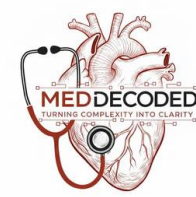


بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



HISTOLOGY

MID | Lecture 7

وَلَقَدْ خَلَقْنَا الْإِنْسَانَ وَنَعَلَهُمَّا تَوْسُوسًا بِهِ نَفْسُهُ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Epithelium

Images part one

Written by : Hala Marei
Dareen Alhababseh



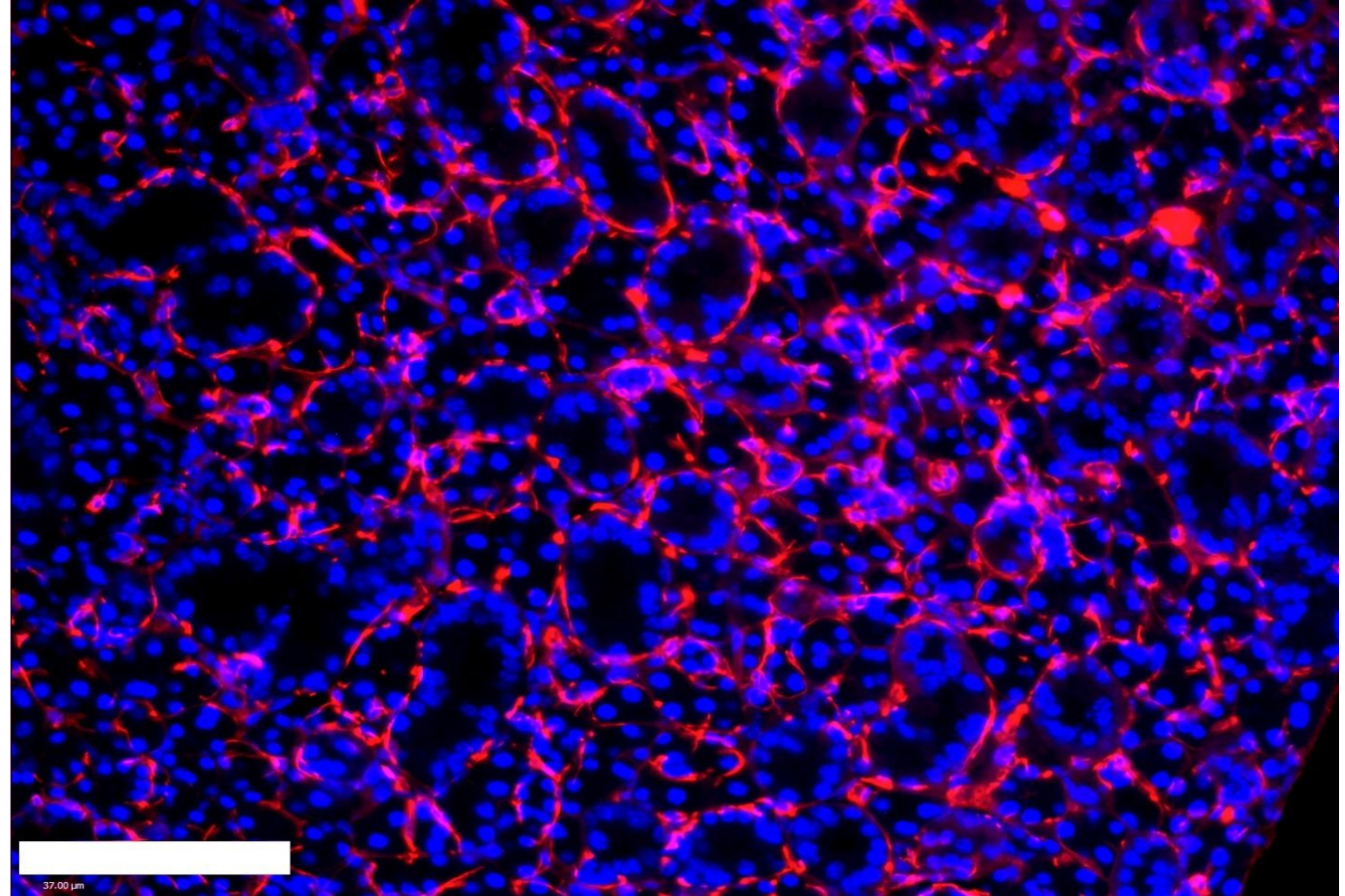
Reviewed by : Joud Alsafadi

Immunofluorescence Staining/Tissue

The nuclei are stained with this blue
DAPI
This type of filaments is called
alpha smooth muscles which is
the red color

This needs a UV lamp and
immunofluorescence microscope with the
refraction of specific wave length
is characterized by a black background
with
colored signals (often blue for nuclei and
red or
green for specific proteins)

DAPI is a specific fluorescent stain that binds to DNA and therefore labels cell nuclei with blue fluorescence. In contrast, red fluorescence typically indicates the presence of specific proteins detected by fluorescent antibodies, such as α -smooth muscle actin, which highlights the actin filaments characteristic of smooth muscle cells

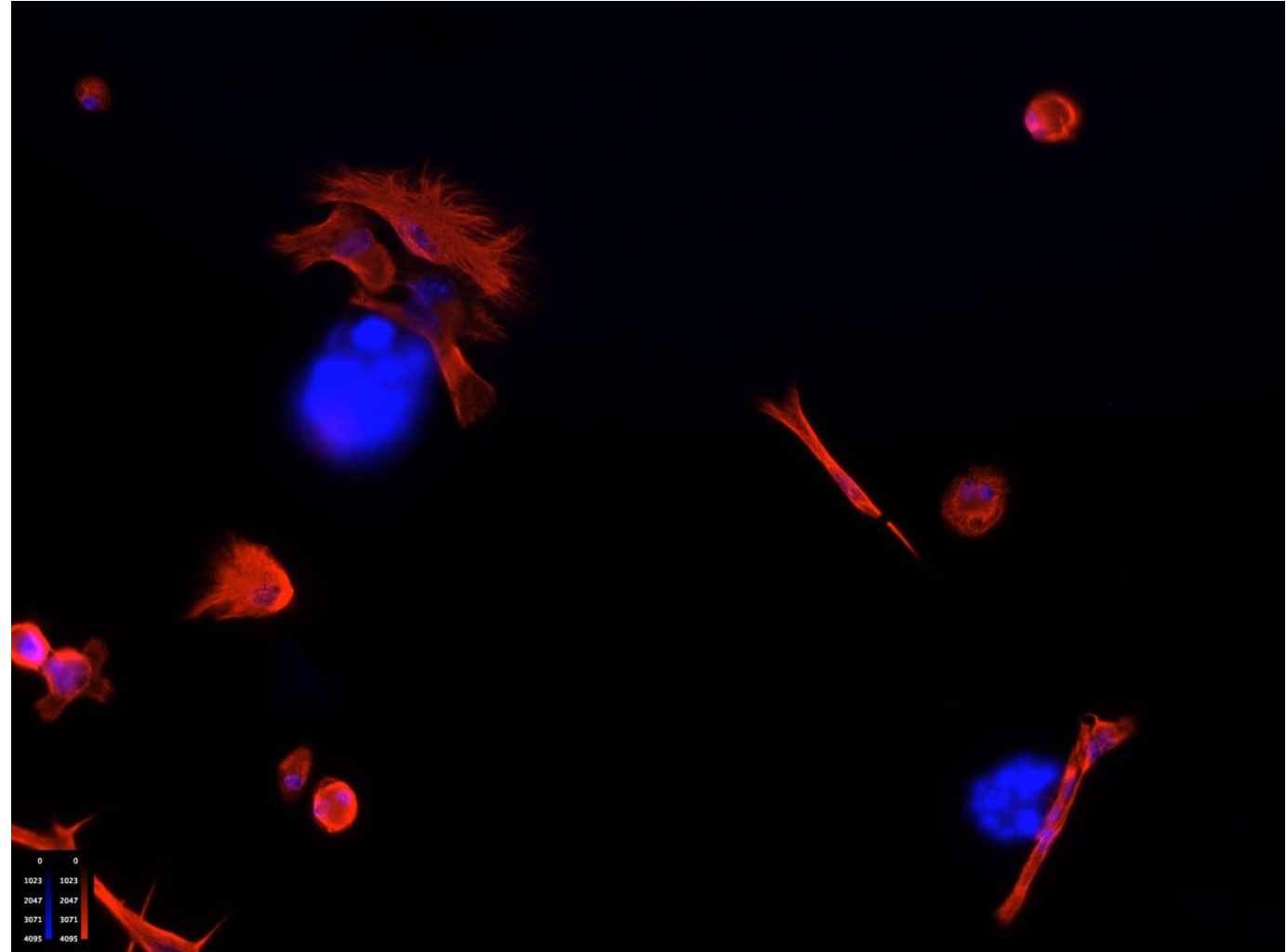


This is a salivary gland that we sectioned
and stained

Immunofluorescence Staining/cultured cells

As you see here we have the same staining in the first two images, but the difference that in the first one we have a section taken of a **tissue** and the next one are a **cultured cells** so you can see lots of nuclei that are positive

In immunofluorescence (IF) staining of cell cultures, the nucleus typically appears as a distinct, sharply defined, and intense central structure, most commonly stained blue (when using DAPI)



Additional information from the doctor

For drug testing, it is important to first test the drug on cells. Then we calculate several parameters regarding the effect of the drug on the cells. After that, the drug is tested on animals because it must be tested at least three times before it can be used in humans. Once it is proven to be safe, we begin the clinical trials.

We also use the same type of marker for both cell types to test whether they respond positively to the medicine.

Question:

When testing the effects of a drug on cultured cells, the cells must be alive during the experiment. However, immunofluorescence staining usually requires fixation, which kills the cells. How can immunofluorescence be used in drug testing if the staining procedure kills the cells?

