PAUL SCHERRER INSTITUT

PSI

Mirko Holler :: Paul Scherrer Institut

High-resolution 3D X-ray Imaging at the Swiss Light Source: Instrumentation and Bio Applications

Motivation and Outline

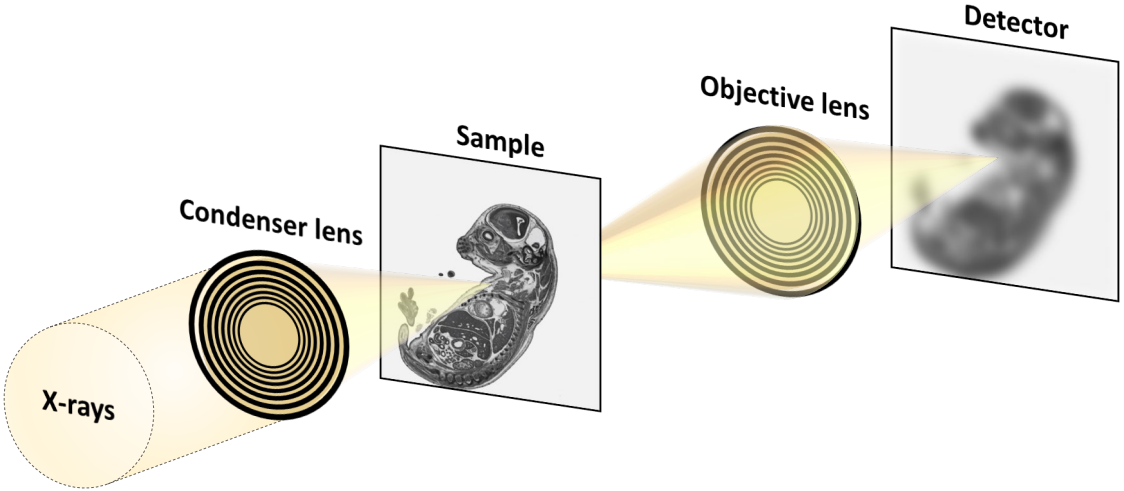
MAIN MOTIVATION:

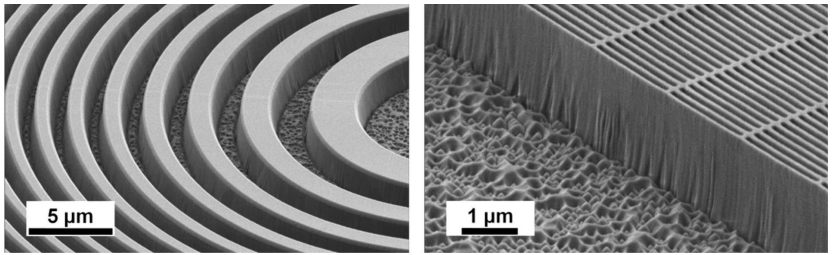
- Can we provide high-resolution 3D images of continuous, densely packed (biological tissues) non-destructively with X-rays?
- What resolution can we achieve?
- What is limiting the resolution?

Outlook of this Presentation - OUR WORK:

- Development of ptychographic tomography at hard X-rays (5-9 keV)
- Instrumentation
- Examples and applications in biology
- Preparing for SLS 2.0

Due to high-penetration of X-rays, lenses are limiting the maximum attainable resolution

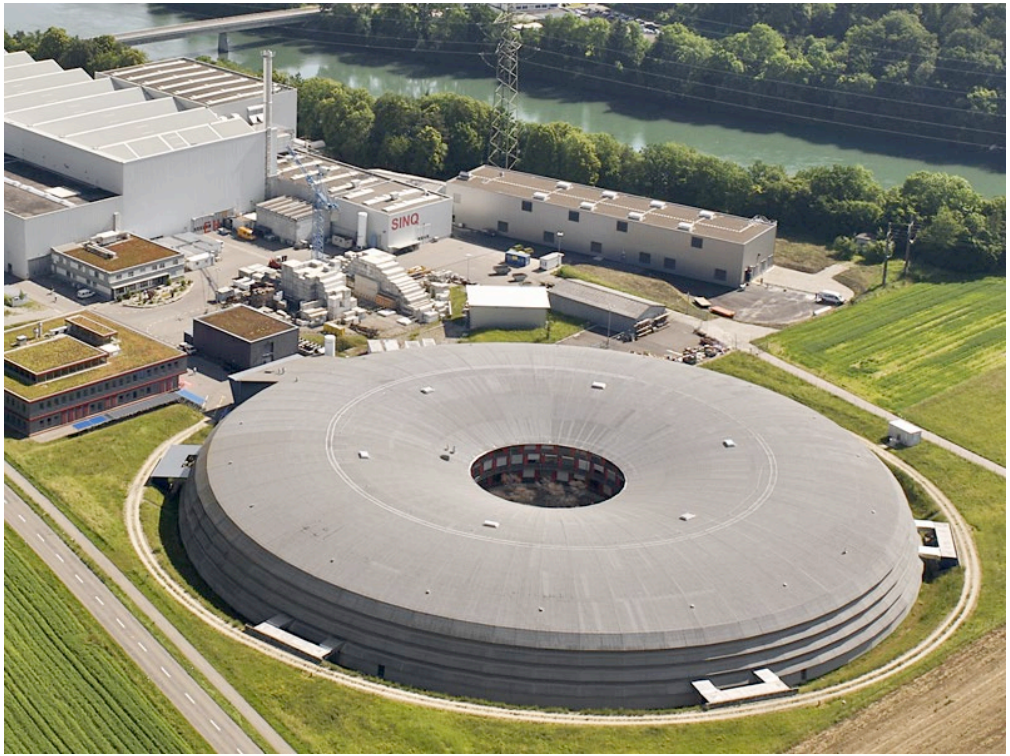




https://www.psi.ch/en/lxn/diamond-fresnel-zone-plates

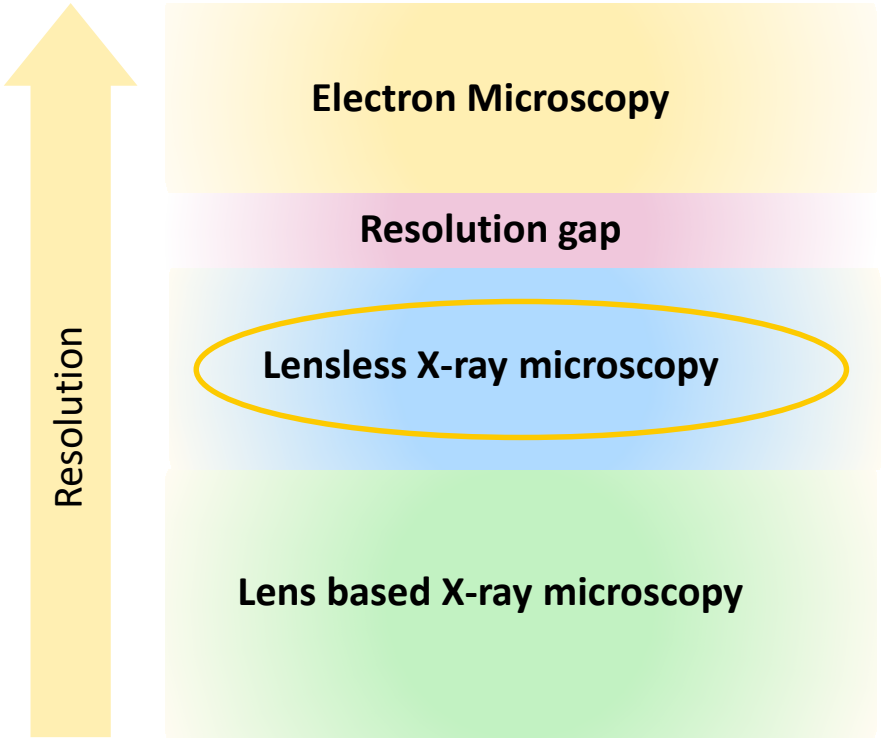
The Swiss Light Source

The Swiss Light Source, at about 5 minutes from here
2.4 GeV electron storage ring



- X-ray radiation with high flux and high brilliance
- Broad X-ray spectrum
- Great advantage for X-ray imaging with high temporal and/or spatial resolution
- User facility
- 18 beamlines, from which 5 do some type of imaging

High-resolution X-ray imaging



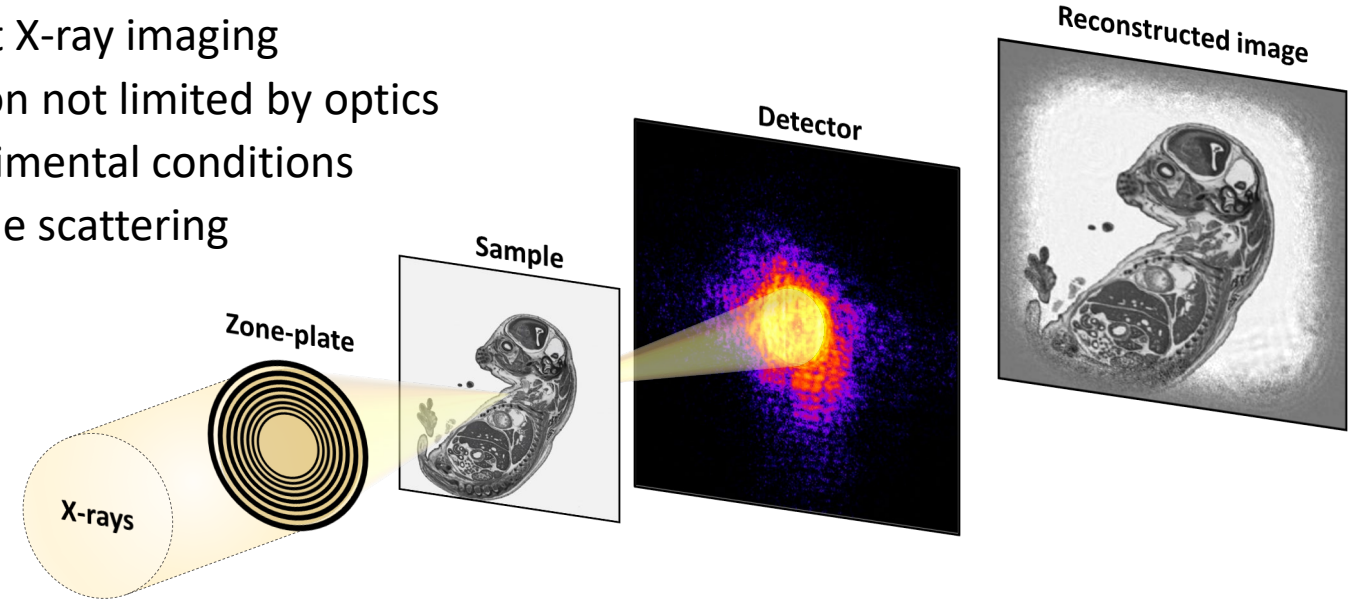
cSAXS
coherent Small Angle X-ray Scattering
Ptychography (5-12 keV)
- high resolution (down to 4 nm)
- sample thickness (up to 100 µm)

