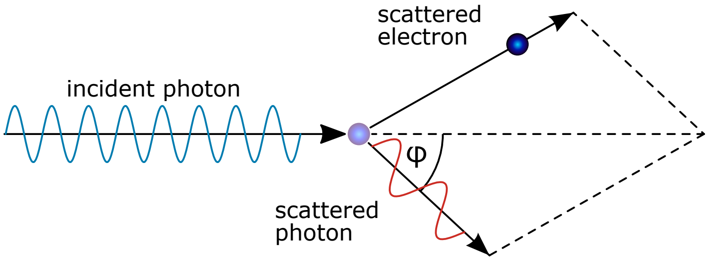
EVERY PHOTON COUNTS

Dose-efficient imaging of biological materials with Compton X-ray microscopy

X-rays are invaluable for studying small structures due to their penetrating power and short wavelengths, which allow high spatial resolution. Many X-ray imaging techniques are based on the elastic scattering of light. However, at the same time some photons are also absorbed, i.e. their energy is deposited into the sample. This causes damage in radiosensitive materials such as biological specimens. For these kinds of materials, researchers have to find an optimum between limiting radiation damage and obtaining a sufficient signal level. A team of researchers from DESY took on this challenge and developed a new type of X-ray microscope that exploits not elastic, but inelastic scattering of photons. So-called Scanning Compton X-ray Microscopy (SCXM) uses photons with an energy of 60 keV, where photoabsorption is greatly reduced and inelastic Compton scattering is the main interaction between the incident photons and the material. In this process, every photon deposits a small part of its energy into the sample – each detected photon comes at the price of radiation dose. However, the method is more dose efficient than coherent (elastic) scattering techniques and a promising technique for biological research.



Compton scattering.
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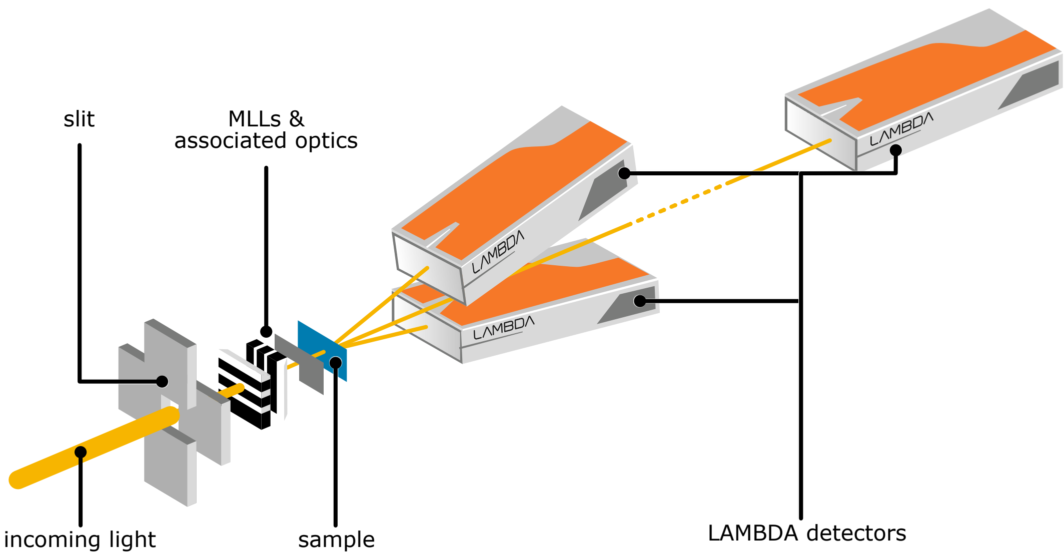
EXPERIMENTAL SETUP

In order to achieve high spatial resolution, the incident X-ray beam at the high-energy material science beamline P07 at PETRA III, DESY, had to be focused down to the nanometer scale. This was done with new volume diffractive optics that are under development at DESY. So-called Multilayer Laue Lenses (MLLs) are made layer by layer with atomic precision and operate with high efficiency at high X-ray energies. The sample was mounted in the focal plane, and the Compton scattering signal was recorded with two LAMBDA 750k GaAs detectors in two scattering directions. Lead foil covered plastic pyramids shielded the detectors from stray photons in order to minimize the background noise. **"We chose the LAMBDA detector for this experiment in order to understand the origin and distribution of all detected photons so that we could optimize data collection and exclude non-specific samples"**, said Saša Bajt, who led the study. **"We also required high quantum efficiency at these photon energies and high frame rates."** A third LAMBDA detector was used for alignment and determination of the total flux downstream of the setup. The biological sample used for testing the technique were species of the cyanobacterium spirulina.



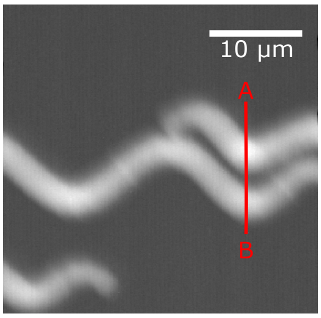
Focusing optics and detector setup for SCXM.

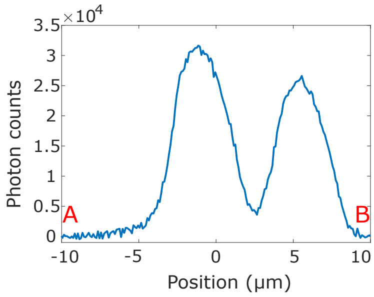
RESULTS

This experimental study was the first proof-of-concept of the novel SCXM technique. In the images of spirulina samples that were recorded with this setup, the maximum contrast between signal and background was 3.1×10^4 photons, using counts from both detectors. This is in agreement with theoretical considerations and validates the theory underlying the method.

The focal spot size achieved with the setup was 240 nm. This also posed the main limitation to the spatial resolution of the images. The authors expect it to improve if even more brilliant X-ray beams can be used. They also propose to enhance detection efficiency by using other sensor materials such as cadmium telluride (CdTe, which is available for LAMBDA cameras as well), or by changing the geometry of the setup in order to cover a larger angle and catch a higher fraction of the scattered photons.

Setup	PETRA III, DESY (Germany), P07 beamline
Camera	LAMBDA 750k GaAs detector
Resolution	786,432 pixels with 55 µm
Acquisition frequency	10 Hz
Photon energy	60 keV





Left: Spirulina SCXM image at 60 keV with 100 nm steps and 100 ms dwell time. Right: Photon counts along the red line. Adapted with permission from Villanueva-Perez et al. (2021) © The Optical Society.

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