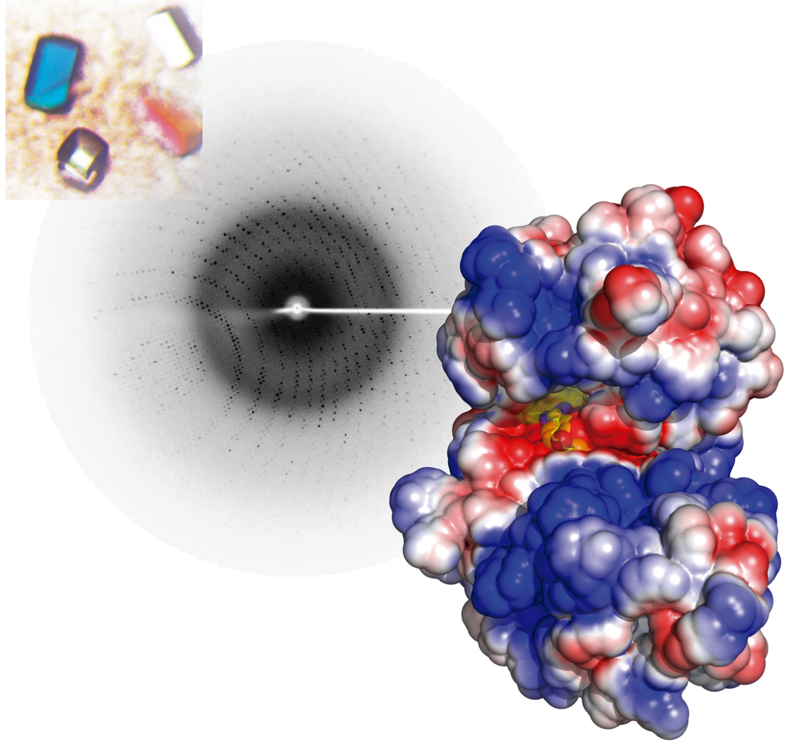
Unit of Protein Crystallography

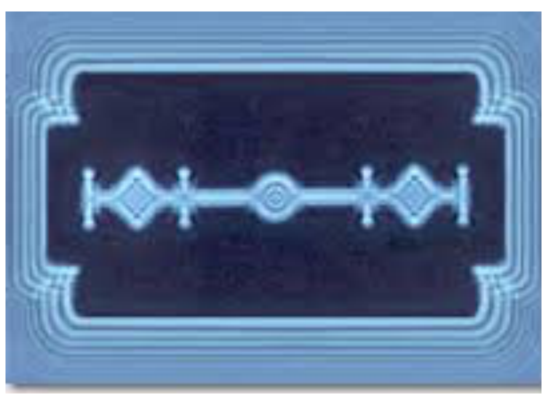
Institut Pasteur de Montevideo

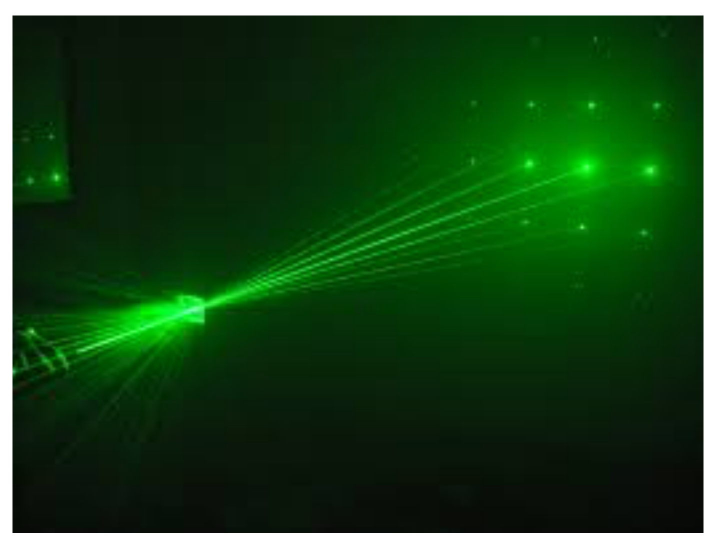
Theory of X-ray diffraction

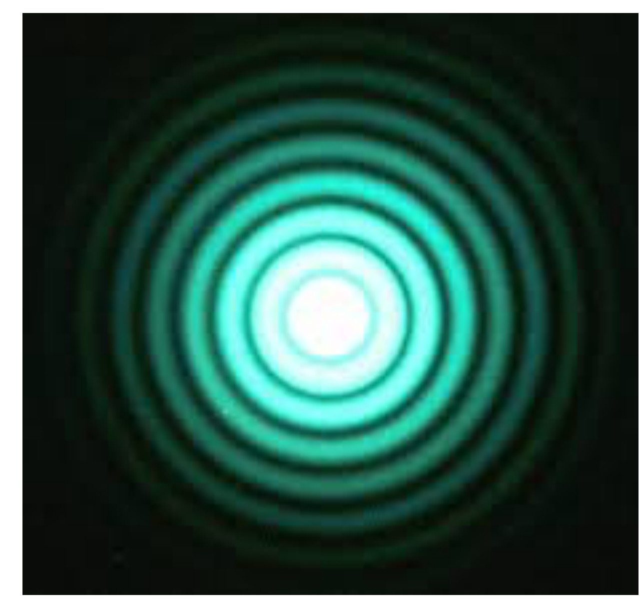


Macromolecular Crystallography School 2018
November 2018 - São Carlos, Brazil

Diffraction manifests itself as the bending of light trajectory around small obstacles and the “unusual” spreading out of waves past small openings



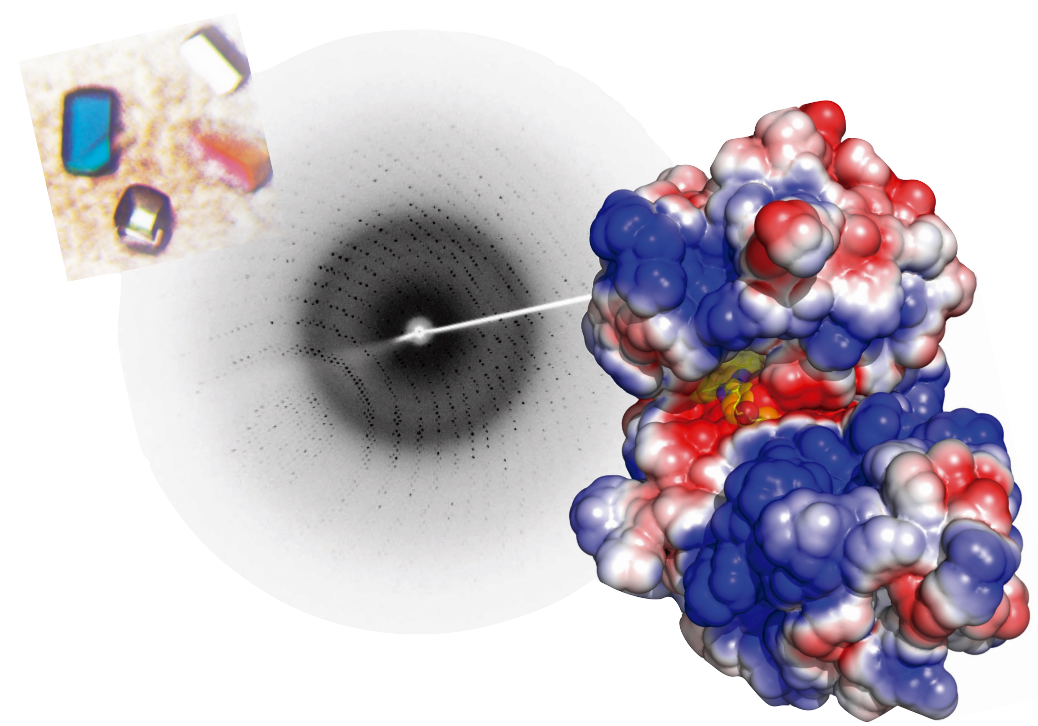


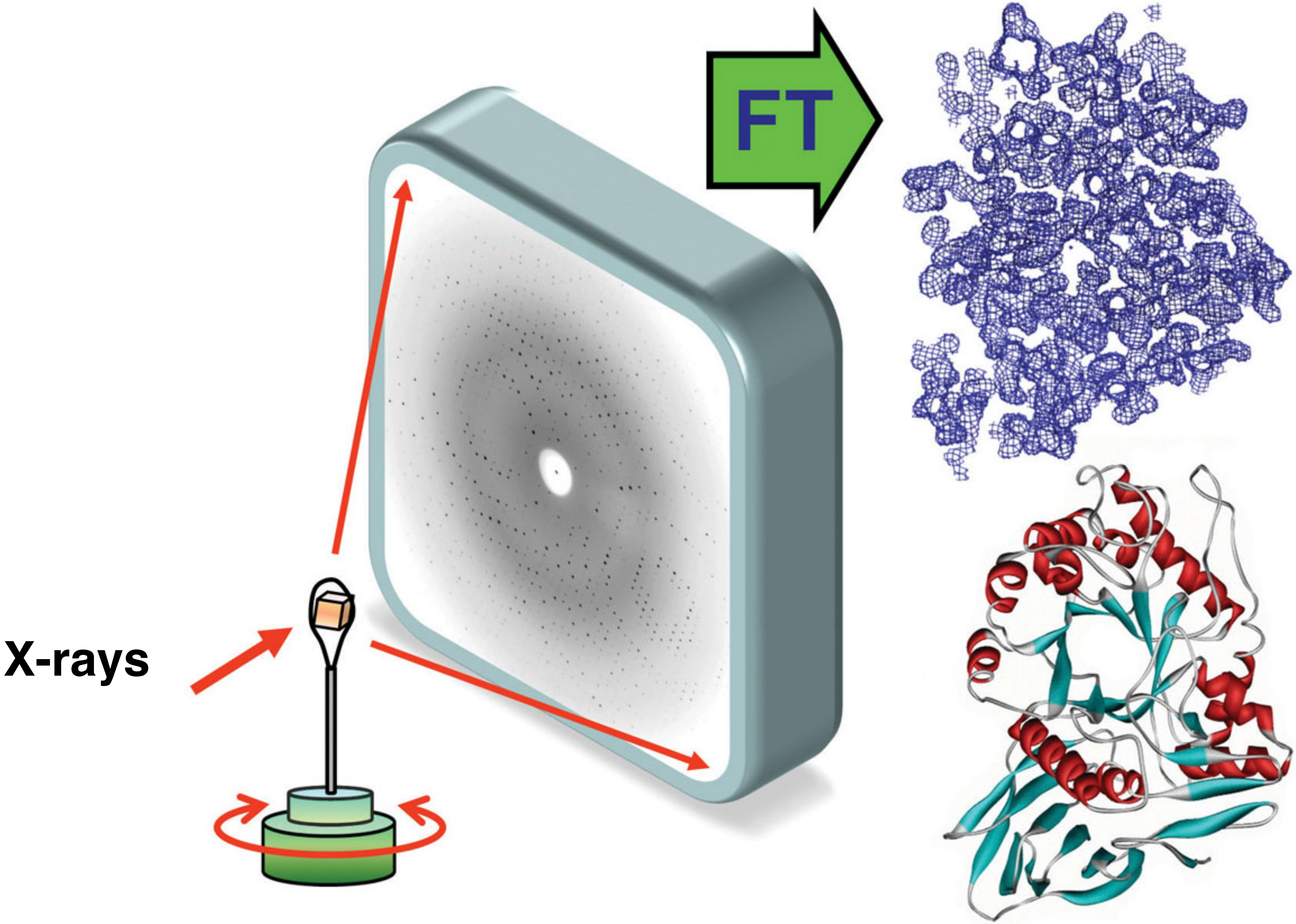


…why would this matter in Biology???

because the pattern produced can be recorded, and gives accurate information about the structure of the material that generated it :

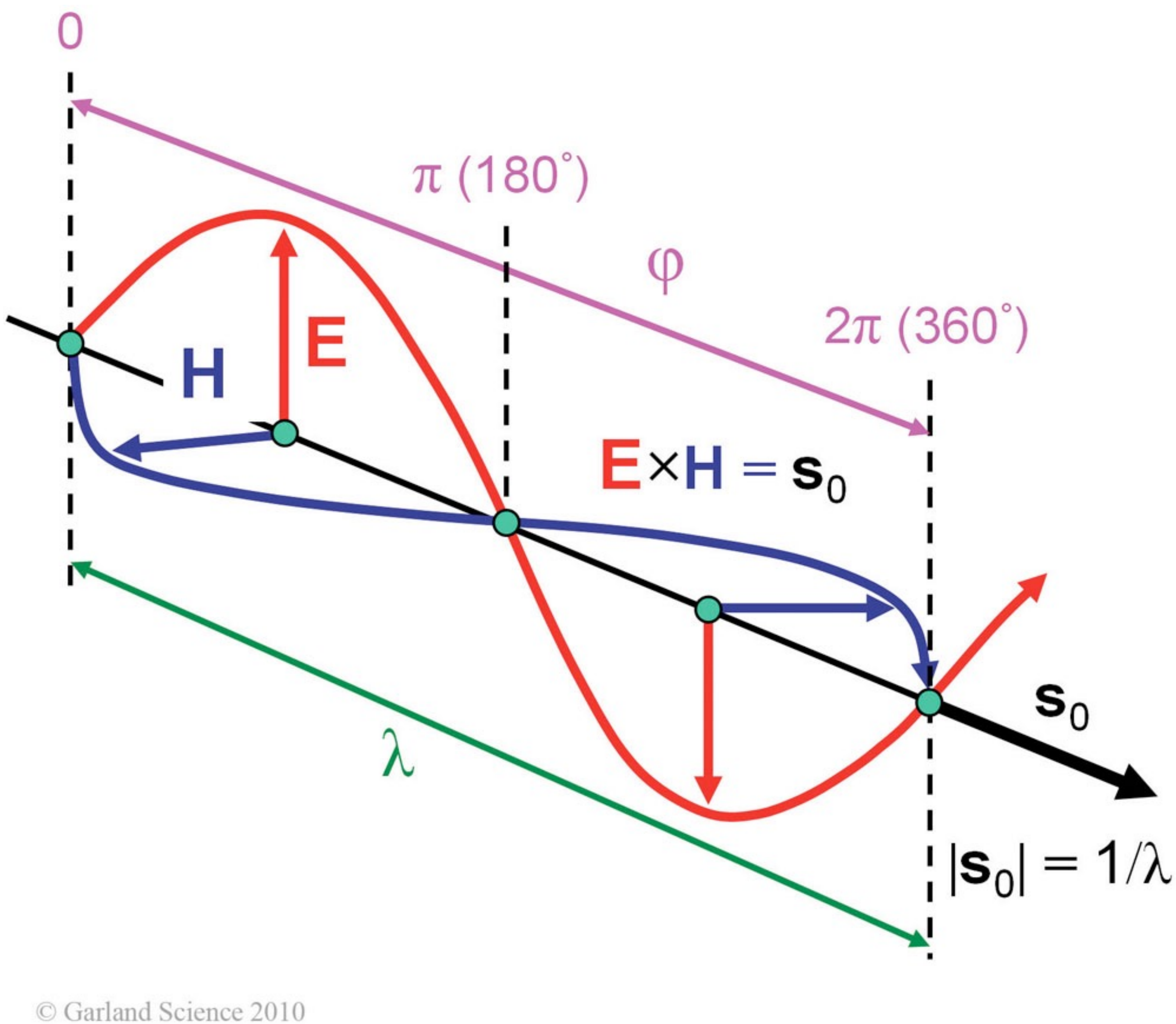
molecules and ordered arrays of molecules (such as in crystals), are known to diffract light, uncovering their three-dimensional structure at the atomic level





from “Biomolecular Crystallography” Bernhard Rupp, Garland Science 2010

Electromagnetic waves



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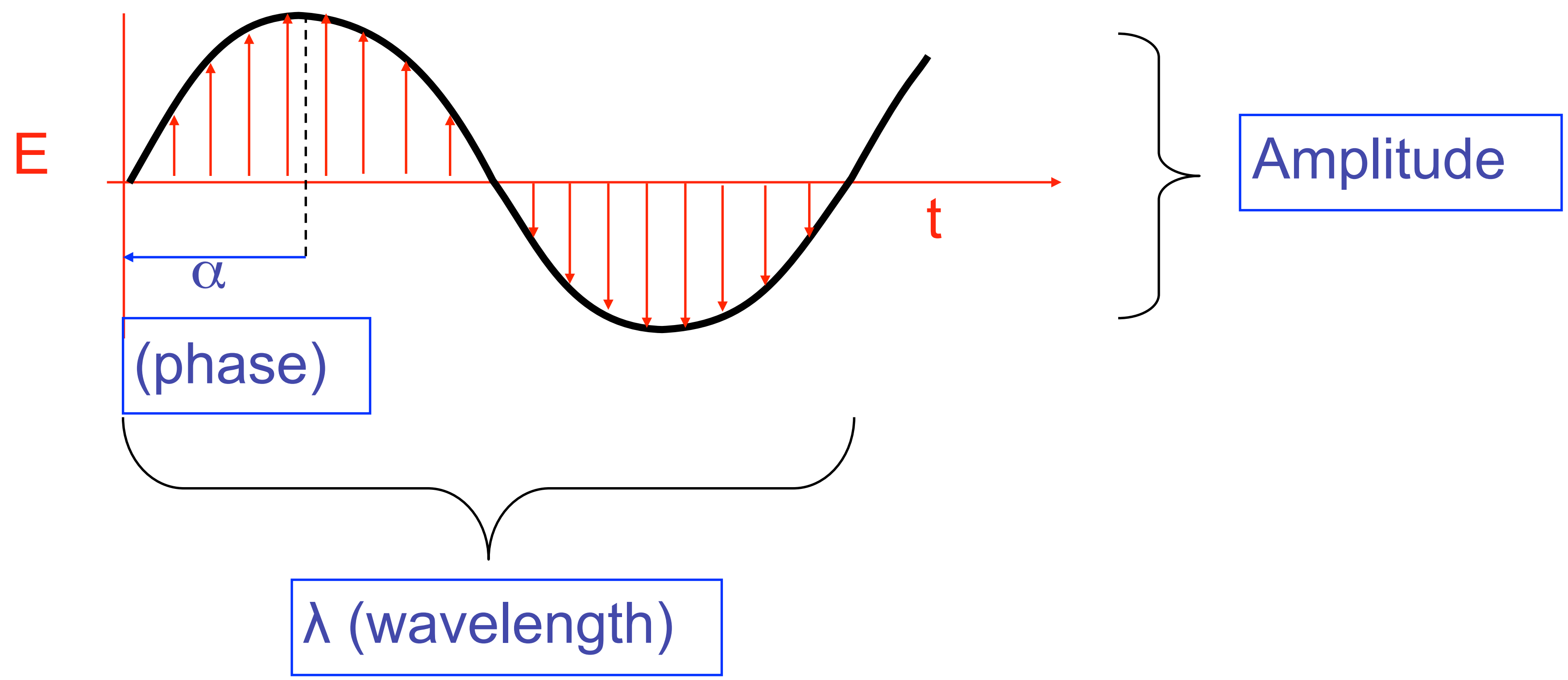
Basic diffraction concepts: waves, interference & reciprocal space

X rays : Photons = particle / wave duality



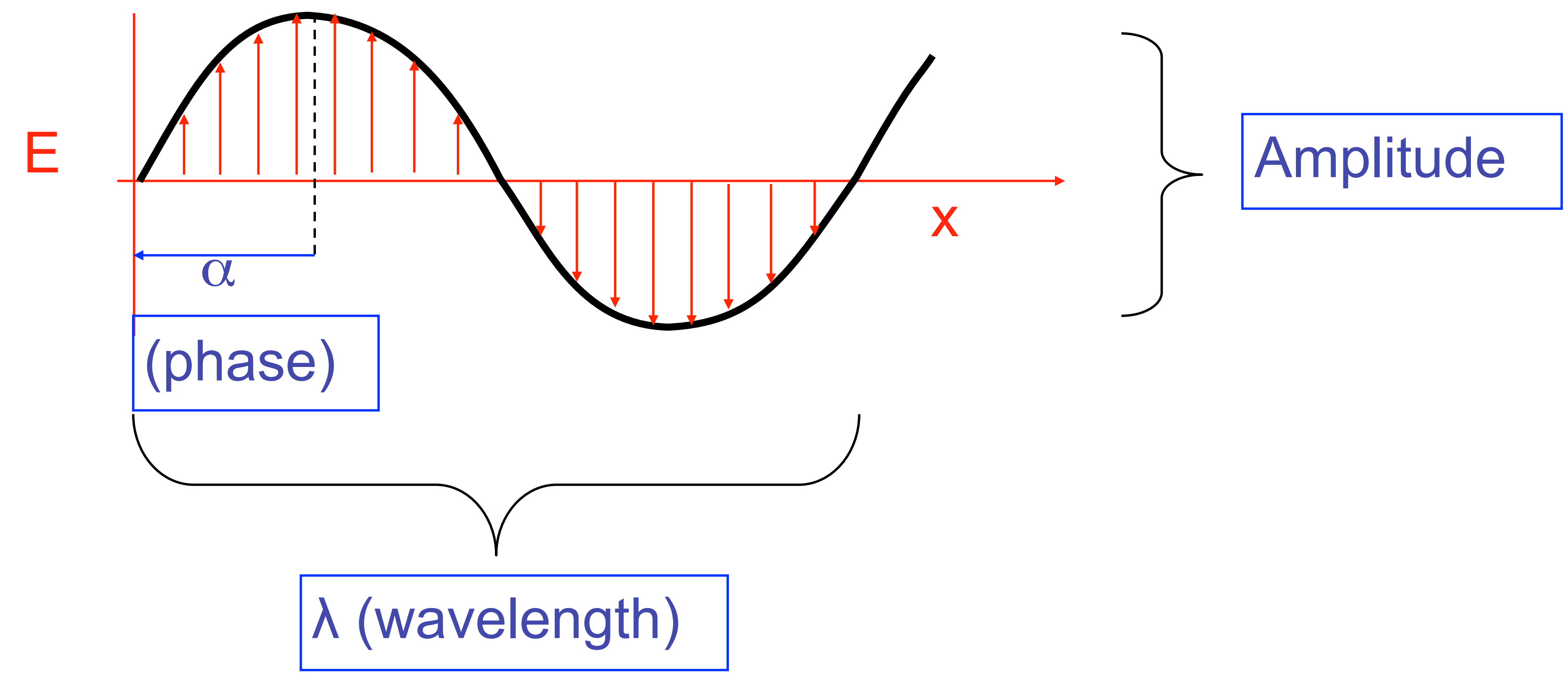
$$E(t) = A\cos(\omega t + \alpha)$$

$\omega = 2\pi\,\nu$

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

Basic diffraction concepts: waves, interference & reciprocal space

X rays : Photons = particle / wave duality



$$E(x) = A\cos(\omega x + \alpha)$$

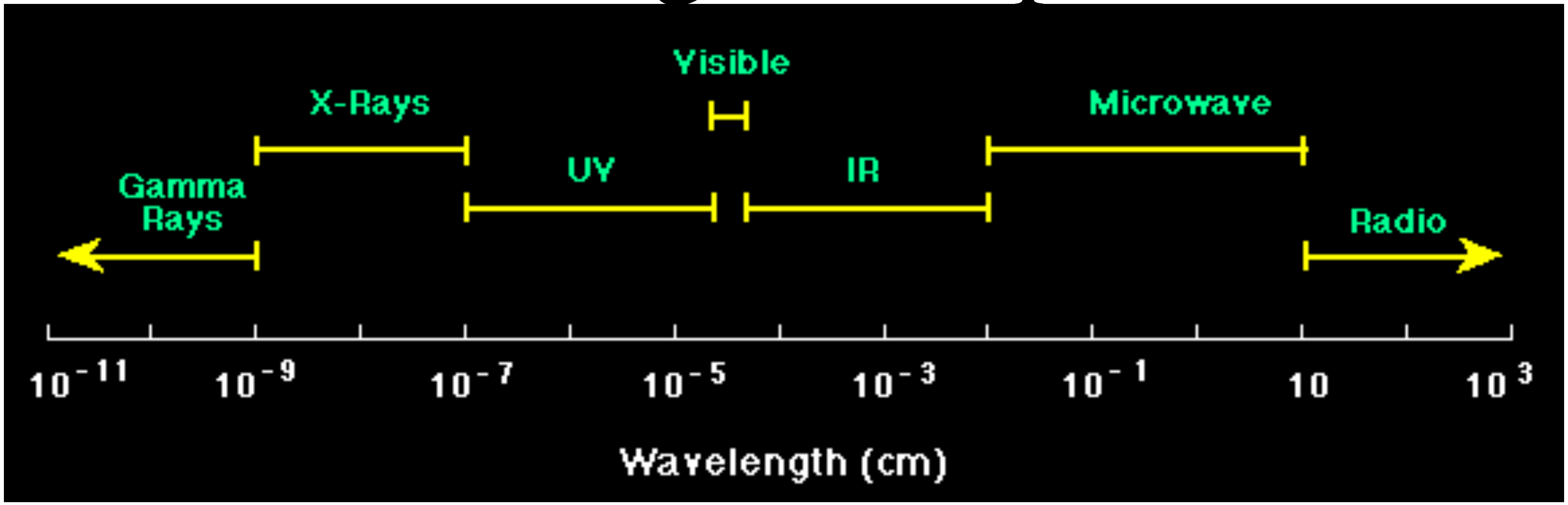
$\omega = 2\pi/\lambda$

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

resolution limit when using light to see!

Relative sizes of the object to study and the wavelength of the illuminating light

Electromagnetic spectrum



The wavelength of X rays is just about right to use in crystallography : 1Å - 3Å (Å = 10^{-8}cm) ; 1.54Å (Cu) often used in the lab

Frecuency = c/λ =(3×10^{10}cm/s) /(1.54×10^{-8}cm) ≈ 2×10^{18} s^{-1}

Interactions of X-rays with matter

Absorption

Elastic scattering (Thomson)

…we will only introduce the classic approach, we leave quantum mechanics for our next edition! 😉

Inelastic scattering (Compton)

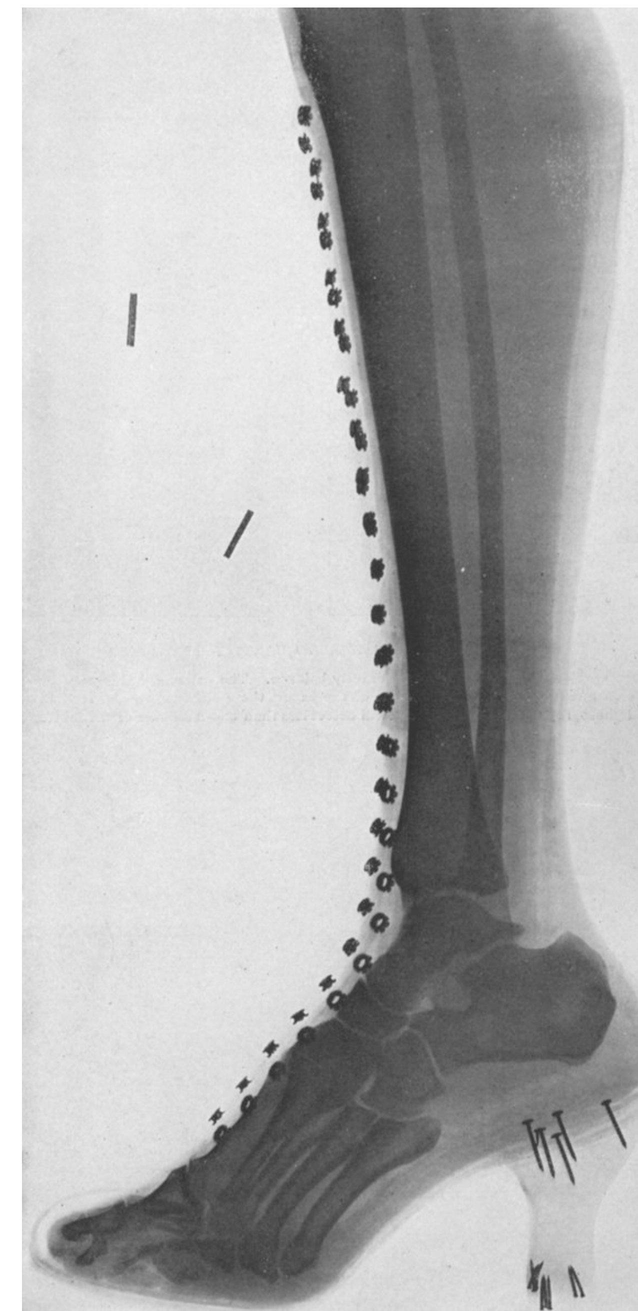
We use X rays, since their wavelengths are just within the right range, to see atoms and interatomic bonds...

but why do we get electron density?

What are X rays?

Photons= an oscillating electric field *

*also a magnetic field of same frequency and phase, but orthogonal



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An electron in an oscillating electric field

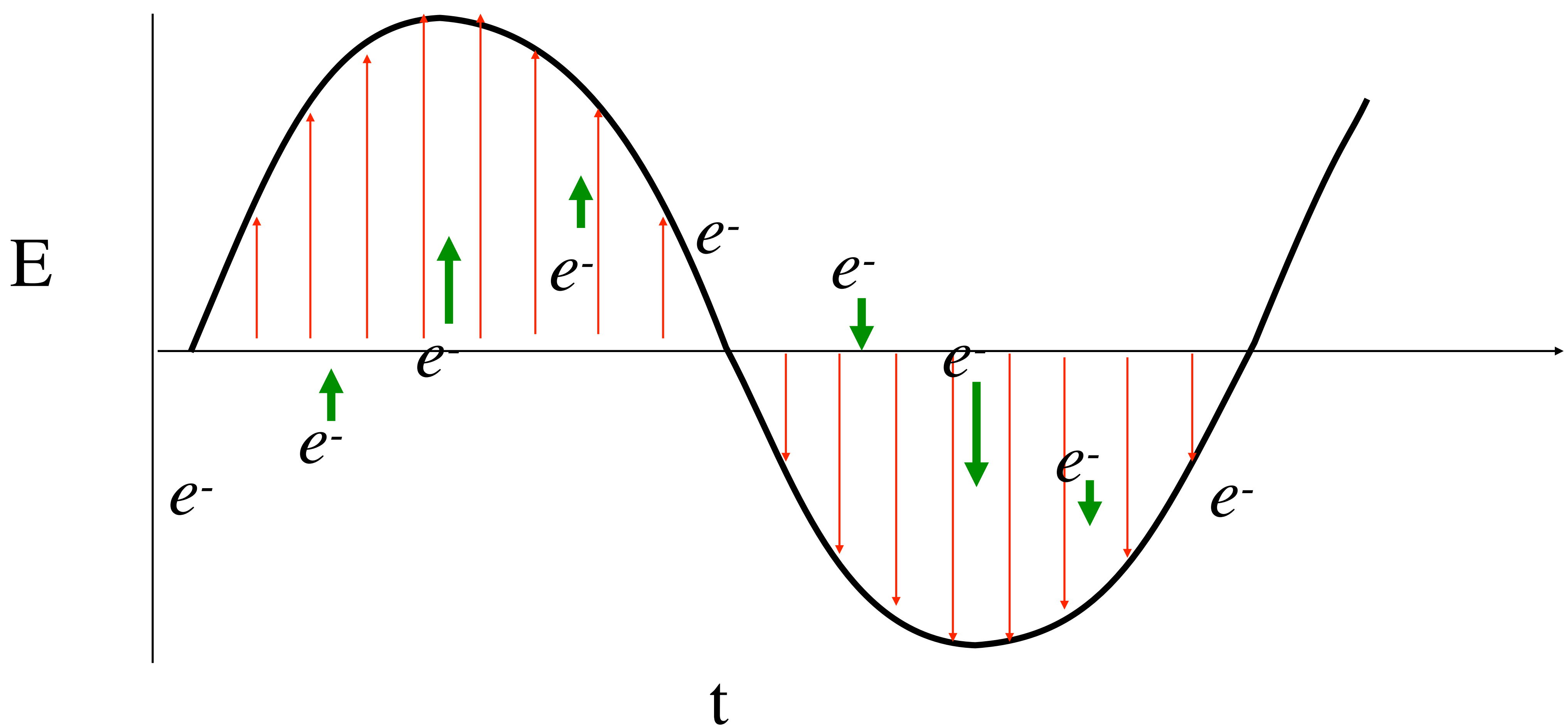
Electrons e^- orbit with a speed of approx 1/100th c ($\approx 2x10^6$m/s),

Hence, in one Rx beam wave cycle, the e^- will travel $2x10^6$m s^{-1} / $2x10^{18}$s^{-1} = 10^{-12}m = 0.01Å (*not much* compared to the size of the atom)

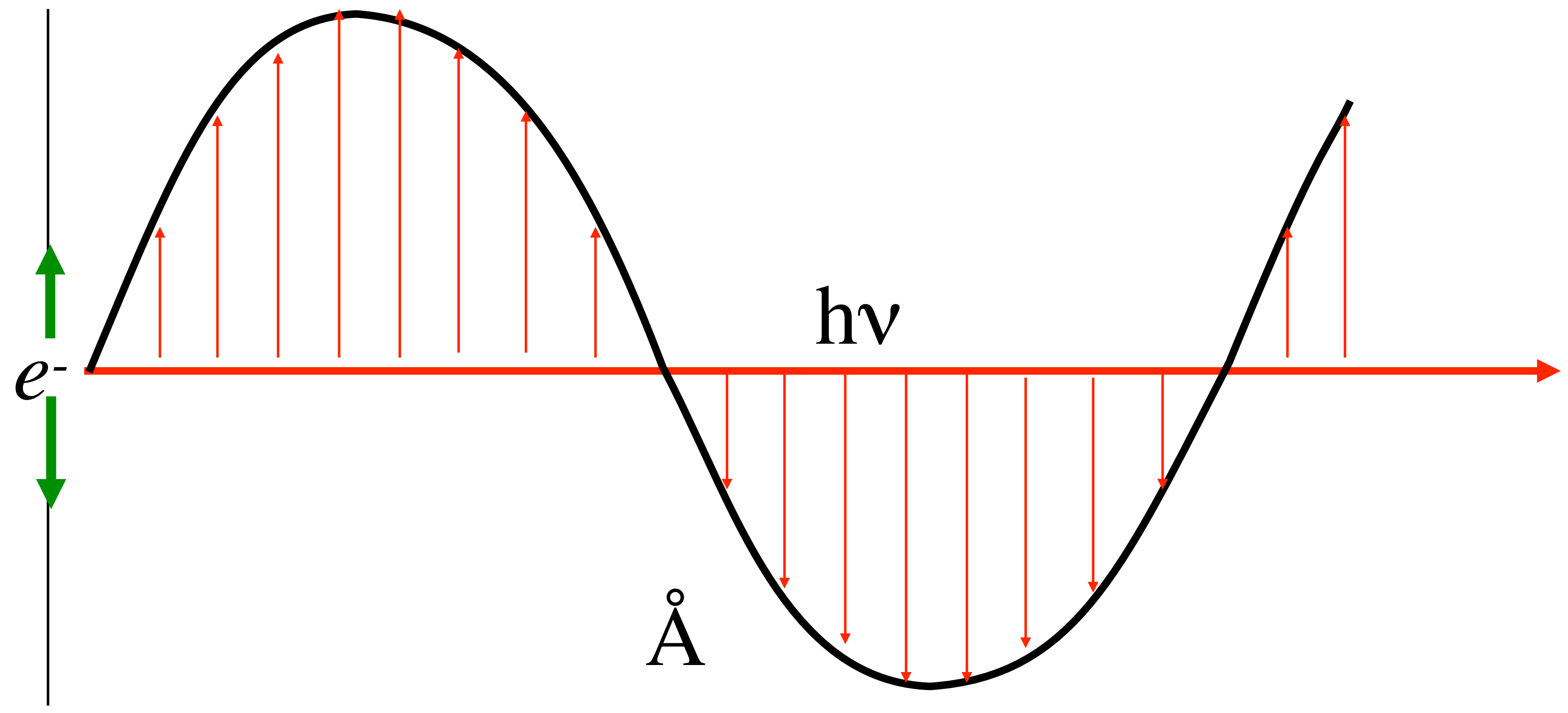
In other words, the X ray beam sees e^- ***as if they were still***.

e^- oscillate in an electric field...

