



Analytical Methods for Materials

Lesson 2

Principles of Optical Microscopy

Suggested Reading

- Y. Leng, *Materials Characterization, 2nd Edition*, (2013), Wiley, Hoboken, NJ – Chapter 1.

Reference

- Goodhew, Humphreys and Beanland, Chapter 1
- Brandon and Kaplan, Chapter 3, pp. 123-177
- K. Geels, D.B. Fowler, W-U. Kopp, and M. Rückert, *Metallographic and Materialographic Specimen Preparation, Light Microscopy, Image Analysis and Hardness Testing*, (2007) ASTM International, West Conshohocken, PA.
- G.F. Vander Voort, *Metallography Principles and Practice*, (1999) ASM International, Materials Park, OH.

Additional Resources

1. ASM Handbook, Volume 9, ASM International, Materials Park, OH (2004).
2. MECH 4430 Lecture notes by Dr. Jingshen Wu and Dr. Yang Leng, Department of Mechanical Engineering, Hong Kong University of Science and Technology (<http://www.me.ust.hk/~mejswu/>). This is the author of the required textbook.
3. Buehler web site (<http://buehler.com>).
4. Nikon web site (<http://www.microscopyu.com>).
5. University of Cambridge, DoITPoMS Teaching and Learning Packages (<http://www.doitpoms.ac.uk/tlplib/optical-microscopy/index.php>).
6. Olympus Microscopy Resource Center (<http://www.olympusmicro.com/>).

Optical Microscopy (OM)

- Most widely utilized method to examine microstructures of materials.
- Used in:
 - Quality control for materials processing, product development, etc.
 - Determining why a material failed
 - Establishing structure-property relationships in materials

Some Types of Optical Microscopes

1. Simple optical microscope → One lens; 25x; 10 μm resolution
2. Stereoscopic microscope → Two lens trains; 6-8x
3. Compound optical microscope → Objective + eyepiece + condenser lenses; 1300x; 1 μm resolution.



Refraction

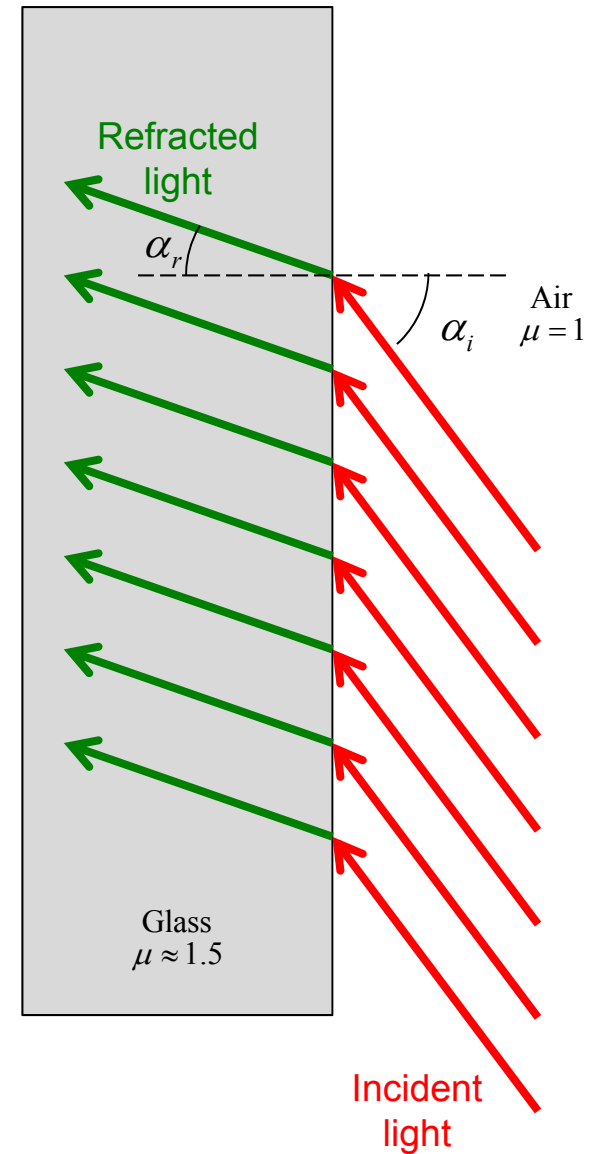
- Provides the physical basis for optical lenses.
- Deflection of light due to density changes in from one substance to the next.
- Defined by refractive index, μ .



where:

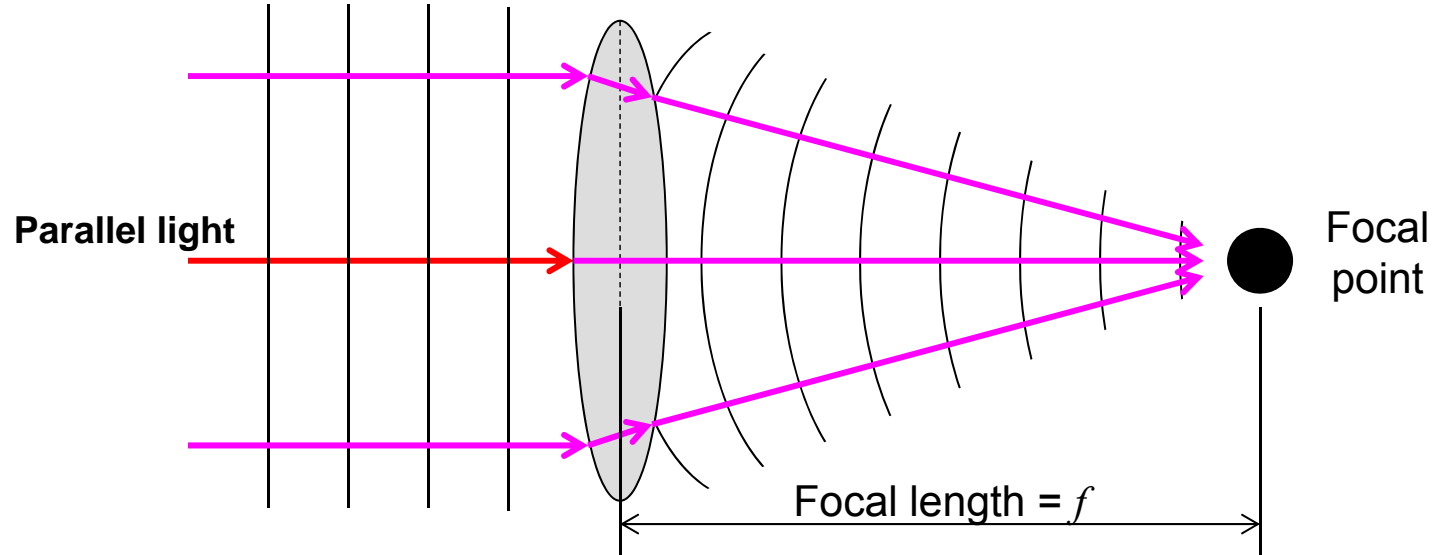
α_i = incident angle

α_r = refracted angle



Effect of Refraction in Lenses

- Because the refracted angle α_r depends on the incident angle α_i , a convex lens can be used to focus light to a point at a specified distance from the lens.



