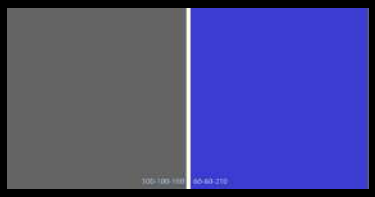
Introduction to microscopy and cell imaging techniques

Prof. Özgür Çınar, M.D.

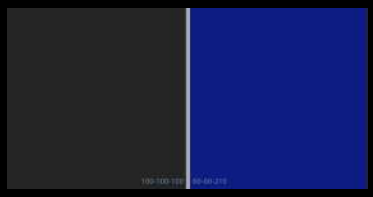
Can we see a cell with naked eyes?

A. Yes, we can
B. No, we can not
C. Sometimes
D. If the cell wants to be seen
E. If we have blue eyes

Factors affecting microscopic observations

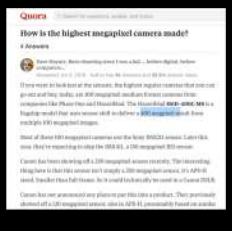


Factors affecting microscopic observations

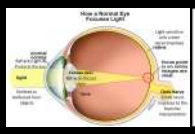


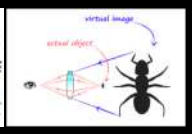
Factors affecting microscopic observations

Do we really need a microscope?



Magnification vs Resolution

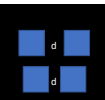




	Distance between resolvable points
Human eye	0.2 mm = 200.000 nm
Bright-field microscope	0.2 µm = 200 nm
SEM	2 nm
TEM - Theoretical	0.05 nm
TEM – Tissue section	1 nm
Atomic force microscopy	50 pm

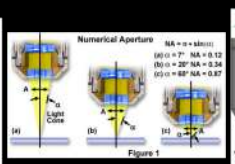
Resolution = d = r

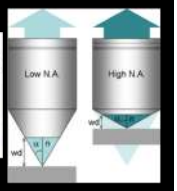
Resolution (r = d)



$$d = \frac{\lambda}{2.n.\sin\alpha} = \frac{\lambda}{N.A.obj + N.A.cond}$$

d = point-to-point distance of resolved detail (in nm),
λ = wavelength of light used
n = refractive index
NA = numerical aperture (or sine of half angle picked up by the objective or condenser of a central specimen point multiplied by the refractive index of the medium between objective or condenser and specimen.)

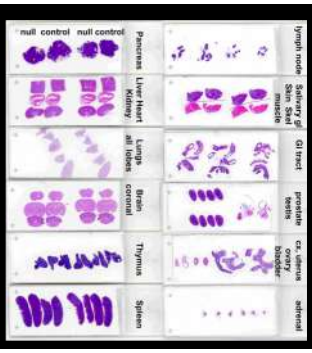




$$d = \frac{\lambda}{2.n.\sin\alpha} = \frac{\lambda}{N.A.obj + N.A.cond}$$

Anatomy of a microscope

Where are the specimens?



How to prepare a tissue section?

- Fixation
- Embedding
  - Cutting
- Staining

To preserve the structure: Fixation

- terminates cell metabolism,
- prevents enzymatic degradation and autolysis
- kills pathogenic microorganisms
- hardens the tissue as a result of either cross-linking or denaturing protein molecules

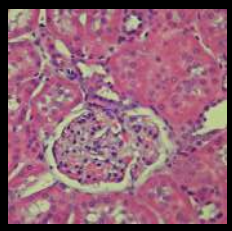
- Formaldehyde -> Formalin

Embedding

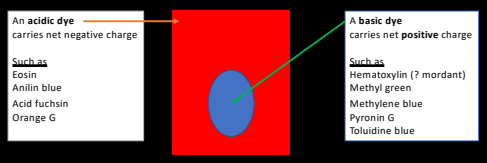
- Washing (washing)
- Dehydration (ascending concentration of alcohol)
- Clearing (Xylene)
- Paraffin
- Cutting/slicing by a microtome
- Mounting on a slide (egg white, acrylic resins)

Most popular dyes: hematoxylin-eosin

- Clearing (Xylene)
- Rehydration (descending concentration of alcohol)
- Washing (water)
- Staining



General approach to cell/tissue staining



What if you want to see something specific in the cell/tissue!

Homework…

- How to adjust a microscope for proper use? Köhler's illumination
- Metachromasia?
- What are the other microscopic techniques (like; fluorescent, confocal, electron, atomic force) and how do they work?
- If you want to analyze transcription, mRNA levels etc.!
- If you want to measure the quantity of protein!

Recommended references

- Histology: A Text and Atlas, Ross and Pawlina
- Histology and Cell Biology: An Introduction to Pathology, Kierszenbaum A.L
- Color Atlas and Text of Histology, Gartner LP
- Junqueira's Basic Histology: Text and Atlas, Mescher A
- Molecular Biology of the Cell, Alberts B
- The Cell: A Molecular Approach, Cooper GM
- *Anatomy & Physiology: The Unity of Form and Function, Saladin KS*