Lensless imaging overcomes the limitations of X-ray lenses

Coherent X-ray imaging

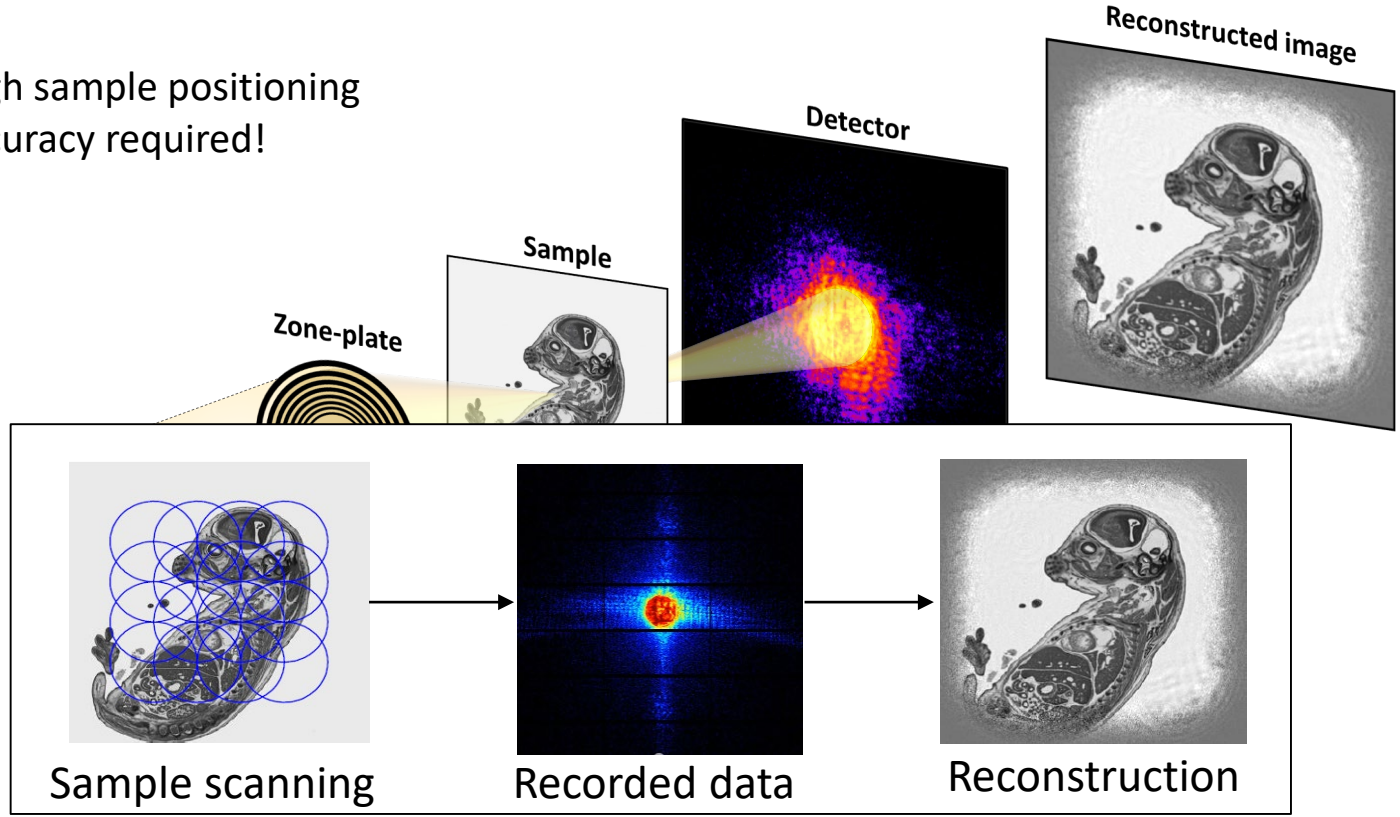
Resolution not limited by optics

- Experimental conditions
- Sample scattering

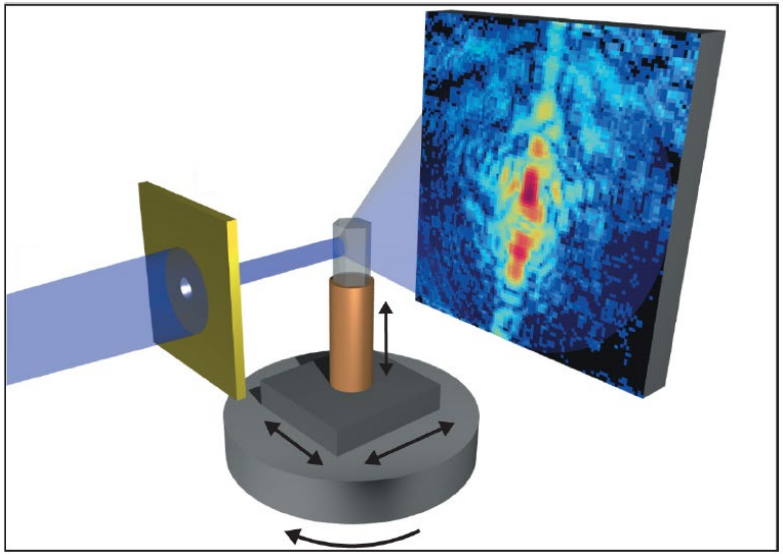


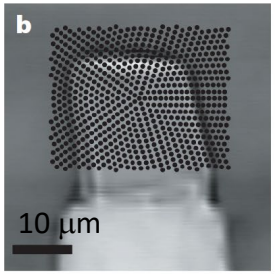
We use ptychography for lensless imaging, where the sample is scanned across an X-ray beam

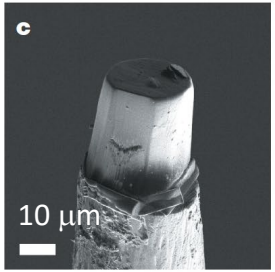
High sample positioning accuracy required!

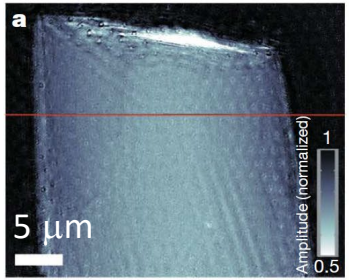


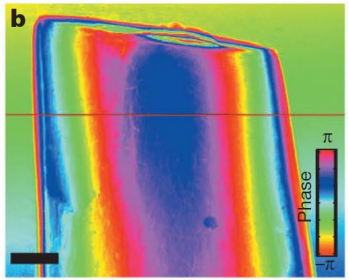
Ptychographic X-ray tomography

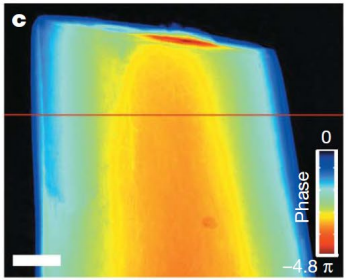




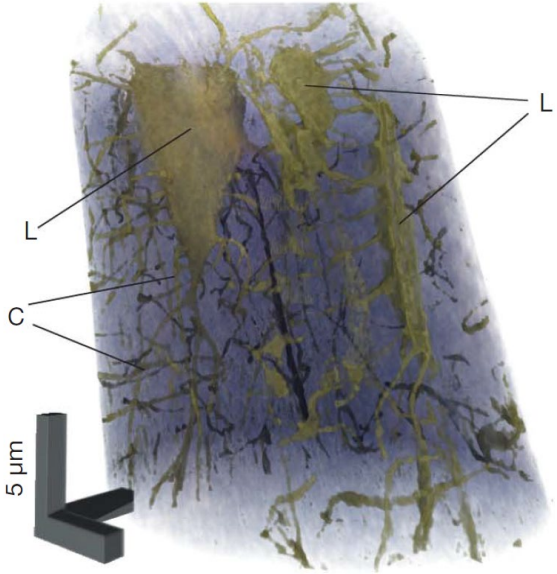








M. Dierolf *et al.*, Nature **467**, 436 (2010)



Mouse bone specimen

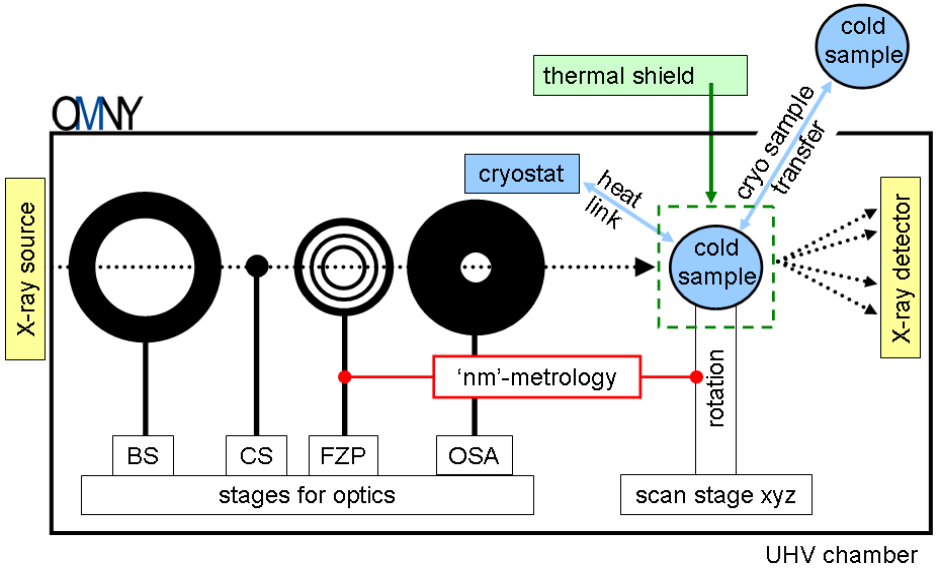
Voxel size: 65 nm
Resolution: 120 nm
Dose: 2MGy

OMNY – tOMography Nano crYo endstation

Goal: Development of a **dedicated instrument** to perform tomography at the **nano-scale** on **biological samples** and condensed matter physics samples using Ptychography in a **controlled sample environment**.

