Ecole d'Oléron 2018

A few physical concepts essential to structural biology

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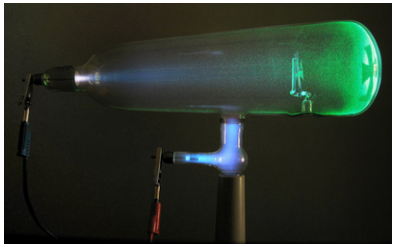
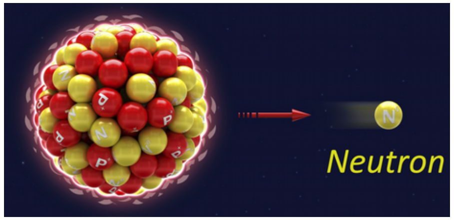
How can we obtain structural information?

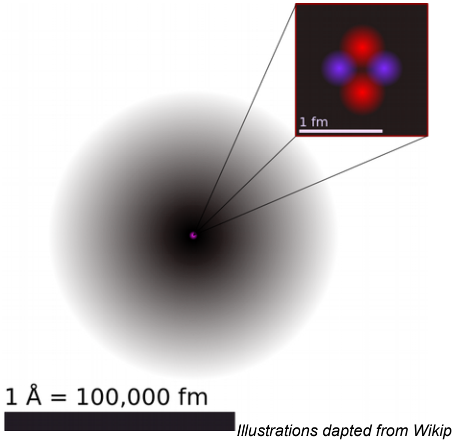
- A question of interaction between radiation and matter

- Radiations can be:
  - Photons (electro-magnetic wave: light, X-rays)
  - Electrons
  - Neutrons

- Matter
  - Your (macro)molecule under study
    - Atoms forming your molecule
      - H, C, N, O, S, ...



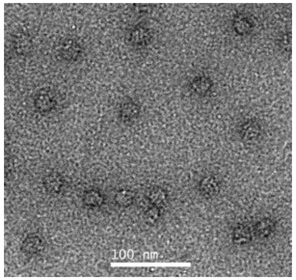


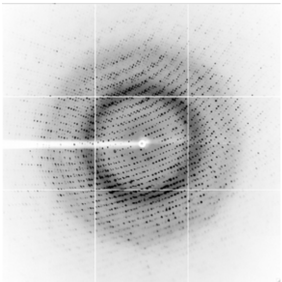


Illustrations dapted from Wikipedia

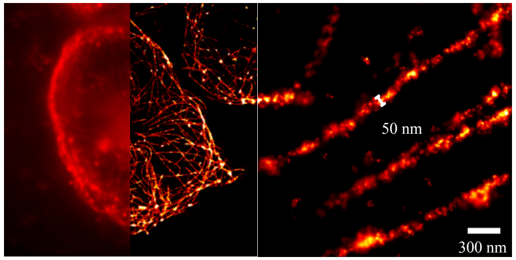
Possible approaches

- "Imaging" techniques
  - Visible light microscopy
  - Electron microscopy
  - X-ray, neutron or electron crystallography

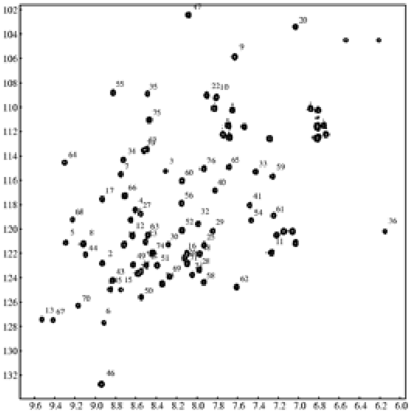


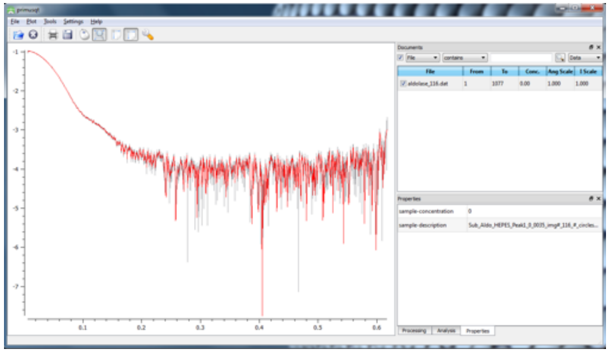


- Localization technique
  - Super-resolution microscopy



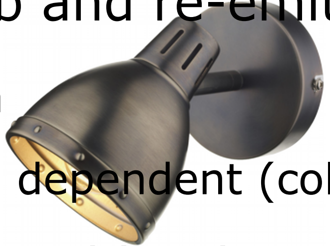
- Spectroscopic techniques
  - NMR
  - SAXS



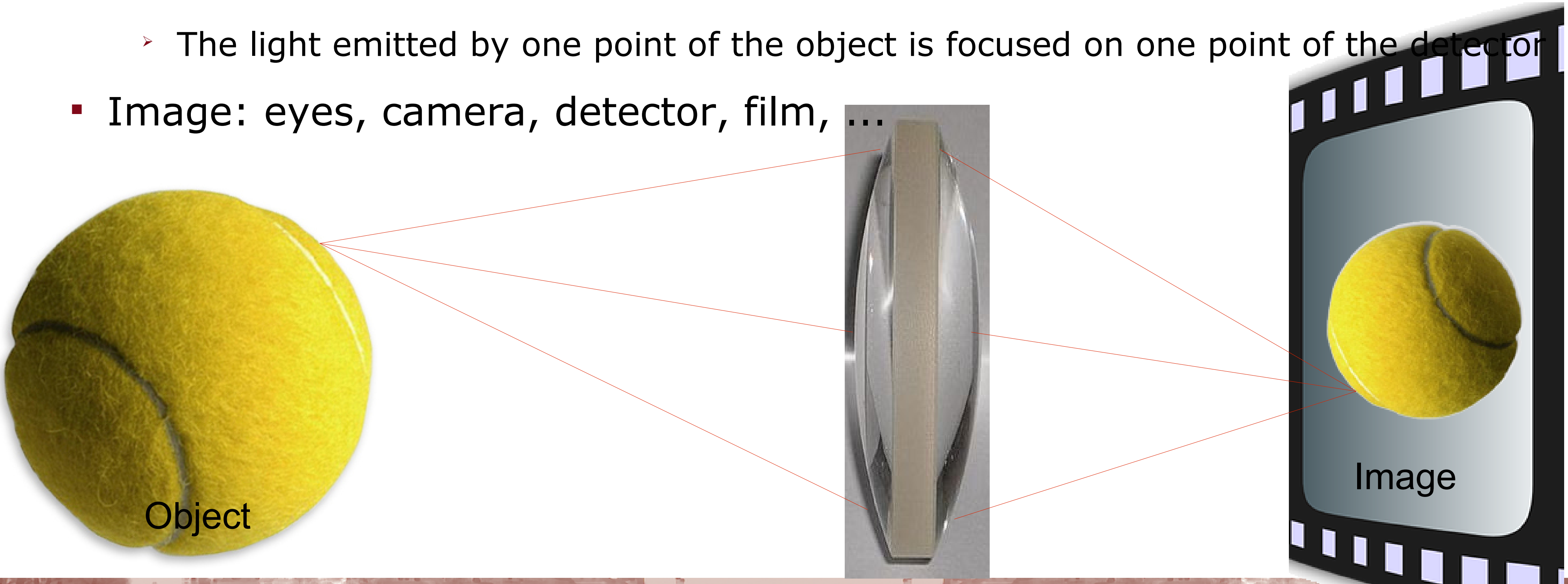


Visible light microscopy

- Source light (radiation): photons or electromagnetic wave
    - Wave length: 0.3 – 0.8 µm
- Object (matter): absorb and re-emit incident light in all directions
    - More or less absorption
    - some time wave length dependent (color)



- Lens: focuses light emitted by the object on the image plane
    - The light emitted by one point of the object is focused on one point of the detector
- Image: eyes, camera, detector, film, ...



Why can't we see molecule with visible light?

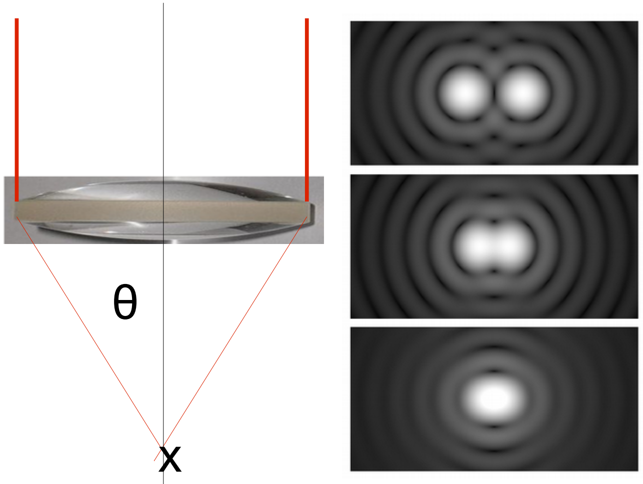
- Be because of the wave nature of light
  - Diffraction of light by a disk when the disk size of the same order of magnitude than the wave length:
    - The angle at which the 1st minimum occurs is given by:

$$\sin\theta \approx 1.22\frac{\lambda}{d} \quad \text{(far from the aperture, d diameter of the aperture)}$$

- Rayleigh criterion

$$d = 1.22\frac{\lambda}{2n\sin\theta} \approx \frac{\lambda}{2\sin\theta} \quad \text{(in air)}$$

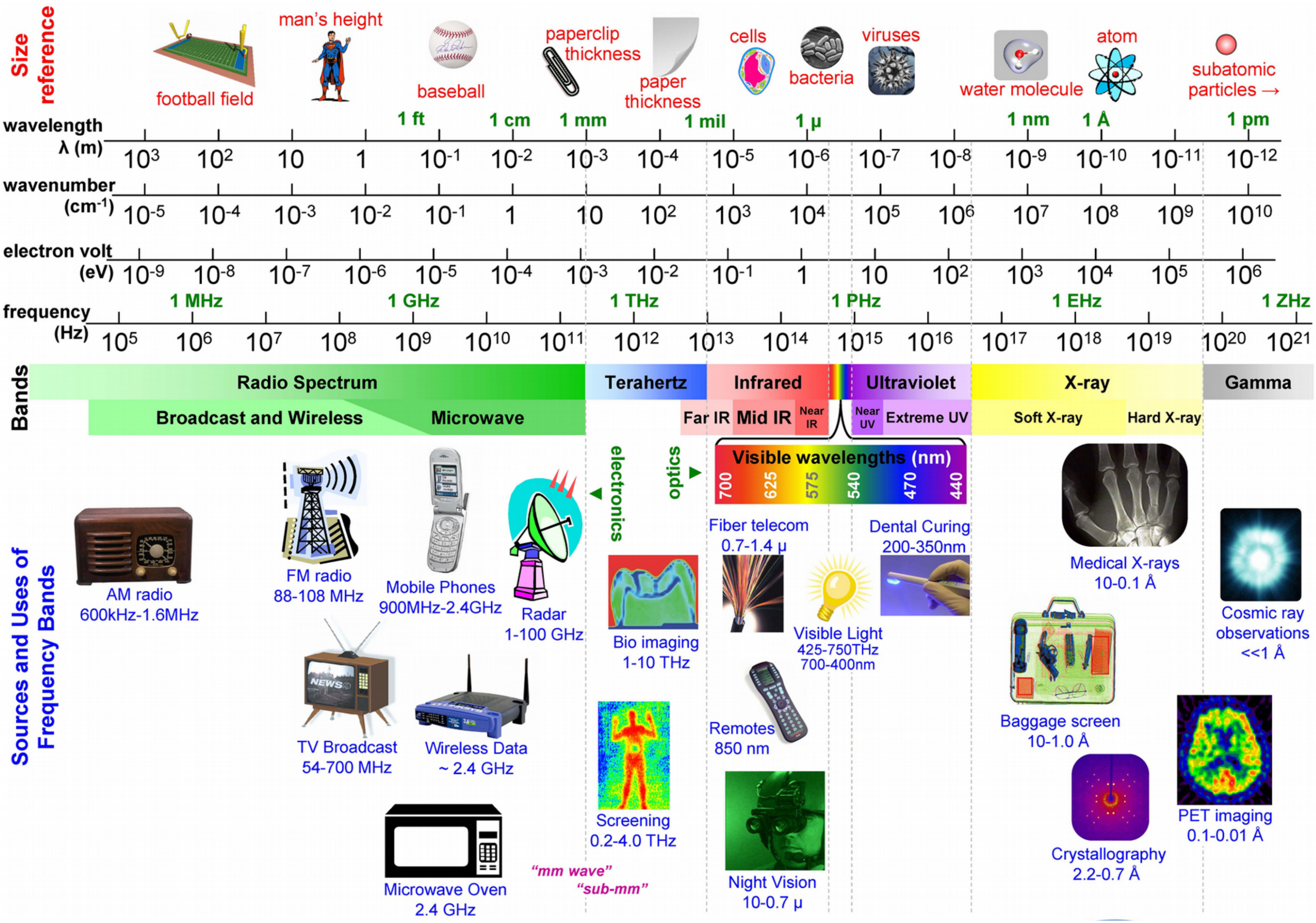
  - n.sinθ is the numerical aperture of the lens
    - Max ≈ 1.4 – 1.6
- Maximum resolution with visible light
    - ≈ 0.25 µm
    - Enough for cells
    - Not enough for molecules



Airy disk

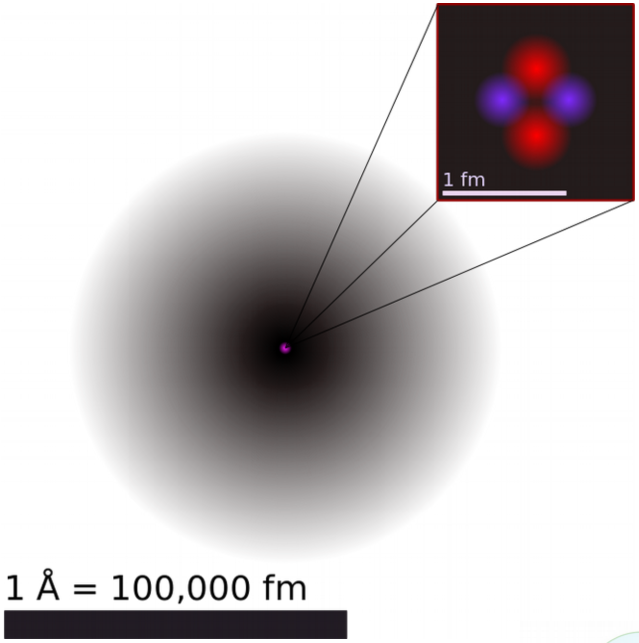
The best focussed spot of light made by a lens of circular aperture is limited by the diffraction light

How to increase resolution?

