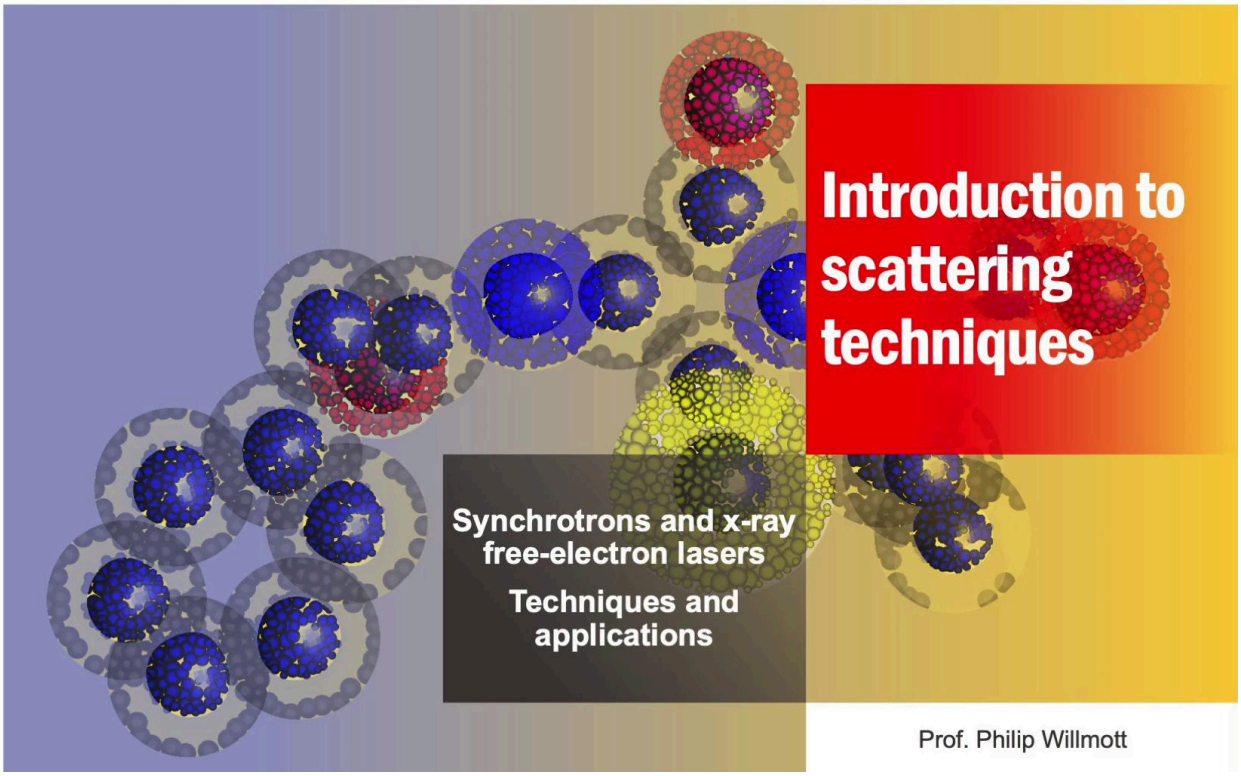
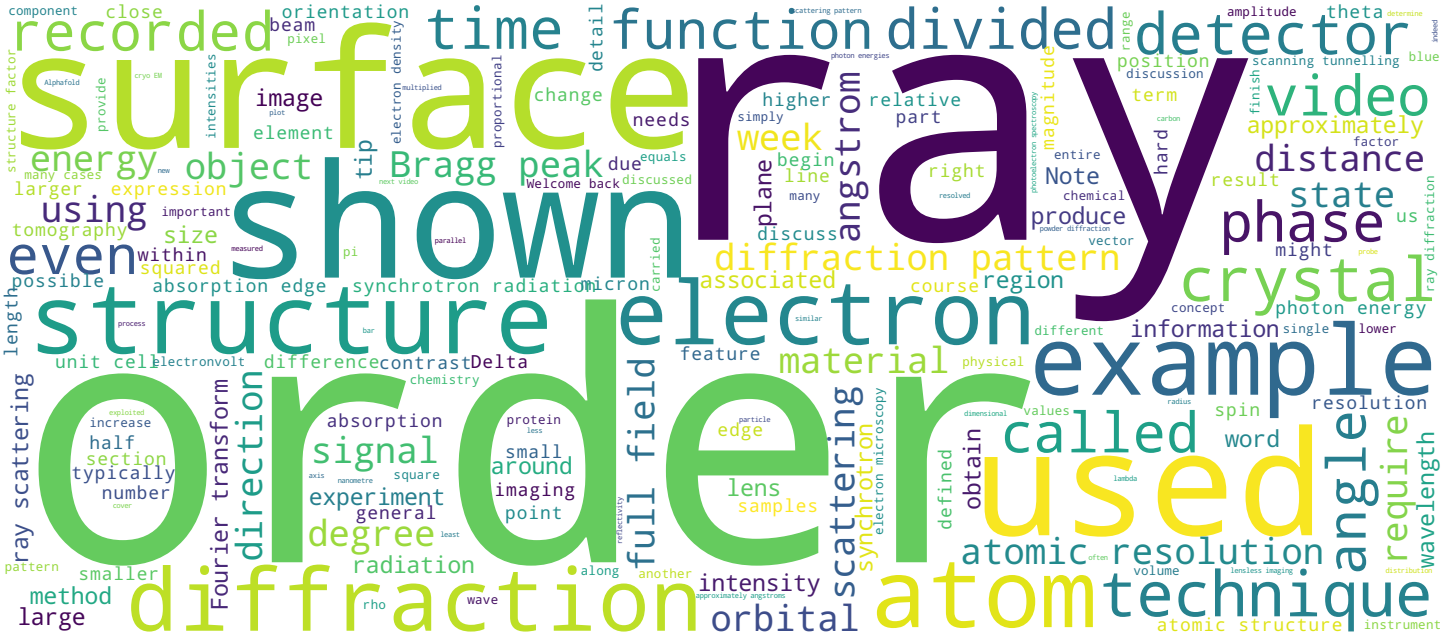


