A few physical concepts essential to structural biology

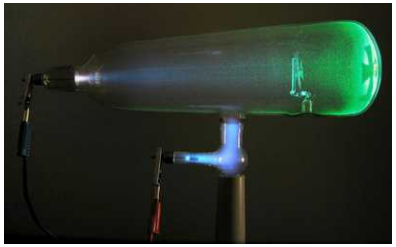
Dominique HOUSSET
E-mail : dominique.housset@ibs.fr

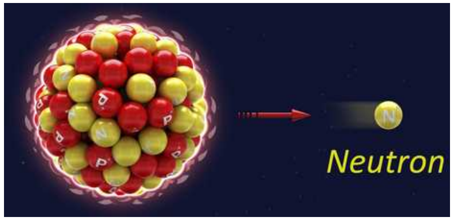
How can we obtain structural information?

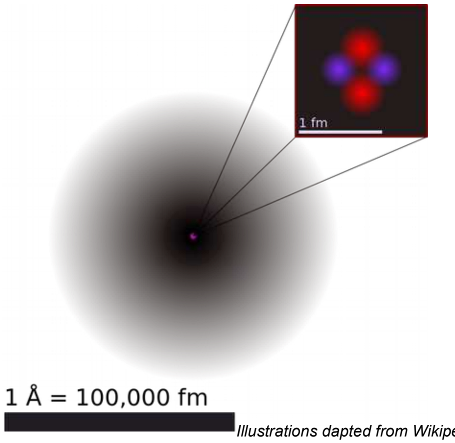
- A question of interaction between radiation and matter

- 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, ...



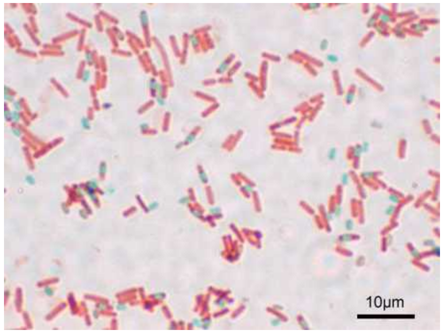


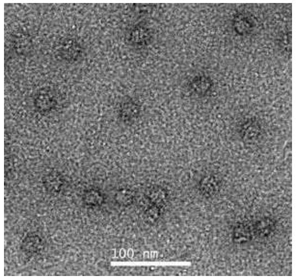


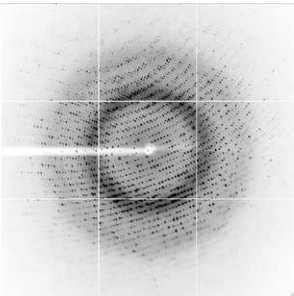
Illustrations dapted from Wikipedia

Possible approaches

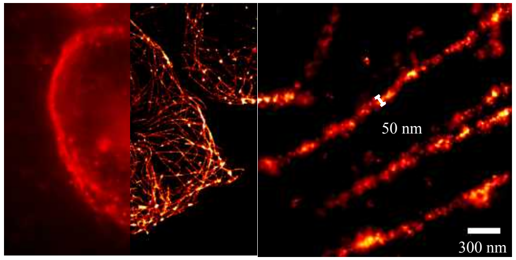
- “Imaging” techniques
  - Visible light microscopy
  - Electron microscopy
  - X-ray, neutron or electron crystallography



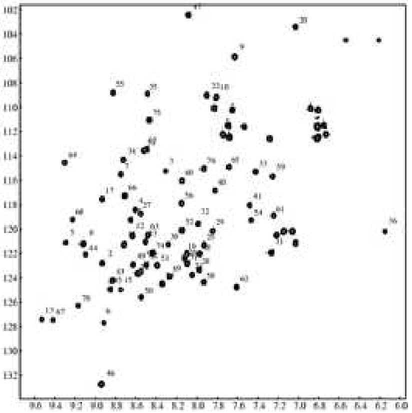
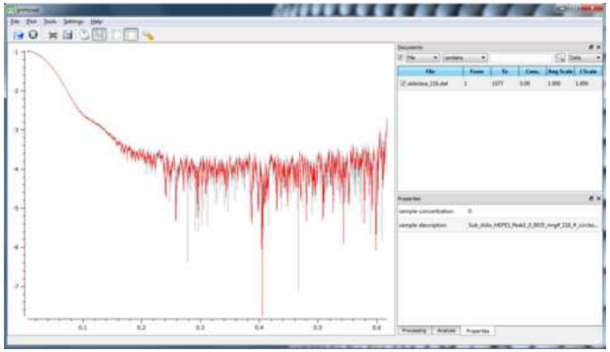




- Localization technique
  - Super-resolution microscopy



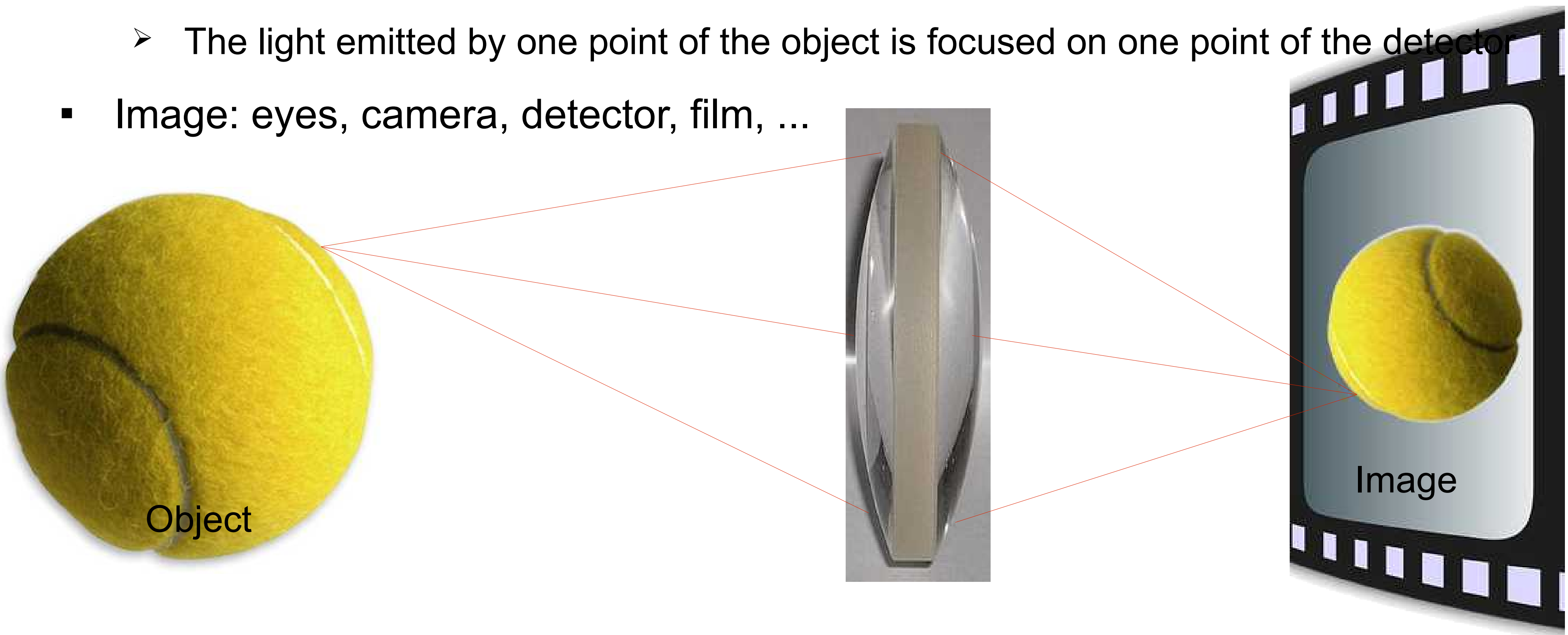
- Spectroscopic techniques
  - NMR
  - SAXS

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?

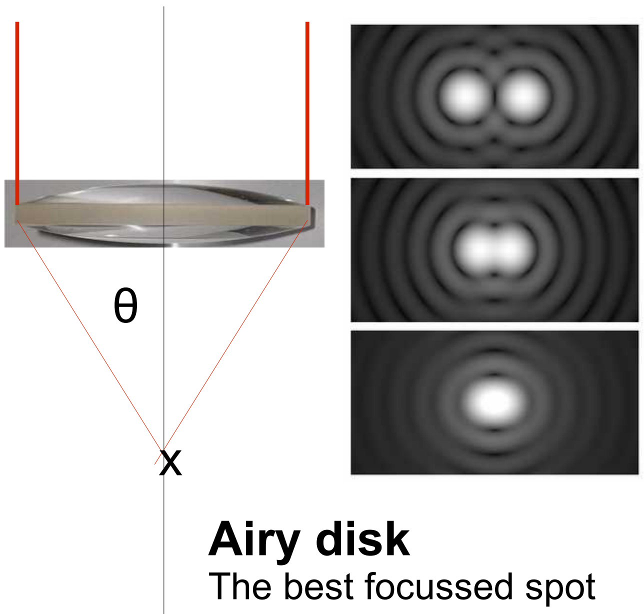
- Be because of the wave nature of light
  - Diffraction of light by a disk when the disk size of the same order of magnitude than the wave length:
    - The angle at which the 1st minimum occurs is given by:

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

- Rayleigh criterion

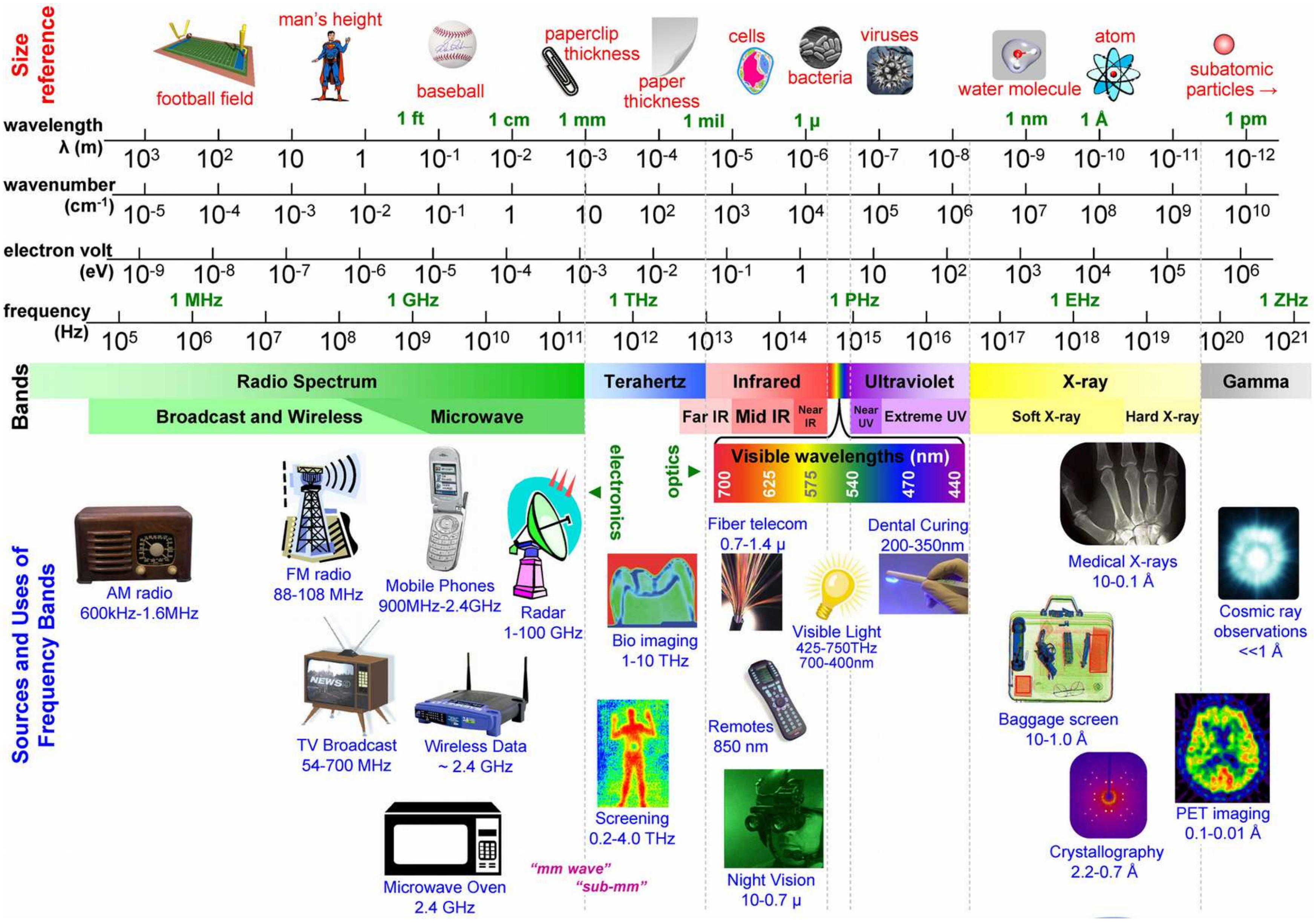
$$d = 1.22\frac{\lambda}{2n\sin\theta} \approx \frac{\lambda}{2\sin\theta} \quad \text{(in air)}$$

  - 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



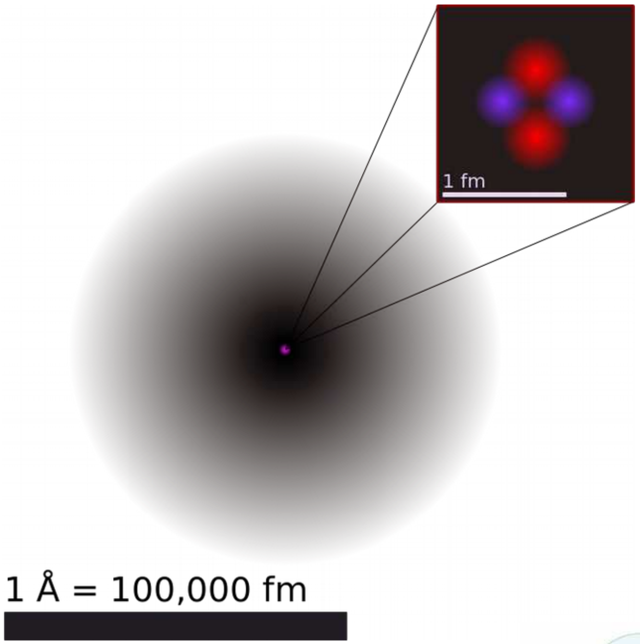
Airy disk
The best focussed spot of light made by a lens of circular aperture is limited by the diffraction light

How to increase resolution?

