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Multiscale Phase Contrast Bio-medical Imaging at the SYRMEP beamline

Giuliana Tromba

on behalf of the SYRMEP team and collaborators

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Advanced Medical Imaging with Synchrotron and Compton X-ray Sources

Bologna, 21-22 November 2019

Synchrotron Radiation and multiscale imaging for bio-medical research

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Spatial coherence
Phase contrast imaging

High Fluxes
Dynamic imaging

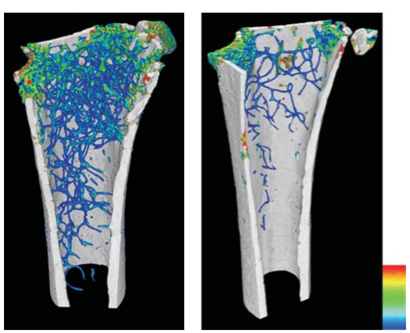
Monochromaticity
Energy tuning/optimization

Collimation
Nearby parallel beam

Synchrotron Radiation

“In vitro” - high res. imaging

Applicable to study tissues, organs, sizes on the order of some millimiters, typical pixel size ~ 0.6-5 µm



Clinical – Applications to patients

Research protocol, selected patients
Find best compromise between dose & image quality, pixel sizes : 50 – 100 µm

Pre-clinical - Imaging of animals

Studies on organs, animal models
Typical pixel size: 9 µm (for ex-vivo) to 100 µm (in-vivo)

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PHase Contrast (PHC) imaging

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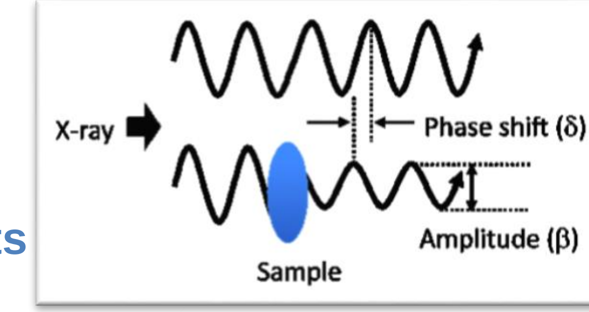
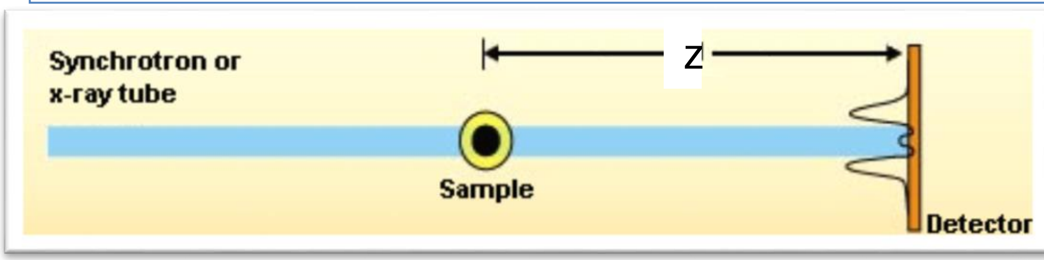


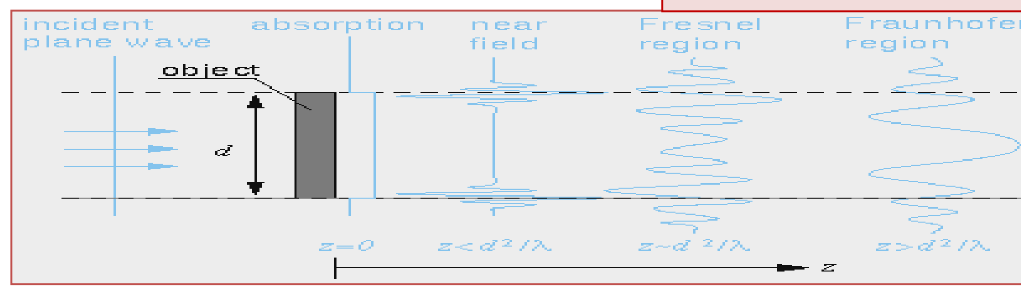
- Conventional imaging relies on **X-ray absorption**
- Phase contrast imaging is based on the detection of **phase shifts** occurring to X-rays crossing the sample

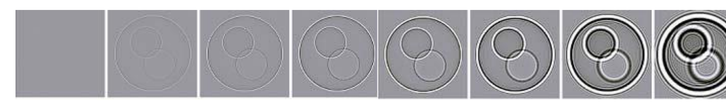
Complex refractive index: $n = 1-\delta+i\beta$
Linear attenuation of X-rays: $\mu = 4\pi\beta/\lambda$
Phase shifts: $\phi = -2\pi\delta t/\lambda$ (t=sample thickness)

Propagation based imaging (PBI) - Simplest approach – no optical element needed.Contrast arises from interference among parts of the wave front differently deviated (or phase shifted) by the sample.
Edge enhancement effects, different regimes according to the selected sample-to-detector distance.



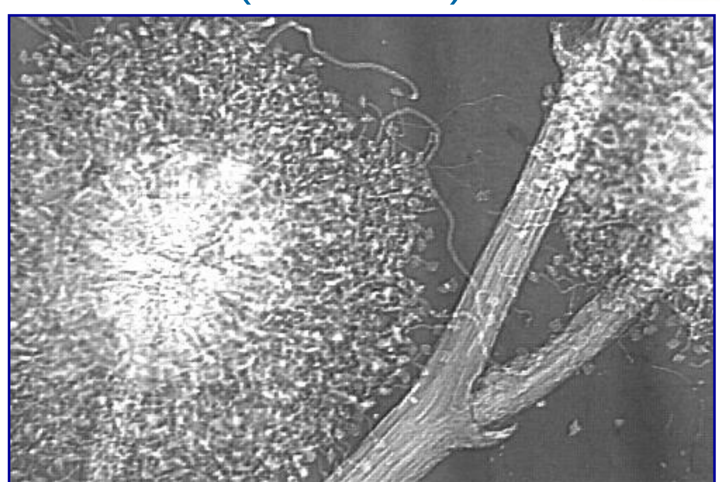
PHC regimes





Absorption (z = 0)

Near field (z = 50 cm)



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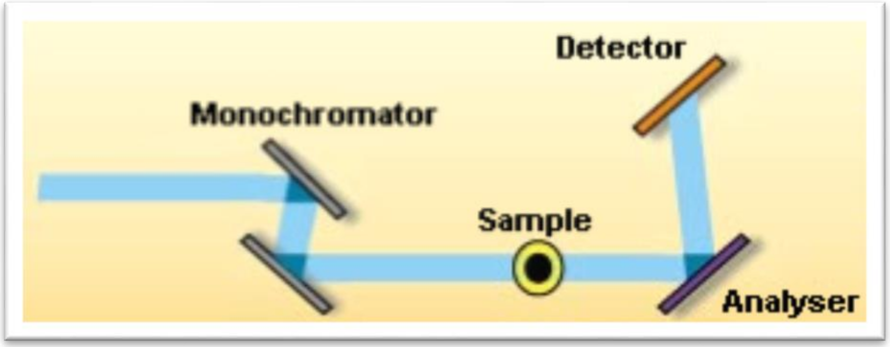
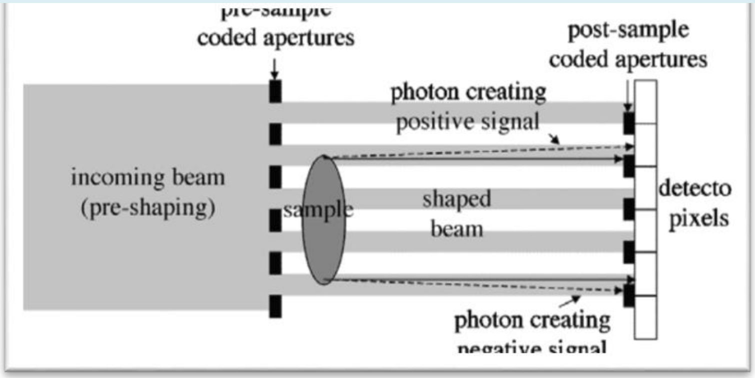
PHC imaging: other approaches

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

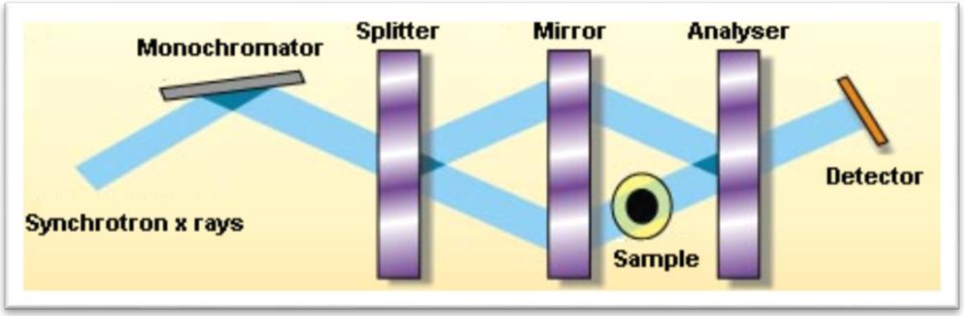


Coded Apertures
Use of coded apertures (masks) to select refraction angles

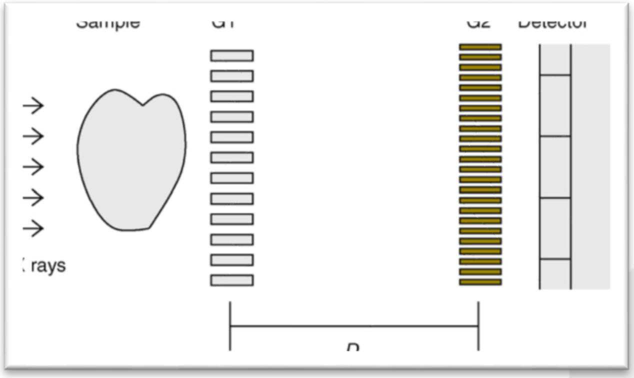


Interferometric approaches - waves are superimposed in order to extract information - direct measure of phase shifts introduced by the sample

Crystal Interferometry



Grating interferometry



SYRMEP Beamline layout

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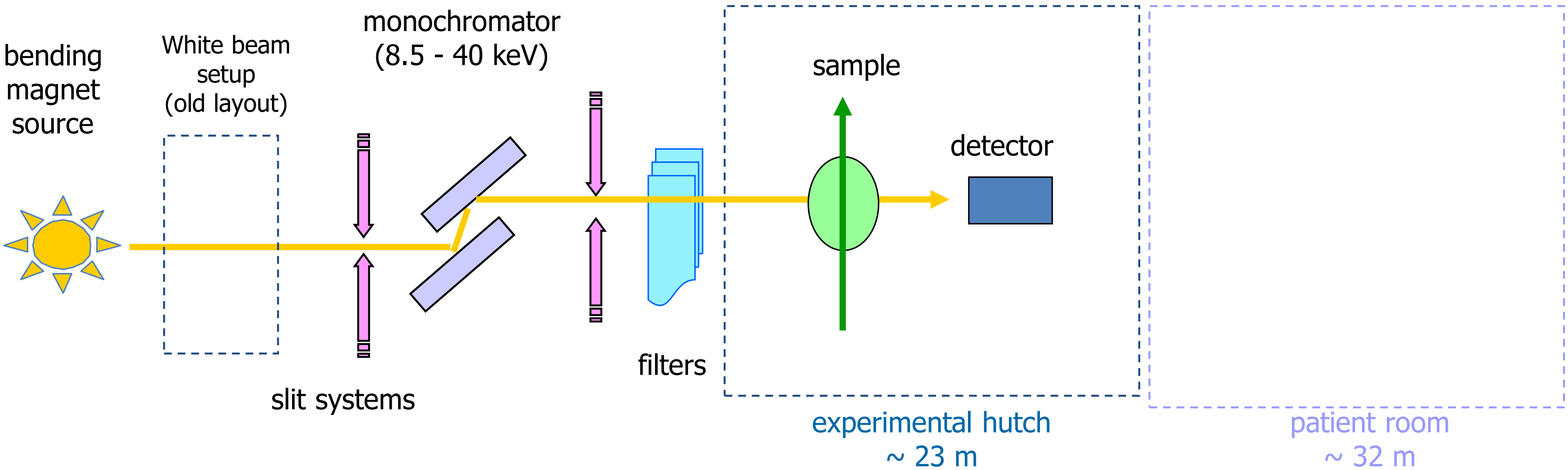
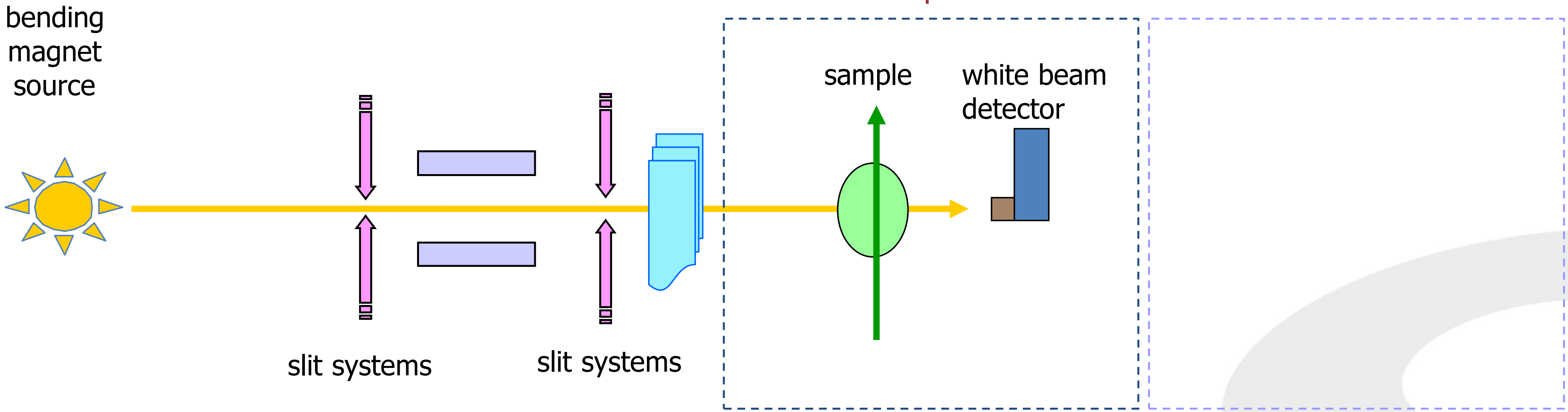
NEW!

EURO-BIOIMAGING

Monochromatic beam in experimental hutch



Use of white/pink beam



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SYRMEP Beamline layout

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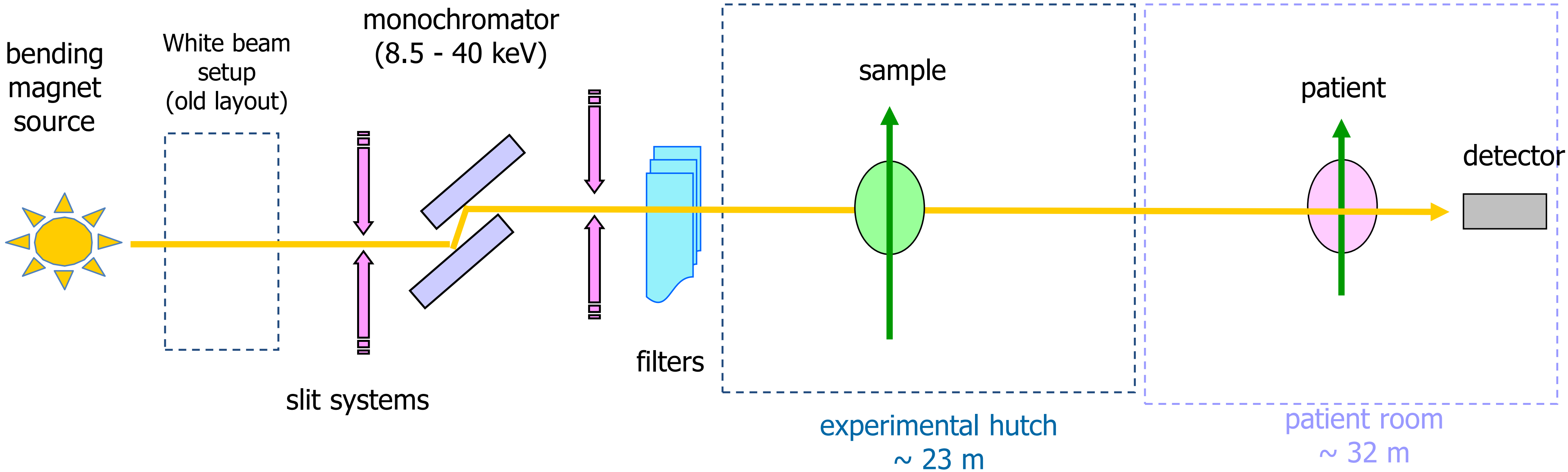
EURO-BIOIMAGING

NEW!

