

A fluorescence microscopy image of a cell. The cell's internal structure is highlighted with green filaments, likely representing the cytoskeleton. Numerous small, bright red puncta are scattered throughout the cell, possibly representing organelles or specific proteins. The background is dark, making the green and red signals stand out.

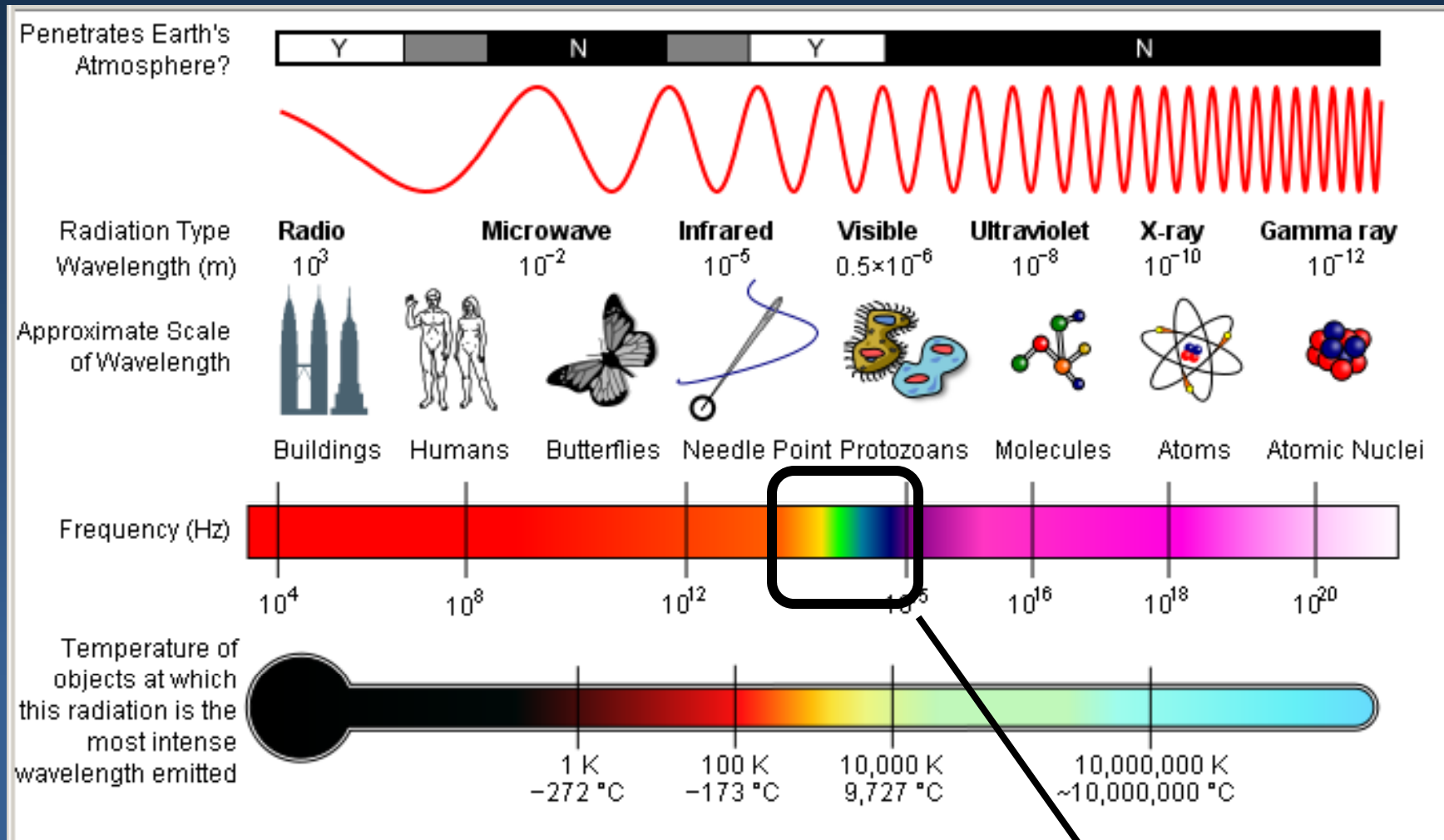
*McGill University Life Sciences  
Complex Imaging Facility*

