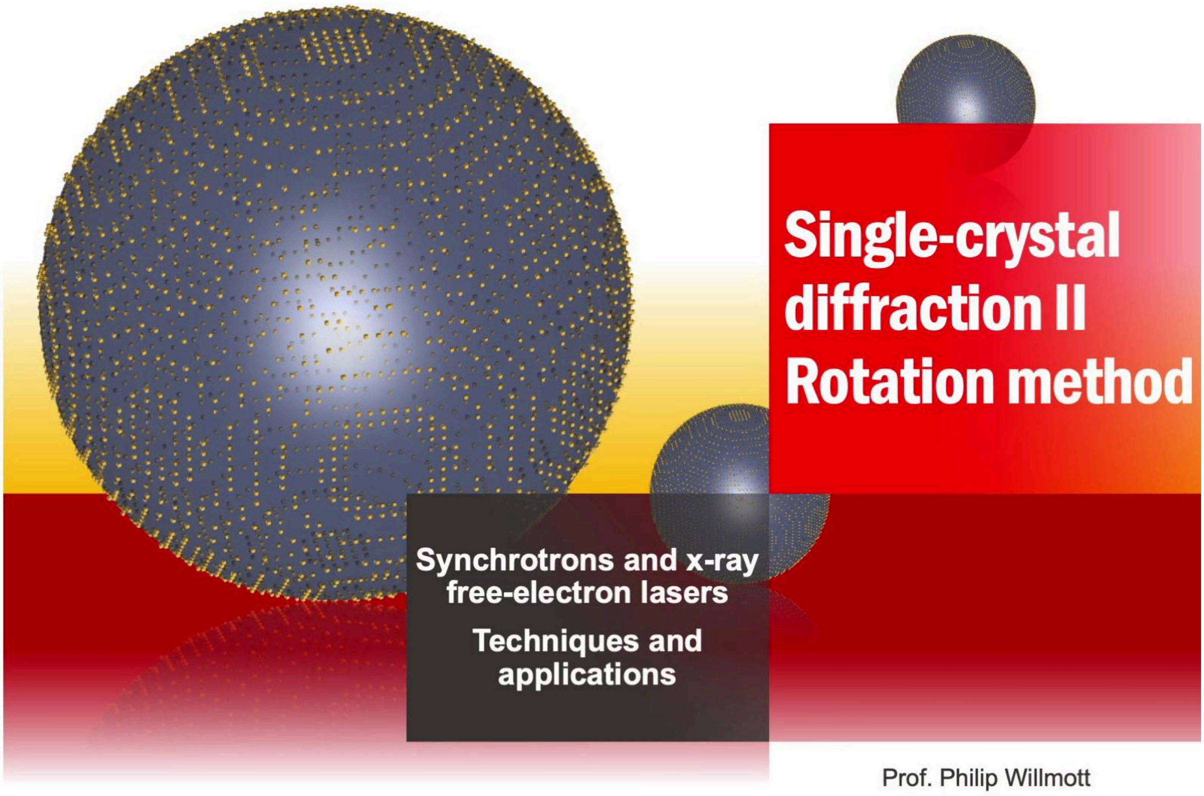
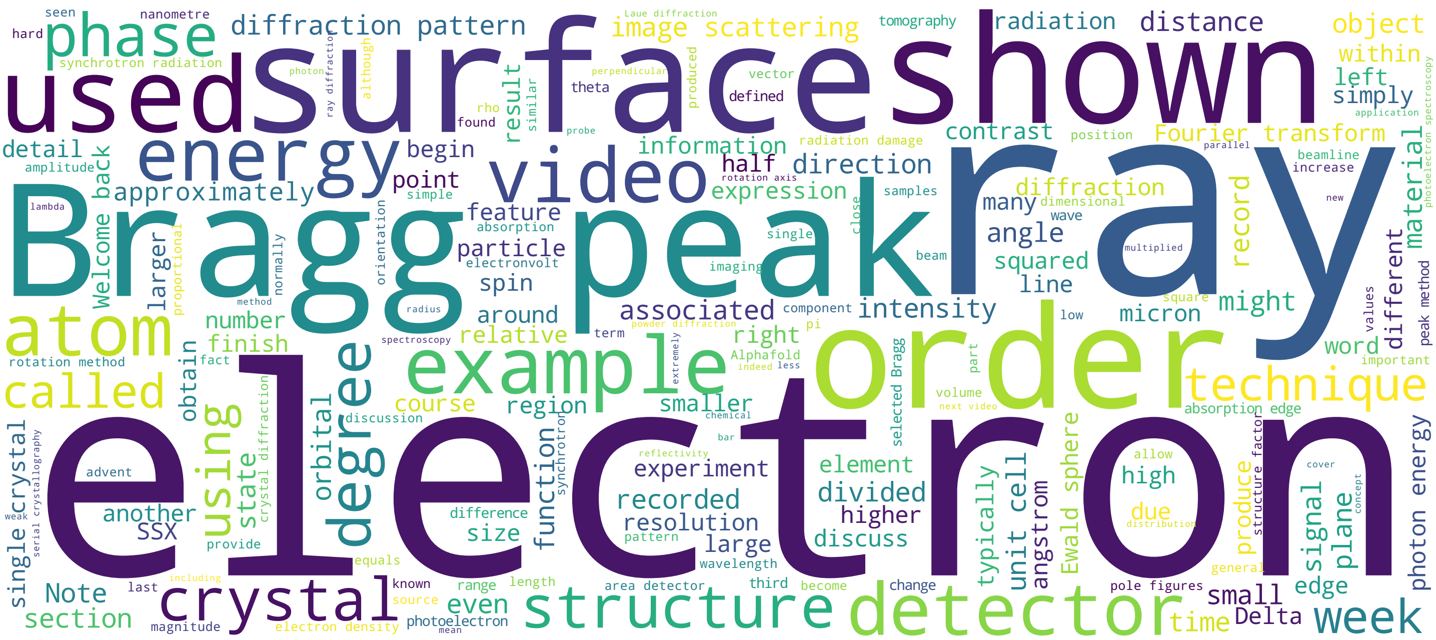
École polytechnique fédérale de Lausanne



