Introduction to Histology and Its Methods of Study

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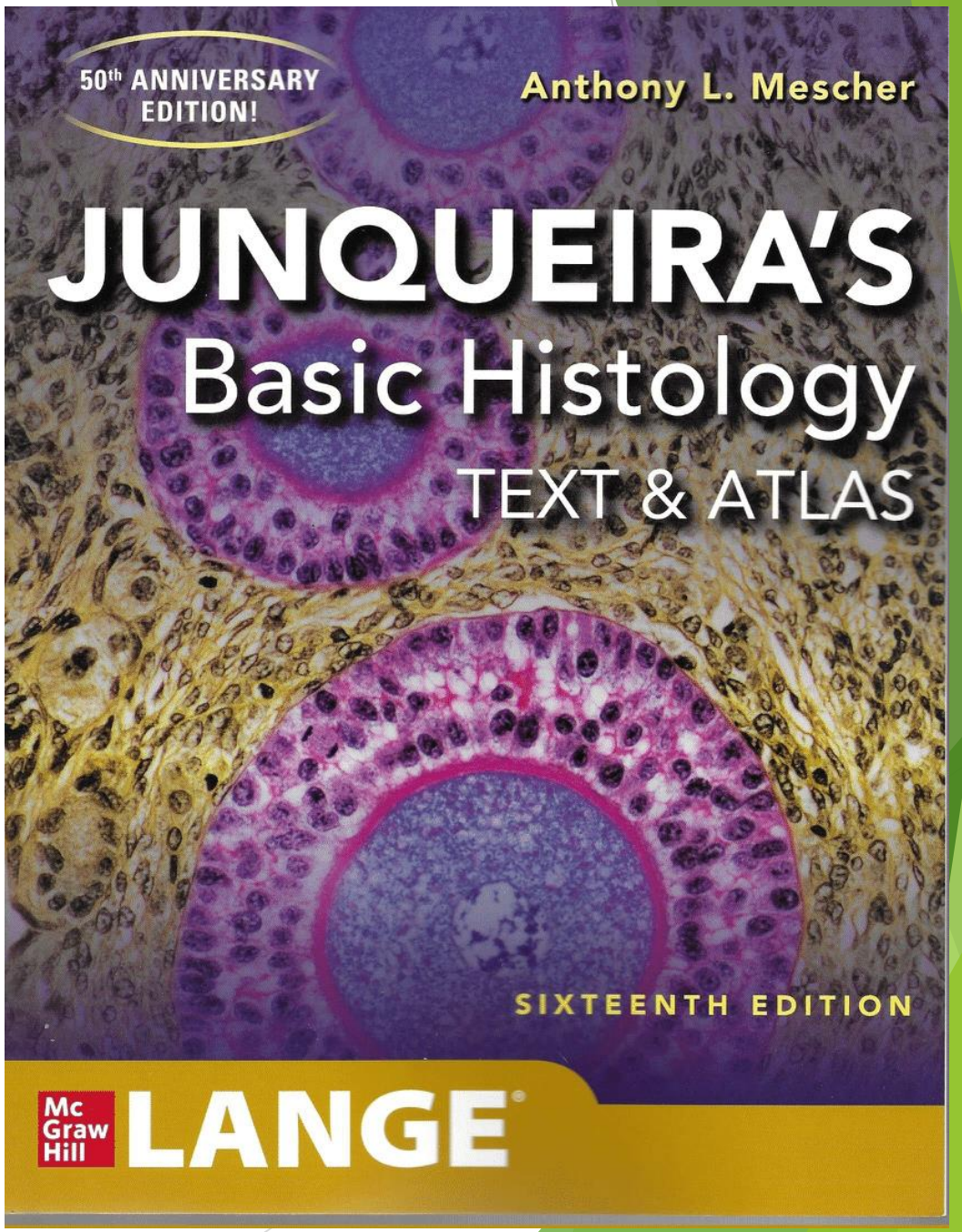
Histology Textbook

Junqueira's Basic Histology

Text and Atlas

16th edition

By Anthony L. Mescher



What is Histology?

- Histology is the study of the tissues of the body and how these tissues are arranged to constitute organs.
- This subject involves all aspects of tissue biology, with the focus on how cells' structure and arrangement optimize functions specific to each organ.

Levels of organization of the body

1-Chemical

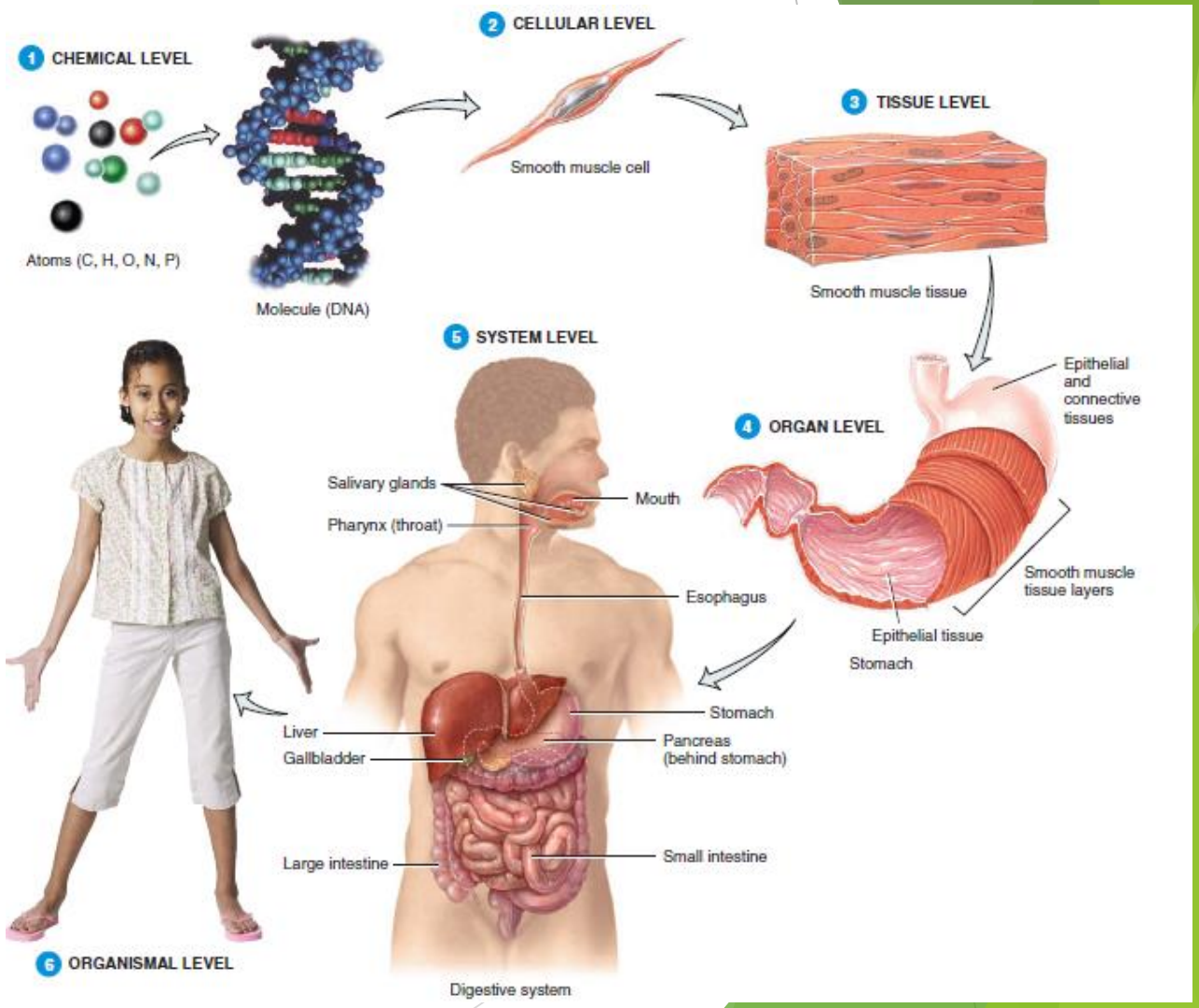
2-Cellular

3-Tissue

4-Organ

5-System

6-Organism



Some definitions….

