ICTP School on Synchrotron Radiation and Application

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Protein Crystallography:
Tutorial on Crystal Structure Determination

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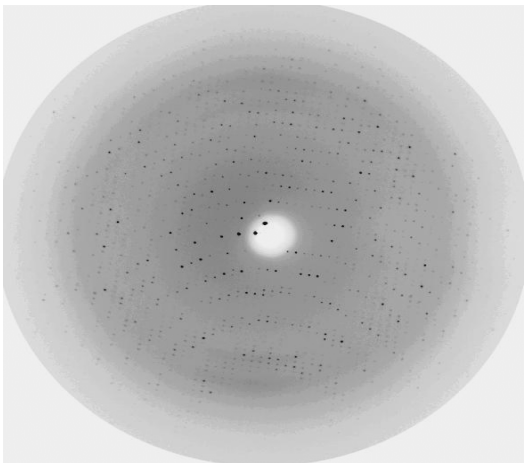
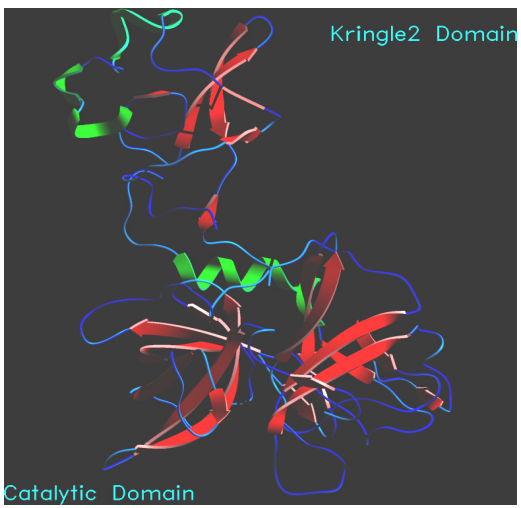
Summary

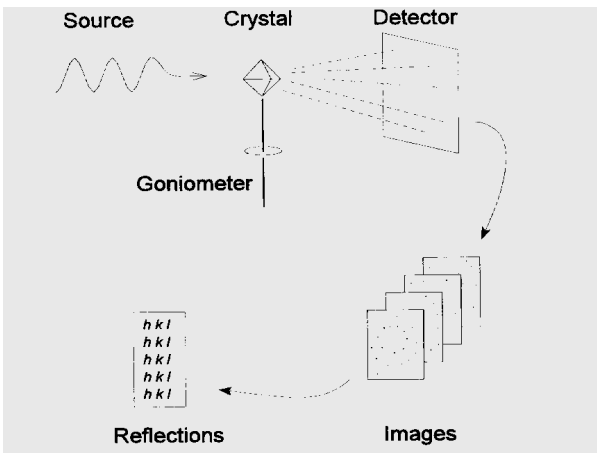
- Diffraction
- Data Collection
- Structure Solution
- Refinement