Single-crystal diffraction II Rotation method

Prof. Philip Willmott



Search MOOC

Video

Contents and objectives of this video

- The rotation method
- Serial crystallography
- The selected Bragg-peak method
- Pole figures

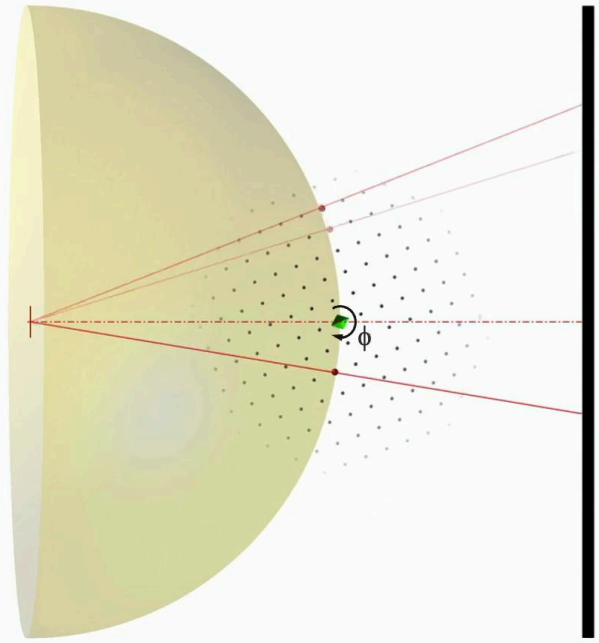
Welcome back to the second video on single crystal diffraction. Now in this video, we'll concentrate on methods that use monochromatic radiation beginning with the classic rotation method, and then discuss serial crystallography, and thereafter, the selected Bragg peak method. We finish by considering pole figures which provide information on preferential orientations in textured samples.

Notes

Summary

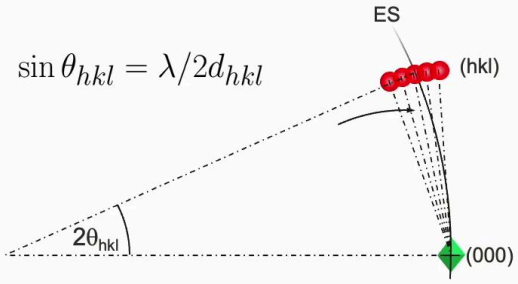
0m 05s

The rotation method



- Monochromatic radiation
- Rotate sample
  - Signal lights up as Bragg peaks pass through the Ewald sphere
  - Signal at angle 2θ relative to direct beam (000)
  - Record at high frame rates (“fine phi-slicing”) to capture passage of Bragg peak through Ewald sphere ⇒ obtain Bragg-peak profile

$$\sin\theta_{hkl} = \lambda/2d_{hkl}$$



M. Müller *et al.*, https://doi.org/10.1107/S0907444911049833

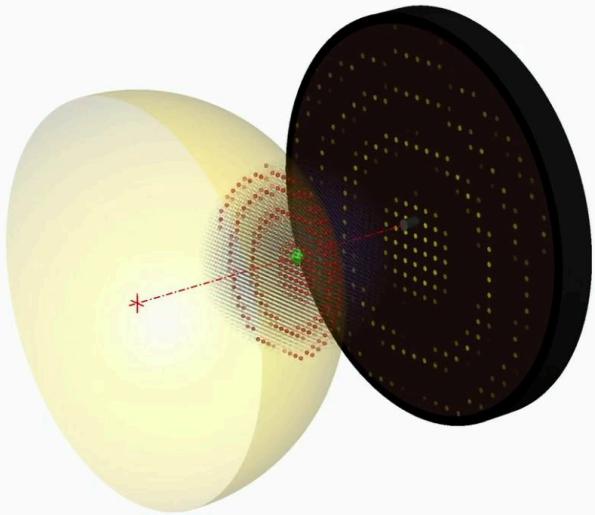
The rotation method is very simple to understand. One rotates a crystal around a certain axis, usually chosen to be perpendicular to the incident radiation, and then record Bragg peaks as they pass across the surface of the Ewald sphere. Each (hkl) Bragg peak is found at an angle two theta_hkl relative to the direct 000 beam, given by Bragg's Law. Since the advent of area detectors with fast readout times beginning with the Pilatus detector in the early 2000s, one can record the progress of the pattern at a frame rate high enough to record the 3D profile of the Bragg peaks as they pass through the Ewald sphere. This has allowed a substantial improvement on intensity, accuracy, and profile fitting, which is especially important for anomalous techniques.

