IIT Guwahati Lecture 30

Course BT 631

Protein Structure, Function and Crystallography

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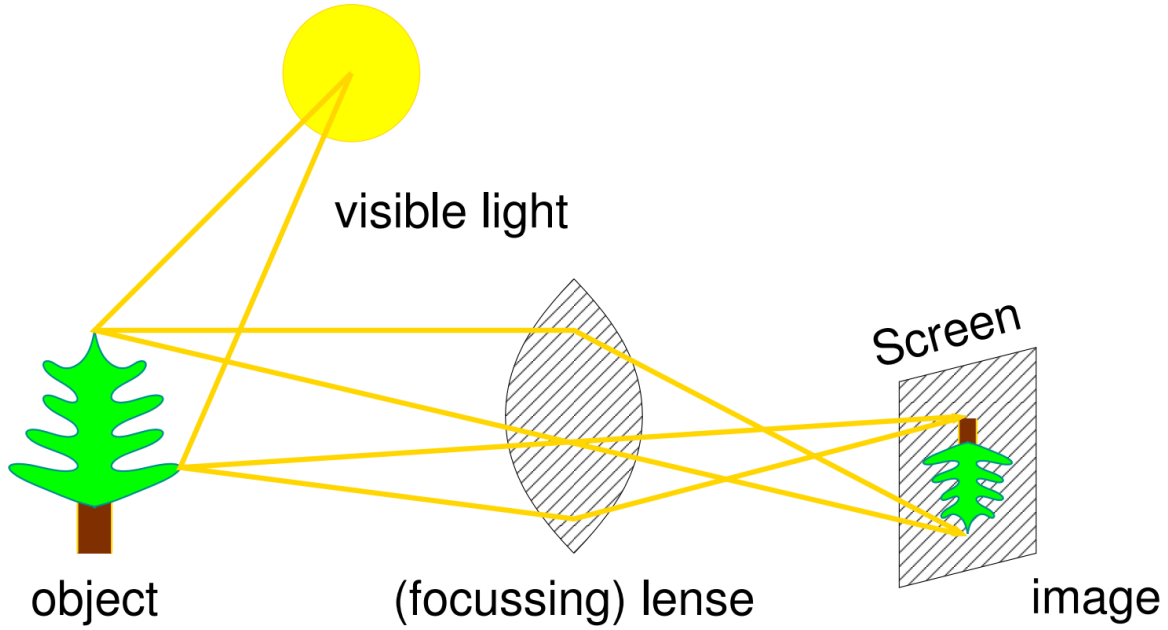
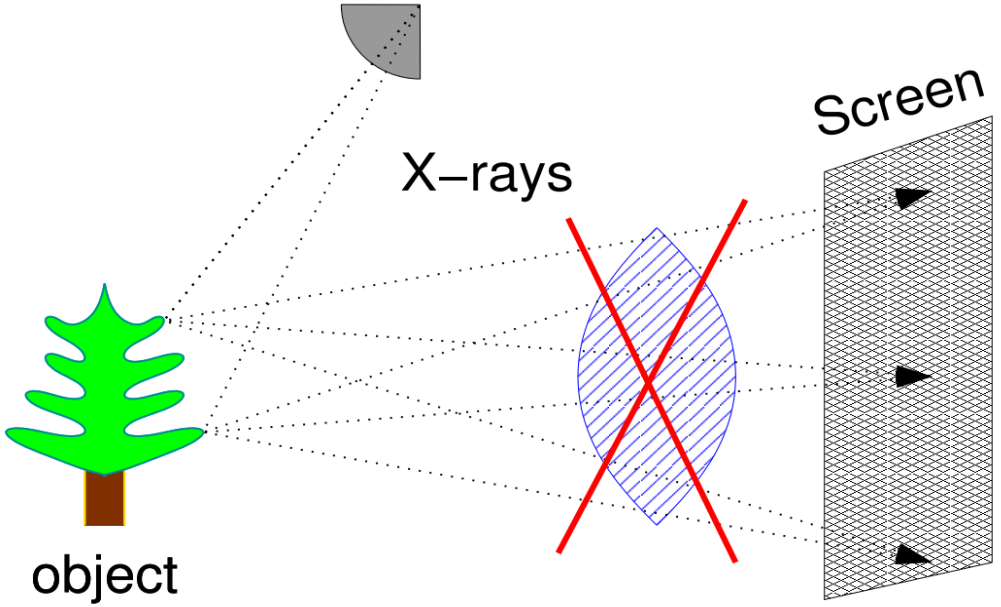
Diffraction (Visible Light vs X-rays)

