Laboratory tomographic microscopy with compact plasma based extreme ultraviolet and soft x-ray sources

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in cooperation with

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EXTATIC Welcome Week

22-24 Sept. 2017, Prague

Outline

- Introduction
- Experimental set-up
- Challenges-activity updates
- Neuron cell imaging
- Current magnification and resolution
- Capillary for biological sample
- Future work
- Conclusions

Soft X-ray (SXR) and Extreme Ultraviolet (EUV) radiation microscopy

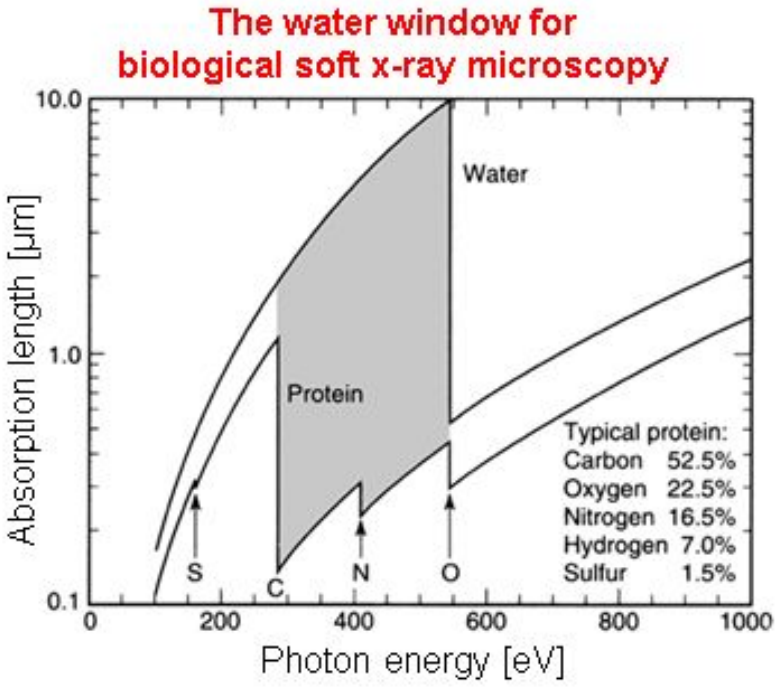


Figure 1: Absorption length in water window [Attwood, D. 1999.]

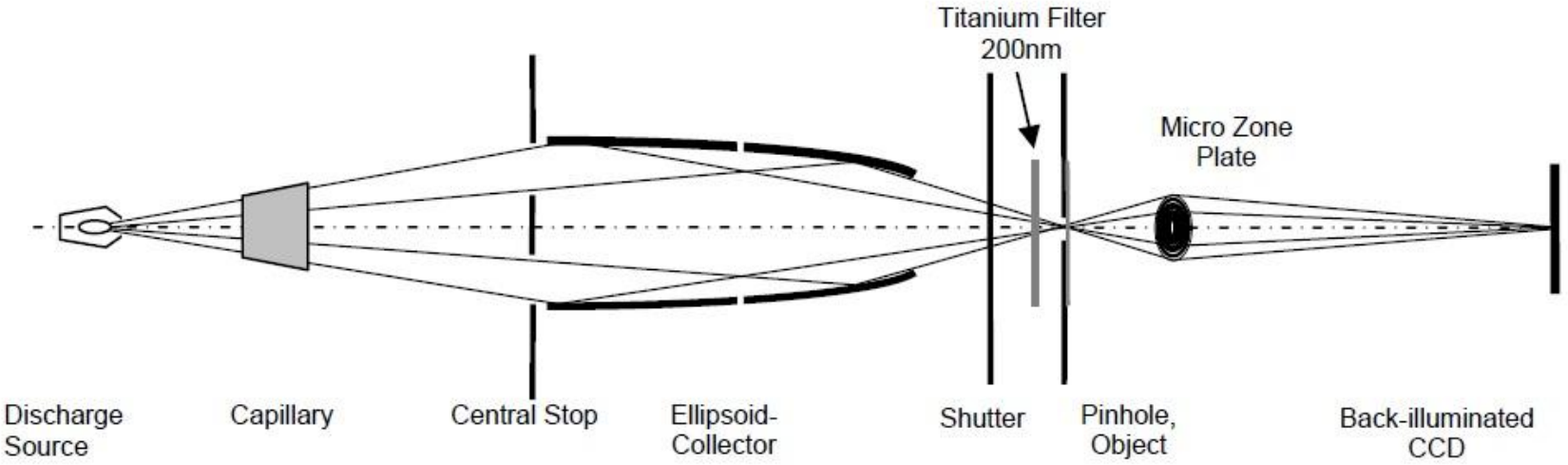
Applications [Attwood, D. 1999; Benk, M., et. al. 2011.; Schafer, D., et. al. 2009]:

- Micro and nano-structure imaging
- Cells and organelles imaging
- Chemistry of proteins and biomolecules

Water window (2.3 – 4.4 nm radiation)

- Water is highly transparent as compared to proteins and other organic compounds.

Experimental set-up



Collector	Ellipsoidal mirror
Collector NAmax/NAmin at source at sample	0.024 / 0.0126 0.046 / 0.025
Collector focus diameter (best focus)	x-axis=1.52 mm; y-axis=1.51 mm
Zoneplate (ZP) outer zone width	40 nm
ZP NA	0.036

Experimental set-up

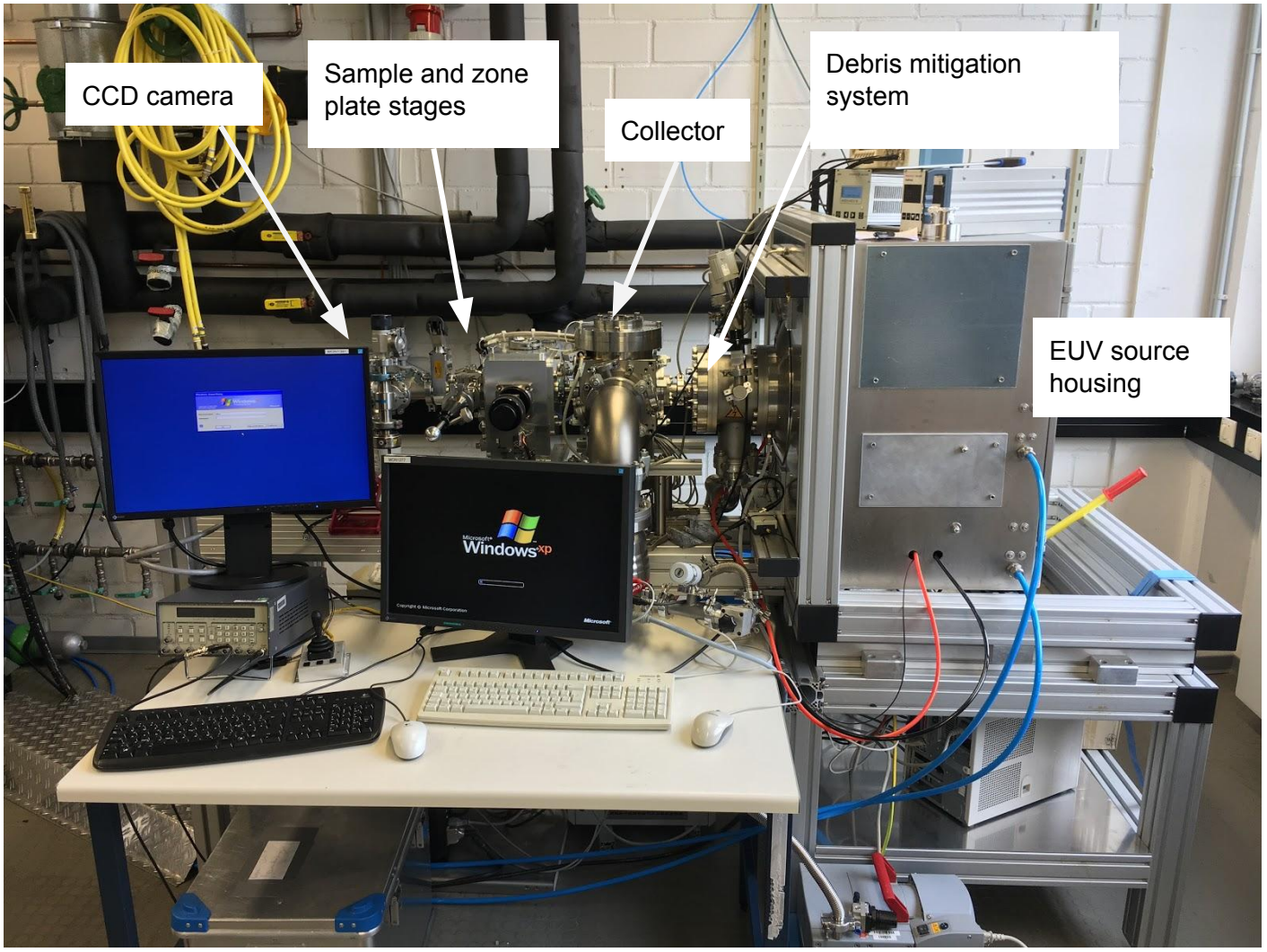


Figure 3: Experimental setup of LTXM

Experimental set-up

EUV source for Tomographic Microscopy Machine Specification:

Model, serial number	*(Prototype)*
Working gas	Nitrogen
Gas pressure during operation	5.0×10^{-2} mbar - 7.0×10^{-2} mbar
Voltage during operation:	2.0 kV - 3.5 kV
Discharge repetition frequency	2 Hz - 3000 Hz
Capacitor (C):	2.25 uF (9 x 0.25 uF)[1]
Wavelength (λ)	2.88 nm (or 430 eV)

Note: [1]There are 18 capacitors available for use with capacitance of 0.25 uF each.

Experimental set-up

Pulsed gas discharge source

- Gas discharge electrode system [Bergmann, K., et. al. 2008.]
  - Radiance on axis: up to 28 W/sr/cm^2 @ single line at 2.88 nm
  - Current output (at the source) 4.12 x 10^{12} photons/(sr pulse)
  - Diameter of emitting area: 1300 um FWHW

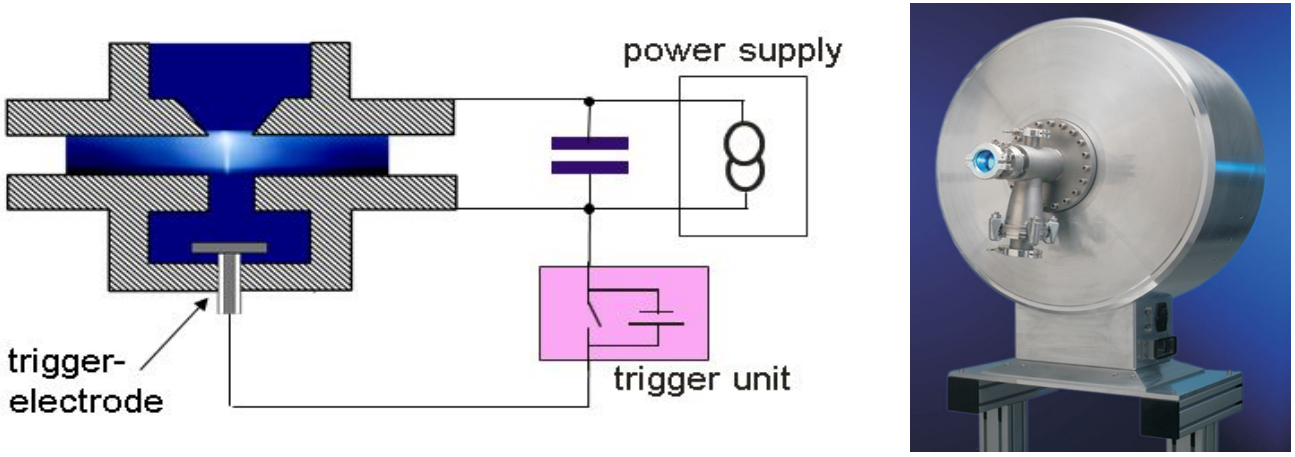
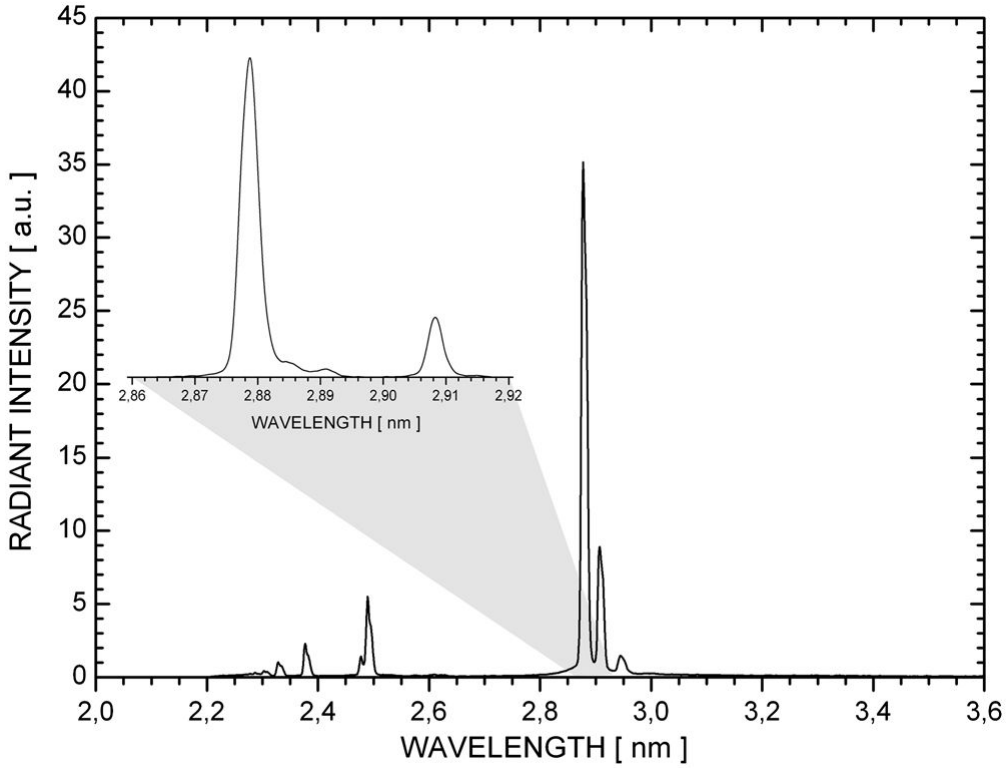


Figure 4: Schematic of the electrode system (left) and the lamp head (right). [*Benk, M. et.al. 2012.*]

Experimental set-up

Emission in the soft x-ray range



- Narrow band emission at 2.88 nm
- Emission spectrum using nitrogen

Figure 5: Spectrum at 2.88 nm emission [Benk, M. et.al. 2012.]

Problems encountered (Feb. 2017)

Problem: The sample and zone plate (ZP) stages do not work properly during operation, i.e. readback, motion (click) indicator, motion steps.

Solution: Proper shielding using Al-foil and grounding

Problems encountered (March 2017)

Problem: The ZP stage controllers do not respond. The circuit was disconnected.

Solution: Replacement and reconstruction of the connectors to fit the setup (L-configuration)

Problems encountered (April 2017)

Problem: Decreased photon counts (radiance intensity of about 10^11 ph/(sr pulse)) as comparable to the previous results of Benk, M. et al. (2008), i.e. 10^13 ph/(sr pulse) order of magnitude.

Solution: Realigning the setup, cleaning and replacement of the source electrodes

Problems encountered (May 2017)

Problem: There are discrepancies with calculations of output measurements and nominal values.

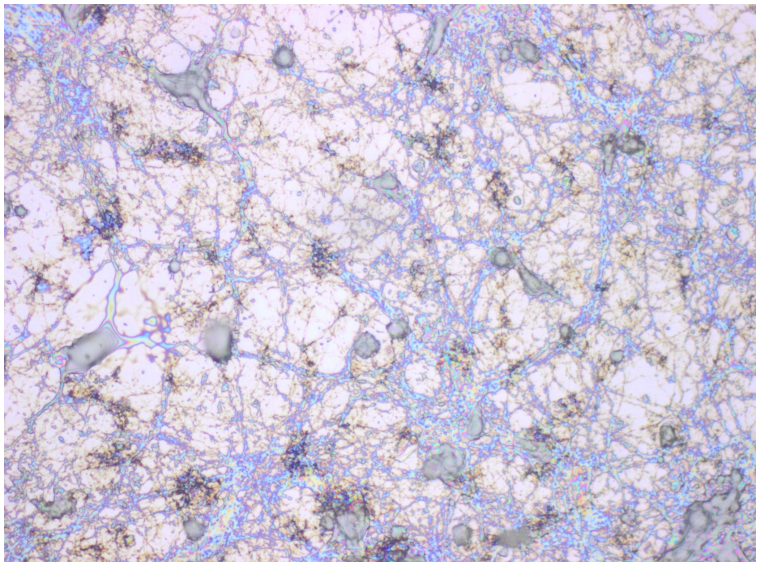
Solution: Verification, determination and re-calibration of transmission factors (collector, debris mitigation system (DeMi), filters). A new collector was installed.

Neuron cell imaging

Objectives:

To study the anatomy of a neuron cells using SXR imaging

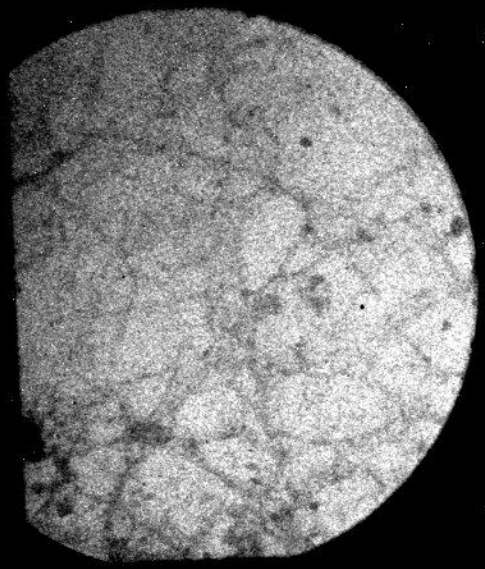
Sample:



- Cultured rat cell on a 50 nm thick Si3N4 membrane
- Dried
- Provided by M. Odstrcil, similar to the samples used by Baksh, P. et. al., 2017

Neuron cell imaging

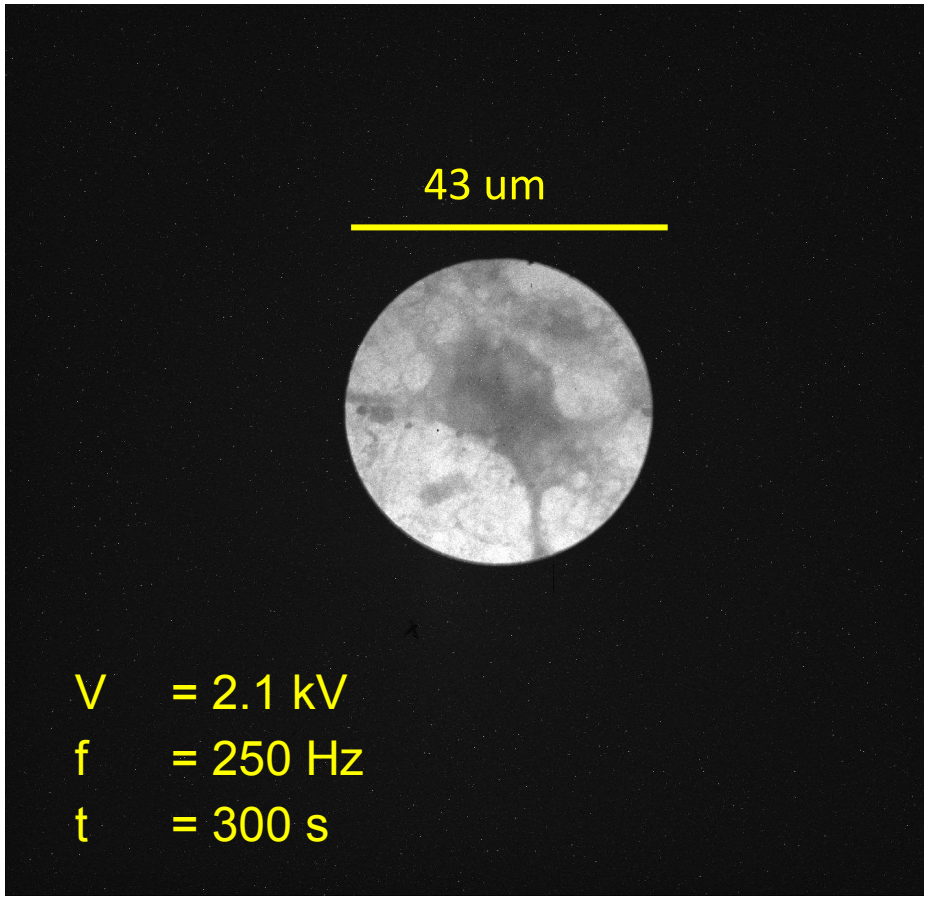
43 um



Magnification: ~ 204

V = 2.1 kV
f = 250 Hz
t = 10 s

Neuron cell imaging



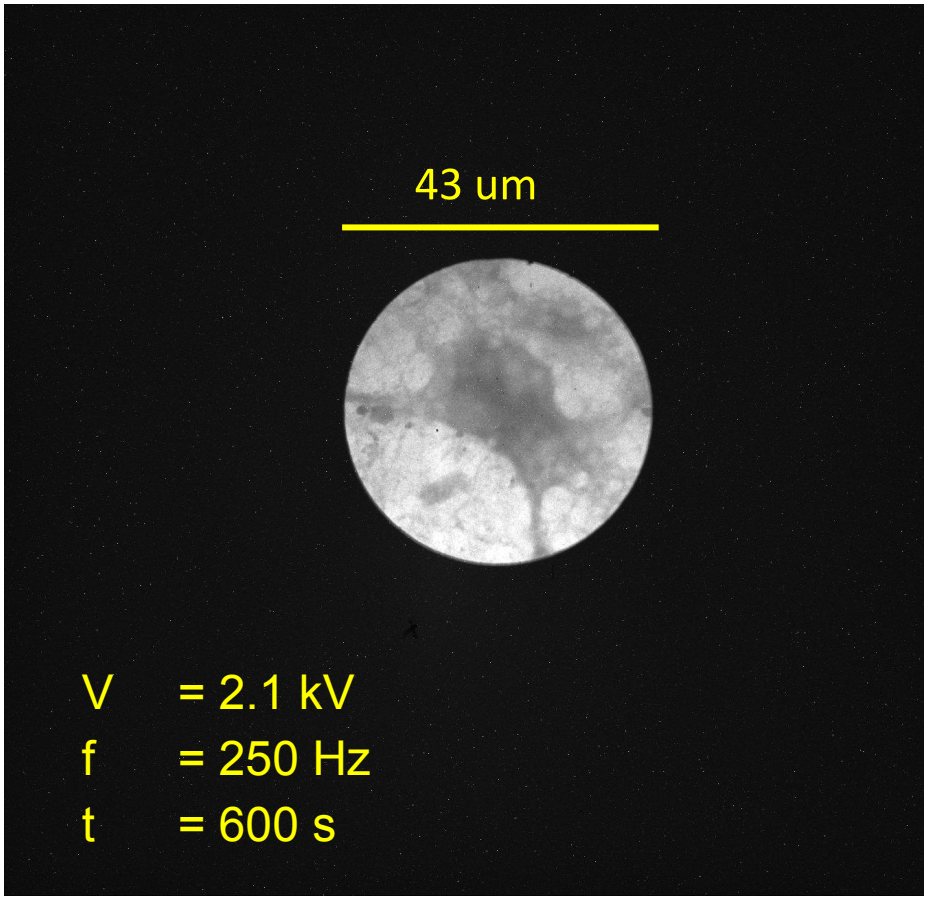
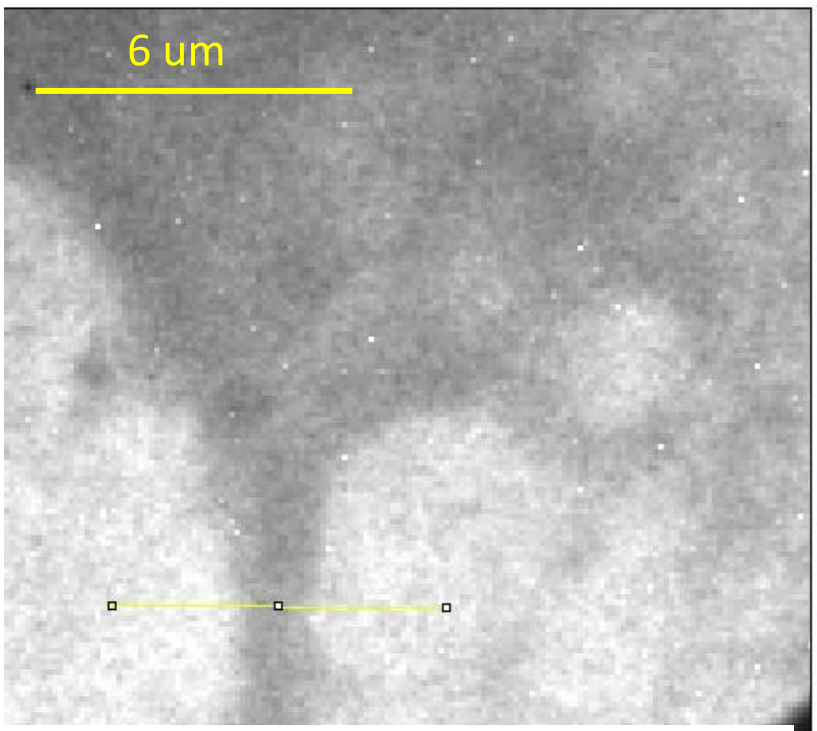
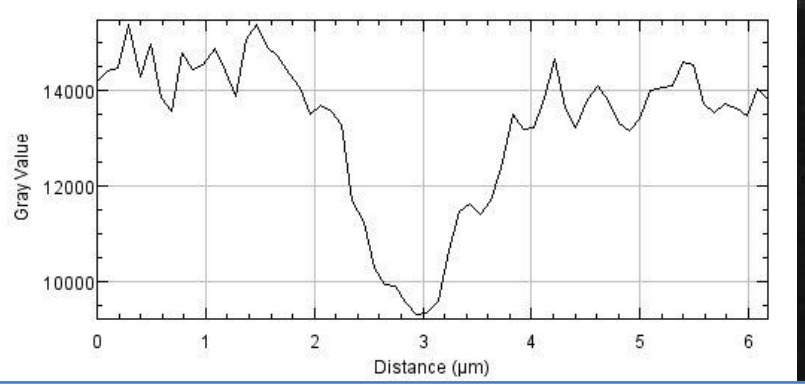
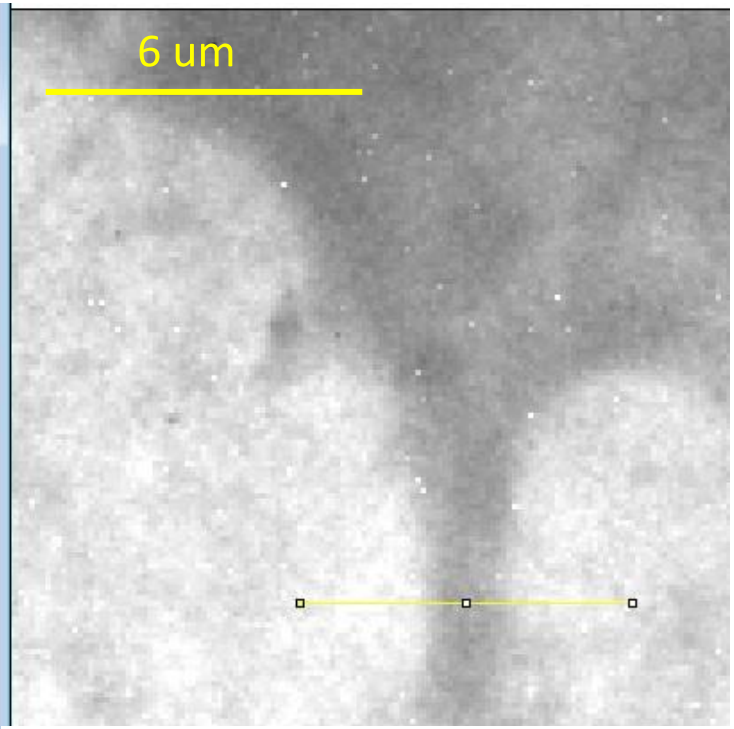


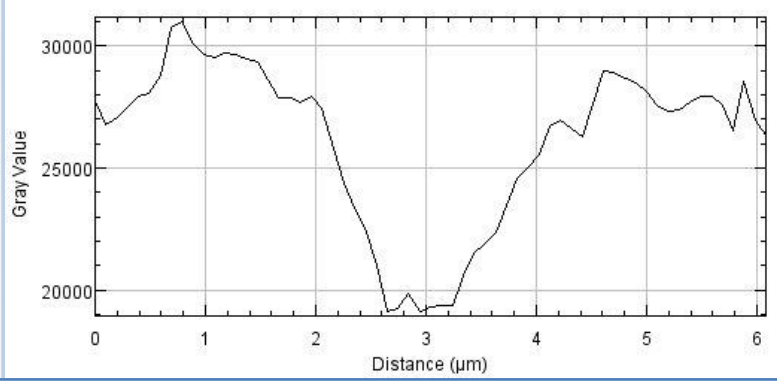
Image quality is almost the same for 5 min. and 10 min. exposure times.

Neuron cell imaging

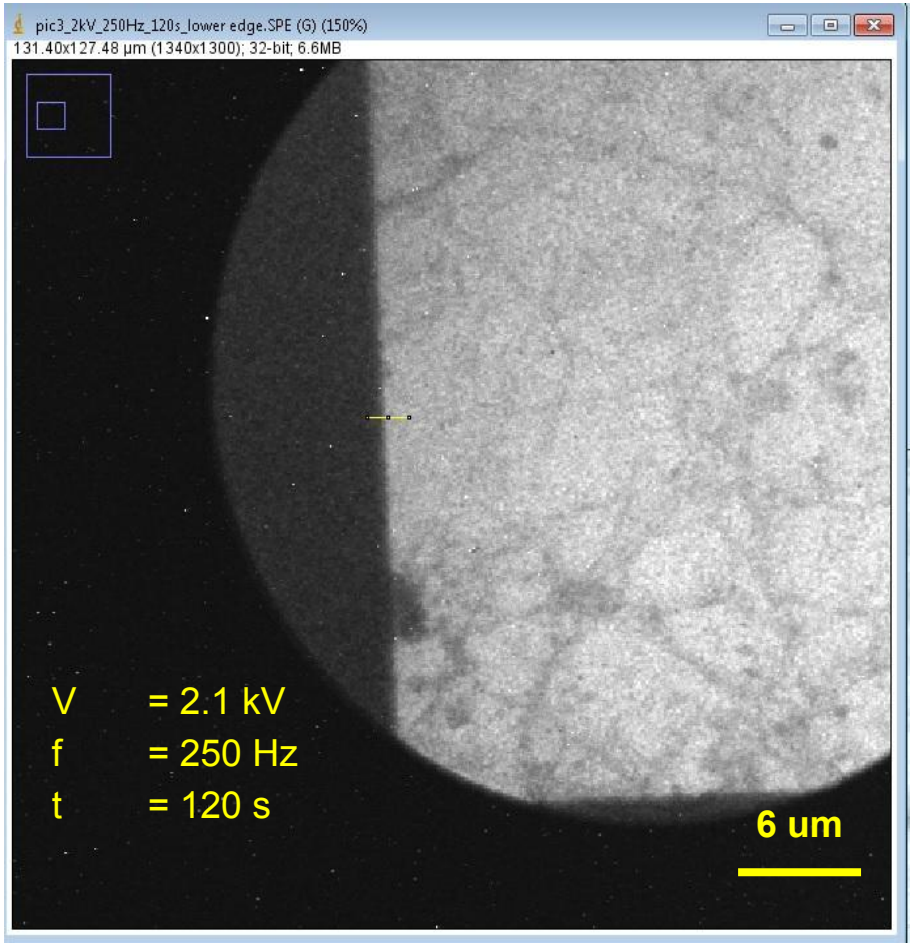






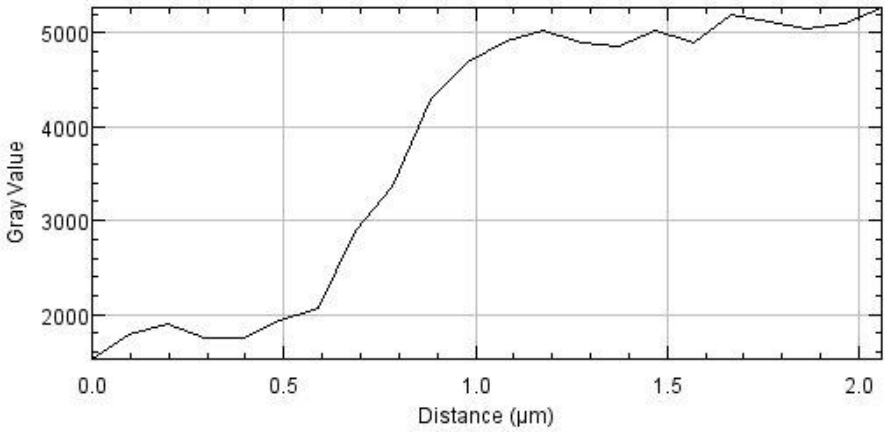


Neuron cell imaging

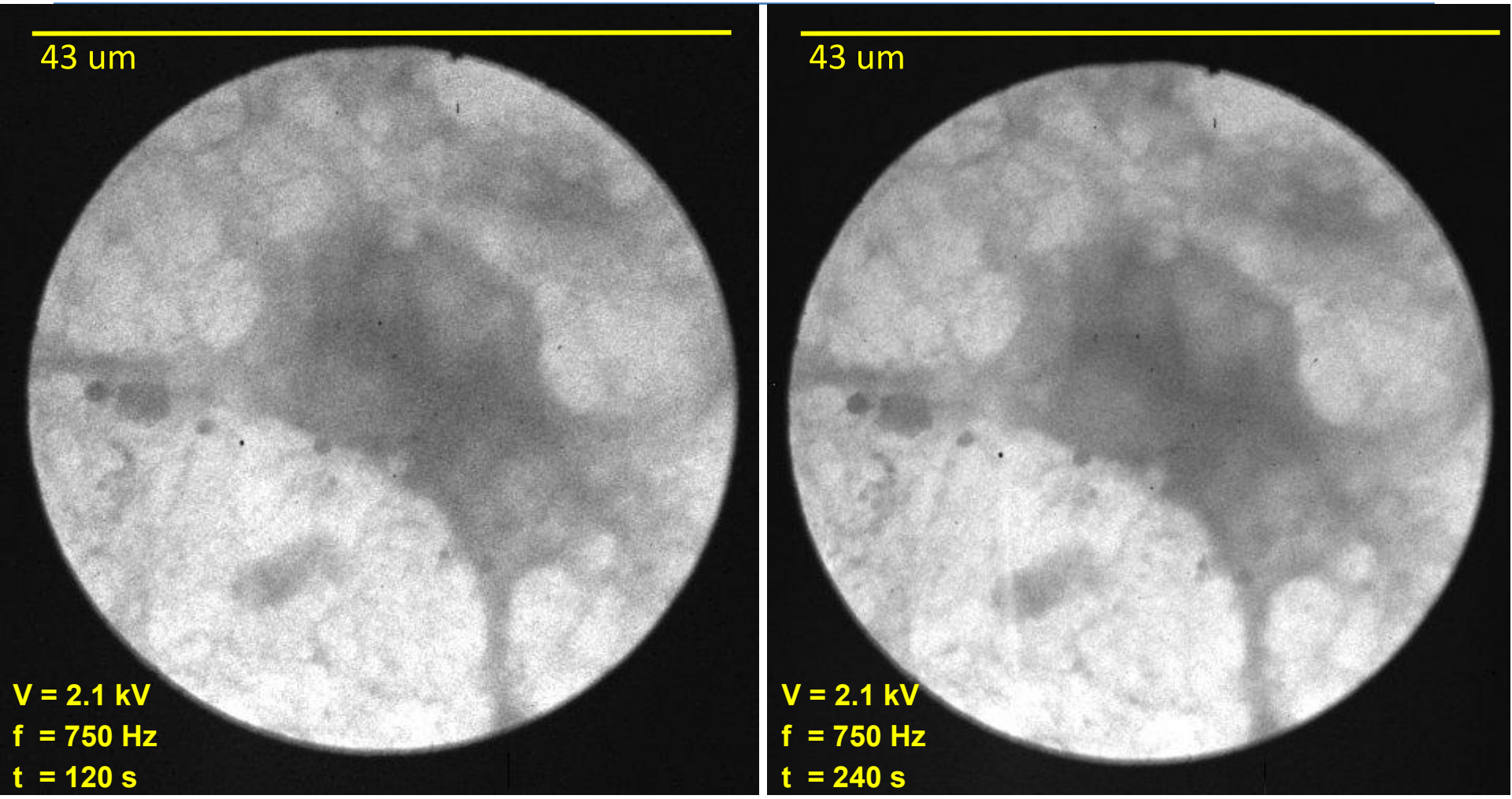


Magnification: ~ 204

Resolution: 310 nm



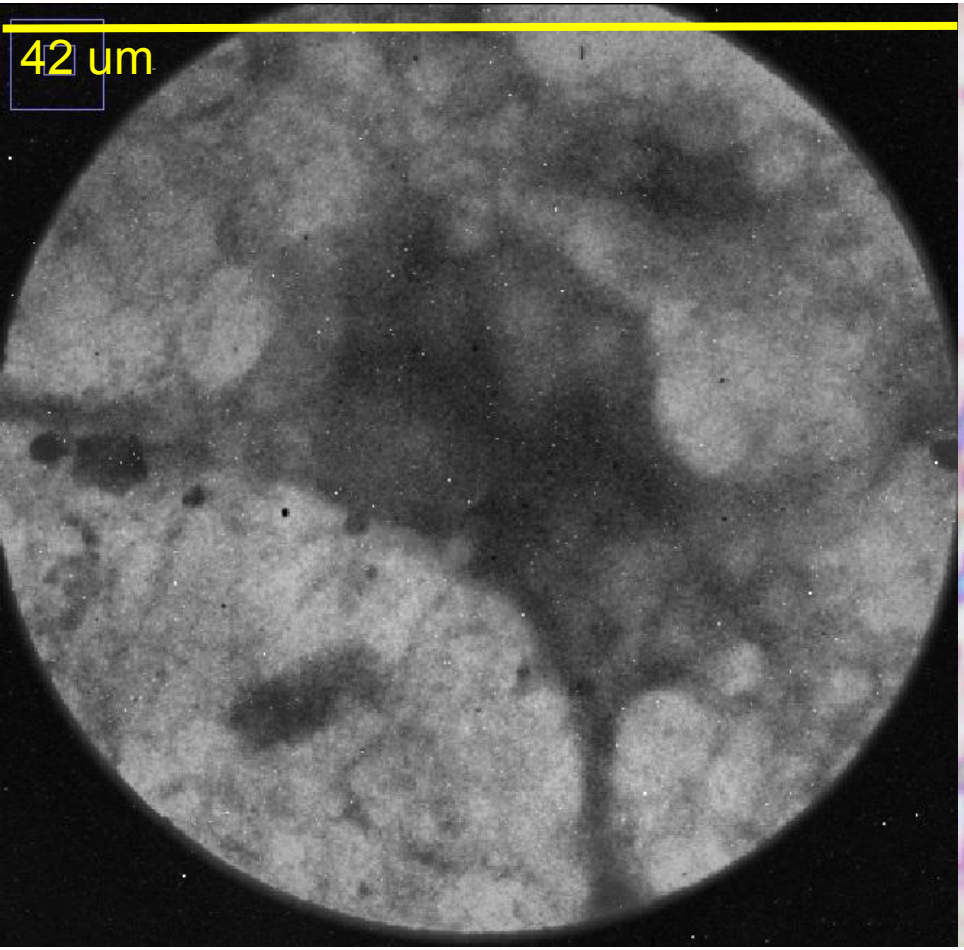
Neuron cell imaging

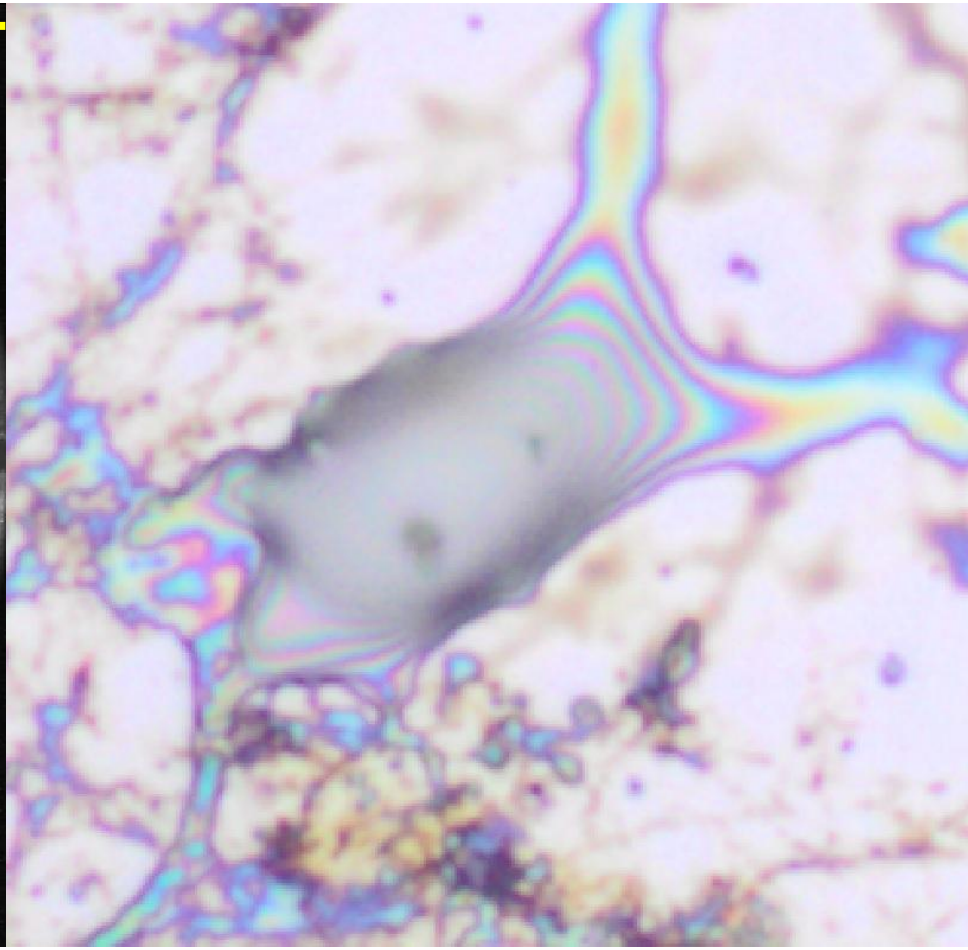


Magnification: ~ 204

Resolution: 310 nm

Neuron cell imaging



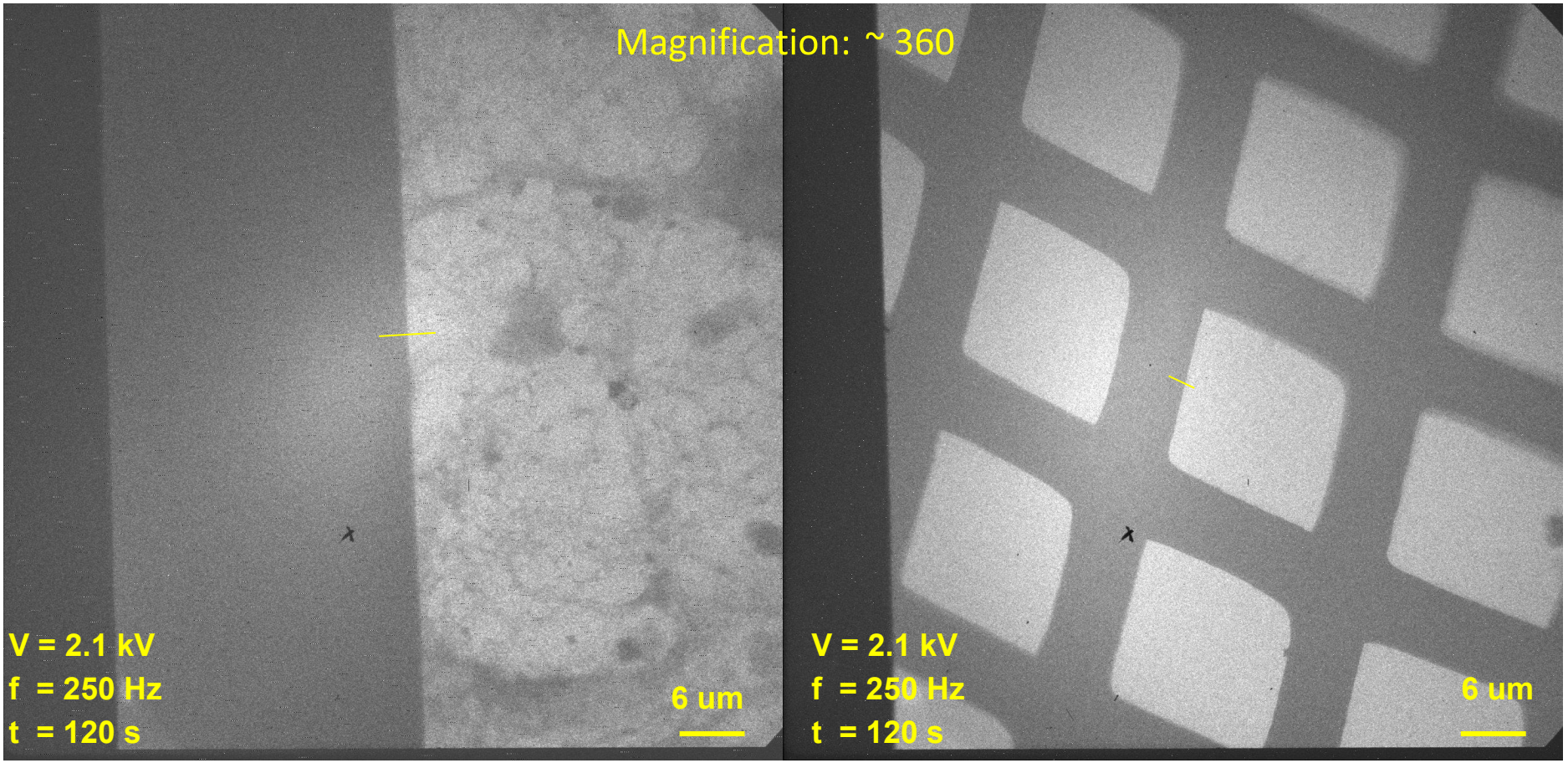


Neuron dimension:

approx. length = 25 um

approx. width = 13 um (at the middle part)

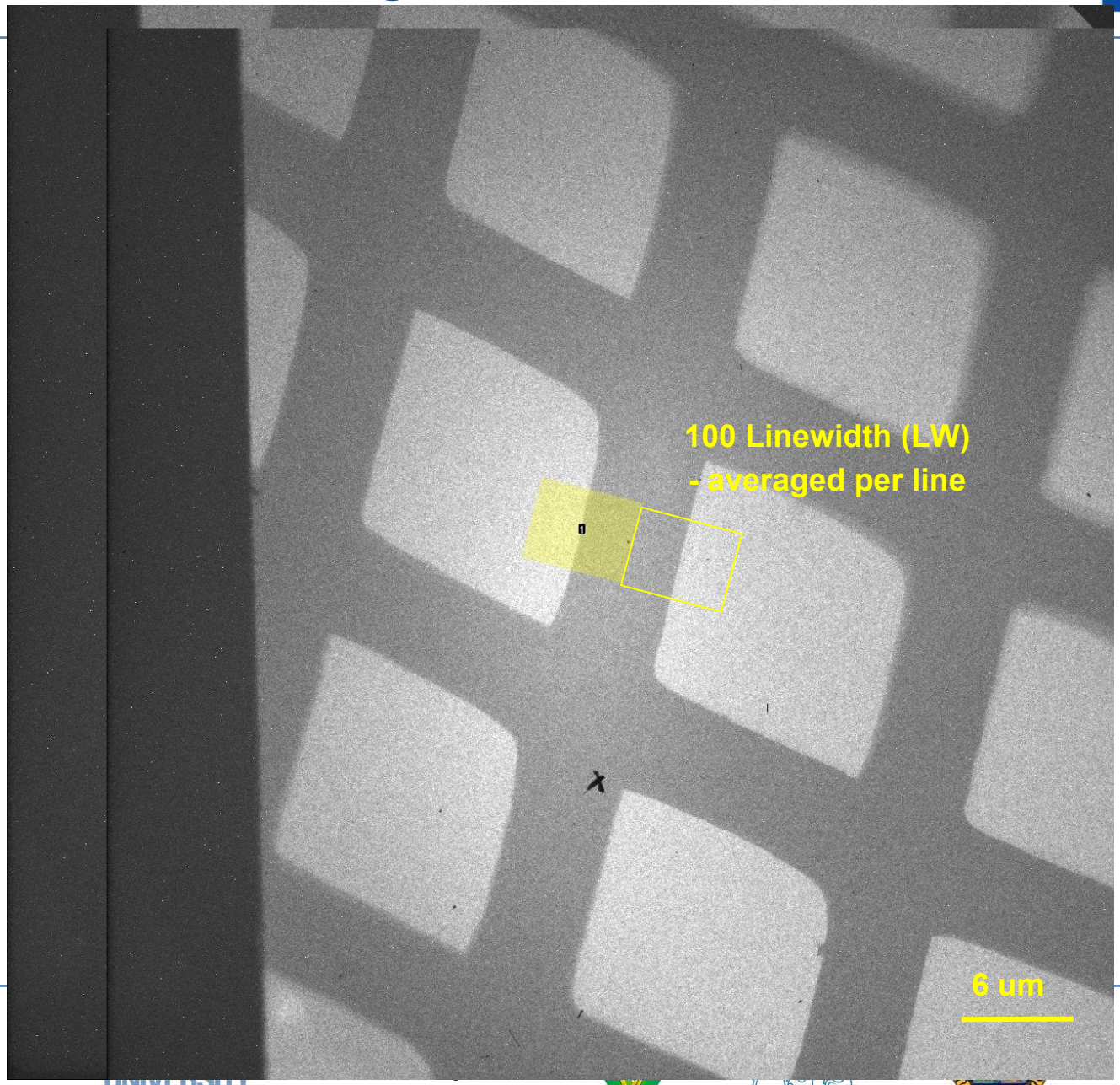
Current Magnification and Resolution



Magnification	=	360
Resolution	=	90 nm

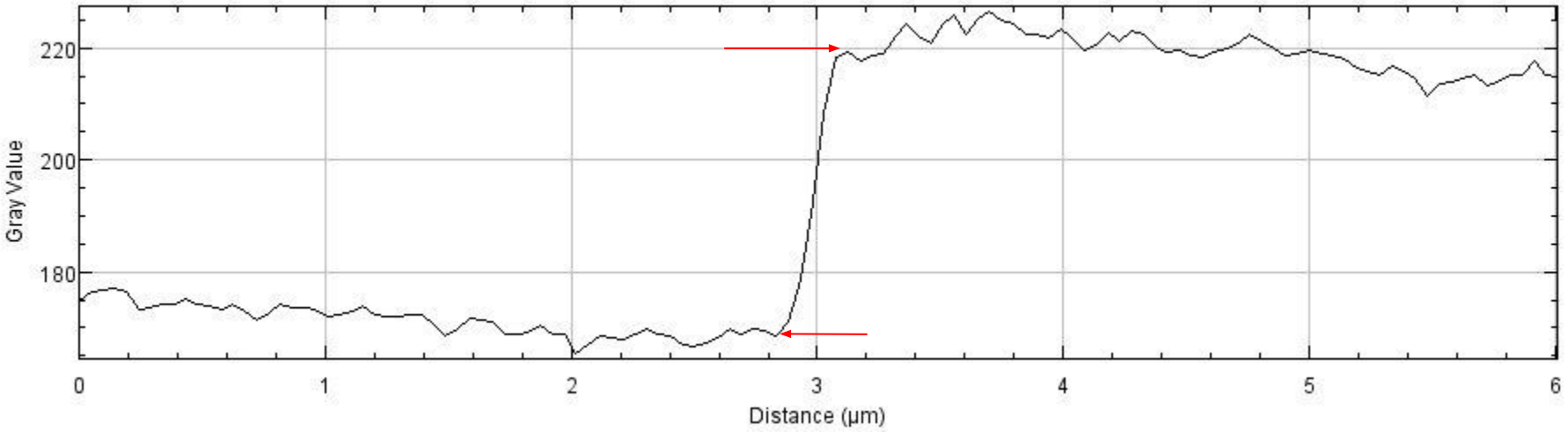
Magnification	=	360
Resolution	=	176 nm

Current Magnification and Resolution



Current Magnification and Resolution

Resolution calculation using Au mesh

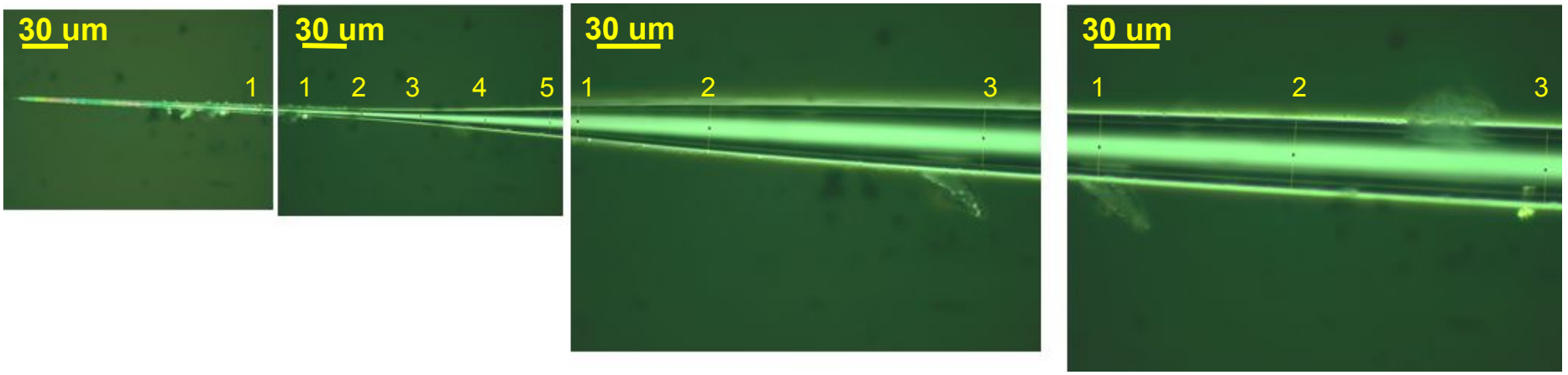


Ave of 100 lines

%	X (um)	Y (GV)
100 %	3.17	218
90 %	1.79	196
10 %	2.29	173
0 %	2.84	168

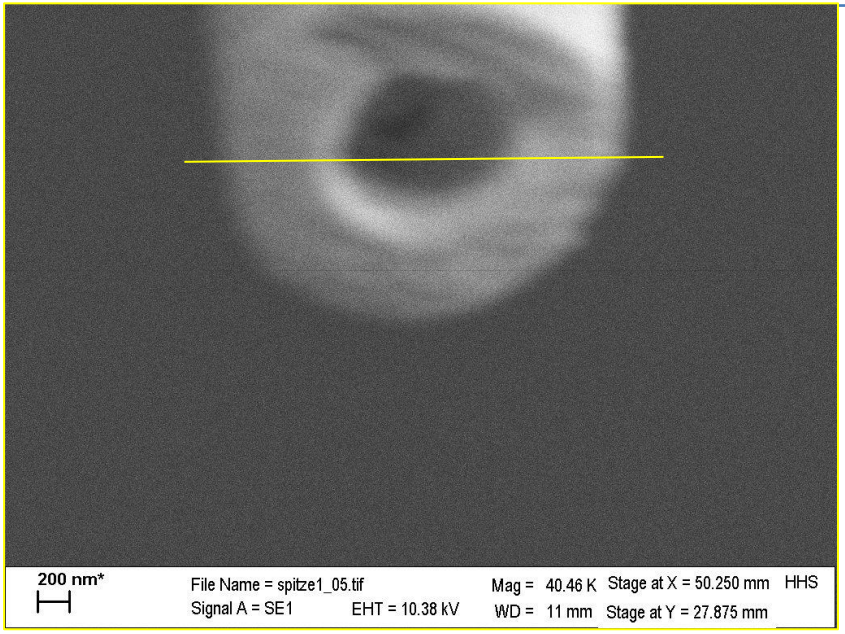
Magnification = 360
Resolution = 91.4 nm

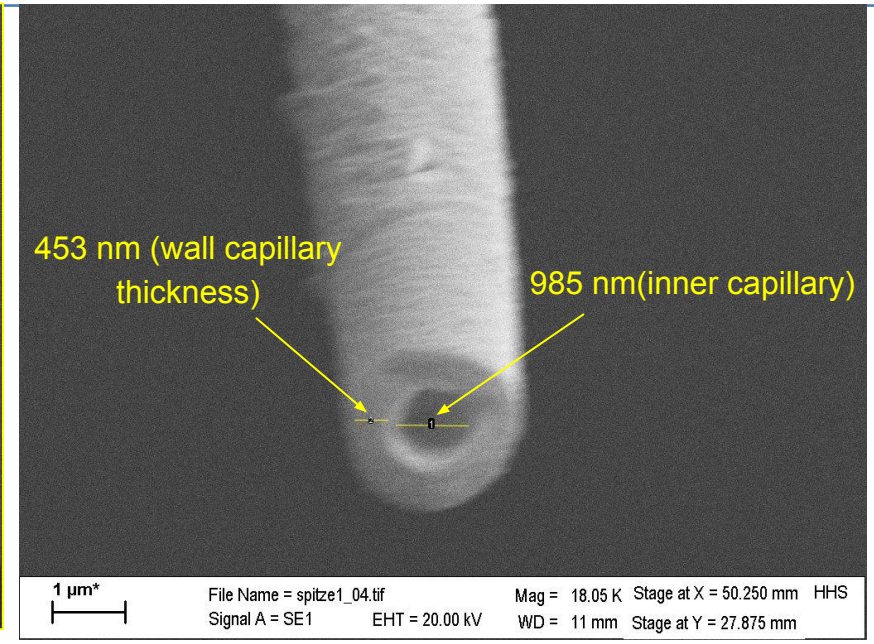
Capillary for biological sample



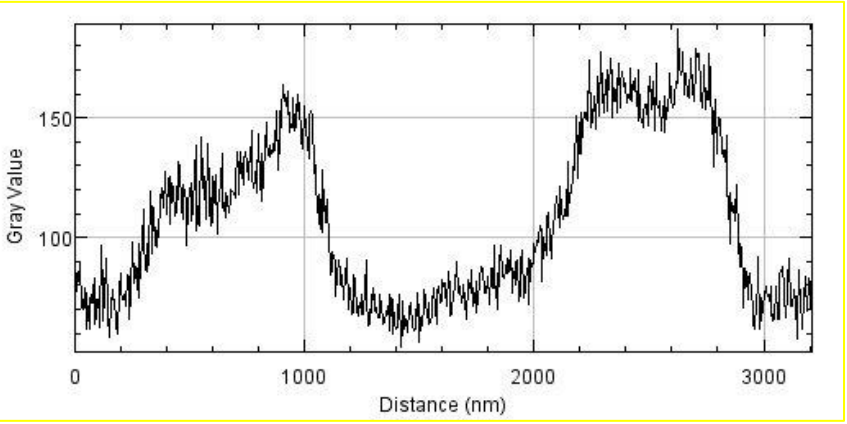
Quartz a		Quartz b		Quartz c		Quartz d	
1	6.81 um	1	4.05 um	1	15.13 um	1	31.33 um
		2	6.4 um	2	21.75 um	2	30.11 um
		3	9.6 um	3	26.85 um	3	35.16 um
		4	14.6 um				
		5	19.6 um				

SEM images of the capillaries





Profile plot of the quartz capillary SEM image



Transmission of Quartz (using CXRO [8])

500 nm - 42 %
400 nm - 50 %
200 nm - 71 %

Transmission of Water

2 um - 70 %
10 um - 17 %

SEM images of the capillaries

- The quartz capillary has appropriate dimensions which can contain biological samples from 2 um to 10 um.
- Using CXRO database, transmission of 3 % to 27 % is possible for our setup.

Future work

- Increase the magnification to about 750 - 1000 x
- Use the micro-/nano-structured samples (CSAR zone plate and Au transmission mask) from Valerie Deuter) to determine the resolution
- Compute the radiation dose to the sample with our current setup
- Create high-dynamic-range images from multiple exposures
- Obtain tomographic projections of a biological sample

Conclusion

- The resolution of 90 nm (from knife-edge test) is currently limited by pixel size and magnification but already better than the resolution of a visible light microscope.
- The neuron image provides more details and some information of the inner anatomy of the cell.
- Capillaries for biological samples are appropriate for 3D imaging.

References

[1] Benk, M., Bergmann, K., Querejeta-Fernandez, A., Srivastava, S., Kotov, N.A., Schaefer, D., and Wilhein, T. (2011). Soft X-ray Microscopic Investigation on Self Assembling Nanocrystals. AIP Conf. Proc. 1365, 433 – 436.

[2] Attwood, David. (1999). Soft X-rays and Extreme Ultraviolet Radiation Principles and Application. Cambridge University Press. Ch. 9, pp. 369 – 379.

[3] Schafer, D., Benk, M., Bergmann, K. Nisius, T., Wiesemann, U. and Wilhein, T. (2009). Optical setup for tabletop soft X-ray microscopy using electrical discharge sources. J. of Phys. Conference Series **186** 012033.

[4] Bergmann, K., Kupper, F., and Benk M. (2008). Soft x-ray emission from a pulsed gas discharge in a psedosparklike electrode geometry. J. Appl. Phys. 103 123304-1 – 103 123304f-8.

[5] Benk, M., Bergmann, K., Schafer, D. and Wilhein T. (2008). Compact soft x-ray microscope using a gas-discharge light source. Opt. Lett. **33** (20), pp. 2359 – 2361.

[6] Kinney, J.H., and Nichols, Monte. (1992). X-ray Tomographic Microscopy (XTM) using Synchrotron Radiation. Annu. Rev. Mater. Sci. 22 pp. 121 – 152.

[7] Baksh, P., Odstrcil, M., et. al. (2017). Quantitative Evaluation of Hard X-ray Damage to Biological Samples using EUV Ptychography. IOP Conf. Series: Journal of Physics: Conf. Series 849 (2017) 012034, pp. 1-4

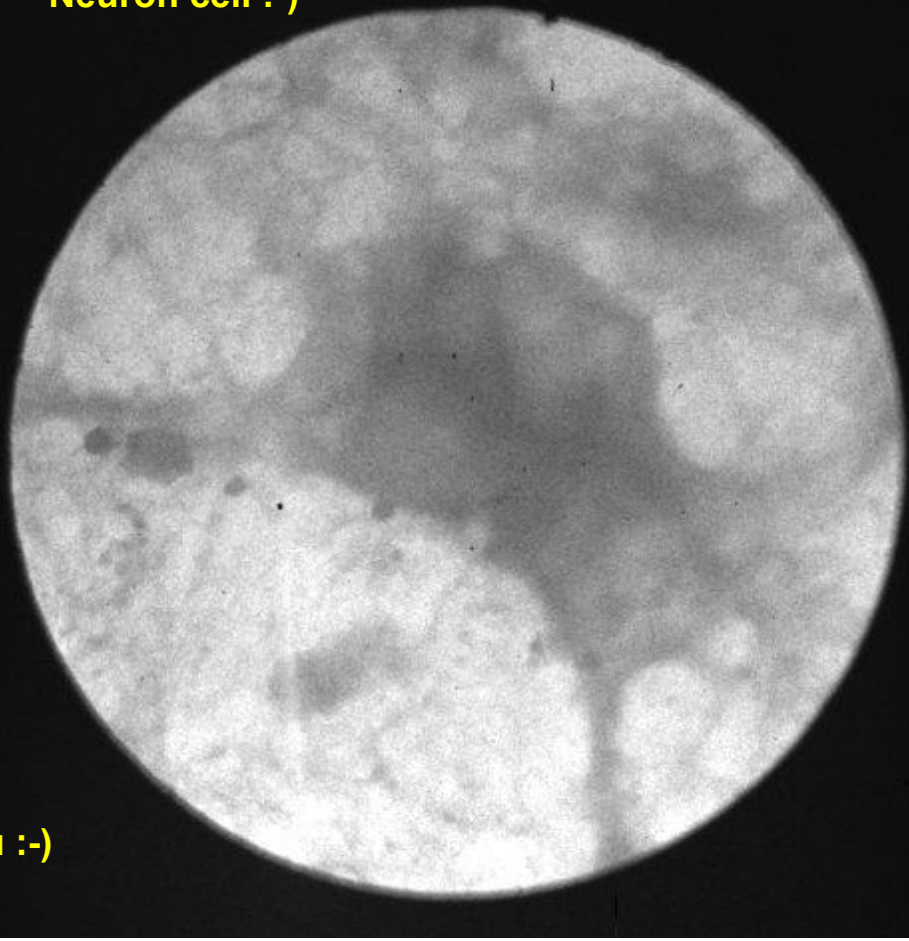
[8] CXRO Database: http://henke.lbl.gov/optical_constants/

Acknowledgement

- Prof. Dr. Larissa Juschkin
- ILT TOS/ RWTH EUV group
- Education, Audiovisual and Culture Executive Agency (EACEA) Erasmus Mundus Joint Doctorate Programme Project No. 2012 – 0033.

New Moon

Neuron cell :-)



Thank you :-)

New moon figure taken from :
https://upload.wikimedia.org/wikipedia/commons/e/e1/FullMoon2010.jpg