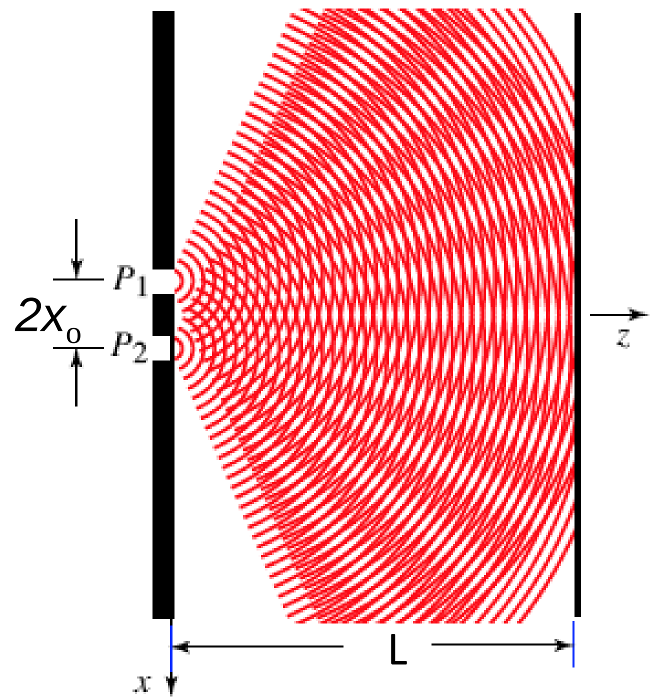
MICRO-561

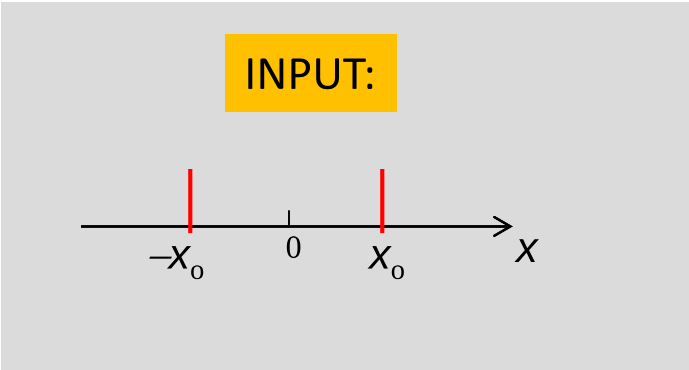
Biomicroscopy I

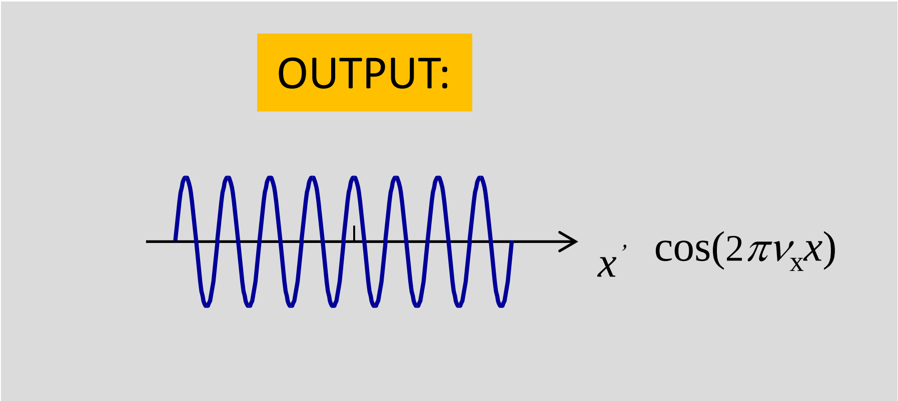
Syllabus (tentative)

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
Lecture 8	Resolution-3
Lecture 9	Resolution-4 & Contrast-1
Lecture 10	Contrast-2 & Fluorescence-1
Lecture 11	Fluorescence-2
Lecture 12	Fluorescence-3, Sources
Lecture 13	Filters & Detectors
Lecture 14	Bio-application Examples

Reminder: Double Slit (with no width) Experiment

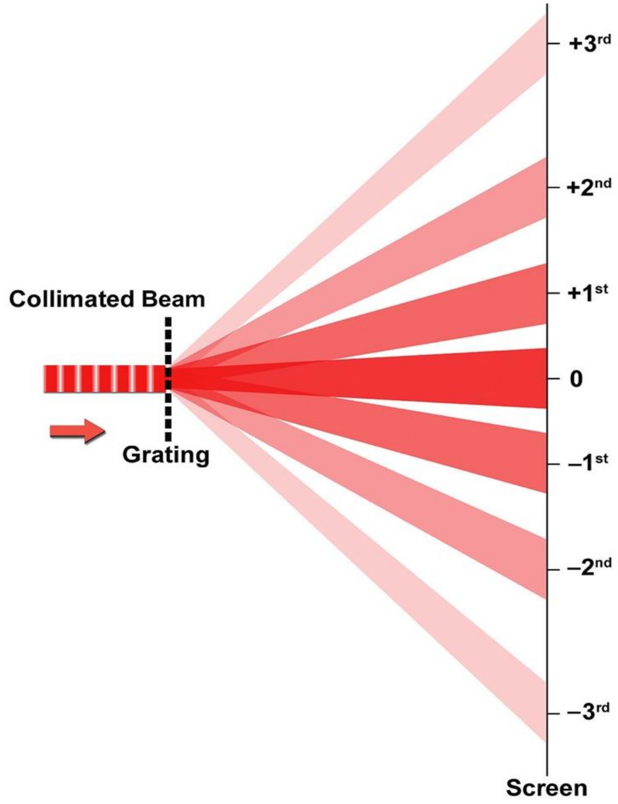






PS: Ignore slit width

Reminder: Diffraction by a **“grating”**



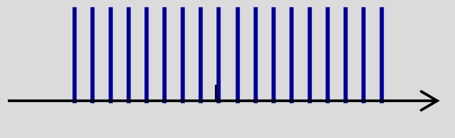
m^{th} order:

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

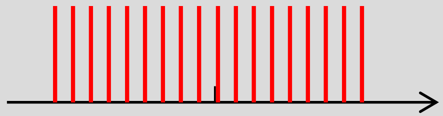
Grating Equation:

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

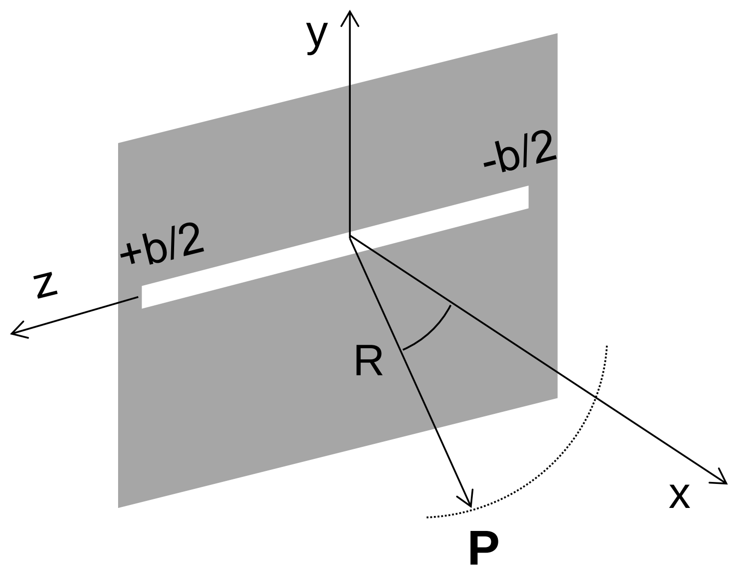
INPUT:



OUTPUT:



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



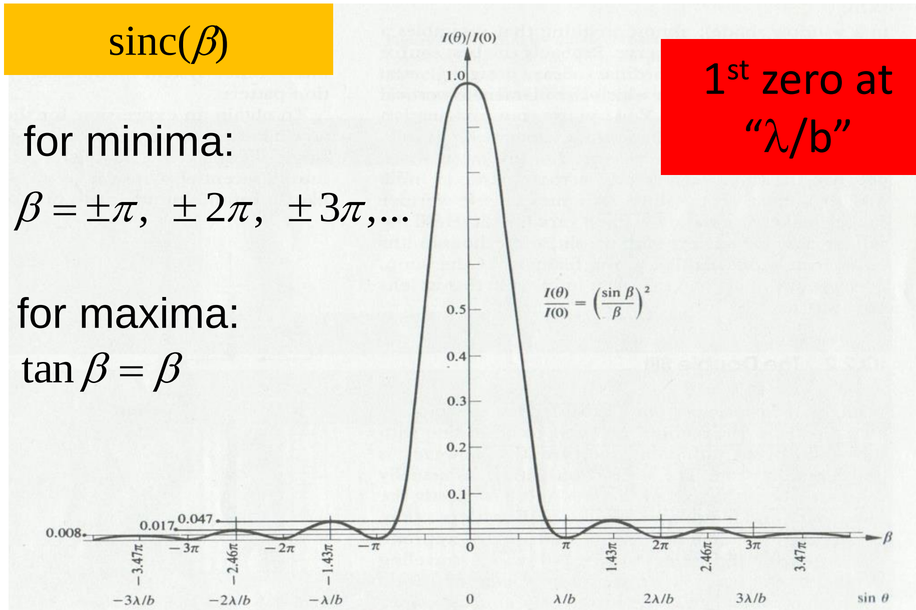
sinc(β)

for minima:

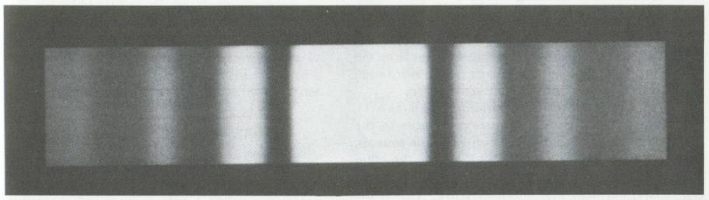
$\beta = \pm\pi,\ \pm 2\pi,\ \pm 3\pi, ...$

for maxima:

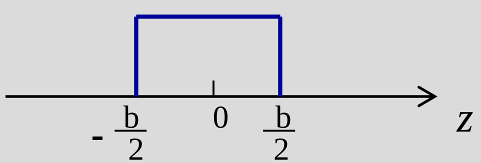
$\tan\beta = \beta$



1st zero at "λ/b"



INPUT: rect(z/b)



