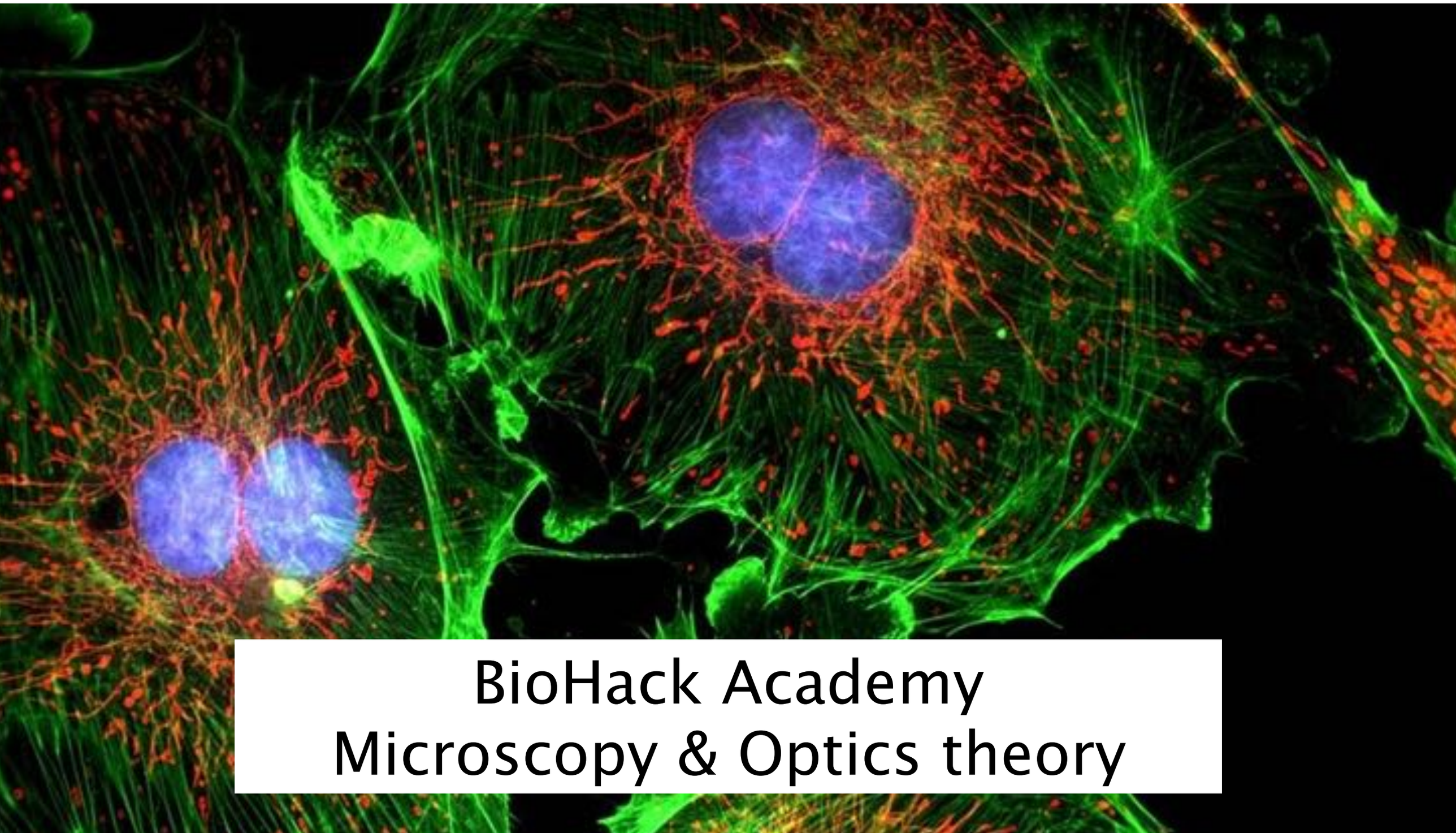
waag
wetlab amsterdam



# BioHack Academy
# Microscopy & Optics theory

Antonie van Leeuwenhoek

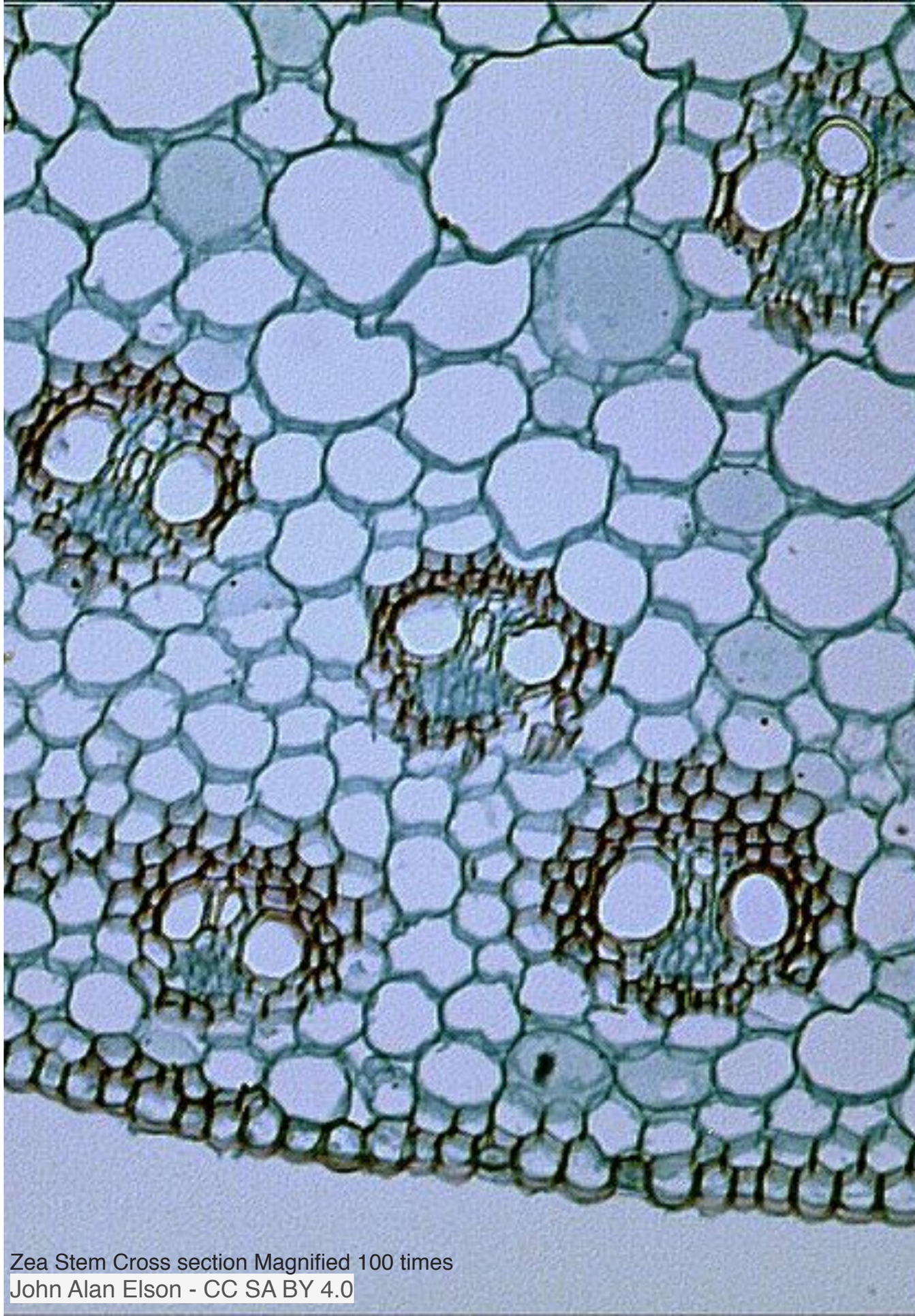
- Businessman
- Amateur biologist
- Lenses made …
- from pulling glass rods apart in a flame?
- Sanding?

Replica of microscope by Leeuwenhoek

Jeroen Rouwkema - CC BY-SA 3.0

Bright Field

- Shadow of the object



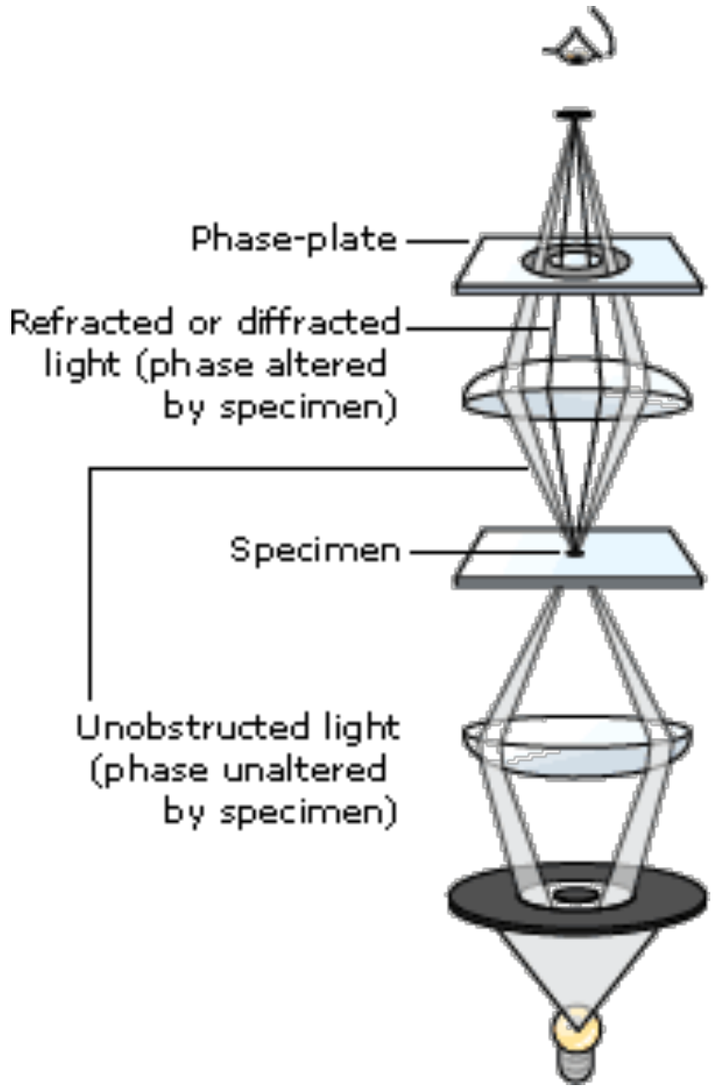
Zea Stem Cross section Magnified 100 times
John Alan Elson - CC SA BY 4.0

