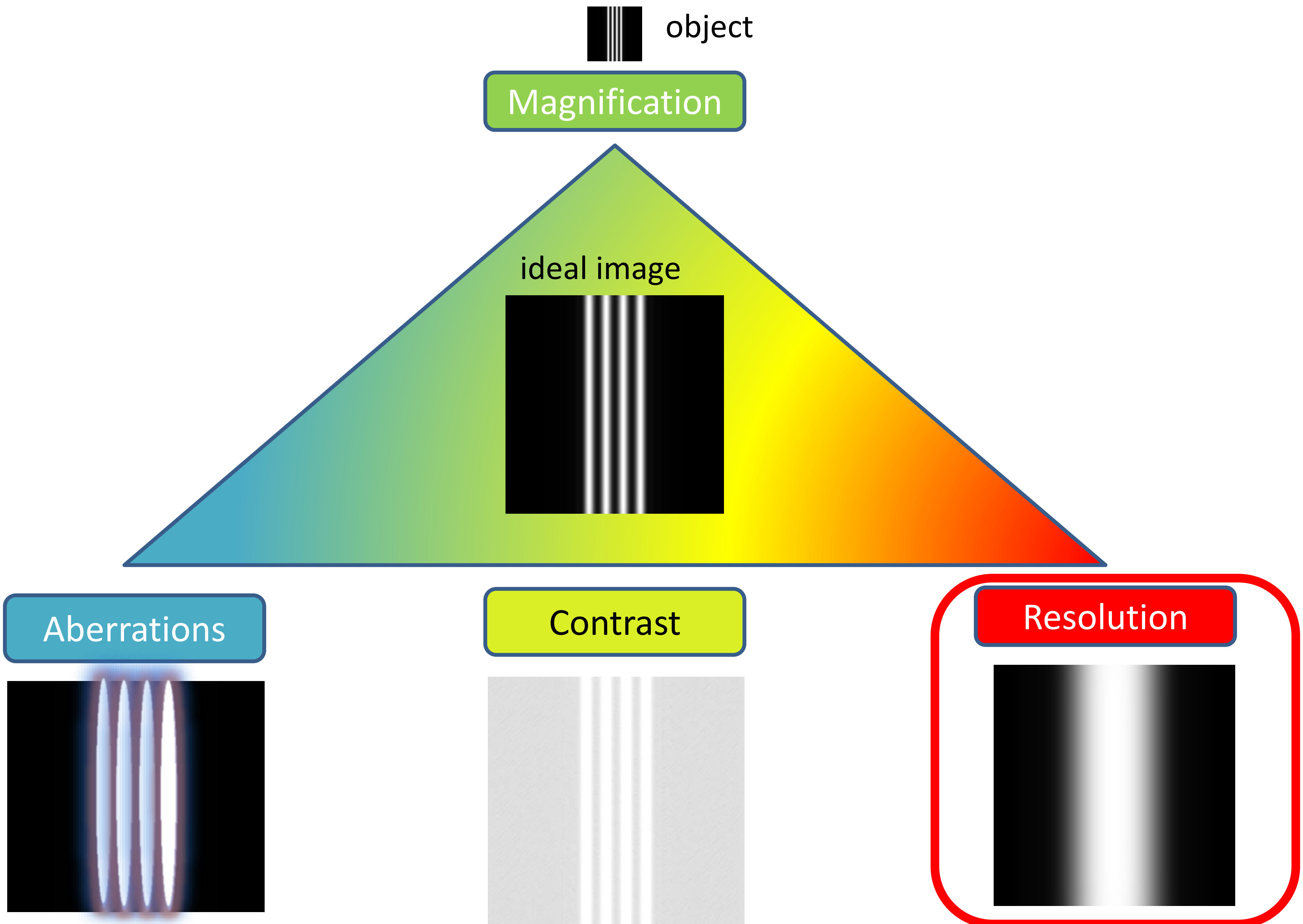
MICRO-561

Fundamentals of Biomicroscopy

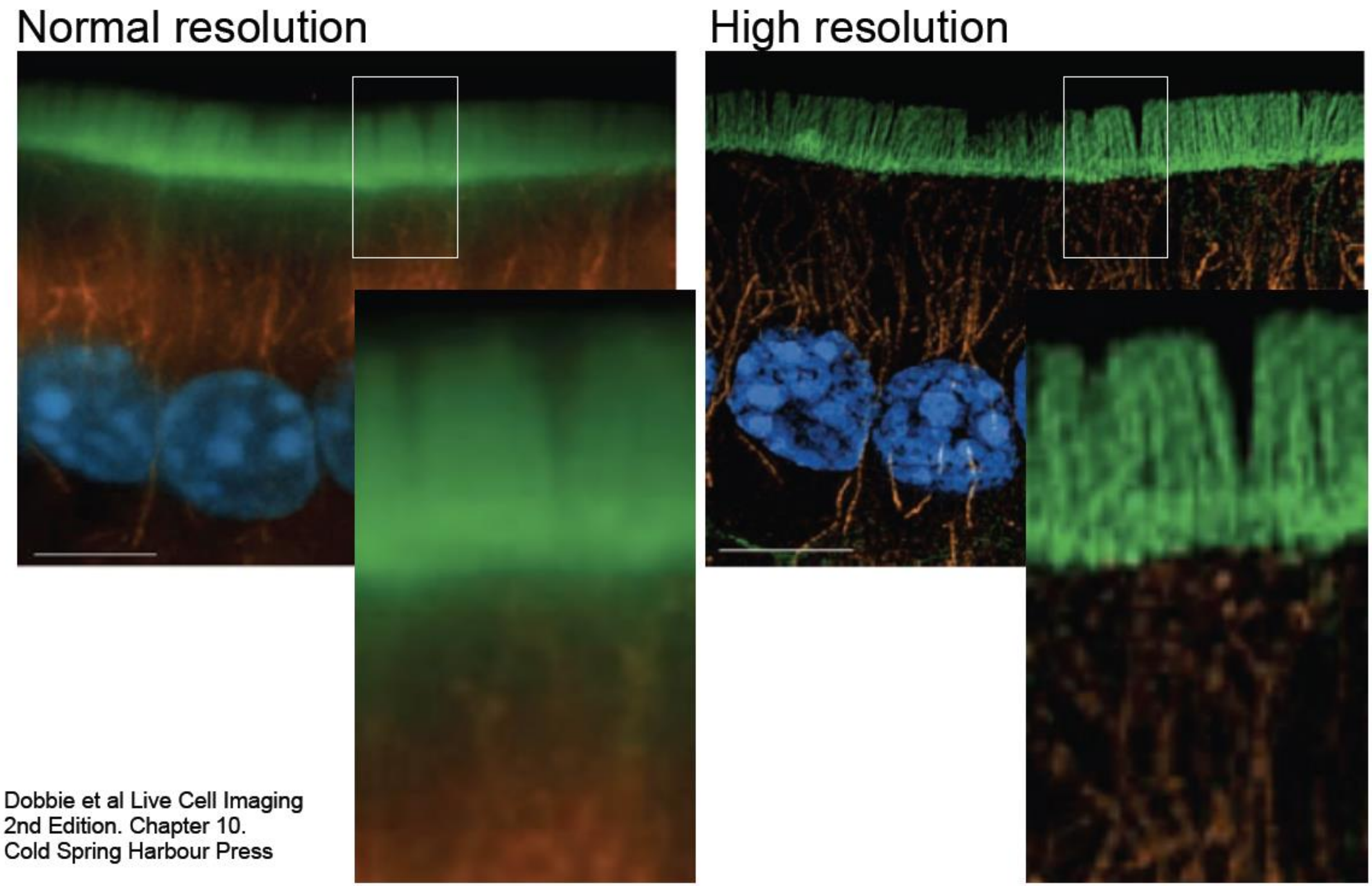
Syllabus (tentative)

Lecture 1	Introduction & Ray Optics-1
Lecture 2	Ray Optics-2 & Matrix Optics-1
Lecture 3	Matrix Optics-2
Lecture 4	Matrix Optics-3 & Microscopy Design-1
Lecture 5	Microscopy Design-2
Lecture 6	Microscopy Design-3 & Resolution -1
Lecture 7	Resolution-2
Lecture 8	Resolution-3
Lecture 9	Contrast
Lecture 10	Fluorescence-1
Lecture 11	Fluorescence-2
Lecture 12	Fluorescence-3, Sources, Filters
Lecture 13	Detectors
Lecture 14	Bio-application Examples

Important aspects for microscopy



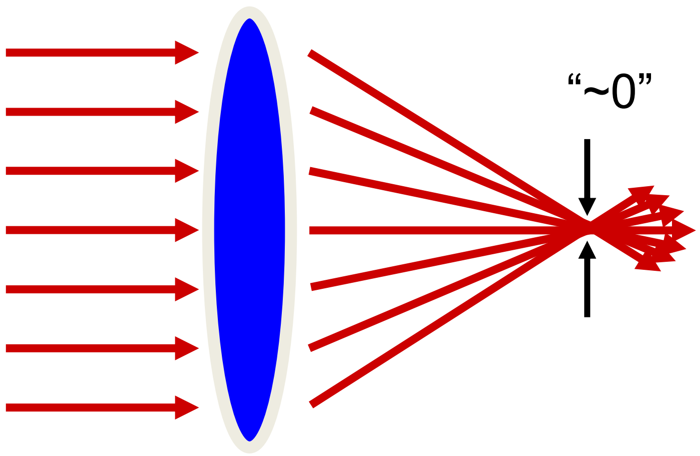
Importance of Resolution in Microscopy



Dobbie et al Live Cell Imaging
2nd Edition. Chapter 10.
Cold Spring Harbour Press

Geometrical optics

Ray (geometrical) optics imply that we can focus a beam to an ideal "point" with zero size.



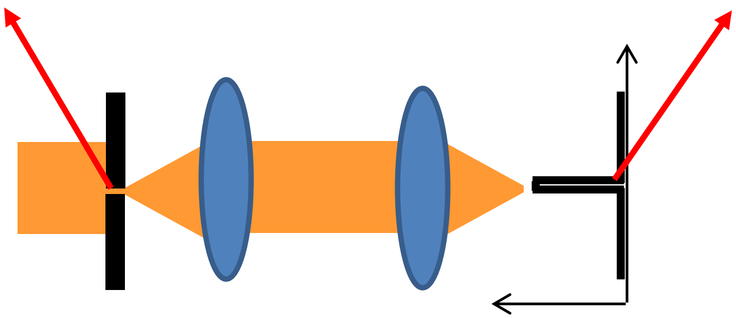
What does this imply for microscopy?