Project started 2010

- Nano positioning of the sample
- Rotation of the sample for tomography (vertical axis)
- Beam conditioning: Fresnel Zone-plate illumination
- Cryogenic temperatures
- UHV environment
- Sample transfer of frozen bio-samples (contamination free)

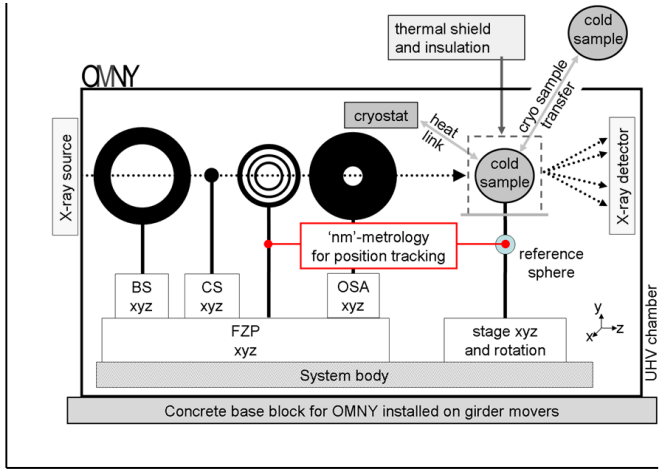


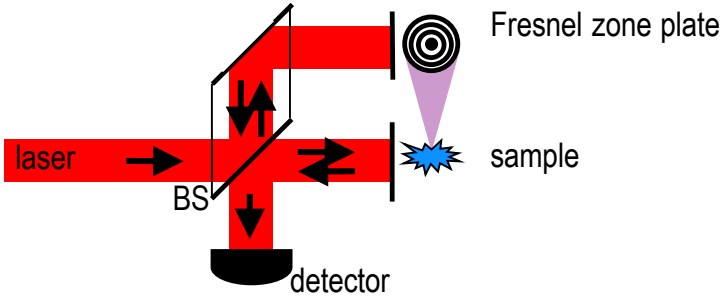
Resolution goal: 10 nm in 3D
to bridge the resolution gap between
high-resolution EM and conventional X-ray imaging

Metrology for OMNY

Accurate sample positioning
→ measure relative position FZP vs. sample

vertical rotation axis
→ for vertical measurement a plane mirror can be used





Heterodyne laser interferometry

- Resolution: 0.3 nm
- non-contact, long range
- exteroceptive:
  include thermal drifts in the measurement
- linear, accurate and stable scale

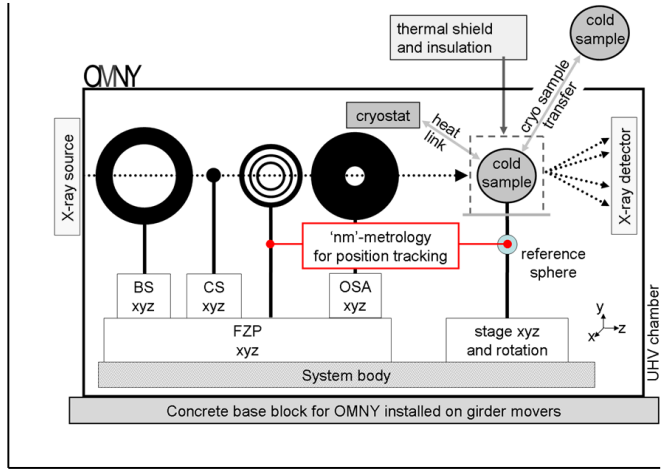
Metrology for OMNY

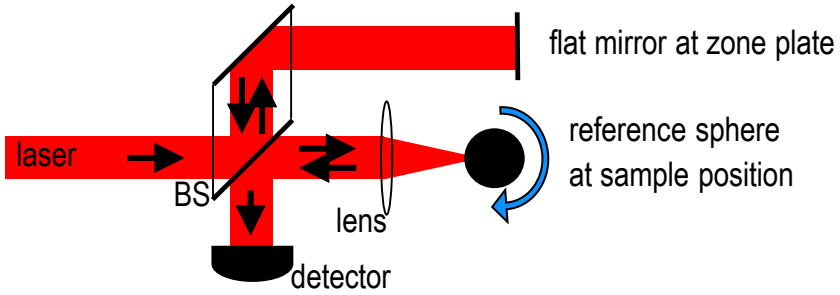
vertical rotation axis

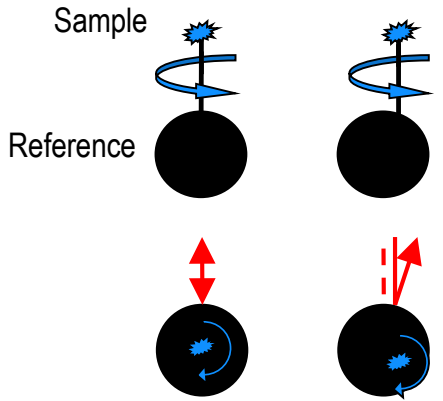
→ for horizontal measurement a spherical mirror is used (equator of a sphere)

BUT

→ no centering mechanics between mirror and sample → wobble of sphere → beam loss



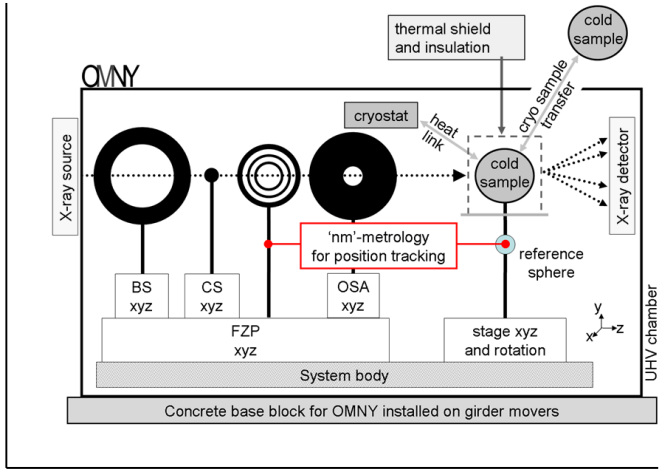


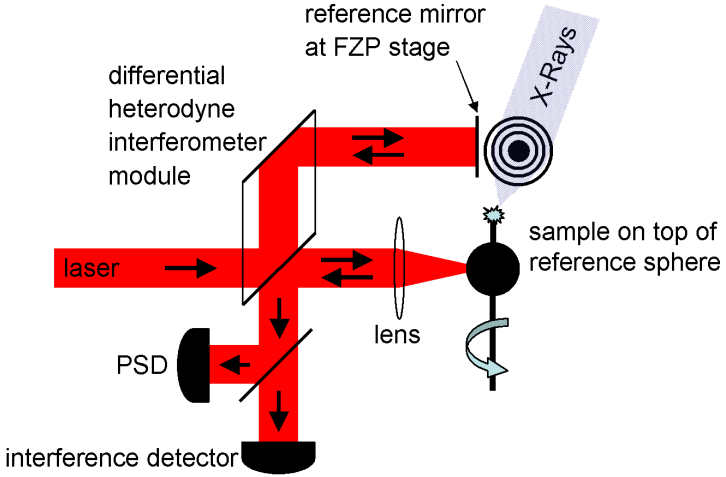


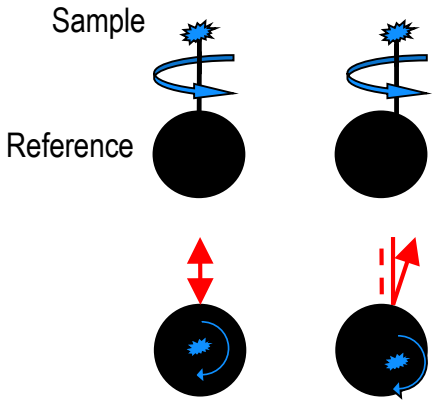
Tracking interferometer

interferometer tracks the reference sphere

maintains alignment automatically



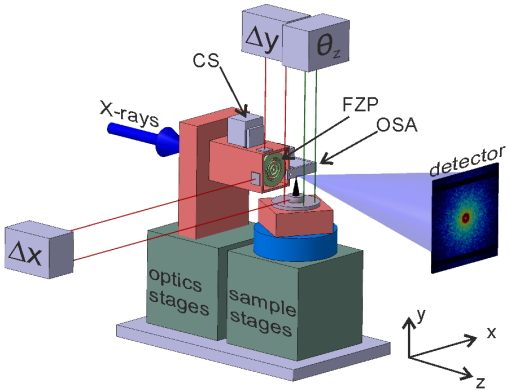




Compensation of mechanical tracking error motion needed – details in
M. Holler and J. Raabe, Opt. Eng. 54(5) 054101 (2015)
Pat. publication no. WO 2012079875 A1

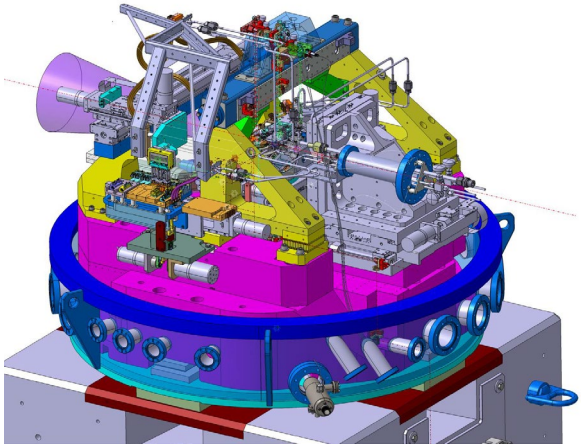
Two instruments with different sample environements

flOMNI (flexible tOMography Nano Imaging)
+ nano-positioning, tomography with interferometric position control
+ no cryogenic sample environment → limited to radiation hard samples
+ atmospheric pressure
+ breadboard style – flexible sample environments



M. Holler, et al., Rev. Sci. Instrum. 83, 073703 (2012)

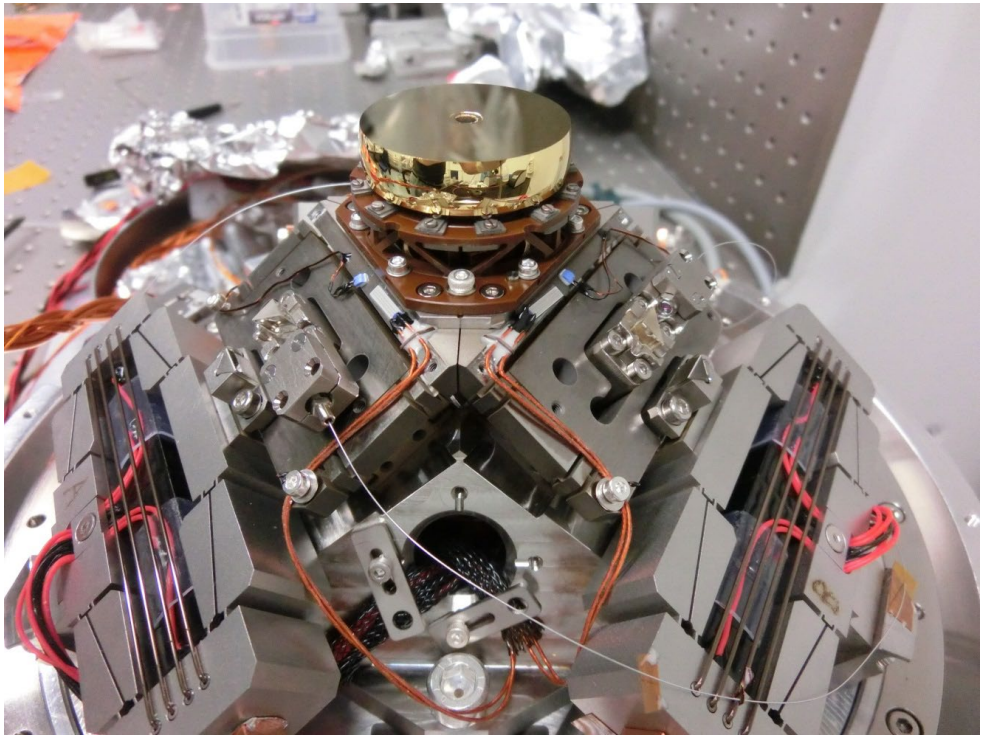
OMNY (tOMography Nano crYo)
+ optimized mechanical structures
+ cryogenic environment and UHV



