Multiscale phase contrast imaging in biomedical research: the experience at Elettra

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CompactLight Complementary Use and Opportunities

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I) Phase contrast CT for virtual histology, guided sectioning and correlative imaging

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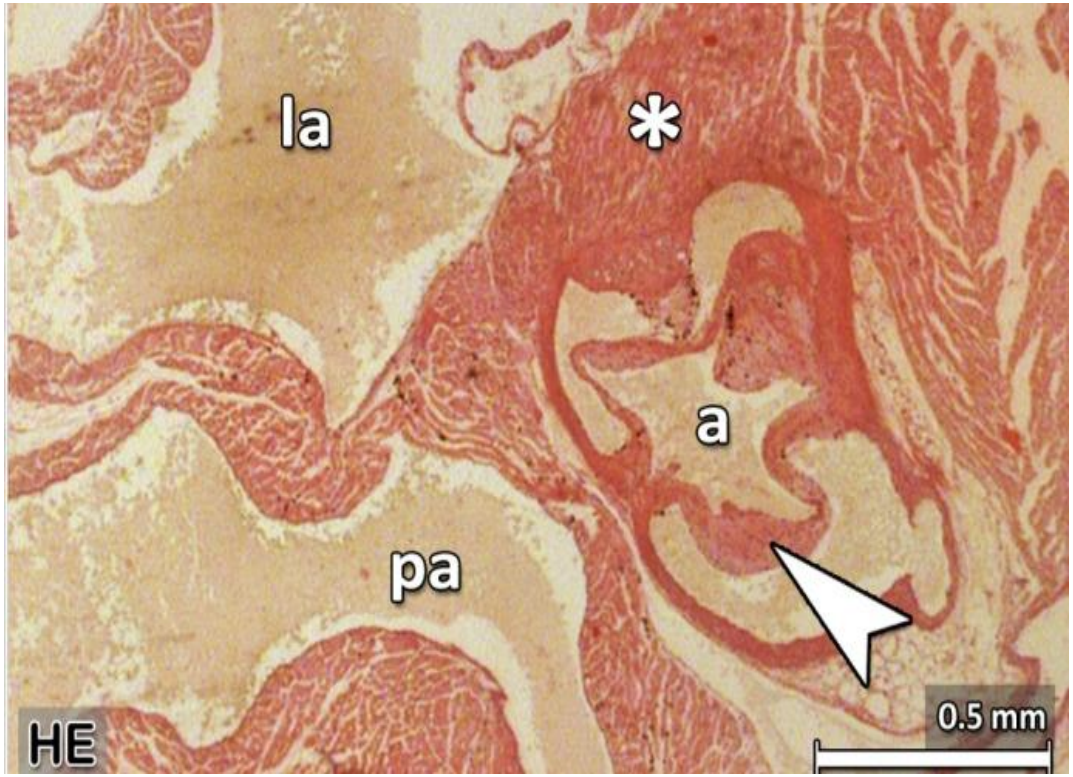
histology

Histology: versatile tool for tissue analysis, resolution only limited by optical microscopy; very specific in combination with immunochemistry



HE stained mouse heart. The following structures are clearly depicted: a, aorta; pa, pulmonary artery; la, left atrium and star, heart muscle.

Disadvantages:

- ✓ sample destroyed within the process
- ✓ only 2D
- ✓ cutting angle fixed
- ✓ preparation steps can introduce artifacts (shrinkage)
- ✓ parameters like volumes of structures, vessel tree architecture hard to assess

µCT for virtual histology

x-ray based imaging:

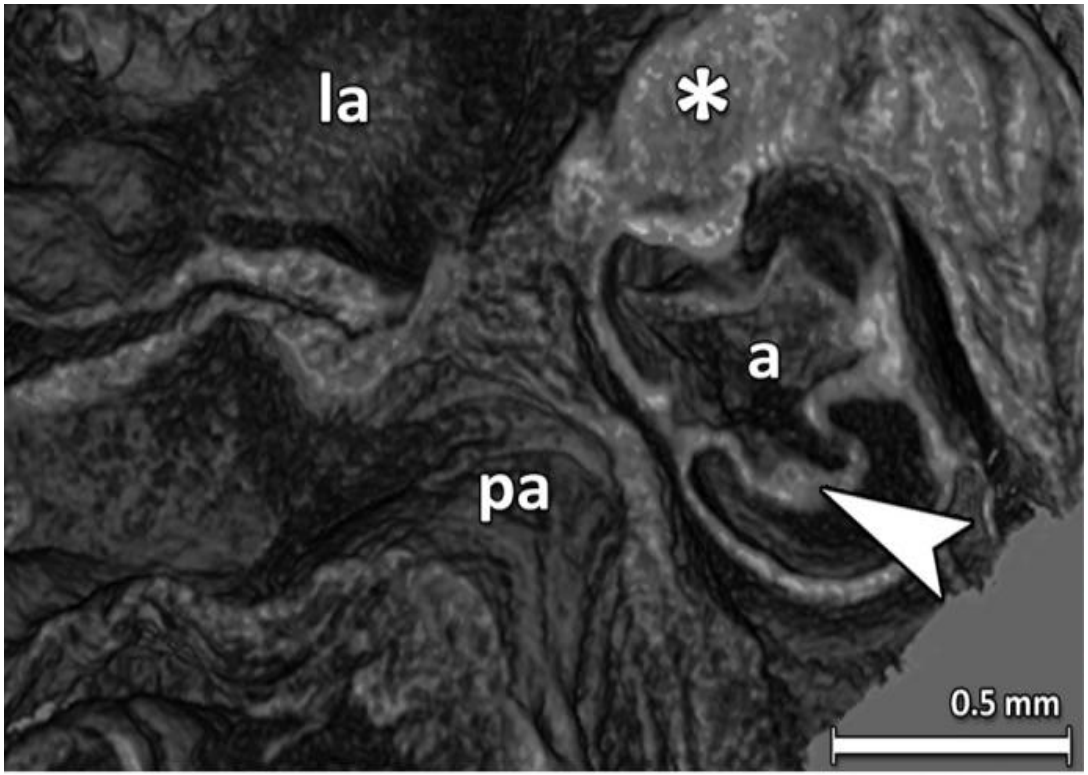
high penetration depth -> no cutting of samples, resolution <1µm possible , 3D images

