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Scanning X-ray microscopy with large solid angle X-ray fluorescence detection at the XUV beamline P04, DESY

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Abstract: The presented scanning transmission X-ray microscope (STXM), built on top of a modular platform, combines soft X-ray transmission and fluorescence microscopy with high detection efficiency and high spatial resolution. The setups user concept as well as the large solid angle (> 1 sr) of the integrated Silicon-drift-detector are unique characteristics of this endstation. In combination with the soft X-ray beamline P04 at PETRA III (DESY), it delivers a new type of nanoscope, providing a high flexibility and very low acquisition times.

Keywords: Instrumentation for synchrotron radiation accelerators, X-ray fluorescence (XRF) systems

Contents

1 Introduction

The P04 Beamline at PETRA III is a variable polarization XUV beamline with small bandpass and an exceptionally high photon flux of up to 10^{12} photons per second [1]. Its design allows to switch between different endstations so that a variety of experiments can be performed at this beamline. The endstation presented in this paper combines scanning and fluorescence X-ray microscopy. Due to its design, the setup can also be converted into a full-field X-ray microscope, which has already successfully been tested [2]. In scanning mode, the transmission signal is detected by a spatially resolved CCD camera, so that several imaging methods such as absorption and phase contrast can be employed [3]. The fluorescence signal is recorded by a Silicon-Drift-Detector (SDD) implemented into the setup, allowing the investigation of the samples lateral elemental distribution. The efficient fluorescence detection results in very short measurement times, thus preventing the samples from structural alterations [4]. The combination of this endstation with the P04 beamline presents a powerful tool to help addressing questions in life and material science.

2 STXM Setup

In contrast to a full-field microscope, where a large area on the sample is illuminated at once, scanning microscopy requires a small illumination spot on the sample. For each scan step, the sample is shifted by a well-defined distance so that that the transmission and, in our case, the fluorescence signal can be recorded at each scanning position. Therefore, a small beam size on the sample as well as small scan steps are required to achieve a high spatial resolution. Since P04 at PETRA III, DESY is a beamline with no fixed endstation, our setup was developed and tested in the laboratory. Therefore, it has to be transported to the beamline and subsequently assembled and aligned for each experiment. Its modular design allows both an easy transport and a high flexibility. The possibility to adapt the system for different light sources (e.g. FLASH, DESY [5]) was an important aspect for the design of the basic setup (vacuum- and positioning system). Figure 1 a) depicts the setup of the endstation at the P04 beamline at PETRA III. The majority of the optical elements are located in a vacuum chamber. The latter is placed on a support frame which can be easily configured and adapted to the different conditions of other light sources. For

the STXM, a positioning system inside the vacuum chamber allows a precise adjustment of the optical components, presented in figure 1. A Fresnel zone plate is used to focus the radiation on a small spot on the sample. In the current STXM setup, a tungsten zone plate with a diameter of 333 μm and an outermost zone width of 45 nm has been integrated. Its central stop is made of gold and has a diameter of 160 μm. Between the zone plate and the sample, an order sorting aperture (OSA) with a diameter of 140 μm ensures the suppression of the zeroth and higher diffraction orders. Behind the sample, the transmitted signal is projected onto a P43 phosphor screen and imaged by a visible light CCD camera. The fluorescence signal from the sample is detected by an SDD (Bruker XFlash FlatQUAD) with an energy resolution of <130 eV @ Mn Kα and a 0.5 μm thick mylar ($C10H8O4$)-window. Four detector segments are arranged in a circle in the center of which a hole allows the excitation radiation from the beamline to pass through (figure 1 d)). This configuration accounts for a very small distance between the fluorescence detector and the sample and thus a large detecting angle. At an optimum distance to the sample, a solid angle of >1 sr can be collected by the detector. The OSA is mounted on a tube thin enough to fit inside the opening of the detector. In this way, shadow effects and scattered fluorescence radiation from the OSA can be avoided. The sample is mounted on a piezo scanner (fine scanning range of 100 μm × 100 μm) which itself is placed on a core stage with a range of 50 mm × 50 mm. In that way, several samples can be investigated successively and without having to open the vacuum chamber, thus providing a more stable and time efficient work flow.

