

## Scanning Transmission X-Ray Microscopy in the Soft Energy Range with Very Large Solid Angle of Detection for X-ray Fluorescence Imaging

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Scanning transmission X-ray microscopy (STXM) in the soft energy range, especially in combination with X-ray fluorescence detection, is becoming an increasingly important tool for life sciences. Biological material generally has a low fluorescence yield and correspondingly a low fluorescence signal. Hence, high detector efficiency (e.g through a large solid angle) is indispensable to avoid long measurement times and radiation damage.

A novel STXM endstation has been installed at the P04 beamline at PETRA III, profiting of its high brilliance [1]. Recently, a 4-channel fluorescence detector offering a solid angle of detection of up to 1.2 sr was integrated. The excitation beam passes through a hole in the flat 4-channel SDD and hits the sample (see **Figure 1**). Fluorescence radiation is detected in backscatter geometry. Since biomedical research often involves samples prone to radiation damage, this unique combination of a high brilliance beamline and the high detection efficiency of the endstation offers new analytical possibilities for this field.

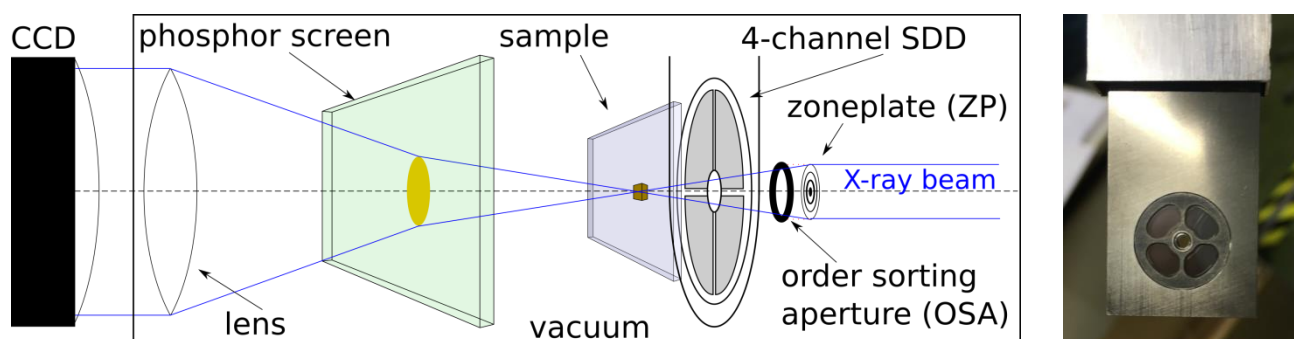
As a first biological application and in collaboration with Prof. Heeren from the University Medical Center of Hamburg-Eppendorf, our endstation was employed to investigate the lipid metabolism in mice cells with respect to the iron distribution in cold-activated brown adipose tissue (BAT). Under cold conditions brown adipose tissue produces heat by burning huge amounts of lipids correcting hyperlipidemia and atherosclerotic plaque progression [2,3]. Up to now, the transport of lipoproteins via the endothelium into the active BAT could not be visualized. The understanding of uptake and intracellular processing by endothelial cells by high-resolution scanning transmission microscopy might open new avenues for the therapy of metabolic disease in the future.

The (living) mice were injected with lipoproteins labeled with superparamagnetic iron oxide nanoparticles (SPIO-lipoproteins) and maintained under thermoneutral (30°C) or cold activation (6°C) conditions for 24 hours. The iron in the tissue could very efficiently be detected with the 4-channel fluorescence detector. The iron distribution in comparison to the carbon distribution is an indicator of the uptake of the injected lipoproteins in to the BAT.