École polytechnique fédérale de Lausanne



Contents and objectives of this video

- Atomic resolution
- Approaches to get atomic resolution
- X-ray full-field microscopes
- X-ray diffraction and scattering
- Scattering at synchrotrons

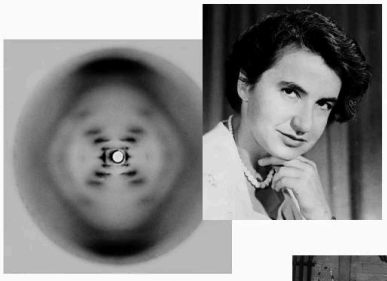
In this first video focusing on scattering and diffraction, we ask what is diffraction and why is it used compared to other approaches of investigating matter at the atomic level? The advantages of X-ray scattering and diffraction at synchrotrons will also be outlined.

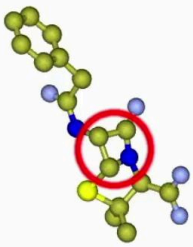
Notes

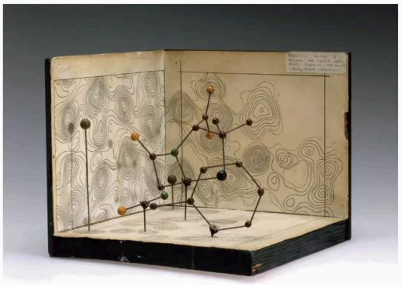
Summary

0m 05s

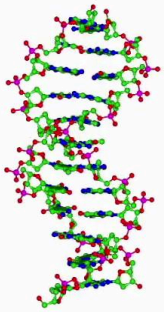
Atomic resolution – who needs it?







Courtesy of The Science Museum, London, UK



It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

All B/W images courtesy Wikimedia commons

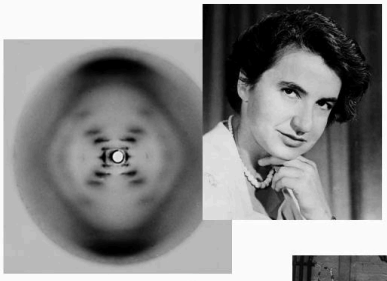
The most obvious answer to the question who needs atomic resolution, is the extremely strong correlation between structure and function, both chemical and physical. In many cases, a knowledge of the atomic structure leads directly to the functionality of the system, as exemplified by the now famous understatement of Watson and Crick in the penultimate paragraph of their Nobel Prize winning Nature paper on the structure of DNA in 1953. "It does not escape to our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." The atomic structure of DNA was gleaned by Watson and Crick to a large extent by simple structural and chemical considerations and arguments. But even prior to this scientific tour de force, Dorothy Crowfoot Hodgkin would determine the 3D structure of penicillin in 1945, thus identifying the four-membered so-called beta-lactam ring C3N shown here, assumed by many chemists until this study to be too chemically unstable to exist. Indeed, this was the key to the mechanism of penicillin in destroying bacteria.

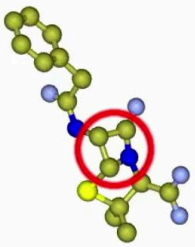
Notes

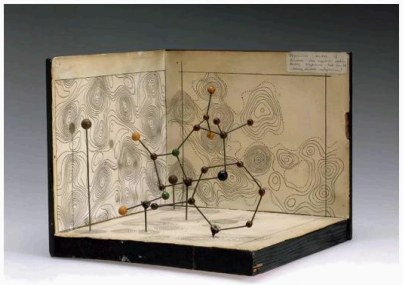
Summary

0m 24s

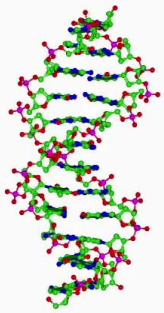
Atomic resolution – who needs it?