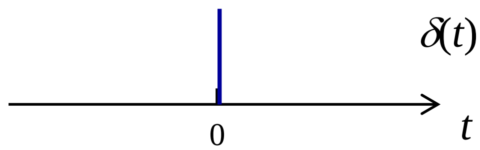
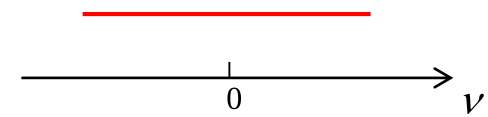
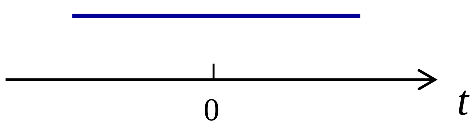
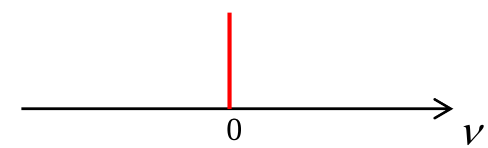
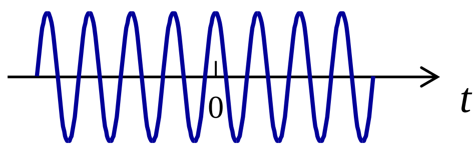
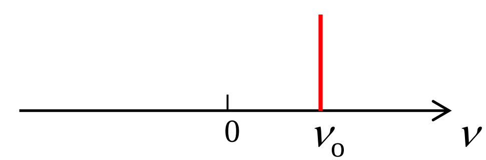
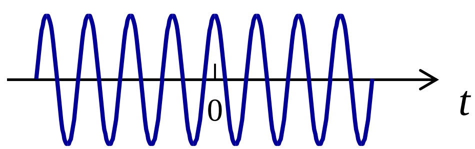
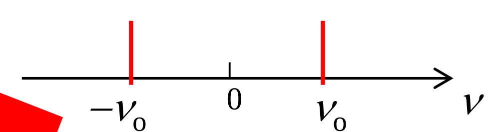
OUTPUT:

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

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

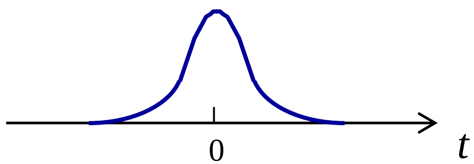
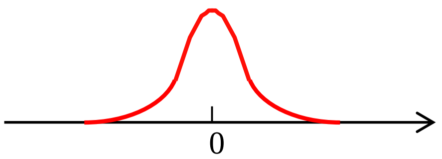
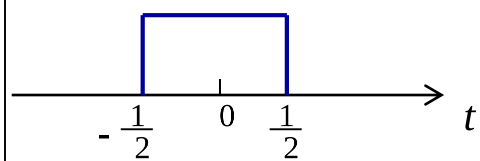
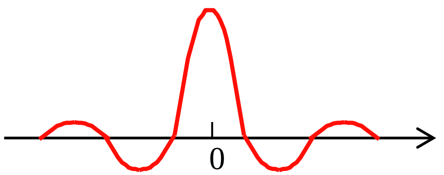
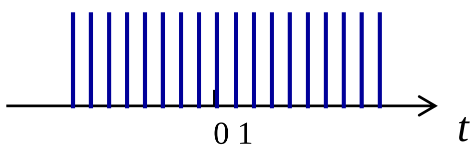
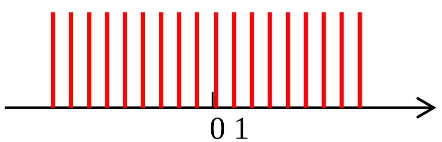
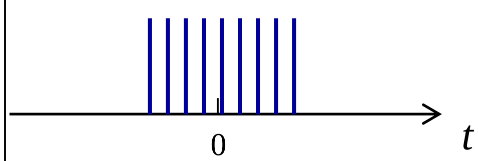
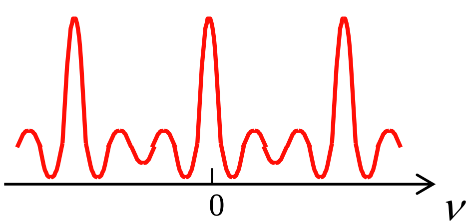
http://micro.magnet.fsu.edu/primer/java/diffraction/basicdiffraction/index.html

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			$\dfrac{\sin(M\pi\nu)}{\sin(\pi\nu)}$	

Rectangular aperture

Grating

Fourier Transform - Temporal

For a temporal function of f(t):

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

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:

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

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

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):

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

Fourier Transform

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

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 also encounter with the following F.T. formula :

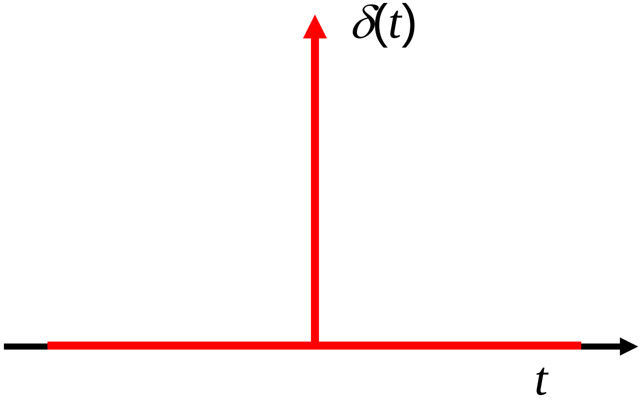
$$\boxed{F.T.\{f(x)\} = F(k) = \int_{-\infty}^{+\infty} f(x)e^{-ik_x x}dx}$$

In optics, k_x is called **wave-number:**

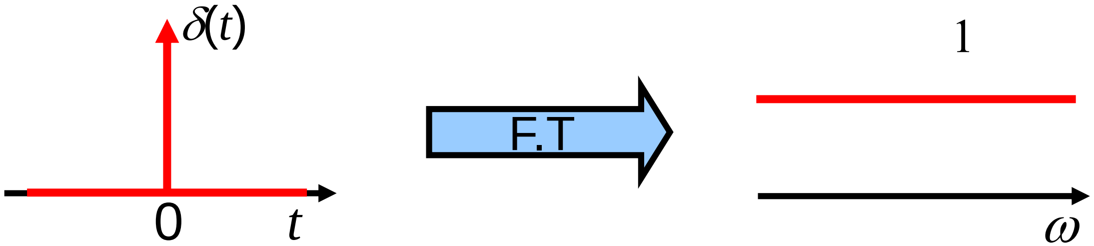
$$k_x = 2\pi p_x$$

Examples: the Dirac delta function, δ(t)

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

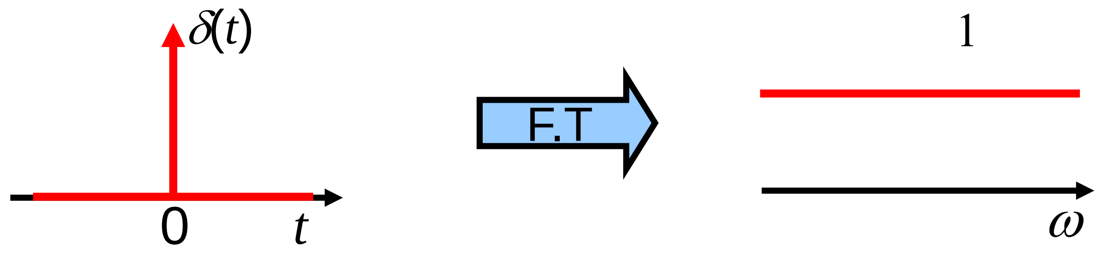


Fourier Transform: $$\int_{-\infty}^{\infty} \delta(t) \exp(-i\omega t)\, dt = \exp(-i\omega[0]) = 1$$

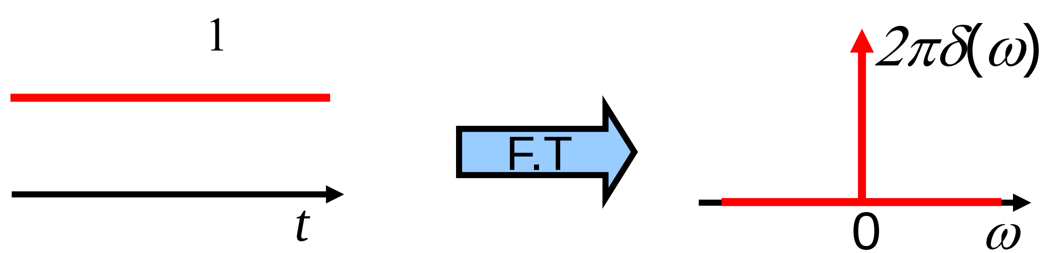


Examples: Fourier Transforms of $\delta(t)$ and 1.

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

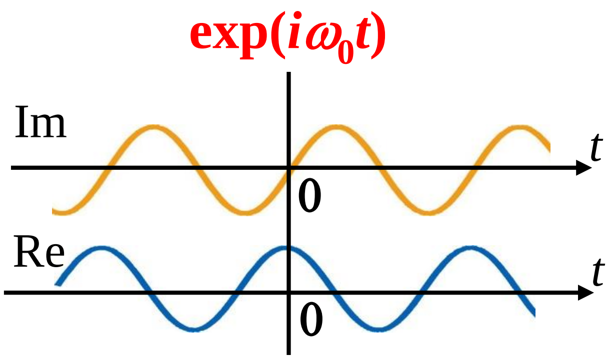


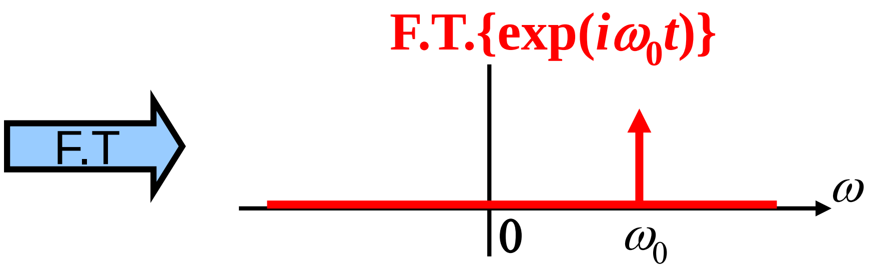
Fourier Transform of "1" is $2\pi\delta(\omega)$: $\displaystyle\int_{-\infty}^{\infty} 1\exp(-i\omega t)\,dt = 2\pi\,\delta(\omega)$



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.

