



Elettra Sincrotrone Trieste

Biomedical imaging with Synchrotron Radiation

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XVIII School on Synchrotron Radiation: Fundamentals, Methods and Applications

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Outline

- Advantages of using SR for biomedical imaging
- SR X-rays imaging techniques
 - K-edge subtraction imaging
 - Phase sensitive* techniques
- Relevant applications and achievements
- The new imaging beamline @Elettra 2.0: layout and research perspectives

Advantages of SR for biomedical imaging

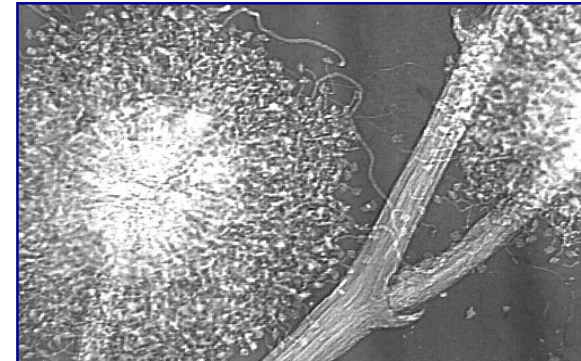
Monochromaticity allows for:

- *optimization* of X-ray energy according to the specific case under study (dose reduction)
- quantitative CT evaluations
- no beam hardening
- convenient use of contrast agent (**K-edge subtraction imaging**)

Spatial coherence

- Phase contrast overcomes the limitation of conventional radiology (it enables the applications of **phase contrast techniques**)
- It brings to a **dose reduction**
- Improved contrast resolution, edges enhancement
- Use of phase retrieval algorithms to separate phase from absorption contribution

PHC image



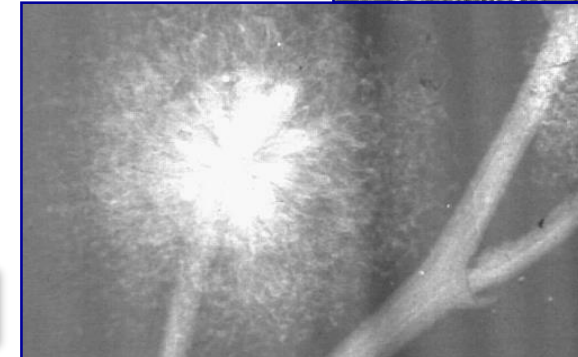
High fluxes

- Short exposure time
- Dynamic studies....

Collimation

- parallel beams, scatter reduction
- beam shaping (micro-beams)

Absorption image

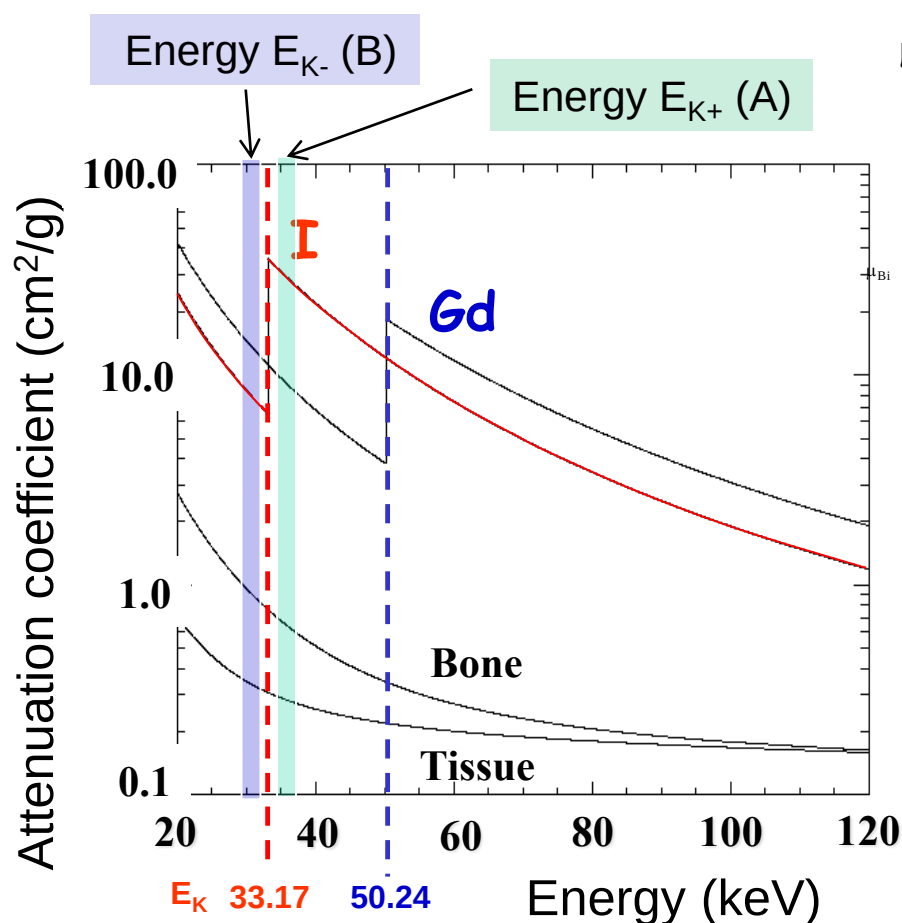


SR X-rays imaging techniques

1) K-edge subtraction imaging

Exploiting the monochromaticity of SR...

1. Contrast agent: **Iodine**, or **Gadolinium**, etc.
2. Two Images are acquired: just Above (A) and just Below (B) the K-edge of Contrast agent
3. From image processing : Iodine and Tissue images can be separated



μ_{xy} : **Attenuation coefficients** : x = energies (A or B),
y = material (tissue (t) or iodine (i)).

$$X_i = \frac{\mu_{Bi} \ln(A) - \mu_{Ai} \ln(B)}{\mu_{Bi} \mu_{At} - \mu_{Ai} \mu_{Bt}}$$

$$X_t = \frac{\mu_{Bi} \ln(A) - \mu_{Ai} \ln(B)}{\mu_{Bi} \mu_{At} + \mu_{Ai} \mu_{Bt}}$$



Below

Above
K-edge

Iodine Image

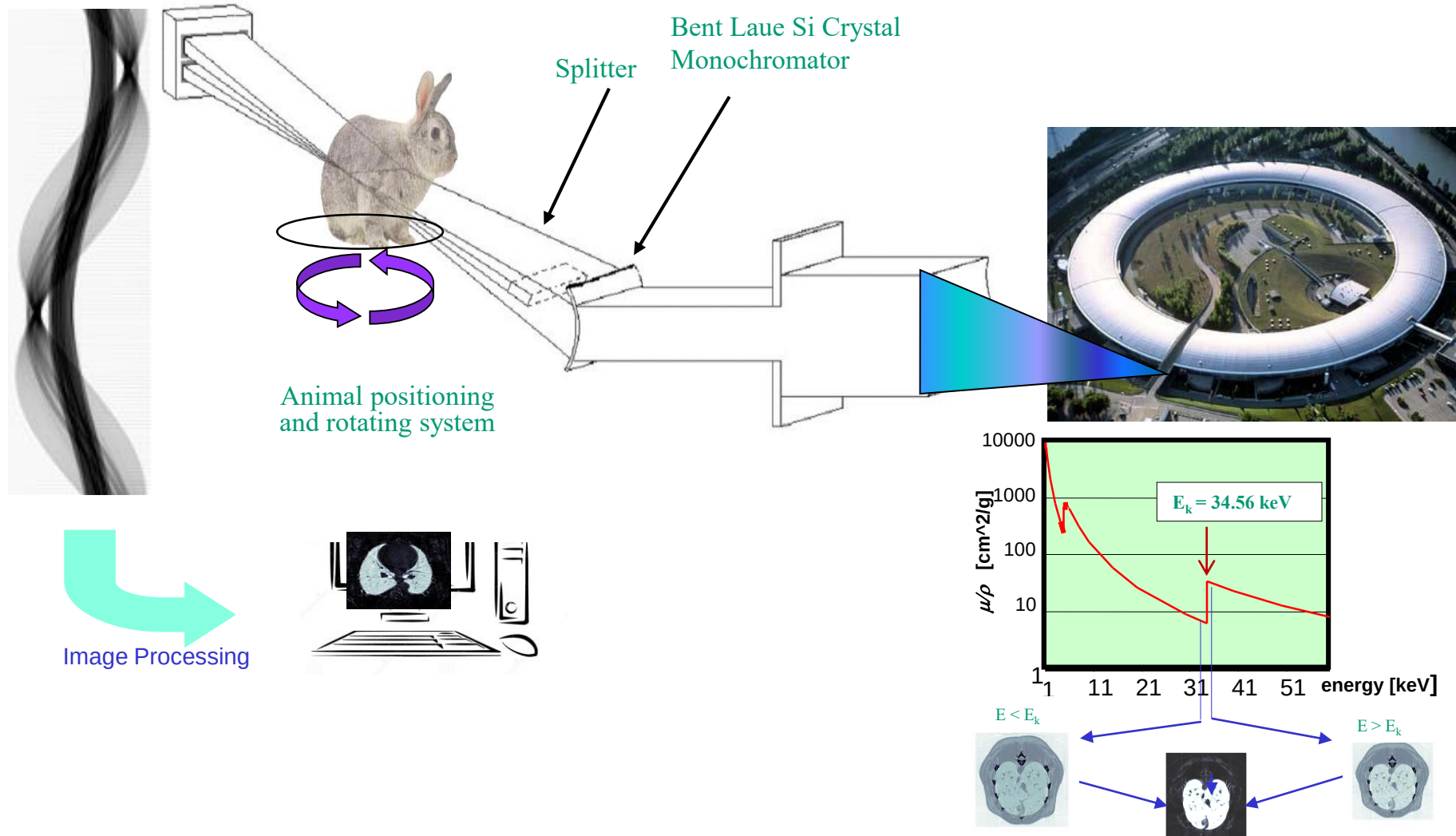


K-Edge absorption imaging: applications

Bronchography/lungs imaging
(pre-clinical – animal model)

Dual Line Ge Detector

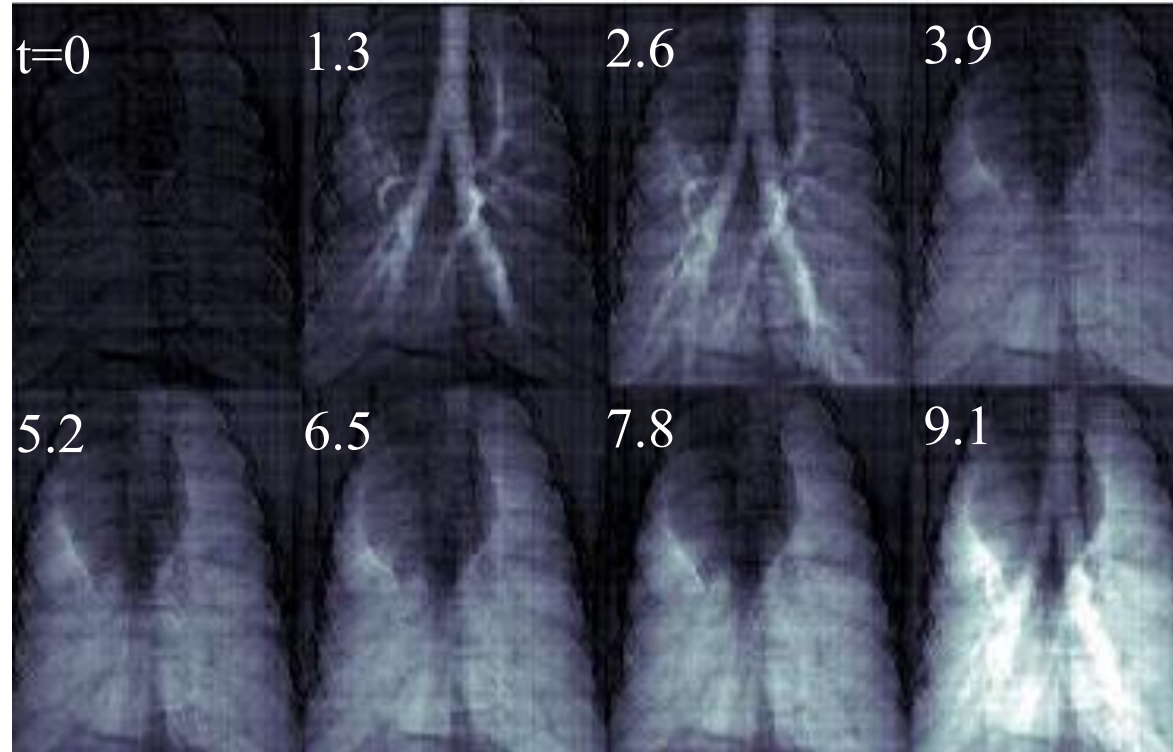
w: 150 mm, 350 μm pitch, beam thickness 700 μm



in-vivo Rabbit Lung Xenon K-Edge Subtraction Imaging and CT

Projection
Images

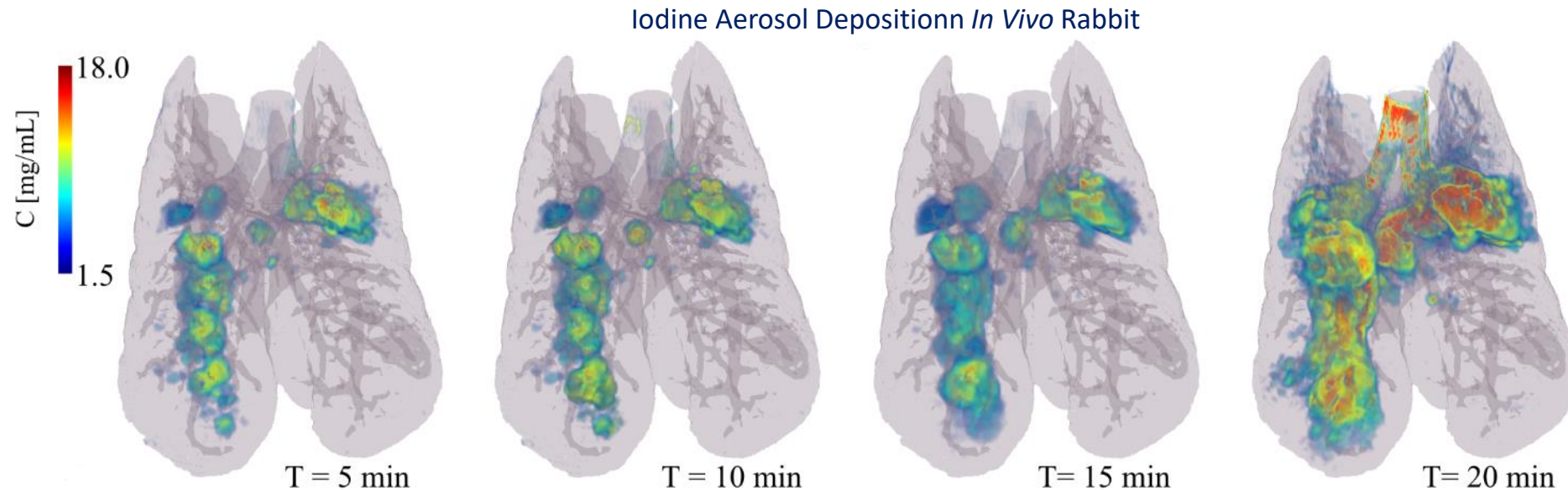
Time between
images = 1.3 s



- Lung ventilation studies are used to evaluate the presence of ventilation heterogeneities
- Dynamic imaging during Xenon wash-in with KES lung CT allows to perform quantitative measurements of regional specific Ventilation (sV) (i.e., ventilation normalized to Voxel volume).

Quantitative imaging of contrast agent: aerosol particle deposition

- In case of inhalation of drug/pollutant **aerosol particles** it is important to evaluate their **regional deposition** against the **lung morphology** and the **regional ventilation**
- KES imaging was used to quantitatively map the regional deposition of **iodine labelled aerosol particles**
- 2 X-ray beams tuned at slightly different energies above and below the K-edge, of **Xe** (34.6 keV) or **Iodine** (33.2 keV)
- Two CT scans are simultaneously acquired during the inhalation of stable **Xe gas** or **iodine-stained aerosol particles**. The density due to the contrast element (Xe, I) can be separated from that of tissue, in each image.
- **“Xe-density” or “I-density” images allow the direct quantitative measurement of these elements within the airways.**
- **A “tissue-density” image obtained from the same data allows the assessment of lung morphology.**



- Aerosol particles 3 μm mass median aerodynamic diameter -> inhomogenous deposition !
- Comprehensive technique for studying biodistribution of inhaled drugs/pollutants

2 - *Phase – sensitive* imaging techniques

An overview of the evolution and key features of various hard X-ray PHC methods in connection with translation to a wide range of imaging applications can be found in the paper:

On the evolution and relative merits of hard X-ray phase-contrast imaging methods

S. W. Wilkins, Ya. I. Nesterets, T. E. Gureyev, S. C. Mayo, A. Pogany and A. W. Stevenson

Phil. Trans. R. Soc. A 2014 **372**, 20130021, published 27 January 2014

Exploiting the spatial coherence of SR...

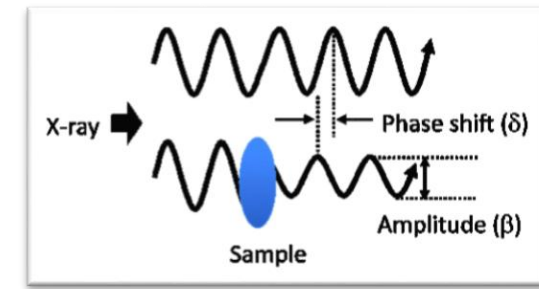
Phase sensitive techniques (Phase Contrast (PHC) imaging)

- X-ray interaction with matter is ruled by the **refractive index (n)**. In the complex representation, it is composed by: $n = 1 - \delta + i\beta$
 - (β), absorption properties of the material, (wave amplitude variation).
 - (δ) phase shifts effects induced on the incoming wave

Linear attenuation of X-rays: $\mu = 4\pi\beta/\lambda$ (λ = X-ray wavelength)

Transmitted beam intensity: $I = I_0 e^{-\mu t}$ (I_0 = incident intensity)

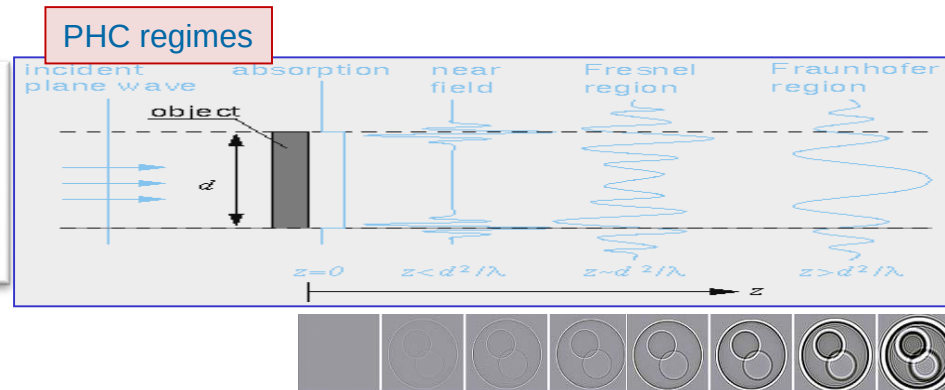
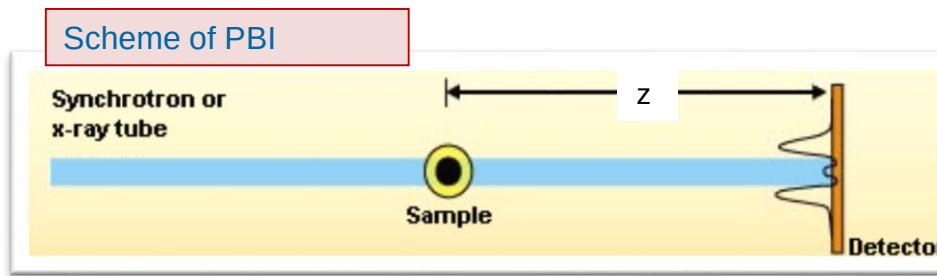
Phase shifts: $\phi = -2\pi\delta t/\lambda$ (t =sample thickness)



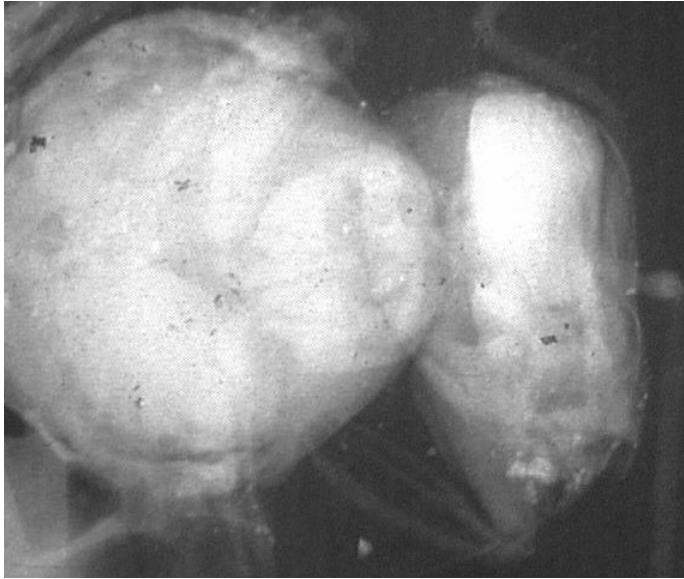
- Conventional imaging relies on **X-ray absorption**
- Phase contrast imaging** is based on the detection of **phase shifts** occurring to X-rays crossing the sample

Propagation based imaging (PBI) - Simplest approach – no optical element needed. Image contrast arises from interference among parts of the wavefront differently deviated (or phase shifted) by the sample.

Edge enhancement effects, different regimes according to the selected sample-to-detector distance.

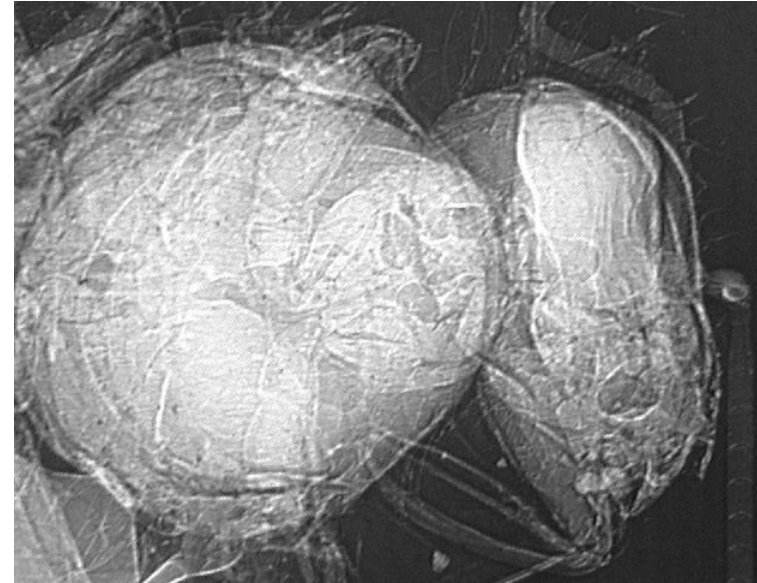


Edge enhancements effects – trend with X-ray energy

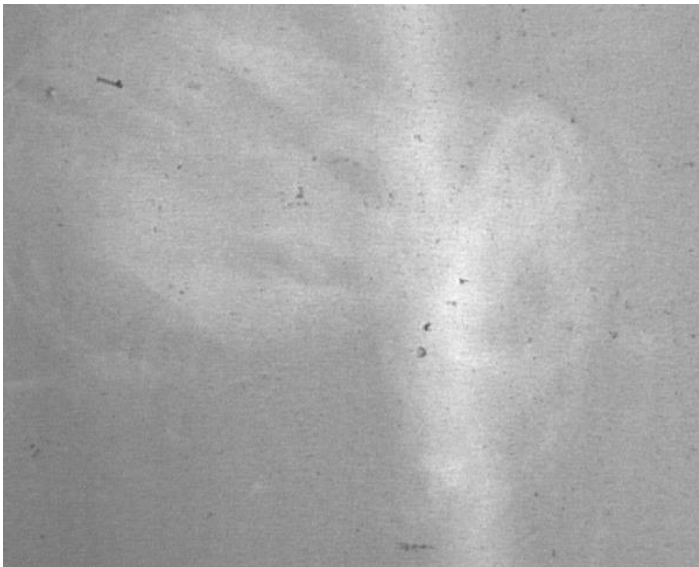


Absorption

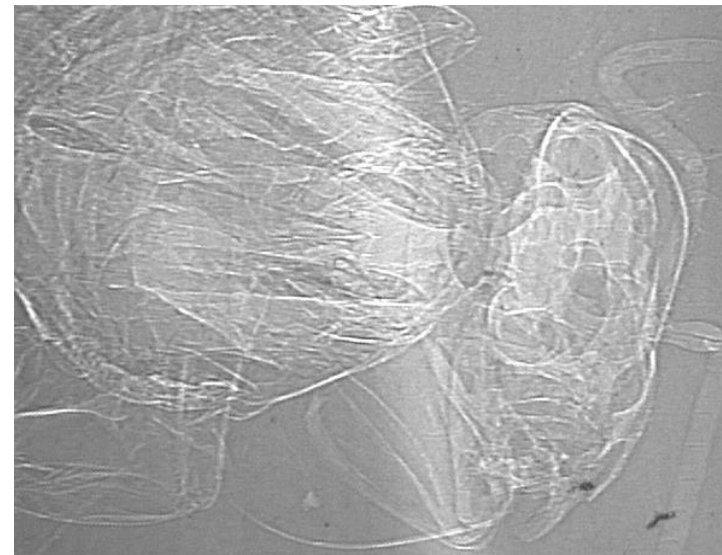
10 keV



Phase Contrast



20 keV

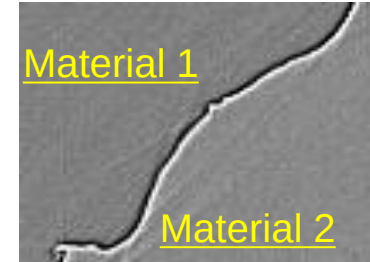


Edge enhancement effect and use of phase retrieval algorithms

An “edge” image obtained by PBI imaging does not resolve unambiguously the structure of the imaged sample

Complex refractive index: $n = 1 - \delta + i\beta$

δ → phase β → attenuation

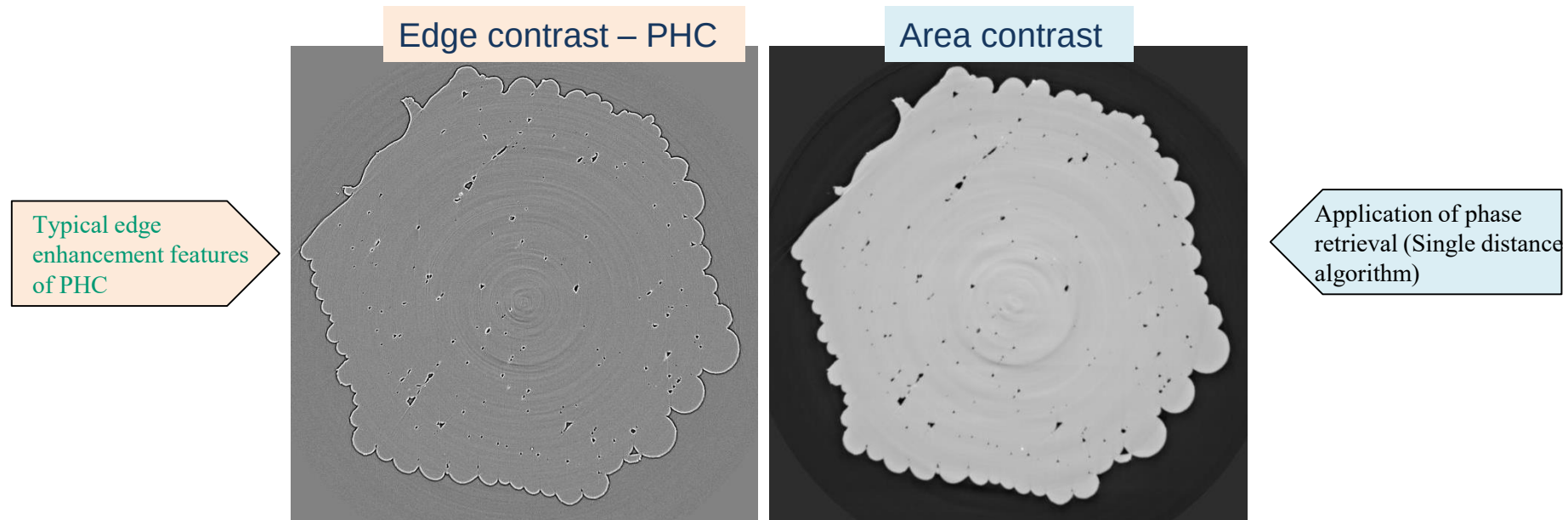


Edge between two materials with negligible absorption

Instead of “edge contrast” we would like “area contrast” (ideally a map of δ)

The approach to get the “area contrast” is called *phase retrieval* and two main approaches exist:

- **Holotomography** - (P. Cloetens et al., ESRF) - quantitative approach with identification of material components, it requires multiple distances acquisition
- **Single-distance** – approximated, working on homogeneous materials in the near field conditions has the advantage to require acquisition at one distance (D. Paganin – Monash Univ.) – preferable for biomedical imaging

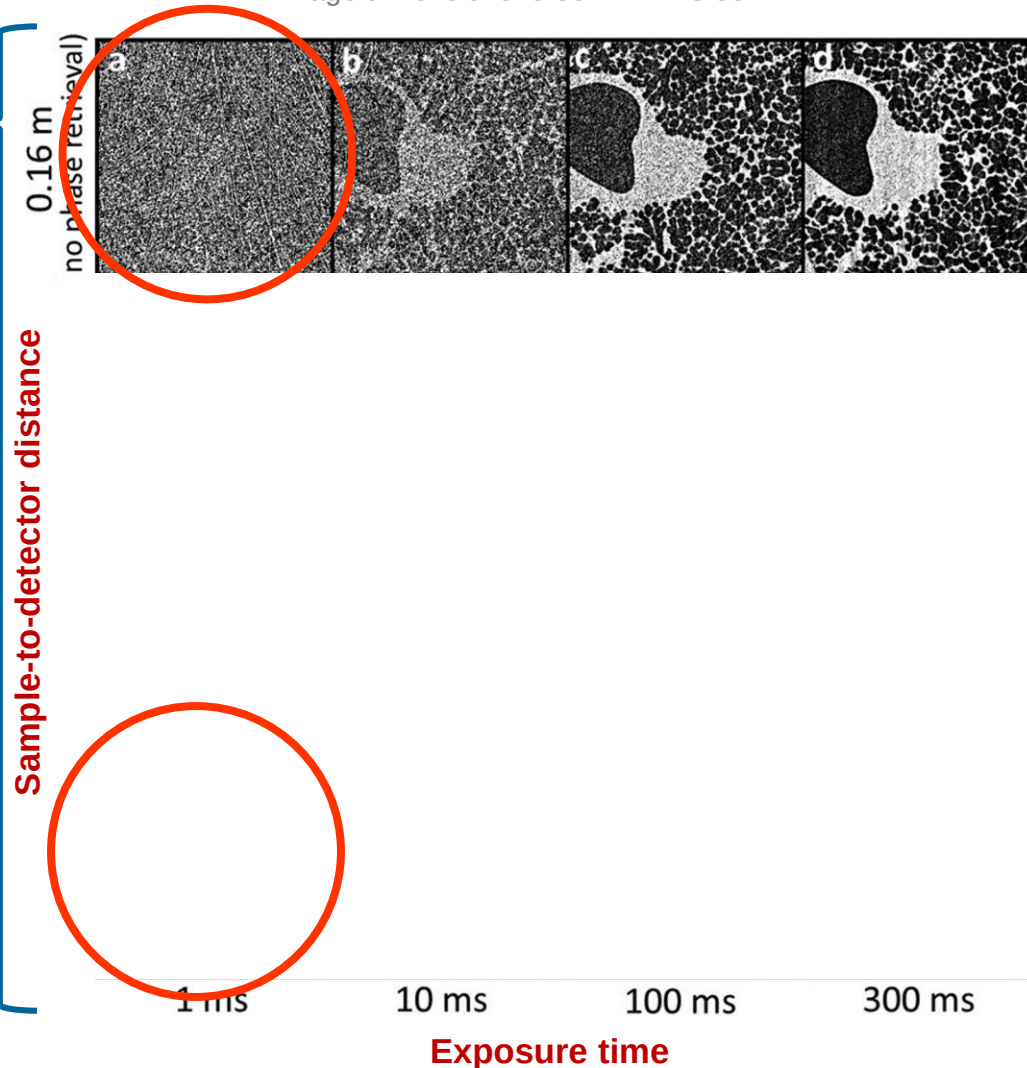


Dose reduction with PBI Imaging using Phase Retrieval

- The phase shift signal (δ) is much stronger than the absorption signal (β) signal -> This means that phase retrieval (PHR) should allow for significant dose reduction

Image dimensions: 3.83 mm $\times$ 3.83 mm.

