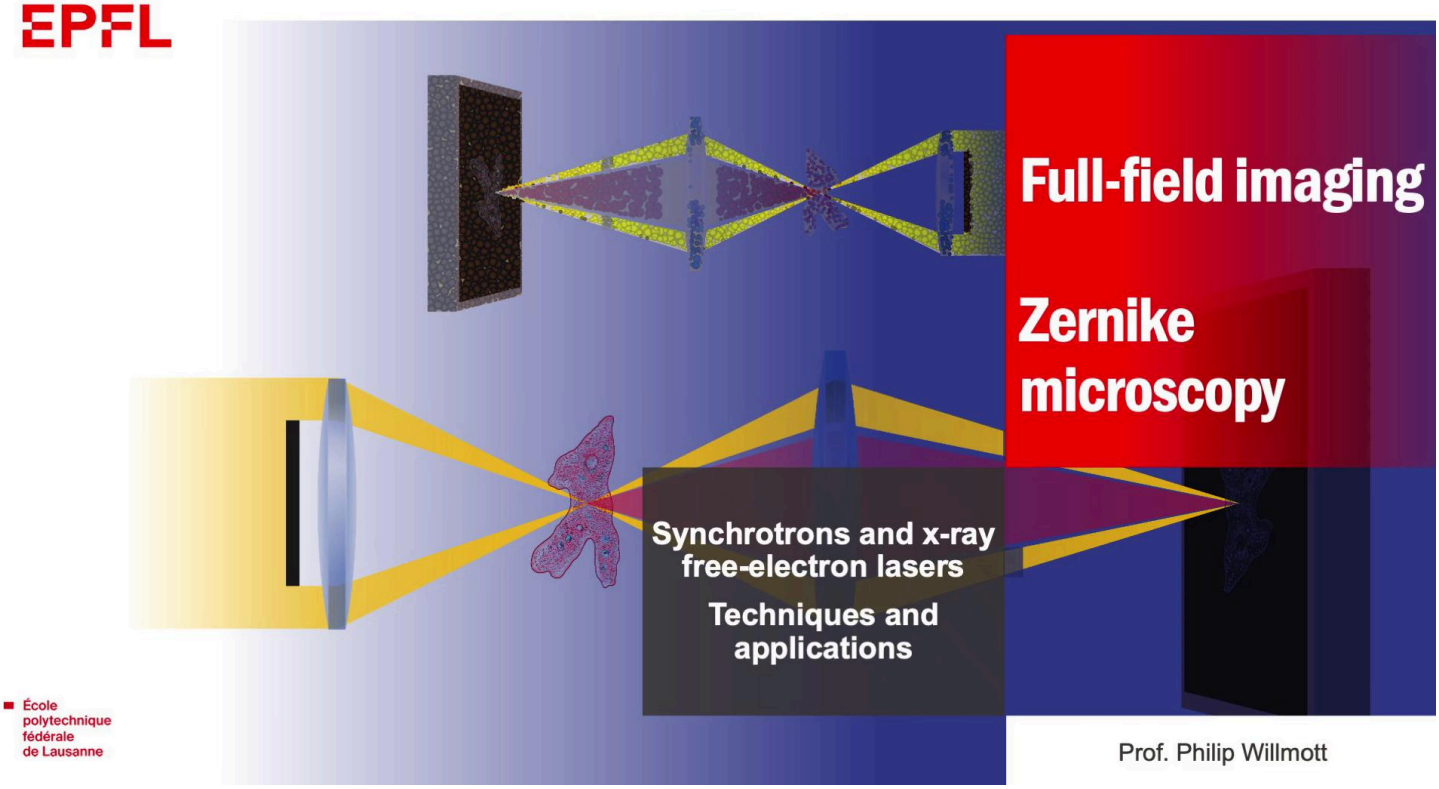


Search MOOC

Video

EPFL

Introductory comments

- Complementary to propagation-based phase-contrast imaging/tomography
- Exploits phase control of reference radiation
- Applications
  - Materials science
  - Biology
  - Nanotechnology
- Ability to provide high-resolution images of internal structures with high contrast ⇒ particularly valuable for studies of weakly absorbing complex biological systems