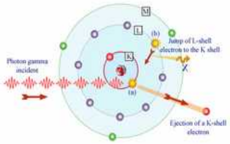


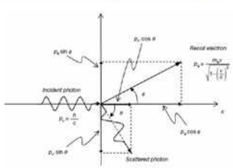
Atomic resolution with photons?

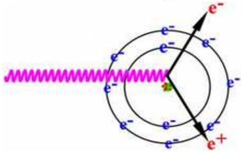
- Decrease the wavelength
  - For atomic resolution
    - d ~ 1 Å => $\lambda \leq 2$ Å
  - Use photons in the domain of X-rays
    - Typically: for $\lambda = 1$ Å, $E = h\nu = hc/\lambda \approx 12.4$ keV

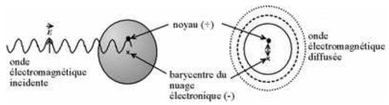


- Do X-rays interact with atoms?
  - Yes, X-ray photons with the electronic cloud of an atom
    - Photoelectric effect (absorption of a photon and emmision of an elctron)
    - Compton Scatering (inelastic scattering between a photon and an electron)
    - Electron/Positron pair production (only high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







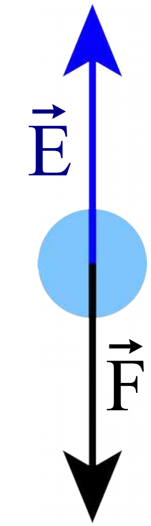


How a photon is scattered by an atom ?

- **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

Oscillating 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: $\vec{d}=Ze.\vec{r}$

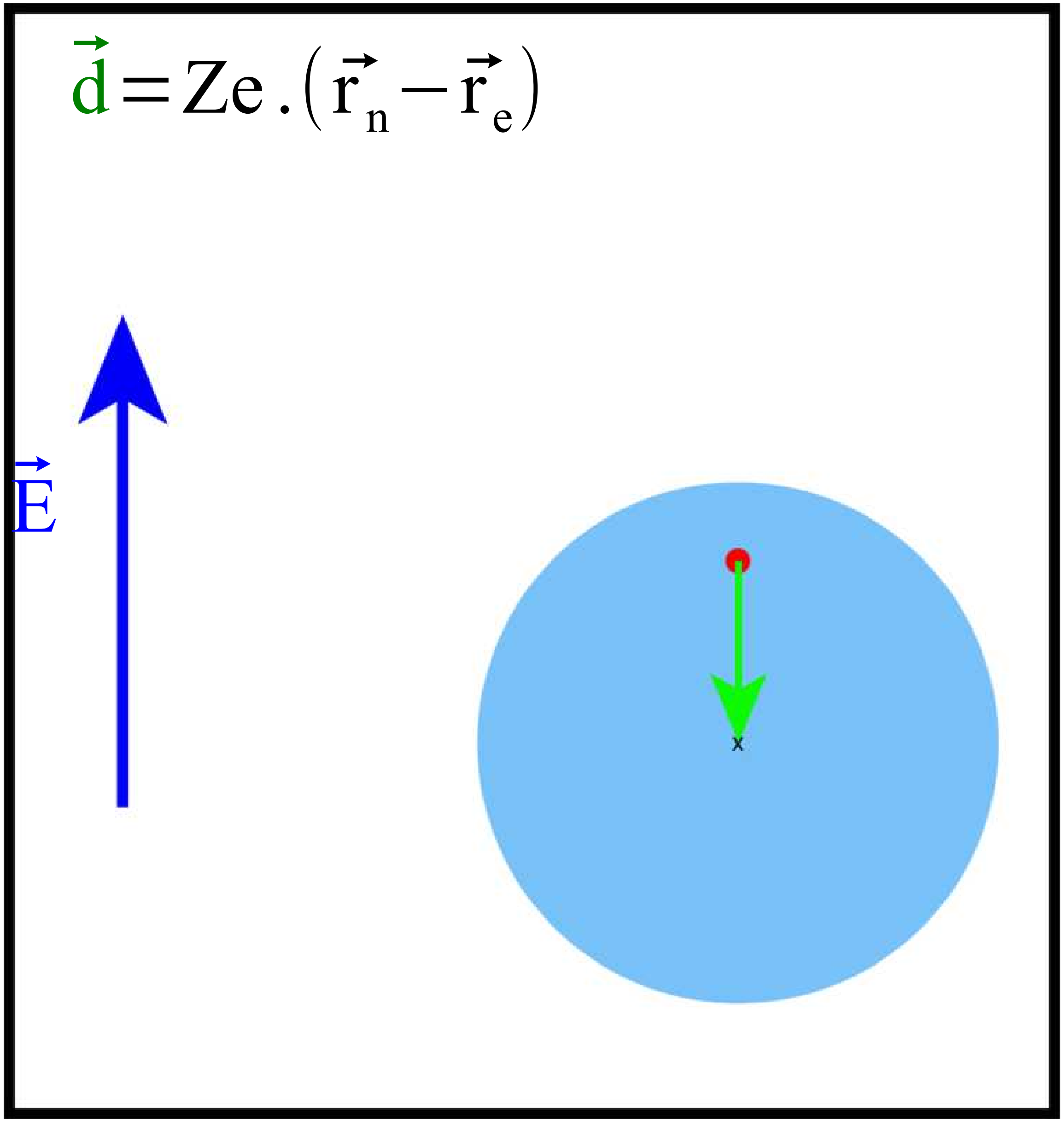
(with $\vec{r}$ vector between center of mass of electrons and nucleus)

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

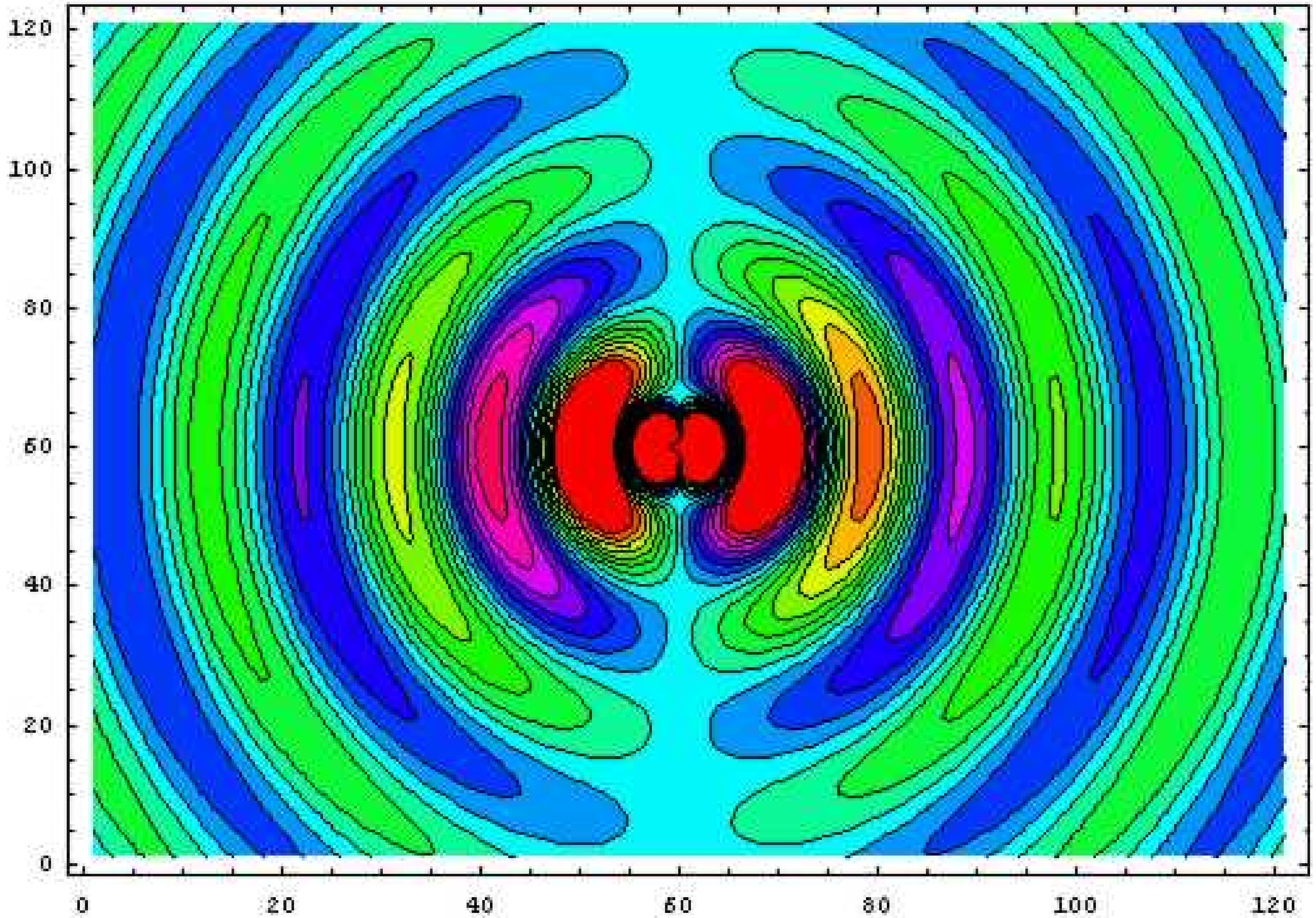
$$\underbrace{\vec{E}=\vec{E}_0\cos[\omega t]}_{\text{oscillating electric field}} \Rightarrow \underbrace{\vec{d}=-\left(Ze^2\frac{\vec{E}_0}{m_e\omega^2}\right)\cos[\omega t]}_{\text{oscillating dipole}}$$

Oscillating dipole and emitted wave

The incident electromagnetic wave induce the oscillation of the electronic cloud



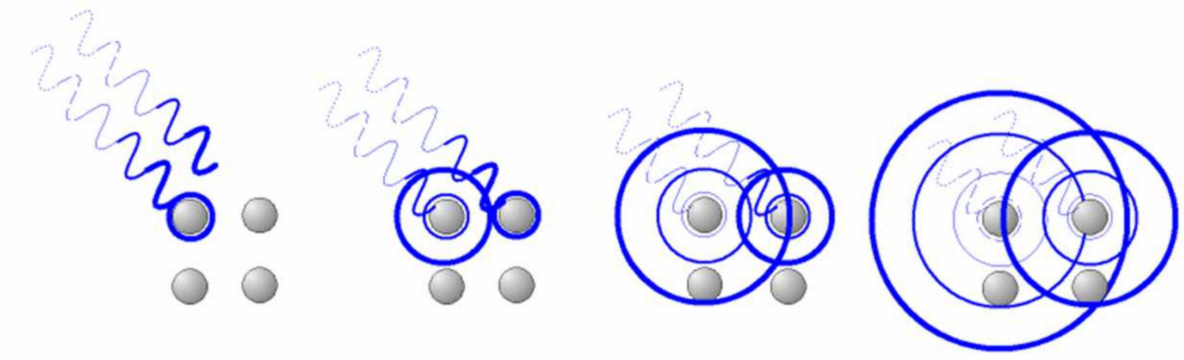
Oscillating dipole and emitted wave



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 : momentum , ν : frequency

Energies and wave length

- 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)