<u>Disadvantages:</u>

contrast is related to the atomic number, therefore soft-tissue shows poor contrast

Stainings are diffusion limited

Typically non-specific

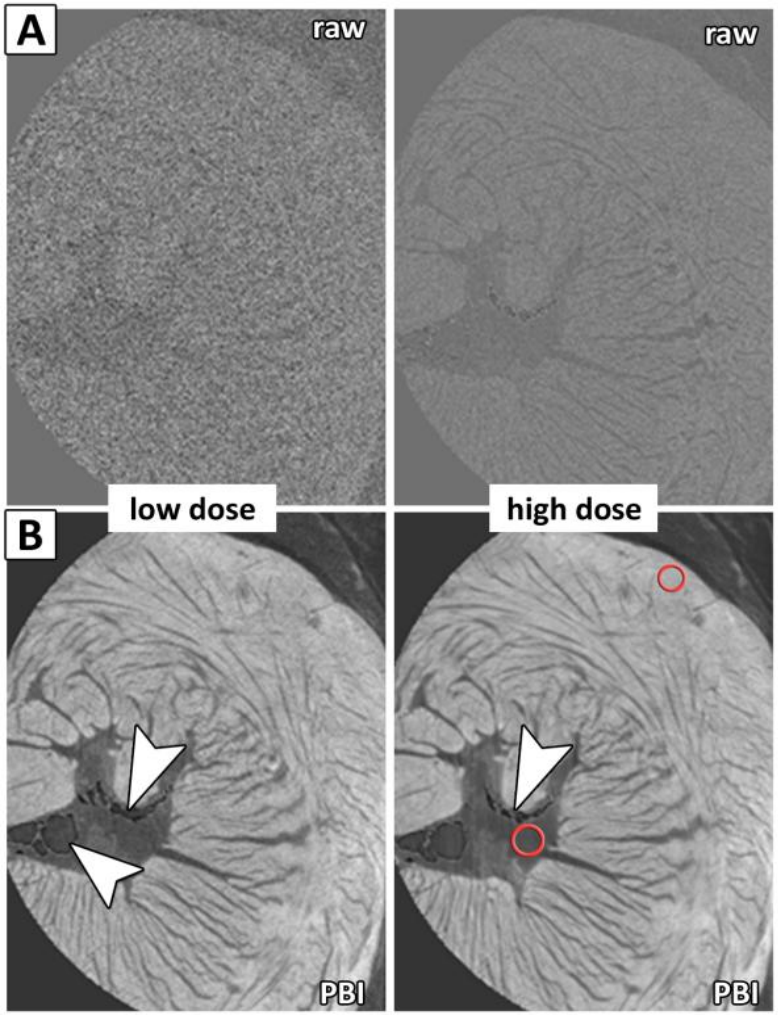


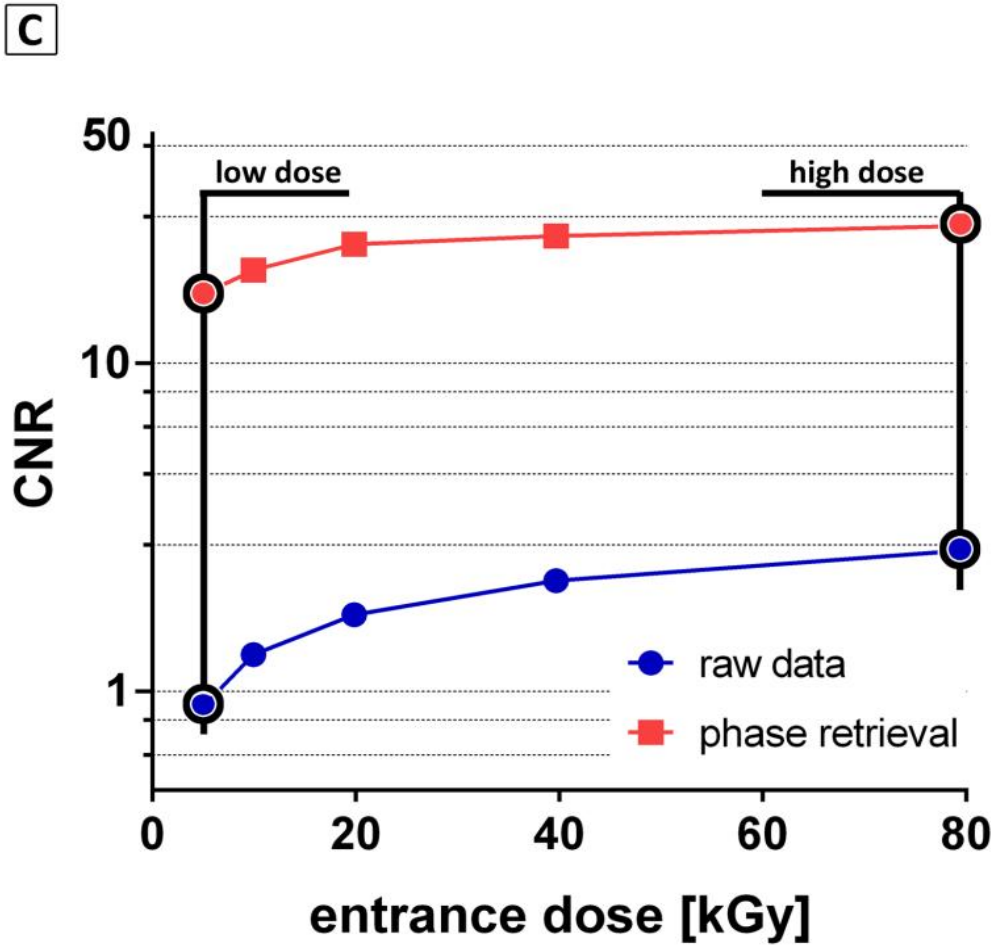
The same heart scanned at the SYRMEP beamline of the Italian Synchrotron (prior histologic sectioning). Resolution ~2µm. Notably, even very small structures like the flaps of the aortic valves (indicated by white arrow heads) can be seen.

