Realtime 4D tomographic microscopy: the SLS experience

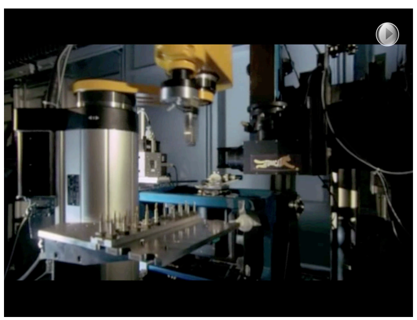
M. Stampanoni[a,b]
on behalf of the TOMCAT team

[a]Swiss Light Source, Paul Scherrer Institut, 5232 Villigen, Switzerland;
[b]Institute for Biomedical Engineering, University and ETH Zürich, 8092 Zürich, Switzerland;

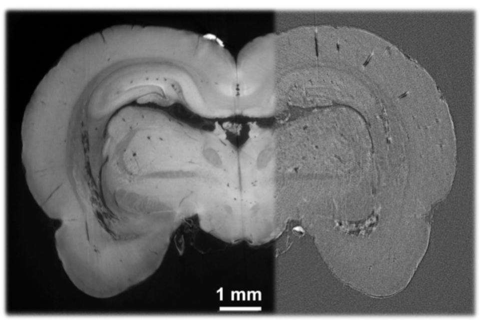
TOMCAT at a glance

Features:

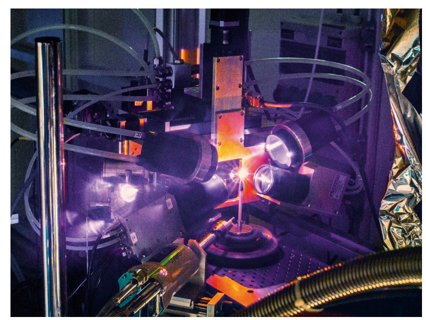
- Wide spatial resolution range: nano-to-meso scales (0.1-10µm)
- Broad range of sample sizes (10µm-20mm)
- High density resolution enhanced by phase contrast
- High temporal resolution (3D data acquisition in less than 1s)



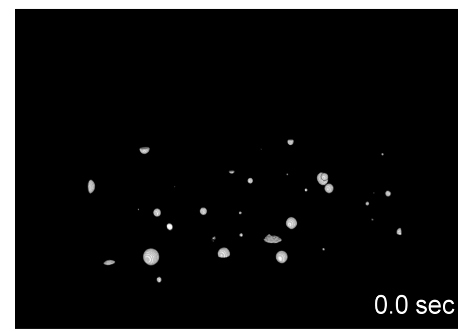
Automation
large scale studies



Density resolution
phase contrast imaging



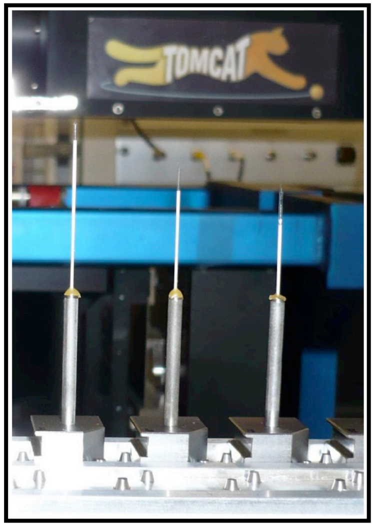
In situ capabilities
temperatures away from ambient

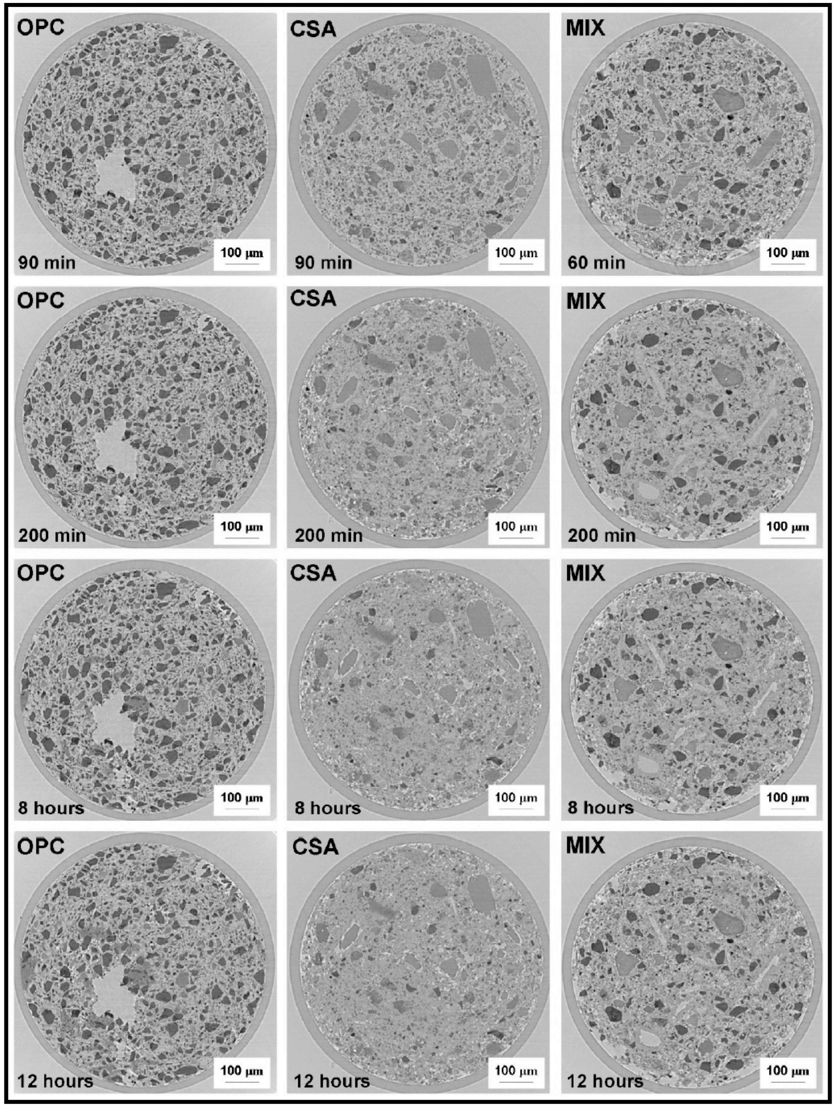


Ultra-fast data acquisition
dynamic studies

Fully automated tomographic microscopy endstation

D. Attenborough's "*First Life*", BBC 2011





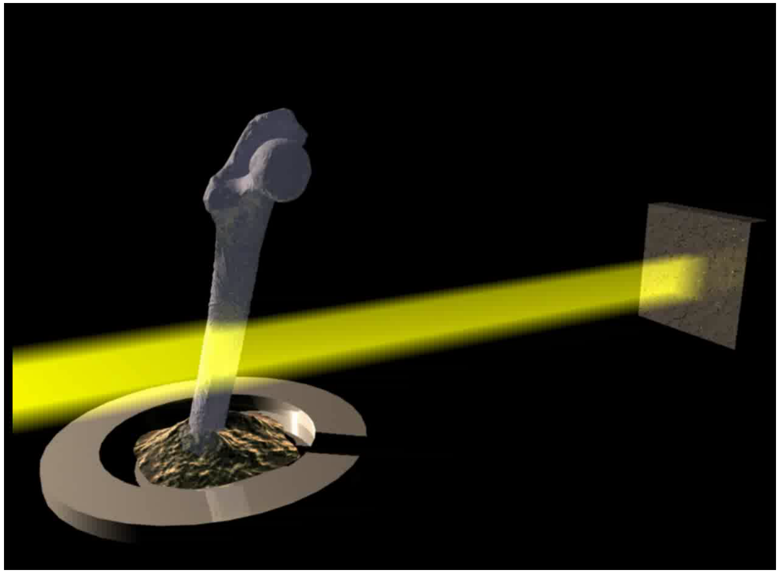
D. Gastaldi, Construction and Building Materials 29 (2012) 284–290

- Cement materials in capillaries of 600 microns diameter
- Study on different binders:
    - Ordinary Portland Cement (OPC)
    - Calcium Sulphoaluminate Cement (CSA)
    - Mixed sample(MIX)
- Sample mounting and sequencing fully automatic

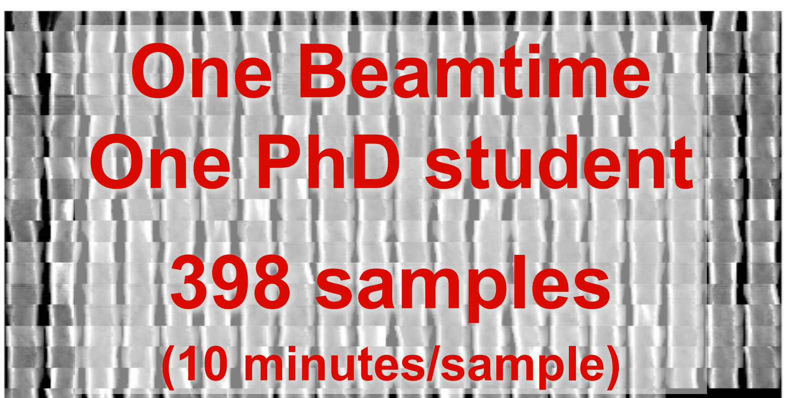
→ **Unattended monitoring of hydration process over 12h**

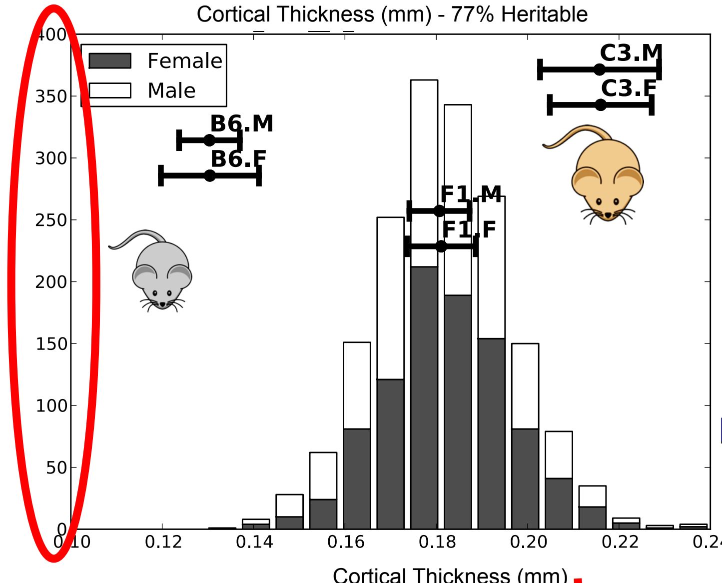
High-throughput: from sample alignment to QTL analysis

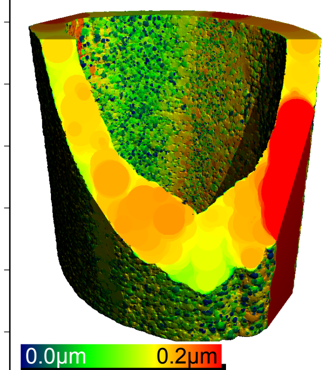
High-throughput: ROI selection and alignment

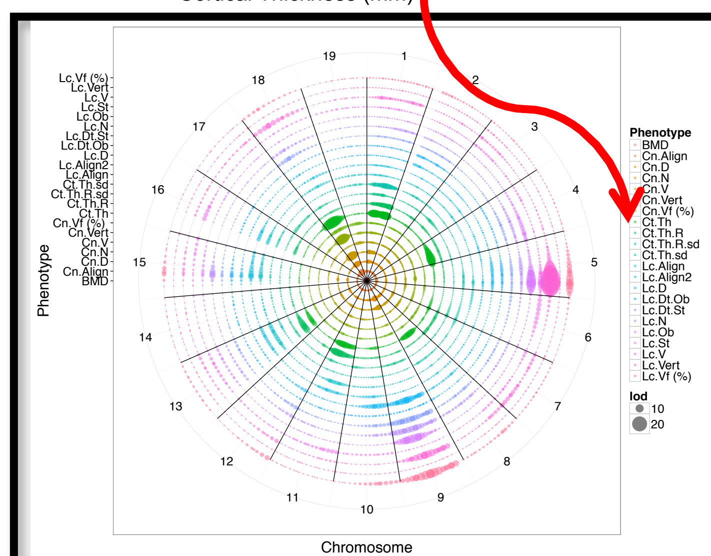


Femur samples automatically aligned using goniometer and moved to region of interest using projections and image processing scripts







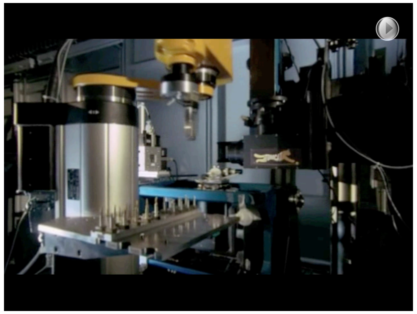


K. Mader, PhD Thesis 2013 and K. Mader et al., BMC Genomics 16:493 (03 Jul 2015)

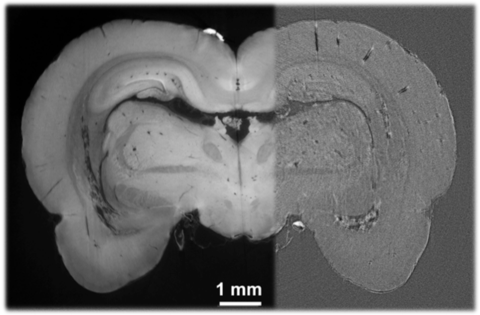
TOMCAT at a glance

Features:

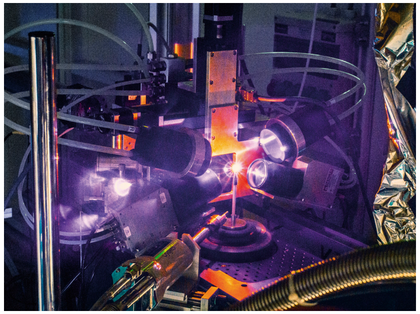
- Wide spatial resolution range: nano-to-meso scales (0.1-10μm)
- Broad range of sample sizes (10μm-20mm)
- High density resolution enhanced by phase contrast
- High temporal resolution (3D data acquisition in less than 1s)



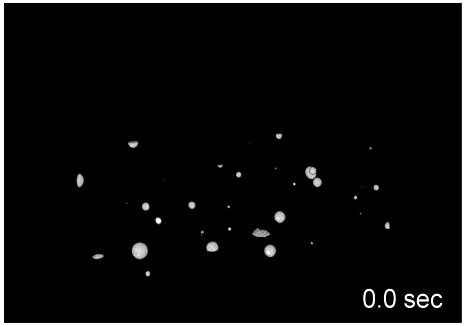
Automation large scale studies



Density resolution phase contrast imaging



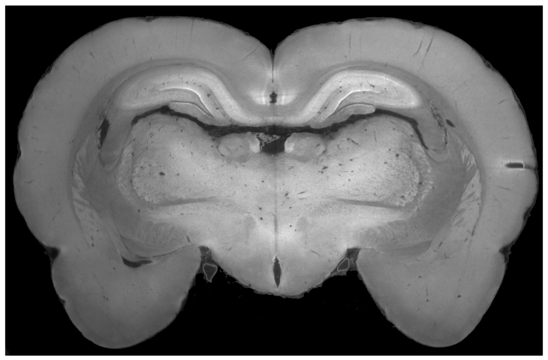
In situ capabilities *temperatures away from ambient*



Ultra-fast data acquisition dynamic studies

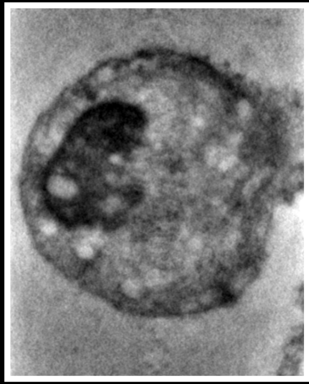
Phase contrast imaging over four length scales