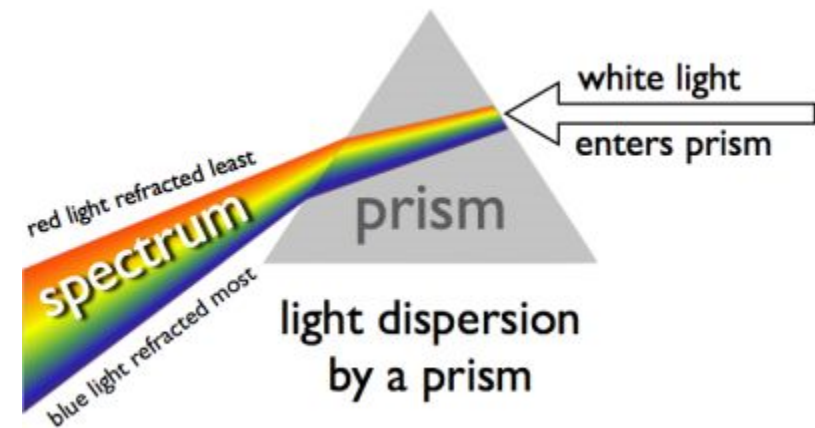
- Front and back faces don't need to be symmetric.
- Can further enhance by modifying μ outside the lens.

Dispersion

- Light with different λ 's refracts differently.
 - E.g., violet light ($\lambda \sim 400$ nm) refracts more than red light ($\lambda \sim 700$ nm).



http://gallery.hd.org/_c/natural-science/prism-and-refraction-of-light-into-rainbow-AJHD.jpg.html

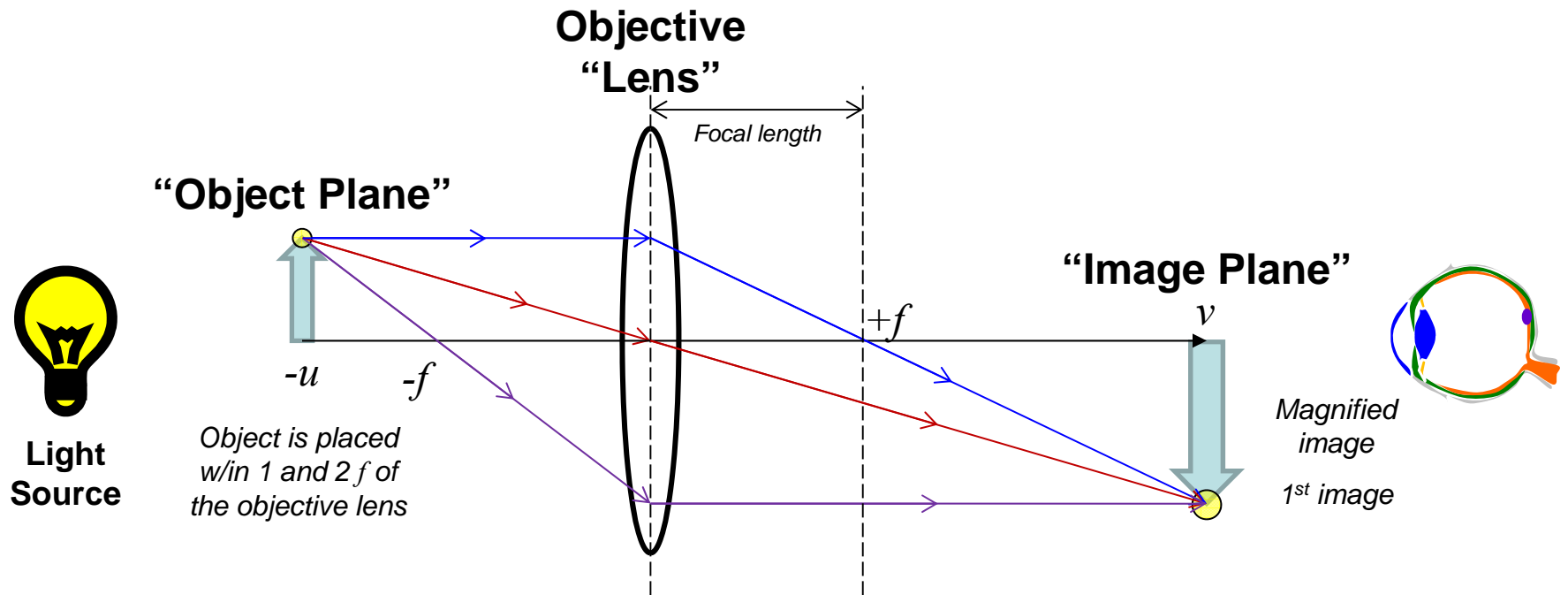


www.rkm.com.au/.../animation-physics-prism.html

<http://science.howstuffworks.com/question41.htm>

- This is why we see rainbows.

Optical Principles (Convex Lens)



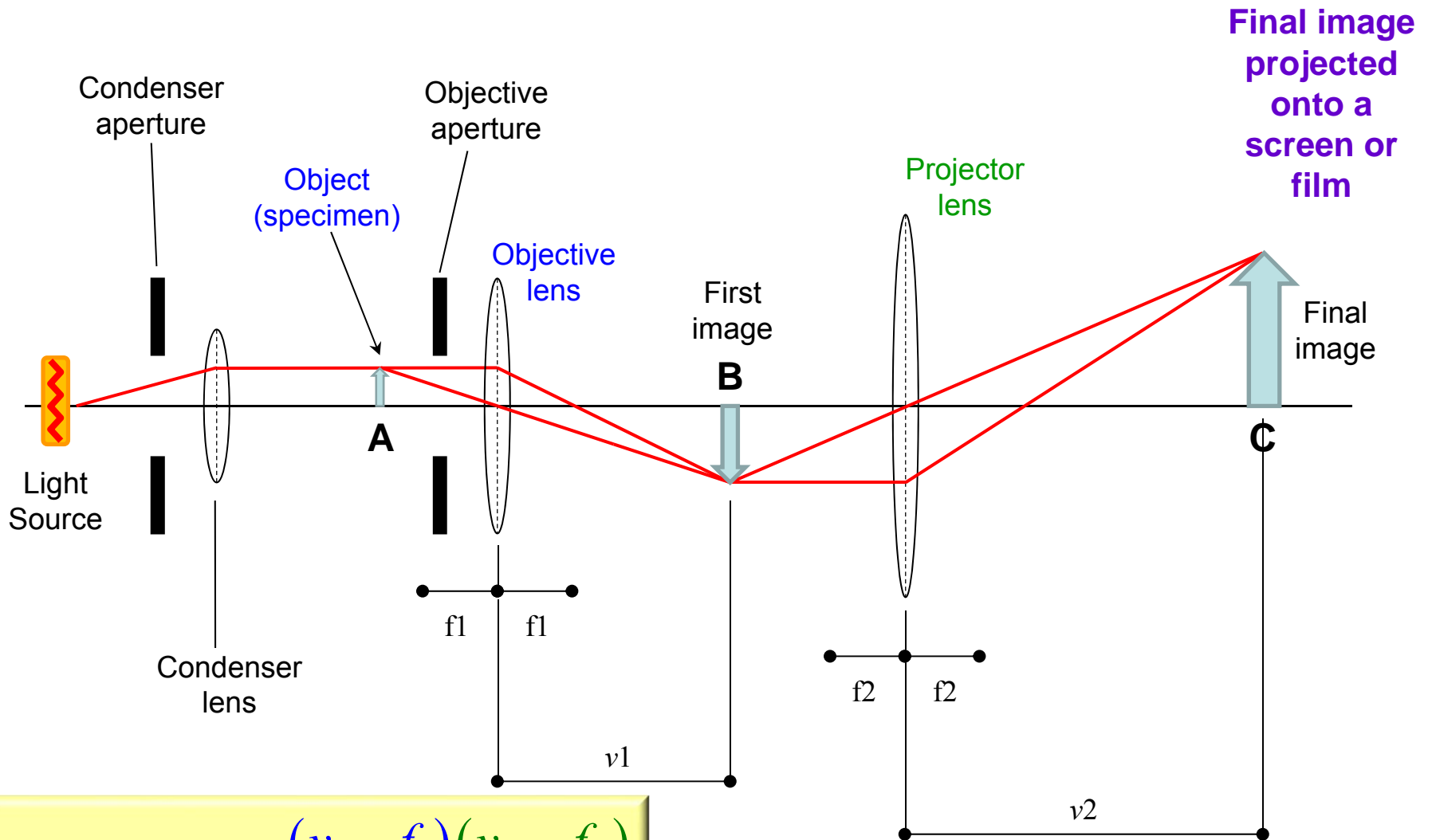
For focal length f , an object at $-u$ gives an image at v which is magnified by a factor M , where:

$$\frac{1}{u} + \frac{1}{v} = \frac{1}{f} \quad \text{and} \quad M = \frac{v-f}{f} = \frac{v}{u}$$

