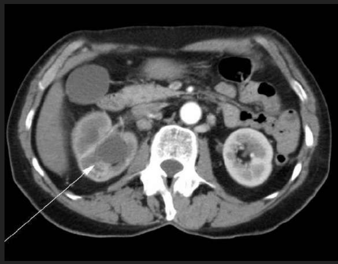
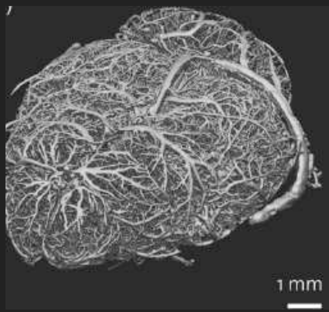
Imaging life with bright X-rays

Rajmund Mokso

Max IV Laboratory

Outline

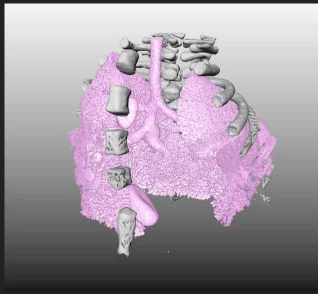


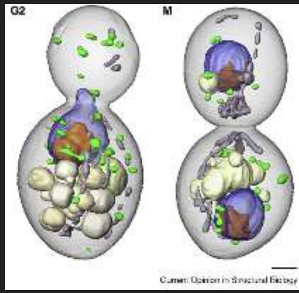


I. Introduction to MAX IV
   I. Source and beamlines

II. Currently available techniques
   I. Nano probe and scattering

III. Selected Science cases (future capabilities at MAX IV)
   I. In vivo small animals, various fixed tissues





Rajmund.Mokso@maxiv.lu.se

The MAX IV Accelerators

Properties:

Wide band

High intensity/Brilliance

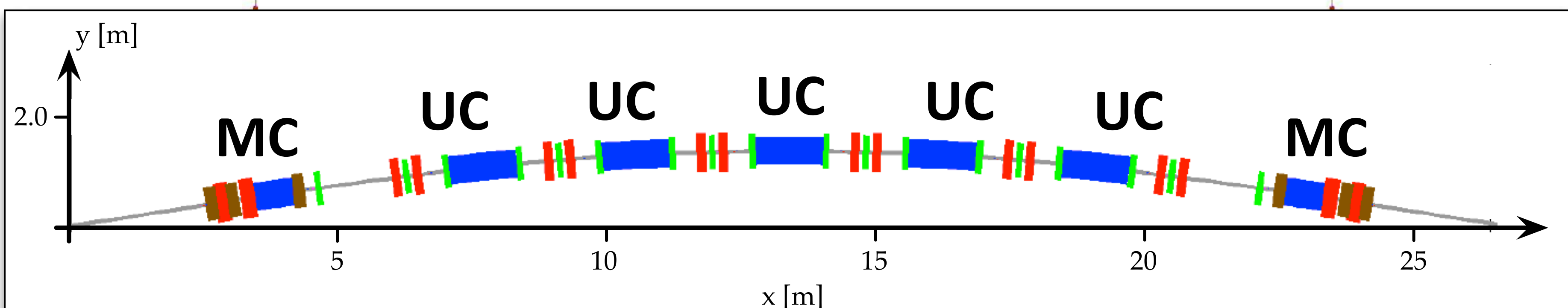
Polarization

Time structure

Pictures and animation by
S.Werin

MAX IV magnettic design

- 528 m circumference, 500 mA with top-up, 20 achromats
- 19 long straights (4.6 m) for users, 1 for injection
- 40 short straights (1.3 m) for RF & diagnostics
- 7-bend achromat: 5 unit cells (3°) & 2 matching cells (1.5° LGB)
- 328 pm rad bare lattice emittance (ε_y adjusted to 2-8 pm rad)



PRST-AB **12**, 120701 (2009)

IPAC'**11**, THPC059, p.3029

JSR **21**, 862-877 (2014)

The 7 bend achromat

Each **achromat** consists of 5 unit cells plus 2 matching cells

MAX IV magnets

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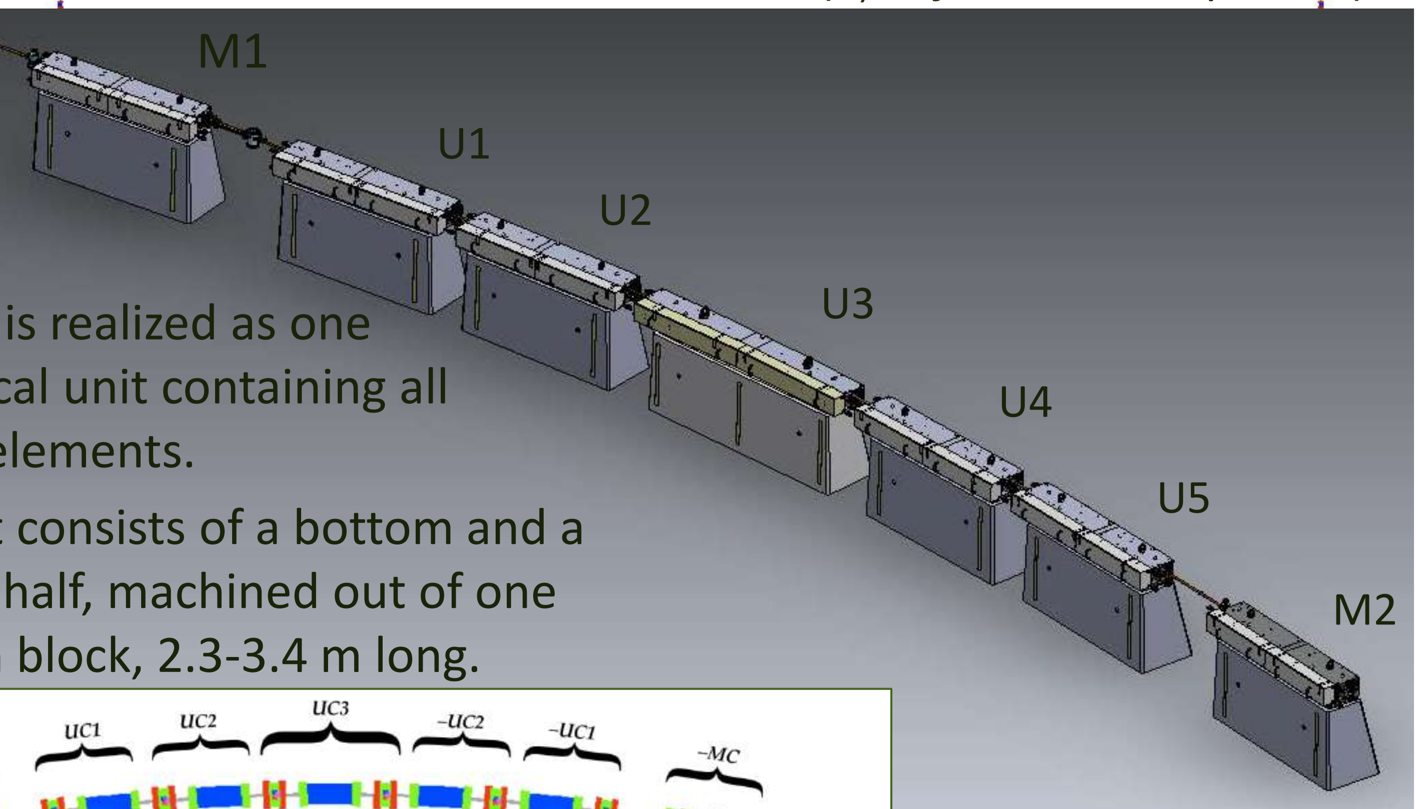
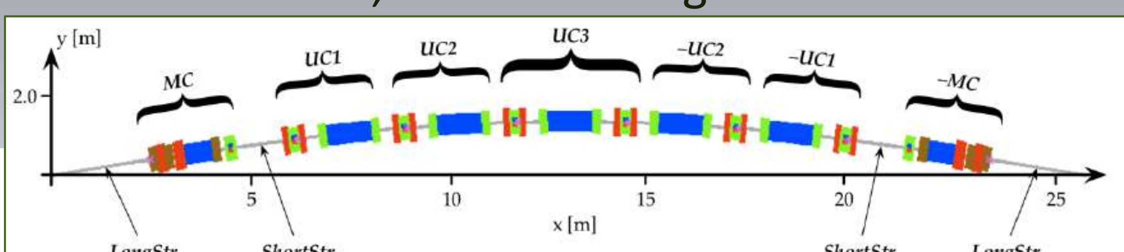
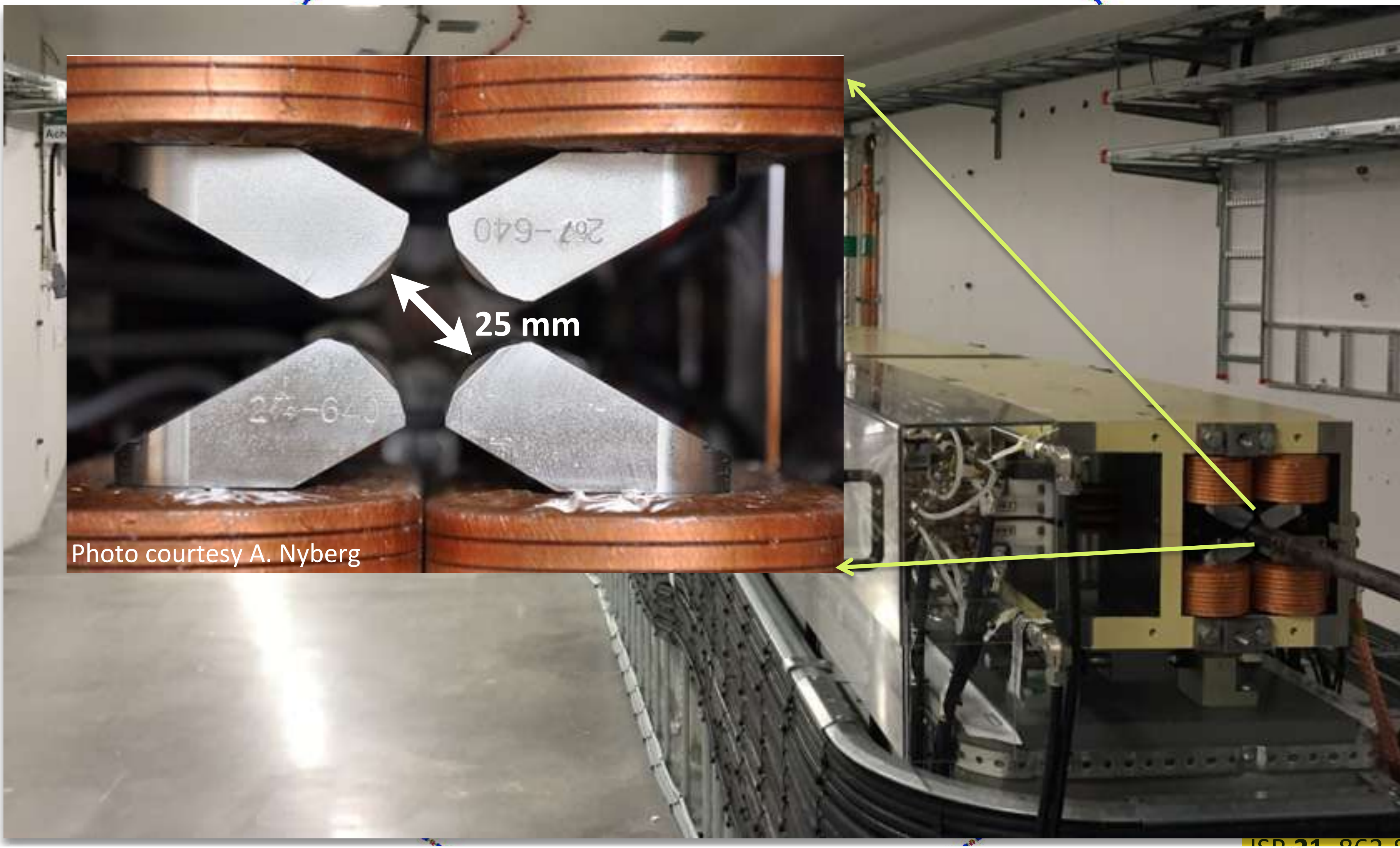


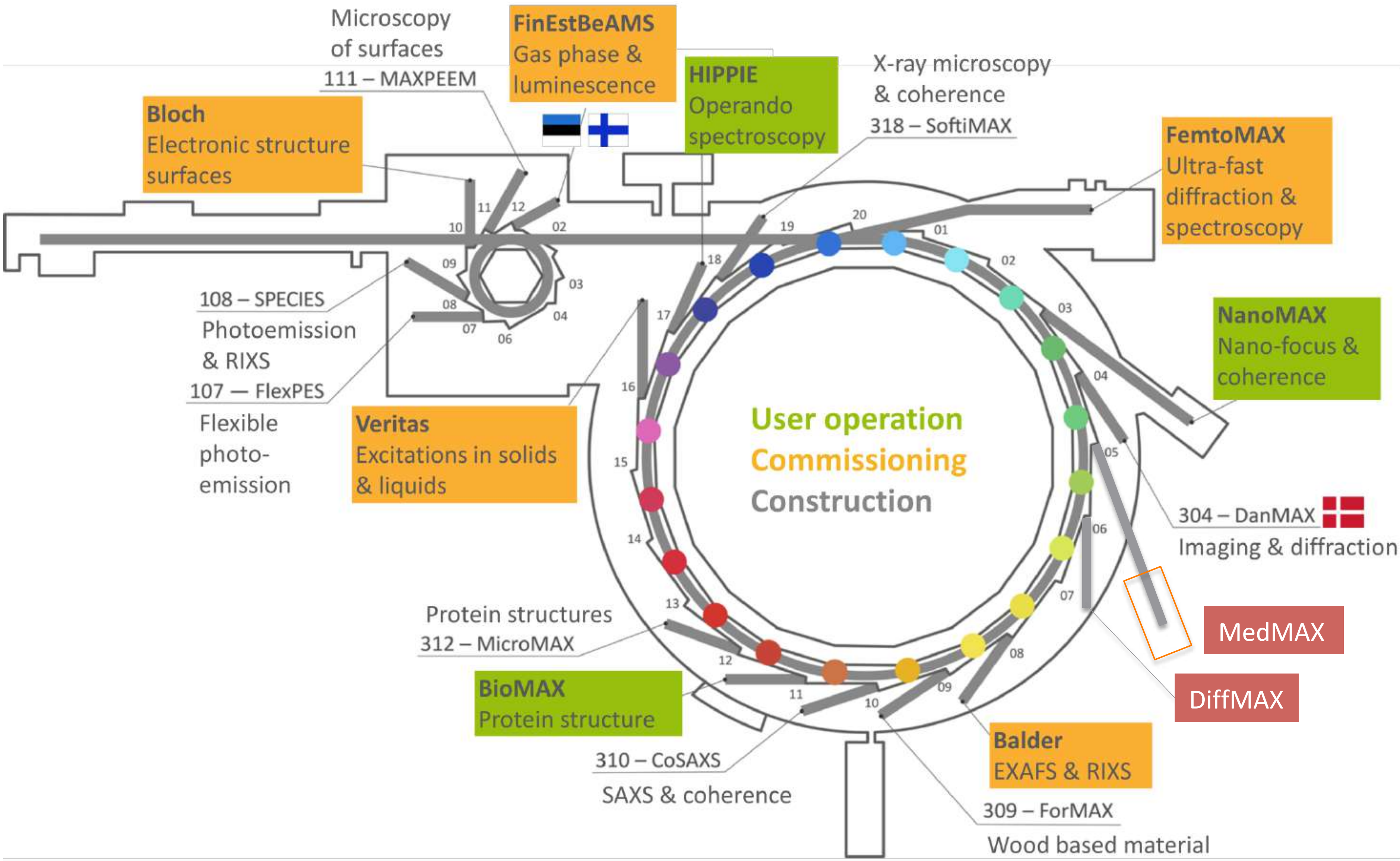
- Each cell is realized as one mechanical unit containing all magnet elements.
- Each unit consists of a bottom and a top yoke half, machined out of one solid iron block, 2.3-3.4 m long.



MAX IV storage ring

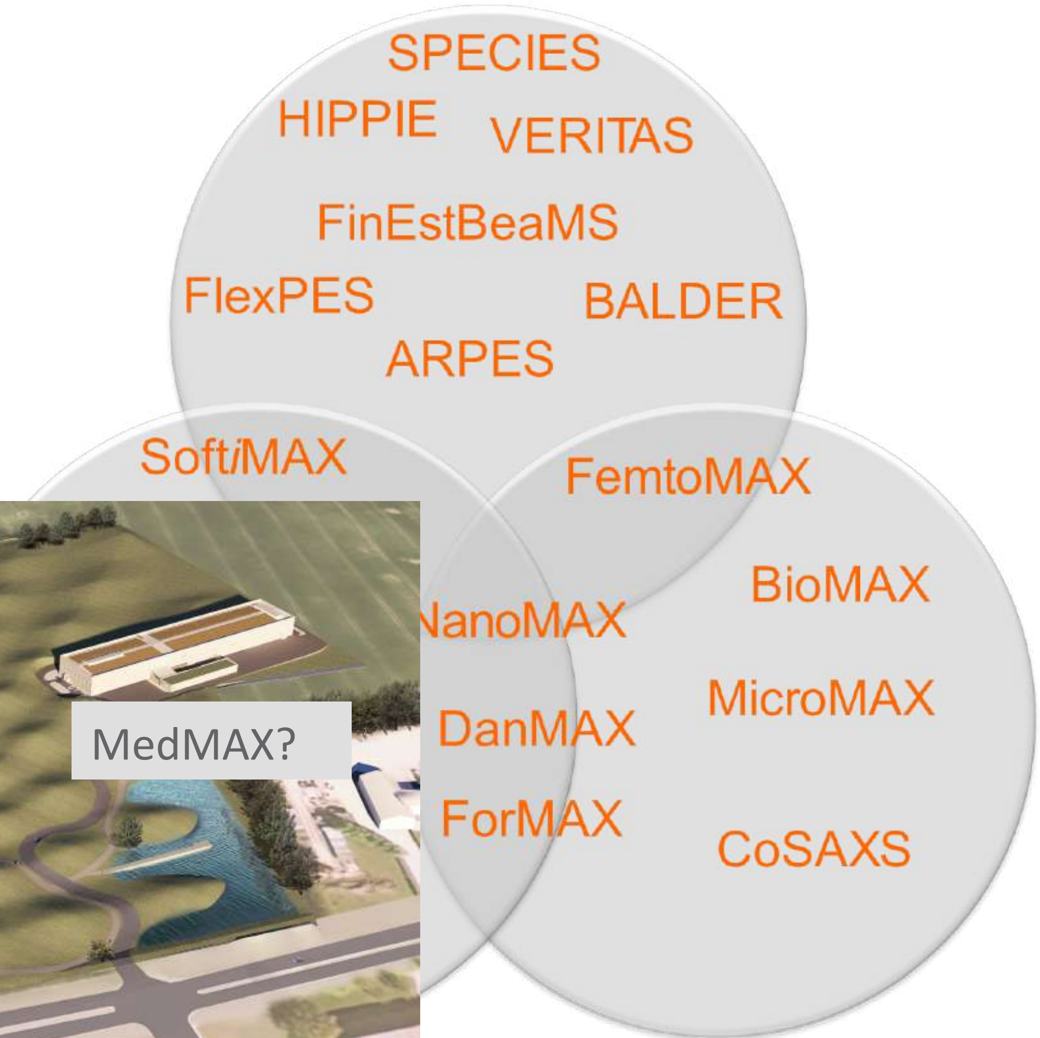
- 528 m circumference, 500 mA with top-up, 20 achromats

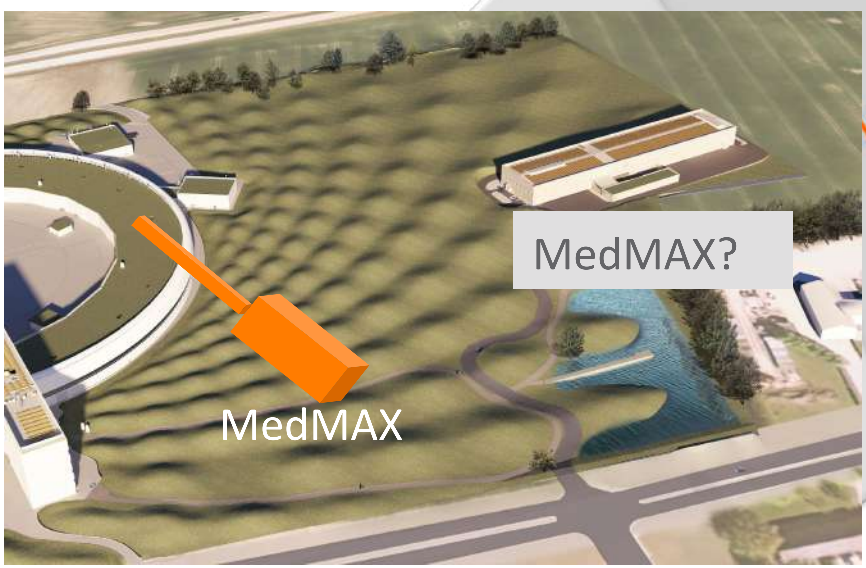




Microscopy of surfaces
111 – MAXPEEM
FinEstBeAMS
Gas phase & luminescence
HIPPIE
Operando spectroscopy
X-ray microscopy & coherence
318 – SoftiMAX
Bloch
Electronic structure surfaces
FemtoMAX
Ultra-fast diffraction & spectroscopy
NanoMAX
Nano-focus & coherence
108 – SPECIES
Photoemission & RIXS
107 — FlexPES
Flexible photo-emission
Veritas
Excitations in solids & liquids
User operation
Commissioning
Construction
304 – DanMAX
Imaging & diffraction
MedMAX
DiffMAX
Protein structures
312 – MicroMAX
BioMAX
Protein structure
310 – CoSAXS
SAXS & coherence
Balder
EXAFS & RIXS
309 – ForMAX
Wood based material

Spectroscopy





FOJAB arkitekter 2017-01-16 FÅGELPERSPEKTIV FRÅN SYDOST FLEXENHET, ÖSTRA TORN 27:12, LUNDS KOMMUN

Imaging

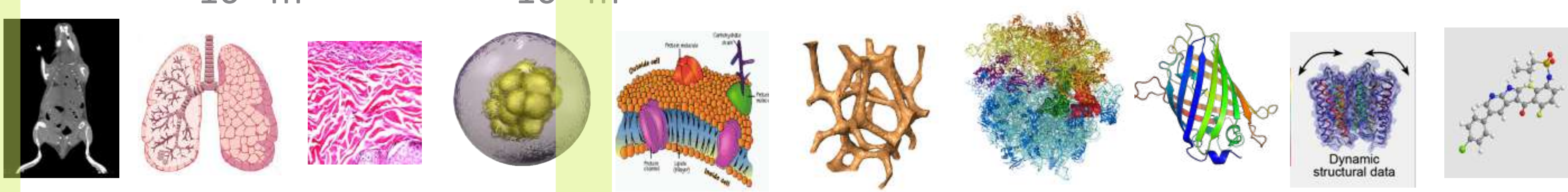
Diffraction/ Scattering

MAX IV LIFE
Biology at different length and time scales

NanoMAX

MedMAX III   MedMAX II   MedMAX I   SoftiMAX   DiffMAX   coSAXS   BioMAX   MicroMAX   FemtoMAX   Balder

