Introduction to Diffraction Theory

DLS-CCP4 School in Macromolecular Crystallography 2025

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10/10/2025
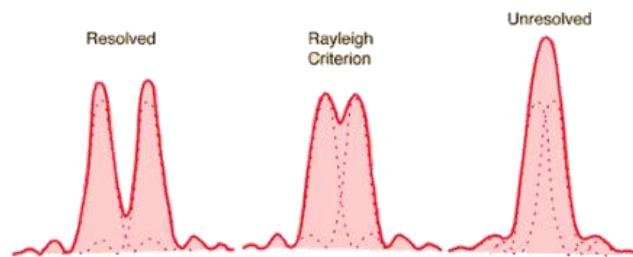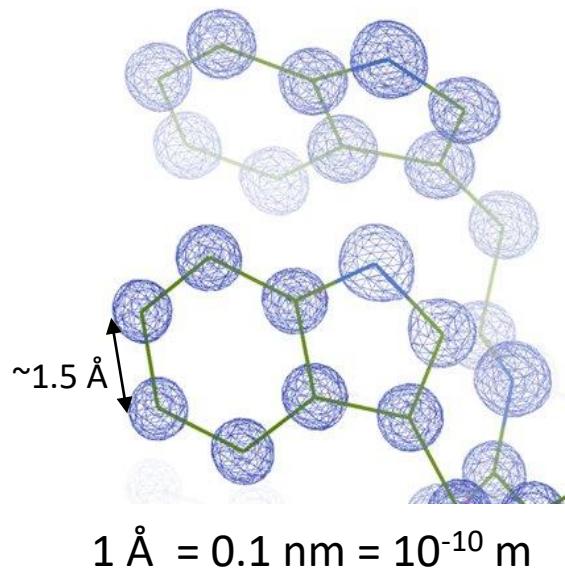
X-ray crystallography experiment

Crystal · Diffraction patterns · Phases · Electron density maps · Model building & refinement · Final model
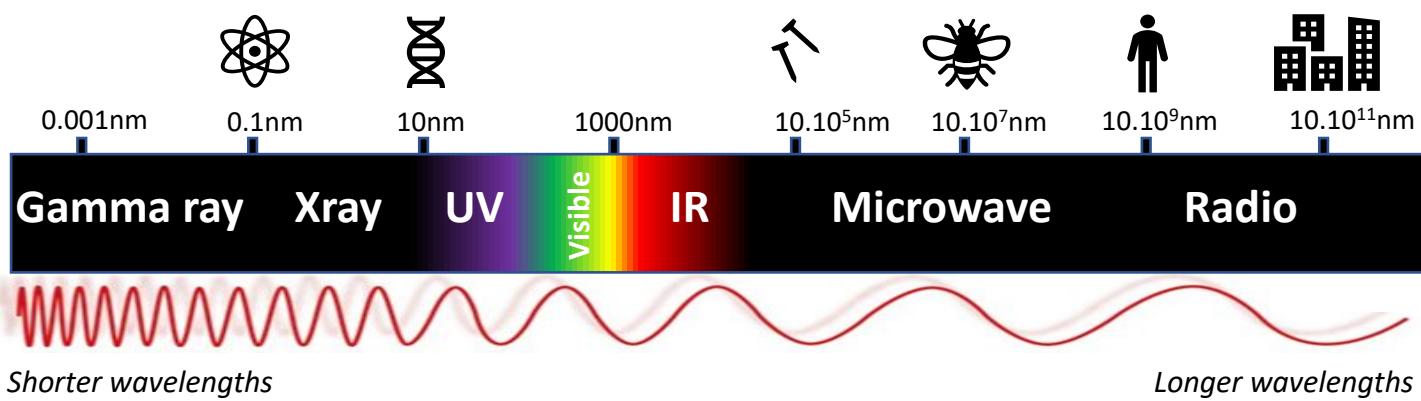
- ➢ Why do we need X-rays and crystals?

- ➢ What is diffraction?

- ➢ What are structure factors?

Why can't we see atoms with visible light?

~1.5 Å

1 Å = 0.1 nm = 10^{-10} m

Resolved

Rayleigh Criterion

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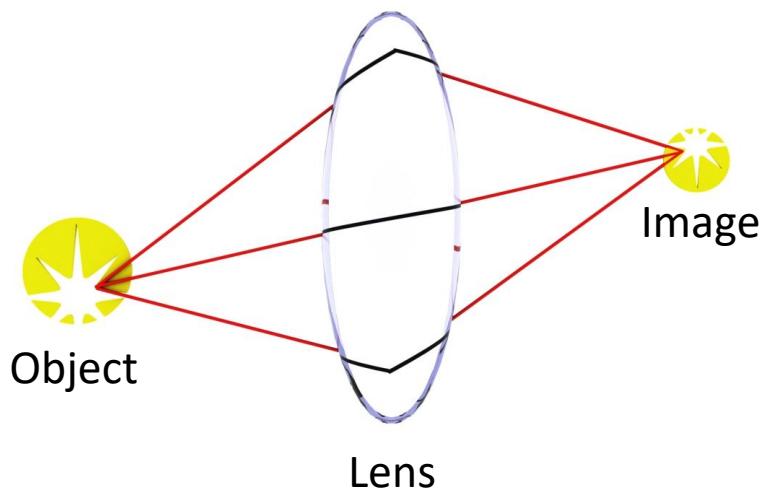
$$r = \frac{\lambda}{2 \cdot NA}$$

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

0.001nm	0.1nm	10nm	1000nm	$10 \cdot 10^5$nm	$10 \cdot 10^7$nm	$10 \cdot 10^9$nm	$10 \cdot 10^{11}$nm

Gamma ray **Xray** **UV** **Visible** **IR** **Microwave** **Radio**

Shorter wavelengths

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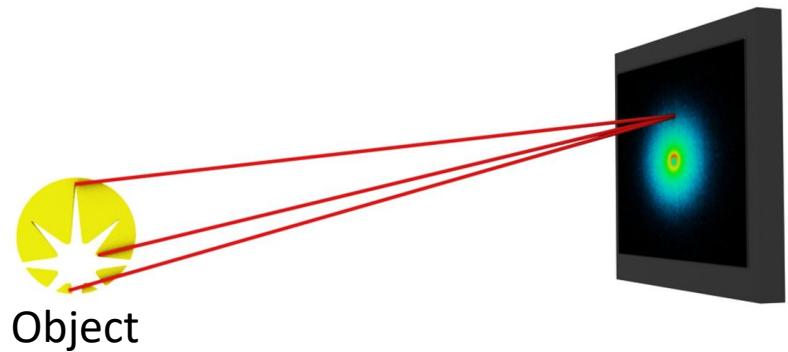
We can't see objects that are much smaller than the wavelength (λ) of the light that is being used.

Light arriving on one point of the image comes from one point on the object
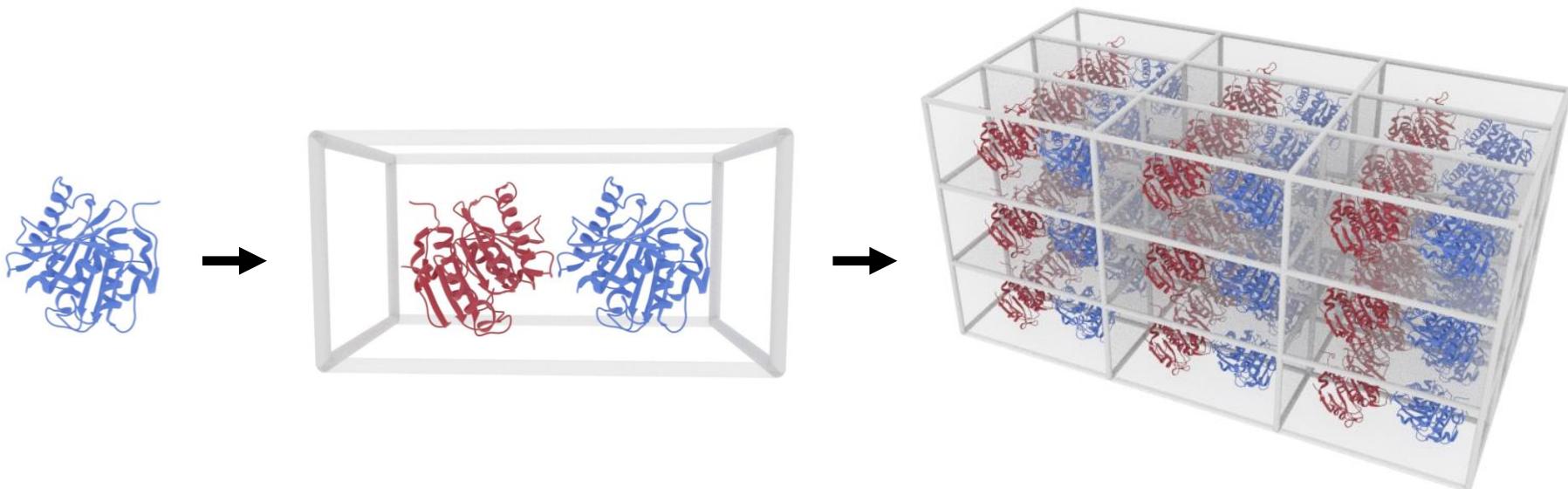
Light arriving on one point of the image comes from all points on the object.

Object

Lens

Image

Object

We can't build X-ray lenses (exception: zone plates), **instead we use computers and FT.**

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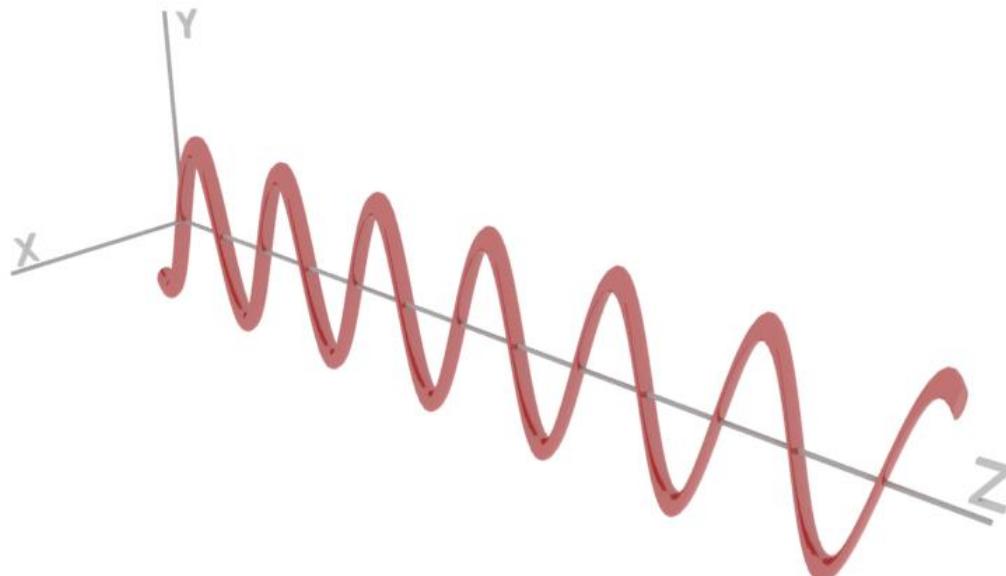
- Diffraction from one molecule is too weak: crystals acts as a **signal amplifier**.
- Crystals are made up of identical building blocks called unit-cells.
- Multiple unit-cells constitute a 3-dimensional translation lattice.
- Crystals are particularly suited for X-ray diffraction (λ ~ atomic spacing).
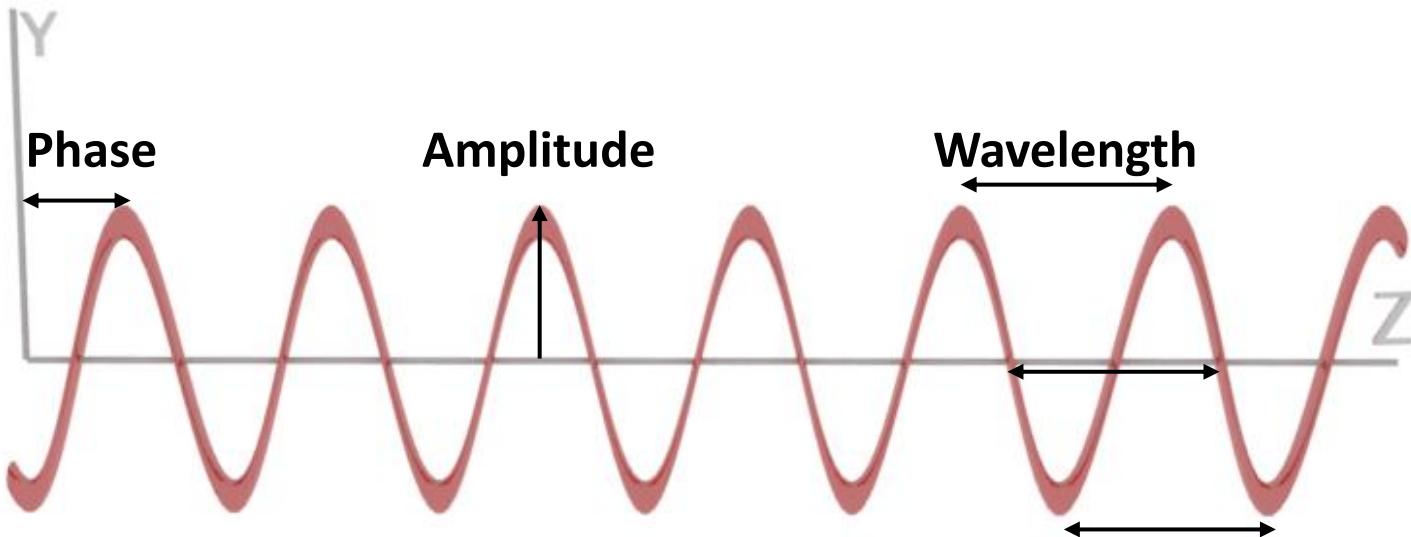
➢ Why do we need X-rays and crystals?