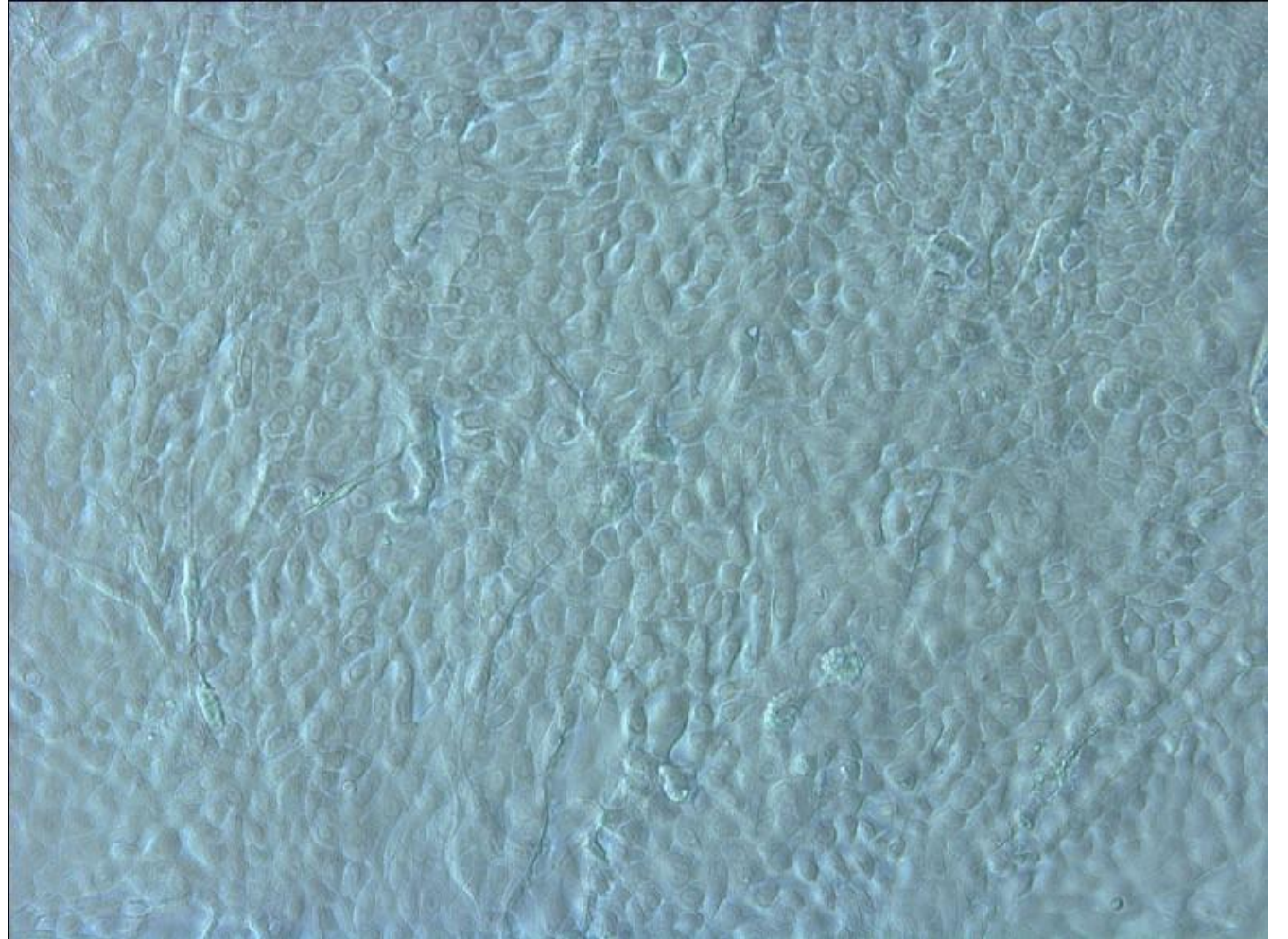
Answer:

In drug testing experiments, cultured cells are first maintained alive and exposed to the drug for a specific period to allow the drug to exert its biological effects. After the treatment phase, the cells are fixed to preserve their structural and molecular state at that exact moment. Immunofluorescence staining is then performed on these fixed cells to detect specific proteins or cellular structures using fluorescent antibodies. Therefore, although immunofluorescence is applied to fixed (non-living) cells, the drug's effects are studied because the treatment occurred while the cells were still alive.

Phase-contrast Microscope

We can't distinguish the nuclei these all are cells so close to each other so it gives the feeling of 3D while actually it is not it is just a refraction the one who made did that so I can see the cells without staining by simply playing with the opticals that are present in the microscope.

If cells appear as "tear drops" and have a 3D feel without staining, it is the result of a genius optical setup (phase contrast) rather than the physical properties of the cells themselves.

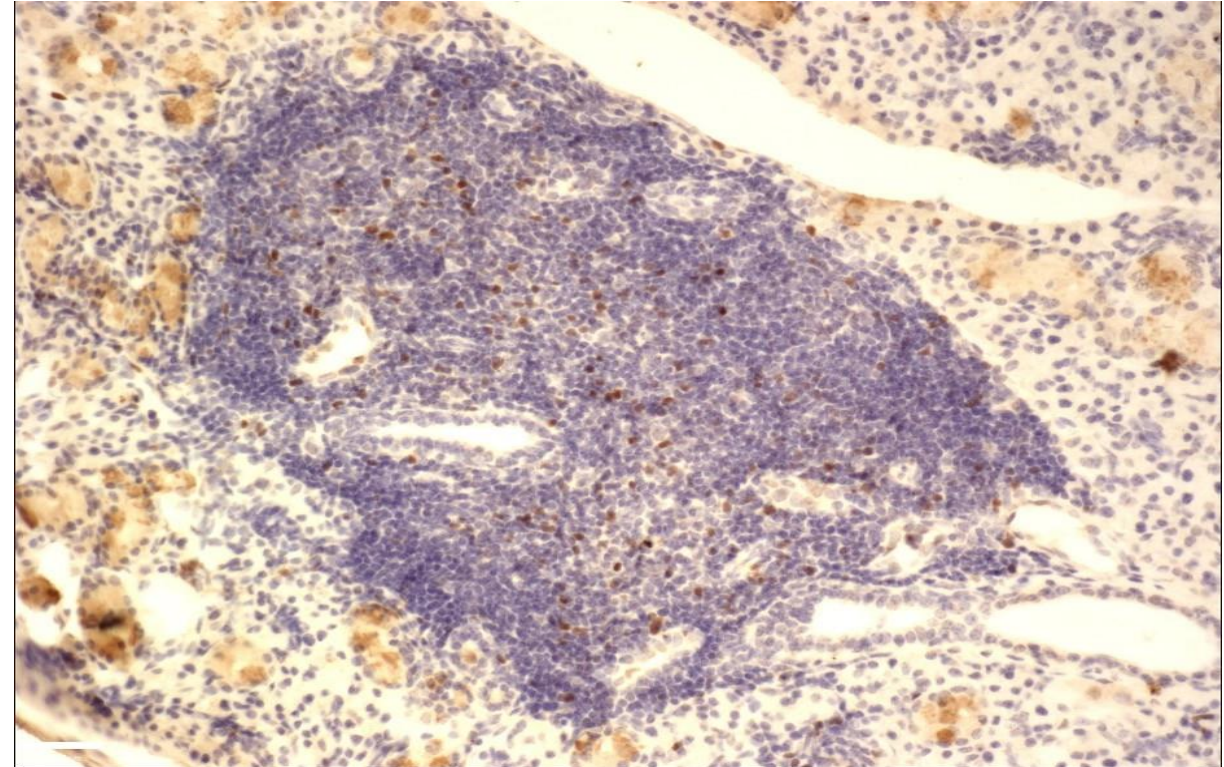


Immunohistochemical Staining/Tissue

This is simple typical bright field microscope
And the enzymes here is the thing that gives the
signal because when we put the substrate it will
work and will give us the brownish color

Just for knowledge (additional
information) Immunohistochemical (IHC) staining is a
technique that uses antibodies to detect specific antigens,
usually proteins, in tissue sections. The antigen-antibody
reaction is visualized using an enzyme that produces a colored
precipitate, commonly brown with DAB (diaminobenzidine),
which can be observed under a light microscope. This
technique is widely used to identify disease markers in tissues.

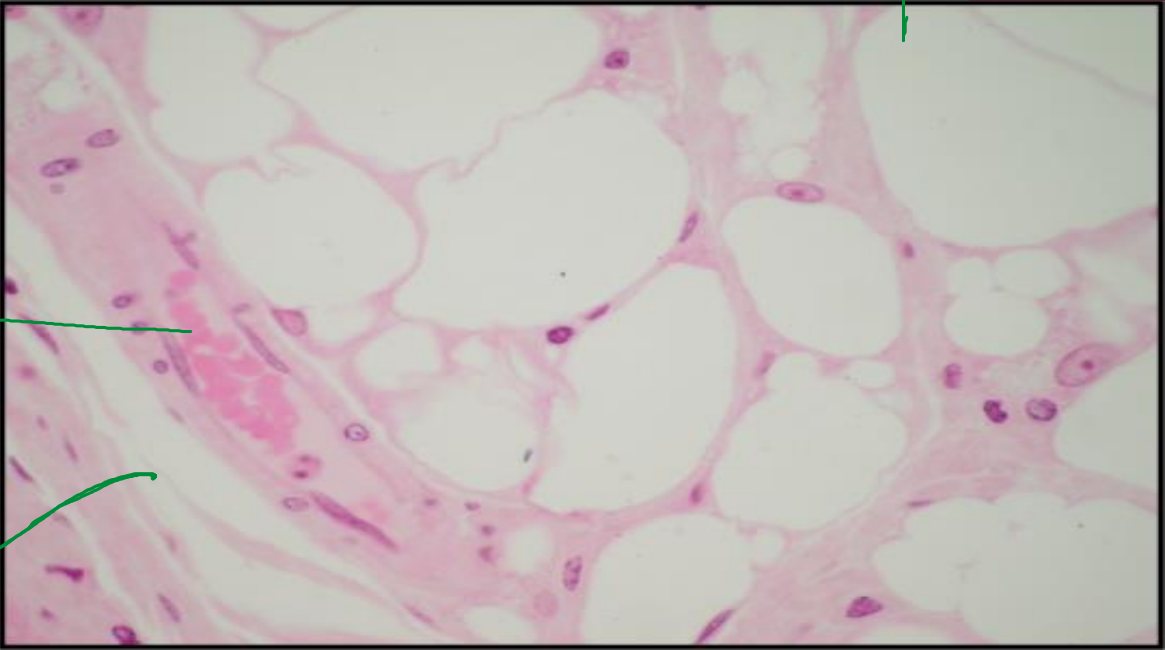
Immunofluorescence (IF) differs from IHC in that it uses
fluorescently labeled antibodies that emit light under
specialized fluorescence microscopy, rather than enzyme-
based color reactions so always the background will appear
blackish .



ENDOTHELIUM

Note: we used a bright field light microscope and this section is taken from a liver

These are fat cells and they are white because you simply dissolved it while preparation (it was removed so These white circular spaces represent fat cells (adipocytes). They appear empty because the lipid content is dissolved and removed during tissue preparation.



RBCs

Additional information: To differentiate the lumen (the open, central cavity of a vessel or tube) from fat (adipose tissue) in microscopy, rely on structural morphology and staining: fat appears as large, empty-looking, round, white polygonal cells with a thin, peripheral nucleus. The lumen is an irregular, open space within a vessel, usually bounded by distinct cell layers (endothelium) and surrounded by connective tissues

Lumen : this is a longitudinal section
This is endothelium which means it has big cells so we can't really see all the nuclei (since the surface area of it is big so the section will pass not necessarily through the entire length of the cell so we'll see the nuclei in some areas and others not
This lumen is for blood vessel and we see no brown color because it doesn't have a nuclei

Endothelium

True lumens in the liver will contain Red Blood Cells (RBCs). RBCs are anucleated and show only eosinophilia (pink color). In contrast, White Blood Cells (WBCs) are nucleated.

