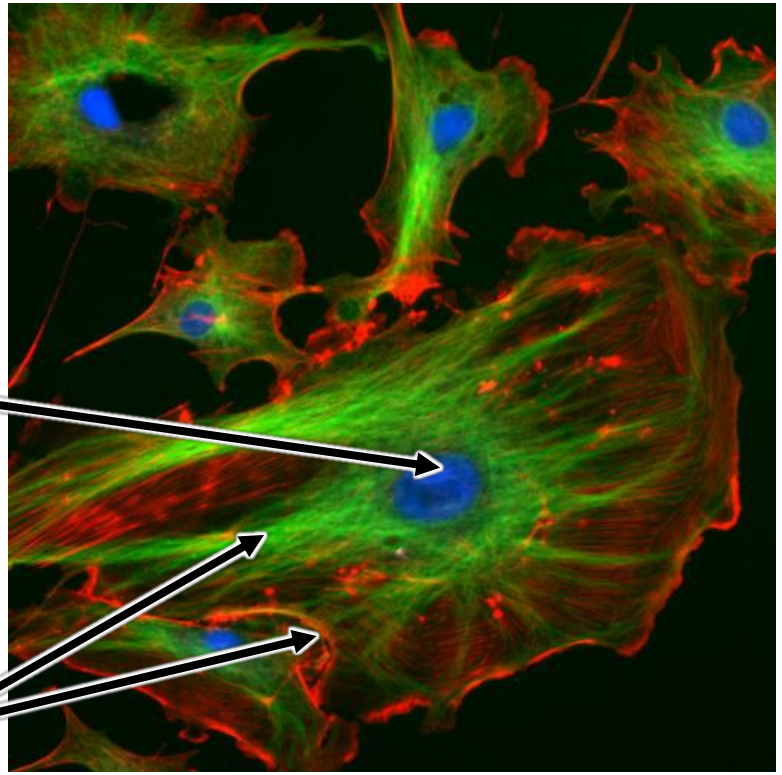
- When certain substances are irradiated by a ray of a certain wavelength, they emit an electromagnetic wave of a, usually, longer wavelength. This is called ***fluorescence***.

- When UV light is used, the emission is in the visible spectrum.

- During tissue preparation, certain substances with this characteristic can be added to the tissue. These will bind to the various structures and make them fluorescent.

- Example of fluorescent substances:

1) Diamidinophenylindole (DAPI) binds to DNA → ***Blue***
2) Phalloidin binds to actin filaments → ***Red***, ***Green***
3) Tetracycline binds to newly formed bone → ***Green***



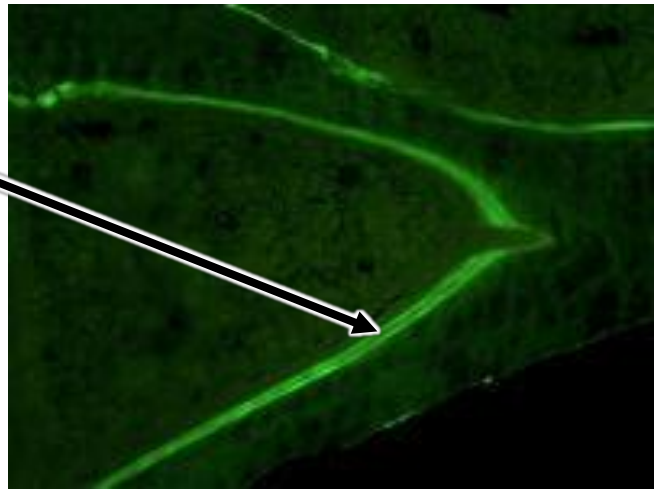


Fig.7: Different colors produced by different fluorescent dyes.

The Electron Microscope

- Uses a beam of electrons instead of light photons.
- It gives a much higher *resolution* than the light microscope (resolving power = 3nm).
- It could be either Transmission Electron Microscope (TEM) or Scanning Electron Microscope (SEM).

1) TEM

- The beam of electrons interact differently with the different parts of the section.
- Some are deflected, some pass through, and some are reflected.
- Electrons passing through the section are detected to produce an image.