Resolution in microscopy

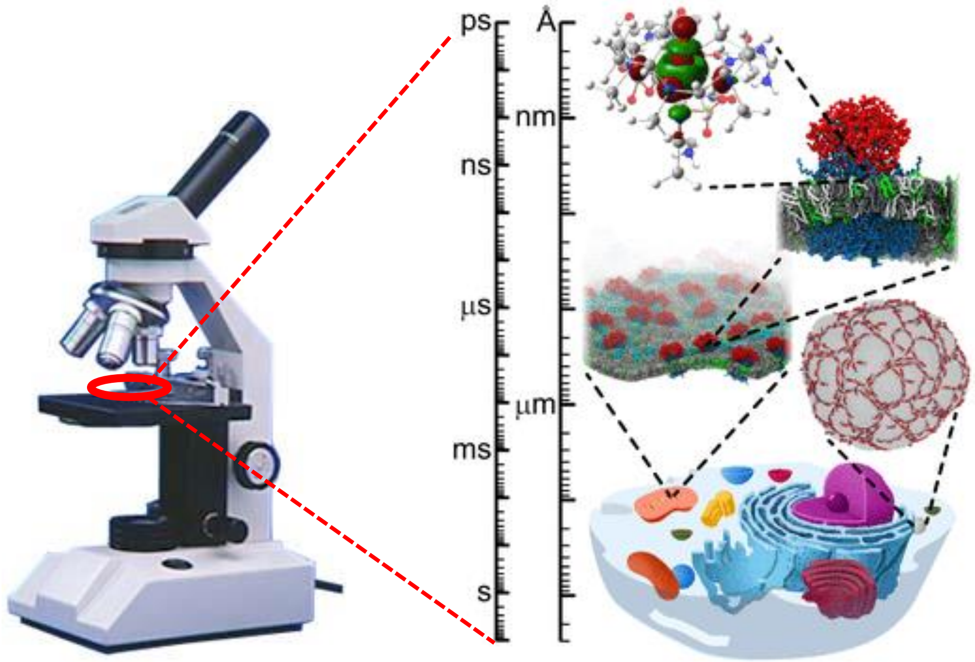
Object/Specimen:
If object is a pin-hole
(which represents a point source)

Image:

Ideally the image will also a point source

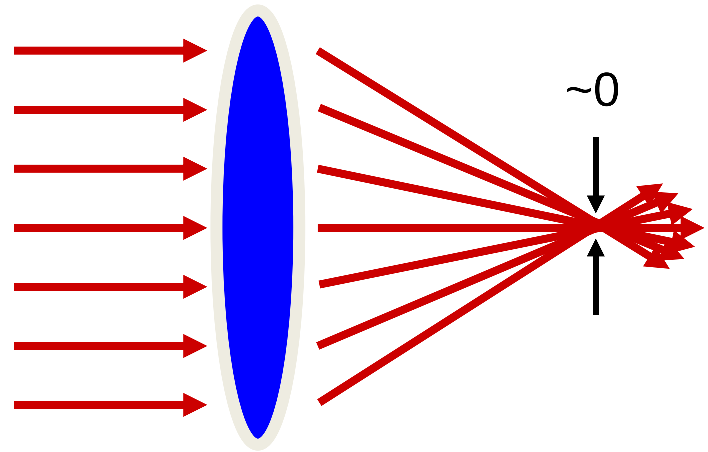


Ability to image an exact copy of a point source (with ~zero dimension) implies that a microscope should able to see **ANY** object, even those with extremely small dimensions such as: proteins, DNA, atoms …



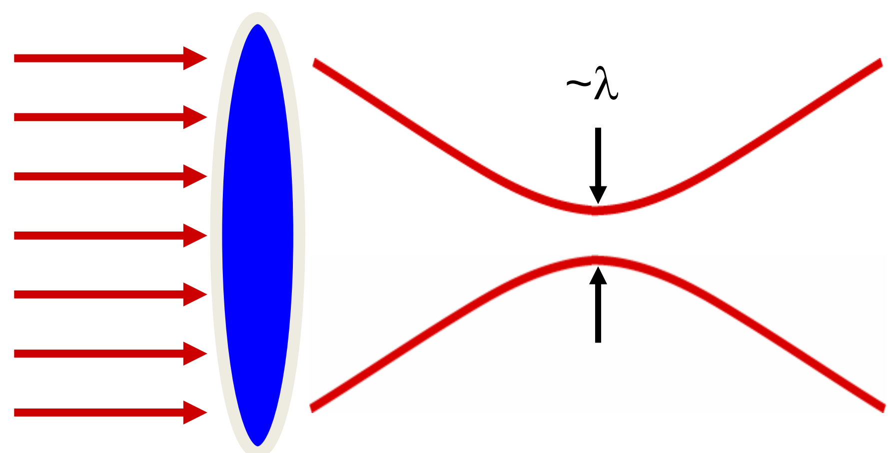
Is geometrical optics the whole story?

Ray optics imply that we could focus a beam to an ideal "point" with zero size.



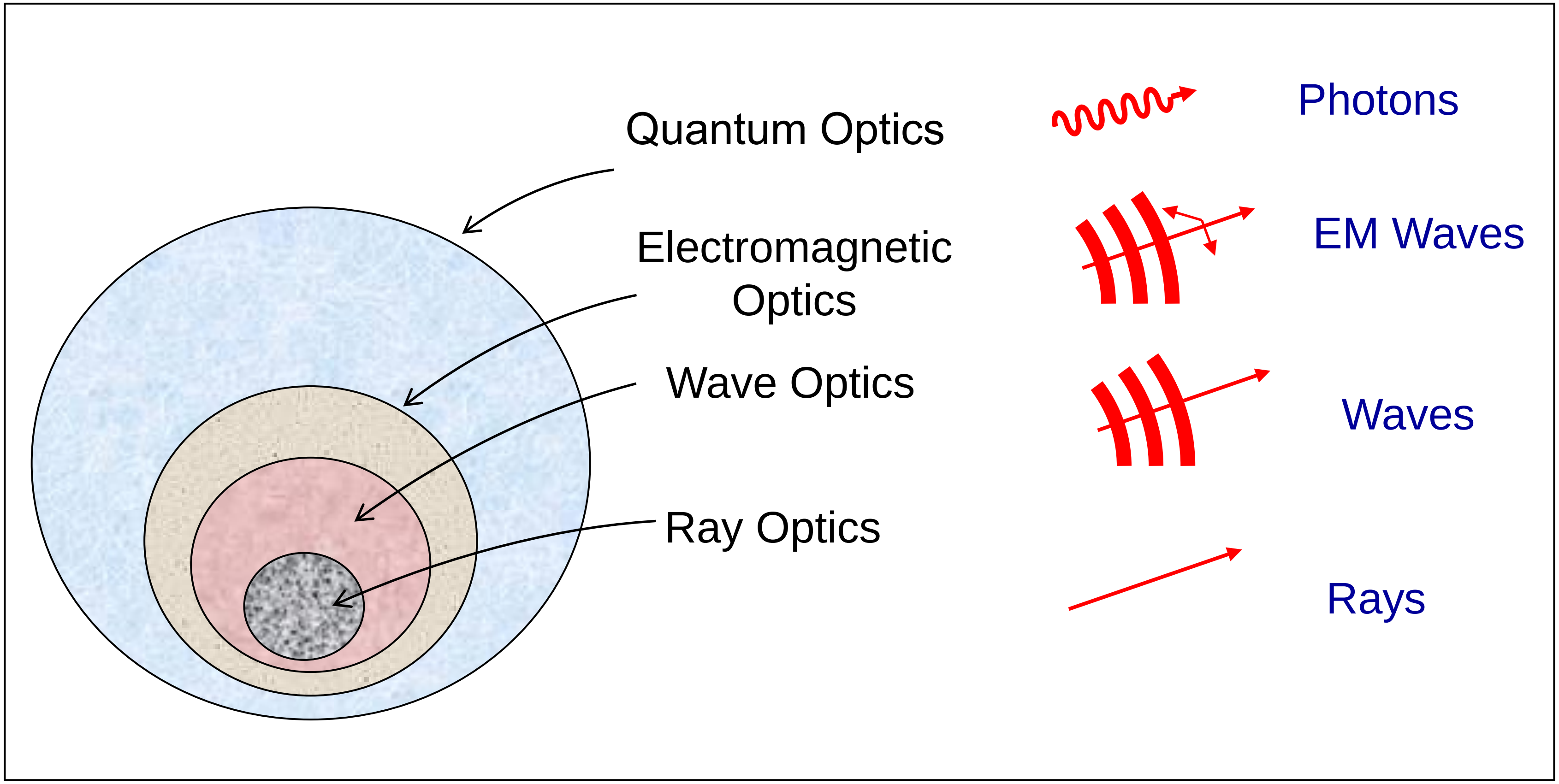
But, this does not hold in reality....

- The minimum possible "spot size" is about one wavelength, λ.
- The finite spot size is due to diffraction, which is not described by ray optics.
- This minimum spot size (i.e. ~λ) also gives the best spatial resolution one can achieve with a microscope.
- In practice, this implies an object having a dimension smaller than λ, will appear at best having a size of λ.



Hierarchy of Theories in Optics

Ray optics cannot explain diffraction → **we need to consider wave optics**



Outline

- **Wave optics**
  - **Wave equation**
  - **Wavelength, period, amplitude & phase of a wave**
  - **Monochromatic waves**
  - **Real waves and complex waves**
  - **Elementary waves: plane wave and spherical wave**

Waves & Optics

An optical wave is described mathematically by the wavefunction $u(\mathbf{r}; t)$, at position $\mathbf{r} = (x, y, z)$ and time t.

Wavefunction satisfies the wave equation:

$$\nabla^2 u - (1/v^2)\partial^2 u/\partial t^2 = 0$$

$$\nabla^2 = \partial^2/\partial x^2 + \partial^2/\partial y^2 + \partial^2/\partial z^2$$

- " v" is the speed of the wave.
- For E&M waves we use "c", for the speed of light in free space.

- Wave equation is general.
- It can be applied to E&M radiation at different spectrum (i.e. light waves, microwaves, radio waves) and also other waves such as water, acoustic, seismic..

