MICRO-561

Biomicroscopy I

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
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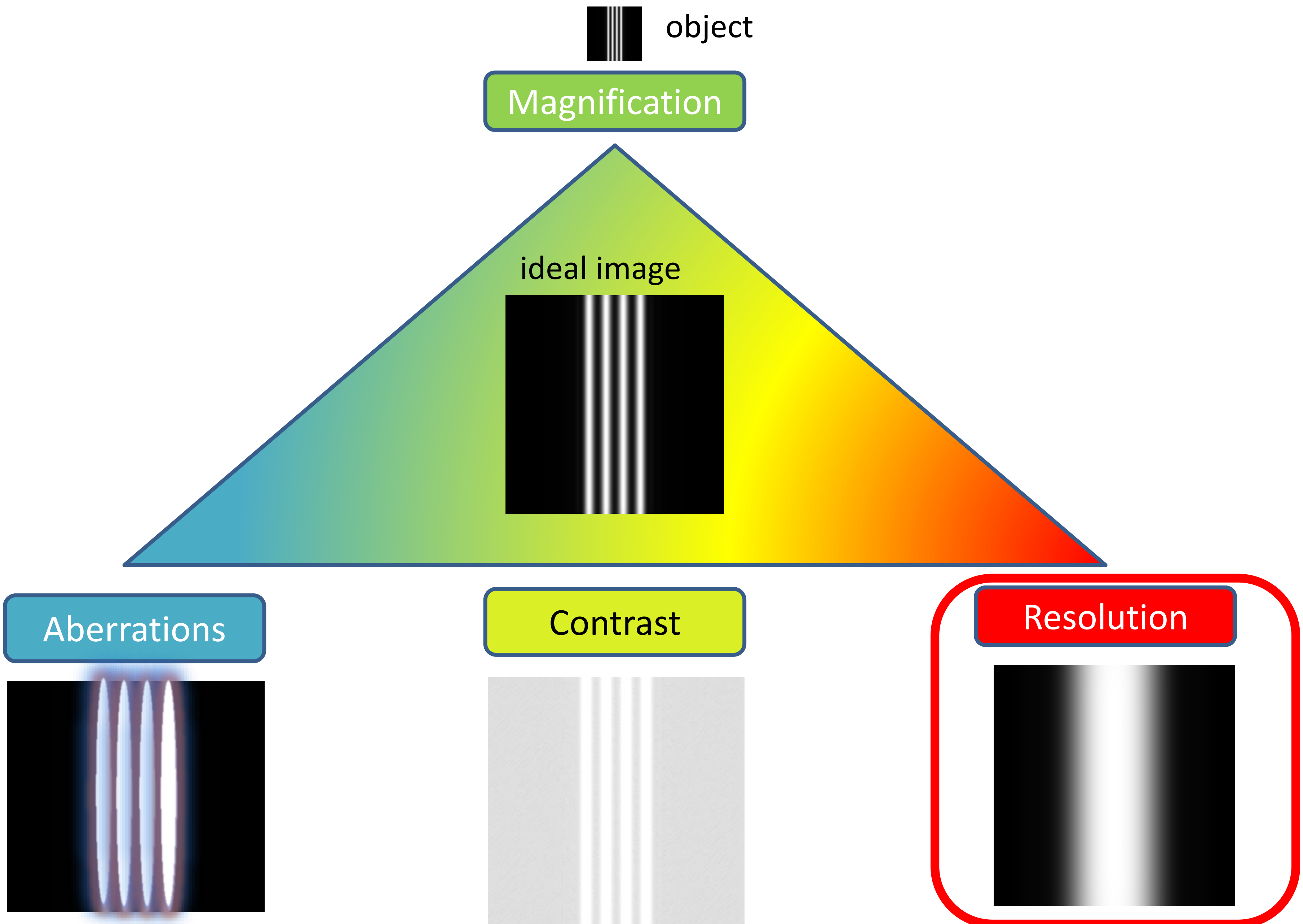
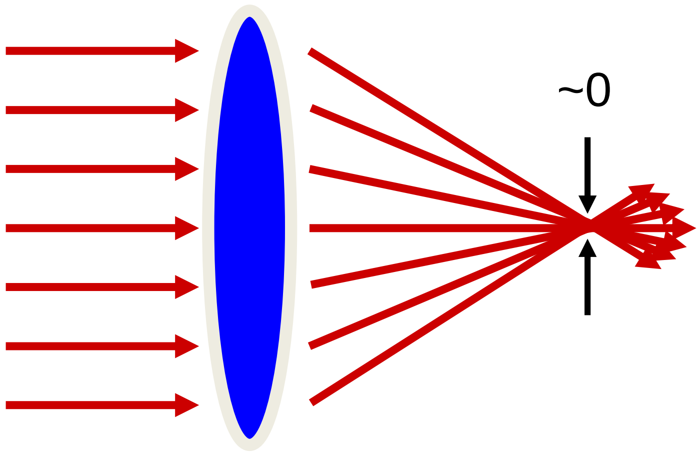
Lecture 9	Contrast
Lecture 10	Fluorescence-1
Lecture 11	Fluorescence-2
Lecture 12	Fluorescence-3, Sources, Filters
Lecture 13	Detectors
Lecture 14	Bio-application Examples

Important aspects for microscopy



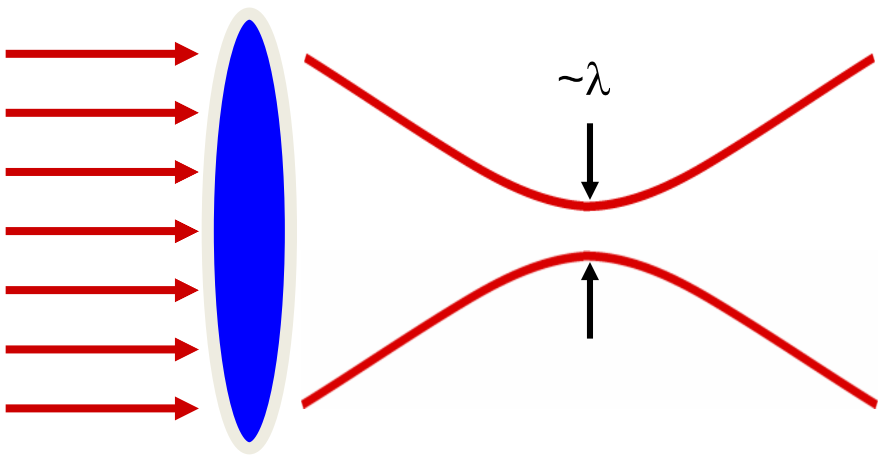
Reminder: Resolution limit

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

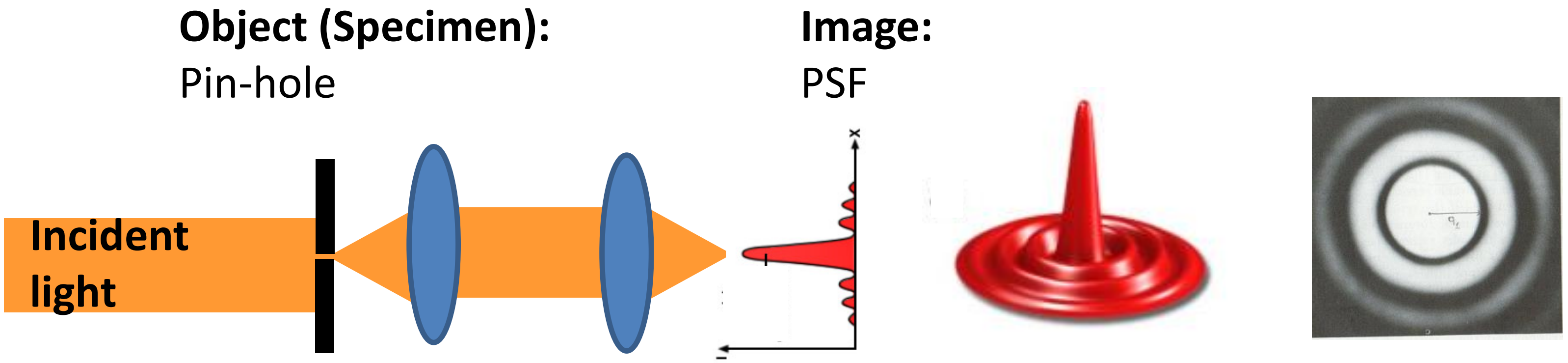


But, this does not hold in reality….

- 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.