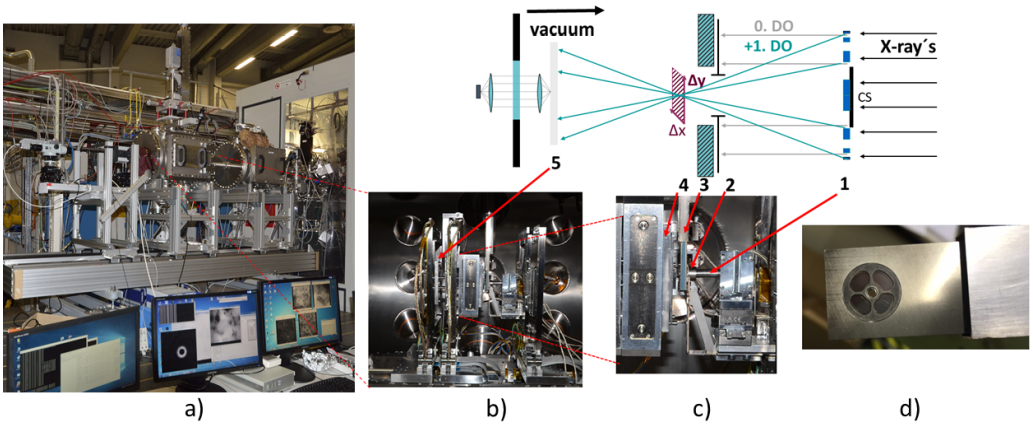

Figure 1. a) Setup overview of the endstation at the P04 beamline platform. **Bottom:** b), c) experimental setup of the STXM inside the vacuum chamber. d) SDD with circularly arranged detector elements. **Top:** Schematic representation of the STXM setup, 1) Fresnel zone plate, 2) OSA (order sorting aperture), 3) Fluorescence detector (SDD), 4) Sample mounted on the scanner, 5) Phosphor screen.

3 First Results

At the beginning of each beamtime the assembling and aligning of the endstation are usually carried out in less than 12 hours. Subsequently, a Siemens star is recorded to assess the spatial resolution of the setup (figure 2). Figure 3 presents images of another test structure (150 nm tungsten deposit on a 200 nm $Si3N4$-window) simultaneously recorded with the transmission and the fluorescence signal.

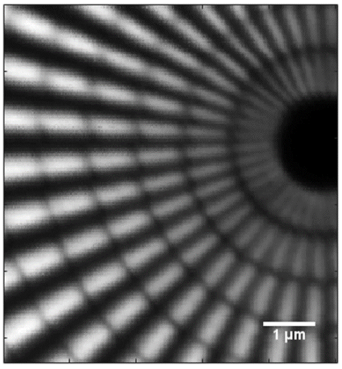

Figure 2. Transmission image of a Siemens star test pattern, recorded with a photon energy of at 720 eV. The smallest structures have a width of 50 nm. On-the-fly scan, step size: 20 nm, field of view: 5.4 μm x 5.4 μm, acquisition time per pixel 200 ms

In the last beamtime the new SDD-detector was implemented for the first time and acquisition time tests where performed, some results are shown in figure 3. For this test we used a reference sample which consists of 150 nm tungsten deposit on a 200 nm thick SiN window.

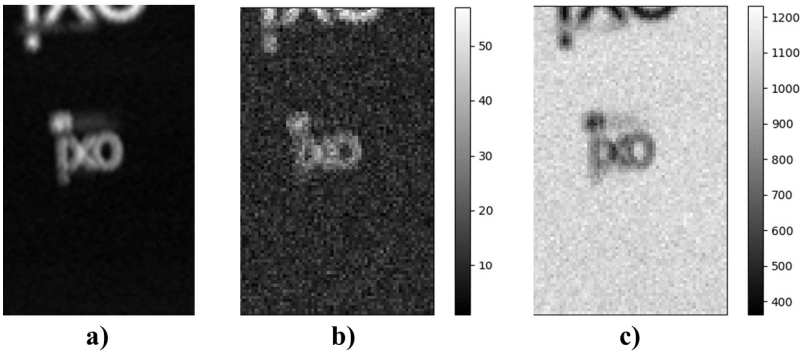

Figure 3. Transmission and fluorescence images of a test pattern, recorded with a photon energy of 1000 eV. a) Transmission image, b) N signal, c) Ni signal. On-the-fly-scan, step size: 1 μm, acquisition time per pixel 5 ms, field of view 100 μm x 100 μm.