Lenses allow us to build microscopes, telescopes, to actually see (with our own eyes' lenses).



No Focusing Lenses = No Image, only "blur"

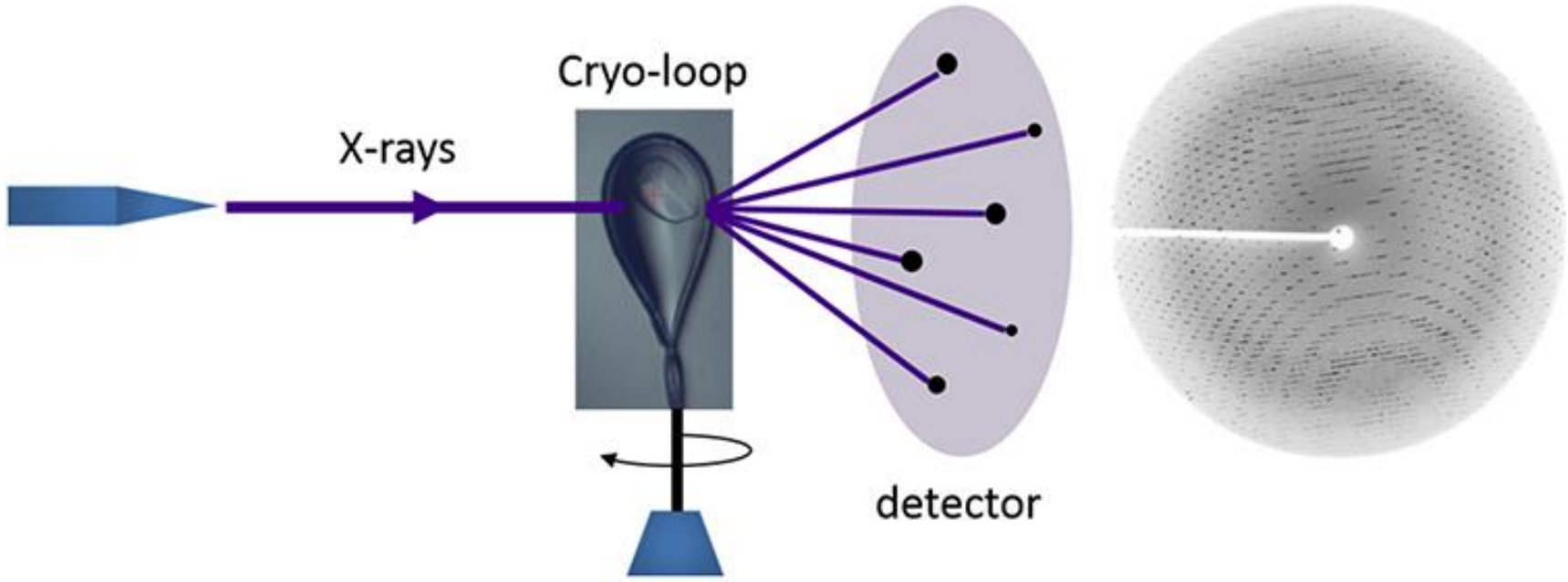


Diffraction

The "blur" contains no useful information that could help us reconstruct the image of the tree.