➢ What is diffraction?

➢ What are structure factors?

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.

- For simplification, electromagnetic waves are often represented only by their electric component (most important one for our experiment).
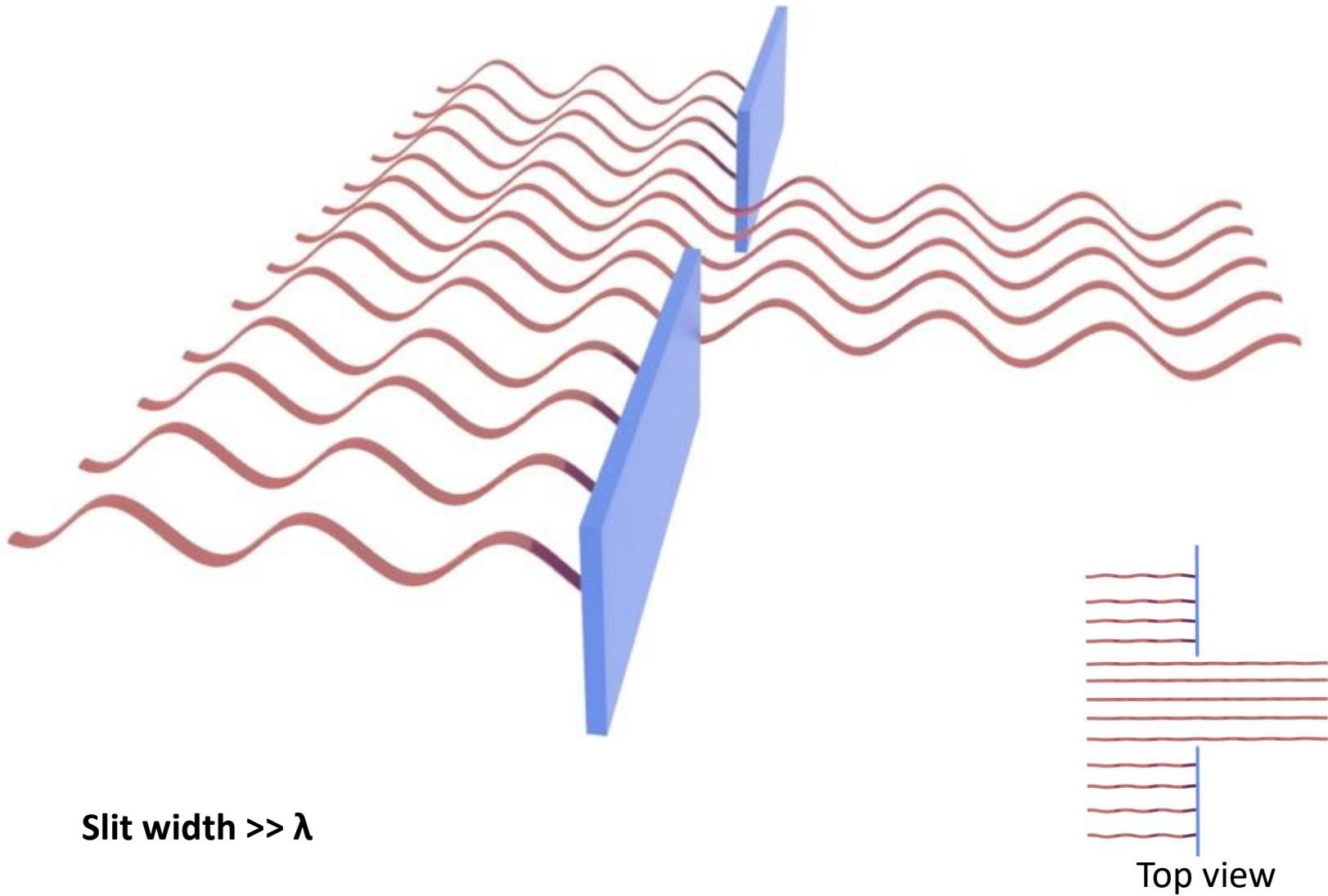
Electromagnetic waves
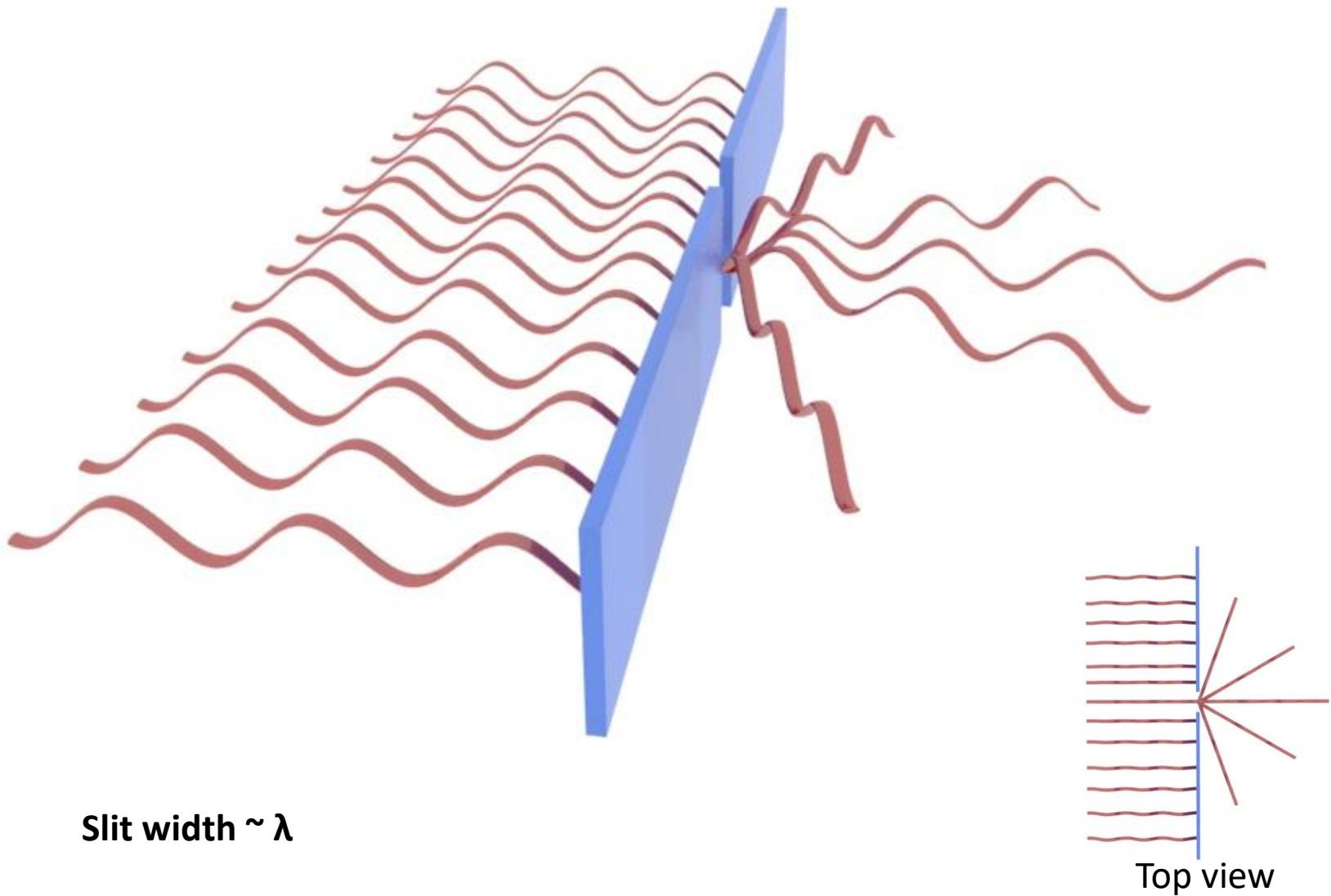
- A wave can be defined by its wavelength, amplitude and phase.
- In XRC experiments, we usually use monochromatic X-rays, so the wavelength (λ) is known and fixed.

# Slit experiment

Slit width >> λ

Top view
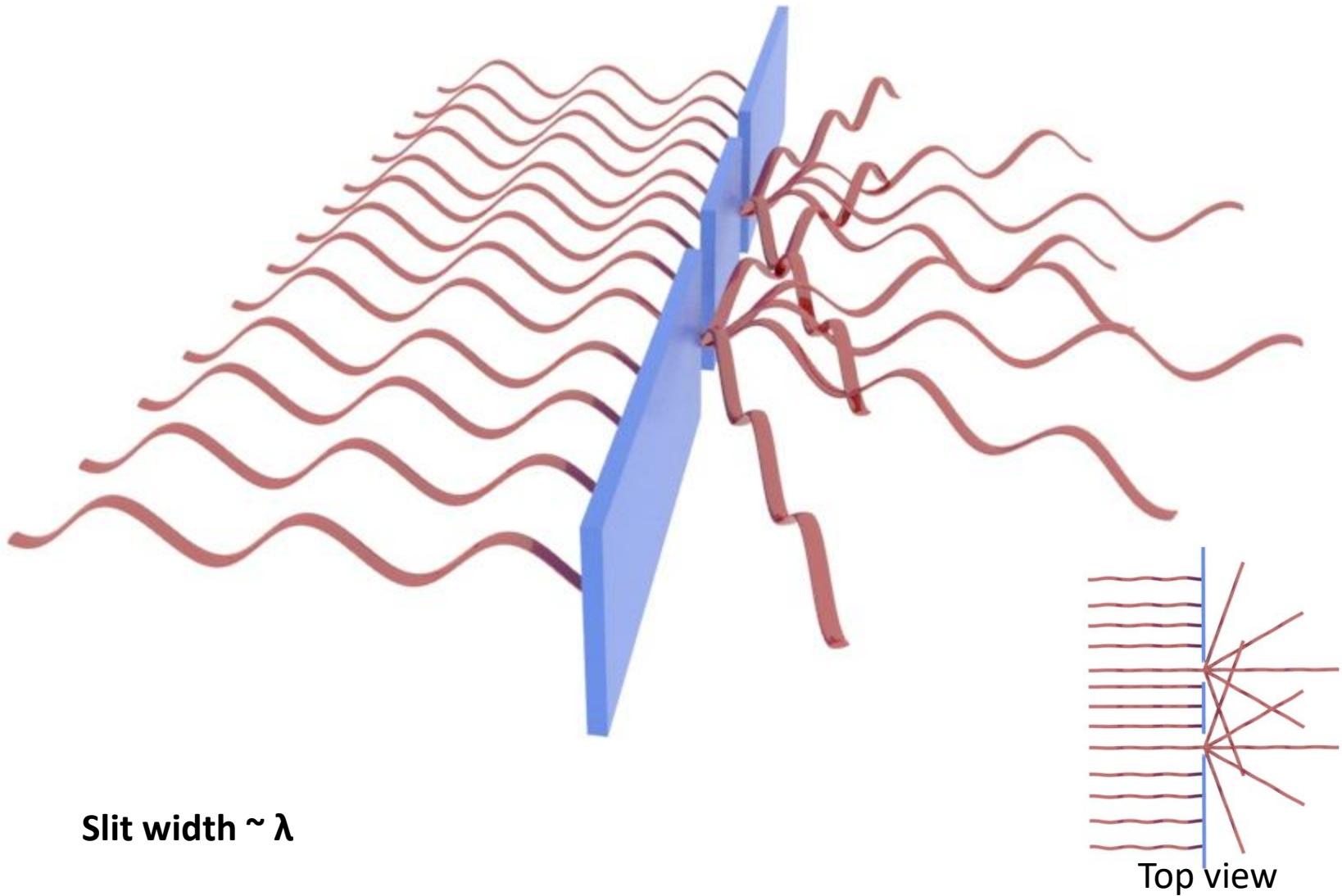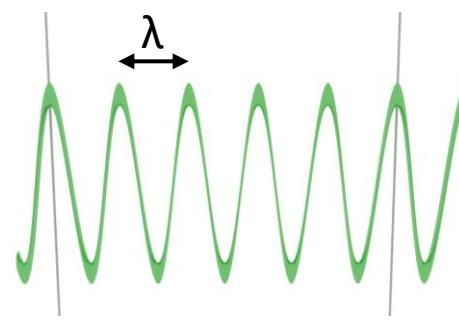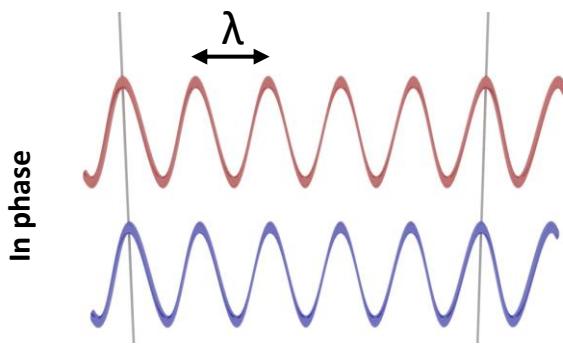
Slit experiment

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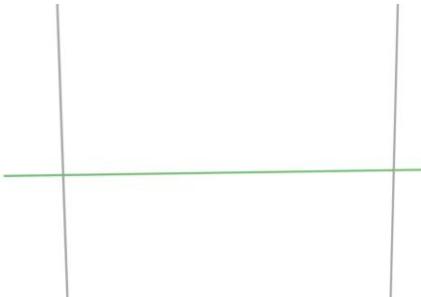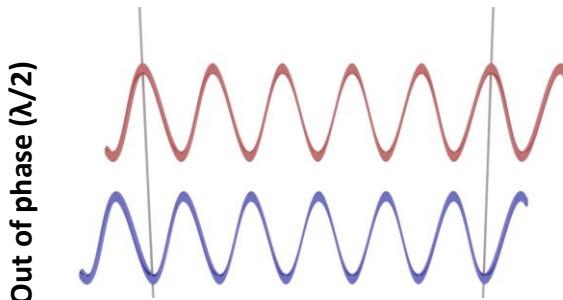

