Lecture 1

Microscopy in Forensics

Microscopy in Forensic Science

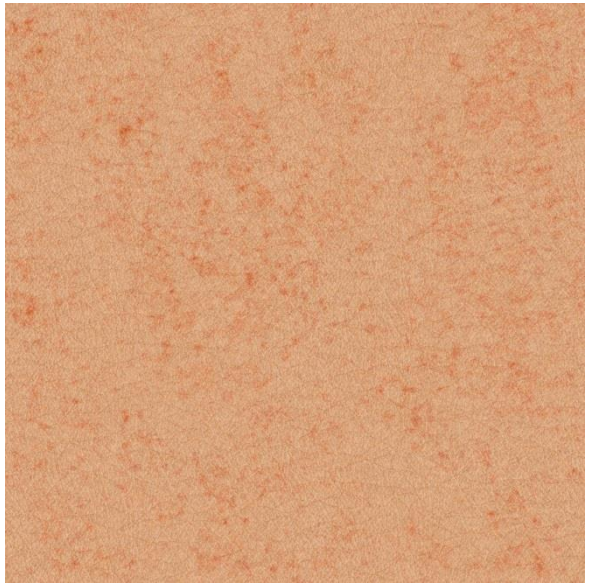
Microscopy in Forensics: References

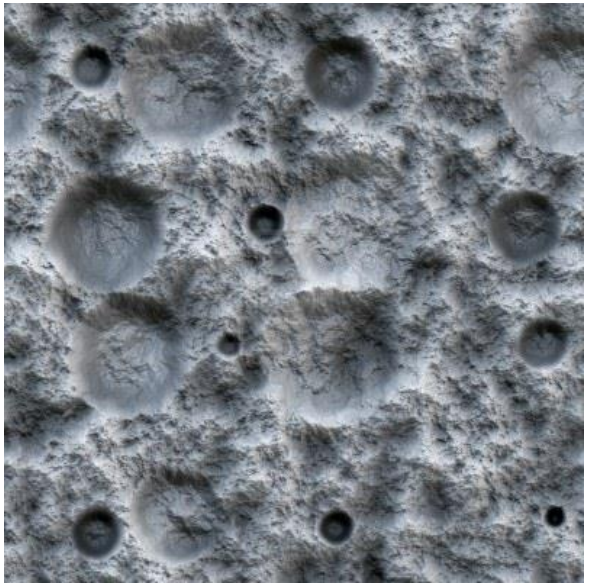
1. *Color Atlas and Manual of Microscopy for Criminalists, Chemists, and Conservators* (N. Petraco and T. Kubic), CRC Press
2. *Physics (any textbooks are okay)*
3. *Optics* (E. Hecht), 4th ed. Addison Wesley Press
4. *Forensic Science Handbook* (Ed. R. Saferstein), Volume 1, Prentice Hall
    1. Chapter 5: Foundations of forensic microscopy
    2. Chapter 6: Visible microscopical spectrometry in the forensic sciences
    3. Chapter 7: The forensic identification and association of human hair
5. *Forensic Science Handbook* (Ed. R. Saferstein), Volume 2, Prentice Hall
    - Chapter 5: Microscopy and microchemstry of physical evidence
    - Chapter 6: an introduction to the forensic aspects of textile fiber examination
6. *Forensic Examination of Fibers* (ed. J. Robertson and M. Grieve), CRC Press
    - Chapter 1: Classification of textile Fibers
    - Chapter 2: The structure of Textiles
    - Chapter 7: Microscopical Examination of Fibers
    - Chapter 9.2: Scanning electron microscopy and elemental analysis
7. *Forensic Examination of Hair* (ed. J. Robertson), Taylor & Francis
    - Chapter 1: Physiology and growth of human hair
    - Chapter 2: Forensic and microscopic examination of human hair
8. *Forensic Examination of Glass and Paint* (ed. B. Caddy), Taylor & Francis
    - Chapter 1: What is trace evidence?
    - Chapter 3: Microscopic techniques for glass examination
    - Chapter 8: The role of color and microscopic techniques for the characterization of paint fragments
    - Chapter 12: SEM/EDS for forensic examination of paints and coatings
9. *Physical Evidence in Forensic Science*, (eds. H. C. Lee and H. A. Harris), Lawyers & Judge
    - Fibers (page 125) and Hair (page 173)

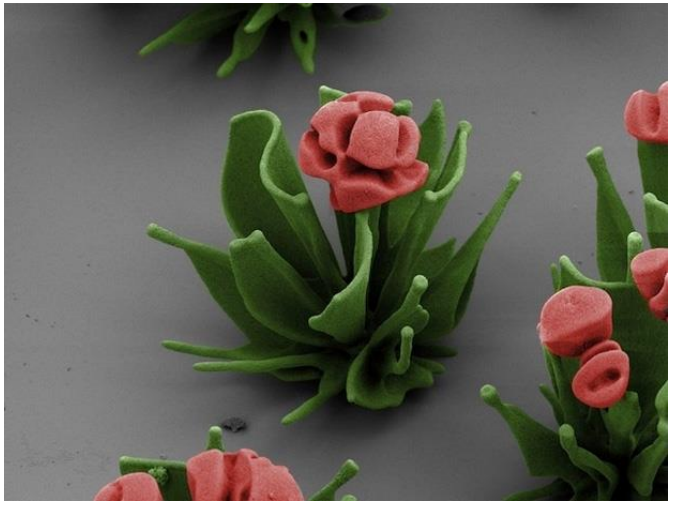
The Scope of Microscopy in Forensic Science

- Preliminary, routine, and easily accessible investigating tool.
- Characterization, identification, and comparison of any physical evidences
- Various samples: drug, paint, soil, minerals, dusts, glass, polymers, fibers (synthetic and natural), paper, starches, wood, hairs, pollens, etc
- Limited chemical information: polarized light source, fluorescence labeling, coupled with infrared spectroscopy, and microprobe analysis (electron microscopy)

What are These Images?







http://petapixel.com/2013/05/17/scientist-creates-and-snaps-photographs-of-microscopic-crystal-flowers/

Applications in Forensic Science

- Investigation for any physical evidences
  1. Fiber and hair investigations
  2. Document examination
  3. Tool mark comparison
  4. Firearm investigation
  5. Serology (scientific study of blood serum)
  6. Drug chemistry
  7. Trace evidence, etc

- Goals
  1. Routine instrument for most preliminary examinations and evaluations
  2. Major tool in hair and fiber investigations

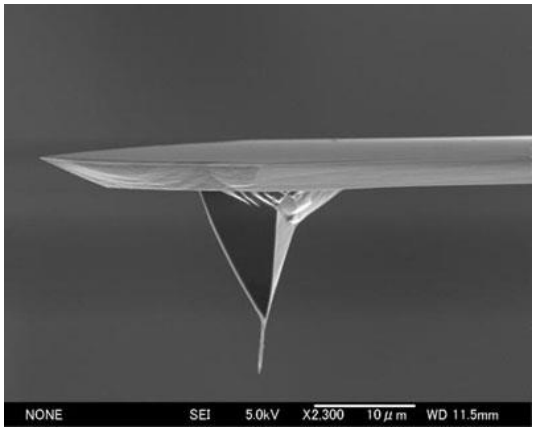
Varieties of Microscopy

- Optical microscopy (OM)
  1. Visible light (400 – 700 nm)
  2. Resolution: ~0.2 μm

- Electron microscopy (EM)
  1. Electron beam
  2. Resolution: ~0.05 nm

- Scanning probe microscopy (SPM)
  1. Probe tips or current or laser, etc
  2. Resolution: ~0.05 nm

probe tip



http://en.wikipedia.org/wiki/Microscopy

Differences in Various Microscopy

	Optical	EM	SPM
Source	visible light	electron beam	probes (tips, current, etc)
Probing	Contrast (differences in light intensities)	Contrast in electron densities (electron-sample interaction)	force (AFM) or current (STM) or laser (SNOM) between tips and sample
Resolution*	0.2 μm	0.05 nm	0.05 nm
Experimental environments	STP or cryogenic	STP or UHV (RT or cryogenic)	STP or UHV (RT or cryogenic)

STP: standard conditions for temperature and pressure (20 °C and 1 atm)

Optical Microscope

1. Employ light source
2. Two lenses: objective and eyepiece
3. Conventional microscope (Compound microscope)
4. Major variants
   - Biological vs. metallurgical microscope: transmitted light illumination or epi-illumination
   - Upright vs. inverted microscope:
   - Dark field, bright field, vs. phase-contrast microscope
   - Plan vs. polarized light microscope: polarized light
   - Stereo microscope: 3-dimensional view
   - Comparison microscope

