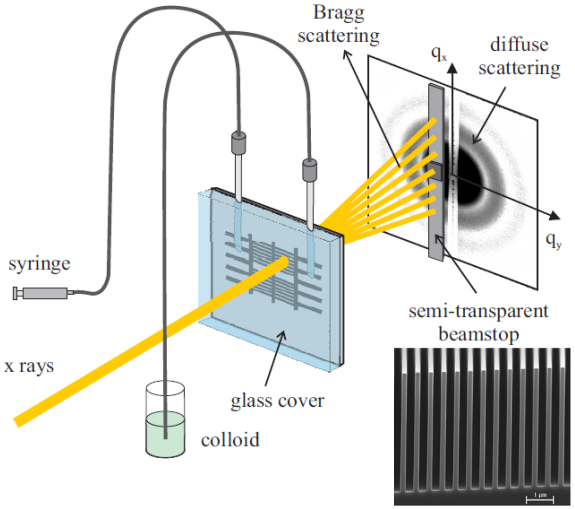
X-ray scattering to probe nanostructures and tissues at the Swiss Light Source

Invited lecture at the EPFL course “Physical optics and advanced imaging” by Prof. Guizar-Sicairos

Ana Diaz
Lausanne, 29th October 2025

Let me introduce myself

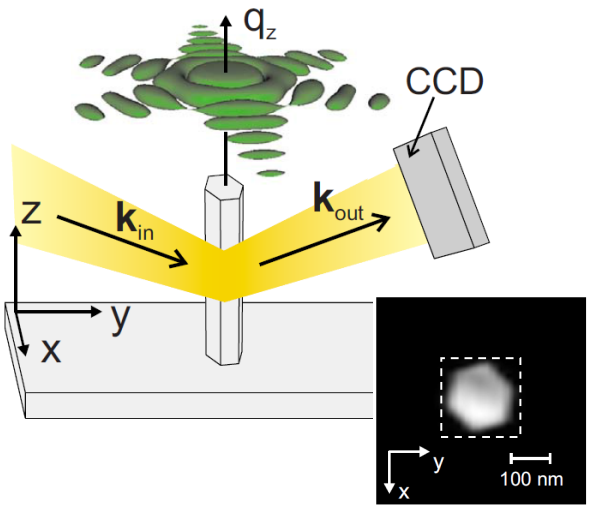


PhD at the Paul Scherrer Institute

Nanoscale ordering of confined fluids

Prof. Friso van der Veen

Christian David



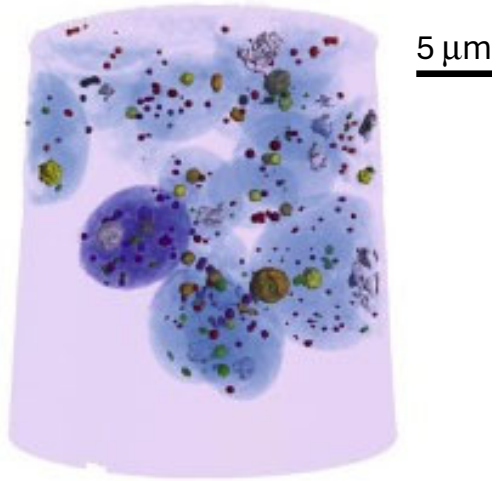
Postdoc at ID01 beamline, ESRF

Bragg coherent diffraction imaging of epitaxial nanostructures

Till Hartmut Metzger

Prof. Bauer (Uni. Linz, Austria)

Julian Stangl (Uni. Linz, Austria)



Beamline scientist at cSAXS, SLS

Paul Scherrer Institute

X-ray ptychography

Andreas Menzel

Manuel Guizar-Sicairos

Mirko Holler

Part I: small-angle X-ray scattering - SAXS

Basic concepts of X-ray scattering

- Coherent vs. incoherent scattering
- Isotropic vs. anisotropic scattering

Classical SAXS

- Average structure of nanoparticles or biomolecules

Scanning SAXS

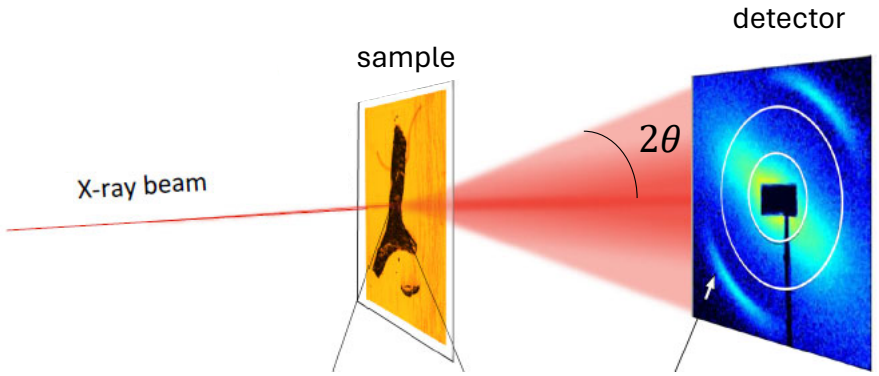
- Structure of hierarchical bio-materials
- Example: collagen in bone or teeth

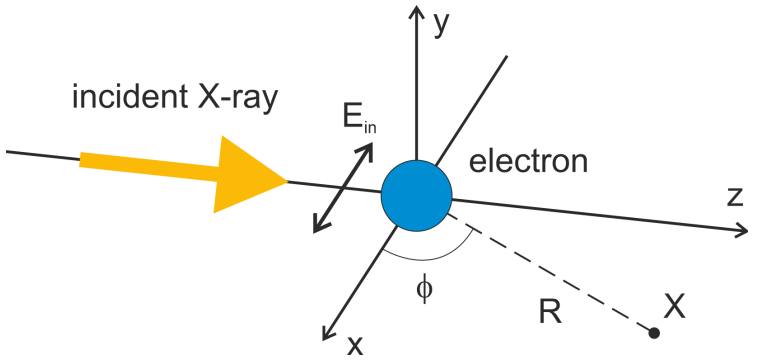
SAXS tensor tomography

- Extension of scanning SAXS to bulk 3D samples
- Bone, brain

Basic concepts of X-ray scattering

X-ray scattering





M. Liebi *et al.*, Nature **527**, 349 (2015)

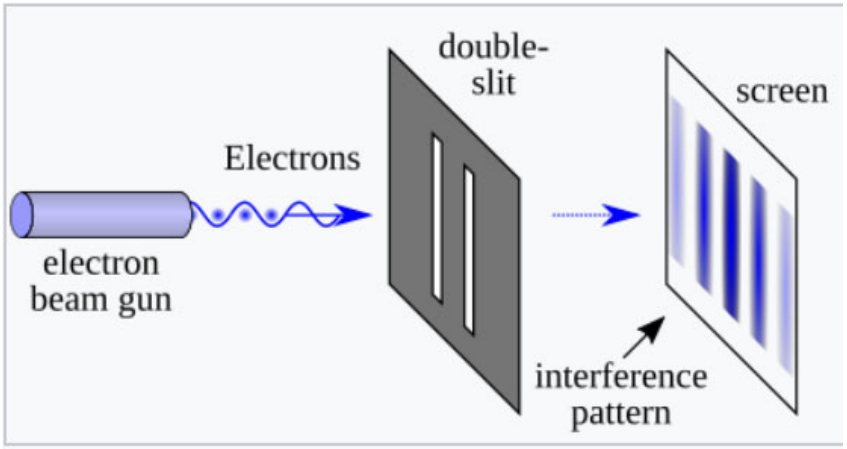
Observation

- X-rays going through a sample in transmission are scattered at different angles
- With a detector placed downstream we record the scattered intensity
- Nanostructures (1-100 nm) $\Rightarrow 2\theta < 4°$

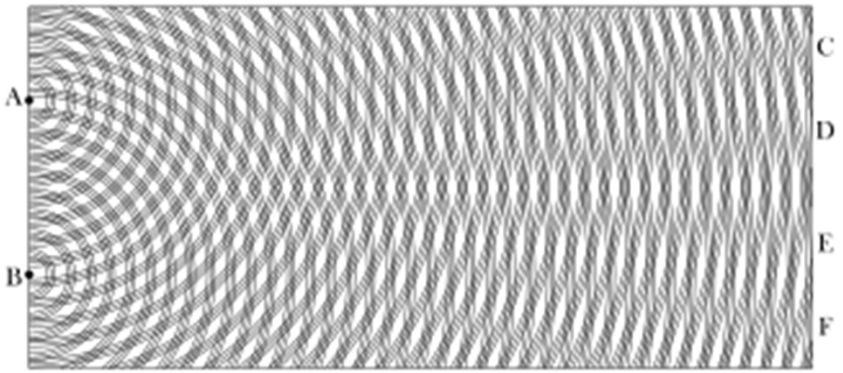
Physics

- **Elastic scattering**: equal incoming and scattered photon energy
- X-rays are scattered by the electrons in the material

Scattering by a double slit



https://en.wikipedia.org/wiki/Double-slit_experiment



Observation

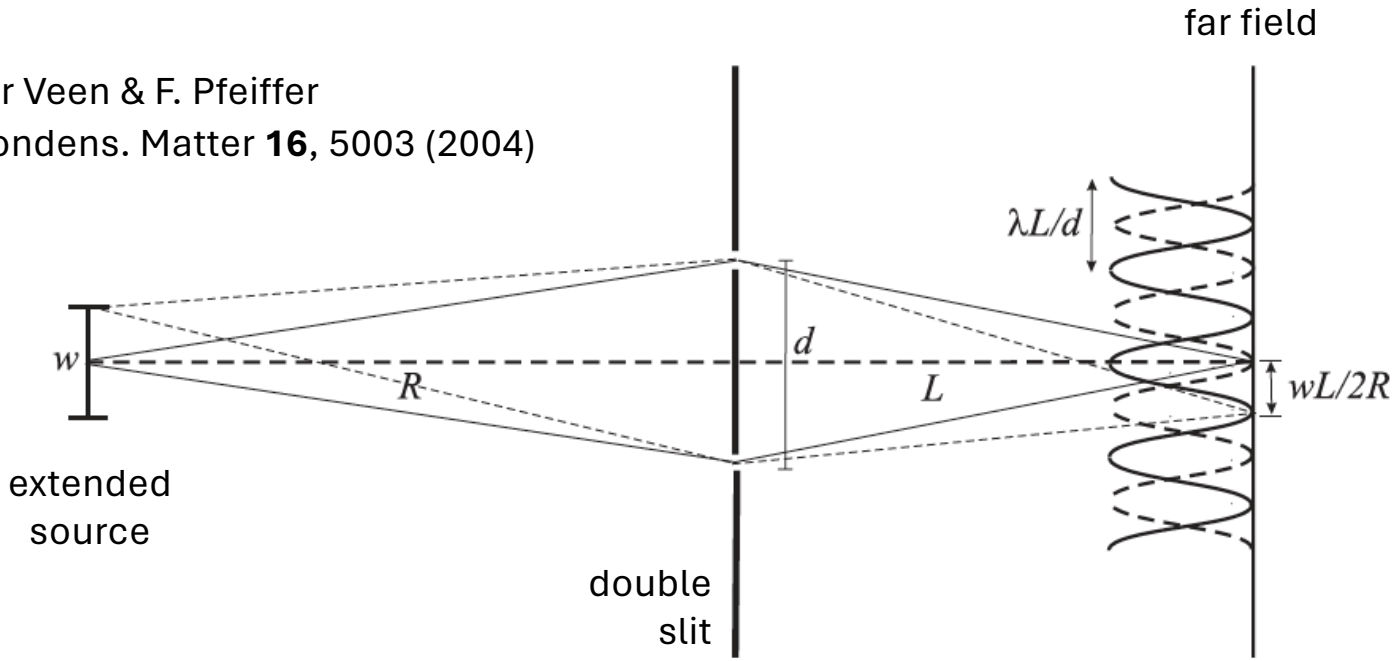
- Particles sent through a double slit produce a periodic intensity pattern on a detector placed downstream
- Particles can be electrons, photons, etc...

Explanation by Thomas Young

- Assume wave nature of particles
- Constructive and destructive **interference**

Transversal coherence

J. F. Van der Veen & F. Pfeiffer
J. Phys.: Condens. Matter **16**, 5003 (2004)

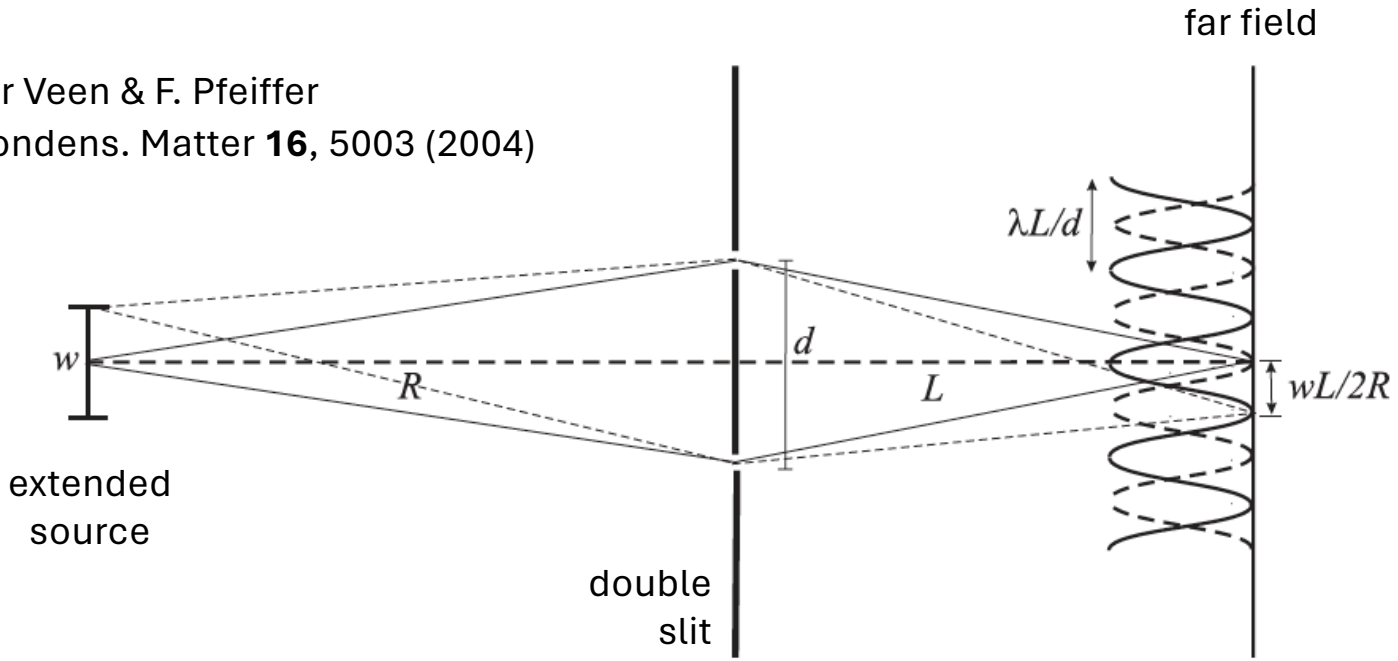


Transversal coherence length: $\xi_t = \frac{\lambda R}{w}$

Maximum distance between two slits such that they produce constructive interference when illuminated by an extended source size

Transversal coherence

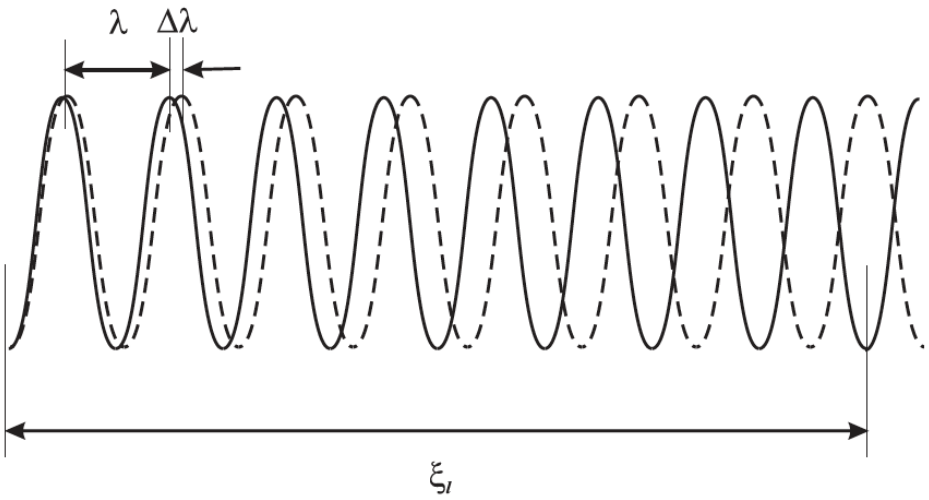
J. F. Van der Veen & F. Pfeiffer
J. Phys.: Condens. Matter **16**, 5003 (2004)



Transversal coherence length: $\xi_t = \frac{\lambda R}{w}$

λ = 1 Å
R = 50 m
w_v = 20 µm ➔ ξ_v = 250 µm
w_h = 200 µm ➔ ξ_h = 25 µm

Longitudinal coherence

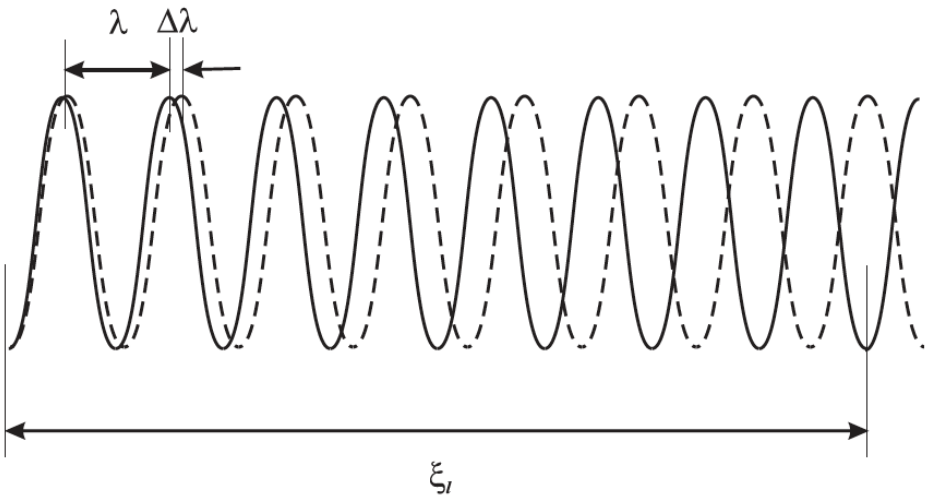


J. F. Van der Veen & F. Pfeiffer
J. Phys.: Condens. Matter **16**, 5003 (2004)

Longitudinal coherence length: $\xi_l \simeq \frac{1}{2}\frac{\lambda^2}{\Delta\lambda}$

"Propagation of two waves with wavelengths λ and λ + Δλ. The longitudinal coherence length is defined as the distance over which the phase difference between the two waves is π."

Longitudinal coherence

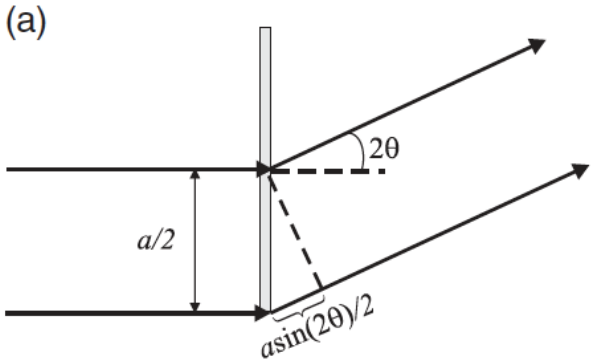


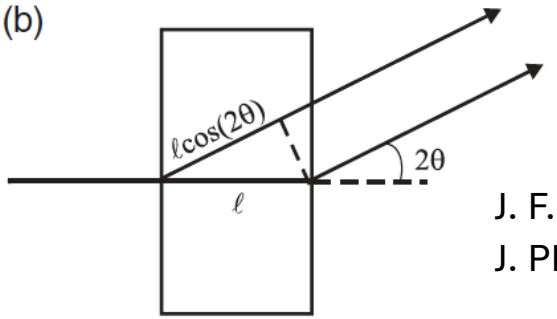
J. F. Van der Veen & F. Pfeiffer
J. Phys.: Condens. Matter **16**, 5003 (2004)

Longitudinal coherence length: $\xi_l \simeq \frac{1}{2}\frac{\lambda^2}{\Delta\lambda}$

Si (111) monochromator: $\Delta\lambda/\lambda = 1.3\times10^{-4}$
λ = 1 Å ➔ $\xi_l \approx$ 400 nm

Implications of longitudinal coherence





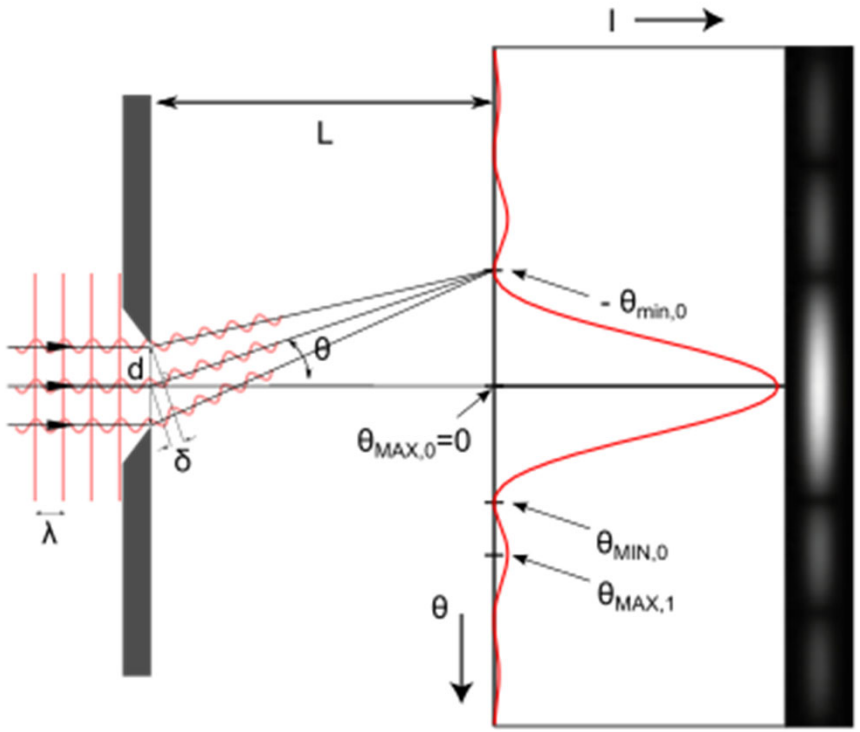
J. F. Van der Veen & F. Pfeiffer
J. Phys.: Condens. Matter **16**, 5003 (2004)

$$PLD = \frac{1}{2} a \sin(2\theta) < \xi_l$$

$$PLD = l - l\cos(2\theta) < \xi_l$$

A condition for coherence is that the path length difference (*PLD*) between points scattered from different points at the sample is smaller than the longitudinal coherence length ξ_l

Physics of X-ray scattering in the far field

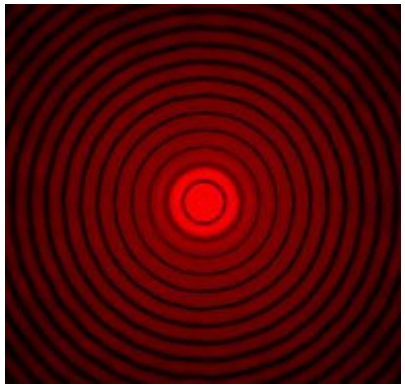


https://en.wikipedia.org/wiki/Diffraction

X-ray scattering of an aperture

The X-ray scattering of a coherently illuminated object in the far field corresponds to the **Fourier transform** of the object

- Far field: $L >> d^2/\lambda$
- Object: electron density distribution



Scattering (or diffraction) from a coherently illuminated circular object

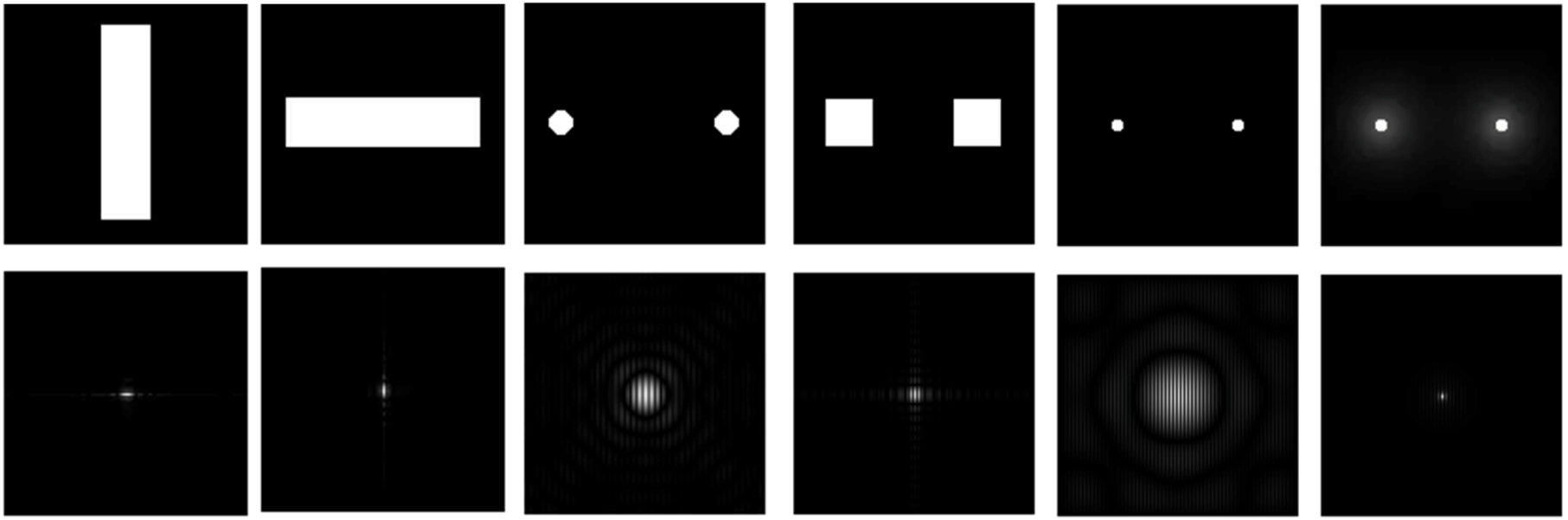
https://en.wikipedia.org/wiki/Airy_disk

Examples of 2D Fourier transforms

PSI

object

Fourier transform

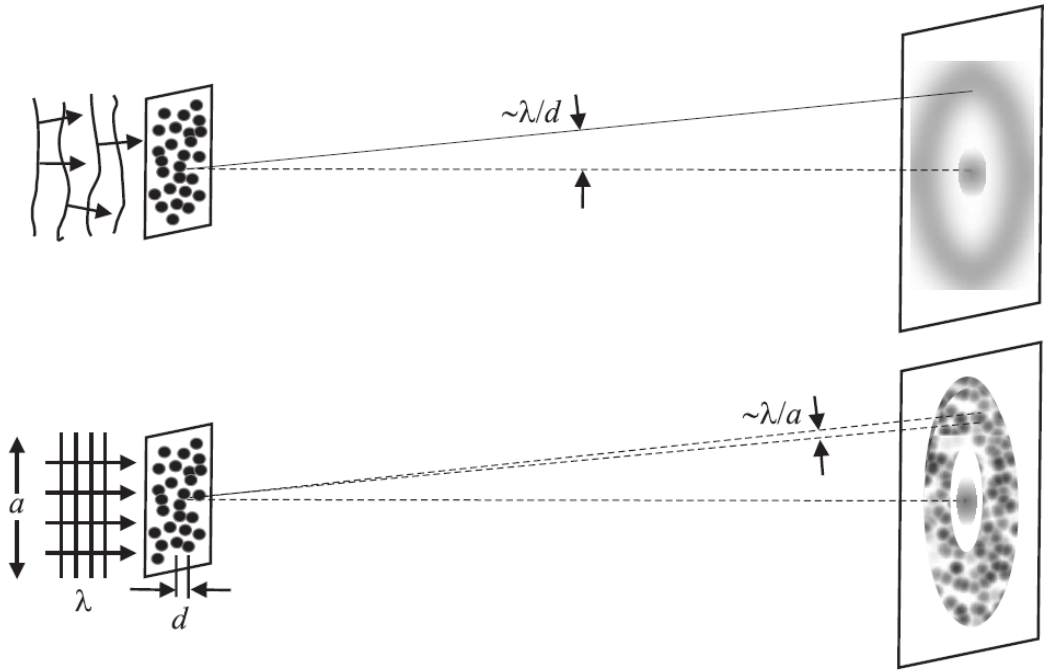


https://albertyumol.github.io/FourierTransformApplication/#

Partially vs “fully” coherent scattering

J. F. Van der Veen & F. Pfeiffer
J. Phys.: Condens. Matter **16**, 5003 (2004)

X-ray scattering from a disordered medium in the far field



$d < \xi_l < a$

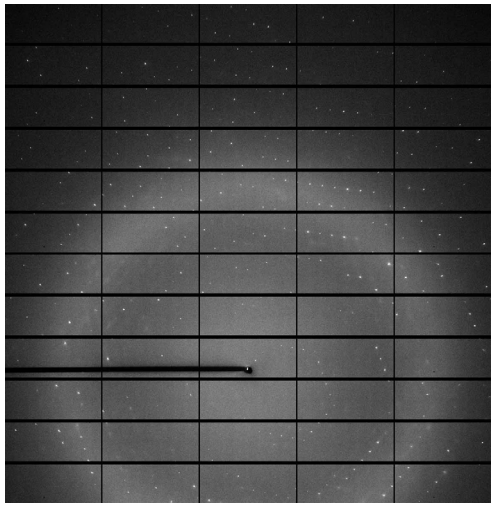
$\xi_l > a$

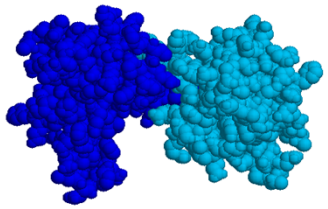
d: average particle distance
a: object size