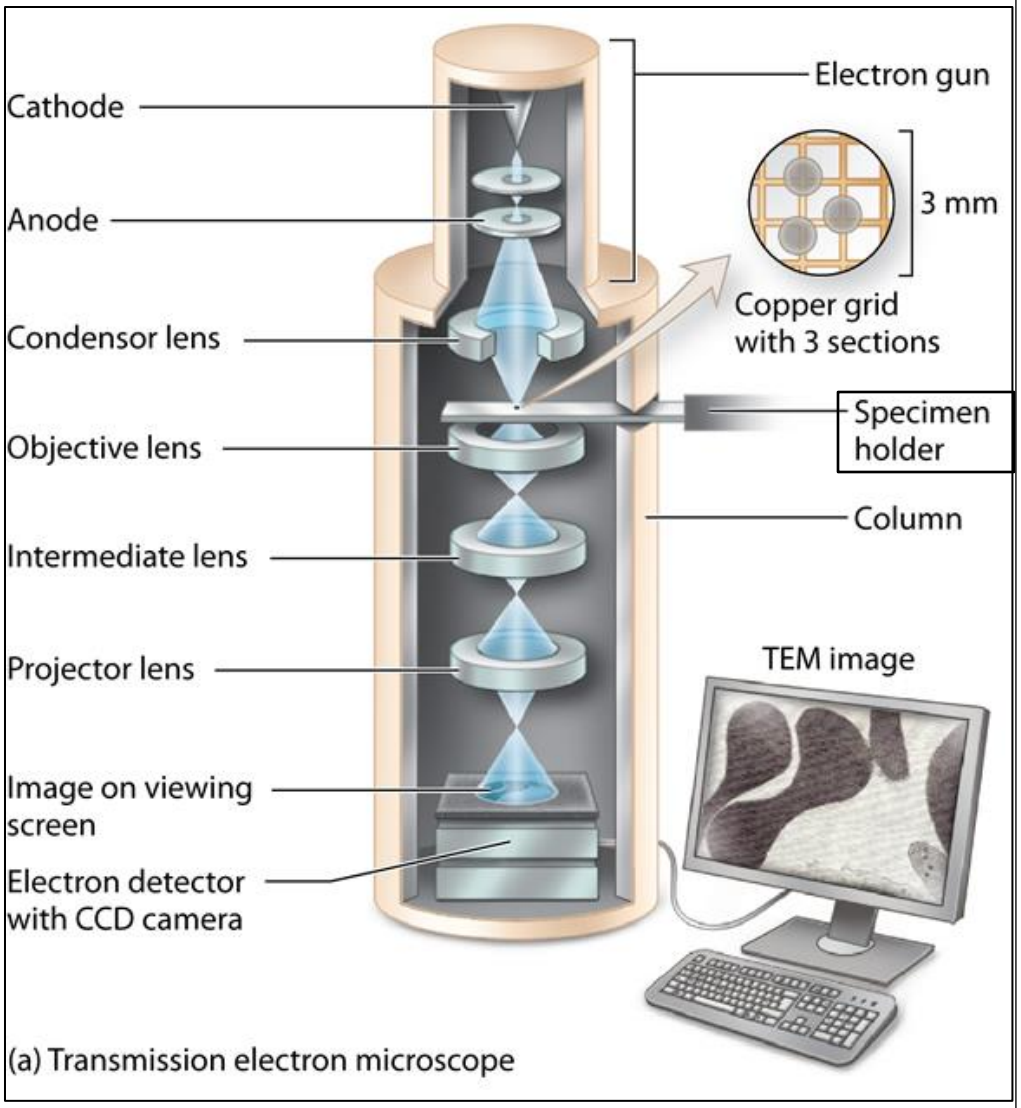
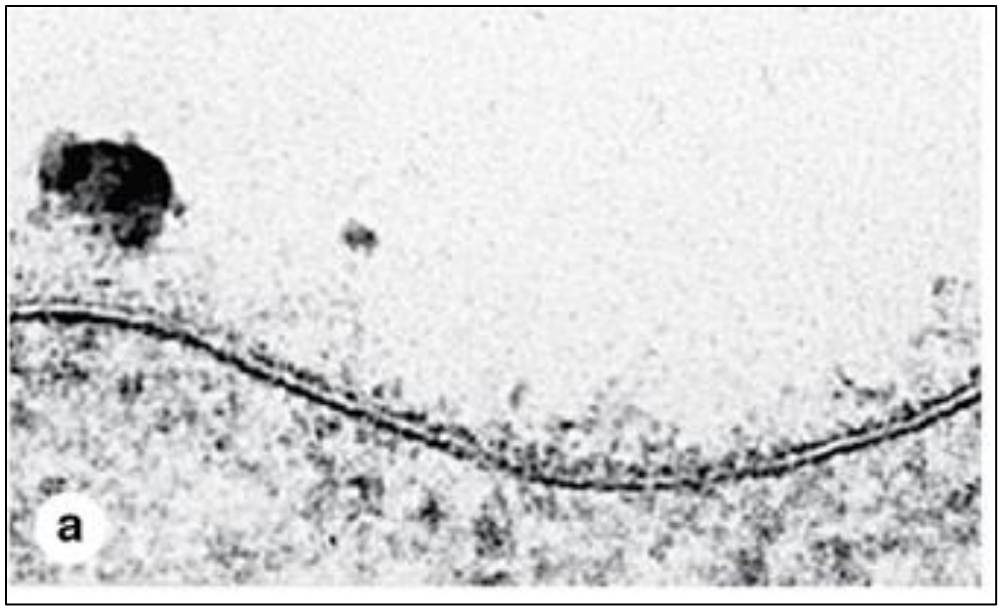


Fig.8: Schematic drawing of TEM.