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

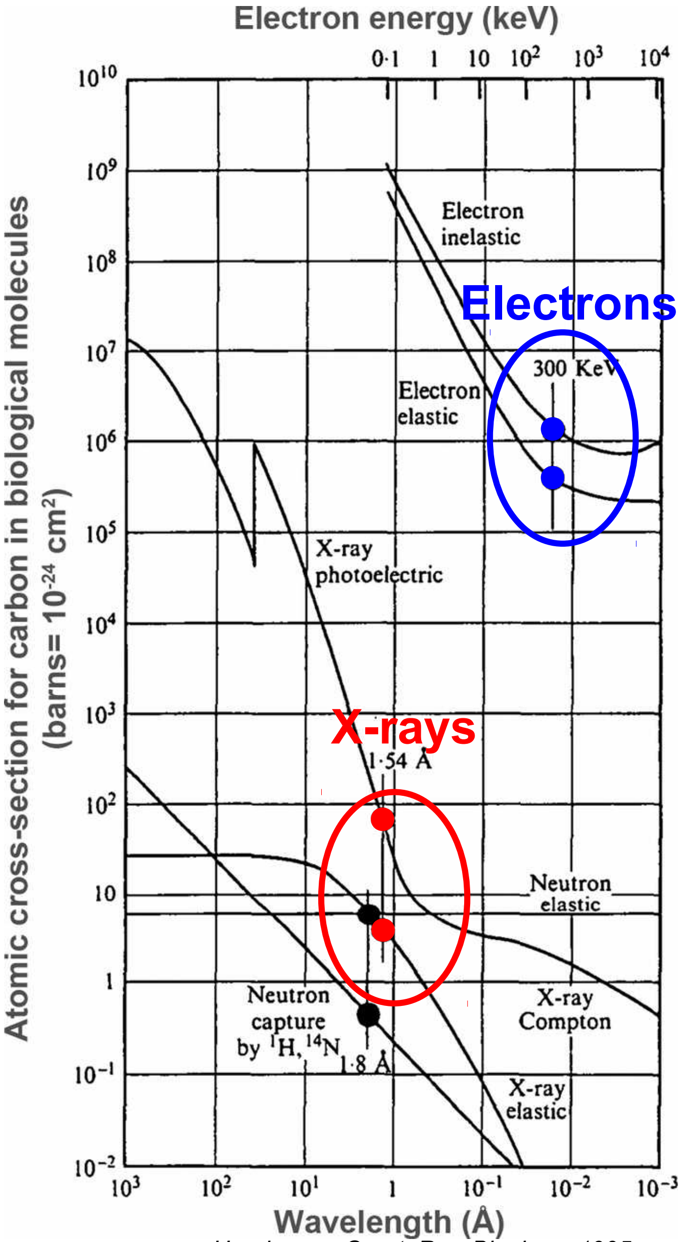
in practice $100\ \text{keV} < E < 300\ \text{keV}$ and $0.037\ \text{Å} > \lambda > 0.019\ \text{Å}$

- 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

- Strongly interact with matter
  - About 10^4 times more than X-rays
- Elastic scattering represents 25% of scattered electrons
  - Only 5% for X-rays
- X-rays are quite inefficient to probe matter
  - 98% of the photons go trough the sample without being scattered (with a typical protein crystal)
  - For the 2% remaining
    - 84% are anihilated
    - 8% are involved in Compton scattering
    - 8% useful for Bragg diffraction (elastic scattering)



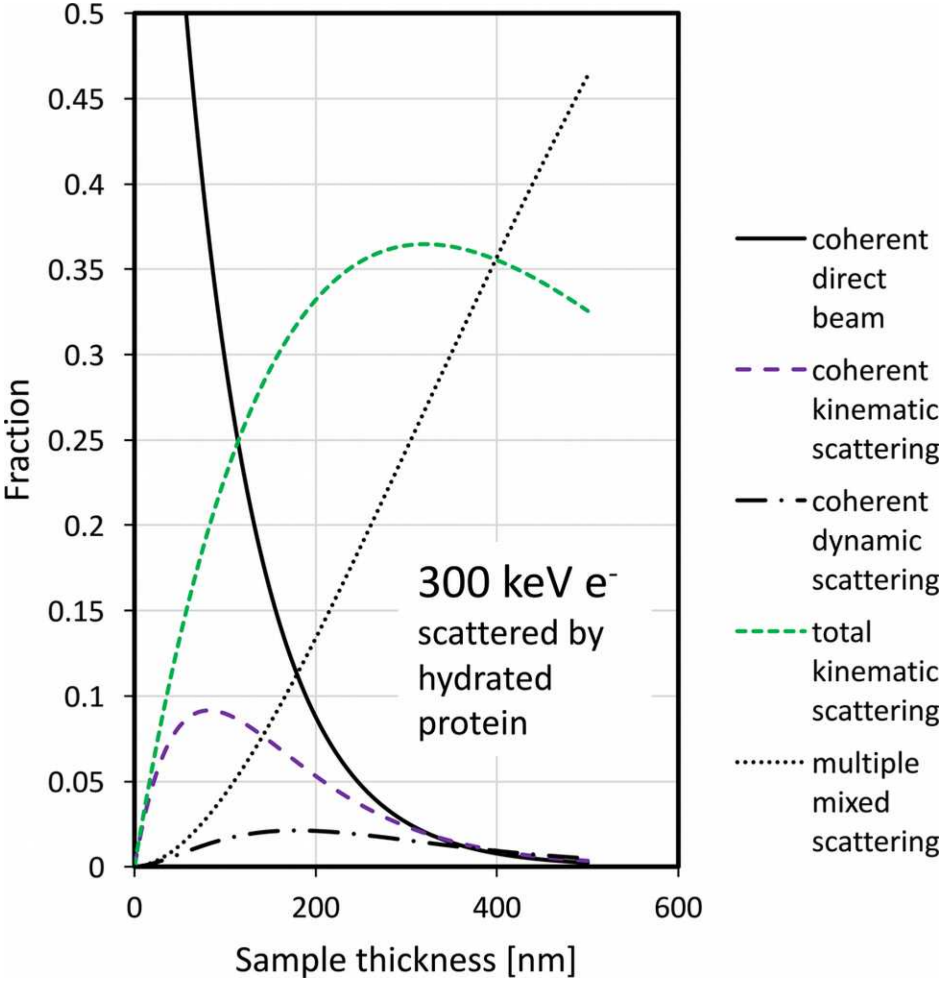
Henderson, Quart. Rev. Biophys., 1995

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
- Mean free path of electrons in ice

	120 keV	300 keV
Λ for $e_{inelastic}$	50 - 200 nm	~ 400 nm
Λ for $e_{elastic}$	300 - 800 nm	~ 1600 nm

Angert et al., Ultramicroscopy, 1996 ; Yonekura et al., J. Struct. Biol., 2006 ; Grim et al., Ultramicroscopy, 1996 ; Feja & Aebi, J. Microsc., 1999

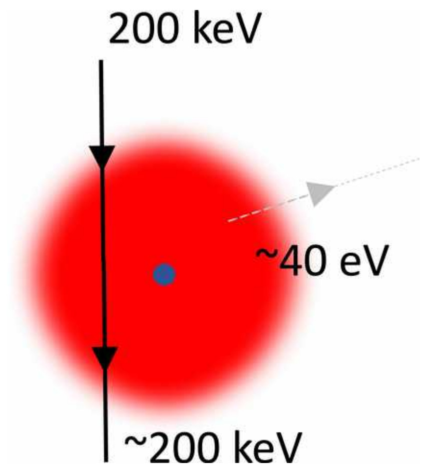


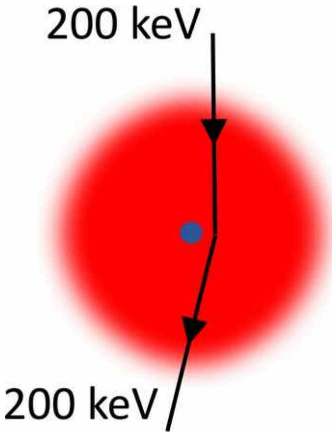
Illustrations taken from J.P. Abrahams

- For crystals optimal thickness is larger
  - I ~ N^2

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)

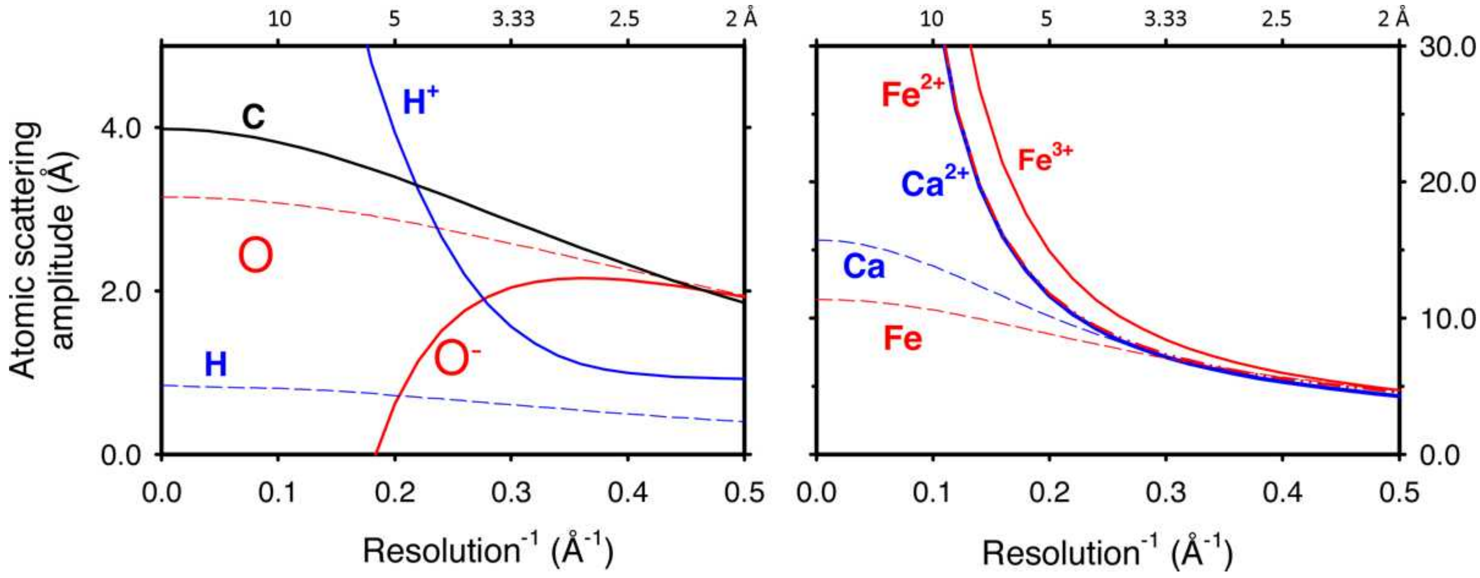




Illustrations taken from J.P. Abrahams

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 the Coulomb potential
  - Depends from both the electron density and charges

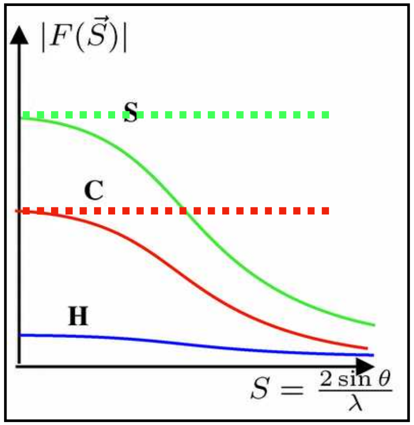
Illustrations taken from K. Yonekura

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

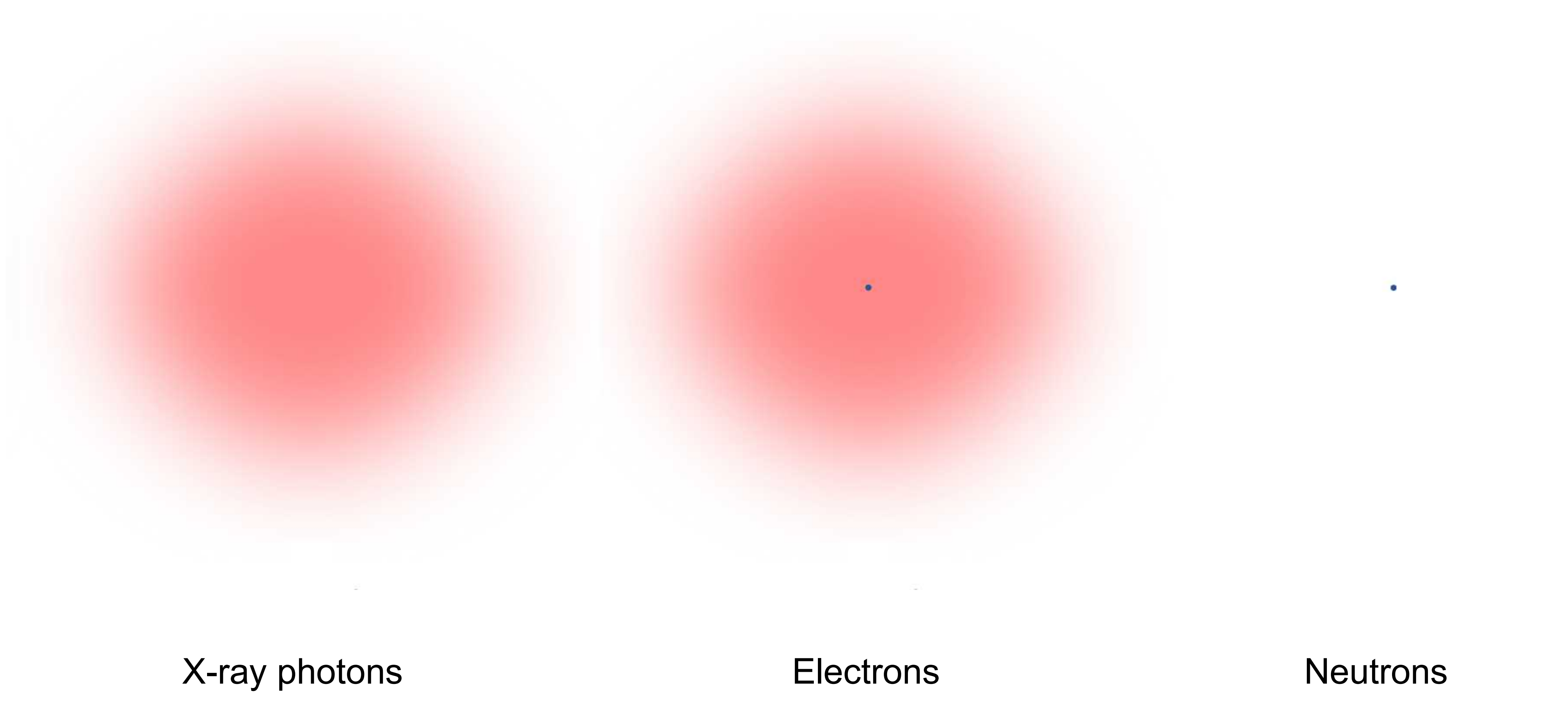
Neutrons: differences and similarities with X-rays