*Systems Biology  
Microscopy Workshop*

*Tuesday December 7<sup>th</sup>, 2010*

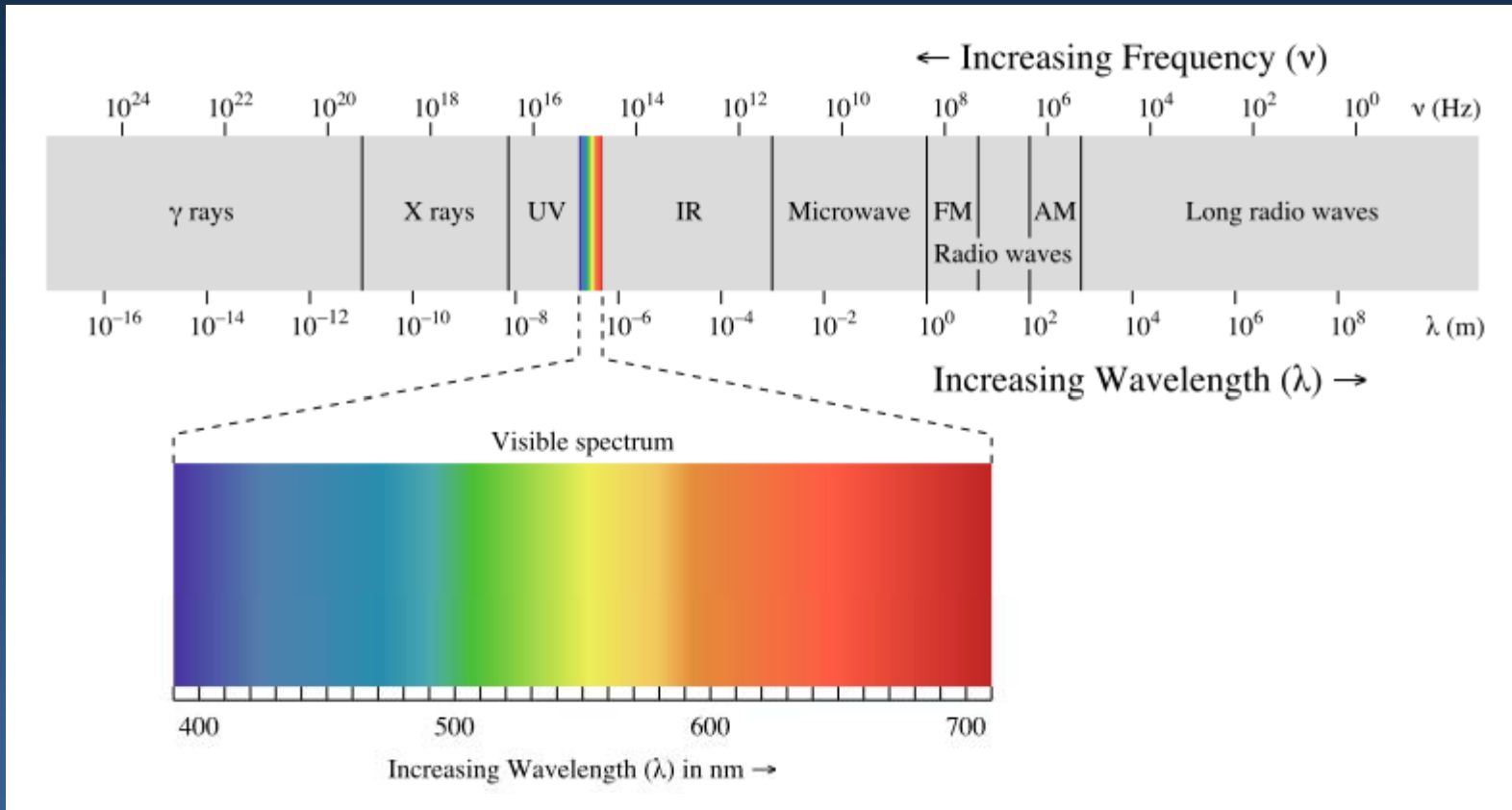
*Light Matter Interactions*

# Electromagnetic Radiation



Visible Electromagnetic Radiation  
LIGHT

# The Light Spectrum



*Light is composed of visible and invisible wavelengths.*

*Light microscopes use visible light wavelengths.*

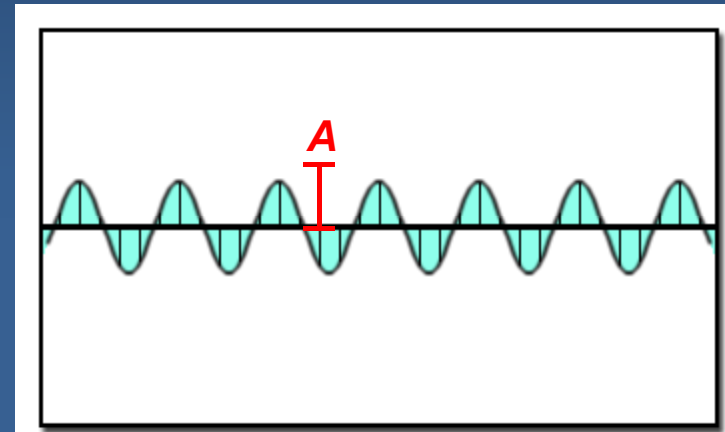
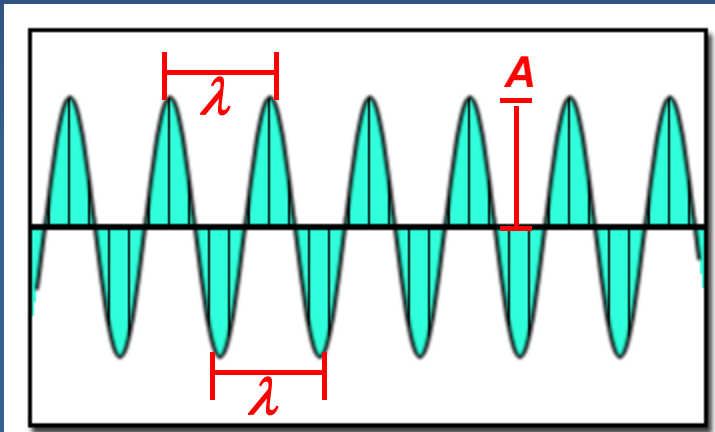
# How Do We Characterize Light?

Wavelength ( $\lambda$ ): distance between two peaks or valleys in the light wave.

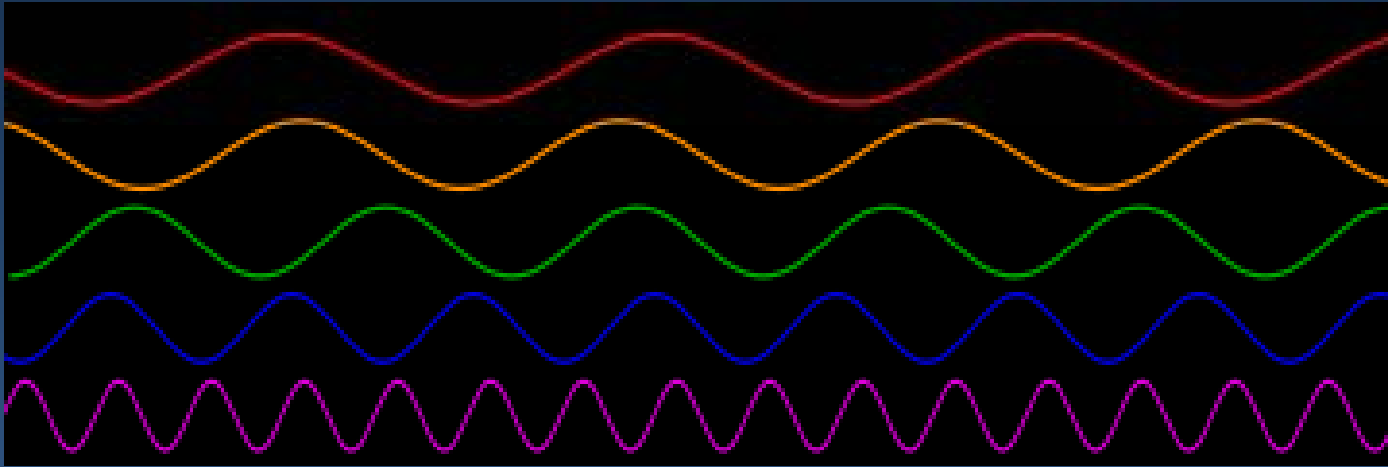
Amplitude (A): height of peaks and valleys in electromagnetic wave.

Frequency (f): number of complete wavelengths that pass a given point per second.

Energy (E): directly related to frequency and inversely related to wavelength.



# How Do We Characterize Light?



$$\text{Frequency } (f) = c/\lambda$$

$c = \text{speed of light}$

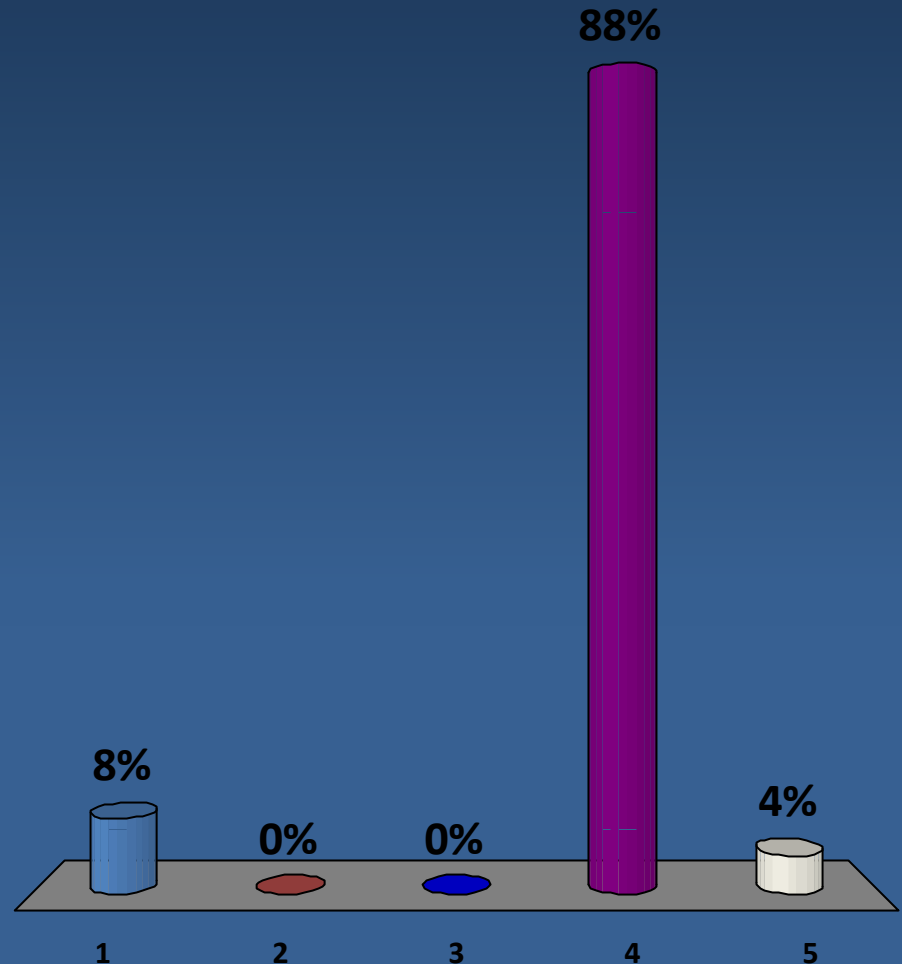
$$E = (h*f) = (h*c)/\lambda$$

$h = \text{Planck's constant}$

[Electromagnetic Wave Tutorial](#)

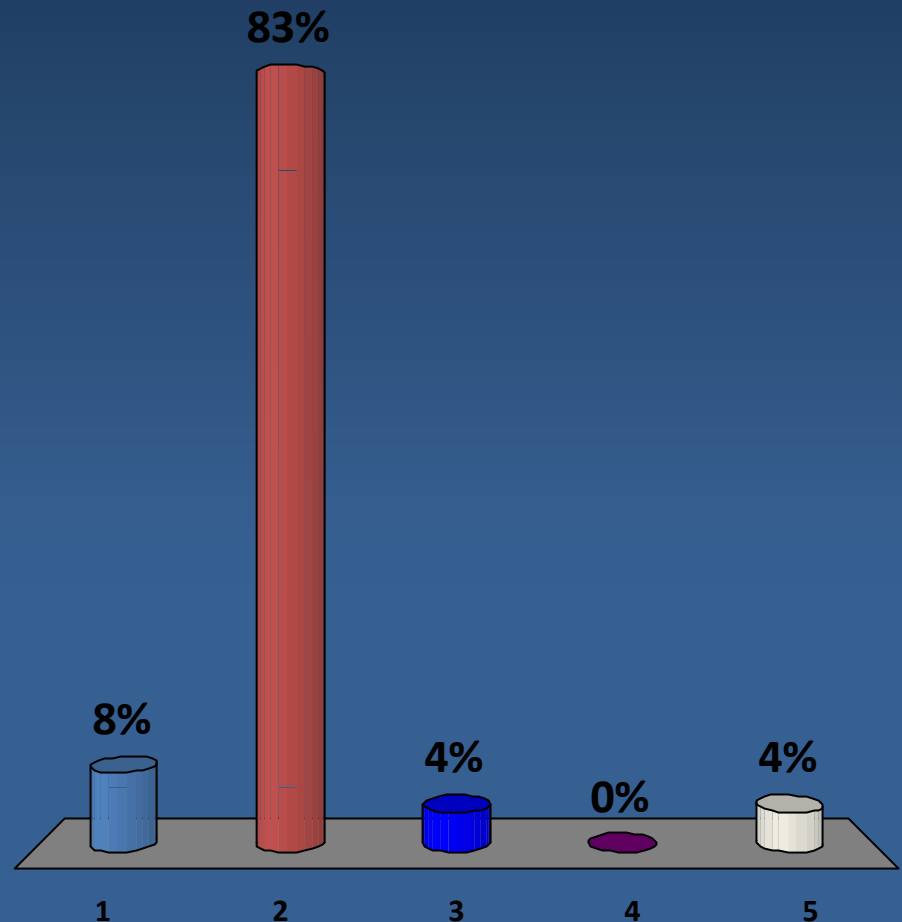
# Which Colour of Light Has the Most Energy?