Reminder: Wave phenomena & microscopy



In a microscope:

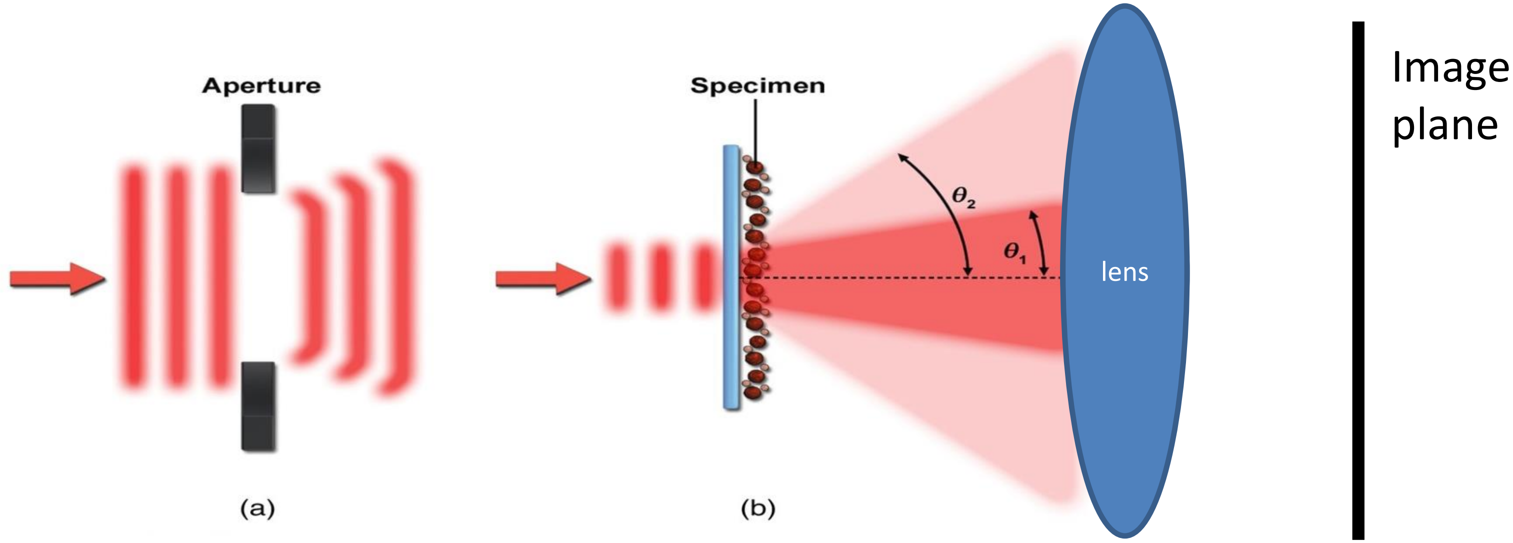
1) Light from the illuminator is **diffracted** (i.e. spread) by the specimen (**object**)

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**

Reminder: Diffraction

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



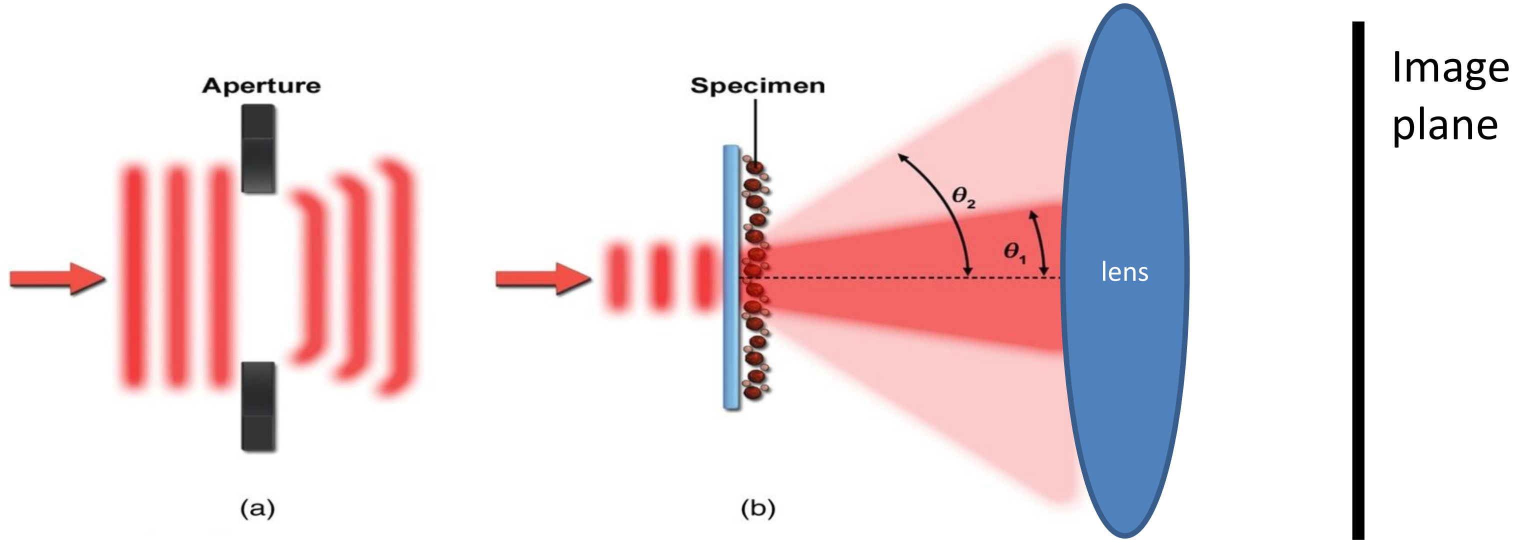
Example-1 (a): When a beam of light is directed to an aperture, light appears to **bend around the edges**. The aperture could be the “stops” in the microscope (or edges of the lenses)

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

Reminder: 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

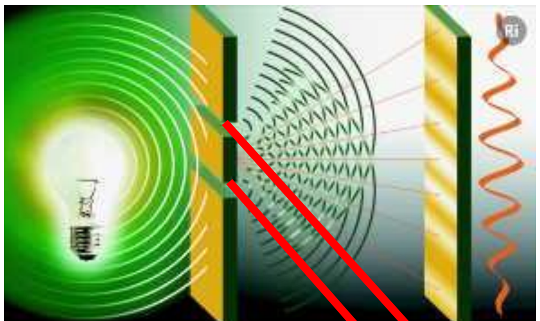
- Augustin-Jean **Fresnel** synthesized the work of Hyugens and Young.
- Championed the wave theory in his 1818 memoir on diffraction.
- All this finally replaced by Newton’s corpuscular (particle) theory .*

Augustin-Jean Fresnel
1788-1827

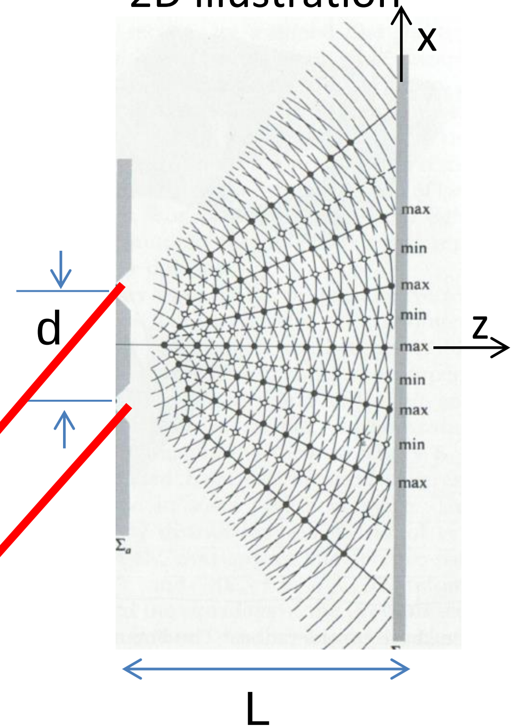
* Until the 20th century (quantum era)

Thomas Young's Double Slit Experiment

3D illustration



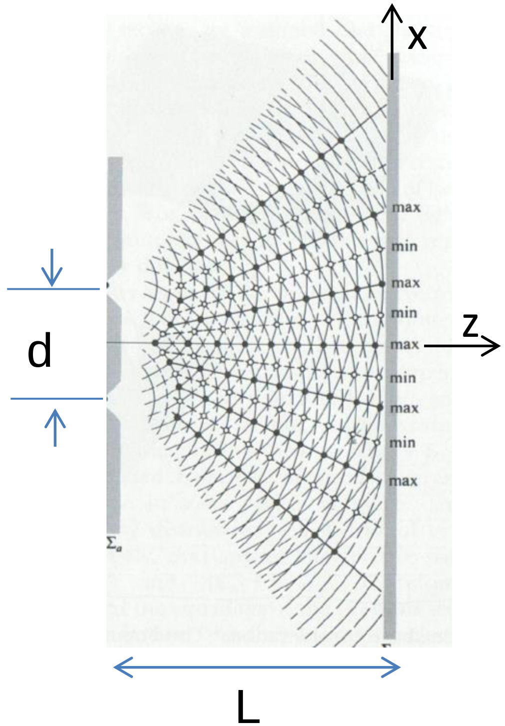
2D illustration

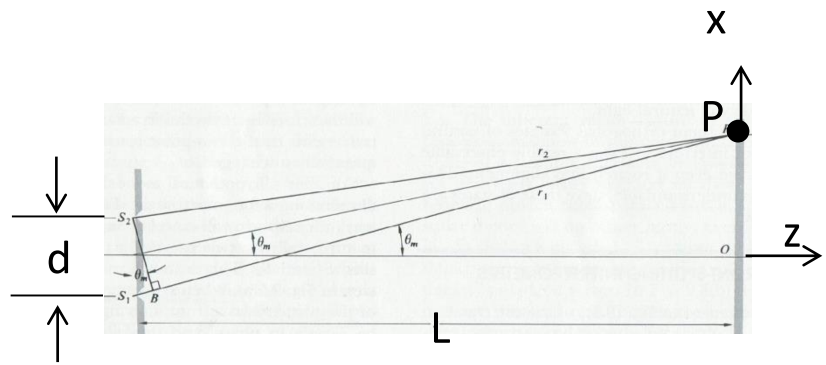


Consider two point sources at the two slit positions (in cross-section view), as shown above.