Typical examples of X-ray scattering

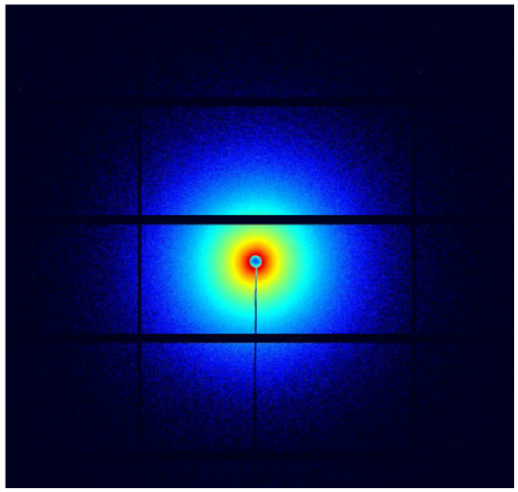
PSI

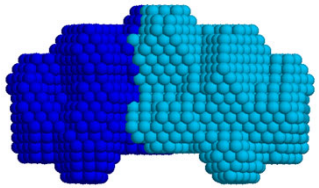
protein crystallography (PX)



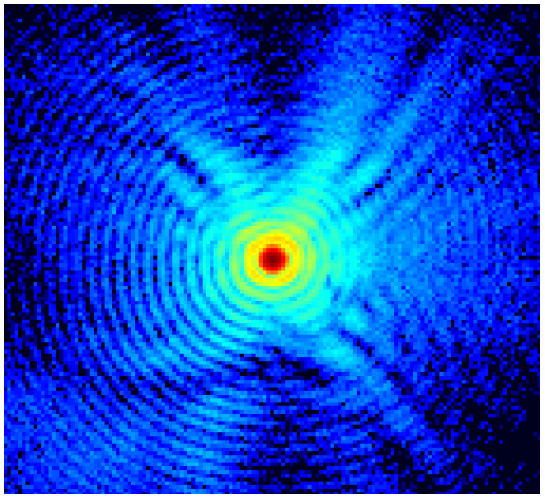


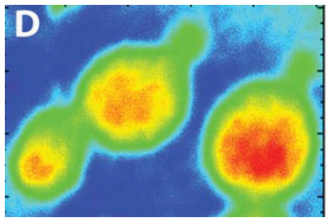
small-angle x-ray scattering (SAXS)





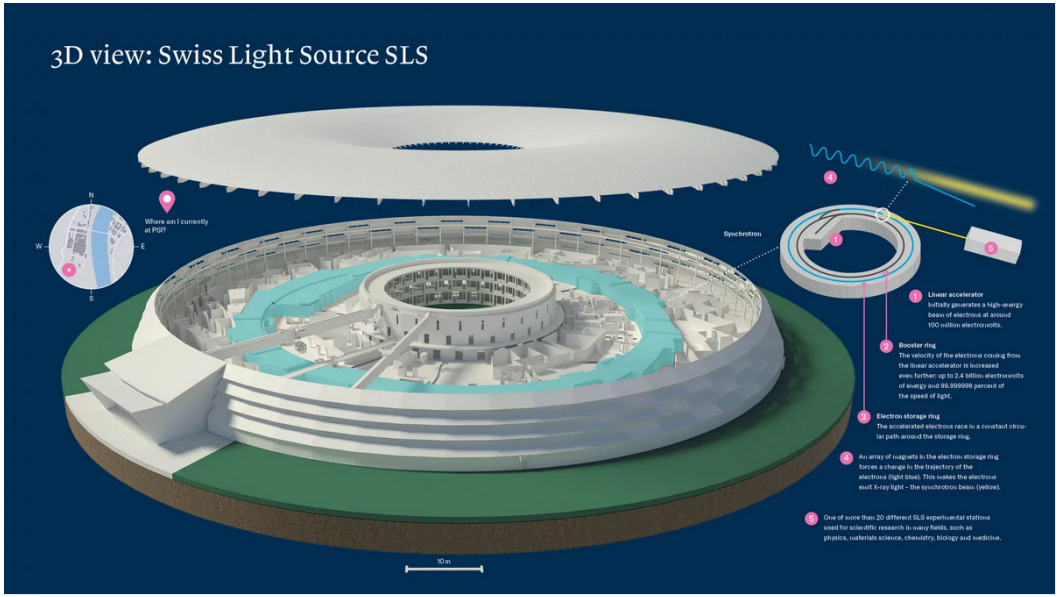
coherent diffraction imaging (CDI)





resolution: ~ 1 Å ~ 1 nm ~ 10 nm

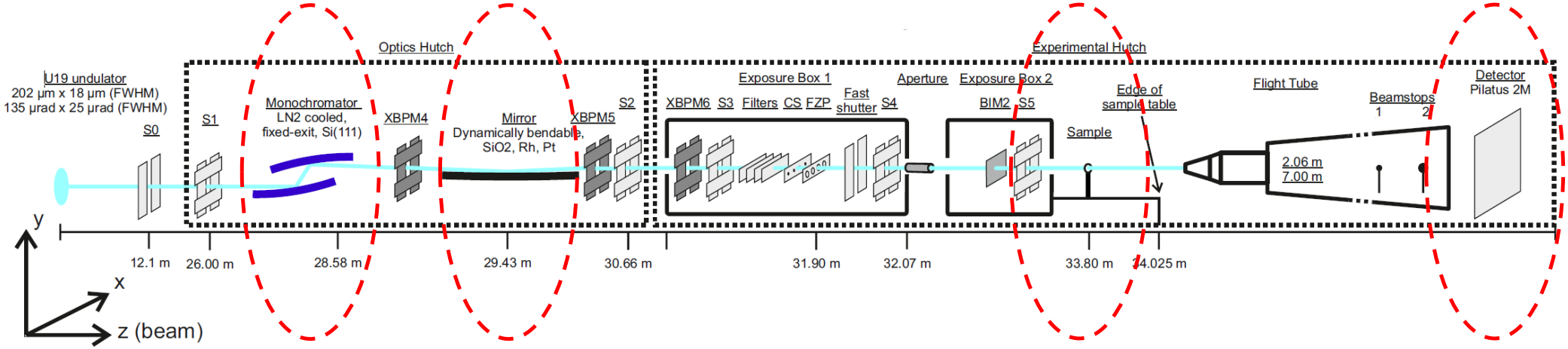
The Swiss Light Source



- The Swiss Light Source
- About 1h from Zurich
- 2.4 GeV electron storage ring

- Laboratory for Macromolecules and Bioimaging:
  - Tomcat
  - PX
  - cSAXS

The cSAXS beamline at the Swiss Light source



Monochromator:
Energy 4.5-18 keV
Horiz. focusing

Mirror:
Vert. focusing

Sample table

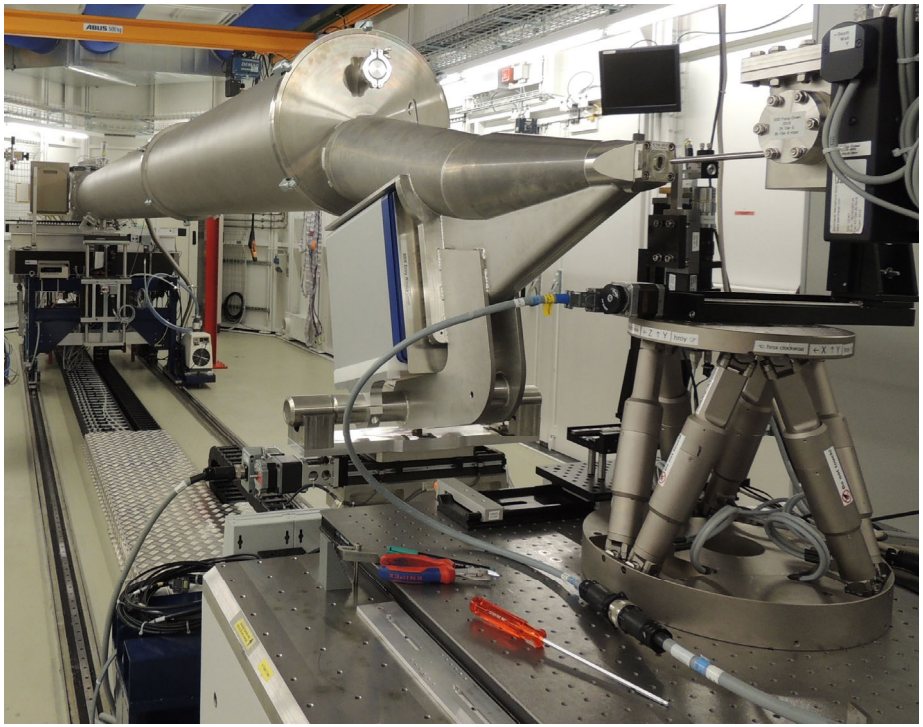
Detector

34 m

2 m or 7 m

The cSAXS beamline at the Swiss Light Source

cSAXS: coherent small-angle X-ray scattering



Photon energy: 5-20 keV

Main techniques:
- Spatially-resolved SAXS
- Ptychography

Very broad applications:
- Biology
- Material science
- Energy
- X-ray optics metrology

The cSAXS team

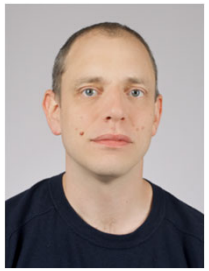

Mads A. Carlsen


Ana Diaz


Xavier Donath


Kazu Hirosawa


Mirko Holler


Andreas Menzel

Huaiyu Chen

Postdoc position

Alumni

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T. Aidukas
N. Phillips
C. Appel
M. Guizar-Sicairos
J. Ihli
Z. Gao
M. Verezhak
K. Wakonig
D. Karpov
M. Odstrcil
V. Lütz-Bueno
E. Tsai
M. Liebi
I. Rajkovic
R. Jacob
J. da Silva
J. Han
O. Bunk
H. Deyhle
T. Ikonen
C. Kewish
F. Pfeiffer
P. Thibault
M. Dierolf

Collaborations within PSI

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C. David (CPS)
E. Föjd (detectors)
M. Guizar-Sicairos (CPS & EPFL)
G. Aeppli (CPS)
M. Liebi (CPS & EPFL)
K. Wakonig (CSD)
C. Appel (CSD)
S. Shahmoradian (CLS)
T. Ishikawa (CLS)
E. Müller (CLS)
A. Wanner (CLS)
G. Schertler (CLS)
V. Panneels (CLS)
B. Rösner (CPS)
H.-C. Stadler (CSD)
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C. Appel (CSD)
K. Wakonig (CSD)

External collaborators (PI's)

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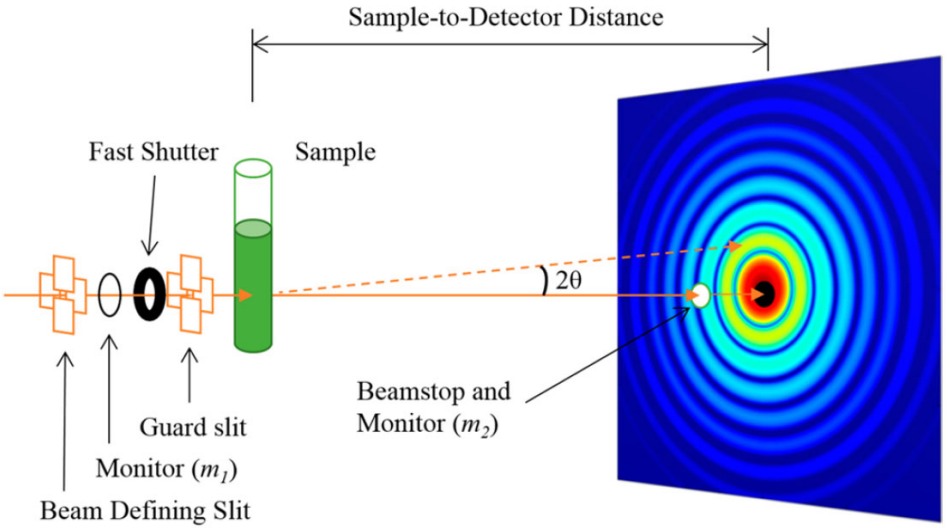
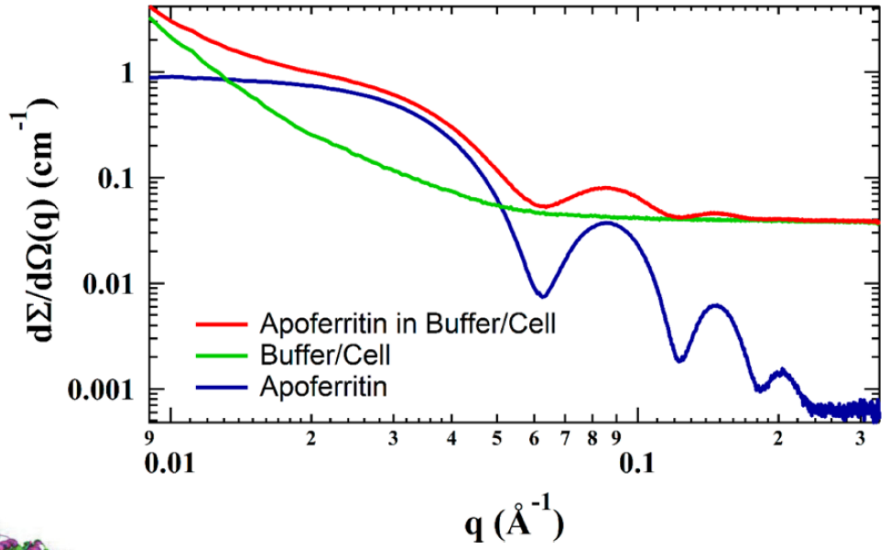
I. Rostami (Uni. Bern, CH)
C. Bosch & A. Schaefer (Francis Crick Institut, UK)
A. Pacureanu (ESRF, France)

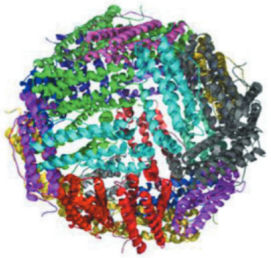
SAXS: average structure of nano-particles

SAXS: small-angle X-ray scattering





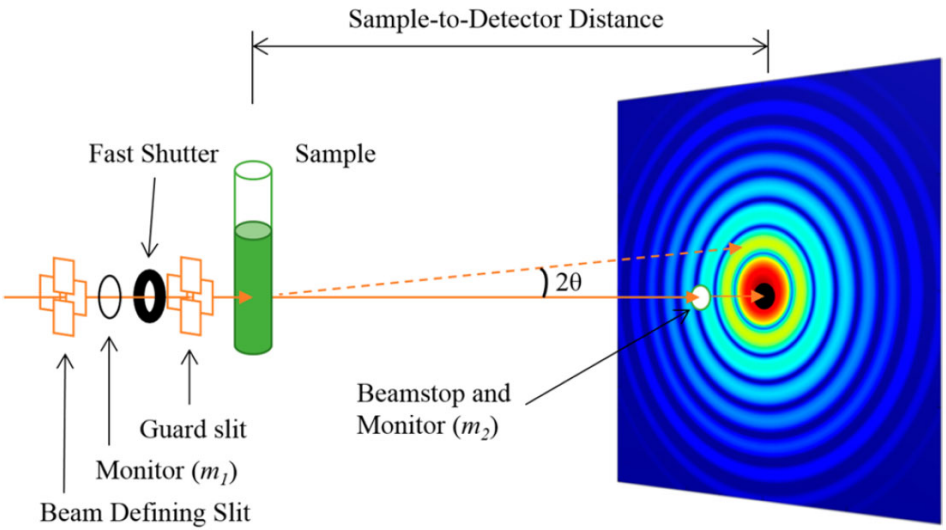
T. Li *et al.*,
Chem. Rev. **116**, 11128-11180 (2016)



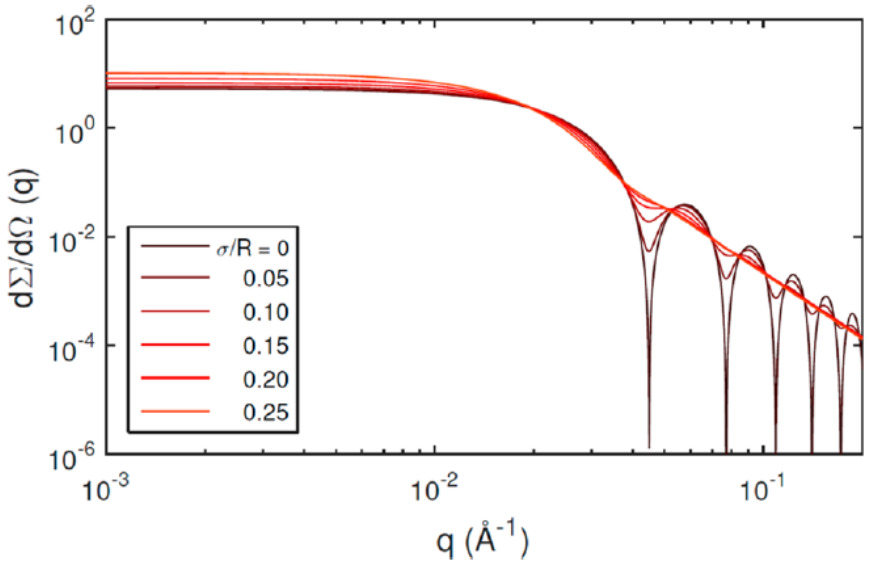
12 nm

Apoferritin structure
ILL Annual Report (2011)

SAXS: small-angle X-ray scattering

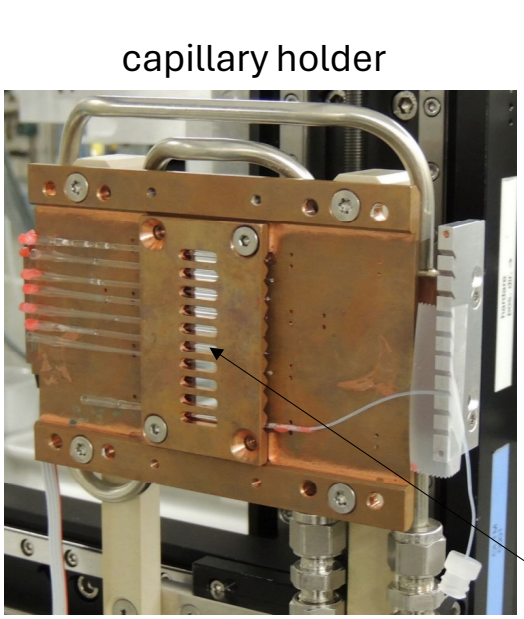


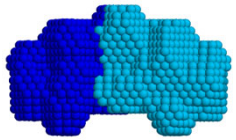
Scattering of spherical particles with varying degree of polydispersity



T. Li *et al.*,
Chem. Rev. **116**, 11128-11180 (2016)

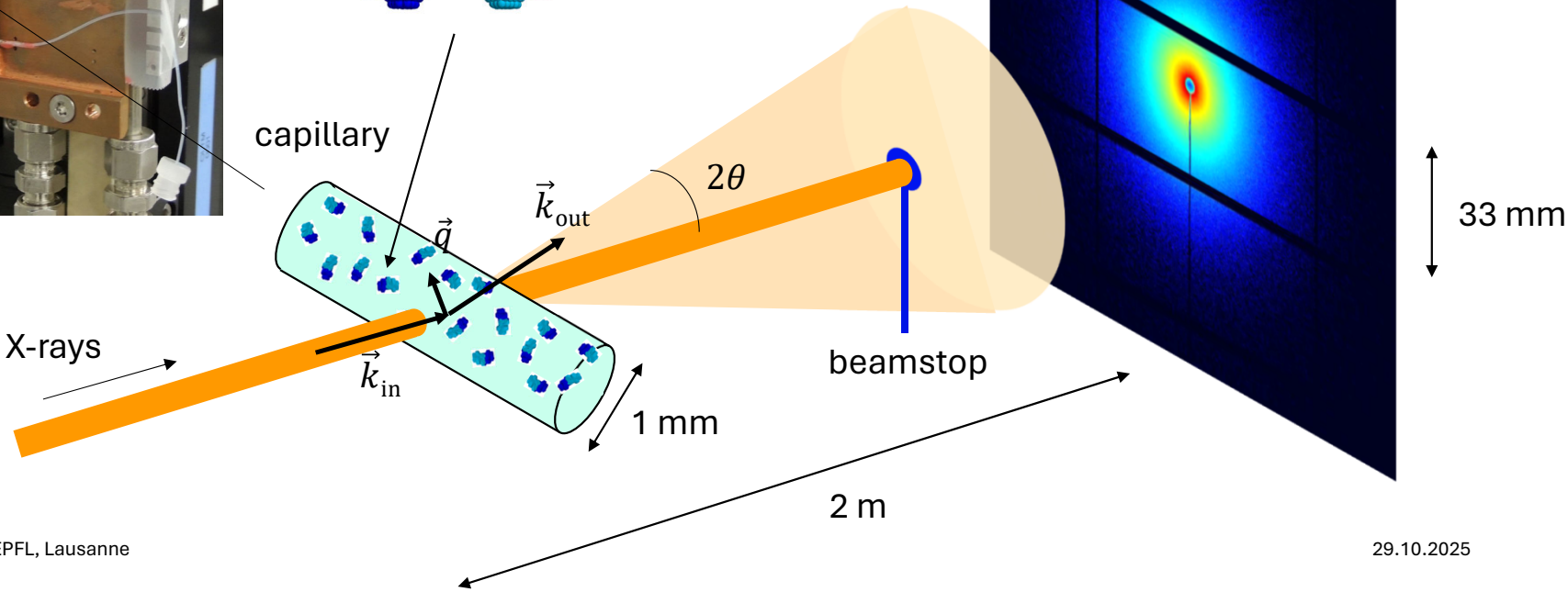
Bio SAXS: protein solutions in glass capillaries



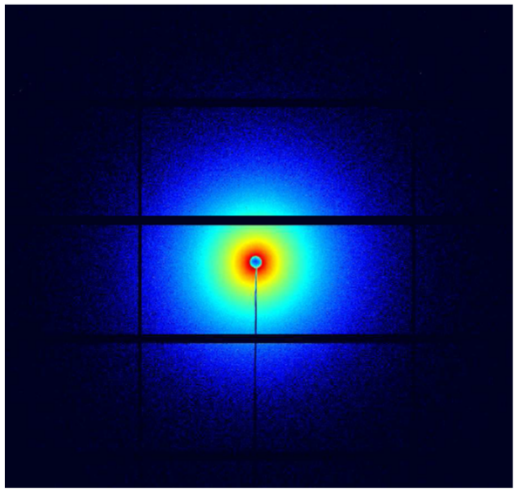


scattering vector:

$$|\vec{q}| = q = \frac{4\pi}{\lambda}\sin\theta$$

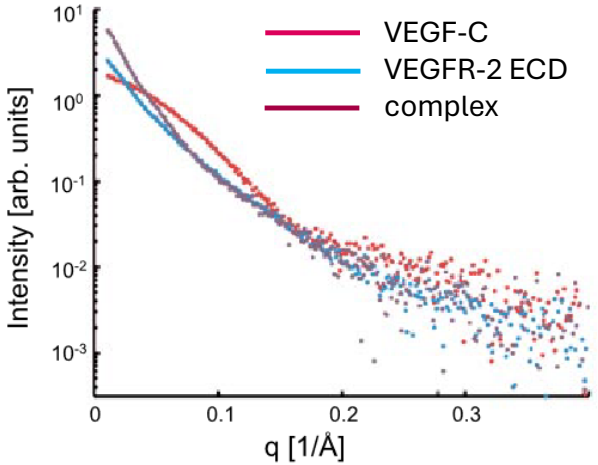


Bio SAXS: data analysis

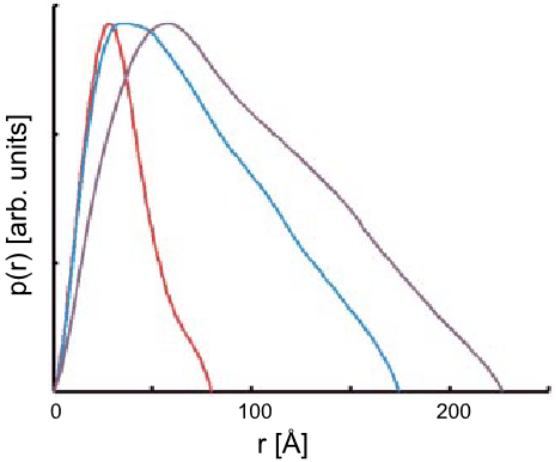


azimuthal symmetry

radially-integrated 1D scattering curves

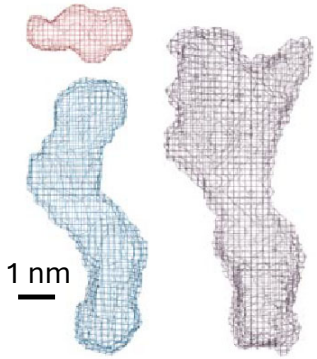


pair distance distribution functions

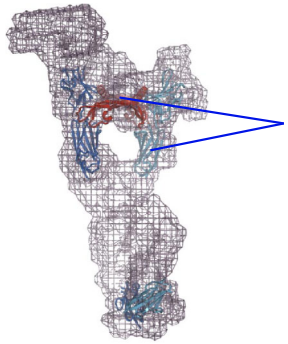


K. Kisko *et al.*,
FASEB J. **25**, 2980-2986 (2011)

Understanding the activation mechanism receptor tyrosine kinases, relevant for clinical applications



Ab initio shape reconstructions



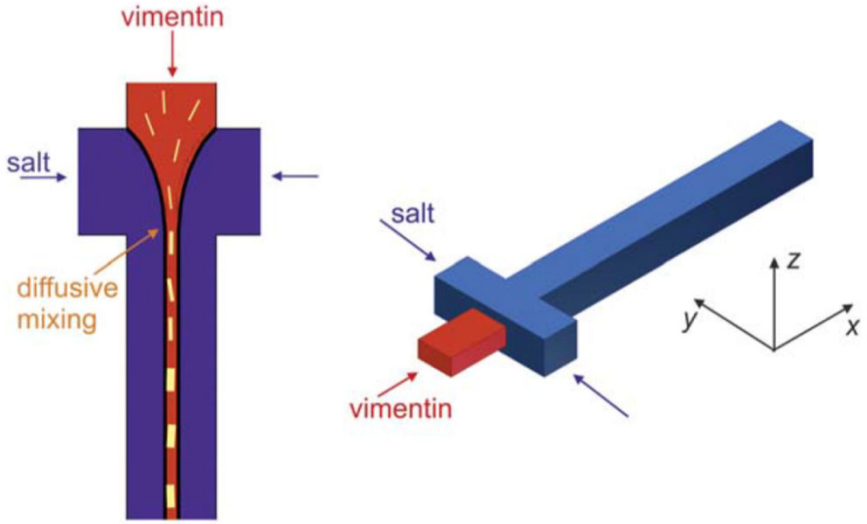
High-resolution structures of some ligand domains from PX

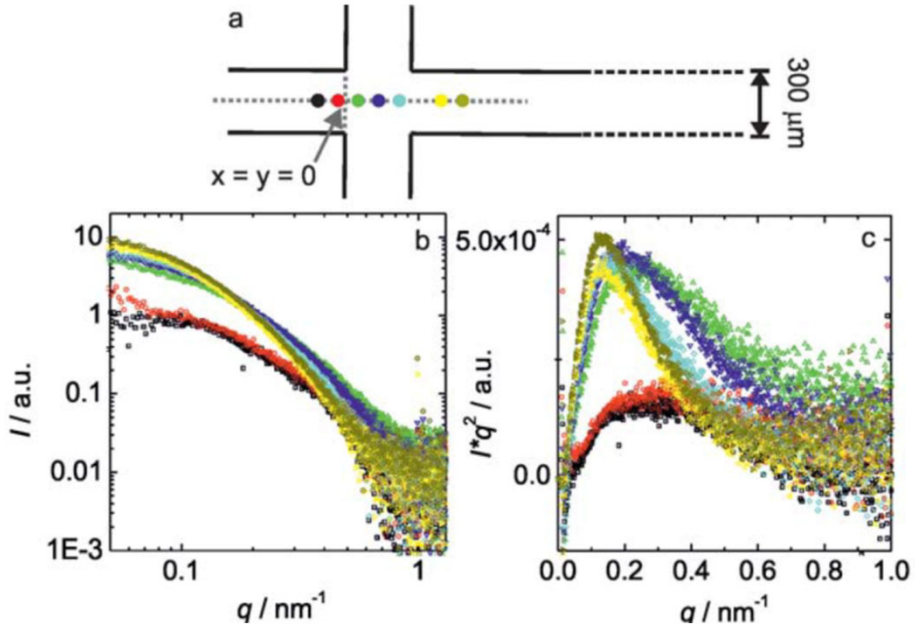
Reaction bio SAXS

Kinetics of intermediate-filaments assembly:

- Sample: human vimentin protein
- Sample environment: microfluidics chip enabling protein jet with varying salt concentrations
- Time scale ~25 msec
- M.E. Brennich, J.-F. Nolting *et al.*, Lab Chip **11**, 708 (2011)

