

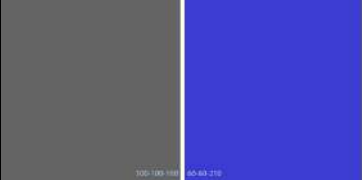
Introduction to microscopy
and cell imaging techniques

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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



100-100-100 50-50-250

Factors affecting microscopic observations



100-100-100 50-50-250

Factors affecting microscopic observations



Do we really need a microscope?



Quora · Question: How is the highest megapixel camera made?
4 Answers

Quora User, Retired Research Scientist, Ballistic Missile Defense Research Institute (1968–2014) · 100+ views · 100+ answers · 100+ views · 100+ views

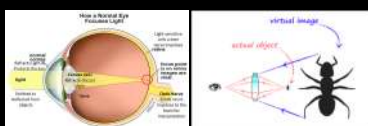
If you were to look out of the aperture, the highest angular resolution that can be seen and they would see 376 megapixel resolution. This resolution is the same as the resolution of the human eye. The resolution of the human eye is 376 megapixel. This resolution is the same as the resolution of the human eye.

Most of these 376 megapixel cameras use the Sony IMX225 sensor. This sensor has a resolution of 376 megapixel. This sensor has a resolution of 376 megapixel. This sensor has a resolution of 376 megapixel.

Can't see from taking of a 376 megapixel camera directly. The resolution of the camera is about the same as the resolution of the human eye. This is a good thing. This is a good thing. This is a good thing.

Can't see any more detail than you can see with a camera. This is a good thing. This is a good thing. This is a good thing.

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 = $d = r$



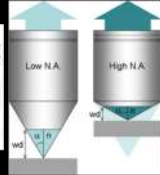
Resolution ($r = d$)

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

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.)






Numerical Aperture $NA = n \cdot \text{sin}(\alpha)$
 (a) $n = 1.7$, $\alpha = 60^\circ$, $NA = 0.92$
 (b) $n = 1.5$, $\alpha = 45^\circ$, $NA = 0.53$
 (c) $n = 1.5$, $\alpha = 60^\circ$, $NA = 0.87$



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

Anatomy of a microscope



Where are the specimens?

Pharynx	all tissues	Lungs	Brain	Thymus	Spleen	lymph nodes	Spleen	ovary	adrenal
control	control	control	control	control	control	control	control	control	control

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

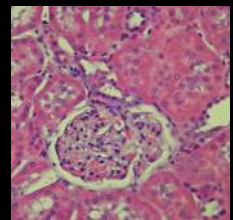
- Formaldehyde -> Formalin

Embedding

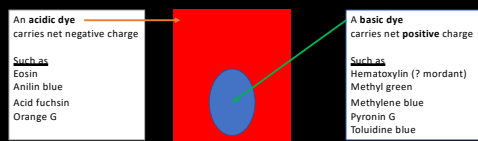
- Washing (washing)
- Dehydration (ascending concentration of alcohol)
- Clearing (Xylene)
- Paraffin
- Cutting/slicing by a microtome
- Mounting on a slide (egg white, acrylic resins)

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