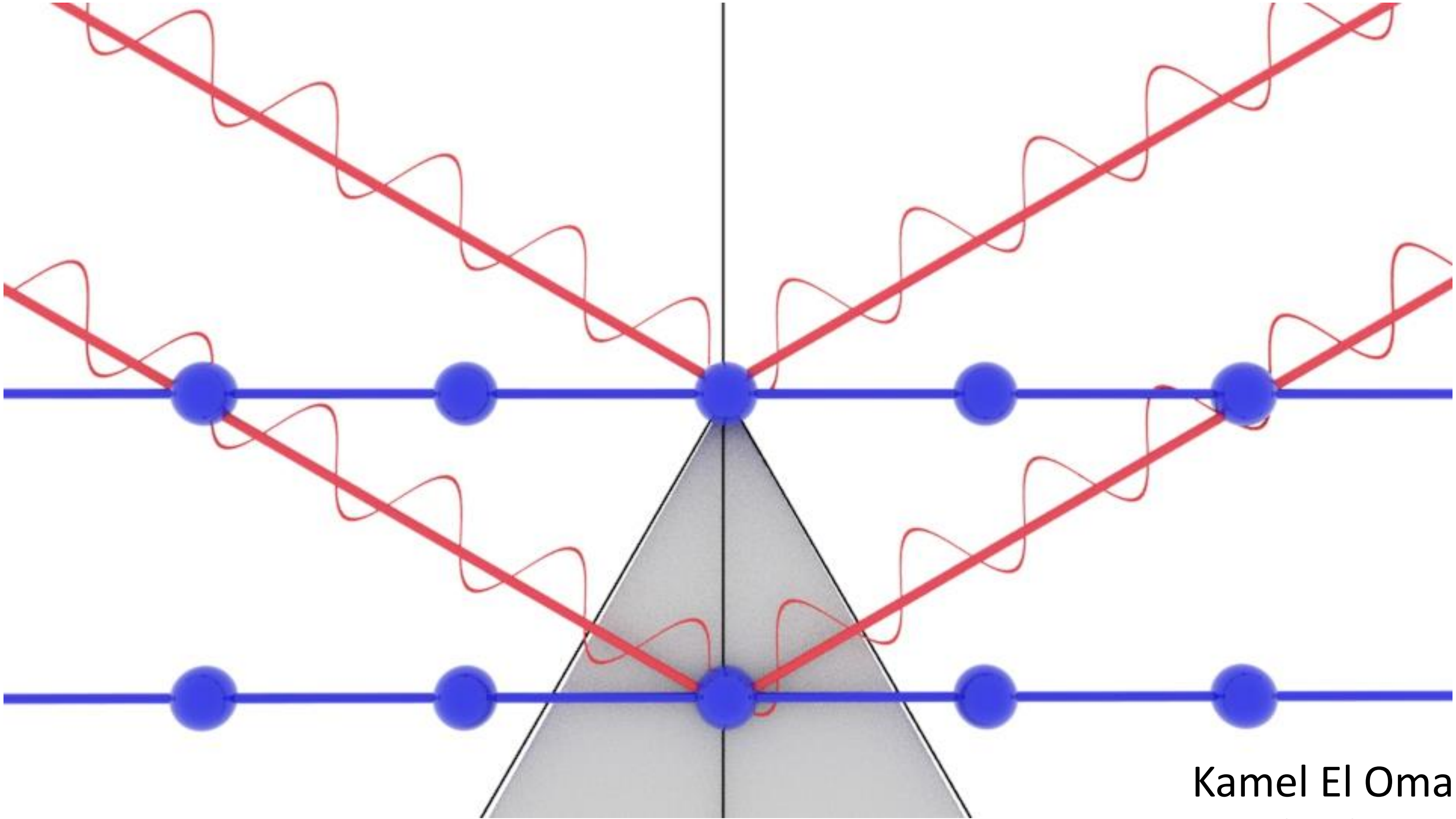
Introduction to Diffraction Theory

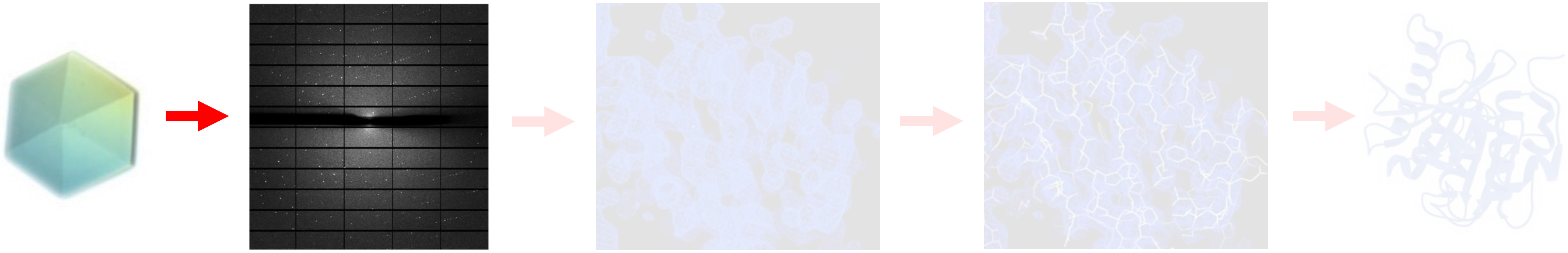


Kamel El Omari
22/11/2022

Diamond-CCP4 Data Collection and Structure Solution Workshop 2022

kamel.el-omari@diamond.ac.uk

X-ray crystallography experiment



Crystal

Diffraction patterns

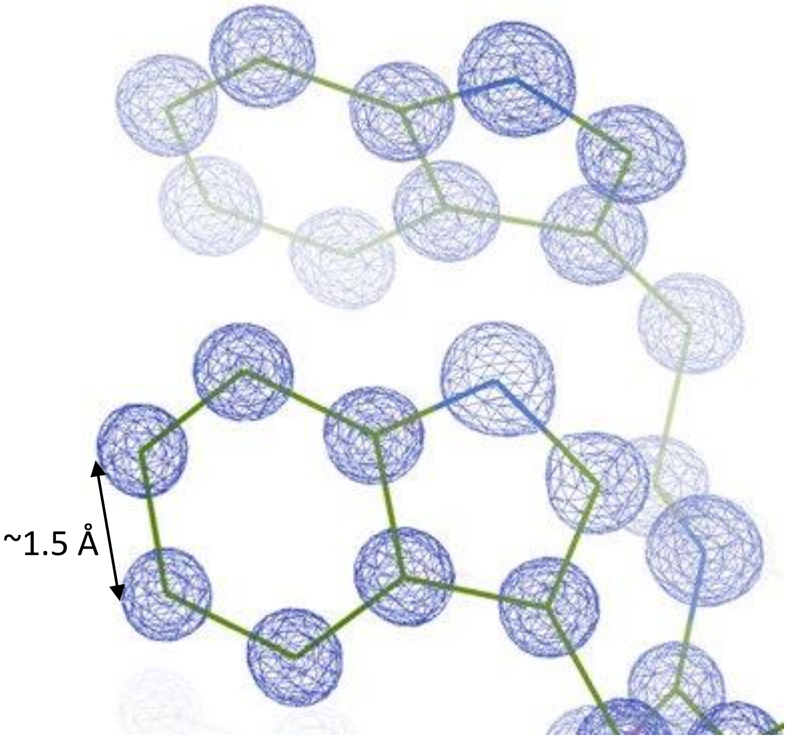
Electron density maps

Model building & refinement

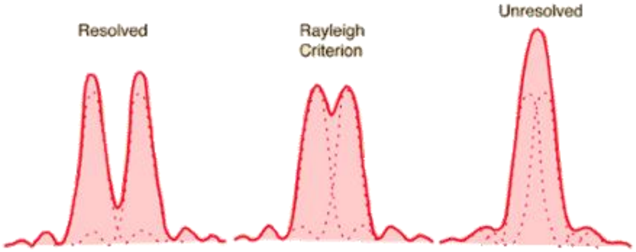
Final model

- Why do we need X-rays?
- Why do we need crystals?
- How are X-rays diffracted by crystals?

Why can’t we see atoms with visible light?



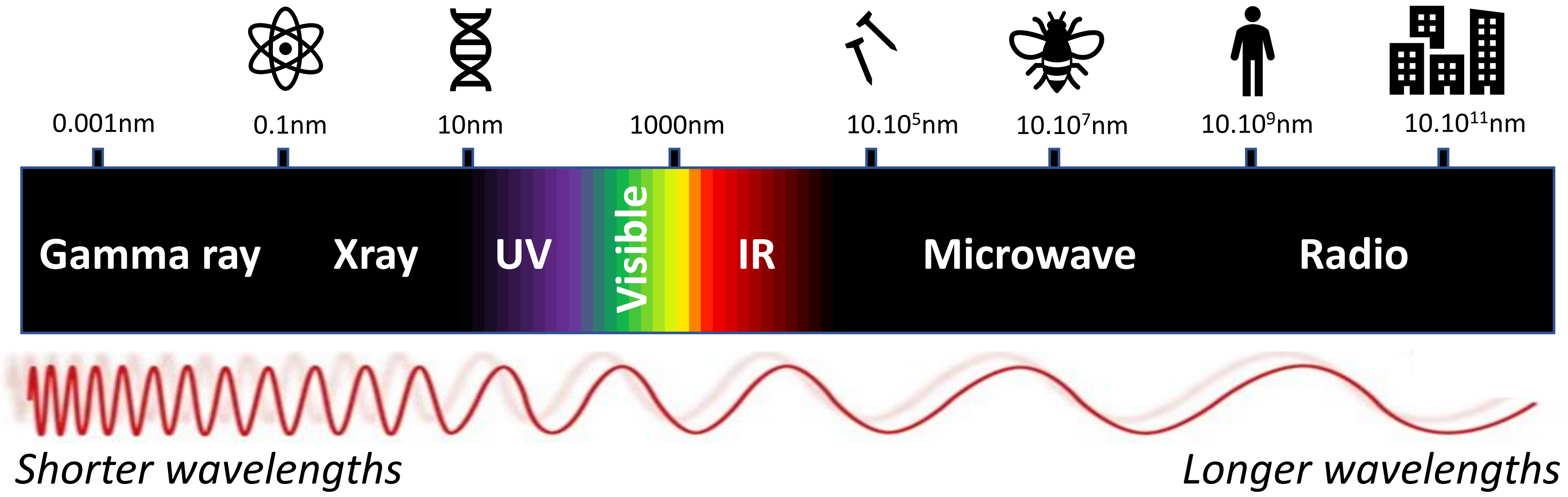
1 Å = 0.1 nm = 10^{-10} m



$$r = \frac{\lambda}{2 \cdot NA}$$

***r** is the minimum distance between resolvable points.*
***λ** is the wavelength of light*
***NA** is the numerical aperture*

We can’t see objects that are much smaller than the wavelength (λ) of the light that is being used.

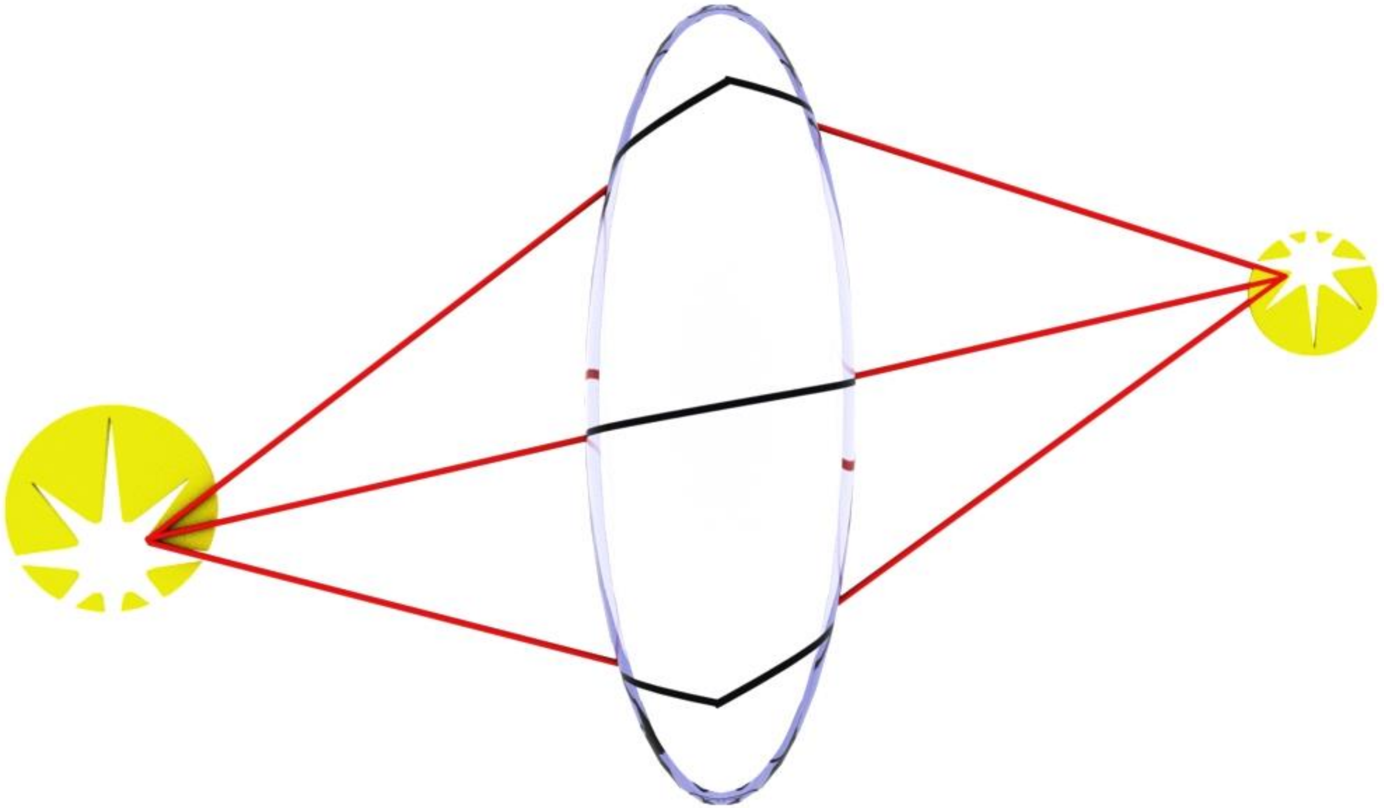


- The wavelength for visible light is measured in 100s of nm, while atoms are separated by distances of the order of 0.1 nm.

- Looking at the electromagnetic spectrum, X-rays are the best suited.

- X-rays are penetrating radiations and can provide a truly 3D image, on contrary to visible light.

Light arriving on one point of the image comes from one point on the object

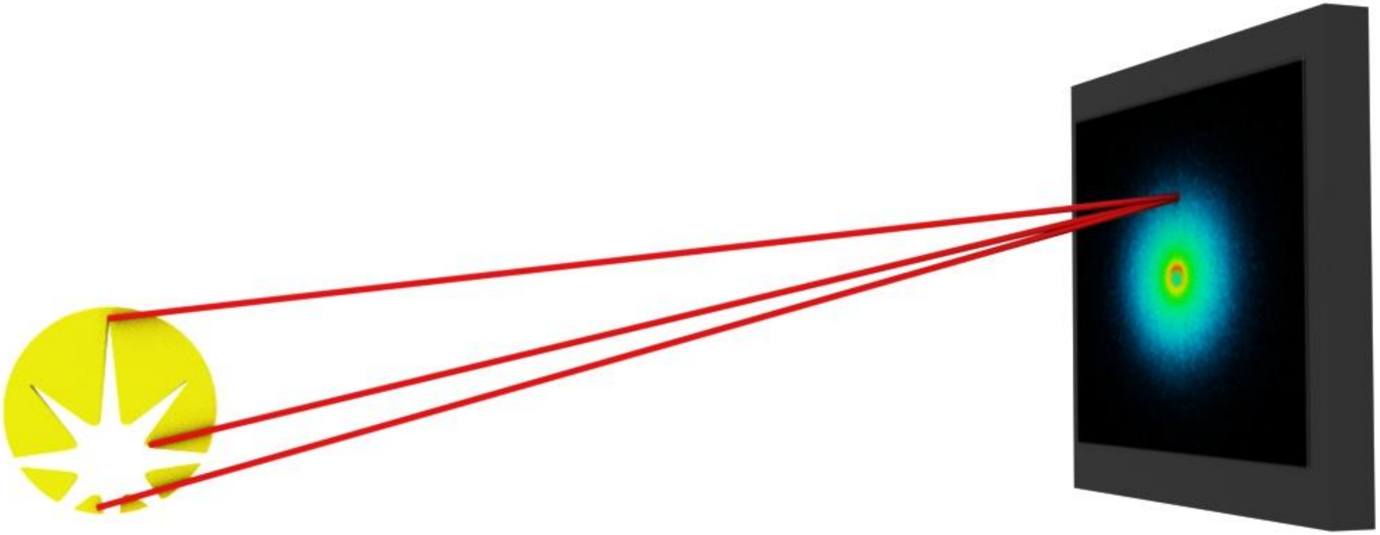


We can't build an X-ray microscope to look at molecules:

- X-ray lenses don’t exist. If they did, they would need tolerances smaller than the distance between two atoms.
- X-rays have refractive index close to 1 so their path does not change much when entering a material. The focal lengths for these lenses become impractically long.

Zone plates can focus X-rays to form an image but resolution is limited to ~ 30-50 nm.

Without a lens, light arriving on one point of the image comes from all points on the object.