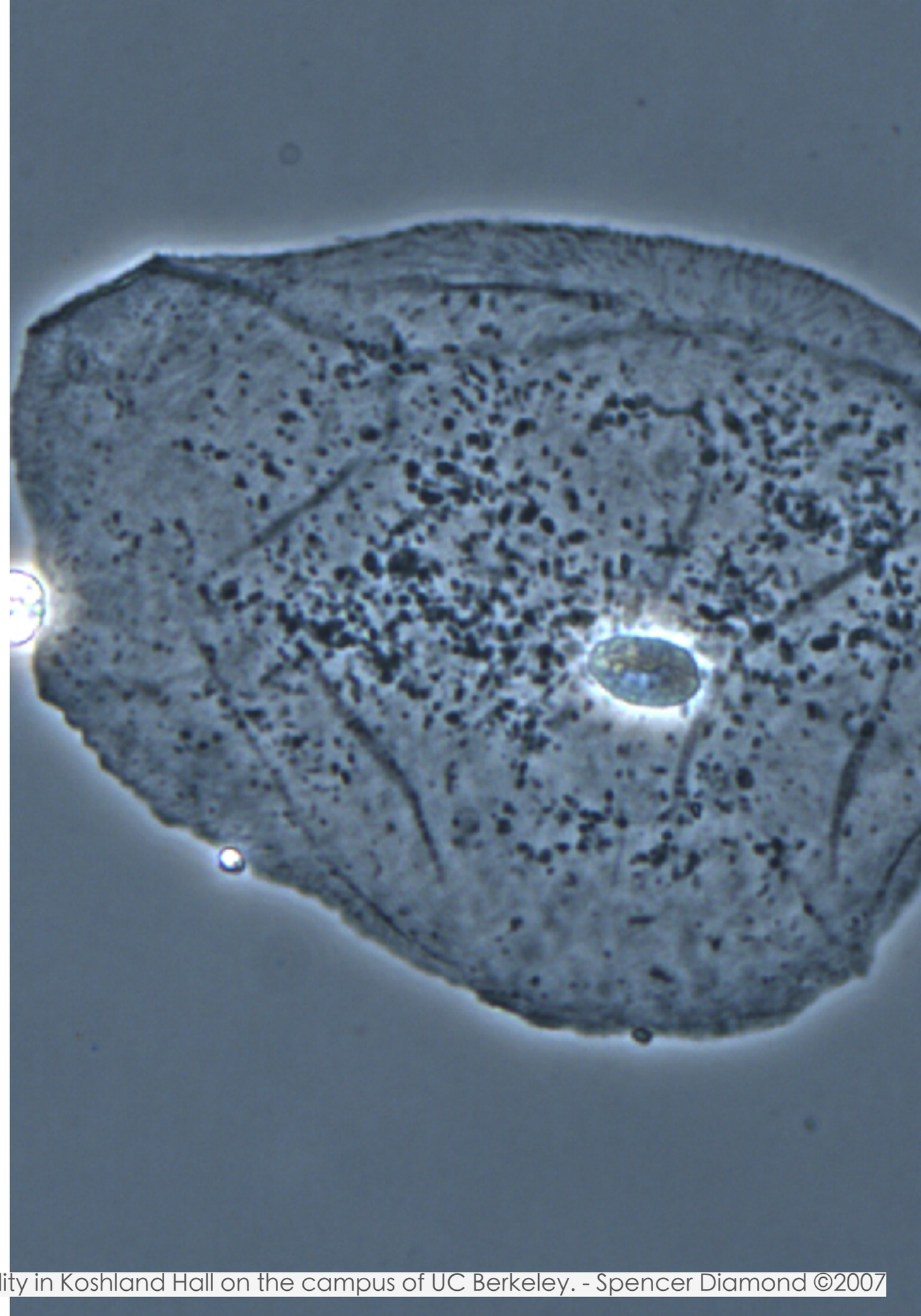
# Dark Field

- Light from the side
- Reflected light
- Better contrast

# Phase Contrast

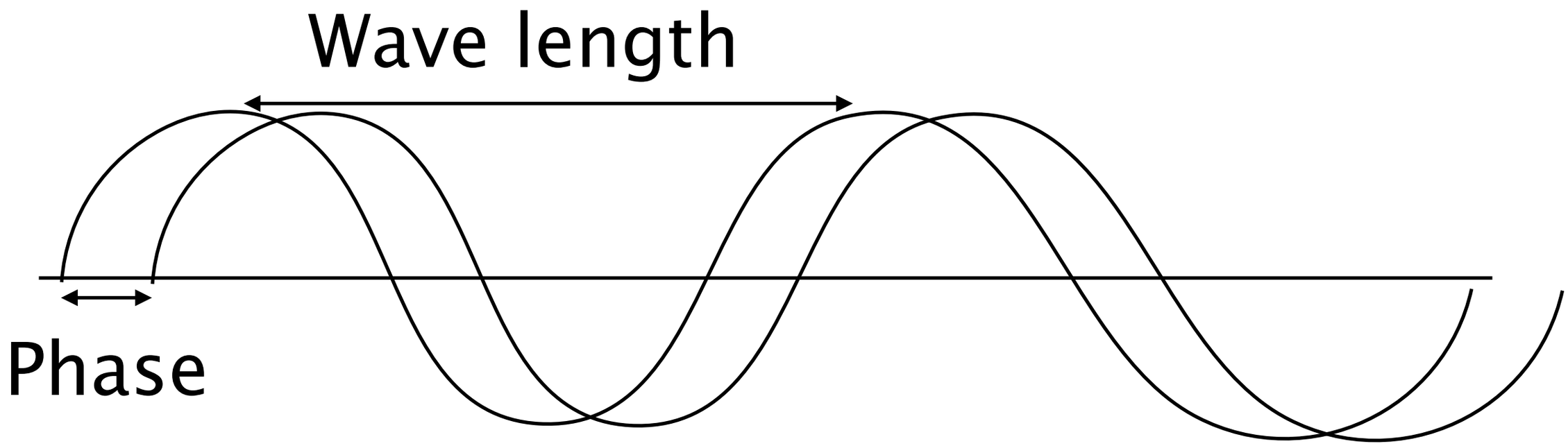
No need for staining



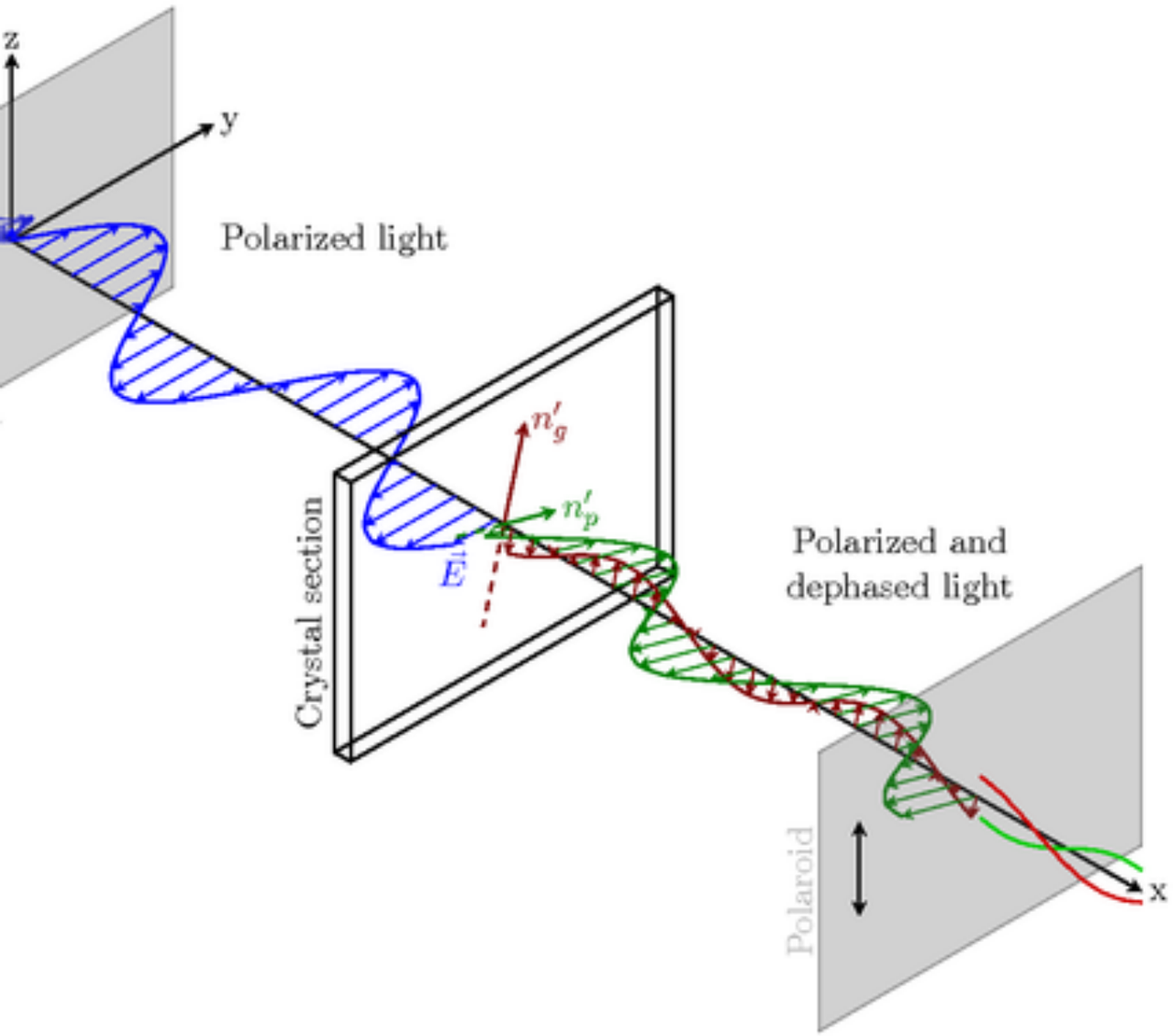



Spencer Diamond at the Biological Imaging Facility in Koshland Hall on the campus of UC Berkeley. - Spencer Diamond ©2007