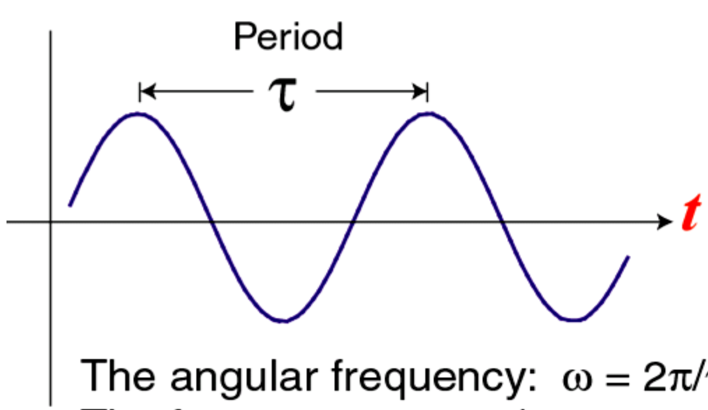
Monochromatic waves

- A monochromatic wave is one of the possible solutions to wave equation.
- It has a harmonic dependence in time and space.

$$u(x,t) = A\cos[(k\,x - \omega\,t) - \theta]$$

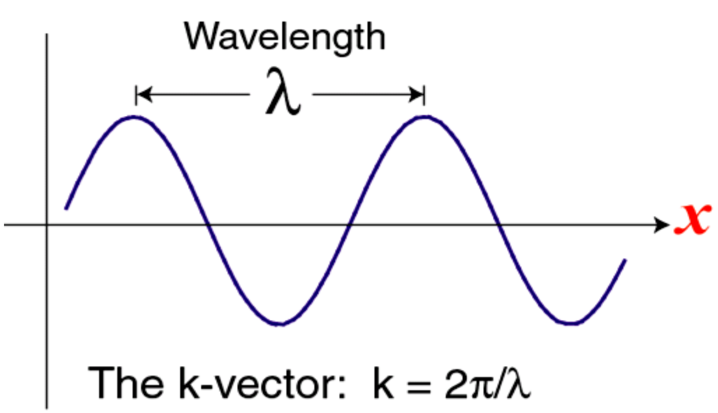
- Monochromatic wave function oscillates periodically in space & time.
- "One" wavelength defines the monochromatic wave (i.e. it is a "mono/single" color wave)

Temporal quantities:



The angular frequency: $\omega = 2\pi/\tau$
The frequency: $\nu = 1/\tau$

Spatial quantities:

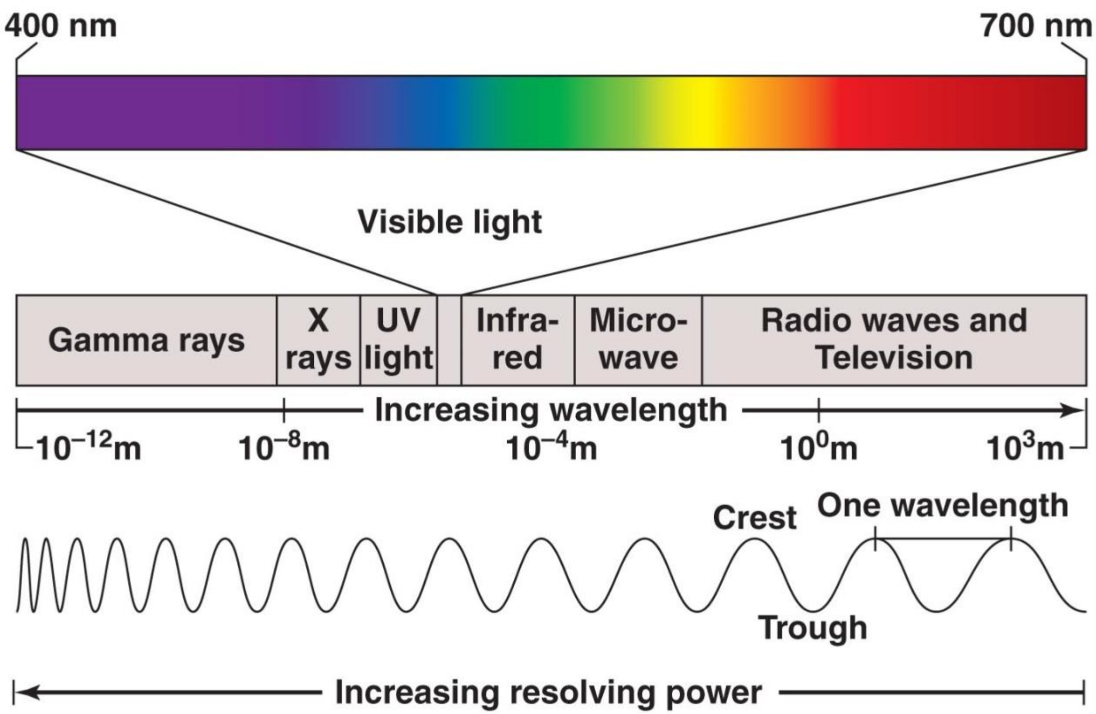


The k-vector: $k = 2\pi/\lambda$
The wave number: $\kappa = 1/\lambda$

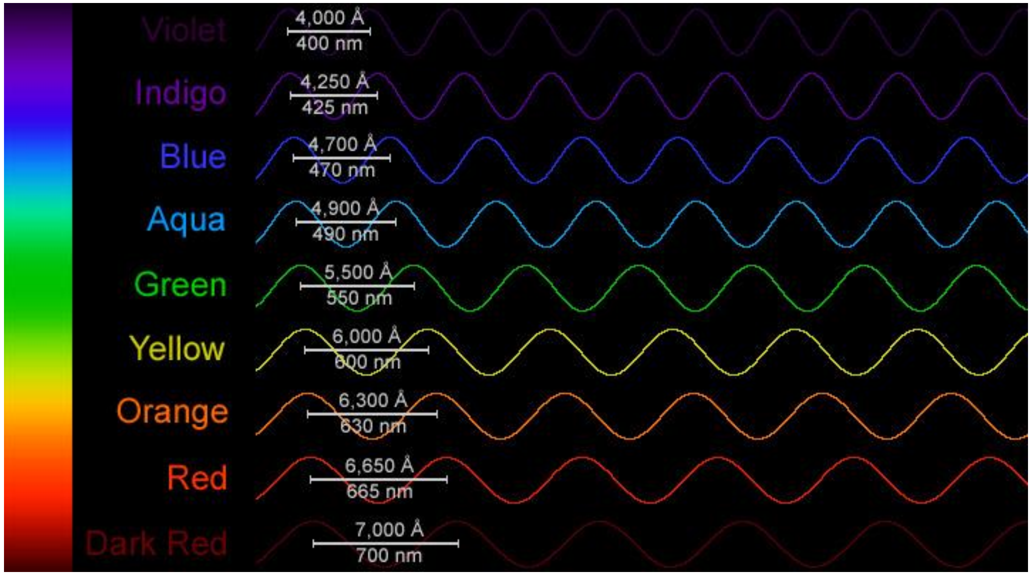
Wavelength of Radiation

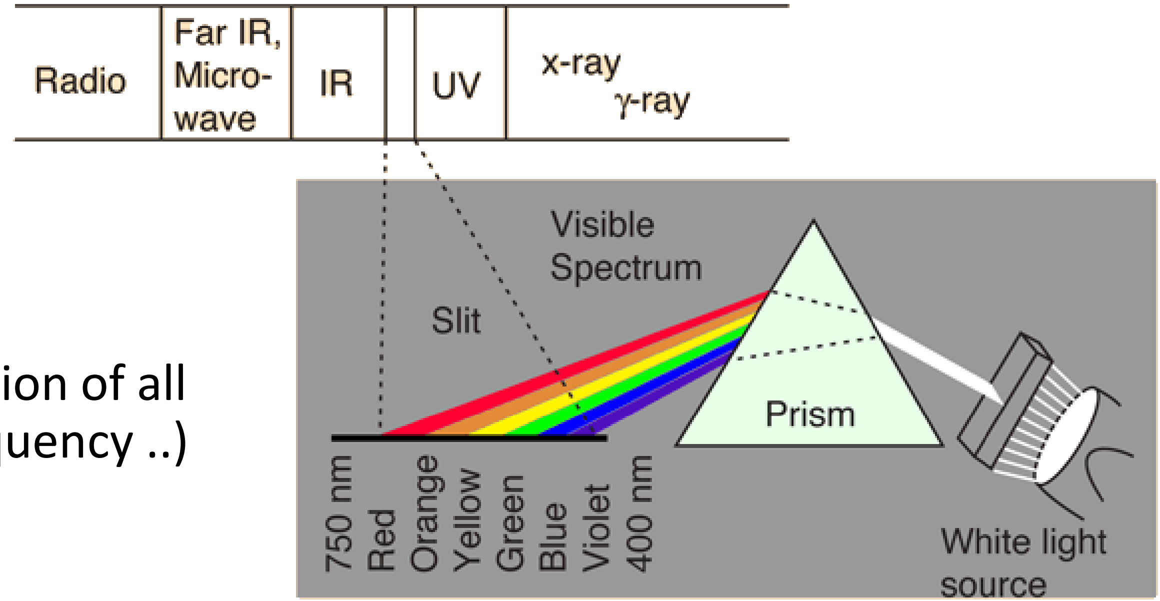
Wave radiation differs in **wavelength**

The electromagnetic spectrum



Visible spectrum

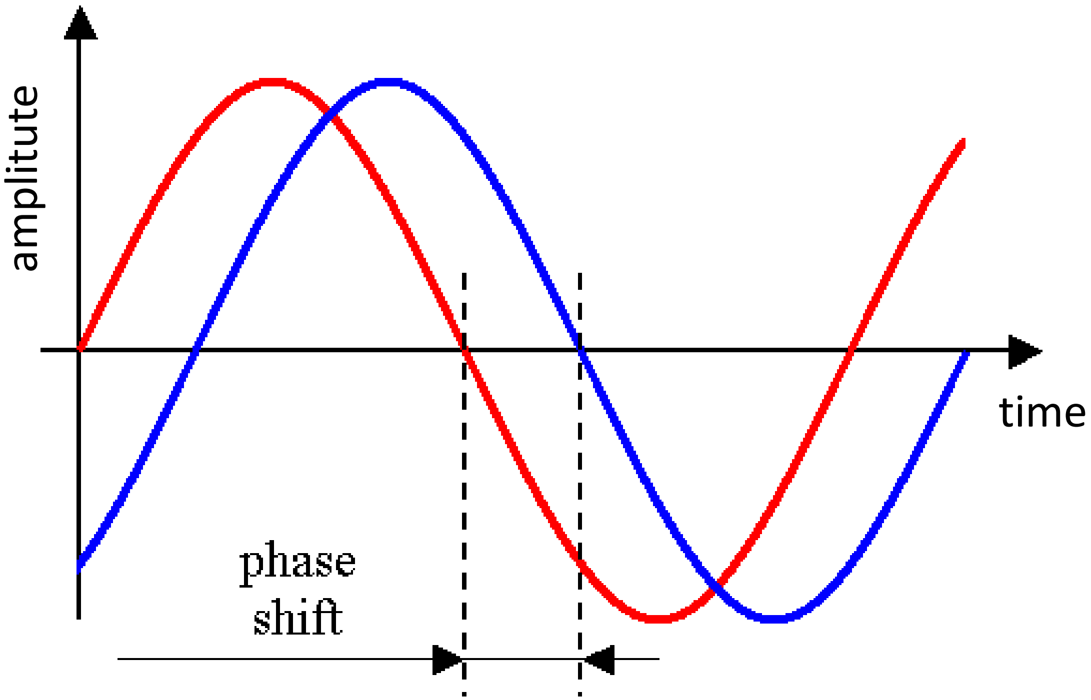




White light is a combination of all wavelengths (colors, frequency ..)