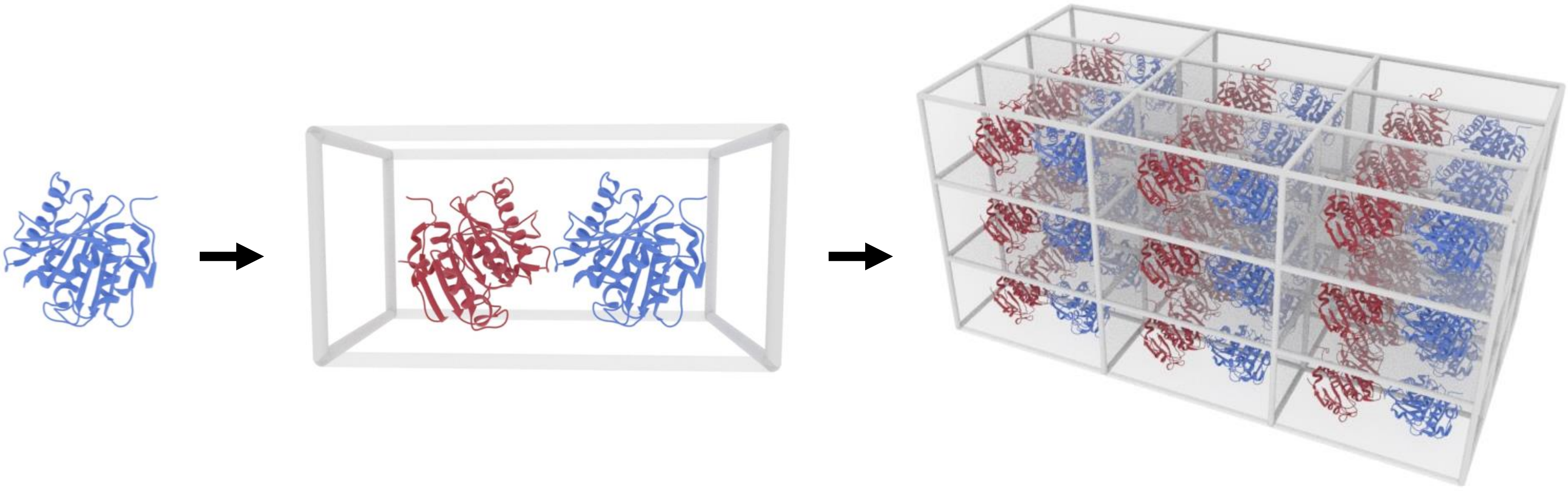
Object

Diffraction spectra

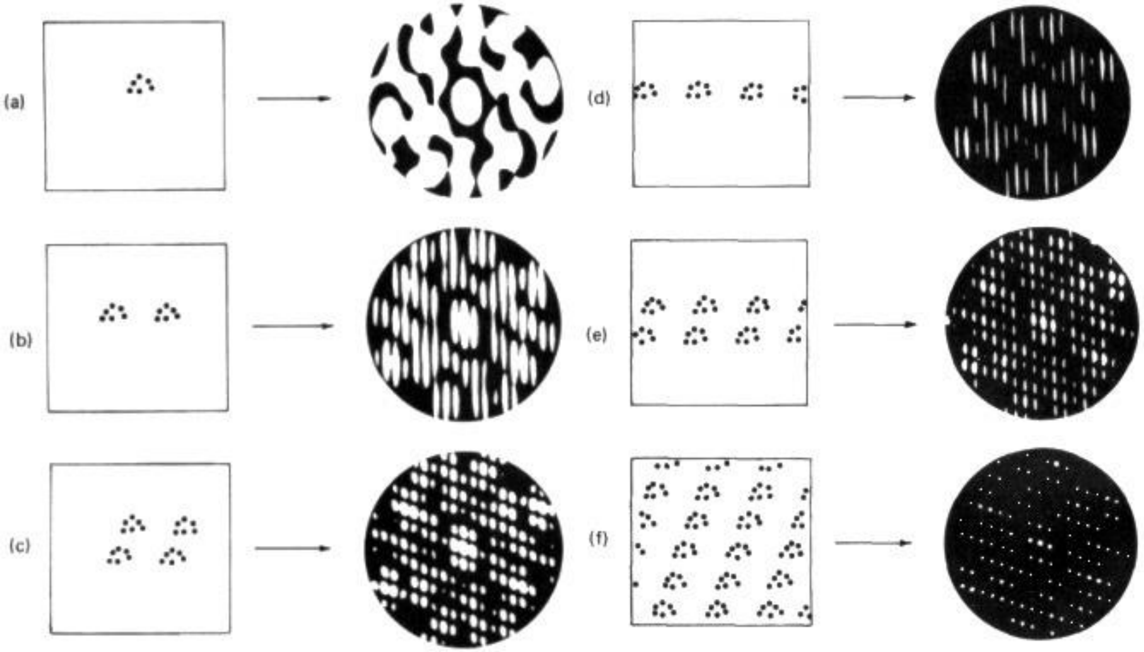
Instead of lenses, we use computers and FT.

Why do we need crystals?

- Diffraction from one molecule is too weak.
- Crystals are made up of identical building blocks called unit-cells.
- Multiple unit-cells constitute a 3-dimensional translation lattice.
- A crystals acts as a signal amplifier and are particularly suited for X-ray diffraction (λ ~ atomic spacing).



Why do we need crystals?



https://www.xtal.iqfr.csic.es/Cristalografia/parte_05-en.html

Diffraction patterns of: (a) a single molecule, (b) two molecules, (c) four molecules, (d) a periodically distributed linear array of molecules, (e) two linear arrays of molecules, and (f) a two-dimensional lattice of molecules.

The discrete sampling at the peaks of a diffraction pattern greatly increases the signal-to-noise ratio in practical measurements of the diffraction intensities.

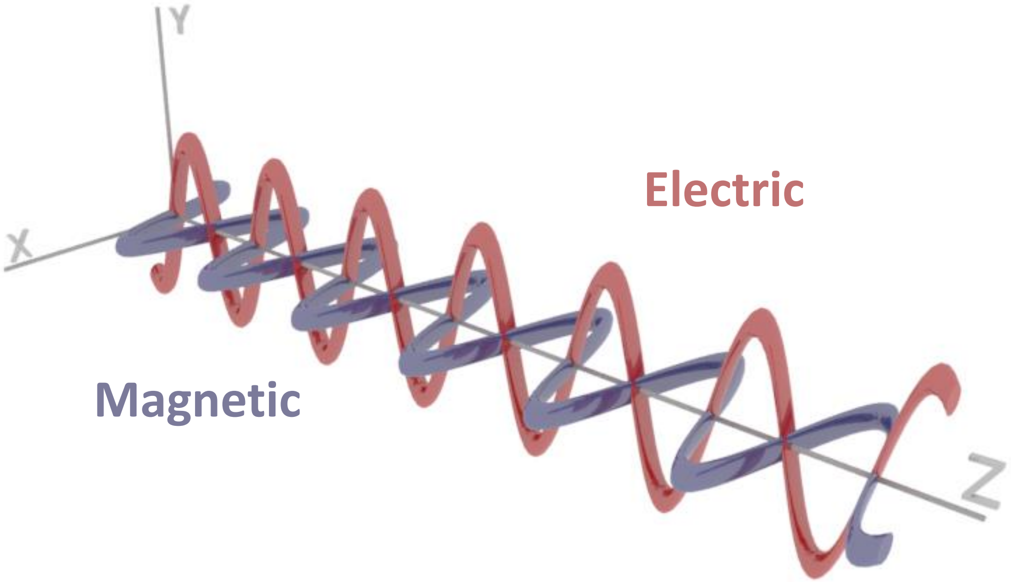
- Why do we need X-rays?

- Why do we need crystals?

- **How are X-rays diffracted by crystals?**

Electromagnetic waves

- X-rays (and visible light) are one type of electromagnetic radiations, they can also be called electromagnetic waves, light or photons (particle without mass).
- Electromagnetic waves consist of 2 waves oscillating perpendicular to one another at the speed of light: magnetic and electric components.



Electromagnetic waves

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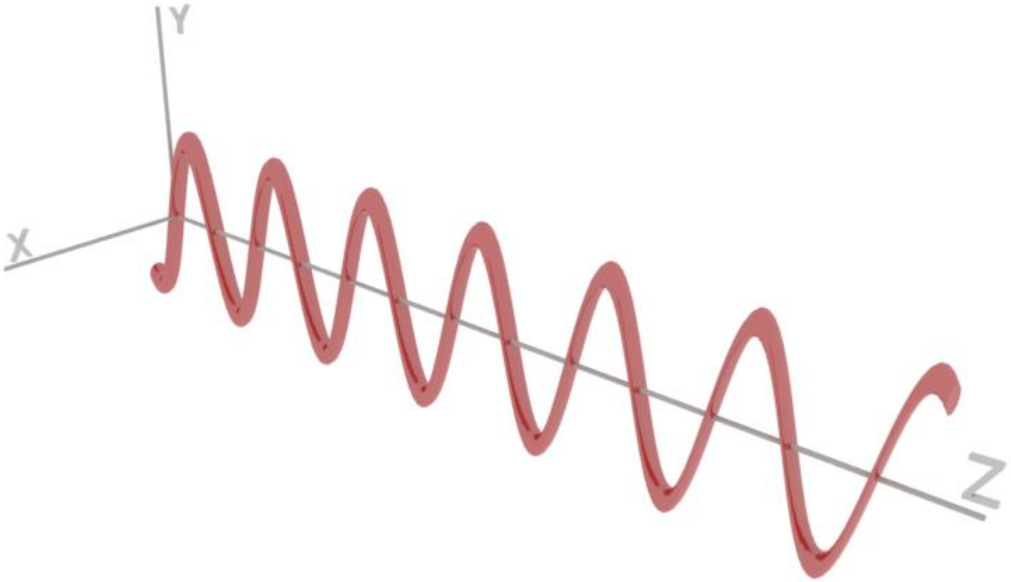
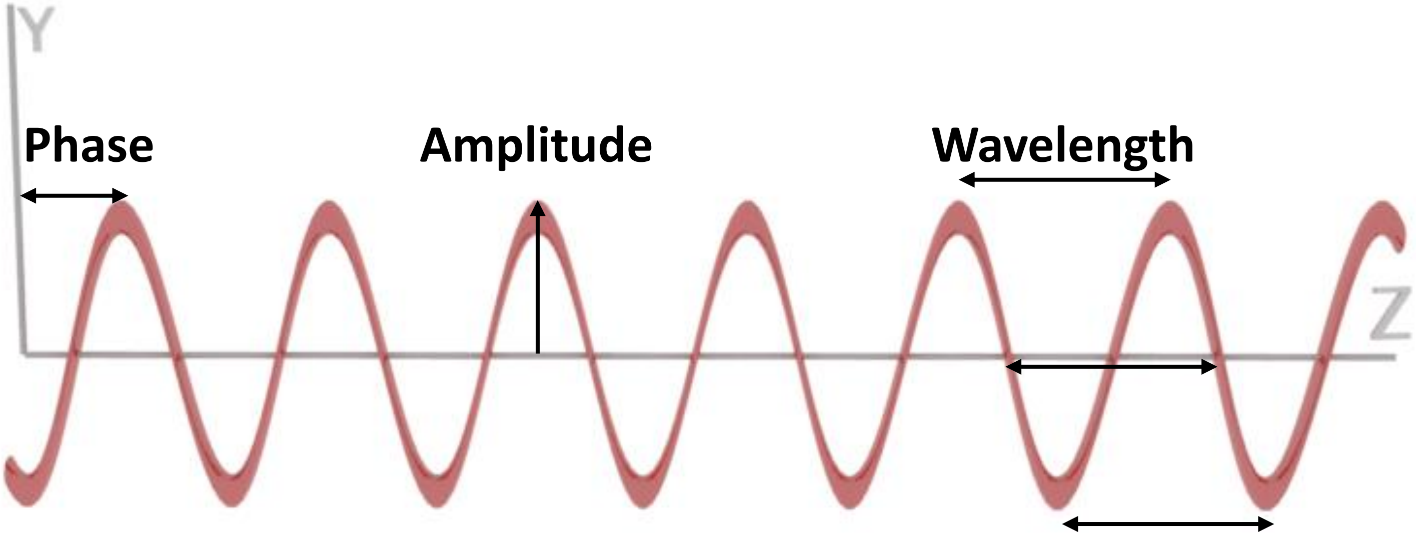
- Electromagnetic waves consist of 2 waves oscillating perpendicular to one another at the speed of light: magnetic and electric components.
- For simplification, electromagnetic waves are often represented only by their electric component (most important one for our experiment).

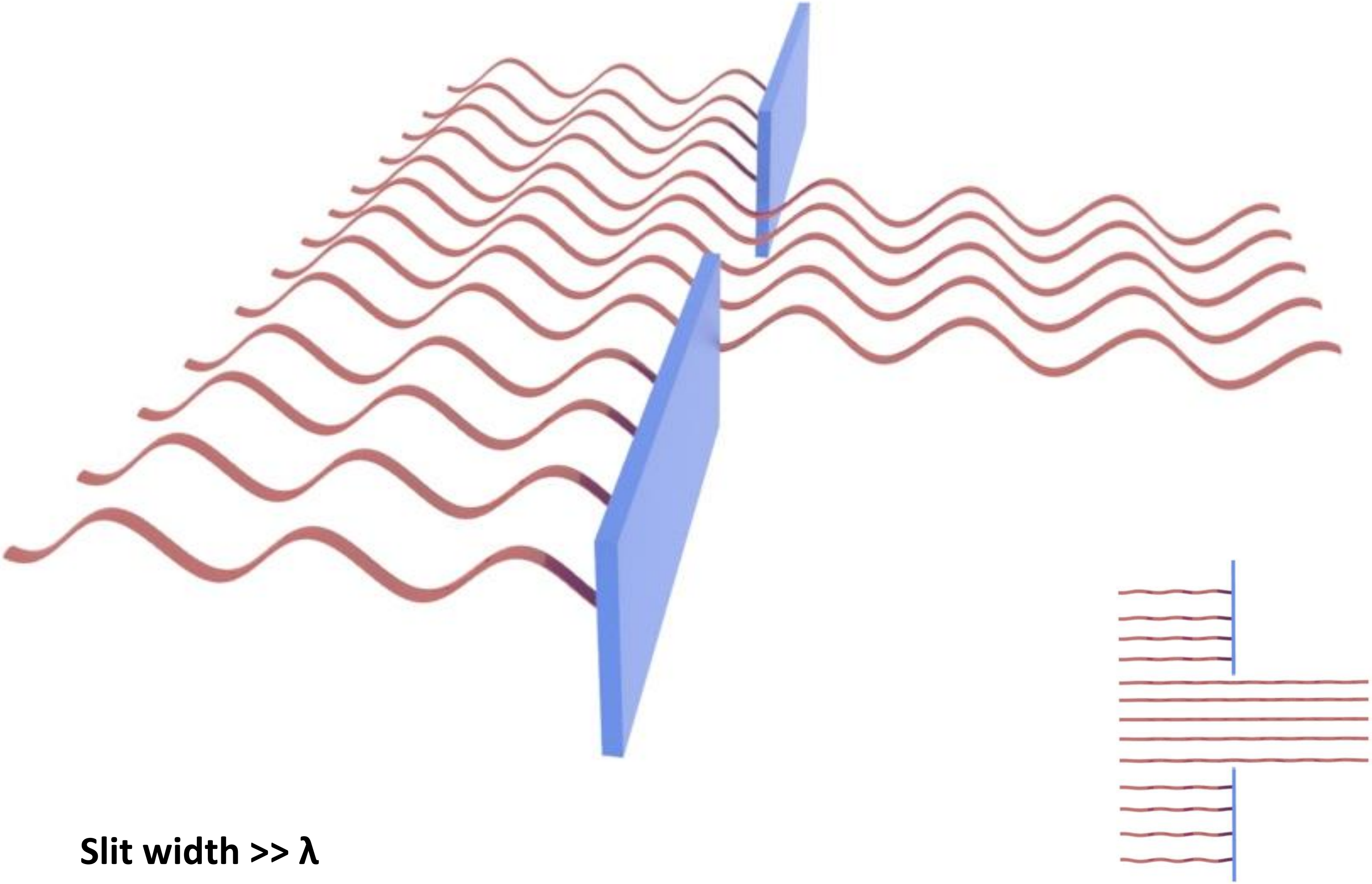


Electromagnetic waves



- A wave can be defined by its wavelength, amplitude and phase.
- In XRC experiments, we usually use monochromatic X-rays, so the wavelength (λ) is known and fixed.