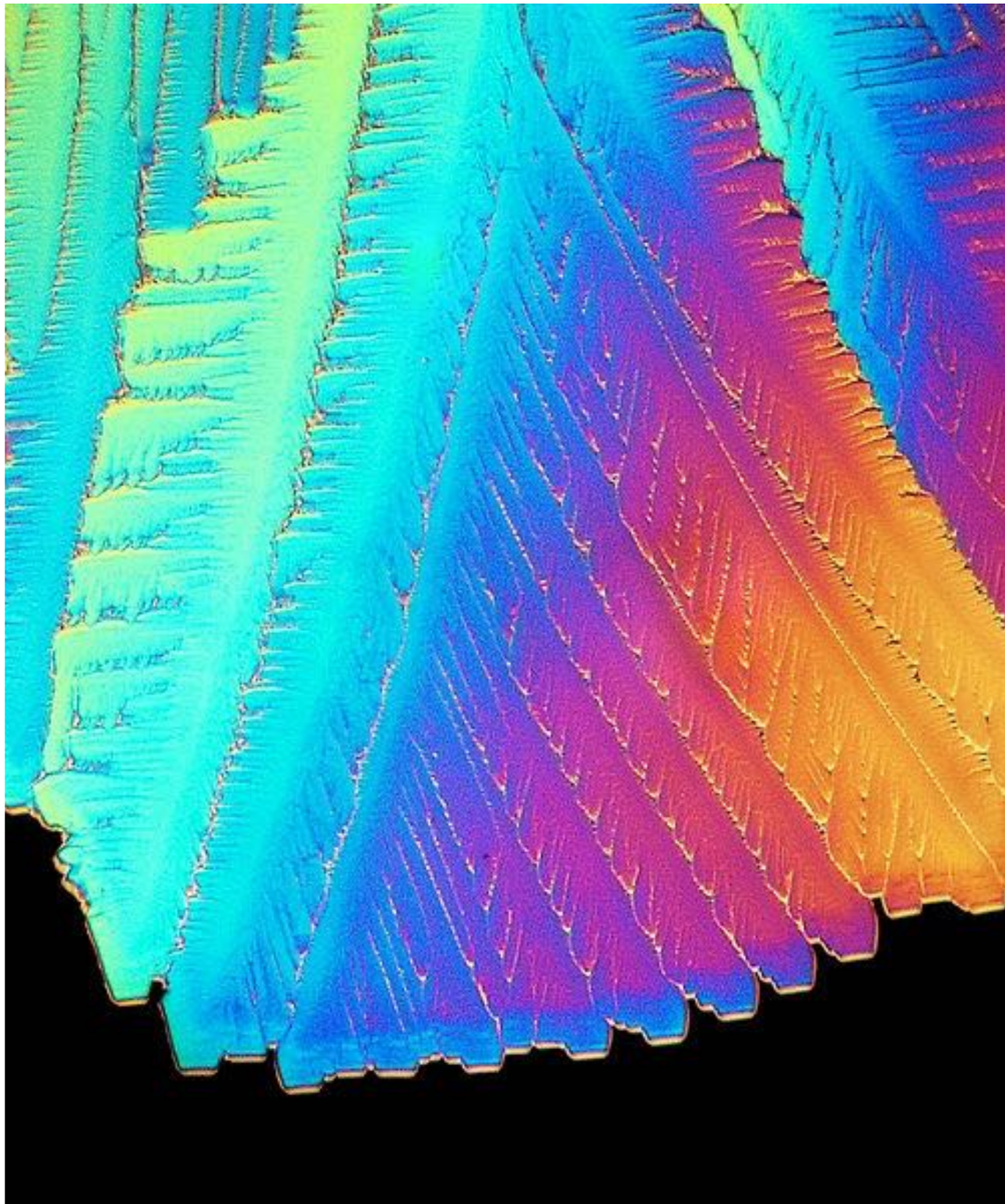
# Light waves



# Polarized microscopy



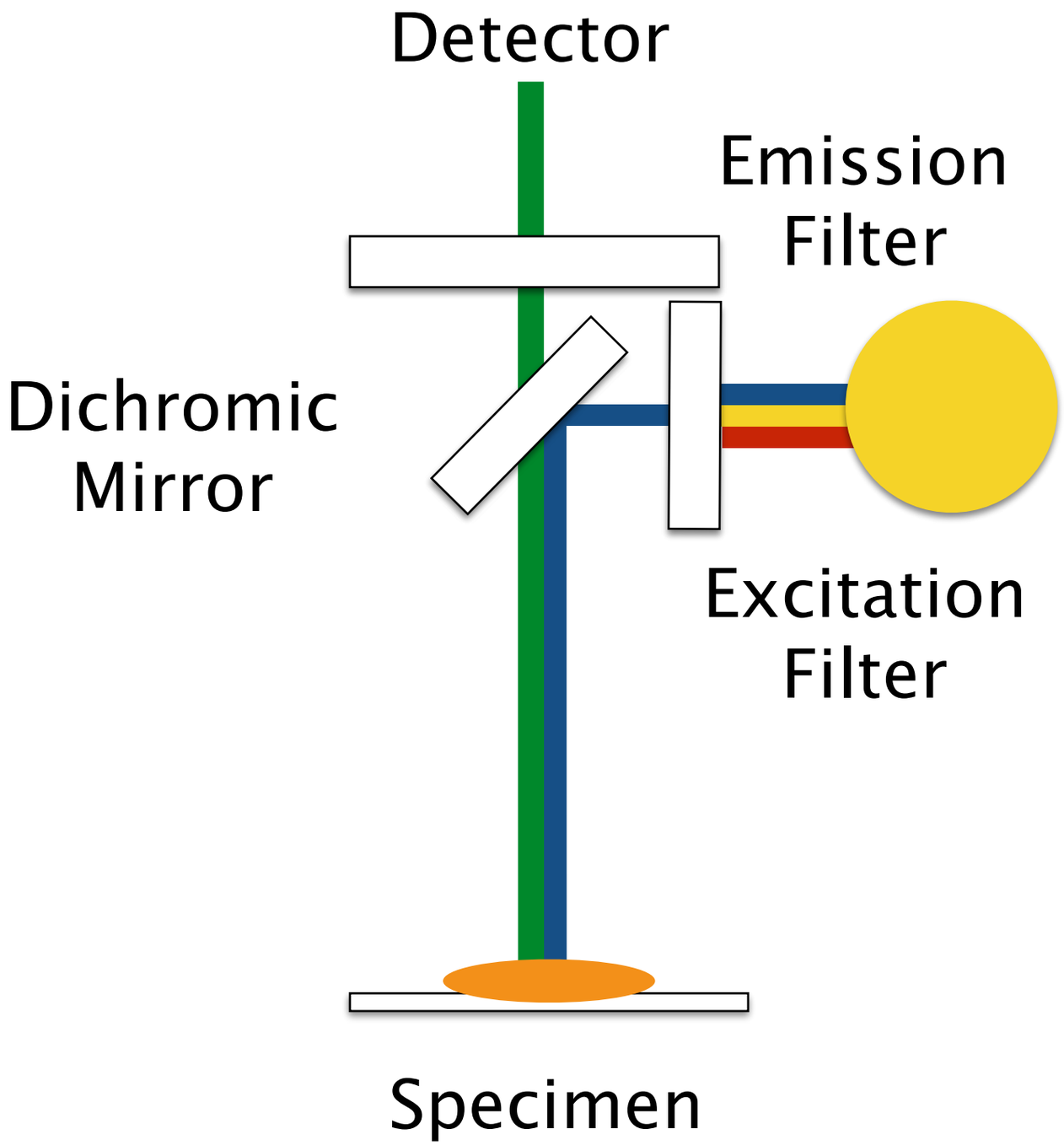

Cyril Langlois CC-BY 2.5

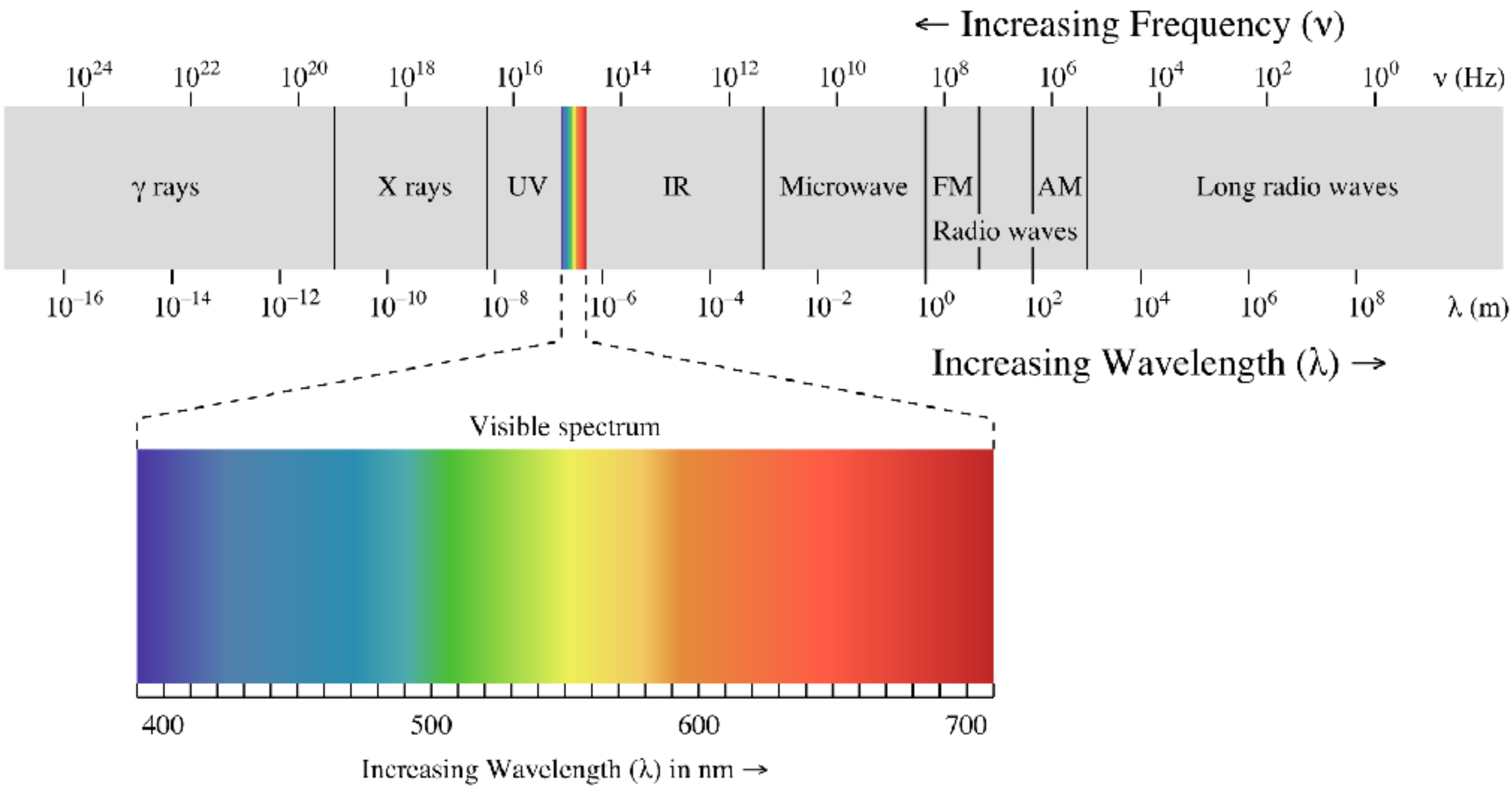


# Fluorescent microscopy

- Excite with low wavelength
- Emits high wavelength

- Most important tool in optic biology

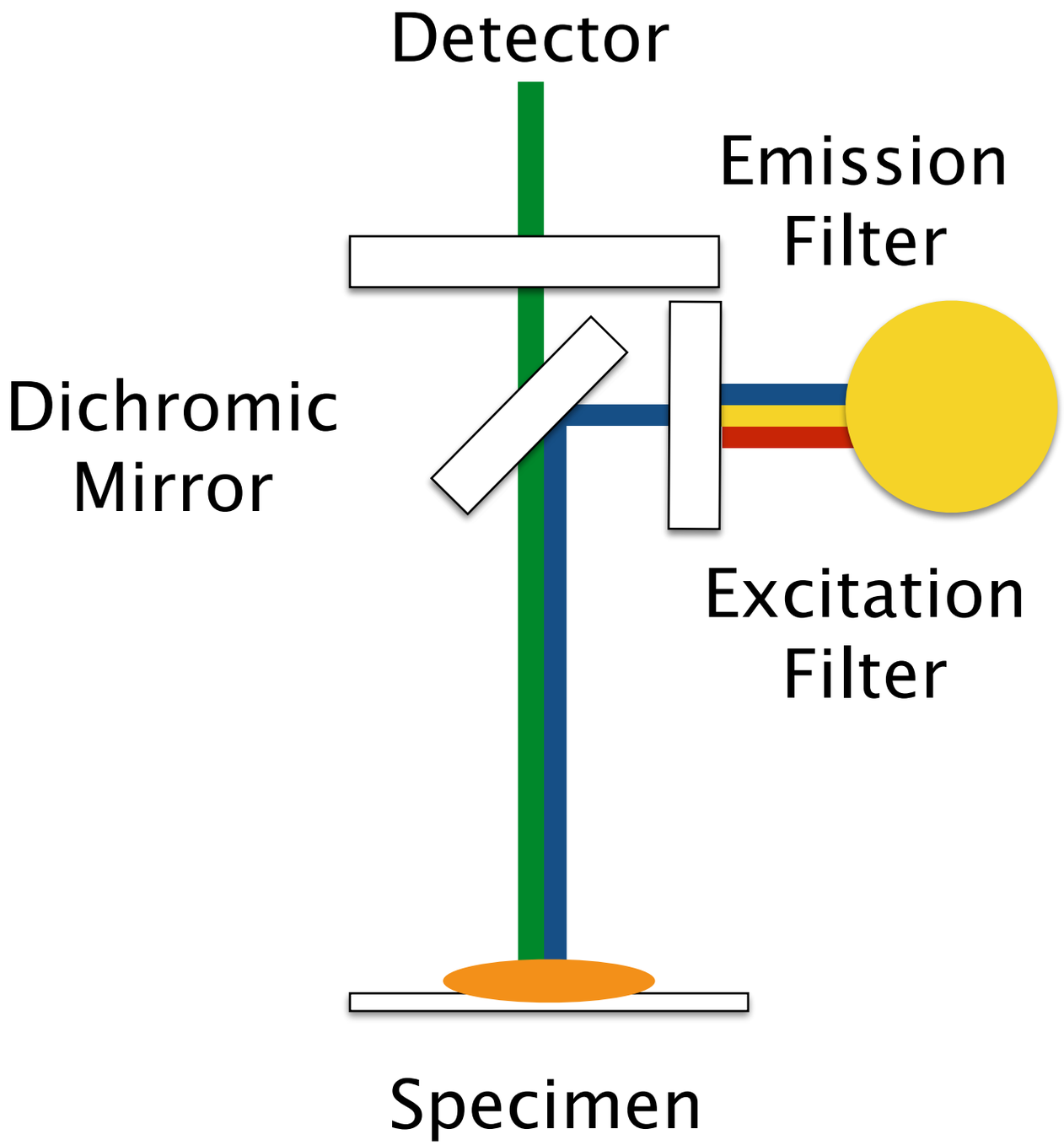



← Increasing Frequency (ν)
$10^{24}$ $10^{22}$ $10^{20}$ $10^{18}$ $10^{16}$ $10^{14}$ $10^{12}$ $10^{10}$ $10^{8}$ $10^{6}$ $10^{4}$ $10^{2}$ $10^{0}$ ν (Hz)
γ rays
X rays
UV
IR
Microwave
FM
AM