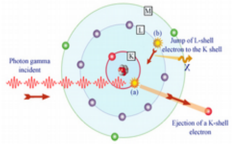


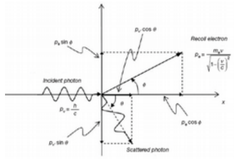
Atomic resolution with photons?

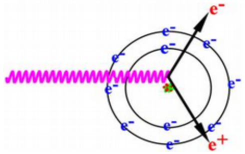
- Decrease the wavelength
  - For atomic resolution
    - d ~ 1 Å => $\lambda \leq 2$ Å
  - Use photons in the domain of X-rays
    - Typically: for $\lambda = 1$ Å, $E = h\nu = hc/\lambda \approx 12.4$ keV

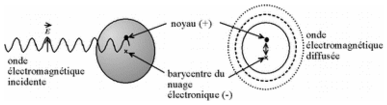


- Do X-rays interact with atoms?
  - Yes, X-ray photons with the electronic cloud of an atom
    - Photoelectric effect (absorption of a photon and emmision of an elctron)
    - Compton Scatering (inelastic scattering between a photon and an electron)
    - Electron/Positron pair production (only high energy photon > 1MeV)
    - Rayleigh scattering (Photon eleastic scattering by atomic electons)







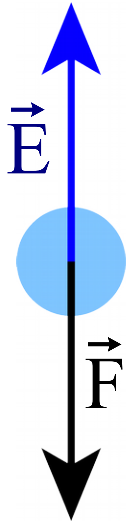


How a photon is scattered by an atom ?

- **Elastic scattering** (no loss of energy, wavelength is conserved)
  - **Rayleigh scattering:** bound atomic electrons
  - **Thomson scattering**: free electrons (photon energy >> electron binding energy)
    - Carbon atom E(1s) = -1013 eV, E(2s,2p) = -36 eV to be compared to 7 to 15 keV for X-ray photons
  - Photon energy should differ from element absorption edges
- The wave description of X-ray photons (electromagnetic wave) is fine to explain the phenomenon (classical model)
  - In an electric field $\vec{E}$ a charge e feels a force: $\vec{F} = e . \vec{E}$
    - Thus, the electric field of the electromagnetic wave will induce movement of nucleus and electrons
    - Due to the non-relativistic velocity of atomic electrons, the Lorentz force induced by the magnetic field of the electromagnetic wave $\vec{F} = e . \vec{v} \wedge \vec{B}$ can be neglected

Ocillating dipole

- In an electric field $\vec{E}$ a charge e feels a force: $\vec{F}=e.\vec{E}$
- The force will induce an acceleration of both the electron and the nucleus
  - $\vec{F}=m.\vec{\gamma} \Rightarrow \vec{\gamma}_e=-e.\frac{\vec{E}}{m_e}$ and $\vec{\gamma}_n=+Ze.\frac{\vec{E}}{(Zm_p+(A-Z)m_n)}$
    - Since m_p and $m_n >> m_e$ one can neglect the movement of the nucleus



- The dipole induced by the electric field is: $\vec{d}=Ze.\vec{r}$

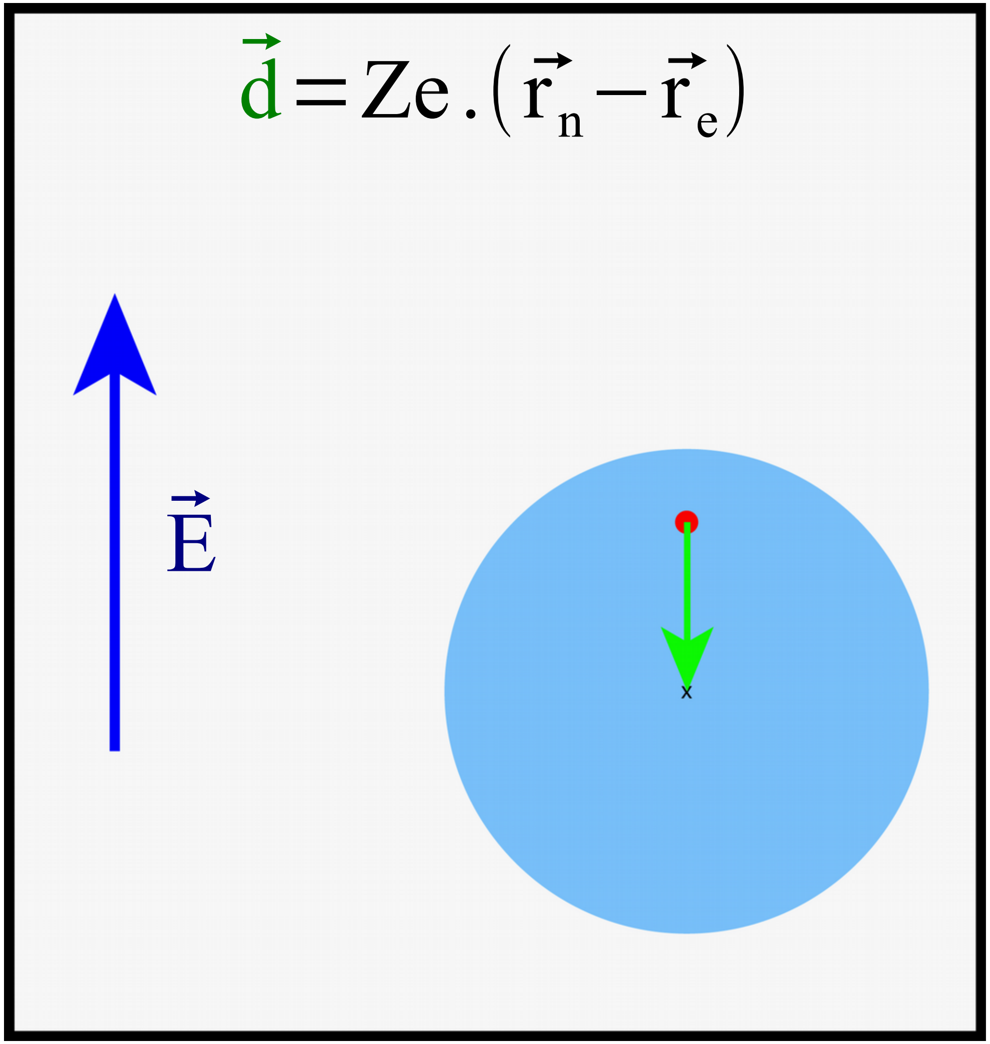
(with $\vec{r}$ vector between center of mass of electrons and nucleus)

$$\frac{\partial^2\vec{d}}{\partial t^2}=-Ze.\vec{\gamma}_e=Ze^2.\frac{\vec{E}}{m_e}$$

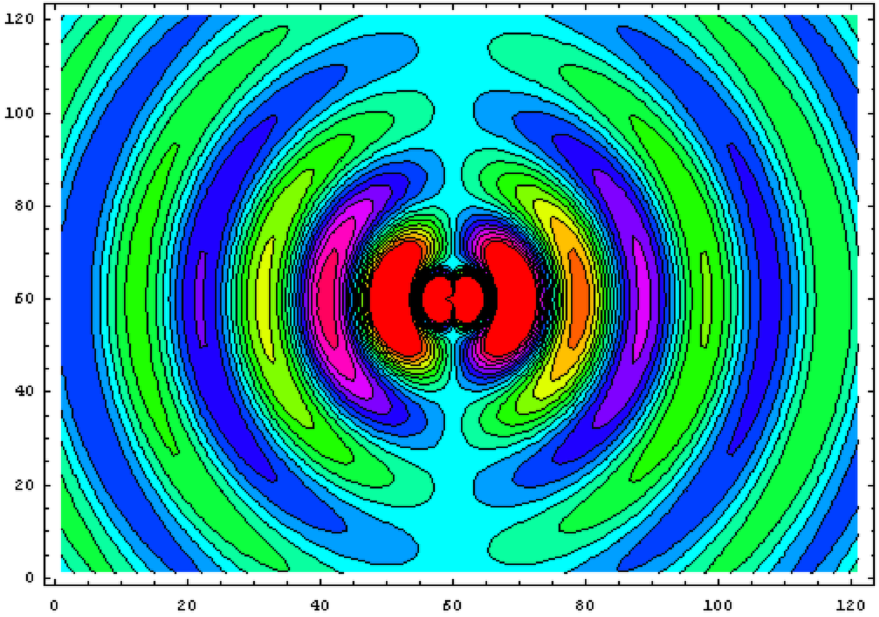
$$\underbrace{\vec{E}=\vec{E}_0\cos[\omega t]}_{\text{ocillating electric field}}\Rightarrow\underbrace{\vec{d}=-(Ze^2\frac{\vec{E}_0}{m_e\omega^2})\cos[\omega t]}_{\text{ocillating dipole}}$$

Oscillating dipole and emitted wave

The incident electromagnetic wave induce the oscillation of the electronic cloud



$$\vec{d} = Ze.(\vec{r}_n - \vec{r}_e)$$



The negatively charged electronic cloud and the positively charged nucleus become an **oscillating dipole**, thus emitting a spherical electromagnetic wave of **same wavelength** with a phase shift of π

Accessible information?

- Each atoms is emitting an electromagnetic wave (photon):
  - Amplitude proportional to Z (number of electrons)
  - Phase determined by the phase of the incident wave, at the position of the atom
- => In principle, access to the **electron density**

- What about other beams?
  - **Electrons**
  - **Neutrons**

Other particles to probe matter?

- Is photon (electromagnetic wave) the unique probe to see molecule?
- In 1924, Louis de Broglie proposed that all elementary particles can behave both as a wave and as a particle
  - Any particle can be used to probe matter if
    - the associated wave length $\lambda = \frac{h}{p}$ is appropriate
    - It interact with matter
  - What is the wave length of an elementary particle?
    - Photon (no mass): $E = h\nu = h\frac{c}{\lambda}$, $p = \frac{h\nu}{c}$
    - Particle (mass ≠ 0): $E = \frac{1}{2}mv^2$, $p = mv$, $\lambda = \frac{h}{mv}$

with $h\,(\text{Plank constant}) = 6.6257\,10^{-34}\,\text{J.s}$, $p:\text{momentum}$, $\nu:\text{frequency}$

Energies and wave length


Wilhelm Röntgen

- Electromagnetic waves / photons (1901, Röntgen)
  - Photon energy: $E = h\nu = h\frac{c}{\lambda}$ → $7\ \text{keV} < E < 17\ \text{keV}$ or $1.7\ \text{Å} > \lambda > 0.7\ \text{Å}$

- Electrons (1897, Thomson)
  - Negatively charged particle (q = 1.6 10^{-19} C, m_e = 9.1091 10^{-31} kg)

$$E = \frac{1}{2} m_e v^2 \ ,\ \lambda = \frac{h}{m_e v} \rightarrow \lambda = 1.2\ \text{Å for } v = 6000\ \text{km/s and } E = 100\ \text{eV}$$

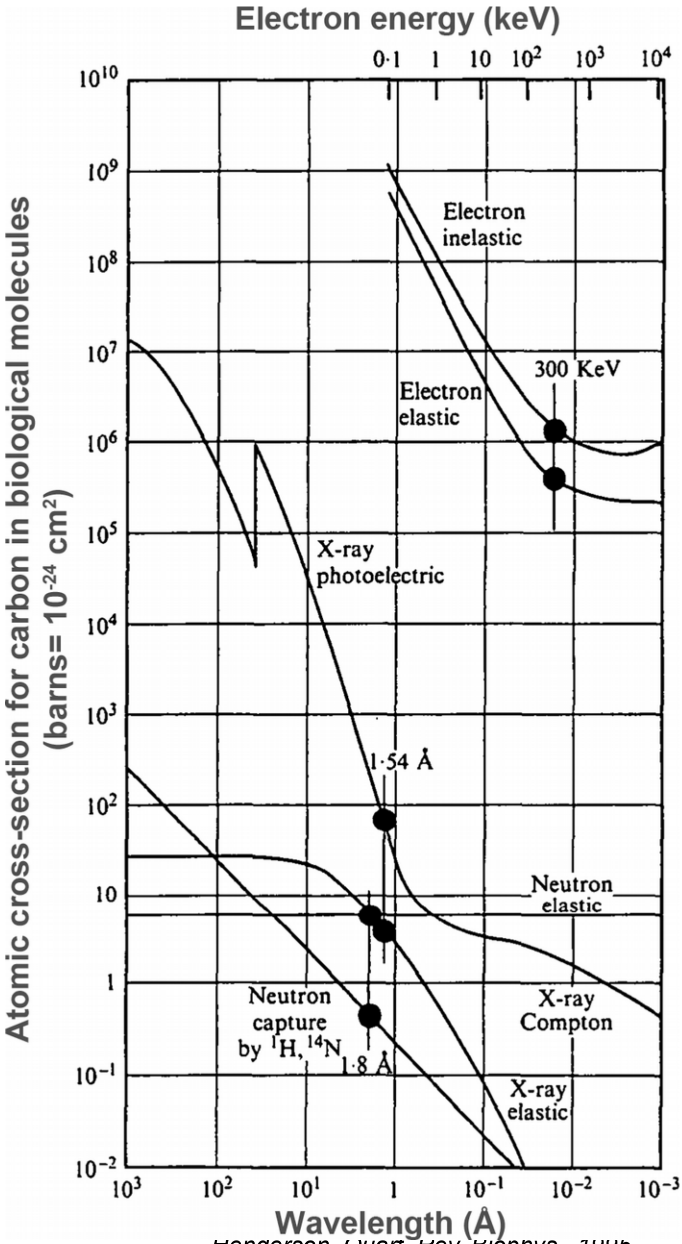
in practice $100\ \text{keV} < E < 300\ \text{keV}$ and $0.037\ \text{Å} > \lambda > 0.019\ \text{Å}$

