2009 spring

Microstructural Characterization of Materials

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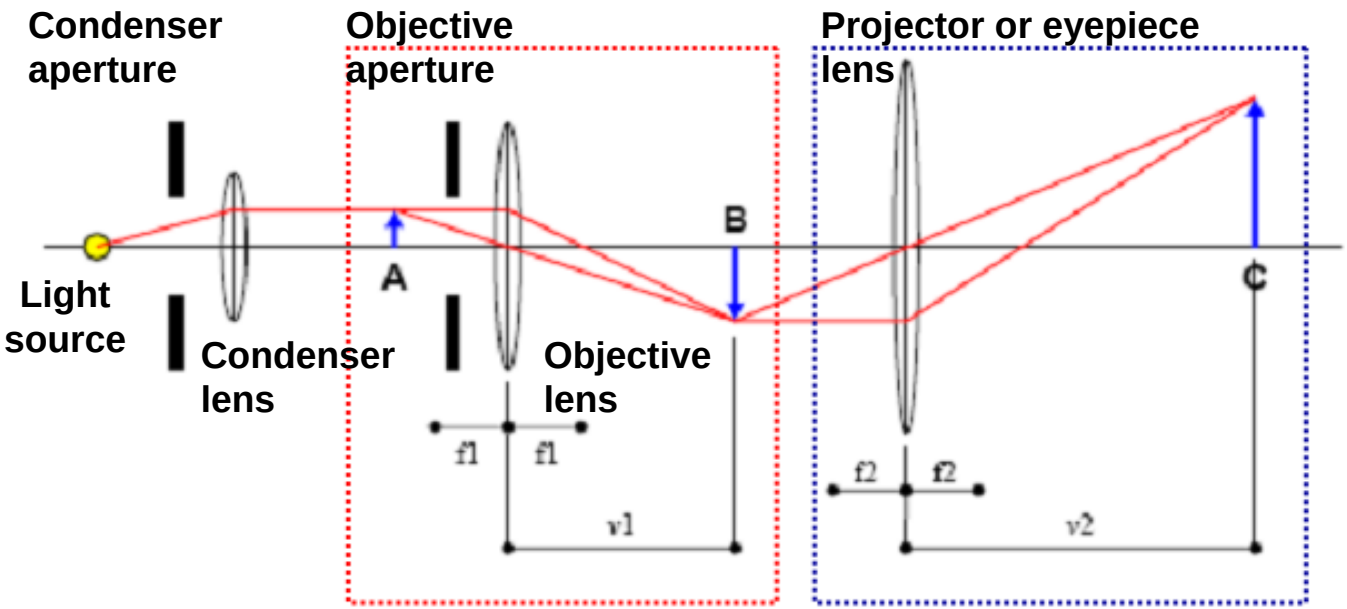
Contents for previous class



- Let v be the distance between the magnified image and the lens (also known as the image distance). Let f be the focal distance

OBJECTIVE LENS MAGNIFICATION

$$M1 = (v1 - f1) / f1$$

PROJECTOR LENS MAGNIFICATION

$$M2 = (v2 - f2) / f2$$

TOTAL MAGNIFICATION

$$M1 \times M2$$

Optical Performance

Resolution

- Recall: the resolution of a system is the smallest distance between two points or lines that can be observed.
- In the case of optical microscopy it is a function of the wavelength of light and the N.A. of the objective.
- Resolution limits magnification in an optical microscope.

$$d = \frac{0.612\lambda}{\text{N.A.}} = \frac{0.612\lambda}{\mu \cdot \sin\alpha}$$

Abbe's Equation

where: d= resolution

λ= wavelength of imaging radiation

μ= index of refraction of medium between point source and lens, relative to free space

α= half the angle of the cone of light from specimen plane accepted by the objective (half aperture angle in radians)

Optical Performance

Resolution & Depth of Field

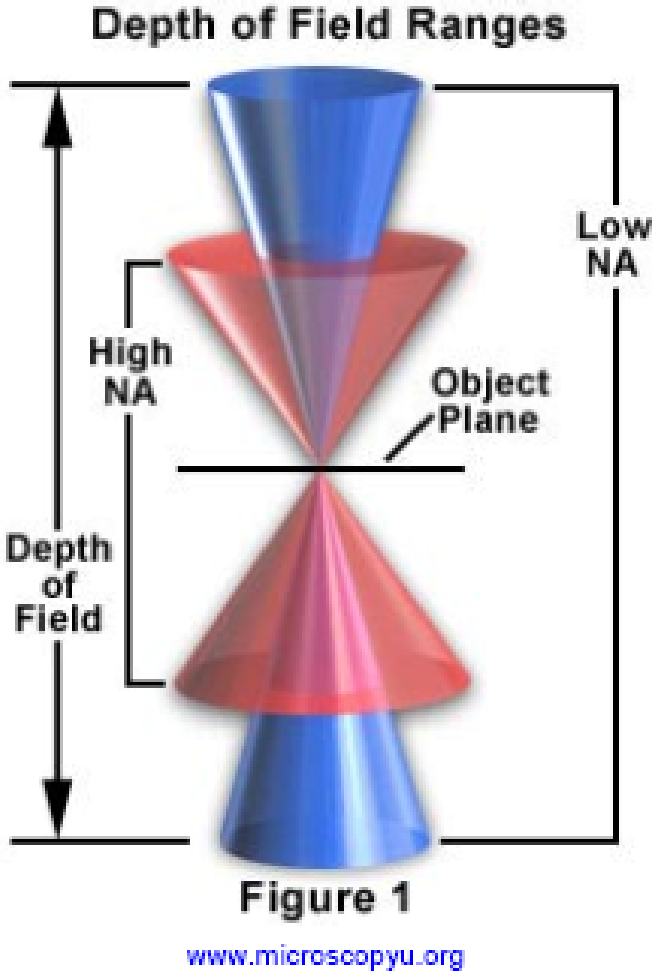
-Resolution

$$r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin\alpha} = \frac{0.612\lambda}{N.A.}$$

-Depth of Field

$$D_f = \frac{1.22\lambda}{\mu \sin\alpha \tan\alpha}$$

- Large D_f and r_1 cannot be obtained simultaneously.
- Large D_f means larger r_1 and worse resolution.

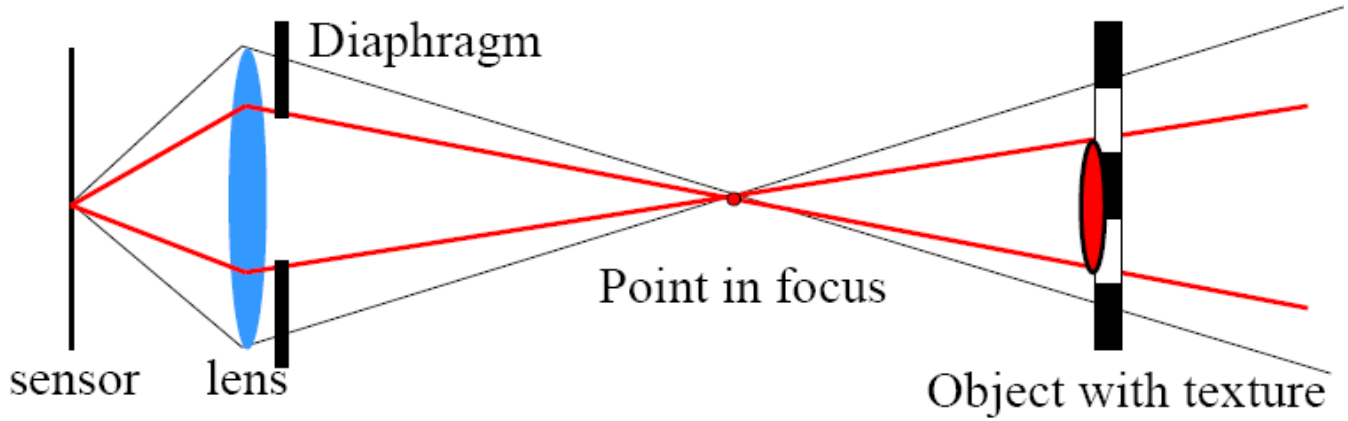


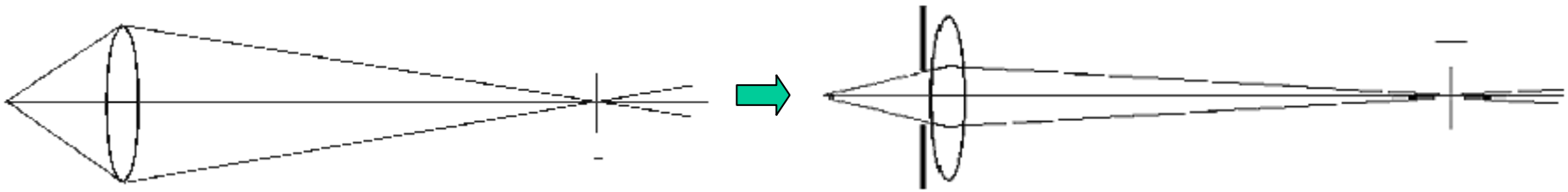
$$D_f = \frac{1.22\lambda}{\mu \sin\alpha \tan\alpha}$$

대부분 광학현미경의 초점 심도는 <1 micrometer>

How to improve depth of field

1. Reduce *N.A.* by closing down aperture diaphragm, or use a lower *N.A.* objective lens. ~ **lower α**

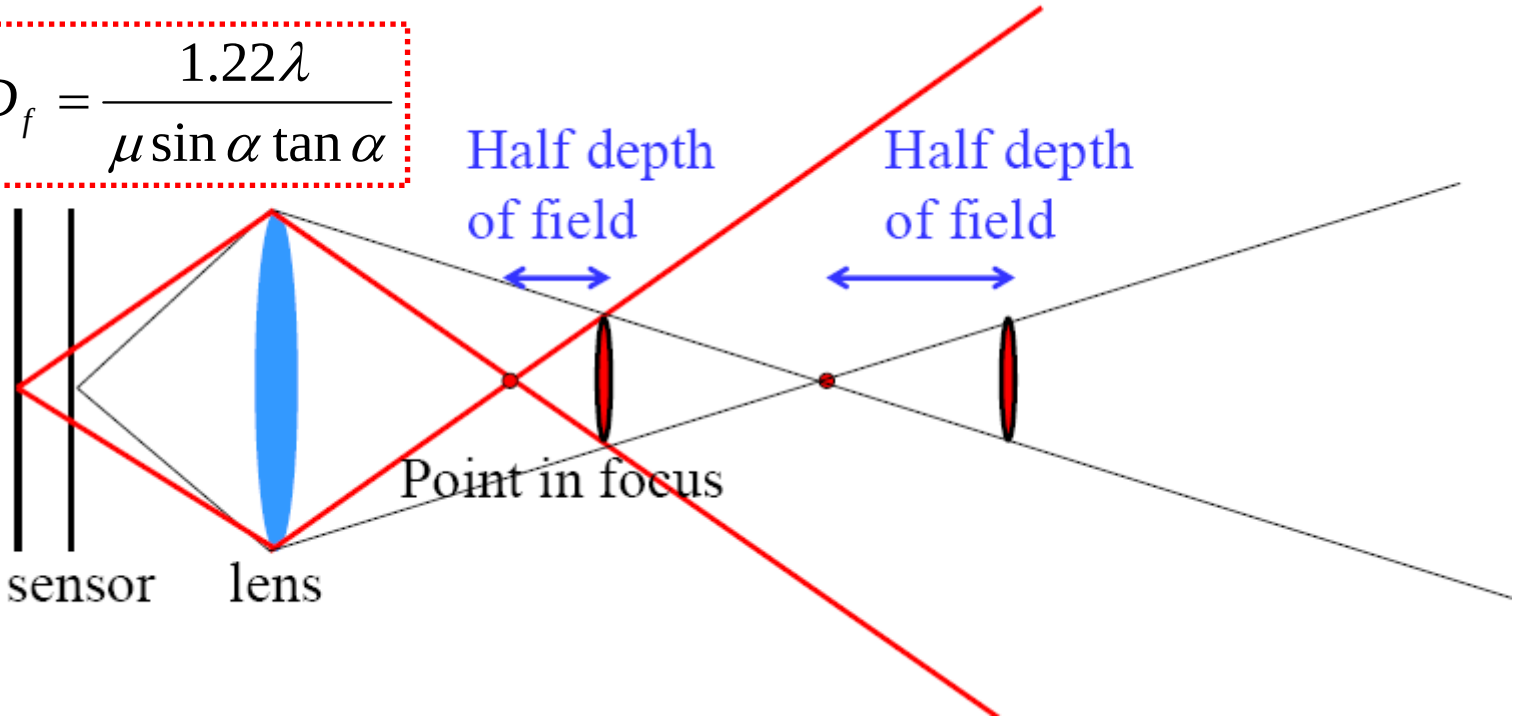


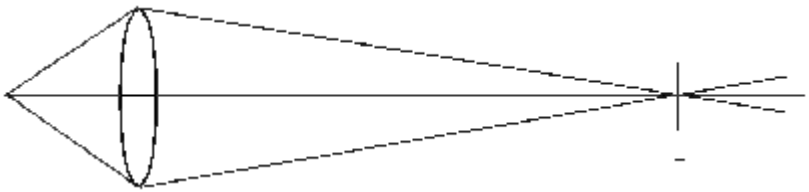


How to improve depth of field

1. Reduce *N.A.* by closing down aperture diaphragm, or use a lower *N.A.* objective lens. ~ **lower α**

$$D_f = \frac{1.22\lambda}{\mu \sin\alpha \tan\alpha}$$



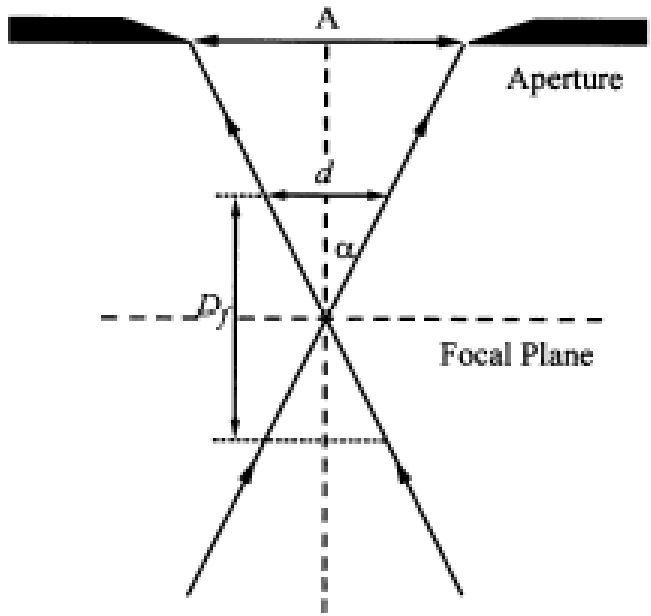


How to improve depth of field

1. Reduce *N.A.* by closing down aperture diaphragm, or use a lower *N.A.* objective lens.

2. Lower the magnification for a given *N.A.*

$$D_f = \frac{1.22\lambda}{\mu \sin\alpha \tan\alpha} = \frac{d}{\tan\alpha}$$



5. Use longest possible wavelength

Depth of Focus

- The range of image plane position at which the image can be viewed without appearing out of focus for a fixed position of object.