Monochromatic beam in experimental hutch or in patient hutch – use of large propagation distances if detector is in the patient hutch



- Source: Bending magnet, front-end **hor. acceptance: 7 mrad, vertical divergence: 0.2 mrad**
- Source-to-sample distance ≅ **23** m (exp. hutch), ≅ **30** m (patient room)
- Beam hor. size at sample ≅ **160** mm (exp. hutch), ≅ **210** mm (patient room)
- Energy range: **8.5 - 40 keV**, B.W. $\Delta E/E \cong 2 * 10^{-3}$

Techniques

- Absorption/Phase Contrast Imaging (free propagation)
- Dual energy imaging (K-edge subtraction)
- Analyzer Based Imaging (ABI)
- Gratings, Coded Aperture (in collaboration with users)

Modes:
- *Planar*
- *Micro-CT*

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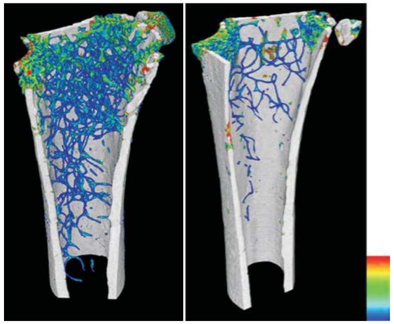
Multiscale applications @SYRMEP:



1- High resolution imaging
(in vitro, ex-vivo)

- Major aims: provide images with maximum achievable spatial and contrast resolution (min. pixel size: **0.9 µm**, future achievement: **0.6 µm**)
- Great advantage in the use of phase contrast techniques
- Combined use of staining and Phase Retrieval algorithms to increase image contrast
- Sample preparation: none, ethanol, paraffin, resin, agarose, formaline
- Applications
  - Imaging of excised tissues/organs, scaffolds
  - Virtual histology – possible combinations with other microscopy analysis

Bone turnover in mice exposed to micro-gravity conditions

- 3 wild type (WT) mice and 3 pleiotrophin-transgenic (PTN-Tg) mice in a special payload (MDS - Mice Drawer System). The transgenic mouse strain over-expressing pleiotrophin (PTN) in bone was selected because of the PTN positive effects on bone turnover.
- **91 days in the International Space Station (ISS) by NASA: Aug. - Nov. 2009.**
- Controls:
  - mice on Earth in the same special payload MDS (*ground* mice)
  - mice in common cages (*vivarium* mice)
- SR µ-CT experiments were performed on femurs and spines
- Being non-destructive, µ-CT is very attractive for these rare specimens

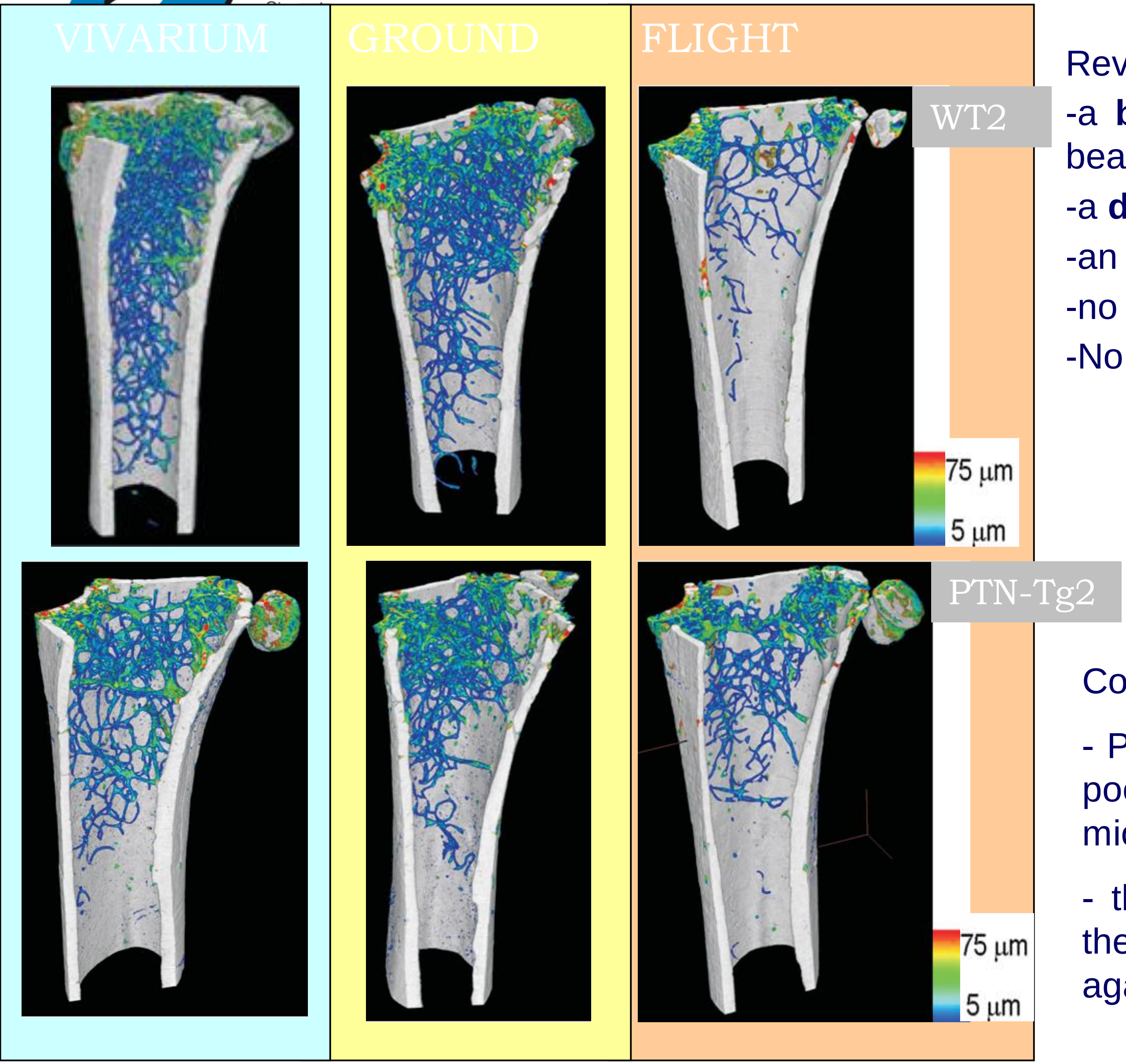
University of Genova

Università Politecnica delle Marche

University of Trieste – Dept. of Engineering

http://www.nasa.gov/mission_pages/station/research/experiments/MDS.html

Analysis of the microarchitecture of the trabecular bone in femurs



Revealed:

-a **bone loss** during spaceflight in the weight-bearing bones

-a **decrease** of the trabecular number

-an **increased** mean trabecular separation

-no significant change in trabecular thickness.

-No effects on not weight-bearing bones.

E = 19 keV
Pixel size = 9 mm
N. Proj = 900
Distance sample-ccd= 3 cm

Comparison WT vs.PTN-Tg2:

- PTN-Tg exposed to normal gravity has a poorer trabecular organization than WT mice

- the expression of the PTN gene during the flight resulted in some protection against microgravity's negative effects.

Color map represents bone trabecular thickness distribution in the femur (red = 75 µm, blue = 5 µm)

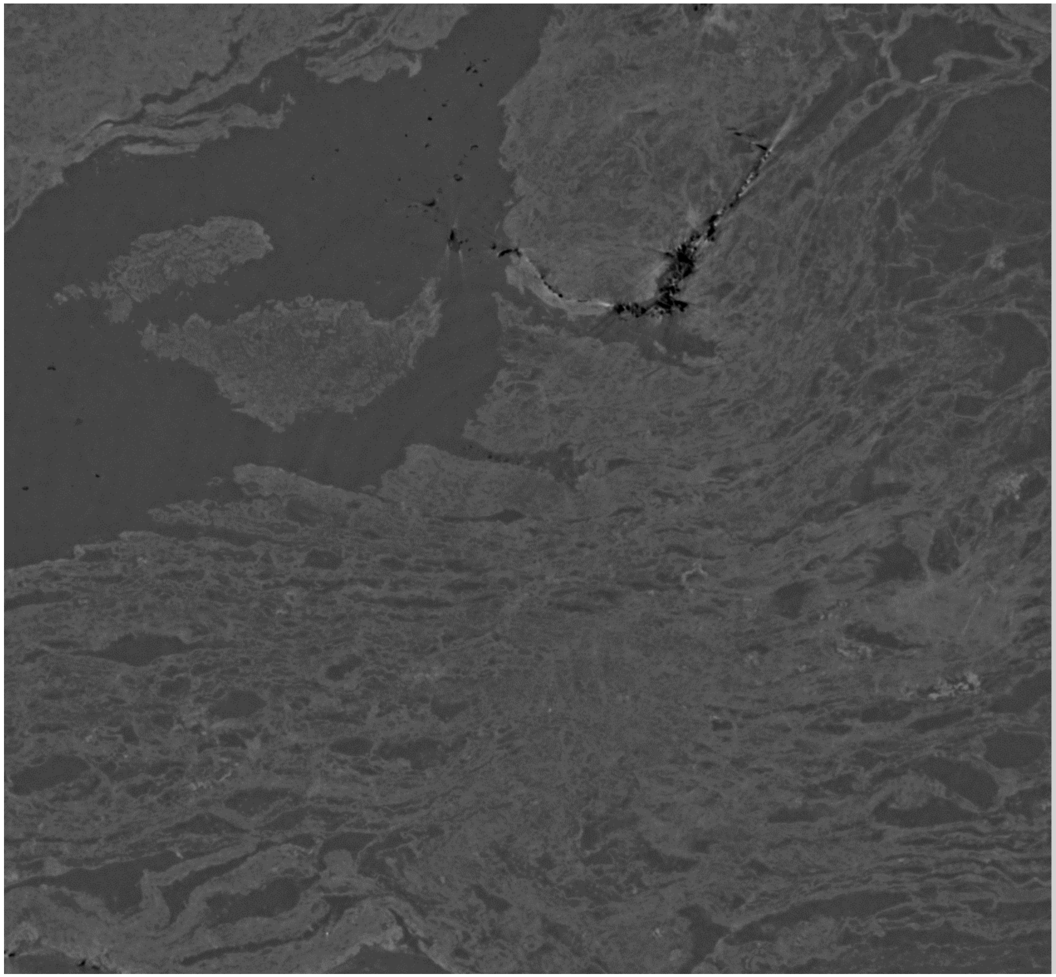
S. Tavella et al "*Bone Turnover in Wild Type and Pleiotrophin-Transgenic Mice Housed for Three Months in the International Space Station (ISS)*", *PlosONE, March 2012.*

Distribution of metal abrasion in Titanium implants for the treatment of spinal deformities in children

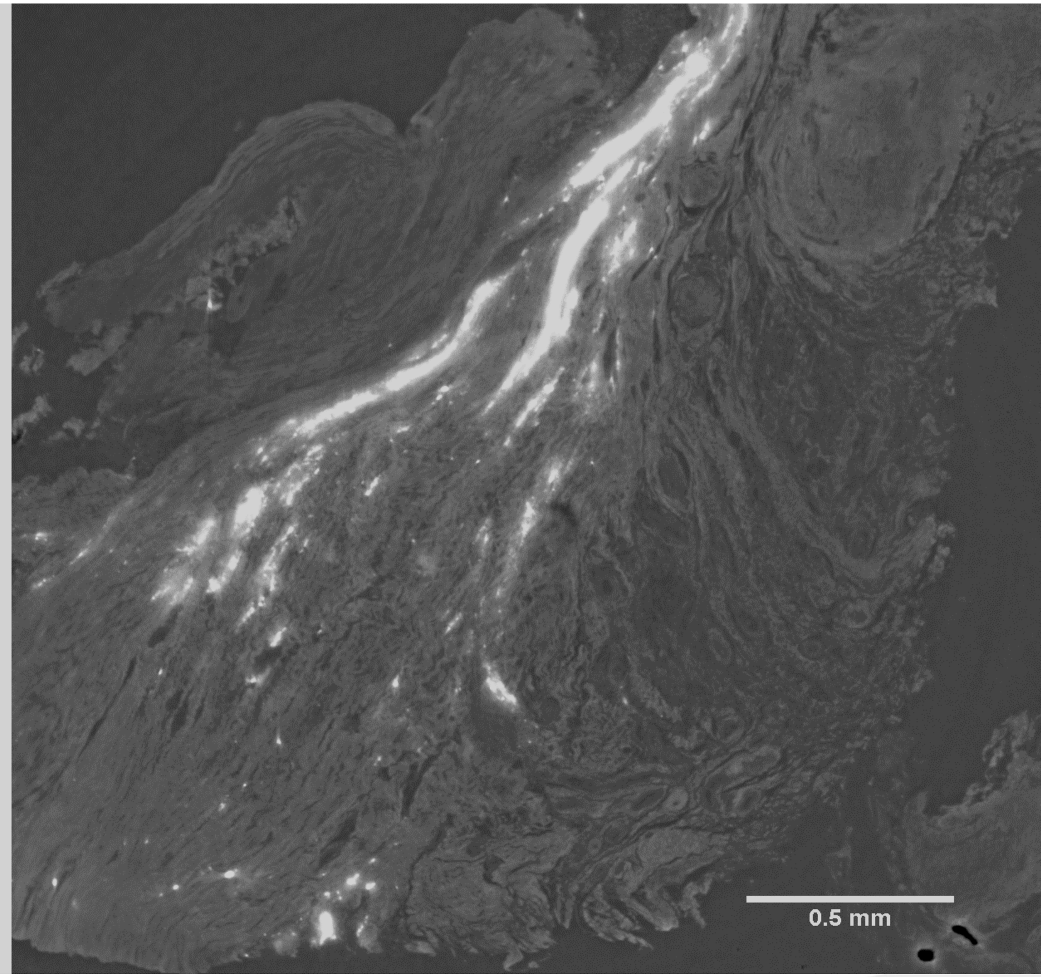
Feasibility study

- Children with progressive spinal deformities receive growth-friendly implant systems that are frequently surgically renewed or extended externally.
- Metal abrasion and corrosion of surgical metal implants in the body can lead to local and systemic reactions.
- Various studies detected microscopically metal particles on the tissue surrounding the implant.
- The particles can activate macrophages that cause inflammatory reactions and bone resorption.
- Special implants, which are widely used in pediatric orthopedics, consisting of a Ti alloy with 6% aluminum and 7% niobium are studied.
- The distribution of Ti particles in small tissues specimens taken in the implants area has been evaluated using phase contrast micro-CT

Sample without Ti contamination



Sample withTi contamination



Pixel size = 2 µm
Tissue samples preparation: chemical dehydration and embedding in paraffin.