- Cells: the basic structural and functional units of an organism
- Tissues: groups of cells and the materials surrounding them that work together to perform a particular function
- Organs are composed of two or more different types of tissues; they have specific functions and usually have recognizable shapes
- A system consists of related organs performing a function

General Arrangement of Tissues

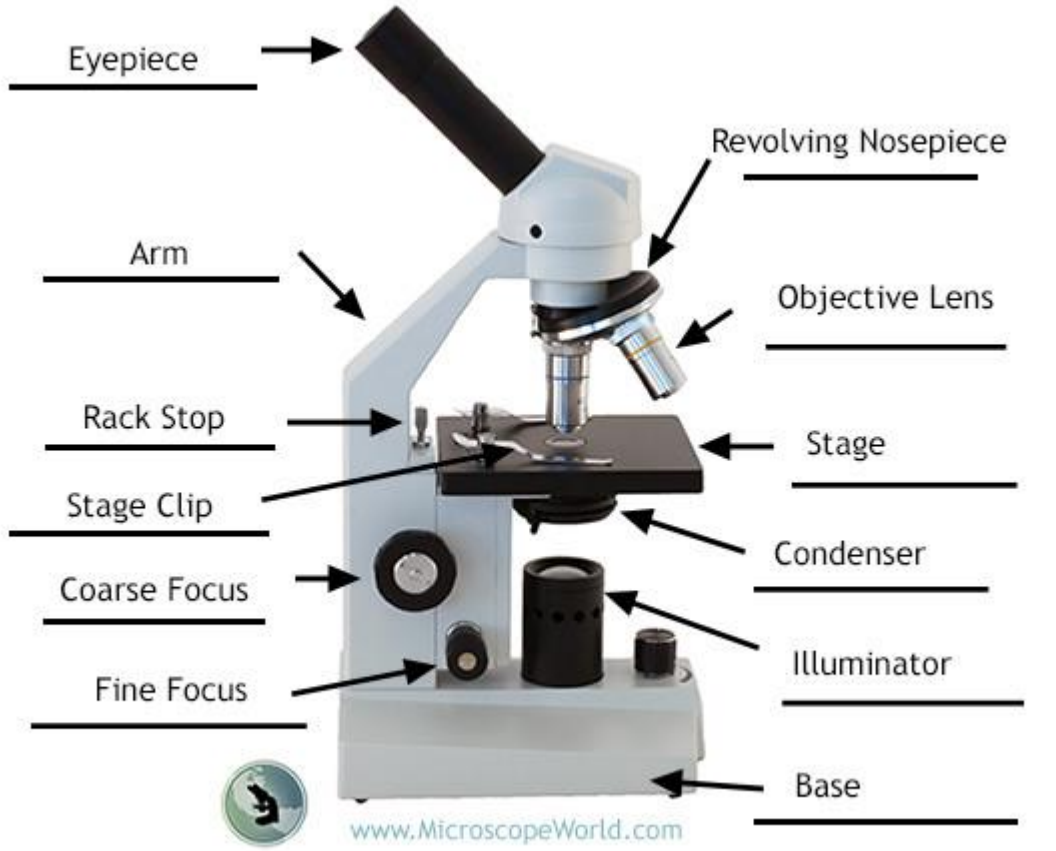
- Tissues have two interacting components: cells and extracellular matrix (ECM).
- The ECM consists of many kinds of macromolecules, most of which form complex structures, such as collagen fibrils.
- The ECM supports the cells and contains the fluid transporting nutrients to the cells, and carrying away their wastes and secretory products.
- Cells produce the ECM locally and are in turn strongly influenced by matrix molecules.

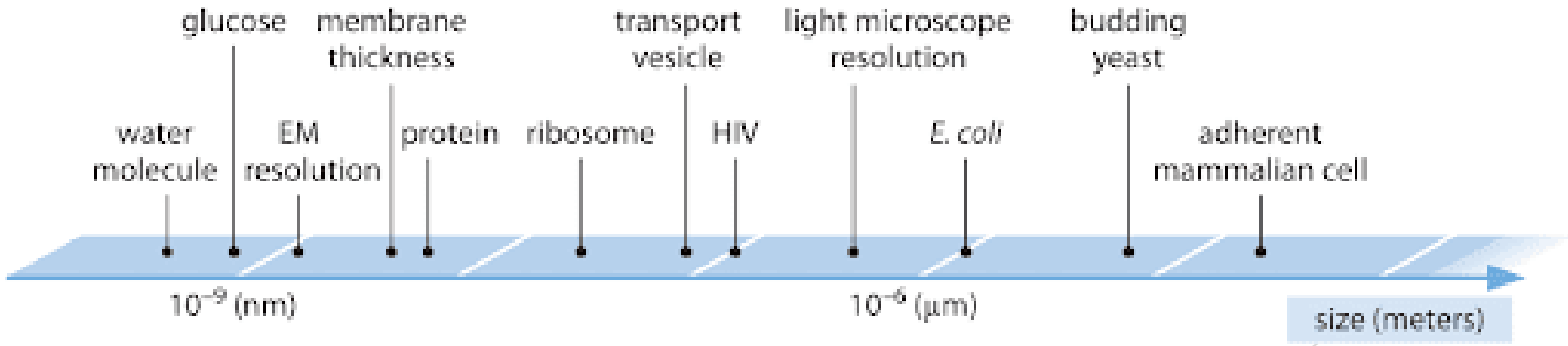
Basic tissues in our body

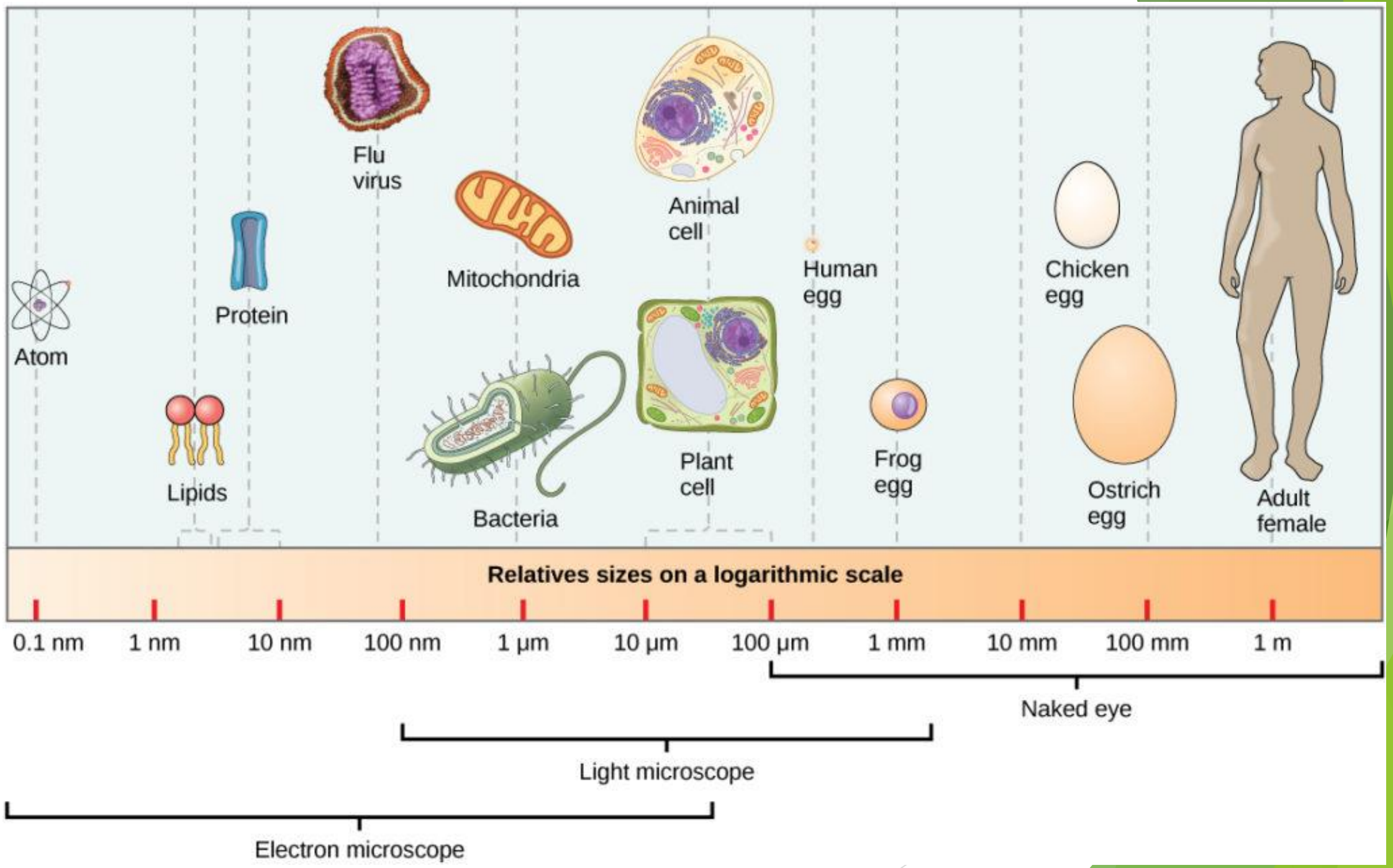
- Epithelial tissue
- Connective tissue
- Muscular tissue
- Nervous Tissue

How to study tissues?

- The small size of cells and matrix components makes histology dependent on the use of microscopes and molecular methods of study.