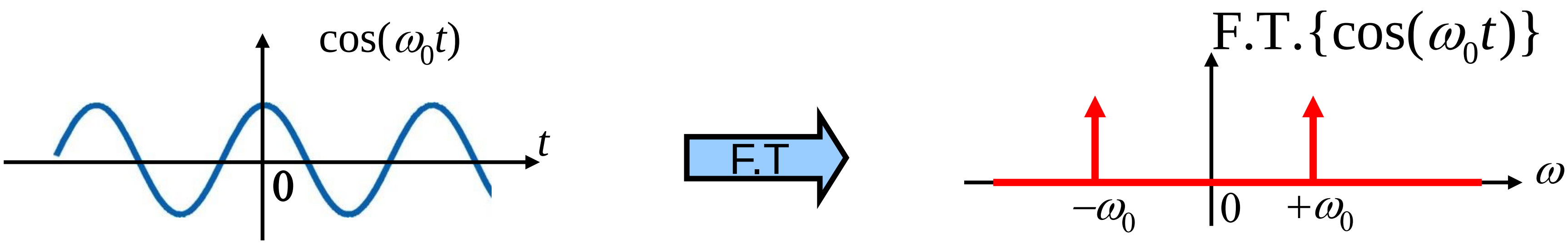




$$F.T.\{\exp(i\omega_0 t)\} = \int_{-\infty}^{\infty} \exp(i\omega_0 t)\exp(-i\,\omega t)\,dt$$

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

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



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

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

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

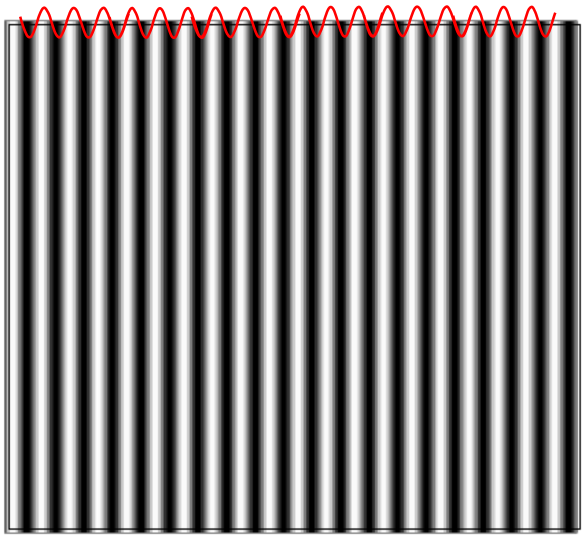
$$= \pi\,\delta(\omega - \omega_0) \quad + \quad \pi\,\delta(\omega + \omega_0)$$

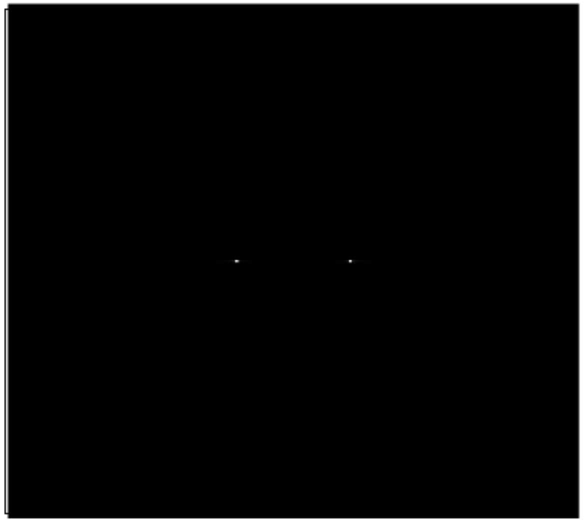
Examples: 1D Fourier Transform - Spatial

Space Domain
(i.e specimen plane)

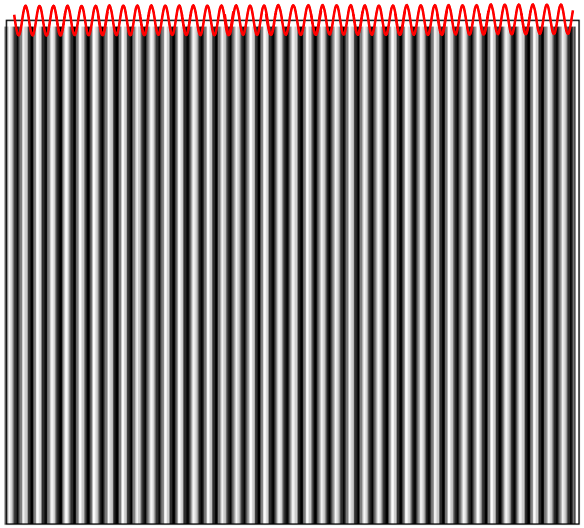
Frequency Domain
(i.e. Fourier plane)

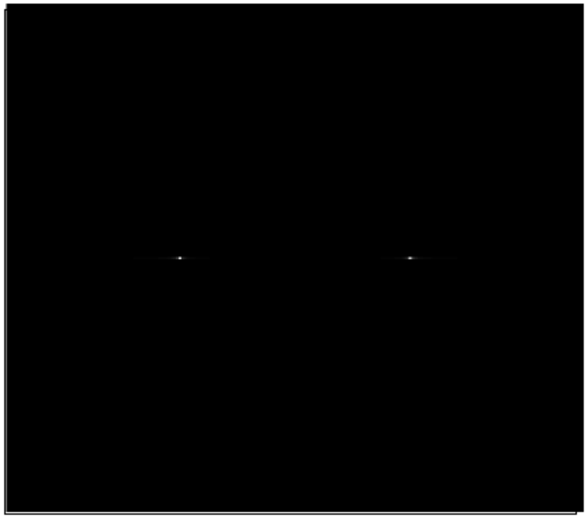
sinusoidal





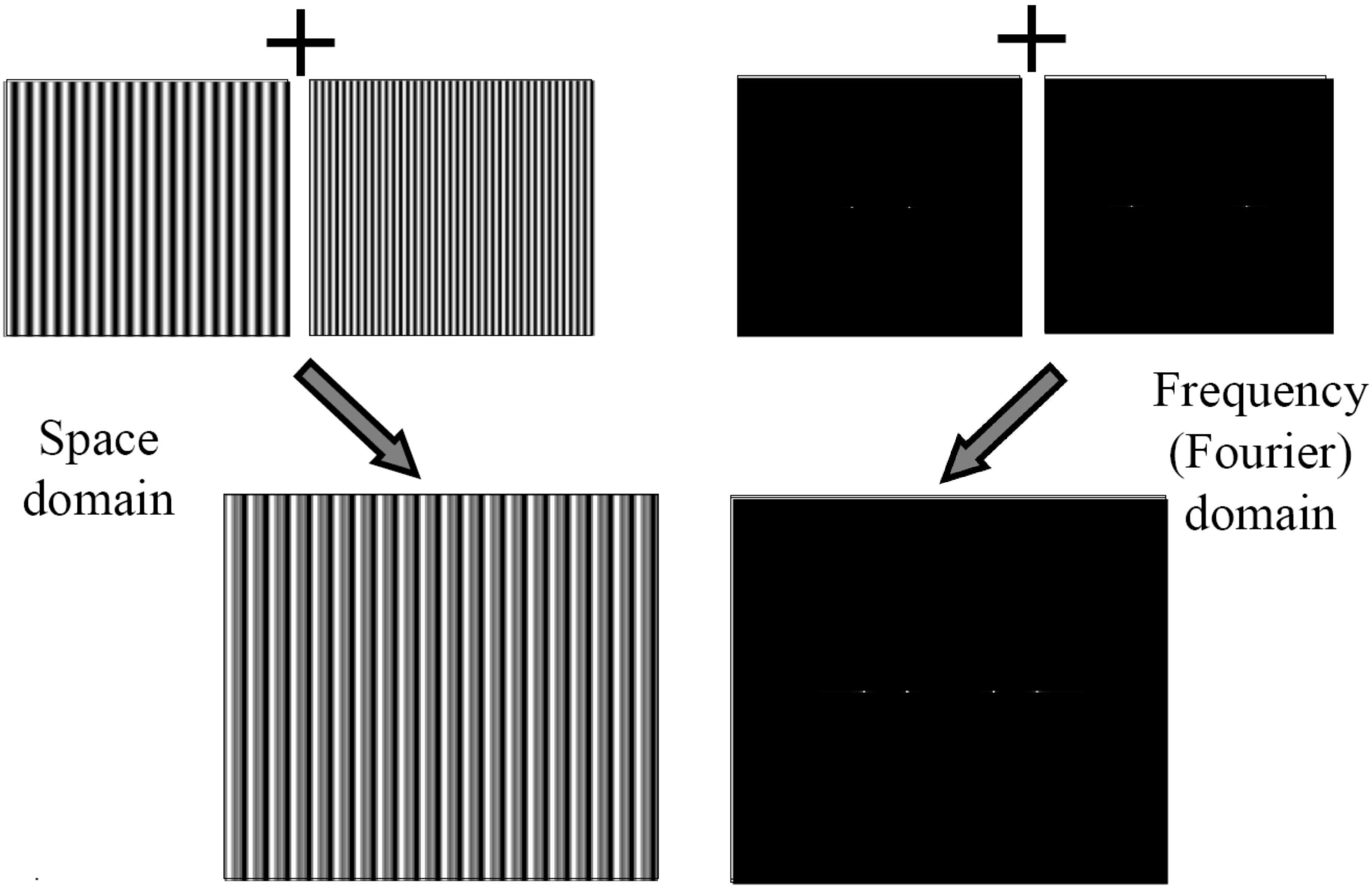
Sinusoidal:
smaller periodicity





Higher frequency

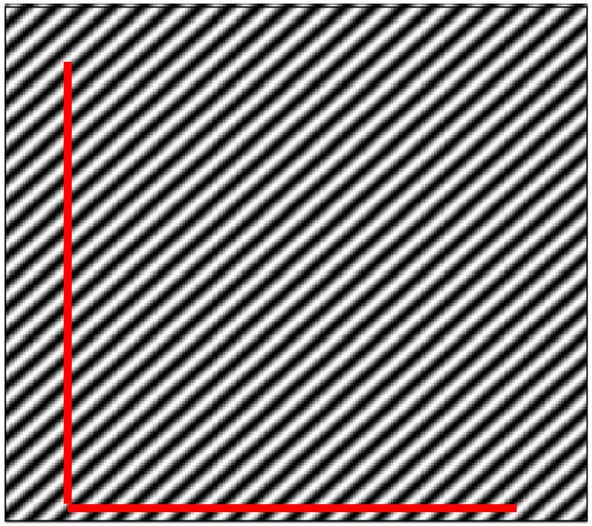
Examples: Superposition Principle



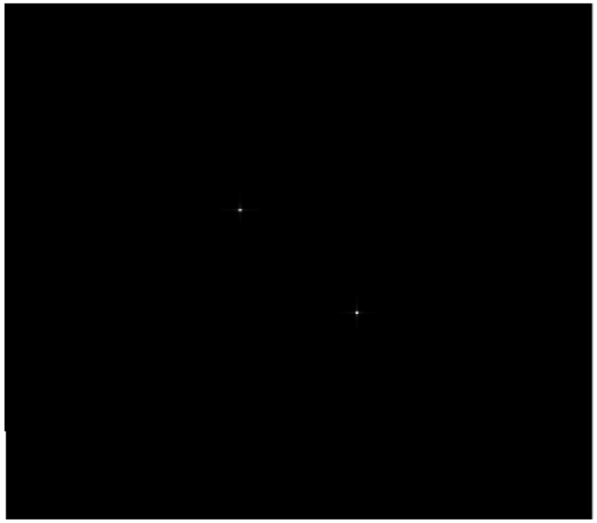
Examples: 2D-Fourier Transform - Spatial

Space Domain
(i.e. specimen plane)