Electron Microscopy

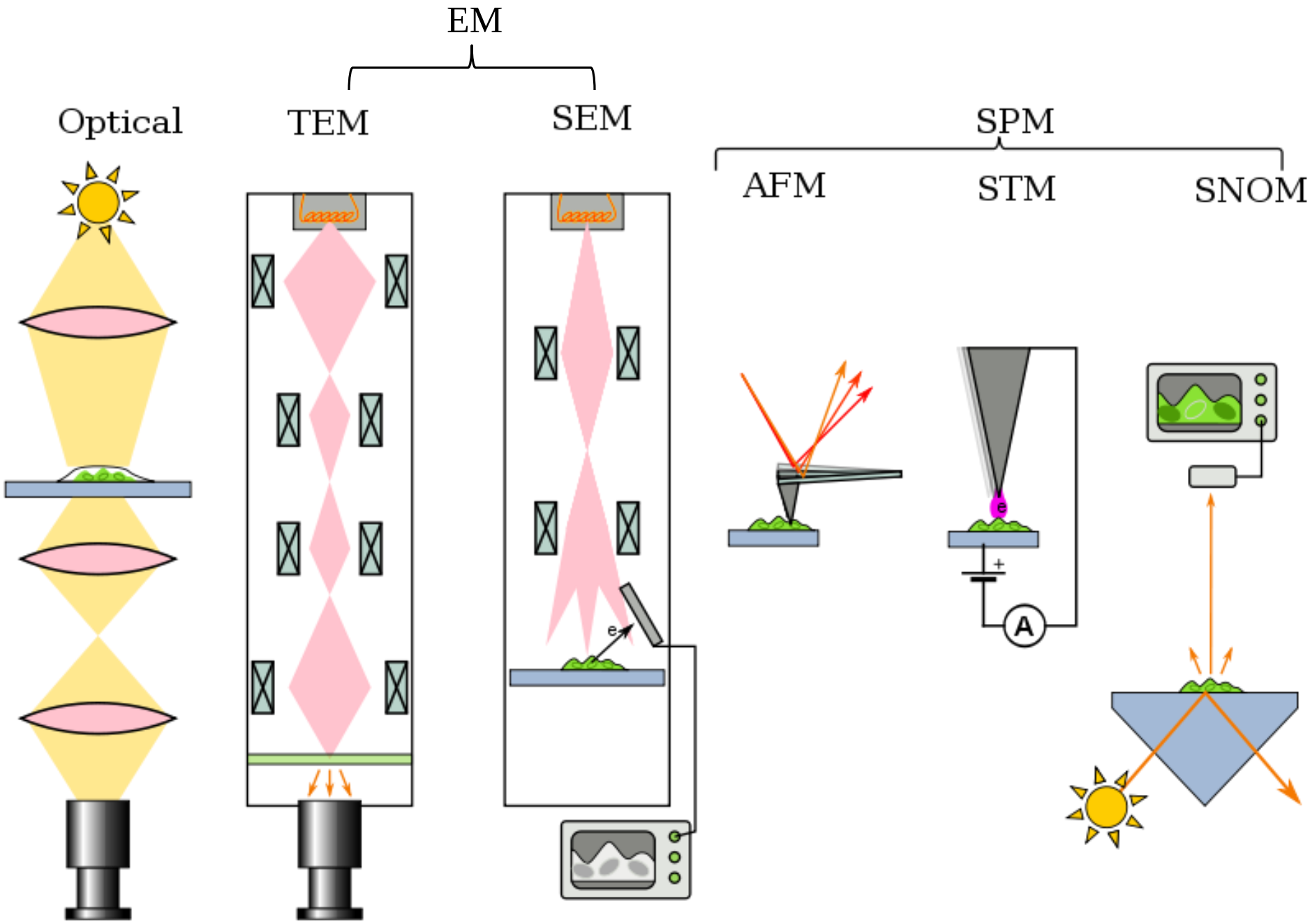
1. Scanning electron microscopy (SEM)
2. Tunneling electron microscopy (TEM)
3. Reflection electron microscopy (REM)
4. Scanning transmission electron microscopy (STEM)
5. Microprobe analysis
   - Energy dispersive x-ray analysis (EDX)
   - Wave dispersive x-ray analysis (WDS)

http://en.wikipedia.org/wiki/Electron_Microscopy

Scanning Probe Microscopy (SPM)

1. Atomic force microscopy (AFM)
2. Scanning tunneling microscopy (STM)
3. Scanning capacitance microscopy (SCM)
4. Scanning Near-field optical microscopy (SNOM)

http://en.wikipedia.org/wiki/Scanning_probe_microscopy


EM
Optical
TEM
SEM
SPM
AFM
STM
SNOM
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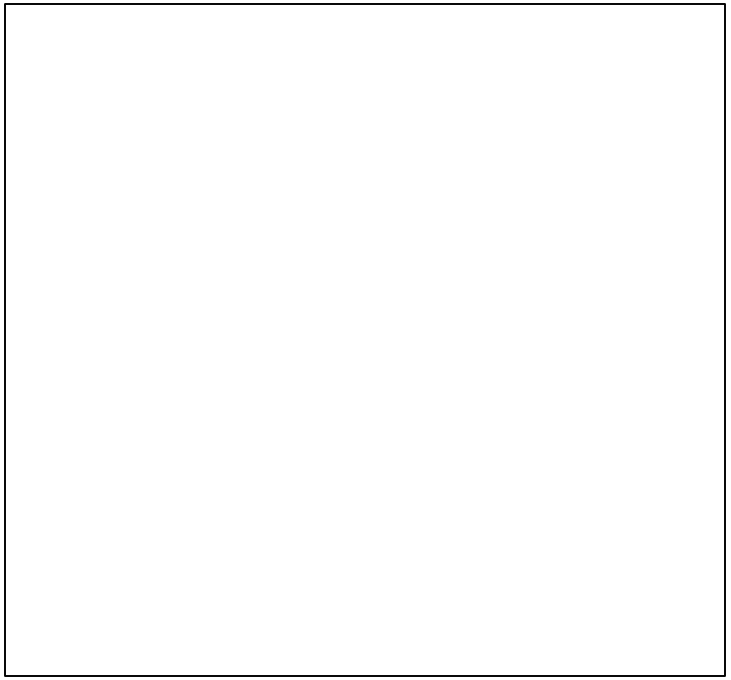
Lecture 2

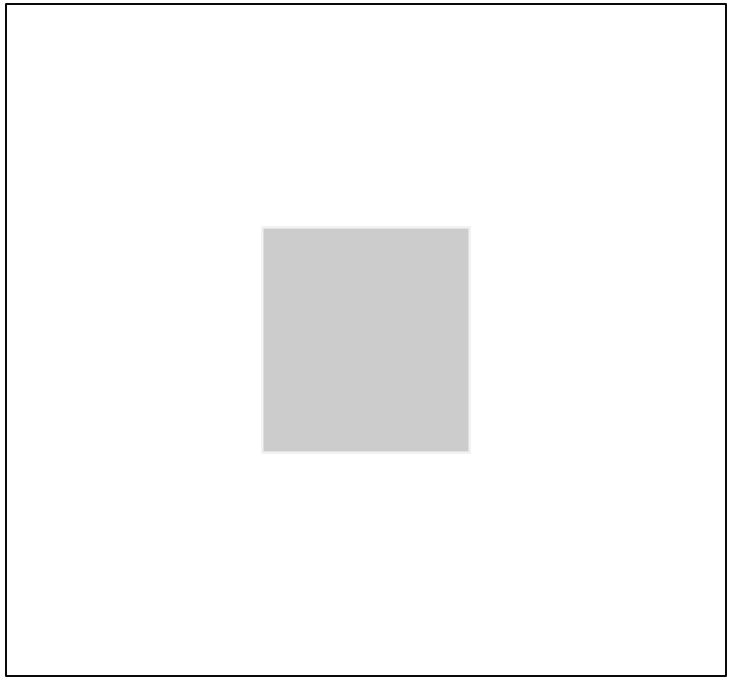
Variants of Optical Microscopy

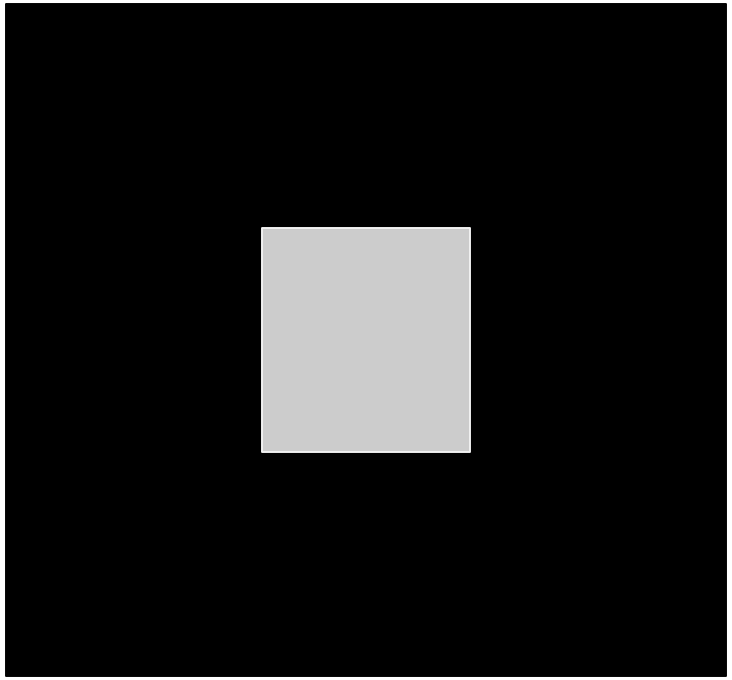
Have You Seen This Old B-W TV?