Slit experiment

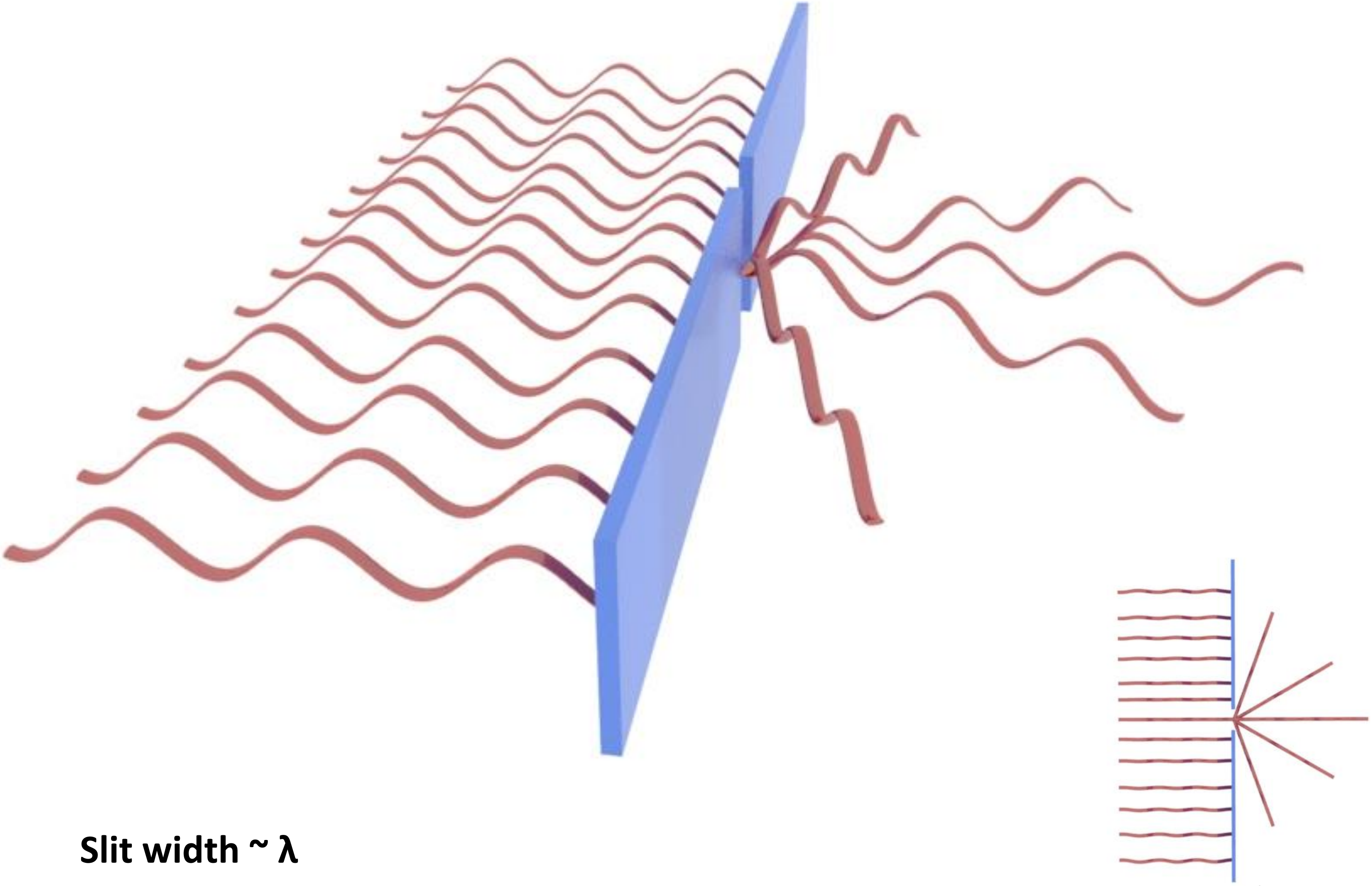


Slit width >> λ

Top view

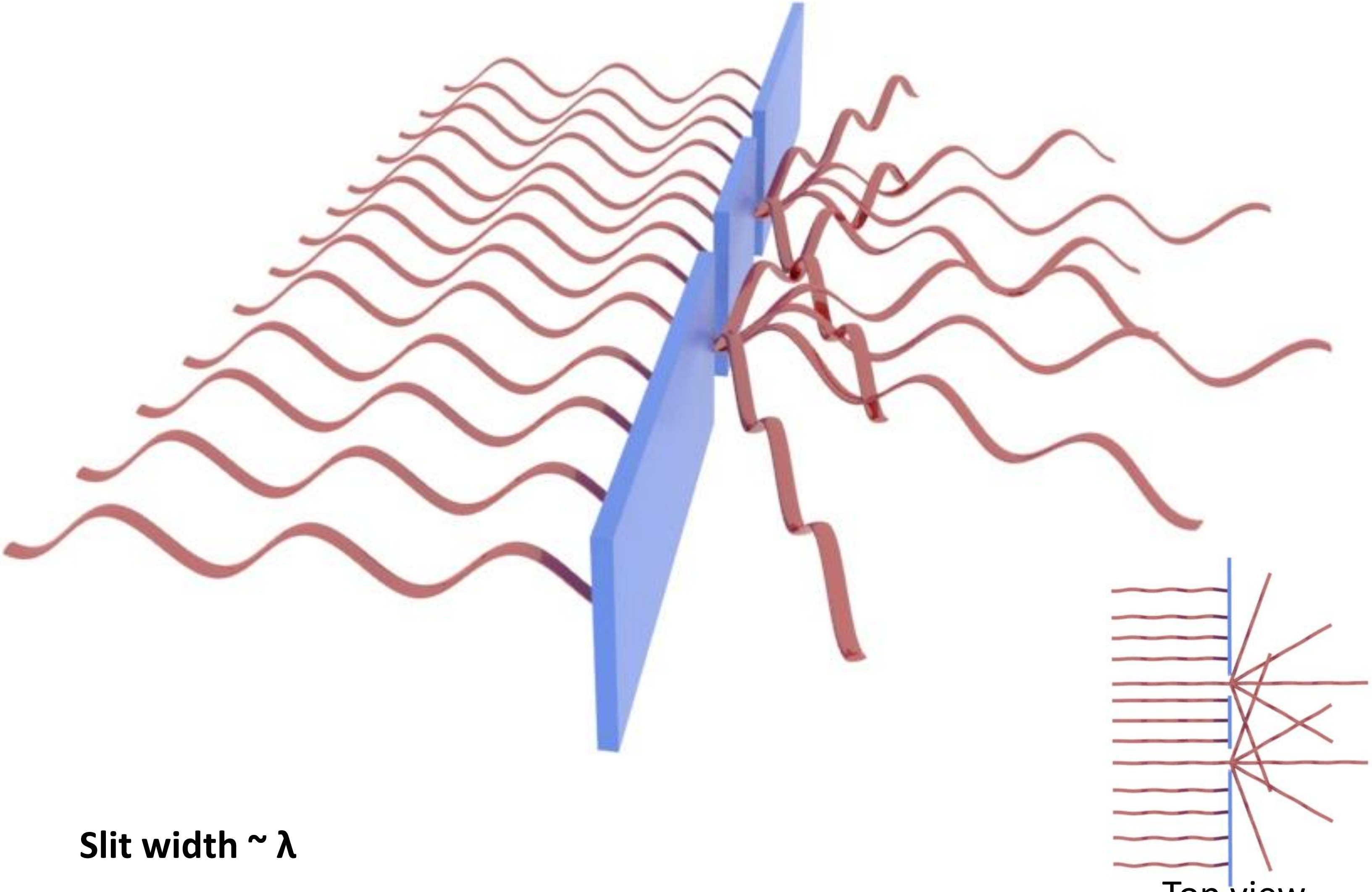
Slit experiment

Diffraction is the spreading out of waves as they pass through an aperture or around objects.



Slit width ~ λ

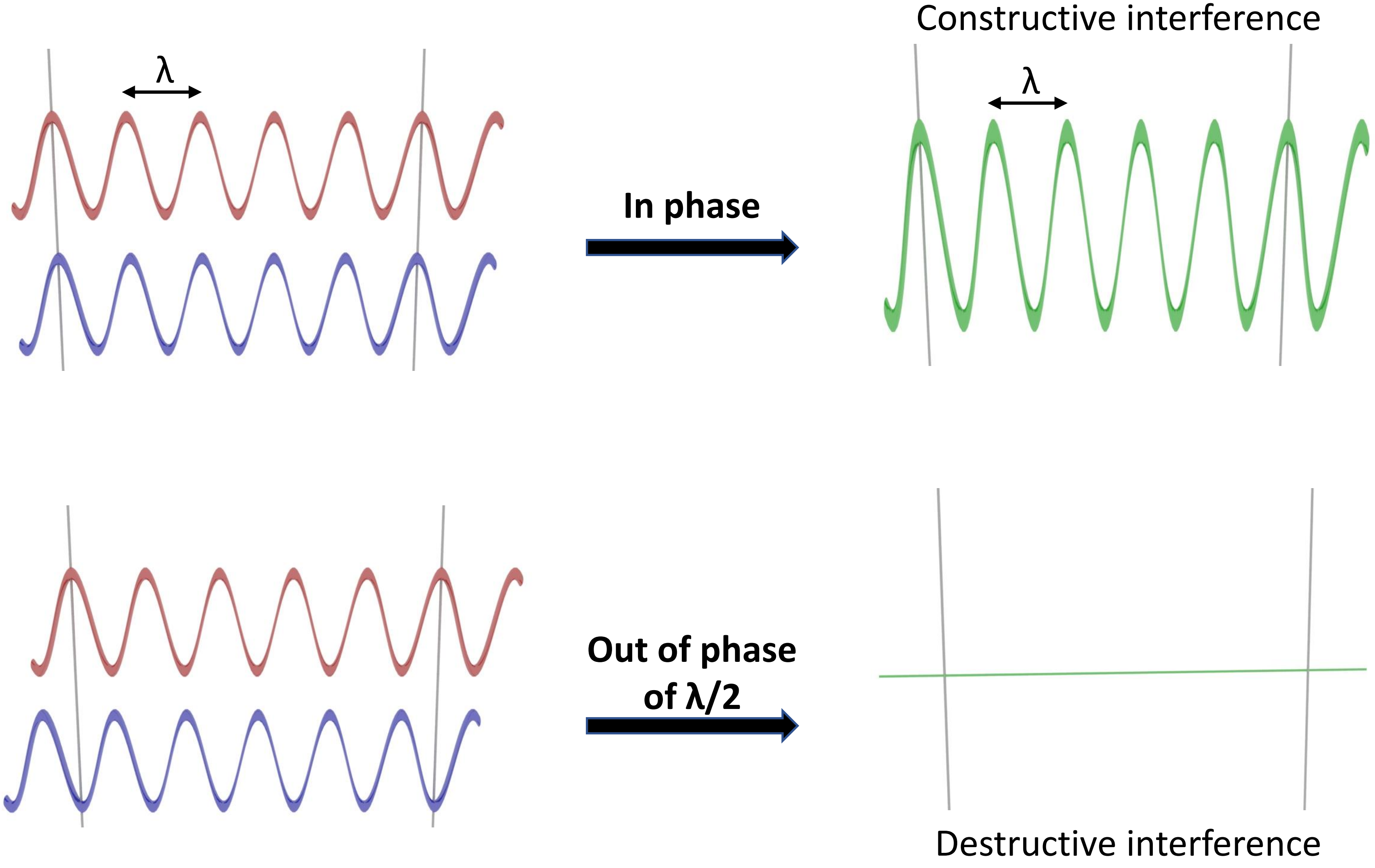
Slit experiment



Slit width ~ λ

Top view

Wave interference



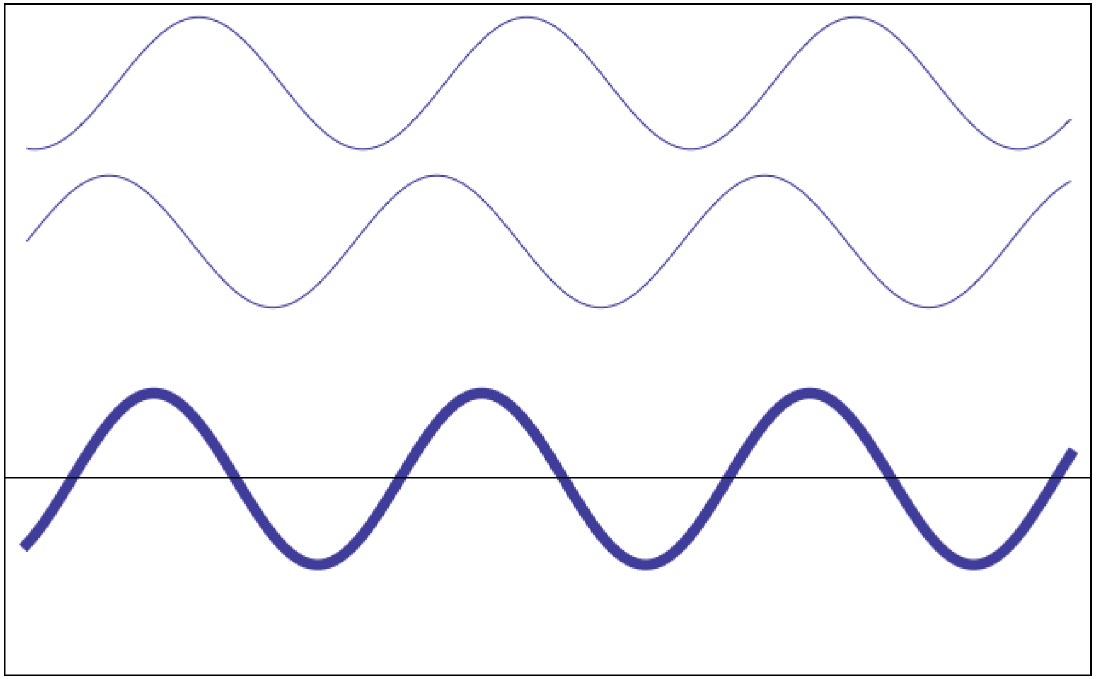
Constructive interference

In phase

Out of phase of $\lambda/2$

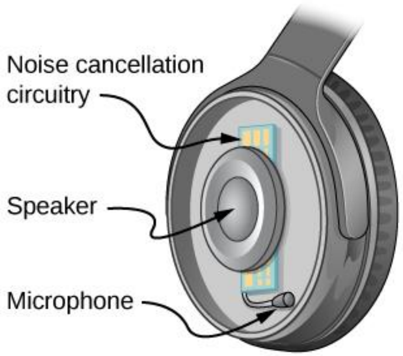
Destructive interference

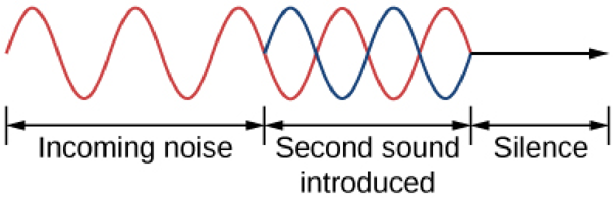
Wave interference



https://www.acs.psu.edu/drussell/Demos/superposition/superposition.html

Wave interference

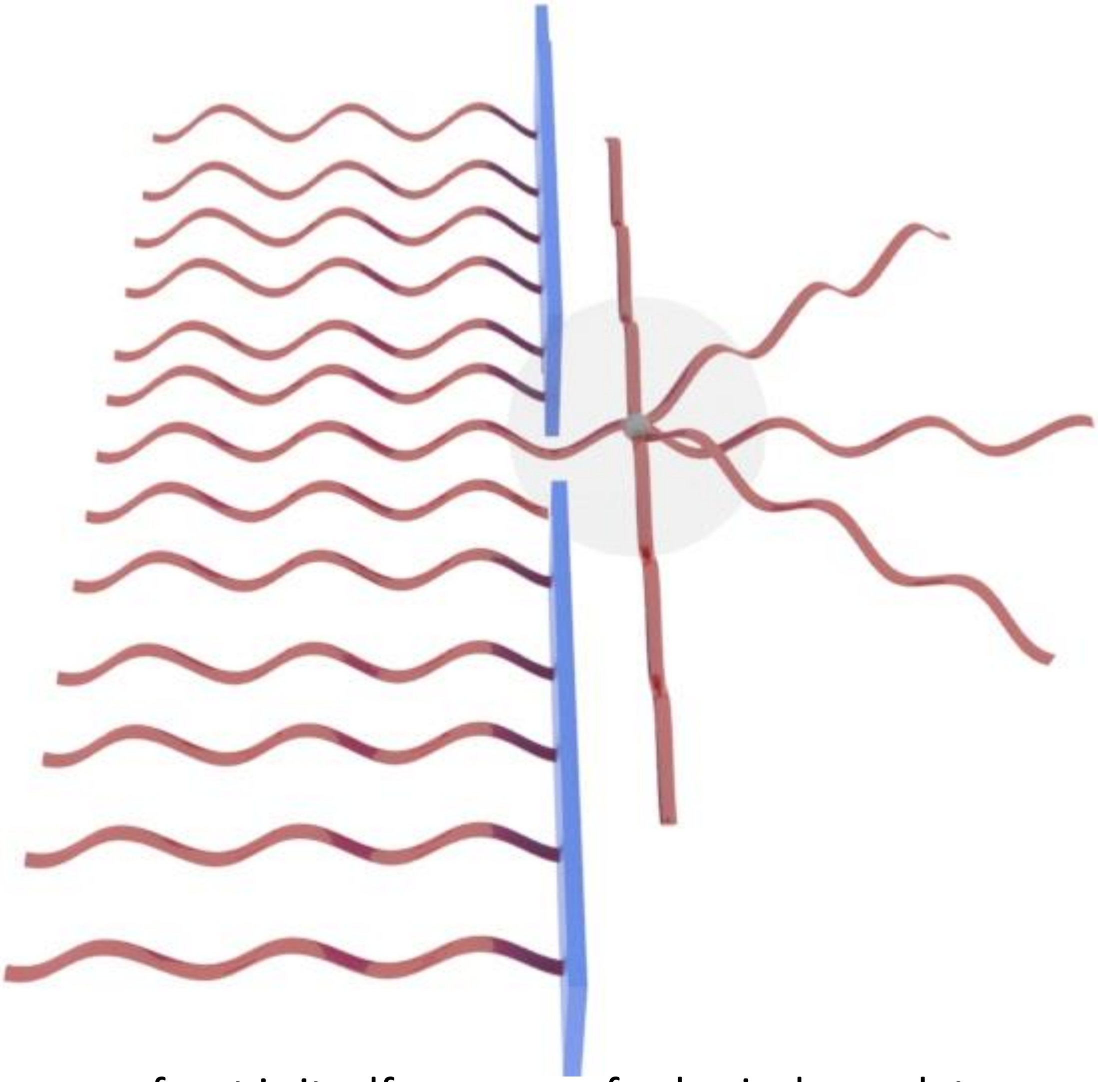




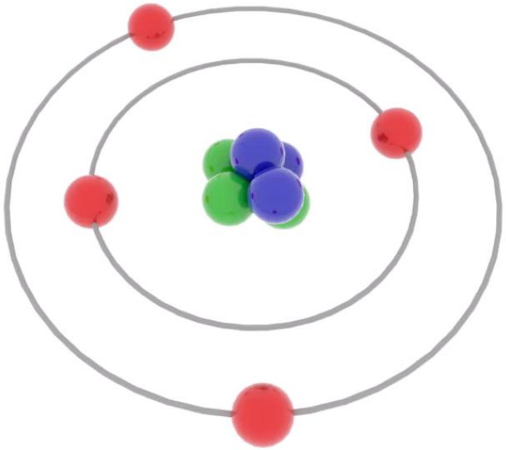
https://courses.lumenlearning.com/suny-osuniversityphysics/chapter/17-4-normal-modes-of-a-standing-sound-wave/

https://www.youtube.com/watch?v=Iuv6hY6zsd0&list=PLDvVtGPo3ANw8upOKjs_FrkE8iqRrAVnT&index=1

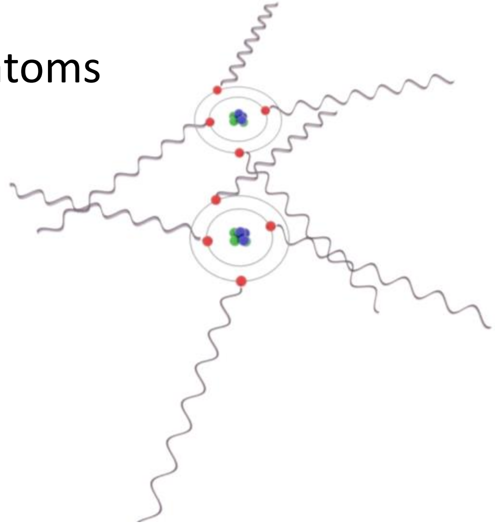
Huygens–Fresnel principle

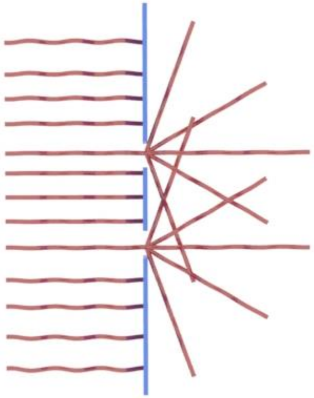


- Every point on a wavefront is itself a source of spherical wavelets.
- The secondary wavelets emanating from different points mutually interfere.
- The sum of these spherical wavelets forms the new wavefront.