Periodic in x & y



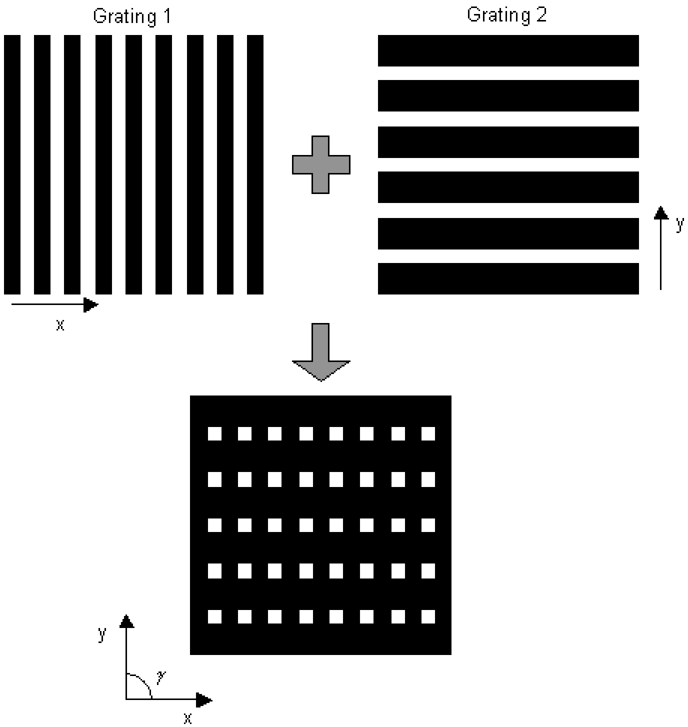
Frequency Domain
(i.e. Fourier plane)

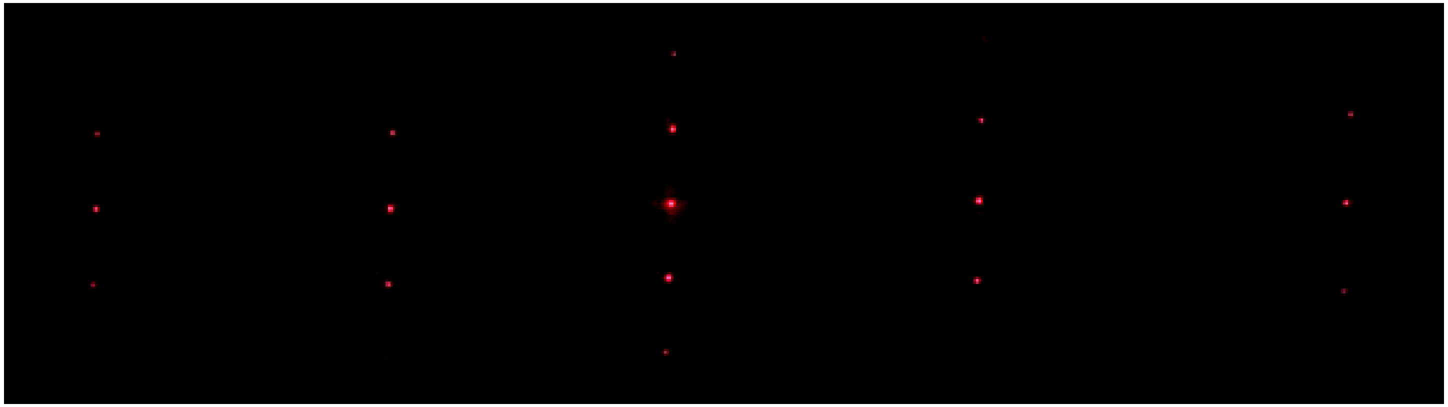


frequency in x & y

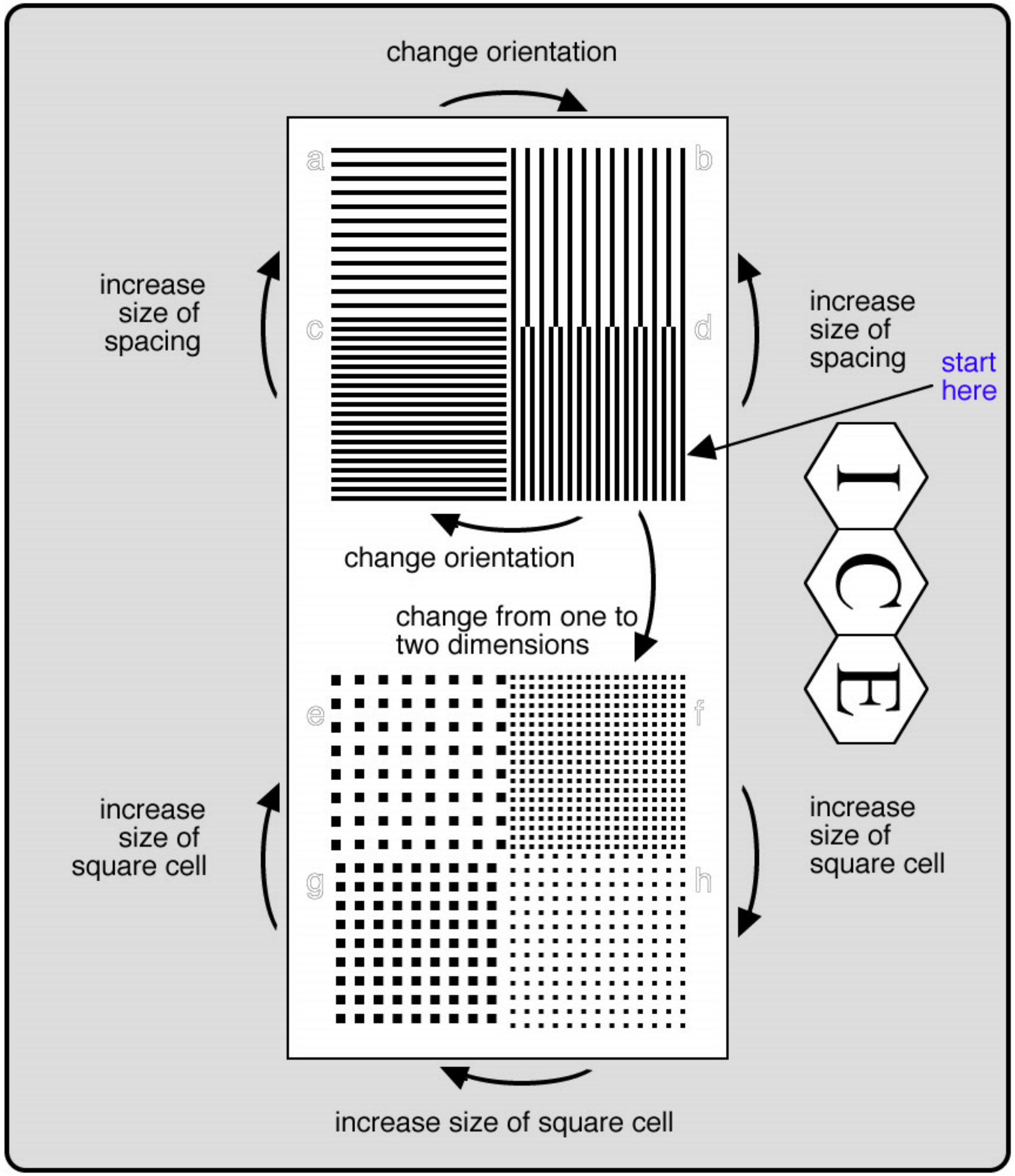
Examples: 2D diffraction of a periodic “pattern”

Moving towards a real object (pattern):

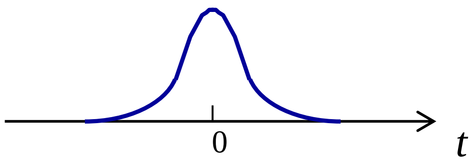
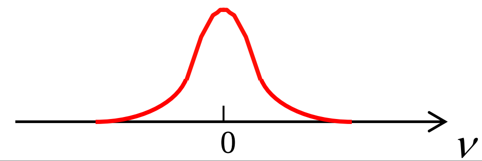
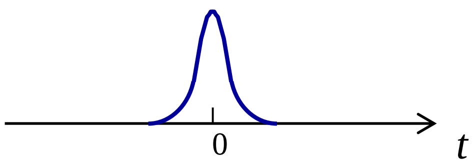
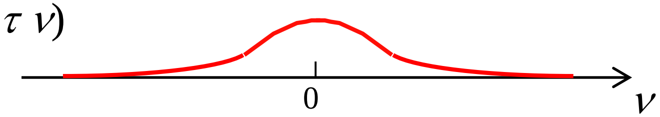




In Class: Experimental Set-up



Properties of the Fourier Transform

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

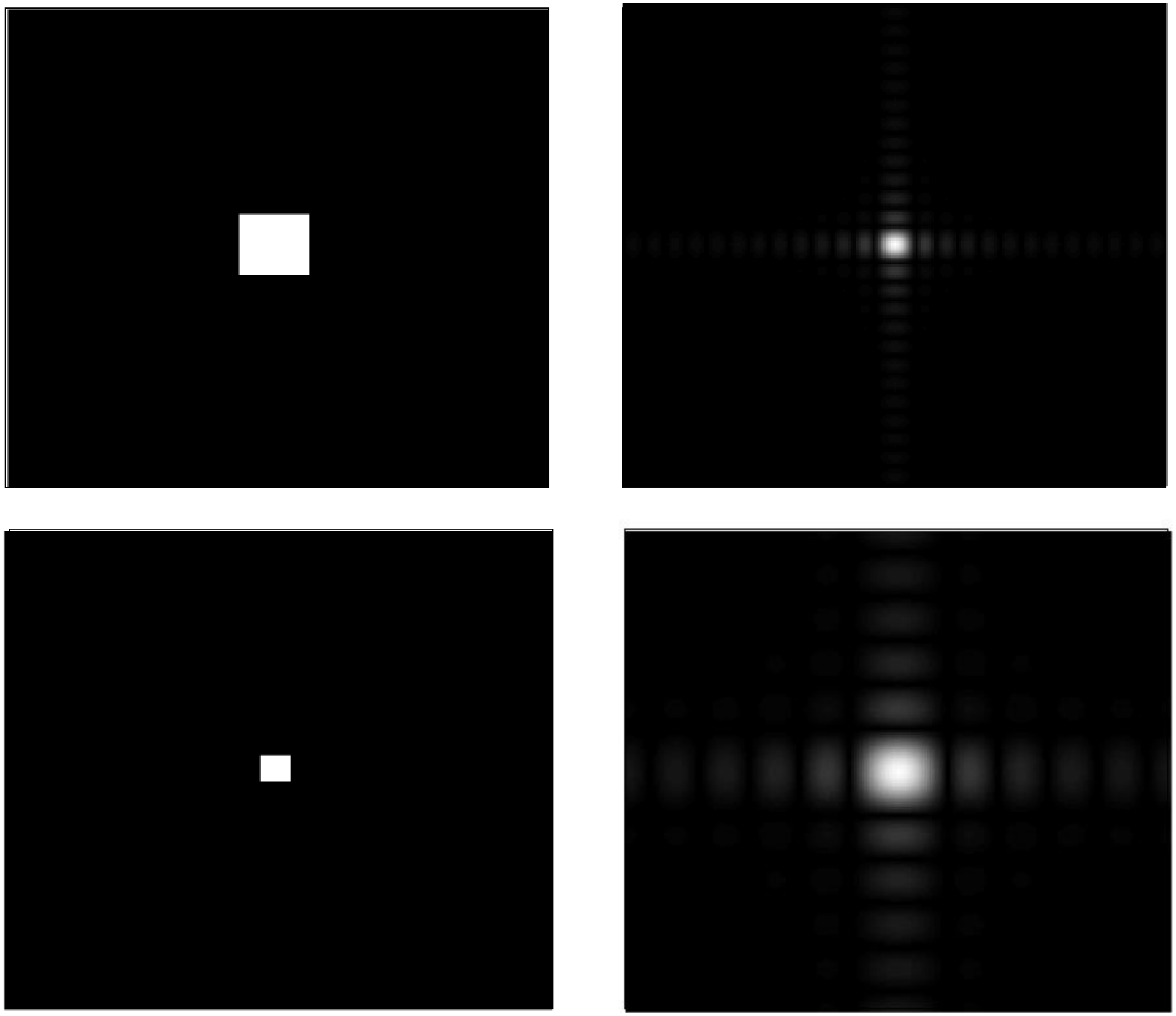
When you scale down a function in time/space domain, its F.T. will spread in the frequency domain