Dullin, C., Ufartes, R., Larsson, E., Martin, S., Lazzarini, M., Tromba, G., ... & Alves, F. (2017). µCT of ex-vivo stained mouse hearts and embryos enables a precise match between 3D virtual histology, classical histology and immunochemistry. PloS one, 12(2), e0170597.

Phase contrast µCT (PBI)

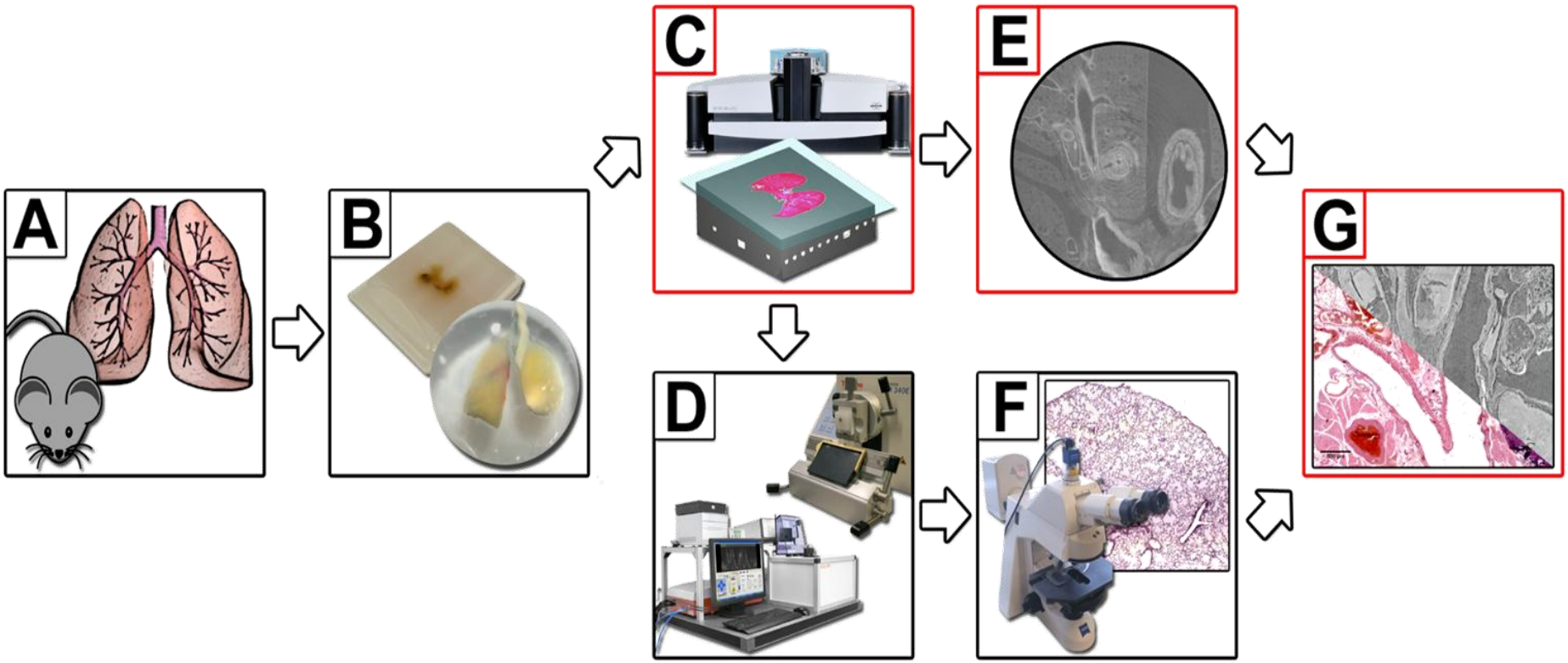
coherent x-rays: phase contrast can be exploited -> more dose efficient, dramatically boosting soft-tissue contrast





Saccomano, M., Albers, J., Tromba, G., Dobrivojević Radmilović, M., Gajović, S., Alves, F., & Dullin, C. (2018). Synchrotron inline phase contrast µCT enables detailed virtual histology of embedded soft-tissue samples with and without staining. Journal of Synchrotron Radiation, 25(4), 1153-1161.

