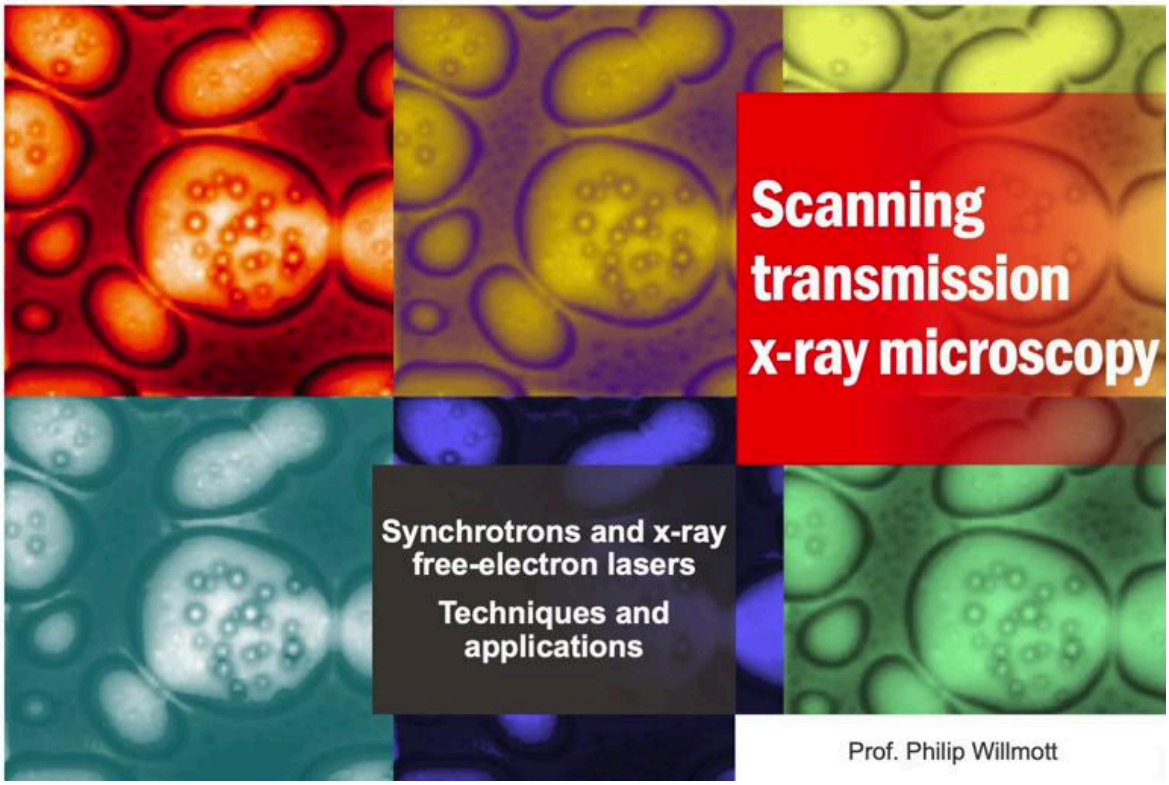
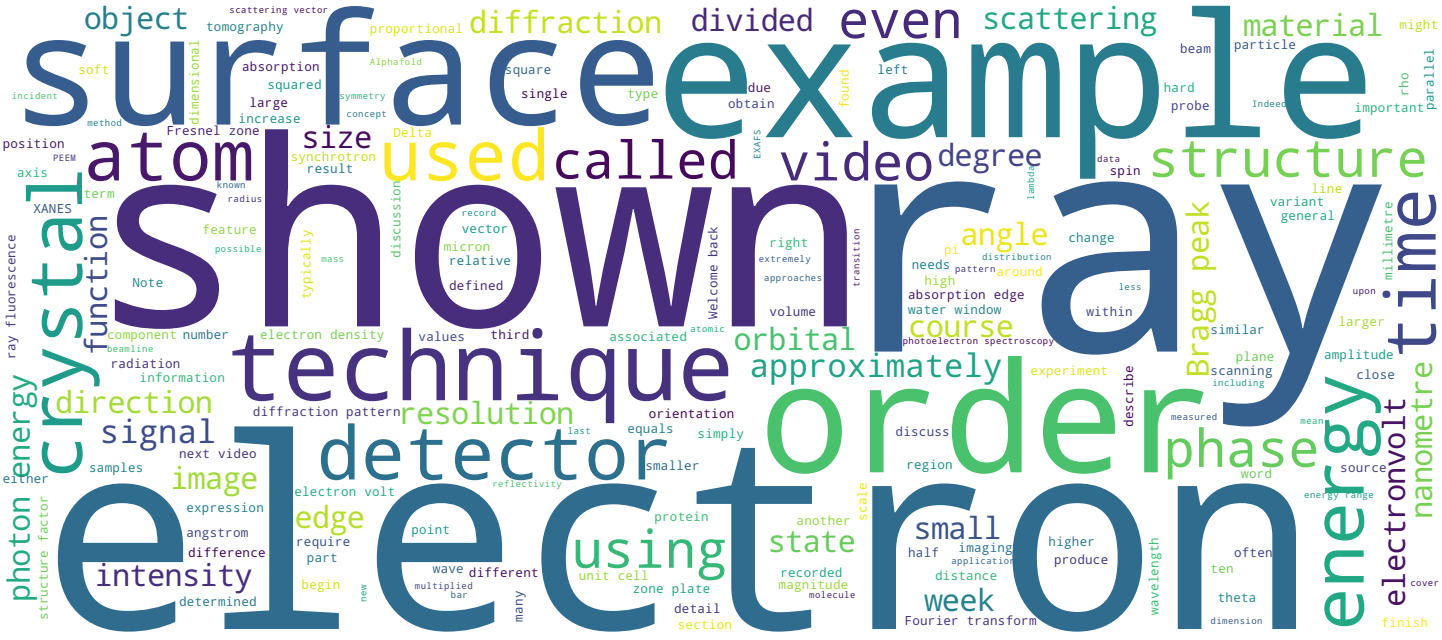