  – Often confused with depth of field

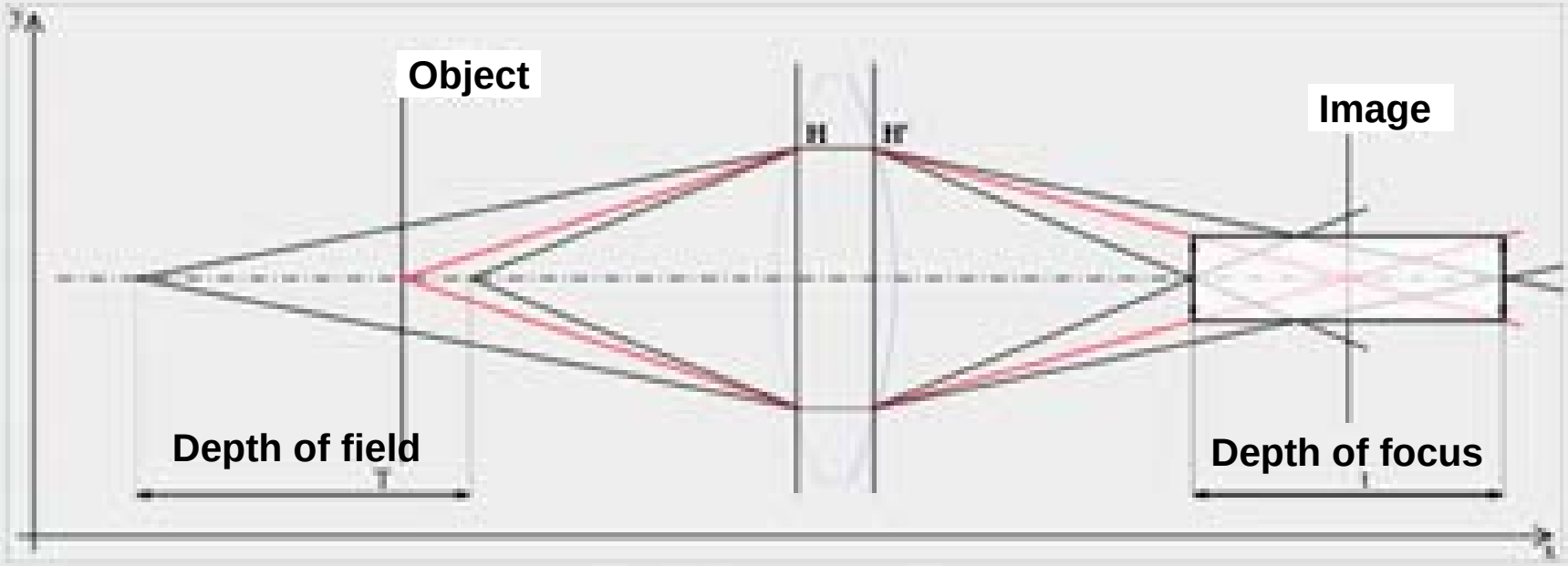
  – Not as important as depth of field

  - Depth of focus is M^2 times depth of field

$$D_{focus} = D_f \times M^2 = \frac{1.22\lambda}{\mu \sin\alpha \tan\alpha} \times M^2$$

고배율에서는 depth of field 값이 짧아지지만 image의 depth of focus 값은 증가한다.

Depth of field and focus



Conjugate relationship between depth of field and depth of focus

Quit

Image Quality

When you look at a specimen using a microscope, the quality of the image you see is assessed by the following:

- **Brightness** - How light or dark is the image?
- **Focus** - Is the image blurry or well-defined?
- **Resolution** - How close can two points in the image be before they are no longer seen as two separate points?
- **Contrast** - What is the difference in lighting between adjacent areas of the specimen?

Brightness

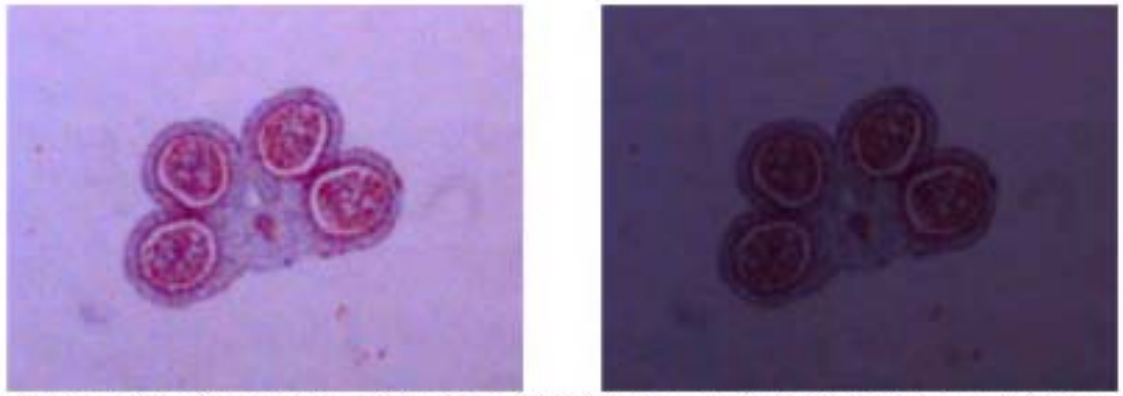


Image of pollen grain under good brightness (left) and poor brightness (right)

Focus

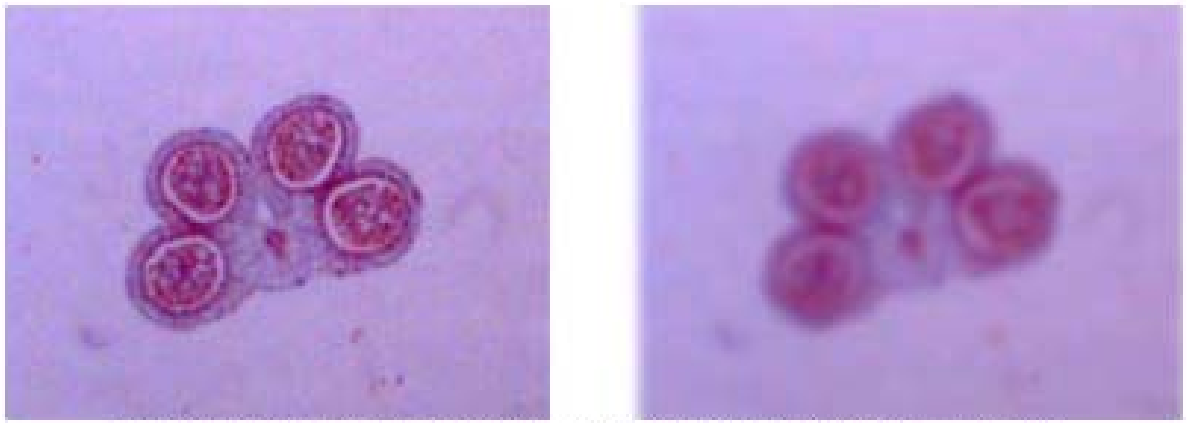


Image of pollen grain in focus (left) and out of focus (right)

Resolution

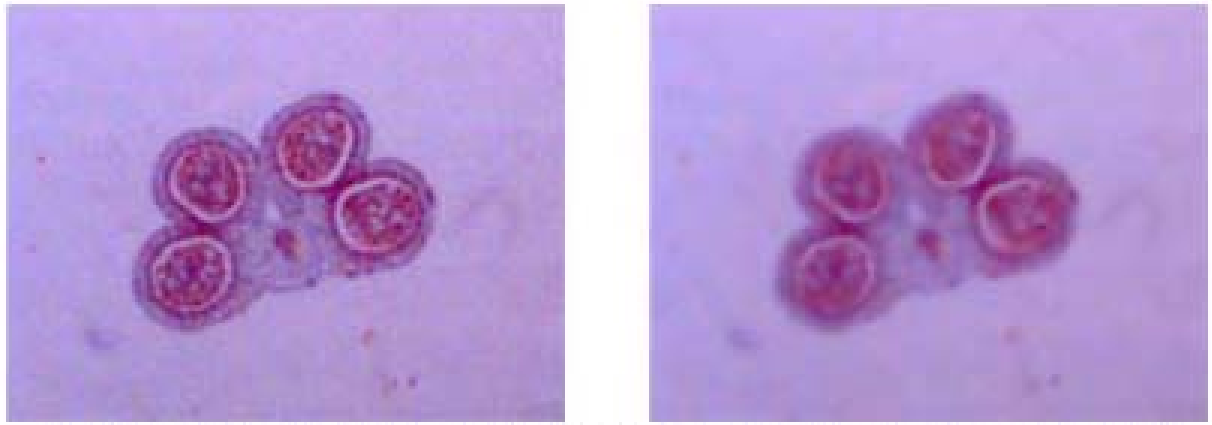


Image of pollen grain with good resolution (left) and poor resolution (right)

Contrast

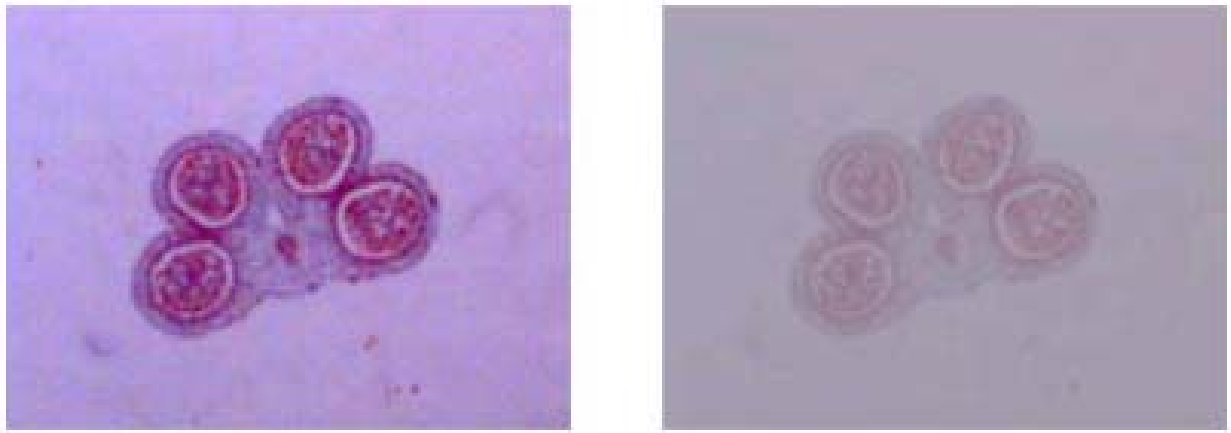


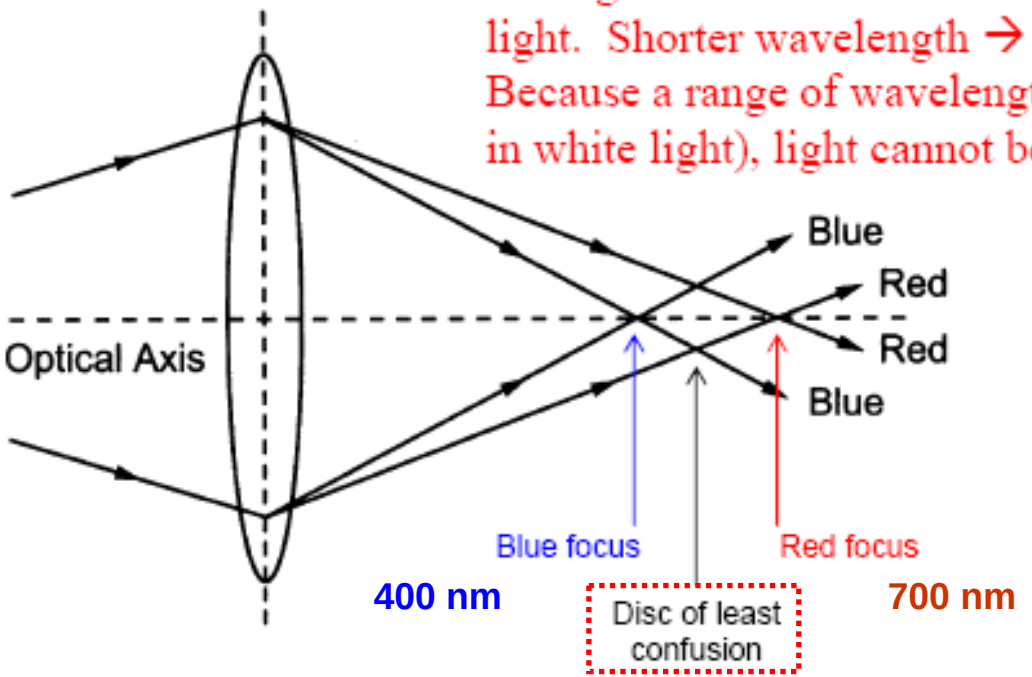
Image of pollen grain with good contrast (left) and poor contrast (right)

• *Aberrations in optical systems*

Resolution and depth of field are based on the assumption that all the components of the microscope are perfect, and that the light from any point on an object focuses at a similar unique point in the image. This is impossible due to *lens aberrations*.

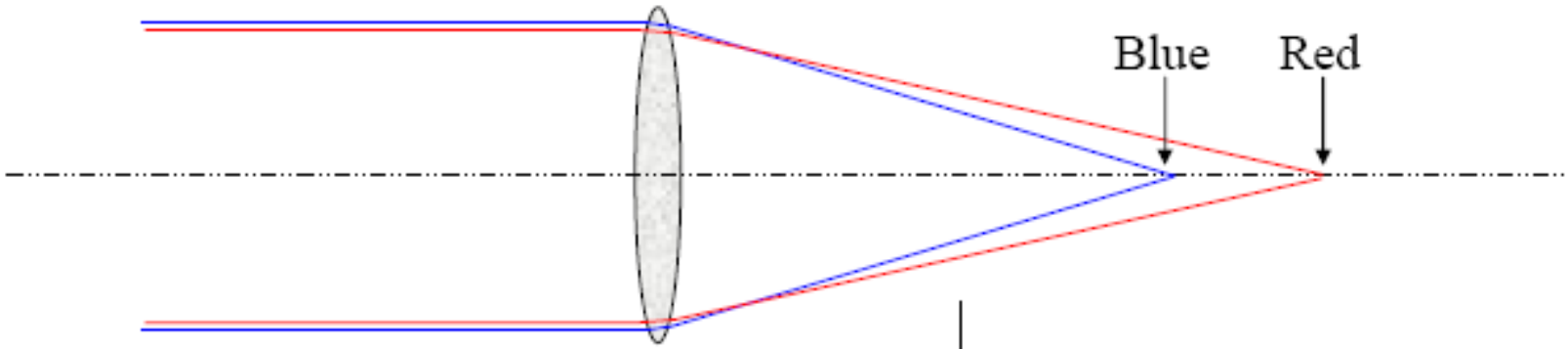
Chromatic aberrations

The light deflection of a lens depends on the wavelength of light. Shorter wavelength → larger degree of deflection. Because a range of wavelengths is present in the light (e.g., in white light), light cannot be focused to a single point.



