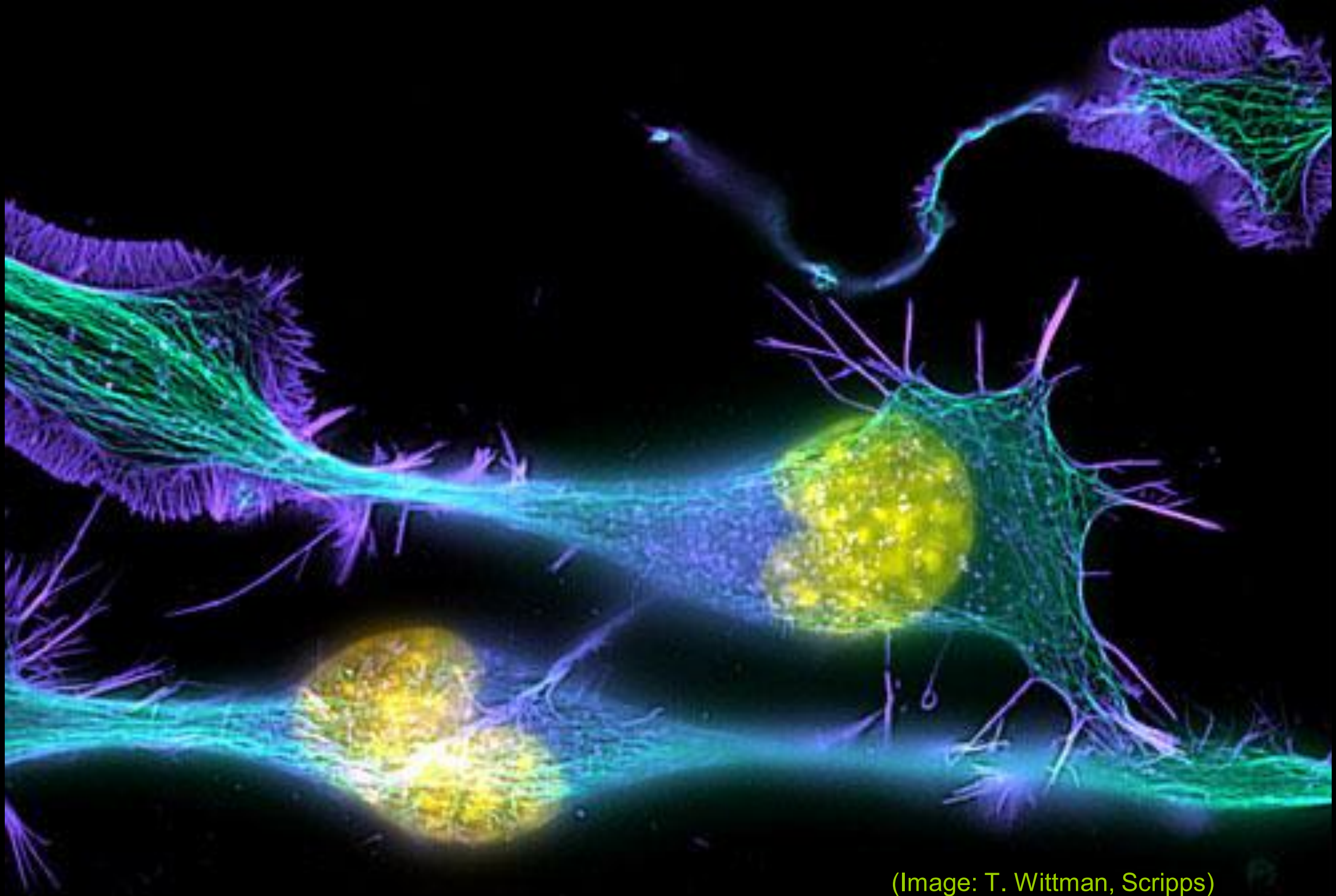
Introduction to Light Microscopy


(Image: T. Wittman, Scripps)

The Light Microscope

- Four centuries of history
- Vibrant current development
- One of the most widely used research tools

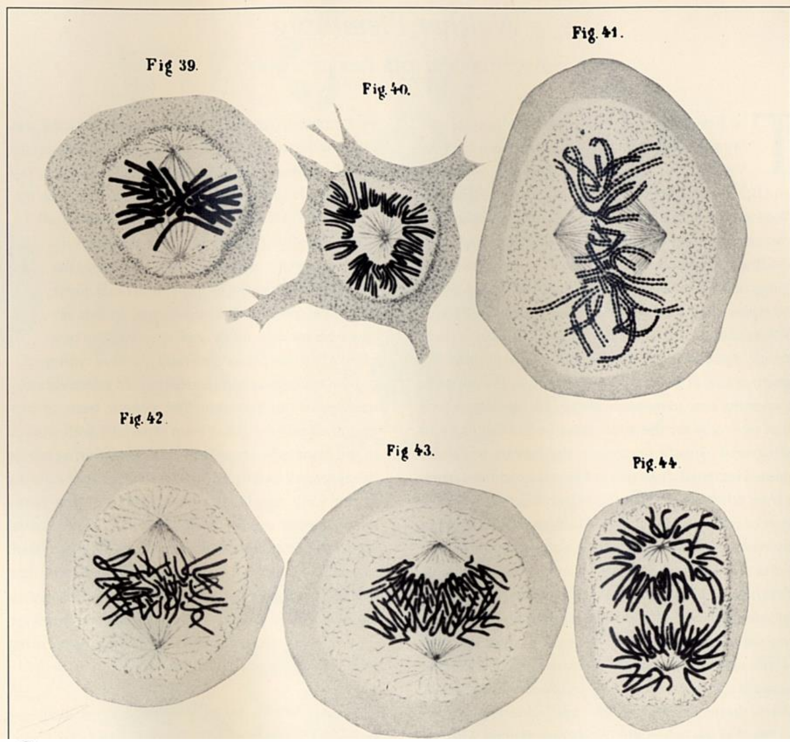
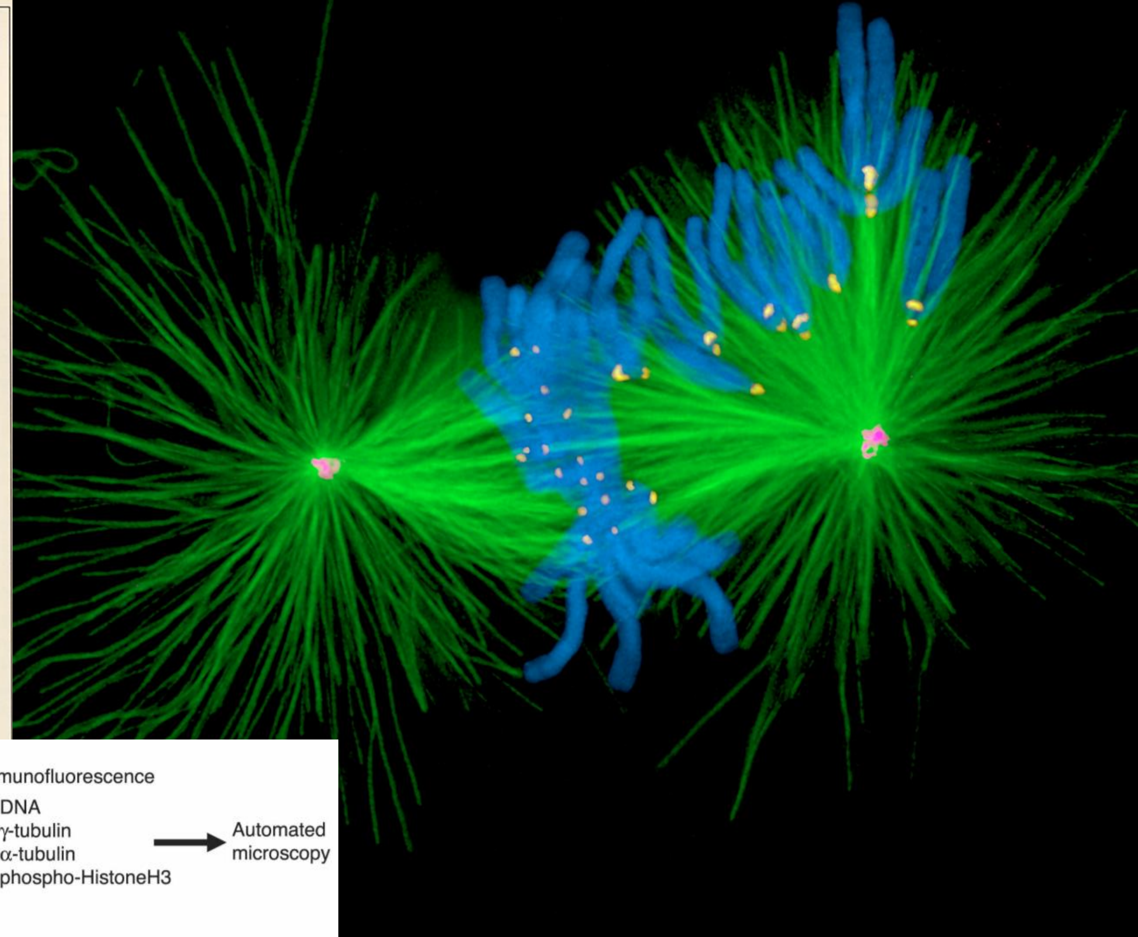
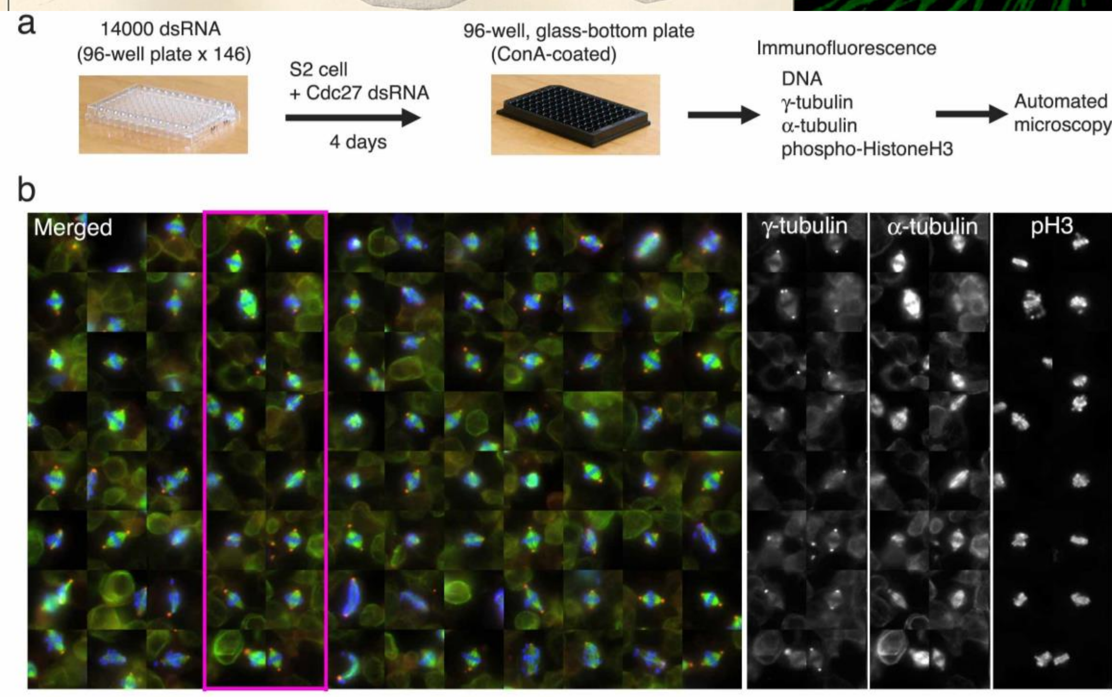


Fig 39.
Fig. 40.
Fig. 41.
Fig. 42.
Fig 43.
Fig. 44.




a
14000 dsRNA
(96-well plate x 146)
S2 cell
+ Cdc27 dsRNA
4 days
96-well, glass-bottom plate
(ConA-coated)
Immunofluorescence
DNA
γ-tubulin
α-tubulin
phospho-HistoneH3
Automated
microscopy
b
Merged
γ-tubulin
α-tubulin
pH3

Major Imaging Functions of the Microscope

- Magnify
- Resolve features
- Generate Contrast
- Capture and Display Images

An Upright Epifluorescence Microscope

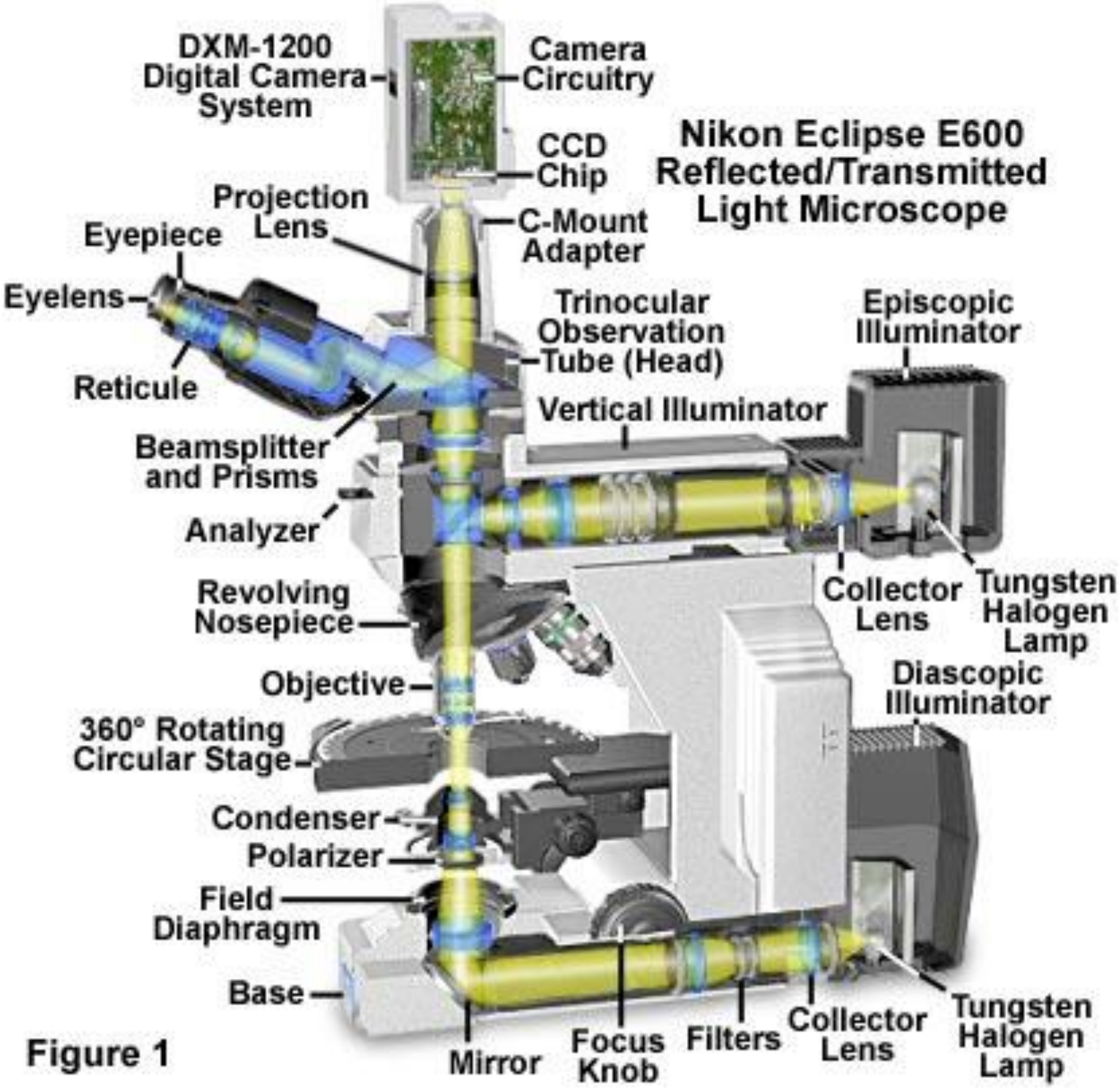
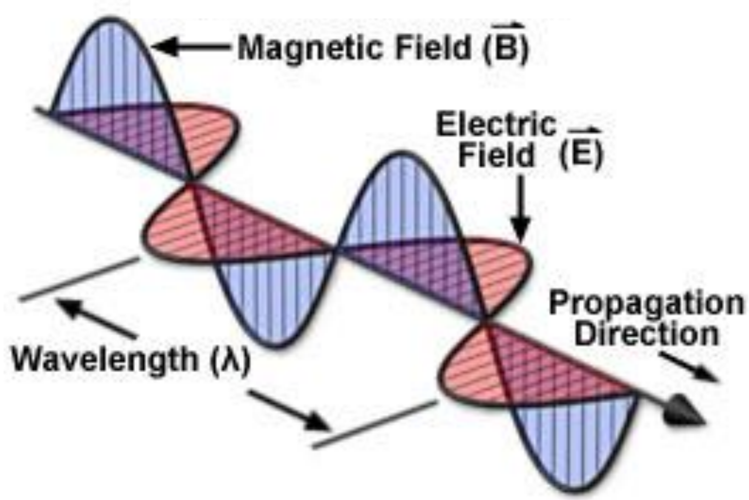


Figure 1

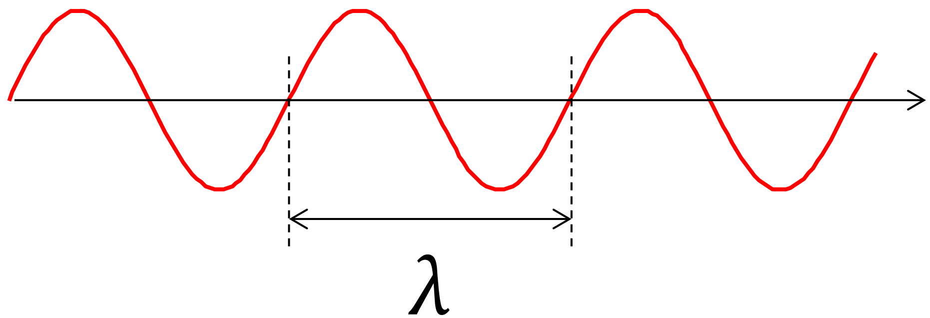
What is light?

