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A compact Laboratory Transmission X-ray Microscope for the water window

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Abstract. In the water window (2.2 - 4.4 nm) the attenuation of radiation in water is significantly smaller than in organic material. Therefore, intact biological specimen (e.g. cells) can be investigated in their natural environment. In order to make this technique accessible to users in a laboratory environment a Full-Field Laboratory Transmission X-ray Microscope (L-TXM) has been developed. The L-TXM is operated with a nitrogen laser plasma source employing an InnoSlab high power laser system for plasma generation. For microscopy the Ly_α emission of highly ionized nitrogen at 2.48 nm is used. A laser plasma brightness of 5×10^{11} photons/(s $\times$ sr $\times$ μm^2 in line at 2.48 nm) at a laser power of 70 W is demonstrated. In combination with a state-of-the-art Cr/V multilayer condenser mirror the sample is illuminated with 10^6 photons/(μm^2 $\times$ s). Using objective zone plates 35 - 40 nm lines can be resolved with exposure times < 60 s. The exposure time can be further reduced to 20 s by the use of new multilayer condenser optics and operating the laser at its full power of 130 W. These exposure times enable cryo tomography in a laboratory environment.

1. Introduction

Water window transmission microscopy is suitable for 3-dimensional microscopy of whole cryogenic biological cells [1]. At synchrotron facilities it was shown that by cryo fixation the specimen can be subjected to high radiation doses without showing visible changes in the image [2]. Shock frozen (cryo fixed) samples can be ideally investigated in the water window because of the high penetration depth of radiation in water in this spectral range. Furthermore, the high absorption contrast between the surrounding water and organic structures in the water window enables imaging cells with high resolution. By using zone plates for imaging, resolutions down to 10 nm

are presently achieved at synchrotron facilities [3].
An immediate and permanent access of this technique in the home laboratory would be advantageous for many researchers. In order to make this technique accessible to researchers in a laboratory environment, key components for laboratory microscope setups were developed in the recent decade [4, 5, 6]. In this article we present a further development step of laboratory transmission x-ray microscopy in the water window. The presented laboratory microscope in this article is based on cryo nitrogen jet laser plasma sources driven by a novel high power 130 W InnoSlab laser system and equipped with a Cr/V condenser multilayer mirror and a micro zone plate for imaging. A similar microscope employing a 200 W InnoSlab laser upgrade was taken into operation at the KTH in Stockholm [7]. It was shown that the higher source power decreases the acquisition times for a high quality microscope image to a level which makes cryo tomography feasible in the home laboratory.

2. Arrangement of the laboratory microscope

The arrangement of the microscope is shown in Fig. 1. The setup consists of a laser plasma source for x-ray generation and the x-ray optical arrangement for imaging including a condenser x-ray mirror for sample illumination. A detailed description of the laboratory microscope can be found in [8].