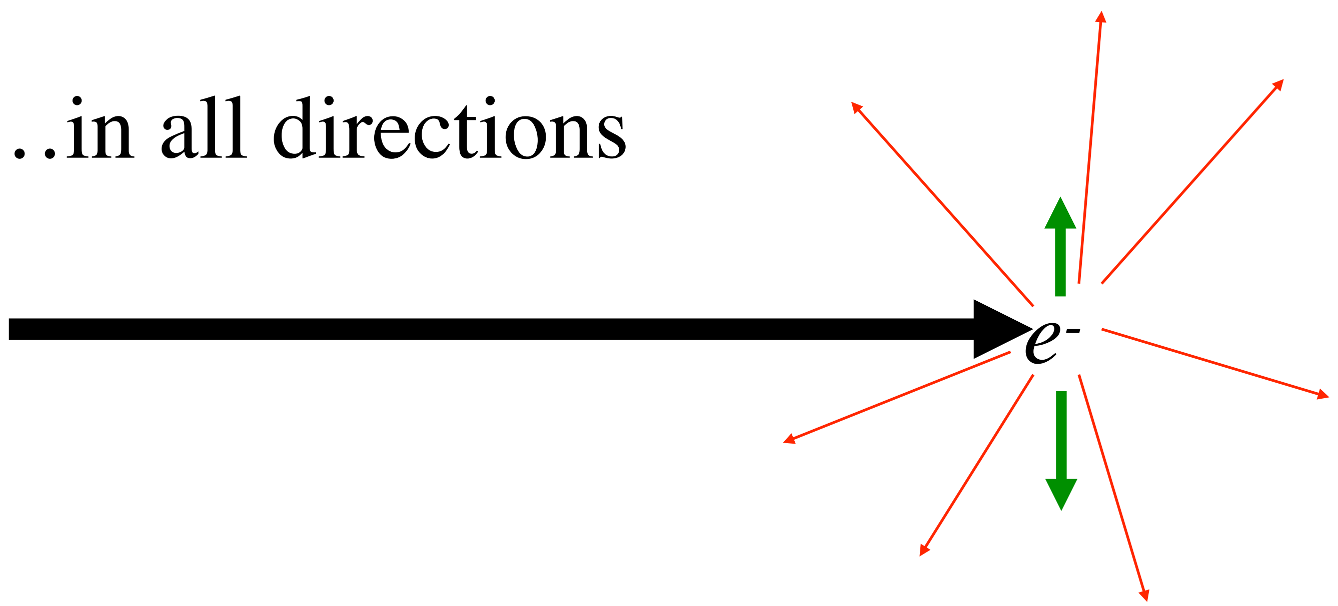
- the oscillation of the e^- has the same frequency as the Rx that hit them : elastic component
- this e^- oscillation is much faster than their orbital movement
- the amplitude of this e^- oscillation is large, because their mass is so small. Atomic nuclei's oscillation is almost undetectable



…charges in oscillation generate photons!

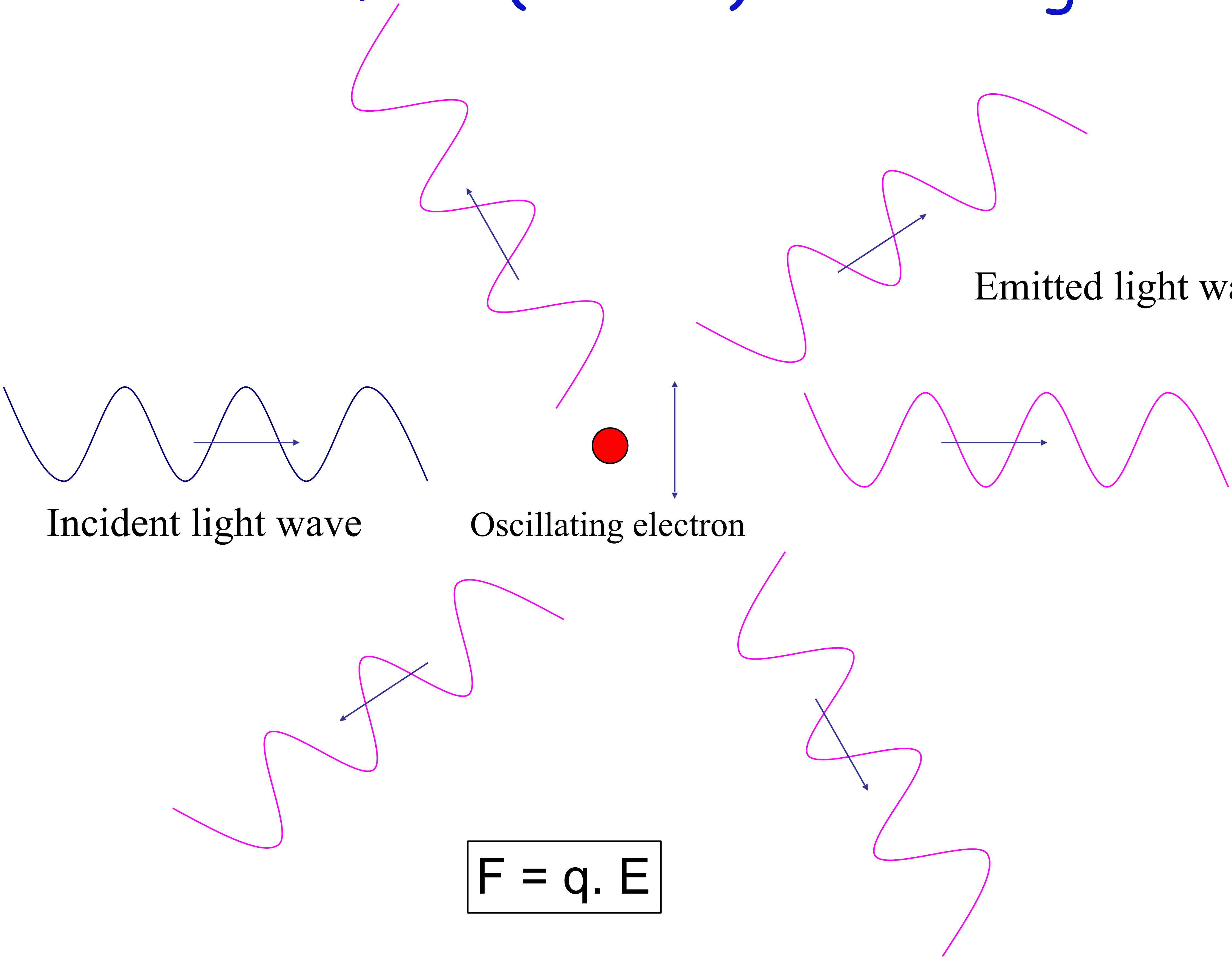


…in all directions



This is called SCATTERING!

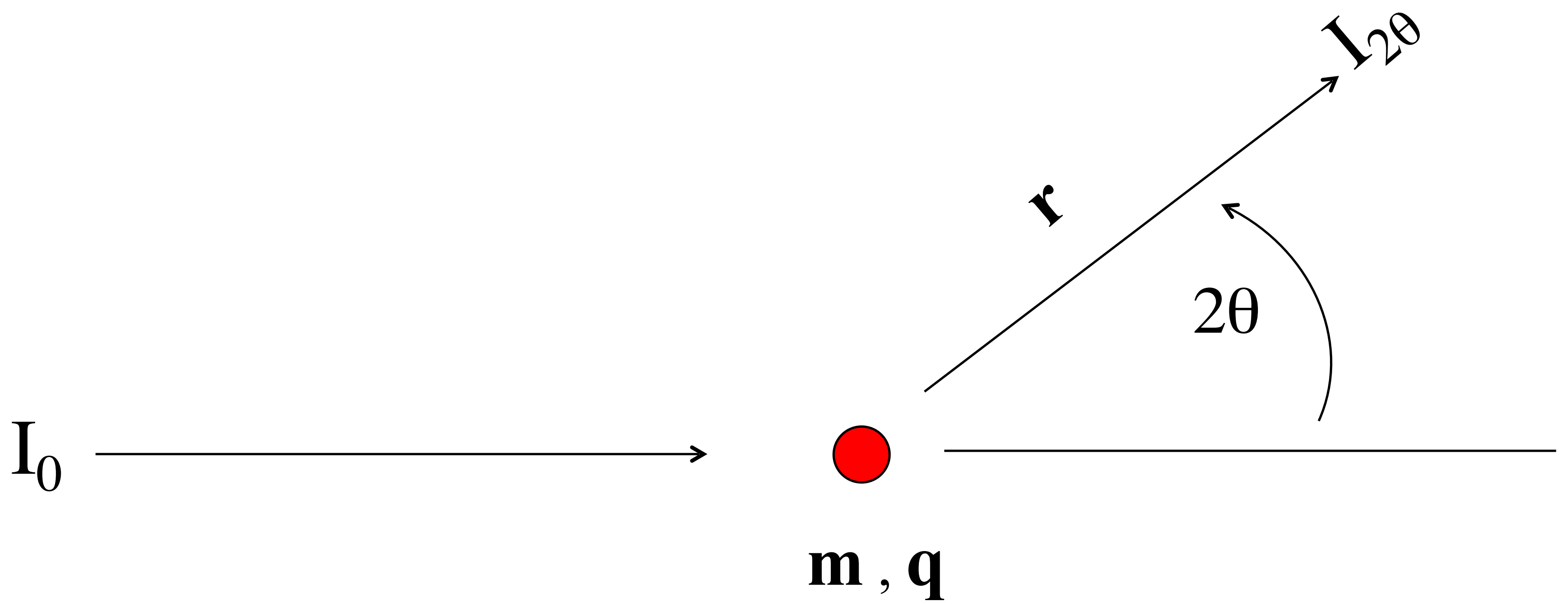
Thomson (elastic) scattering



$$\boxed{F = q.\ E}$$

Thomson scattering

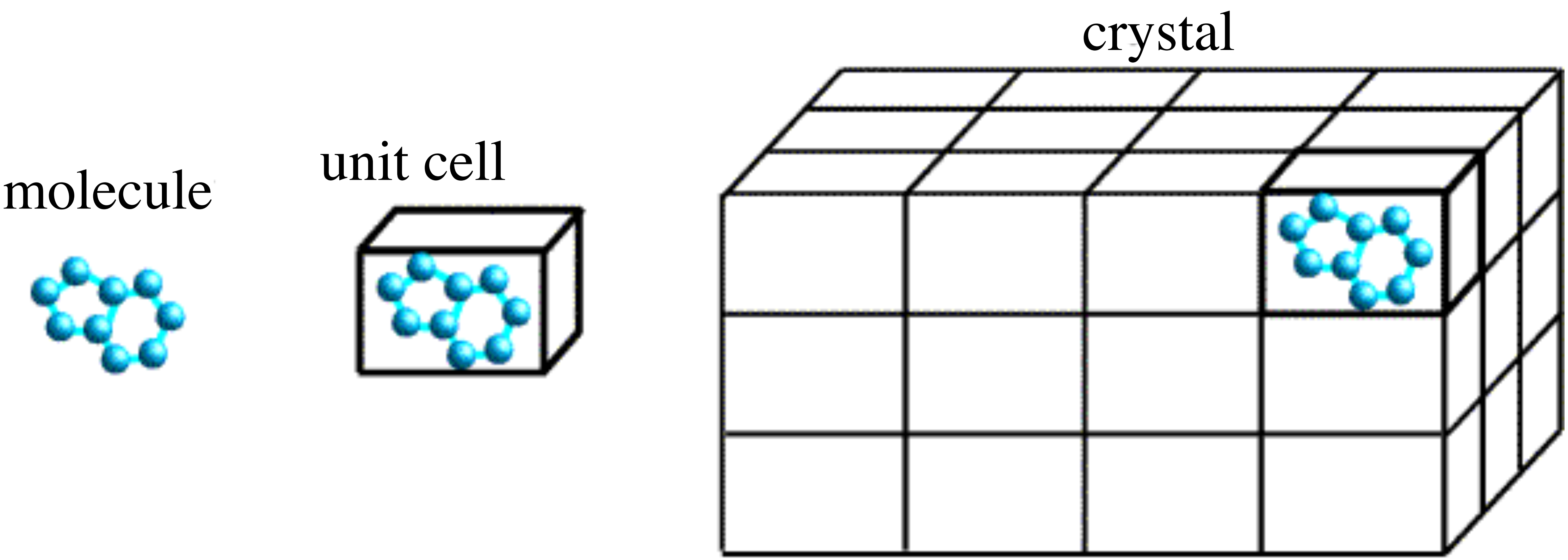
$$I_{2\theta} = I_0 \frac{q^4}{r^2 m^2 c^4} \left(\frac{1 + \cos^2 2\theta}{2} \right)$$



Solid state long-range order

Crystal structure = motif * crystal lattice

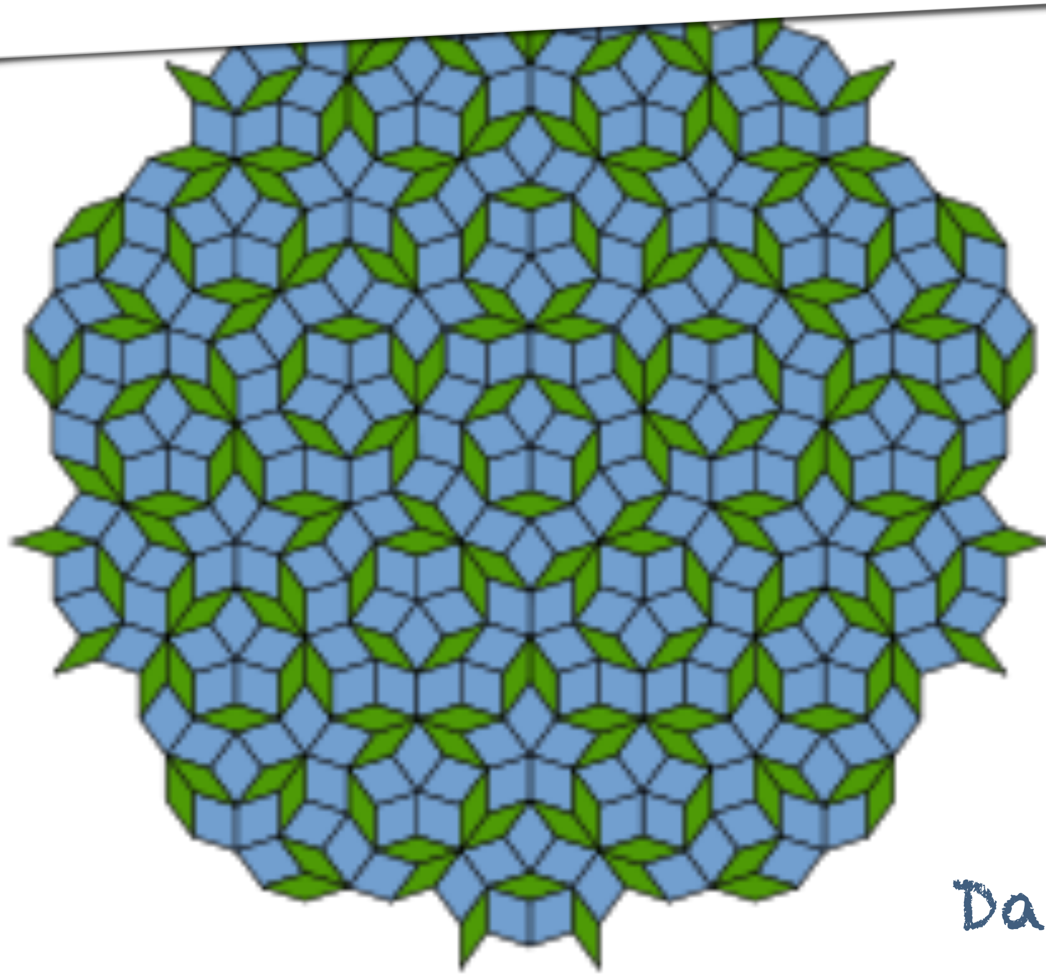
The crystal works as a « signal amplifier » : many molecules, all with the same orientation



Solid state long-range order

Crystal structure = motif * crystal lattice

aperiodic forms of order = quasicrystals



Dan Shechtman (nobel prize 2011)

Diffraction:

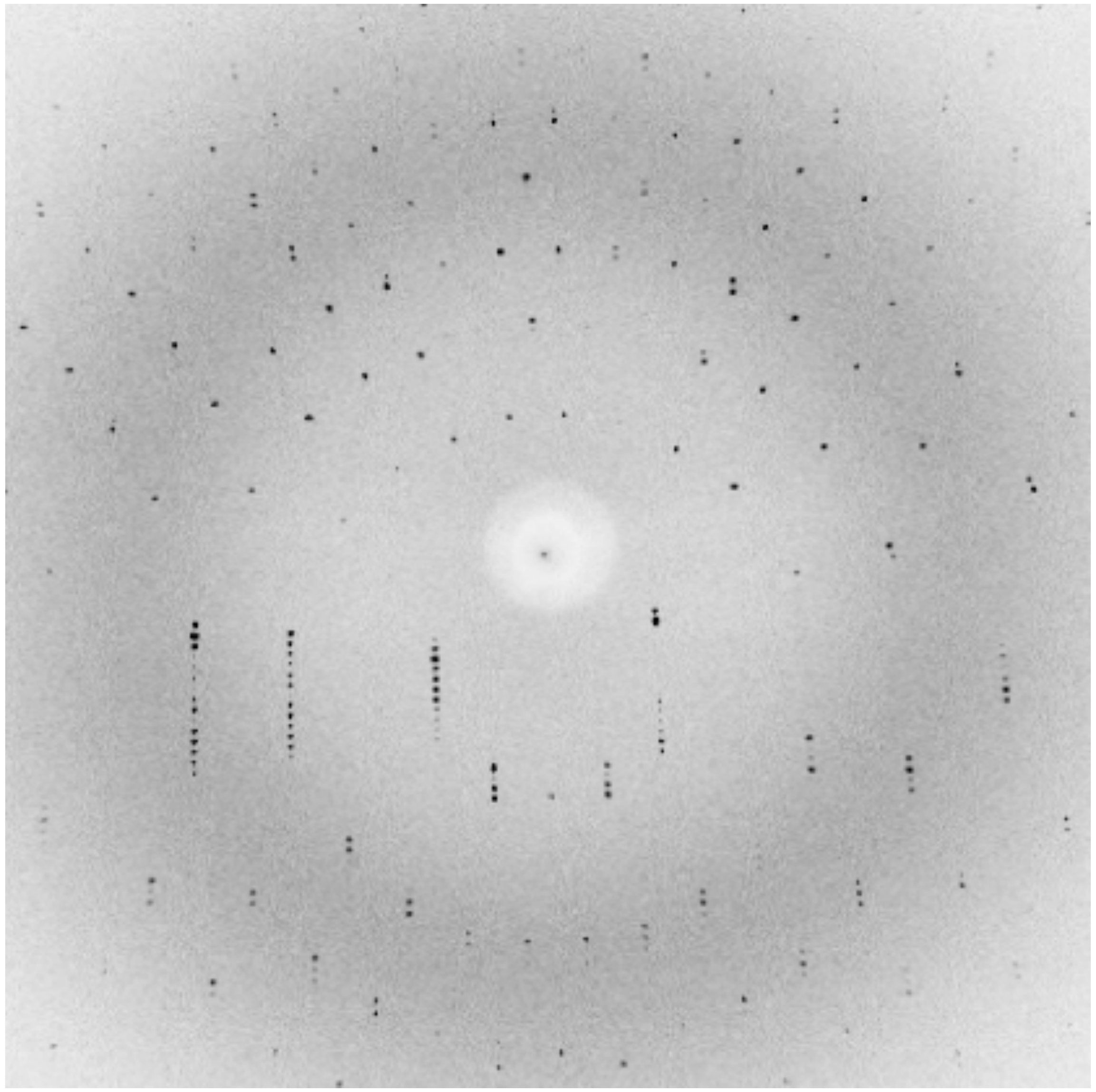
Each electron scatters

The emitted waves add up … and get subtracted!!

The final result depends on the relative phases of the waves that scatter in each direction

Bragg's law :

$n\lambda = 2d \sin \theta$



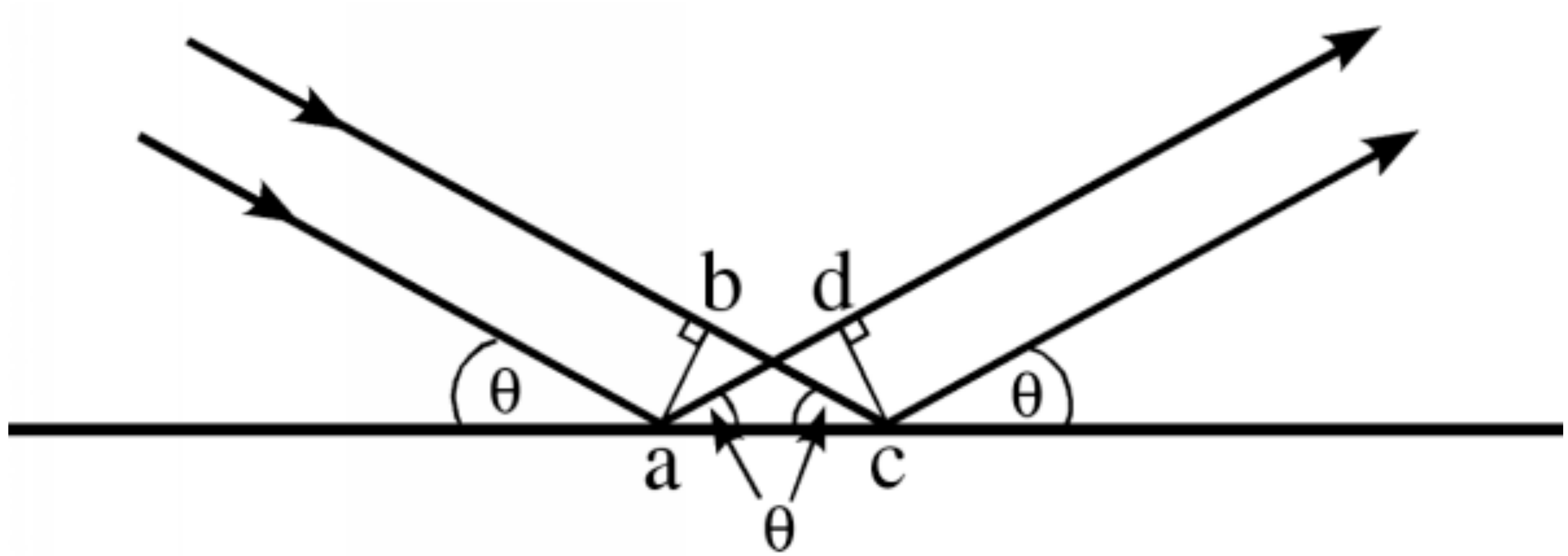
Try the interactive site

www.mpi.stonybrook.edu/SummerScholars/2003/Projects/RGonzalez/BraggsLawApplet/index.html