- 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





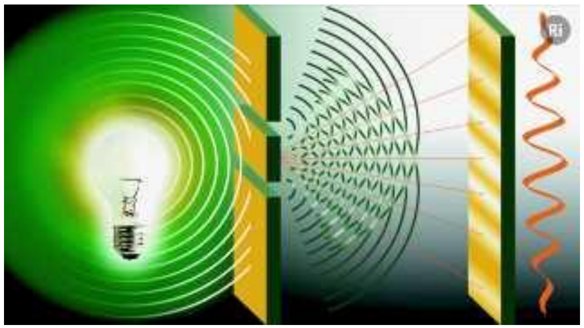
Consider two point sources at the two slit positions, as shown above.

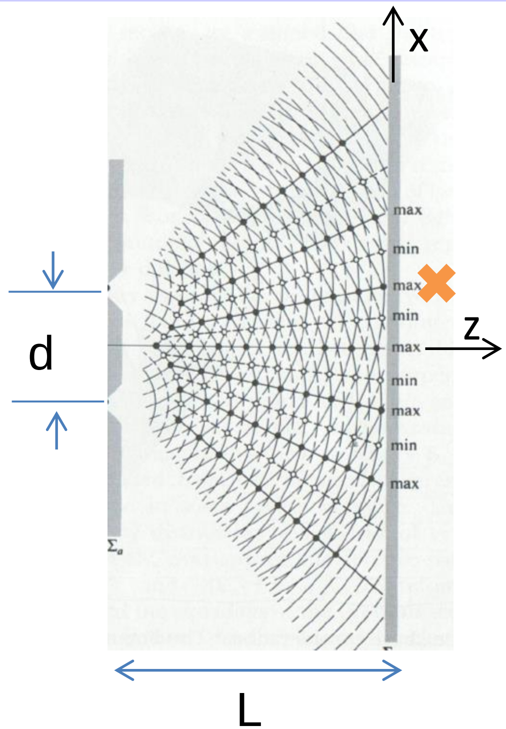
- Two sources emit waves with spherical wave-fronts.
- Let's put an observation screen **"L" away** from the double slit plane **at the z-axis**. Note that **L >> d,** λ
- **Along x-axis** of the observation screen (let's take **point P**, on the right figure), there will be a **phase difference (**φ**)** 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 a **sinusoidal intensity fluctuation in the x-axis**.
- This intensity pattern is governed by the following interference equation:

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

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

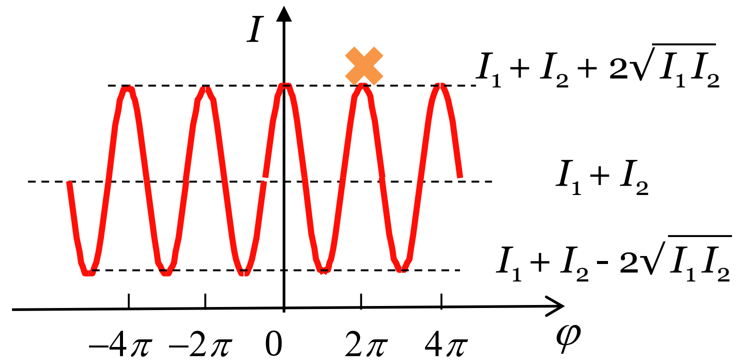
Thomas Young's Double Slit Experiment





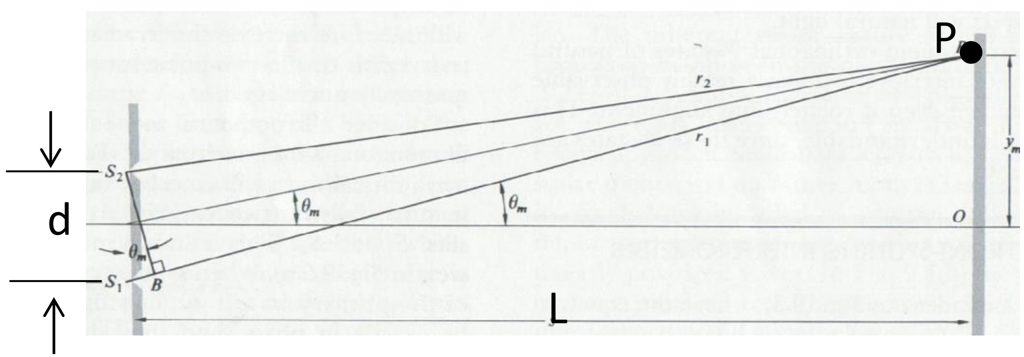
$$I = I_1 + I_2 + 2(I_1 I_2)^{1/2} \cos\varphi$$

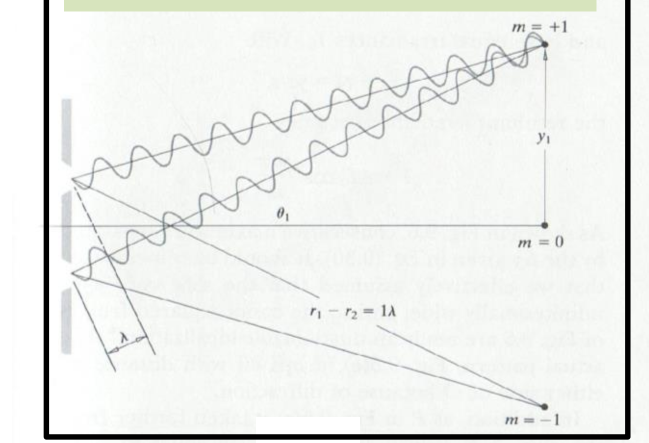
$$\varphi = \varphi_2 - \varphi_1$$



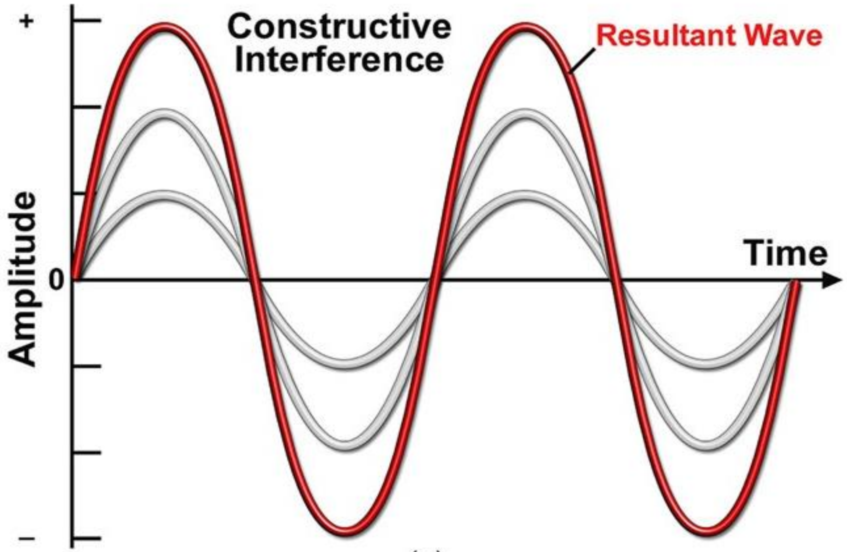
1st max (m=+1):
- **Phase Difference (φ) is $+2\pi$**
- **Path Difference (S_1B) is λ**

➔ Constructive Interference

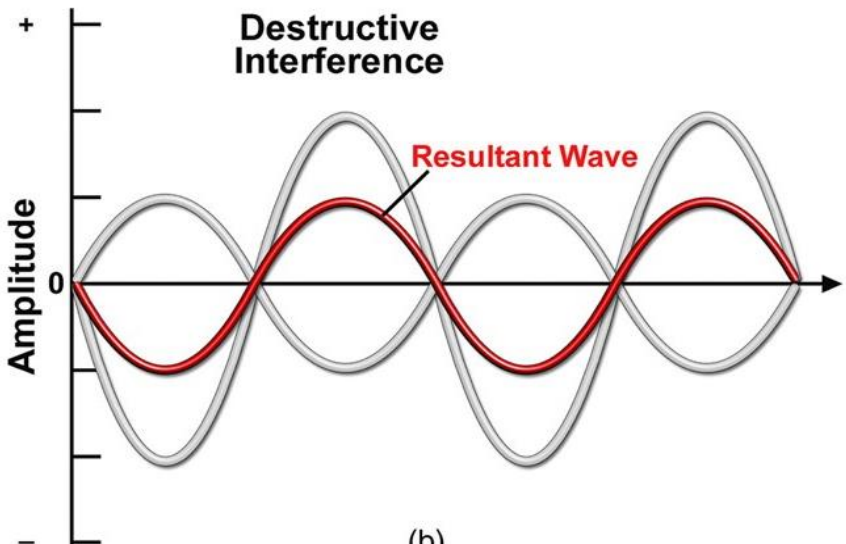




Waves interference



(a)