Magnified mouse lung tissue reconstructions as a function of propagation distance and exposure time



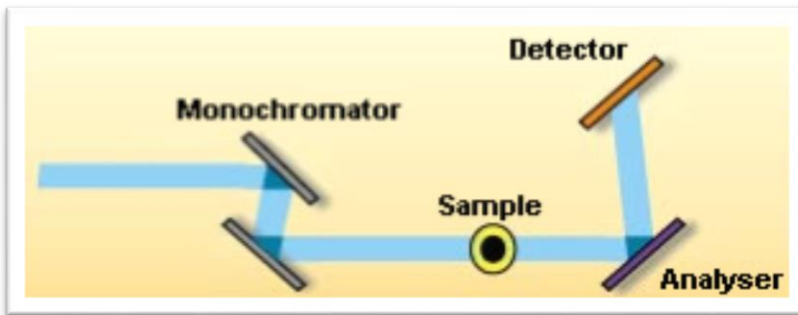
M.J.Kitchen et al., *Sci.Rep.* | 7: 15953 | (2017).
Paganin, D., et al. *J Microsc* **206**, 33–40 (2002).

PHC imaging: other approaches

Methods exploiting the particle nature of photons – measure of X-ray refraction angles

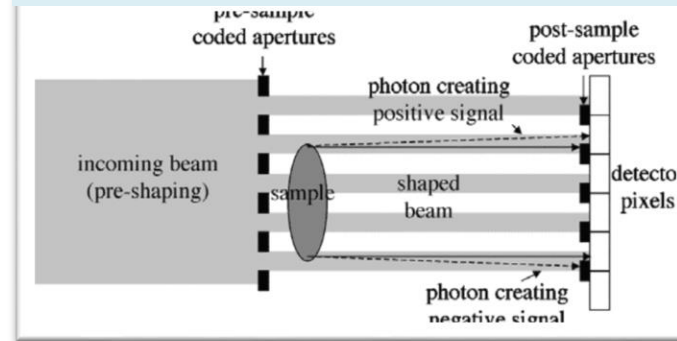
Analyzer Based Imaging

Use of perfect crystals to select angular directions of X-rays exiting the sample



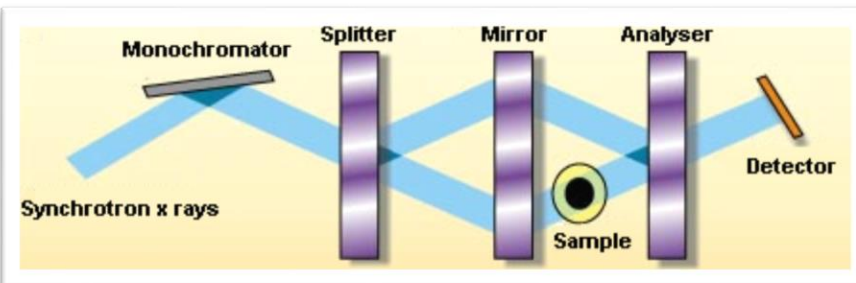
Coded Apertures

Use of coded apertures (masks) to select refraction angles

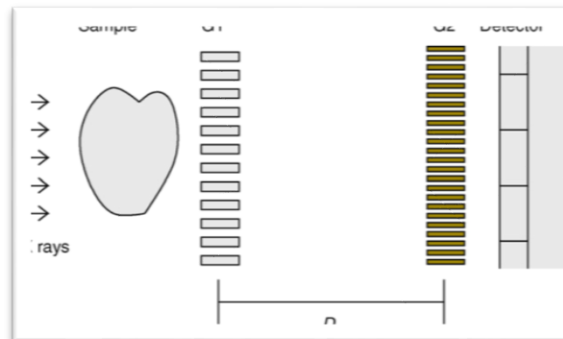


Interferometric approaches - waves are superimposed in order to extract information - direct measure of phase shifts introduced by the sample

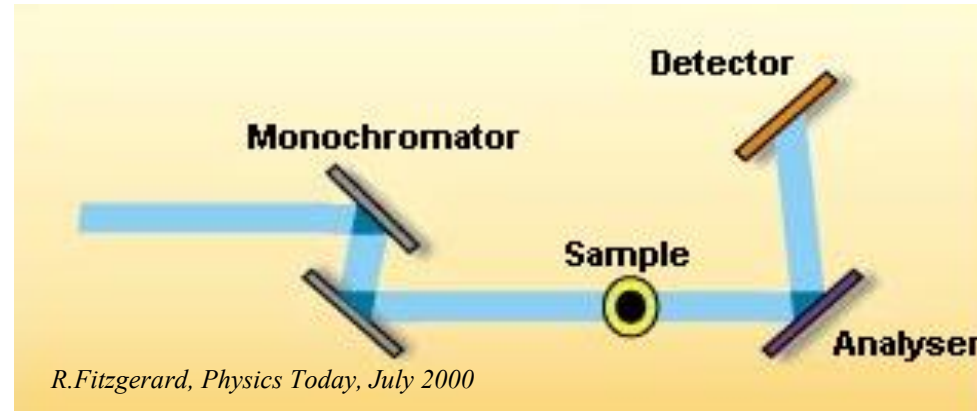
Crystal Interferometry



Grating interferometry



Analyzer Based Imaging (ABI)



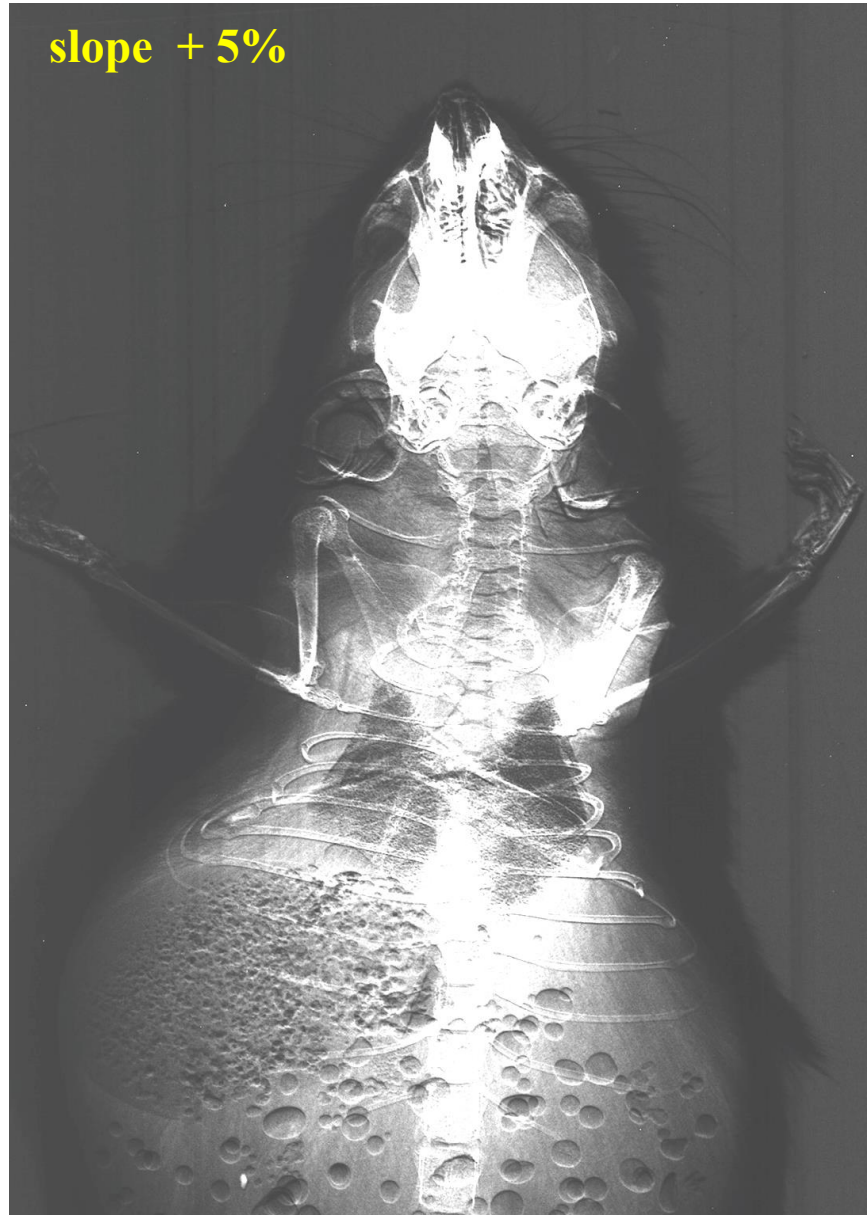
- A perfect crystal is used as an angular filter to select angular emission of X-rays. The filtering function is the rocking curve (FWHM: 1-20 μrad)
- Image formation with ABI is sensitive to a variation of δ in the sample. Indeed, **refraction angle is roughly proportional to the gradient of δ**
- Analyzer and monochromator aligned $\rightarrow$ X-ray scattered by more than some tens μrad are rejected
- Small misalignments $\rightarrow$ investigation of phase shift effects
- With greater misalignments the primary beam is almost totally rejected and pure refraction images are obtained
- Sensitive to $\nabla\Phi(x,y)$
- The technique requires the beam monochromaticity.

Podurets K. M. et al., Sov. Phys. Tech. Phys. 34(6), 1989

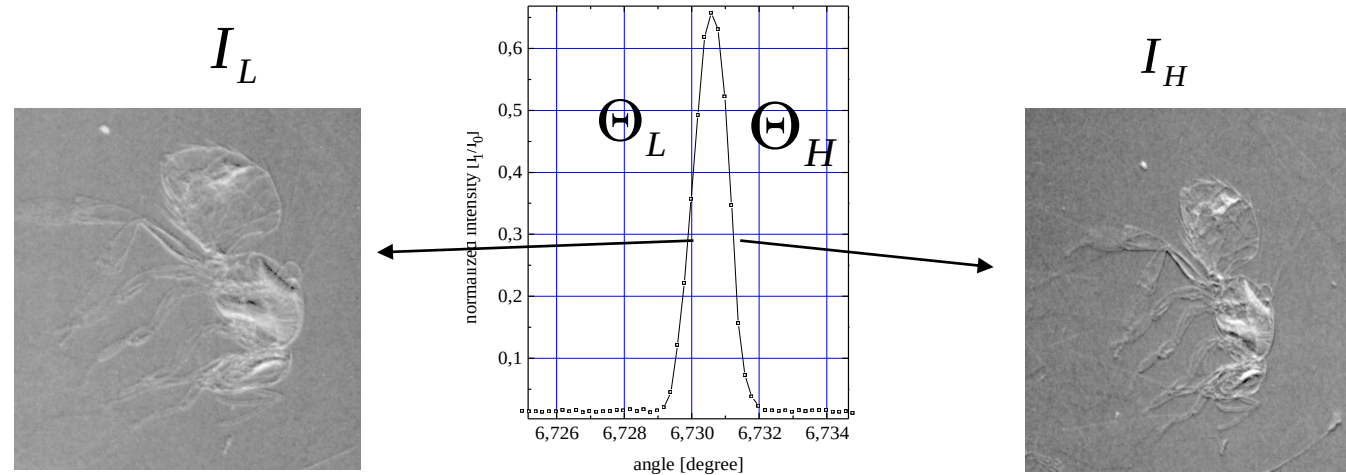
V. N. Ingal and E. A. Beliaevskaya, J. Phys. D: Appl. Phys. 28, 1995

Chapman D et al., Phys. Med. Biol. 42, 1997

ABI images for different analyzer positions



ABI image manipulation (original algorithm)



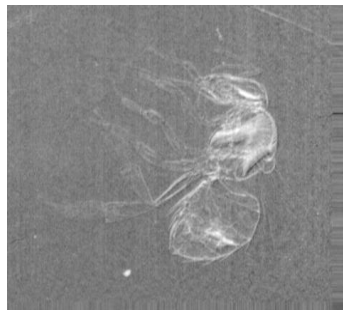
Linear approximation of rocking curve at half values (I_R and I_L)

$$I_L = I_R \left(R(\Theta_L) + \frac{\partial R}{\partial \Theta}(\Theta_L) \Delta \Theta_z \right)$$

$$I_H = I_R \left(R(\Theta_H) + \frac{\partial R}{\partial \Theta}(\Theta_H) \Delta \Theta_z \right)$$

Θ_z = refraction Image

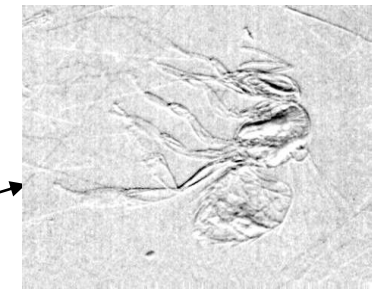
I_R = apparent absorption image (absorption+extinction)



Apparent Absorption Image

$$I_R = \frac{I_L \cdot \frac{dR}{d\Theta} \Big|_{\Theta_H} - I_H \cdot \frac{dR}{d\Theta} \Big|_{\Theta_L}}{R(\Theta_L) \cdot \frac{dR}{d\Theta} \Big|_{\Theta_H} - R(\Theta_H) \cdot \frac{dR}{d\Theta} \Big|_{\Theta_L}}$$

$$\Theta_z = \frac{I_H \cdot R(\Theta_L) - I_L \cdot R(\Theta_H)}{I_L \cdot \frac{dR}{d\Theta} \Big|_{\Theta_H} - I_H \cdot \frac{dR}{d\Theta} \Big|_{\Theta_L}}$$



Refraction Image

Apparent absorption and refraction images

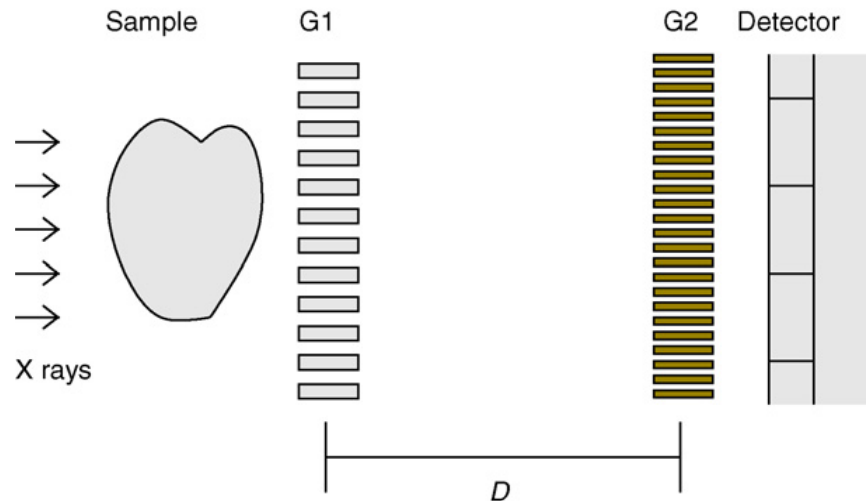
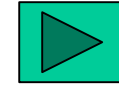


Apparent absorption



Refraction image

Grating interferometric imaging (GI)

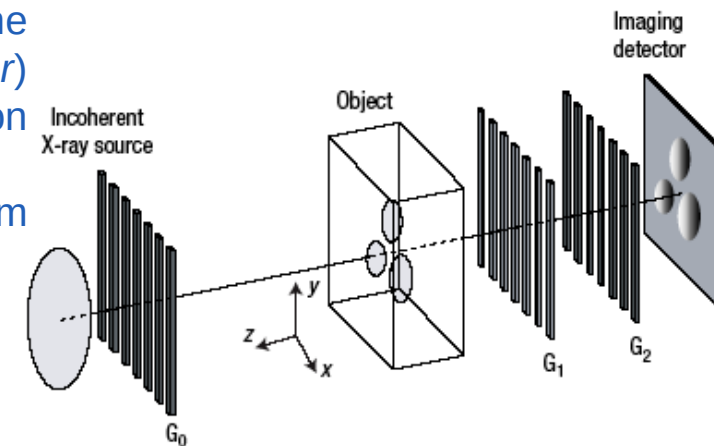


Based on an optical phenomenon discovered by Talbot (1936) and explained by Rayleigh (1881).

With a coherent radiation, the image of the grating is repeated at regular distances behind the grating, $D = 2d^2/\lambda$ (d =grating period, λ =wavelength).

The beam is split and analyzed by means of two gratings, introduced between the object and the detector:

- The X-ray wavefront transmitted by the sample go through **a linear diffraction grating G1** (*beam splitter*).
- Downstream G1, a pattern of interference fringes is formed. The local distortions of the fringe pattern from its ideal regular shape contain information on the sample structure.
- Since the fringes are too closely spaced to be resolved by the pixel detector, an additional **absorption grid G2** (called *analyzer*) in front of the detector is needed to transform fringe-position information into intensity values on the detector.
- A modified set-up can be applied to polychromatic spectrum from an X-ray tube. 

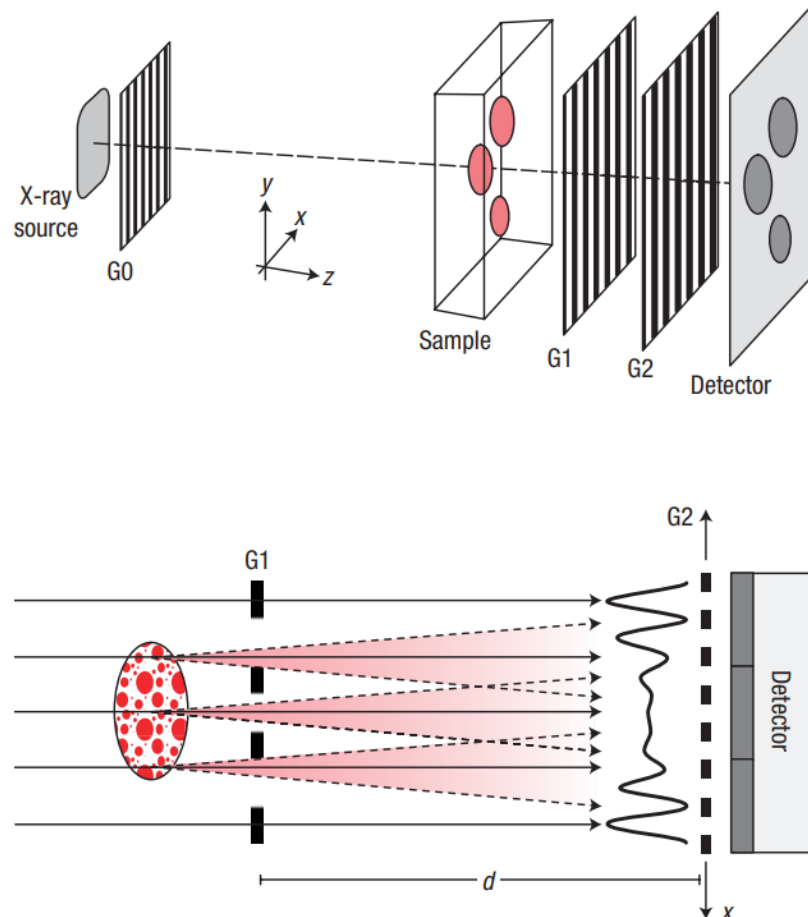


How to choose the best Phase Contrast Approach ?

Technique class	PROS	CONS
Propagation based imaging Sensitive to $\nabla^2\Phi(x,y)$	- Simple and straightforward implementation - Approximated methods (Paganin) allow a reasonable separation between the phase information and absorption, allowing improved image contrast.	- It does not allow a direct separation between diffracted and transmitted photons - It needs multiple acquisition in case of highly quantitative measurements (holotomography) - It requires a high coherent source and high resolution detectors
Gratings, Coded apertures, Analyzer Based Imaging Sensitive to $\nabla\Phi(x,y)$ or $\Phi(x,y)$	- Source coherence is not critical - They allow to separate the detection of diffracted, refracted and transmitted signal. - They allow the access of phase information	- Need of implementing and control complex optics components between source and sample and/or between sample and detector - They require multiple expositions for each study

Dark field imaging of a chicken wing

Implementation on a conventional X-ray tube



Transmission image



Dark-field image



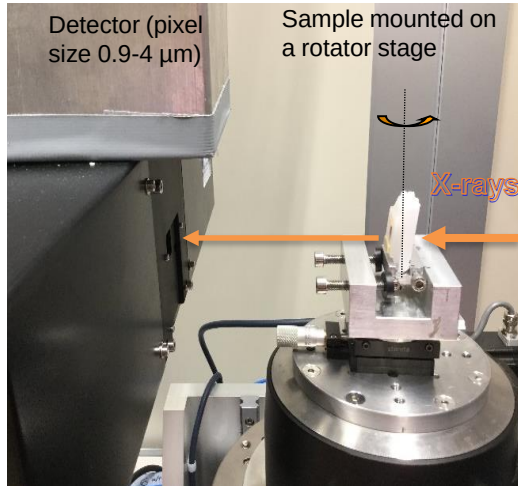
The X-ray scattering due to the porous bones microstructure and the reflection at internal or external interfaces produce a strong signal in the dark-field image.

Applications

High resolution imaging

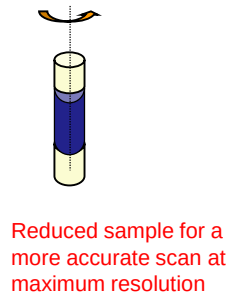
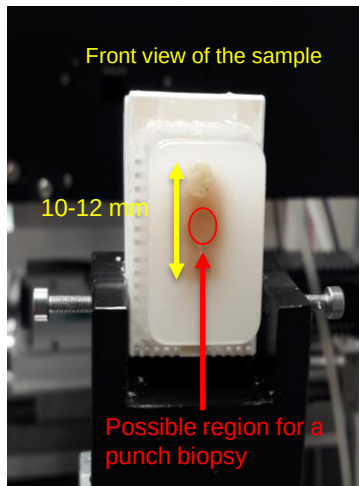
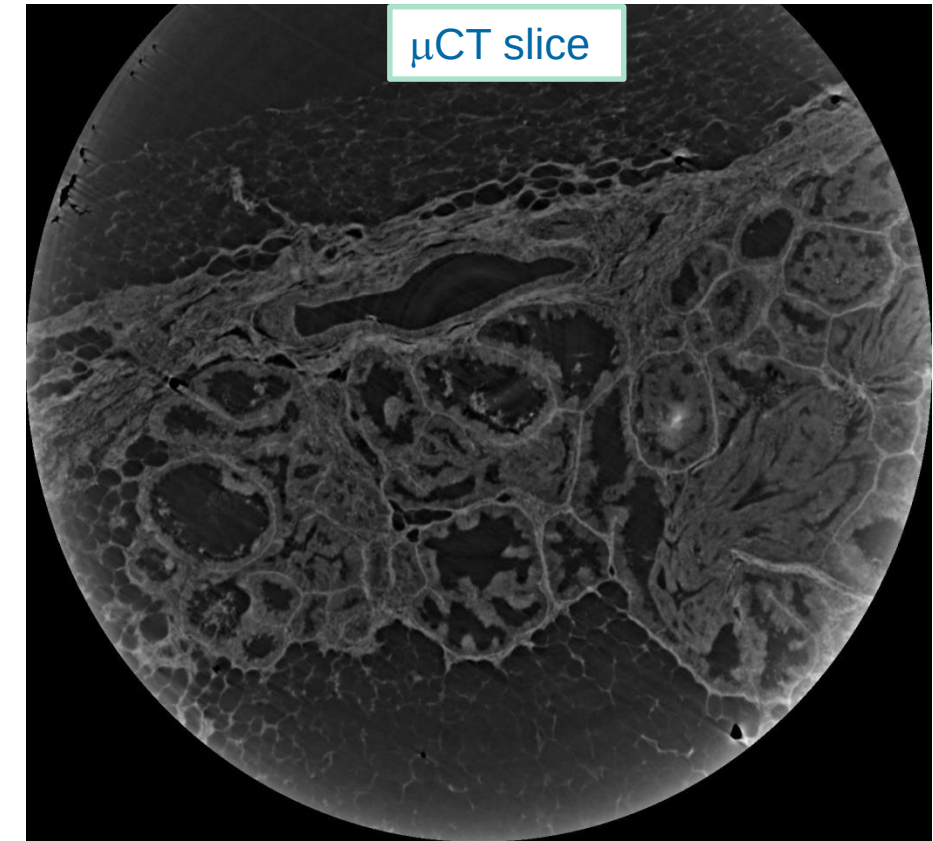
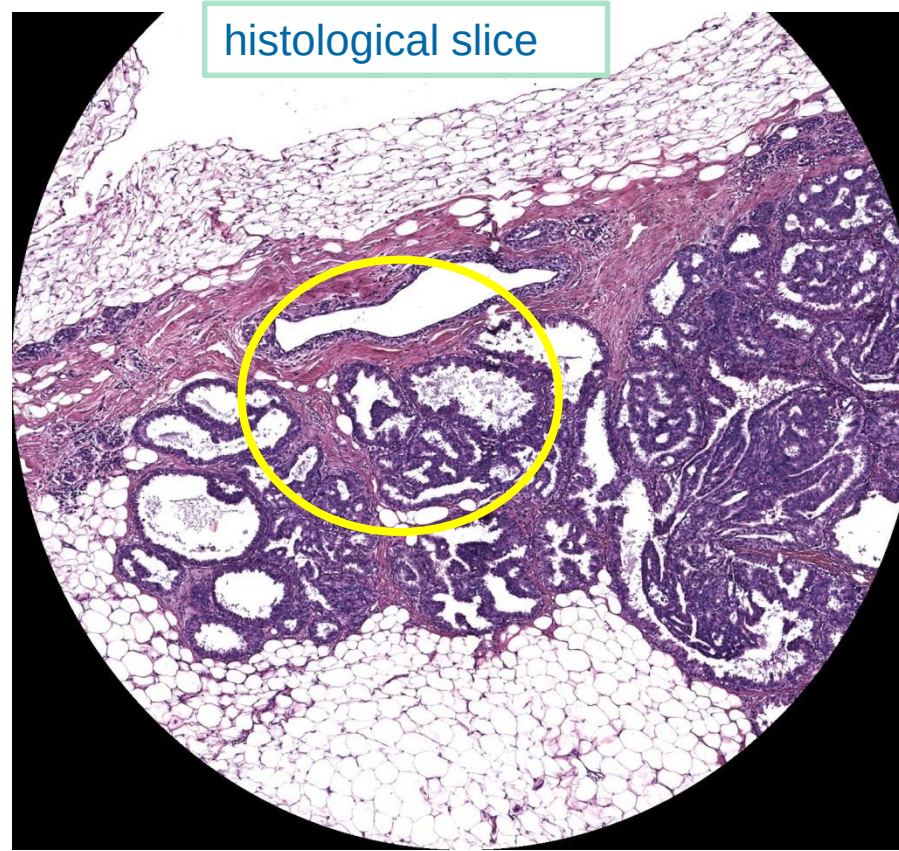
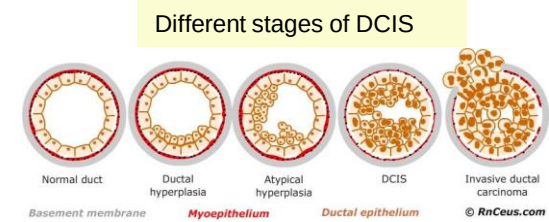
- “In vitro” imaging: high resolution morphological studies (es. micro-CT studies of tissues, organs, biomaterials - virtual histology)
High resolution required, no dose constraints, main limitation is radiation damage