Courtesy of The Science Museum, London, UK



It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

All B/W images courtesy Wikimedia commons

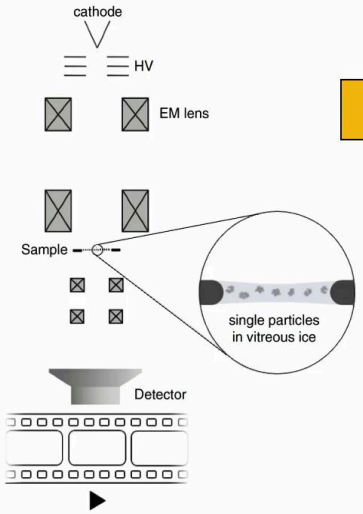
The instability of the carbon three N ring allowed it to be easily prized open, become highly reactive and thereby deactivate enzymes responsible for cell-wall growth in bacteria, causing them to rapidly expire. These are just two classic examples of the importance of unveiling the atomic structure, examples which incidentally use X-ray diffraction as a central tool. This correlation between structure and function is not limited to biology, however, and many insights to physical and chemical properties have been made in the fields of electronics, for example, in the strongly correlated electron systems based on perovskite structures or in energy catalysis, and the role of open structures such as zeolites.

Notes

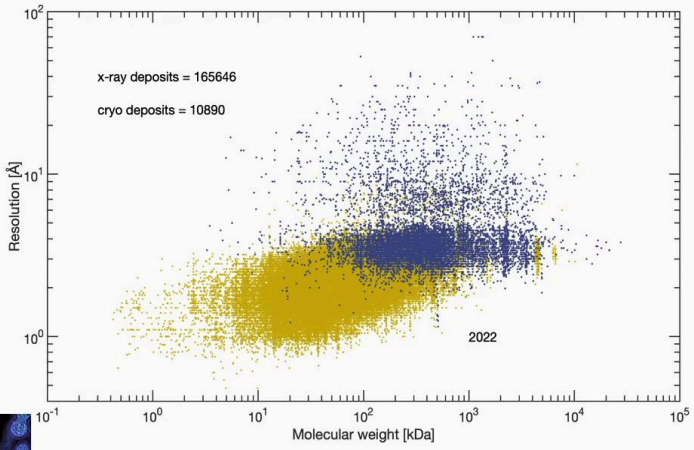
Summary

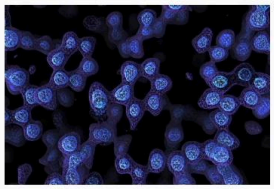
1m 53s

Techniques that provide atomic resolution



Cryo-EM





Credit: Paul Emsley
MRC Laboratory of Molecular Biology

Nobel Prize, Chemistry, 2017
Jacques Dubochet, Joachim Frank and Richard Henderson

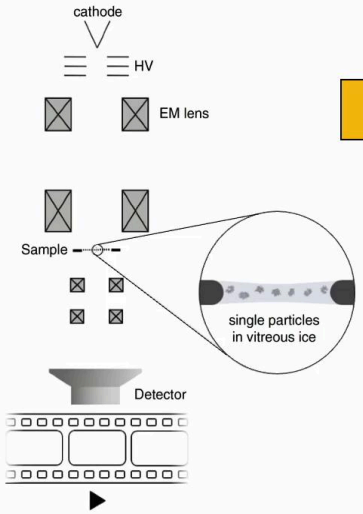
A technique which has gained prominence in recent years and garnered the 2017 Nobel Prize in chemistry is cryogenic electron microscopy, or cryo-EM. It has been shown to provide, in many cases, atomic resolution of large macromolecules using ensembles of individual particles trapped in vitreous ice in an electron microscope setup. Importantly, it does not require the macromolecule to form a crystal, oftentimes a bottleneck in diffraction approaches to biomolecular structure. It requires also only extremely small volumes of material measured in microlitres and concentrations of micromoles per litre, in other words, only picomoles of concentrated material. Moreover, it delivers direct images rather than diffraction patterns which need to be analysed. On the other hand, sample preparation is nontrivial and the signal to noise ratio can be a major obstacle, especially for smaller particles. Data mining is complex and extremely time and CPU consuming, and the equipment is expensive. It only operates in ultra-high vacuum and provides little or no chemical information. A plot of the deposited structures in the protein data bank up until August 2022, for X-ray derived structures in gold and cryo-EM structures in blue is shown here.

Notes

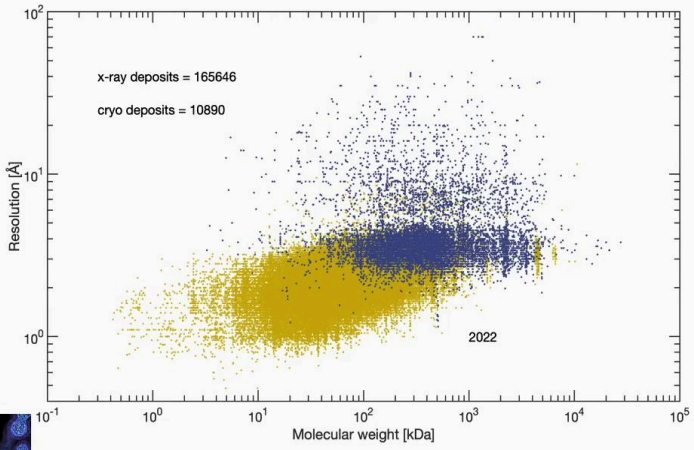
Summary

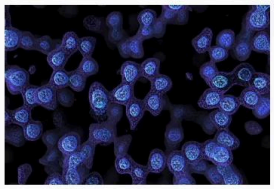
2m 50s

Techniques that provide atomic resolution



Cryo-EM





Credit: Paul Emsley
MRC Laboratory of Molecular Biology

Ultimate resolution 2 – 3 Å
More commonly 4 – 5 Å

Nobel Prize, Chemistry, 2017
Jacques Dubochet, Joachim Frank and Richard Henderson