(b)

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

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

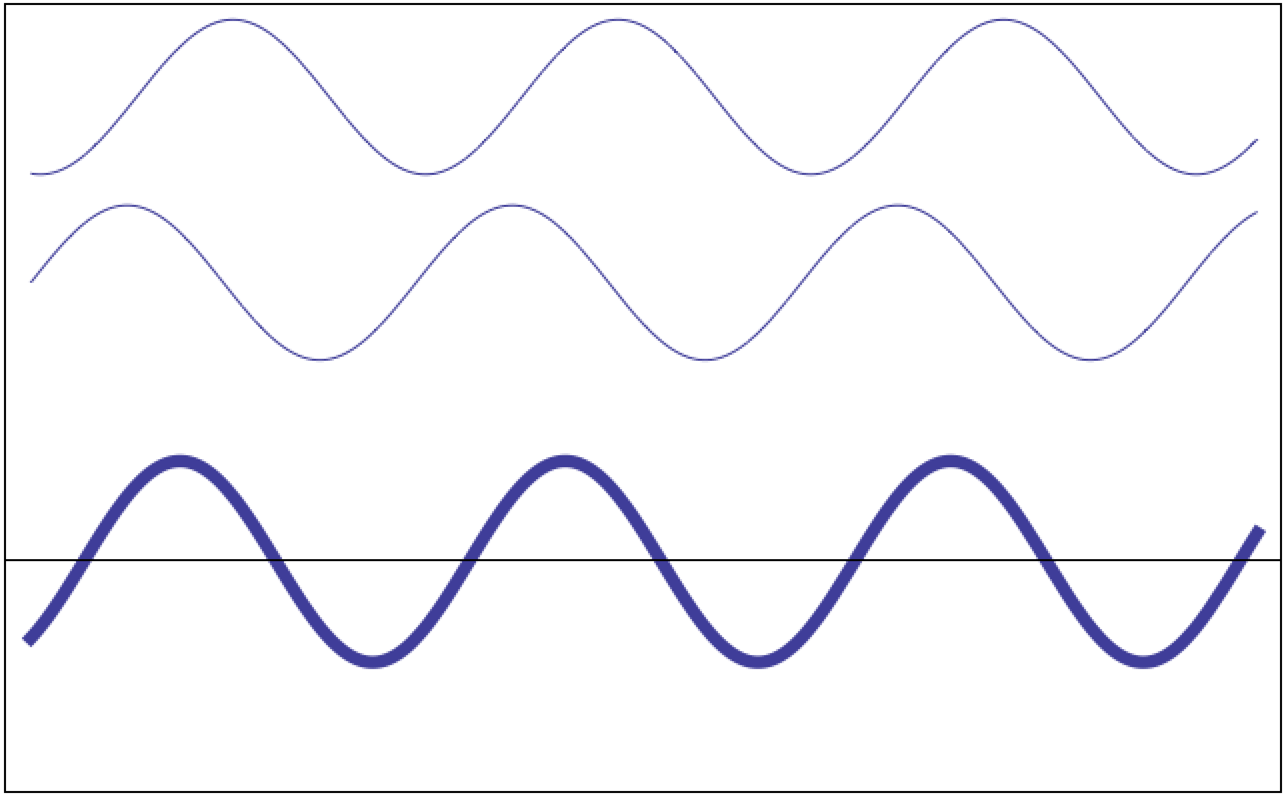
- The two waves (tinted gray) have the same wavelength, but vary in amplitude.
- The wave resulting from their interference is shown with red curve.

(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".

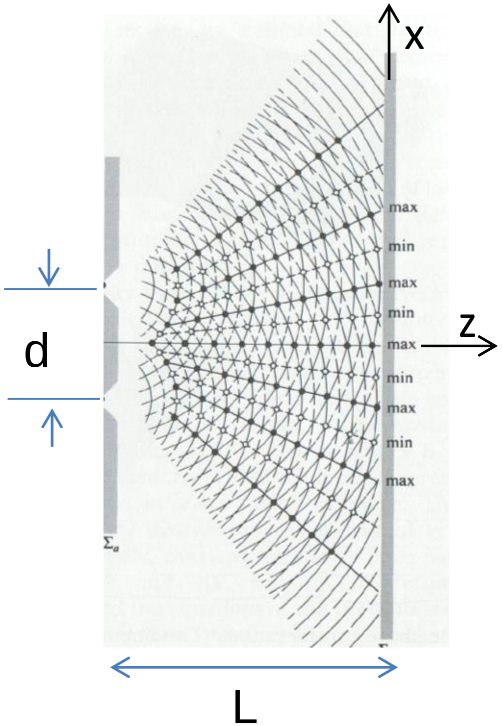
Waves Interference

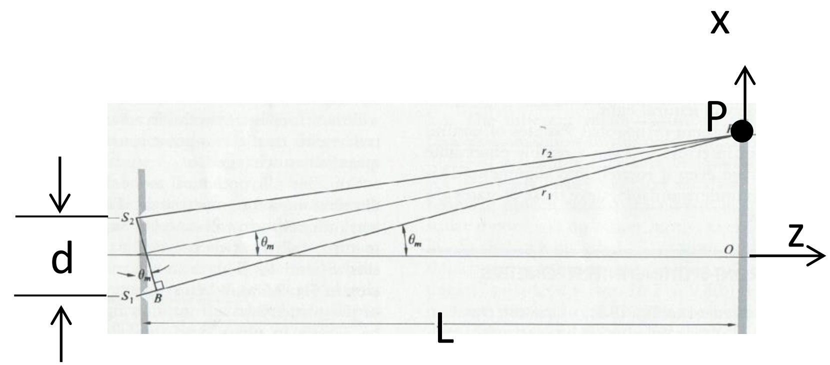
- Two waves (thin blue) are shown to oscillate in the plane of the page. They have same wavelength (frequency) and same amplitude.
- The wave resulting from their interference is shown with thick blue curve.



- **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





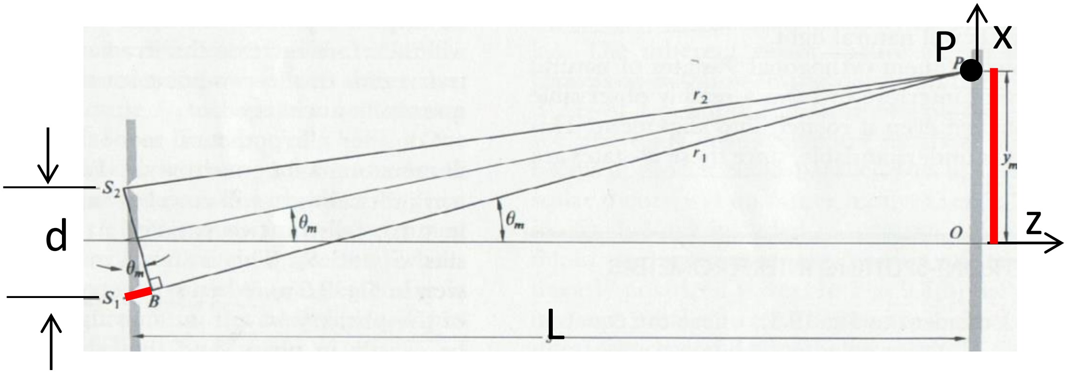
For two point sources at the two slit positions & observation plane “L” away from the slits:

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

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

- The 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



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

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

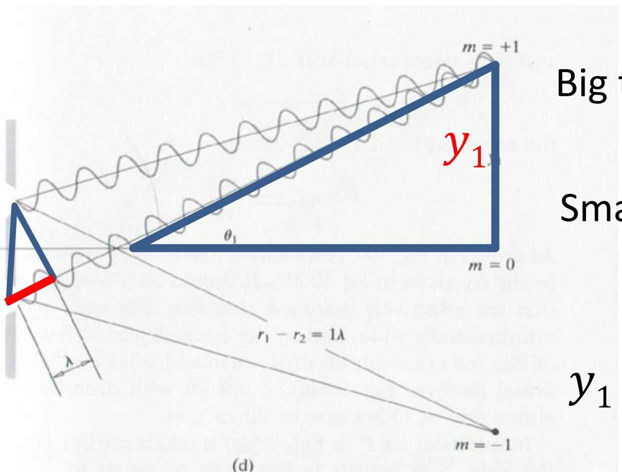
$$Phase\ difference \rightarrow \varphi = \varphi_2 - \varphi_1$$

$$Path\ difference \rightarrow |S_1B| = r_2 - r_1$$

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

For 1st max (y_1):