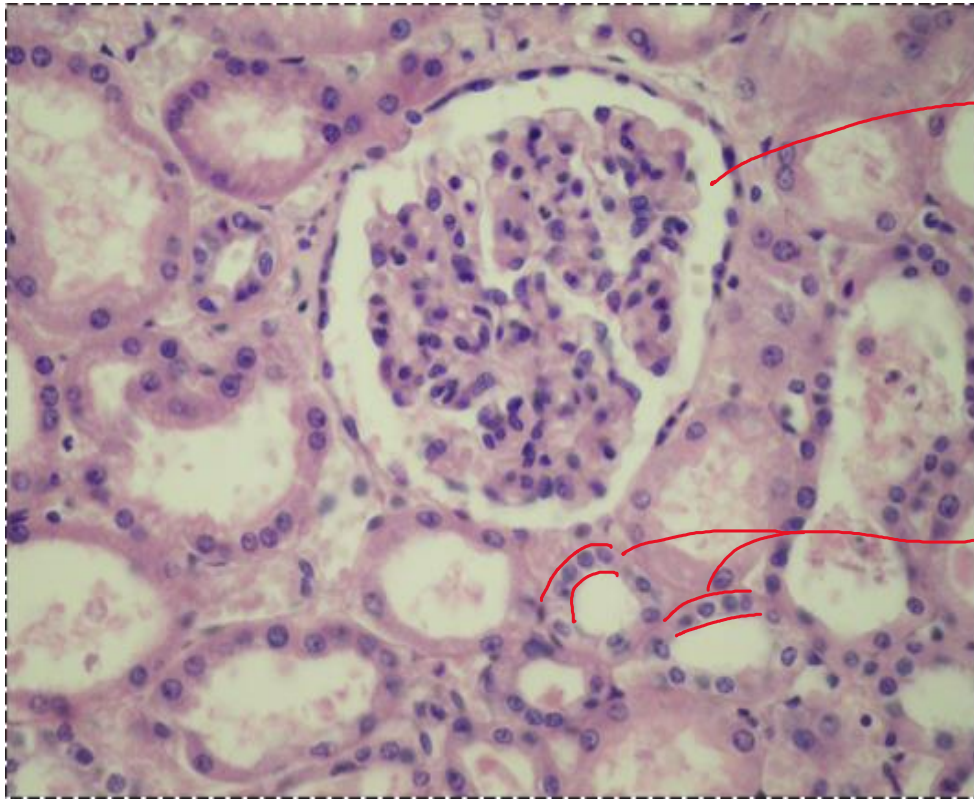


Note: every green circle is a lumen we also used bright field light microscope

Note: There is two types of sections: cross (Structures usually look **round or circular.**) longitudinal (Structures look **elongated or tube-like**).

The staining is H&E and the microscope is bright field light microscope

SIMPLE SQUAMOUS EPITHELIUM



Lumen (it is a nephron there is a glomeruli which are capillaries covered with cells and the space is called capsular space)

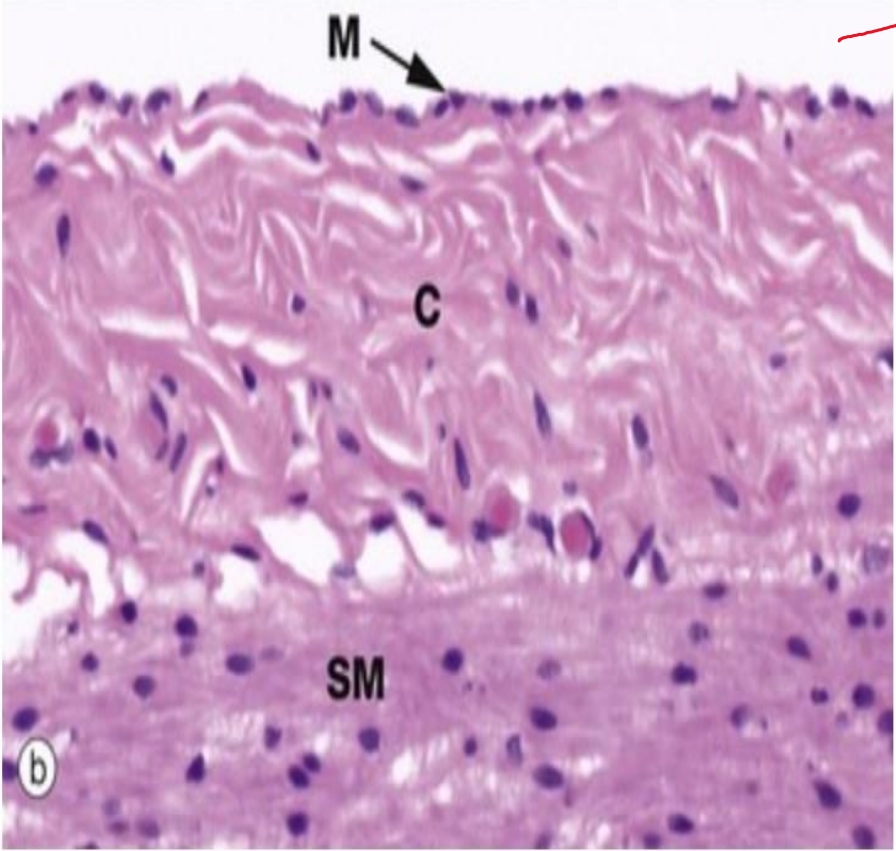
Simple cuboidal

This is from cortex kidney (you are not required to know the section at this stage)

If you're not sure about the type of epithelium then look at the cytoplasm you will find that it is not on the same level of the nucleus it is actually tapering on both sides of the nuclei which means it's squamous

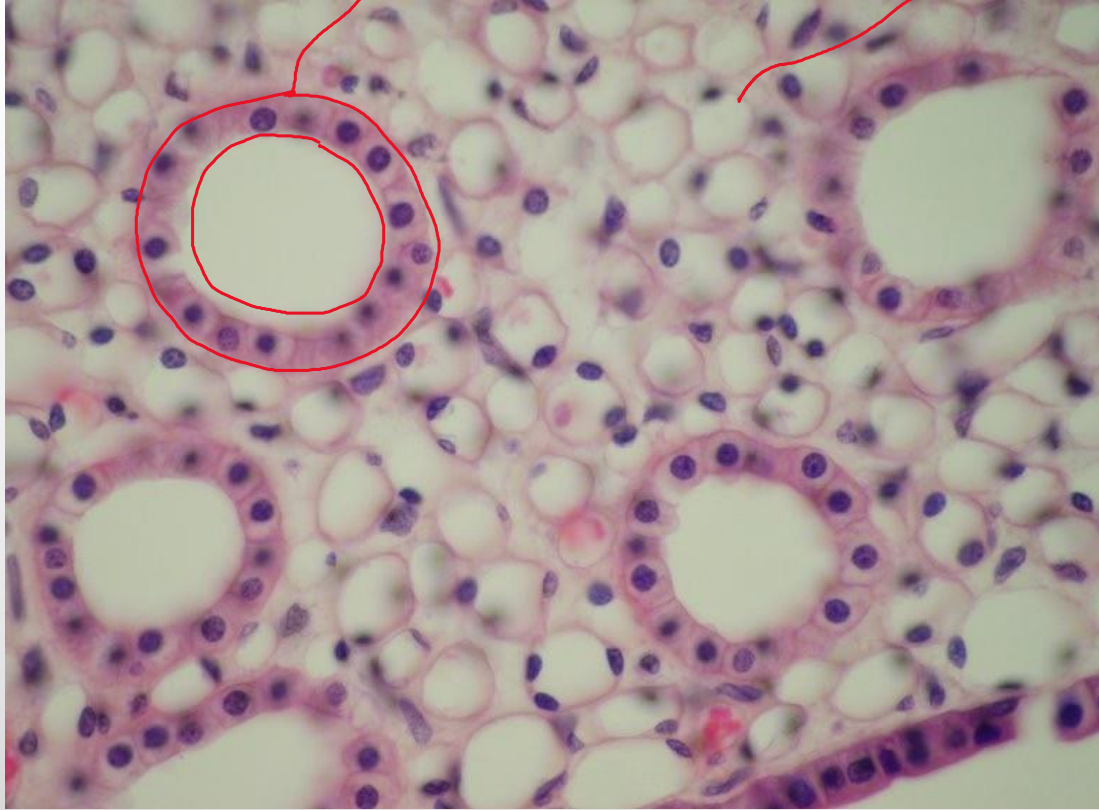
You can see the nucleus which mean the cells are small and other cells where you can't see them seems to be big cells (in big cells you will not see all the nucleus side by side)

SIMPLE SQUAMOUS EPITHELIUM

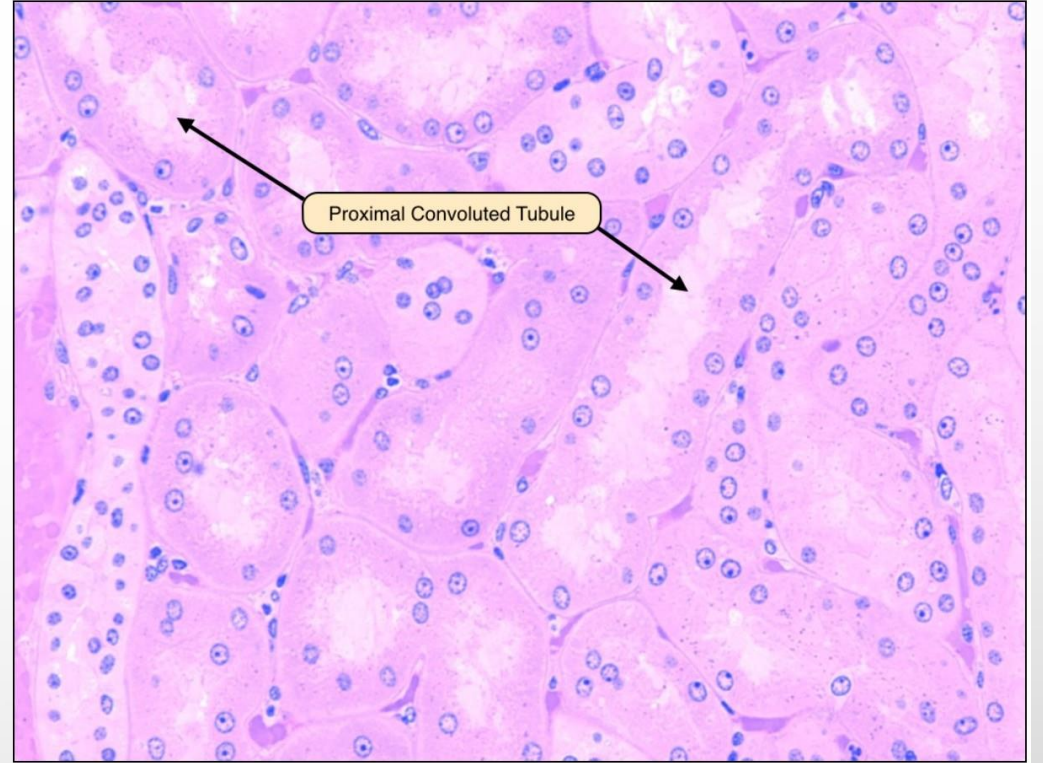


You can't actually use the term because the lumen must be an internal space (this is a space on an external surface)