10^{-6} m 10^{-9} m 10^{-10} m



Animals   Organs   Tissues   Cells   Membranes Surfaces   Micro-structures   Molecular complexes   Biomolecules   Atoms

"in/ex vivo"   "In solution"

Small animals/ disease models

Histology/ Histopathology

Cellbiology

Molecular Medicine/ Chemical Biology

Molecular Biology/ Biochemistry

The Max IV imaging beamlines

NanoMAX
The nanofocus beamline
(users in 2017)

SoftiMAX
Soft X-rays
(in construction -> 2018)

DanMAX
Imaging & diffraction
(in construction -> 2018)

MedMAX
The biomedical imaging beamline
(preparing CDR)

2016 — 2017 — 2018 — 2019-20

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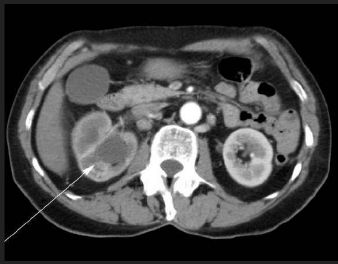

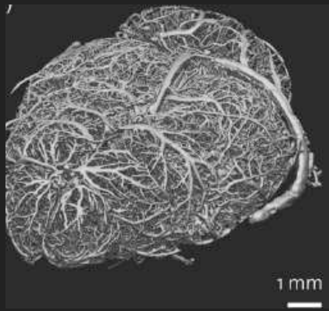

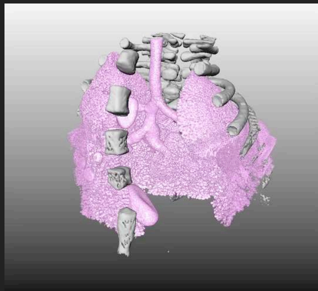

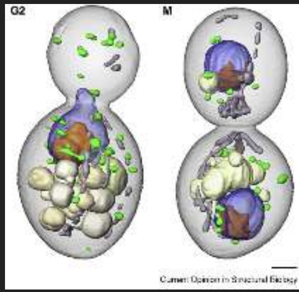

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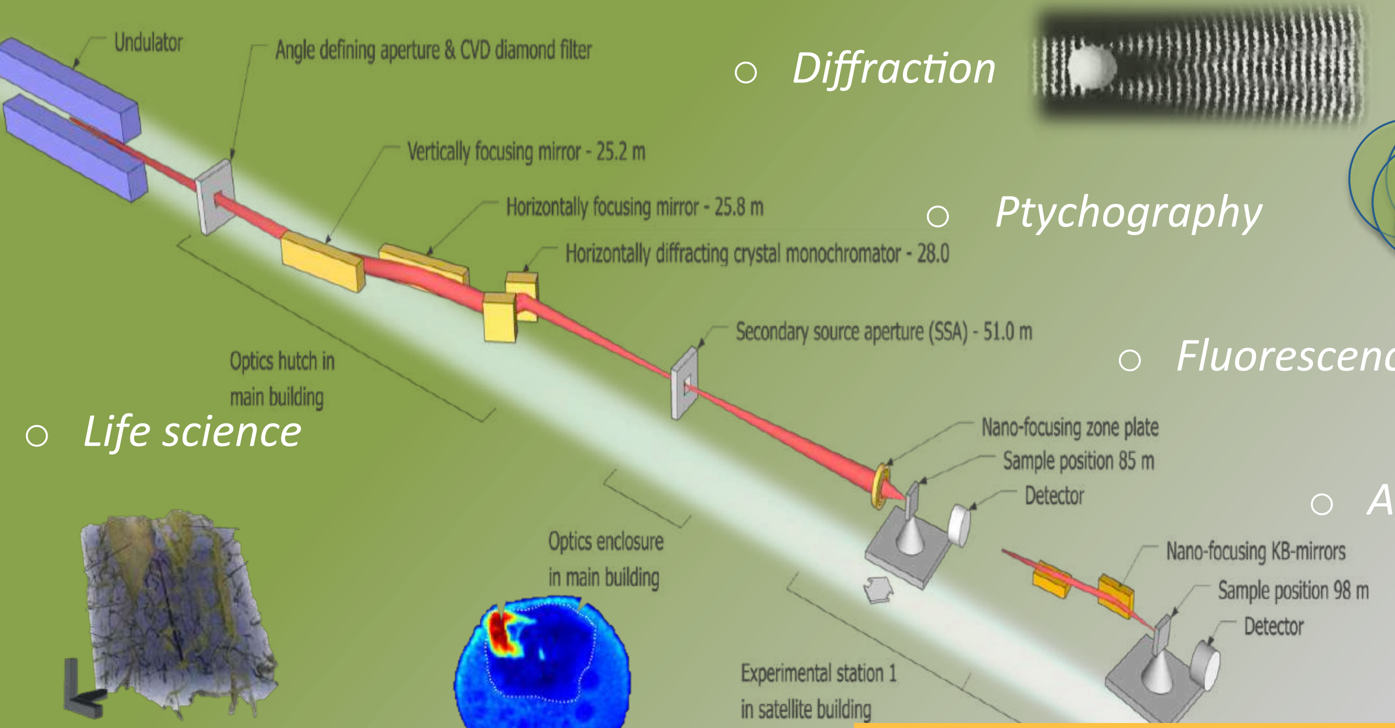
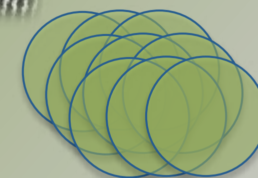
NanoMAX: scanning X-ray nano-probe

2D & 3D
Structure & strain
Elemental distribution
Morphology

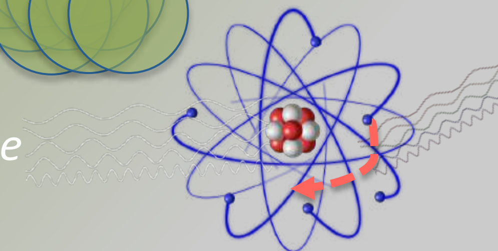


- *Diffraction*

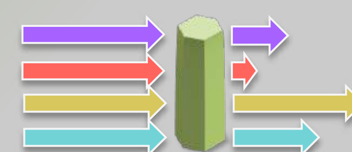
- *Ptychography*



- *Fluorescence*



- *Absorption*

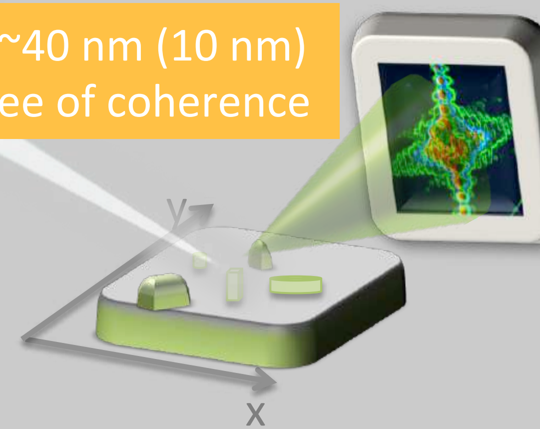


- *Life science*

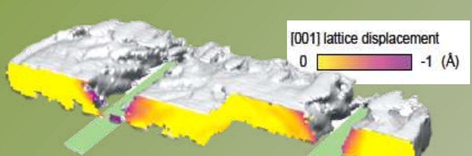
Bone inner structure by Ptychography
M Dierolf *et al. Nature* 2010 467, 436-439

Malaria pigment in red blood cell by XRF
F. Dubasr et al. Chem. Commun 2012 48, 910

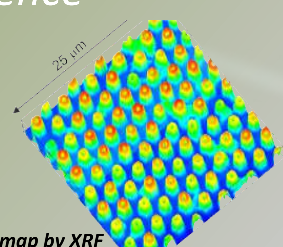
Focusing beam to ~40 nm (10 nm)
Clean and with high degree of coherence



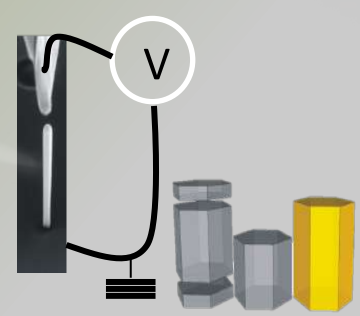
- *Materials science*



Strain in SiGe device by Bragg ptychography
S. O. Hruszkewycz et al. arXiv:1506.01262v1 [cond-mat.mtrl-sci]



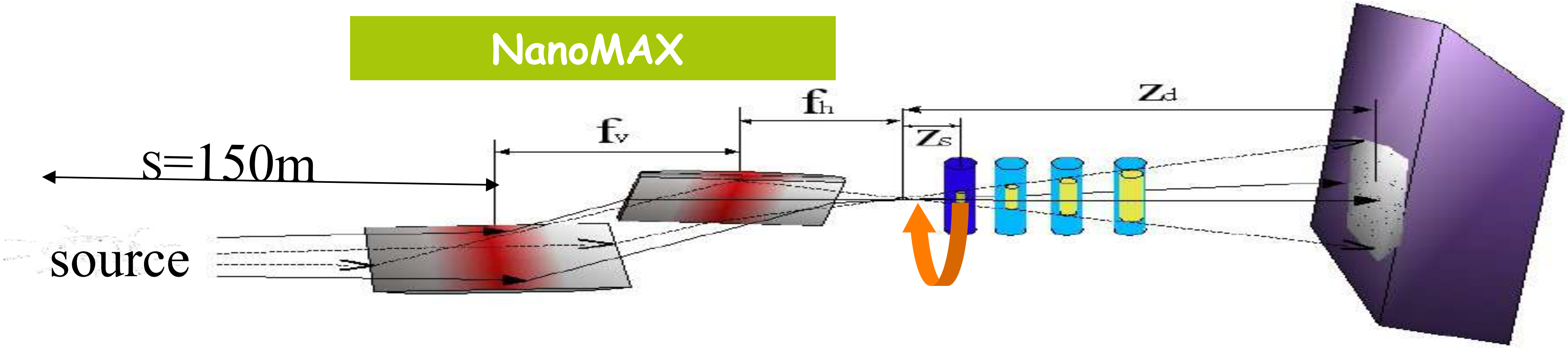
Ga density map by XRF
G. Martinez-Criado *et al*
Nano Lett. (2012) **12**, 5829



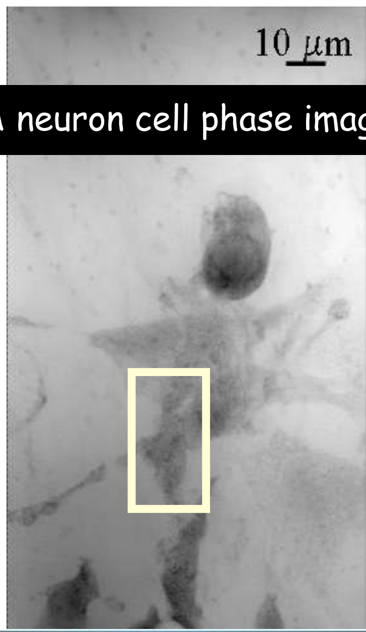
Structure and transport in GaAsby In-Situ XRD
G. Bussone te al. *Nano Lett.* 2015, 15 981

Nanoimaging, Fluorescence spectroscopy

NanoMAX

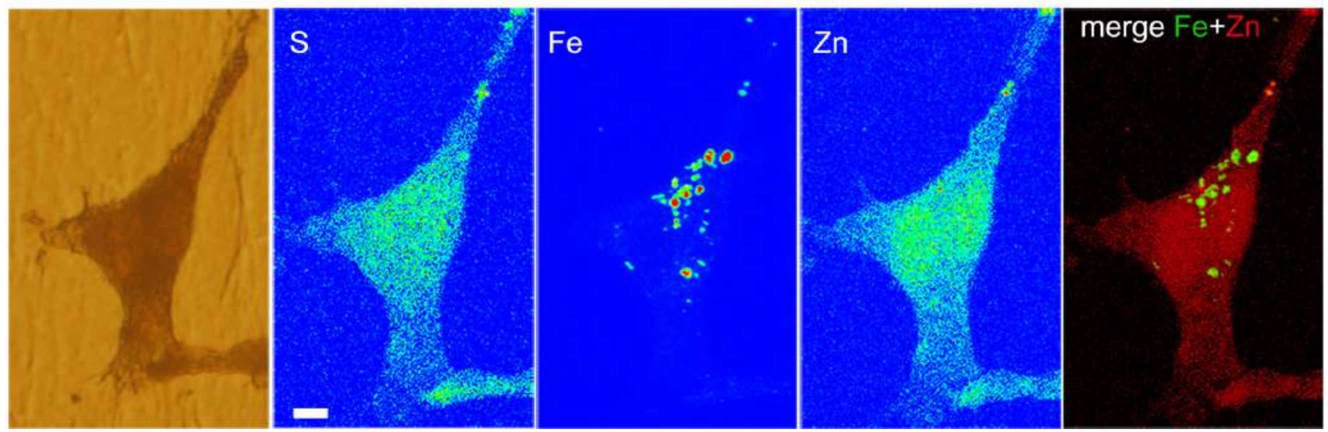


A neuron cell phase image



Mokso, Cloetens et al. , APL 2007

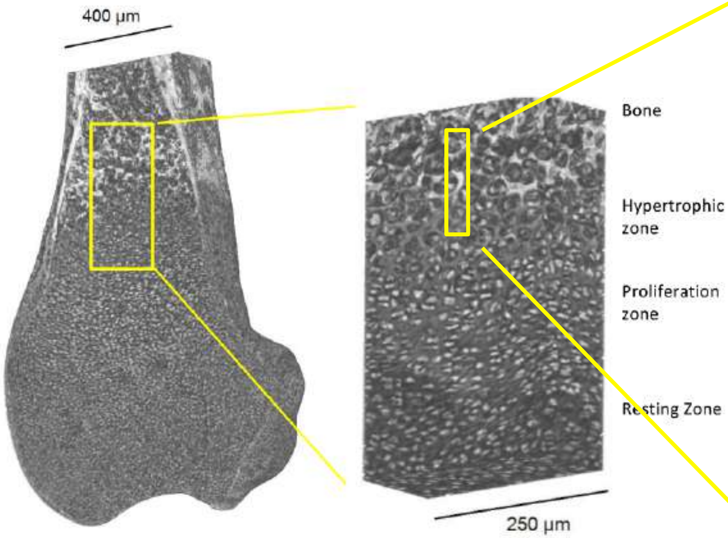
Chemical element distribution



Ortega. , Mol. Neurobiol. 2016

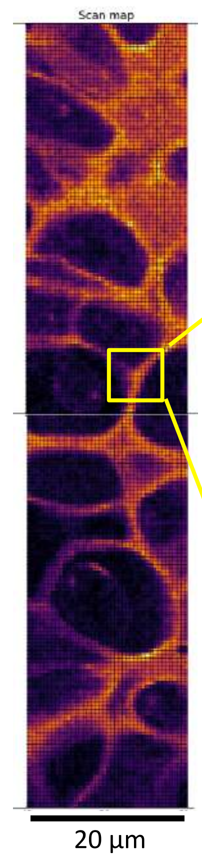
NanoMAX: first users

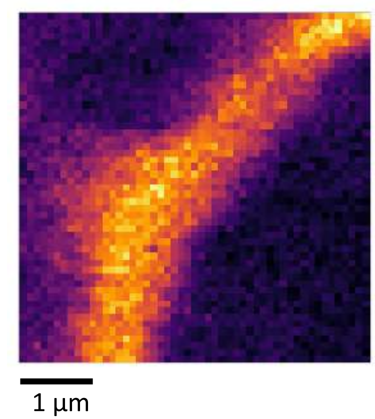
Embryonic mouse growth plate



Phase contrast x-ray tomography (TOMCAT, PSI)
Distal humerus, mouse TS24

NanoMAX
Fluorescence Ca mapping

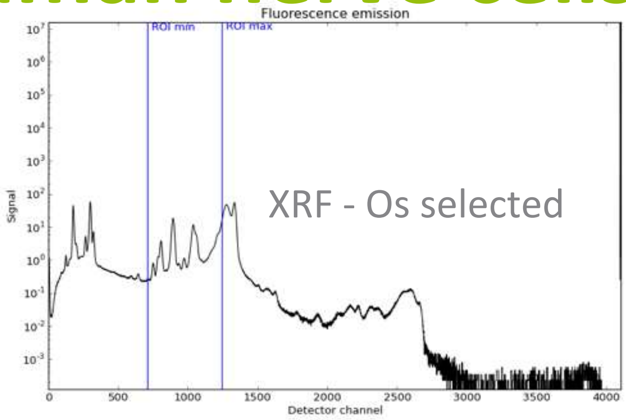


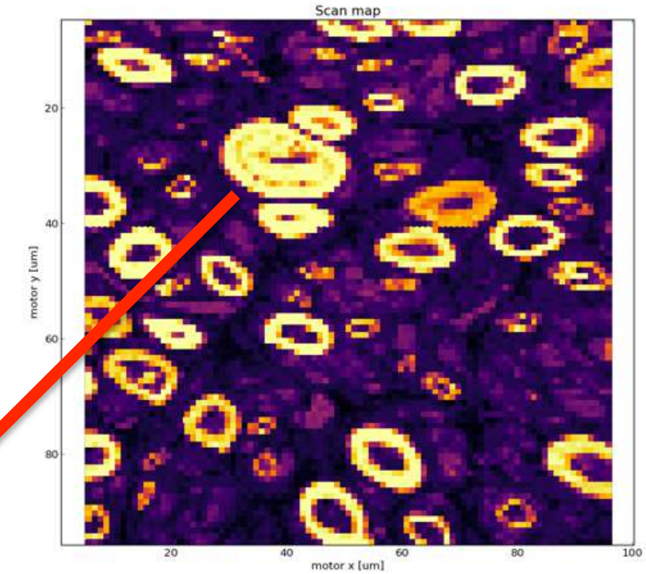


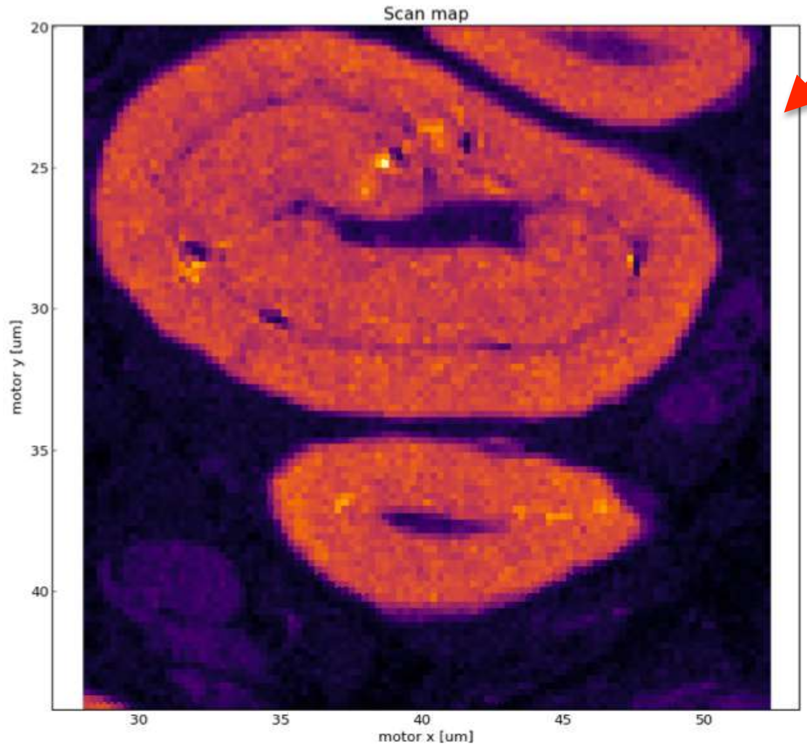
Growth plate, hypertrophic zone, Mouse TS 23

Hanna Isaksson, Lund University
Niamh Nowlan, Imperial Collage, London

Human nerve cells – diabetes type 2





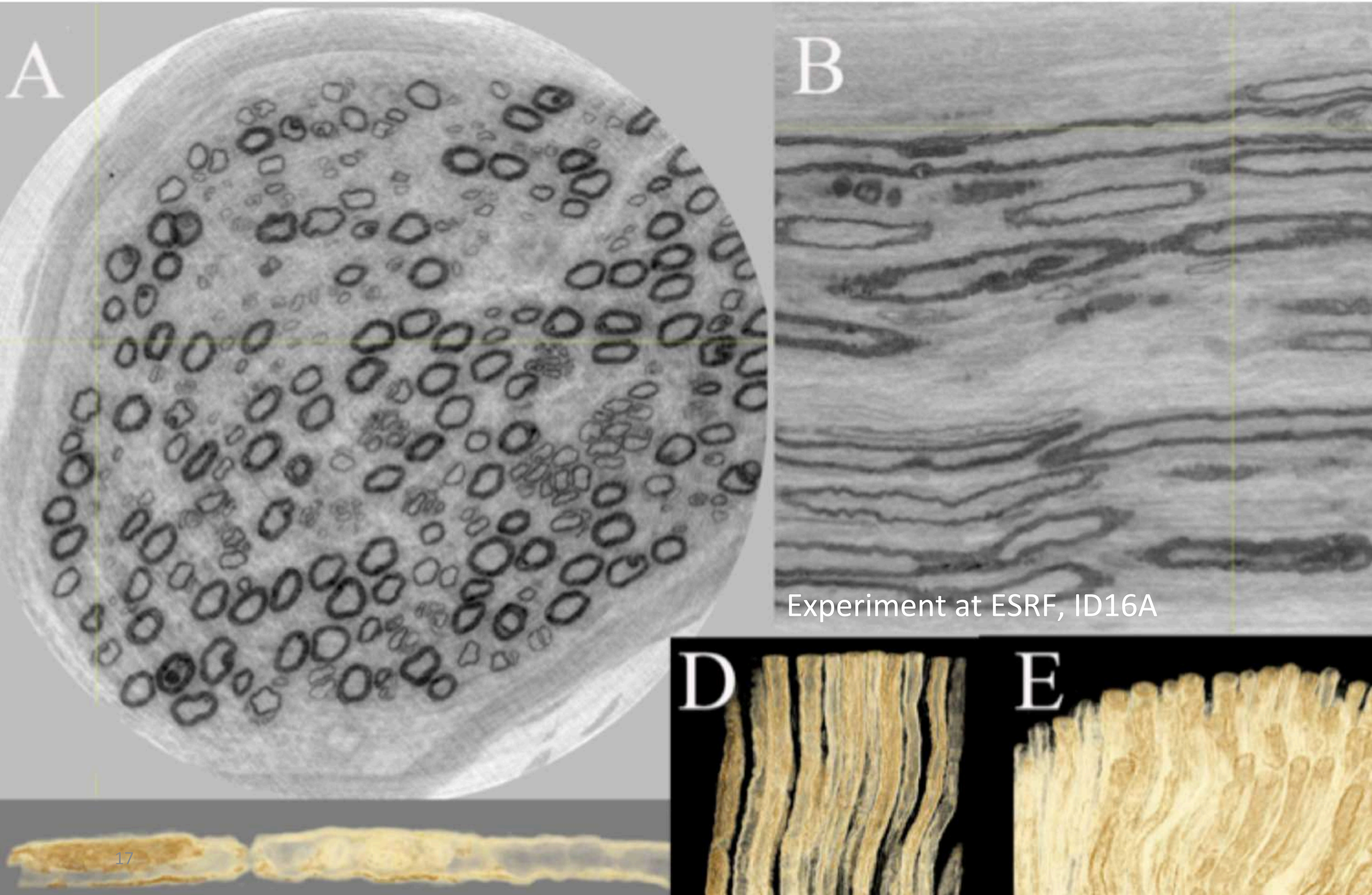


XRF imaging of human nerve cells of diabetes type 2 person. Samples stained with Osmium.
Left image 25x20 um^2.