Example: Scaling of an 2D spatial object

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

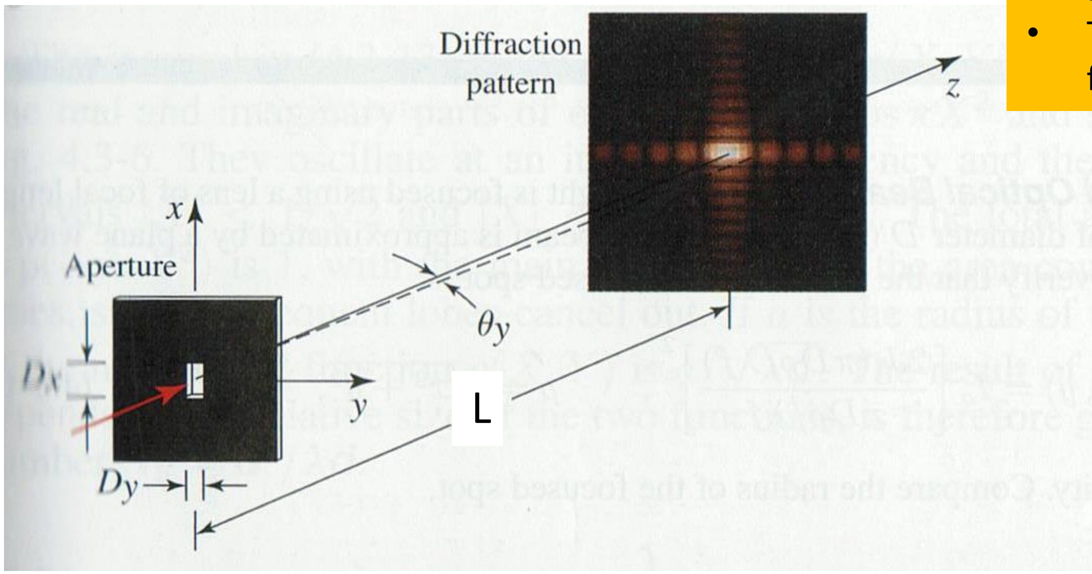
Specimen Plane

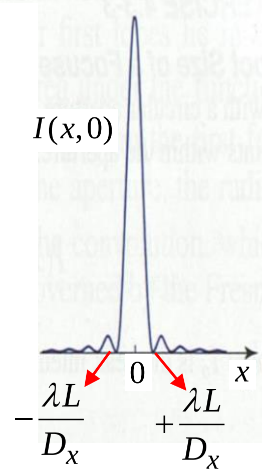
Fourier Plane

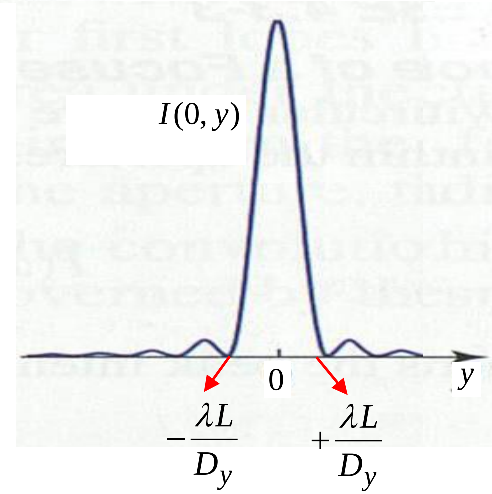


Diffraction of a 2D-Rectangular Aperture

- 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:

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

OUTPUT:

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

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

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

Diffraction of a Circular Aperture

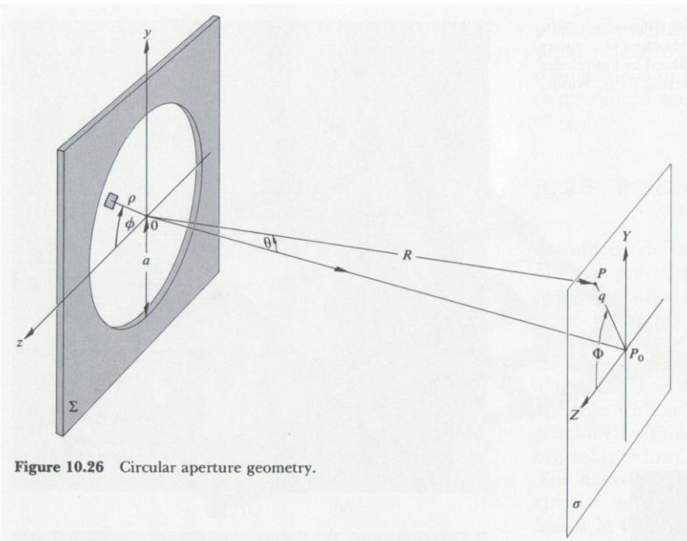
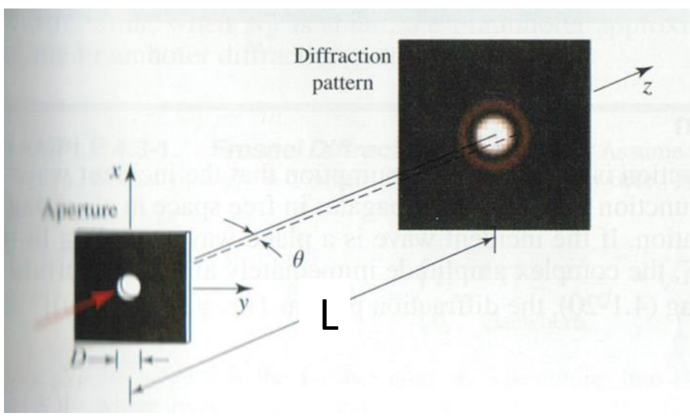


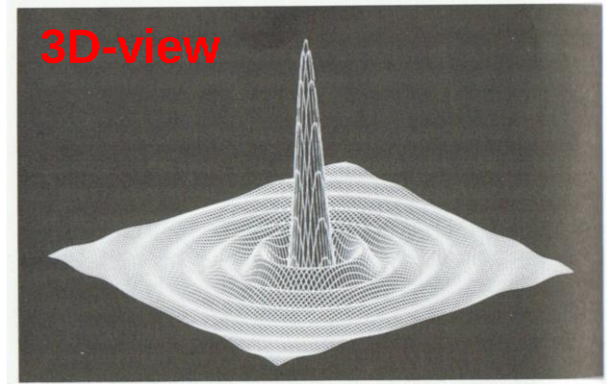
Figure 10.26 Circular aperture geometry.

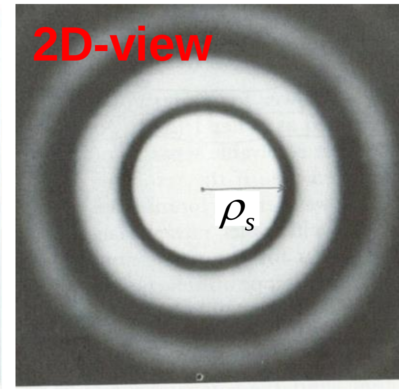


INPUT:

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

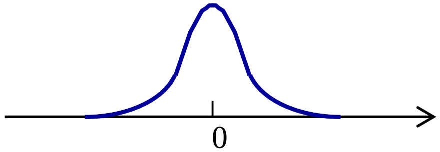
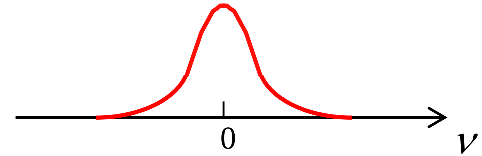
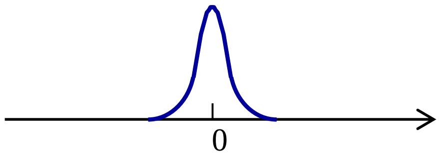
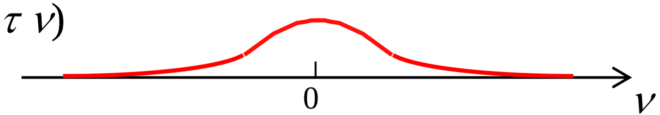
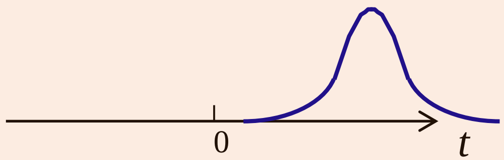
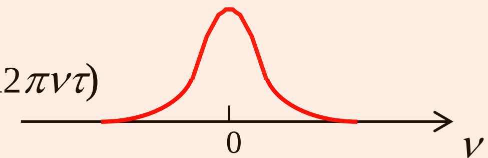
OUTPUT:

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

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

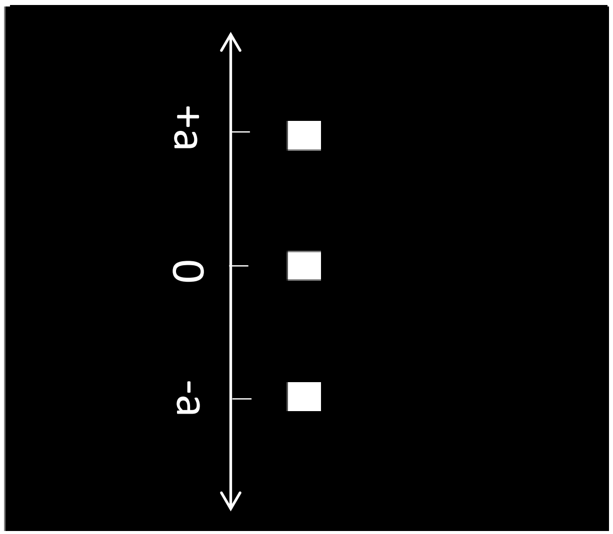
$$angle \quad \theta = 1.22\frac{\lambda}{D}$$

