There is 3 major staining techniques

1. Histo chemistry : staining by reactions between tissue components & chemicals

includes (the most common)

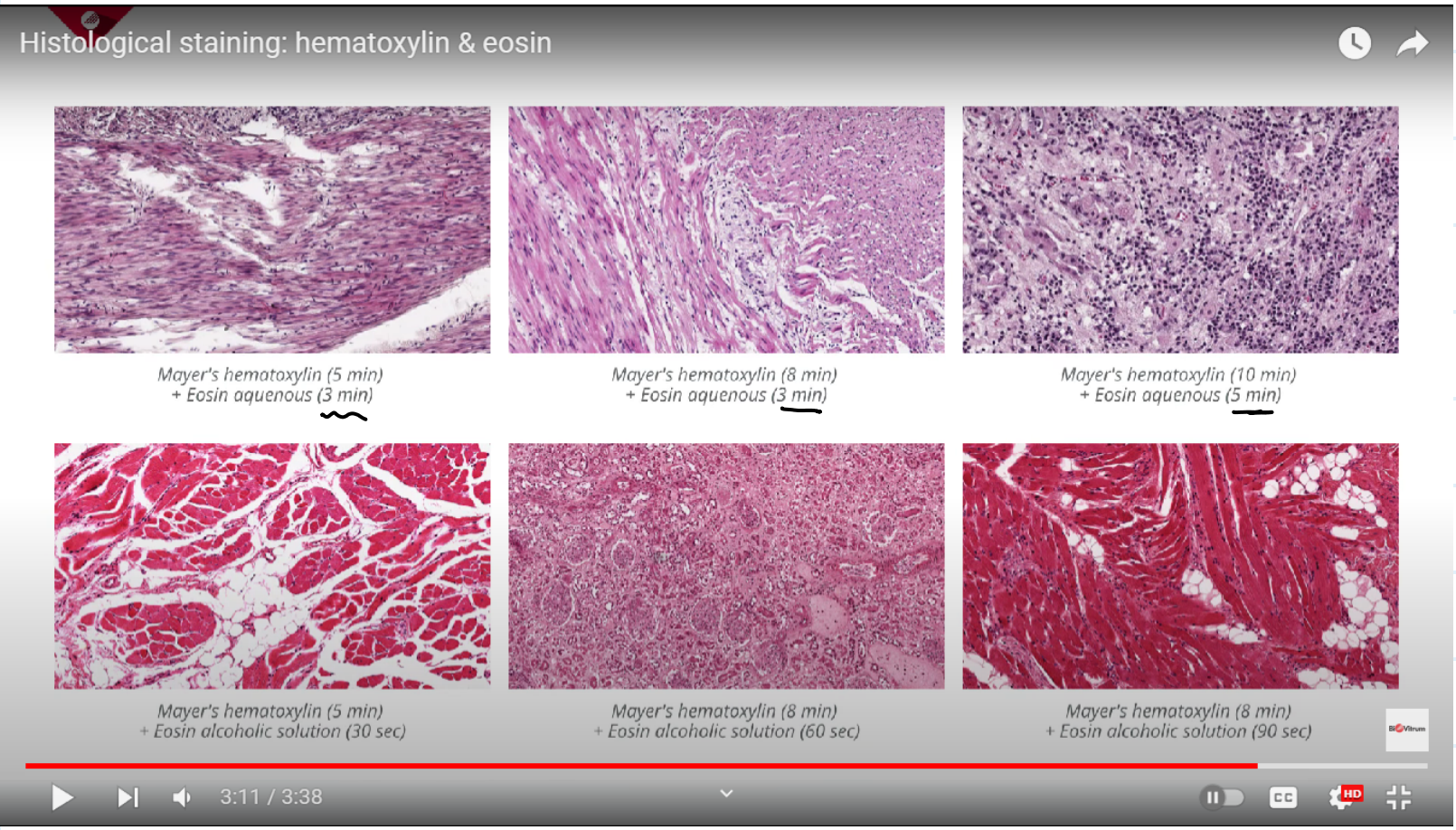
- H&E (Hematoxylin & Eosin)

Staines nucleus Blue (cause Hematoxylin is Base and the nucleus contains Acids (DNA))

Stains cytoplasm pink (cause Eosin is Acidic and the cytoplasm contains many Basic proteins)

note: Eosin varies (pink to orange including red) / Hematoxylin (blue to purple)