- Neutrons (1932, Chadwick)
  - Neutral particle (m_n = 1.6749 10^{-27} kg)

$$E = \frac{1}{2} m_n v^2 \ ,\ \lambda = \frac{h}{m_n v} \rightarrow \lambda = 1.5\ \text{Å for } v = 2600\ \text{m/s and } E = 3.6\,10^{-2}\ \text{eV}$$

Electrons

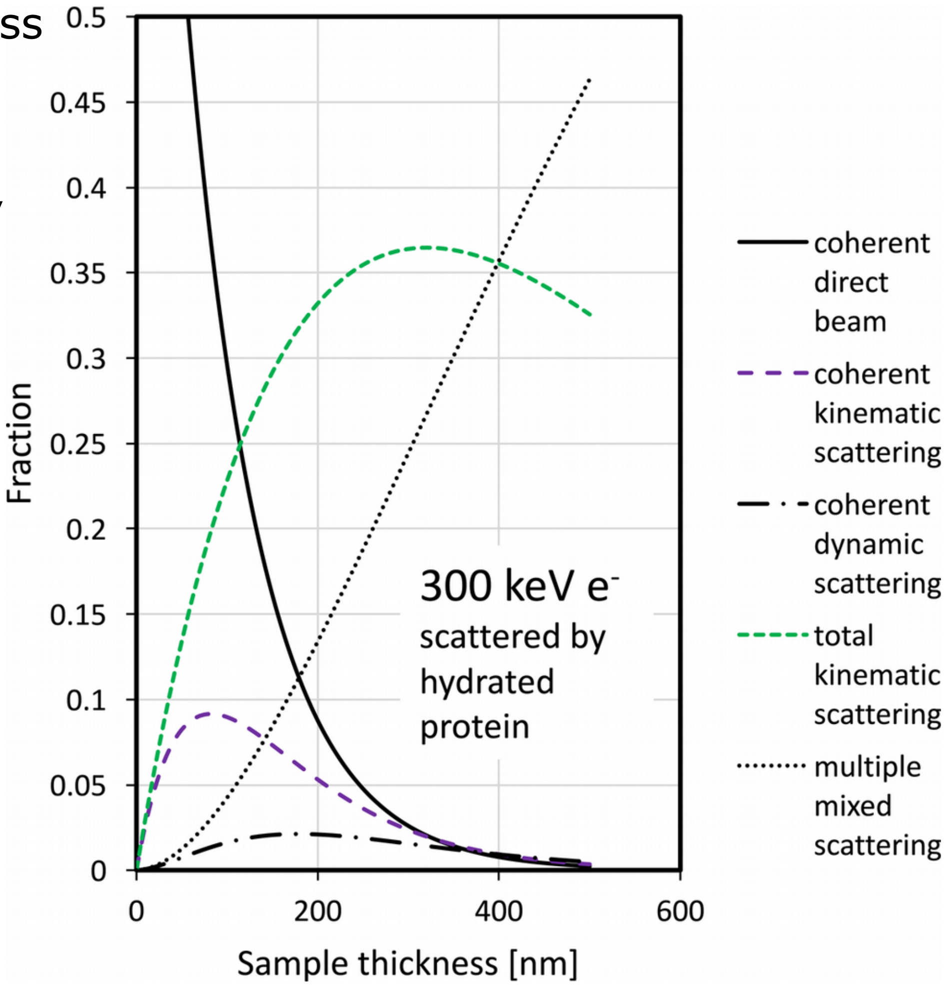
- Charged particle
  - “Sense” the electrostatic potential
- Strongly interact with matter
  - About 10^4 times more than X-rays
- Elastic scattering represents 25% of scattered electrons
  - Only 5% for X-rays



Henderson, Quart. Rev. Biophys., 1995

Electrons

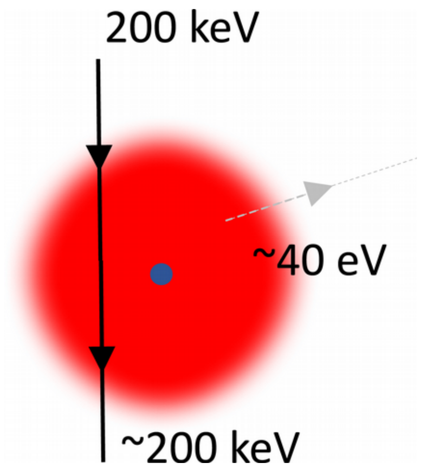
- Optimal thickness of sample
  - All electrons are absorbed if sample thickness exceed the µm
  - For X-rays, about 98% of the photons go through a 100 µm thick sample without any interaction

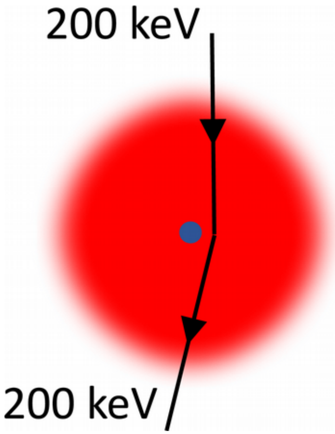


Illustrations taken from J.P. Abrahams

Electrons

- Strongly interact with matter
  - Elastic interaction with:
    - Atomic electron (small energy transfer)
      - No change in trajectory
    - Nucleus (Rutherford or Coulomb scattering)
      - Main contribution to elastic scattering
  - Inelastic interaction
    - Bremsstrahlung (higher enrgies)
    - Absorption (lower energies)

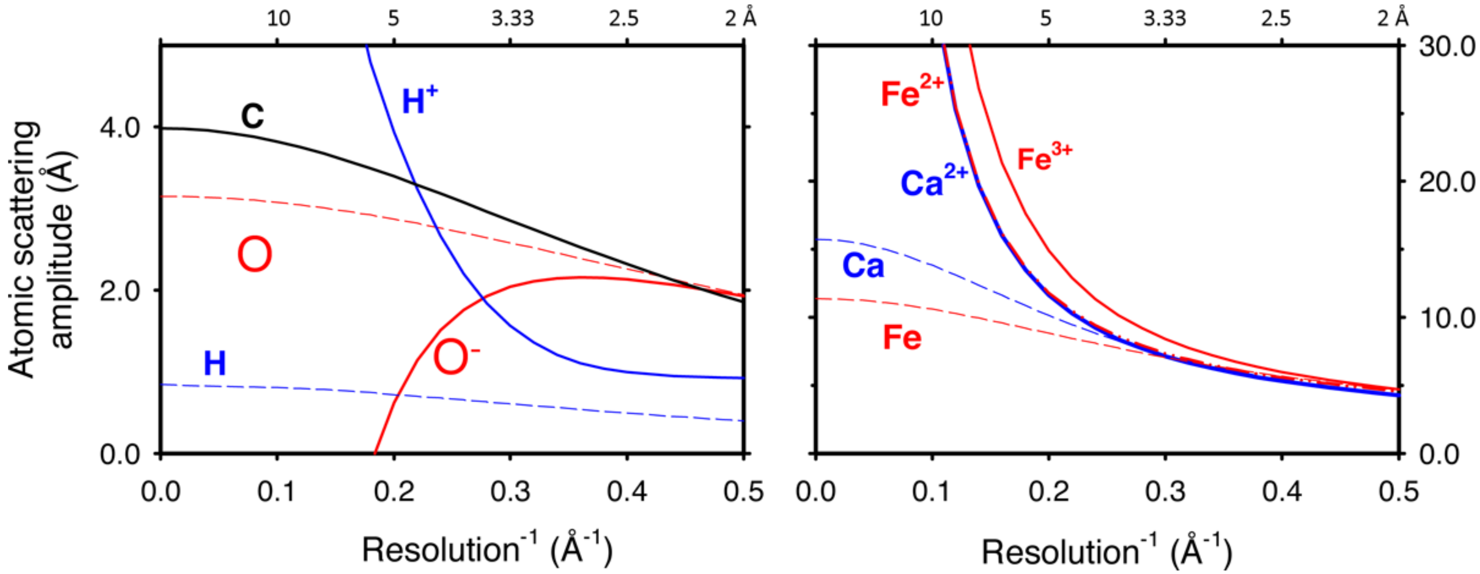




Illustrations taken from J.P. Abrahams

Electrons

- Atomic scattering factor for electrons depends on Z
  - Scattering probability ~ $Z^{4/3}$ (for X-rays, scattering probability ~Z^2)
- Also strongly depends on the atomic charge



- => access to a combination of electron density and charge information

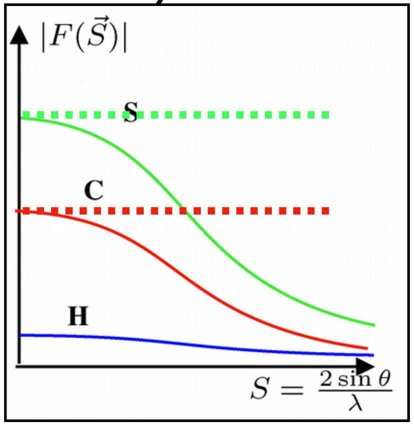
Illustrations taken from K. Yonekura

Neutrons

- No electromagnetic interaction
  - Penetrate matter easily
- Different type of neutrons
    - Cold neutrons: E<0.0038 eV
    - Thermal neutrons: 0.0038 eV < E < 0.5 eV => used for diffraction and SANS experiment
    - Epithermal or resonance neutrons: 0.5 eV<E<100 keV
    - Fast neutrons: 100 keV<E<10MeV
    - Relativistic neutrons: E>10MeV
  - Elastic interaction with nucleus: E < 1MeV
  - Inelastic scattering by nucleus: E > 1MeV
  - Induced fission

Neutrons: differences and similarities with X-rays

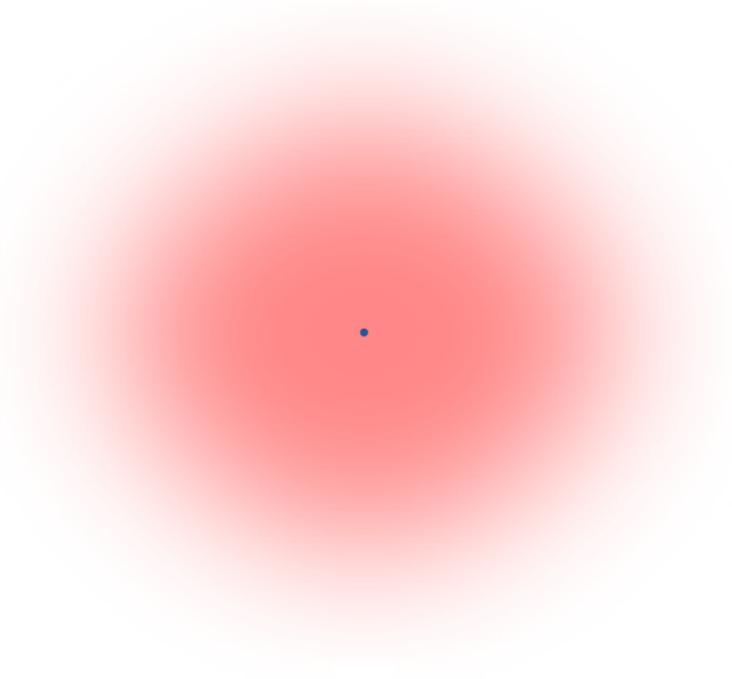
- Neutrons interact with nucleus
  - We observe nucleus and not electron density
    - Proton can be observed
  - Scattering cross-section comparable to that of X-rays
  - Variable scattering cross section depending on the type of atom
- Nuclei are very small (10^{-15}m) compare to the wave length (10^{-10} m)
  - Quasi a point
    - Impact on the atomic form factor
      - Spherical electron density $f_{at}(\vec{s})=f_{at}(|\vec{s}|)=2\int_{r=0}^{\infty}\rho(r)\sin\frac{(2\pi sr)}{s}dr$
    - $f_{at}(0)=Z$
    - For neutron $f_{nuc}(s)=\sigma_{scat}$
    - No decrease with s or resolution