Virtual histology of breast malignant lesions: *in situ* cribriform Ductal Carcinoma



Unstained sample embedded in paraffine (3-4 mm diameter)

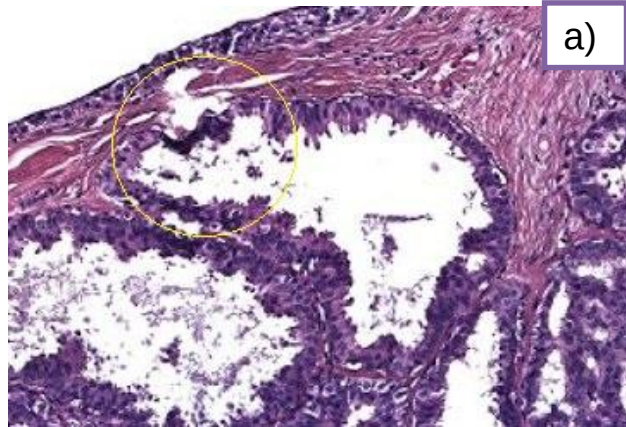
- White/pink scan, mean x-ray energy: 19 keV, 0.9-1.2 μm pixel
- sample-detector dist. = 19 cm, Paganin phase retrieval algorithm



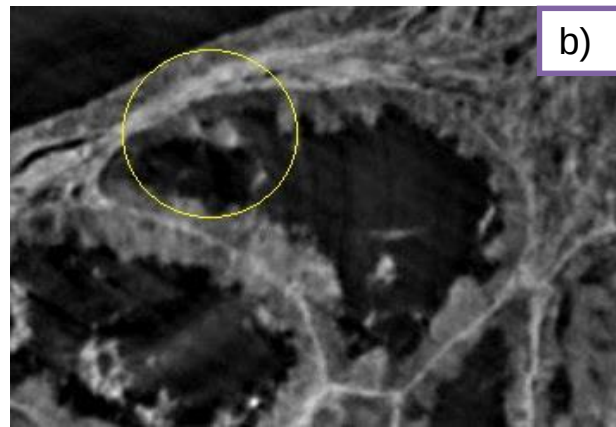
<https://cdn.knightlab.com/libs/juxtapose/latest/embed/index.html?uid=38bfc344-96f9-11eb-83c8-ebb5d6f907df>

Breast malignant lesion: duct detail

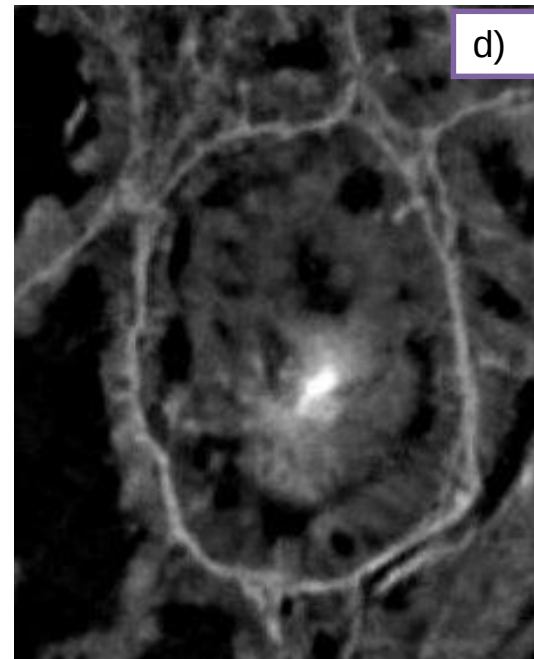
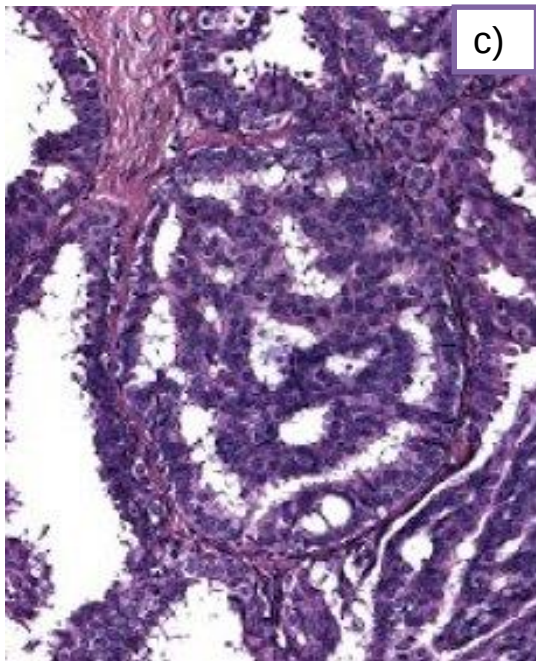
histological slice - detail



μ CT slice - detail



Close-up of a duct including micro-calcifications (yellow circle). On μ CT image (b) calcifications are well visible, on the histology image (a) they are completely lost due to the cutting process causing an hyperchromasia (dark purple zone inside the circle).

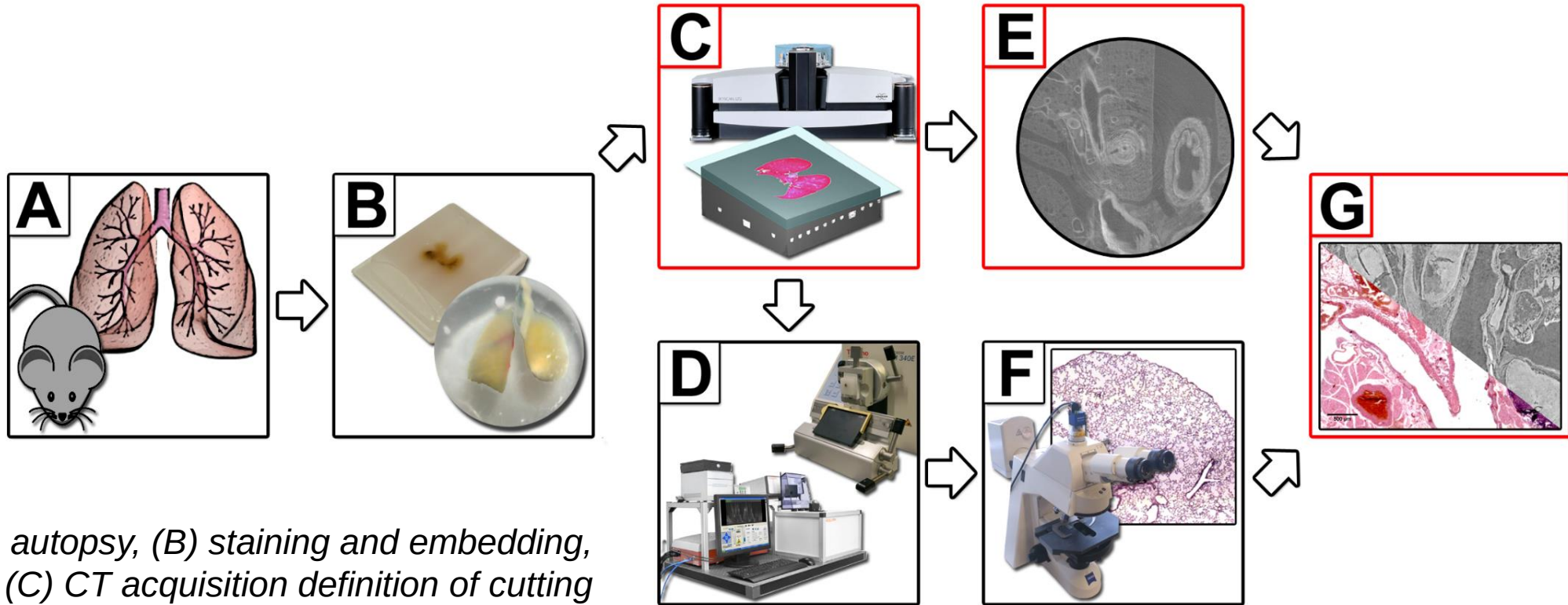


Duct with typical cribriform features. Basement membrane and calcification better visible in d)

PBI μ CT can be helpful for:

- deciding the cutting orientation of the histology
- evaluate presence of microcalcifications
- highlight interfaces (membranes, etc.)

MicroCT based guided sectioning

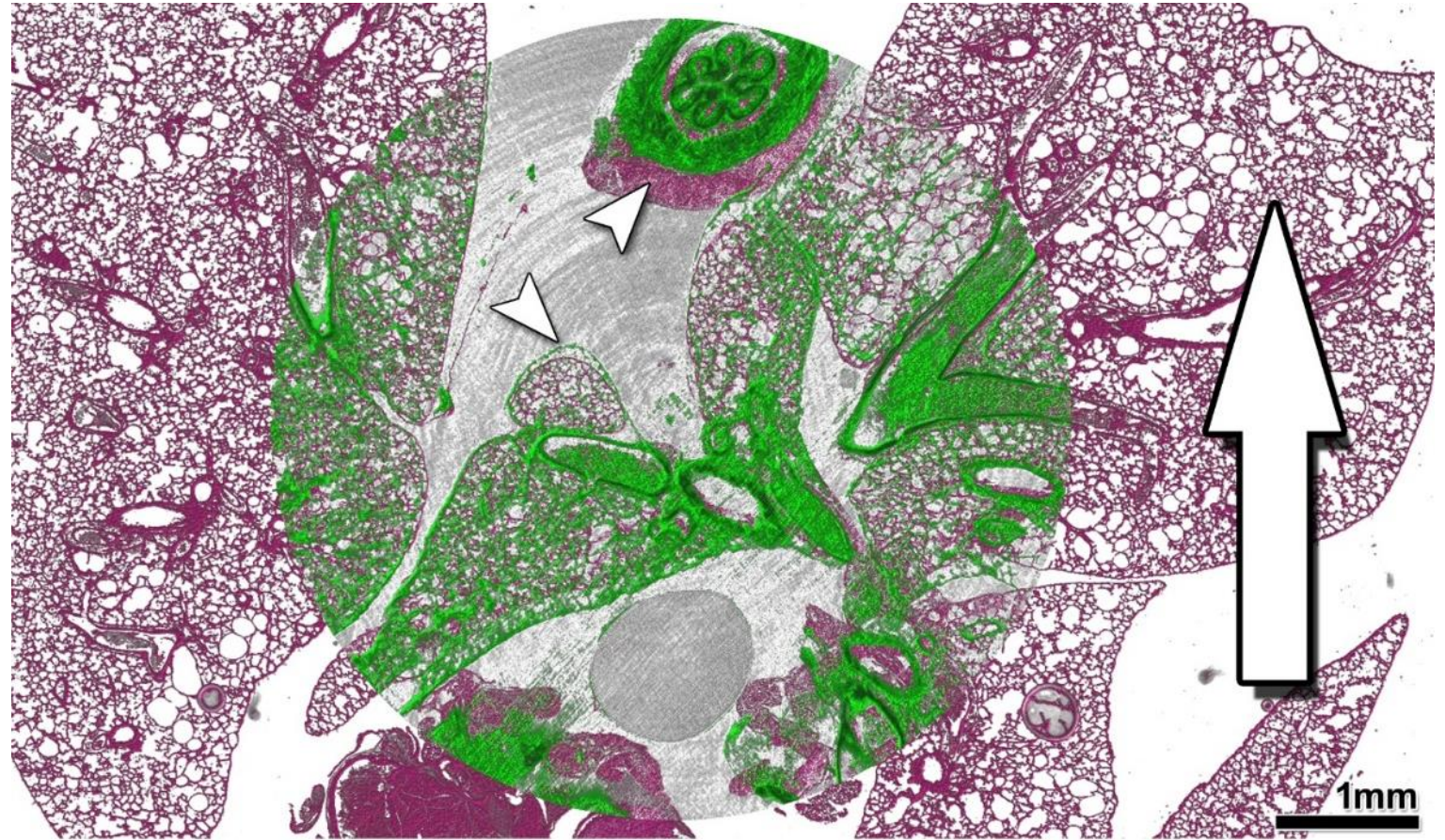


(A) autopsy, (B) staining and embedding,
(C) CT acquisition definition of cutting
position and angle, (D) cutting with a
microtome, (E-G) comparison and
overlay for quality assessment

Elastic imaging registration

Cutting: introduces non-linear deformations that need to be correct for reliable image fusion
Raises questions about precision of morphometry in histology

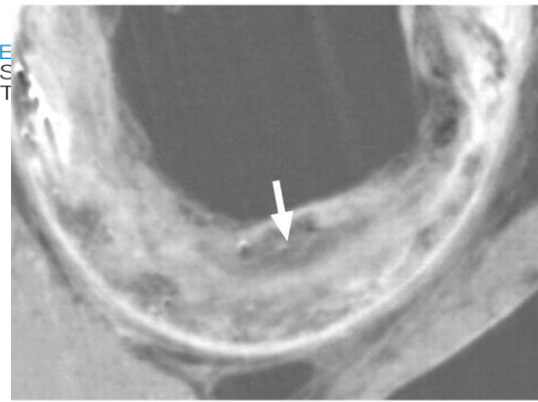
*overlay of classical histology (red, HE staining) and high resolution CT (green, 1 μ m).
Mechanical sectioning introduces deformation (white arrow heads).*



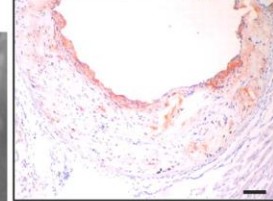
Albers, J., Markus, M. A., Alves, F., & Dullin, C. (2018). X-ray based virtual histology allows guided sectioning of heavy ion stained murine lungs for histological analysis. Scientific reports, 8(1), 1-10.

Atherosclerotic plaque imaging using Grating Interferometry

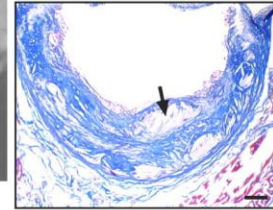
- Reliable imaging modalities to characterize plaque components are clinically desirable for detecting **unstable coronary atherosclerotic (ATH) plaques**, which cause acute coronary syndrome.
- Although recent clinical developments in CT have enabled the visualization of luminal narrowing and calcified plaques in coronary arteries, the identification of **non-calcified plaque components** remains difficult.
- ATH plaque components are important predictors of plaque stability and clinical events. The risk of plaque rupture appears to depend on the plaque components rather than the severity of stenosis.
- A model of Apolipoprotein E -deficient [knockout] mice (ApoE-KO mice) developing ATH in a short time was used. They were fed with a normal diet or a high cholesterol diet.
- GI based micro-CT studies were conducted on **excised aorta samples**.



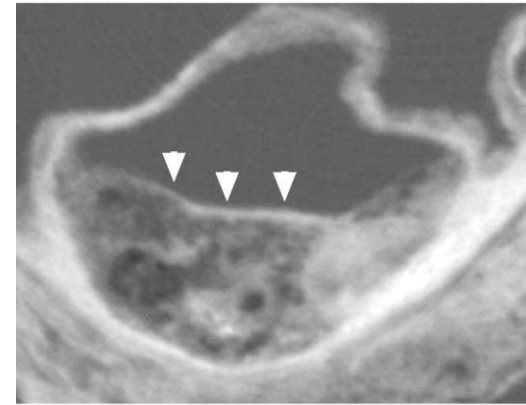
Phase contrast CT



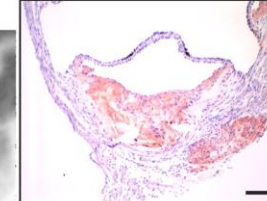
Lipid (Sudan-III)



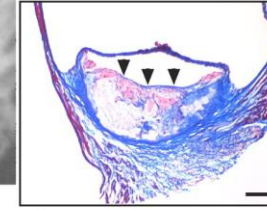
Collagen (Masson's trichrome)



Phase contrast CT

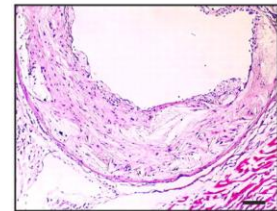


Lipid (Sudan-III)

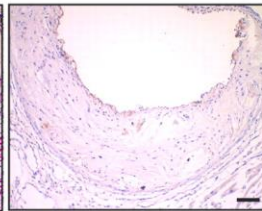


Collagen (Masson's trichrome)

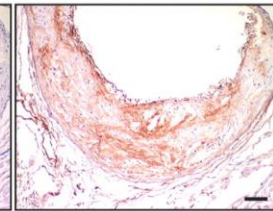
ATH lesion in the aortic sinus of a ApoE-KO mouse, **12 wk of age**, fed with a **high-cholesterol diet** for **8 wk**.



HE



Macrophage (MOMA-II)



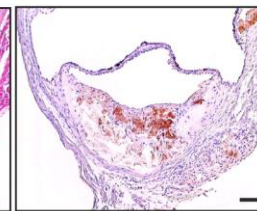
Smooth muscle (1A4)

ATH lesion in the aortic sinus of ApoE-KO mouse, **36 wk of age**, fed with a **normal diet**.

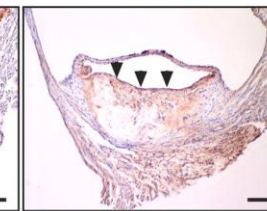
Scale bar = 100 μ m



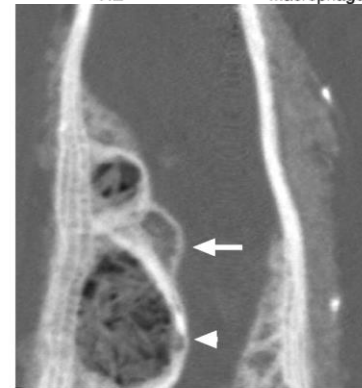
HE



Macrophage (MOMA-II)

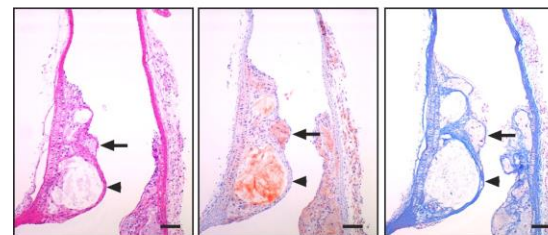


Smooth muscle (1A4)

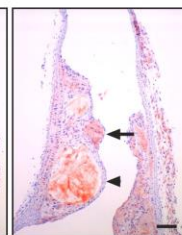


Phase contrast CT

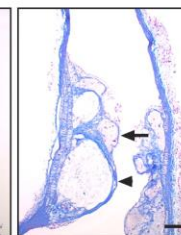
ATH lesion in the right common carotid artery of a ApoE-KO mouse, **12 wk of age**, fed with a **high-cholesterol diet** for **8 wk**.



HE



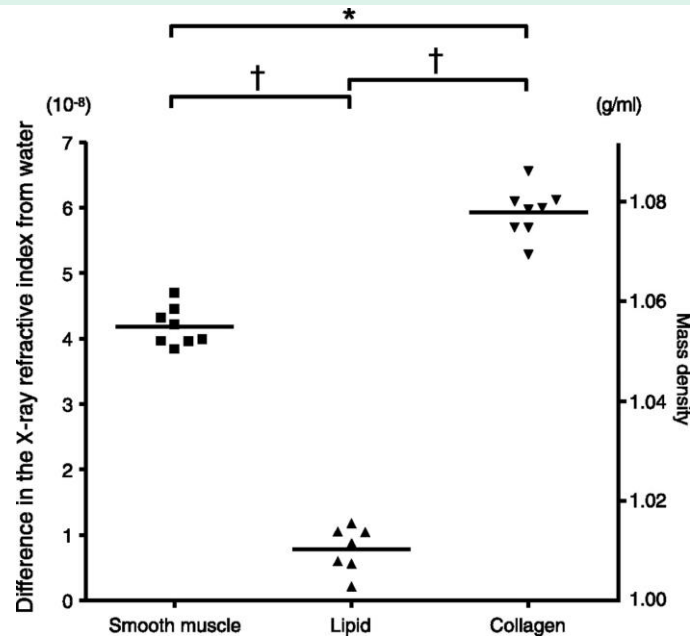
Lipid (Sudan-III)



Collagen (Masson's trichrome)

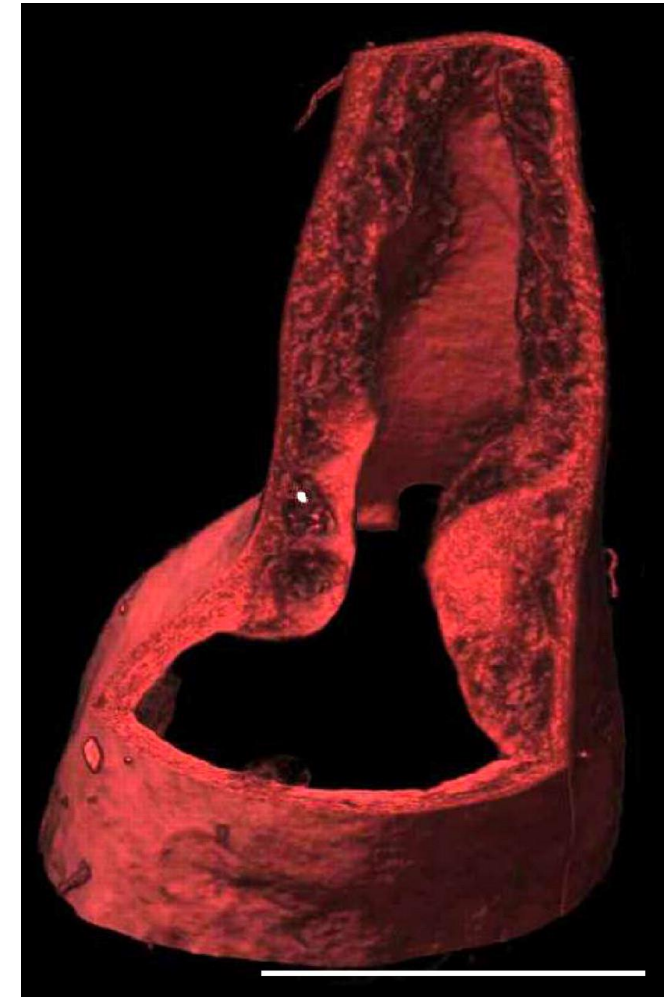
Shinohara M et al. Am J Physiol Heart Circ Physiol; 294:, 2008

- The different plaques components have been recognized in the GI CT studies as it was demonstrated in the comparison with histological data.
- Quantitative measurements of refraction indexes have been provided.



Mean dδ values and specific mass densities of the various components of plaques. The lipid area shows a low dδ ($0.79 \pm 0.13 \times 10^{-8}$) and mass density (1.011 ± 0.001766 g/ml). The smooth muscle and collagen areas show high dδ (4.18 ± 0.10 and $5.93 \pm 0.13 \times 10^{-8}$, respectively) and mass density (1.057 ± 0.001407 and 1.08 ± 0.001794 g/ml, respectively).

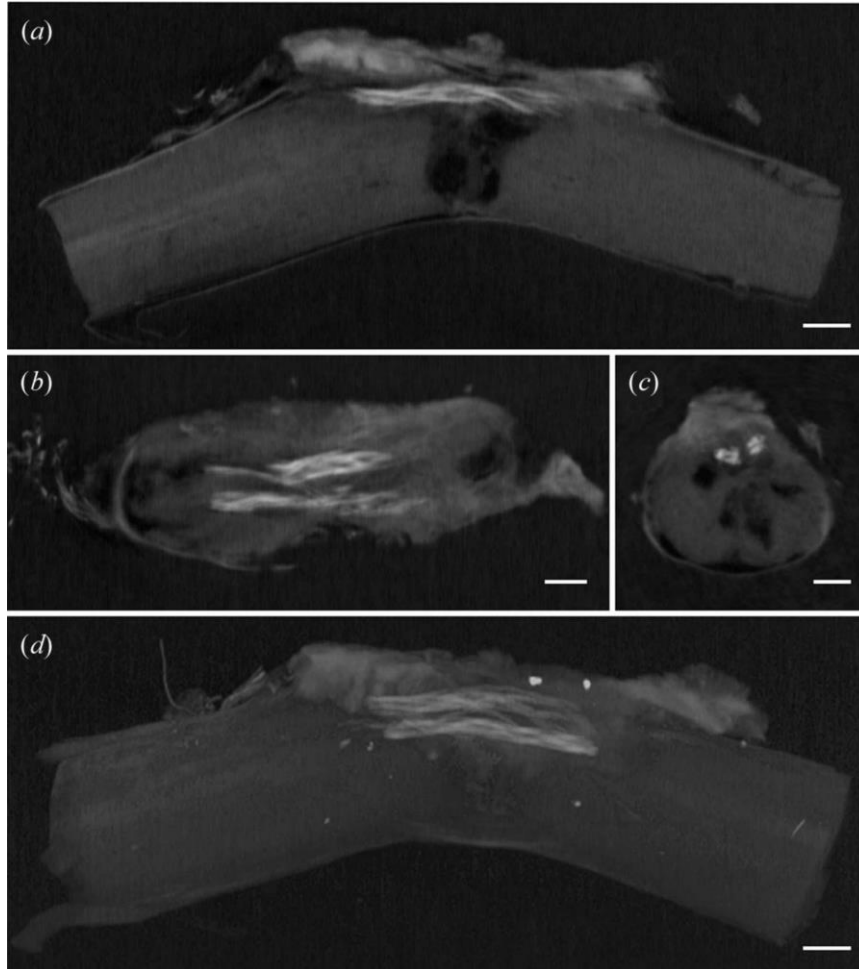
* $P < .05$; † $P < .01$.



3D rendering of the right common carotid artery of the ApoE-KO mouse fed a high-cholesterol diet.

Scale bar = 1 mm.

Use of gratings-interferometry (GI) phase contrast imaging to study the microstructure and degradation of implanted biodegradable scaffolds after spinal cord injury

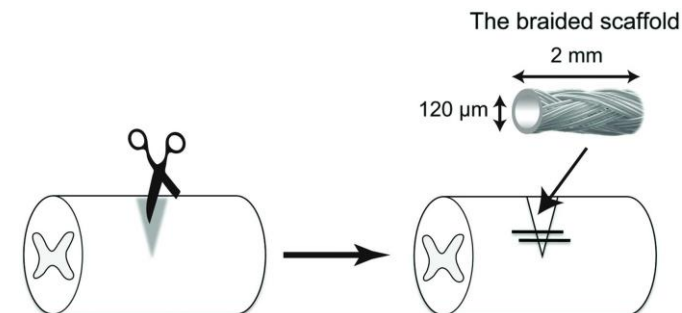


Braided PGA scaffolds in the injured spinal cord 28 days after implantation. a-c) 2D views. (d) 3D volume rendering
Scale bar = 500 μm .

Tissue engineering strategies for spinal cord repair after spinal cord injury (SCI) are fundamental issues

There is the need to develop an imaging protocol capable to visualize *in-vivo*, either the **microstructure** of 3D scaffolds and their **degradation process** to treat SCI.

GI has been used at Spring 8 on mice animal model to visualize the **polyglycolic acid (PGA) scaffold**, including its microfibers, after implantation into the injured spinal cord.



Quantification of braided scaffolds degradation in the injured spinal cord.

