



INTRODUCTORY LECTURE

LIGHT MICROSCOPY

Curriculum: Phase 1/ Semester2/ TOB/ Session 1

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SELECTED REFERENCES



- **Histology Textbooks ‘Basic Histology’, Junqueira, 13 th Edition.**
- **‘Colour Atlas of Histology’ Gartner and Hiatt**



LECTURE OBJECTIVES

- ❖ *state the relationship between milli-, micro- and nanometers.*
- ❖ *state the meaning of the term “tissue”.*
- ❖ *explain the value of histology in diagnosis.*
- ❖ *describe common biopsy techniques (e.g. curettage, needle, transvascular etc) giving examples of tissues which can be sampled by each method.*
- ❖ *explain why tissue needs to be fixed and state which fixatives are commonly used.*



- ❖ *describe how tissue processing can lead to the formation of shrinkage artefacts.*
- ❖ *discuss the value of histological staining and state the components of tissue stained by the Periodic acid Schiff reaction (PAS) and by Haematoxylin and Eosin (H & E) staining.*
- ❖ *outline the advantages conferred by phase contrast, dark field, fluorescence, and confocal light microscopy.*



STATE THE MEANING OF THE TERM TISSUE



Tissue : a collection of cells specialized to perform a particular function .

Tissues are made of two interacting components:

- cells
- extracellular matrix.

Histology :Is the study of the tissues of the body and how these tissues are arranged to constitute organs, by means of special staining techniques combined with light and electron microscopy.



TISSUE CLASSIFICATION

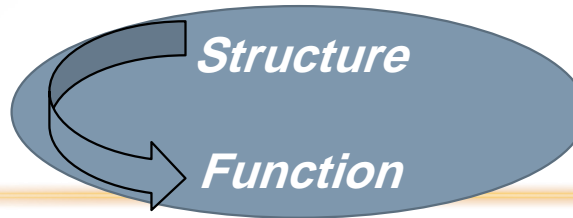


1. Epithelial tissue
2. Connective (Support) tissue
3. Muscle tissue
4. Nervous tissue



ANATOMY (macroanatomy)
(form & gross structure)

HISTOLOGY
(microanatomy)
(tissue/cell structure)



PHYSIOLOGY
(functioning of
body)

BIOCHEMISTRY
(chemical processes
substances)



THE RELATIONSHIP BETWEEN MILLI-, MICRO AND NANOMETERS.