Slit width ~ λ

Top view

Slit experiment

Slit width ~ λ

Top view

Wave interference

In phase

λ

→ Constructive interference

Out of phase (λ/2)

λ

→ Destructive interference

Noise cancellation circuitry

Speaker

Microphone
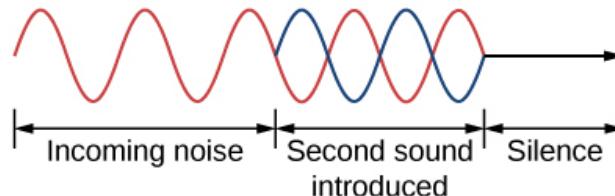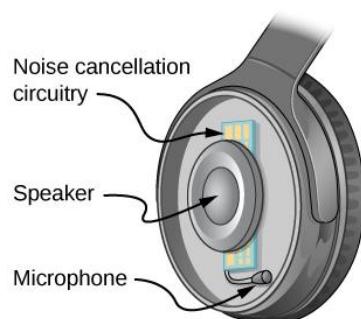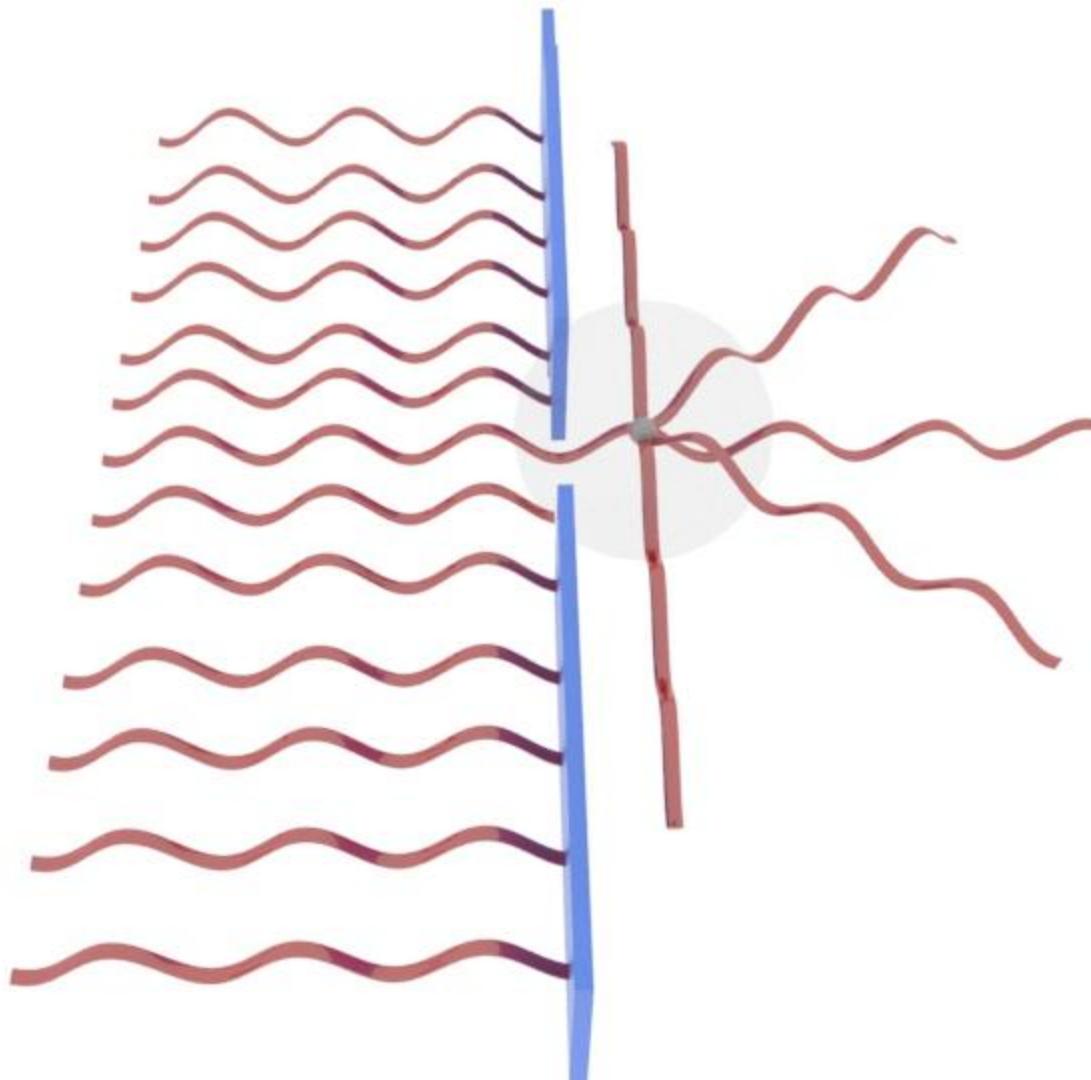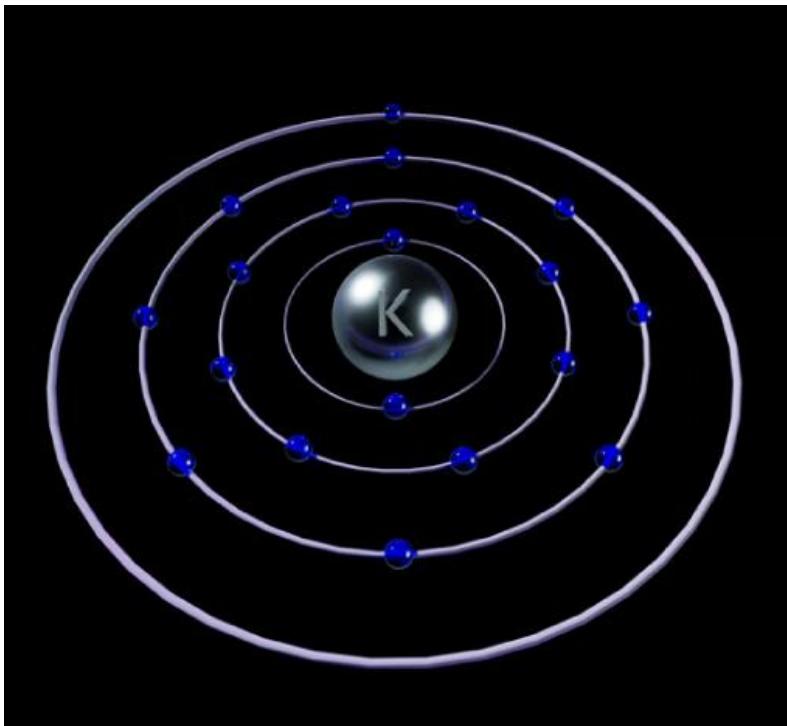
Incoming noise | Second sound introduced | Silence

Huygens–Fresnel principle
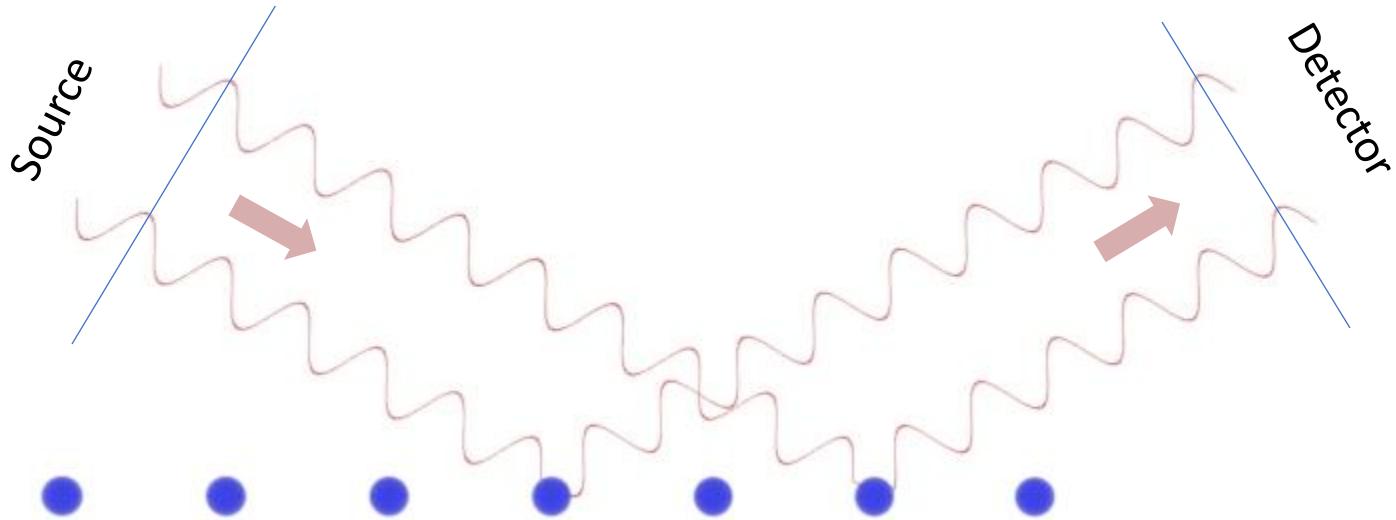
- Every point on a wavefront is itself a source of spherical wavelets.
- The secondary wavelets emanating from different points mutually interfere.
- The sum of these spherical wavelets forms the new wavefront.
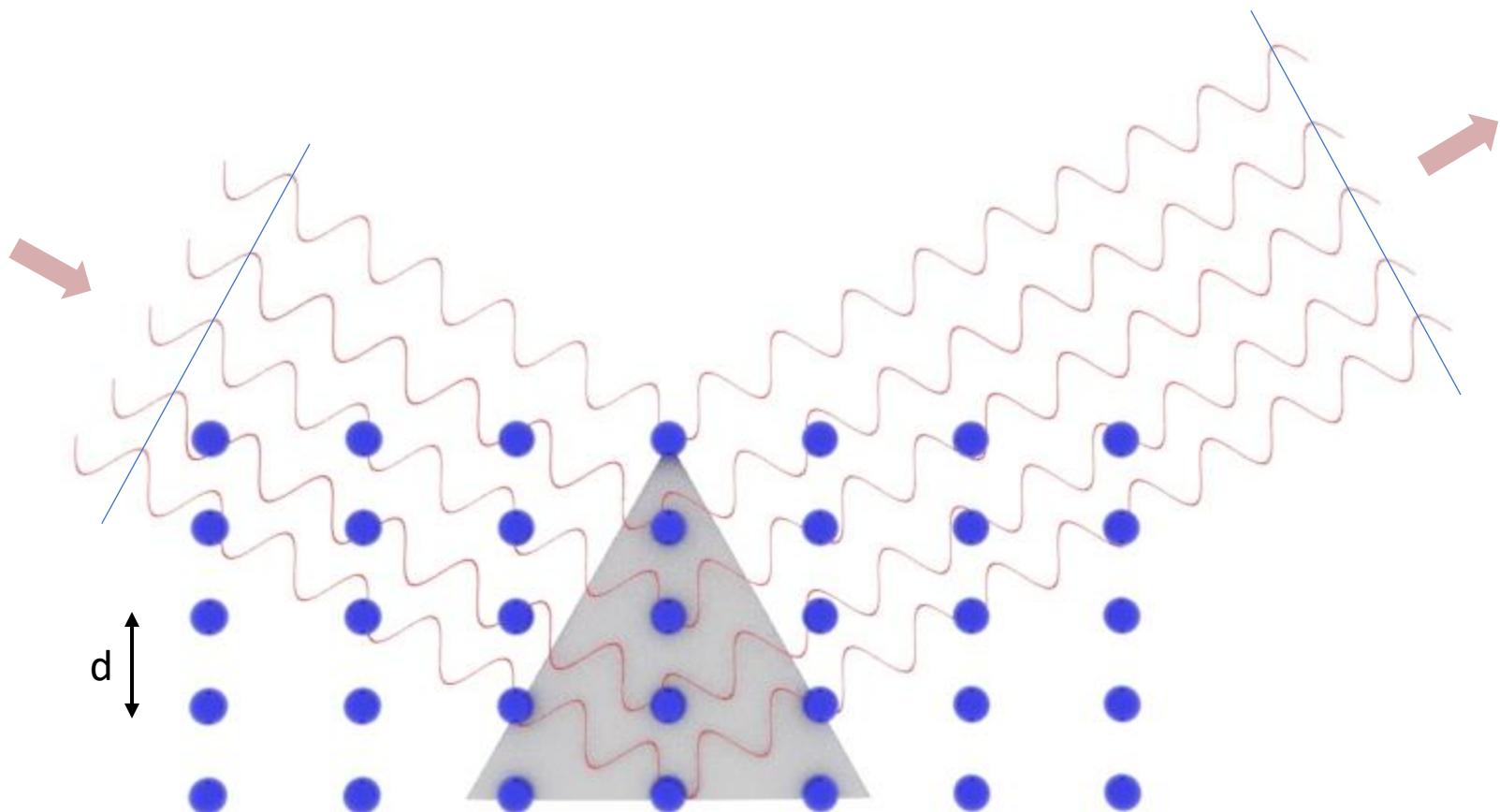
- When X-rays interact with electrons, they set the electron oscillating with the X-ray wavelength (excited state).

- 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.

- At specific angles, the scattered waves add up constructively, producing strong diffraction peaks.