Changes of the mean square radius of gyration perpendicular to filament from 13 nm^2 to 58 nm^2

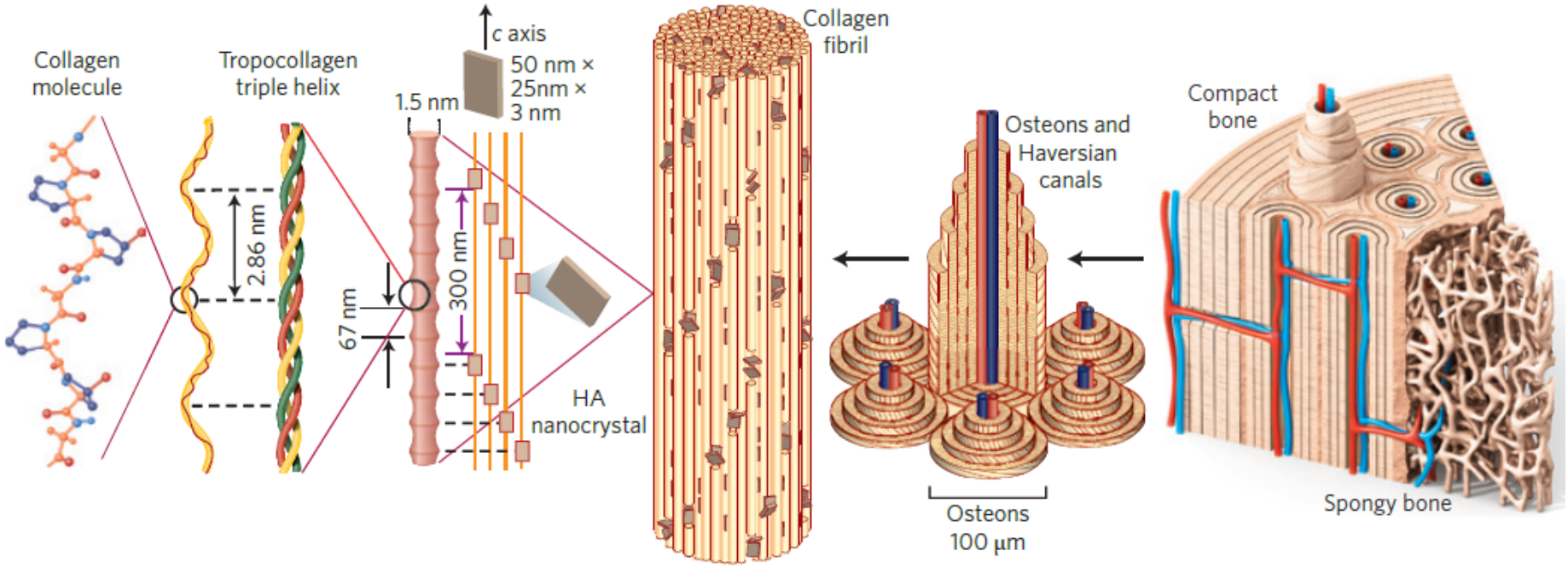




Scanning SAXS: structure of hierarchical bio-materials

Hierarchical structures in biological materials

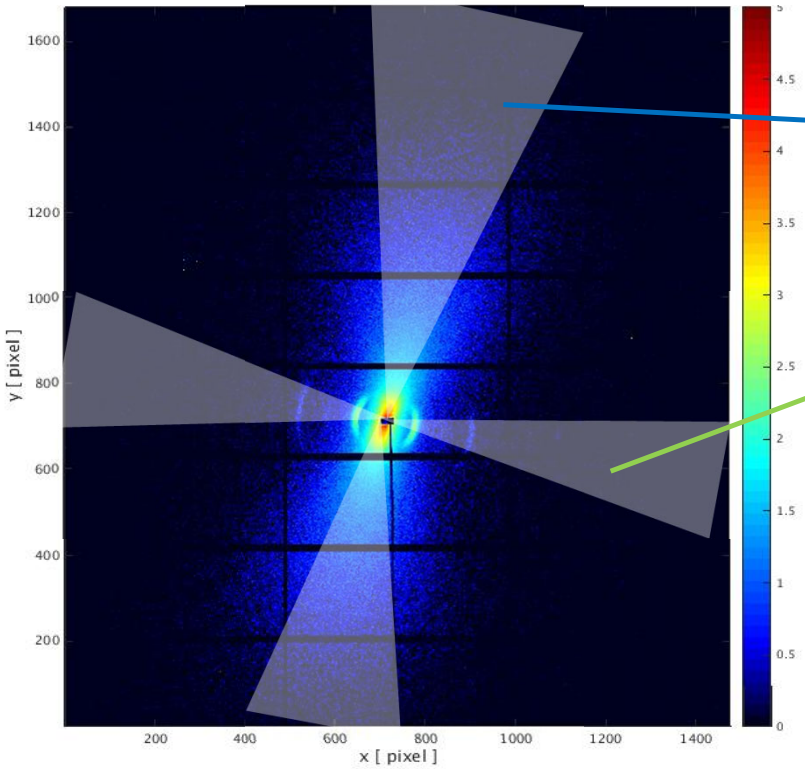
Example: bone structure



U. G. K. Wegst *et al.*, Nat. Mater. **14** (2014) 23

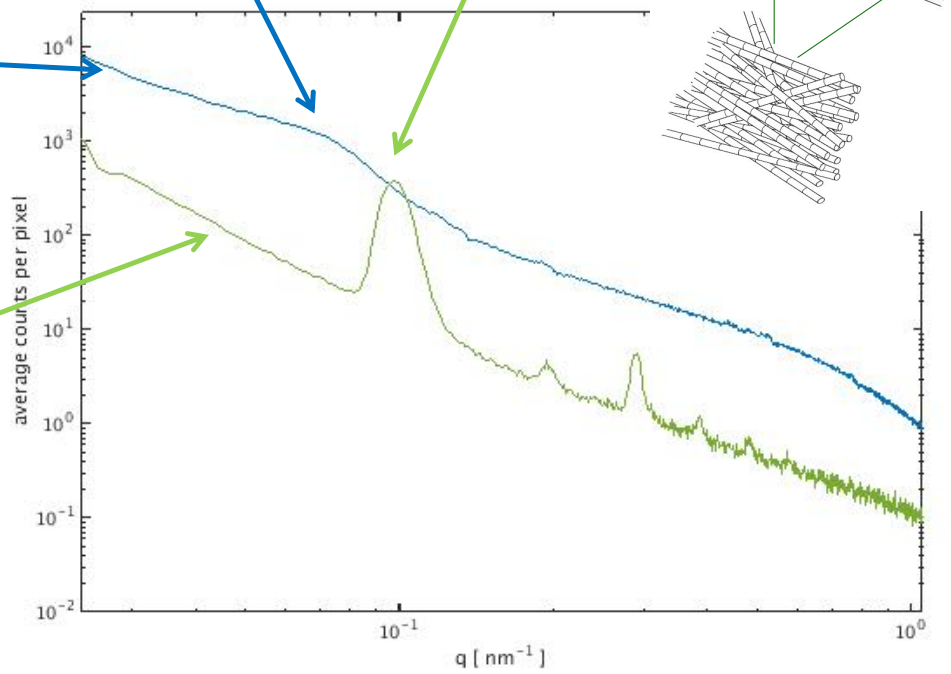
SAXS of bone

SAXS signal from mineralized collagen in human bone



50 – 200 nm diameter of collagen fibrils

spacing of gaps along collagen fibrils

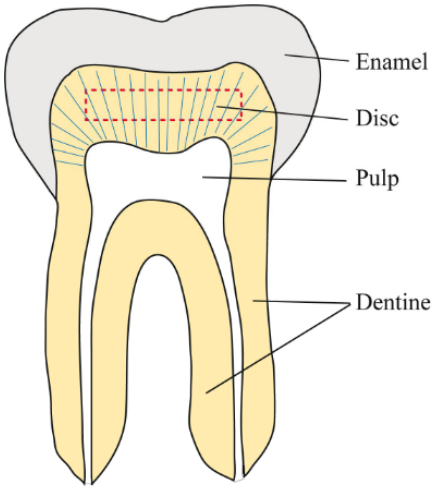


Slide courtesy of Marianne Liebi

Hierarchical structures in biological materials

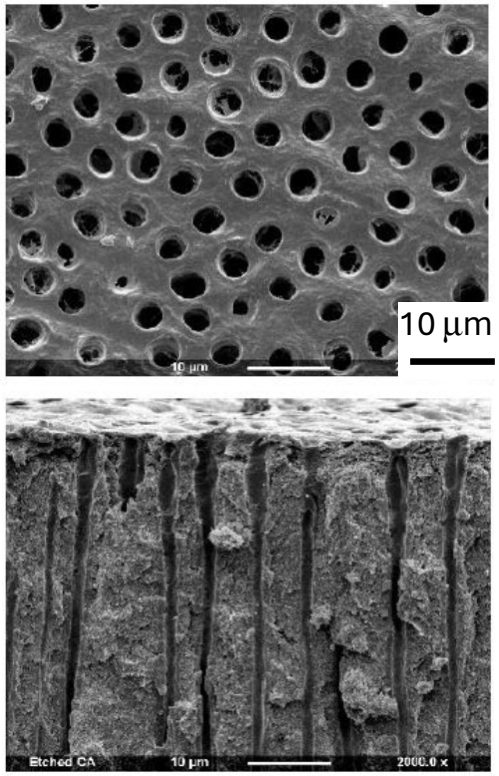
PSI

Example: tooth



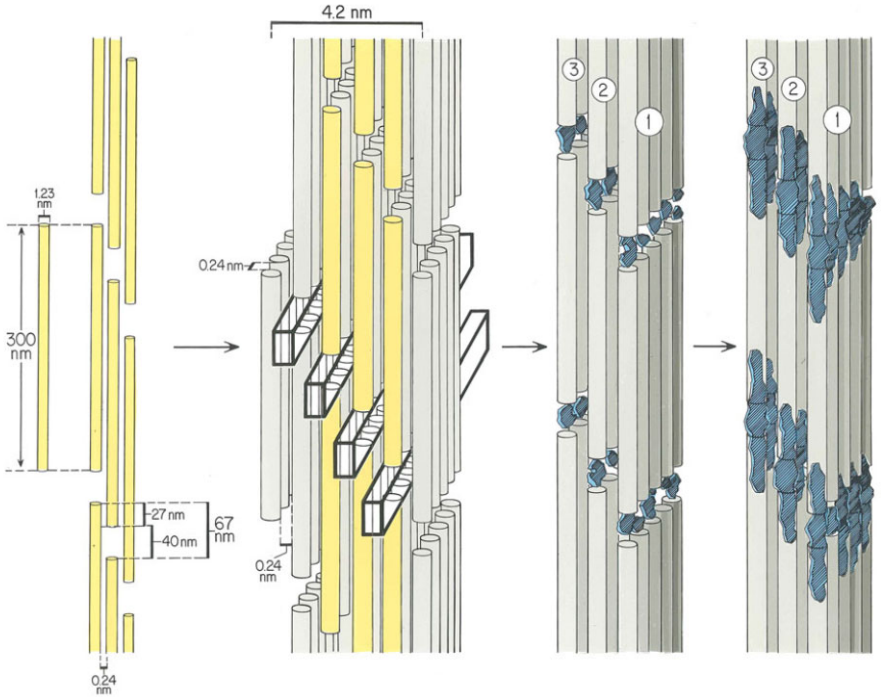
F. Kernen *et al.*,
Proc. of SPIE **7078**, 70780M (2008)

Microscale: microtubules



A.D. Sinodinou *et al.*, J. Dent. Maxillofacial **6**, 1-8 (2022)

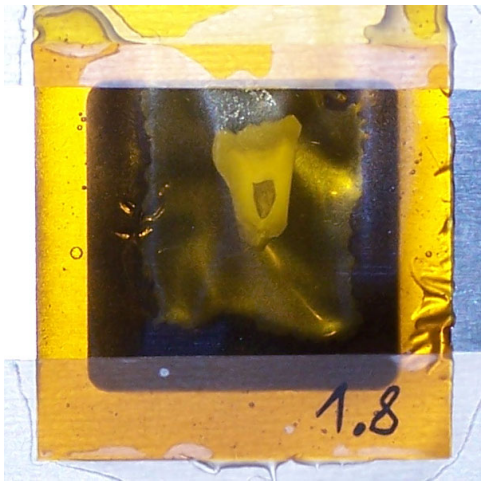
Nanoscale: mineralized collagen



W.J. Landis & R. Jacquet,
Calcif. Tissue Int. **93**, 329-337 (2013)

Spatially-resolved SAXS (scanning SAXS)

H. Deyhle, B. Müller, O. Bunk



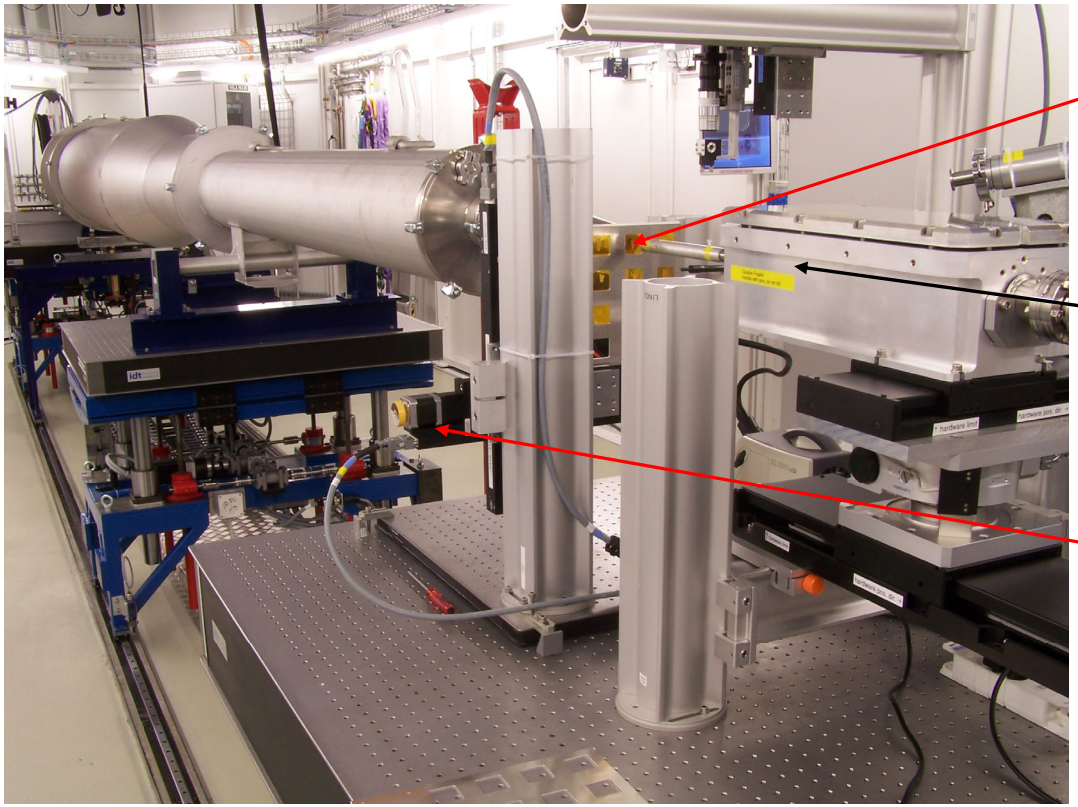
~ 0.5 mm thick slices
of human tooth

2D detector

Beam size on
sample ~ 25x20 µm^2

X-ray beam focused
on sample

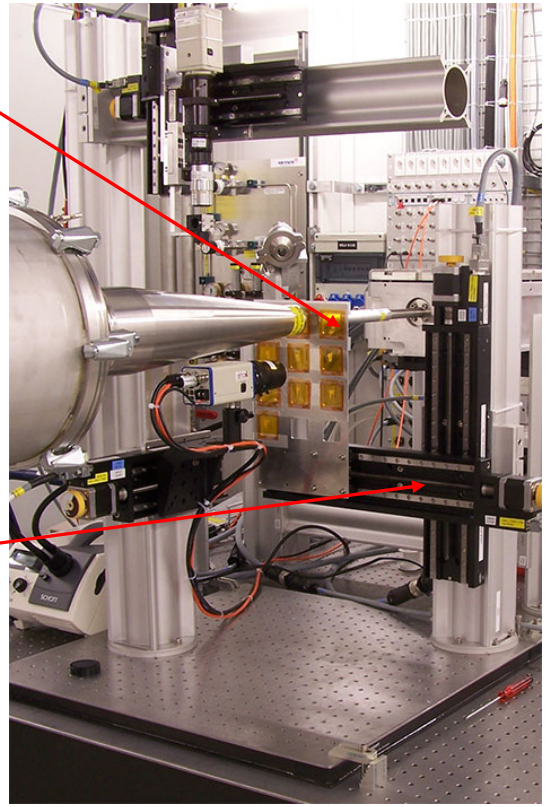
Setup for 2D scanning SAXS at cSAXS



Sample holder

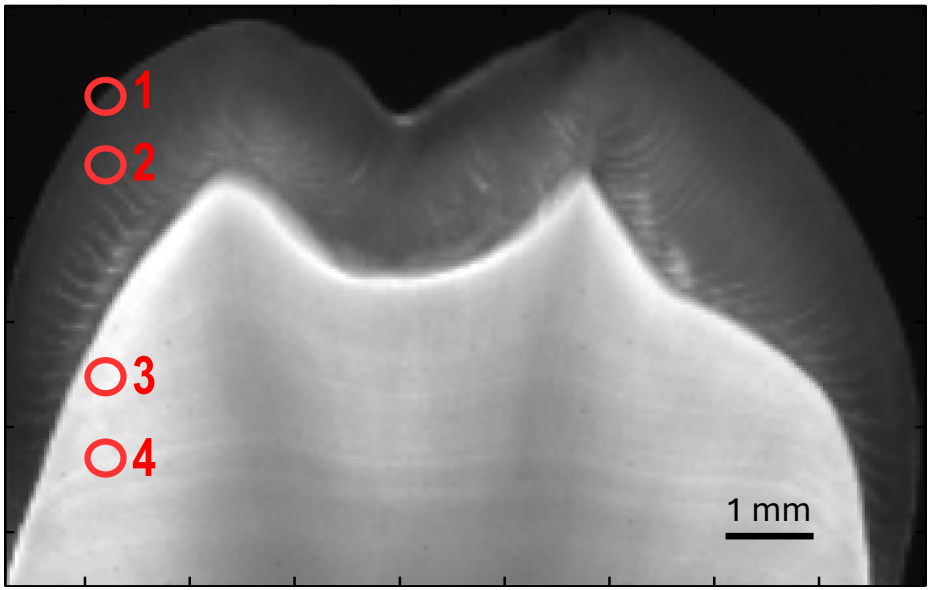
X rays

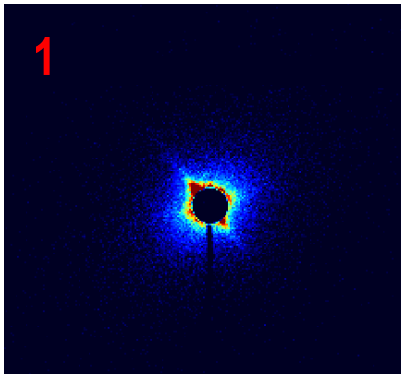
Translation stages

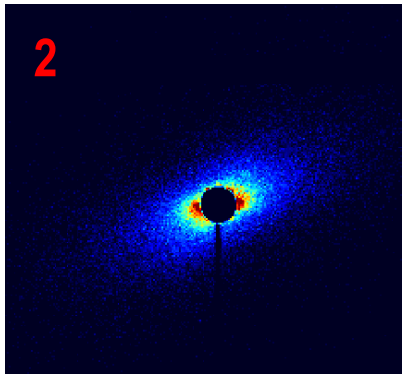


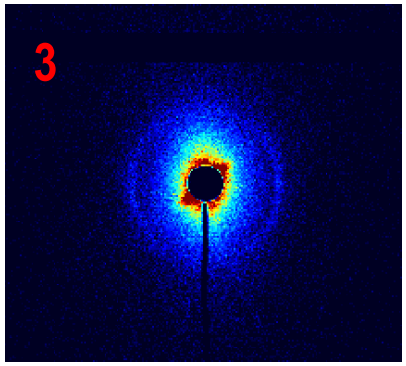
Spatially-resolved SAXS (scanning SAXS)

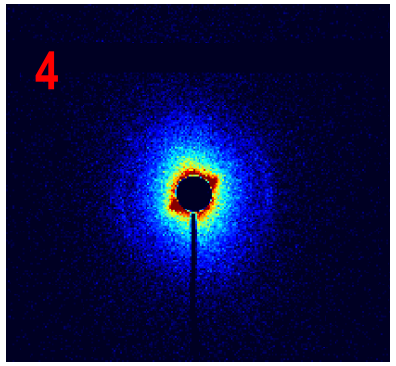
H. Deyhle et al,
Proc. of SPIE **7401**,74010E (2009)





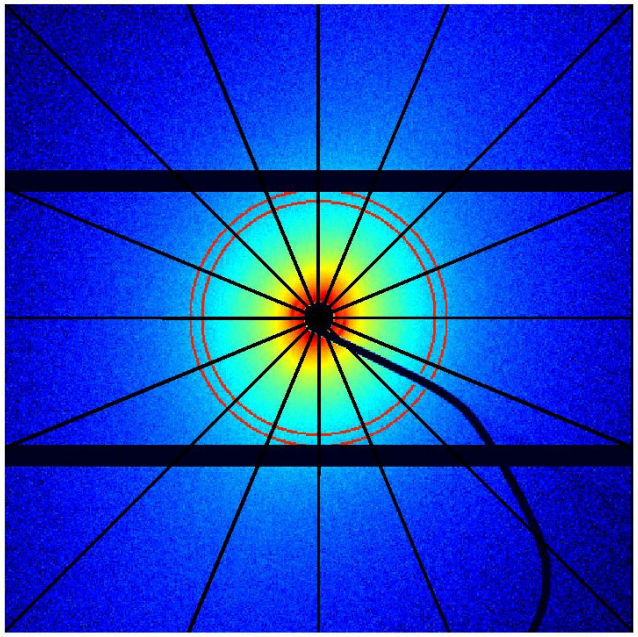


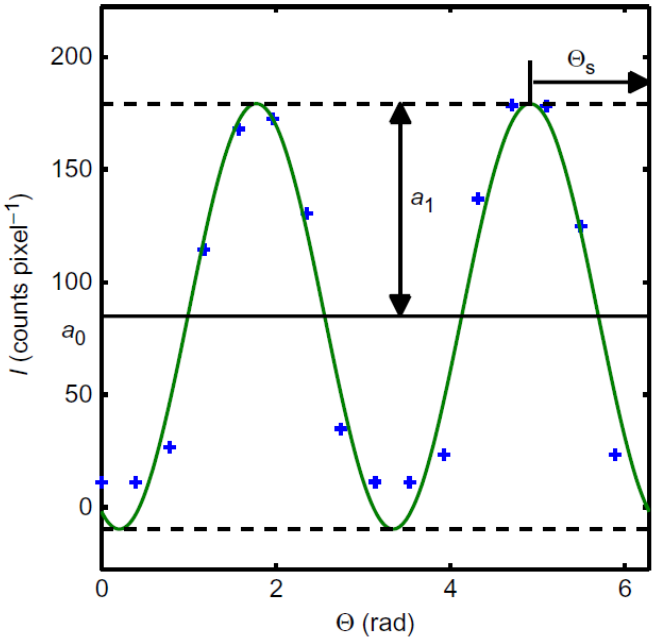




Scanning SAXS – data analysis

O. Bunk *et al.*, New J. Phys. **11**, 123016 (2009)





$$I(n_\theta) \approx a_0 + a_1 \cos\left(\frac{2\pi n_\theta}{N_\theta} - \Theta_s\right)$$

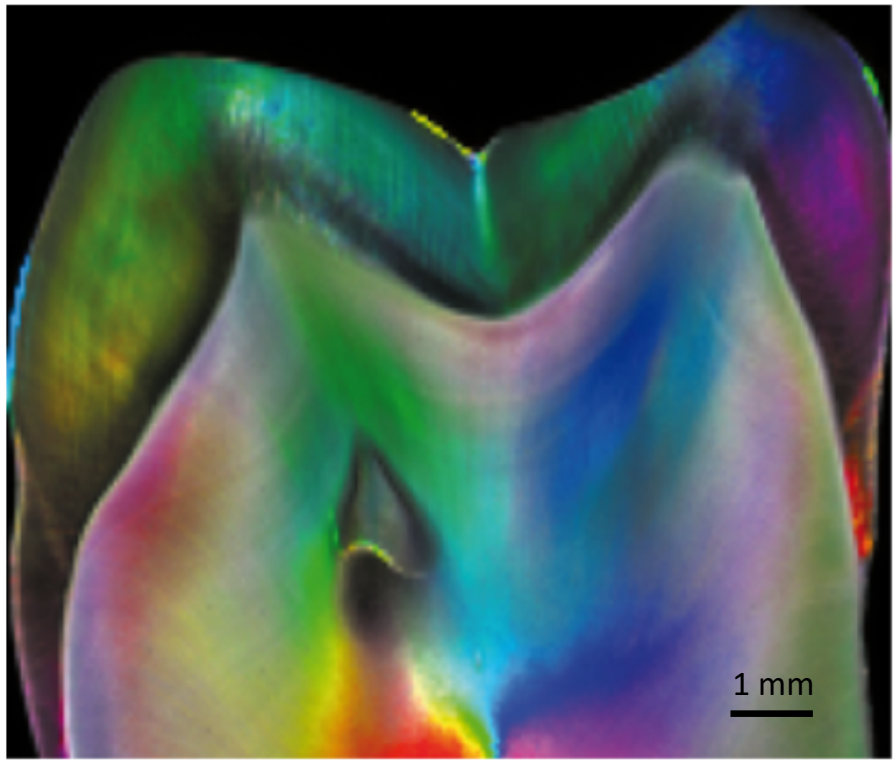
a_0 : average scattering (abundance of nanostructures)

a_1 : oriented part of scattering

$\frac{a_1}{a_0}$: degree of orientation

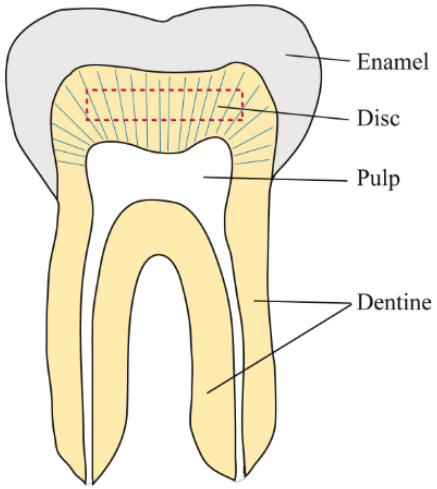
Scanning SAXS results on human tooth

Length scale from 14 to 28 nm



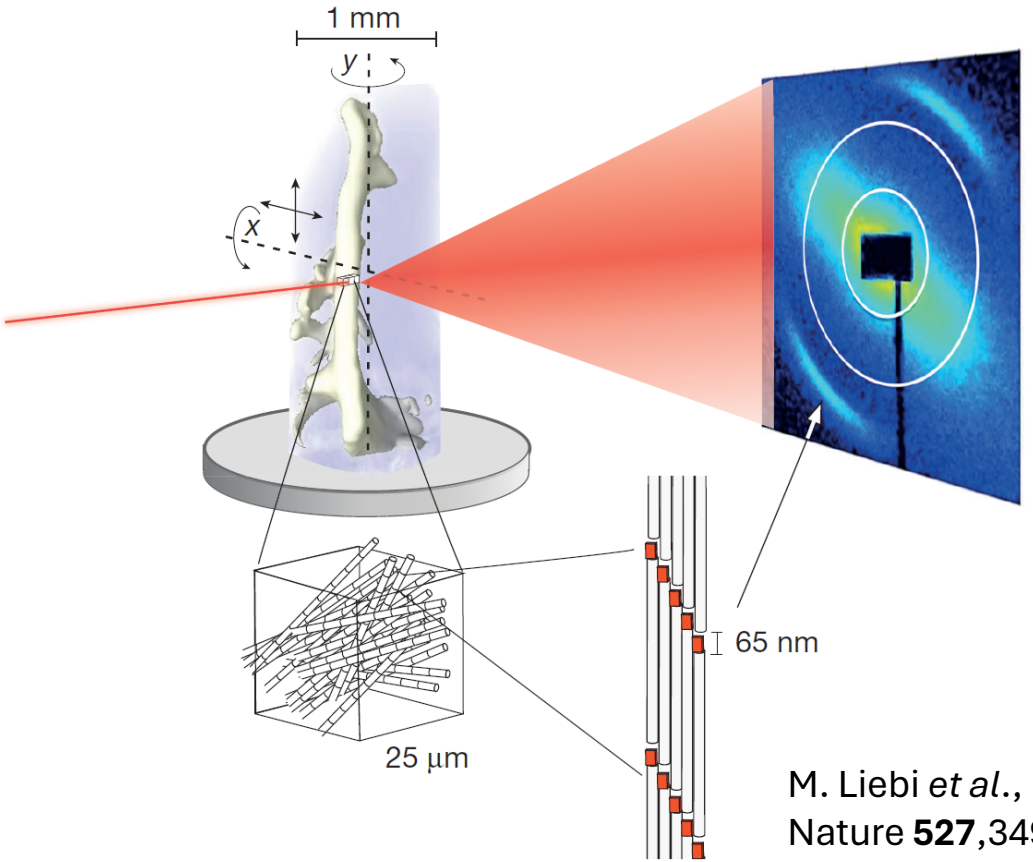
- Orientation of nanostructures (14 to 28 nm)
- Image of entire tooth section
- Resolution determined by beam size (~ 20 µm)
- Collagen seems to align radially around the dentine-enamel junction – like micro-tubules
- The alignment is not identical in dentine and enamel

