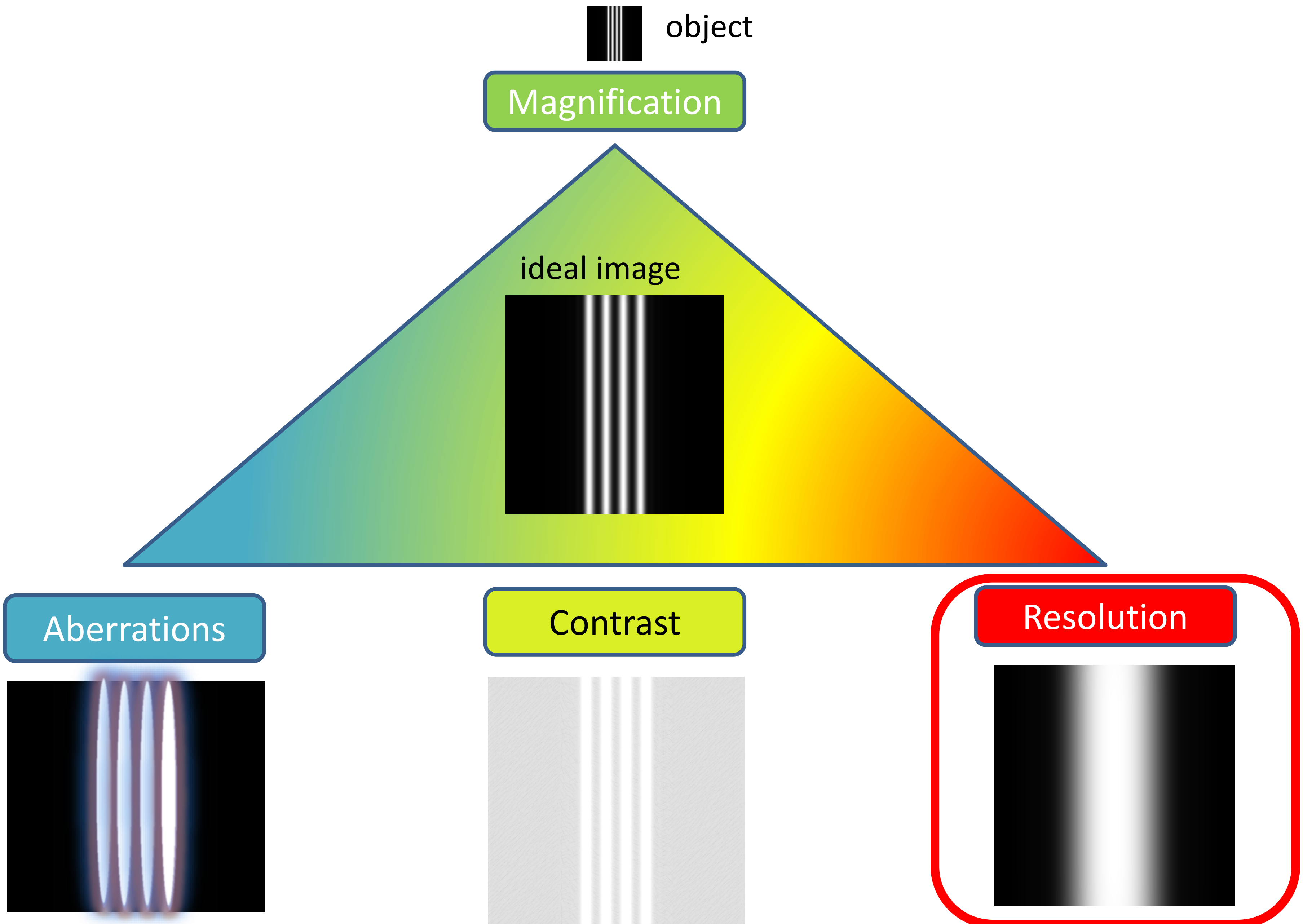
MICRO-561

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

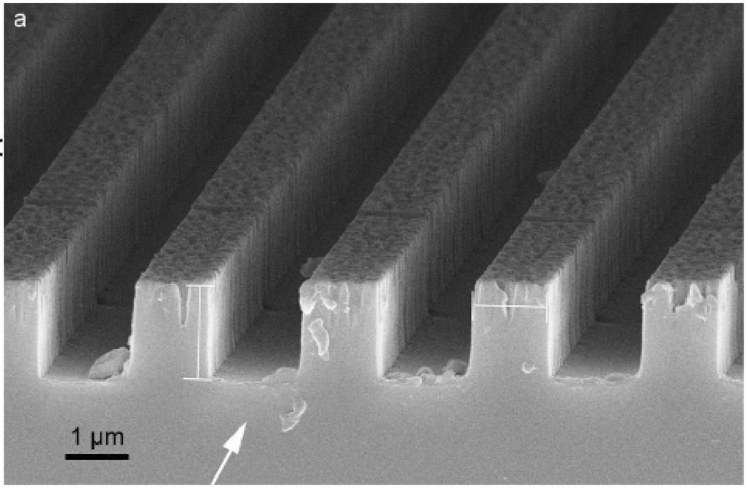
Important aspects for microscopy



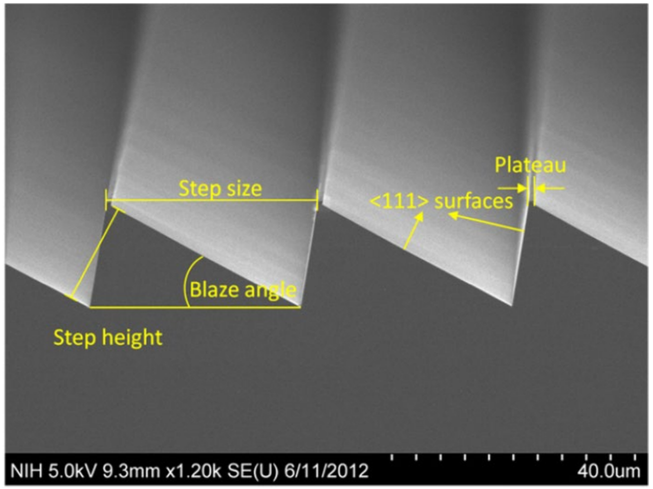
Diffraction by a **“grating”**

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

Examples:



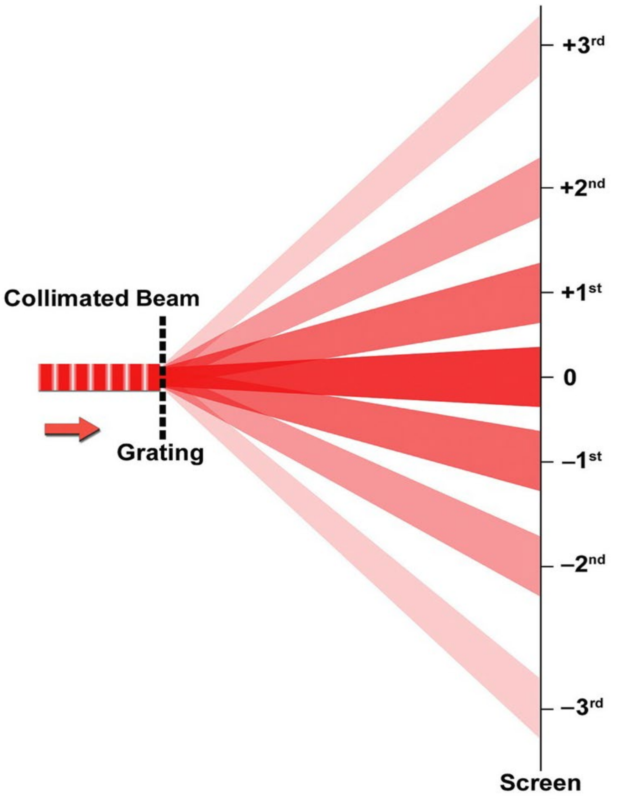
Binary grating



Blazed grating

Diffraction by a **"grating"**

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



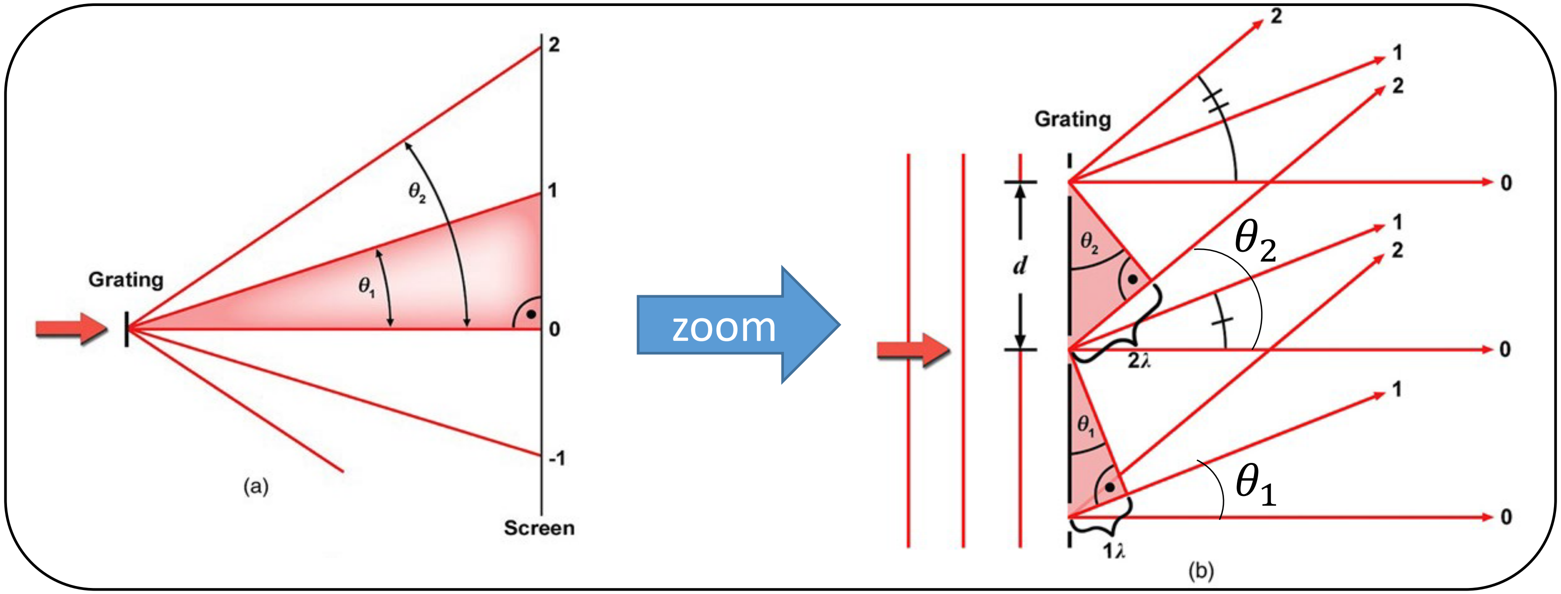
- Illuminate with a collimated monochromatic beam
- When we project the diffracted light on a screen at far distance, we obtain:

  ➔ A bright, central "0^{th}" order spot

  ➔ And, higher order bright (1^{st}, 2^{nd}, 3^{rd}, ..) 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:

$$sin\theta_1 = \frac{1.\,\square}{d}$$

2nd order:

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

generalize

mth order:

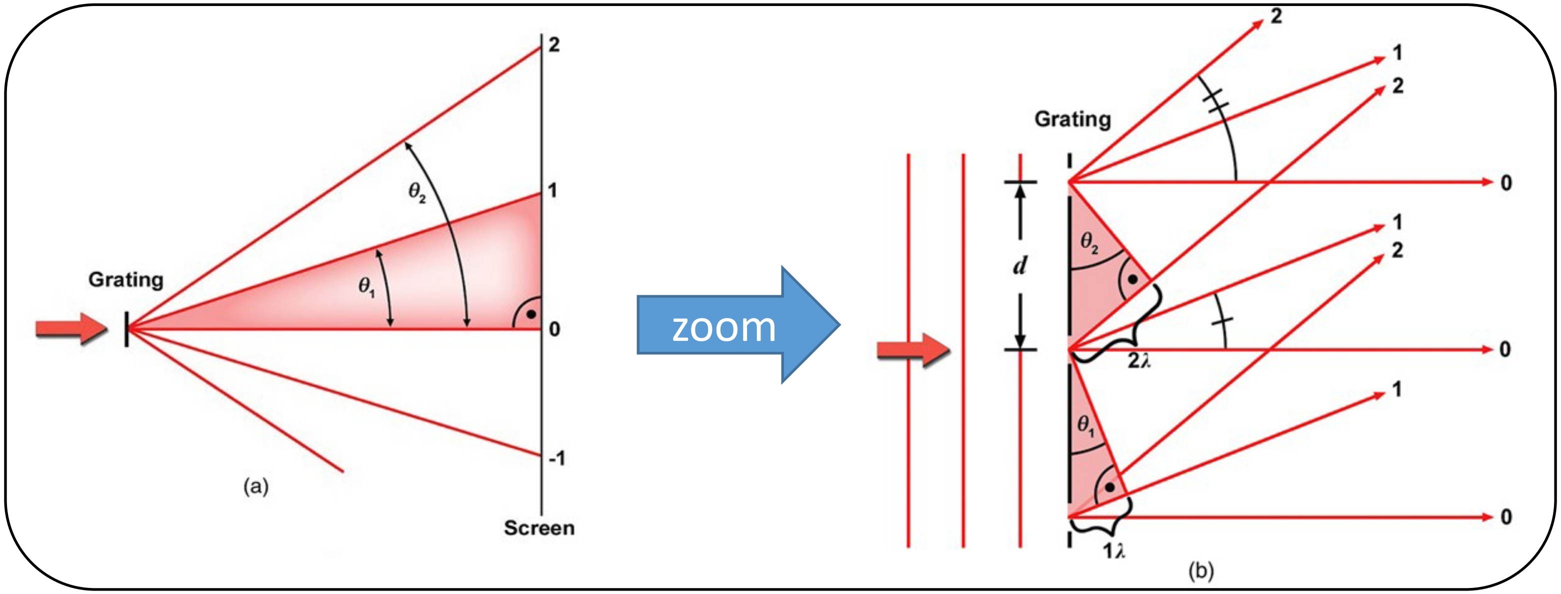
$$sin\theta_m = \frac{m\lambda}{d}$$

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.**
- These waves **interfere constructively** to form the bright **diffraction spots.**