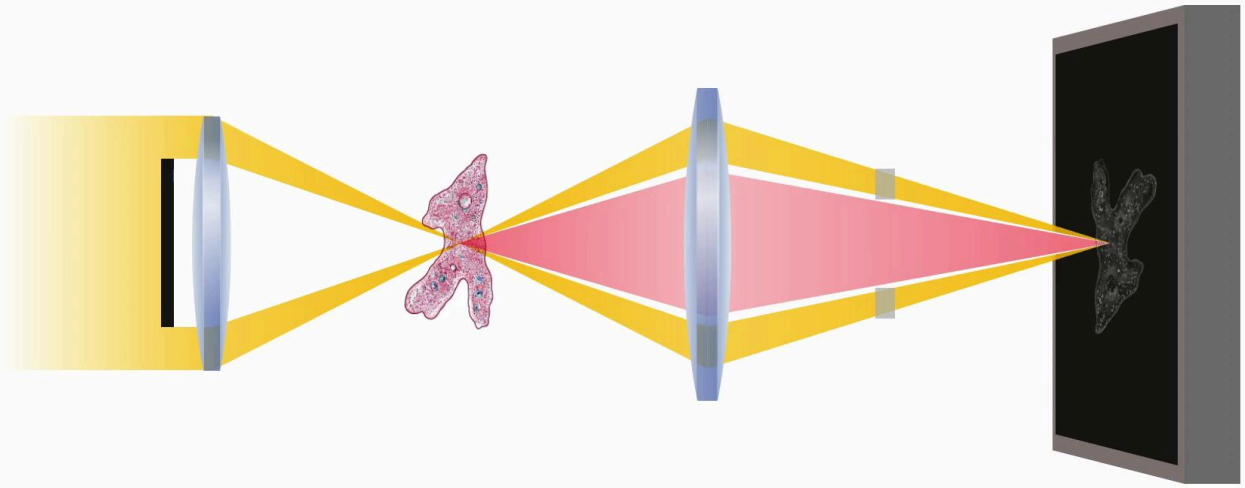
Welcome back. In this second video of the last section of the fifth week, we discuss Zernike microscopy as used for hard X-rays. After looking at the basic principles behind Zernike microscopy, we will consider how one might optimise the image contrast, and we will show a recent example. X-ray Zernike microscopy is complementary to the propagation-based phase contrast techniques described in the last section. In its appearance, it's very similar in setup to dark field microscopy described in the previous video. Zernike microscopy is defined by its controlling the phase of reference radiation. It has applications in broad areas of the natural and engineering sciences, perhaps foremost in biology, as it is able to provide high resolution images to better than 100 nanometres of internal structures with high contrast, even for low-density soft matter samples.

Notes

Summary

0m 04s

Working principle of Zernike phase-contrast imaging



See also: https://en.wikipedia.org/wiki/File:Dark_field_and_phase_contrast_microscopies.ogv