H. Deyhle et al,
Proc. of SPIE
7401,74010E (2009)



SAXS tensor tomography: extension of scanning SAXS to bulk samples

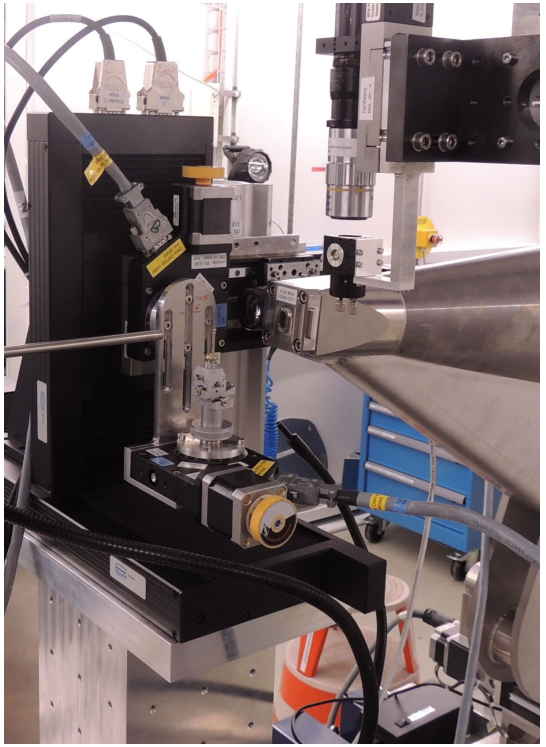
SAXS tensor tomography



M. Liebi *et al.*,
Nature **527**,349 (2015)

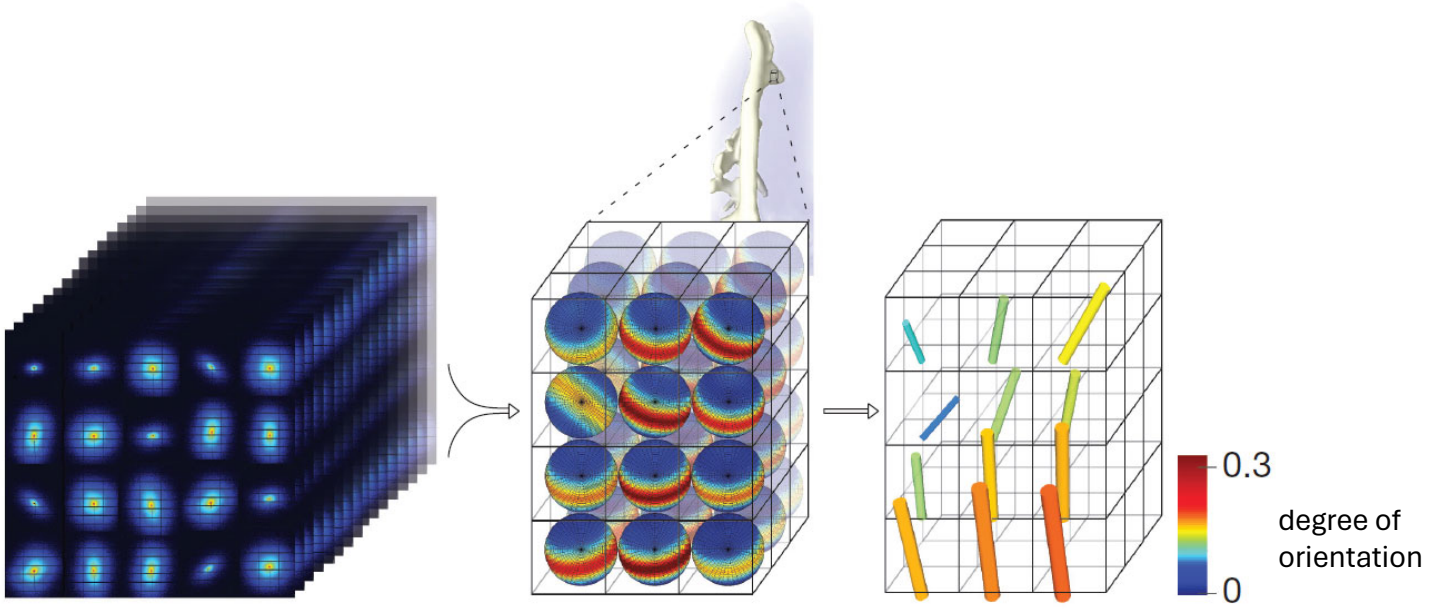
Setup at cSAXS

X-rays



SAXS tensor tomography reconstruction

M. Liebi *et al.*, Nature **527**,349 (2015)



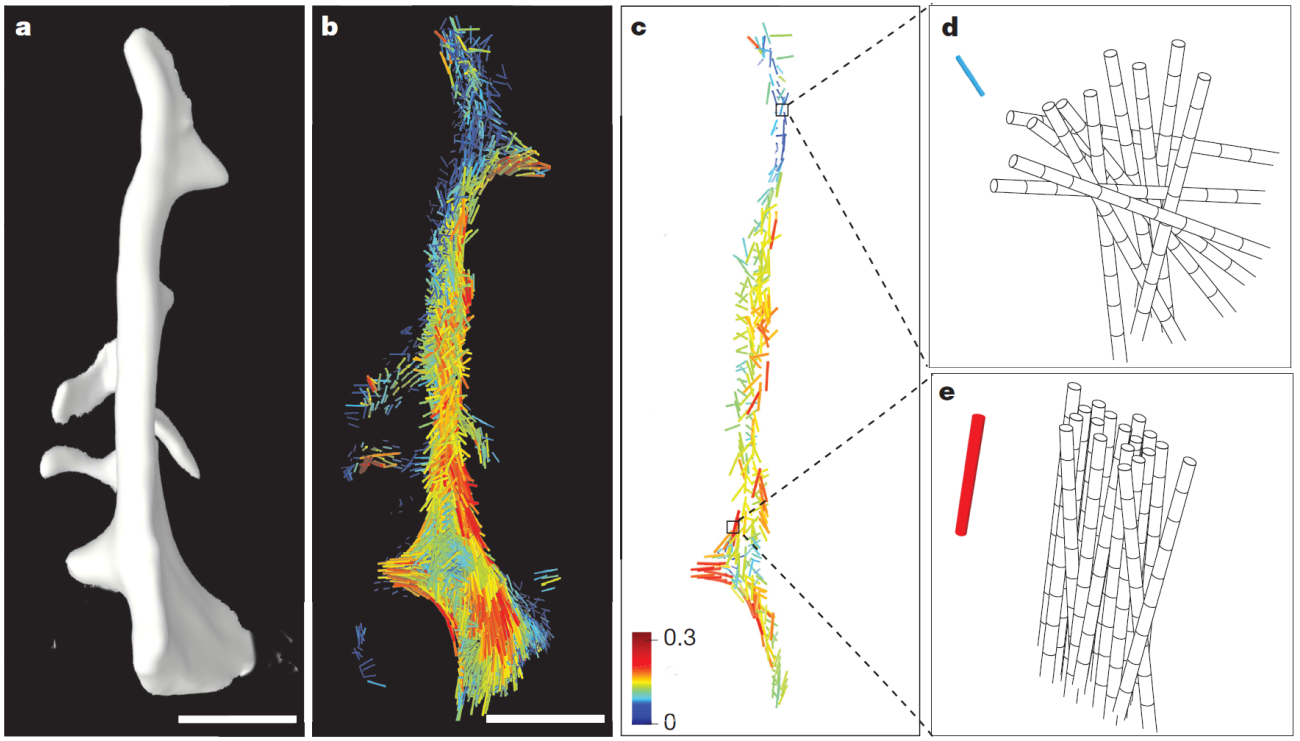
> 100,000 scattering patterns

Spherical harmonics to model the 3D reciprocal space of each voxel

Result: 3D orientation of nanostructures in each voxel

SAXS tensor tomography

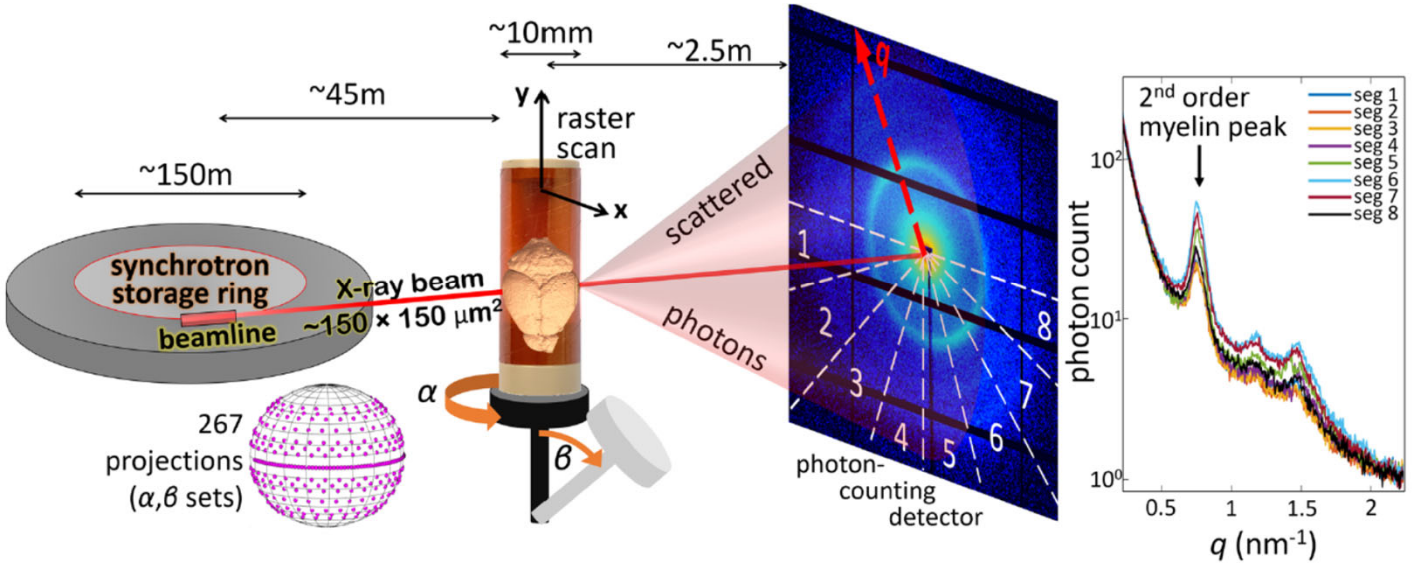
M. Liebi *et al.*, Nature **527**,349 (2015)



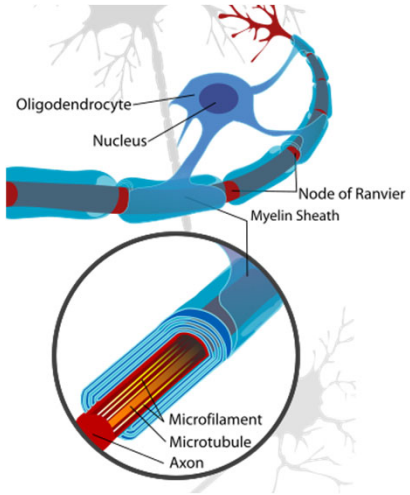
Scale bar: 0.5 mm

Myelin localization and orientation in a full mouse brain

M. Georgiadis *et al.*, Nat. Commun. **12**, 2941 (2021)



https://en.wikipedia.org/wiki/Myelin

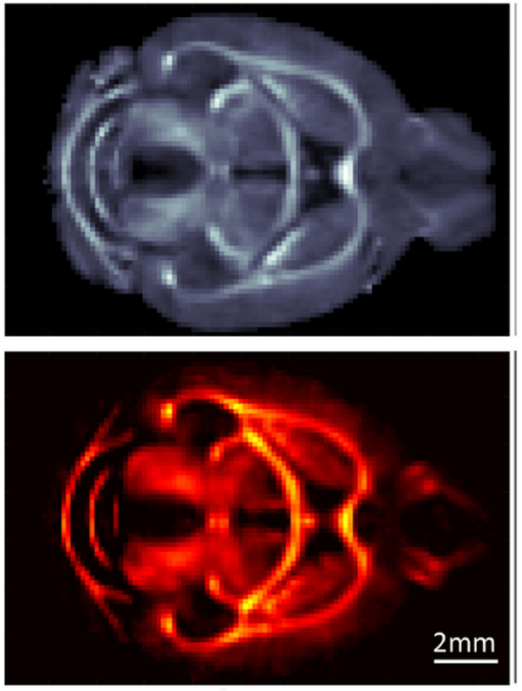


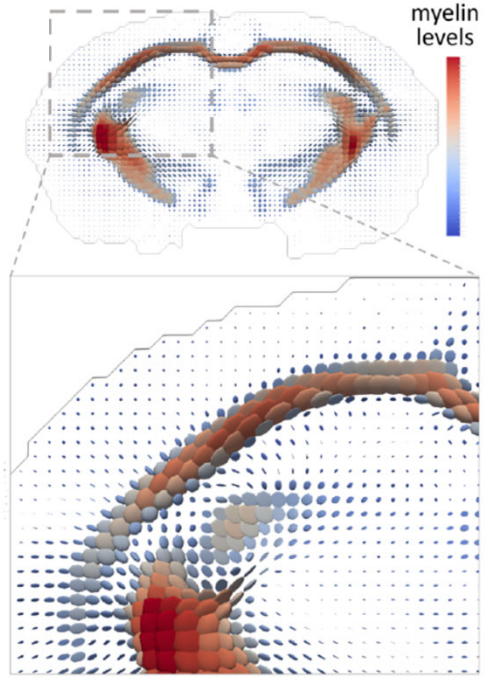
Myelin localization and orientation in a full mouse brain

M. Georgiadis *et al.*, Nat. Commun. **12**, 2941 (2021)

Unspecific scattering

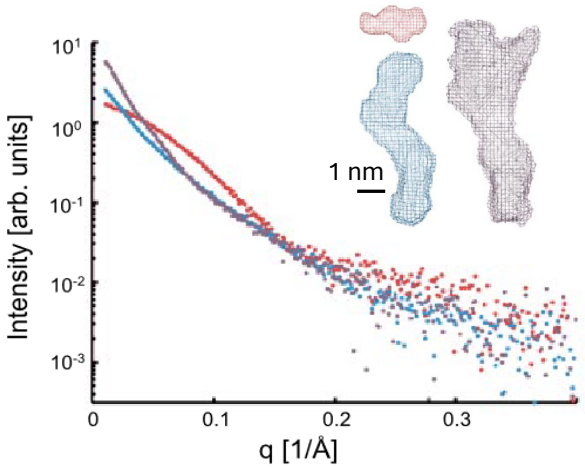
Myelin-specific scattering





Ellipsoids representing myelinated fiber orientation at each voxel

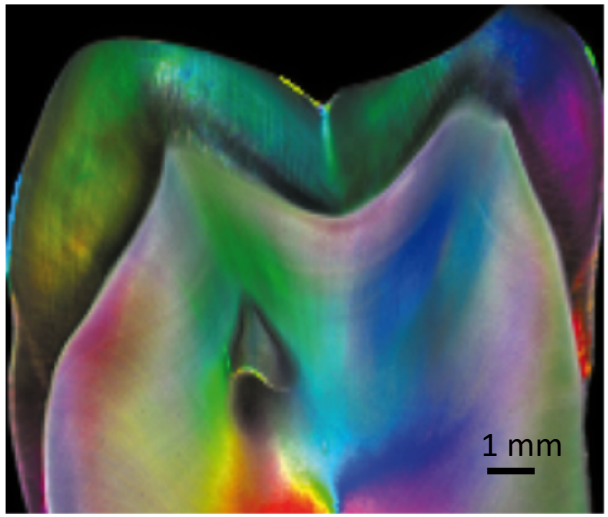
Summary of Part I: probing nanostructures with SAXS



SAXS

Identical nanostructures

Average shape and size

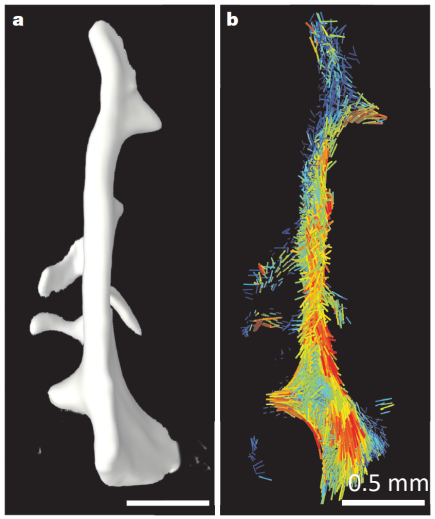


Scanning SAXS

Images of 2D slices of tissues

Spatial resolution limited by beam size (here ~ 25 µm)

Information about nanostructures (here collagen orientation)



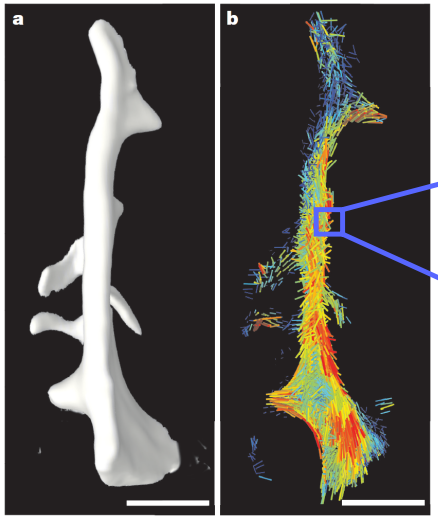
SAXS tensor tomography

Bulk 3D tissues

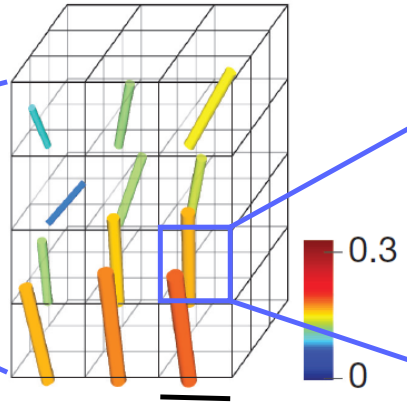
Information about 3D nanostructure orientation

Scanning SAXS vs X-ray ptychography

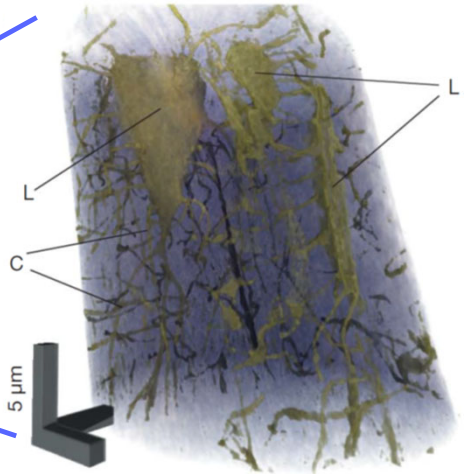
M. Liebi *et al.*, Nature **527**,349 (2015)



Scale bar: 0.5 mm



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



Part II: X-ray ptychography

PSI

Motivation

- The challenge of high-resolution hard X-ray microscopy

X-ray ptychography

- Implementation at the synchrotron
- Advantages with respect to other imaging techniques

Applications

- High-resolution nano-tomography
- Biological tissues

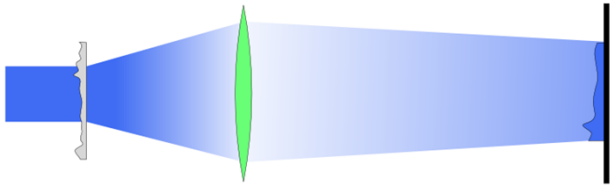
The future at 4th generation synchrotron sources

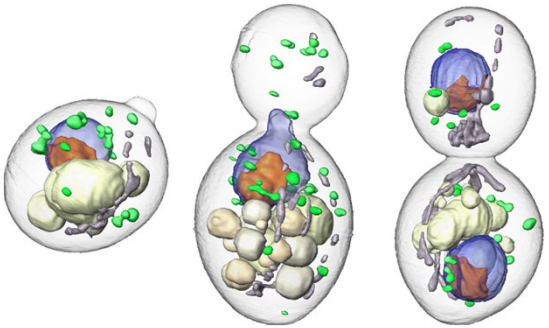
- Faster, larger volumes, higher resolution…

Motivation for X-ray ptychography

Soft X-ray microscopy

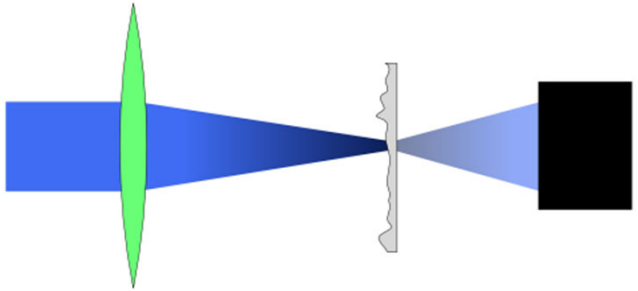
full-field microscopy:



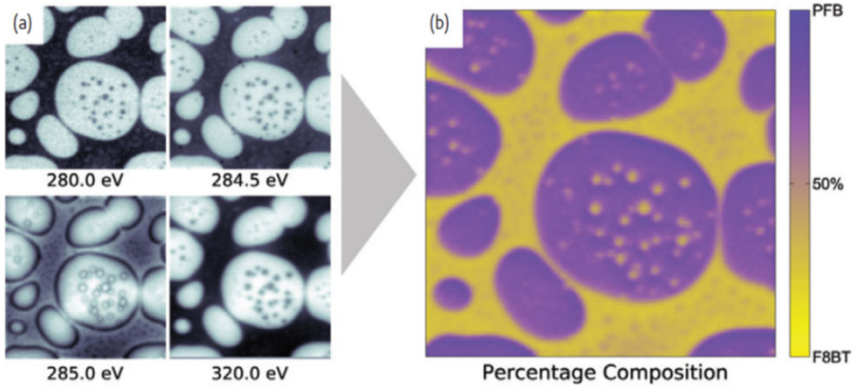


M. Uchida *et al.*,
Yeast **28**, 227 (2011)

scanning transmission microscopy (STXM):



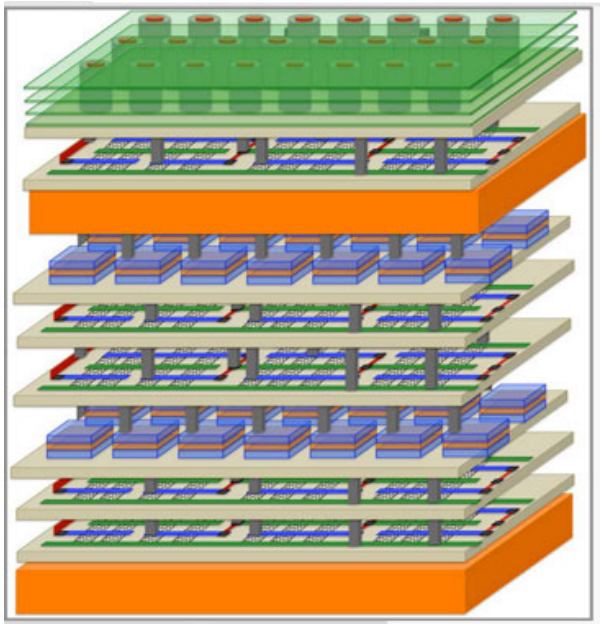
B. Watts *et al.*,
Materials Today
15, 148 (2012)



- Typically with Fresnel zone plates in the soft X-ray regime (e.g. in the water window)
- Resolution can reach 25-30 nm, but depth of focus limited to about 1 µm

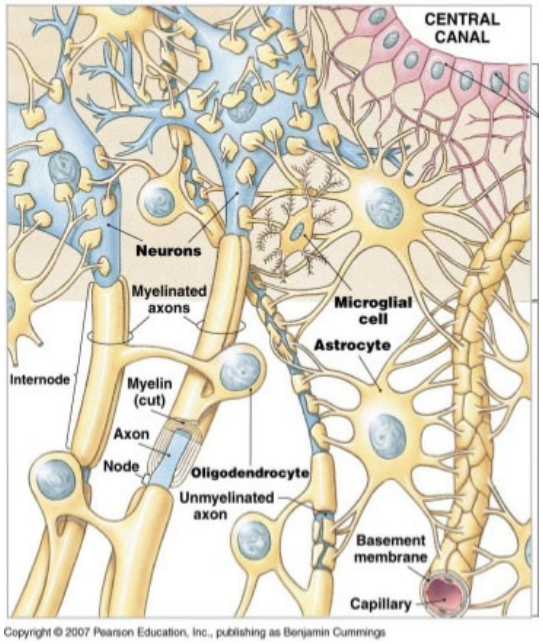
Hard X-ray microscopy

Computer chip



https://www.livescience.com/52207-faster-3d-computer-chip.html

Nervous tissue



Hierarchical structures

3D imaging of bulk samples

Thickness from 10 to 100 µm

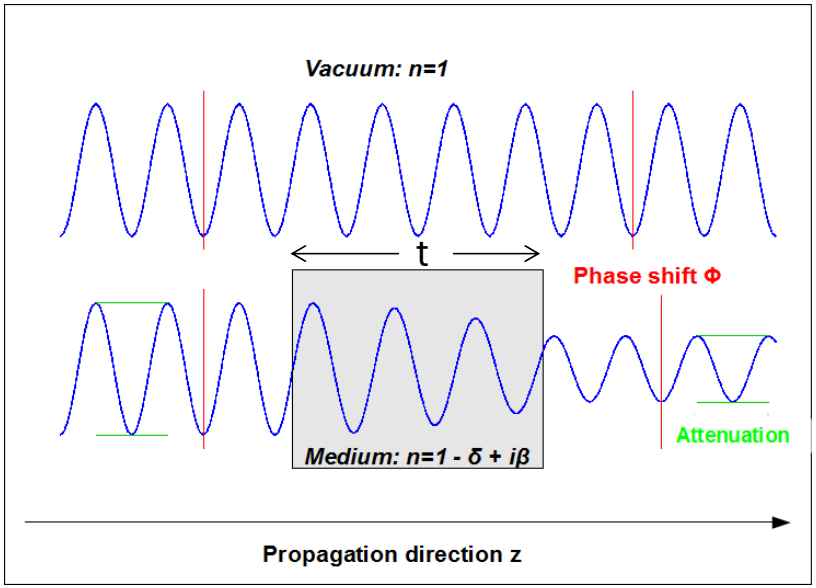
Resolution from 10 to 100 nm

Energy > 2 keV

Challenges:

- Low absorption
- Fabrication aberration-free, high-resolution lenses

Absorption and phase contrast



pixelated detector

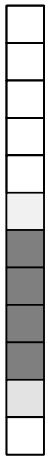
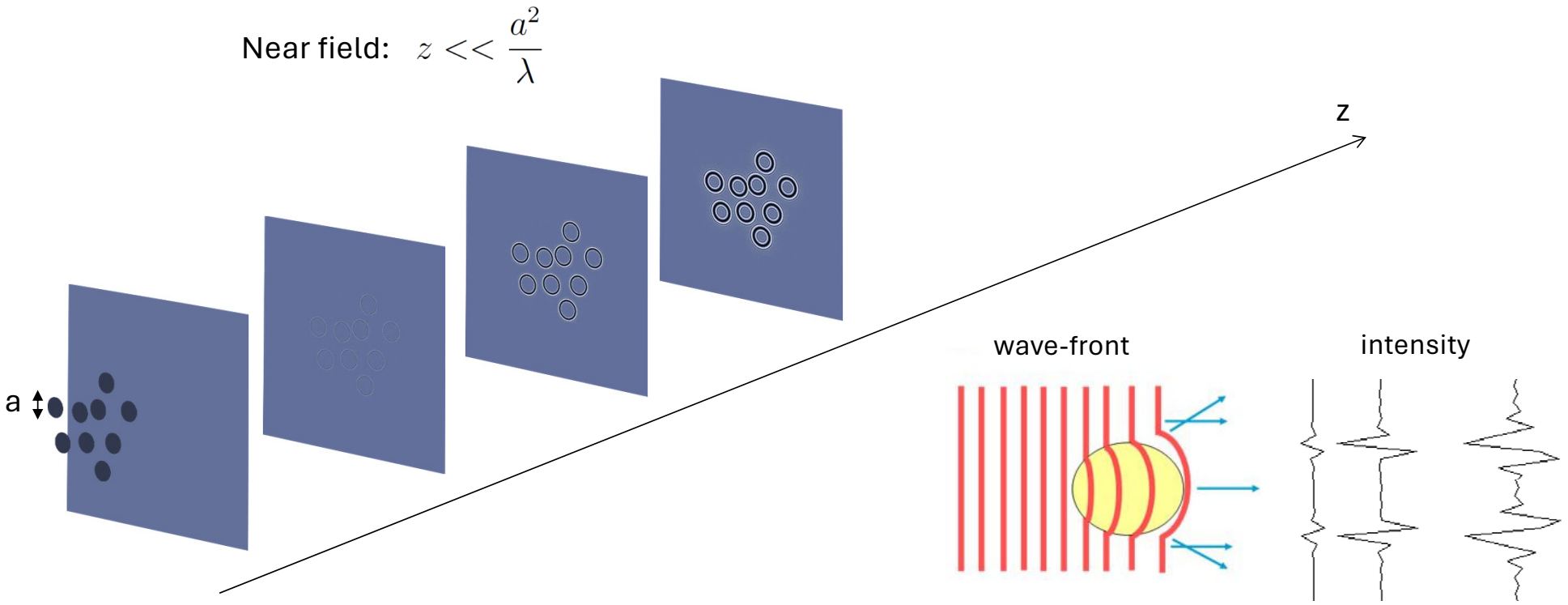