Courtesy of L. Braunschweig and C.Dullin (Univ Hosp. Goettingen)

Use of staining and Phase Retrieval algorithm - Imaging of PTA stained mouse heart embedded in paraffin

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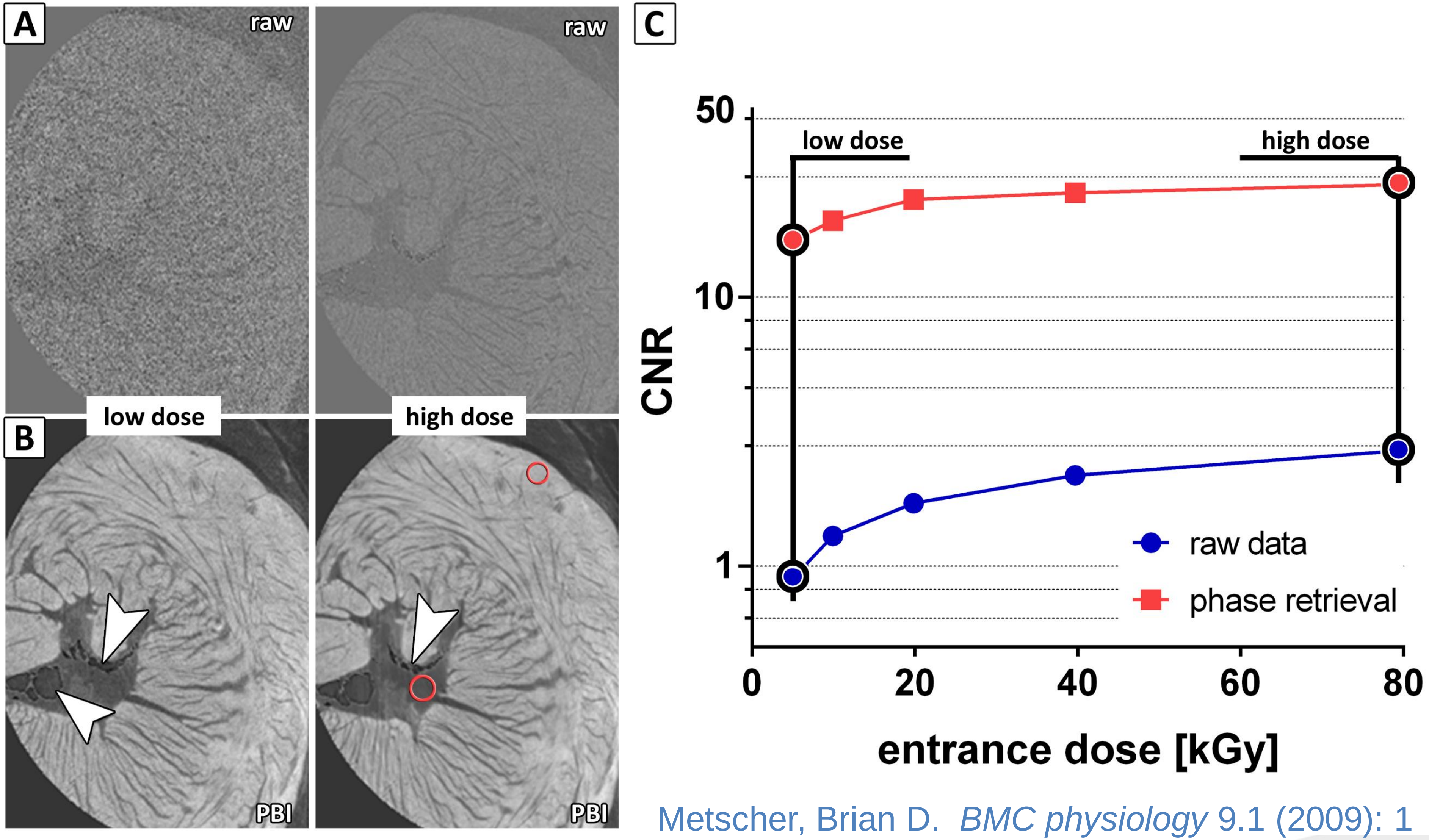
Standard FBP reconstruction

Application of Phase retrieval algorithm



Metscher, Brian D. *BMC physiology* 9.1 (2009): 1

PBI + phase retrieval dramatically **increases** contrast-to-noise ratio in PTA stained mouse hearts -> possible **dose reduction** or **shorter acquisition times**

M.Saccomanno et al., J. Synchrotron Rad. (2018). 25

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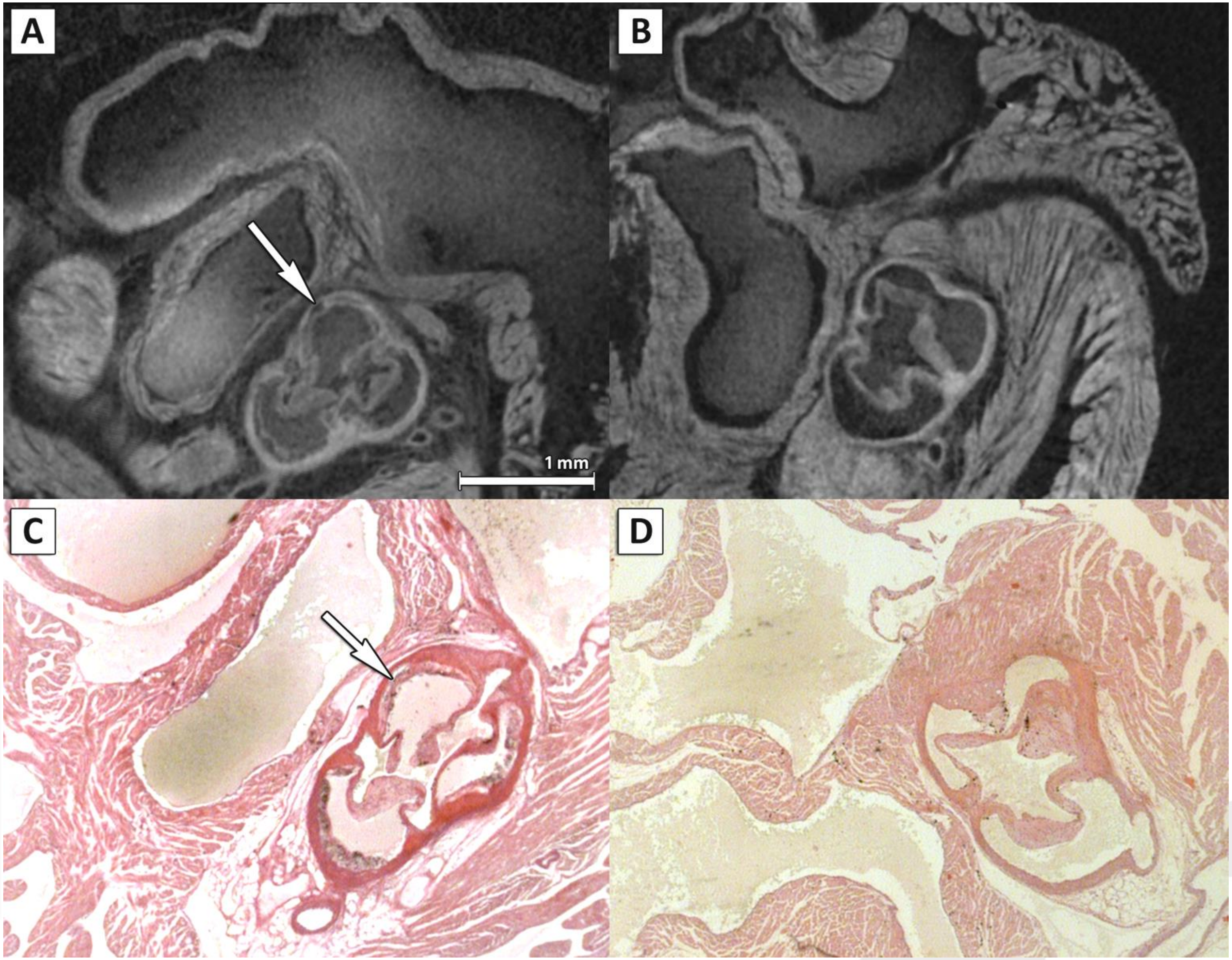
Imaging of atherosclerotic mouse: comparing µCT slice with histology

Animal model: atherosclerotic mouse
Apolipoprotein E-deficient (apo) mouse (deficient transgenic mice demonstrates a strong tendency to develop hyper-cholesterolemia)

Aim: evaluate the capability of µCT to highlight the formation of atherosclerotic plaques in normal and Apo mice. All mice were fed with a high fat diet for 70 days

Combination of soft tissue staining by phosphotungstic acid (PTA) and sample embedding in paraffin or agarose gel allows direct **overlay of µCT data sets and microscopy after immunochemical staining**

Atherosclerotic | Normal

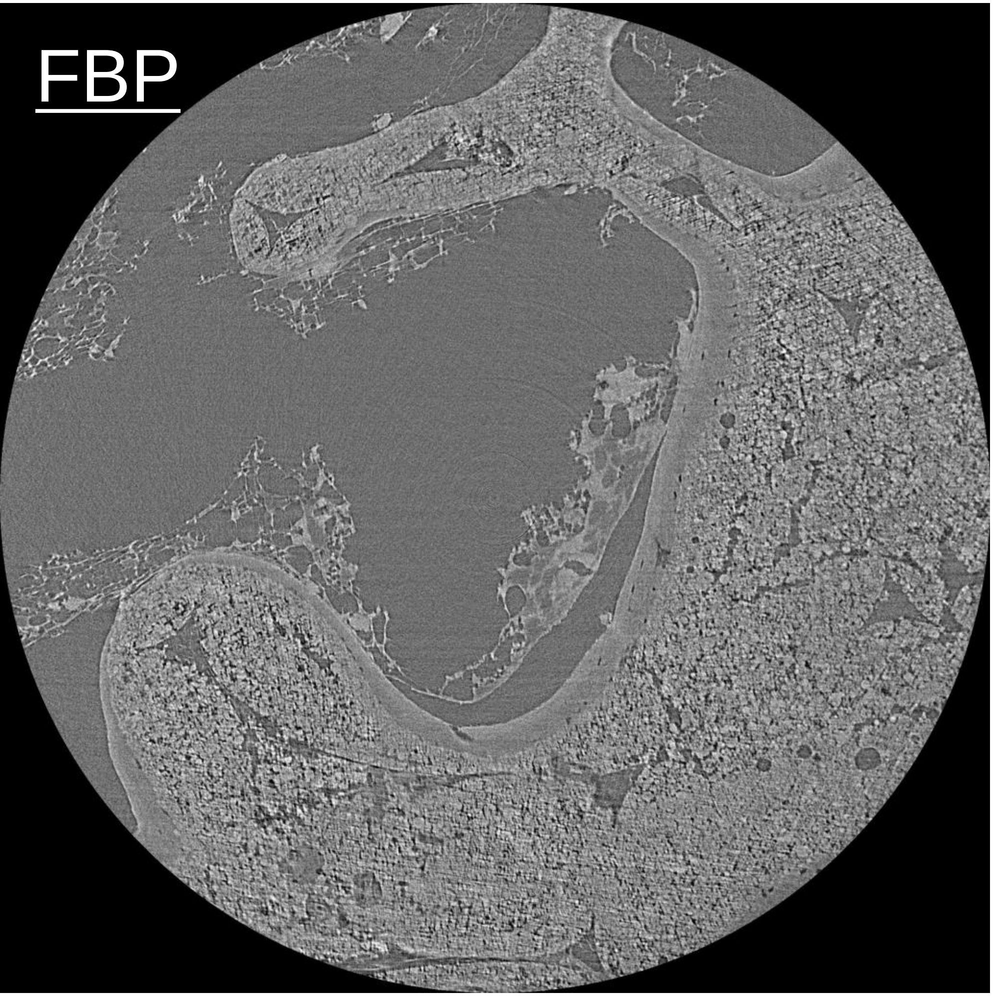


Dullin C. et al., PLoS ONE 12(2): e0170597 (2017)

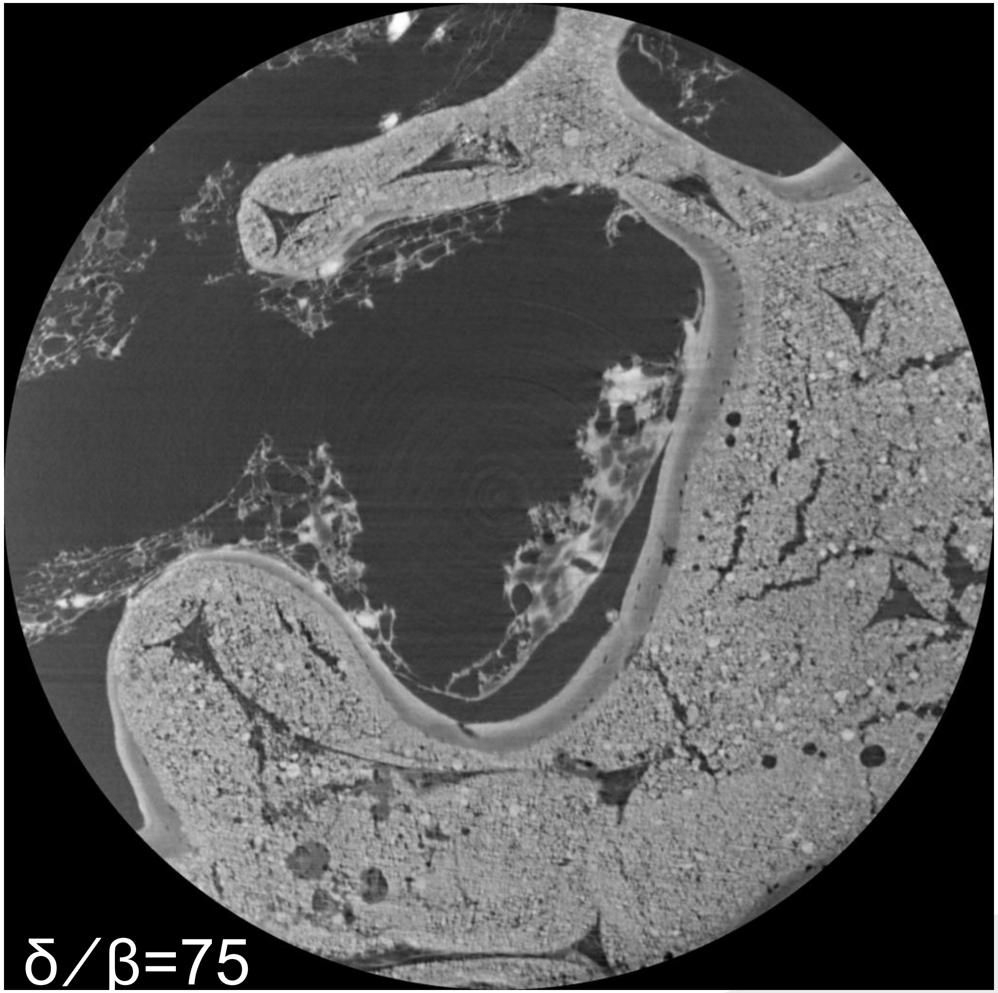
Imaging of scaffolds

PHC imaging and phase retrieval algorithms enhance the visualisation of new bone formation and vascularization at micron scale resolution.

PHC slice



Phase Retrieved slice



Collab: M.Mastrogiacomo, R.Cancedda (Uni Genova), A.Cedola, G. Campi, M.Fratini et al. (CNR – Roma)

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2 - Pre-clinical imaging

- Major aims: provide images of excised organs or on small animals at different dose levels/resolution scales. Pixel size: **4.5 - 9 µm** (ex-vivo), **25 - 100 µm** (in-vivo).
- Use of Phase Retrieval algorithms dramatically increases image contrast (allowing strong dose reduction)
- Possibility for in-vivo low dose phase contrast CT and dynamic imaging
- Applications:
  - Functional and morphological imaging in small animal models
  - Cell tracking (tracking cancer cells in animal models, …)
  - Vascular imaging (efficient use of contrast agents, k-edge subtraction imaging)
  - Multi resolution CT studies (Zoom CT)
  - Low dose *in-vivo* imaging of lung function