Application

Since soft X-rays are particularly well suited to examine biological samples, one beamtime was dedicated to the investigation of the transport mechanism of lipids through the blood circuit in adipose tissue as a proof of principle experiment. We analyzed samples consisting of brown adipose tissue extracted from different mice. Before extraction, the mice were either part of a control group or kept at low temperature in order to activate the transport of lipids (activated mice). Both groups received an injection of lipids marked with iron nanoparticles (superparamagnetic iron oxide, SPIO) so that the iron distribution within the tissue would reveal the location of those lipids and provide an insight into their transport mechanism. The sample slices ($\approx$ 3 μm thickness) were deposited on a Si3N4-window (200 nm thickness, standard sample holders in transmission electron microscopy) and fixated with 2.5 % formaldehyde. After air drying and transfer to the

vacuum chamber, the measurements were carried out at a photon energy of 1000 eV. Figure 4 shows a selection of the results. However, further measurements on a larger set of samples have to be carried out in order to come to obtain conclusive results for this biomedical research question.

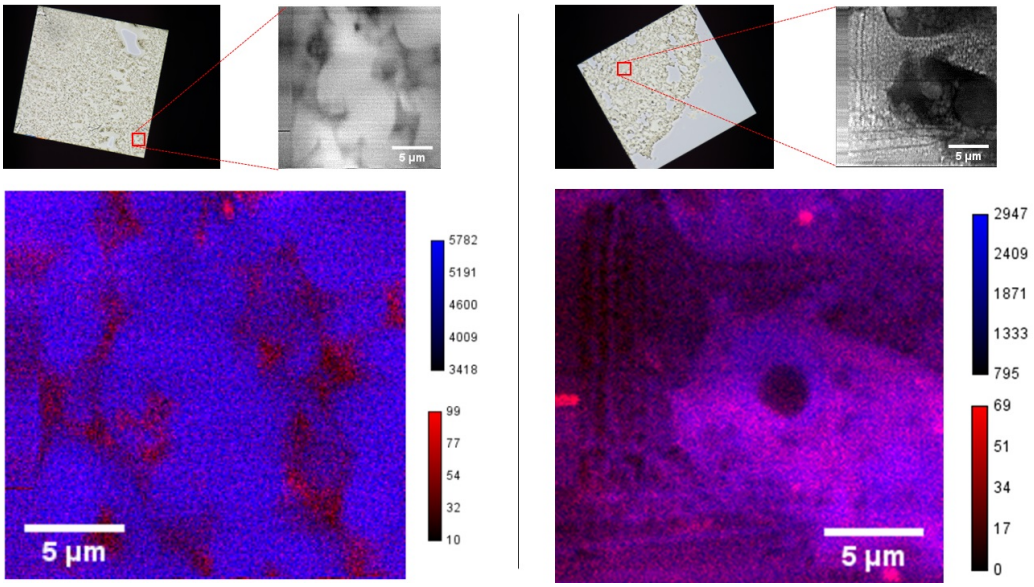

Figure 4. Sample slices from brown adipose mice tissue. The tissue is marked with iron nanoparticles (SPIO). **Left:** control group sample. Top/left: visible light microscopy image. Top/right: STXM absorption image. Bottom: fluorescence with C (blue) and Fe (red). **Right:** activated group sample. Top/left: visible light microscopy image. Top/right: STXM absorption image. Bottom: fluorescence image with C (blue) and Fe (red). The scans were performed in an on-the-fly mode with a step size of 100 nm, a field of view of 20 μm $\times$ 20 μm and an acquisition time per pixel of 200 ms.

4 Outlook

In order to reduce radiation damage for biological samples, a setup able to provide a cryo-environment, including a transfer system for the sample into the vacuum chamber, is under construction and will soon be implemented. Furthermore, a rotary stage for tomographic acquisition will also be added to the setup. For the acquired fluorescence spectra, a semi-quantitative approach will be developed.

Acknowledgments

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