- When X-rays interact with electrons, they set the electron oscillating with the X-ray wavelength (excited state).
- To return to its unexcited state, the oscillating electron radiate X-rays with the same wavelength, in all directions (elastic scattering, Thomson scattering).
- Electrons become an X-ray source.





Crystals are regular arrays of electrons, that behave similarly to slits, they will radiate X-rays in all directions with the same wavelength but different amplitudes and phases.

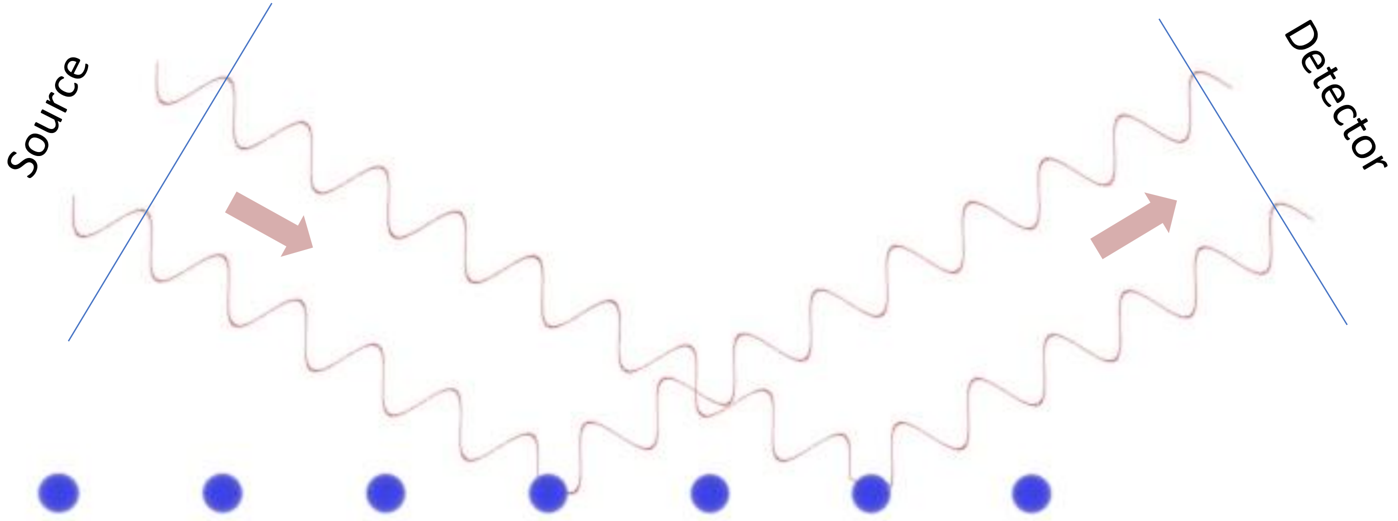
Waves in phase

If we shine monochromatic X-ray source on a crystal, two waves are in phase when:

- They travel the same distance.
  - Direct beam
  - Single plane

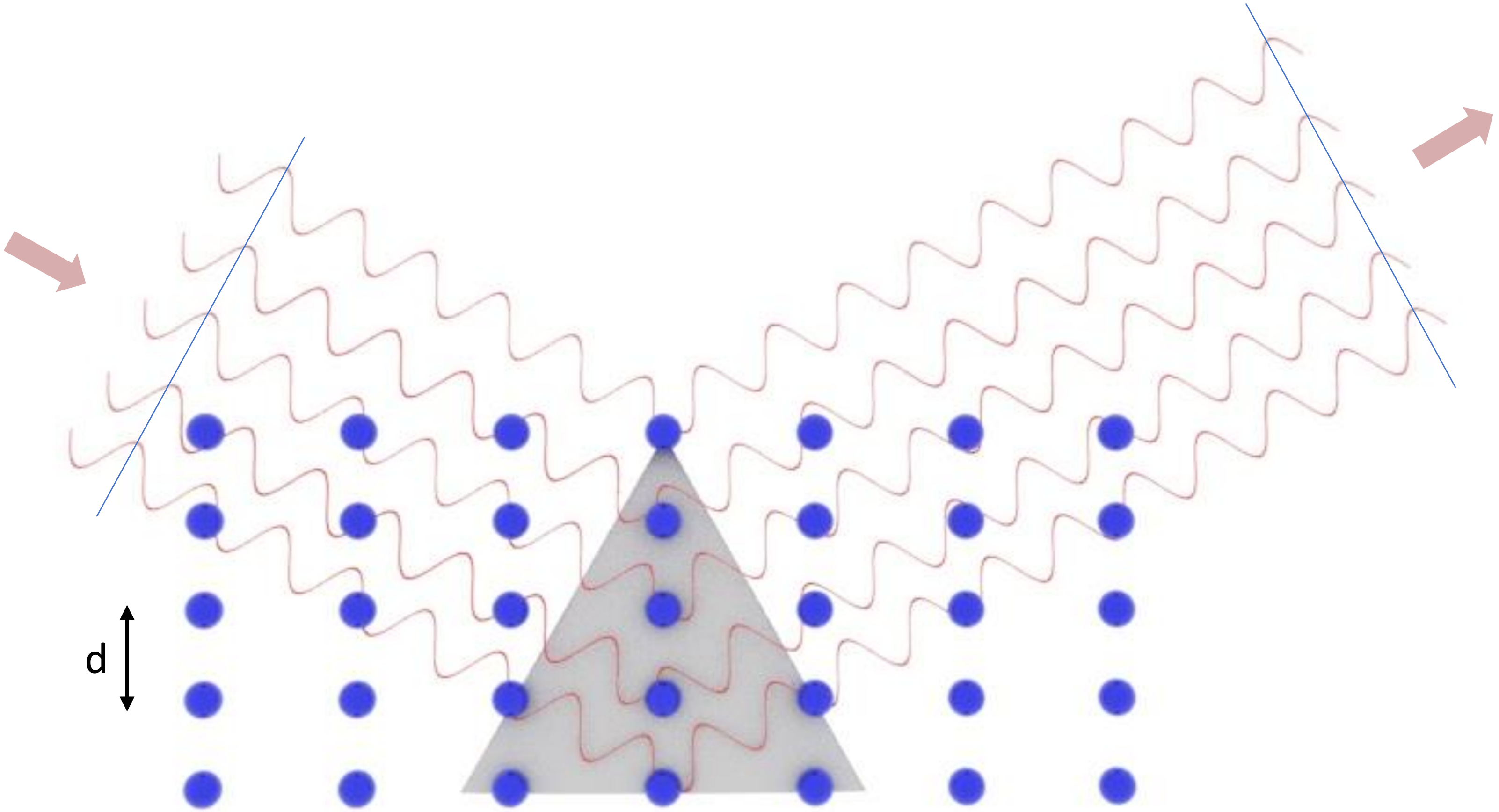
- The difference between the path lengths is equal to an integer multiple of the wavelength.

Waves in phase



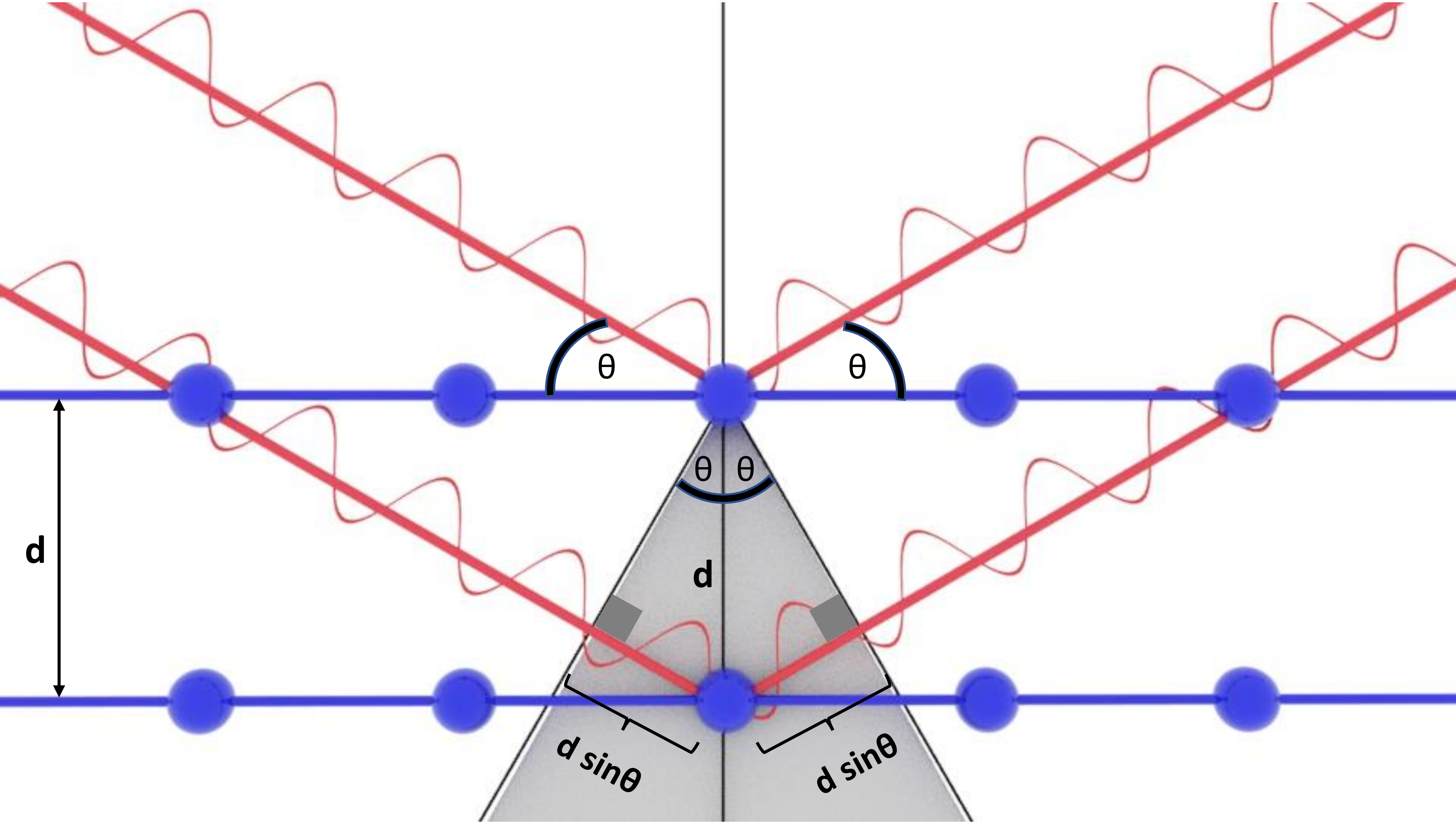
When their path length is the same.

Waves in phase



When their path length differ by an integer number of wavelength.
(The path lengths differ by exactly one wavelength, it is a first order reflection (n=1).

Waves in phase



The extra path travelled by the wave is (d.sin θ + d.sinθ).

Waves in phase

- In order for the waves to interfere constructively, the differences in the travel path must be equal to integer multiples of the wavelength.
- When this constructive interference occurs, a diffracted beam of X-rays will leave the crystal at an angle equal to that of the incident beam.

$$\mathbf{n\lambda = 2dSin\theta}$$

λ is the wavelength of the radiation used,

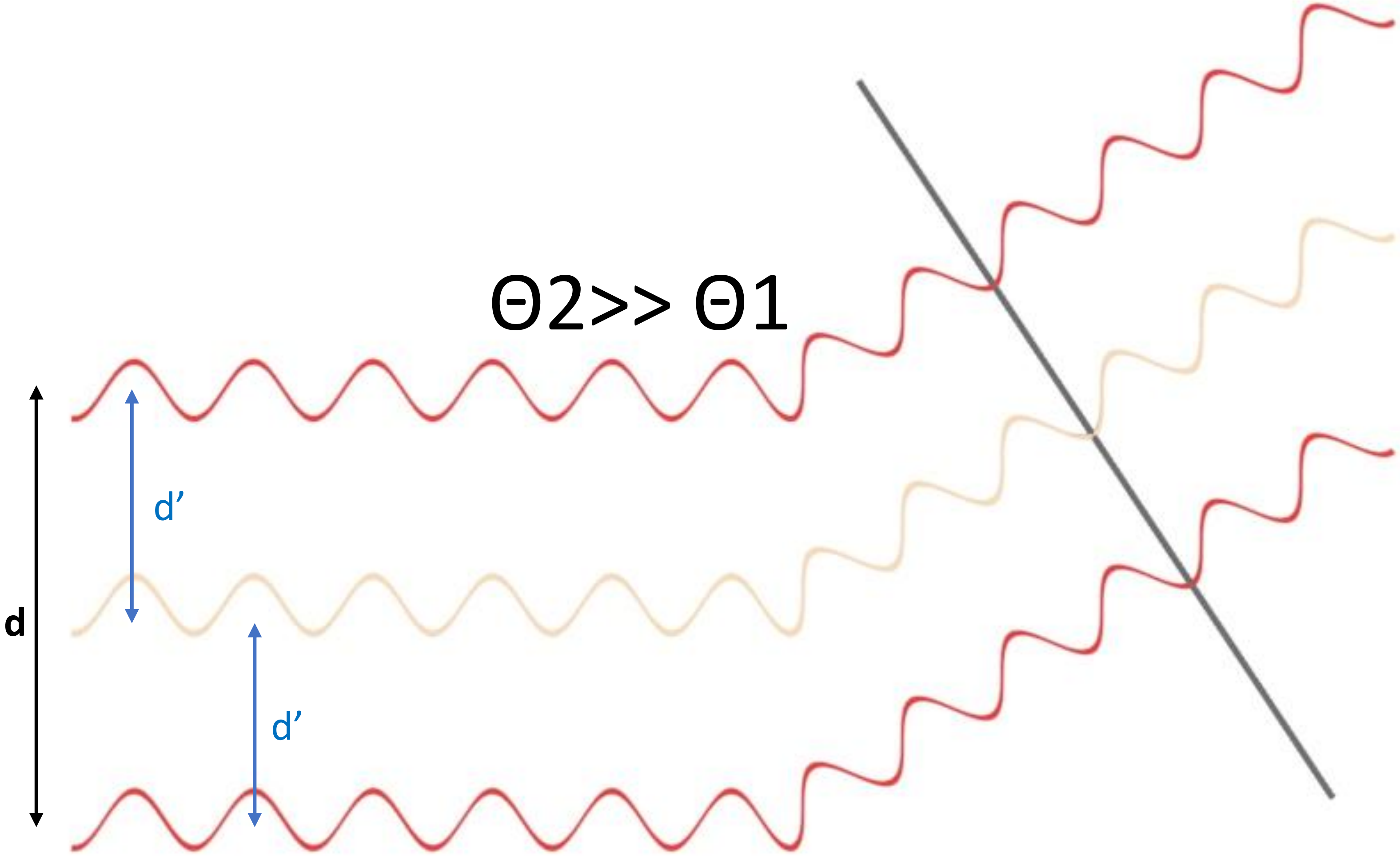
d is the inter-planar spacing involved,

θ is the angle between the incident (or diffracted) ray and the crystal planes,

n is an integer, referred to as the order of diffraction.

- Because Bragg's planes behaves like 'mirrors', the diffracted X-rays form a diffraction spot on the detector, this is also called: a reflection.
- Since we know, n, λ and θ, we can find d the distance between planes and start to get some information about distances.

Waves in phase



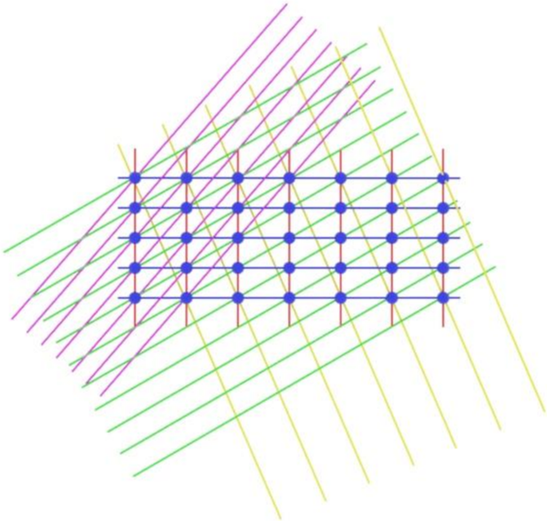
The smaller the spacing between planes, the larger the diffraction angles are.

Miller indices

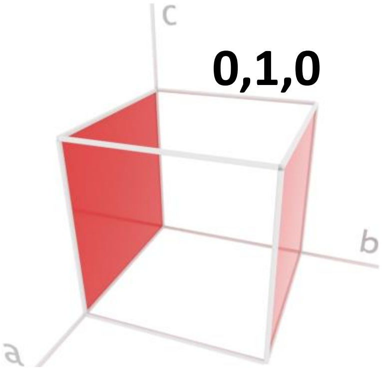
- We need to define the planes of atoms responsible for constructive interferences.

- A family of lattice planes is defined by 3 integers *h*, *k*, *l*, called the *Miller indices*.

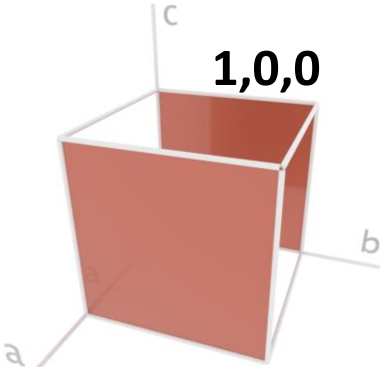
- The orientation of the lattice planes is described in term of their intercept on the axes a, b and c from the unit cell.