Courtesy of Lars Dahlin & Martin Bech, BMC Lund University.

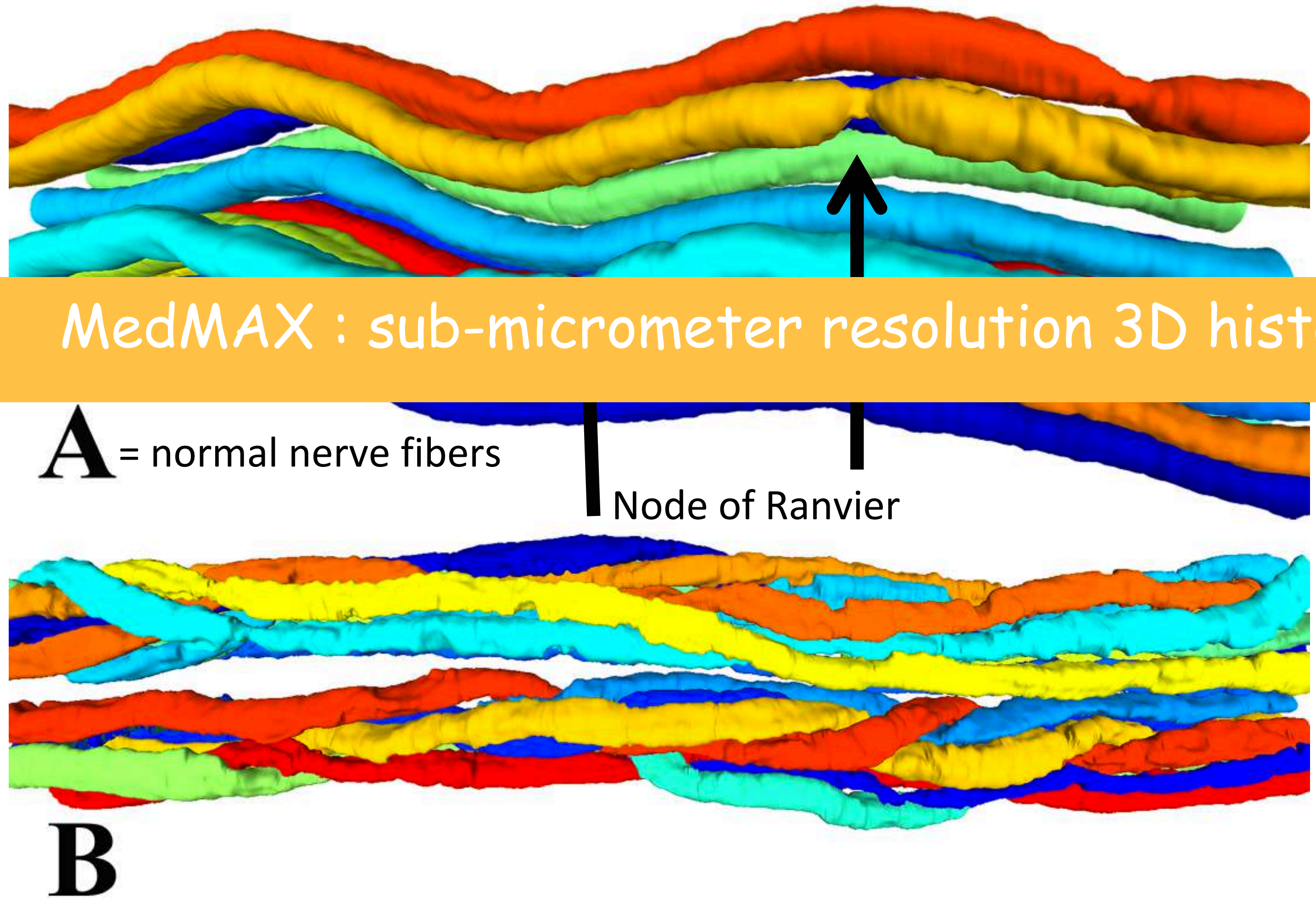
MAX IV

Human peripheral nerves



Human peripheral nerves (diabetes)

MedMAX : sub-micrometer resolution 3D histology



= regenerative clusters

Lars Dahlin and Martin Bech, Medical Faculty, Lund University

Flu [illegible] 3D

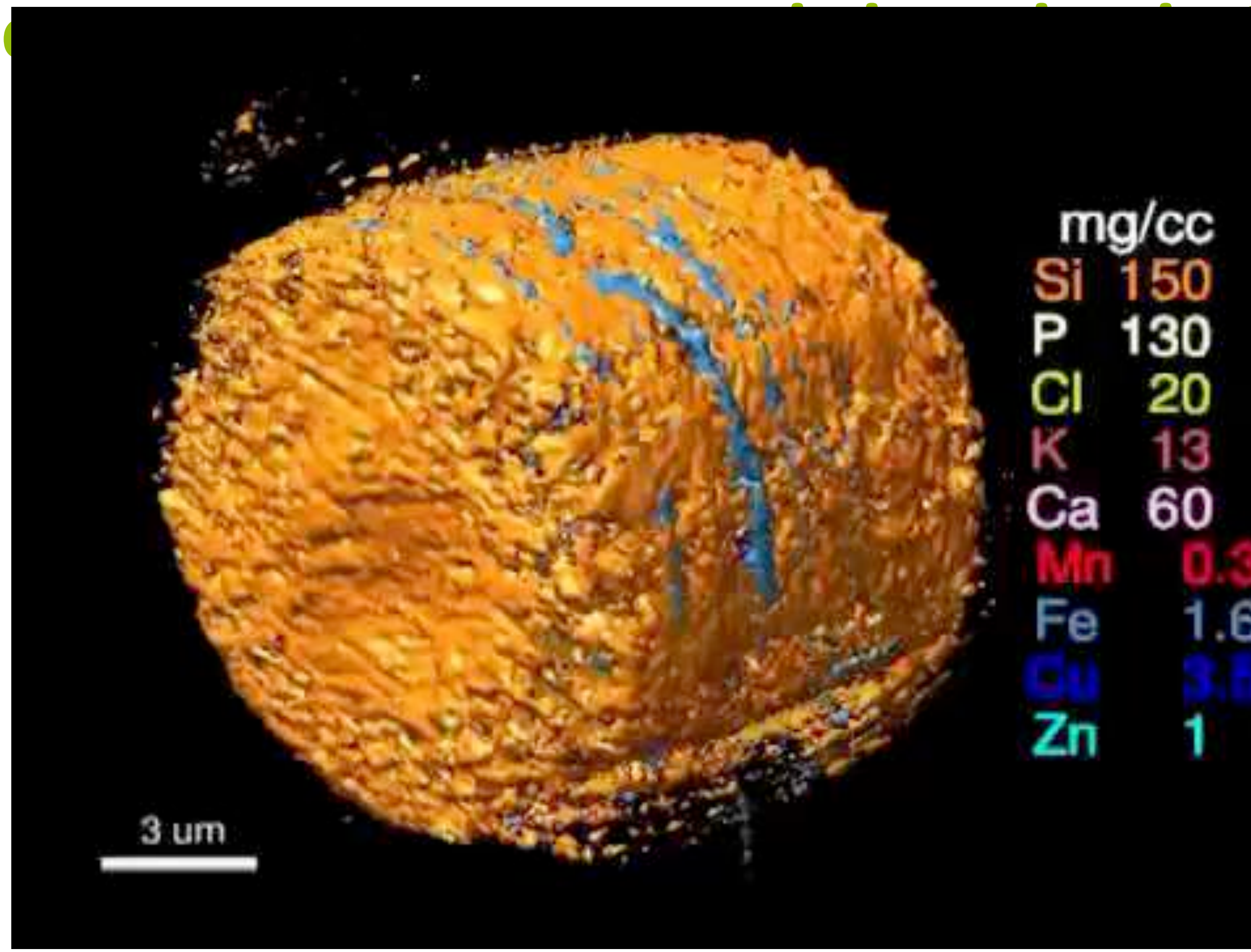
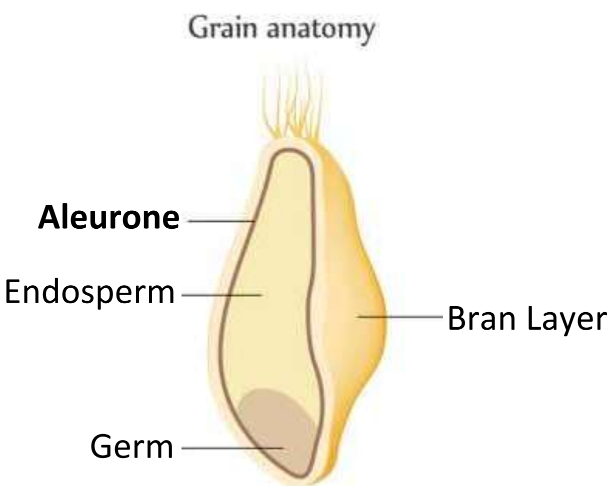


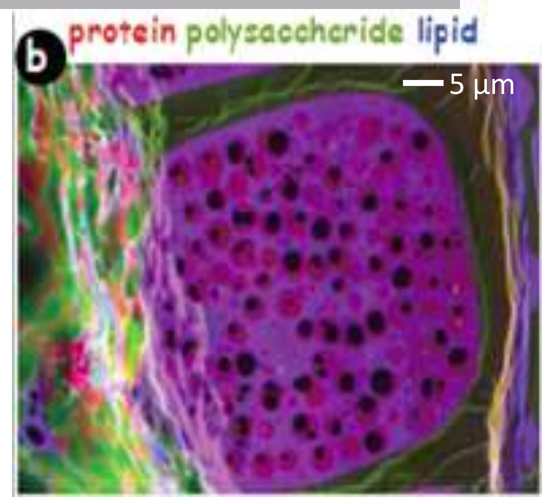
Figure 24. X-ray fluorescence tomography of an air-dried single-celled freshwater diatom *Cyclotella meneghiniana*. The silicacious frustule has been removed to show inner detail. Adapted with permission from ref 76. Copyright 2010 National Academy of Sciences.

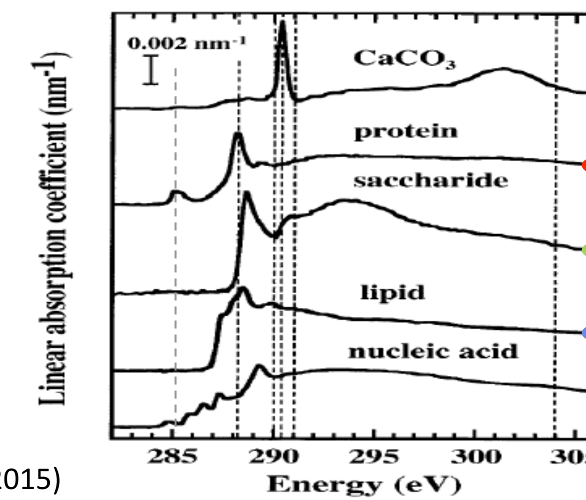
SoftiMAX: Soft X-ray scanning microscopy

Currently finalizing the design of the scanning transmission microscopy endstation

A grain's anatomy by chemical contrast: different forms of carbon





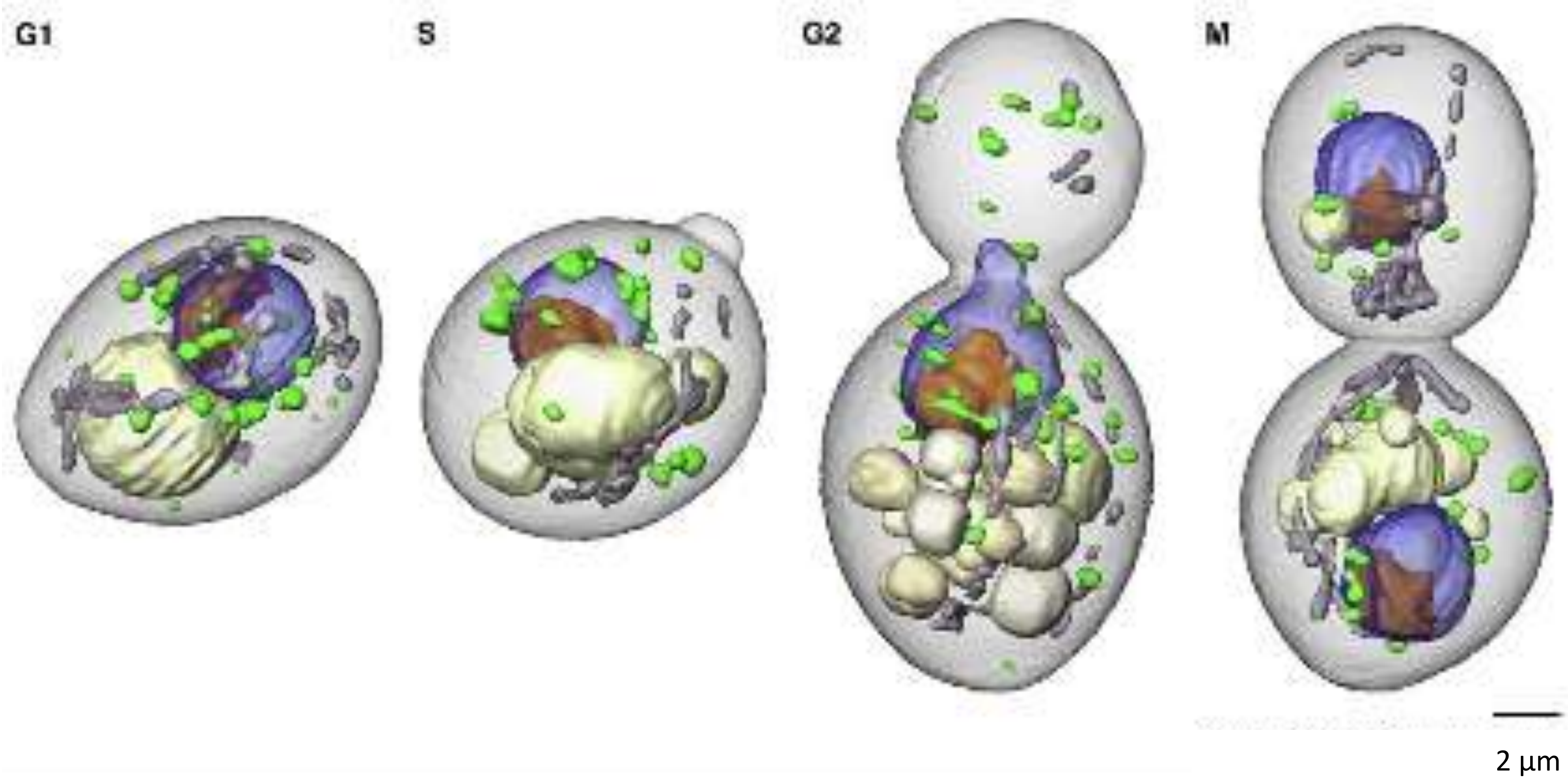


C. Karunakaran, *et al.*, PLoS One 10, e0122959 (2015)

Imaging cells with hard X-rays still a big challenge.
While it is almost a routine with soft X-rays

Soft X-ray microscopy

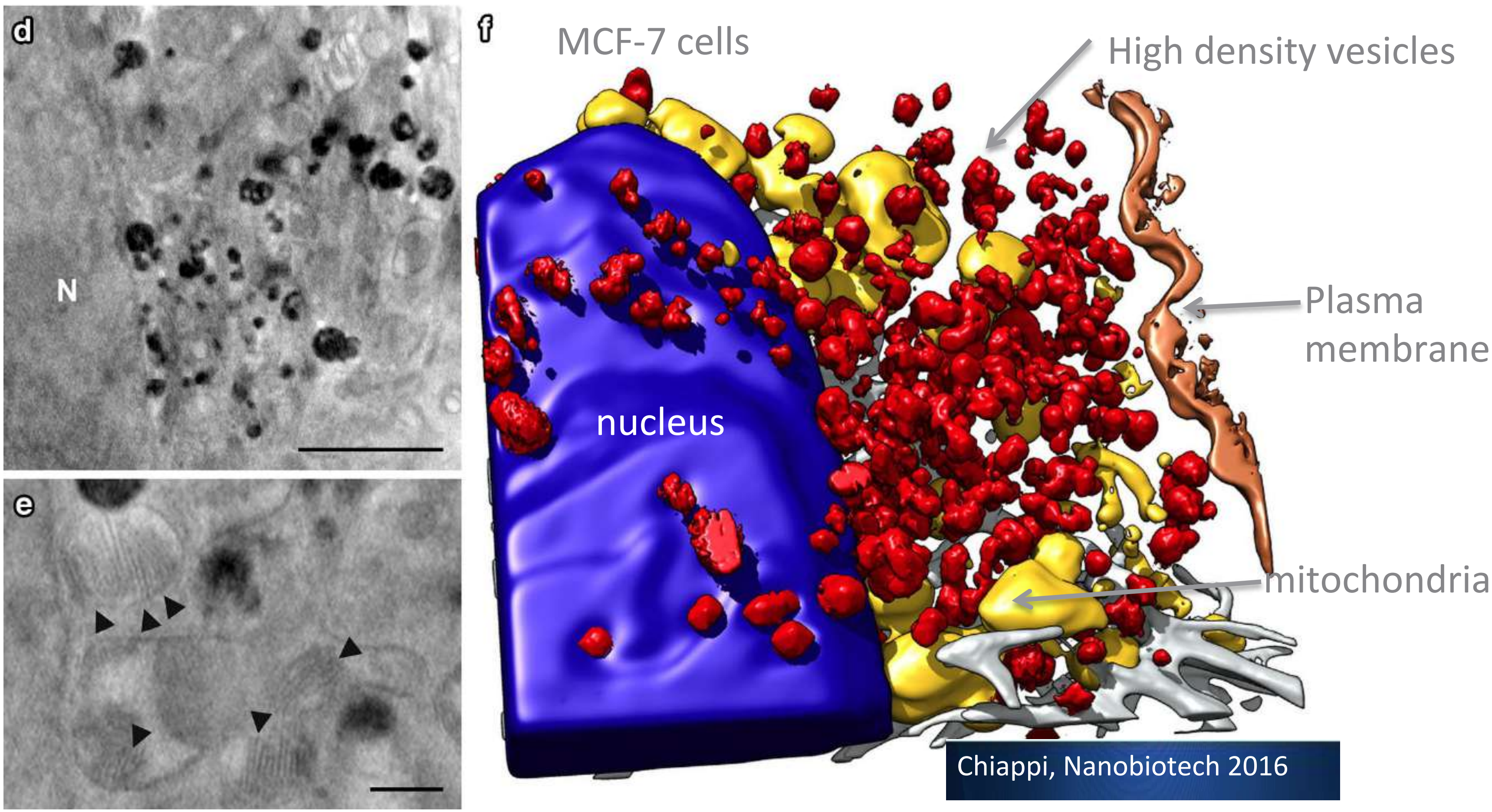
spatial resolution ~ 30 nm



Larabell & Nugent, Current Opinion in Structural Biology (2010)

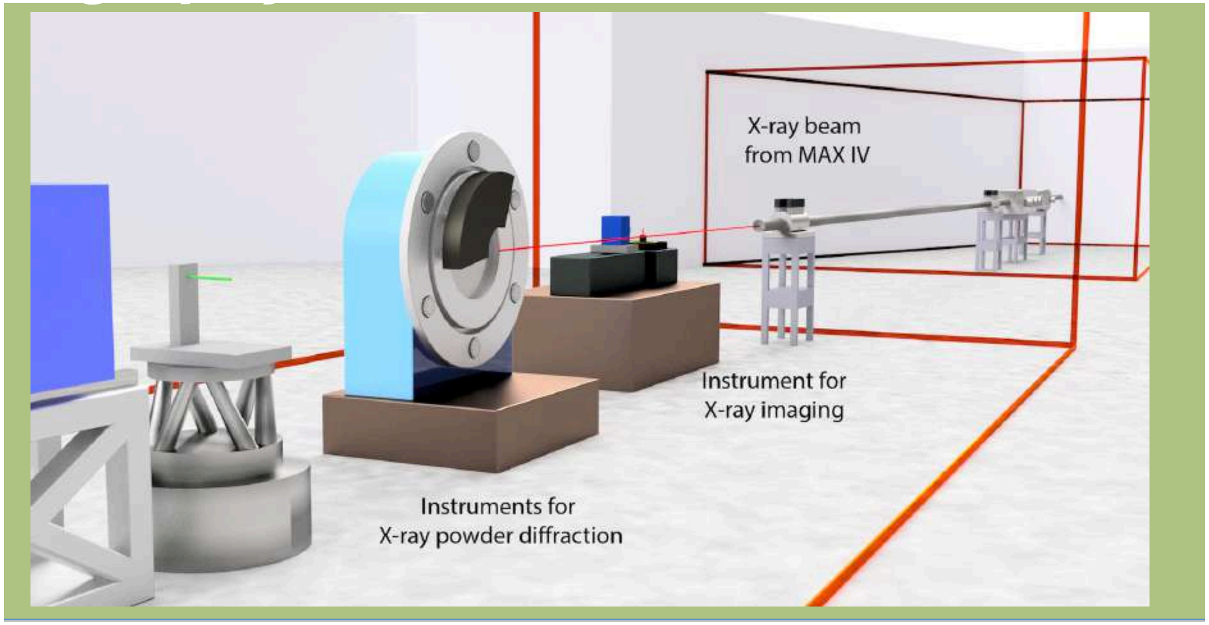
Soft X-ray tomography of rapidly frozen Saccharomyces cerevisiae cells imaged at each phase of cell cycle – G1, S, M, and G2. Organelles are color- coded as follows: blue, nucleus; orange, nucleolus; gray, mitochondria; ivory, vacuoles; green, lipid bodies.