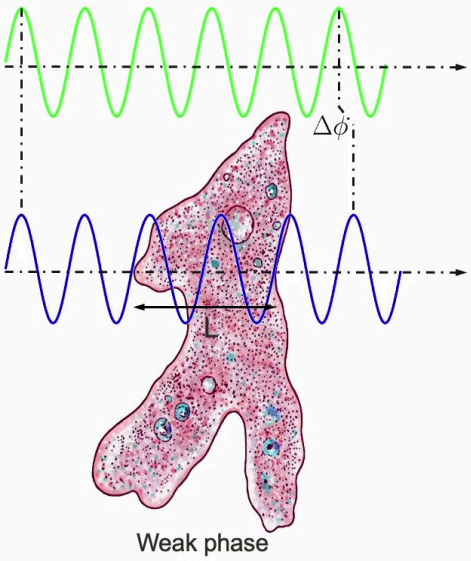
So, hopefully you remember the optical setup for dark field microscopy described in the previous video, and which is re-shown here. Zernike microscopy differs from dark field microscopy in how it handles the unscattered or so-called reference beam. Firstly, as can be seen, it dispenses with the opaque rings and allows through the annular reference radiation shown in yellow. This alone isn't very helpful because it will flood the detector with unscattered radiation via the lensing, which is liable to wash out the scattered signal from the sample. shown here in pink. However, crucially, a different ring is inserted in the path of the reference radiation. This ring is made to have a thickness so that the reference radiation is advanced by a phase of pi upon 2, or 90 degrees.

Notes

Summary

1m 07s

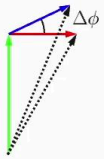
Working principle of Zernike phase-contrast imaging



$$\Delta\phi = \frac{2\pi L\delta}{\lambda}$$

No phase ring

$\pi/2$ phase ring



$\pi/2$ phase ring partially absorbing

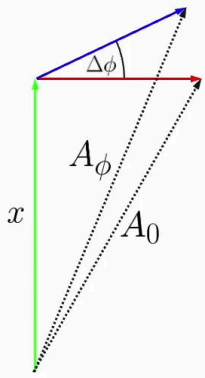
So if we consider a weak phase object, then some small fraction of the beam will travel through it and induce a small phase advance. Delta phi given by 2Pi L Delta divided by lambda, whereby L is the distance the radiation passes through it. The difference in amplitude of this vector added to the unscattered radiation, the blue plus green arrows, compared to the totally unscattered radiation, the red plus green arrows is very small and hence, contrast is low. Okay, but now, if we include that phase ring, the unscattered radiation changes its angle by 90 degrees, and the amplitude difference becomes significantly larger. What we also can do is increase this contrast still further by making the ring not only induce a phase advance of 90 degrees, but also it should partially absorb the unscattered radiation, thus reducing the amplitude of the reference wave.

Notes

Summary

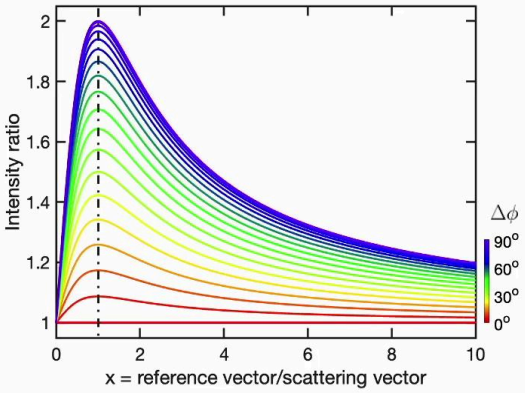
2m 05s

Optimizing the contrast



$$I_0 = |A_0|^2$$
$$I_\phi = |A_\phi|^2$$

- Attenuate reference vector to a value x times the sample vector to maximize contrast between shifted and unshifted cases
  - Limiting cases:
    - x = 0, no contrast $(I_\phi/I_0 = 1)$
    - $x \gg 1$, also no or little contrast
  - $$\frac{I_\phi}{I_0} = \frac{1 + x^2 + 2x\sin(\Delta\phi)}{1 + x^2}$$
  - Maximum contrast @ $x = 1$
  - x-value independent of $\Delta\phi$!!!
  - $$\left.\frac{I_\phi}{I_0}\right|_{x=1} = 1 + \sin(\Delta\phi)$$



Okay, but by how much should we attenuate? We use the parameter X, which is the ratio of the reference vector length in green to the scattered vector length in blue. So too much absorption makes X extremely small, and the length of the green arrow will become much smaller than the blue or red vectors, and we have overegged the whole absorption. So there must be an optimal value. And this turns out to be when the reference green vector is the same size as the red or blue vectors. Note that this is independent of Delta phi, in other words, how much the sample phase shifts the scattered radiation.