Search MOOC

Video

EPFL

Contents and objectives of this video

- STXM – introductory comments
- The water window
- Experimental details
- STXM example

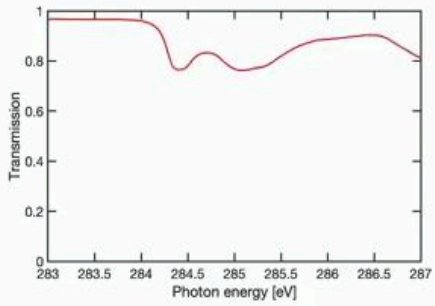
Welcome back to our discussion of absorption spectroscopies. In this video, we will look at Scanning X-ray Transmission Microscopy, or STXM. STXM is most commonly, if not exclusively, used in the energy range defined by the so-called water window between approximately 200 and 530 electronvolts. Before looking at a recent example of STXM, we will consider some experimental aspects related to STXM studies.

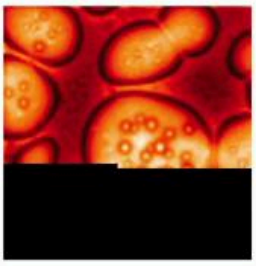
Notes

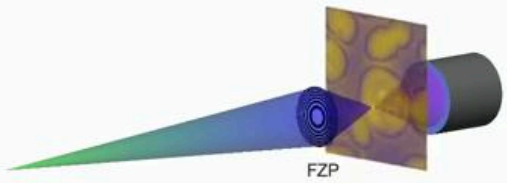
Summary

0m 05s

Introductory comments







- Scanning transmission x-ray microscopy, STXM
- Type of XANES
  - Probing absorption spectra around and just above selected absorption edge(s)
- Features
  - Very tight focus (FZP) ~ 10 – 50 nm typical
  - Record spectrum
  - Raster scan the sample step-by-step or on-the-fly
  - STXM is thus a microspectroscopy
  - Can be combined with XRF and/or Auger
- Most commonly used in soft x-ray regime, especially in so-called “water window”
- Applications
  - Polymer technologies
  - Biological imaging
  - Soil science