meter

m

millimeter

mm

10^{-3}m

micrometer

mm

10^{-6}m

nanometer

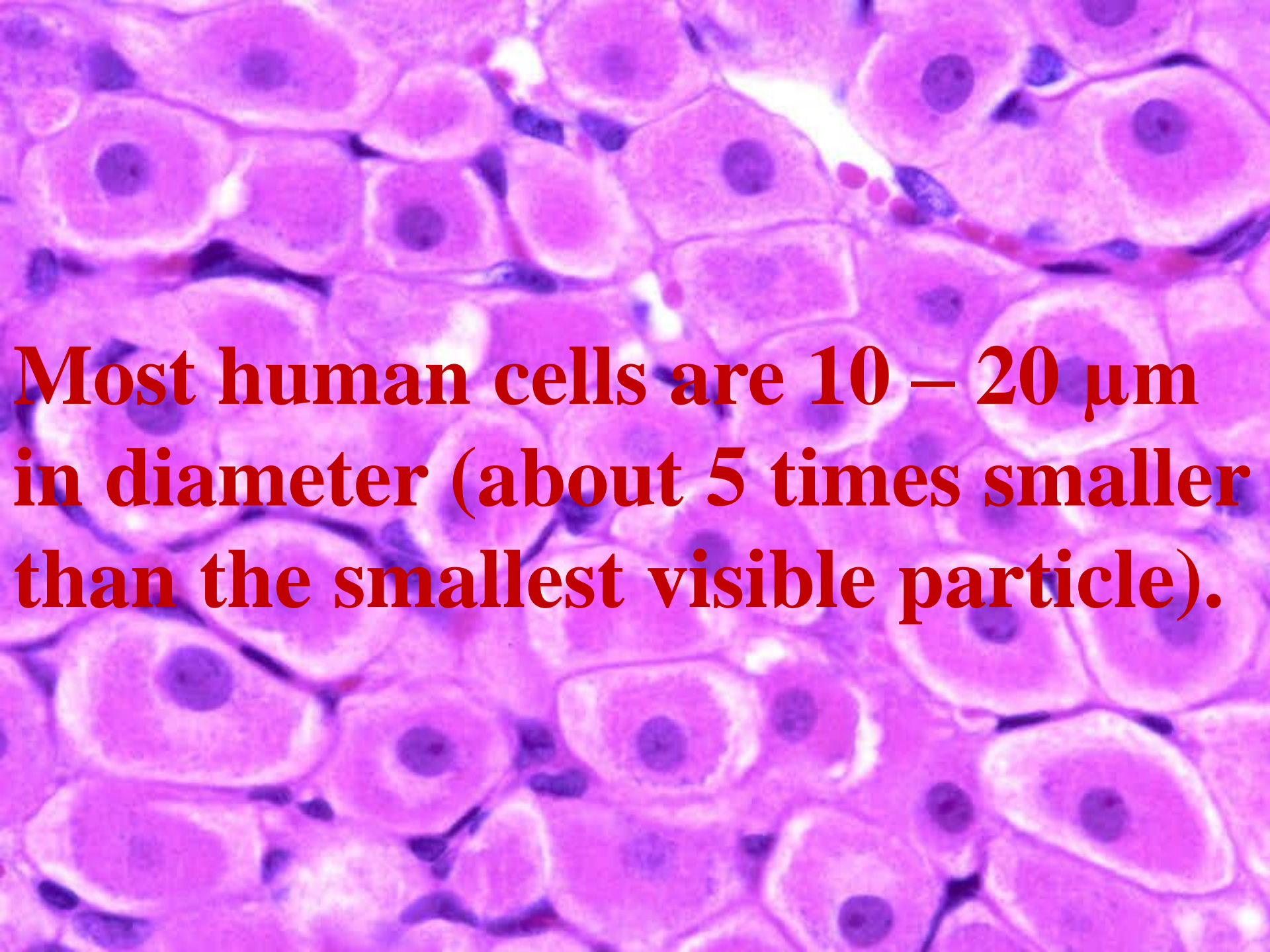
nm

10^{-9}m

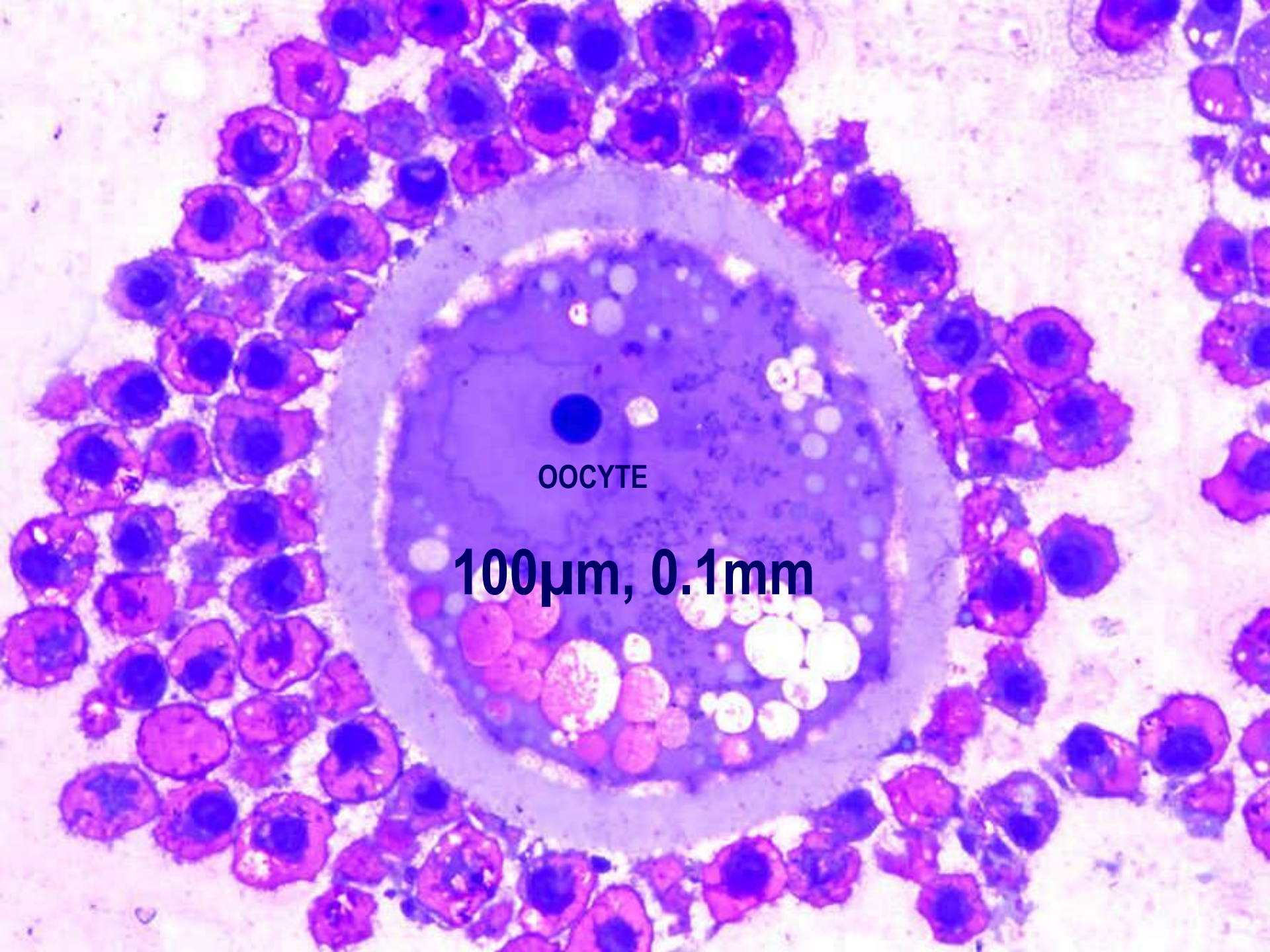
Angstrom Unit

Å

10^{-10}m

A microscopic image showing a dense field of human cells, likely epithelial cells, stained with hematoxylin and eosin (H&E). The cells are roughly circular to polygonal in shape, with prominent, dark purple nuclei and lighter pink cytoplasm and cell membranes. The cells are closely packed together, with some showing clear cell boundaries. The overall color palette is dominated by shades of pink and purple.

Most human cells are 10 – 20 μm in diameter (about 5 times smaller than the smallest visible particle).



OOCYTE

100 μ m, 0.1mm



**describe common biopsy techniques
(e.g. curettage, needle, transvascular
etc) giving examples of tissues which
can be sampled by each method.**



Biopsy – the removal of a small piece of tissue from an organ or part of the body for microscopic examination.



Types of biopsy

Smear – e.g. *cervix*

Curettage – e.g. *endometrial lining of uterus*

Needle – e.g. *brain, breast, liver, kidney, muscle*

Direct incision – e.g. *skin, mouth, larynx*

Endoscopic – e.g. *lung, intestine, bladder*

Transvascular – e.g. *heart, liver*



why tissue needs to be fixed and state which fixatives are commonly used. describe how tissue processing can lead to be formation of shrinkage artifacts.



Tissue Processing Procedure

1-Fixation

2- Dehydration and clearing

3- Wax embedding

4- Cutting and Mounting section

5- Staining

6- Mounting

Biopsy $\xrightarrow{\text{Tissue processing}}$ Microscopy



Fresh biopsy (i.e. needle biopsy – wet and bloody)



FIXATION (glutaraldehyde, formaldehyde)



Fixed biopsy (macromolecules cross-linked, cellular structure preserved, no autolysis or putrefaction)

Fixed biopsy

Water

Dehydration

water

ethanol

Ethanol

Clearing

ethanol

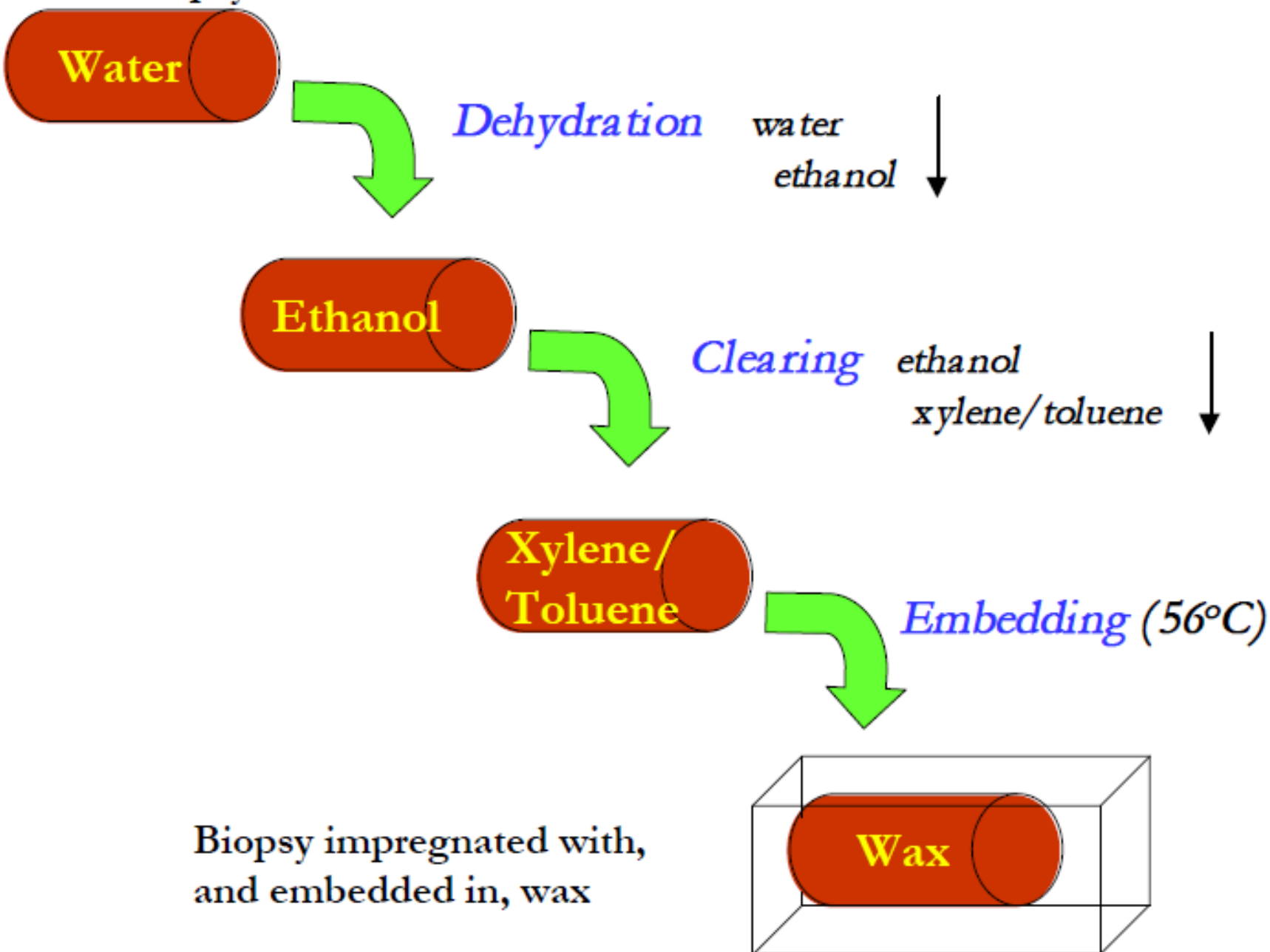
xylene/toluene

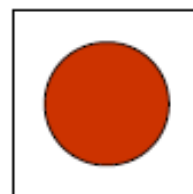
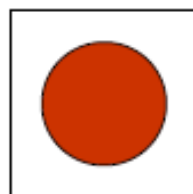
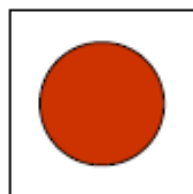
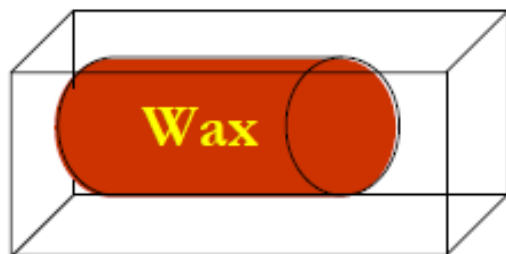
Xylene/
Toluene

Embedding (56°C)

Biopsy impregnated with,
and embedded in, wax

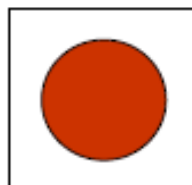
Wax





Sectioning

Section in wax on
microscope slide



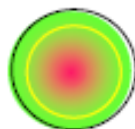
Rehydration

(xylene
ethanol
water)



Staining

(most stains
water soluble)



Stained section on slide



Dehydration (water
ethanol
xylene)