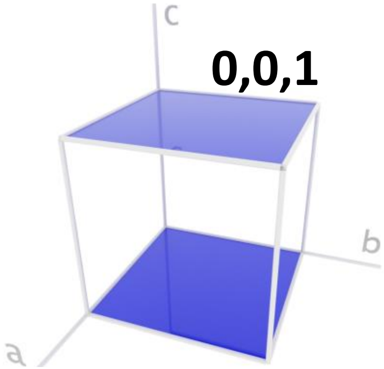
Miller indices



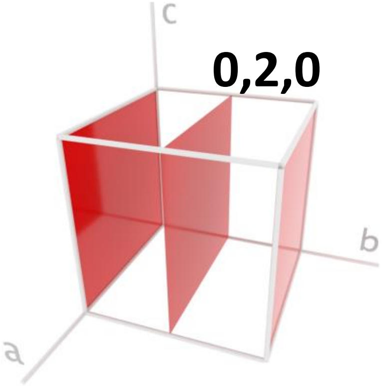
0,1,0



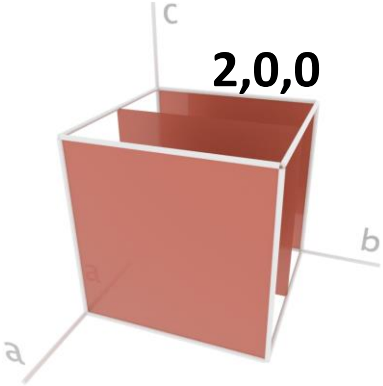
1,0,0



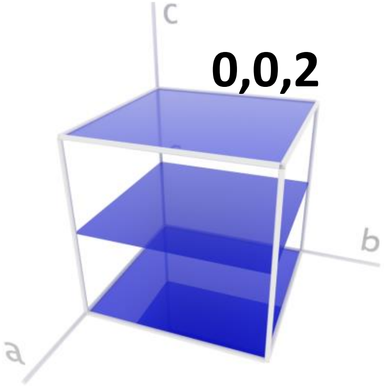
0,0,1



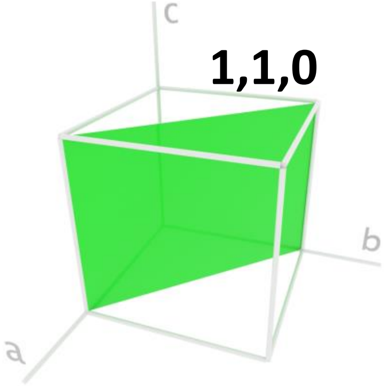
0,2,0



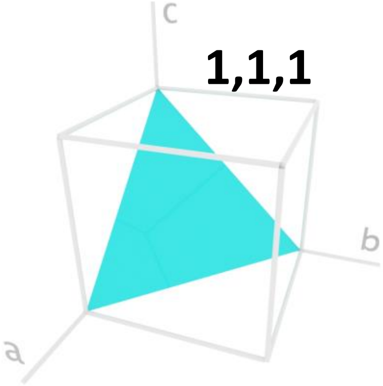
2,0,0



0,0,2

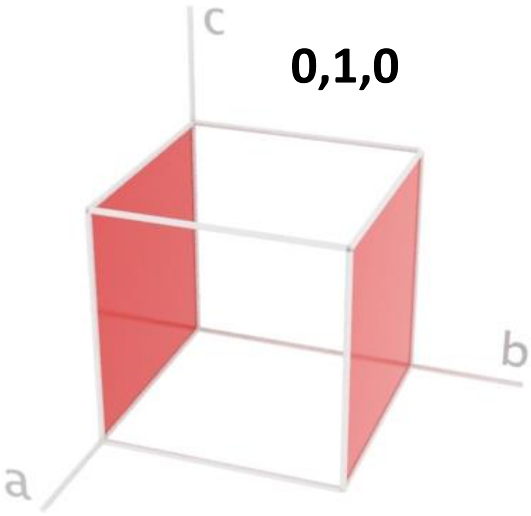


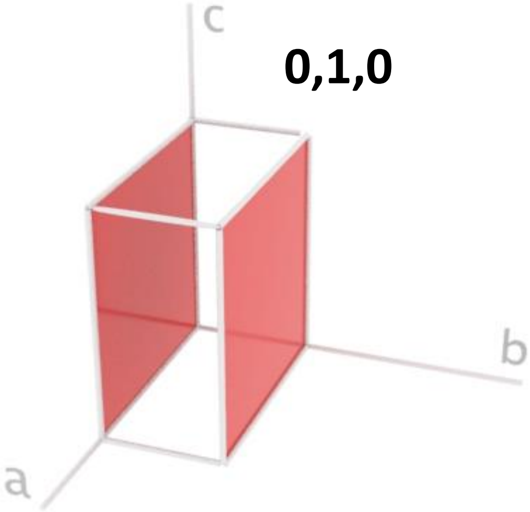
1,1,0



1,1,1

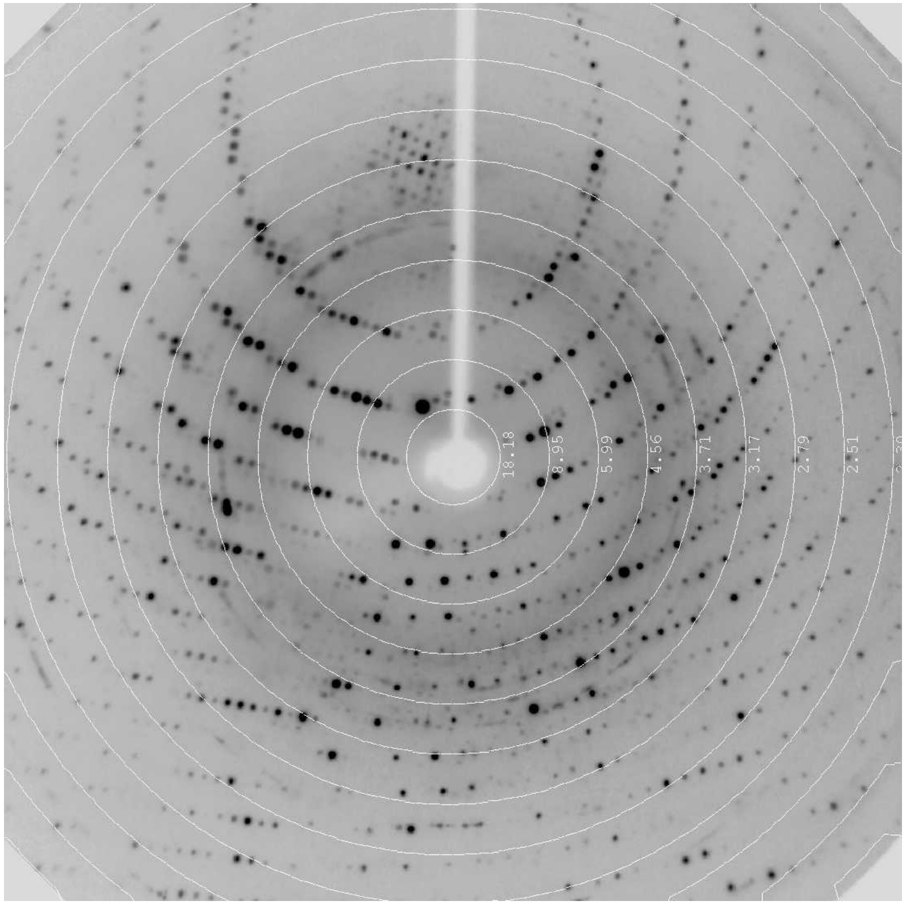
Miller indices



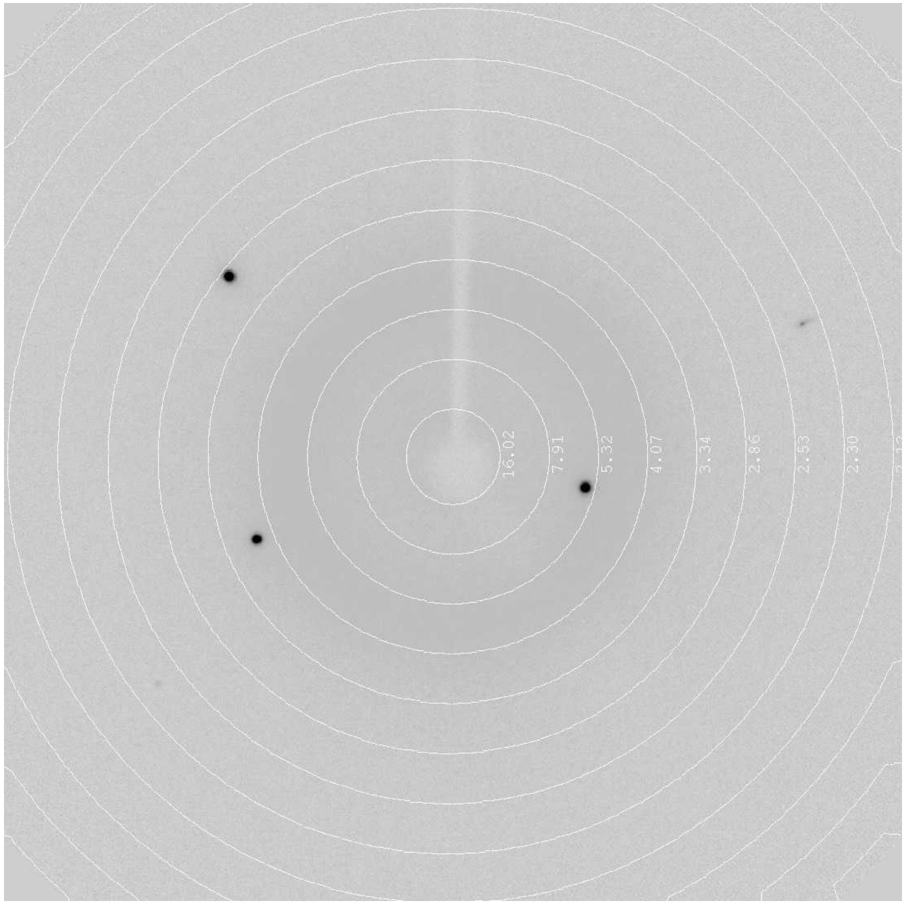


Smaller unit cells, have smaller d spacing and larger diffraction angles.

Miller indices



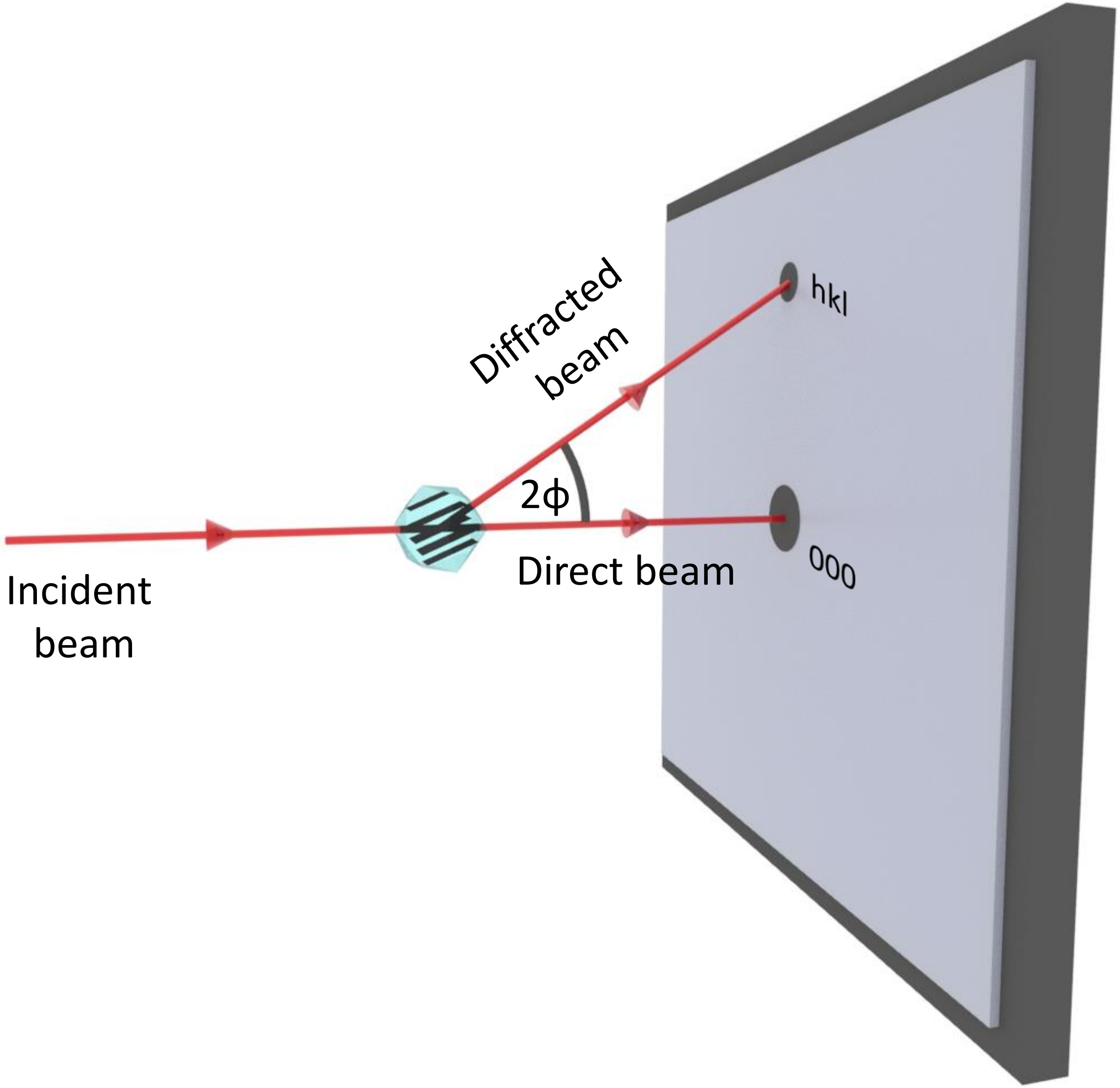
Diffraction spots are closer for large unit cells.



Diffraction spots are more spaced for small unit cell.

https://steemkr.com/steemstem/@suesa/what-biologists-do-all-day-structural-biology-again

Miller indice



Reciprocal lattice

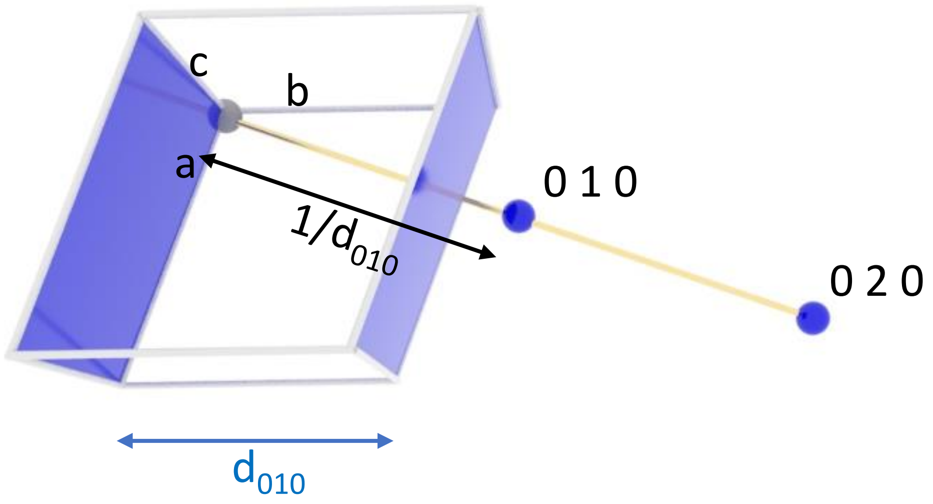
- Because of the reciprocal nature of d spacings and θ from Bragg's Law, the pattern of the diffraction we observe can be related to the crystal lattice by a mathematical construct called the reciprocal lattice.

- For each lattice plan in the real space, we can construct a point in the reciprocal lattice.

  1. We define the origin in the crystal lattice.
  2. We select a set lattice planes (h,k,l), and draw a vector with:
     - a length $1/d_{hkl}$
     - a direction normal to the set of lattice planes (h,k,l)