M. Holler, et al., Rev. Sci. Instrum. 89, 043706 (2018)

PAUL SCHERRER INSTITUT

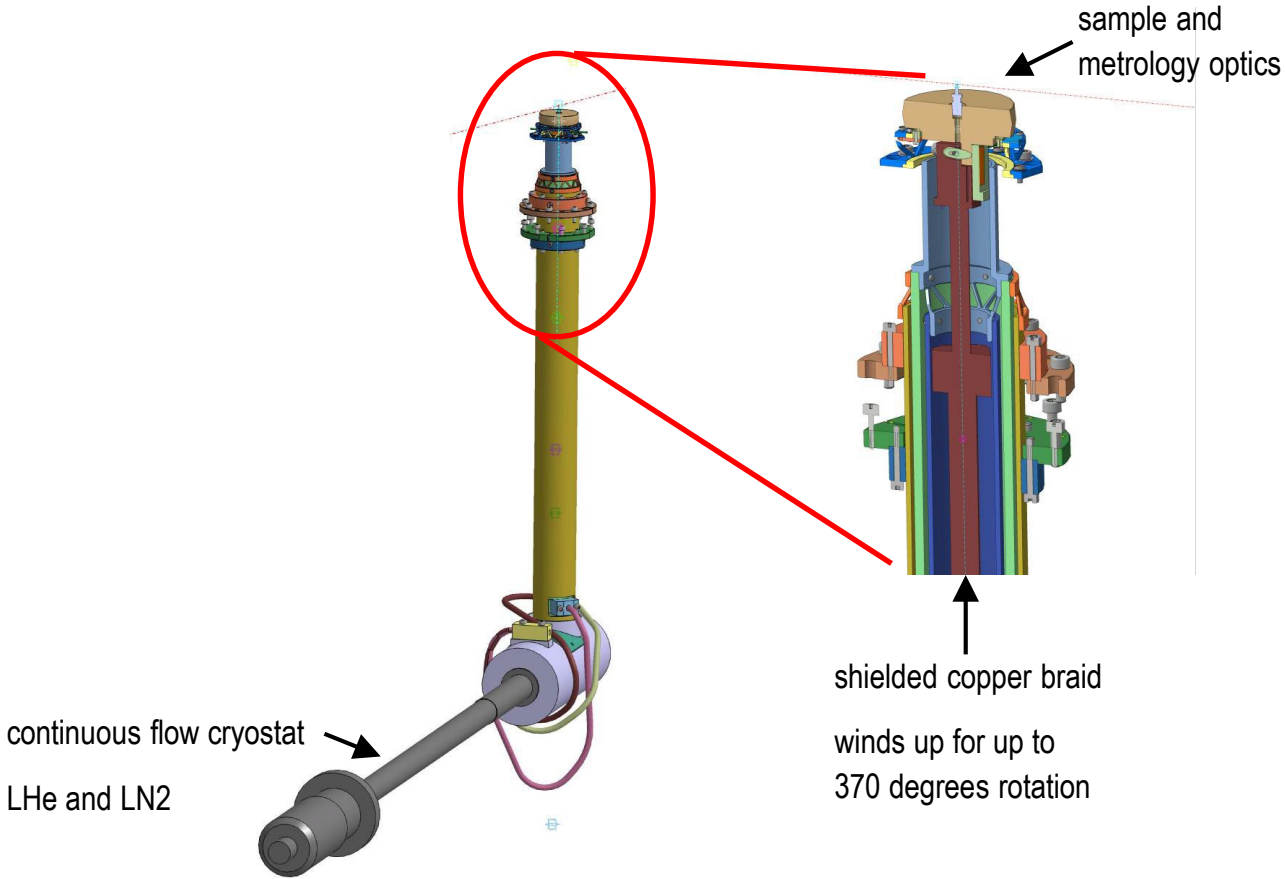
sample mount installed on scanner



large range and high resolution

→ local metrology based on fiber interferometry

OMNY – cryogenic sample conditions

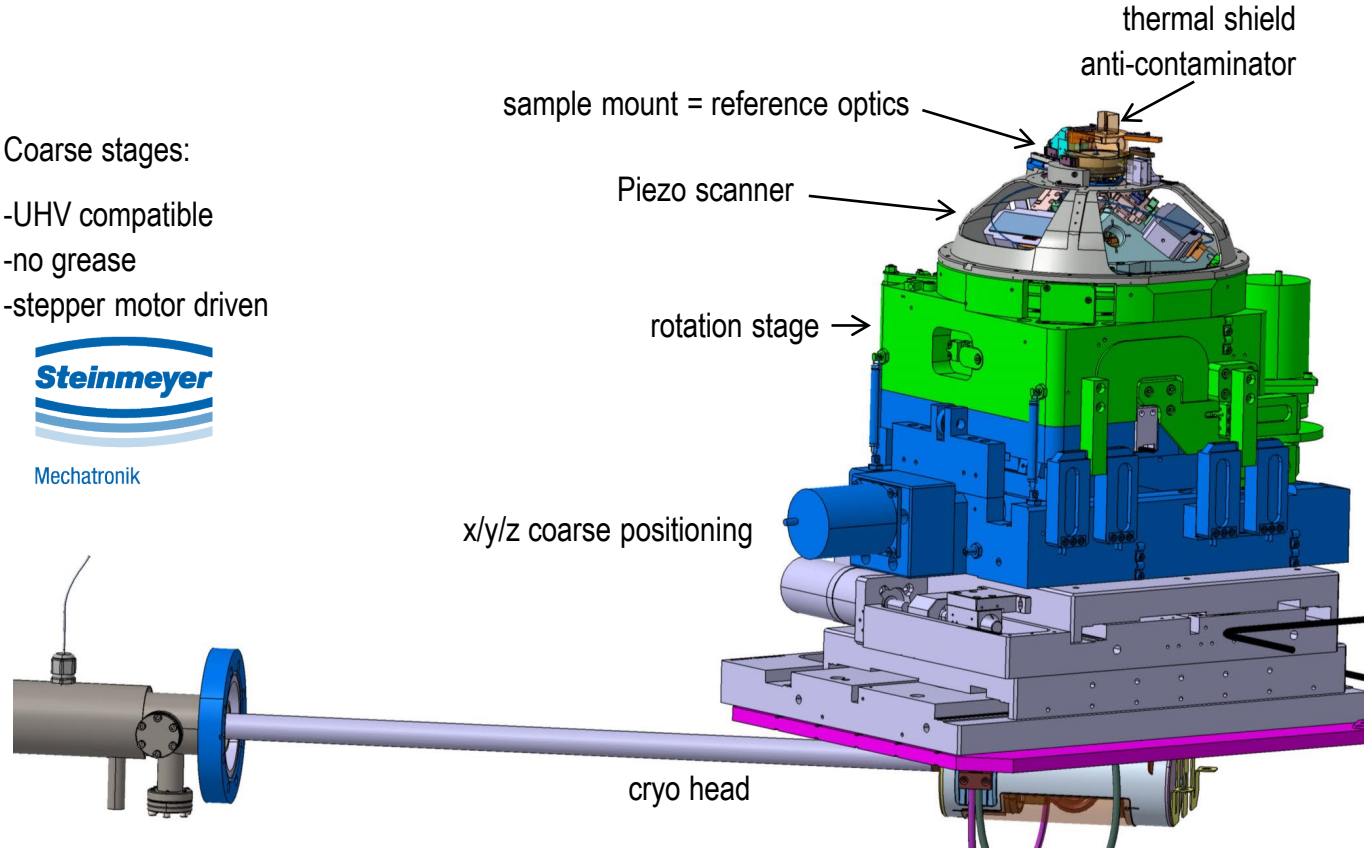


Complete sample stage

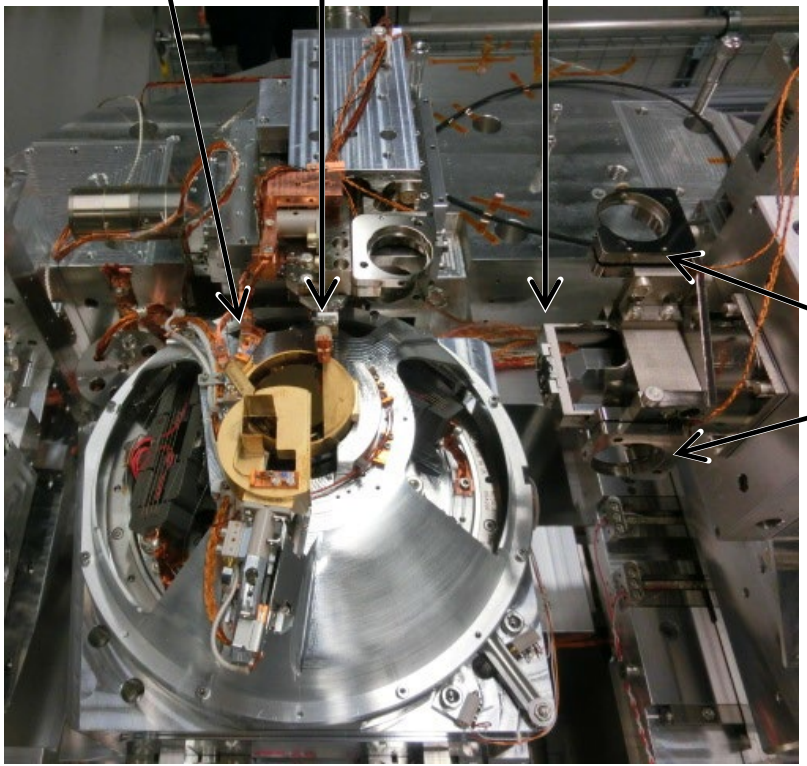
Coarse stages:

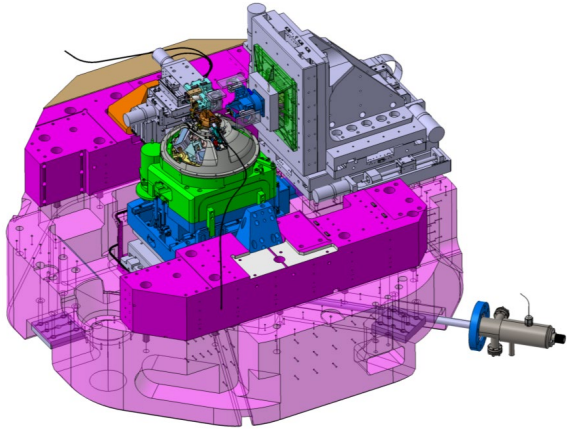
-UHV compatible
-no grease
-stepper motor driven