Guided sectioning



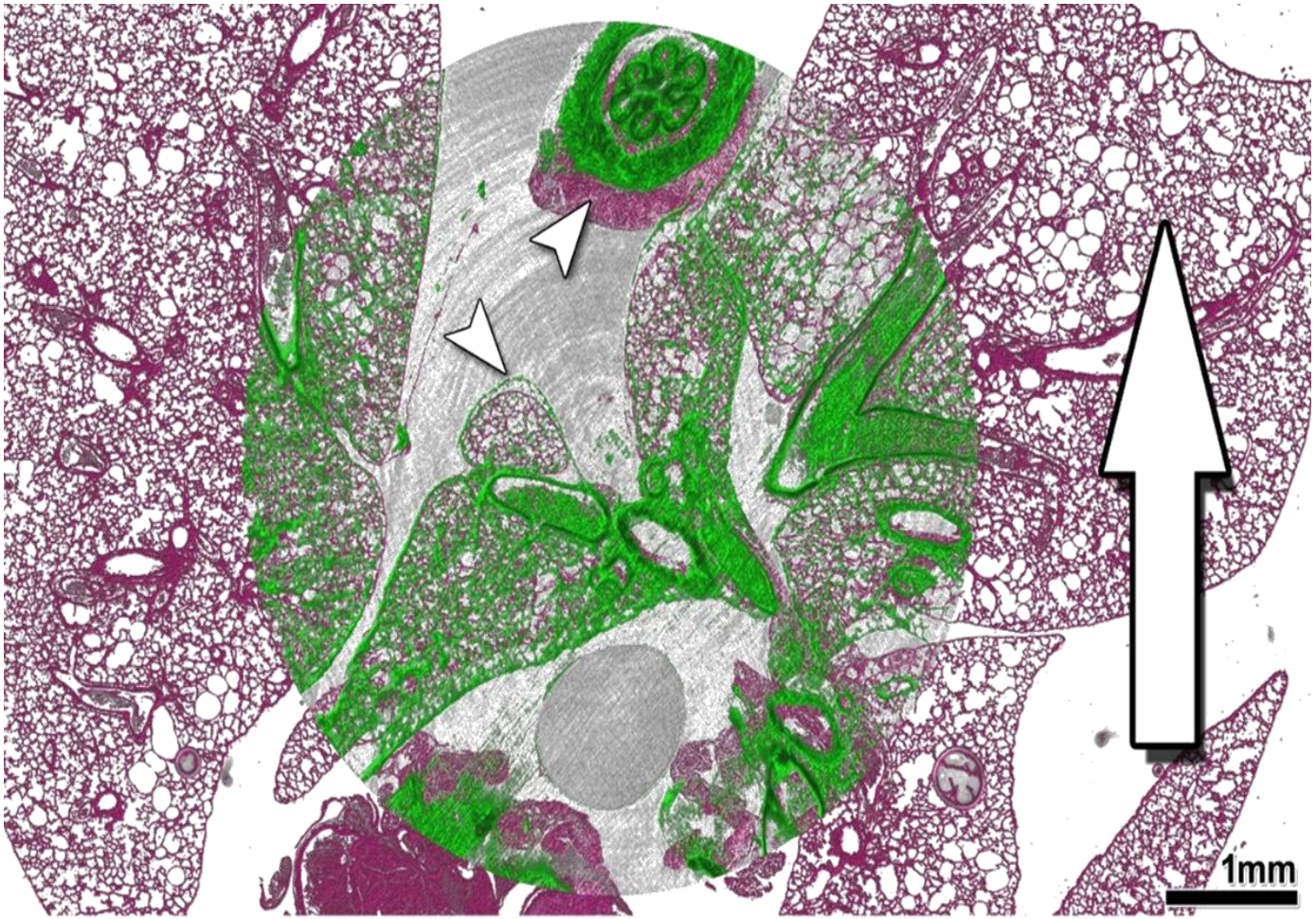
(A) autopsy, (B) staining and embedding, (C) CT acquisition definition of cutting position and angle, (D) cutting with a microtome, (E-G) comparison and overlay for quality assessment

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.

Correlation of CT and histology

Cutting: introduces non-linear deformations that need to be correct for reliable image fusion