Diffraction : waves in phase

1. When do two or more waves scatter in phase?

When they travel the same trajectory



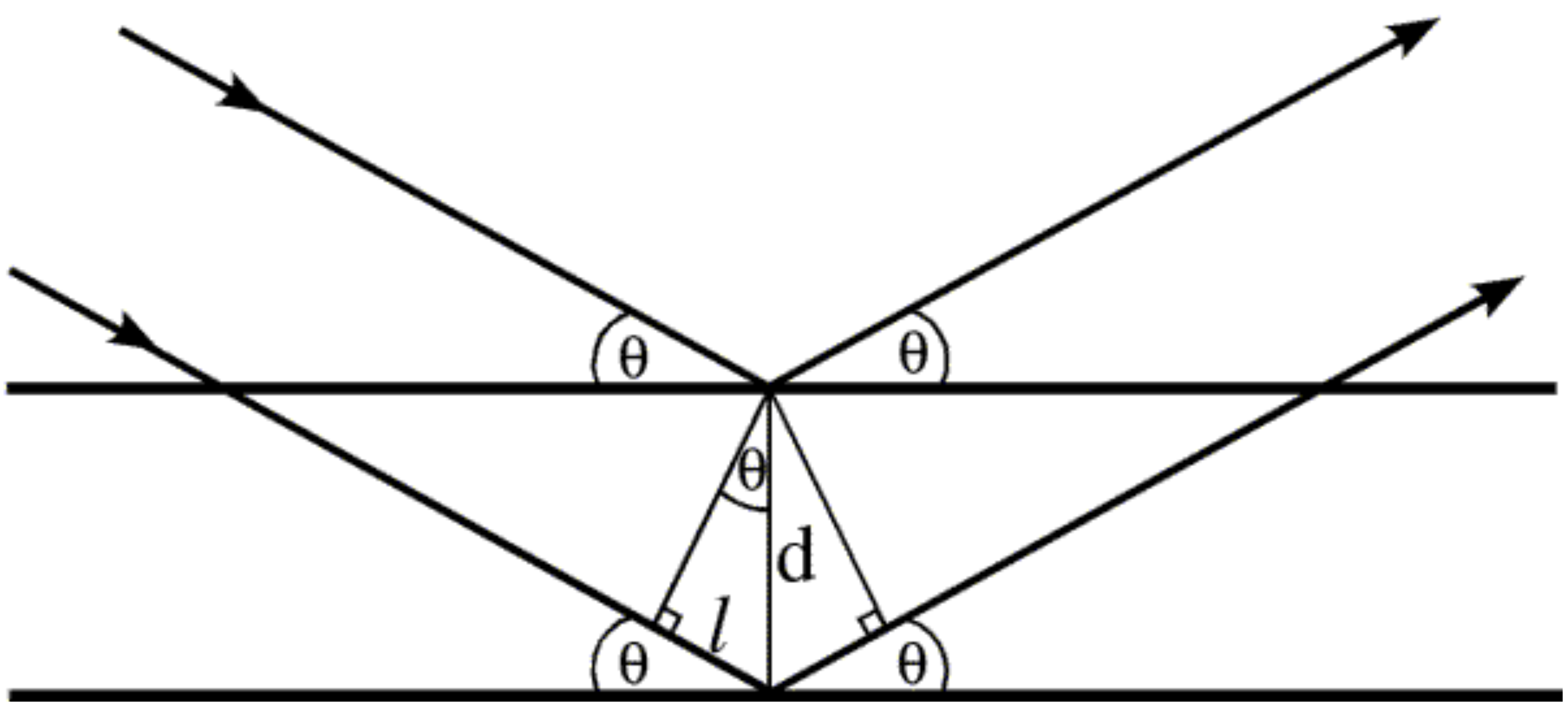
… like in light reflection

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

Diffraction : waves in phase

2. When do two or more waves scatter in phase?

When their trajectories differ by an integer number of wavelengths

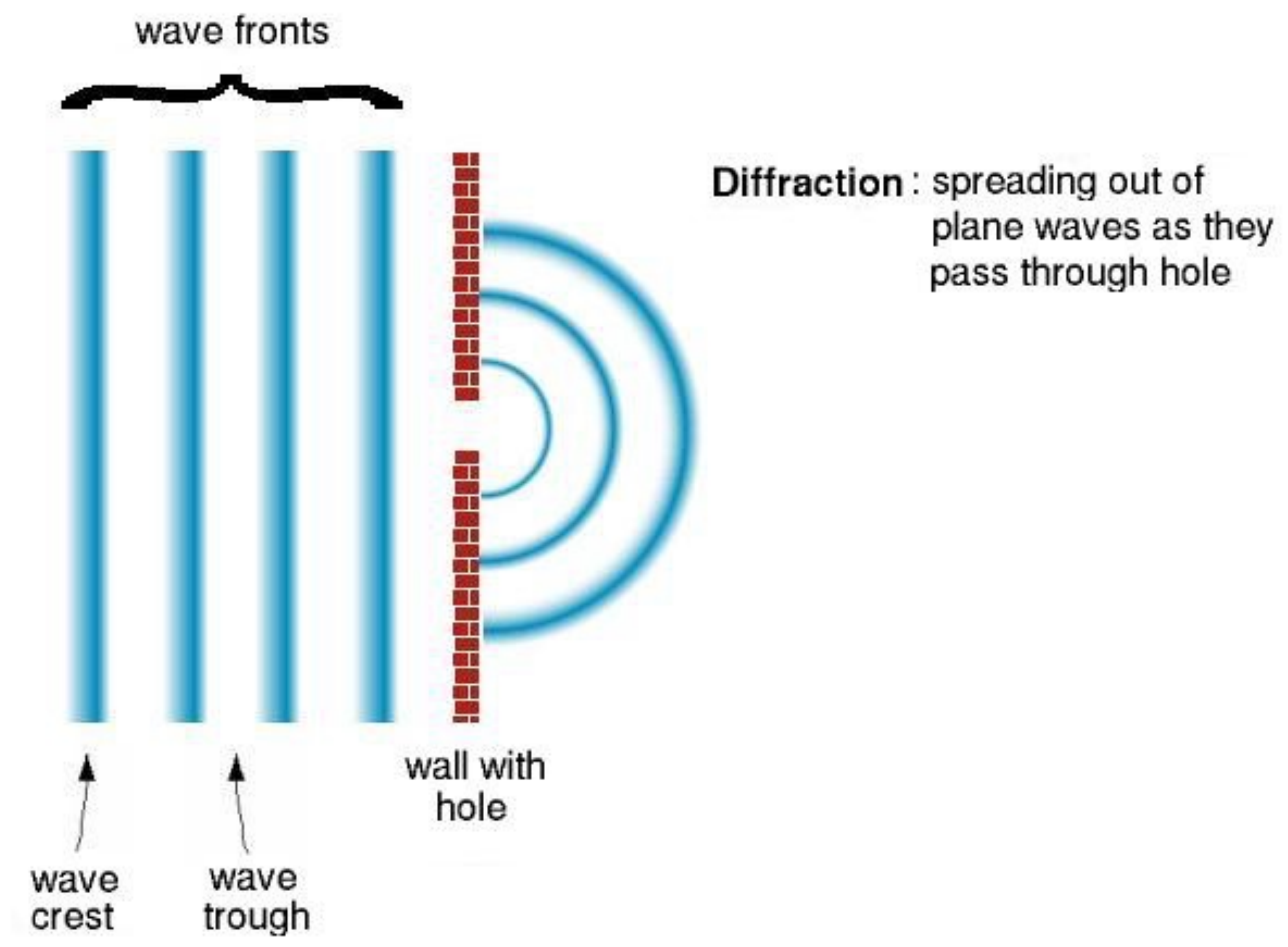


$n\lambda = 2d \sin \theta$

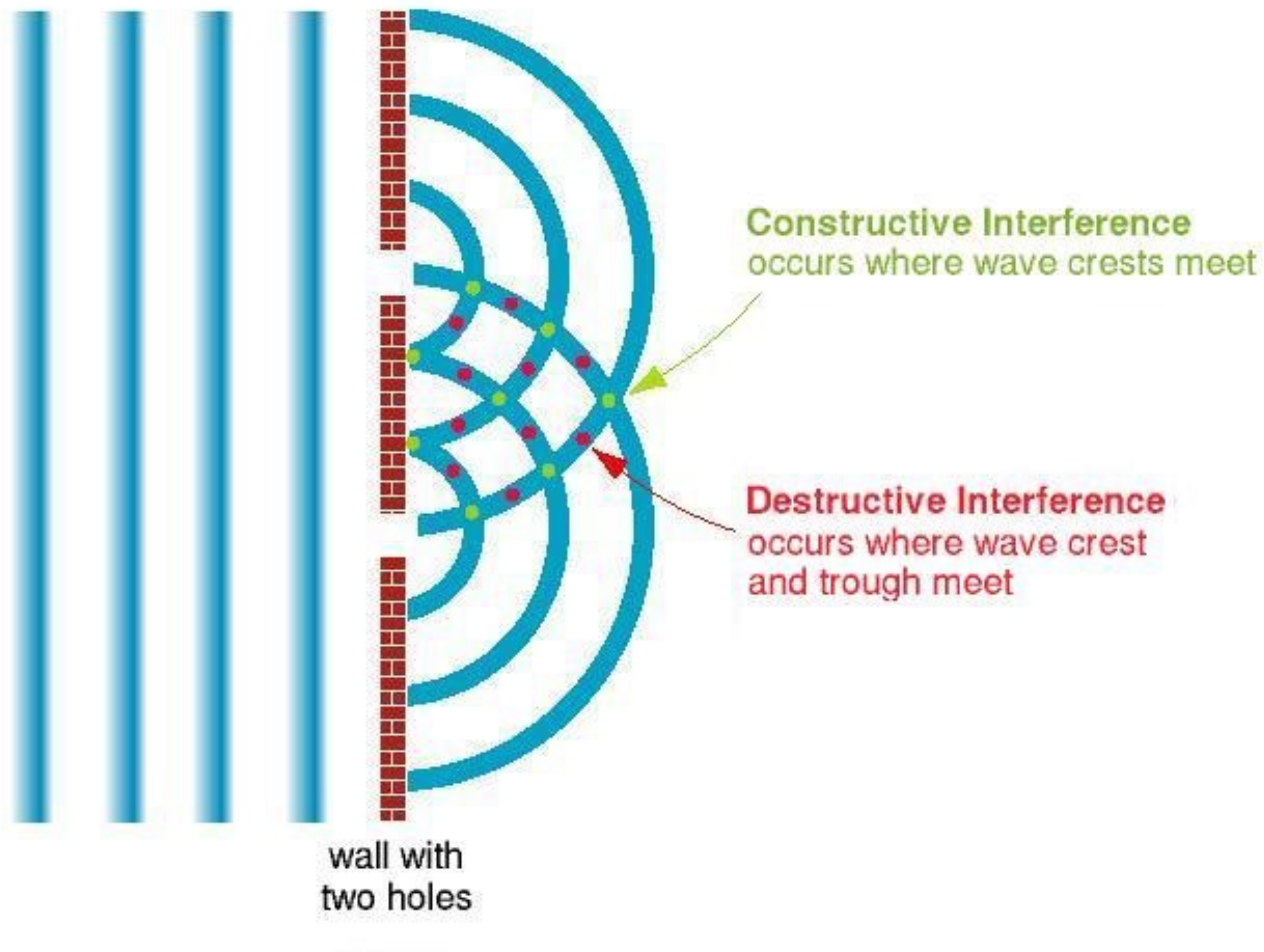
… like in light diffraction

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

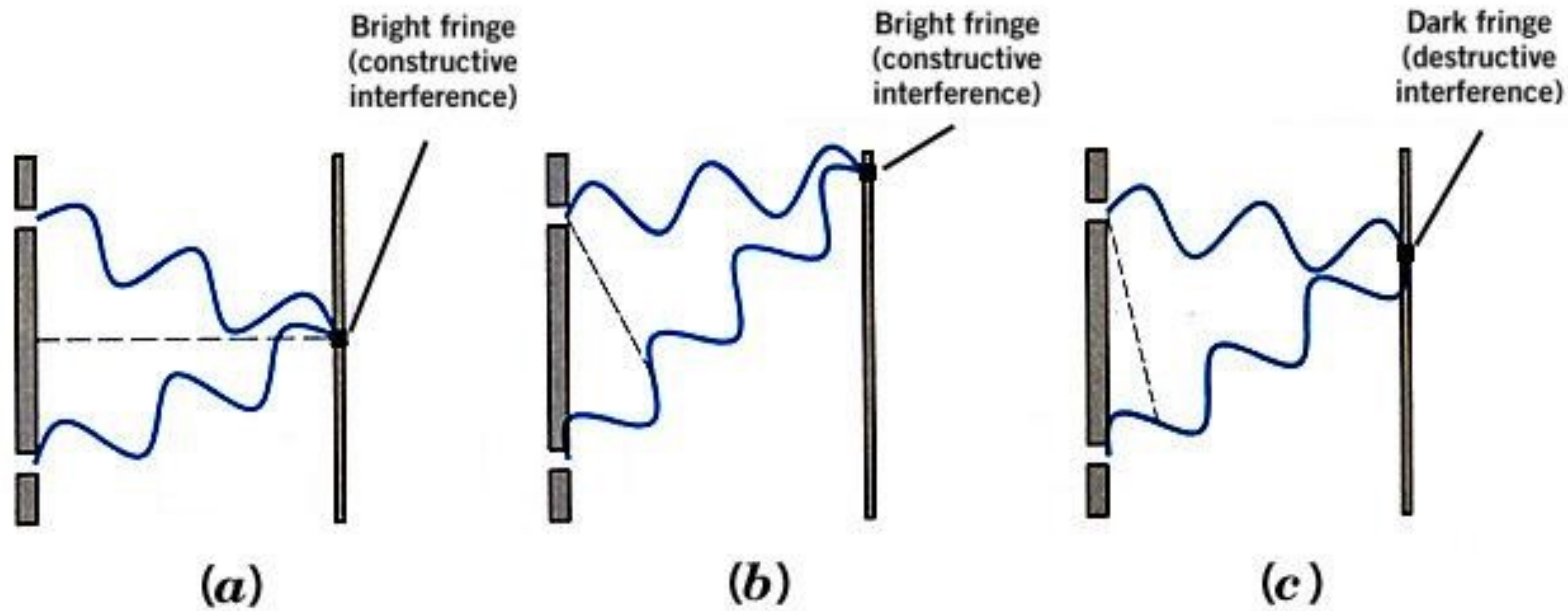
Diffraction



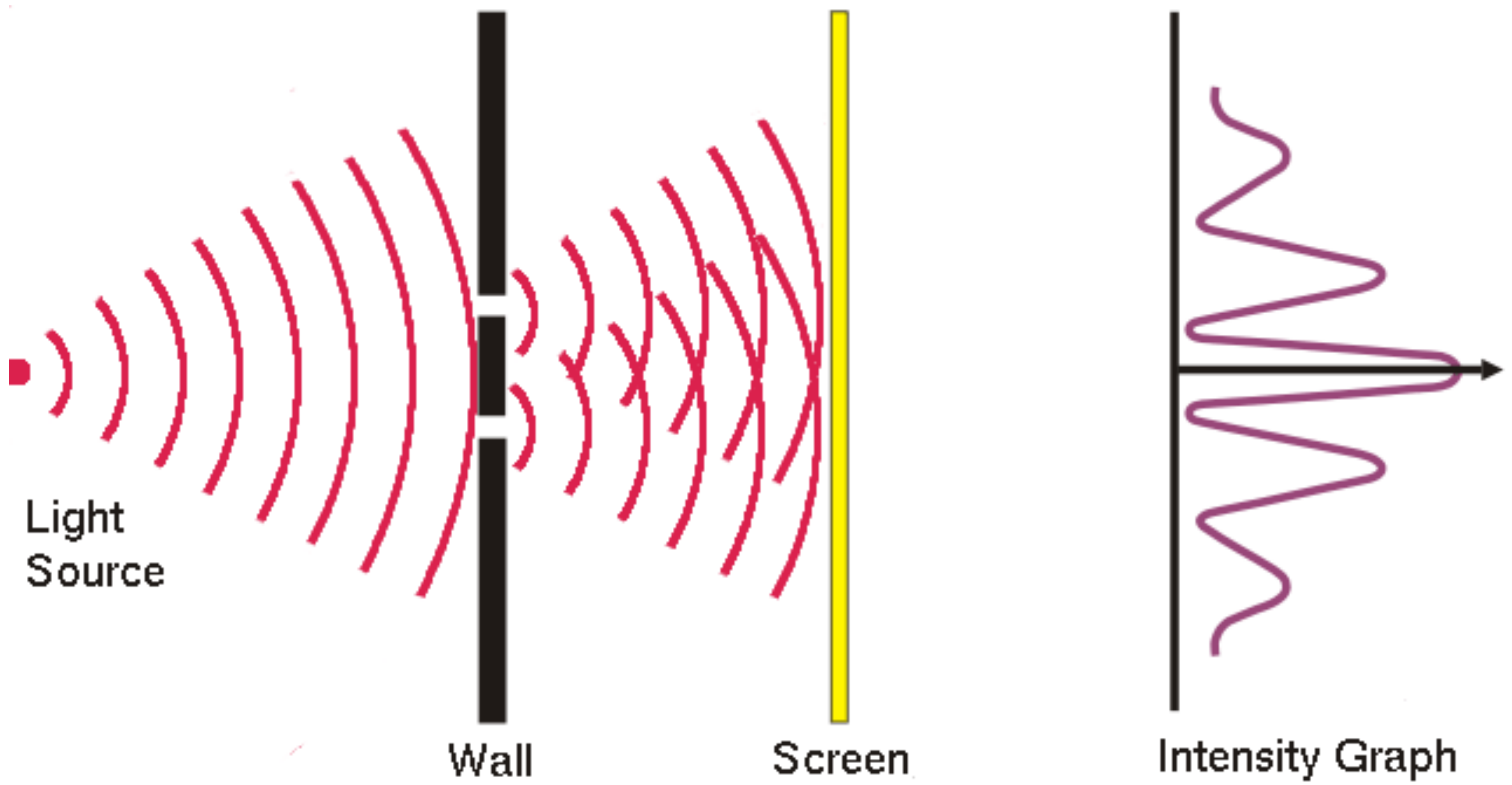
Interference



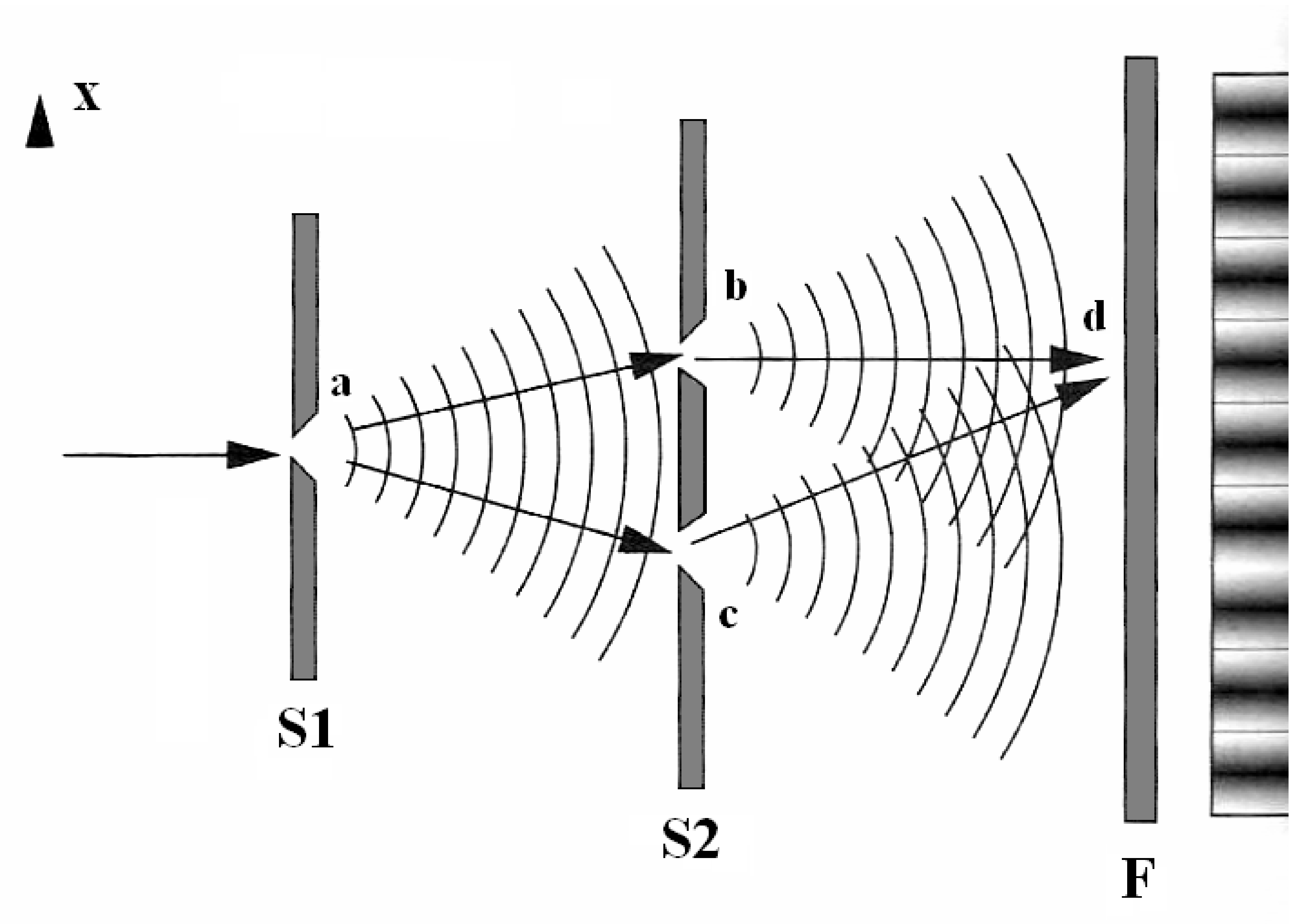
Interference Fringes on a Screen



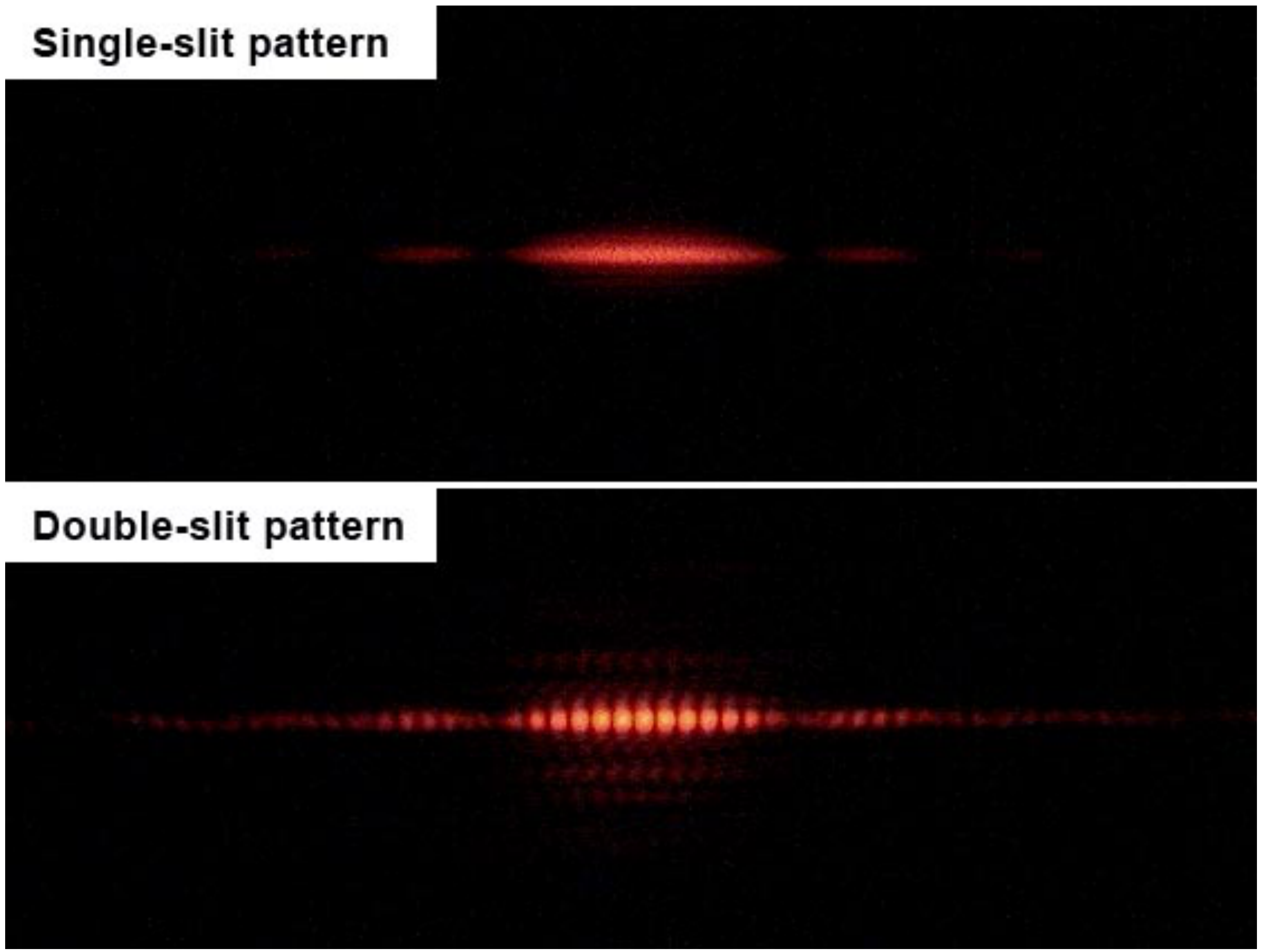
Double-Slit Experiment



Double slit experiment (Young 1801)



Diffraction and interference (one and two slits…)



Diffraction and interference (two slits…)

$$\frac{n\lambda}{d} = \frac{x}{L} \quad \Leftrightarrow \quad n\lambda = \frac{xd}{L} \, ,$$

$a^2/L\lambda \geq 1$ Fresnel diff

$a^2/L\lambda << 1$ Fraunhofer diff

n = integer (diffraction order)

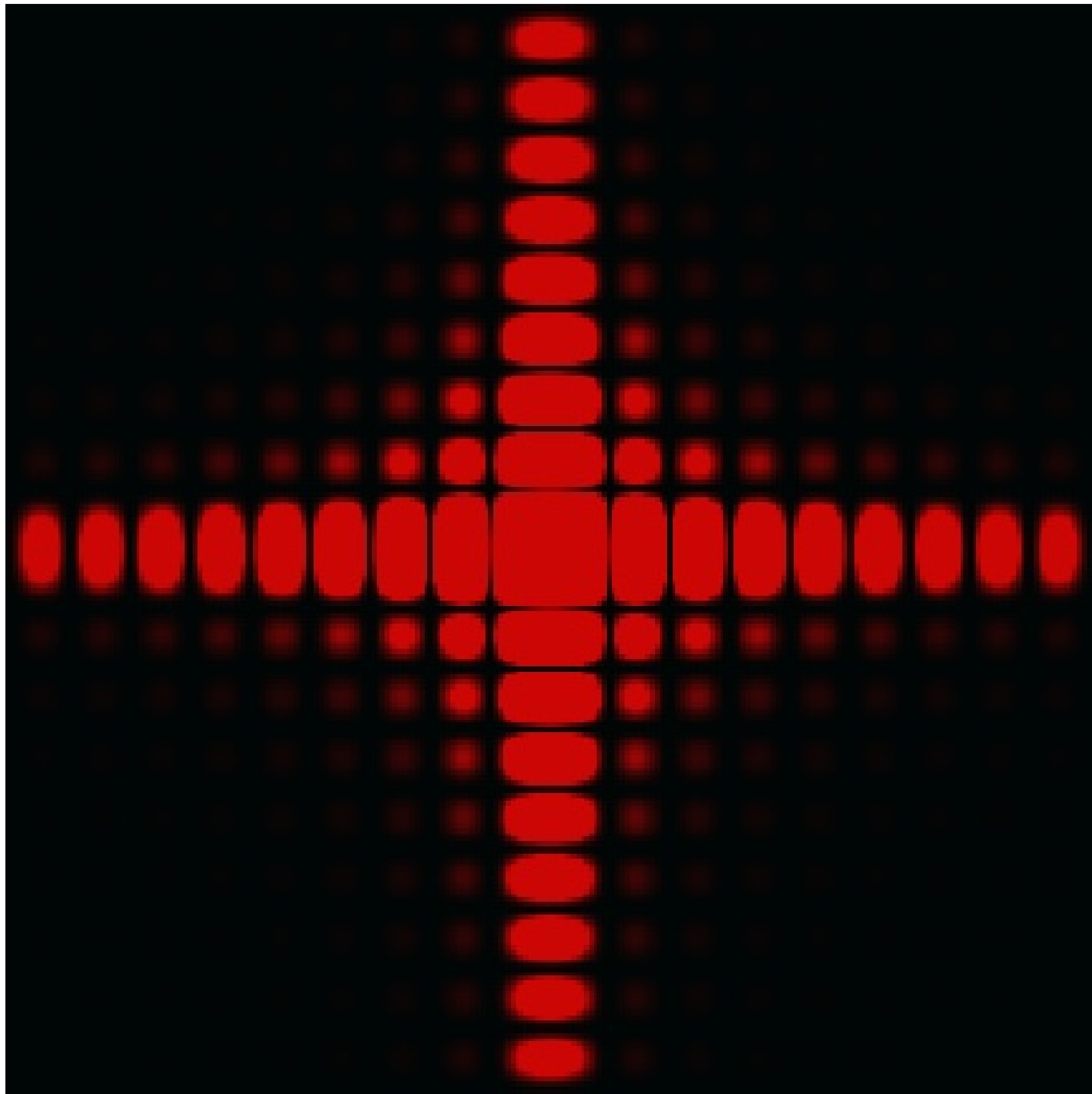
λ = wavelength

d = distance between slits

x = distance of diffracted positions wrt origin

L = distance between slits and detector

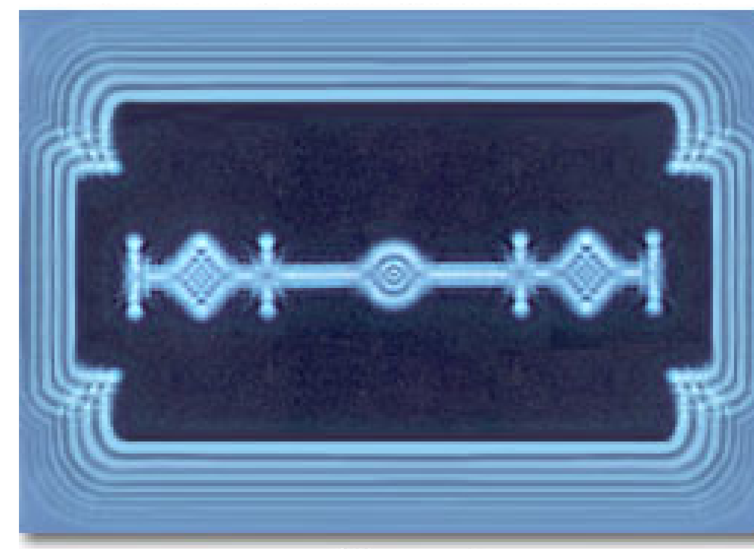
a = slit size

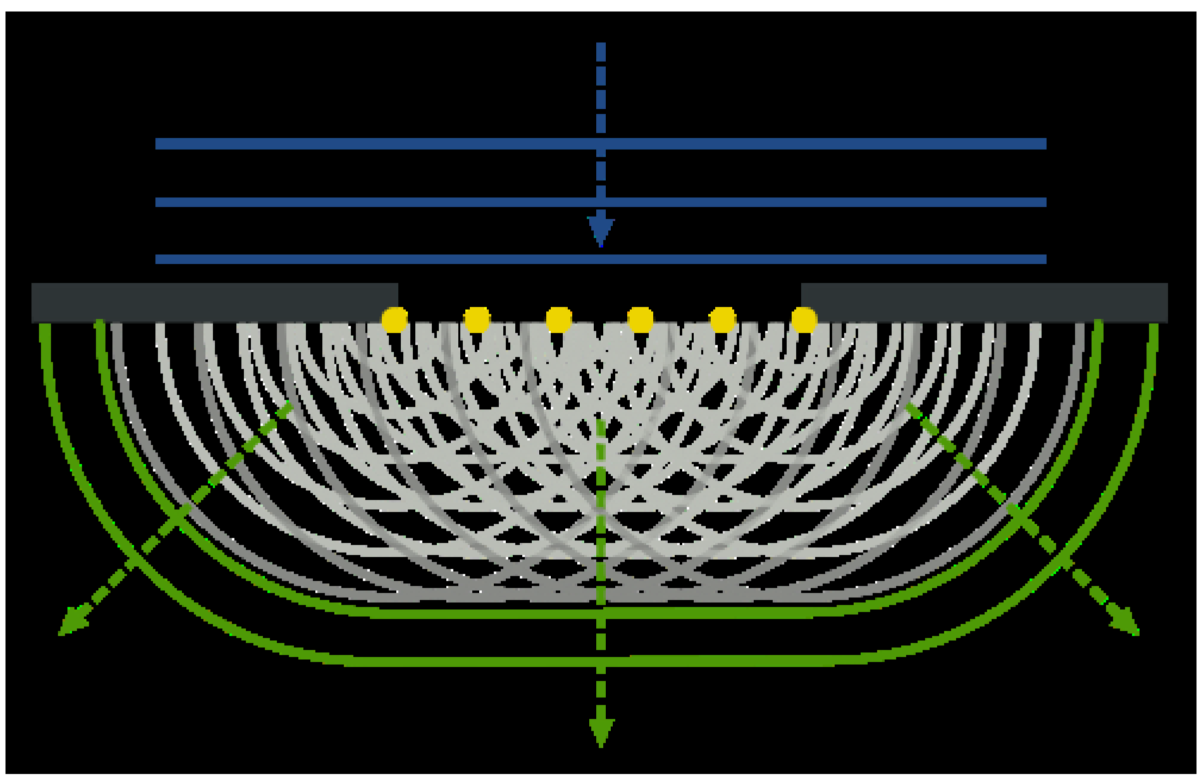


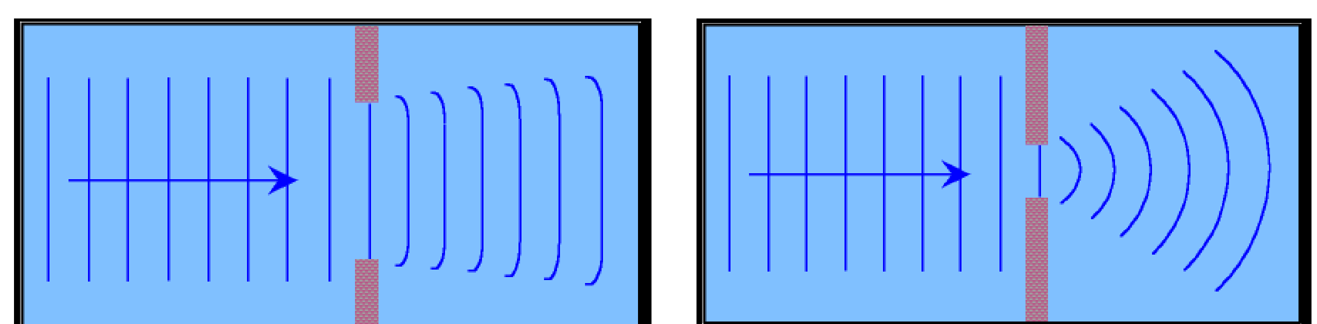
So, why is there diffraction with only one slit???

