IOP Conf. Series: Journal of Physics: Conf. Series **849** (2017) 012007 doi:10.1088/1742-6596/849/1/012007

Scanning Transmission X-ray Microscopy with X-ray Fluorescence Detection at the XUV Beamline P04, PETRA III, DESY

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Abstract. The presented scanning transmission x-ray microscope (STXM), build on top of our existing modular platform (FlexIX) for high resolution imaging experiments, allows versatile investigations of different samples. The FlexIX endstation allows to switch between a Full Field and a STXM mode. For the STXM mode we use a spatialy resolved detector together with an energy dispersive detector, this allows to investigate the morphology and the chemical or elemental distribution of the sample simultaneous. The combination of the nanoscopy endstation and the XUV beamline P04 results in a powerful tool for investigations of life science samples.

1. Motivation

Classical optical microscopy techniques are fundamentally limited by the diffraction limit, which implies that spatial resolutions below 200 nm can only be achieved by the use of short-wavelength radiation. While electron microscopy enables two- and three-dimensional imaging of materials in the sub-nanometer range [1], there is a gap in spatial resolution in the 10^{th} nm range, which can be covered with X-ray Microscopy (XRM). For state of the art investigations in biomedicine this spatial resolution range is of a great interest [2]. In addition, the penetration depth of XRM is much higher as compared to electron microscopy, which facilitates sample preparation.

The $P04$ beamline of Petra III is a variable polarisation XUV beamline with an energy range of 250 eV up to 3000 eV [3]. This energy range is suitable to identify most of the interesting elements typically found in biological samples [2]. The beamline provides exceptionally high photon flux of up to 10^{12} photons per second in combination with a very high resolving power ($E/\Delta E > 10.000$) [3]. In contrast to most other beamlines, $P04$ has no fixed endstation and the experimental setups are provided by the user. In order to perform XRM at the $P04$ beamline the variable FlexIX endstation was developed and tested in the laboratory.

The FlexIX endstaion consists out of up to three separate vacuum chambers and custom designed high resolution piezo stages. These piezo stages are able to move precisely in x-, y- and z-direction, which makes it possible to adjust components for different imaging modes.

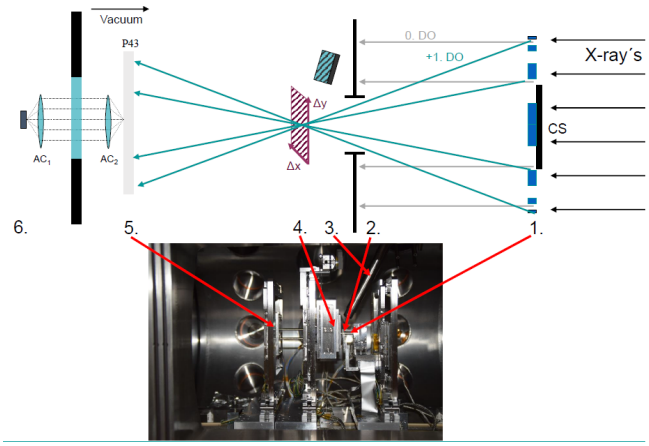

Figure 1. Experimental setup of the scanning microscope. Top: Sketch of the setup, bottom: real setup insight the vacuum chamber. 1. Fresnel zone plate; 2. Order sorting aperture (OSA); 3. Fluorescence detector; 4. Sample on scanner; 5. Phosphor screen; 6. CCD

The endstation is designed for quick changes between full-field (FF) mode and STXM/ fluorescence mode.
The main advantage of FF mode is that a large area of the sample can be investigated with very high spatial resolution $\approx 30\ nm$ with a single exposure. During recent experiments the endstation was operated as a transmission x-ray microscope (TXM) in FF mode [4].