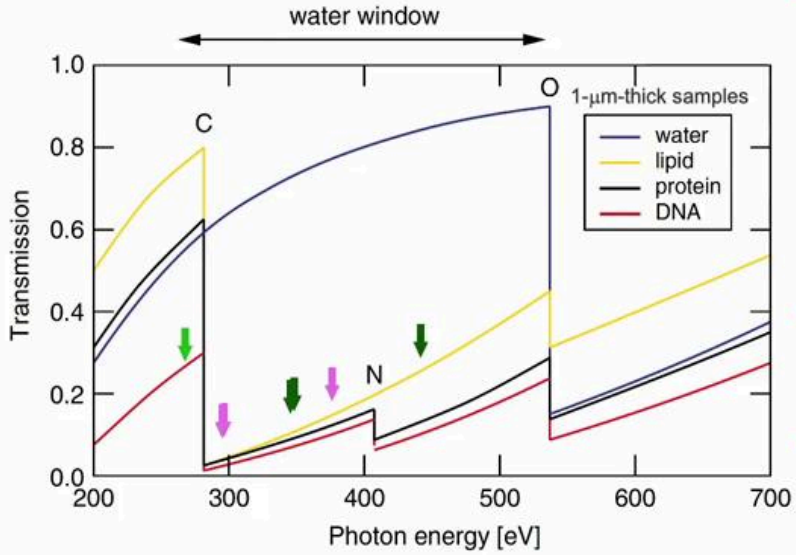
STXM is a type of XANES experiment in that it probes the absorption spectra around and just above the absorption edge of interest. It is a non-destructive method that, as its name suggests, is a scanning technique in which a sample is rastered in the plane perpendicular to the X-ray beam. The resolution is determined by the size of the focus, which can be as small as 10 nanometres, plus any inaccuracies in sample positioning. STXM is therefore a type of microspectroscopy. Note that it can also be combined in parallel with other probes, such as X-ray fluorescence and/or Auger electron spectroscopy. There are many problems in biology, organic chemistry, and polymer physics, which require detailed chemical analysis at a sub-micron scale but mapped over macroscopic areas. Although traditional methods such as infrared spectroscopy and nuclear magnetic resonance can differentiate chemical species by observing subtle differences in bond strengths caused by the local chemical environment, their spatial resolution is limited to the millimetre scale. STXM has sub-micron to few nanometre resolution. It's most commonly used in the soft X-ray regime, defined by the water window, which we'll describe in detail in just a moment. Its primary applications are in polymer technologies, biological imaging of cells or tissues in their natural hydrated state, and in soil science.

Notes

Summary

0m 35s

The water window



- Water transparent below oxygen K-edge down to ca. 200 eV
  - At lower photon energies, transmission drops due to $\Lambda_a \propto (h\nu)^3$
  - Organic compounds containing C and N have high absorption contrast
  - L-edges of other biologically relevant elements accessible, especially K, Ca
- XANES spectra fingerprints
  - Pre-edges
  - Quasibound signal above edge
- In-vivo or cryo-experiments possible
  - DNA, RNA, histone, protein
  - Easily distinguishable (c.f. hard x-ray phase-contrast methods)

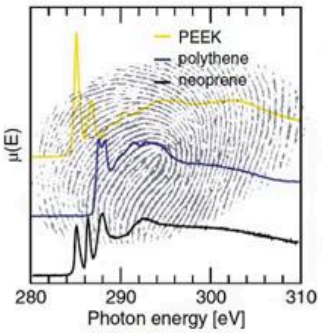
Oxygen at approximately 63% by mass is the predominant element in most living tissue. With carbon, hydrogen, and nitrogen coming in second, third, and fourth place at 19%, 9% and 5%, respectively. The main reason for this is, of course, that most living matter is composed of approximately 60-90% water, depending on whether you're a human or a jellyfish. Importantly, the K-edge of oxygen at approximately 530 electronvolts is above those of carbon at about 280 electronvolts and nitrogen at 410 electronvolts. Much below 200 electron volts, the transmission becomes uncomfortably low due to the absorption length varying as the third power of the photon energy. Hence, organic compounds, be they living tissue or polymers, have a high absorption contrast within the water window. Moreover, this energy range contains the L-edges of the biologically relevant elements, potassium, three edges at approximately 295, 297, and 379 electronvolts, calcium at 346, 350, and 438 electronvolts, and even the admittedly weak L1 edge of chlorine at 270 electronvolts.