- 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 $f_{at}(\vec{s}) = f_{at}(|\vec{s}|) = 2\int_{r=0}^{\infty} \rho(r) \sin\frac{(2\pi s r)}{s} dr$
    - $f_{at}(0) = Z$
    - For neutron $f_{nuc}(s) = \sigma_{scat}$
    - No decrease with s or resolution

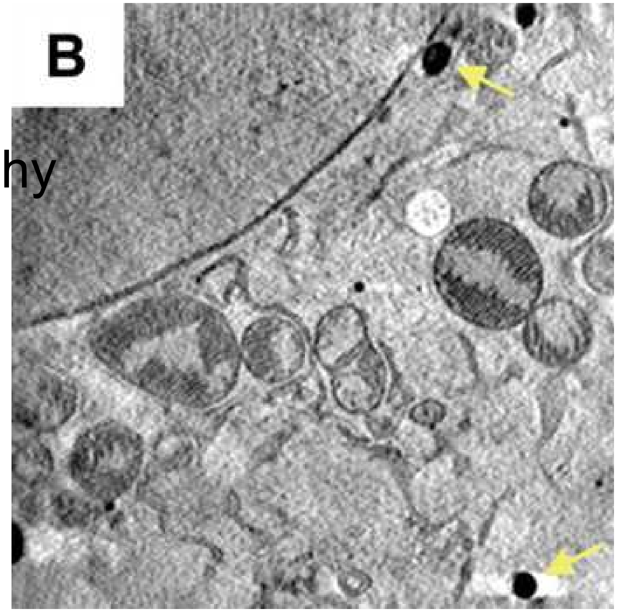


- **=> access to spatial distribution of nuclei**

Different point of views

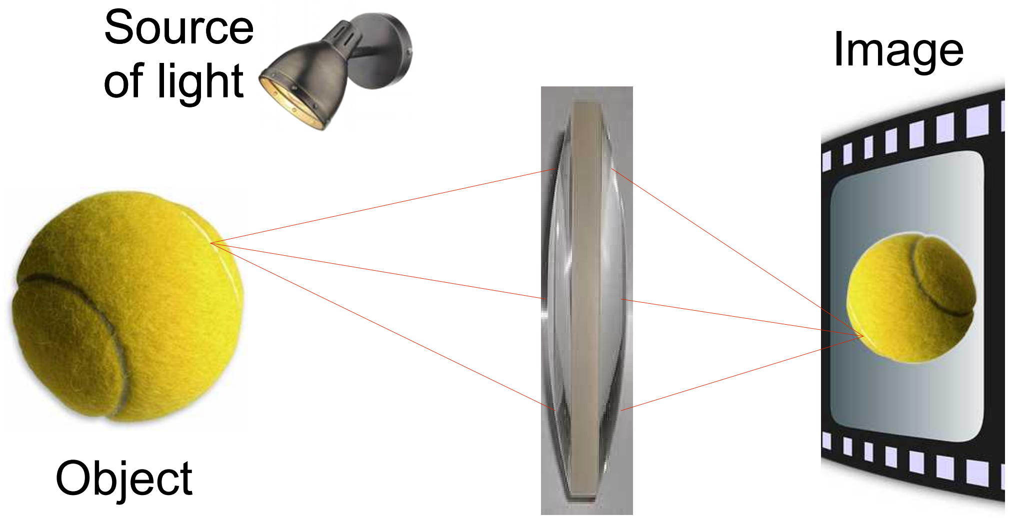


Getting the image of the molecule with X-rays?



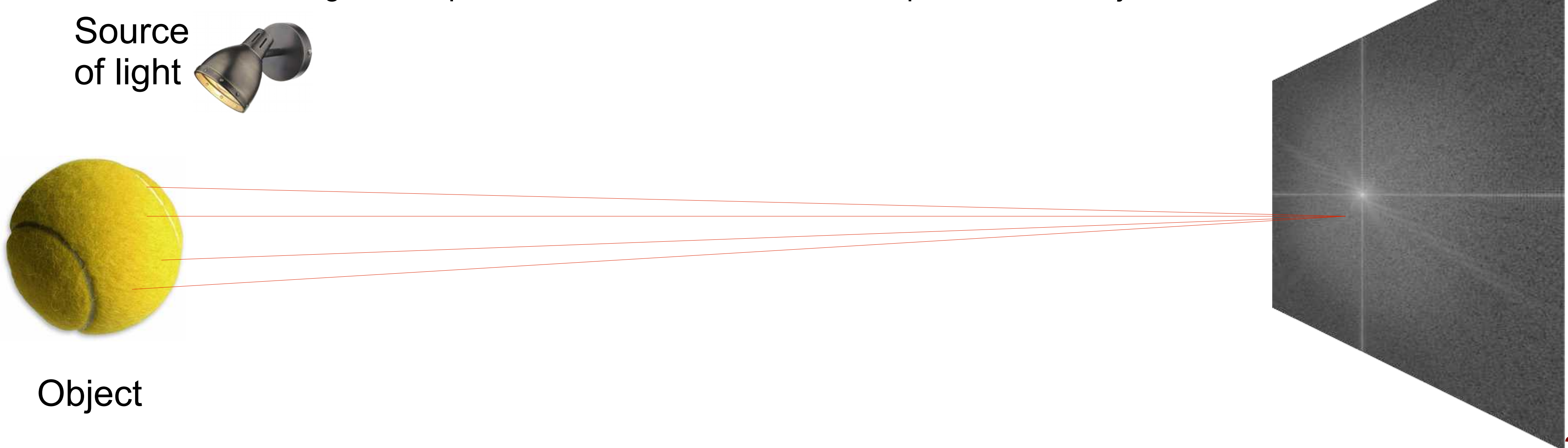
- Do we have lenses for X-ray ?
- Yes for soft X-rays : Soft X-ray microscopy or tomography
  - Energy in the range 200 eV - 1000 eV
  - Fresnel zone plate objective
  - Resolution limited to ≈ 50 nm
    - OK for cellular imaging
- No lens for 7 – 17 keV X-ray photons
  - No possibility to form an image at atomic or quasi-atomic resolution
-
- No image on the detector, but a **scattering spectra**

Scheme for a scattering spectrum



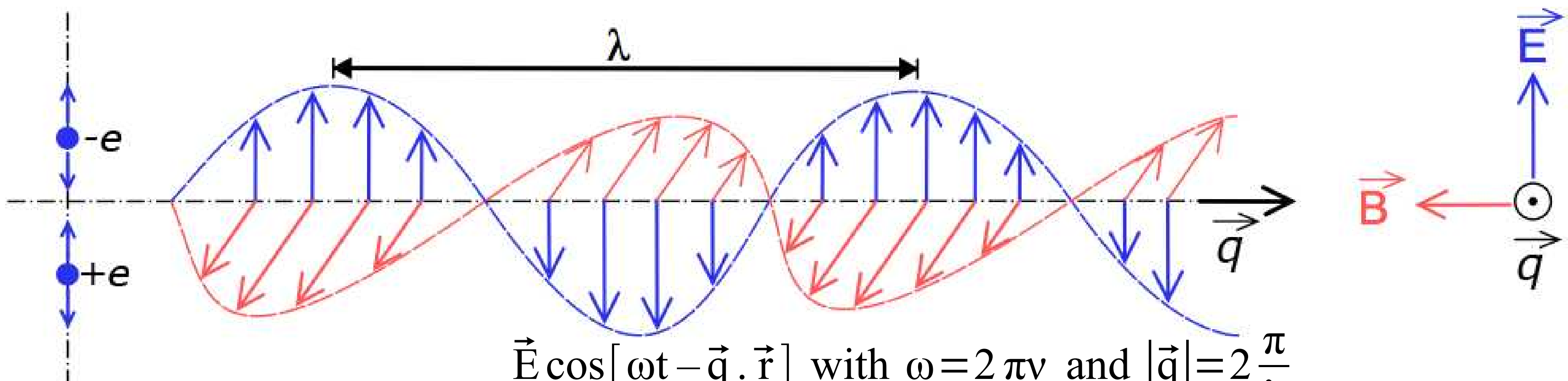
- With a lens
  - All radiation arriving on one point of the detector come from one point on the object

- Without a lens
  - Radiation arriving on one point of the detector come from all points on the object



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

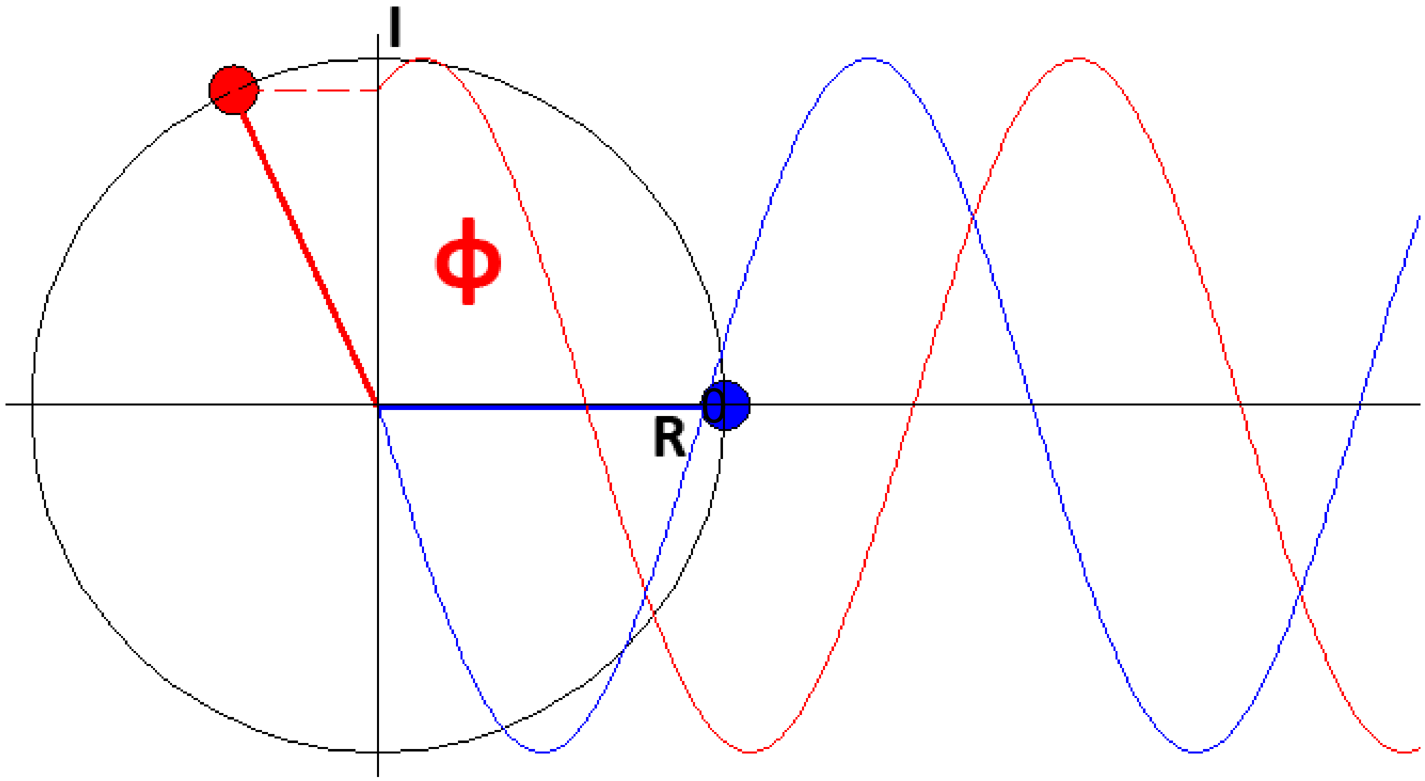


$$\vec{E}\cos[\omega t - \vec{q}.\vec{r}] \text{ with } \omega = 2\pi\nu \text{ and } |\vec{q}| = 2\frac{\pi}{\lambda}$$