E. M. Friis, M. Stampanoni et al., Nature 2007
Modified Transport of Intensity



S. McDonald, M. Stampanoni et al., JSR 2009
Talbot interferometry

T .Thüring et al, Skeletal Radiolgy,2013
Talbot-Lau interferometry

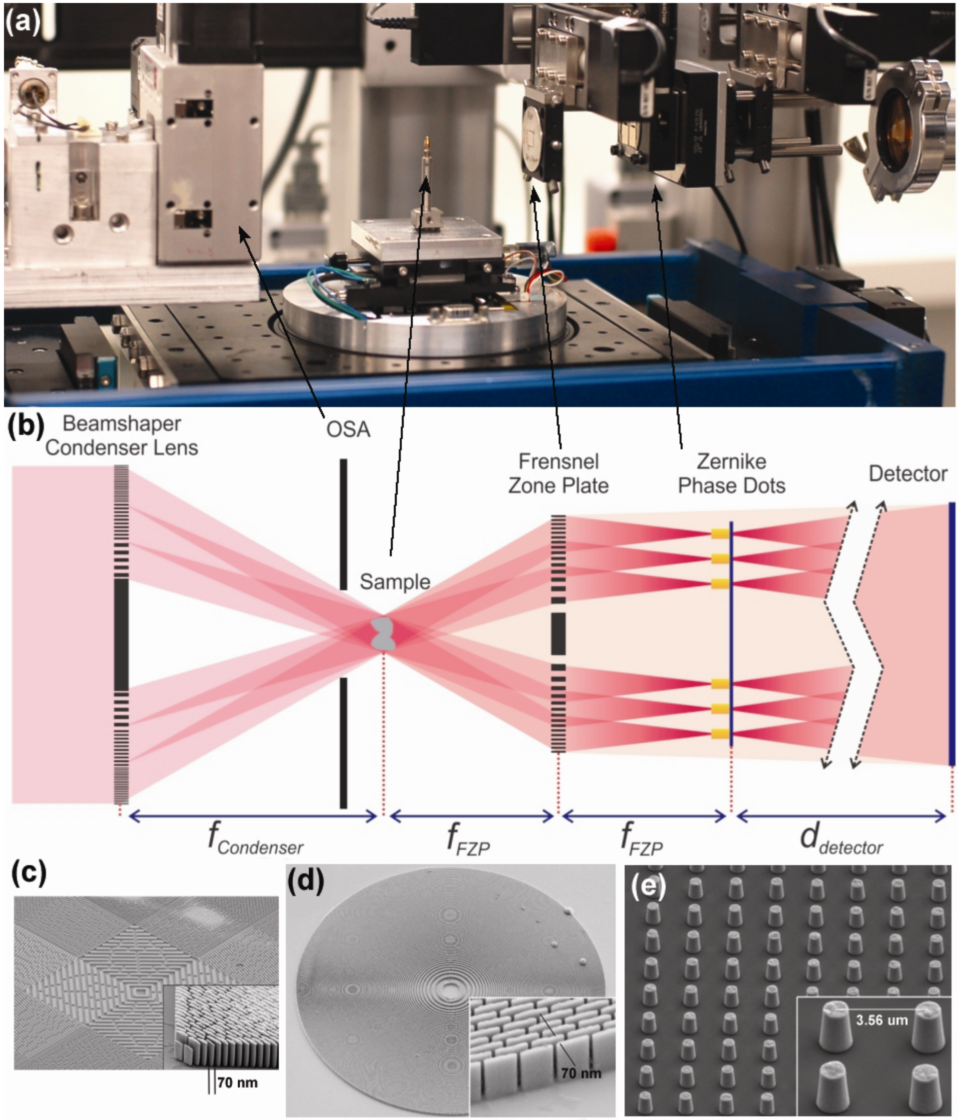


M. Stampanoni et al, PRB 2010
Hard X-ray Zernike Phase Contrast

0.1 micron | **1 micron** | **10 microns** | **100 microns**

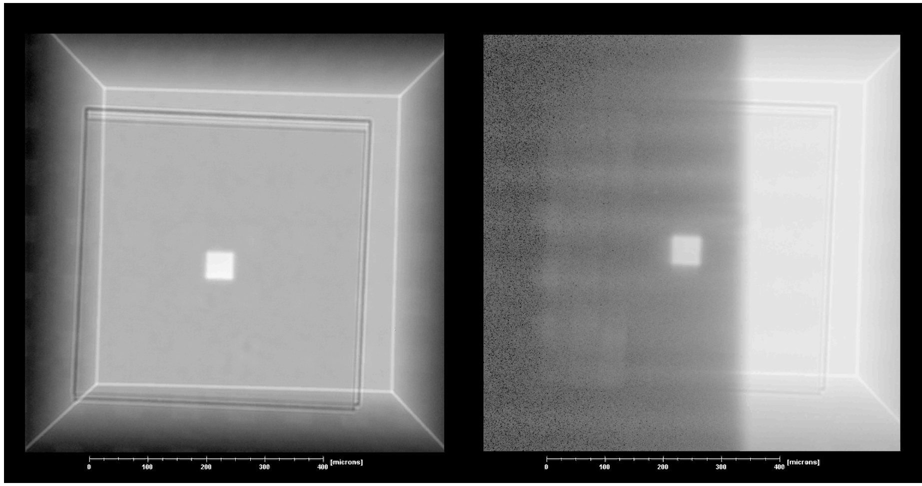
cells | **small animals** | **humans**

TOMCAT nanoscope (operating at 150-200nm)

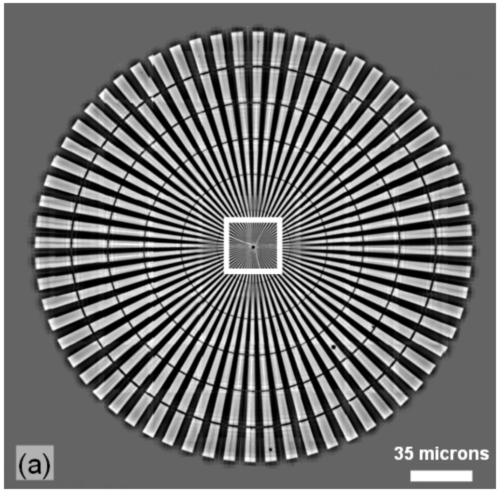


M. Stampanoni et al., PRB2010 ; R. Mokso et al., JSB2012

Square, top-flat, illumination



Sensitivity and resolution

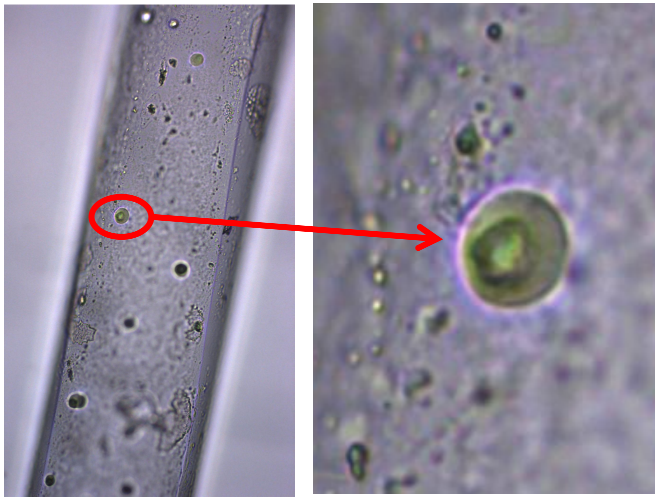


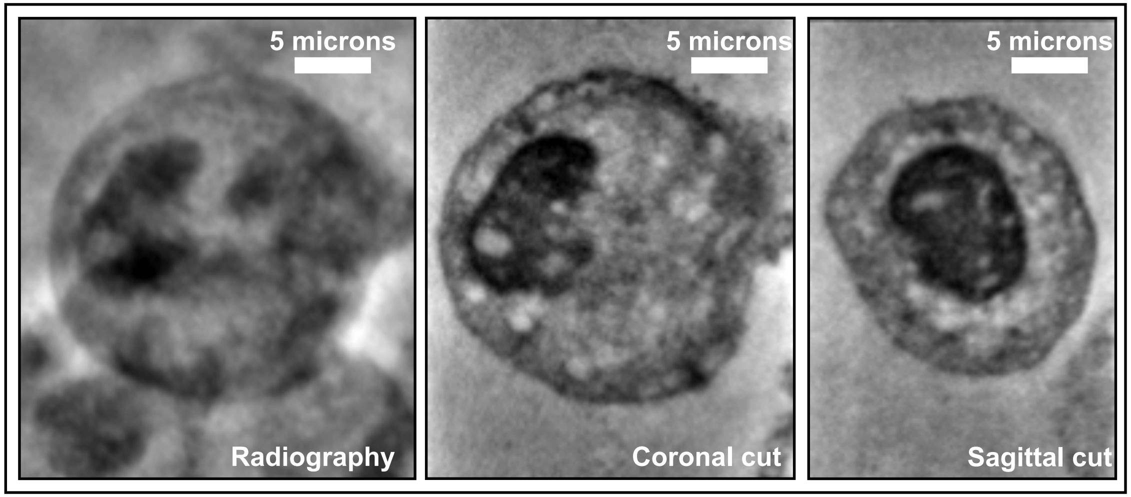
Si - Siemens star

- 300 microns diameter
- 1 microns depth structure
- Phase shift: π/15

- Rayleigh limit : 122 nm
- Measured: 133 nm (2D)
- Energy: 10 keV

Nanotomography of a MC3-preosteoblast cell

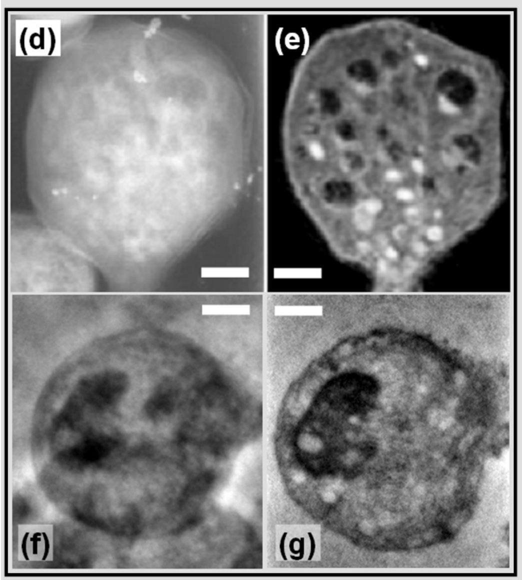




TOMCAT Nanoscope

- 10 keV
- Pixel Size 70 nm
- True 3D res: ~ 200 nm
- High penetration power !
- High depth of focus !
- No cooling
- Sample in large capillary
- Lower dose-deposition

M. Stampanoni et al., PRB2010

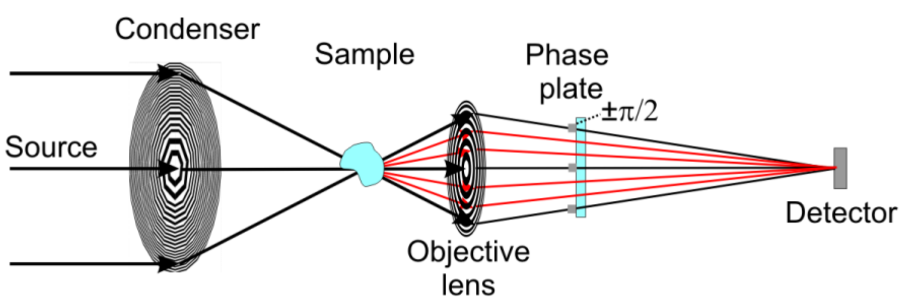


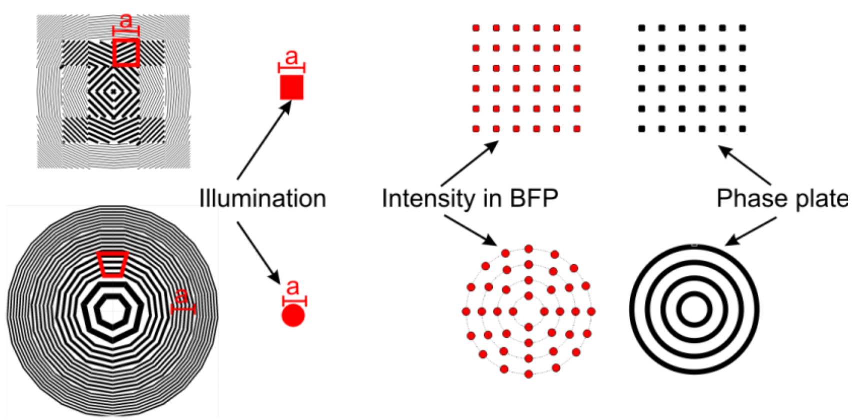
ALS: XM-1 Microscope

- Water window operation
- Pixel size: 50 nm
- True resolution: >100 nm
- Optics in vacuum
- Sample cryo-cooled
- Single cells in 20 microns capillary

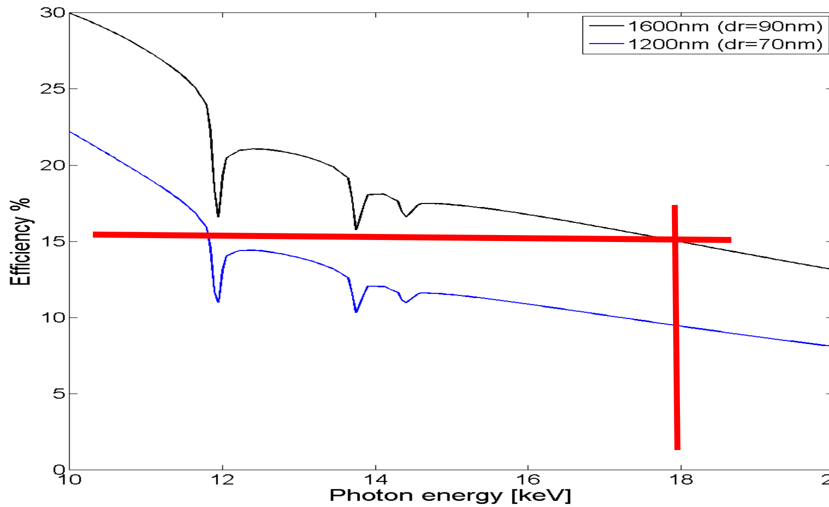
C.A. Larabell et al., Molecular Biology of the Cell, 15(3), 956-962, 2004

Improving efficiency → faster acquisition

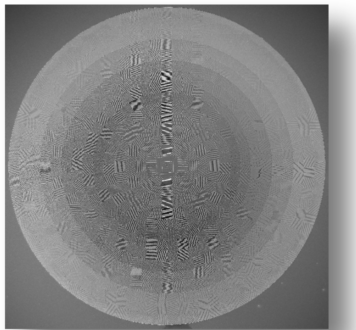




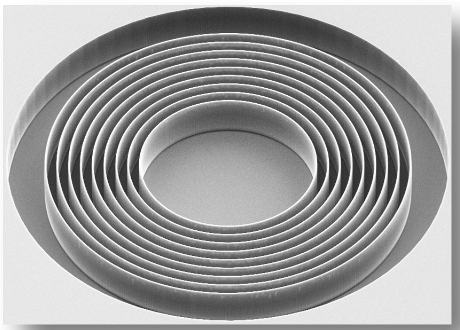
SEM-image of the FZP. Inset shows the thickness of the zones tilted at 45 degrees.



I. Vartiainen et al, Opt. Letters 2014

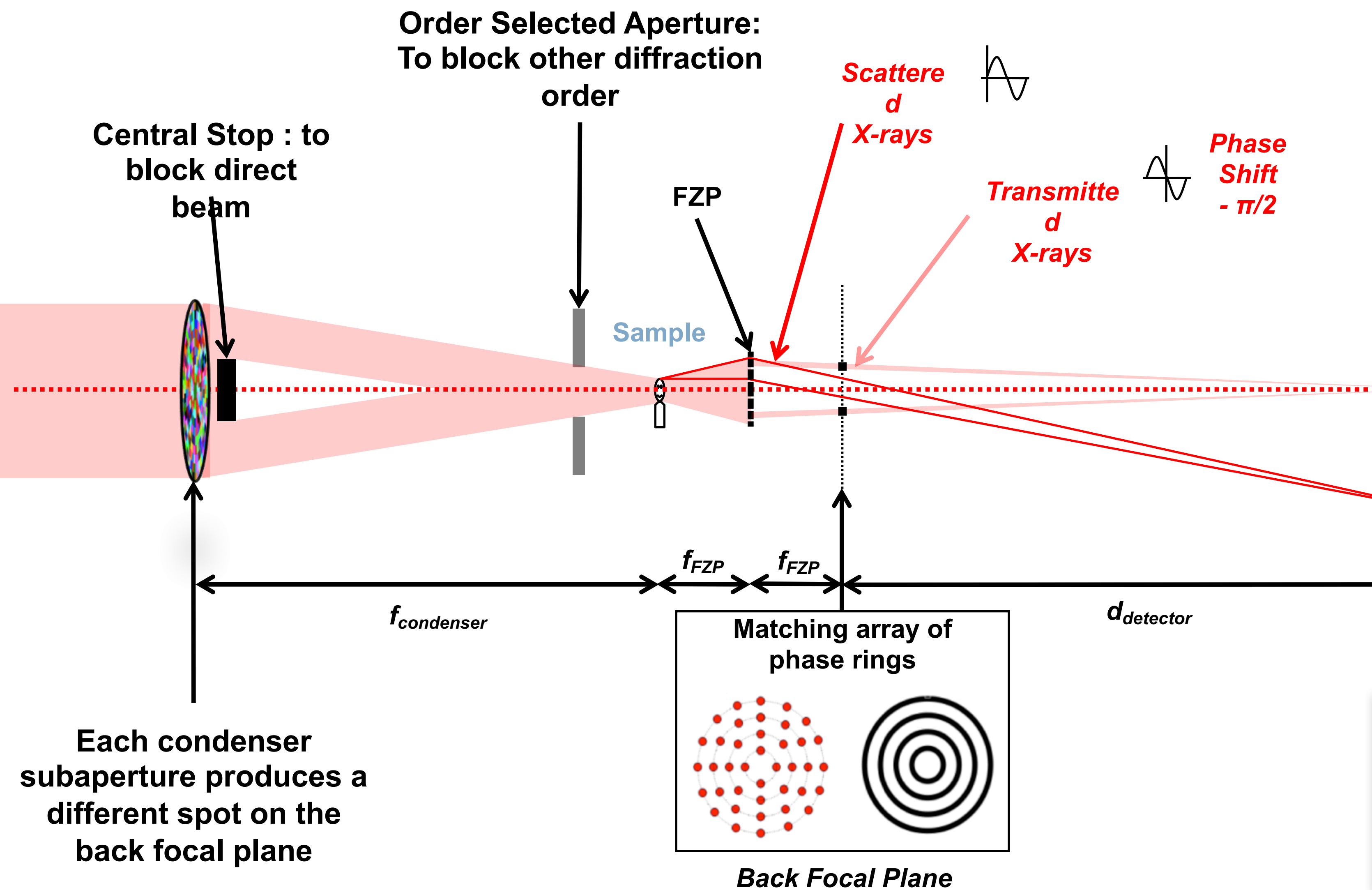


SEM-image of the condenser.