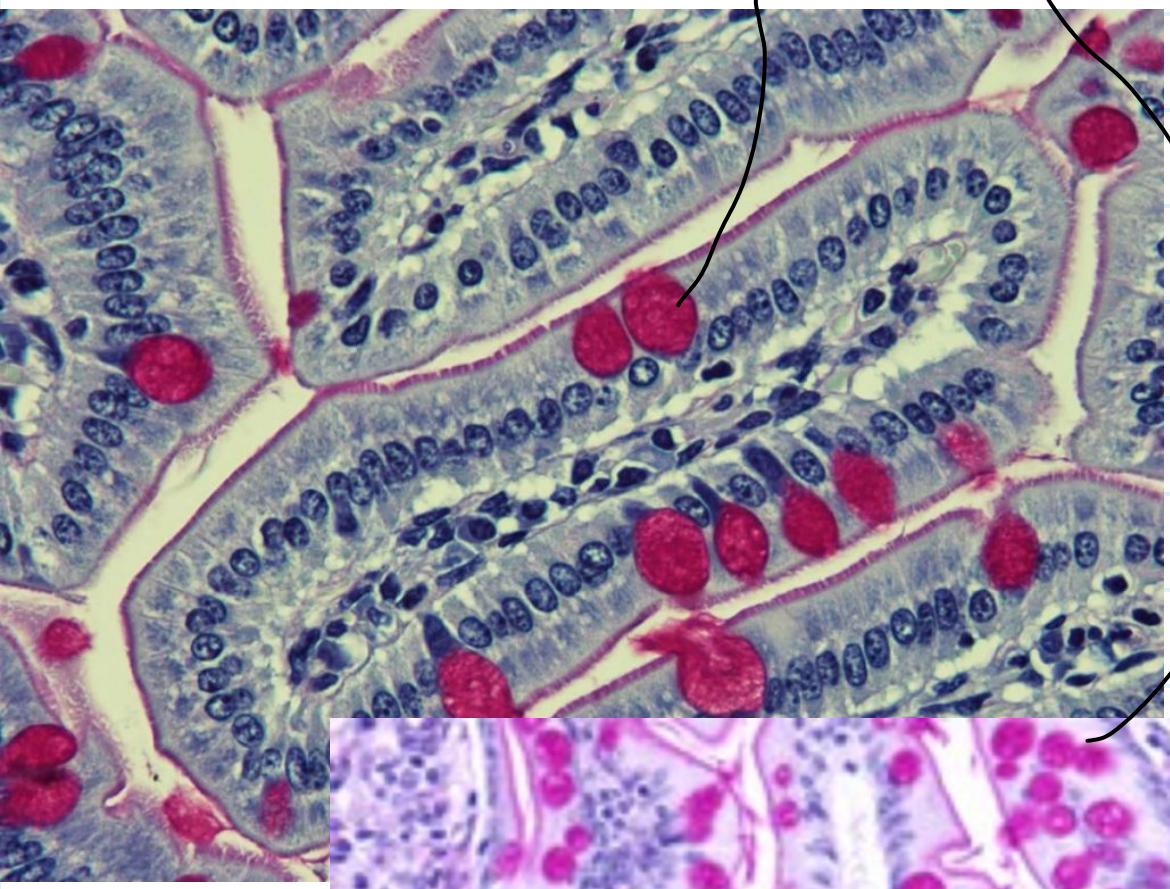
According to: ① How long the specimen has been preserved
② How long it stayed in the stain solution