Radio waves
Long radio waves
$10^{-16}$ $10^{-14}$ $10^{-12}$ $10^{-10}$ $10^{-8}$ $10^{-6}$ $10^{-4}$ $10^{-2}$ $10^{0}$ $10^{2}$ $10^{4}$ $10^{6}$ $10^{8}$ λ (m)
Increasing Wavelength (λ) →
Visible spectrum
400
500
600
700
Increasing Wavelength (λ) in nm →

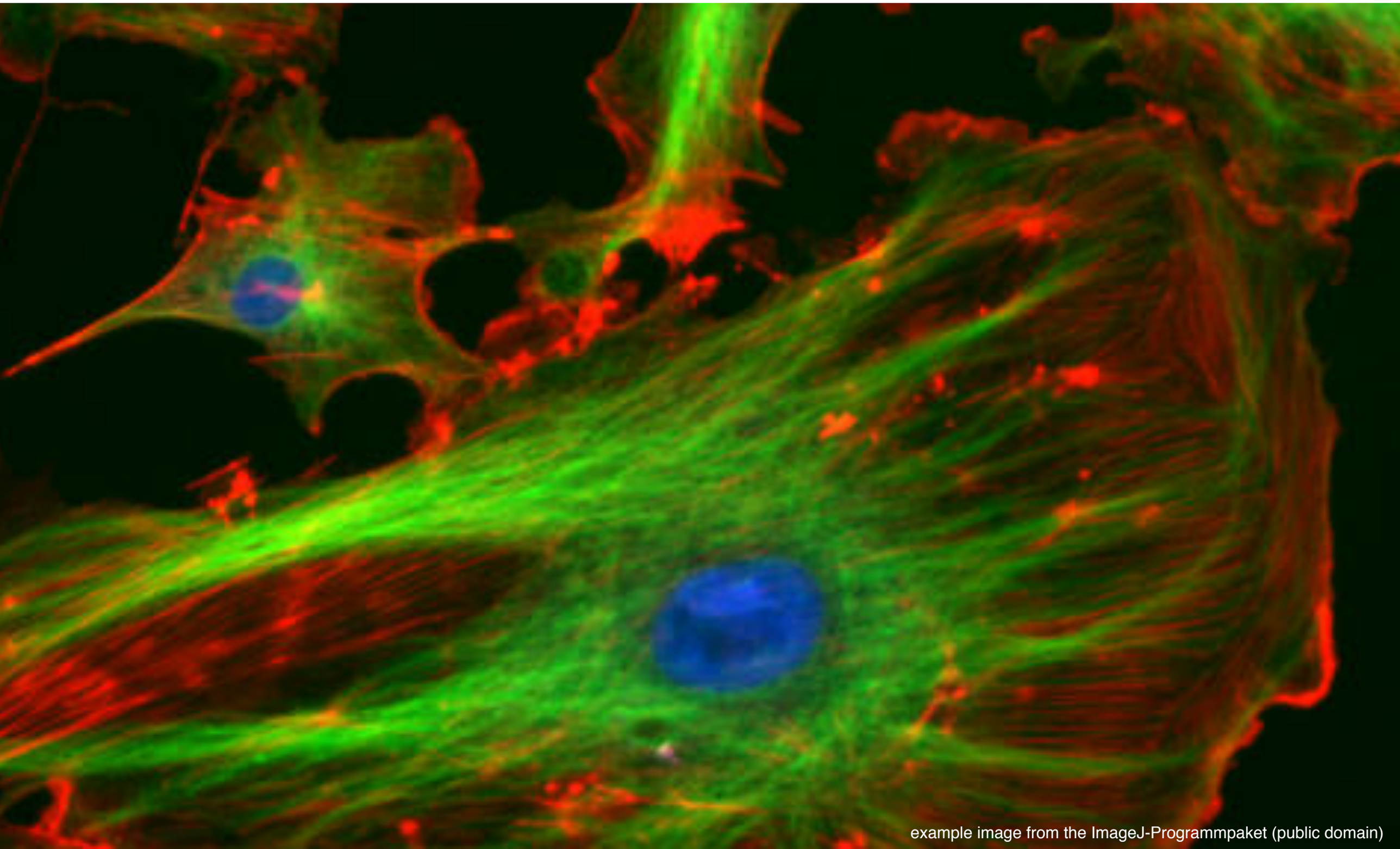
# Fluorescent microscopy

- Excite with low wavelength
- Emits high wavelength

- Most important tool in optic biology



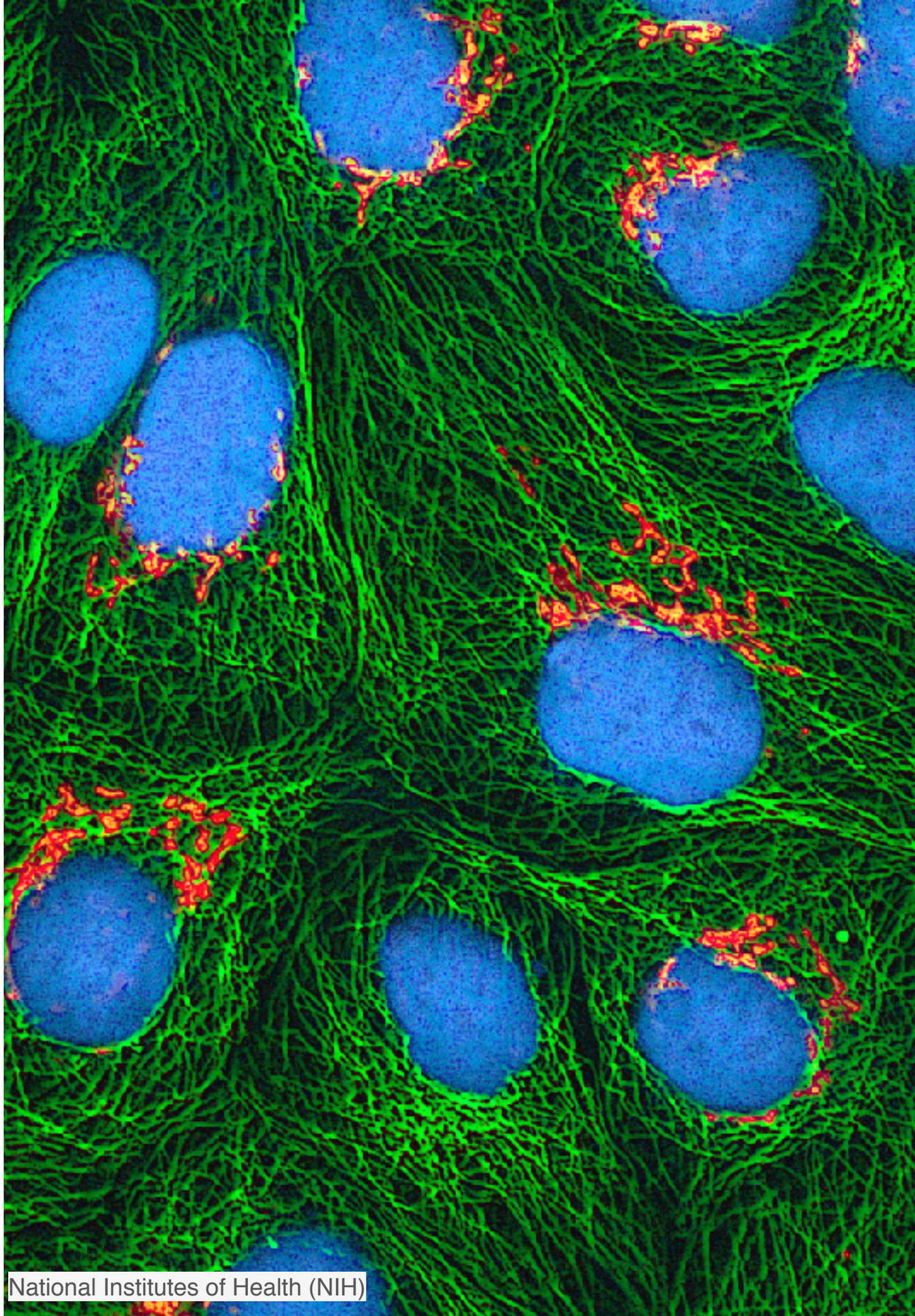
# Fluorescence microscopy



example image from the ImageJ-Programmpaket (public domain)