Characteristics of a wave - PHASE

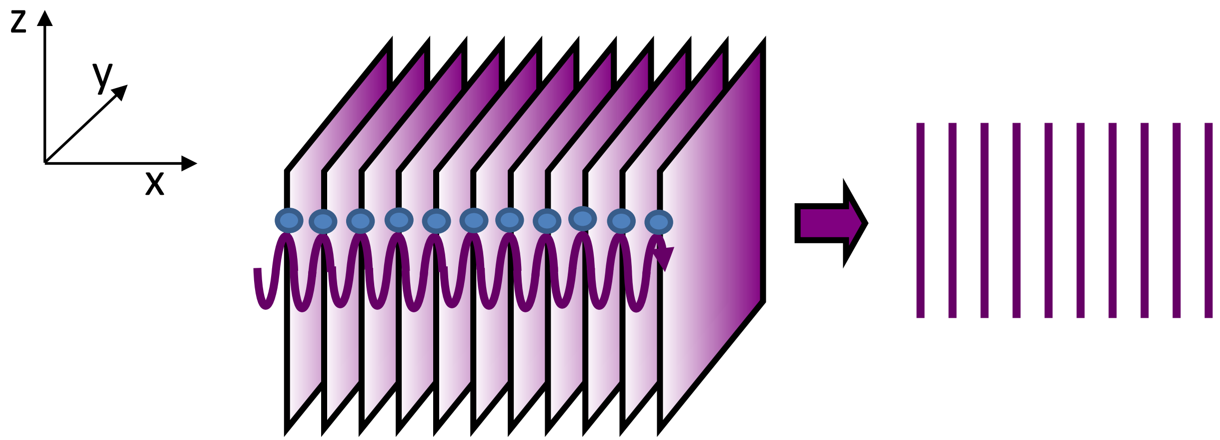
Below figure shows two waves with same amplitude & frequency (i.e. wavelength, period) but they differ in **PHASE**:



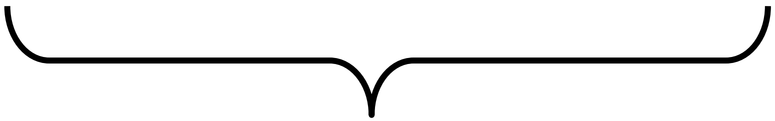
- Phase concept is introduced in wave optics.
- It does not exist in ray optics.

Monochromatic "plane wave" function

This equation represents a monochromatic **"plane"** wave: $u(x,t) = A\cos[(\omega t - k x) - \theta]$



Contours of maximum field, called wave-fronts or phase-fronts, are planes.



Usually we just draw lines

- They extend over all space (y-z).
- Wave-fronts are equally spaced: one wavelength apart.
- They're perpendicular to the propagation direction (x).

Monochromatic wave function

This equation represents a monochromatic **"plane"** wave: $u(x,t) = A\cos[(\omega t - kx) - \theta]$

If we generalize this equation to "**any**" monochromatic wave (planar, spherical, etc…), then the expression becomes:

$$u(r,t) = a(r)\cos\left[\omega t + \varphi(r)\right]$$

This represents a real wavefunction for a monochromatic wave with harmonic time dependence.

Complex representation of a monochromatic wave function

In optics (and E&M), it is convenient to work with **complex wave** formalism

Real wave function is: $u(r,t) = a(r)\cos\left[\omega t + \varphi(r)\right]$

Complex wave function representation of u(r,t) is: $U(r,t) = a(r)e^{i\varphi(r)}e^{i\omega t}$

$$U(r,t) = U(r)e^{i\omega t}$$

Here, U(r) is called complex amplitude.

Mathematically, U(r,t) describes the wave completely, and the wavefunction u(r,t) is its real part:

$$u(r,t) = \mathrm{Re}\{U(r,t)\} = \frac{1}{2}\left[U(r,t) + U^*(r,t)\right] = \frac{1}{2}[U(r)e^{i\omega t} + U^*(r)e^{-i\omega t}]$$

Here, sign * signifies complex conjugation.

Complex wave function also satisfies the wave equation: $\nabla^2 U - \frac{1}{c^2}\frac{\partial^2 U}{\partial t^2} = 0$

Amplitude, intensity and phase of a monochromatic wave

$$u(r,t) = a(r)\cos\left[\omega t + \varphi(r)\right]$$

← this defines the real monochromatic wave, u(r,t)

$$U(r,t) = a(r)e^{i\varphi(r)}e^{i\omega t}$$

← This is the complex wave function representation of u(r,t)

$$U(r,t) = U(r)e^{i\omega t}$$

← Here, U(r) is called complex amplitude.

Real part of U(r) gives the **amplitude** of the wave**:**

$$\left|U(r)\right| = a(r)$$

Intensity of the wave is the square of its amplitude**:**

$$I(r) = \left|U(r)\right|^2 = a(r)^2$$

Imaginary part of U(r) gives the **PHASE** of the wave**:**

$$\arg\{U(r)\} = \varphi(r)$$

- "Phase" is introduced in wave optics. It does not exist in ray optics.
- "Phase" explains the "**interference & diffraction**" phenomena.

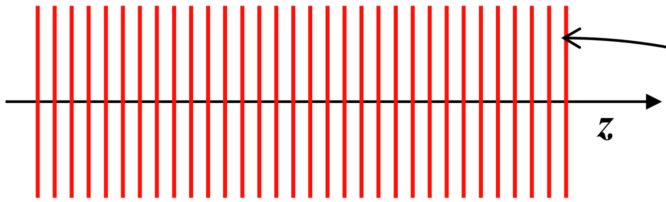
1st example for "elementary wave": Plane wave

- Plane wave traveling in z-direction

$$u(\mathbf{r};t) = a\cos[\omega t - kz + \varphi_o]$$

$$U(\mathbf{r}) = a\exp(i\varphi_o)\exp(-ikz)$$

$$U(\mathbf{r}) = A\exp(-ikz)$$



Wavefronts $\varphi_o - kz = 2\pi m$

$kz = 2\pi m + \text{constant}$

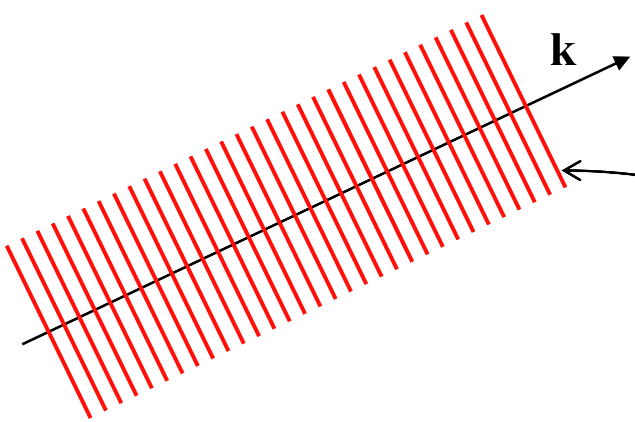
$$U(r) = Ae^{\pm ikz}$$

Propagation direction (left or right)

- Plane wave traveling in arbitrary direction

$$U(\mathbf{r}) = A\exp(-i\mathbf{k}.\mathbf{r})$$

$$= A\exp[-i(k_x x + k_y y + k_z z)]$$

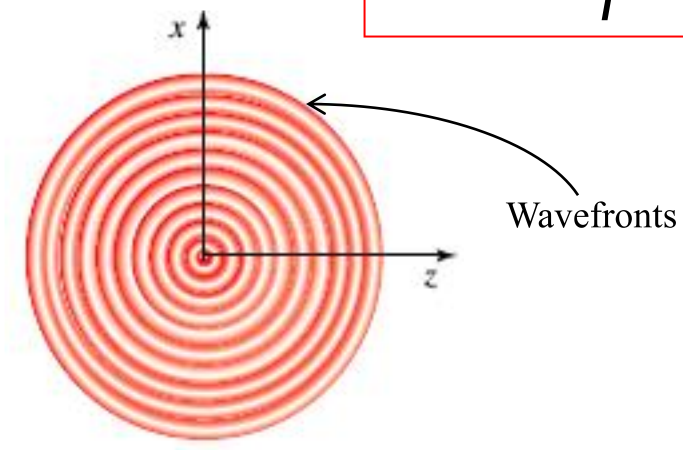


Wavefronts $k_x x + k_y y + k_z z = 2\pi m + \text{constant}$

2nd example for "elementary wave": spherical wave

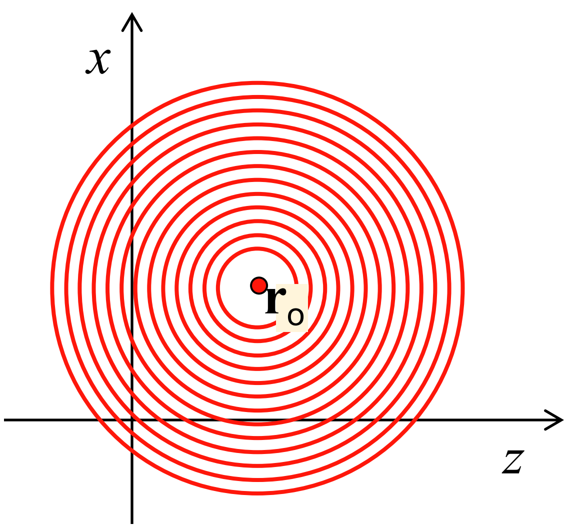
$$U(r) = \frac{A}{r} e^{\pm ikr}$$

Propagation direction (inward or outwards)



$$kr = 2\pi m + \text{constant}$$

$$I(\mathbf{r}) = |A|^2 / r^2$$

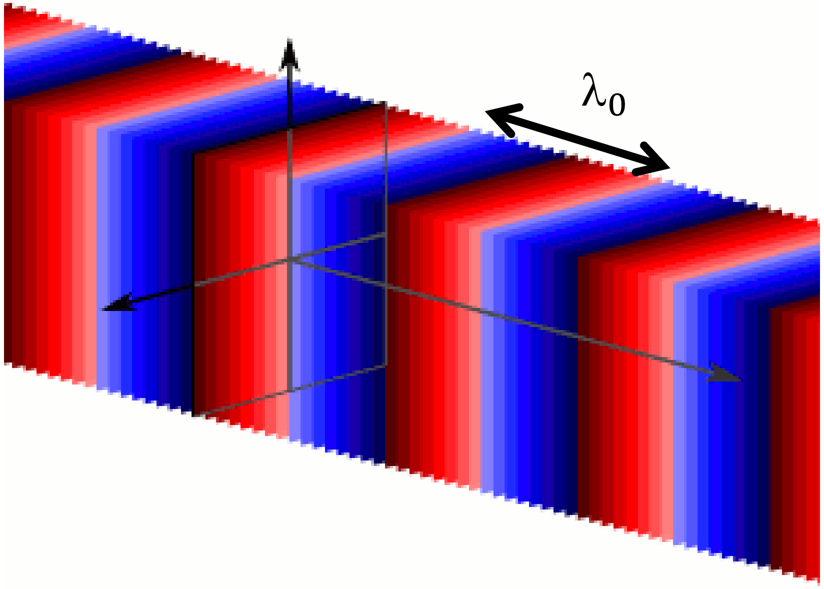


$$U(r) = \left(\frac{A}{|r - r_o|}\right) e^{-ik|r - r_o|}$$

Spherical wave centered at $\mathbf{r}_o$

Wave Propagation

Example:
Propagating plane wave in free space



- In free space, one wavelength (λ_0) is the distance that light travels over one period in time (τ) at a speed of *c:*

