IOP Conf. Series: Journal of Physics: Conf. Series **849** (2017) 012050 doi:10.1088/1742-6596/849/1/012050

Nanoscale imaging applications of soft X-ray microscope based on a gas-puff target source

P. W. Wachulak*[a], A. Torrisi[a], A. Bartnik[a], L. Wegrzynski[a], T. Fok[a], H. Fiedorowicz[a]

[a] Institute of Optoelectronics, Military University of Technology, Kaliskiego 2 Str., 00-908 Warsaw, Poland

* wachulak@gmail.com

Abstract. A compact microscope based on nitrogen double stream gas puff target soft X-ray source, which emits radiation in the water-window spectral range, at the wavelength of λ = 2.88 nm, is presented. The microscope, employing ellipsoidal grazing incidence condenser and a Fresnel zone plate objective, is capable of capturing images with a 60 nm spatial resolution and exposure time as low as a few seconds. Examples of different applications of the SXR microscopy, and its applicability for various fields of science, are presented and discussed.

1. Introduction

The water-window spectral range (λ = 2.3 - 4.4 nm) [1] is particularly suitable for biological imaging (of cells, membranes, lipids, DNA plasmids, etc.) because it is possible to obtain high contrast images due to a significant difference in absorption of water (oxygen) and carbon - biological sample constituents. Soft X-ray (SXR) microscopy has been successfully employed with different techniques, mainly in transmission mode, using diffractive optics, such as zone-plates [2], raster scanning of the sample by focused SXR beam [3] or as a contact microscopy [4]. Short wavelength sources, such as synchrotrons, allow for imaging in the water-window with spatial resolution of 10 nm [5]. However, for a worldwide spread and possible commercial applications, the development and use of a compact high resolution imaging systems is of major importance. In such systems the tradeoff between the exposure time and the size and complexity of the system is still challenging [6–8]. In this work we try to partially overcome presented limitations showing a SXR microscopy system, based on a double stream nitrogen gas puff target [9], capable of resolving 60 nm features, with a desk-top footprint.

2. Description of the SXR microscope

The experimental setup can be seen in figure 2. A nitrogen plasma was produced by focusing Nd:YAG laser pulses 4ns/ 0.74J (NL303HT, from EKSPLA) with an f = 25 mm focal length lens, onto a double stream gas puff target. The gaseous target is formed by two circularly symmetric nozzles. The inner and outer nozzles inject nitrogen (at pressure of 8 bar) and helium (6 bar), respectively. The nozzle timing and position in respect to the laser focal spot were optimized for highest photon flux at the sample plane [10]. The SXR radiation from the nitrogen plasma is collected and focused by an ellipsoidal, axi-symmetrical nickel coated condenser mirror (Rigaku Innovative Technologies Europe s.r.o.) that can efficiently reflect radiation from the SXR region. The nitrogen plasma emission was monochromatized

using a 200 nm thick, free-standing titanium filter (Lebow). The sample is imaged using a Fresnel zone plate objective, Δr=30 nm, (Zoneplates Ltd., UK) onto a SXR sensitive back-illuminated CCD camera (1 Mpix, i-Kon, DO-934N, from Andor). The geometrical magnification of the system is ~220x (sample to CCD). The source photon flux at the sample plane was equal to $(7.9 \pm 0.2)\cdot 10^9$ photons/pulse at 2.88 nm wavelength, theoretically $(8 \pm 0.2)\cdot 10^{11}$ photons/(sec·0.1%BW). This high value allows to achieve short exposure times and high spatial resolution. The half-pitch resolution of the microscope was assessed by a well established knife edge (KE) test resulting equal to 60 nm. More details can be found in [10,11].

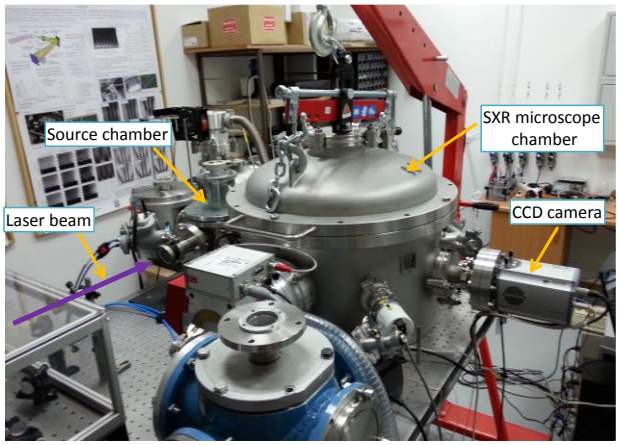

Figure 1. Photograph of the SXR microscope.

3. Imaging results and applications

In case of microscopy, the most important factor is the quality of images of real samples and its possible applications. Some examples of water-window images of various objects are depicted in figs. 2 and 3. Exposures of 1 to 600 SXR pulses were required, at 10 Hz source repetition rate, to acquire the SXR images. During the image acquisition, the CCD camera was cooled down to -20 ºC to decrease its intrinsic thermal noise. Below we present some recent applications of this system.