- Crystals are regular arrays of electrons, that behave similarly to slits.

Waves in phase
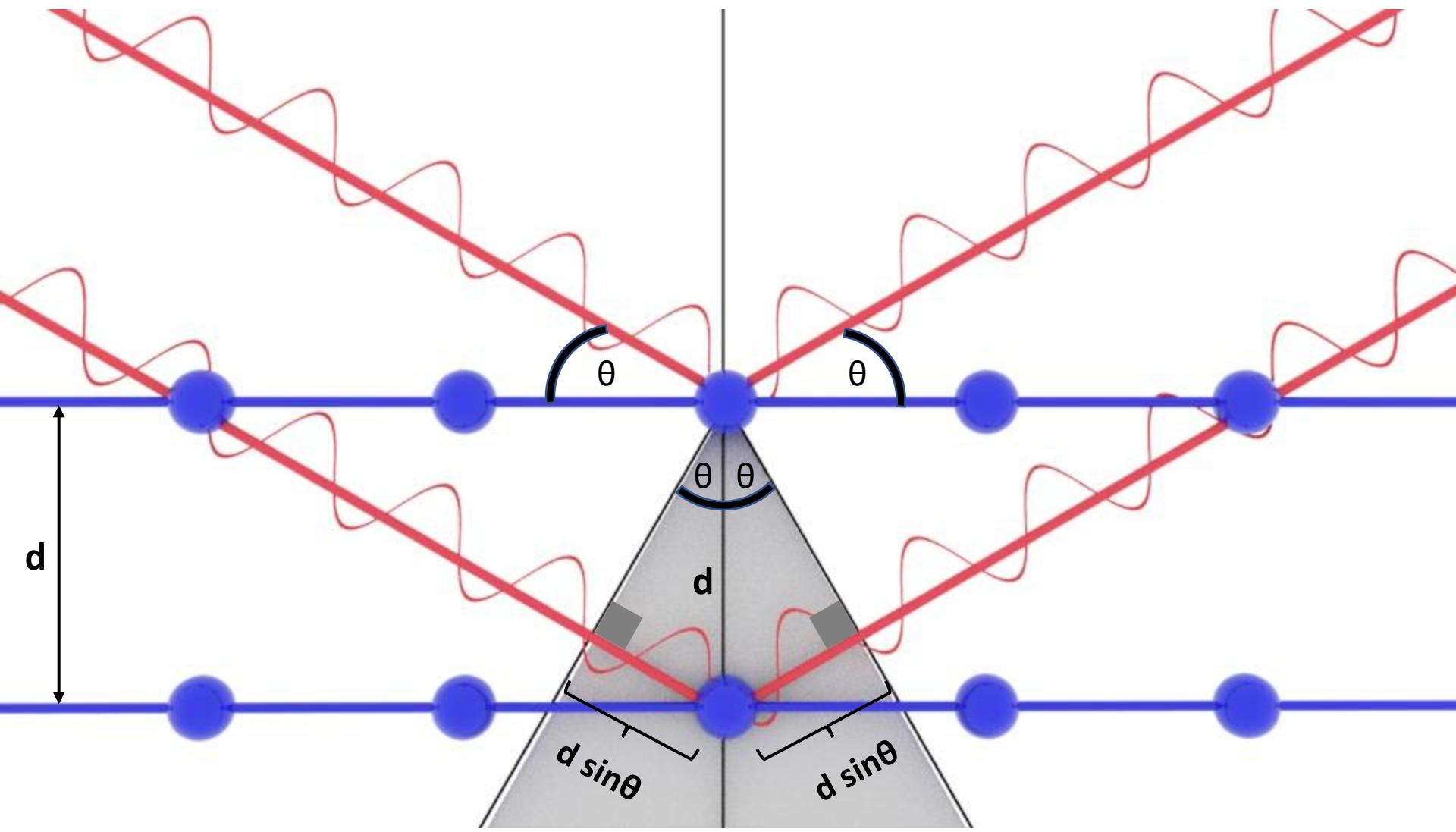
When the path length is the same.

Waves in phase

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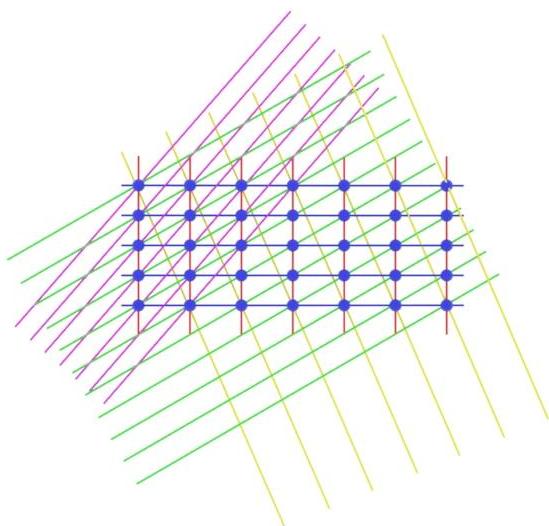
The extra path travelled by the wave is (d.sin θ + d.sinθ).

Waves in phase
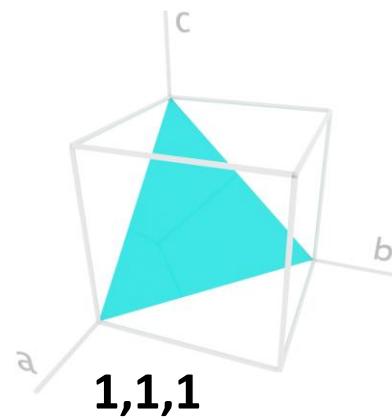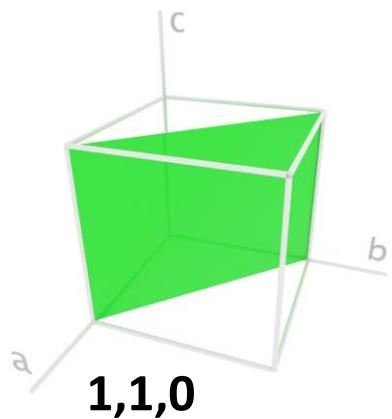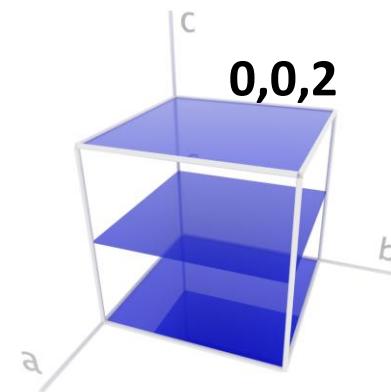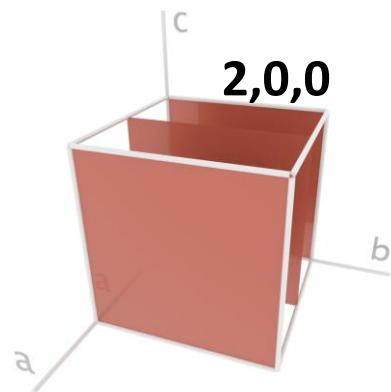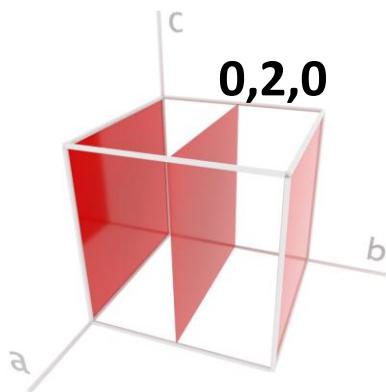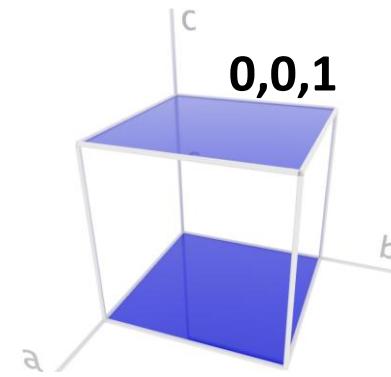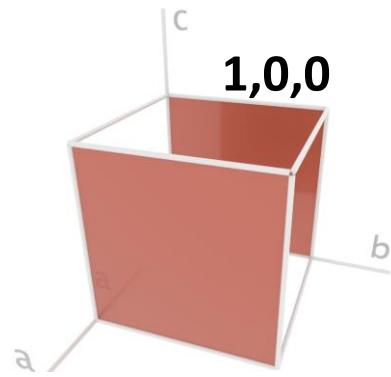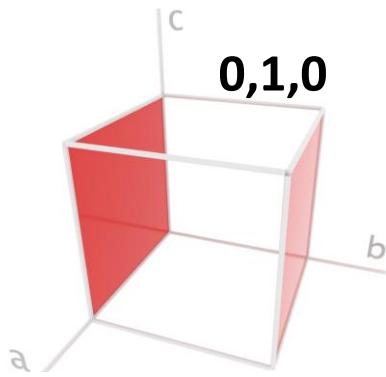
- In order for the waves to interfere constructively:
  - The differences in the travel path must be equal to integer multiples of the wavelength: **nλ = 2dSinθ** (Braggs law).
  - The diffracted beam of X-rays will leave the crystal at an angle equal to that of the incident beam.

- 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.

- From Bragg law:
  - Large diffracting angles θ correspond to small d values (higher resolution).
  - Small diffracting angles θ correspond to large d values (lower resolution).

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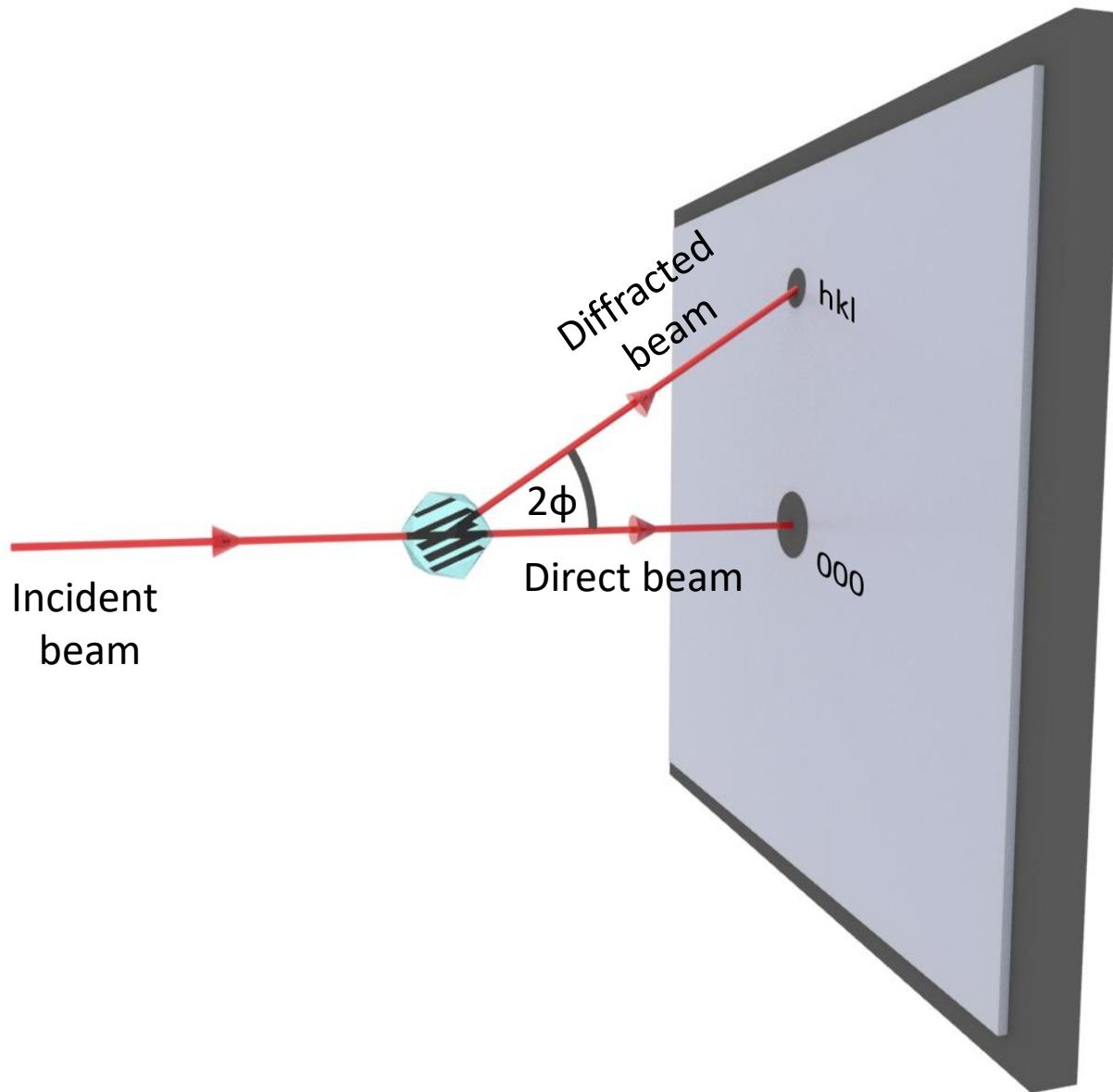
- We need to define the planes of atoms responsible for constructive interferences.