Raises questions about precision of morphometry in histology

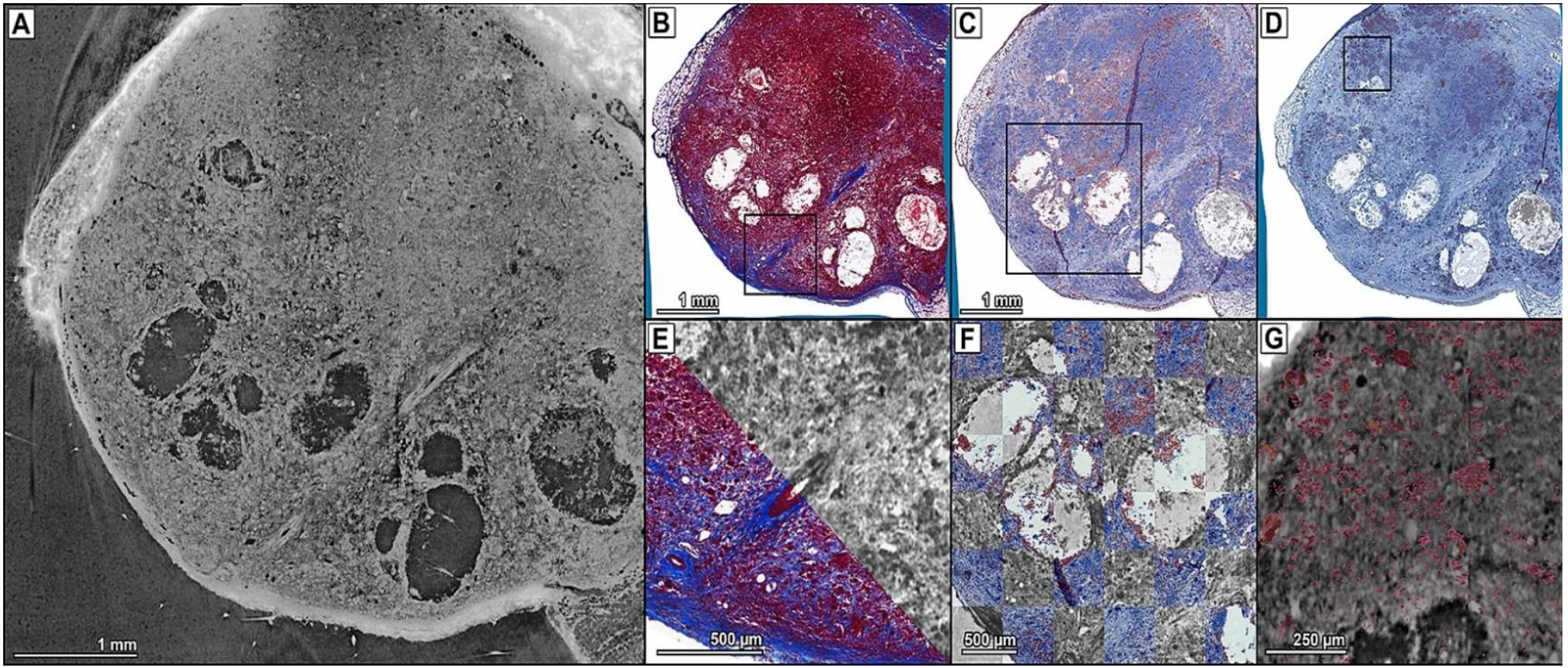


overlay of classical histology (red, HE staining) and high resolution CT (green, 1µm). Mechanical sectioning introduces deformation (white arrow heads).

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.

Elastic image registration

Label free phase contrast CT based virtual histology – mouse breast tumor model embedded in paraffin



(A) Virtual cut SRµCT shown in (B). (B-D) MTS, Anti-CD68 and Anti-TAg staining. (E-G) overlaid CT

https://github.com/xPITcoding/Fuxlastix

Current limitation: corresponding virtual slice in CT data must be selected manually.

Albers, J., Svetlove, A., Alves, J., Kraupner, A., di Lillo, F., Markus, M. A., ... & Dullin, C. (2021). Elastic transformation of histological slices allows precise co-registration with microCT data sets for a refined virtual histology approach. Scientific reports, 11(1), 1-13.

Summary part I

- ✓ Phase contrast boost soft-tissue contrast and allows for label-free virtual histology
- ✓ No additional staining needed -> µCT can be integrated into the standard pipeline of histological tissue analysis
- ✓ Specimens can be scanned in few minutes allowing high-throughput
- ✓ 3D information can be used for guided histological cutting
- ✓ Elastic image registration allows combining µCT and subsequent histology

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II) Low dose phase contrast CT for in-vivo lung imaging in preclinical mouse models of lung disease

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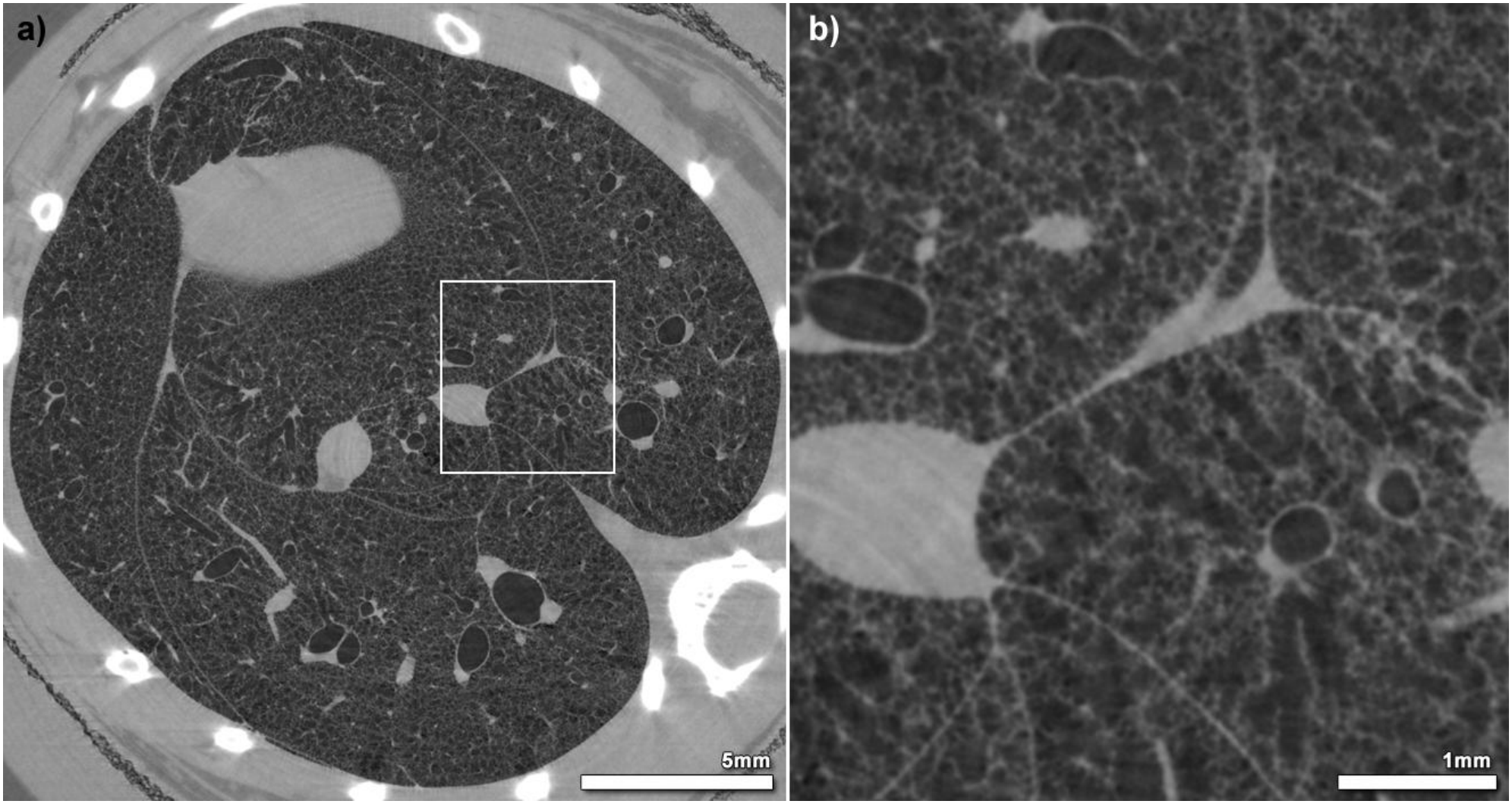
Achievable image quality (in-situ) 2012



a) Cross-section through a PBI scan of a mouse lung (E=22keV, SDD=30cm, resolution 9µm, z-coverage ~4mm, 30 min acquisition time per scan (2012)
b) Detail view shows nearly cellular resolution (problem: thermal stability of the specimen)