Diffraction – The method

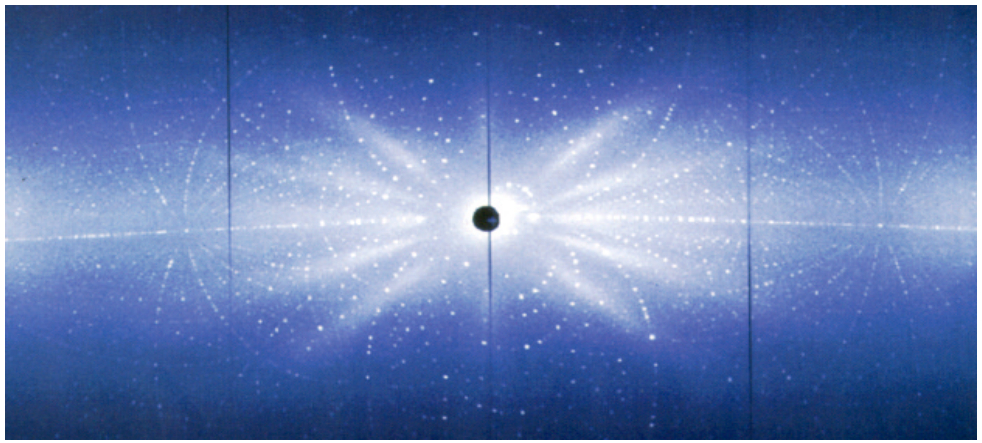


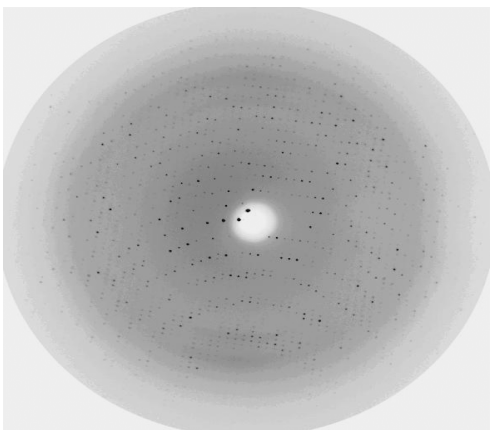


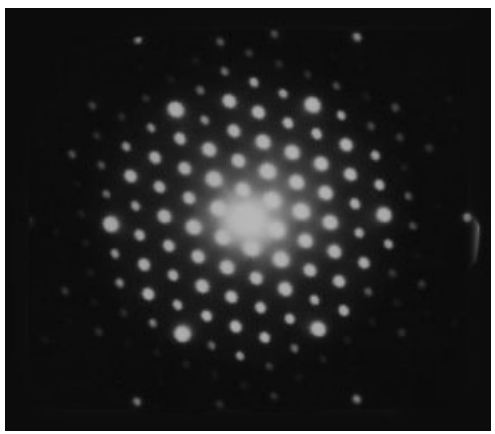
- X-ray diffraction from single crystal is still the main method for proteins structure determination.



Diffraction – Diffraction and Crystals







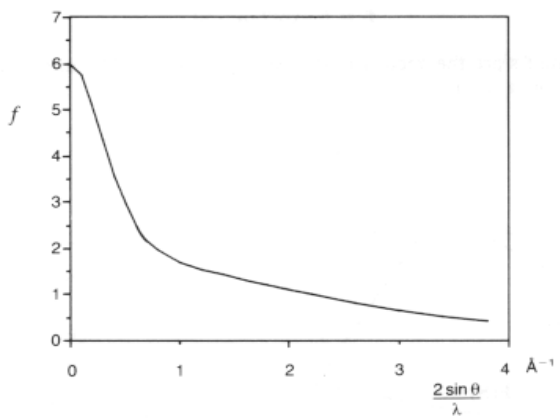
- Crystals behave as a three-dimensional diffraction net.

- The interaction between waves of a given wavelength such as X-rays, but even neutrons and electrons give rise to diffraction phenomena.

Diffraction – X-ray electrons interaction

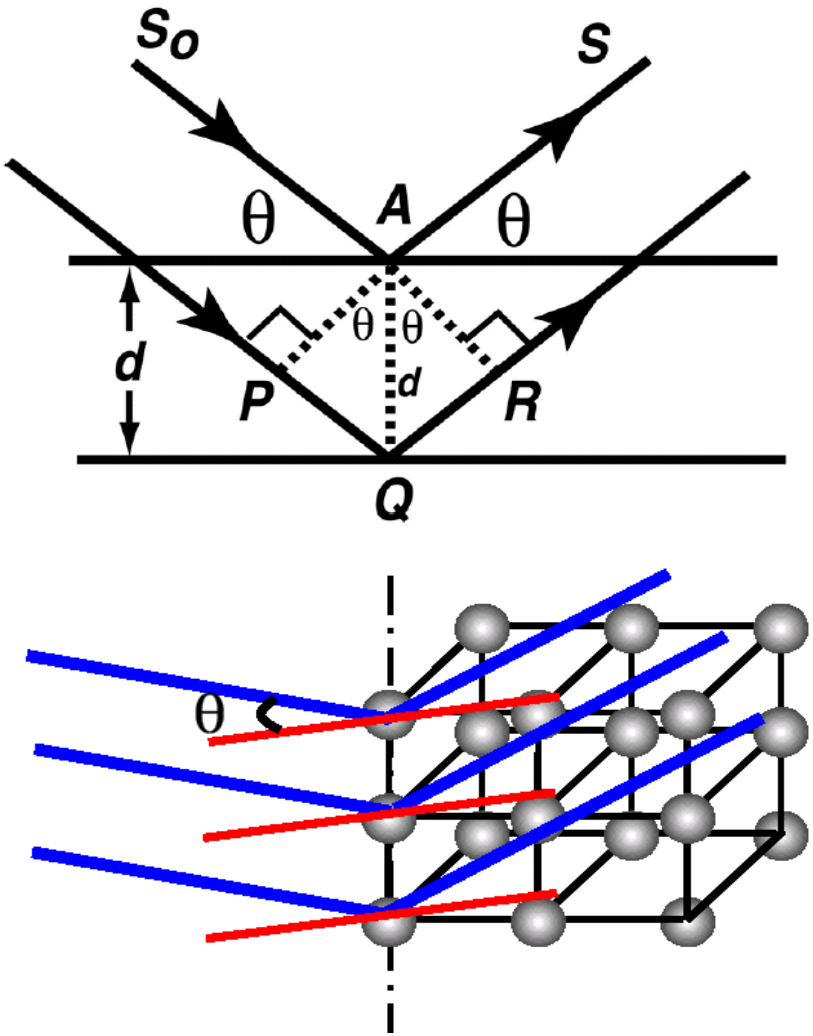
- X-rays interact with electrons, which absorb X-ray photons starting to oscillate in phase with the incident wave. Oscillating electrons emit "secondary" wave which conserve energy and original phase difference (elastic scattering).