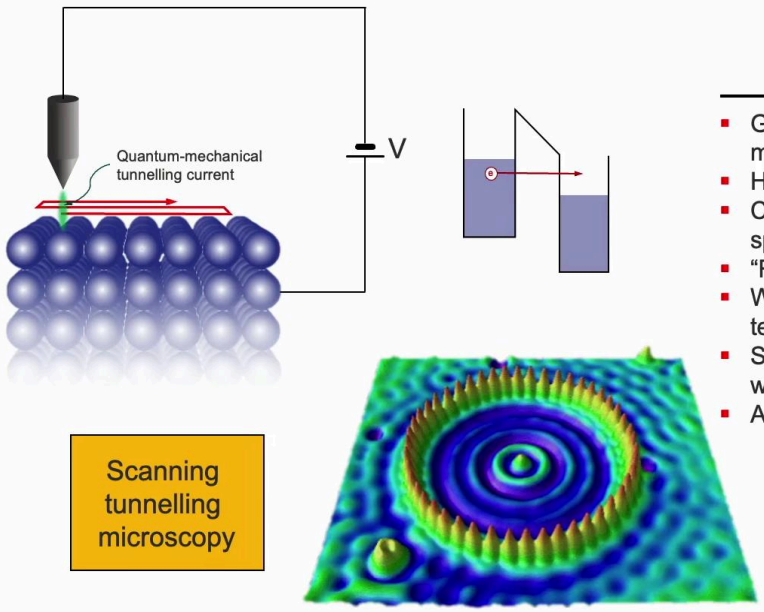
One sees that the cryo-EM structures commonly have resolutions of approximately 4 angstroms, and essentially all are above 2 angstroms, and for structures with molecular weights above 100 kilodaltons. The X-ray structures can in many cases be resolved to better than one angstrom, and can easily, indeed most easily, be applied to structures with molecular masses below 2,000 daltons.

Notes

Summary

4m 36s

Techniques that provide atomic resolution



Scanning tunnelling microscopy

- Good for electronic materials
- High resolution
- Can be element specific (“STS”)
- “Real space” images
- Wide sample temperature range
- Samples electron wavefunctions
- Atom manipulation

Nobel Prize, Physics, 1986
Gerd Binnig and Heinrich Rohrer

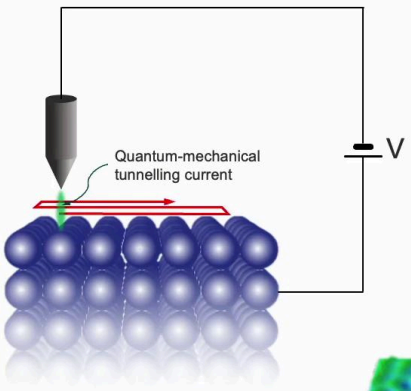
Another breakthrough in imaging of surfaces with atomic resolution was heralded in the early 1980s with the invention of scanning tunnelling microscopy, or STM. A conducting tip with an atomically sharp end is allowed to scan across the surface of a clean object in vacuum. A voltage applied between the tip and surface makes the potential of the surface lower than that of the tip. There is a nonzero probability that electrons in the tip can quantum mechanically tunnel across the narrow gap through to the surface, thereby generating a tunnelling current. The strength of this tunnelling current increases with decreasing separation between the tip and surface. Using this method, the famous silicon(111) 7 times 7 surface reconstruction involving no fewer than 147 atoms, could be ascertained for the first time with atomic resolution. It can even be used as an atomic tweezer to nudge atoms into specific configurations. STM is a powerful probe to investigate electronic materials. It is even capable of being elements specific in scanning tunnelling spectroscopy, by tuning the voltage between tip and surface. It delivers real space images across a wide temperature range.

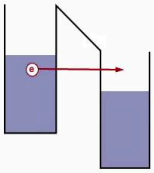
Notes

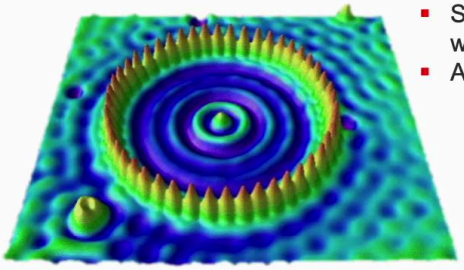
Summary

5m 06s

Techniques that provide atomic resolution







Scanning tunnelling microscopy

▪ Good for electronic materials ▪ High resolution ▪ Can be element specific (“STS”) ▪ “Real space” images ▪ Wide sample temperature range ▪ Samples electron wavefunctions ▪ Atom manipulation	▪ Requires UHV ▪ Sample preparation extremely demanding ▪ Sample must be (semi)conducting ▪ Only probes surfaces ▪ Slow scanning speed ▪ Local (representative?) properties ▪ Vibrations ▪ Tip quality critical