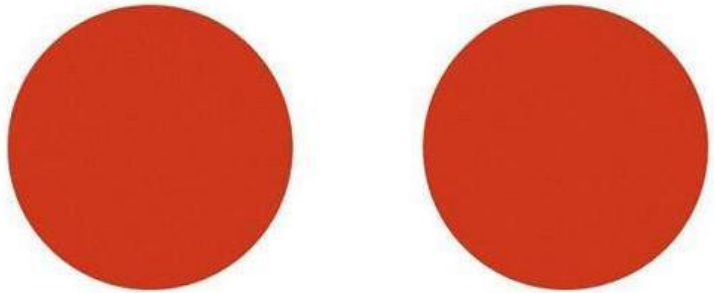



Flu virus
Animal cell
Mitochondria
Protein
Human egg
Chicken egg
Atom
Lipids
Bacteria
Plant cell
Frog egg
Ostrich egg
Adult female
Relatives sizes on a logarithmic scale
0.1 nm
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm
10 mm
100 mm
1 m
Naked eye
Light microscope
Electron microscope

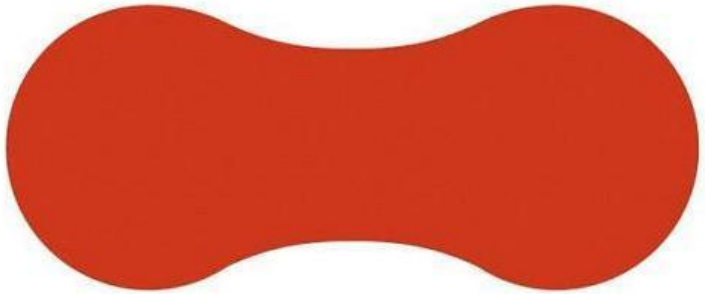
Terminology

- **Magnification**: is the degree to which a lens, mirror, or other device can **enlarge** an object, or the degree to which the object is **enlarged**.
- **Resolution**: is the **shortest** distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities. (dependent on wavelength "λ")
- **Contrast**: is the difference in light **intensity** between the image and the adjacent background relative to the overall background intensity

Resolution

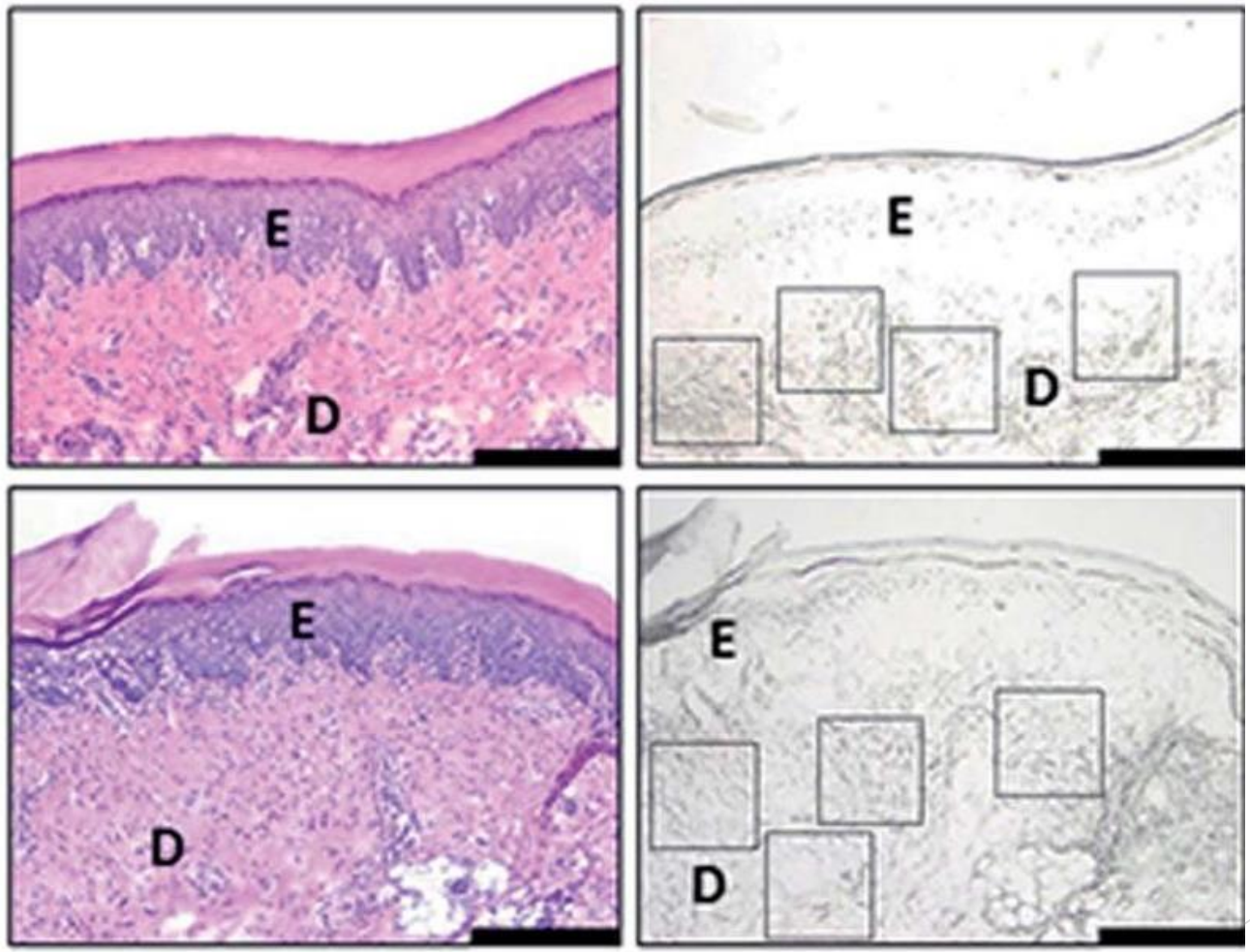


(a) The two dots are resolved--that is, they can clearly be seen as separate structures.



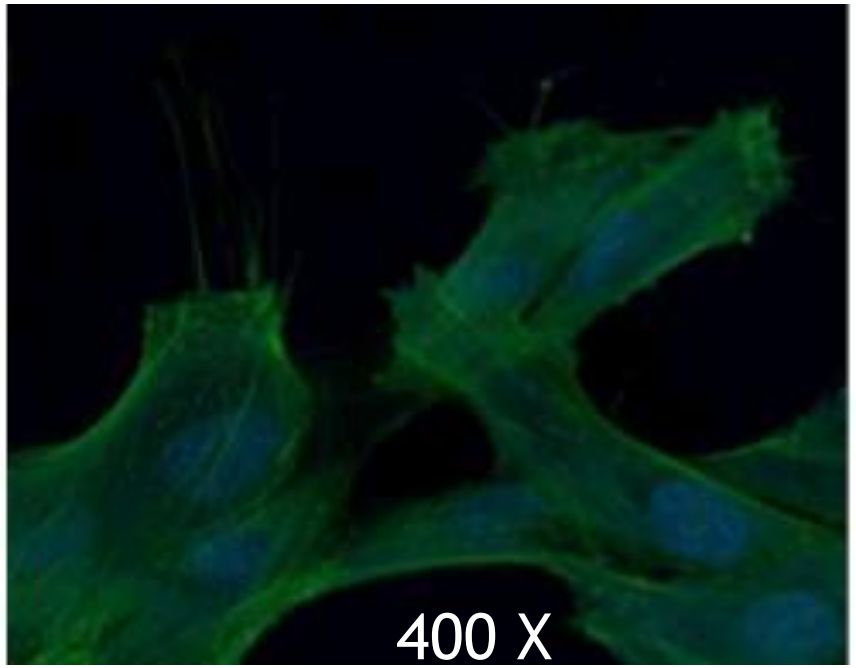
(b) These two dots are not resolved--they appear to be fused.