- Scattered waves from electrons of all atoms inside the crystal interfere, generating diffracted waves



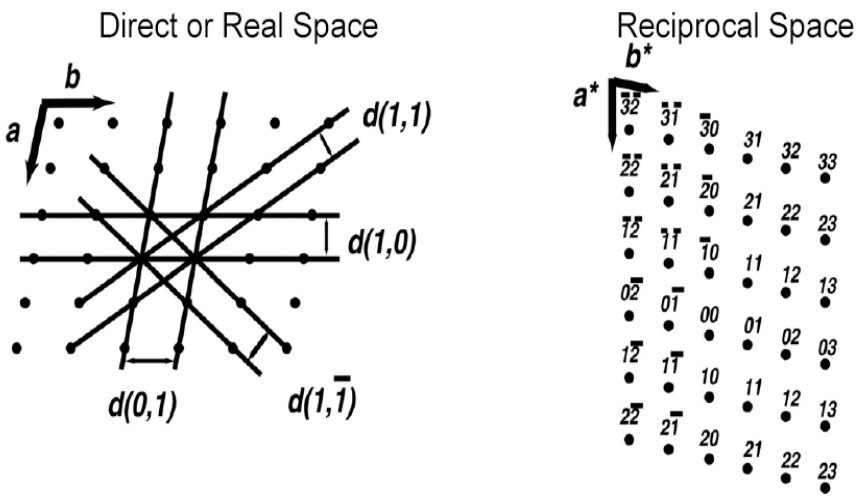
The intensity of X-ray scattered waves from atoms increases with the atomic number and decreases as the scattering angle increase

Diffraction – Bragg Planes



- Diffraction of X-ray by crystals can be viewed as the reflection by families of planes, containing atoms, inside the crystal itself.
- Each family of planes is characterized by 3 numbers (Miller indexes) which identify the Bragg plane orientation relative to the crystal axes.
- The angle between the incident (or scattered) wave and the plane is called Bragg angle.