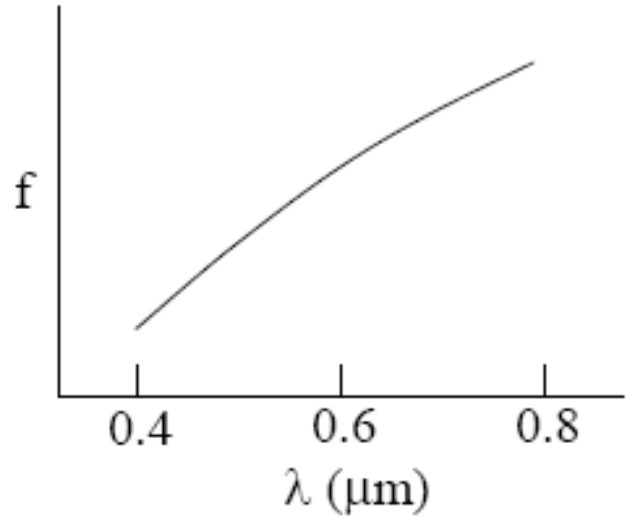
Chromatic Aberration

- Spherical and chromatic aberrations are the only "on-axis" aberrations.
- Chromatic aberration occurs for lenses only.



$$\frac{1}{f} = (n-1)\left[\frac{1}{r_1} - \frac{1}{r_2}\right]$$

$$n = n(\lambda)$$



Spherical Aberrations(구면수차)

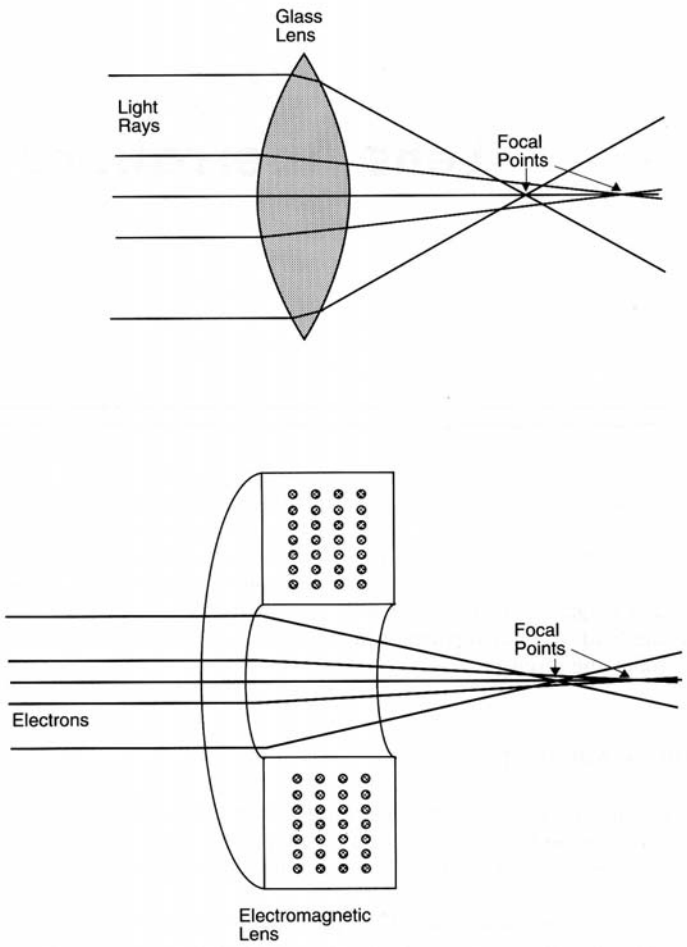


Figure 4-1 Spherical aberration arises when light rays or electrons passing through a lens are brought to different focal points. The differential bending of the light rays or electrons is due to varying lens strength.

빛이나 전자가 렌즈의 중앙(center)을 지날 때와 모서리(edge) 부분을 지날 때 각각 다른 focal length를 가진다.

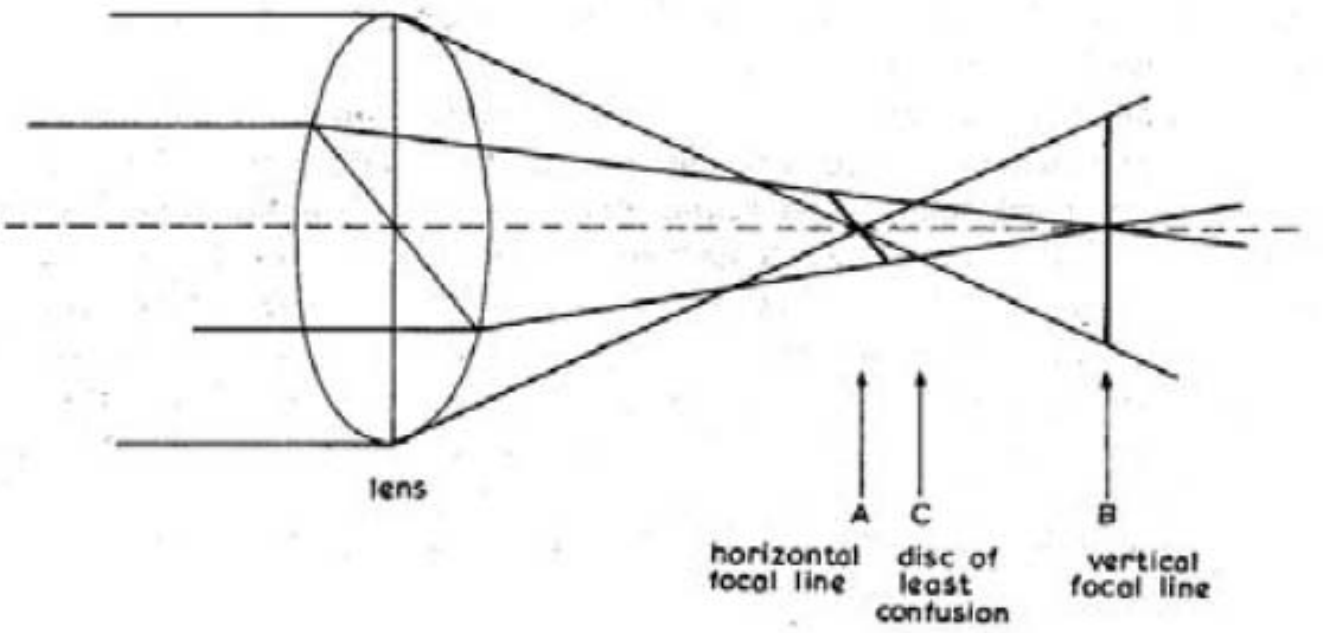
Center: long focal length

Edge: short focal length

Peripheral 빛과 전자는 렌즈의 중앙을 지나는 빛과 전자보다 더 굽어진다.

- *Astigmatism* (비점수차)

Astigmatism is related to spherical aberrations. A lens, which does not have identical properties in the horizontal and vertical planes, generates the aberrations as shown in the figure.



Ray diagram illustrating the formation of ***astigmatism*** for a lens with slightly different optical properties in the horizontal and vertical directions. In this illustration the lens is more powerful in the vertical plane.

Astigmatism(비점수차)

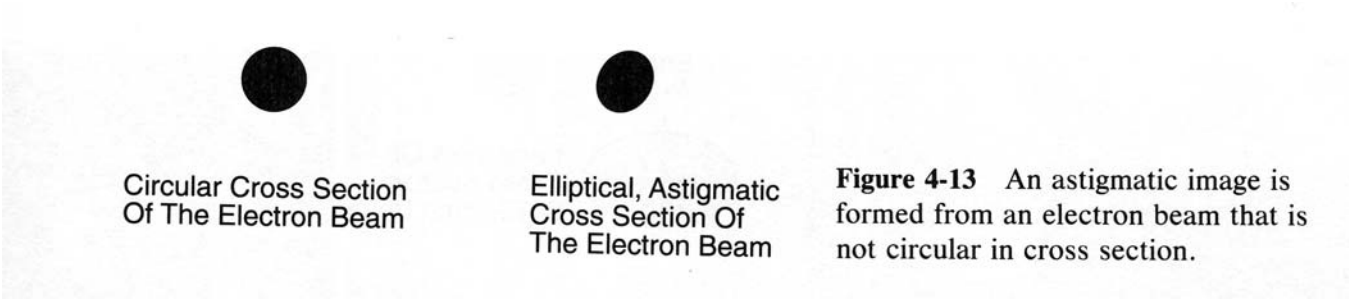


Figure 4-13 An astigmatic image is formed from an electron beam that is not circular in cross section.

Stigma: point

Astigmatism: 렌즈가 빛이나 전자를 point로 만들 수 없는 정도, 즉 전자빔의 단면이 완전한 구가 아니고 타원일 때 생김.

Cause of Astigmatism

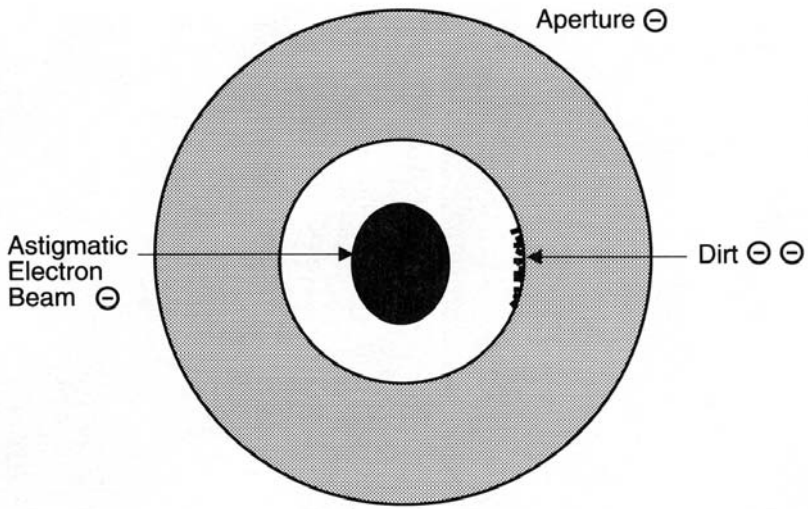


Figure 4-18 A dirty aperture charges, resulting in a symmetrical electrostatic field that produces an astigmatic electron beam.

1. Nonsymmetrical fields in electron lens

- 완전한 대칭적인 **field strength**를 갖는 렌즈제작 불가능

- 렌즈구멍 가공시의 불완전성**, winding**의 비대칭성**, pole piece**의 **iron**의 불균일성 등

2. 오염된 **aperture, lens**

- column, aperture, lens 등이 오염되면 비점이 심해진다**.**

Astigmatism(비점수차)

Figure 4-16 A circular object appears elongated in an astigmatic image.

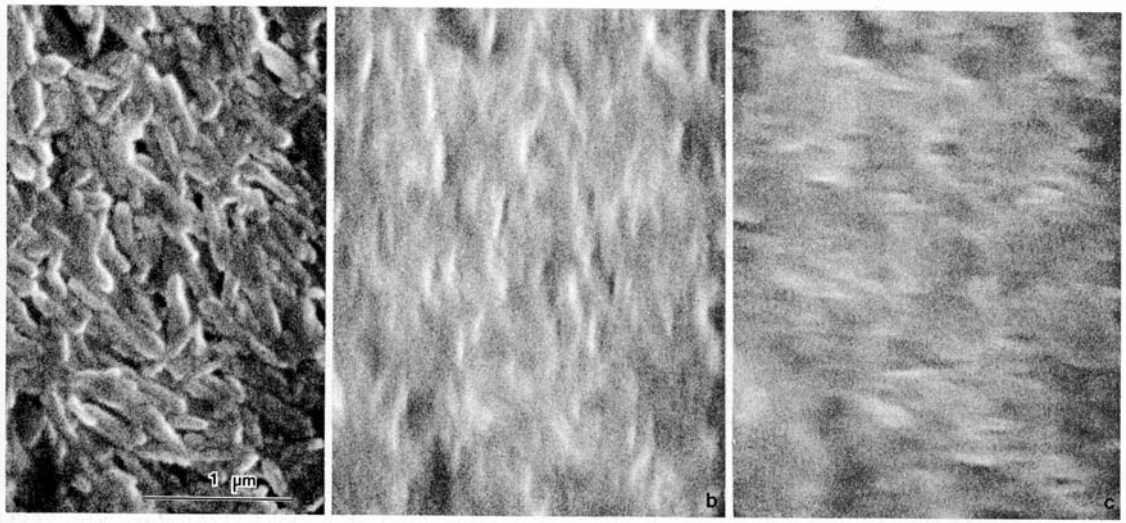


Figure 4-17 Scanning electron micrographs of the same area of magnetic tape taken at an accelerating voltage of 30,000 V. (a) An in-focus micrograph showing no astigmatism. (b) and (c) Images taken with an astigmatic electron beam showing how the orientation of details of the specimen changes 90° as the operator goes from overfocus to underfocus. (b) An astigmatic overfocused image showing the orientation of details primarily in the vertical plane. (c) An underfocused astigmatic image showing the orientation of details primarily in the horizontal direction.

Correction of aberrations

All aberration corrections are designed to reduce in size of the disc of least confusion.

Corrections of chromatic aberration

- Combining lenses of different shapes and refractive indices
- Eliminating the variation in wavelength from the light source by using filters and special lamps.

Corrections of astigmatism

It can be corrected relatively easy in electron microscope, because the magnetic lens strength can be adjusted in two perpendicular planes.

Chromatic Aberrations

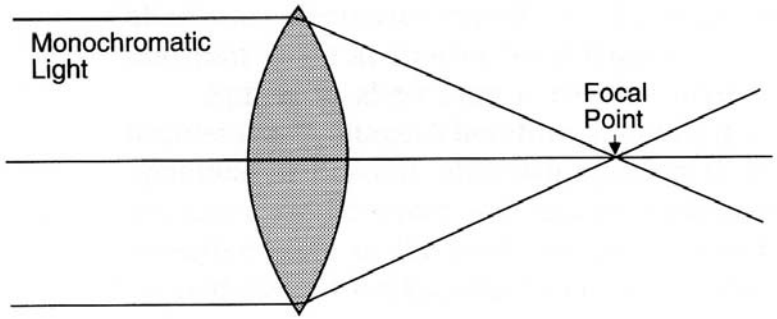


Figure 4-9 Monochromatic light rays are brought to a single focal point by a perfectly constructed glass lens.