Cryo soft X-ray microscopy of cells



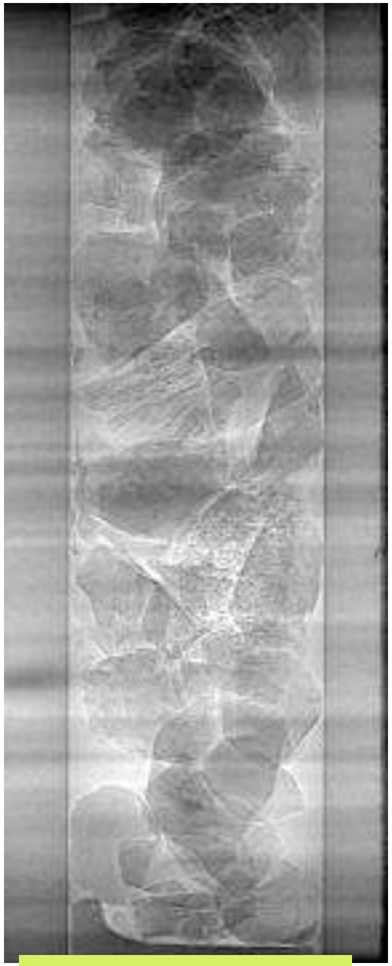
Chiappi, Nanobiotech 2016

DanMAX: tomography and diffraction for materials

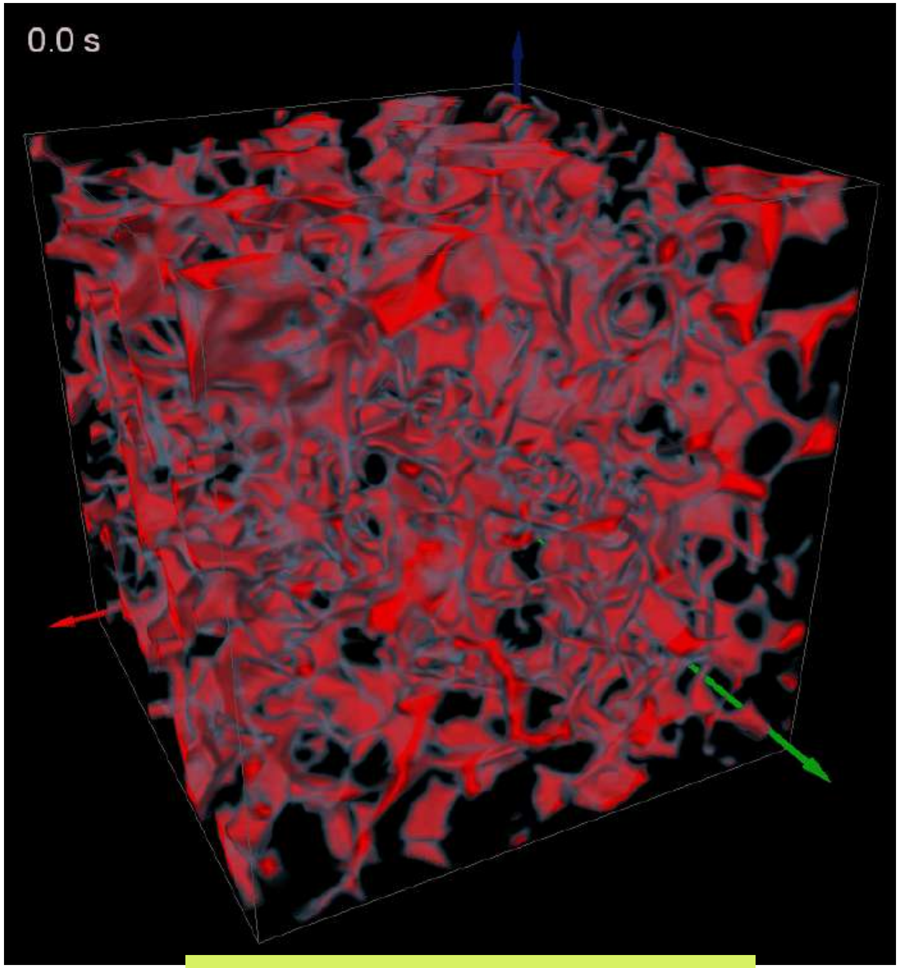


- study real materials at real conditions at real timescales.
- Combining powder X-ray diffraction (PXRD) and full field imaging

DanMAX: 1/2 tomographic microscopy



Crystal melting



Exploiting oil from the rock

Seeing the dynamics of matter in 3D at the micrometer scale

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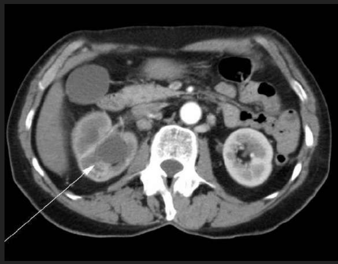

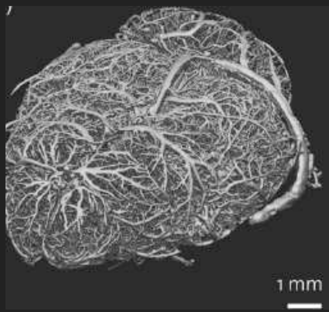

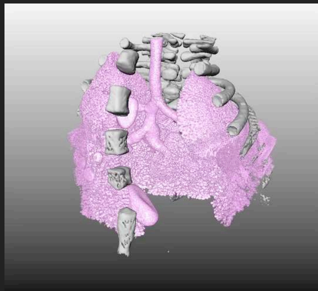

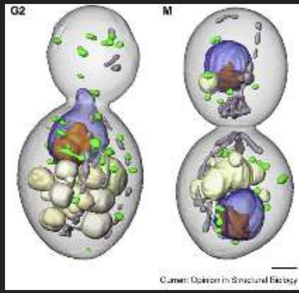

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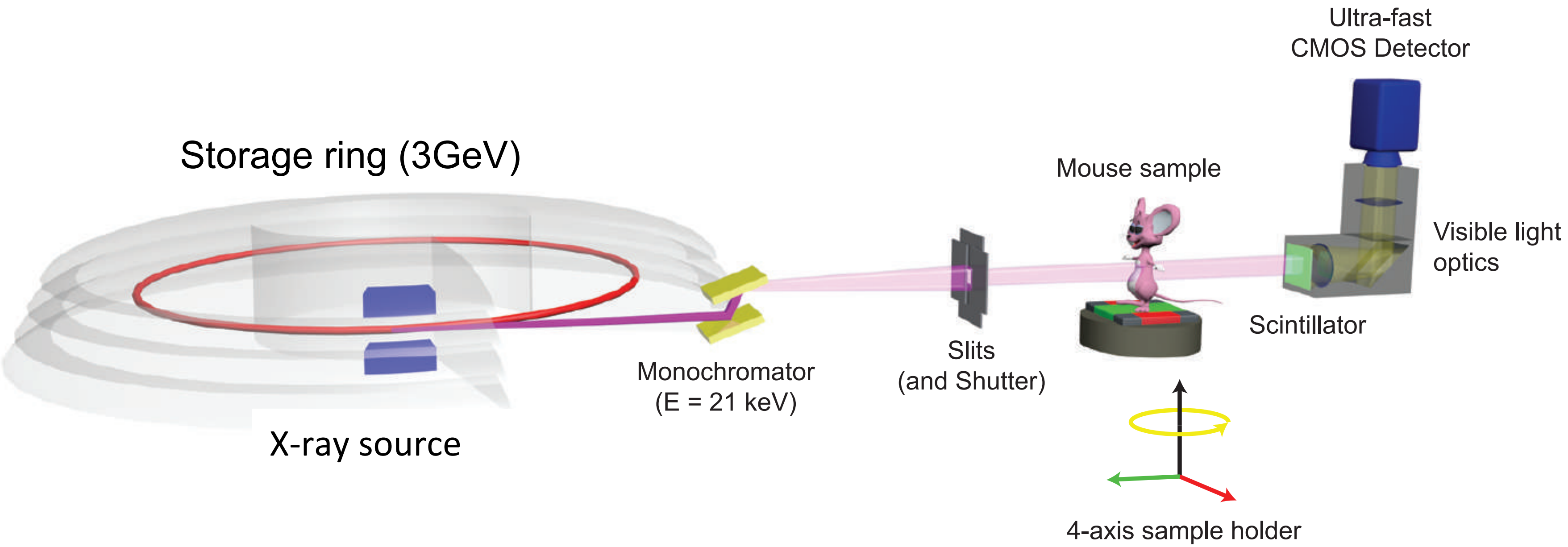
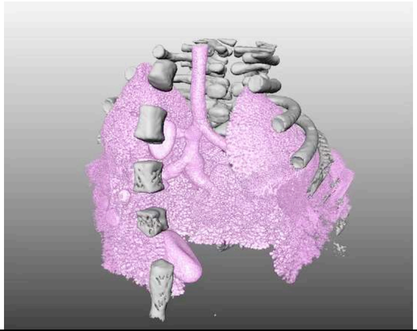
Tomographic microscopy at synchrotron



Radiographic projection clinics



Tomographic reconstruction Synchrotron

Pixel size: 11 -> 0.3 µm

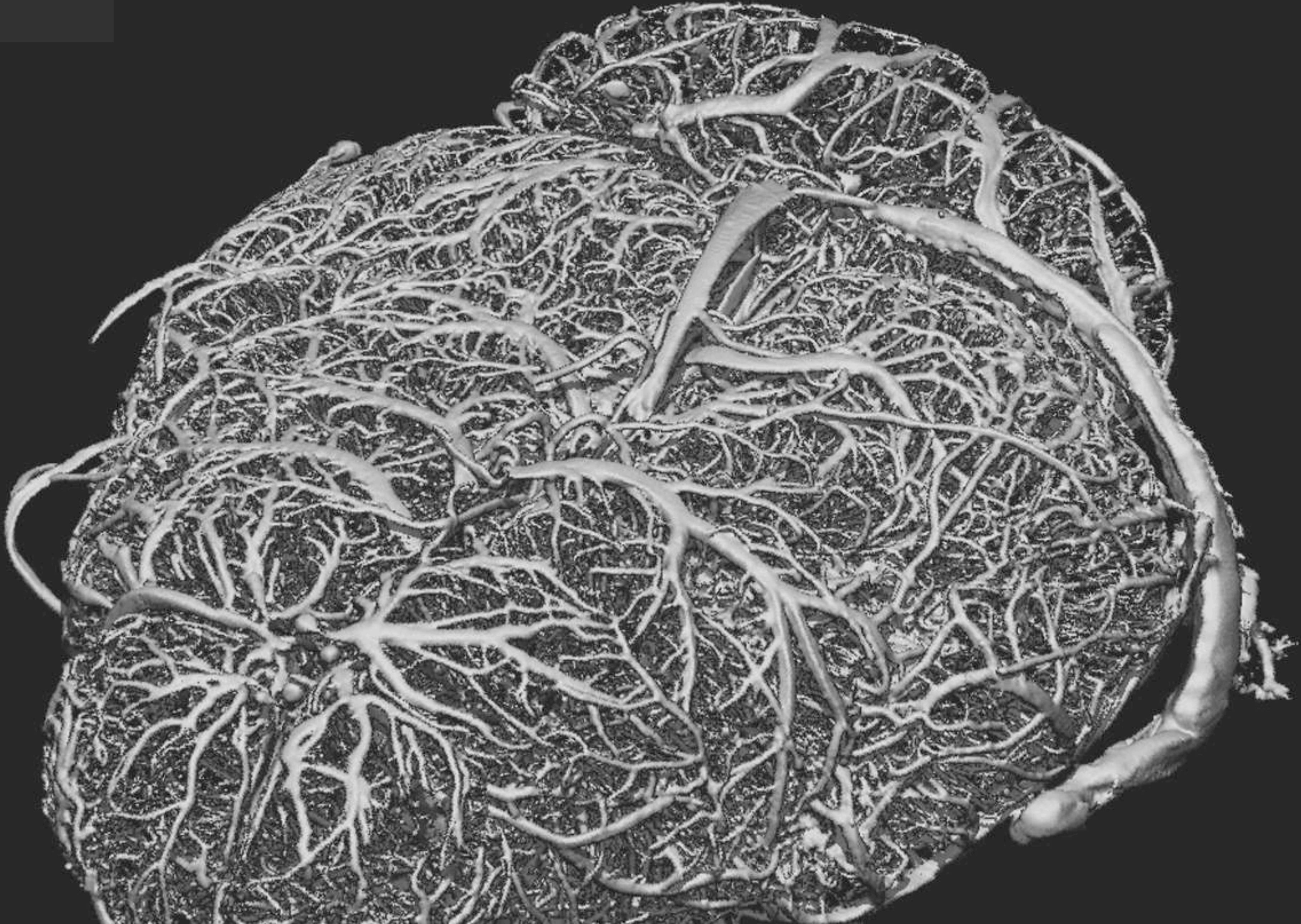
FOV: 22 x 22 -> 1 x 1 mm^2

Projections: 300–2000

Exposure times: 0.1–300 ms

Total scan time: 0.05 to 500 s

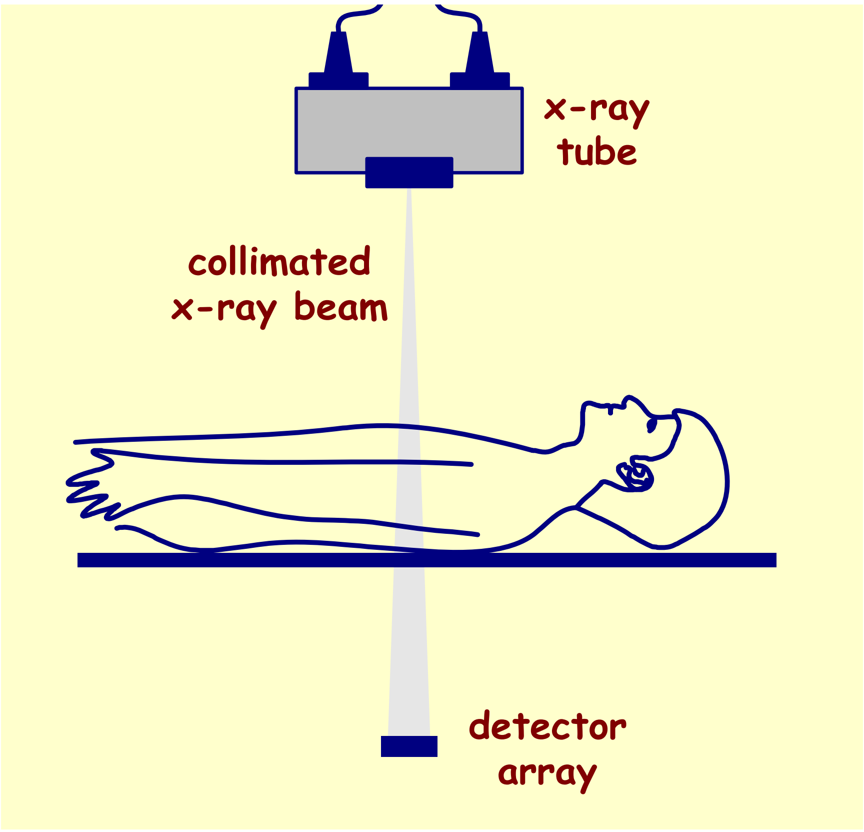
The MedMAX project

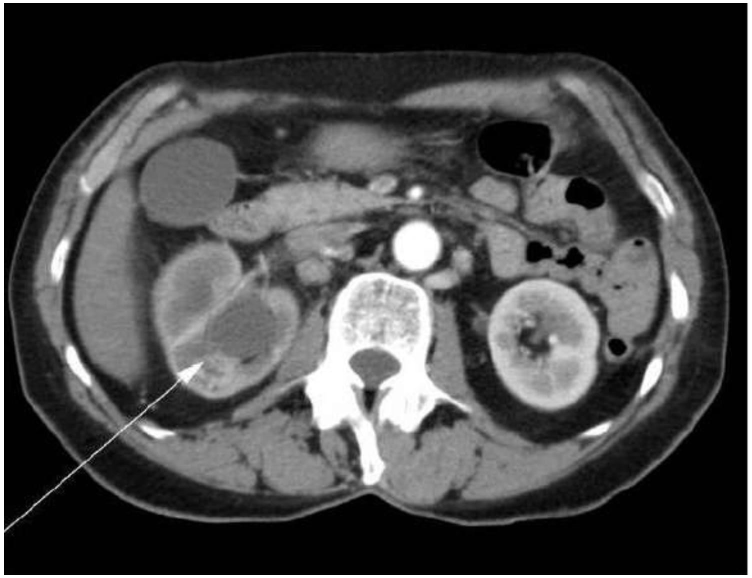


Medical imaging: CT

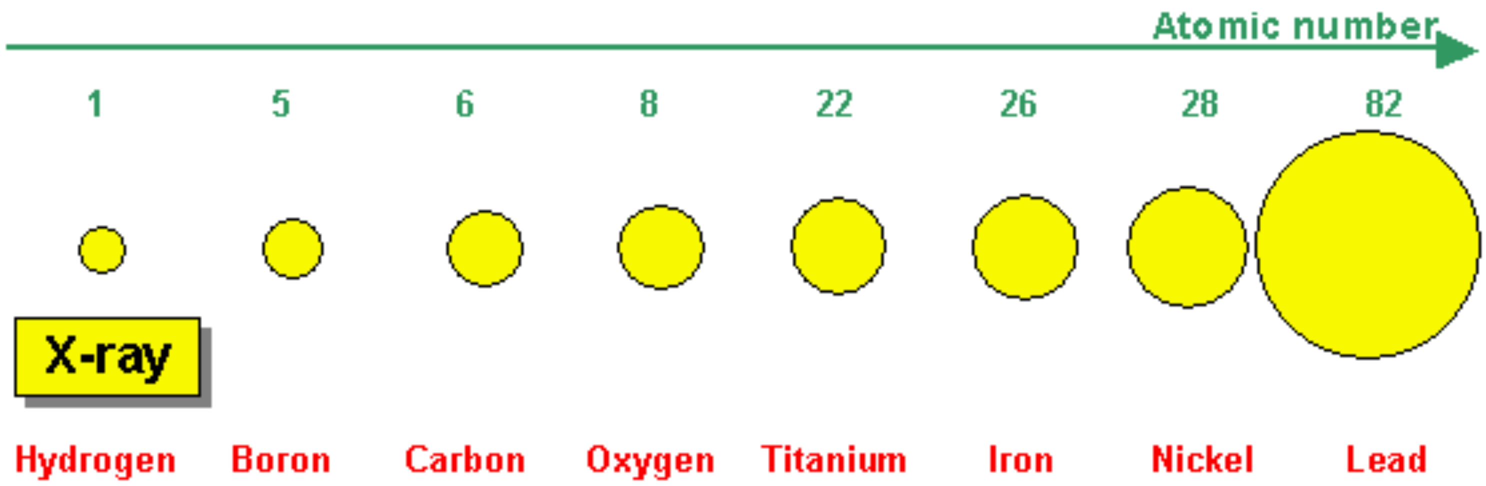
Resolution: 0.5 mm
Speed: seconds
+ high resolution
- Radiation dose

Radiation dose per CT scan ~ 3-5 mGy

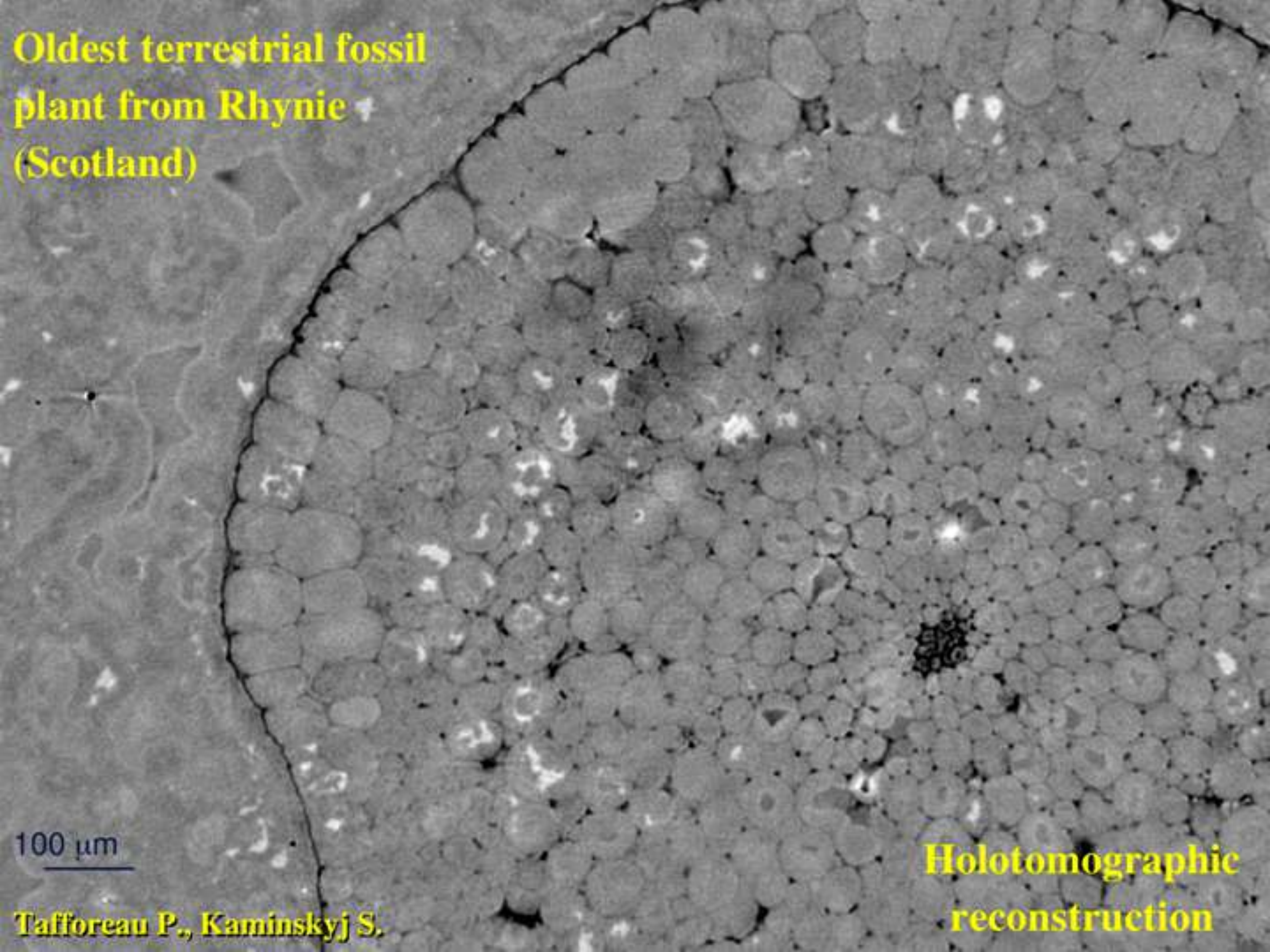




Probing matter with X-rays



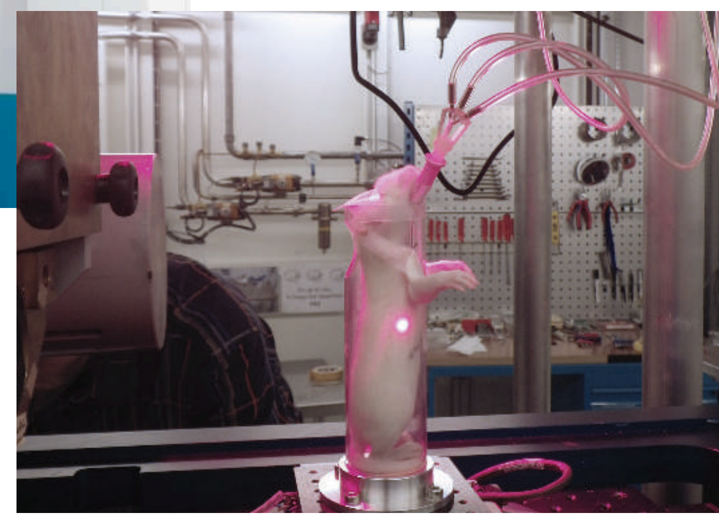
X-rays $$\sigma_{abs} \propto Z^4 \cdot (\hbar\omega)^{-3}$$


Oldest terrestrial fossil plant from Rhynie (Scotland)
100 µm
Holotomographic reconstruction
Tafforeau P., Kaminskyj S.

