248
doi:10.1017/S1431927618013594
Microsc. Microanal. 24 (Suppl 2), 2018

Towards High Performance Soft X-ray Cryo-Tomography in the Laboratory

Aurélie Dehlinger[1,2,*], Julia Braenzel[3], Daniel Groetzsch[1,2], Torsten Feigl[4], Robert Jung[1,3], Birgit Kanngießer[1,2], Stefan Rehbein[5], Christian Seim[6], Holger Stiel[1,3]

[1.] Berlin Laboratory for innovative X-ray Technologies (BLiX), Berlin, Germany
[2.] Technical University of Berlin, Institute for Optics and Atomic Physics, Berlin, Germany
[3.] Max-Born-Institut, Berlin, Germany
[4.] optiX fab GmbH, Jena, Germany
[5.] Helmholtz-Zentrum für Materialien und Energie GmbH, Institute for Soft Matter and Functional Materials, Berlin, Germany
[6.] Physikalisch-Technische Bundesanstalt, X-ray Spectrometry, Berlin, Germany
* a.dehlinger@tu-berlin.de

Soft X-ray microscopy is an ideal tool to fill the spatial resolution gap between visible light microscopy and electron microscopy. Transmission electron microscopy, due to its small penetration depth, requires extensive and highly invasive sample preparation, such as embedding and sectioning. Soft X-ray microscopy (XRM) in the water-window, in contrast, allows the structural investigation of samples in an aqueous environment with a penetration depth of up to 10 µm and resolutions of a few ten nanometers which is achieved by utilizing radiation between the absorption edges of carbon and oxygen. Therefore, tomographic imaging of a sample can easily be realized using appropriate stages and adequate sample holders for vitrified, non-embedded samples. Whereas most soft X-ray microscopes rely on synchrotron radiation, this contribution presents a laboratory setup based on a highly brilliant laser produced plasma (LPP) source, aiming to increase the availability of water window XRM to a broader scientific community and at the same time offering a higher flexibility to the users [1]. A similar laboratory setup can be found at the KTH Stockholm [2].

A detailed treatise of the setup of our full-field laboratory soft X-ray transmission microscope (LTXM) can be found in [3]. Even tough laboratory setups have proven to achieve similar spatial resolutions as synchrotron-based instruments, there are still differences in terms of stability and exposure time. In principle there are two ways to reduce exposure times in laboratory X-ray tomography: i) increase the average power of the pump laser and ii) optimize the optical setup. High average power lasers that would be suitable pump sources for the LPP are already commercially available, but occasional instabilities of the LPP source as well as debris issues at very high average pump power are still not fully mastered. Hence, our primary focus has shifted to optimizing the optical setup which on one hand can be achieved by increasing the reflectivity of the condenser optic and on the other hand by improving the preadjustment. In the following, we will discuss these aspects in detail and present improved operational performance in the first results.

In order to carry out tomographic measurements, it is crucial to reduce the measuring time by at least one half and the current prevailing view is that water-window condenser optics with a low reflectivity of 0.5% as reported in [1] possess the highest potential for improvement. For this reason, optiX fab GmbH has developed a new set of multilayer mirrors that can achieve a higher reflectivity due to improvements of the manufacturing process. An optimized substrate and new multilayer design in combination with improved coating parameters (resulting in a better lateral uniformity essential for a good wavelength matching) can lead to a reflectivity of more than 4%, as shown in figure 1 (top). However, it is also apparent that the reflectivity of this particular mirror is not uniform over the whole area, thus compromising the average reflectivity. In figure 1 (bottom), a new multilayer model has overcome this limitation and provides a high reflectivity at every point of the mirror, thus enabling a highly efficient Köhler illumination. Adding this mirror to our LTXM setup greatly reduces exposure times and facilitates tomographic measurements.