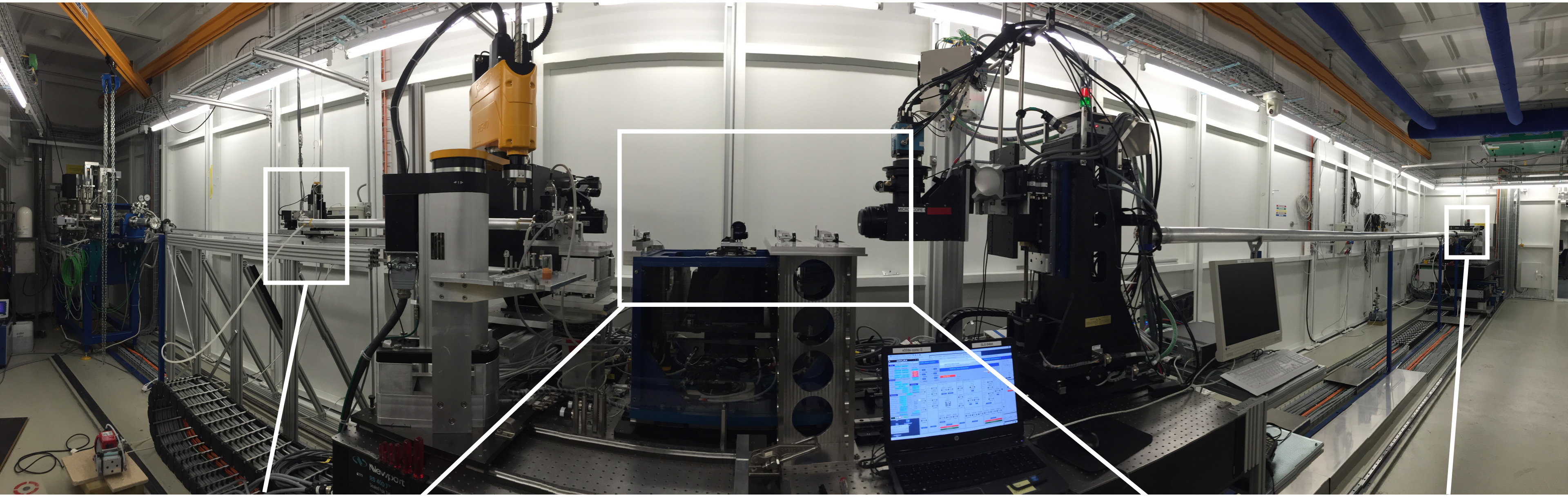
SEM-image of the phase rings.

“Energy tunable” TOMCAT nanoscope (8-20 keV)

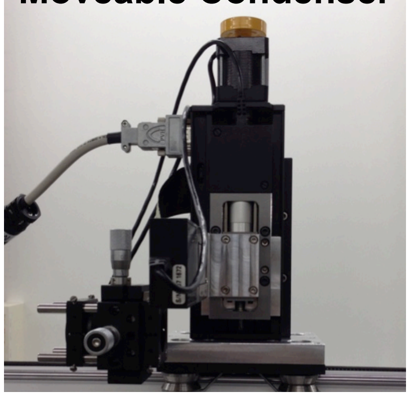


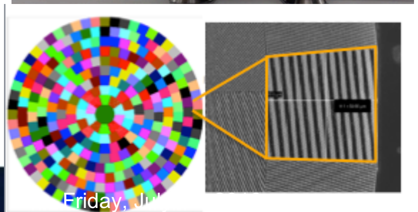
A. Bonnin

The “energy tunable” TOMCAT nanoscope

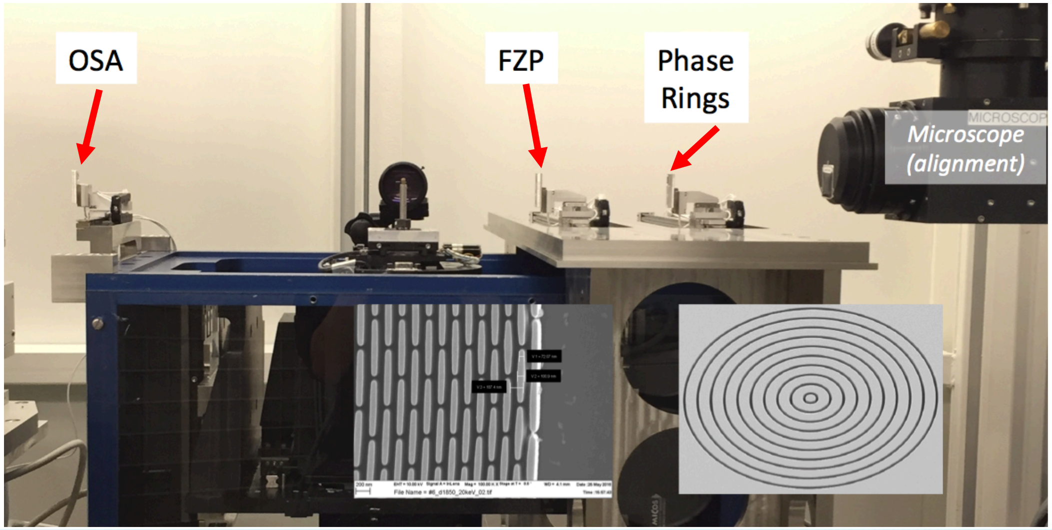


Moveable Condenser

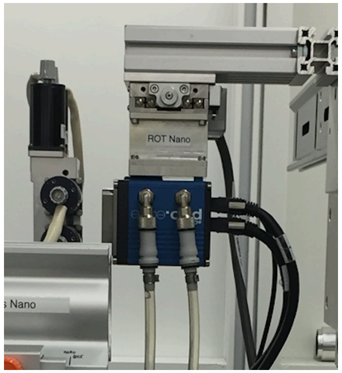




Nanoscope optics



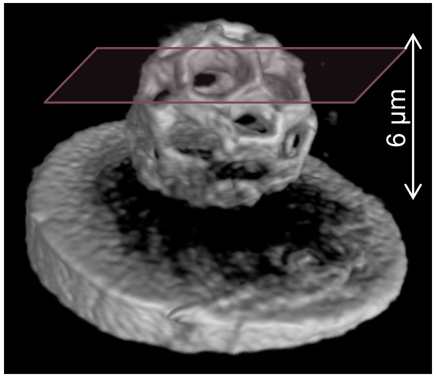
Detector

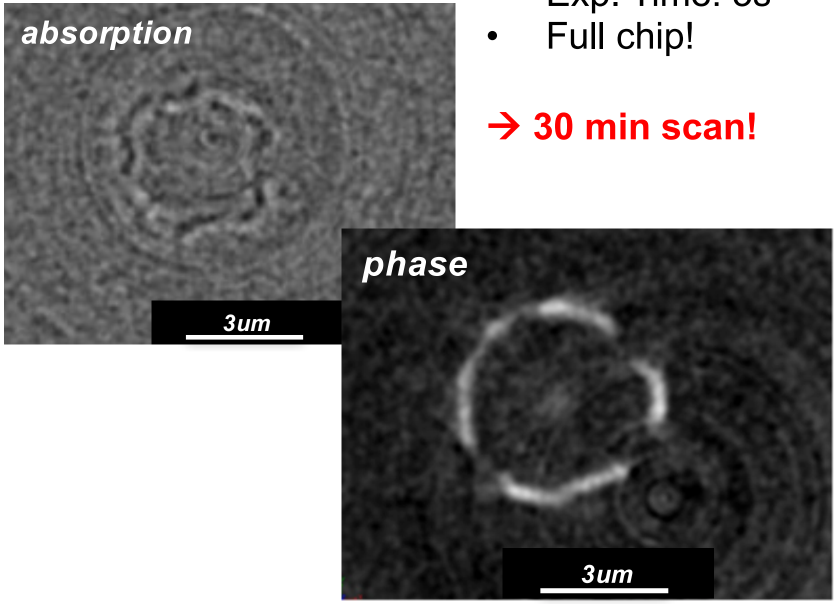


Custom PCO Edge 4.2
@ 10 m from FZP
magnification 80x
Effective pixel size 80nm

Cobalt-coated artificial buckyball polymer scaffold

3D artificial material composite: cobalt-coated buckyball with about 270nm wall width.





TOMCAT Nanoscope

Tomography scan:

- Si111 @ 12 keV
- Proj: 720
- Exp. Time: 3s
- Full chip!

→ 30 min scan!

PRL **114,** 115501 (2015) PHYSICAL REVIEW LETTERS week ending 20 MARCH 2015

Element-Specific X-Ray Phase Tomography of 3D Structures at the Nanoscale

Claire Donnelly,[1,2] Manuel Guizar-Sicairos,[2,*] Valerio Scagnoli,[1,2] Mirko Holler,[2] Thomas Huthwelker,[2] Andreas Menzel,[2] Ismo Vartiainen,[2] Elisabeth Müller,[2] Eugenie Kirk,[1,2] Sebastian Gliga,[1,2] Jörg Raabe,[2] and Laura J. Heyderman[1,2,†]

[1]*Laboratory for Mesoscopic Systems, Department of Materials, ETH Zurich, 8093 Zurich, Switzerland*

[2]*Paul Scherrer Institute, 5232 Villigen PSI, Switzerland*

(Received 23 October 2014; published 16 March 2015)

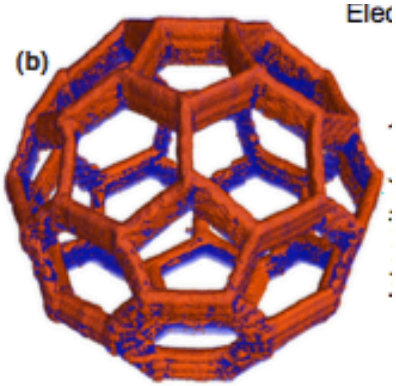
Recent advances in fabrication techniques to create mesoscopic 3D structures have led to significant developments in a variety of fields including biology, photonics, and magnetism. Further progress in these areas benefits from their full quantitative and structural characterization. We present resonant ptychographic tomography, combining quantitative hard x-ray phase imaging and resonant elastic scattering to achieve *ab initio* element-specific 3D characterization of a cobalt-coated artificial buckyball polymer scaffold at the nanoscale. By performing ptychographic x-ray tomography at and far from the Co *K* edge, we are able to locate and quantify the Co layer in our sample to a 3D spatial resolution of 25 nm. With a quantitative determination of the electron density we can determine that the Co layer is oxidized, which is confirmed with microfluorescence experiments.

DOI: 10.1103/PhysRevLett.114.115501 PACS numbers: 81.07.-b, 42.30.Rx, 68.37.Yz, 81.70.Tx

Ptychographic Scan on cSAXS

- 3 min per projection
- 160 projections over 180°

→ 8 h scan

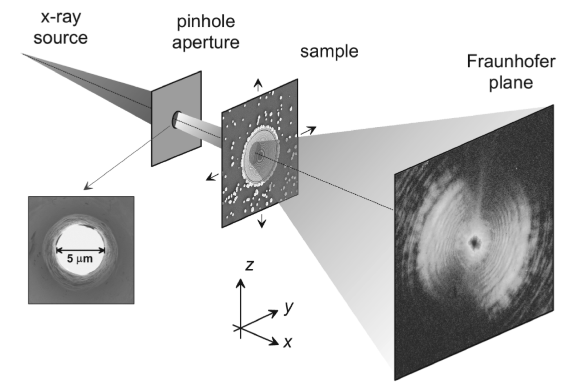


3D rendering with elemental contrast, where the Co is rendered in orange and the resist in blue.

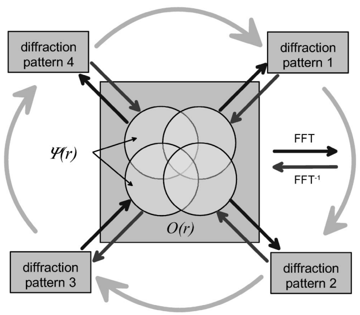
Courtesy of C.Donnelly (LMN)

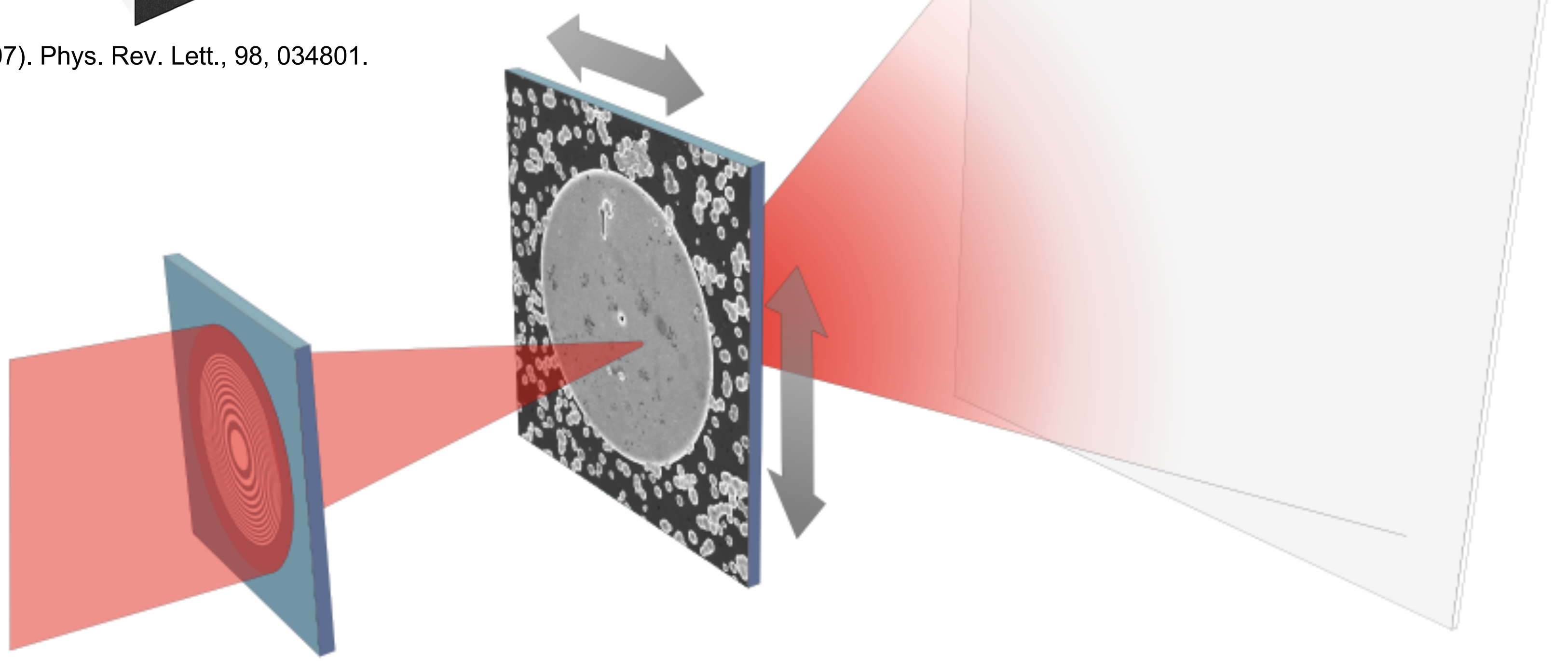
Scanning X-ray diffraction microscopy

X-ray ptychography with a focused probe



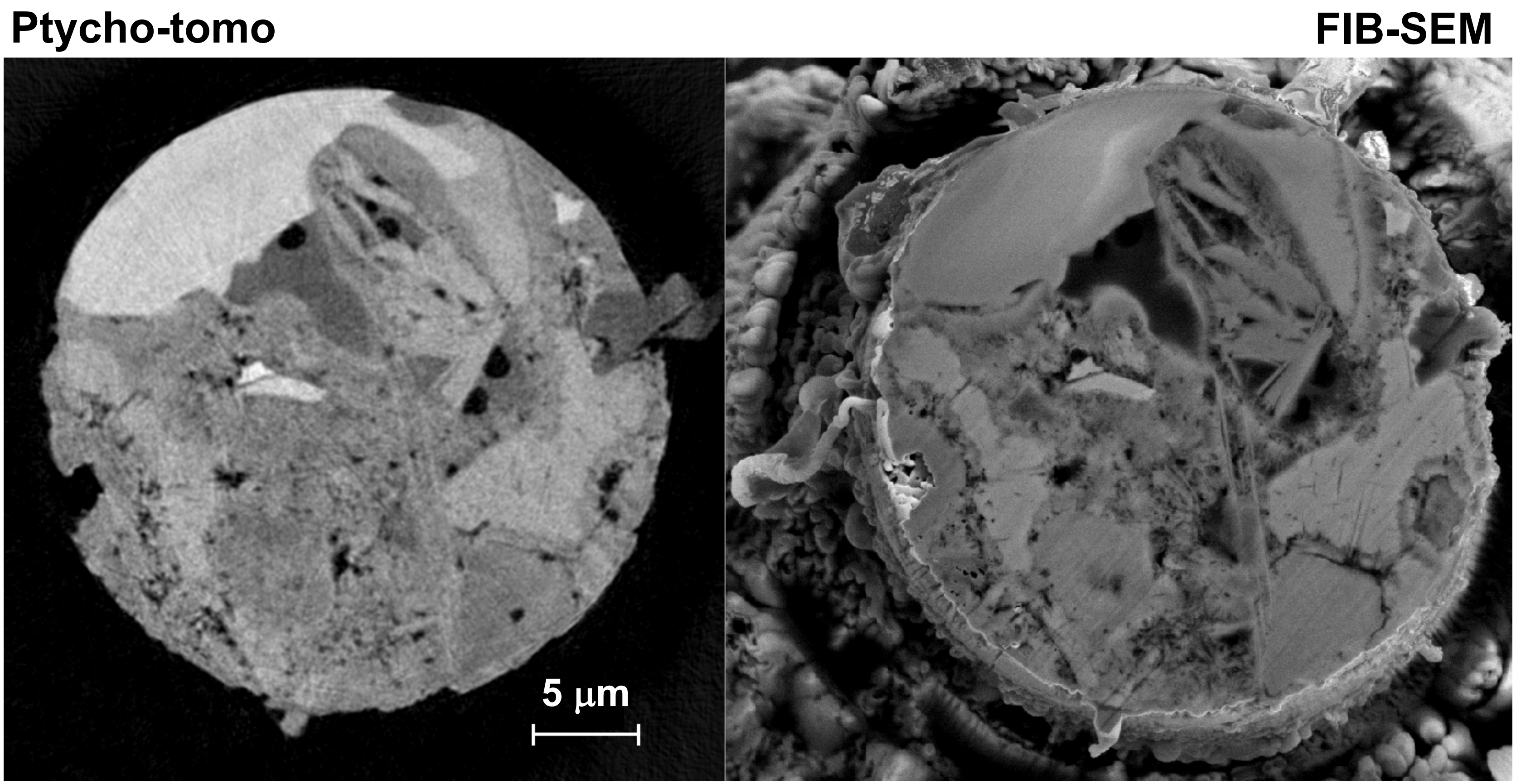
Rodenburg *et al.* (2007). Phys. Rev. Lett., 98, 034801.