Notes

Summary

0m 32s

The rotation method



Shown here is a simulation of the diffraction patterns seen on an area detector as the crystal is rotated for a crystal with a unit cell size which is 12 times larger than the x-ray wavelength. Those of you who have already performed protein crystallography experiments will be familiar with such patterns. Those Bragg peaks that are passing through the Ewald sphere at any one moment in time are highlighted in red and are projected onto the detector.

Notes

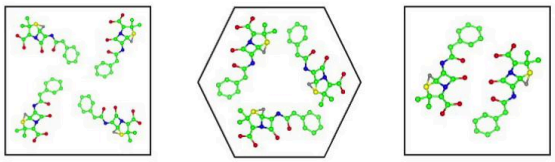
Summary

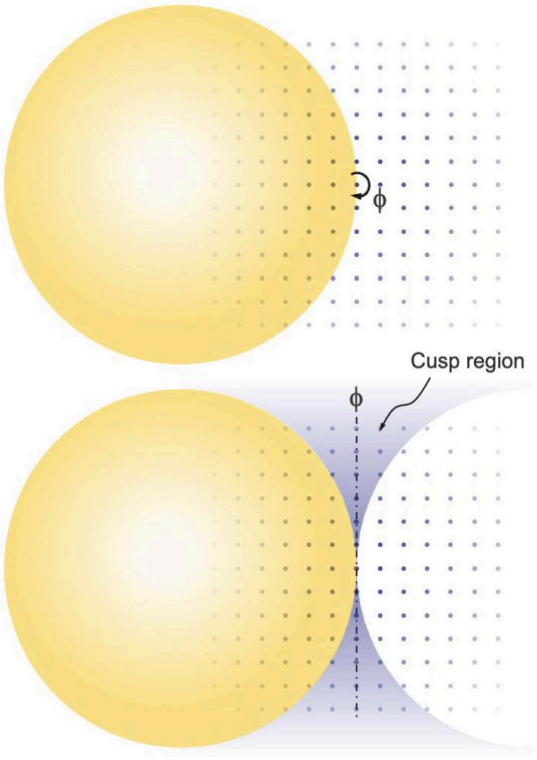
1m 34s

The rotation method

- Rotate around ϕ
- Data close to ϕ-axis ("cusp", or "blind" region) not recorded
- Rotate ϕ (normally through 90°) to capture this missing data

- High-symmetry unit cells require reduced range of ϕ-rotation (90°, 120°, 180°)





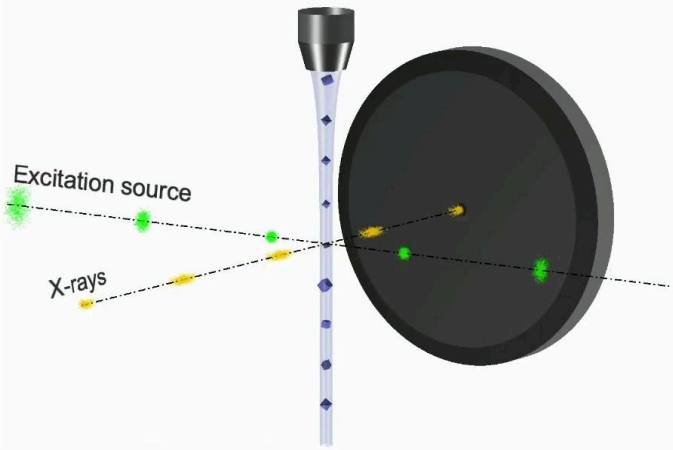
Now, a closer inspection of the rotation method experimental setup should make it clear that for a given rotation axis of the crystal, there will be a set of Bragg peaks, which are known as the cusp region, that will never pass across the surface of the Ewald sphere. In order to record these, a new rotation axis for the crystal needs to be chosen, and this is often taken to be at 90 degrees to the first axis. Note also that high-symmetry unit cells will require a reduced range of crystal rotation to record a complete dataset.