Waves vs. Photons vs. Rays

- Quantum wave-particle duality
- Rays: photon trajectories
- Rays: propagation direction of waves

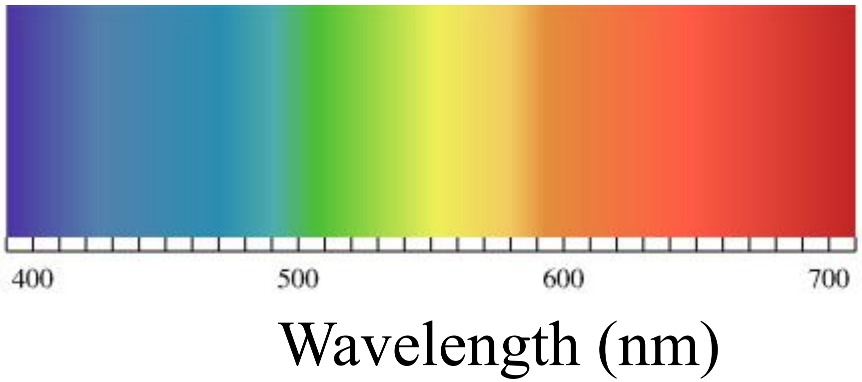


Wavelength and frequency



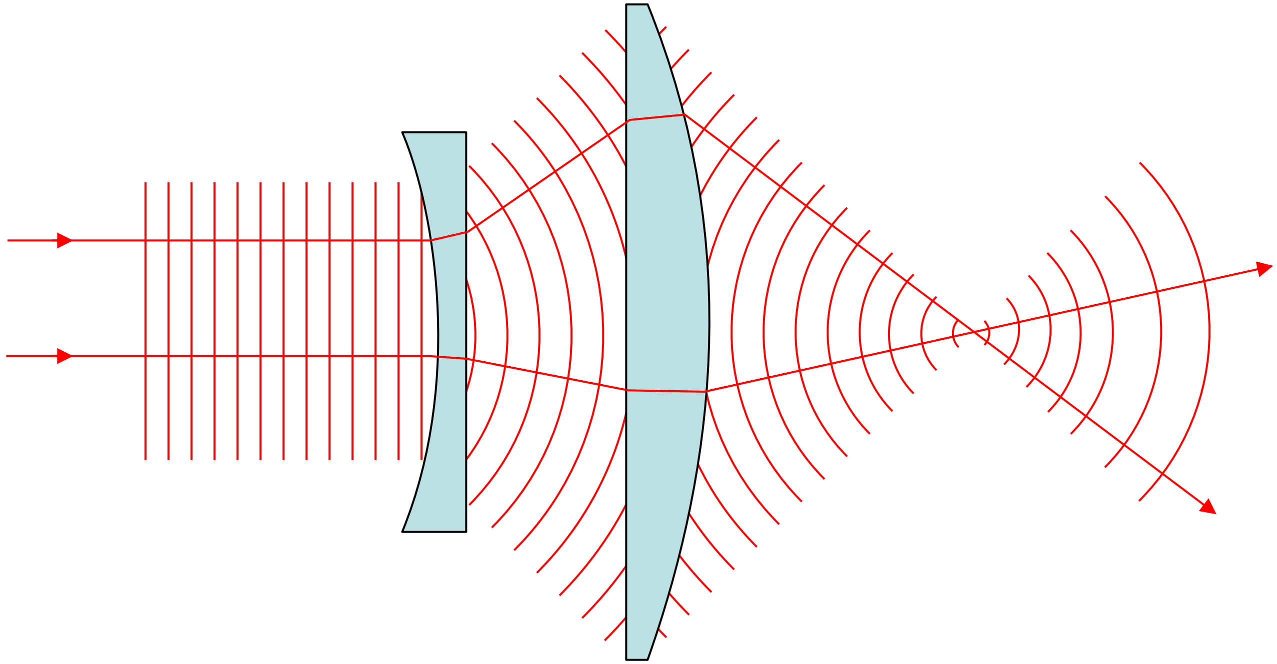
$$c = \lambda\nu$$

$$= 299792458 \text{ m/s in vacuum}$$



$\nu = 10^{14} - 10^{15}$ Hz

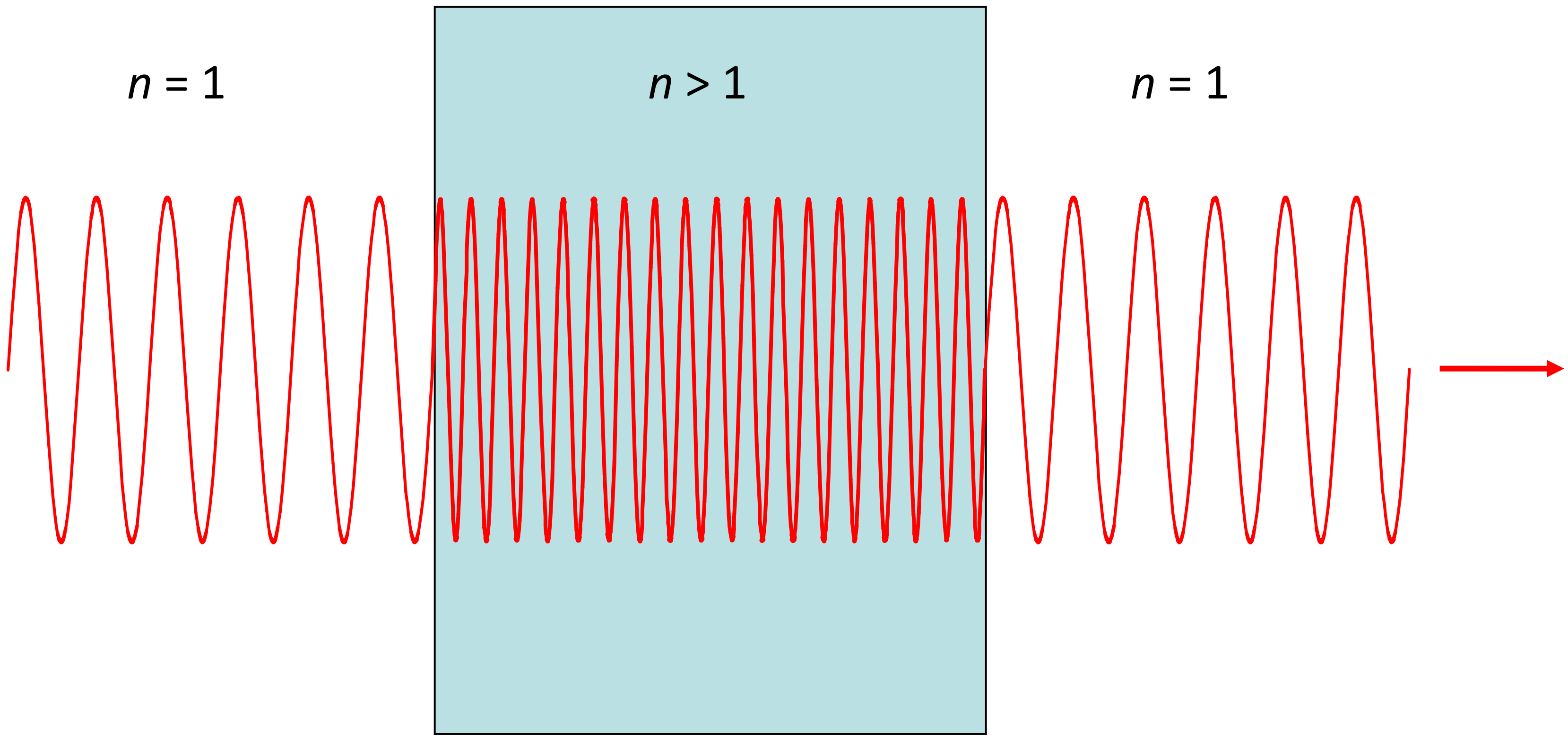
Rays are perpendicular to wavefronts



Light travels more slowly in matter

The speed ratio is the ***Index of Refraction, n***

$$\boxed{v = c/n}$$

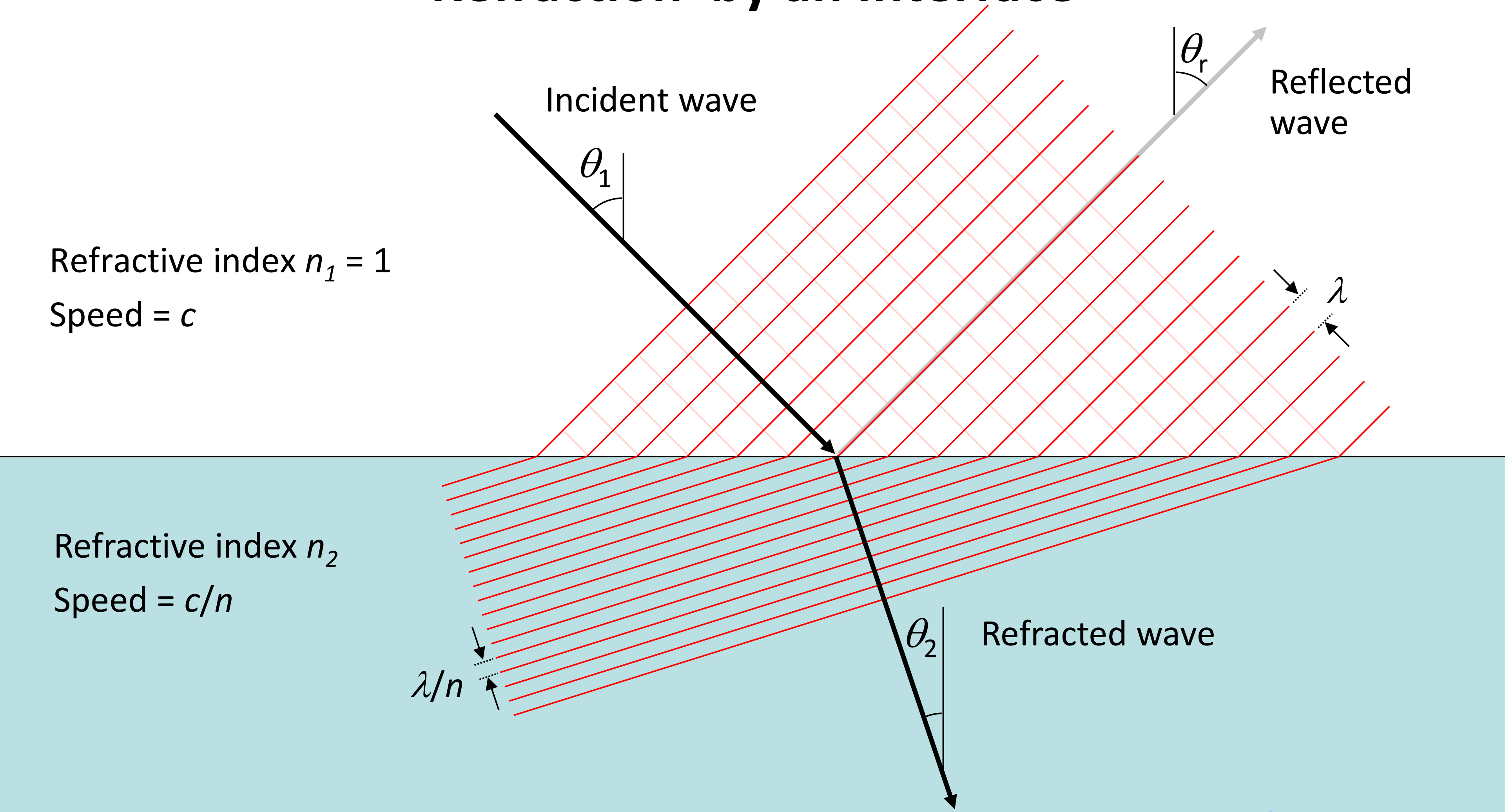


Refractive Index Examples

• Vacuum	1
• Air	1.0003
• Water	1.333
• Cytoplasm	1.35–1.38 ?
• Glycerol	1.475 (anhydrous)
• Immersion oil	1.515
• Fused silica	1.46
• Optical glasses	1.5–1.9
• Diamond	2.417

Depends on wavelength and temperature

Refraction by an Interface



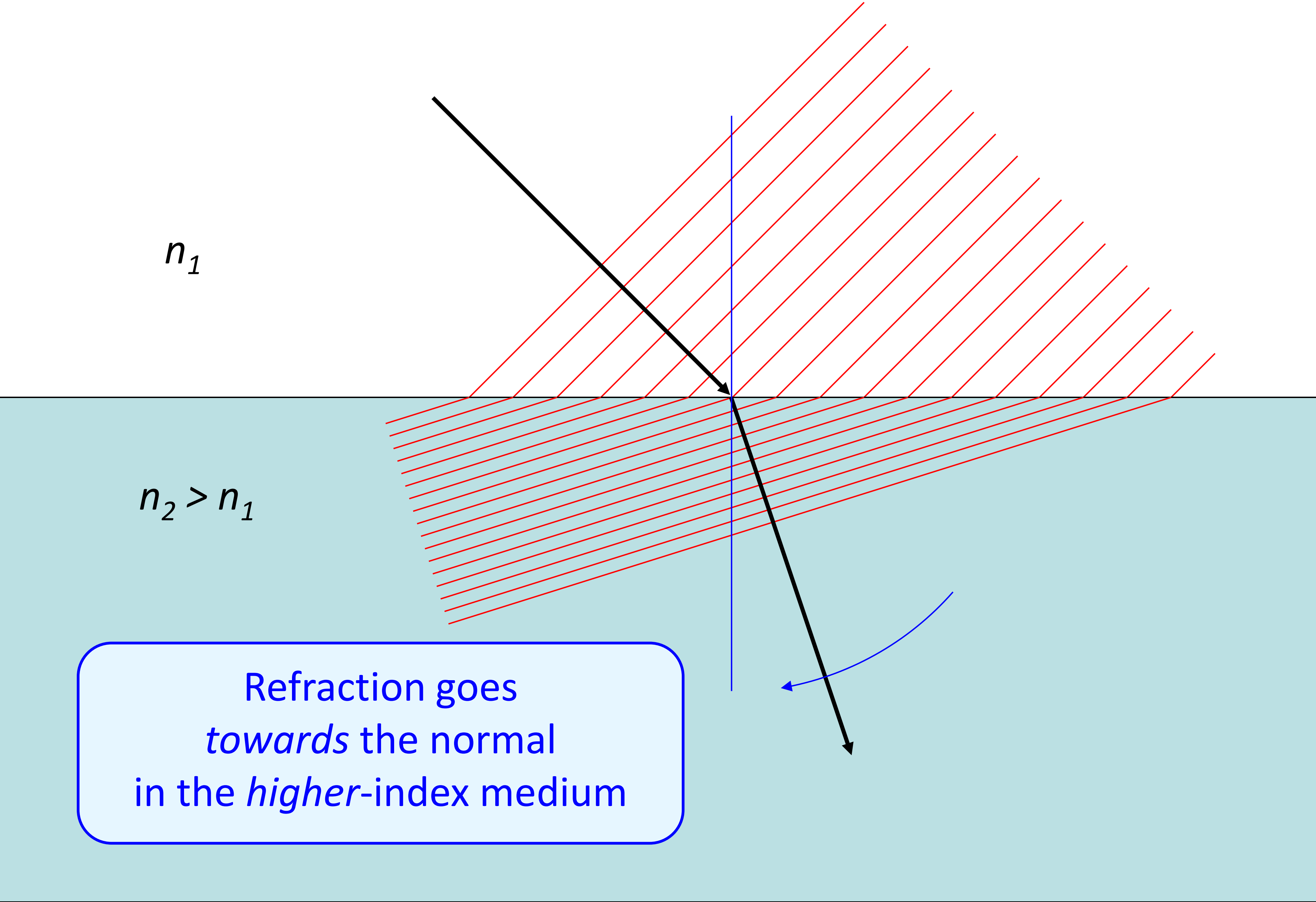
$\Rightarrow$ Snell's law:

$$\boxed{n_1 \, \mathrm{Sin}(\theta_1) = n_2 \, \mathrm{Sin}(\theta_2)}$$

Mirror law:

