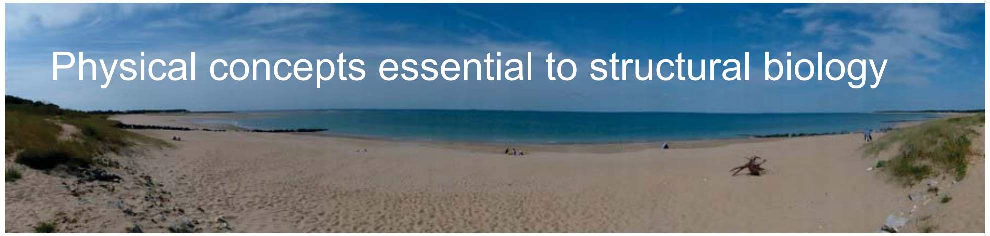
Ecole d'Oléron 2016



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 molecule under study

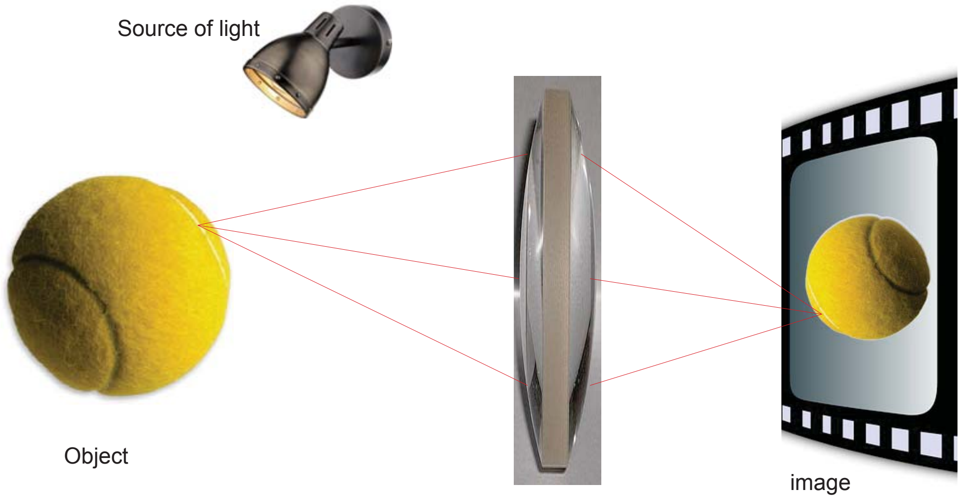
Possible approaches

- “Imaging” techniques
  - Visible light microscopy
  - Electron microscopy
  - X-ray or neutron crystallography
- Localization technique
  - Super-resolution microscopy
- Spectroscopic techniques
  - NMR
  - SAXS

Visible light microscopy

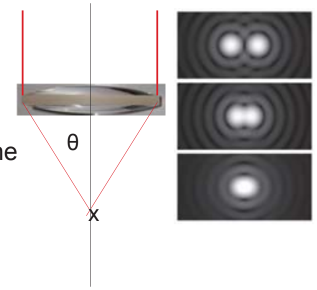
- Source light (radiation): photons or electromagnetic wave
  - Wave length: 0.3 – 0.8 µm
- Object (matter): absorb and re-emit incident light in all directions
  - More or less absorption
  - some time wave length dependent (color)
- Lens: focuses light emitted by the object
  - The light emitted by one point of the object is focused on one point of the detector

A scheme to explain



Why can't we see molecule with visible light?