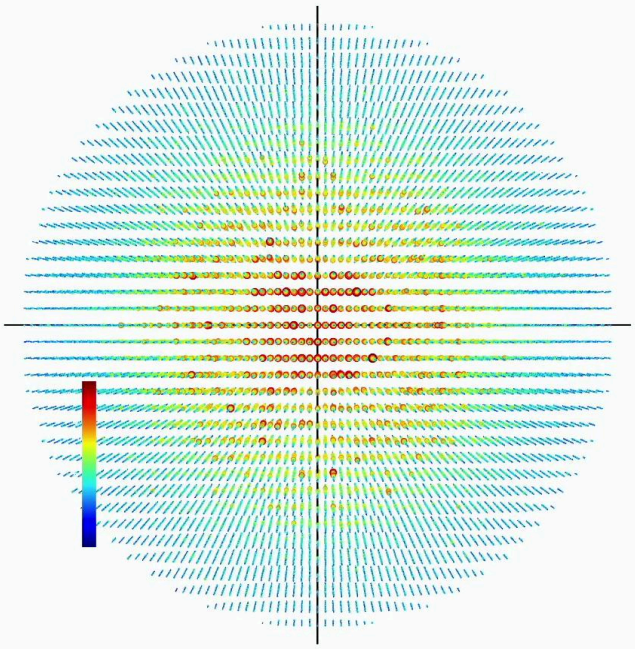
Notes

Summary

2m 07s

Serial crystallography





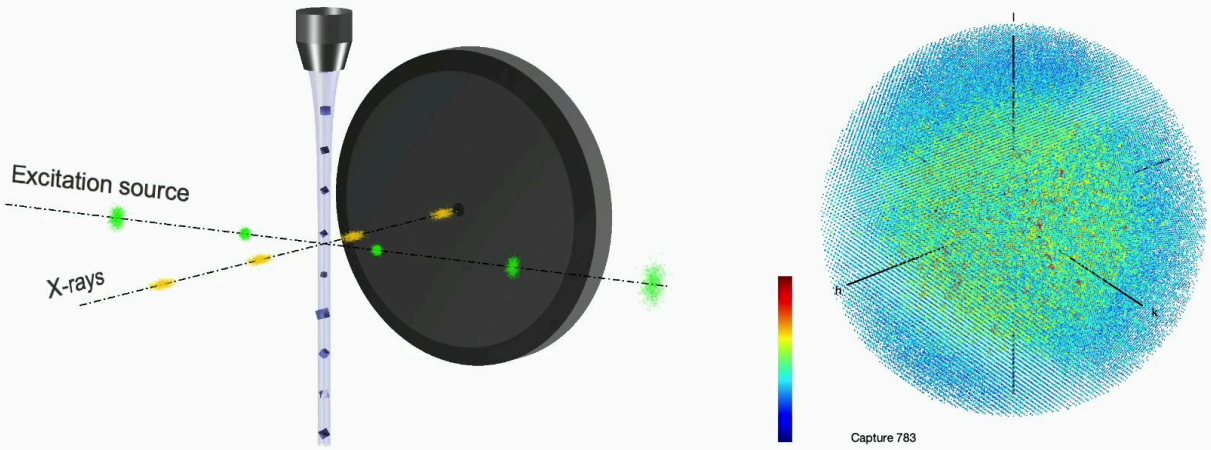
Despite the fact that up until today, well over 160,000 atomic resolution structures of biological molecules and their complexes have been deposited in the protein databank, one important class of protein named membrane proteins, which accounts for one-third of all proteins and two-thirds of medicinal drug targets, is extremely underrepresented, about 1 to 2%. This is in large part due to the hydrophobic nature of membrane proteins, which makes their crystallisation difficult and often limits the crystal size to the micron scale. This problem can be mitigated to an extent by using so-called lipidic cubic phases, or LCPs, as the crystallisation medium.

Notes

Summary

2m 47s

Serial crystallography



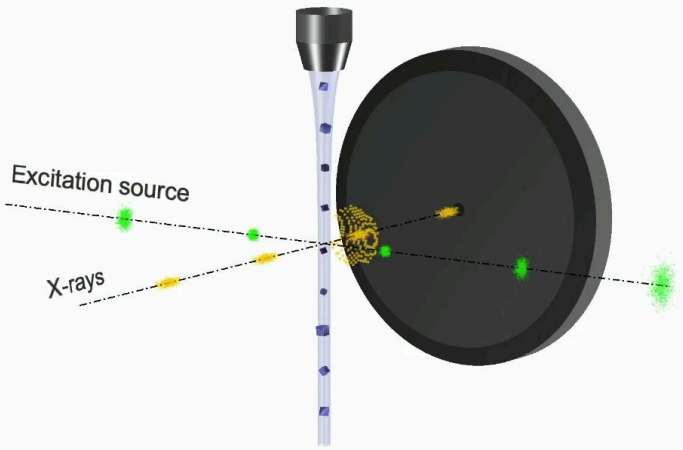
Such small crystals require micron or submicron beam focusing, which until the advent of DLSRs meant divergences could be about 0.2 degrees, or even larger in the horizontal plane, weakening the diffraction signal at high resolution. Only a limited amount of the diffraction signal can be obtained from any given crystal before radiation damage sets in. Individual microcrystals are insufficient for a complete data set. Hence, diffraction data from multiple crystals must be merged for structure determination. Prompted by successes in similar approaches at XFELs there has been a concerted effort in the last five years towards crystal delivery via the use of streams of randomly oriented microcrystals illuminated by synchrotron radiation in a technique coined Synchrotron Serial Crystallography, or SSX. The motivation of SSX has been to exploit very small crystals of linear dimensions, not much more than a micron, and which would otherwise be extremely difficult to mount on a conventional crystal mount and would frazzle up in a second or in a fraction of a second, indeed, due to radiation damage, thus necessitating an unrealistically large number of crystals for a single dataset.