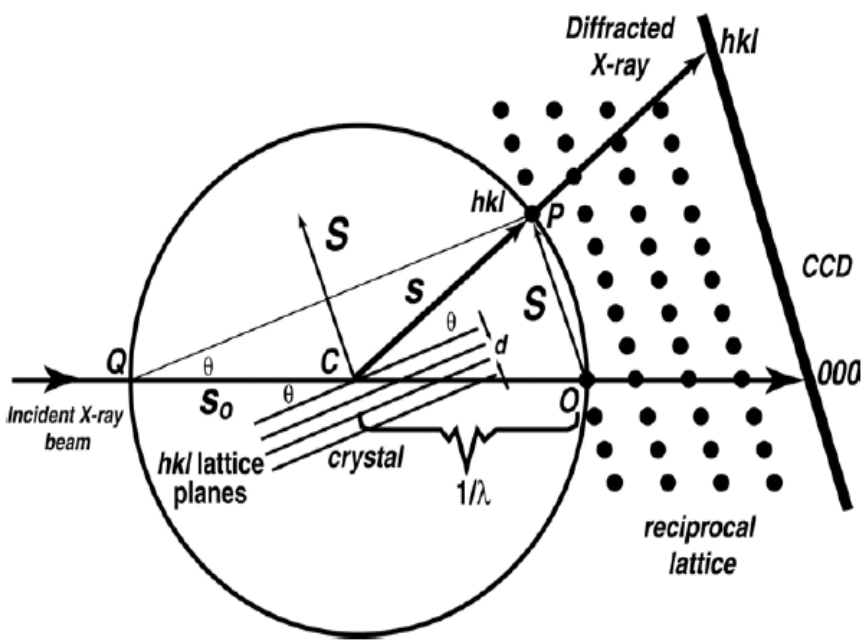
Diffraction – The Reciprocal Lattice



- We can associate at every family of Bragg planes a vector orthogonal to the planes themselves.

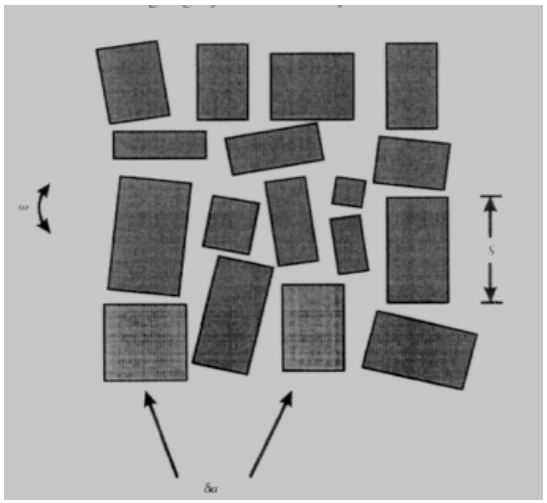
- We can construct in this way a new net called reciprocal lattice, and every discrete point inside it is called reciprocal lattice point (rlp).

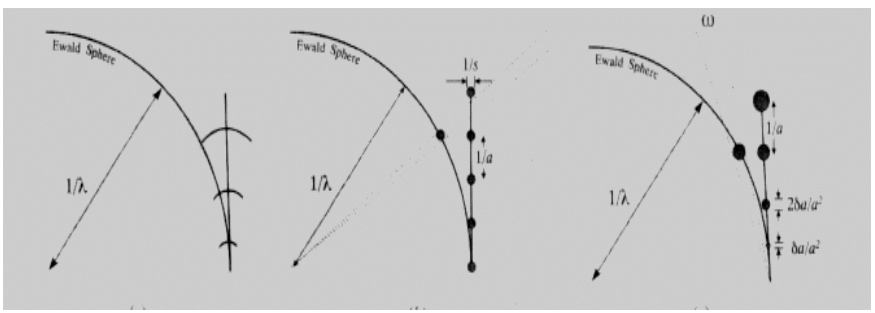
Diffraction – The Ewald Sphere



- A useful tool when considering diffraction by crystals, is the Ewald sphere.
- Every time a reciprocal lattice point lies on the Ewald sphere, that point (or its related Bragg Plane) originates a diffracted ray.