Can You See Something in the Box?



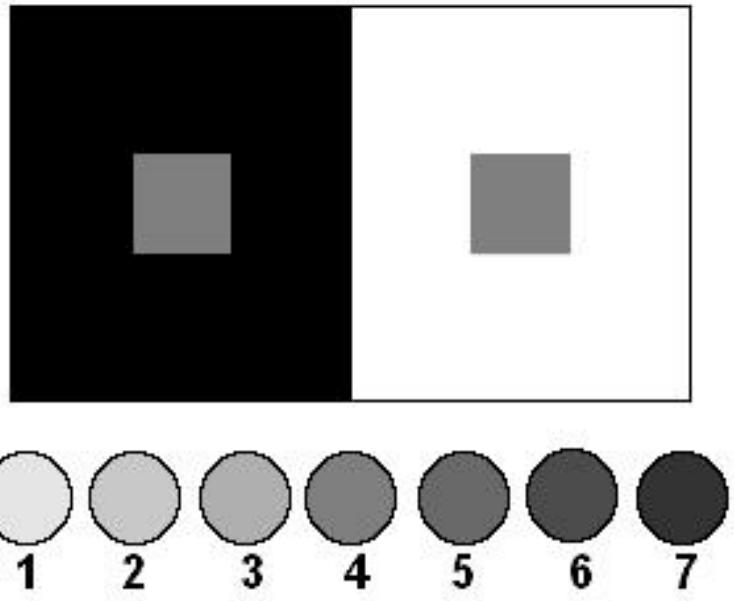
Can You See Something in the Box Now?

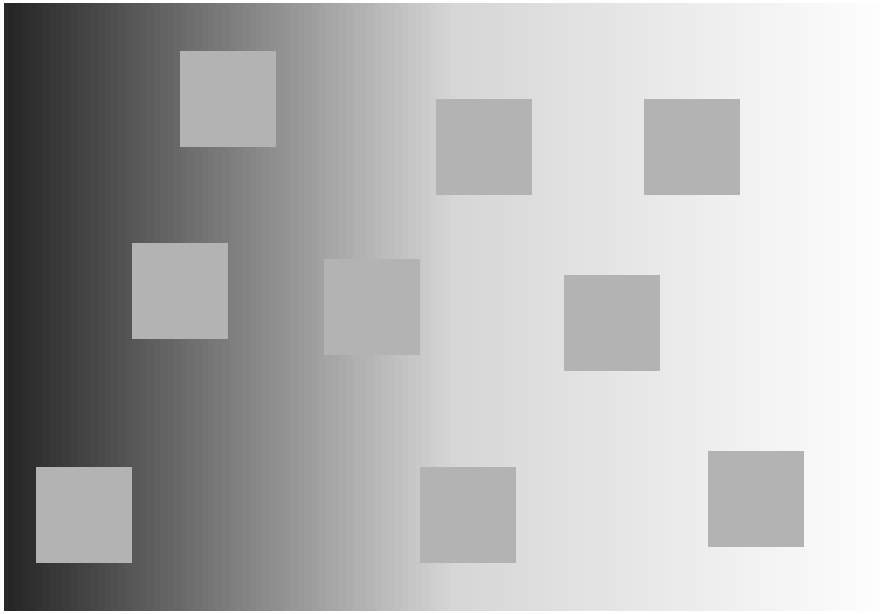




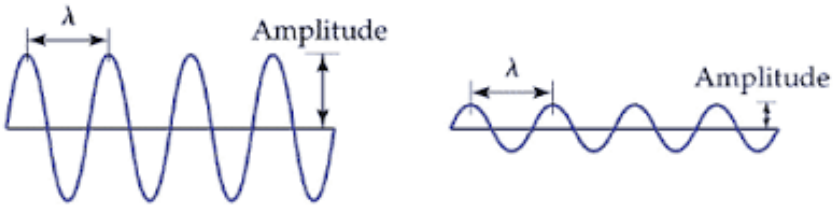
What's the Contrast?

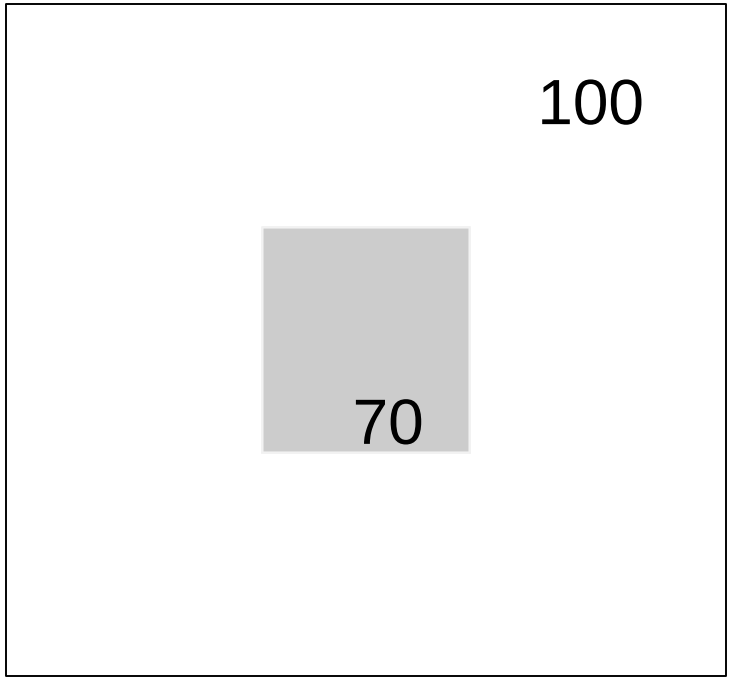
- The difference in visual properties (lightness and intensity) that makes an object (or its representation in an image) distinguishable from other objects and the background.
- In visual perception of the real world, contrast is determined by the difference in the color and brightness of the object and other objects within the same field of view