3.1 Material science and nanotechnology applications

A silicon nitride membrane, 50 nm thick, was used as a support for a thin layer of saccharose ($C_{12}H_{22}O_{11}$), as depicted in figure 2a). Because of the use of monochromatic radiation, it was possible to measure the transmittance of a single (71.8 %) and double (52.3 %) saccharose layers, yielding the layer thickness of ~160 nm. More details about this work can be found in [10].

A SXR image of gold microcracks is depicted in figure 2b). A high resolution image was obtained with features visible beyond the diffraction limit of the ordinary visible light microscope. A small, contrast-enhanced inset shows a branched nanocrack in more detail due to higher spatial resolution achievable with the SXR radiation. The width of the crack was ~100 nm. More details is in [11].

3.2 Biological applications

Images of dehydrated adherent CT 26 fibroblasts, derived from colon carcinoma Mus musculus (strain BALB/c) are depicted in figure 3a-c). The cells, 20-30 μm in size, were imaged using a visible light microscope a) and are directly compared to the SXR image b). Their internal structure is visible due to a phase contrast in figure 3a) and as a modulation of the sample absorption in the SXR image –b). figure 3c) shows comparison of small areas of figure 3a) and 3b). The SXR image shows enhanced spatial resolution due to much shorter wavelength, beyond the capabilities of visible light microscopes. The internal and external structures of the cells, of the order of ~60 nm, are clearly visible in the SXR image, as indicated with arrows in figure 3c), while in the visible light micrograph they cannot be resolved. More details can be found in [11].

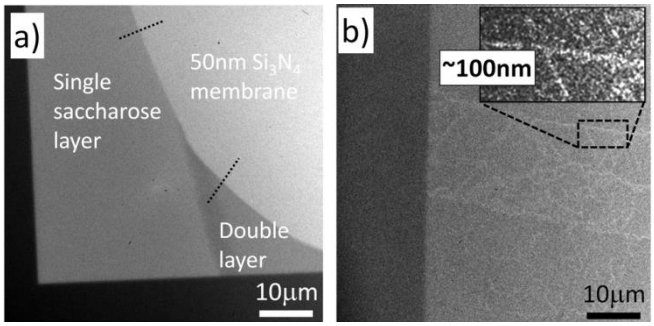

Figure 2. A 50 nm thick silicon nitride membrane with ~160 nm thick saccharose layer – a). A 100 nm thick silicon nitride membrane with 100 nm thick gold layer with microcracks - b).

CT 26 fibroblast, prepared without fixing procedure, are depicted in figure 3d-e). The first image was obtained with a traditional optical microscope (NA=0.7, 40x) and the second one shows a SXR image, acquired with 200 SXR pulses (22 sec exposure). The sample was prepared with a gradual dehydration in ethanol series (final concentration 70% EtOH), without any fixation procedure. Also in this case it is possible distinguish internal and external structures of the cells. The dehydration procedure by a carbon-rich ethanol washes causes, however, the sample to absorb too much SXR radiation.

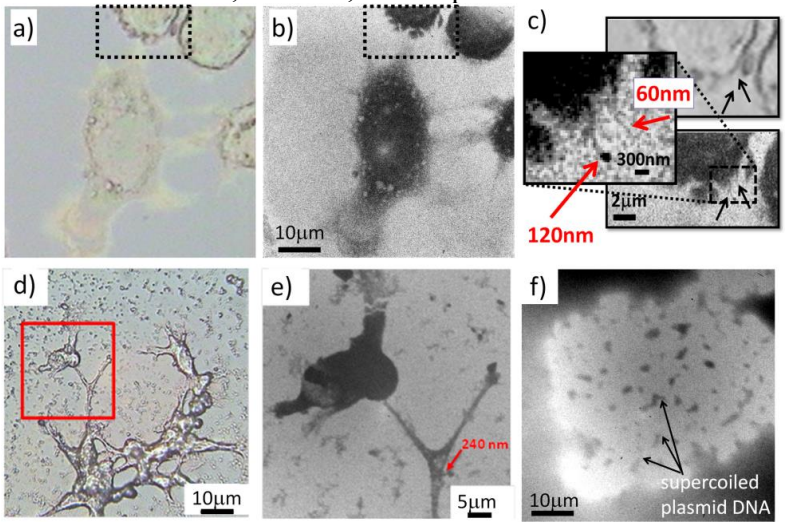

Figure 3. Comparison of visible light microscopy image – a, d) and SXR images – b, e) of dehydrated CT 26 fibroblasts, with and without fixing procedure, respectively. Image c) shows comparison of small areas and smallest features in the visible light microscopy image and the SXR image. The SXR image e) was obtained in the region indicated by a red square in figure d). A supercoiled pBR322 plasmid DNA is imaged in – f). All images, except c), the field of view (FOV) is 60x60 μm^2.