- => access to spatial distribution of nuclei

Different point of views

X-ray photons

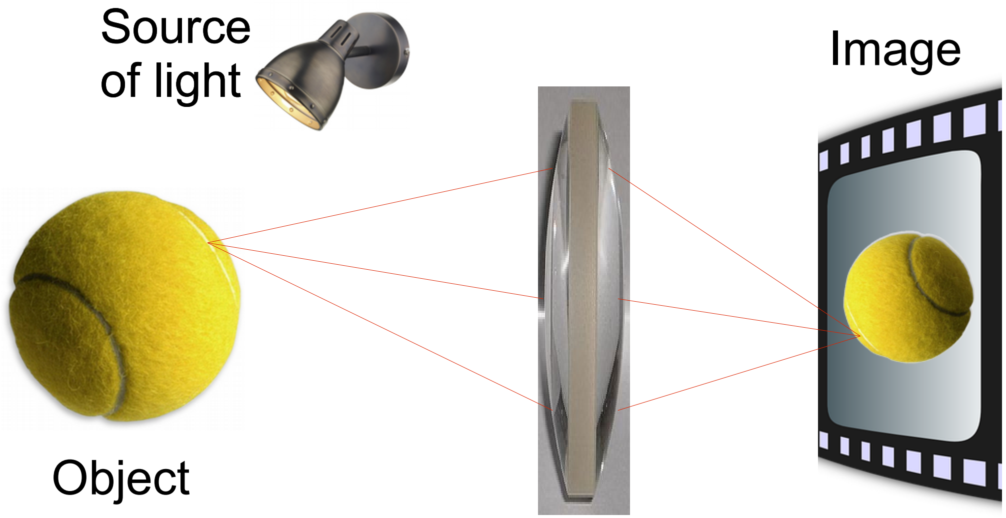


Electrons

Neutrons

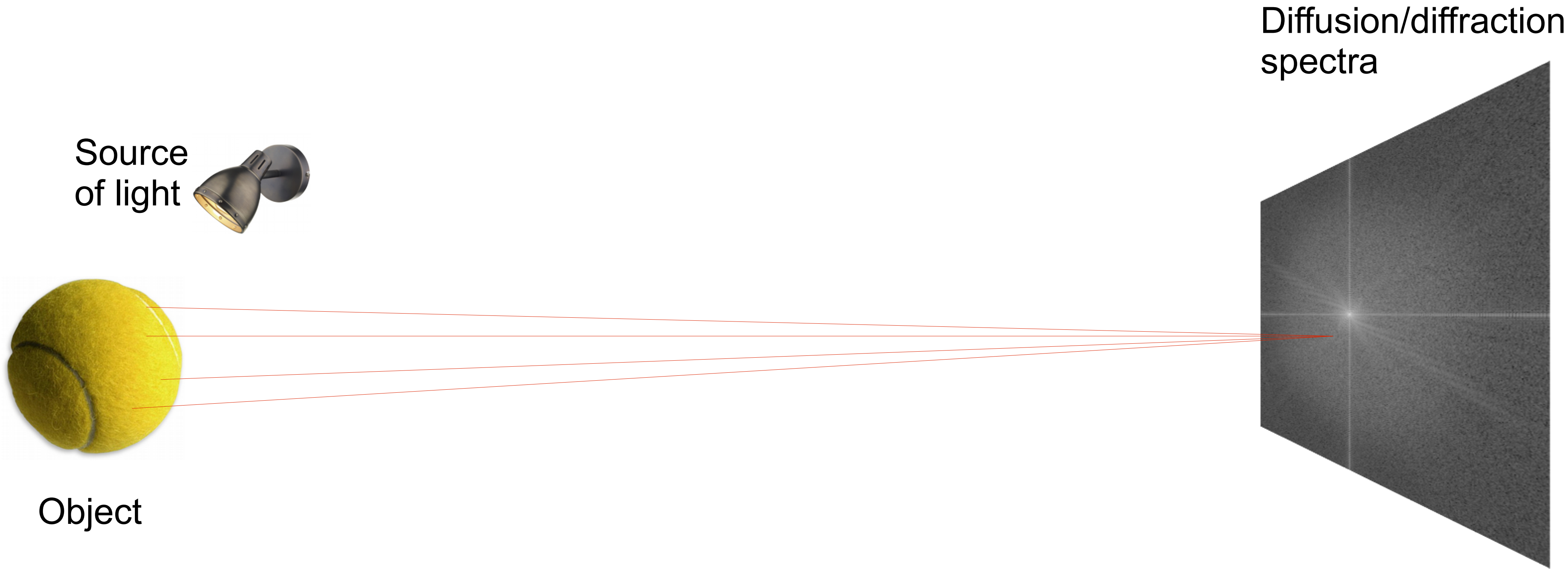
Why can't we get directly the image of the molecule with X-rays?

- Problem: we have **no lens for X-ray** photons
  - No image on the detector, but a scattering spectra
- With a lens
  - All radiation arriving on one point of the detector come from one point on the object



Scheme for a scattering spectrum

- Without a lens
  - Radiation arriving on one point of the detector come from all points on the object
    - It the sum of waves emitted by each point of the object



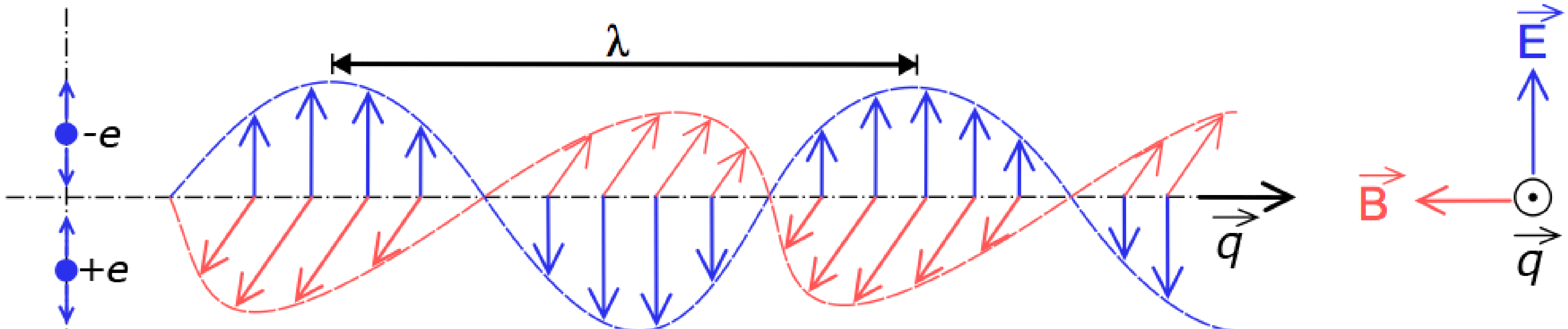
Diffusion/diffraction spectra

Source of light

Object

What can we do with a scattering spectra?

- What is the link between the scattering spectra and the molecule?
- Can we still get a image of the molecule?
- Mathematical representation of an electromagnetic wave?
  - Wave generated by an oscillating dipole

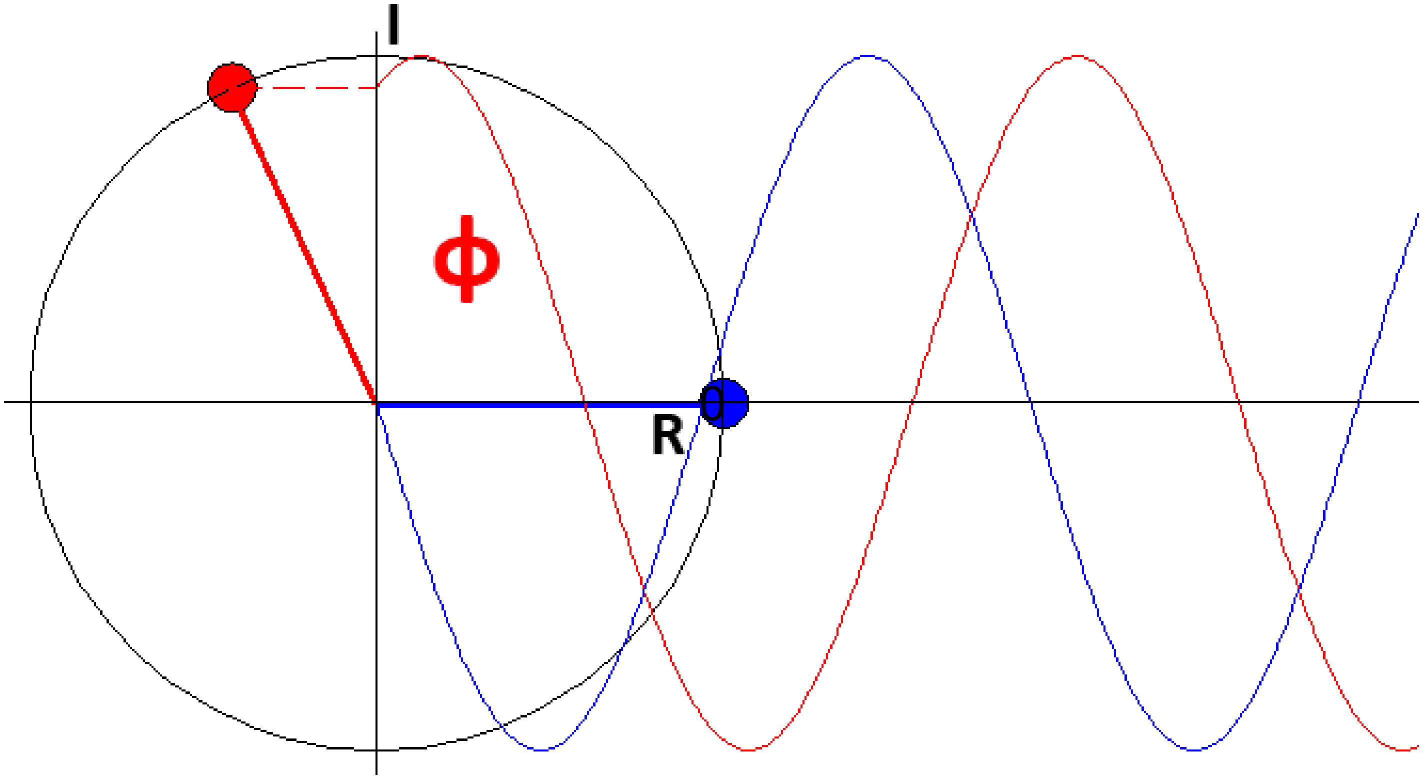


$$\vec{E}\cos[\omega t - \vec{q}.\vec{r}] \text{ with } \omega = 2\pi\nu \text{ and } |\vec{q}| = 2\frac{\pi}{\lambda}$$

ω: angular frequency, ν: frequency, λ: wave length, $\vec{q}$: wave vector or momentum

Reminder about waves

- Fresnel representation of waves
  - Electric field: $\vec{E}_0 \cos[\omega t - \vec{q}_0 . \vec{r}] = \vec{E}_0 \exp[i(\omega t - \vec{q}_0 . \vec{r})]$
  - A phase shift φ: $\vec{E}_0 \exp[i(\omega t - \vec{q}_0 . \vec{r} + \varphi)] = \vec{E}_0 \exp[i(\omega t - \vec{q}_0 . \vec{r})] . \exp[i\varphi]$