First experiments with the STXM mode were done in October 2015 and June 2016. In STXM mode the spot size on the sample defines the spatial resolution of the system. Therefore a zone plate (ZP) is used to focus the radiation on a small spot. Then the sample is moved through the focus point in a raster grid or in a continuous on-the-fly scan to retrieve an image.
In STXM similar resolutions with respect to FF can be achieved, but since scanning is needed, investigations of large areas are quite time consuming. However, in contrast to FF different imaging options such as phase-contrast or dark-field are easier to implement [5] and elemental contrast by the detection of characteristic fluorescence radiation is accessible.

2. Experimental Setup

A sketch of the STXM setup is shown in Figure 1. X-Ray radiation from the beamline is focussed by a zone plate with 333 μm in diameter and an outer most zone width of 40 nm onto the sample. The zone plate consists out of 150 nm tungsten deposited on a 100 nm thick SiN membrane and possesses a central stop of gold with 160 μm in diameter. Between ZP and sample is an order sorting aperture (OSA) made of 100 μm thick stainless steel with a 150 μm hole. It is mounted on a small aluminium tube in order to prevent the fluorescence radiation from the OSA to reach the detector.
The sample is scanned by a $P733$ scanning stage from Physik Instruments (PI). It has a travel range of 100 μm x 100 μm with a resolution of 0.1 nm.
The sample holder is designed to have space for up to eight samples prepared on common transmission electron microscopy (TEM) SiN membranes or grids.
The detection of the transmitted intensity distribution is done indirect with a $P43$ phosphor screen which is used to convert the X-rays into visible light. An optical path is used to transfer the visible light out of the vacuum chamber and an objective is used to image the signal from the phosphor screen on the Andor iXon3 EMCCD detector with 128 x 128 pixel and a pixel size of 24μm x 24 μm.

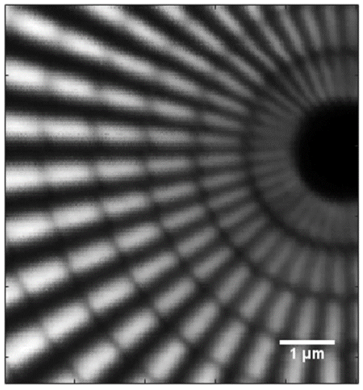

Figure 2. Transmission image of a test pattern, taken at 720 eV photon energy, smallest structures 50 nm. On-the-fly scan, step size: 20 nm, field of view: 5.4 μm x 5.4 μm, scan time: 72 min. (without electron multiplier)

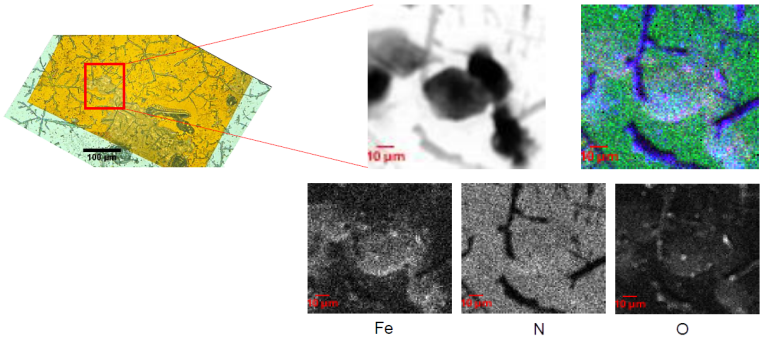

Figure 3. Mouse liver cells grown on a 200 nm thick SiN membrane, after air drying and fixation the sample was investigated by 720 eV photon energy, top left: Light microscope image of the sample; top middle: Transmission image, top right: Fluorescence image, (red) iron, (blue) oxygen, (green) nitrogen; bottom: Fluorescence signal of (Fe) iron, (N) nitrogen, (O) oxygen; Step size: 1 μm, field of view: 100 μm x 100 μm, scan time: 34 min. (without electron multiplier)