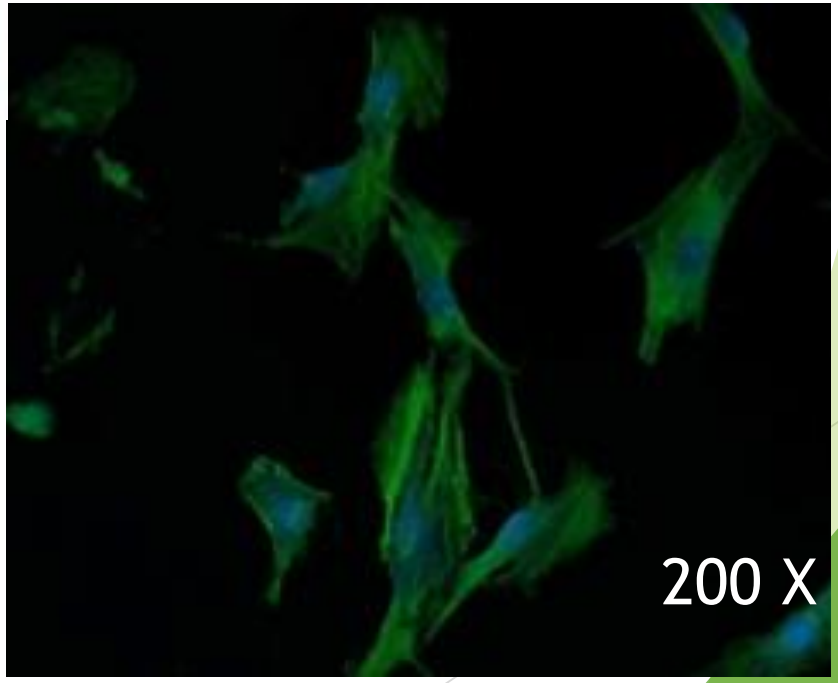
Contrast



Contrast

Magnification





Techniques used in histology

- Light microscopy
- Electron microscopy
- Immunohistochemistry

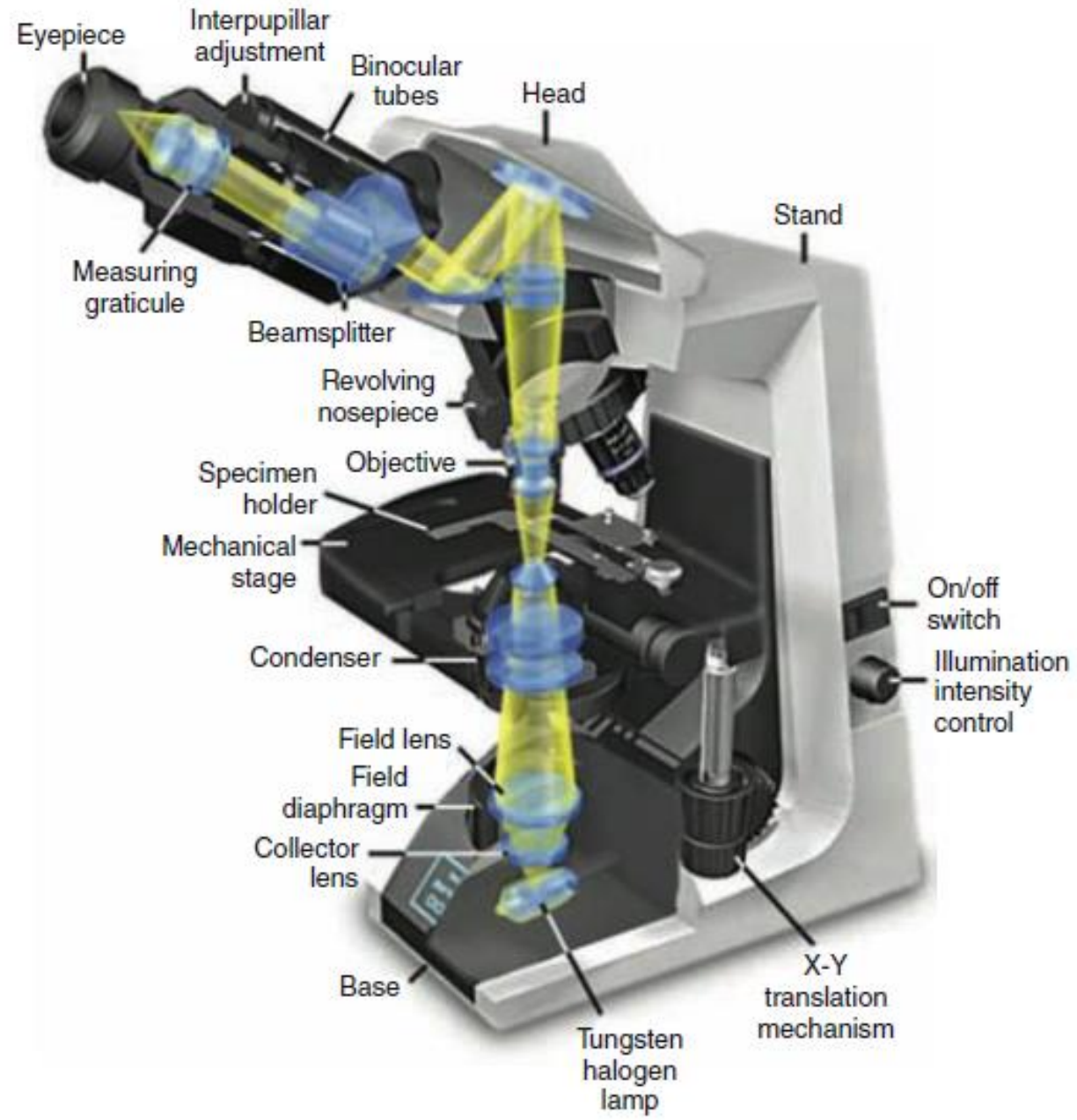
Light microscopy applications

- Bright-field microscopy
- Fluorescence microscopy
- Confocal microscopy
- Phase-contrast microscopy
- Polarizing microscopy

They are all based on the interaction of light with tissue components and are used to reveal and study tissue features.

Bright Field Light microscopy

- The basic functional unit consists of a tube; having an objective lens at one end and an ocular lens at the other
- The condenser focuses light on the object to be studied
- The objective lens enlarges the image of the object in the direction of the ocular lens
- The ocular lens further magnifies this image toward the observer’s eye
- The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses

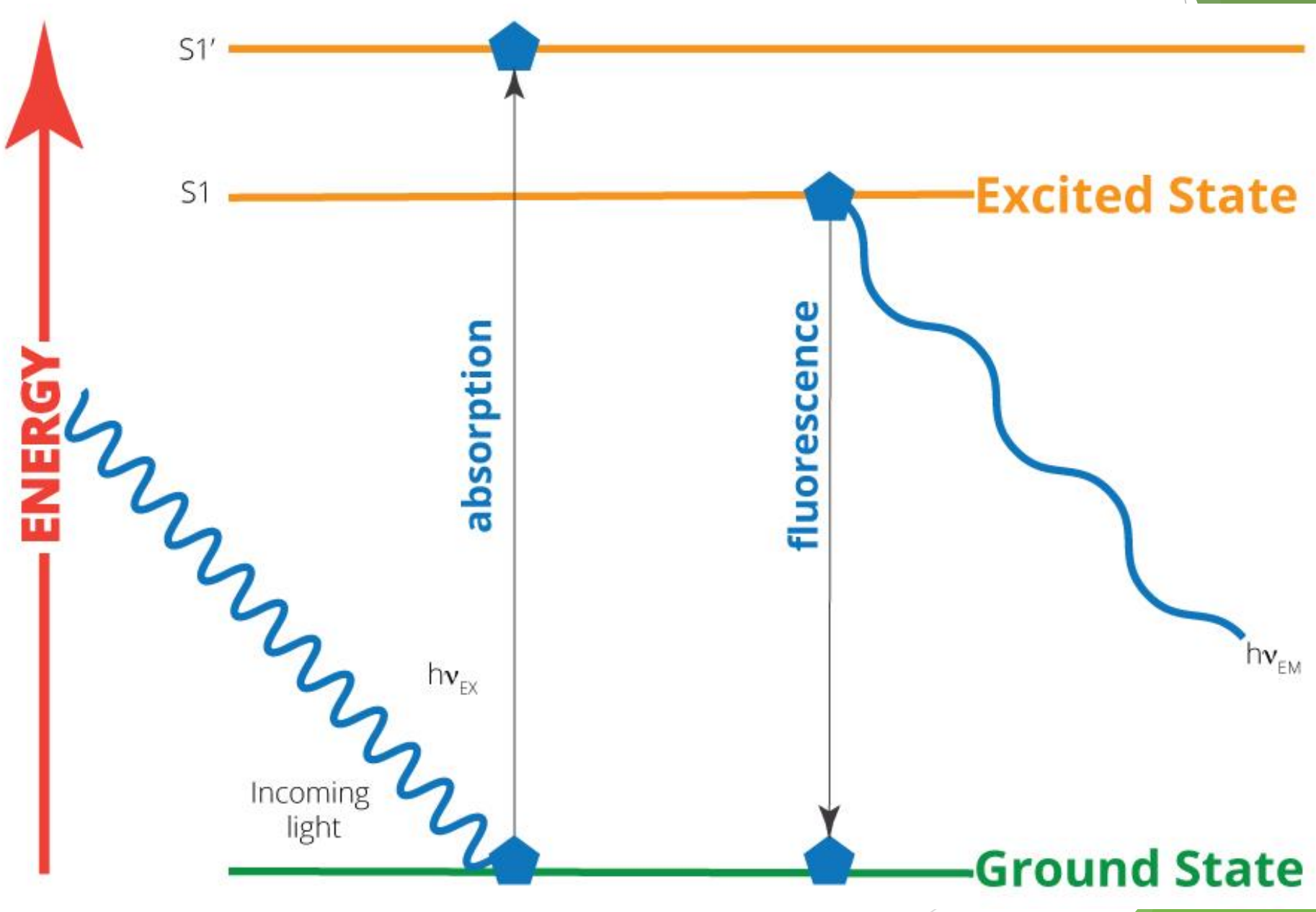

Eyepiece
Interpupillar adjustment
Binocular tubes
Head
Stand
Measuring graticule
Beamsplitter
Revolving nosepiece
Objective
Specimen holder
Mechanical stage
Condenser
On/off switch
Illumination intensity control
Field lens
Field diaphragm
Collector lens
Base
Tungsten halogen lamp
X-Y translation mechanism