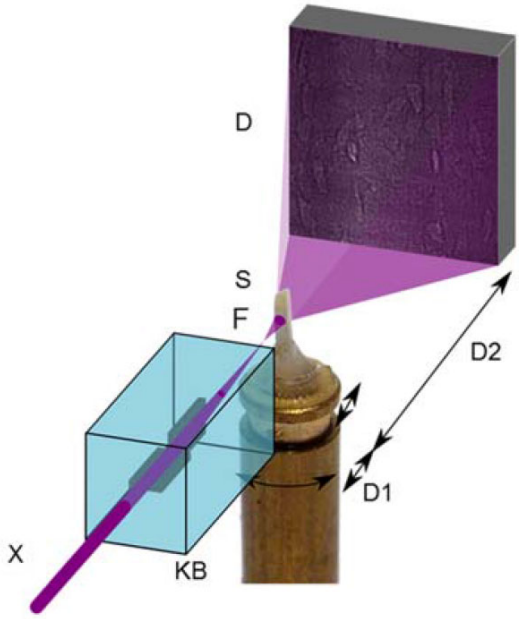
Image from “Phase-contrast X-ray imaging” in Wikipedia:
https://en.wikipedia.org/wiki/Phase-contrast_X-ray_imaging

Wave propagation reveals phase contrast

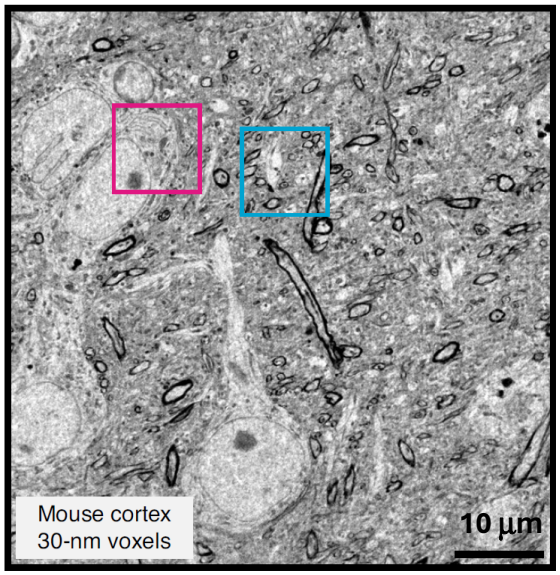
Figure adapted from P. Willmott, “An Introduction to Synchrotron Radiation”, Wiley (2011) p. 314



Propagation-based imaging with magnification



M. Langer *et al.*, PLOS ONE **7**, e35691 (2012)
R. Mokso *et al.*, Appl. Phys. Lett. **90**, 144104 (2007)



A. T. Kuan et al.,
Nat. Neurosci. **23**, 1637-1643 (2020)

Also known as **nano-holotomography**

Wave propagation reveals phase contrast

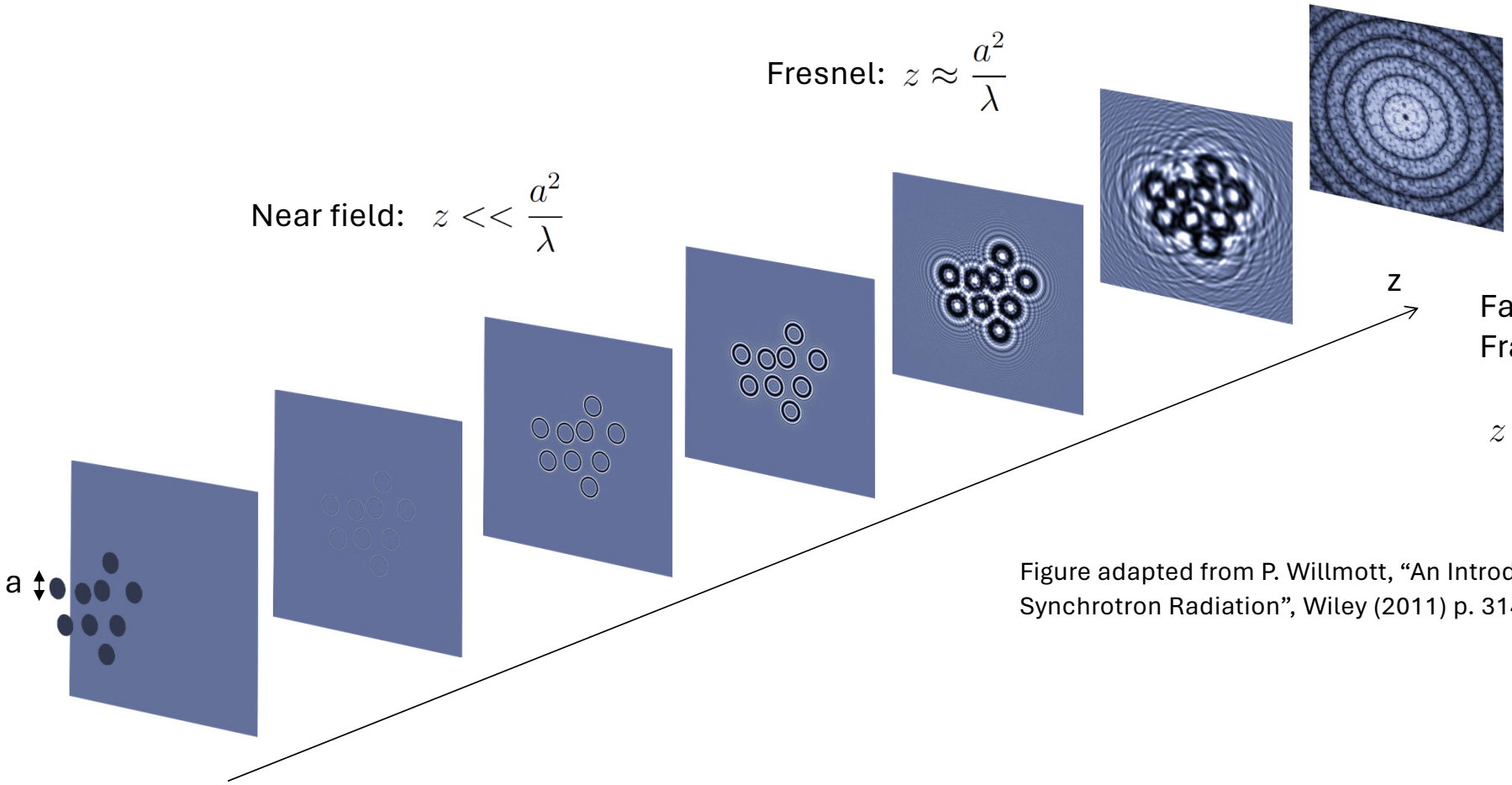
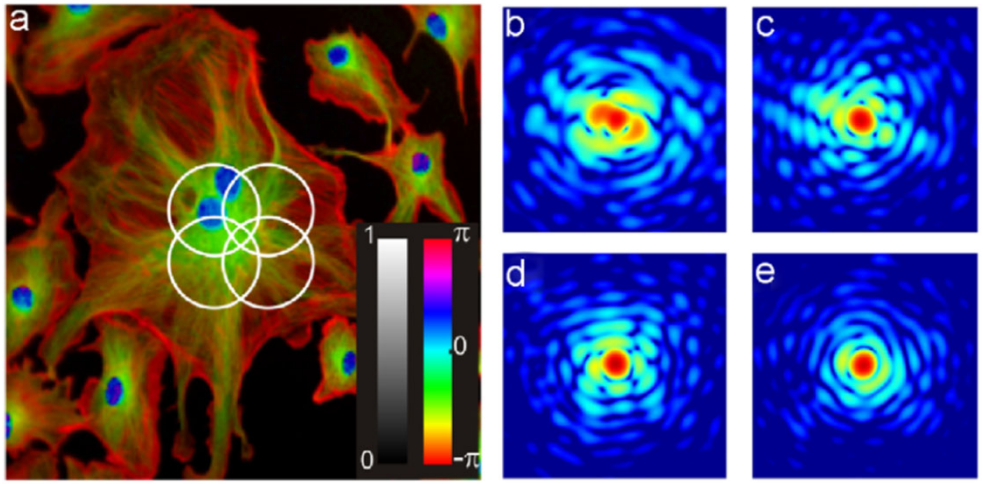


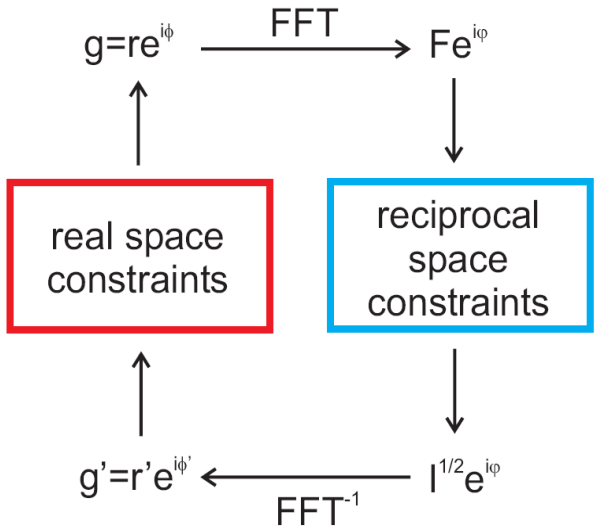
Figure adapted from P. Willmott, “An Introduction to Synchrotron Radiation”, Wiley (2011) p. 314

X-ray ptychography

Coherent diffraction patterns from overlapping illuminated areas



Iterative phase retrieval algorithms to reconstruct complex-valued transmissivity

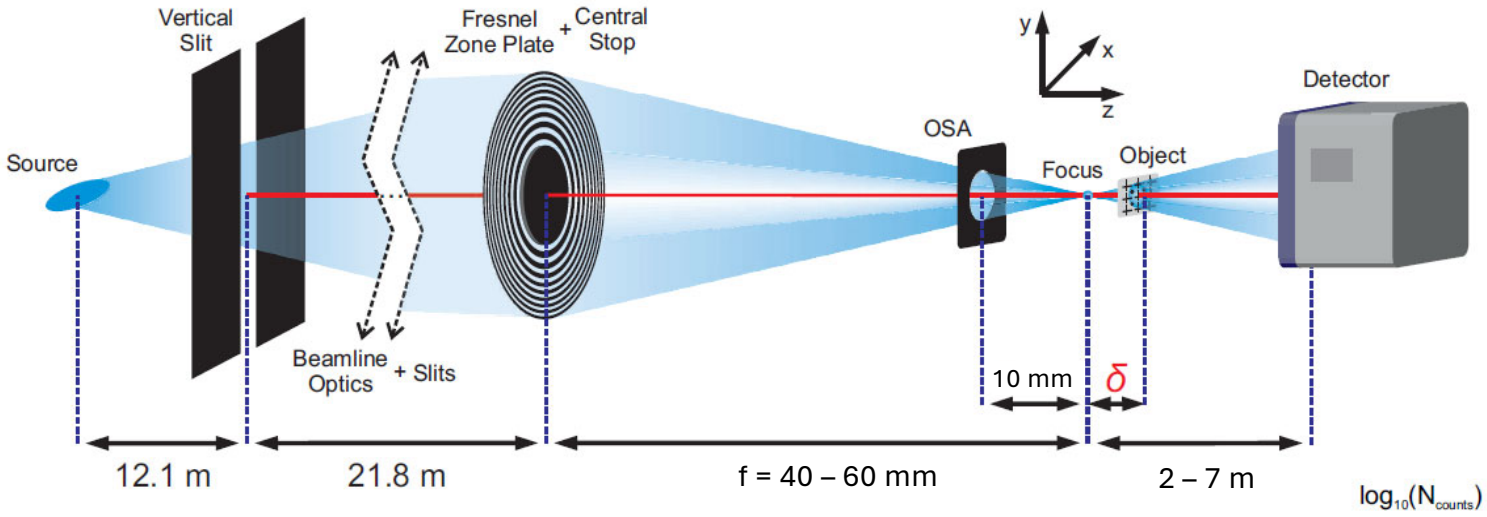


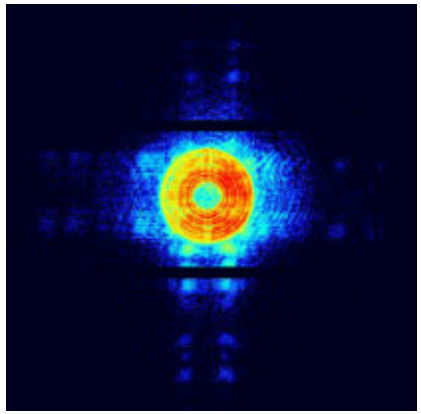
H. M. L. Faulkner & J. M. Rodenburg, Phys. Rev. Lett. **93**, 023903 (2004)

- Absorption and phase contrast
- Resolution not limited by a lens!
- In practice limited by mechanical stability and thermal drifts

Implementation and advantages of X-ray ptychography

A typical X-ray ptychography setup



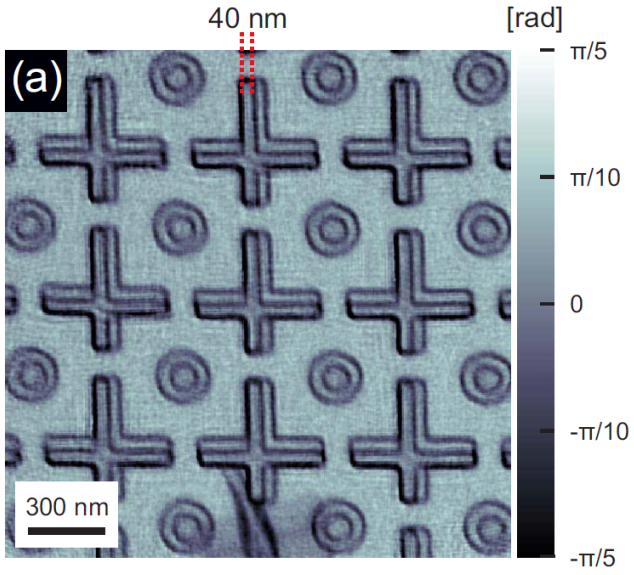


coherent flux:
5×10^8 photons/s
@ 6.2 keV

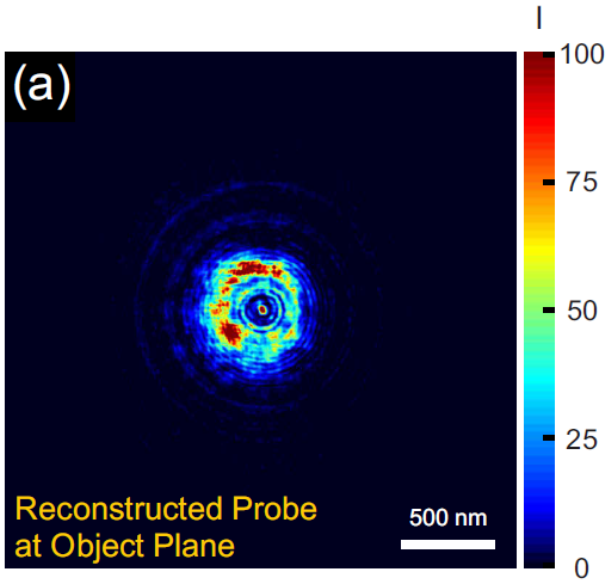
J. Vila-Comamala *et al.*, Opt. Express **19**, 21333 (2011)

Simultaneous probe reconstruction with ptychography

Phase image



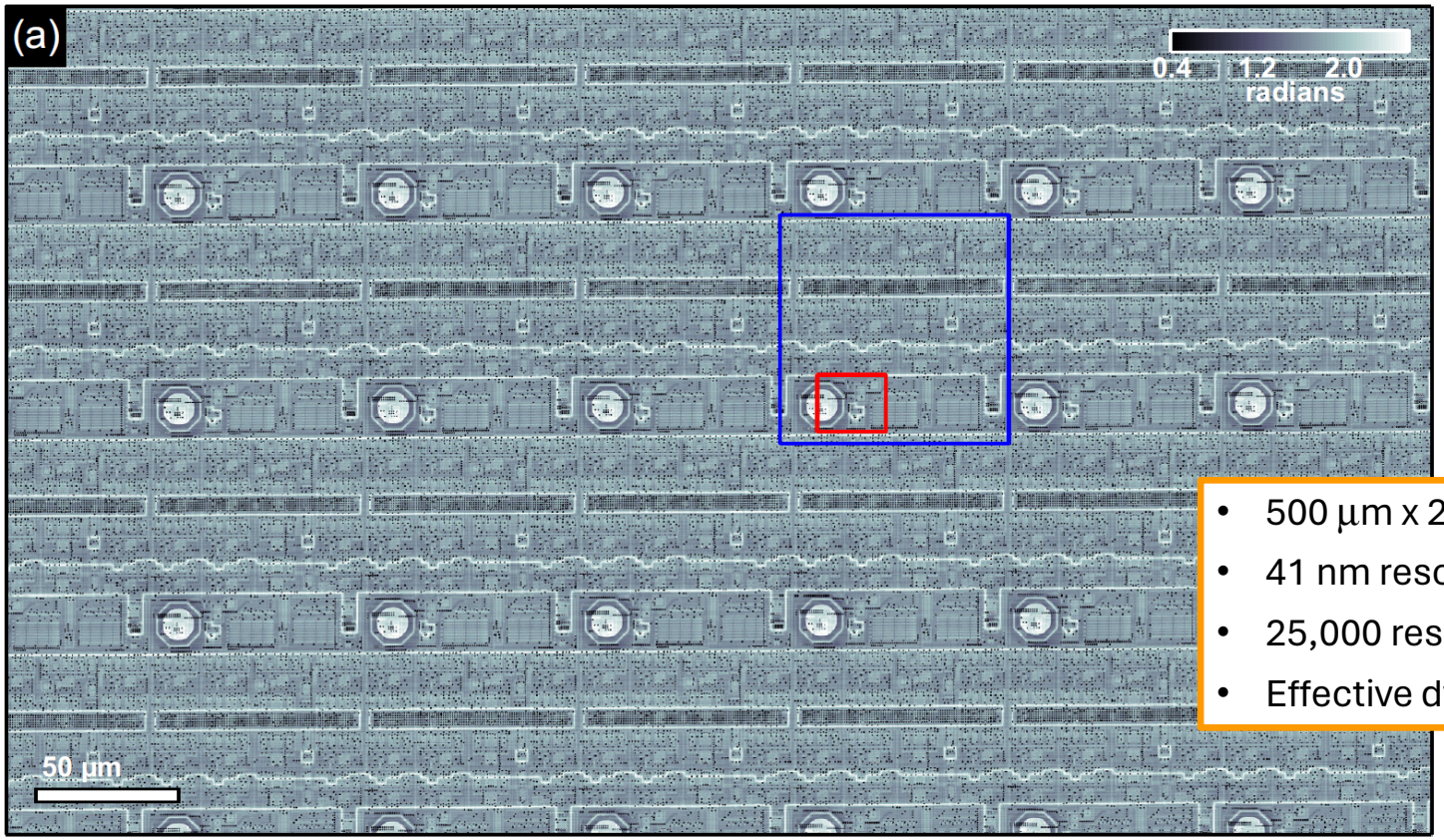
Illumination beam

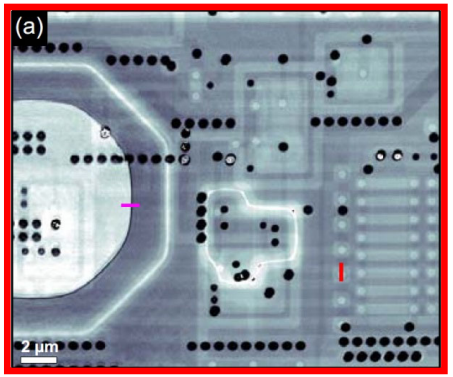


8 nm resolution
2×2 µm area
4 min

High-throughput X-ray ptychography

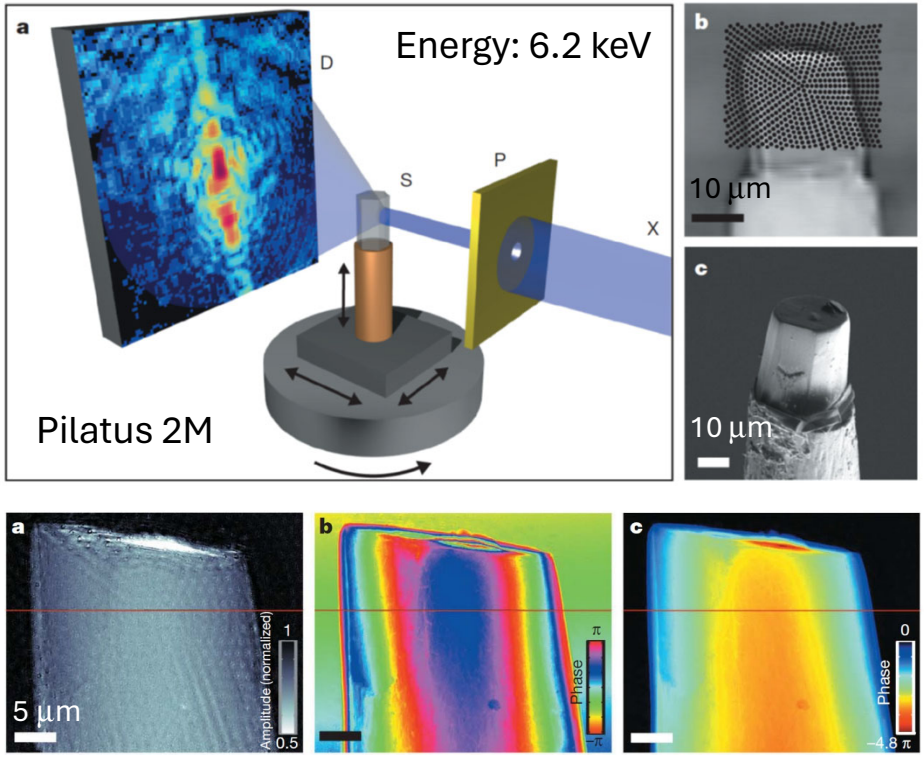
The EIGER "selfie" M. Guizar-Sicairos *et al.*, Opt. Express **22** (2014) 14859



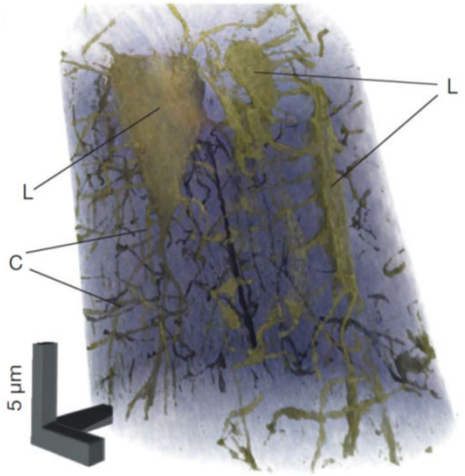


- 500 µm x 290 µm image
- 41 nm resolution
- 25,000 resolution elements / second
- Effective dwell time of 40 µs / resel

Ptychographic X-ray computed tomography (PXCT)



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

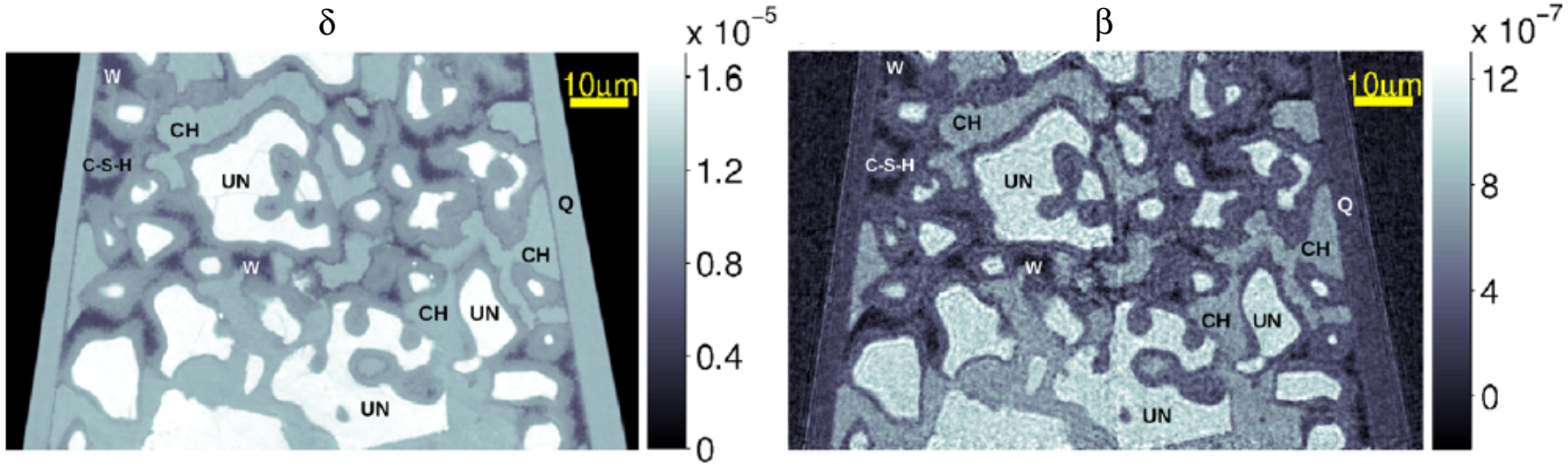


Mouse bone specimen

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

Quantitative contrast

- Hydrated cement phase
- 3D distribution of refractive index: $n(\mathbf{r}) = 1-\delta(\mathbf{r})+i\beta(\mathbf{r})$

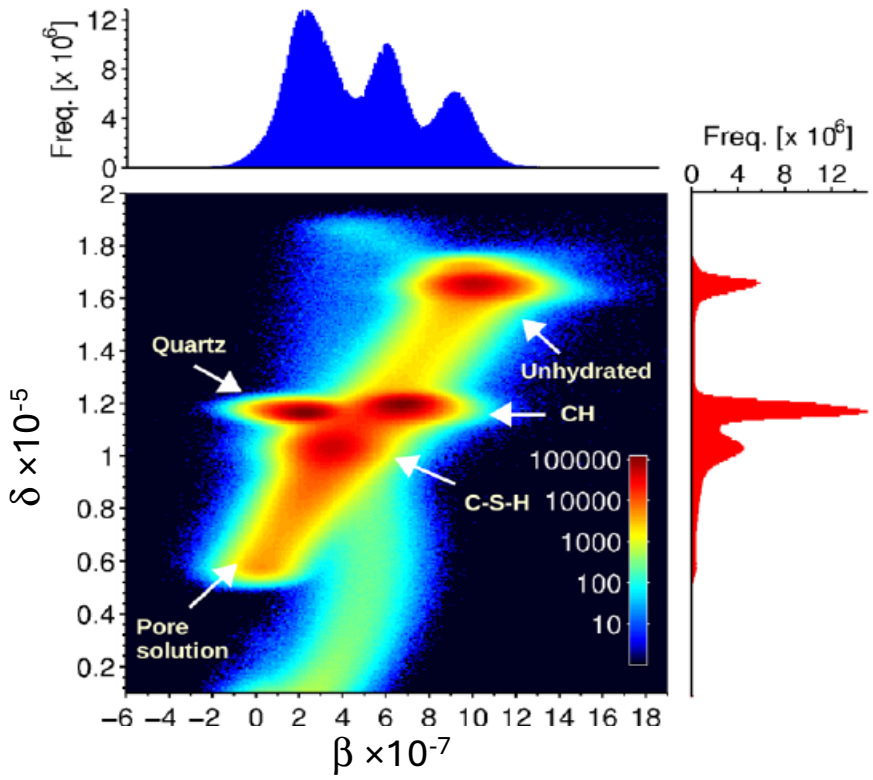


J. C. da Silva *et al.*,
Langmuir **31**, 3779 (2015)

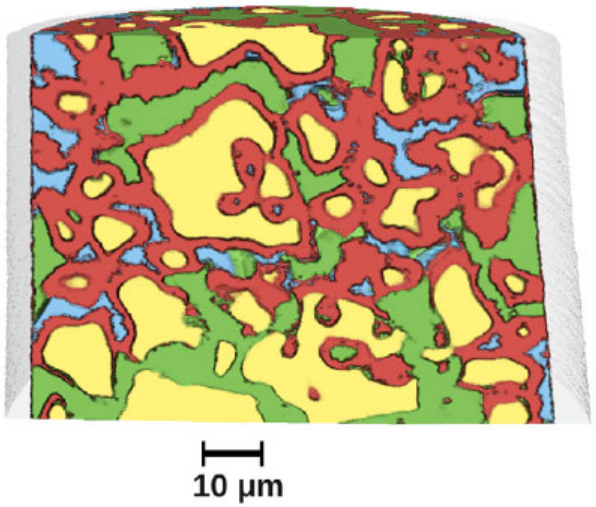
Identification of material phases:

UN: unhydrated alite particles
W: porosity (mostly water)
CH: calcium hydroxide
C-S-H: calcium silicate hydrates

Quantitative contrast



J. C. da Silva *et al.*, Langmuir **31**, 3779 (2015)

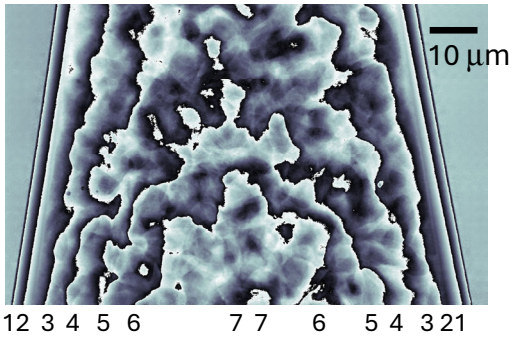


UN: **unhydrated alite particles**
W: **porosity (mostly water)**
CH: **calcium hydroxide**
C-S-H: **calcium silicate hydrates**

