

Article

Table-Top Water-Window Microscope Using a Capillary Discharge Plasma Source with Spatial Resolution 75 nm

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Received: 31 July 2020; Accepted: 11 September 2020; Published: 13 September 2020



Abstract: We present a design of a compact transmission water-window microscope based on the Z-pinching capillary discharge nitrogen plasma source. The microscope operates at wavelength of 2.88 nm (430 eV), and with its table-top dimensions provides an alternative to large-scale soft X-ray (SXR) microscope systems based on synchrotrons and free-electron lasers. The emitted soft X-ray radiation is filtered by a titanium foil and focused by an ellipsoidal condenser mirror into the sample plane. A Fresnel zone plate was used to create a transmission image of the sample onto a charge-coupled device (CCD) camera. To assess the resolution of the microscope, we imaged a standard sample-copper mesh. The spatial resolution of the microscope is 75 nm at half-pitch, calculated via a 10–90% intensity knife-edge test. The applicability of the microscope is demonstrated by the imaging of green algae-*Desmodesmus communis*. This paper describes the principle of capillary discharge source, design of the microscope, and experimental imaging results of Cu mesh and biological sample.

Keywords: SXR microscopy; water window; Z-pinch; discharge source; Fresnel zone plate; imaging

1. Introduction

Imaging is a crucial factor in a fundamental understanding of the environment surrounding us as well as the insight of ourselves. New imaging methods have been explored during the last couple of decades to continue research in cell biology. One of the emerging imaging methods for cell biology is soft X-ray microcopy with its spatial resolution in the order of tens nm covering the gap between transmission electron microscopy (TEM) and visible light microscopy. The soft X-ray microscopic images captured by employing the Fresnel zone plate as the objective optics with 10 nm spatial resolution have been presented [1], and the soft X-ray ptychographic microscope demonstrated spatial resolution below 5 nm [2] as ptychography removed the resolution limit imposed by the characteristics of the X-ray optics. Soft X-ray microscopes typically operate within the “water-window” spectral region, between the K-absorption edges of oxygen (2.34 nm; 530 eV) and carbon (4.4 nm; 280 eV) [3,4] and have three advantages over light and electron microscopes. Firstly, SXR radiation penetrates biological materials much more easily than electrons, which allows specimens up to 10 µm thick to be imaged. Unlike electron microscopy, there is no need for sectioning eukaryotic cells with an ultramicrotome before imaging [5]. Secondly, the short-wavelength allows image cells with higher spatial resolution to be compared to light sources [4]. Finally, image contrast is obtained from the absorption of X-rays by

the specimen [5]; thus, there is no need to insert any fluorescence markers or other substances into the sample. This absorption is described by the Beer–Lambert Law, therefore is linear with thickness and concentration [4]. By imaging in the “water-window,” organic matter containing carbon and nitrogen absorbs photons at an order of magnitude more than water, which creates inherent contrast to visualize cellular structure [6]. The in-depth comparison between images taken by the soft X-ray microscope (scanning transmission X-ray and X-ray ptychography) and the electron microscope can be found in [7].

A major part of SXR microscopes is based on large-scale facilities such as synchrotron or free-electron lasers, and their advantage is a short exposure time and high spatial resolution contrary to their vastness and the price demands (construction and operation), which does not allow access to this imaging technique for the broader scientific community. Therefore, in the mid-1990s the first effort to develop a compact, laboratory-scale source appeared. In recent years, considerable progress has been achieved, and several transmission SXR microscopes operating in the water-window region based on table-top sources were reported. Compact transmission SXR microscopic systems based on laser-produced plasma sources—using liquid-jet target [8–10], gaseous target [11,12], double-stream gas target [13–15], or ultra-thin metal target [16]—were developed. The second branch of laboratory-scale transmission SXR microscopes are based on discharge-produced plasma sources using pseudo sparks such as electrode geometry [17], an electrode-less design (Energetiq, Inc., WI, MA, USA) [18,19], or Z-pinching capillary discharge [20]. A compact transmission SXR microscope based on an electron-impact source, utilizing the oxygen K-alpha emission line at 525 eV (2.36 nm), was reported [21]. Nevertheless, the reported photon flux of these sources is about a few orders lower compare to laser-produced and discharge-produced plasma sources. Coherent laboratory sources such as plasma-based X-ray lasers (XRLs), sources based on high harmonics generation (HHG), or betatron radiation produced by laser-wakefield accelerators (LWFAs) are due to the high spatial coherence more suitable for other imaging techniques than transmission water-window microscopy, such as coherent diffraction imaging, ptychography, etc. [22]. The main limitations of using coherent sources for the transmission water-window microscopy are the huge and expensive pump lasers in the case of XRL and LWFA, and low flux in the case of HHG [23,24]. However, a newly developed TW-class mid-infrared laser, together with a loose focusing geometry for HHG, resulted in increased photon flux, opening a new possibility for the transmission water-window imaging [25]. However, the laser-produced and discharge-produced plasma sources remain the more compact solution. The up-to-date overview of laboratory water-window X-ray microscopy is described in the review article [22], where the table-top sources, optics, detectors, and sample preparation for SXR microscopy are discussed. Although table-top sources are accessible and easy to operate, they come with limitations in terms of the required exposure times, and in terms of the comparable spatial resolution with large-scale facilities. To improve the brilliance of the compact sources, a jet with a so-called barrel shock system was introduced [26], enhancement of the soft X-ray radiation by inserting a low-pressure nitrogen atmosphere to increase the conversion efficiency of the laser-produced plasma sources was demonstrated [27,28]. Nevertheless, the future development of laboratory-scale sources is necessary to perform a single-shot imaging experiment as the photon flux of these sources is a few orders of magnitude below the requirements for a single shot imaging micrometer-sized specimen [29]; a current perspective of a single shot imaging using compact SXR sources is discussed in [29].