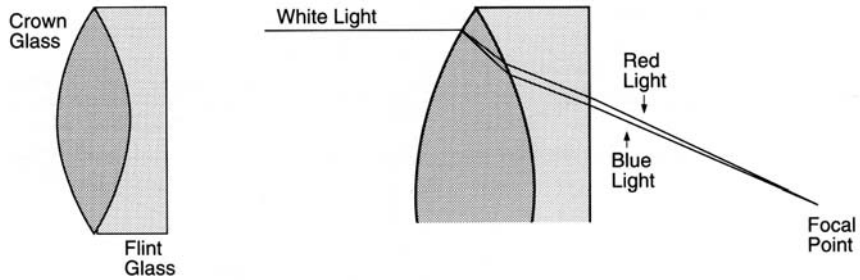


Figure 4-10 Chromatic aberration in a glass lens can be minimized by combining a converging and diverging lens of the correct strengths so that the light rays are brought to a single focal point.

광학현미경의 색수차 보정

1. 단색광 장치(monochromator)를 사용하여 단색 혹은 좁은 파장의 빛만 렌즈를 통하면 색수차가 보정된다.

2. 서로 다른 굴절률을 가지는 렌즈들 (converging and diverging lens)을 함께 사용하여 색수차 보정

Spherical Aberrations(구면수차)

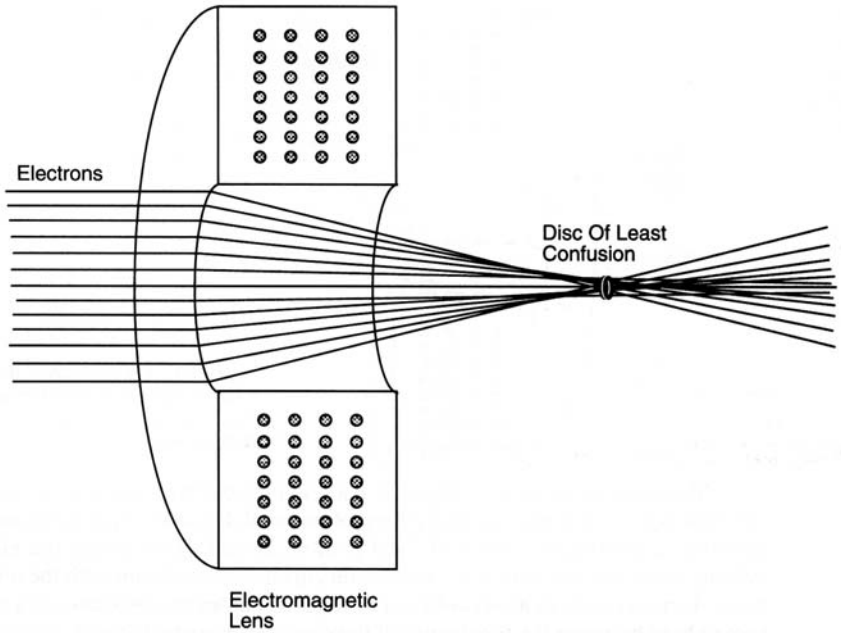


Figure 4-3 Electrons of equal energy passing through an electromagnetic lens are brought to different focal points. The closer the electrons are to the windings, the more the electrons are deflected by the magnetic field and the closer the focal point is to the lens.

구면수차는 연속적인 **focal point** 를 가지며 이들 **focal point** 중 가장 작은 **electron beam diameter**를 가지는 부분을 **disc of least confusion(circle of minimum confusion)**이라 한다

전자현미경의 해상도는 **electron beam** 이 가장 작은 **diameter**의 **focal point**를 가질 때 가장 크다.

Electrostatic Stigmator

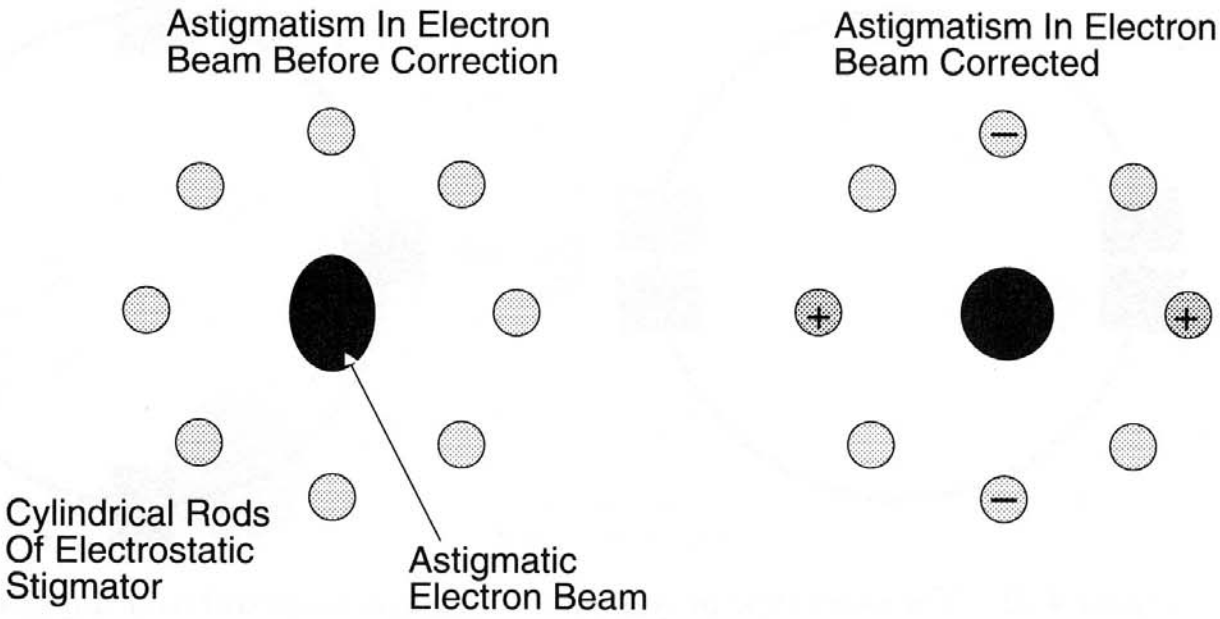


Figure 4-19 An electrostatic stigmator used to correct astigmatism of the electron beam by applying an electrostatic field to push and pull the electron beam into a circular cross section.

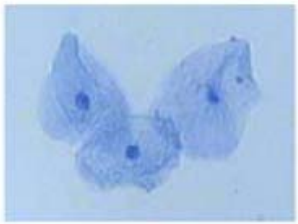
Stigmator: 전자빔의 비점수차**(astigmatism)**를 보정하는 장치

-찌그러진 전자빔을 **circular beam**으로 만든다**.**

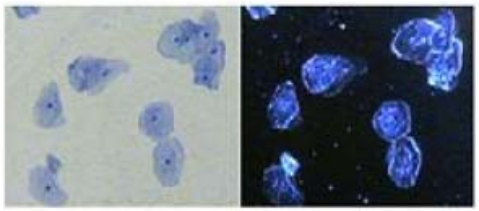
-정전기적**(electrostatic),** 자기적**(magnetic) stigmator**로 나누어짐

Contrast is number of shades found in an image. A high contrast picture will have only two shades, black and white. The more shades you have, the less contrast, but more information.

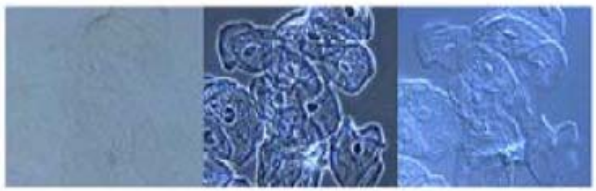
Color is also considered a form of contrast.



Absorption contrast



Diffraction contrast



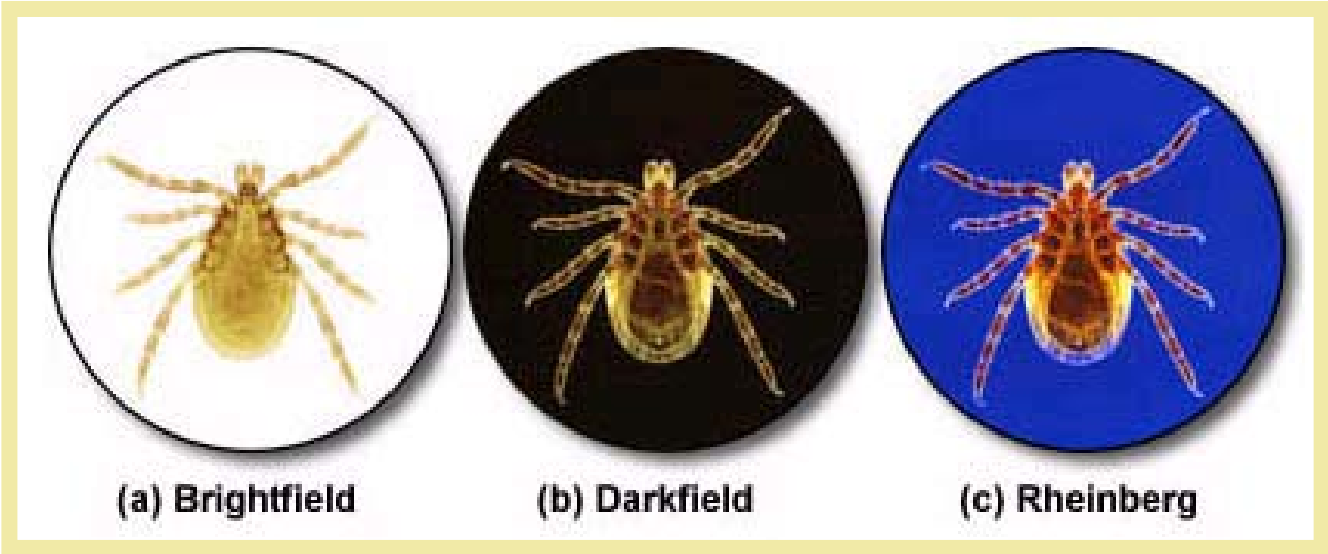
Interference contrast

Contrast & Visibility

Absorption
Reflection
Interference
Polarization
Fluorescence
…..

Observation Modes

- Brightfield - This is the basic microscope configuration This technique has very little contrast.
- Darkfield - This configuration enhances contrast.
- Rheinberg illumination - This set-up is similar to darkfield, but uses a series of filters to produce an "optical staining" of the specimen.



Imaging Modes

- Bright-field and dark-field are the most commonly used examination modes.

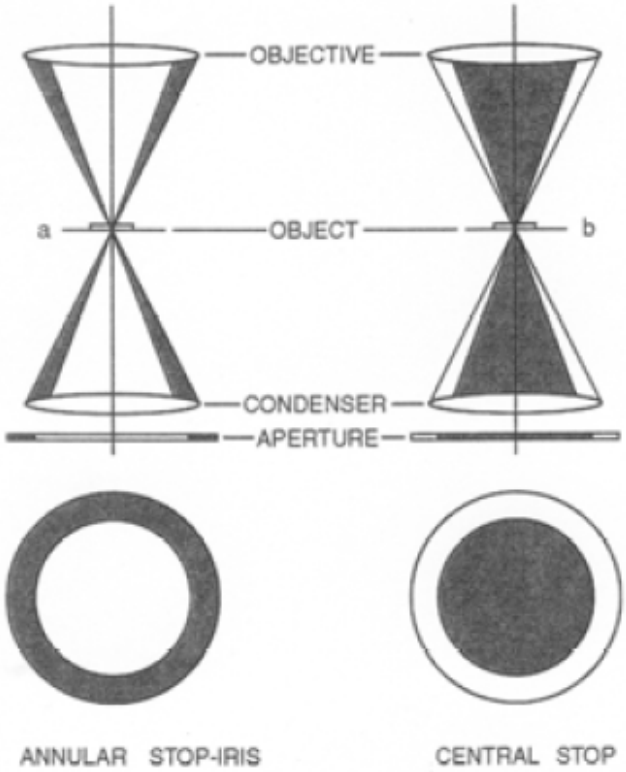
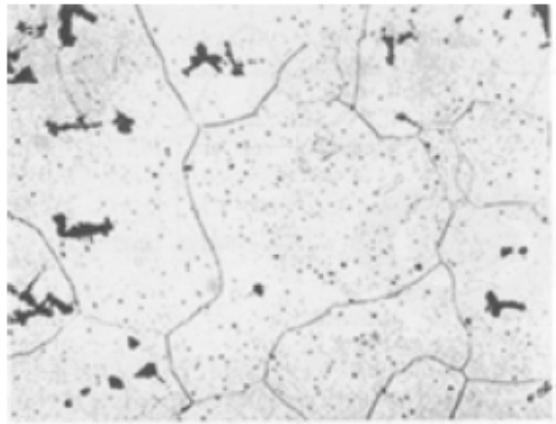


Figure 1.28 (a) Bright-field illumination; and (b) dark-field illumination in transmitted mode. Shaded areas indicate where the light is blocked.

BF



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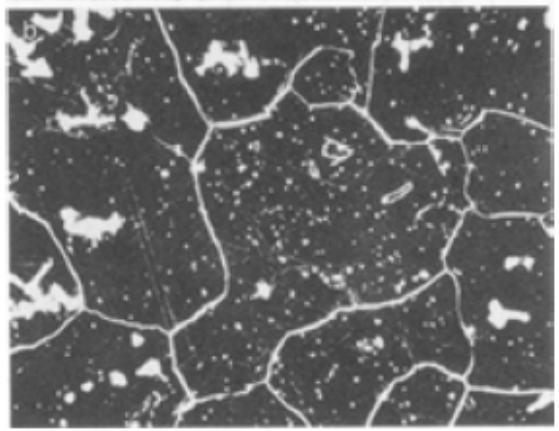


Examination Modes
Bright Field

- Bright-field examination is the most widely used method of observation.

- Surfaces of the sample that are normal to the incident light appear to be bright. Surfaces oblique to the incident light reflect less back and appear darker.

- The natural color of the material can also be seen in this mode. For example, if there is a color difference in the phases present it would add contrast to the image.

Examination Modes
Dark Field

- Dark field microscopy is usually use to examine things such as cracks, pores, voids, and inclusions.

- It works opposite of bright-field microscopy in that no light that is directly reflected from the sample contributes to the image.