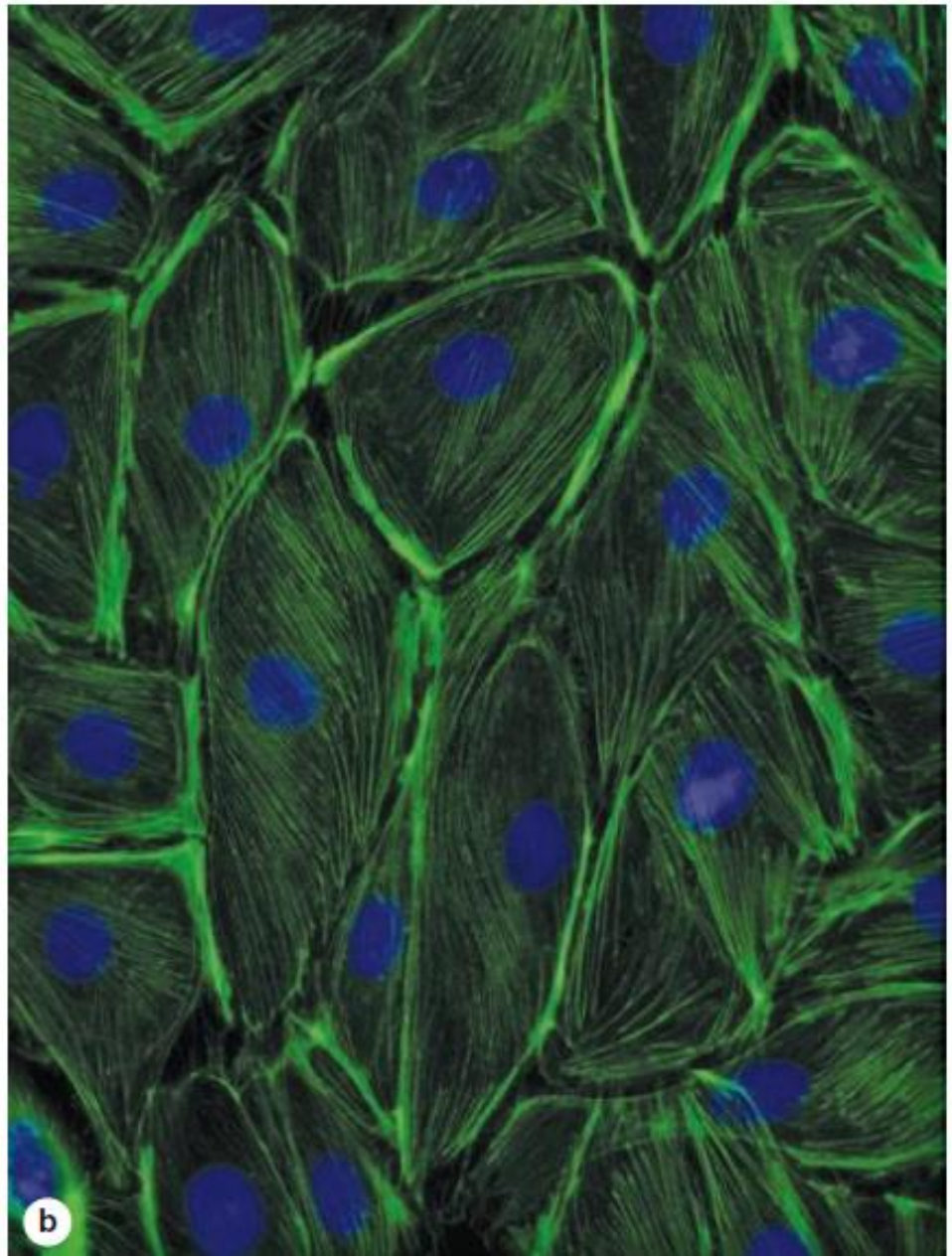
LM limits

- The critical factor in obtaining a crisp, detailed image with a light microscope is its resolving power, defined as the smallest distance between two structures at which they can be seen as separate objects.
- The maximal resolving power of the light microscope is approximately 0.2 μm, which can permit clear images magnified 1000-1500 times.
- Objects smaller or thinner than 0.2 μm (such as a single ribosome or cytoplasmic microfilament) cannot be distinguished with this instrument.

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 bright on a dark background.
- For fluorescent microscopy the instrument has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized.


S1'
S1
Excited State
ENERGY
absorption
fluorescence
hν EX
hν EM
Incoming
light
Ground State



Blue: DAPI stain (which binds DNA)

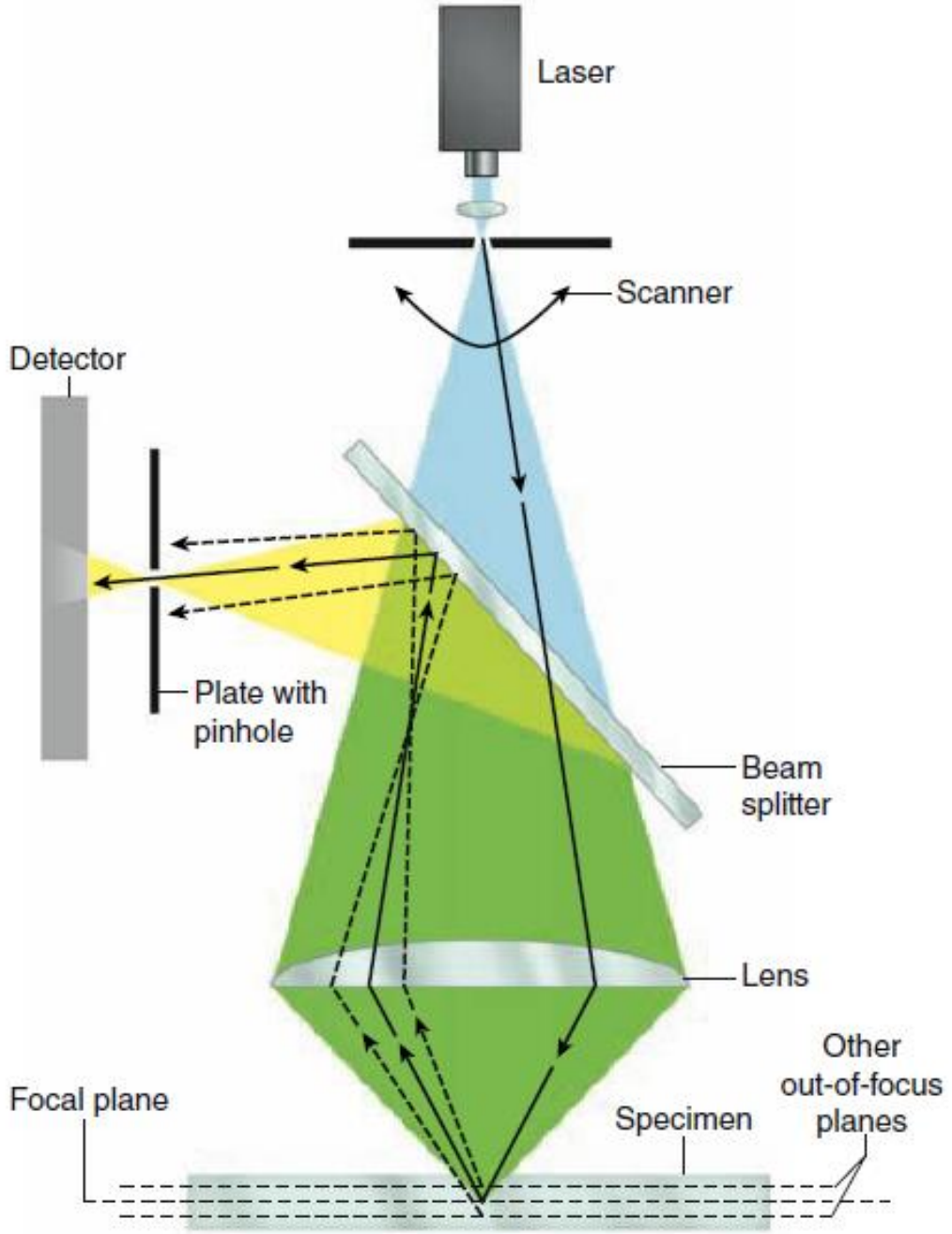
Green: fluorescein-phalloidin stain (which binds actin filaments)