SIMPLE CUBOIDAL EPITHELIUM

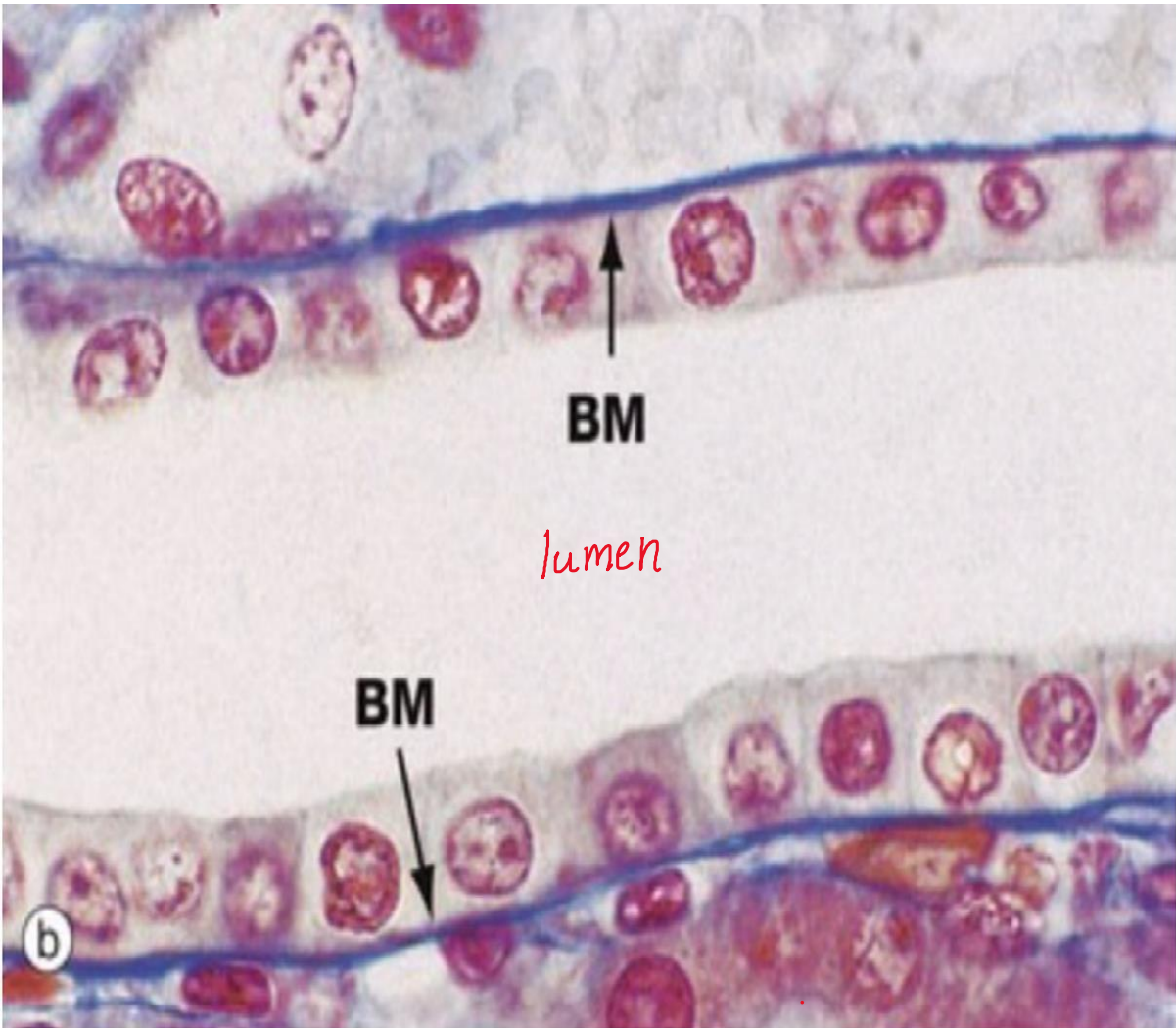


This is a cross section



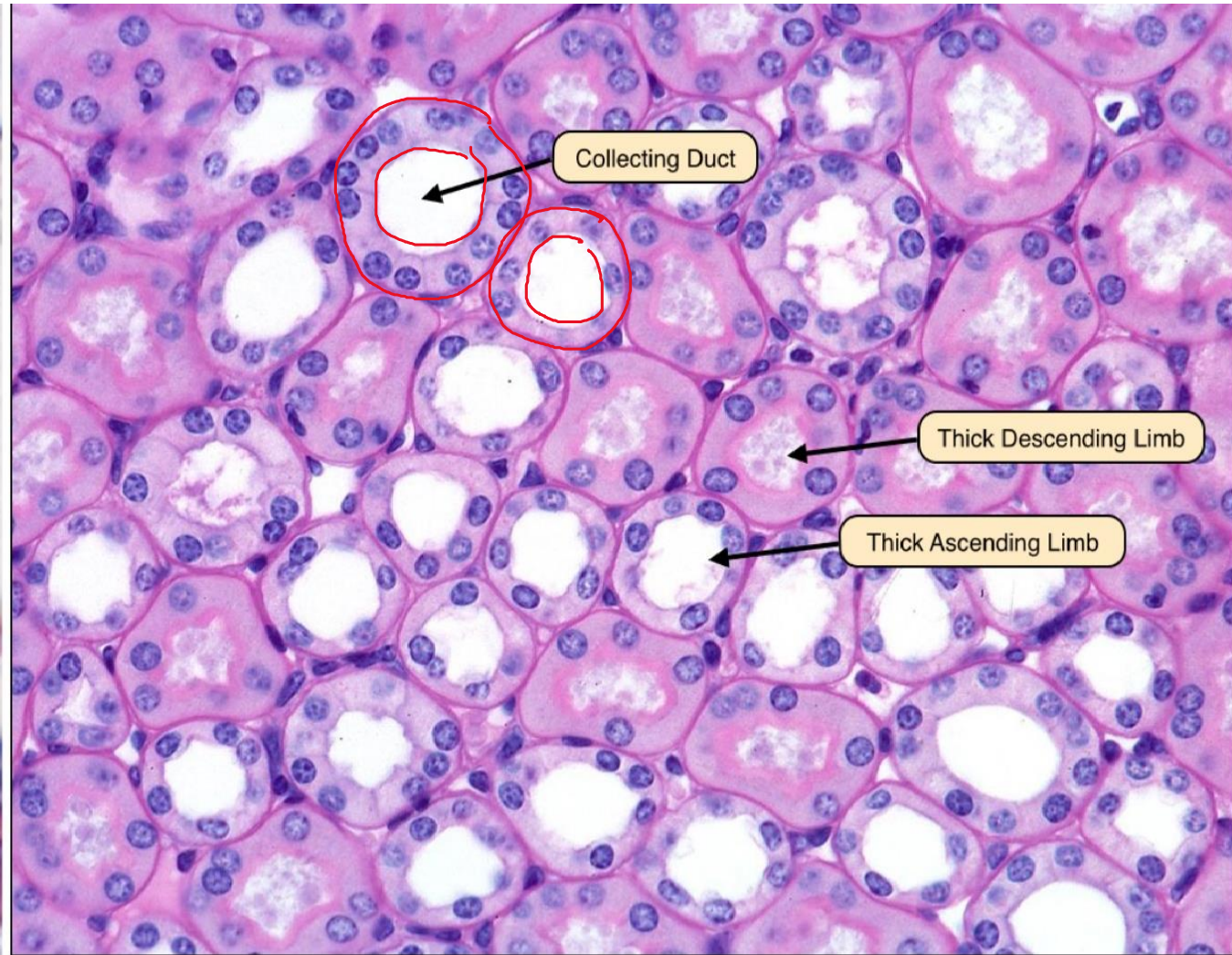
This is a longitudinal section (these are small cells)

Simple cuboidal epithelium



Trichrome stain

Don't be confused because of the amount of the epical cytoplasm we don't one perfect structure for the cuboidal



PAS stain

The basal membrane is well outlined because we used the PASS stain

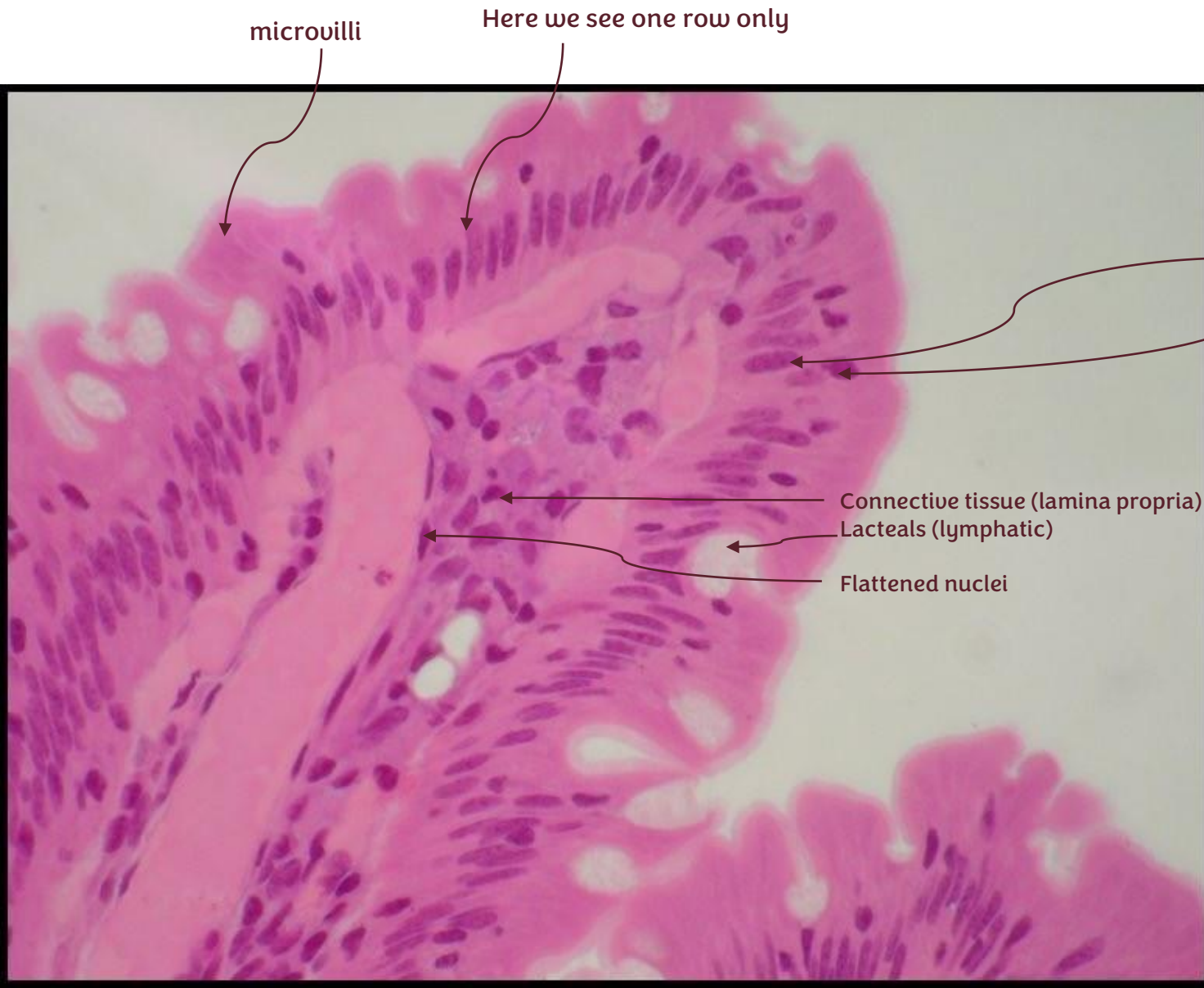
SIMPLE COLUMNAR EPITHELIUM

How did we know it's cilia not microvilli? Microvilli one complete line on the surface and its length compared to the cell itself is relatively short, but cilia compared to the cell is relatively long and there is a little space between them because they are motile so they can't be close to each other like microvilli

The nuclei are arranged in a single row, but because they are very close to each other and the cell dimensions are relatively small, they appear to overlap