The form factor -->> a Fourier transform of the obstacle!

only detectable according to ratio between λ and a (size of the obstacle)

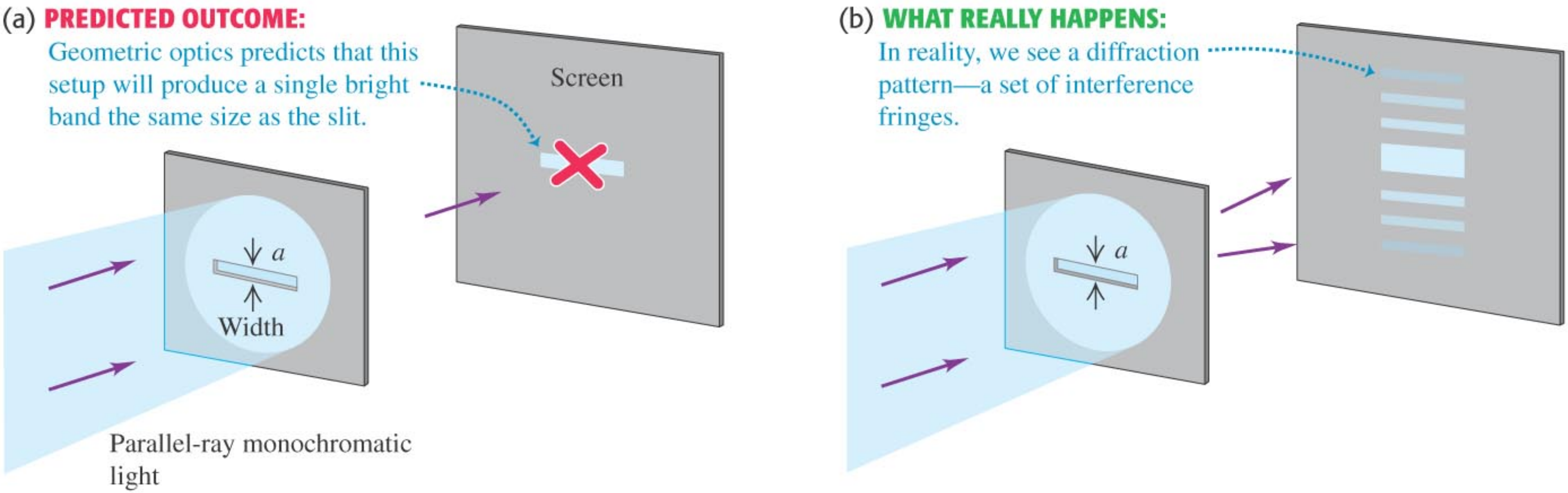


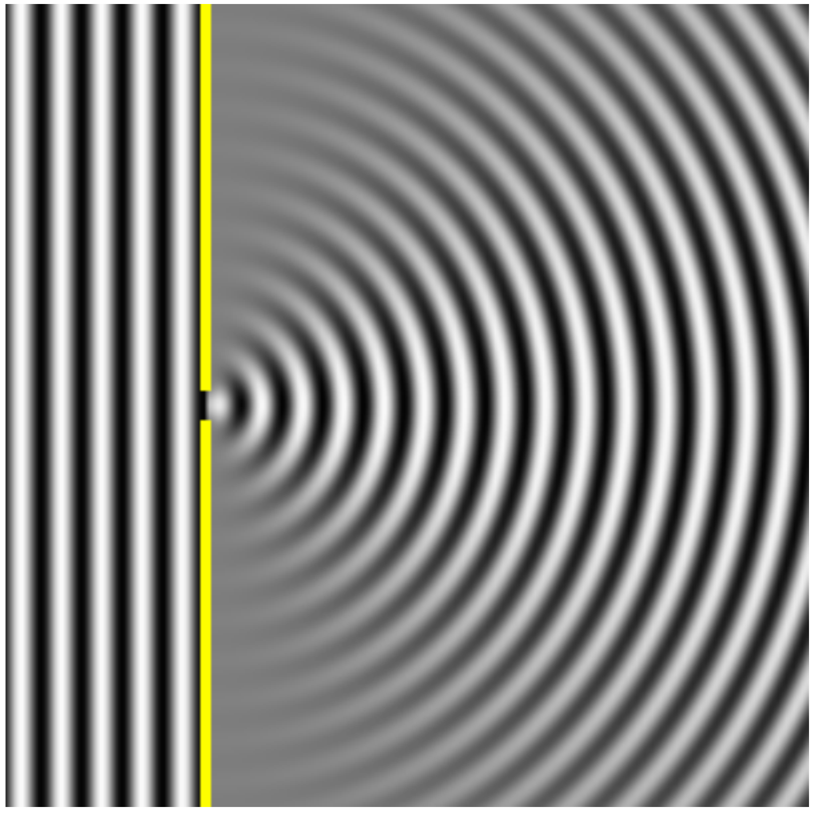


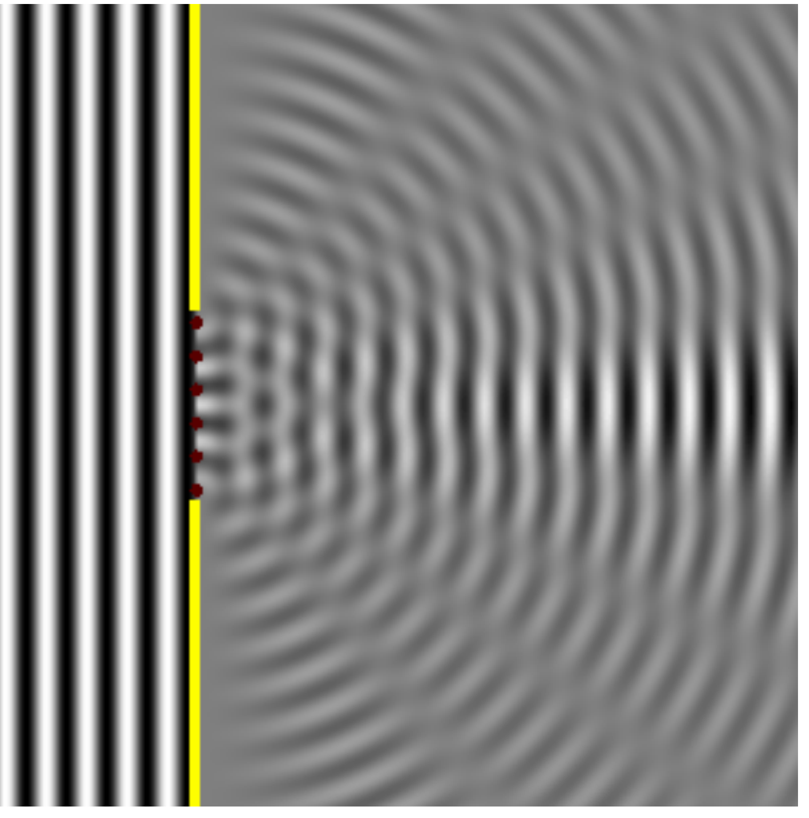


Wave propagation Huygens-Fresnel principle

Diffraction and interference (one and two slits…)





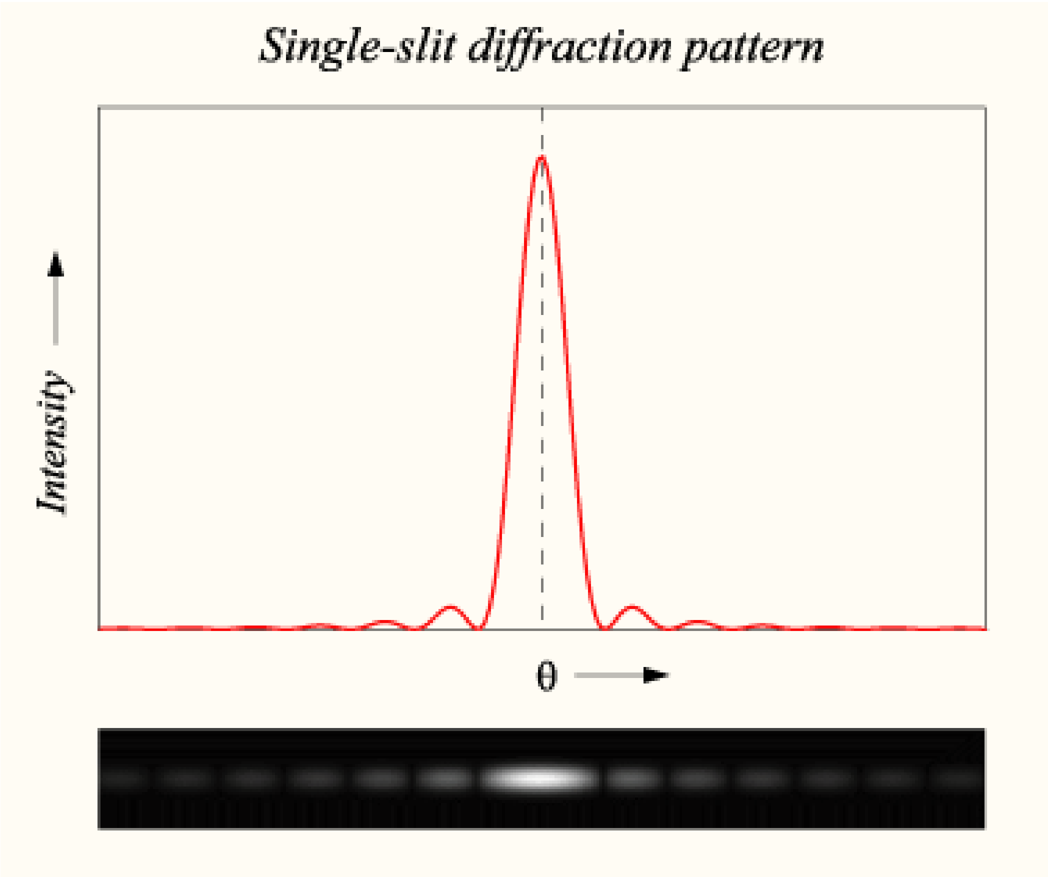


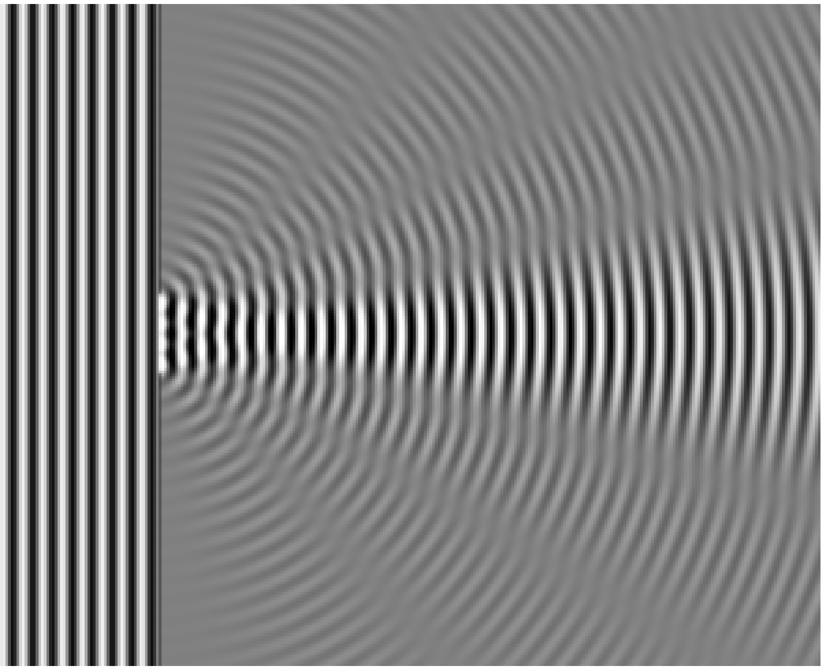
So, why is there diffraction with only one slit???

Huygens-Fresnel principle

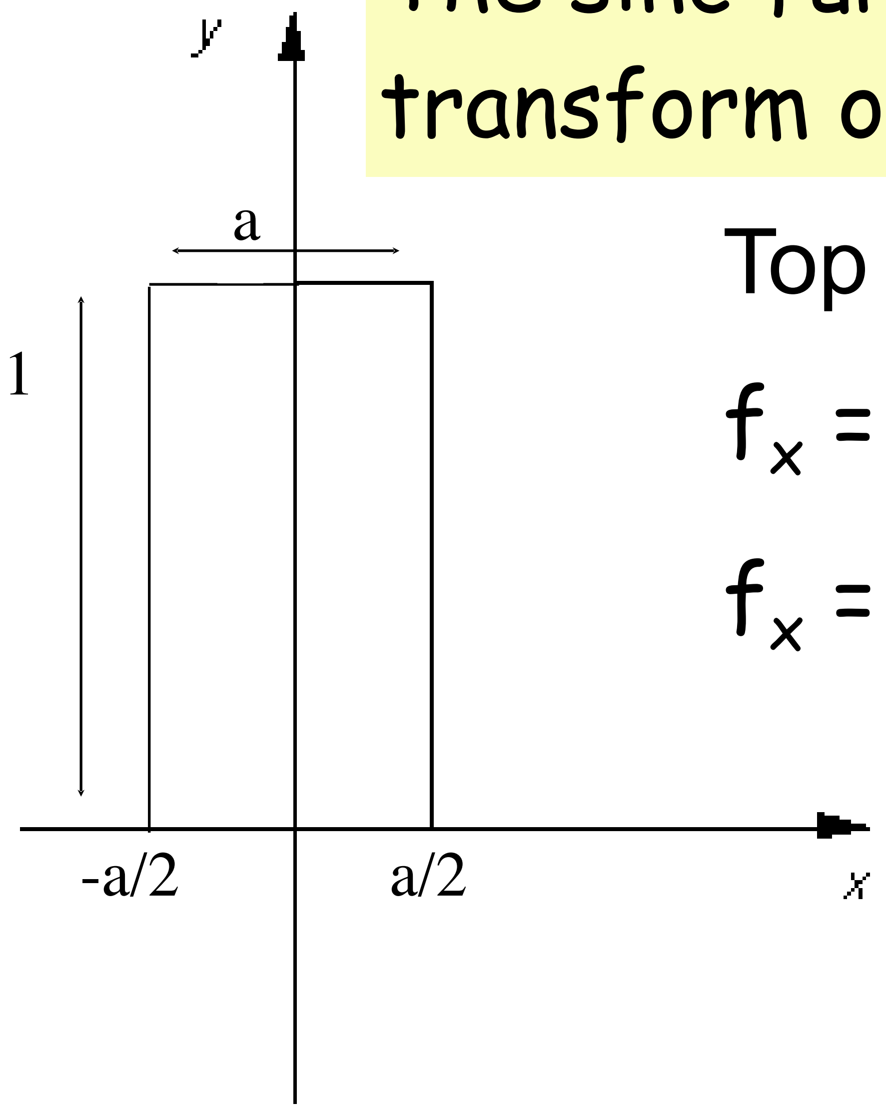
$$\mathrm{sinc}(x) = \frac{\sin(\pi x)}{\pi x}$$

$$I(\theta) = I_0 \left[\mathrm{sinc}\left(\frac{\pi a}{\lambda} \sin\theta \right) \right]^2$$





The sinc function is nothing but the Fourier transform of the top hat function!!



Top hat:

$f_x = 1$; if $|x| \le a/2$

$f_x = 0$; if $|x| > a/2$

FT:

$$F_h = \int f_x \, e^{-2\pi i h x} \, \delta x$$

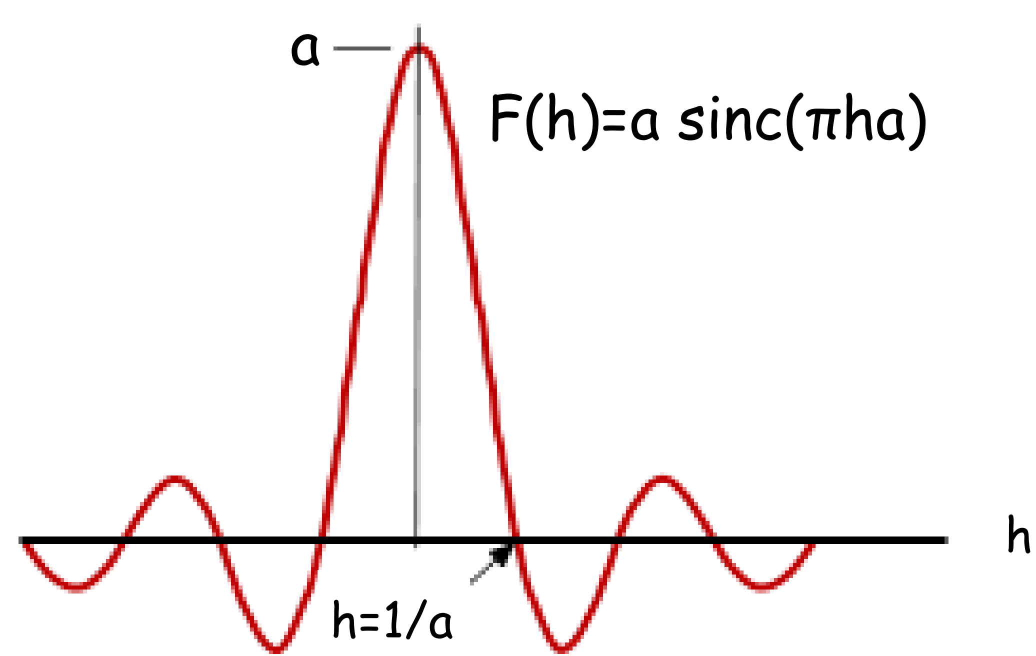
$$F_h = \int_{-a/2}^{a/2} 1 \, . \, e^{-2\pi i h x} \, \delta x$$

$$1/(2\pi i h) \, . \, (e^{[a/2]2\pi i h} - e^{-[a/2]2\pi i h})$$

$\cos x = (e^{ix} + e^{-ix}) / 2$

$\sin x = (e^{ix} - e^{-ix}) / 2i$

$$1 \, . \, a \, . \, \underbrace{\sin(\pi h a) / (\pi h a)}_{\text{sinc function}}$$



…when going to several N slits separated by d (or x_0) :

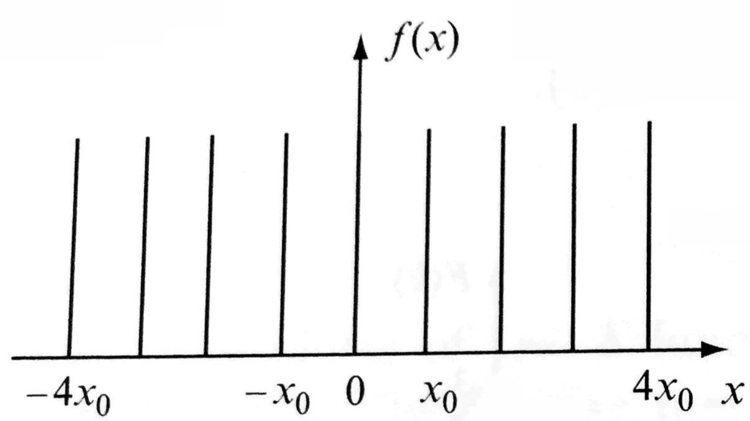
Huygens-Fresnel principle $\mathrm{sinc}(x) = \frac{\sin(\pi x)}{\pi x}$

$$I(\theta) = I_0 \left[\mathrm{sinc}\left(\frac{\pi a}{\lambda}\sin\theta\right)\right]^2 \cdot \left[\frac{\sin\left(\frac{N\pi d}{\lambda}\sin\theta\right)}{\sin\left(\frac{\pi d}{\lambda}\sin\theta\right)}\right]^2$$

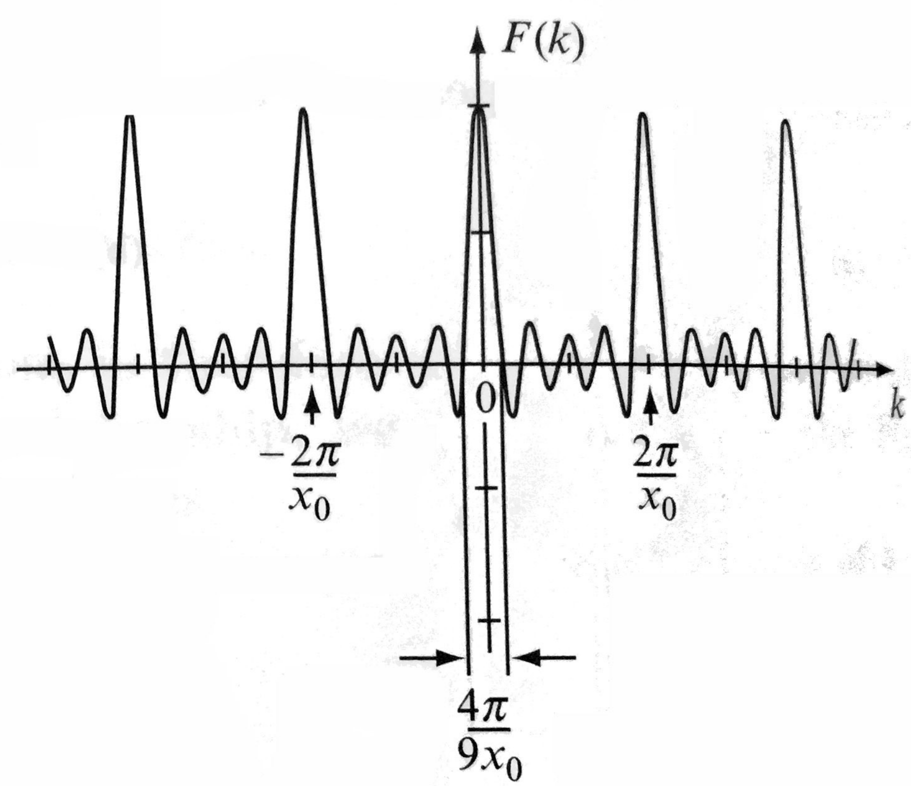
…when going to several N slits separated by d (or x_0) :

Huygens-Fresnel principle $$\mathrm{sinc}(x) = \frac{\sin(\pi x)}{\pi x}$$

$$I(\theta) = I_0 \left[\mathrm{sinc}\left(\frac{\pi a}{\lambda}\sin\theta\right)\right]^2 \cdot \left[\frac{\sin\left(\frac{N\pi d}{\lambda}\sin\theta\right)}{\sin\left(\frac{\pi d}{\lambda}\sin\theta\right)}\right]^2$$

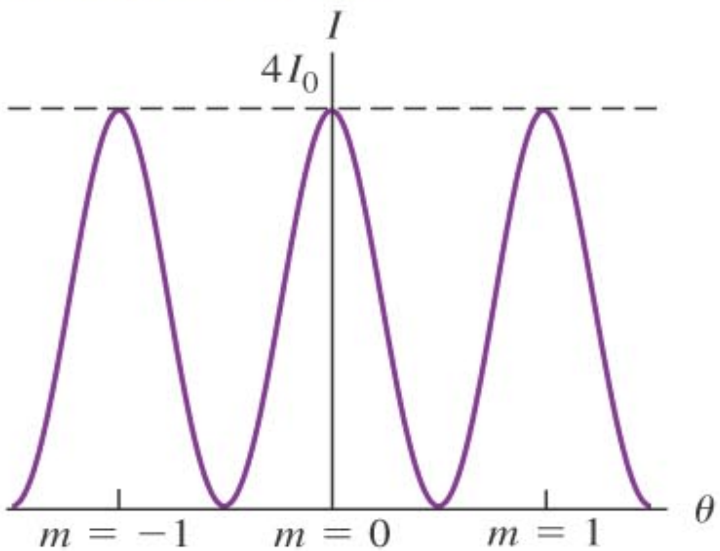


$$f(x) = \sum_{n=-4}^{n=4} \delta(x - nx_0)$$

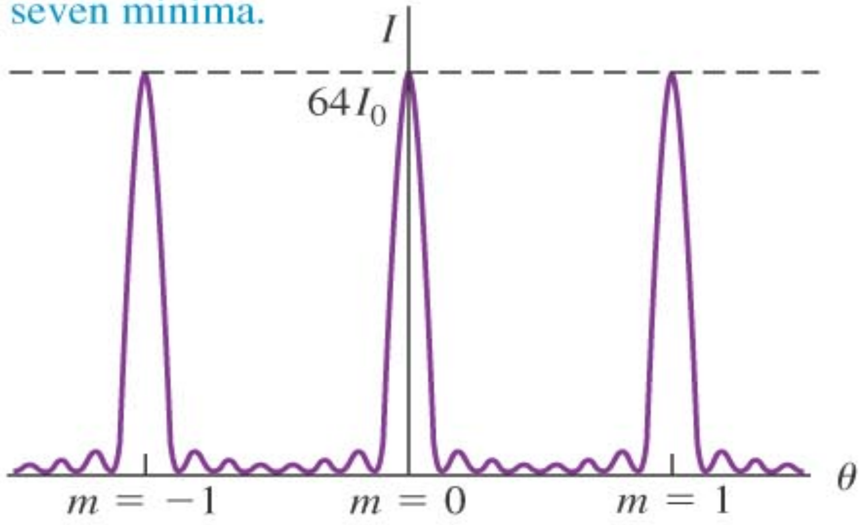


$$F(k) = \frac{\sin\dfrac{Nkx_0}{2}}{\sin\dfrac{kx_0}{2}} \quad (N=9)$$

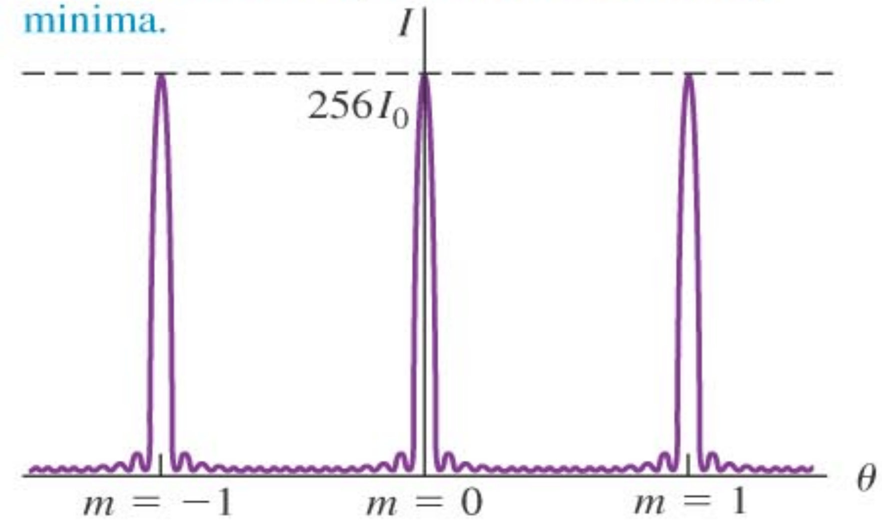
interference patterns for 2, 8, and 16 equally spaced narrow slits.



(a) $N = 2$: two slits produce one minimum between adjacent maxima.

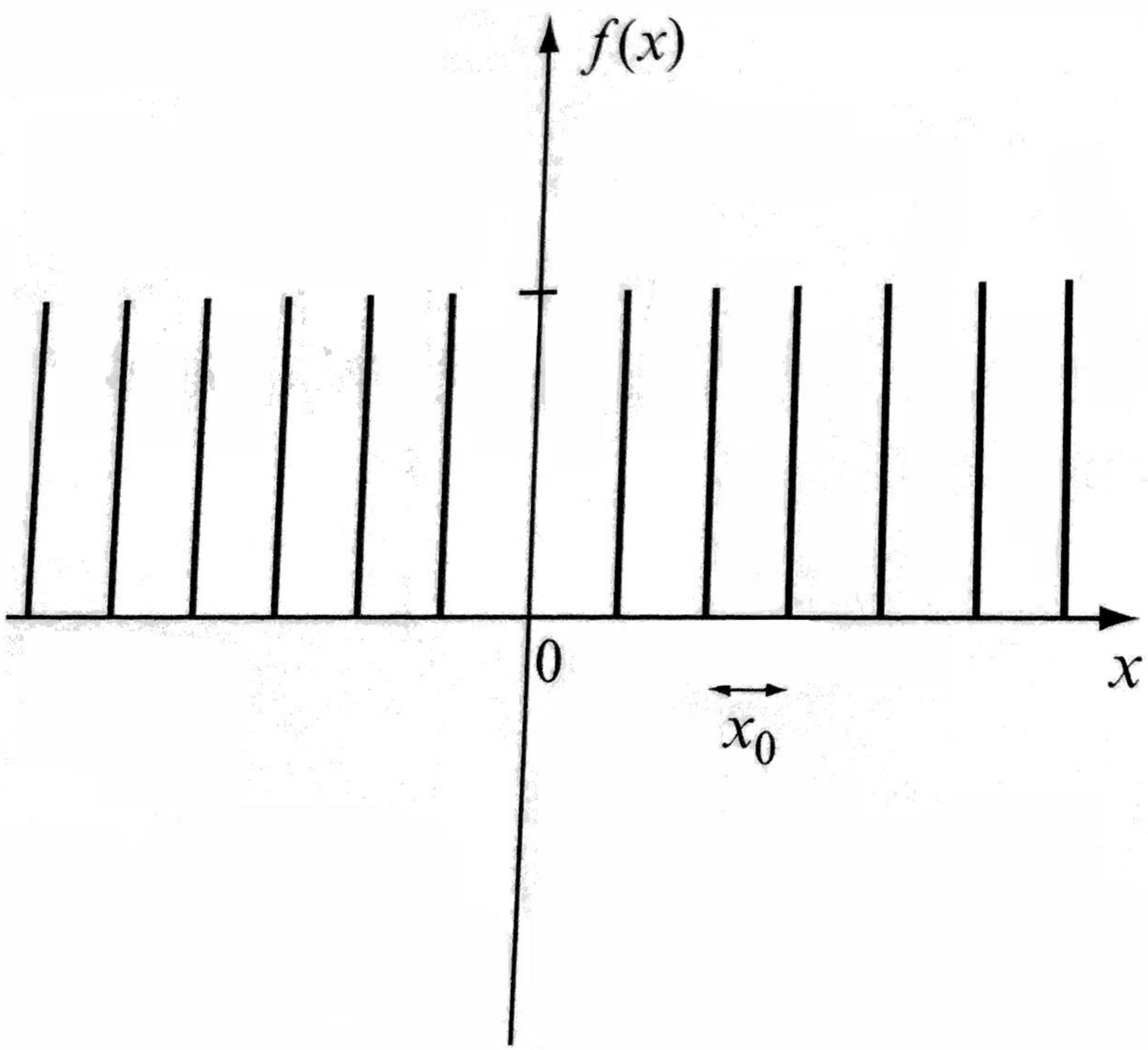


(b) $N = 8$: eight slits produce taller, narrower maxima in the same locations, separated by seven minima.

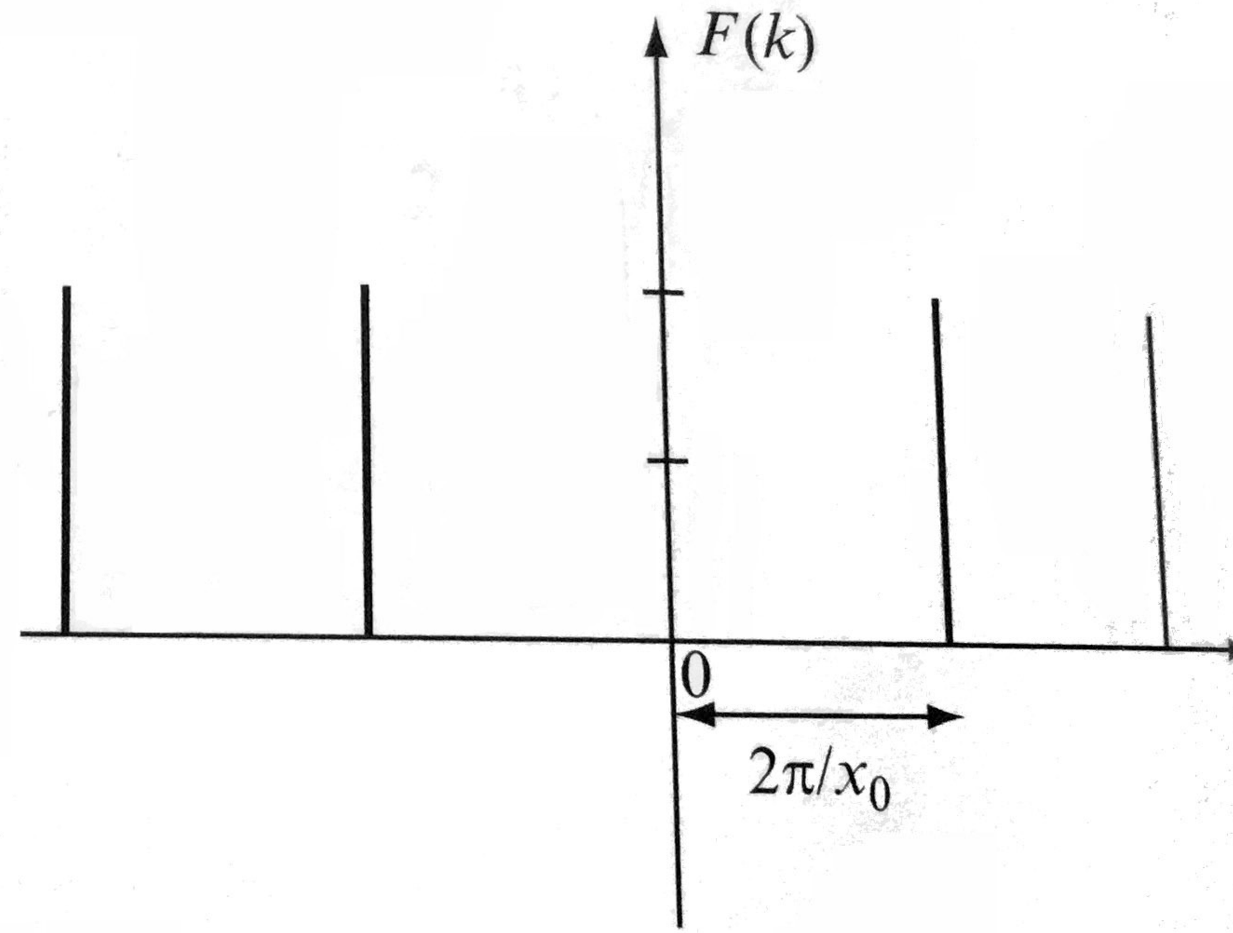


(c) $N = 16$: with 16 slits, the maxima are even taller and narrower, with more intervening minima.