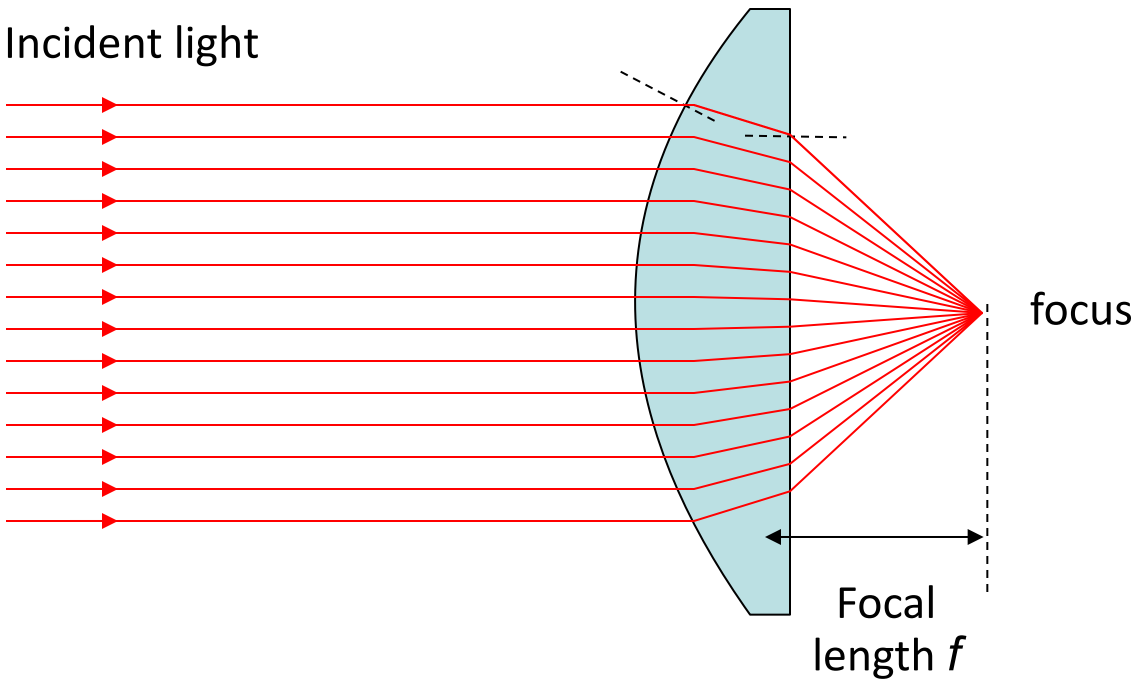
$$\boxed{\theta_r = \theta_1}$$

Which Direction?



Refraction goes
towards the normal
in the *higher*-index medium

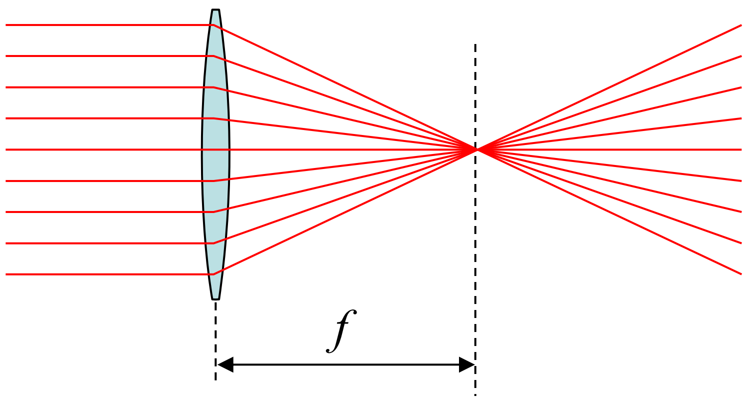
Lenses work by refraction



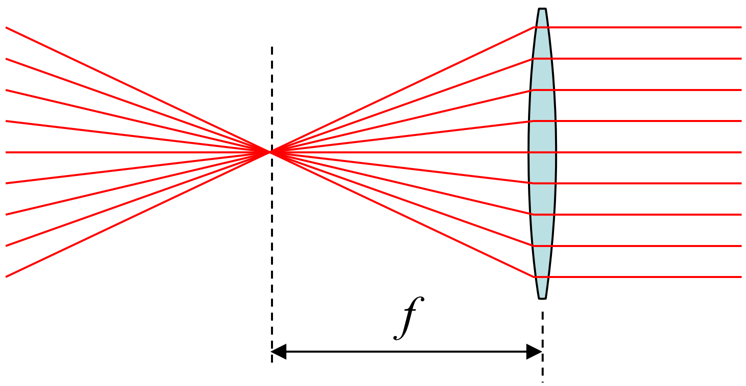
Ray Tracing Rules of Thumb

(for thin ideal lenses)

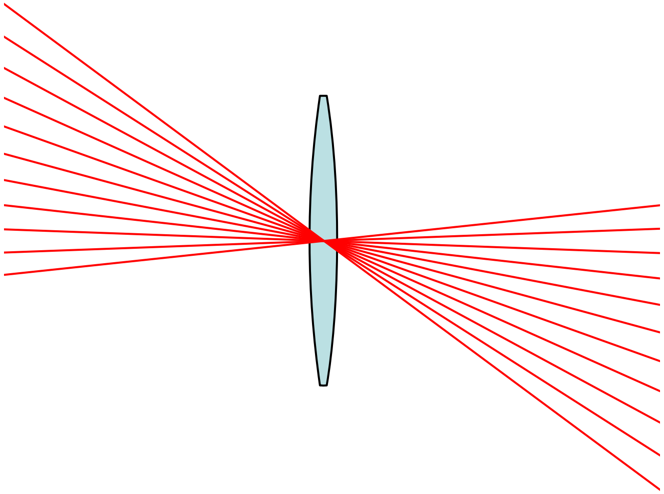
Parallel rays converge at the focal plane



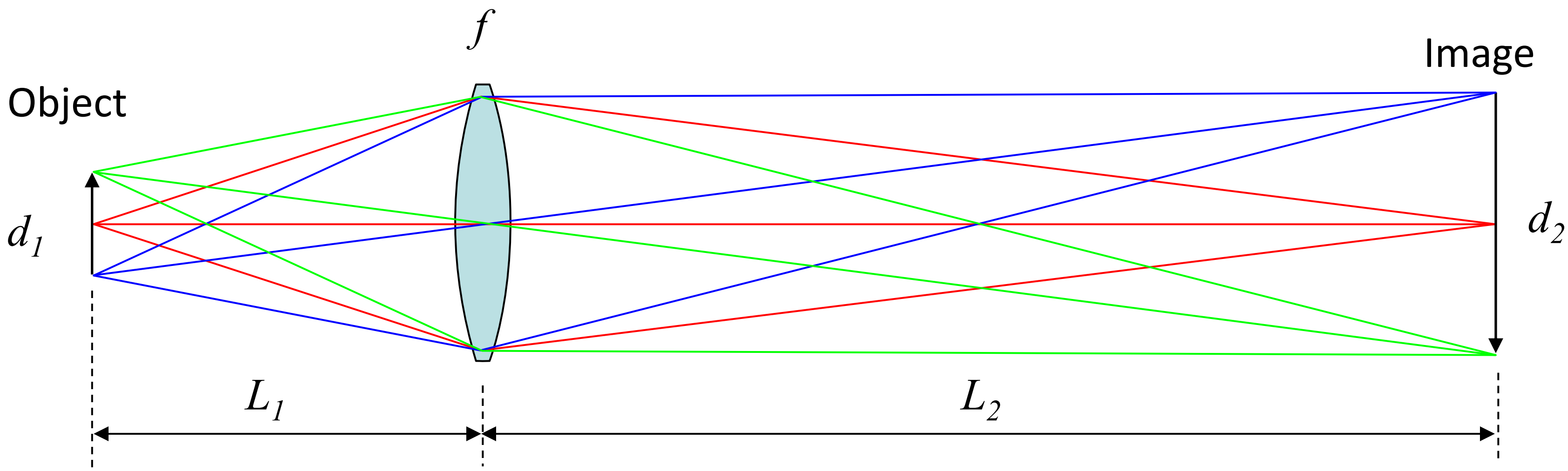
Rays that cross in the focal plane end up parallel



Rays through the lens center are unaffected



Imaging

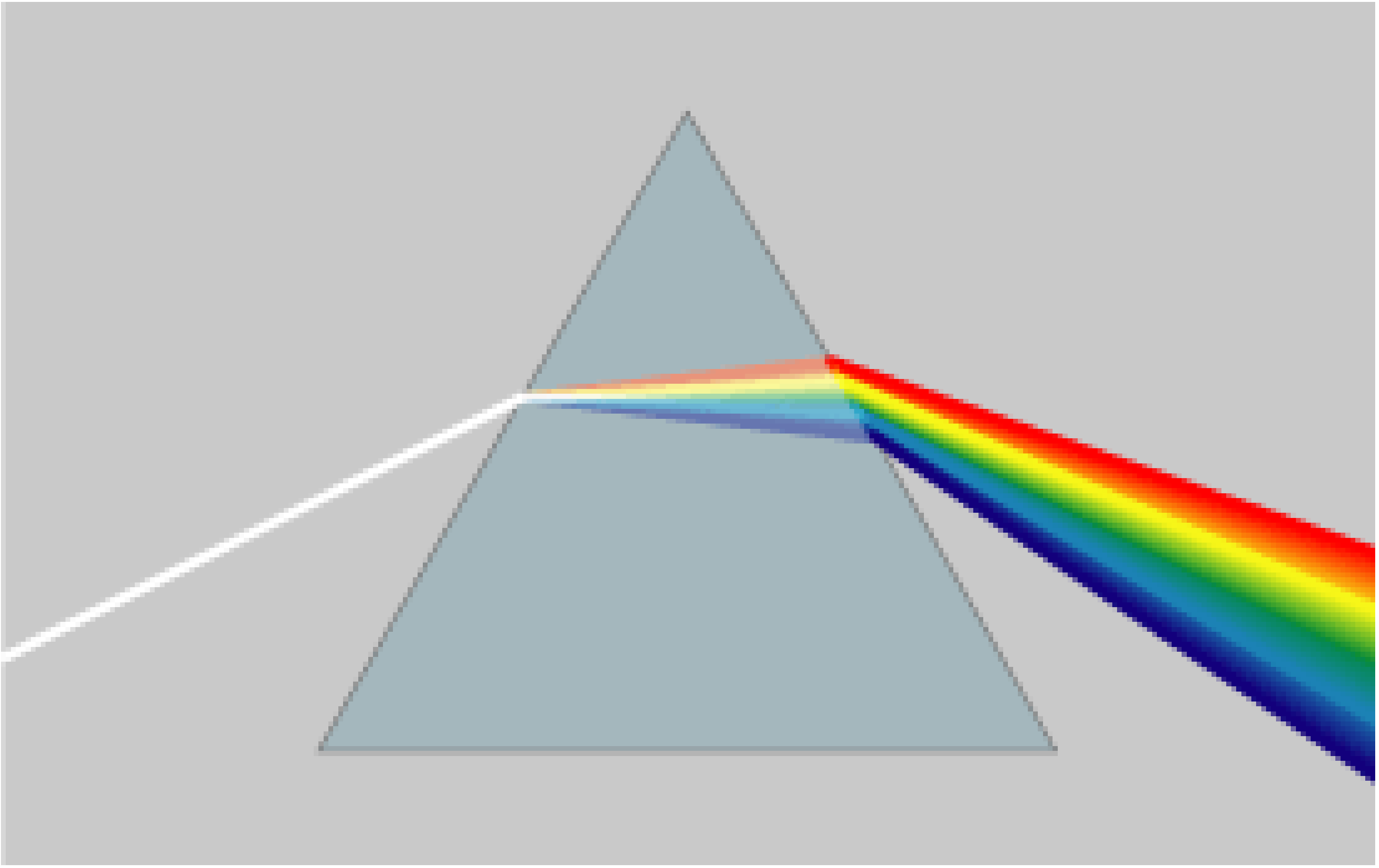


The lens law: $$\frac{1}{L_1}+\frac{1}{L_2}=\frac{1}{f}$$

Magnification: $$M=\frac{d_2}{d_1}=\frac{L_2}{L_1}$$

Dispersion

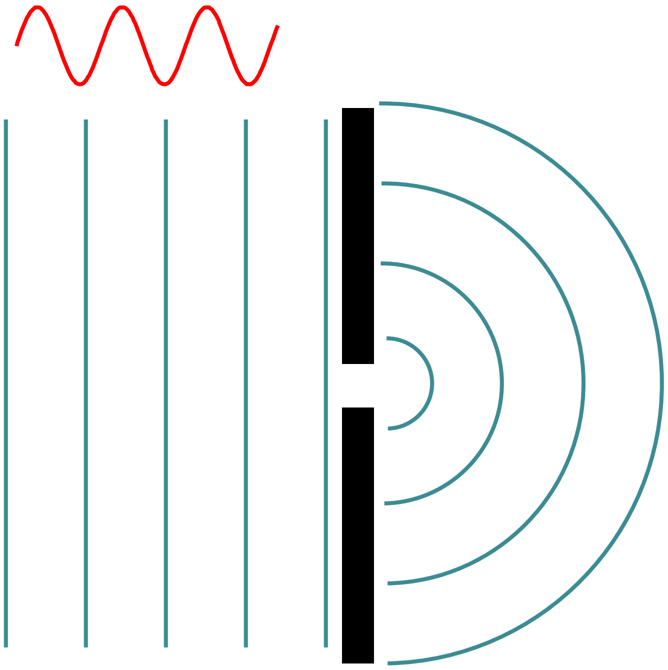
Refractive index is wavelength dependent