P. Thibault et al., Science, 321, 379-382 (2008)

Ptychography on hardened cement paste

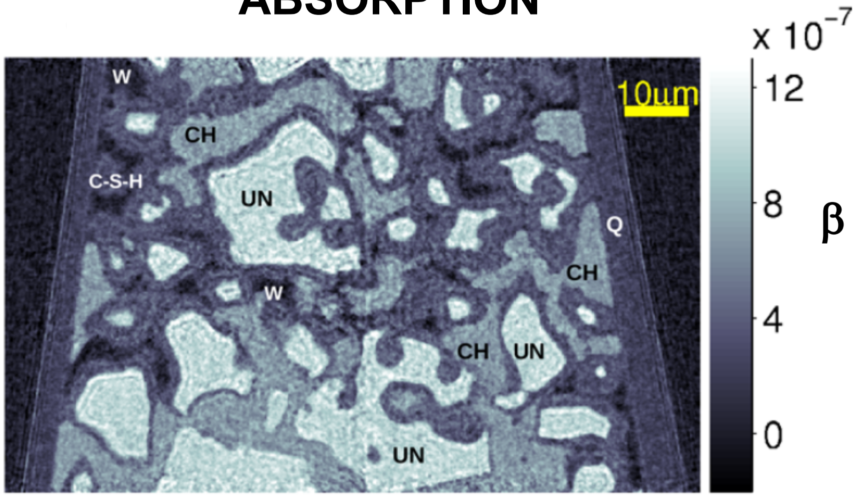


P. Trtik et al, Cement & Concrete Composites 36 (2013) 71

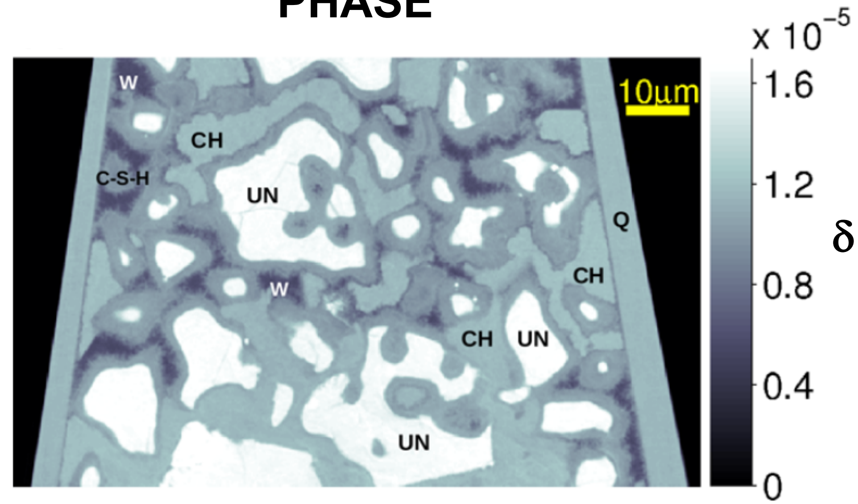
Slide courtesy of A. Diaz, cSAXS

Hydrated cement paste

ABSORPTION



PHASE



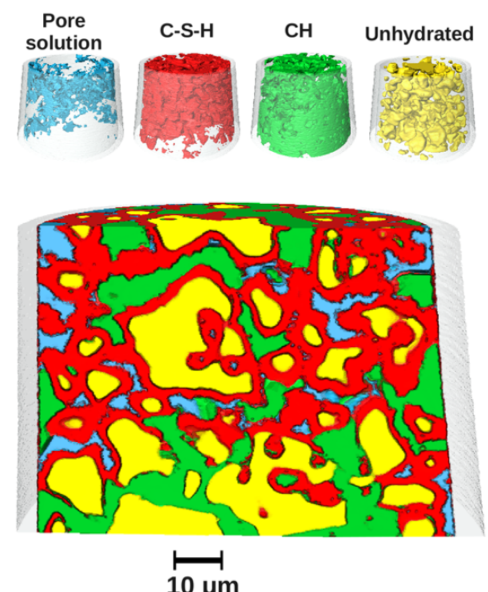
- Glass micro-capillary filled with hydrated cement phase
- Identification and segmentation of material phases:

  UN: unhydrated alite particles

  W: porosity comprising mostly water

  CH: calcium hydroxide

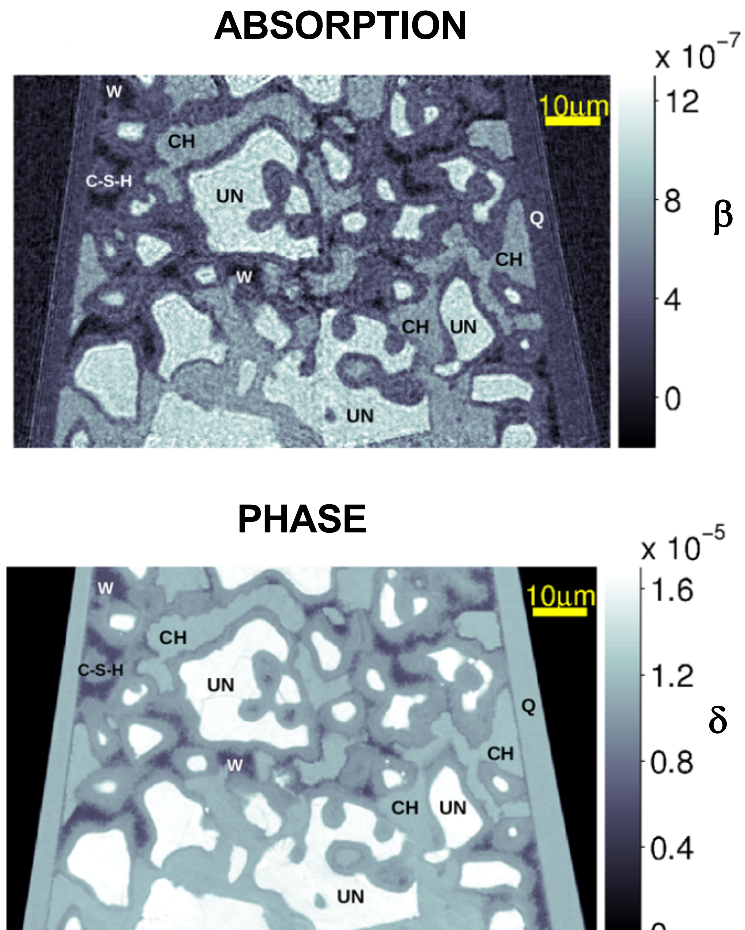
  C-S-H: calcium silicate hydrates
- Average mass density of C-S-H: 1.828 **g/cm^3**

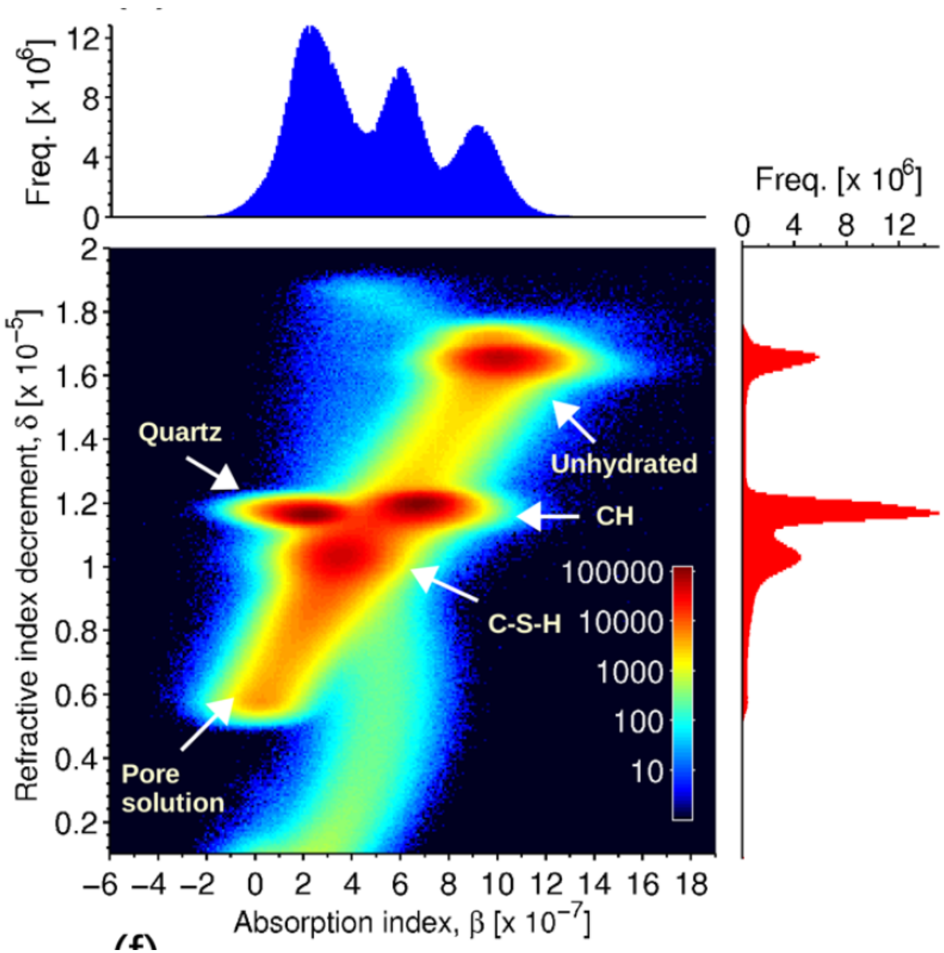


J. C. da Silva *et al*., Langmuir **31** (2015) 3779

Slide courtesy of A. Diaz, cSAXS

Water content in hydrated cement paste



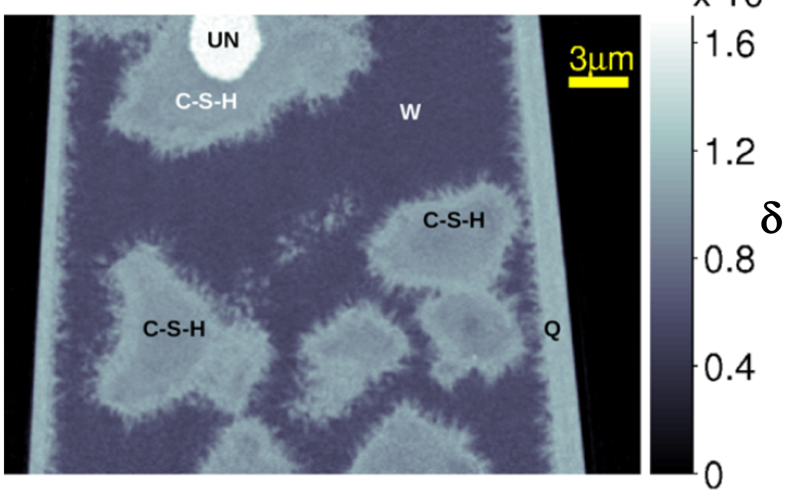


J. C. da Silva *et al*., Langmuir **31** (2015) 3779

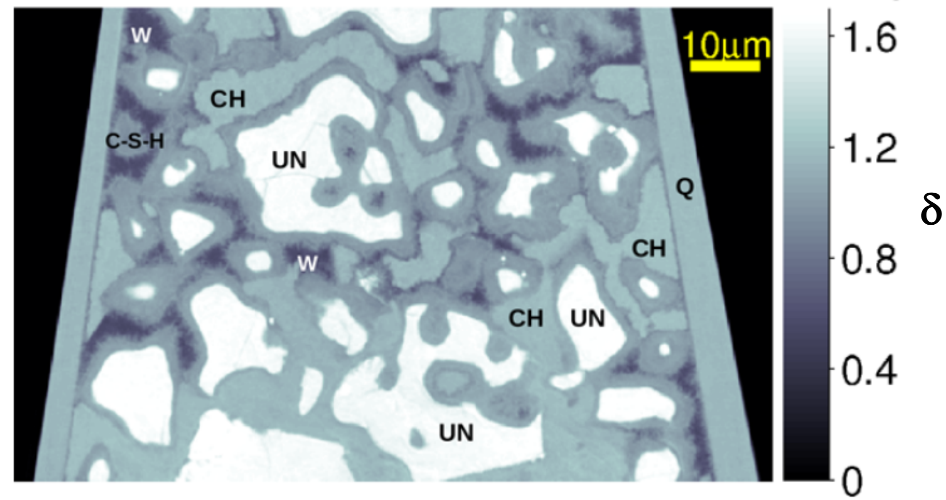
Slide courtesy of A. Diaz, cSAXS

Hydrated cement paste

TOP PART ~25 μm diameter



BOTTOM PART ~90 μm diameter



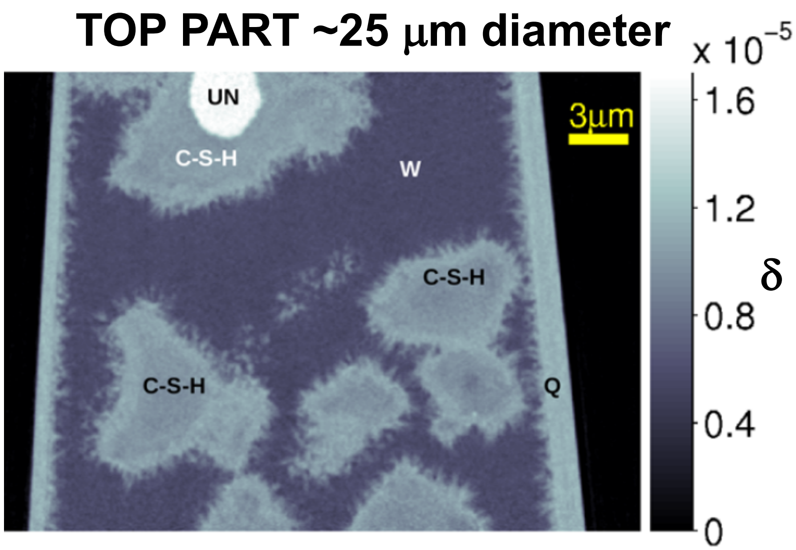
$$\underbrace{2\,Ca_3SiO_5}_{\text{alite}} + \underbrace{6H_2O}_{\text{water}} \rightarrow \underbrace{3CaO\cdot 2SiO_2\cdot 3H_2O}_{\text{calcium-silicate hydrates}} + \underbrace{3Ca(OH)_2}_{\text{calcium hydroxide}}$$

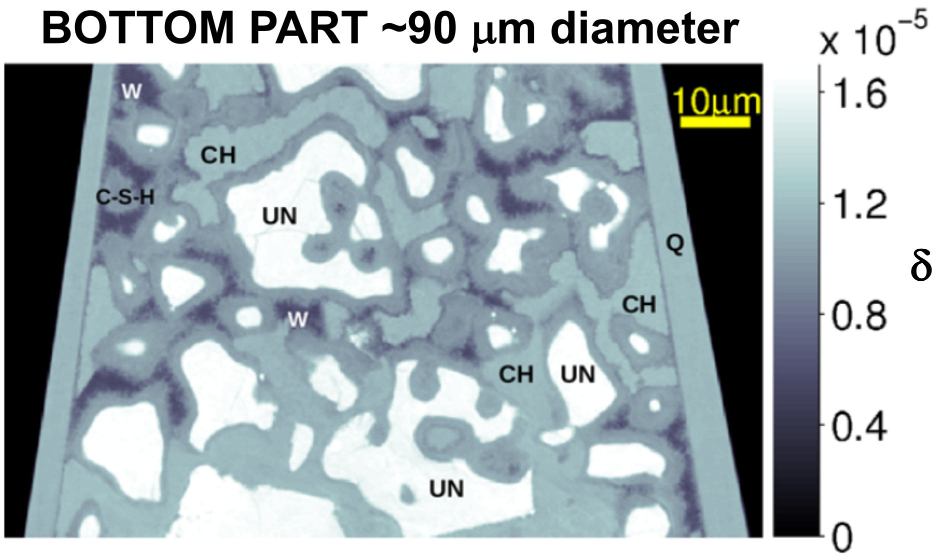
Glass micro-capillary filled with alite (C_3S) particles, flushed with water, sealed, and cured for 16 days

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

Slide courtesy of A. Diaz, cSAXS

Hydrated cement paste





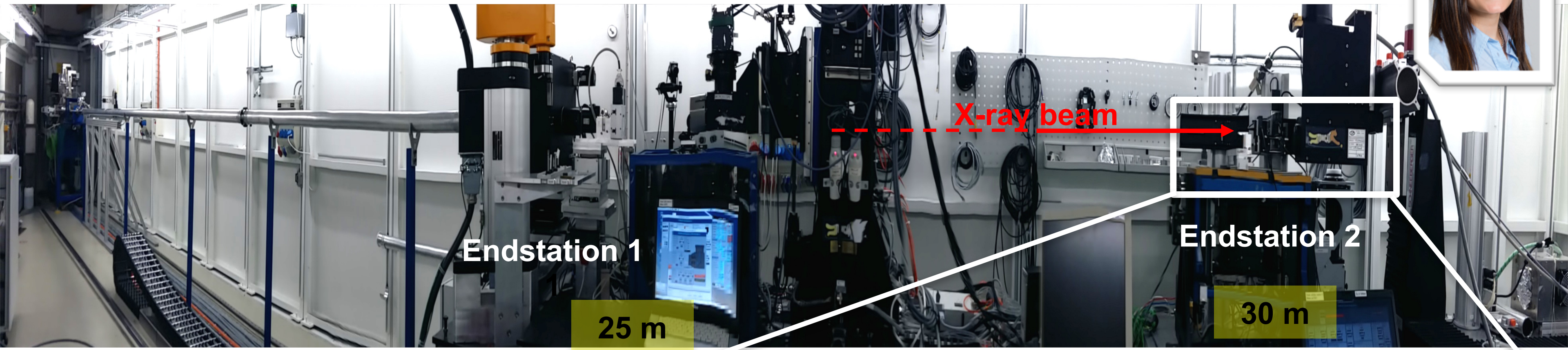
CH: N/A
W: (0.969±0.005) g/cm^3, theory: 1.000 g/cm^3
Q: (2.158±0.002) g/cm^3, theory: 2.2 g/cm^3
C-S-H fully hydrated, inner: (1.726±0.006) g/cm^3
C-S-H fully hydrated, outer: (1.909±0.005) g/cm^3
C-S-H partly hydrated, inner: (1.860±0.004) g/cm^3
C-S-H partly hydrated, outer: (1.96±0.04) g/cm^3

CH: (2.184±0.004) g/cm^3, theory: 2.251 g/cm^3
W: (0.994±0.005) g/cm^3, theory: 1.000 g/cm^3
Q: (2.188±0.002) g/cm^3, theory: 2.2 g/cm^3
C-S-H, partly hydrated: (1.828±0.005) g/cm^3