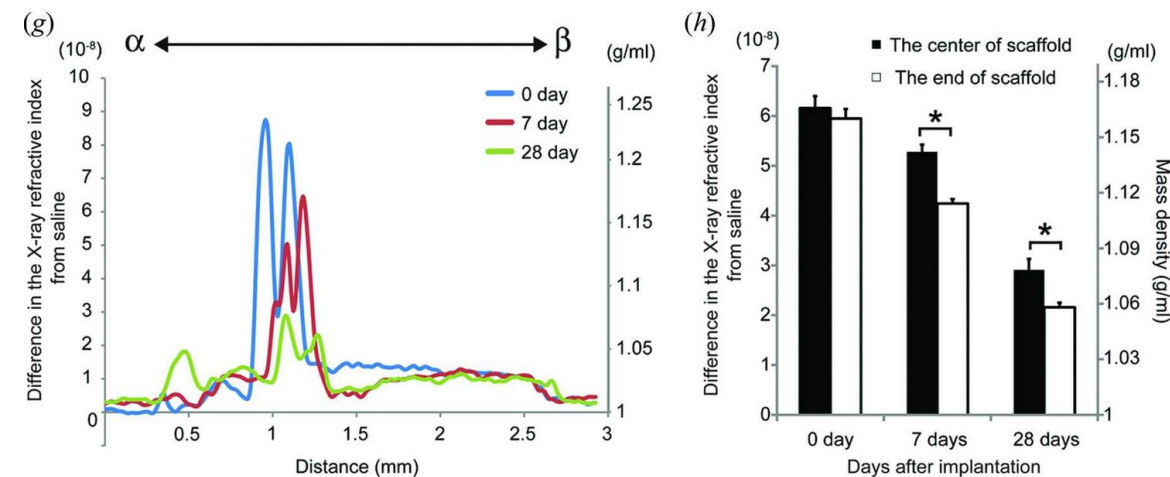
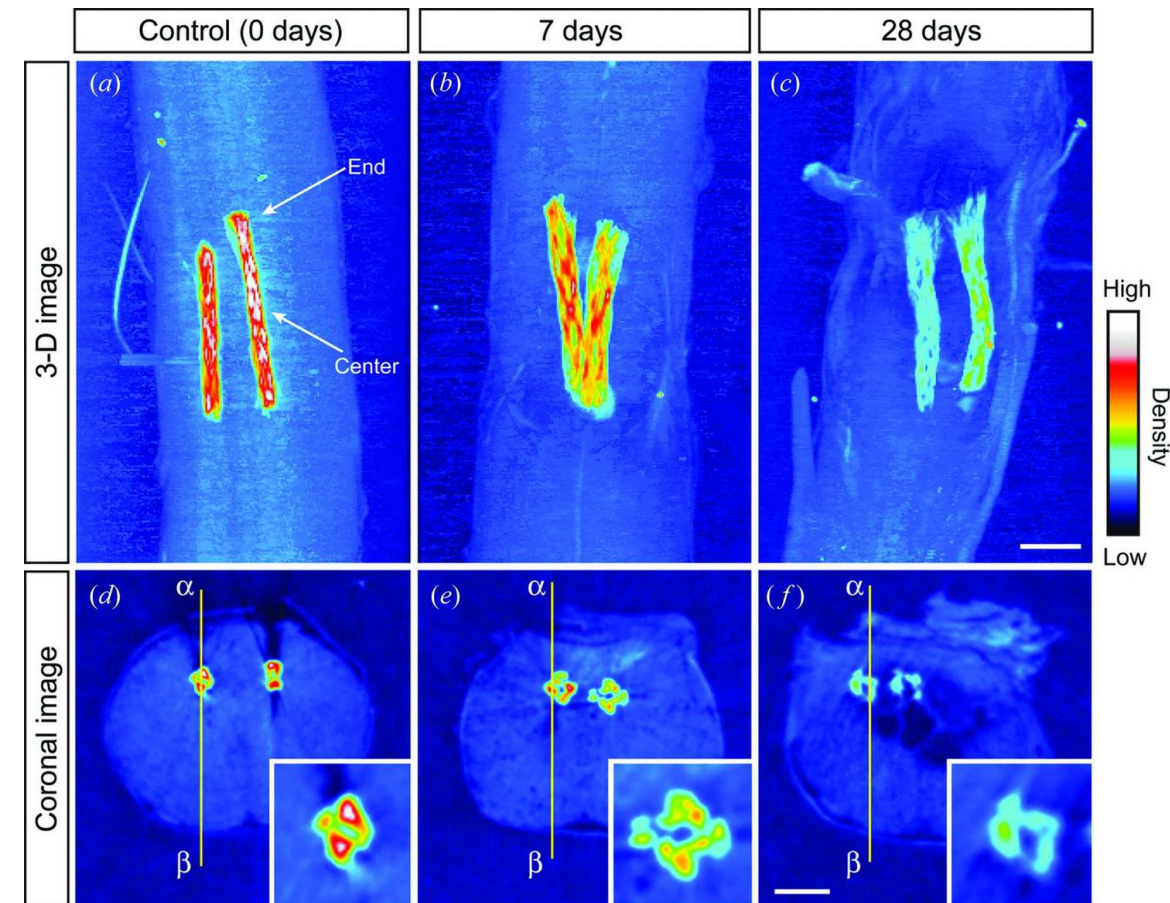
(a–f) Relative mass density maps acquired with GI PhC-CT

At 7 and 28 days after implantation the density in the central portion of the scaffold was higher than that in the end portion, indicating faster degradation in the peripheral portion.

(g) The X-ray refractive index difference from saline ($\Delta\delta$ value) and estimated mass density through the spinal cord from α to β

(h) Mass density in the central and end portions of the scaffold after implantation into the spinal cord.

Scale bar = 500 μm .



Differently from PB PhC, GI PhC is capable to access the direct measure of refractive index, allowing to quantitative determine the scaffold biodegradable profile after implantation

Pre-clinical imaging

- Imaging of small animals, tissues and organs: applied for different purposes in the development of ***animal models*** (*ex vivo*, *in-vivo*)

Research protocols, pixel size: 4.5 - 9 μm (ex-vivo) up to 100 μm (in-vivo).

2D dynamic Imaging of lungs Function & morphology (I)

- Animal model: rabbit pups
- Imaged pups with PBI, either before the first breath (fetus) and at fixed intervals after birth (up to 2h)

Exp. time: 80 ms

Interval: 0.8 s

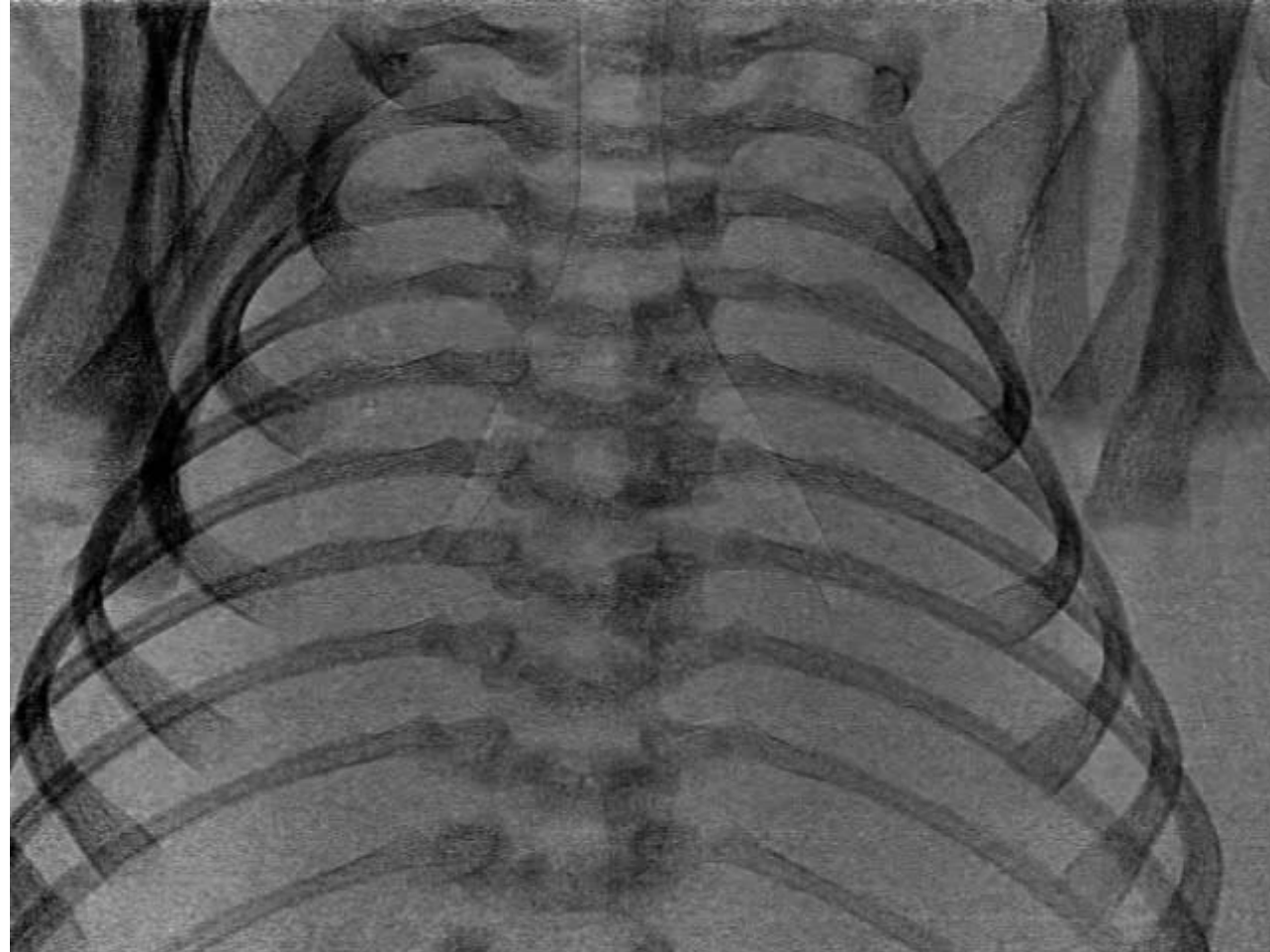
Skin Dose: ~ 0.15mGy/f

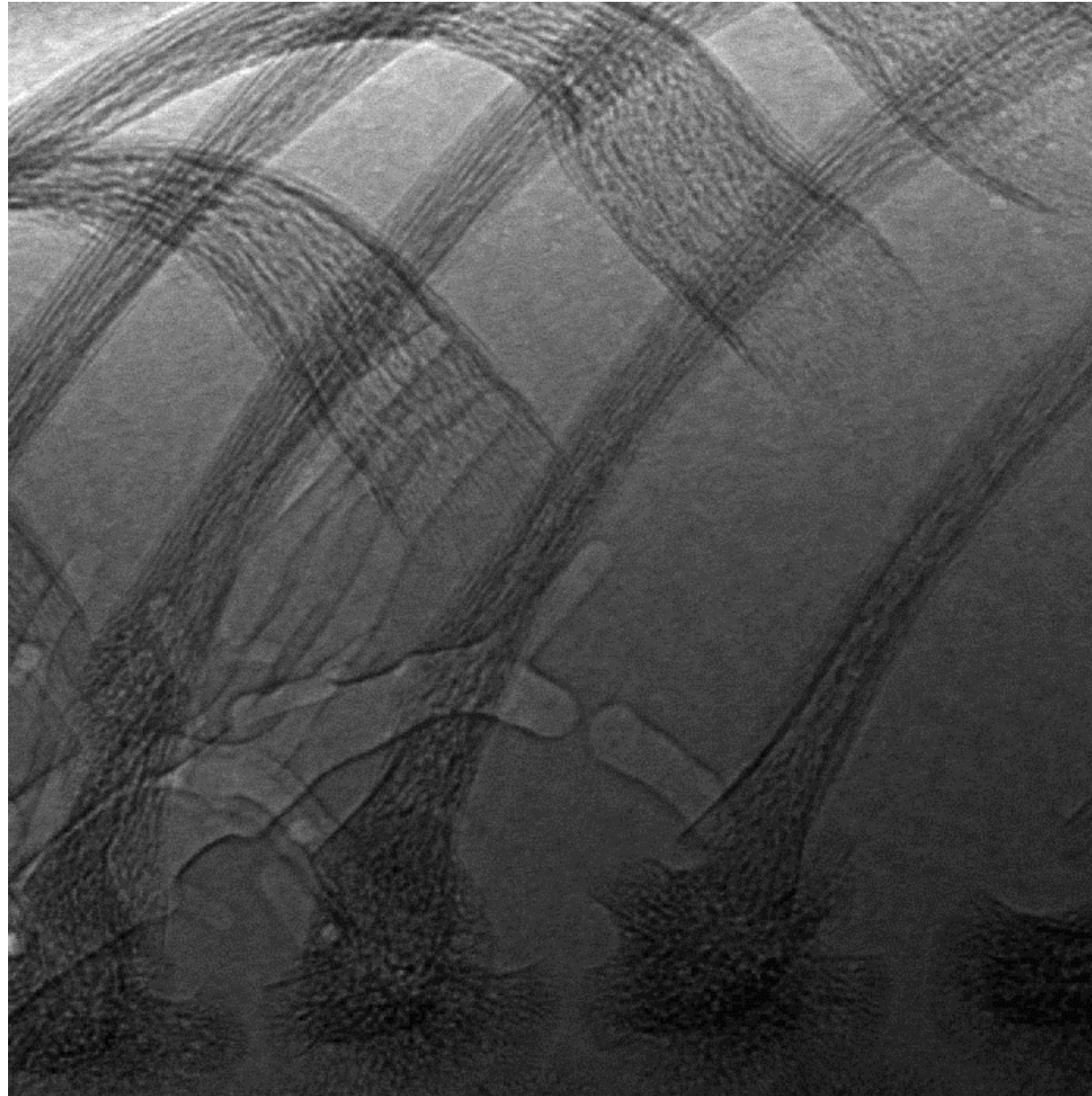
Pixel Size: 22.5 μm

E = 25 keV

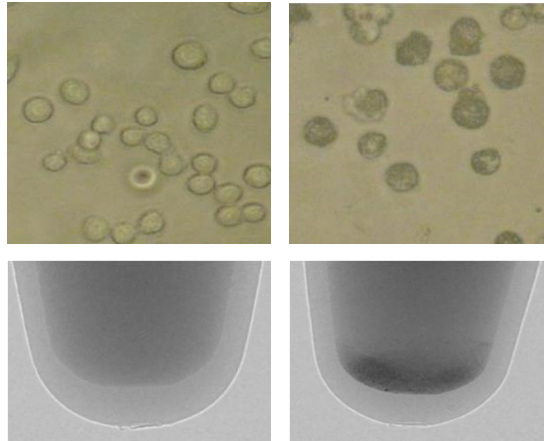


Effects of Ventilation on Lung Liquid Clearance at Birth
Aim: to observe lung aeration on a breath-by-breath basis.





Imaging of inflammation in asthmatic mice: use of macrophages with double staining

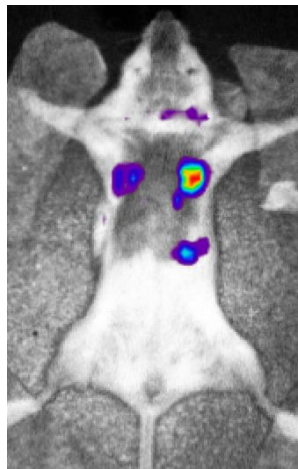


Unlabeled MΦ

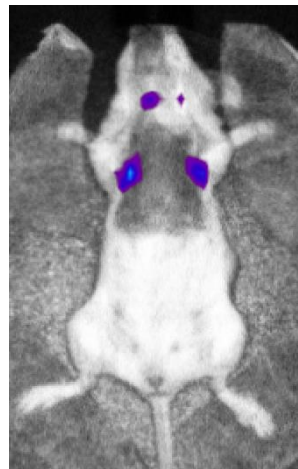
Ba labeled MΦ

- Animal model of **allergic asthma** induced by ovalbumin based on balb/c mice
- Aim: evaluate the potential of SR-based technique for **functional** and **morphological** imaging of mice lungs
- Available techniques: optical imaging and PBI micro-CT
- Use of immortalized Murine Alveolar Macrophage Cell line (MΦ) with double staining:
 - Barium sulfate (clinical contrast agent) for microCT
 - DiD fluorescent dye for optical imaging.

In vivo validation of homing of MΦ to inflammation sites.
Images performed 24 hours after MΦ administration.



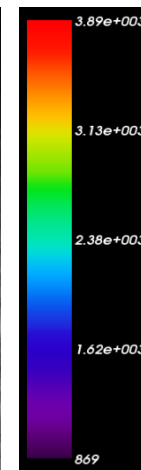
Asthmatic mouse
treated with MΦ



Normal mouse
treated with MΦ



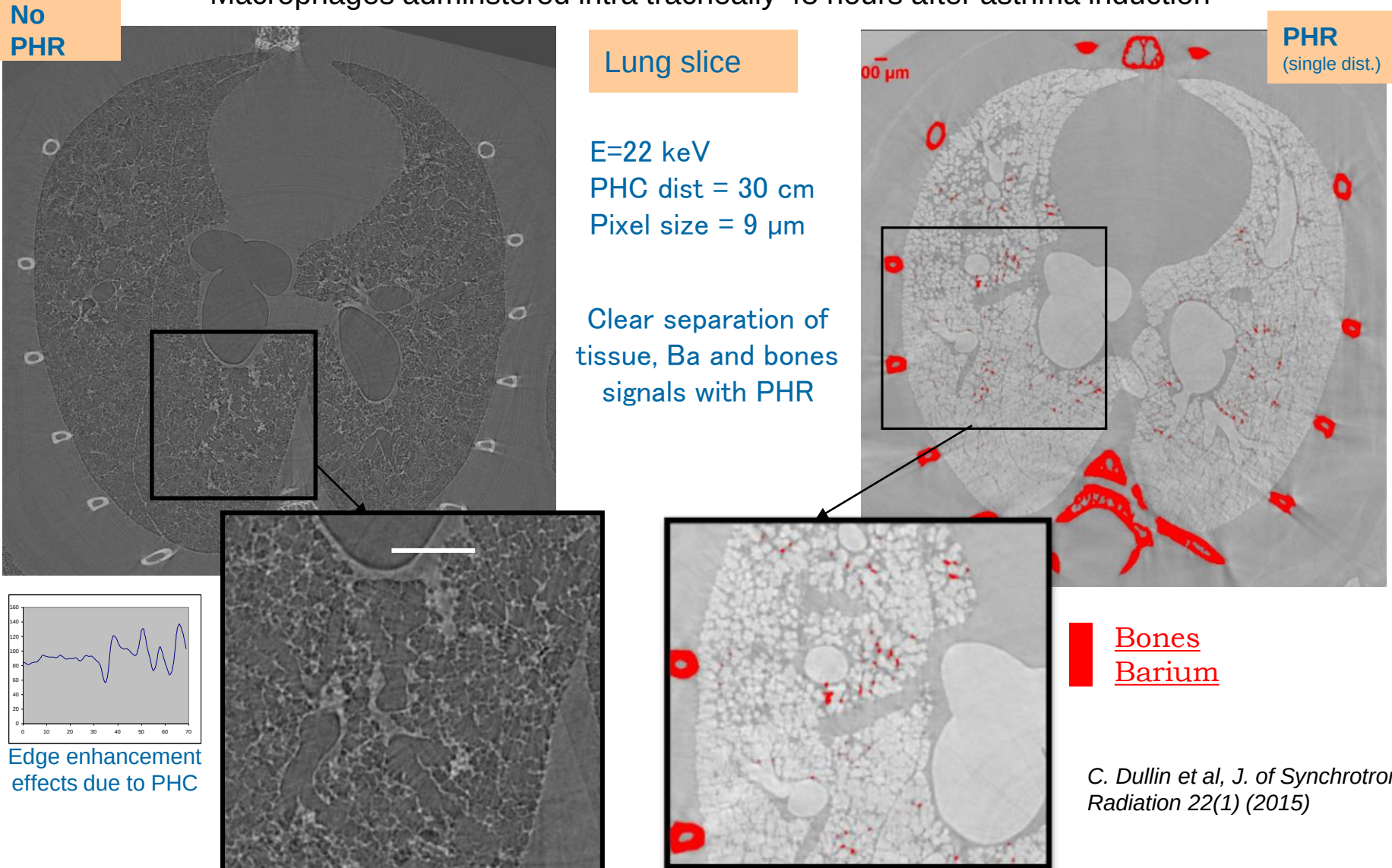
Native mouse
untreated



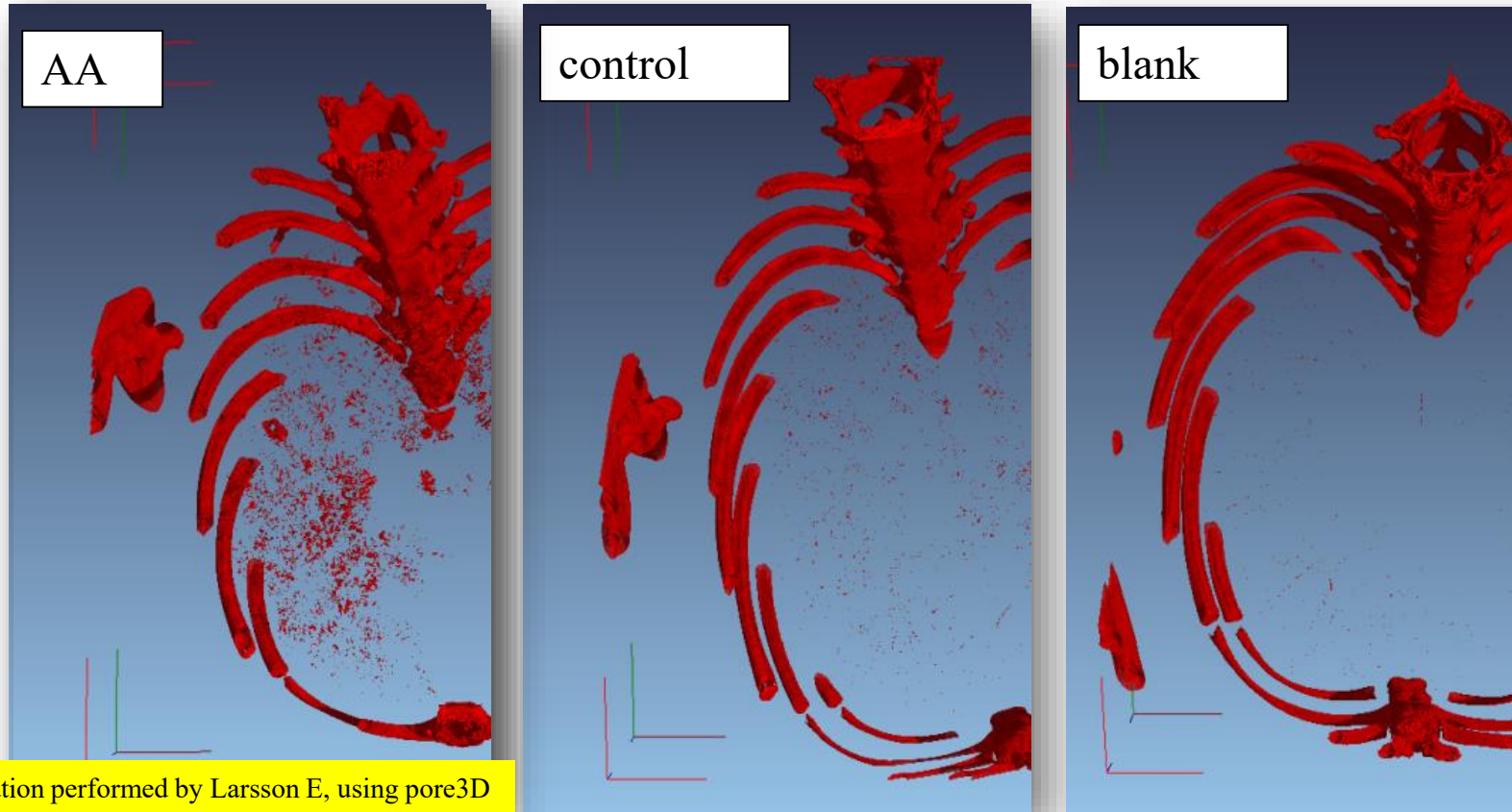
Macrophages were administered
intra tracheally 48 hours after
asthma induction

Imaging of inflammation in asthmatic mice: potentials of PBI

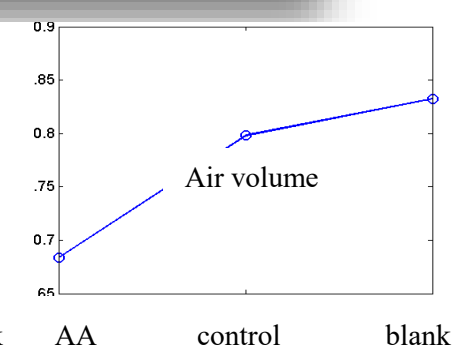
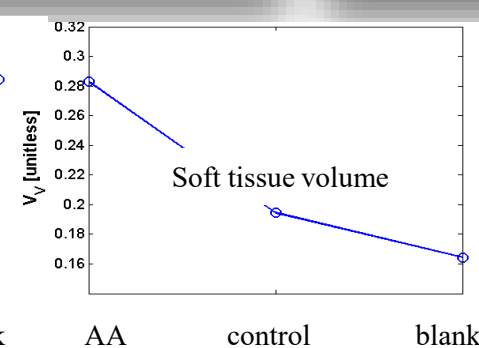
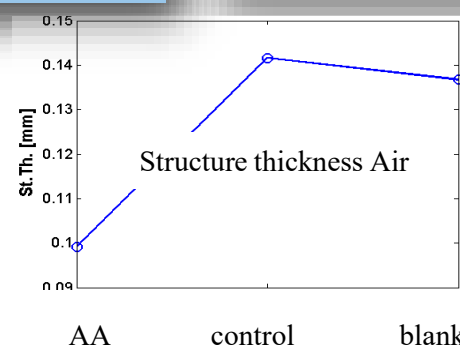
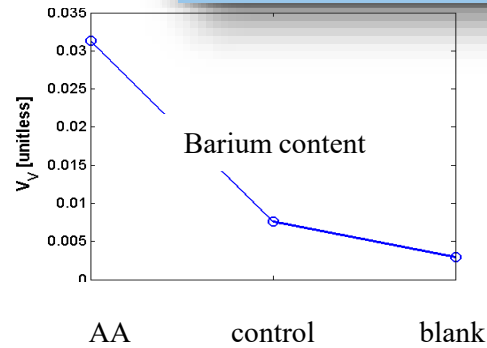
- Animal model of allergic asthma induced by ovalbumin based on balb/c mice
- Murine Alveolar Macrophage Cells stained with Barium sulfate (Guerbet, F)
- Macrophages administered intra tracheally 48 hours after asthma induction