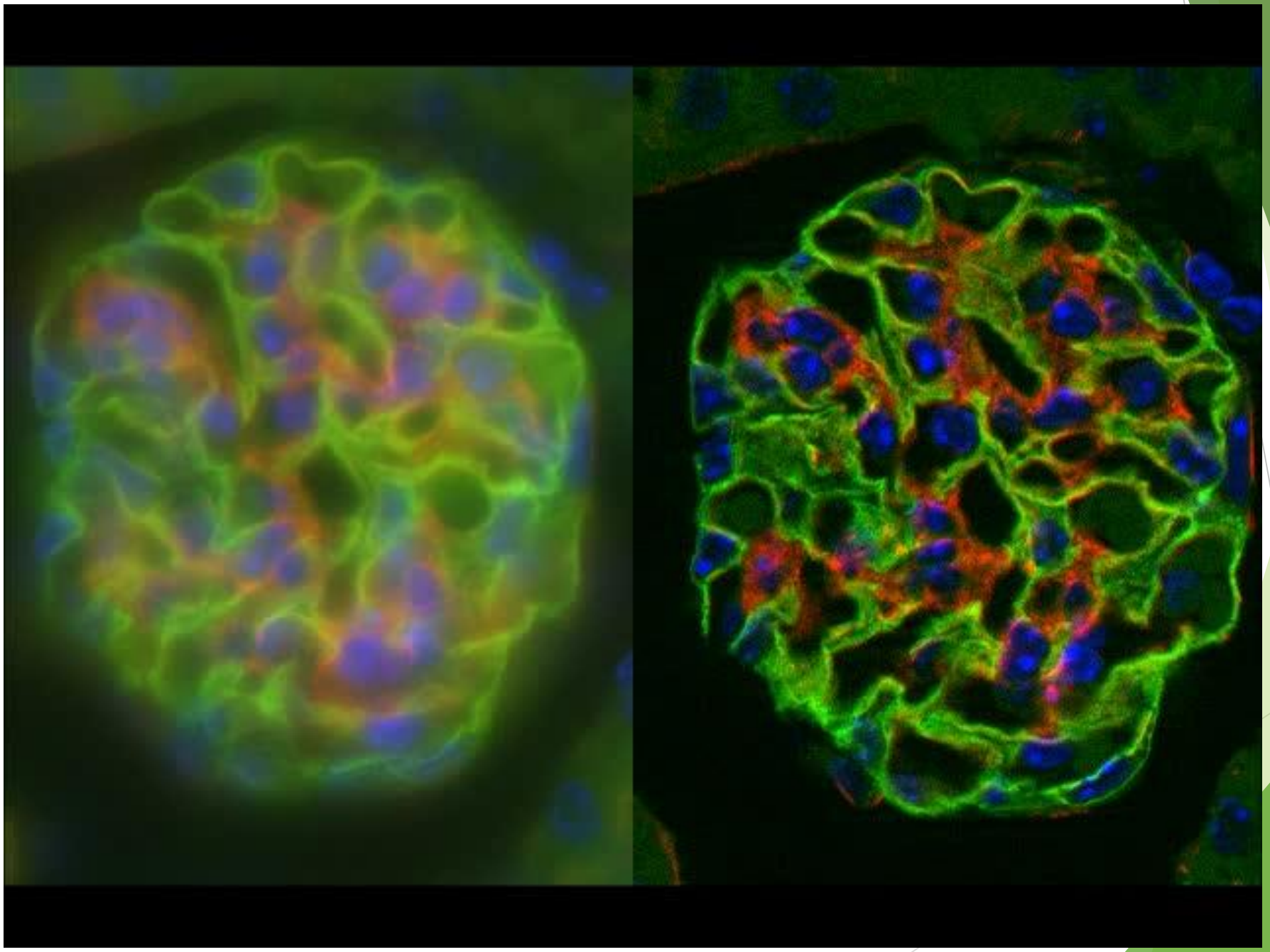
Confocal Microscopy

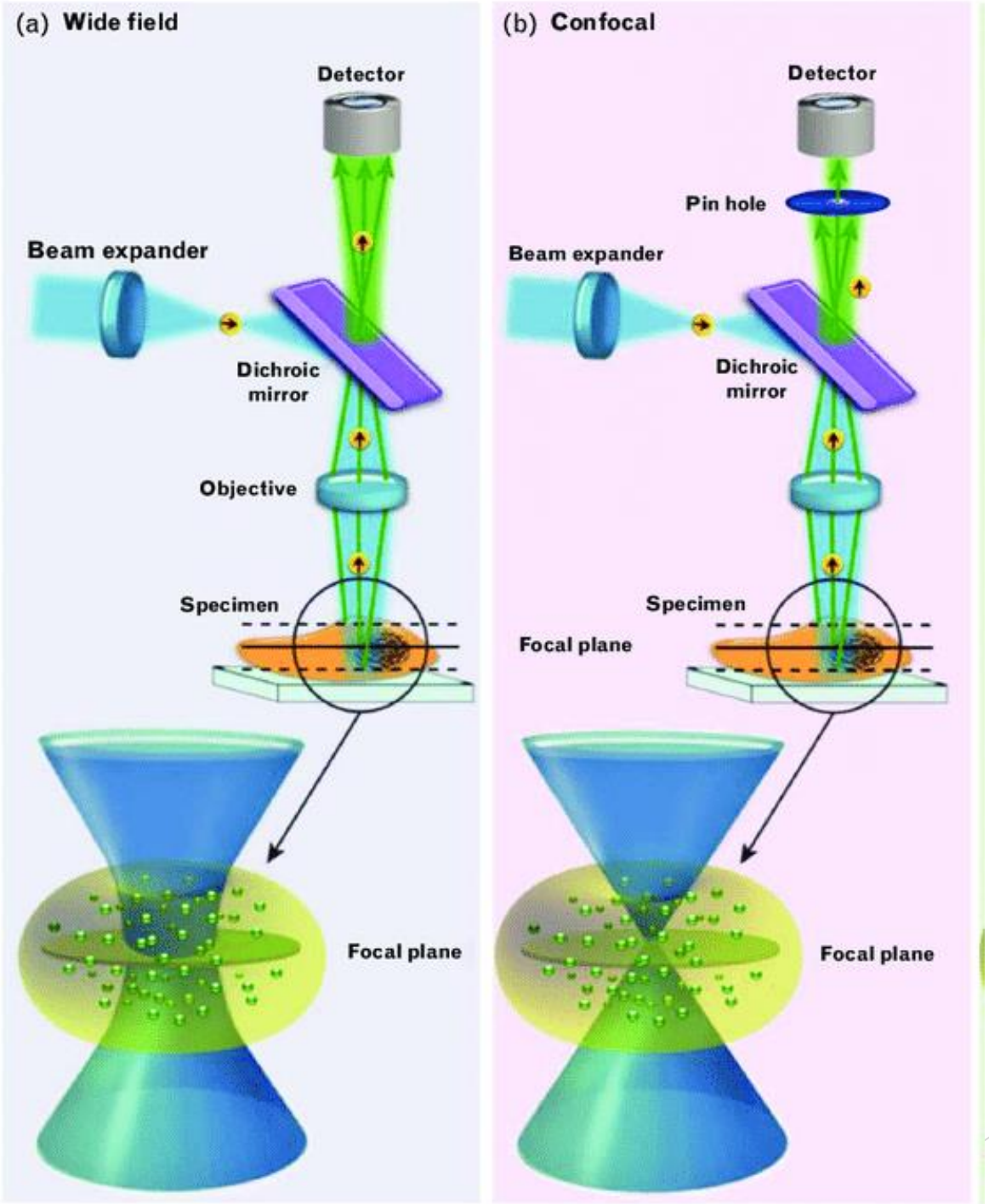
- Confocal microscopy achieves high resolution and sharp focus by using

  (1) a small point of high-intensity light, often from a laser

  (2) a plate with a pinhole aperture in front of the image detector.





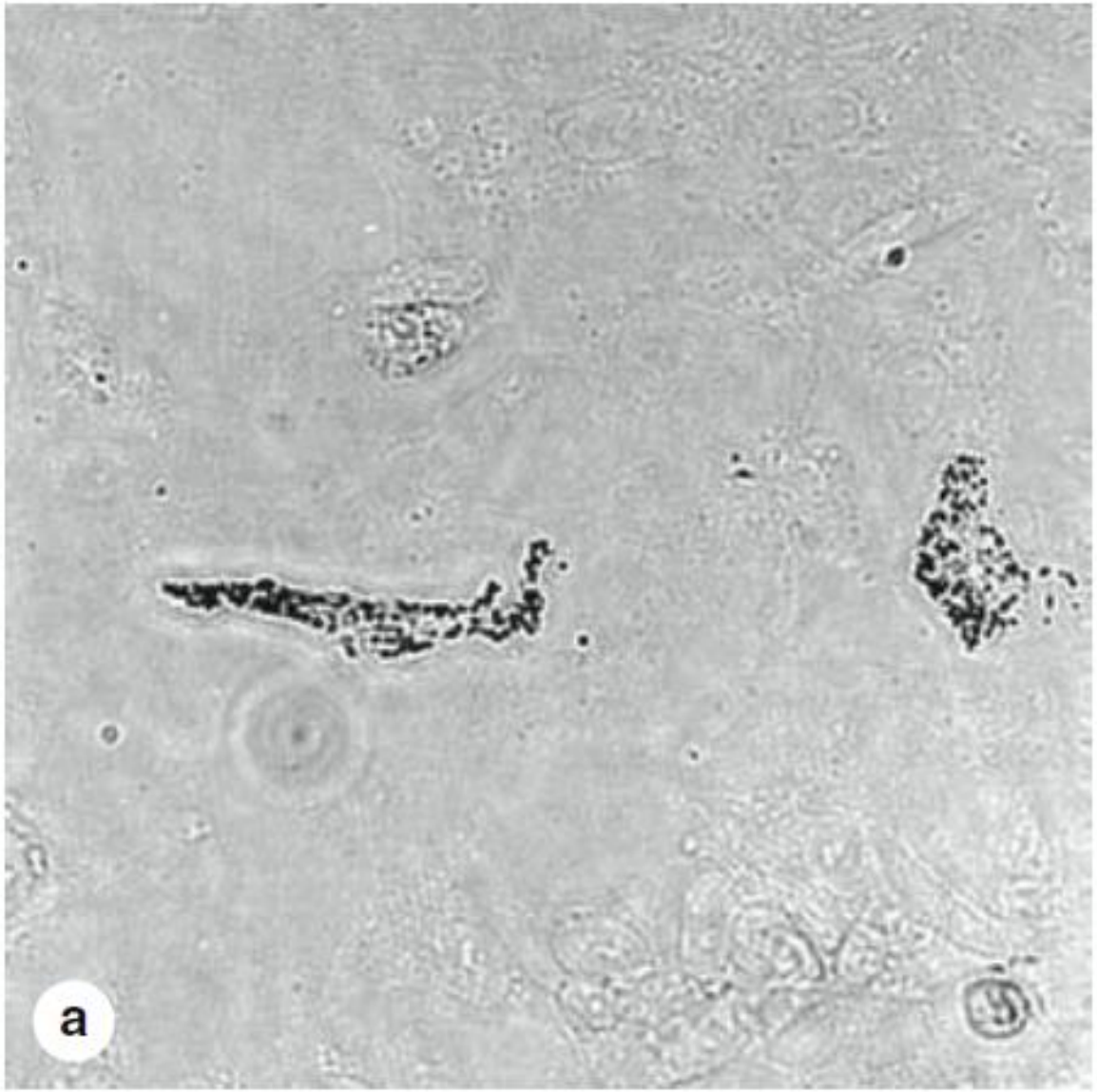

(a) Wide field
Detector
Beam expander
Dichroic mirror
Objective
Specimen
Focal plane
(b) Confocal
Detector
Pin hole
Beam expander
Dichroic mirror
Specimen
Focal plane
Focal plane