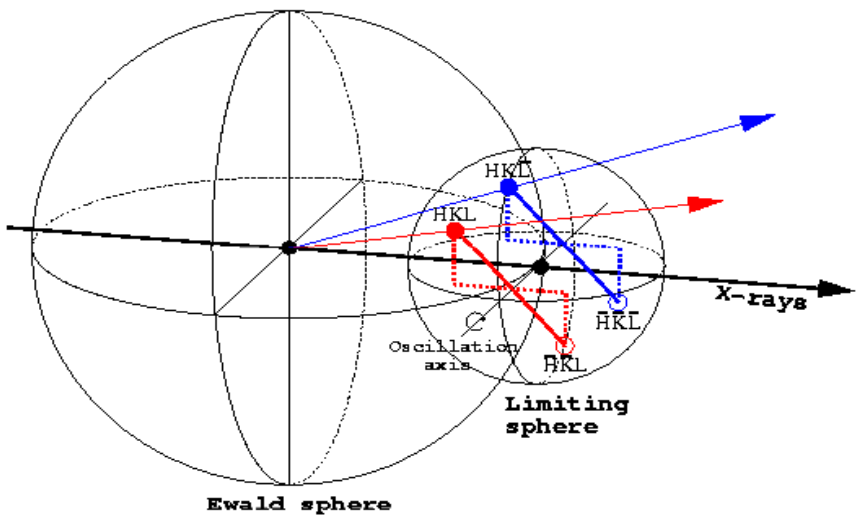
Diffraction – Mosaic Spread





- In principle reciprocal lattice points are zero dimensional, in practice they are not.
- A single crystal can be seen as composed of several blocks, each other misaligned by a more or less small extent.
- The different blocks will be brought in diffraction condition for different but contiguous angles ϕ (while Bragg angle θ does not change), so the diffracted spot will have a distribution (assumed gaussian) around the theoretical position.

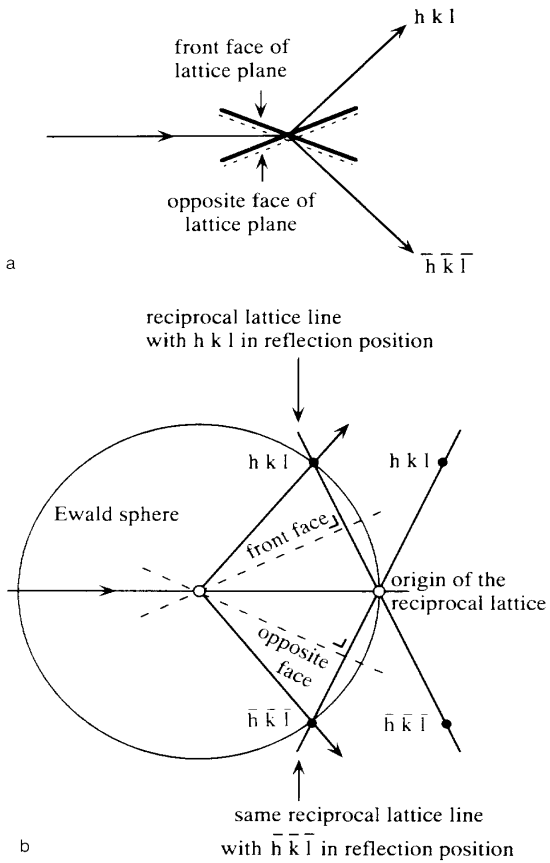
Diffraction – Limiting Sphere



- The intensity of the scattered wave from an atom decreases as the Bragg angle θ increases.
- The internal disorder of the crystal and the thermal motion of the atoms have the same effect on diffraction intensity.
- In practice a maximum angle θ exists above which diffraction is not observed anymore. This angle is related to the level of detail of the final crystal structure (Resolution).

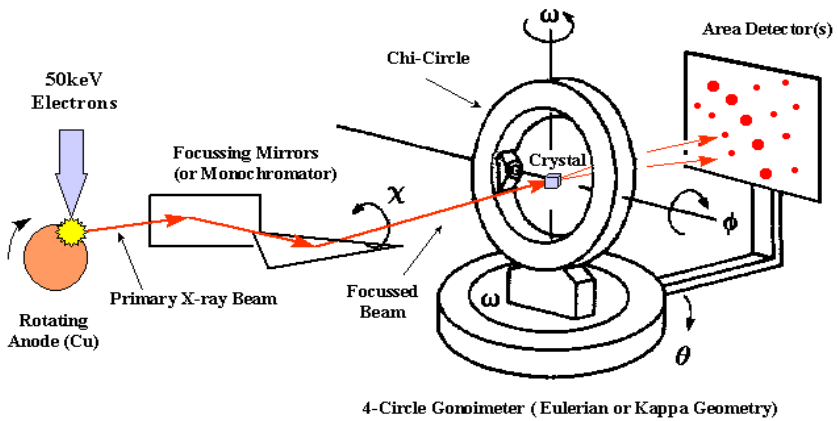
$$\rho(x\ y\ z) = 1/V\ \Sigma_{hkl}\ F(h\ k\ l)\ \exp[-2\pi i(hx+ky+lz) + i\alpha(h\ k\ l)]$$

Diffraction - Friedel Pairs



- The interaction between X-ray and crystals is such that planes related by centrosimmetry, which is planes (h k l) and planes (-h –k –l), have the same diffracted intensity (Friedel law).
- Friedel Law does not hold anymore, if the wavelength of the incident X-ray is near or equal to an absorption edge of an element inside the crystal. This is due to the anomalous scattering contribution.