Steinmeyer Mechatronik

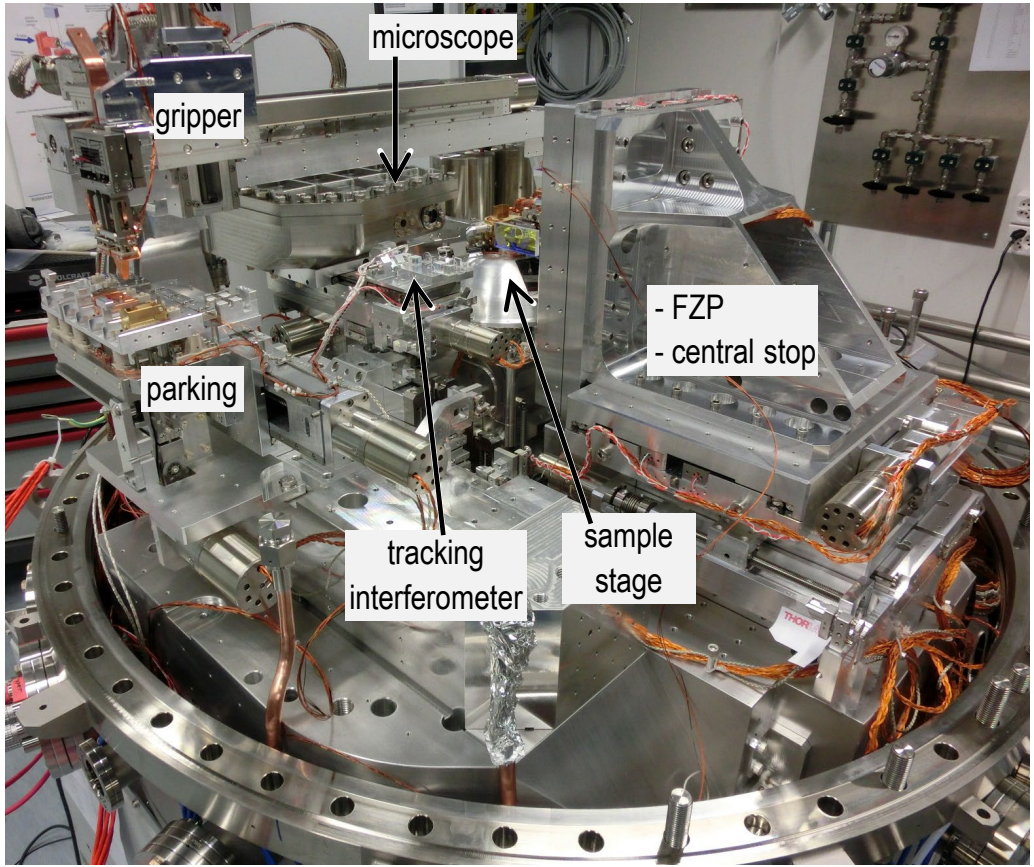


OSA and FZP added



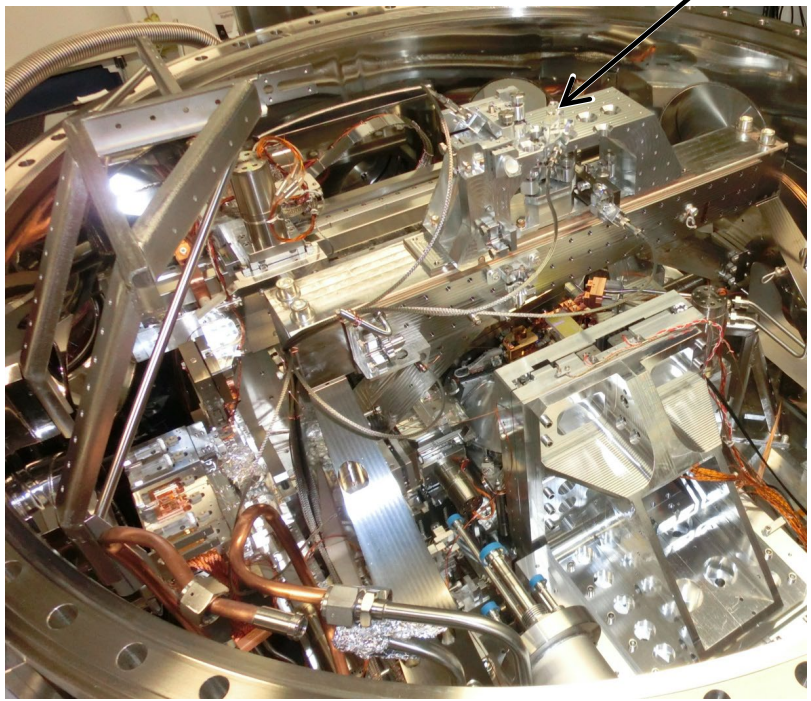


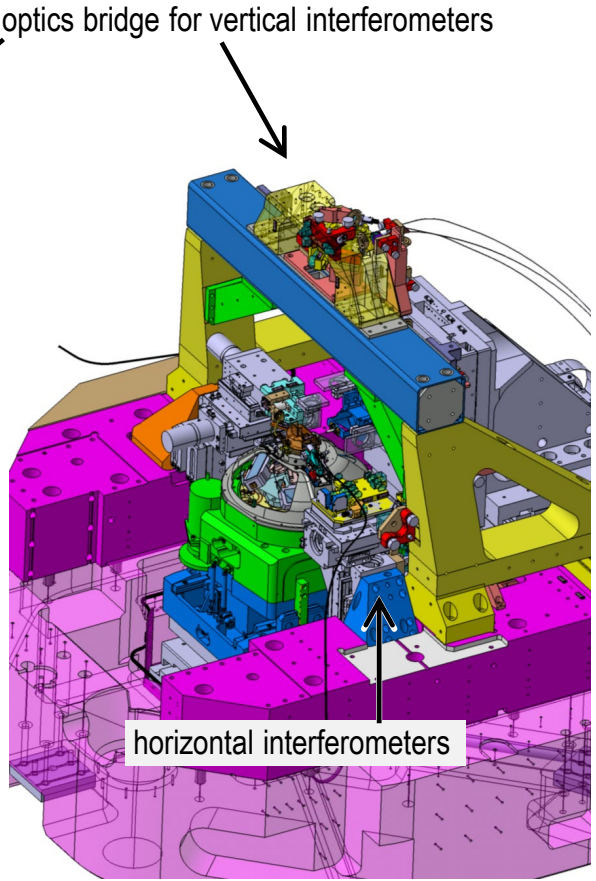
View into the chamber



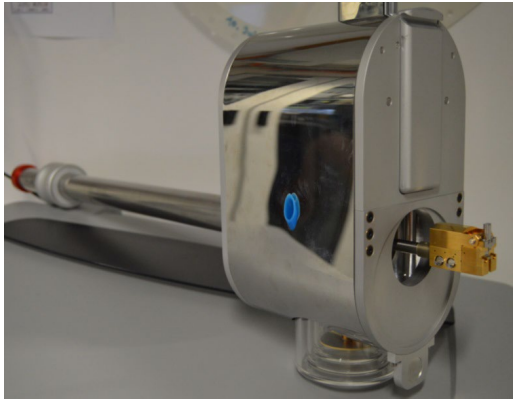
PAUL SCHERRER INSTITUT

Interferometry





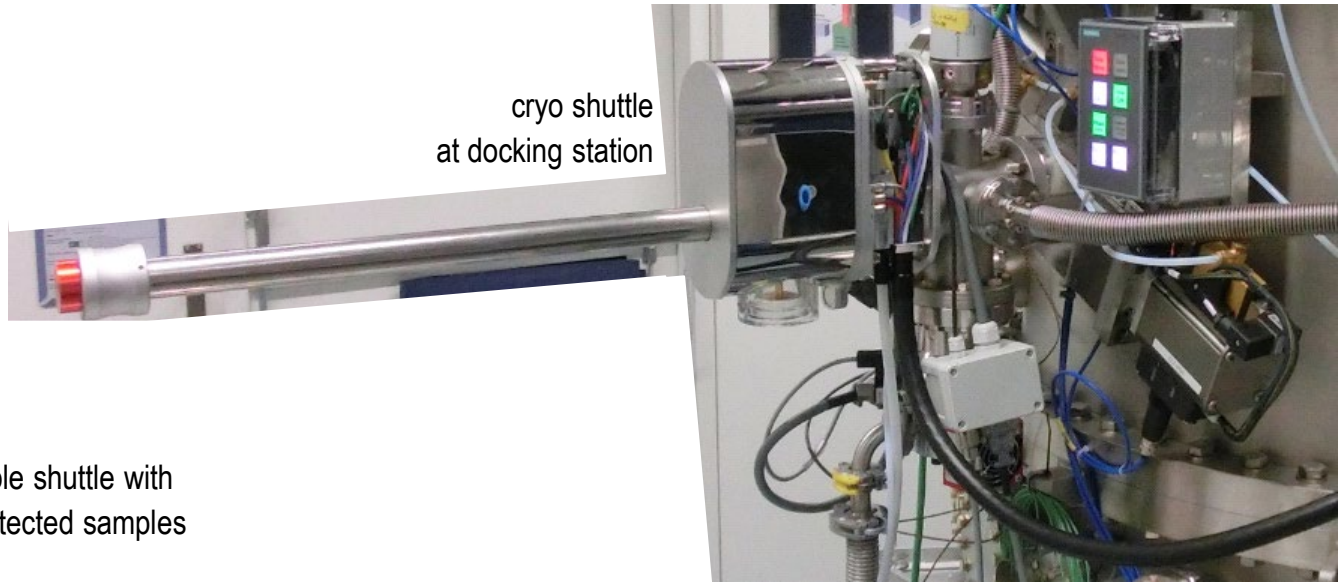
Sample transfer

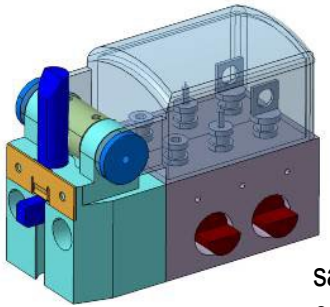


Based on Leica VCT 100

→ connectivity to existing cryo-equipment at PSI and ETH Zurich such as cryo FIB and SEM