Distance = Speed x Time

$$\lambda_0 = c \times \tau$$

- Velocity in vacuum (i.e. "*c*") = 2.99792458 • 10^8 m/sec

Wave Propagation in Free Space

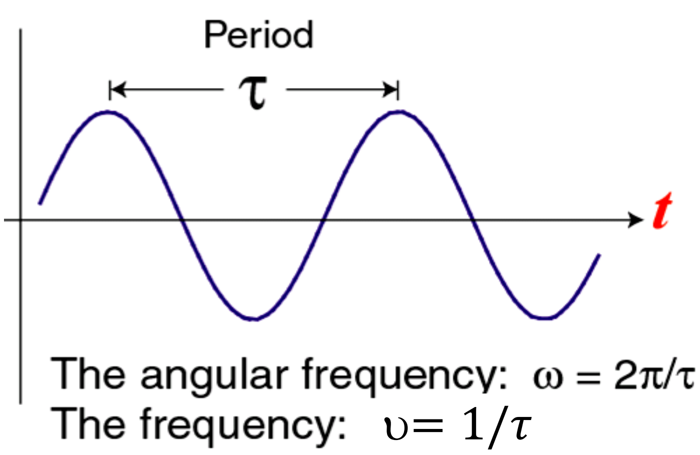
- In free space, one wavelength (λ_o) is the distance that light travels over one period in time (τ) at a speed of *c:*

$$\lambda_0 = c \times \tau$$

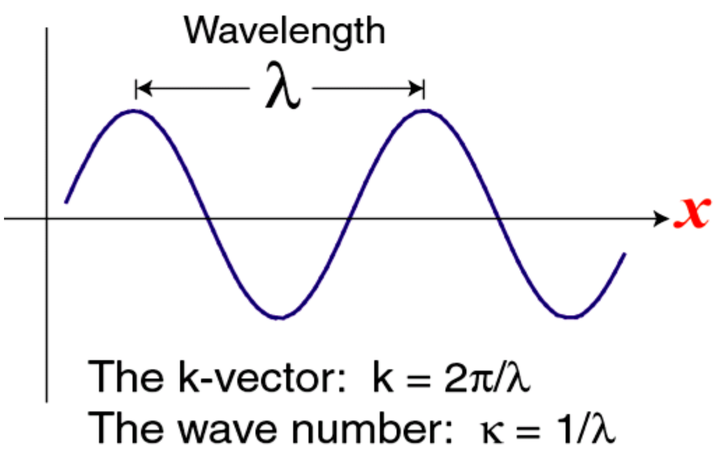
- Velocity in vacuum (i.e. "*c*") = 2.99792458 • 10^8 m/sec
- Recall the definition of frequency & period in time: $\upsilon = 1/\tau$
- Thus, velocity and wavelength of light are linked to each other as:

$$c = \upsilon\, \lambda_0$$

Temporal quantities:



Spatial quantities:



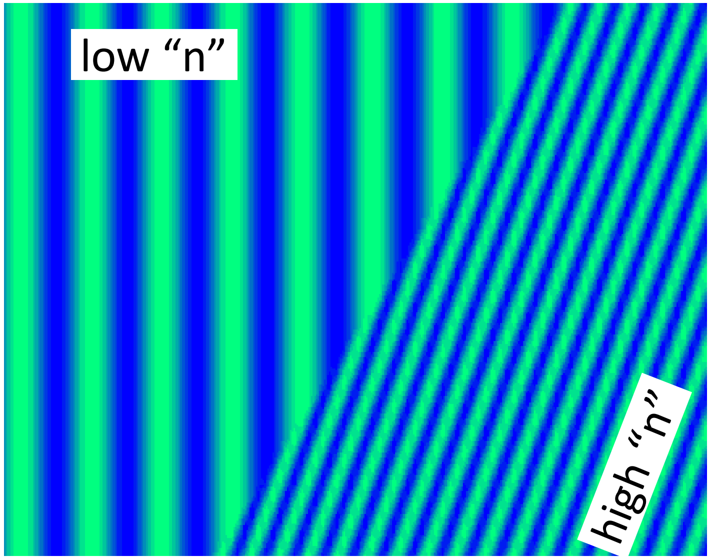
Wave Propagation in Medium

- In free scape: $c = \upsilon \lambda_0$

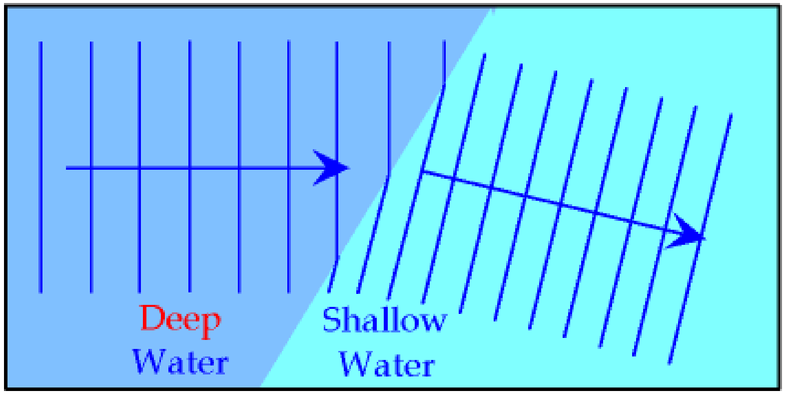
- When light enters into a medium (with index n), its frequency (which is linked to energy) remains constant but its wavelength and speed (v) change as follows: $v = \upsilon \lambda$

- Both wavelength and speed of light scale down linearly with the refractive index n:

$$v = c/n \qquad \lambda = \lambda_0 / n$$



Analogy with "water" waves



Wave Propagation Through Objects

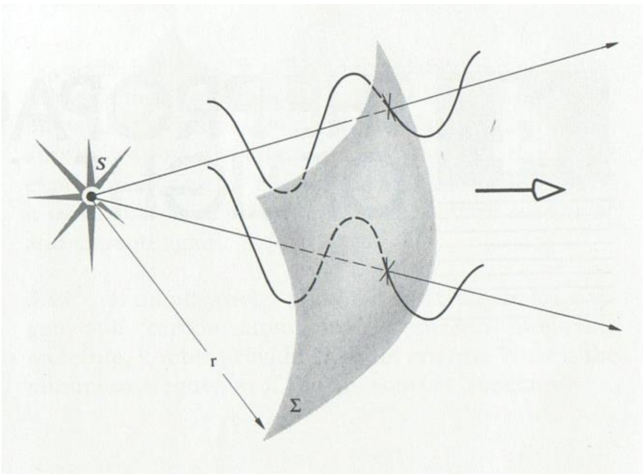


Figure 4.1 A segment of a spherical wave.

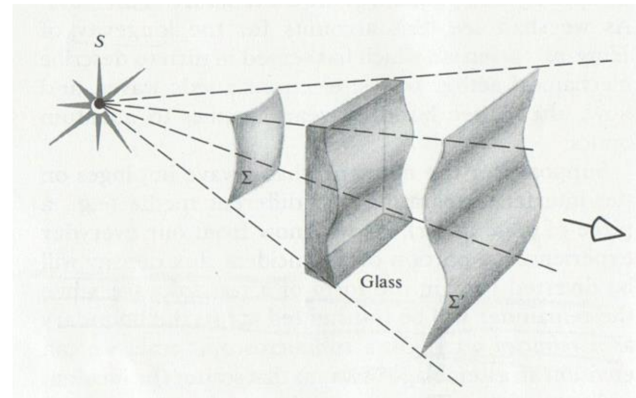


Figure 4.2 Distortion of a portion of a wavefront on passing through a material of nonuniform thickness.

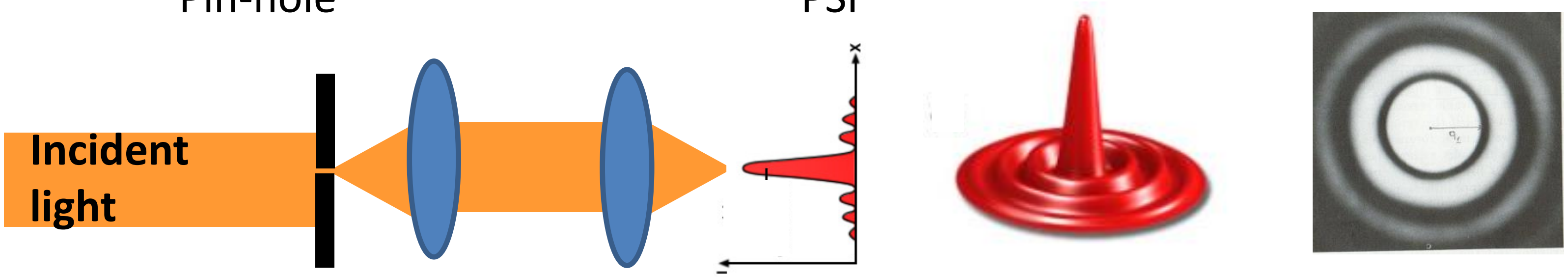
consider a spherical weave interacts with an object (such as a lens, aperture, slit)

its wave fronts gets distorted with this interaction

Wave phenomena & microscopy

Object (Specimen):
Pin-hole

Image:
PSF



In a microscope:

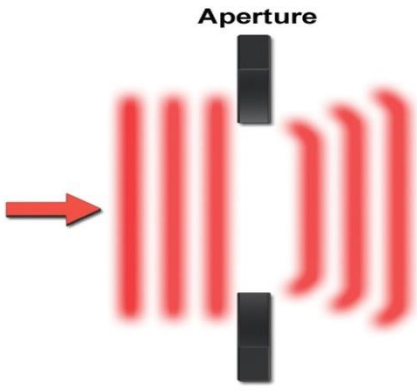
1) Light from the illuminator is **diffracted** (i.e. spread) by the specimen (**object**)

2) Then, diffracted light is **collected** by the **objective lens**

3) And **focused** by the following optical components in the image plane where the propagating light waves **constructively & destructively interfere** to form the **image**

Wave phenomena : diffraction

Depending on the specimen type, diffracted light can be perceived in different ways