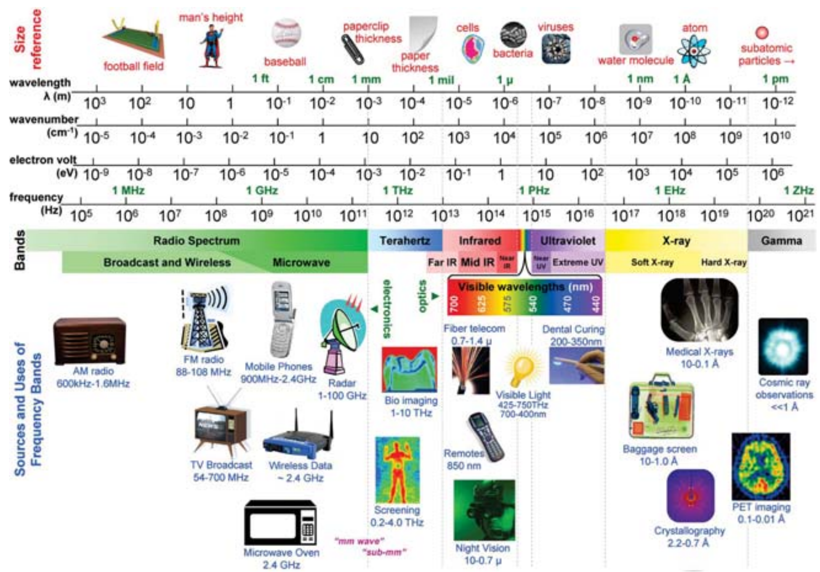
- Be because of the wave nature of light
  - Diffraction of light by a disk when the disk size of the same order of magnitude than the wave length
- Rayleigh criterion
  - $d = 1.22 \dfrac{\lambda}{2\, n\, sin\theta} \approx \dfrac{\lambda}{2\, sin\theta}$ (in air)
    - λ is the wave length, n is the refraction index, θ the
  - 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



How to increase resolution?

- Atomic resolution
  - d ~ 1 Å => λ ≤ 2 Å
  - Use photons in the domain of X-rays
    - Typically: for λ = 1 Å, E = hν = hc/λ ≈ 12.4 keV

How to increase resolution?



How to increase resolution?

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  - Use photons in the domain of X-rays
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-
- Do X-rays interact with atoms?
  - Yes, X-ray photons can be elastically scattered by the electronic cloud of an atom

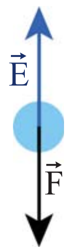
How a photon is scattered by an atom ?

- Elastic scattering (no loss of energy, wavelength is conserved)
  - 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
- $\vec{F}=m.\vec{\gamma}$ => $\vec{\gamma}_e=-e.\dfrac{\vec{E}}{m_e}$ and $\vec{\gamma}_n=+Ze.\dfrac{\vec{E}}{(Zm_p+(A-Z)m_n)}$
  -
  - 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)



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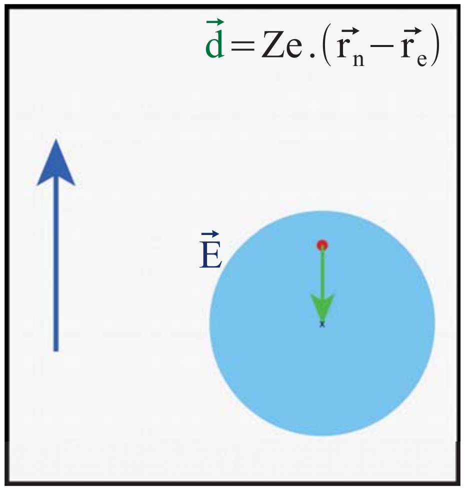
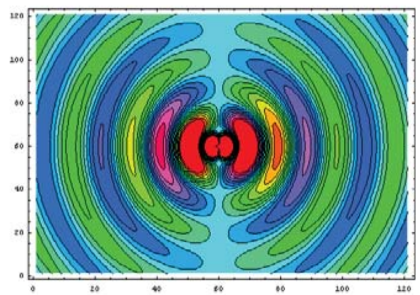
$$\frac{\partial^2\vec{d}}{\partial t^2}=-Ze.\vec{\gamma}_e=Ze^2.\frac{\vec{E}}{m_e}$$

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

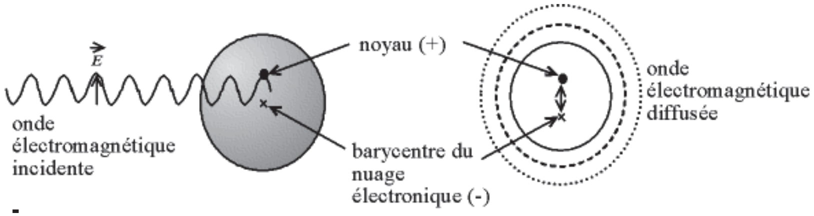
Oscillating dipole and emitted wave





How a photon is scattered by an atom ?