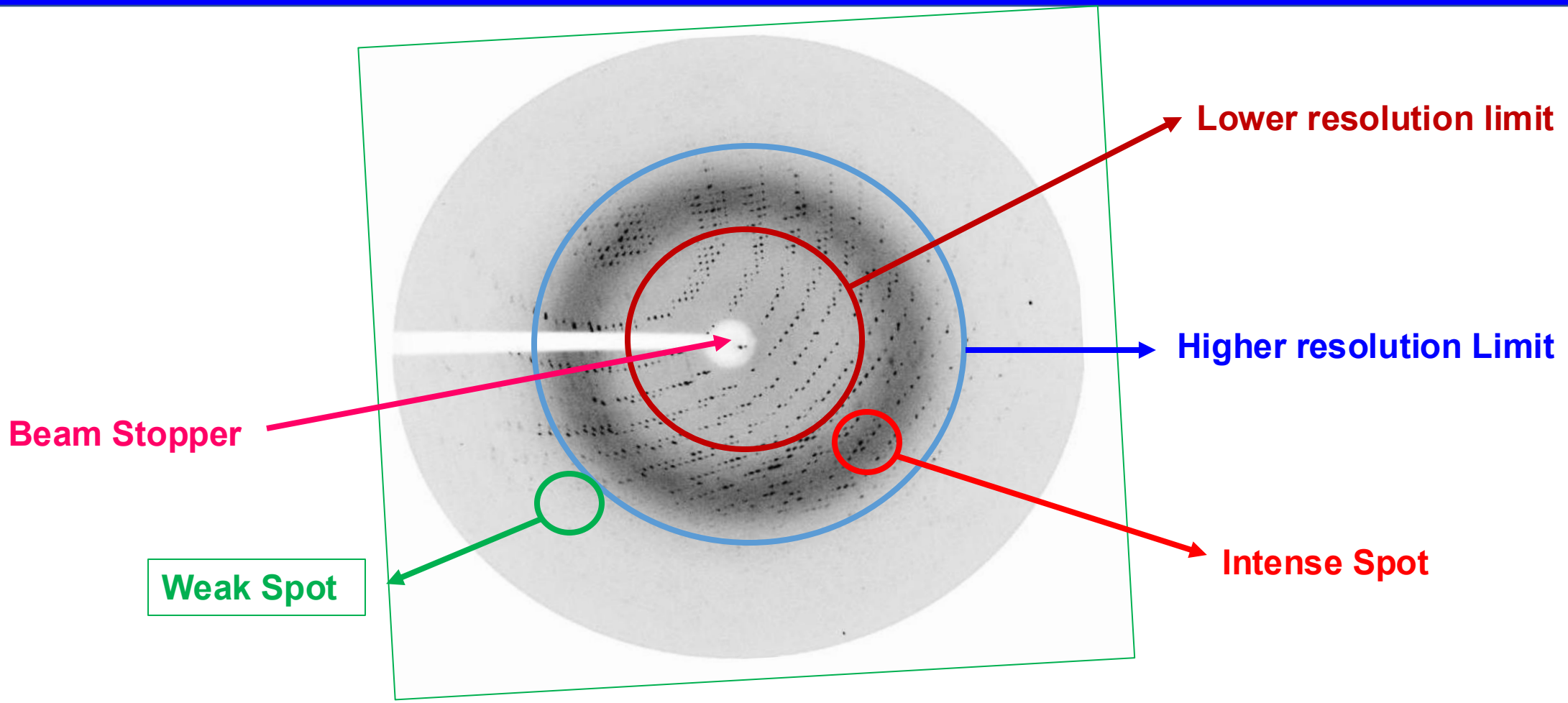
Crystal and X-ray Diffraction

While in case of crystals: Their periodic composition- made up of myriads of unit cells causes spots (reflections) to appear on top of the "blur".