- 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
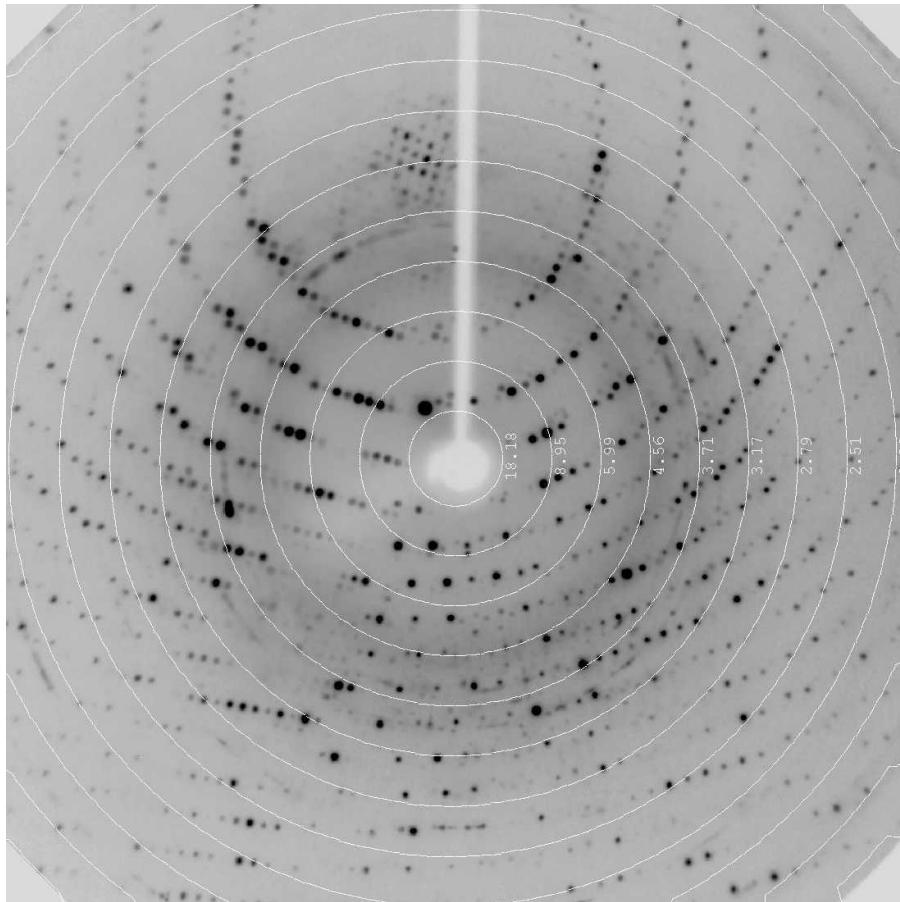
Miller indices
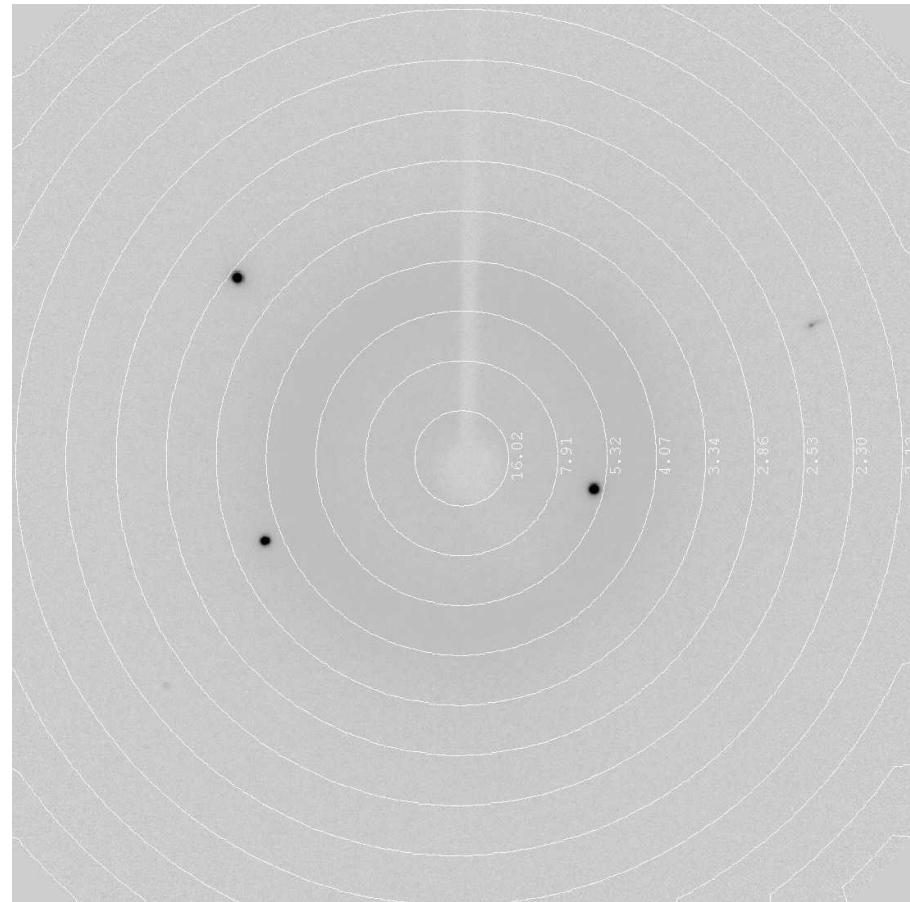
Miller indices

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

Diffraction spots are closer for large unit cells.
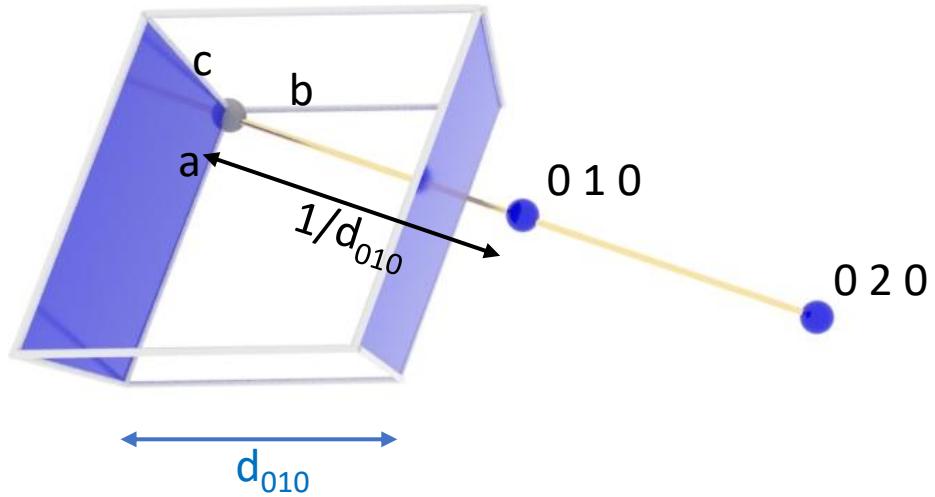
Diffraction spots are more spaced for small unit cell.

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)
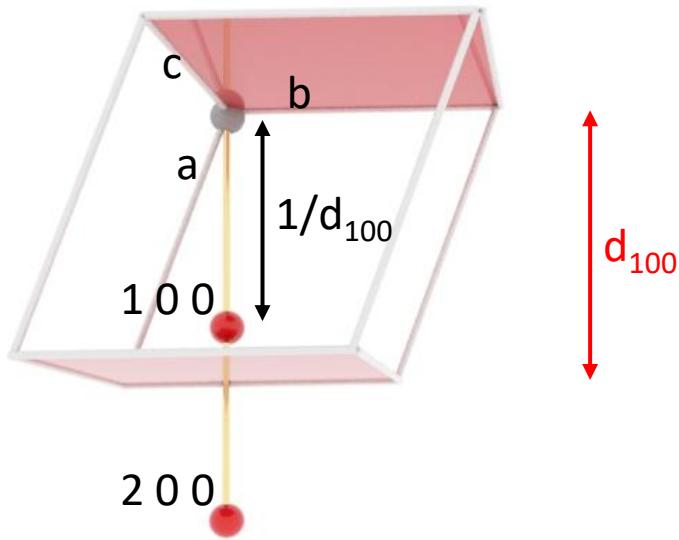
Reciprocal lattice

Planes: 0 1 0

$1/d_{010}$

0 1 0

0 2 0

d_{010}

Not to scale

Reciprocal lattice

Planes: 1 0 0

c

b

a

$1/d_{100}$

1 0 0

2 0 0

d_{100}

Not to scale

Reciprocal lattice

0 0 0

0 1 0

1 0 0

0 2 0

b*

1 1 0

2 0 0

1 2 0

2 1 0

a*

2 2 0
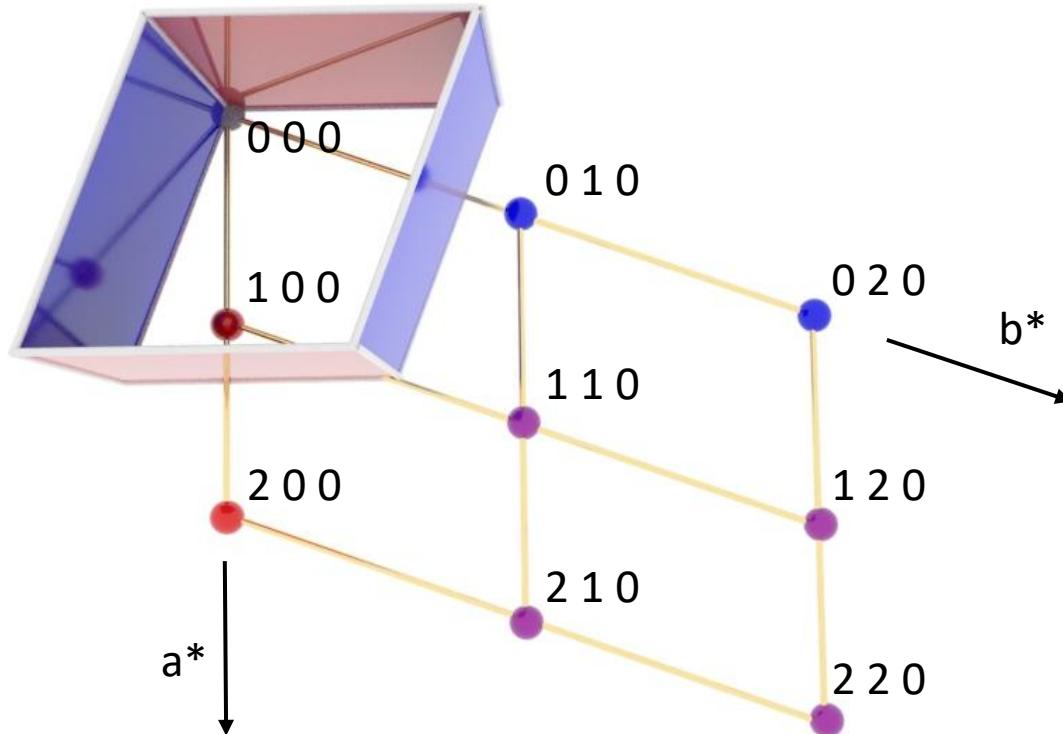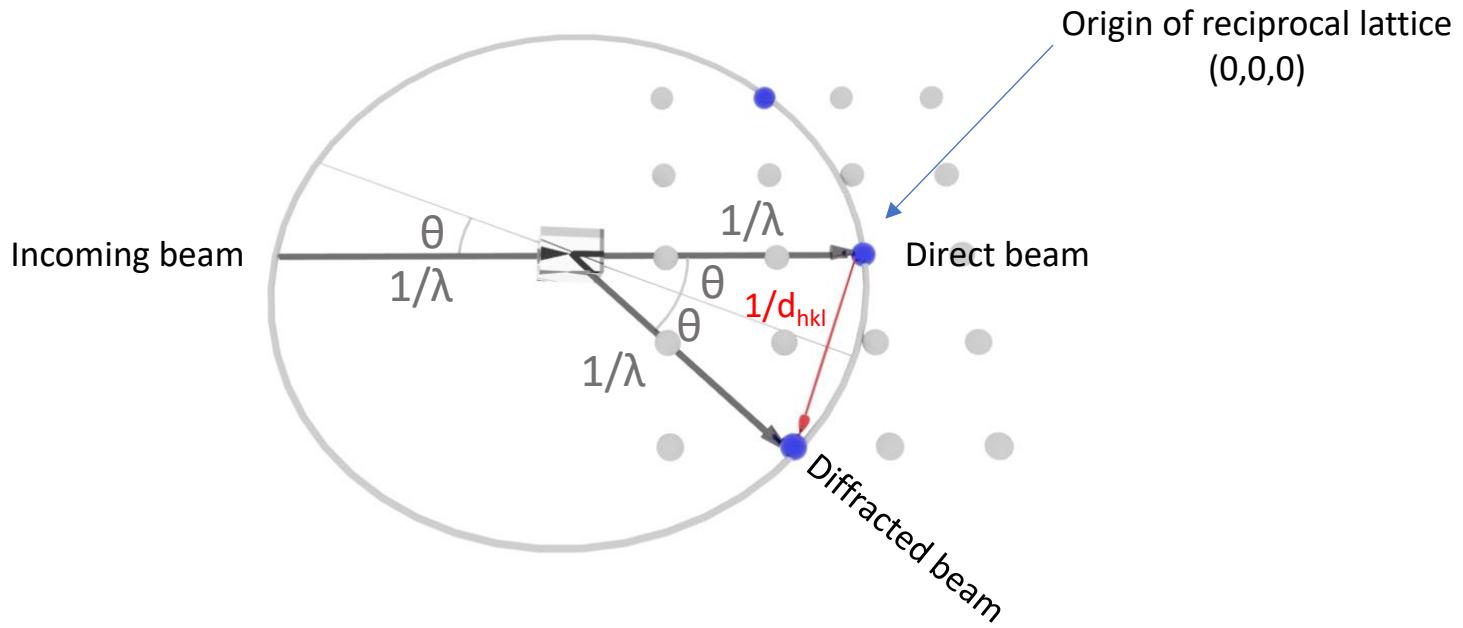
Not to scale