- The incident electromagnetic wave induce the oscillation of the electronic cloud
- The negatively charged electronic cloud et the positively charged nucleus become an oscillating dipole, thus emitting a spherical electromagnetic wave of same wavelength and a phase difference of π.

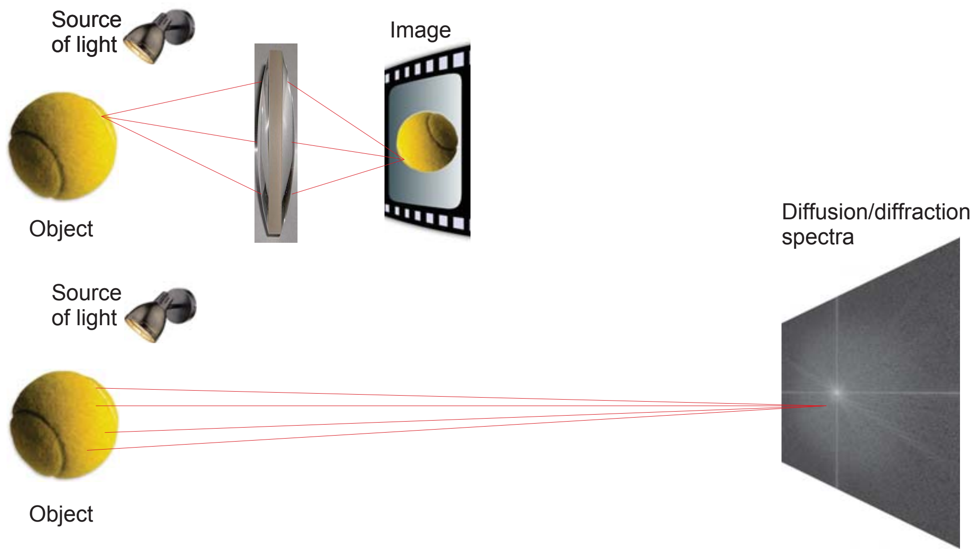


- The atom becomes a source of photon, with the same wave length.

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

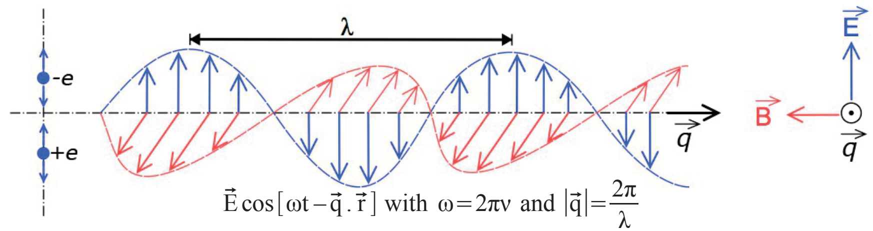
- Problem: we have no lens for X-ray photons
  - No image on the detector, but a scattering spectra
- With a lens
  - All radiation arriving on one point of the detector come from one point on the object
- Without a lens
  - Radiation arriving on one point of the detector come from all points on the object
    - It the sum of waves emitted by each point of the object

Scheme for a scattering spectrum



What is a scattering spectra?

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

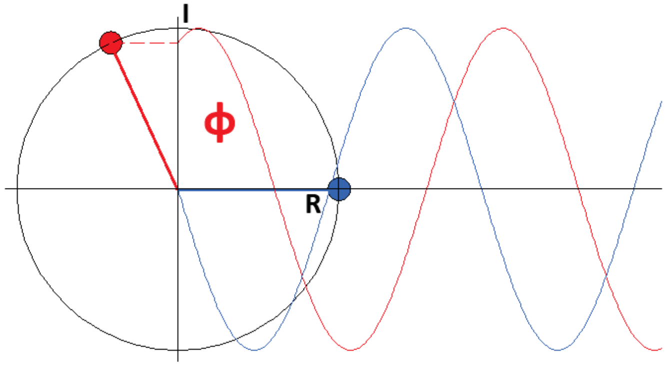


$\vec{E}\cos[\omega t - \vec{q}.\vec{r}]$ with $\omega = 2\pi\nu$ and $|\vec{q}| = \frac{2\pi}{\lambda}$