- Only the light ray that are deflected by diffuse scattering enter back into the objective lens for image formation

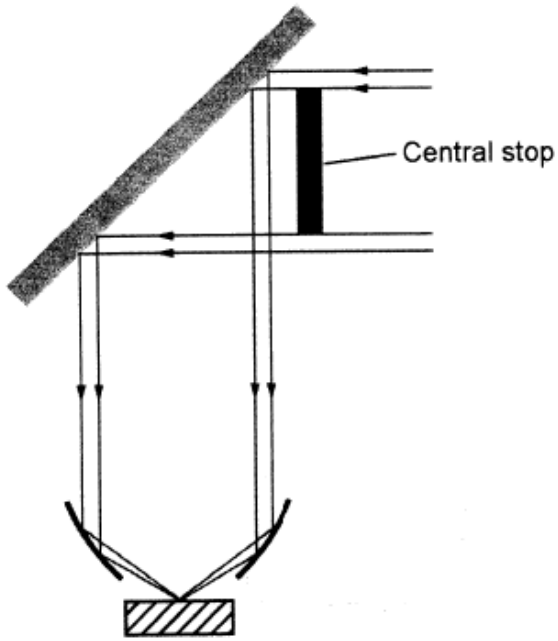
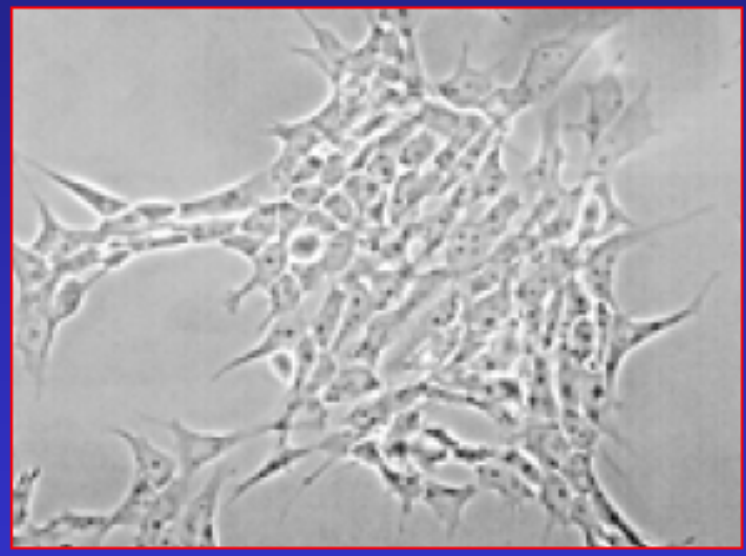


Figure 1.29 Dark-field illumination in a reflected light microscope.

Phase contrast

Many objects show only very little absorption but can be visualized in a phase contrast microscope

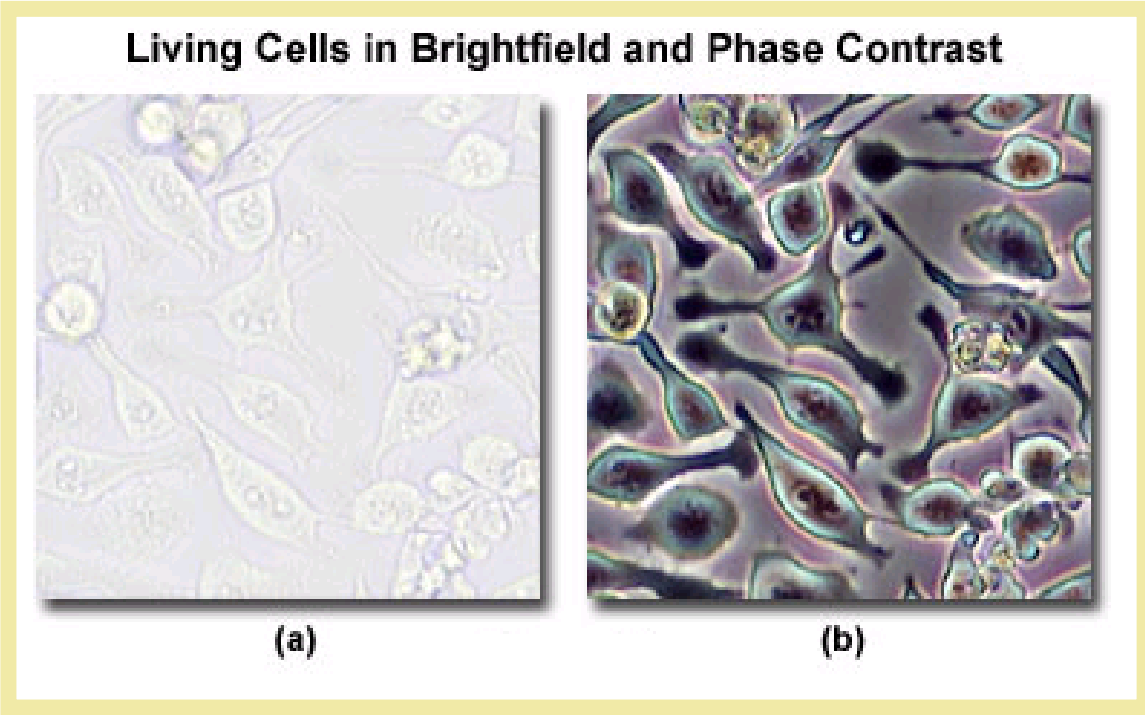
Bright field microscope



Phase contrast microscope

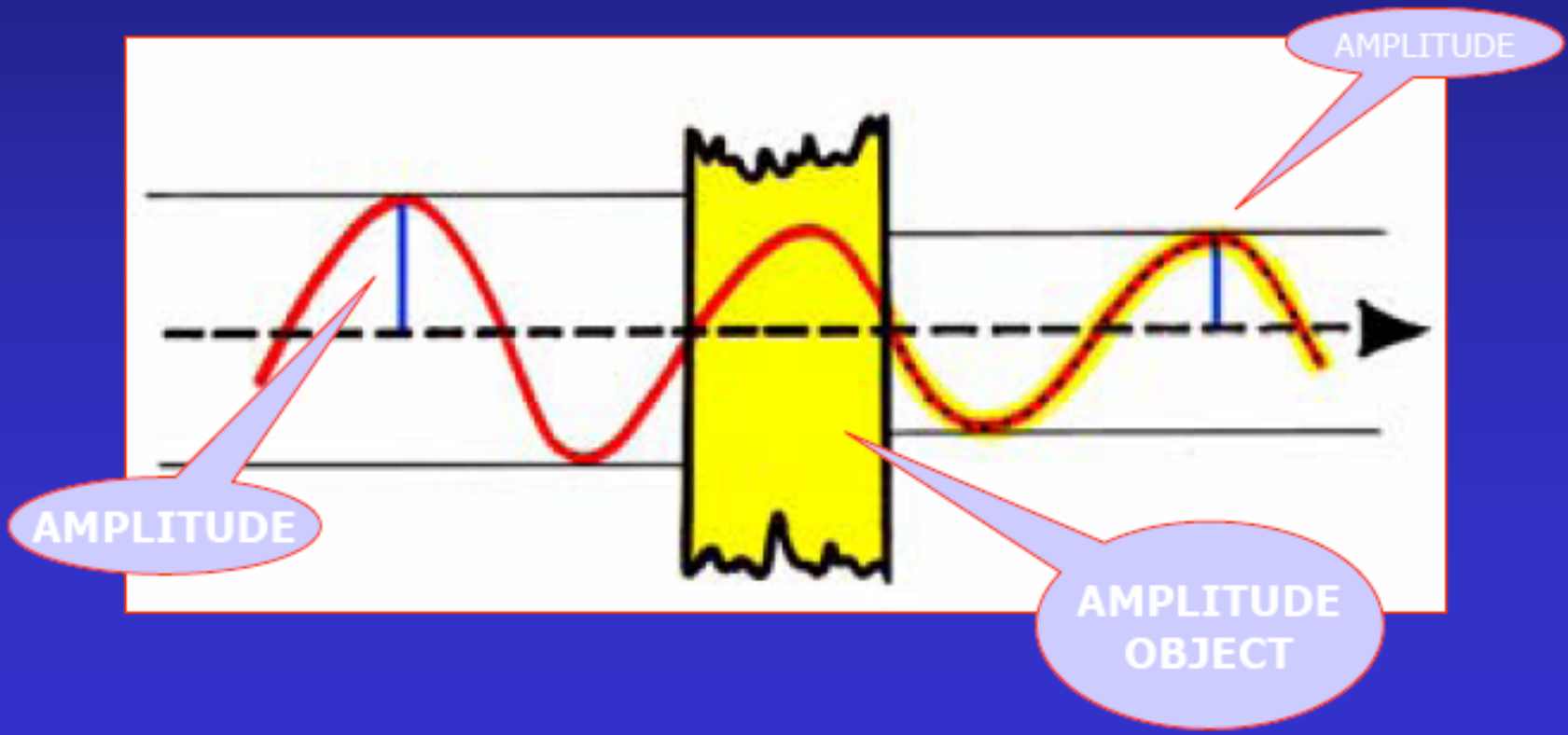
Observation Modes

•**Phase contrast** - In a phase-contrast microscope, the annular rings in the objective lens and the condenser separate the light. The light that passes through the central part of the light path is recombined with the light that travels around the periphery of the specimen. The interference produced by these two paths produces images in which the dense structures appear darker than the background.



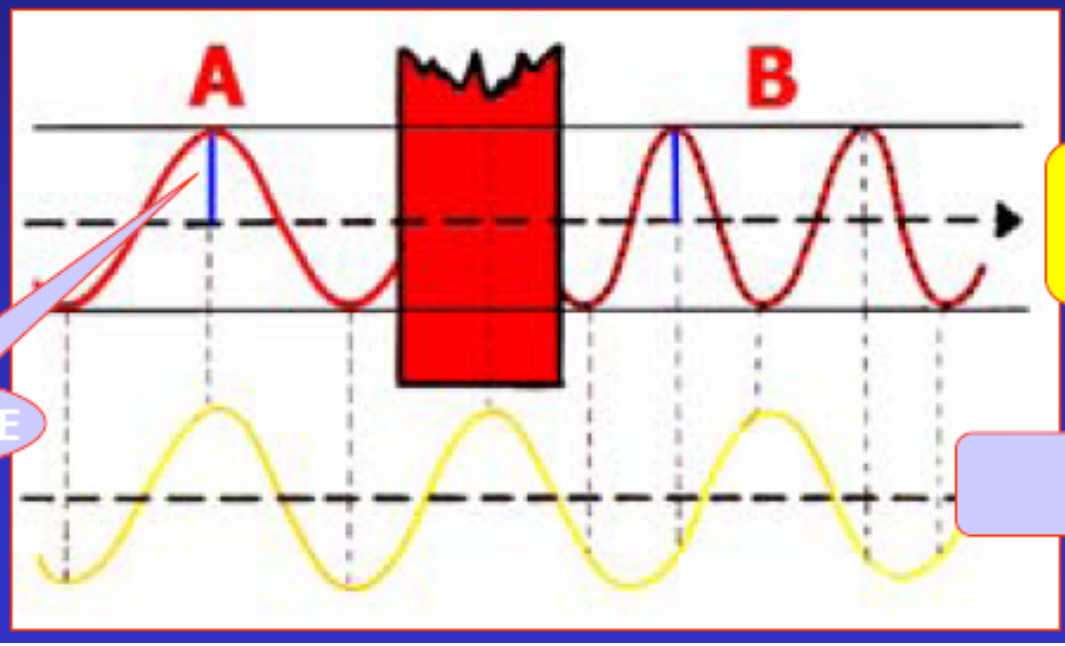
Amplitude object

Absorbtion of light → reduction of the amplitude of the light waves.



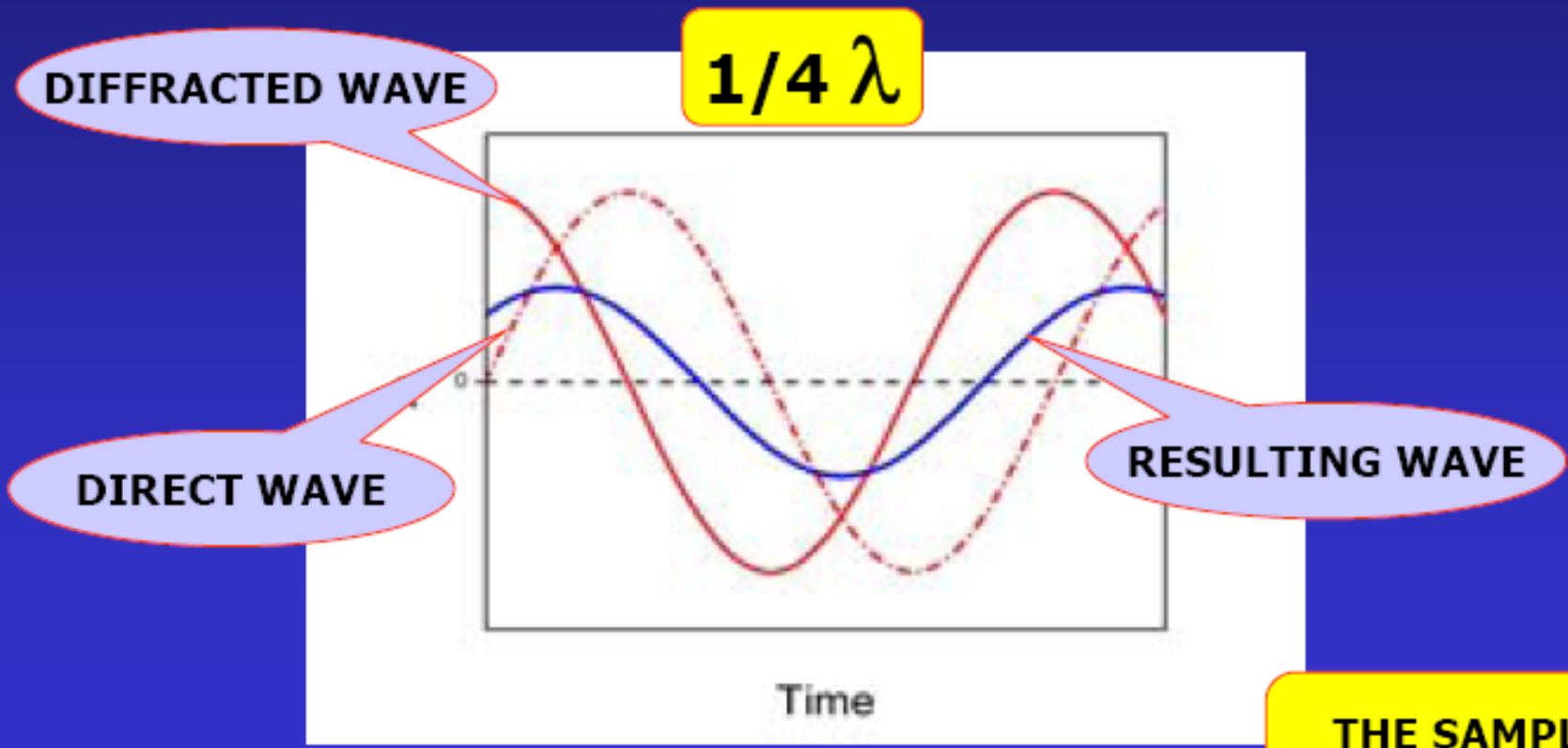
Phase objects

Difference in index of refraction
→ change of phase of the light wave + scattering



Phase contrast

The diffracted waves B have a phase difference of about 1/4 λ compared with the direct waves A → little difference in brightness



THE SAMPLE IS NOT VISIBLE

Phase Contrast

- For specimens with little inherent contrast in bright-field.