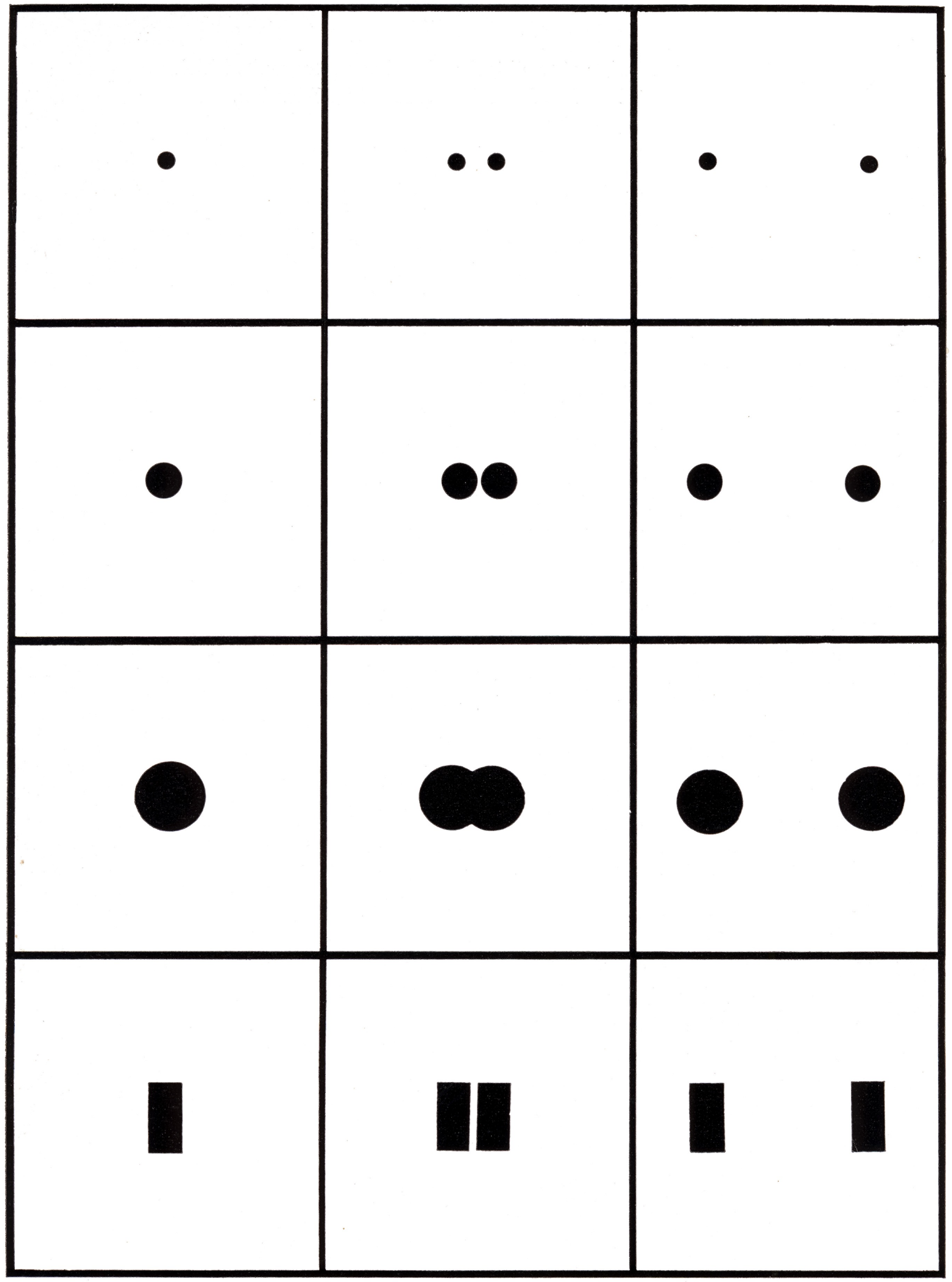
…and infinite N slits :

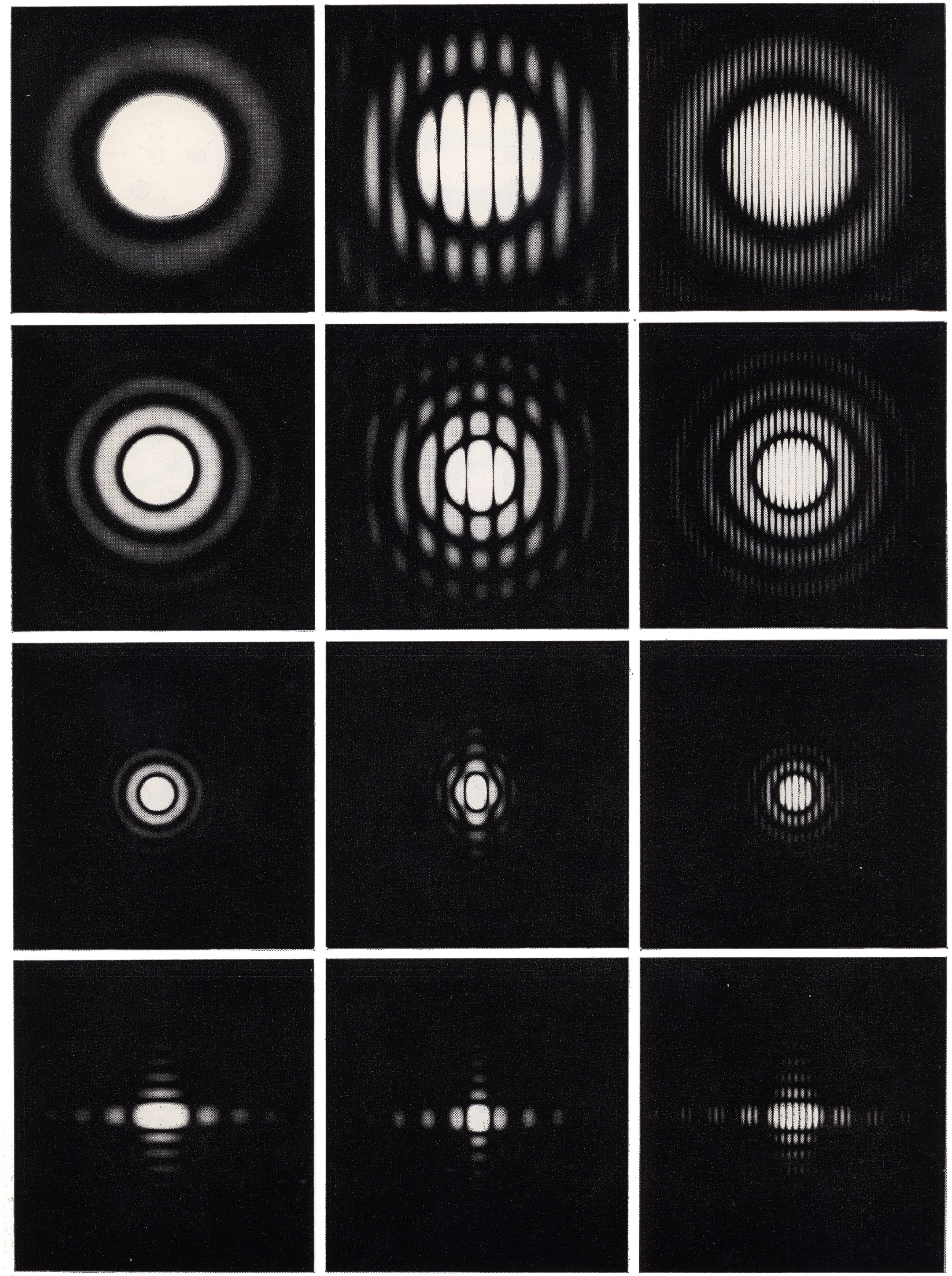


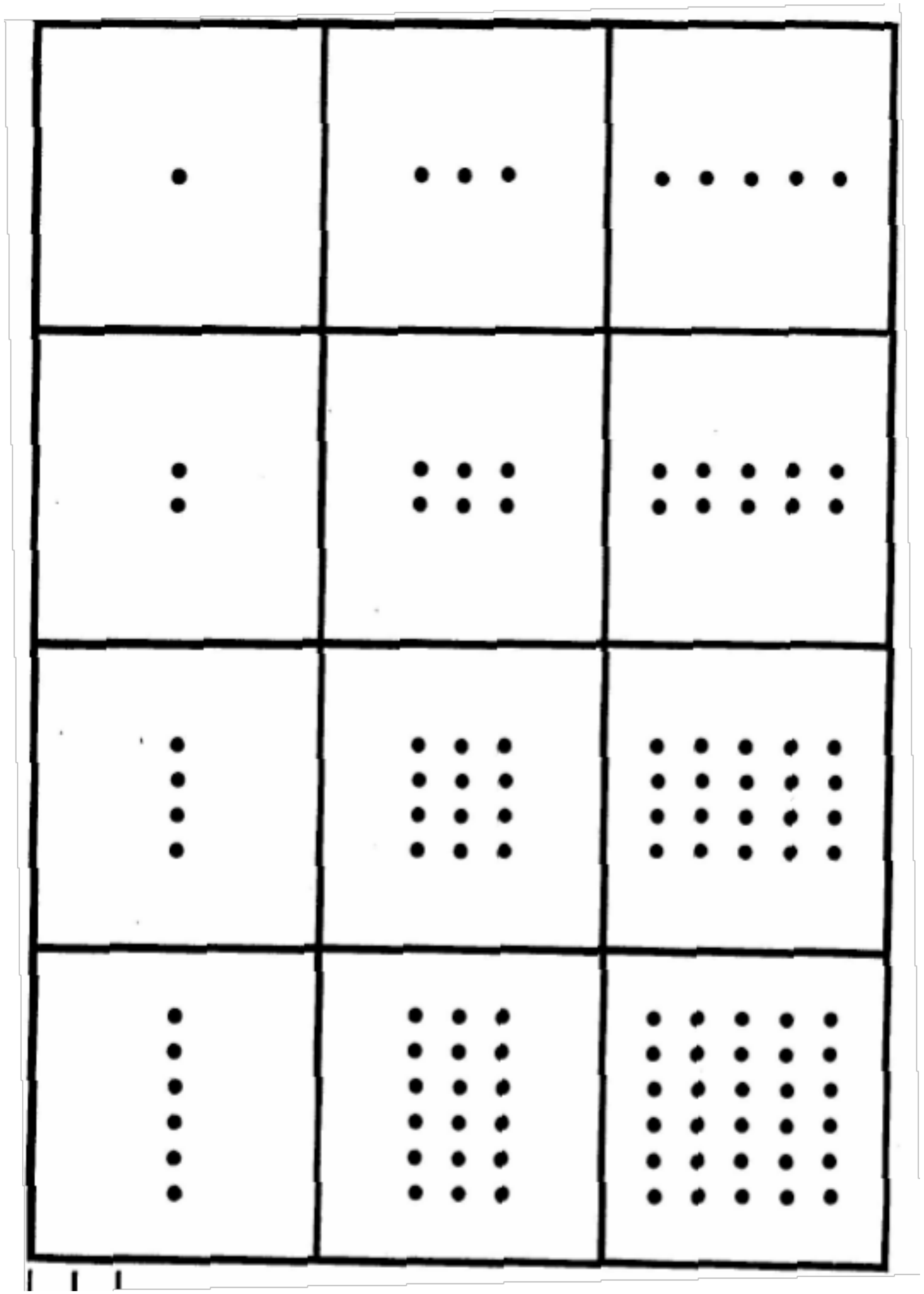
$$f(x)=\sum_{n=-\infty}^{n=+\infty} \delta(x-nx_0)$$

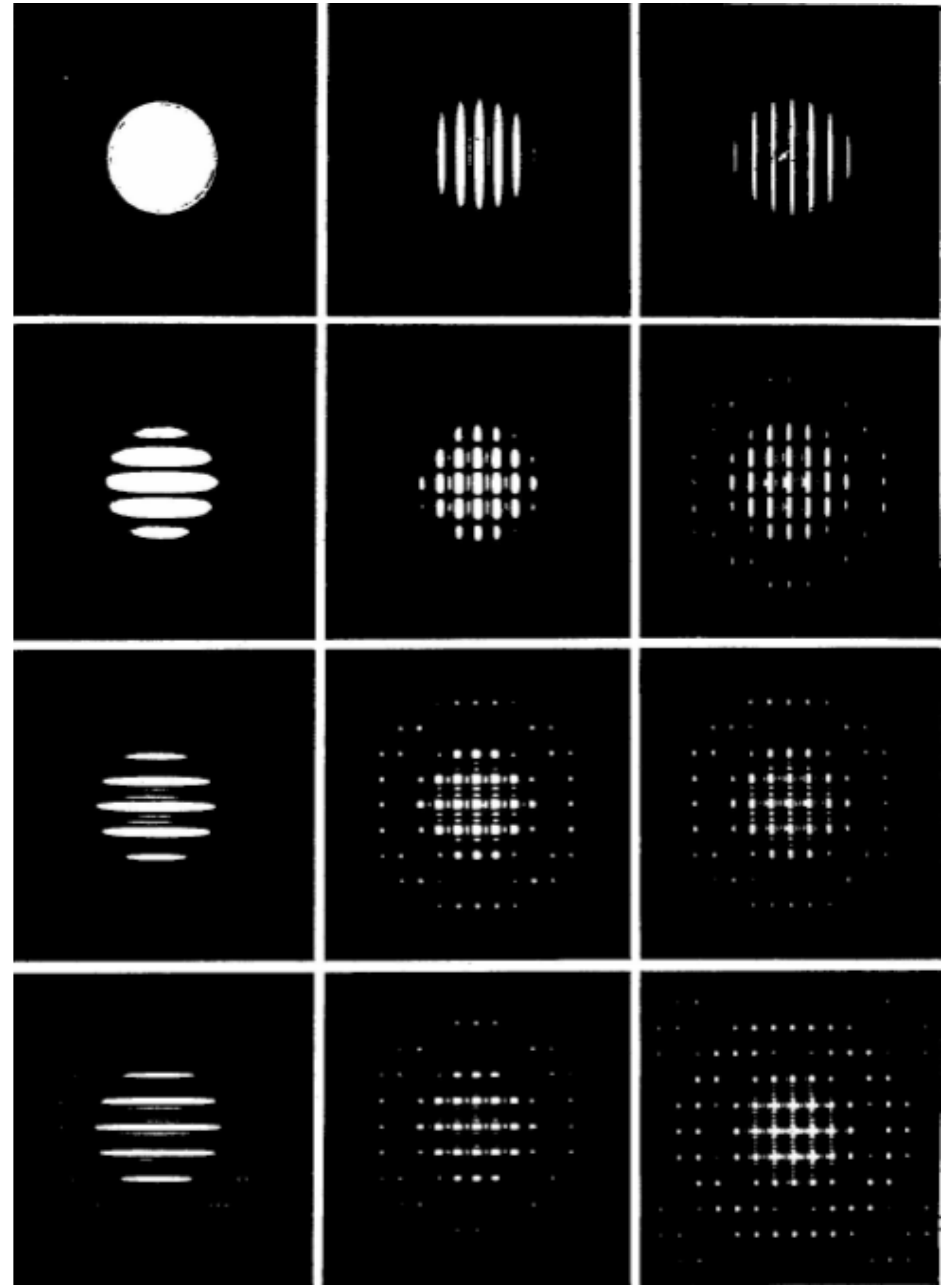


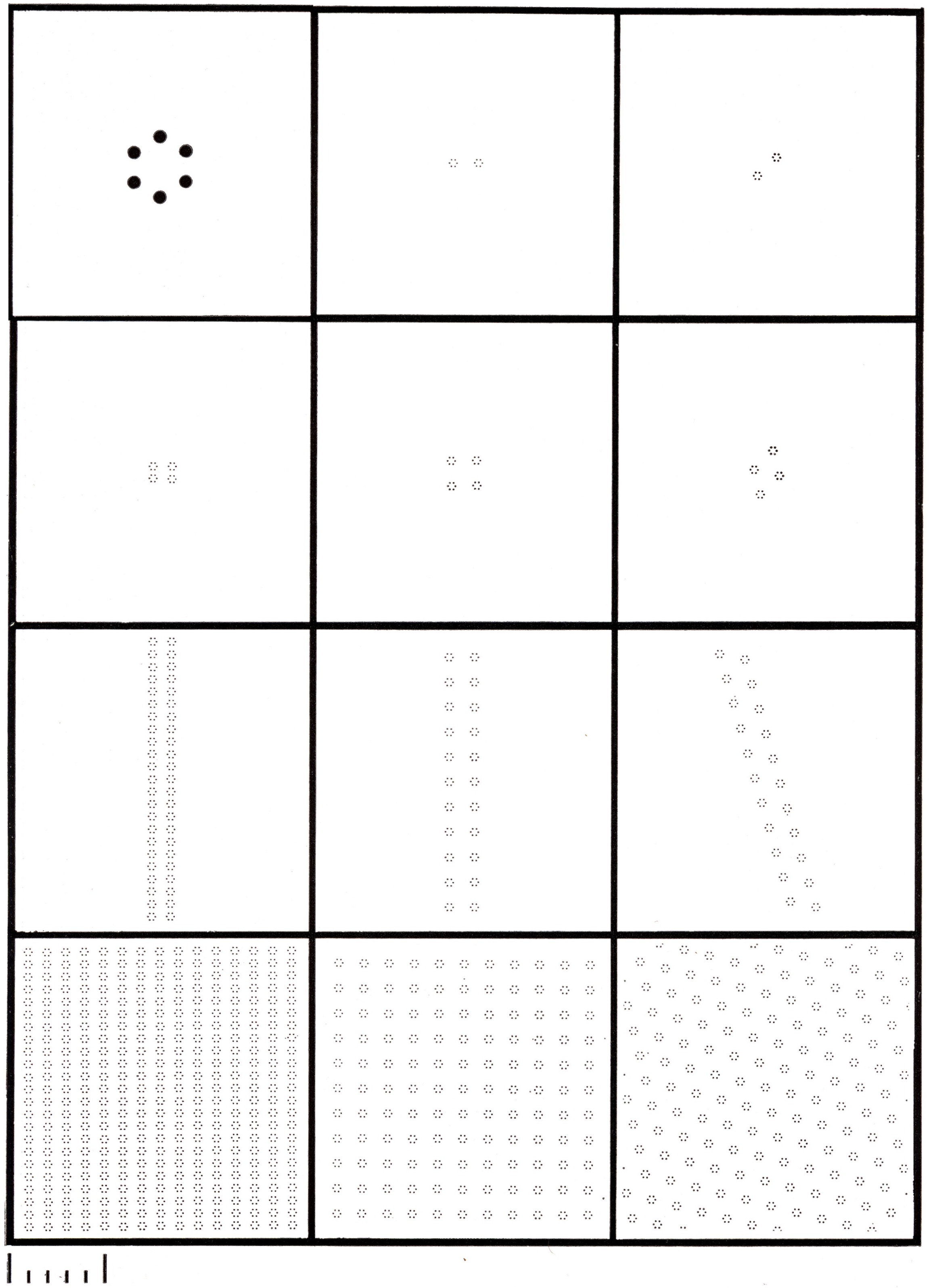
$$F(k)=\sum_{n=-\infty}^{n=+\infty} \delta\left(k-2n\frac{\pi}{x_0}\right)$$