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

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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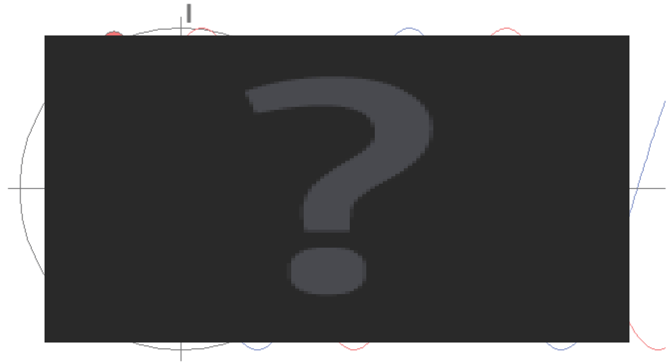
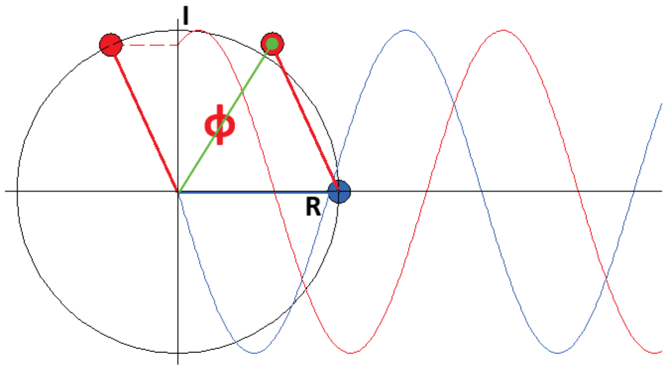


Reminder about waves

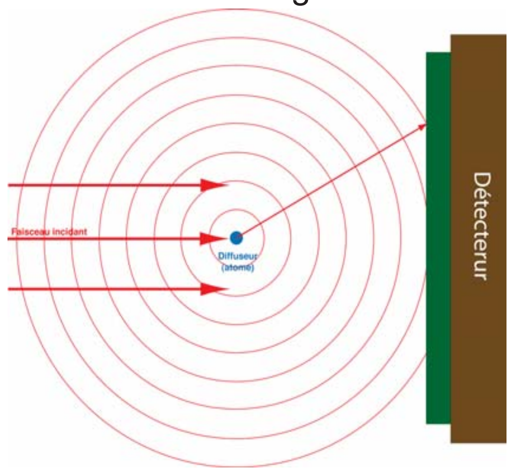
- Sum of two waves are simple to express