Lateral resolution 1 – 2 Å
Height resolution ~ 0.1 Å

Nobel Prize, Physics, 1986
Gerd Binnig and Heinrich Rohrer

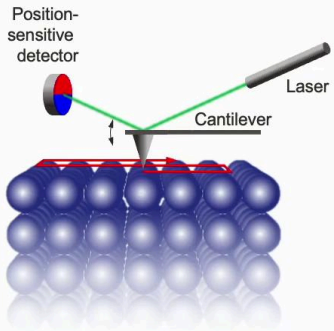
On the downside, it requires UHV conditions, and sample preparation is, in general, very demanding. Samples must be at least semiconducting, and image recording is measured in minutes. There is sometimes the danger that a local feature is misinterpreted as being representative of the entire sample, hence, multiple measurements should be carried out before any statistical statements can be made. Lastly, the technique is extremely sensitive to external vibrations, and the tip quality is critical to the resolution. Tips can also be very easily blunted or even destroyed. STM offers lateral resolutions of the order of an angstrom and height resolutions often superior to 0.1 angstroms.

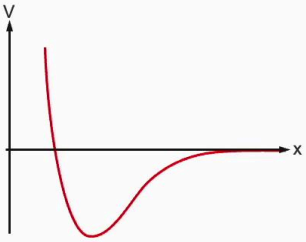
Notes

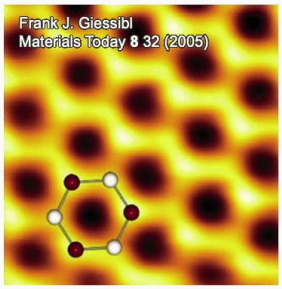
Summary

6m 46s

Techniques that provide atomic resolution







Atomic force microscopy

- Insulating materials also possible
- Modest sample environment
- "Real space" images
- Wide sample temperature range

- Cantilever/tip quality critical
- Only probes surfaces
- Local (representative?) properties
- Slow scanning speed
- Vibrations
- No chemical information
- Steep edges difficult to record

Lateral resolution 2 – 3 Å
Height resolution 0.1 Å

Now, a similar technique to scanning tunnelling microscopy, which can be thought of as the younger sister, is that of atomic force microscopy, or AFM. It exploits the well-known Lennard-Jones potential describing interatomic forces. An atomically sharp tip is attached to the end of a microscopic cantilever and is scanned across the surface. The cantilever flexes according to the profile of the surface. A narrow laser beam reflected off the upper surface of the cantilever hits a position-sensitive photo detector, thereby providing the height-sensitive signal. Using AFMs, many different surfaces can be imaged. In contrast to STM, AFM can also sample insulating materials and does not require the very strict sample environment of UHV. The samples can be heated or cooled at will. Like STM, the performance of AFM depends critically on the quality of the tip and suffers from most of the other limitations already listed for scanning tunneling microscopy. The lateral resolution of AFM is approximately 2-3 angstroms, while height profiles can be resolved down to approximately 0.1 angstroms, similar to the performance of STM.

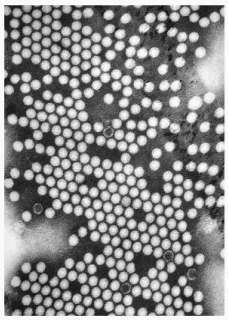
Notes

Summary

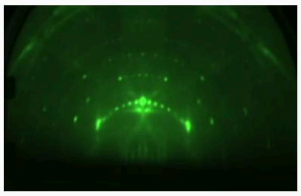
7m 50s

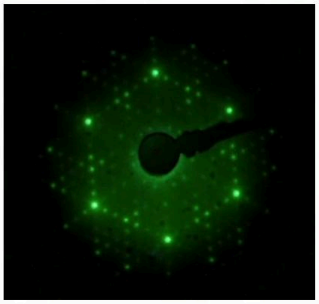
Other techniques that provide (near) atomic resolution