- Phase changes caused by light diffraction by an object is converted into an amplitude change..

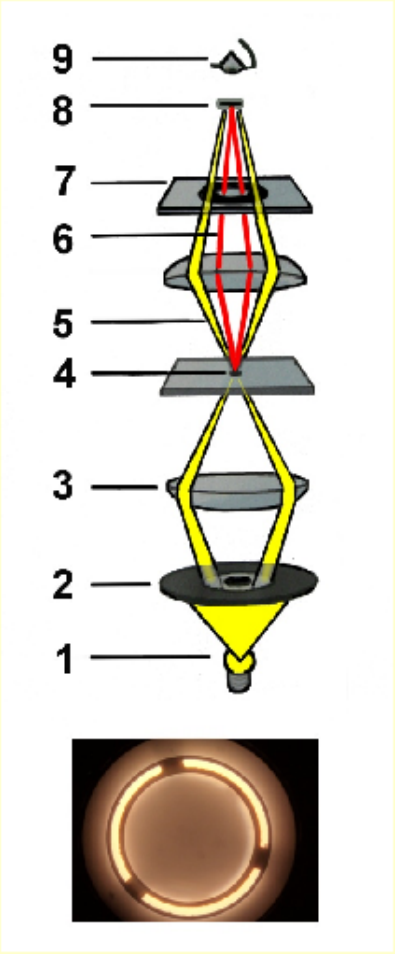


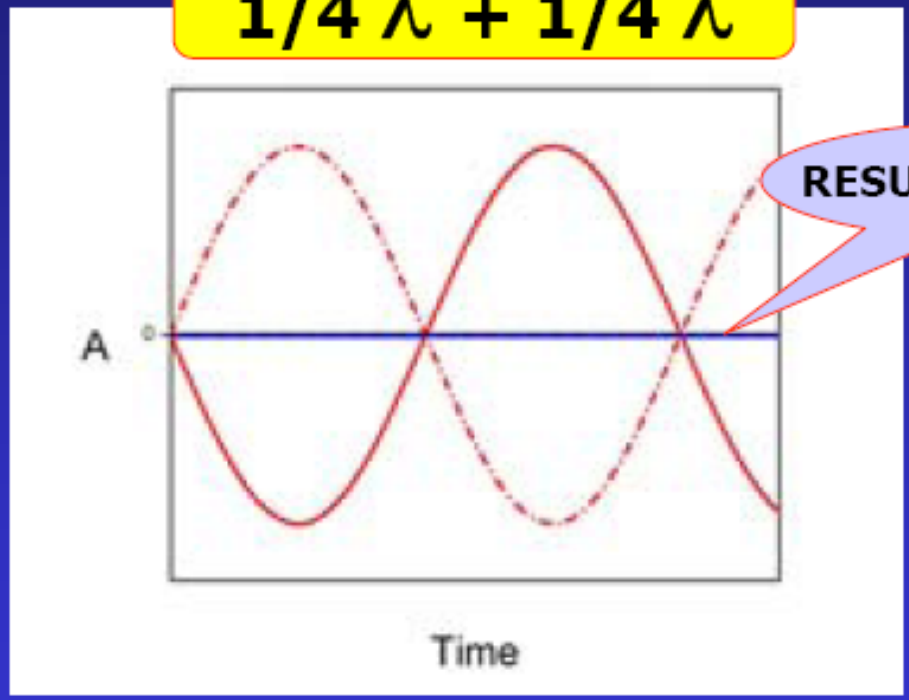
Fig. 1:
Simplified optical pathway for phase contrast microscopy (modified from 5)
Alignment of condenser annulus (bright) and phase ring (dark)

1 = light source
2 = annular shaped light mask (condenser annulus)
3 = condenser
4 = specimen
5 = background light
6 = light bent by the specimen
7 = phase ring
8 = eyepiece with intermediate image
9 = eye

Phase contrast

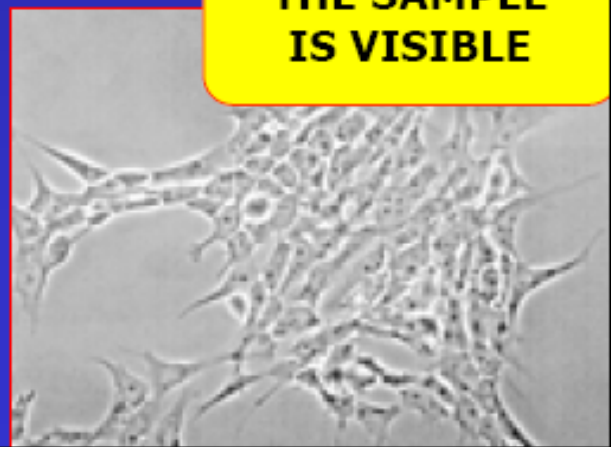
Add 1/4 λ extra phase shift → destructive interference

1/4 λ + 1/4 λ



RESULTING WAVE

THE SAMPLE IS VISIBLE

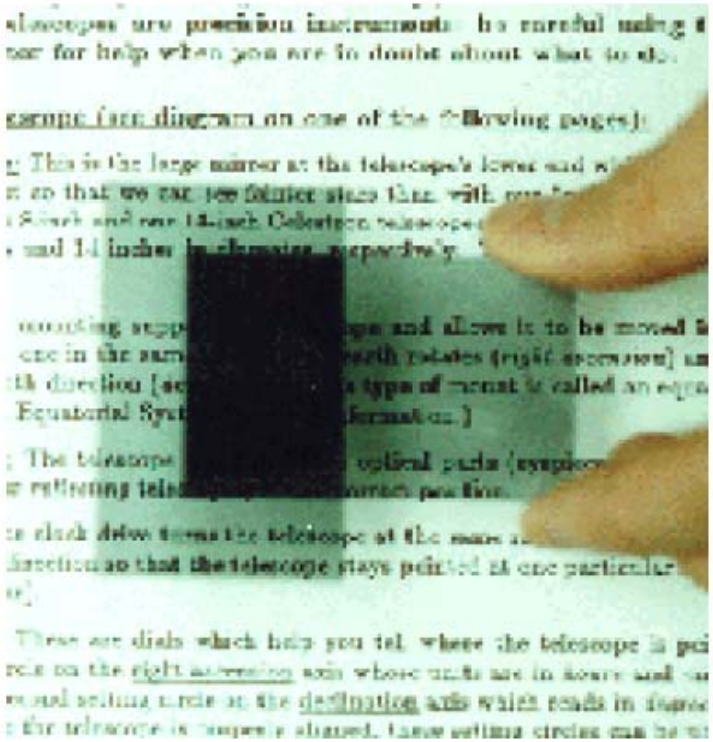


Polarized Light

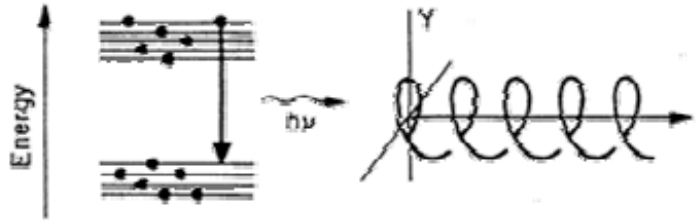
- Polarized light waves are light waves in which the vibrations occur in a single plane.

- It is used to examine materials that are optically anisotropic.

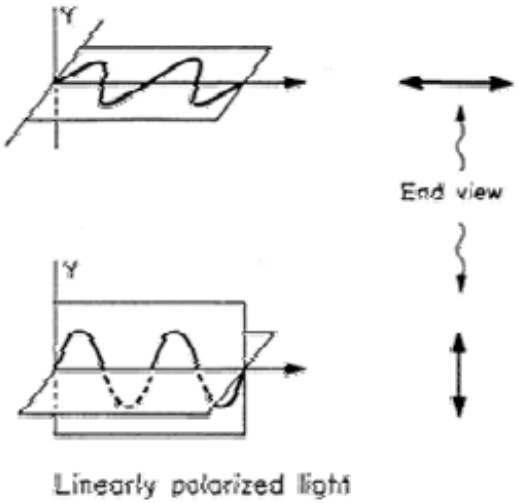
Polarized light



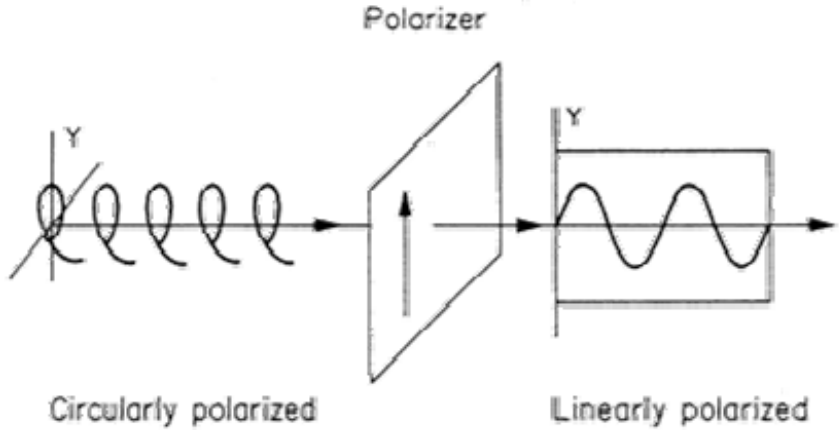
Polarized light



Light is emitted from an atom when an electron makes a transition from a higher energy state to a lower energy state.

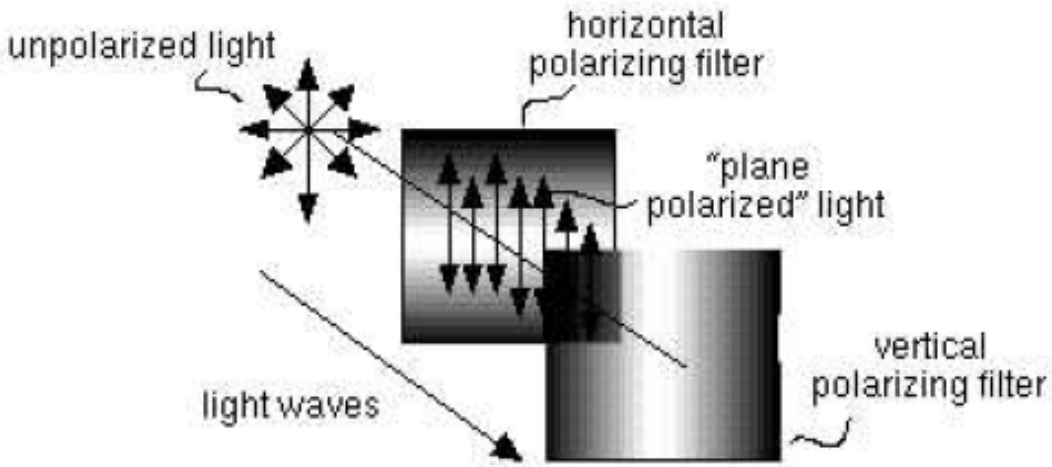


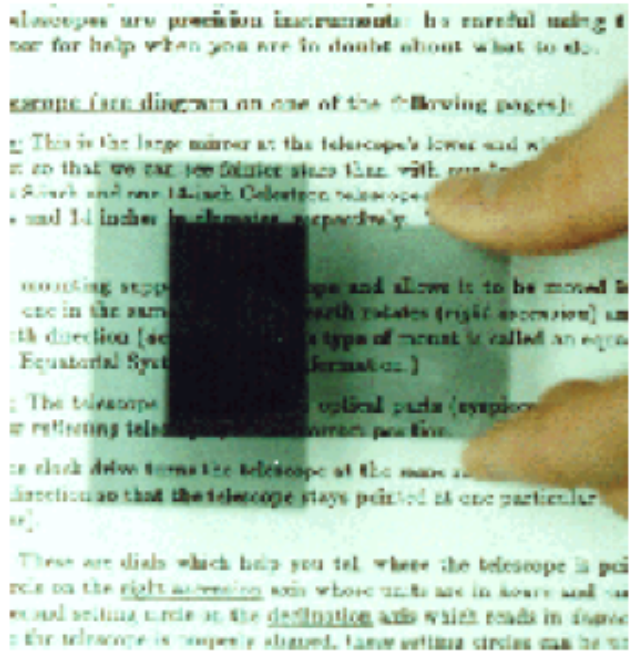
Two examples of linearly polarized light where the electric vector of the light wave vibrates in one plane.



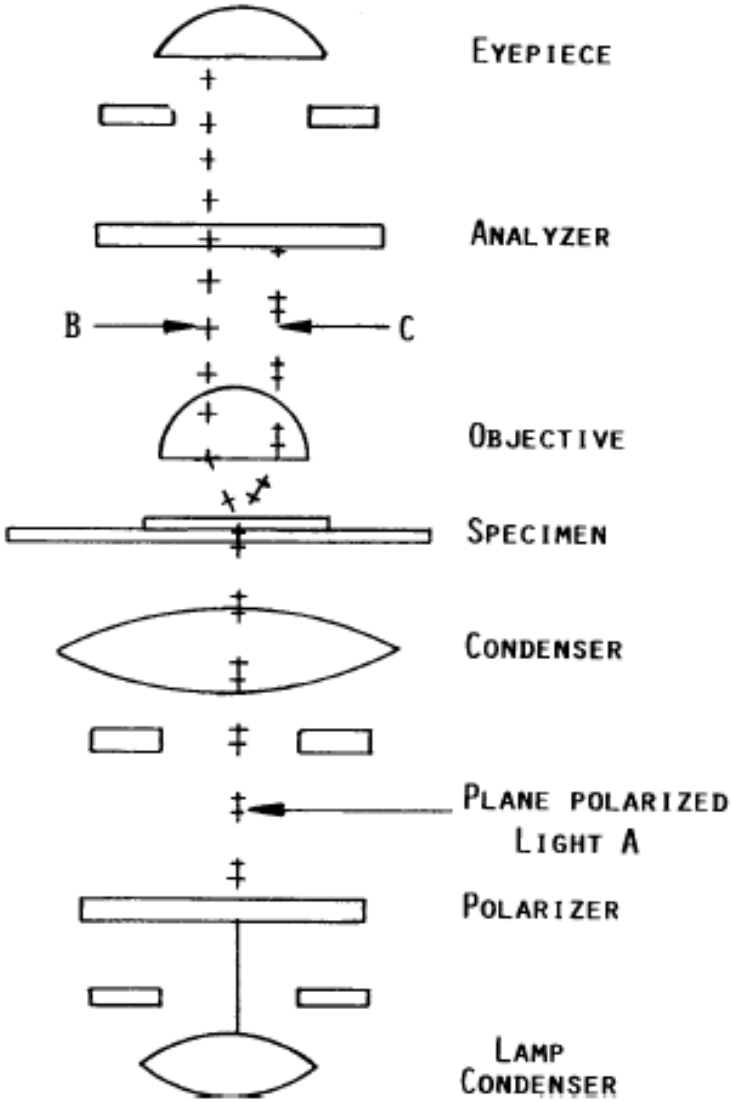
Circularly polarized light incident on a polarizer is transmitted as linearly polarized light.