1. Red
2. Orange
3. Yellow
- 😊 4. Violet
5. Green



# Which Colour of Light Has the Longest Wavelength?

1. Blue
- 😊 2. Orange
3. Yellow
4. Violet
5. Green



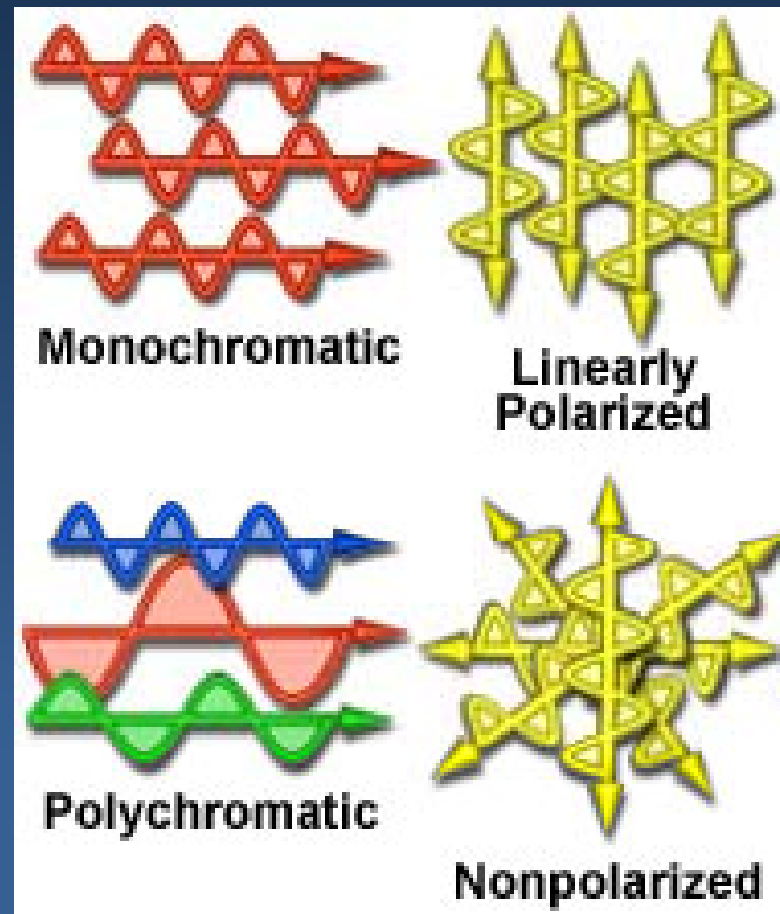
# Types of Light Waves

**Monochromatic:** Light of one wavelength or colour.

**Polychromatic:** Light made up of many wavelengths or colours.

**Polarized Light (Linear):** Isolation or selection of light from one direction or electromagnetic plane.

**Non-polarized Light:** Light waves going in all directions.





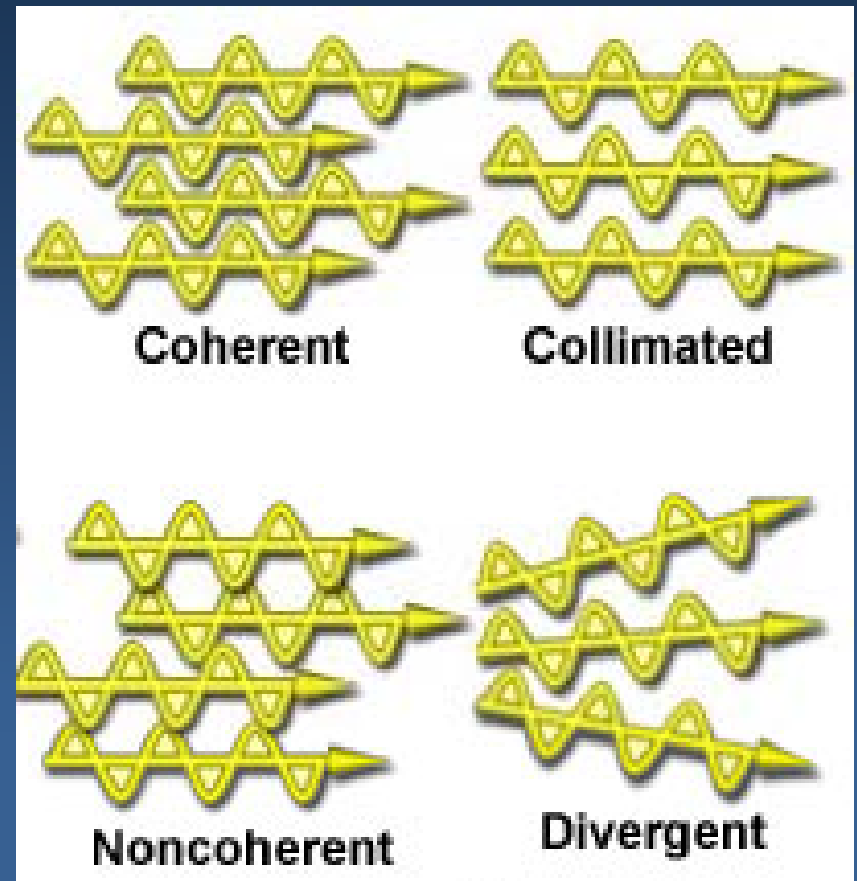
# Types of Light Waves

**Coherent Light:** Waves of a given wavelength that have the same phase relationship.

**Non-Coherent Light:** Waves that displays a variety of phase relations for different wavelengths.

**Collimated Light:** Waves having the same path of propagation, not convergent or divergent, but not necessarily the same wavelength, phase or polarization.

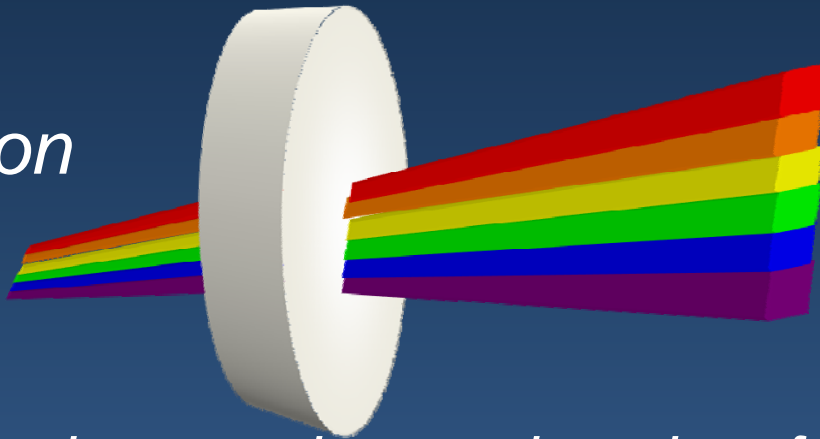
**Divergent Light:** Waves that propagate along different paths diverging from one another.



# Absorption

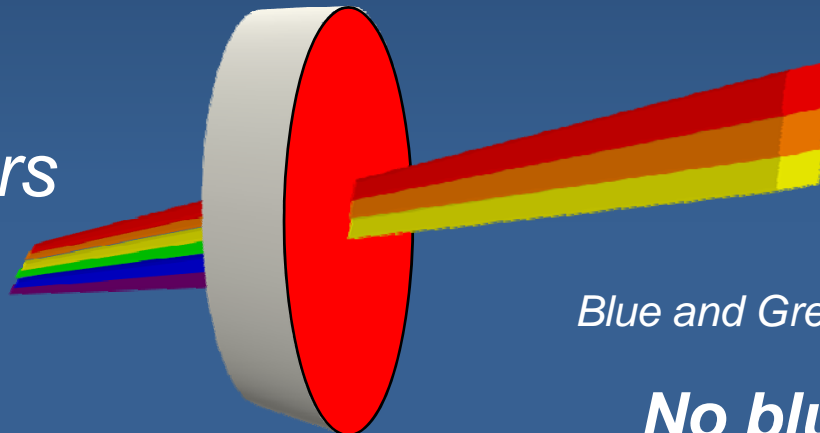
Absorption: Blocking or reduction of one or more wavelengths of light.