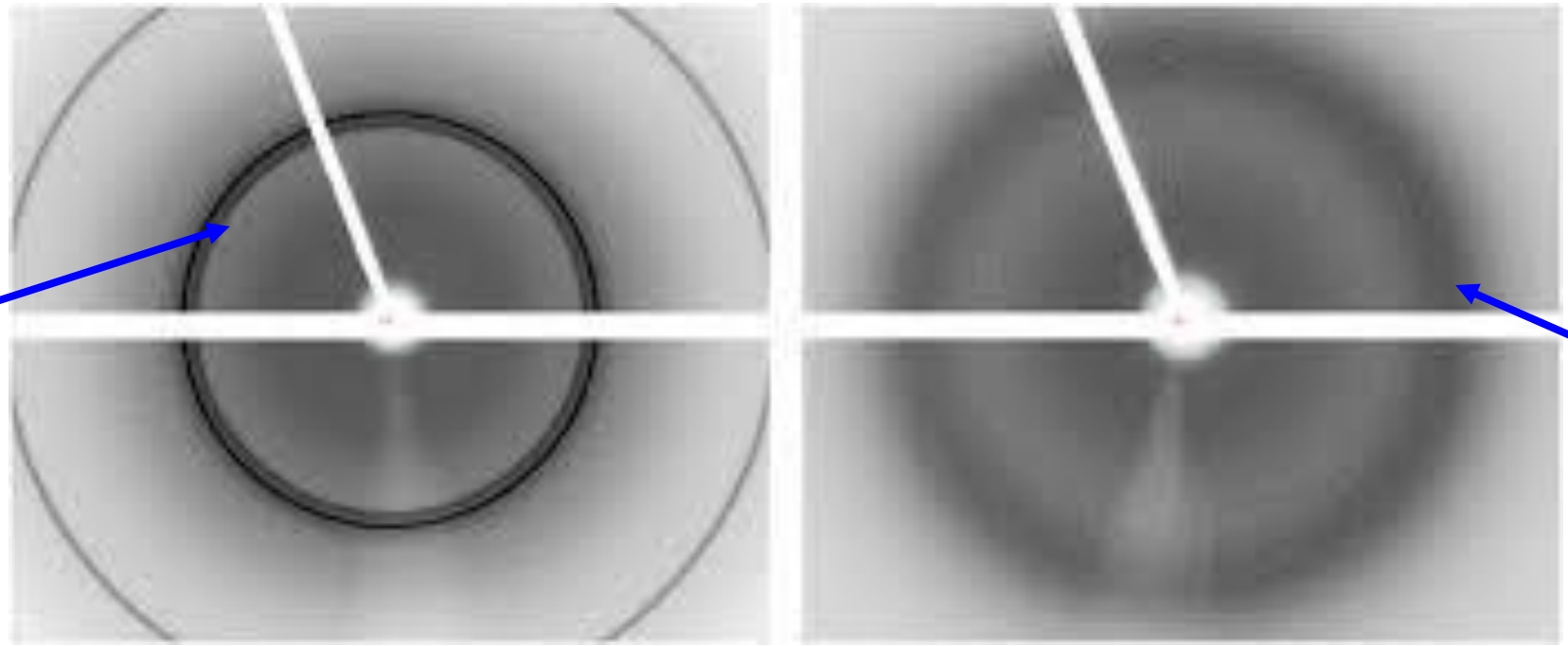
To resolve atoms with bond distances around 1.5 Å, X-rays of (λ = 0.5 - 2.0 Å) are needed.

What information we can obtain from Diffraction pattern?