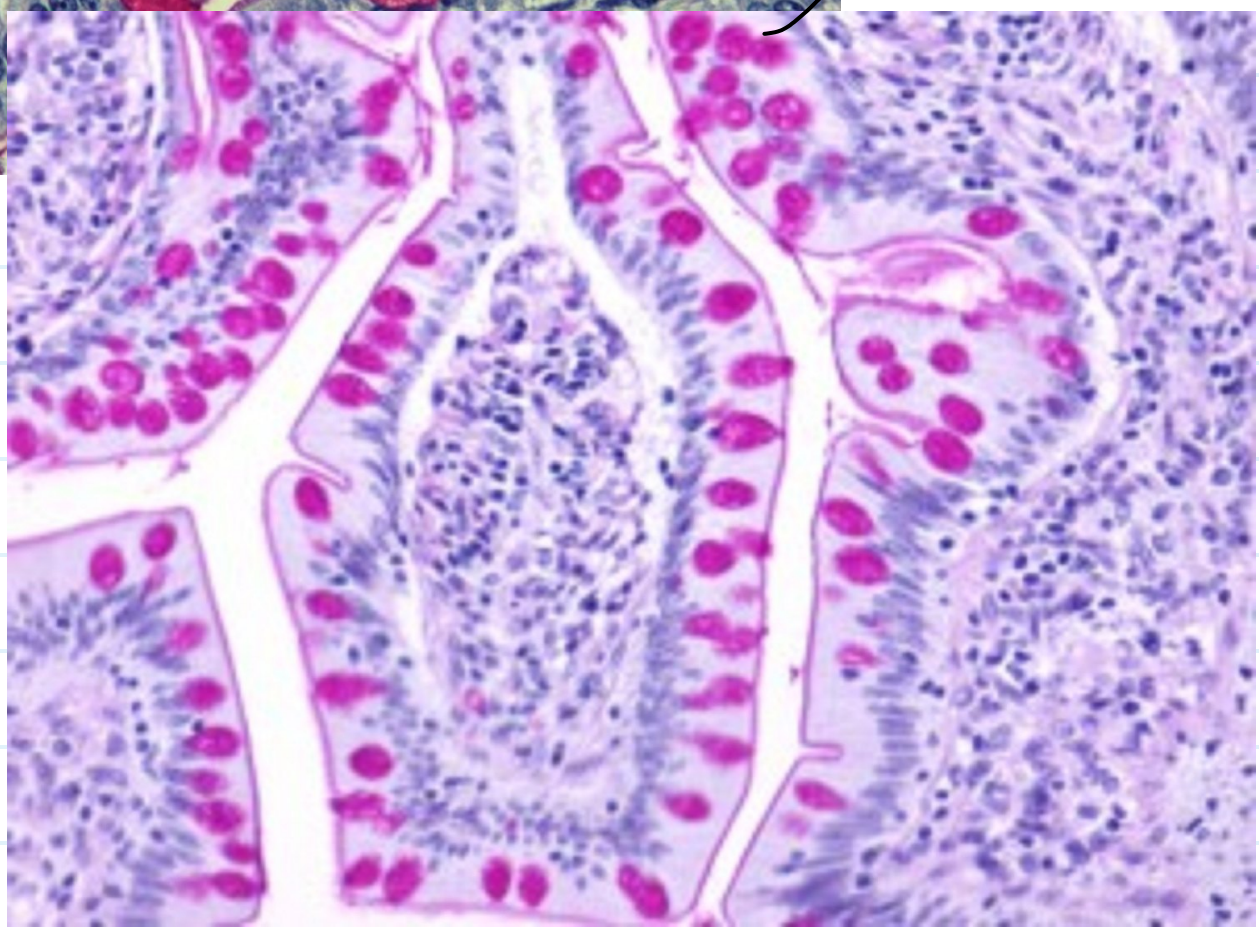


PAS (Periodic Acid schiff): Usually reacts with

. carbohydrates to visubize it

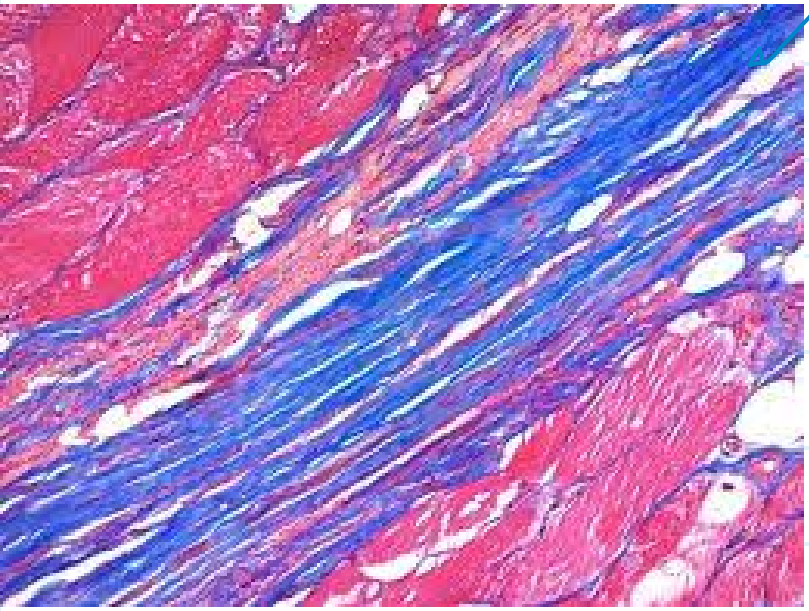
and it appears Red or Magneta

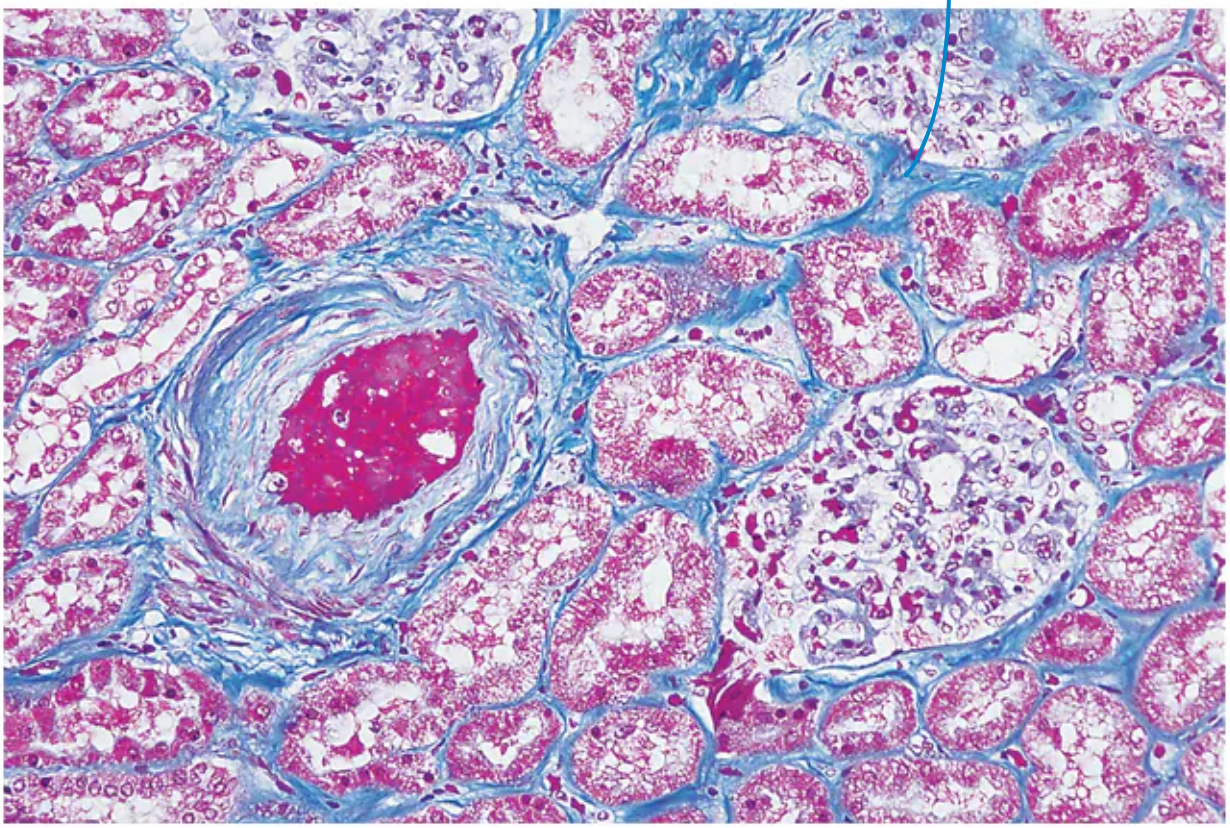




Trichrome: combination of dyes often used to visualize collagen (which appear blue)

3 colors





2. Immuno Histo Chemistry:

using specific interactions between Antigens & Antibodies

* Used to stain certain protiens

How it is done:

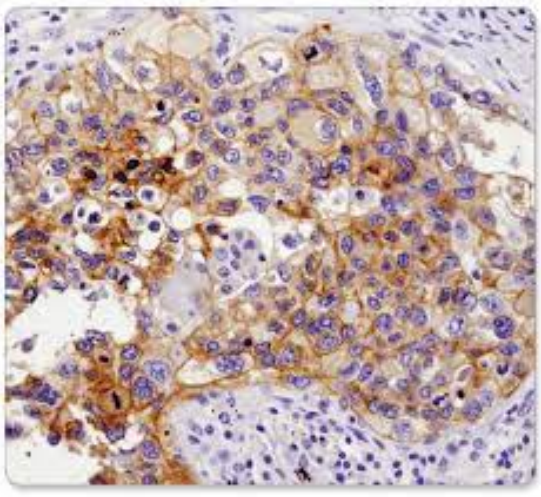
Directly: ① An Antibody with chromogen (color generating Antigen) is attached to the protien after recognizing it

② certain chemical is applied to react with chromogen to turn the tagged region → to Brown color

In Directly: here an aditional step happens:

Rather than using the same Antibody to recognize the protien & hold the chromogen, primary Antibody recognize and a secondary Antibody with chromogen attach to the primary Antibody.