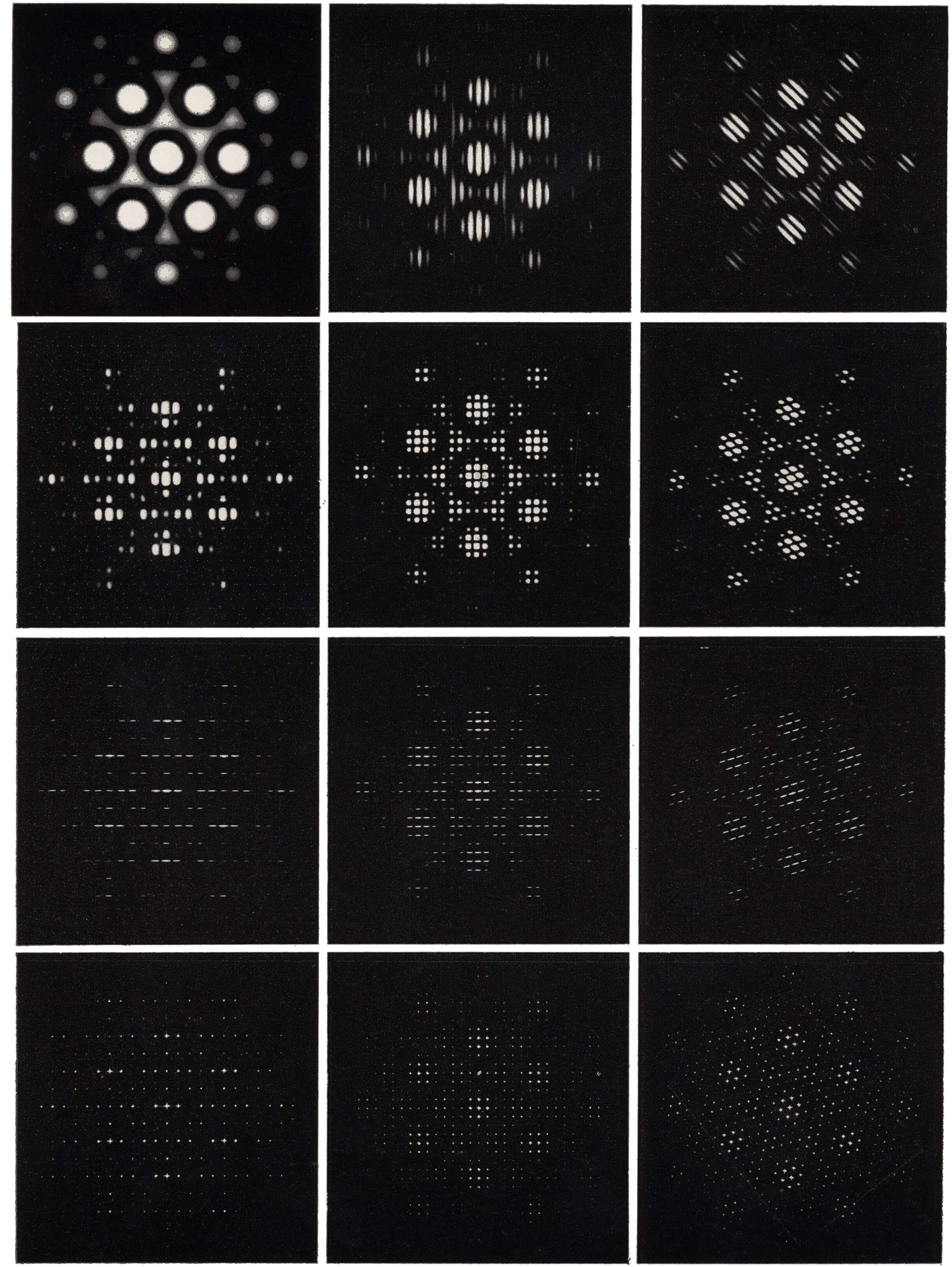


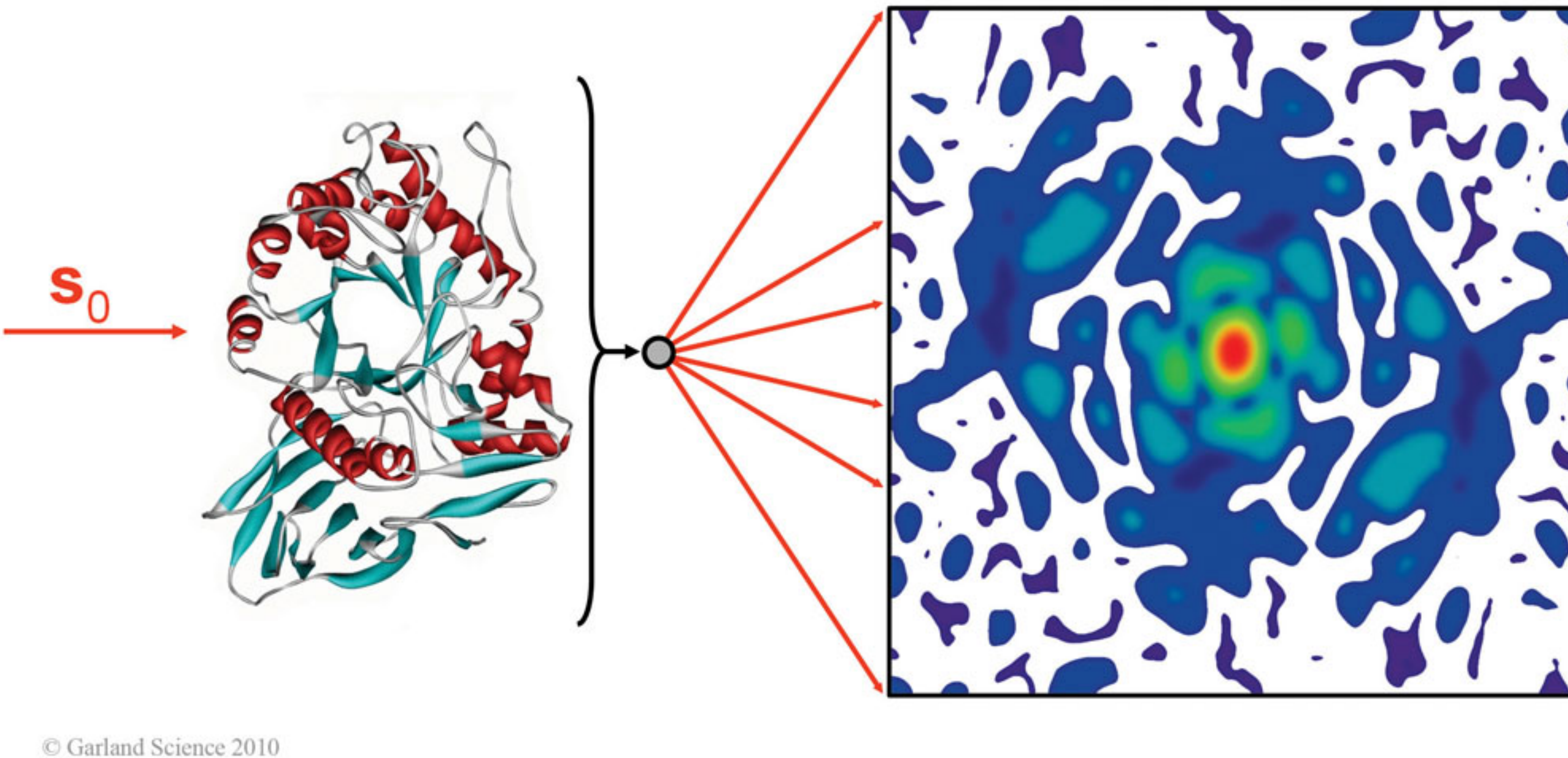




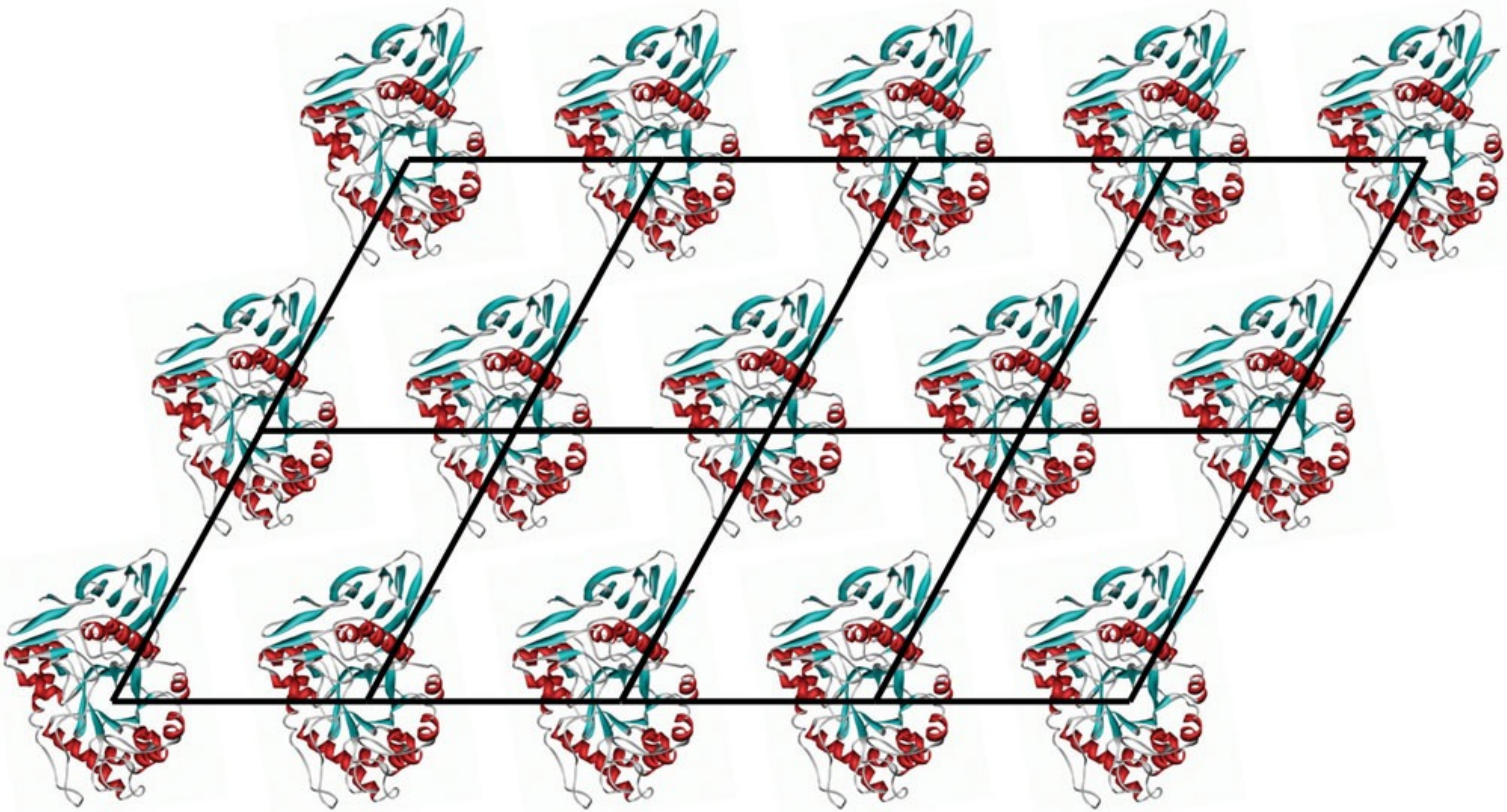








from “Biomolecular Crystallography” Bernhard Rupp, Garland Science 2010



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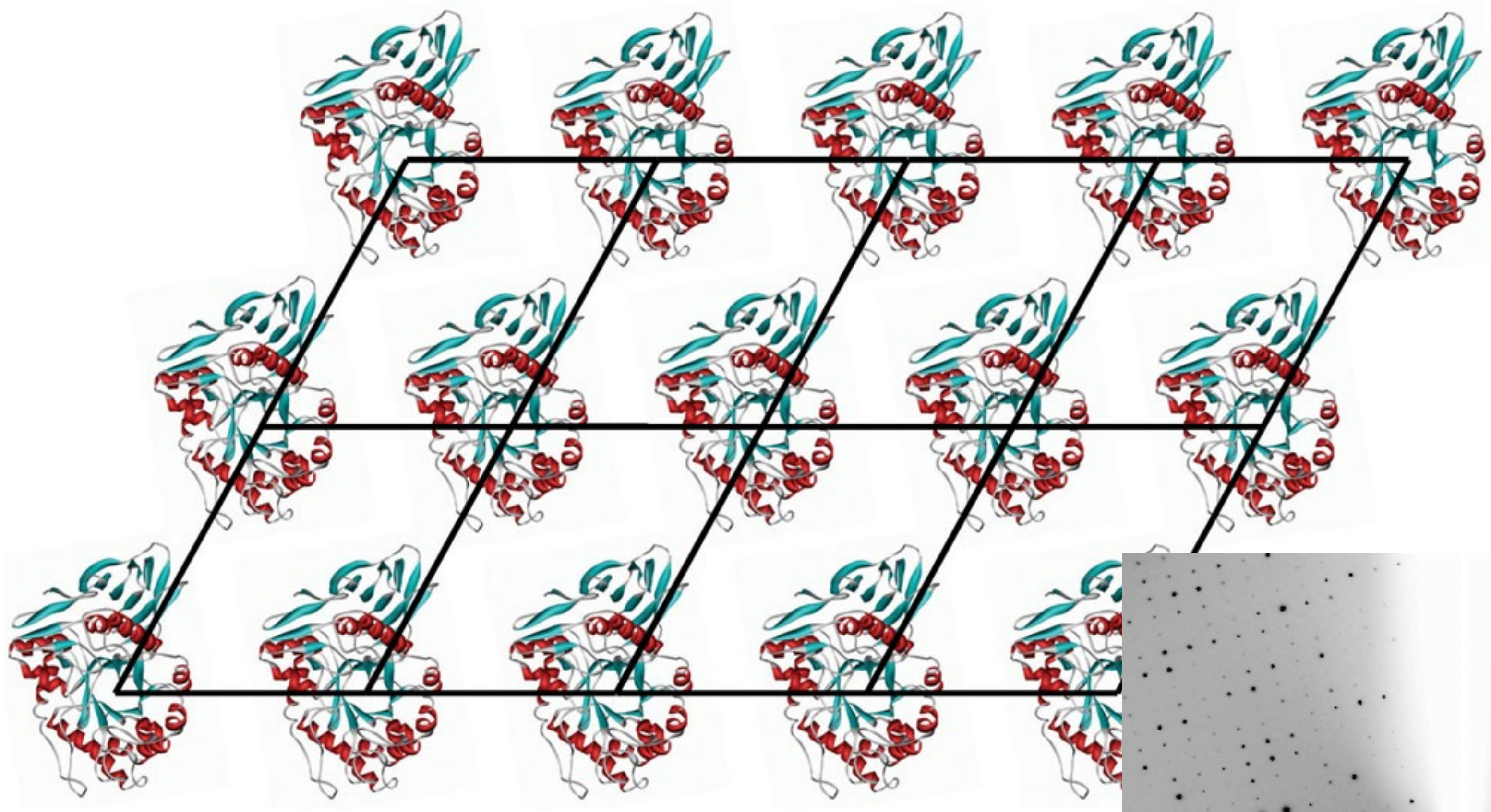
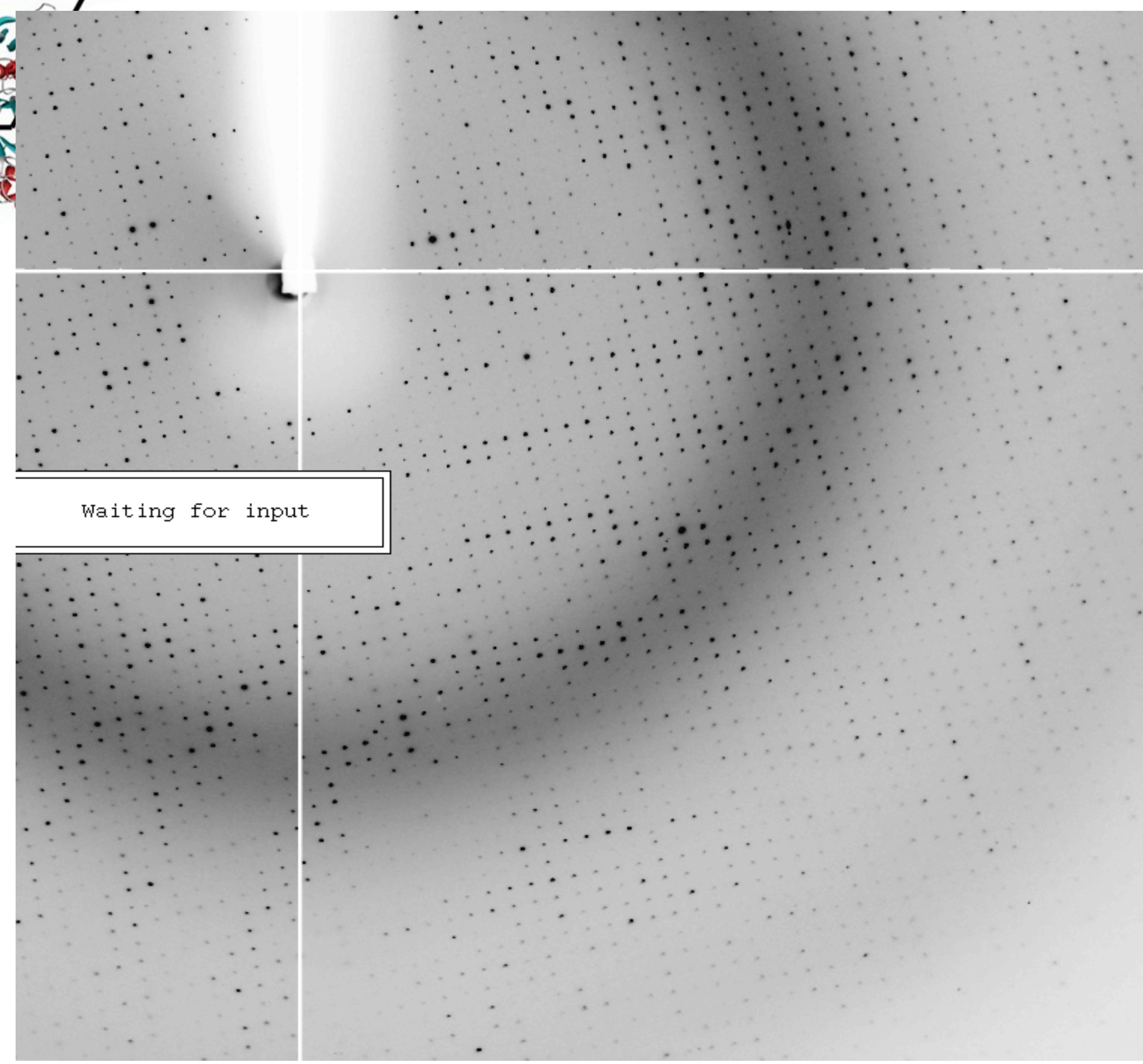
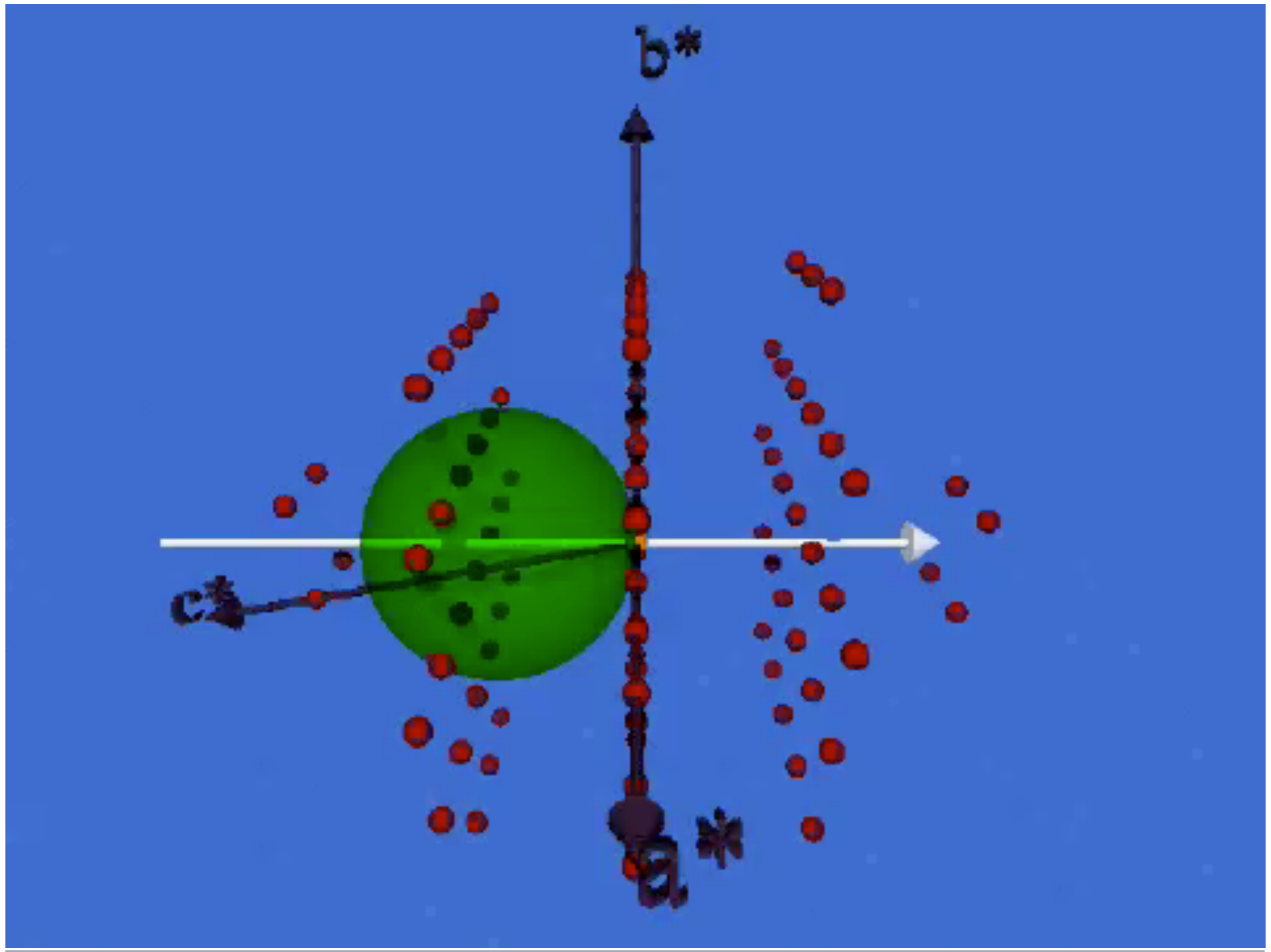




crystal
periodicity

discrete diffraction pattern

(diffraction spots are only observed
for particular directions $\mathbf{S}=\mathbf{H}_j$)

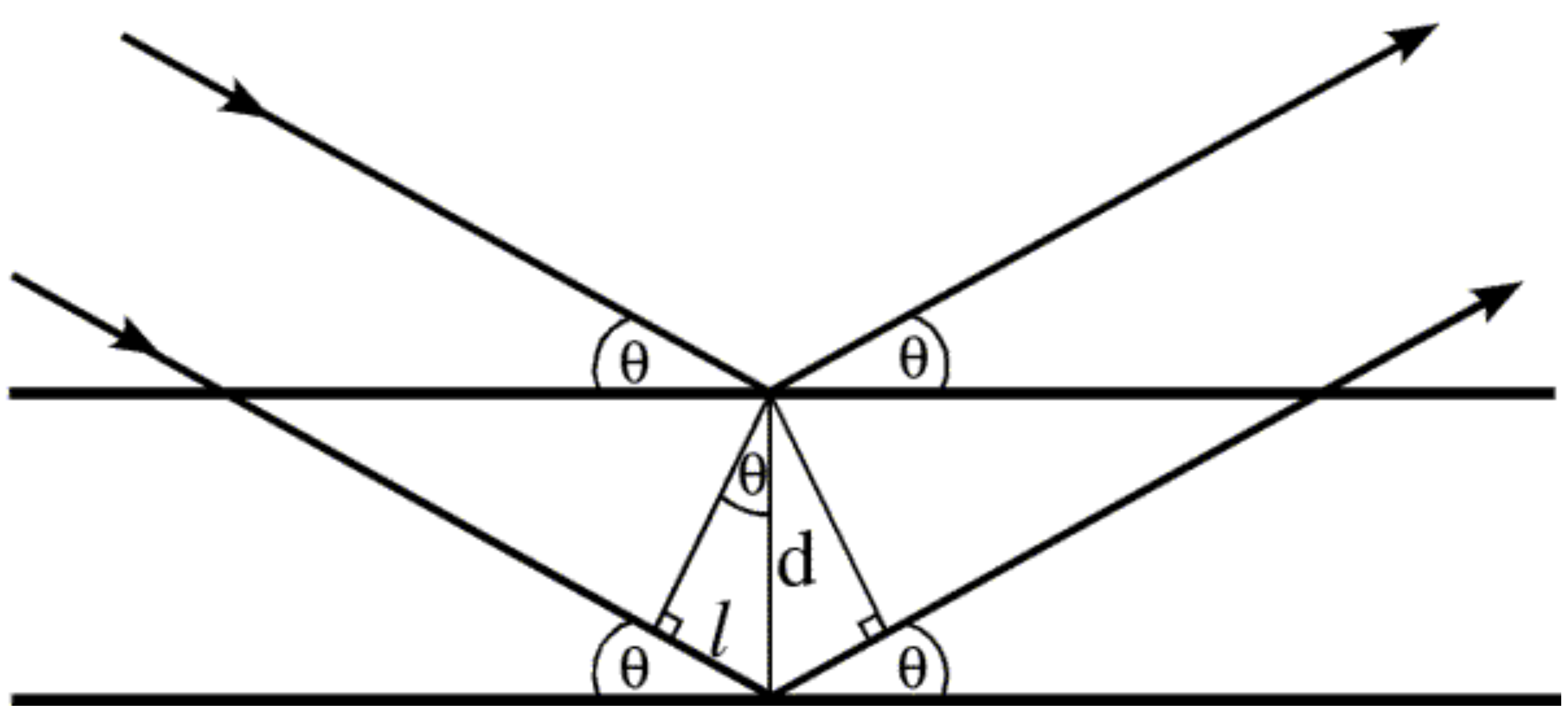


for similar animations see https://www.youtube.com/watch?v=YvJod1B-Irc

Diffraction : waves in phase

2. When do two or more waves scatter in phase?

When their trajectories differ by an integer number of wavelengths



$$n\lambda = 2d \sin \theta$$

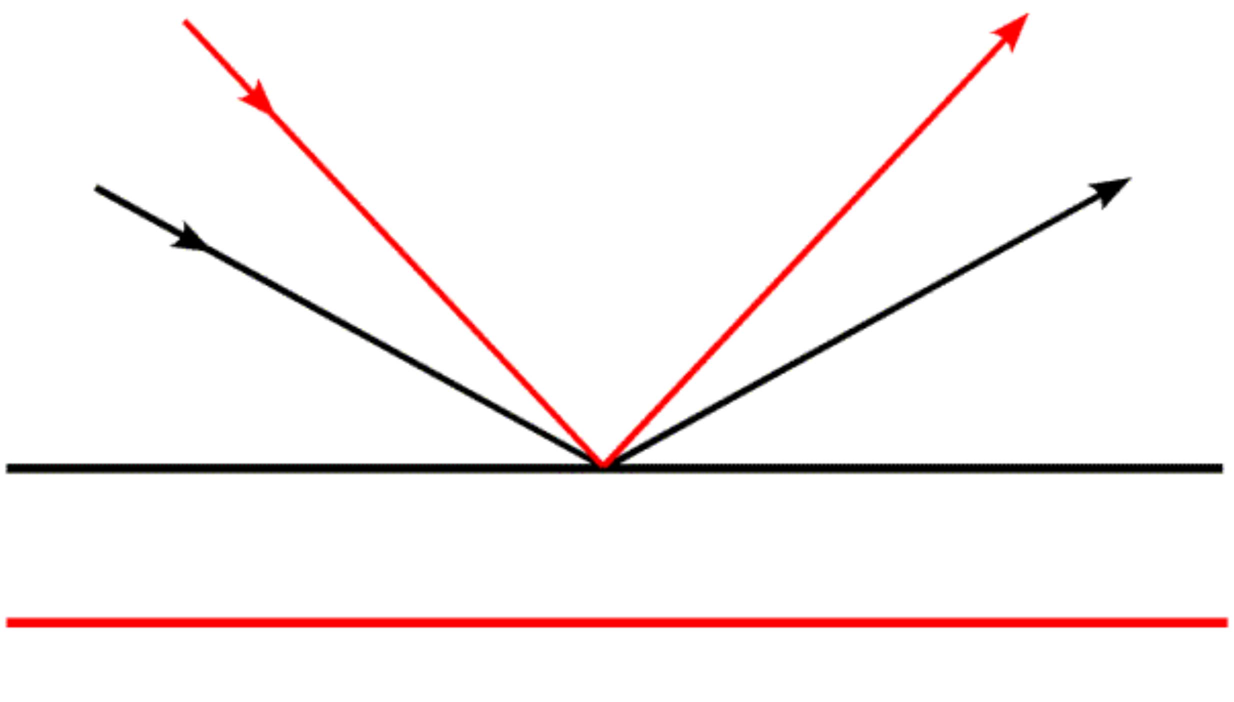
… like in light diffraction

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

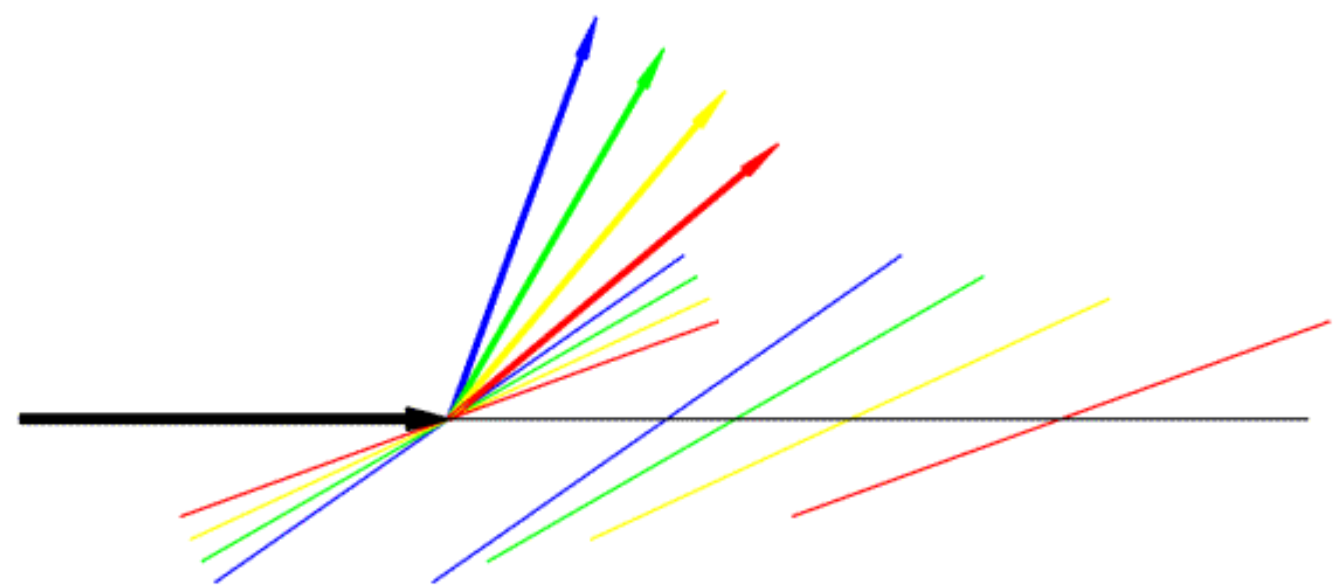
Diffraction: waves in phase

The bigger the angle of diffraction, the smaller the spacing to which the diffraction pattern is sensitive

$$n\lambda = 2d \sin \theta \qquad \sin \theta / n\lambda = 1 / 2d$$



Changing the incoming beam direction

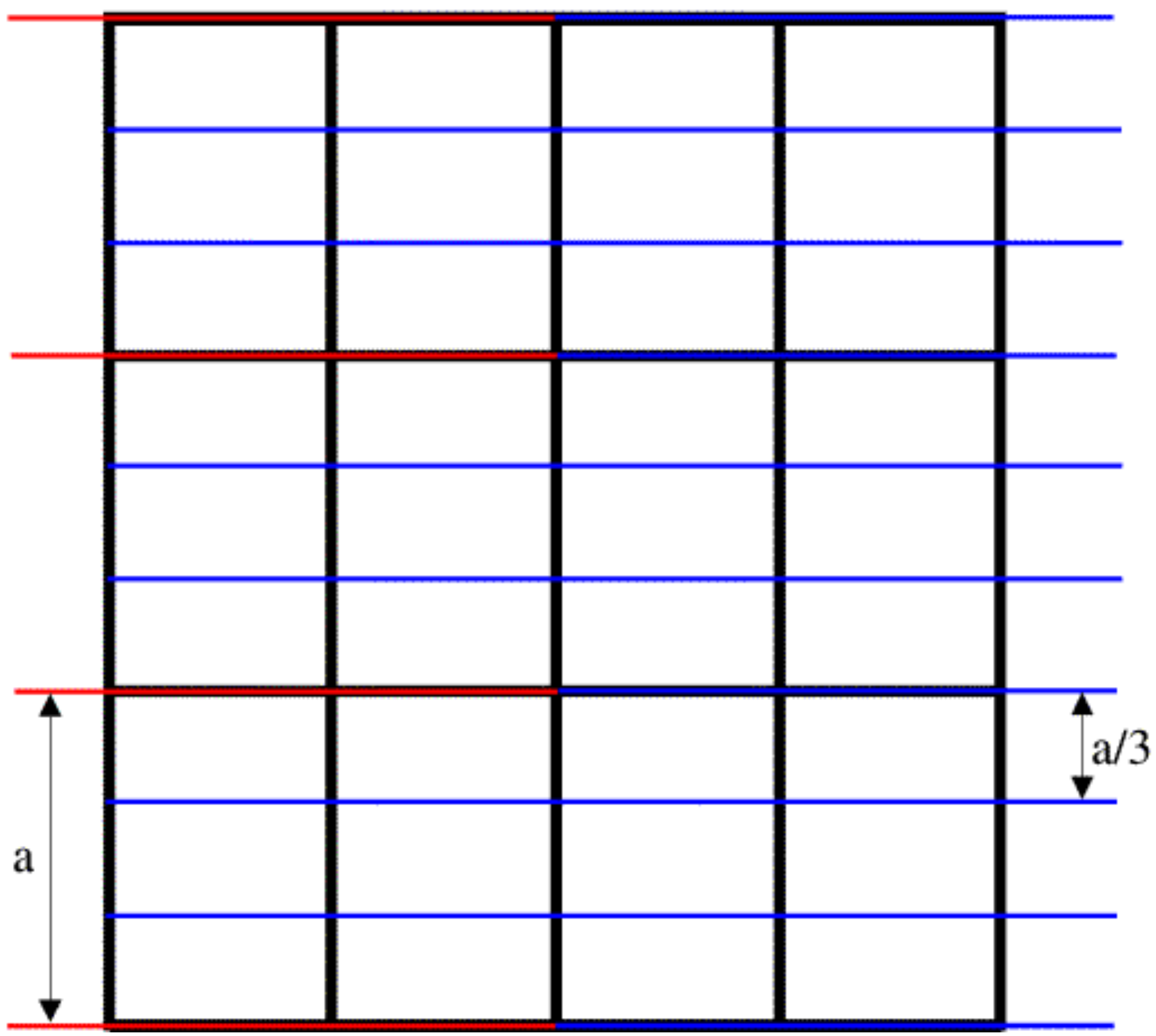


Looking at different planes in the crystal

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

diffraction from a crystal…

- only repetitive planes can diffract in phase
  owing to the crystal symmetry (repetition of a unit cell),
  they have integer ratios with the unit cell axes!!



a b c : the three crystal axes

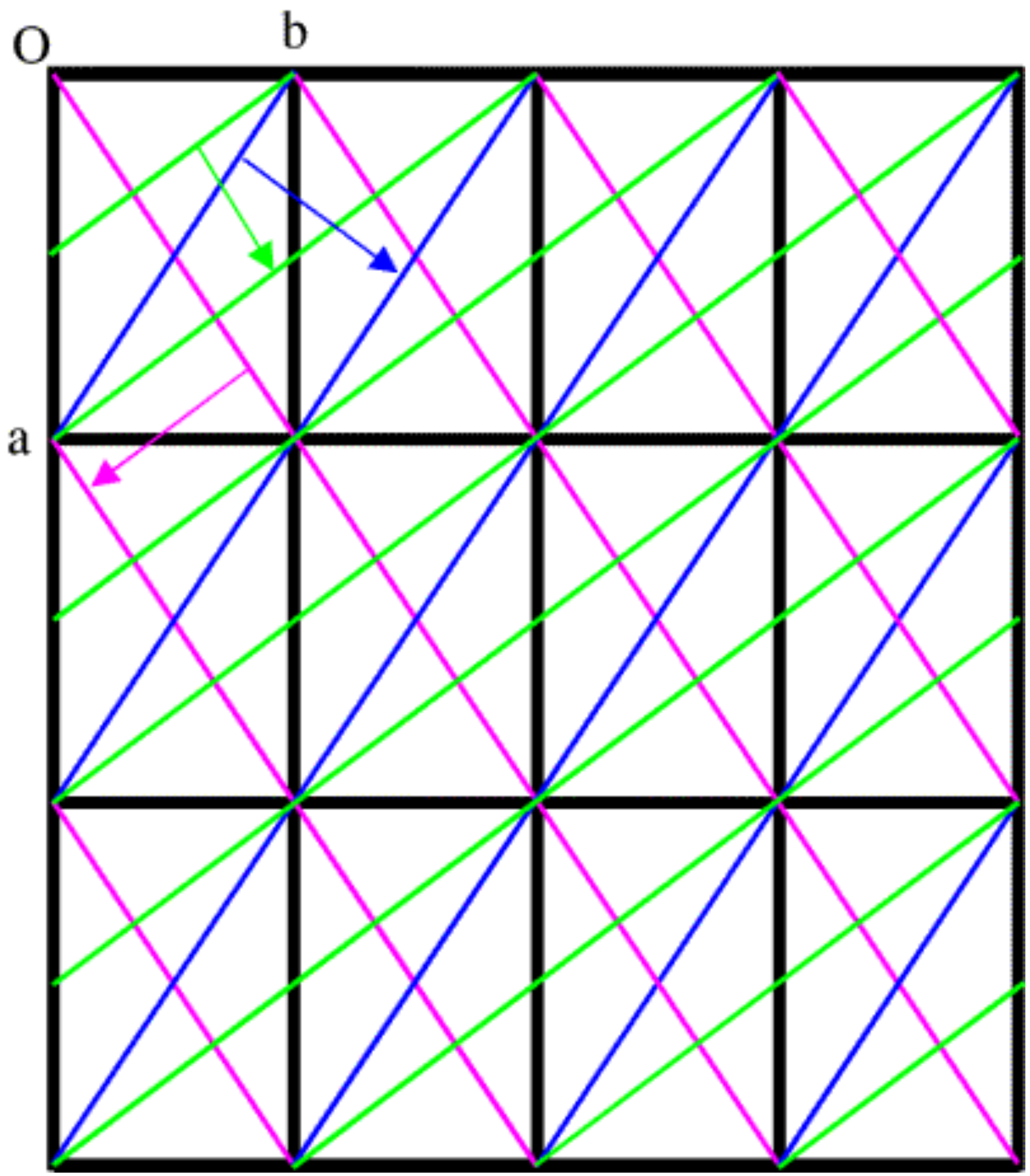
Bragg planes connect integer divisions of each axis

plane 1 0 0

plane 3 0 0

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

diffraction from a crystal…



Now looking at different orientations in 2 dimensions

plane 1 1 0

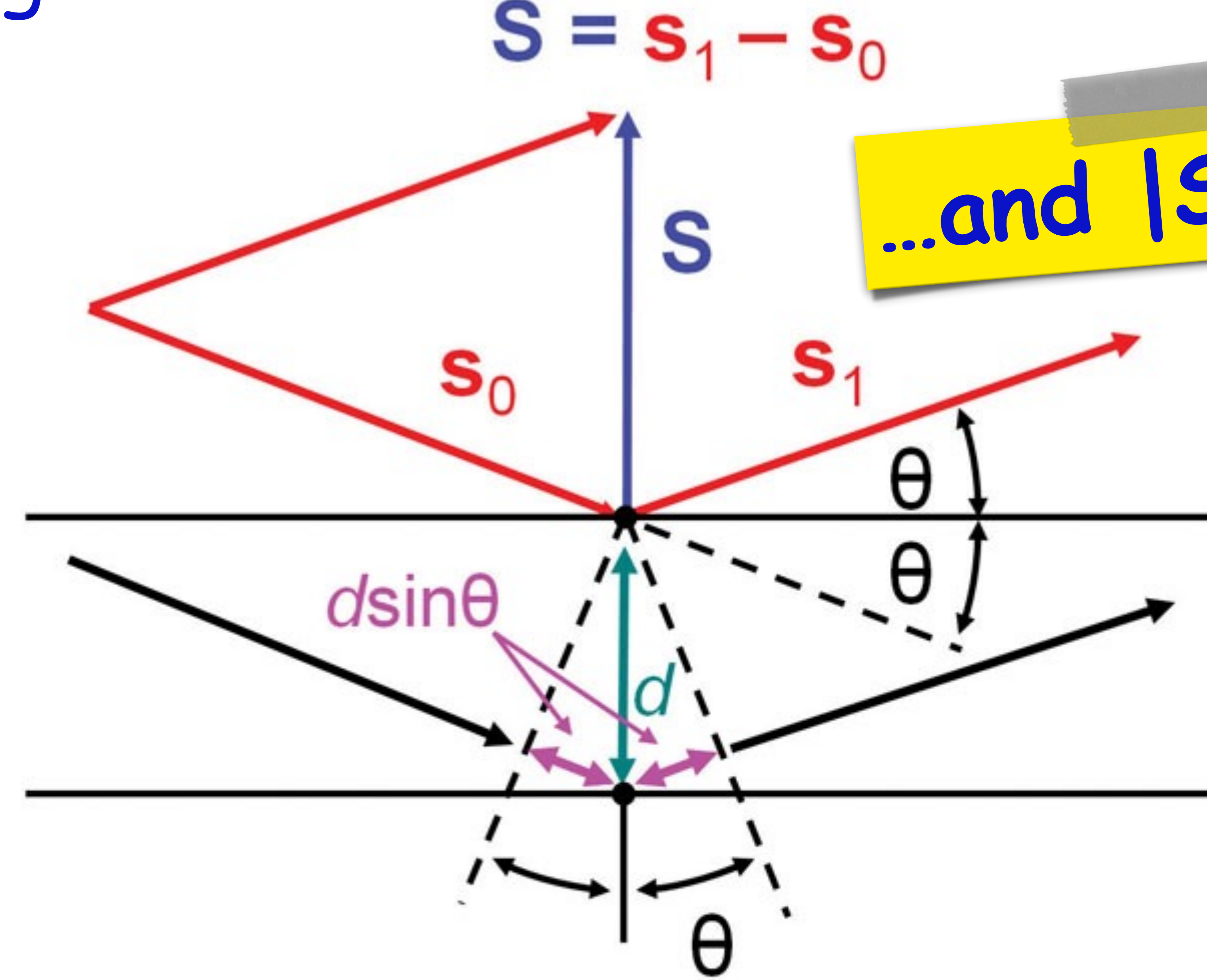
plane 2 1 0

plane 1 -1 0

Miller indices h k l

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

Bragg's law



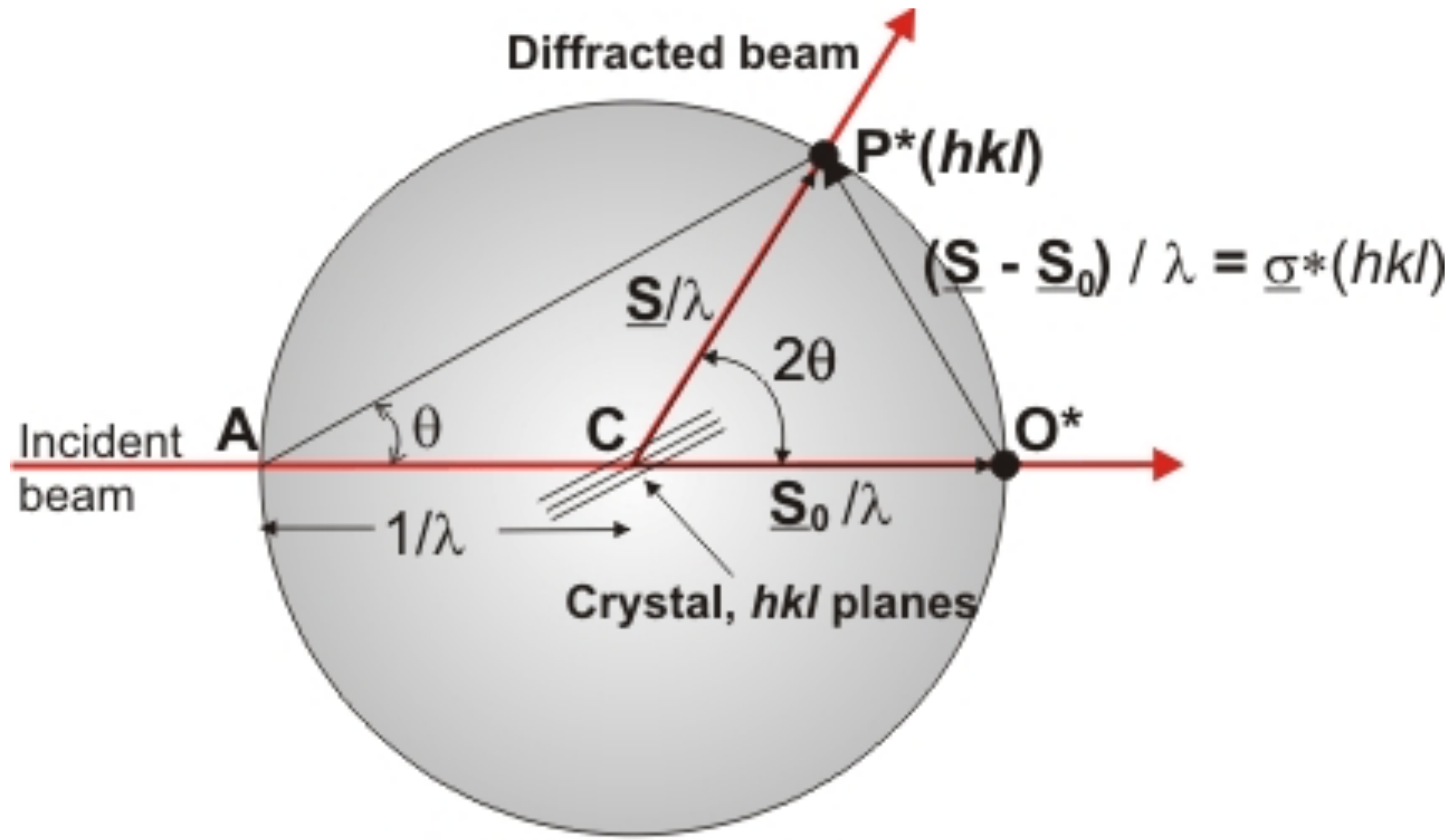
...and |S|=1/d !