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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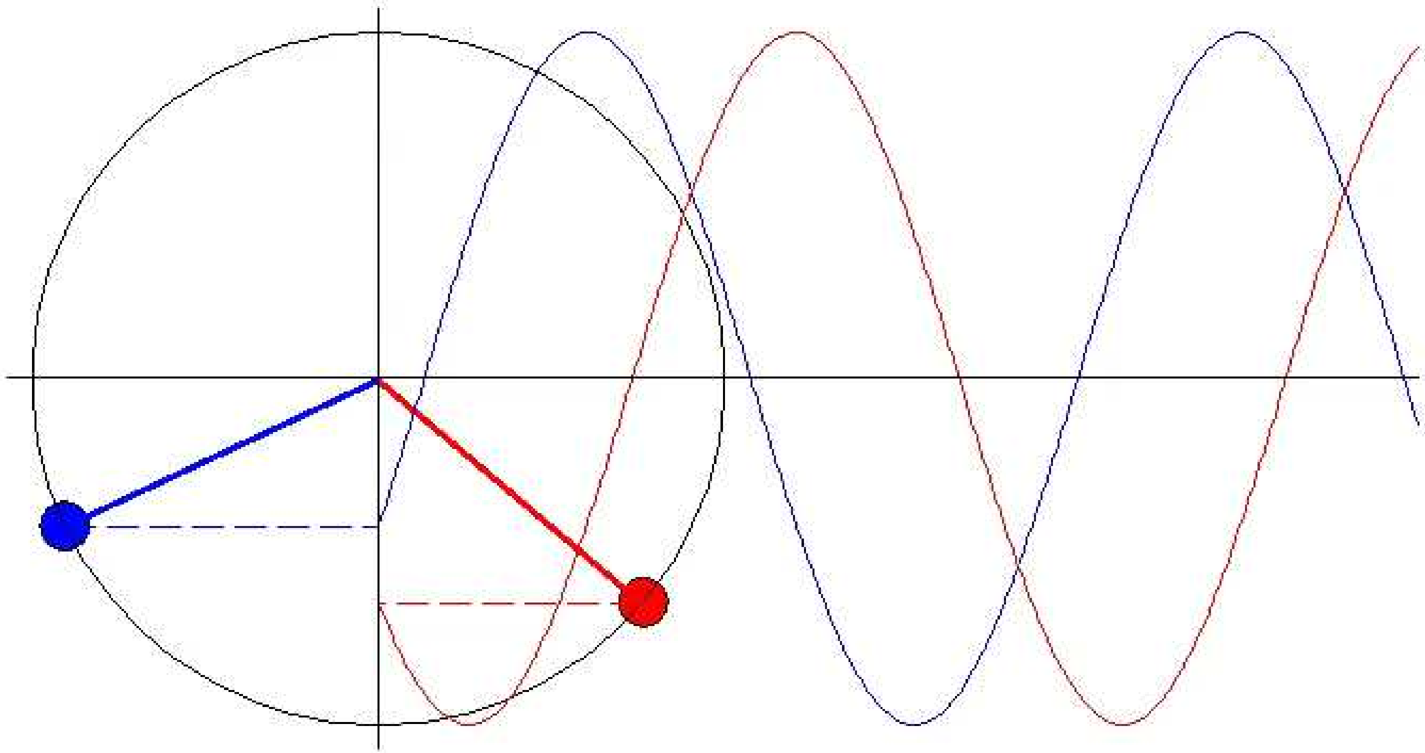
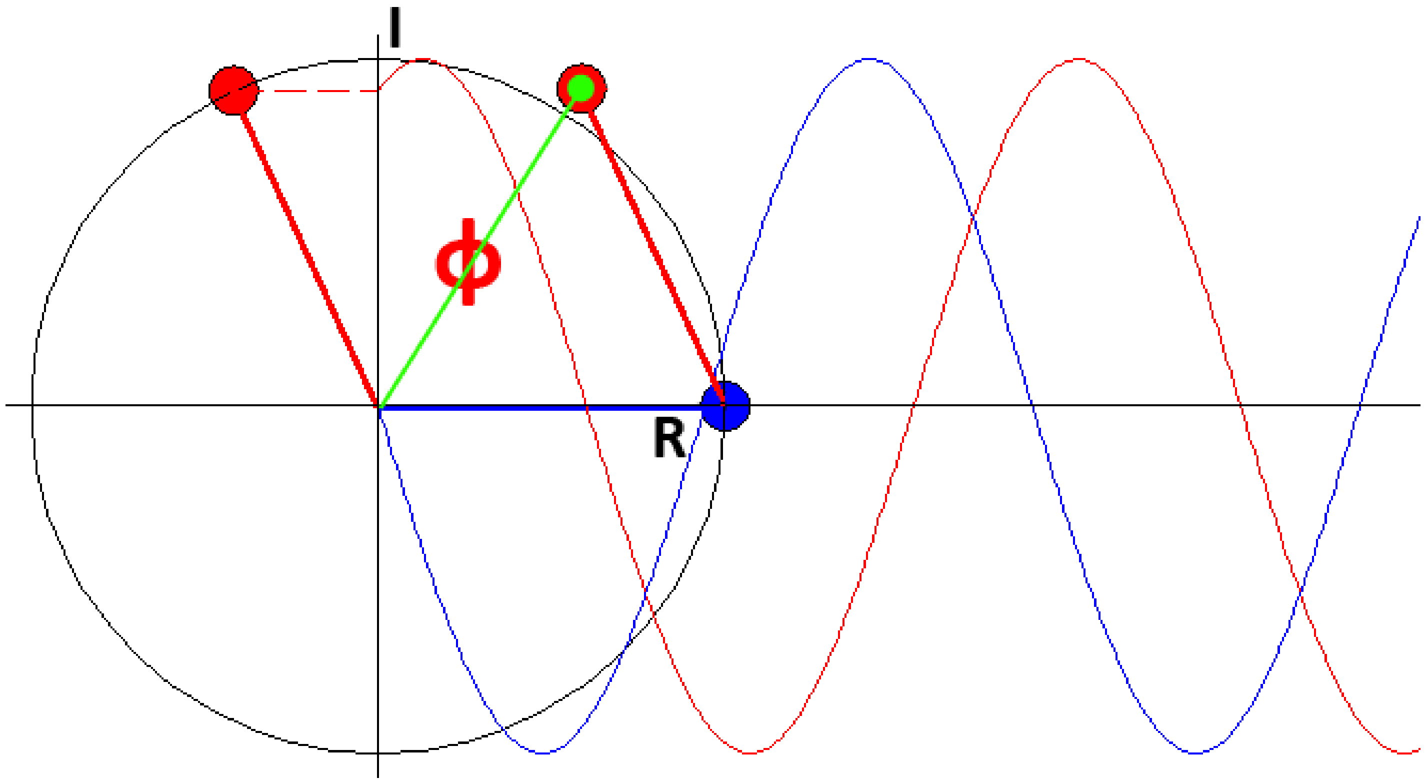


Reminder about waves

- Sum of two waves are simple to express

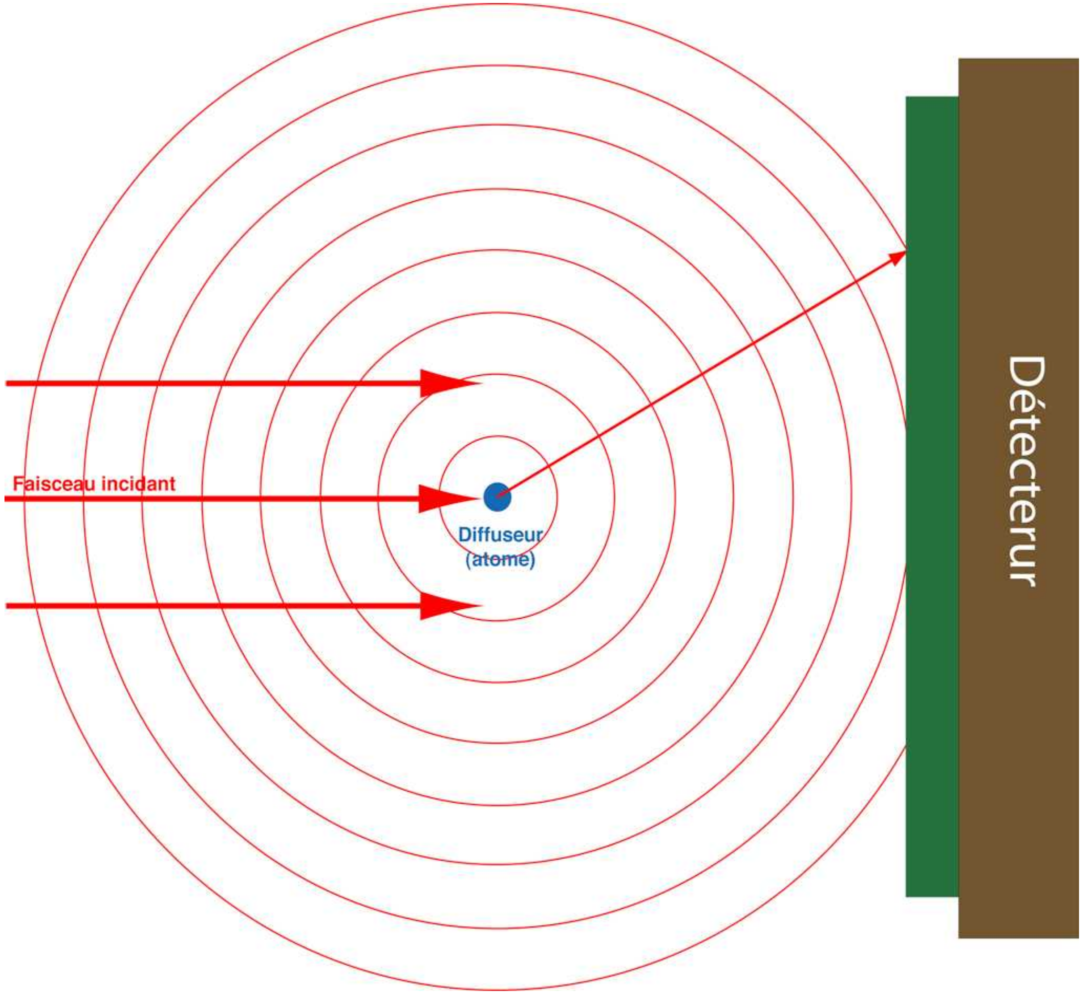
$$\vec{E}_0 \exp[i(\omega t - \vec{q}_0 . \vec{r})] + \vec{E}_0 \exp[i(\omega t - \vec{q}_0 . \vec{r})] . \exp[i\varphi] = \vec{E}_0 \exp[i(\omega t - \vec{q}_0 . \vec{r})] . (1 + \exp[i\varphi])$$