No Absorption  
(Glass)



Blocking or reducing certain wavelengths of light:

Absorption  
Colour Filters



Blue and Green are Absorbed

**No blue/green light**

**Red filter**

# Absorption

Absorption: Blocking or reduction of one or more wavelengths of light.

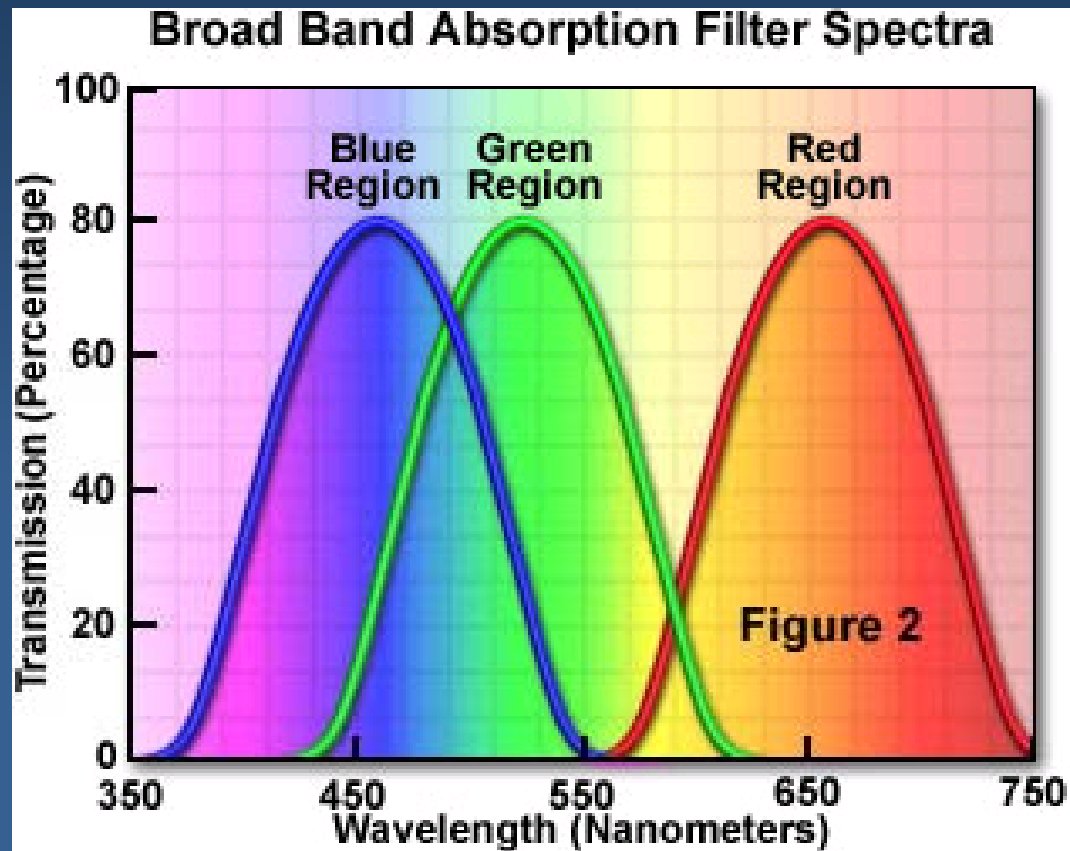
## Colour filters

$$A=1/T$$

*A = Absorption*

*T = Transmission*

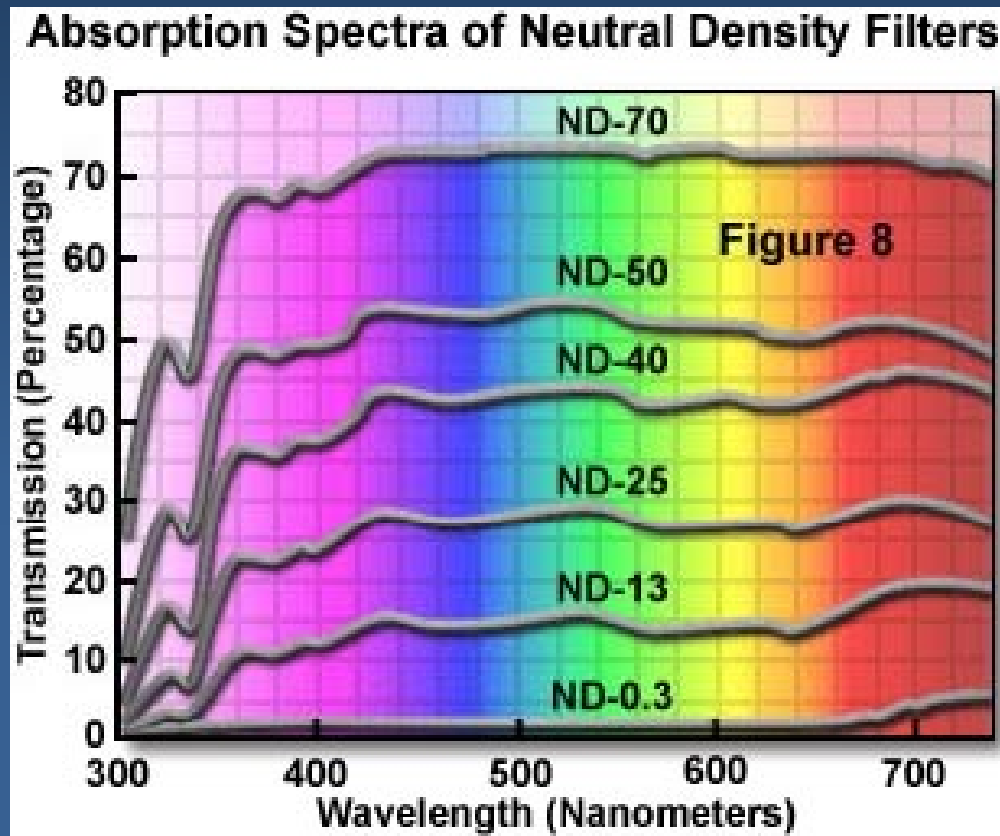
[Absorption Tutorial](#)



# Absorption

Absorption: Blocking or reduction of one or more wavelengths of light.

Blocking or reducing ALL wavelengths of light –  
**Neutral Density (nd) filters**



# Reflection

Reflection: *Bouncing of light off a reflective surface with the absence of absorption.*



**Smooth Water Surface**



**Wavy Water Surface**

- 1) *Smooth Surface generates perfect image of objects.*
- 2) *Rough surface causes light scattering and the image is not clear.*