Ewald sphere

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

- We define:
    - A sphere of radius = 1/λ
    - 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.
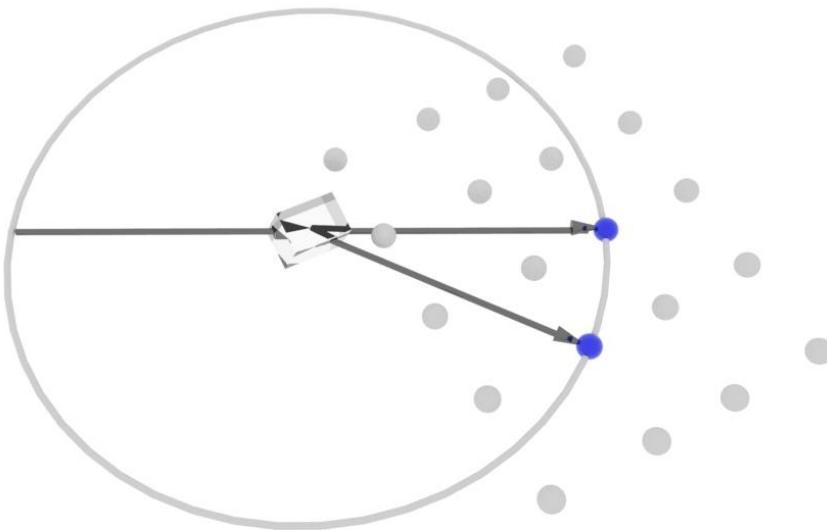
Ewald sphere

- 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 $1/d = (2/\lambda) \sin\Theta$ is fulfilled).
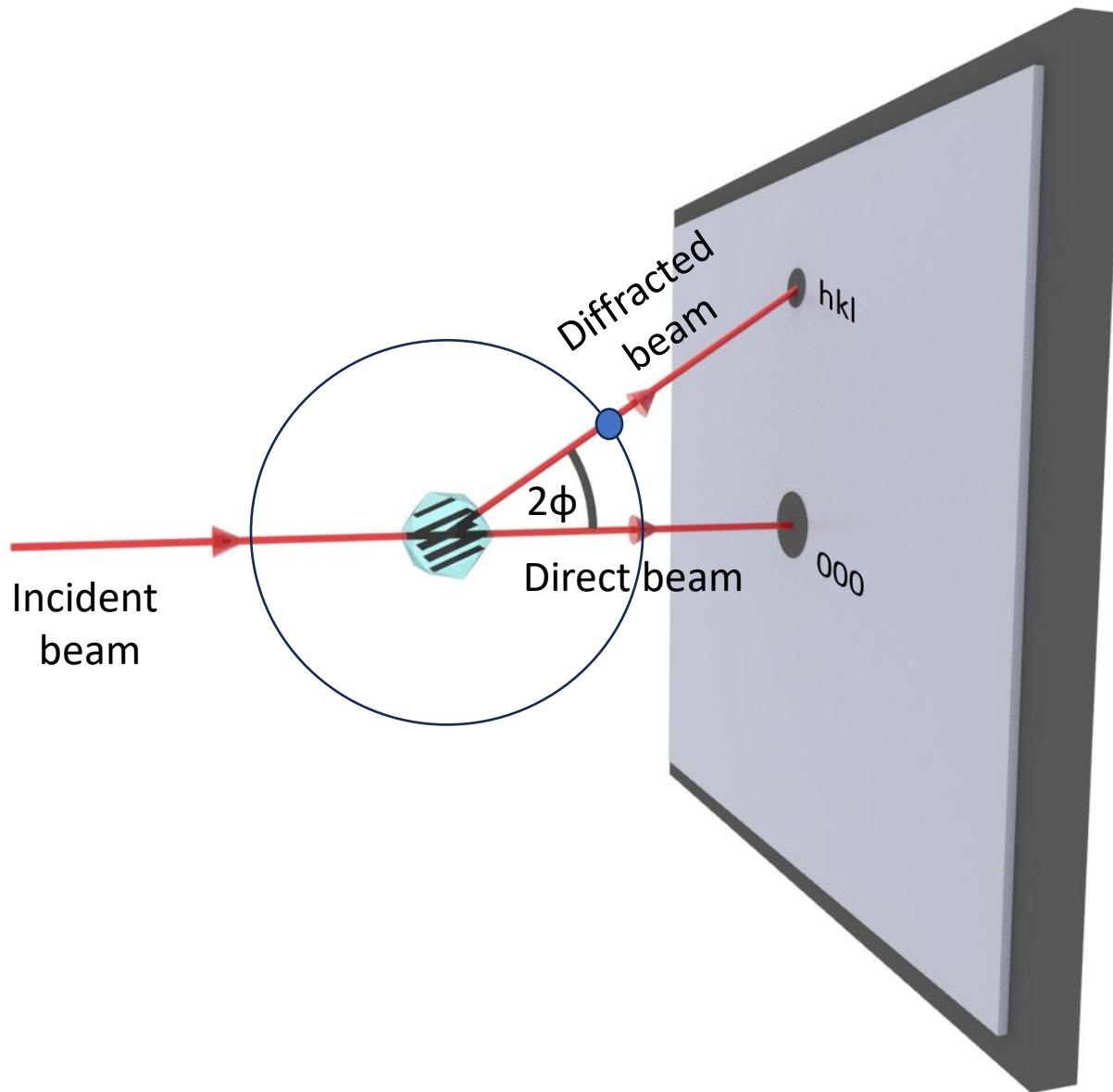

- Conversely, if the direct beam does not strike the planes with the correct angle θ, the reciprocal space vector will not be on the surface of the Ewald sphere.

Ewald sphere
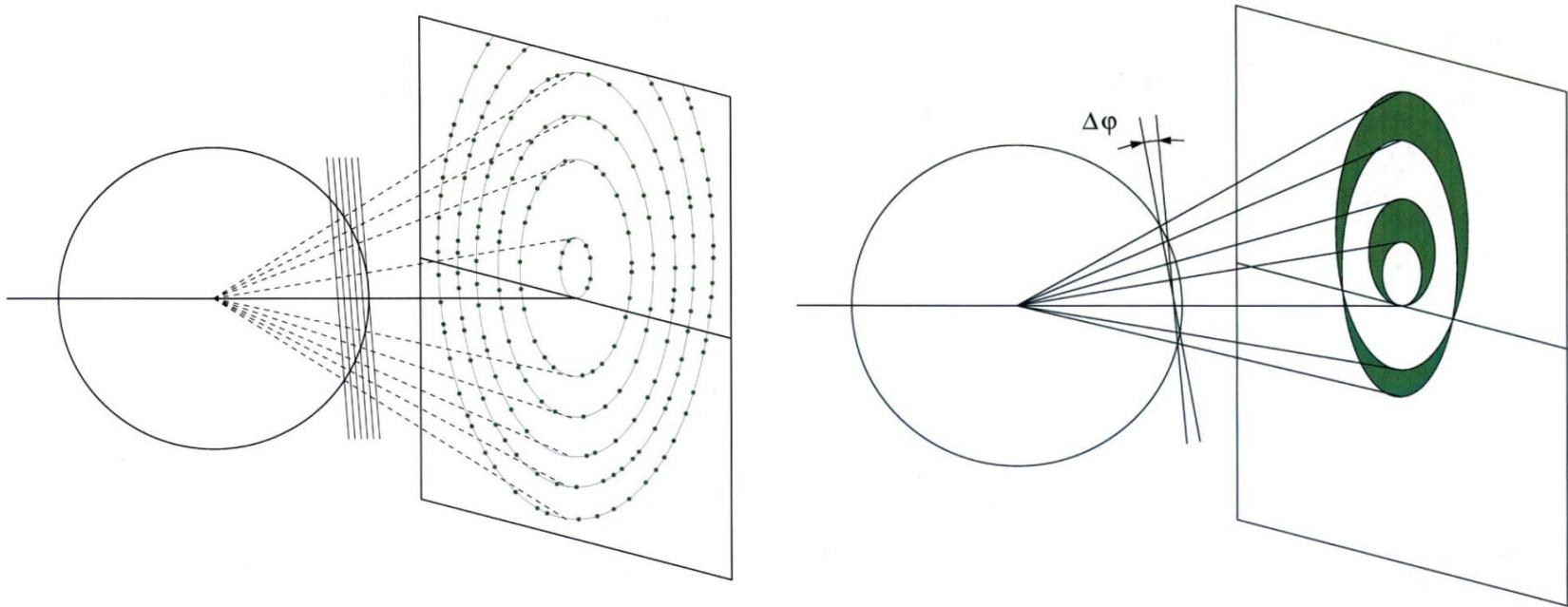
Ewald sphere

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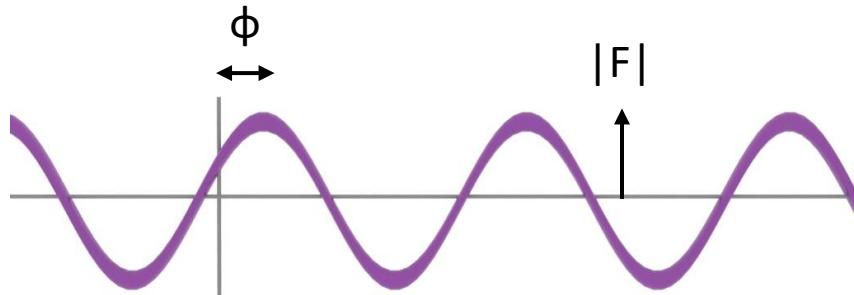
By observing the spacing and pattern of reflections on the diffraction pattern, we can determine the lengths and angles of the unit cells as well as some information on the space group.

So how do we find what is inside the unit cell?
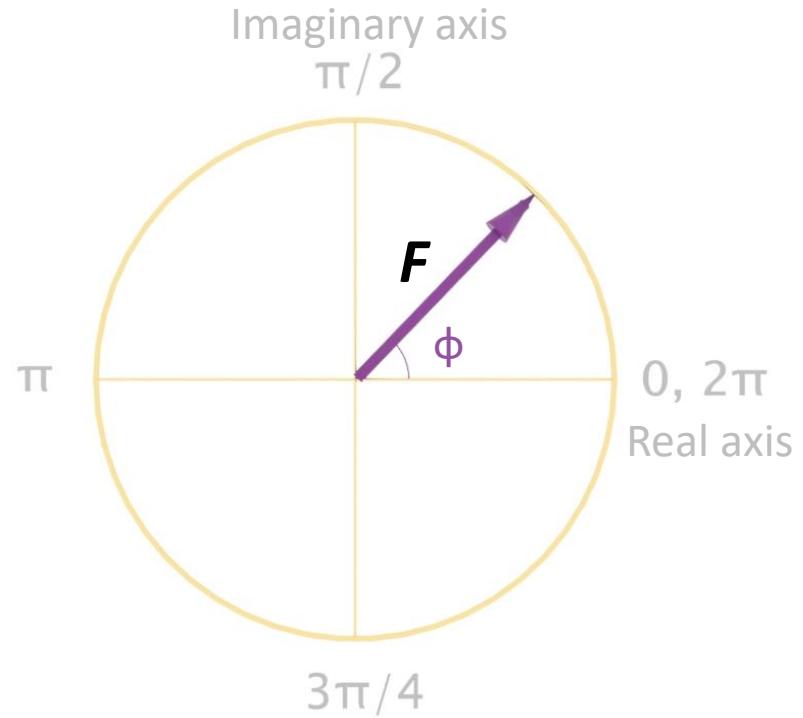
➢ Why do we need X-rays and crystals?

➢ What is diffraction?

➢ What are structure factors?

Argand diagram

- Y axis imaginary component and x axis: real component.
- Waves (F and φ) are represented by vectors.