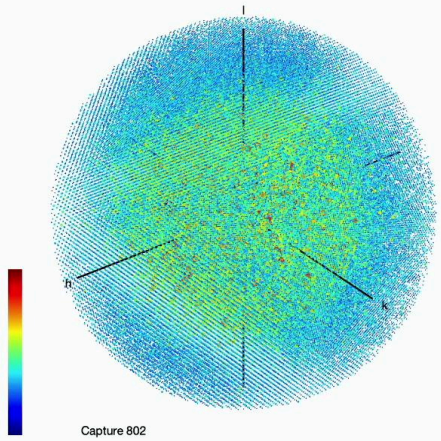
Notes

Summary

3m 38s

Serial crystallography





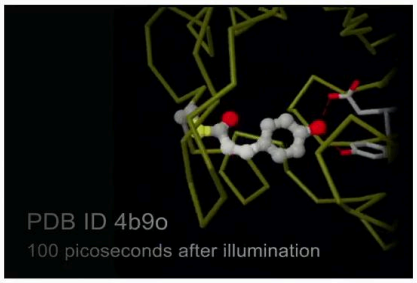
SSX can be carried out at both room temperature and cryogenic conditions, requiring novel techniques in sample preparation, delivery, data collection, and processing. The use of small micron-sized crystals furnishes one further benefit. It appears that radiation damage per unit volume is further reduced compared to larger crystals as the photoelectrons which are produced by the initial absorption process and then the subsequent secondary electrons can escape with a significant probability before they destroy or ionise parts of the crystal. The threshold size below which this begins to bestow an advantage appears to be a few microns. In SSX, the microcrystals are delivered to the beam via one of several methods: by a fast-moving liquid jet, a slow-moving LCP toothpaste extrusion, mounted on a membrane, or as a collection of crystals in a crystallisation well. Fresh material can then be delivered at controlled speeds, adjusted to administer the maximum tolerable dose for each crystal. A large number of hits are required in order to provide a statistically reliable full dataset from the random slices in reciprocal space collected from the ensemble of randomly oriented crystals, as can be seen from the first thousand of 15,000 hits from an SSX experiment conducted on lysozyme on the right.

Notes

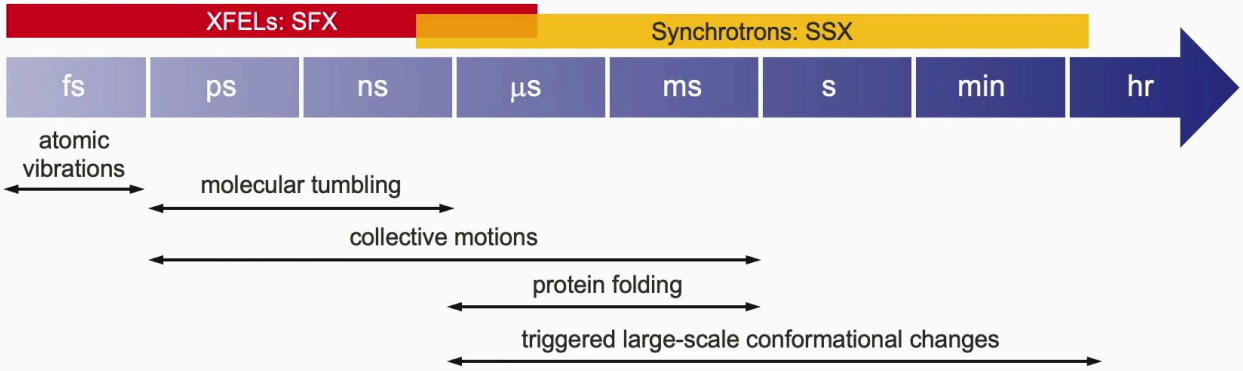
Summary

5m 08s

Serial crystallography



- Why bother? We now have Alphafold2!!
- Incentive is not equilibrium structure
- Incentive is
  - biological function
  - mechanistic studies
  - macromolecular dynamics



One might ask, why bother with these complex experiments when we now have AlphaFold 2? The incentive is actually clear as we are interested in biological action, not simply an equilibrium structure. SSX, in conjunction with serial femtosecond crystallography carried out at XFELs covers the whole gamut from atomic vibrations on the femtosecond time scale to triggered large-scale conformational changes of protein structures which can span anything between microseconds and hours.

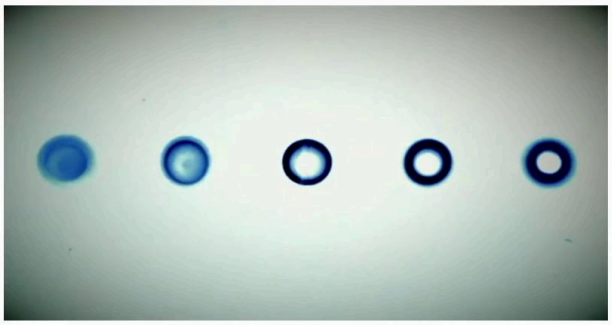
Notes

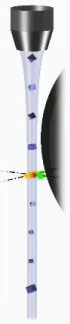
Summary

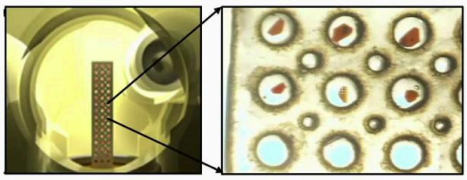
6m 50s

Serial femtosecond crystallography

- SFX @ XFELs
- Deposited energy in microscopic crystals causes them to “Coulomb explode”
  - Replace with new crystal for each XFEL pulse
    - Liquid jet
    - LCP “toothpaste”
    - Membrane array
    - …
  - Repetition rate of XFEL pulses limited due to time needed to insert new crystal into beam
- Electrons (mainly) remain in ground state longer than pulse duration
  - Diffraction signal survives
  - “Diffraction before destruction”
- N.B. requires charge-integrating detectors, e.g. Jungfrau detector (with variable gain)