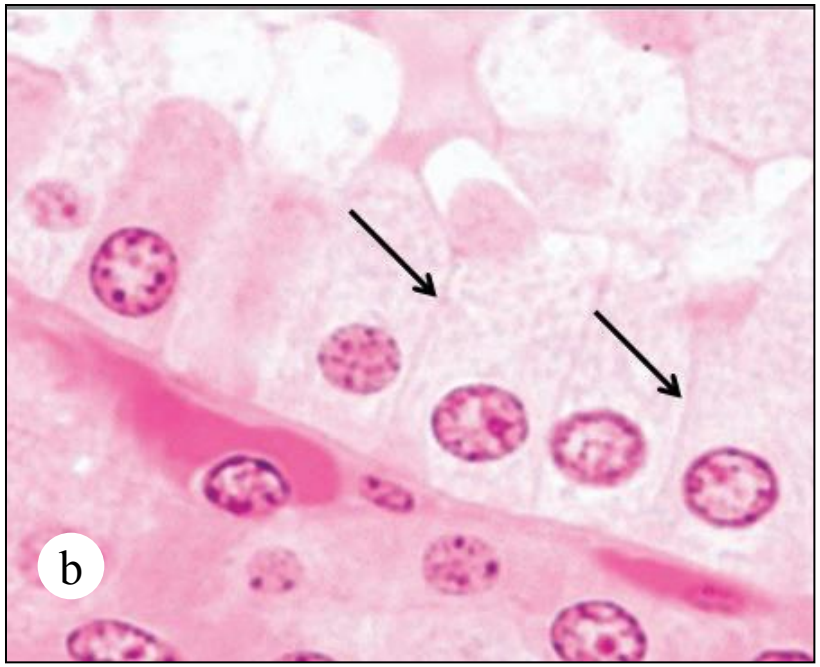


Fig.9: (a) A TEM image of the cell membrane. Note how it appears to be formed of a white line between two dark lines. In the light microscope image (b), the cell membrane appears as a very thin line (arrows). With the electron microscope, we obtained an image with a higher resolution giving us more details about the structure studied.

2) SEM

- The specimen is first coated with a metal that reflects electrons.
- The electron beam scans the specimen from end to end.
- The reflected electrons are captured to produce a *pseudo-3D* image of the coated *surface*.