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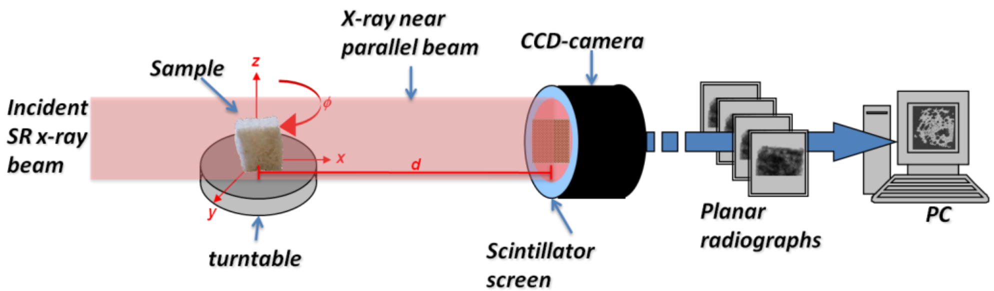
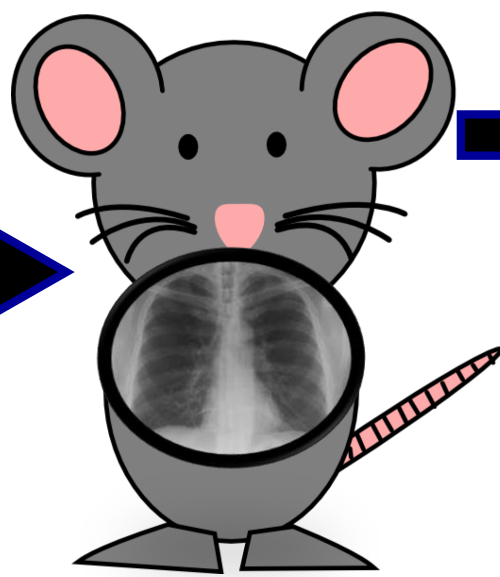
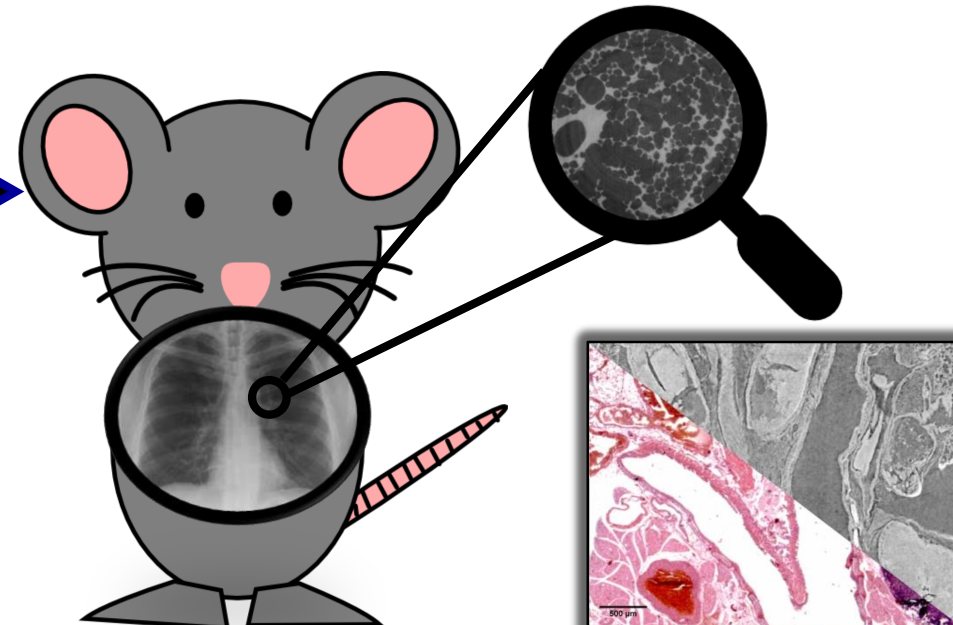
Multi-resolution CT: Zoom CT



Agarose embedded mice



9 µm voxel size overview scan



1-2 µm voxel size region of interest scan

Combined CT / histology analysis

Zoom CT - Visualization of lung methastasis in mice

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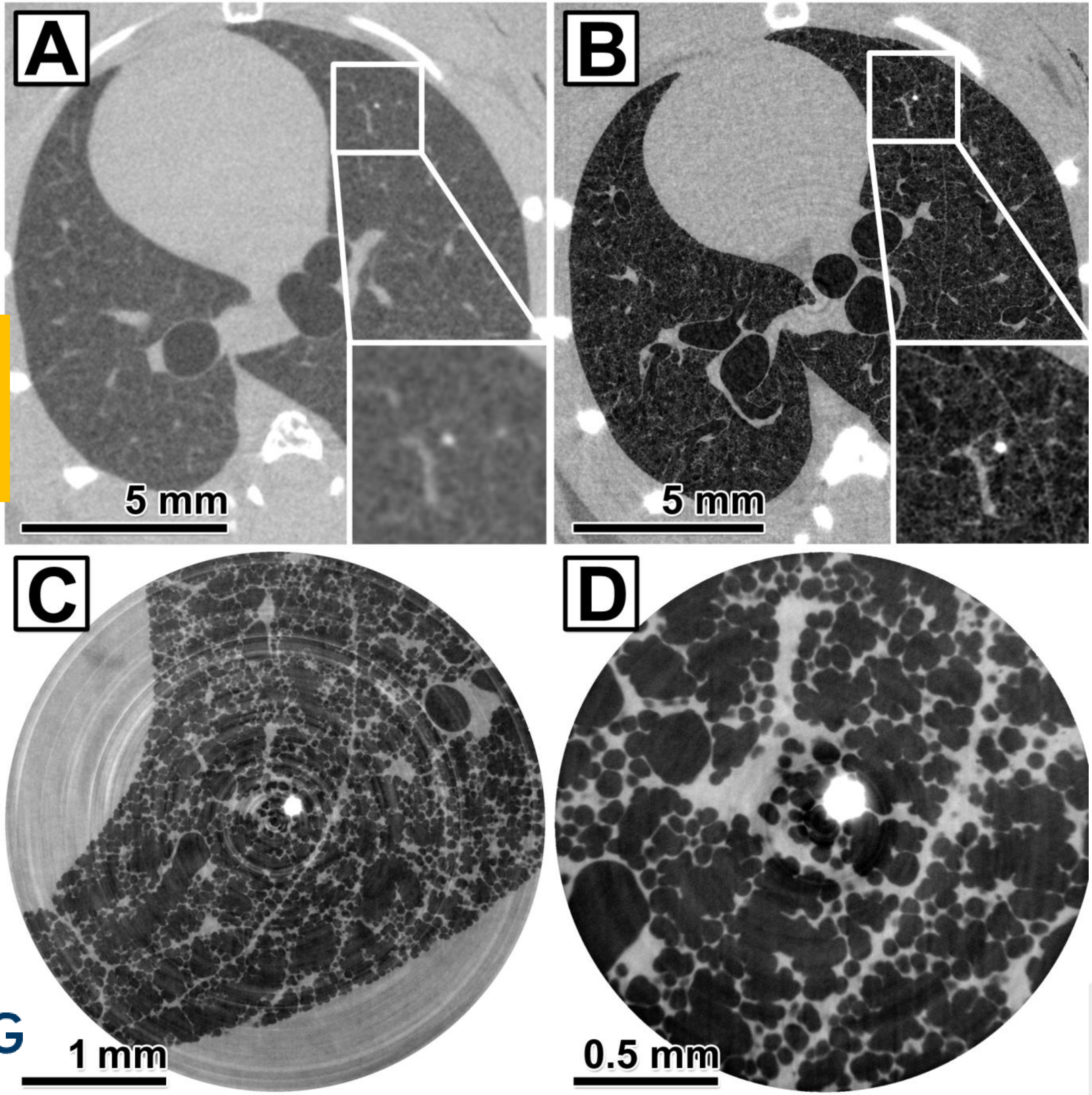
E = 22 keV,
(monochr. Beam)
pixel size = 9 µm
Slice of the entire lung

Lesion produced by cancer cells labeled by Ba np injected in blood stream



E = 22 keV,
pixel size = 9 µm
Phase retrieval,
$\delta/\beta = 1950$

Pink beam,
pixel size = 2 µm
Phase retrieval,
$\delta/\beta = 1950$

Pink beam,
pixel size = 1 µm
Phase retrieval,
$\delta/\beta = 1950$

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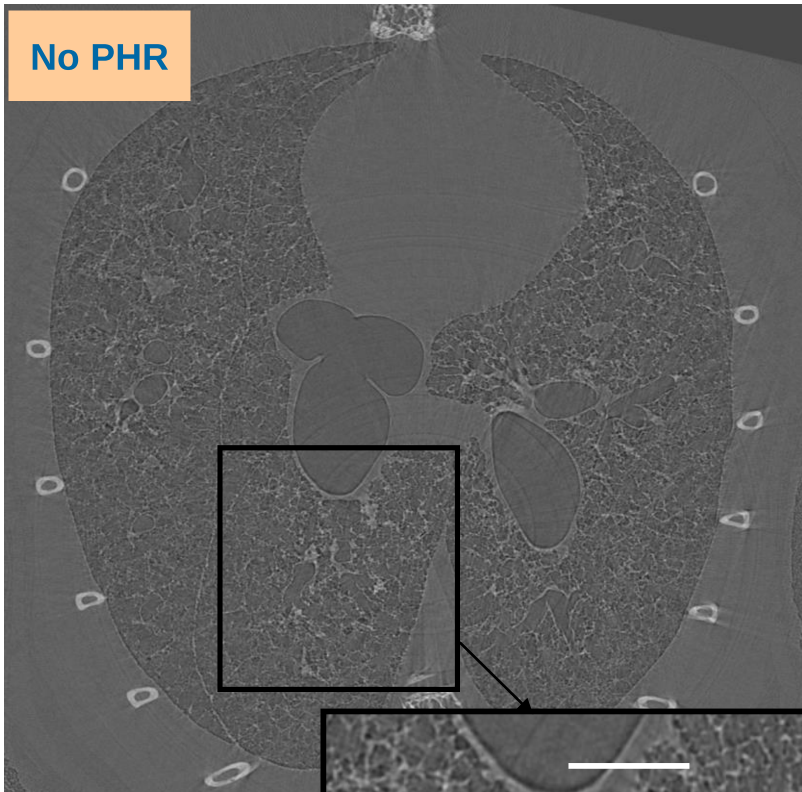
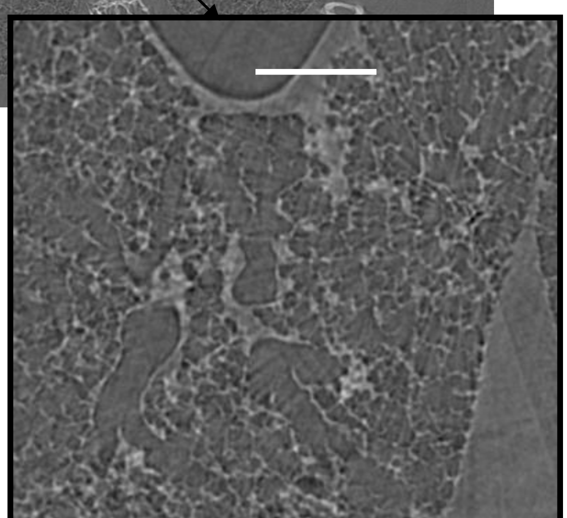
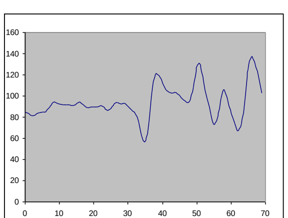
(Courtesy of J. Albers)

PBI potentials in tissues visualization: imaging of inflammation in asthmatic mice

- Animal model of allergic asthma induced by ovalbumin based on balb/c mice
- Murine Alveolar Macrophage Cells stained with Barium sulfate used to target inflammation sites
- Macrophages were adminstered intra-tracheally 48 hours after asthma induction





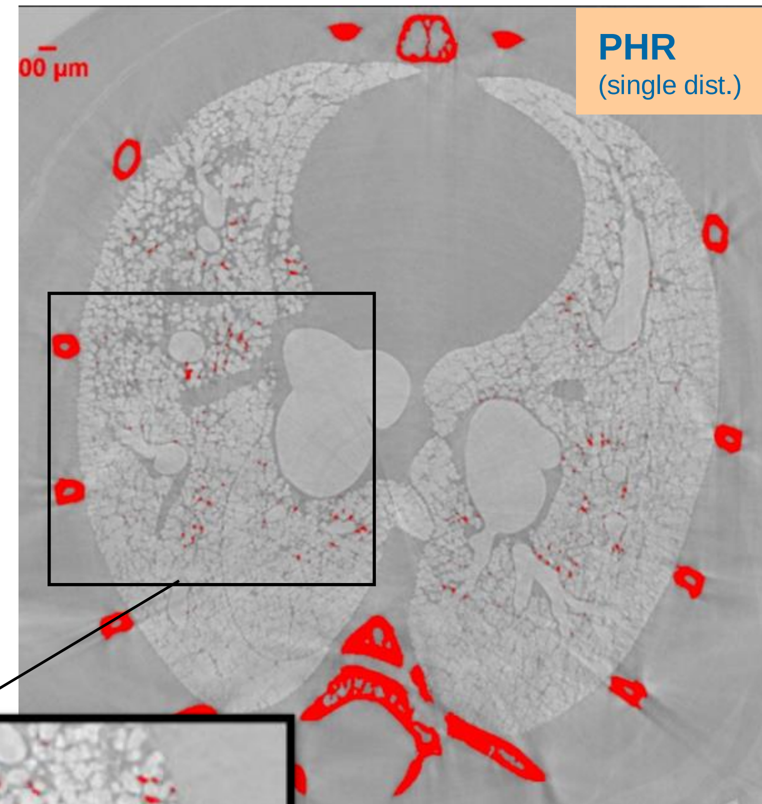


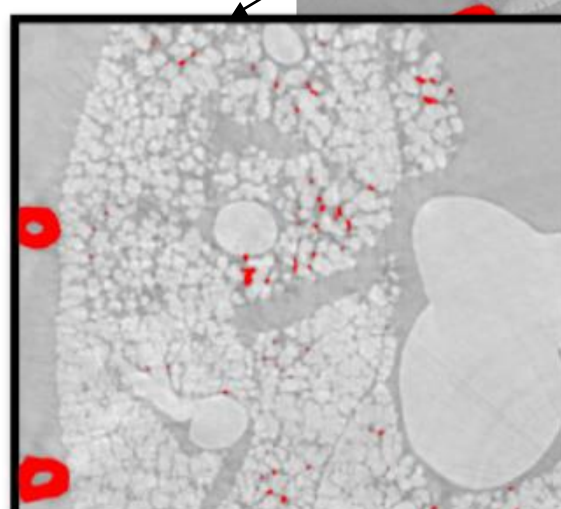
Edge enhancement effects due to PHC

Lung slice

E=22 keV
PHC dist = 30 cm
Pixel size = 9 µm

Clear separation of tissue, Ba and bones signals with PHR





Bones
Barium

C. Dullin et al, J. of Synchrotron Radiation 22(1) (2015)

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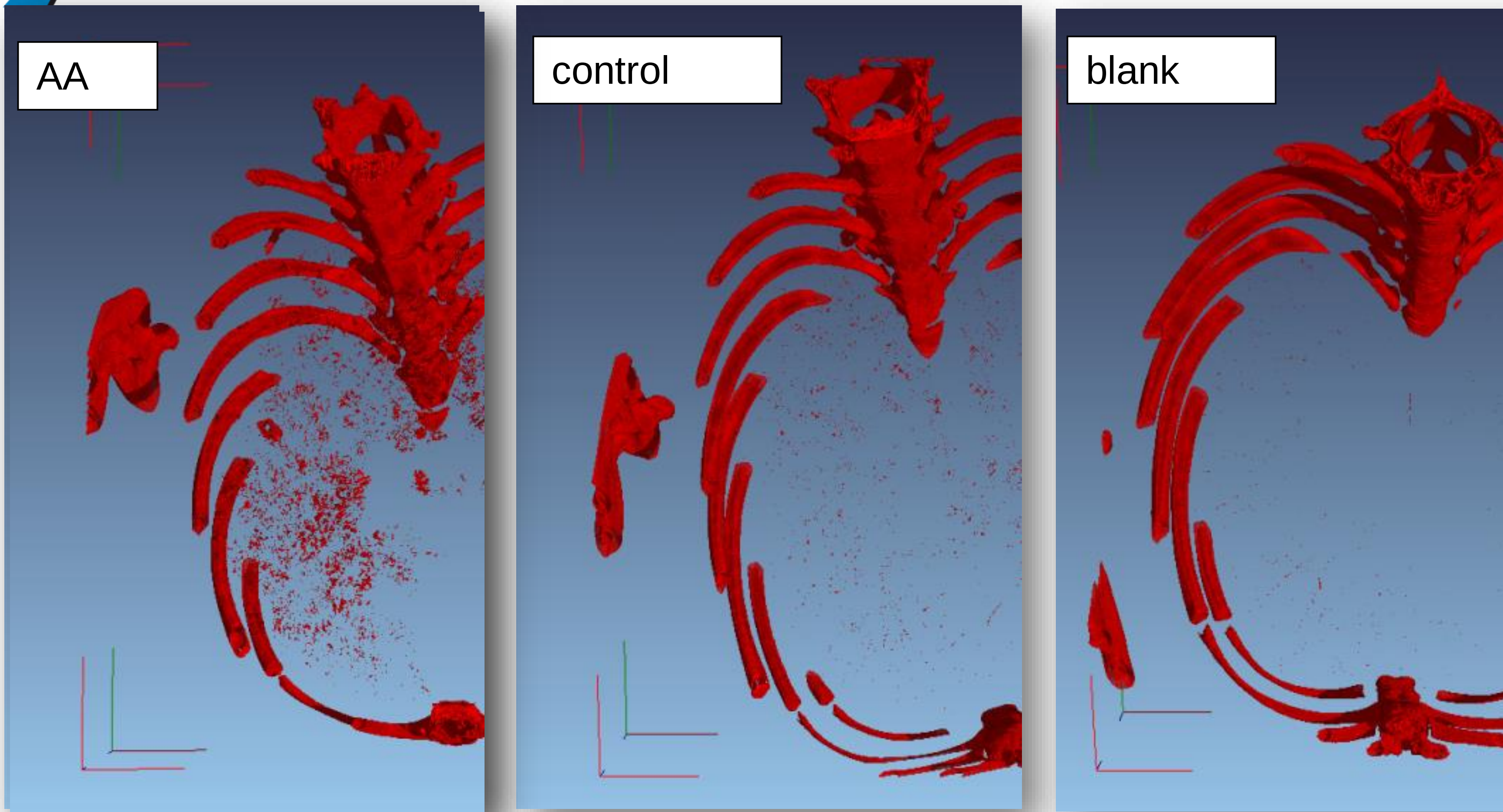
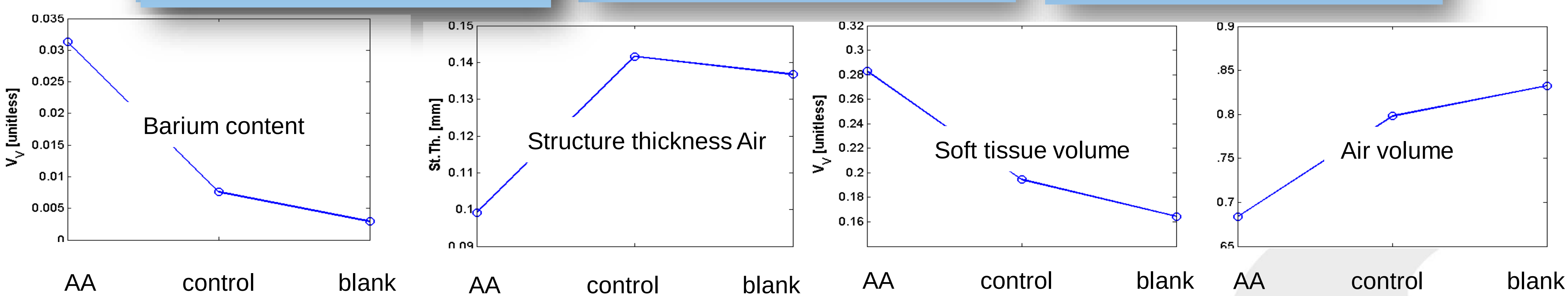
3D Visualization of labeled macrophages