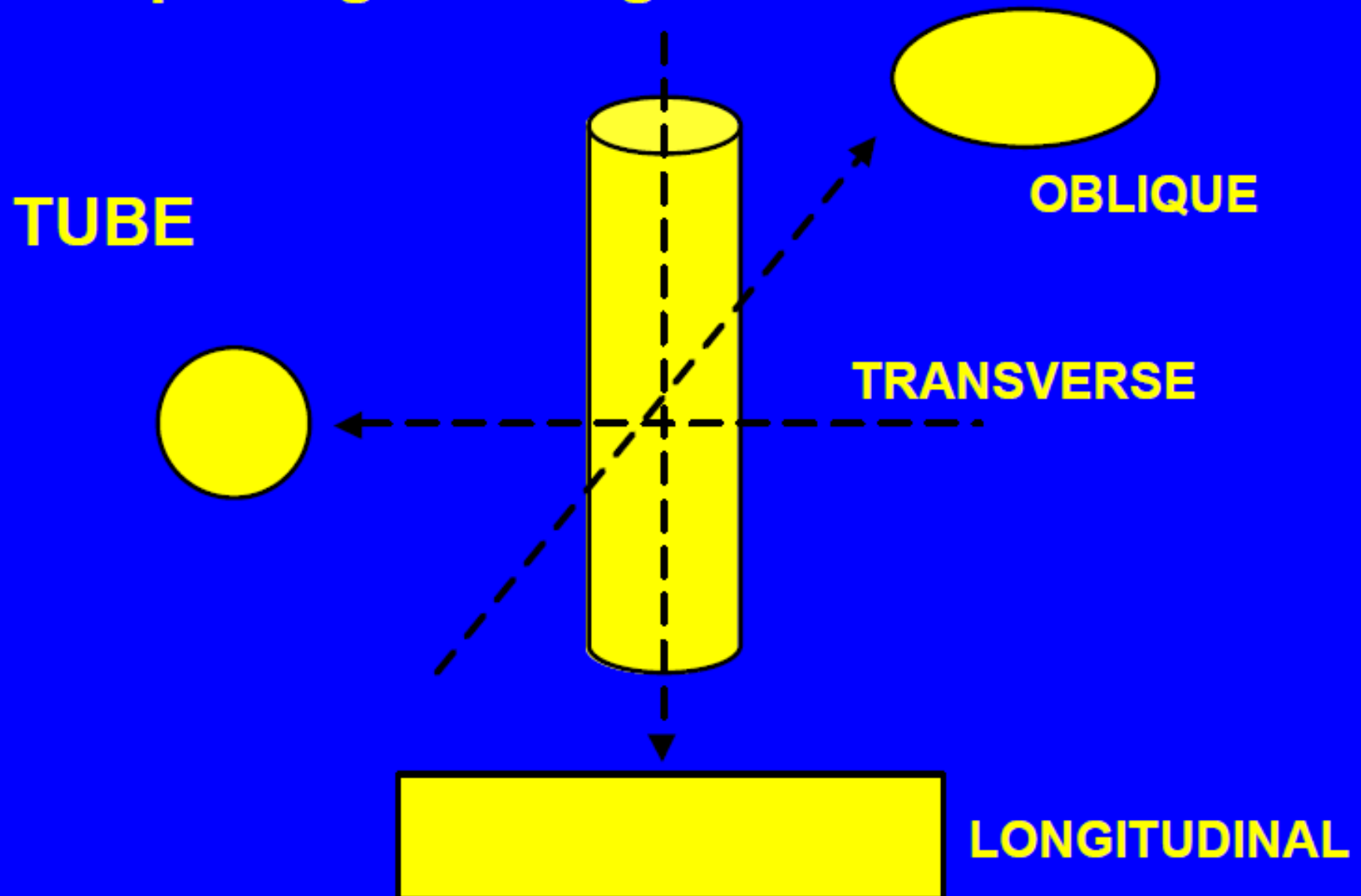


Mounting (DPX dries
under
coverslip)

Section ready for
microscopy

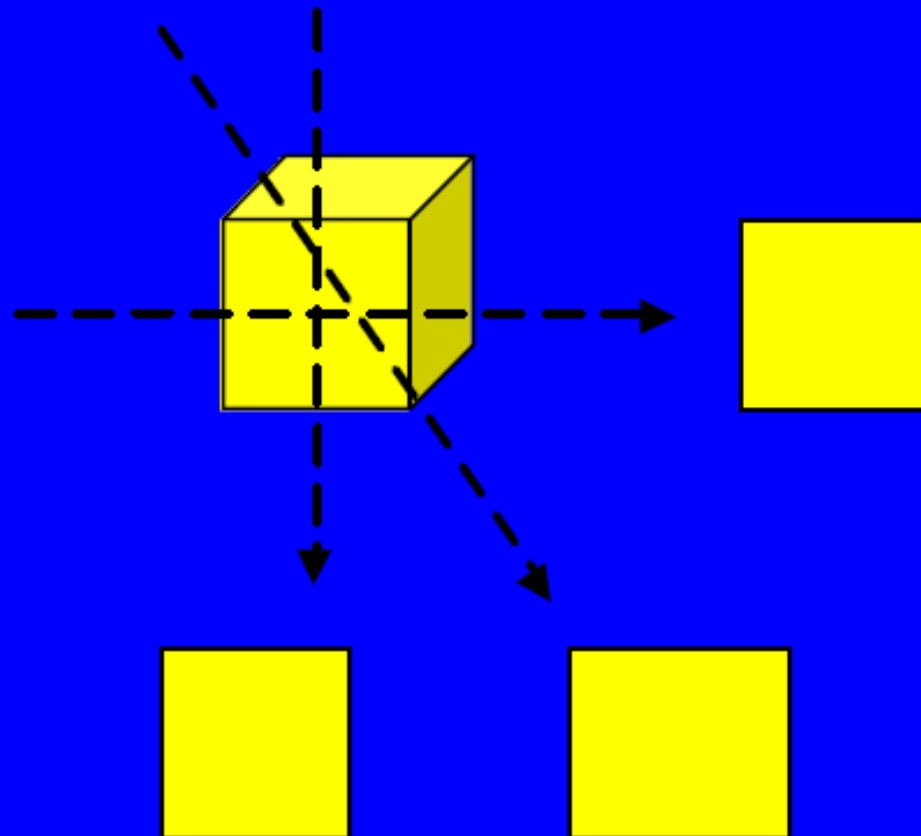


Think about the plane of section when interpreting 2D images.



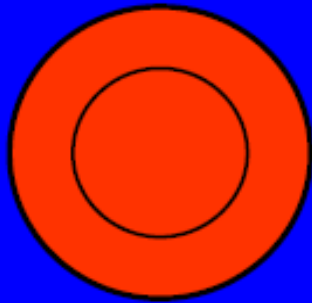
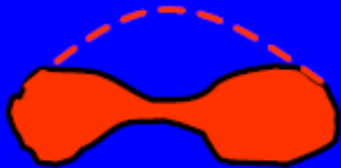
Think about the plane of section when interpreting 2D images.

CUBE

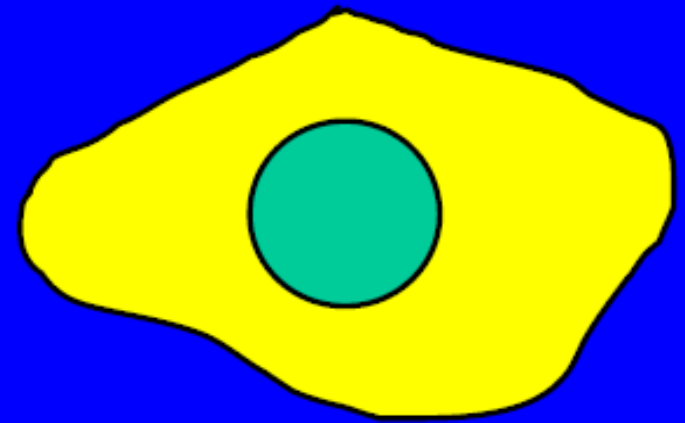


Think about the plane of section when interpreting 2D images.

Red blood cell



Squamous cell





the value of histological staining, and state the components of tissue stained by the Periodic Schiff reaction and by Haematoxylin and Eosin staining