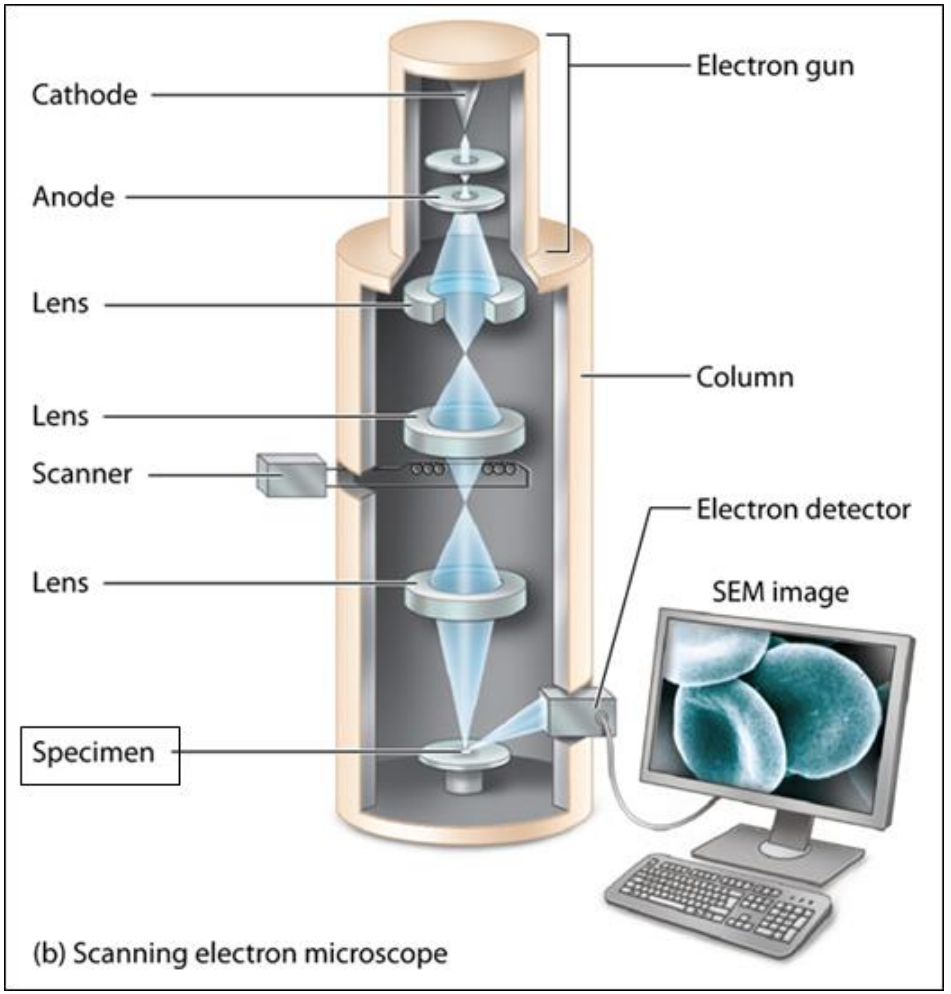


Fig.10: Above: schematic drawing of SEM. Left: Ant seen by SEM.

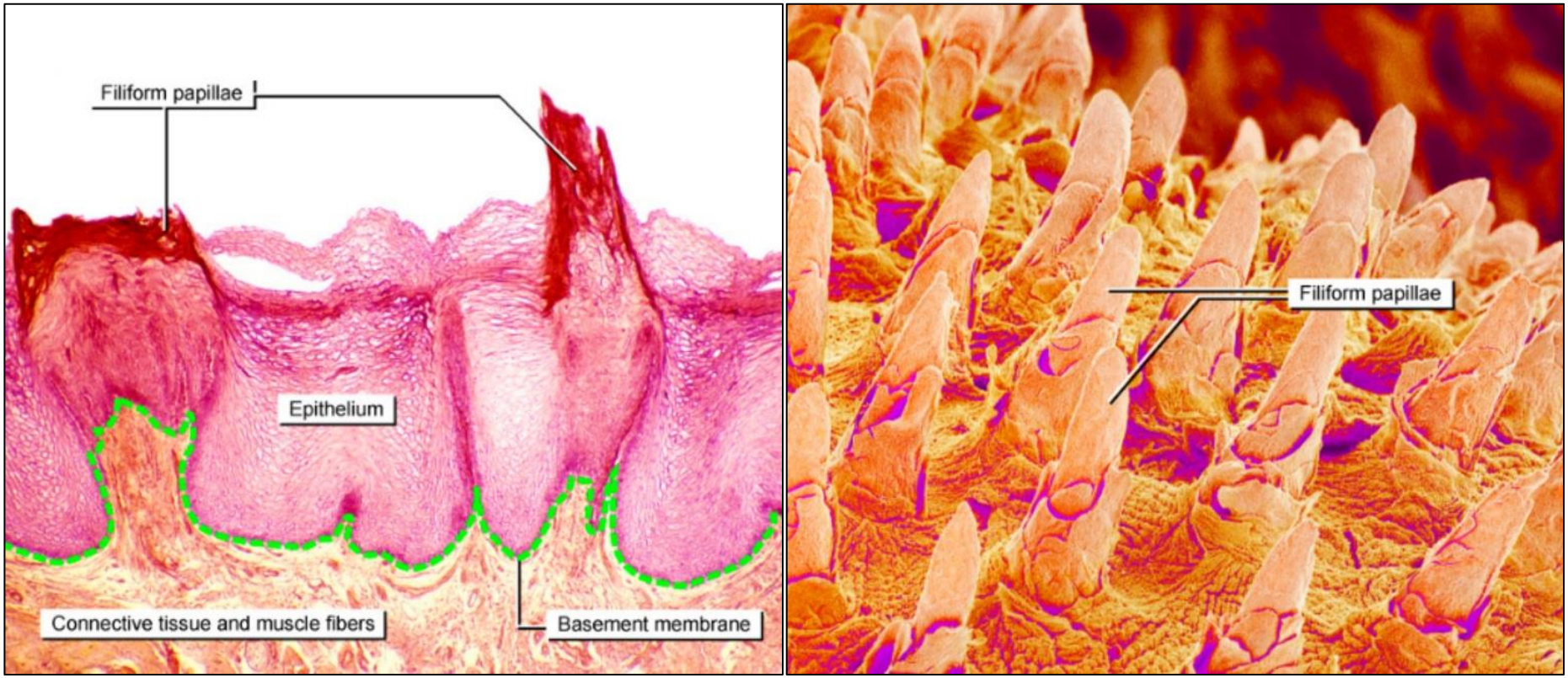


Fig.11: Comparison between light microscope and scanning electron microscope. Left: Filiform papillae seen under the light microscope. Right: Filiform papillae seen under the scanning electron microscope.