See C. Stan *et al.*, Nature Physics

Serial femtosecond crystallography at XFELs is used to investigate dynamics typically between the picosecond and microsecond time scale. Now, it should be noted that although the diffraction pattern propagates from the crystal marginally before photoelectrons are fully ejected, the resulting Coulomb forces thereafter cause an explosion of the absorbing volume in the case of delivery of crystals by liquid or LCP jets. This causes them to be locally destroyed, requiring a healing time measured in milliseconds before the next pulse can be used. This is not normally a problem for XFELs running at a few tens or hundreds of hertz, but is a major drawback for high rep rate XFELs such as the European XFEL. Note also that all such XFEL experiments require charge-integrating detectors such as the Jungfrau detector.

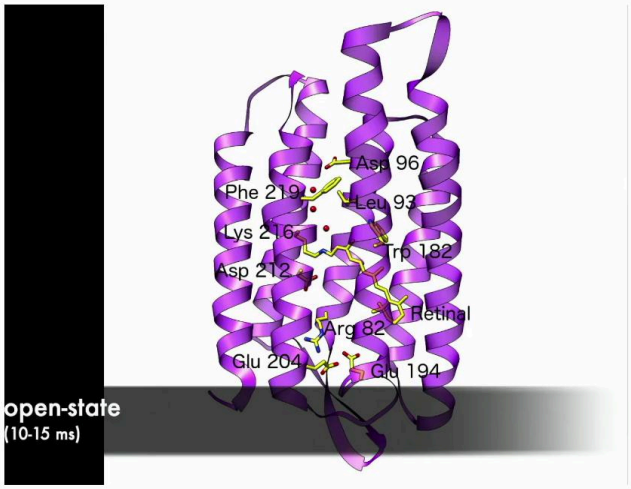
Notes

Summary

7m 23s

Serial synchrotron crystallography

- SSX @ synchrotrons
- Sub μs – s resolution
  - Complementary to XFEL investigations
- DLSRs tight focus
  - Allows investigation of micron-sized crystals
    - Membrane proteins
    - G-coupled protein receptors
  - Higher Bragg-peak intensities
    - Approach μs regime from ms regime of 3rd-generation facilities
  - Potentially combine with pink-beam mode for SS-Laue-X
    - Towards ns resolution



See: "Proton uptake mechanism in bacteriorhodopsin captured by serial synchrotron crystallography", Weinert *et al.* DOI: 10.1126/science.aaw8634

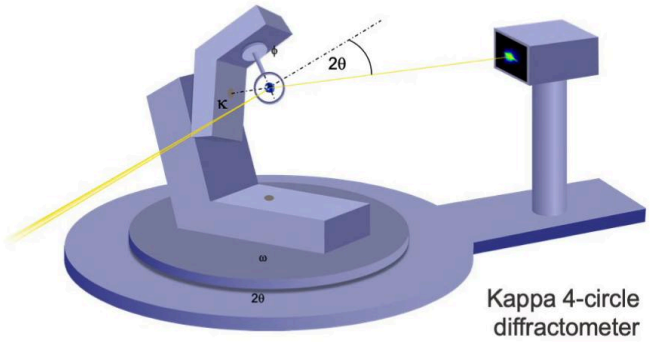
Serial synchrotron crystallography, on the other hand, is best suited to characteristic reaction times measured in the microseconds to seconds range and is thus complementary to time-resolved protein crystallography at XFELs. DLSRs are particularly useful as they allow the use of high-flux beams with very tight focuses of under a micron while maintaining a very low divergence. Moreover, Laue diffraction is experiencing something of a renaissance in SSX when the beamline is operated in pink beam mode. A recent example of SSX in which the mechanism of proton uptake in bacteriorhodopsin was revealed and is shown here and in detail in the provided link.

Notes

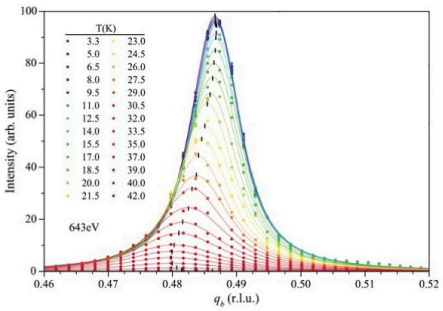
Summary

8m 24s

The selected Bragg peak method



Kappa 4-circle diffractometer



Magnetic order in multiferroic $LuMnO_3$ produces a weak Bragg peak close to (0 ½ 0), enhanced using 643-eV radiation tuned to the L-edge of Mn

Y.W. Windsor *et al.*, Phys. Rev. Lett. **113** 167202 (2014)