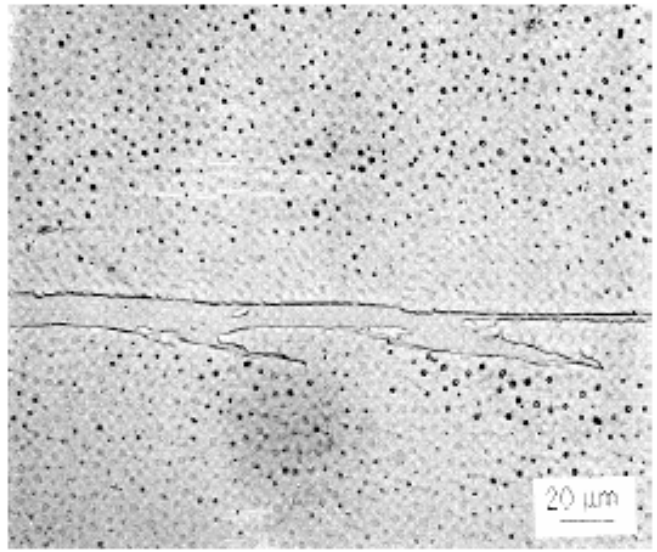
Polarized light

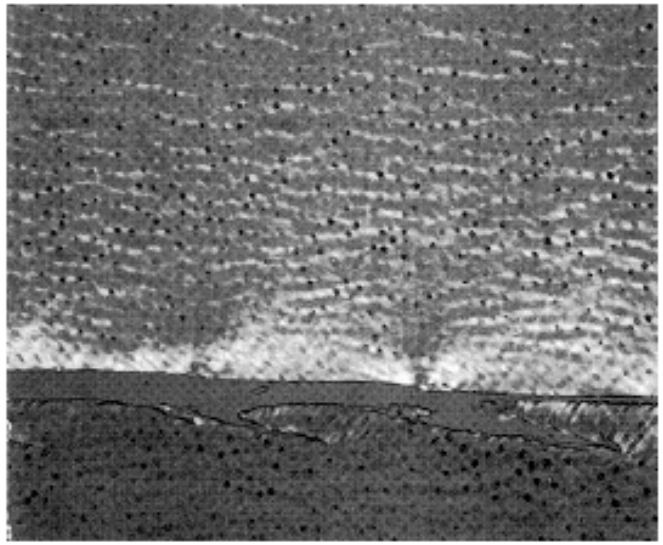


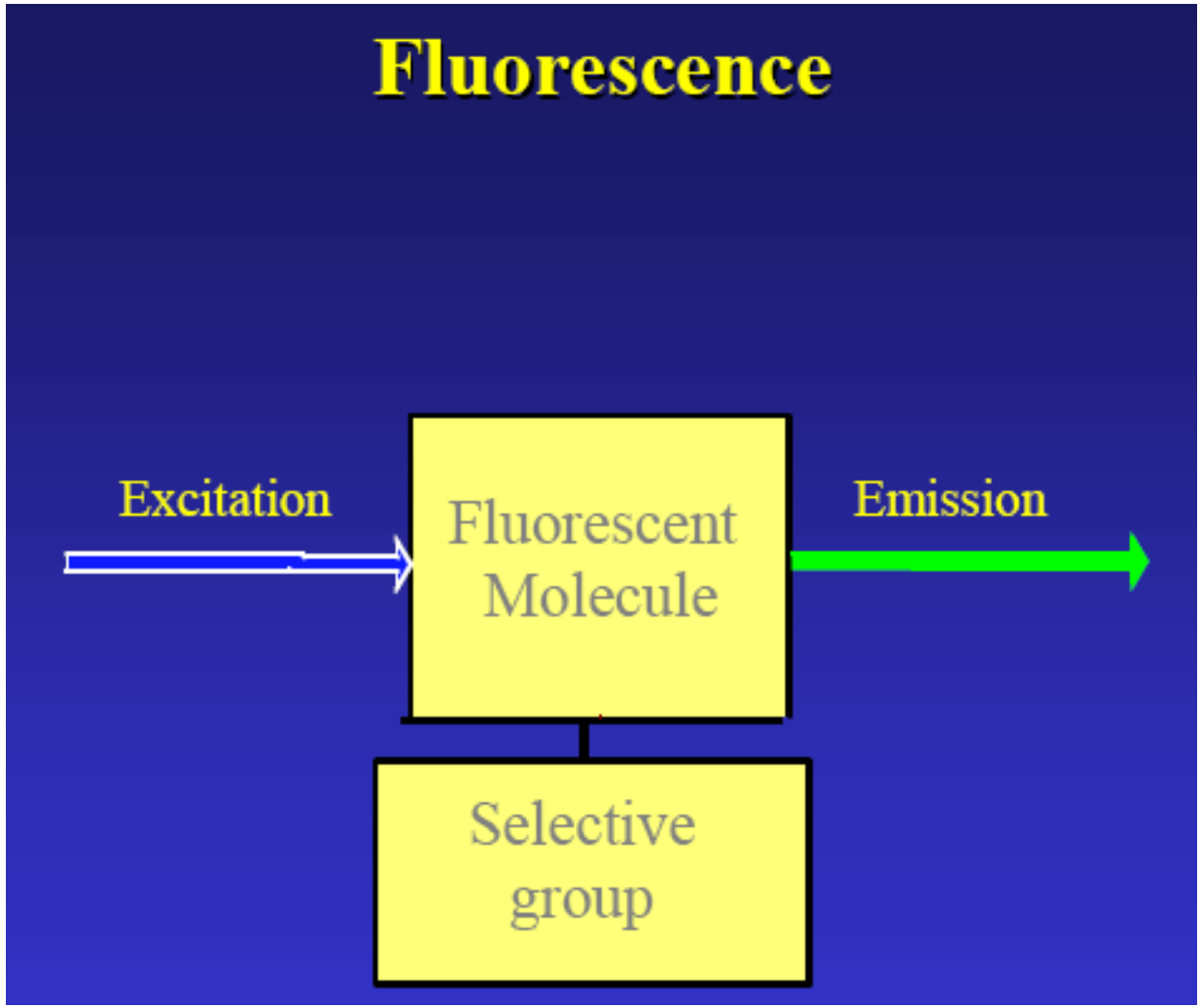


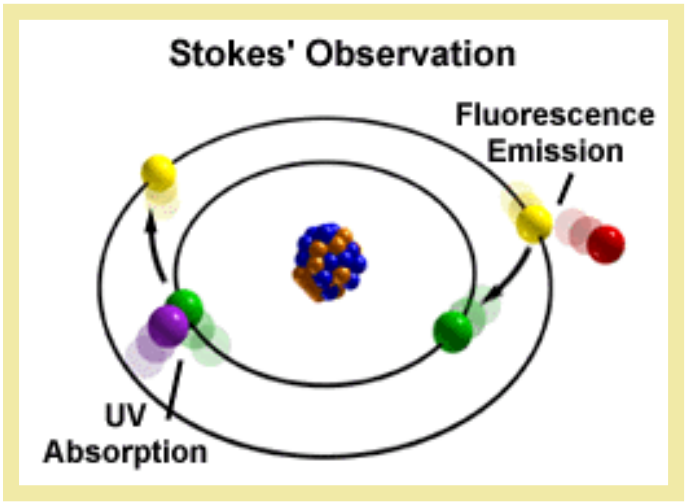
Polarized light microscopy

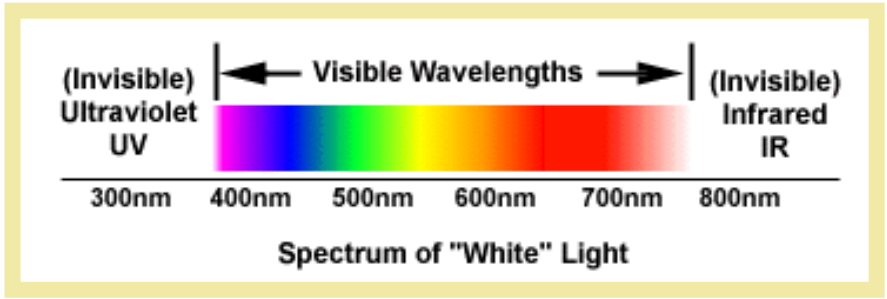


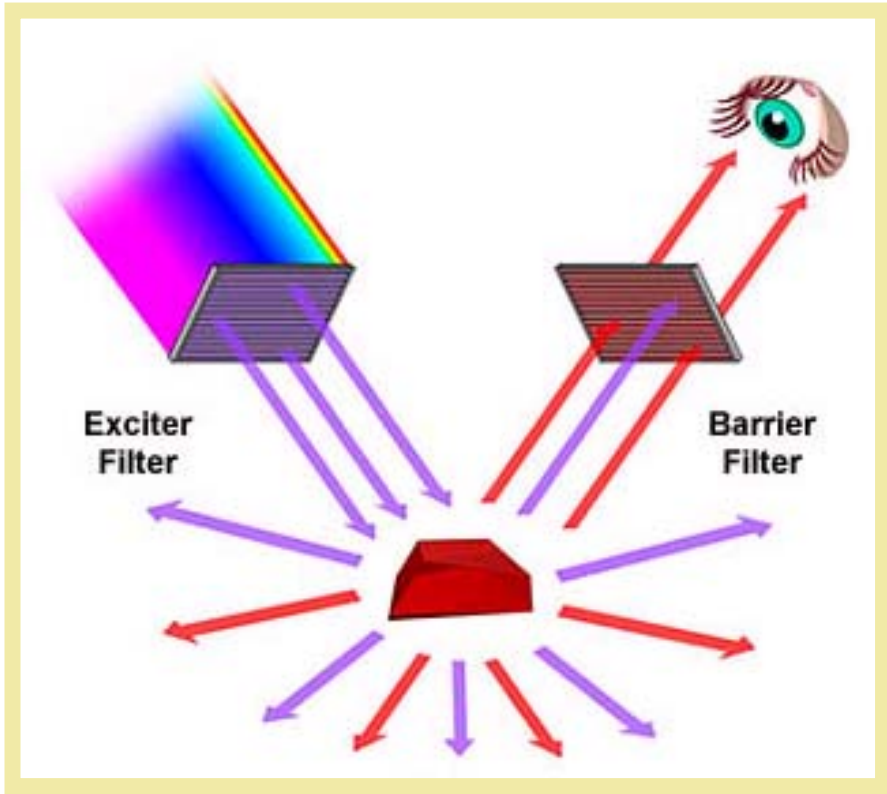




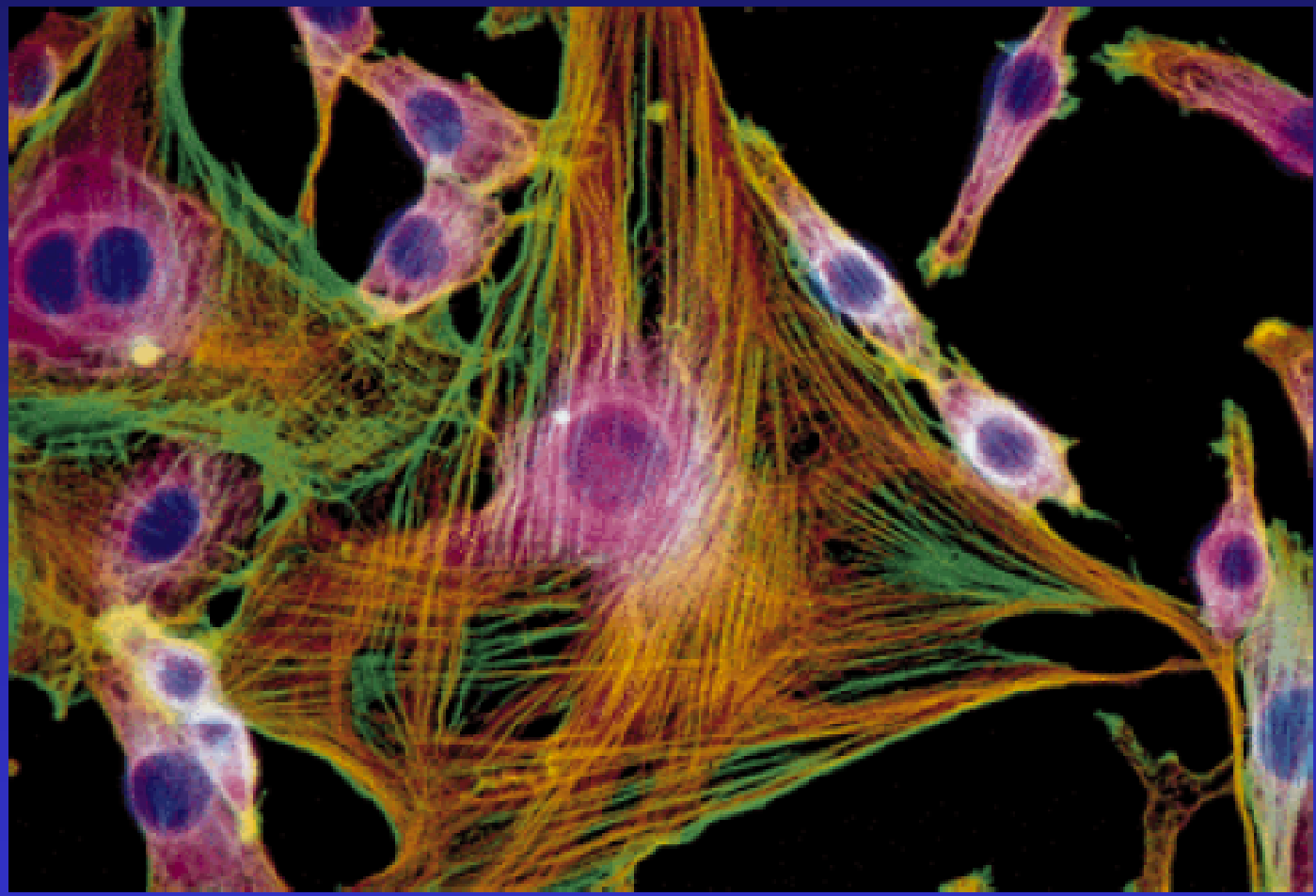

Fluorescence
Excitation
Fluorescent Molecule
Emission
Selective group


Stokes' Observation
Fluorescence
Emission
UV
Absorption


(Invisible)
Ultraviolet
UV
Visible Wavelengths
(Invisible)
Infrared
IR
300nm
400nm
500nm
600nm
700nm
800nm
Spectrum of "White" Light