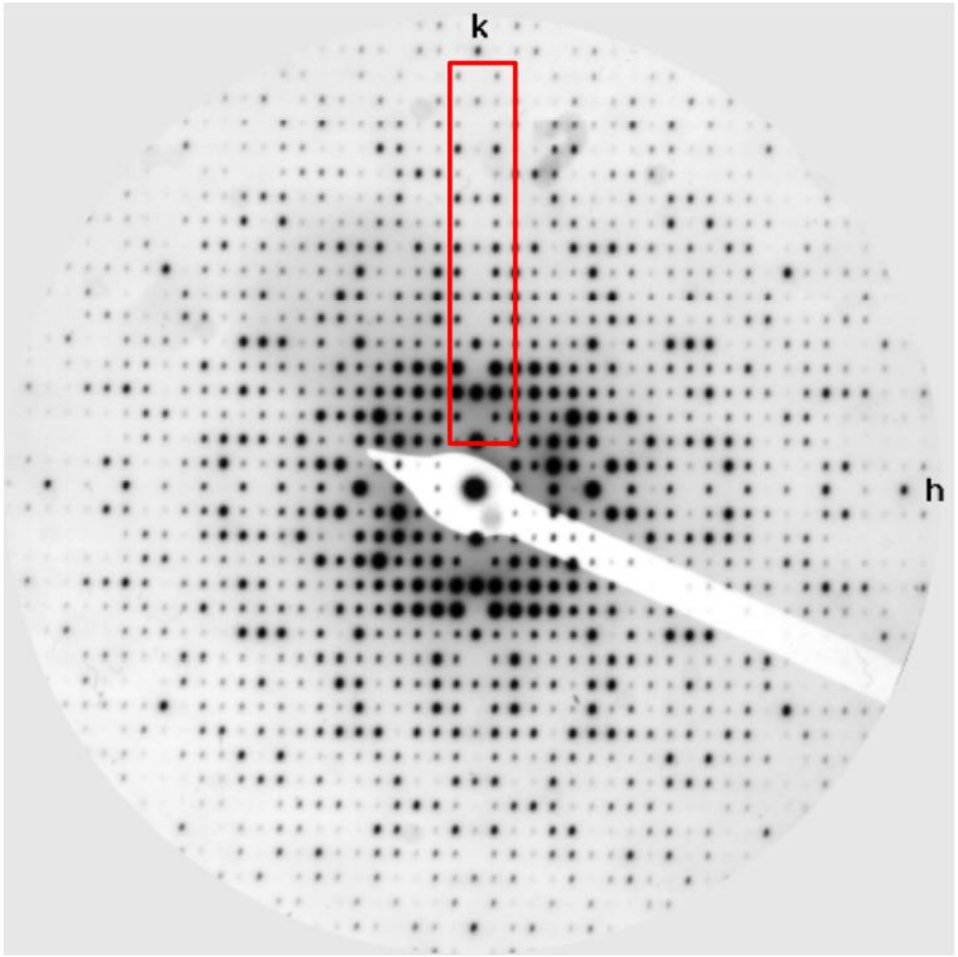
What information we can obtain from Diffraction pattern?

When the protein crystal size is large, it contains water in the form of hexagonal crystals (ice) which, causes the quality deterioration or collapse of the protein crystals by forming the ice ring during diffraction.

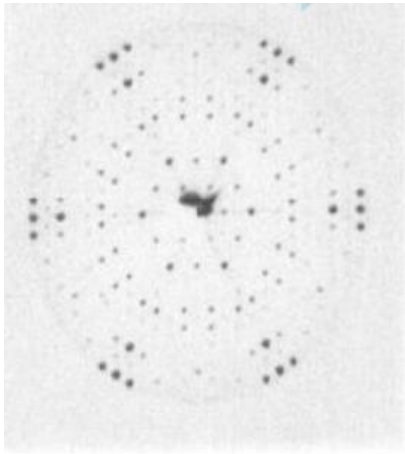


Therefore, the utilization of cryoprotectant such as glycerol, sucrose, polyethylene glycol etc. enhances to avoid such condition during x-ray diffraction,