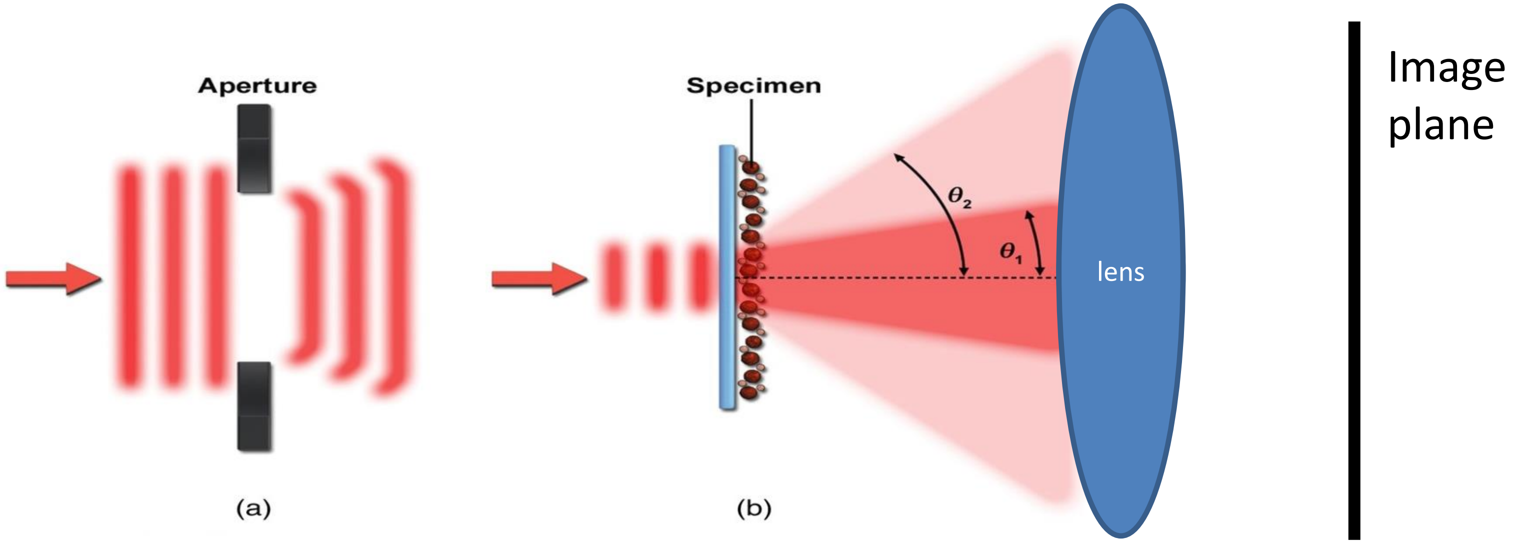


(a)

Example-1 (a): When a beam of light is directed to an aperture, light appears to **bend around the edges**. The aperture could be the "stops" in the microscope (or the edges of the lenses)

Wave phenomena : diffraction

Depending on the specimen type, diffracted light can be perceived in different ways



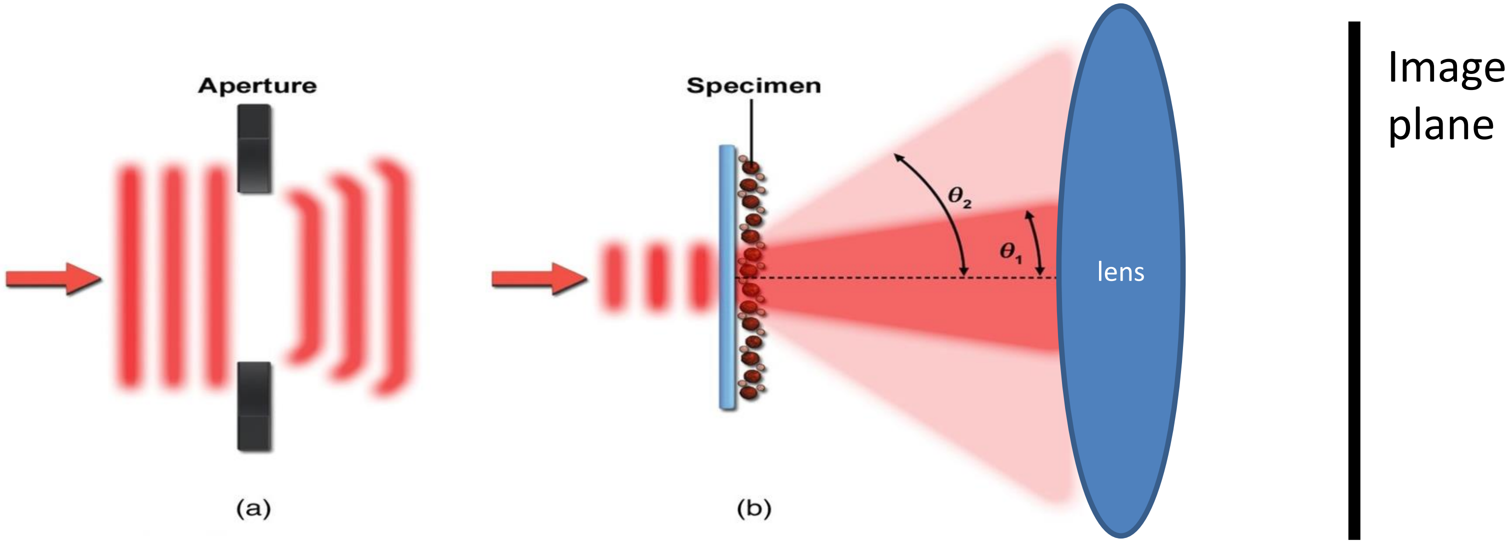
Example-1 (a): When a beam of light is directed to an aperture, light appears to **bend around the edges**. The aperture could be the "stops" in the microscope (or edges of the lenses)

Example-2 (b): Diffraction also occurs when light illuminates a microscope slide covered with **small particles**. The amount of light **scattering** & the angle of spreading depend on the size & density of the diffracting particles on the slide.

In the right drawing, let's assume a mixture of 0.2 um & 2 um diameter particles as specimen.

The angle of spreading is inversely proportional to the particle size.
➔ Larger angle (θ_2) corresponds to light diffraction by the smaller particles

Wave phenomena : diffraction & interference



Diffraction - In microscopy, there are two primary sites for diffraction:

1- the specimen itself

2- the most limiting aperture of the microscope system

Interference can be seen as the combination of diffracted waves.

➔ This is also the process responsible for creating images.

Wave theory & wave phenomena (diffraction, interference …)

- First deduced by Robert Hooke and mathematically formulated by Christiaan **Hyugens**
- Thomas **Young** demonstrated that wave theory best explained interference phenomenon.

Christiaan Hyugens
1629-1695

Thomas Young
1773-1829

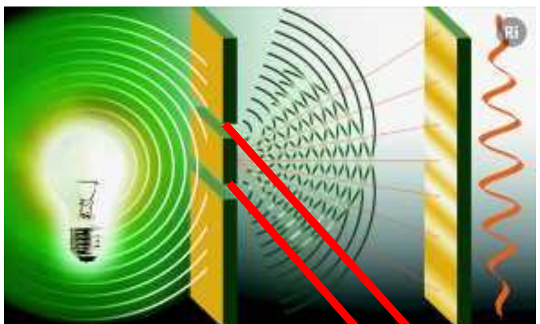
- Augustin-Jean **Fresnel** synthesized the work of Hyugens and Young.
- Championed the wave theory in his 1818 memoir on diffraction.
- All this finally replaced by Newton's corpuscular (particle) theory .*

Augustin-Jean Fresnel
1788-1827

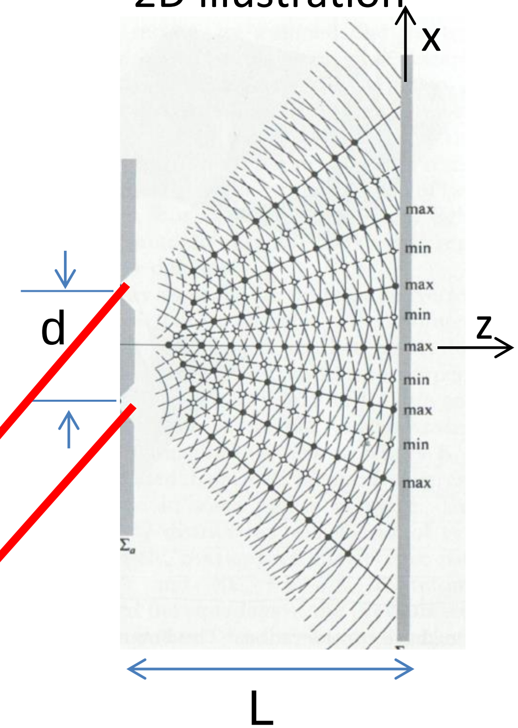
* Until the 20^{th} century (quantum era)

Thomas Young's Double Slit Experiment

3D illustration



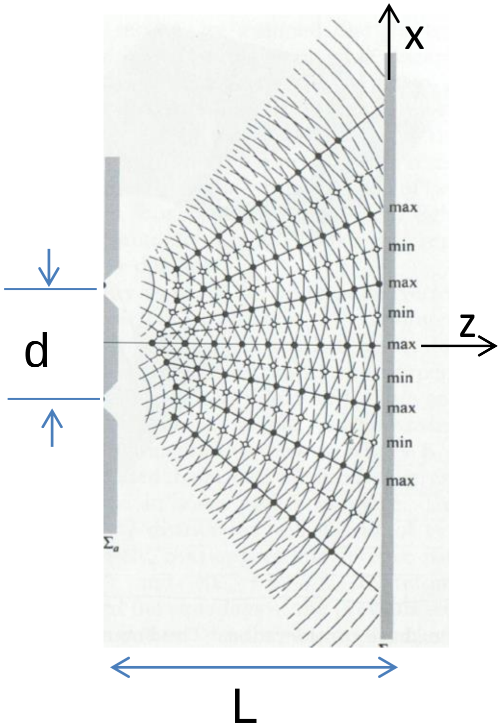
2D illustration

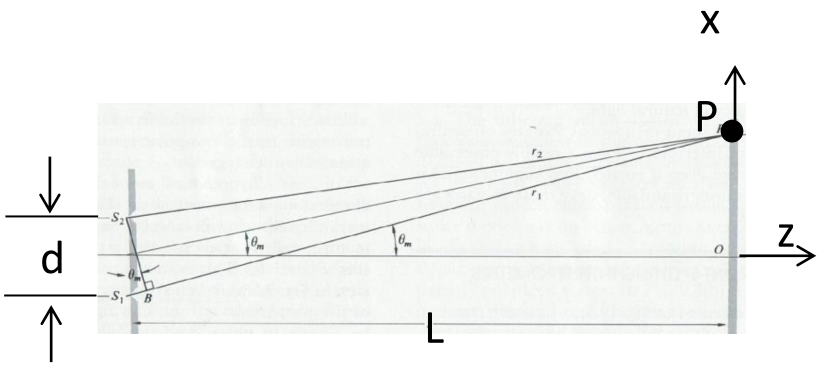


Consider having two point sources at the two slit positions (in cross-section view), as shown above.

- They are separated from each other by a distance d (right figure)
- The sources emit light waves with spherical wave-fronts
- These light waves (with spherical wave-fronts) interfere as they propagate in free space.

Thomas Young's Double Slit Experiment





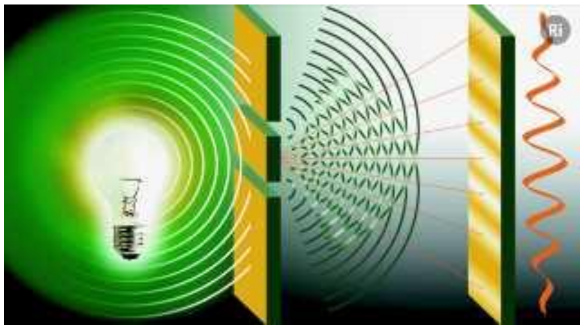
Consider having two point sources at the two slit positions, as shown above.