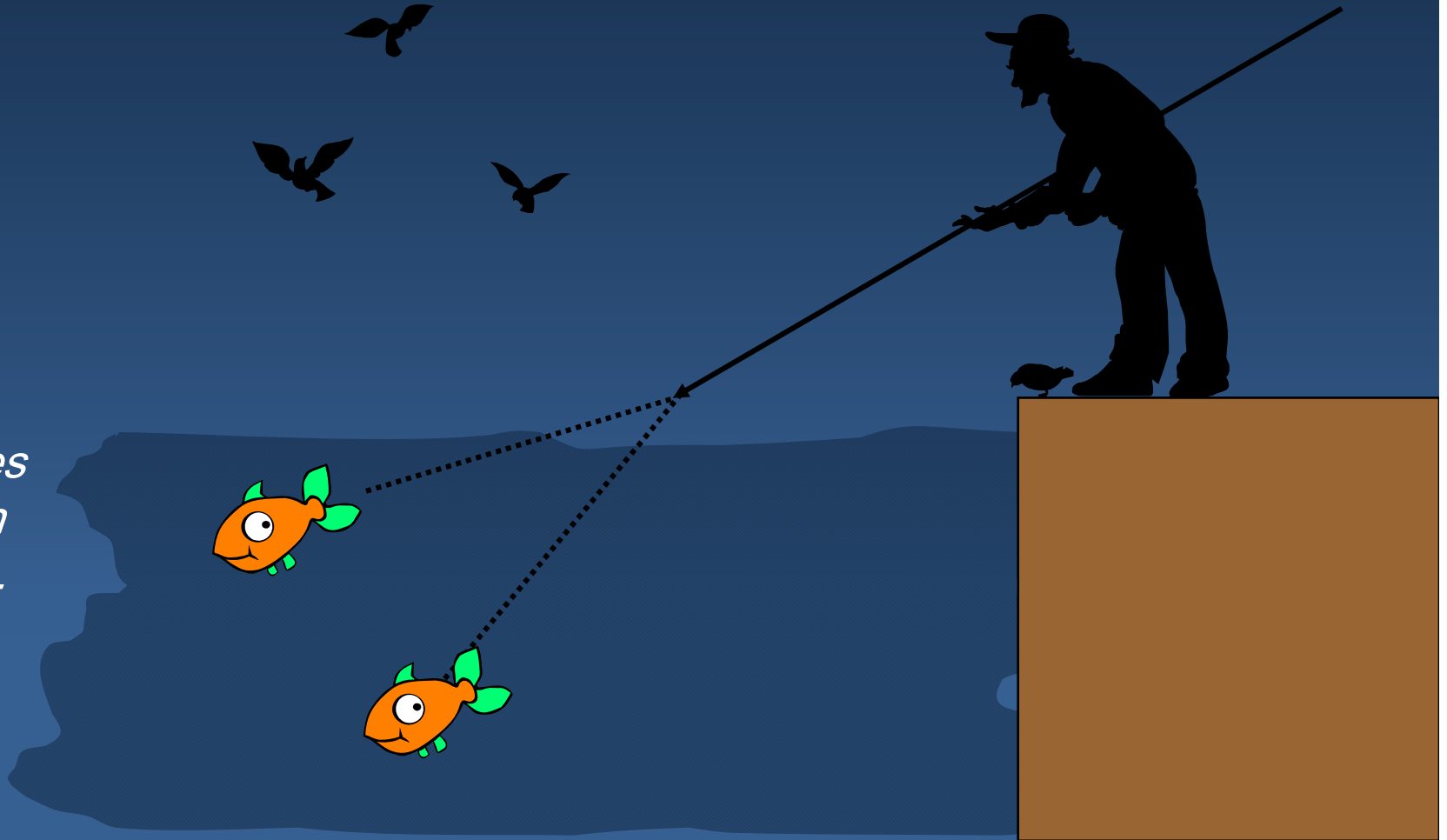
# Refraction

**Refraction:** *Bending of light due to a change in speed as it passes, at an angle, from one material to another. These two materials must have different refractive index or optical densities for refraction to occur.*



# Refraction

*He sees  
the fish  
here....*



*But it is really here!!*

# Refractive Index

Refractive Index (R.I. or  $n$ ) or Index of Refraction: Measure of how much the speed of light is reduced in a medium relative to the speed in a vacuum.

*R.I. in a vacuum is set to 1.0 and all other measures are made relative to this.*

Material	Refractive Index
Air	1.0003
Water	1.333
Glycerin	1.473
Immersion Oil	1.515
Glass (Crown)	1.520
Glass (Flint)	1.656
Zircon	1.920
Diamond	2.417
Lead Sulfide	3.910



# Refraction

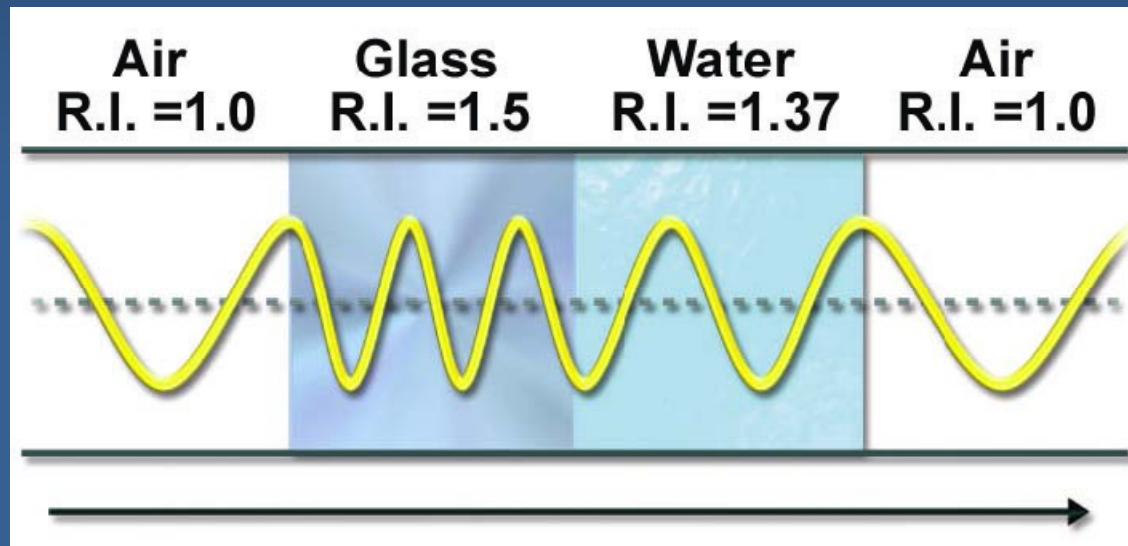
## What causes the light to refract (bend)?

*The change in speed of light as it moves from one medium to another.*

$$\text{Refractive Index} = n = c/v$$

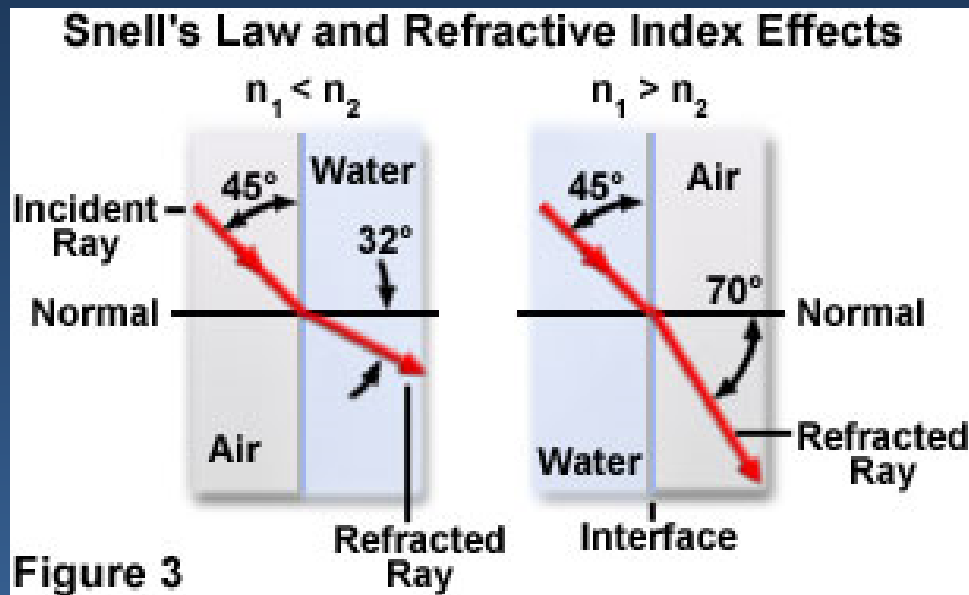
*c = speed of light in vacuum*

*v = speed of light in medium*



# Refraction

Snell's Law: Relates the refractive index difference between two materials to the angle of refraction.



*If  $n_1 < n_2$  then  $q_1 > q_2$*

*If  $n_1 > n_2$  then  $q_1 < q_2$*

*Direction of bending – roller skater.*

$$n_1 \times \sin(q_1) = n_2 \times \sin(q_2)$$

$n_1$  = refractive index of material incident ray is coming from

$n_2$  = refractive index of material incident ray is entering

$q_1$  = incident angle

$q_2$  = refracted angle

[Refraction  
Tutorial](#)

# Dispersion

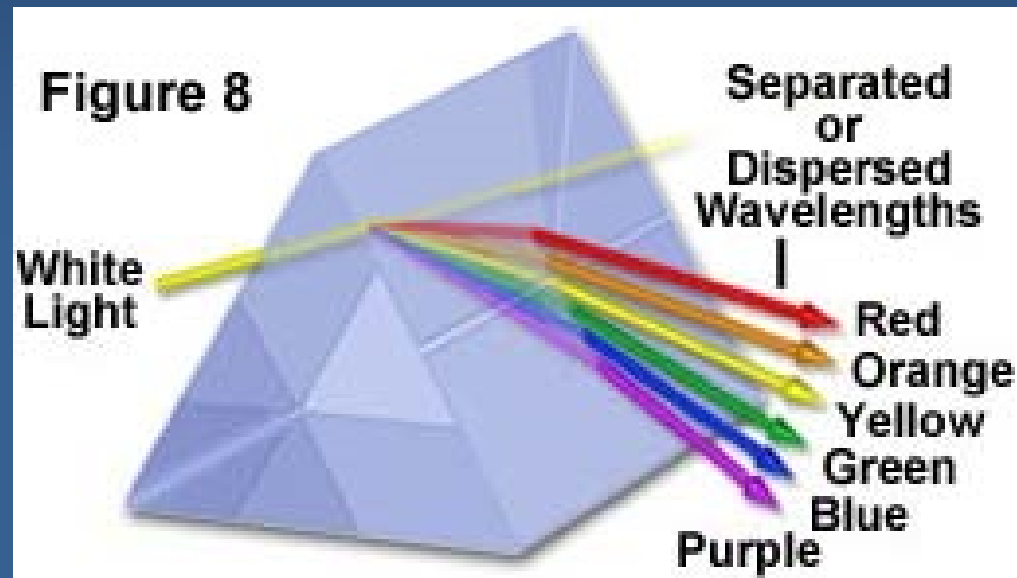
**Dispersion:** Wavelength dependent deviation in the angle of light refraction.