cryo shuttle
at docking station

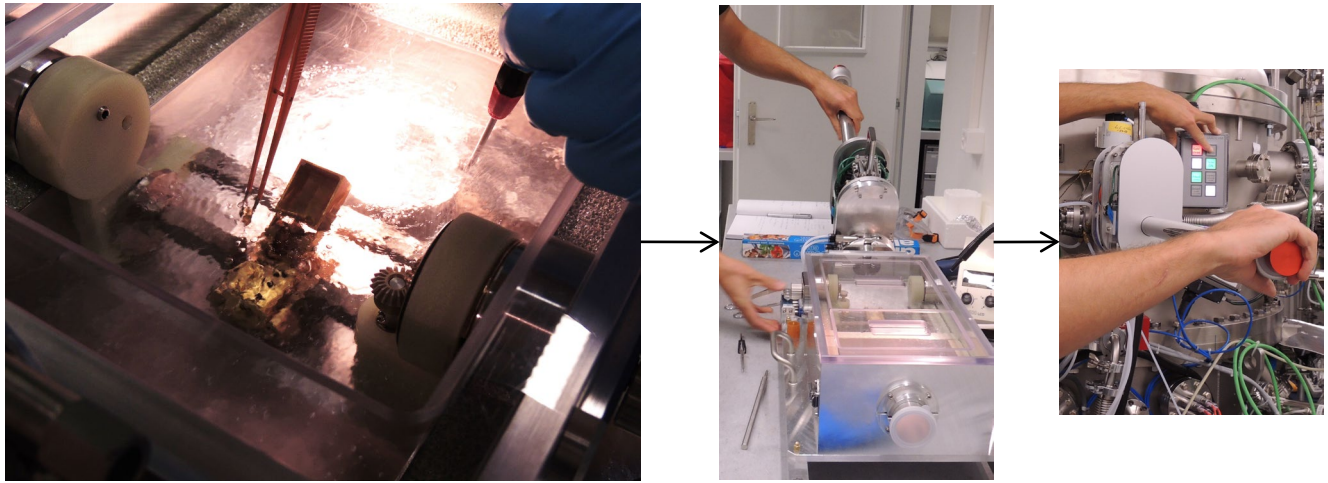




sample shuttle with
6 protected samples

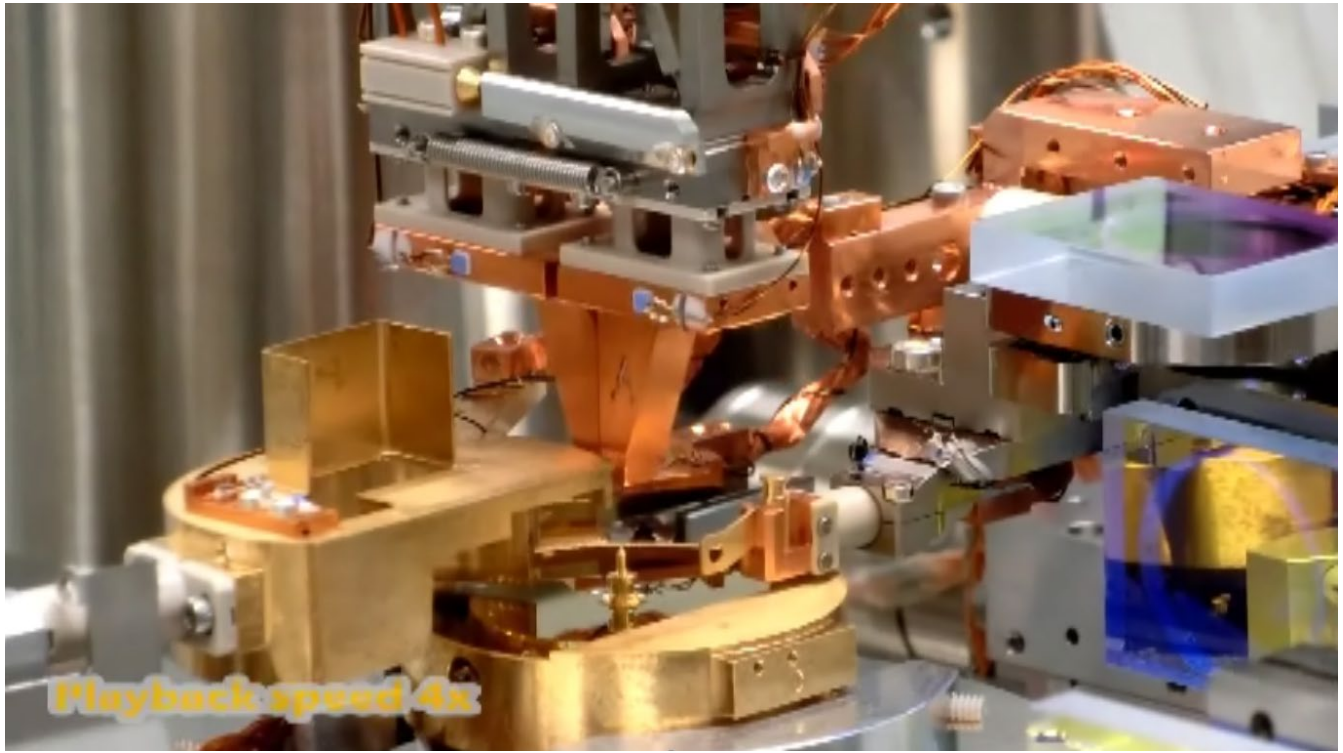
Loading biological samples

Ice crystals destroy biological samples. Freezing at high cooling rate and cryogenic protectants prevents the formation of crystalline ice. After that the sample has to remain below -130 degC!



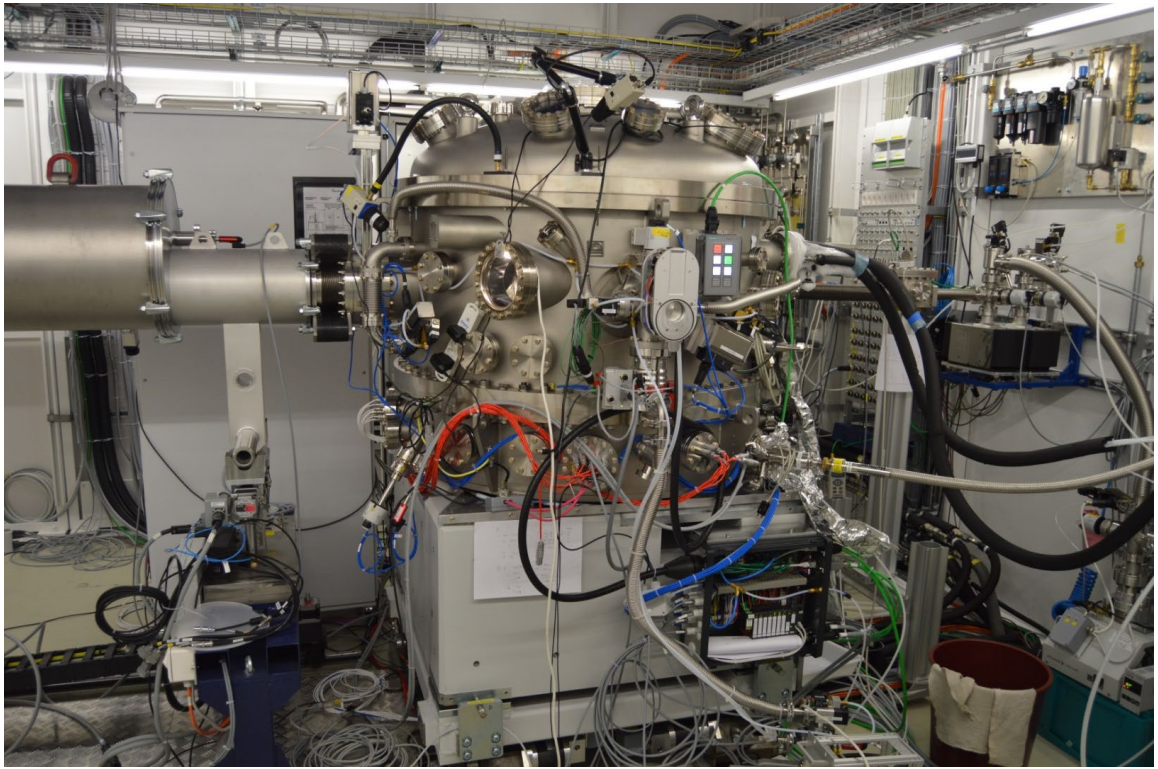
Cryogenic sample transfer

The gripper and parking lot are cooled to ~110 K.



PAUL SCHERRER INSTITUT

OMNY at the beamline



Beetle scale structure: optimized by evolution

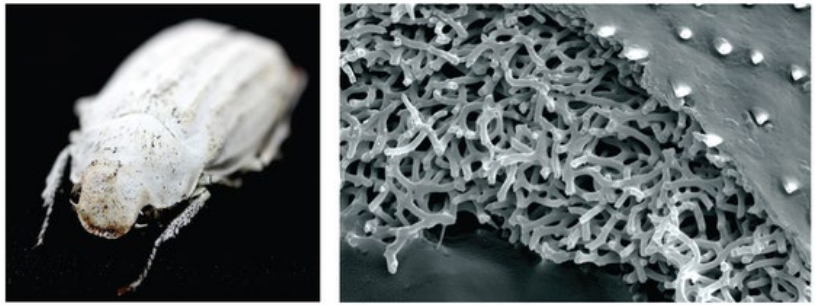
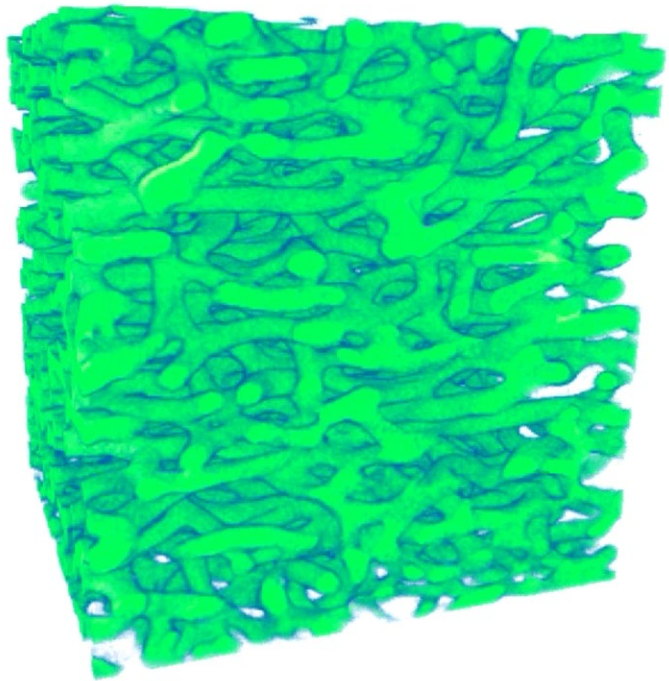


Figure from D. S. Wiersma, Nat. Photonics **7** (2013) 188

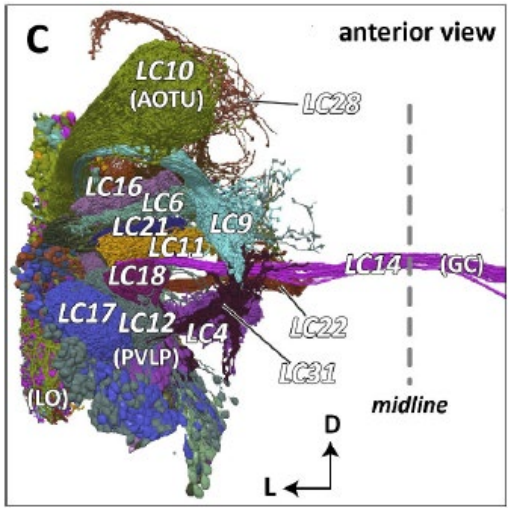
- *Cyphochilus* beetle scale specimen prepared by focus ion beam milling
- OMNY cryo stage at 92 K in vacuum
- 3D resolution: 28 nm
- Nanophotonic simulations confirm that the structure is optimized by evolution