Dispersion

Diffraction and interference

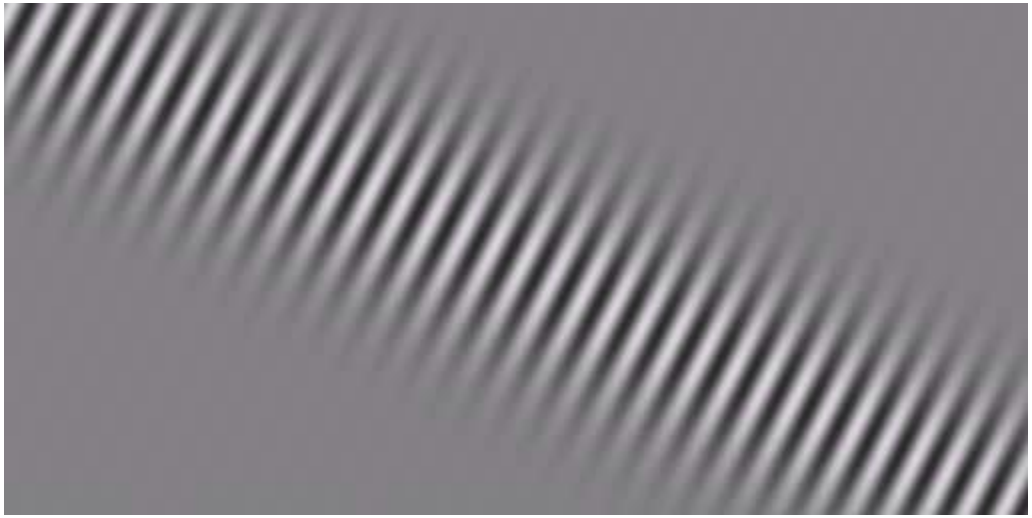
Diffraction

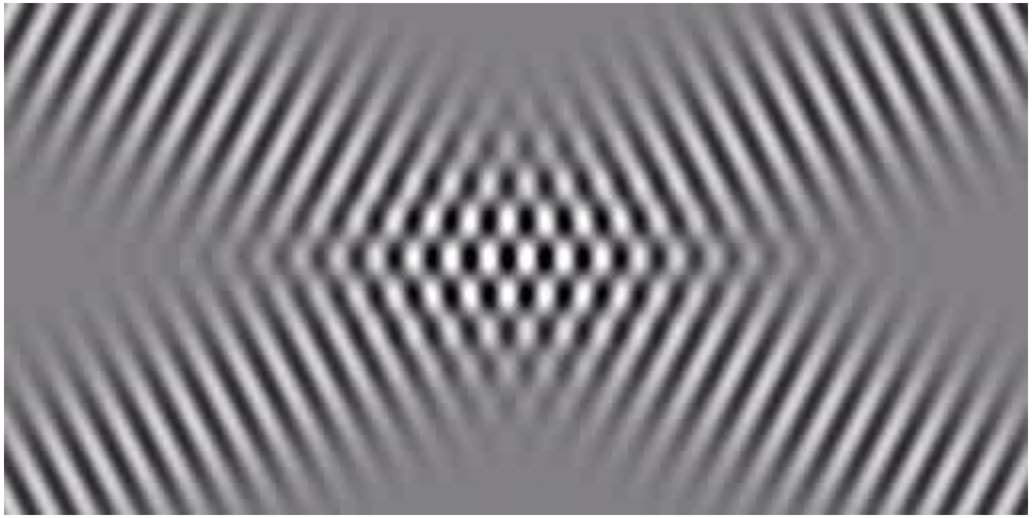


Diffraction by an aperture

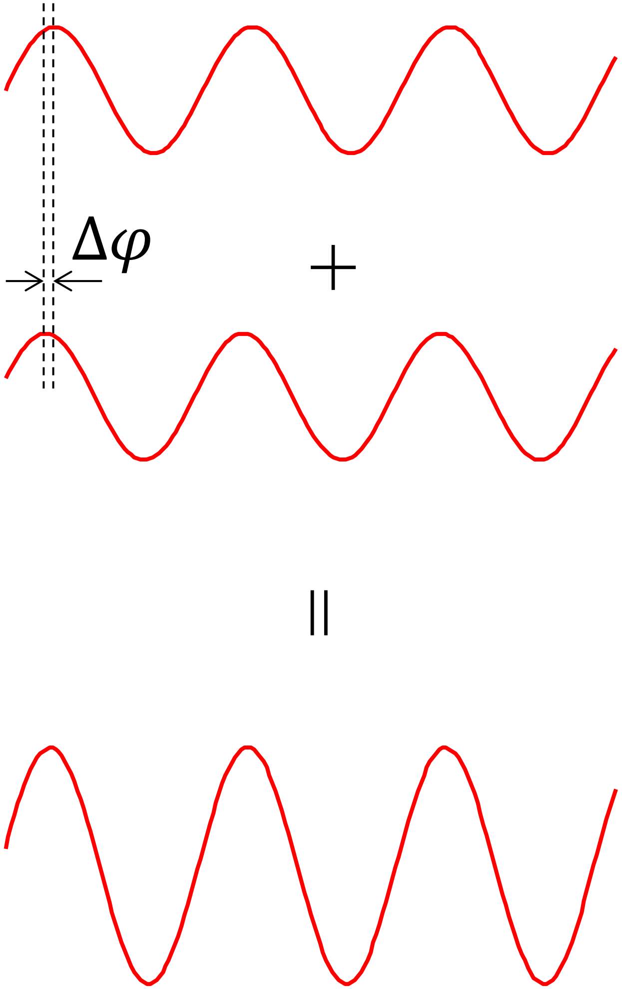
See "Teaching Waves with Google Earth"
http://arxiv.org/pdf/1201.0001v1.pdf for more

Interference

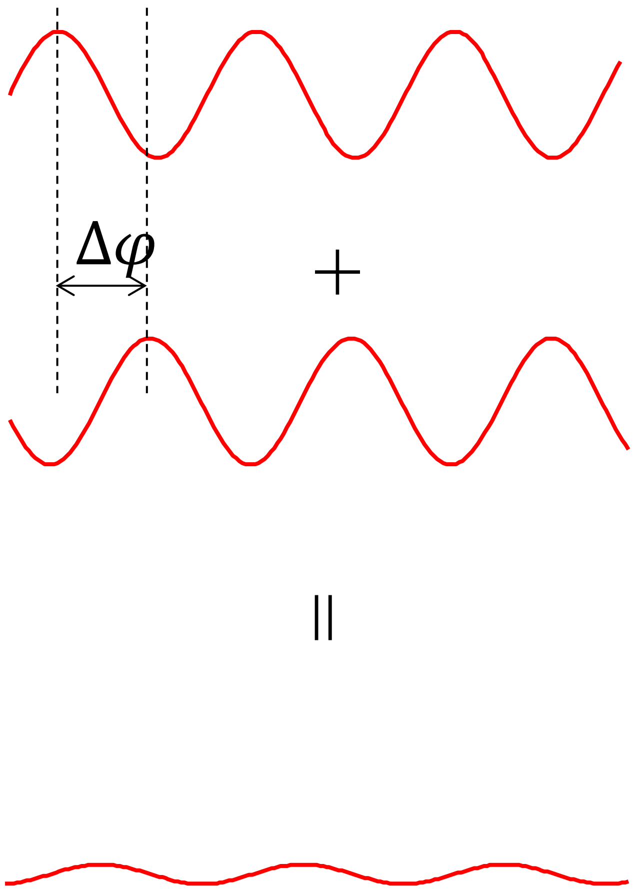




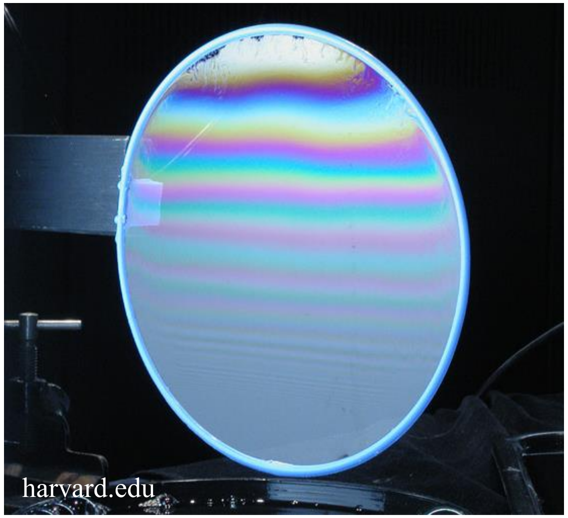
Constructive interference

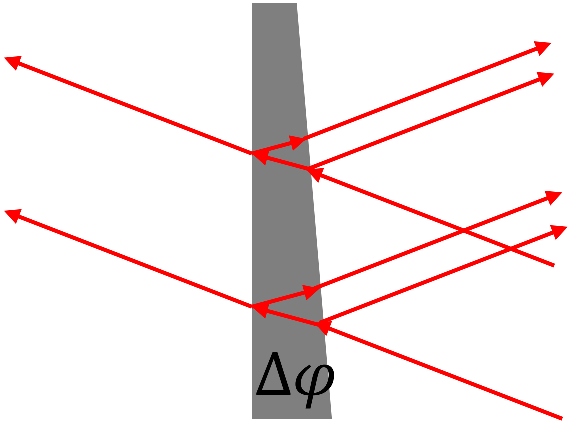


Destructive interference

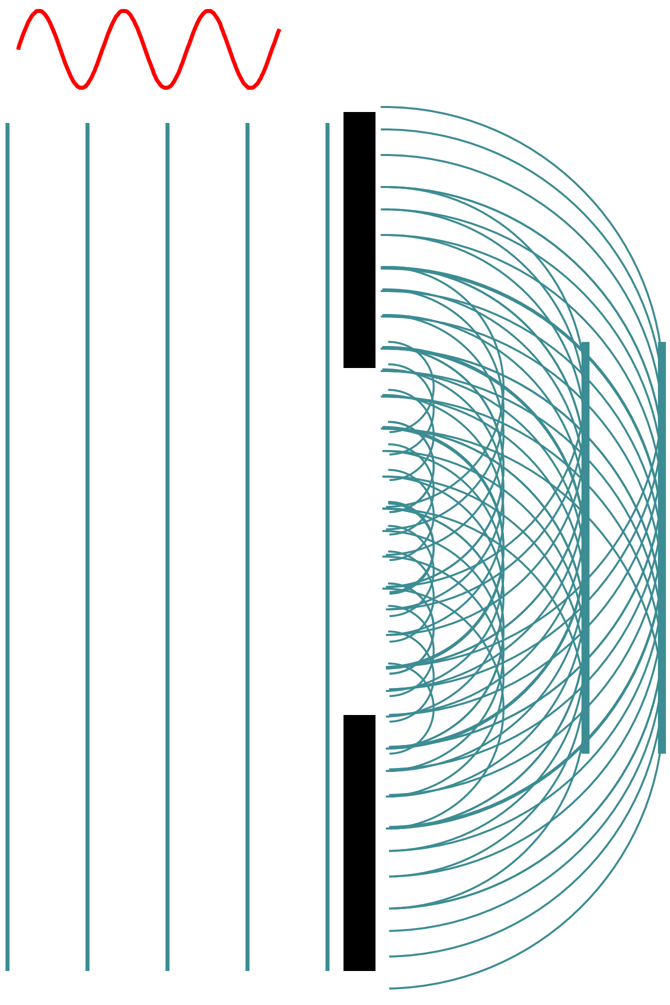


Thin film interference

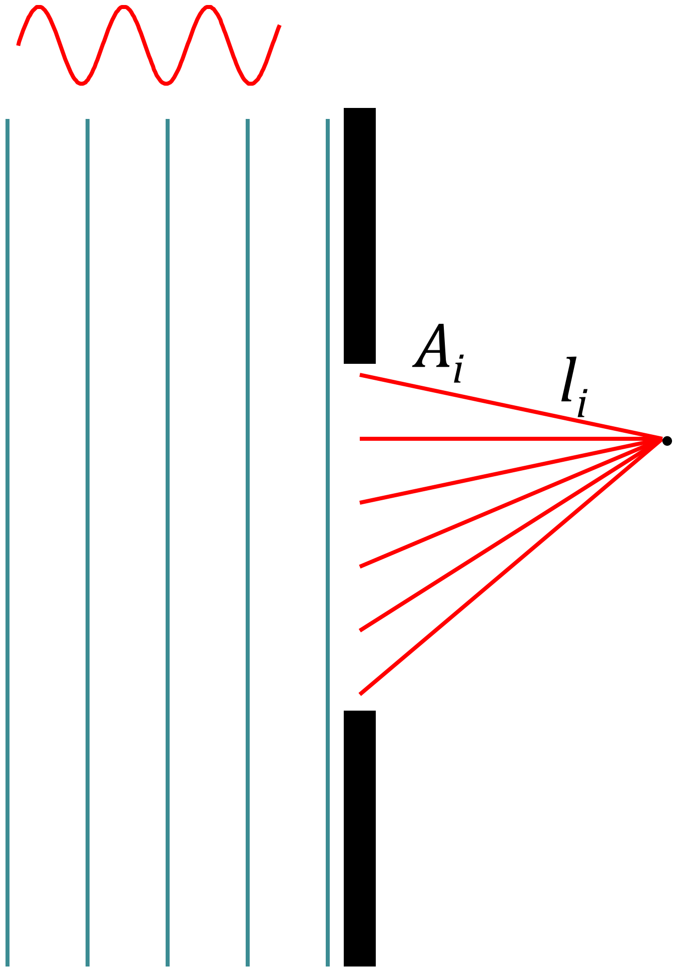




Light propagation = diffraction + interference



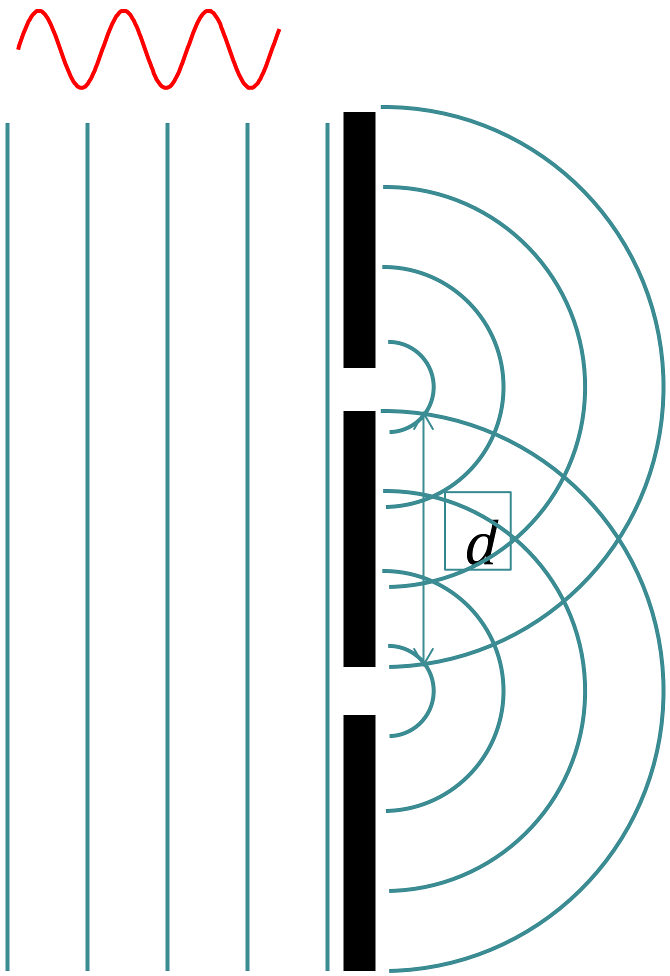
Light propagation = diffraction + interference