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

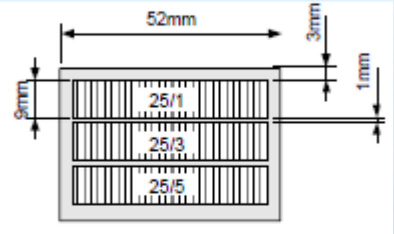
Slide courtesy of A. Diaz, cSAXS

Grating interferometry at TOMCAT

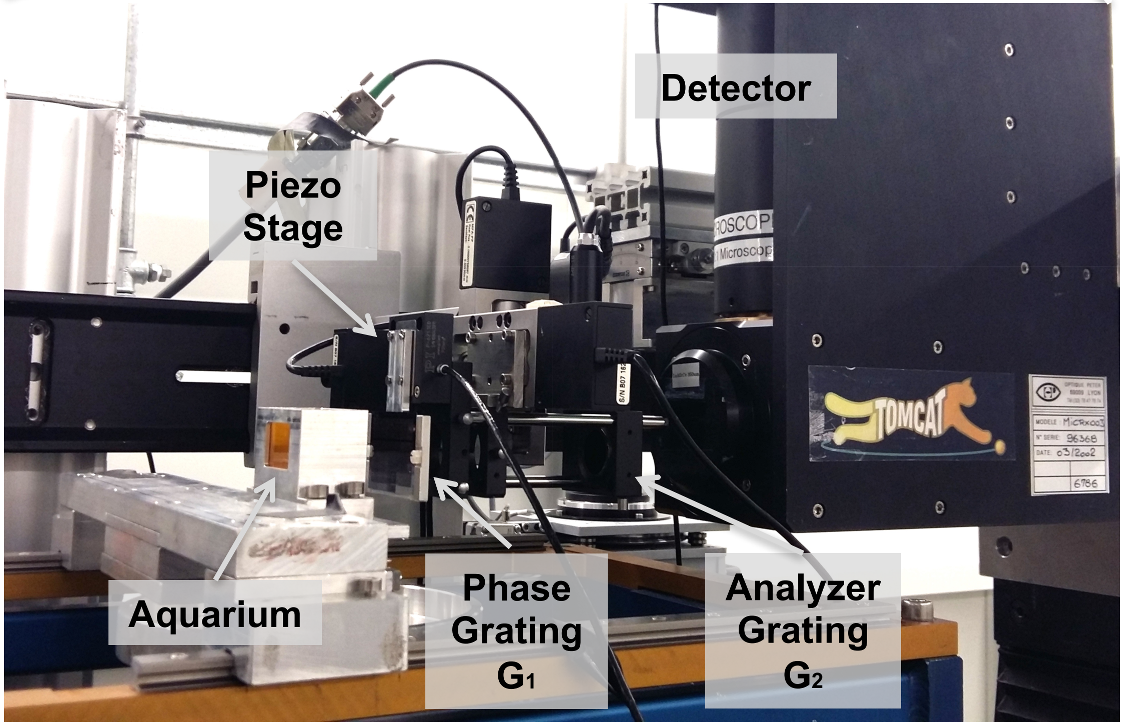


Phase Grating G_1

TO	Pitches [µm]
1	3.994
3	3.983
5	3.972



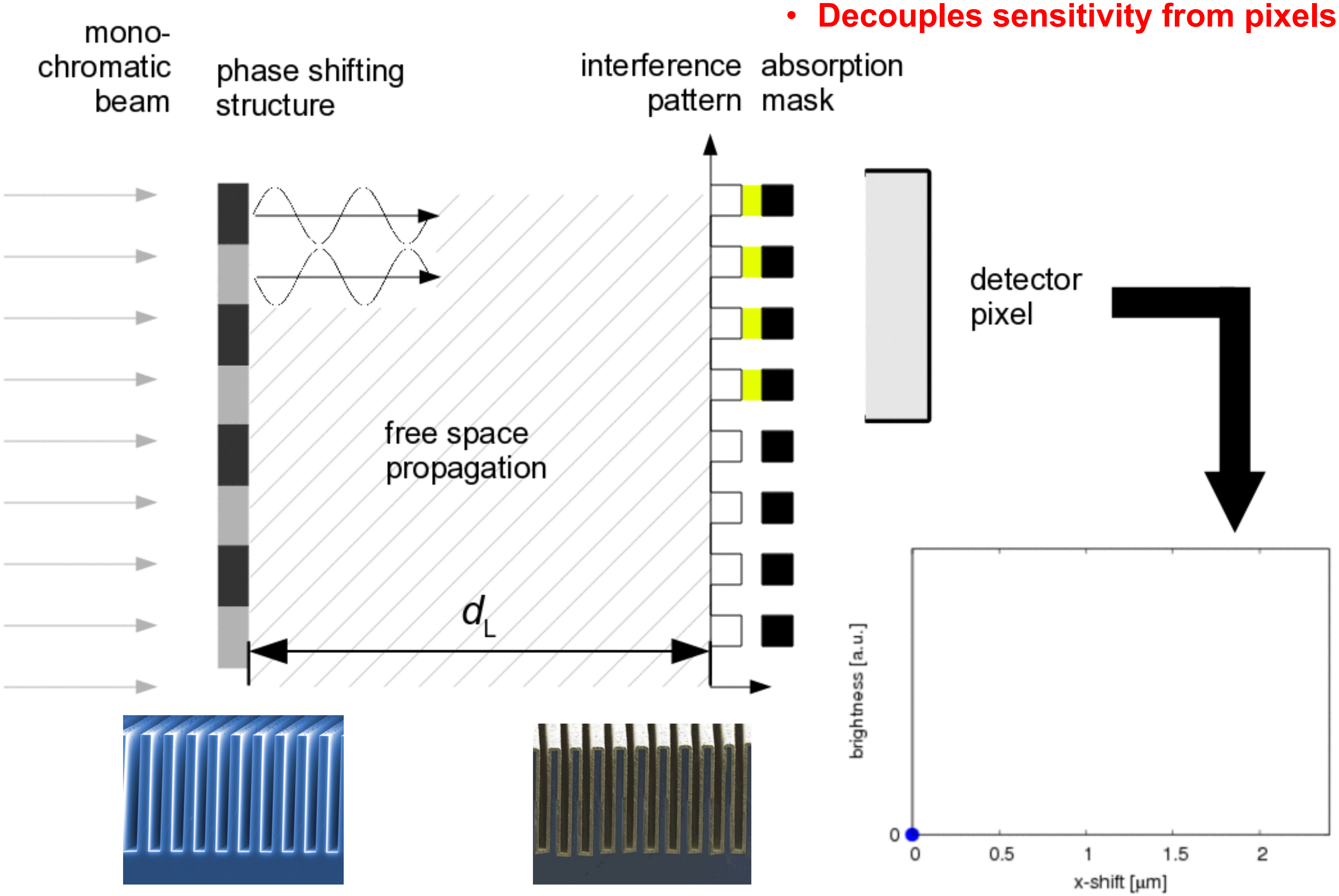
Phase shift of π.
Wafer thickness 250 um.



Design energy = 25 keV
Other energies available on request

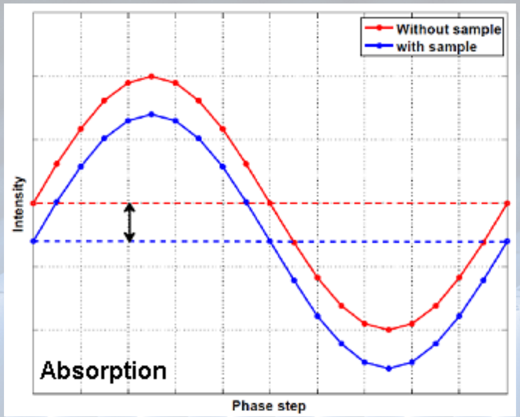
Grating interferometry in a nutshell

- **Very sensitive on short distances**
- **Decouples sensitivity from pixelsize**



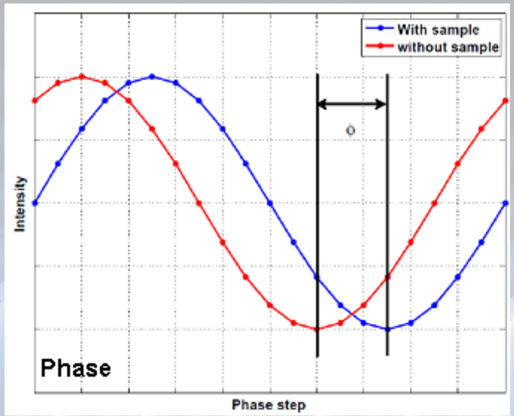
Sensing the wavefront with grating interferometry

Absorption contrast



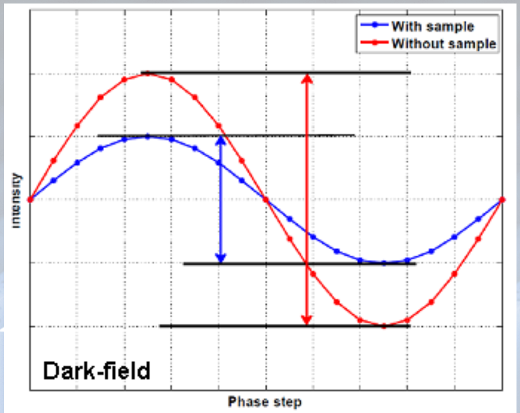
$$-log\left(\frac{I_s}{I_b}\right)$$

Differential phase contrast



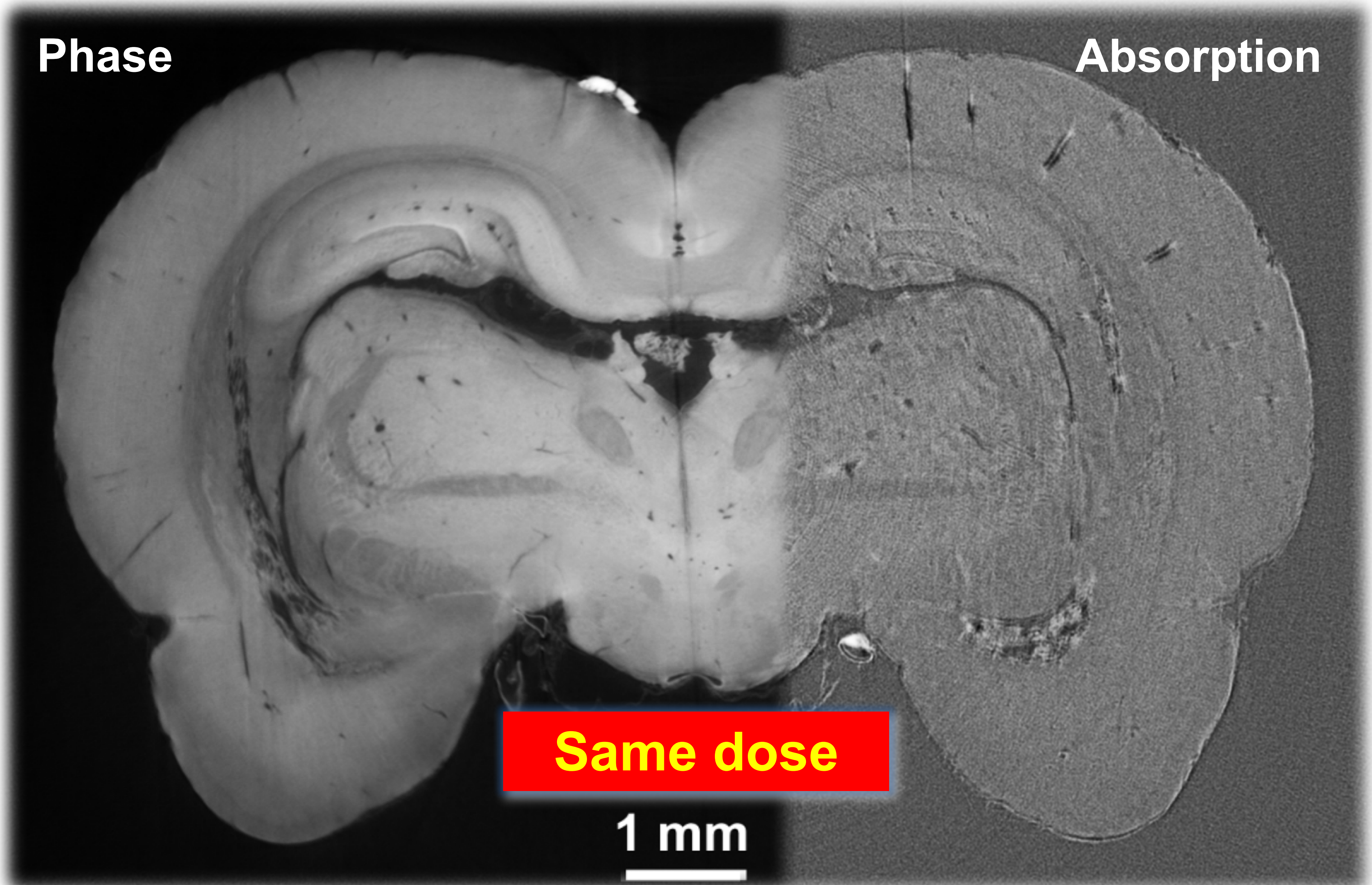
$$\Phi_s - \Phi_b$$

Dark-field contrast

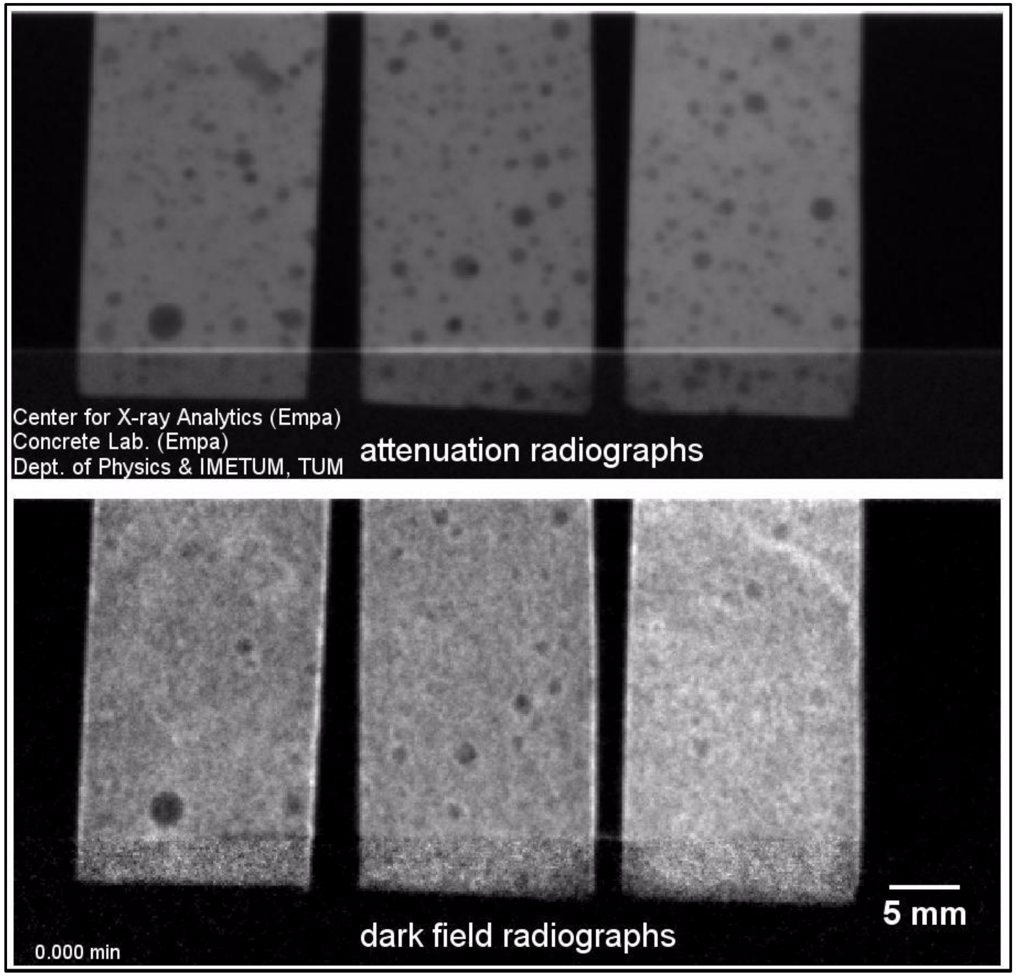


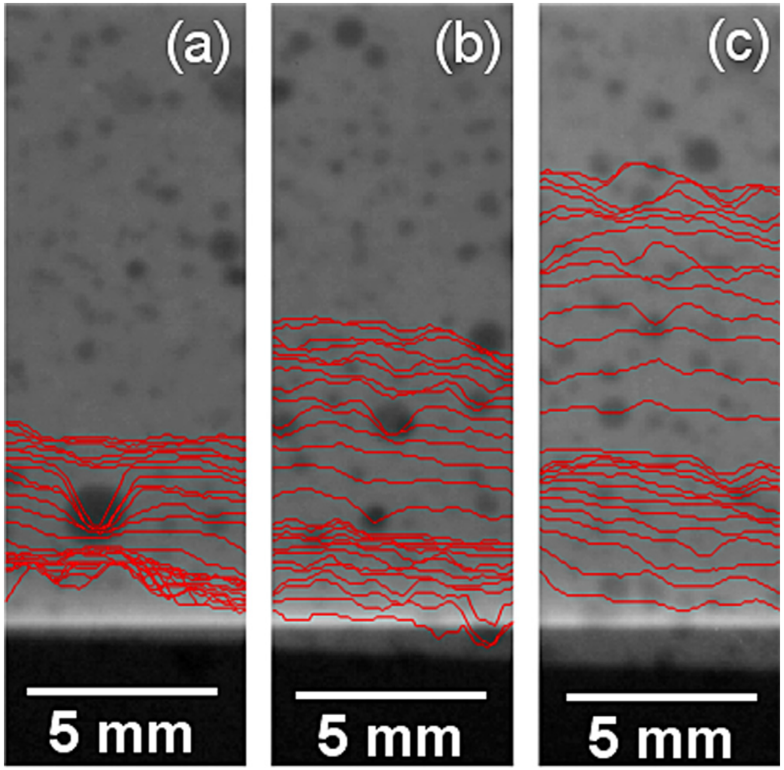
$$-log\left(\frac{V_s}{V_b}\right) \quad V = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

Improved soft tissue contrast



Dark-field X-ray imaging of water capillary uptake in mortars



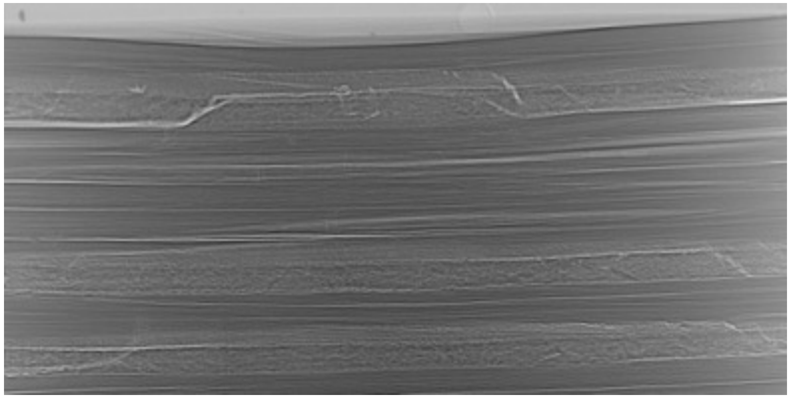


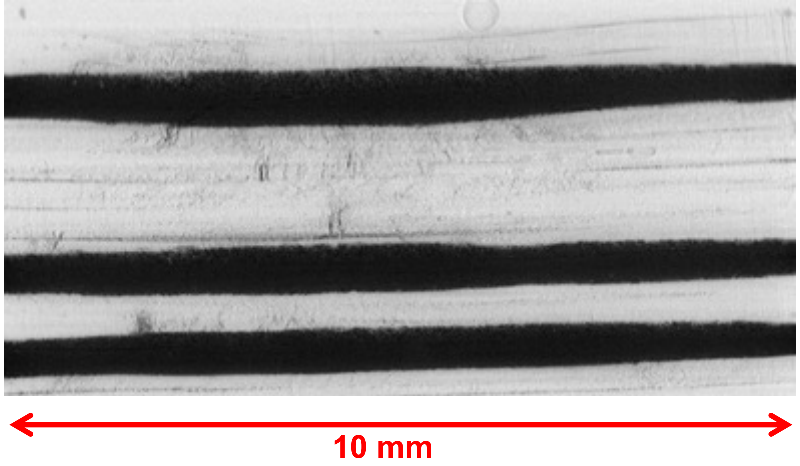
Wetting front positions (red lines) for three differently threated (heat load) samples.