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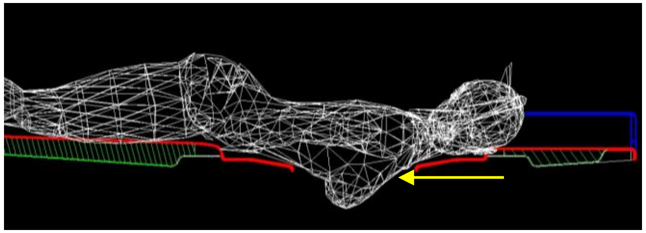
3 - Clinical imaging



- Major aims: implement research protocols on selected patients. Pixel sizes : **50 – 100 µm**
- Possibility for *in-vivo* low dose phase contrast CT and dynamic imaging
- Use of Phase Retrieval algorithms dramatically increases image contrast (allowing strong dose reduction).
- Analyzer Based Imaging allows for the visualization of cartilage
- Optimization of X-ray energy (dose reduction, convenient use of contrast agents)
- Applications:
  - Breast imaging
  - Potentials for imaging of cartilages
  - Proof-of-principle study for low dose lung CT

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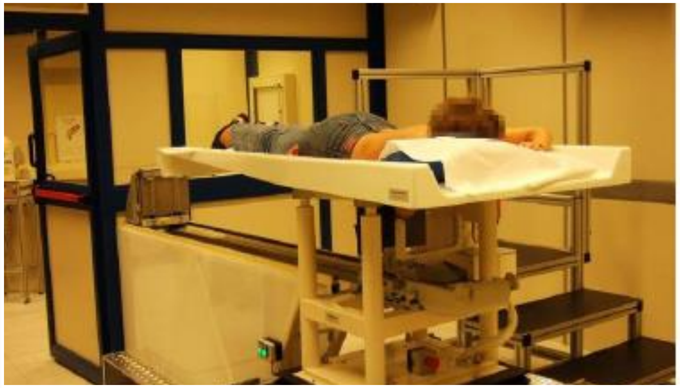
Towards low dose phase contrast breast CT – the SYRMA-CT project

Motivations

- Projection X-ray mammography has important limitations in breast cancer detection, especially in the dense breasts.
- 3D imaging approach implemented in additional breast imaging modalities: breast tomosynthesis, breast ultrasound, breast MRI, all used in combination.
- First dedicated breast CT systems are obtaining promising results.
- Our previous experience: SR phase contrast mammography outperforms conventional mammography

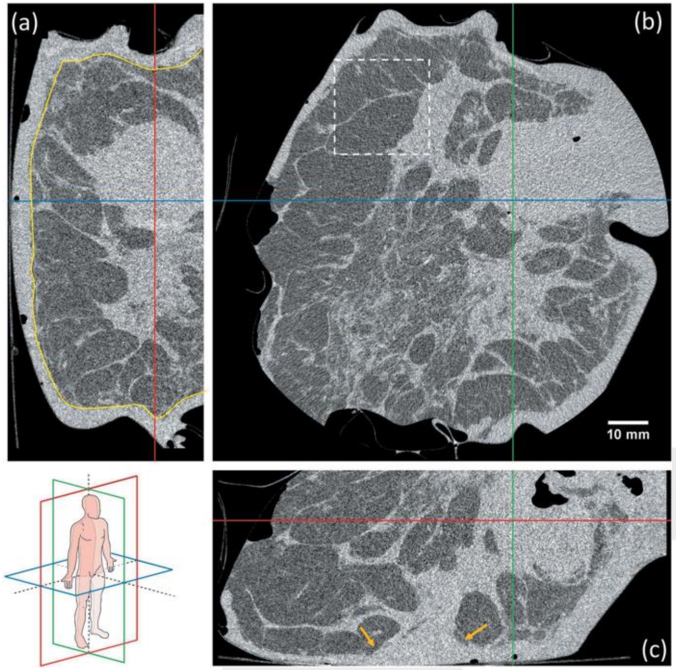


Aims

- Evaluate the effectiveness of **low dose phase contrast breast CT** on selected patients
- Reduction of Mean Glandular Dose (MGD) up to 5 or 2 mGy

Methods

- Use of sensitive Single photon counting detector (Pixirad)
- Optimization of X-ray Energy: 26-38 keV
- Application of propagation based phase contrast imaging and phase retrieval algorithms
- Optimization of reconstruction algorithms



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UNIVERSITÀ DEGLI STUDI DI TRIESTE

INFN Istituto Nazionale di Fisica Nucleare

Azienda Sanitaria Universitaria Integrata di Trieste

Sezioni di: Trieste, Pisa, Cagliari, Napoli, Ferrara

R.Longo et al. :J. Synchrotron Rad. (2019). N. 26

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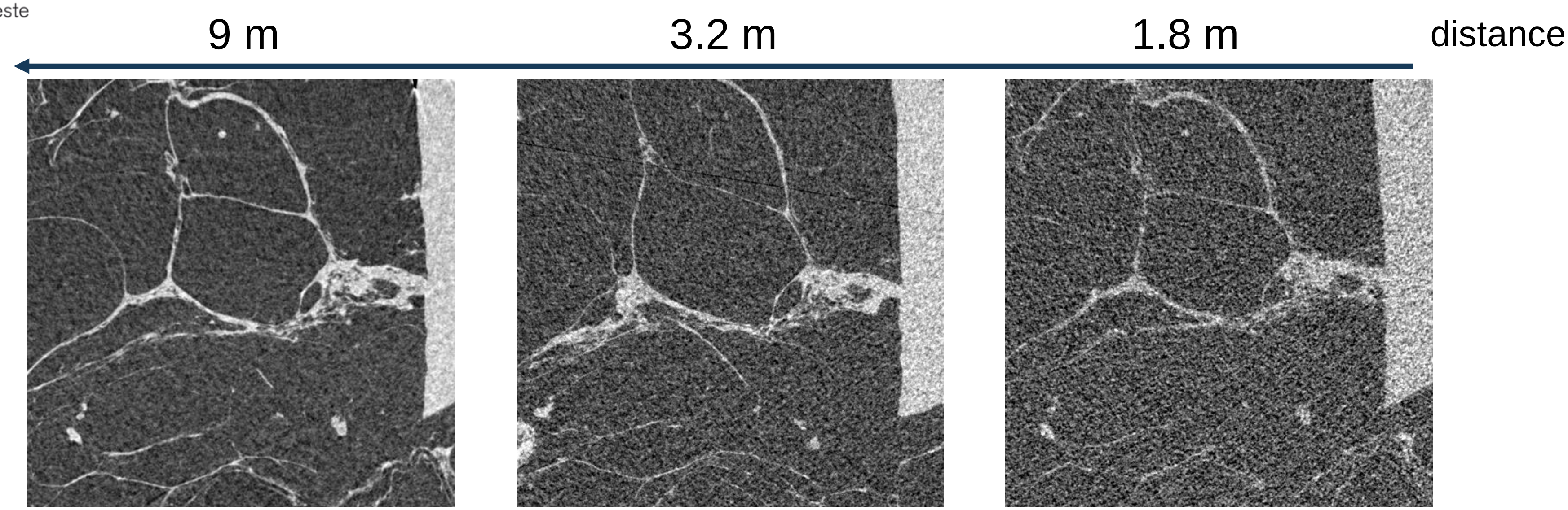
Low dose CT - Effect of long propagation distance

Mastectomy detail
approx. size:1.5 x 1.5 cm
E = 32 keV
Pixirad detector
MGD ~ 5 mGy

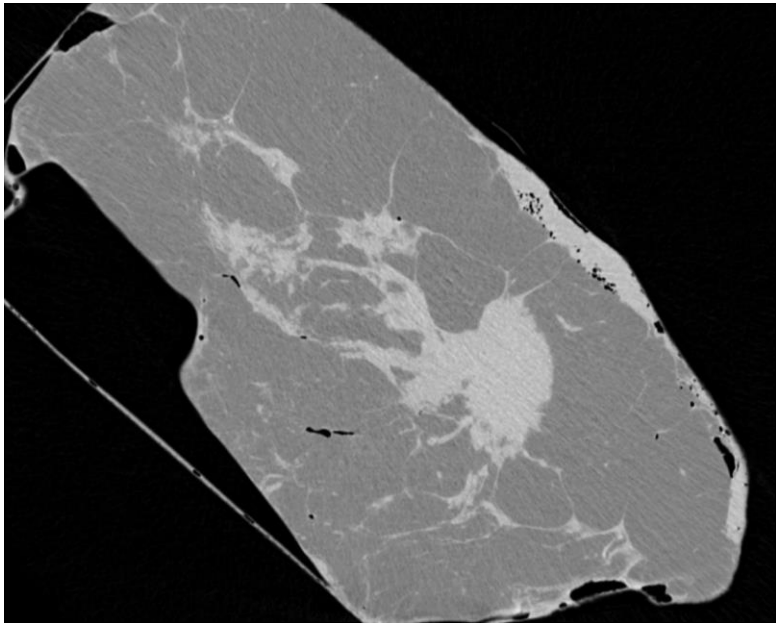
L.Brombal et al.:, Phys. Med. Biol.63, 2018

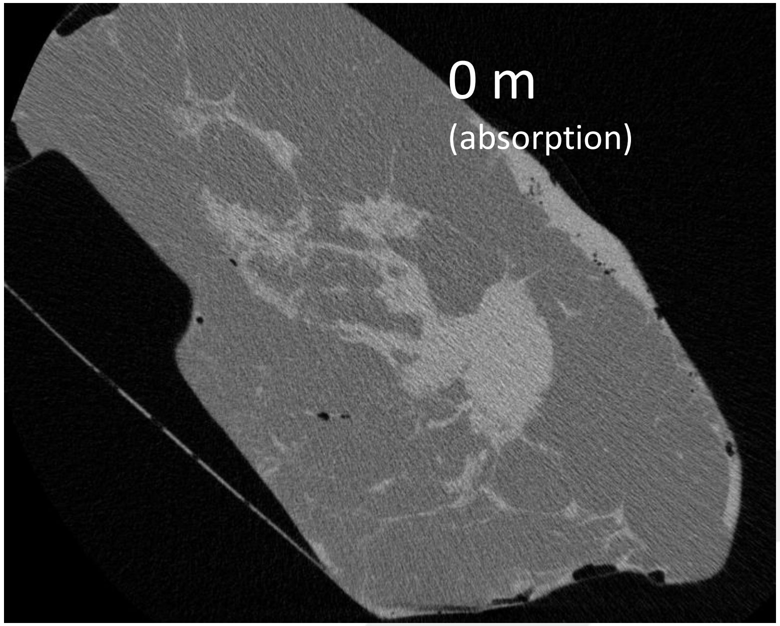


Reconstruction of low dose CT slice with application of phase retrieval algorithm (Paganin 2002)

Mastectomy slice
Size:7 x 3 cm
E = 32 keV
XCounter detector
MGD ~ 5 mGy

Baran et al.: Phys. Med. Biol. 62, 2017





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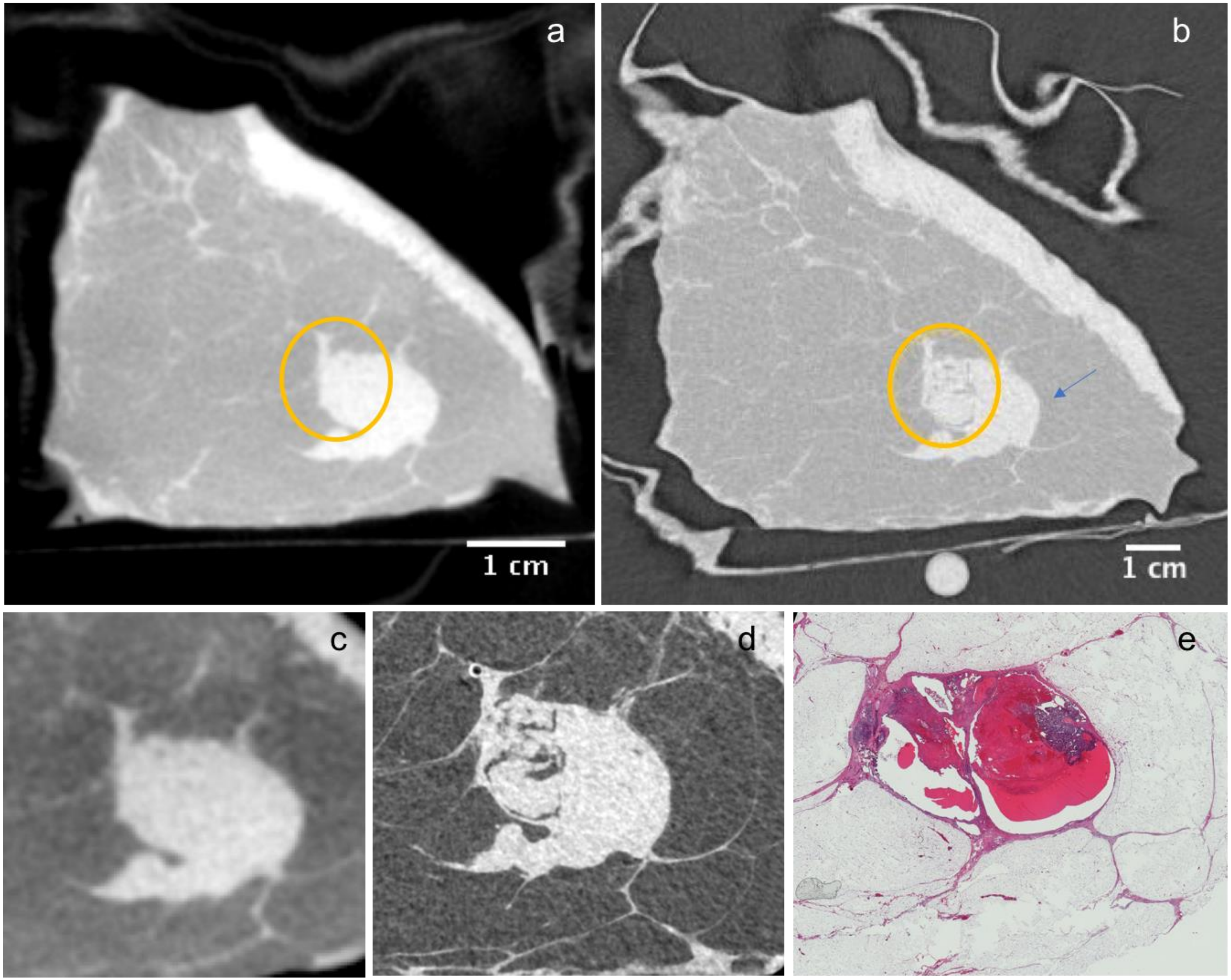
Comparison between Propagation Based-CT (PB-CT) and Dedicated Breast Cone Beam-CT (CB-CT)

Conventional | Synchrotron



Excised breast sample from an 80 year old woman, including an in situ intraductal papillary carcinoma.

a) image obtained with the dedicated CB-CT system (Konig).
b) image obtained with PB-CT technique.