Data Collection



We collect Intensities of the diffracted waves, but not their phases!

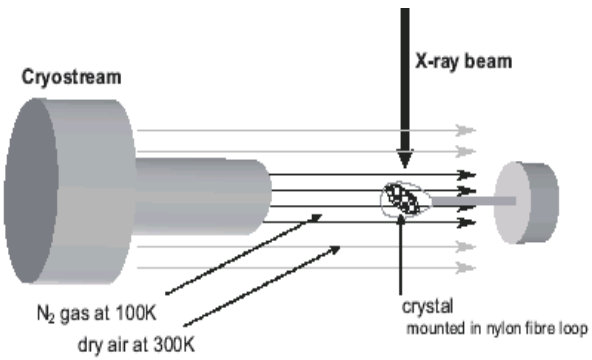
$$I_{hkl}=KI_0\lambda^3LPAV_x|F_{hkl}|^2/V^2_0$$

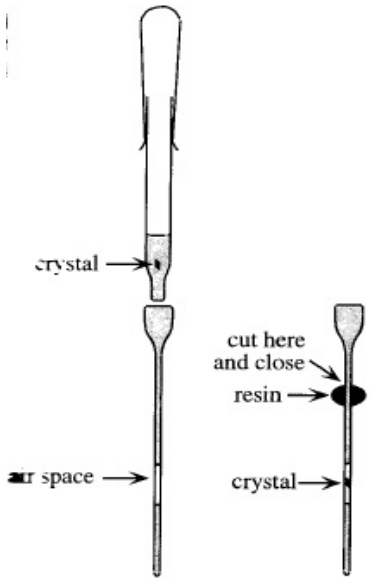
- In a Single Crystal diffraction experiment we have to collect as much as possible diffracted waves, generally indicated as reflections. To do that a general setup consist of:
  - A source of X-rays
  - A goniostat to orientate and rotate the crystal so that a certain number of Bragg planes can be brought in diffraction conditions
  - A detector to acquire the diffracted rays

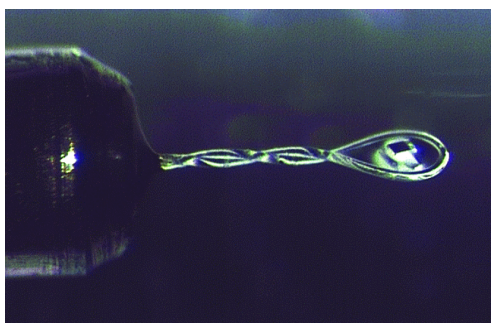
Data Collection – Radiation Damage

- Protein crystals are damaged from the exposure to the X-ray beam
- Radiation damage causes a reduction in lifetime of crystals, which becomes apparent as a reduction of the diffraction pattern maximum resolution, and worsening of the diffracted spot shape.
- At the final electron density map level,disulfide bonds can be broken, and carboxylic groups of exposed aspartic or glutamic acid may be lost.
- The extent of the damage depends on the X-ray dose absorbed, and on the energy of the incoming photons.
- X-rays promote the radiolysis of water molecules, the radicals generated chemically damage the proteins. Migration of radicals can be stopped by freezing protein crystals with an appropriate cryoprotectant.
- X-ray absorption causes the emission of photoelectrons with formation of ions. With an extremely intense X-ray beam (3^{rd} Generation Synchrotron undulators) this effect may become apparent. It is insensitive to low temperature data collection.