- Follow specific Bragg points
  - Observe physical phenomena through structural changes
  - e.g., change in symmetry and/or unit cell dimensions with…
    - temperature
    - pressure
    - applied magnetic field
    - …
- Signal often weak
- SR needed
- Used in
  - resonant soft x-ray scattering
  - surface x-ray diffraction

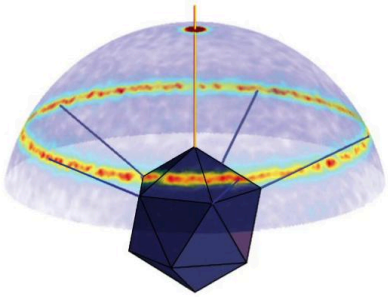
Now, some experiments use the so-called selected Bragg peak method in which individual Bragg peaks are recorded. Depending on the details of the setup, there are typically four rotational degrees of freedom in order to achieve the Bragg condition. In the shown kappa four-circle configuration here, the sample can be rotated through three axes while the detector through only one. The selected Bragg peak method is often used to observe changes in physical properties depending on the local environment such as pressure, temperature, applied magnetic or electric fields and so on and so forth. Often, signal can be very weak, such as in surface x-ray diffraction discussed later this week, for which synchrotron radiation is completely indispensable.

Notes

Summary

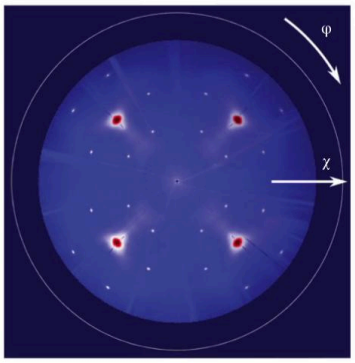
9m 14s

Pole figures



Pole figure of an icosahedral Ti-Ni-Zr quasicrystal film grown on $Al_2O_3(0001)$ mapped on a spherical surface. A fivefold symmetry axis points perpendicularly out of the film, while the five other fivefold axes at 63.435° relative to each other and the out-of-plane axis, produce the ring feature. This texturing is induced by the 5-fold planes having the lowest surface energy

P.R. Willmott *et al.*,
Phys. Rev. B
71 094203 (2005)



Pole figure acquired over a range of Q = 2.25 to 2.35 $Å^{-1}$ and at a photon energy of 16.49 keV of heteroepitaxial β-Fe_2O_3 thin film. The pattern encompasses three families of reflections associated with the film.

J.D. Emery *et al.*,
ACS Appl. Mater. Interf. **6**, 21894–21900
(2014)

- Fix θ-2θ
- Rotate sample
  - azimuthally (ϕ) by up to 2π
  - polar coordinate (χ) up to π
- Modern detectors can record range of 2θ
  - Vary θ and obtain multiple pole figures in one image
- Suitable for single-crystal and some textured samples

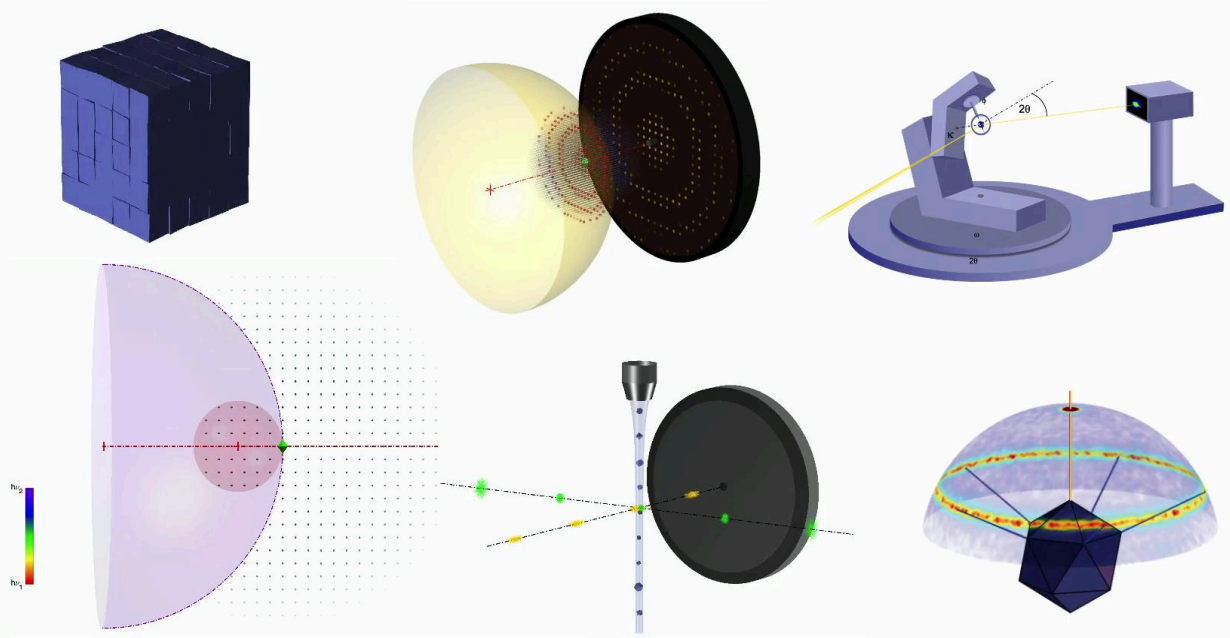
We finish this video by considering textured samples rather than single crystals. The aim here is to see if there might be preferred orientations of crystallites in what might otherwise be assumed to be a powder sample with random orientations. The Bragg reflection of interest is selected by fixing the two theta angles and then rotating the sample through all possible orientations via a matrix of azimuthal and polar angles. In the case of modern area detectors, a range of two theta angles can be recorded in a single image and thus obtain multiple pole figures in a single dataset. Two examples of pole figures are shown here.