Then the same chemical is applied



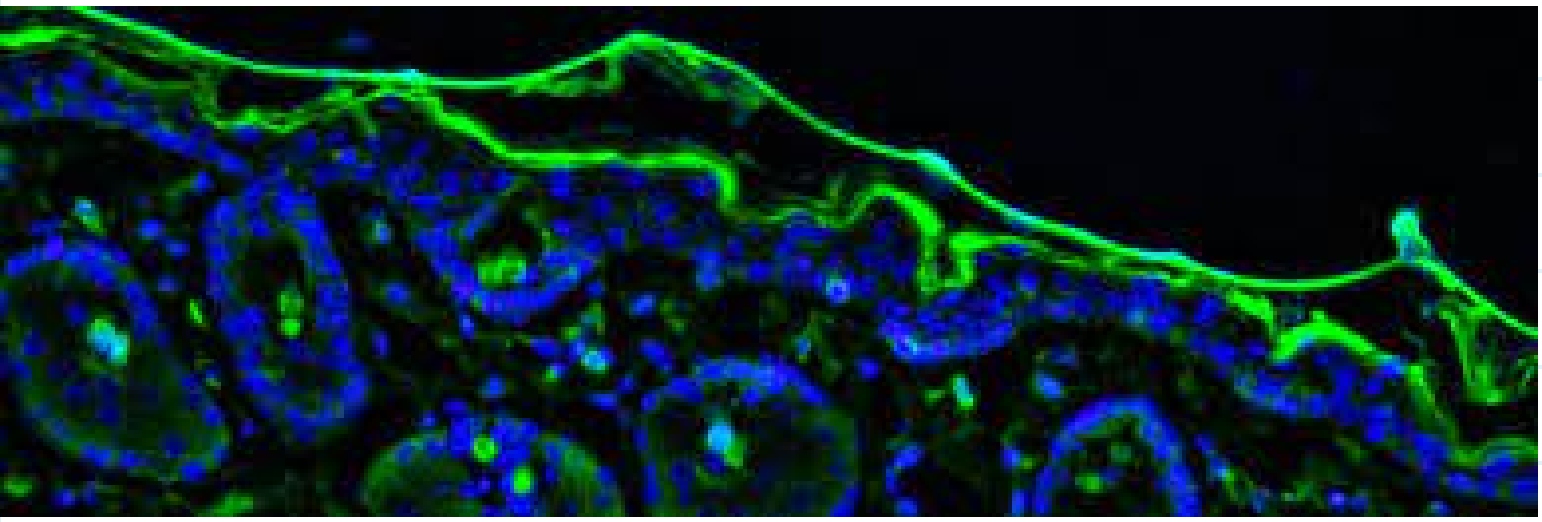
3 Immunofluorescence:

Just like the previous one with (fluorescent Tag) instead of chromogen.

Also the fluorescent Tag is excited by UV light or Laser not a chemical substance

This excitation causes the fluorescent Tag to emit specific frequency in the light spectrum

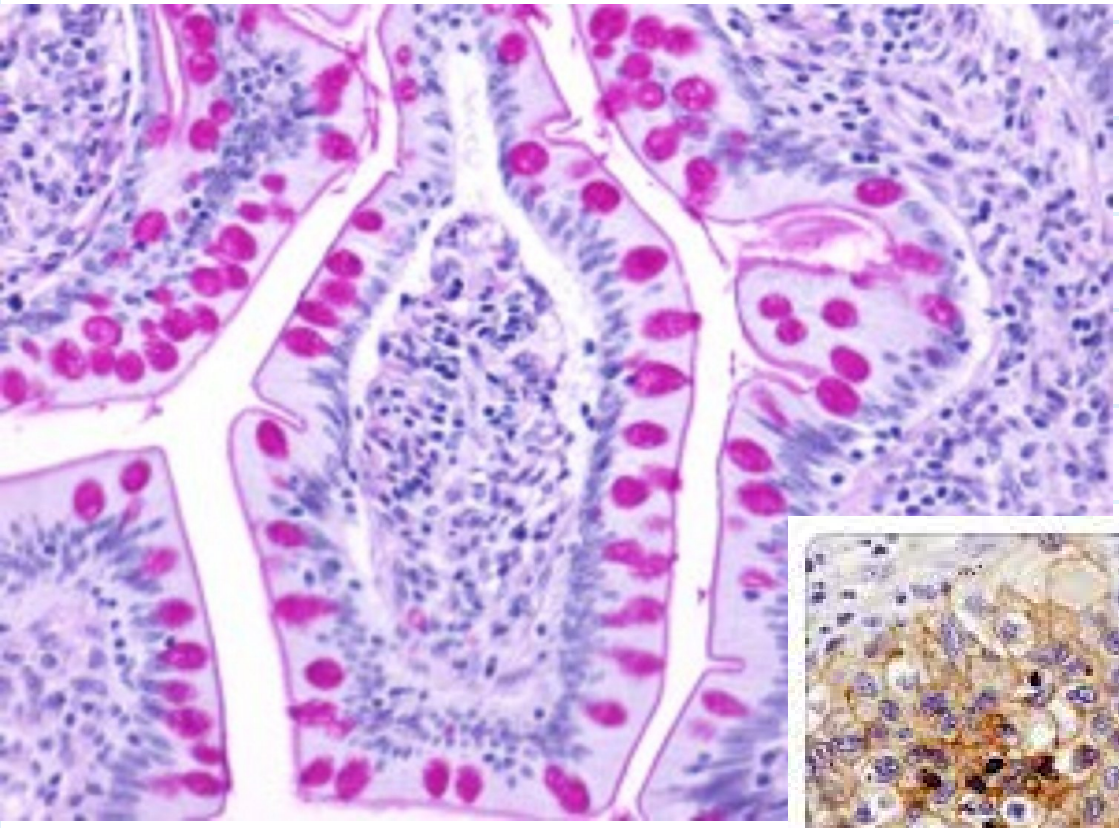
* Common colors are Green (microtubules) & Blue (nuclear protiens) on black ground