In this paper, we present a new design of a table-top water-window transmission microscope using a Z-pinching capillary discharge source, operating at 2.88 nm (430 eV) corresponding to the $1s^2$ - $1s2p$ quantum transition of helium-like nitrogen ion. The working principle of the source is described, as well as the sample preparation. Images of *Desmodemus communis* (green algae) showing their organelles are demonstrated.

2. Experimental Setup

The laboratory-scale microscope is based on table-top discharge produced plasma source, and employs an ellipsoidal nickel-coated condenser mirror, a Fresnel zone plate objective, and an

SXR-sensitive back-illuminated CCD camera. The dimensions of the whole system are roughly 0.5×2 m; depending on the actual magnification of the microscope, which is adjustable from 190 $\times$ up to 400 $\times$.

2.1. Z-Pinching Capillary Discharge Plasma Source

Figure 1 depicts the schematic of the source and the charging circuit. Plasma is generated by a current discharge through a 10 cm long, 3.2 mm inner diameter ceramic capillary (Al_2O_3) filled with nitrogen gas. A ceramic capacitor bank with a maximum capacity of 21 nF is pulse-charged by a March-Fitch generator up to 90 kV, and switched by a self-breakdown spark-gap. The discharge voltage is regulated with nitrogen pressure in the spark-gap and by March-Fitch generator charging voltage. Before the main discharge, a 35 A, 3 μs long current pulse pre-ionizes the gas in the capillary and prepares a uniform conducting channel. The main current pulse has a damped sinus shape, with a half-period of 150 ns and a maximum amplitude of 30 kA. The capillary is filled with nitrogen gas through a hollow in the electrode on its grounded side. Radiation is also emitted through this hole. The system is enclosed in duralumin housing in order to reduce electromagnetic noise. The photon flux of this incoherent source is 5.5×10^{13} photons/sr/pulse, the source size at full width half maximum (FWHM) is 360 μm , and the beam divergence at FWHM is 30 mrad [20,30]. More details about the source and its optimization and characterization can be found in [20,30–32].

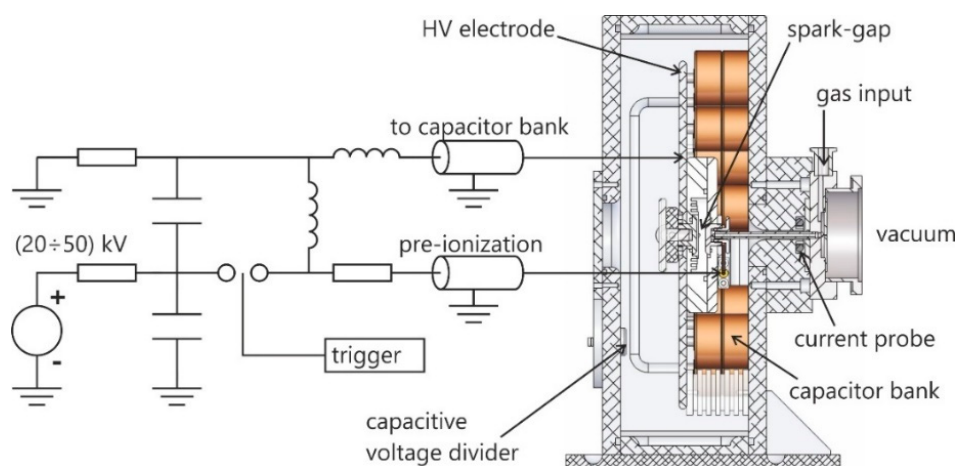


Figure 1. Schematic of capillary plasma driver and charging circuit.