Reciprocal lattice

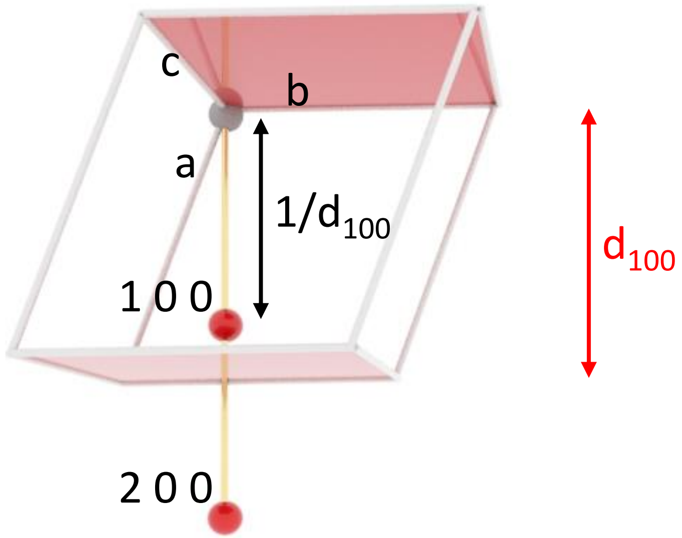
Planes: 0 1 0



Not to scale

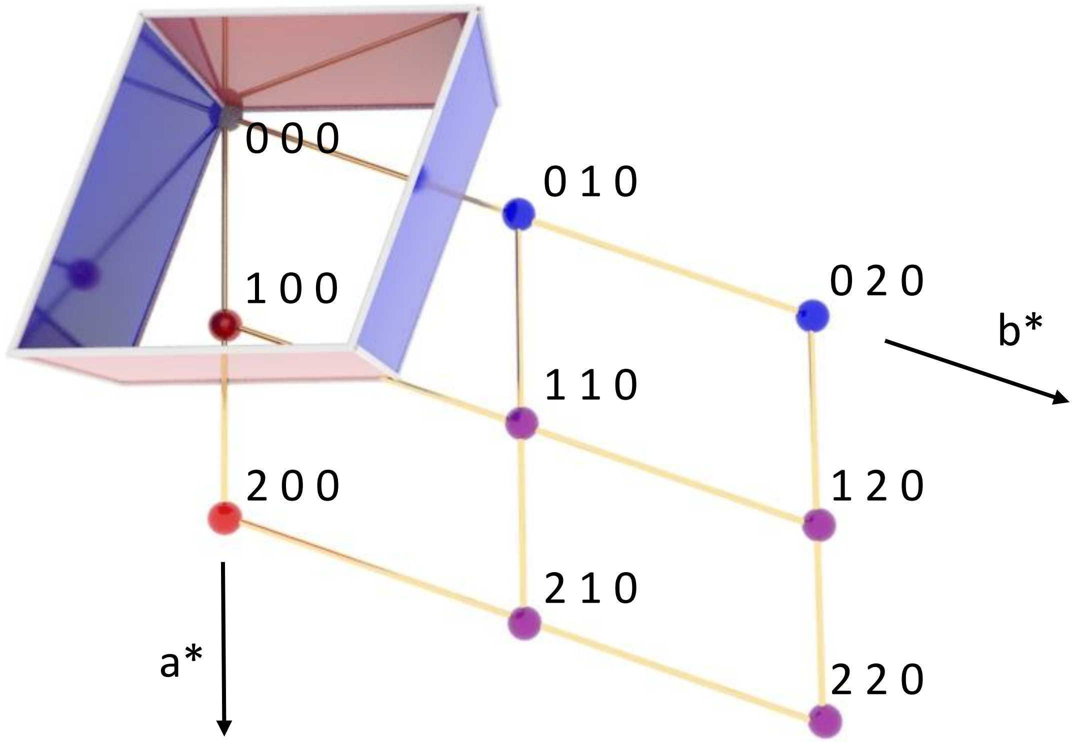
Reciprocal lattice

Planes: 1 0 0



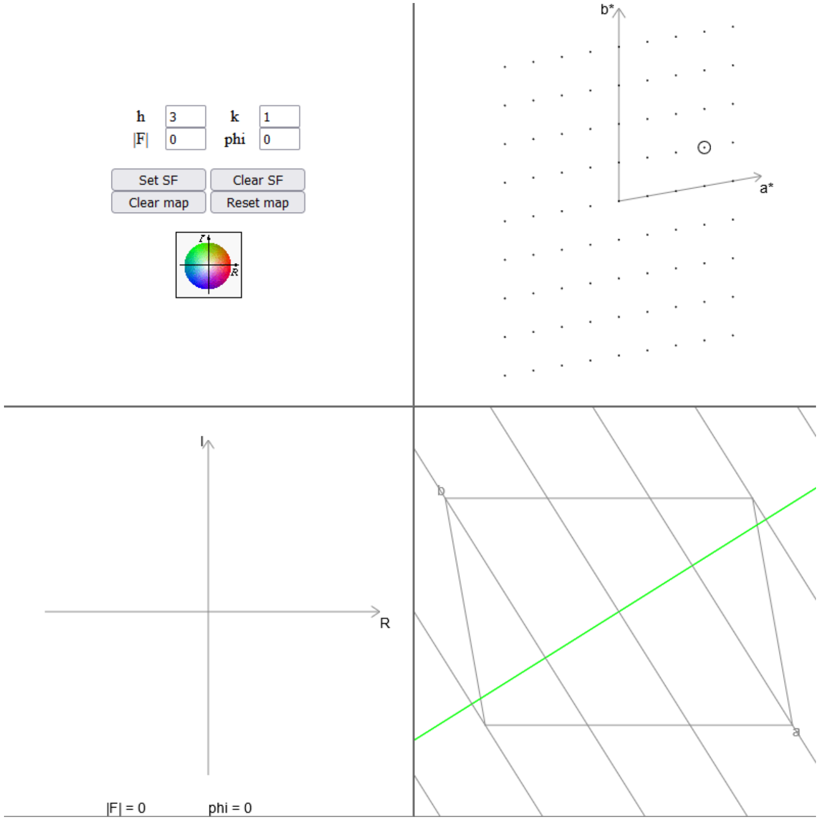
Not to scale

Reciprocal lattice



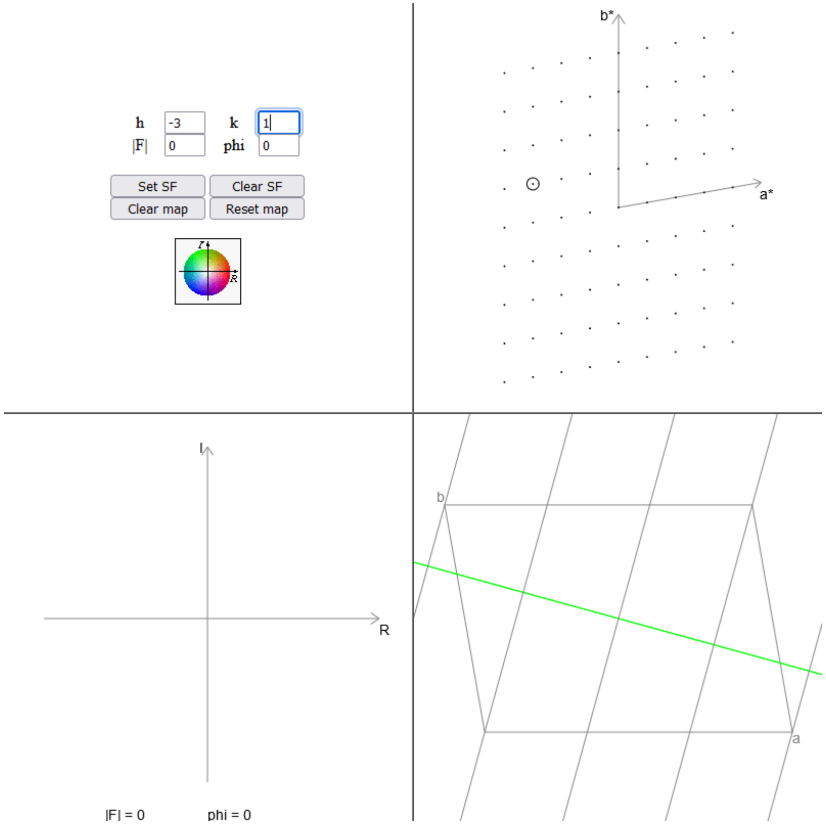
Not to scale

Reciprocal lattice



h 3 k 1
|F| 0 phi 0

|F| = 0 phi = 0



h -3 k 1
|F| 0 phi 0

|F| = 0 phi = 0

http://www.ysbl.york.ac.uk/~cowtan/sfapplet/sftut1.html

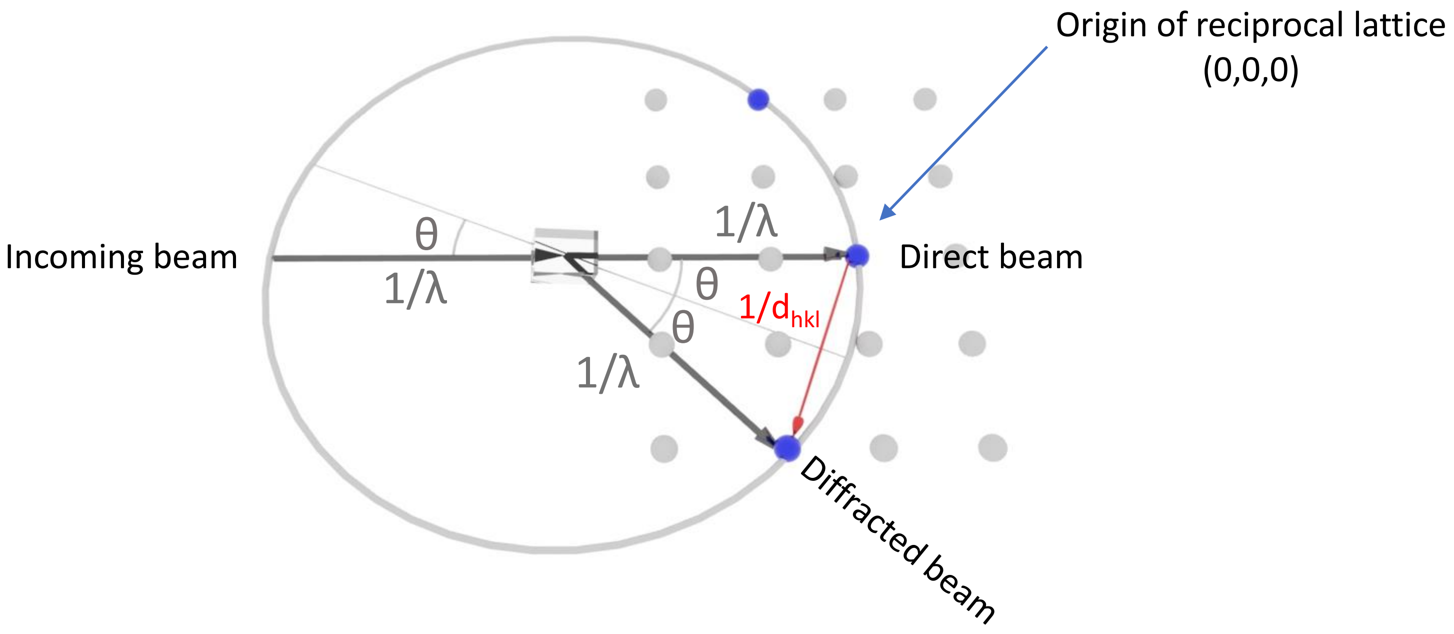
Ewald sphere

- Geometrical construction to visualise which Bragg planes are in the correct orientation to diffract.

- We define:
  - A sphere of radius = $1/\lambda$
  - The origin of the crystal is at the centre of the Ewald sphere,
  - The origin of reciprocal space is at the point where the incoming X-ray beam exits the Ewald sphere.
  - The vector S between the exit of the direct beam and the exit of the diffracted ray.

Ewald sphere

- If a set of planes is in the diffracting condition, the corresponding reciprocal space vector has to end on the surface of the Ewald sphere (the trigonometric condition 1/d = (2/λ) sinΘ is fulfilled).



Origin of reciprocal lattice (0,0,0)

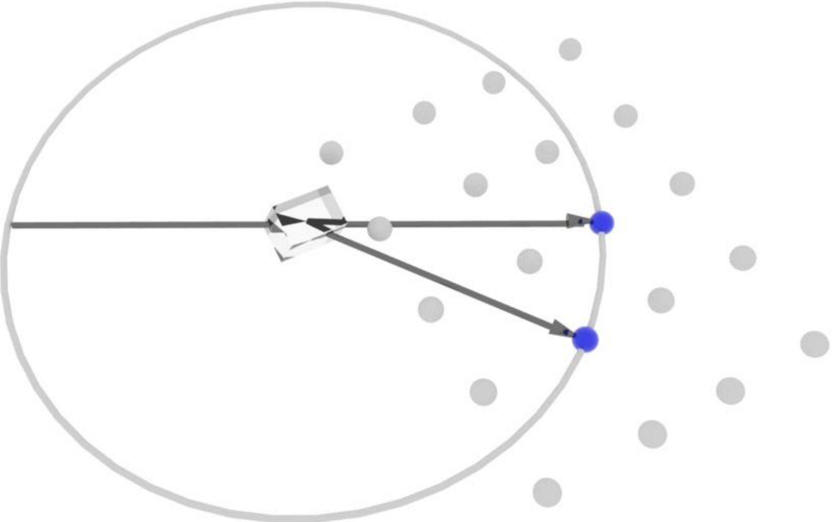
Incoming beam

Direct beam

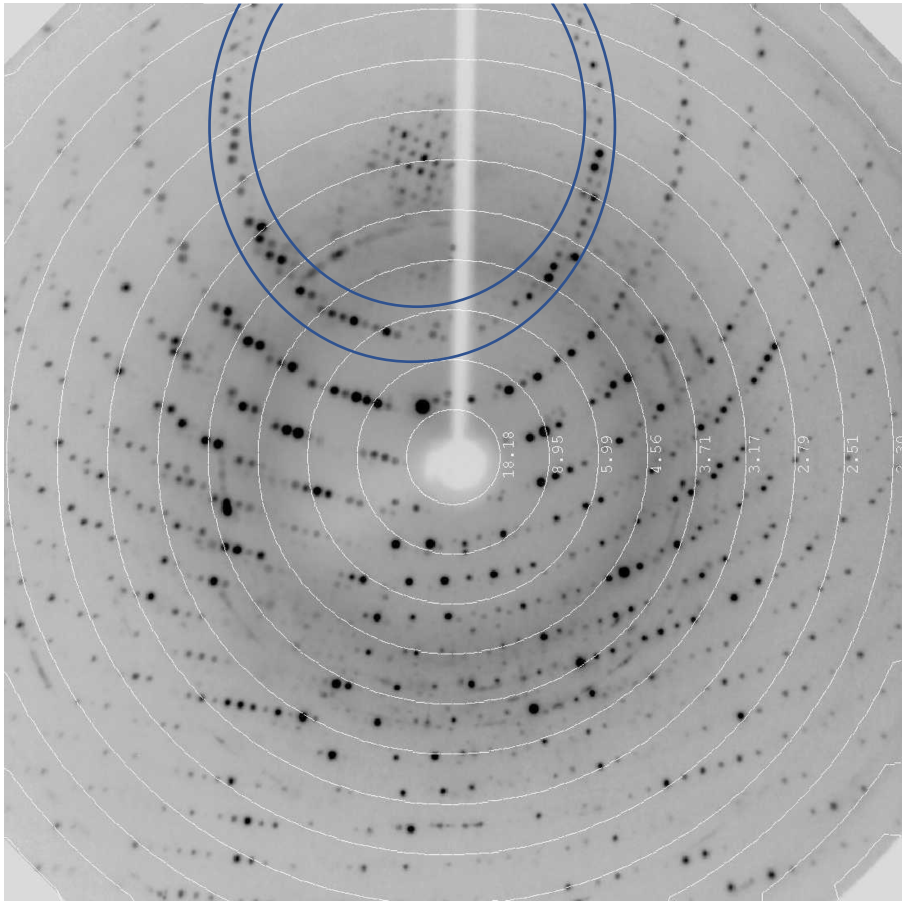
Diffracted beam

- Conversely, if the direct beam does not strike the planes with the correct angle θ, the reciprocal space vector will not be on the surface of the Ewald sphere.

Ewald sphere



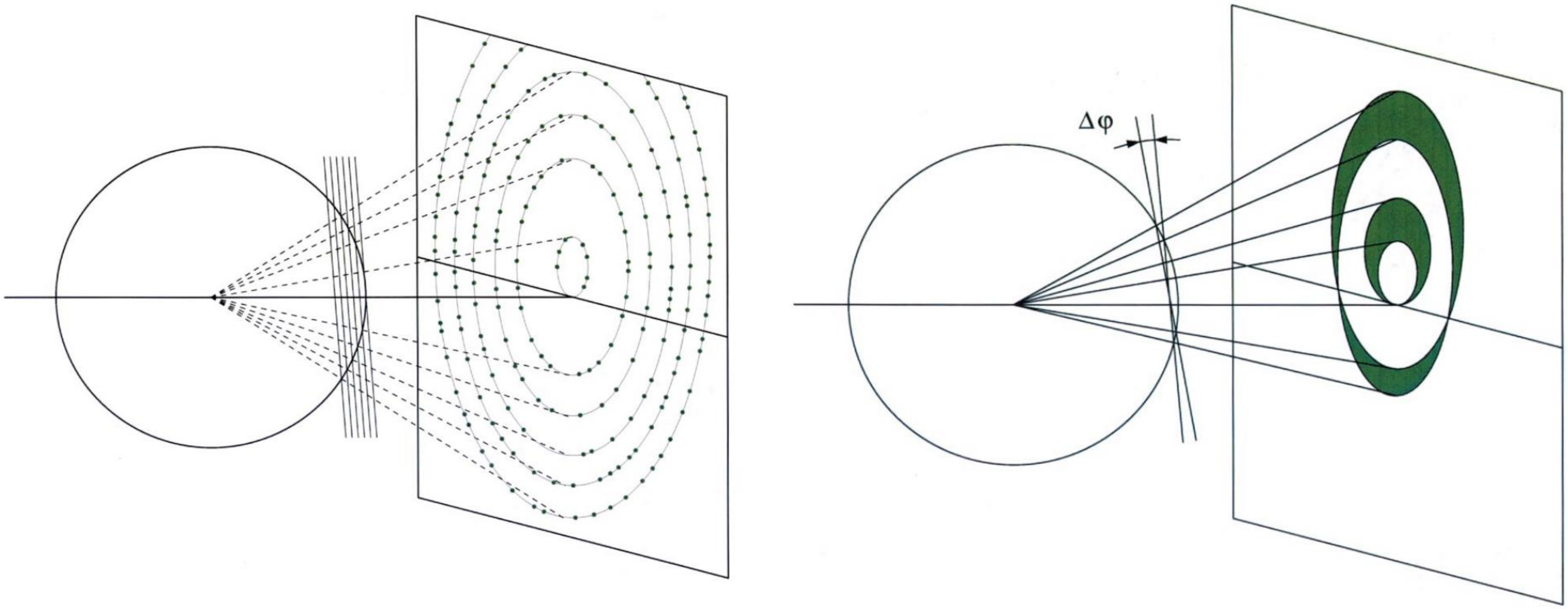
Ewald sphere



Lunes:
Reflections appear in rings or circles in the diffraction image.

Ewald sphere

Reciprocal lattice points lie in layers (planes). Each plane intersects the sphere in a circle, and the spots projected on the detector lie in ellipses.



- A still exposure with a stationary crystal: contains only a small number of reflections arranged in a set of narrow ellipses.
- When the crystal is rotated, reflections from the same reciprocal lattice plane form a lune, limited by two ellipses corresponding to the start and end positions.