Medical and preclinical CT

The X-ray dose scales with resolution^3

Resolution:	300 µm -> 3 µm
Dose:	3 mGy -> 3 kGy



1 mm

50 µm

Spatial resolution

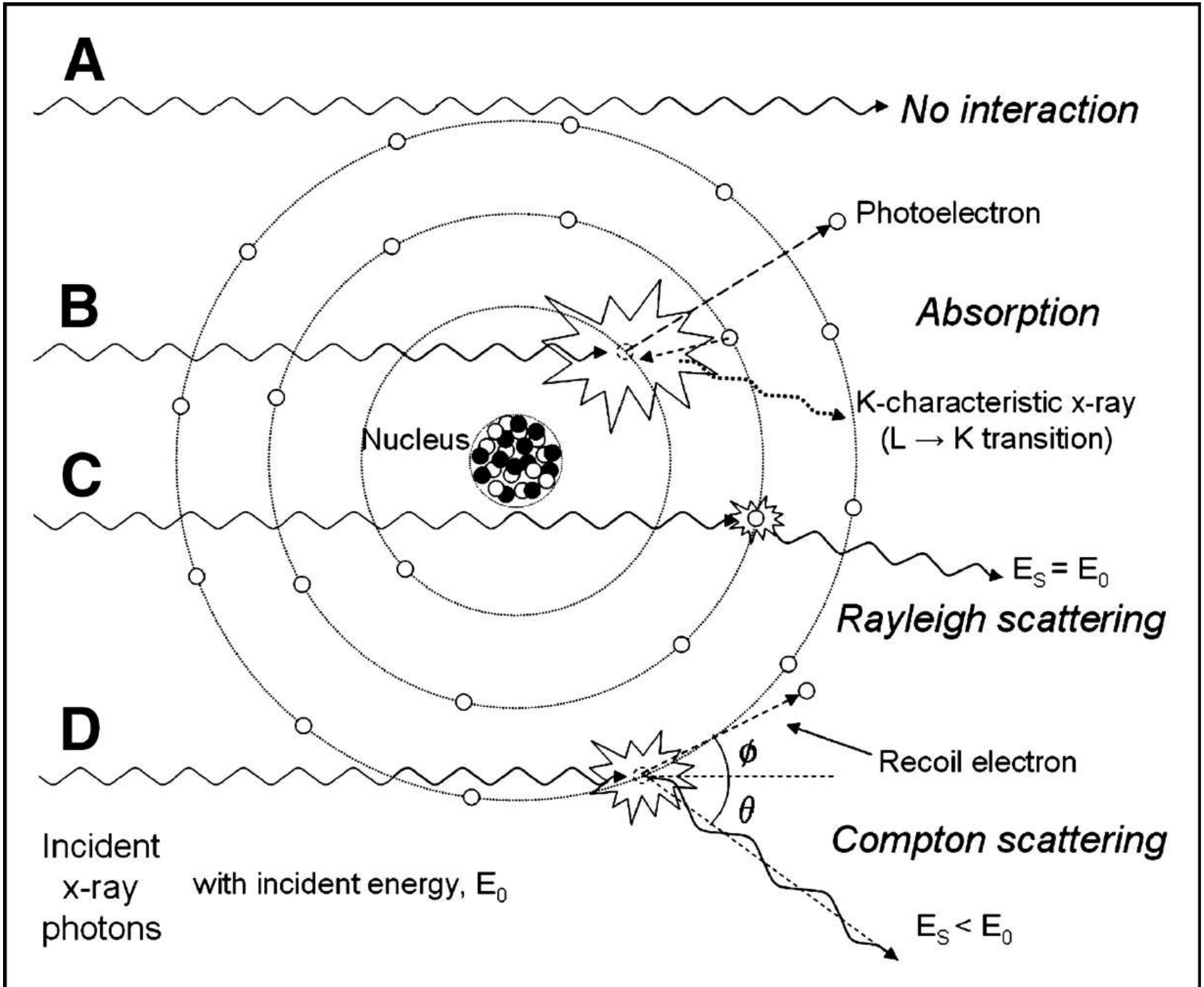
1 µm

Absorbed dose:

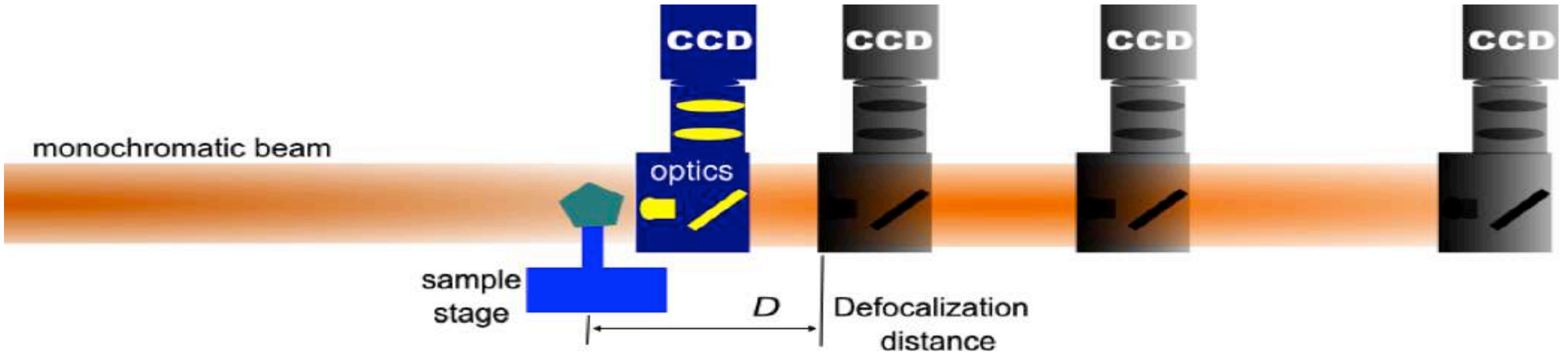
$$D = \frac{E_{\mathrm{abs}}}{m} = \frac{\Delta I h\nu}{V\varrho} = \frac{(I_0 - I_1)h\nu}{\varrho A \Delta x}$$

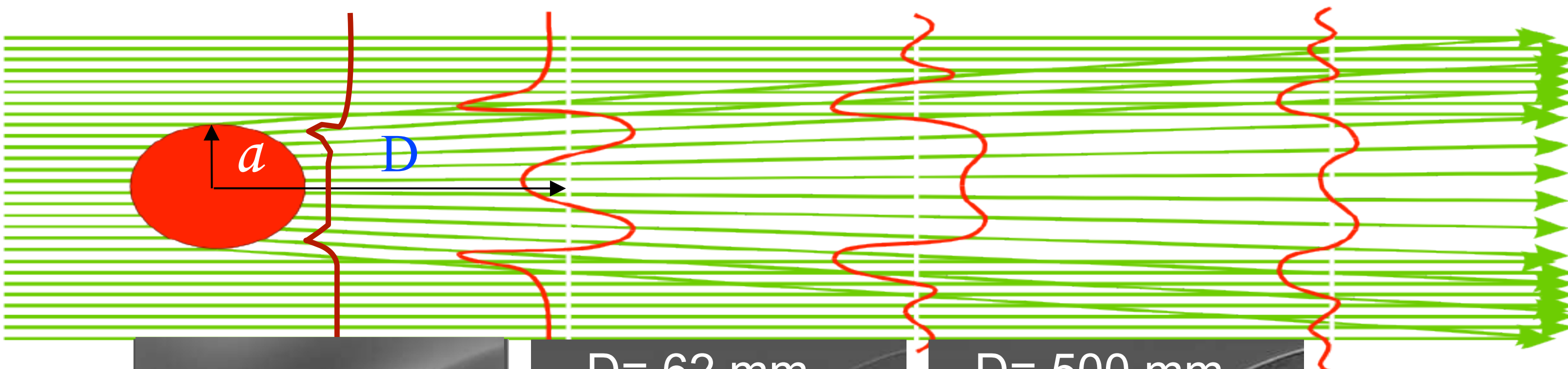
10 mGy Radiation dose 1 Gy 100 Gy

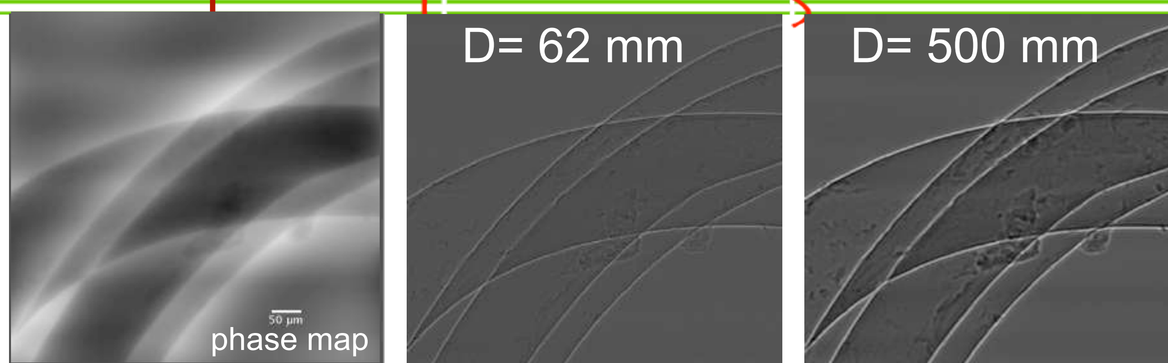
Absorption and scattering



Phase contrast in free space propagation







Three phase fibers

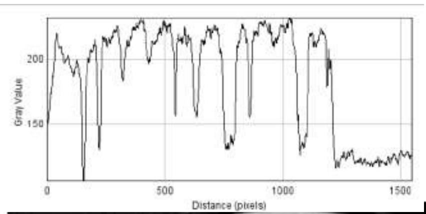
Fresnel diffraction
Partially coherent illumination

Cloetens et al. J. Phys. 1999

MAX IV

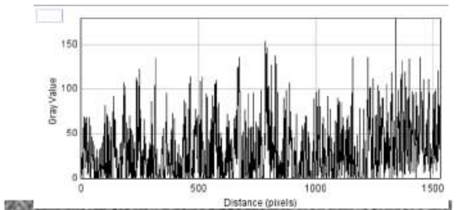
Phase tomography

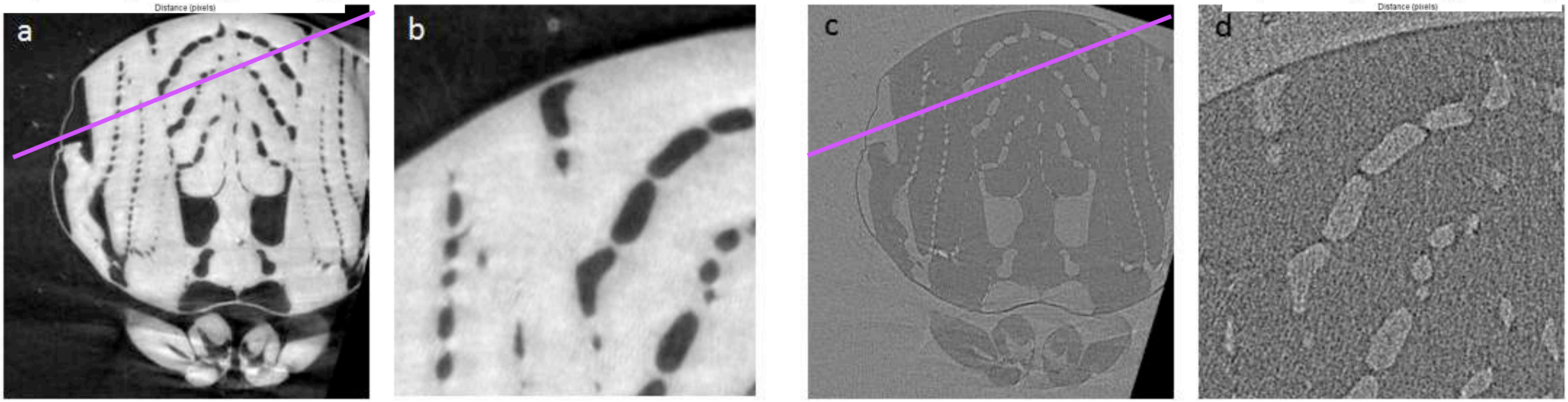
- Refractive index n $\boxed{n = 1 - \delta + i\beta}$ $\qquad \delta \gg \beta$



fast tomography
30% of detector dynamic range

flight muscles of a fly





phase map

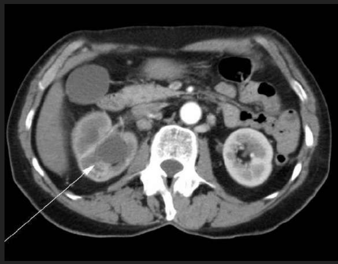
absorption & edge

Phase tomography

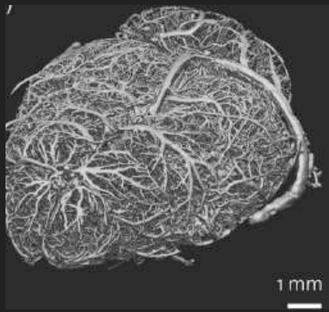
Phase contrast tomography

Mokso et al., J. Phys. D, 2013

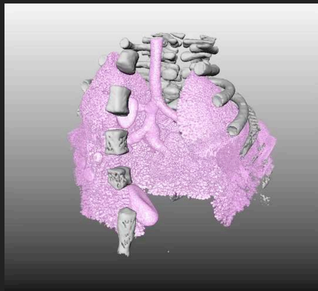
Outline



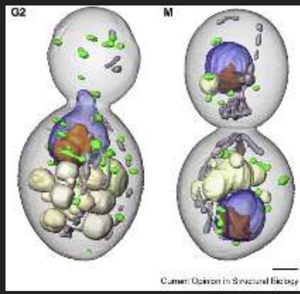
I. Existing and soon available instruments
   I. NanoMAX, SoftiMAX, DanMAX

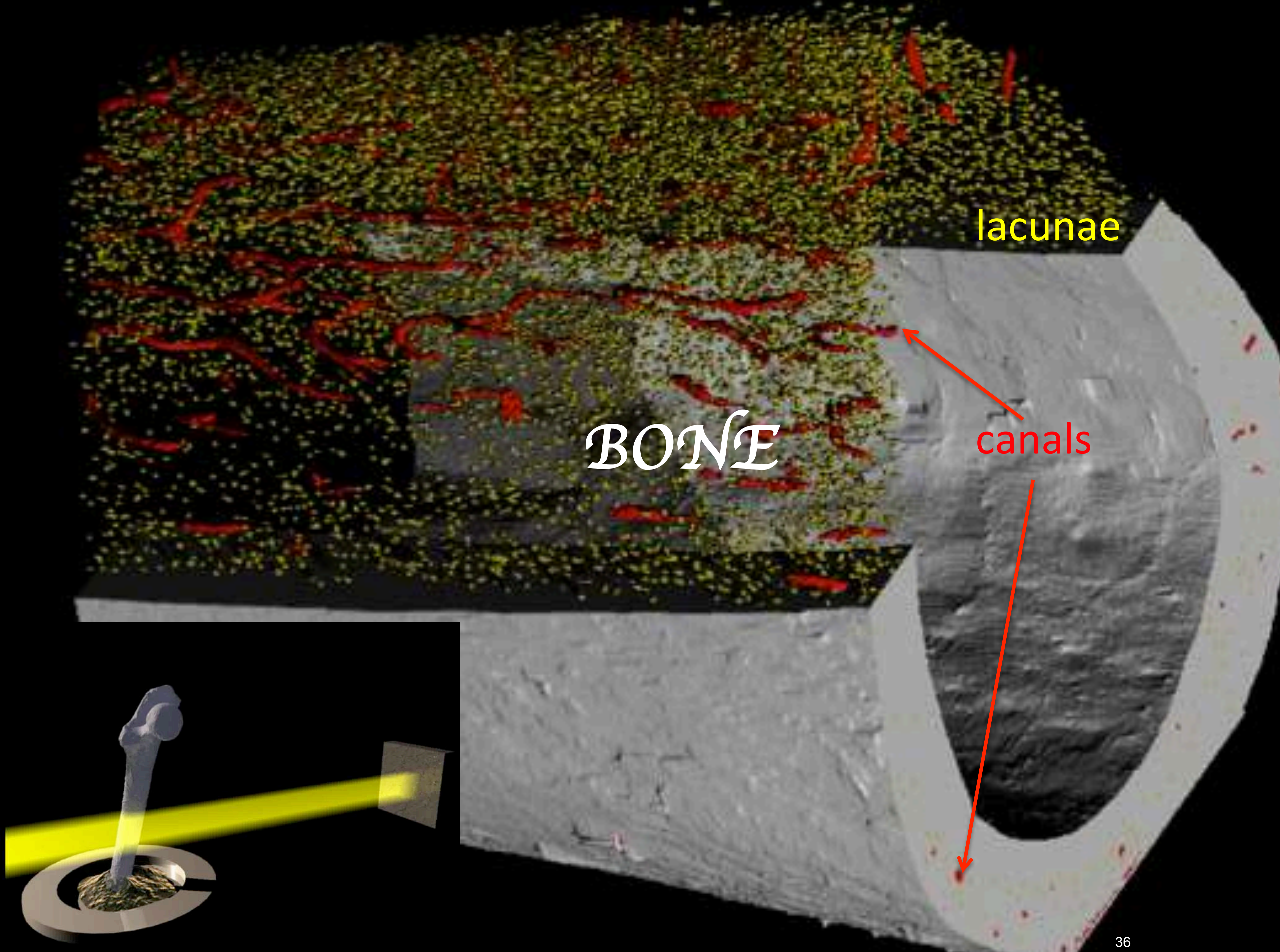


II. Biomedical tomographic microscopy
   I. The MedMAX project

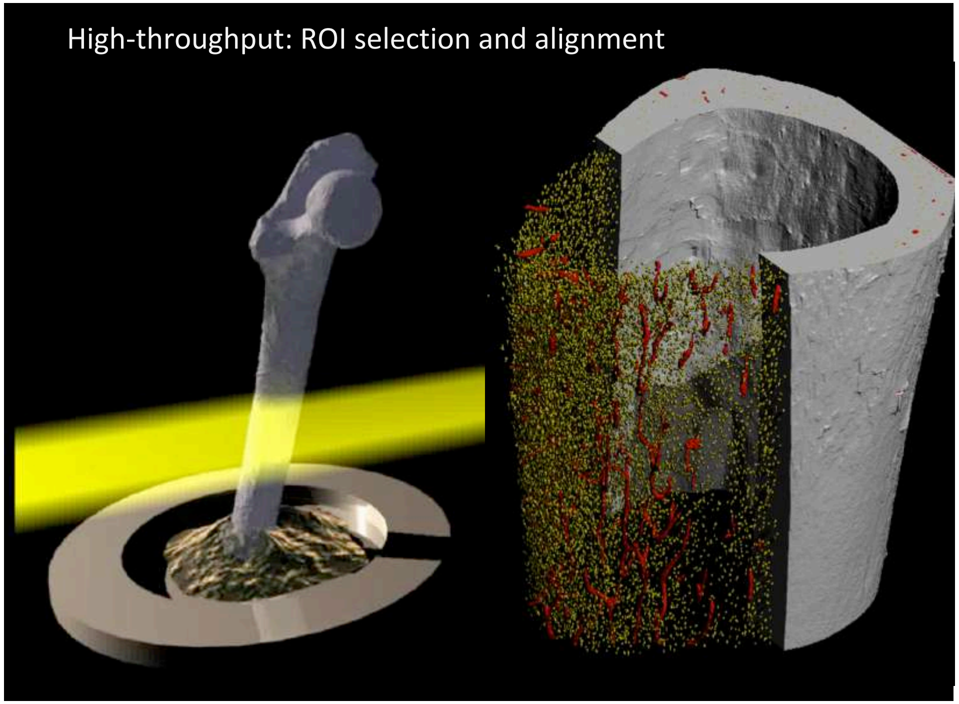


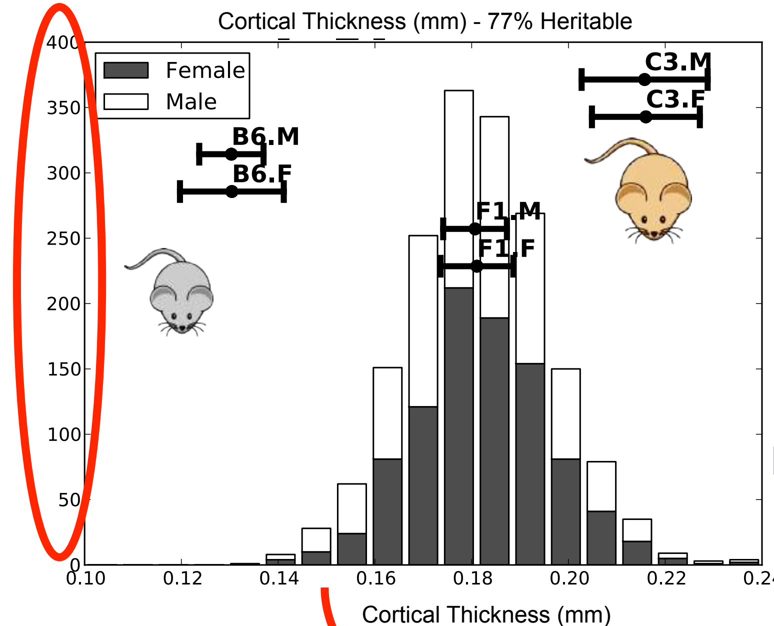
III. Examples
   I. In vivo small animals, various fixed tissues