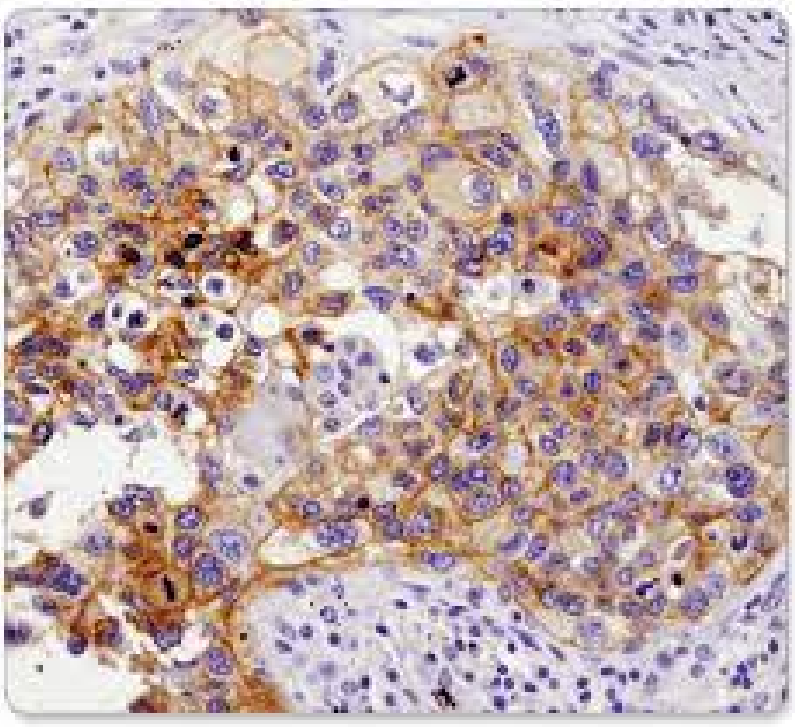


Light microscopes

1 Bright-field :

*white background
*used to observe tissue stained with histochemistry (H&E, PAS, Trichrome)
*also it is used to observe tissue stained with Immunohistochemistry