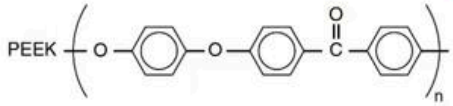
Notes

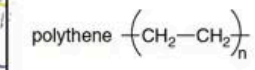
Summary

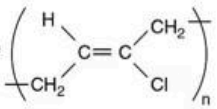
2m 21s

The water window



PEEK 

polythene 

neoprene 

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Reference spectra: O. Dhez *et al.*, "Calibrated NEXAFS spectra of some common polymers" J. Electron Spectrosc. Rel. Phenom., **128**, 85–96 (2003) and linked spectra

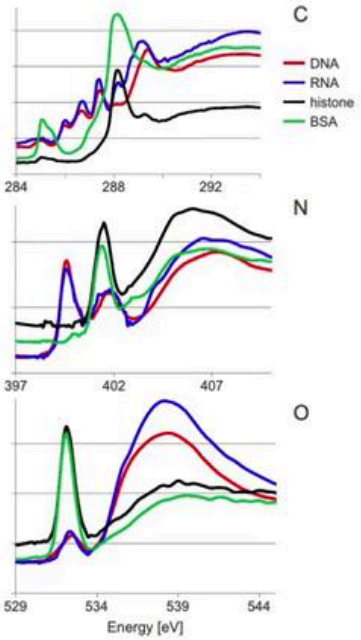
The featureless curves shown in the previous slide, belie the rich information provided by XANES in reality. Indeed, these are so detailed that they can act as excellent fingerprints or signatures of specific compounds, such as shown here for the three polymers of polyether ether ketone, polythene, and neoprene. A large database of reference spectra can be found in the link provided here and other links within that link.

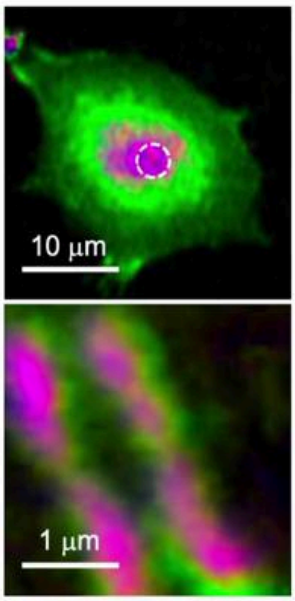
Notes

Summary

3m 51s

The water window





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Adapted from: K. Shinohara *et al.*, Cells **8** 164 (2019) https://doi.org/10.3390/cells8020164

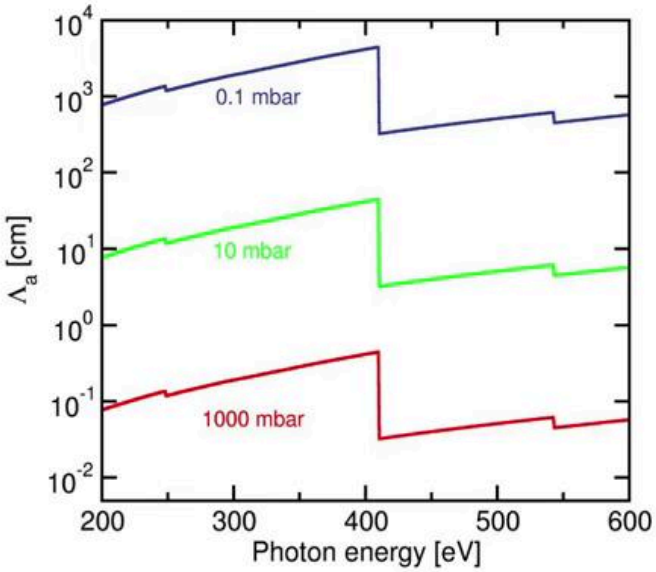
Indeed, this can be applied at all three K-edges of carbon, nitrogen, and oxygen for in-vivo or cryogenic experiments with resolutions of just a few tens of nanometre. These approaches are complementary to hard X-ray phase-contrast methods discussed in weeks five and six. STXM provides immediate and clearly distinguishable chemical contrasts through the XANES fingerprint, but at the cost of high absorption and dose rates. Hard X-ray experiments can sample larger volumes with comparable resolution but struggle to distinguish between chemically and electron-density similar materials such as DNA and RNA.