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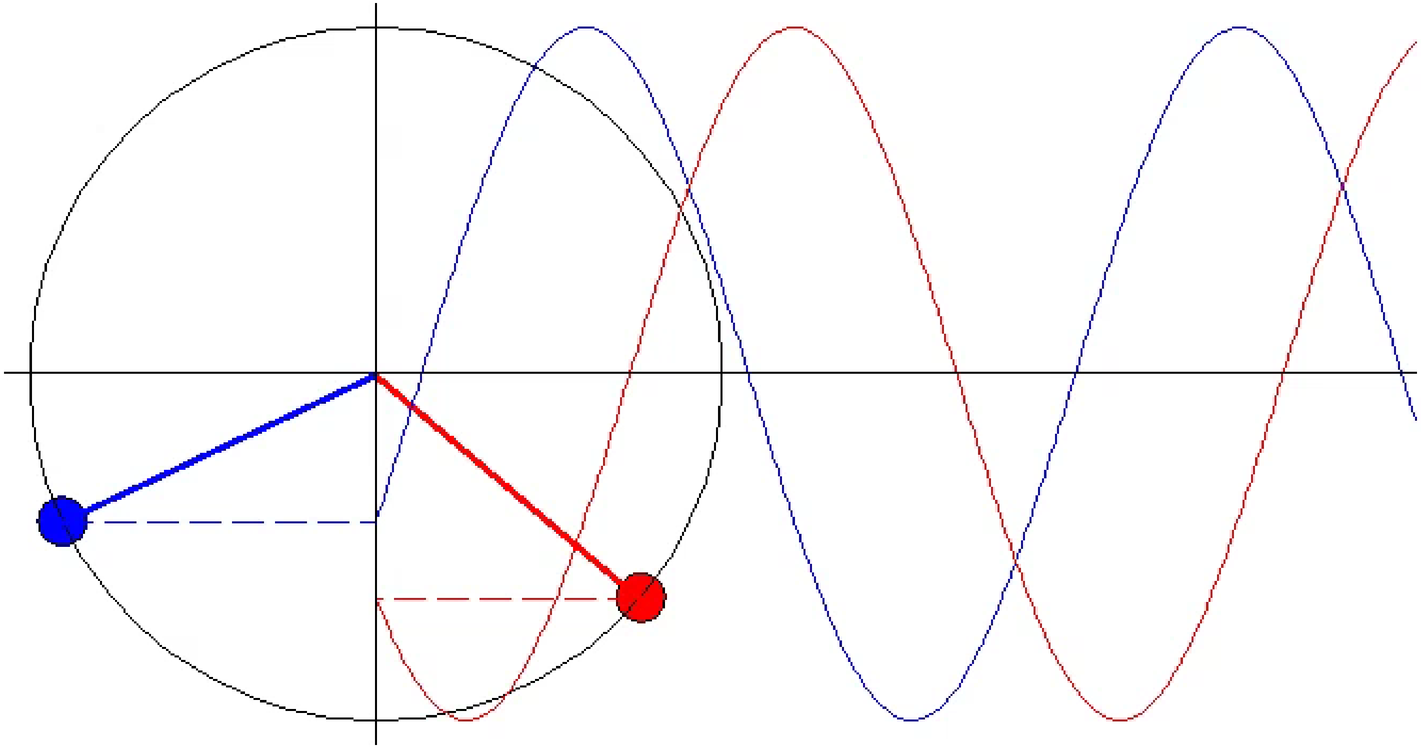
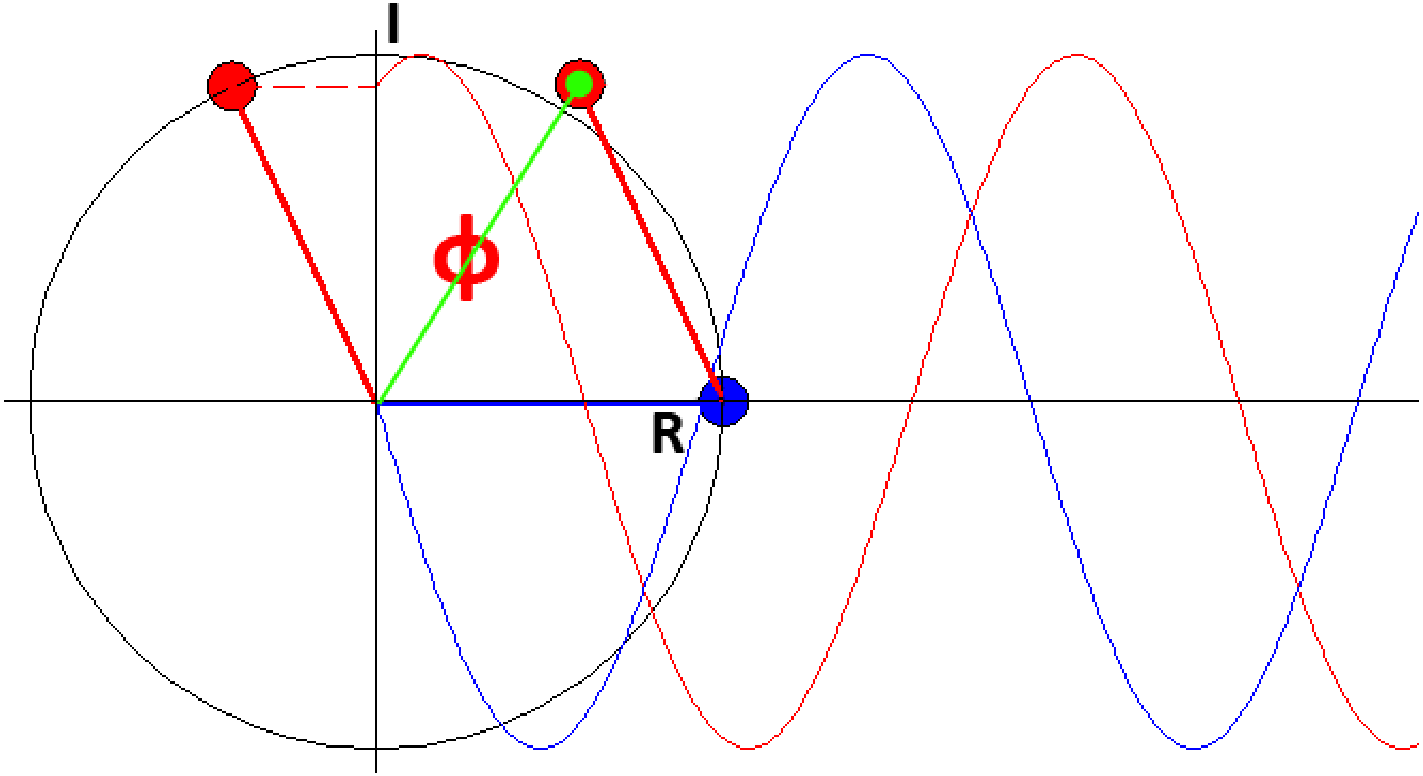


Reminder about waves

- Sum of two waves are simple to express

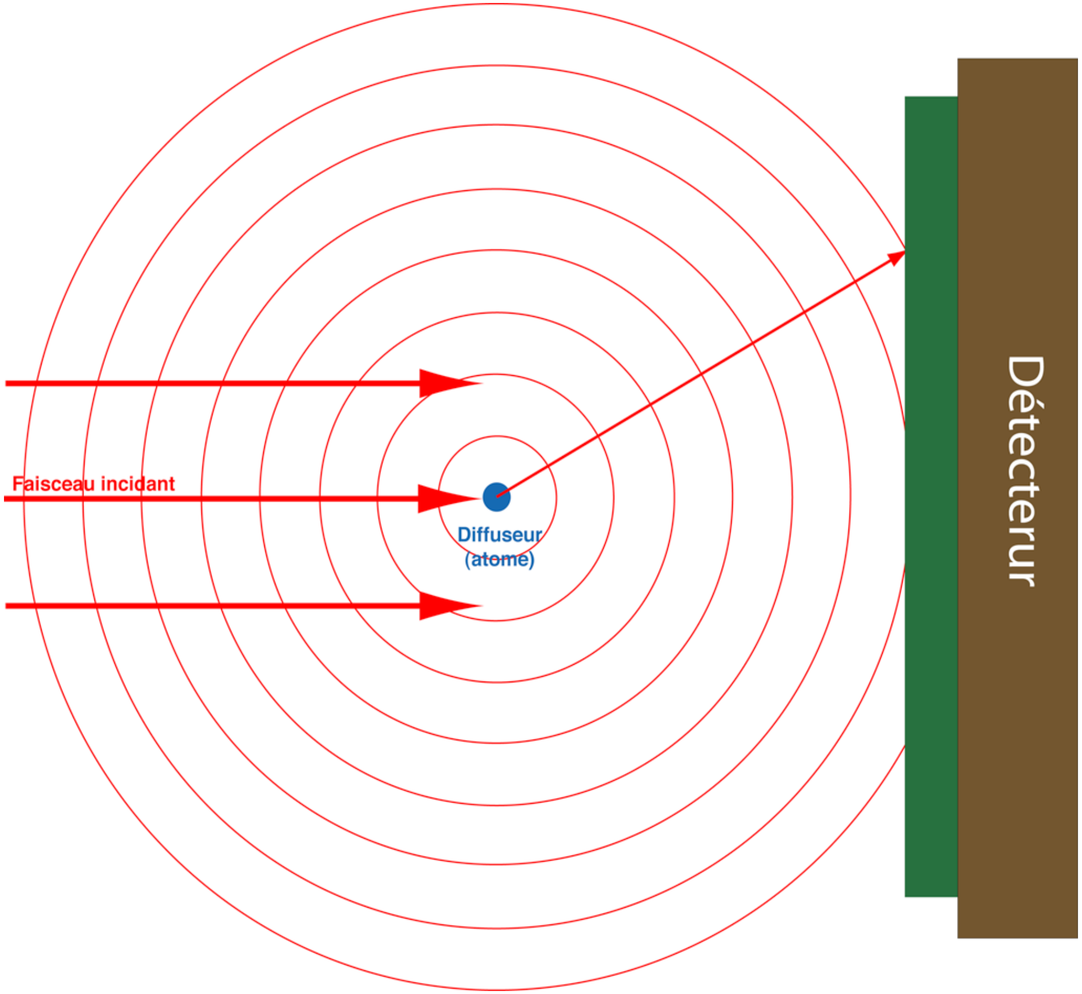
$$\vec{E}_0 \exp\left[i\left(\omega t-\vec{q}_0.\vec{r}\right)\right]+\vec{E}_0 \exp\left[i\left(\omega t-\vec{q}_0.\vec{r}\right)\right].\exp\left[i\varphi\right]=\vec{E}_0 \exp\left[i\left(\omega t-\vec{q}_0.\vec{r}\right)\right].\left(1+\exp\left[i\varphi\right]\right)$$



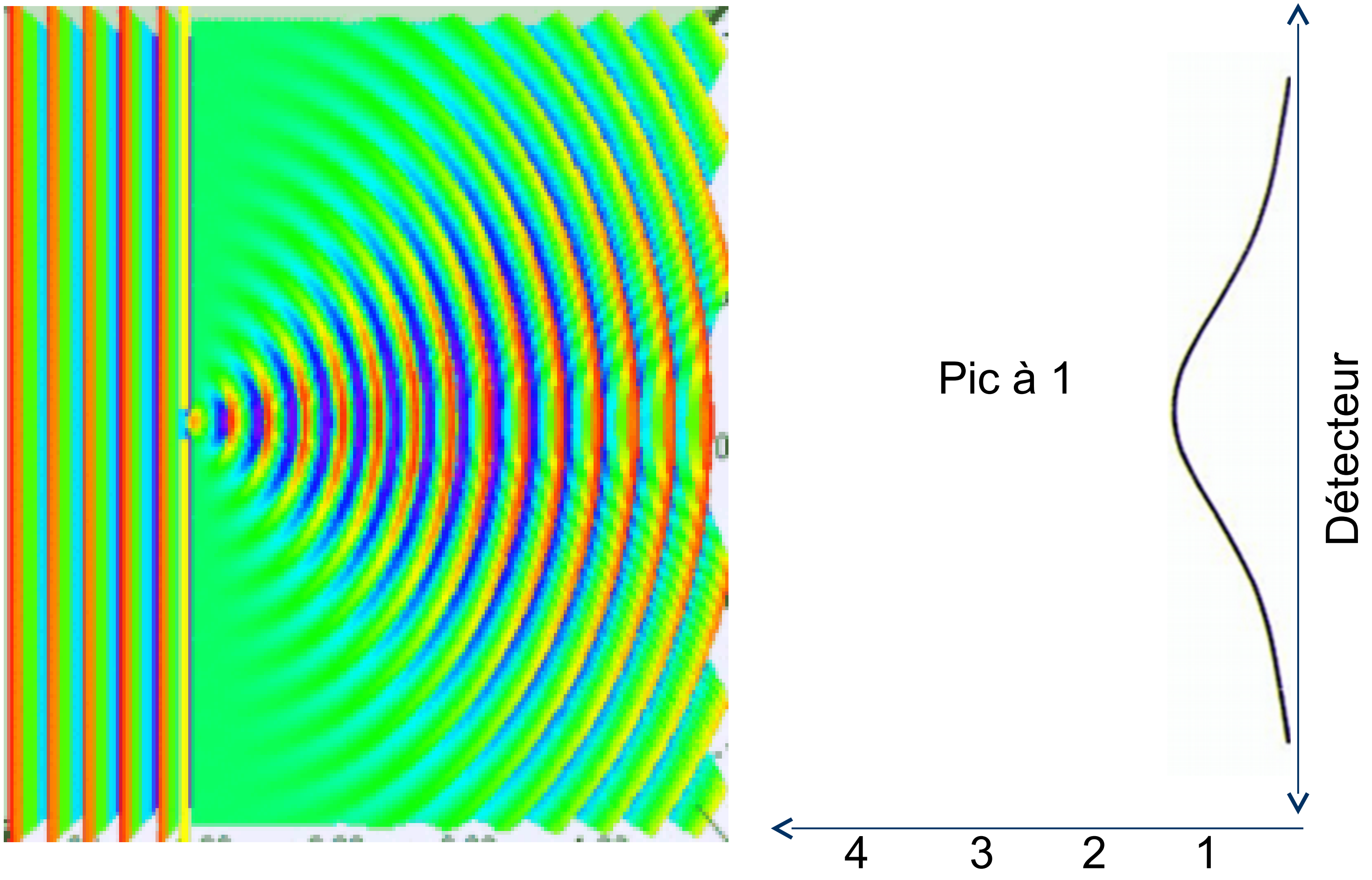
- φ = 0° (or 2π) => in phase => constructive interference
- φ = 180° (or π) => out of phase => destructive interference

Scattering by one atom

- The planar incident wave induces the emission of a spherical wave of same wavelength and with a π phase shift