The blue arrow indicates the part of the lesion with regular borders, the yellow circle highlights the infiltrating part.

c) Close-up CB-CT
d) Close-up PB-CT
e) Histological Image

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S.Pacilè et al., accepted for publ. on Sci. Rep.

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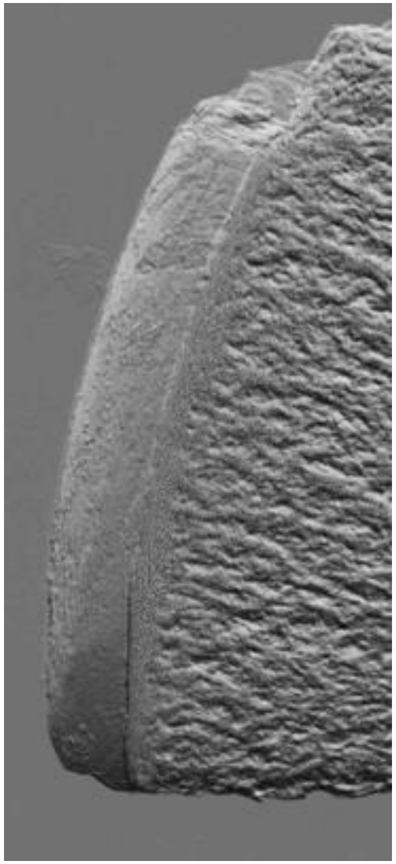
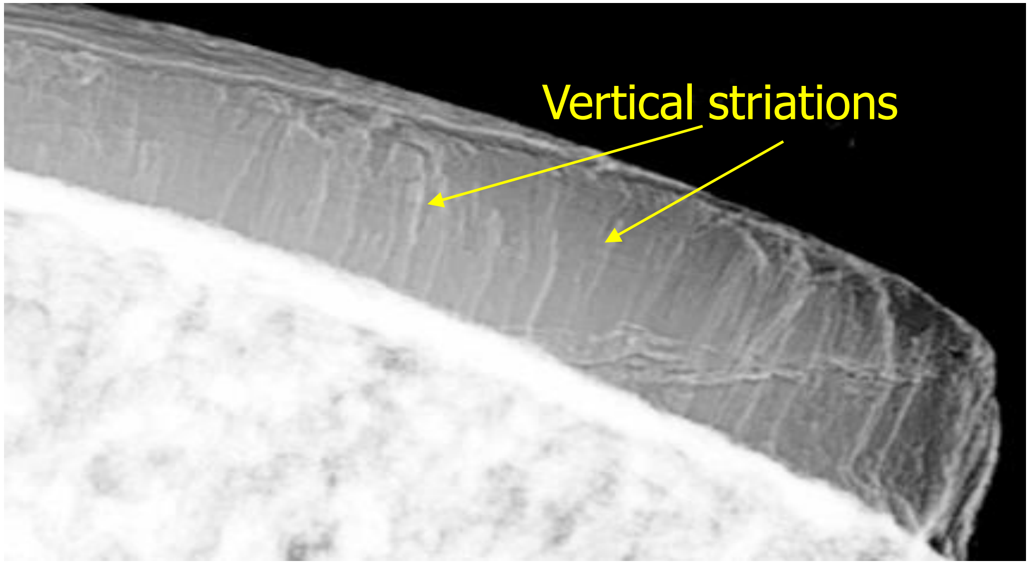
Imaging of cartilage and bone interface



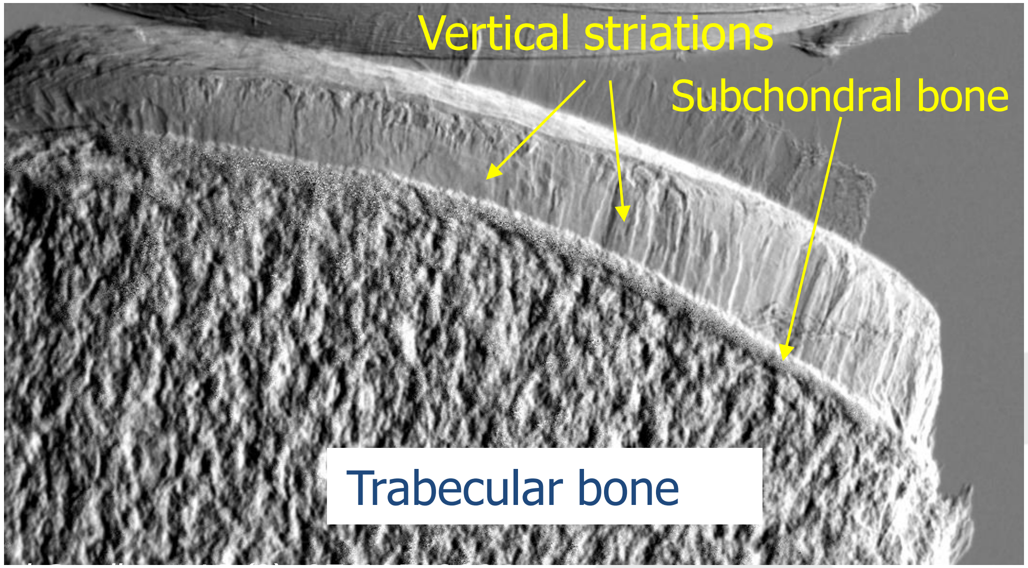
Sample: human femur head

Need to study:
- cartilage
- cartilage-bone interfaces
- changes in the bone structure



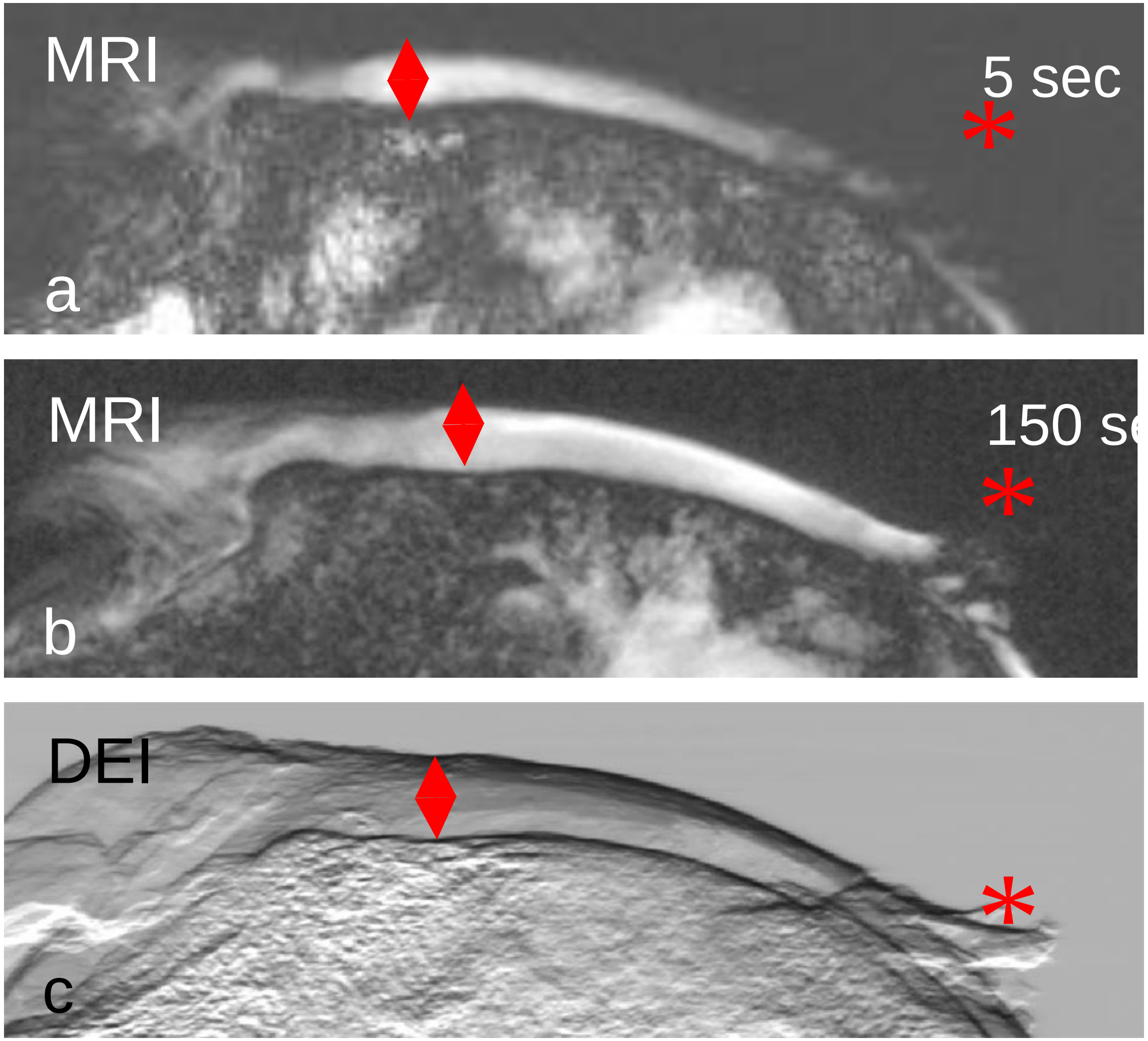
Apparent absorption image

Refraction image



- The ABI technique allows to visualize the discontinuities in the sample and the inner structures invisibles by means of abs 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

Femur head core cuts: comparison with MRI

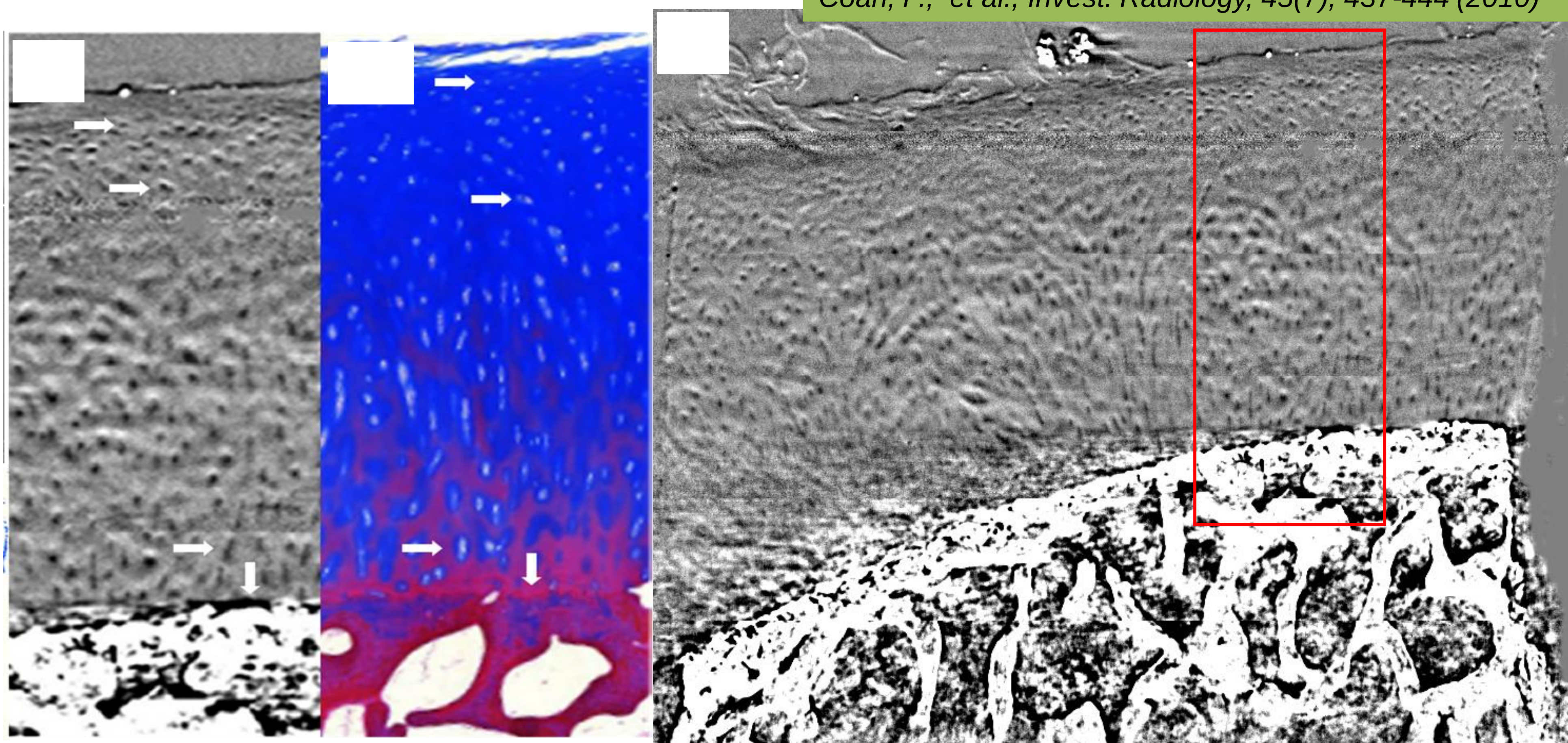


A.Wagner et al., Nucl. Instrum. Methods A 548, 47 (2005).

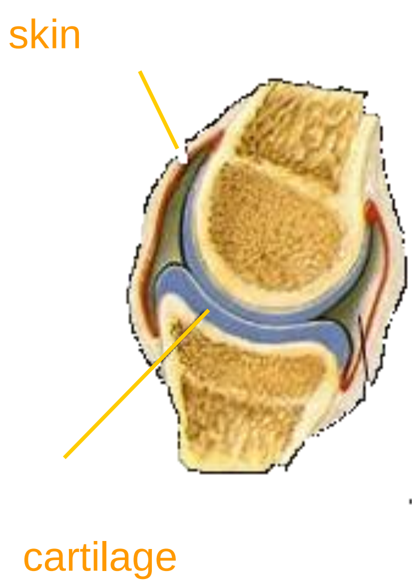
Specimen of normal cartilage (A), Coronal plane extracted from the reconstructed CT volume (B), Magnified portion identified by the ROI (C), Corresponding section from histologic preparation (D).
E = 26 keV, pixel size = 8 x 8 μm^2.