Other methods of study

1) Autoradiography

- Molecules (amino acids, sugars, nucleotides, …) labeled with radioactive isotopes (usually tritium, ^{3}H) are added to the living tissue prior to preparation.
- These are taken up by the tissue. Tissue will give off radiation.
- The slide is covered by an emulsion containing **Silver Bromide** to detect the radiation.
- The slide is developed in a dark box and the areas of tissue containing the radioactive molecules appear as black dots.

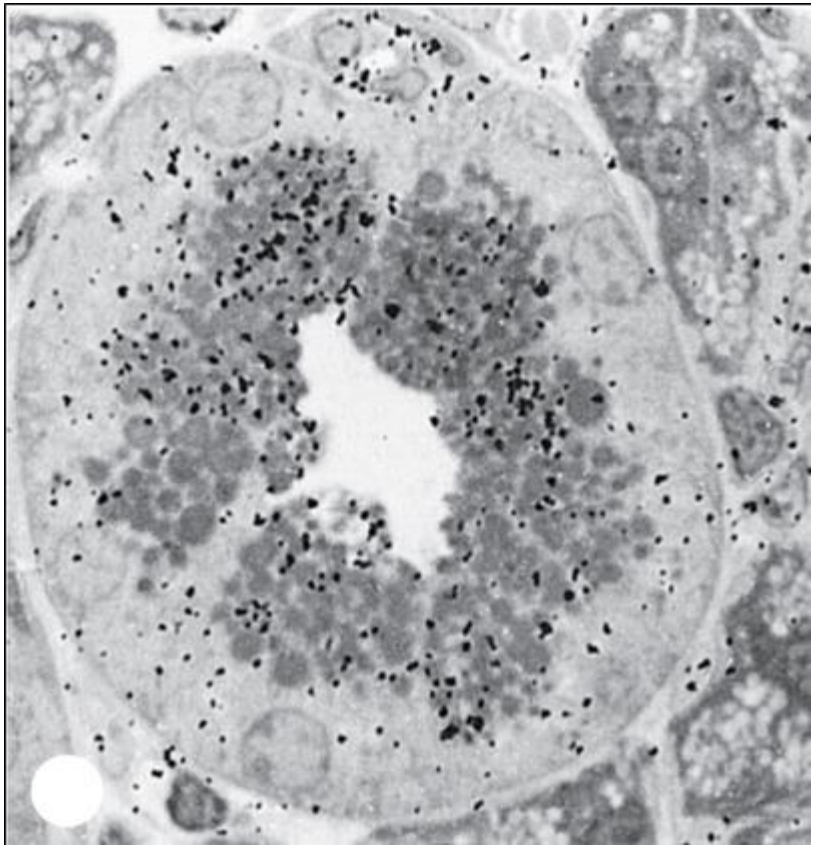


Fig.12: Mouse salivary gland injected with radioactive fucose which was used in the synthesis of saliva. The black dots indicate the site of synthesis.

2) Histochemistry

- Chemical reactions occur throughout the body. These reactions produce soluble, thus, invisible substances.

- In histochemistry, certain ***Markers*** are added to the tissue that will convert the reaction products into insoluble and, therefore, visible substances that can be detected.