Phase Contrast Microscopy

- Unstained cells and tissue sections, which are usually transparent and colorless, can be studied with these modified light microscopes.
- Phase-contrast microscopy uses a lens system that produces visible images from transparent objects and, importantly, can be used with living, cultured cells

Principle of phase contrast microscopy

- Phase-contrast microscopy is based on the principle that light changes its speed when passing through cellular and extracellular 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.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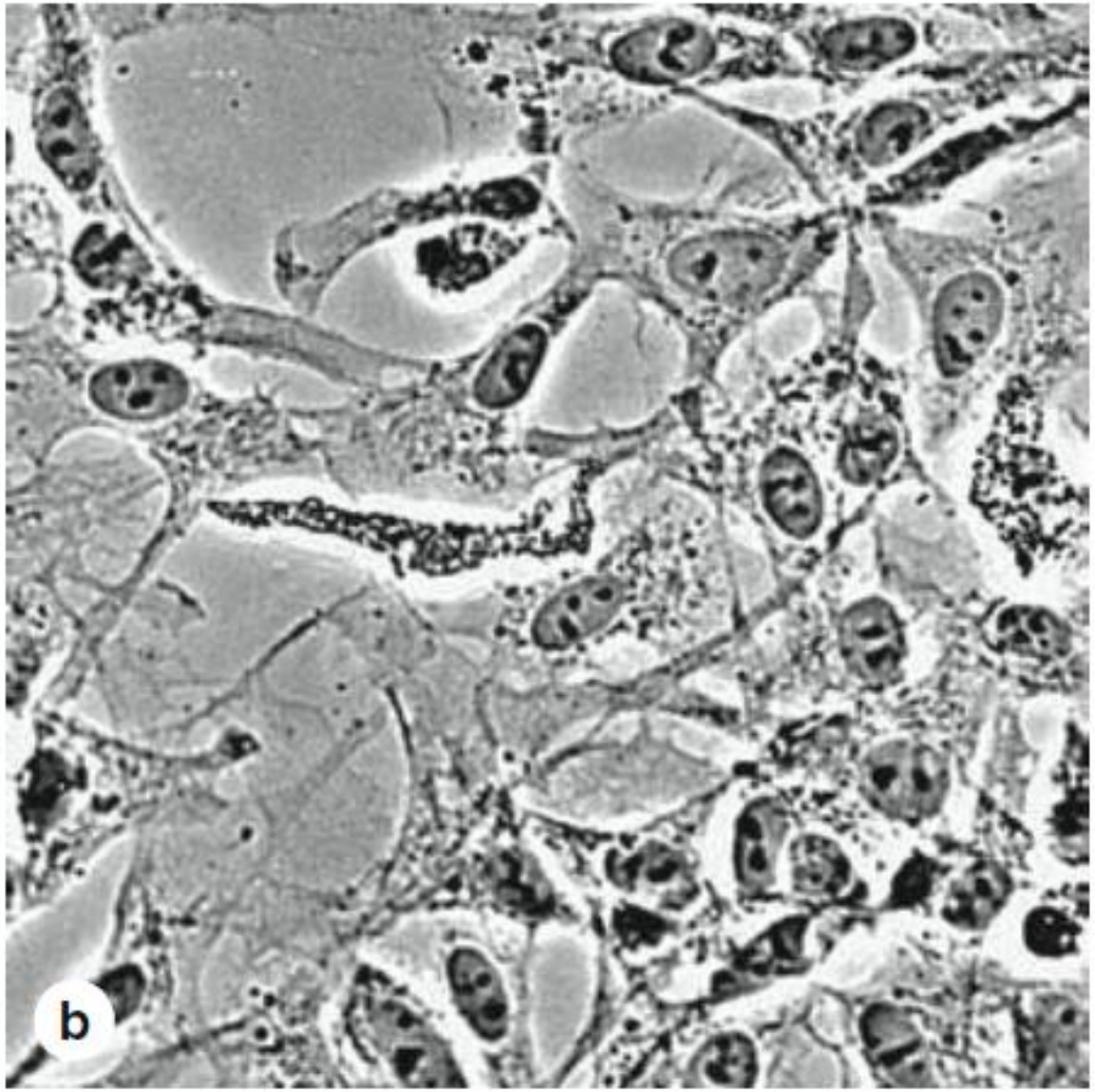
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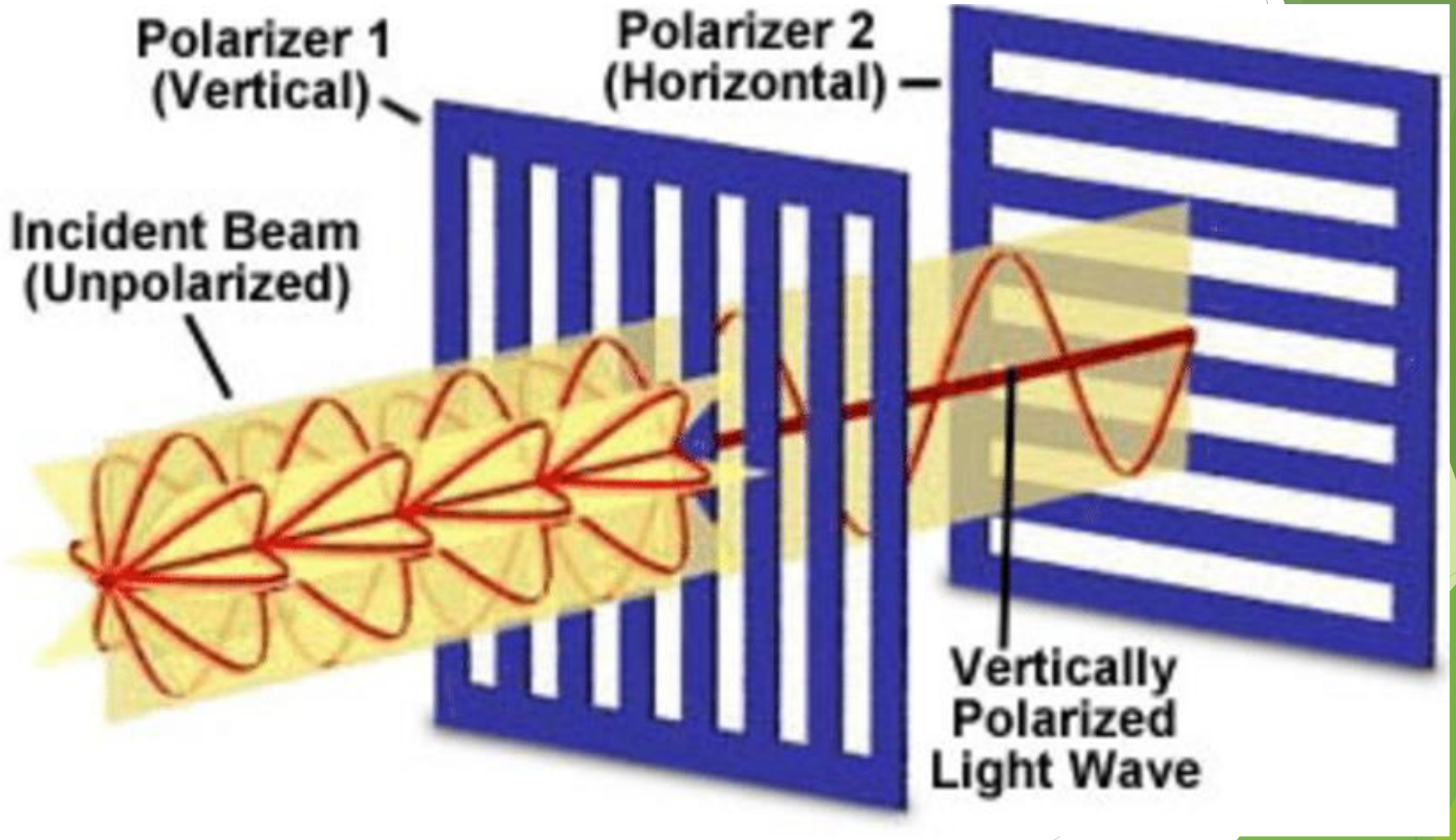
Polarizing Microscopy

- Polarizing microscopy allows the recognition of stained or unstained structures made of highly organized subunits.
- When normal light passes through a **polarizing** filter, it exits vibrating in only one direction. If a second filter is placed in the microscope above the first one, with its main axis perpendicular to the first filter, no light passes through.
- If, however, tissue structures containing oriented macromolecules are located between the two polarizing filters, their repetitive structure rotates the axis of the light emerging from the polarizer and they appear as bright structures against a dark background
- The ability to rotate the direction of vibration of polarized light is called **birefringence** and is a feature of crystalline substances or substances containing highly oriented molecules, such as cellulose, collagen, microtubules, and actin filaments.