Notes

Summary

10m 03s

Summary of this section

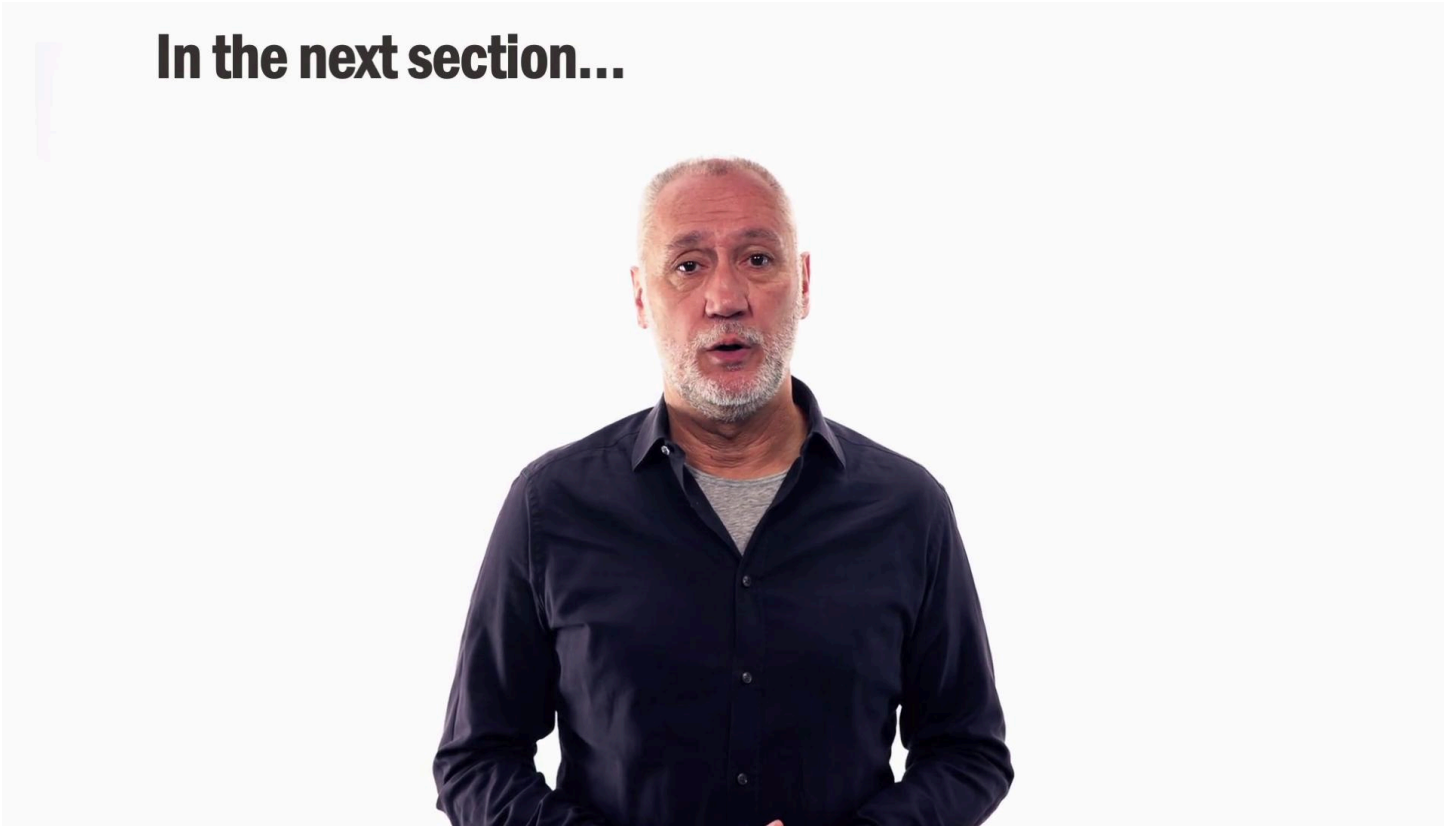


This first section of week two was concerned primarily with single crystal diffraction. We began by defining different crystal types, then went on to look at Laue diffraction and how this is experiencing a renaissance with the advent of fast readout and sensitive photon counting detectors. We then moved on to the rotation method using monochromatic x-rays and the related technique of serial crystallography, both using synchrotron and XFEL radiation. We finished with a brief description of the selected Bragg peak method and pole figures.

Notes

Summary

10m 50s



In the next section we will look at powder diffraction, a powerful technique that takes advantage of collapsing the 3D information of a single crystal diffraction pattern into one degree of freedom only.

Notes

Summary

11m 31s