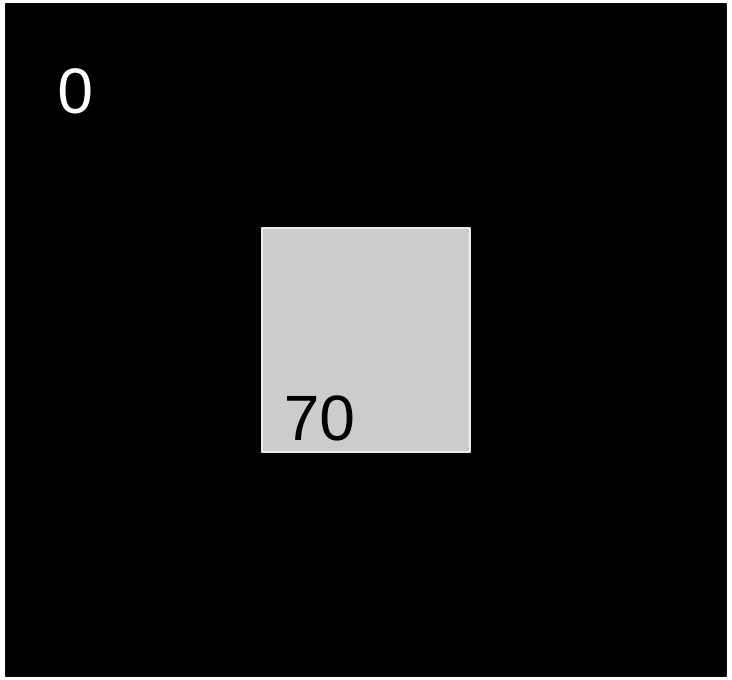




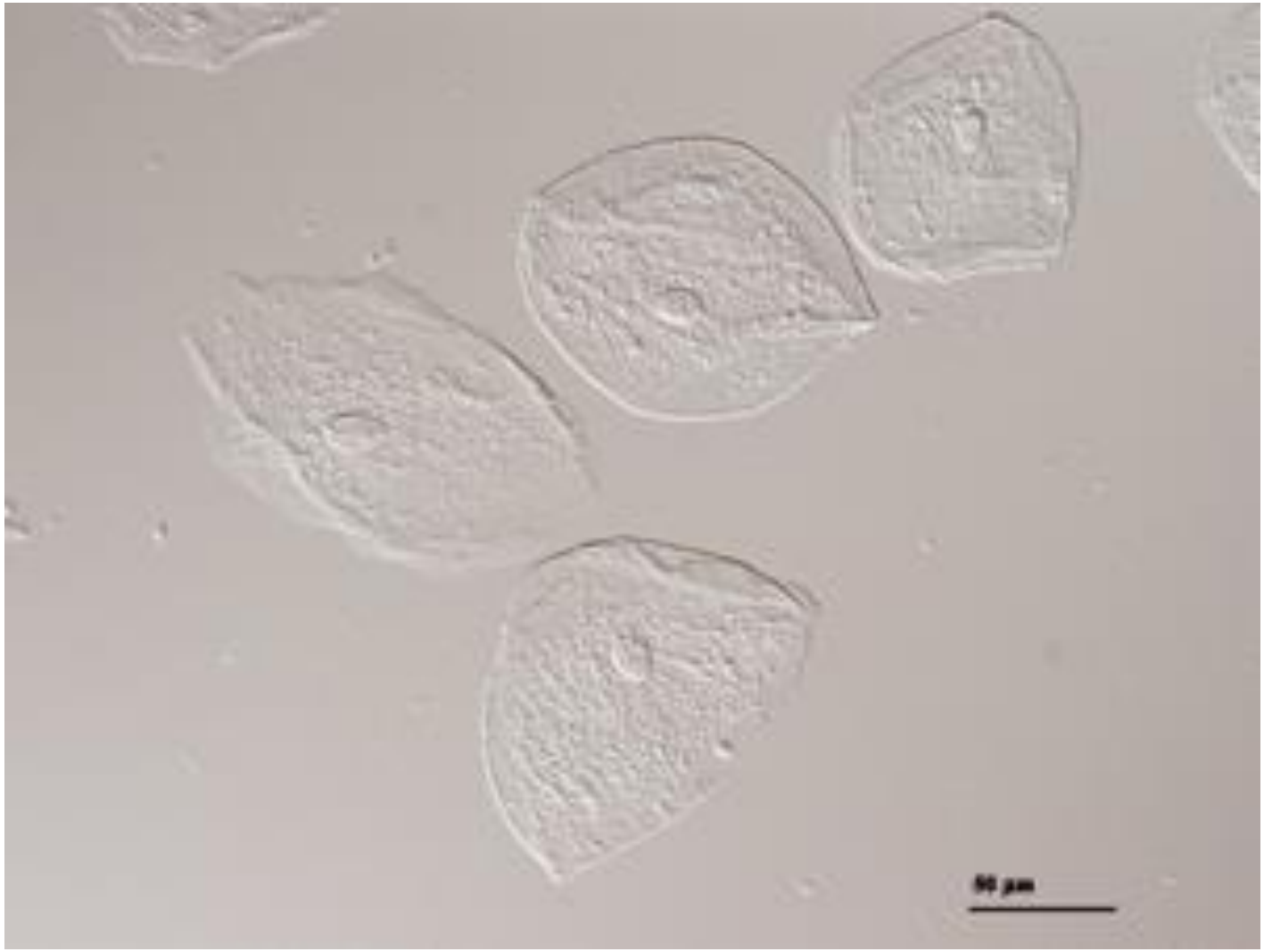
Light Intensity (Amplitude) vs Contrast







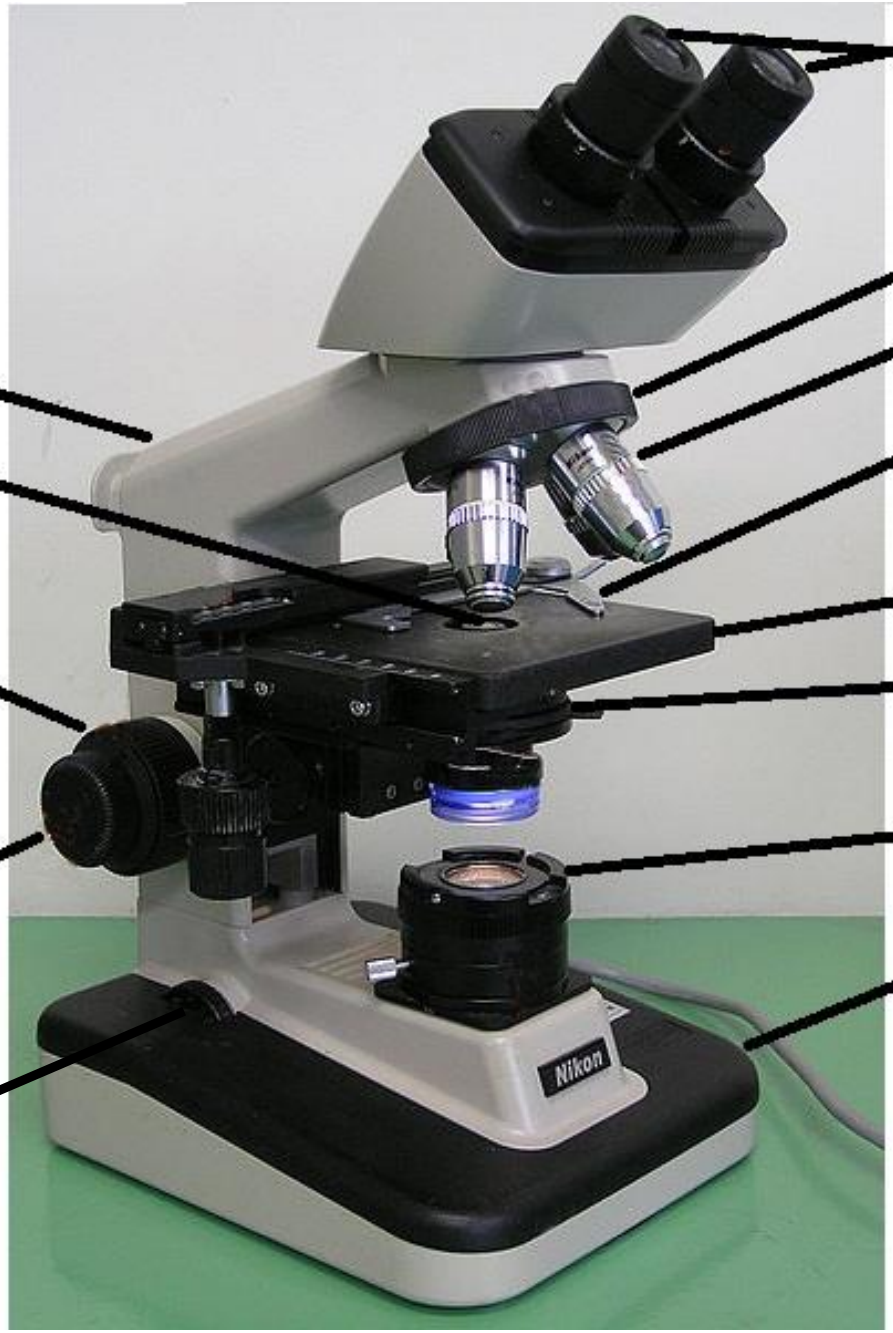
Contrast Produces Image



Light Path and Illumination Methods

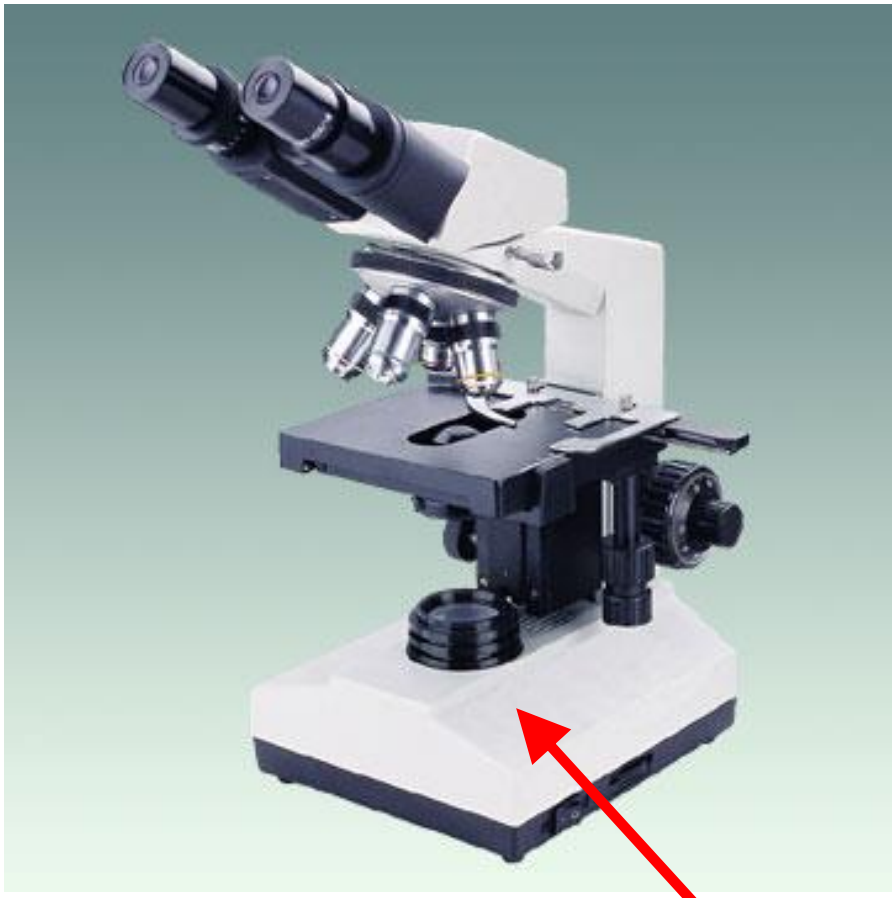
1. Compound microscope (one light path)
   - Two stage of magnification via two lenses (objective and eyepiece)
   - Conventional microscope
   - Trans-illumination: transparent biological samples
   - Epi-illumination or reflected illumination: opaque or nontransparent samples
   - Upright vs. inverted microscope
   - Dark field, bright field, vs. phase-contrast microscope
2. Stereo microscope (two light paths)
   - Reflected illumination
   - Limited magnification (X100)
   - 3-dimensional view via two light paths