Data Collection – Crystal Mounting





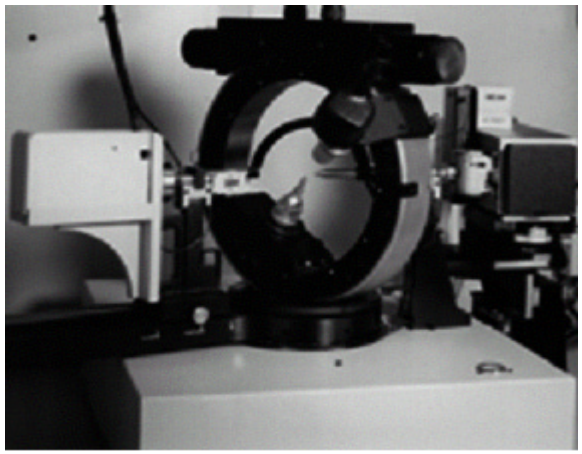


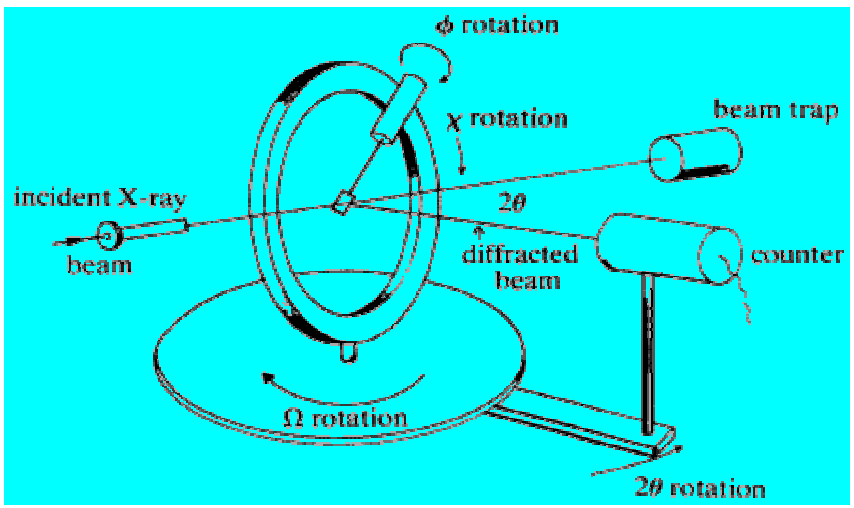
- If crystal decay is not a problem, capillary mounting is an option.
- For Synchrotron data collection, the safest option is to flash freeze crystals soaked with a cryoprotectant.
- Finally crystals are mounted on goniometer head and centered, so that they do not give rise to precession upon spindle axis rotation.

Data Collection - Methods

- Different methods exist for collecting diffraction data, depending on:
  - The incident beam is monochromatic or not
  - The Geometry of diffraction experiment
  - The Detector can be zero, one or bidimensional
- Polychromatic
  - Laue Method
- Monochromatic
  - Single Crystal Diffractometer (with photon counter)
  - Precession Method
  - Rotation Method

Data Collection – Diffractometer

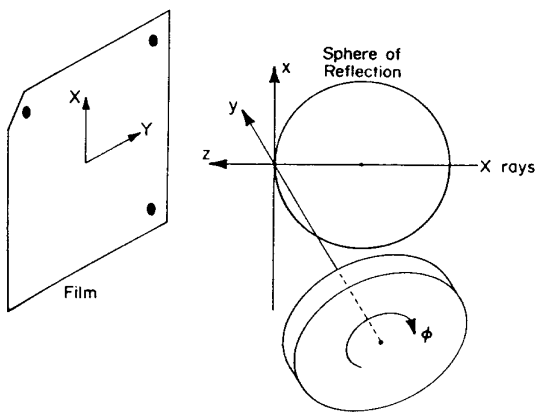




- The Bragg planes are brought into diffraction condition one at time. The diffracted intensity is collected with a zero dimensional detector (photo multiplier)
- Advantage: Data are accurate, semi-empirical absorption correction is possible.
- Disadvantages: Extremely slow! In practice it is not used anymore for Protein crystallography.

Data Collection – Rotation Method

- The crystal is rotated around a generic crystallographic direction so that a certain number of rlps are brought into diffraction conditions.
- Advantages: Fast
- Disadvantages: Distorted Image of rlp planes.
- Rotation Method is the Standard method of data collection for macromolecules.