Laser scanning microscopy

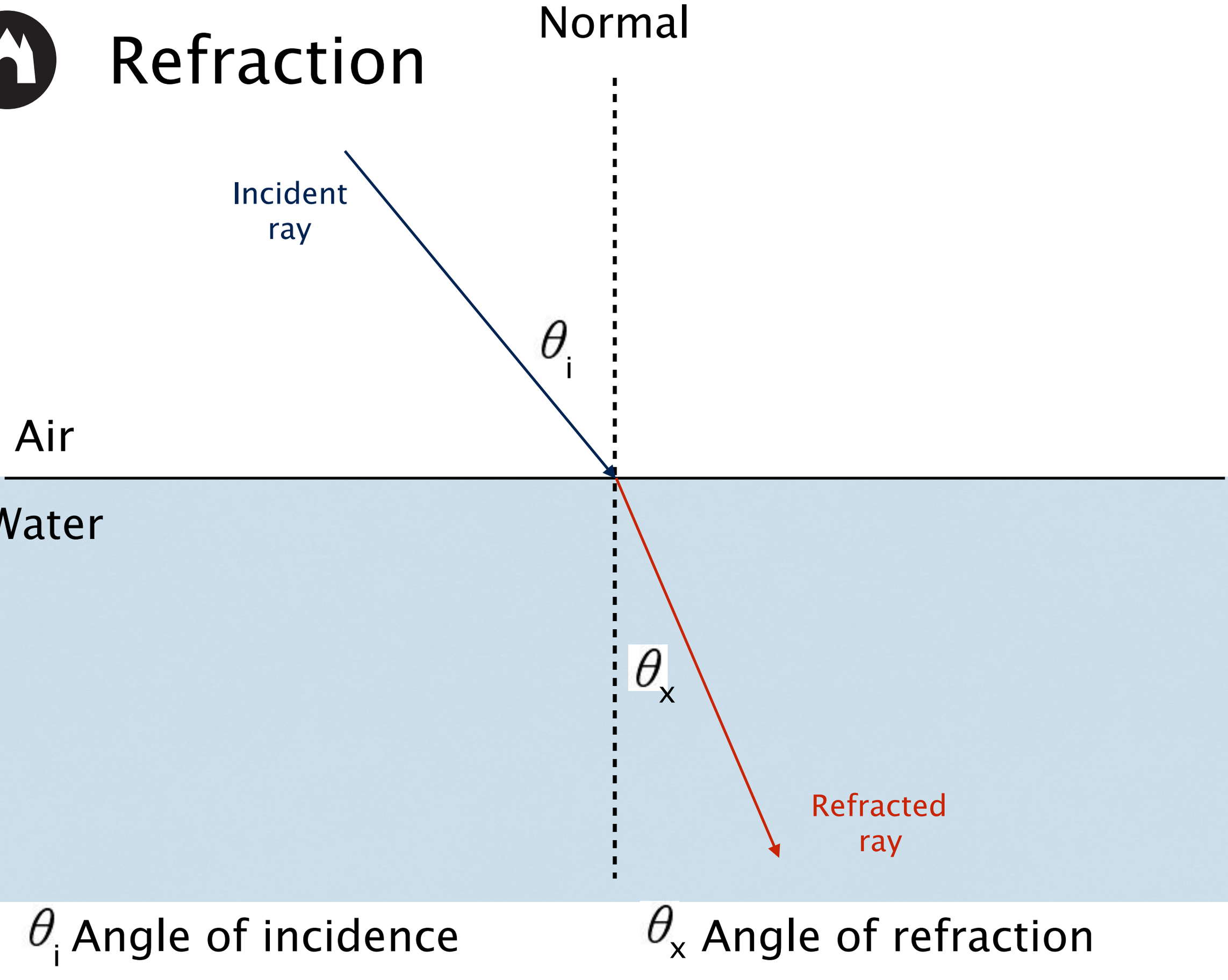
- Scan an image point by point
- Much better focus than other techniques


National Institutes of Health (NIH)