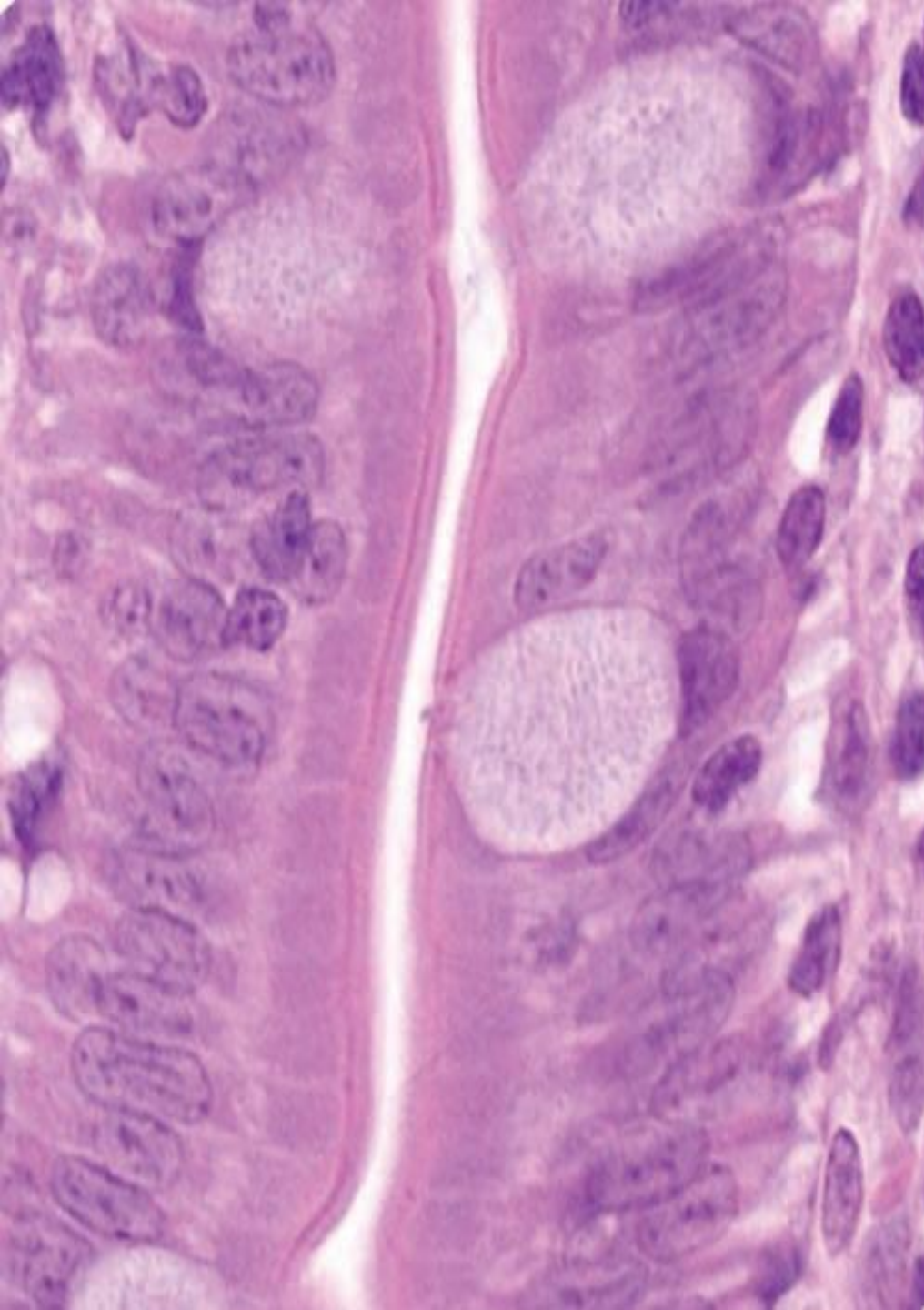


Haematoxylin

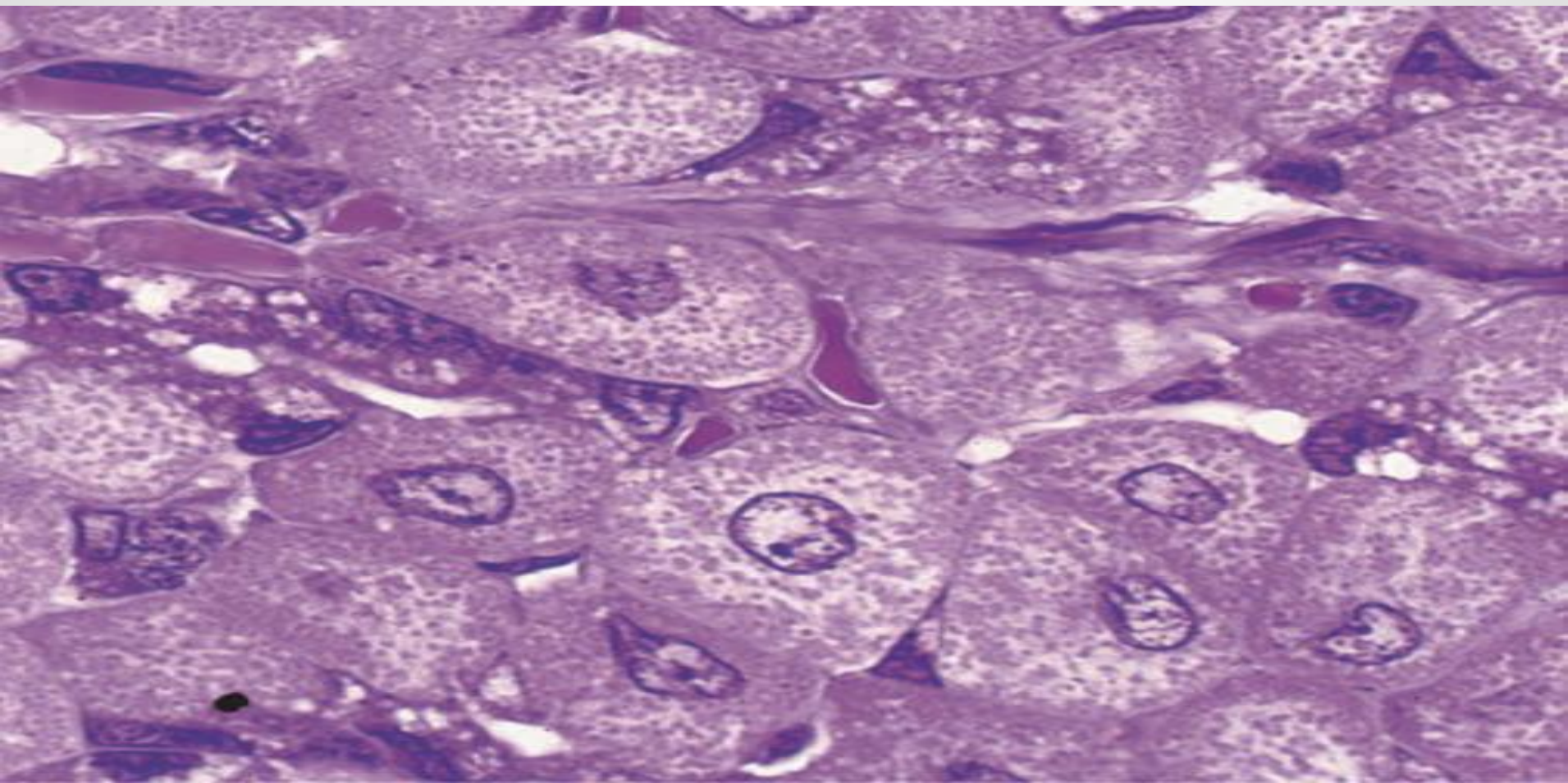
Stains acidic components of cells- purple/blue e.g. Nucleolus (RNA) and chromatin (DNA).

Eosin

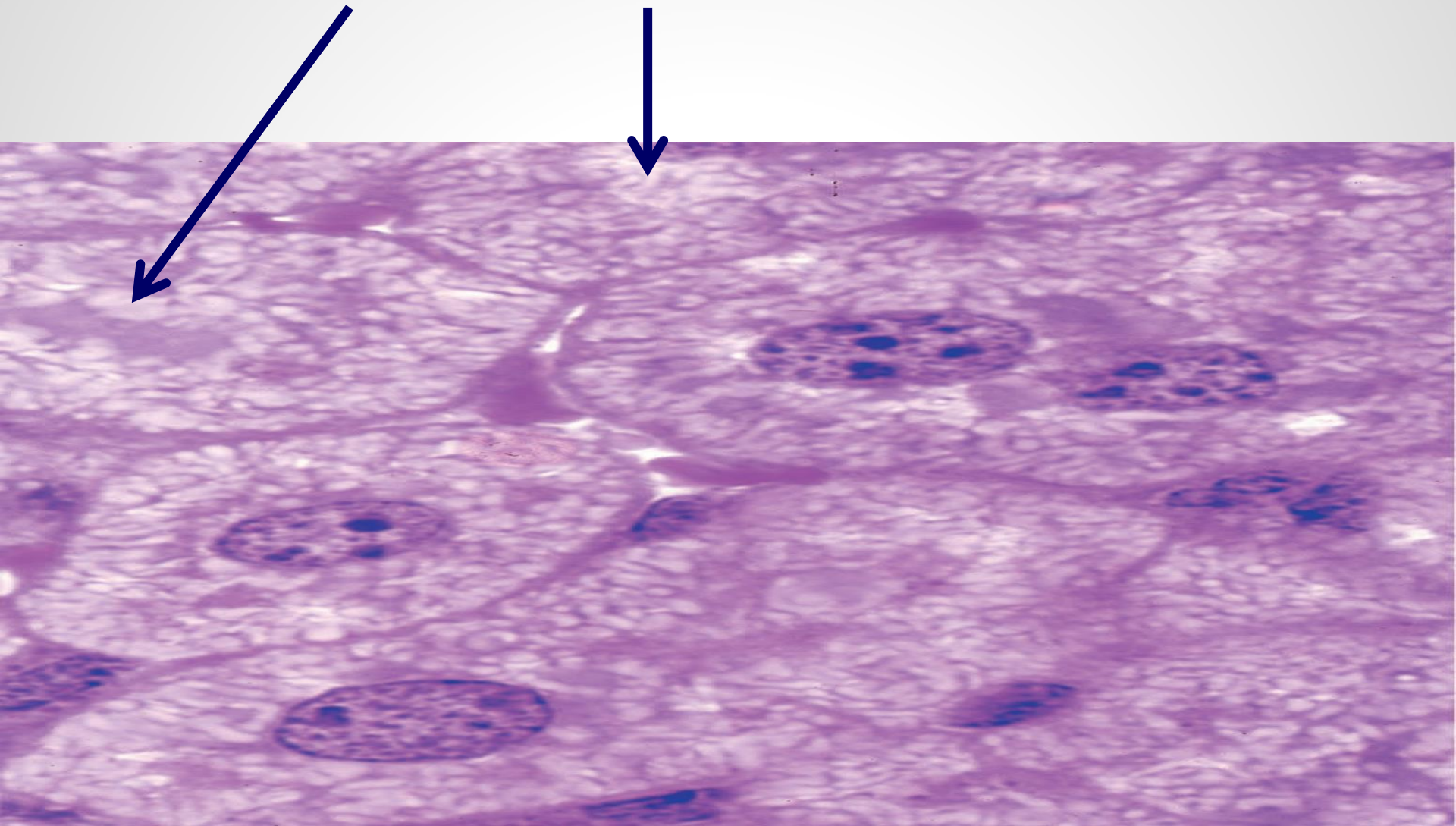
Stains basic components of cells- pink e.g. most cytoplasmic protein and extracellular fiber