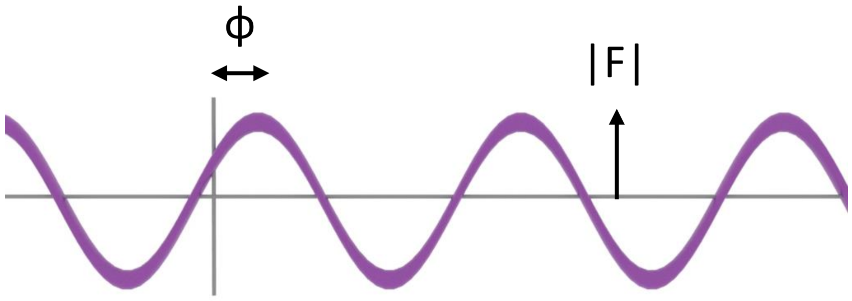
Dauter, ActaD, 1999

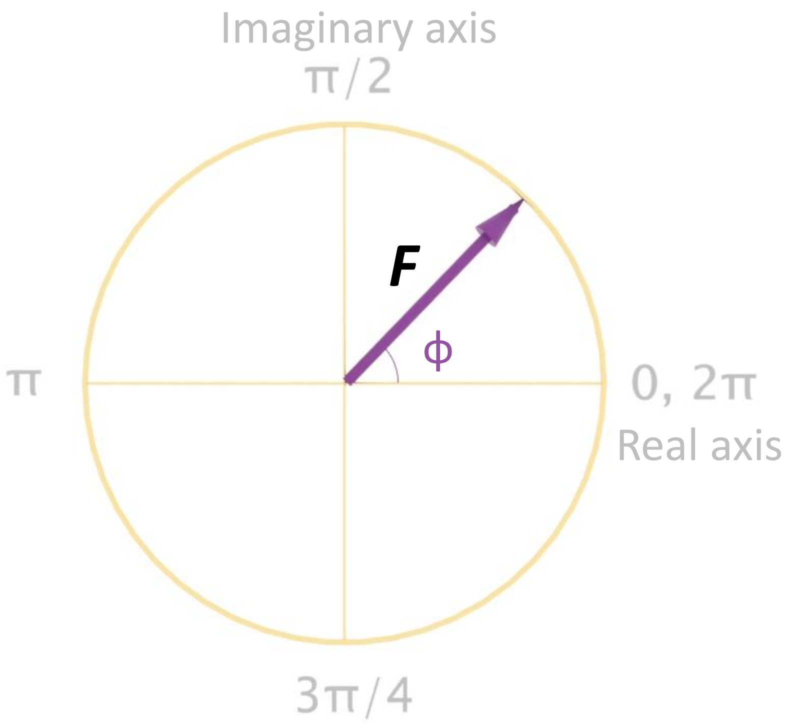
By observing the spacing and pattern of reflections on the diffraction pattern, we can determine the lengths and angles of the unit cells as well as some information on the space group.

So how do we find what is inside the unit cell?

Argand diagram

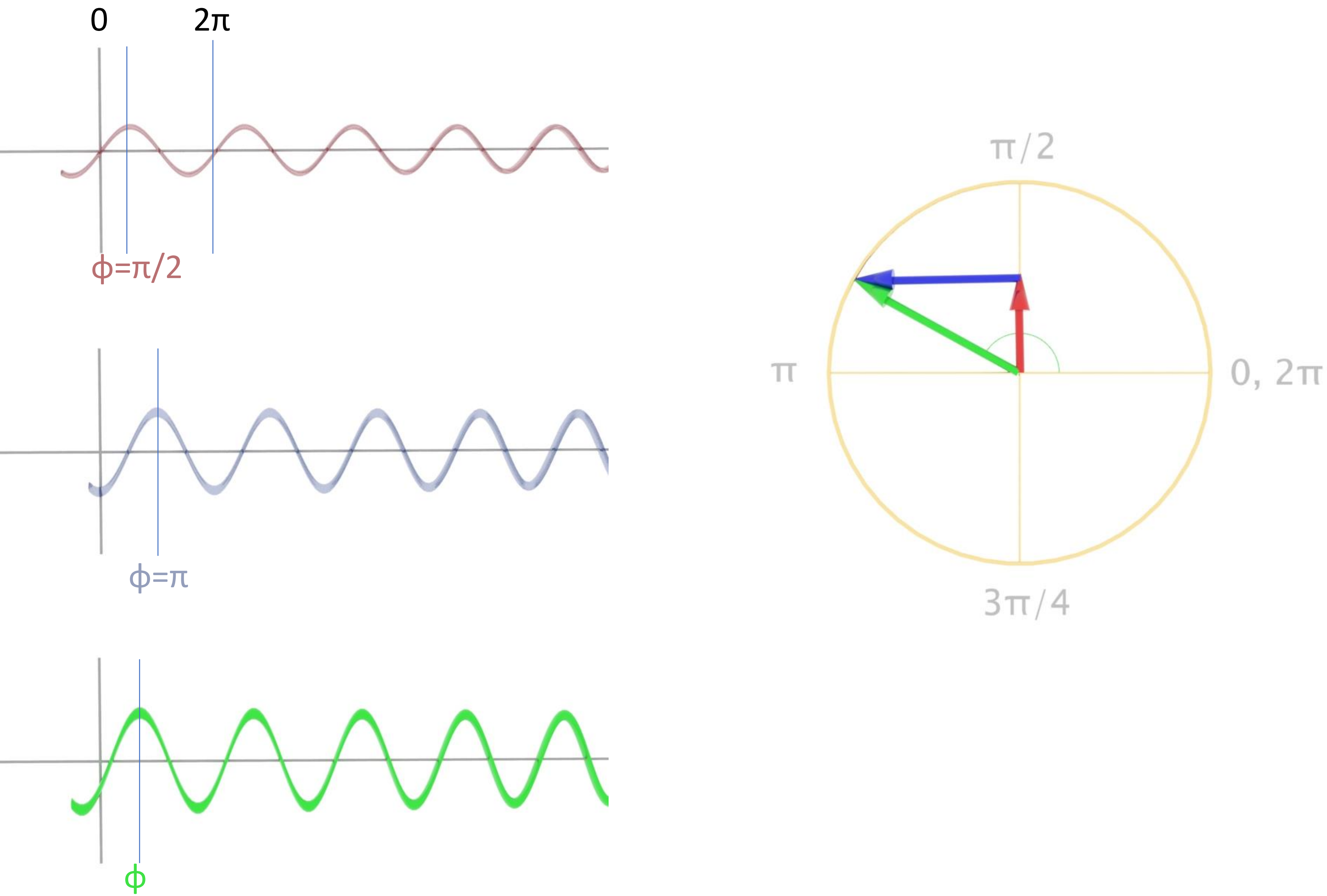
- Y axis imaginary component and x axis: real component.
- Waves (F and ϕ) are represented by vectors.
- Easier way to add waves.





$$F = |F|.(\cos(\phi t)+i.\sin(\phi\ t)) = |F|.e^{i\phi}$$

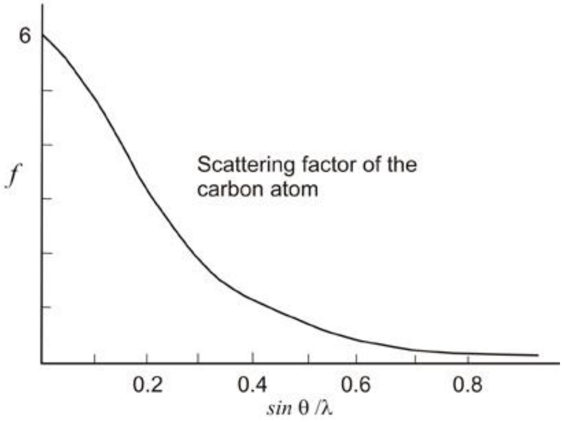
Argand diagram

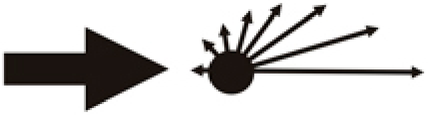


Atomic scattering factors

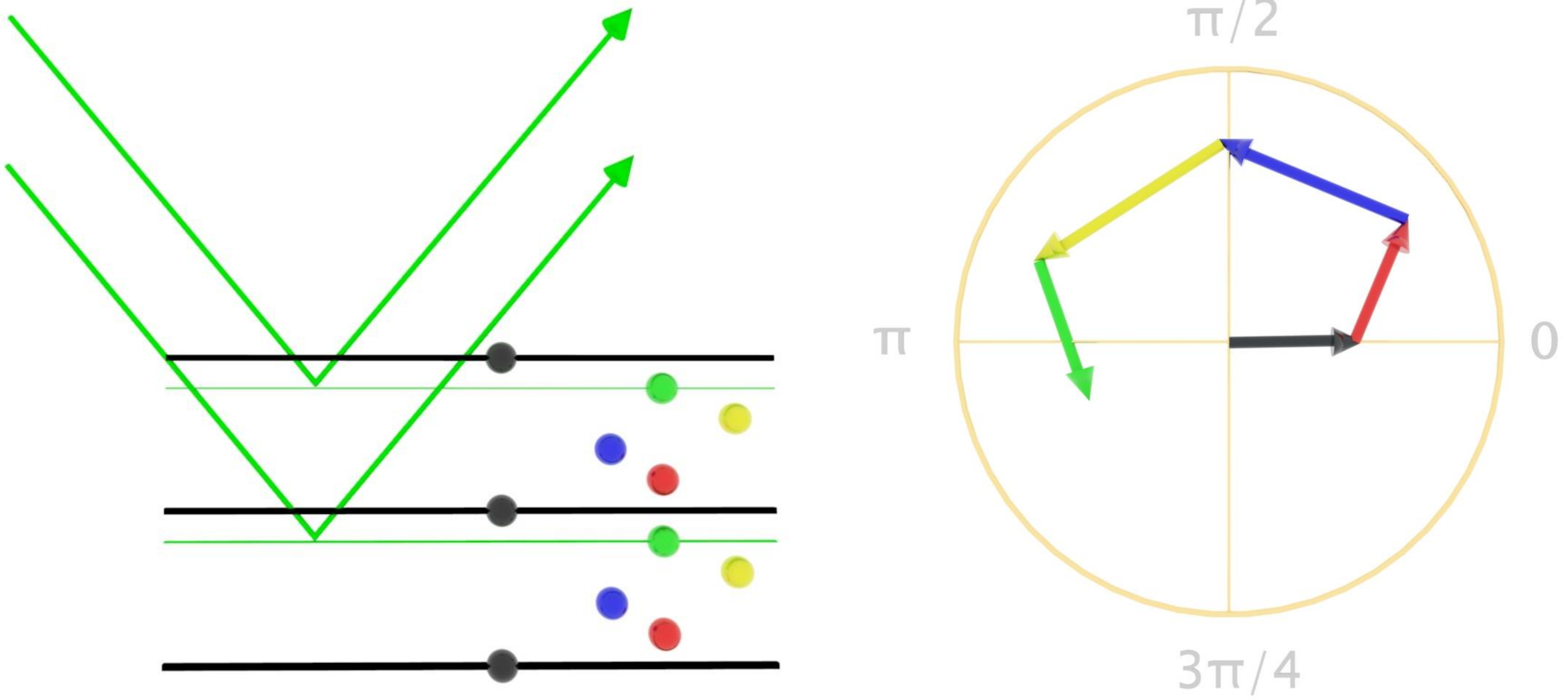
The individual atomic components (f_j) are called atomic scattering factors.

- Provide a measure of how efficiently an atom scatters X-rays compared to an electron.
- Depend on nature of atom, direction of scattering, and X-ray wavelength.
- Listed as a function of sin(θ)/λ for each atom in the International Tables.
- Maximum value of f_j is Z_j, the number of electrons of the j^{th} atom
- The atomic scattering factor is independent of the position of the atom in the unit cell.



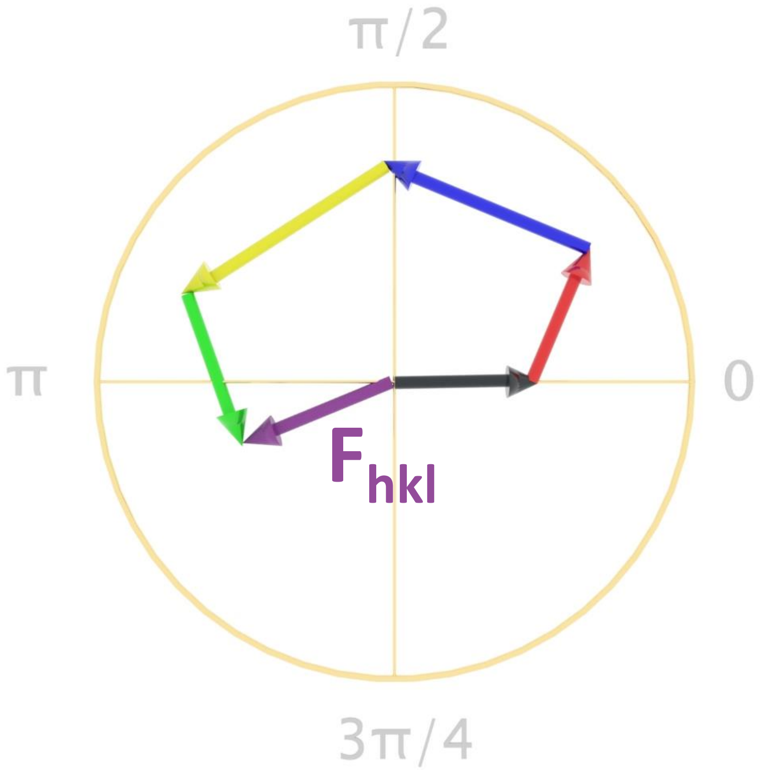


Structure factors



Adapted from Ed Lowe

Structure factors



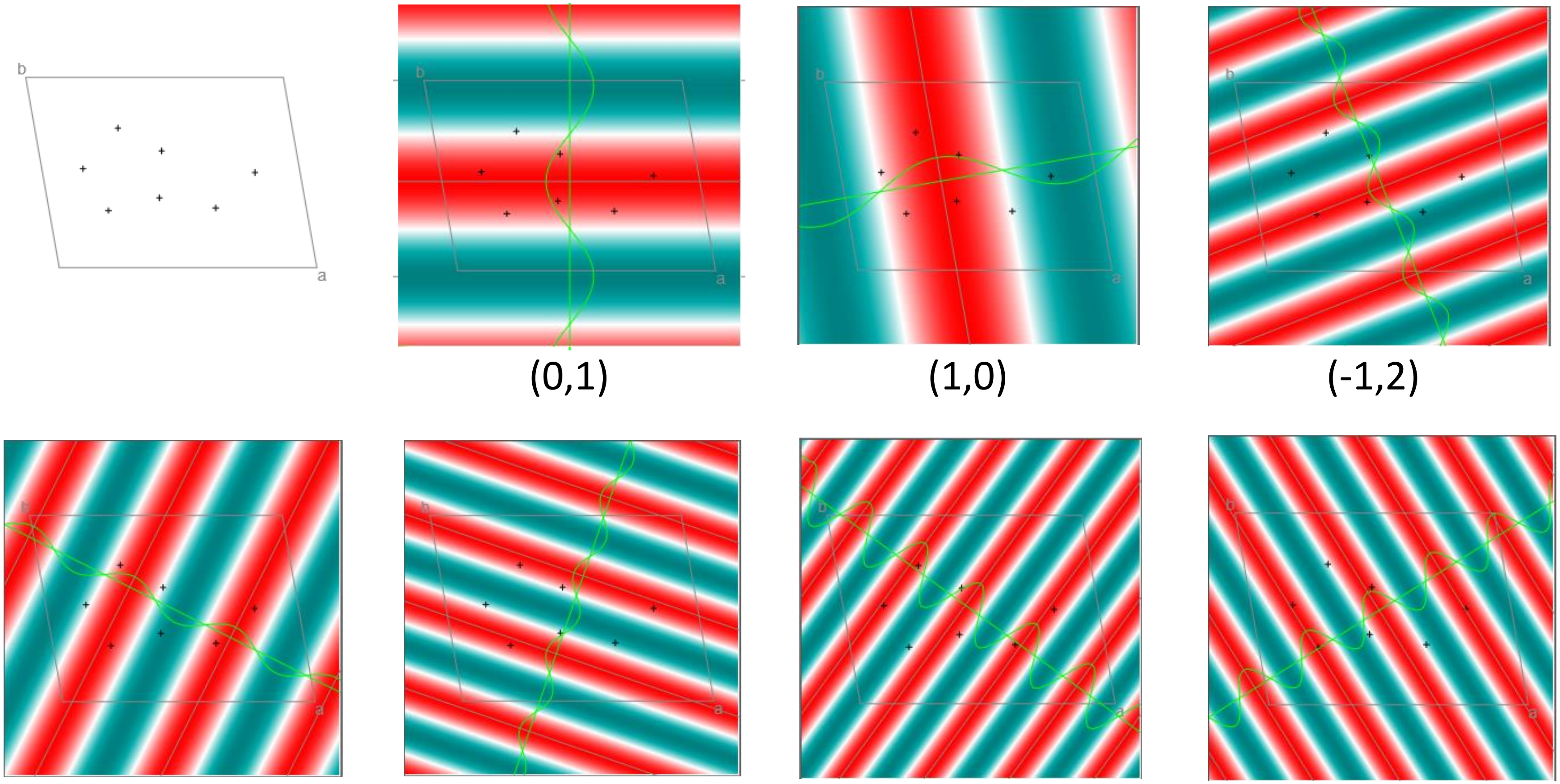
- The total scattering **F_{hkl}** is the vector sum of the individual atomic scattering vectors.

- **F_{hkl}** is called the structure factor because it is dependant on the structure of atoms in the unit cell.

- The structure factor F_{hkl} is a mathematical function describing the amplitude and phase of a wave diffracted from crystal lattice planes h,k,l by the n atoms contained in the unit cell.

How structure factors relate to electron density?

Atoms in a unit cell, structure factors and electron density.



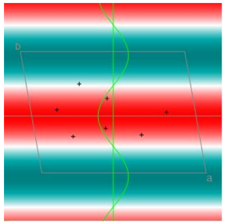
(0,1) (1,0) (-1,2)

(-2,1) (1,2) (3,-2) (3,1)

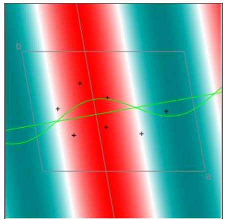
Electron density maps: dark red representing the most positive density and dark cyan representing the most negative, with white as zero.

http://www.ysbl.york.ac.uk/~cowtan/sfapplet/sftut3.html

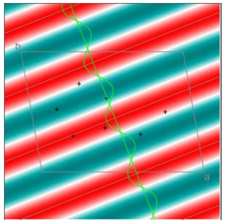
Atoms in a unit cell, structure factors and electron density.