Multiple lenses can be used to increase magnification

Magnification in Compound Microscope

(transmission illumination)



$$M = M_1 M_2 = \frac{(v_1 - f_1)(v_2 - f_2)}{f_1 f_2}$$

Total mag. is product of mags. for each lens.

Microscope with an eyepiece

- Light path goes through the eyepiece rather than through the projector lens to form a virtual image on the retina.
- Virtual image is usually taken as 25 cm from the eyepiece.
- In modern microscopes you can switch between eyepiece and projector lens for digital recording.

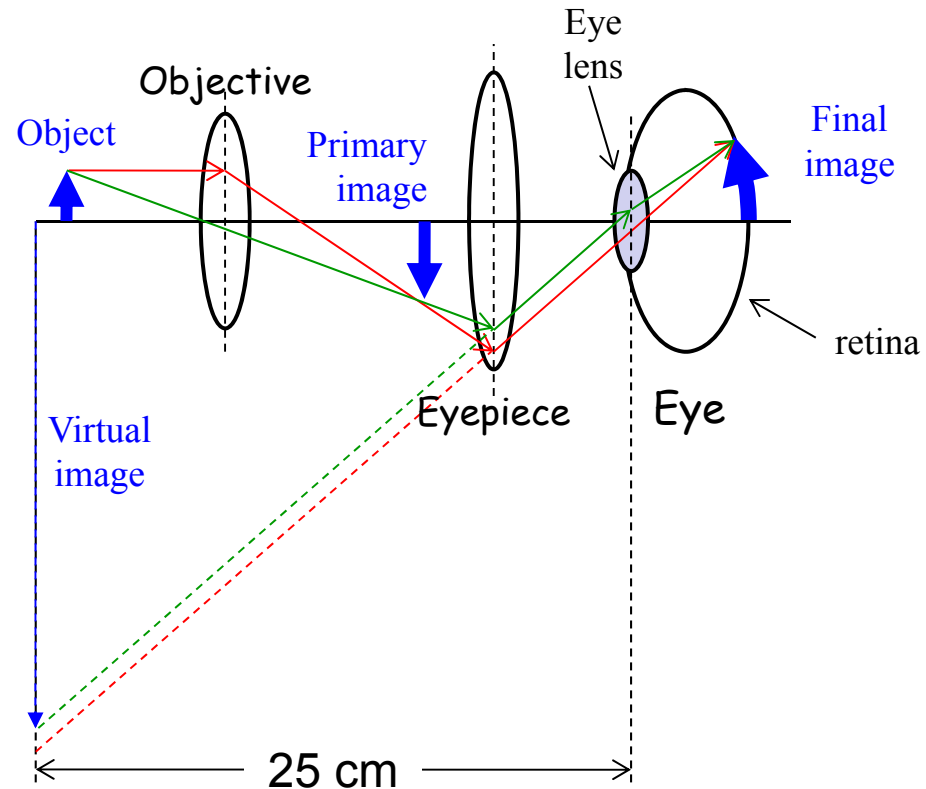
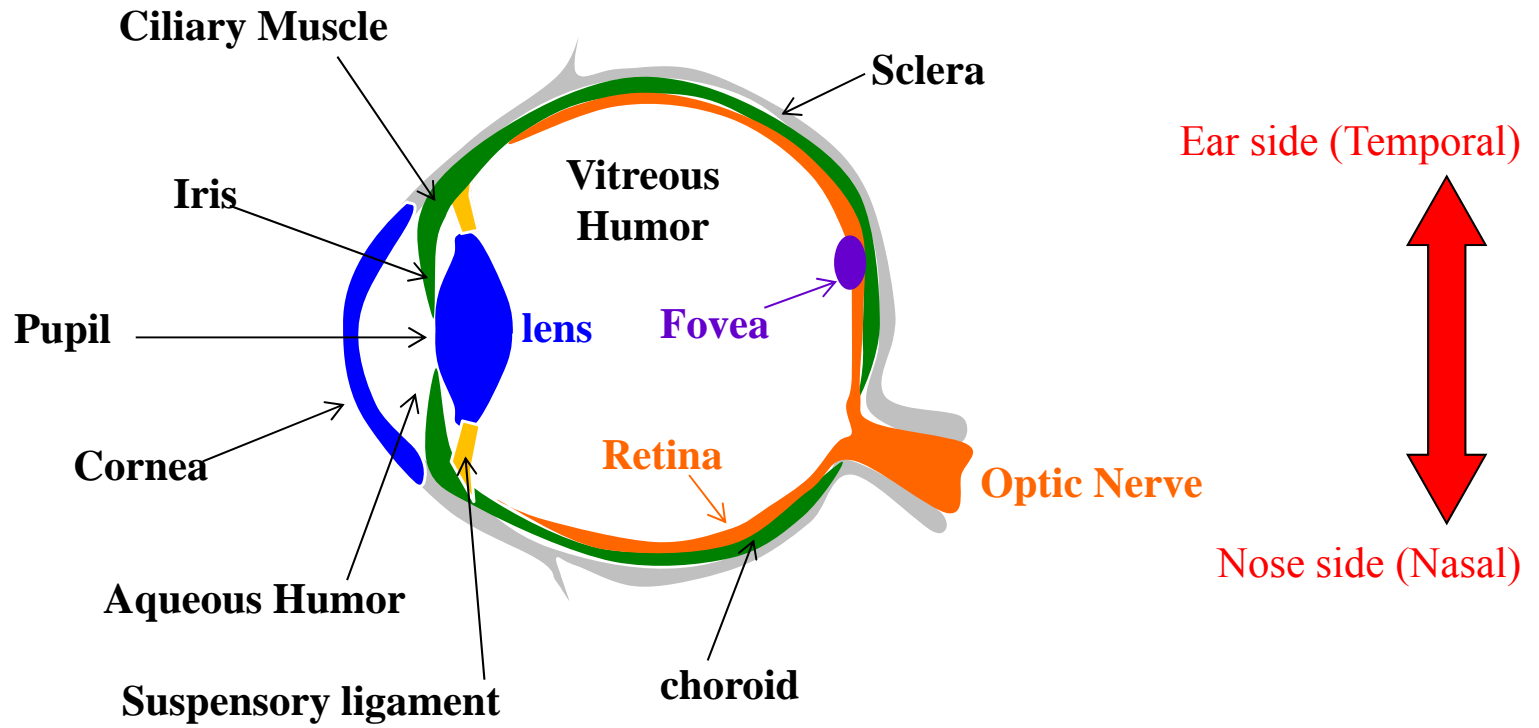


Figure 1.2 Schematic path of light in a microscope with eyepiece. The virtual image is reviewed by a human eye composed of an eye lens and retina. Adapted from Y. Leng, *Materials Characterization*, Wiley (2008).