Full-field (conventional) transmission electron microscopy

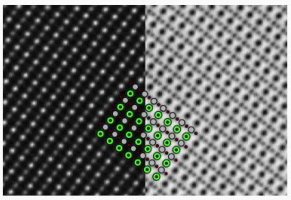


(SPA-)LEED
RHEED





Scanning transmission electron microscopy



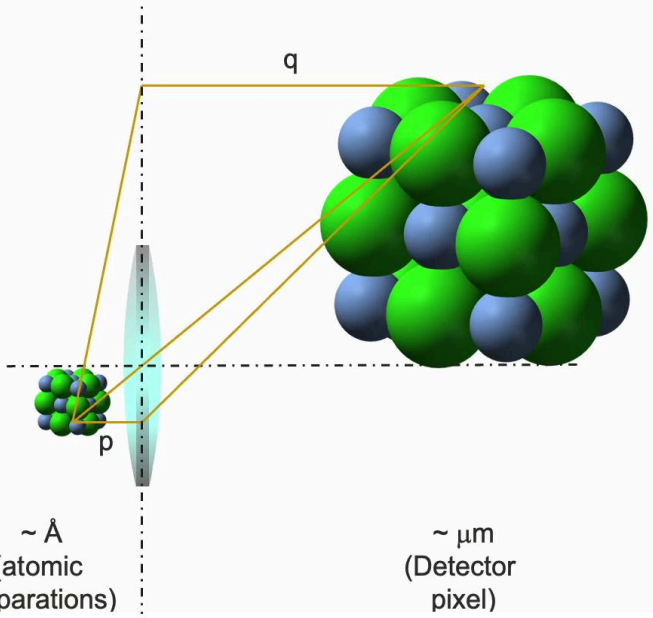
Briefly, other electron beam techniques that provide atomic or near atomic resolution include full-field transmission electron microscopy, scanning tunnelling electron microscopy, low-energy electron diffraction, and reflection high-energy electron diffraction. In the case of LEED and RHEED, a quantitative description of the surface is possible to extract in a manner similar to that used in X-ray diffraction, but with the serious disadvantage that multiple scattering must be considered, making analysis much more demanding.

Notes

Summary

9m 19s

Why not full-field x-ray microscope?



- Magnification q/p ~ 10^4
  - ⇒ f ≃ p
- f_{min} ~ 50 mm
  - ⇒ q ~ 1 km!!
- Refraction (CRLs)
  - $n_R = 1 - \delta$, very small refraction effect
  - ⇒ very poor light-gathering power (NA)
- Reflection (mirrors)
  - Only very shallow angles
- Diffraction (FZPs)
  - Best choice regarding magnification

- e.g. Matsuyama *et al.*, Scientific Reports **7** 46358 (2017)

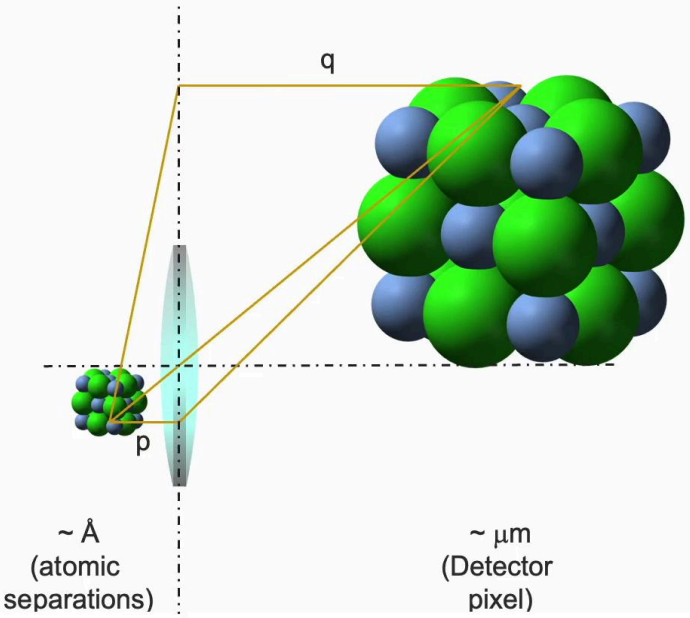
Okay, what's stopping us from building a full-field X-ray microscope similar in concept to a full-field electron microscope? Well, the simple answer is that no lens can be fabricated with sufficient power to achieve the necessary magnification. Firstly, we should recognise that in order to image an object with atomic resolution, no matter by which method, we need to use radiation with wavelengths of the same order of magnitude or smaller. In other words, in the hard X-ray regime with wavelengths of about an angstrom. Let us consider the basic specifications that such an instrument would need to have. We want a magnification factor m equal to q divided by p, such that a sample, a distance p from the lens is imaged onto a detector at distance q from the lens, so that atomic features are resolved. Such an area detector would have pixels with linear dimensions measured in microns, meaning that m needs to be at least of the order of 10 to the 4, 10,000 and the sample lens distance p is very close to the focal length of the lens. This requires that the lens detected distance is of the order of one kilometre, for p is equal to 50 millimetres.

Notes

Summary

10m 02s

Why not full-field x-ray microscope?



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- f_{min} ~ 50 mm
  - ⇒ q ~ 1 km!!
- Refraction (CRLs)
  - $n_R = 1 - \delta$, very small refraction effect
  - ⇒ very poor light-gathering power (NA)
- Reflection (mirrors)
  - Only very shallow angles
- Diffraction (FZPs)
  - Best choice regarding magnification

- Resolution ~ 500 Å (50 nm)?
- Full-field x-ray microscope possible!
  - Still ~ 50 m long!

- e.g. Matsuyama *et al.*, Scientific Reports **7** 46358 (2017)

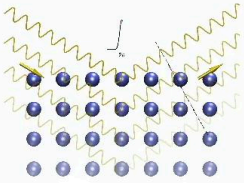
Refraction-based lenses such as CRLs are far too weak to service such a lens and have very poor light gathering powers in any case. Similarly, focusing via reflection from a curved mirror is impractical due to the very shallow maximum reflection angles of hard X-rays. The best choice regarding magnification would be diffraction-based lenses such as Fresnel zone plates, but even these cannot hope to achieve such a large magnification factor. Thus, full-field direct imaging on the angstrom scale is completely excluded. If one only needs a resolution of a few tens of nanometers, full-field X-ray microscopy is possible, though only for very long beamlines with lengths of the order of 100 metres. Such an instrument is described in the reference shown here.