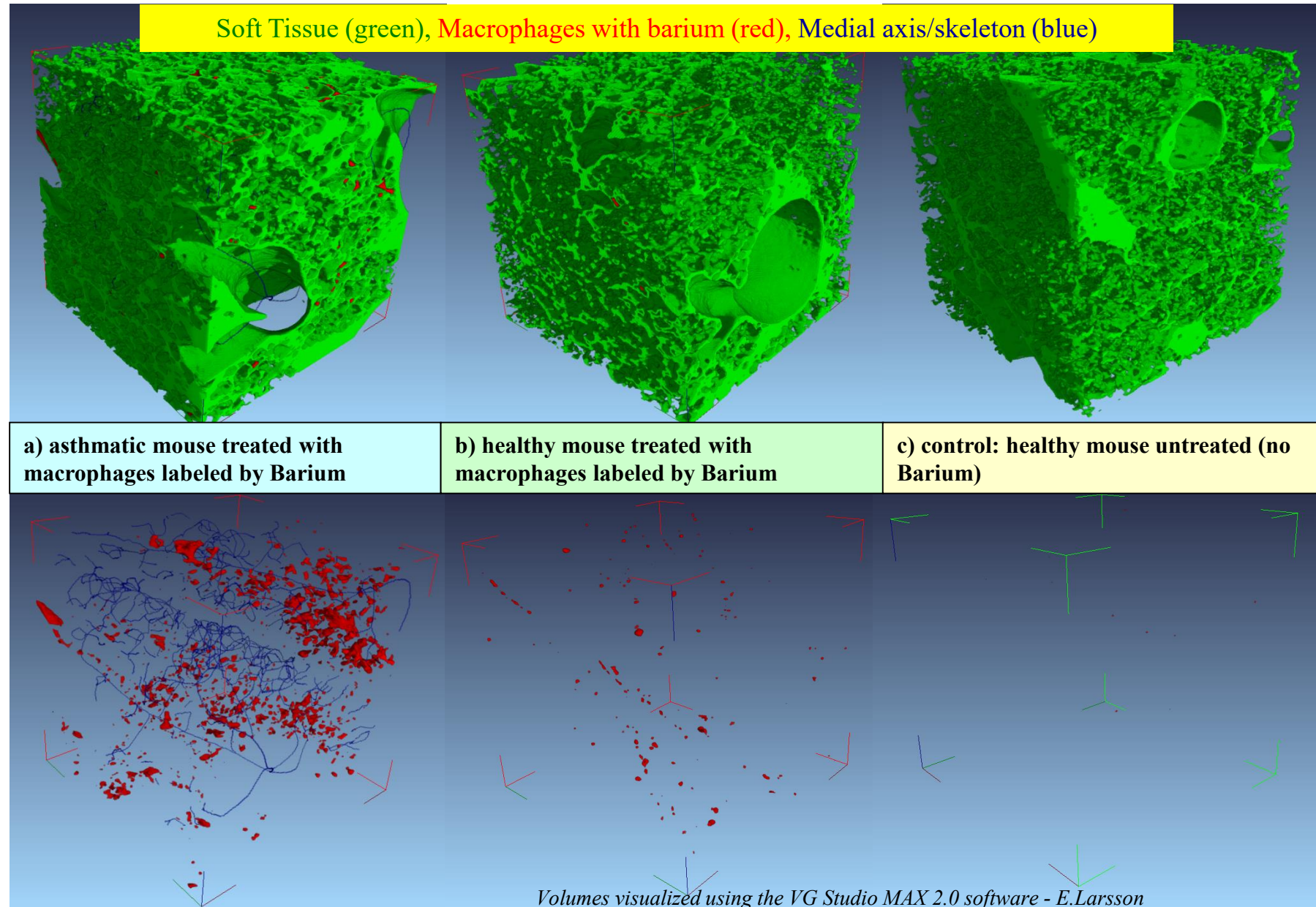
Visualization of labeled macrophages



Quantification performed by Larsson E, using pore3D

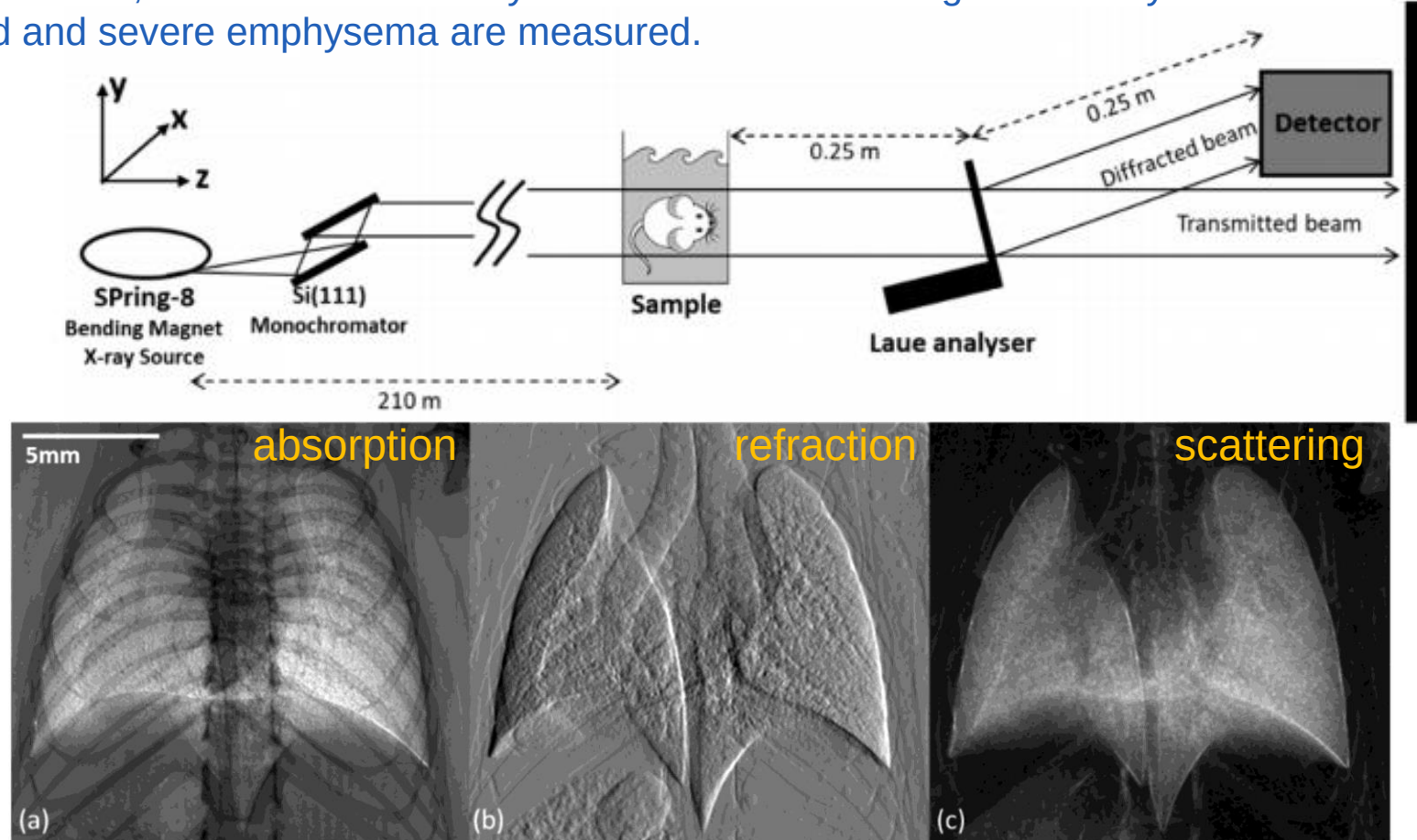


VOI of soft lung tissue



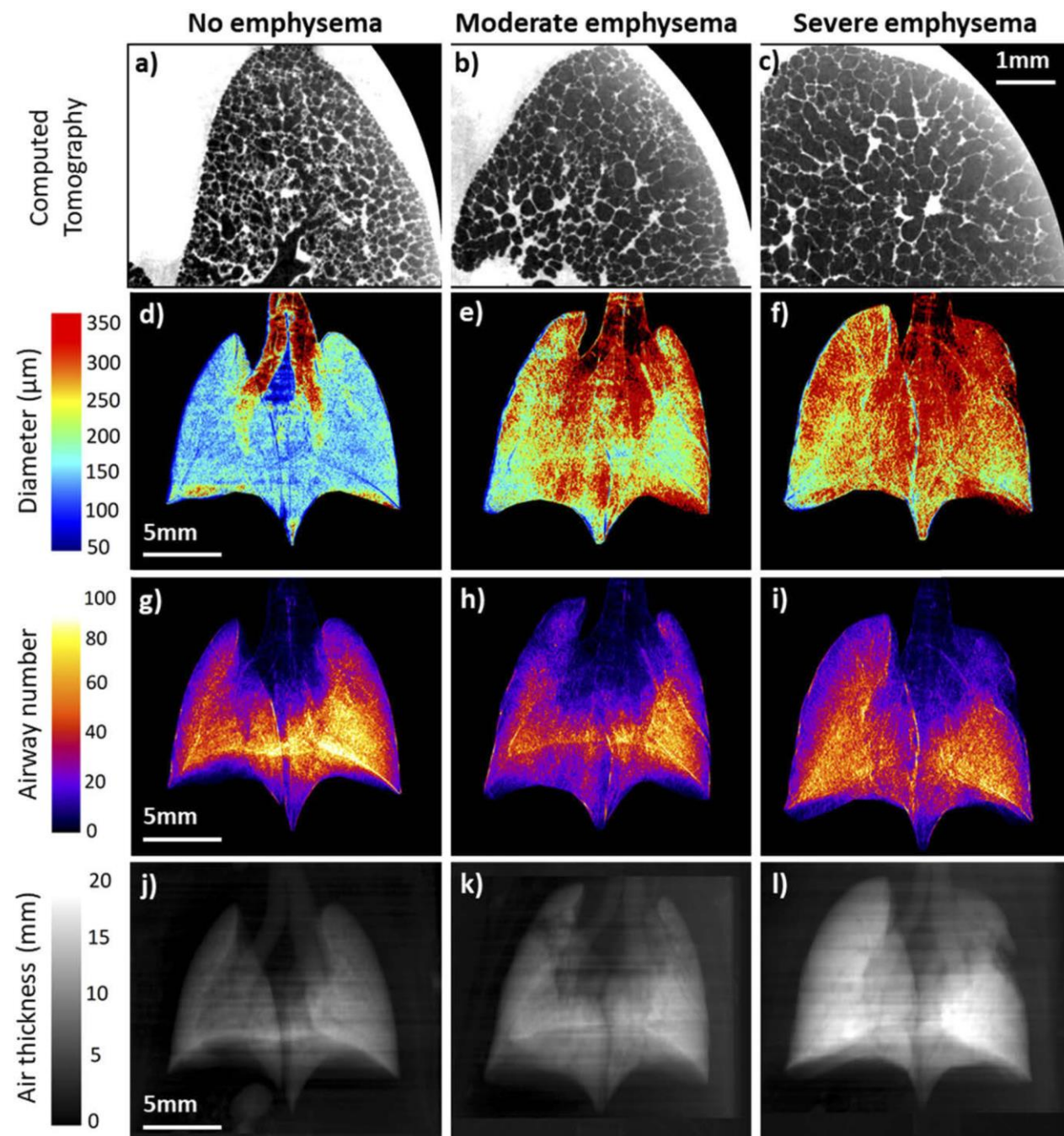
Emphysema quantified: mapping regional airway dimensions using 2D ABI

- ABI can be used to measure the mean length scale of pores or particles that cannot be resolved directly by the system.
- By combining **attenuation**, **phase** and **ultra-small angle X-ray scattering (USAXS)** information, differences in airway dimension between lungs of healthy mice and those with mild and severe emphysema are measured.



Row 1: CT slices. The alveoli of the emphysematous mice are enlarged compared with those of the control mouse.

Rows 2, 3 and 4: images of airway diameter (d-f), the number of airways in projection (g-i), and the projected air thickness maps (j-l) of the lungs



Cell tracking brain tumors in rats

C6 glioma cells were cultured and some of the cultures were exposed to colloidal **Gold Nano Particles** (GNP) for 22 hrs.

Cells were implanted into the brain of adult male Wistar rats with animal under anesthesia.

The animals were sacrificed two weeks later.

The detection of labeled cells is **enhanced by the higher absorption of gold** with respect to tissue and by PHC effects.

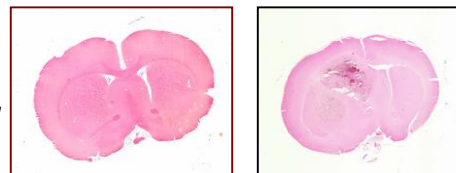
Aims for cell tracking:

- to monitor the dynamic of tumour growth
- to follow the migration of tumour cells
- to understand the metastasis spread dynamic

E = 24 keV

Num. proj. = 720, Pixel size = 14µm

Histologies – 2 weeks after implant.



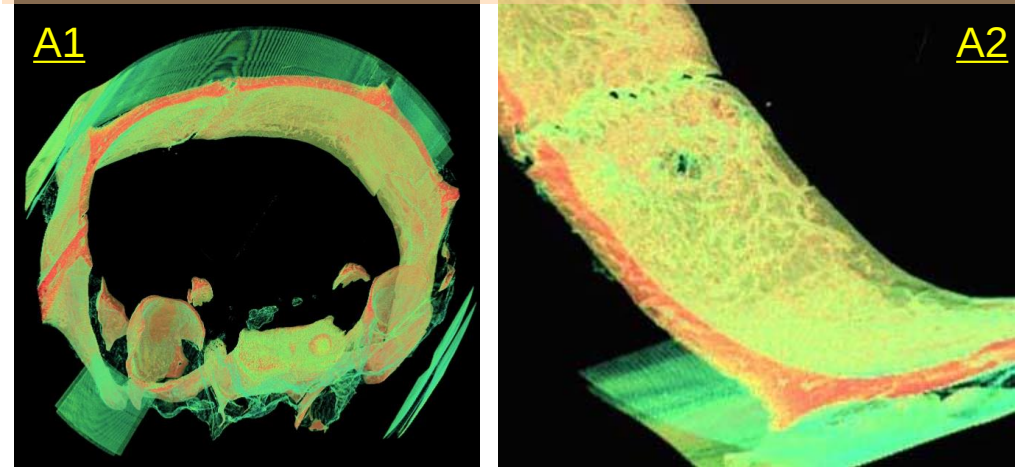
Healthy

Brain with C6 cells

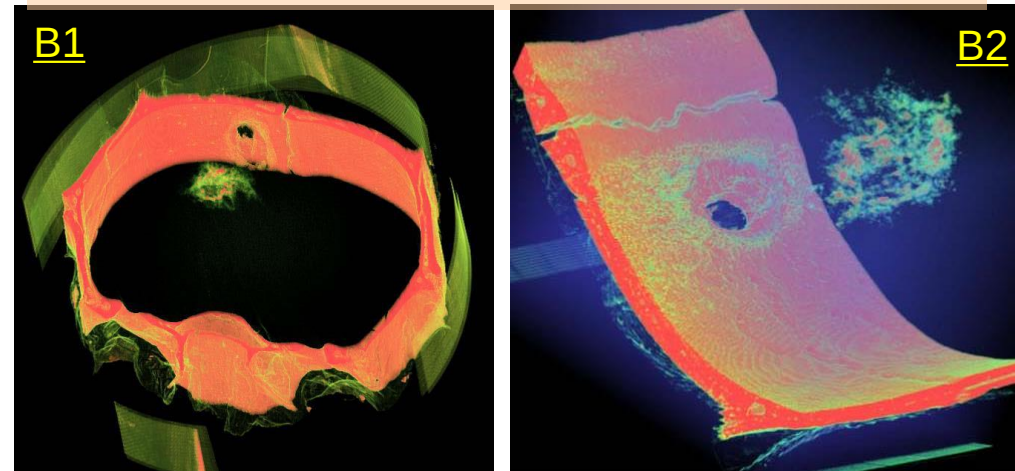
C,J,Hall et al., Eur. J. of Radiol.,
Volume 68, Issue 3 (2008)

3D rendering of 3 mm height skull portion

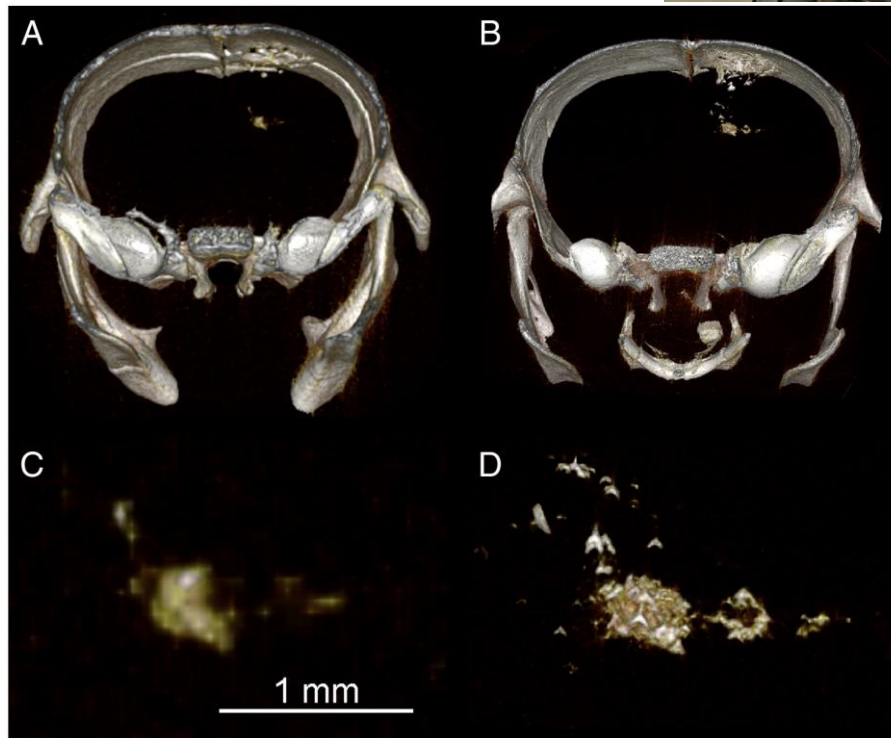
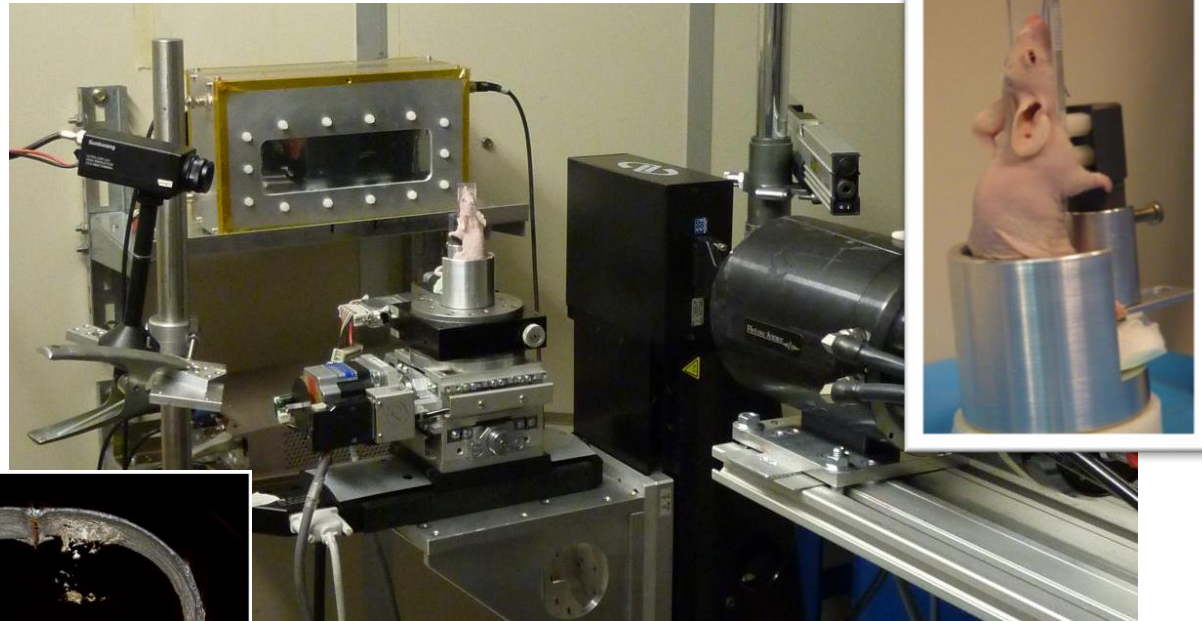
A1 and A2: Tumor with 300,000 cells – not labelled



B 1 and B 2: Tumor with 300,000 colloidal gold-loaded cells



In-vivo study at low dose



Comparison of two 3D renderings of a CT of a mouse injected with 100,000 GNP-loaded F98 cells depicts:

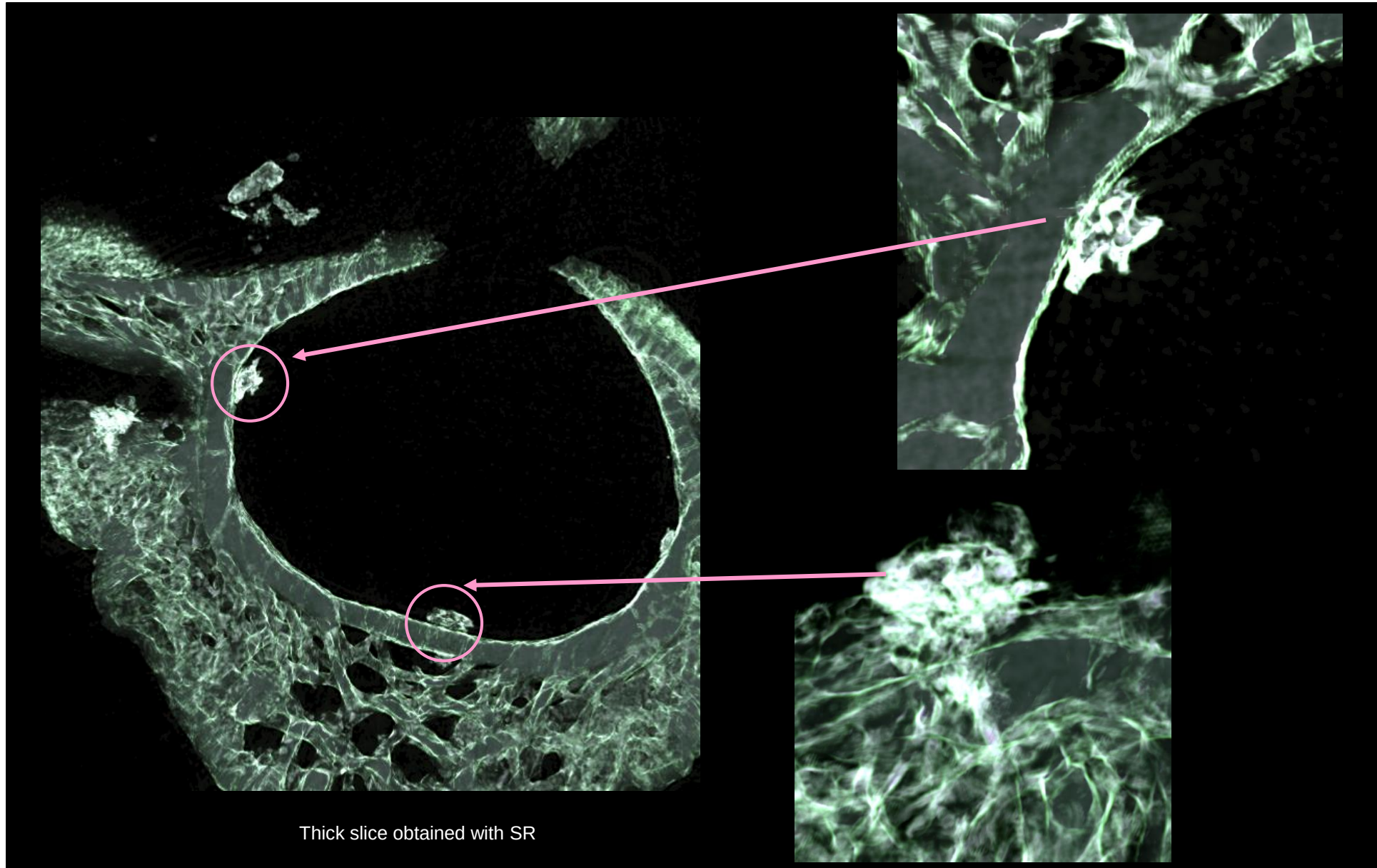
(A–C) - low x-ray dose *in vivo* data

(B–D) the high x-ray dose *ex vivo* data

The images in panels C and D are enlargements at full system resolution of the developed tumor depicted in panels A and B, respectively.

First experiment *in vivo*: lesions are visible also at low doses

Rat 706: Metastasis spread in the spine



Clinical applications

- Imaging on patients
 - *Need to **limit** radiation dose. Strict research protocol for selected patients. Find best compromise between dose and image quality, pixel sizes : 50 – 100 μm*

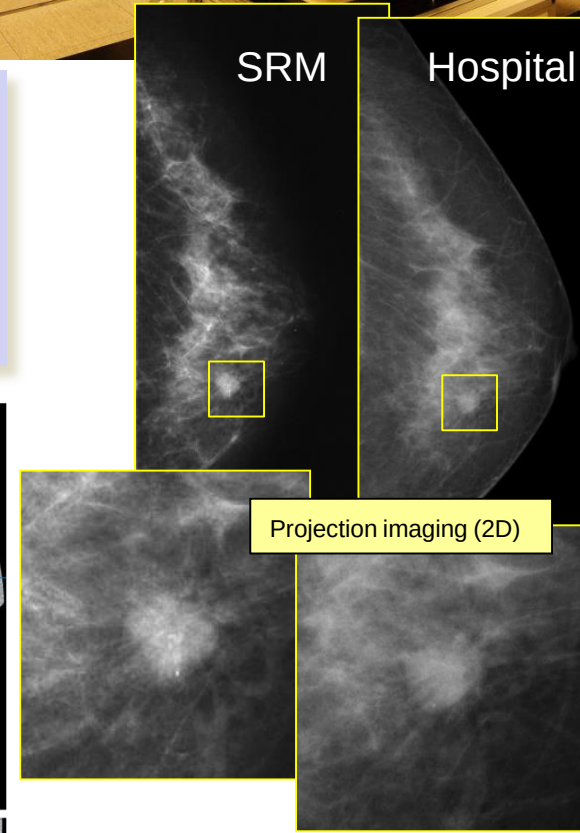
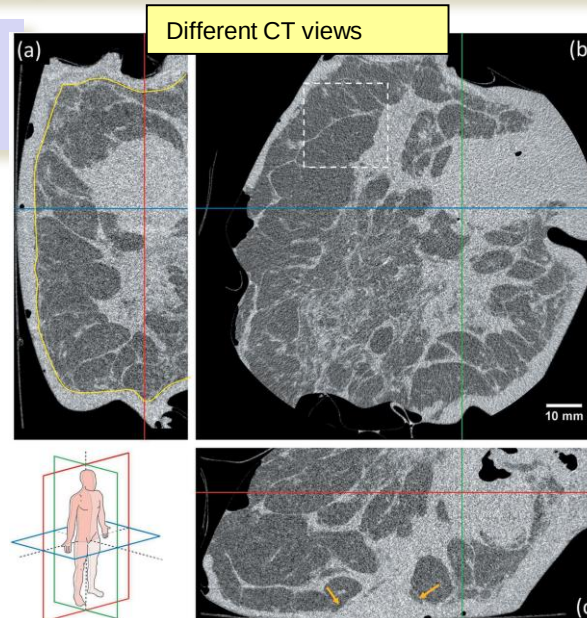
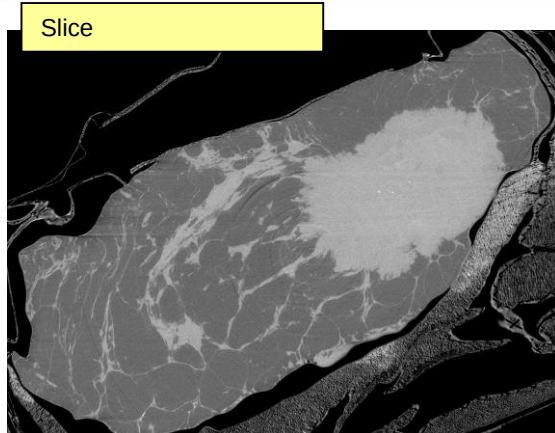
Breast imaging – first mammography protocol with patients at Elettra

Outcomes of first protocol

Clinical images with SR have:

- *higher specificity,*
- *better agreement with the golden standard (biopsy),*
- *improved image quality,*
- *strong reduction of X-ray doses.*

Next step: 3D imaging: Low dose phase
contrast breast CT (BCT) protocol



Breast CT program @ the Australian source

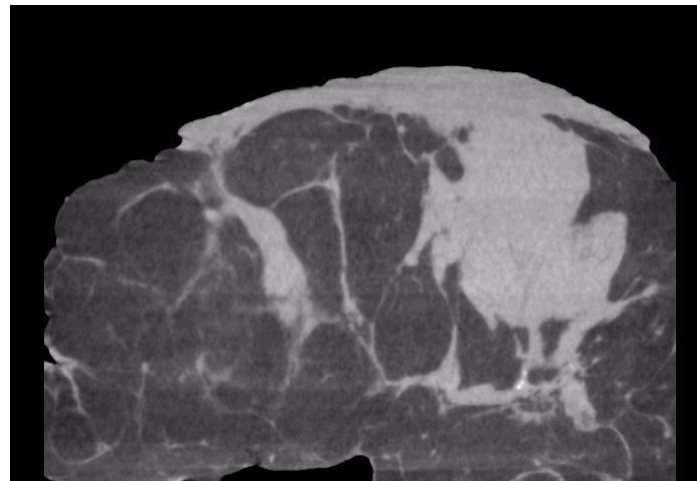
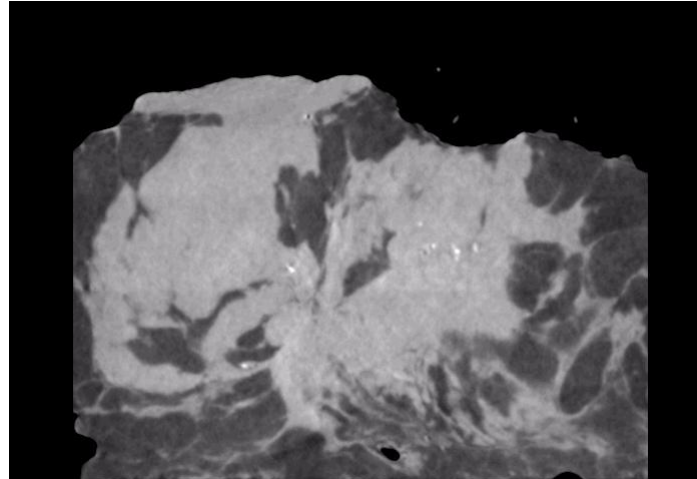
- A program for breast CT (BCT) is ongoing at the Australian synchrotron
- Excised breast tissue containing a multifocal invasive papillary carcinoma with skin invasion. The tumor is composed of multiple nodules through the scar tissue.

- **Parameters**

Detector pixel size = 100 μm

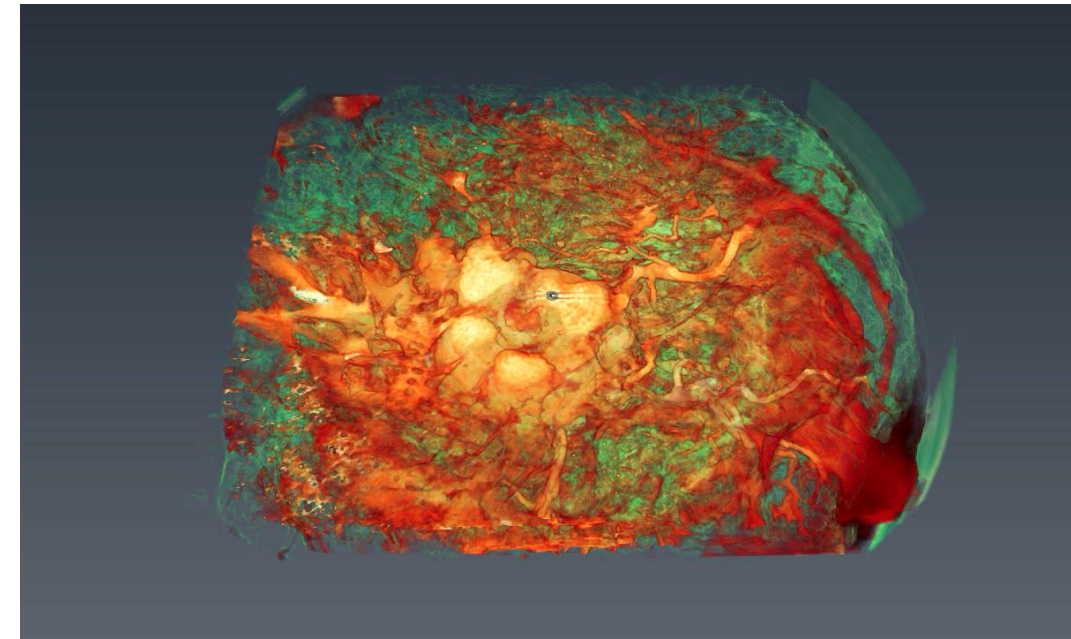
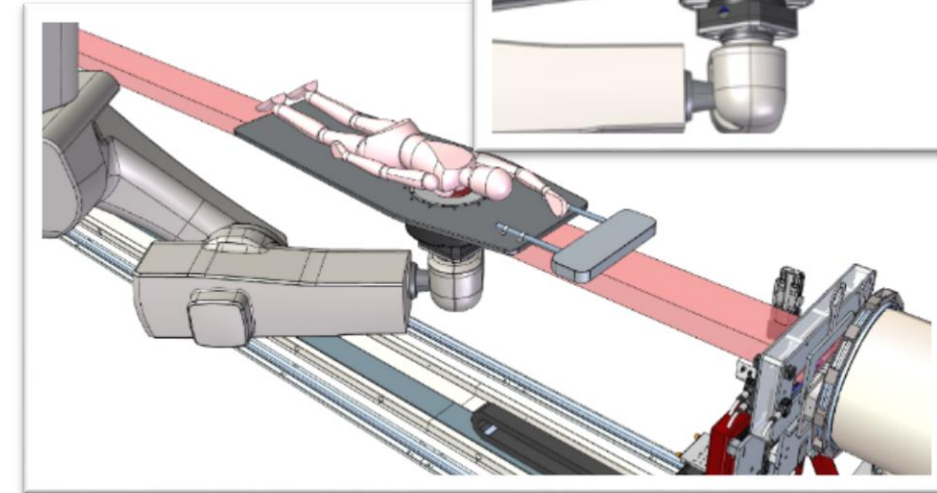
MGD $\sim$ 5 mGy

Energies = 38 keV, Distance = 5,7 m



Feasibility studies carried out on breast mastectomies demonstrated that BCT has a **high potential** for improving the quality and diagnostic value of images obtained using conventional mammography and digital breast-tomosynthesis.