Using a spatially resolved detector for the transmitted intensity allows to gather several imaging modes such absorption contrast and phase contrast simultaneously [5]. This is done without any change in the optical path only by different treatment of the experimental data from the transmission detector. For each of the several imaging modes specific evaluation of the data is required to generate the differnet images.
The STXM is also suitable to investigate the elemental distribution. Characteristic X-ray fluorescence radiation from the sample is detected by a windowless 30 mm^2 SDD detector (Brucker Xflash 5030) with an enrgy resolution of < 127 eV FWHM @ $MnK\alpha$.
For each beamtime the endstation has to be assembled and aligned, this takes typically one day. After the alignment procedure is completed, usually a resolution test is performed. An exemplary STXM result is shown in Figure 2. The used test object was a Siemens star with 50 nm smallest structures consisting of 200 nm thick layer of tungsten on a 100 nm thick SiN membrane.
During the last phase of the commissioning of the experimental setup, it was possible to perform on-the-fly scans. This was a great and important step, since it reduces scanning time significantly.

3. Biological imaging

The STXM setup was used for biological samples with interest to the transport mechanism of lipids through the blood circuit into adipose tissue.
In first tests mouse liver cells marked with iron nano particless (SPIO) grown on 200 nm thick SiN windows were investigated. After air drying and fixation with 2.5 % formaldehyde, the samples were investigated with the STXM endstation with a photon energy of 720 eV (Figure 3). During the first beamtime the resolution was limited, so it was not possible to resolve the agglomerations of iron particles which are expected to be in the 100 nm range. Nevertheless, the combination of fluorescence and transmission microscopy is working, as can be seen in figure 3.
In the second beamtime tissue slices from brown adipose tissues of a mouse were investigated.

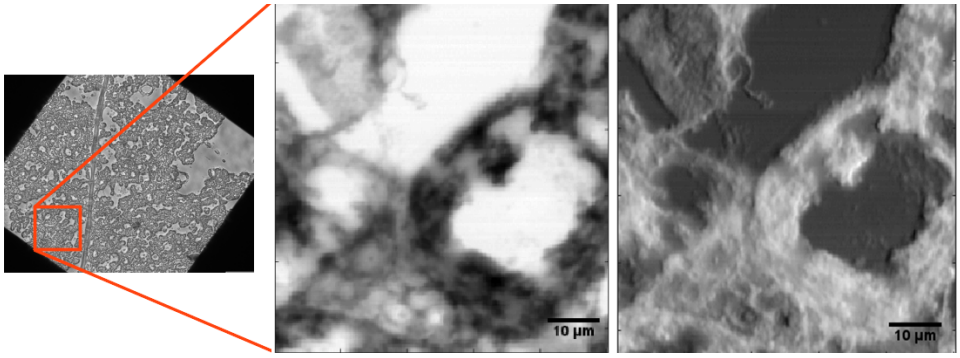

Figure 4. Tissue slices from brown adipose tissues of a mouse. The tissue is marked with iron nano particles (SPIOs). The slices are $\approx 3\ \mu m$ thick, placed on a 200 nm thik SiN membrane and fixed with 2.5 % formaldehyde. After air drying the samples were investigated with a photon energy of 750 eV. Left: Light microscope image of the sample; Midle: Transmission image; Right: Differential phase contrast (horizontal); On-the-fly scan; 270 x 270 Pixel; Field of view: 54 μm x 54 μm; Scan time: 72 min. (without electron multiplier)

The $\approx 3\ \mu m$ thick slices were deposited on 200 nm thick SiN windows (TEM windows) and were fixated with 2.5 % formaldehyde. After air drying, the samples were investigated using a photon energy of 750 eV. Some results are shown in figure 4.

4. Outloock

In order to reduce radiation damages for biological samples, a cryo environment including a transfer system will be implemented. The bottleneck of the system with respect to faster scanning times is the solid angle of the fluorescence detector. A new detector with approximately ten times larger solid angle of detection will be implemented. For this detector a semi-quantitative approach for the analysis of the fluorescence data will be developed.

Acknowledgments

This project is financially supported by the German Federal Ministry for Education and Research (BMBF) at the DLR (05K13UL1).

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