Dispersion effect of the "**grating**"



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

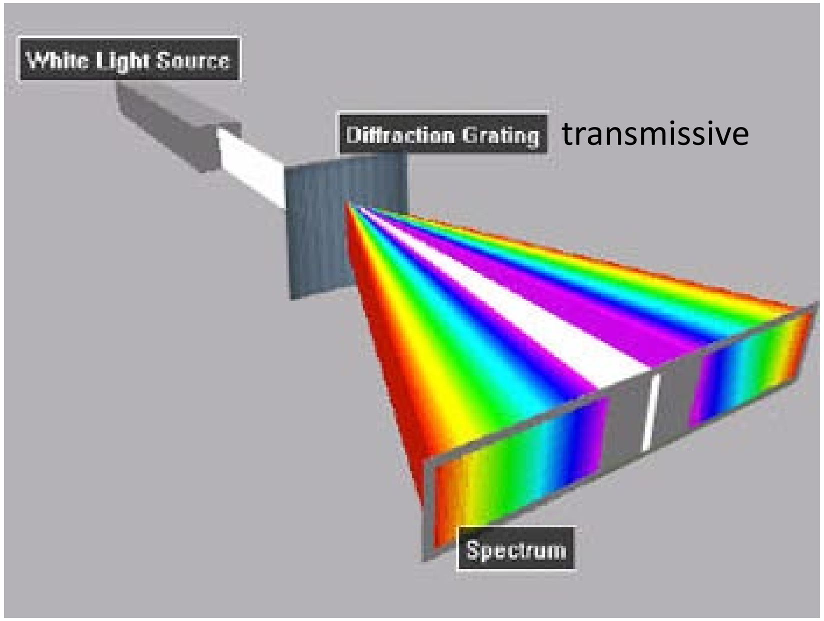
Grating Equation:

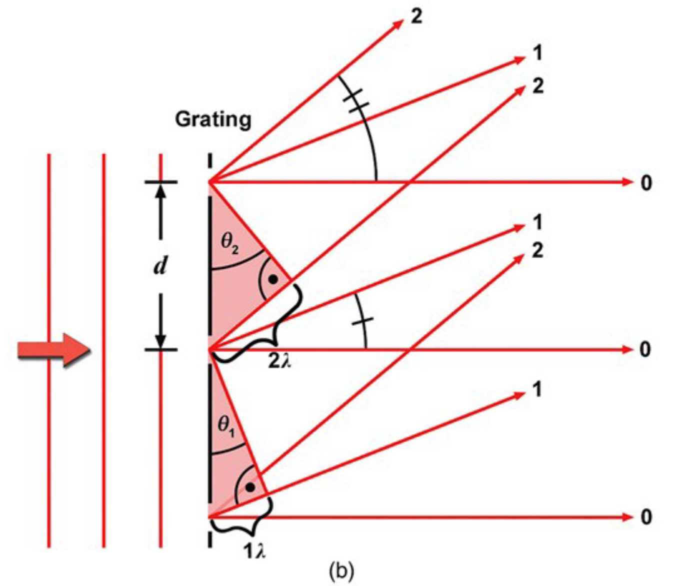
$$d\ sin\theta_m = m\,\lambda$$

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”**





mth order:

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

Grating Equation:

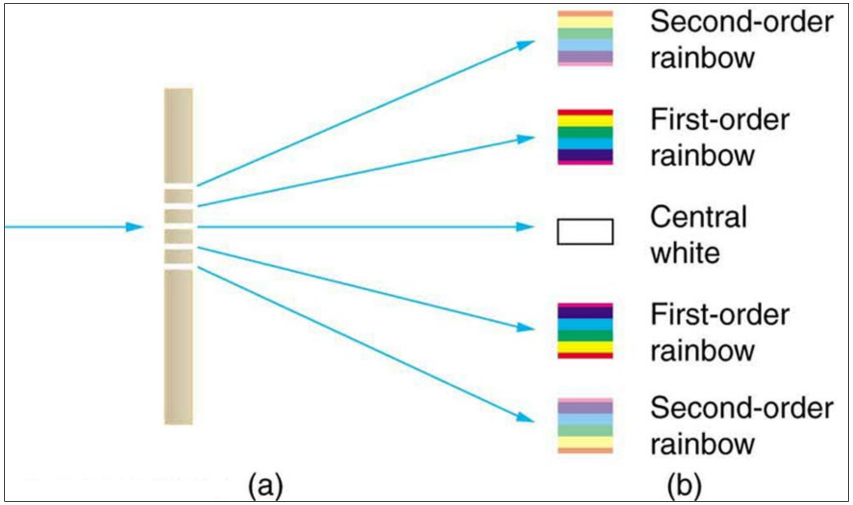
$$\boldsymbol{d\ sin\theta_m = m\,\lambda}$$

Diffraction angle θ increases as the wavelength λ increases

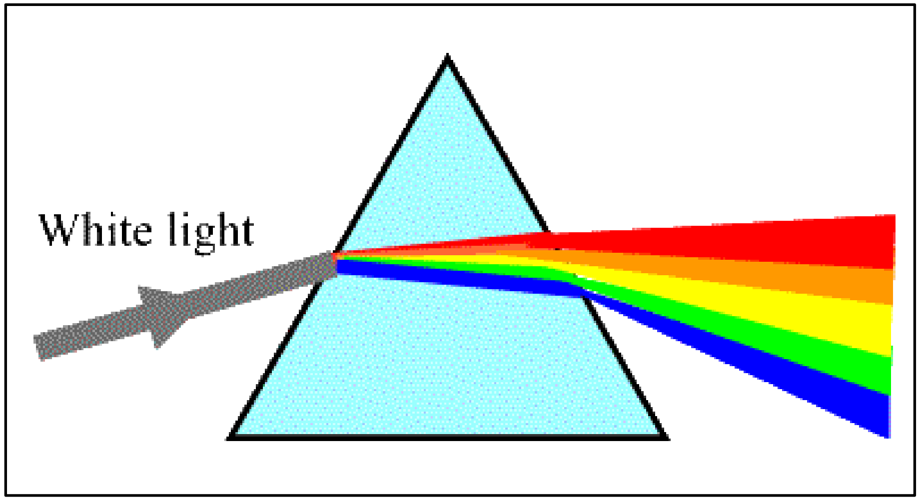
➔ A diffraction grating can be used to **disperse** the white light!

Dispersion by two components: grading and prism

Dispersion by grading

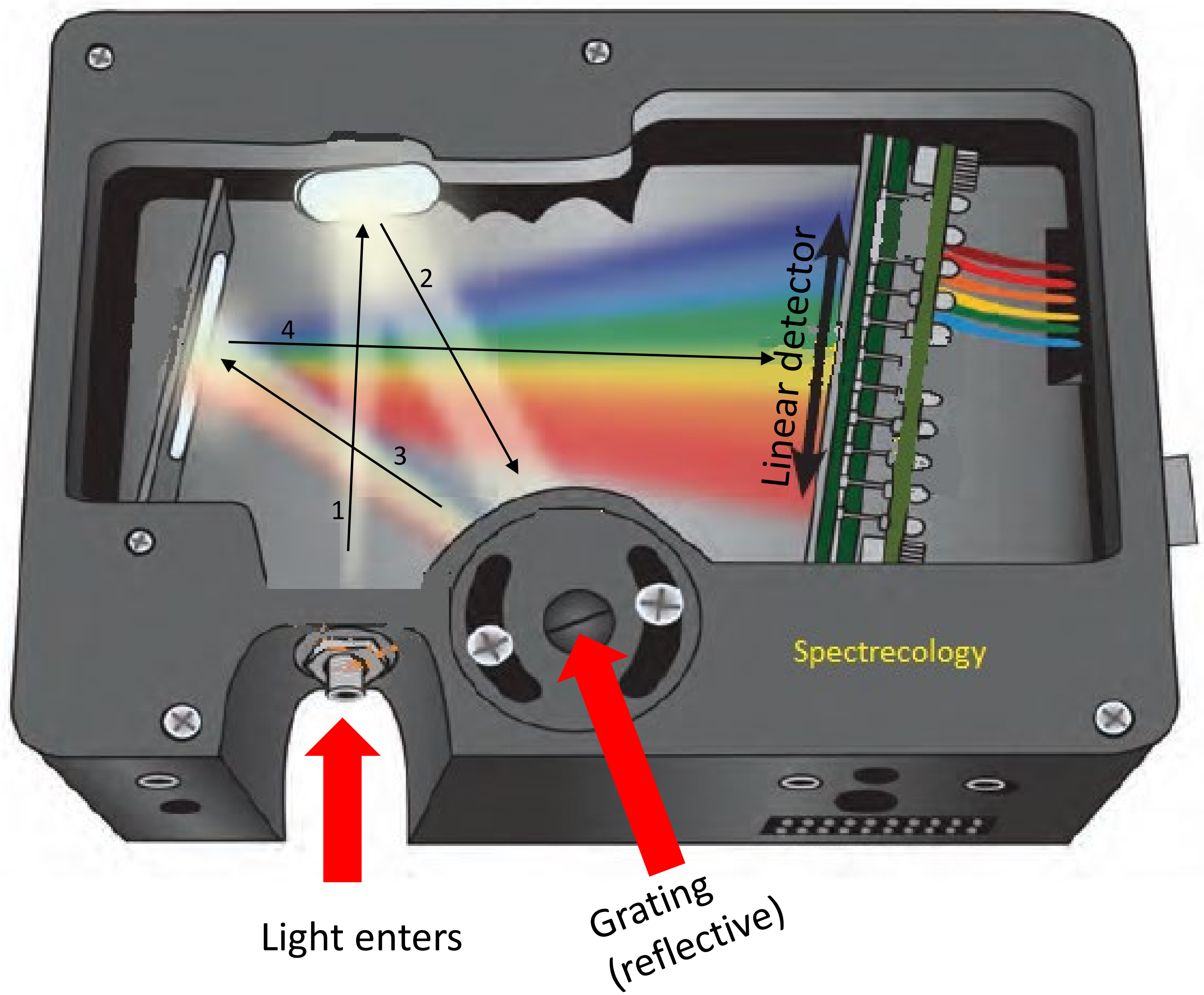


Dispersion by prism



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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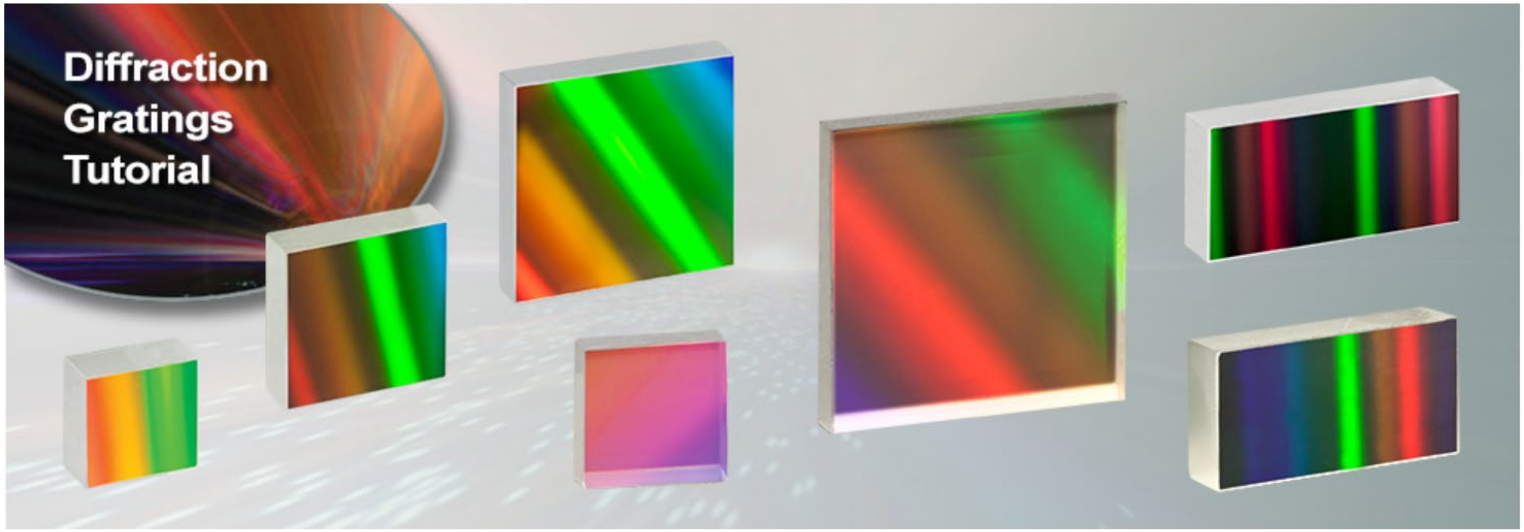
Diffraction Gratings Tutorial



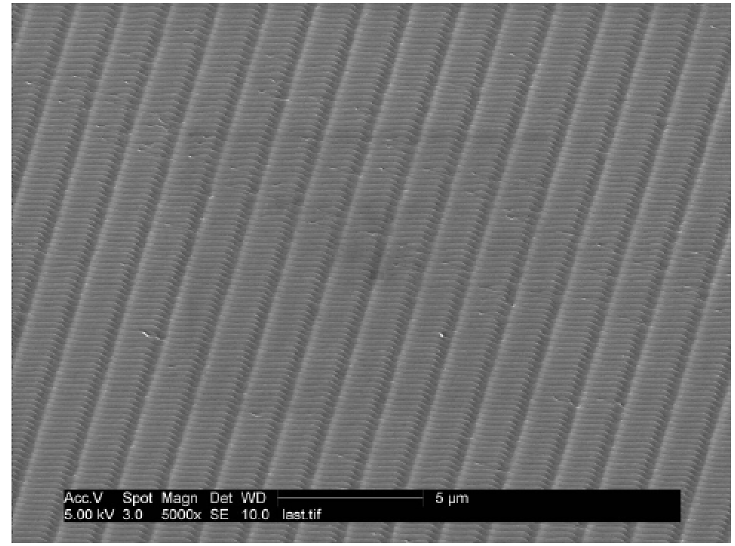
Gratings Tutorial | Gratings Guide | Feedback