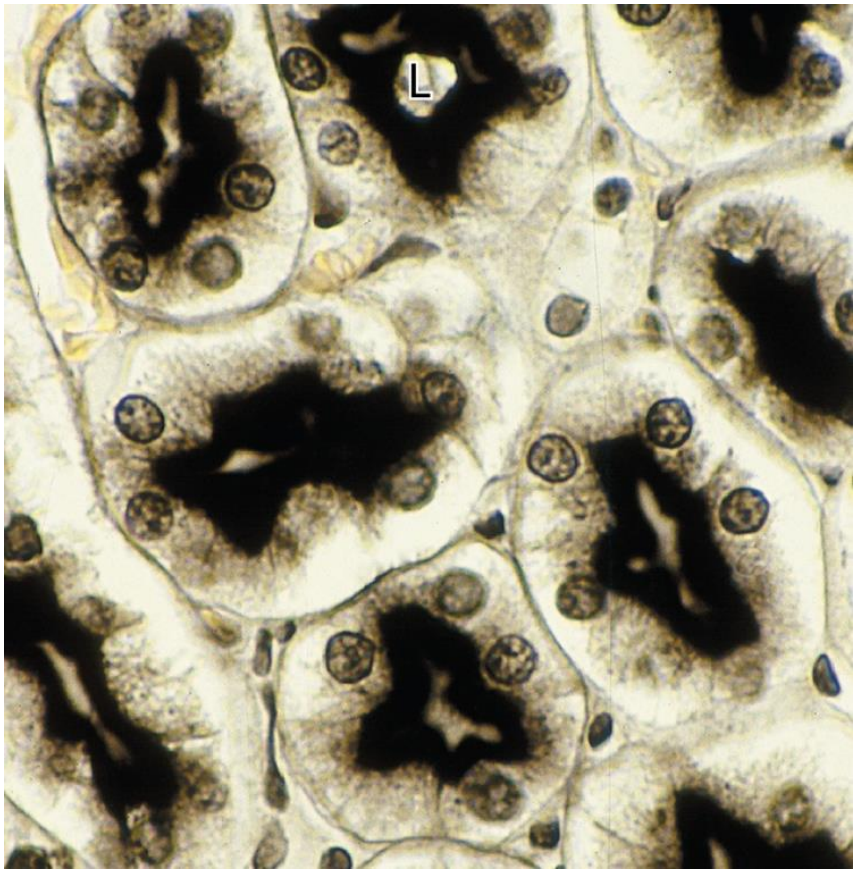


Fig.13: Renal tubules. A histochemical method was used to localize areas with high alkaline phosphatase activity.

3) Immunocytochemistry

- ***Tagged antibodies*** specific against a certain part of a tissue are used.
- These bind to the tissue causing their staining.

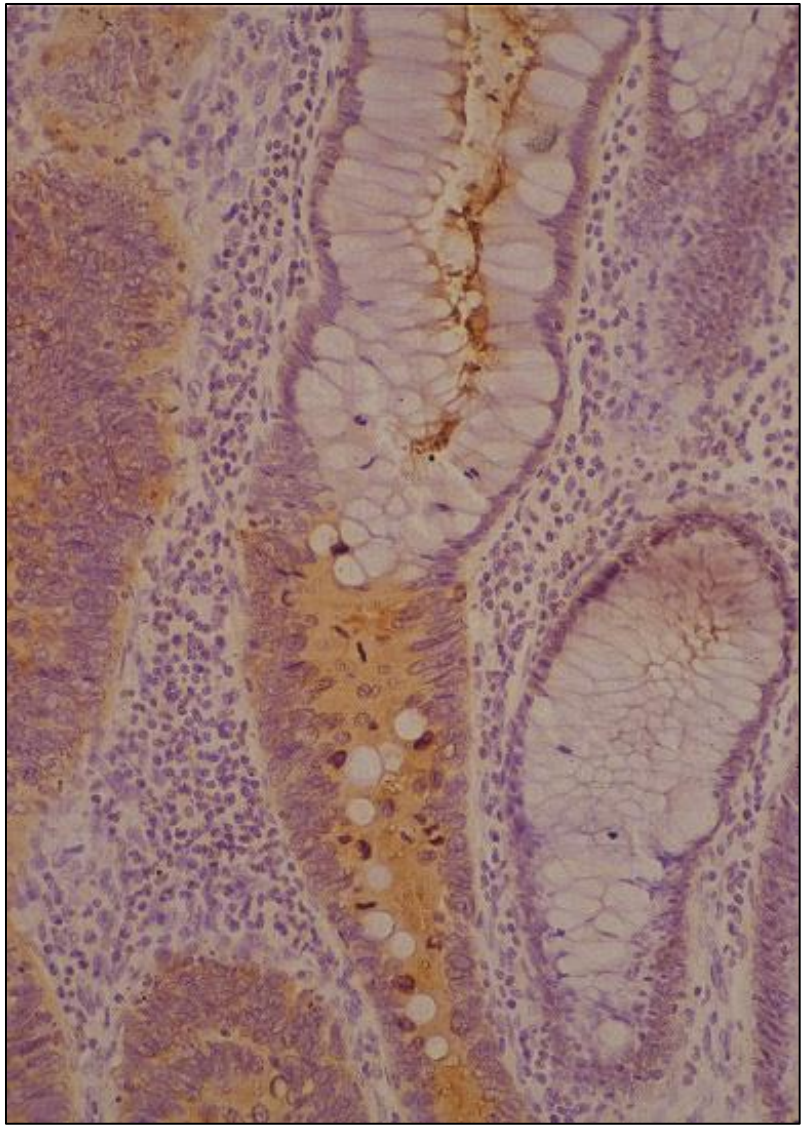


Fig.14: Adenocarcinoma of the intestine stained using an antibody against a specific substance produced by the tumor. Cancer cells are stained brown.