For an interactive description, see the following web page:

<http://www.wonderwhizkids.com/Physics/Light/Optical+Instruments/Optical+instruments.html>



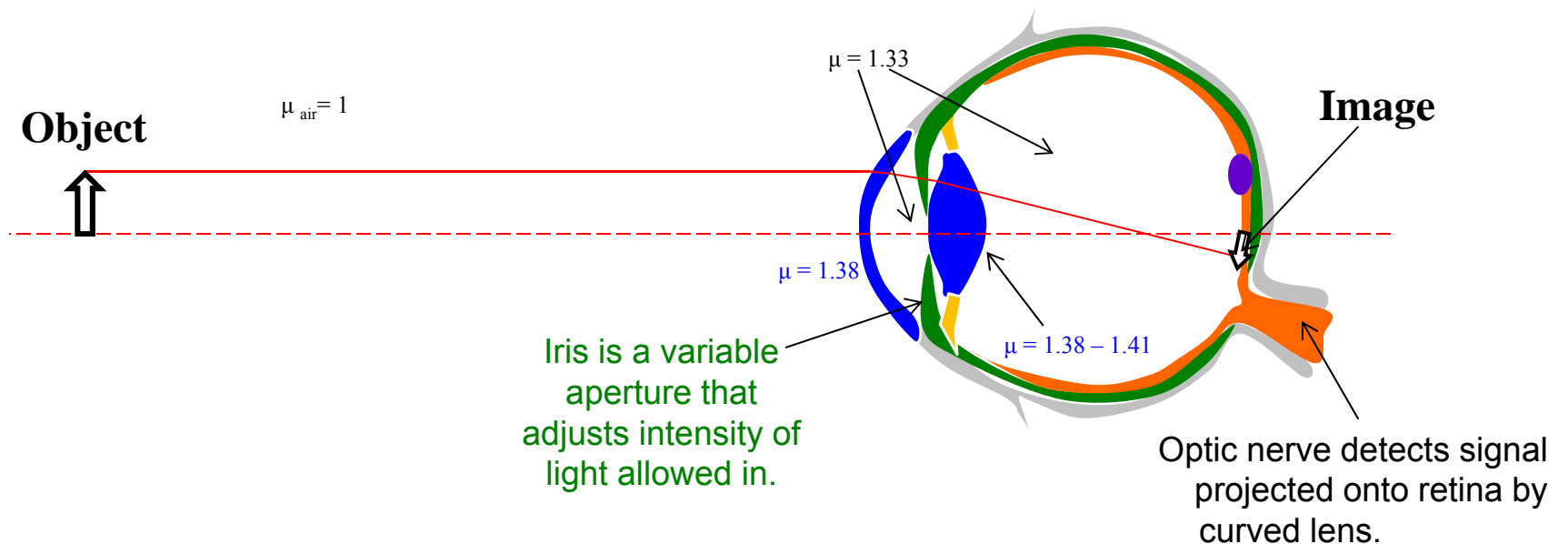
The human eye is a complete imaging system.

Figure modeled after 'Imaging Science Fundamentals' produced by the Chester F. Carlson Center for Imaging Science

<http://www.cis.rit.edu/info/HighSchool/pdf/HumanEye1.pdf>

Image formation in the eye (simplified)

- Regions with higher refractive indices than air bend light towards normal.



- The curved surfaces of the eye focus the image onto the back surface of the eye.

Figure modeled after 'Imaging Science Fundamentals' produced by the Chester F. Carlson Center for Imaging Science

<http://www.cis.rit.edu/info/HighSchool/pdf/HumanEye1.pdf>

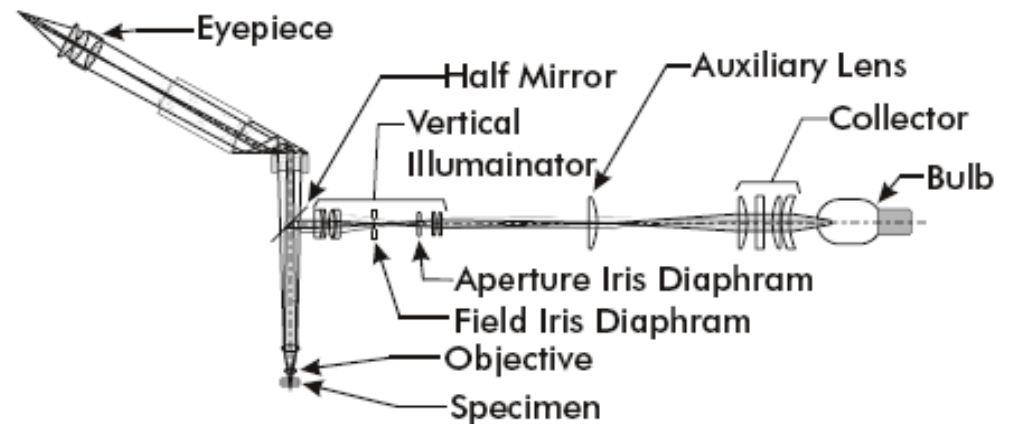
Metallographic Microscope

- Inverted metallurgical microscope (metallograph).
- Essentially a compound microscope set up to handle reflected light as opposed to transmitted light.
- Prisms and apertures for doing polarized light microscopy.