Properties of the Fourier Transform

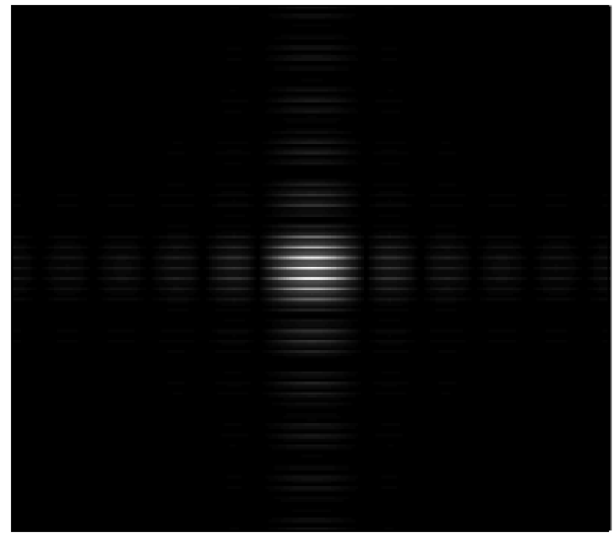
	 $f(t)$	$F(\nu)$ 
Scaling	 $f(t/\tau)$	$\|\tau\|F(\tau\,\nu)$ 
Translation	 $f(t-\ \tau)$	$F(\nu)\ \exp(-i2\pi\nu\tau)$ 

Example: Superimpose Shifted Objects

Specimen Plane



Fourier Plane



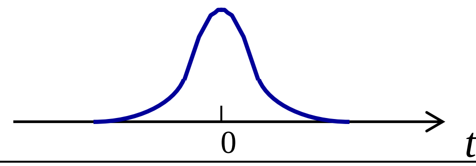
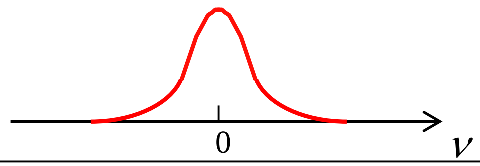
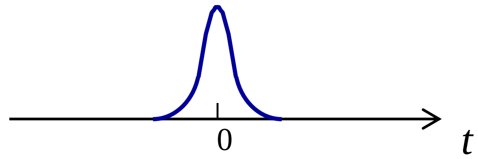
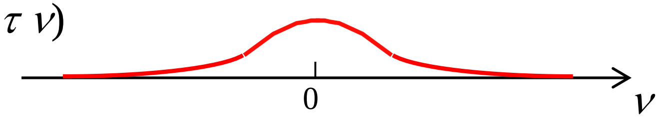
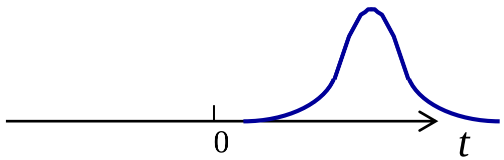
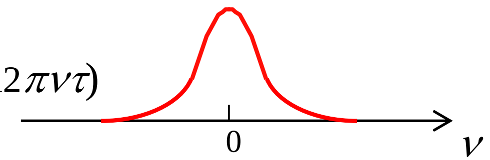
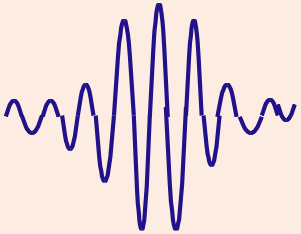
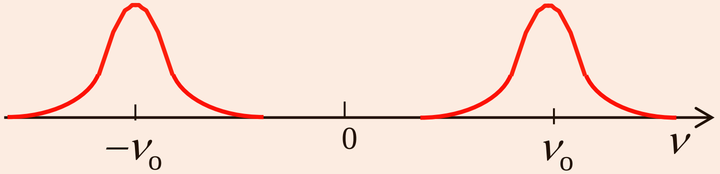
If we assume a 1D case, then input function is:

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

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

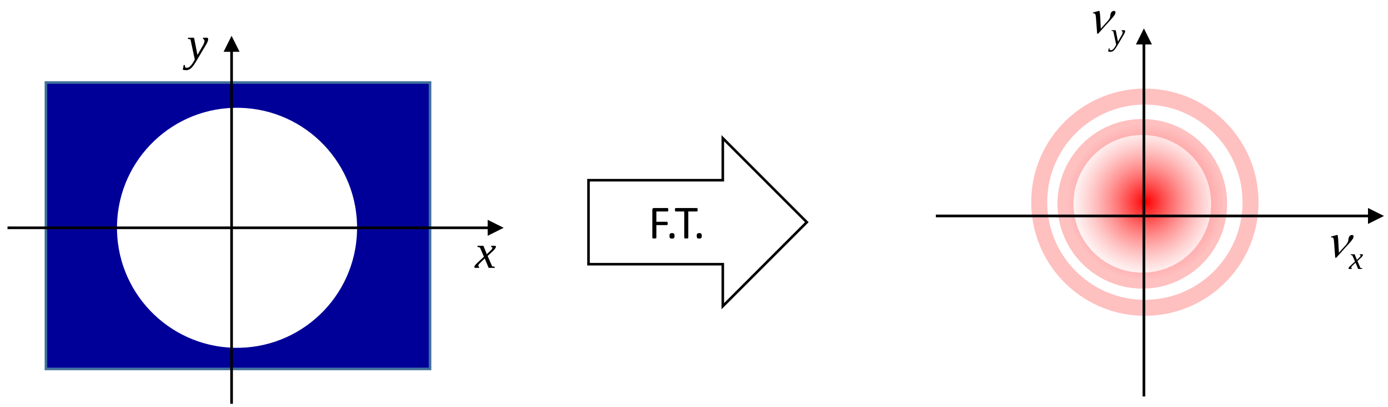
$F(\nu) = sinc(\nu)\ [1 + \exp(-i2\pi\nu a) + \exp(+i2\pi\nu a)]$

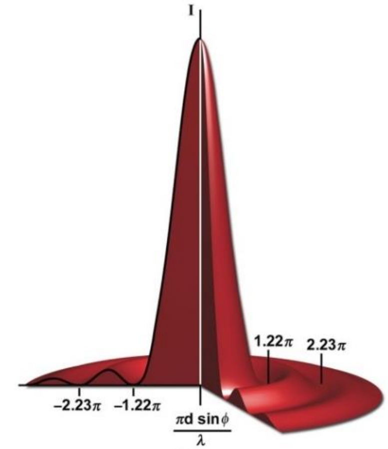
Properties of the Fourier Transform

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

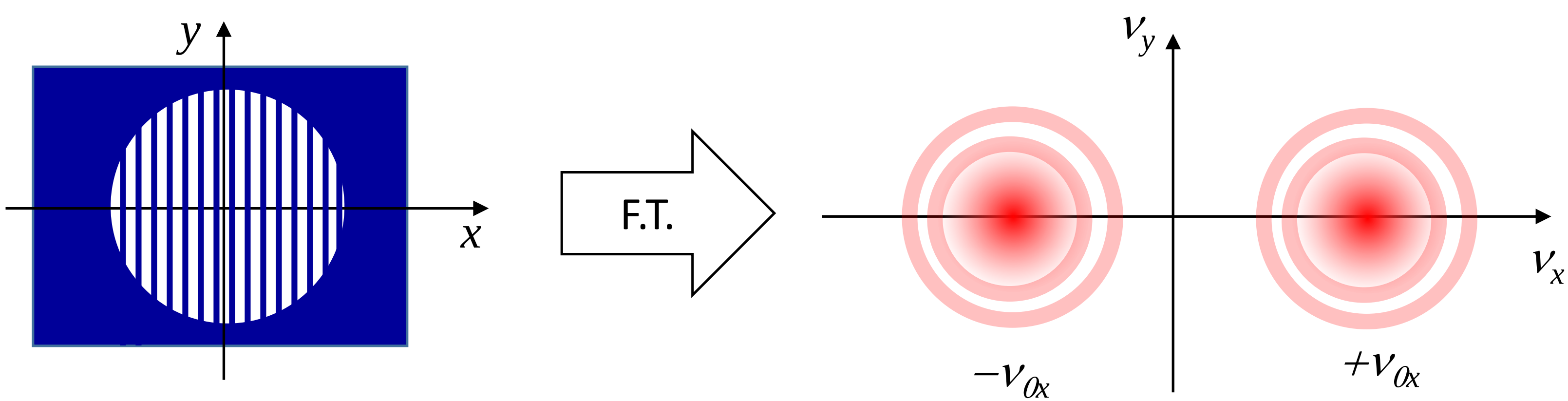
Example: Spatially Modulated 2D Object

$f(x,y)$ $\qquad$ $F(\nu_x, \nu_y)$

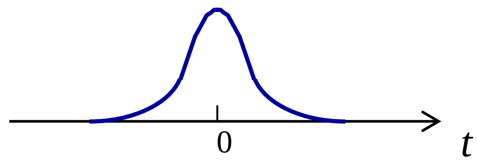
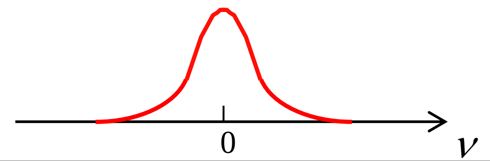
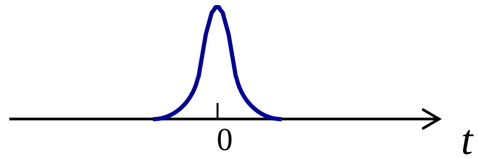
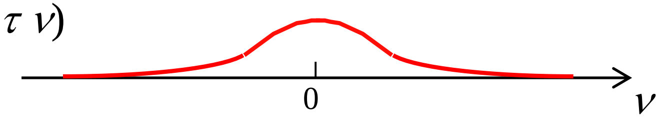
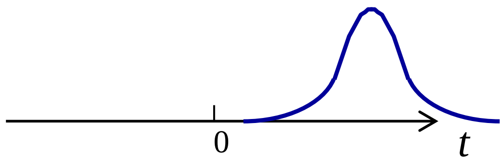
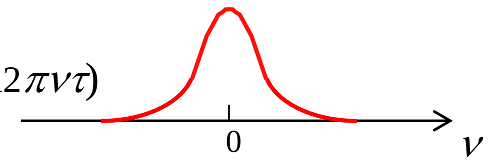
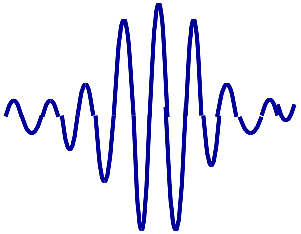
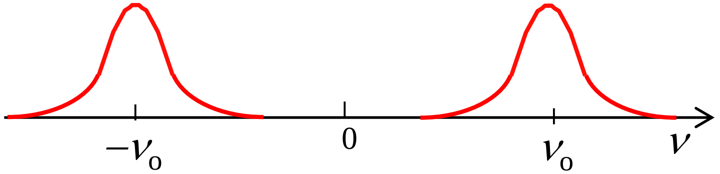




$f(x,y)\cos(2\pi\nu_{0x}x)$ $\qquad$ $F(\nu_x - \nu_{0x}, \nu_y) + F(\nu_x + \nu_{0x}, \nu_y)$