SIMPLE COLUMNAR EPITHELIUM



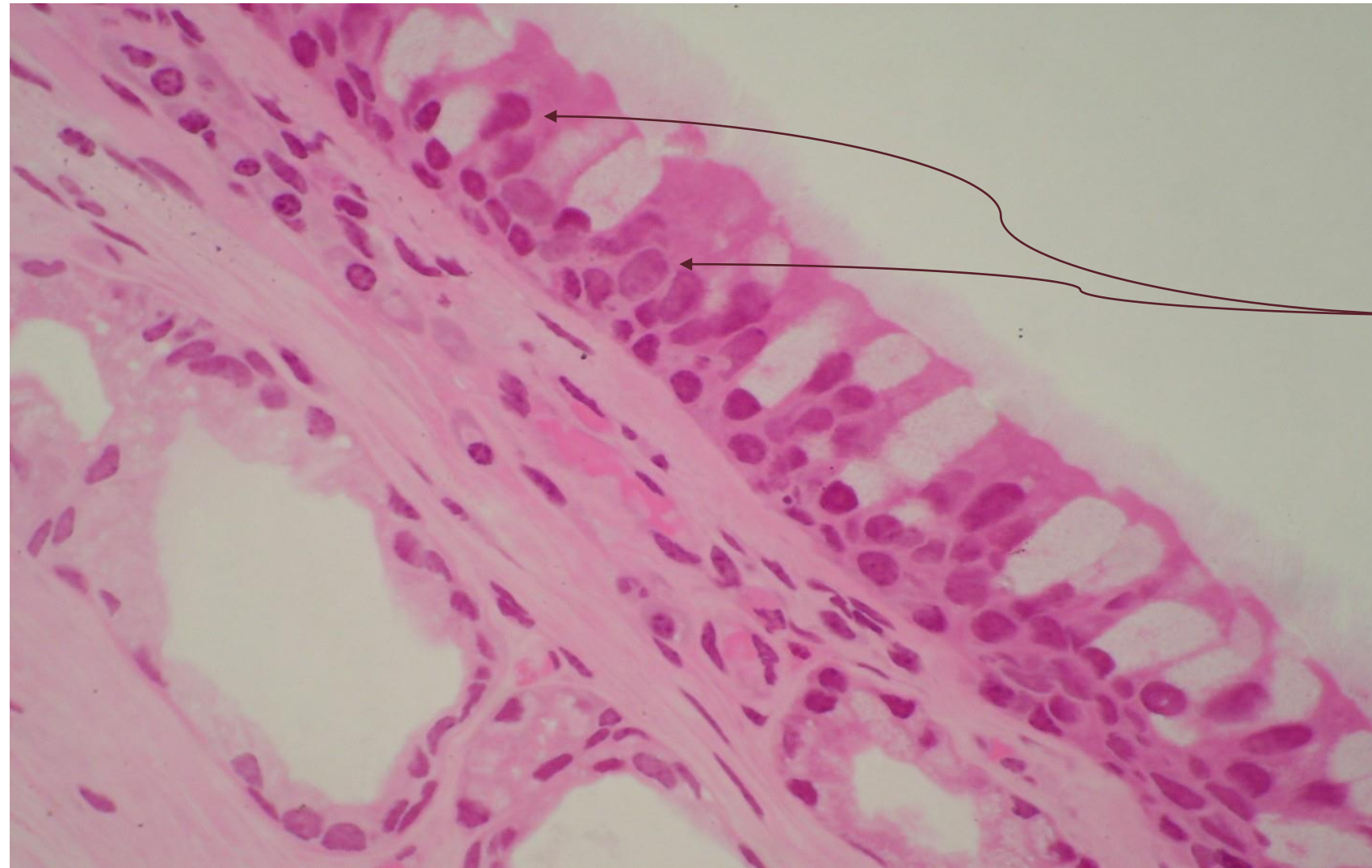
In simple epithelium, no matter how the tissue is cut, it will still appear simple. In contrast, stratified epithelium will always appear stratified regardless of how the section is taken. Here, someone might convince themselves that this is one row and that next to it is another row, especially if the section is slightly folded. However, when we examine the entire section carefully, we notice that there is only one row of cells and no row above it. Therefore, this is simple columnar epithelium. At this view, we should not focus only on small parts and conclude that it is stratified. Instead, we must look at the whole section. When we find a view showing one row of elongated cells resting on the basement membrane, this clearly indicates simple columnar epithelium. If the tissue were stratified, we would never see such an image. Stratified epithelium, no matter how it is cut, will always show more than one row of cells. In contrast, simple epithelium—even if the section is tilted or slightly thicker—can still show this appearance of a single row of cells.

Ciliated **PSEUDOSTRATIFIED COLUMNAR EPITHELIUM** With goblet cells

Goblet cells have a foamy or “goblet-like” appearance because their cytoplasm is filled with mucin. During tissue dehydration, most of the water in the mucin is lost, leaving behind streaks, which are remnants of the protein component of the mucin.

To identify pseudostratified epithelium, first look at the nuclei, then follow them up toward the surface and down toward the base to see that all cells are attached to the basement membrane. This confirms that it is pseudostratified columnar epithelium.

The full name is ciliated pseudostratified columnar epithelium with goblet cells, which can be abbreviated as respiratory epithelium.



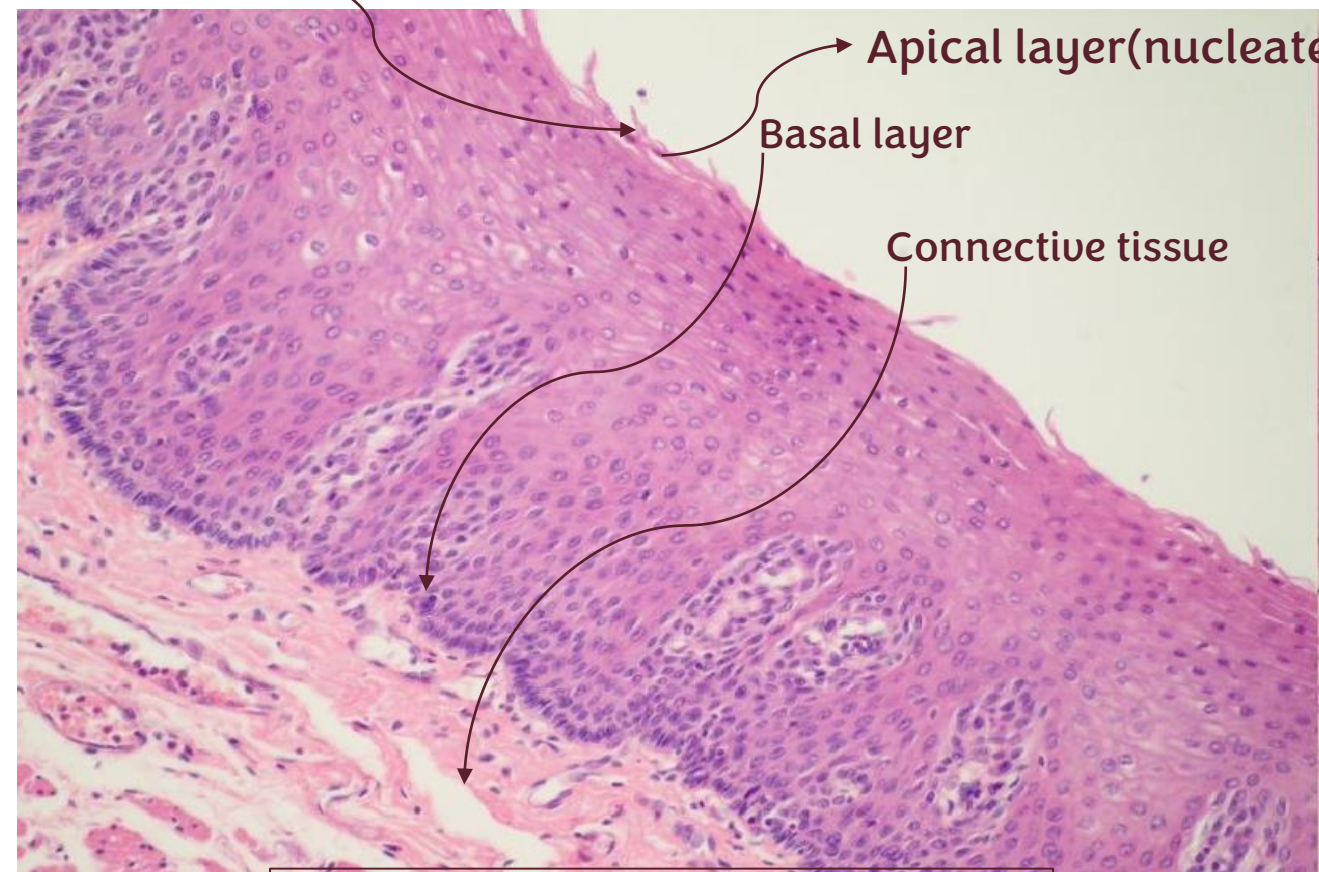
STRATIFIED SQUAMOUS

Stratified squamous epithelium can be either keratinized or non-keratinized, and these are the most common types of stratified epithelium. Stratified cuboidal and stratified columnar epithelium are less commonly seen, with stratified columnar being the rarest. Almost the entire urinary tract is lined by transitional epithelium.

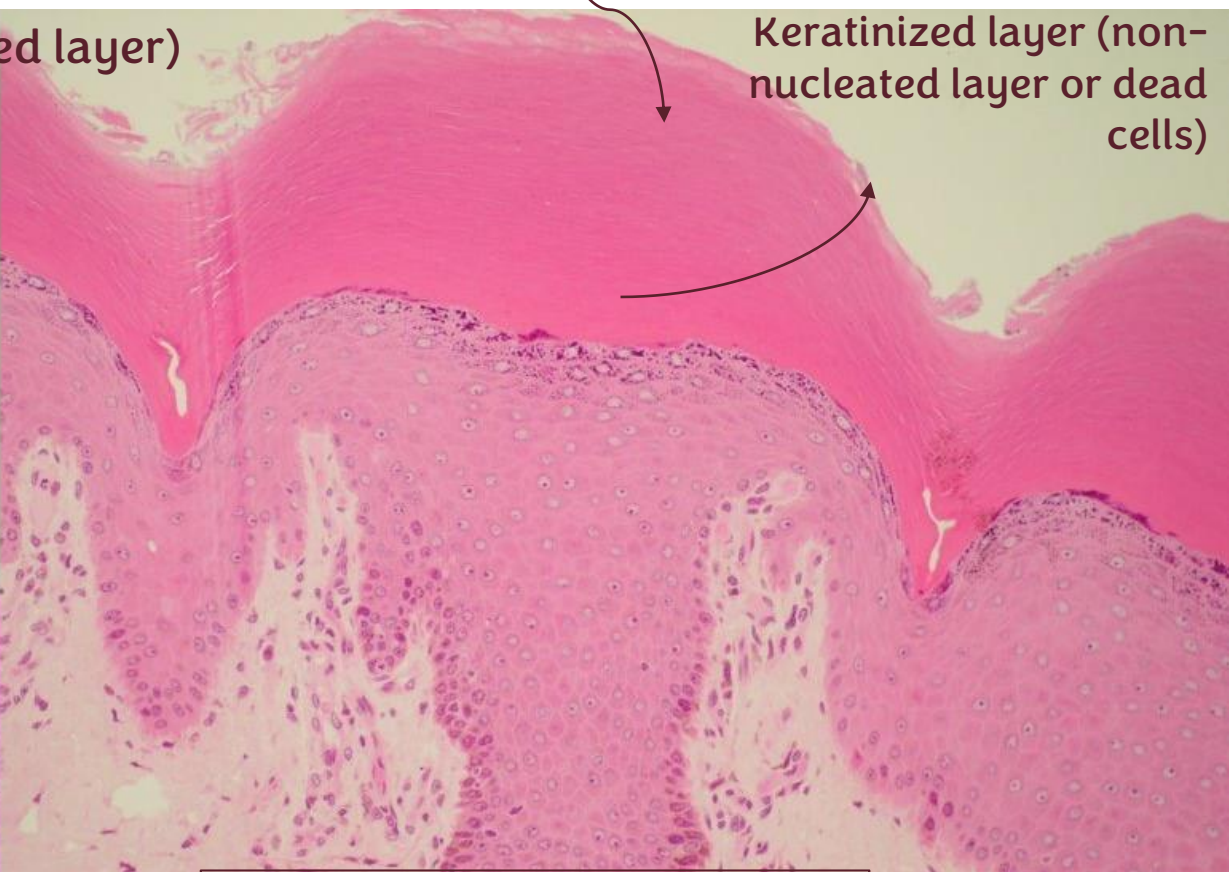
You will never see stratified epithelium with many layers this is just for squamous epithelium. However, squamous epithelium does not always have many layers as in the image; for example, in the cornea it has only a four layers, while in the oral cavity it is prominently stratified, as in the image.

