Table-Top Water-Window Microscope Using Z-Pinching Capillary Discharge Source

T. Parkman[1]*, M. Nevrkla[2], M. F. Nawaz[3], A. Jancarek[2], D. Panek[1], J. Turnova[1], M. Vrbova[1]

[1]*Czech Technical University in Prague, Faculty of Biomedical Engineering, nam. Sitna 3105, 272 01 Kladno, Czech Republic*
[2]*Czech Technical University in Prague, Faculty of Nuclear Sciences and Physical Engineering,* V Holesovickach 2, *180 00 Prague 8, Czech Republic*
[3]*ASML, De Run 6501,5504 DR, Veldhoven, The Netherlands*
*tomas.parkman@fbmi.cvut.cz

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Introduction

We present a design of a compact transmission water-window microscope based on nitrogen plasma induced by the Z-pinching capillary discharge. Nitrogen plasma is ideal for its quasi-monochromatic radiation with wavelength λ = 2.88 nm, which corresponds to the quantum transition $1s^2$-1s2p of helium-like nitrogen ion. This wavelength falls within the so-called water-window wavelength region (λ = 2.3 – 4.4 nm) and thus provides the natural contrast between water and carbon-based substances, e.g. proteins.

Capillary discharge source

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 pulsed charged by March-Fitch generator up to 100 kV, and switched by self-breakdown spark-gap. The discharge voltage is regulated by 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 has a damped sinus shape, with a half-period of 150 ns and 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.

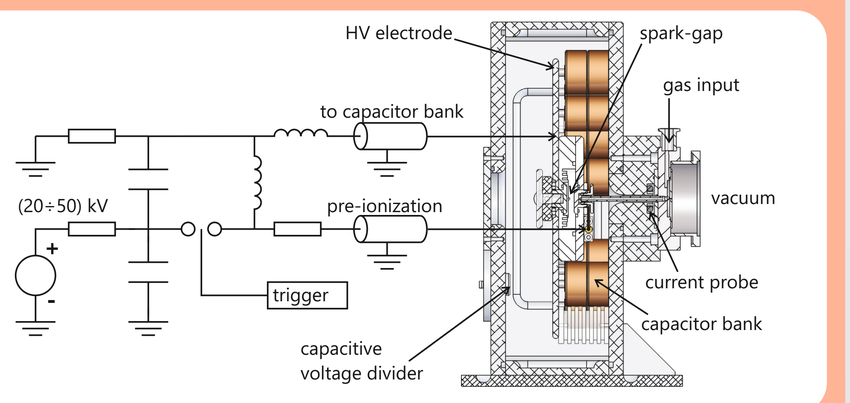

Fig. 1: Schematic of capillary plasma driver and charging circuit.

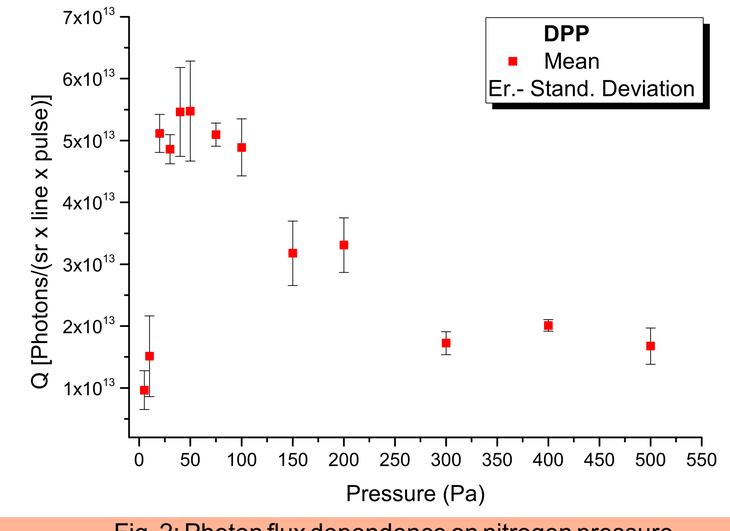

Fig. 2: Photon flux dependence on nitrogen pressure. Discharge current fixed at 26-27.5 kA.

Sample preparation

Desmodesmus communis is a Green algae (Chlorophyta). These cells are forming in a coenobia. The algae were cultured in DY-V medium. A drop of the algal suspension (10 μl) was placed onto a Si_3N_4 membrane. The sample was fixed with the mix solution 1:1 of 2% glutaraldehyde in 0.1M Na-cacodylate buffer and 4% paraformaldehyde in PBS for 3 h. After fixation the sample was rinsed in 0.1M 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 HMDS (hexamethyldisilazane) treatment: the sample was immersed in 50:50 mixture of 100% ethanol and 100% HDMS for 5 min and two times in pure HDMS for 30 min and then dried on air.

Conclusion

The transmission water-window microscope based on nitrogen plasma generated by a Z-pinching capillary discharge source is presented, together with the descriptions of the employed optical system.
The initial images of copper mesh are demonstrated with an achieved spatial resolution of 75 nm at half-pitch. The images of Desmodesmus cells are presented as well as the description of the sample preparation process. The organelles of Desmodesmus are visible, and features in order of hundred nm are distinguishable.

References

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Design of water-window microscope

The spectrum of soft X-ray radiation produced by the source is filtered by titanium filter (200 nm, Lebow) to achieve monochromatic radiation with wavelength λ = 2.88 nm. Filtered radiation is focused by an ellipsoidal nickel coated condenser mirror (Rigaku). We used a Fresnel zone plate (ZonePlates) to create an image of a sample transmission on a SXR-sensitive CCD camera (Greateyes). The magnification of the microscope is adjustable from 190x up to 400x.

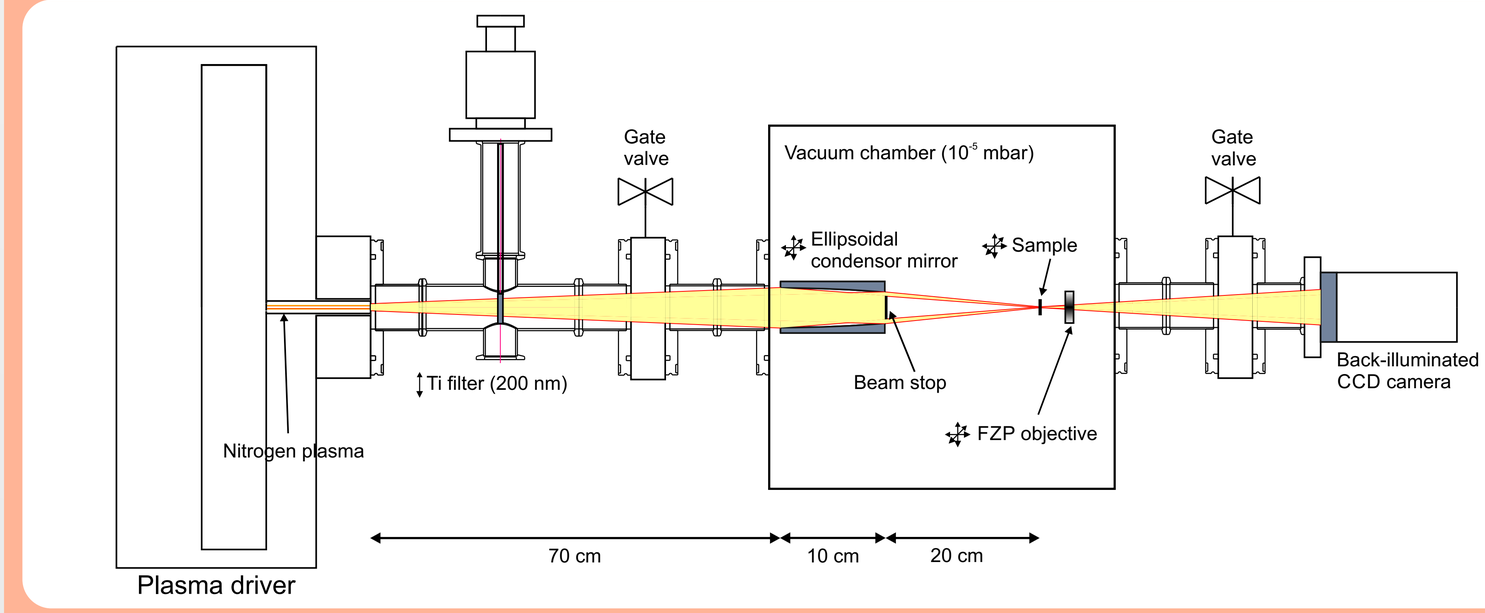

Fig. 3: Schematic of a water-window microscope based on a nitrogen plasma capillary discharge source.

Optics

Ellipsoidal condenser mirror	
Input numerical aperture	0.014
Output numerical aperture	0.043
Mirror length	100 mm
Coated by	nickel
Focal length	200 mm

Fresnel zone plate	
Width of the outer-most zone	30 nm
Numerical aperture	0.048
Focal length	1.87 mm
Diameter of zone plate	180 μm
Depth of focus	1.25 μm

Back-illuminated CCD camera	
Total number of pixels	2048 x 2048
Pixel size	13.5 x 13.5 μm
Image active area	27.6 x 27.6 mm
Quantum efficiency @ 2.88 nm	83 %

Experimental results

The first experiments were performed with a copper TEM mesh (SPI, USA) with 1000 lines/inch (bar width 6 μm, hole width 19 μm). The measured and calculated magnification is around 340x for this microscope layout and thus the corresponding field of view is ~80 x 80 μm. The image of Cu mesh shown in Fig. 4. E) was acquired with an exposure time of 3 min and repetition rate of the capillary discharge source of 2 Hz, which means 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, half-pitch spatial resolution is equal to 75 nm, measured from the image of the mesh and indicated by a red solid line.

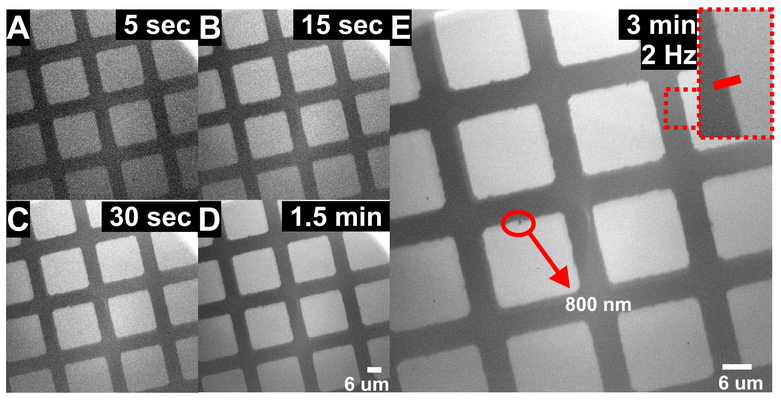

Fig. 4: Images of the copper 1000 lines/inch mesh with different exposure times. A)10 SXR pulses / 5 sec, B) 30 SXR pulses / 15 sec, C) 60 SXR pulses / 30 sec, D) 180 SXR pulses / 90 sec, E) 360 SXR pulses / 3 min. The imperfection of the Cu mesh is highlighted. The dashed rectangle represents the spot where the spatial resolution was calculated. Scale bar is 6 um.

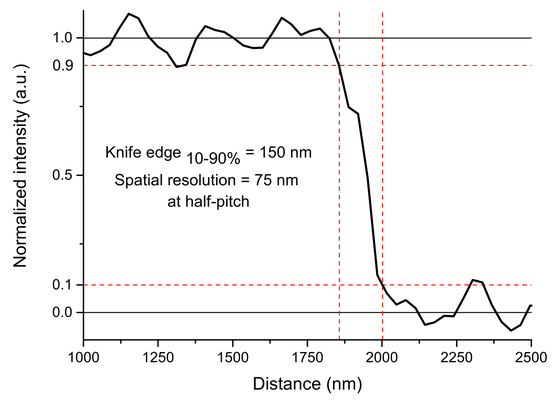

Fig. 5: Knife-edge resolution test result showing normalized intensity profile across a sharp edge. A 10–90 % intensity transition, corre- sponding to Rayleigh resolution, is equal to 150 nm, half-pitch spatial resolution equal to 75 nm, measured from the image of the Cu mesh.

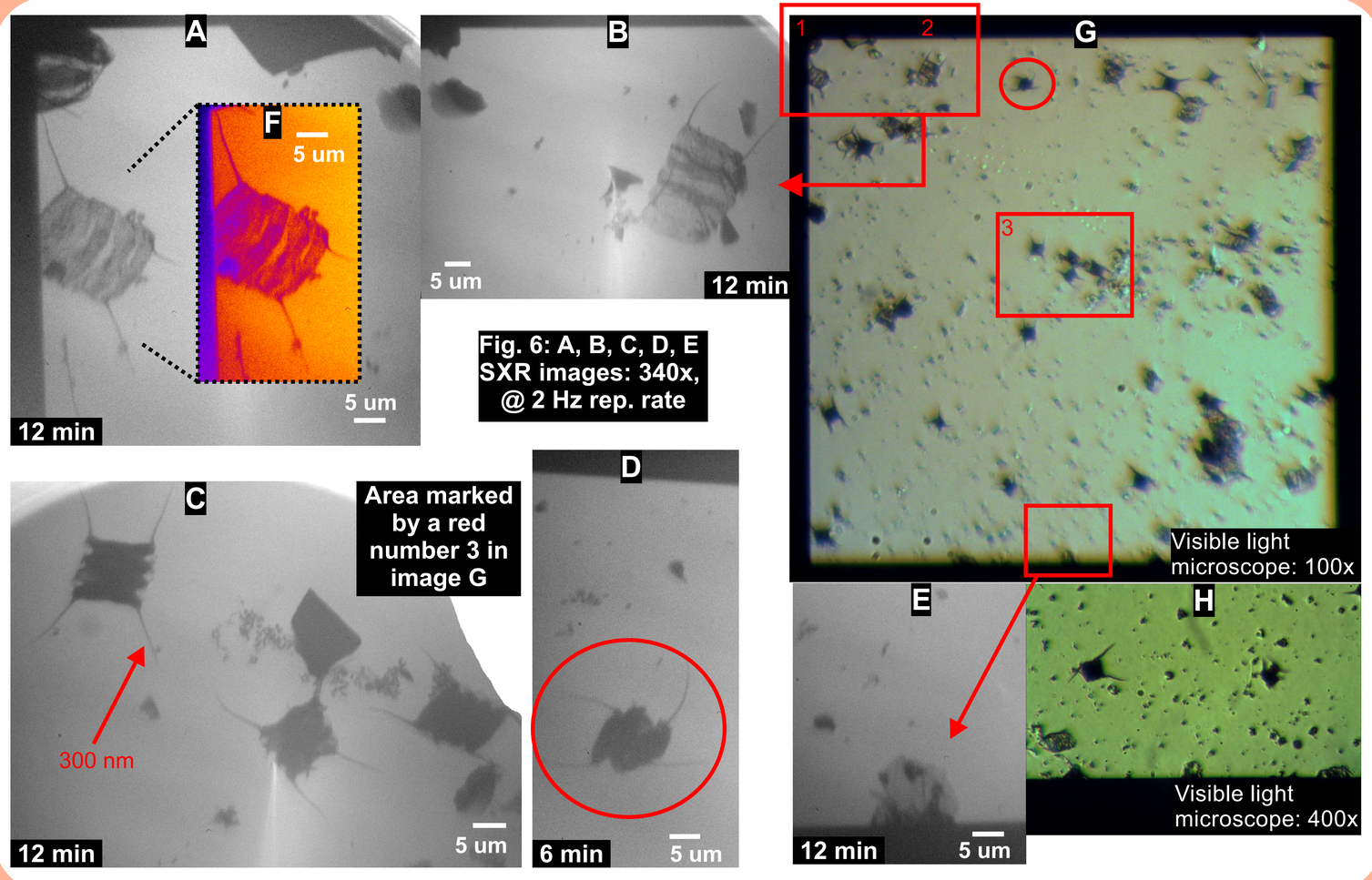

Fig. 6: A, B, C, D, E SXR images: 340x, @ 2 Hz rep. rate

Fig. 6: A comparison between images taken by the SXR water-window microscope (A - E) and visible light microscope (G, H) of dried biological sample (Desmodesmus) deposited on 50 nm thick Si_3N_4 membrane. Image F) represents the density distribution in a “Fire” lookup table mode.