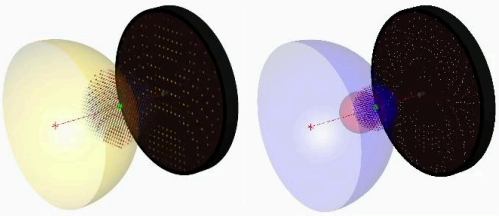
Notes

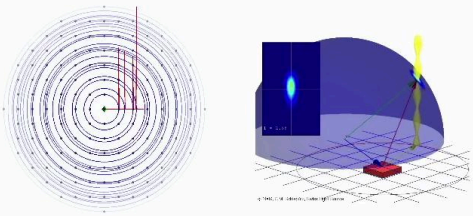
Summary

11m 37s

… and finally, x-ray scattering and diffraction







- Diffraction
  - Bulk single crystals
  - Powder/polycrystalline material
  - Thin films/heterostructures
- SAXS/WAXS
  - Cells, viruses, nanoparticles
- CXDI/ptychography
  - Single nanocrystals
  - Nanoscale-structured extended objects
- …

Given that a full-field microscope with atomic resolution seems to be impossible, how do we then obtain atomic-scale images? The answer is diffraction, which one can think of as a lensless imaging technique, although this term is used for a more specific scattering application. Diffraction and elastic X-ray scattering in general, allow one to investigate an enormous variety of materials and structures, including not only crystalline objects, but also nonperiodic structures such as cells and other biological objects.

Notes

Summary

12m 40s

Imaging without a lens

Instead of imaging with a lens, in X-ray scattering and diffraction, one collects the elastically scattered X-rays directly. This information is fed into a computer, where so-called phase retrieval, or phasing, is carried out using one or more of many different approaches. Phase retrieval describes the need to obtain not just the intensities and positions of the diffracted signal components, but also their relative phases. Remember, these are X-ray waves. We need this in order to reconstruct the image that produces the scattering pattern.

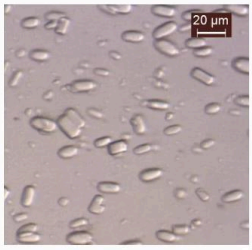
Notes

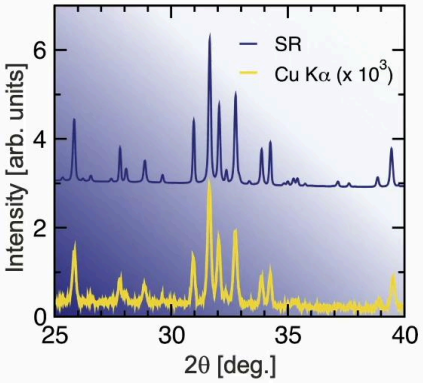
Summary

13m 23s

X-ray scattering and diffraction at synchrotrons

- **HIGH BRILLIANCE**
  - Low divergence ⇒ high angular resolution scattering/diffraction patterns
  - Tight focus ⇒ small sample sizes e.g., protein crystals ~ 1 μm^3
  - Low emittance ⇒ large working distance between focussing optics and sample ⇒ bulky sample environments
  - High flux ⇒ rapid data acquisition, time-resolved studies down to μs regime or shorter





Diffraction and scattering experiments at synchrotrons profit from synchrotron radiation for several reasons. The low divergence and high flux of synchrotron radiation compared to laboratory sources means that better angularly resolved and much higher intensity patterns can be recorded, much facilitating the process of phasing and obtaining an atomic structure. The two powder diffraction patterns on the right highlight this difference. The top one was recorded in 5 seconds using synchrotron radiation and a one-dimensional Mythen detector, while the lower one, which has been blown up by a factor of 1,000 along the ordinate axis here, was recorded over nearly 3 hours at a lab-based source and exhibits a significantly broader signal by approximately a factor of 2. The low emittance of synchrotrons can also be exploited to obtain tight focuses of the order of a micron for investigation of very small crystalline samples such as the lysozyme crystals shown in the upper left image. The low emittance can otherwise be exploited by allowing a large working distance by parallelising the beam as much as possible in order to accommodate complex experimental setups, such as that shown here in the lower left image.

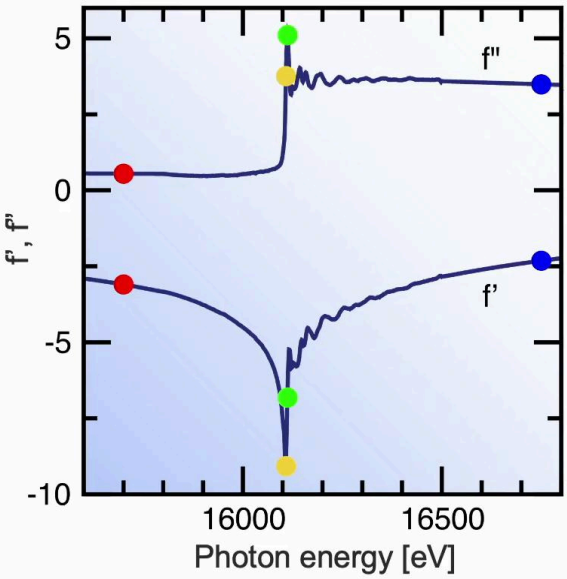
Notes

Summary

14m 06s

X-ray scattering and diffraction at synchrotrons

- **ACCESS TO HIGH PHOTON ENERGIES**
  - Penetrate deep into samples, e.g., aeronautical components, large fossils, concrete, etc.
- **TUNABILITY**
  - Abrupt changes to atomic scattering amplitudes as one crosses an absorption edge
    - "Anomalous" signal
    - Phasing (MAD, SAD)
  - Unique to synchrotrons