To determine the type, should I look at the basal layer or trace upward?
you should trace upward. In this image, the apical cells are flattened, so it is stratified squamous epithelium.

This is usually found on the skin, but not all skin looks like this. The skin in this image is called thick skin. Look at the thickness of this layer; it is called the stratum corneum.



Stratified squamous non-keratinized



Stratified squamous keratinized

STRATIFIED SQUAMOUS

Look at these pictures. They are different from the previous ones because the thickness is different. For example, a section through the oral cavity, a section through the GI tract, the vagina, or the lower part of the anal canal—you will not have the same vision, view, or thickness all around.

What matters is to look closely and count the layers.

There are many layers in stratified squamous epithelium.

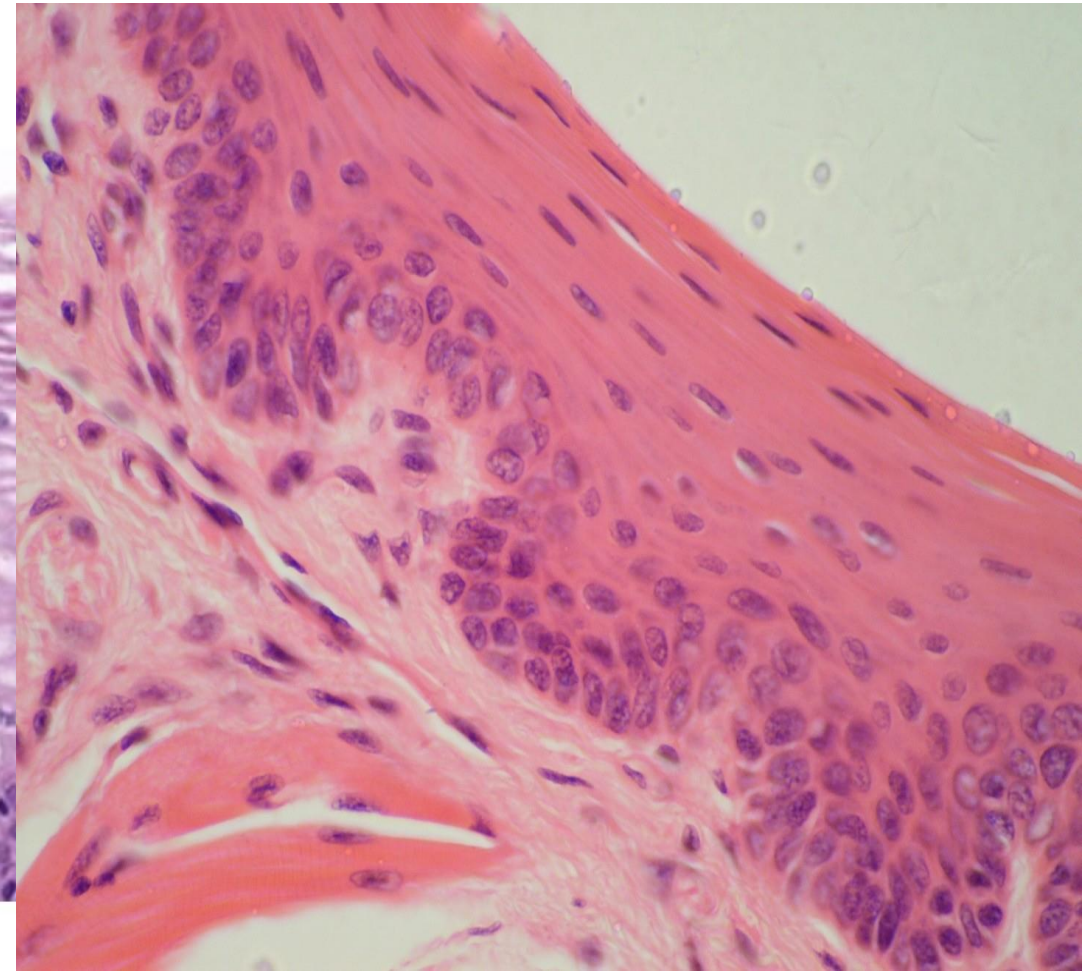
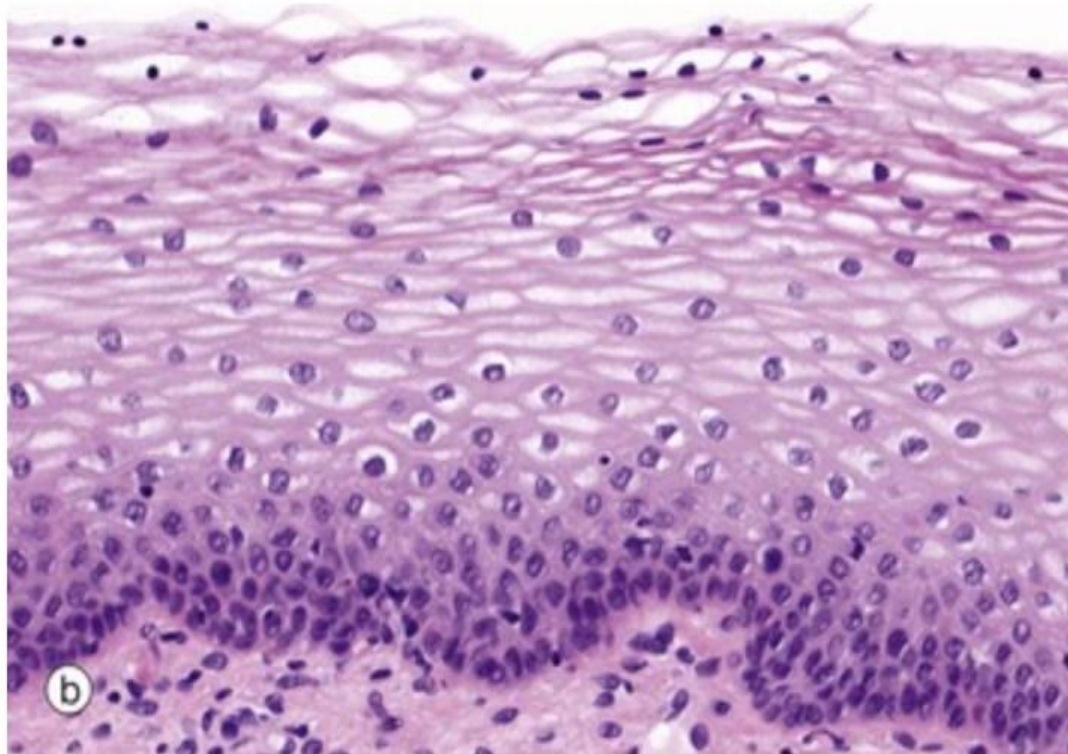
In stratified cuboidal epithelium, we usually see two layers, rarely more.

Stratified columnar epithelium is similar, with approximately two layers wherever it occurs.

Here, we see many layers, so we suspect stratified squamous epithelium. To confirm:

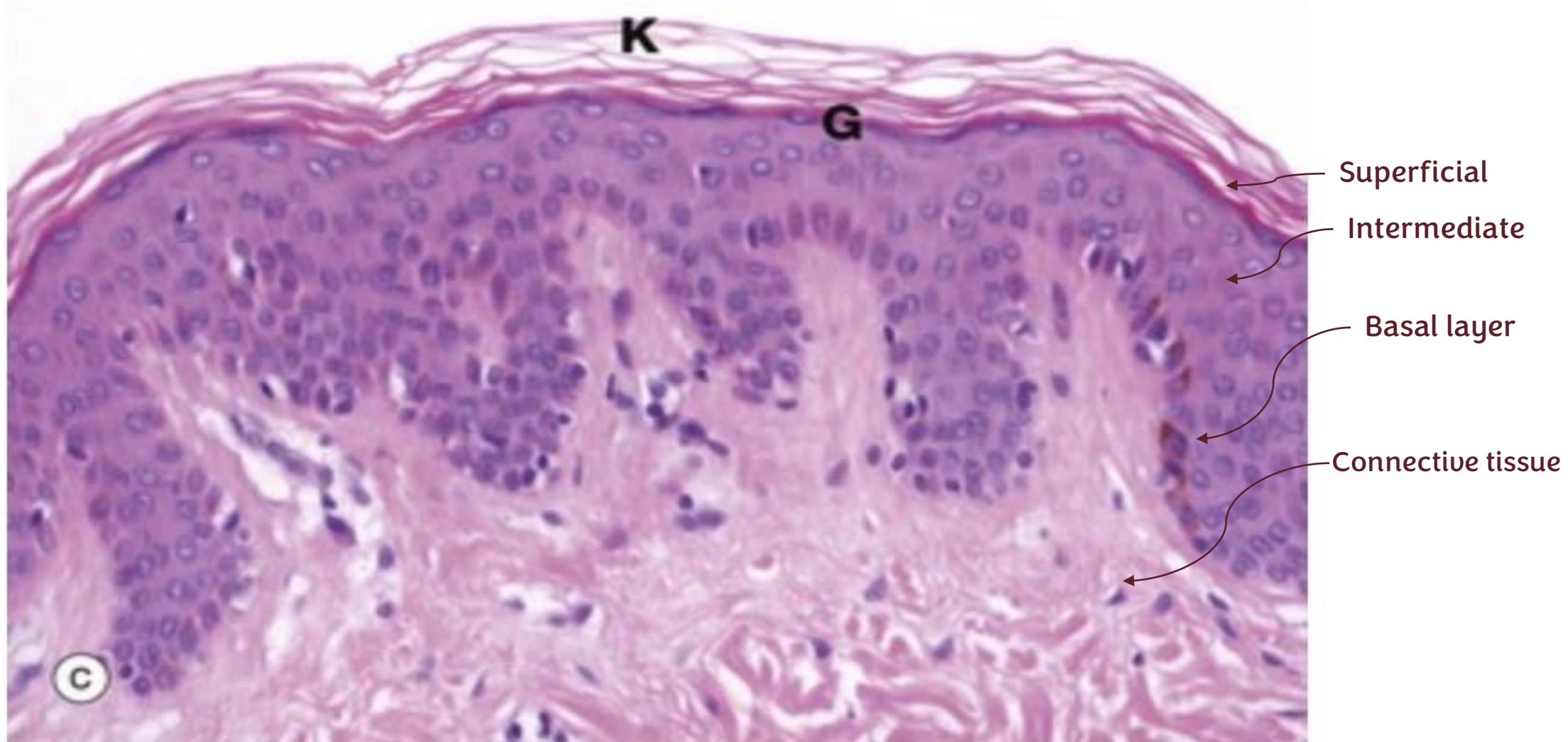
If the surface cells have nuclei, it is non-keratinized.