Notes

Summary

4m 20s

Experimental details



- Synchrotron-only technique
  - Scan photon energy
- Strict vacuum requirements between source and sample/detector
  - Transmission for 1-mm air = 6% above N-edge @ 410 eV
  - Typically requires 0.1 mbar or better
- Long-term accumulation of carbon contamination on optics (cryo worse!)
  - Compromises "real" sample C-XANES
  - Incident radiation "cracks" CO_2 on surface
  - Remove using low-pressure O_2 leak
- FZP
  - Focal length ∝ hν: requires axial scanning
  - OSA

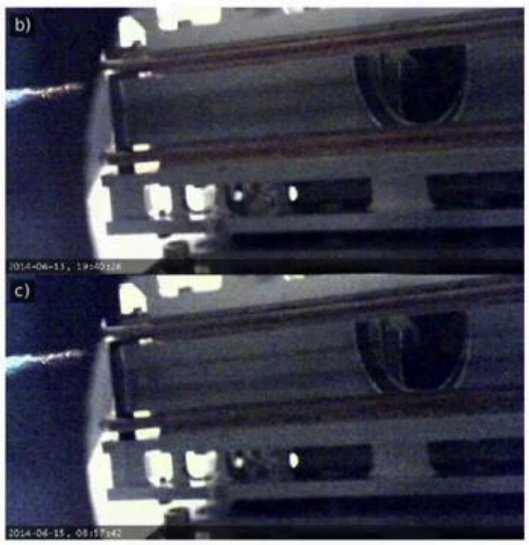
Regarding experimental aspects of STXM, the first thing to mention is that this is a technique that can only be carried out at a synchrotron facility, on account of it requiring one to scan the photon energy. Because STXM normally operates in the water window, there are, in general, very strict vacuum requirements for the flight path between source and sample and sample and detector. For example, just one millimetre of air will absorb 94% of radiation above the nitrogen edge at normal pressure. Typically, one needs to keep the pressure from the sample to the detector to 0.1 millibars or better.

Notes

Summary

5m 06s

Experimental details



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See B. Watts *et al.* https://doi.org/10.1088/1748-0221/13/04/C04001

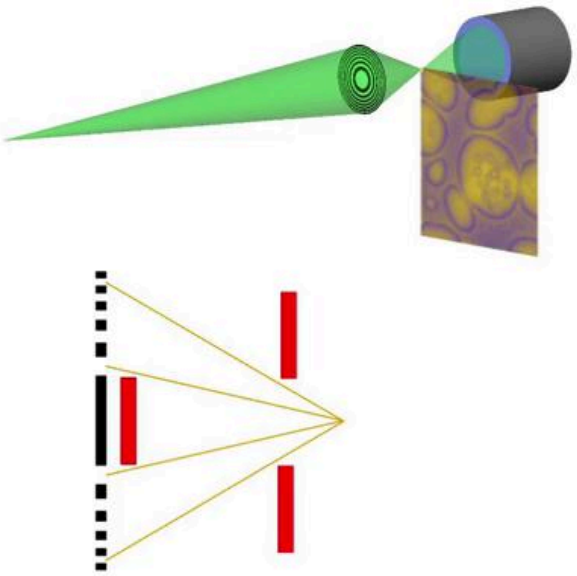
The vacuum requirements from the source to the optics and then the sample are even more stringent, as this path length is measured in tens of metres. A highly detrimental phenomenon is the accumulation of carbon on optics elements such as mirrors or monochromators. This can happen if the optical element is cryo-cooled in order to remove heat resulting from the incident X-ray beam. The element acts as a pump for any residual gases such as carbon monoxide, CO2, or methane. Moreover, the X-rays can be absorbed by surface species on optical elements, even those that are not cooled, causing these to crack and leave solid carbon behind. It's not uncommon to see X-ray mirrors after they have been in operation for several months that have stripes of graphitic carbon along their surface, where the footprint of the X-ray is. A common way to remove this is to use a low-pressure oxygen leak or even an oxygen plasma discharge.