B. D. Wilts *et al.*, Adv. Mater. **30**, 1702057 (2018)



About 7 x 7 x 7 μm^3

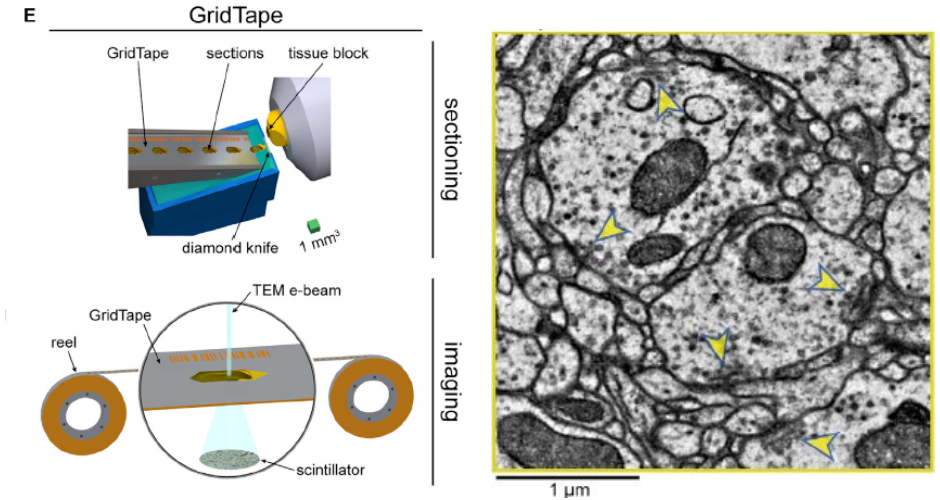
Image the connectome to understand the brain



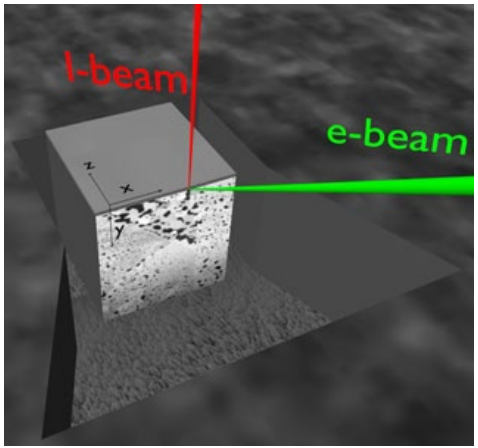
A large part of a fly brain
~250 μm size
L.K. Scheffer *et al.*, eLife **9**, e57443 (2020)

- The connectome is the map of neural connections in the brain
- The connectome is to neuroscience what the genome is to molecular biology
- A full connectome requires imaging a full brain at synaptic resolution (< 20 nm)
- The technology is under development and relies on electron microscopy

Electron microscopy on brain tissues



J.S. Phelps *et al.*, Cell **184**, 759-774 (2021)

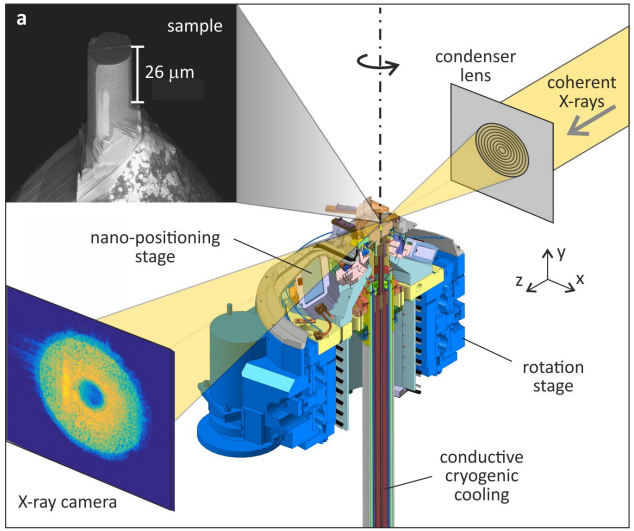


FIB techniques
CIME - EPFL

CHALLENGES:

- Destructive
- Missing data due to cutting issues
- Scalability

X-ray ptychographic tomography @ cSAXS



55 nm resolution @ 3e8 Gy

Resolution limited by radiation-induced deformations and mass loss at dose >5e8 Gy

→ Development of new resins

Collaboration with:

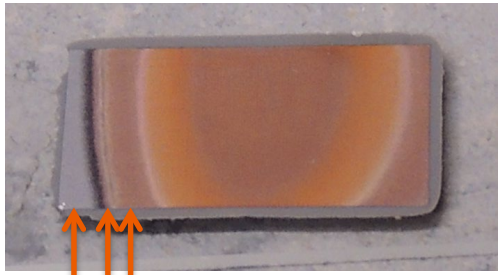
C. Bosch and A. Schaefer (Francis Crick Institute, UK)

A. Pacureanu (ESRF, France)

Artificial intelligence - Intel Core Pentium G3260

Socket LGA 1150, 3MB Cache, **22nm**, 53Watt, inkl. GMA HD Grafikkern (350/1100 MHz GPU), Intel HD, inkl. Cooler

Inhomogeneous polishing trying to remove copper layer and interconnects



Copper & interconnects

Active layer

Silicon

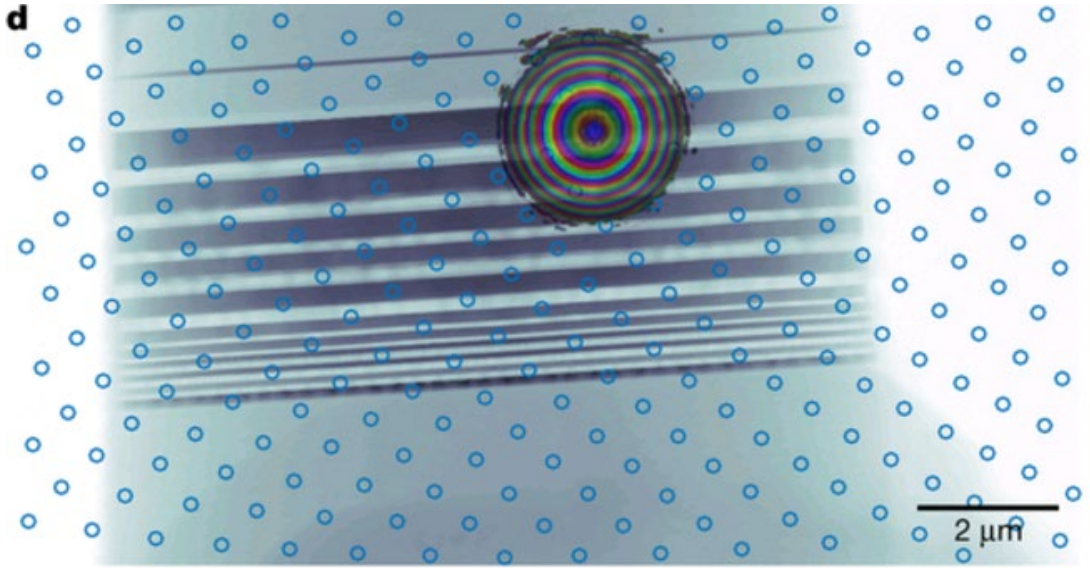
10 micron diameter pillar

Holler *et al.,* Nature 543, 402–406 (16 March 2017)

Intel Core Pentium G3260 (3300) Dual Core

- Scanning step 0.5 µm
- Reconstructed beam about Ø 2 µm

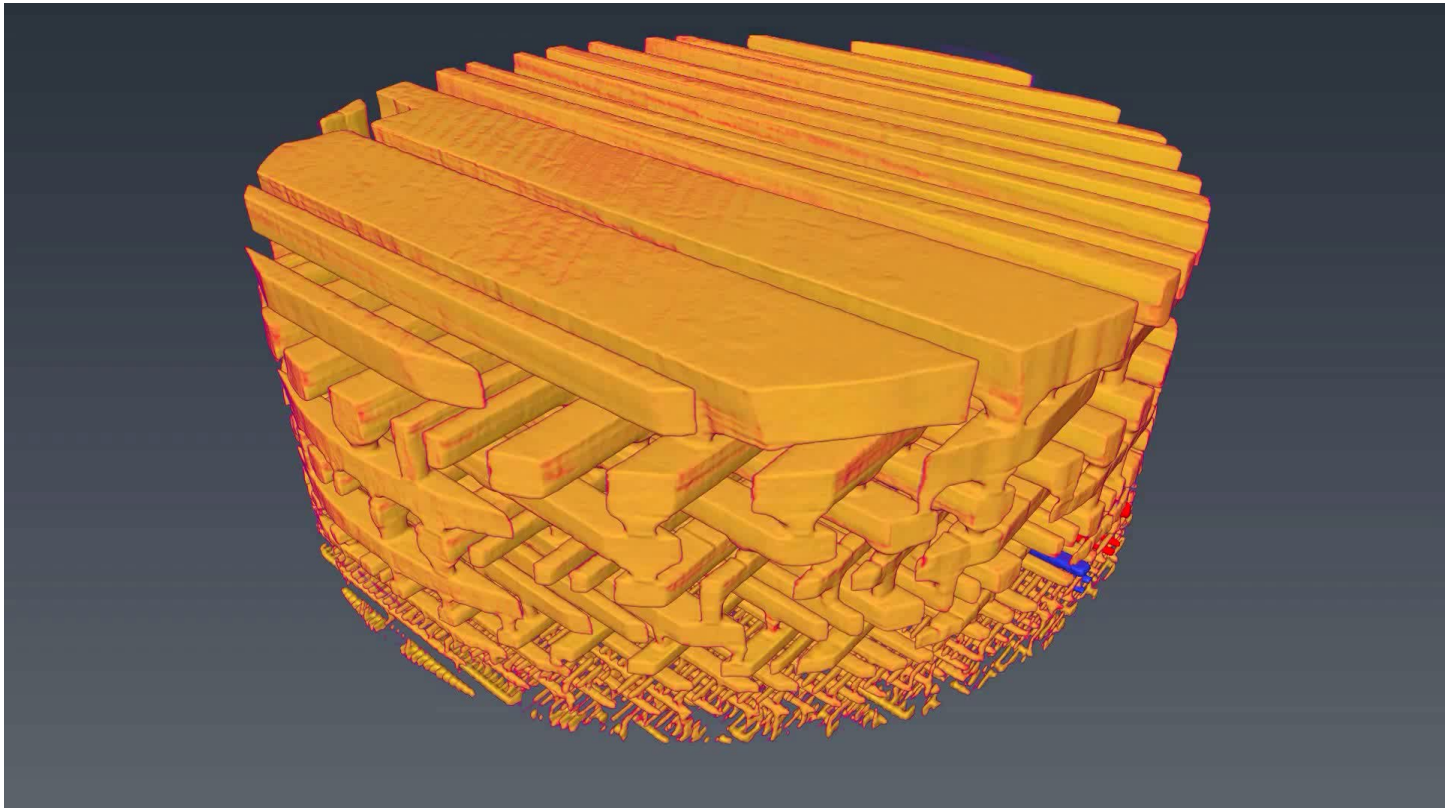
- Projection resolution ~14 nm



For the tomogram 1200 of such projections were acquired

Holler *et al.,* Nature 543, 402–406 (16 March 2017)