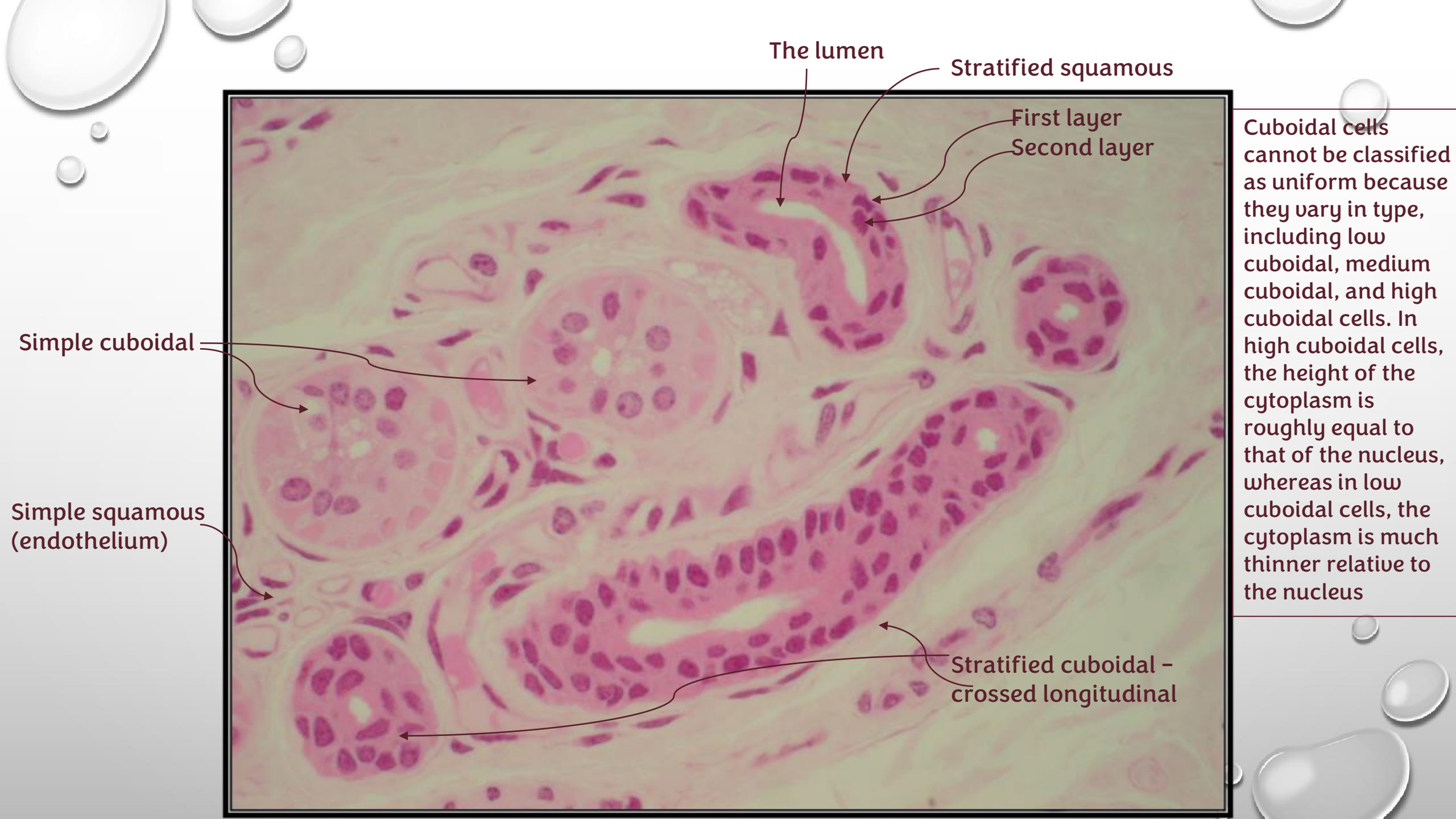
If the surface cells lack nuclei, it is keratinized.



KERATINIZED-STRATIFIED SQUAMOUS

Here, it is also keratinized, but the amount of keratinization is low. This sample is taken from what we call it the thin skin.





The lumen

Stratified squamous

First layer

Second layer

Simple cuboidal

Simple squamous
(endothelium)

Stratified cuboidal -
crossed longitudinal

Cuboidal cells cannot be classified as uniform because they vary in type, including low cuboidal, medium cuboidal, and high cuboidal cells. In high cuboidal cells, the height of the cytoplasm is roughly equal to that of the nucleus, whereas in low cuboidal cells, the cytoplasm is much thinner relative to the nucleus

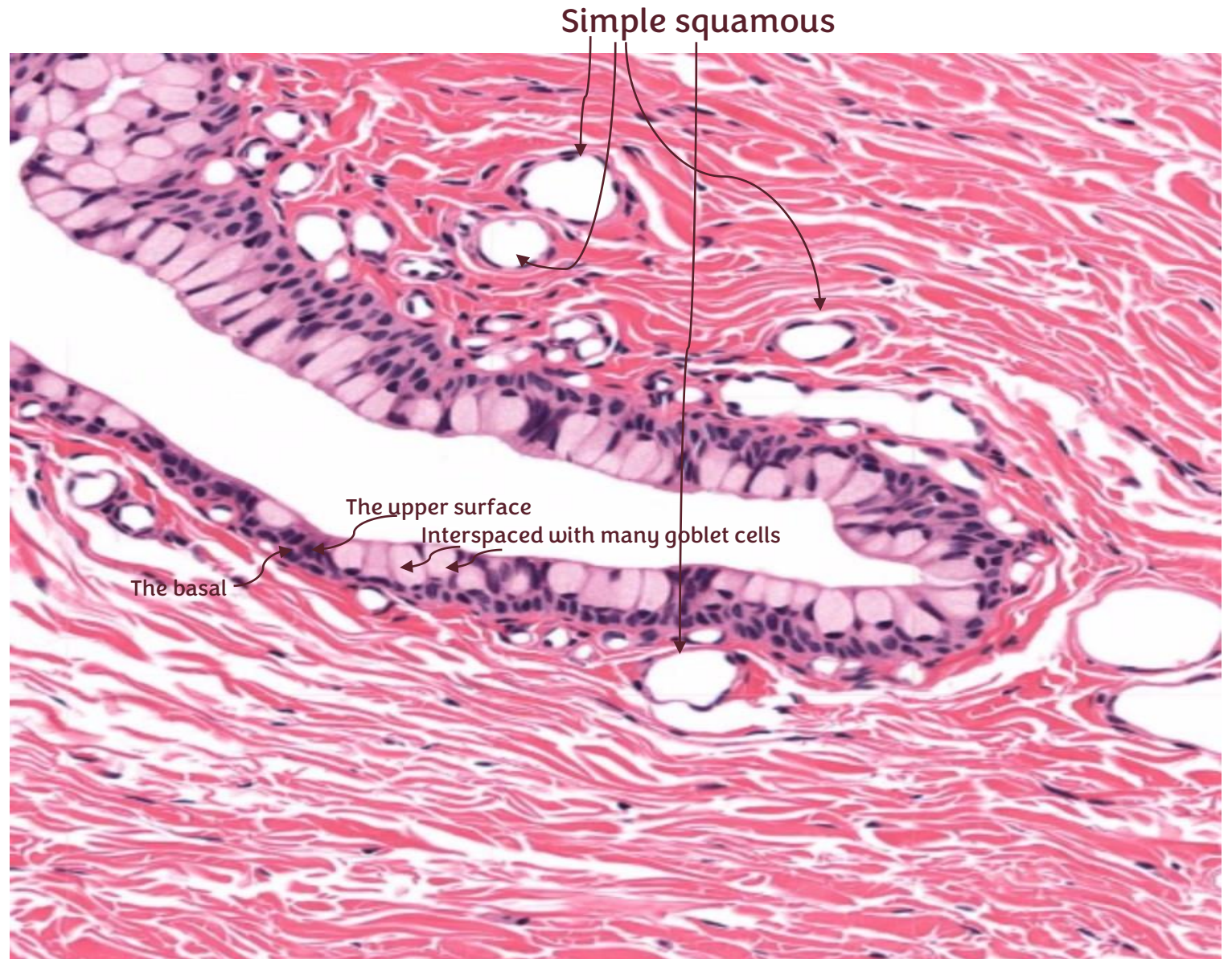
STRATIFIED COLUMNAR With goblet cells

The rarest type

This section is taken from the conjunctiva of the eye.

When we first look at the eye, the first protective layer we see is the cornea. The white part (sclera) also has a surface that needs to be covered by epithelium, which is stratified columnar.

The conjunctiva covers the white part of the eye and is also present on the inner surfaces of the upper and lower eyelids, as well as on the plains of the inner surface of the iris.

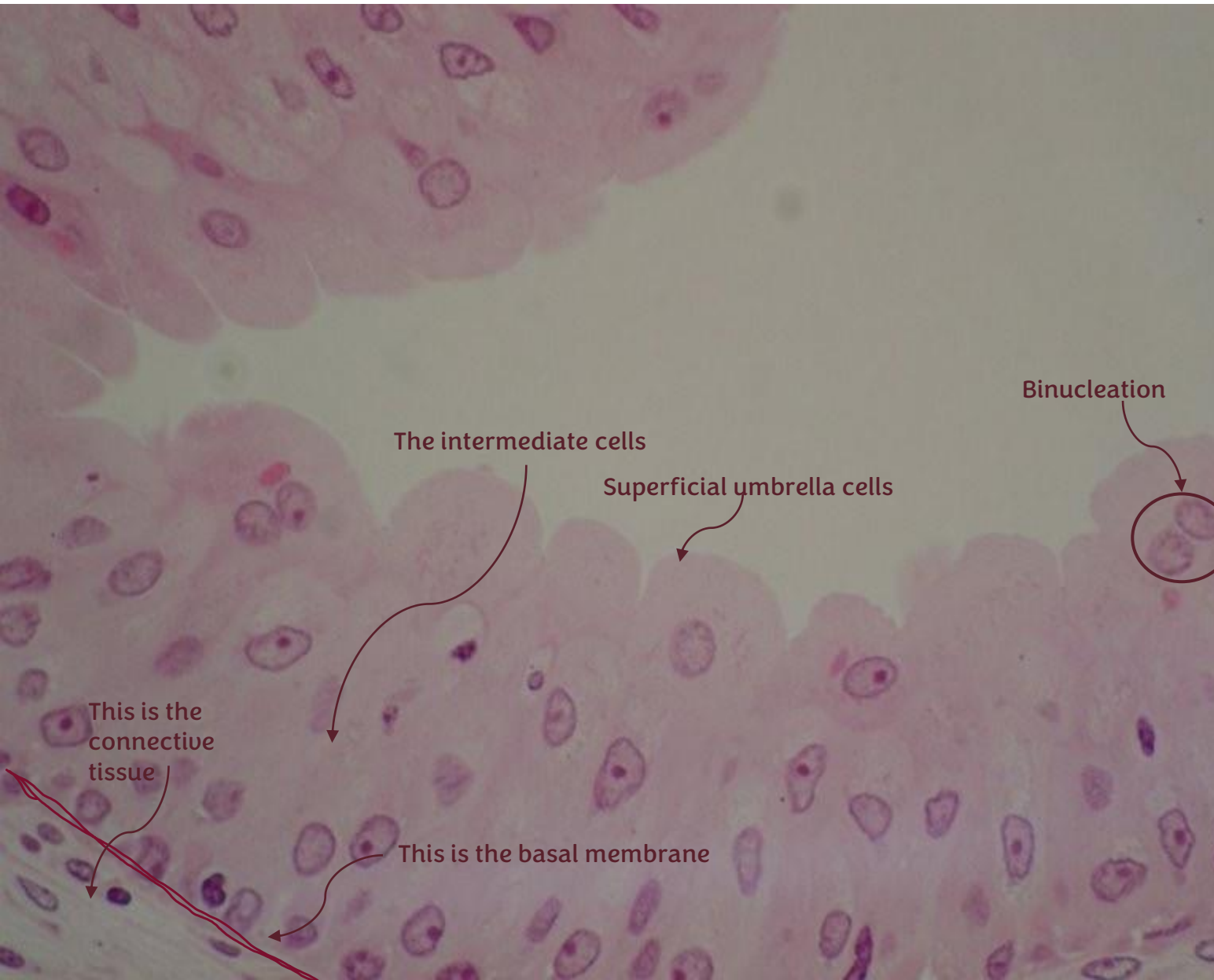


TRANSITIONAL EPITHELIUM

How do we know that this epithelium is transitional?