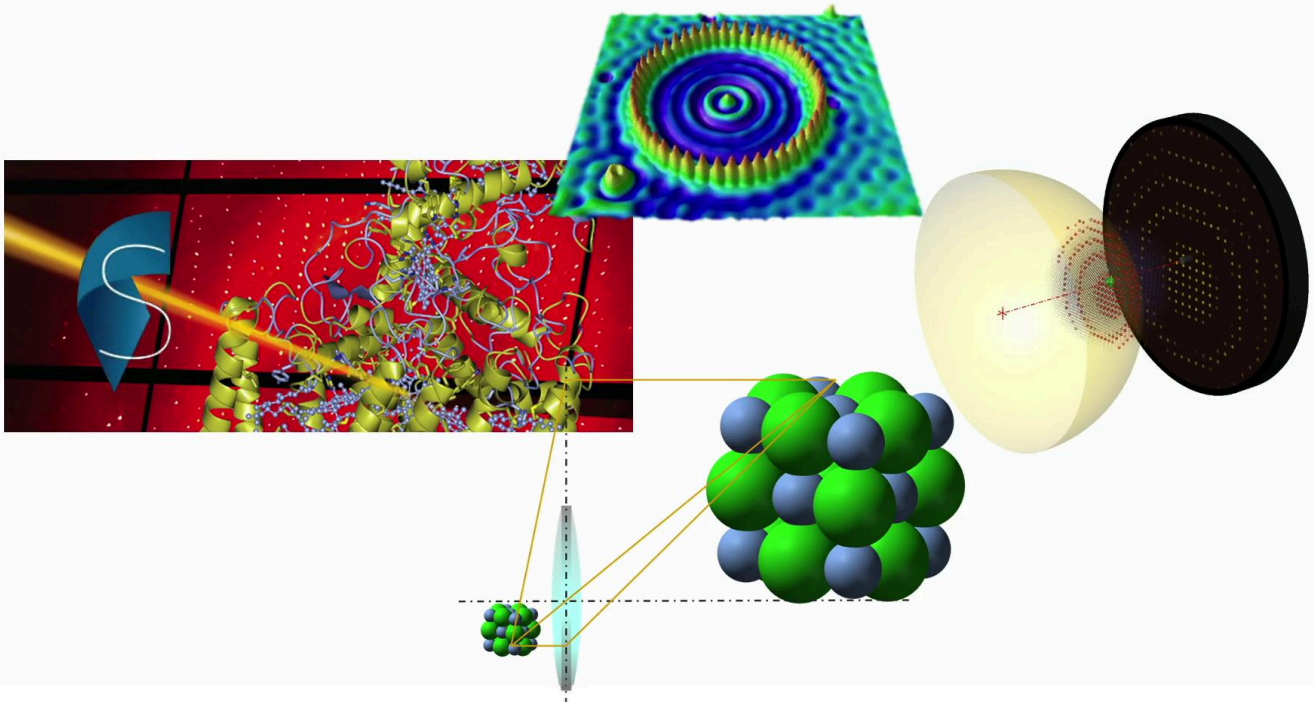
Now, synchrotron radiation isn't attractive only because of the high fluxes it provides. High-photon energies up to a megaelectronvolt are accessible at high-energy synchrotron storage rings, such as the ESRF in France, APS in the US and SPring-8 in Japan. The large penetration depths of this radiation means that larger objects such as aeronautical components or large fossil specimens can be probed. A unique property of synchrotron light is its tunability. As we discussed in detail in the sister course, the scattering amplitude and phase of a given atom type changes abruptly and sometimes very strongly close to an absorption edge of that atom, that is, at an energy equating to the binding energy of one of the atom's electrons, normally a core electron. By recording diffraction data in this region, the phase problem can be unambiguously solved. Moreover, in some techniques, this change in response is able to light up selected elements or chemical compounds.

Notes

Summary

15m 42s

Summary of this section



To summarise this short introductory section, we reviewed the contents of both this and the sister course. We also considered different approaches to imaging material on the atomic scale before discussing the requirements needed to build a full-field hard X-ray microscope with atomic resolution, concluding that such an instrument is impossible to construct and why, instead, X-ray scattering and diffraction are suitable alternative solutions.

Notes

Summary

17m 04s

In the next section

In the next section, we begin by looking at what defines a crystal and the terminology we use for them. We then look at simple interference and diffraction phenomena, including Bragg's law and general rules which are always followed in diffraction.

Notes

Summary

17m 42s