Intel Core Pentium G3260 (3300) Dual Core

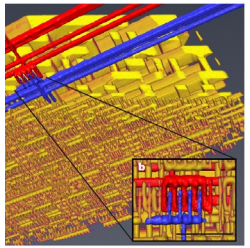


3D resolution: 14.6 nm

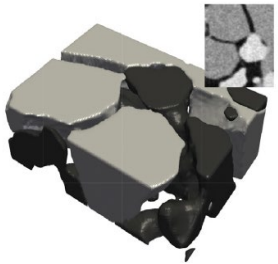
Holler *et al.,* Nature 543, 402–406 (16 March 2017)

Summary for ptychographic tomography

Hard samples

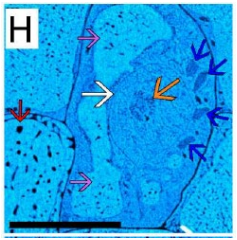


- Radiation-hard samples with small, high-contrast features
- <5 nm resolution

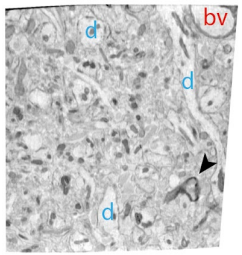


- Ex-situ experiment
- 50 nm resolution
- 4h per tomogram
- Limited by time

Soft samples



- Native frozen hydrated tissue
- Dose of 1.5×10^{7} Gy
- 90 nm resolution
- Limited by coherent flux

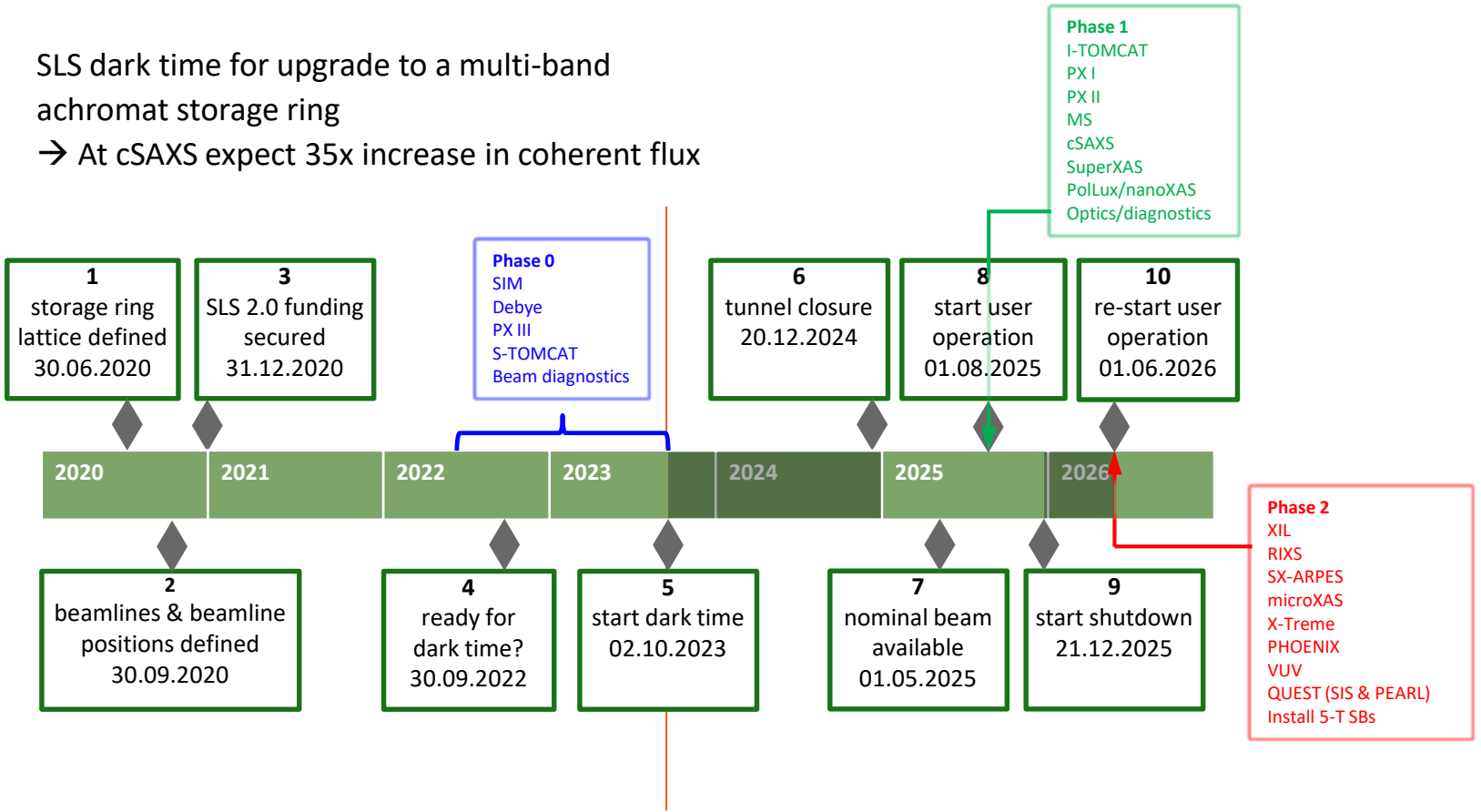


- resin-embedded, stained biological tissue
- Dose of 2×10^{8} Gy
- 54 nm resolution
- Limited by radiation damage

SLS 2.0 upgrade timeline

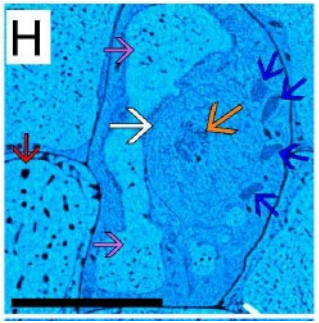
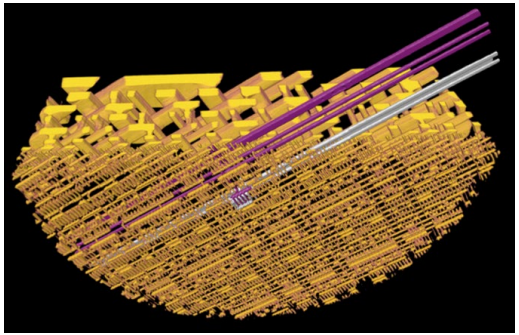
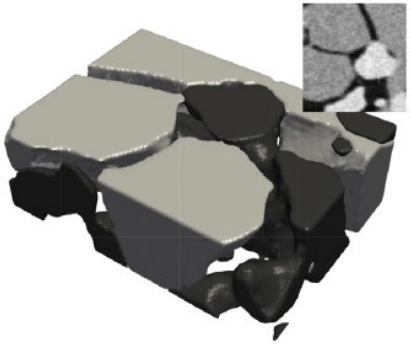
SLS dark time for upgrade to a multi-band achromat storage ring

→ At cSAXS expect 35x increase in coherent flux



Slide courtesy of Phil Willmott

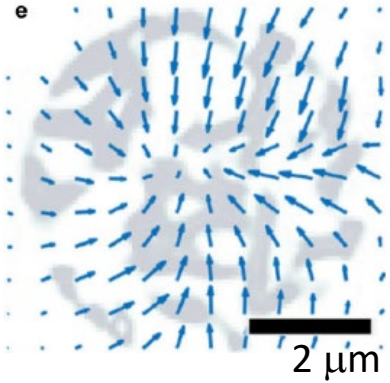
Feasible in SLS 2.0?

BETTER RESOLUTION	LARGER VOLUMES	3D MOVIES
Biological tissue imaging at 30 nm 3D resolution	Chip imaging with 5 nm res. over 150×150 μm^2 area	*In-operando* SOC electrode degradation in 2 min shots
		
H. Help *et al.*, BioRXiv https://doi.org/10.1101/767558	M. Holler *et al.*, Nature **543**, 402 (2017)	S. De Angelis *et al.*, J. Power Sources **360**, 520 (2017)
10^2 times more dose than current dose ~ 10^7 Gy	10^2 times faster scanning and laminography	10^2 times faster scanning, could be improved with sparsity?
Radiation damage?	Stability over large areas?	Scanning speed?

Challenges and developments

Deformation of sample during acquisition

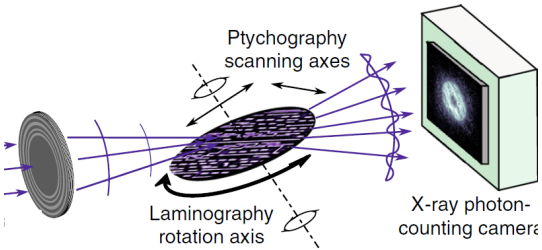
Non-rigid tomographic reconstruction



M. Odstrcil *et al.*, Nat. Commun. **10**, 2600 (2019)

Extension to large sample volume

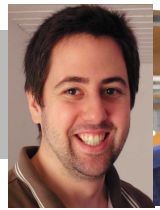
Laminography



M. Holler *et al.*, Nat. Electronics. **2**, 464 (2019)

extended samples in 2D
10-100 µm thick

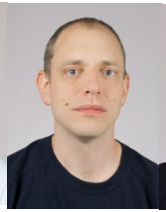
Thanks to all contributors!


Mirko Holler


Manuel Guizar


Ana Diaz


Christian Appel


Andreas Menzel


Nicholas William Phillips


Xavier Donath


Zirui Gao

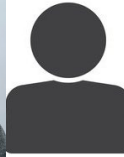

Michal Odstrcil

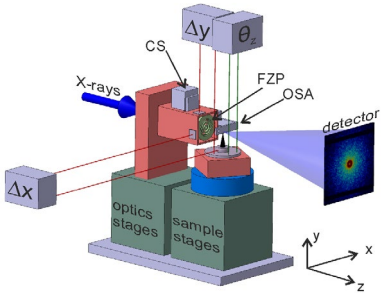
ZEISS

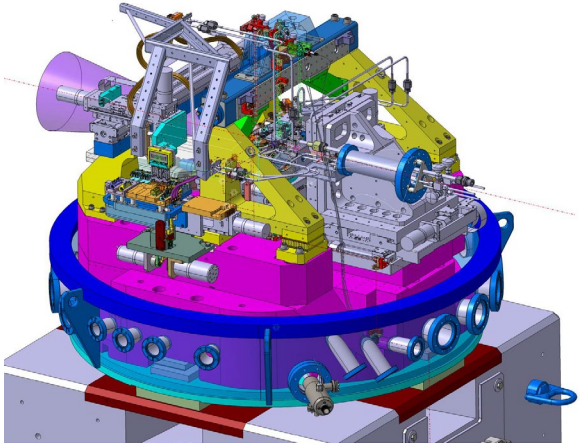

Jörg Raabe

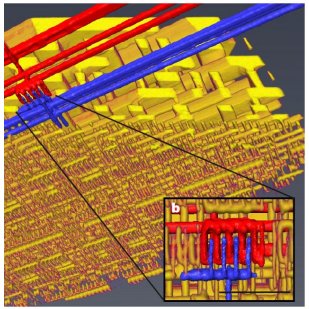

Oliver Bunk


Gabriel Aeppli


and many more







THANK YOU FOR YOUR ATTENTION!