Mass density of C-S-H: 1.828 g/cm^3

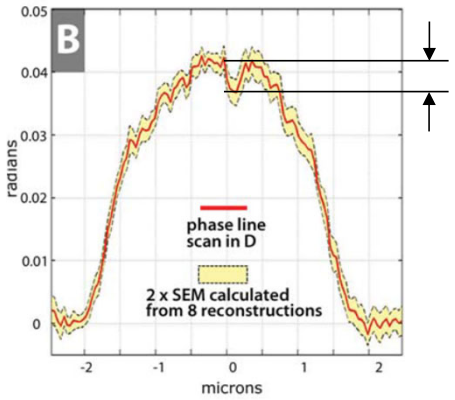
Advantages of X-ray ptychography

- Optically thick samples



Cement paste in capillary
J. da Silva *et al.*,
Langmuir **31** (2015) 3779

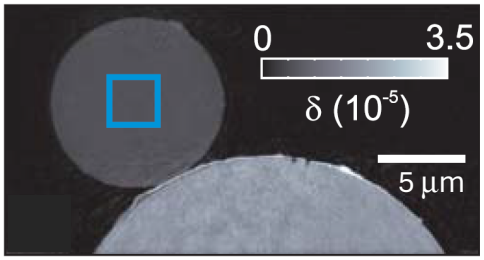
- High phase sensitivity



0.005 rad

Line profile across cell in ice
E. Lima *et al.*,
J. Microscopy **249** (2013) 1

- Quantitative contrast

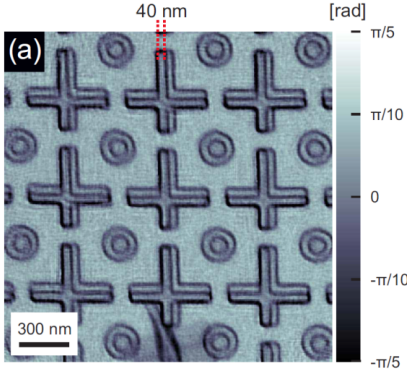


1.048±0.008 g/cm^3
Nominal: 1.055 g/cm^3

Polystyrene sphere in 3D

A. Diaz *et al.*, Phys. Rev. B **85** (2012) 020104(R)

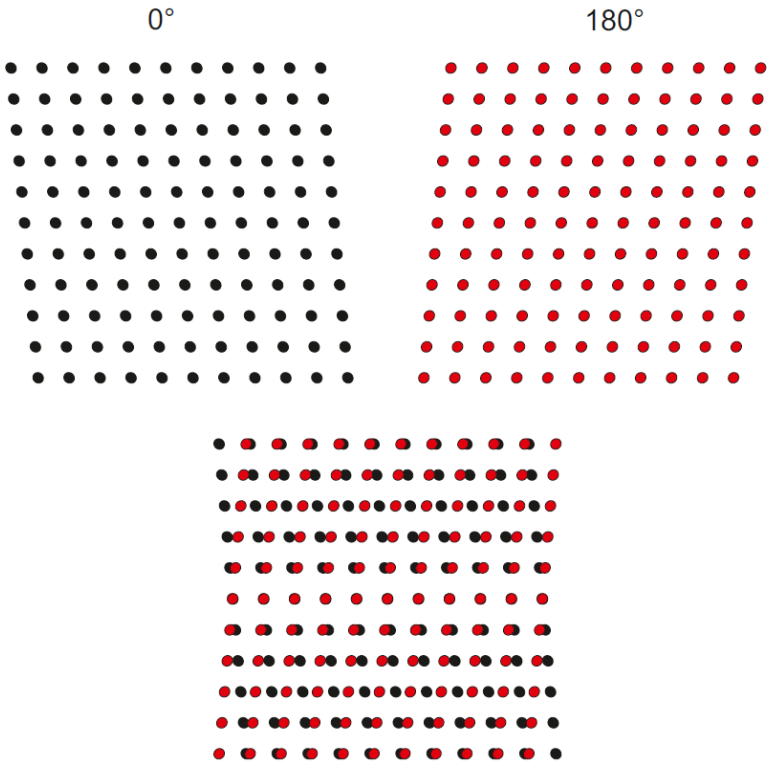
- High resolution



8 nm @ 6.2 keV

J. Vila-Comamala *et al.*,
Opt. Express **19** (2011) 21333

Challenges of PXCT: scanning position errors



- Piezo scanner error motions and thermal drifts effectively map different positions at different angles
- Distorted positions result in distorted 2D images, also in ptychography
- The 3D resolution is effectively smeared out after tomographic reconstruction

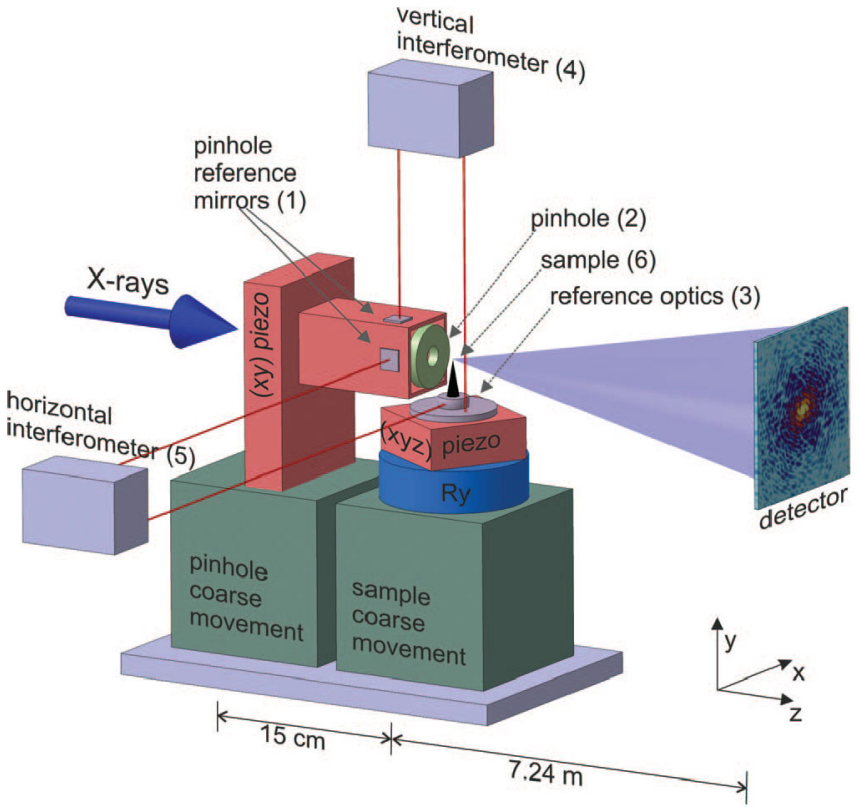
Instrumentation for PXCT

PSI

OMNY: tOMography Nano crYo stage

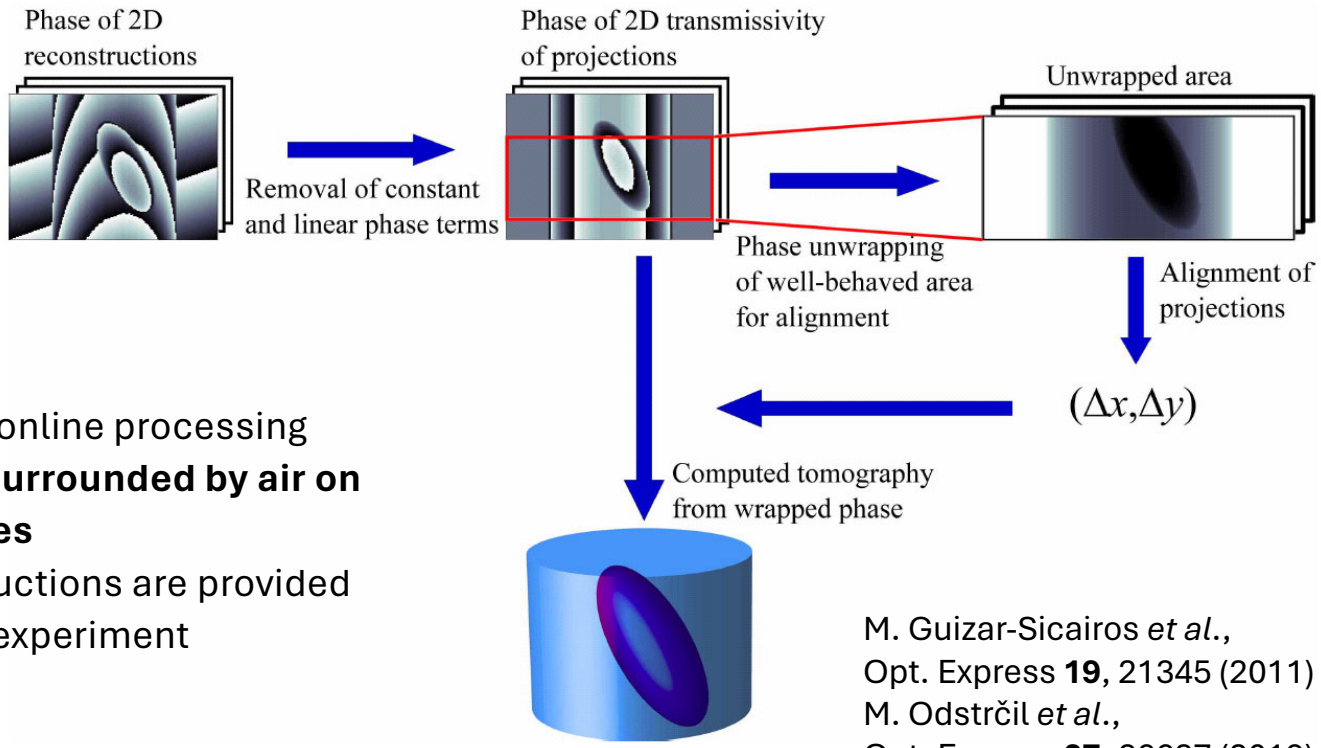
M. Holler and J. Raabe

- Laser interferometry for relative positioning of sample and illumination optics
- Aimed 3D resolution: 10 nm
- Cryo stage in ultra-high vacuum
- First test setup in air at room temperature, still in user operation



M. Holler *et al.*, Rev. Sci. Instrum. **83**, 073703 (2012)
M. Holler *et al.*, Rev. Sci. Instrum. **89**, 043706 (2018)

Image processing for PXCT

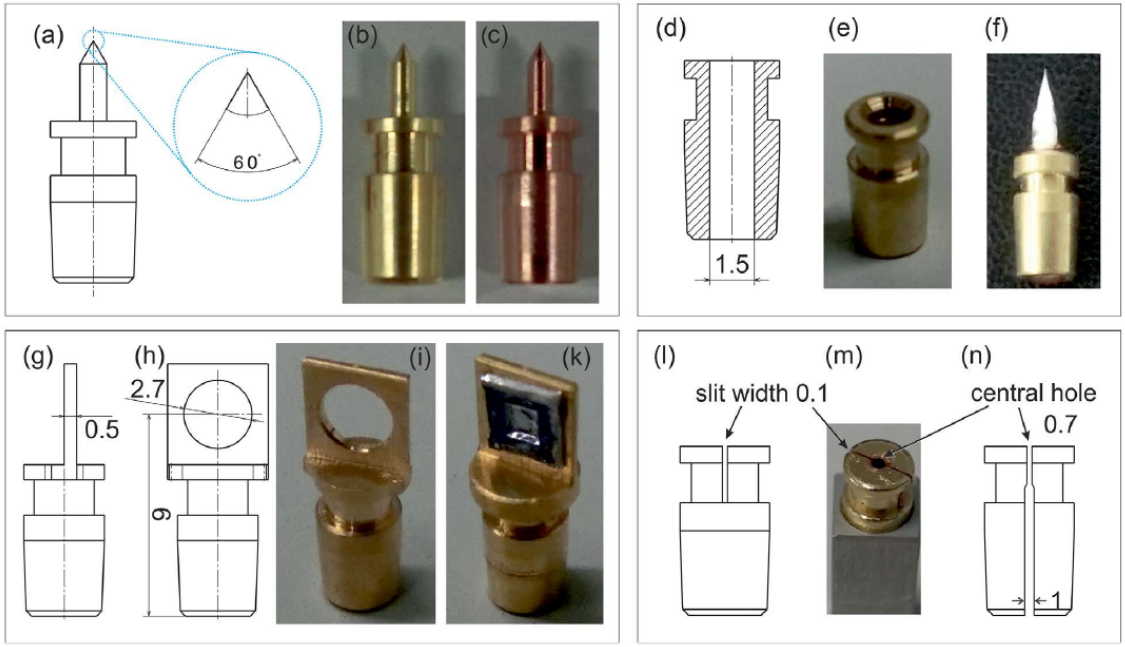


- Robust algorithms for online processing
- **Sample needs to be surrounded by air on both sides at all angles**
- Tomographic reconstructions are provided to the user during the experiment

M. Guizar-Sicairos *et al.*, Opt. Express **19**, 21345 (2011)
M. Odstrčil *et al.*, Opt. Express **27**, 36637 (2019)

Sample mount: the OMNY pin

Manual or FIB cut samples



2D membranes

Micro-capillaries
Need centering!

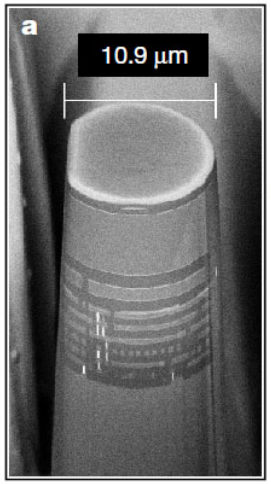
High-pressure freezing tubes

M. Holler *et al.*, Rev. Sci. Instrum. **88**, 113701 (2017)

Applications

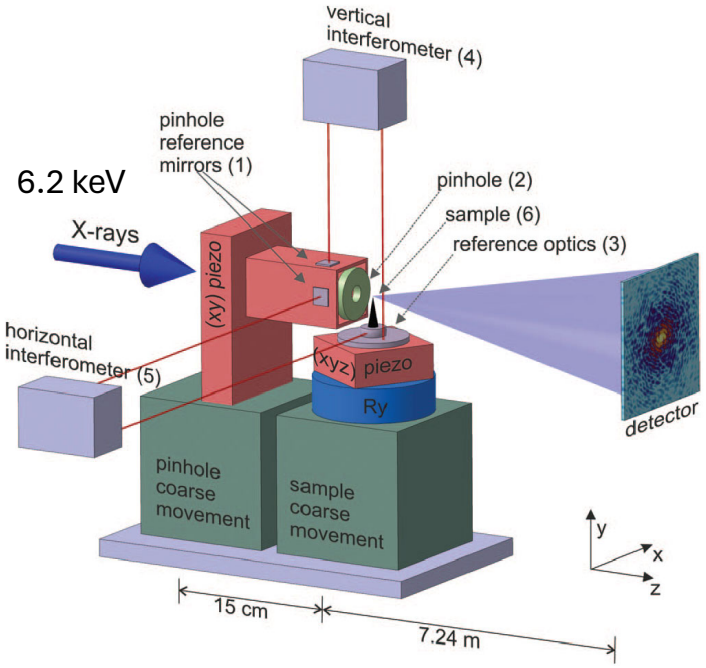
PXCT on integrated circuit

Sample



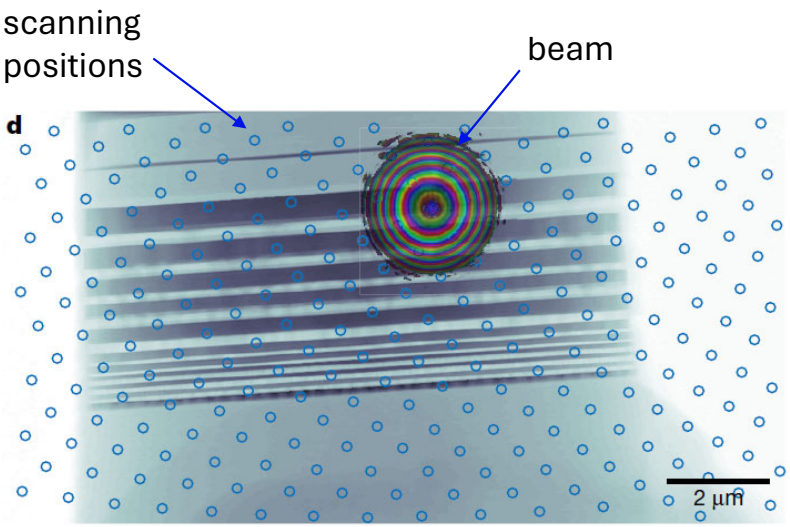
IC sample prepared by focused ion beam (FIB)

flOMNI instrument at cSAXS



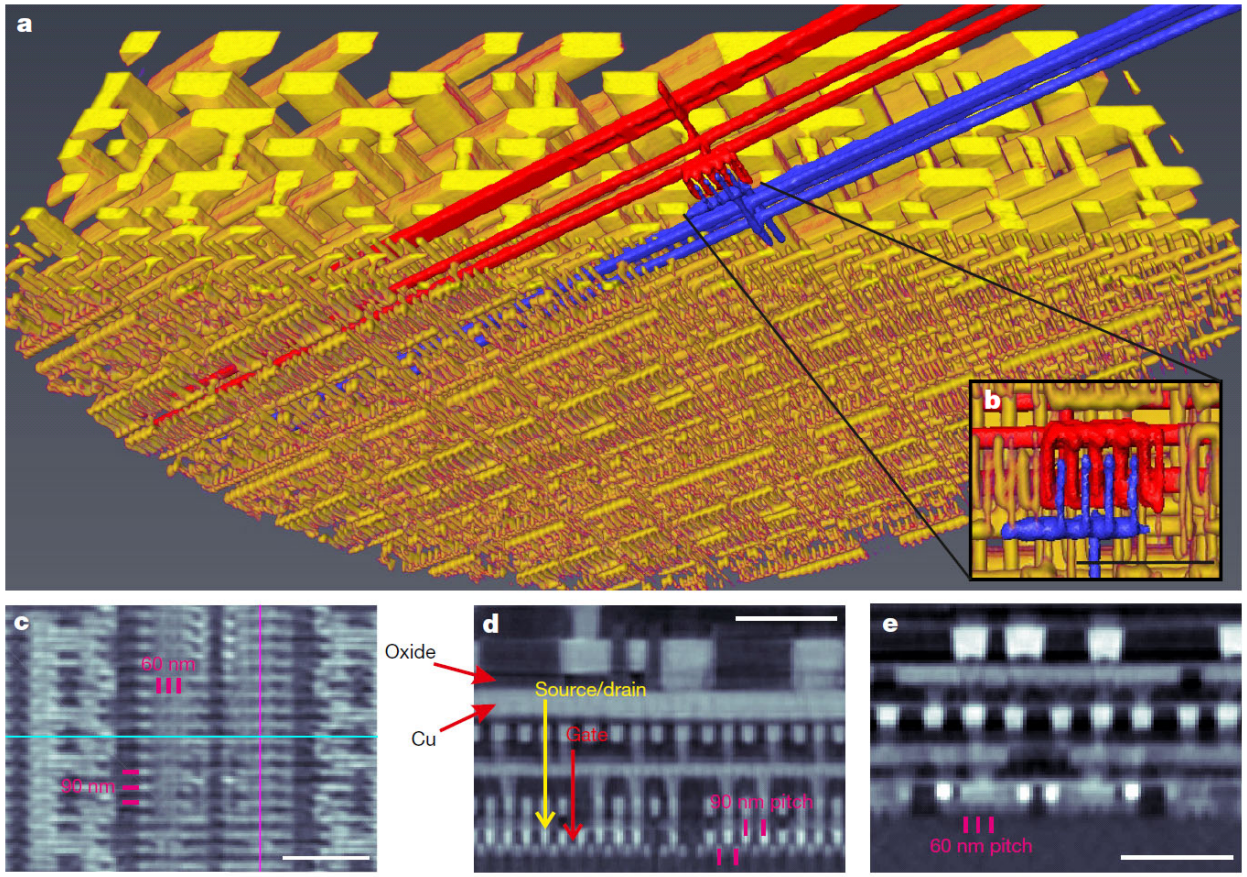
High-precision scanner
Differential laser interferometry between sample and beam-defining optics
Rotation for tomography

2D phase projection



Contrast: phase shift of X-rays
Proportional to integrated density along beam

PXCT on integrated circuit

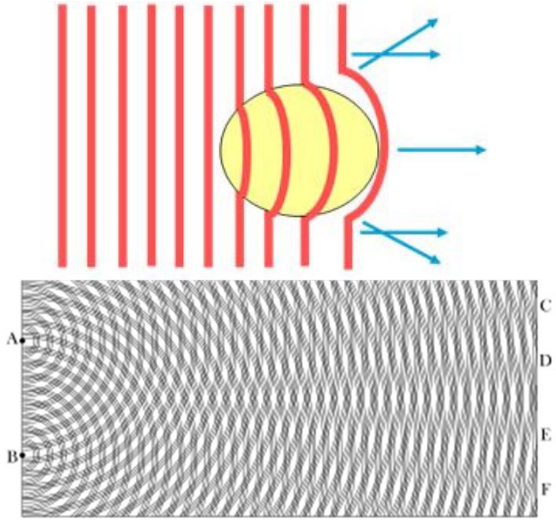


Intel chip,
22 nm technology

M. Holler *et al.*,
Nature **543**, 402 (2017)

Resolution: 14.6 nm
Scale bars: 500 nm

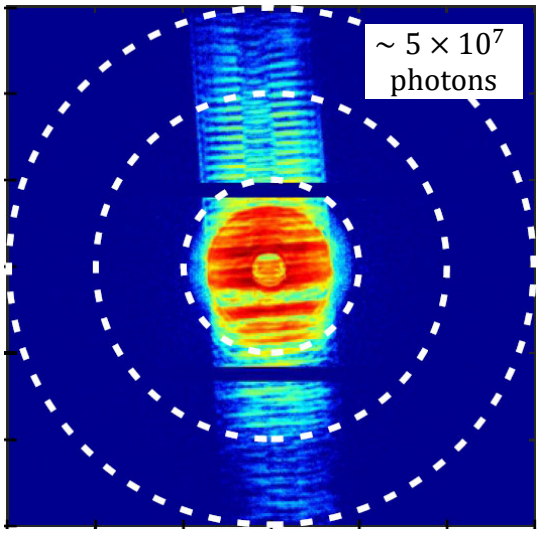
Relevant parameters for high-resolution X-ray ptychography



Contrast and feature density

Contrast: $\delta_1 - \delta_2 \sim \rho_1 - \rho_2$

Many small features $\Rightarrow$ high-frequency scattering



Coherent flux

Total number of photons in the coherent illumination

Resolution depends on dose: energy deposited on sample per mass unit, measured in Gray (Gy)

Resistance to radiation

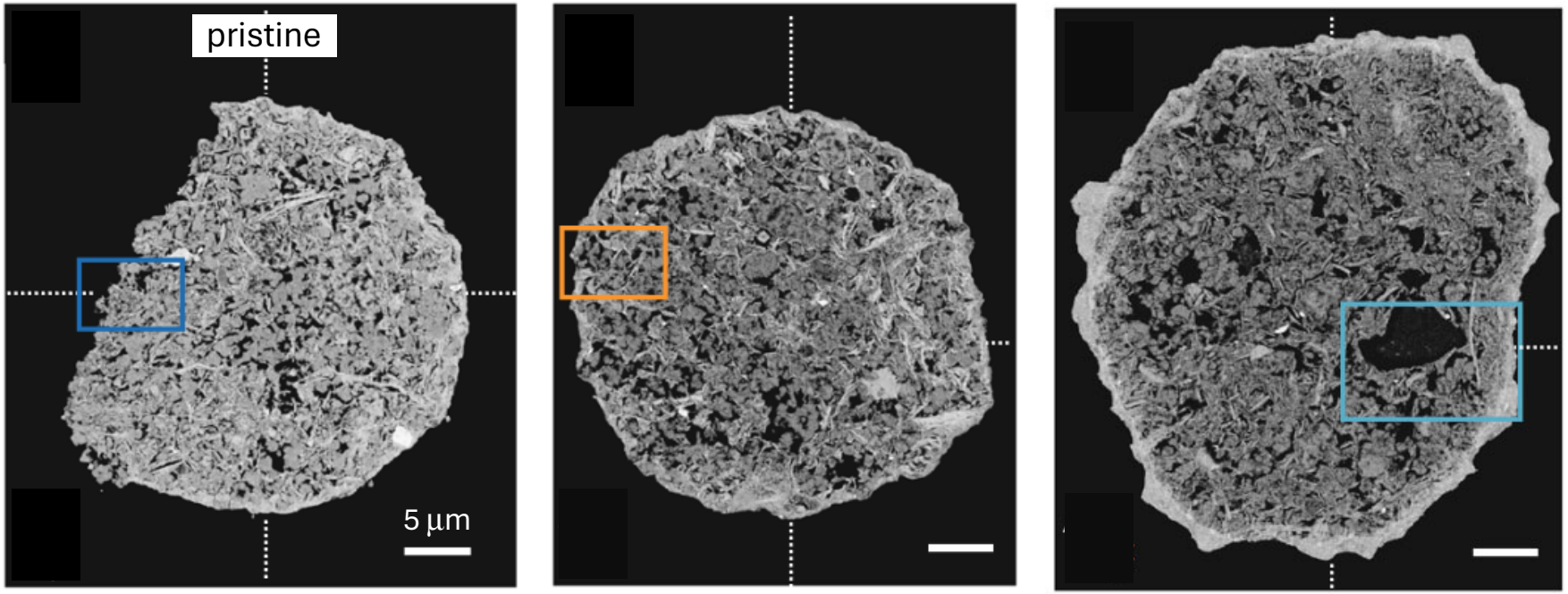
Conductive hard materials like metals or pure carbon are usually resistant

Polymers and biological tissues can show deformations or mass loss

Deactivation of fluid catalytic cracking catalysts

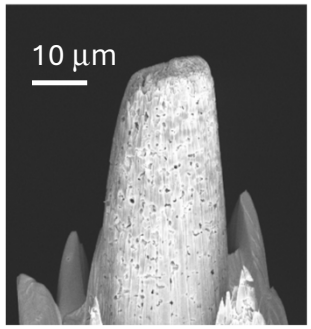
PSI

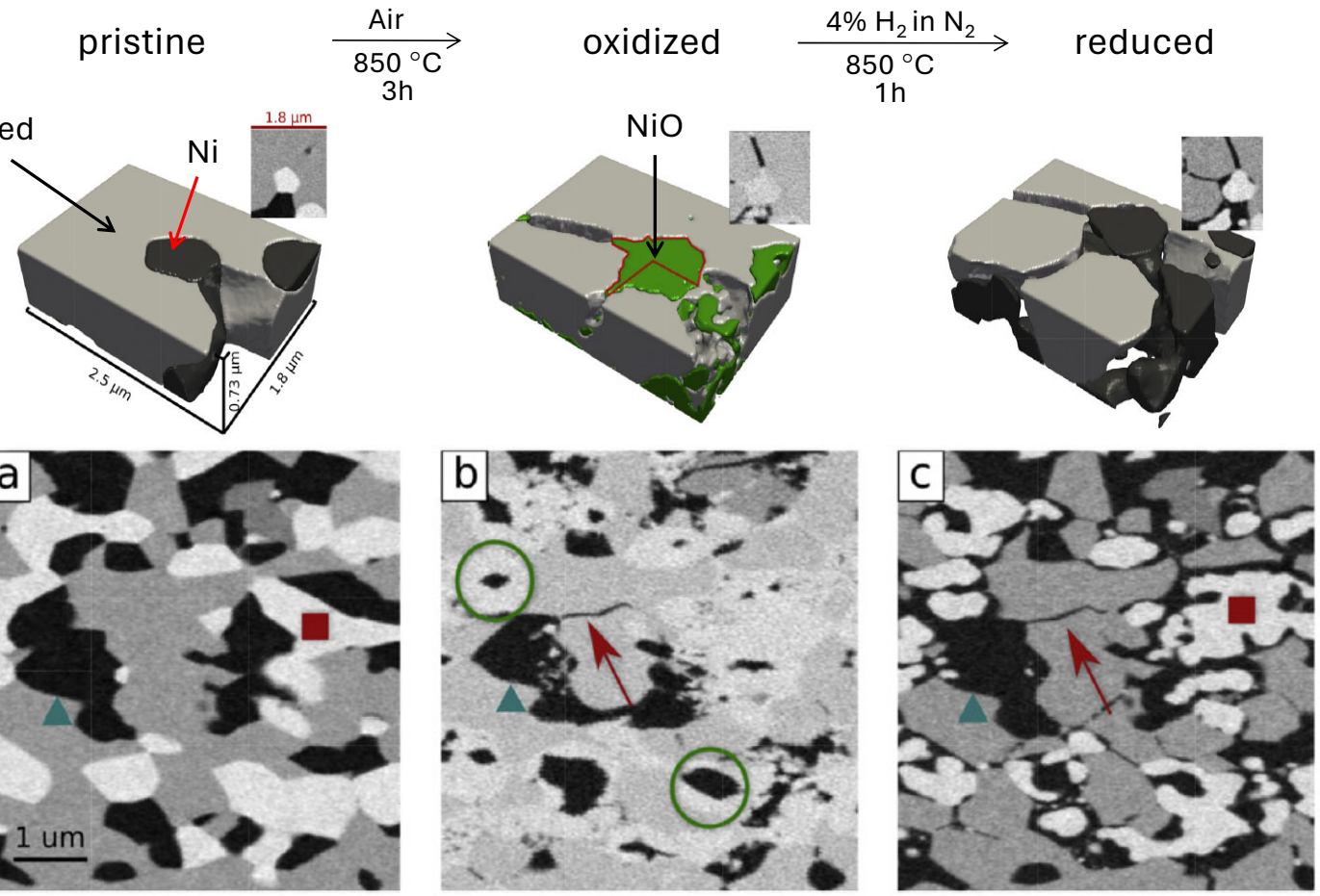
Degree of deactivation (or time spent in the reactor)