Dispersion is responsible for the “rainbow” effect seen when light is separated into composite colours when passing through an equilateral prism.

Shorter wavelengths (blue light) are refracted at greater angles than longer wavelengths (red light).

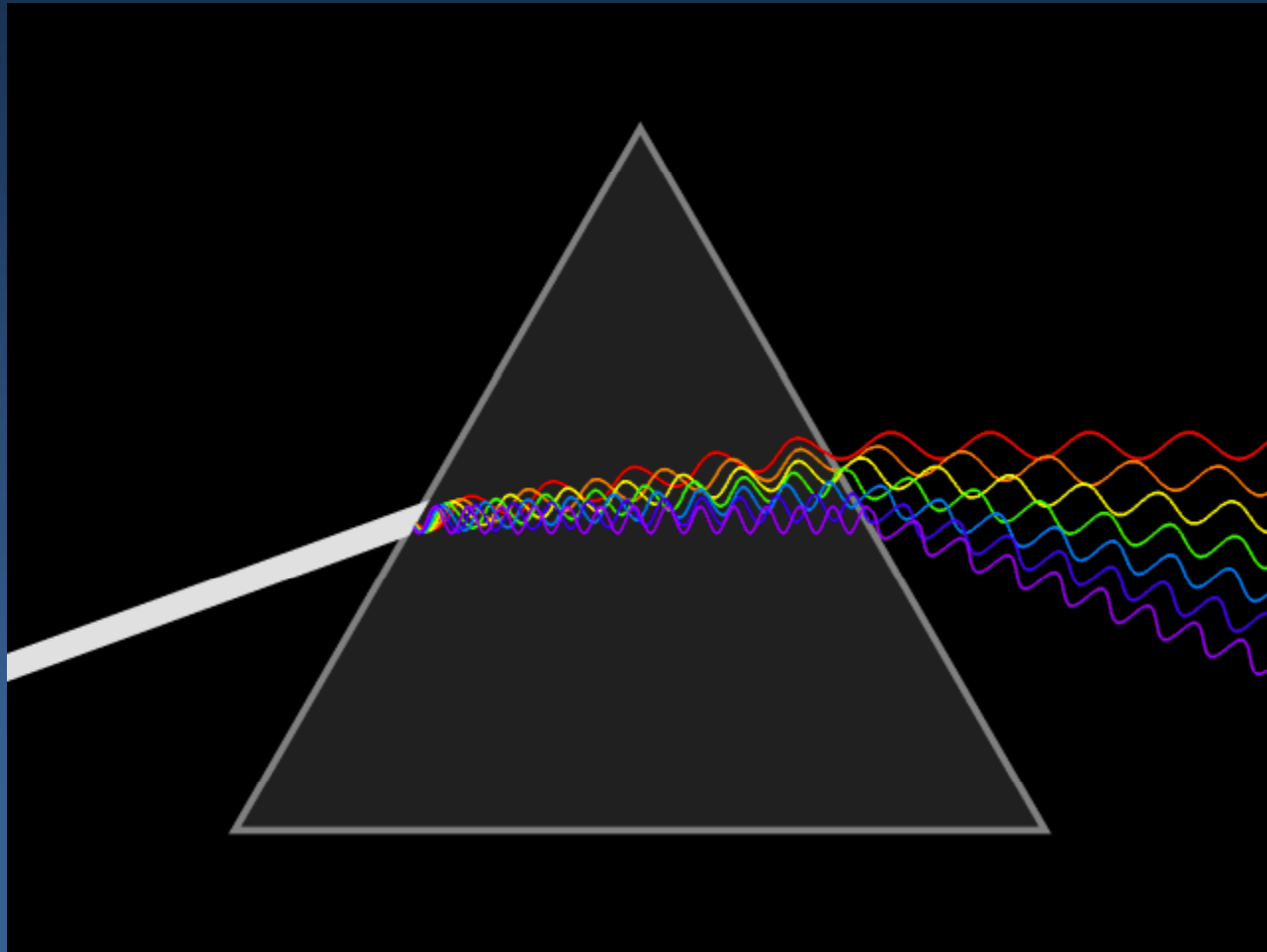
**RED resists refraction.**

Dispersion depends on R.I. of medium and incident angle.



[Dispersion](#)  
[Tutorial](#)

# *Dispersion*



*Dispersion Animation*

# Diffraction

Diffraction: The bending of light as it passes a corner, an edge, or through an opening or slit that is physically the same size or smaller than the wavelength of the light.



*Light is diffracted by the water droplets in the clouds.*

# Diffraction

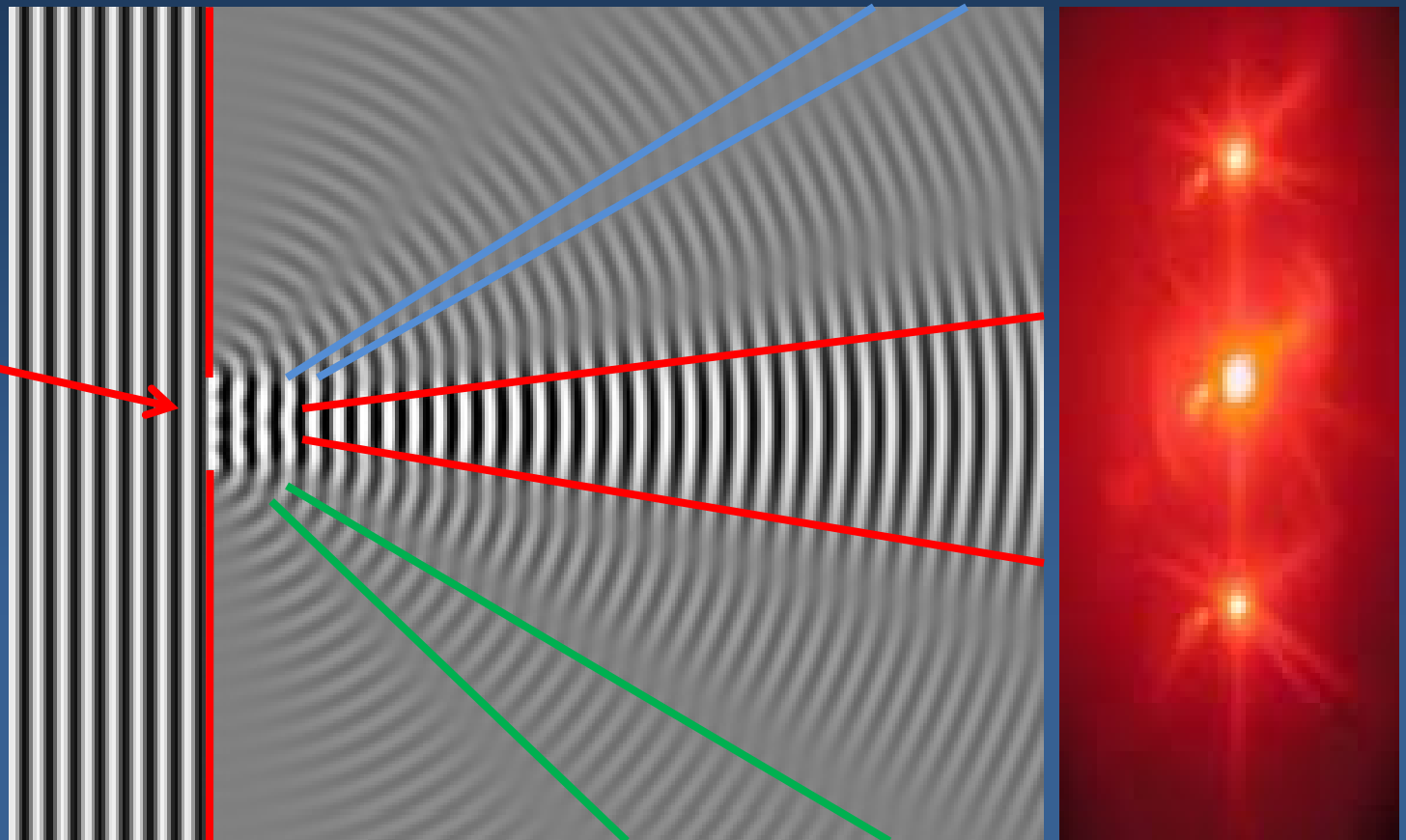
Diffraction: The bending of light as it passes a corner, an edge, or through an opening or slit that is physically the same size or smaller than the wavelength of the light.