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



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

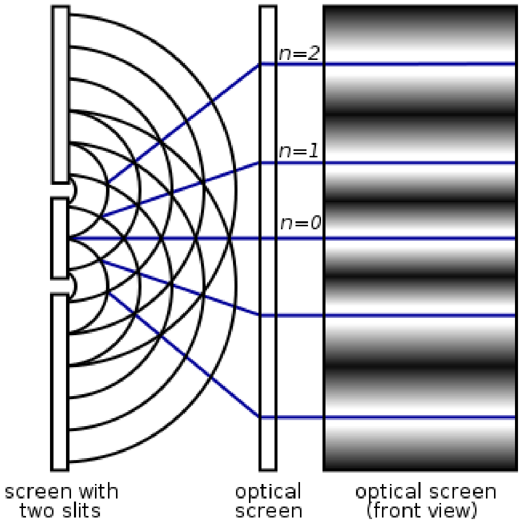
For mth max (y_m):

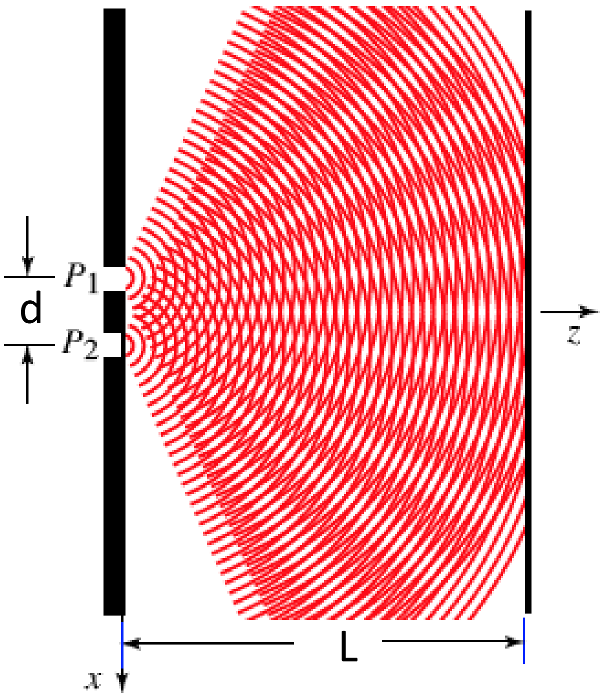
- **Path difference is m × λ**
- **Constructive Interference**

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

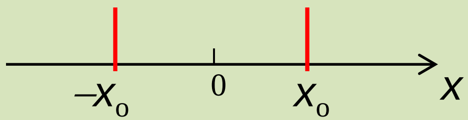
$$\lambda = \frac{y_m d}{m.L}$$

Double Slit (with no width) Experiment

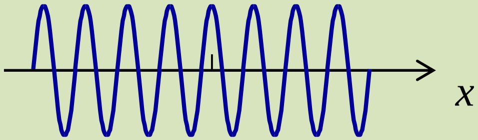




INPUT:



OUTPUT:

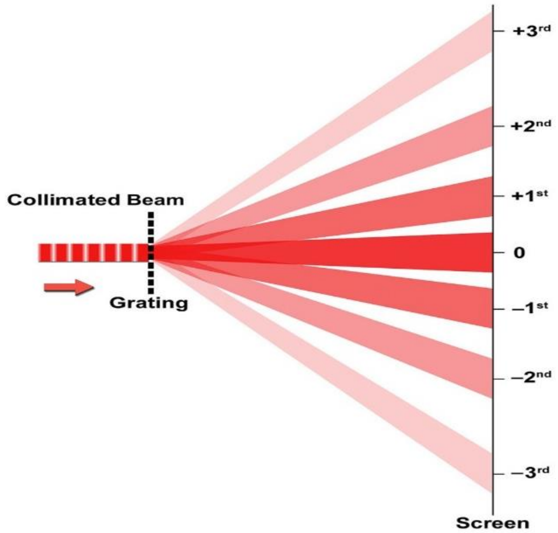


$\cos(2\pi\nu_x x)$

PS: Ignore the slit width

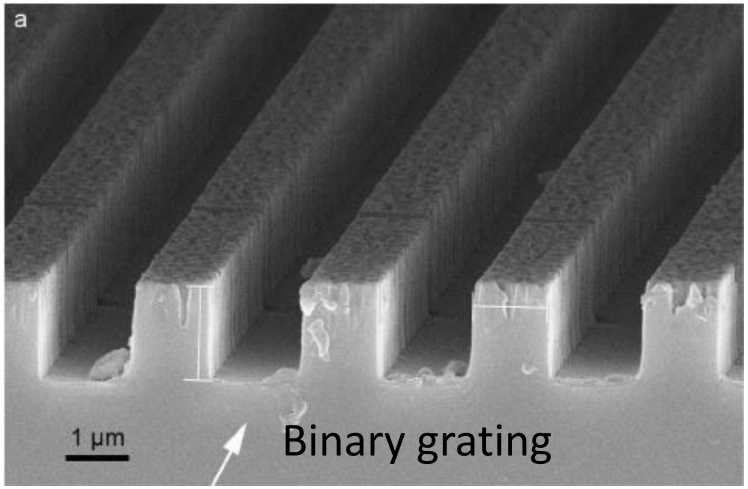
Diffraction by a **"grating"**

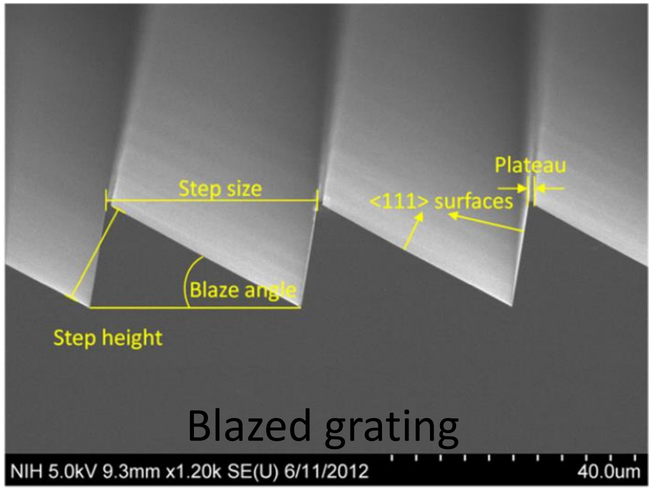
Diffraction grating is an optical element containing periodic groves/rulings.
The period or the feature size is comparable to ~λ.



- Illuminate with a collimated monochromatic beam

Examples:





Diffraction by a "**grating**"

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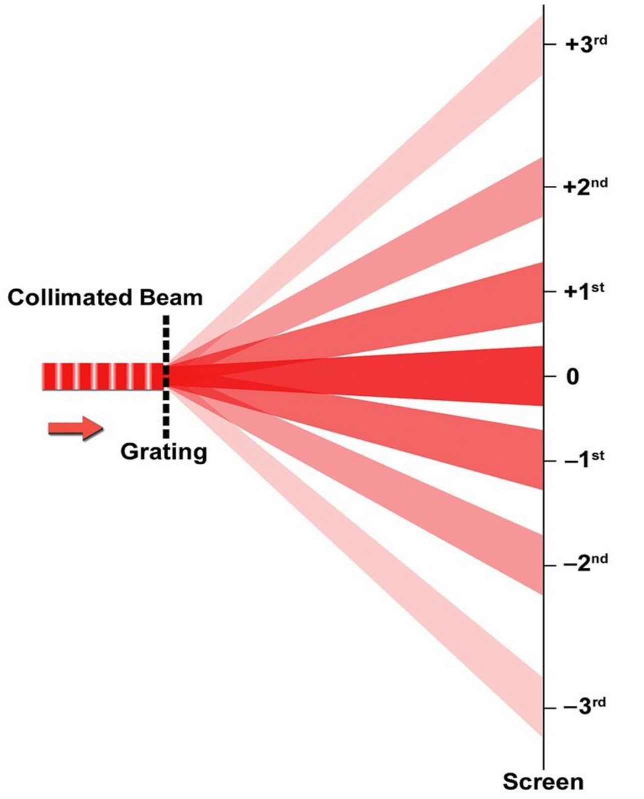