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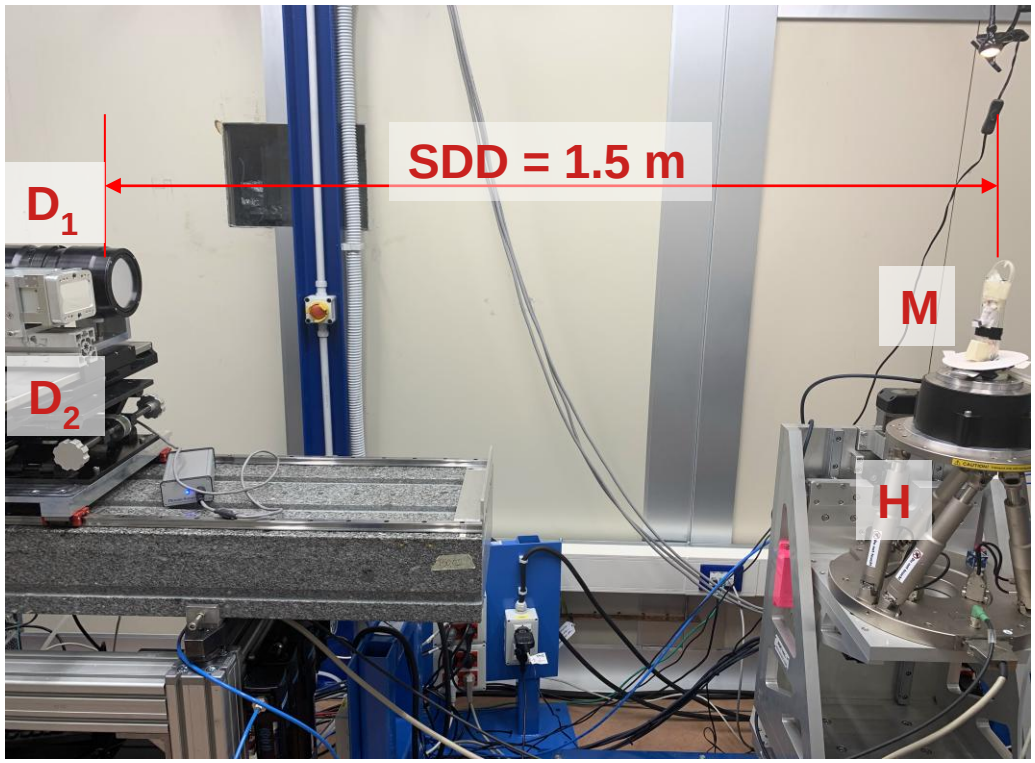
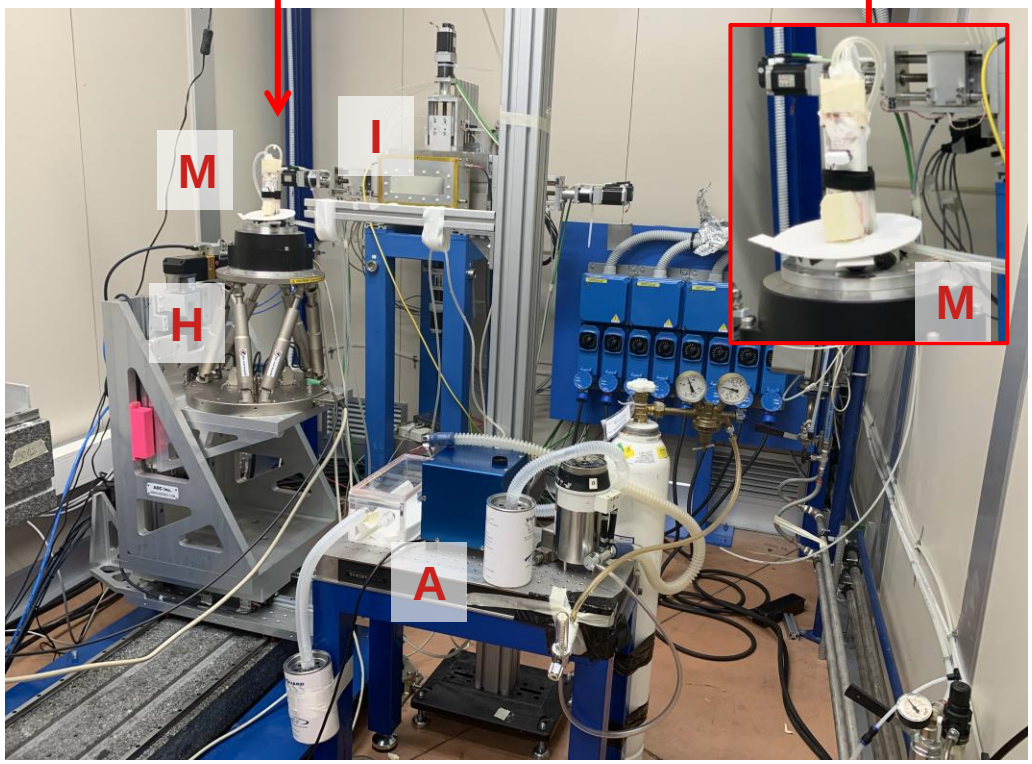
In-vivo low dose mouse lung imaging using Synchrotron phase contrast CT