For constructive interference, we need:

$$2d \sin \theta = n \lambda$$

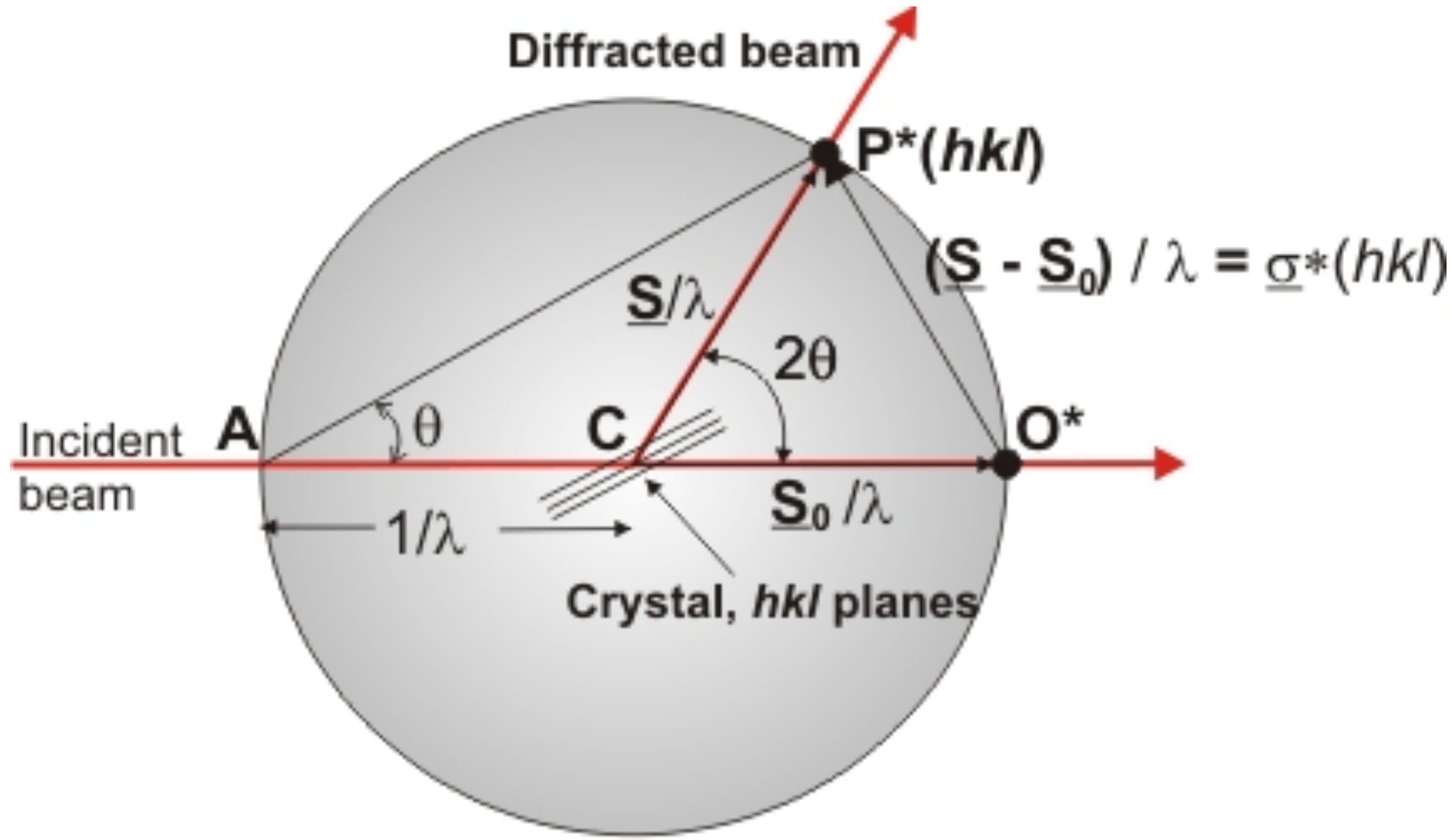
adapted from "Biomolecular Crystallography" Bernhard Rupp, Garland Science 2010

Ewald's sphere to visualize the diffracted beam vector S_1 (Rx frequency in elastic scattering, $|S_1|=1/\lambda$); and, the scattering vector S ($|S|=1/d$, signifying an hkl frequency linked to spatial frequency of crystal planes)



Ewald's sphere

At each direction in diffraction condition (hkl reflections), the diffracted beam will have a measurable intensity ∝ [amplitude]^2 of the structure factor, and that amplitude depends on the 3D organization of scatterers in the xtal



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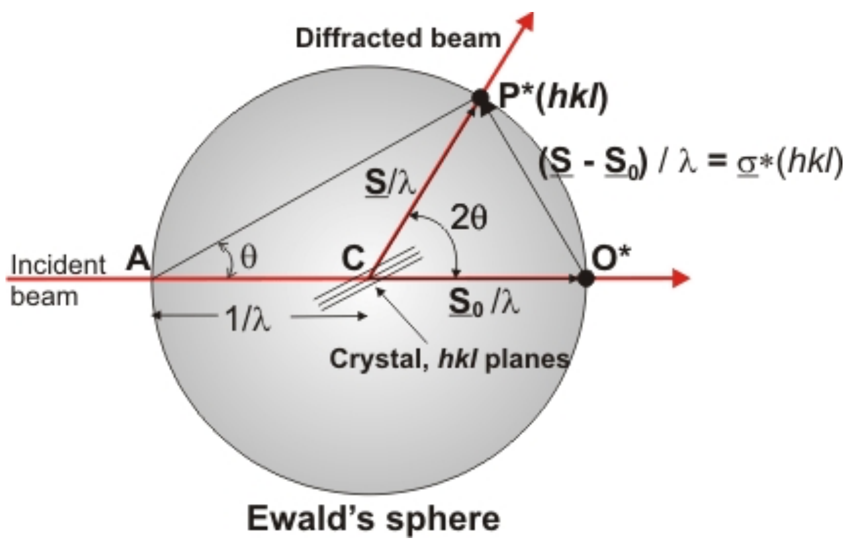

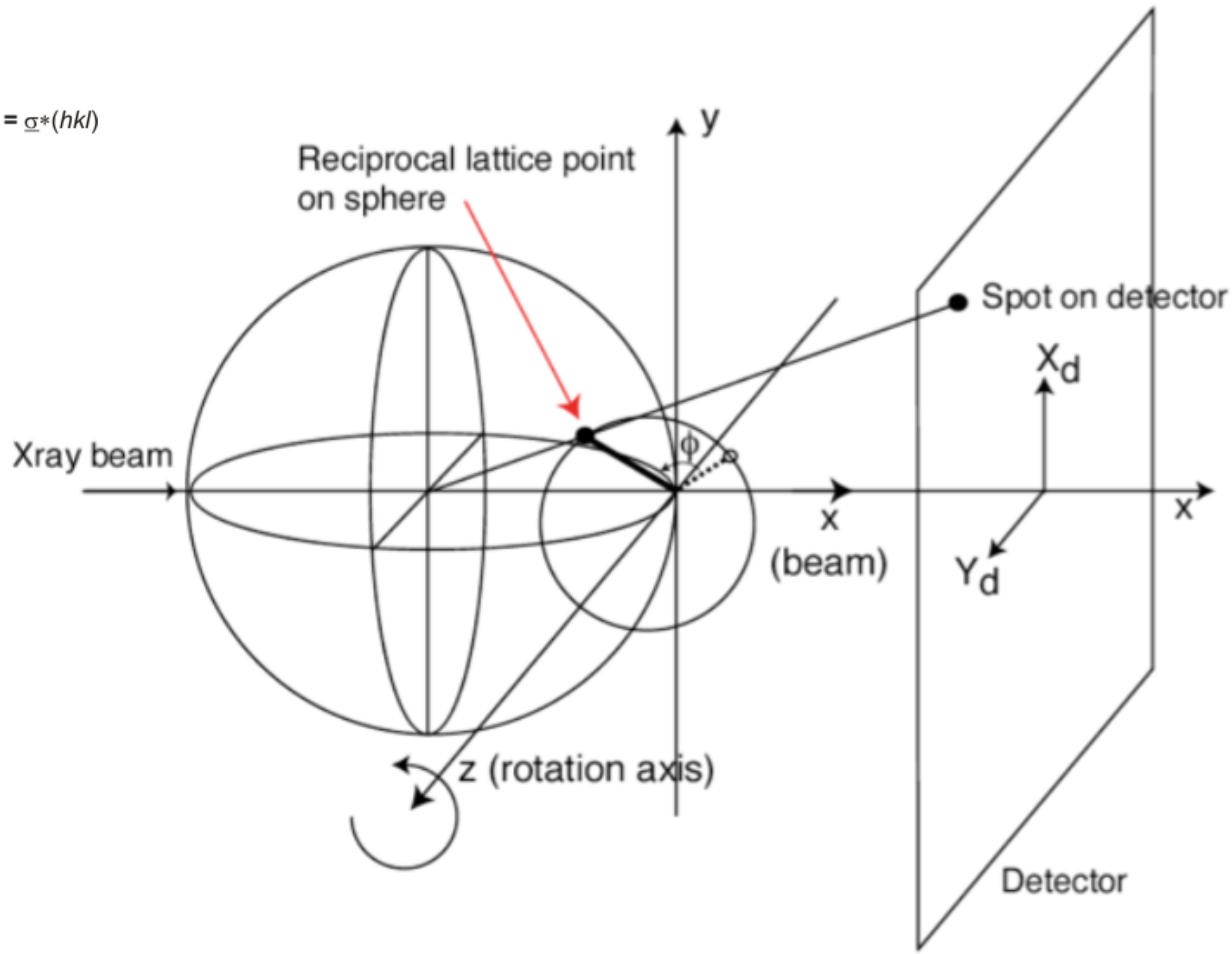

...so, diffraction physically performs the Fourier transform of an object (or of the convolution of that object with a 3D lattice array = a crystal), analyzing it

$$\mathbf{F}(\mathbf{S}) = V \int_{V} \rho(\mathbf{r}) \; e^{2\pi i \, \mathbf{S}.\mathbf{r}} \, d\mathbf{r}$$

Unit cell

these are simple sinusoidal waves, each with

frequency (defined by **S**)

amplitude $\propto$ [intensity]$^{1/2}$

phase (relative to a defined origin)

...so, diffraction physically performs the Fourier transform of an object (or of the convolution of that object with a 3D lattice array = a crystal), analyzing it

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Unit cell

??

these are simple sinusoidal waves, each with

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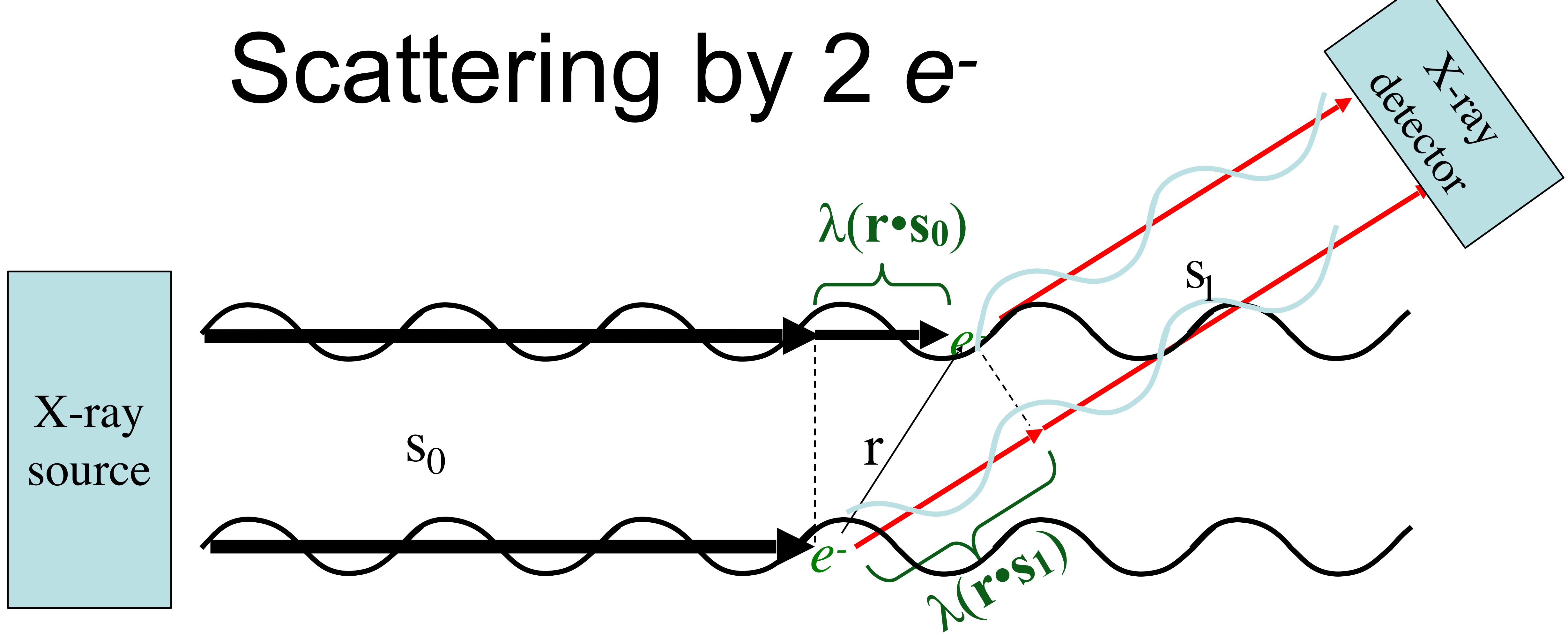
amplitude ∝ [intensity]$^{1/2}$

phase (relative to a defined origin)

Scattering by 2 e^-



Difference in path = $\lambda\ (r \bullet s_1 - r \bullet s_0)$

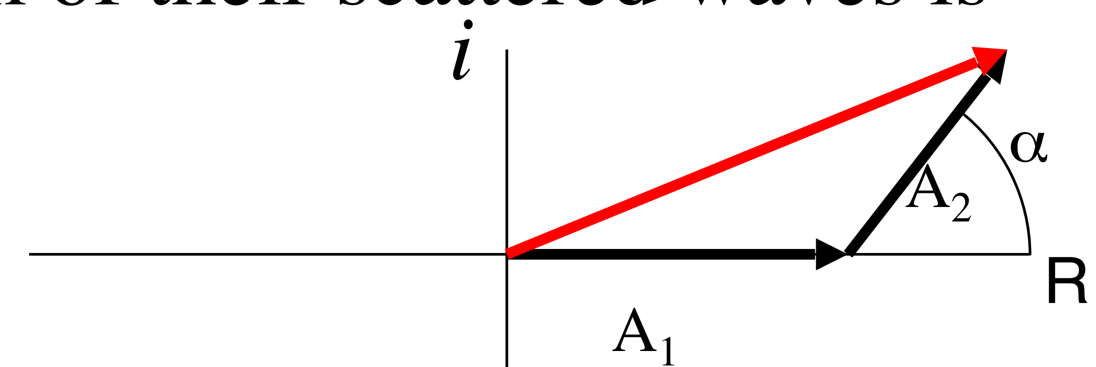
Relative phase: $\alpha = 2\pi(r \bullet s_1 - r \bullet s_0)$

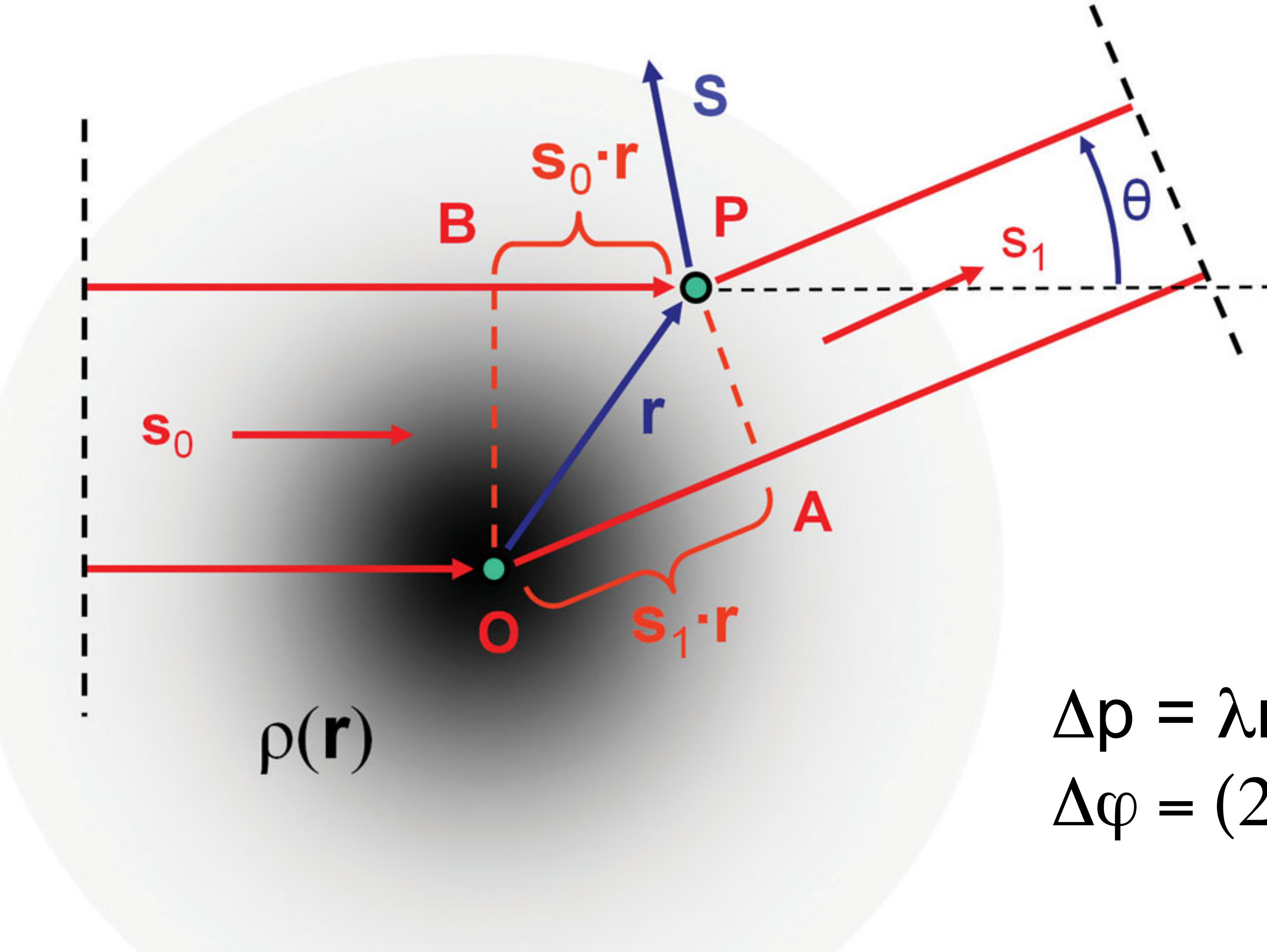
remember that $|s_0| = |s_1| = 1/\lambda$

if $(s_1 - s_0) = S$, then $\alpha = 2\pi(r \bullet S)$

If $e^-(1)$ scatters with amplitude A_1, and $e^-(2)$ scatters with amplitude A_2, then the sum of their scattered waves is

$$A_1 + A_2 e^{i\alpha}$$





$$\Delta p = \lambda \mathbf{r}.\mathbf{s_1} - \lambda \mathbf{r}.\mathbf{s_0} = \lambda \mathbf{S}.\mathbf{r}$$

$$\Delta\varphi = (2\pi/\lambda)\ \Delta p = 2\pi\ \mathbf{S}.\mathbf{r}$$

$$A = A_0 + A_P\ e^{\ i\Delta\varphi}$$

$$A = A_0 + A_P\ e^{\ 2\pi i\ \mathbf{S}.\mathbf{r}}$$

from “Biomolecular Crystallography” Bernhard Rupp, Garland Science 2010

Structure factor F from a single electron

Represents the wave that results from diffraction : it's a complex number with amplitude and phase.

To put F on absolute scale, the diffraction from a single electron at a point is defined as having an amplitude of 1e.

$$\mathbf{F}(\mathbf{s}) = 1e\ \exp(2\pi i\mathbf{s}\cdot\mathbf{r})$$

F from a number of electrons…

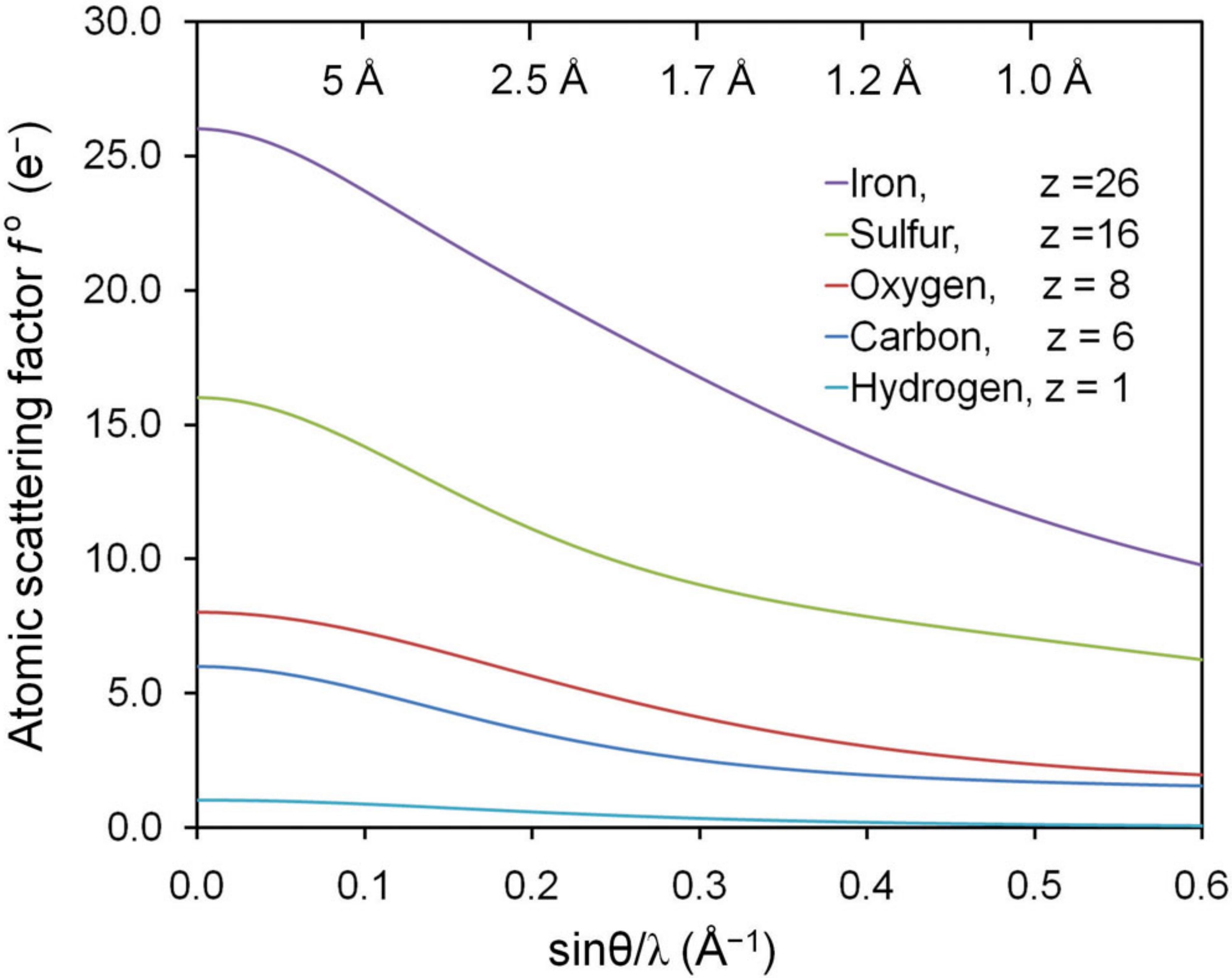
$$\mathbf{F}(\mathbf{s}) = \sum_j \exp\left(2\pi i\mathbf{s}\cdot\mathbf{r}_j\right)$$

…or a continuous distribution of electrons…

$$\mathbf{F}(\mathbf{s}) = \int_{space} \rho(\mathbf{r})\exp(2\pi i\mathbf{s}\cdot\mathbf{r})d\mathbf{r}$$

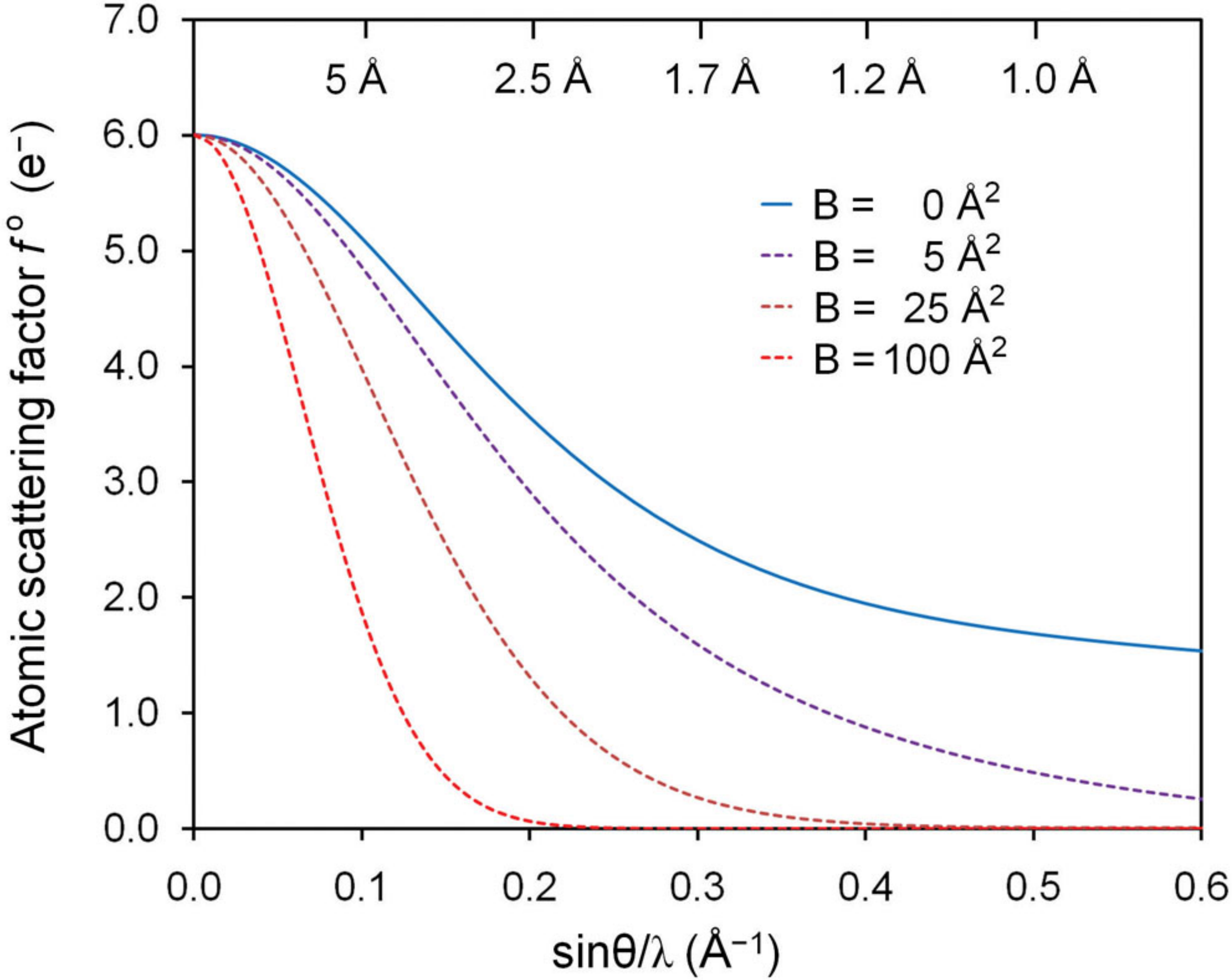
the dot product (r•S) of two 3D vectors,
can be substituted by its computed value :

$$\mathbf{F}_{hkl} = V \int_{xyz} \rho_{xyz}\, e^{[2\pi i(hx+ky+lz)]}\, \delta xyz$$



$$f_{(|s|)} = V \int_{xyz} \rho_{xyz}\, e^{[2\pi i\, S\bullet r]}\, \delta xyz$$

from “Biomolecular Crystallography” Bernhard Rupp, Garland Science 2010

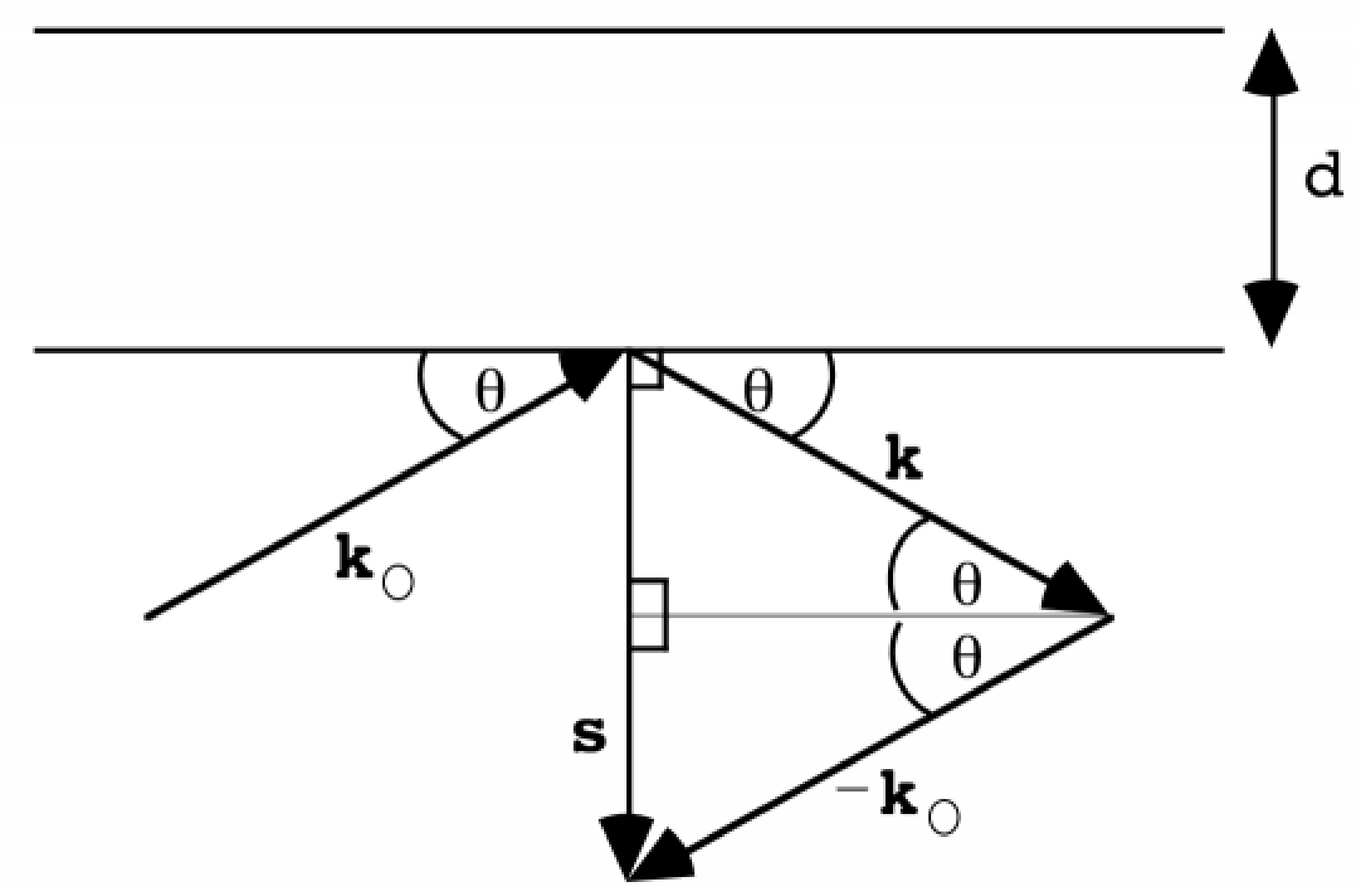


$$f_{(|s|)} = f_{(0)}\, e^{\left[-B_{iso}\left(\frac{\sin\theta}{\lambda}\right)^2\right]}$$

from "Biomolecular Crystallography" Bernhard Rupp, Garland Science 2010

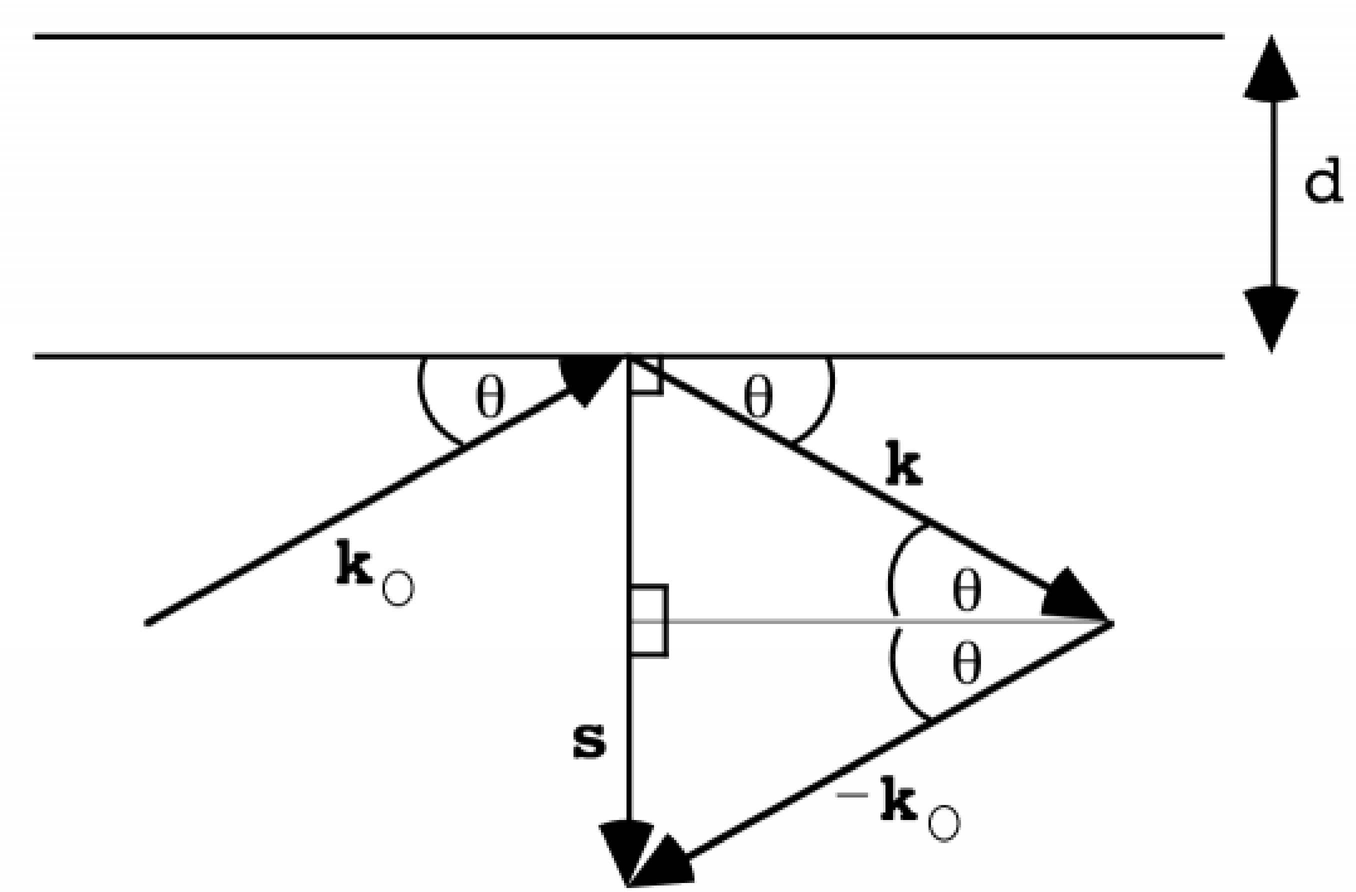
What matters for the phase of diffraction is the component of **r** in the direction of the diffraction vector **s**

All points **r** with the same value of **s•r** (i.e. satisfying the equation **s•r**=c) lie on a plane perpendicular to **s** and diffract with the same phase.



adapted from http://www-structmed.cimr.cam.ac.uk/Course/

We can use this picture to work out Bragg's law again. The phase relative to diffraction from the origin depends on the value of **s•r**, or the component of **r** parallel to **s**.