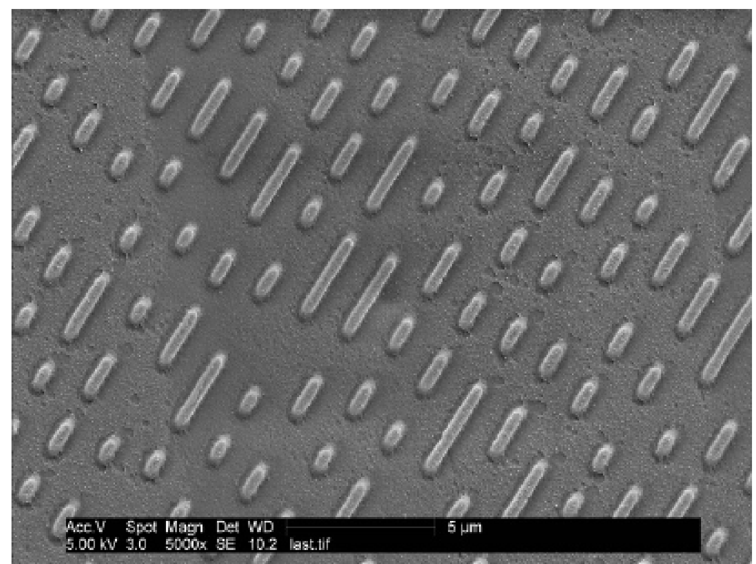
Diffraction Gratings Tutorial

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 $\theta_i = 0°$,

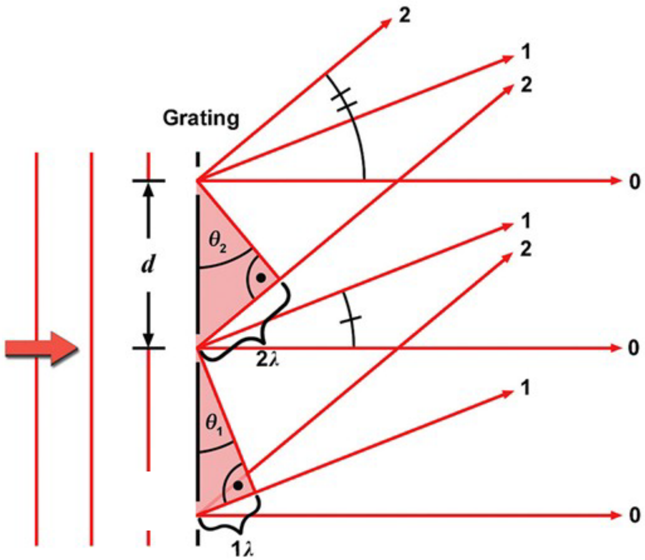
$$a \sin(\theta_m) = m\lambda \qquad (1)$$

Browse Our Selection of Diffraction Gratings

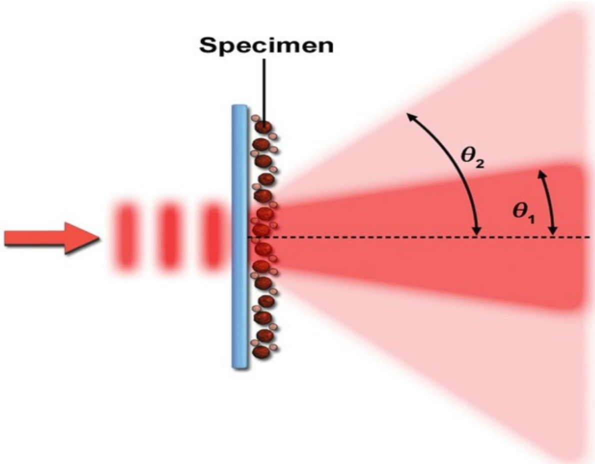

Acc.V Spot Magn Det WD 5 µm
5.00 kV 3.0 5000x SE 10.0 last.tif


Acc.V Spot Magn Det WD 5 µm
5.00 kV 3.0 5000x SE 10.2 last.tif

Diffraction by a **"grating"**



Reminder from previous lecture



mth order:

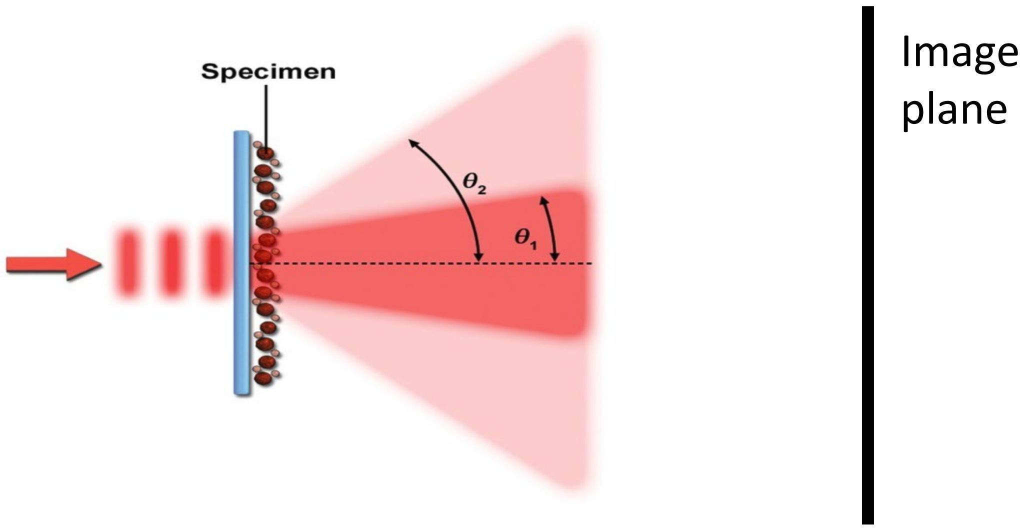
$$sin\theta_m = \frac{m\lambda}{d}$$

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

under paraxial approximation

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

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

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

.

.

.

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

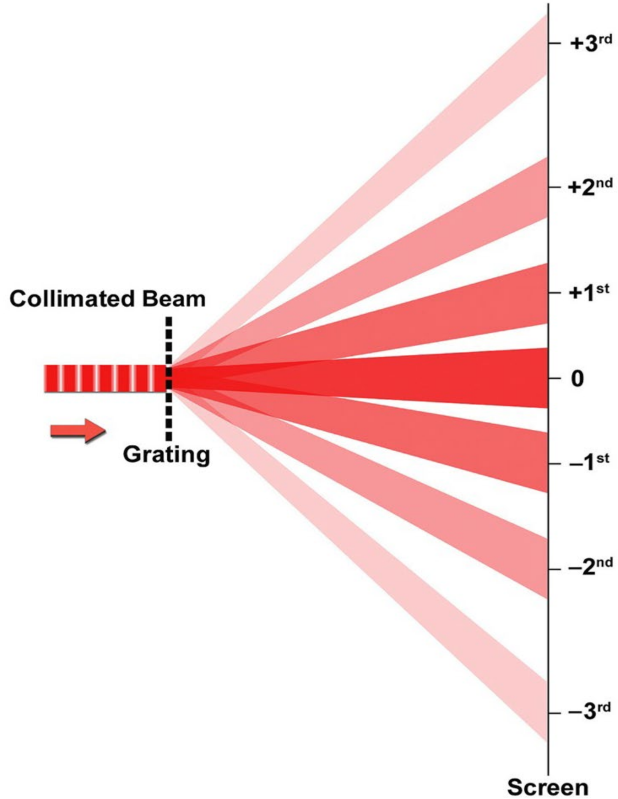
$$\theta_i \approx \lambda\nu_i$$

angle (θ_i)

spatial frequency (ν_i)

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

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



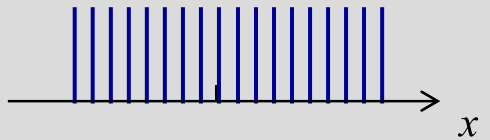
m^{th} order:

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

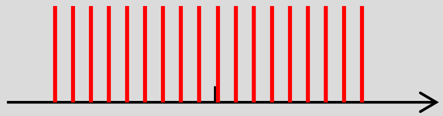
Grating Equation:

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

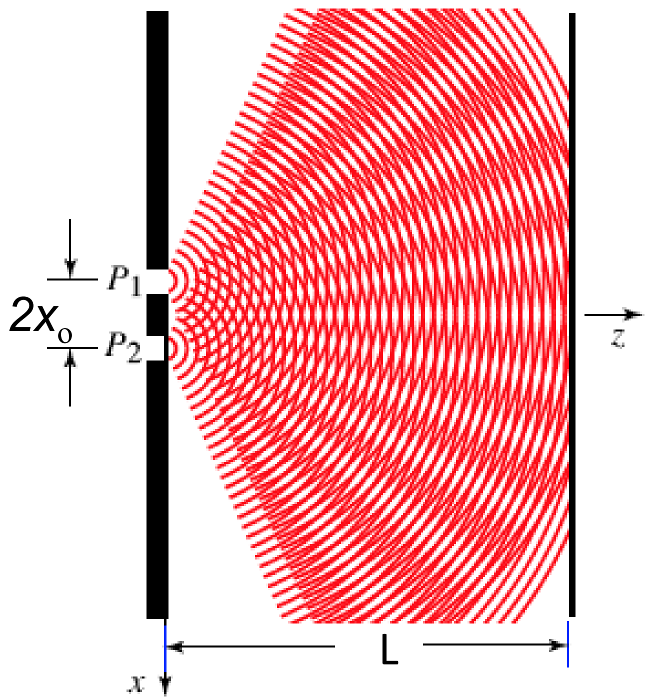
INPUT:



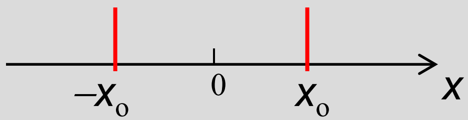
OUTPUT:



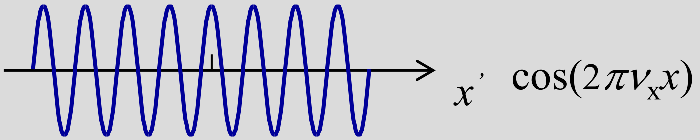
Reminder: Double Slit (with no width) Experiment



INPUT:

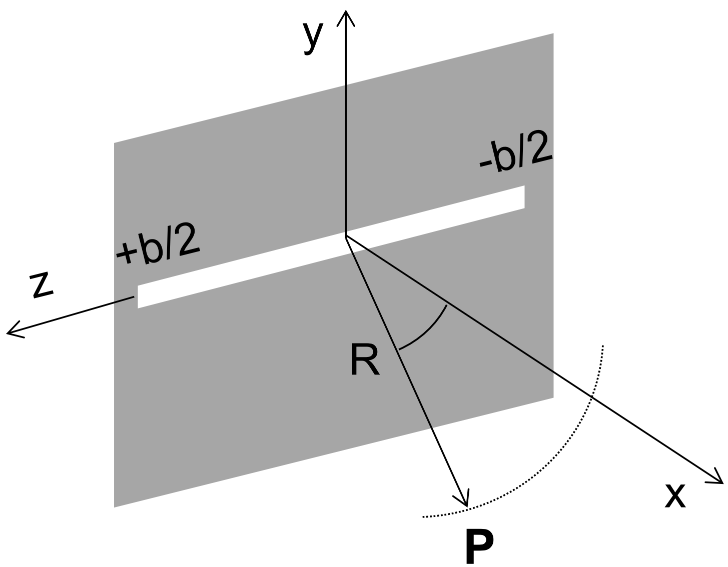


OUTPUT:

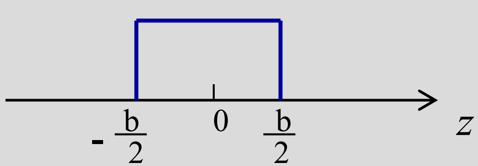


PS: Ignore slit width

Diffraction of a 1D single slit(*aperture) with a width* of "*b*"



INPUT: $\mathrm{rect}(z/b)$



$\mathrm{sinc}(\beta)$

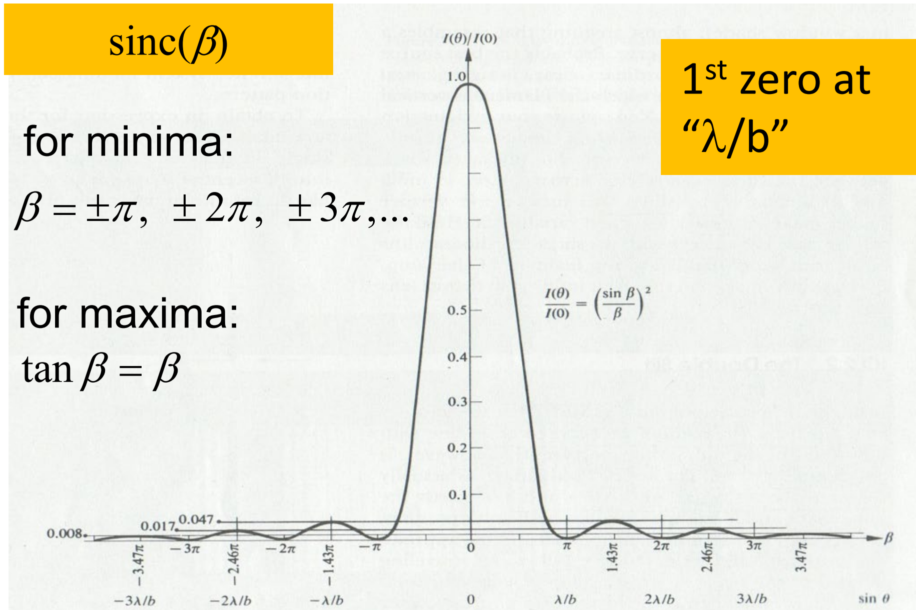
1st zero at "λ/b"