2.2. Table-Top Water-Window Microscope

The sketch of the microscope is shown in Figure 2, and Figure 3 illustrates a real photo of the microscopic system. The emitted SXR radiation is filtered through a titanium filter (Lebow, Goleta, CA, USA; 200 nm) to achieve monochromatic radiation with wavelength $\lambda = 2.88$ nm. The filtered radiation is focused by an ellipsoidal, grazing incidence, nickel-coated condenser mirror (Rigaku Inc., Auburn Hills, MI, USA; focal length 200 mm, mirror length 100 mm) into the sample plane. The focal spot has a circular shape with a diameter of 200 μm at FWHM, observed by YAG scintillator, and recorded onto a CCD camera (Chameleon, Point Grey Research Inc., Richmond, BC, Canada). A Fresnel zone plate (ZonePlates Ltd., London, UK; width of the outer-most zone 30 nm, diameter 180 μm , focal length 1.87 mm) was employed to create an image of a specimen transmission on an SXR-sensitive back-illuminated CCD camera (Greateyes GmbH, Berlin, Germany; 2048 $\times$ 2048 pixels, 13.5 $\times$ 13.5 μm pixel size, quantum efficiency at 2.88 nm 83%). The images were acquired with a magnification of up to 400 $\times$, and the CCD camera was cooled down to minus 30 $^{\circ}\text{C}$ to minimize its thermal noise.

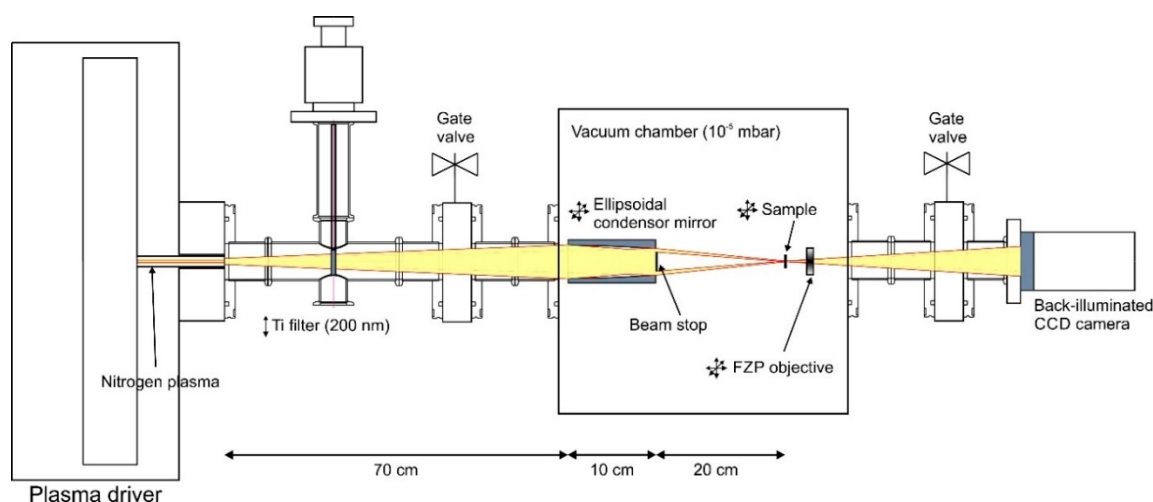


Figure 2. Schematic of a water-window microscope based on a nitrogen plasma capillary discharge source.