Diffuse purple tinted cytoplasm is indicative of the presence of cytoplasmic RNA in the form of ribosomes, and thus active protein synthesis.



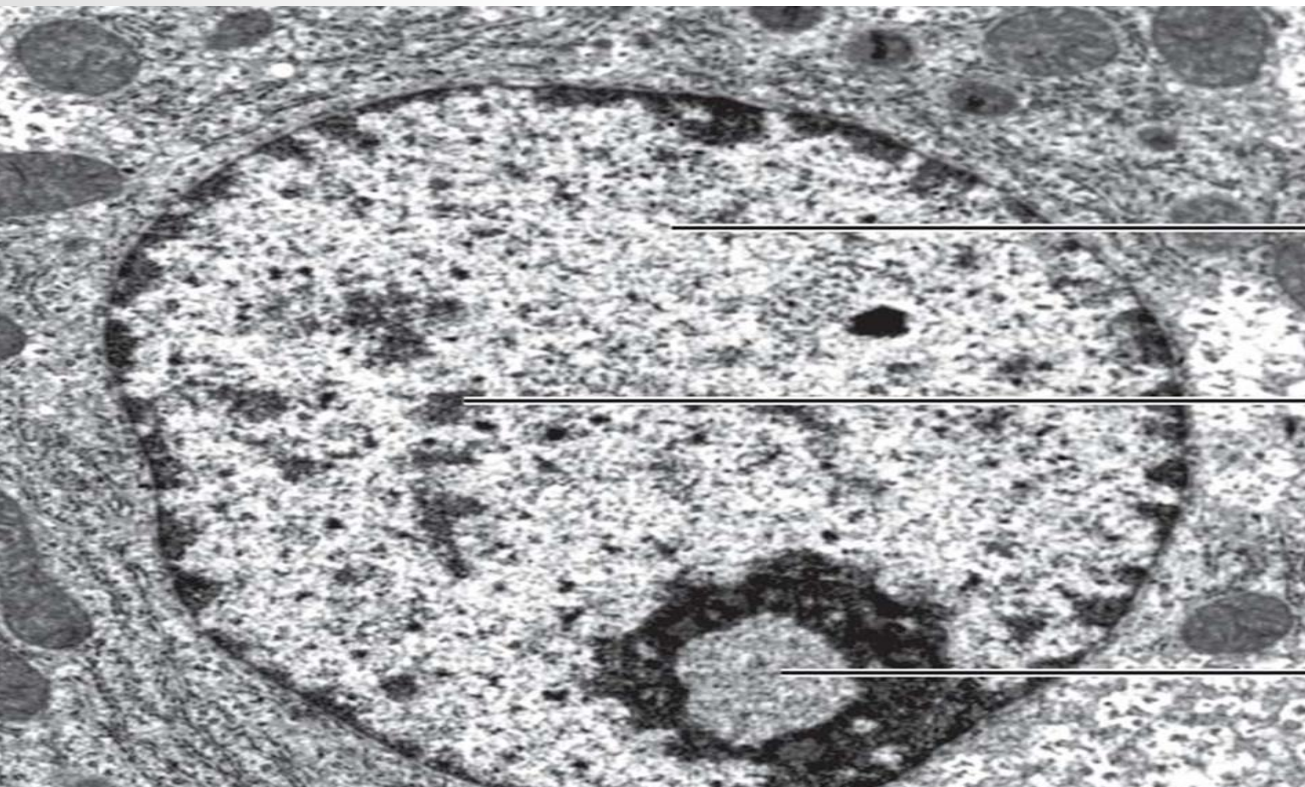
Non-staining areas may indicate that the cell contains mucous secretory vacuoles or fat droplets.





Nucleus

A cell that is actively synthesizing large quantities of protein usually has a large, pale staining nucleus (due to active transcription of chromatin) with prominent nucleoli (sites of active ribosomal RNA synthesis).