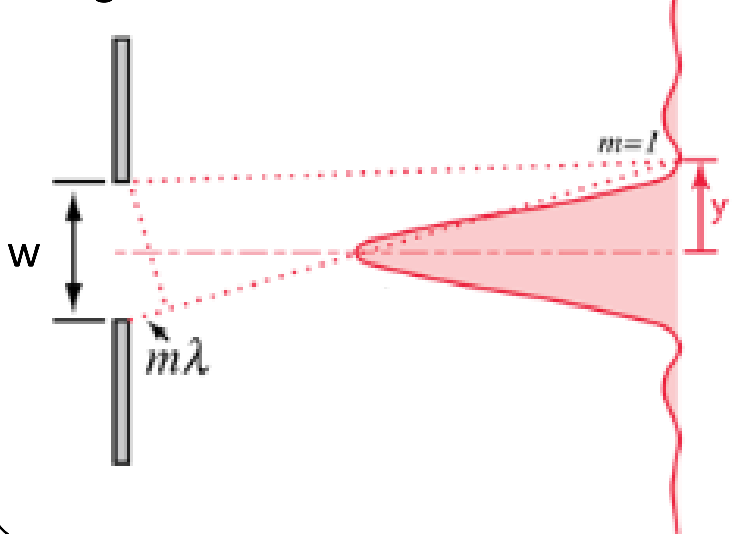


Properties of the Fourier Transform

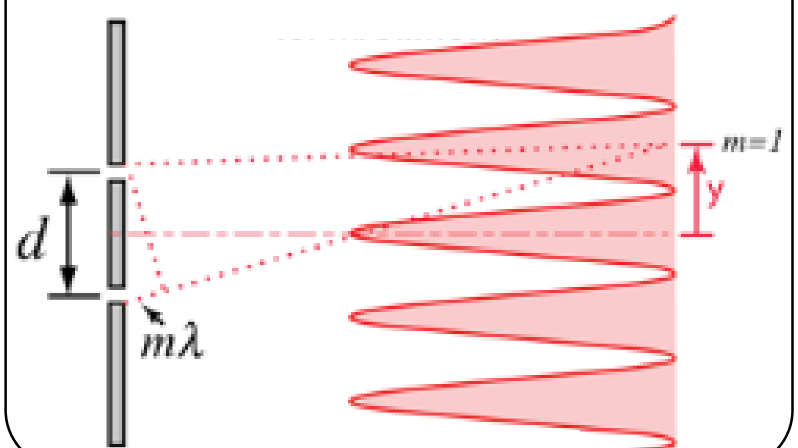
	 $f(t)$	$F(\nu)$ 
Scaling	 $f(t/\tau)$	$\|\tau\|F(\tau\,\nu)$ 
Translation	 $f(t- \tau)$	$F(\nu)$ exp$(-i2\pi\nu\tau)$ 
Modulation	 $f(t)\cos(2\pi\nu_o t)$	 $F(\nu+\nu_o)/2 + F(\nu-\nu_o)/2$
Convolution	$f(t) * g(t)$	$F(\nu)\,.\,G(\nu)$

Example: Diffraction with single slit and double slits

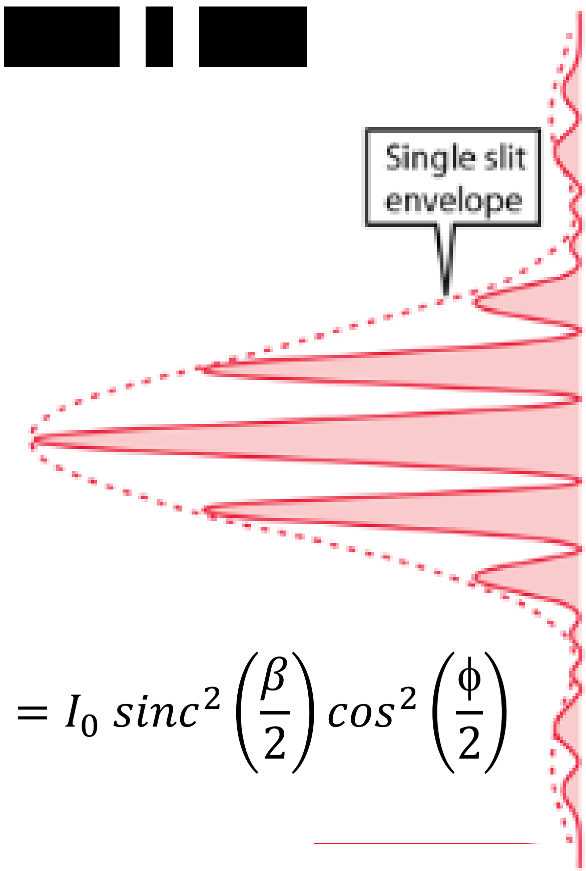
Single slit with a finite width "w"



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



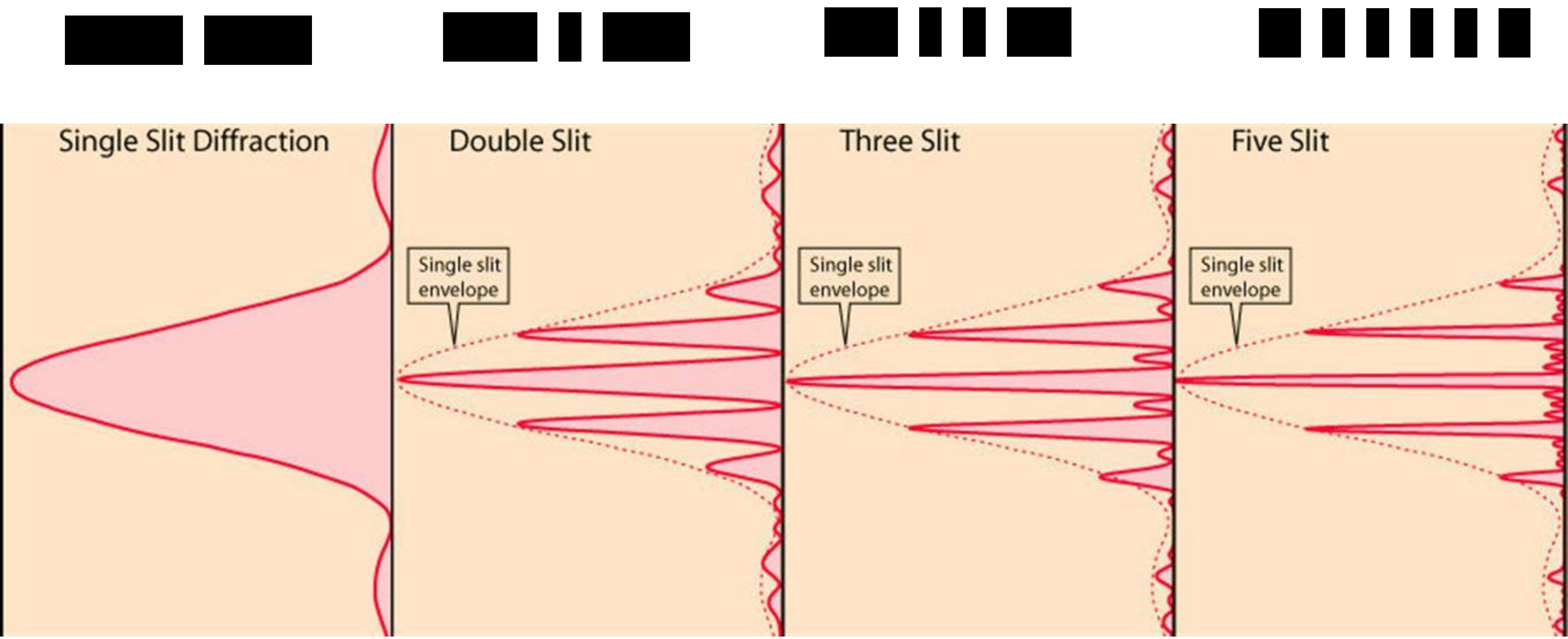
Convolution of these two yields:
Double slit with a finite width (w) and separation (d)



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

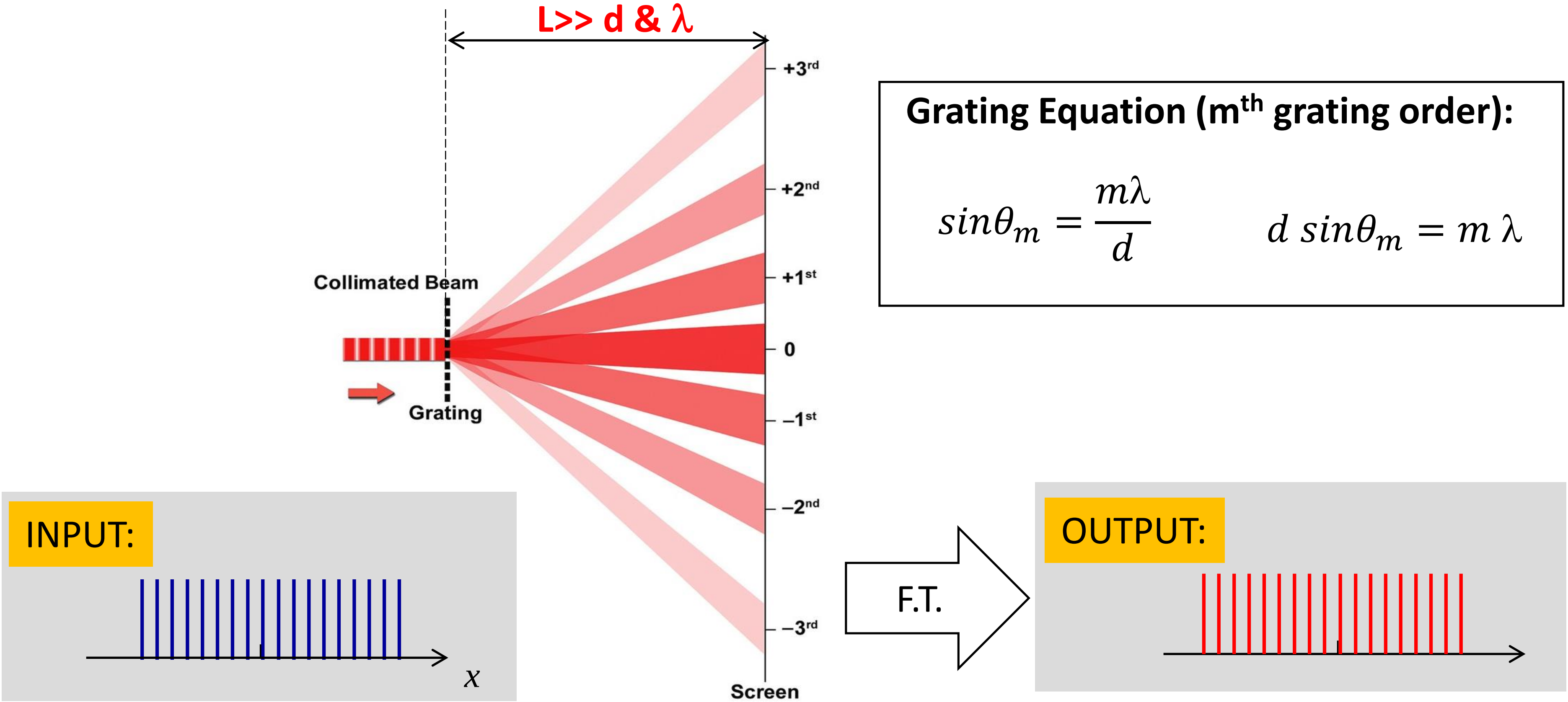
$$\beta = \frac{2\pi w}{\lambda} sin\theta \qquad \phi = \frac{2\pi d}{\lambda} sin\theta$$