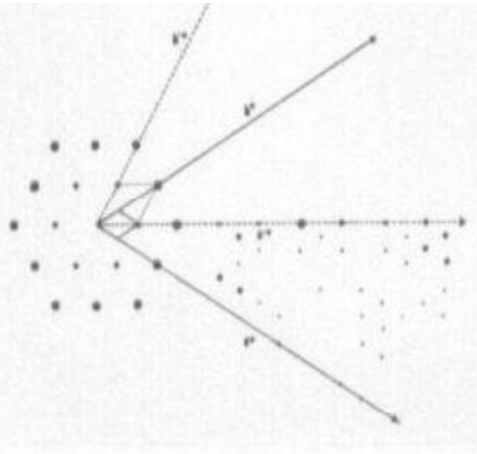
What information we can obtain from Diffraction pattern?



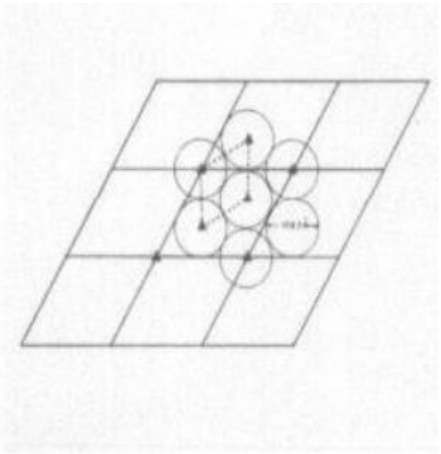
Diffraction pattern reveals- Internal symmetry of the particular crystal and space group.