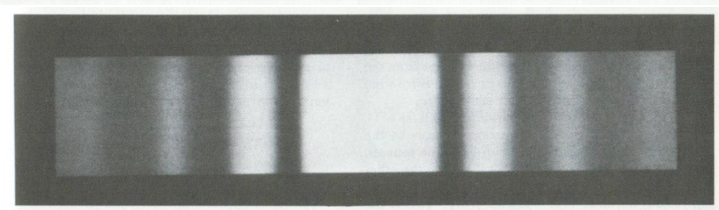
for minima:

$\beta = \pm\pi,\ \pm 2\pi,\ \pm 3\pi, \ldots$

for maxima:

$\tan\beta = \beta$





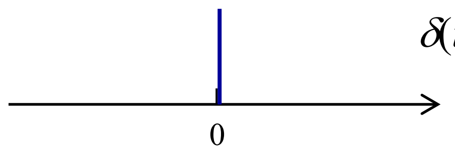
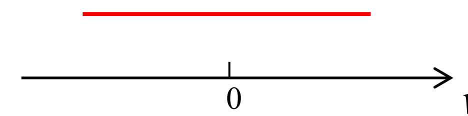
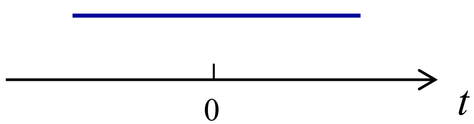
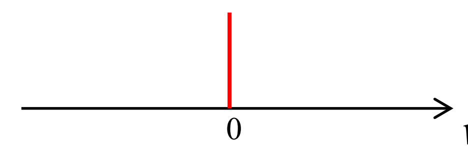
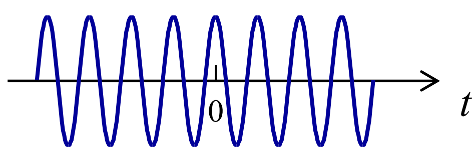
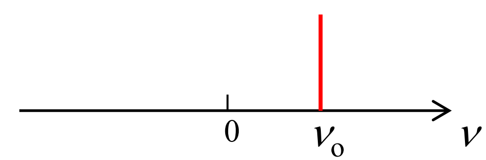
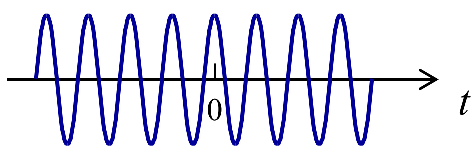
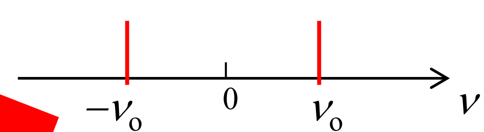
OUTPUT:

$$I(\theta) = I(0)\left(\frac{\sin\beta}{\beta}\right)^2$$

$$\beta = (kb/2)\sin\theta$$

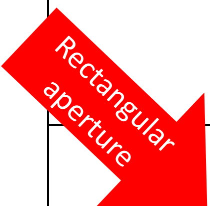
http://micro.magnet.fsu.edu/primer/java/diffraction/basicdiffraction/index.html

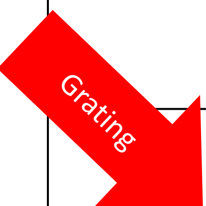
Fourier Transform Pairs - Basic Functions

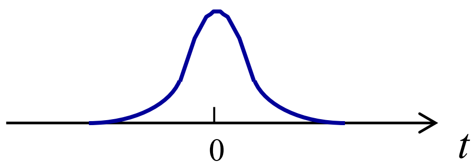
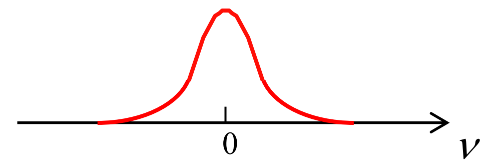
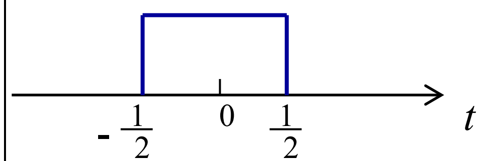
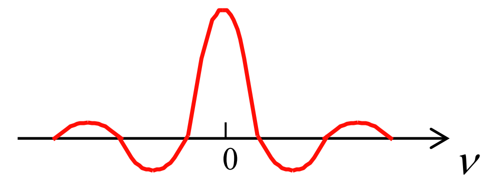
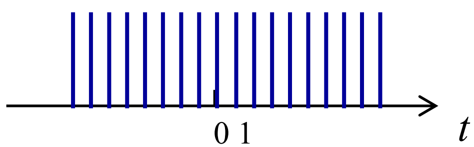
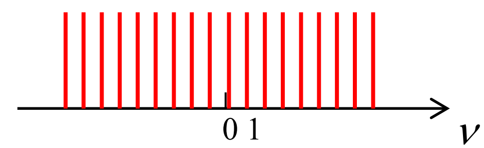
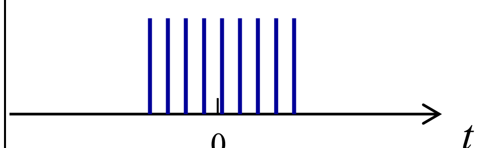
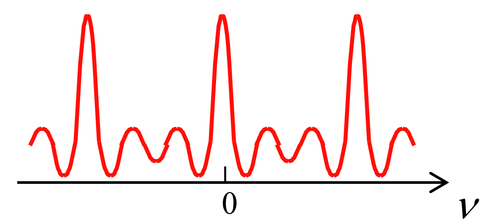
		$f(t)$	$F(\nu)$	
Impulse		$\delta(t)$	1	
Uniform		1	$2\pi\delta(\nu)$	
Harmonic Function		$\exp(i2\pi\nu_o t)$	$\delta(\nu-\nu_o)$	
Harmonic Function		$\cos(2\pi\nu_o t)$	$\frac{1}{2}\,\delta(\nu-\nu_o) + \frac{1}{2}\,\delta(\nu+\nu_o)$	

Double-slit (with no-width)

Fourier Transform Pairs - Basic Functions





		$f(t)$	$F(\nu)$	
Gaussian		$\exp(-\pi t^2)$	$\exp(-\pi \nu^2)$	
Rectangular		$\mathrm{rect}(t)$	$\mathrm{sinc}(\nu)$	
Comb				
M **Multiple Impulses**			$\frac{\sin(M\pi\nu)}{\sin(\pi\nu)}$	

Fourier Transform - Temporal

For a temporal function of f(t):

$$F.T.\{f(t)\} = F(v) = \int_{-\infty}^{+\infty} f(t)e^{-i2\pi vt}dt$$

Fourier Transform

$$I.F.T.\{F(v)\} = f(t) = \int_{-\infty}^{+\infty} F(v)e^{+i2\pi t}dv$$

Inverse Fourier Transform

ν is called as **temporal frequency** and has units of 1/time

Example: [ν]=1/sec=hertz

Fourier Transform - Temporal

For a temporal function f(t), you may also encounter with the following F.T. formula:

$$F.T.\{f(t)\} = F(\omega) = \int_{-\infty}^{+\infty} f(t)e^{-i\omega t}dt$$

$$I.F.T.\{F(\omega)\} = f(t) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} F(\omega)e^{+i\omega t}d\omega$$

Here, ω is called as **angular frequency:** $\omega = 2\pi v$

Its units is radians/time , such as 2π/sec.

Fourier Transform - Spatial

For a spatial function of f(x):

$$F.T.\{f(x)\} = F(p_x) = \int_{-\infty}^{+\infty} f(x) e^{-i2\pi p_x x} dx$$

Fourier Transform

$$I.F.T.\{F(p_x)\} = f(x) = \int_{-\infty}^{+\infty} F(p_x) e^{+i2\pi x} dp_x$$

Inverse Fourier Transform

p_x is called **spatial frequency** and has units of [1/length]

Fourier Transform - Spatial

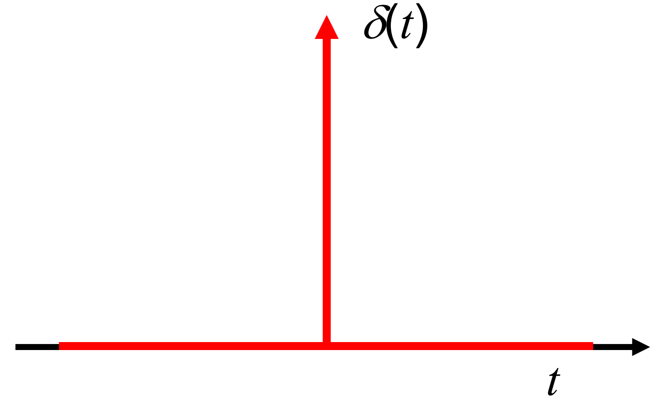
For a spatial function f(x), you may encounter with the following F.T. formula :

$$F.T.\{f(x)\} = F(k) = \int_{-\infty}^{+\infty} f(x) e^{-ik_x x} dx$$

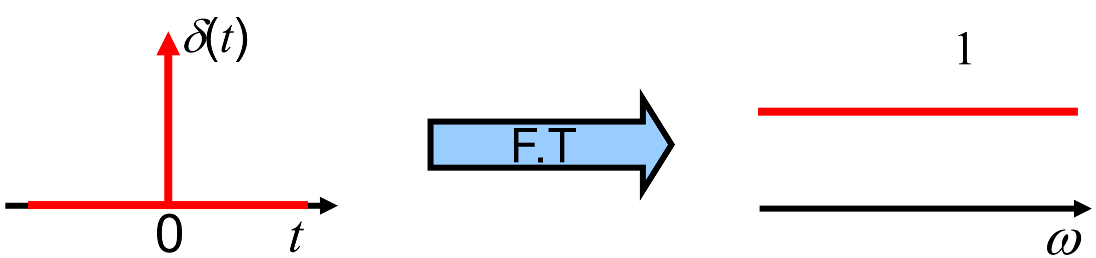
In optics, k_x is called **wave-number:** $k_x = 2\pi p_x$

Examples: the Dirac delta function, δ(t)

$$\delta(t) \equiv \begin{cases} \infty \text{ if } t = 0 \\ 0 \text{ if } t \neq 0 \end{cases}$$

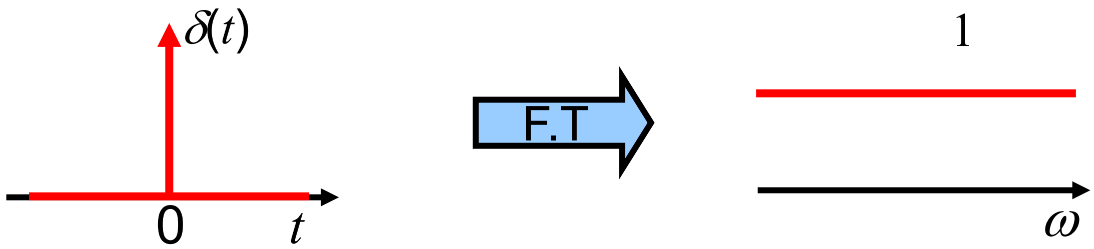


Fourier Transform: $$\int_{-\infty}^{\infty} \delta(t)\exp(-i\omega t)\,dt = \exp(-i\omega[0]) = 1$$

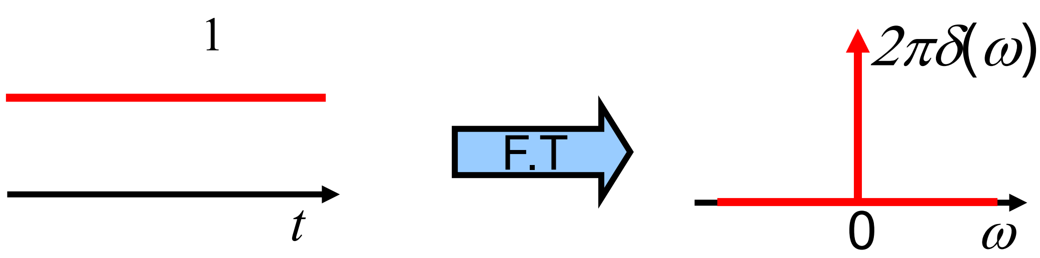


Examples: Fourier Transforms of $\delta(t)$ and 1.

$$\int_{-\infty}^{\infty} \delta(t)\exp(-i\omega t)\,dt = \exp(-i\omega[0]) = 1$$

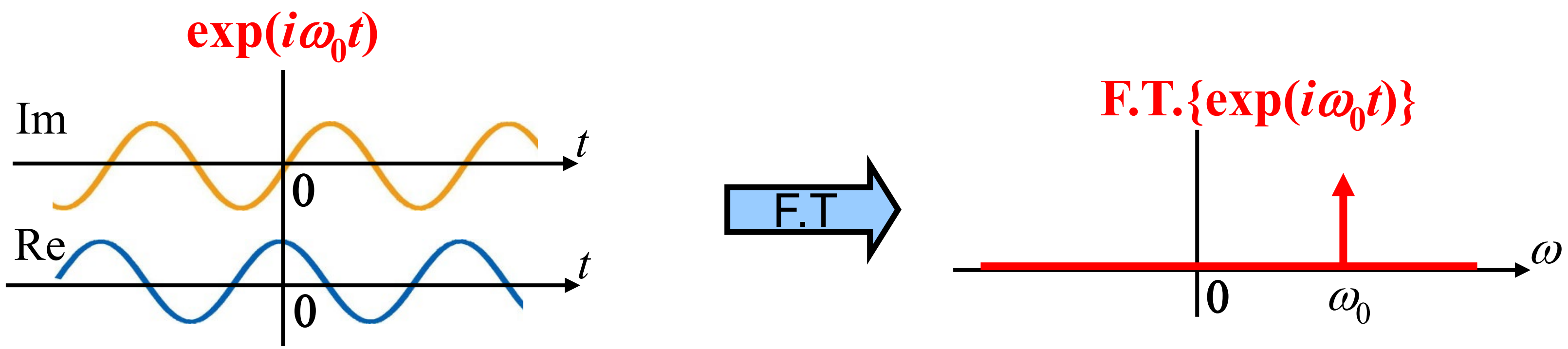


Fourier Transform of "1" is $2\pi\delta(\omega)$: $\int_{-\infty}^{\infty} 1\exp(-i\omega t)\,dt = 2\pi\,\delta(\omega)$