<http://www.Nikon.com>

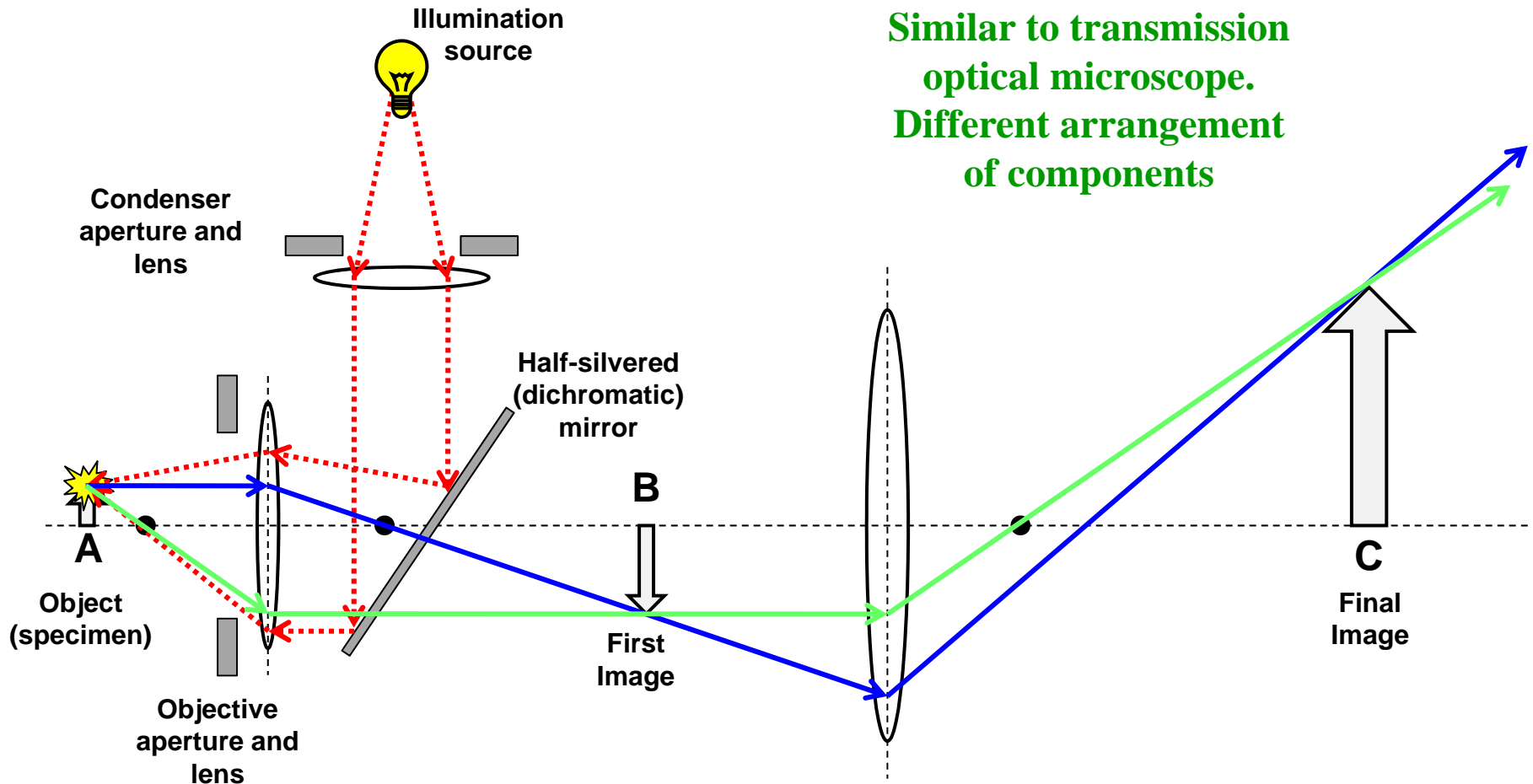
Reflected Light Path



<http://www.leco.com>

Image formation in a reflected light microscope

Similar to transmission optical microscope.
Different arrangement of components

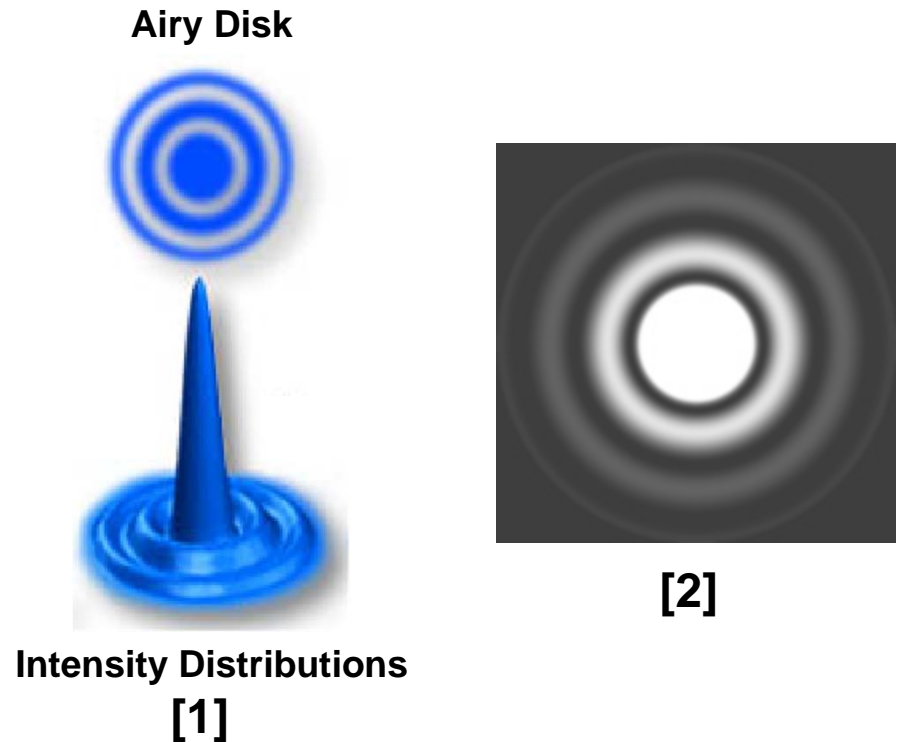


How do we increase magnification?

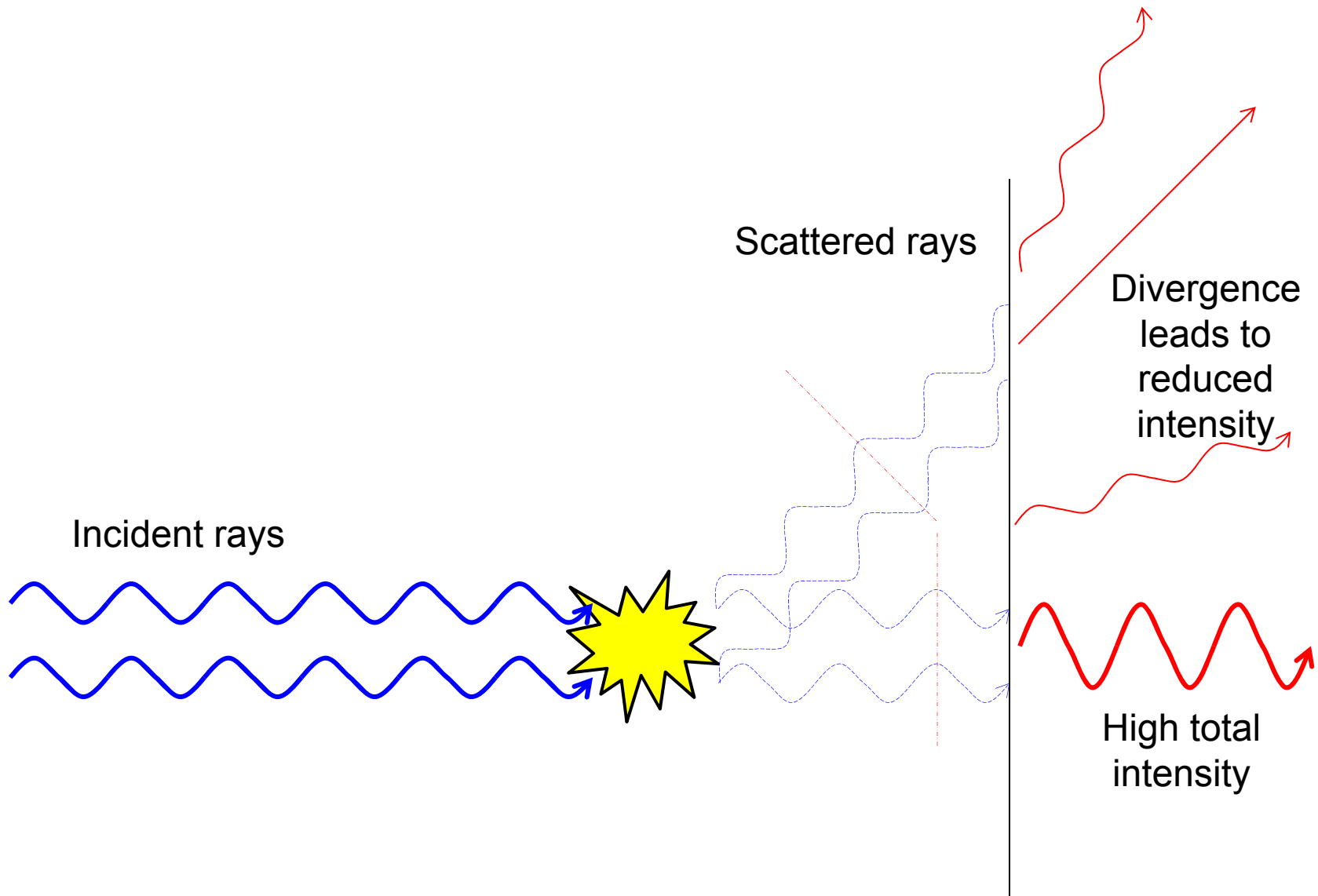
- The image distance is more or less fixed.
- We usually change the focal distance by **changing (objective) lenses.**
- Higher magnification lenses have shorter focal distances.