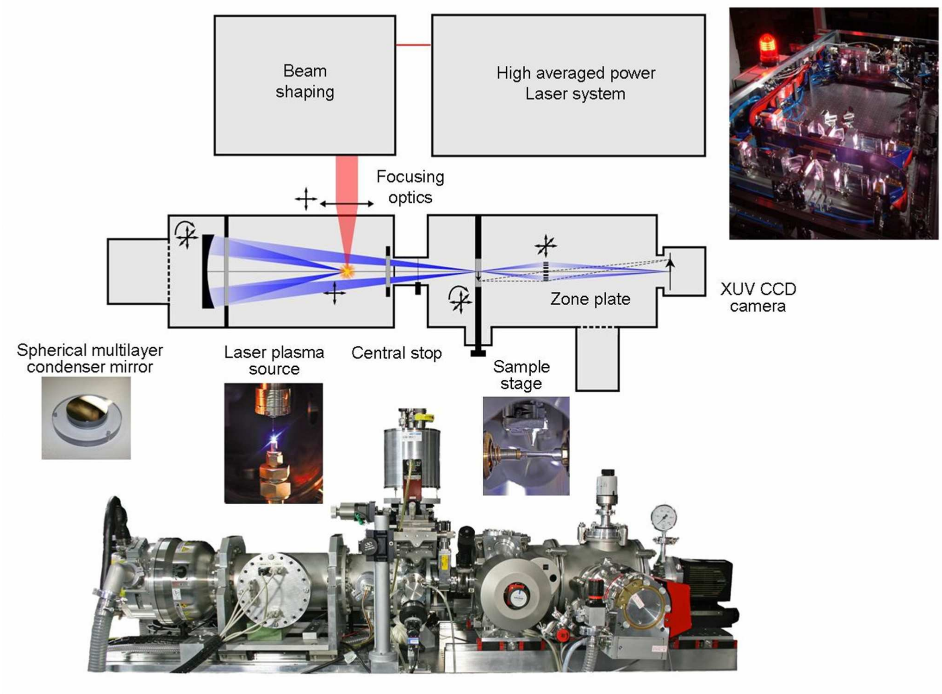

Figure 1. Scheme of the Laboratory Transmission X-ray Microsocpe (L-TXM).

The plasma is generated by focusing the laser beam on a liquid nitrogen cryo jet target. For microscopy the Ly_α emission of highly ionized nitrogen at 2.48 nm is used (Fig. 2). The sample is illuminated by a spherical Cr/V multilayer condenser mirror. The peak reflectivity of the condenser mirror in normal incidence geometry is up to 0.6 % and the bandwidth is 0.008 nm (FWHM). The usage of a multilayer condenser in normal incidence geometry is advantageous for the following reasons: It allows a monochromatization of the plasma light, an easy matching of the zone plate aperture, aberration free imaging of the source into the sample plane and most important, a higher resistance to damages due to debris from the laser plasma source in comparison to condenser zone plates. A central beam stop at the exit of the plasma source unit, placed between source and sample, blocks radiation emitted by the source into the direction of the sample. Scattered laser radiation is eliminated in front of the sample by a 200 nm Al filter behind the laser plasma source. Using an objective zone plate an enlarged image of the specimen is projected onto a CCD camera. Zone plate and sample positioning are integrated in a compact microscopy unit. Samples can be quickly transferred into vacuum with a load lock and are positioned with high accuracy. Cryo tomography of quick-frozen samples can be performed using a cryo sample holder. The characteristic parameters of the L-TXM are summarized in Table 1.

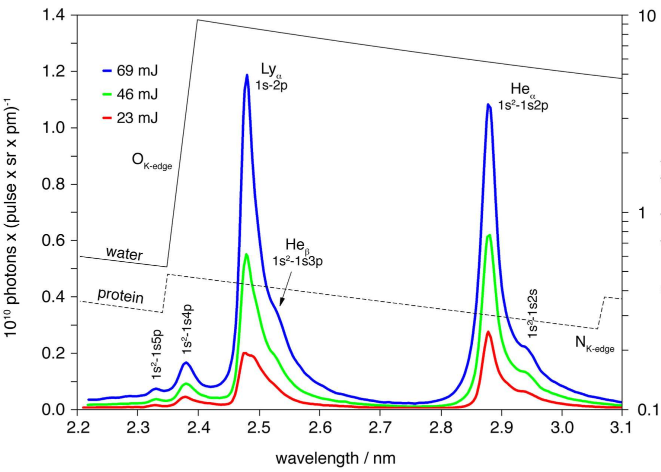

Figure 2. Emission spectra of the liquid-nitrogen plasma. For comparison the attenuation length of radiation within the water window is shown.

Table 1. Characteristics of the L-TXM at a laser power of 70 W. The intensity on the sample is given for 0.1% BW, which is estimated from the measured line width of ≈ 2 pm for the Ly_α emission [9].