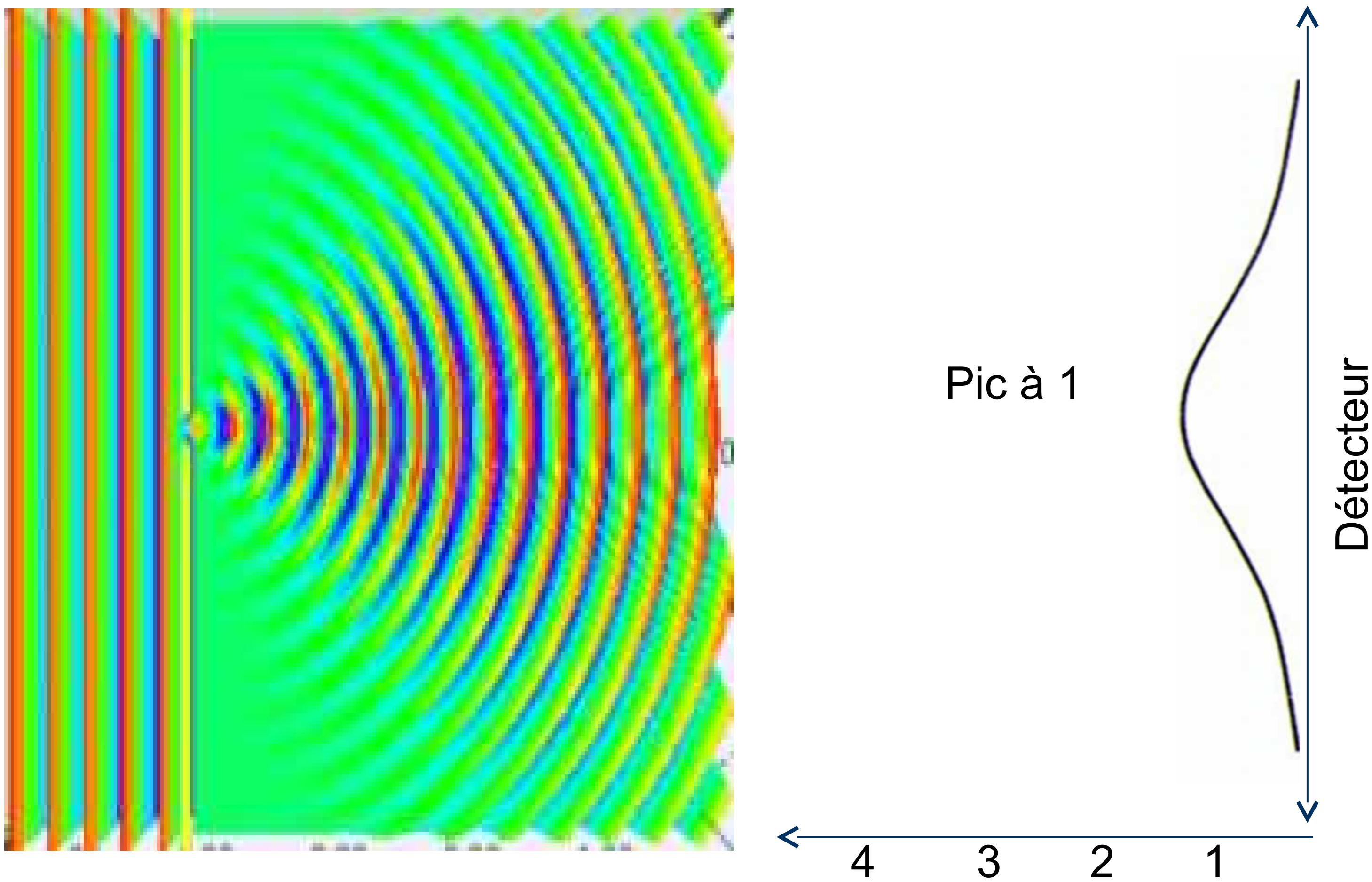
- φ = 0° (or 2π) => in phase => constructive interference
- φ = 180° (or π) => out of phase => destructive interference