Examples: Fourier transform of $\exp(i\omega_0 t)$

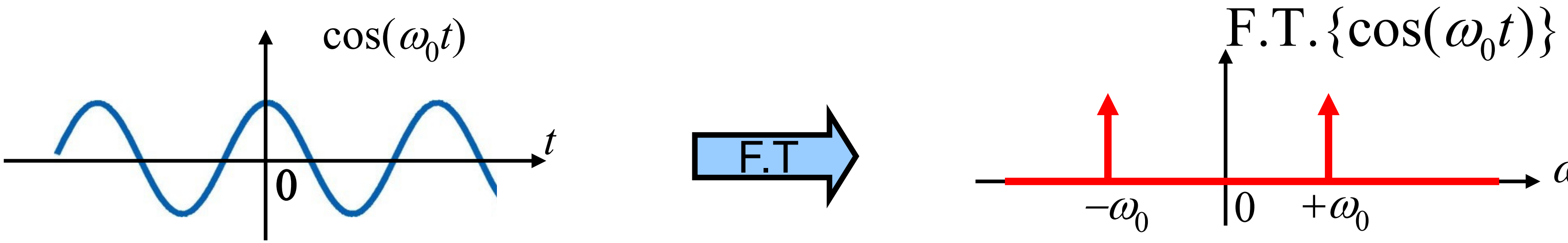
- The harmonic function $\exp(i\omega_0 t)$ is the basis of Fourier analysis.
- It corresponds to a **single** frequency, ω_0.



$$F.T.\{\exp(i\omega_0 t)\} = \int_{-\infty}^{\infty} \exp(i\omega_0 t)\exp(-i\omega t)\,dt$$

$$= \int_{-\infty}^{\infty} \exp(-i[\omega-\omega_0]t)\,dt = 2\pi\,\delta(\omega-\omega_0)$$

Basic Examples: Fourier transform of $\cos(\omega_0 t)$



$$F.T.\{\cos(\omega_0 t)\} = \int_{-\infty}^{\infty} \cos(\omega_0 t)\exp(-i\,\omega t)\,dt$$

$$= \frac{1}{2}\int_{-\infty}^{\infty} \left[\exp(i\,\omega_0\,t) + \exp(-i\,\omega_0\,t)\right]\exp(-i\,\omega t)\,dt$$

$$= \frac{1}{2}\int_{-\infty}^{\infty} \exp(-i[\omega-\omega_0]t)\,dt \quad + \quad \frac{1}{2}\int_{-\infty}^{\infty} \exp(-i[\omega+\omega_0]t)\,dt$$

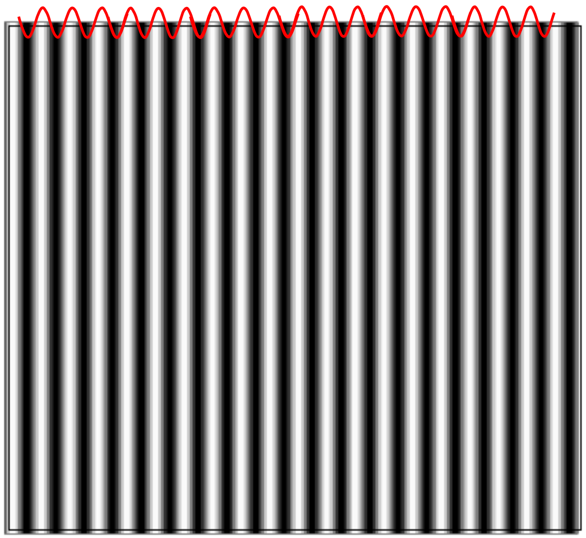
$$= \pi\,\delta(\omega-\omega_0) \quad + \quad \pi\,\delta(\omega+\omega_0)$$

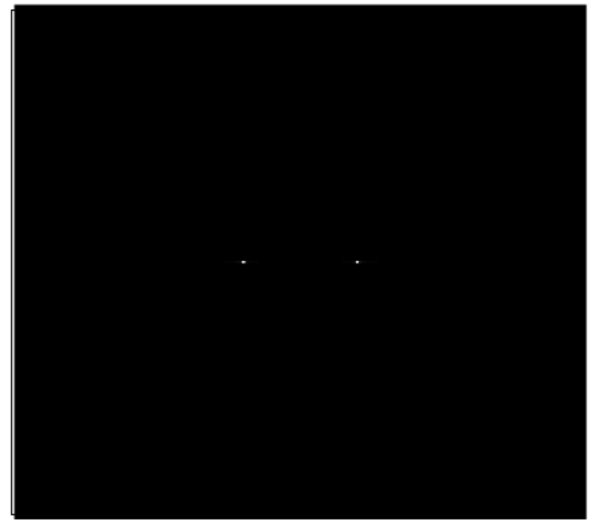
Examples: 1D Fourier Transform - Spatial

Space Domain
(i.e specimen plane)

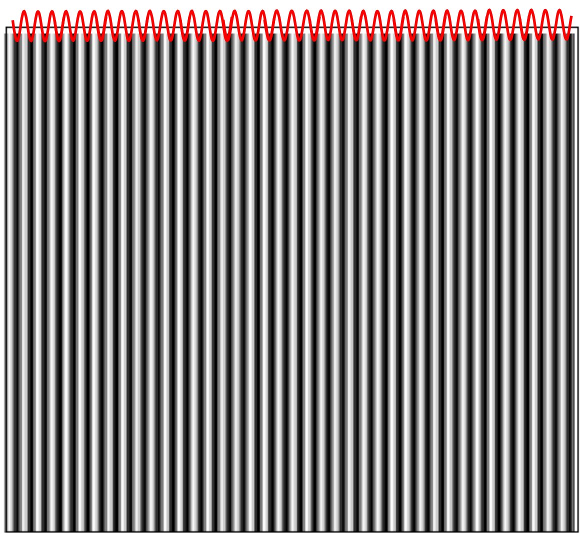
Frequency Domain
(i.e. Fourier plane)

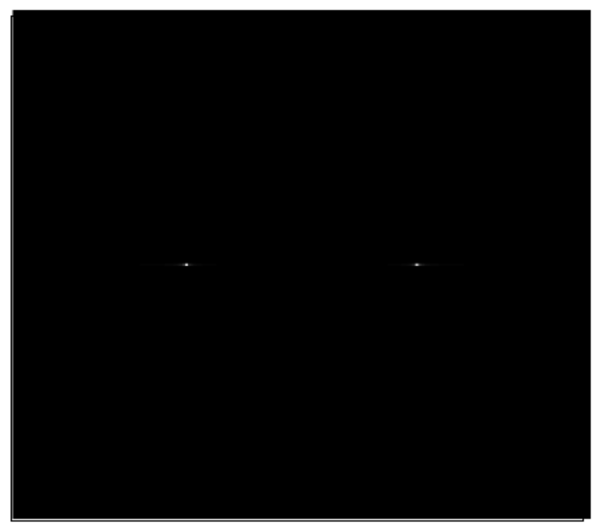
sinusoidal





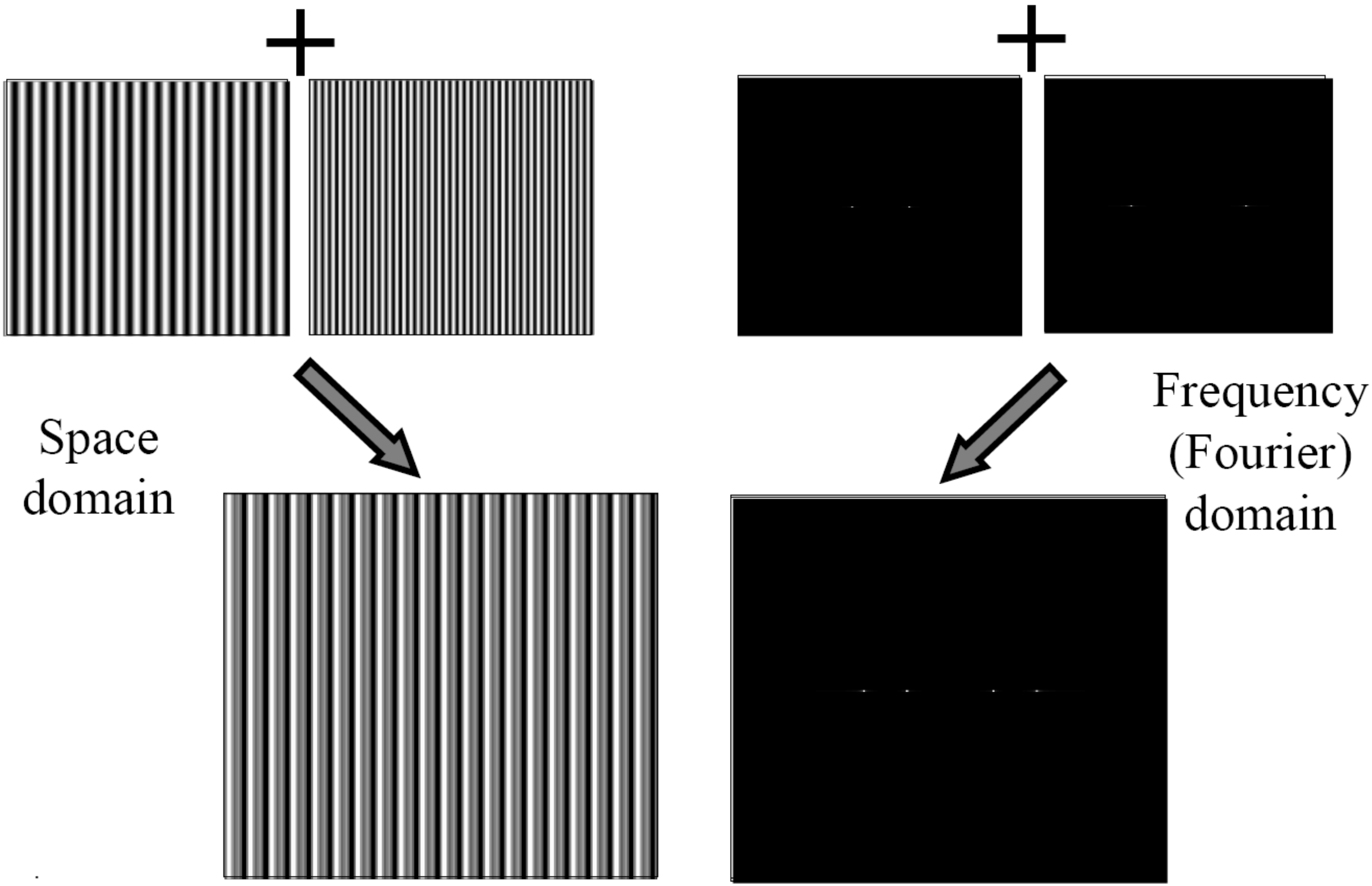
Sinusoidal:
smaller periodicity





Higher frequency

Examples: Superposition Principle

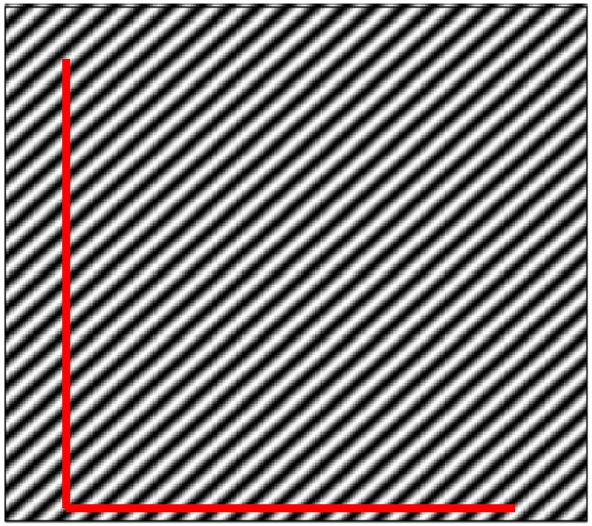


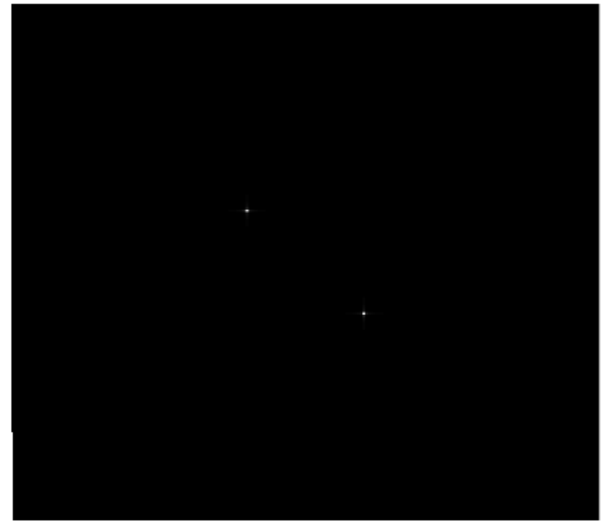
Examples: 2D-Fourier Transform - Spatial

Space Domain
(i.e. specimen plane)

Frequency Domain
(i.e. Fourier plane)