waag
wetlab amsterdam

# Optics

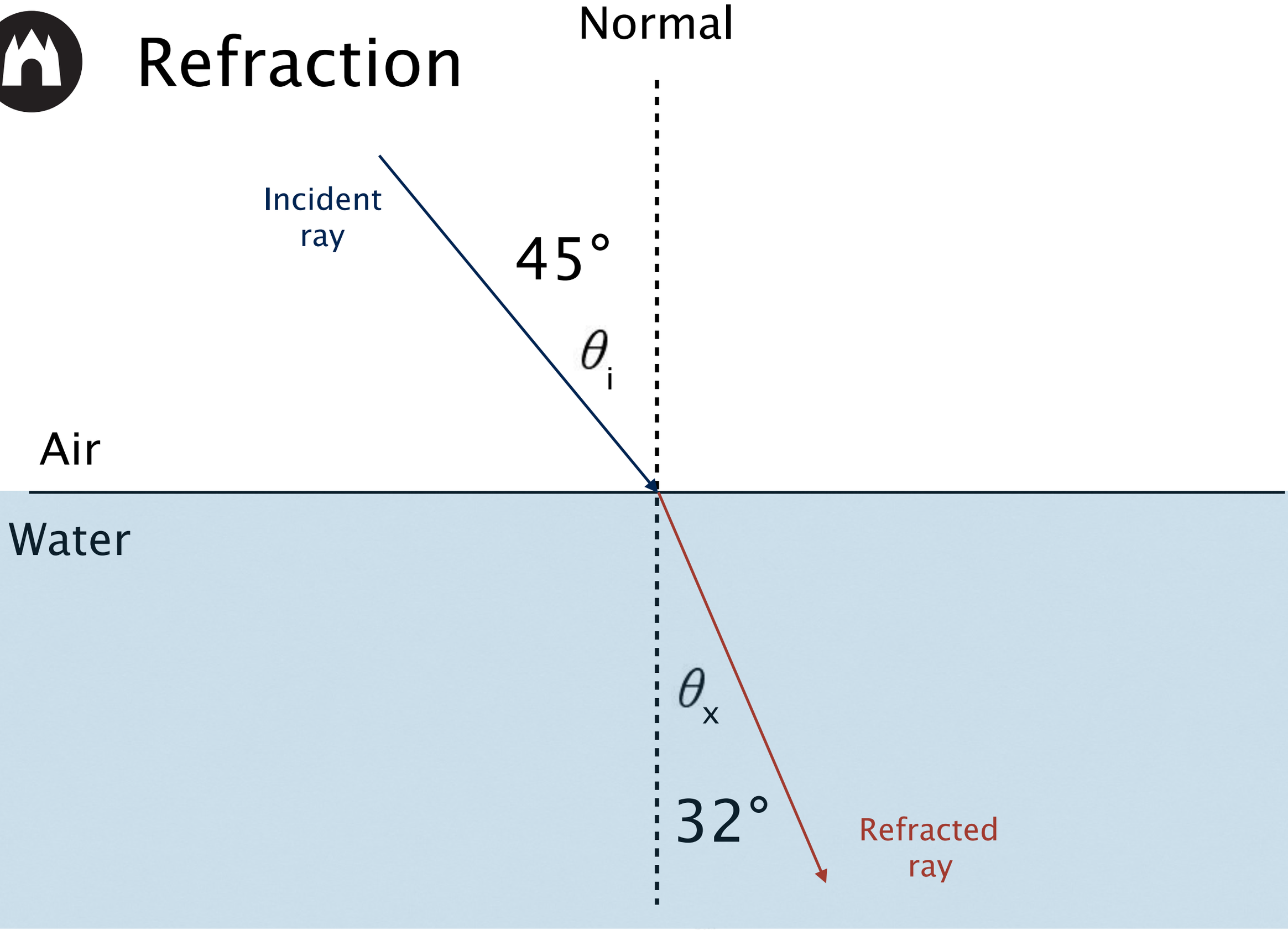
# Refraction




$\theta_i$ Angle of incidence

$\theta_x$ Angle of refraction

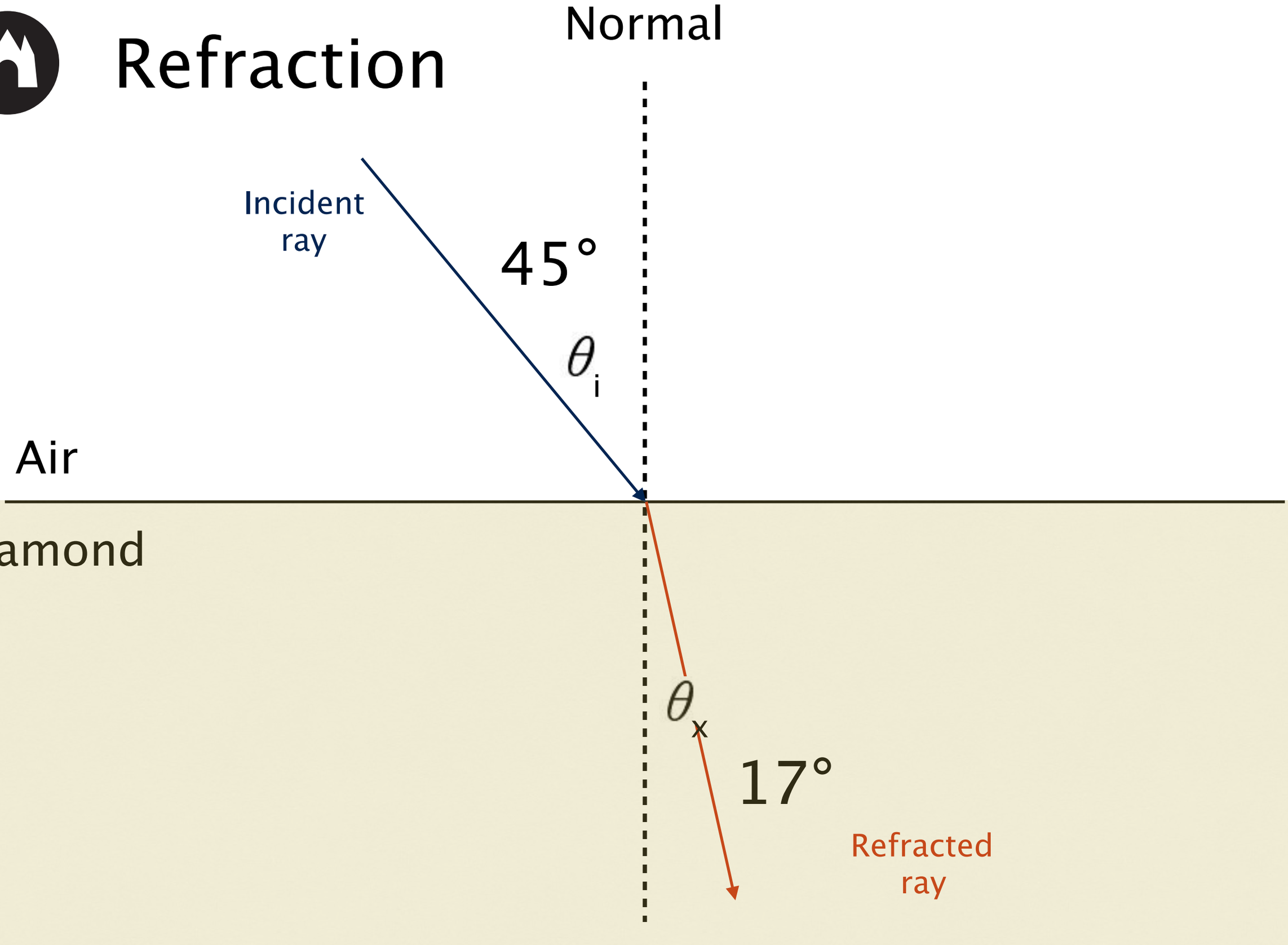
# Refraction




$\theta_i$ Angle of incidence

$\theta_x$ Angle of refraction

# Refraction



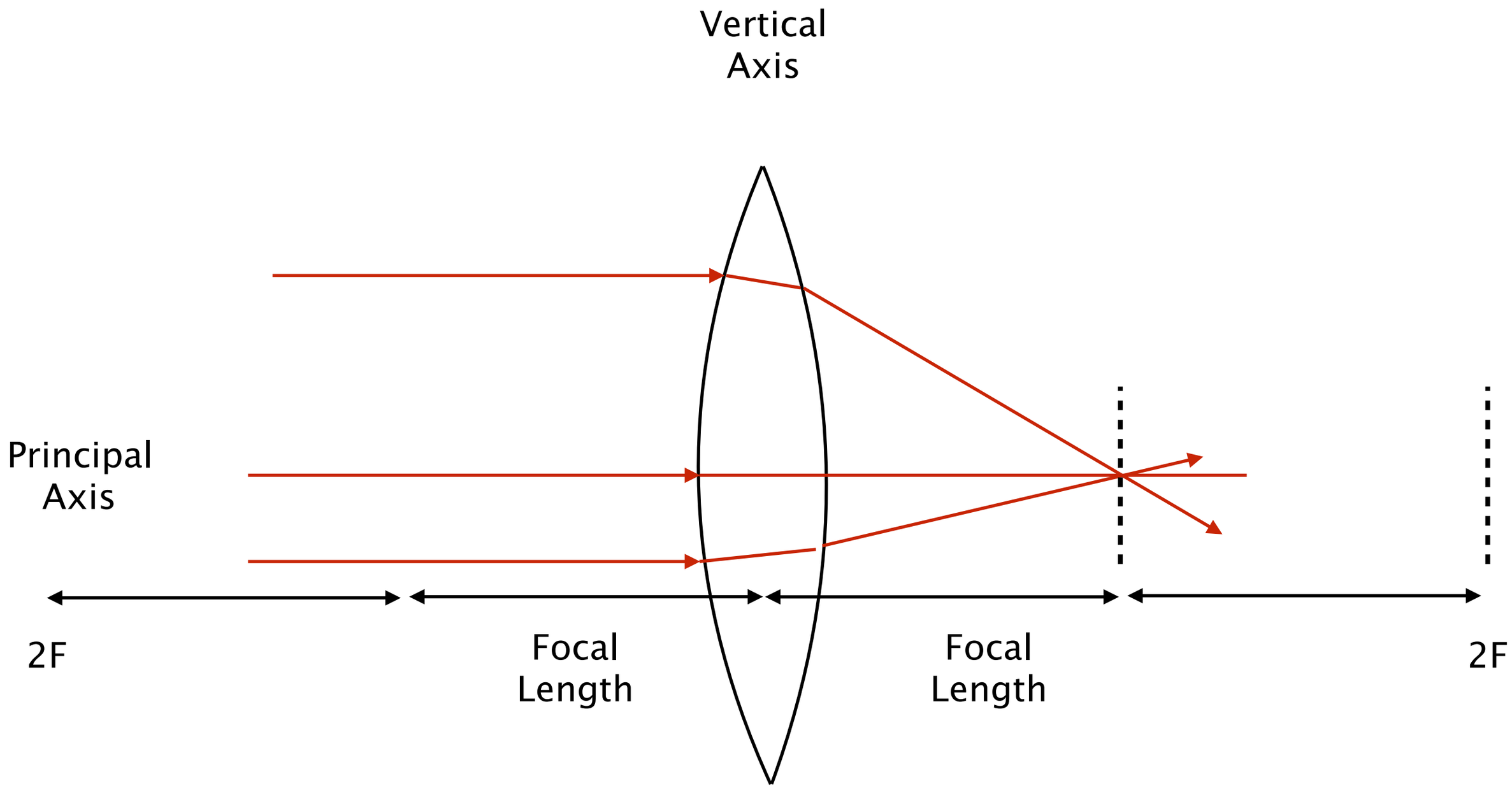

$\theta_i$ Angle of incidence

$\theta_x$ Angle of refraction

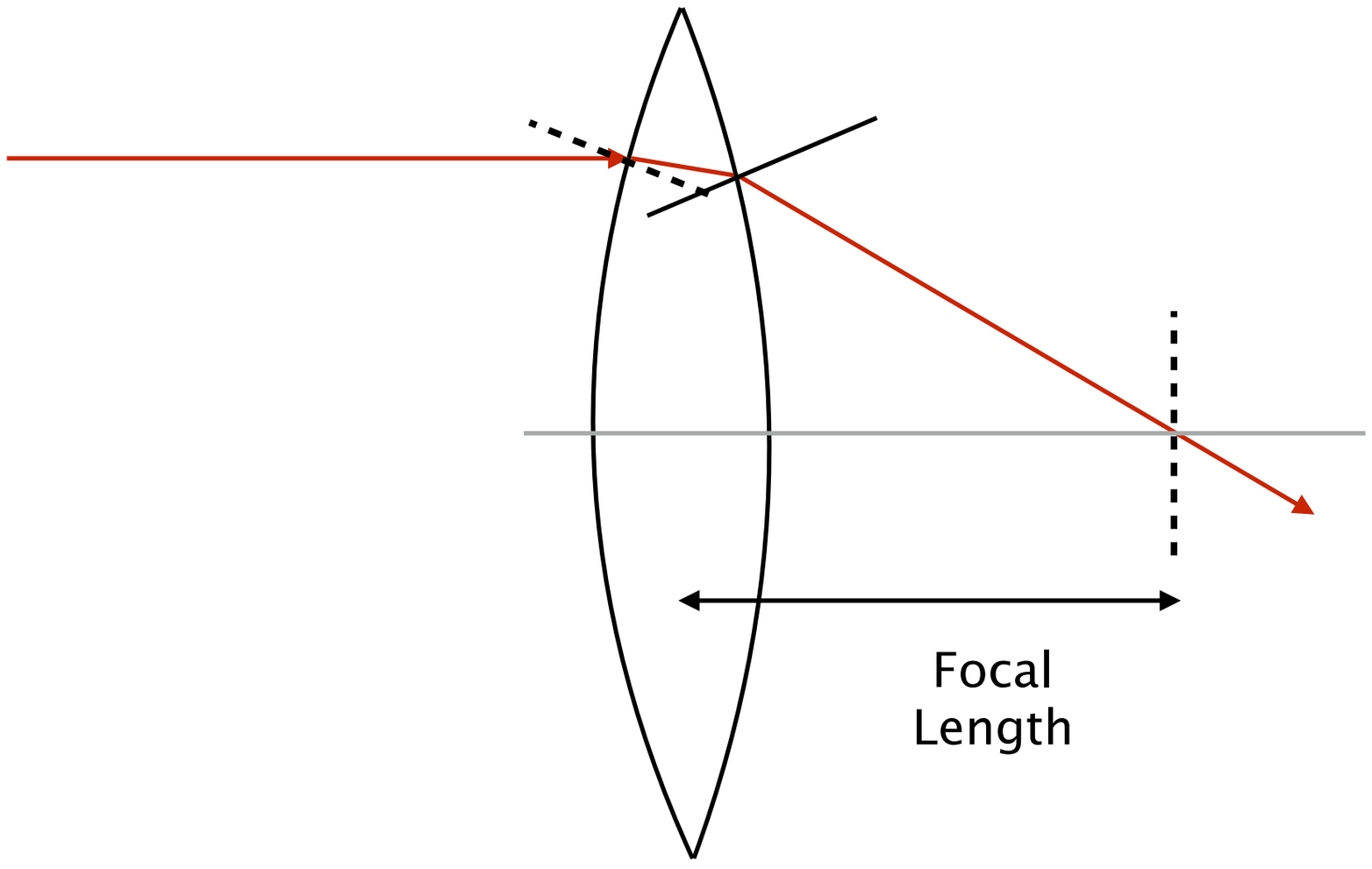
# Snell's Law

$$\sin(\theta_i) = n_{material} \times \sin(\theta_x)$$

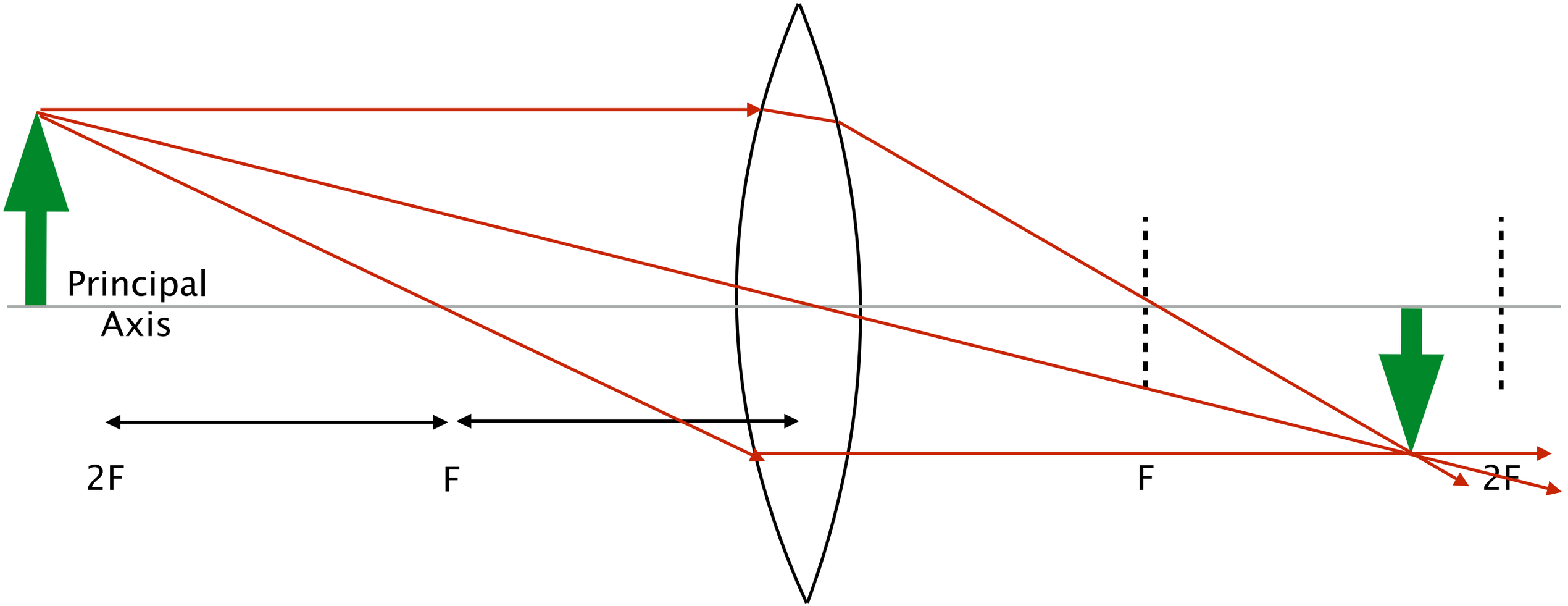
# Anatomy of a Lens



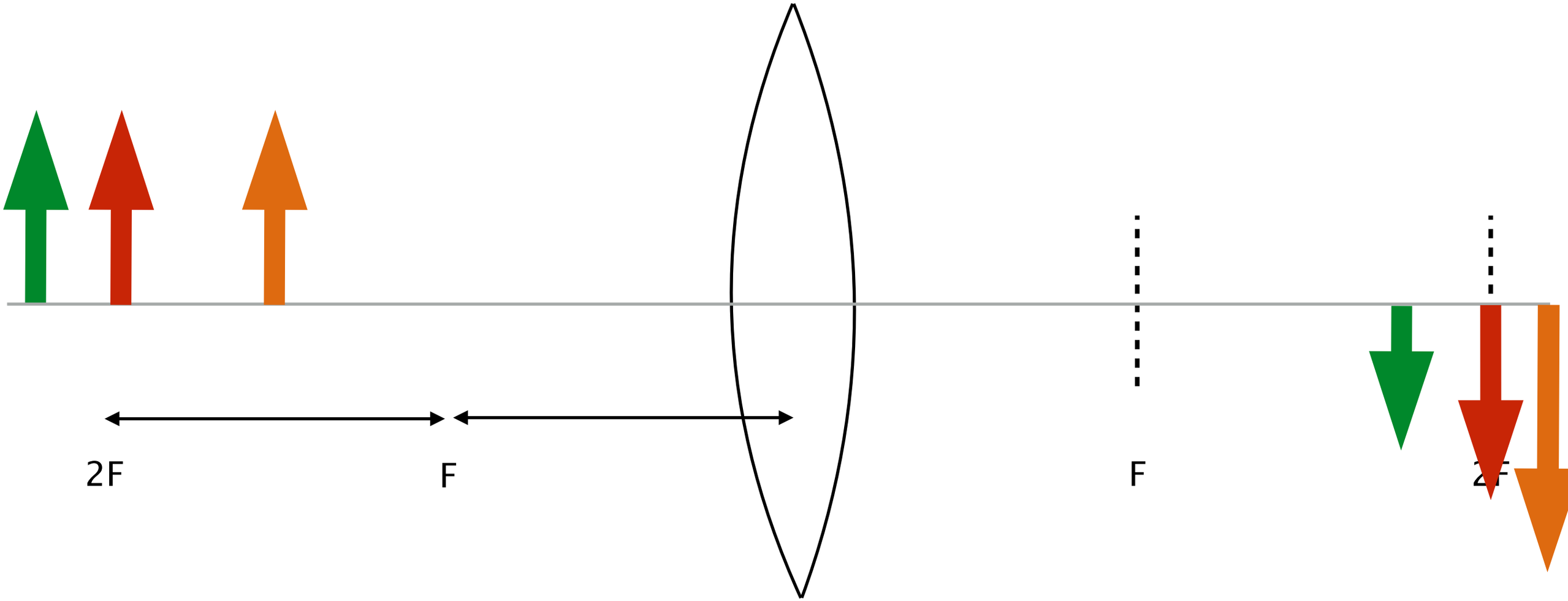