Diffraction by slits with finite width



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

Recall: Diffraction of a "**grating**"



Grating Equation (m^th grating order):

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

Diffraction angle θ_m increases as the period *d* decreases

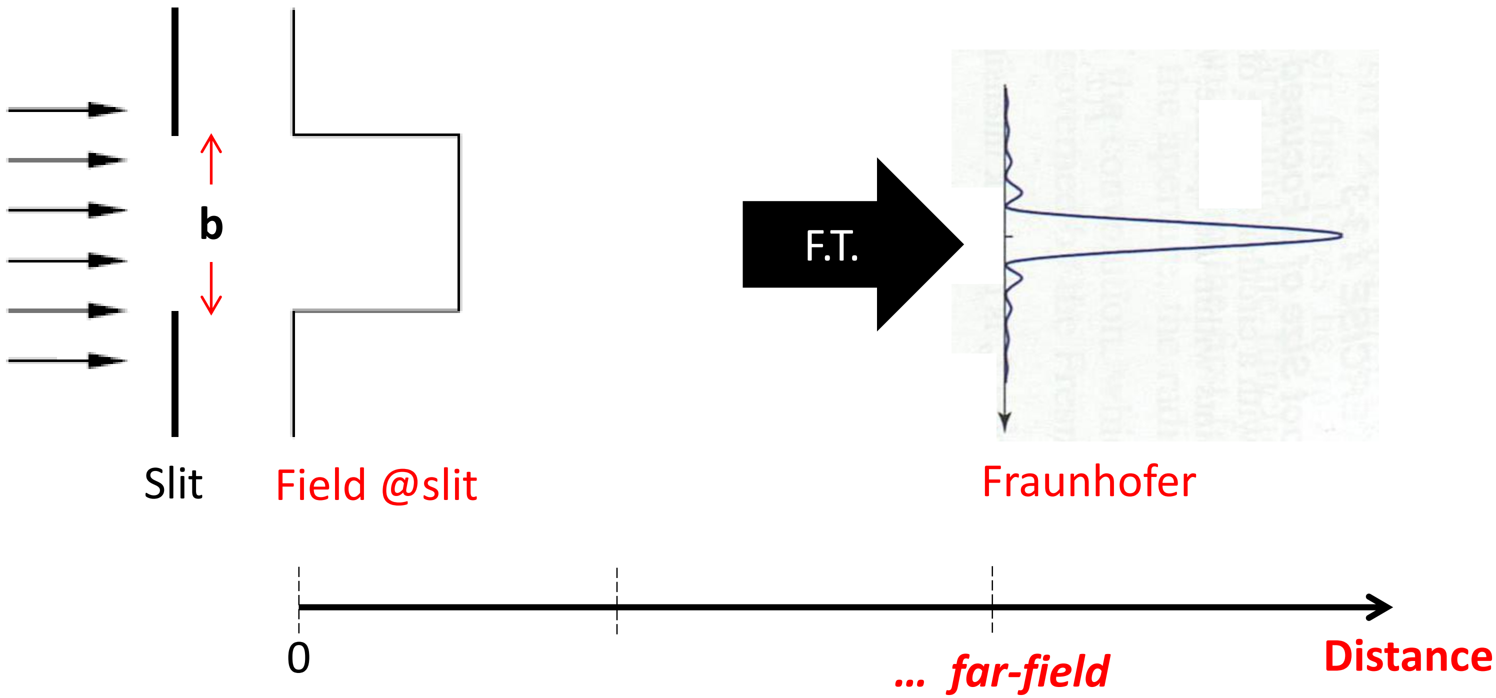
➔ Smaller features diffracts more!

Diffraction angle θ_m increases as the wavelength λ increases

➔ Diffraction grating can be used to **disperse** white light!

- For a given input function, we obtain the output pattern (which corresponds to the F.T. of the input function) at far distance….
- What is the criteria for being at far distance?

Diffraction of a 1D Rectangular Aperture



Fourier transform plane is at the far-field:

➔ Far-field observation condition is given by *Fraunhofer* Approximation, which is **$b^2/\lambda L \ll 1$**

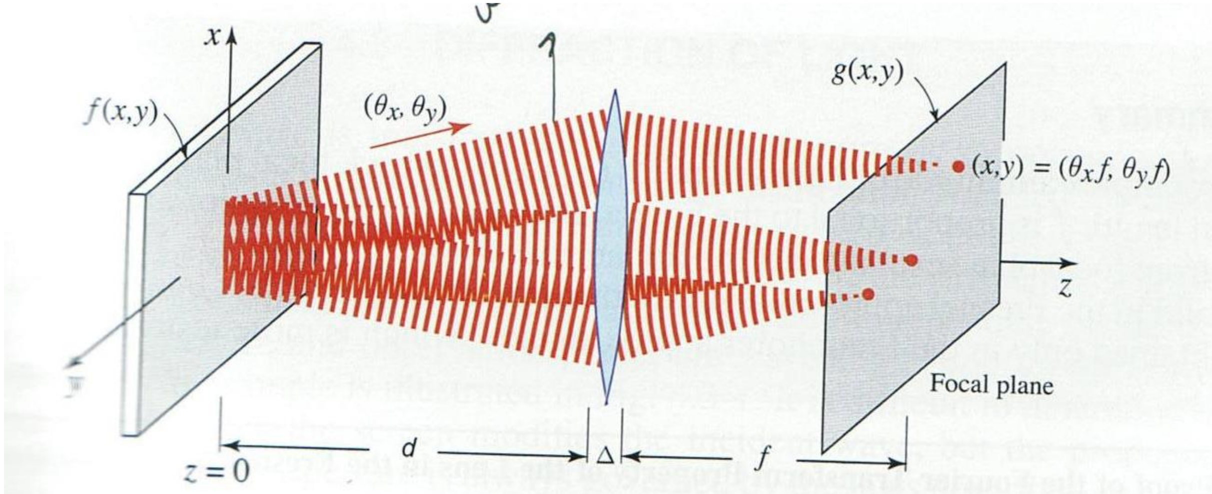
For λ = 500nm (green)

i) b =2.5 cm ➔ L >> 1.9 km

ii) b =1 mm ➔ L >> 6 m

iii) b =2 μm ➔ L >> 0.15 mm

A lens can bring the F.T. plane from far-field to a closer distance



Input:
f(x,y)

Output at the back-focal plane of the lens:
F(ν_x, ν_y)

Recall 1-to-1 relationship between:

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

angle spatial frequency

Back Focal Plane of Lens = Fourier Plane

Each wave is focused in Fourier plane at:

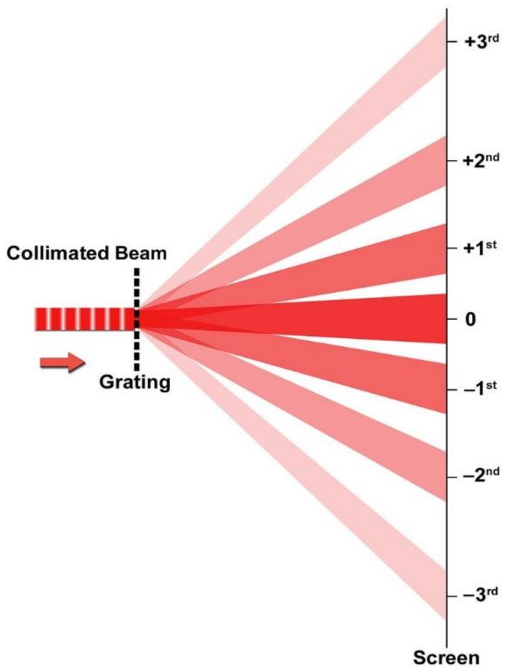
$$x = \theta_x f$$

$$x = \lambda \nu_x f$$

Derivation is given in Saleh & Teich, Ch. 4

Reminder: Spatial Frequency & Angles

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



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

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

.
.
.

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

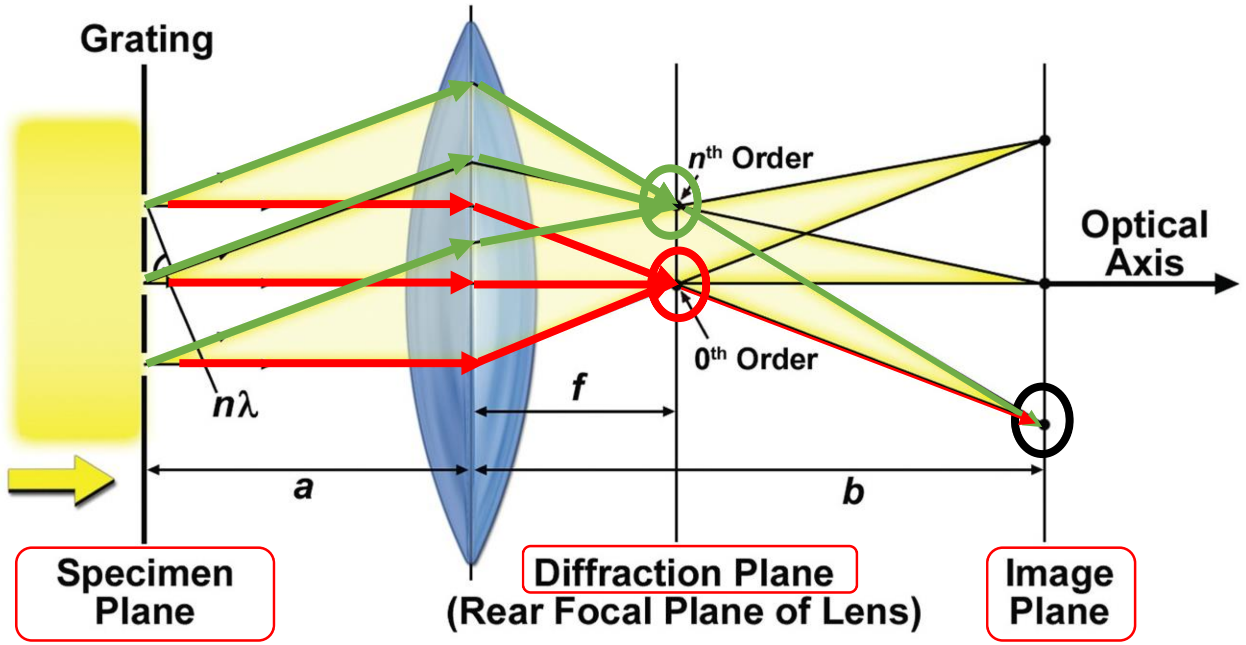
$$\theta_i \approx \lambda\nu_i$$

angle

spatial frequency

- "**Larger angle**" means "**higher spatial frequency**"
- "**Higher frequency**" corresponds to "**smaller feature**"
  ➔ THUS, smaller features contain higher frequency

An Example: Fourier (a.k.a Diffraction) Plane



- Let's assume that our object is a grating, and it is placed on specimen plane, which is located between f & 2f (2f>a>f).

- The objective lens produces a magnified real image of the grating (i.e. object) in the image plane.

- **The Fourier (or diffraction) plane is located at the back focal plane of the lens.**

- The rays of different orders are separated at the *diffraction plane*

- But, the rays of different orders are merged (interfere) at the *image plane*