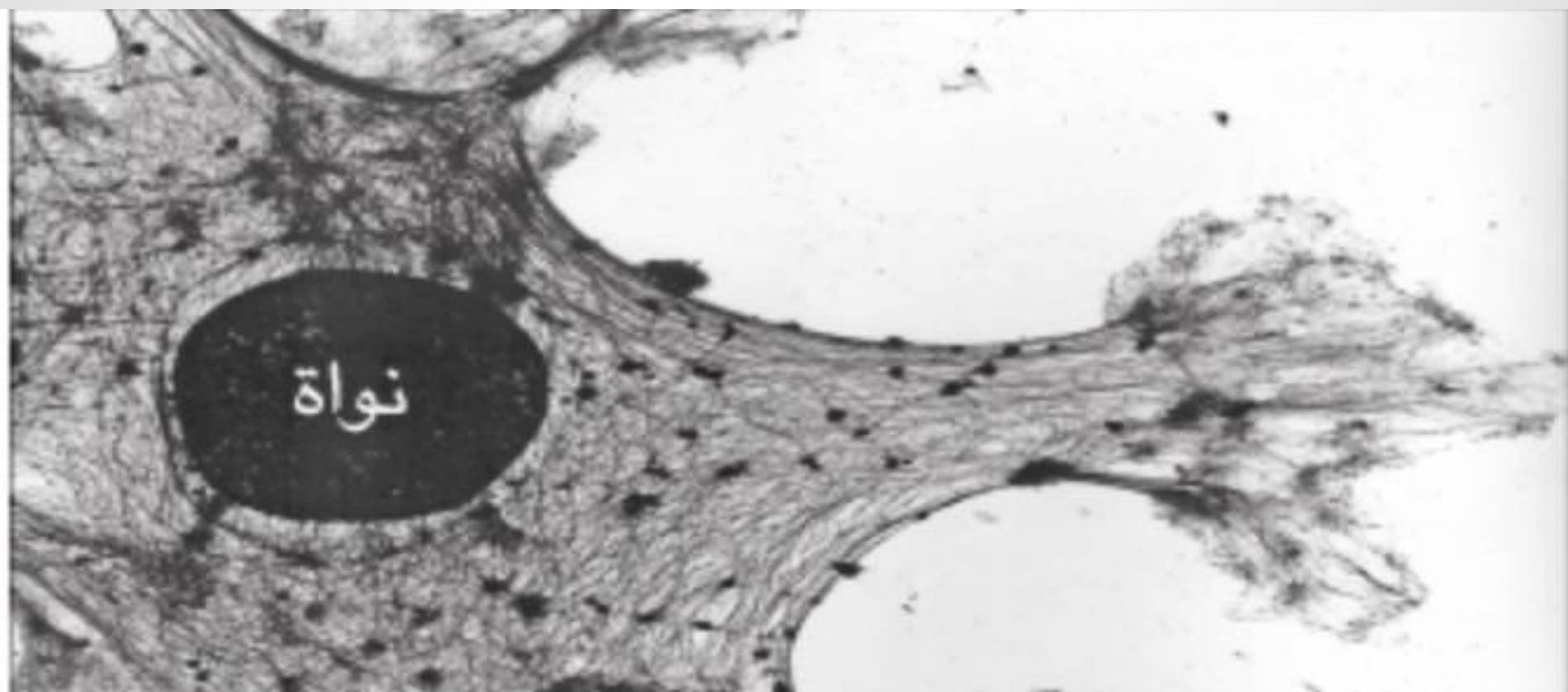
euchromatin

heterochromatin

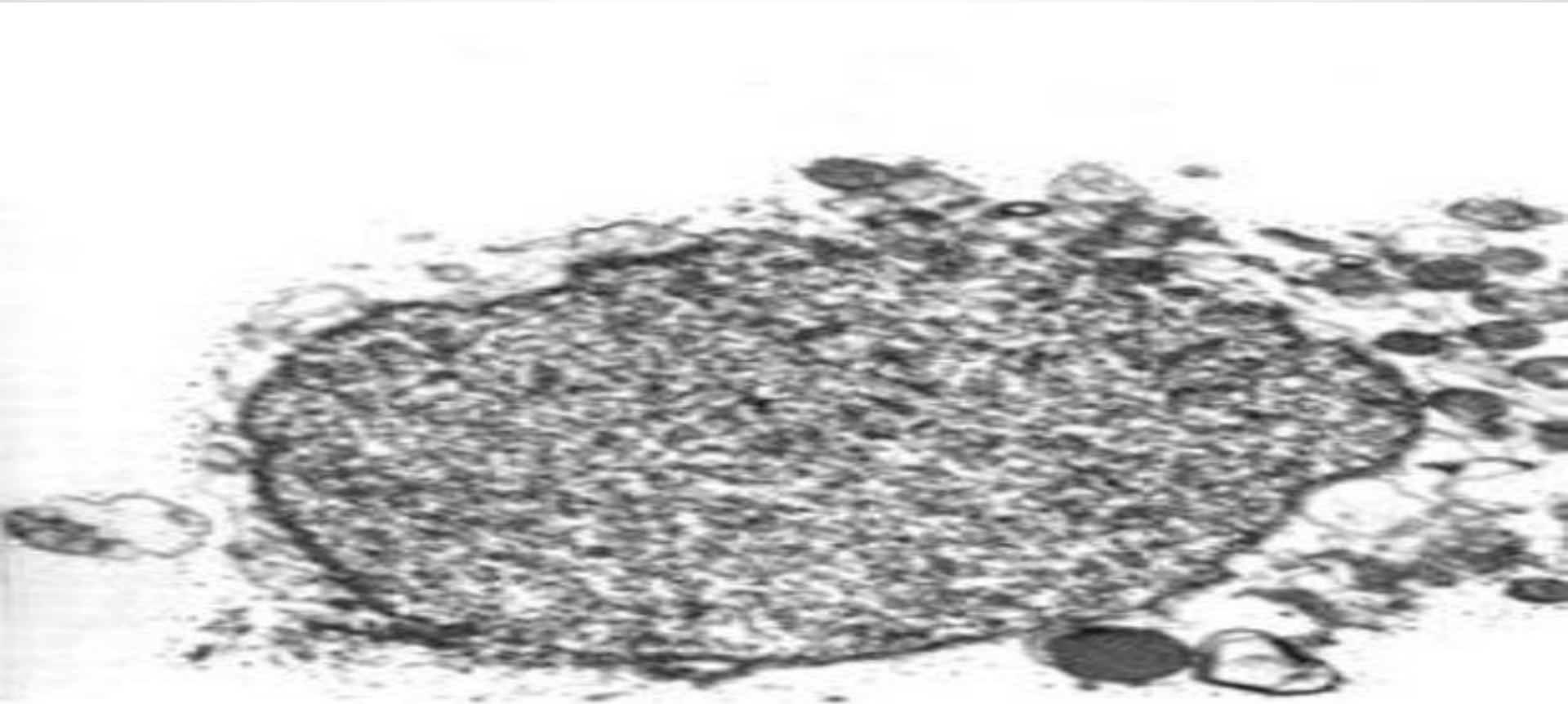
nucleolus



An inactive cell has a compact deep-staining nucleus (little chromatin being transcribed) which lacks visible nucleoli (minimal ribosome production).

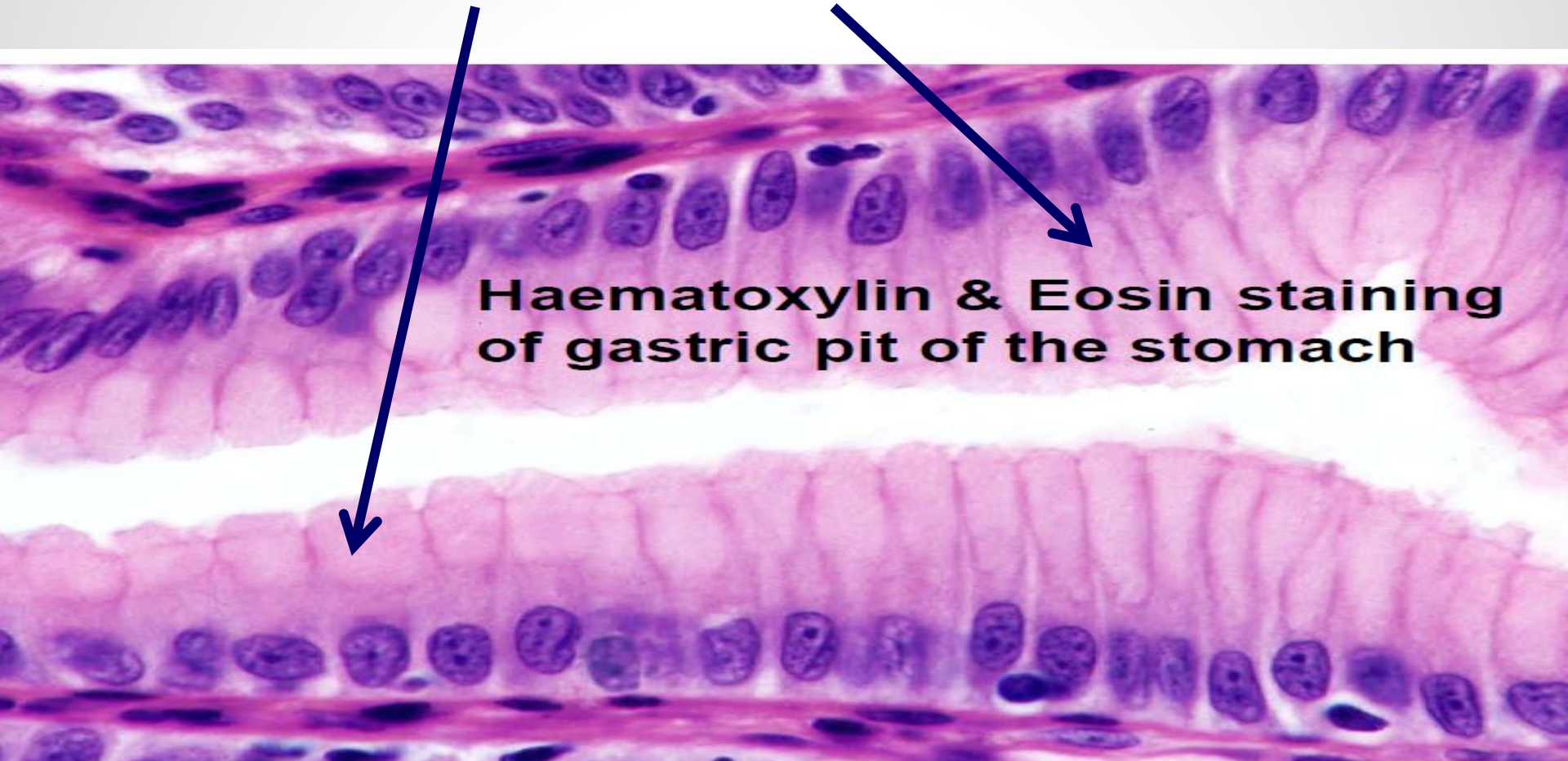


A dead cell often has an intensely staining, shrunken nucleus, although sometimes the nucleus may be fragmented or absent due to lysis of nuclear material.