Periodic in x & y

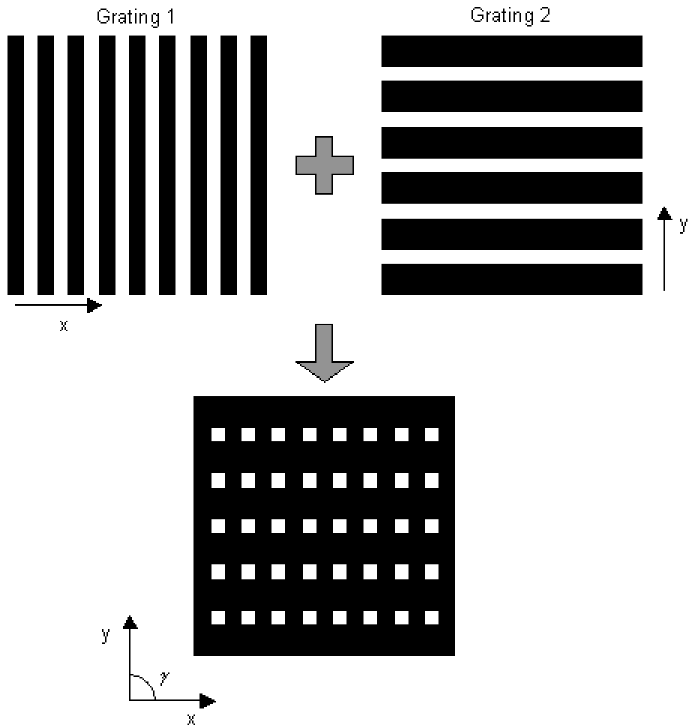


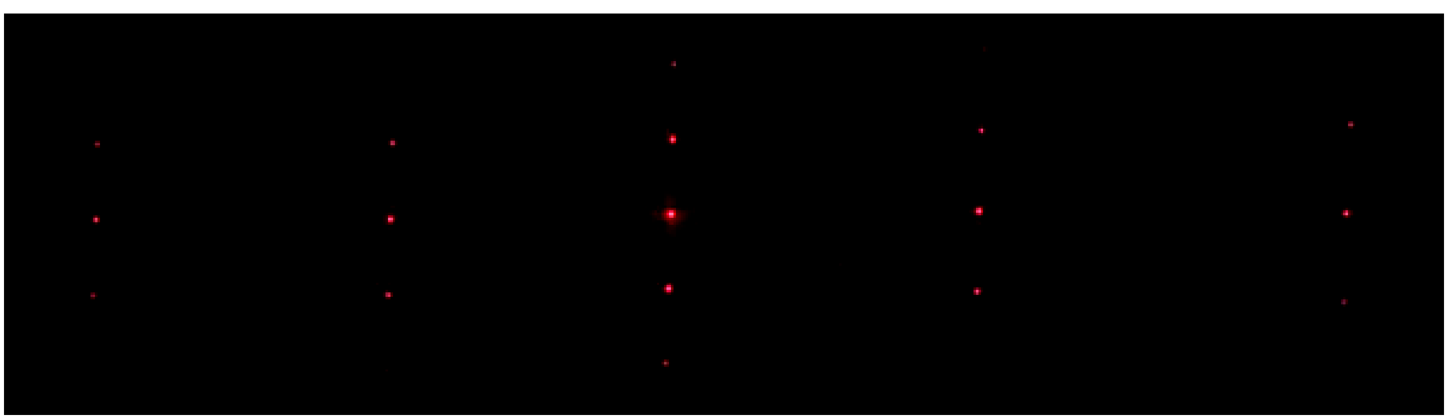


frequency in x & y

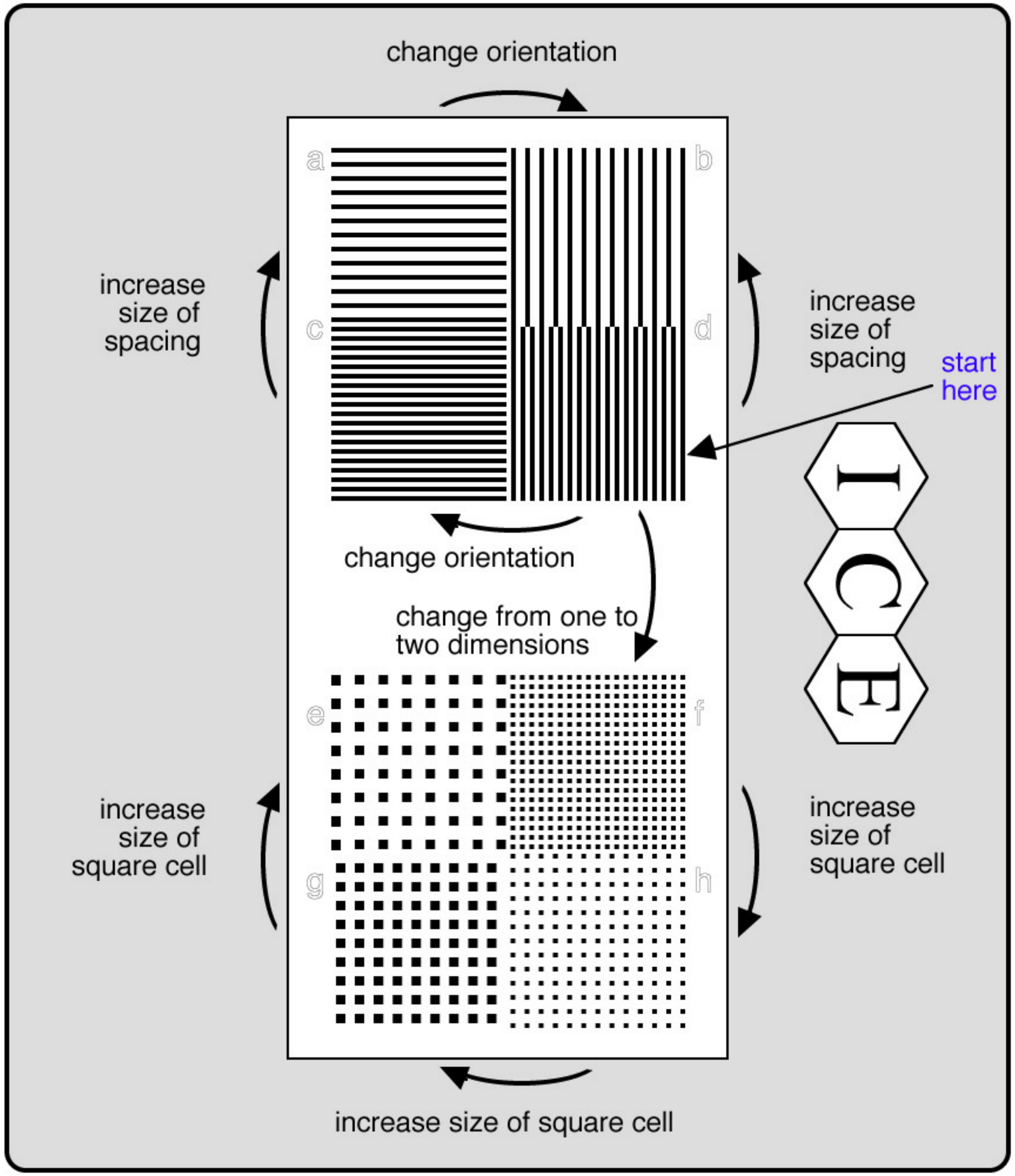
Examples: 2D diffraction of periodic structures

Towards real object:

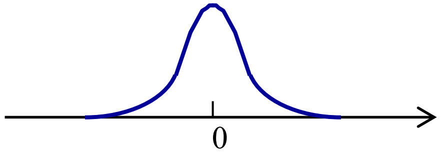
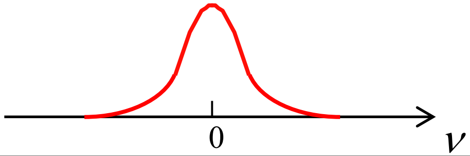
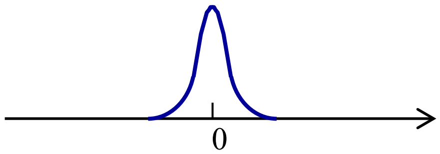
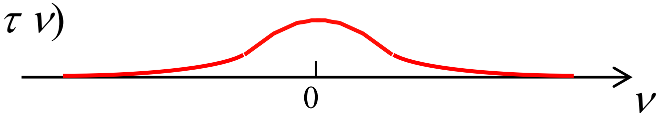




In Class: Experimental Set-up



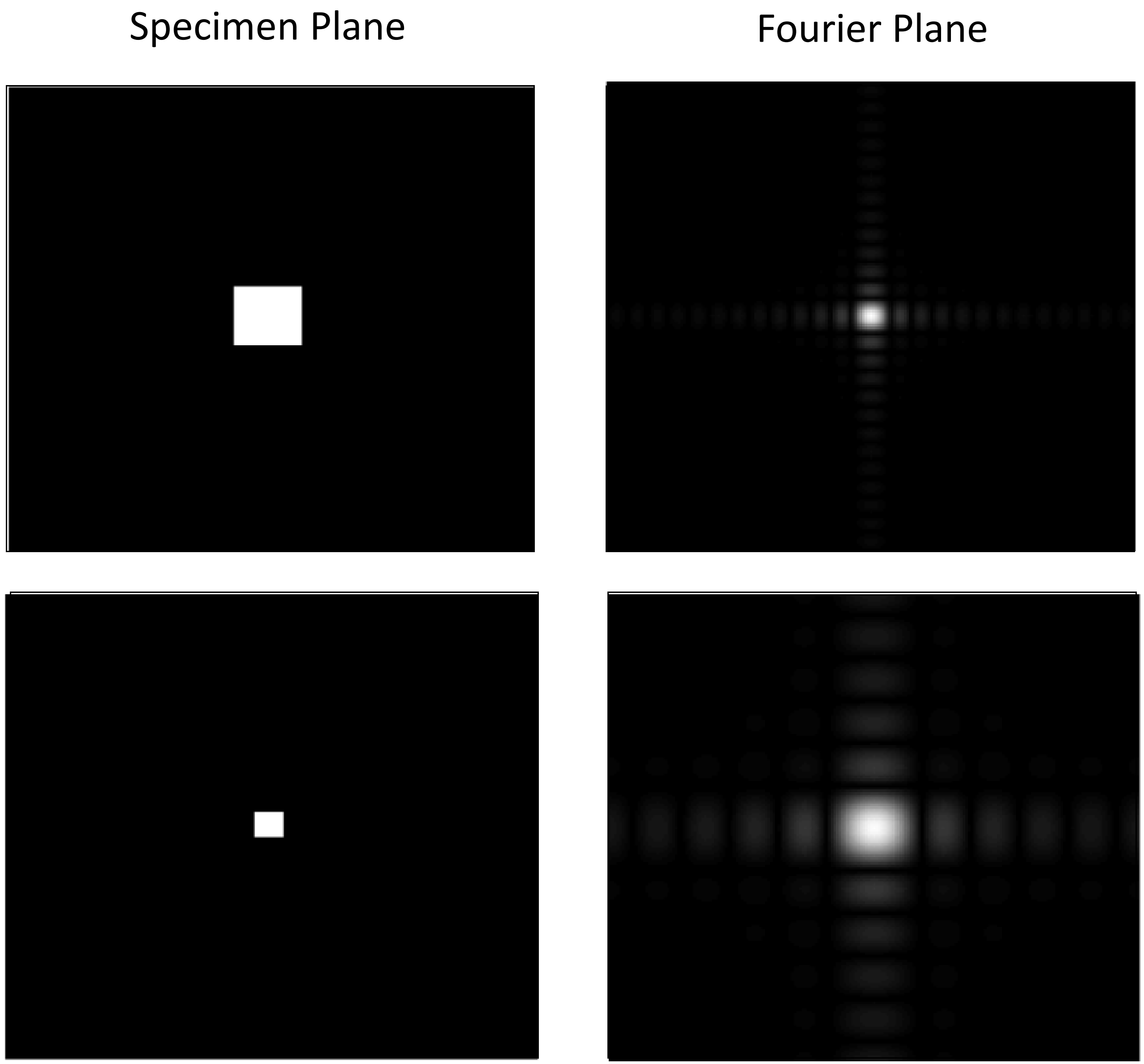
Properties of the Fourier Transform

	 $f(t)$	$F(\nu)$ 
Scaling	 $f(t/\tau)$	$\|\tau\| F(\tau \nu)$ 

When you scale down a function in time/space domain, its F.T. will spread in the frequency domain

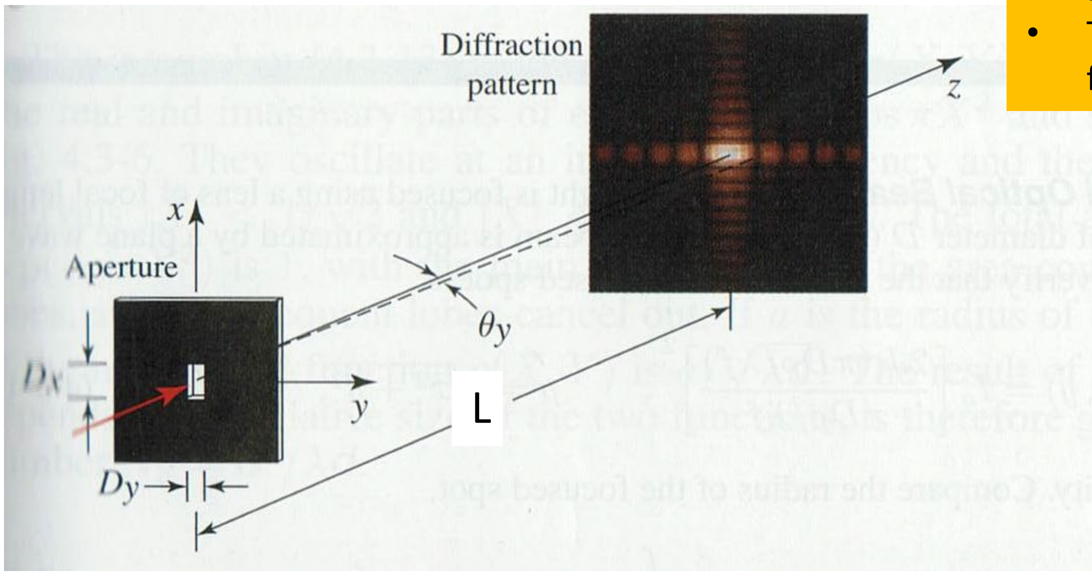
Example: Scaling of an 2D spatial object

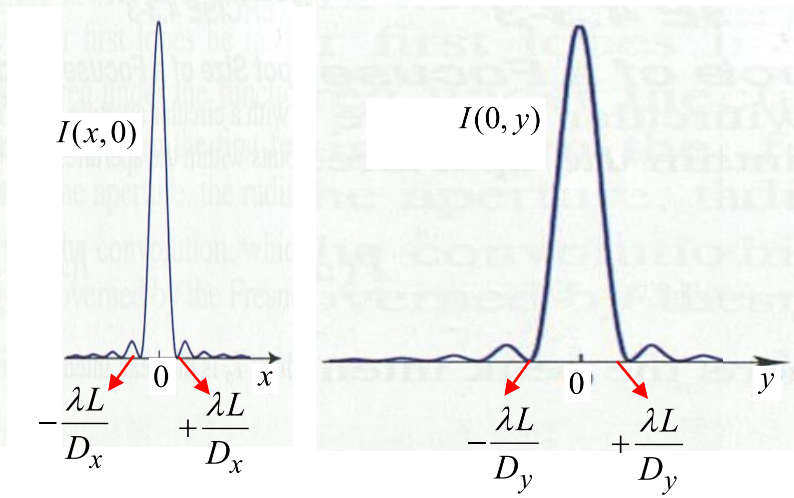
Relationship between the "object size" and the "frequency content"



Diffraction of a 2D-Rectangular Aperture

- In this example D_y is smaller than D_x
- Thus, diffraction pattern spreads more (i.e. higher frequencies) along the y-axis compared to the x-axis.