(D_1) Photonics detector, (D_2) Mönch detector, (I) Ion chamber, (A) gas anaesthesia setup, (H) hexapod for positioning, (M) mouse holder with living mouse

Aim: low dose lung imaging in mice to get anatomical and functional information

Challenges: resolution vs. dose, infrastructure

Monochromatic, E=22keV

Status: 2D movies for lung function measurement (100fps), CT data sets for quantification of anatomical changes (25µm resolution, <170mGy)

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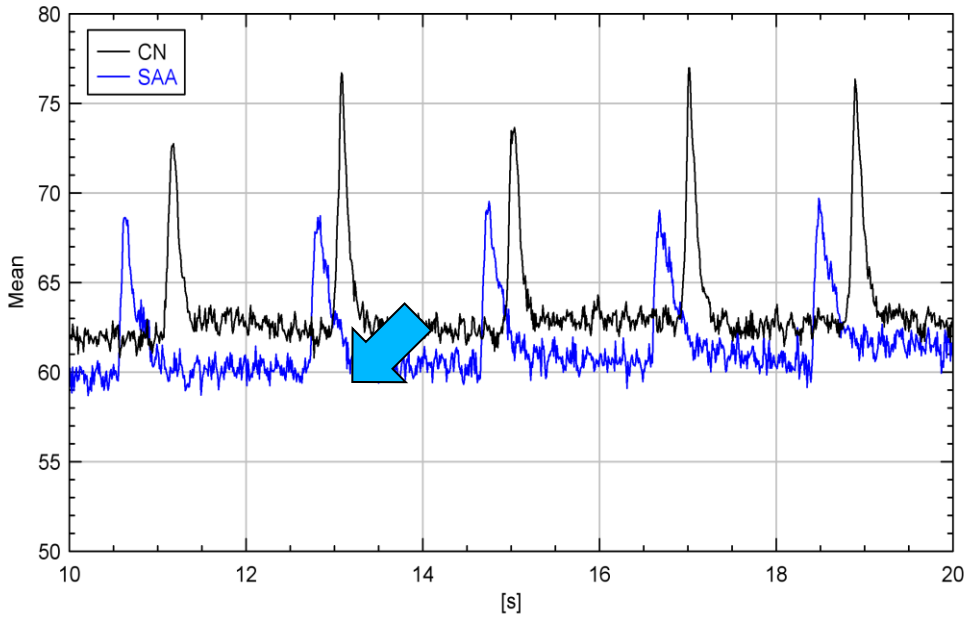
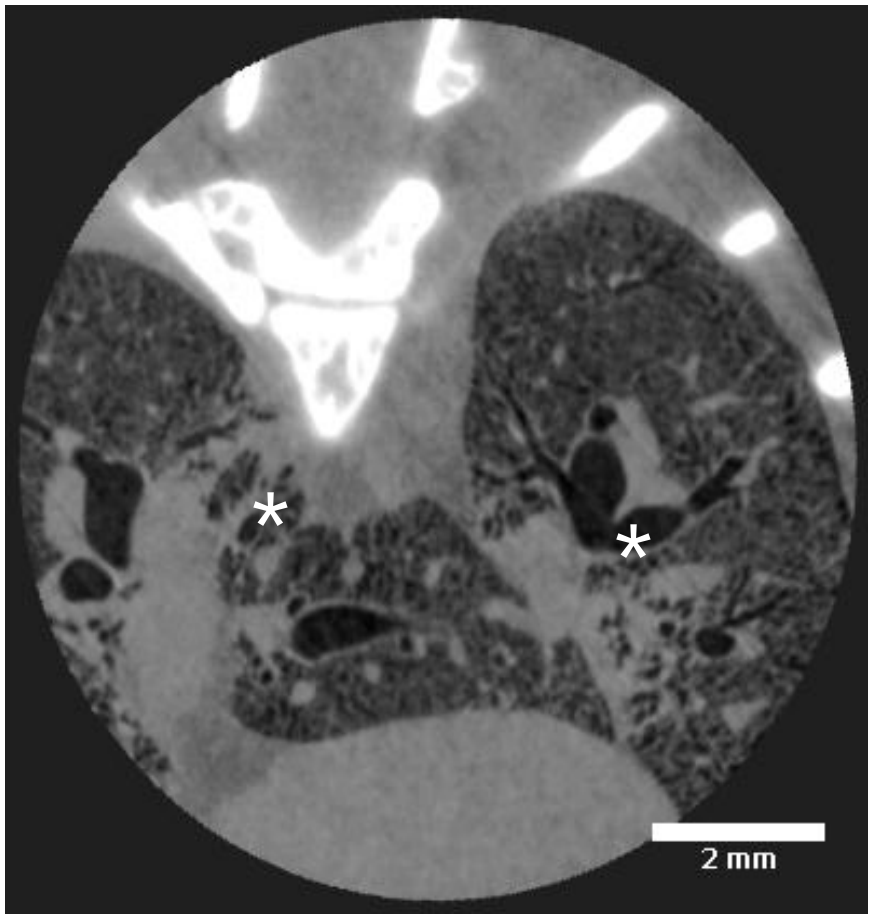
In-vivo low dose mouse lung imaging using Synchrotron phase contrast CT

an average dose of 84 mGy for planar lung function measurements and 168 mGy for pSR-µCT was measured with the dose rate being 2.8 $mGys^{-1}$
Based on implanted TLDs



Average x-ray attenuation over the chest show typical signs of asthma:

- ✓ Reduced transmission due to the presence of inflammation (*)
- ✓ Prolonged expiration phase due to reduced elastic recoil of the lung tissue (arrow)