Notes

Summary

5m 49s

Experimental details



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- FZP
  - Focal length ∝ hν: requires axial scanning
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Focusing in STXM to a few nanometres or a few of tens of nanometres is normally carried out using a Fresnel zone plate. The focal length of a Fresnel zone plate is proportional to the photon energy, hence there needs to be a concerted movement of either the Fresnel zone plate or the sample in order to maintain a tight focus. This might typically be 10-20% of the Fresnel zone plate's focal length. This axial motion needs to be very well-controlled in order to maintain the same position of the focus on the sample. One also needs to use an order-sorting aperture for the Fresnel zone plate in order to cut out the zeroth order radiation from that zone plate.

Notes

Summary

6m 56s

STXM example – colour selection by butterflies

https://commons.wikimedia.org/wiki/File:European_peacock_butterfly_%2836693337710%29.jpg

- Laminography + STXM
- Butterfly wing scale (BWS)
  - Many colours
  - Not only pigments!
  - Natural photonic crystal selects narrow band of visible radiation
  - 711 eV
    - Moderate contrast
    - Good working distance
    - High photon flux

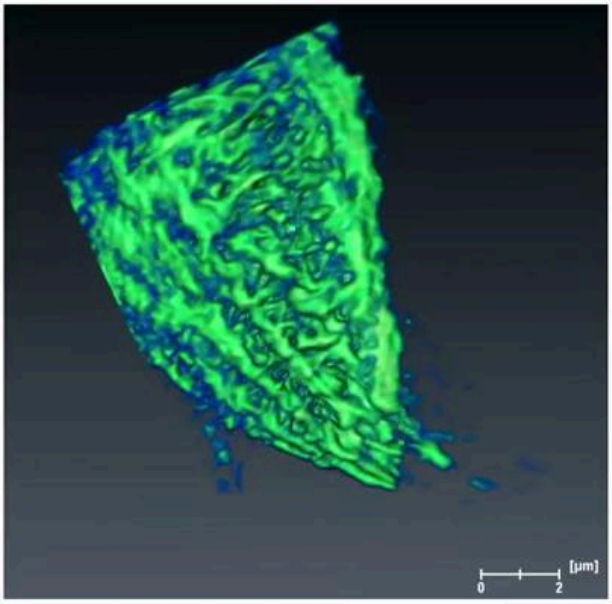
We finish with an example of STXM used in combination with laminography, a variant of tomographic methods, which we will encounter in Week Six of this course. This is a study of the architecture of the scales of a butterfly wing, in this example, that of the European peacock butterfly. Butterflies can exhibit intense colours. These are only partly due to pigmentation, but more often, a narrow range of colours is selected via diffraction from the natural repetitive structures embedded in the scales. A single scale from a European peacock butterfly was investigated at 711 electronvolts, rather than at the carbon edge. This was a compromise between obtaining sufficient contrast, having a comfortably large working distance, and keeping a high photon flux.

Notes

Summary

7m 41s

STXM example – colour selection by butterflies



- Lamellae 1200 nm separation
- Crossribs 150 ± 50 nm diameter
- Ridges ca. 1000 nm width

Optical image of single scale

K. Witte *et al.*, Nanoletters

A volume of approximately 10 by 10 by 10 microns is shown here. The regular repetition in one direction between lamellae of around 1.2 microns is clearly identifiable. These are separated by narrow crossribs to provide rigidity. Depending on the dominant colour, the detailed architecture of the wing scales will vary.

Notes

Summary

8m 37s

In the next video...

In the next video, we will look at the XANES technique of Photoelectron Emission Microscopy, or PEEM, a technique which images the photoelectric emission using electron optics very similar to those used in conventional electron microscopy. The most commonly used application of PEEM is in magnetic contrast measurements of samples with distinct magnetic domains.

Notes

Summary

9m 02s