2 Phase-contrast:

* Gray background

*used to observe (non-stained) tissues

so it can be living tissue or cultured cells

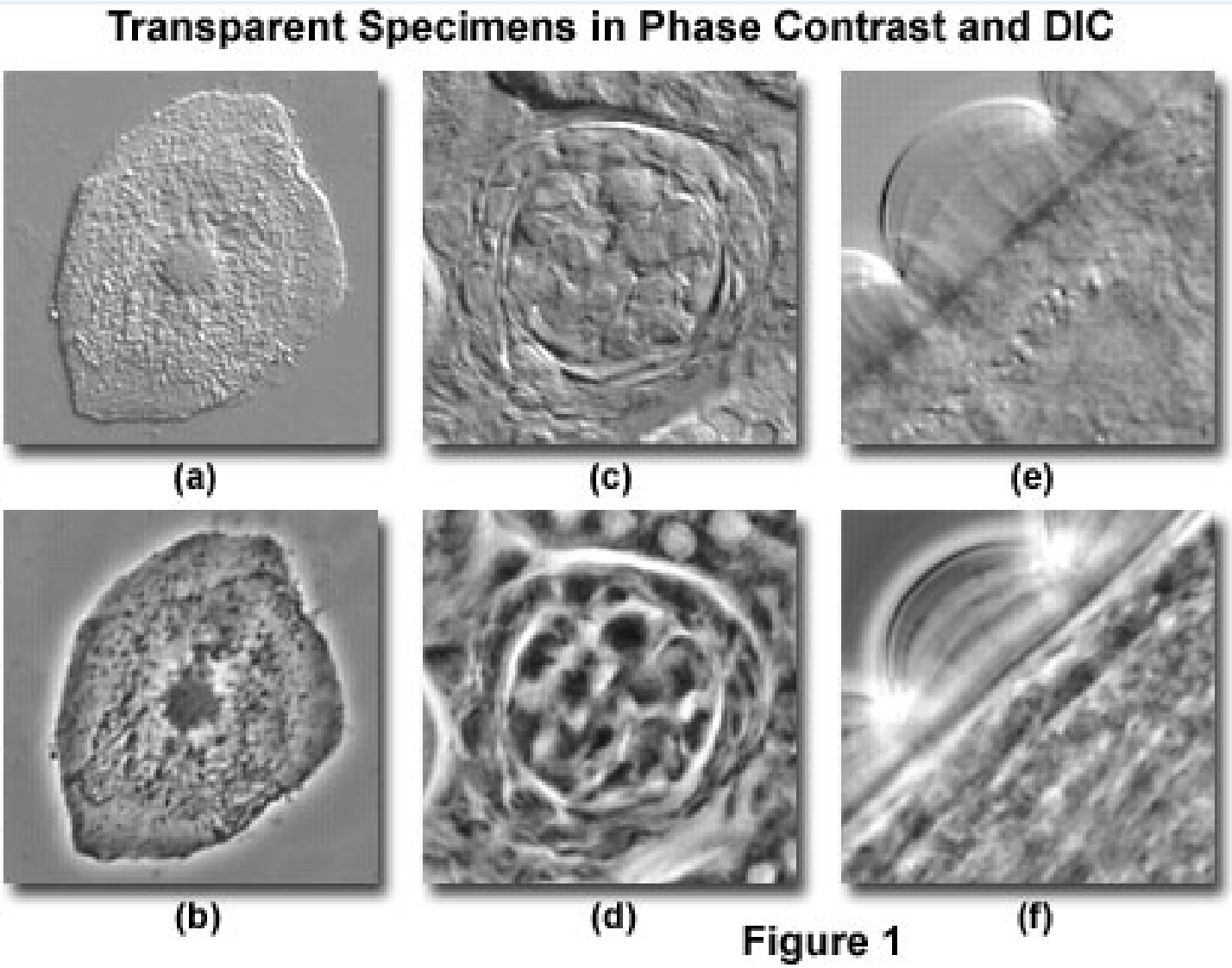
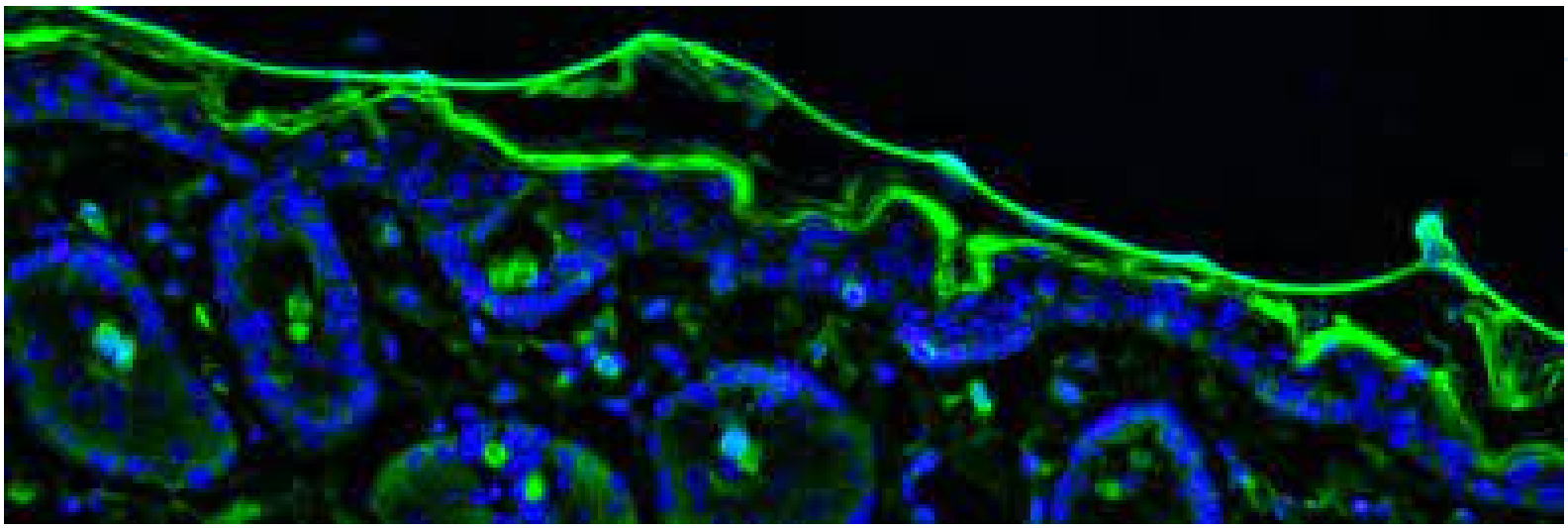