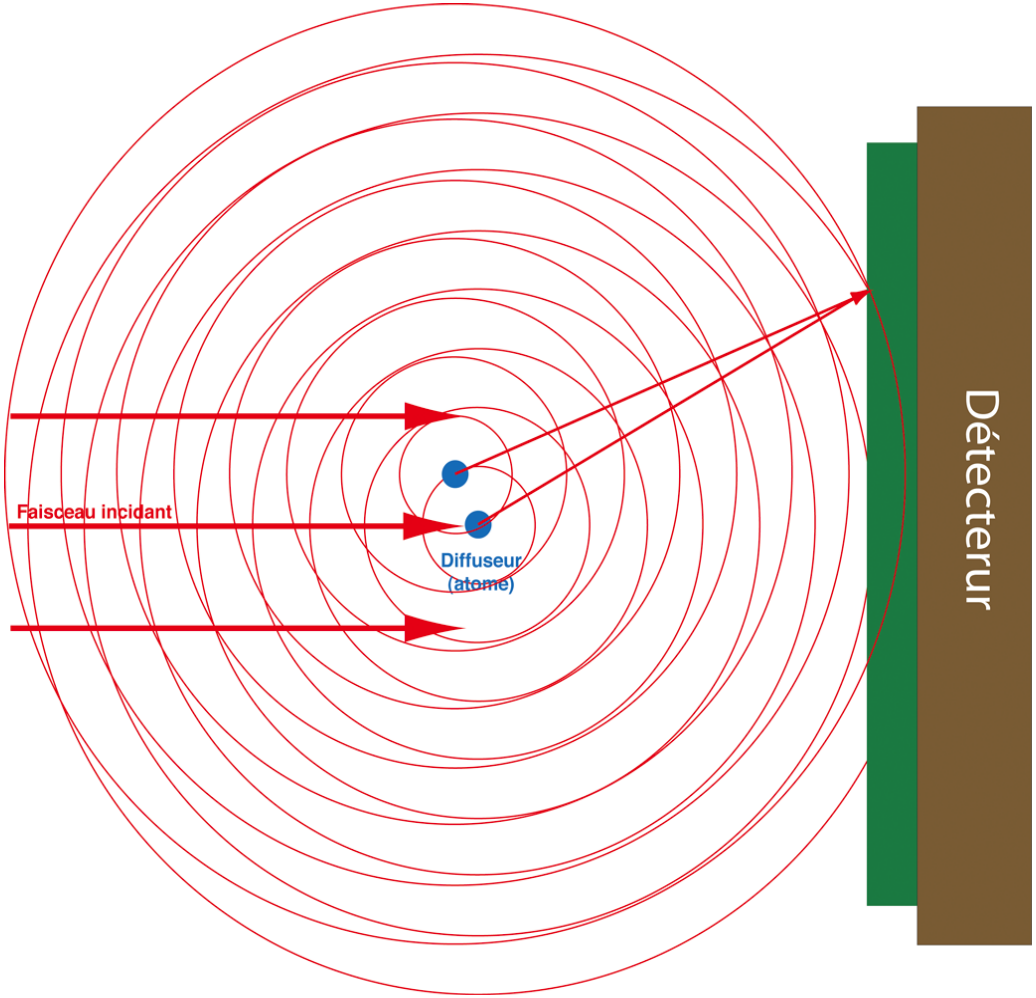


Scattering by one atom

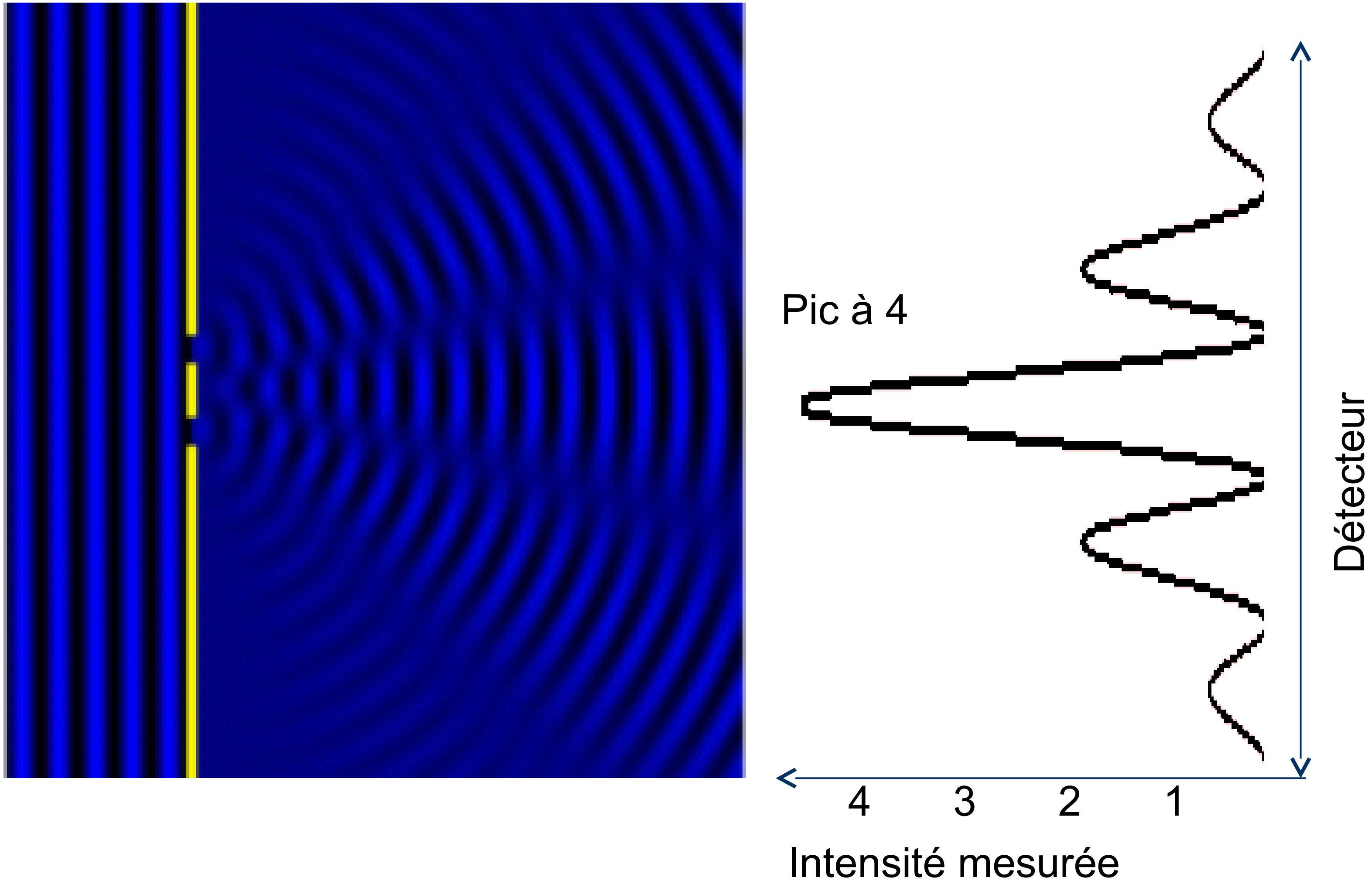


Scattering by two atoms

- The two atoms are emitting a spherical electromagnetic wave. If the two atoms are not mobile and close by, the two waves interfere

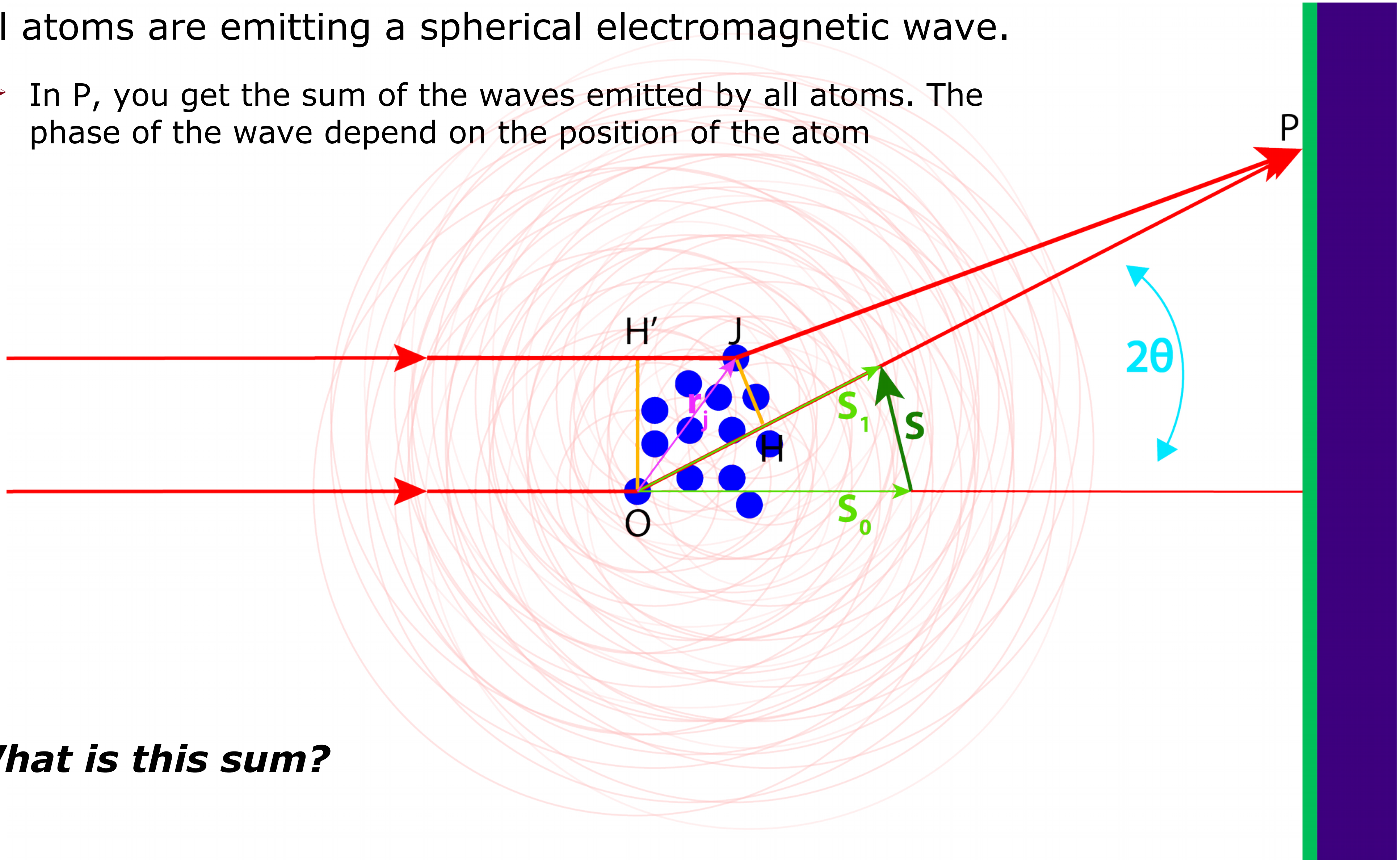


Scattering by two atoms



Scattering by numerous atoms

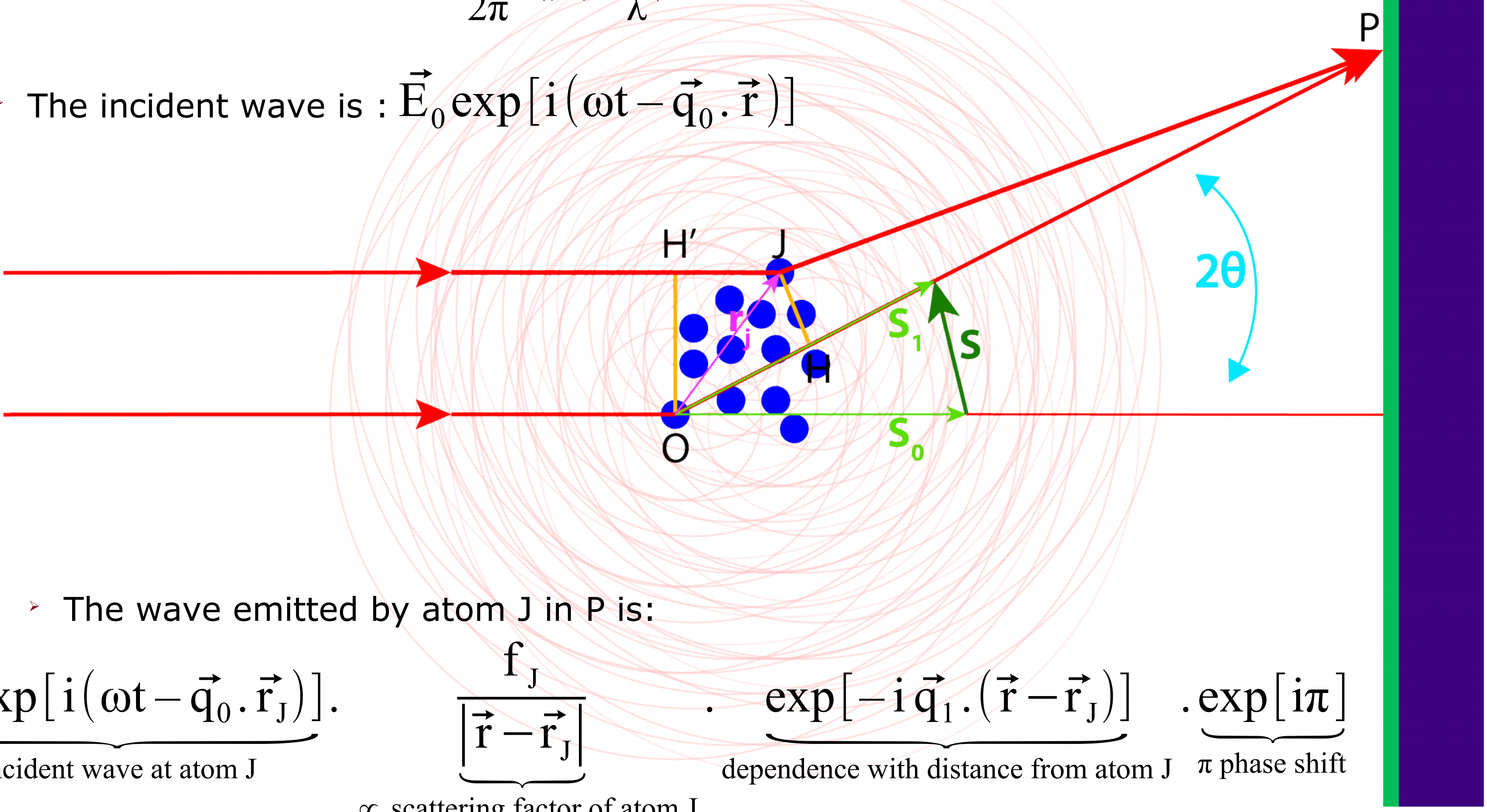
- All atoms are emitting a spherical electromagnetic wave.
  - In P, you get the sum of the waves emitted by all atoms. The phase of the wave depend on the position of the atom



- ***What is this sum?***

Scattering by numerous atoms

- A wave is described by its wave vector $\vec{q}$ $\left(|\vec{q}|=\frac{2\pi}{\lambda}\right)$
- or is scattering vector $\vec{s}=\frac{\vec{q}}{2\pi}$ $\left(|\vec{s}|=\frac{1}{\lambda}\right)$
- The incident wave is : $\vec{E}_0 \exp[i(\omega t-\vec{q}_0.\vec{r})]$



- The wave emitted by atom J in P is:

$$\underbrace{\vec{E}_0 \exp[i(\omega t-\vec{q}_0.\vec{r}_J)]}_{\text{incident wave at atom J}} . \frac{f_J}{\underbrace{|\vec{r}-\vec{r}_J|}_{\propto\ \text{scattering factor of atom J}}} . \underbrace{\exp[-i\vec{q}_1.(\vec{r}-\vec{r}_J)]}_{\text{dependence with distance from atom J}} . \underbrace{\exp[i\pi]}_{\pi\ \text{phase shift}}$$