Another measure that has already been proven to successfully increase the LTXM's performance is to thoroughly plan and perform the preadjustment routine prior to LTXM measurements. Furthermore, the integration of apertures to limit stray light has recently led to higher contrast and more uniformly illuminated images. Figure 2 shows a comparison between the recording of the same Siemens Star with a 25-nm zone plate objective before and after the advanced preadjustment procedure and the corresponding calculated contrast transfer function for both recordings. Not only has the visible contrast been improved due to the preadjustment and reduction of stray light effects, but also the resulting spatial resolution. The LTXM now achieves a better contrast transfer over the whole range of spatial frequencies and hence the half-pitch resolution, corresponding to a decrease of the contrast transfer to 10%, has been pushed from (35±3) nm to (30±3) nm, resulting in a full-period resolution of (60±6) nm.

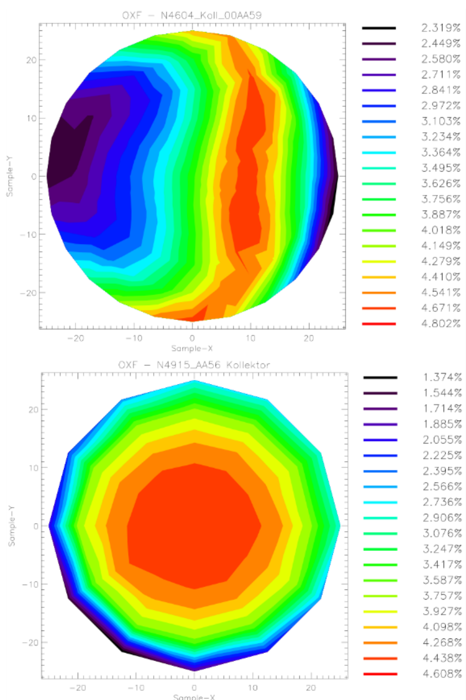

Figure. 1. Improvements in multilayer technology led to shorter exposure times. Top: Reflectivity of the first prototype multilayer condenser optic. Bottom: Advanced technology delivers a more uniform and higher reflectivity. (Measurements performed at PTB).

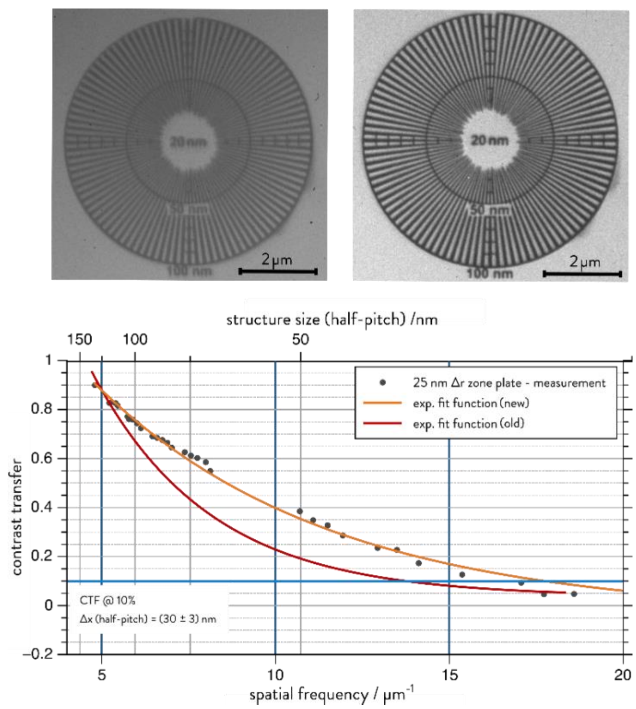

Figure. 2. LTXM recording of a Siemens Star before (left) and after (right) preadjustment routine. The resulting contrast transfer functions (bottom) show that with this method, a higher spatial resolution can be achieved.

By using the described advanced alignment procedure together with highly efficient optics the LTXM's throughput can be improved by at least a factor of 3, reducing the exposure time for one single projection in cryo-tomography from 60 s [4] to less than 20 s at an average pump laser power of only 50 W.

[1] H. Legall, G. Blobel, H. Stiel, Optics Express **20(16)** (2012), 18362
[2] E. Fogelqvist, M. Kördel, V. Carannante, Scientific Reports **7** (2016), 13433
[3] Dehlinger *et al.* SPIE (2015) **9589**: 95890M-1
[4] Stiel *et al.* SPIE (2017) **10243**: 1024309-1