Resolution

- Consider an optical microscope.
 - Point sources of light from a specimen will appear as *Airy disks* (i.e., *diffraction patterns*) in the intermediate image plane of the microscope.
 - In physics lab you used a laser and a diffraction grating to produce these.

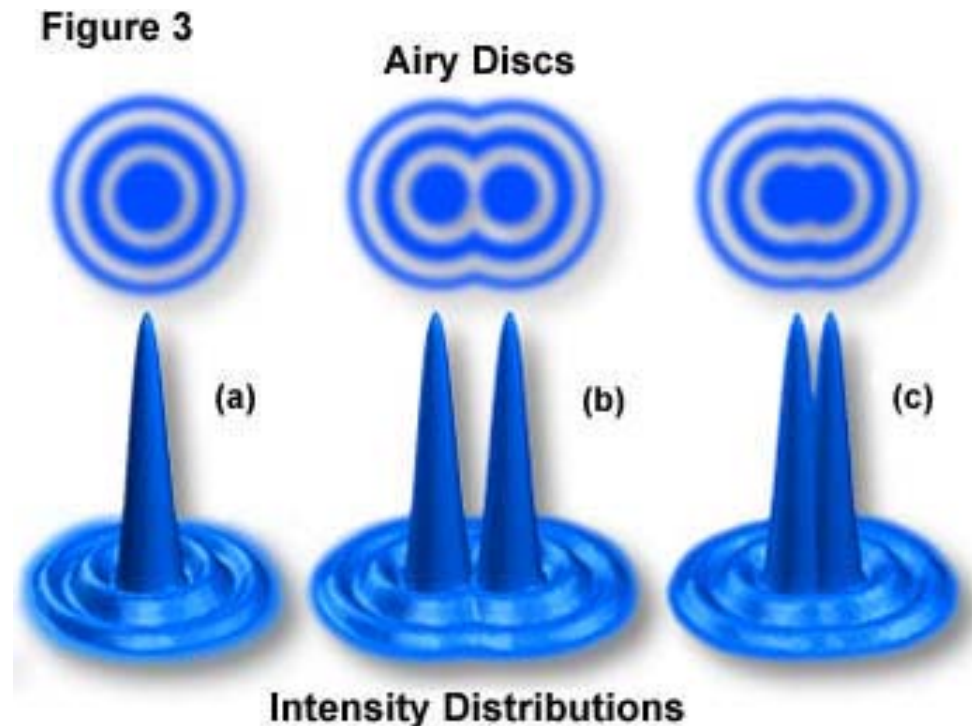


1. <http://micro.magnet.fsu.edu/primer/anatomy/numaperture.html>
2. <http://www.cambridgeincolour.com/tutorials/diffraction-photography.htm>



Resolution as you learned in Physics

- The resolution limit of an optical microscope objective refers to its ability to distinguish between two closely spaced Airy disks in a diffraction pattern.



<http://micro.magnet.fsu.edu/primer/anatomy/numaperture.html>

Abbe equation

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

μ = refractive index of medium btw. object and objective lens

$\mu \sin \alpha$ = numerical aperture (*N.A.*)

- Used to calculate the resolution as a function of microscope parameters.
- What is the resolution limit of an optical microscope?

Resolution Limit of Light Microscope

- You can decrease λ to 400 nm (violet light).
- $N.A.$ is limited to ~ 1.6 .

$$\text{LOM } r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} = \frac{0.612\lambda}{N.A.} = \frac{0.612 \times 400}{1.6} = 152 \text{ nm}$$

- The maximum resolution in an optical microscope is around ~ 150 nm ($0.15 \mu\text{m}$).

How can you improve resolution?

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

- Improved resolving power means smaller r_1 or d .
- Increase $N.A.$ (not feasible in optical microscopy).
- Reduce λ of light (limited by electromagnetic spectrum (~400 – 700 nm)).



Improving Resolution

- Reduce λ for better optical imaging (i.e., incr.resolution).
 - UV (10-400 nm) - absorption problems
 - X-ray (20 pm - 10 nm) - lens problems
 - Fast electrons (1 pm - 1 nm)

All required indirect observation (e.g., CCD camera)

- Use scanned images where resolution is given by excitation volume and step size.
- Both approaches are used in electron microscopy.

Resolution Limits of Light & Electron Microscopes

- Moments ago we calculated the resolution limit of a light optical microscope:

$$\text{LOM } r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} = \frac{0.612\lambda}{N.A.} = \frac{0.612 \times 400}{1.6} = 152 \text{ nm}$$

- The maximum resolution is around $\sim 150 \text{ nm}$ ($0.15 \mu\text{m}$).
- In comparison, in an electron microscope λ can decrease to 0.001 nm and $N.A.$ is much smaller (~ 0.1 radians)

$$\text{EM } r_1 = \frac{d}{2} \geq \frac{0.612\lambda}{\mu \sin \alpha} \approx \frac{0.612\lambda}{\alpha} = 0.02 \text{ nm}$$

Magnification

- For a compound microscope, magnification is the product of the magnifications for each lens.

- Effective magnification (M_{eff}):

$$= \frac{\text{resolution of eye}}{\text{max. resolution of LOM}} = \frac{0.2 \text{ mm}}{0.00015 \text{ mm}} \approx 1333 \times$$

- $M's > M_{eff}$ make the image bigger, but do not provide any additional details. This is termed “empty magnification.”

Brightness and Contrast

- Brightness = the intensity of light.
Related to the *N.A.* and the magnification (*M*).

$$B_{transmission} = \frac{(N.A.)^2}{M^2}$$

$$B_{reflected} = \frac{(N.A.)^4}{M^2}$$

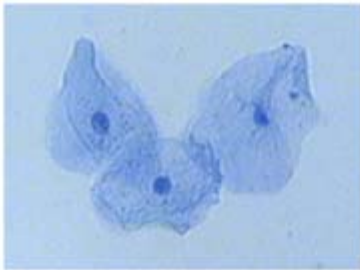
- Contrast = the relative change in light intensity (*I*) between an object and its background.

$$Contrast = \frac{I_{object} - I_{background}}{I_{background}}$$

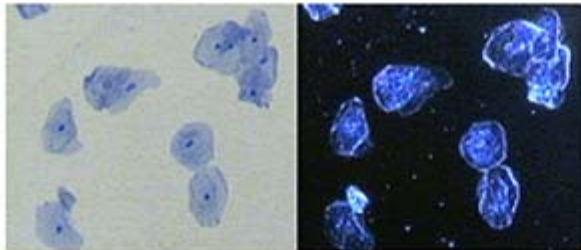
To be visible, an object must exceed the contrast threshold.

Contrast

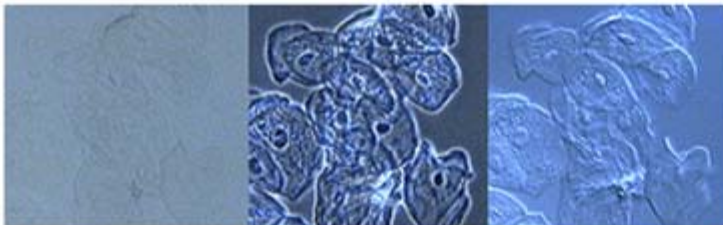
- The number of shades found in an image. Contrast provides information!
- High contrast images have two shades, black and white.
- More shades means lower the contrast (but more info.).
- Color is a form of contrast.