Scattering by one atom

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

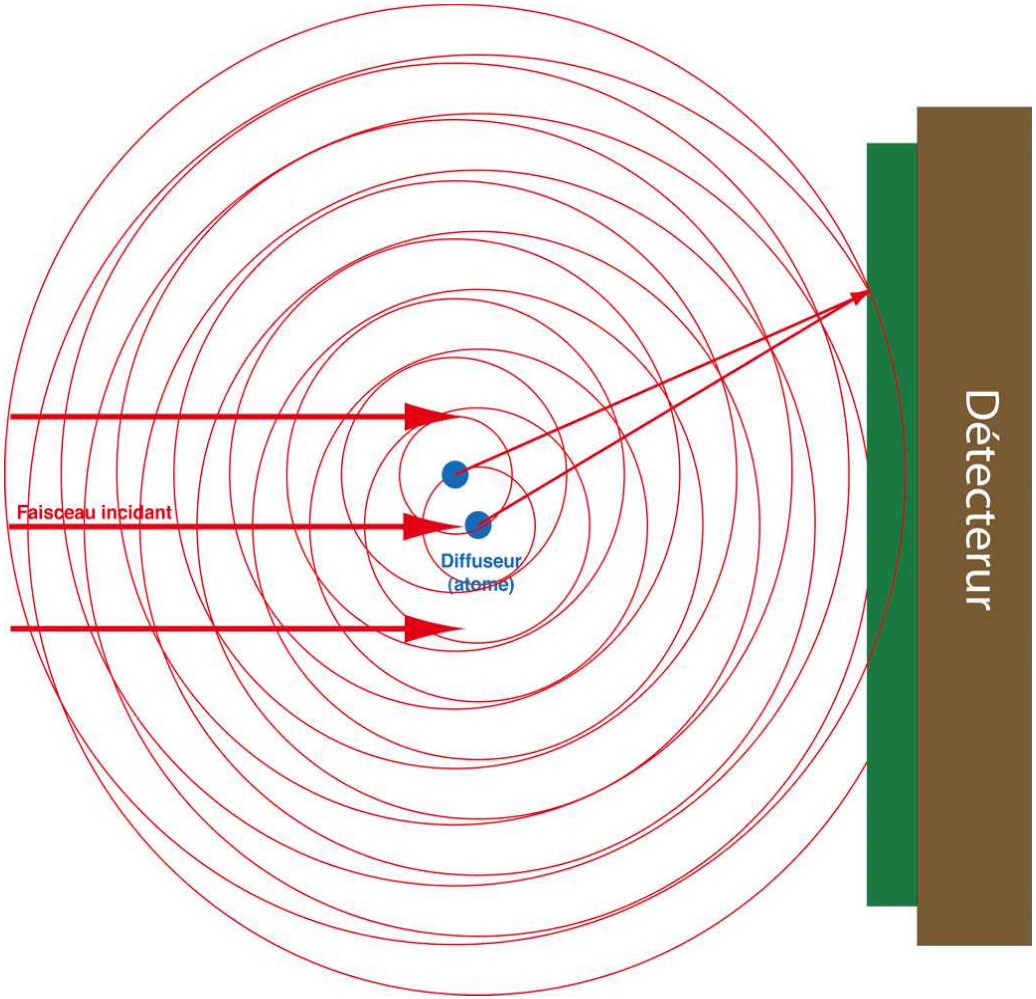


Scattering by one atom

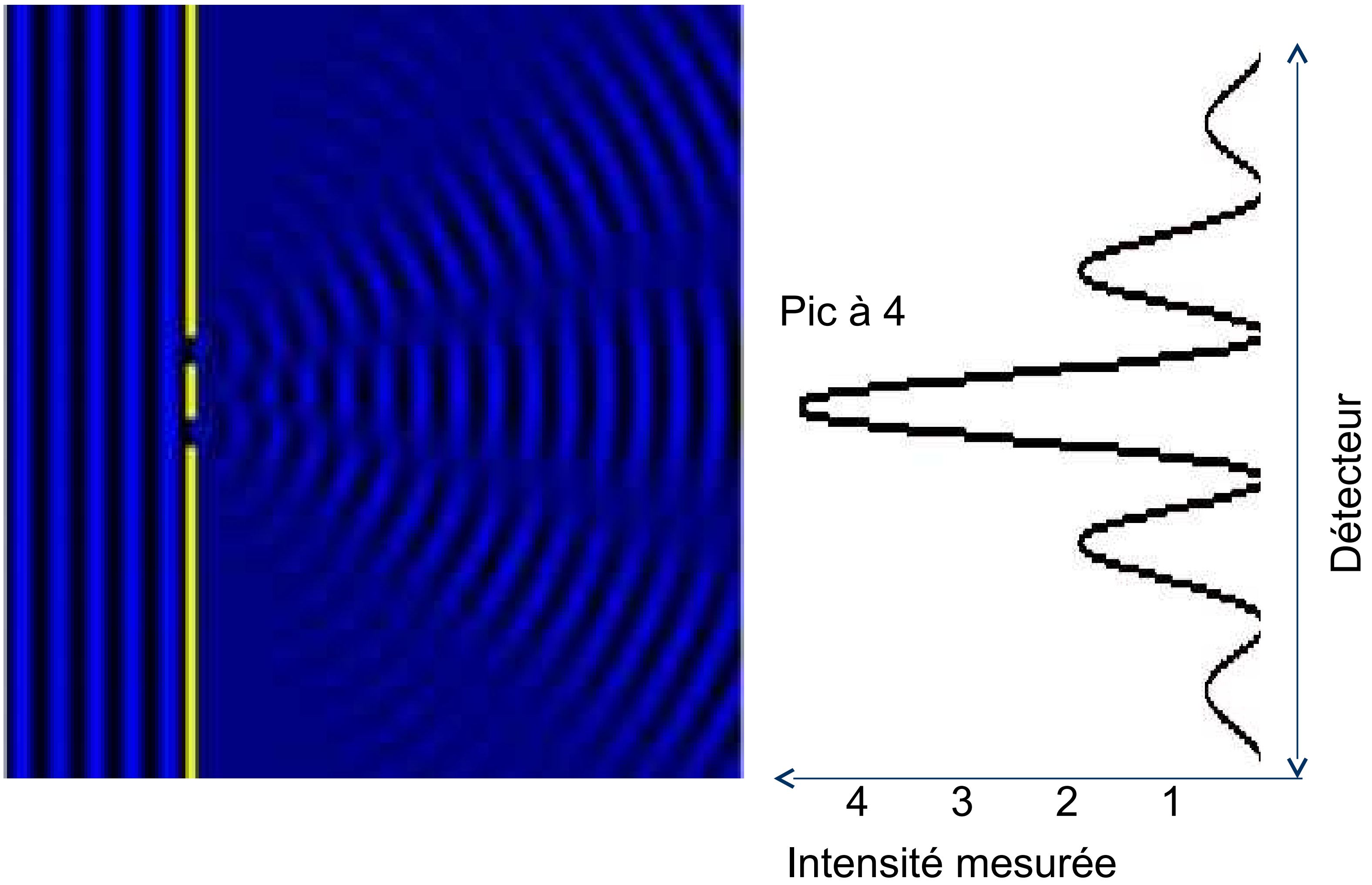


Scattering by two atoms

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

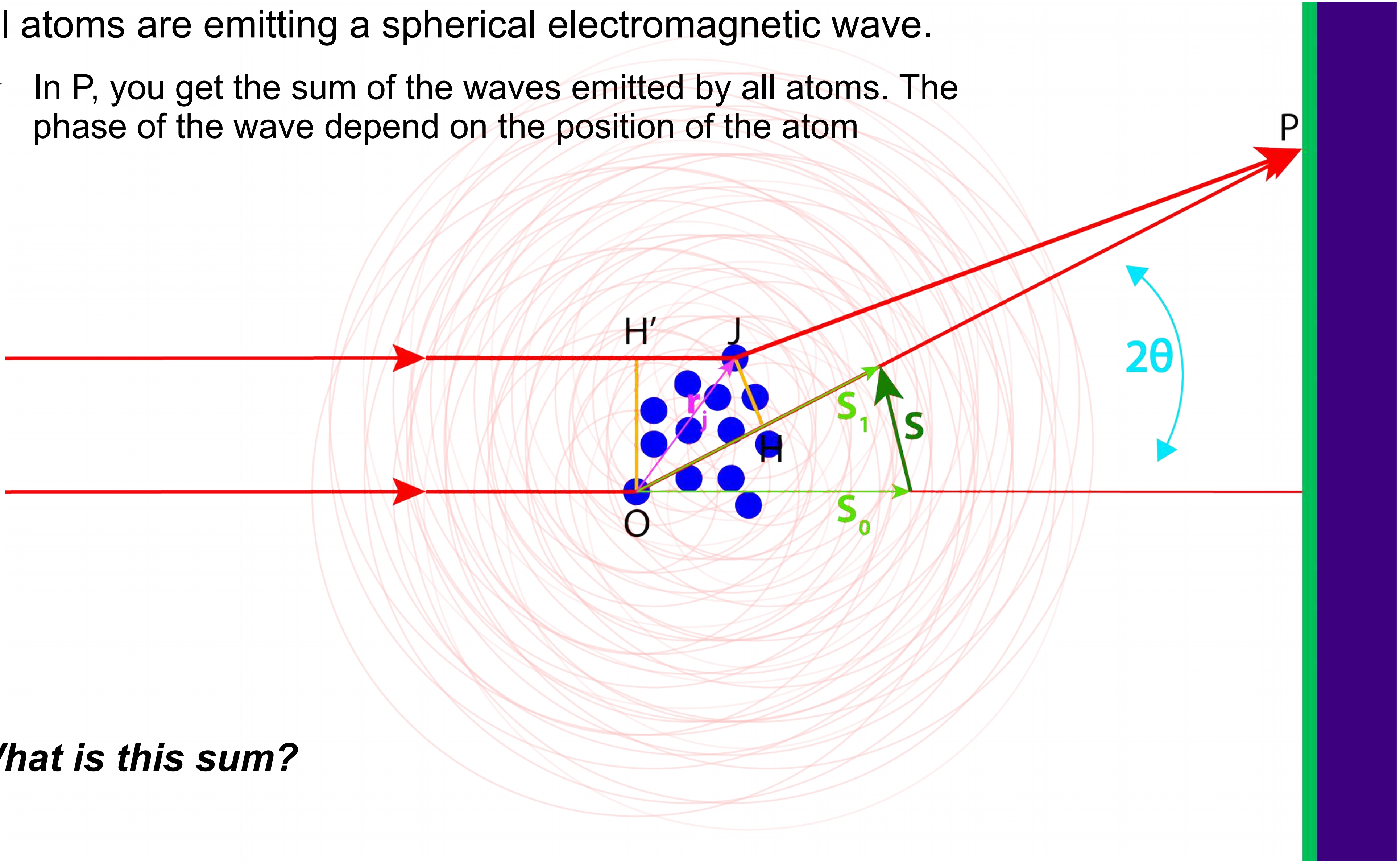


Scattering by two atoms



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

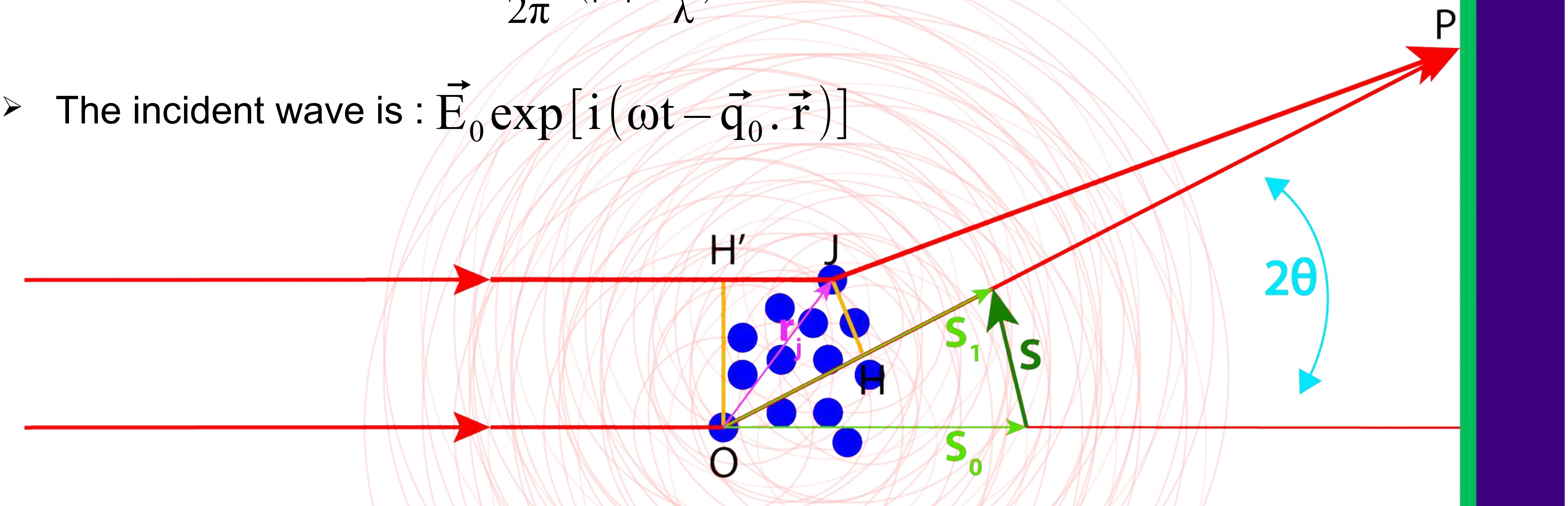


- ***What is this sum?***

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



- The wave emitted by atom J in P is:

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

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 $(|\vec{r} - \vec{r}_J| \approx |\vec{r} - \vec{r}_0|)$ 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: $\vec{s} = \vec{s}_1 - \vec{s}_0 = \frac{1}{2\pi} . (\vec{q}_1 - \vec{q}_0)$

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

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:

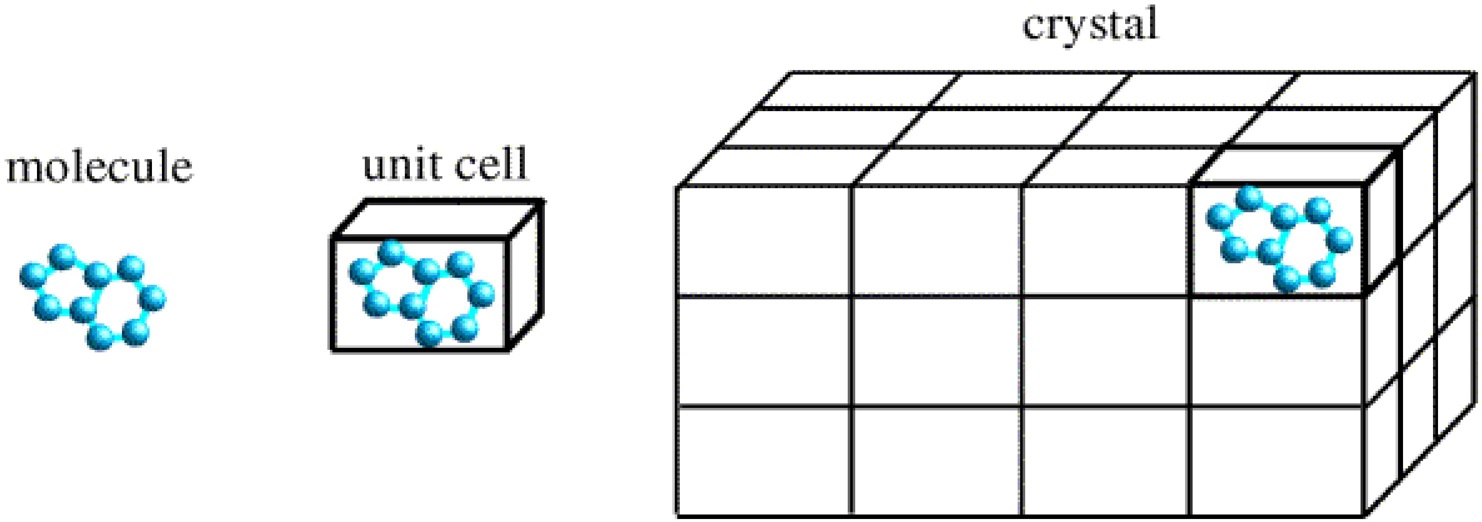
$$\rho(\vec{r}) = \sum_J f_J . \delta(\vec{r} - \vec{r}_J) = \int_{rec.vol.} F(\vec{s}) \exp[-2i\pi \vec{r}.\vec{s}].d\vec{s}$$

- The 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?

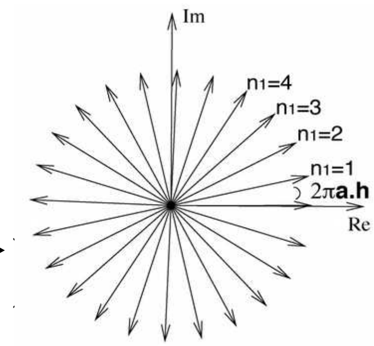
- The general form of the structure factor is:

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

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

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

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



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

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

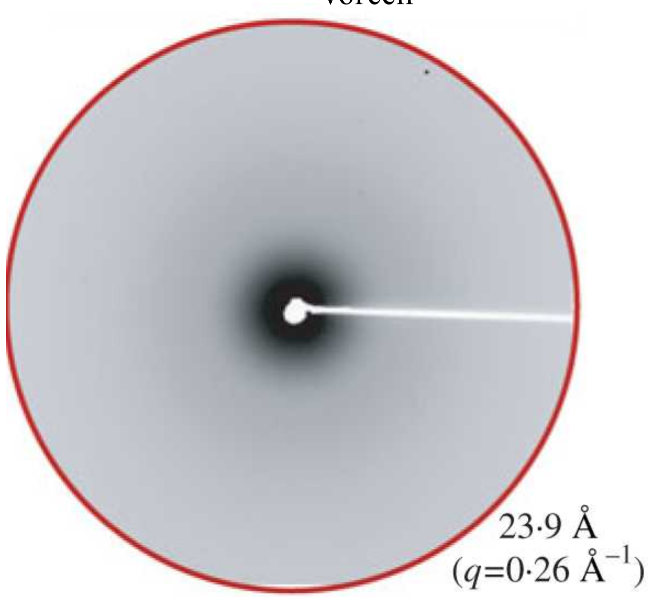
X-ray scattering by a crystal

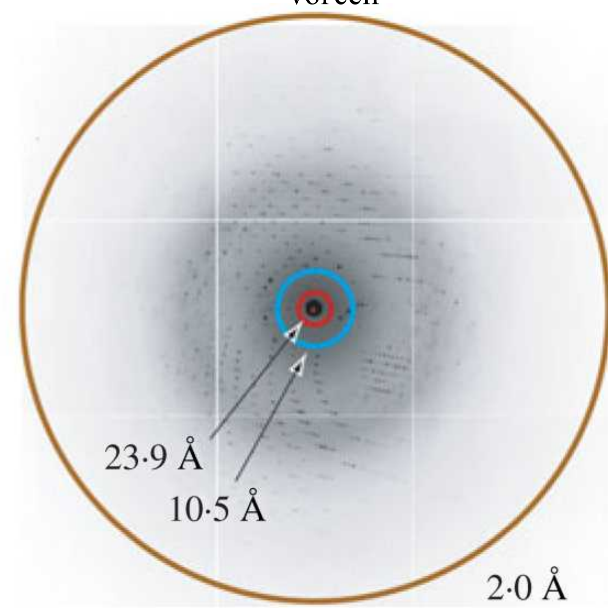
- In directions that satisfy Laue equation, the structure factor is the one of the unit cell, multiplied by the number of cells in the crystal.

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

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

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





Laue equation and Bragg's law

- In the case of a crystal:
- $\vec{s}$ satisfies 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**

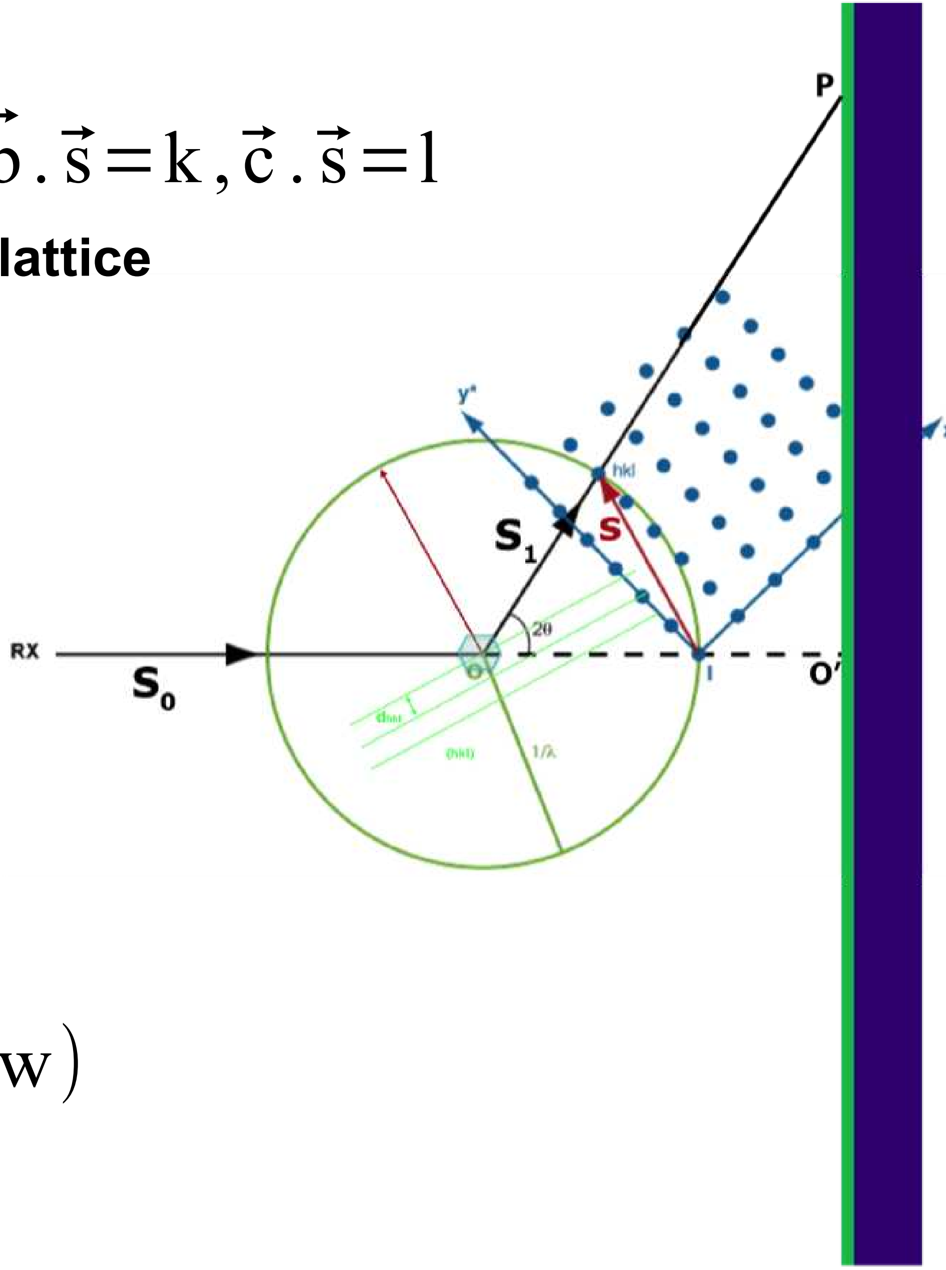
$$\vec{s}=h.\vec{a}^*+k.\vec{b}^*+l.\vec{c}^*$$