A supercoiled pBR322 plasmid DNA (4361bp), circular double-stranded DNA from Inspiralis, UK, was deposited on top of 50 nm thick Si_3N_4 membrane, from 100 ng / μl solution, dried in nitrogen atmosphere for 20 minutes and imaged - figure 3f). The aggregations are clearly visible; some of them are separated from the "DNA bulk" and vary in size from a few microns down to hundreds of nanometers. More information about those measurements is presented in [10].

4. Conclusions

In conclusion, we presented a water-window compact, desk-top microscope, based on nitrogen double stream gas puff target SXR source and Fresnel zone plate objective. The microscope allows capturing magnified images of the objects, with 60 nm half-pitch spatial resolution, exposure time as low as a few seconds, desk-top footprint, easy accessibility and simple operation. Such system does not require

sample preparation, such as gold coating in case of SEM, exploits natural, optical absorption contrast in the water-window spectral range, is adequate for biological imaging and may be considered as a complementary imaging tool to the already well established techniques. It was also shown that this compact SXR microscope is capable of imaging variety of different samples from various fields of science and technology, including, but not limited to, biology, material science and nanotechnology. The versatility of such microscope may open the possibility of widespread and commercialization of such systems in the near future.

Acknowledgments
The research was supported by the Laserlab Europe Project (EU-H2020 654148), the EMJD Programme EXTATIC (No 2012-0033), the National Science Centre; award numbers UMO-2015/17/B/ST7/03718, UMO-2015/19/B/ST3/00435 and the National Centre for Research and Development, LIDER programme, award # LIDER/004/410/L-4/12/NCBR/2013. We would like thank to Dr. Daniel Adjei, Laboratoire d'Optique Appliquée, ENSTA Paris Tech, Ecole Polytechnique, CNRS, for preparing the supercoiled pBR322 plasmid DNA (4361bp). We thank also to Šárka Vondrová, Jana Turňová and Prof. Miroslava Vrbová from Czech Technical University in Prague, Czech Republic, for preparing the cell samples investigated with the SXR full field microscope.

References

[1] Da Silva L B, Trebes J E, Balhorn R, Mrowka S, Anderson E, Attwood D T, Barbee T W, Brase J, Corzett M and Gray J 1992 X-ray laser microscopy of rat sperm nuclei. *Science (New York, N.Y.)* **258** 269–71

[2] Schneider G, Niemann B, Guttmann P, Rudolph D and Schmahl G 1995 Cryo X-ray microscopy *Synchrotron Radiation News* **8** 19–28

[3] Kirz J, Jacobsen C, Lindaas S, Williams S, Zhang X A E and H M 1994 *Synchrotron Radiation in the Biosciences* (Oxford: Oxford University Press)

[4] Poletti G, Orsini F and Batani D 2005 Study of Multicellular Living Organisms by SXCM (Soft X-Ray Contact Microscopy) *Solid State Phenomena* **107** 7–10

[5] Chao W, Fischer P, Tyliszczak T, Rekawa S, Anderson E and Naulleau P 2012 Real space soft x-ray imaging at 10 nm spatial resolution *Optics Express* **20** 9777

[6] Takman P A C, Stollberg H, Johansson G A, Holmberg A, Lindblom M and Hertz H M 2007 High-resolution compact X-ray microscopy *Journal of Microscopy* **226** 175–81

[7] Johansson G A, Holmberg A, Hertz H M and Berglund M 2002 Design and performance of a laser-plasma-based compact soft x-ray microscope *Review of Scientific Instruments* **73** 1193

[8] Legall H, Blobel G, Stiel H, Sandner W, Seim C, Takman P, Martz D H, Selin M, Vogt U, Hertz H M, Esser D, Sipma H, Luttmann J, Hofer M, Hoffmann H D, Yulin S, Feigl T, Rehbein S, Guttmann P, Schneider G, Wiesemann U, Wirtz M and Diete W 2012 Compact x-ray microscope for the water window based on a high brightness laser plasma source *Optics Express* **20** 18362–9

[9] Wachulak P W, Bartnik A, Skorupka M, Kostecki J, Jarocki R, Szczurek M M, Wegrzynski L, Fok T and Fiedorowicz H 2013 Water-window microscopy using a compact, laser-plasma SXR source based on a double-stream gas-puff target *Applied Physics B: Lasers and Optics* **111** 239–47

[10] Wachulak P W, Torrisi A, Bartnik A, Adjei D, Kostecki J, Wegrzynski L, Jarocki R, Szczurek M and Fiedorowicz H 2015 Desktop water window microscope using a double-stream gas puff target source *Applied Physics B* **118** 573–8

[11] Wachulak P, Torrisi A, Nawaz M F, Bartnik A, Adjei D, Vondrová Š, Turňová J, Jančarek A, Limpouch J, Vrbová M and Fiedorowicz H 2015 A Compact "Water Window" Microscope with 60 nm Spatial Resolution for Applications in Biology and Nanotechnology *Microscopy and Microanalysis* **21** 1214–23