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)



ABI studies of the finger joint

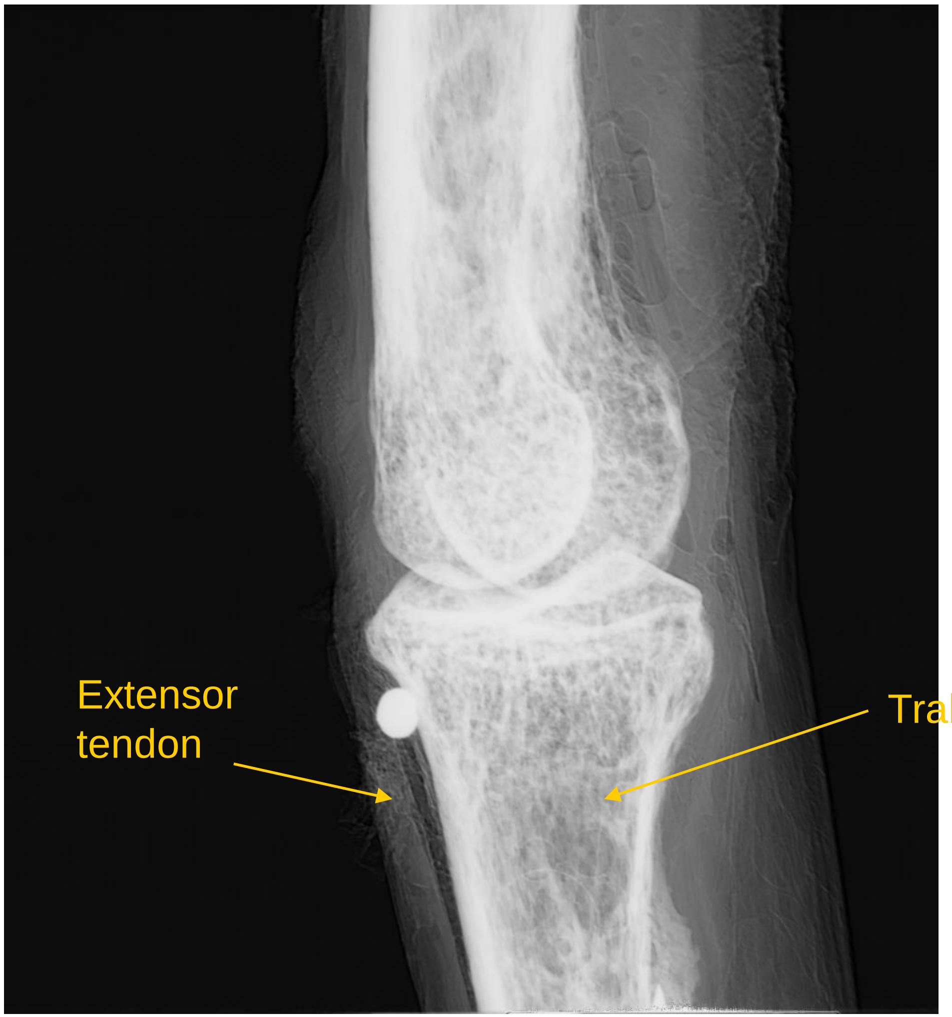


Conventional radiograph

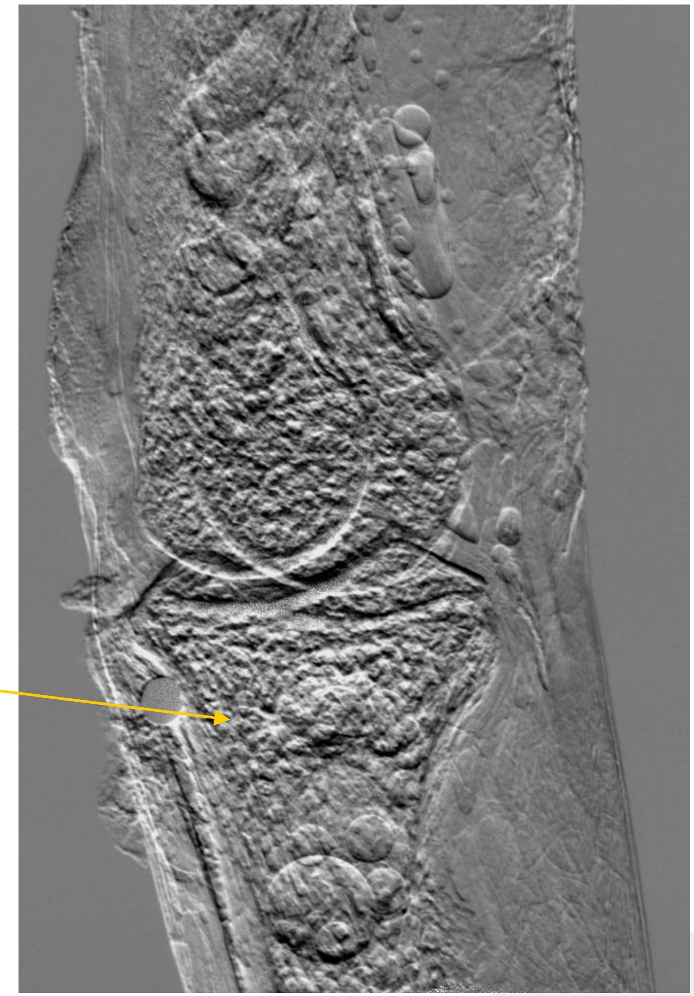
Apparent absorption image @ 20 keV at ELETTRA

Lewis, R. A., et al., British J. Rad., 76(905), 301-308 (2003)

Index finger proximal interphalangeal joint



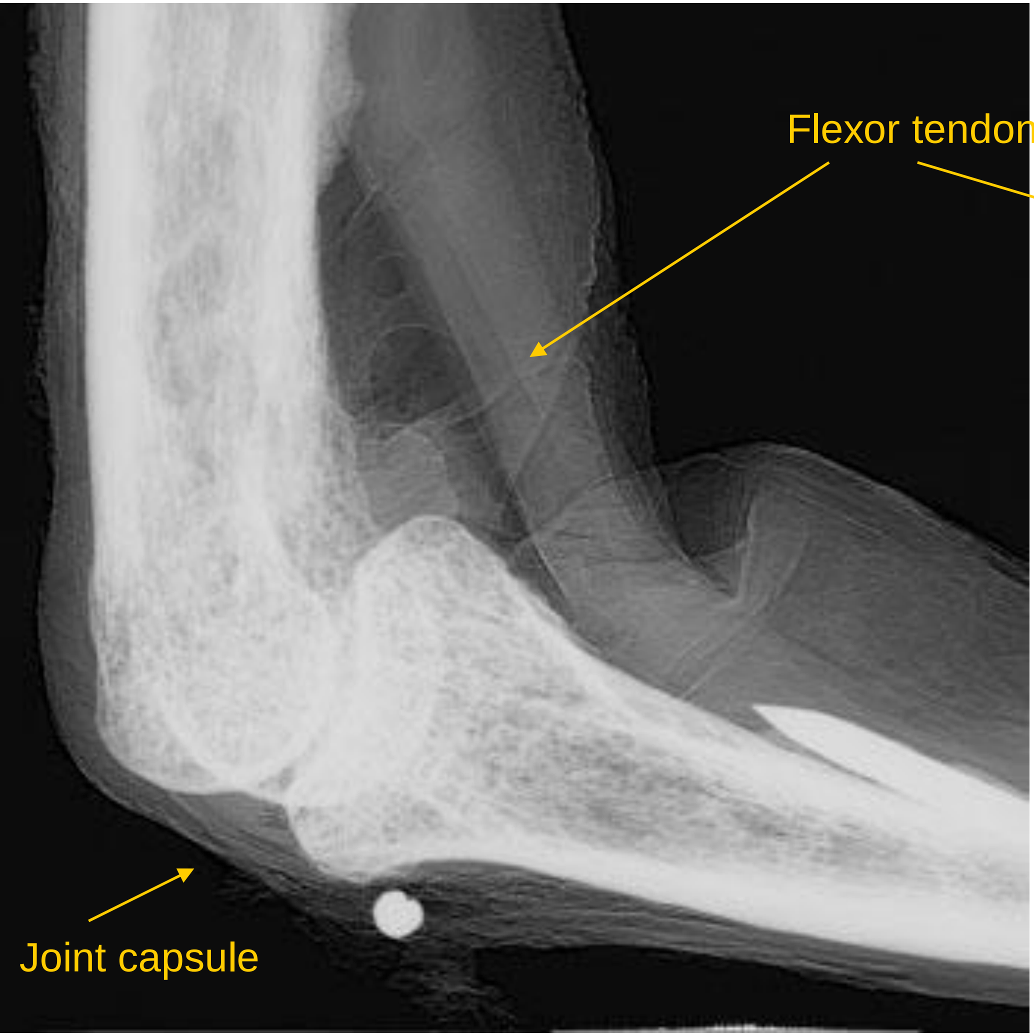
Apparent absorption Image



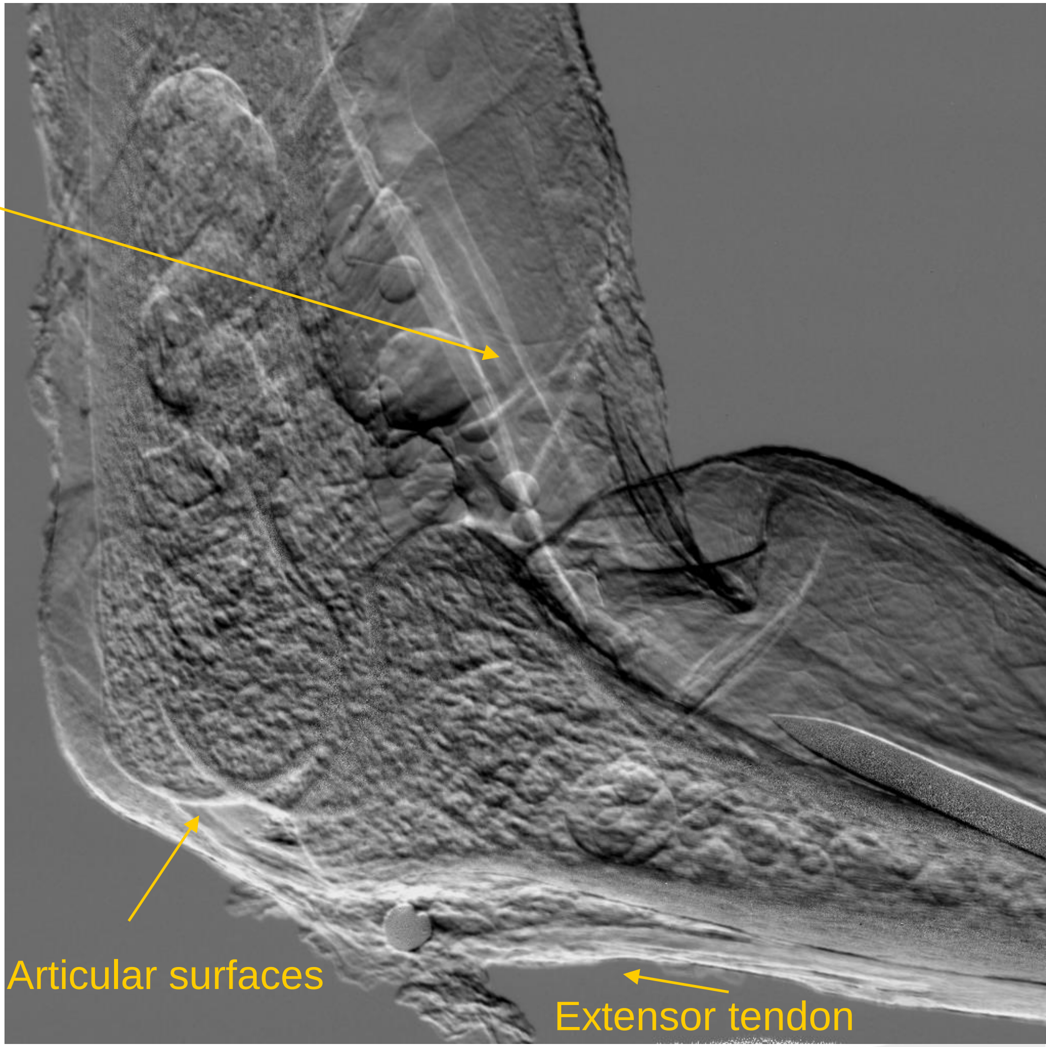
Refraction Image

Lewis, R. A., et al., British J. Rad., 76(905), 301-308 (2003)

Index finger proximal interphalangeal joint



Apparent absorption Image



Refraction Image

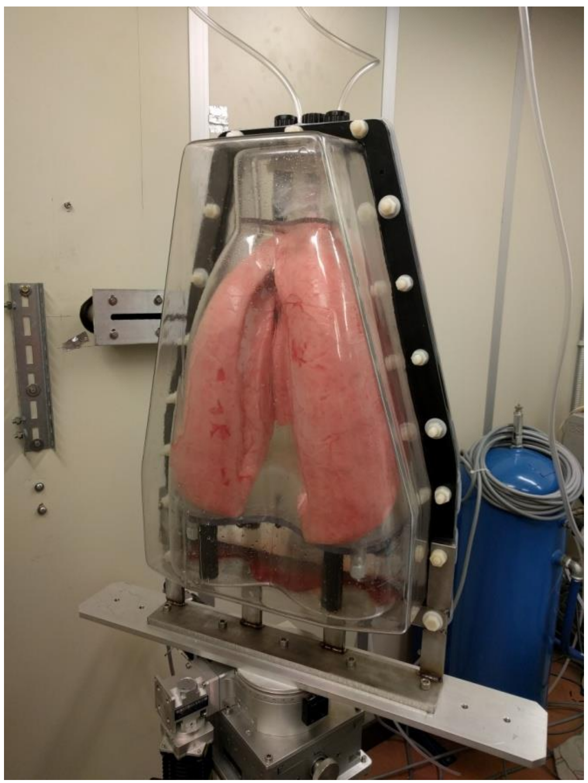
Elettra Sincrotrone Trieste

Low dose phase contrast Lung CT - proof-of-principle study on porcine lungs

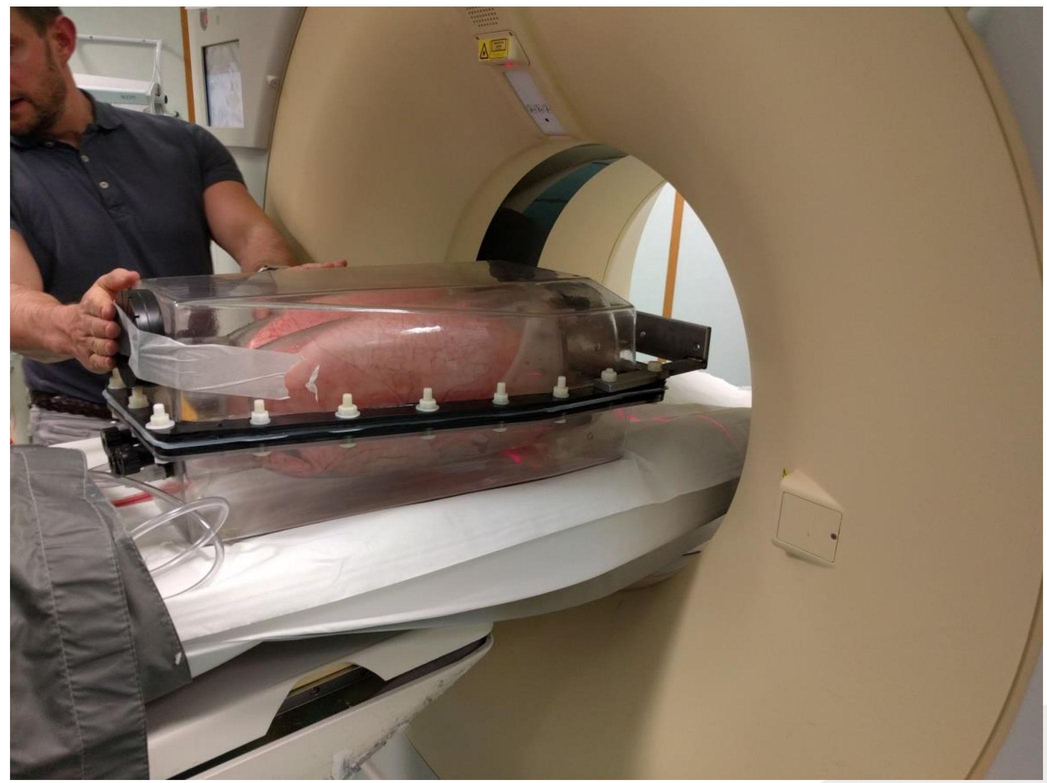
Aim: evaluate the potentials of lungs CT in the early detection of **lung cancer** and/or **Idipathic Lung fibrosis**

- samples: porcine lungs in the artiCHEST training phantom (they mimik human lungs)
- SR imaging: E = 40 keV, prop dist = 2.5 m, air entrance dose ~ 13 mGy
- Reconstruction: conventional FBP, phase retrieval pre-processing

SYRMEP beamline



Cattinara hospital Trieste



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DZL Deutsches Zentrum für Lungenforschung