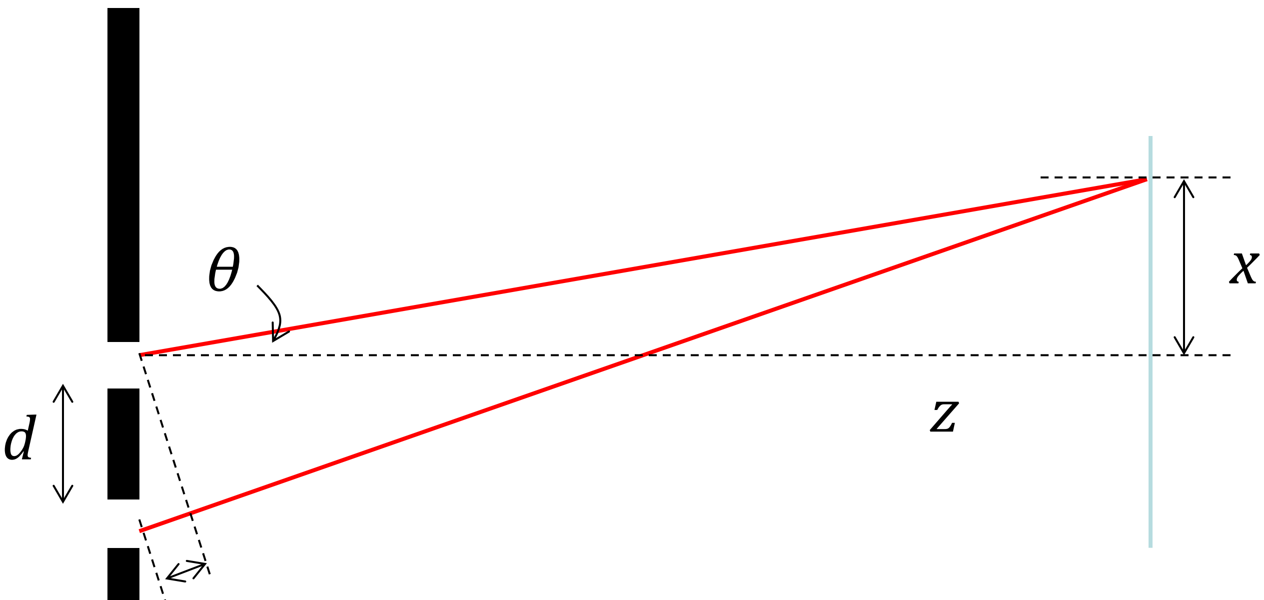
$$A = \sum A_i \sin(2\pi l/\lambda)$$

$$I = A^2$$

Double slit interference



Double slit interference

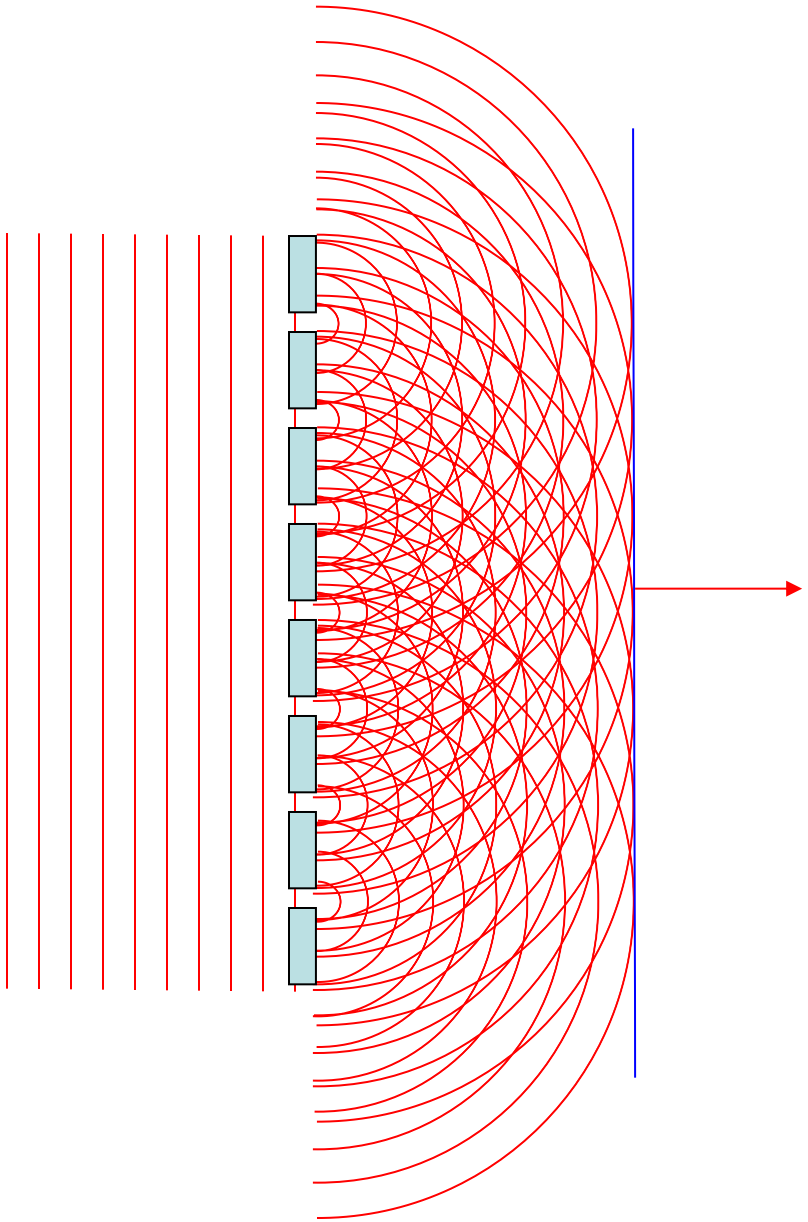


$\Delta = d\,\sin\theta \approx dx/z$

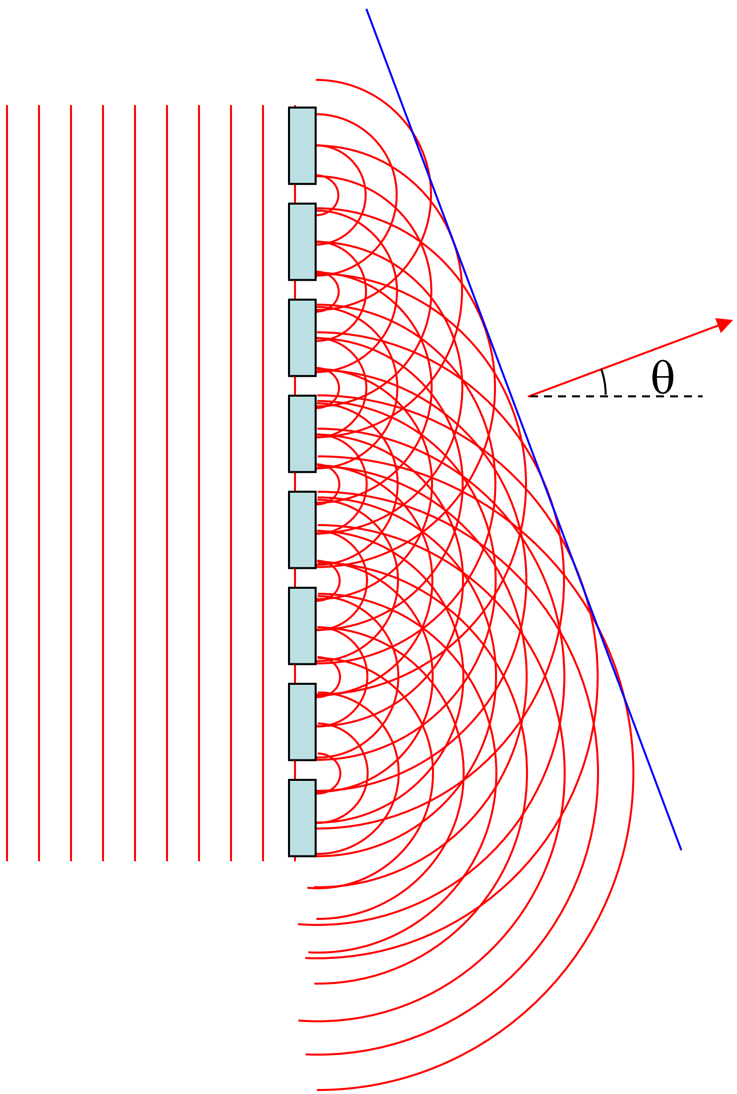
$\Delta = k\lambda$ for constructive interference

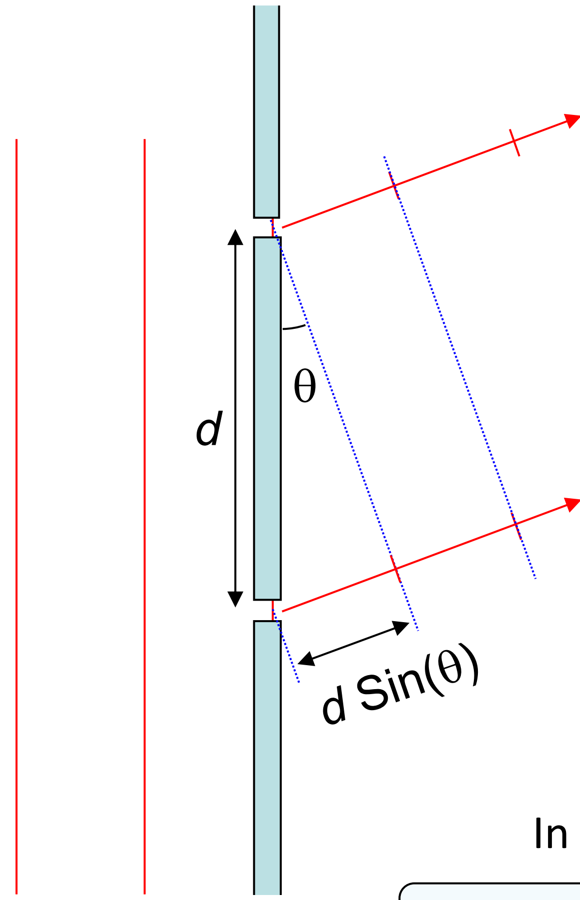
Stripe distance $= \dfrac{z}{d} \cdot \lambda$

Diffraction by a periodic structure (grating)



Diffraction by a periodic structure (grating)





In phase if:

$$\boxed{d \sin(\theta) = m \lambda}$$

for some integer m

Light as electromagnetic waves

Maxwell's equations

$$\nabla \cdot \boldsymbol{E} = \frac{\rho}{\varepsilon_0}$$

$$\nabla \cdot \boldsymbol{B} = 0$$

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

$$\nabla \times \boldsymbol{B} = \mu_0 \boldsymbol{J} + \mu_0 \varepsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}$$

Light as electromagnetic waves

Maxwell's equations

$$\nabla \cdot \boldsymbol{E} = \frac{\rho}{\varepsilon_0}$$

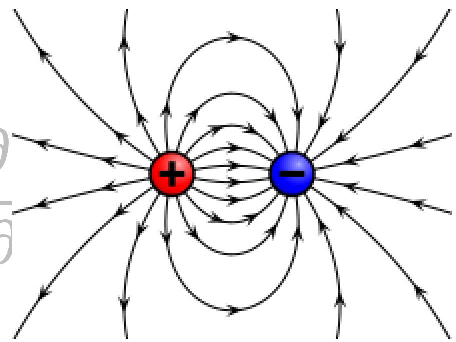
Charge density

Electric field

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

$$\nabla \times \boldsymbol{B} = \mu_0 \boldsymbol{J} + \mu_0 \varepsilon_0 \frac{\partial}{\partial}$$

Static electric field generated by charges



wikipedia

Light as electromagnetic waves

Maxwell's equations

$$\nabla \cdot \boldsymbol{E} = \frac{\rho}{\varepsilon_0}$$

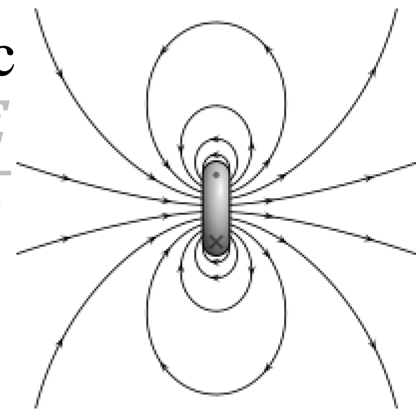
$$\nabla \cdot \boldsymbol{B} = 0$$

Magnetic field

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

$$\nabla \times \boldsymbol{B} = \mu_0 \boldsymbol{J} + \mu_0 \varepsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}$$

Magnetic force lines form closed c



wikipedia

Light as electromagnetic waves

Maxwell's equations

$$\nabla \cdot \boldsymbol{E} = \frac{\rho}{\varepsilon_0}$$

$$\nabla \cdot \boldsymbol{B} = 0$$

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

Rate of change

$$\nabla \times \boldsymbol{B} = \mu_0 \varepsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}$$

A changing magnetic field generates electric field

Light as electromagnetic waves

Maxwell's equations

$$\nabla \cdot \boldsymbol{E} = \frac{\rho}{\varepsilon_0}$$

$$\nabla \cdot \boldsymbol{B} = 0$$

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

$$\nabla \times \boldsymbol{B} = \mu_0 \boldsymbol{J} + \mu_0 \varepsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}$$

Electric current

Electric current and changing electric field generate magnetic field

Light as electromagnetic waves

Maxwell's equations

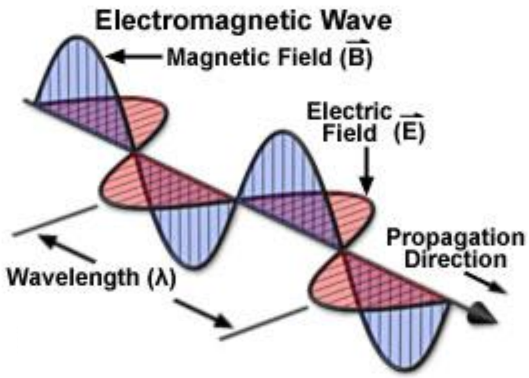
$$\nabla \cdot \boldsymbol{E} = \frac{\rho}{\varepsilon_0}$$

$$\nabla \cdot \boldsymbol{B} = 0$$

$$\nabla \times \boldsymbol{E} = -\frac{\partial \boldsymbol{B}}{\partial t}$$

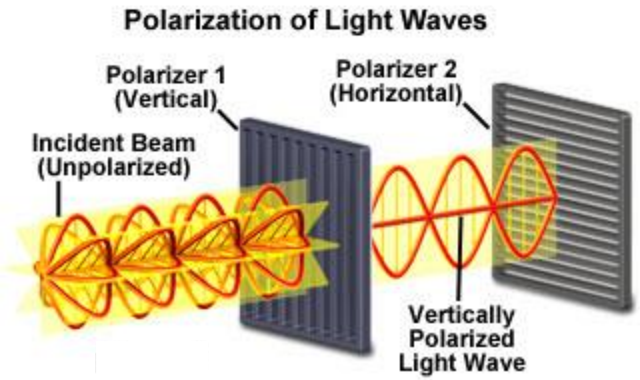
$$\nabla \times \boldsymbol{B} = \mu_0 \boldsymbol{J} + \mu_0 \varepsilon_0 \frac{\partial \boldsymbol{E}}{\partial t}$$

Speed of light $= 1/\sqrt{\mu_0 \varepsilon_0}$



Michael Davidson

Polarization



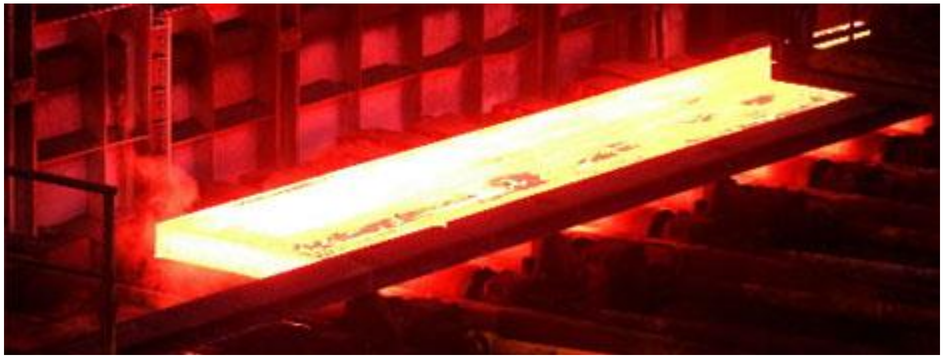
Michael Davidson

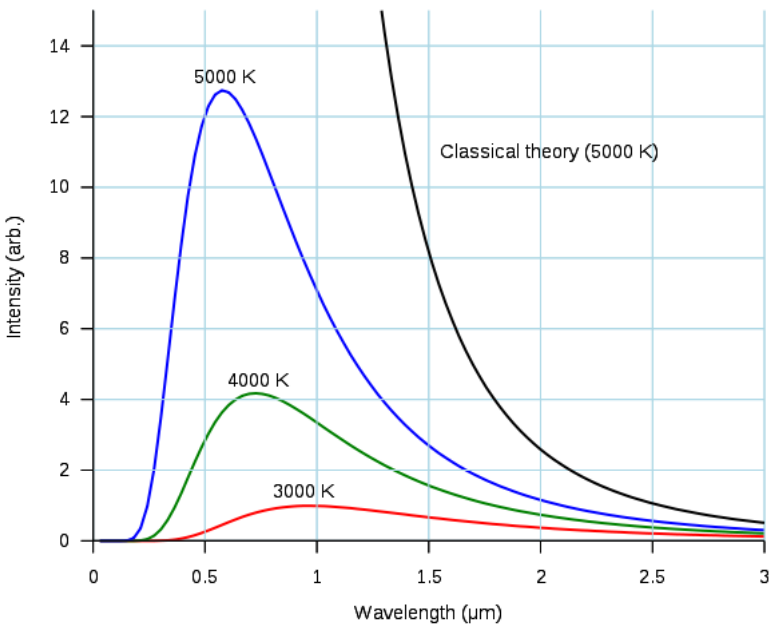
wikipedia

What about energy?

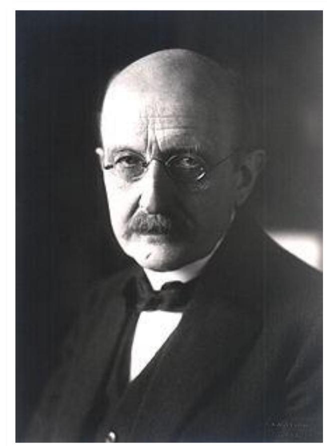
- Classically, the energy of light was just the amplitude of the electric and magnetic fields.
- But this led to problems….