Compound Microscope and Components

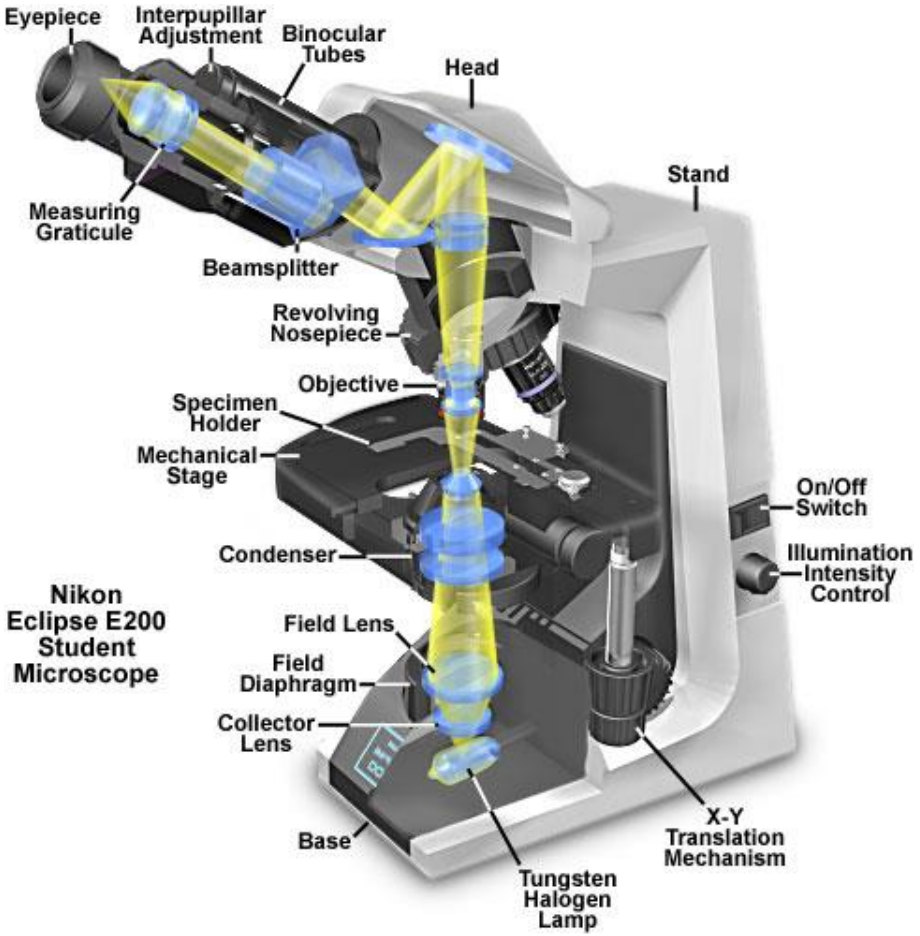


1) Eyepiece
2) Nosepiece
3) Objective Lenses
4) Stage clips
5) Stage
6) Condenser diaphragm
7) Field diaphragm
8) Base
9) Arm
10) Condenser Lens
11) Coarse Adjustment Knob
12) Fine Adjustment Knob
13) Illumination switch

Biological Microscope: Trans-illumination for Transparent Samples



Light source



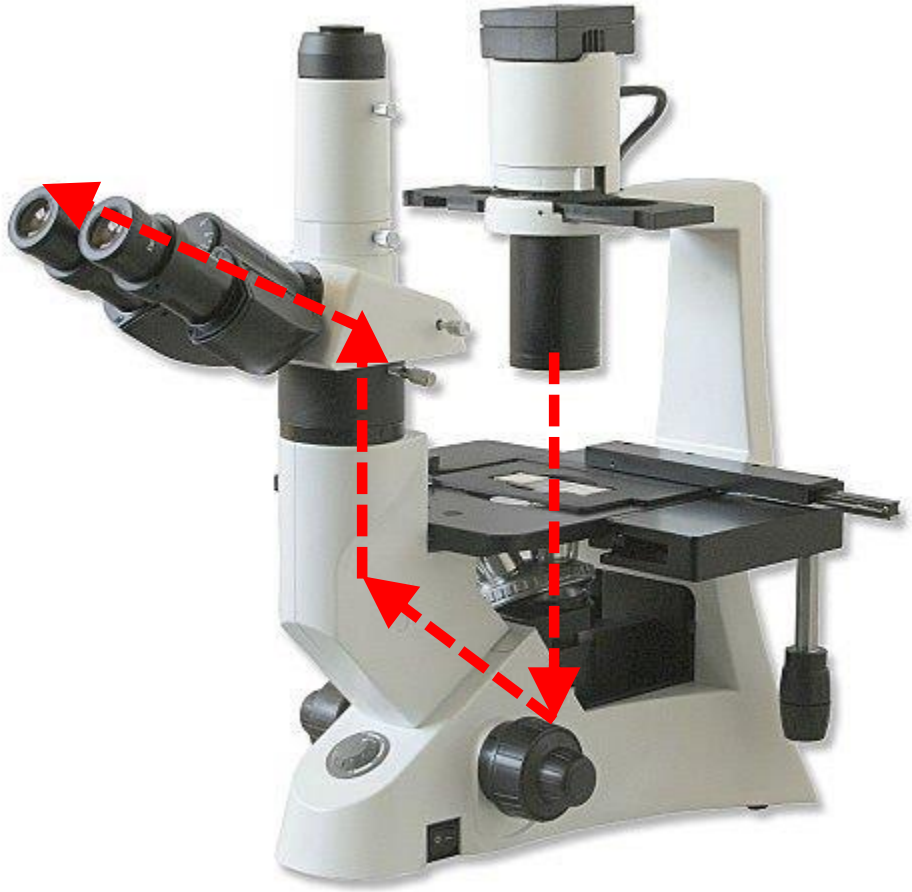
Metallurgical Microscope: Epi-Illumination for Non-Transparent Samples

Please notice the difference in the location of light source

Upright and Inverted Microscope

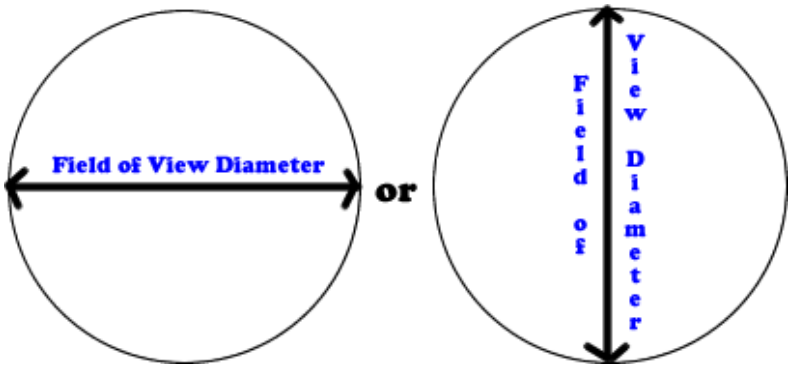
Top Down

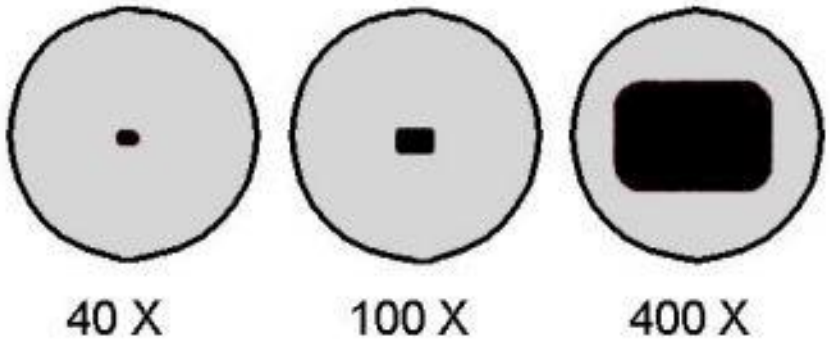
Bottom Up



Field of View (Field)

- When you look through the lens of a microscope you **see** a circular area, the diameter of which is known as the **field of view**.
- It depends of magnifications