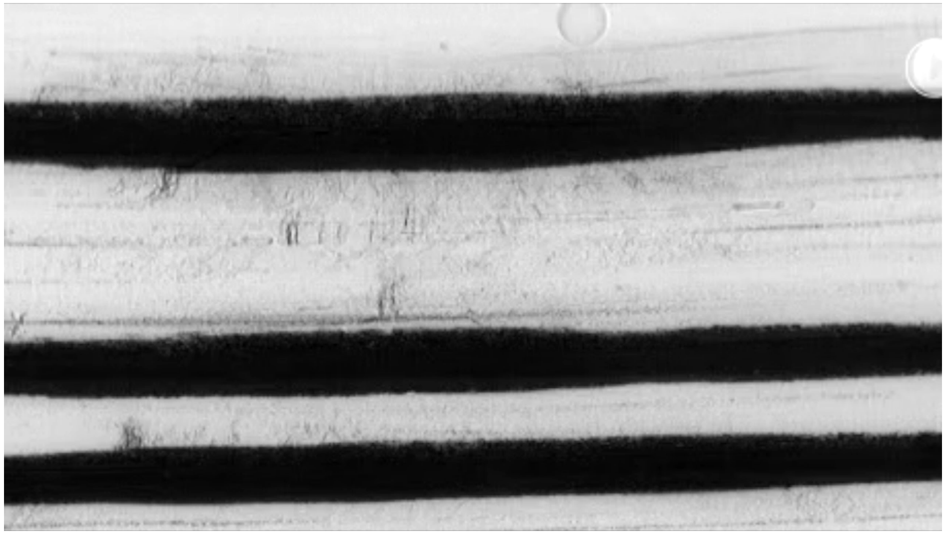
F. Yang, et al. Appl. Phys. Lett. 105, 154105 (2014)

Darkfield radiology

CFRP laminated structure consisting of alternate layers of plastic matrix and fiber reinforcement



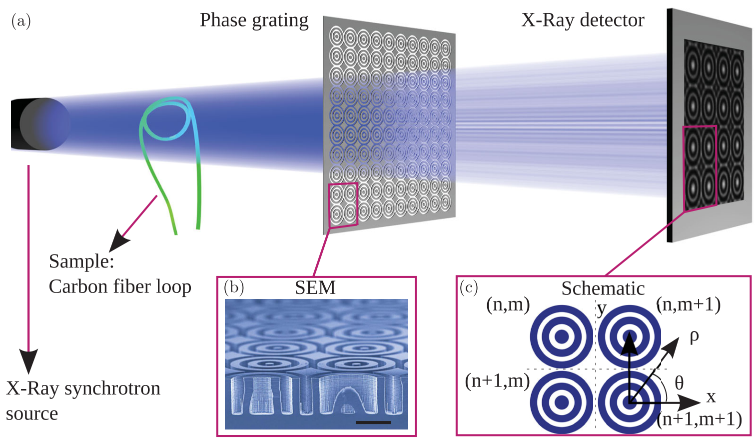


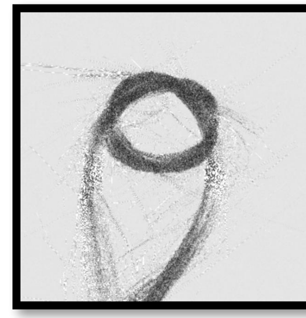


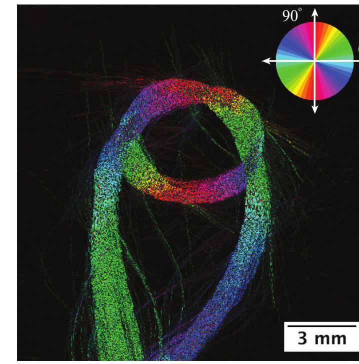
Directional dependency of the darkfield signal.
Issues with the tomographic reconstruction…

S. McDonald, M. Stampanoni et al. , Journal of Synchrotron Radiation 16, 562-572,(2009)

Single-shot 2D-omnidirectional hard X-ray scattering (dark-field) sensitivity







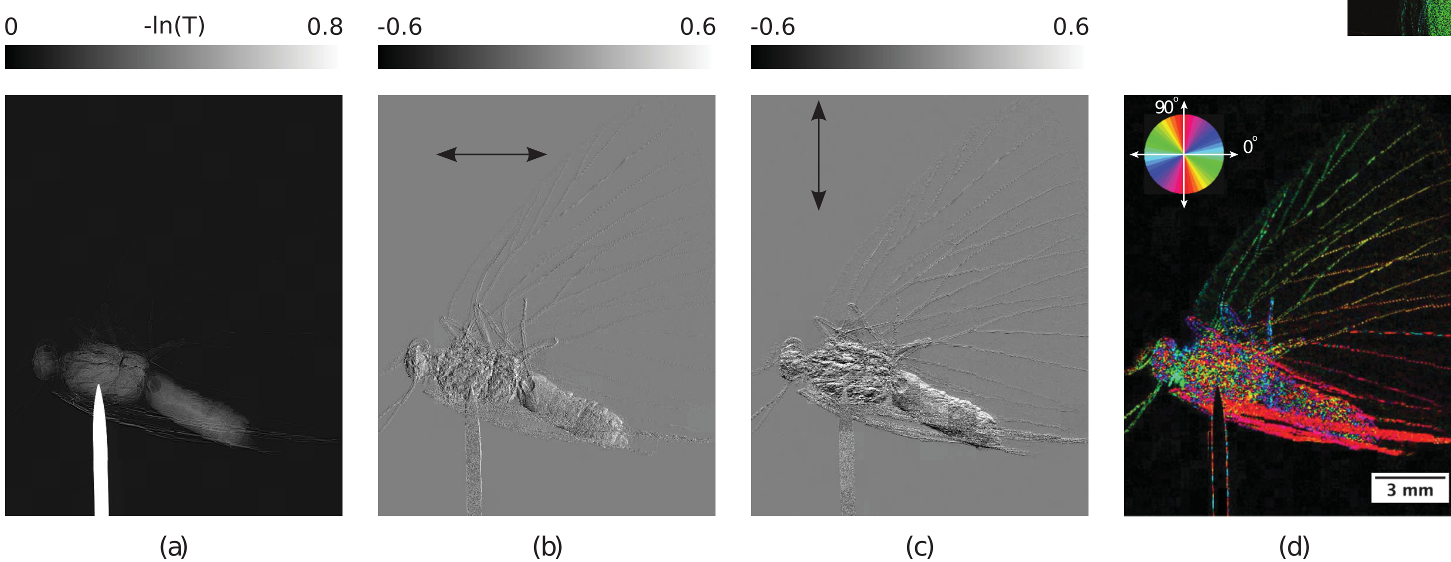


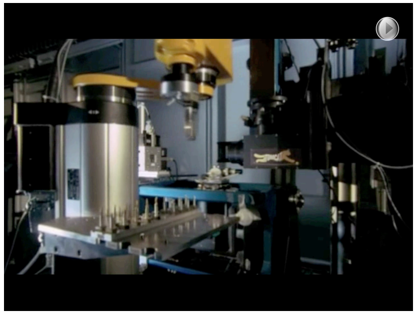
FIG. 3. (a) Absorption, (b) differential phase along x, (c) differential phase along y, and (d) directional scattering image of a butterfly fixed on a steel needle. The extracted scattering orientations are related to structures in the size range of the autocorrelation length of the system, which in our case is 2.5 μm.

M. Kagias *et al.*, Phys. Rev. Lett. 116, 093902 (2016)

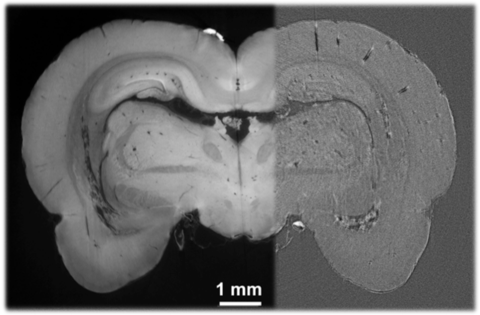
TOMCAT at a glance

Features:

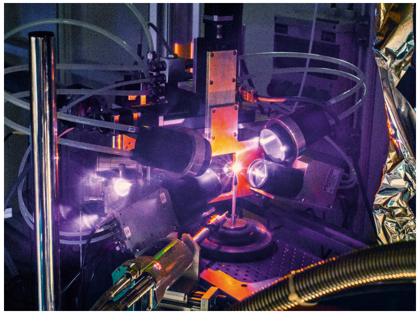
- Wide spatial resolution range: nano-to-meso scales (0.1-10µm)
- Broad range of sample sizes (10µm-20mm)
- High density resolution enhanced by phase contrast
- High temporal resolution (3D data acquisition in less than 1s)



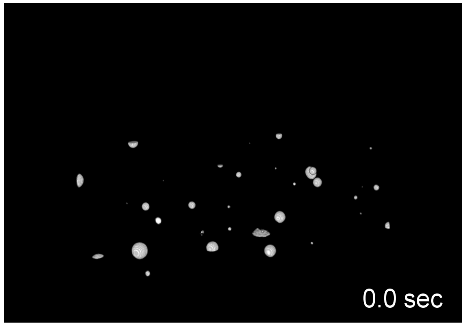
Automation large scale studies



Density resolution phase contrast imaging

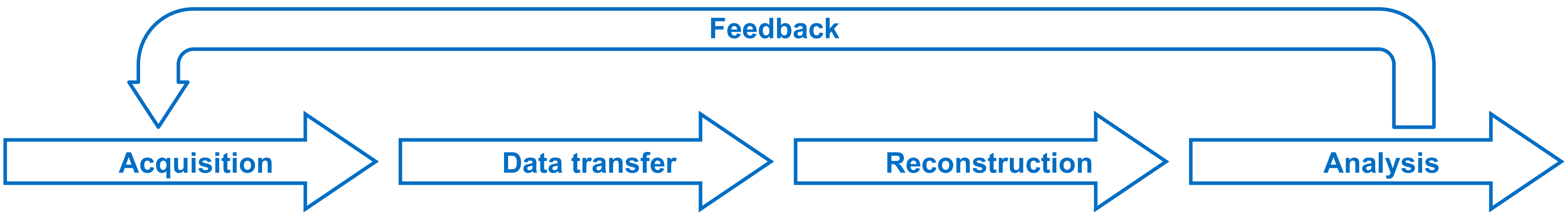


In situ capabilities *temperatures away from ambient*



Ultra-fast data acquisition dynamic studies

Requirements for fast tomography



Acquisition

Bright source: synchrotron

Fast detectors:
1000 – 100.000 fps

Efficient optics

Precision hardware

Flexible synchronization and triggering protocols

In situ, in vivo, in operando sample environments

Data transfer

Continuous data stream

Sustainable at maxiumum detector frame rate (several GB/s)

Store only useful data

On-line preview capabilities

Reconstruction

Synchronous reconstruction on subsets of data

Use of phase information to enhance contrast

New reconstruction algorithms:

- Incomplete or interlaced data
- Iterative approaches

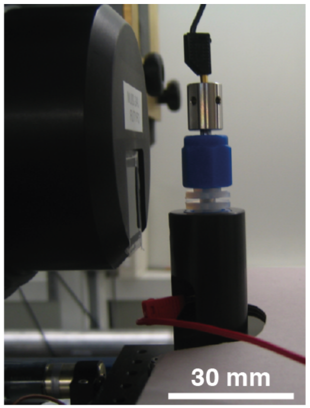
Analysis

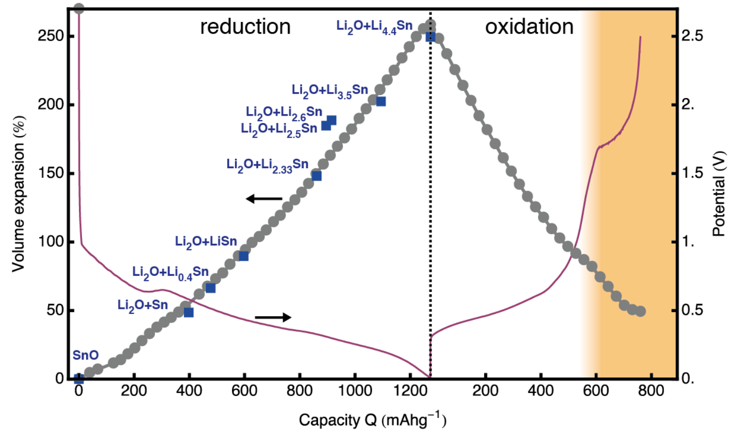
Automated quantitative data analysis on large datasets (TB):

- Segmentation
- Labelling
- Tracking

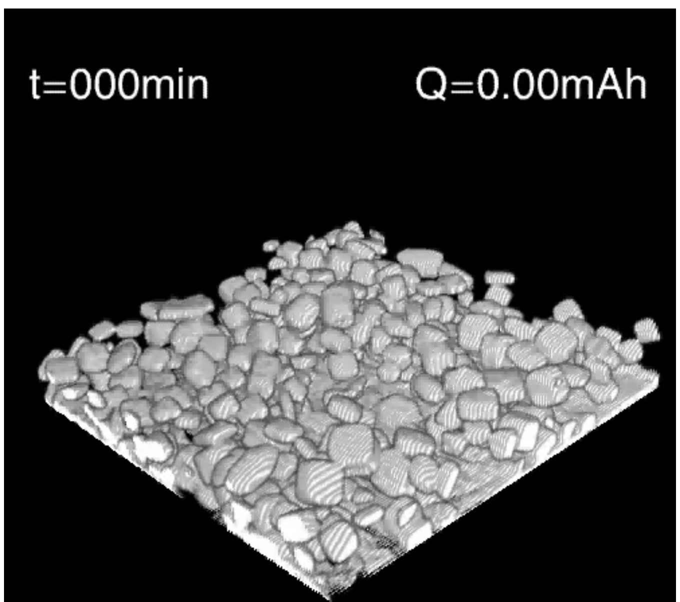
On-the-fly computation of sample characteristics

In-operando tomography: catch the dynamics of your sample

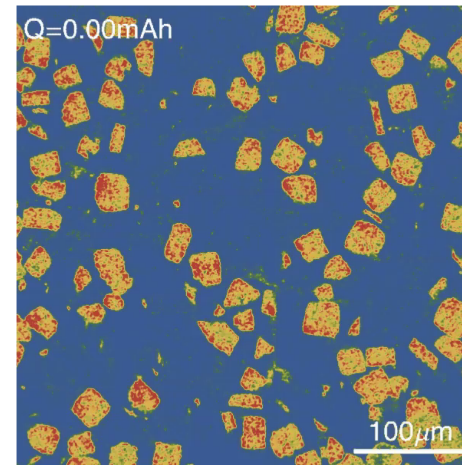


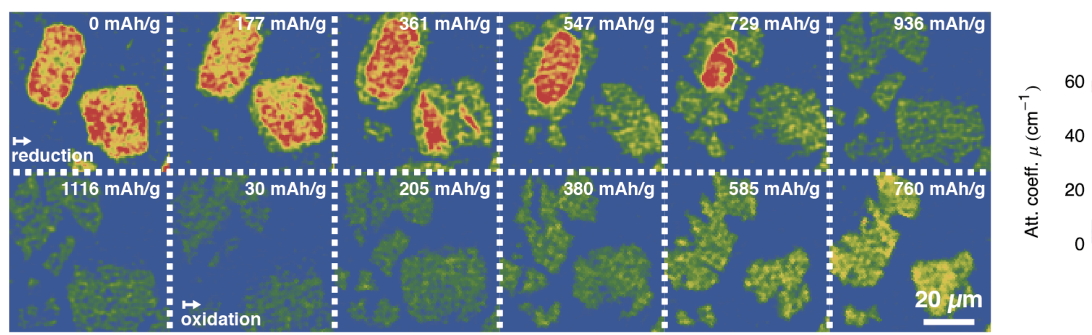


$$SnO \xrightarrow{conversion} Li_2O + Sn \xleftrightarrow{(de)alloying} Li_2O + Li_xSn$$