Diffraction pattern



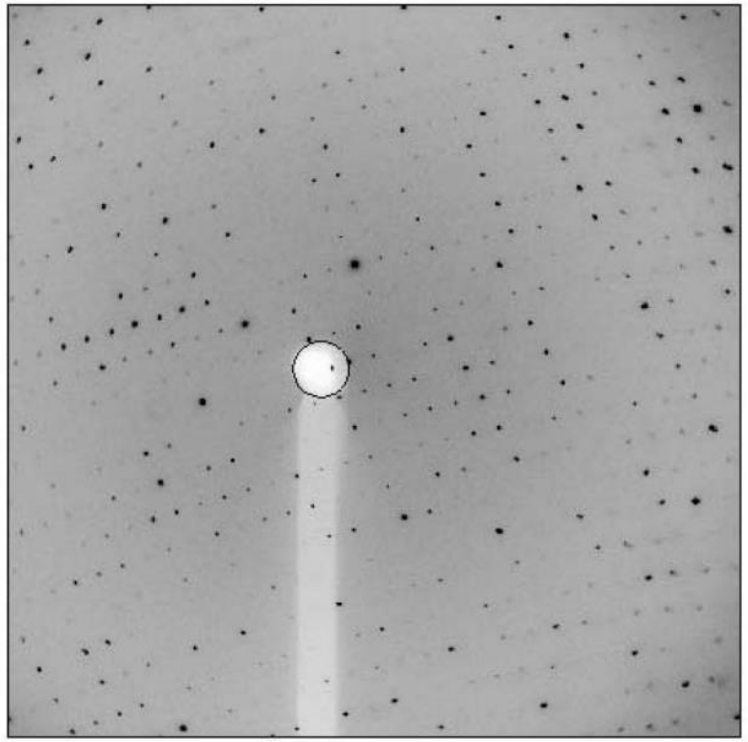
Analysis



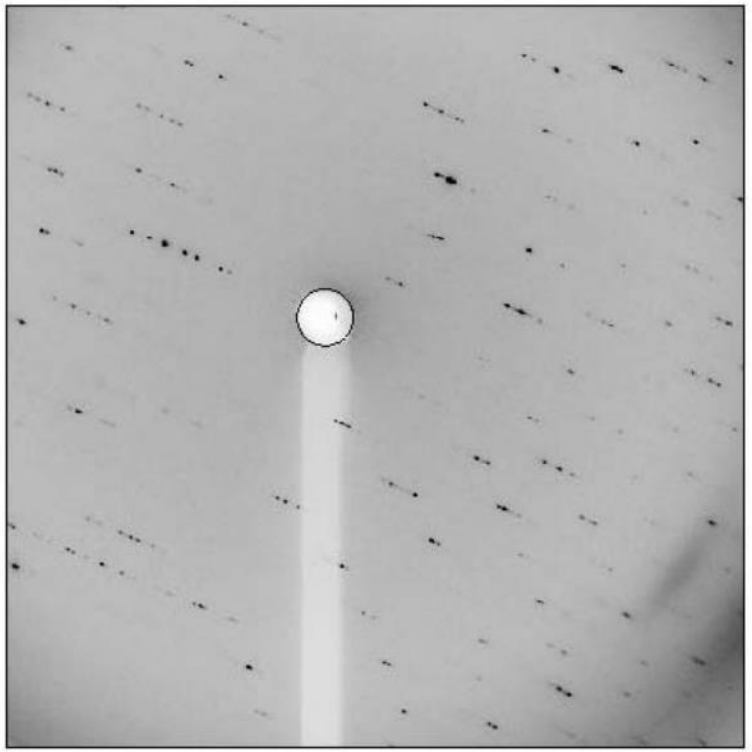
Hexagonal Packing

What information we can obtain from Diffraction pattern?

Quality of Crystal- Internal order of the crystal, vibration or motions of atoms, purity of the protein sample.



Well ordered and Sharp Spots



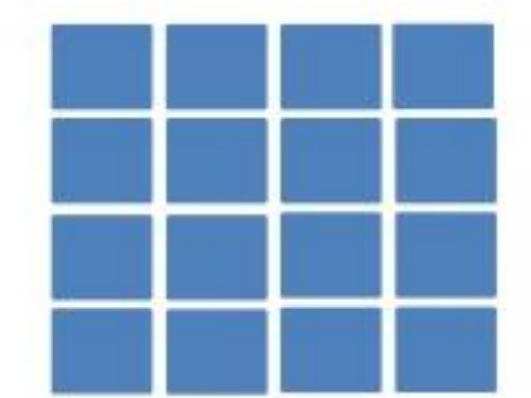
Poorly ordered and Streaky Spots

Quality indicators obtained from crystal Diffraction pattern

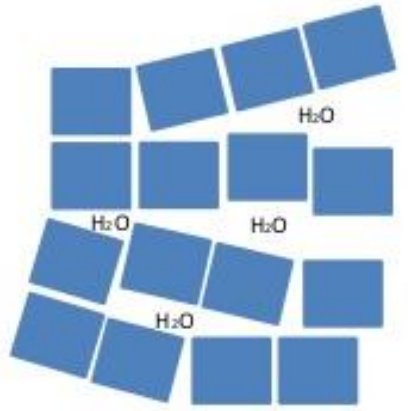
Crystal Mosaicity– **Protein crystals are imperfect, consisting of a mosaic of blocks that are slightly misaligned which causes the problem in diffraction pattern.**

Mosaicity is a measure of the spread of crystal plane orientations.

A mosaic crystal is an idealized model of an imperfect crystal, imagined to be consisted of numerous small perfect crystals (crystallites) that are to some extent randomly misoriented.



Perfectly ordered crystals



Mosaic crystals

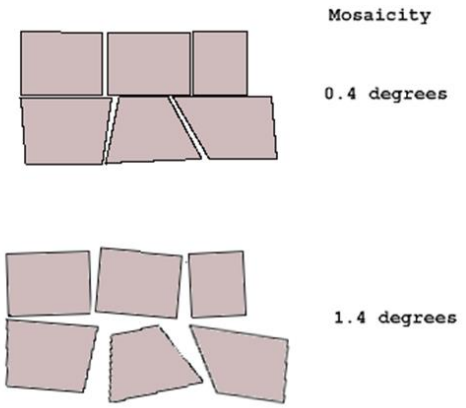
The quality of a crystal is described by the parameter, mosaicity (also known as mosaic spread, rocking angle), involves the degree of perfection of the lattice translations throughout the crystal.