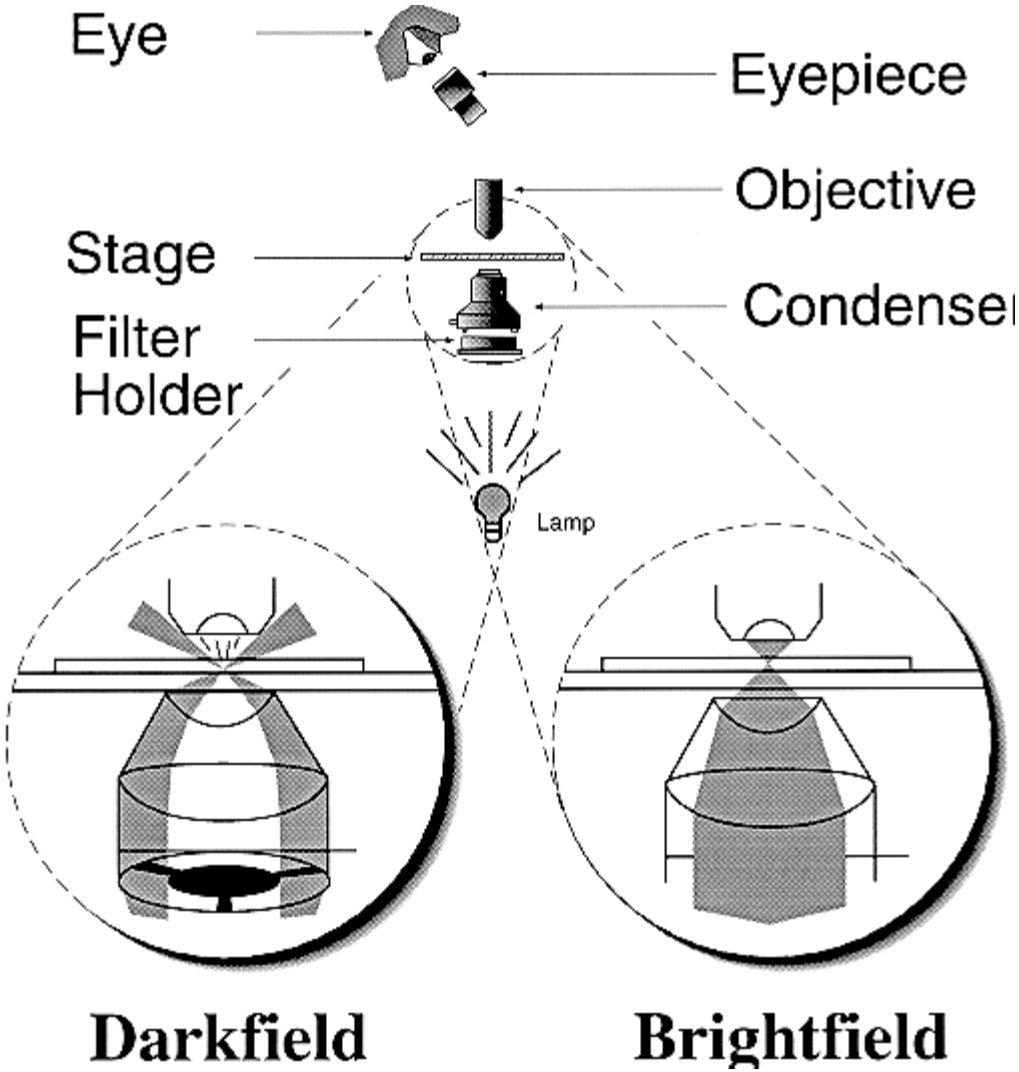


Dark Field vs. Bright Field Microscope

1. Bright field optical microscope
   a) Most common type
   b) Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light.
2. Dark field optical microscope (see next page)
   a) exclude the unscattered beam from the image. As a result, the field around the specimen (i.e. where there is no specimen to scatter the beam) is generally dark.
3. Phase contrast optical microscope
   a) small phase shifts in the light passing through a transparent specimen are converted into amplitude or contrast changes in the image.