Blackbody emission and the UV catastrophe





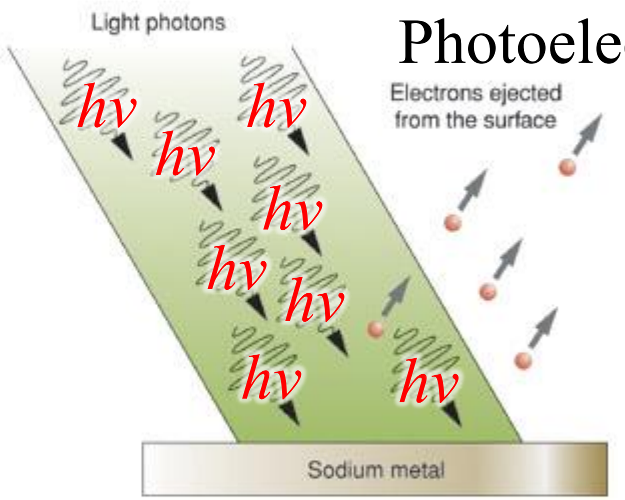
Max Planck



$$E = h\nu$$

Photoelectric effect

Photoelectrons



The Encyclopedia of Science

Albert Einstein

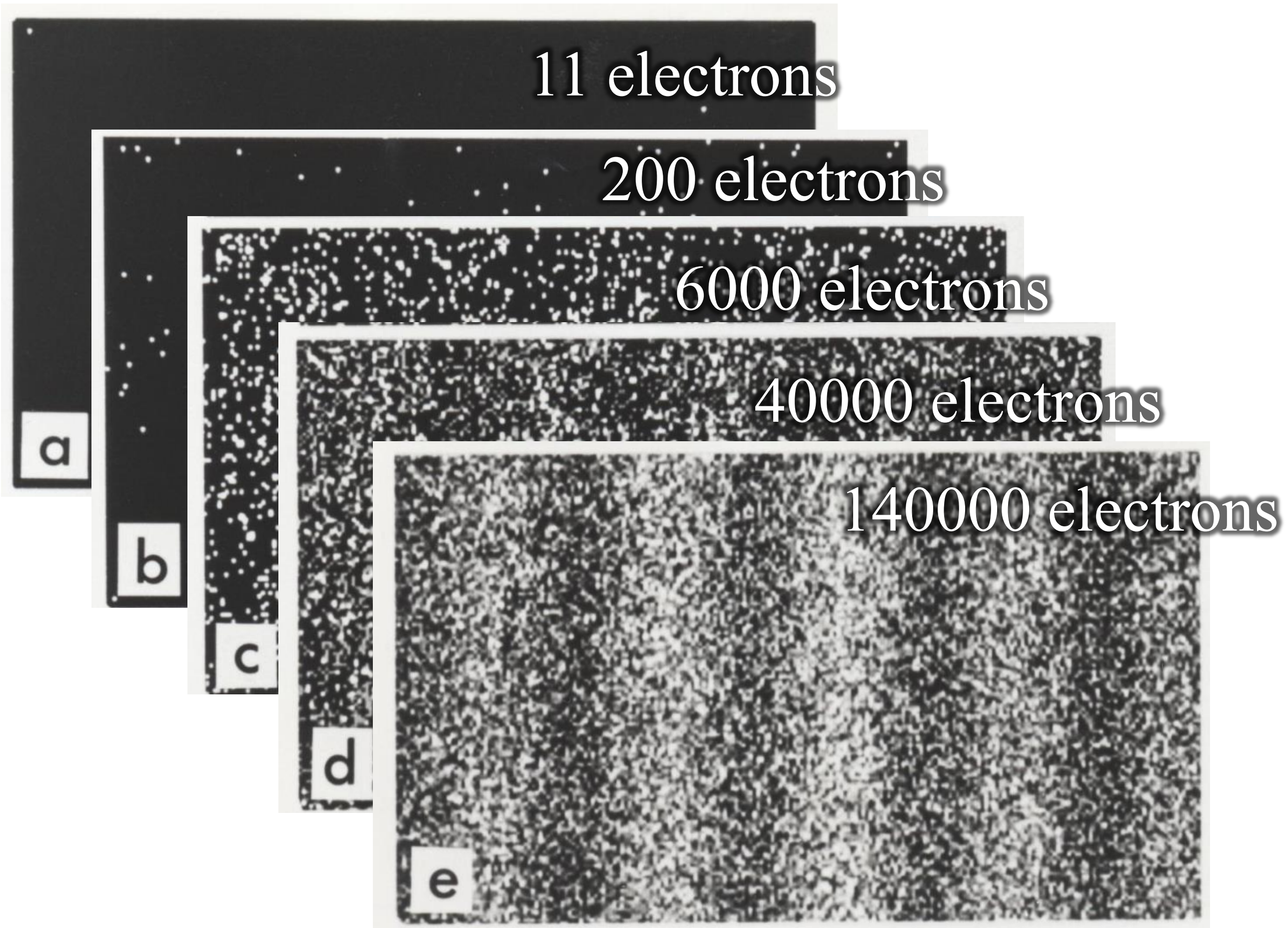
Photons are light particles with $E = h\nu$

Properties of Light

- Speed: c in vacuum, $v = c/\lambda$ in matter
- Wavelength: λ
- Frequency: ν
- Energy: $E = h\nu$
- $\nu = c/\lambda$

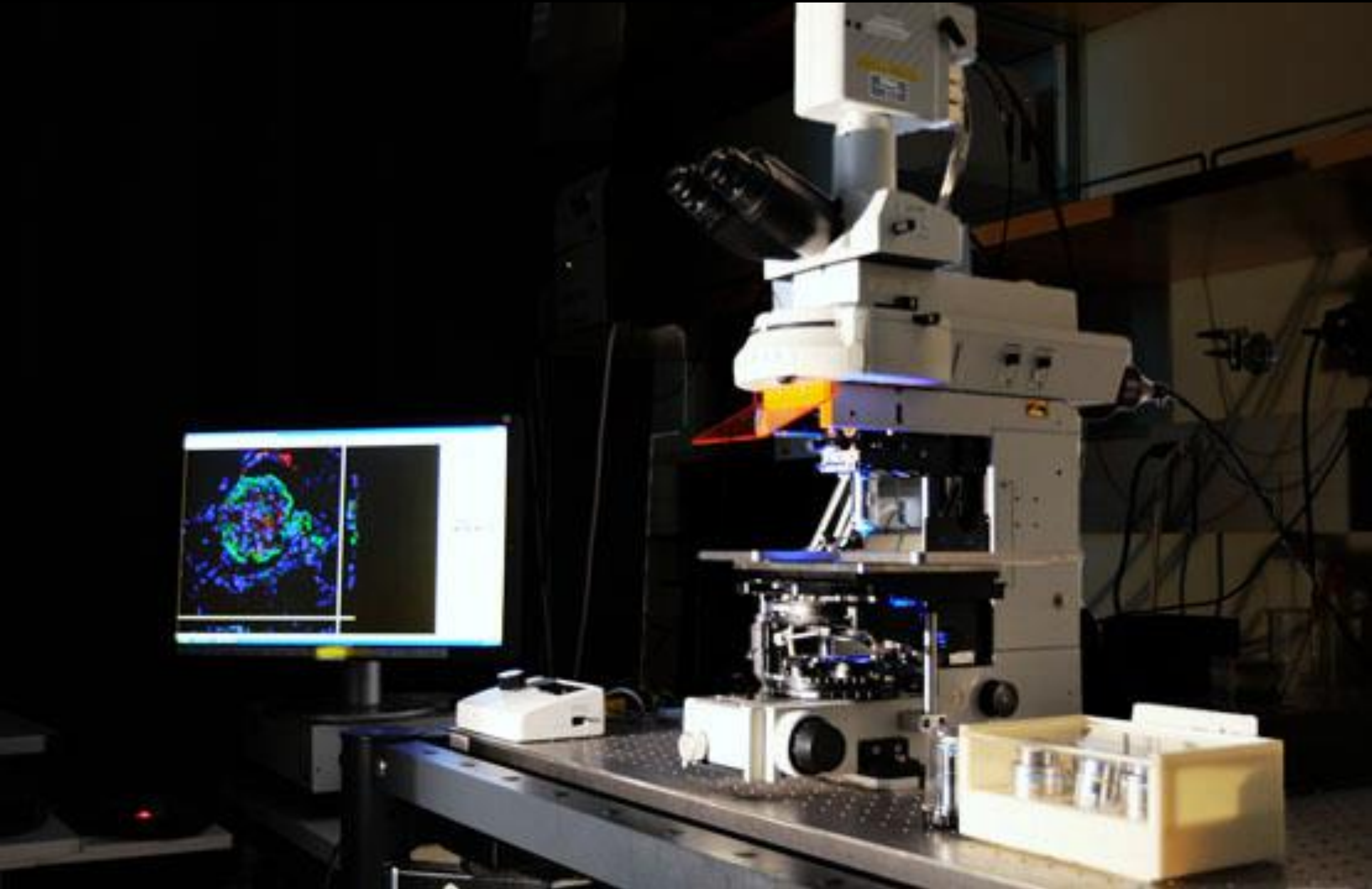
Wave-particle duality

Double-slit experiment of electrons

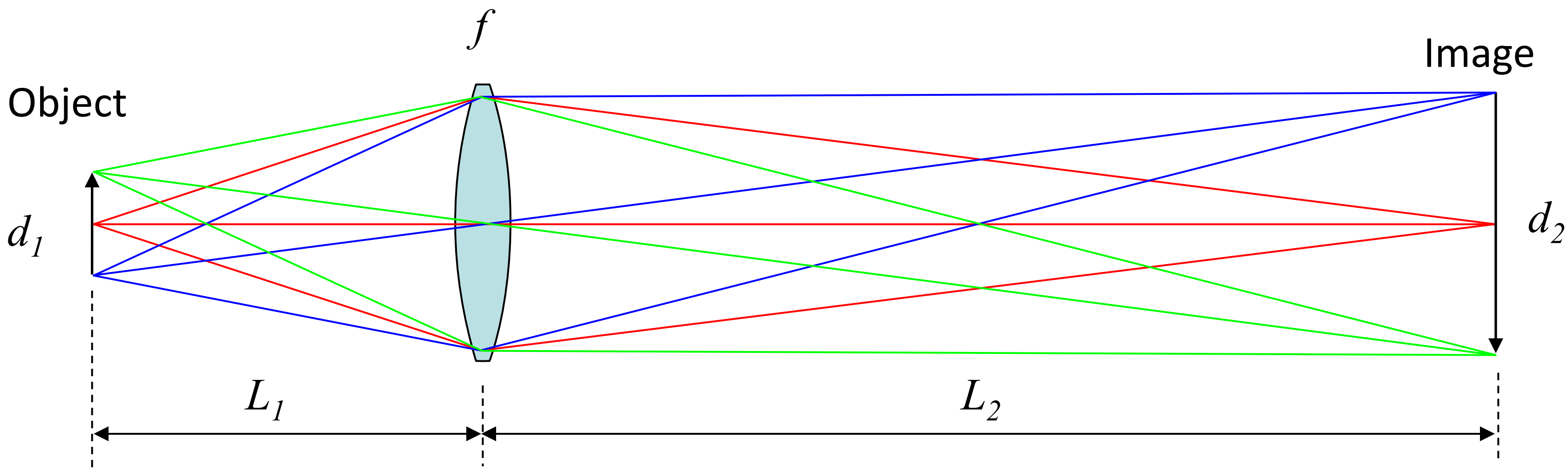


Akira Tonomura, PNAS, 2005

Back to Microscopy



Imaging

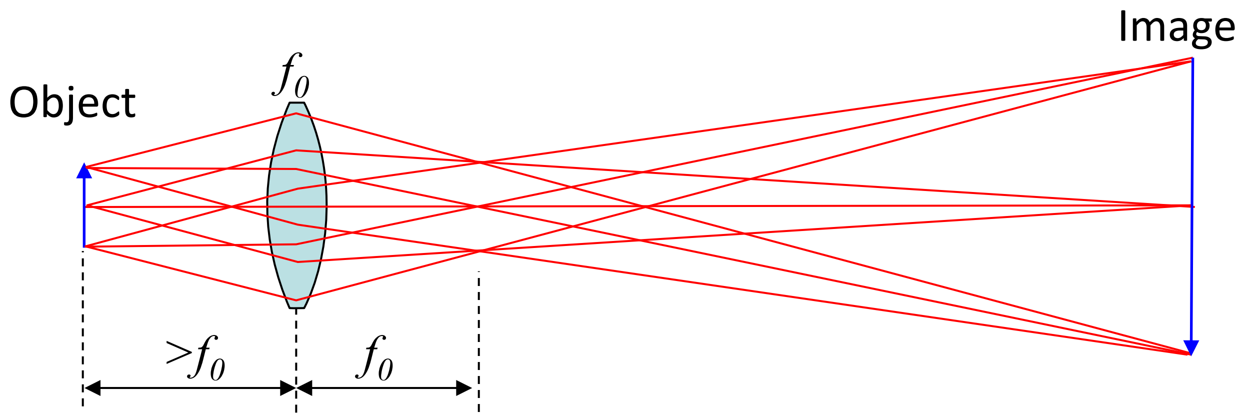


The lens law: $$\frac{1}{L_1} + \frac{1}{L_2} = \frac{1}{f}$$

Magnification: $$M = \frac{d_2}{d_1} = \frac{L_2}{L_1}$$

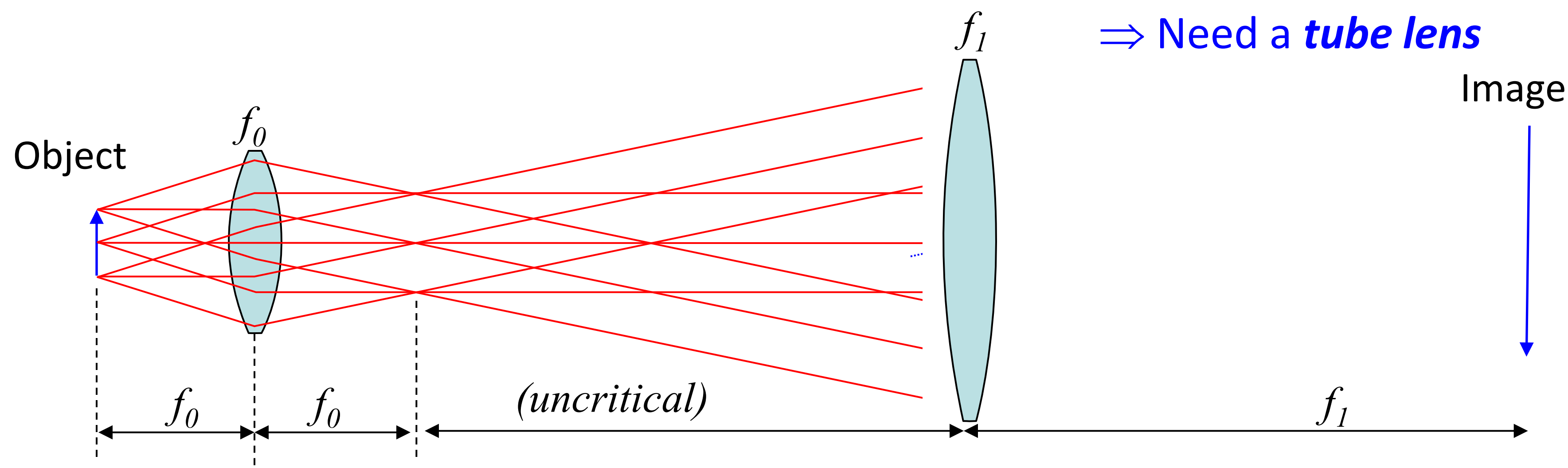
Finite vs. Infinite Conjugate Imaging

- Finite conjugate imaging (older objectives)