Furthermore, BCT is a painless process as no breast compression is required



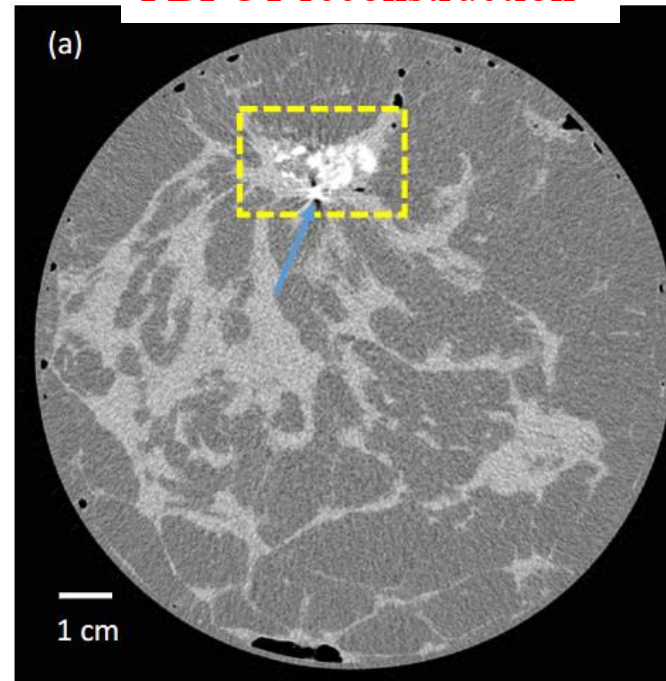
Dark field in PBI breast CT

Dark field signal can be extracted from PBI images using an approximated method (considering samples made predominantly of a single material, or several different materials with similar ratios of δ/β).

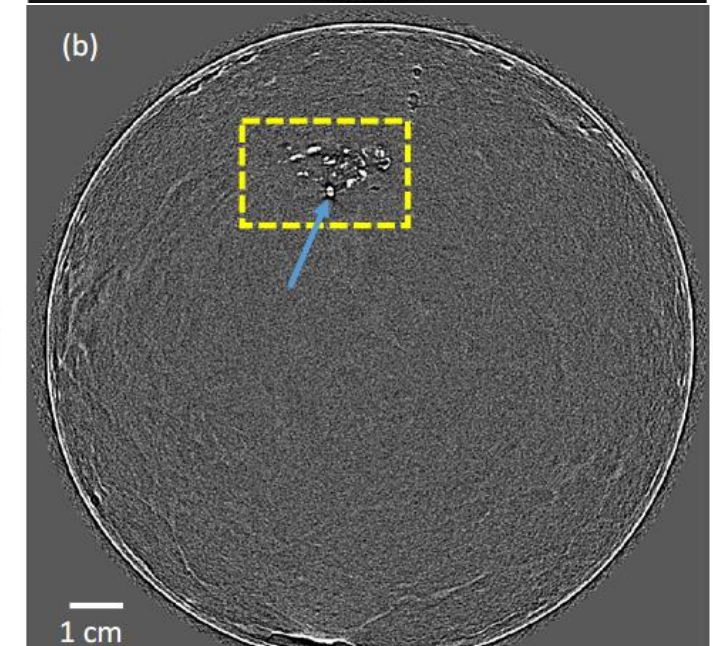
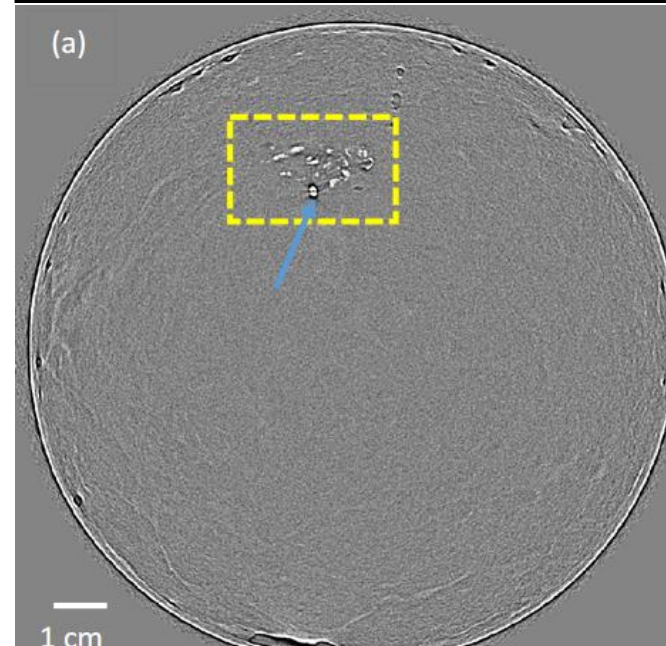
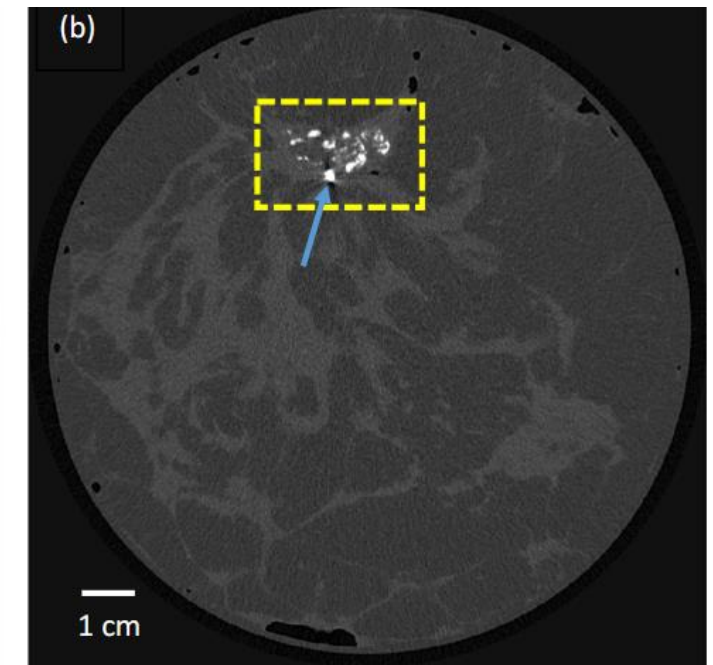
Dark-field is capable of delivering better-quality images showing the shape and quantity of microcalcifications which are often used to differentiate between benign and malignant tissues

T E Gureyev *et al* 2020 *Phys. Med. Biol.* **65** 215029

PBI CT reconstruction



PBI CT reconstruction optimized grayscale

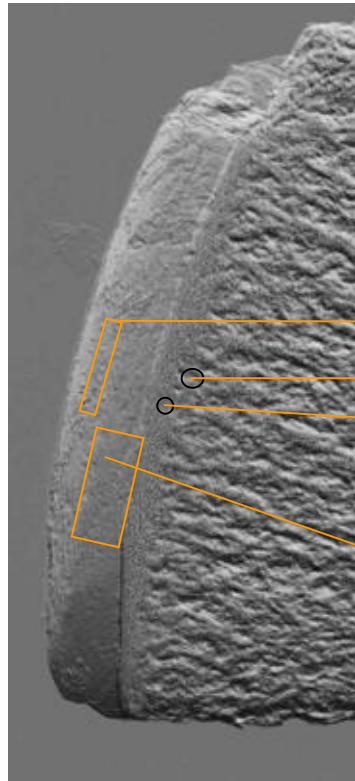


DF CT reconstruction

DF CT reconstruction optimized grayscale

ABI studies of Cartilage and bone interface

Osteoarthritis (OA) is a disease characterized by the progressive degeneration of articular cartilage and the development of altered joint congruency. It has a high incidence in the adult population. Affecting mainly the elderly population, it is one of the main causes of disability worldwide. Conventional radiography detects only **important osseous changes**, at advanced OA or RA stages, when therapeutic strategies are less effective. **Early changes** in the **cartilage** and other **articular tissues** are **not** directly visible. MRI imaging works better but the maximum achievable spatial resolution is not always adequate.



Need to study:

- cartilage
- cartilage-bone interfaces
- changes in the bone structure

Superficial Layer (Zone of horizontal collagen fibers with flat cells)

Subchondral Bone Plate (**Important for diagnostic purposes in OA**)

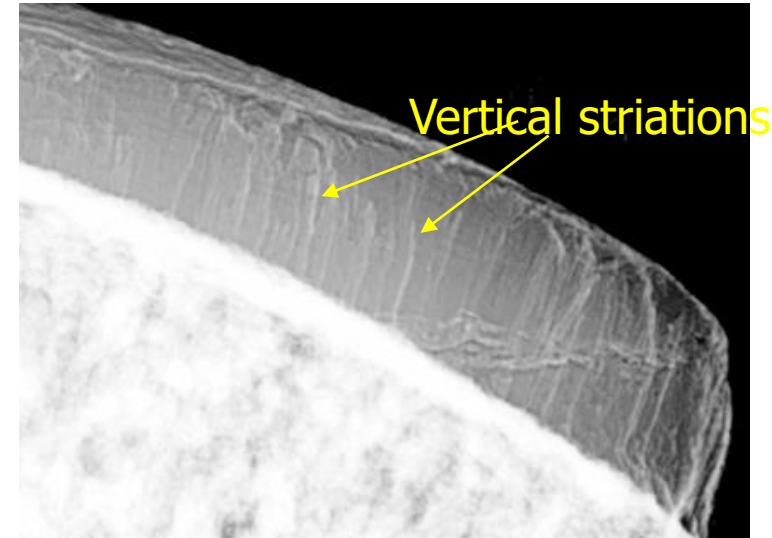
Tidemark (Border between normal and mineralized cartilage)

Transitional and Deep Layer (round cells, collagen fiber switches from horizontal to vertical orientation, increasing stiffness and material density)

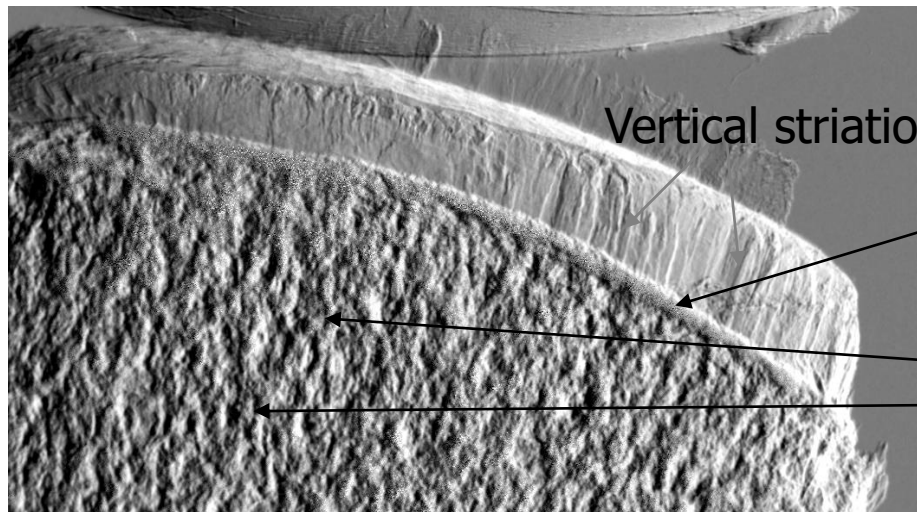
Aim: detect the architectural arrangement of collagen within cartilage and evaluate how the cartilage degeneration affects the underlying subchondral and trabecular bone.

Femur head core cuts: collagen arcades structure

- The ABI technique allows to visualize the discontinuities in the sample and the inner structures invisibles by means of conventional X-Ray imaging.
- The transition bone-cartilage is emphasized.
- The articular cartilage striations are well visible due to X-ray diffraction at edges of fibers



Refraction image



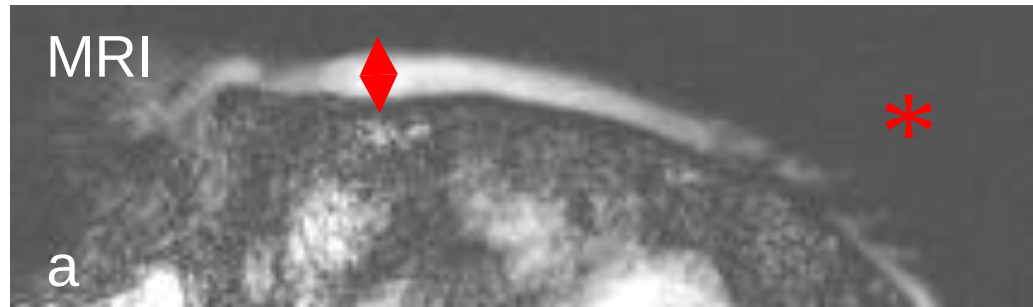
Apparent absorption image

Elettra
25 keV

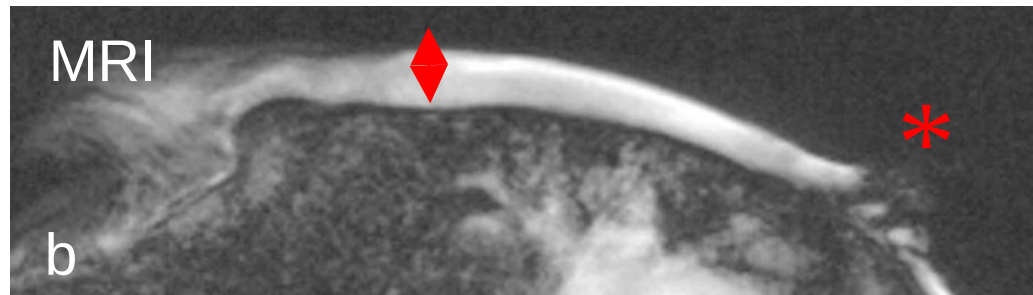
Subchondral
bone

Trabecular bone

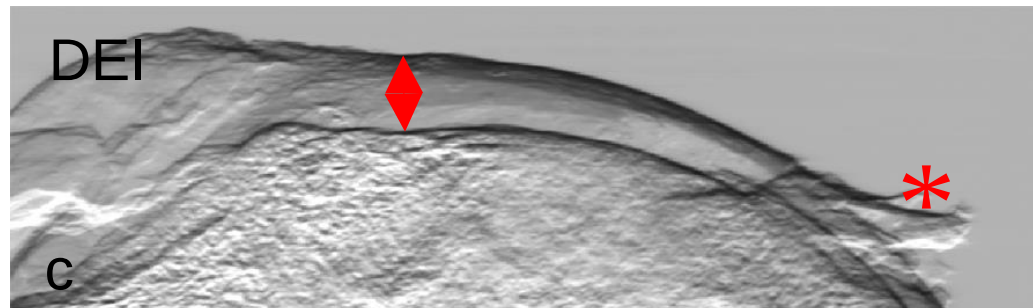
Femur head core cuts: comparison with MRI

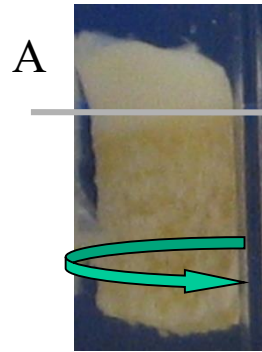


5 sec



150 sec

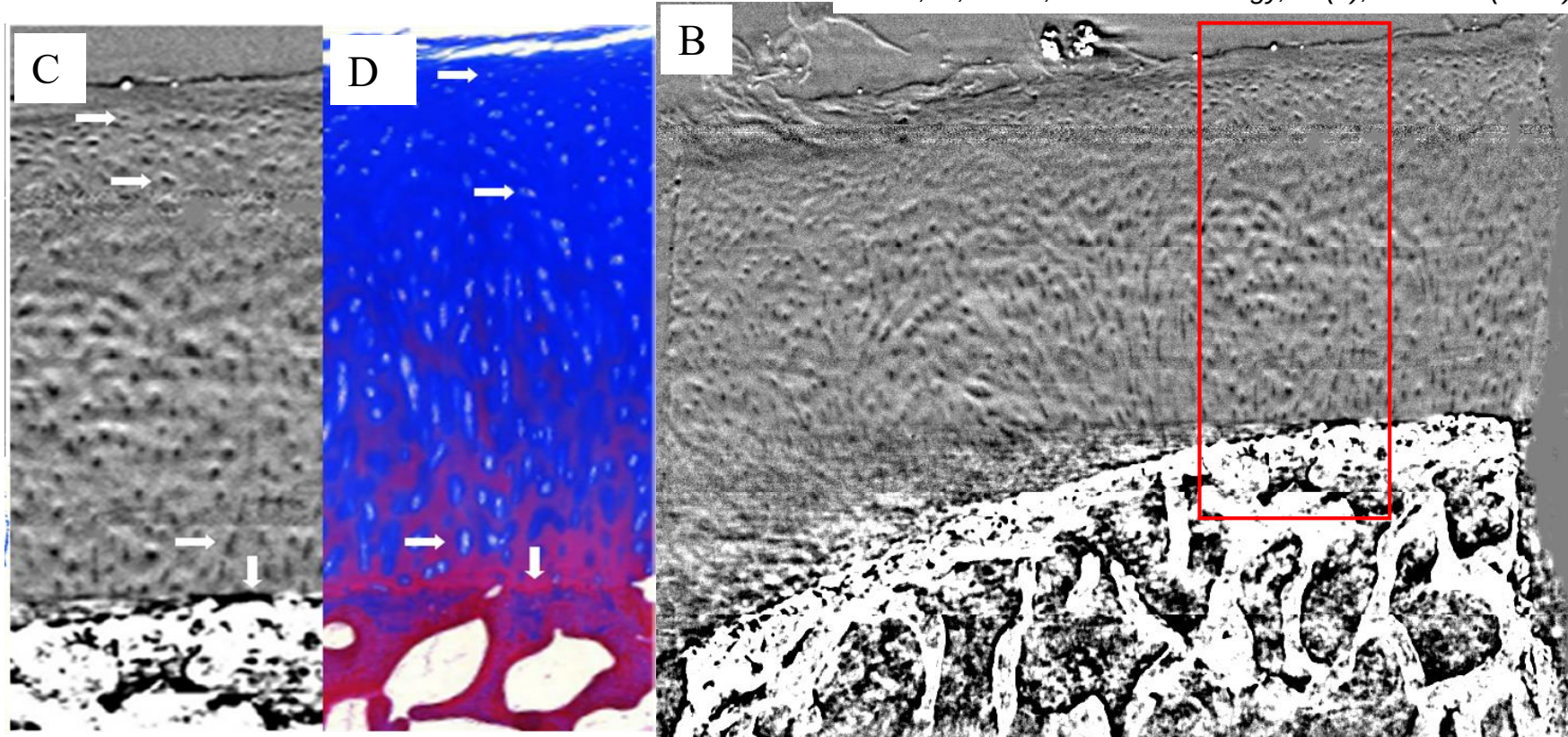




Specimen of normal cartilage (A), Coronal plane extracted from the reconstructed CT volume (B), Magnified portion identified by the ROI (C), Corresponding section from histologic preparation (D).
E = 26 keV, pixel size = $8 \times 8 \mu\text{m}^2$.

ABI in planar and tomographic modes was performed *in vivo* on articular joints of guinea pigs. Images showed the potential of technique in revealing initial lesions. Images with high spatial resolution and with an acceptable radiation dose.

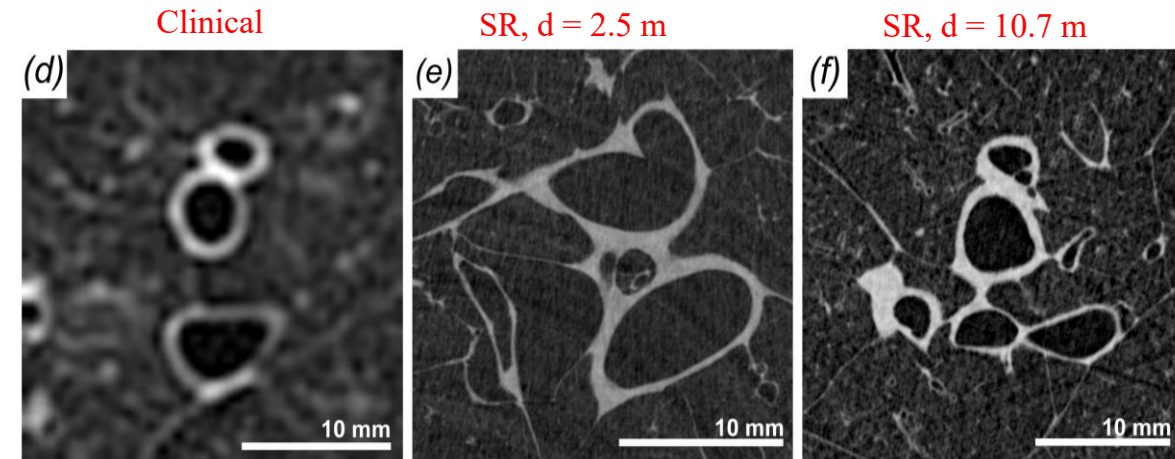
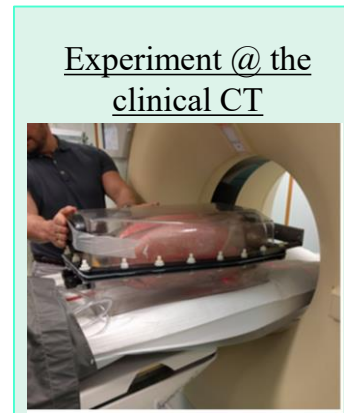
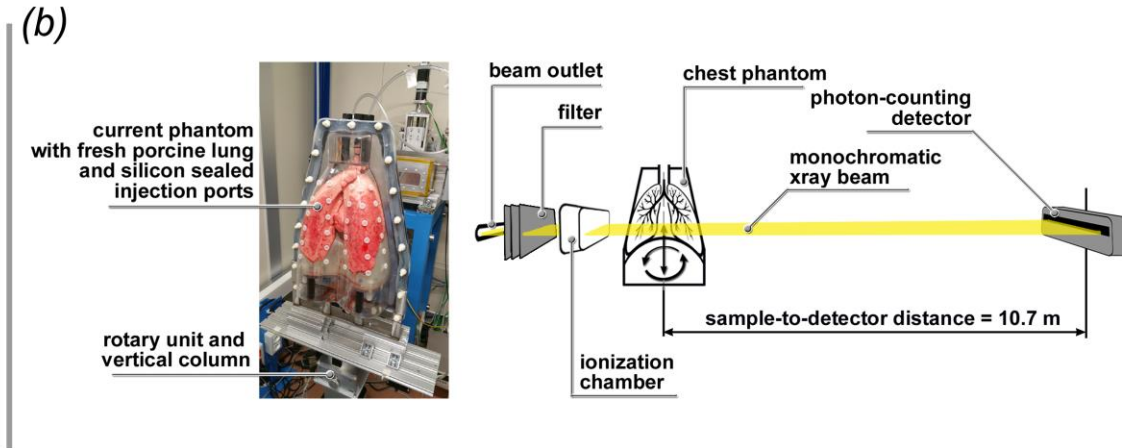
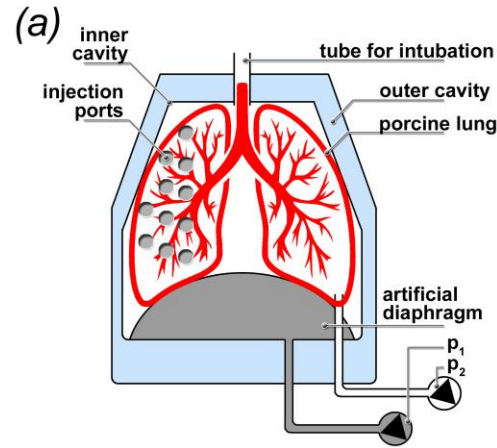
Coan, P., et al., *Invest. Radiology*, 45(7), 437-444 (2010)



The LUNG-CT project at Elettra

Aim: evaluate the potentials of propagation based lung CT in the early detection of **lung cancer** and **Idiopathic Lung fibrosis**

- Samples: porcine lungs in the artiCHEST phantom
- $E = 40$ keV, prop. Dist. up to 10.7 m, air entrance dose ~ 13 mGy
- Reconstruction: conventional FBP, phase retrieval pre-processing
- Resolution $\sim 67 \mu\text{m}$
- Comparison with conventional clinical CT (Cattinara Hospital, Trieste)



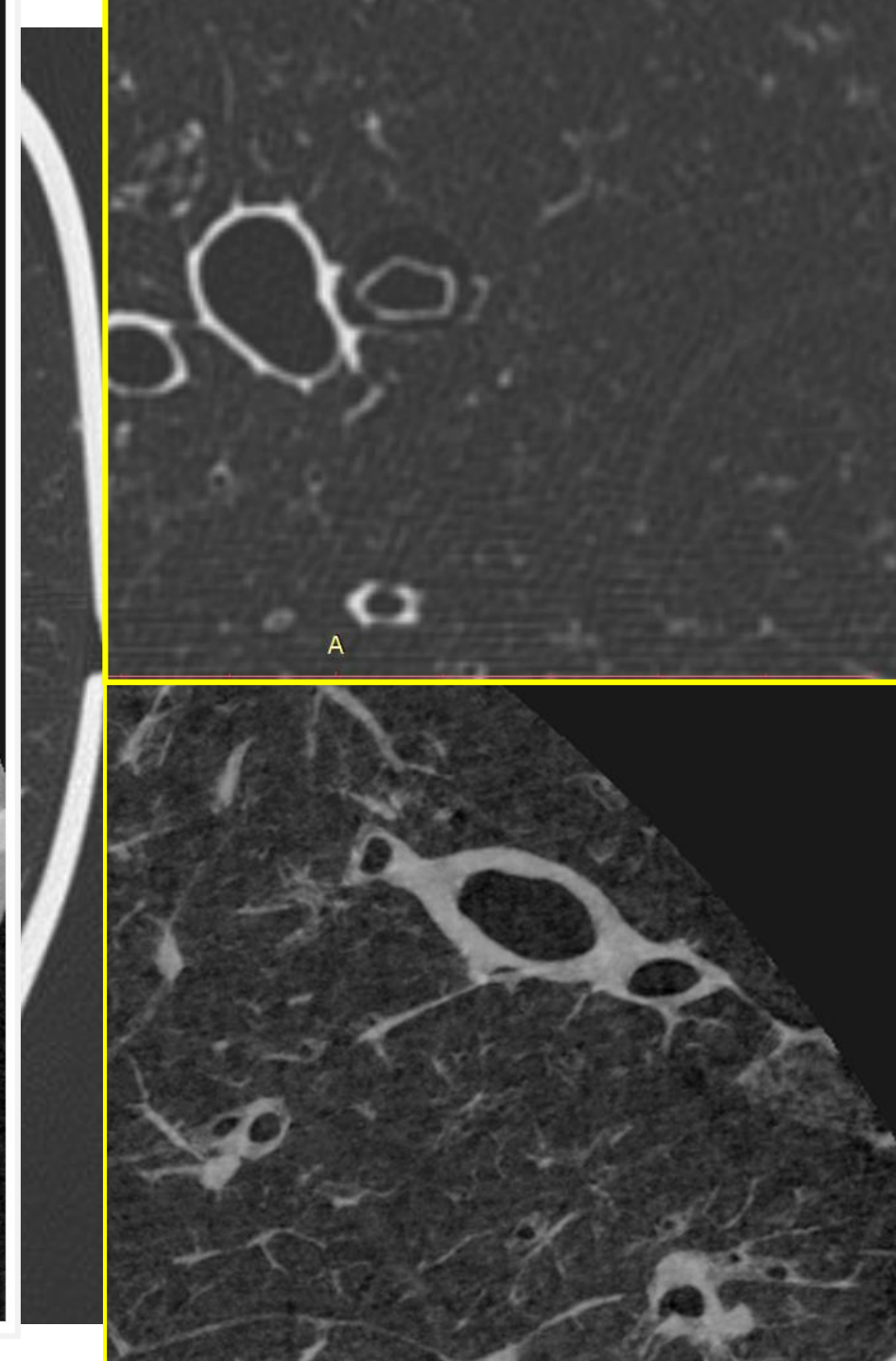
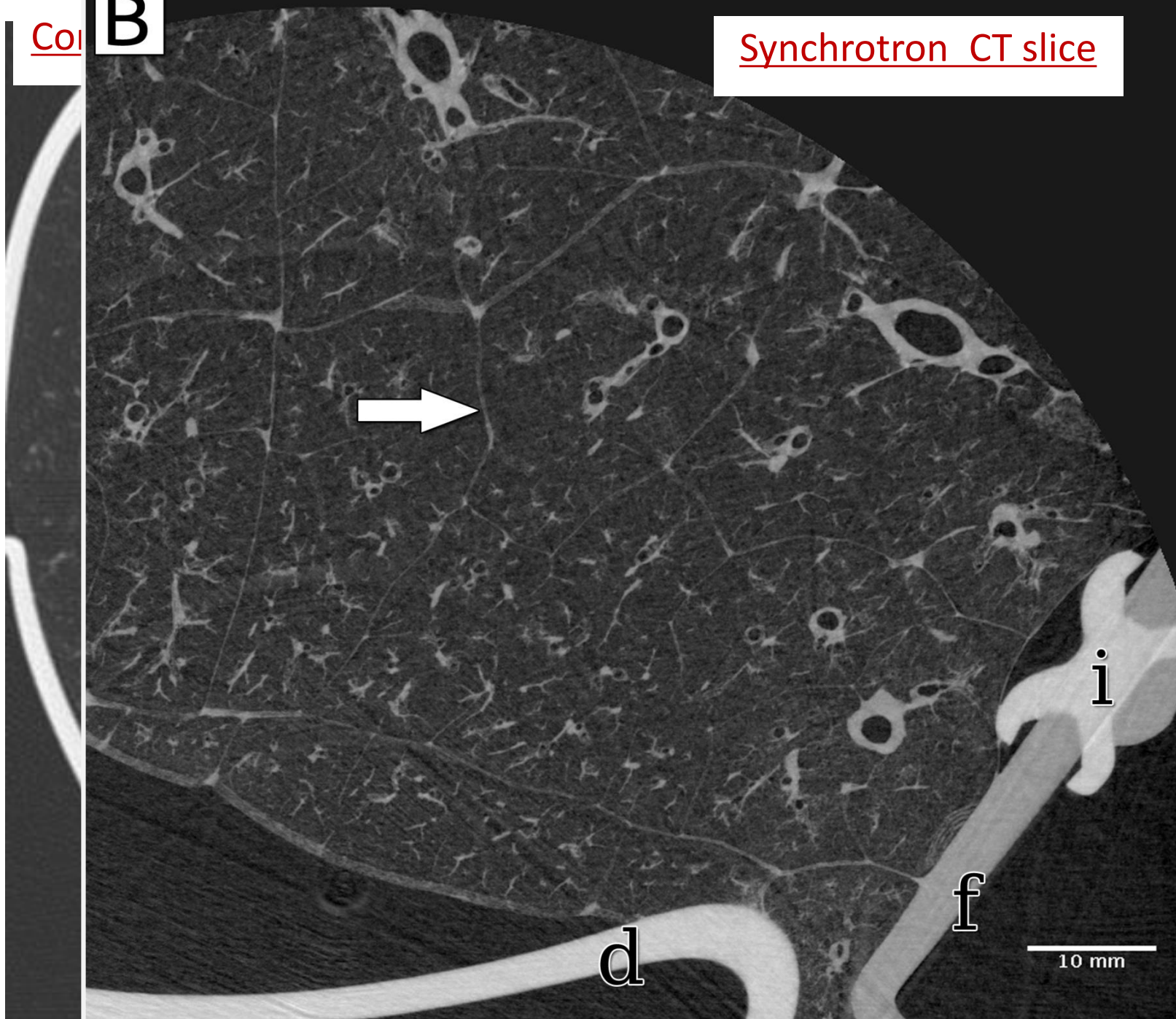
SR image quality is higher increasing the propagation distance from 2.5 to 10.7 m even a lower dose level

Clinical CT (d, resolution $450 \times 450 \times 900 \mu\text{m}$) shows similar details rather blurred.