De-lithiation process in battery operation
- **Particle expansion**
- **Monitoring of the reaction mechanism**



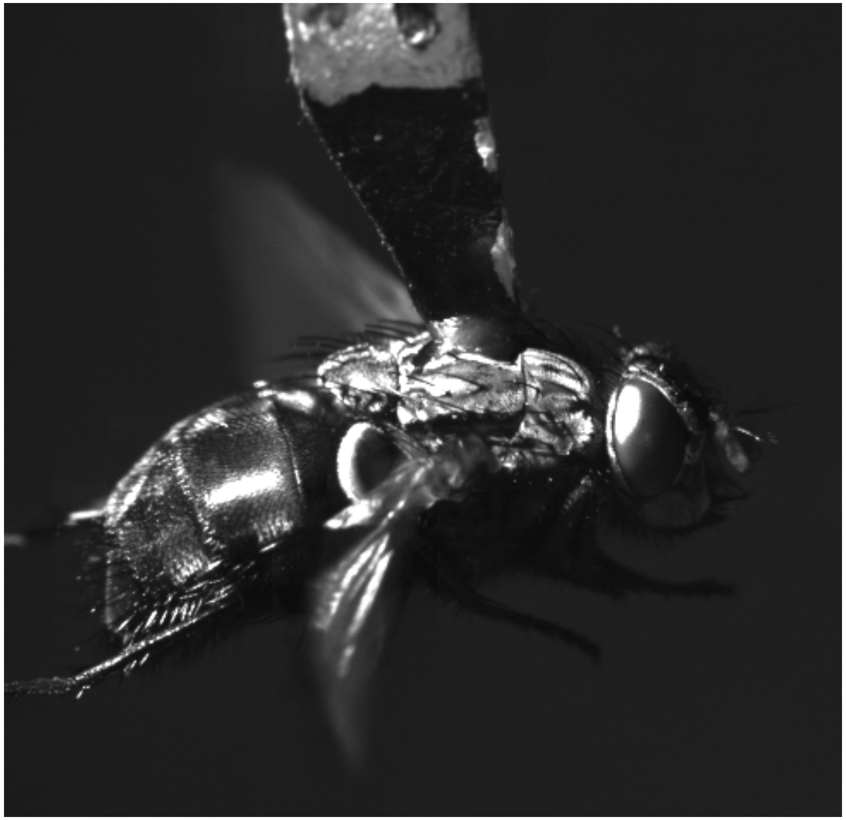


M. Ebner, F. Marone, M. Stampanoni, V. Wood, *Science* 342, 716 (2013)

Get insights in insect flight control

Investigate the biomechanics underlying
flight manoeuvres and gaze shifts

→**CT following the dynamics of <u>100+ Hz</u> wing beat!**

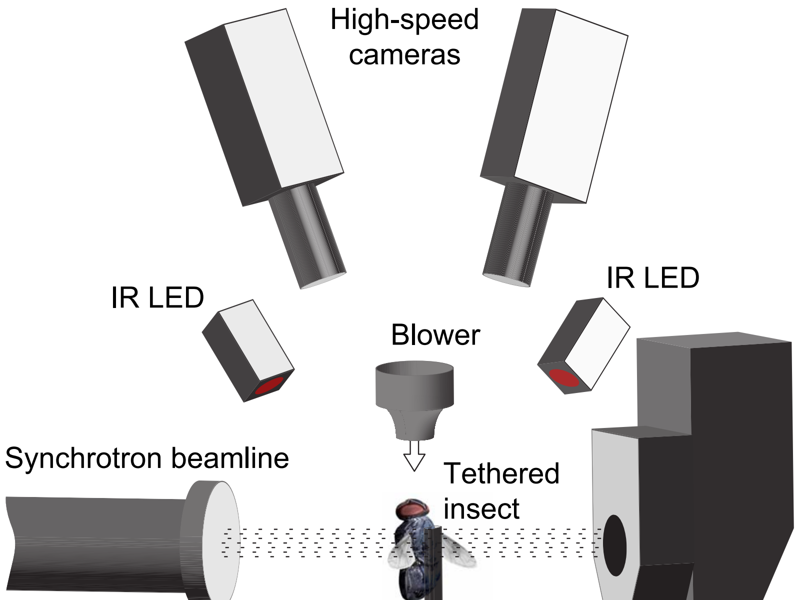


- Time resolution way too demanding for standard CT data acquisition
- BUT: Motion is quasi-periodic!
- Resolve a time-averaged motion pattern over multiple wing beat periods?

→ Gated kHz tomography

- kHz time resolution
- Averaged motion over many cycles

The “fly” experiment



T

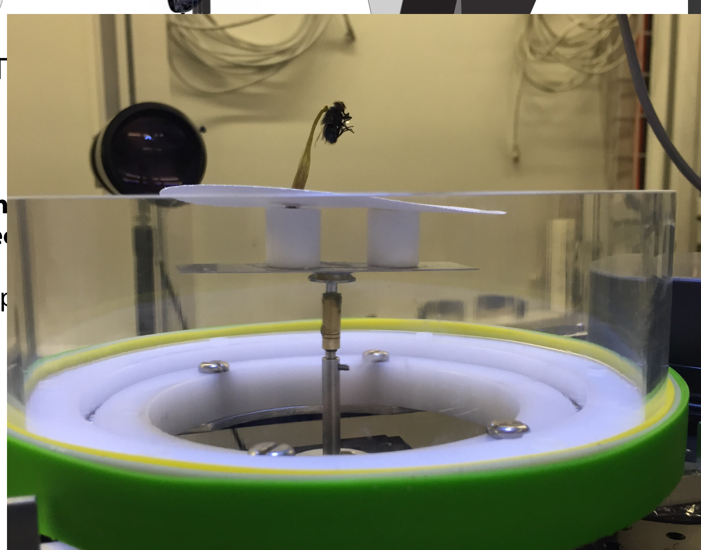
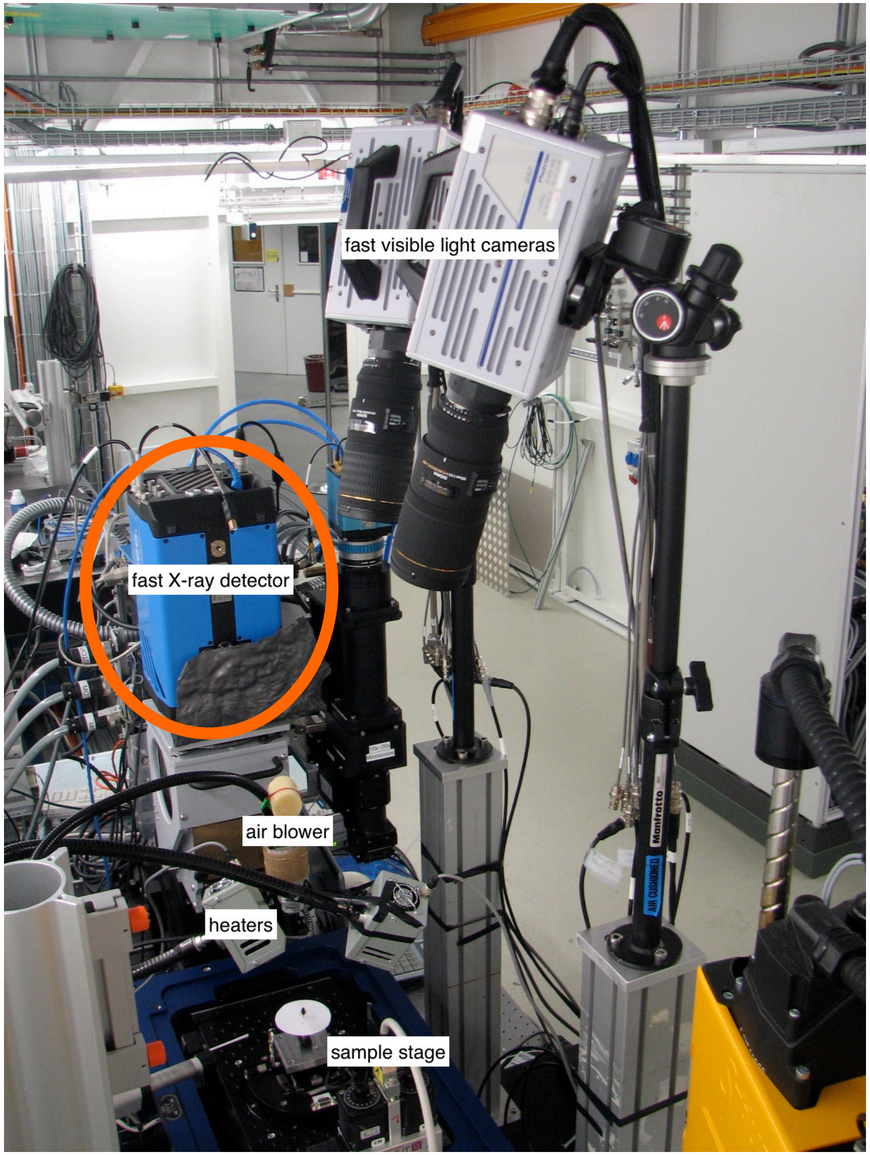


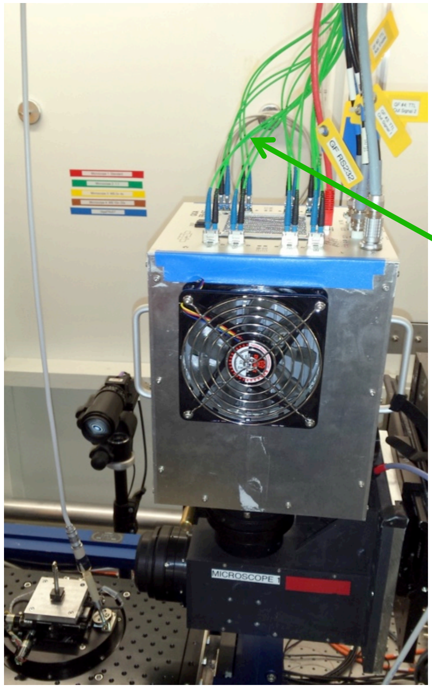
Figure 1. Schem
showing the dire
rotational stimuli
doi:10.1371/journal.p

The fly's flight recorder…



- Limitations:
  - Fast acquisition into onboard memory
  - Total number of frames limited by memory
  - Data transfer to network storage MUCH slower than measurement
    (Dimax: few seconds dataset, 45 min transfer)
- Implications:
  - Fast acquisition only for short periods
  - Blind acquistion → Need to know exactly when to look
  - Reduce FOV to increase number of frames
  - Many phenomena cannot be studied in full length

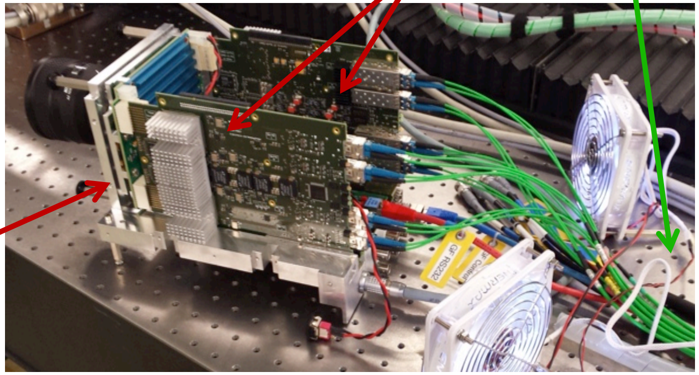
GigaFRoST: Gigabit Fast Read-Out System for Tomography



fiber-optic connections

- PSI in-house development
- In user operation since September 2015
- pco.Dimax fast imaging sensor
- Custom readout electronics
- No on-board memory
- 8 parallel fiber-optic connections, continuous direct data streaming to server:
  8 GB/s → 1 TB/2min!
- 1.25 kHz full frame rate (2016x2016 @ 12 bit)
  10 kHz @ 576x575,12 bit
- Live preview from subset of streamed images

GigaFRoST data boards

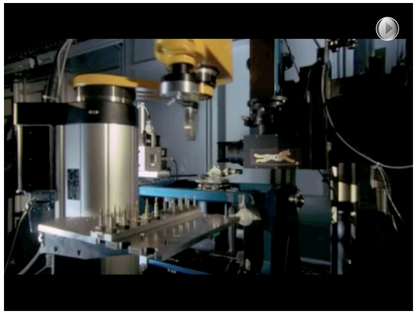


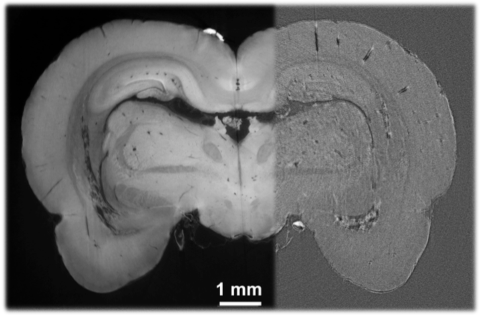
pco.Dimax headboard

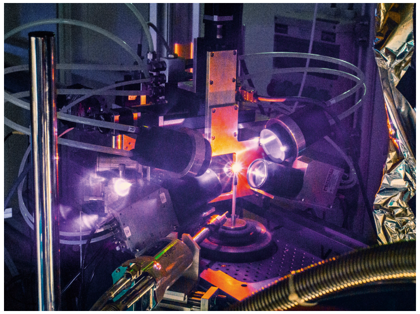
TOMCAT at a glance

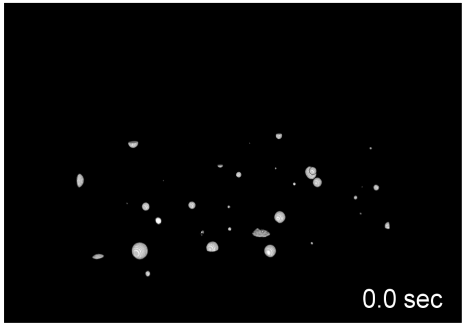
Features:

- Wide spatial resolution range: nano-to-meso scales (0.1-10μm)
- Broad range of sample sizes (10μm-20mm)
- High density resolution enhanced by phase contrast
- High temporal resolution (3D data acquisition in less than 1s)









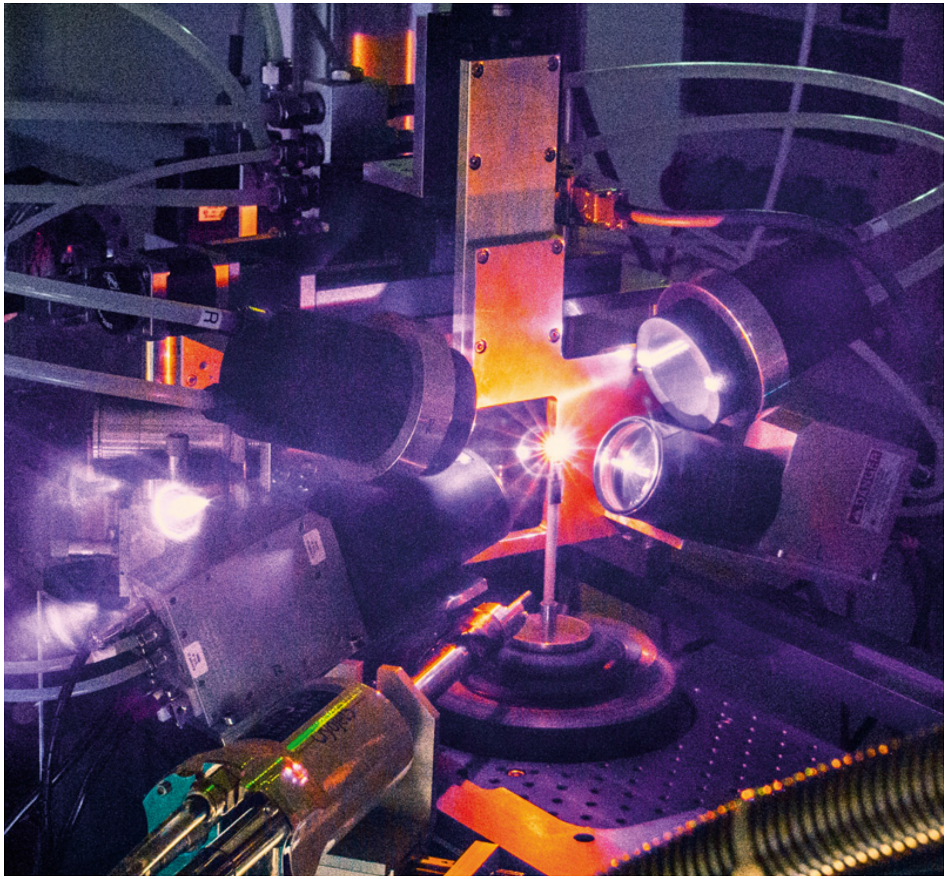
Automation
large scale studies

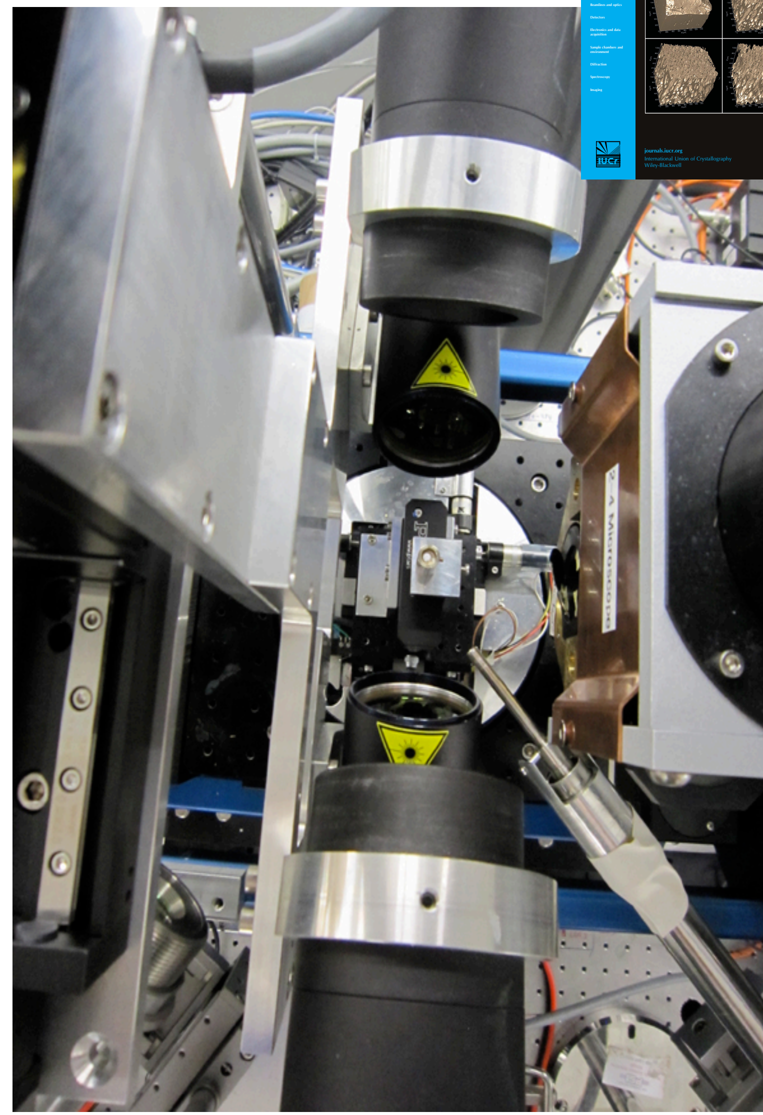
Density resolution
phase contrast imaging

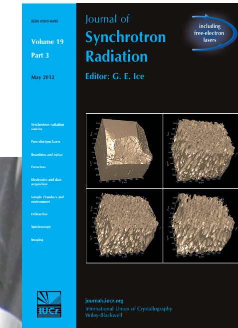
In situ capabilities
temperatures away from ambient

Ultra-fast data acquisition
dynamic studies

Laser Furnace







- 2 cross firing 150W, 980nm cw, class 4 lasers
- Temperature feedback with pyrometer
- Laser power and temperature control fully integrated.