Polarizer 1
(Vertical)
Polarizer 2
(Horizontal)
Incident Beam
(Unpolarized)
Vertically
Polarized
Light Wave

Electron Microscopy

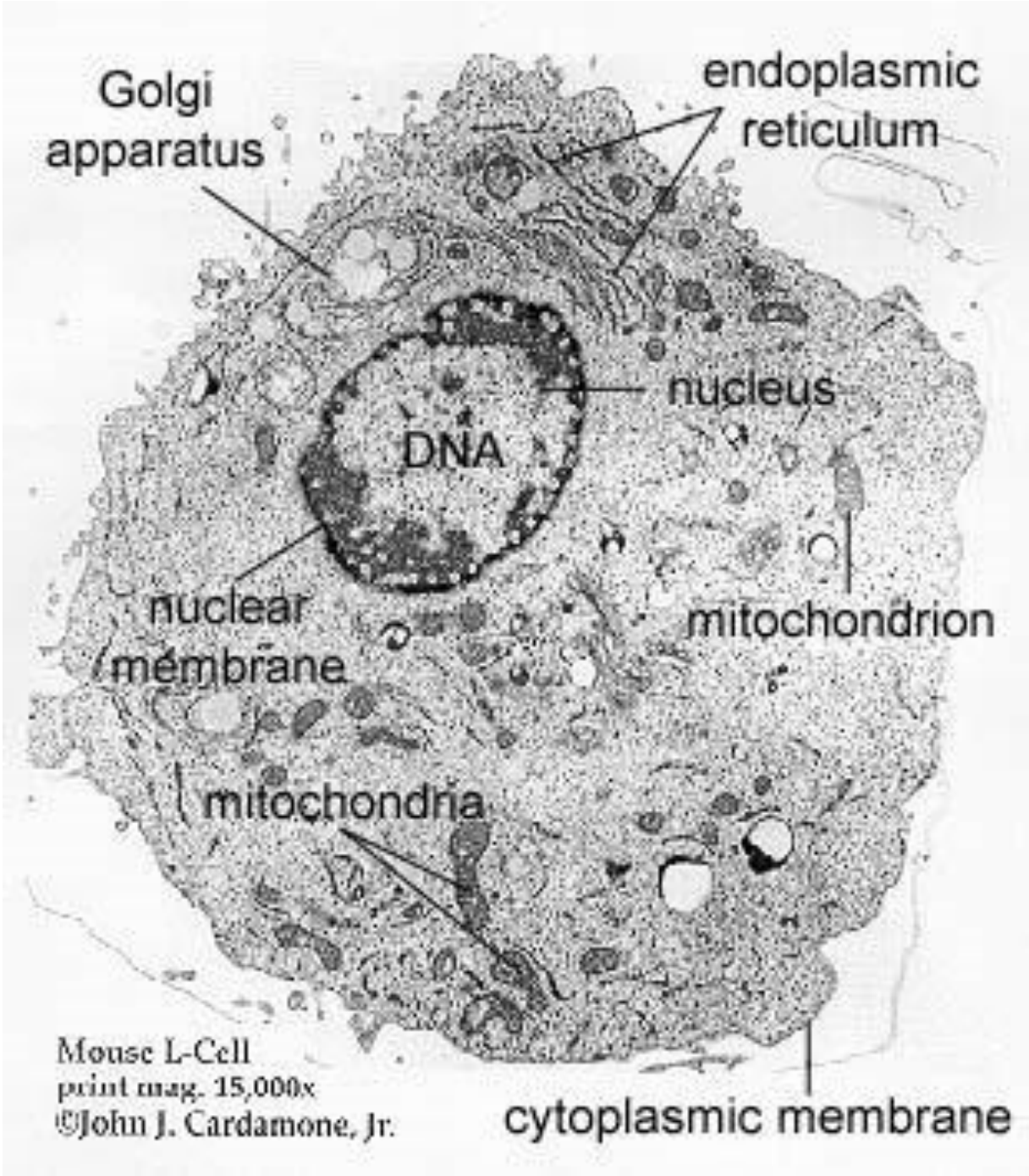
- **Transmission** and **scanning** electron microscopes are based on the interaction of tissue components with beams of electrons.
- The wavelength in an electron beam is much shorter than that of light, allowing a 1000-fold increase in resolution.

Transmission Electron Microscopy

- The **transmission electron microscope (TEM)** is an imaging system that permits resolution around 3 nm.
- This high resolution allows isolated particles magnified as much as 400,000 times to be viewed in detail.
- Very thin (40-90 nm), resin-embedded tissue sections are typically studied by TEM at magnifications up to approximately 120,000 times.

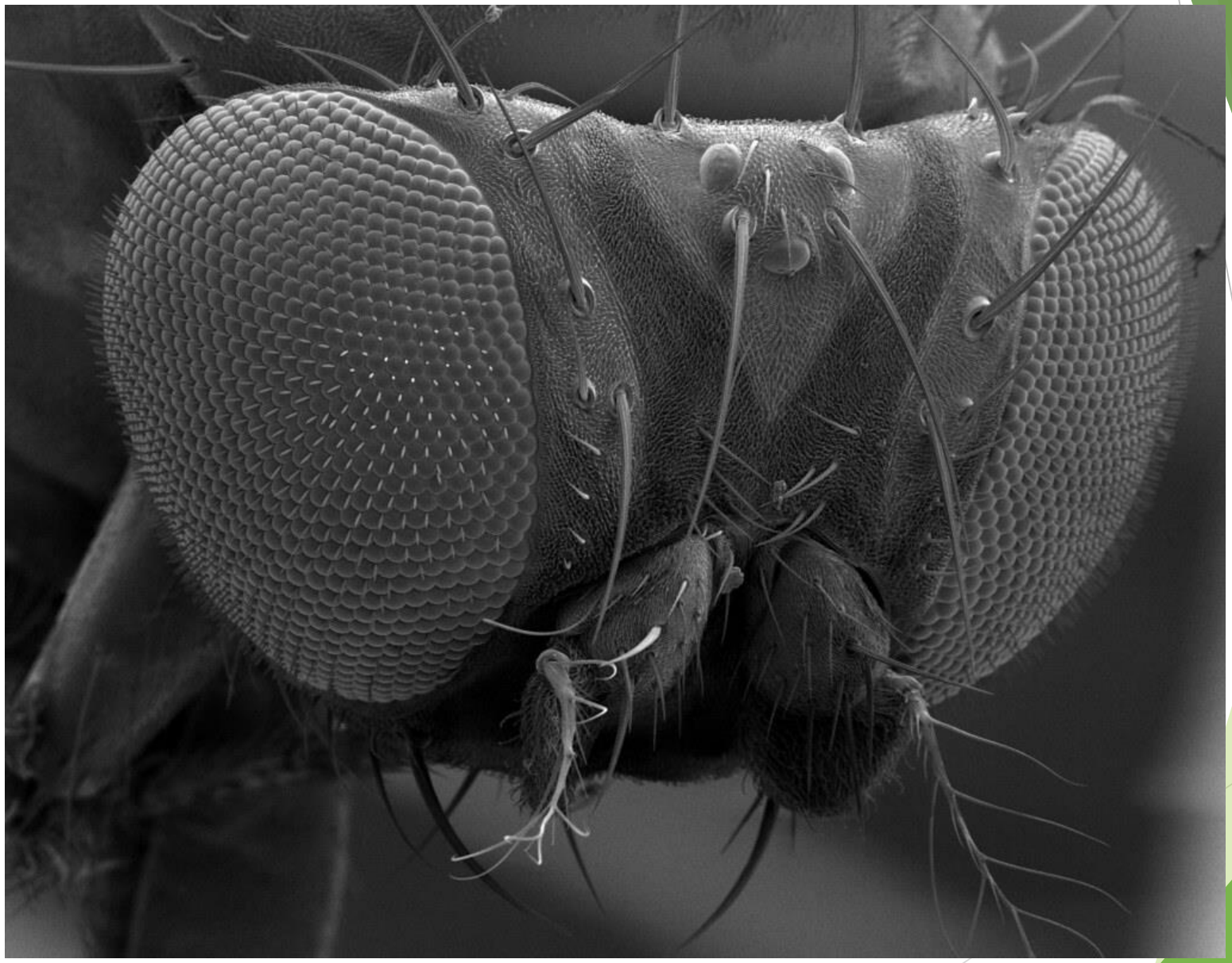
TEM principle

- the components of a TEM and the basic principles of its operation: a beam of electrons focused using electromagnetic “lenses” passes through the tissue section to produce an image with black, white, and intermediate shades of gray regions.
- These regions of an electron micrograph correspond to tissue areas through which electrons passed readily (appearing brighter or electron-lucent) and areas where electrons were absorbed or deflected (appearing darker or more electron-dense).


Golgi apparatus
endoplasmic reticulum
nucleus
DNA
nuclear membrane
mitochondrion
mitochondria
Mouse L-Cell
print mag. 15,000x
©John J. Cardamone, Jr.
cytoplasmic membrane

Scanning Electron Microscopy

- **Scanning electron microscopy (SEM)** provides a high resolution view of the surfaces of cells, tissues, and organs.
- Like the TEM, this microscope produces and focuses a very narrow beam of electrons, but in this instrument the beam does not pass through the specimen.
- Instead, the surface of the specimen is first dried and spray-coated with a very thin layer of heavy metal (often gold) which reflects electrons in a beam scanning the specimen.
- The reflected electrons are captured by a detector, producing signals that are processed to produce a black-and-white image.
- SEM images are usually easy to interpret because they present a three-dimensional view that appears to be illuminated in the same way that large objects are seen with highlights and shadows caused by light.




ERAPY
NTER
HV
10.00 kV
WD
10.1 mm
HFW
59.2 µm
det
ETD
mode
SE
mag
3 500 x
spot
4.5
tilt
52 °
curr
0.13 nA
10 µm
Versa 3D