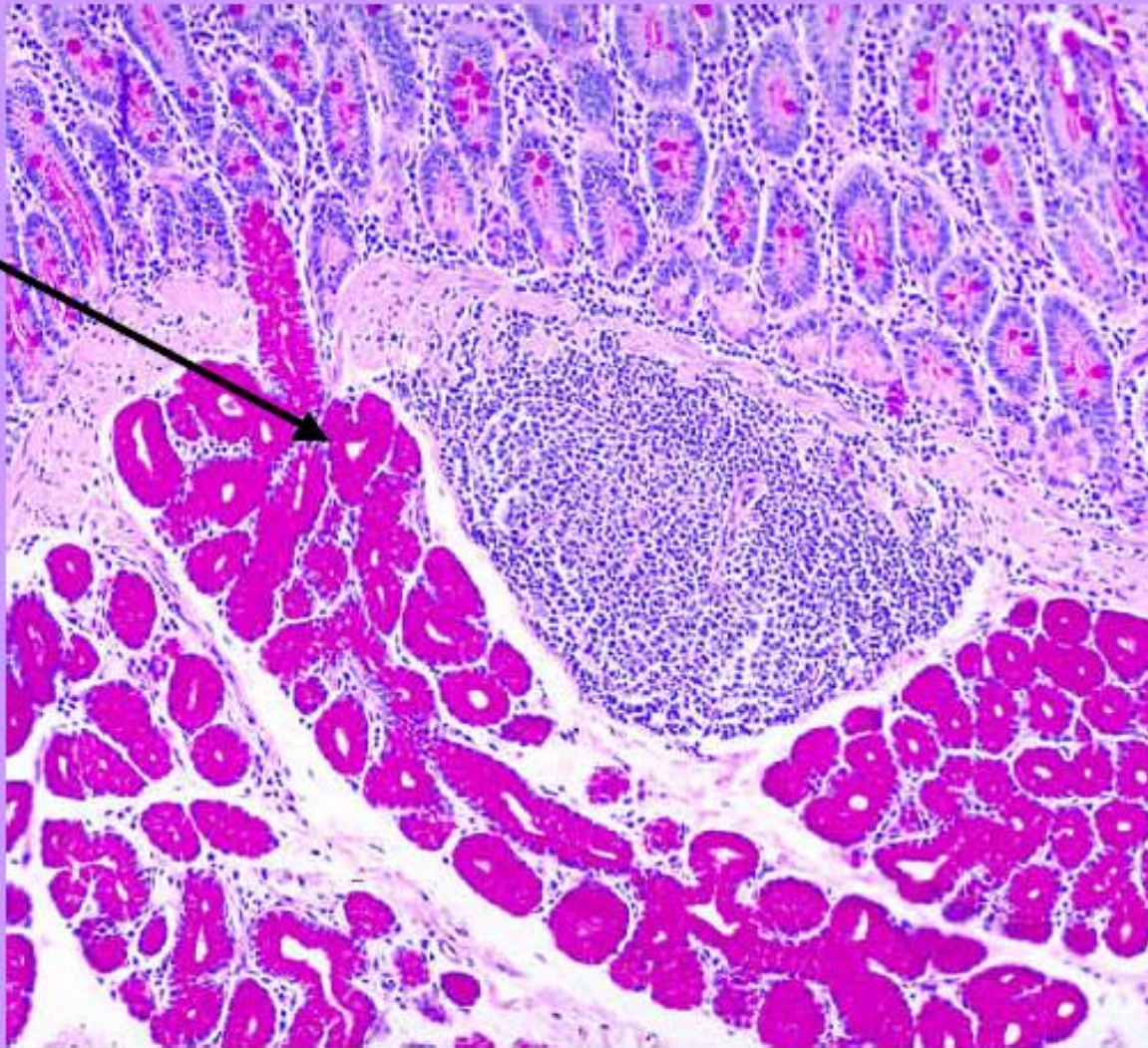
necrosis

Pink-staining granular cytoplasm often contains accumulations of organelles (mitochondria or secretory granules) that take up acidic dye.

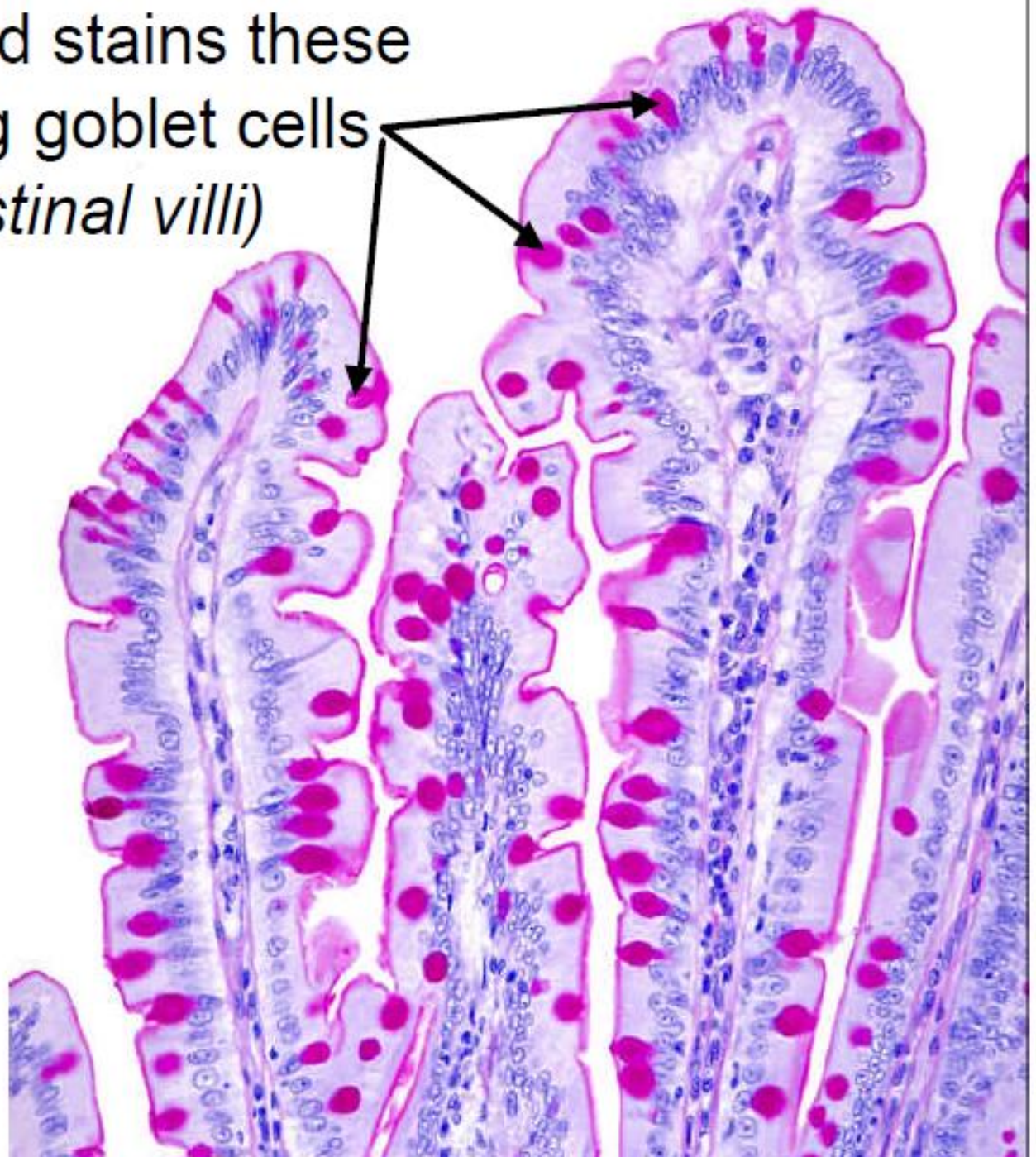


The Periodic Acid-Schiff (PAS) method stains carbohydrates and glycoproteins magenta

(as in this slide of mucus-secreting Brunner's Glands in the duodenal wall)



The PAS method stains these mucus-secreting goblet cells magenta. (*Intestinal villi*)





MICROSCOPE



A microscope is an instrument for viewing objects that are too small to be seen by our naked eyes, The microscope includes an optical system and mechanisms to move and focus the specimen.



TYPES OF THE MICROSCOPE



There are many types of microscopes , ranging from simple , single – lens instruments (magnifying glasses) to compound microscope and high- powered electron . two basic types of microscopes that are used in biological studies : the compound light microscope and the electron microscope



parts of the microscope

Parts	Function
Eyepiece (ocular)	Contains lenses for magnification. Where you look through to see the image of your specimen .
Arm	Supports the body tube and lenses . Use the arm to carry your microscope .
Body tube (Barrel)	Maintains a set distance between the eyepiece and the objective lenses .
Course adjustment knob	Moves the body tube or stage up and down to focus the image ; course focusing .

Fine adjustment knob	Sharpens the image ; fine focusing .
Revolving Nosepiece (Turret)	Contain objective lenses . A rotating device to which objective lenses are attached
stage	The horizontal platform upon which the slide rests supports the slide being viewed.
Stage (slide) clips , or mechanical stage	Clips hold the slide in place on the stage . A mechanical stage aids in centering the specimen .

Sub stage condenser	Lens found beneath the stage that concentrates light before it passes through the specimen to be viewed .
Diaphragm	Open holes on a disk under the stage that regulates the amount of light passing through the specimen .
Base	Supports the microscope ; give the instrument stability .
Illuminator (light source) or mirror	Usually found near the base of the microscope ; the light source makes the specimen easier to see .



Magnification



Your microscope has 3 magnifications : Scanning , Low and High . each objective will have written the magnification . In addition to this , the ocular lens (eye piece) has a magnification . The total magnification is the Ocular × Objective .

Objective lenses	Magnification	Ocular lens	Total magnification
Scanning	4 x Rad	10 x	40 x
Low power	10 x Blue	10 x	100 x
High power	40 x Yellow	10 x	400 x
Oil immersion	100 x White	10 x	1000 x



Electron Microscope

Is a type of microscope that uses a beam of electrons to illuminate the specimen and produce a magnified image. because electrons have wavelengths about 100,000 times shorter than visible light (photons), and can achieve better than 50 pm resolution and magnifications of up to about 10,000,000x.