Notes

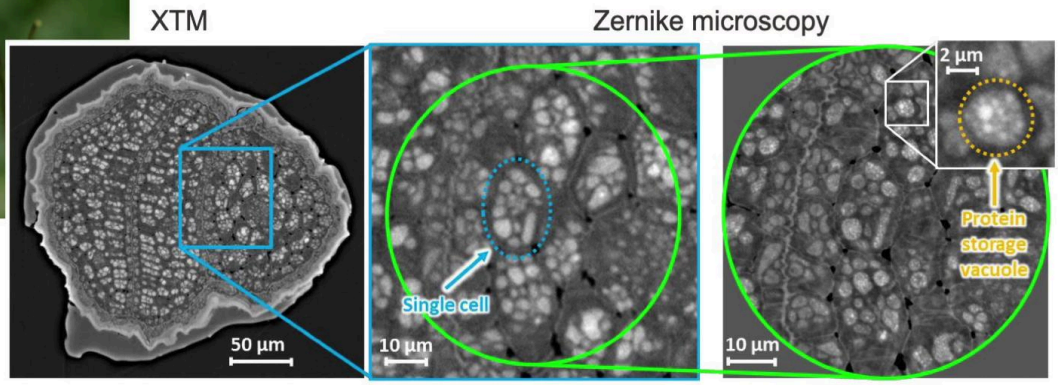
Summary

3m 11s

Example

Creative Commons

- Thale cress (Arabidopsis thaliana) seed
- Zernike microscopy @ 10 keV



See M. Scheel *et al.*, doi:10.1088/1742-6596/2380/1/012045

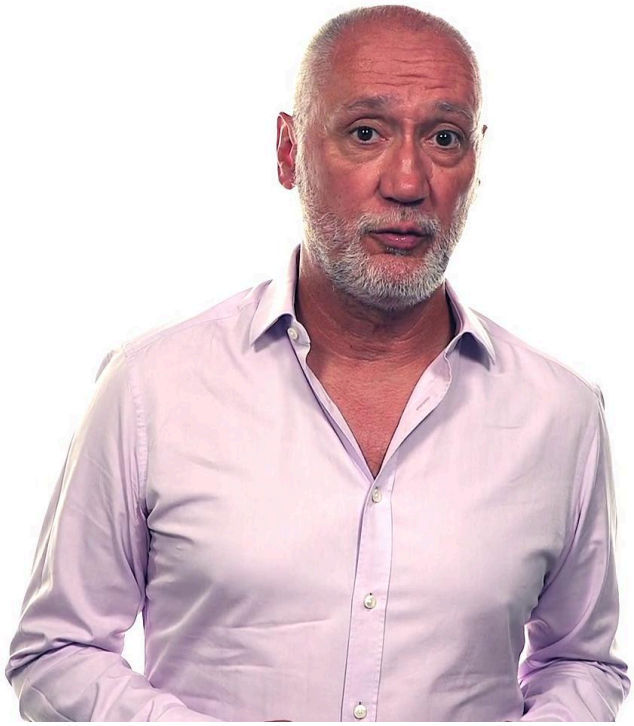
We finish this short video with a quick example of Zernike microscopy at 10 keV of a Zernike nanoscopic tomography of the internal architecture of a Thale cress seed. Virtual slices at increasing resolution are shown, with the highest resolution image on the right having a 95 nanometre pixel size in which the form of protein storage vacuoles can be identified.

Notes

Summary

3m 54s

In the next video...



In the final video of this week, we briefly discussed zoom tomography and exploiting chemical contrast and the variant of the tomography called laminography used for samples in which one dimension is much smaller than the other two.

Notes

Summary

4m 22s