Exciter
Filter
Barrier
Filter

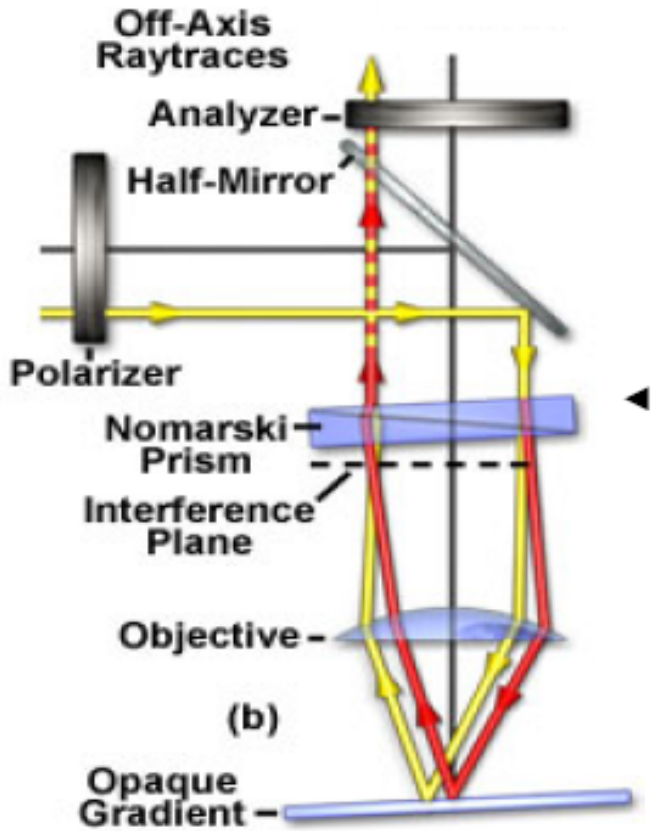
Fluorescence microscopy



Differential Interference Contrast (DIC)

- DIC produces images with emphasized topographic details.
- DIC requires several optical components, therefore it can be very expensive to set up.
- Light from an incandescent source is passed through a polarizer, so that all of the light getting through must vibrate in a single plane. The beam is then passed through a prism that separates it into components that are separated by a very small distance - equal to the resolution of the objective lens. The beams pass through the condenser, then the specimen
- In any part of the specimen in which adjacent regions differ in refractive index the two beams are delayed or refracted differently. When they are recombined by a second prism in the objective lens there are differences in brightness corresponding to differences in refractive index or height of the feature.

Differential Interference Contrast (DIC)



◄ Separates linearly polarized light into two perpendicular components and produces a phase shift in each wavefront.

Another nice description of things can be found on Wikipedia

http://www.microscopyu.com/articles/dic/reflecteddic.html

Examination Modes

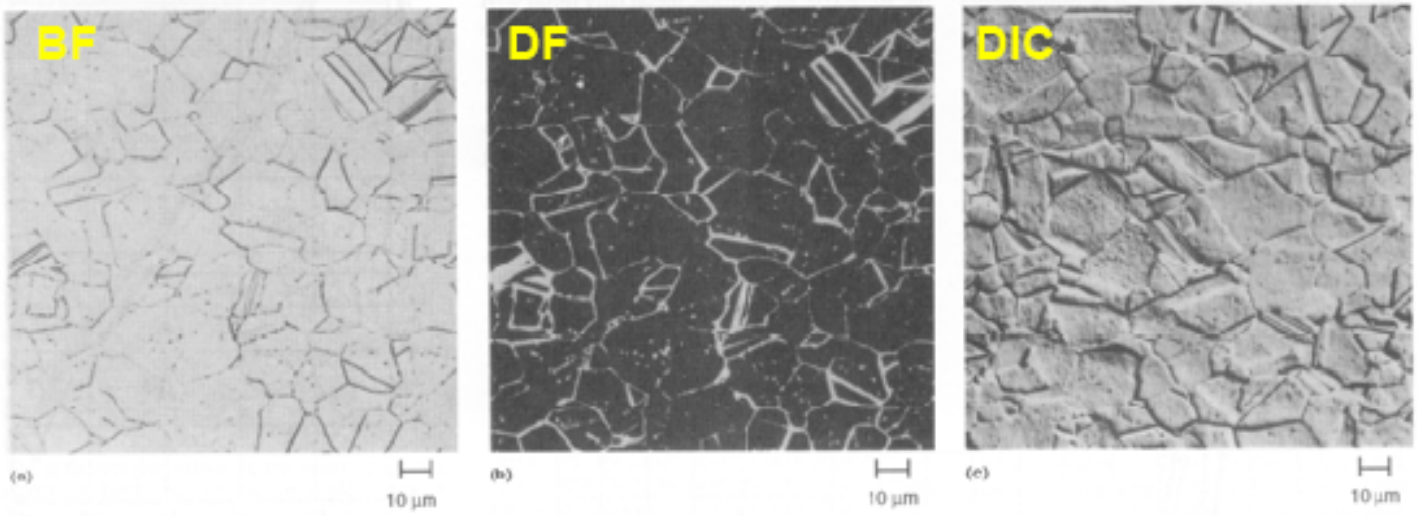


Fig. 19 Austenitic stainless steel (Fe-20Cr-13Ni-2.5Mo-3.5Cu and Nb + Ta), solution annealed. (a) Bright-field illumination. (b) Dark-field illumination. (c) Differential interference-contrast illumination. 15 mL HCl, 10 mL acetic acid, 10 mL HNO_3, and 2 drops glycerol. 400×

ASM Handbook ,Vol. 9, *Metallography and Microstructures*, ASM International, Materials Park, OH (2004), pg. 340

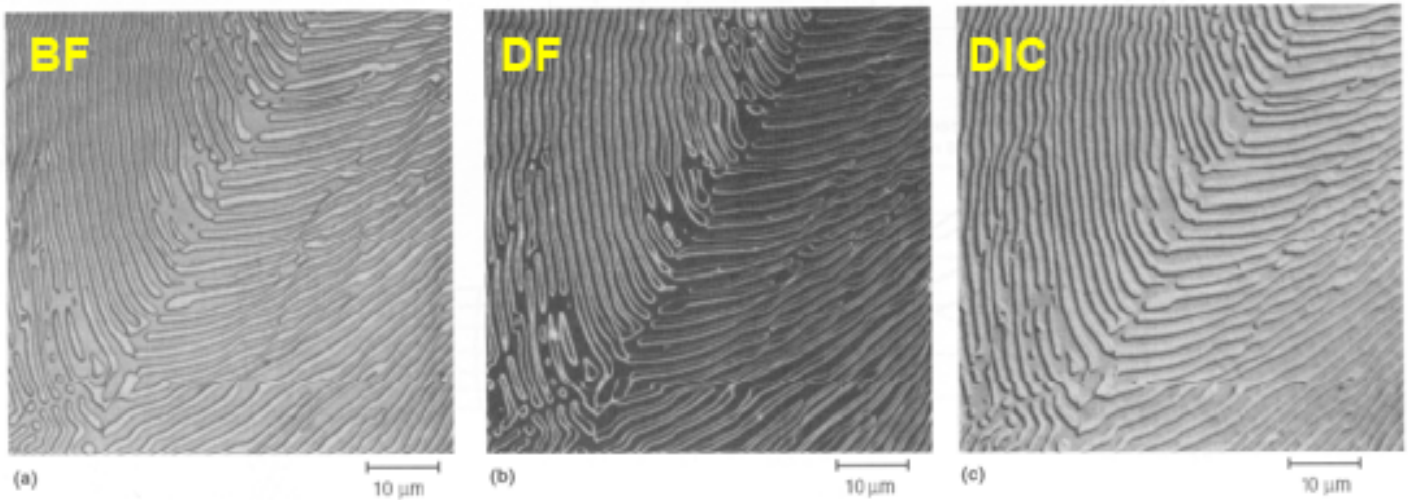


Fig. 20 Cu-8.9P sand cast alloy showing the α + Cu_3P eutectic. (a) Bright-field illumination. (b) Dark-field illumination. (c) Differential interference-contrast illumination. Swab etched using an aqueous solution of 3% $(NH_4)_2S_2O_8$ and 1% NH_4OH. 1000×

Examination Modes

Contrast Enhancing Techniques	
Specimen Type	**Imaging Technique**
Specular (Reflecting) Surface Thin Films, Mirrors Polished Metallurgical Samples Integrated Circuits	Brightfield Illumination Phase Contrast, DIC Darkfield Illumination
Diffuse (Non-Reflecting) Surface Thin and Thick Films Rocks and Minerals	Brightfield Illumination Phase Contrast, DIC Darkfield Illumination
Amplitude Surface Features Dyed Fibers Diffuse Metallic Specimens Composite Materials Polymers	Brightfield Illumination Darkfield Illumination
Birefringent Specimens Single Crystals Oriented Films	Polarized Illumination

Recording The Image
Film

- The best method for capturing an image is film.
- Fine grain film yields the best resolution although they require longer exposure time.
- Detail are preserved upon enlarging.
- Does require a steady stage to eliminate vibrations.

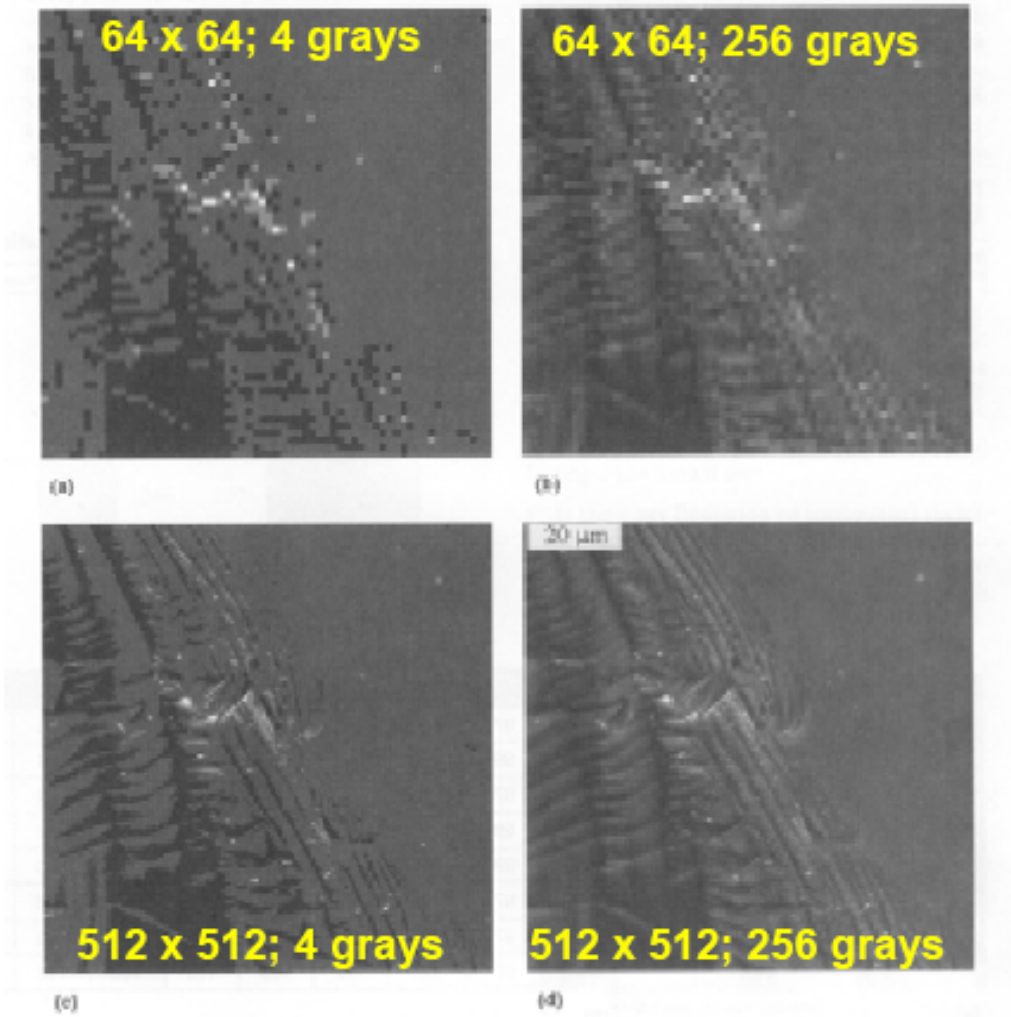
Recording The Image

Digital

- The use of digital photography has become a popular choice because it saves a lot of time.
- Even so digital imaging if done improperly can ruin the quality of an image.
- Care must be taken to ensure the resolution and quantization of a digital image is high enough that it adequately show all of the features of the sample.

Recording The Image

Digital



ASM Handbook ,Vol. 9, *Metallography and Microstructures*, ASM International, Materials Park, OH (2004), pg. 369

Fig. 3 The effect of resolution and quantization on a digital image. The same image as Fig. 2 in different levels of resolution and quantization. (a) 64 x 64 pixels and four gray levels. (b) 64 x 64 pixels and 256 gray levels. (c) 512 x 512 pixels and four gray levels. (d) 512 x 512 pixels and 256 gray levels.

•**Differential interference contrast** (DIC) - DIC uses polarizing filters and prisms to separate and recombine the light paths, giving a 3-D appearance to the specimen (DIC is also called **Nomarski** after the man who invented it).

•**Hoffman modulation contrast** – this contrast is similar to DIC except that it uses plates with small slits in both the axis and the off-axis of the light path to produce two sets of light waves passing through the specimen.

•**Polarization** - The polarized-light microscope uses two polarizers, one on either side of the specimen, positioned perpendicular to each other so that only light that passes through the specimen reaches the eyepiece. Light is polarized in one plane as it passes through the first filter and reaches the specimen. Regularly-spaced, patterned or crystalline portions of the specimen rotate the light that passes through. Some of this rotated light passes through the second polarizing filter, so these regularly spaced areas show up bright against a black background.

•**Fluorescence** - This type of microscope uses high-energy, short-wavelength light (usually ultraviolet) to excite electrons within certain molecules inside a specimen, causing those electrons to shift to higher orbits. When they fall back to their original energy levels, they emit lower-energy, longer-wavelength light (usually in the visible spectrum), which forms the image