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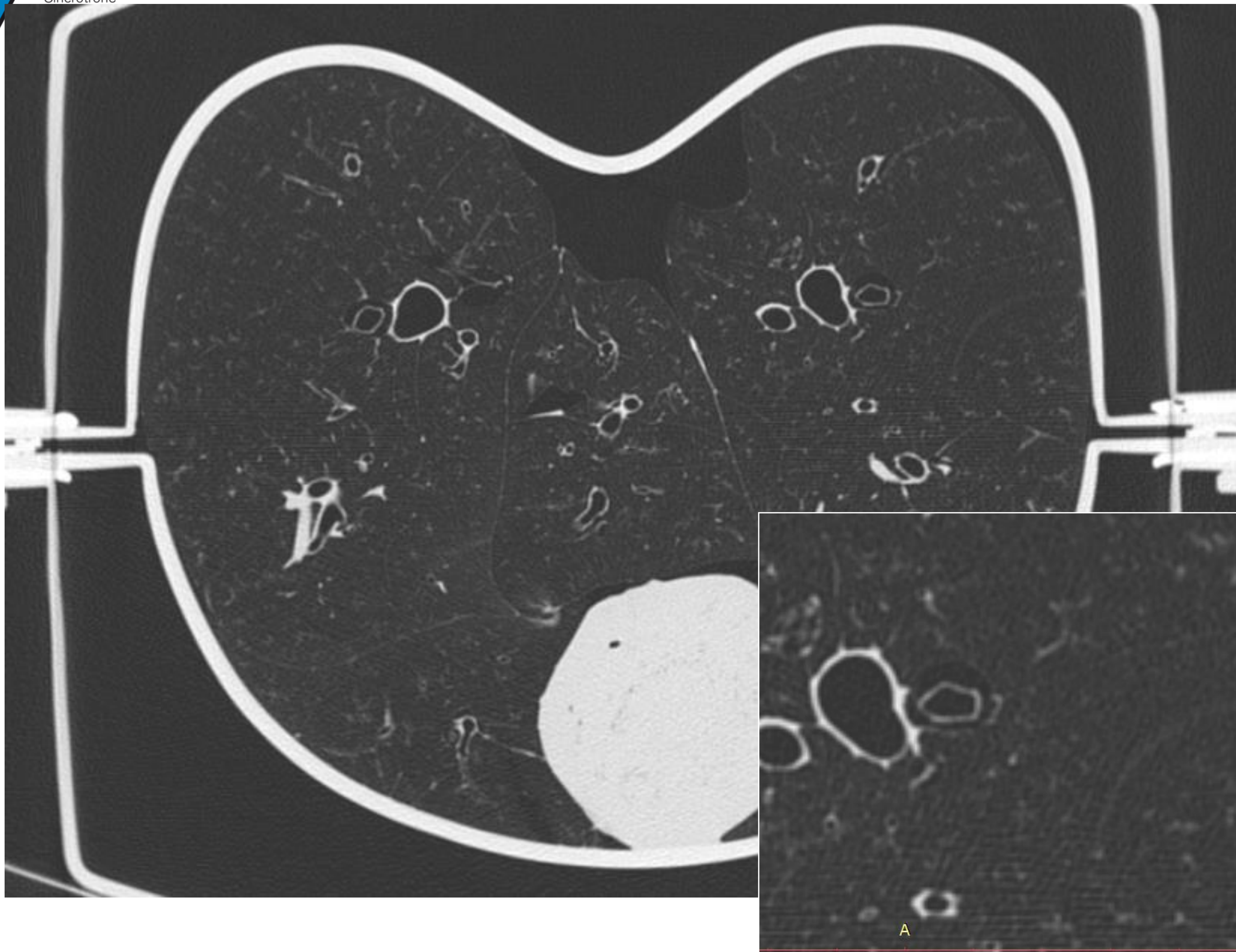
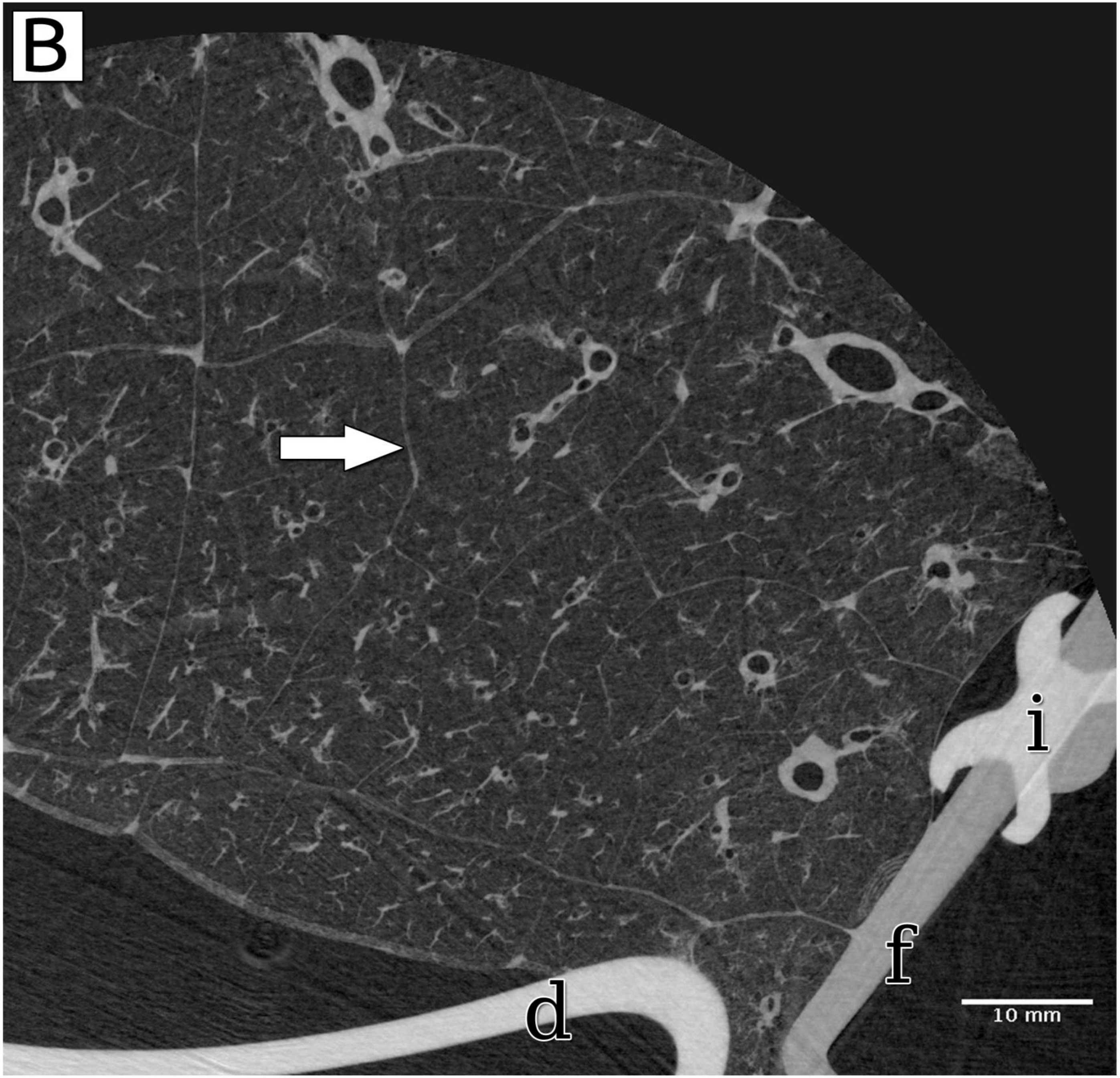
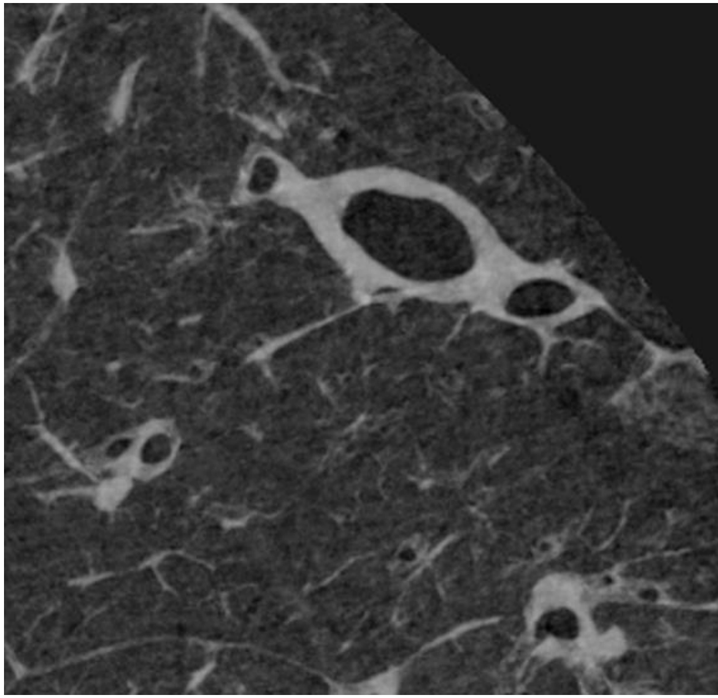
G.Tromba

Conventional CT slice



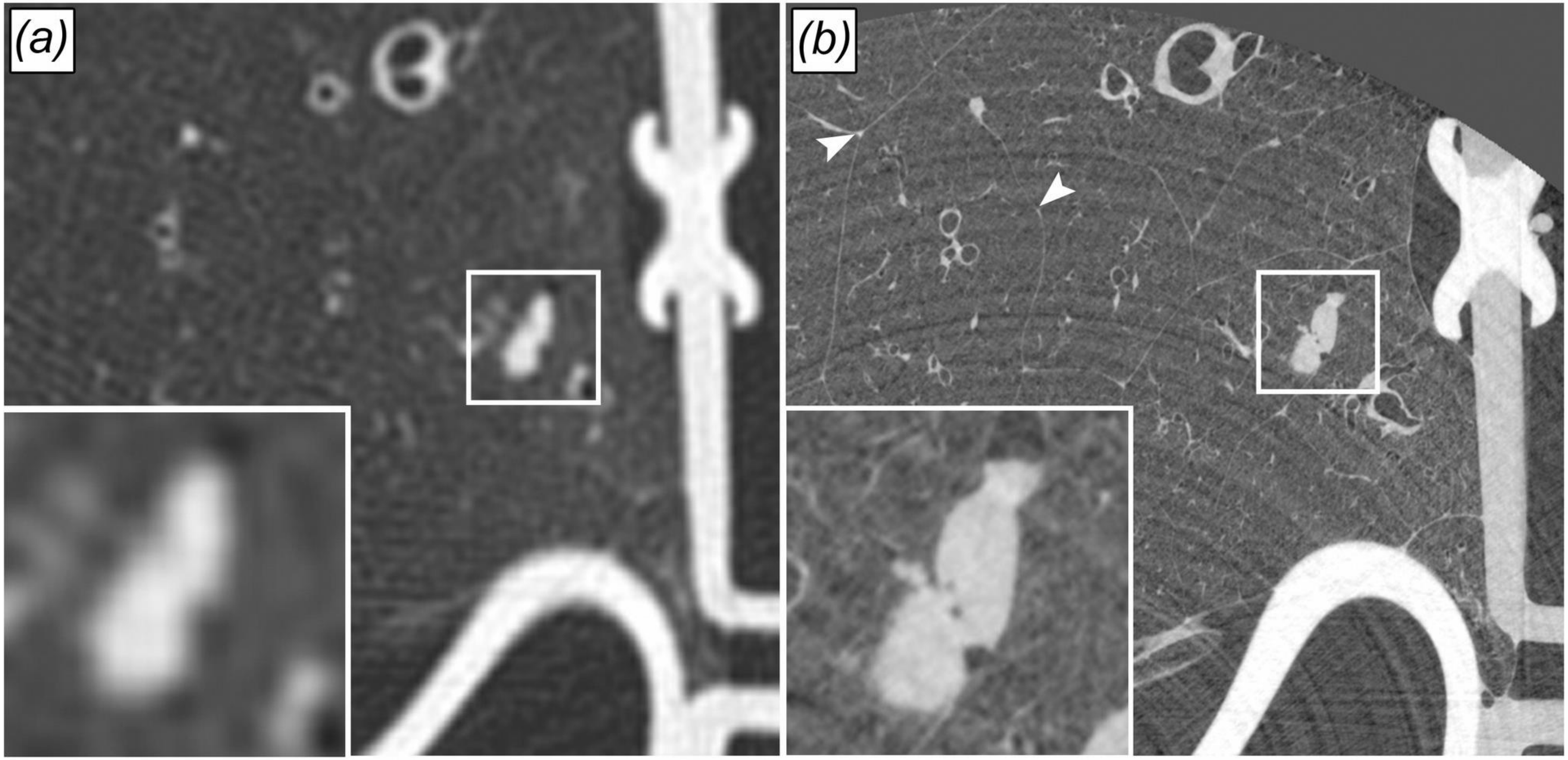
SR CT slice





G.Tromba

Lesions visualization

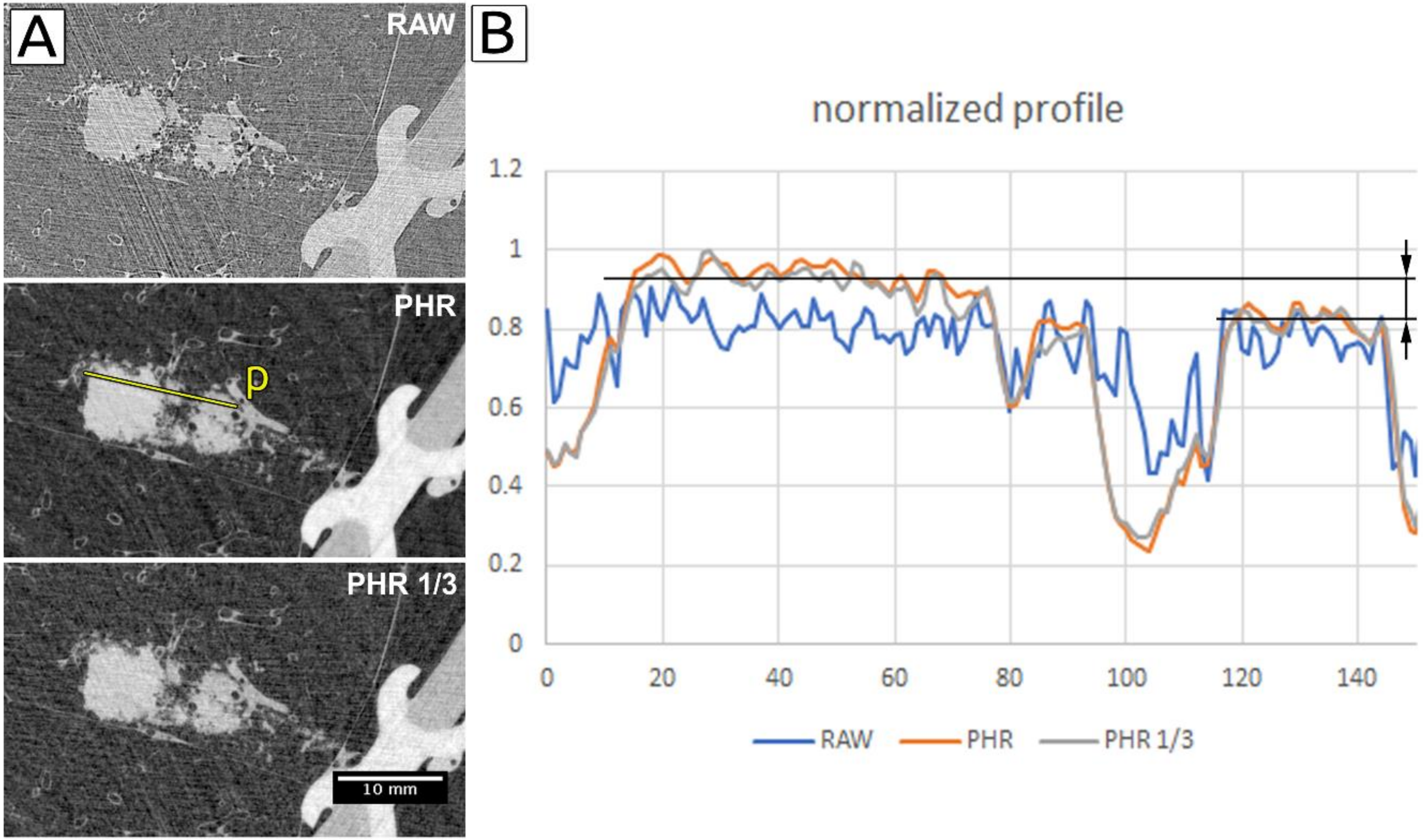


(a) clinical HRCT - air kerma ~ 33 mGy, voxel size 0.45 x 0.45 x 0.9 mm^3
(b) SYRMEP - air kerma ~13 mGy, voxel size 0.1 x 0.1 x 0.1 mm^3

W.Wagner et al.: J.Synchrotron Rad. 25, (2018)

Lesions density assessment

- Artificial nodules created by injecting in lungs of agarose at different concentrations
- Without phase retrieval no density difference between the nodules can be detected (RAW). Phase retrieval (PHR) allowed the differentiability between the nodules density.



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Thanks for your attention!

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