One clue is the umbrella cells (the superficial cells). If the epithelium is in a relaxed state, these cells appear large and rounded.

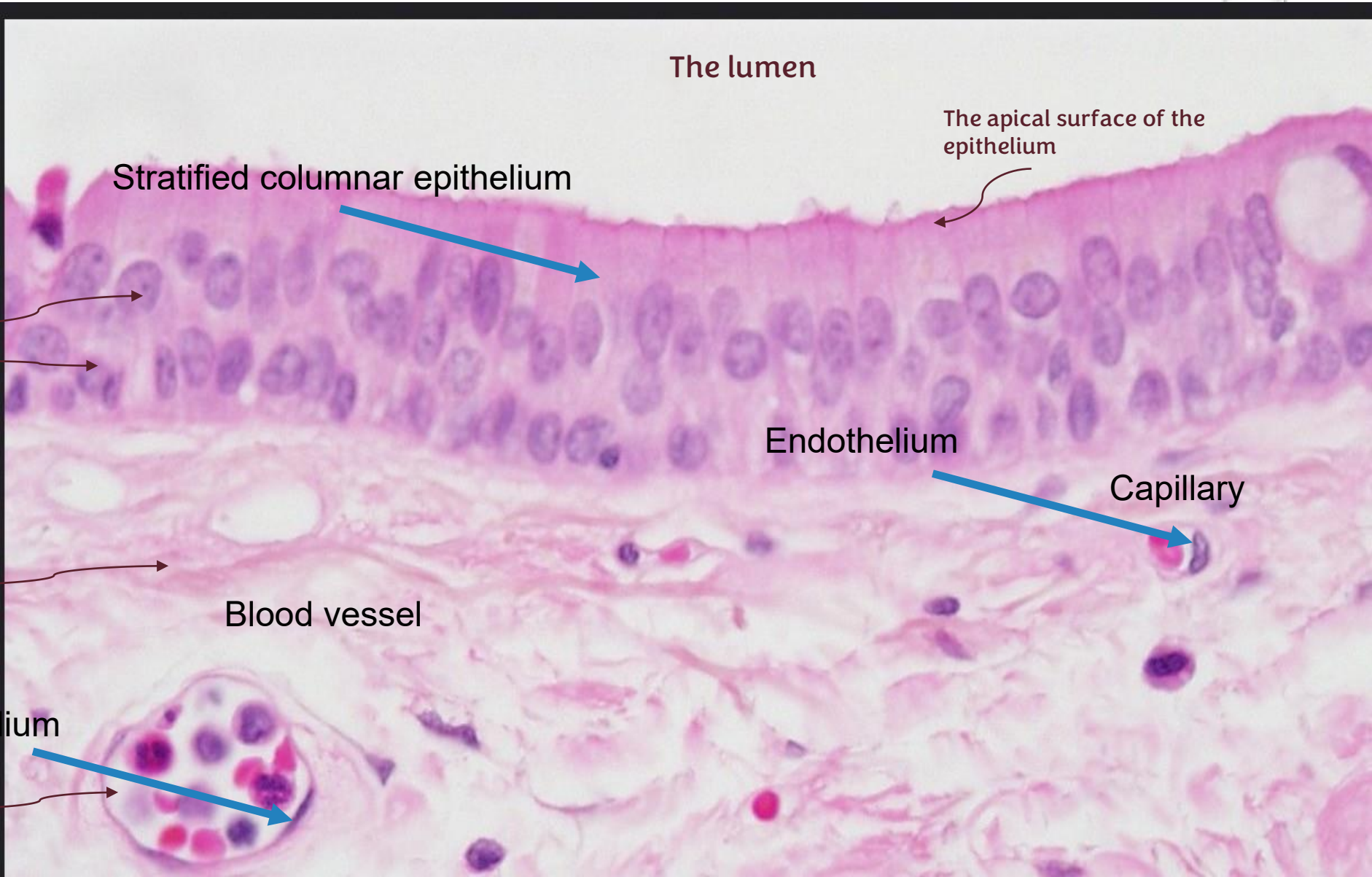
If the epithelium is stretched, a helpful hint is binucleation—seeing cells with two nuclei indicates transitional epithelium adapting to stretching.



This epithelium is in the relaxed state, so it is easy to identify the dome-shaped superficial cells (umbrella cells).

****Here in this image we have two types of tissue , epithelial and connective tissue**

IDENTIFY



The lumen

The apical surface of the epithelium

Stratified columnar epithelium

The first row

The second row

Endothelium

Capillary

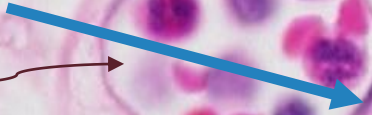
Connective tissue

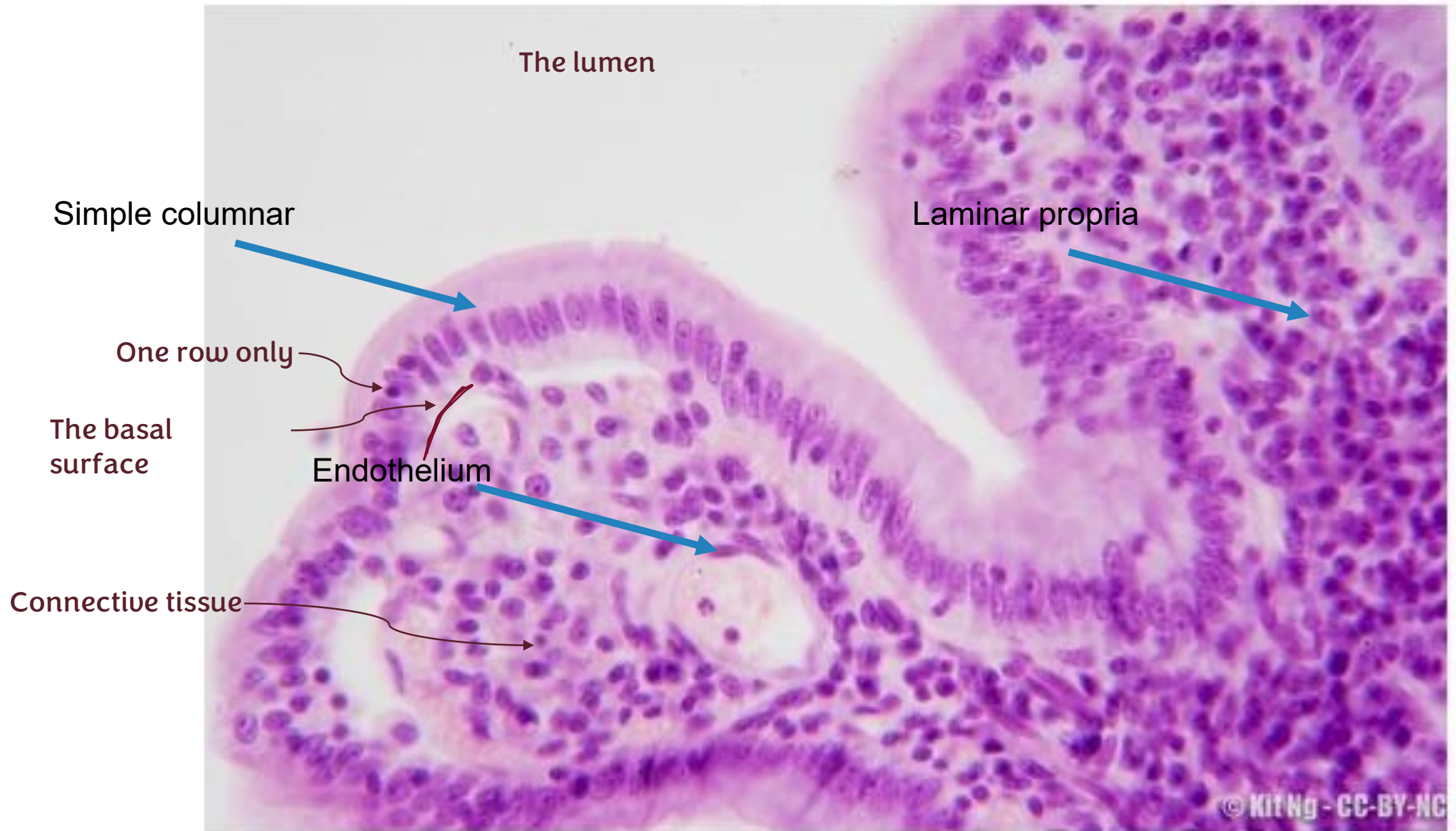
Most of the pink colours are collagen fibers

Blood vessel

Endothelium

Here we have RBCs and WBCs





This is a large duct

The lumen

The apical surface

The basal surface

**Stratified
cuboidal**

Here we have two layers

Here we have two clear layers, and the cells are large and rounded. But why don't we see the nuclei in some cells? Because the cells are relatively large, and sometimes the section does not pass through the nucleus, so it is not visible in that particular cell.



This quiz is intended to assess your knowledge of histological structures

https://docs.google.com/forms/d/e/1FAIpQLSc1SzRTRINyKKc6GU7fbkDgWwOfuPJDvGQvKlb_Hsj4_vWeQ/viewform?usp=publish-editor

For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			


Additional Resources:

رسالة من الفريق العلمي:

Reference Used:

(numbered in order as cited in the text)

Dr. Ghada Abu Al-Ghanem's lecture

رمضان ذهب معظمه وبقي أعظمه، اللهم اغفر لنا ما مضى و أعنا على ما بقى ولا تجعلنا من
الذين ضل سعيهم في رمضان وهم يحسبون أنهم يحسنون صنعا.
اللهم اجعلنا ممن يستجاب دعاؤهم و تغشى قلوبهم رحمتك و انفعنا اللهم بعلمنا و ارزقنا اللهم
حفظ كتابك و اتباع سنة نبيك 

(قد ينال المرء بدعاء أخيه ما لا يناله بدعائه لنفسه فلا تنسوننا من صالح دعائكم في هذه

الأيام المباركة )