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

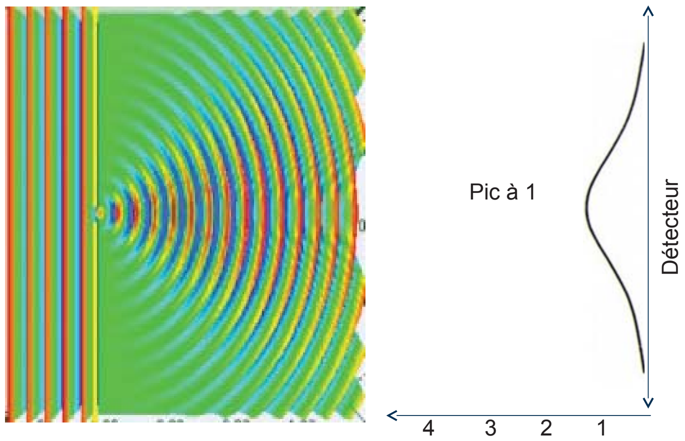


Scattering by one atom

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

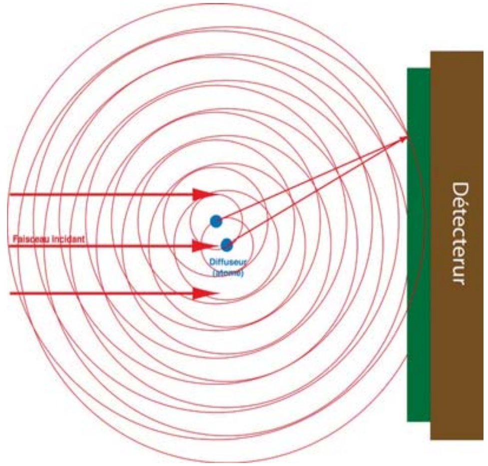


Scattering by one atom

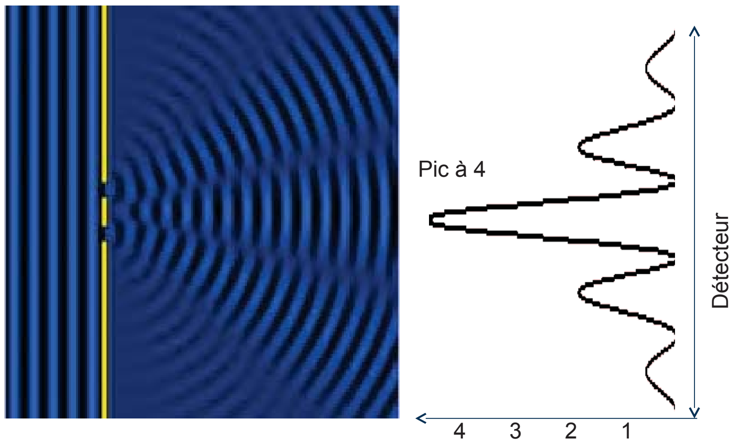


Scattering by two atoms

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

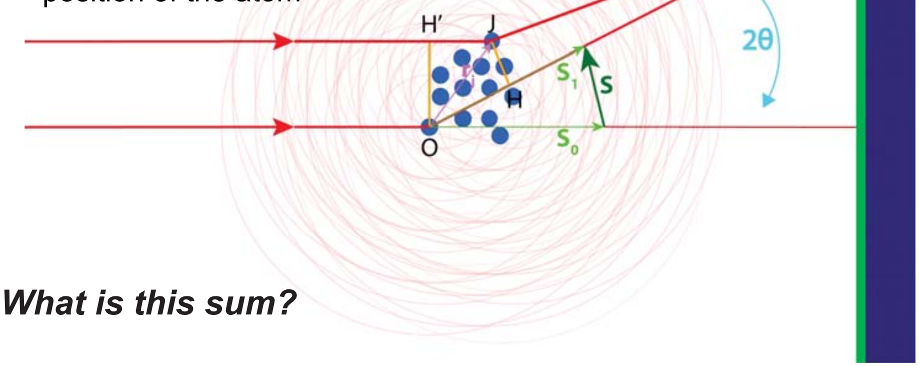


Scattering by two atoms



Scattering by numerous atoms

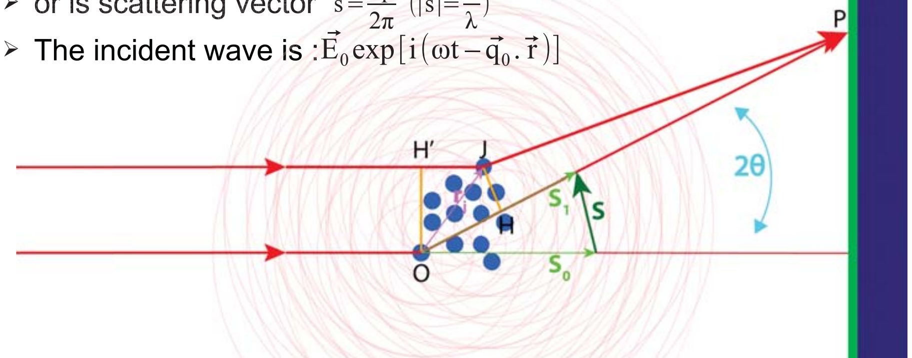
- All atoms are emitting a spherical electromagnetic wave.
  - In P, you get the sum of the waves emitted by all atoms. The phase of the wave depend on the position of the atom



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

Scattering by numerous atoms

- A wave is described by its wave vector $\vec{q}$ ($|\vec{q}|=\frac{2\pi}{\lambda}$)
- or is scattering vector $\vec{s}=\frac{\vec{q}}{2\pi}$ ($|\vec{s}|=\frac{1}{\lambda}$)
- 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:

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

Scattering by numerous atoms

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

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

  - If sample size << distance sample-detector it becomes:

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

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

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

Scattering by numerous atoms

- The structure factor 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:
  - $$F(\vec{s})=\sum_J f_J.\exp[2i\pi(\vec{s}).\vec{r}_J]=\int_{vol}\rho(\vec{r})\exp[2i\pi\vec{r}.\vec{s}].d\vec{r}$$
  - It is a complex number (amplitude and phase)
- The electron density can be calculated by the reverse Fourier transform:

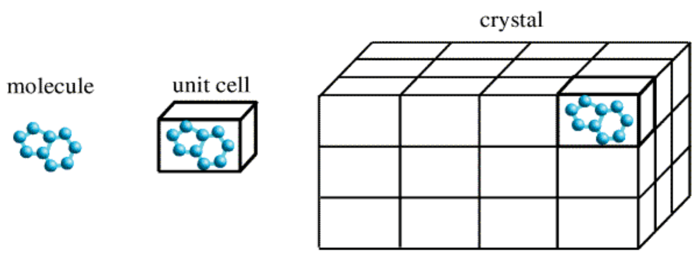
$$\rho(\vec{r})=\sum_J f_J.\delta(\vec{r}-\vec{r}_J)=\int_{rec.vol.}F(\vec{s})\exp[-2i\pi\vec{r}.\vec{s}].d\vec{s}$$

  - The dectector 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?

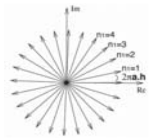
- The general form a the structure factor is:

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

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

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

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

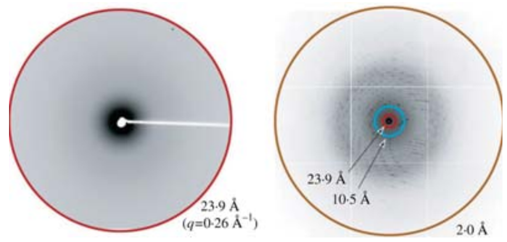


$$F(\vec{s}) = \underbrace{\sum_{n=1}^{N_{cell}} \exp[2i\pi n_1.\vec{a}.\vec{s}]\exp[2i\pi n_2.\vec{b}.\vec{s}]\exp[2i\pi n_3.\vec{c}.\vec{s}]}_{\text{factor} \approx 0\text{, except if } \vec{s} \text{ satisfy Laue equations : } \vec{a}.\vec{s}=h,\ \vec{b}.\vec{s}=k,\ \vec{c}.\vec{s}=l \Rightarrow \text{factor}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

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

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. $F(\vec{s}) = N_{cell}.\underbrace{\int_{\text{vol cell}} \rho(\vec{r}) \exp[2i\pi \vec{r}.\vec{s}].d\vec{r}}_{\text{Fourier transform of electron density of the unit cell}}$
-
- The crystal is a signal amplifier, in direction where you have signal: $I(\vec{s}) \propto N_{cell}^2.(\int_{\text{vol cell}} \rho(\vec{r}) \exp[2i\pi \vec{r}.\vec{s}].d\vec{r}).(\int_{\text{vol cell}} \rho(\vec{r}) \exp[-2i\pi \vec{r}.\vec{s}].d\vec{r})$



How we get the "image" from a diffraction spectra

- If $\vec{s}$ statisfy the Laue equations $\vec{a}.\vec{s}=h, \vec{b}.\vec{s}=k, \vec{c}.\vec{s}=l$
- $\vec{s}$ is a vector a lattice, named reciprocal lattice

$$\vec{s} = h.\vec{a}^* + k.\vec{b}^* + l.\vec{c}^* \qquad \text{with } \vec{a}^* = \frac{\vec{b}\wedge\vec{c}}{\vec{a}.\vec{b}\wedge\vec{c}},\ \vec{b}^* = \frac{\vec{c}\wedge\vec{a}}{\vec{a}.\vec{b}\wedge\vec{c}},\ \vec{c}^* = \frac{\vec{a}\wedge\vec{b}}{\vec{a}.\vec{b}\wedge\vec{c}}$$

- Electronic density calculation

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

↓

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

Phase ?