J. Ihli *et al.*, Nat. Commun. **8**, 809 (2017)

Ex-situ SOC electrode microstructure evolution

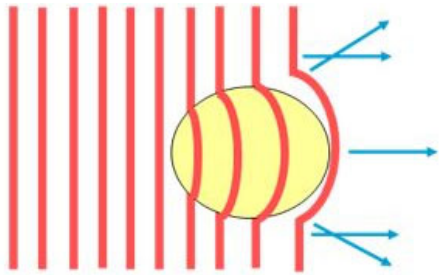




18.4 nm thick slices through 3D dataset

S. De Angelis *et al.*, J. Power Sources **360**, 520 (2017)

The challenge of biological tissues

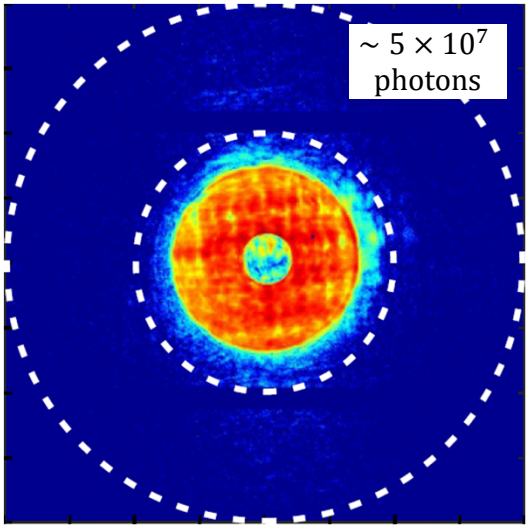


Poor contrast

Contrast: $\delta_1 - \delta_2 \sim \rho_1 - \rho_2$

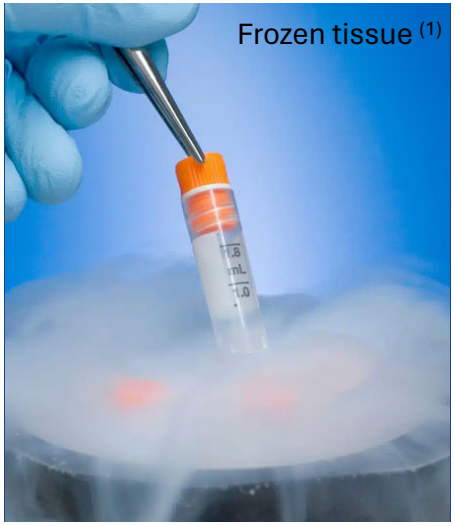
$\rho_{protein}$ - ρ_{water} ≈ 0.35 g/cm^3

($\rho_{Cu} - \rho_{SiO2}$ ≈ 6 g/cm^3)



Need higher coherent flux

to achieve the same resolution compared to a computer chip



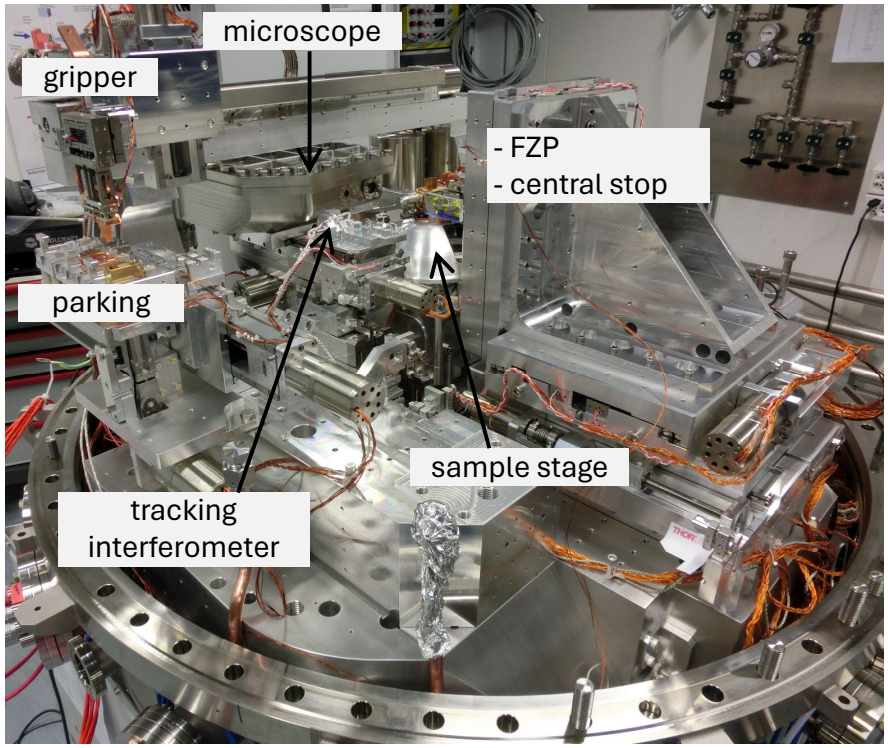
Poor resistance to radiation

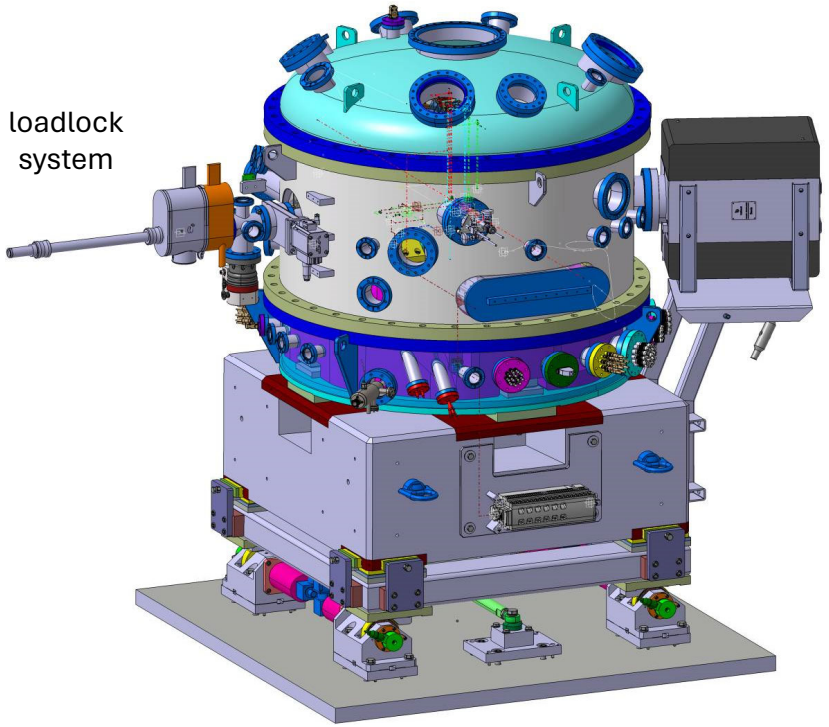
- Need measurements in cryogenic condition to mitigate the effects
- Need complicated sample preparation, either via dehydration or freezing

(1) https://www.ispecimen.com/human-biospecimens/tissue-samples/fresh-frozen-tissue/

OMNY: The cryo-stage instrument

M. Holler, J. Raabe, and engineer team at PSI





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

Beetle scale structure: optimized by evolution

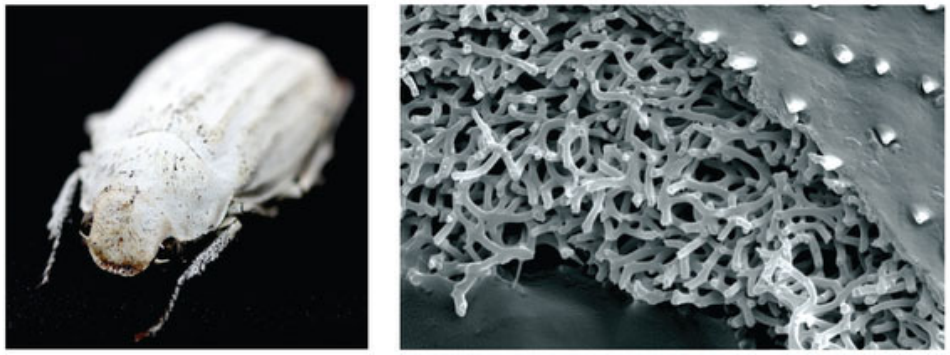
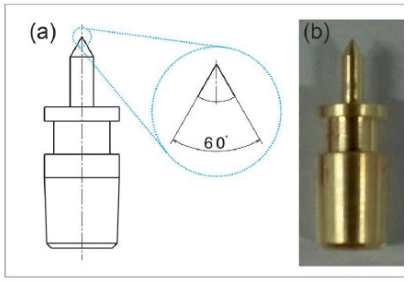
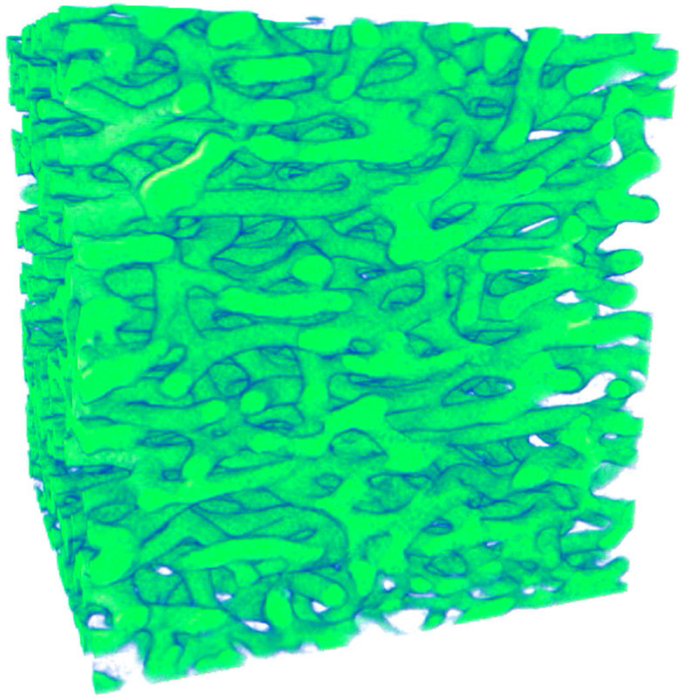


Figure from D. S. Wiersma, Nat. Photonics **7**, 188 (2013)

- *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

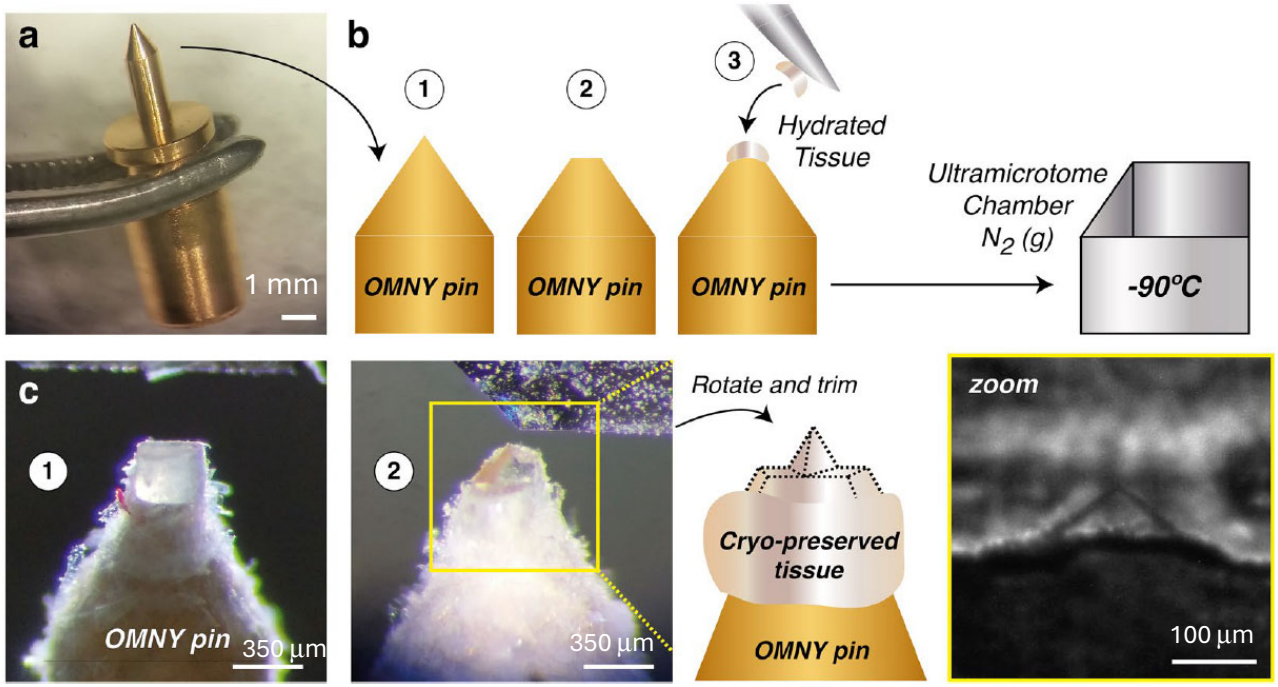


B. D. Wilts *et al.*, Adv. Mater. **30**, 1702057 (2018)



About 7 x 7 x 7 µm^3

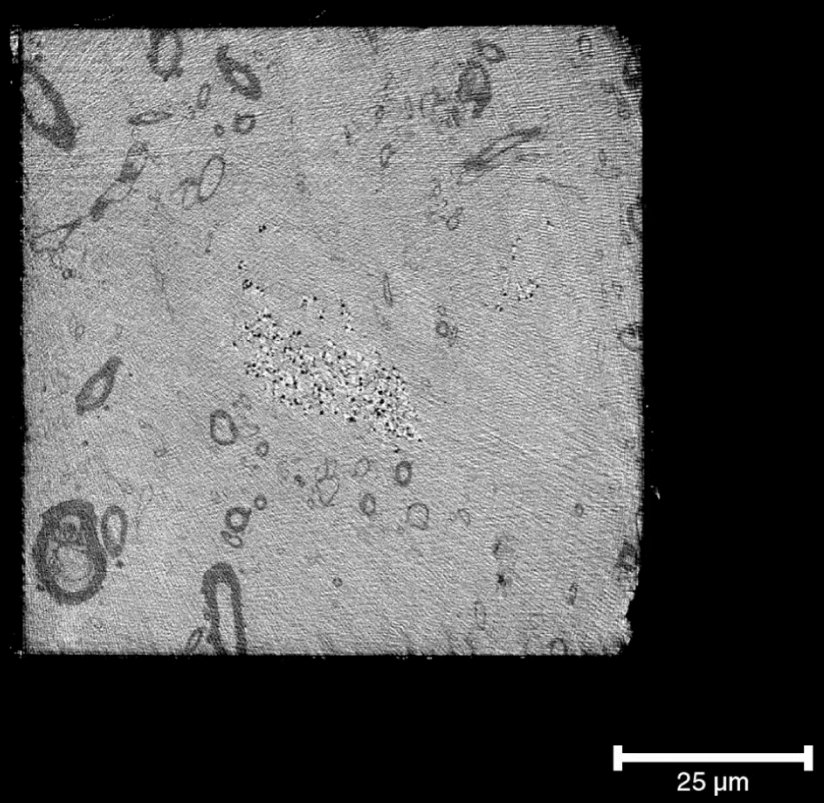
Preparation of brain tissue



- Tissue previously immersed in cocktail of cryo-protectants
- Chemically fixed
- Hydrated, unstained, no organic solvents
- Compatible with post-biochemistry analysis (i.e. localization of specific proteins/antigens)

S. Shahmoradian *et al.*, Sci. Rep. **7**, 6291 (2017)

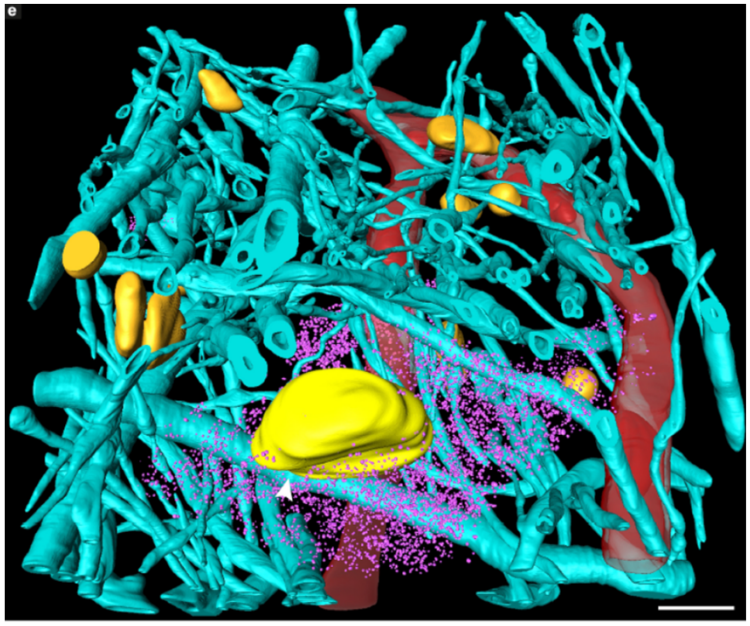
Human brain tissue



H. Tri Tran *et al*., Front. in Neurosci. **14**, 570019 (2020)

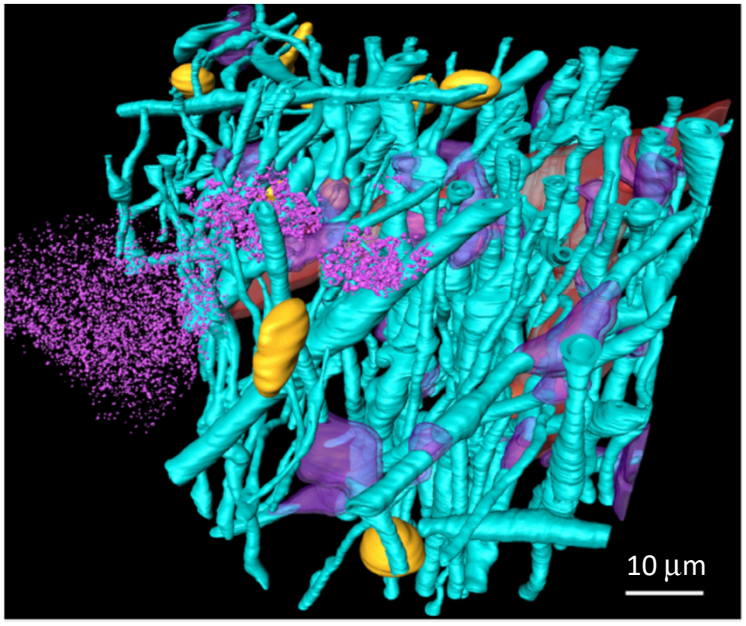
Human brain tissue

healthy



5 specimens imaged from a healthy individual as control

Parkinson-diseased

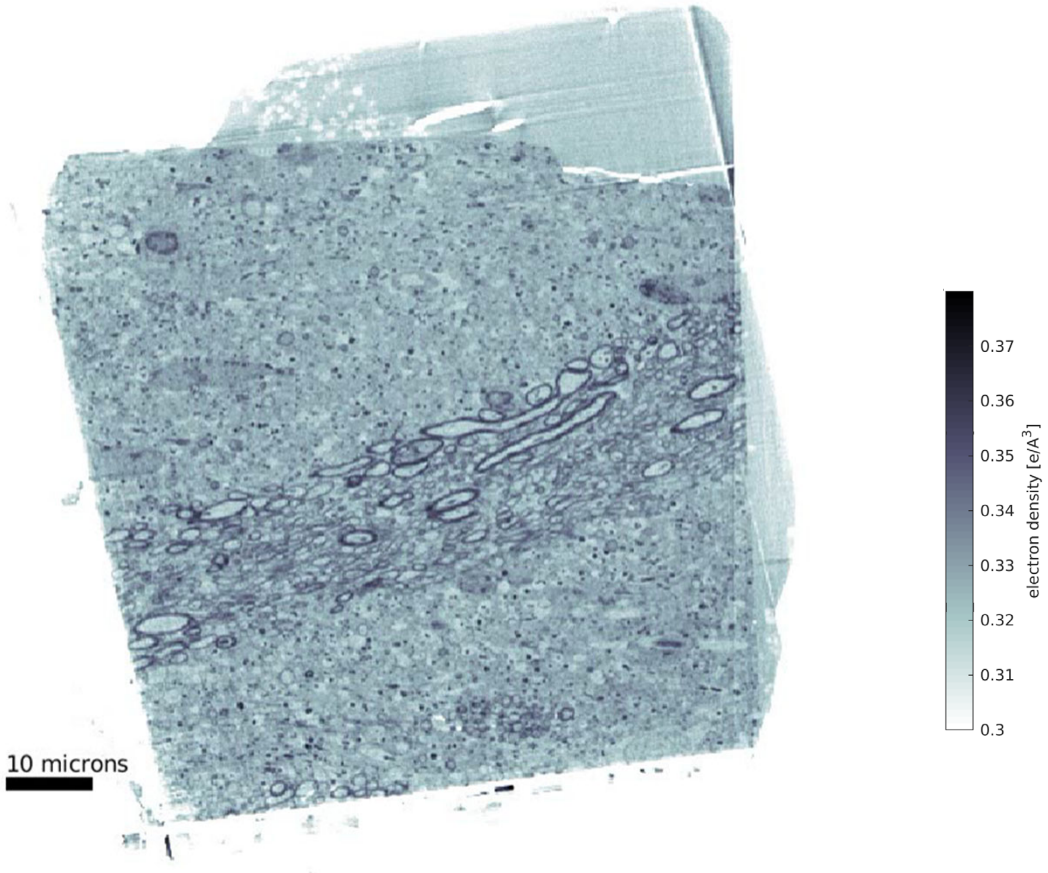


4 specimens imaged from a Parkinson-affected individual

Myelinated axons
Swellings along axons
Cell nuclei
Neuromelanin-containing organelles
Blood vessels
Blood cells

H. Tri Tran *et al.*, Front. in Neurosci. **14**, 570019 (2020)

High pressure frozen mouse tissue



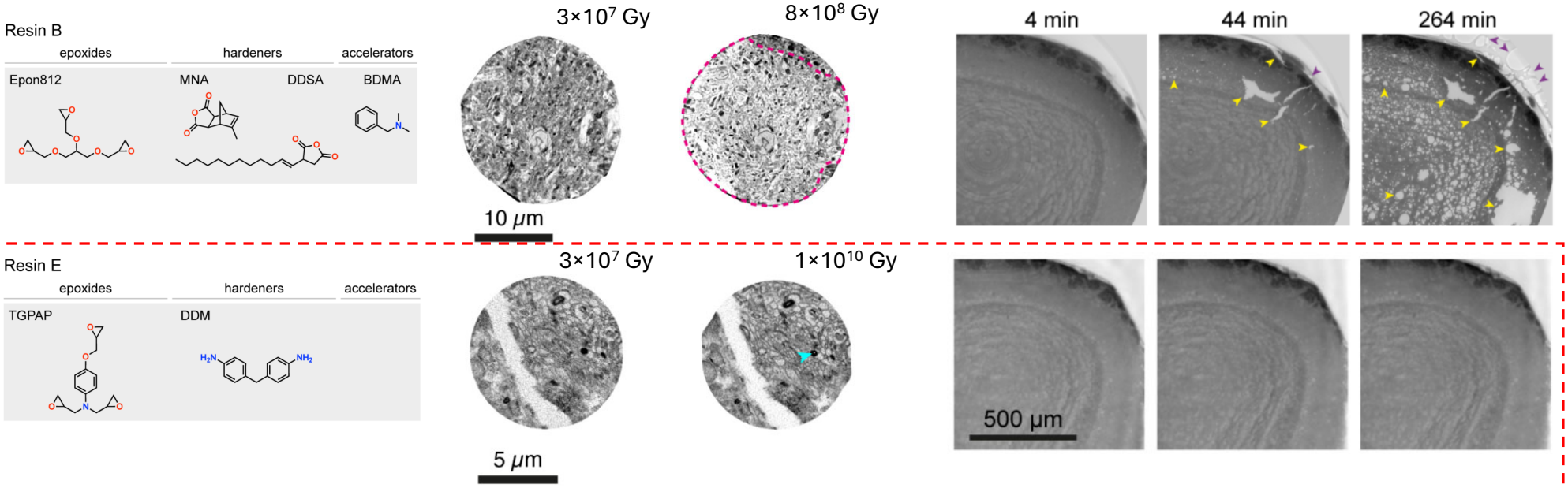
Iman Rostami *et al*. (EPFL Lausanne)
Prepared quicky after sacrificing the mouse: almost intact
Cryo-protectant: PVP

Resolution: 126 nm
Dose: 1.3e7 Gy

Resin embedded, stained brain tissue

C. Bosch *et al.*, bioRxiv (2024) doi: 10.1101/2023.11.16.567403

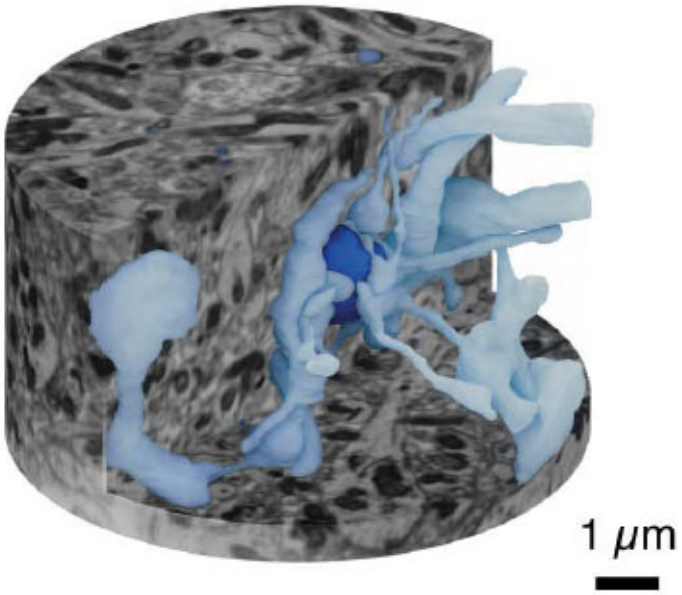
Measurements at BM05 (ESRF)