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

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

$$|\vec{s}|=|\vec{s}_1-\vec{s}_0|=\frac{2\sin\theta}{\lambda}$$

$$2\frac{1}{|\vec{s}|}\sin\theta=2d\sin\theta=\lambda\,(\text{Bragg's law})$$



How we get the "image" from a diffraction spectra

- **Electron density** calculation
  - A Fourier transform
  - General case: $$\rho(\vec{r}) = \sum_J f_J . \delta(\vec{r} - \vec{r}_J) = \int_{rec.vol.} F(\vec{s}) \exp[-2i\pi \vec{r}.\vec{s}].d\vec{s}$$
  - Crystal: $$\rho(\vec{r}) = \sum_J f_J . \delta(\vec{r} - \vec{r}_J) = \sum_{h,k,l} F(\vec{s}) \exp[-2i\pi \vec{r}.\vec{s}]$$

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

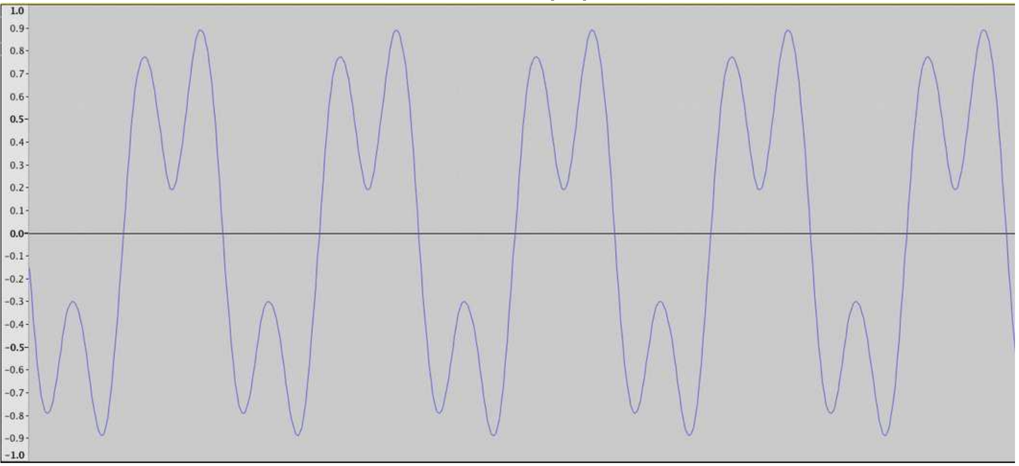
What is the meaning of F(s)?

- 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

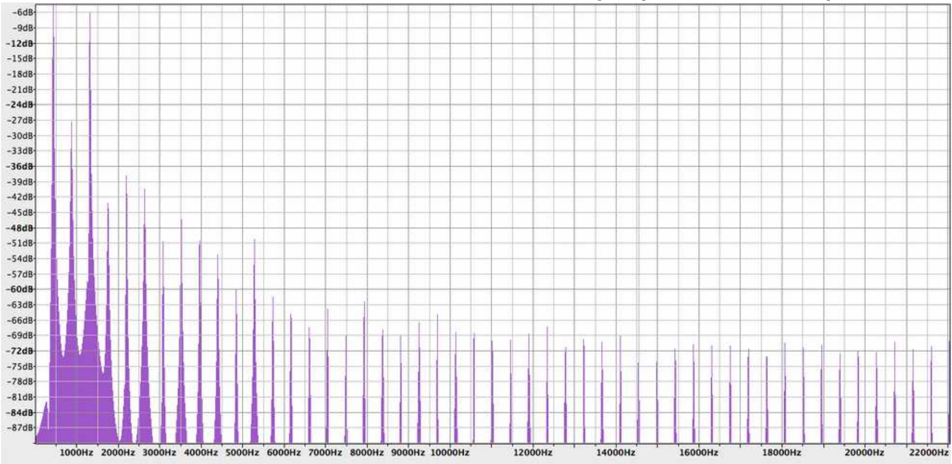
Parallels between sound and electron density

Sound

Time (s)

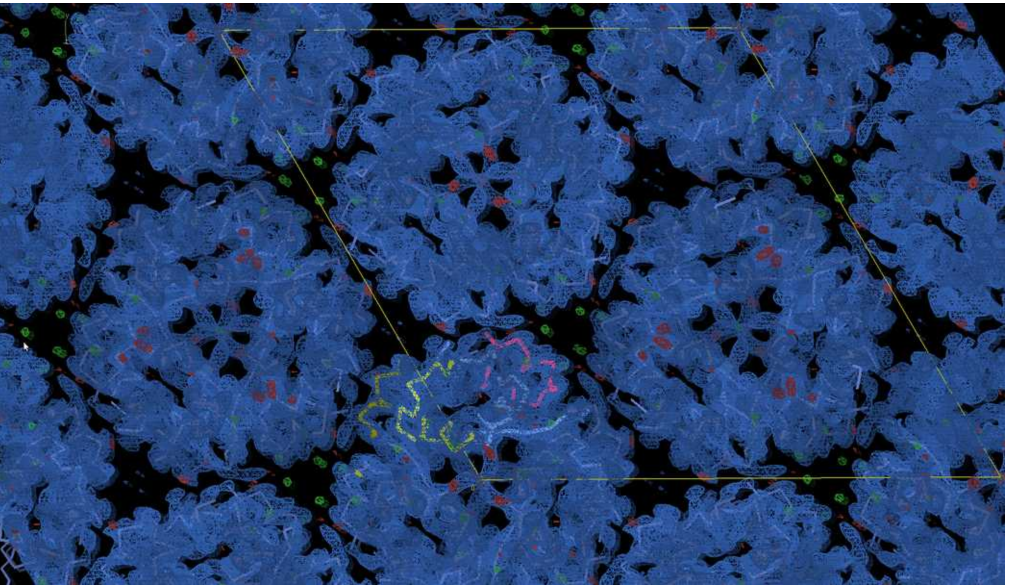


Frequency (s^{-1} or Hz)

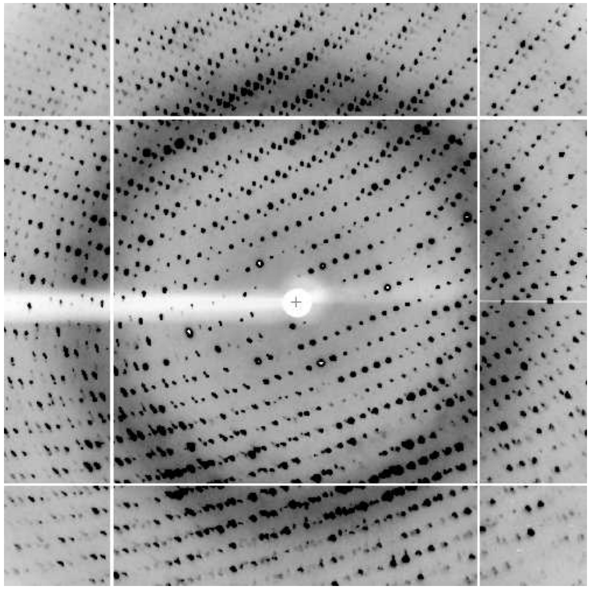


Electron density

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

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

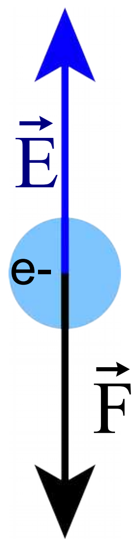
- The reflection (1,0,0) is the coefficient for or sinusoidal function, the period of which is the a axis
- Higher indices correspond to higher spatial frequencies, i.e. to periods which are fractions of the a axis
- The higher you go in indices (h,k,l), the higher is the resolution (1/d):

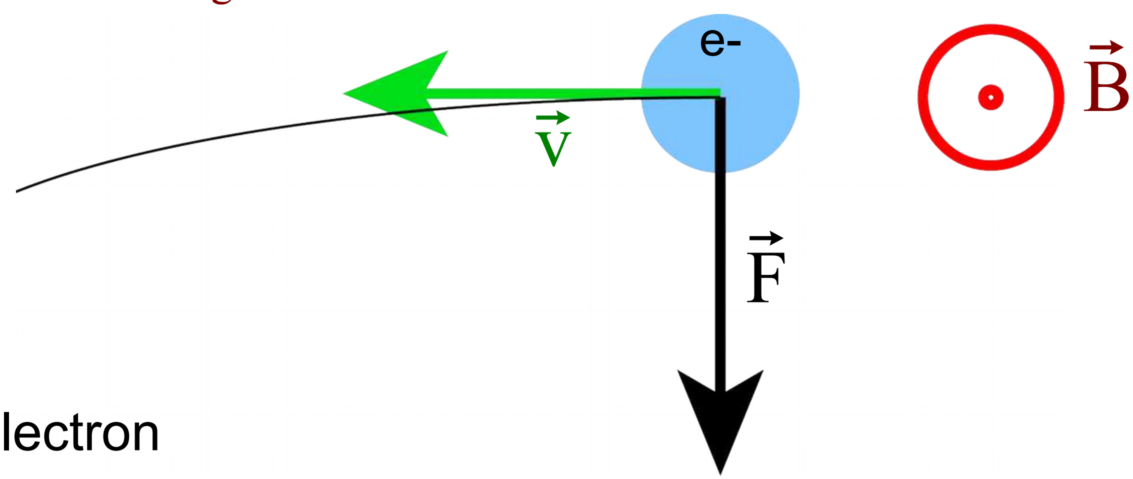
$$|\vec{s}| = |h.\vec{a}^* + k.\vec{b}^* + l.\vec{c}^*| = \frac{1}{d}$$

Electrons: Imaging or diffraction?

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

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

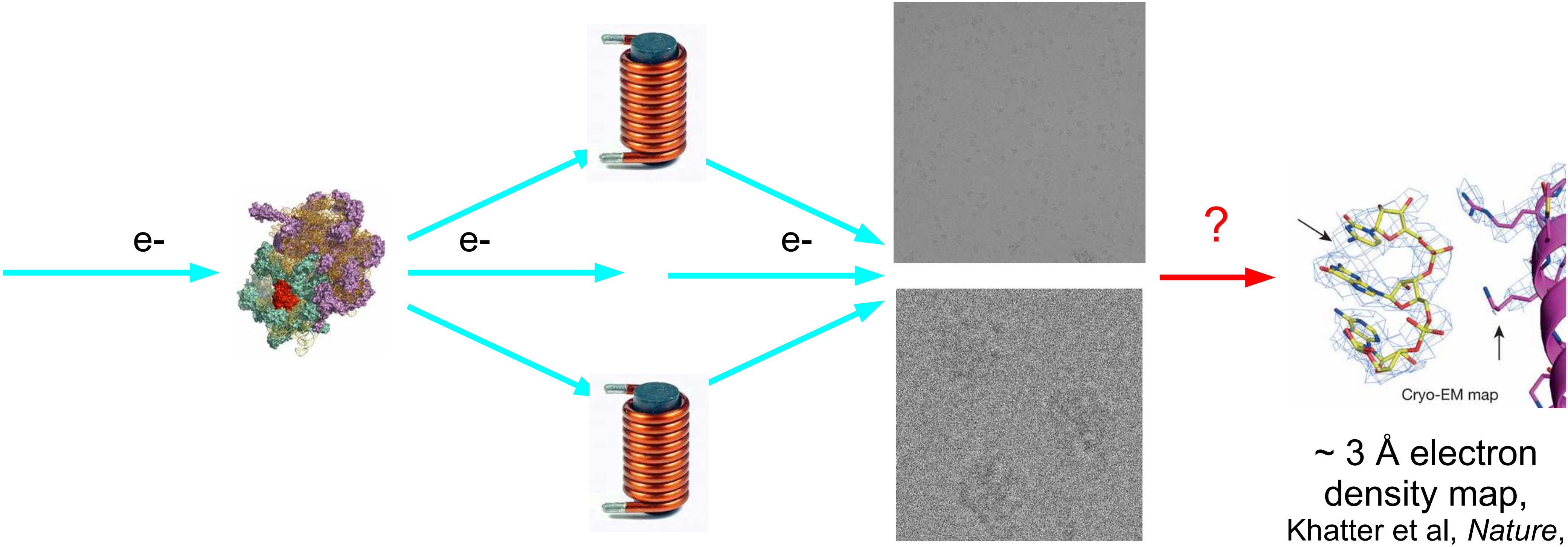




- A magnetic field can deviate an electron

- 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 => Coulomb potential map
  - Diffraction => F(hkl) + φ(hkl) => Coulomb potential map
- NMR
  - Gather structural information (local interatomic distance, …) => search for models that satisfy the data.