Figure 1

3 Fluorescence microscopy :

* Black background

*used to visualize Immunofluorescence stained tissues



Electron microscopes:

1 TEM:

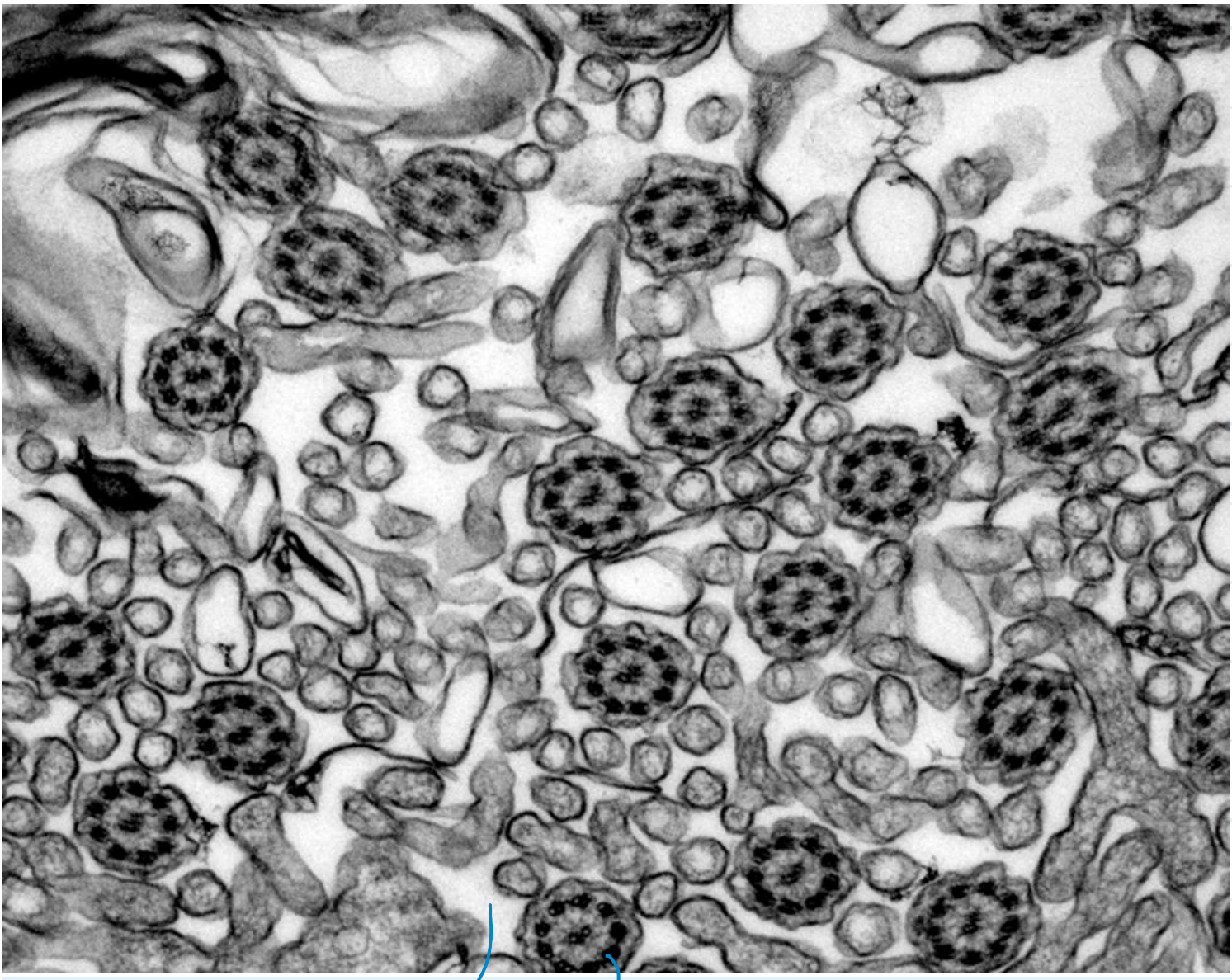
*Greatest magnification &resolution
*electron beam passes through the tissue (the specimen)
*Images colors are shades of Gray :

- the Darker Areas (dense electron area)
not enough electrons were able to pass
so cells or tissues are concentrated

- the lighter Areas (electron light area)

a lot of electrons were able to pass

so fewer cells or tissues are concentrated there
(so it is most likely to be fluid)



Light Areas

Dark Areas

2 SEM:

*electron beam does not pass through the tissue (the specimen)
*The surface of cells & tissues are coated with heavy metals (usually gold) which reflect electrons
* Generate 3D images
they are originally colorless but they can be colored using computer programs