INPUT:

$f(x,y) = \mathrm{rect}(x/D_x)\,\mathrm{rect}(y/D_y)$

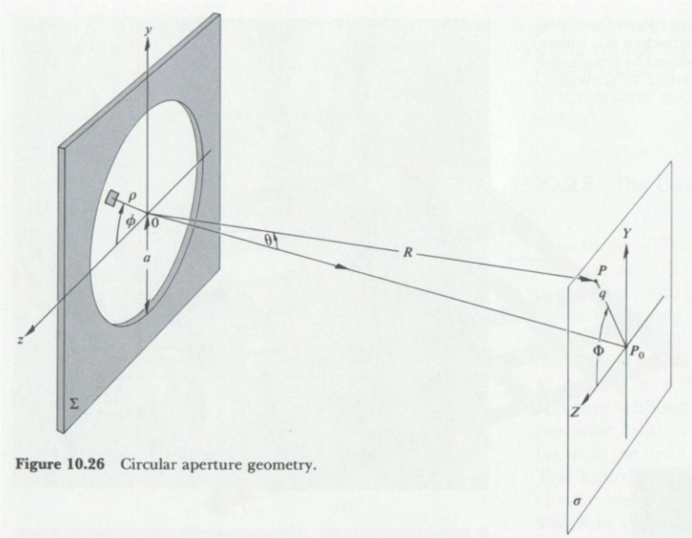
OUTPUT:

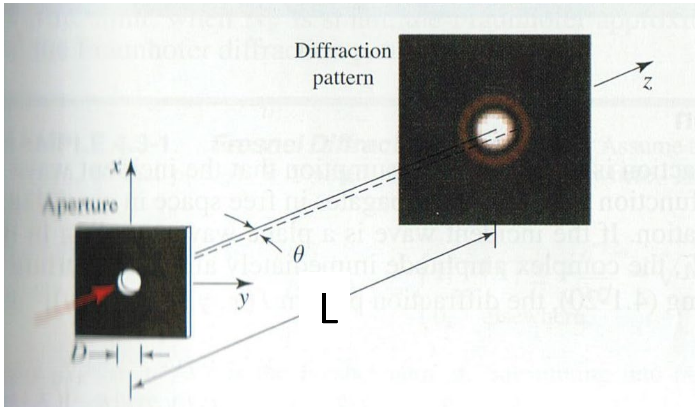
$$I(x,y) = I_o \operatorname{sinc}^2 \frac{D_x x}{\lambda L} \operatorname{sinc}^2 \frac{D_y y}{\lambda L}$$

$$first \quad zeros \quad at \quad x = \pm \lambda L / D_x, y = \pm \lambda L / D_y$$

$$with \quad angles \quad \theta_x = \frac{\lambda}{D_x}, \qquad \theta_y = \frac{\lambda}{D_y}$$

Diffraction of a Circular Aperture

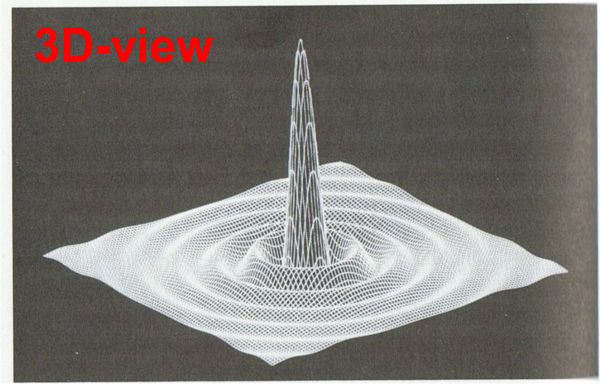


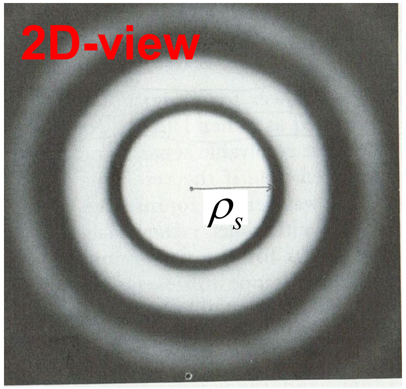


INPUT:

f(ρ) = circ(ρ/D)

$\rho = sqrt\ (x^2+y^2)$





- Spreading of θ (thus frequency) has inverse relation on the radius size (D)

AIRY RINGS

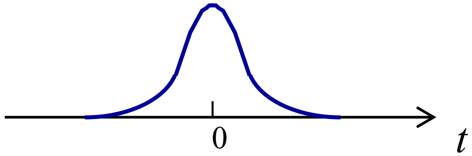
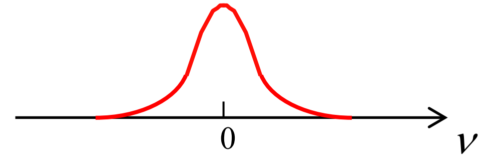
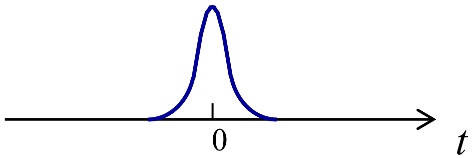
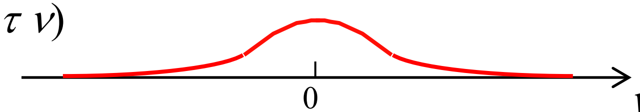
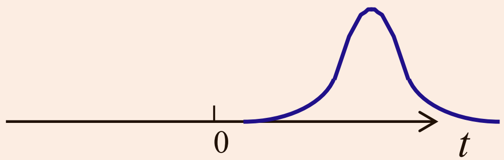
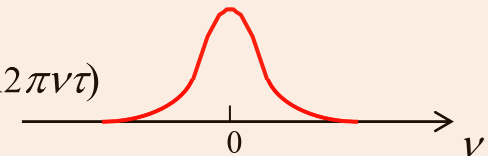
OUTPUT:

$$I(x,y) = I_o \left[\frac{2J_1(\pi D\rho / \lambda L)}{\pi D\rho / \lambda L} \right]^2, \rho = \sqrt{x^2 + y^2}$$

$$first \quad zero \quad at \quad \rho_s = 1.22 \frac{\lambda}{D} L$$

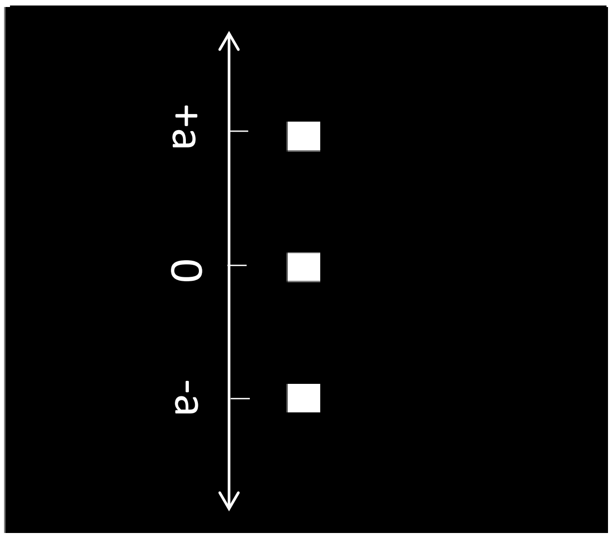
$$angle \quad \theta = 1.22 \frac{\lambda}{D}$$

Properties of the Fourier Transform

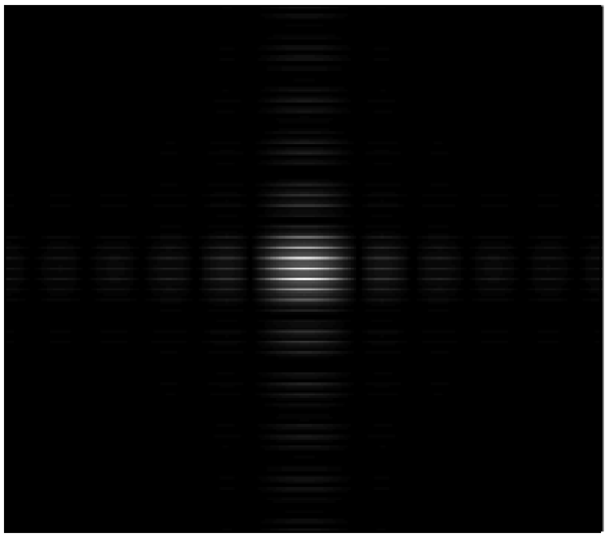
	 $f(t)$	$F(\nu)$ 
Scaling	 $f(t/\tau)$	$\lvert\tau\rvert F(\tau\,\nu)$ 
Translation	 $f(t-\tau)$	$F(\nu)\ \exp(-i2\pi\nu\tau)$ 

Example: Superimpose Shifted Objects

Specimen Plane



Fourier Plane



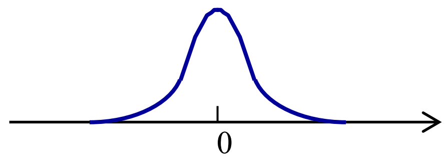
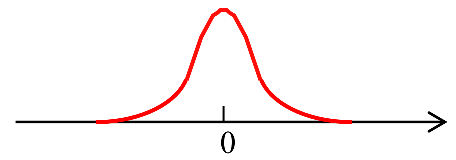
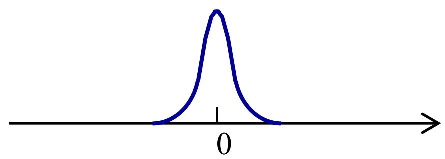
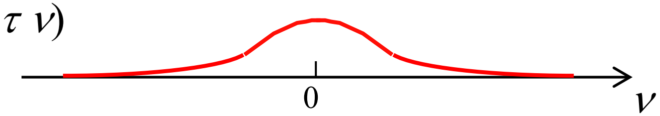
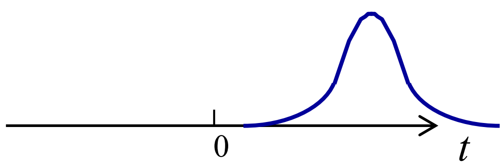
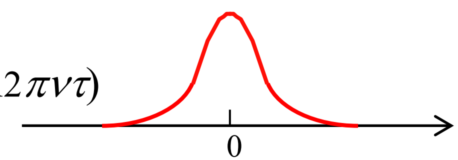
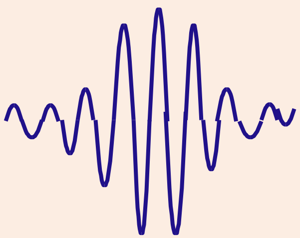
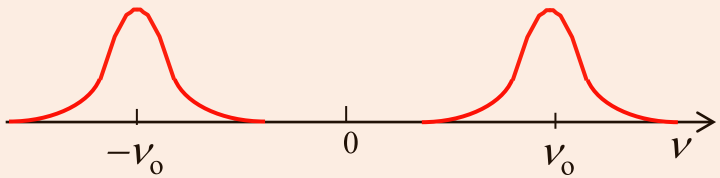
If we assume a 1D case, then input function is:

$f(x) = \mathrm{rect}[(x\text{-}0)/D_x] + \mathrm{rect}[(x\text{-}a)/D_x] + \mathrm{rect}[(x+a)/D_x]$

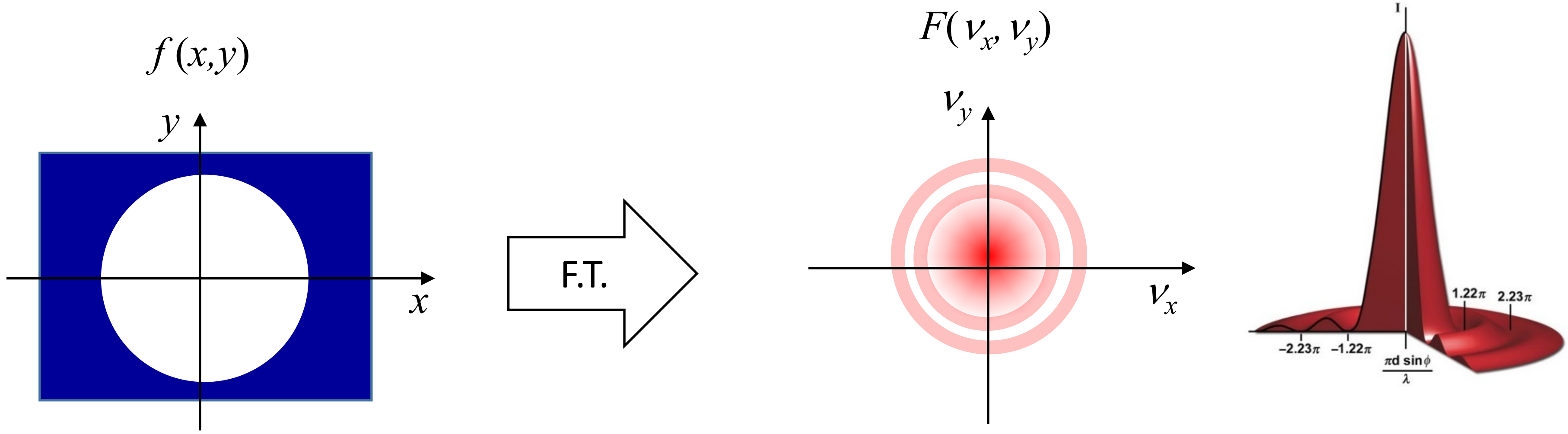
The diffraction pattern at the far-field (Fourier plane) is proportional to the square of:

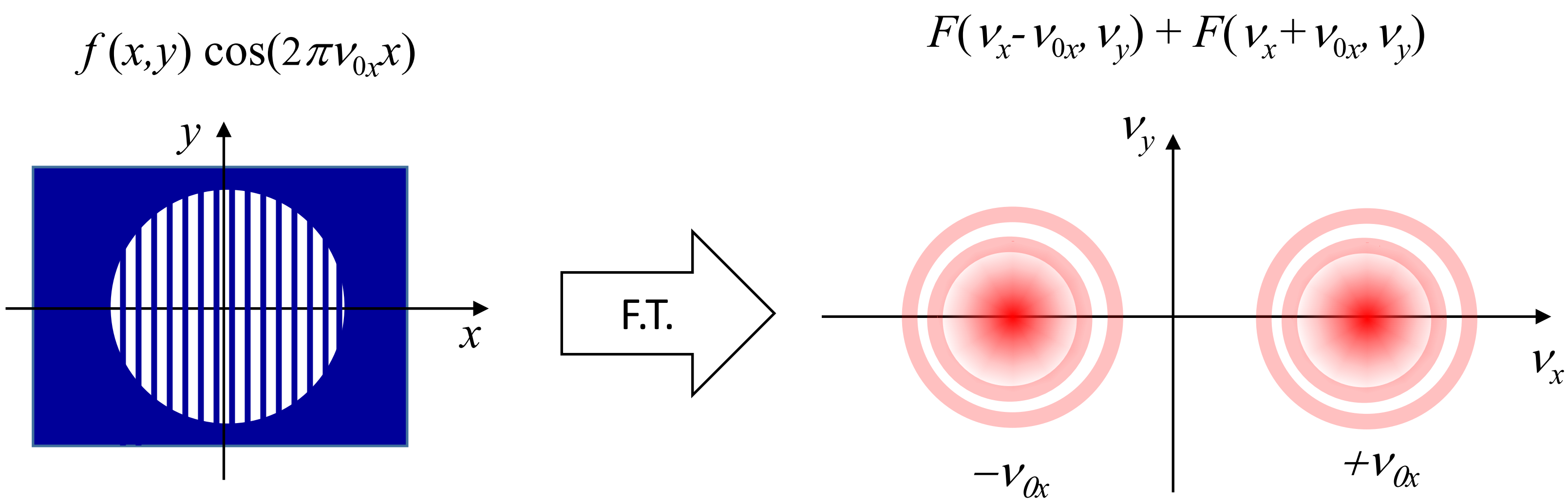
$F(\nu) = sinc(\nu)\ [1 + \exp(-i2\pi\nu a)+\exp(+i2\pi\nu a)]$

Properties of the Fourier Transform

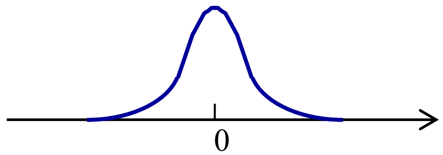
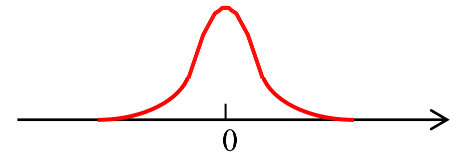
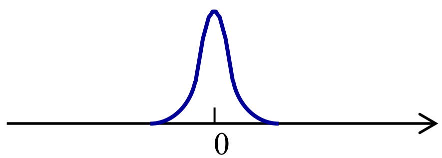
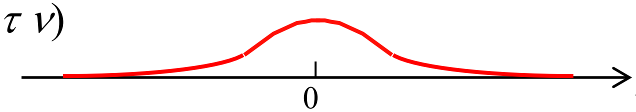
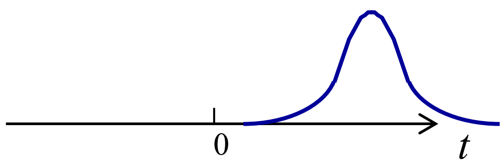
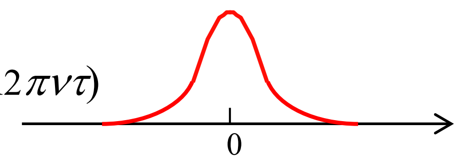
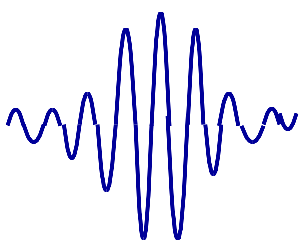
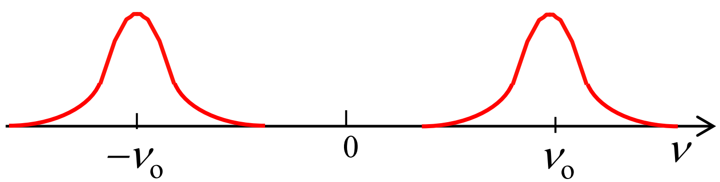
	 $f(t)$	$F(\nu)$ 
Scaling	 $f(t/\tau)$	$\lvert\tau\rvert F(\tau\,\nu)$ 
Translation	 $f(t-\ \tau)$	$F(\nu)\ \exp(-i2\pi\nu\tau)$ 
Modulation	 $f(t)\ \cos(2\pi\nu_o t)$	 $F(\nu+\nu_o)/2 + F(\nu-\nu_o)/2$

Example: Spatially Modulated 2D Object



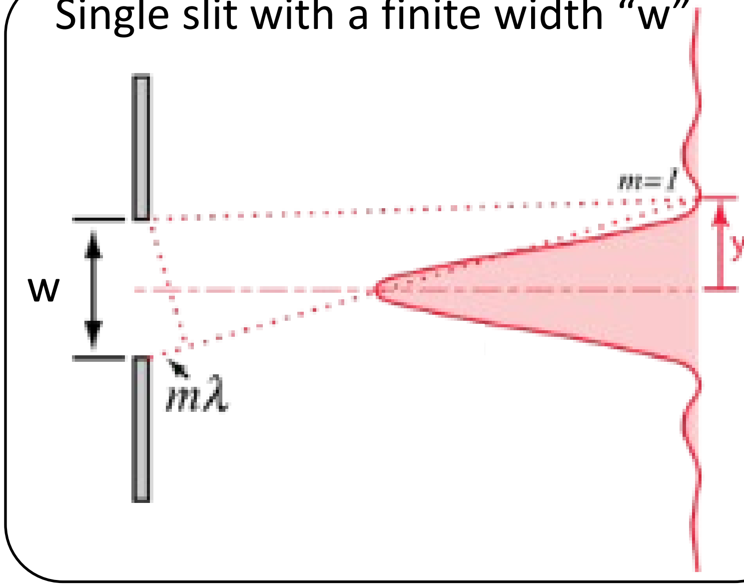


Properties of the Fourier Transform

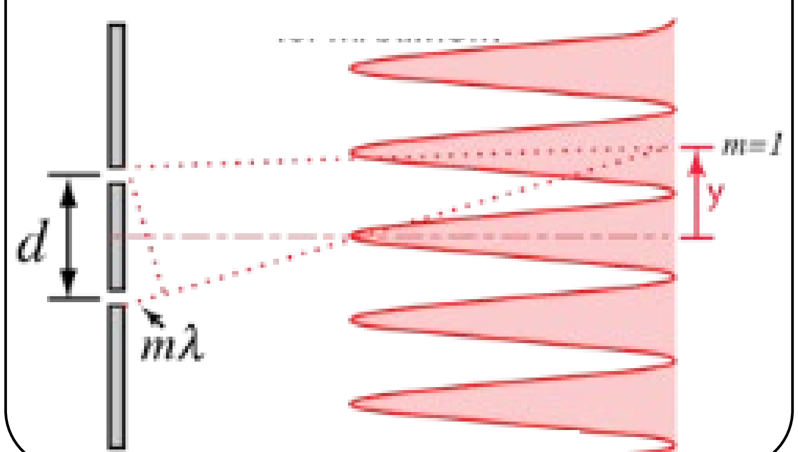
	 $f(t)$	$F(\nu)$ 
Scaling	 $f(t/\tau)$	$\lvert\tau\rvert F(\tau\,\nu)$ 
Translation	 $f(t-\tau)$	$F(\nu)\exp(-i2\pi\nu\tau)$ 
Modulation	 $f(t)\cos(2\pi\nu_o t)$	 $F(\nu+\nu_o)/2 + F(\nu-\nu_o)/2$
Convolution	$f(t) * g(t)$	$F(\nu)\,.\,G(\nu)$

Example: Diffraction with single slit and double slits

Single slit with a finite width "w"

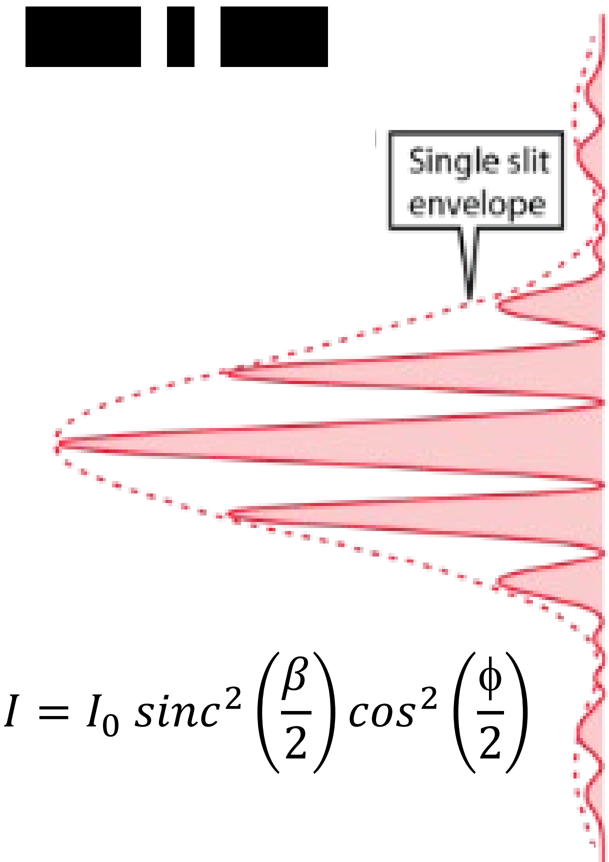


Double slit (without a width) separated by "d"



X

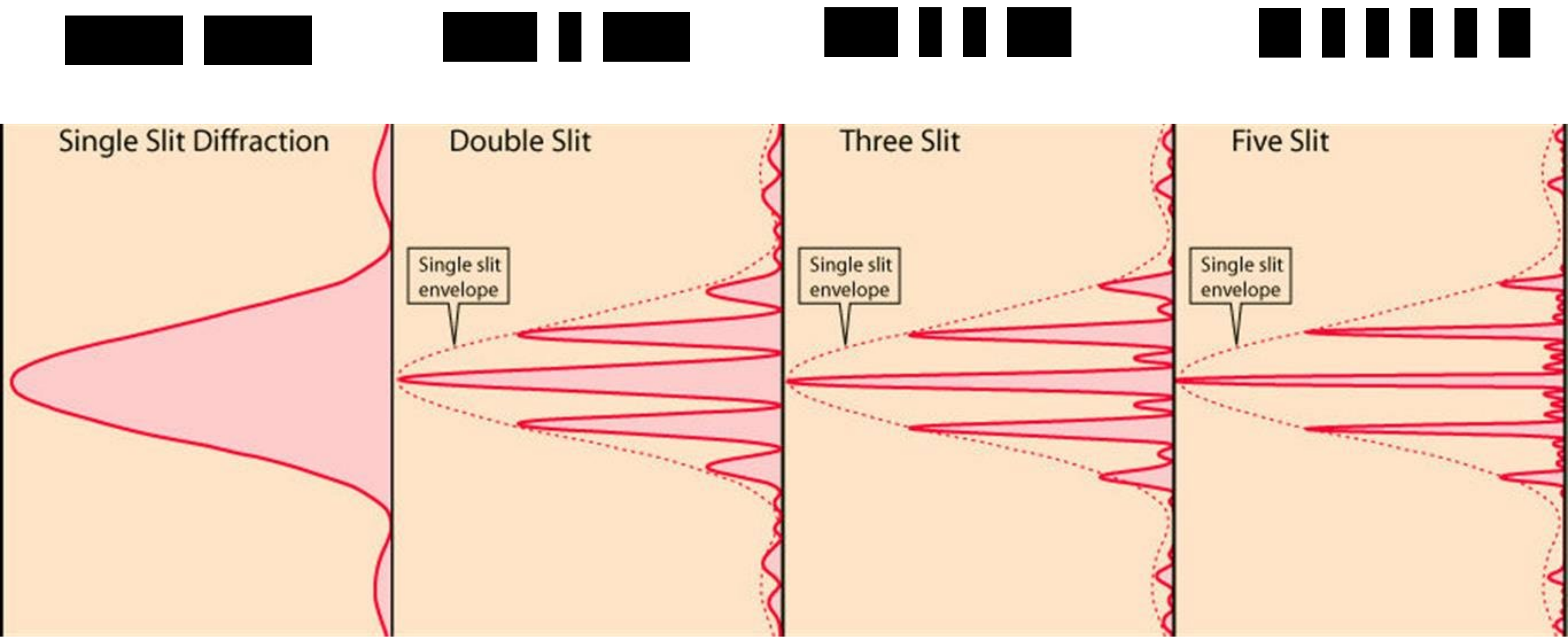
Convolution of these two yields:
Double slit with a finite width (w) and separation (d)



$$I = I_0\, sinc^2\left(\frac{\beta}{2}\right) cos^2\left(\frac{\phi}{2}\right)$$

$$\beta = \frac{2\pi w}{\square} sin\theta \qquad \phi = \frac{2\pi d}{\square} sin\theta$$

Diffraction by slits with finite width



➔ With increasing number of slits (to infinity), the system will gradually transition to a "grating"