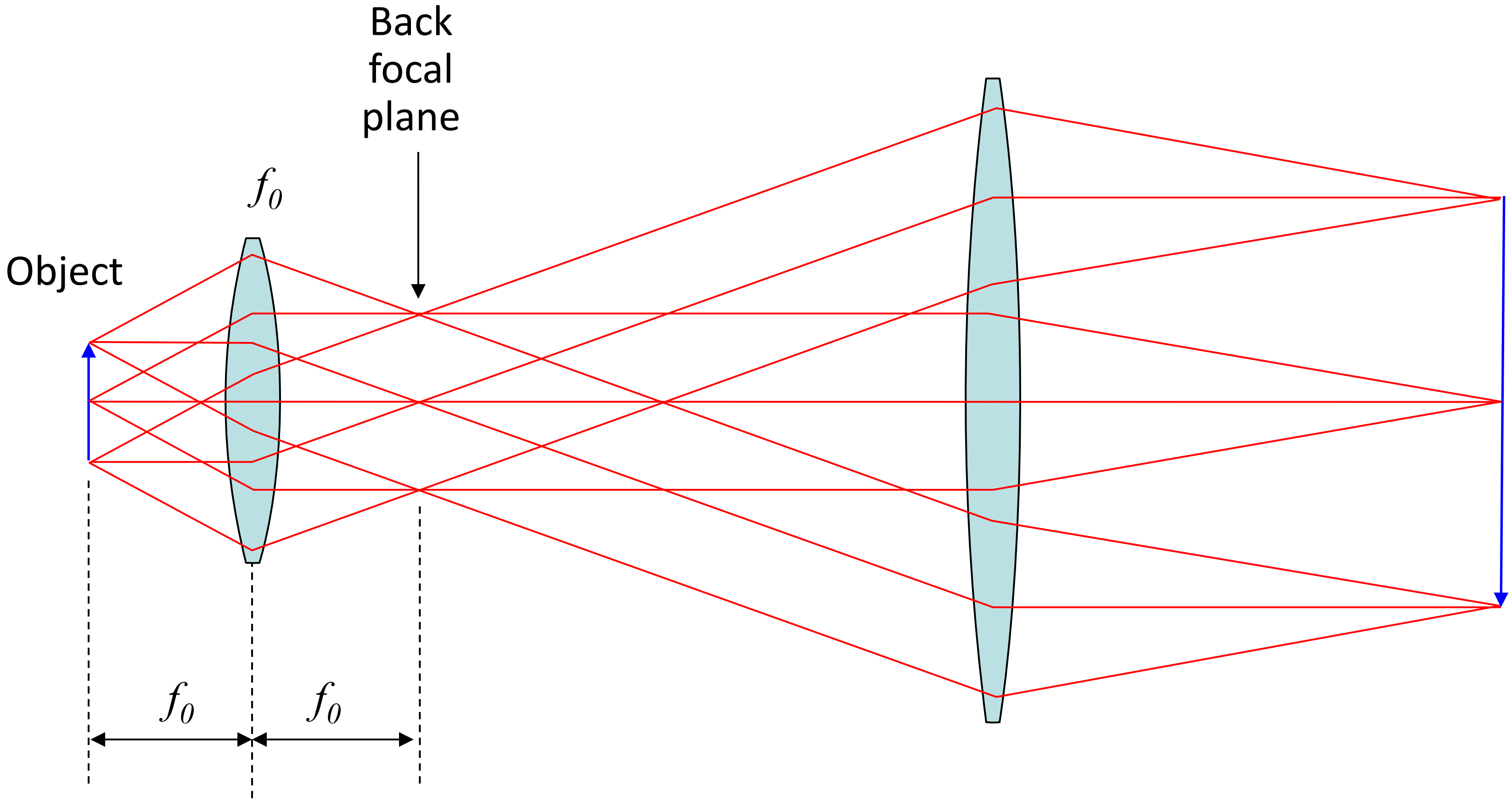
- Infinite conjugate imaging (modern objectives).

Image at infinity
$\Rightarrow$ Need a ***tube lens***



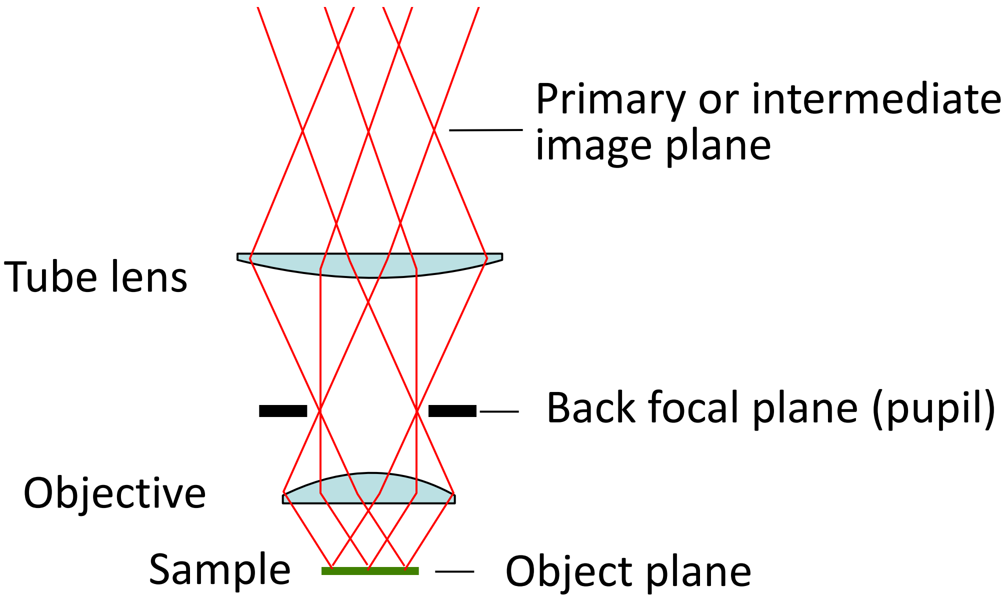
Magnification: $M = \frac{f_1}{f_o}$

Back focal plane

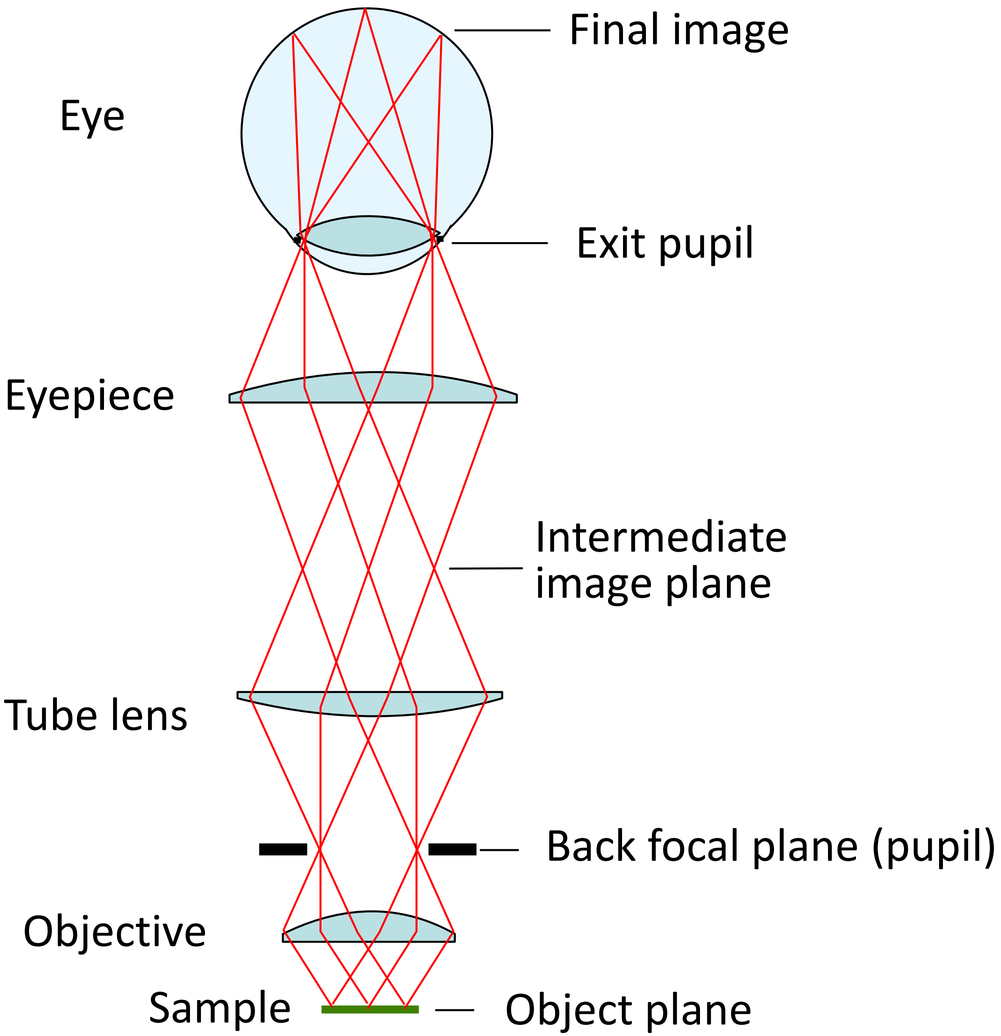


Rays that leave the object with the same angle meet in the objective's *back focal plane*

The Compound Microscope



The Compound Microscope



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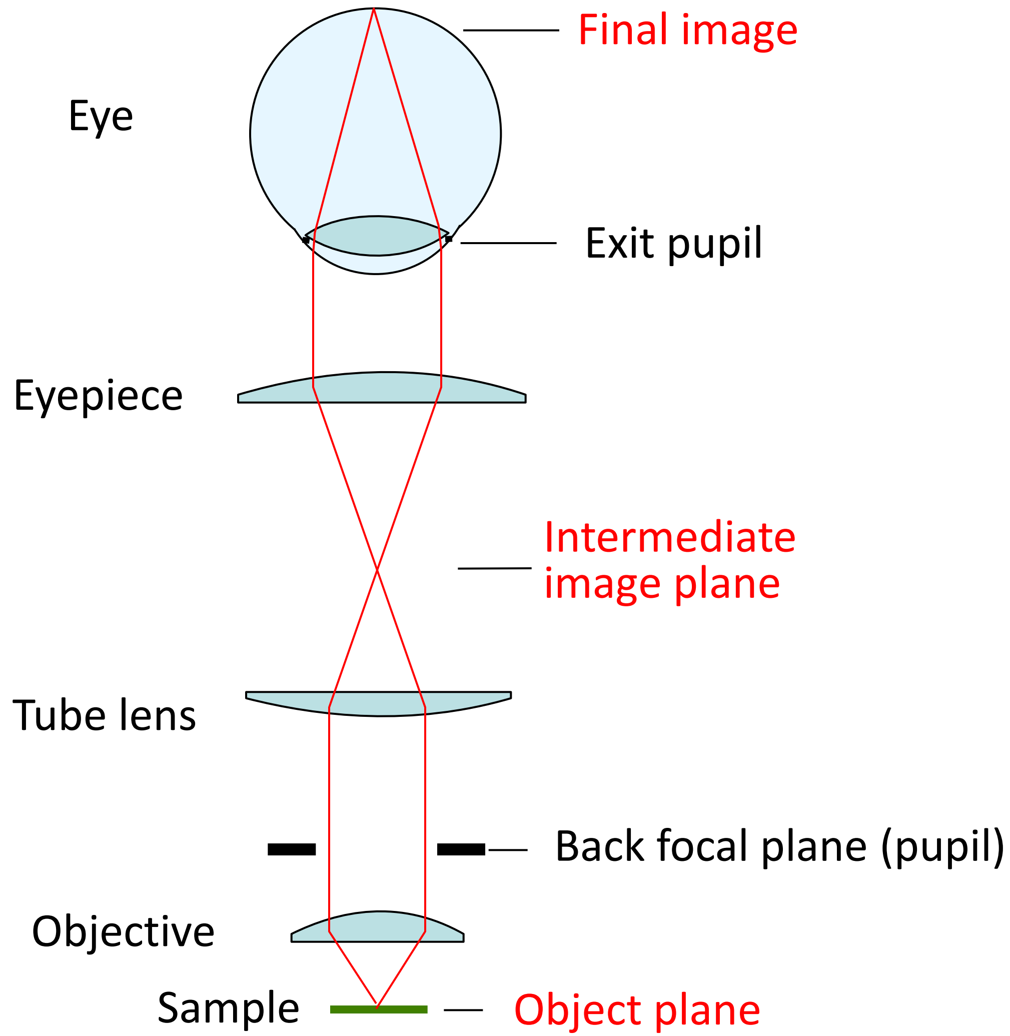
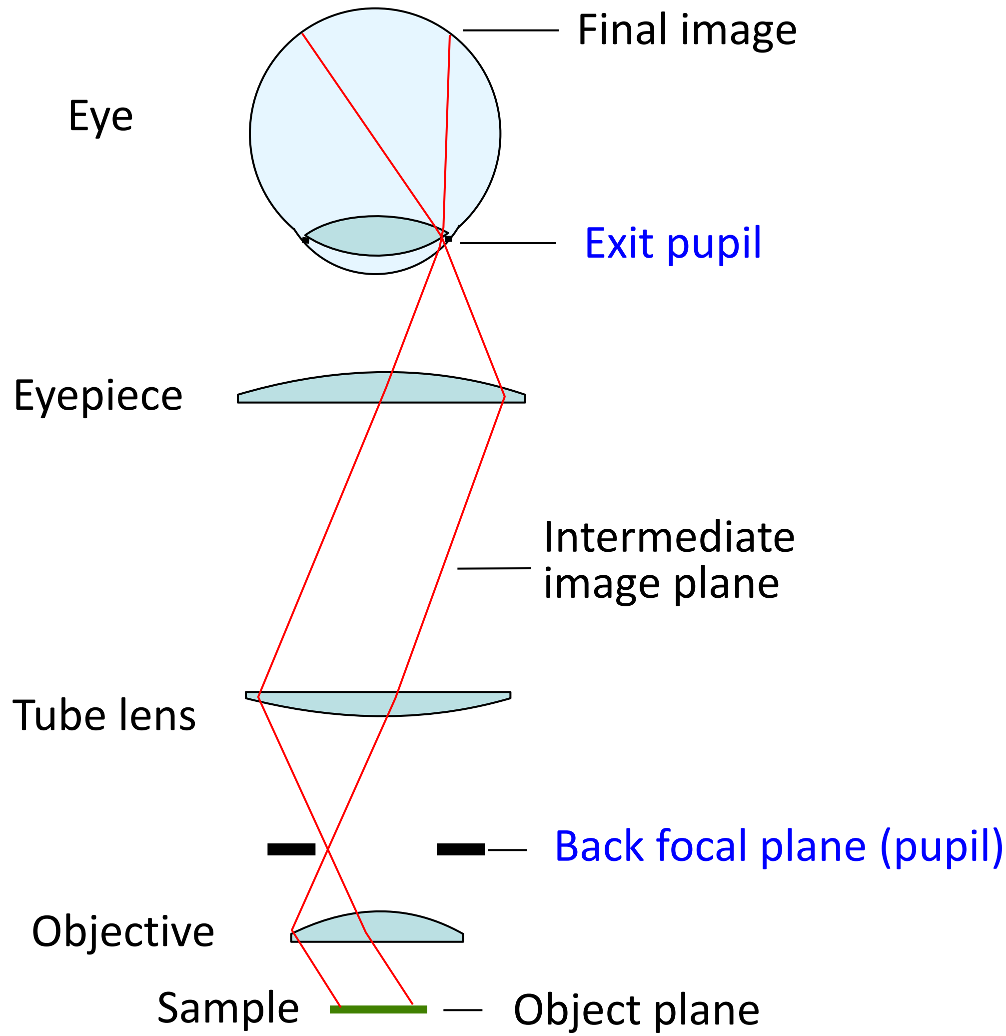
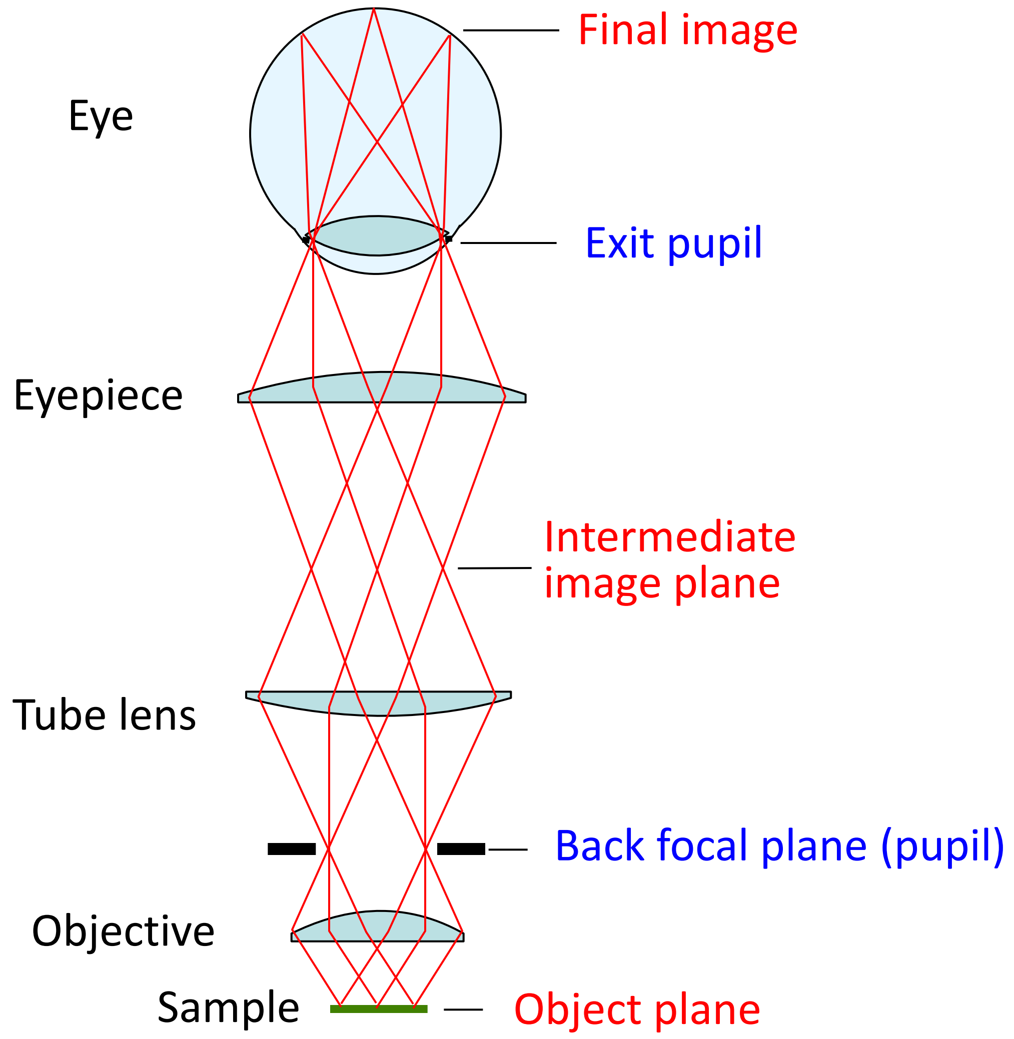