Laser system	130 W, 1.3 kHz, 0.4 .. 1 ns, 1.064 μm (InnoSlab)
Source brightness	5×10^{11} photons/(s $\times$ sr $\times$ μm^2) in line at 2.48 nm
Intensity on sample	10^6 photons/ (s $\times$ μm^2 $\times$ 0.1 % BW at 2.48 nm), $R_{ML} = 0.3\%$
Image field	40×40 μm^2
Resolution	35 - 40 nm
Acquisition times	< 60 s (dry samples), $R_{ML} = 0.3\%$, $\eta_{ZP} = 5\%$

3. Results and discussion

In Fig. 3 a long term acquisition of a Siemens star is shown. The resolution (half-pitch) of this image is 41 nm. This high resolution even at longer exposure times emphasizes the stability of the laboratory microscope arrangement. The latter is a precondition for tomography. In Fig. 4 an image of a diatom is shown. This image was collected with 60 W laser power. The acquisition times of this image is 1 min, the pixel size is 26 nm and the resolution is 50 nm (half pitch). Without sample the detector is illuminated at 60 W with averaged 12 ±2 ph/s per 26 nm pixel. Images at higher laser power were not taken so far. Based on the results at 60 W the recording time can be decreased to < 20 s, if the laser works at full power of 130 W and a multilayer condenser mirror with a reflectivity of 0.6% is used.

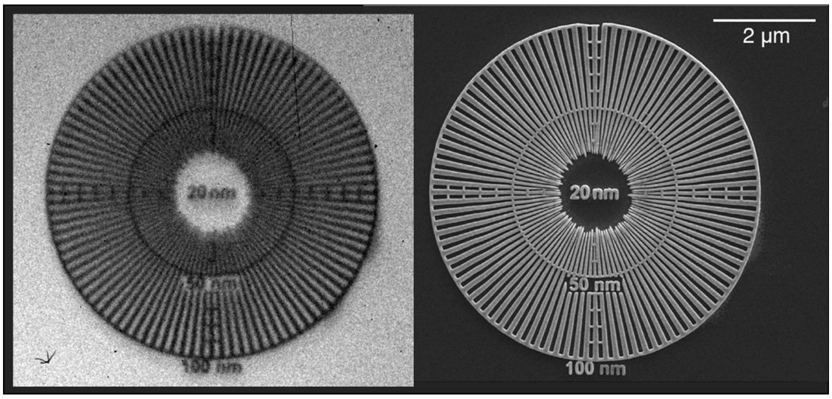

Figure 3. X-ray microscope (left) and SEM (right) image of a Siemens star. The microscope image was taken at a laser power of 10 W and a pulse width of 1 ns with a multilayer reflectivity of 0.45%. The pixel size is 11 nm. The exposure time was 15 min. The efficiency of the used zone plate with an outermost zone width of 25 nm is estimated to be 5%.

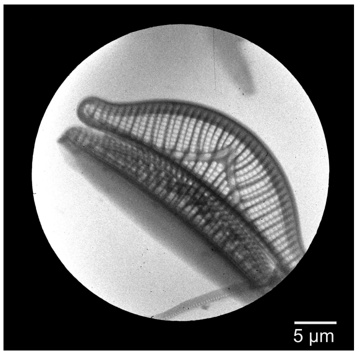

Figure 4. Microscope image of a diatom, taken at a laser power of 60 W, a pulse width of 450 ps and a multilayer reflectivity of 0.3%. The pixel size is 26 nm. The exposure time was 1 min.

4. Summary and perspectives

A novel laboratory x-ray microscope has been developed. Employing a high power InnoSlab laser system, microscopy with exposure times of < 20 s is feasible. This will enable cryo tomography with reduced resolution and sample size in the home laboratory. Further development of the laboratory microscope is in progress, mainly an improvement of the multilayer condenser reflectivity. A reflectivity of 1-2% is envisaged. This would push the performance of the laboratory microscopy to the synchrotron level.

The L-TXM is available for applications at the Berlin Laboratory for innovative X-ray technologies (BLiX).

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