# Refraction in a lens



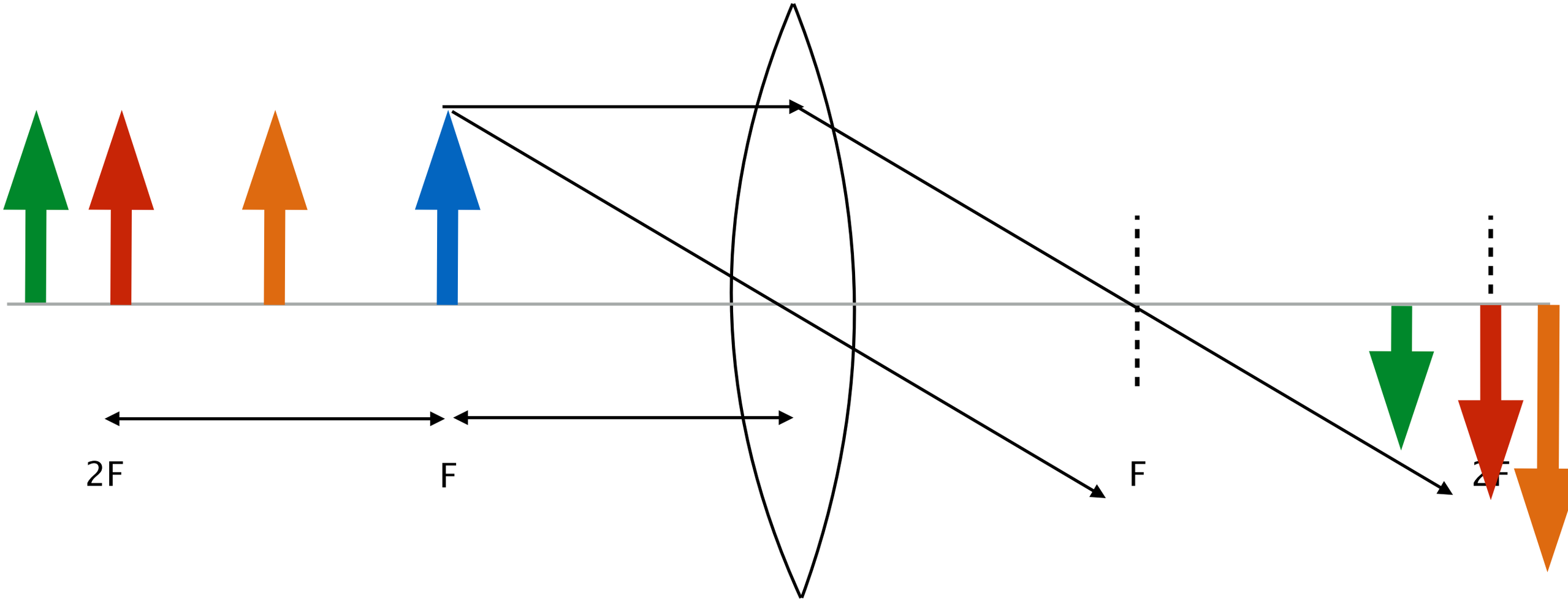
# Image construction



# Object / Image positions



# Object on focal point



# Lens formula

Lens equation

$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q}$$

f = focal length
p = distance to object
q = distance to image

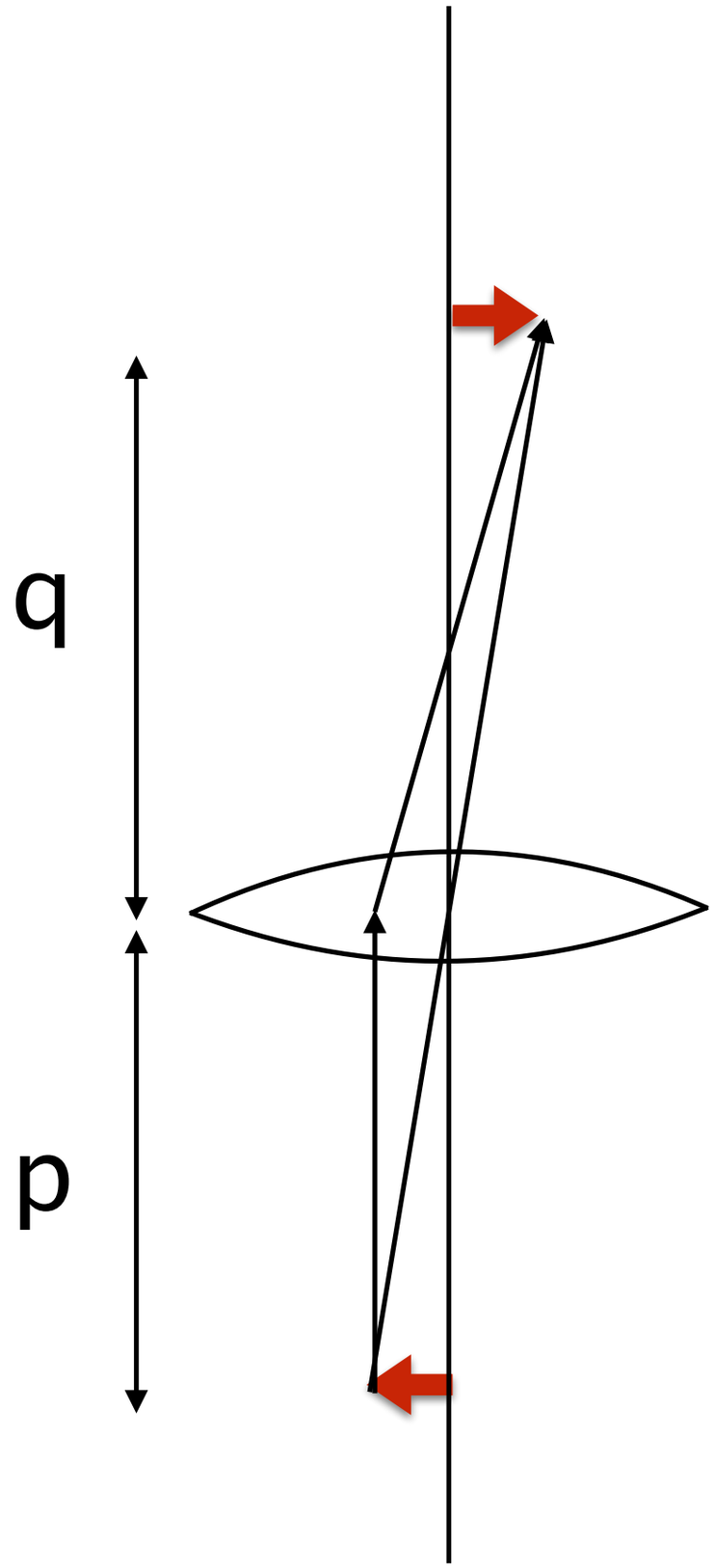
# Microscope formulas

f = 18.2

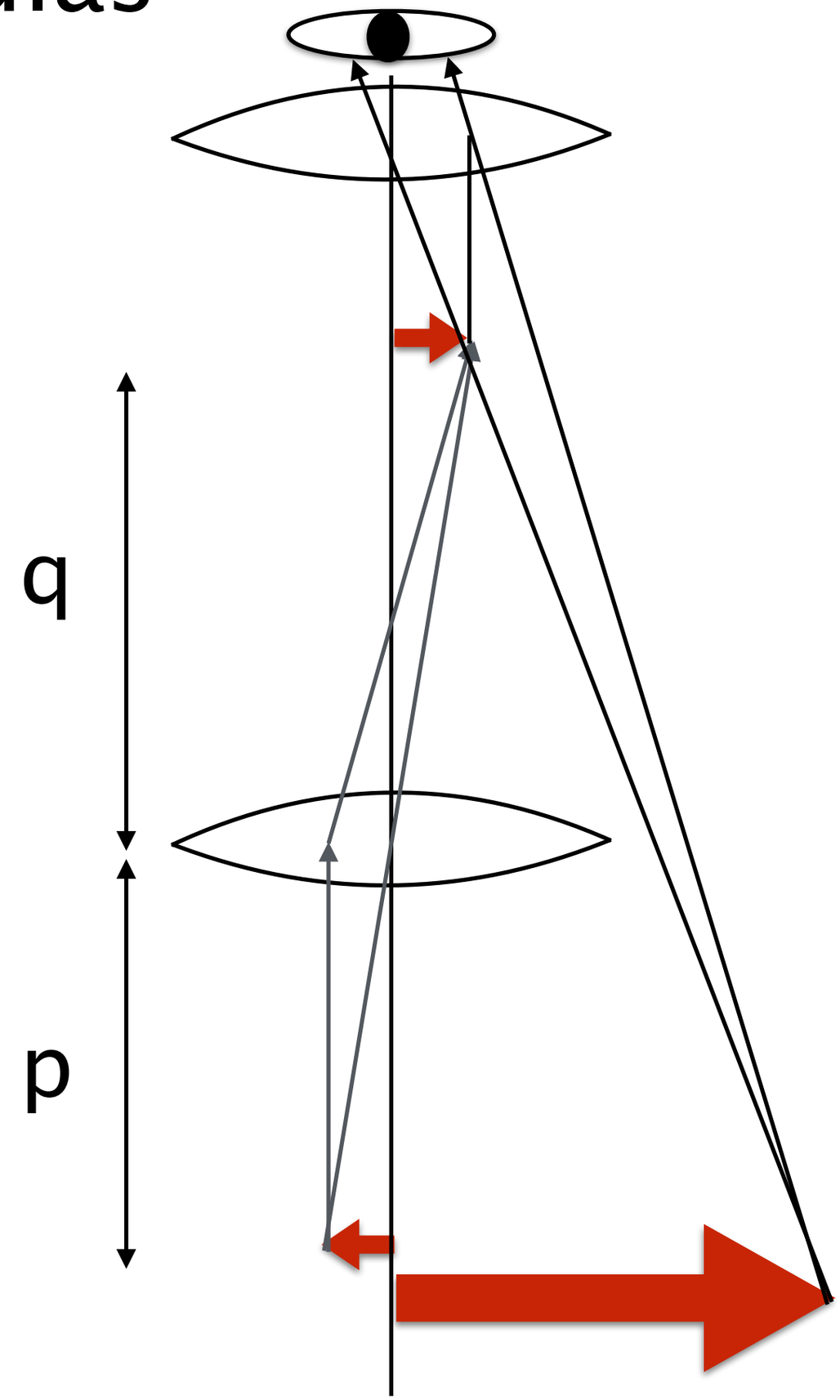
$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q}$$

q = 160 mm

p = 20.6 mm



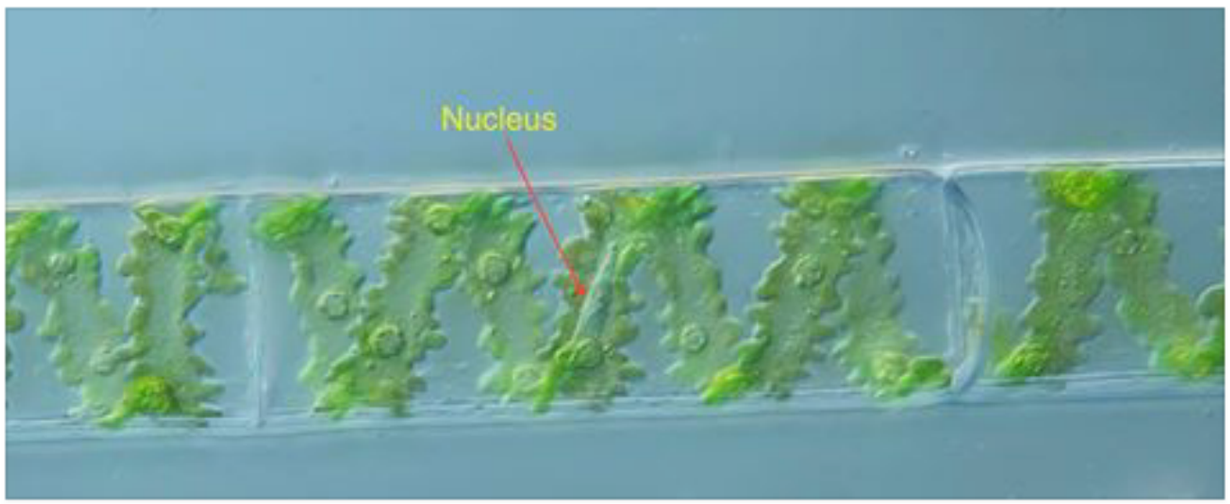