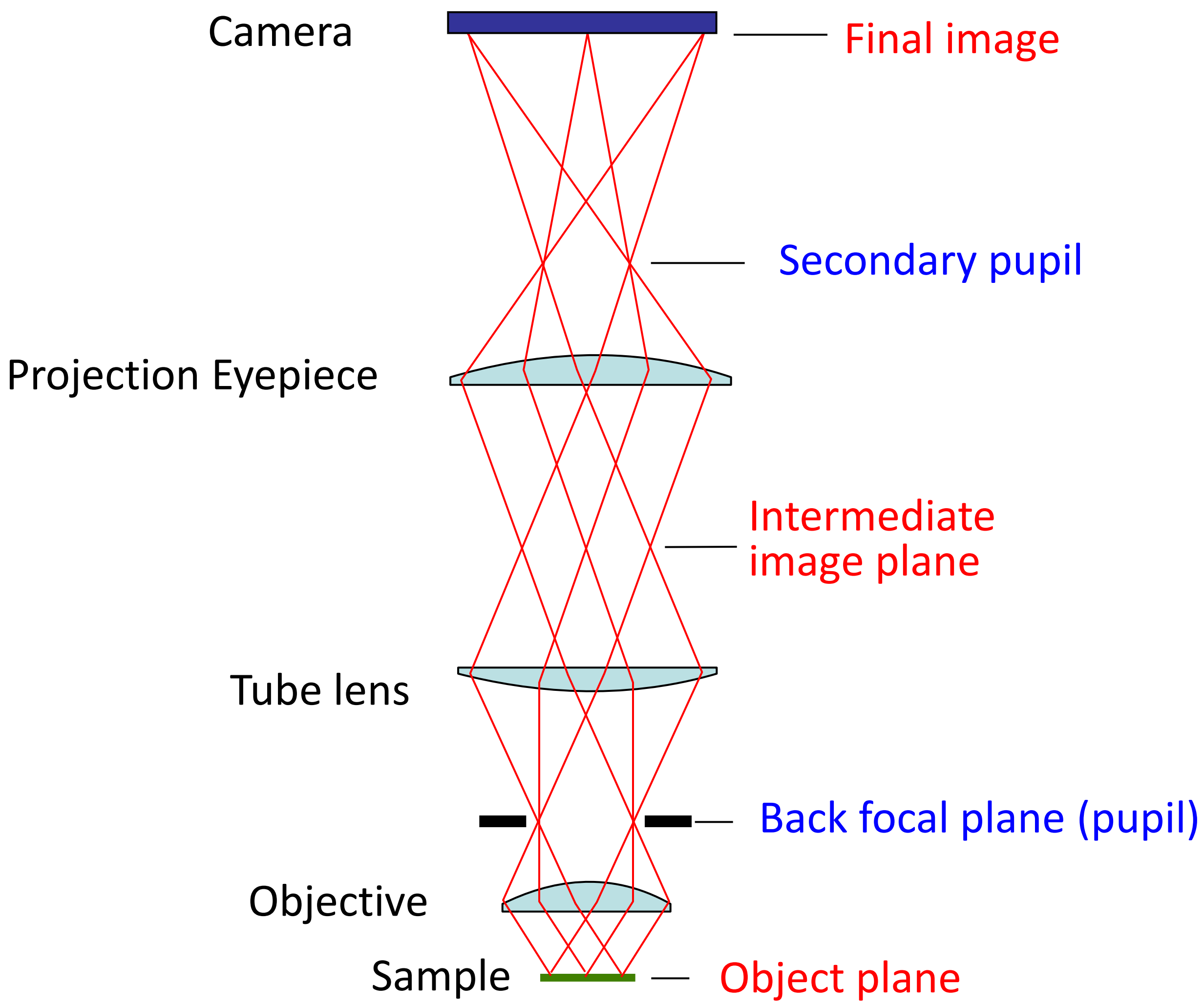
The Compound Microscope



The Compound Microscope



The Compound Microscope

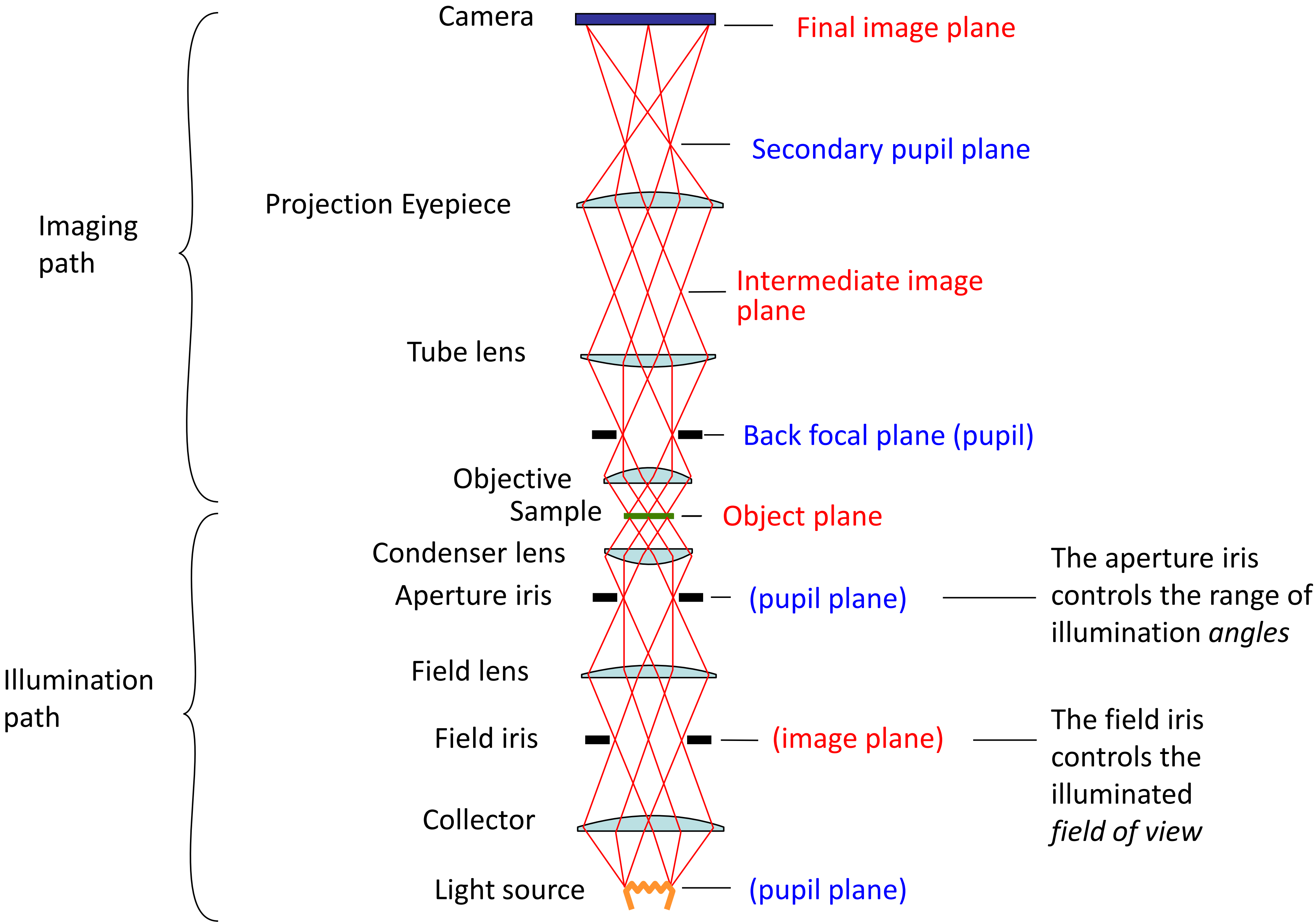


Eyepieces (Oculars)

Features

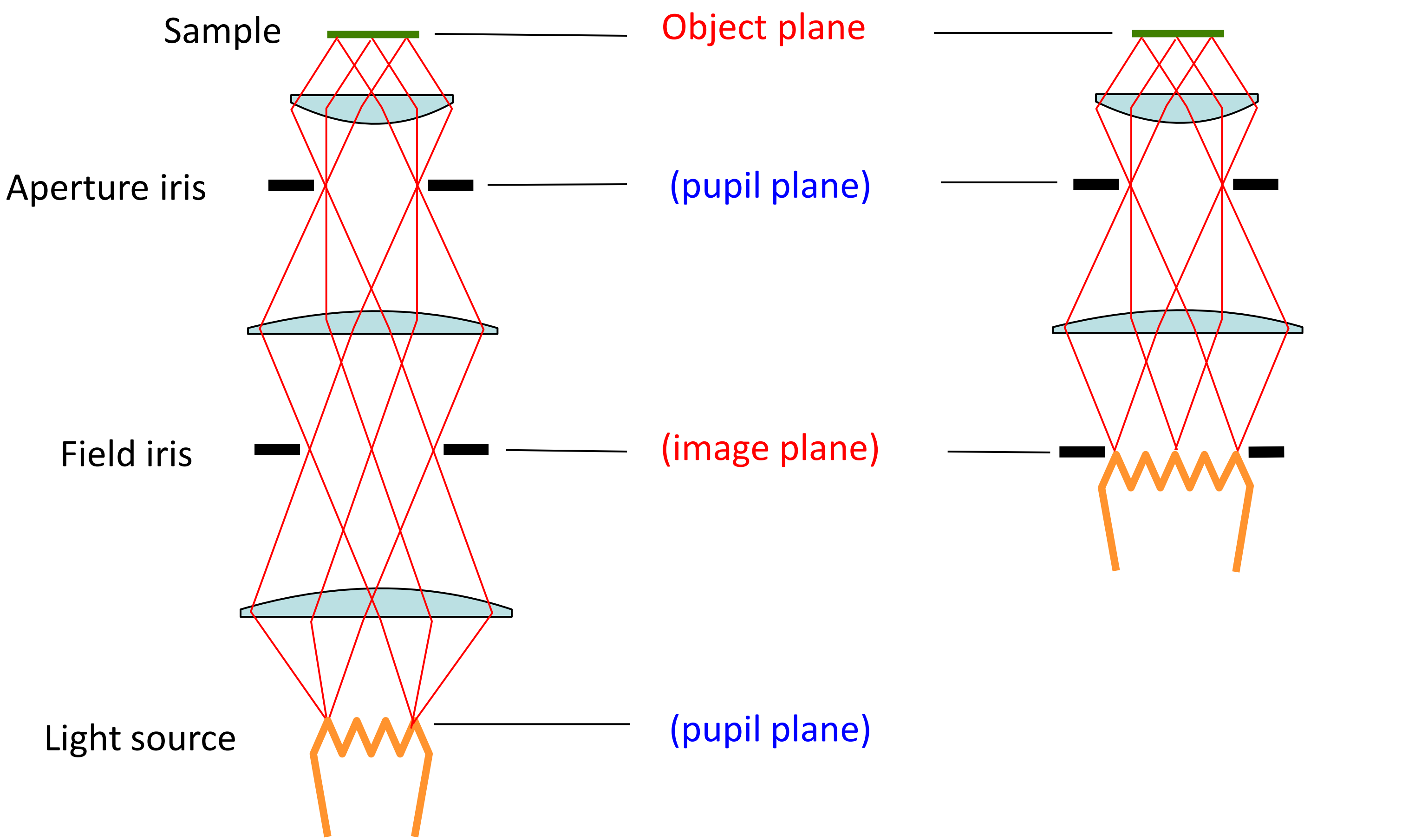
- Magnification (10x typical)
- “High eye point” (exit pupil high enough to allow eyeglasses)
- Diopter adjust (at least *one* must have this)
- Reticle or fitting for one
- Eye cups

Trans-illumination Microscope



Köhler Illumination

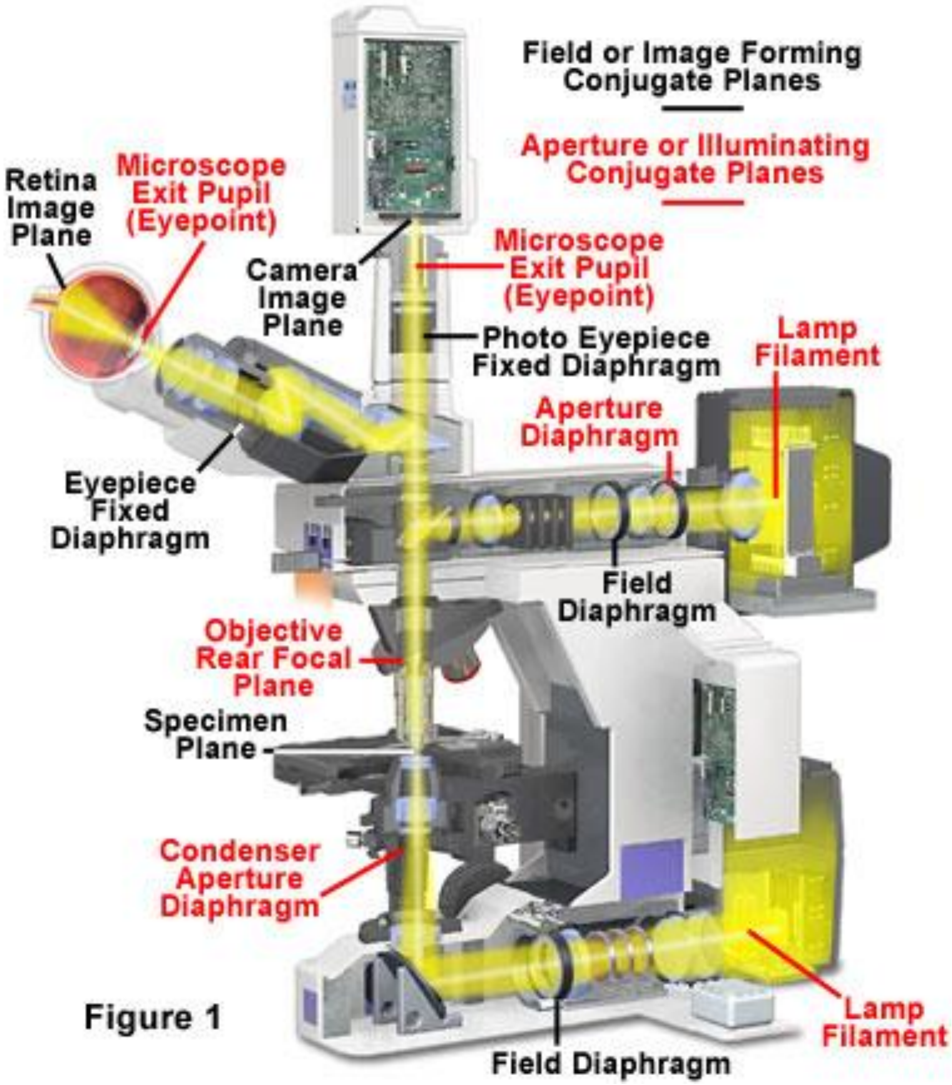
Critical Illumination



- Each light source point produces a parallel beam of light at the sample
- Uniform light intensity at the sample even if the light source is “ugly” (e.g. a filament)

- The source is imaged onto the sample
- Usable only if the light source is perfectly uniform

Conjugate Planes in A Research Microscope



How view the pupil planes?

Two ways:

- “Eyepiece telescope”
- “Bertrand lens”