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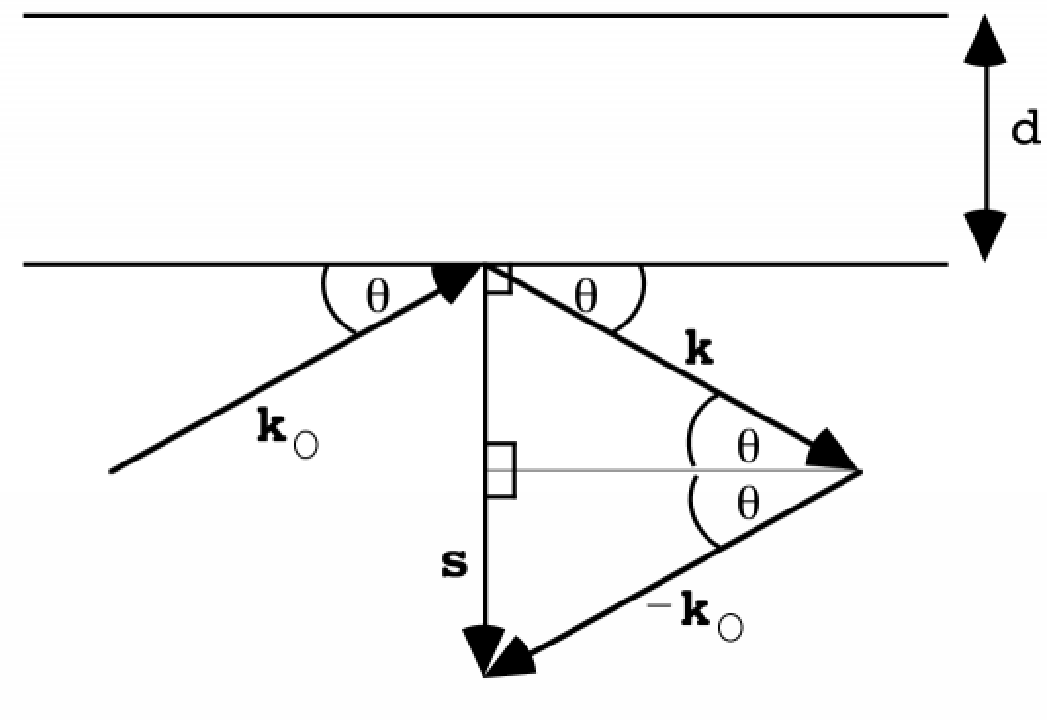


We can use this picture to work out Bragg's law again. The phase relative to diffraction from the origin depends on the value of **s•r**, or the component of **r** parallel to **s**.

From the figure, we see that

$$|\mathbf{s}| = 2 \sin\theta \, |\mathbf{k}| = 2 \sin\theta \, / \, \lambda$$

When **s•r**=1, the phase of diffraction is shifted by 2π and the component of **r** in the direction of **s** is equal to the d-spacing for this diffraction angle.

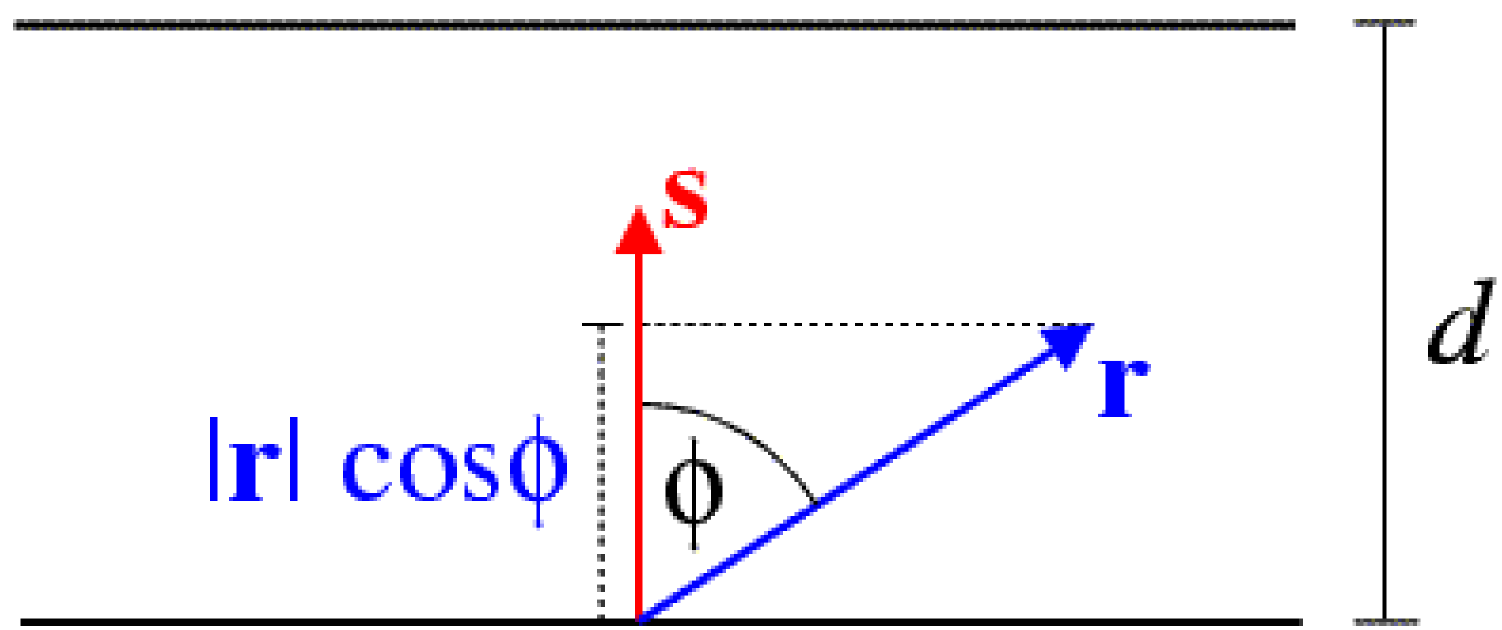
This means that

$$\boxed{|\mathbf{s}| \, d = 1, \text{ or } |\mathbf{s}| = 1/d}$$

We can combine the two equations and rearrange to get Bragg's law: $\lambda = 2 \, d \sin\theta$

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

Since the length of the diffraction vector, |**s**|, is equal to 1/d, **s•r** is equal to the fraction of the distance from one Bragg plane to the next that the position vector **r** has travelled from the origin.



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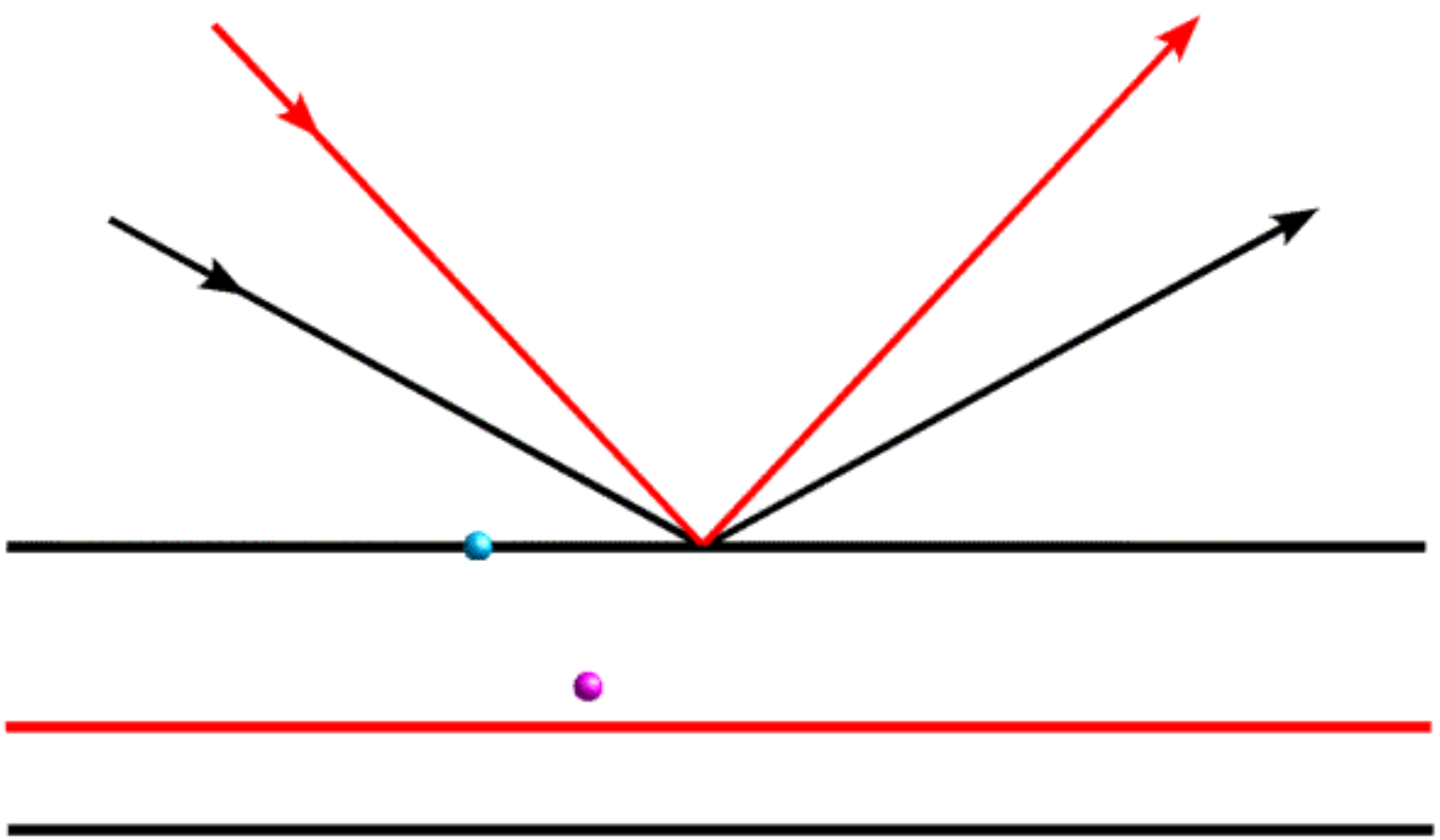
Diffraction: waves in phase

What class of information we get from this?

~relative position of scatterers in the perpendicular direction to the considered planes

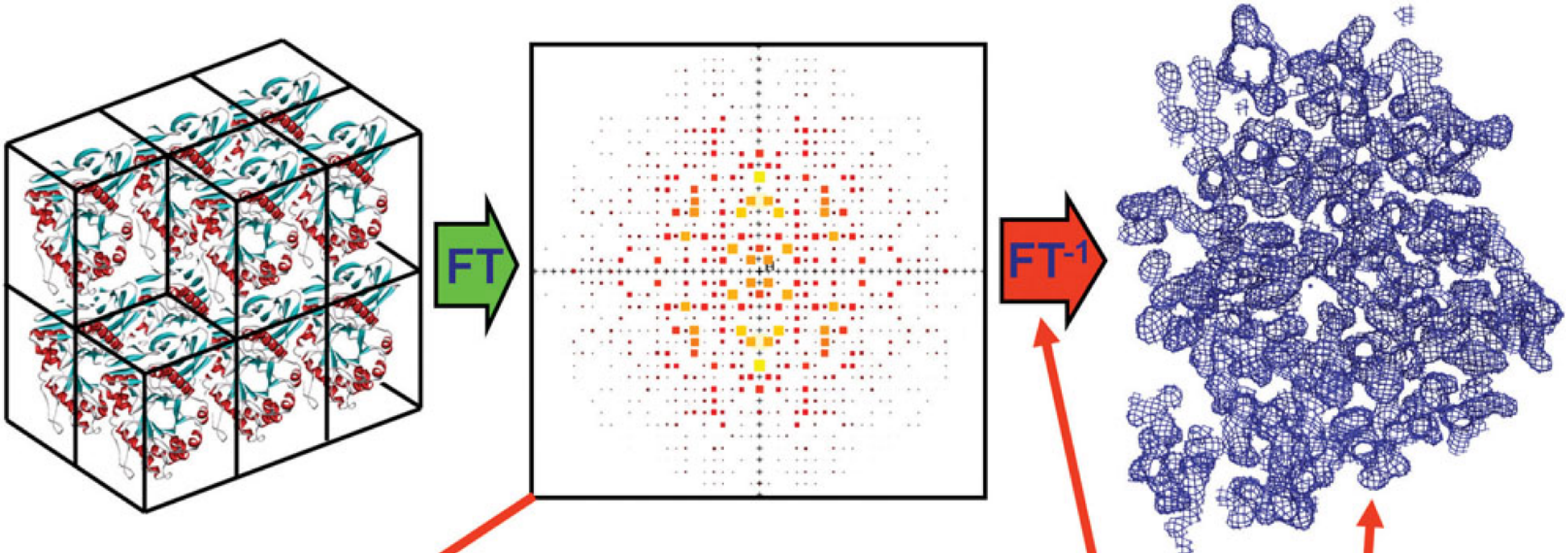


The black angle will give low diffracted intensity

The red one, higher

Why?

adapted from http://www-structmed.cimr.cam.ac.uk/Course/



$$\sum_{\mathbf{h}=-\infty}^{+\infty} F(\mathbf{h})\cdot\exp[-2\pi i(\mathbf{h}\cdot\mathbf{r})+i\varphi(\mathbf{h})]=\rho(\mathbf{r})$$

we can derive amplitudes from measured intensities…

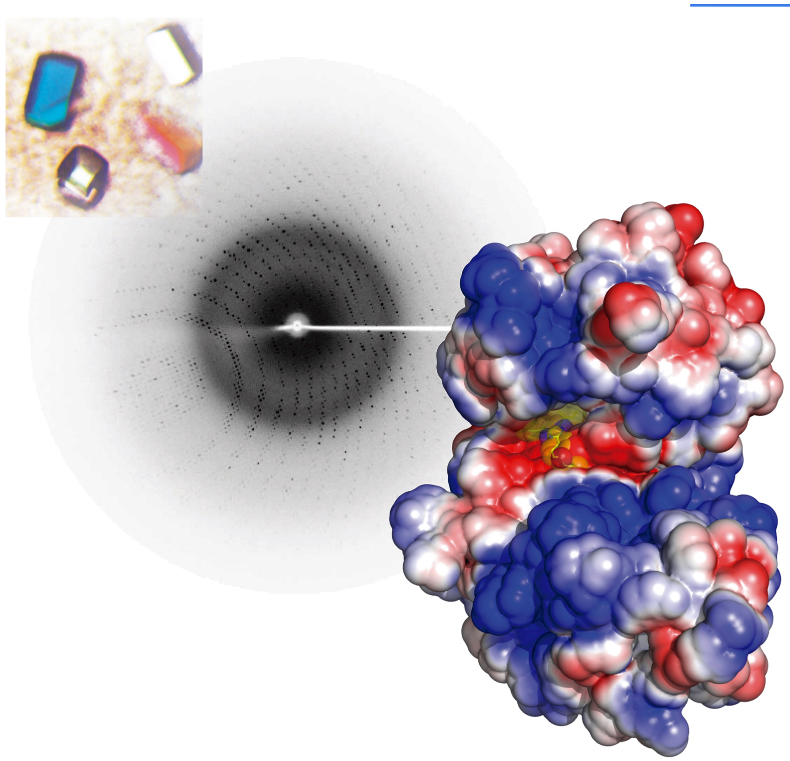
…but the phases are not directly measured!

adapted from “Biomolecular Crystallography” Bernhard Rupp, Garland Science 2010

Unit of Protein Crystallography

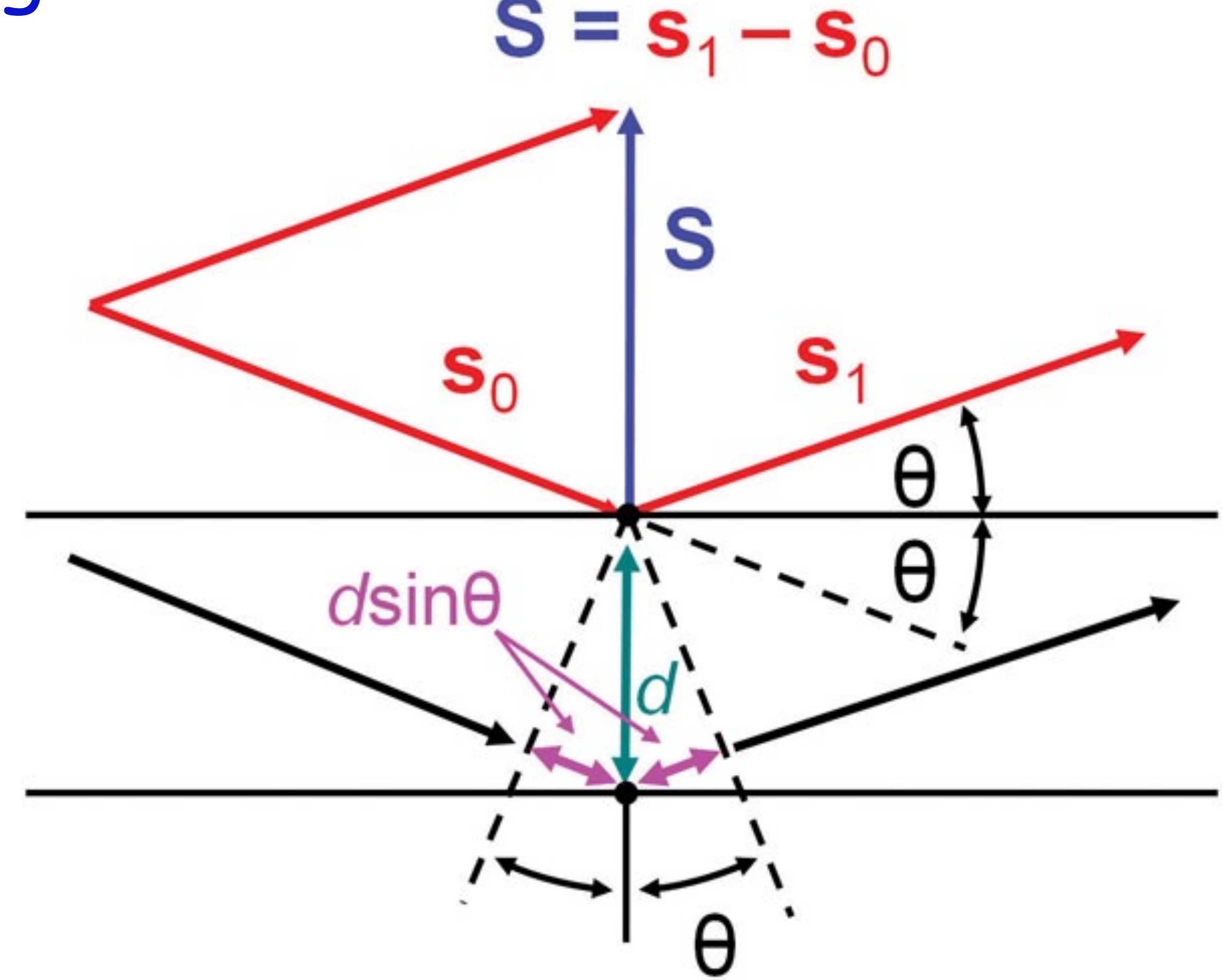
Institut Pasteur de Montevideo

thank you



Macromolecular Crystallography School 2018
November 2018 - São Carlos, Brazil

Bragg's law

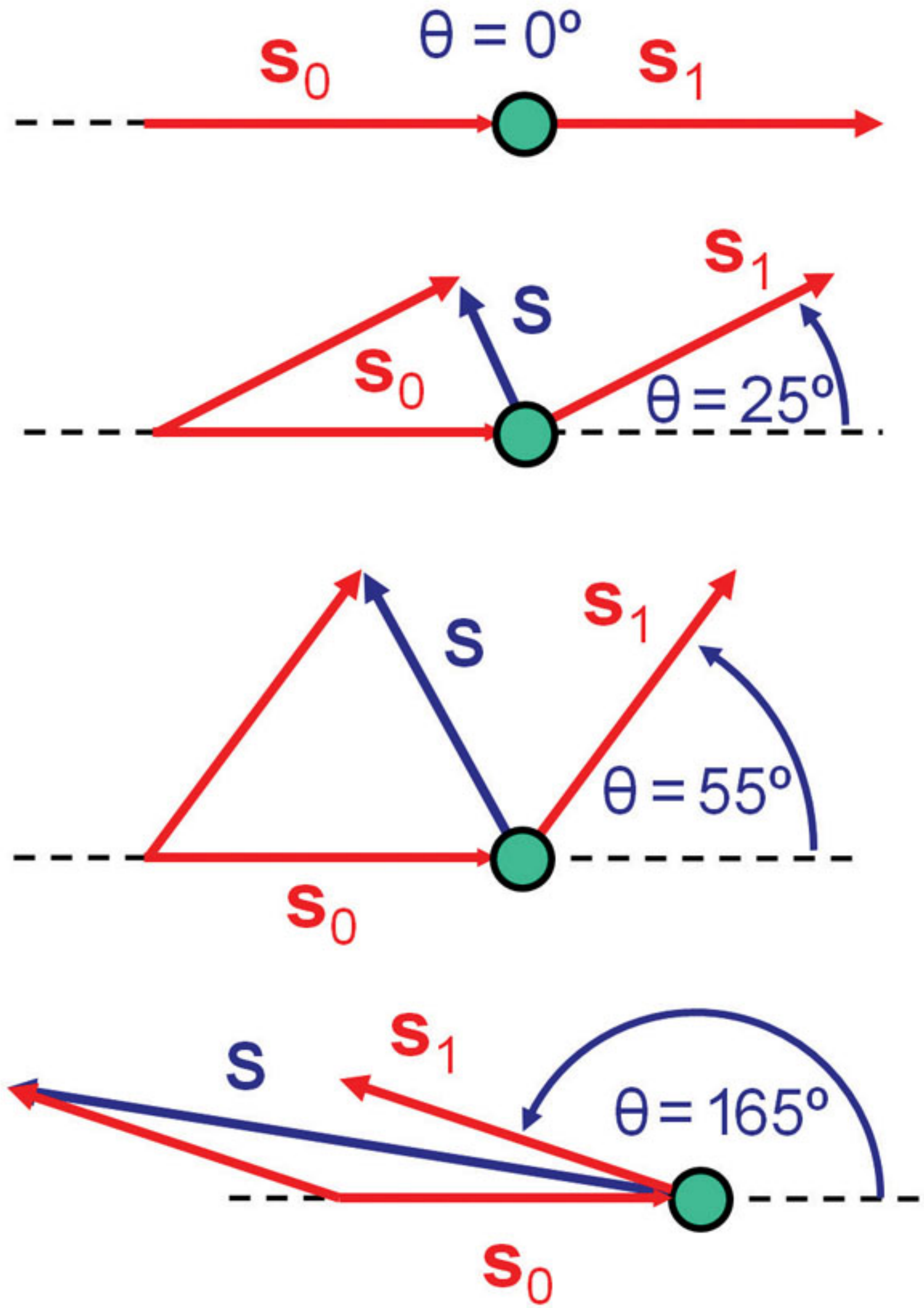


For constructive interference, we need:

$$2d \sin \theta = n \lambda$$

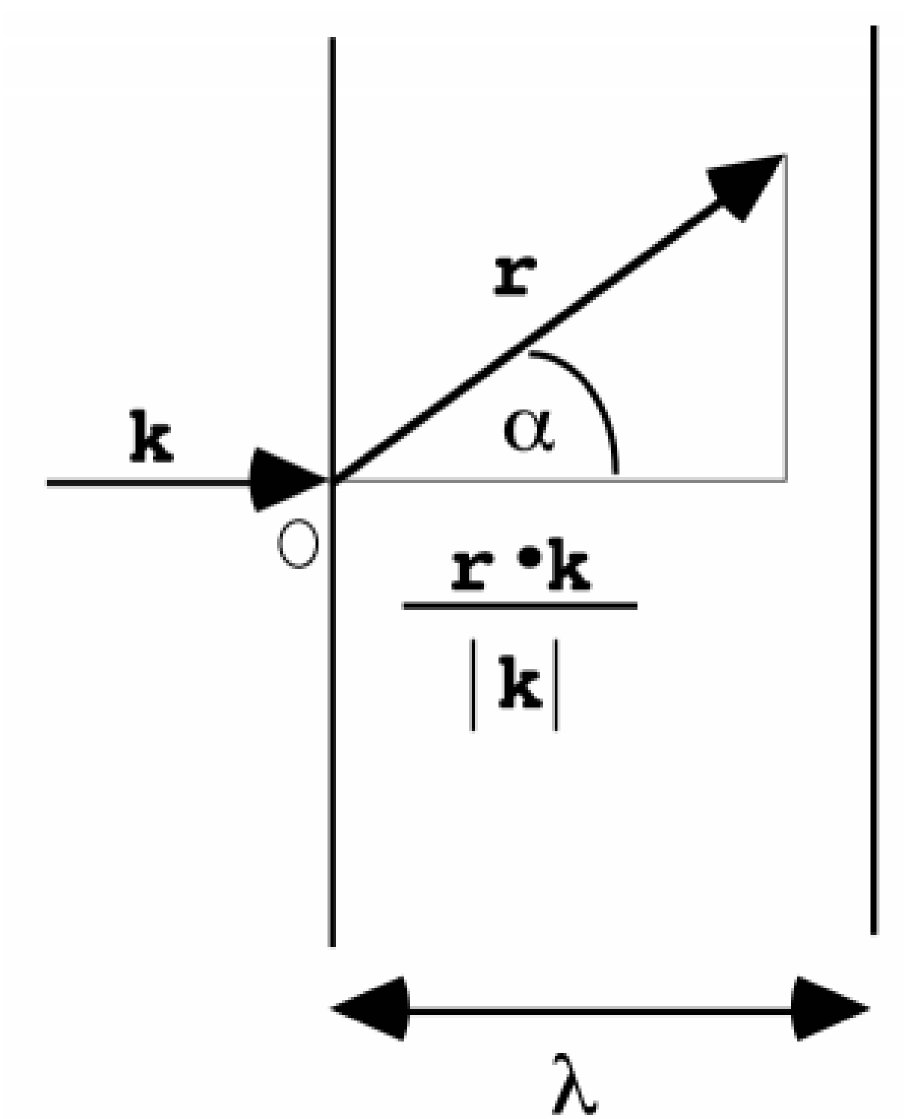
from "Biomolecular Crystallography" Bernhard Rupp, Garland Science 2010

$\mathbf{S} = \mathbf{s}_1 - \mathbf{s}_0$



from "Biomolecular Crystallography" Bernhard Rupp, Garland Science 2010

Again, to look at from a (slightly) different point of view…



Incoming : plane wave (vector k)

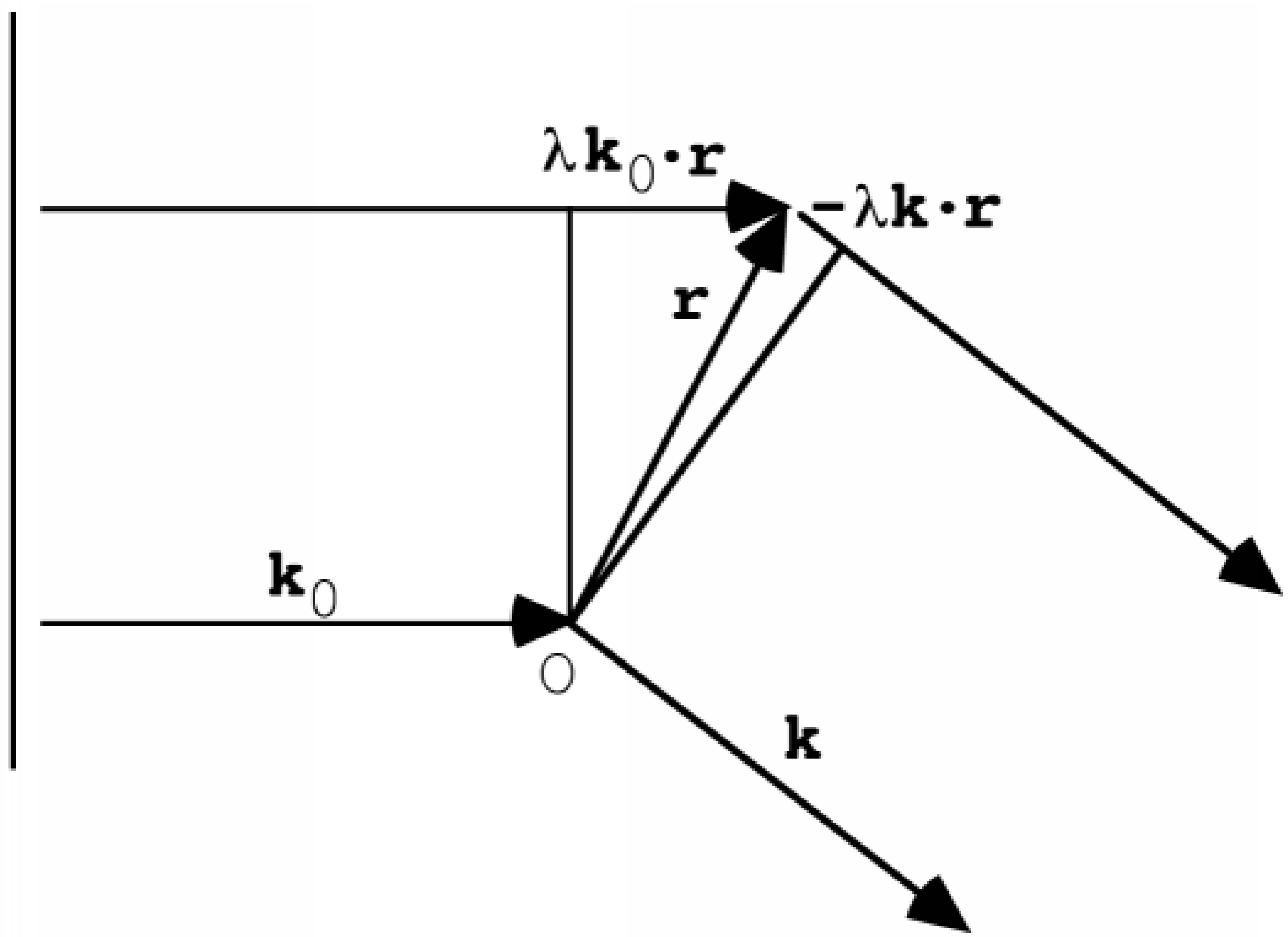
the question is, what is the path in fraction of wavelengths, to point r ?

$-2\pi\ (r \cdot k)\ /\ (|k| . \lambda)$ -->>

$-2\pi\ (r \cdot k)$ …if $|k|=1/\lambda$

adapted from http://www-structmed.cimr.cam.ac.uk/Course/

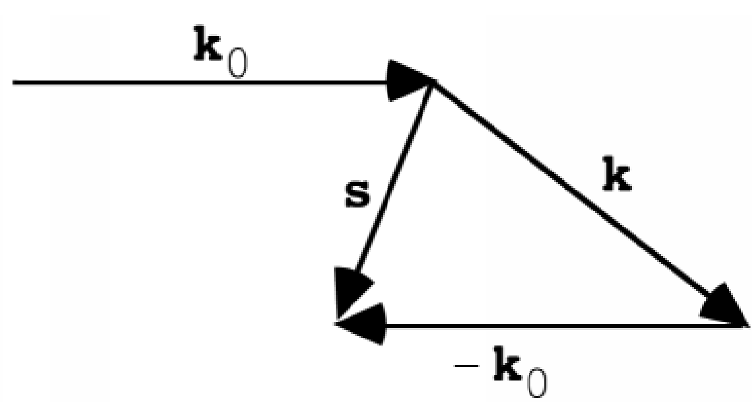
...so phase of diffraction from a single electron at r



$-2\pi\ (r \cdot k_0 - r \cdot k)$

or

$2\pi\ r \cdot (k - k_0)$



$k - k_0 = S$

(diffraction vector!!!)

adapted from http://www-structmed.cimr.cam.ac.uk/Course/