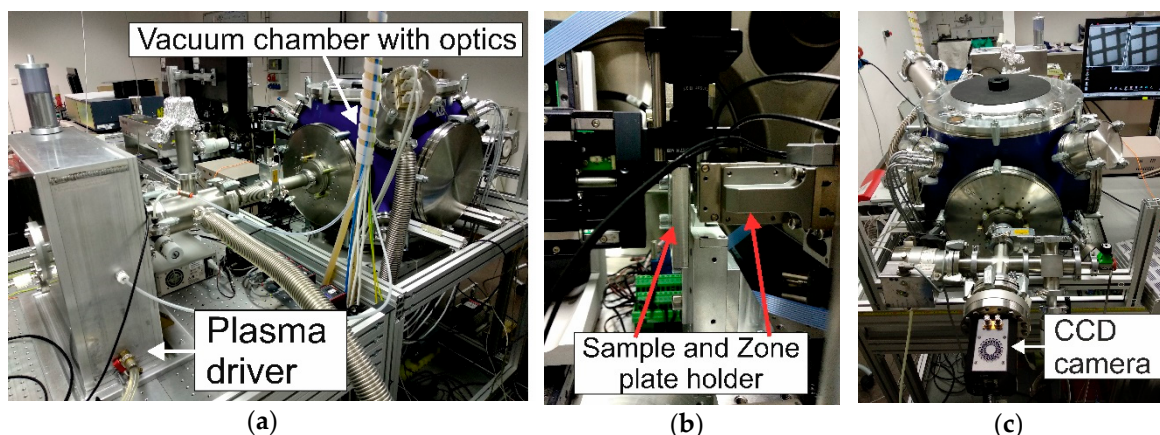


Figure 3. Photographs of the soft X-ray (SXR) imaging system: (a) The “source” view showing plasma driver and vacuum chamber with imaging optics, (b) the internal photograph of a vacuum chamber with sample and zone plate holder, (c) photograph of the “imaging” part of the SXR microscope.

2.3. Sample Preparation of *Desmodesmus Communis*

Desmodesmus communis is a green alga (Chlorophyta); these cells form in a coenobia. The algae were cultured in the DY-V medium. A drop of the algal suspension (10 μ L) was placed onto an Si_3N_4 membrane (Norcada Inc., Edmonton, AB, Canada; thickness 50 nm). The sample was fixed 1:1 with the mixed solution of 2% glutaraldehyde in 0.1 M Na-cacodylate buffer and 4% paraformaldehyde in phosphate-buffered saline (PBS) for three hours. After fixation, the sample was rinsed in 0.1 M Na-cacodylate buffer in PBS for 5 min. Then, it was dehydrated in ethanol solutions of 70%, 80%, 90% for 5 min, and three times in 100% for 10 min each. After the 100% ethanol step, it was dried with a hexamethyldisilazane (HMDS) treatment: The sample was immersed in a 50:50 mixture of 100% ethanol and 100% HDMS for 5 min, and twice in pure HDMS for 30 min before being dried in air.

3. Results

The experiments with a copper TEM mesh (SPI, West Chester, PA, USA) with 1000 lines/inch (bar width 6 μ m, hole width 19 μ m) were performed to determine the spatial resolution of the microscope. The measured and calculated magnification was around 340 $\times$ for this microscope layout, and, thus, the corresponding field of view is $\sim 80 \times 80 \mu\text{m}$. The image of Cu mesh shown in Figure 4a was acquired with an exposure time of 3 min and the repetition rate of the capillary discharge source

of 2 Hz, which allowed the acquisition of 360 SXR pulses. The spatial resolution was calculated via a knife-edge test across a sharp edge. A 10–90% intensity transition, corresponding to the Rayleigh resolution limit, is equal to 150 nm; a half-pitch spatial resolution is equal to 75 nm (Figure 4b), measured from the image of the mesh and highlighted by a red, dashed rectangle. The spatial resolution was calculated from the average of many pixels along the edge to remove the effect of noise.

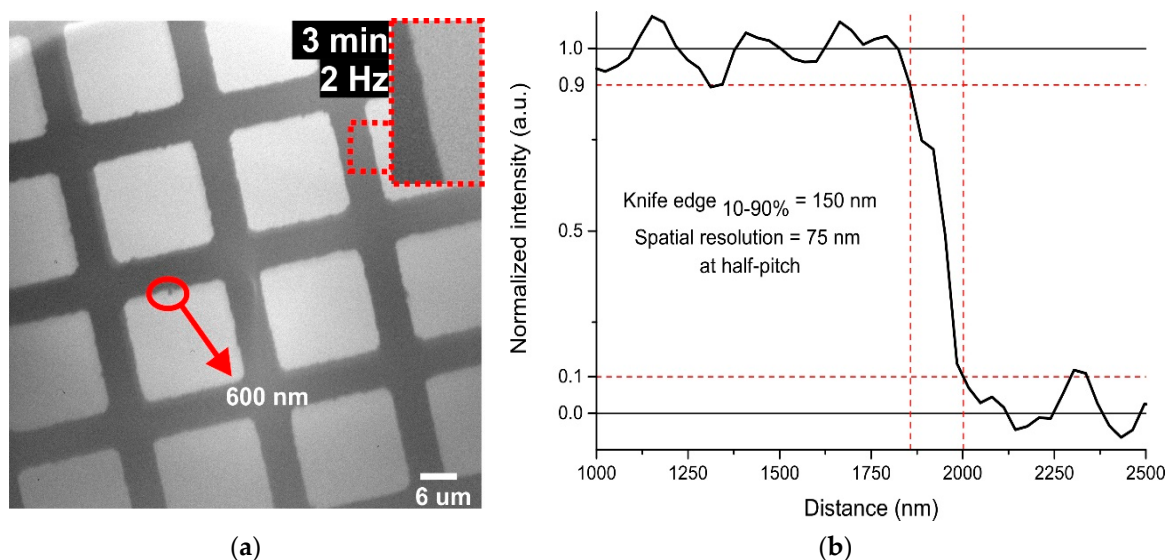


Figure 4. (a) Soft X-ray image of copper mesh 1000 lines/inch acquired with 3 min exposure time (360 SXR pulses). A red, dashed rectangle highlights a sharp edge representing the place where the spatial resolution was calculated. Scale bar is 6 μm . (b) Knife-edge resolution test results showing normalized intensity profile across a sharp edge. A 10–90% intensity transition corresponding to Rayleigh resolution is equal to 150 nm, half-pitch spatial resolution equal to 75 nm, measured from the image of the Cu mesh.

To compare the quality of soft X-ray images of Cu mesh, several images were taken with different exposure times. Figure 5 depicts the outcome of this experiment, demonstrating the differences among the soft X-ray images of Cu mesh taken with 10, 30, 60, and 180 SXR pulses, respectively, at a 2 Hz repetition rate of the source. Some imperfections of the Cu mesh are still visible down to a 5 s exposure time.

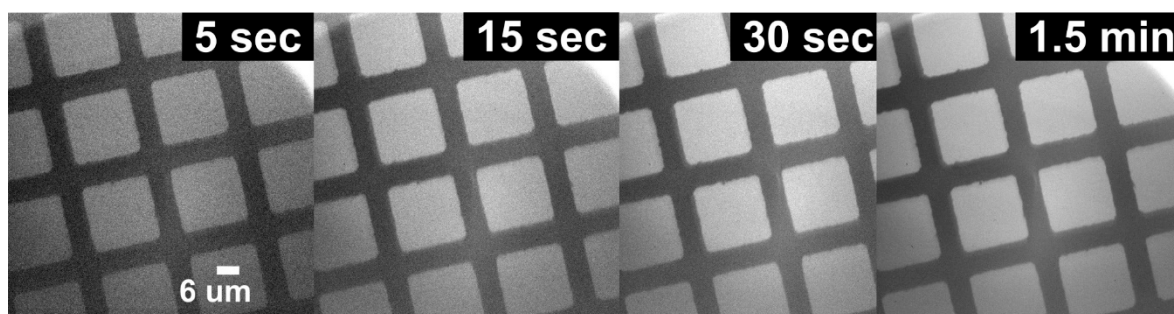


Figure 5. Comparison of soft X-ray images of copper mesh (1000 lines/inch) with different exposure times. The scale bar is 6 μm and is the same for each image.

Finally, the green algae-*Desmodesmus communis* was imaged. Cells were deposited onto an Si_3N_4 membrane (see Section 2.3 “Sample Preparation”). The soft X-ray image (Figure 6a) was taken with a 390 $\times$ magnification and an exposure time of 12 min with a 2 Hz repetition rate of the source. The inner structure (organelle) of the cell is visible and distinguishable. No post-processing was made with a

soft X-ray image except for CCD background removal and brightness adjustment. For comparison, an image of the same sample was taken with the visible light microscope (Figure 6b), the image was captured with a 400 $\times$ magnification, and the white, dashed rectangle represents the field of view in Figure 6a. The SXR image exhibits a superior spatial resolution compared to visible light microscopy (Figure 6b), owing to a much shorter wavelength beyond the capabilities of diffraction-limited visible light microscopes. Small features in the order of hundreds of nanometers can be easily observed, as demonstrated in a zoomed view in Figure 6a.