Dark Region

Slit or aperture

Show laser pointer and diffraction film

[Diffraction Tutorial](#)

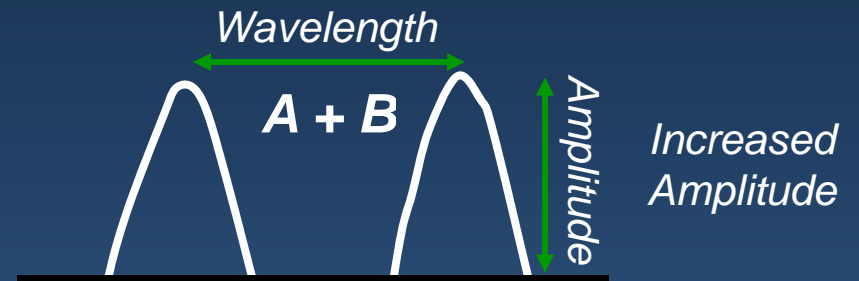
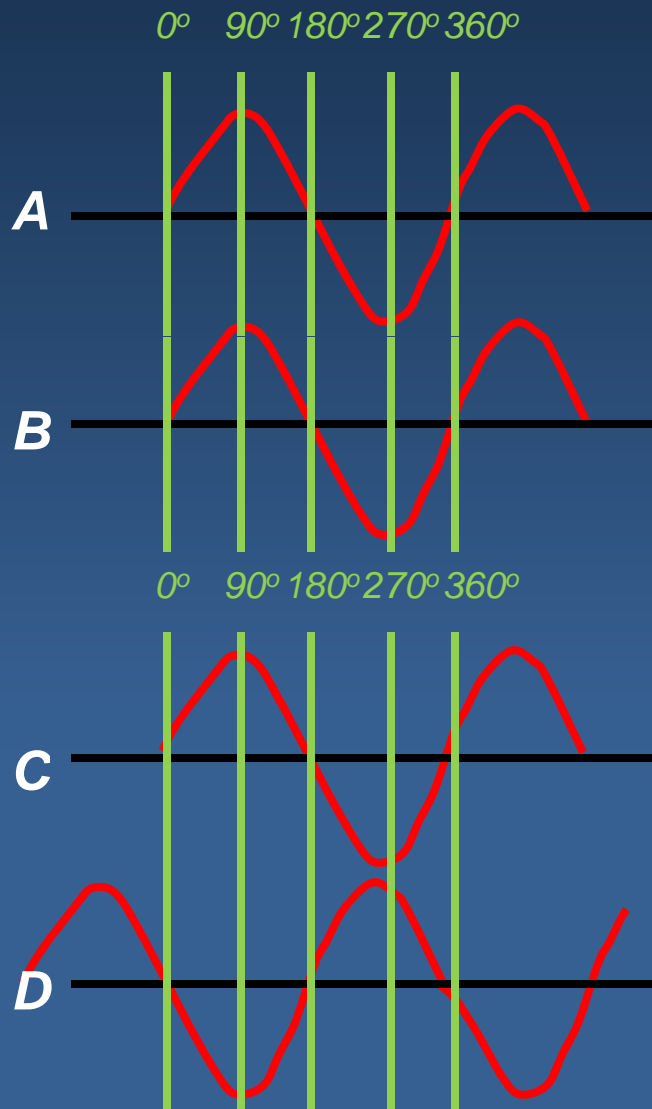


Bright Region

# Interference

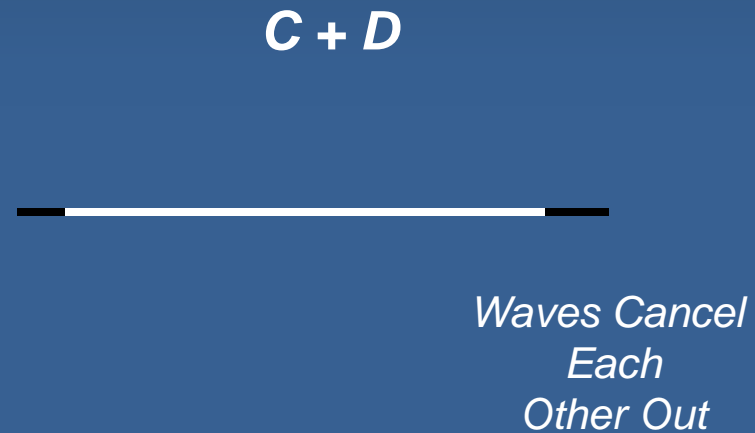
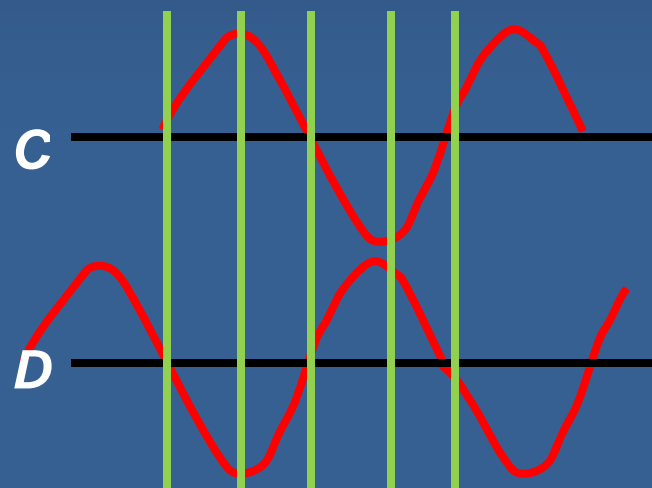
Interference: Addition of two or more waves resulting in a new wave form.

*Constructive Interference*

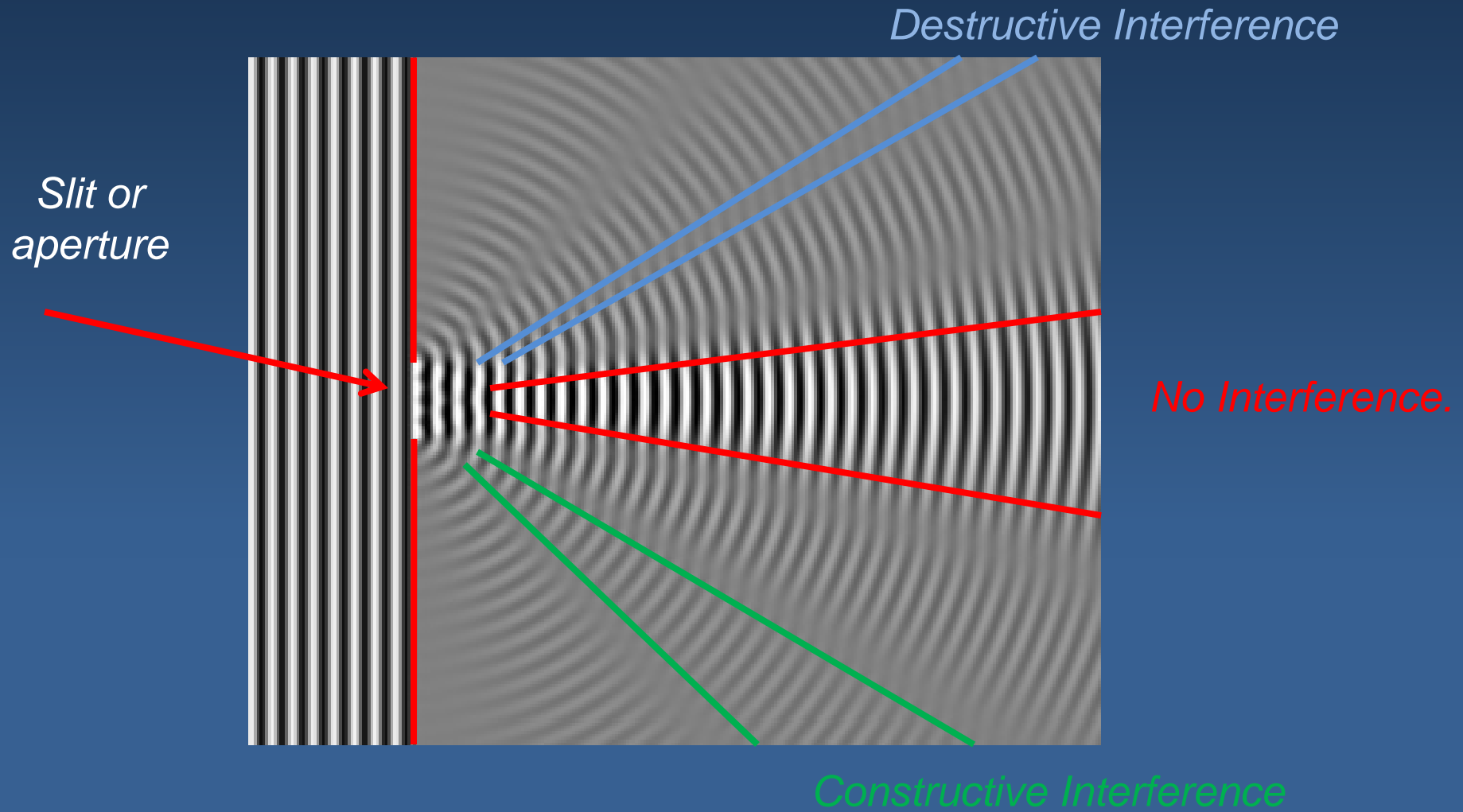


[Interference Tutorial](#)