Scattering by numerous atoms

- The wave emitted by all atoms in P is jus the sum:

$$\sum_{J} \vec{E}_0 \exp\left[i\left(\omega t-\vec{q}_0.\vec{r}_J\right)\right].\frac{f_J}{\left|\vec{r}-\vec{r}_J\right|}.\exp\left[-i\,\vec{q}_1.\left(\vec{r}-\vec{r}_J\right)\right].\exp[i\pi]$$

  - If sample size << distance sample-detector $\left(\left|\vec{r}-\vec{r}_J\right| \approx \left|\vec{r}-\vec{r}_0\right|\right)$ it becomes:

$$\frac{\vec{E}_0}{\left|\vec{r}-\vec{r}_0\right|}\exp\left[i\left(\omega t-\vec{q}_1.\vec{r}\right)\right].\exp[i\pi].\sum_{J} f_J.\exp\left[i\left(\vec{q}_1-\vec{q}_0\right).\vec{r}_J\right]$$

- If we define the scattering vector: $\vec{s}=\vec{s}_1-\vec{s}_0=\frac{1}{2\pi}.\left(\vec{q}_1-\vec{q}_0\right)$

$$\underbrace{\frac{\vec{E}_0}{\left|\vec{r}-\vec{r}_0\right|}\exp\left[2\,i\pi\left(\nu t-\vec{s}_1.\vec{r}\right)\right].\exp[i\pi].}_{\text{depend on the incident wave and position P}} \qquad \underbrace{\sum_{J} f_J.\exp\left[2\,i\pi\,\vec{s}.\vec{r}_J\right]}_{\text{Fourier transform of the distribution of scattering factors}}$$

Scattering by numerous atoms

- The <u>structure factor</u> is $F(\vec{s})=\sum_{J} f_{J}.\exp[2i\pi(\vec{s}).\vec{r}_{J}]$
  - It is the Fourier transform of the distribution of electron, *i.e.* the electron density:

$$F(\vec{s})=\sum_{J} f_{J}.\exp[2i\pi(\vec{s}).\vec{r}_{J}]=\int_{\mathrm{vol}} \rho(\vec{r})\exp[2i\pi\vec{r}.\vec{s}].d\vec{r}$$

  - It is a complex number (amplitude and phase)
- The <u>electron density</u> can be calculated by the reverse Fourier transform:

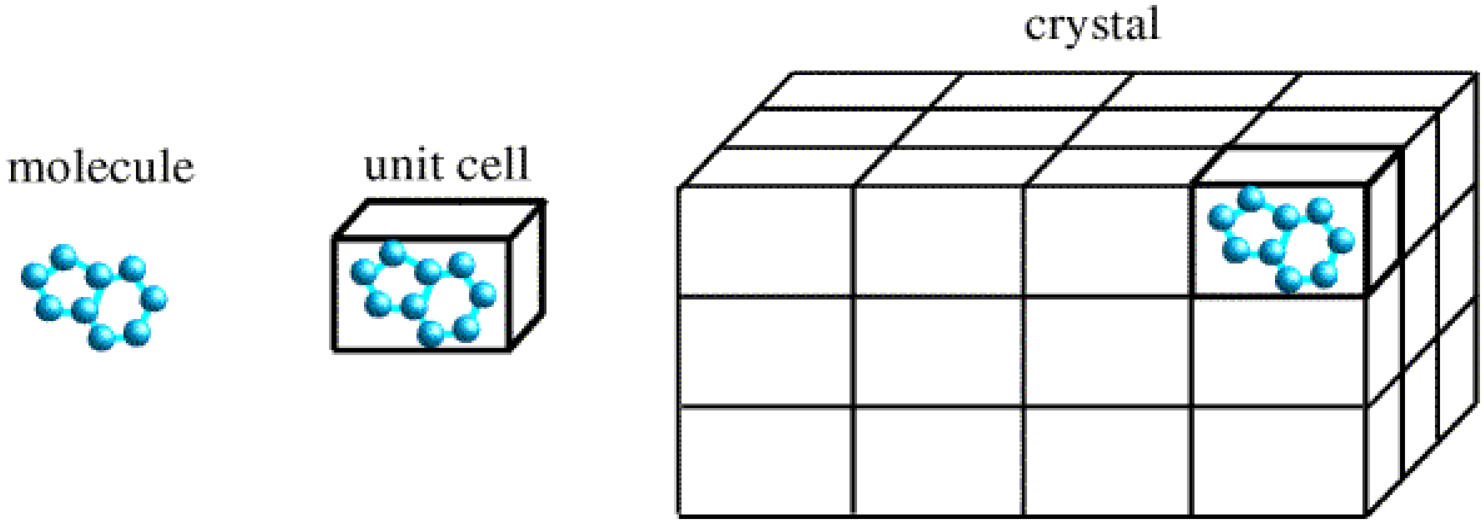
$$\rho(\vec{r})=\sum_{J} f_{J}.\delta(\vec{r}-\vec{r}_{J})=\int_{\mathrm{rec.vol.}} F(\vec{s})\exp[-2i\pi\vec{r}.\vec{s}].d\vec{s}$$

  - The detector measures the intensity of the scattered wave
    - This intensity is proportional to the square modulus of structure factor

$$I(\vec{s})\propto|F(\vec{s})|^{2}$$

What if the sample is a crystal?

- A crystal can be described by a unit cell
  - Three vector $\vec{a}, \vec{b}, \vec{c}$ define this unit cell
- Unit cells (identical content) are piled up in the 3 directions of space



What if the sample is a crystal?

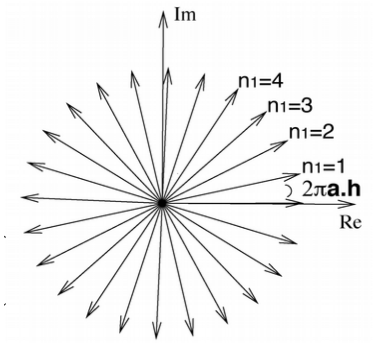
- The general form of the structure factor is:

$$F(\vec{s}) = \int_{\text{vol sample}} \rho(\vec{r}) \exp[2i\pi \vec{r}.\vec{s}].d\vec{r}$$

- If the sample is a crystal, it can be described as a pile of N_{cell} unit cells

$$F(\vec{s}) = \sum_{n=1}^{N_{\text{cell}}} \int_{\text{vol cell}} \rho(\vec{r}+\vec{r}_n) \exp[2i\pi(\vec{r}+\vec{r}_n).\vec{s}].d\vec{r}$$

$$\text{with : } \vec{r}_n = n_1.\vec{a} + n_2.\vec{b} + n_3.\vec{c} \text{ and } \rho(\vec{r}+\vec{r}_n) = \rho(\vec{r}$$



$$F(\vec{s}) = \underbrace{\sum_{n=1}^{N_{\text{cell}}} \exp[2i\pi\, n_1.\vec{a}.\vec{s}] \exp[2i\pi\, n_2.\vec{b}.\vec{s}] \exp[2i\pi\, n_3.\vec{c}.\vec{s}]}_{\text{factor} \approx 0\text{, except if } \vec{s} \text{ satisfy Laue equations : } \vec{a}.\vec{s}=h,\, \vec{b}.\vec{s}=k,\, \vec{c}.\vec{s}=1 \Rightarrow \text{factor} = N_{\text{cell}}} . \underbrace{\int_{\text{vol cell}} \rho(\vec{r}) \exp[2i\pi\, \vec{r}.\vec{s}].d\vec{r}}_{\text{Fourier transform of electron density of the unit cell}}$$

- There is significant X-ray scattering only in specific, discrete direction => <u>diffraction phenomenon</u>

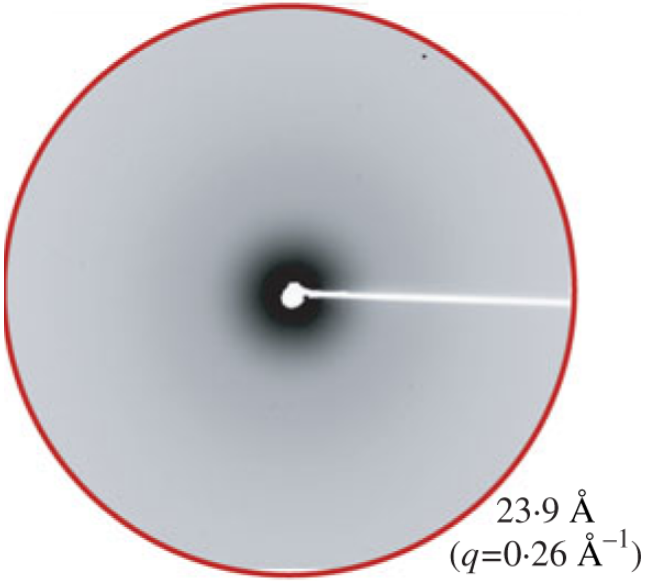
X-ray scattering by a crystal

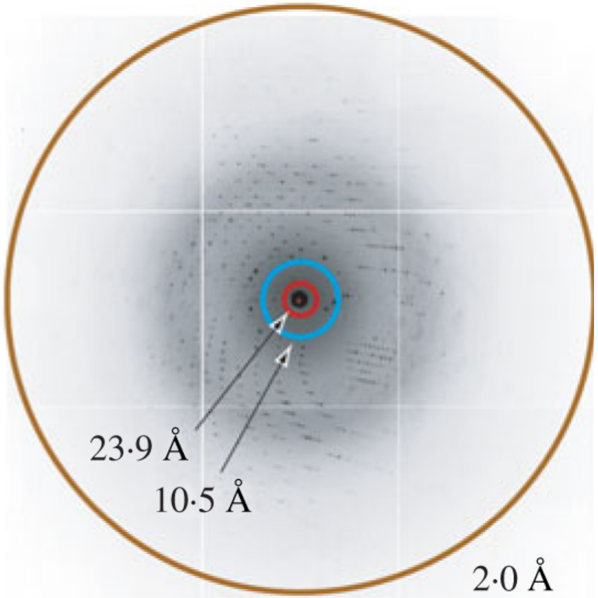
- In directions that satisfy Laue equation, the structure factor is the one of the unit cell, multiplied by the number of cells in the crystal.

$$F(\vec{s})=N_{cell}\,.\,\underbrace{\int_{vol\,cell}\rho(\vec{r})\exp[2i\pi\,\vec{r}\,.\,\vec{s}]\,.\,d\,\vec{r}}_{\text{Fourier transform of electron density of the unit cell}}$$

- The crystal is a **signal amplifier**, in direction where you have signal:

$$I(\vec{s})\propto N_{cell}^{2}\,.\left(\int_{vol\,cell}\rho(\vec{r})\exp[2i\pi\,\vec{r}\,.\,\vec{s}]\,.\,d\,\vec{r}\right).\left(\int_{vol\,cell}\rho(\vec{r})\exp[-2i\pi\,\vec{r}\,.\,\vec{s}]\,.\,d\,\vec{r}\right)$$





How we get the "image" from a diffraction spectra

- In the case of a crystal:
- $\vec{s}$ statisfies the **Laue equations** $\vec{a}.\vec{s}=h, \vec{b}.\vec{s}=k, \vec{c}.\vec{s}=l$
- $\vec{s}$ is a vector of a lattice, named **reciprocal lattice**

$$\vec{s}=h.\vec{a}^*+k.\vec{b}^*+l.\vec{c}^*$$

$$\text{with } \vec{a}^*=\frac{\vec{b}\wedge\vec{c}}{\vec{a}.\vec{b}\wedge\vec{c}},\ \vec{b}^*=\frac{\vec{c}\wedge\vec{a}}{\vec{a}.\vec{b}\wedge\vec{c}},\ \vec{c}^*=\frac{\vec{a}\wedge\vec{b}}{\vec{a}.\vec{b}\wedge\vec{c}}$$

$\vec{a}.\vec{b}\wedge\vec{c}$: cell volume

- **Electron density** calculation

$$\rho(\vec{r})=\sum_J f_J.\delta(\vec{r}-\vec{r}_J)=\int_{\text{rec.vol.}} F(\vec{s})\exp[-2i\pi\vec{r}.\vec{s}].d\vec{s}$$

$$\downarrow$$

$$\rho(\vec{r})=\sum_J f_J.\delta(\vec{r}-\vec{r}_J)=\sum_{h,k,l} F(\vec{s})\exp[-2i\pi\vec{r}.\vec{s}]$$

What is a Fourier transform?

- Example of a crystal

$$\rho(\vec{r}) = \sum_{J} f_J . \delta(\vec{r} - \vec{r}_J) = \sum_{h,k,l} F(\vec{s}) \exp[-2i\pi \vec{r} . \vec{s}]$$

- The electron density is a complex function depending on the nature of your molecule
  - If the molecule is in a crystal, the electron density is periodical

- A way to describe it as a sum of well known functions
  - Sinus or cosinus
  - Structure factors represent the coefficients of these sinus/cosinus functions

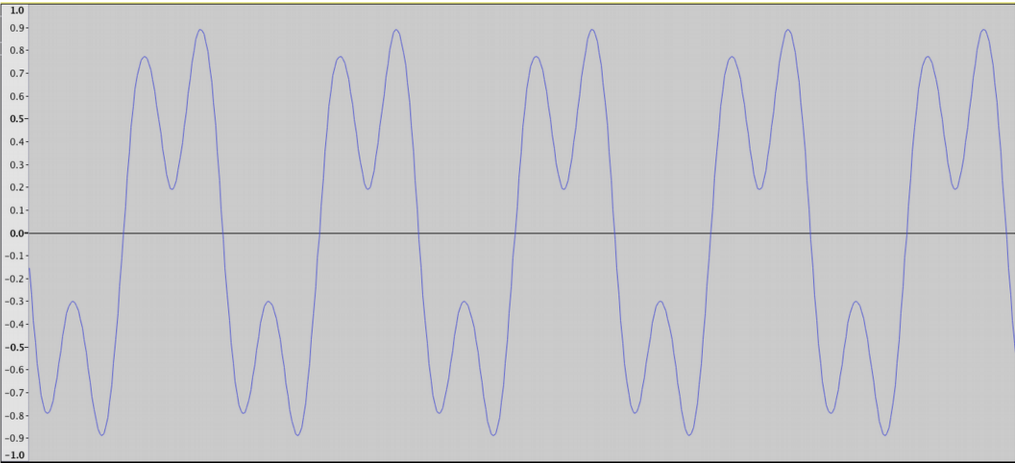
What is the meaning of F(s)?