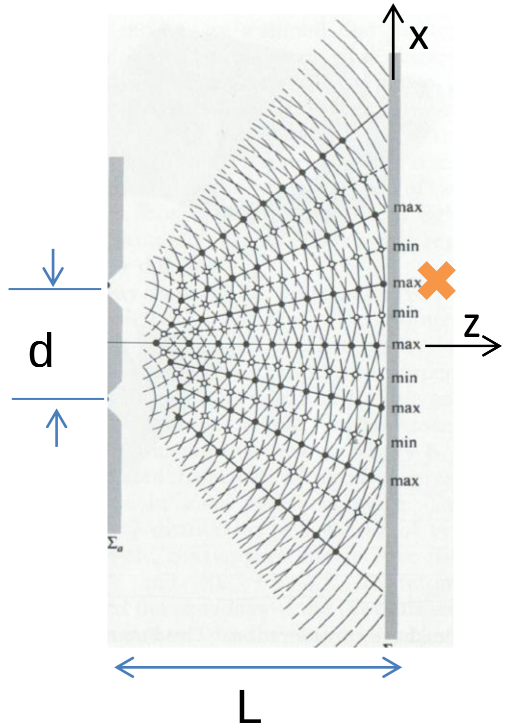
- Two sources emit waves with spherical wave-fronts.
- Let's put an observation screen **"L" away** from the double slit plane **at the z-axis**. Note that L >> d, λ
- **Along the x-axis** of the observation screen (lets take **point P**, on the right figure), there will be a **phase difference (φ)** between the two waves because each wave **travels over a different distance (r_1 or r_2)** (see right figure).
- The interference of these waves with phase delay will led to an **interference pattern** on the observation screen
- The interference pattern will have **sinusoidal intensity fluctuations in the x-axis**.
- Intensity pattern is governed by the following interference equation:

$$I = I_1 + I_2 + 2(I_1 I_2)^{1/2} \cos\varphi \qquad \varphi = \varphi_2 - \varphi_1$$

- Amount of the **phase difference (φ)** dictates the final intensity at the observation plane.

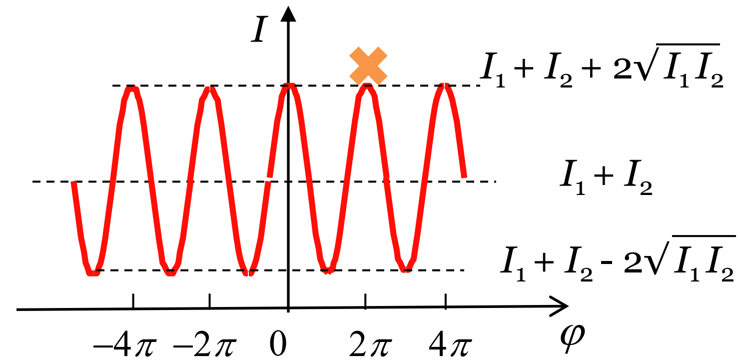
Thomas Young's Double Slit Experiment

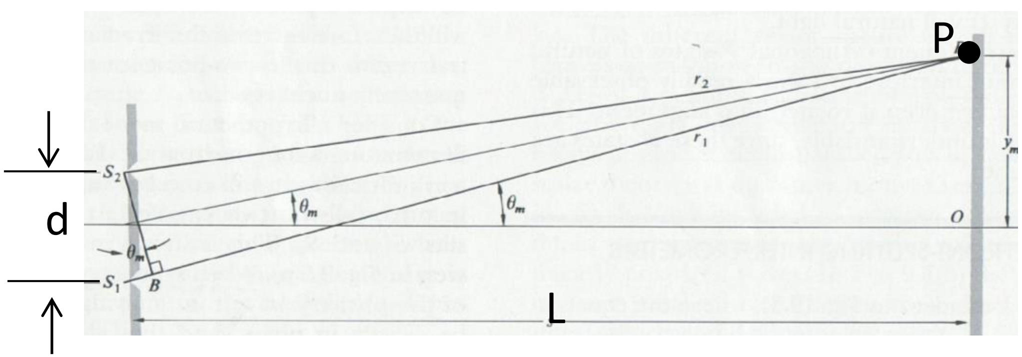




$$I = I_1 + I_2 + 2(I_1 I_2)^{1/2} \cos\varphi$$

$$\varphi = \varphi_2 - \varphi_1$$

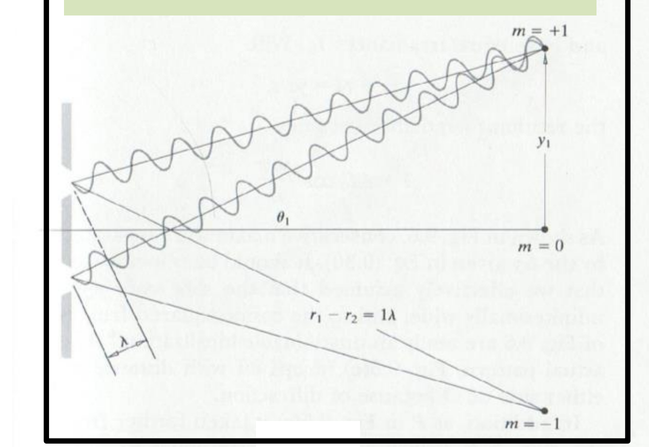




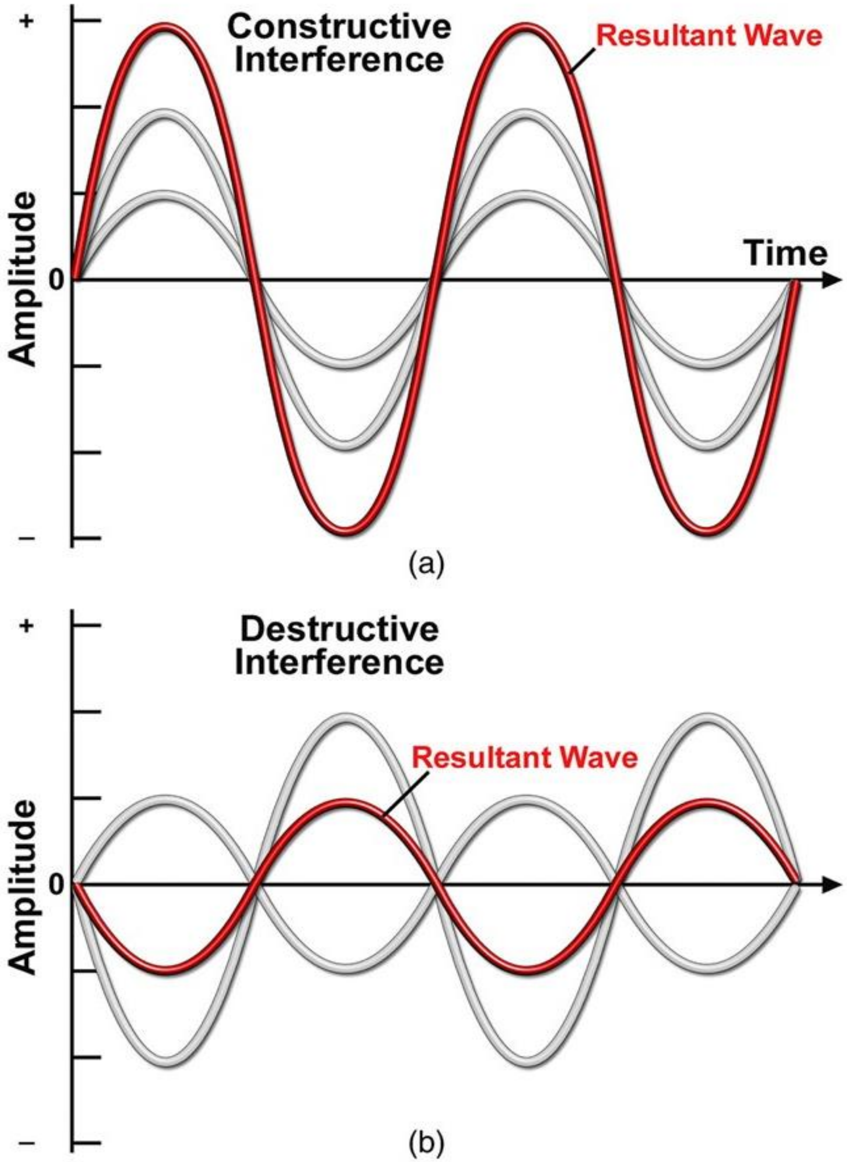
1[st] **max (m=+1):**

- **Phase Difference (φ) is +2π**
- **Path Difference (S_1B) is λ**

➔ Constructive Interference



Waves interference



Two waves are shown that oscillate in the plane of the page.

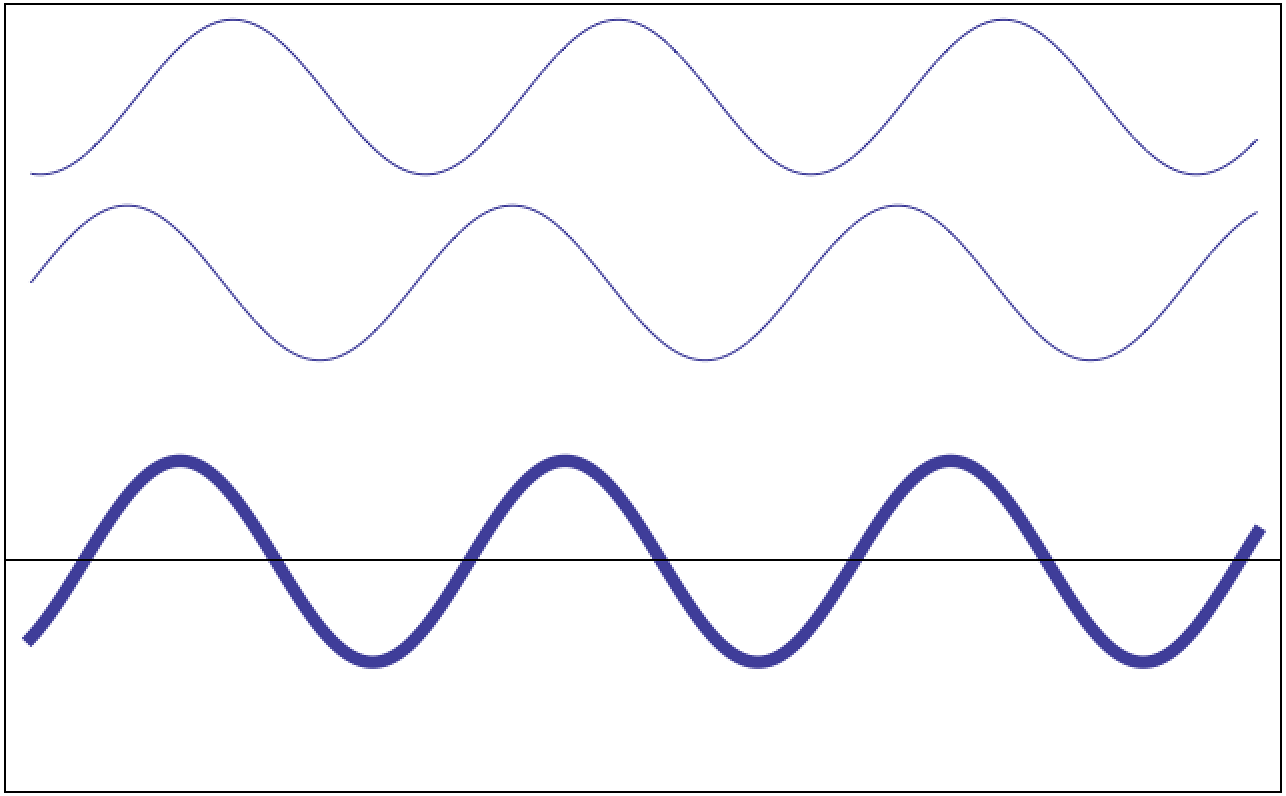
In these examples **(a)** and **(b)**:

- The two waves (tinted gray) have the same wavelength, but vary in amplitude.
- The wave resulting from their interference is shown with red curve.