*Destructive Interference*

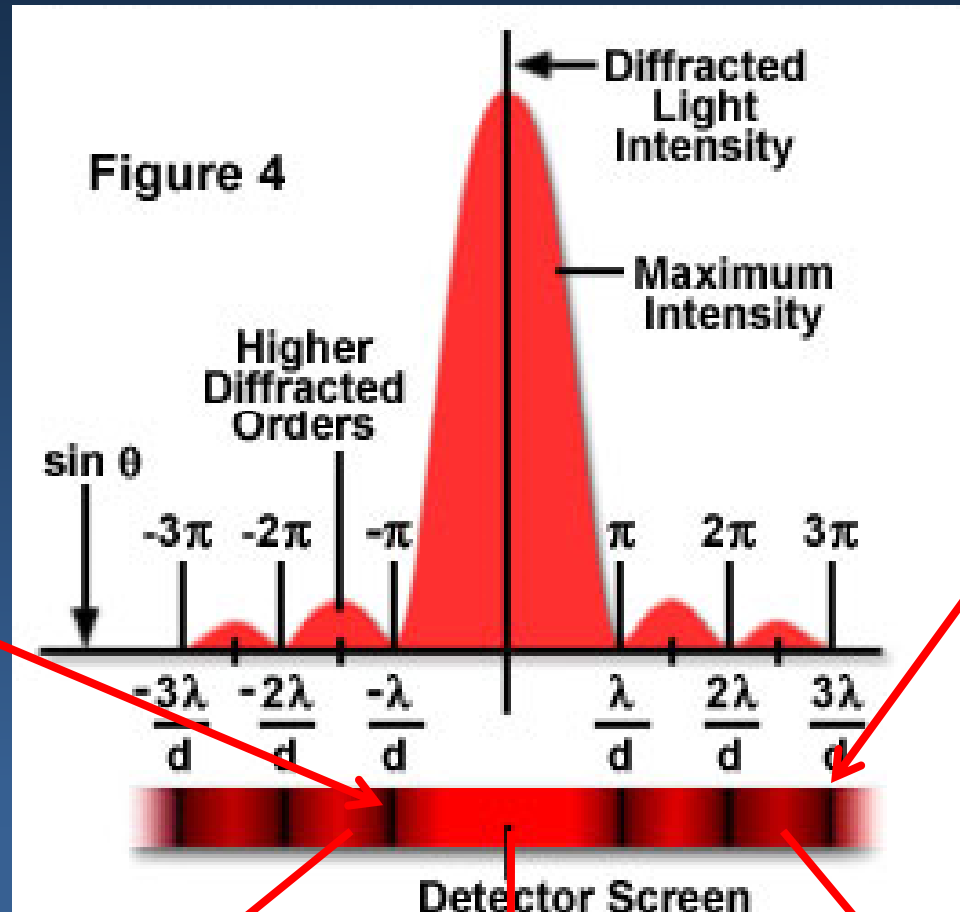


# *Interference and Diffraction Patterns*





# Interference & Diffraction Patterns



*1<sup>st</sup> Order  
Destructive  
Interference*

*3<sup>rd</sup> Order  
Destructive  
Interference*

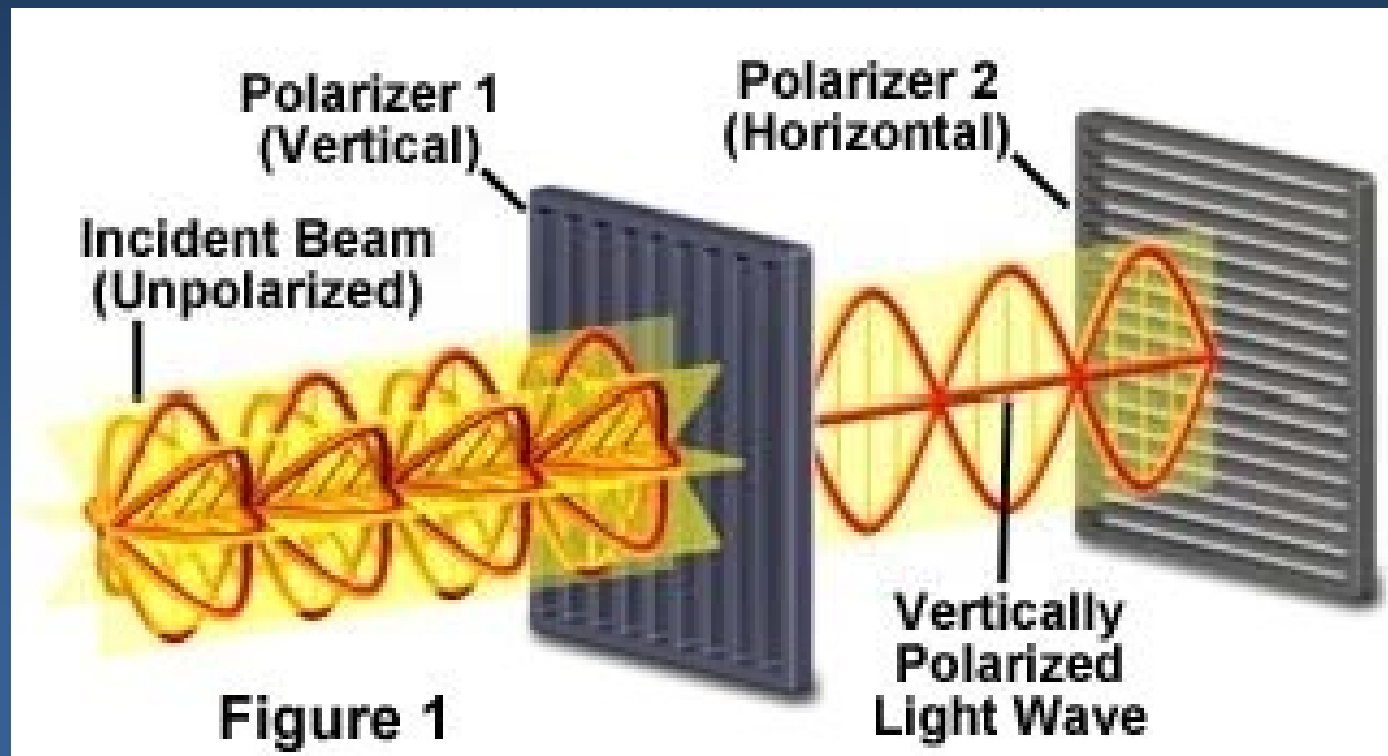
*1<sup>st</sup> order  
Diffracted  
Constructive  
Interference*

*Zero Order  
not Diffracted*

*2<sup>nd</sup> order  
Diffracted  
Constructive  
Interference*

# Polarization

**Polarization:** Isolation or selection of light from one direction or electromagnetic plane.



# Scatter

Scatter: A combination of many light-matter interactions sending light off in a variety of directions.



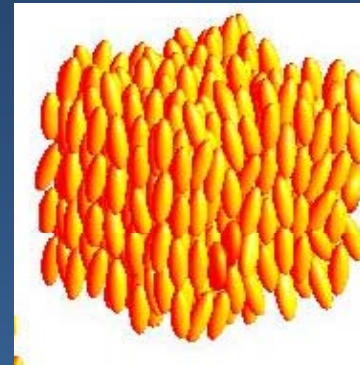
*Sunlight scattered by dew.*

# Types of Matter

**Isotropic:** same optical properties in all directions, homogeneous material.



**Anisotropic:** optical properties depend on direction (crystal).



**Birefringence (double refraction):** decomposition of a ray of light into two rays (ordinary and extraordinary rays) when it passes through certain types of materials.