- A problem …
  - On the intensity of the wave is measured
    - The amplitude of the structure factor can be calculated
    -
  - The phase information is lost !
- No direct calculation of the electron density can be done from the data collected on the detector

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
  - Discrete Fourier transform

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

Go back to 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 (h,0,0) is the coefficient for or sinusoidal function, the period of which is the a axis
- Higher indices correspond to higher indices
- The higher you go, the highest is the resolution

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): $E=h\nu=h\frac{c}{\lambda}\ ,\ p=\frac{h\nu}{c}$
    - with $h(\text{Plank constant})=6.6257\,10^{-34}\,\text{J.s}\ ,\ p:\text{momentum}\ ,\ \nu:\text{frequency}$
    -
    - Particle (mass ≠ 0): $E=\frac{1}{2}mv^2\ ,\ p=mv\ ,\ \lambda=\frac{h}{mv}$

Energies and wave length

- Electromagnetic waves / photons (1901, Röntgen)
  - Photon energy: $E = h\nu = h\frac{c}{\lambda} \rightarrow 7\text{ keV} < E < 17\text{ keV or } 1.7\text{ Å} > \lambda > 0.7\text{ Å}$
- 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 (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 $100\text{ keV} < E < 300\text{ keV and } 0.004\text{ Å} > \lambda > 0.0009\text{ Å}$

Neutron

- 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

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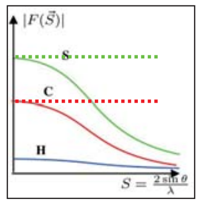
Differences and similarities with X-rays

- Neutrons interact with nucleus
  - ➢ We observe nucleus and not electron density
    - Proton can be observed
- 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 $f_{at}(\vec{s}) = f_{at}(|\vec{s}|) = 2\int_{r=0}^{\infty} \rho(r) \sin\frac{(2\pi s r)}{s} dr$
    - $f_{at}(0) = Z$
    - For neutron $f_{nuc}(s) = \sigma_{scat}$
    - No decrease with s or resolution



Differences and similarities with X-rays

- 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

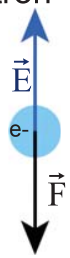
Electrons

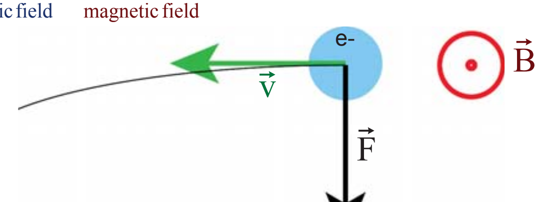
- Charged particle ($q = 1.6\ 10^{-19}$ C, $m_e = 9.1091\ 10^{-31}$ kg)
- Strongly interact with matter
  - Elastic interaction with
    - atomic electron (small energy transfer)
    - Nucleus (Ruthrford scattering)
  - “Sense” the electrstatic potential

Imaging or diffraction?

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

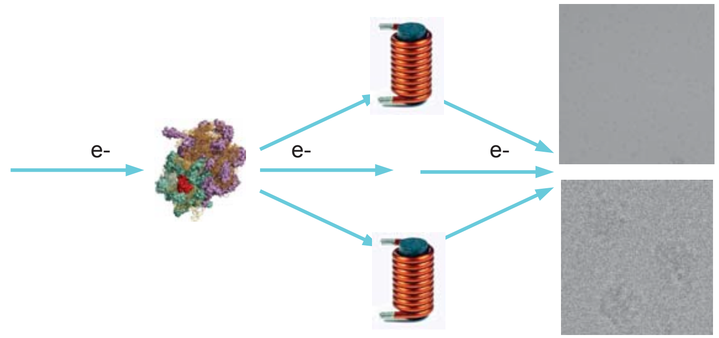
$$\vec{F} = \underbrace{\bar{e}.\vec{E}}_{\text{electric field}} + \underbrace{\bar{e}.\vec{v} \wedge \vec{B}}_{\text{magnetic field}}$$

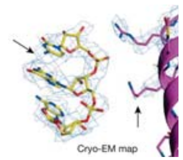




- A magnetic field can deviate an electron
- A lens can be made for electrons with magnet
  - First one made in 1929 (Ruska & Knoll)

Direct imaging of the molecule





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

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 is also possible
- NMR
  - Gather structural information (local interatomic distance, …) => search for models that satisfy the data.
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