http://en.wikipedia.org/wiki/Optical_microscopy

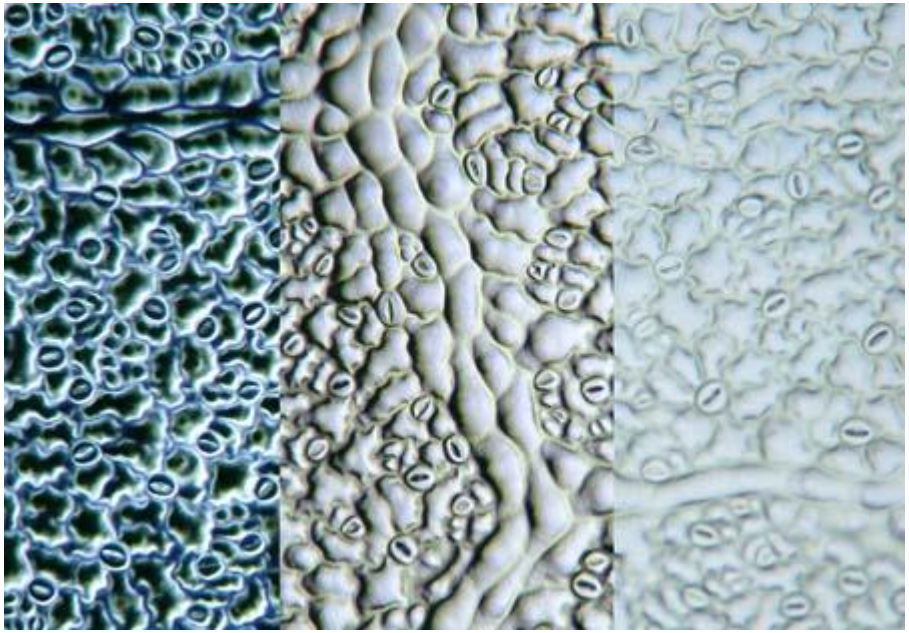
Dark Field vs. Bright Field Microscope



Indirect illumination

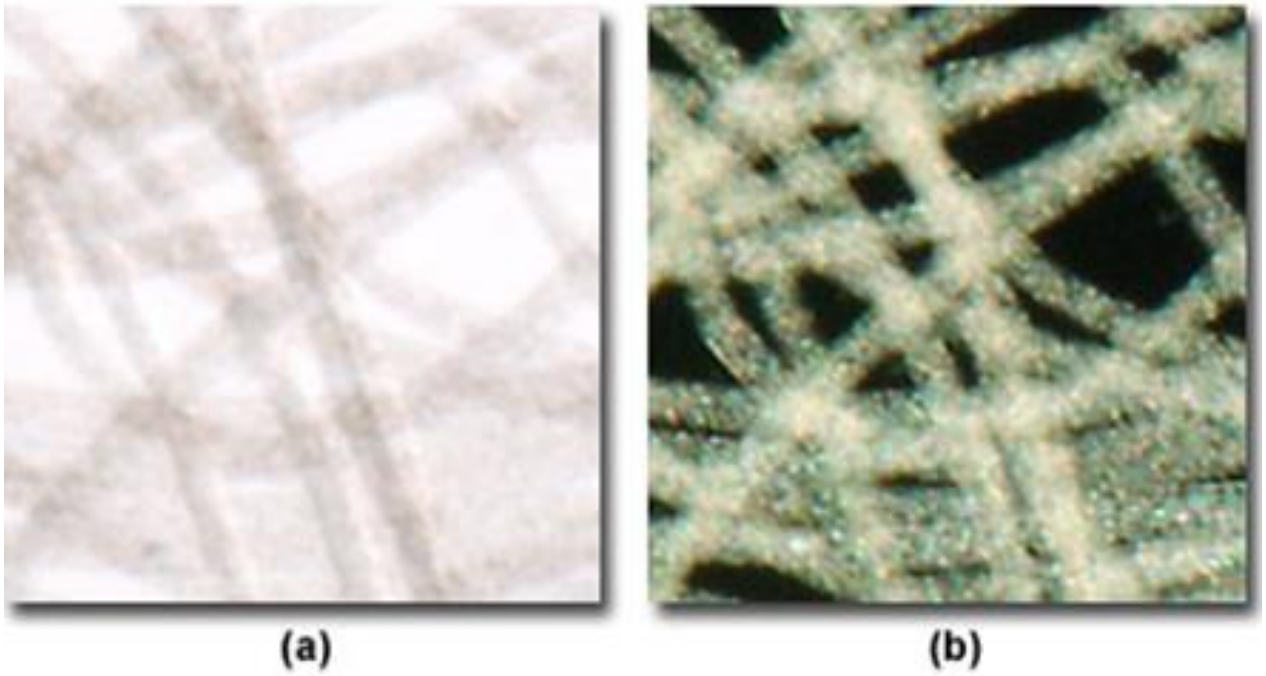
Direct illumination

Dark Field vs. Bright Field Microscope



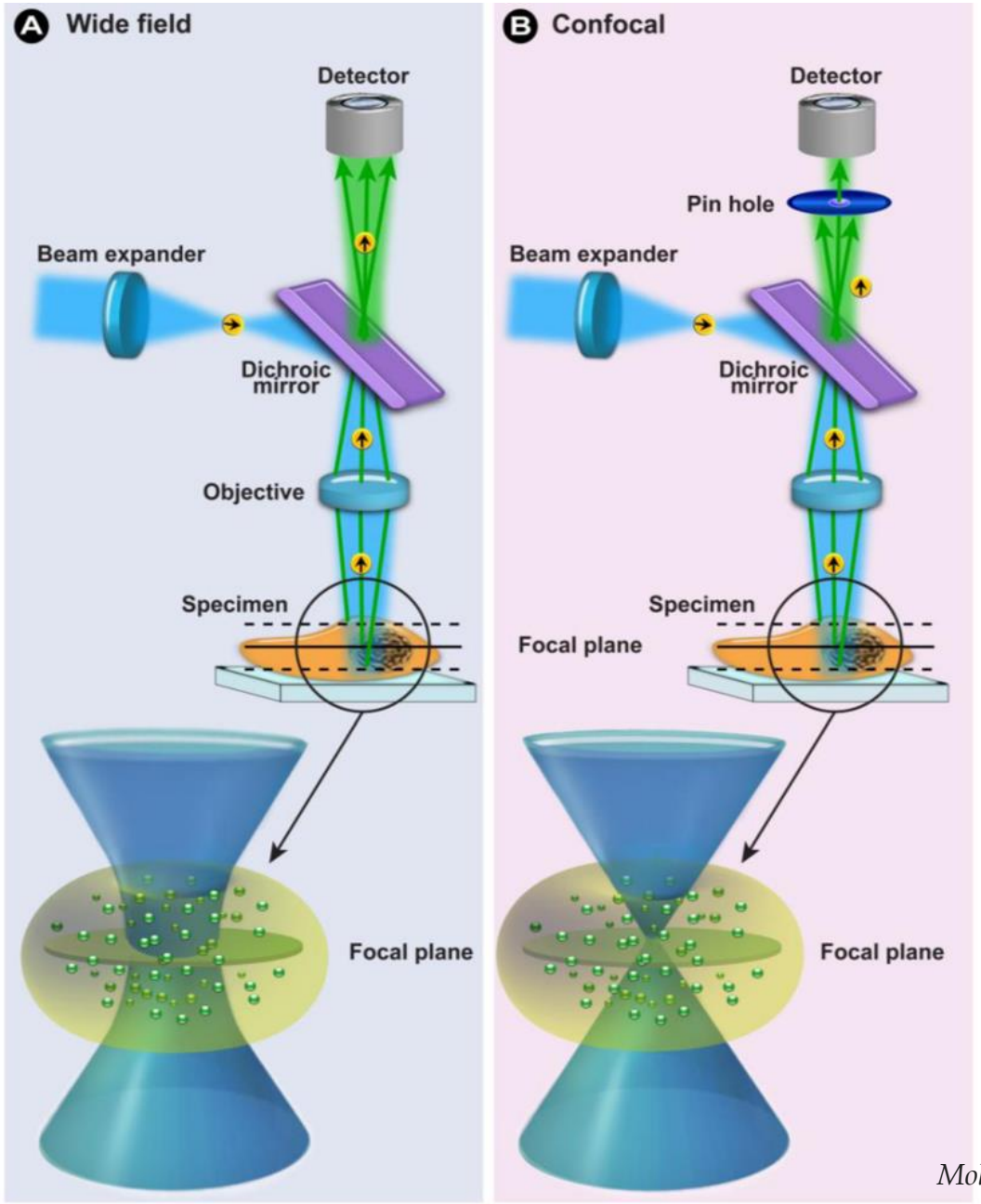
Dark Oblique Bright

Issues in Dark Field and Bright Field Microscope



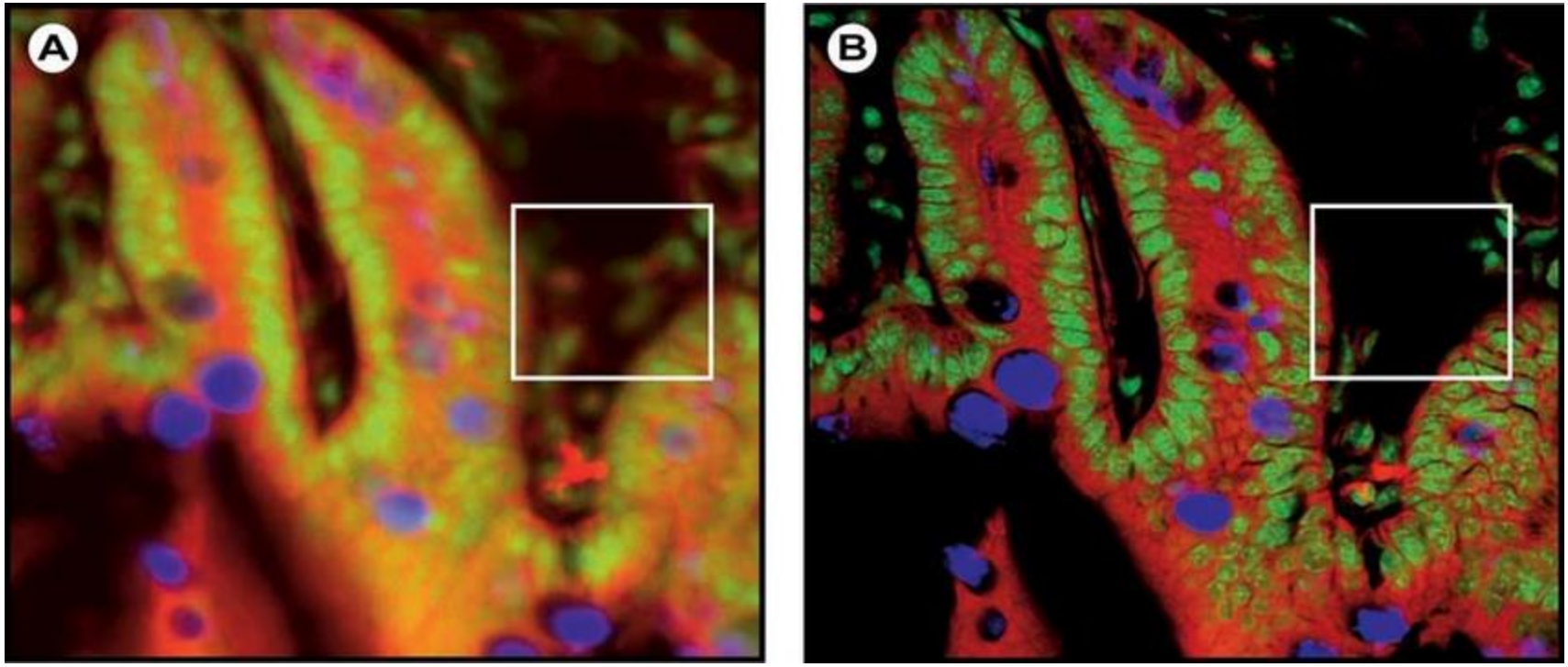
(a) Lack in contrast (not clear, details are not clear)
(b) Lack in contrast (not clear)

Wide Field vs Confocal Microscope



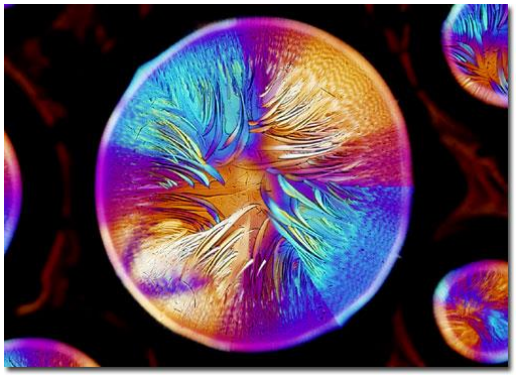
Molecules **2012**, *17*(4), 4047-4132

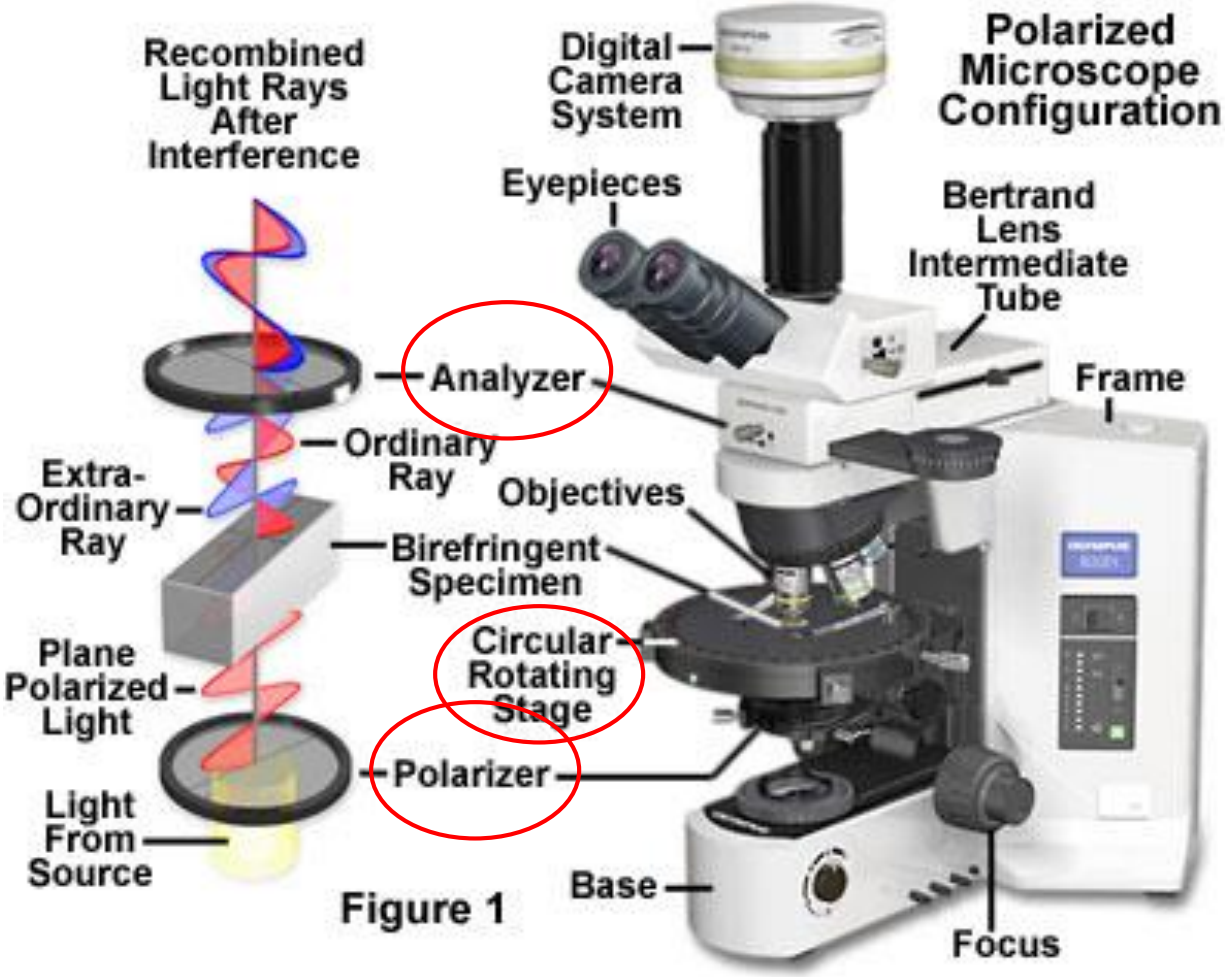
Wide field (A) and confocal (B) image of a triple-labeled cell aggregate (mouse intestine section)