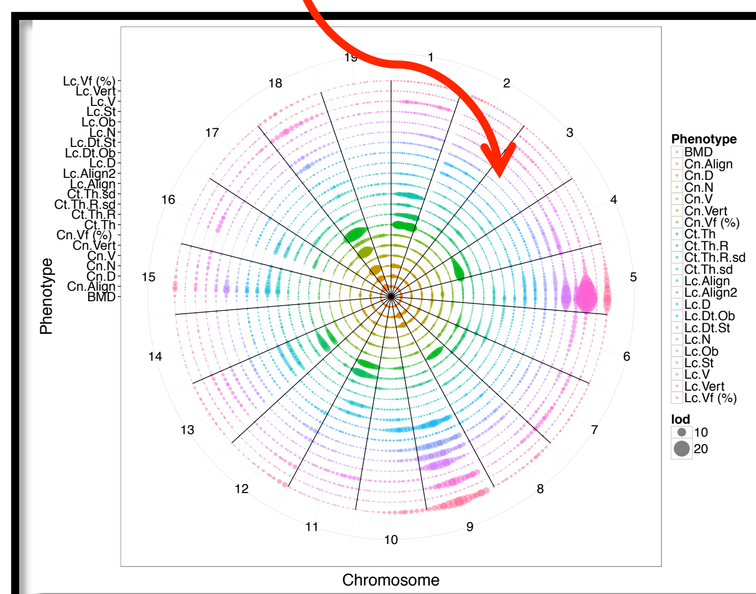



lacunae
BONE
canals

High-throughput imaging: from sample alignment to QTL analysis







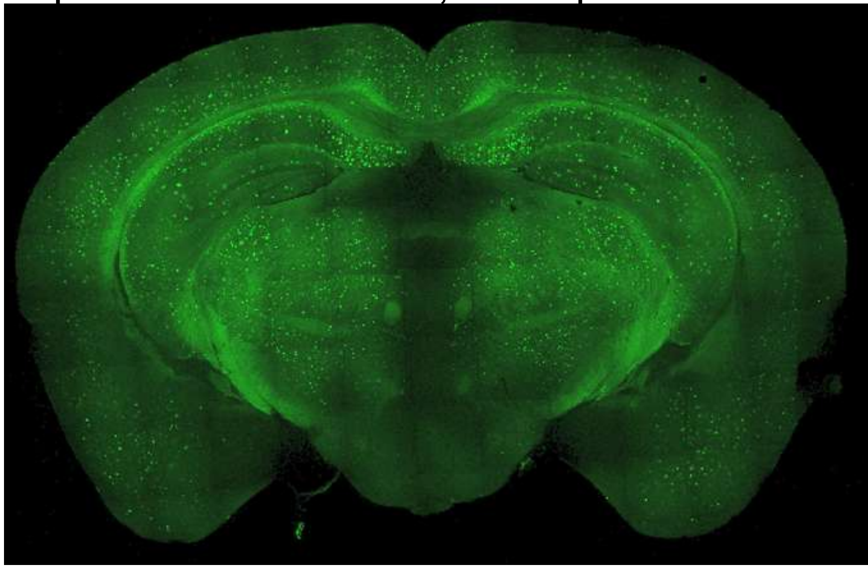
Femur samples automatically aligned using goniometer and moved to region of interest using projections and image processing scripts

K. Mader, PhD Thesis 2013 and K. Mader et al., BMC Genomics 2015

Alzheimer plaques

X-ray DPC scan, $(7.4\mu m)^3$ voxel size

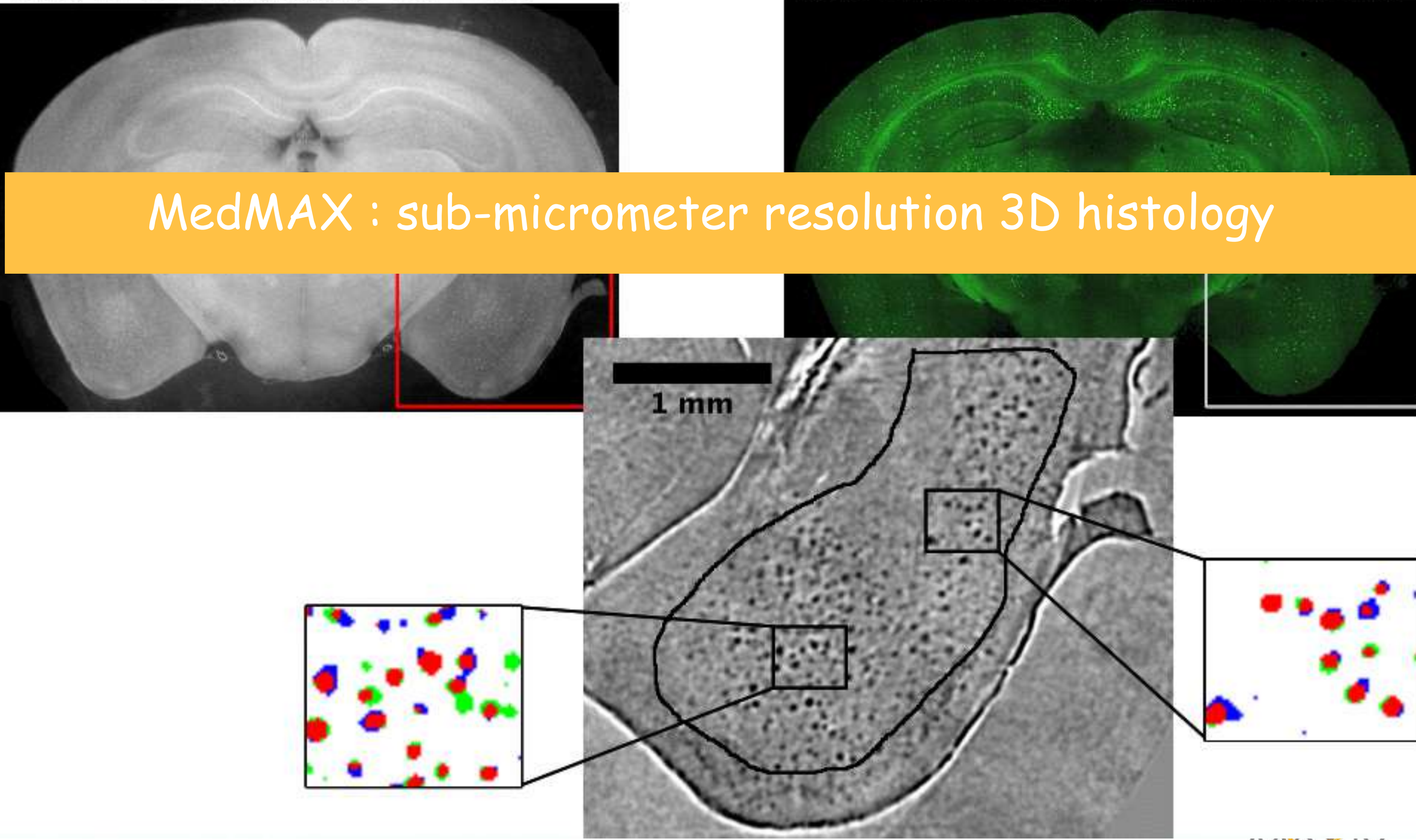
2-photon fluorescence, $2x2x4\mu m^3$ voxel size



Alzheimer plaques

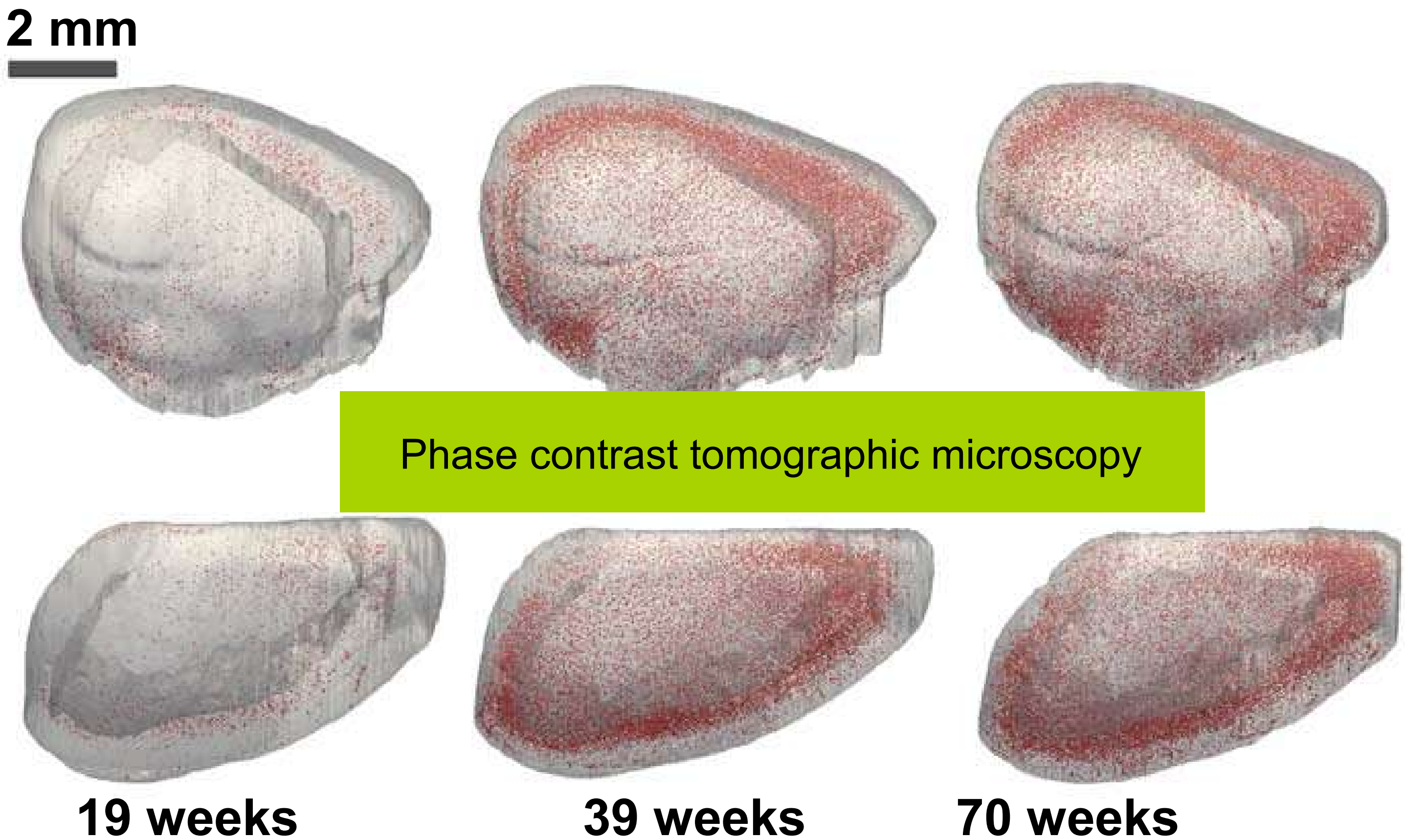
X-ray DPC scan, $(7.4\,\mu m)^3$ voxel size

2-photon fluorescence, $2x2x4\,\mu m^3$ voxel size



MedMAX : sub-micrometer resolution 3D histology

Alzheimer plaques



- Full 3D structural analysis is possible:
  - Size distribution
  - Plaque load (volumetric density)
  - Nearest neighbour distance (and much more)

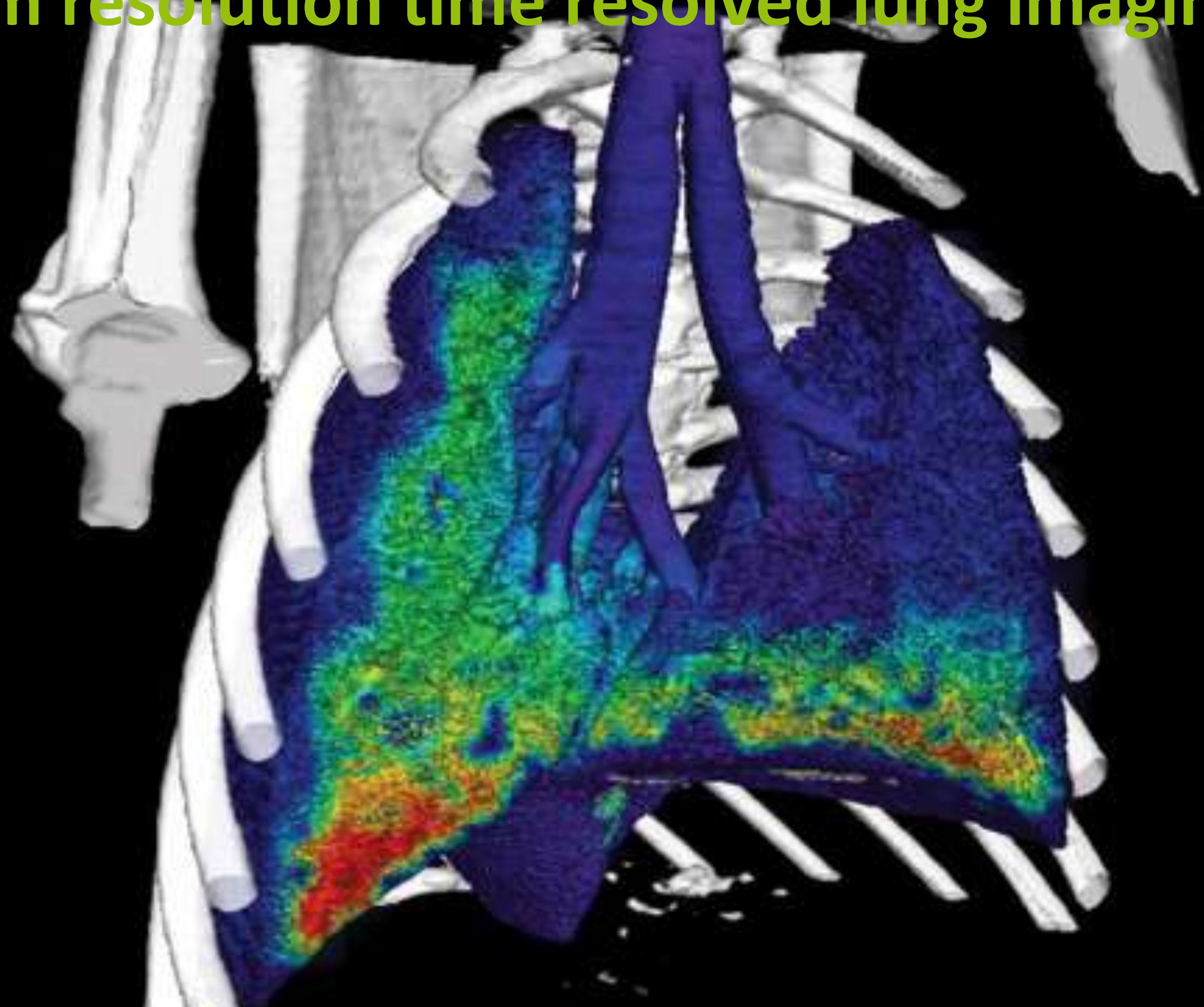
In-vivo lung imaging

exposure time= 1.1 ms
projections= 500
total scan time= 0.57 s
voxel size= 11 um
E= 20 keV

Rajmund.Mokso@maxiv.lu.se

R. Mokso et al AIP Conf. Proc. 1365 (2011)

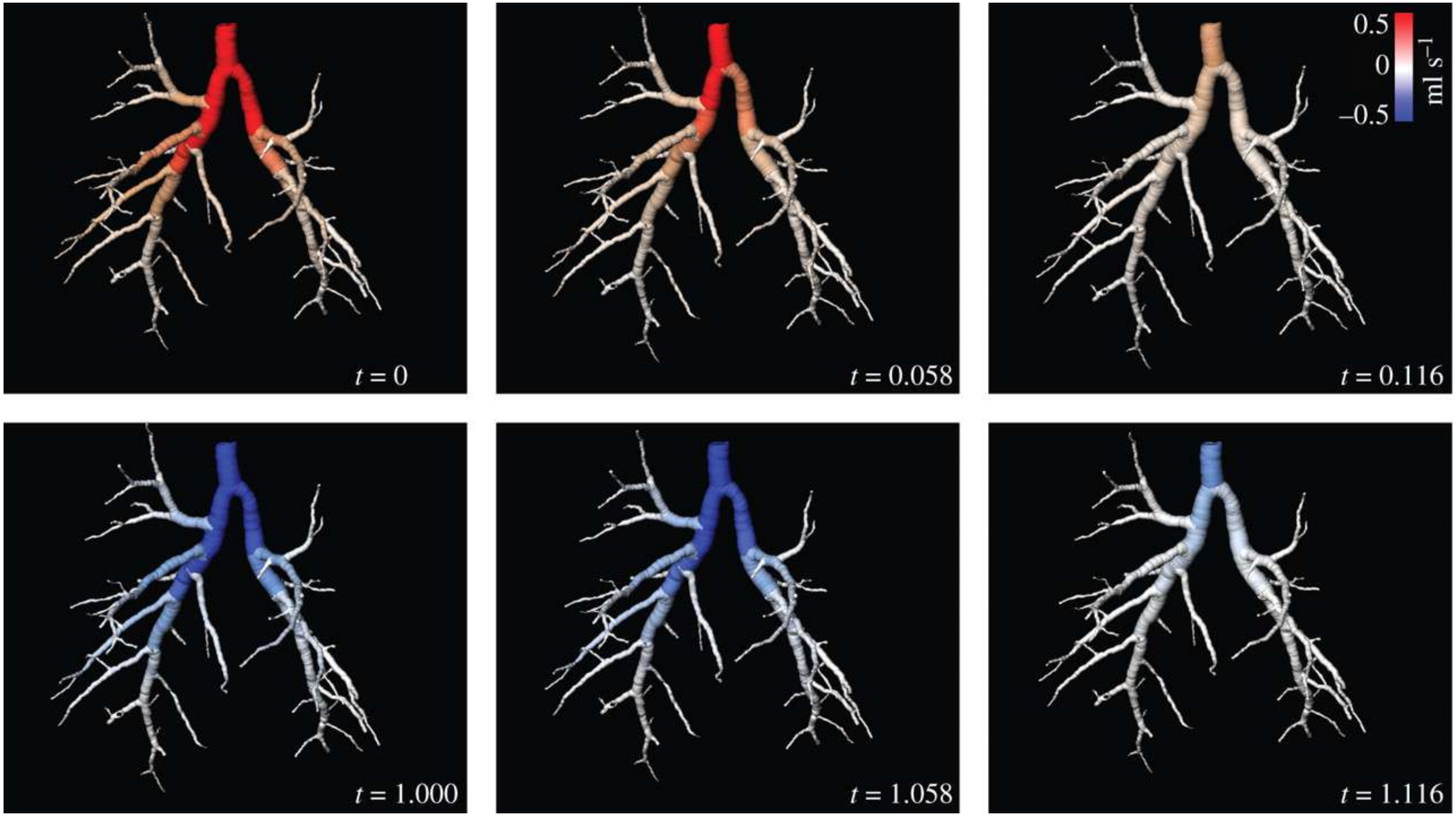
Medium resolution time resolved lung imaging



Three-dimensional representation of the total lung tissue displacement at the end-inspiration for a single time point (end-inspiration) of the four-dimensional dataset for mouse M1.

Stephen Dubsky et al. J. R. Soc. Interface 2012;9:2213-2224

Distribution of air flow through an airway tree in rabbit lungs

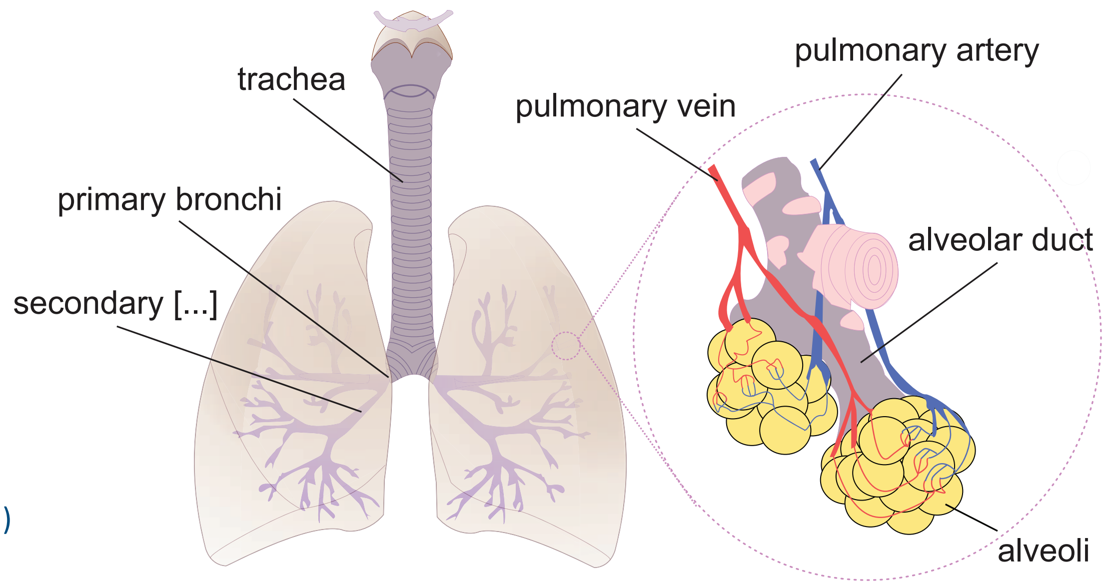


Stephen Dubsky et al. J. R. Soc. Interface 2012;9:2213-2224

Dynamic imaging of lungs at the micrometer scale: motivation

Ventilator-induced lung injury (VILI)

- Overextension of lung tissue in certain lung regions (mechanical damage, biotrauma)
- Still unclear how ventilation induces its deleterious effect [4]
- Hypothesis: local strains in the alveolar wall cause hotspots (overstretching regions)



▸ Human lungs (Source: Wikipedia)

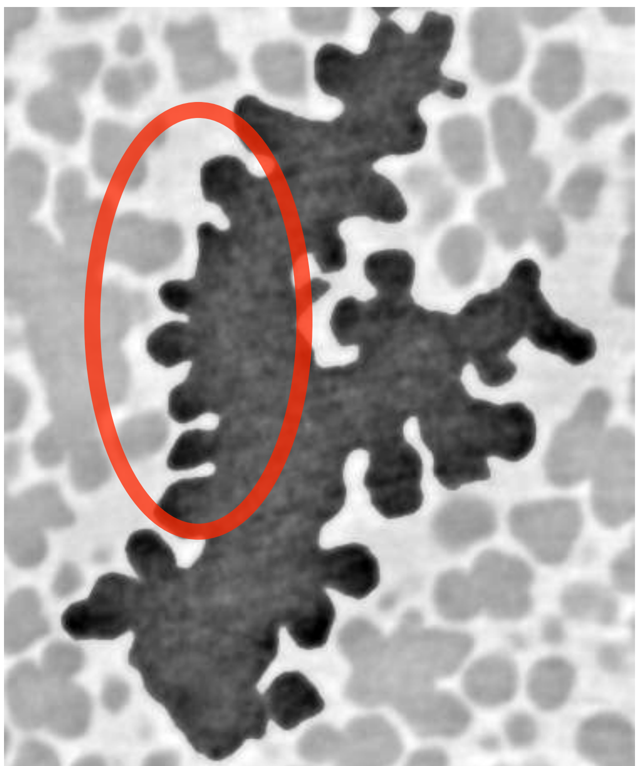
[4] Rausch, S. M. K., Haberthür, D., Stampanoni *et al.*, Ann Biomed Eng **39** (11), 2835 (2011).