# Microscope formulas



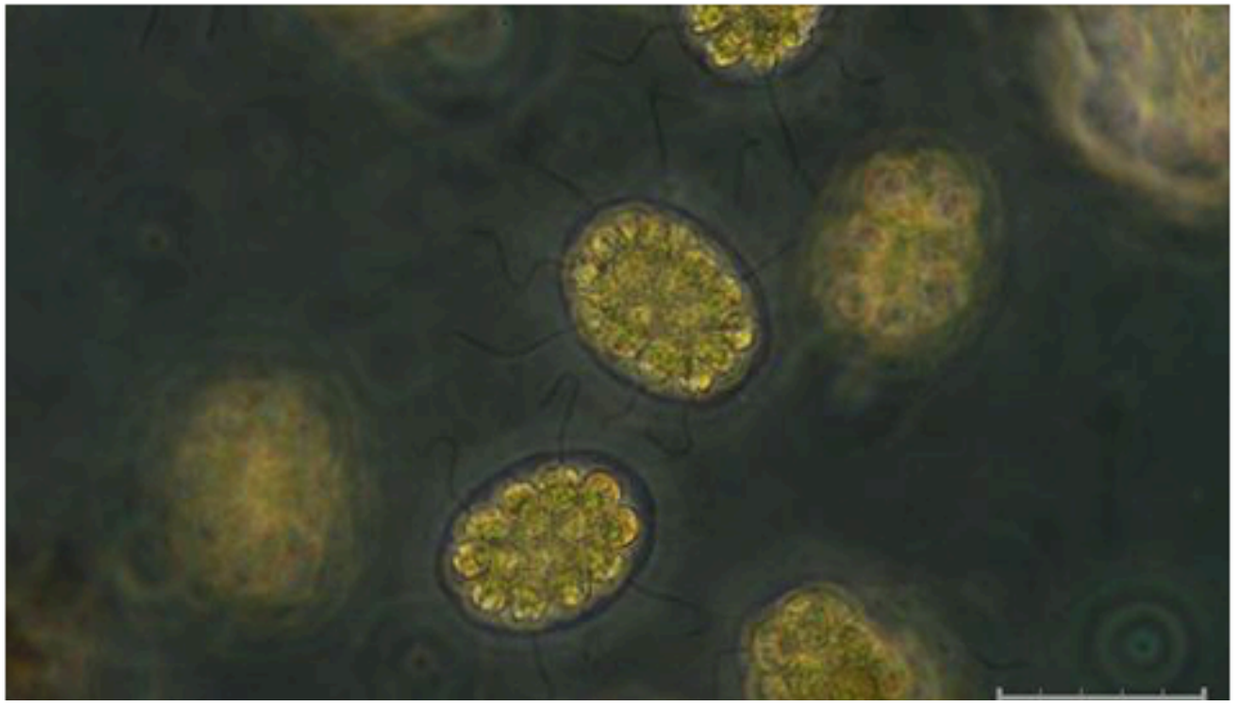
# Online microscopy communities

https://www.facebook.com/groups/Amateur.Microscopy/

This is a photograph of the alga Spirogyra. It is a rare sight here in southern California--even rarer if you do not have a microscope! But when I find see one, it always brings a smile to my face. The spirally arranged chloroplasts in a clear cellulose tube always inspire me. This is a focus stack of 30 sections, put together with Helicon focus.




Eudorina, CdF 40x



flys leg..4 images stacked with czp..

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