J. Fife et al., Journal of Synchrotron Radiation 19(3), 352–358 (2012)

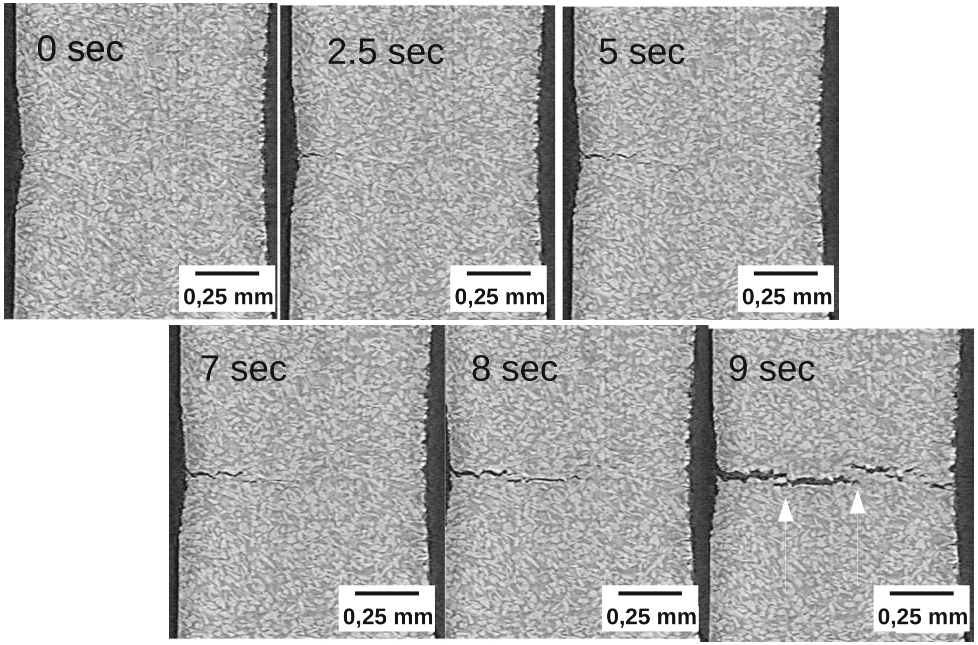
Crack propagation dynamics under tensile load @ 20 Hz

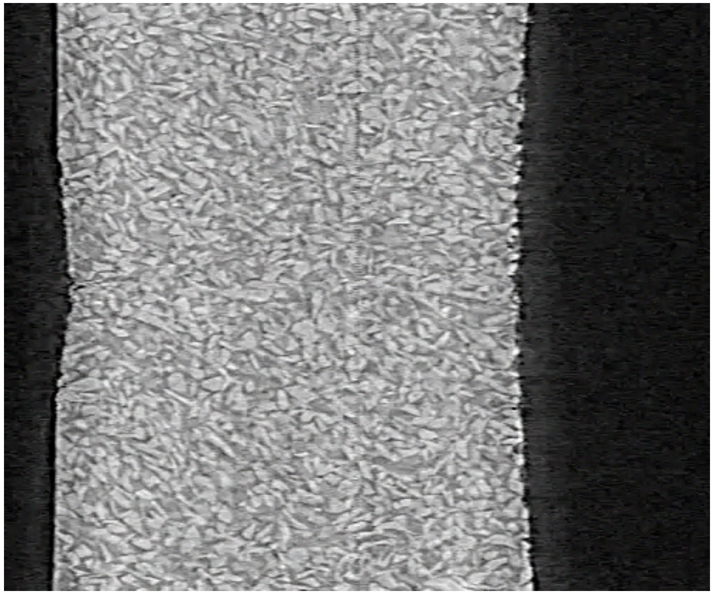
- Custom tensile rig
- Alumina particle reinforced aluminium composite
- Tomography:
  - Polychromatic beam (50% filter, mean energy 30 keV)
  - 100 μs exposure time
  - 500 projections / scan
  - 50 ms scan time (20 Hz)
  - Sample rotating at 600 rpm

- **Fastest scans at TOMCAT to date!**

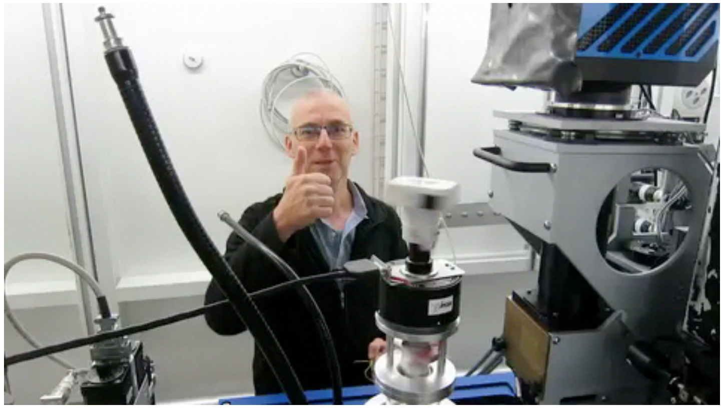
E. Maire, et. al., Int J Fract 1 (2016)

In-situ 20 Hz tomographic imaging



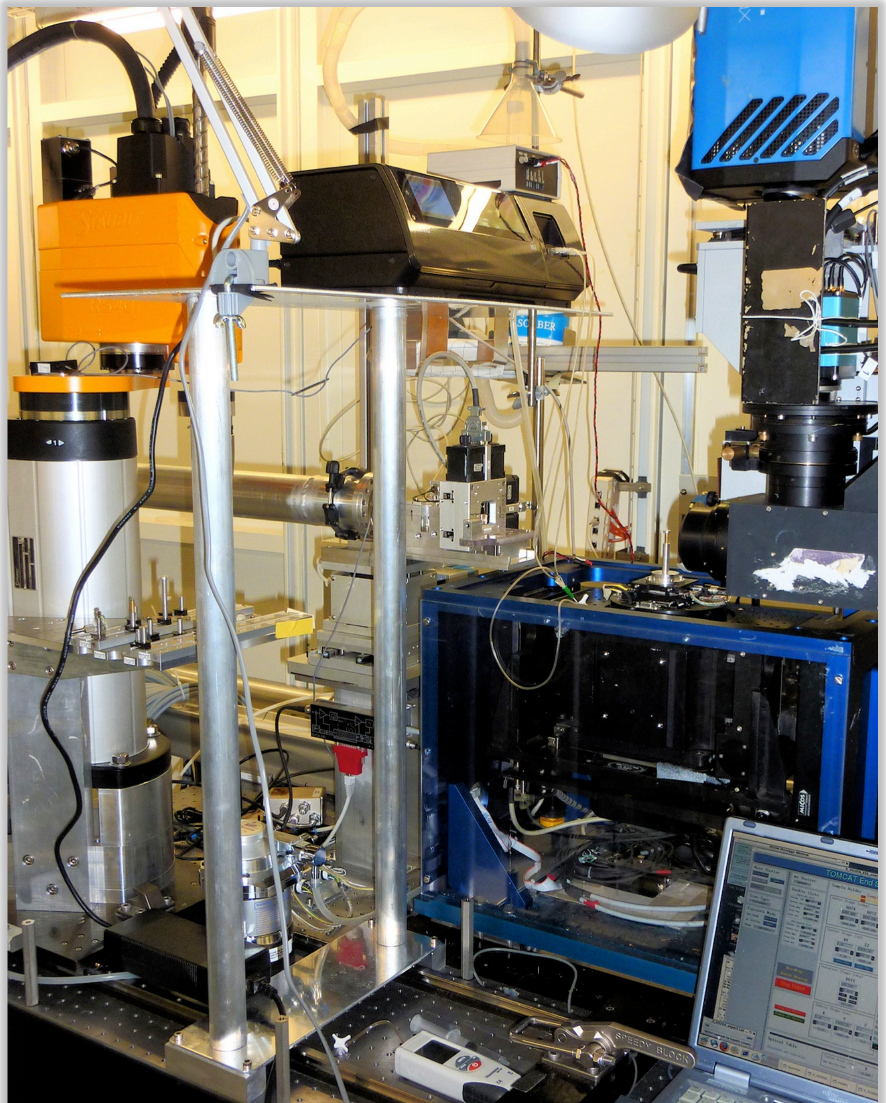


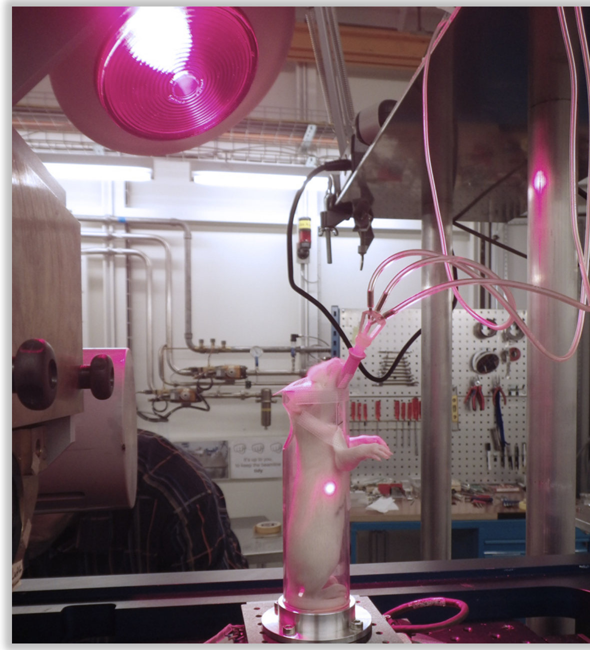
Movie playing in real time (9 seconds, 180 frames)

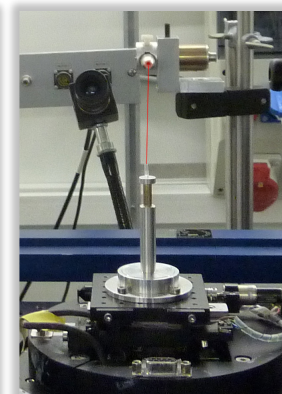


E. Maire, INSA-Lyon, UCBL, CNRS, MATEIS
Université de Lyon France

Dynamic 3D imaging in-vivo: complex triggering







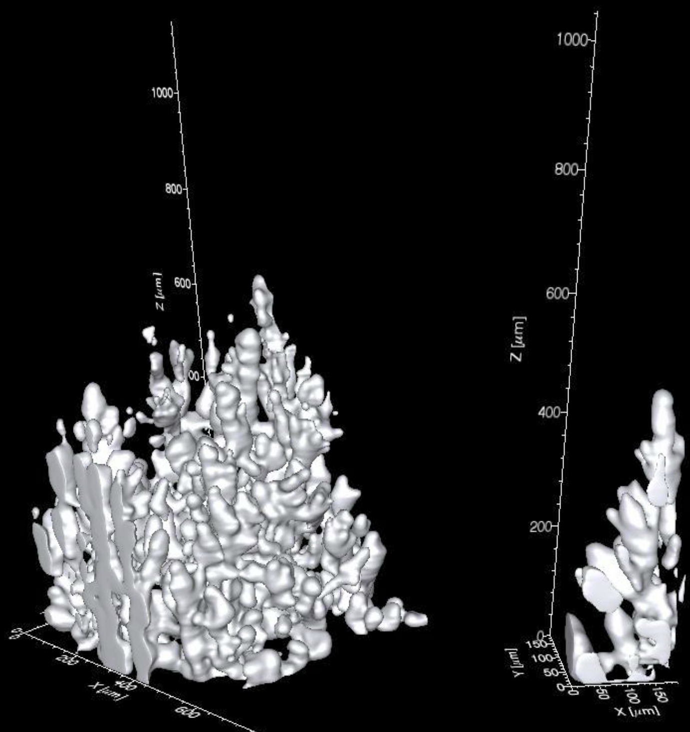
Lovric et al., Physica Medica, 2016

Summary

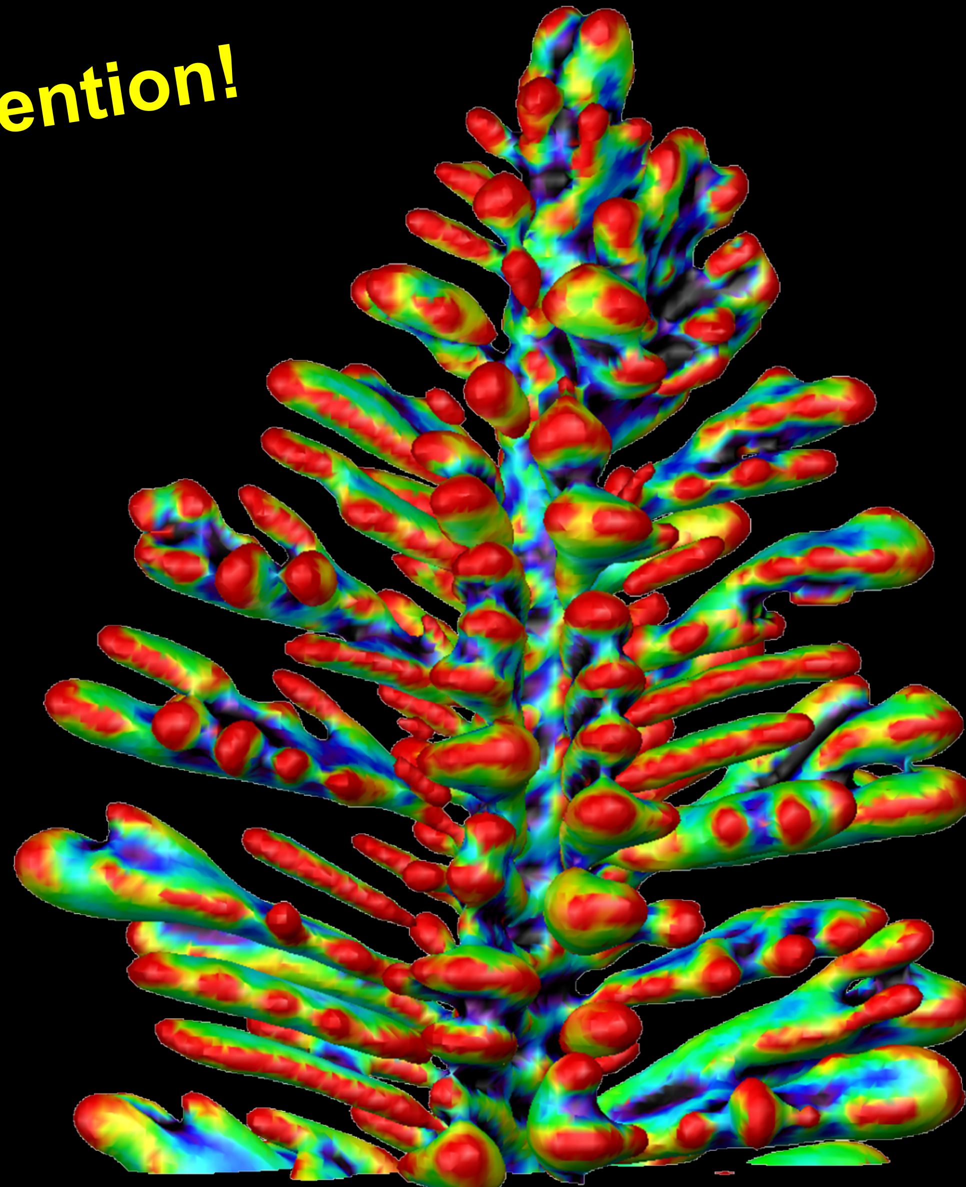
- TOMCAT offers high spatial and temporal resolutions in multiple modalities over a range of length scales
- "Unique" capabilities coupling sub-second AND continuous 3D data acquisition (GIGAFROST detector)
  - Removes hardware limitations that are present in other detector systems for observation of true dynamic phenomena
- Setup is ideal for time-resolved (4D) in-situ materials and in-vivo biological applications
- Cutting-edge grating-interferometry endstation for new microscopy applications and benchmarking purposes
- Outlook: SLS2.0 with more flux , higher energies, round source.

TOMCAT (flying) team

Thank you for your attention!



4D directionally solidified dendrites



Mean curvature colored dendrite (Al/Cu alloy)

J. Fife *et al., in preparation*