Absorption contrast



Diffraction contrast



Interference contrast

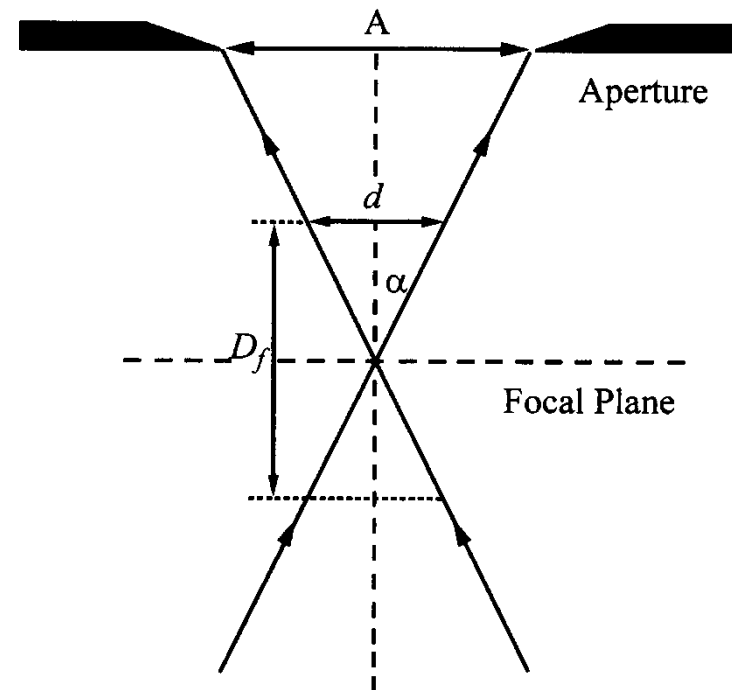
Adapted from MECH 4430 Lecture notes by Dr. Jingshen Wu and Dr. Yang Leng, Department of Mechanical Engineering, Hong Kong University of Science and Technology (<http://www.me.ust.hk/~mejswu/>).

Depth of Field

- The distance along the optic axis over which image details can be observed with acceptable clarity.
- The same factors that effect resolution effect the depth of field but in the opposite way; therefore, a compromise must be reached between these two factors.

$$D_f = \frac{d}{\tan \alpha} = \frac{2r}{\tan \alpha} = \frac{1.22 \lambda}{\mu \sin \alpha \tan \alpha}$$

- Object will be sharp if it is anywhere within the range D_f .



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

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

- Cannot get large depth of field and high resolution.
- Large $D_f \rightarrow$ larger r_1 and worse resolution.

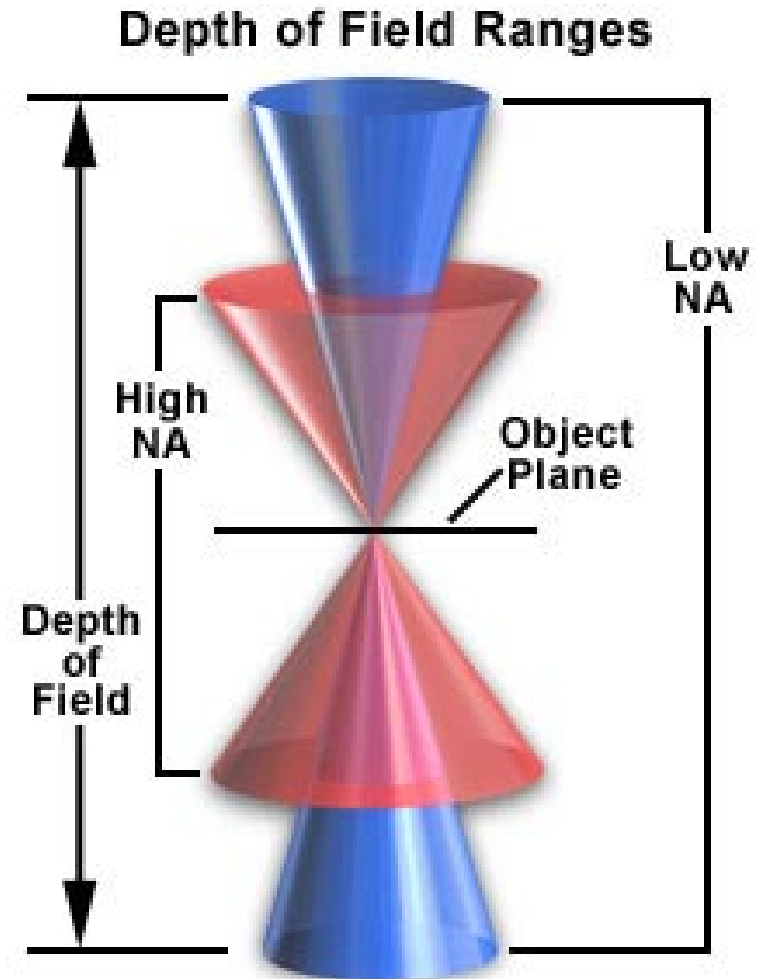


Figure 1

www.microscopyu.org



Optical Performance

Resolution & Depth of Field – cont'd

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

- In a light microscope, α is around 45° . Thus, D_f is not much different from resolution.
- In an electron microscope, α and λ are much smaller.

$$D_f = \frac{0.61\lambda}{\alpha^2}$$

- In an electron microscope, D_f is nearly $10\times$ resolution.

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 $N.A.$
3. Use a high-power eyepiece with a low-power, high- $N.A.$ objective lens.
4. Reduce zoom factor (i.e., “zoom out”).
5. Use longest possible wavelength light.

Depth of Focus

- The range of image plane positions at which the image can be viewed without appearing out of focus for a fixed position of object.
 - Often confused with depth of field but not the same.
 - Not as important as depth of field.
 - Depth of focus is M^2 times depth of field.

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

Aberrations in Optical Systems

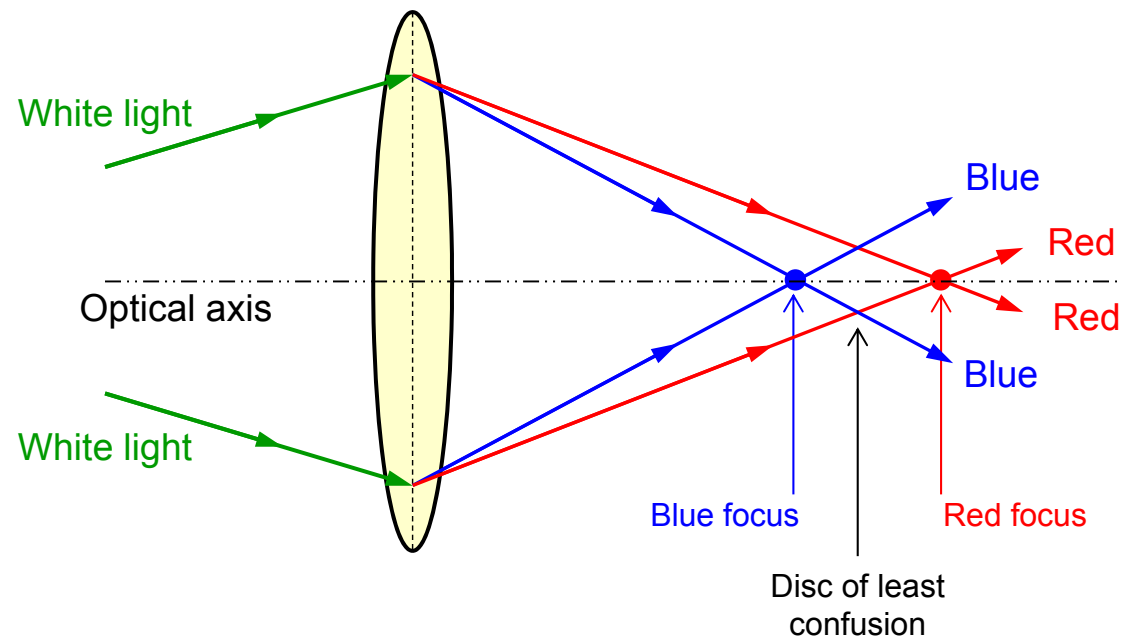
- Resolution and depth of field *assume*:
 - All components of a microscope are perfect,
 - Light from any point on an object focuses at a similar unique point in the image.
- NOT POSSIBLE due to *lens aberrations*.