Problems with tissue preparation

A. ***Artifacts:*** (1)Precipitation of stains, (2)breakage in the tissue, and (3)shrinkage of tissues producing artificial spaces.

B. ***Differential staining:*** It's not feasible to differentially stain different parts of cells or tissues using a single dye. Therefore, several dyes or different methodologies may be used.

C. ***3D vs 2D:*** A section will give us a 2D image of a 3D object. A sphere appears as a circle and a tube may appear as a ring. Different planes of sectioning will give the same object different appearances in the section. It's necessary to create sections in different planes to get the true shape of the object.

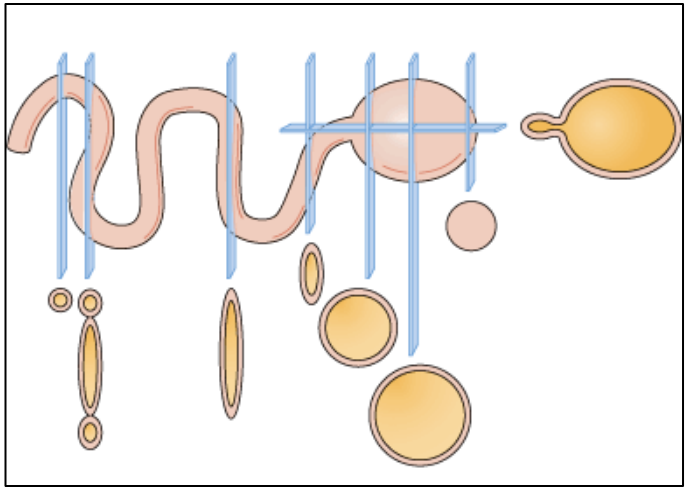
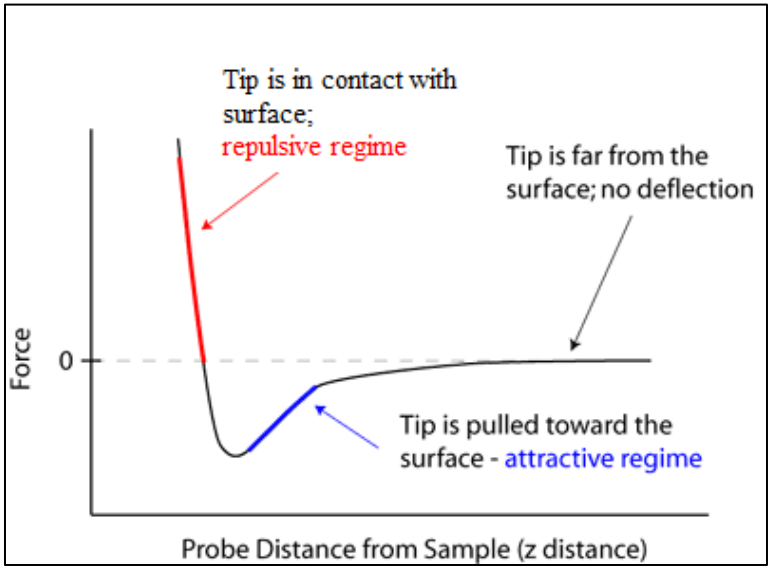


Fig.15: A tube sectioned in several planes.

Atomic Force Microscope (AFM*)

- In this type of microscopes, the surface of the specimen is scanned by a nano-sized probe at very short distances (few nanometers). At such distances, the surface and the probe interact with each mainly through van der Waals force.
- The probe consist of a spring (cantilever) and a tip. As the tip moves over the specimen, the cantilever will bend according to the contours of the surface.