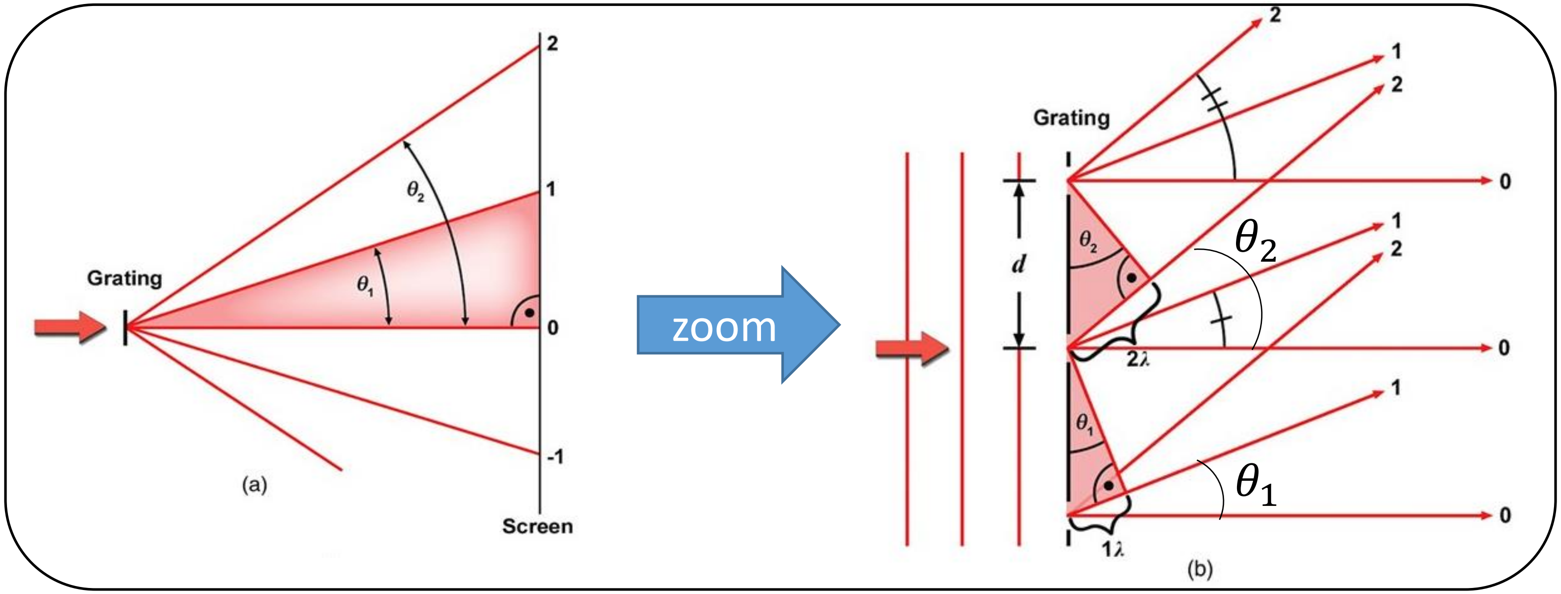
- Illuminate with a collimated monochromatic beam
- When we project the diffracted light on a screen at far distance, we obtain:

➔ A bright, central "0th" order spot

➔ And, higher order bright (1st, 2nd, 3rd, ..) spots

- **Diffraction spots** (a.k.a. diffraction **maxima**) identify **unique directions** (i.e. diffraction angles) along which the **waves** emitted from the grating are **in same phase.**
- These waves **interfere constructively** to form the bright **diffraction spots.**

Diffraction by a “grating”



1st order:

$$\sin\theta_1 = \frac{1.\lambda}{d}$$

2nd order:

$$\sin\theta_2 = \frac{2\lambda}{d}$$

generalize

mth order:

$$\sin\theta_m = \frac{m\lambda}{d}$$

Grating Equation:

$$\boldsymbol{d \sin\theta_m = m\,\lambda}$$

- **Diffraction spots** (a.k.a. diffraction **maxima**) identify **unique directions** (i.e. diffraction angles) along which the **waves** emitted from the grating are **in same phase.**
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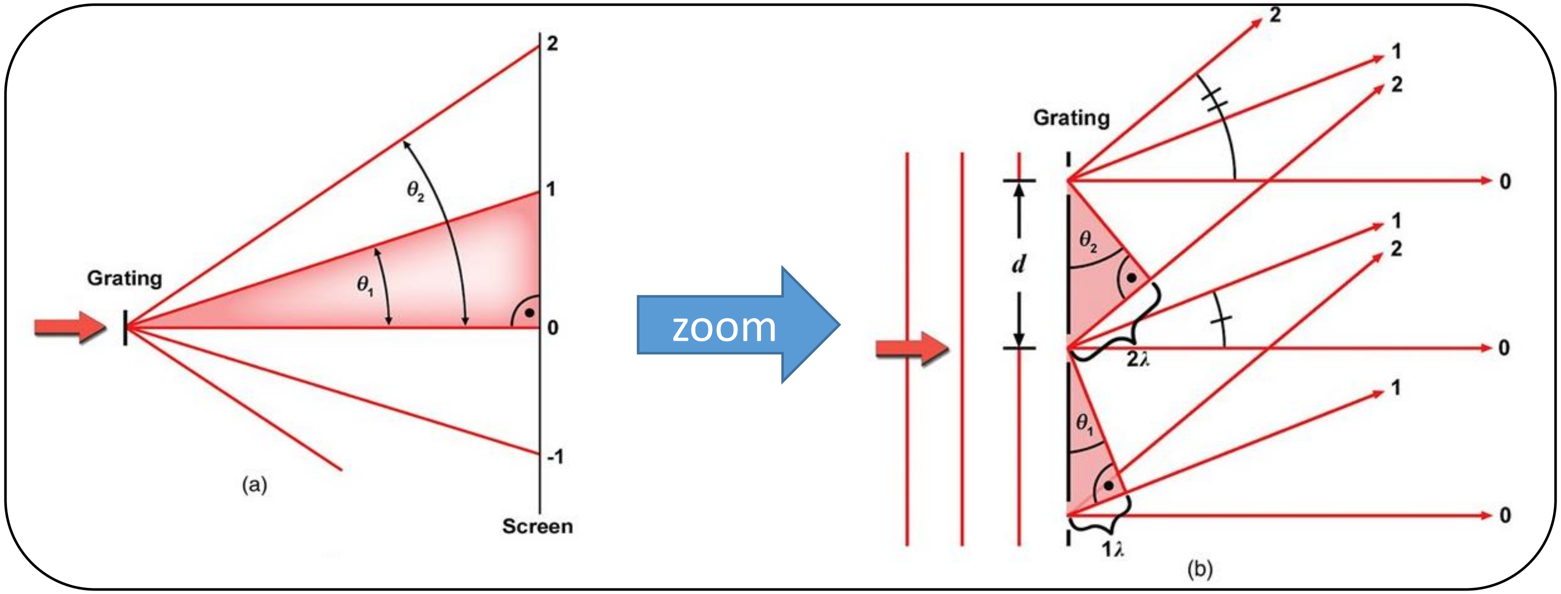
Dispersion effect of the "**grating**"



1st order:	2nd order:	generalize	mth order:
$sin\theta_1 = \frac{\lambda}{d}$	$sin\theta_2 = \frac{2\lambda}{d}$		$sin\theta_m = \frac{m\lambda}{d}$

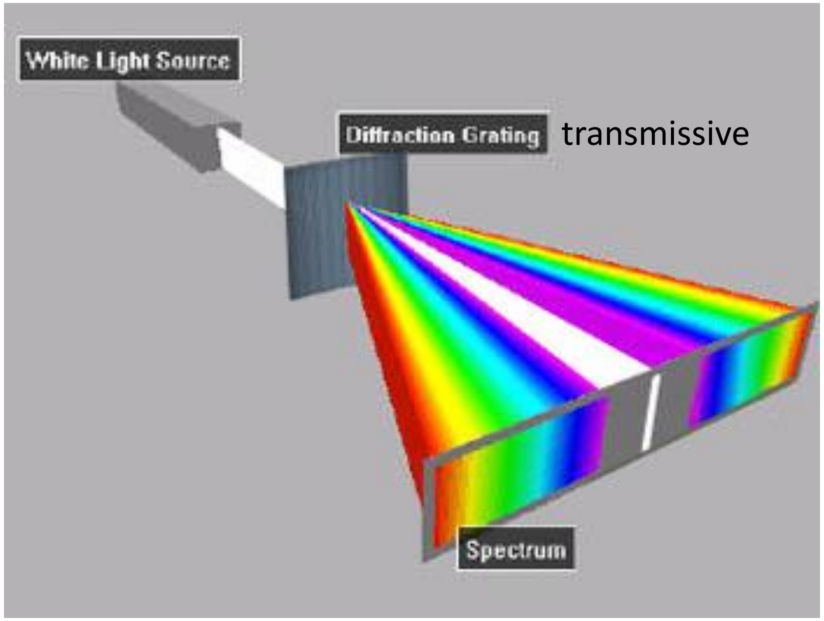
Grating Equation:

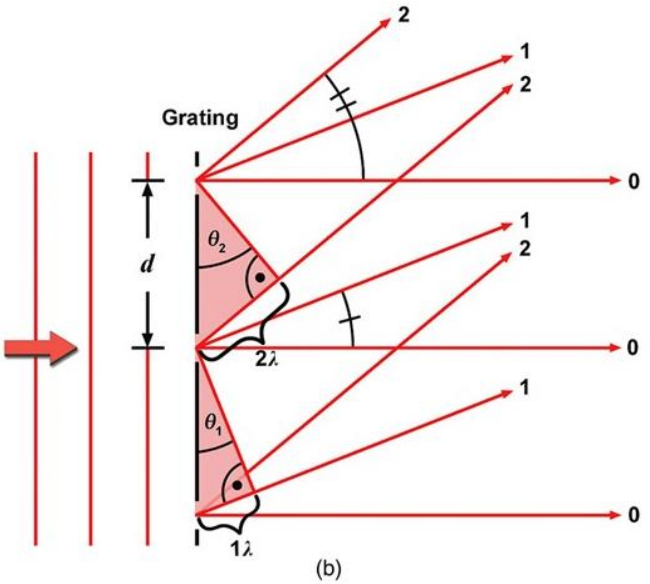
$$\boldsymbol{d\ sin\theta_m = m\,\lambda}$$

Diffraction angle θ increases as the wavelength λ increases

➔ A diffraction grating can be used to **disperse** the white light!