(a) **Constructive interference** occurs when these two waves have the same phase (a.k.a “in phase”).
(b) **Destructive interference** occurs when these two waves are “out-of-phase”.

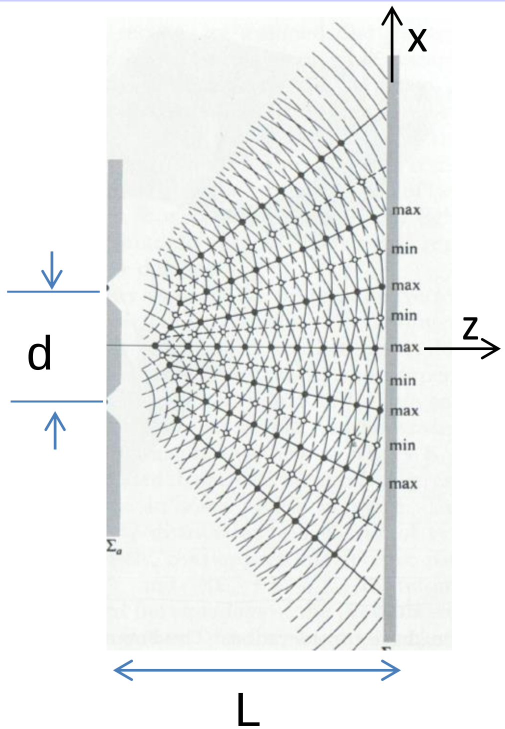
Waves Interference

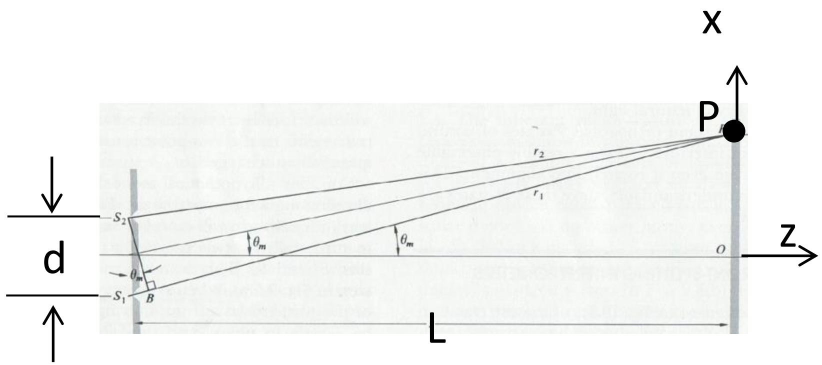
- Two waves (thin blue) are shown to oscillate in the plane of the page. They have same wavelength (frequency) and same amplitude.
- The wave resulting from their interference is shown with thick blue curve.



- **Constructive interference** occurs when these two waves have the same phase (a.k.a "in phase"). It leads to maximum intensity
- **Destructive interference** occurs when these two waves are "out-of-phase". It leads to minimum intensity

Thomas Young's Double Slit Experiment





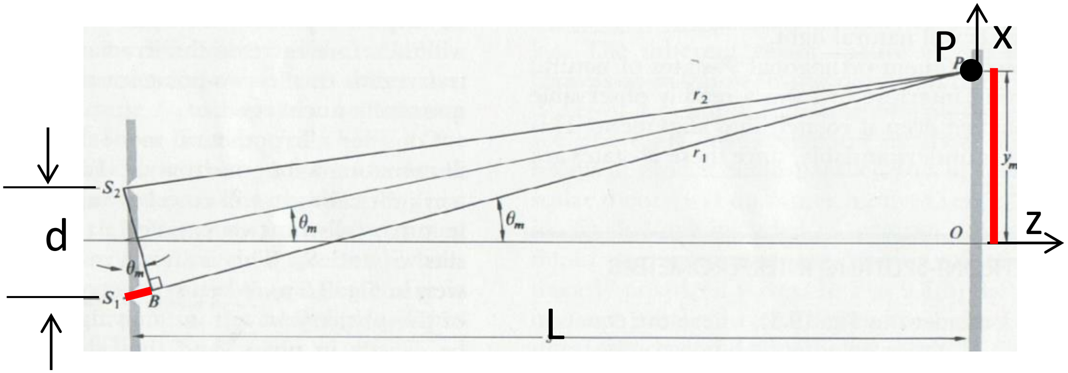
For two point sources at the two slit positions & observation plane "L" away from the slits:

- The interference of the waves with phase delays will led to an **interference pattern** on the observation screen
- The interference pattern will have **sinusoidal intensity fluctuations in the x-axis**.
- Intensity pattern is governed by the following interference equation:

$$I = I_1 + I_2 + 2(I_1 I_2)^{1/2} \cos\varphi \qquad \varphi = \varphi_2 - \varphi_1$$

- Amount of phase difference (φ) dictates the final intensity at the observation plane.
- **Max** intensity points corresponds to **constructive** interference, where $\varphi = 0, 2\pi, 4\pi, 6\pi$ ... in the above equation
- **Min** intensity points corresponds to **destructive** interference, where $\varphi = \pi, 3\pi, 5\pi, 7\pi$ ... in the above equation

Thomas Young's Double Slit Experiment



$$Phase \rightarrow \varphi_1 = k\boldsymbol{r_1} = \frac{2\pi}{\lambda} r_1$$

$$\varphi_2 = k\boldsymbol{r_2} = \frac{2\pi}{\lambda} r_2$$

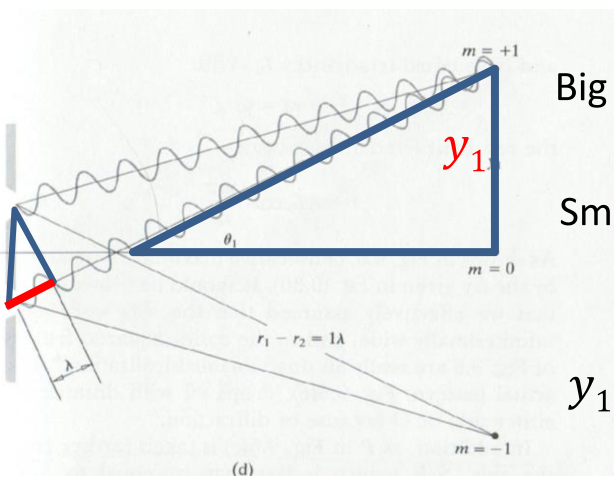
$$Phase\ difference \rightarrow \varphi = \varphi_2 - \varphi_1$$

$$Path\ difference \rightarrow |S_1 B| = r_2 - r_1$$

$$Location\ of\ the\ observation \rightarrow y_m$$

For 1st max (y_1):

- **Constructive Interference**
- **Path difference is** $1 \times \lambda$



Big triangle → $Sin\theta_1 \sim \frac{y_1}{L}$

Small triangle → $Sin\theta_1 = \frac{\lambda}{d}$

$$y_1 = \frac{\lambda L}{d} \Leftrightarrow \lambda = \frac{y_1 d}{1.L}$$

generalize

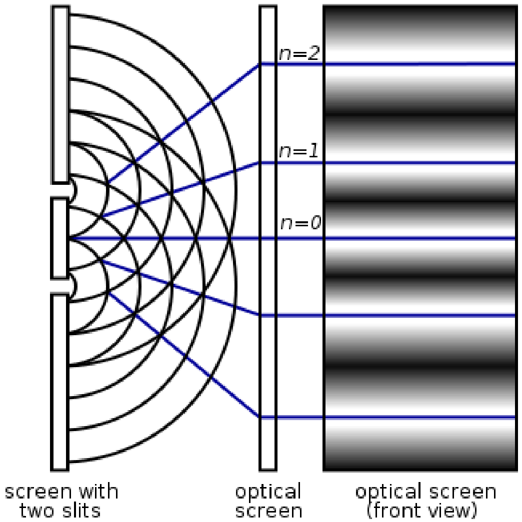
For mth max (y_m):

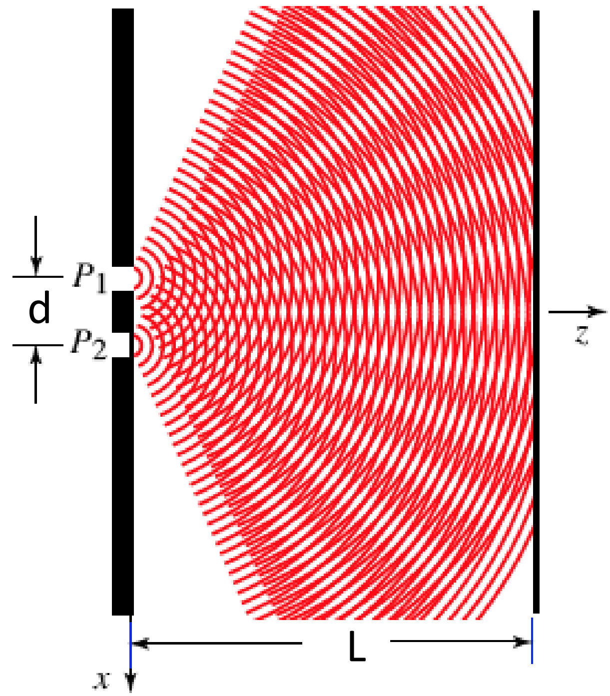
- **Path difference is m × λ**
- **Constructive Interference**

$$y_m = m\frac{\lambda L}{d}$$

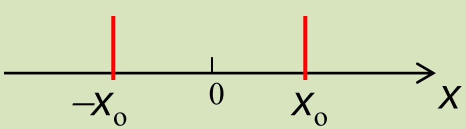
$$\lambda = \frac{y_m d}{m.L}$$

Double Slit (with no width) Experiment

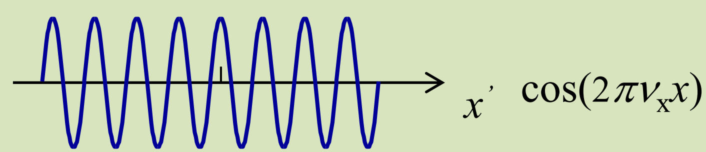




INPUT:



OUTPUT:



PS: Ignore the slit width