The quality parameters for

i) Perfectly ordered crystals= 0.2 - 0.5° ; ii) Imperfectly ordered crystals = 1.0°

Quality indicators obtained from crystal Diffraction pattern

Crystal Mosaicity



A protein crystal may contain a variety of imperfections in packing and heterogeneity of contents. The traditional way of describing mosaicity is by considering the crystal to be composed of mosaic blocks, each block being a perfect crystal. If all blocks are perfectly aligned, the diffraction from each block will be perfectly contiguous and the mosaicity will be 0.

As the mosaic blocks become disordered (as may happen during flash-cooling), the resulting diffraction is spread out. In the rotation technique of diffraction data collection, this is seen as broadening the rotation angle required to collect the intensity of a given reflection. Thus, mosaicity is given in degrees. Mosaicity is also referred to as a "rocking angle". This measurement requires specialized equipment and refers to the angle through which a diffractometer must be swung to encompass a reflection.

In normal usage, as reported by data reduction software, mosaicity incorporates contributions from the instrument as well as from crystal quality. Instrumental contributions include beam divergence and spectral quality of the X-rays.

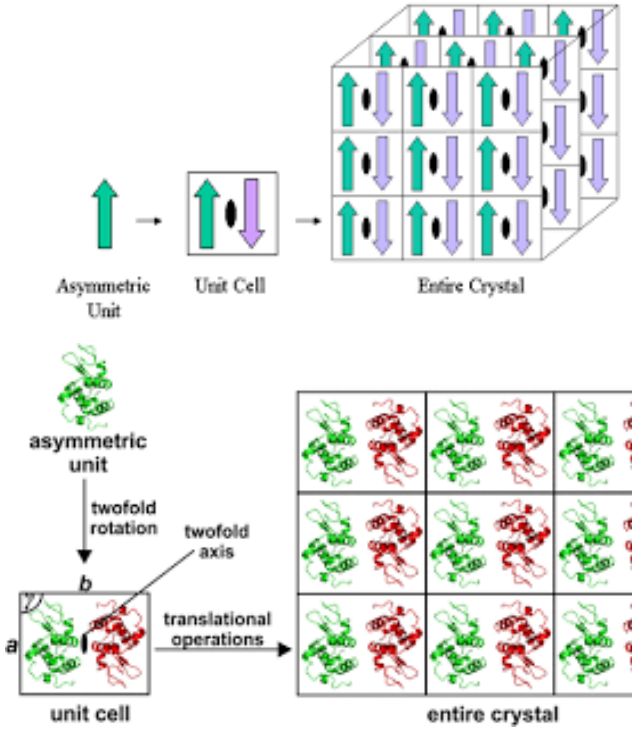
What is an acceptable mosaicity?

A very good crystal will have a mosaicity in the range of 0.2 to 0.5 degrees. However, structures have been solved using crystals with mosaic spread well above 1.0 degree.

Quality indicators obtained from crystal Diffraction pattern

Completeness

- A complete data set should contain all reflections within the asymmetric unit of the crystal. The asymmetric unit is the smallest portion of a crystal structure to which the symmetry operations can be applied in order to generate the complete unit cell (the smallest crystal repeating unit). (Figure)
- This results in the getting proper phases for the determination of protein structure.
- To check the data completeness, 2 diffraction patterns at 90° angle should be collected.
- Processing of these 2 diffraction patterns will give an idea how many diffraction patterns and what degree rotation are to be collected.



Resolution

It is the smallest distance between crystal lattice planes that is resolved in the diffraction pattern.

High numeric values of resolution, such as 3.0-4.0 Å, means poor resolution, while Low numeric values, such as 1.0-2.5 Å, means good resolution.