Dispersion by a diffraction **“grating”**





m^{th} order:

$$sin\theta_m = \frac{m\lambda}{d}$$

Grating Equation:

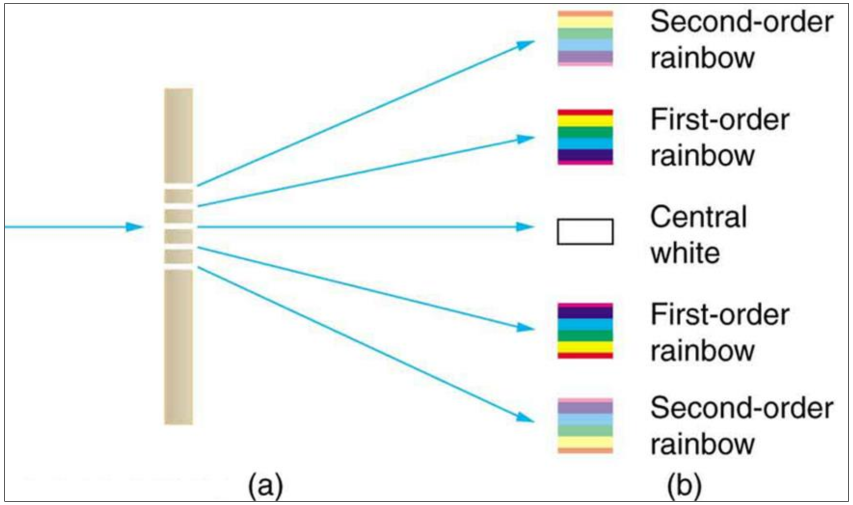
$$\boldsymbol{d\ sin\theta_m = m\ \lambda}$$

Diffraction angle θ increases as the wavelength λ increases

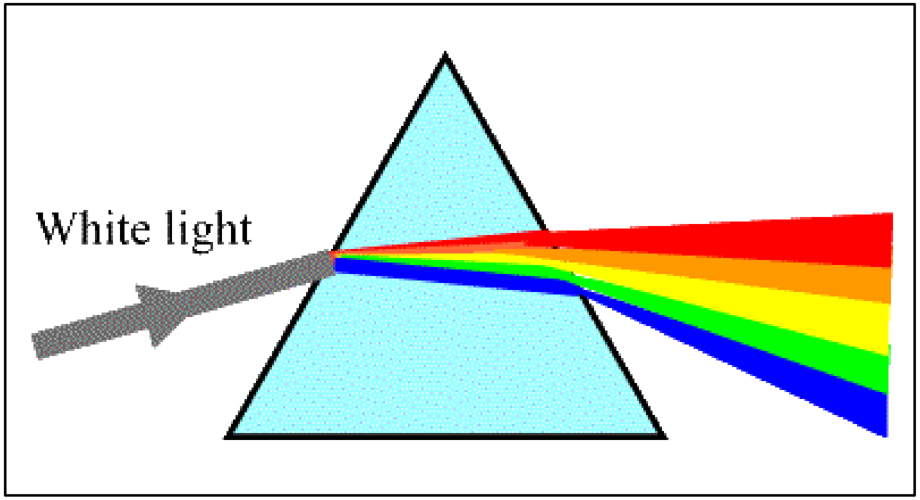
➔ A diffraction grating can be used to **disperse** the white light!

Dispersion

Dispersion by grading

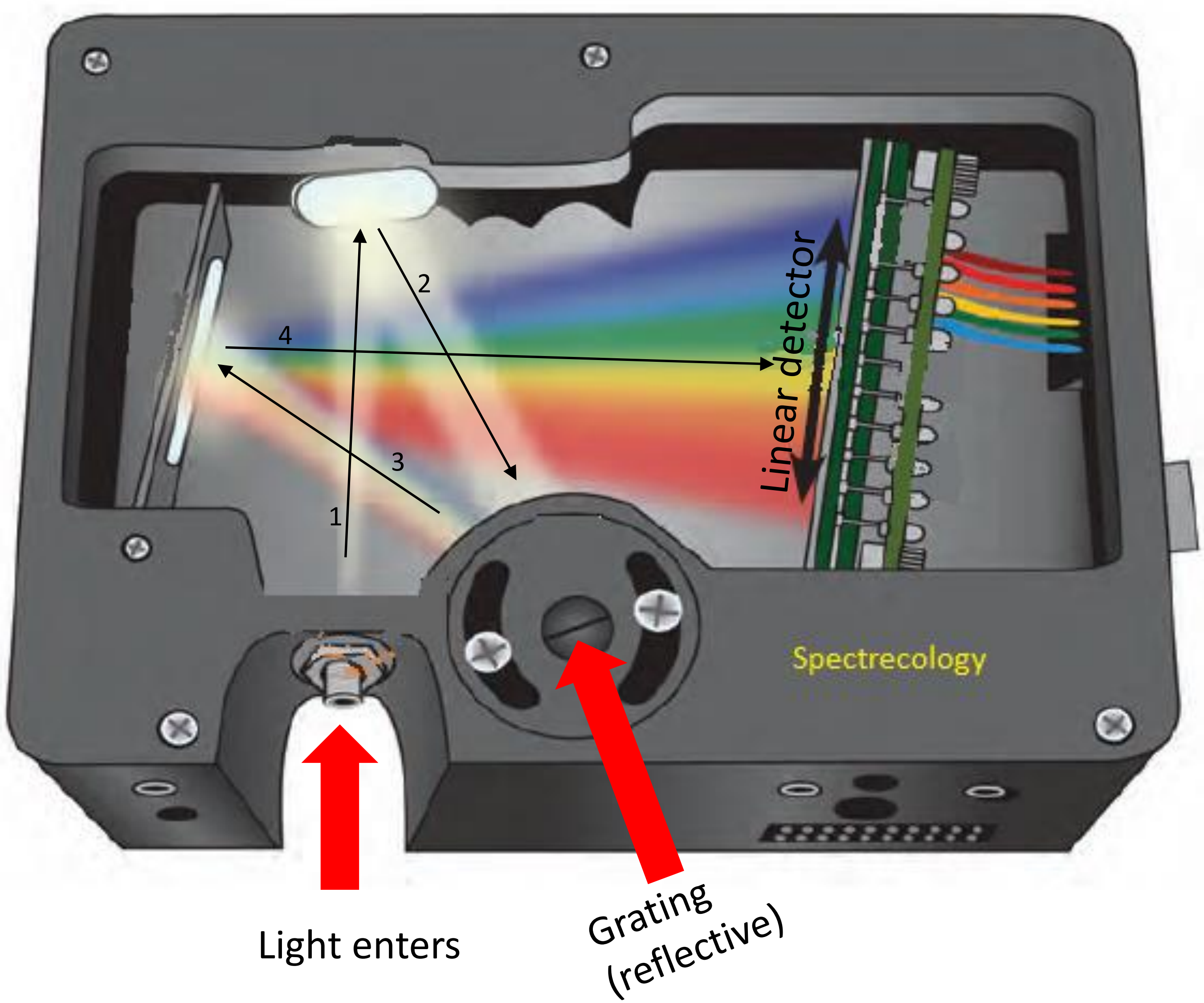


Dispersion by prism



What is the main difference in the diffraction response for these optical elements?

UV-Vis Spectroscopy to analyze samples



Dispersion by a diffraction **“grating”**

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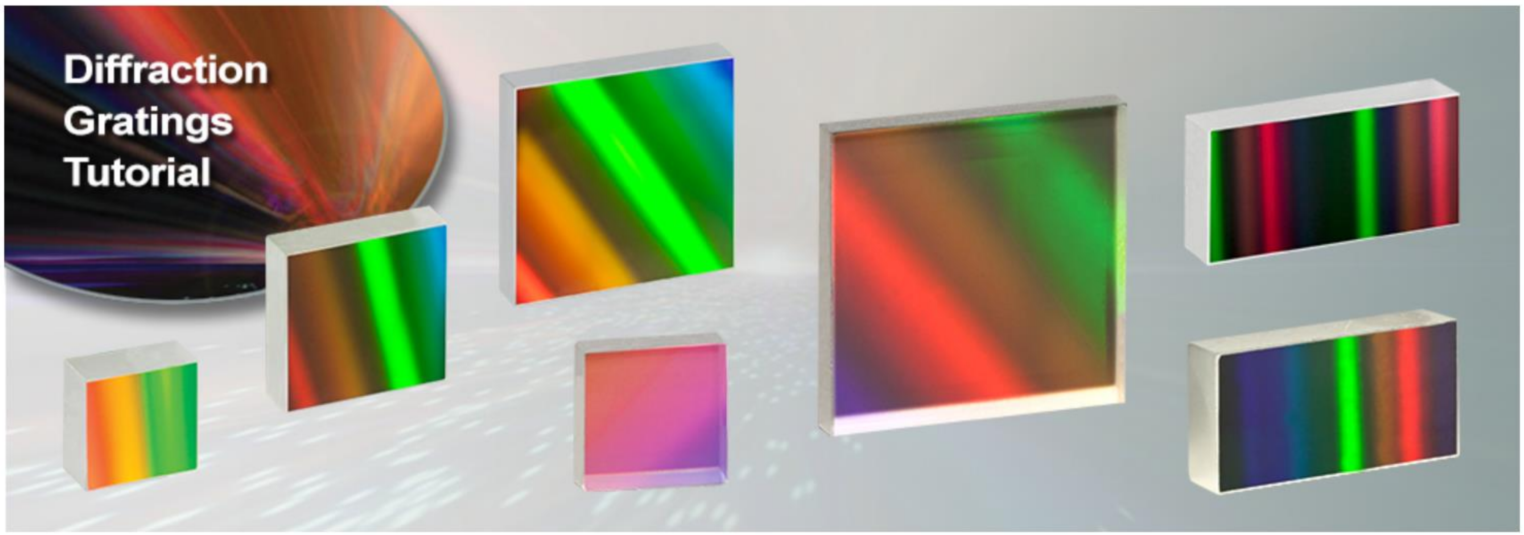
Diffraction Gratings Tutorial