Polarized Light Microscope (PLM)

Polarizer
Analyzer
Compensator





http://www.olympusmicro.com/primer/techniques/polarized/polarizedintro.html

Stereo Microscope

- The stereo microscope uses two separate optical paths with two objectives and two eyepieces to provide slightly different viewing angles to the left and right eyes.

- Will be used for cases where three-dimensional observation and perception of depth and contrast is critical to the interpretation of specimen structure.

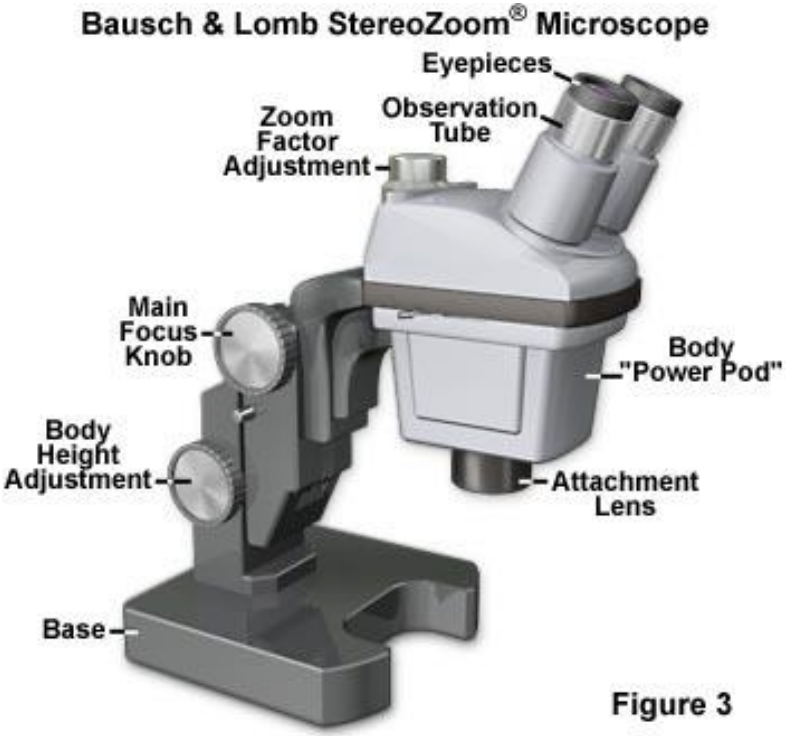
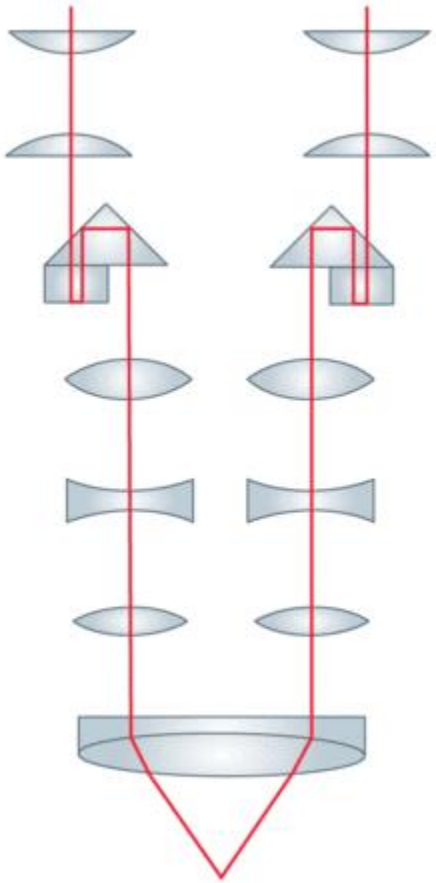


Figure 3

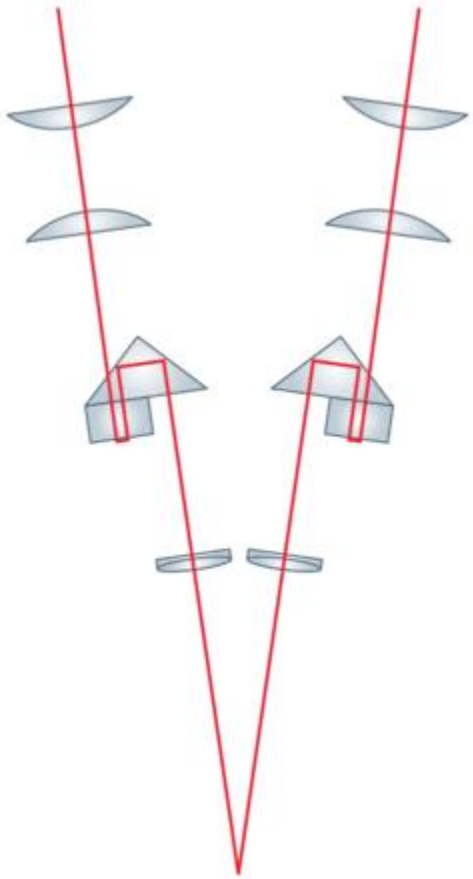
Figure 5

http://www.microscopyu.com/articles/stereomicroscopy/stereointro.html

Stereo Microscope: Two Light Paths



Common main objective principle

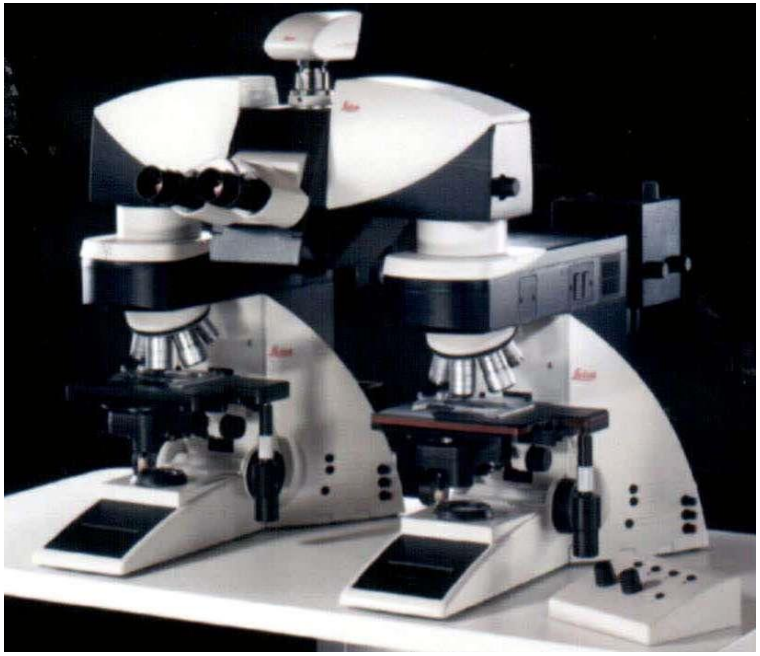


Greenough principle

Comparison Microscope

- The comparison microscope is used to compare two materials under the same optical conditions.

- The bridge connects the two identical microscopes and allows a split field of view that permits a side-by-side comparison of both images.



human hair (left) that has tested positive for cosmetic bleaching using a staining technique (right).

http://en.wikipedia.org/wiki/Comparison_Microscope