Lung tissue quantification

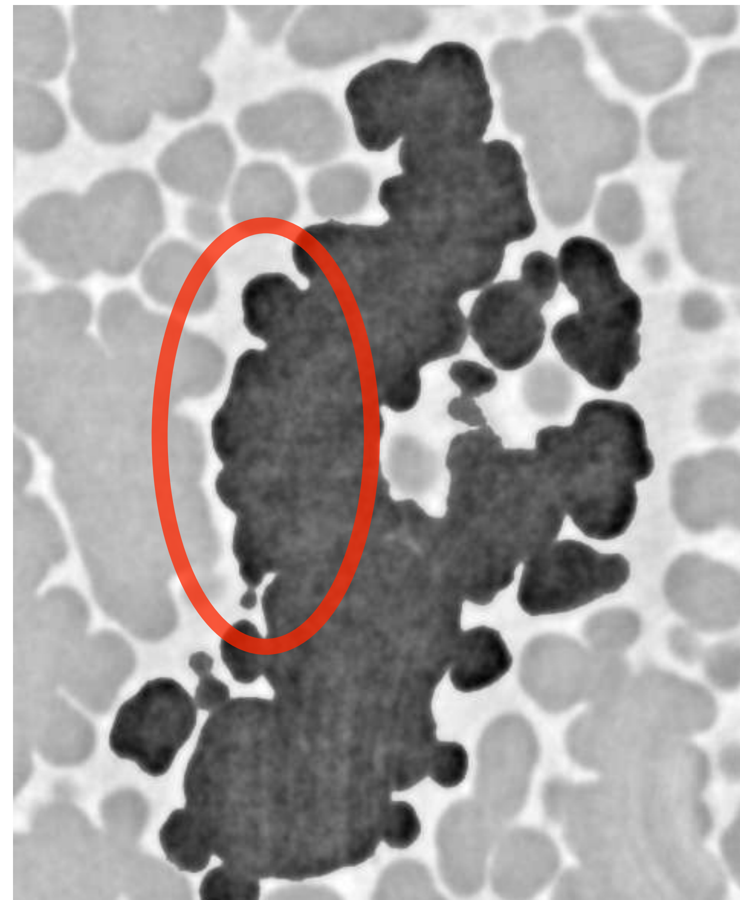
Challenges in lung tissue analysis

- How to detect non-linear and regional changes in the lung?
- How to quantify them?

G. Lovric , PhD thesis



$$\Delta p = 5\,\mathrm{cmH_2O}$$

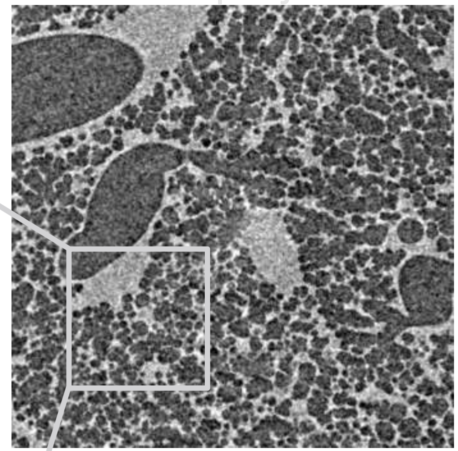


G. Lovric et al., J. Appl. Cryst. 46 (4) 2013

Image quality vs. radiation damage

2.9 µm pixel-size optics

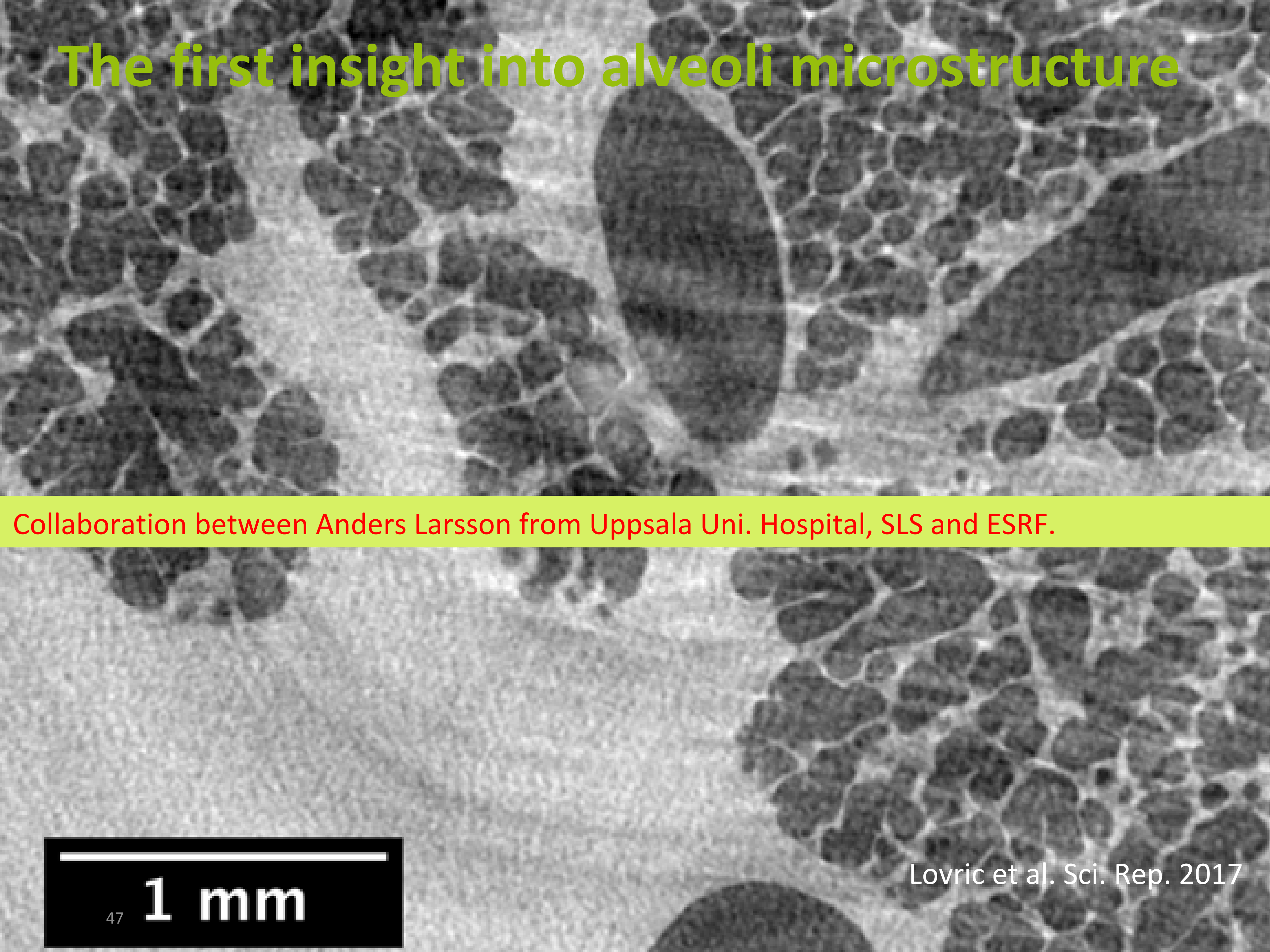
901 projections | 901 projections | 901 projections | 361 projections



	901 projections	361 projections
Scan time	0.24 s	0.17 s
CNR	2.3	1.5
Entrance dose	12 Gy	9 Gy

Rajmund.Mokso@maxiv.lu.se

The first insight into alveoli microstructure



Collaboration between Anders Larsson from Uppsala Uni. Hospital, SLS and ESRF.

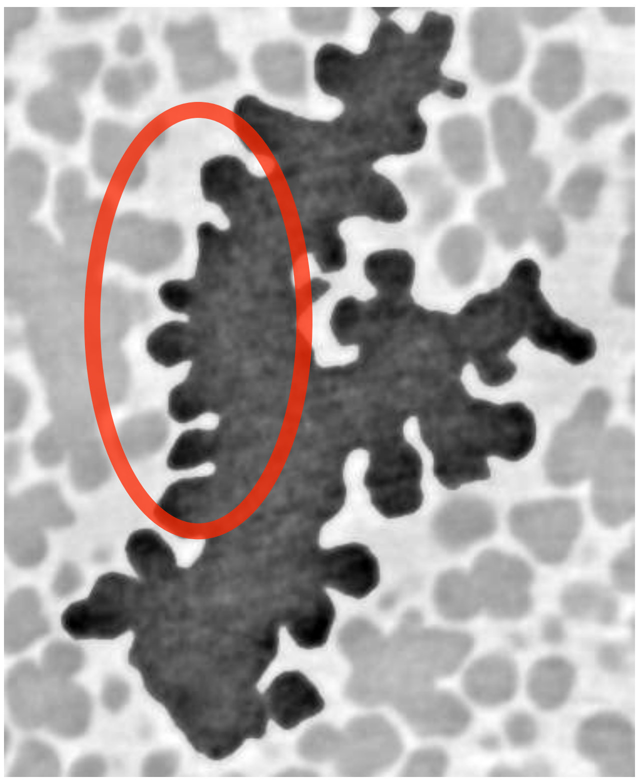
Lovric et al. Sci. Rep. 2017

Lung tissue quantification

Challenges in lung tissue analysis

- How to detect non-linear and regional changes in the lung?
- How to quantify them?

G. Lovric , PhD thesis

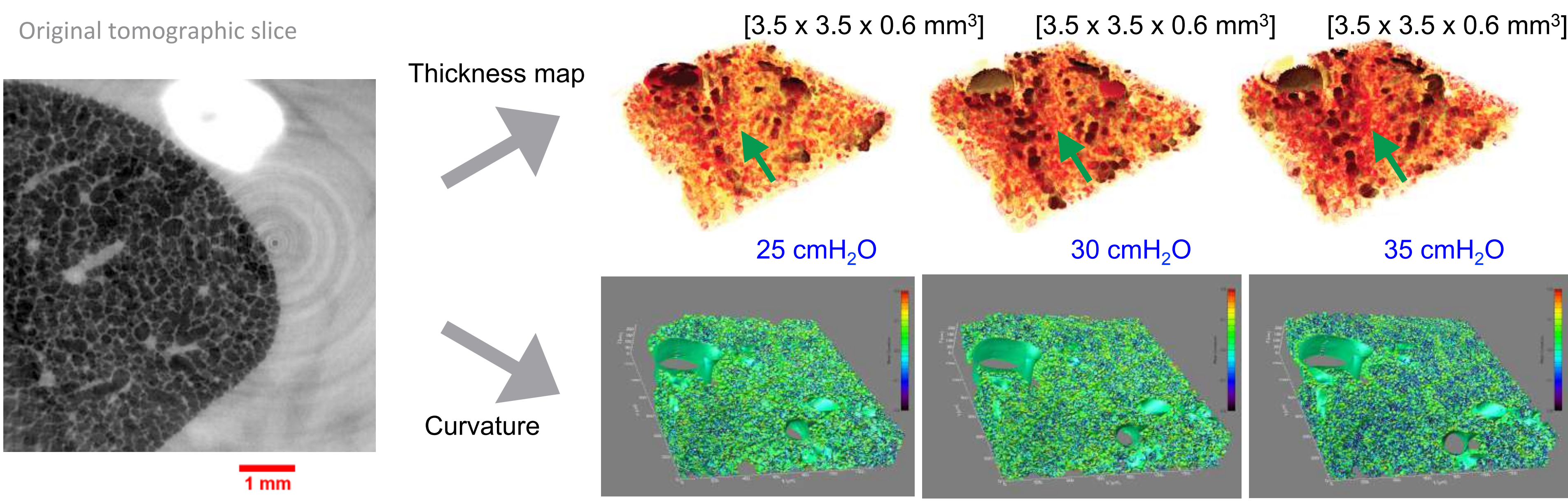


$$\Delta p = 5\,\mathrm{cmH_2O}$$

Alveoli dynamics quantification

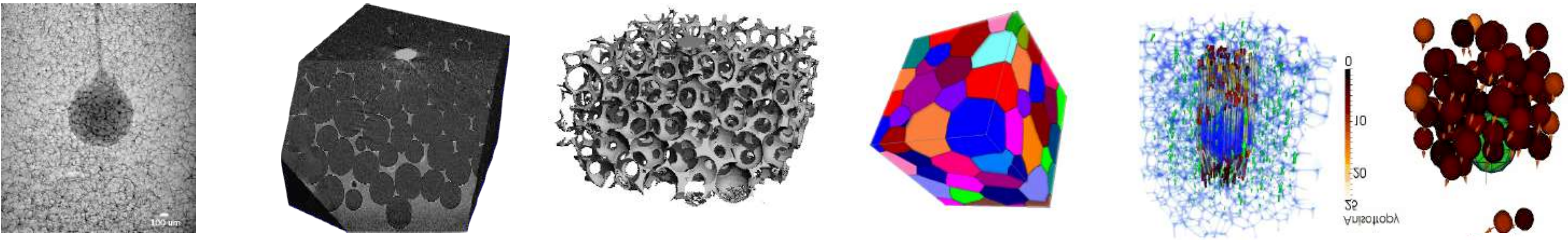
A detailed insight into the lung

- Apply *quantification* and *labeling* tools, previously used for foam and bone data [14] and dendritic microstructures [15]