Gratings Tutorial | Gratings Guide | Feedback

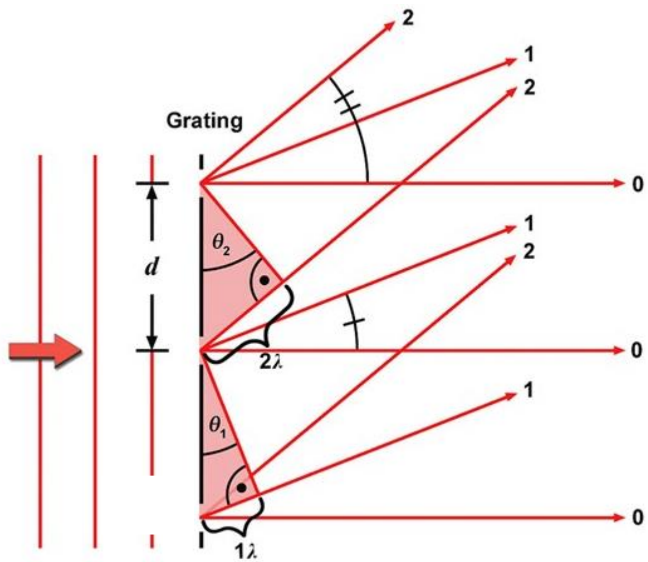
Diffraction Gratings Tutorial

Diffraction gratings, either transmissive or reflective, can separate different wavelengths of light using a repetitive structure embedded within the grating. The structure affects the amplitude and/or phase of the incident wave, causing interference in the output wave. In the transmissive case, the repetitive structure can be thought of as many tightly spaced, thin slits. Solving for the irradiance as a function wavelength and position of this multi-slit situation, we get a general expression that can be applied to all diffractive gratings when $\theta_i = 0°$,

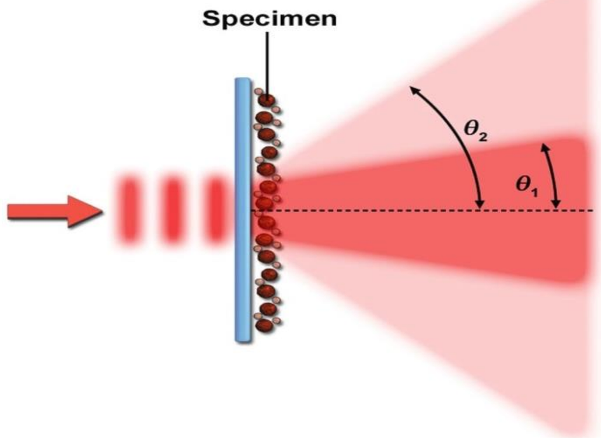
$$a \sin(\theta_m) = m\lambda \qquad (1)$$

Browse Our Selection of Diffraction Gratings

Diffraction by a "**grating**"



Reminder from previous lecture



mth order:

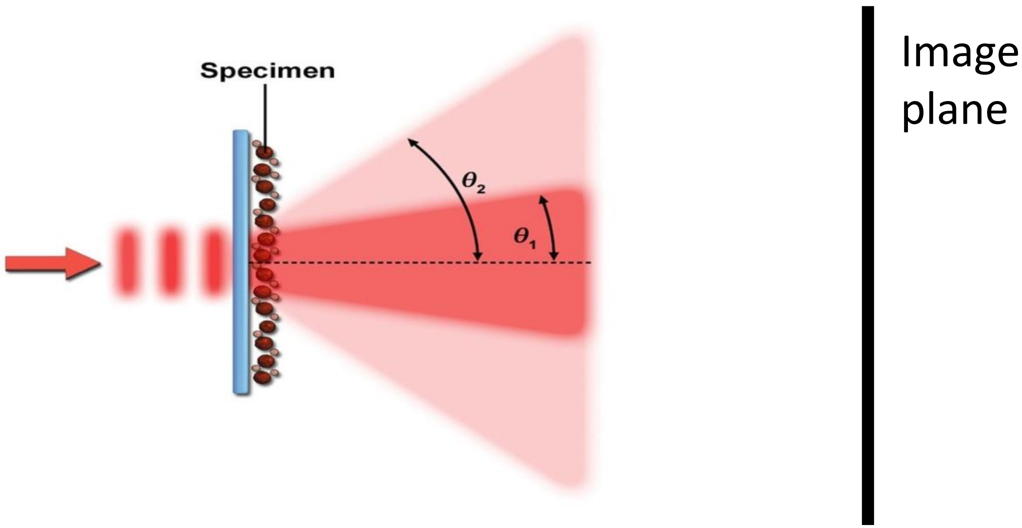
$$sin\theta_m = \frac{m\lambda}{d}$$

Grating Equation:

$$\boldsymbol{d\ sin\theta_m = m\,\lambda}$$

Diffraction angle θ increases as the period d decreases
➔ Smaller features diffract more!!

Reminder: Diffraction



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

- In the above drawing, assume a mixture of 0.2 & 2 um diameter particles.
  - The angle of spreading is inversely proportional to the particle size.
  - Larger angle (θ_2) corresponds to light diffraction by the smaller particles

Spatial Frequency & Angles

$$\sin\theta_m = \frac{m\lambda}{d} \xrightarrow{\text{under paraxial approximation}} \theta_m \approx \frac{m\lambda}{d}$$

$$For\ m = 1 \ \rightarrow \ \theta_1 \approx \lambda\frac{1}{d}$$

$$For\ m = 2 \ \rightarrow \ \theta_2 \approx \lambda\frac{2}{d}$$

.
.
.

$$For\ m = i \ \rightarrow \ \theta_i \approx \lambda\frac{i}{d}$$

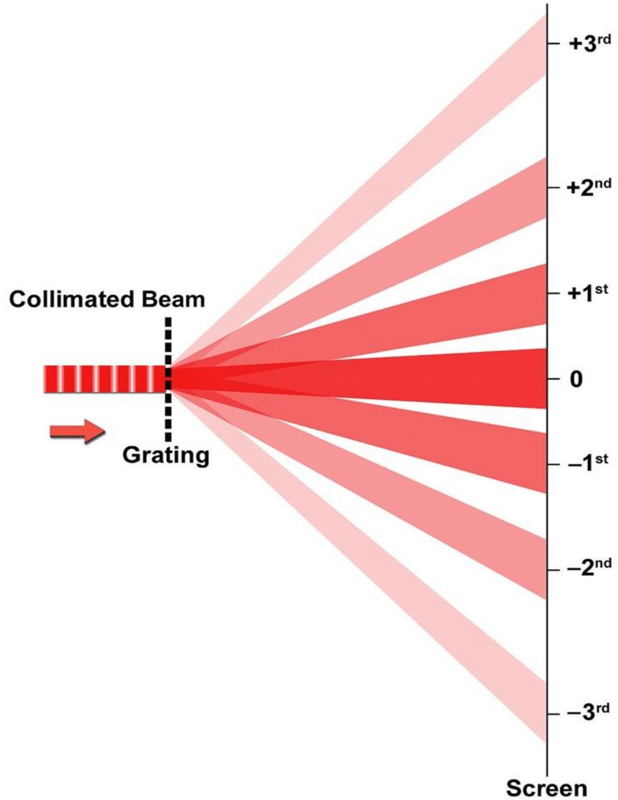
$$\theta_i \approx \lambda\nu_i$$

angle

spatial frequency

- "Larger angle" means "higher spatial frequency"
- "Higher frequency" corresponds to "smaller feature"

Summary: Diffraction by a "**grating**"



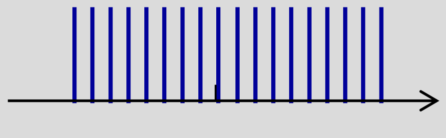
m^{th} order:

$$sin\theta_m = \frac{m\lambda}{d}$$

Grating Equation:

$$d\ sin\theta_m = m\ \lambda$$

INPUT:



OUTPUT: