

Lab-based Correlative Fluorescence and Soft X-ray Microscopy

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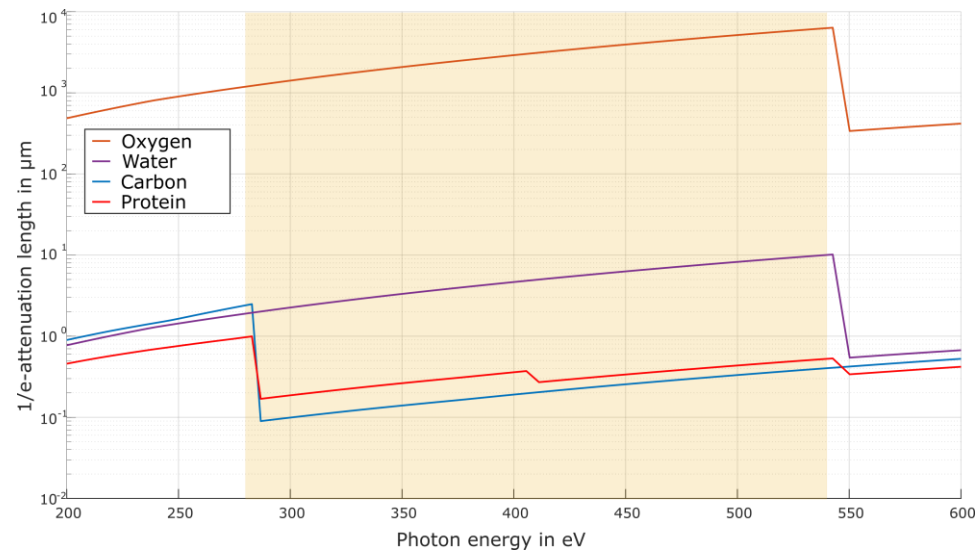


Ministerium
für Bildung,
Wissenschaft und Kultur

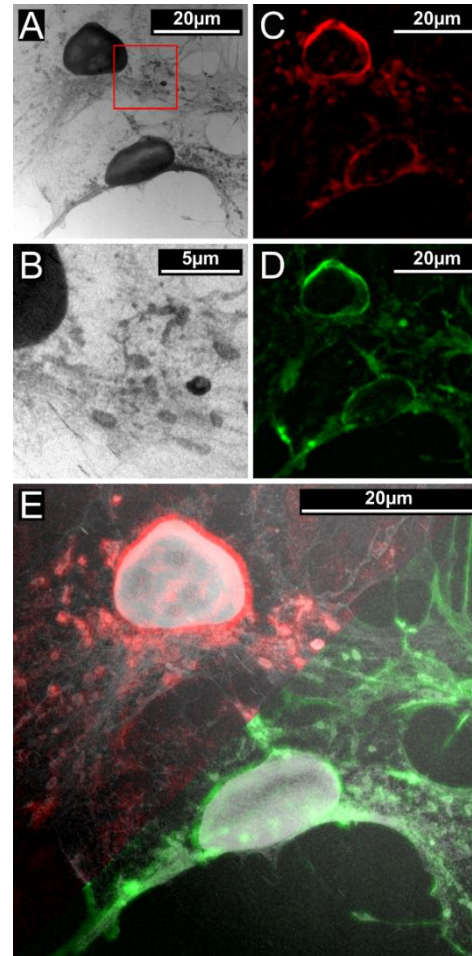
Correlative Microscopy

X-ray microscopy

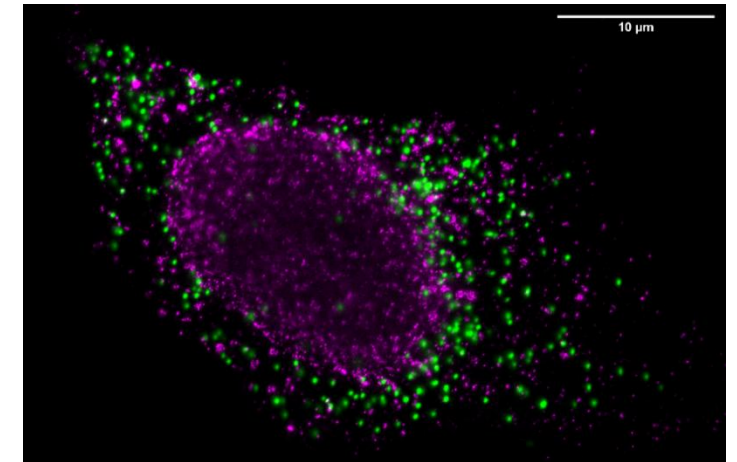
$$d = \frac{\lambda}{2 \sin \alpha}$$



- high resolution
- natural **structural** contrast
- up to 10 μm penetration



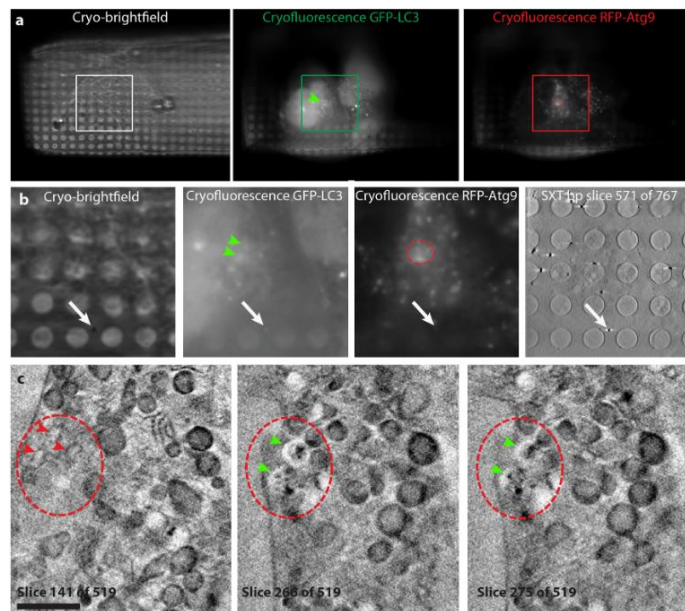
Fluorescence microscopy



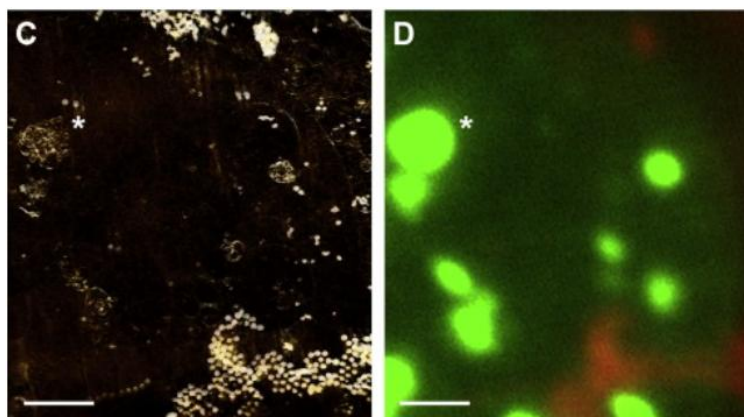
- established Method
- **functional** contrast

➡ novel insights in correlation!

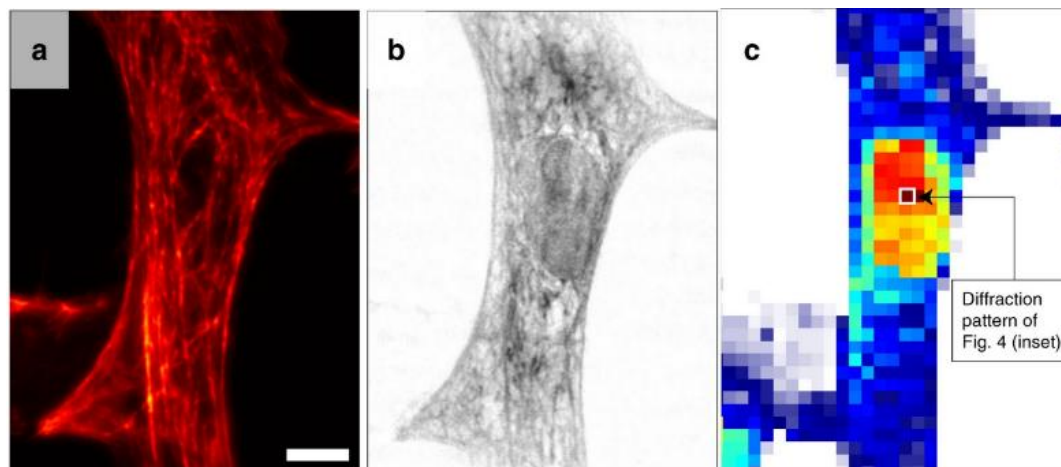
Correlative Microscopy



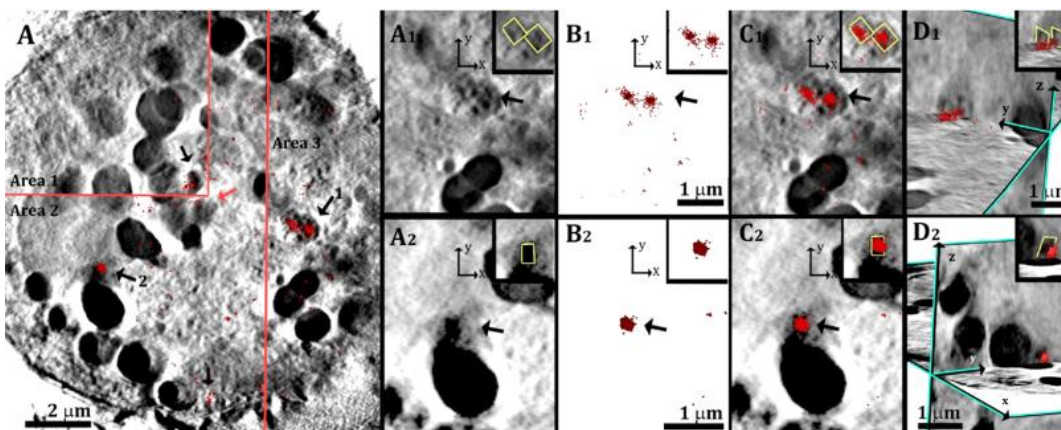
Duke et al.,
Ultramicroscopy 143 (2014)



Hagen et al.,
Jour. of struct. biol.
177.2 (2012)



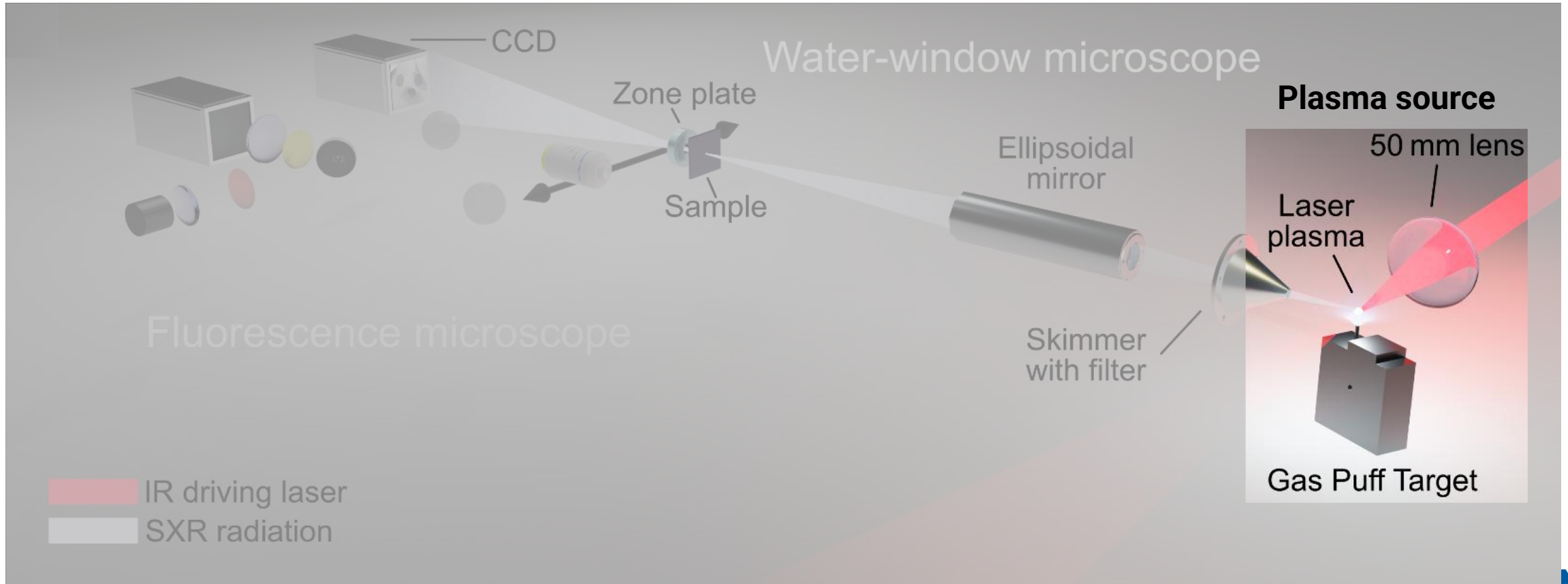
Bernhardt et al.,
Nat. Comm. 9.1 (2018)



Varsano et al.,
JACS 138.45 (2016)

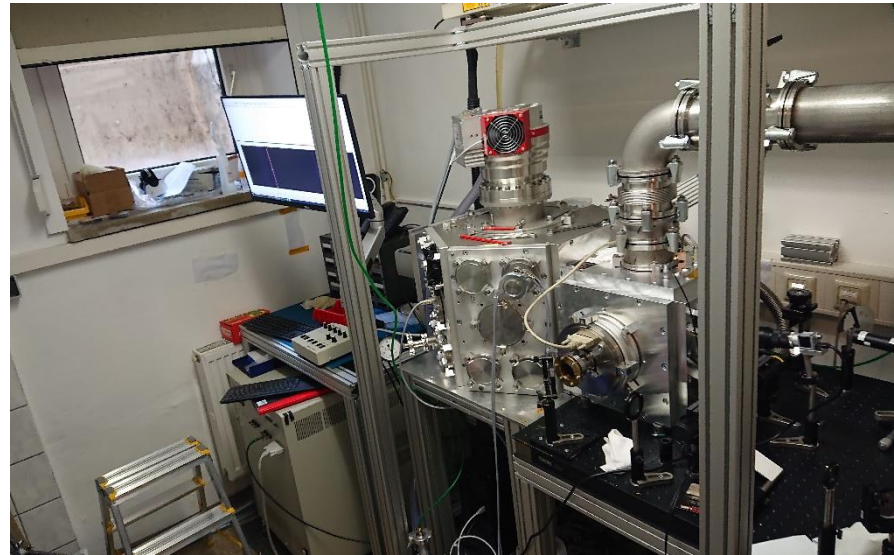
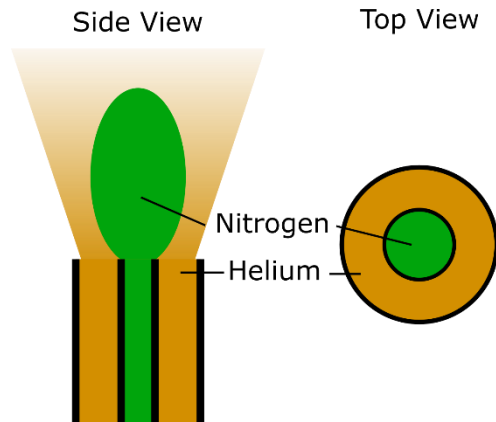
➡ not done in a lab-based setup
(so far)!

Full setup



Plasma Source – Gas Puff Target

- gas driven plasma source
- strong, single line emission from Nitrogen in WW
- gas puff target for high density
- moderate laser requirements



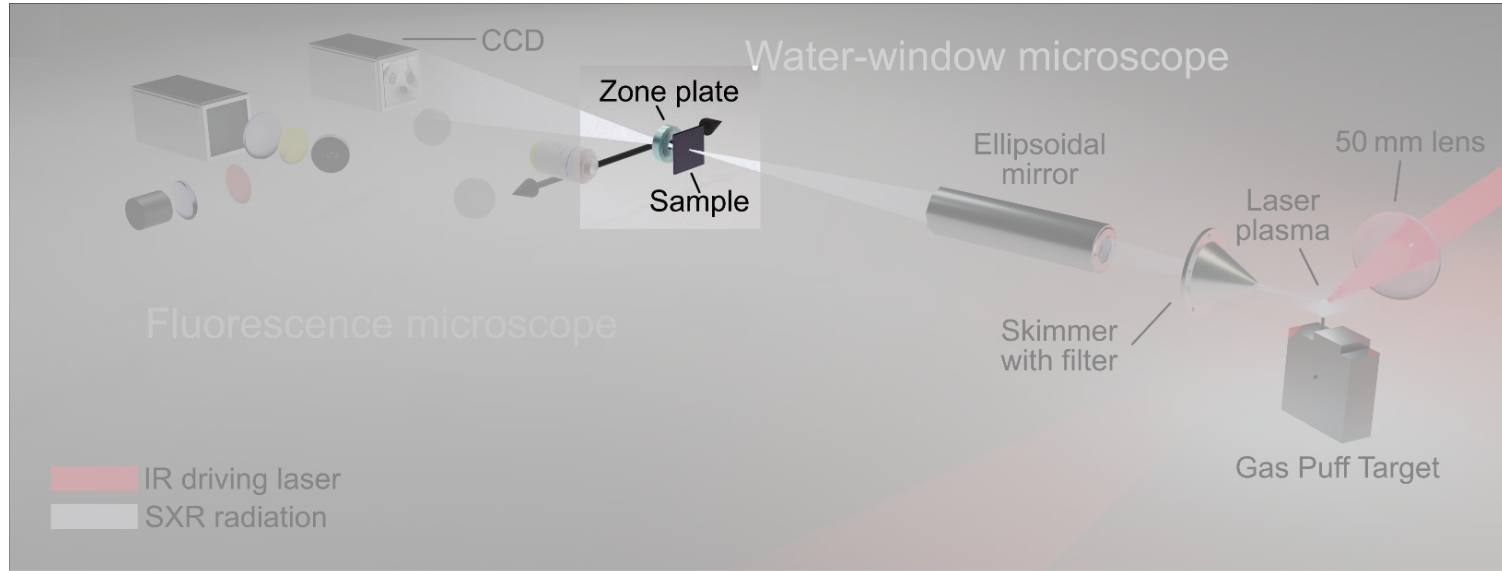
laser specifications

λ	E_p	τ_p	rep
1064nm	2.5J	10ns	10Hz

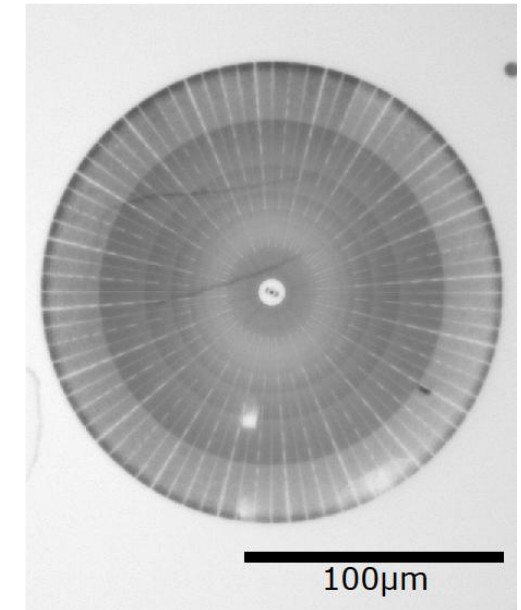


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Sample Holder + Zone Plate



Fresnel zone plate



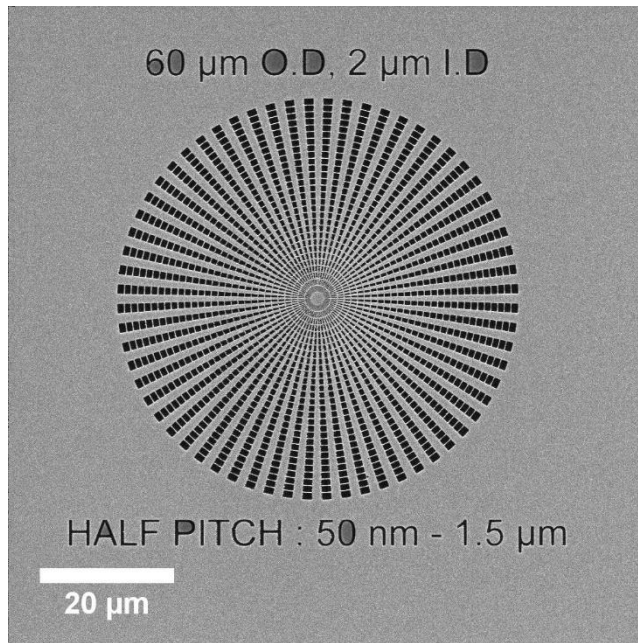
Silicon nitride membranes
→ Typical thickness: 50nm



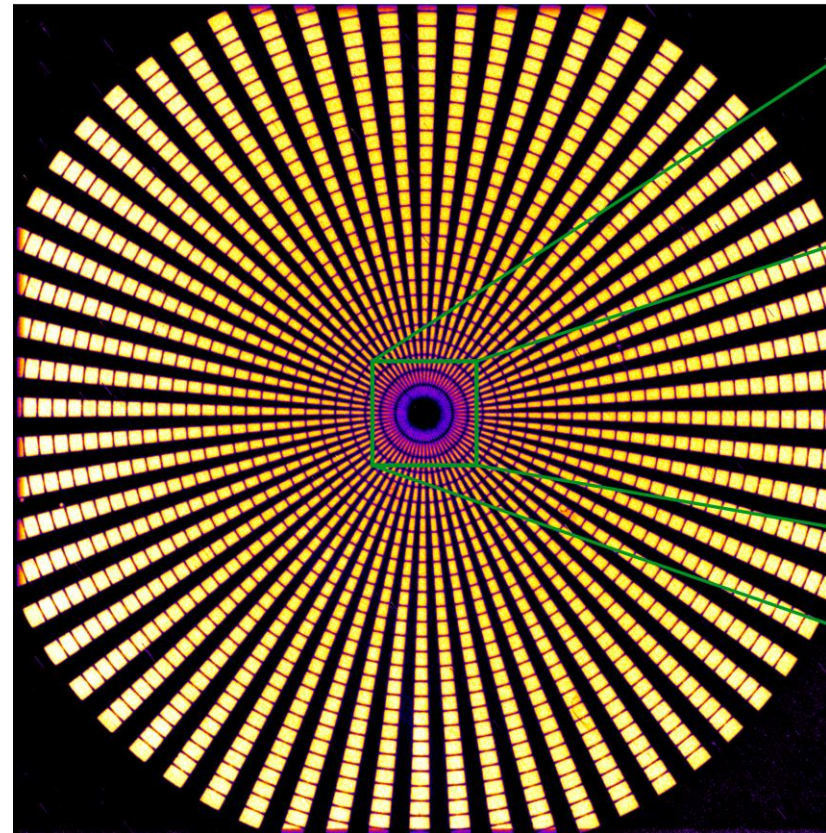
$D=180\mu\text{m}$
 $\Delta r_N=33\text{nm}$
 $f=2.06\text{mm @}430\text{eV}$
→ 1360 Zones

X-ray Microscopy – Siemens Star

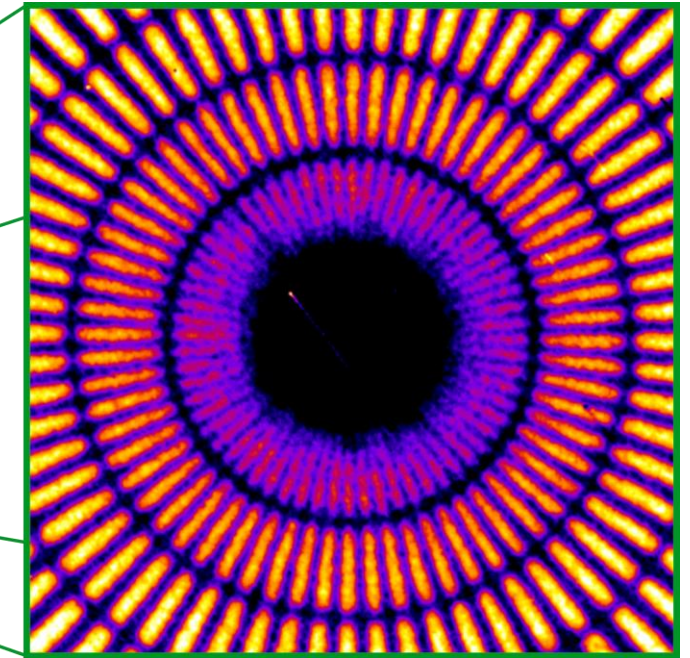
175nm W on
100nm Si_3N_4
60 μm diameter
50nm half pitch features



SEM image



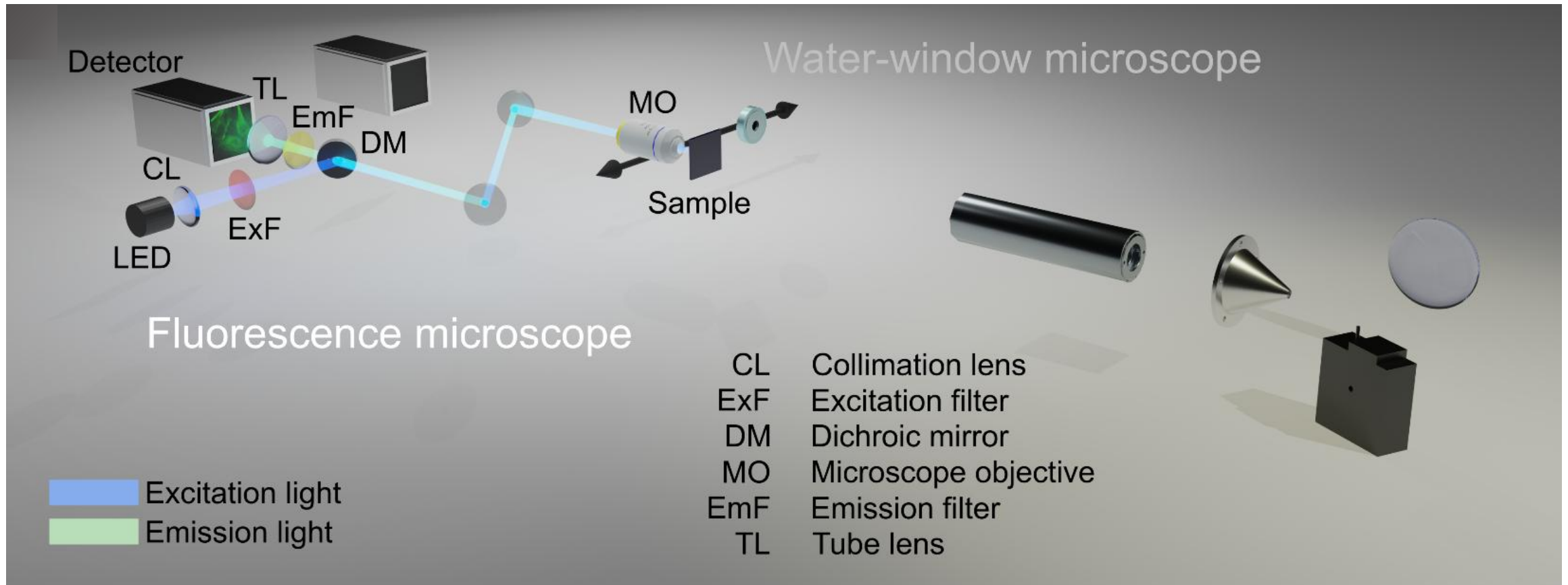
53x80s exposure time



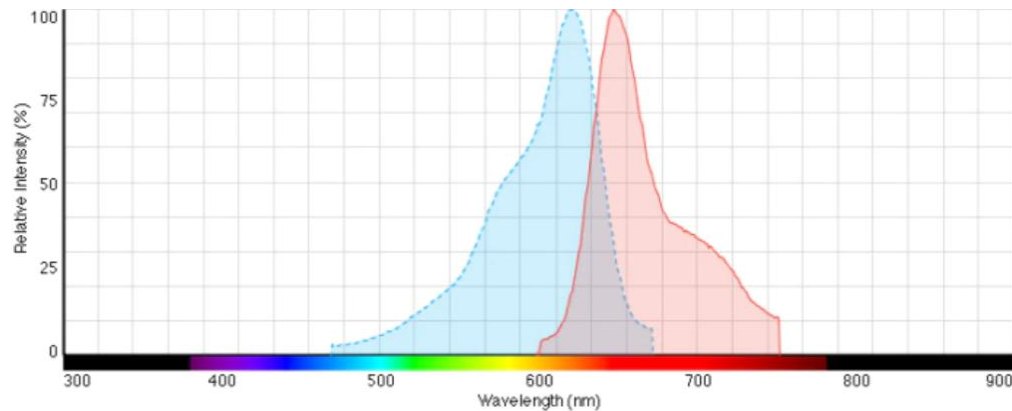
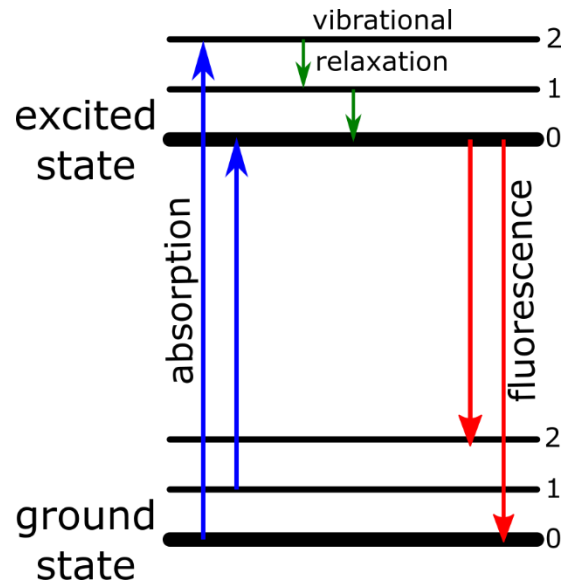
➡ $\approx 50\text{nm}$ half-pitch res.

➡ FOV 50 μm

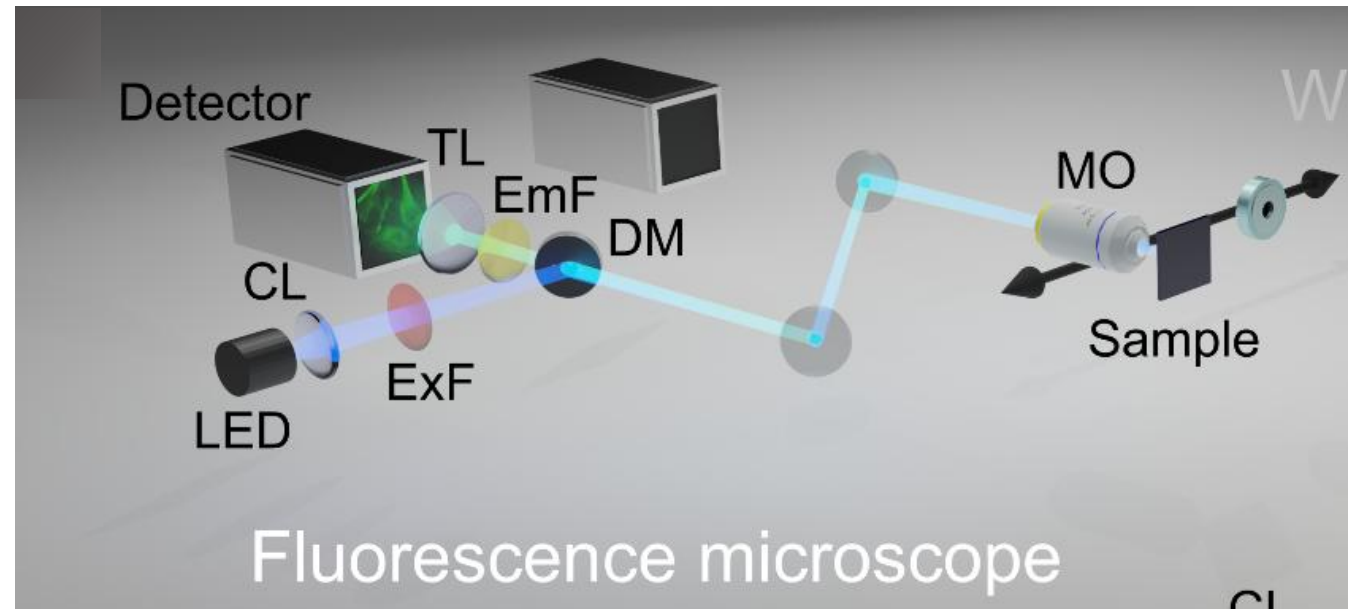
Fluorescence Microscopy



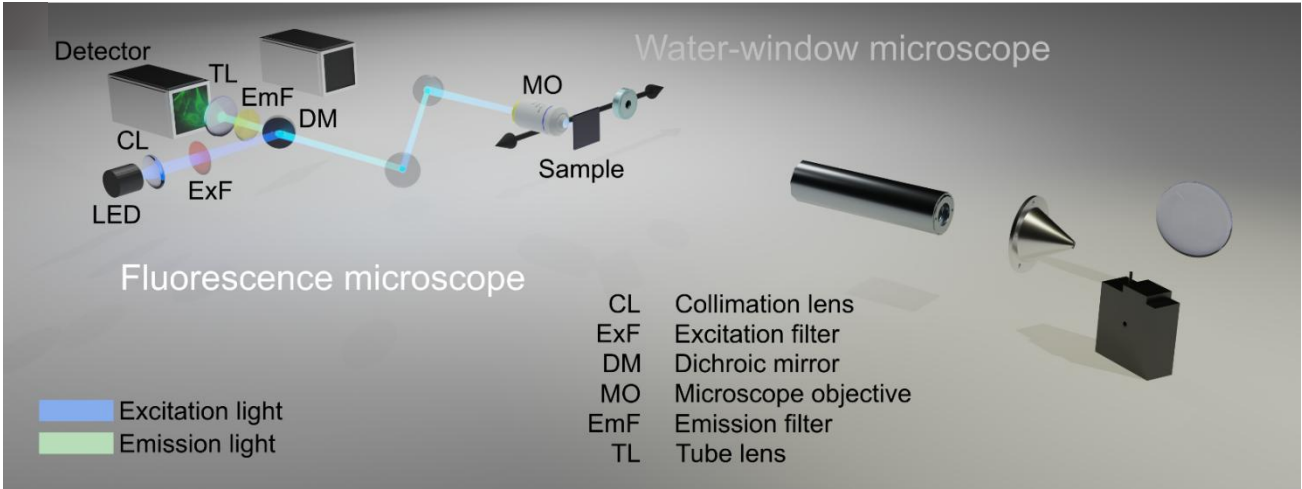
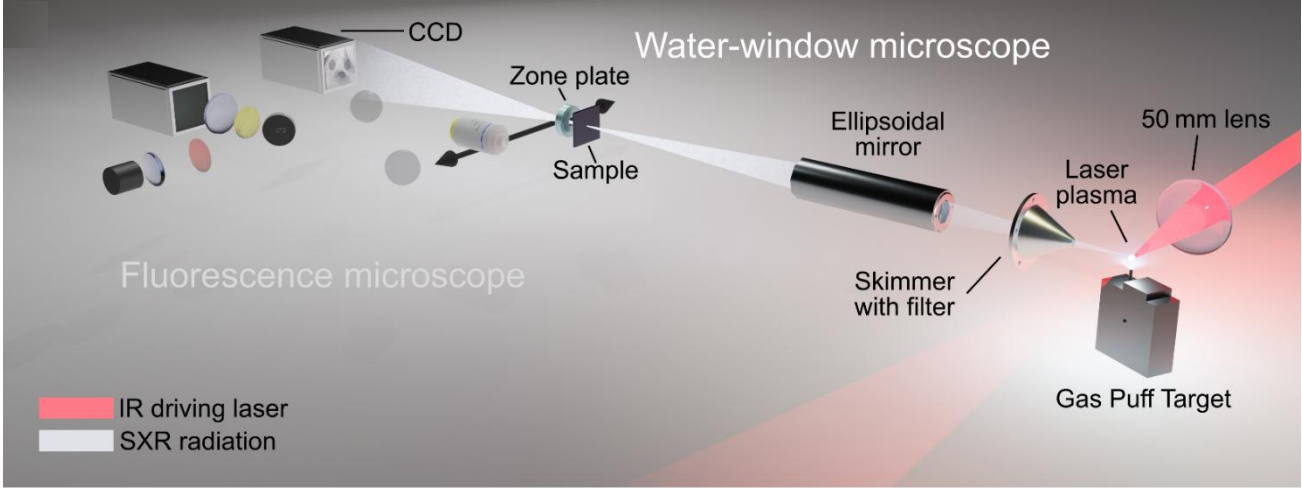
Fluorescence Microscopy



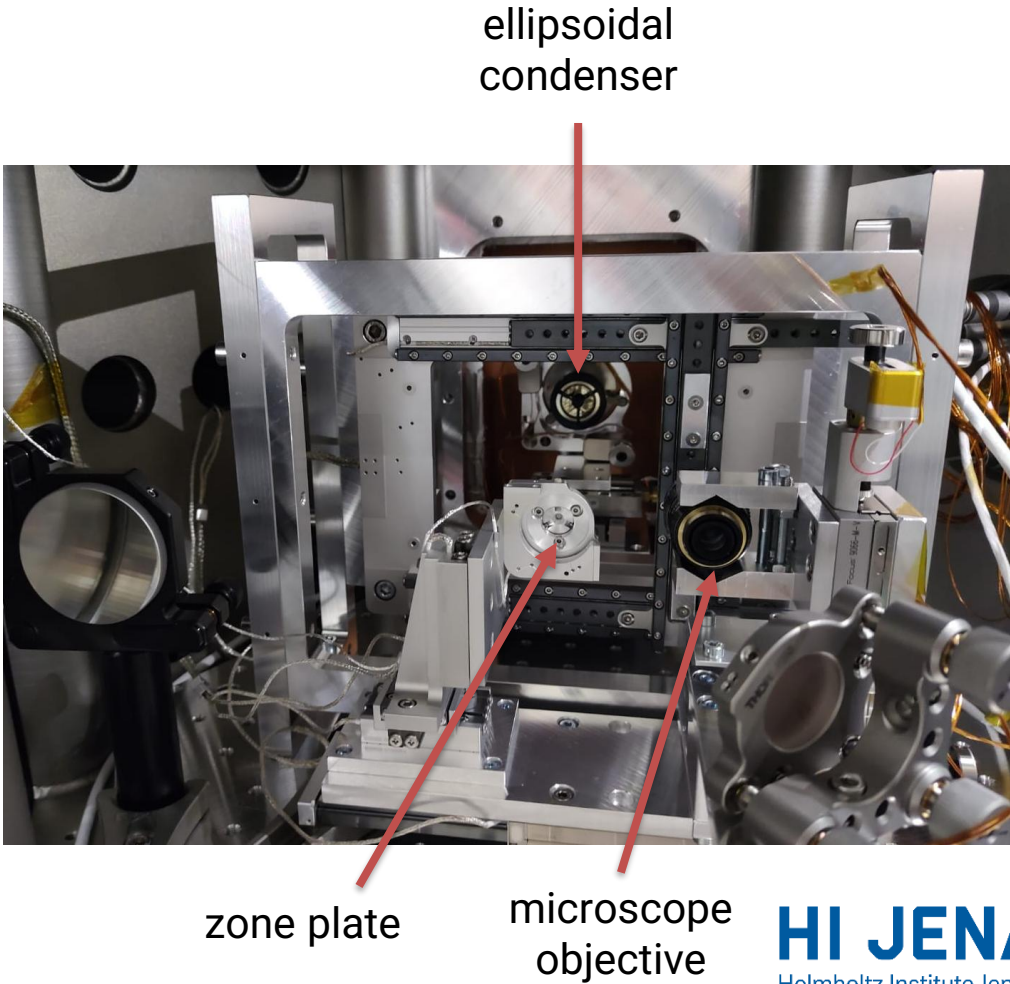
[<https://www.thermofisher.com/content/dam/LifeTech/Documents/spectra/images/8806H2O.jpg>]



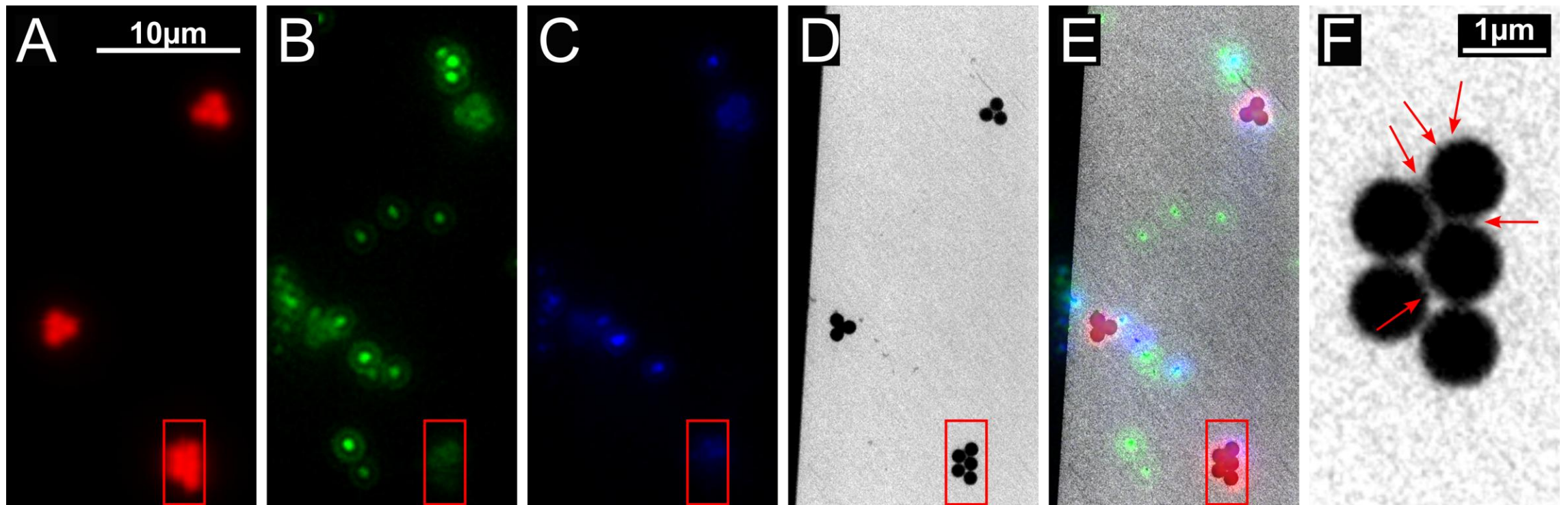
Correlative Setup



 In situ correlation possible!



Fluorescent Nanobeads



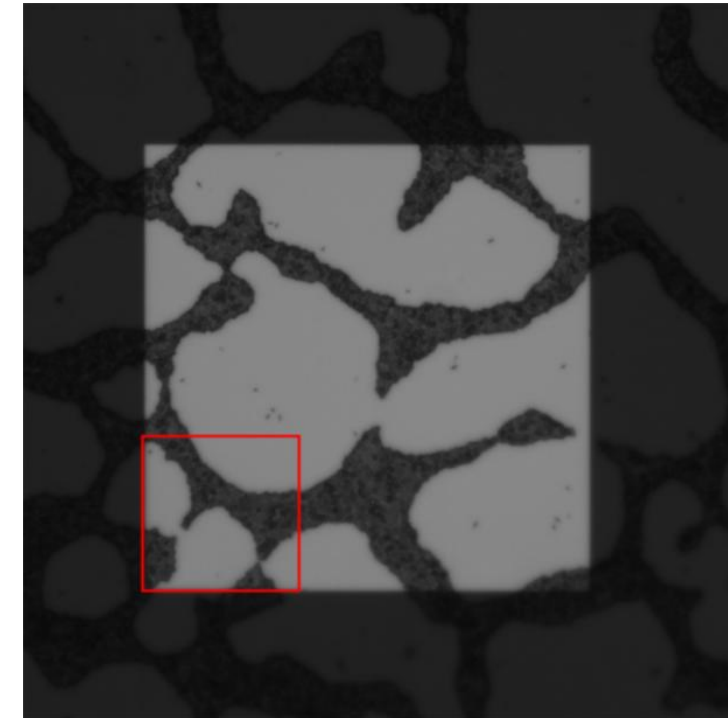
Cyanobacteria



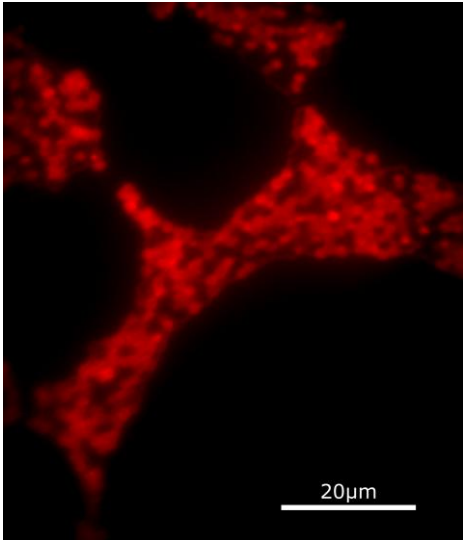
- autofluorescent (Chlorophyll)
- excitation possible over broad range
- emission $>650\text{nm}$
- easy to grow and prepare



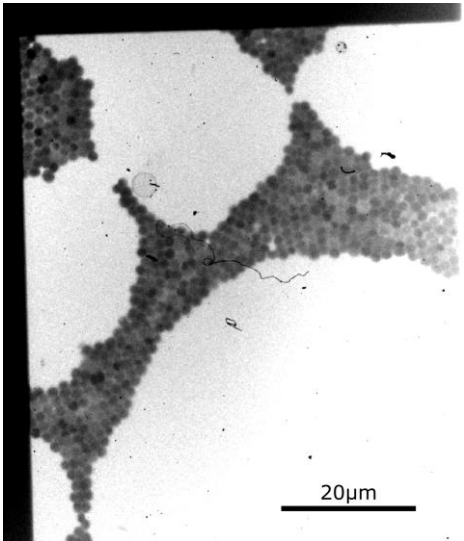
visible light



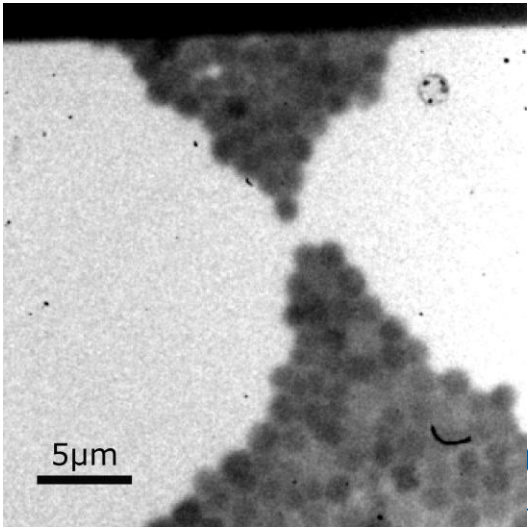
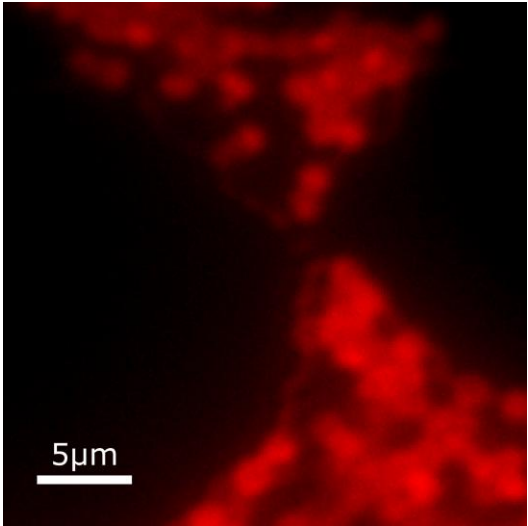
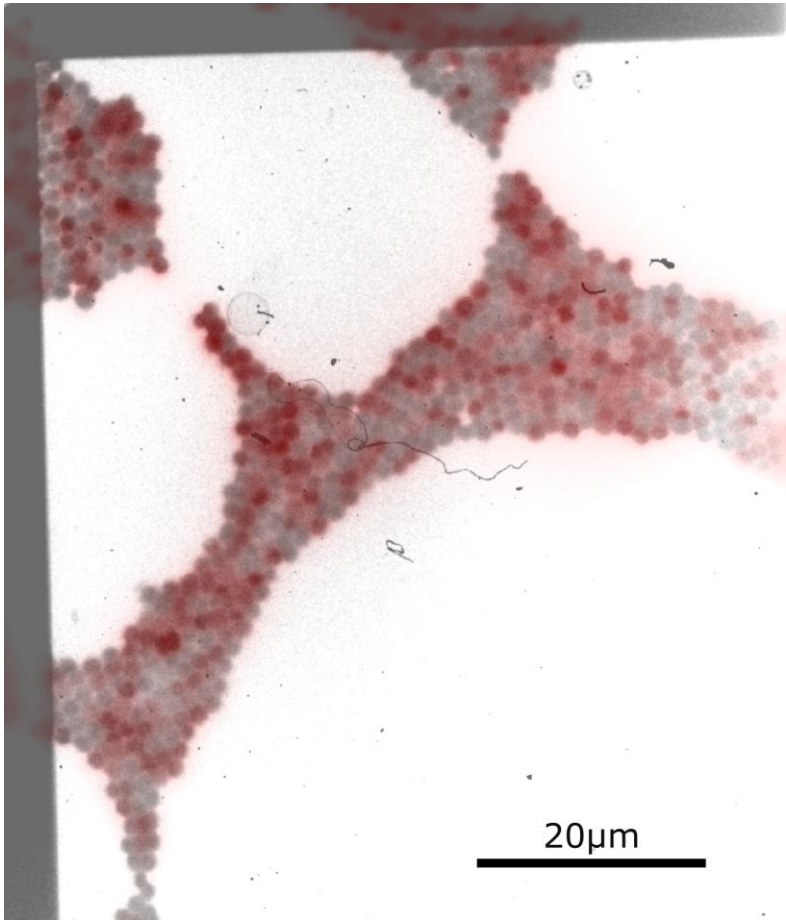
fluorescence



SXR



correlation

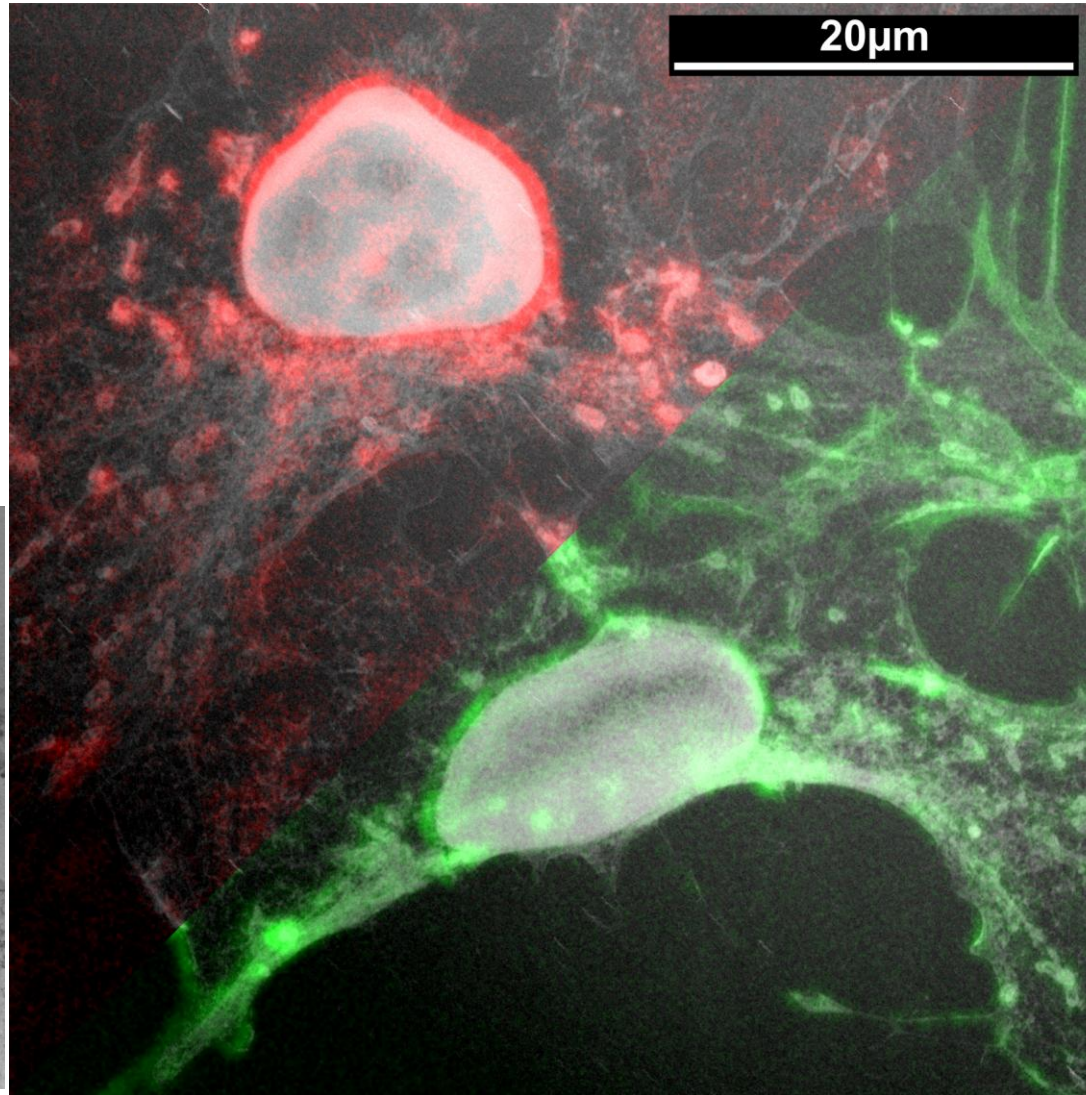
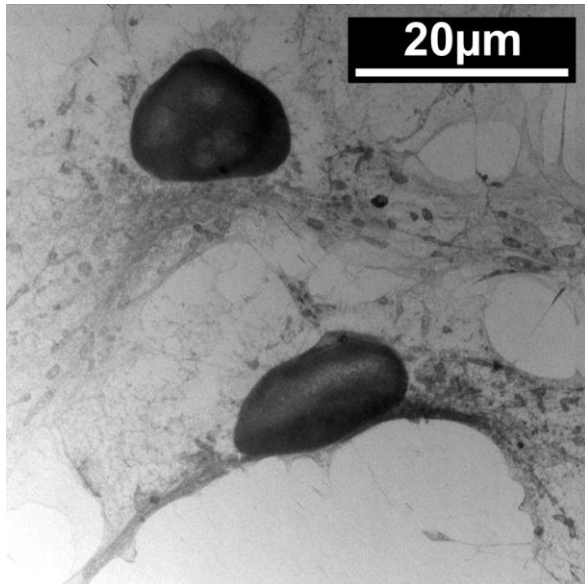


Correlative Images – COS7 Cells

preparation:

- chemical fixation
- critical point drying

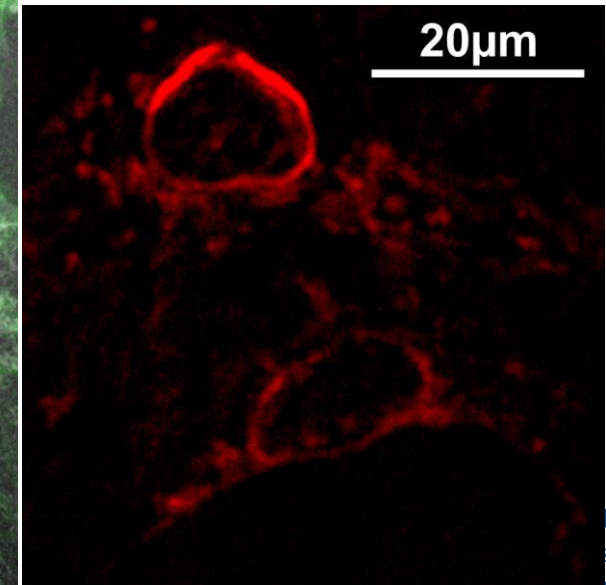
SXR image (81x45s)



cell type:

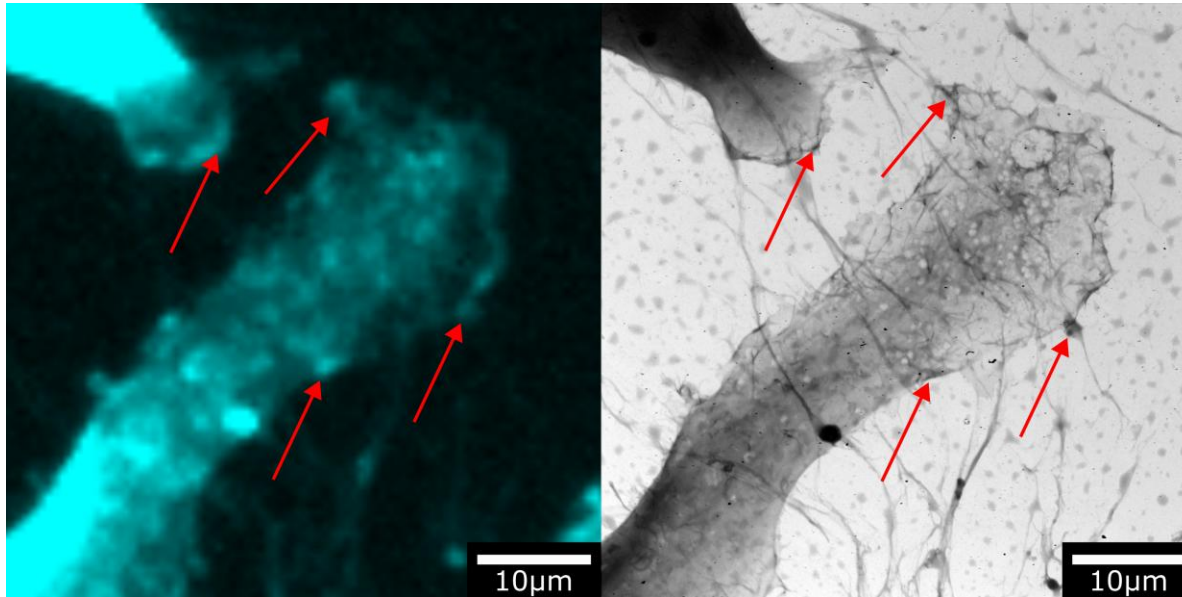
- Monkey kidney fibroblasts
- adherent cells
- cultivated on SiN-membranes

image – mitochondria labelled

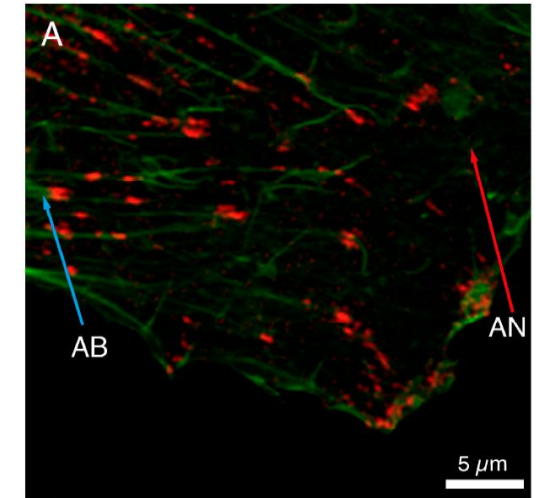


Science Case: Cancer Cell Adhesion

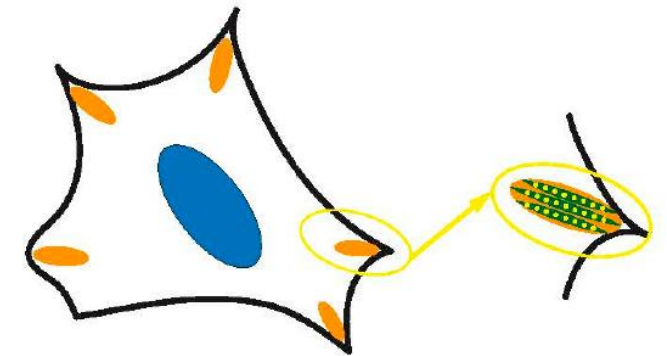
- Collaboration with MUT Warsaw
- **Investigate metastatic mechanisms** in triple-negative breast cancer cells (TNBC)
 - TNBC is occurring in 15-20% of breast cancer patients
 - Poor prognosis due to lack of targeted therapies
- **Size and distribution of FAs** directly determine cell's adhesion strength and migration potential



P. N. Osuchowska, S. Kaleta et al., in preparation

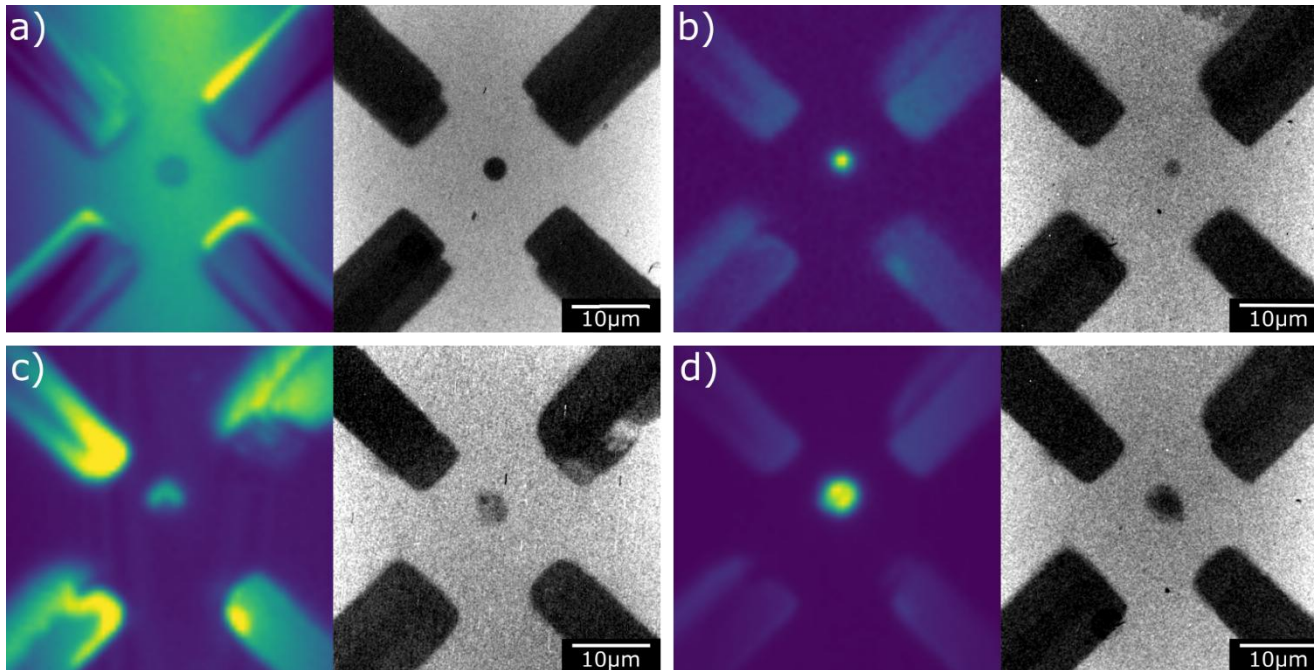


P. N. Osuchowska et al., Int. J. Mol. Sci. 22, 7279 (2021)



SXR Microscopy of Cells in an Aqueous Environment – Microfluidic Cell Device

- Collaboration with Fraunhofer IZI-BB and AG Rothhardt
- Core concept: liquid (water /cell medium) flows between Si_3N_4 -membranes to **maintain aqueous environment inside vacuum chamber** → Microfluidic Cell (MFC)
- Key advantage: **Sequential** introduction of living cells **without breaking the vacuum!**



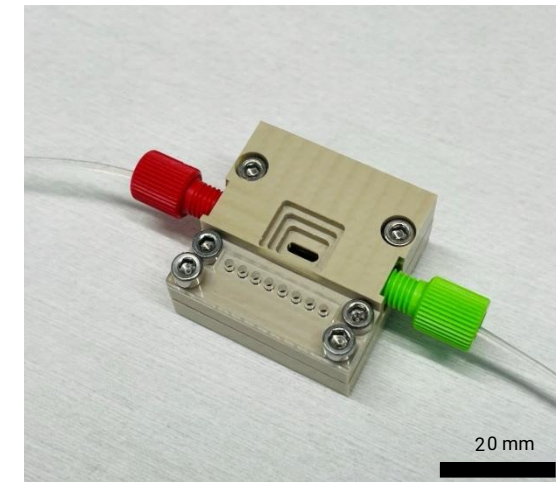
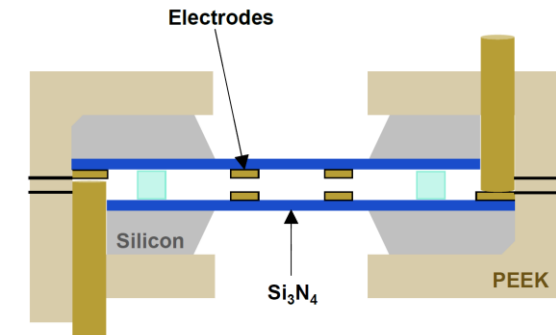
a) Nanobead (exp. time 2h)

c) *Tisochrysis lutea* RCC 1349

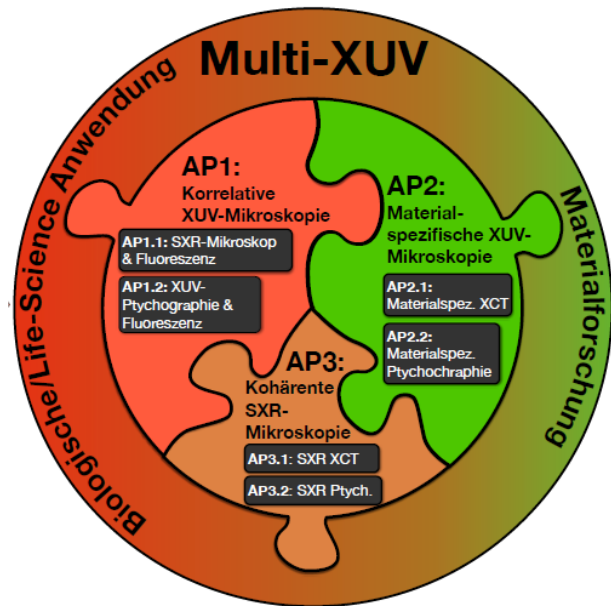
Exposure time b-d: 75 min

b) *Synechocystis* PCC 6803

d) *Chlamydomonas Euryale* SAG 25.89



Research Group Multimodal Nanoscale XUV Imaging



HELMHOLTZ

Helmholtz-Institut Jena



**FRIEDRICH-SCHILLER-
UNIVERSITÄT
JENA**



Thüringer Aufbaubank

- **Profile:** Interdisciplinary research group pioneering multimodal nanoscale XUV imaging from lab breakthroughs to industrial application
- **Technology:** Lab-scale coherent XUV and soft X-ray sources (resolution down to tens of nanometer range, high penetration depth)
- **Biology/Life-Science:** (label-free) nanoscale imaging of cellular ultrastructure and dynamics
- **Materials Science:** Non-destructive nanoscale elemental/density mapping and depth profiling of buried interfaces
- **Collaborators:** Groups of Gerhard G. Paulus, Jan Rothhardt, Christian Eggeling and Stephanie Lippmann



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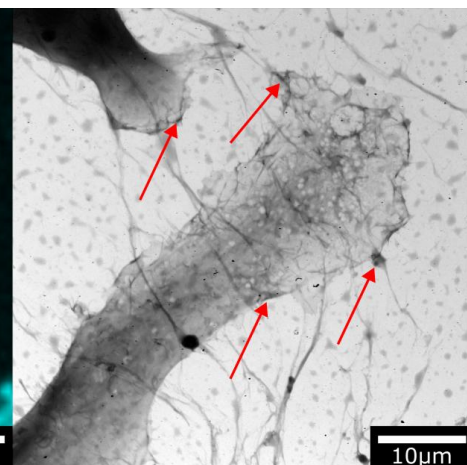
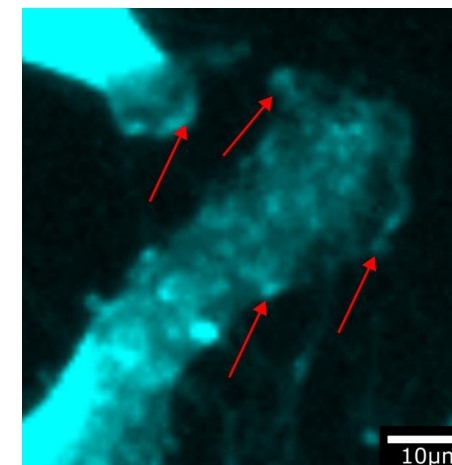
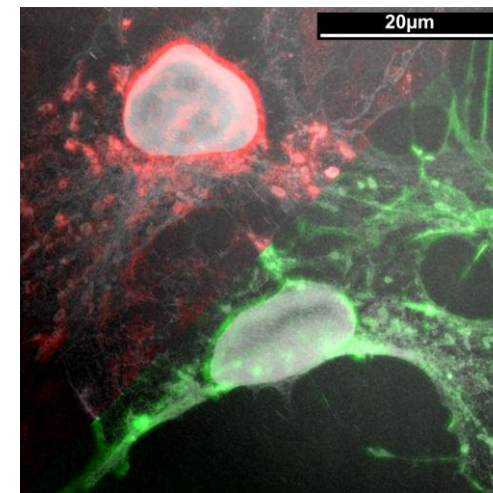
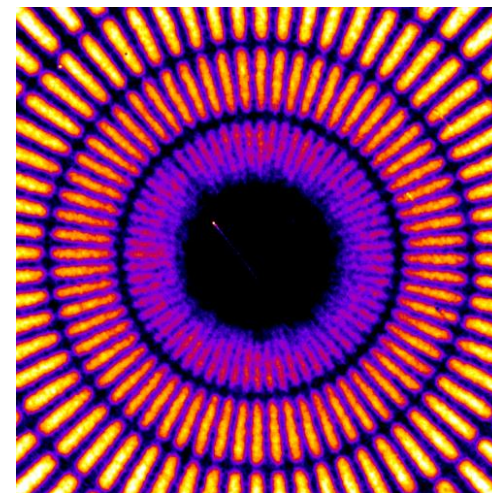
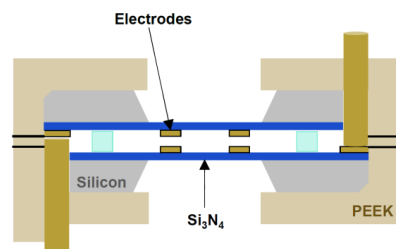
HI JENA

Helmholtz Institute Jena

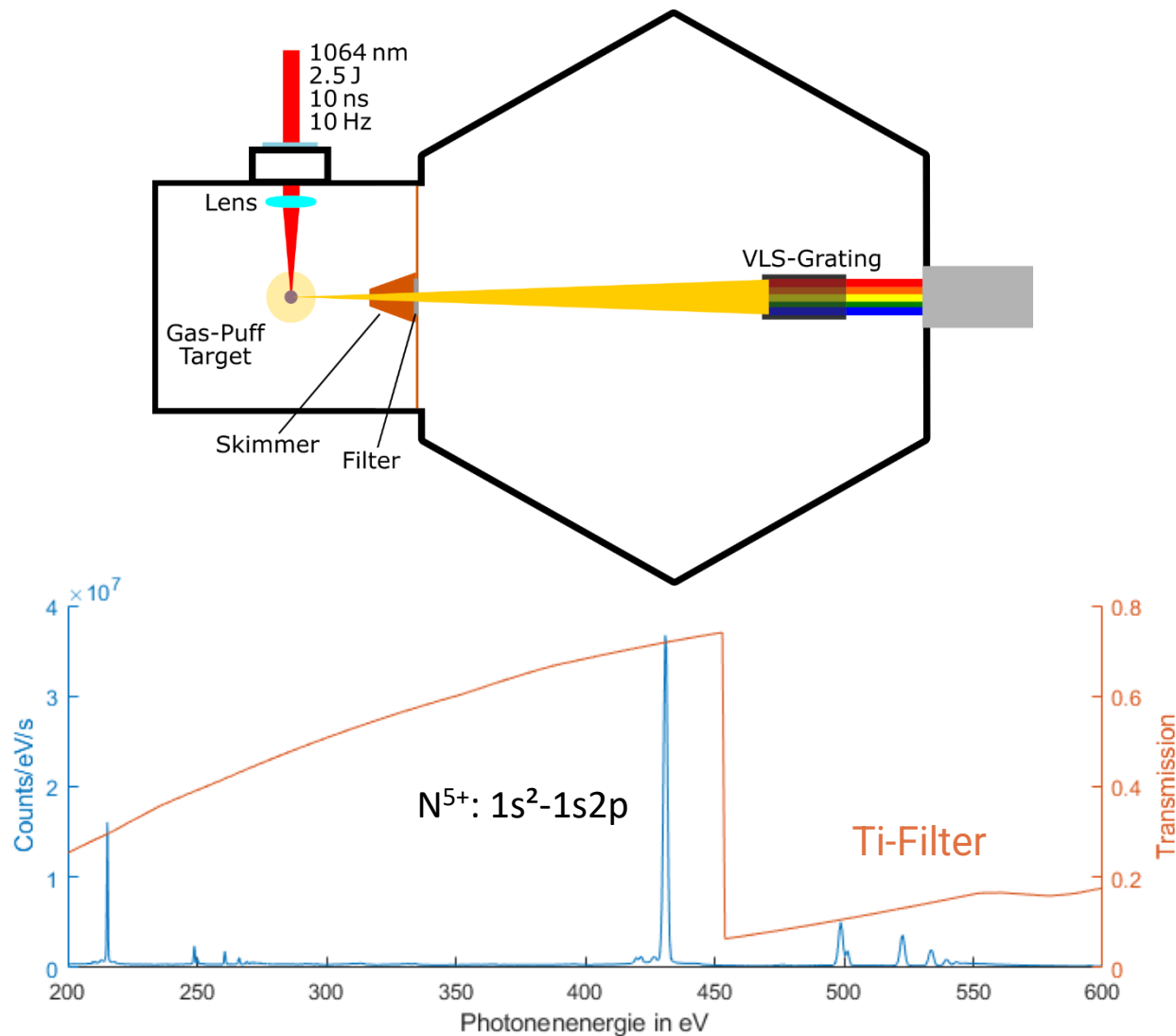
www.hi-jena.de

Summary and Outlook

- Successfully built lab-based SXR microscope with 50nm half-pitch resolution
- Integrated fluorescence microscope
- In situ correlative microscopy demonstrated
- Science case: cancer cell adhesion
- Microfluidic cell device allows imaging in aqueous environment
- In the future:
 - Work on more science cases
 - Advance imaging methods for aqueous environments
 - Upgrade fluorescence microscopy to super-resolution



Spectral Characterization



photon numbers

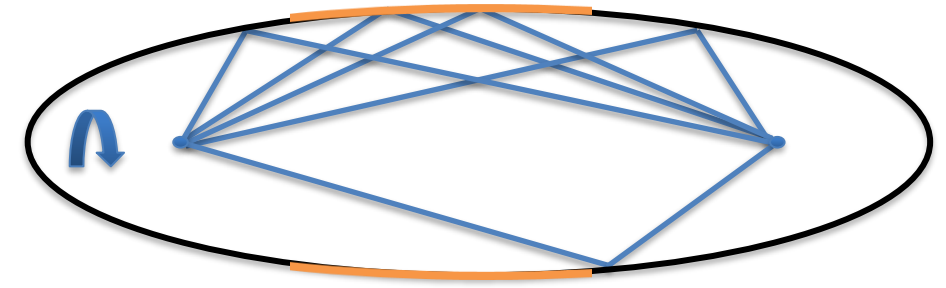
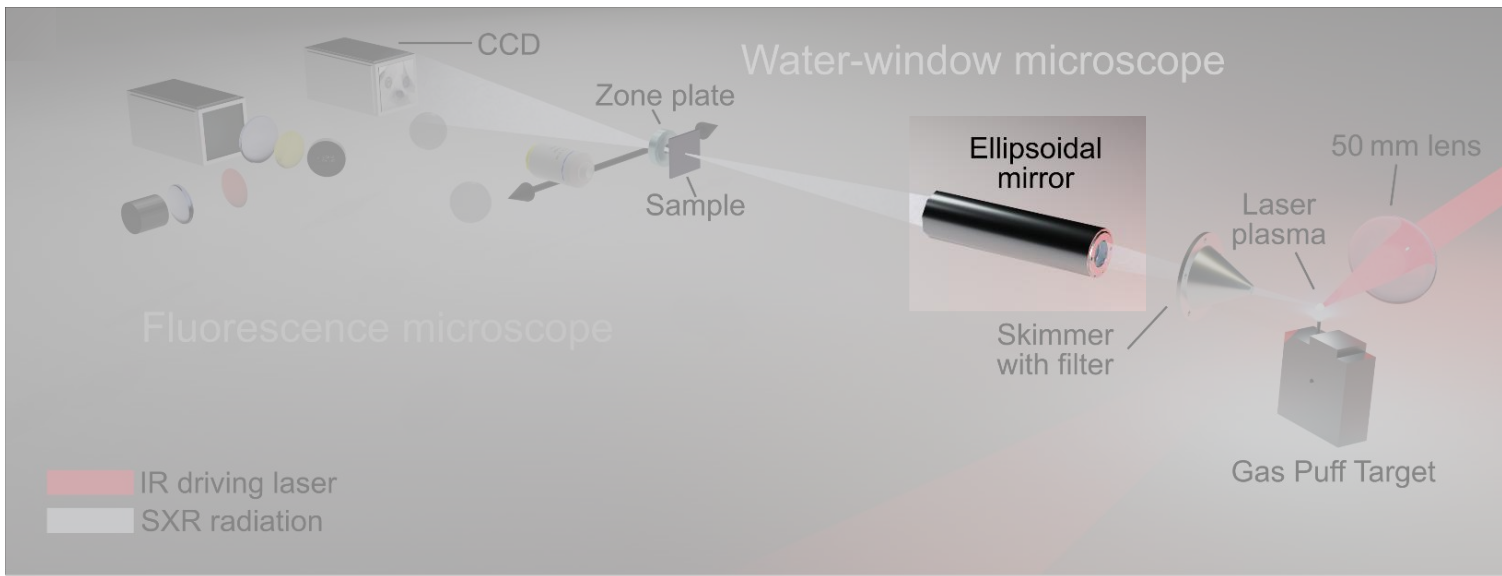
➔ $3 \cdot 10^{11}$ ph/sr/pulse @430 eV
➔ $1.5 \cdot 10^{10}$ ph/pulse on target

comparison:

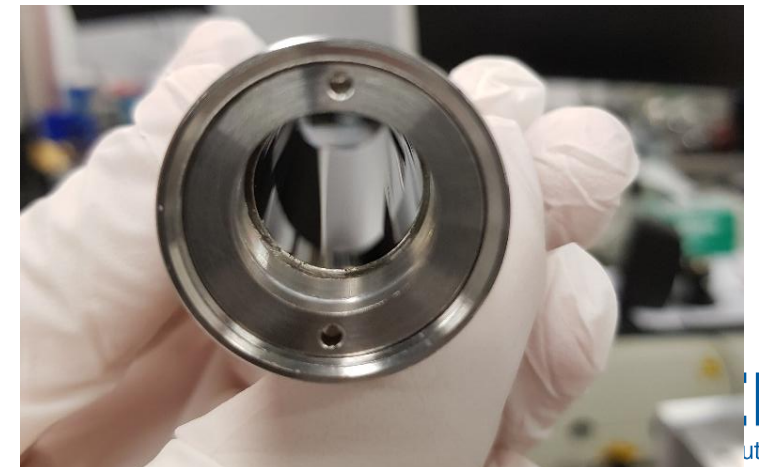
MUT: $8 \cdot 10^9$ ph/pulse on target
[Wachulak et al., *Microscopy and Microanalysis* 21, (2015)]

Stockholm group: $5.5 \cdot 10^{11}$ ph/sr/pulse
[Martz et al., *Optics Letters* 37.21, (2012)]

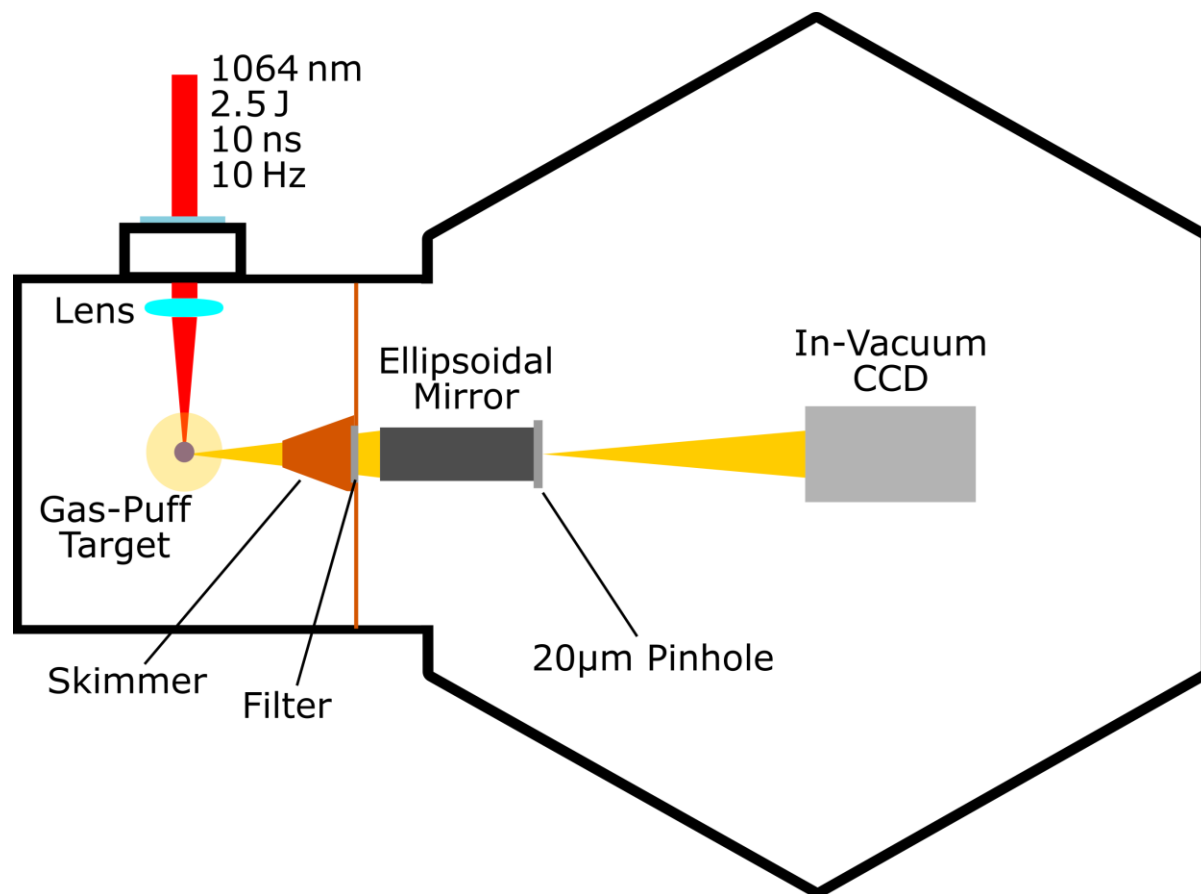
Ellipsoidal Condensor



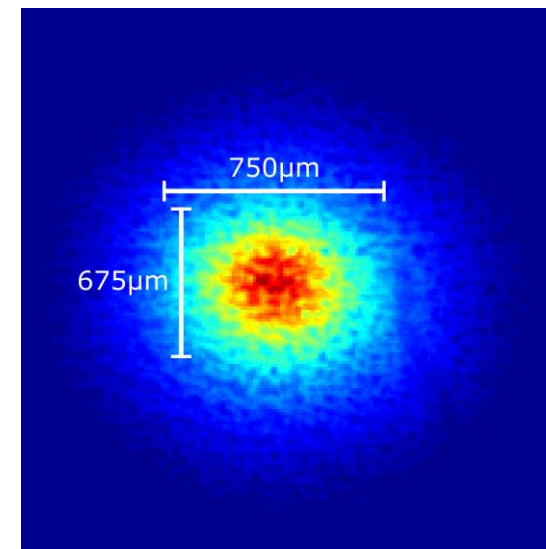
Ni-coating:
76% reflectivity @3° @430eV



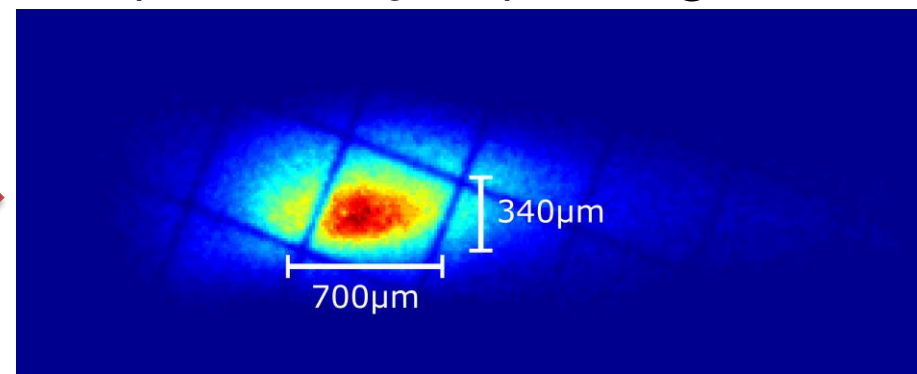
Focus and Plasma Analysis



focal spot of ellipsoidal mirror @430eV

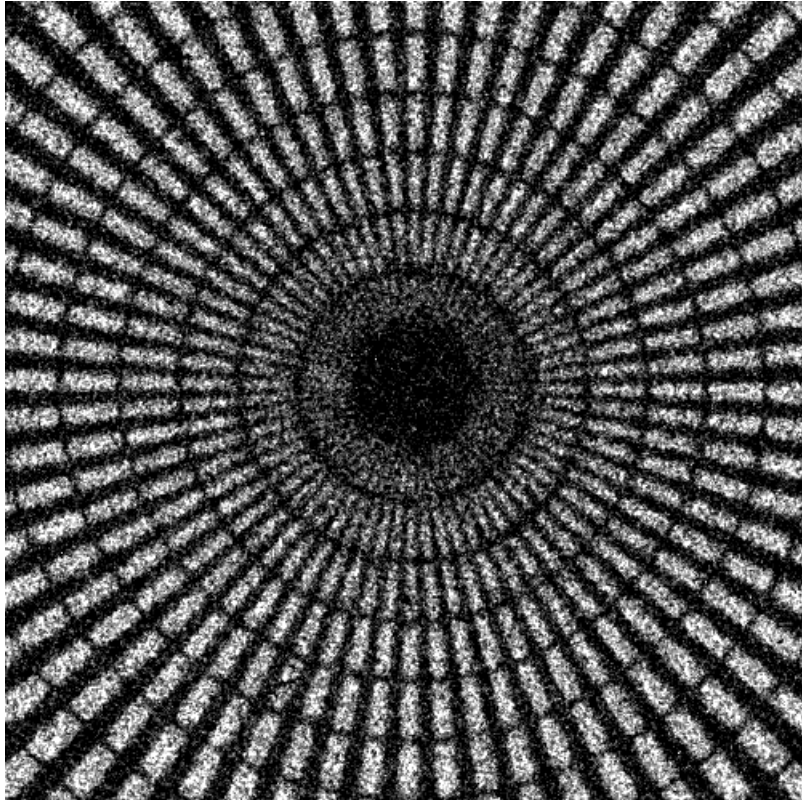


pinhole image of plasma @430eV

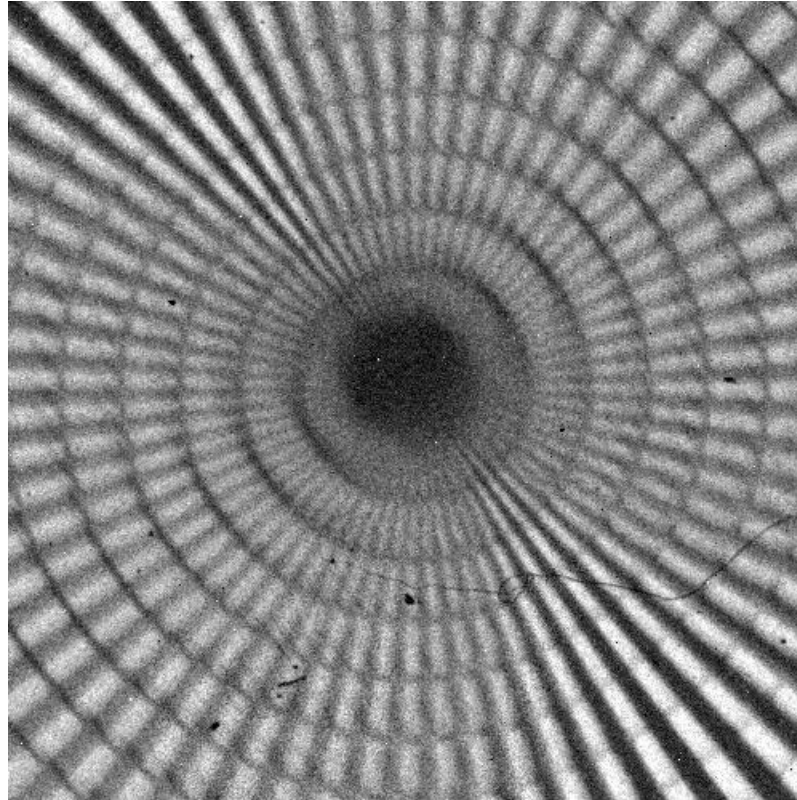


Drift Correction

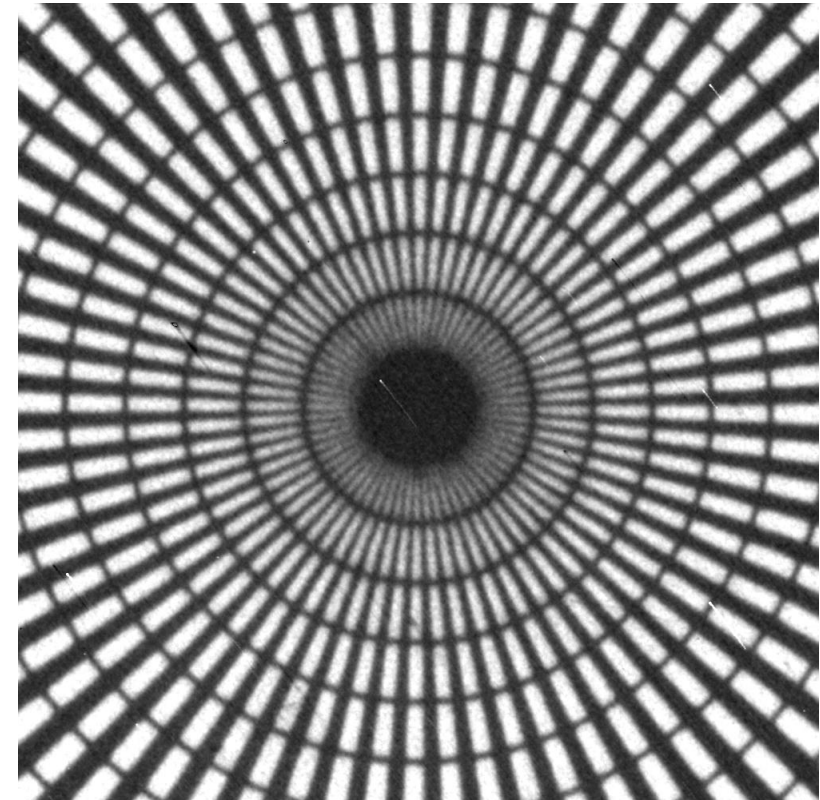
single images 80s



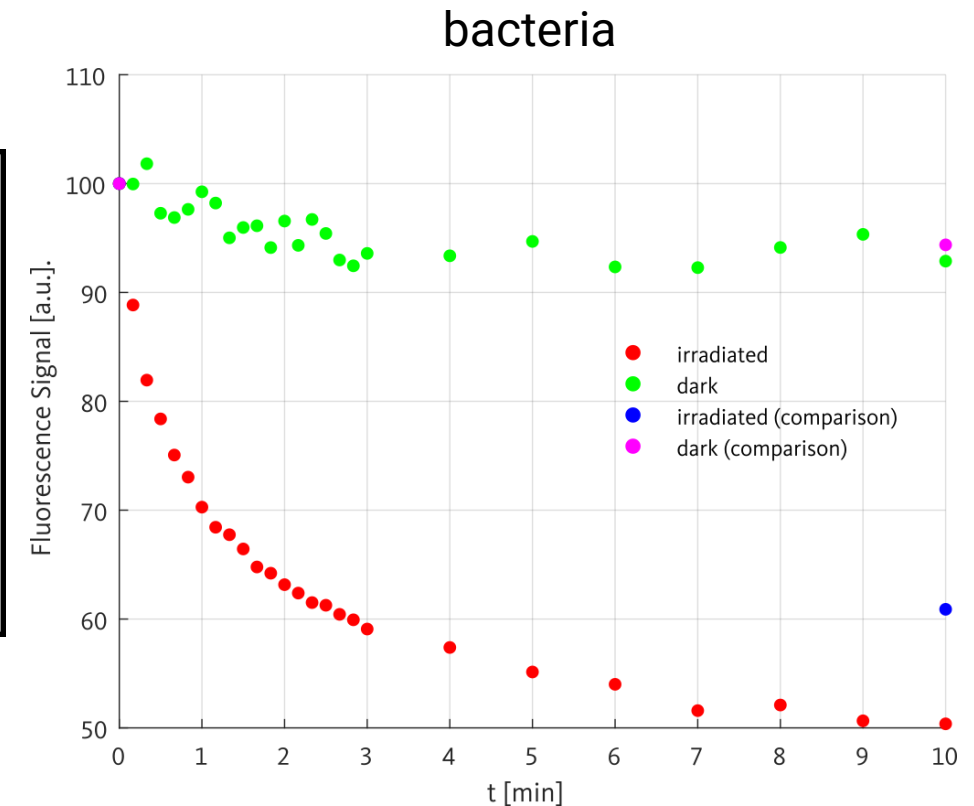
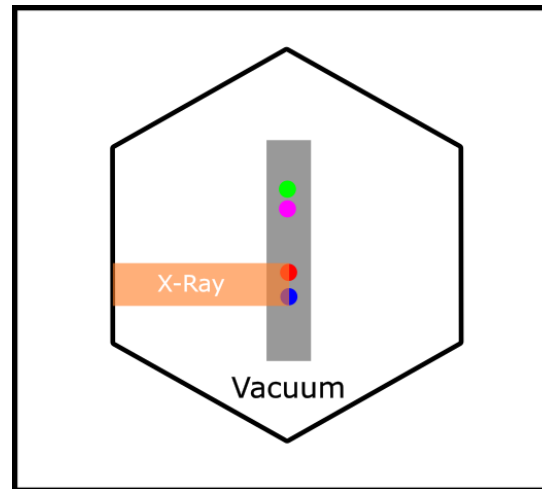
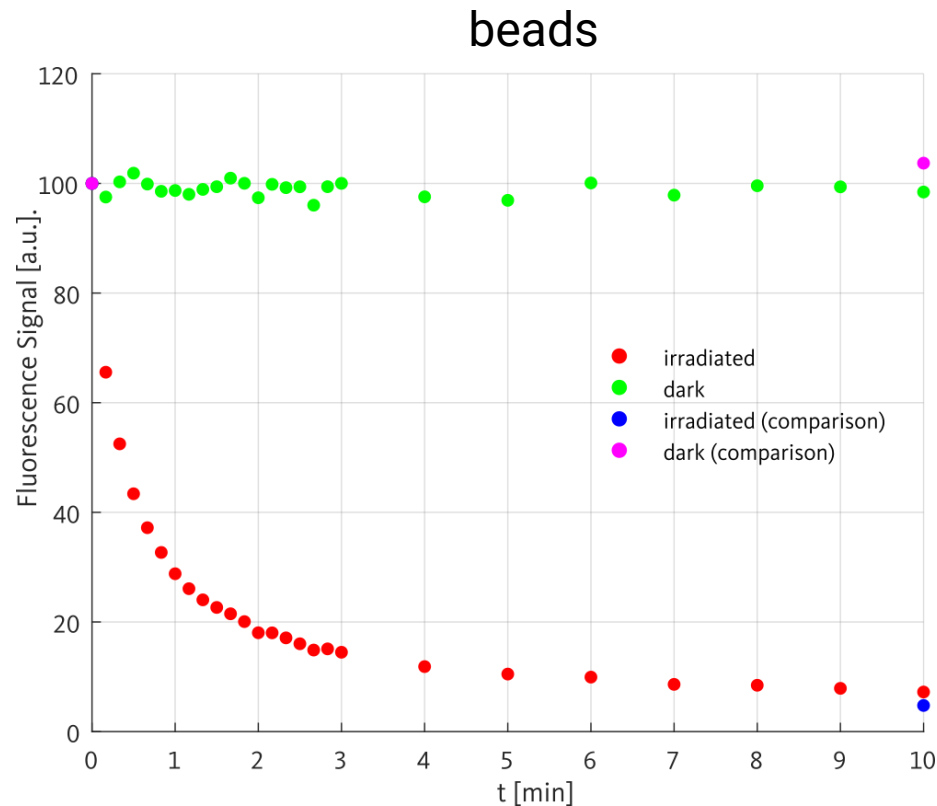
simple sum



drift correction via
cross correlation



Quantitative Measurements - Phototoxicity



- high loss in fluorescence intensity in short time
- fluorescence signal survives hours of SXR-irradiation!