- A parallel with sound
  - The sound can be described as the acoustic pressure as a function of time
  - The Fourier transform is the analysis of the frequencies present in your sound
    - One can describe the sound as a sum of different frequencies
    - The higher frequencies, the more detailed is the sound
    - Parallel with resolution
- Let try a real time analysis
  - Live with AudioXporer

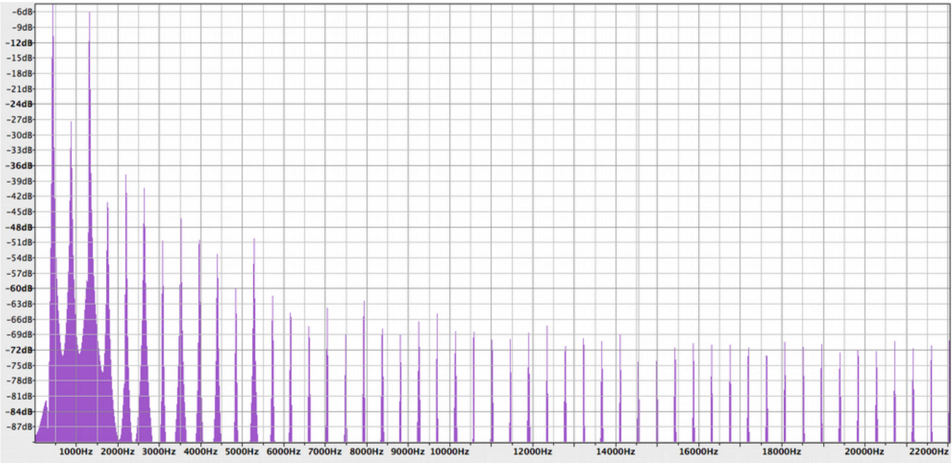
Parallels between sound and electron density

Sound

Time (s)

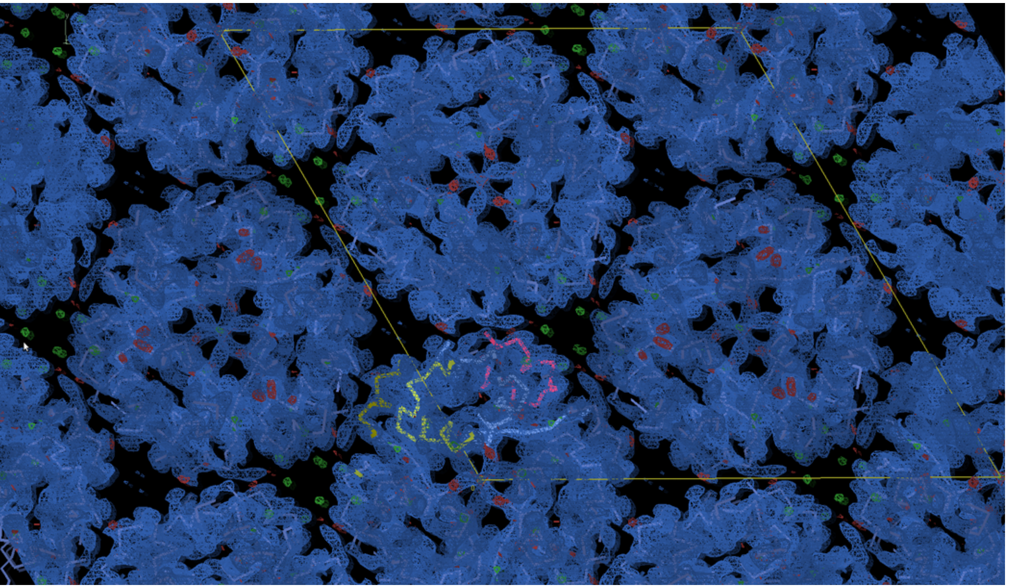


Frequency (s^{-1} or Hz)

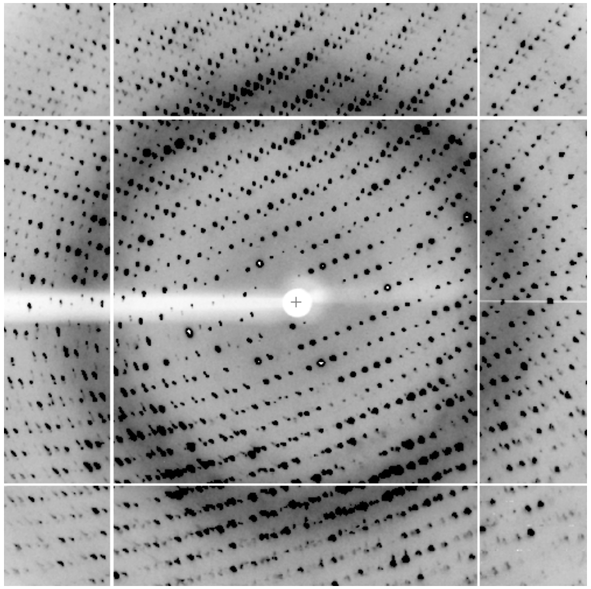


Electron density

Space (Å)



Reciprocal space ($Å^{-1}$)



Go back to electron density

- Equivalence Sound <=> Electron density
    - Pitch <=> unit cell dimensions
    - Instrument sound <=> molecule's electron density

$$\rho(\vec{r}) = \sum_{h,k,l} F(h.\vec{a}^* + k.\vec{b}^* + l.\vec{c}^*)\exp[-2i\pi\vec{r}.(h.\vec{a}^* + k.\vec{b}^* + l.\vec{c}^*)]$$

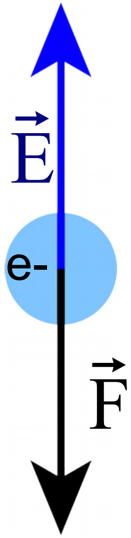
- The reflection (1,0,0) is the coefficient for or sinusoidal function, the period of which is the a axis
- Higher indices correspond to higher spatial frequencies, i.e. to periods which are fractions of the a axis
- The higher you go in indices (h,k,l), the higher is the resolution (1/d):

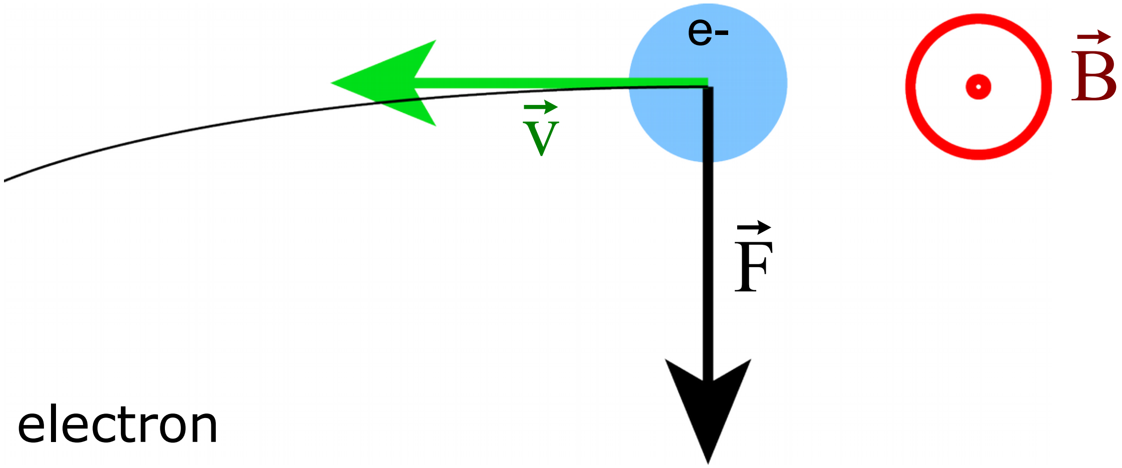
$$|\vec{s}| = |h.\vec{a}^* + k.\vec{b}^* + l.\vec{c}^*| = \frac{1}{d}$$

Electrons: Imaging or diffraction?

- Both the electric and the magnetic field induce force on the electron

$$\vec{F} = \underbrace{\bar{e}.\vec{E}}_{\text{electric field}} + \underbrace{\bar{e}.\vec{v} \wedge \vec{B}}_{\text{magnetic field}}$$

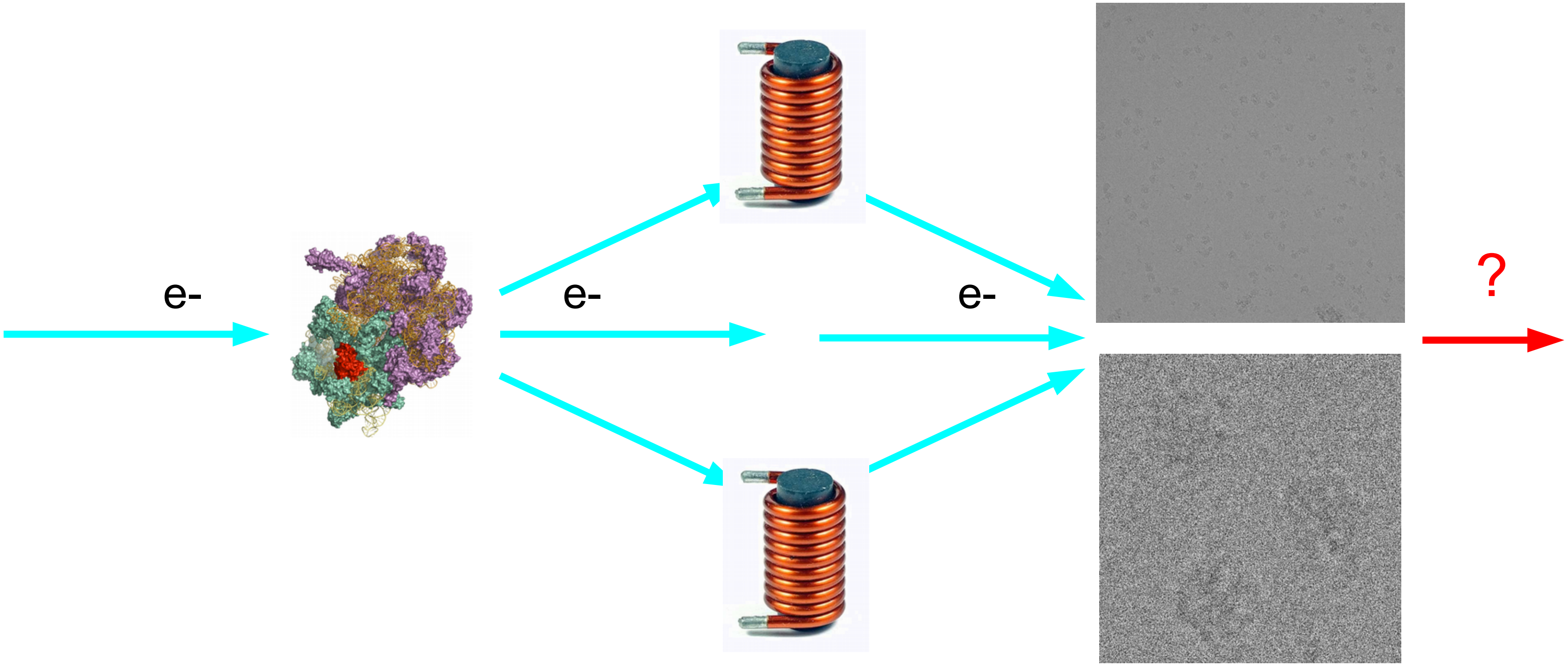


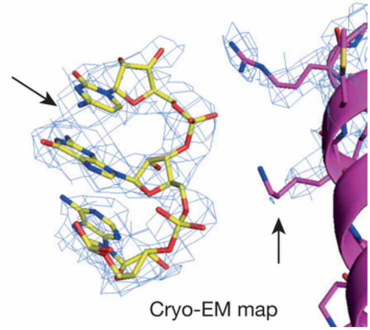


- A magnetic field can deviate an electron

- A lens can be made for electrons with magnet
  - First one made in 1929 (Ruska & Knoll)

Direct imaging of the molecule





~ 3 Å electron density map,
Khatter et al, *Nature*, **520**:640, 2015

Not possible for neutrons

- No lens available for neutrons
  - Diffraction spectra
- If you manage to measure the amplitude and to get the phase of the wave for each reflection on the detector
  - A Fourier transform enable to calculate the distribution of nucleus of your molecule

$$\sum_{J} \sigma_{J} . \delta(\vec{r} - \vec{r}_{J}) = \sum_{h,k,l} F(\vec{s}) \exp[-2i\pi \vec{r} . \vec{s}]$$

- Scattering cross section for H and D are very different
  - Deuteration can be useful

To conclude

- X-rays & Neutrons
  - Diffraction => F(hkl) + φ(hkl) => electron density map
  - Small angle scattering => ab-initio modeling (fit whith scattering curve)
- Electrons
  - Direct imaging => “electron density” map
  - Diffraction => F(hkl) + φ(hkl) => “electron density” map
- NMR
  - Gather structural information (local interatomic distance, …) => search for models that satisfy the data.