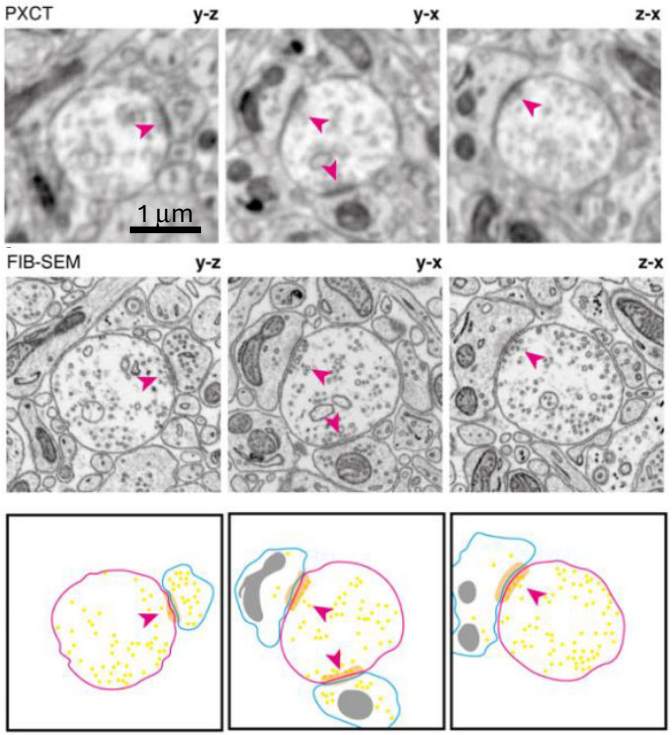
The tough resin

Resin-embedded, stained brain tissue

3D electron density map

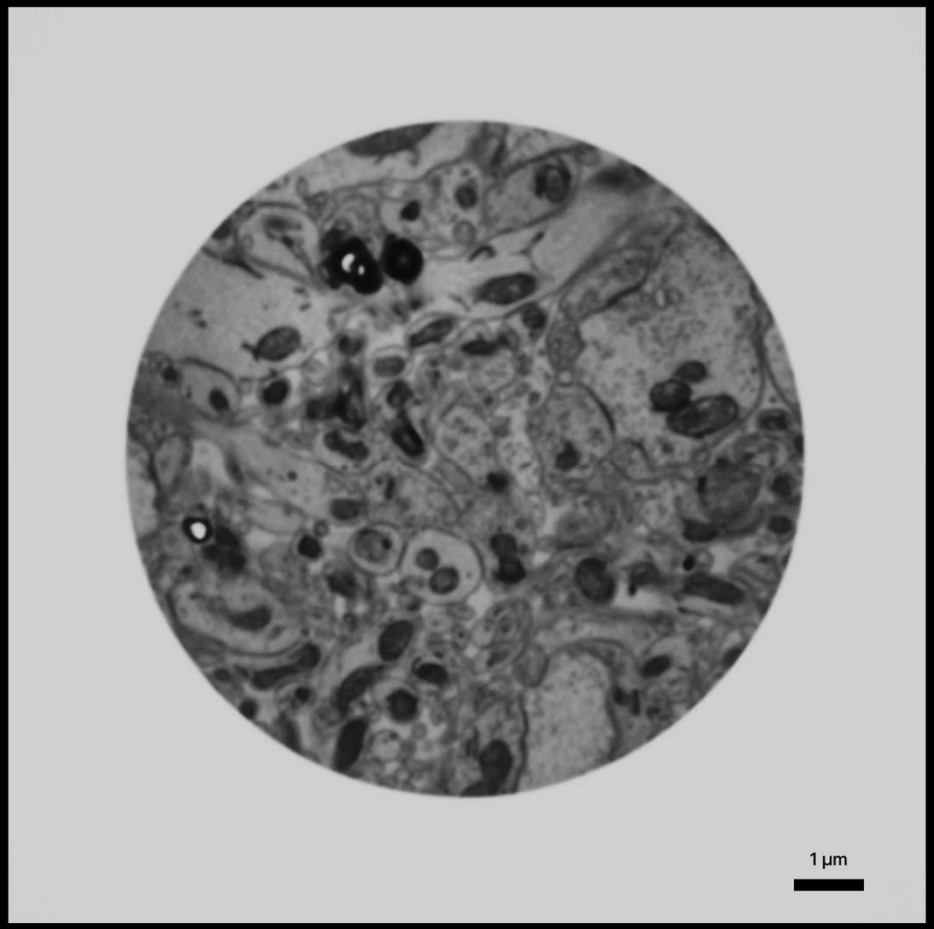


38 nm resolution
absorbed dose: 2.5×10^9 Gy



C. Bosch *et al.*, bioRxiv (2024) doi: 10.1101/2023.11.16.567403

High-resolution 3D imaging of brain tissue

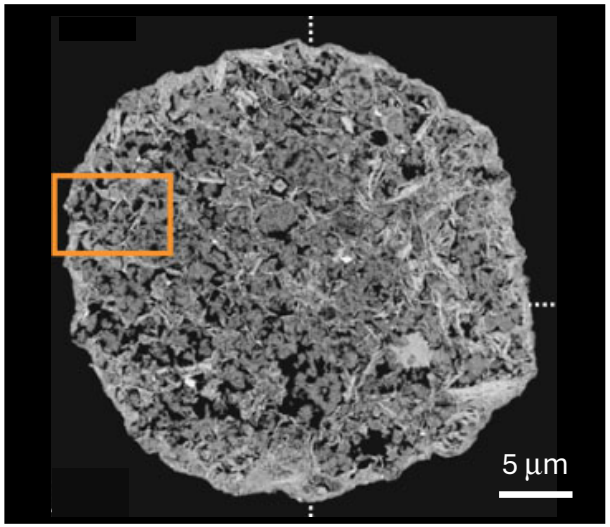


Dose: 2.5×10^9 Gy
38 nm resolution

C. Bosch *et al.*,
bioRxiv (2024) doi:
10.1101/2023.11.16.567403

Summary of part II: PXCT

J. Ihli *et al*. (2017)

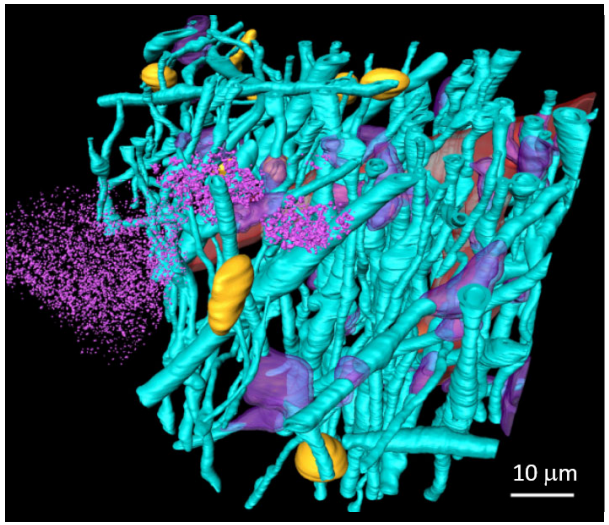


Hard materials

Computer chip, catalyst

High resolution depending on contrast and density of features

H. Tri Tran *et al*. (2020)

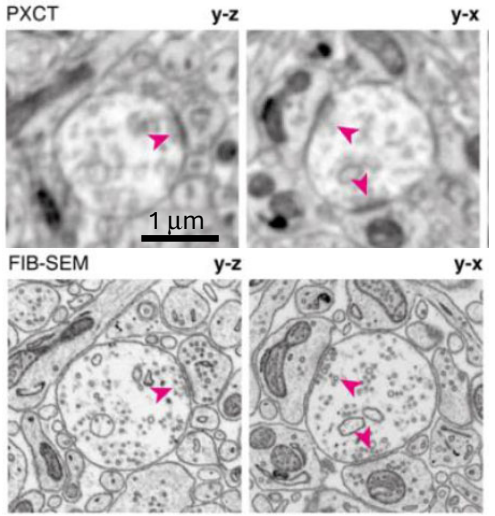


Frozen hydrated biological tissues

Field of view of many tens of microns

Resolution 100 nm, limited by dose

C. Bosch *et al*. (2023)



Resin-embedded, stained tissues

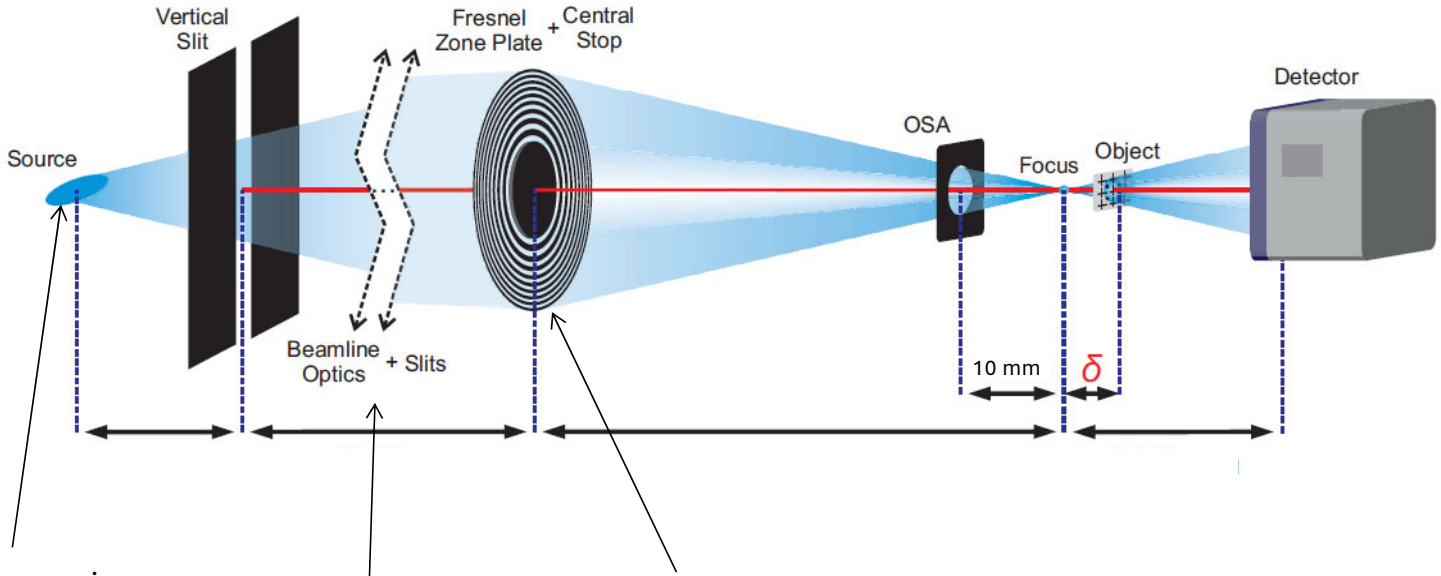
Resolution up to 38 nm

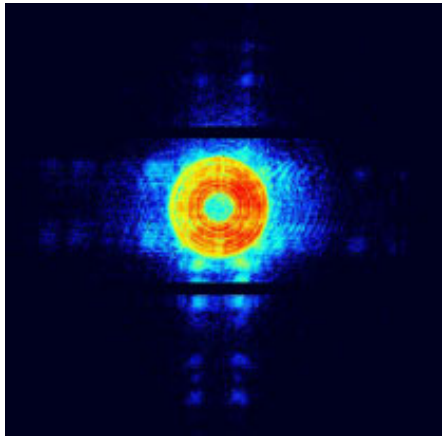
Limit to resolution unknown

Future perspectives

Experimental improvements

J. Vila-Comamala *et al.*, Opt. Express **19** (2011) 21333





coherent flux:
5×10^8 photons/s @ 6.2 keV

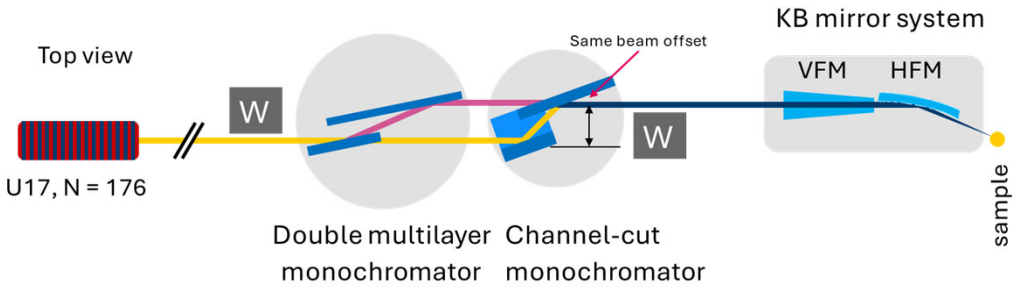
Storage ring upgrade, new undulator ×60

Broader bandwidth ×10

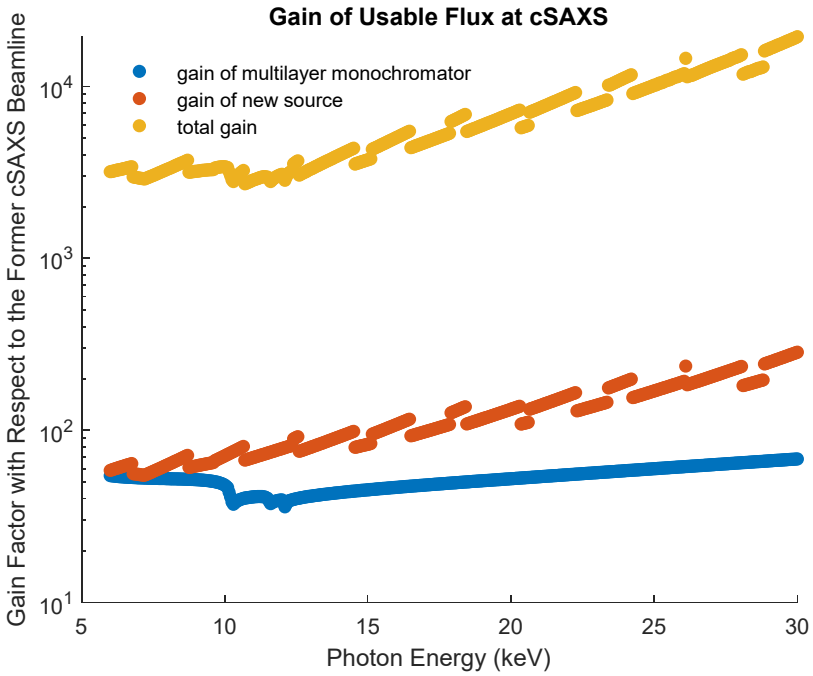
Efficient optics ×10

gain in (coherent) flux

After the upgrade: SLS 2.0 and cSAXS 2.0



- Expected focus on sample ~ 5×5 µm²
- ~ 60× more flux with SLS 2.0 + new undulator
- > 1000× more flux in combination with broadband
- Better beam stability
- Better spatial resolution and/or faster scans



Slide courtesy of Benedikt Rösner

Benefits for experiments after the upgrade

PSI

Higher resolution

- Smaller beam focus for scanning SAXS
- Higher coherent flux for ptychography
- For samples that are radiation resistant
- Example: high-resolution cryo-imaging of biological tissues (as far as samples are resistant)

Faster measurements

- Relies on fast scanning implementation
- More realistic for scanning SAXS with continuous scans
- Relevant e.g. for medical studies or dynamics

Larger volumes

- Relevant e. g. for brain tissues

Exercise

Exercise

Make groups of 2 or 3 people. Each group works on the exercise together as a team.

Part I:

Choose one of the following experiments. For the chosen experiment, propose a measurement (or several measurements) at the cSAXS beamline at SLS 2.0. Please answer the following questions:

- Which technique(s) would you choose to do this measurement? Why?
- How would you prepare the sample? (give details about the approximate size, how would you prepare it, special container if needed…)
- Indicate the required spatial resolution and/or contrast, explain why.
- If appropriate, suggest one of the instruments available at cSAXS to perform the measurements, and explain your choice
- Perform a risk assessment of your proposal: are there any uncertainties for its success?

Part II:

Explain your experiment proposal in about 5 minutes to the rest of the students in the class. Be ready for questions from your colleagues.

Exercise

PSI

Experiment 1

A solid composite material is made of a polymer matrix with silica (SiO_2) spherical nano-particles embedded in it. When breaking the material, a scanning electron microscope (SEM) image reveals that the particles are approximately spherical and have a diameter of around 50 nm. To establish the applicability of this composite for certain applications, we would like to know what is the average size of the silica nanoparticles and what is the variability in particle size, i.e. its polydispersity.

Experiment 2

Biologists would like to know if SiO_2 nanoparticles of 50 nm diameter are being taken up by the cells in mammalian tissues. They are performing some tests on skin tissues that have cells of about 20 micron size and are exposed to a solution of SiO_2 nanoparticles for some time. They would like to know if the nanoparticles penetrate inside the cell walls, and if they do, how do they distribute inside the cell: do they distribute evenly within the cell? Do they cluster in groups? Do they accumulate preferentially around certain organelles like mitochondria or the cell nucleus? This will help them understand the toxicity effects of these particles in humans.

Exercise

PSI

Experiment 3

Caries is one of the most common disease in humans. It causes damage in our teeth and, if not treated, the affected tooth can be lost. Understanding how it affects the nanostructures in teeth may help to prevent it. One of the questions is how it affects the collagen nanostructures: are the collagen nanostructures different in areas of the tooth affected by caries, compared to unaffected areas on the same tooth? Possible differences could be the orientation of the nanostructures or the abundance of nanostructures.

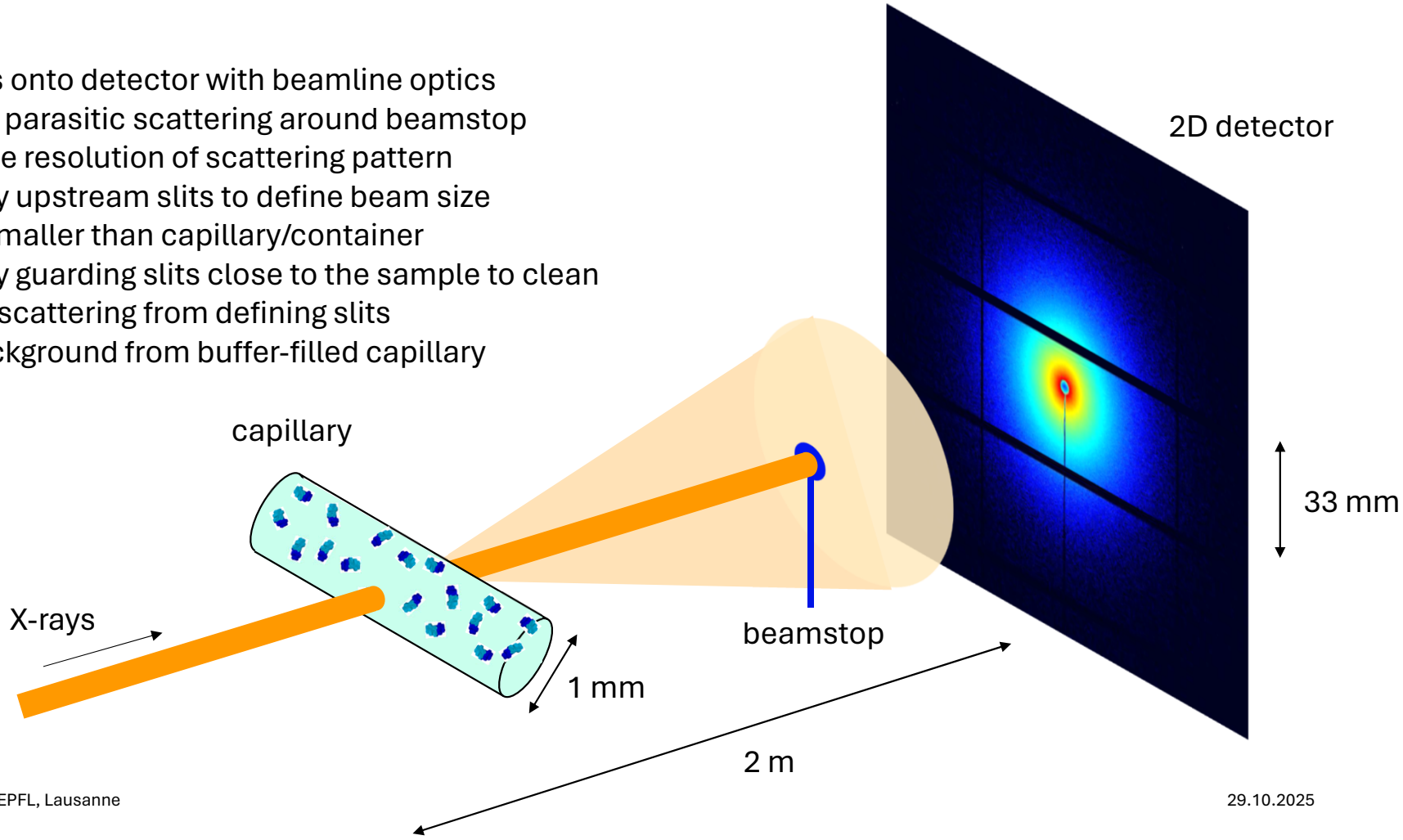
Experiment 4

Perhaps in your current scientific project during your PhD or postdoc you can think of one of the techniques used at cSAXS to characterize your samples. You can discuss this with your team and propose together an experiment to help you unravel the nanostructures in your samples.

Extra slides

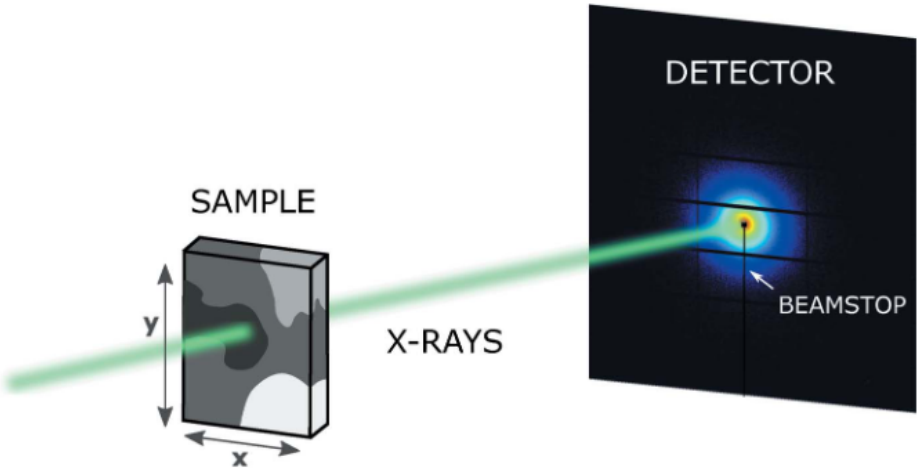
How to acquire good SAXS data

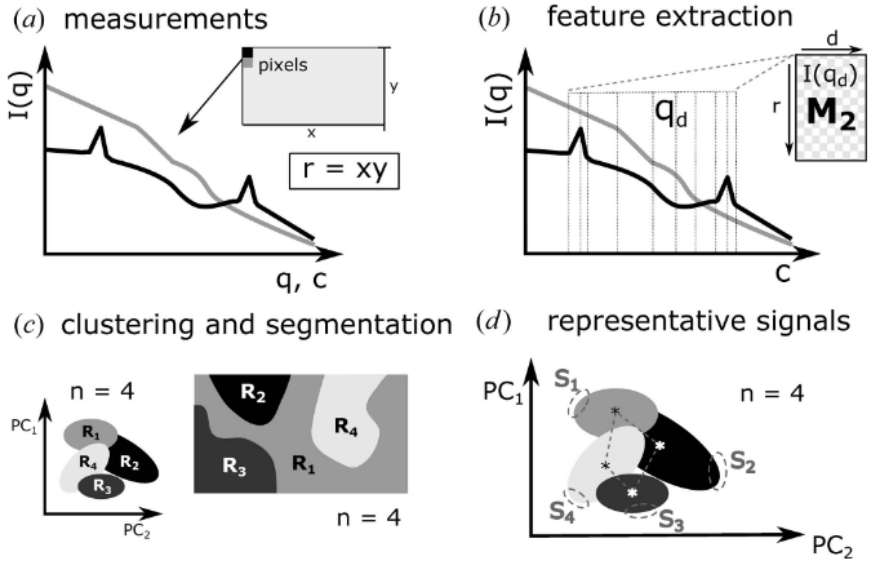
- Focus X-rays onto detector with beamline optics
  - Reduce parasitic scattering around beamstop
  - Optimize resolution of scattering pattern
- Good-quality upstream slits to define beam size
  - Beam smaller than capillary/container
- Good-quality guarding slits close to the sample to clean up parasitic scattering from defining slits
- Subtract background from buffer-filled capillary



Classification of SAXS patterns in tissues

- Tissues: phase with mixtures of different structure
- Aim: model-free classification and segmentation
- Classification of selected components identified by principal component analysis (PCA)



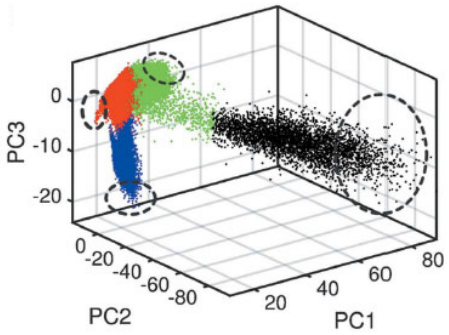


V. Lutz-Bueno *et al.*, J. Appl. Cryst. **51**,1378-1386 (2018)

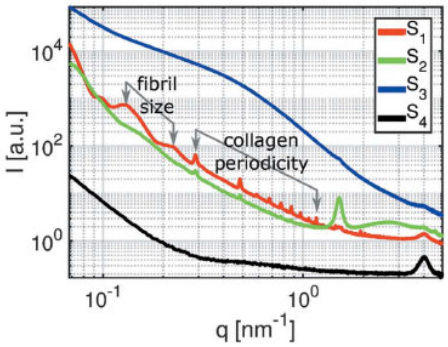
Model-free image segmentation based on scattering signals

Microcalcifications in breast tissue

data classification in clusters

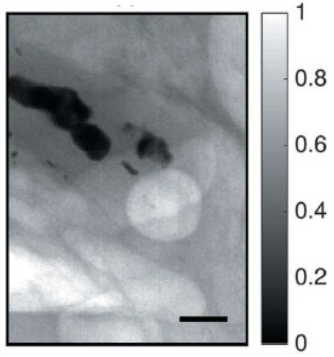


representative signals

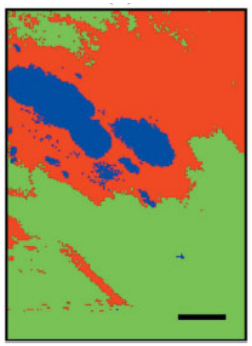


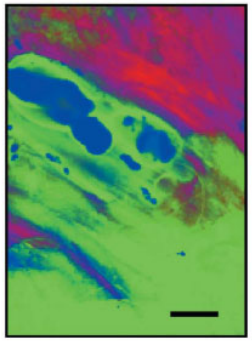
- Collagen
- Lipids
- Microcalcifications
- Kapton

transmission



segmentation





correlation map indicative of misclassifications

V. Lutz-Bueno *et al.*,J. Appl. Cryst. **51**,1378-1386 (2018)

Compare *Chlamydomonas* measurements

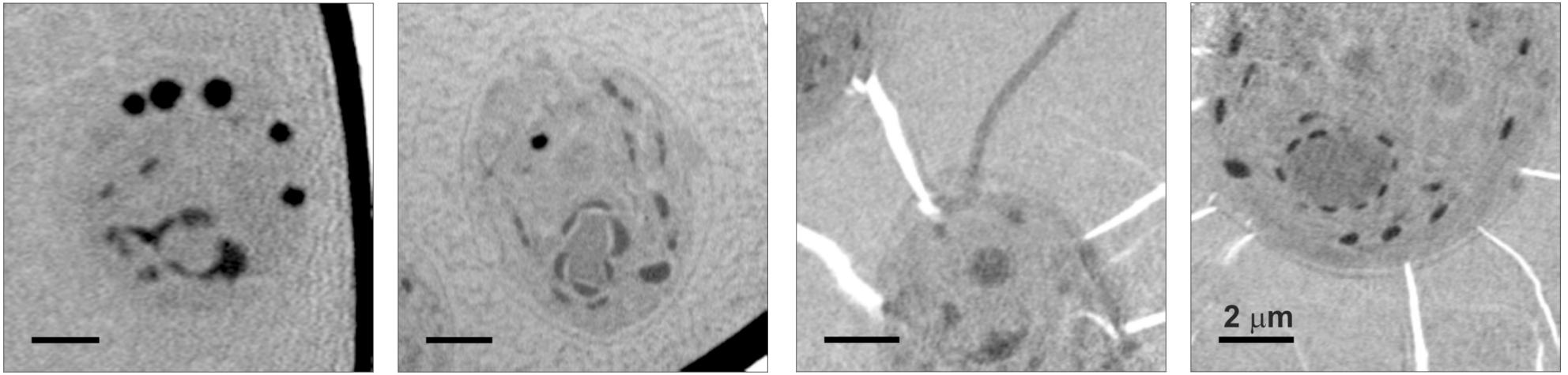
PSI

Plunge frozen

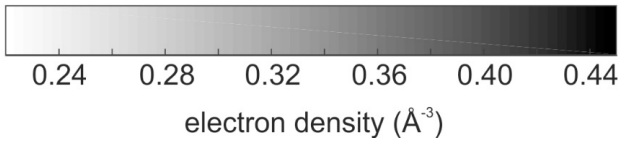
10% glycerol
cryo-jet (1)

10% DMSO
OMNY (2)

High pressure frozen
no cryoprotectant
OMNY (3)



Resolution: ~ 200 nm ~ 100 nm ~ 100 nm

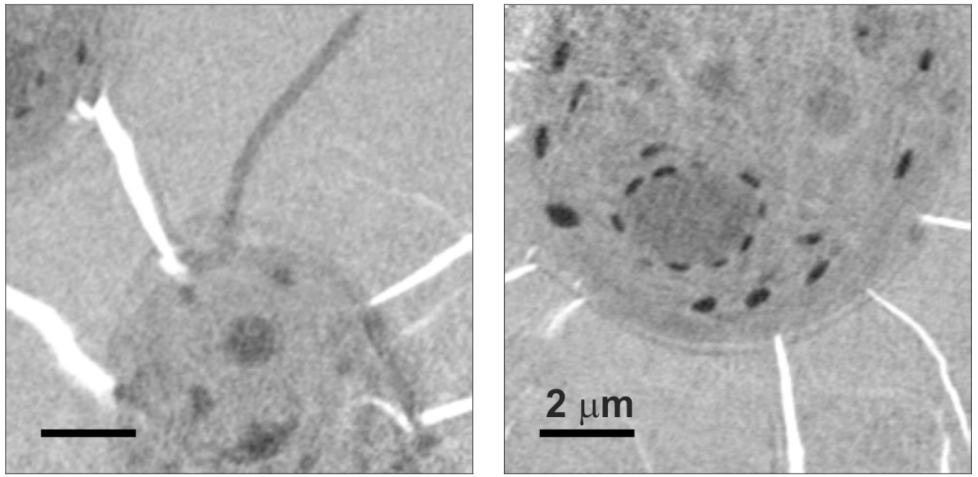


(1) A. Diaz et al., J. Struct. Biol. **192**, 461 (2015)
(2) M. Holler *et al.*, Rev. Sci. Instrum. **89**, 043706 (2018)

Compare PXCT with soft X-ray microscopy

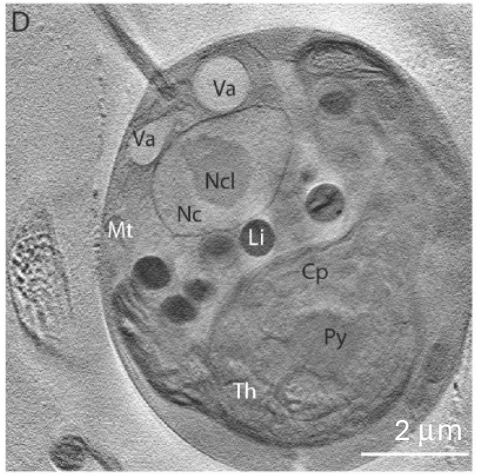
PSI

PXCT
100 nm resolution
2×10^7 Gy



M. Holler *et al.*,
Rev. Sci. Instrum. **88**, 113701 (2017)

Soft X-ray microscopy
30 nm resolution



E. Hummel *et al.*, PLOS One **7**, e53293 (2012)