Col

B

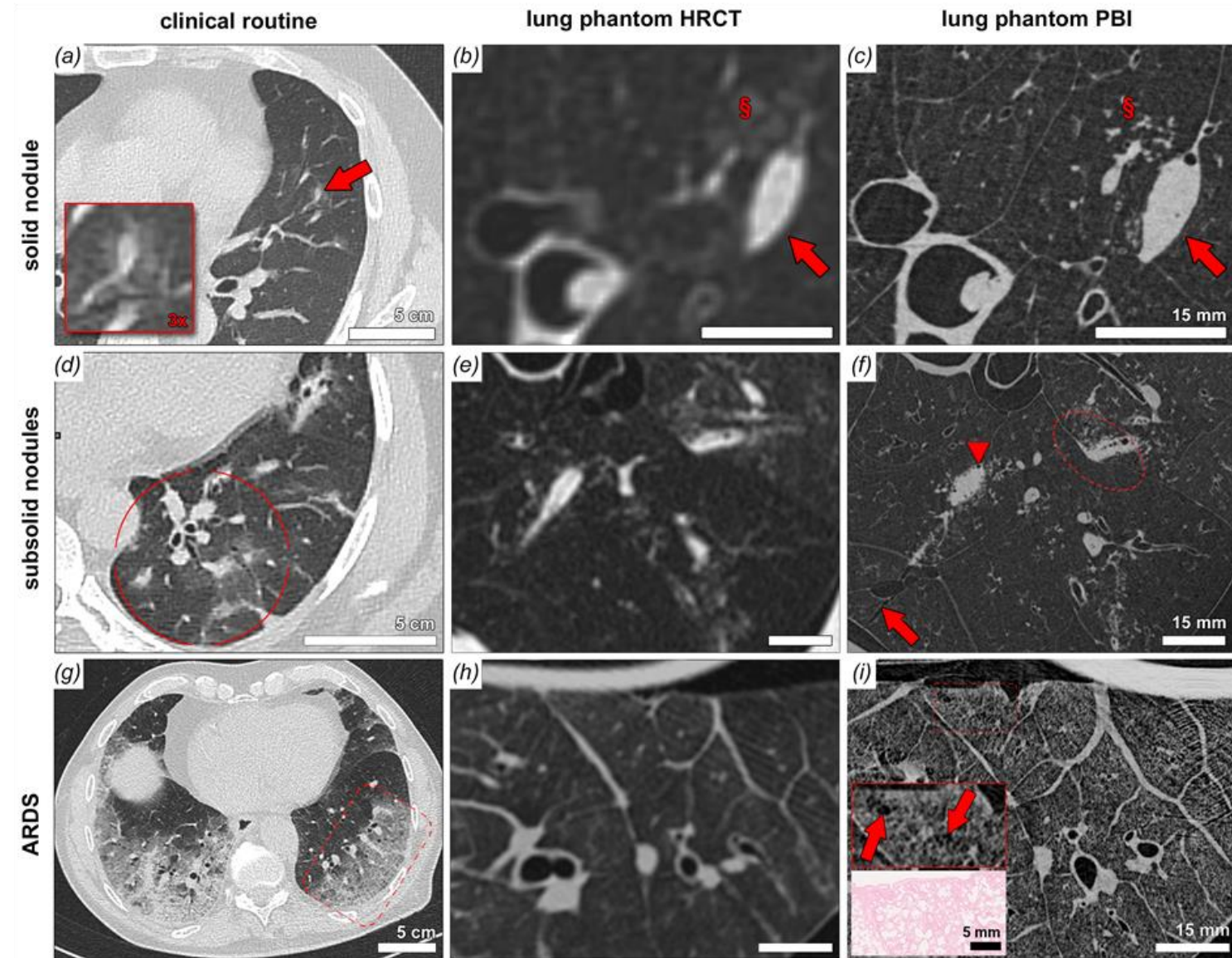
Synchrotron CT slice



What would be the clinical benefit?

a, d and g comparative clinical examples of pulmonary damage patterns from cases of viral pneumonia. Patterns of solid nodules (a), subsolid nodules (b) and acute interstitial pneumonitis/ acute respiratory distress syndrome (g)

Artificial lesions mimicking those patterns were imaged by clinical HRCT (b,e and h) and the same lung regions were imaged by PBI-based local tomography (c, f and i).

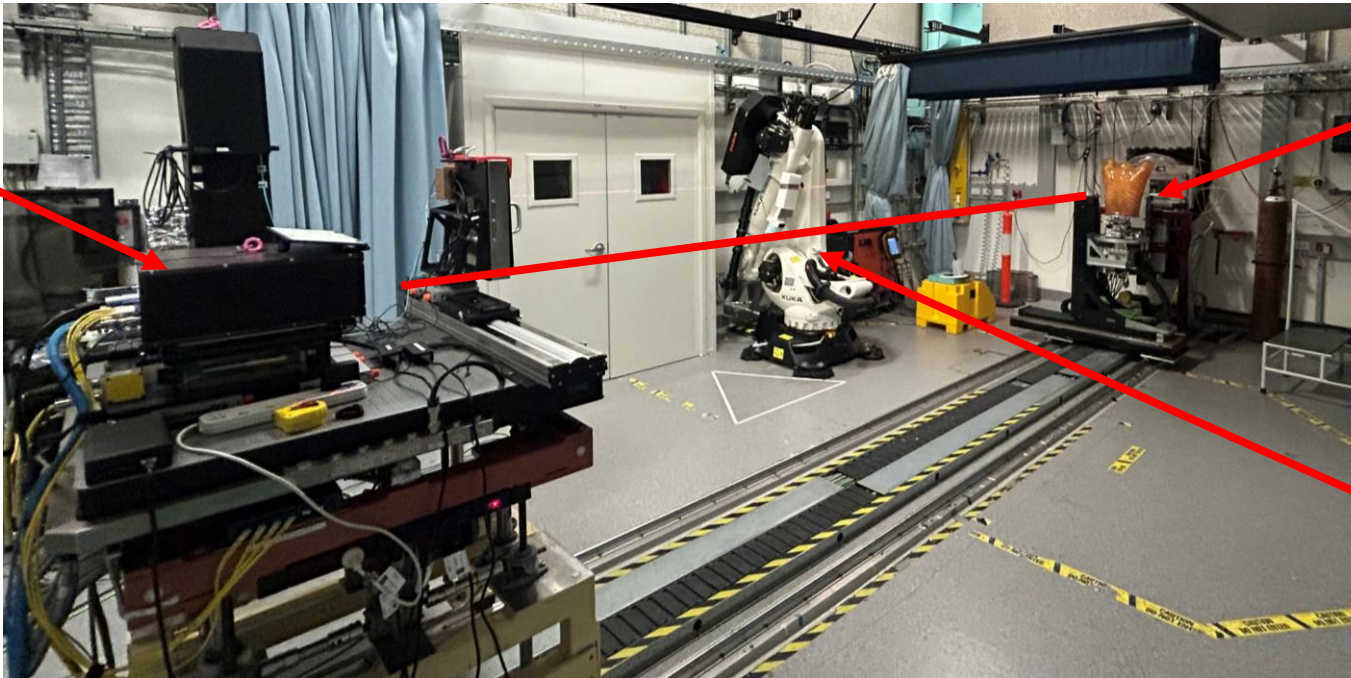


Lung project at the Melbourne Imaging and Medical Beamline (IMBL)

Usable energy range 40-100 keV



Detector

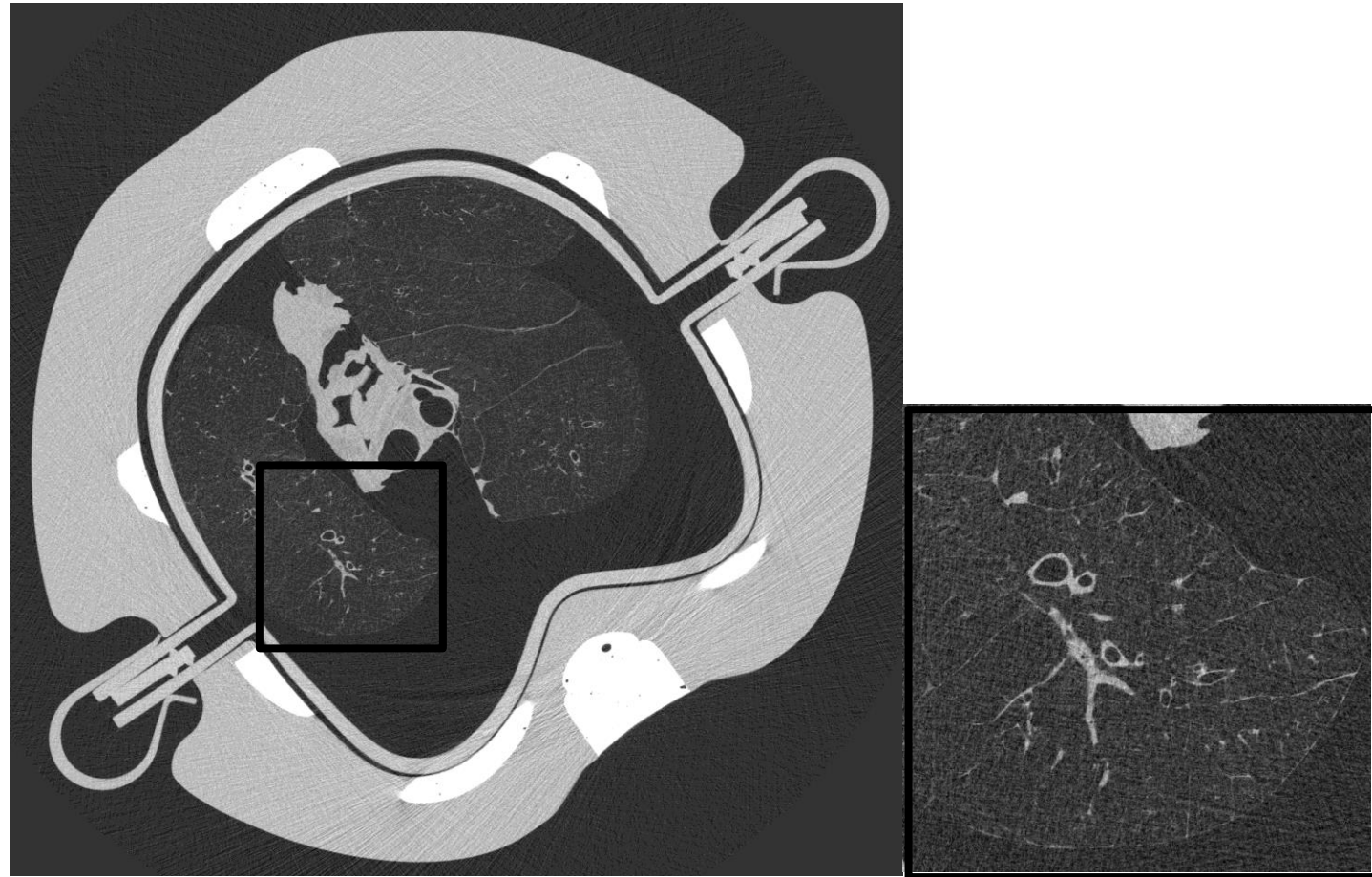


Sample on the rotator stage

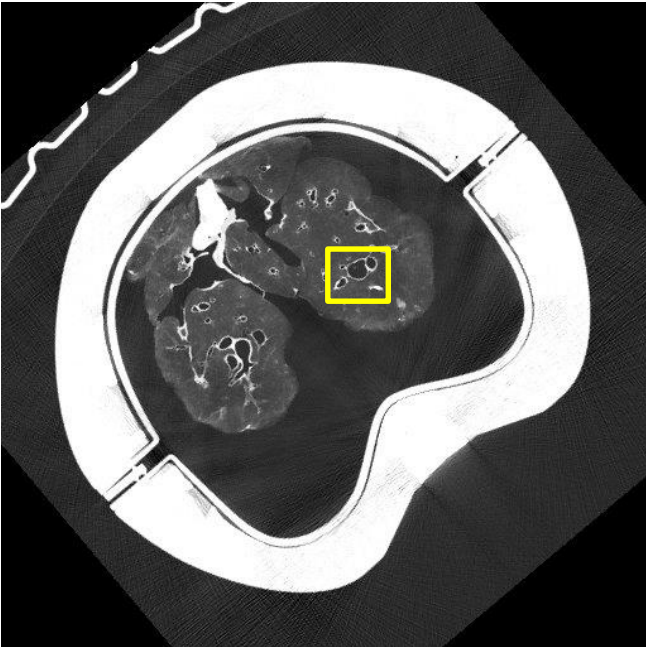
Propagation distance

Use of the anthropomorphic phantom at Melbourne

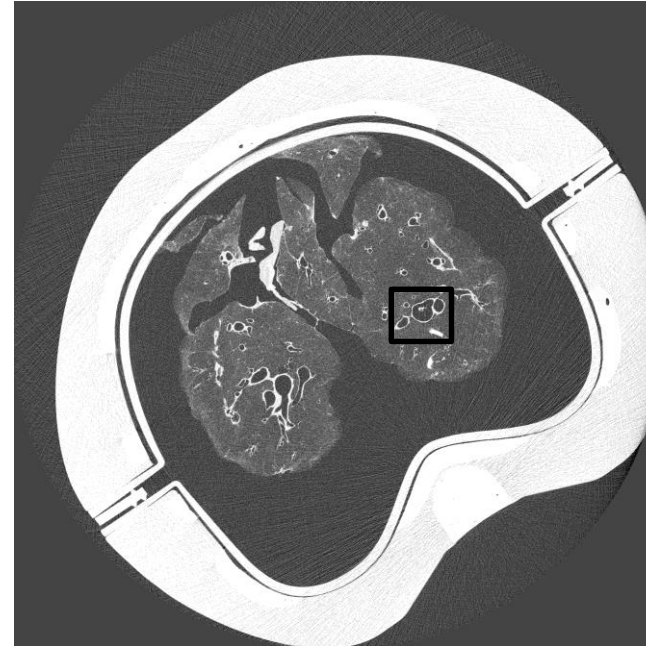
- Energy: 70 keV
- Distance sample-detector: 7.5m
- Exposure time per proj: 10 ms
- Total acquisition time: 36s
- Incident dose: 11.5 mGy



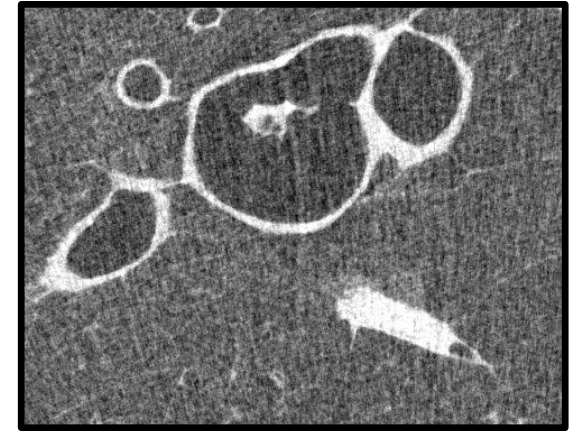
Visualization of agarose nodules (clinical CT vs. Synchrotron)



HR SCAN (0.7 mm)



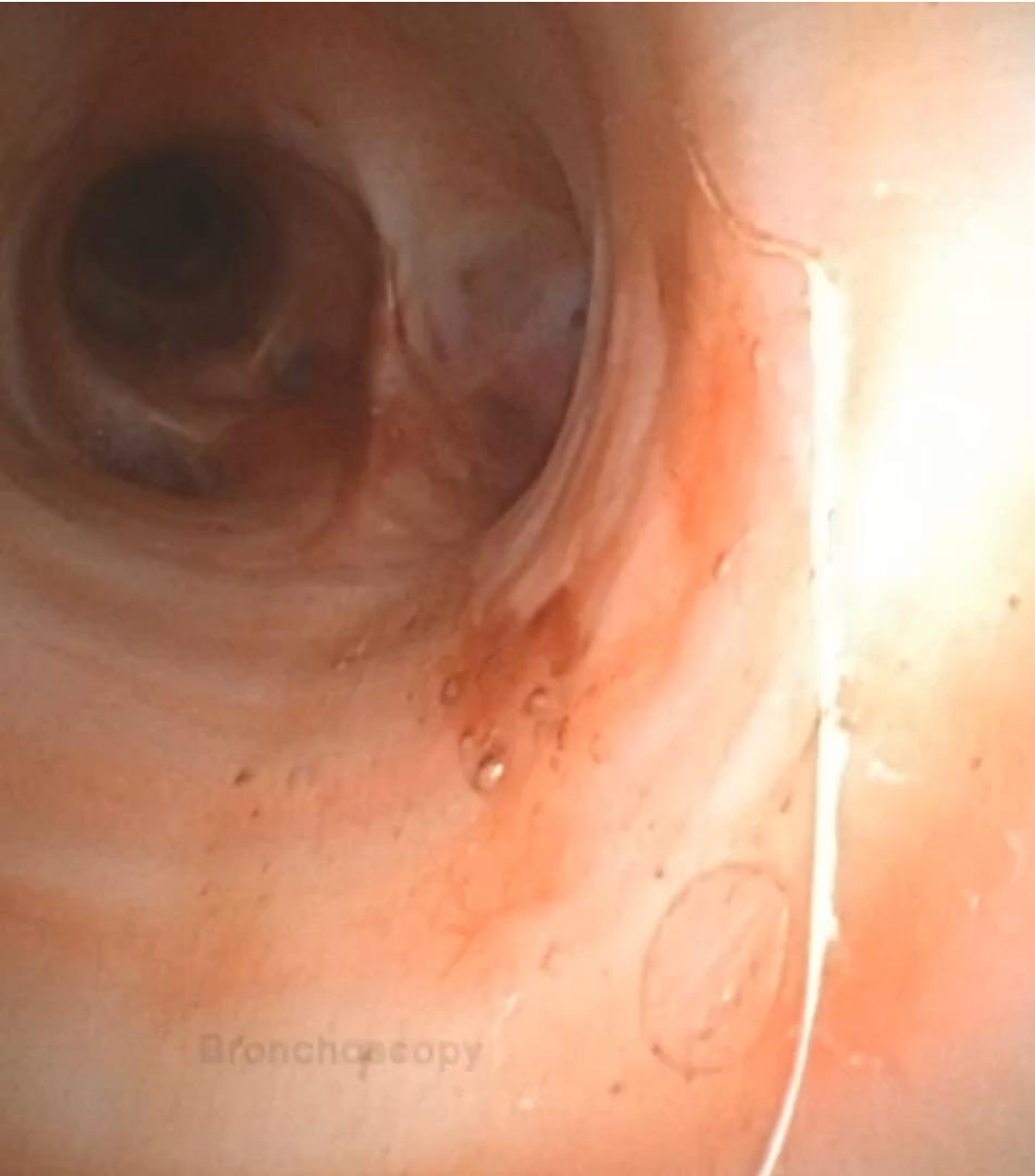
PBI (0.075 mm)





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Correlation with bronchoscopy and HRCT (video by Claudia Benke UHH)



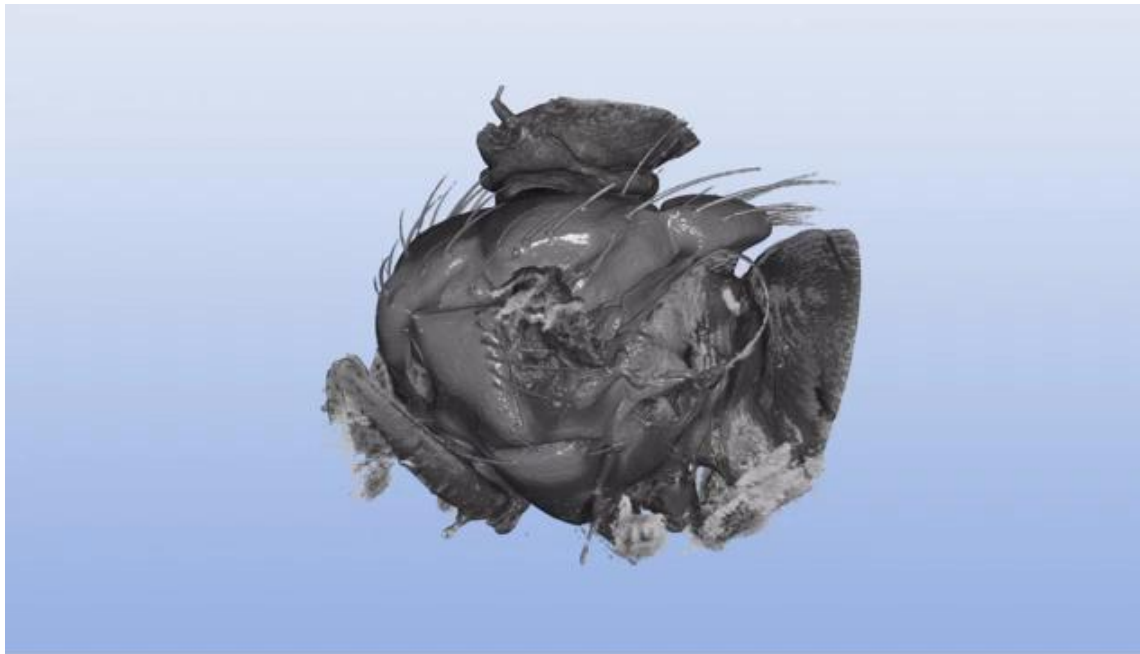
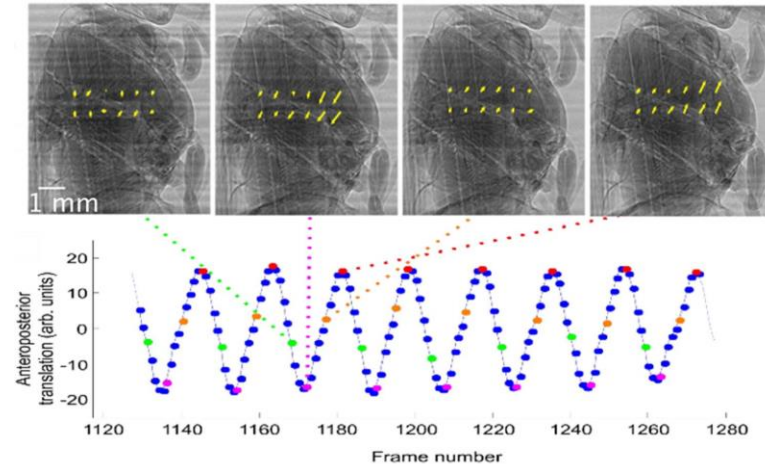
Bronchoscopy and HRCT (grey) both Cattinara
registered and synchronized to PBI (pink,
SYRMEP)

New challenges:

Dynamic studies, multitechnique approach and multiscale
micro-CT

4DCT: *in vivo* X-ray microscopy with projection-guided gating

- Visualizing fast micrometer scale internal movements of small animals
- Application of phase contrast microCT ($\sim 3.3 \mu\text{m}$ voxel size) with retrospective, projection-based gating
- 20 CT scans selected through the 150 Hz oscillations of the blowfly flight
- It is a key challenge for functional anatomy, physiology and biomechanics

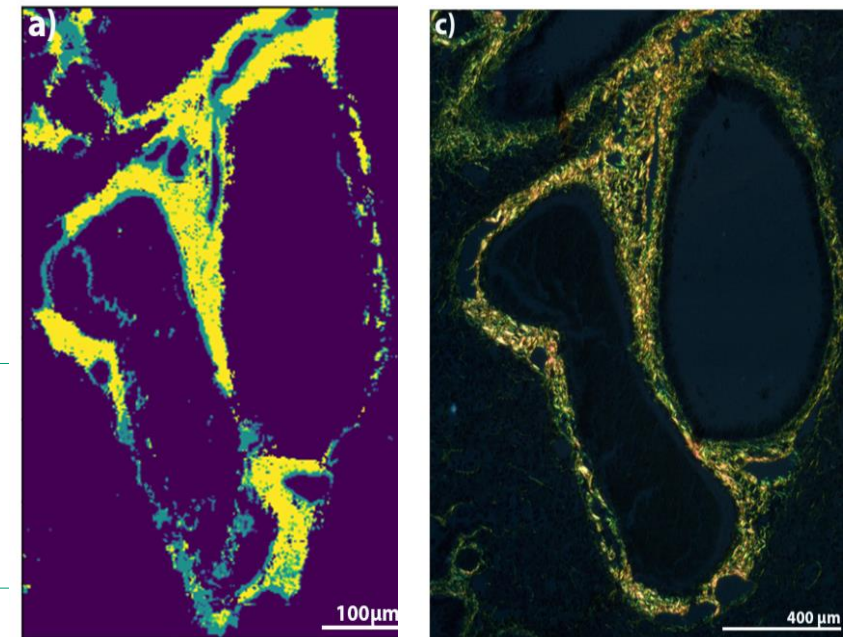
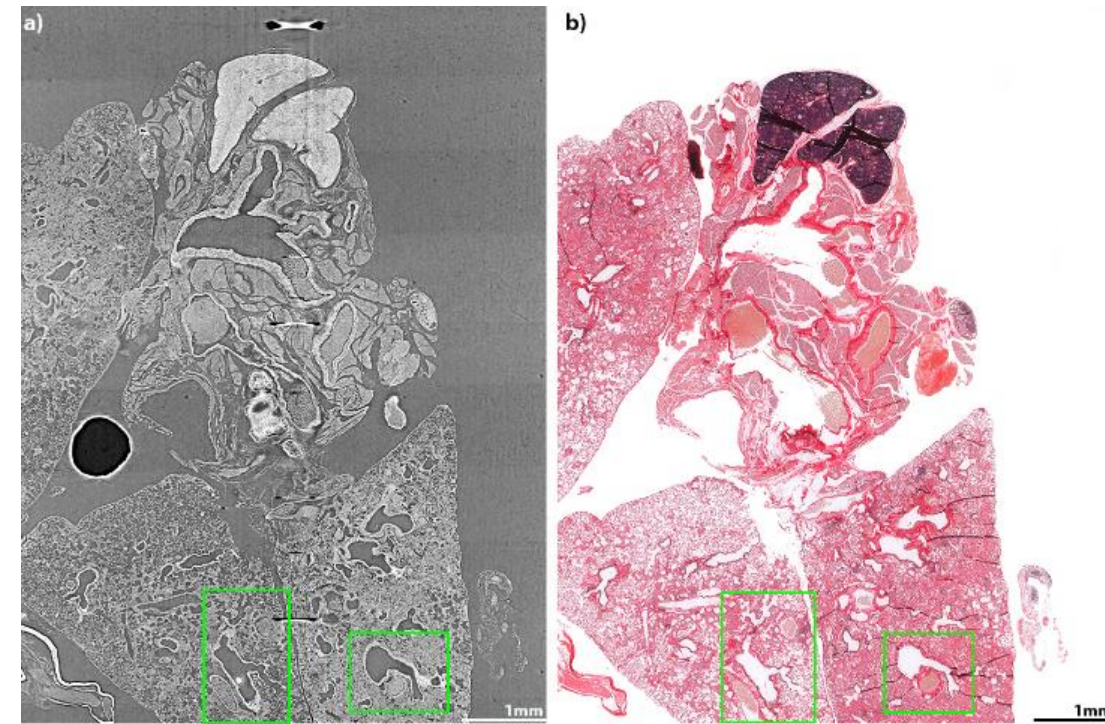