$$F = |F|.(\cos(\phi t)+i.\sin(\phi t)) = |F|.e^{i\phi}$$
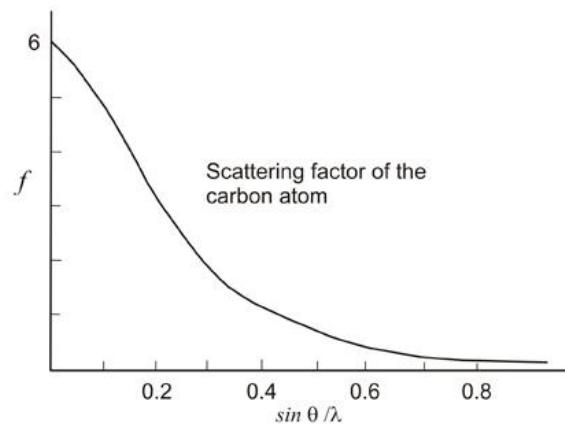
It's easier to add waves when they are represented as vectors.
What are the amplitude and phase for each atom?
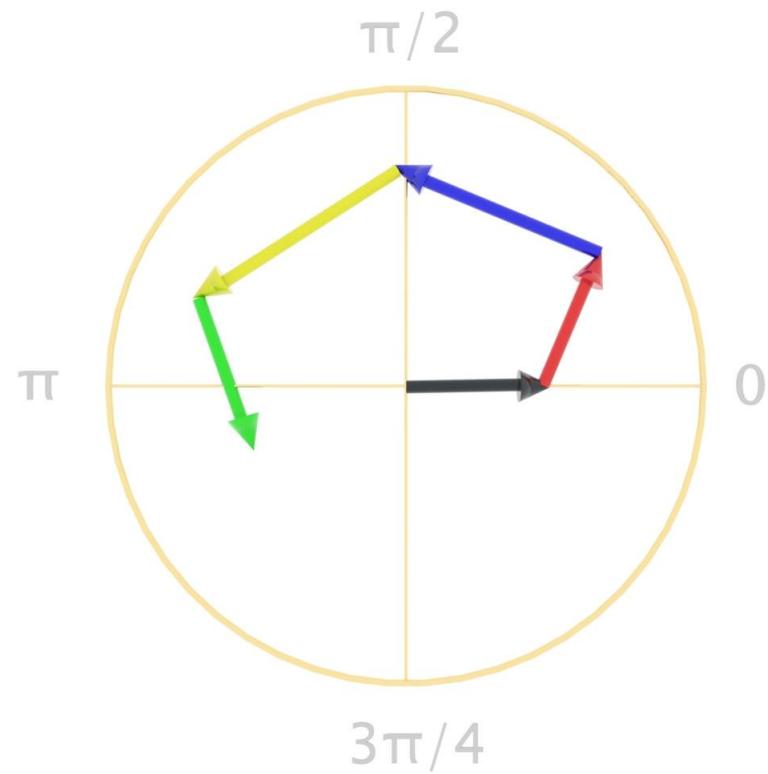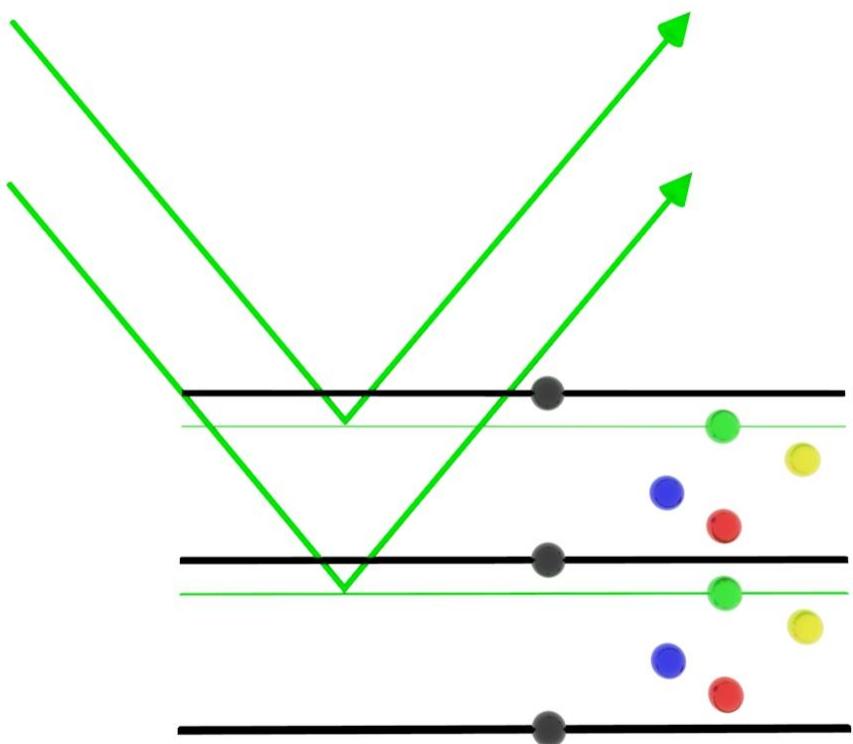
Atomic scattering factors

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 jth atom
- The atomic scattering factor is independent of the position of the atom in the unit cell.

Scattering factor of the carbon atom
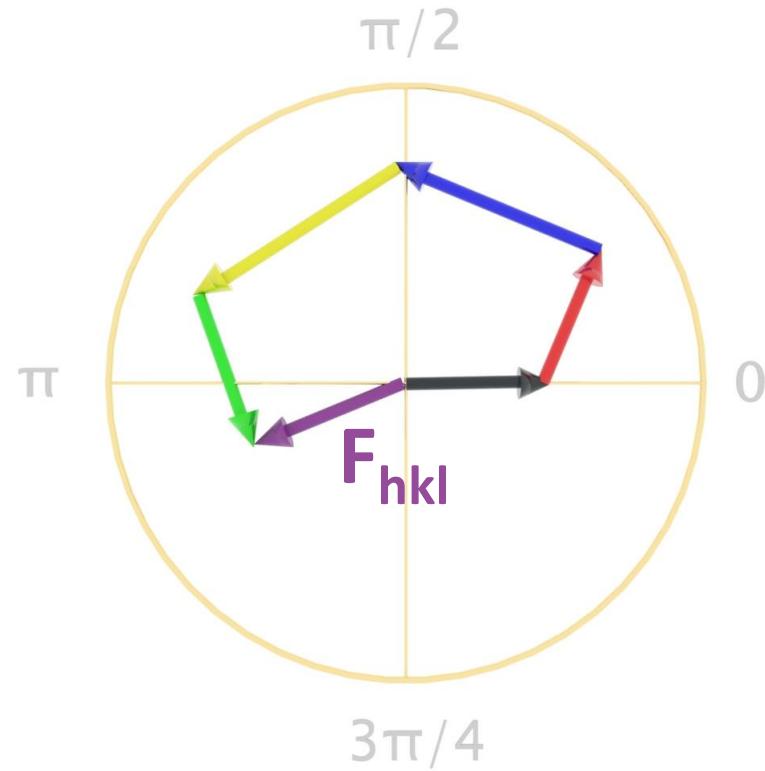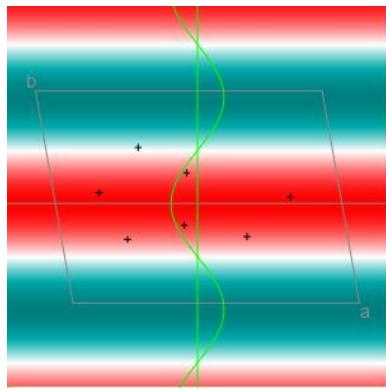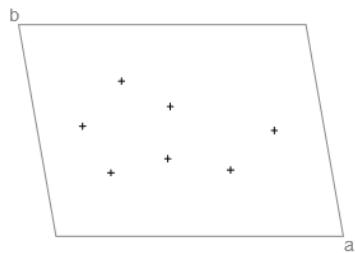
Structure factors

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 dependent 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.
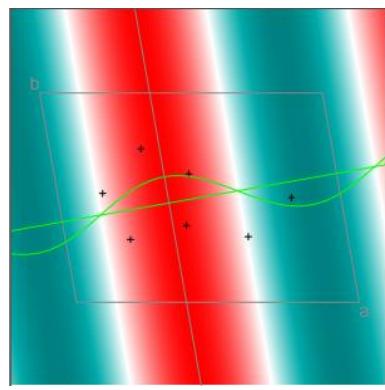
How structure factors relate to electron density?
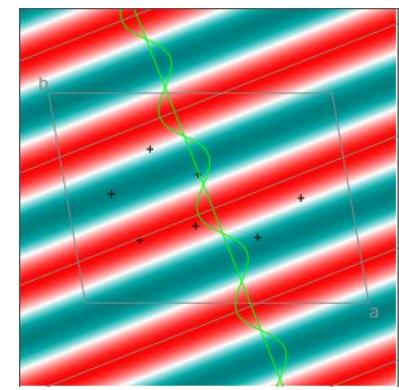
# Atoms in a unit cell, structure factors and electron density.
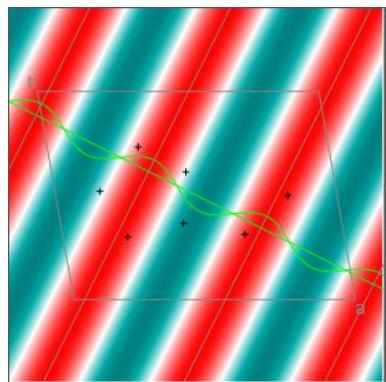
(0,1)
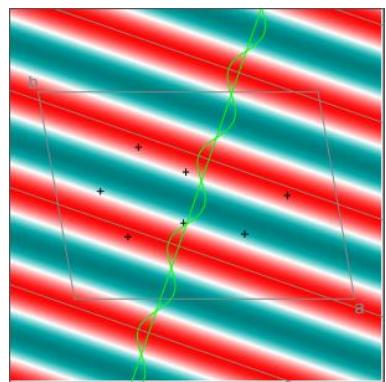
(1,0)
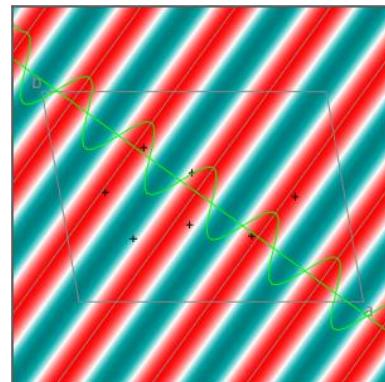
(-1,2)
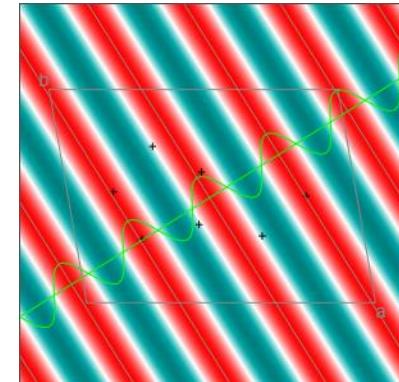
(-2,1)

(1,2)
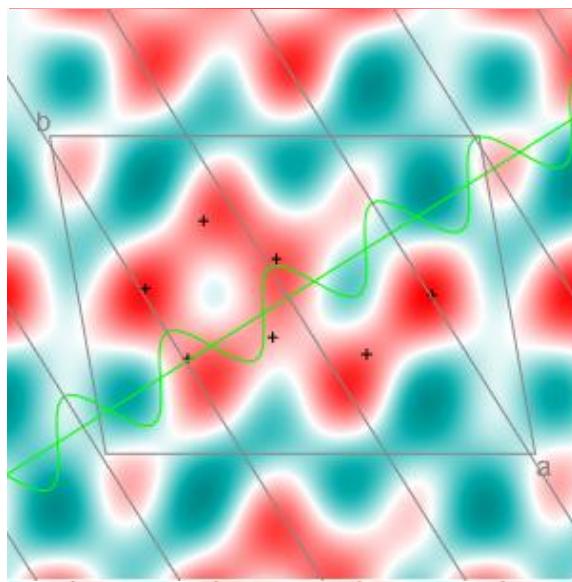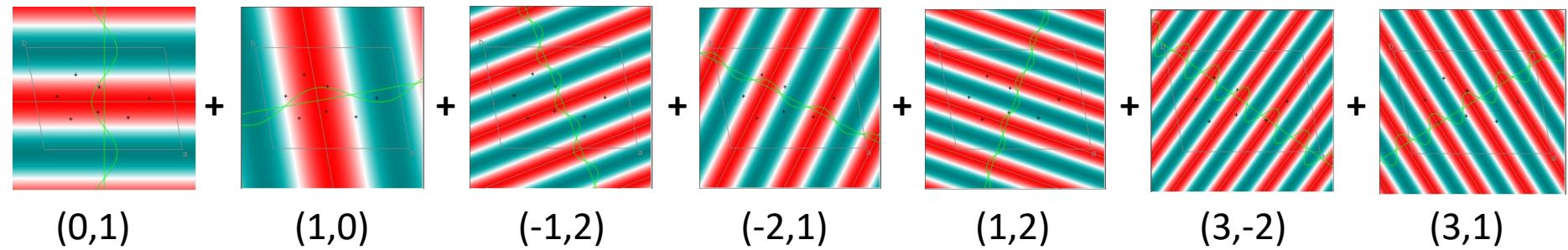
(3,-2)

(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

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

$$F(h,k,l) = V \int_{x=0}^{1} \int_{y=0}^{1} \int_{z=0}^{1} \rho(x,y,z). \exp\left[2\pi i(hx+ky+lz)\right] dx\,dy\,dz$$

$\underbrace{}_{\text{Wave}}$ $\underbrace{}_{\text{function}}$

FT

Electron density equation: Every F_{hkl} contribute to every atom in the unit cell.
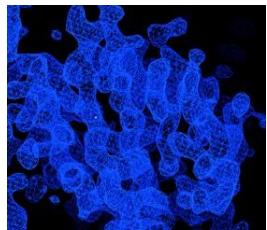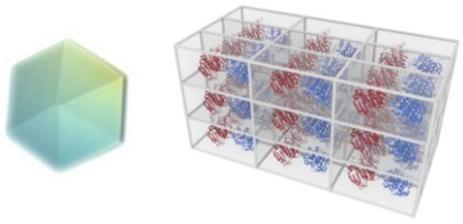
$$\rho(x,y,z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} |F(h,k,l)| \exp\left[-2\pi i(hx+ky+lz) + i\alpha(h,k,l)\right]$$

$\underbrace{}_{\text{function}}$ $\underbrace{\phantom{\frac{1}{V}\sum\sum\sum|F(h,k,l)|\exp[-2\pi i(hx+ky+lz)+i\alpha(h,k,l)]}}_{\text{Wave}}$