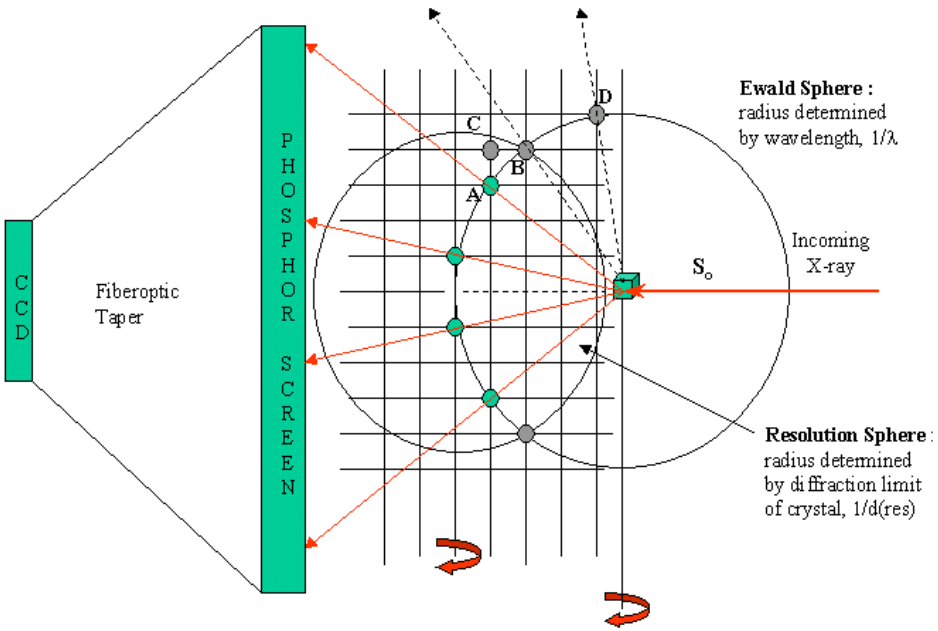
•The simplest setup consist of:

–monochromatic X-ray beam

–single axis goniometer orthogonal to the incident beam

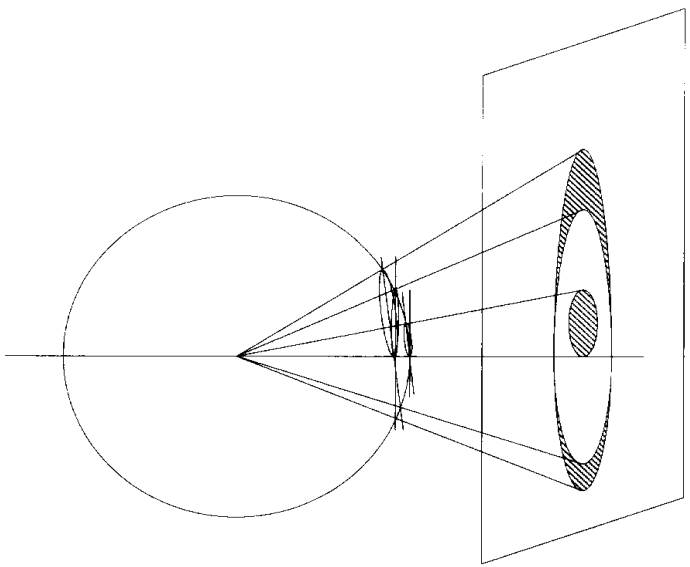
–flat bidimensional detector parallel to the rotation axis. The detector is normally orthogonal to the incident beam, but not necessarily.

Rotation Method - Geometry



- The crystal is rotated around the goniometer axis of a certain angle so that several rlps will be in diffraction condition during the rotation. The diffracted X-rays are collected from the detector behind the crystal, without any intercepting screen between them.
- The rotation is repeated for contiguous angles until, given the orientation of the crystal, the independent (at least) part of the reciprocal lattice is completely scanned.

Rotation Method – Diffraction Pattern



- The diffraction pattern shows typical diffraction figures called “lunes”, due to the sweep through the Ewald sphere of the reciprocal lattice, during the crystal rotation
- The rotation angle must be small enough in order to avoid spots superposition. In practice the lunes must be well resolved.