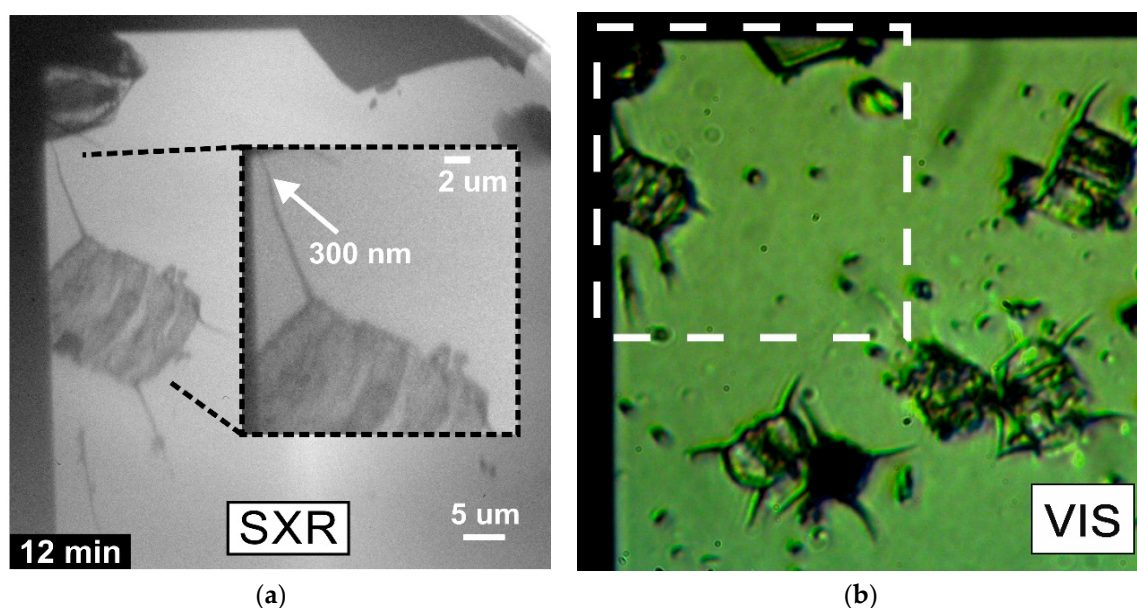


Figure 6. Comparison of the images of dried *Desmodesmus communis* deposited on a 50 nm thick Si_3N_4 membrane. (a) A soft X-ray (SXR) image was acquired with 1400 SXR pulses (12 min). Scale bar is 5 μm . The black, dashed rectangle represents a zoomed area with a 2 μm scale bar. (b) The image was taken with a visible light microscope (VIS). The area marked by a white, dashed rectangle represents the field of view in image a.

4. Discussion and Conclusions

In this paper, we presented a compact, table-top transmission water-window microscope, based on a Z-pinch plasma source operating at 2.88 nm. The microscope allows the capture of images of a specimen with a magnification between 190 $\times$ and 400 $\times$ with a 75 nm half-pitch spatial resolution. In terms of its spatial resolution, it provides a complementary imaging technique between conventional visible light microscopes and transmission electron microscopes, with the advantage of imaging relatively thick samples (up to 10 μm), and does not require fluorescent markers or staining that modifies the morphology of the sample. Although the spatial resolution of this microscope cannot compete with the electron-based system, it provides a much better spatial resolution than conventional light microscopes, as is demonstrated by the imaging of the green algae (*Desmodesmus communis*) in Figure 6.

This microscopic system is easy to operate, affordable, and accessible—providing an alternative to large-scale facilities—although it has a longer exposure time and lower spatial resolution by a factor of 5 compared to synchrotron-based SXR microscopes. Nevertheless, compact SXR microscopic systems have already found applications, not only in biomedicine, but also in material science, nanotechnology, and microelectronics, among others. These cost-effective table-top sources allow access to this imaging technique for the broader scientific community, and the future development of compact, table-top sources promises to broaden access to soft X-ray imaging in other laboratories.

Author Contributions: T.P. designed and constructed the water-window microscope, performed the imaging experiments, and wrote the paper; M.N. and A.J. developed and constructed the discharge source; J.T. prepared the biological samples; D.P. cooperated on the development of the water-window microscope, and M.V. conceived the water-window microscope. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the Ministry of Education, Youth and Sports of the Czech Republic LTT17015 Research in the Frame of the International Center for Dense Magnetized Plasmas, by the CZ.02.1.01/0.0/0.0/16-019/0000778 Centre of Advanced Applied Sciences-CAAS grant, and by the CTU Student grant competition SGS19/084/OHK4/1T/17.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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