1- Scanning electron microscope

2- transmission electron microscope



Transmission electron microscope (TEM)

The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beam to create an image.





Scanning electron microscope

Unlike the TEM, where electrons of the high voltage beam carry the image of the specimen, the electron beam of the scanning electron microscope (SEM) does not at any time carry a complete image of the specimen. The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen.

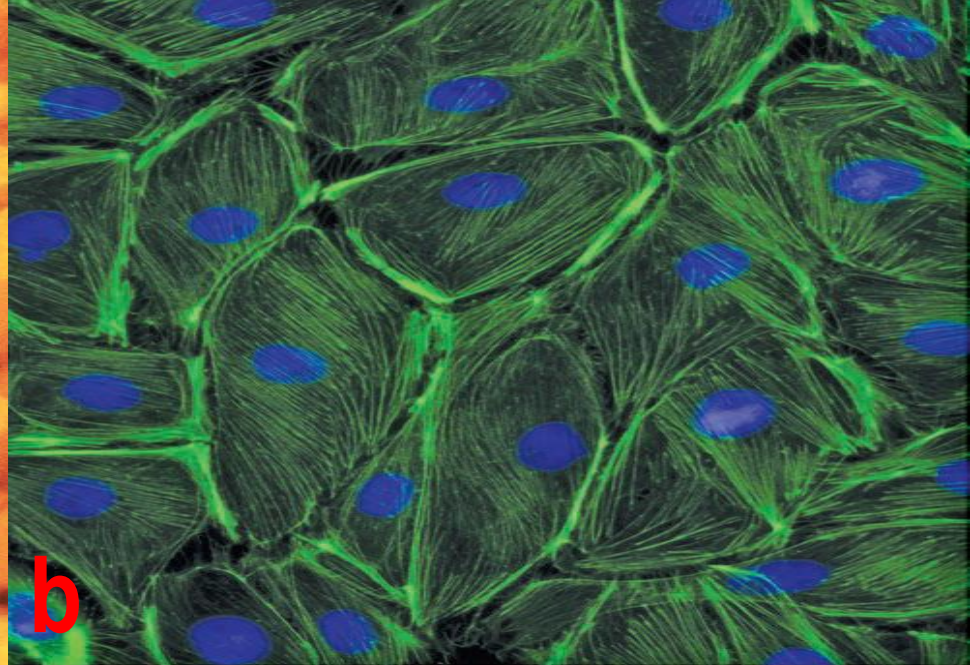
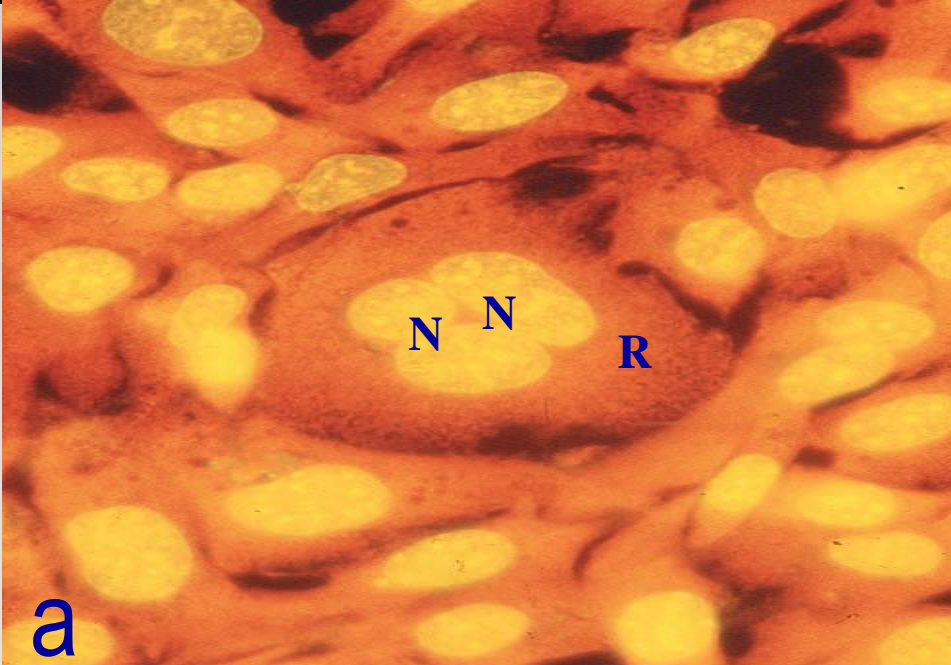


***outline the advantages conferred by
phase contrast, dark field,
fluorescence, and confocal light
microscopy.***



Fluorescence Microscopy

When certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength a phenomenon called fluorescence. In fluorescence microscopy, tissue sections are usually irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum. The fluorescent substances appear brilliant on a dark background. For this method, the microscope has a strong UV light source and special filters that select rays of different wavelengths emitted by the substances.



Components of cells are often stained with compounds visible by fluorescence microscopy. (a) Acridine orange binds nucleic acids and causes DNA in cell nuclei (N) to emit yellow light and the RNA-rich cytoplasm (R) to appear orange in these cells of a kidney tubule.

(b) Cultured cells stained with DAPI (4',6-diamino-2-phenylindole) that binds DNA and with fluorescein-phalloidin that binds actin filaments show nuclei with blue fluorescence and actin filaments stained green. Important information such as the greater density of microfilaments at the cell periphery is readily apparent. Both X500. (Figure 1–4b, contributed with permission, from Drs Claire E. Walczak and Rania Risk, Indiana University School of Medicine, Bloomington.)



Phase-contrast microscopy & differential interference microscopy

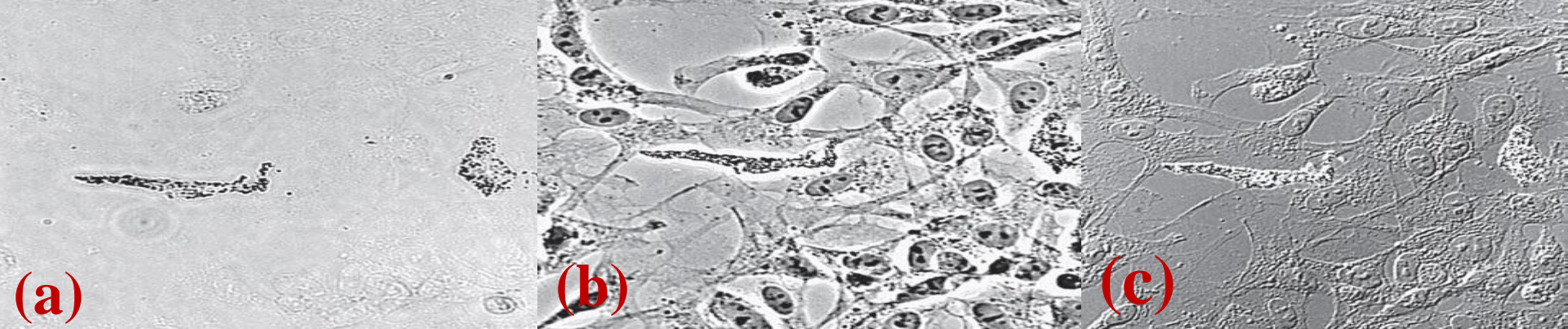
Phase-contrast microscopy uses a lens system that produces visible images from transparent objects and, importantly, can be used with living, cultures cells. **Phase-contrast microscopy** is based on the principle that light changes its speed when passing through cellular anextracellular structures with **different refractive indices**. These changes are used by the phase-contrast system to cause the structures to appear lighter or darker in relation to each other. Because they allow the examination of cells without fixation or staining, phase-contrast microscopes are prominent tools in all cell culture laboratories. A modification of phase-contrast microscopy is **differential interference microscopy** with Nomarski optics, which produces an image of living cells with a more apparent three-dimensional (3D) aspect



Confocal microscopy

avoids Stray (excess) light reduces contrast within the image and compromises the resolving power of the objective lens. Confocal microscopy achieves high resolution and sharp focus by using

- (1) a small point of high-intensity light, often from a laser, and
- (2) a plate with a pinhole aperture in front of the image detector.



Living neural crest cells growing in culture appear differently with various techniques of light microscopy. Here the *same field* of unstained cells, including two differentiating pigment cells, is shown using three different methods. **(a) Bright-field microscopy:** Without fixation and staining, only the two pigment cells can be seen.

(b) Phase-contrast microscopy: Cell boundaries, nuclei, and cytoplasmic structures with different refractive indices affect in-phase light differently and produce an image of these features in *all* the cells. **(c) Differential interference microscopy:** Cellular details are highlighted in a different manner using Nomarski optics. Phase-contrast microscopy, with or without differential interference, is widely used to observe live cells grown in tissue culture.