Aberrations in Optical Systems

- Chromatic aberration
- Spherical aberration
- Astigmatism

Chromatic Aberration

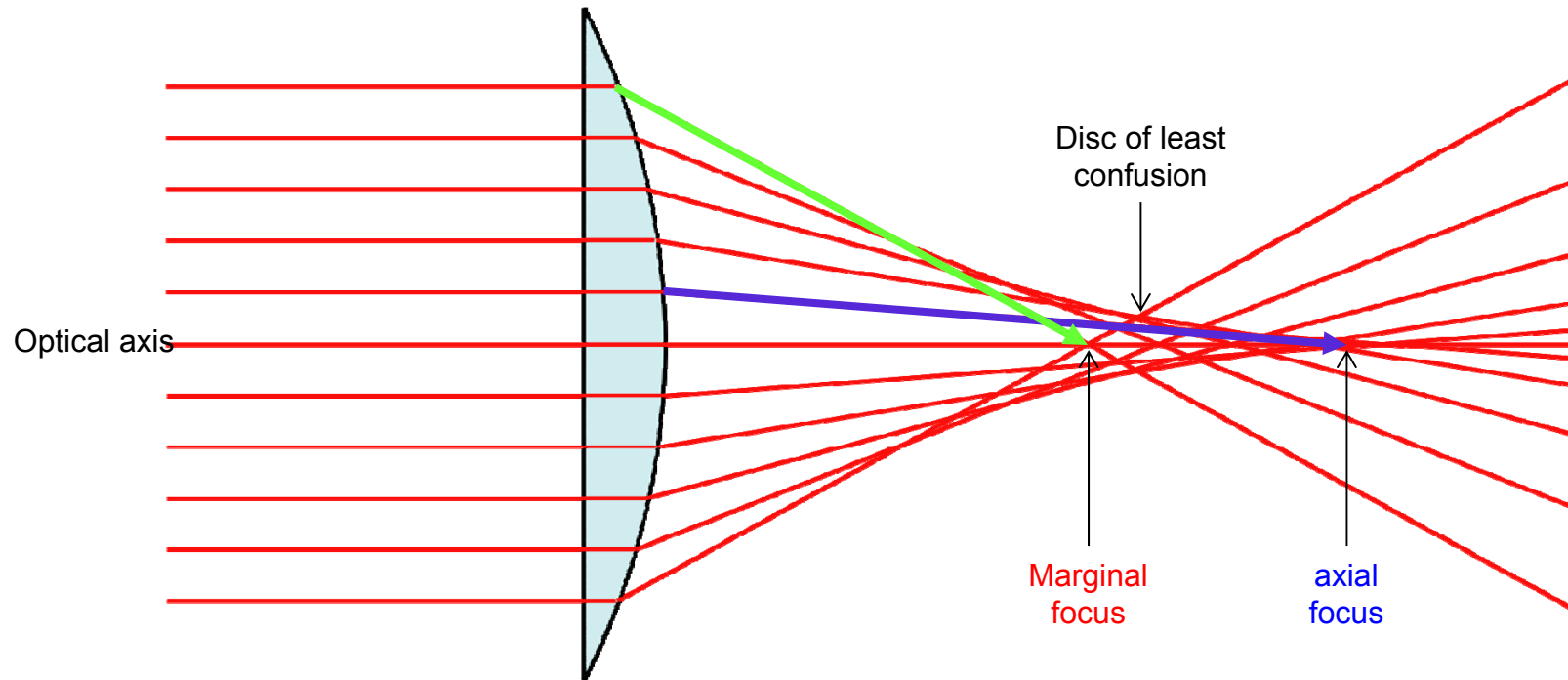
- Light deflection by a lens dep. on λ of light.
- Shorter $\lambda \rightarrow$ larger degree of deflection.
- Range of λ 's present in the white light:
 - \therefore you can't focused light to a single point.



Recall: red light is refracted less than blue light.

Spherical Aberration

- Portion of the lens furthest from the optical axis brings rays into focus closer than the central portion.

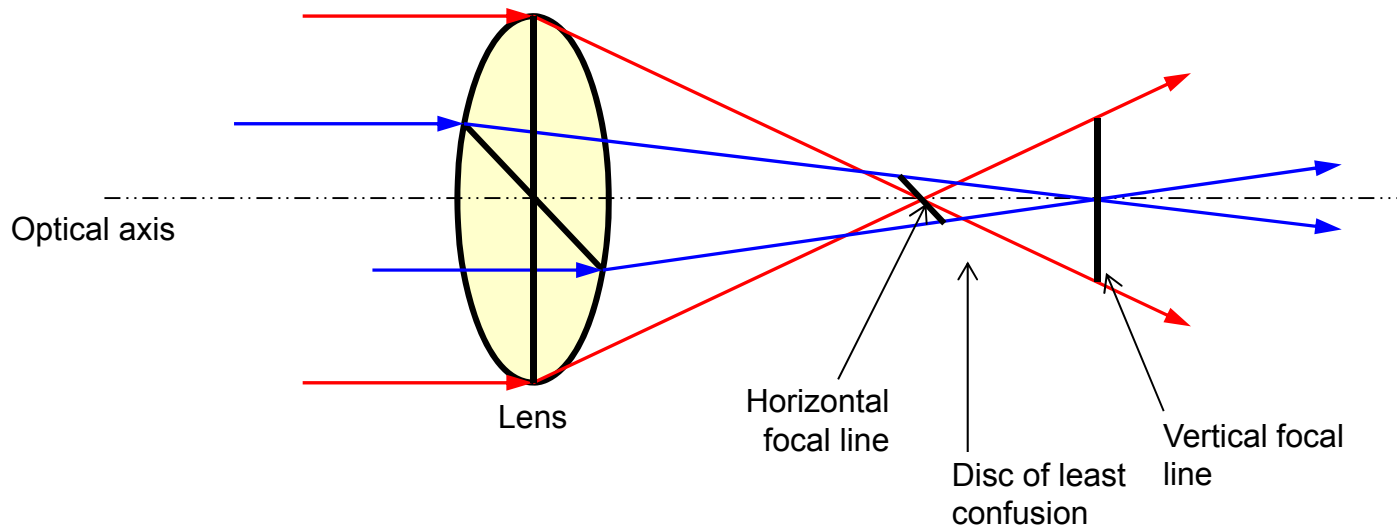


- This is because the optical ray path length from object to focused image should always be the same.

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

Astigmatism

- Caused by light rays passing through vertical diameters of a lens that are not focused on the same image plane as rays passing through horizontal diameters.



Ray diagram illustrating the formation of *astigmatism* in a lens with different optical properties in the horizontal and vertical directions. The illustrated lens is more powerful in the vertical plane. Adapted from Goodhew and Humphreys, p. 16.

Reduce Aberrations

- Combine lenses with different shapes and μ 's to correct spherical and chromatic aberrations.
- Select single λ illumination source to eliminate chromatic aberrations.
- Less aberrations means more \$\$\$.