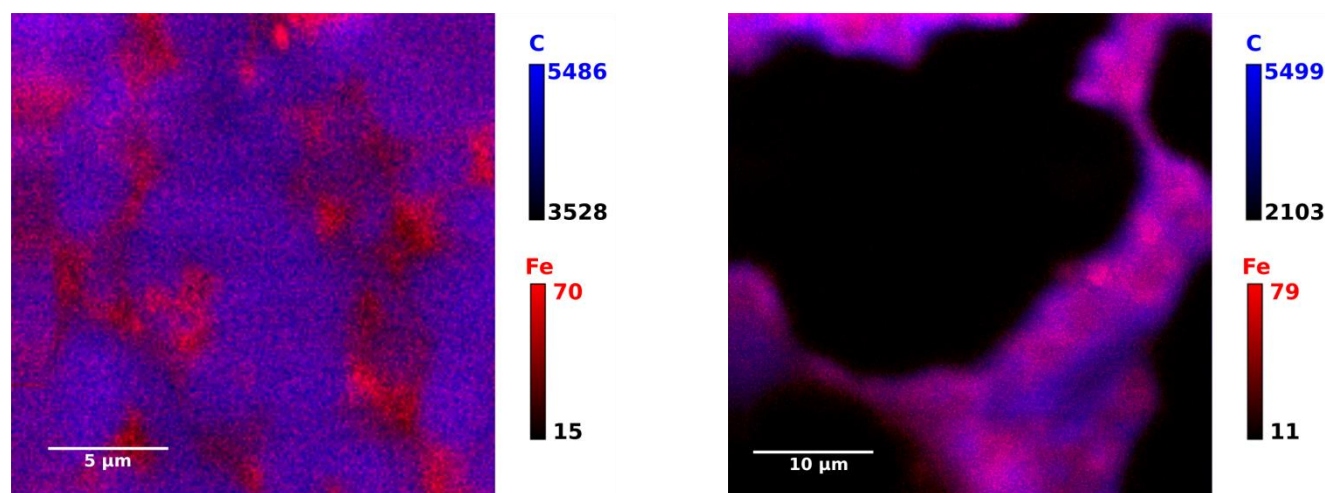
**Figure 2** shows the first results of measurements with an excitation energy of 1000 eV, a scan step size of 100 nm and respective image sizes of 200 x 200 (left) px and 400 x 400 px (right). With measurement times of down to 50 ms we have performed, to the best of our knowledge, the fastest scans up to now in the investigation of trace elements in biological samples with soft X-rays. The statistics of the iron signal can still be enhanced, but even for this short measurement time with iron only present as trace element, imaging is possible and first conclusions can be drawn. The blood vessels show less content of carbon (blue) than the BAT. In the tissue of the mouse that was kept in a thermoneutral environment (**Figure 2**, left) the iron content (red) is higher in the blood vessels compared to the BAT. Whereas for

the mouse stored under cold activation conditions (**Figure 2**, right), the iron content (red) is high in both, the blood vessels and the BAT. First results indicate an enhanced lipid transport into the BAT for cold activated mice. Nevertheless, these results have to be confirmed by more statistics with respect to higher iron content and more samples of tissue from mice kept in thermoneutral and cold activated conditions.

In addition to the investigations of the lipid metabolism, which can help to understand type 2 diabetes mellitus and cardio vascular diseases, various other collaborations have been initiated. Those include the investigation of the inactivation process of bacterial endospores in food, protein particles doped with nanoparticles to understand their potential as drug carrier and the potential risk of carbon black in tattoos. This indicates a high interest in the field of biomedicine for soft X-ray STXM.



**Figure 1:** Left: Sketch of the STXM setup. Right: Front view of 4-channel SDD.



**Figure 2:** False color maps of iron (red) and carbon (blue) in mouse tissue. The mice were maintained under thermoneutral (left) or cold activation (right) conditions. For details see text.

#### References:

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- [3] A. Bartelt A *et al*, Nature medicine 17 (2011) 200-205
- [4] This project is financially supported by the German Federal Ministry for Education and Research (BMBF) at the DLR (05K2016). Parts of this research were carried out at PETRA III at DESY.