Air-filled tracheal network
spanning the
dorsal longitudinal muscles

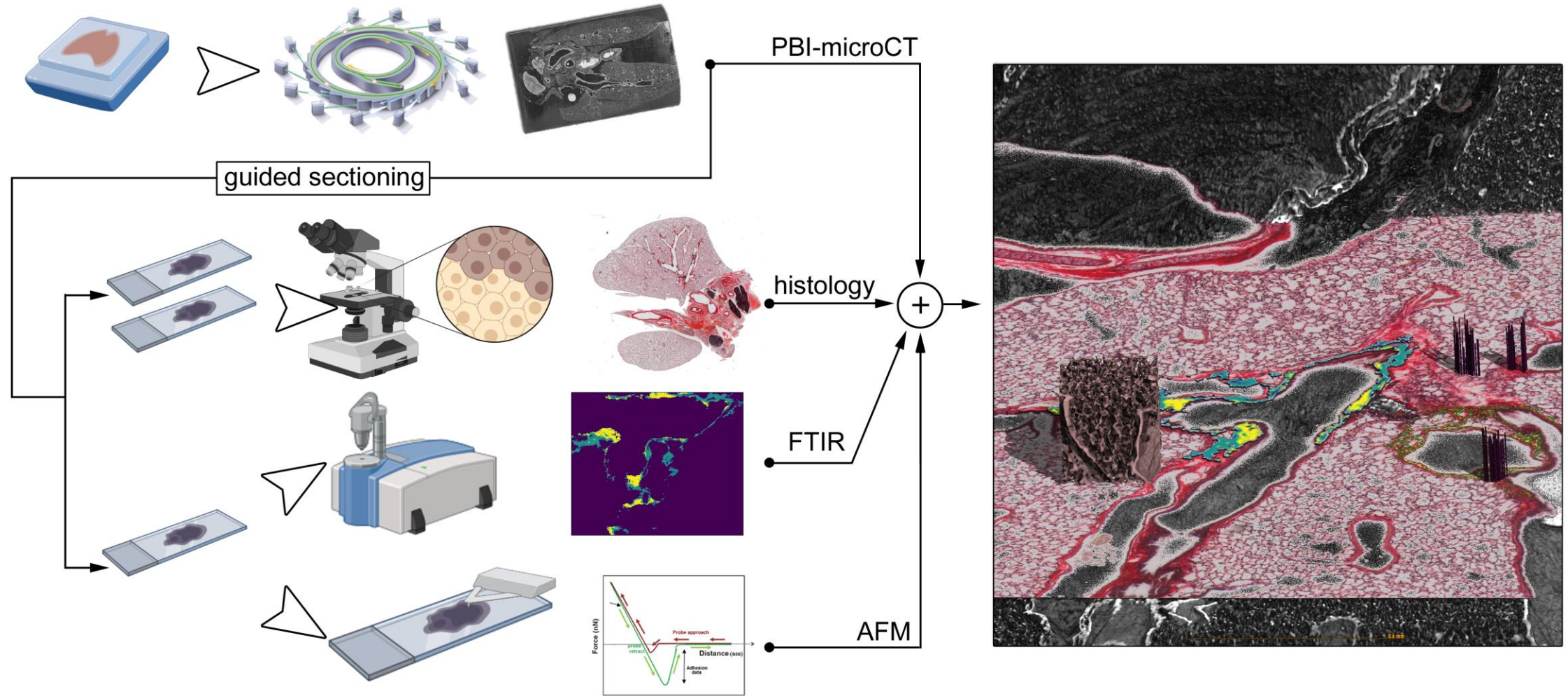
Lung Fibrosis characterization using a multi-technique approach

- Phase contrast micro-CT at different resolution scales
 - Histology
 - Atomic Force microscopy (AFM)
 - Fourier-Transform Infrared Spectroscopy (FTIR)
-
- **Morphological characterization** using micro-CT verified using **histology** (used staining: Haematoxylin and eosin, Masson's trichrome and Picrosirius)
 - Measurements of tissue **mechanical properties** using AFM
 - Identification of **collagen I and III** using **FTIR** and in histology using Picrosirius stained lung sections imaged with polarized light

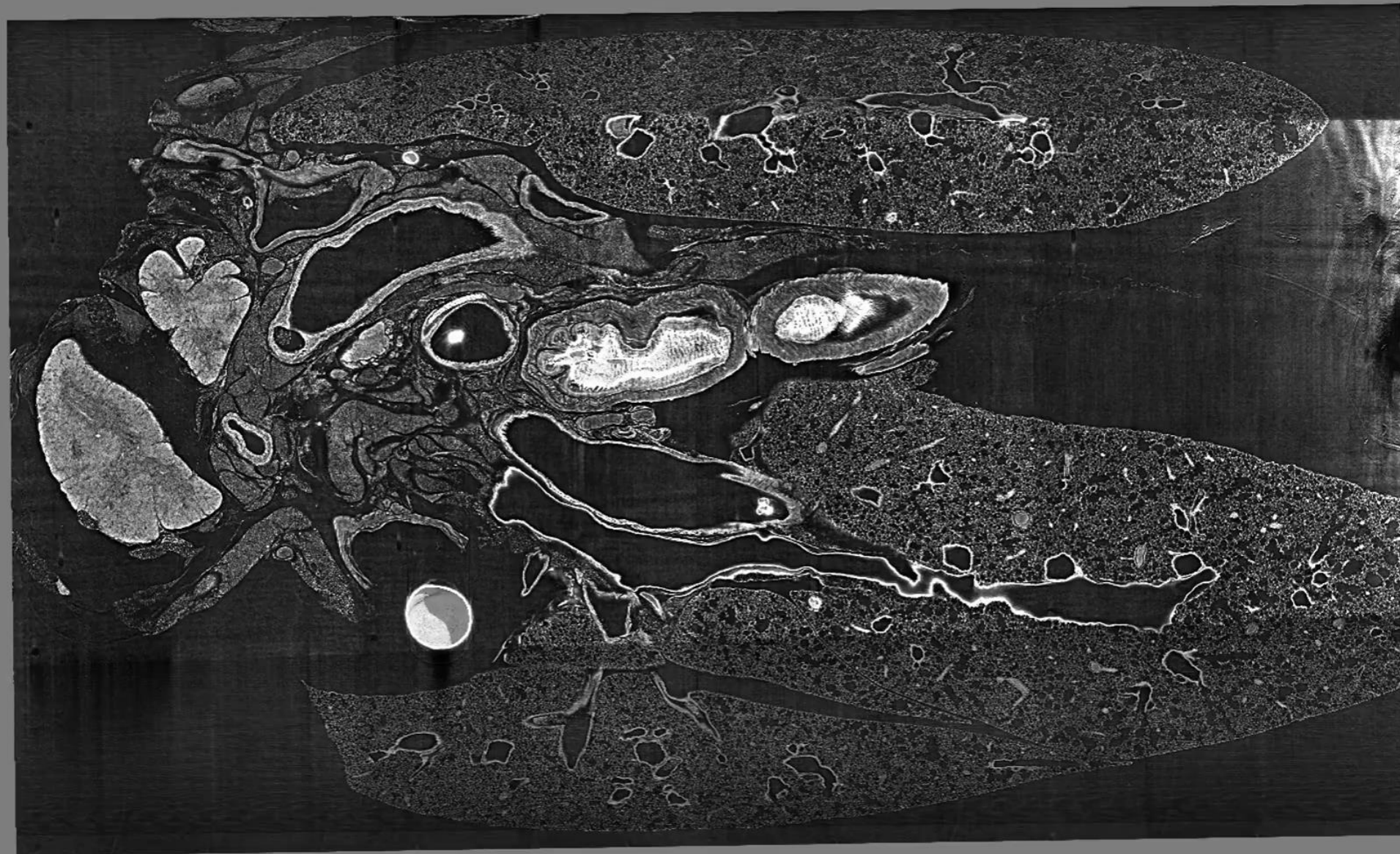
a) FTIR can be used to derive maps for collagen I and III distribution. b) maps correspond to picrosirius stained histology (polarized light microscopy), but in a more quantitative way



Lung Fibrosis characterization using a multi-technique approach - pipeline

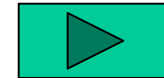


3D registration of all imaging modalities (video by Lorenzo D'Amico (SYRMEP))



2.5 mm

Spatially registered PBI (2 μ m resolution), Picrosirius red, Picrosirius red with polarized light, FTIR and AFM



Multiscale microCT studies: whole organ zoom-in virtual histology

Zoom-In label free microCT in whole human organs in ethanol
Long scanning times (several hours)

Aim: Imaging intact human organs from the organ to the cellular scale in 3D

Development of hierarchical phase-contrast tomography (HiP-CT), free propagation technique

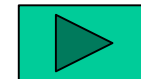
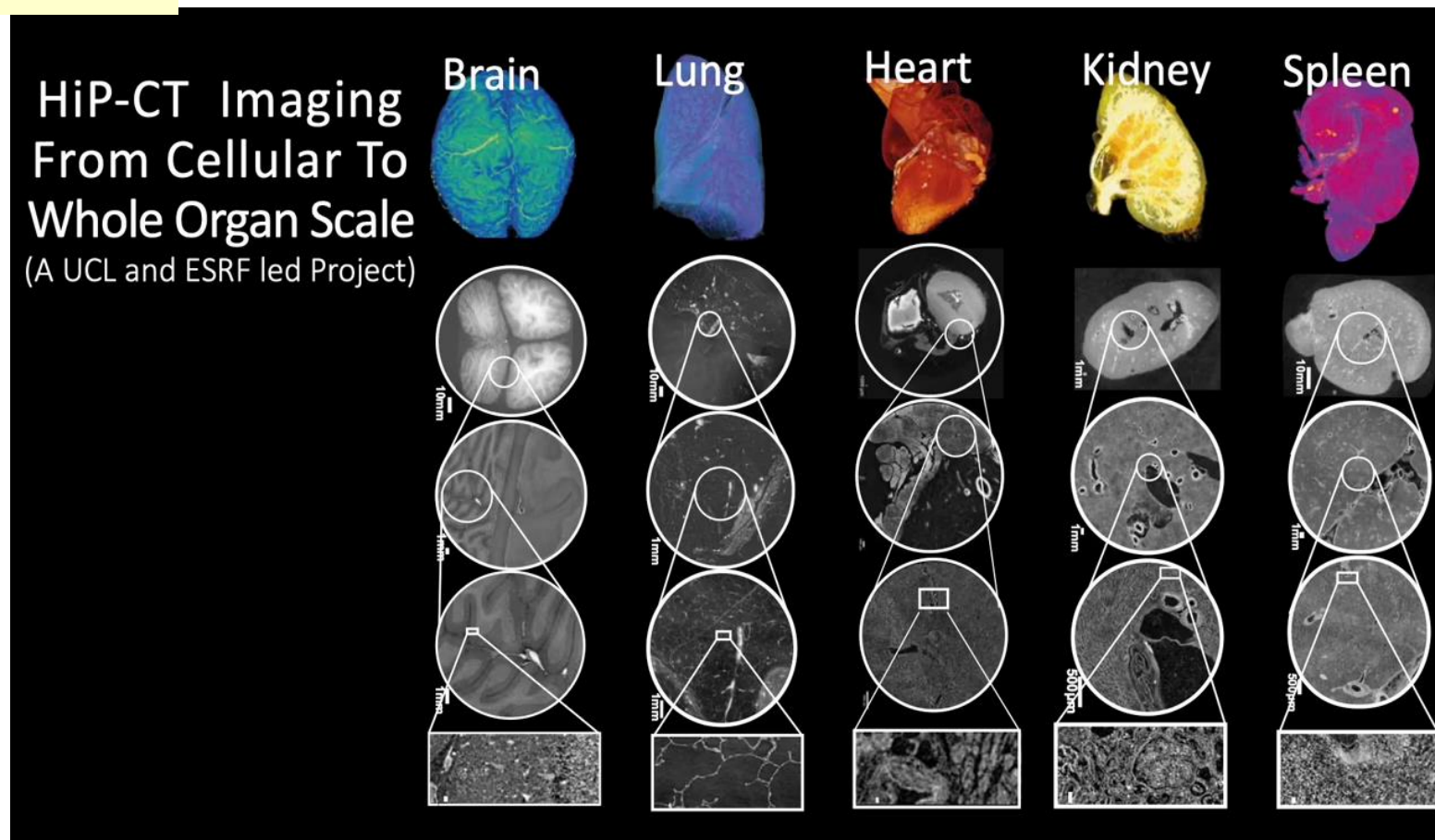
Local area tomography or zoom tomography is an established synchrotron technique that enables this hierarchical approach.

Typical pixel sizes combinations:

- 25 μm for the entire organ and entire volume rendering
 - 6 μm , 1.3 μm
 - 6.5 μm , 2.5, 1.3 μm
- } for Zoom in

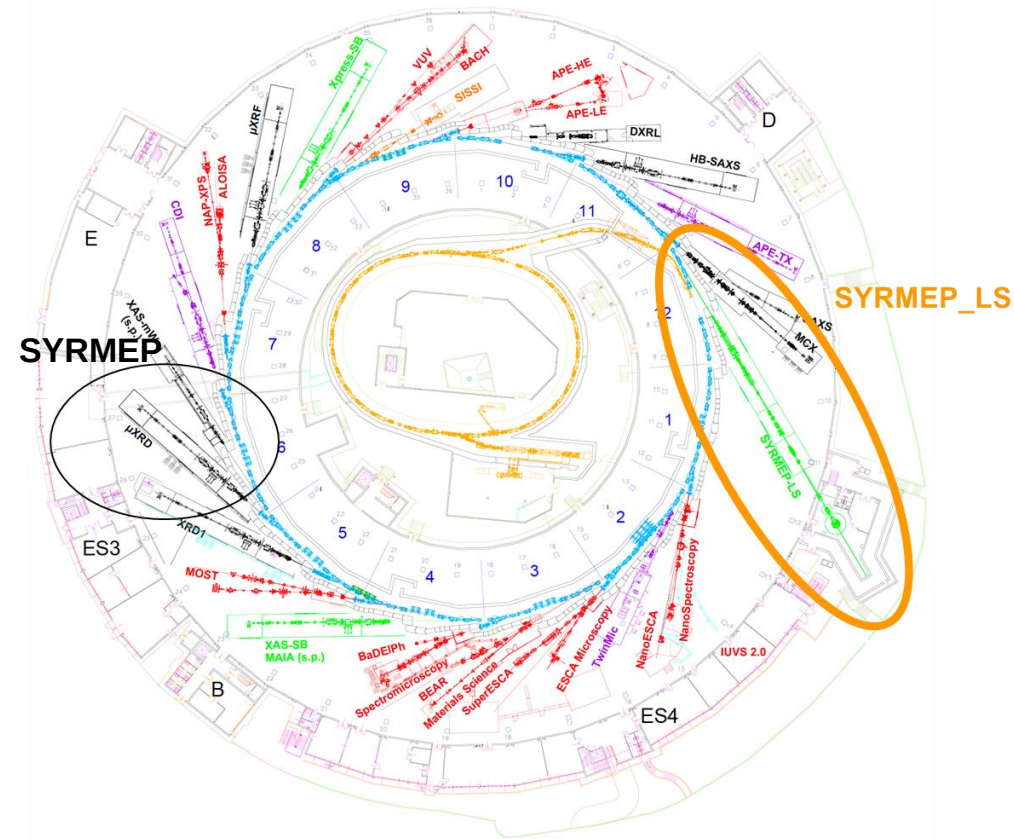
Stitching is performed to merge local areas scans at higher resolution

Walsh, C. L., Tafforeau, P., et al. (2021). Imaging intact human organs with local resolution of cellular structures using hierarchical phase-contrast tomography. *Nature methods*, 18(12), 1532-1541.



The new SYRMEP_LS beamline – aims & key features

Provide a new, state of art beamline for hard X-ray imaging beamline on Elettra 2.0 machine capable to meet the LS users' community demands.



- **Fast:** High fluxes in monochromatic and white/pink modality (usable for dynamic imaging as well)
- **Wide beam** (for big samples): 7 mrad hor. acceptance
- **Broad energy range:** 10 – 130 keV for monochromatic imaging, X-ray spectrum extended up to 300 keV for white/pink imaging
- **Versatile setup:** to allow for implementation of other phase contrast approaches
- **Clinical applications:** high safety standards, dosimetry, ecc.

Optics hutch layout for beam preparation



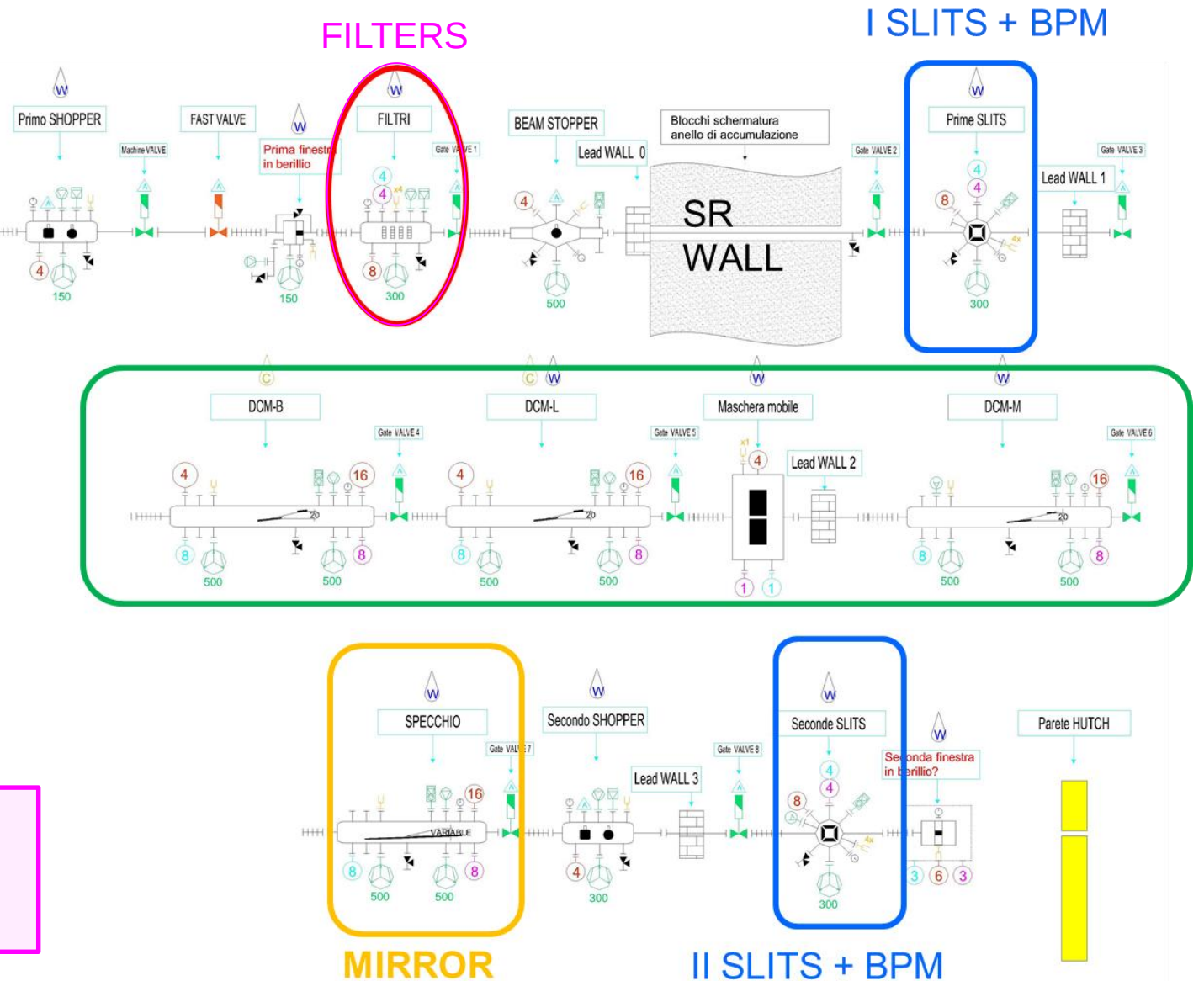
Monochromatic imaging

1. DCM (Bragg) 10 - 40 keV
2. DLM (Laue) 40 - 120 keV
3. DMM Multilayer 10 - 40 keV

White/pink imaging

Use of adequate filters, monochromators removed from the beam, spectrum up to 300 keV is available

MONOs



General SYRMEP-LS features

- Key points: high flux, broader energy range, wide beam

Optics Hutch

Source

Bending Magnet 1.45 T at 2.4 GeV
Super Bending magnet **6 T** in 2028

10^{12} Ph/sec/0.1% BW at 145 keV

Experimental Hutch (approx. 12 m long) Sample stages + Patient stage

	I sample stage	Patient Stage
Distance from the source (m)	30	40
Phase contrast prop. distances, SDDs(m)	0-7, 11-31	15-22
Techniques	PBI, multiscale, other phase sensitive techniques	PBI
Beamsize (mm ²)	210 x 8	280 x 12.5

Max acceptance 7×0.8 mrad²



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SYRMEP_LS

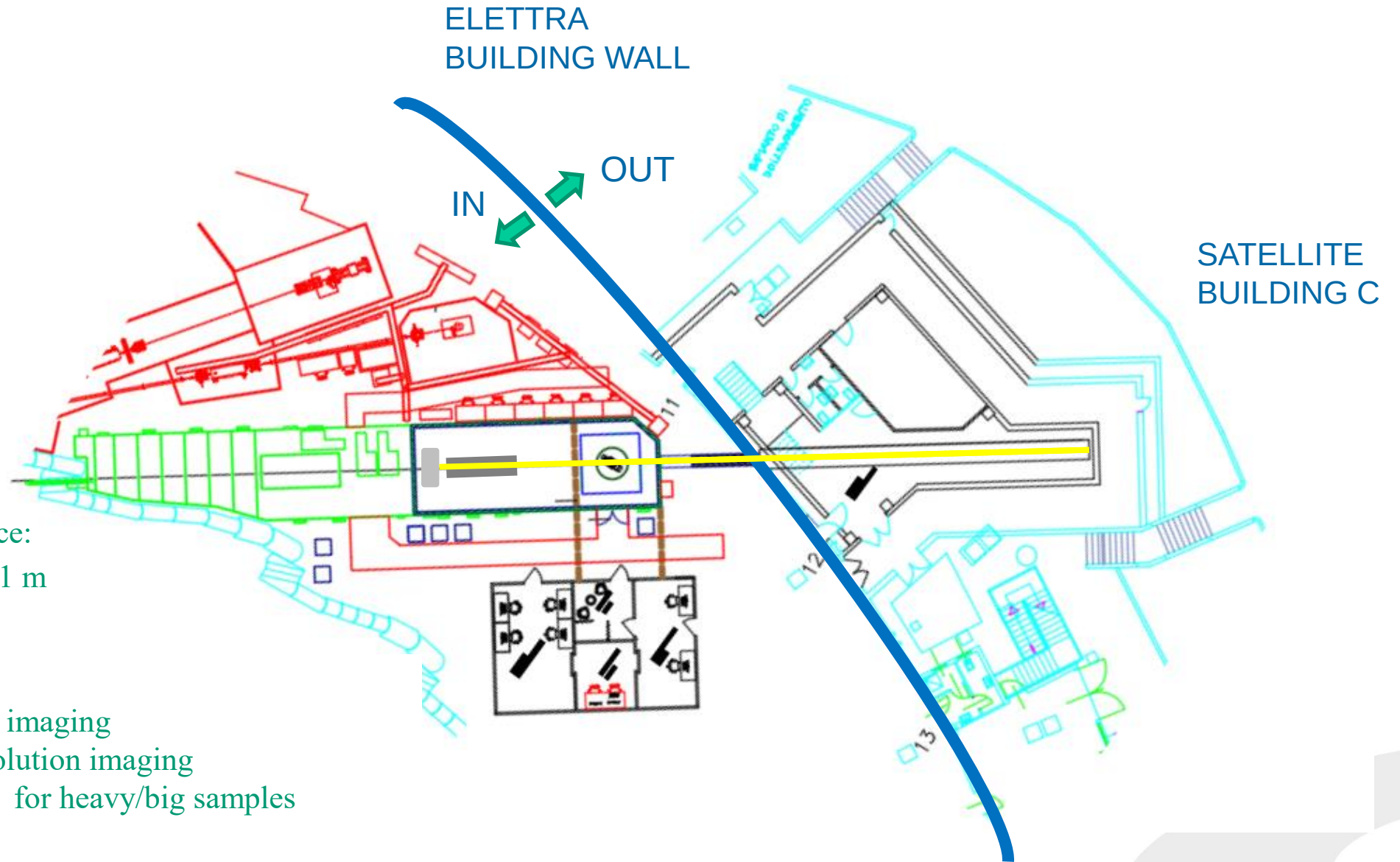
Distances from the source:

Start first exp. setup = 31 m

Patient support = 40 m

Propagation distances:

- ~ 3 - 22 m for clinical imaging
- ~ 0 - 2 m for high resolution imaging
- ~ 0.1 - 5 m, 11 - 31 m for heavy/big samples imaging



Imaging modalities and techniques

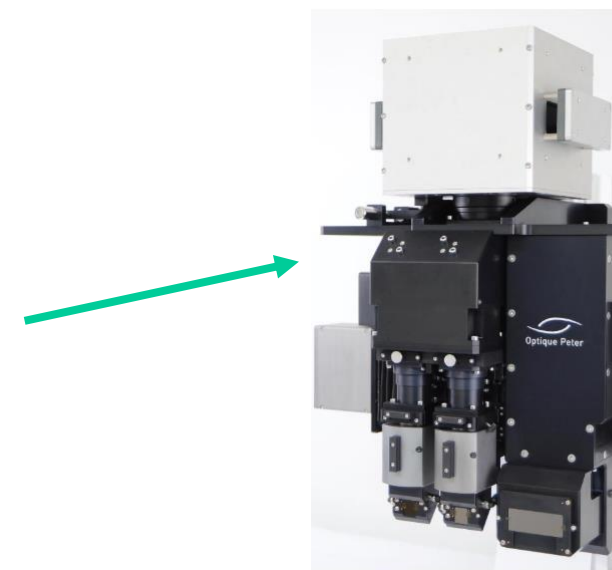
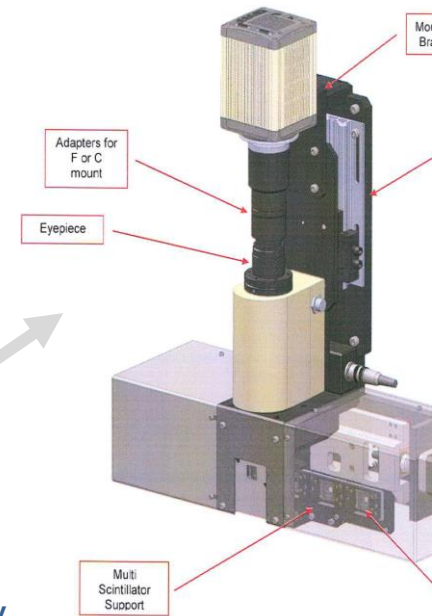
Imaging techniques (Planar and CT modalities)

- Propagation Based Phase Contrast Imaging (or free propagation)
- Analyzer Based Imaging
- Gratings, edge illumination, speckle - in collaboration with users
- Absorption, K-edge, L-edge subtraction imaging

Typical pixel sizes in Multiscale CT

- White/pink beam setup: 0.9 - 5 μm (SCMOS Hamamatsu Orca Flash 6.5 μm pixel size), optically coupled with variable zoom optics)
- Monochromatic beam: 4.5 or 9 μm , 27 μm (CCDs), 100 μm (X-Counter Single photon Counting)

White Beam Detectors	Physical pixel size	0.285x to 1.80x	5x	10x	20x
Hamamatsu Orca Flash (2048 x 2048 pixels)	6.5 μm	22.81 μm to 3.61 μm	1.30 μm	0.65 μm	0.33 μm
PCO.edge 5.5 (2560 x 2160 pixels)	6.5 μm	22.81 μm to 3.61 μm	1.30 μm	0.65 μm	0.33 μm
PCO.edge 10bi (4432 x 2368 pixels)	4.6 μm	16.14 μm to 2.56 μm	0.92 μm	0.46 μm	0.23 μm





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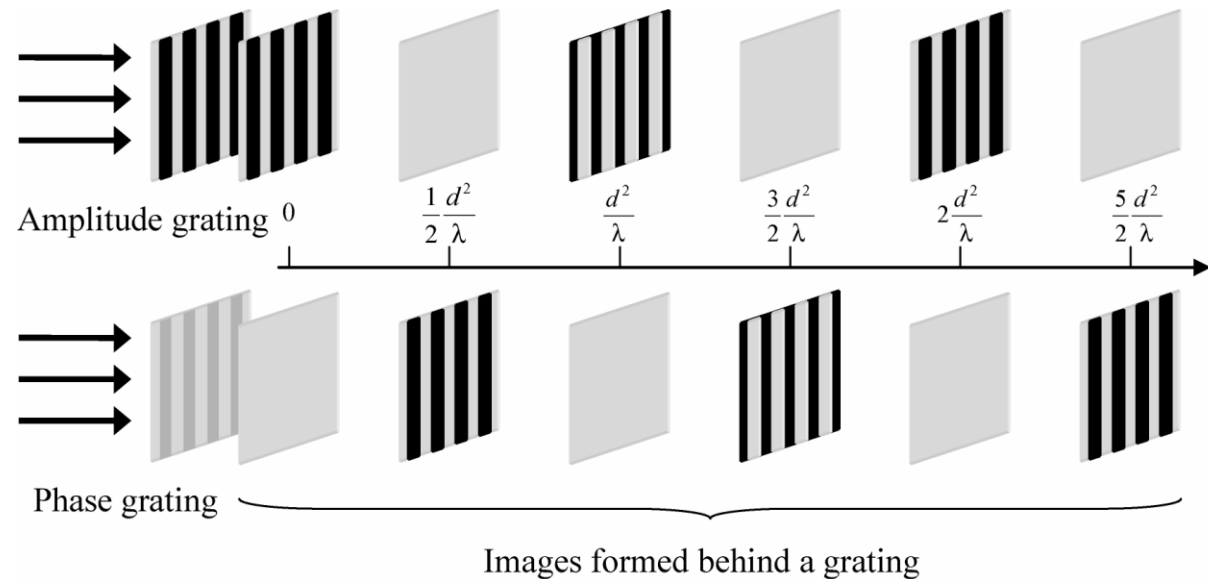
Contact: giuliana.tromba@elettra.eu

Thanks for your attention

www.elettra.eu

Talbot interferometry is based on the Talbot effect (1836). When a plane wave is incident upon a periodic diffraction grating the image of the grating is repeated at regular distances downstream the grating plane (self-imaging effect).

The distances z_T between the object and self-imaging planes are determined by the light wavelength λ and the period d of the structure



Talbot effect in the case of plane-wave illumination. For **an amplitude grating**, self-images are generated (repeated) at $z_T = 0, d^2/\lambda, 2d^2/\lambda$, and so on. (d is the period of the grating, λ is the wavelength). For a **phase grating**, similar patterns are observed at intermediate positions.