(0,1)

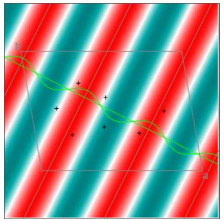
+


(1,0)

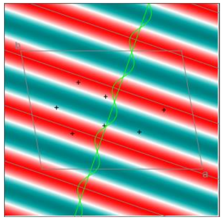
+


(-1,2)

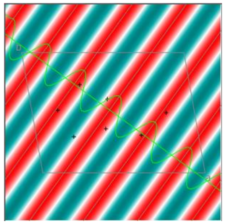
+


(-2,1)

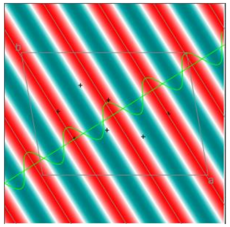
+

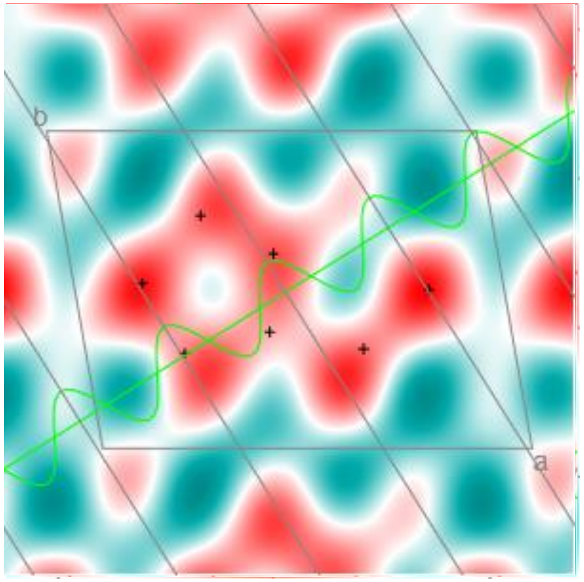

(1,2)

+


(3,-2)

+


(3,1)



http://www.ysbl.york.ac.uk/~cowtan/sfapplet/sftut3.html

$$\underbrace{\boldsymbol{F}(\boldsymbol{h},\boldsymbol{k},\boldsymbol{l})}_{\text{Wave}} = \underbrace{V\int_{x=0}^{1}\int_{y=0}^{1}\int_{z=0}^{1}\rho(x,y,z).\exp\left[2\pi i(hx+ky+lz)\right]dxdydz}_{\text{(Periodic) function}}$$

Structure factor equation

Every atom in the unit cell contribute to a every structure factor F_{hkl}.

$$\underbrace{\rho(x,y,z)}_{\text{(Periodic) function}} = \underbrace{\frac{1}{V}\sum_{h}\sum_{k}\sum_{l}\left|F(h,k,l)\right|\exp\left[-2\pi i(hx+ky+lz)+i\alpha(h,k,l)\right]}_{\text{Wave}}$$

Electron density equation

Every F_{hkl} contribute to every atom in the unit cell.

Fourier transform and synthesis

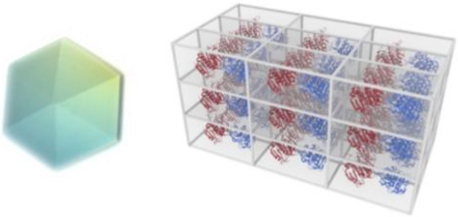
- The decomposition of a (periodic) function *(electron density)* into sine waves *(structure factors)* is called a Fourier transformation.
    - The structure factor equation is a Fourier transform.
    - *Data collection*

- The construction of a (periodic) function *(electron density)* by the summation of sine waves *(structure factors)* is called a Fourier synthesis.
    - The electron density equation is a Fourier synthesis
    - *Structure solution*

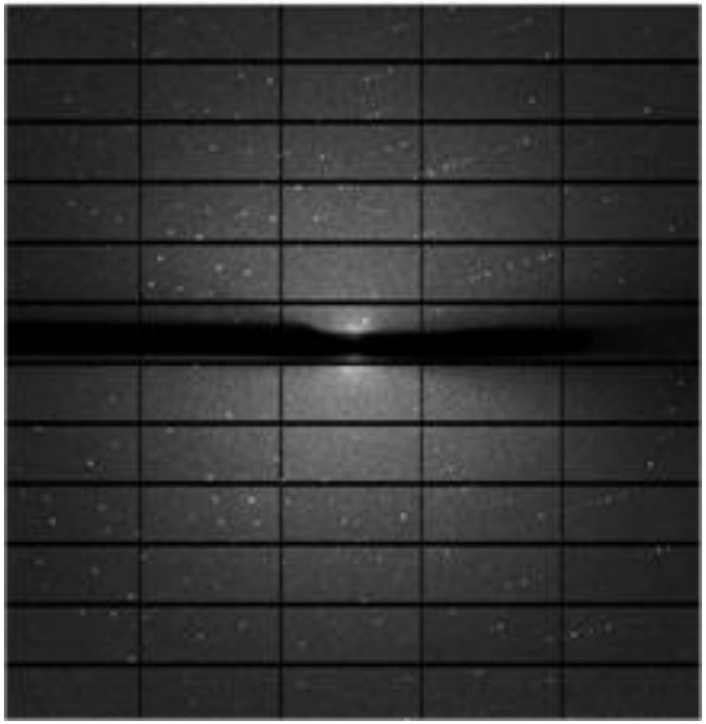
Fourier transform and synthesis

Real Space

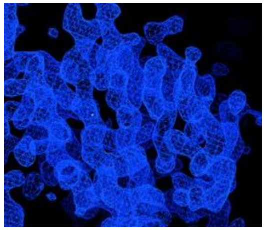
Reciprocal Space



Fourier Transformation



Inversion theorem



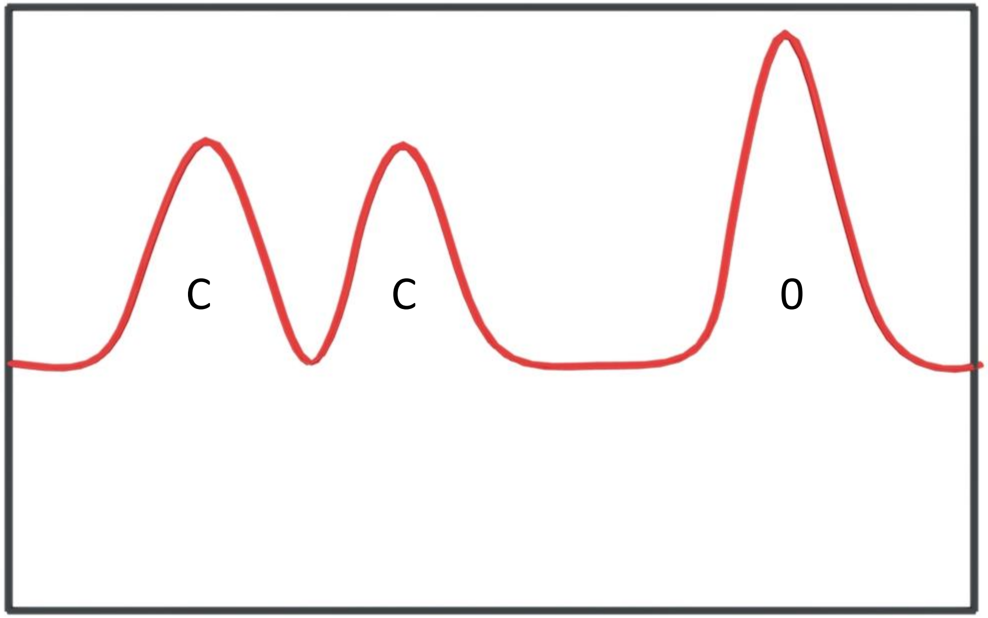
Fourier Synthesis

Electron density

Structure factors

Lens

Fourier transform and synthesis

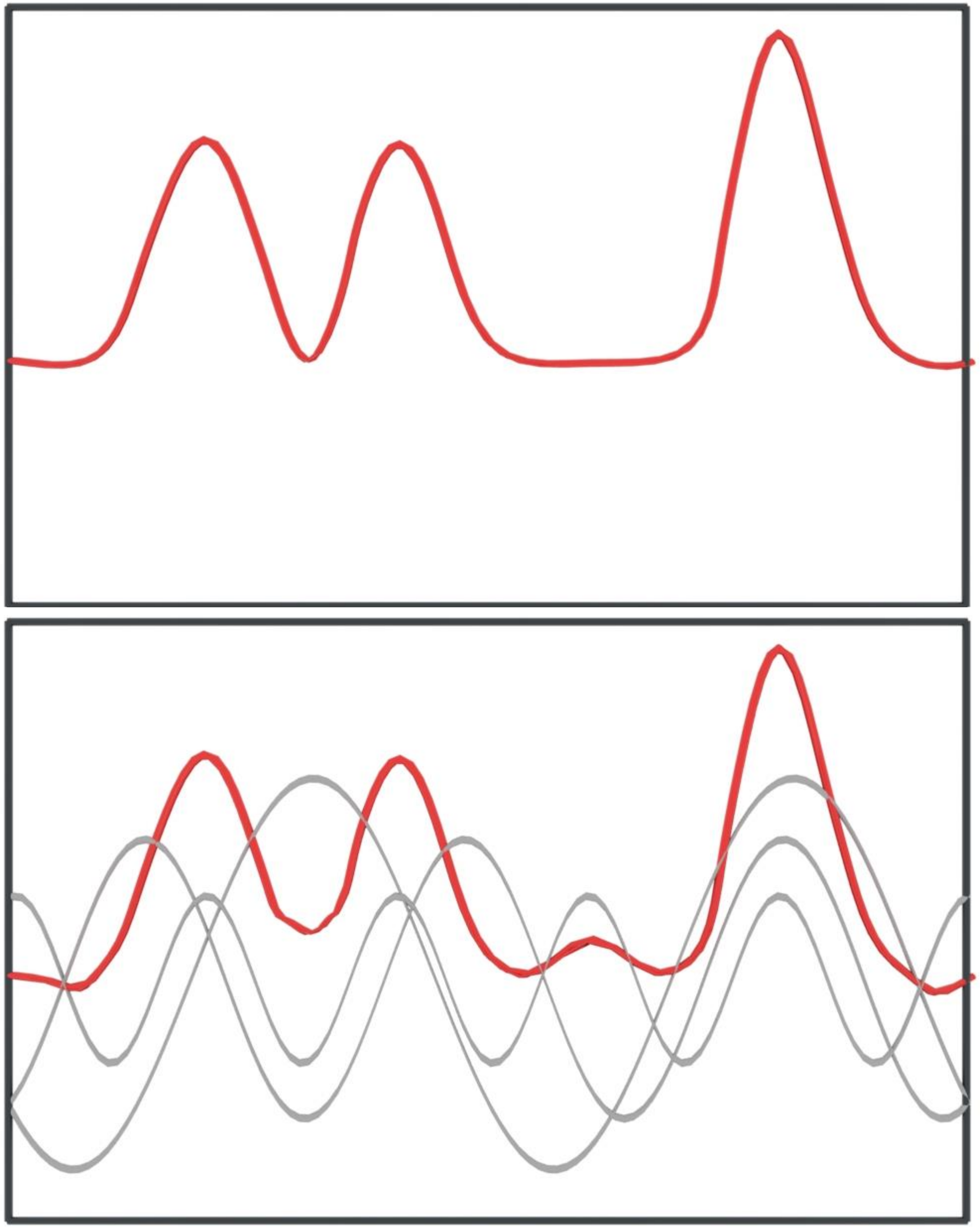


- Consider an imaginary one-dimensional crystal with 3 atoms in the unit cell. The electron density in the unit cell looks like above.

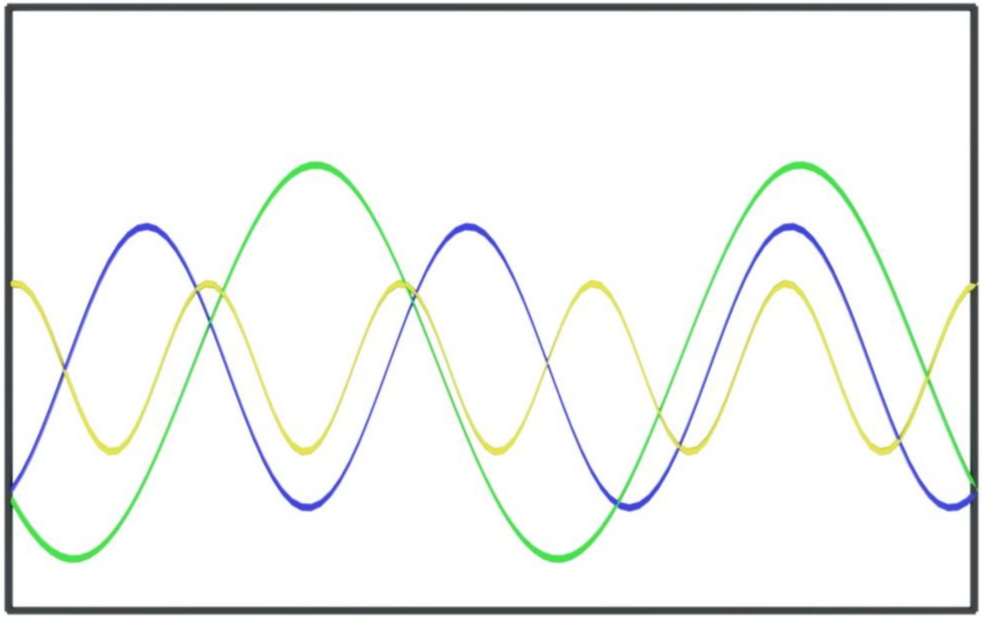
- We will represent this function in terms of sine waves with different frequency.

Adapted from http://www.ysbl.york.ac.uk/~cowtan/fourier/ftheory.html

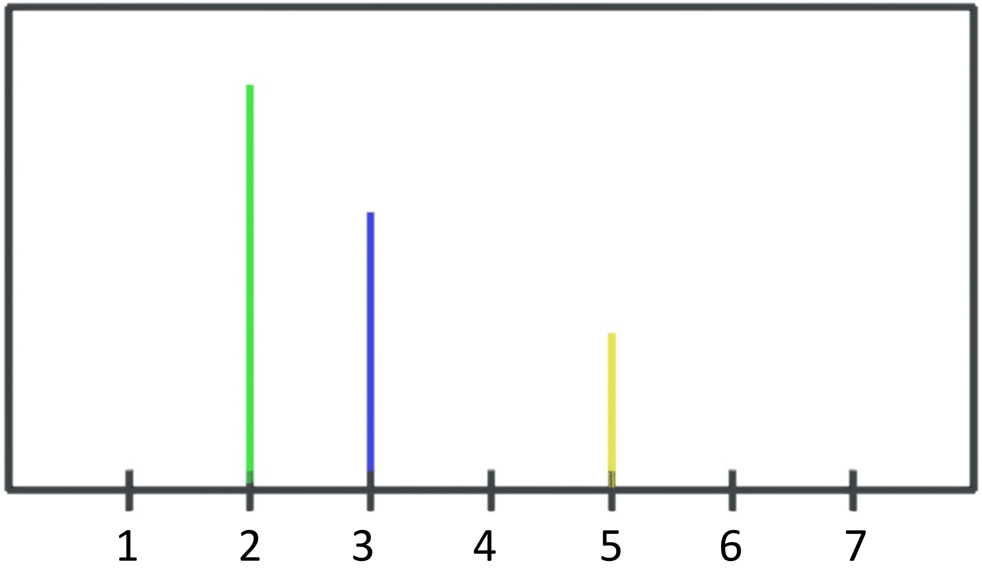
Fourier transform and synthesis



Fourier transform and synthesis



The complete set of components that are necessary to describe a periodic function is called a Fourier series.

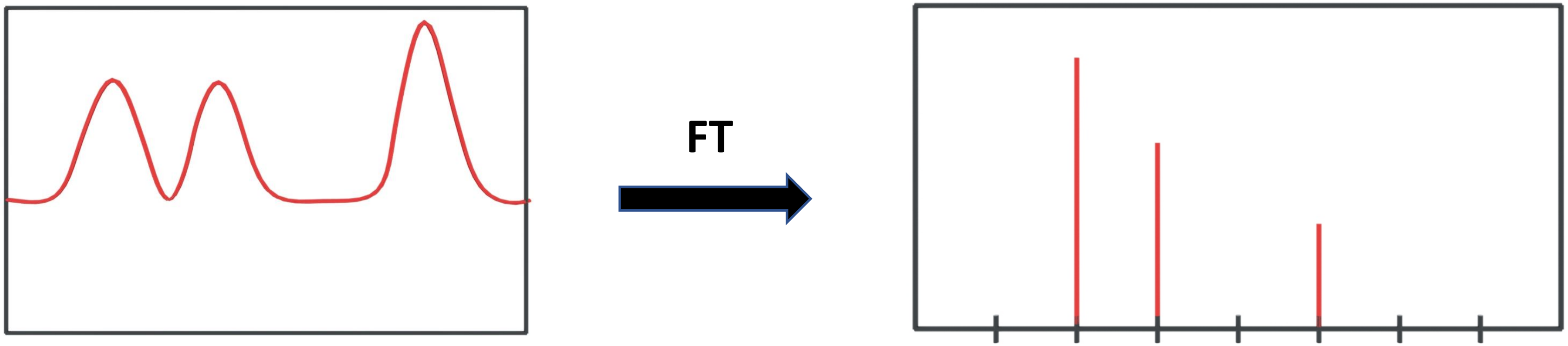


Freq = 2

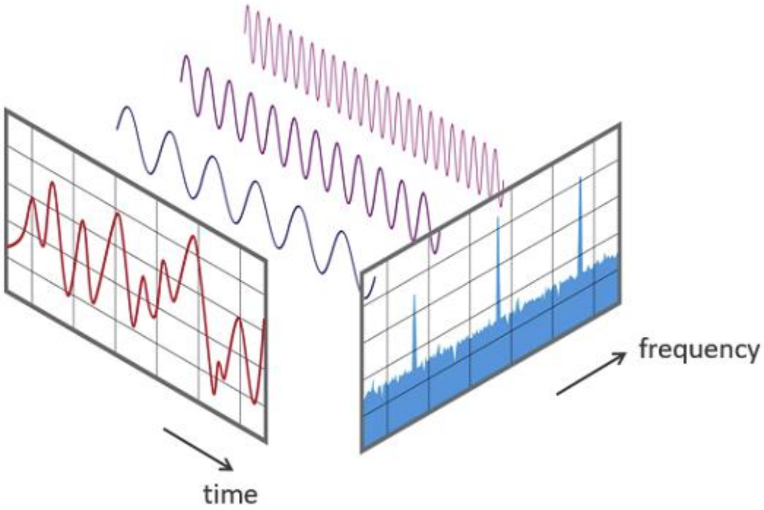
Freq = 3

Freq = 5

Fourier transform and synthesis



The Fourier Transform tells us what mixture of sine-waves is required to make up any function.



Conclusion on diffraction patterns:

- Position of reflections on diffraction images gives information about unit cell dimensions, translational symmetry elements and resolution.

- The amplitude |F| of a particular structure factor indicates the extent to which the electron density is concentrated on planes parallel to the Bragg planes ($I_{hkl} \alpha |F_{hkl}|^2$).

- The phase indicates the position of planes of high electron density relative to the Bragg planes: but lost in our experiment!

Thank you for you attention!