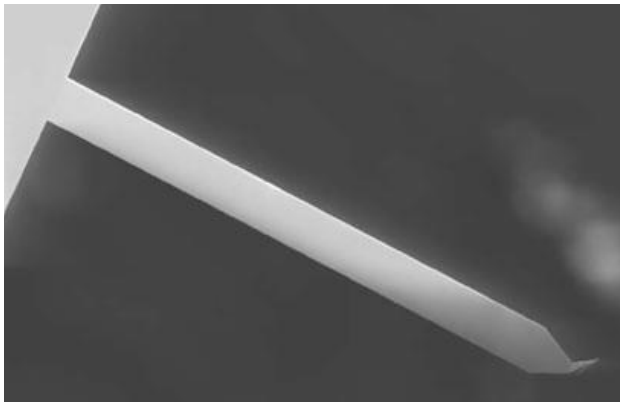
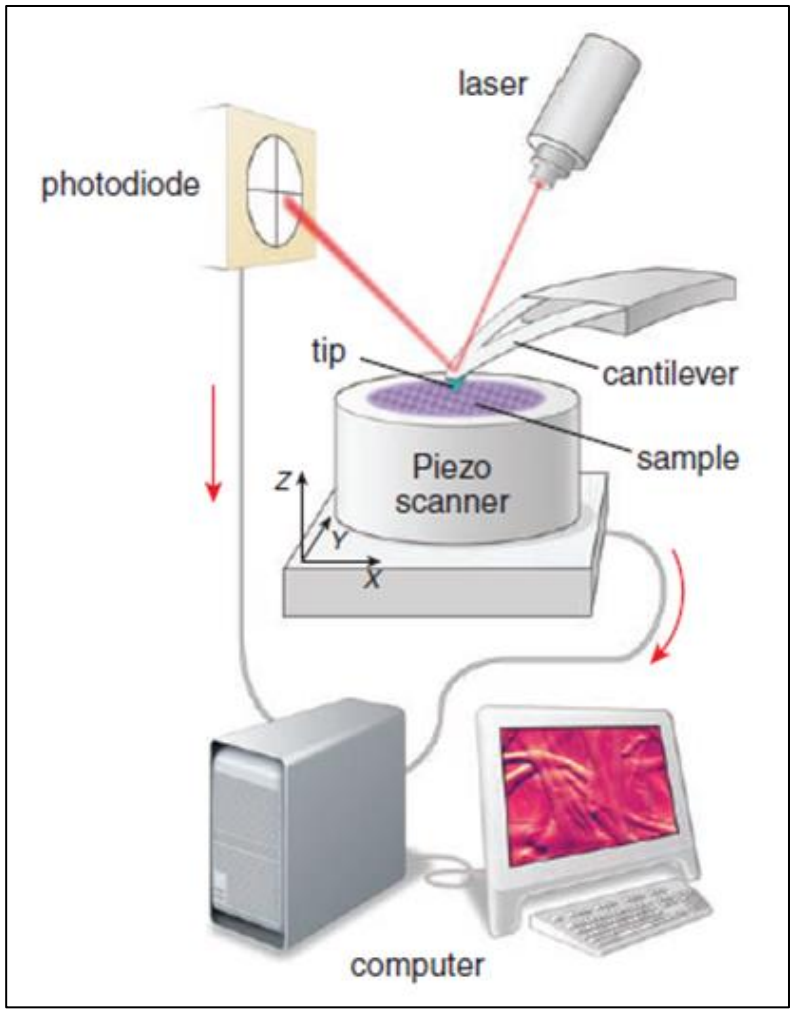


Fig.16: Top: Relation between force and distance in an AFM. Bottom: SEM image of cantilever and tip.

* AFM was invented at IBM.

- A laser directed at the back-surface of the cantilever is reflected onto a photodiode. The position of the laser depends on the position of the cantilever.
- A computer will create an image of the surface based on the varying position of the laser.
- The image obtained is a true 3D image of the surface with a resolving power of 0.1nm (1Å).



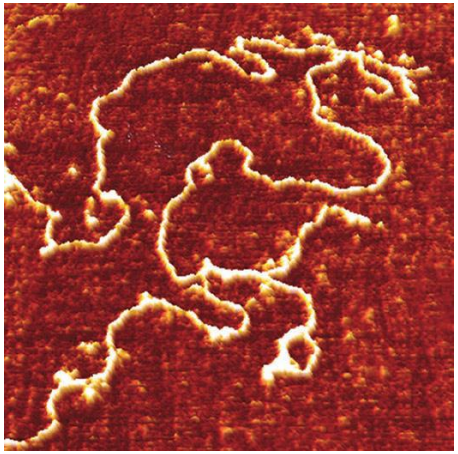


Fig.17: Above: Components of AFM. Left: Image of a single molecule of DNA seen by AFM.

Thank You

" Some men see things as they are and say: WHY?
I dream things that never were and say: WHY NOT? "

For your information:

With the continuous advancement of technology, newer methods of visualization are being invented. In 2013, the electron cloud of the hydrogen atom was visualized using what has been called a 'quantum microscope'. This has no biological use, yet, but who knows what the future holds?

https://doi.org/10.1103/PhysRevLett.110.213001