G. Lovric , PhD thesis, Lovric et al. PLOS On2017e

Image-based quantitative information retrieval



acquisition

Scanning
Sample conditioning

reconstruction

segmentation

feedback

labeling

quantification

visualization

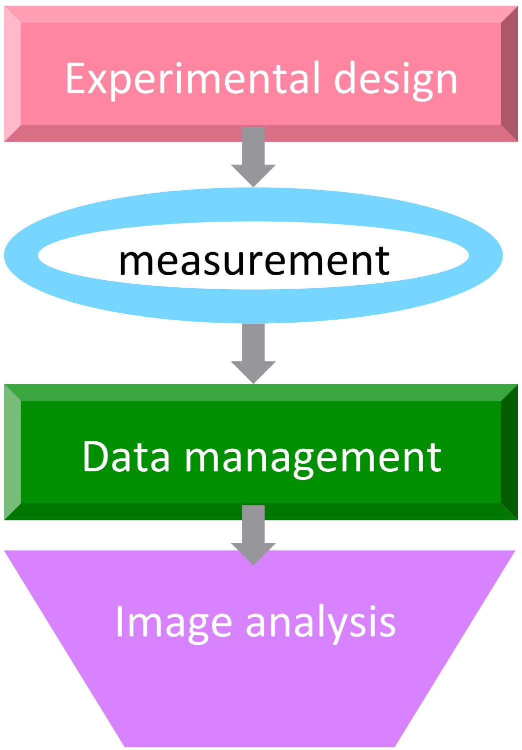
1TB / 2 min

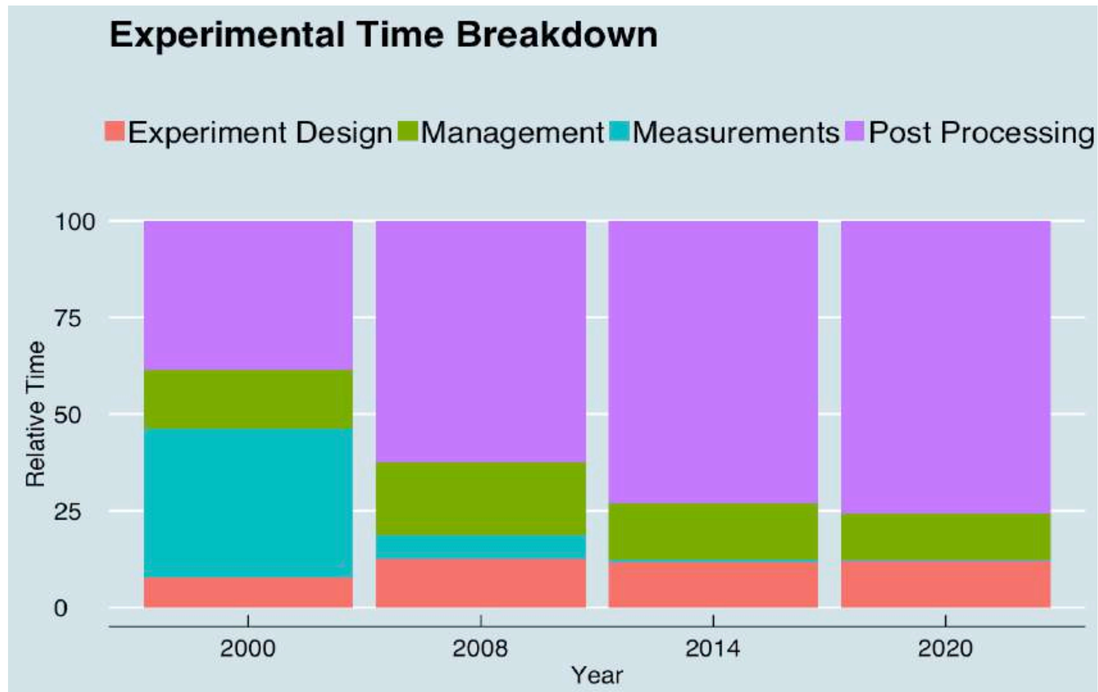
robust, simple & universal

Customized & flexible

Rajmund.Mokso@maxiv.lu.se

High throughput quantitative imaging



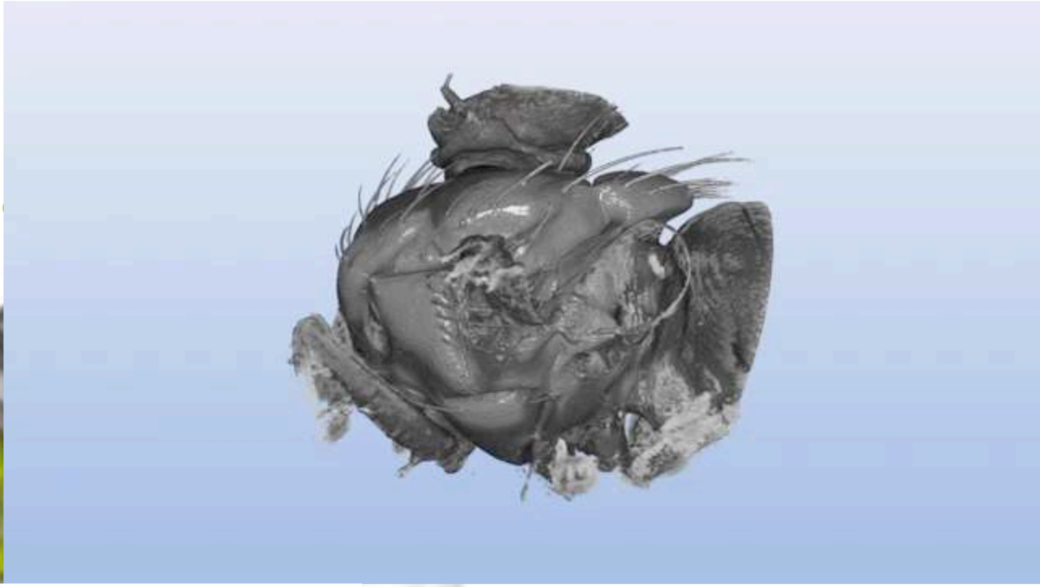


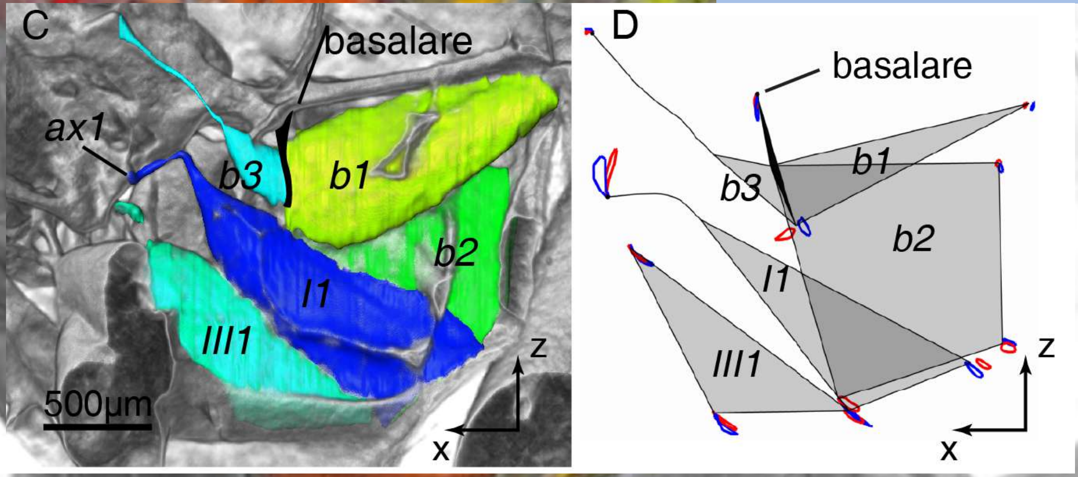
Curtesy of Kevin Mader

1TB / 2 min

Survival ~ 3 s

t of insec





3 kHz effective temporal resolution
10-20 µm spatial resolution

Daniel Schwyn
Imperial College London

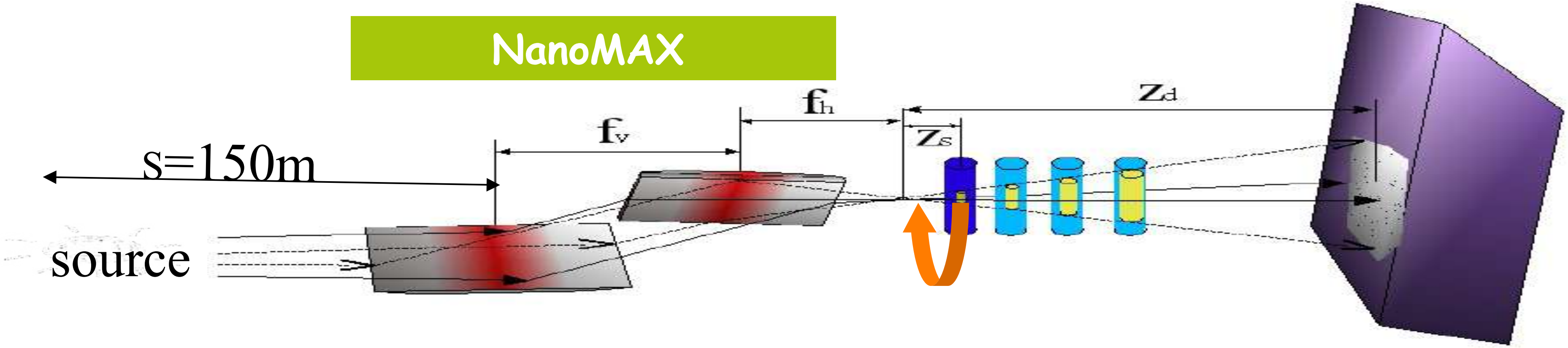
Rajmund Mokso
TOMCAT

Simon Walker
UNIVERSITY OF OXFORD

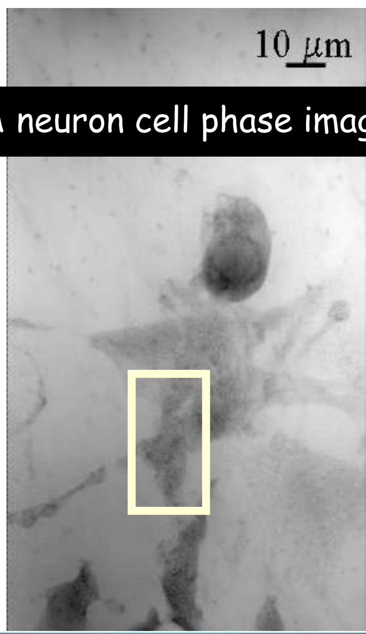
Rajmund.Mokso@maxiv.lu.se

Nanoimaging, Fluorescence spectroscopy

NanoMAX

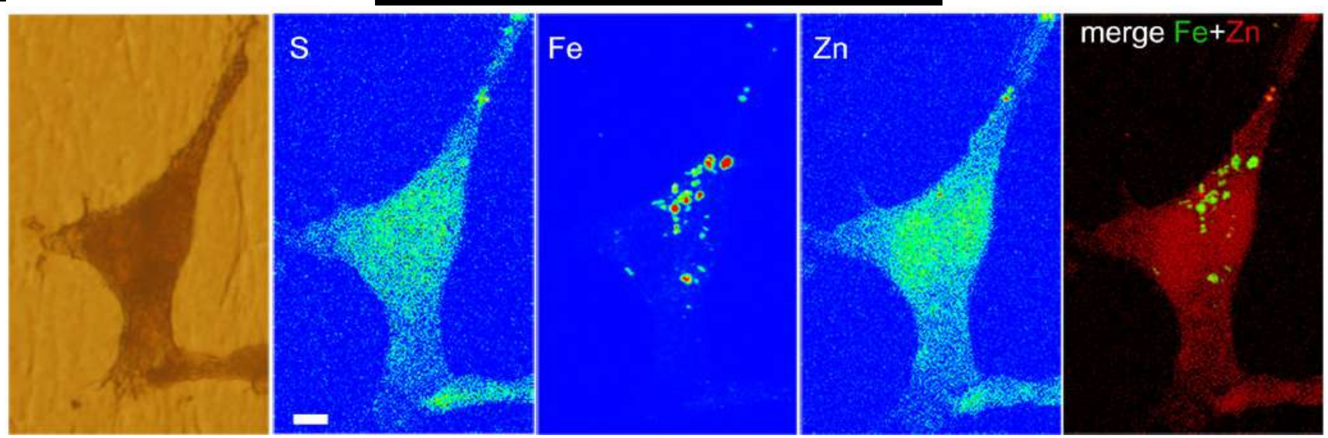


A neuron cell phase image



Mokso, Cloetens et al. , APL 2007

Chemical element distribution



Ortega. , Mol. Neurobiol. 2016

Holographic nanotomography of human cerebellum

FULL PAPER
Nanotomography
ADVANCED SCIENCE
www.advancedscience.cor

Hard X-Ray Nanoholotomography: Large-Scale, Label-Free, 3D Neuroimaging beyond Optical Limit

*Anna Khimchenko, Christos Bikis, Alexandra Pacureanu, Simone E. Hieber, Peter Thalmann, Hans Deyhle, Gabriel Schweighauser, Jürgen Hench, Stephan Frank, Magdalena Müller-Gerbl, Georg Schulz, Peter Cloetens, and Bert Müller**

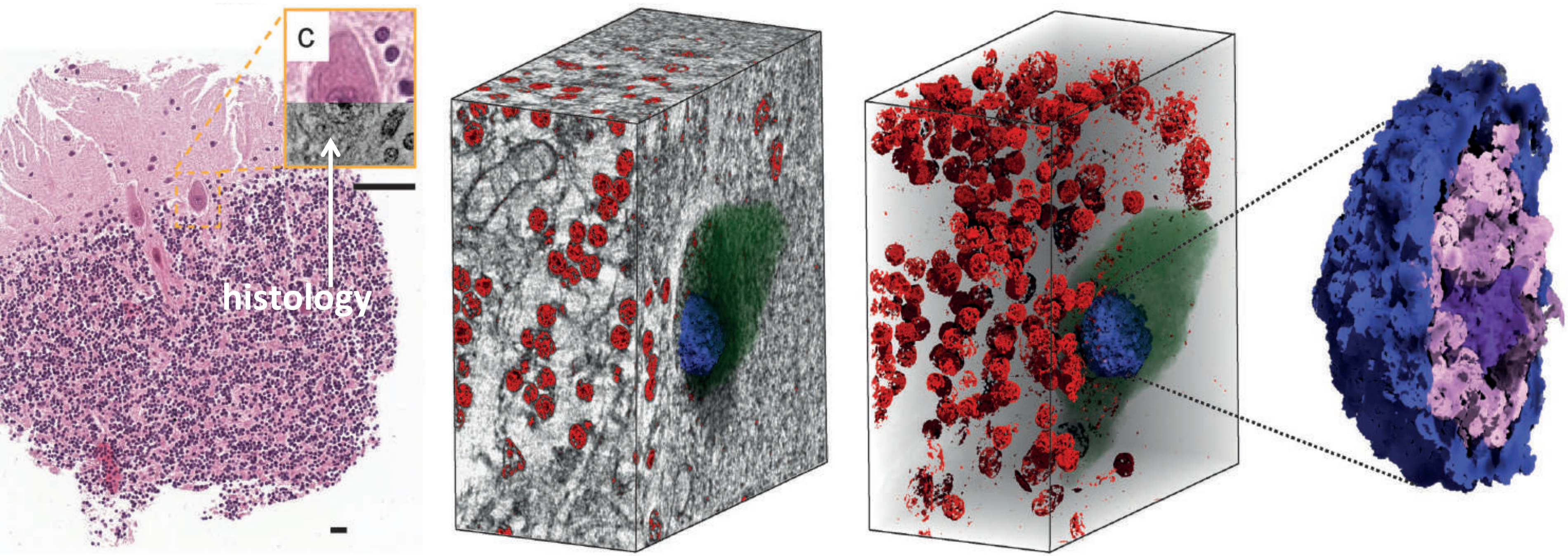
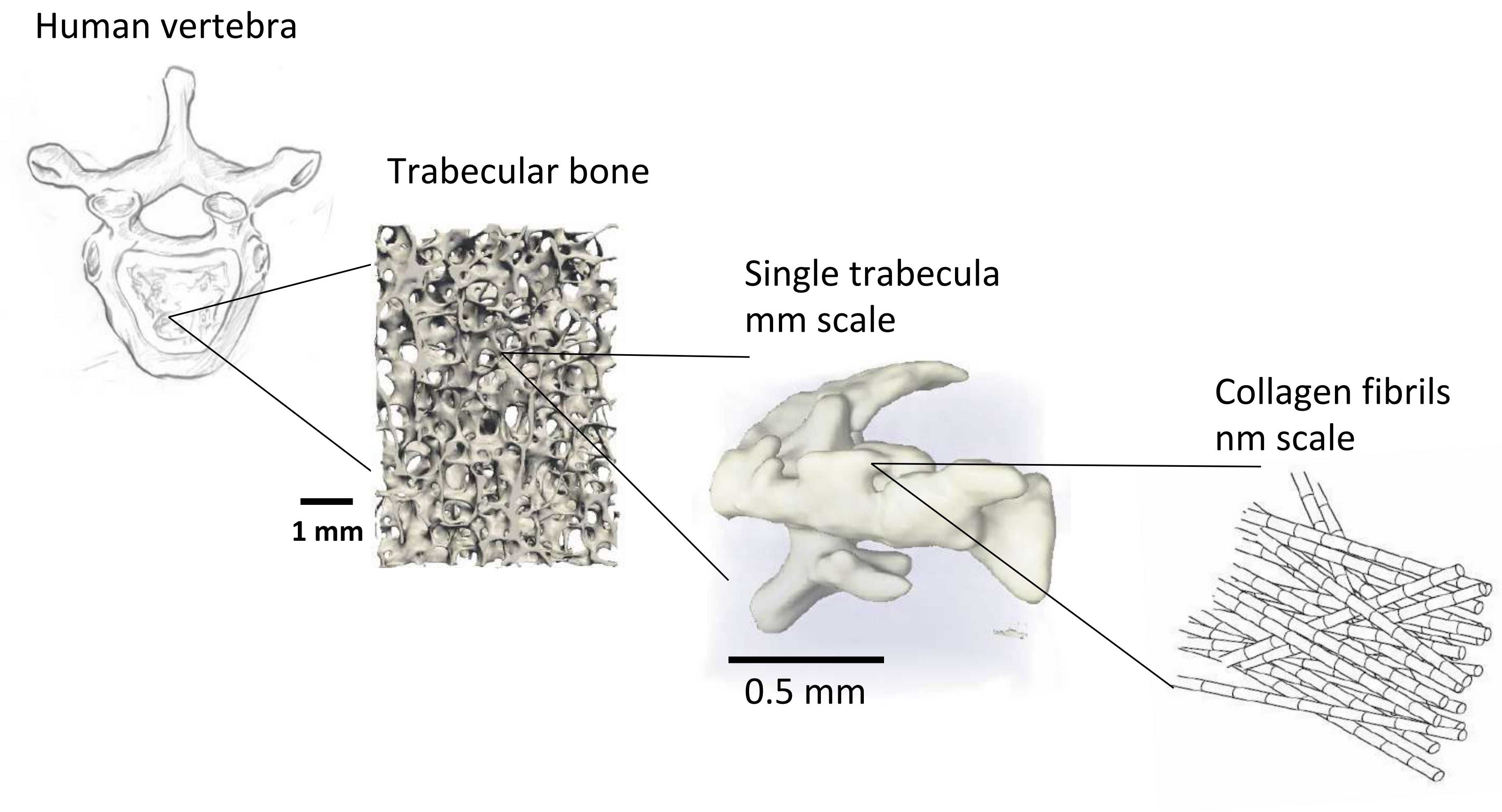
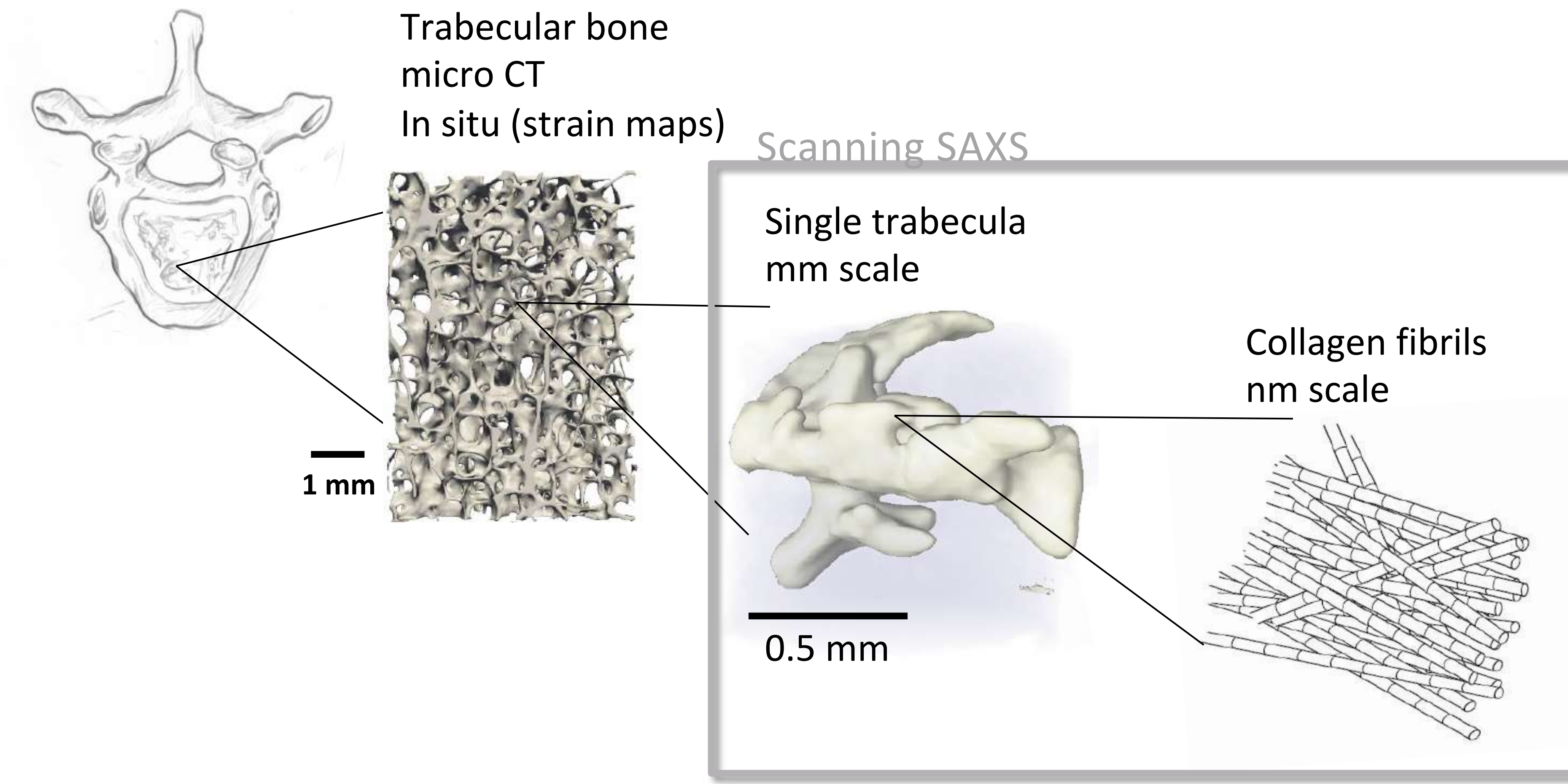


Figure 5. A 3D rendering of (sub)cellular structures within a Purkinje cell, measured with an effective pixel size of 100 nm. Region-growing segmentation allows for the extraction of cell soma (green), nuclear envelope (blue), nuclear content (pink), and nucleolus (violet). Intensity thresholding (red) enables the discrimination of granule cell nuclei. Average diameter of Purkinje cell soma: 54 µm; average diameter of Purkinje cell nucleolus 3.5 µm.

Bone nanostructure (Small Angle X-ray scattering)



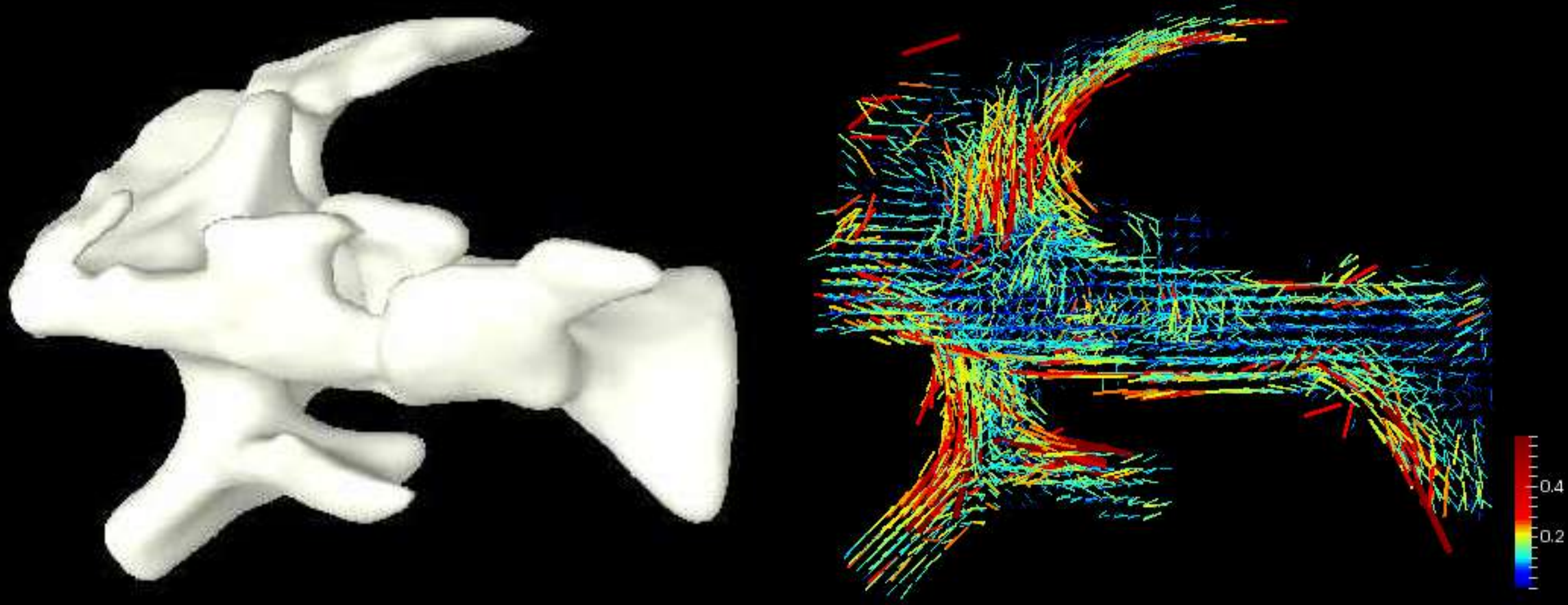
Bone nanostructure (Small Angle X-ray scattering)



vertebra drawing Paloma Ayala

Bone nanostructure (SAXS tensor tomography)

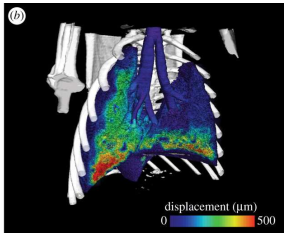
CoSAX : in operation 2019
ForMAX: project phase 2017-2021



Liebi et al. Nature 2016

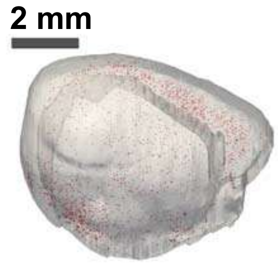
Hierarchical imaging of tissues

Medical imaging and therapy
(in vivo, medium resolution >30 um, low dose, large beam , large FOV)

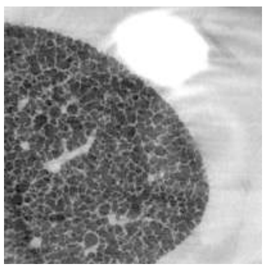


Longitudinal in vivo

The whole organ

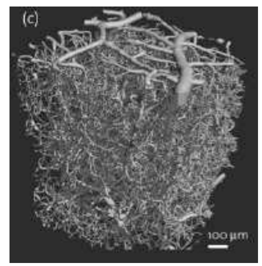


Down to a micrometer resolution
(rarely in vivo, high resolution ~ 1um, higher dose, small FOV)



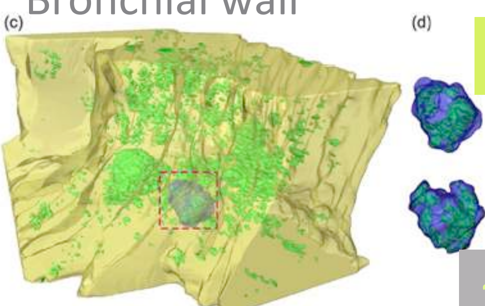
Accute in vivo

Zooming into organs



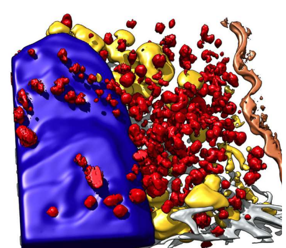
Nano-scale imaging beamlines
(fixed tissue, resolution ~ 100nm, high dose, small samples ~100um)

Bronchial wall



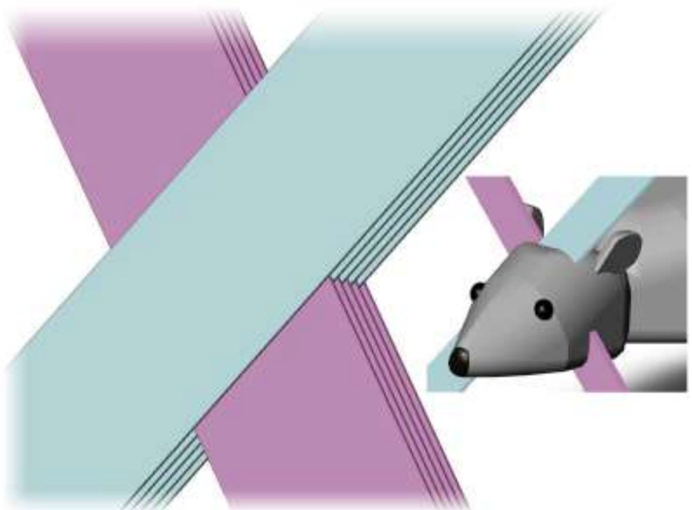
Fixed tissue

Cell level



3 orders of magnitude in spatial resolution

Microbeam radiation therapy



Microbeam irradiated normal CNS of weaned piglets (1.5cm x 1.5cm ~28 mm-wide beams ~210 mm on center, 625 Gy). The histological sections look normal, except for "stripes" due to the dropout of neuronal/astroglial nuclei. This sharp spatial fractionation is preserved throughout the cerebellum. No tissue necrosis, hemorrhage or demyelination was observed.

E. Bräuer-Krisch, et al. New irradiation geometry for microbeam radiation therapy, Phys Med Biol 50 (2005) 3103-3111.


Thank you