FT^{-1}

Fourier transform and synthesis

Real Space
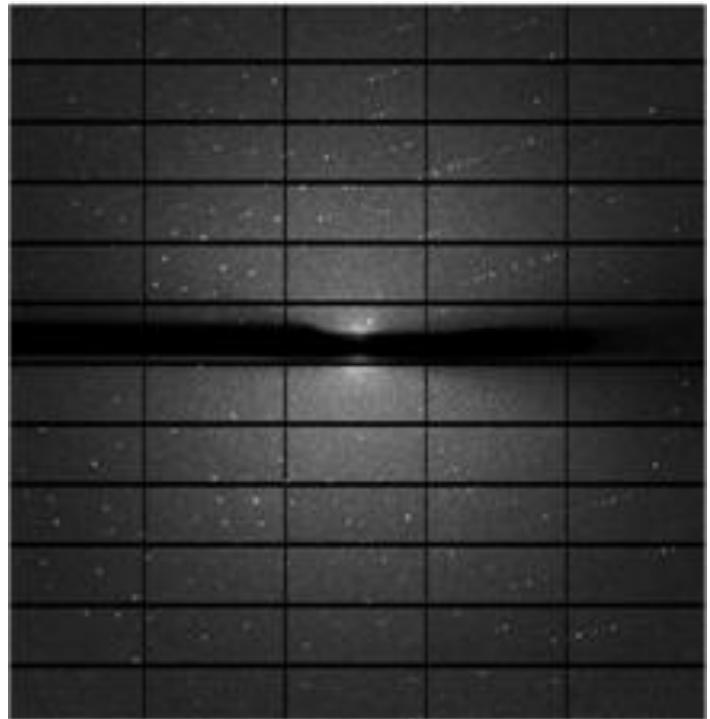
Reciprocal Space

Fourier
Transformation

*Inversion
theorem*

Fourier
Synthesis
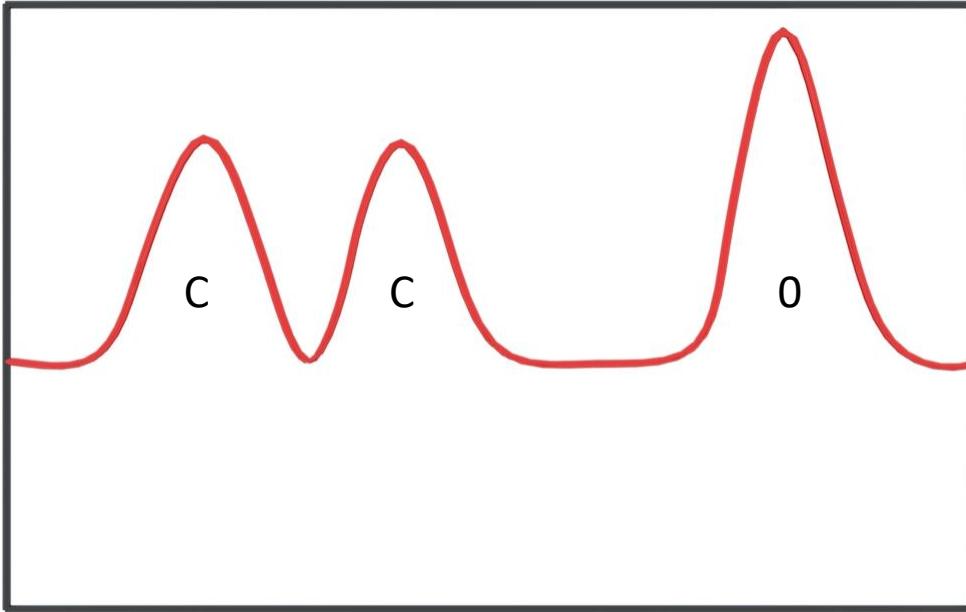
Electron density

Structure factors

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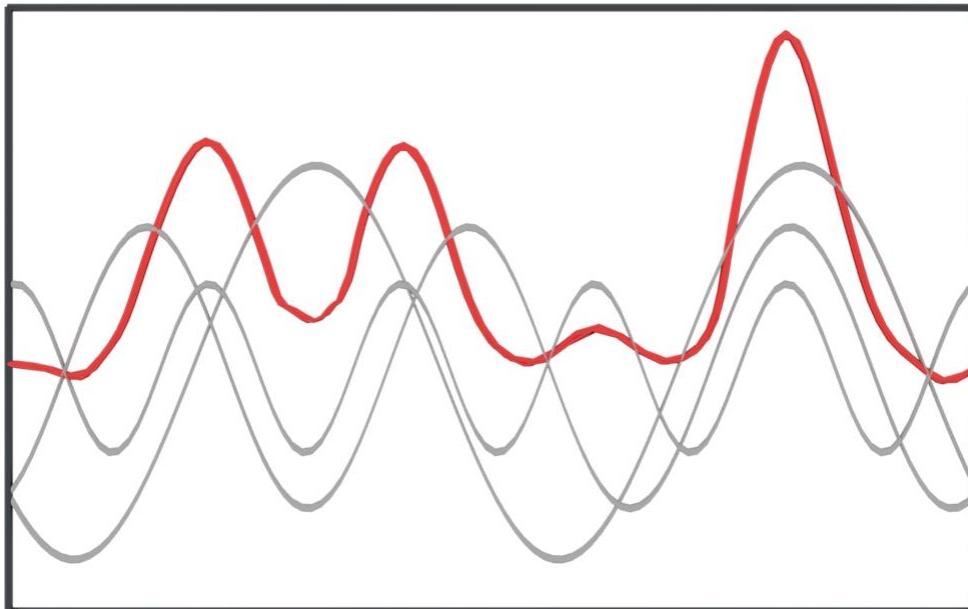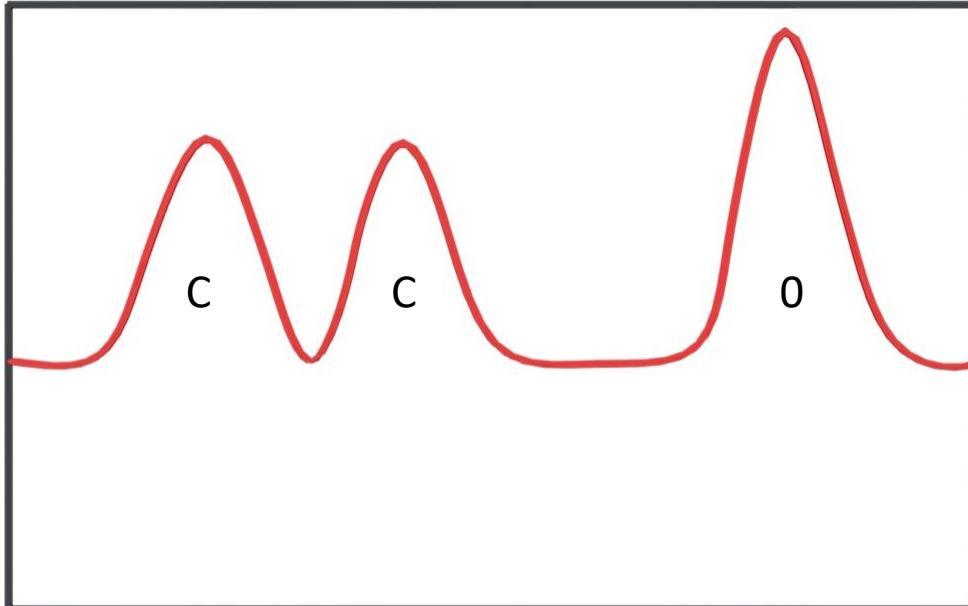
Fourier transform and synthesis
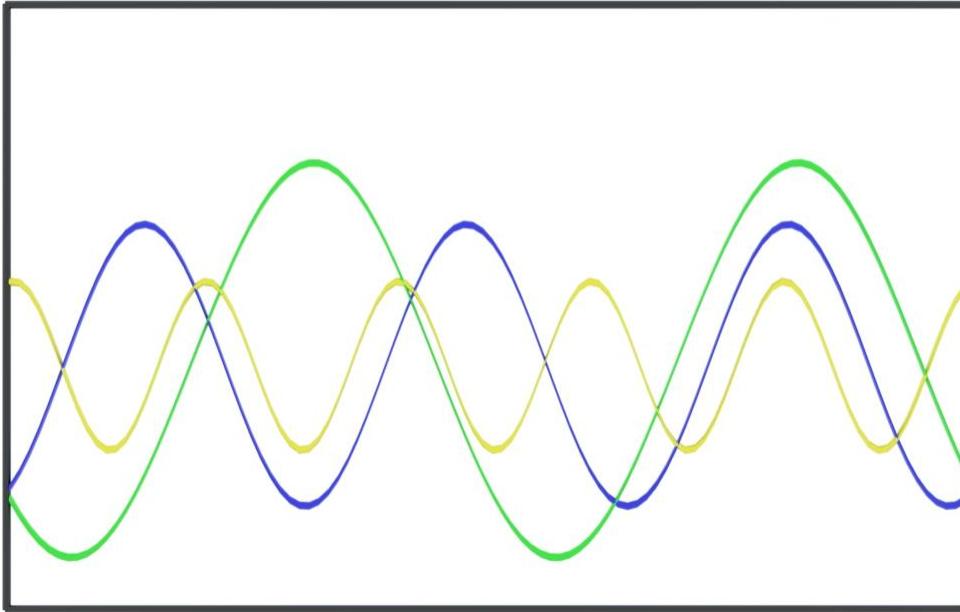
- Consider an imaginary one-dimensional crystal with 3 atoms in the unit cell. The electron density in the unit cell looks like above.

- We will represent this function in terms of sine waves with different frequency.
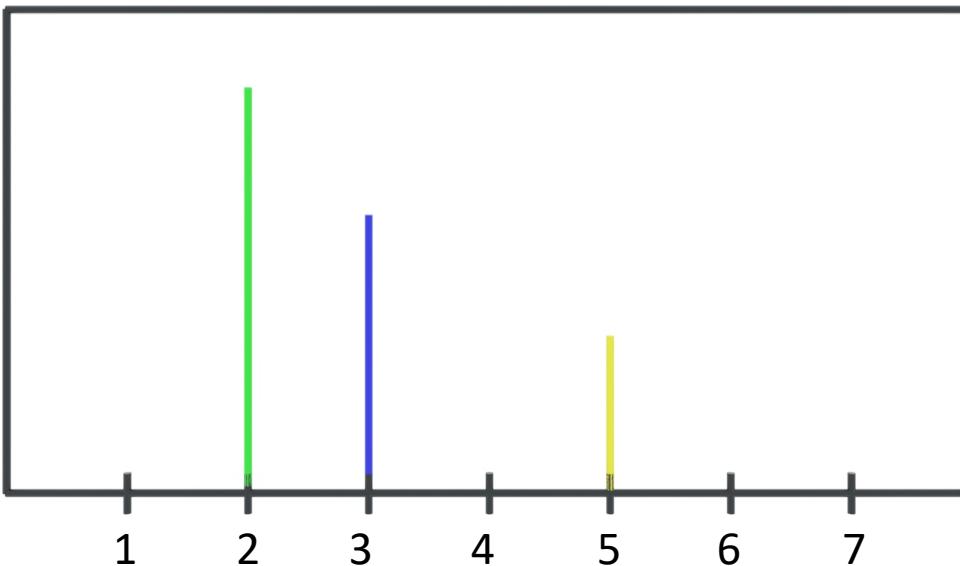
Fourier transform and synthesis

Fourier transform and synthesis

The complete set of components that are necessary to describe a periodic function is called a Fourier series.
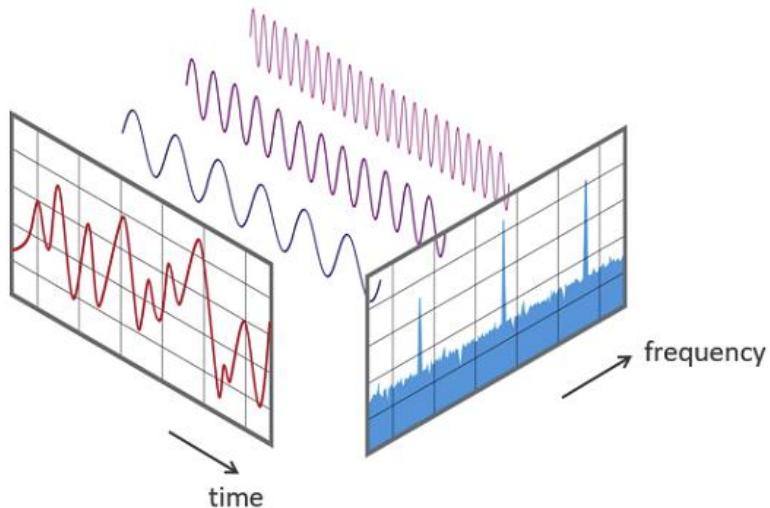
Freq = 2

Freq = 3

Freq = 5

Fourier transform and synthesis

https://www.quantamagazine.org/what-is-the-fourier-transform-20250903/

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

Thank you for you attention!

- Bragg's law, $n\lambda = 2d\sin(\theta)$.

- Here, n is the order of the reflection, and corresponds to the path length difference between X-rays diffracted from two different layers of atoms, in terms of the number of wavelengths. By convention, we treat all reflections as first order.

- So if the path lengths differ by exactly one wavelength, it is a first order reflection.

- if you consider a 2nd order reflection from (100) planes, the equation is: $2\lambda = 2d_{100}\sin(\theta)$.

- A 1st order reflection from (200) planes is: $\lambda = 2d_{200}\sin(\theta)$.

- But we know that $2d_{200} = d_{100}$, so these two conditions both correspond to the same diffraction angle in the material.

- So: $(\lambda = 2d_{200}\sin(\theta))$ = $(\lambda = d_{100}\sin(\theta))$ = $(2\lambda = 2d_{100}\sin(\theta))$.

Reciprocal lattice

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

Panel 1 (top-left):

h `1` k `2`
|F| `10` phi `10`

Set SF   Clear SF
Clear map   Reset map

b*
a*

l
R

|F| = 10   phi = 10

b

a

Panel 2 (top-right):

h `-1` k `2`
|F| `0` phi `0`

Set SF   Clear SF
Clear map   Reset map

b*
a*

l
R

|F| = 0   phi = 0

b

a

Panel 3 (bottom-left):

h `1` k `-2`
|F| `0` phi `0`

Set SF   Clear SF
Clear map   Reset map

b*
a*

l
R

|F| = 0   phi = 0

b

a

Panel 4 (bottom-right):

h `-1` k `-2`
|F| `0` phi `0`

Set SF   Clear SF
Clear map   Reset map

b*
a*

l
R

|F| = 0   phi = 0

b

a