Example image of an asthmatic mouse (MÖNCH detector, 25µm voxel size)

unpublished

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Summary part II

- ✓ Phase contrast allows to perform lung CT and lung function measurements at high spatial and temporal resolution at comparable low dose rates

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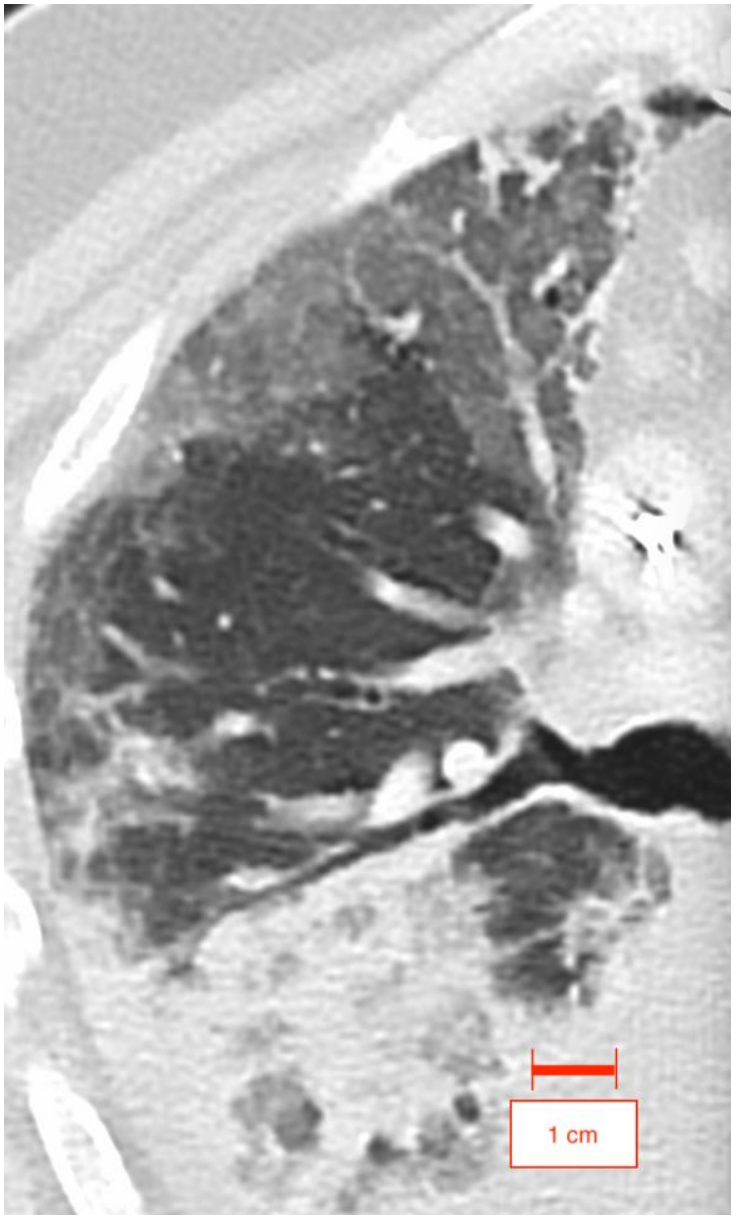
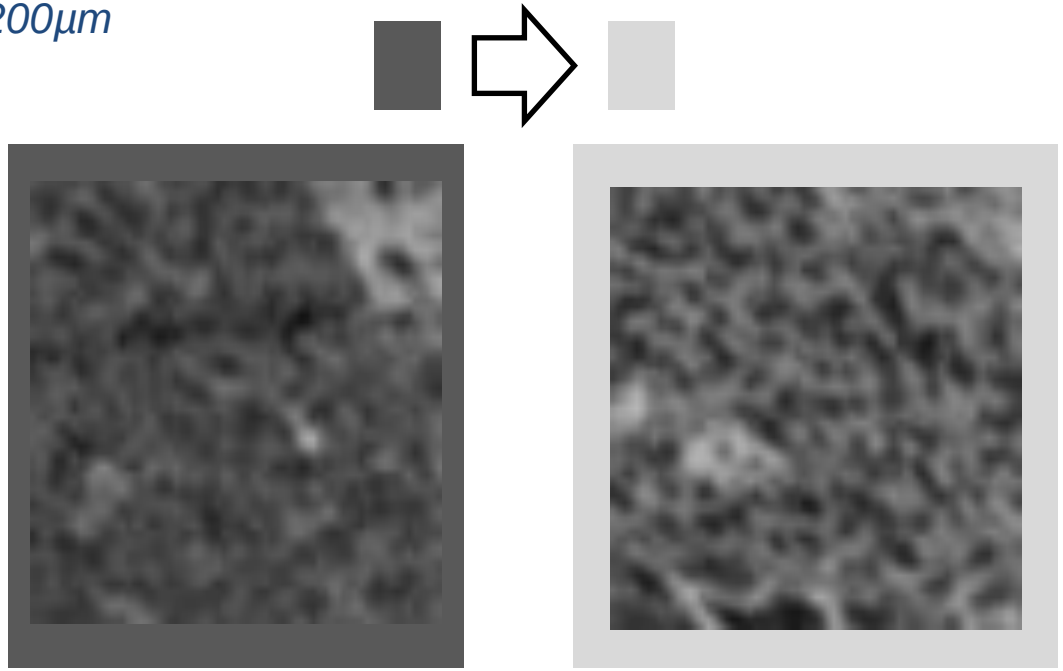
III) Towards phase contrast lung CT in patients

Limitation of clinical CT



ground glass – a result of insufficient resolution, *alveoli*

~200µm



Different potential reasons:

- ✓ Reduced air content / collapse
- ✓ Increased tissue content / swelling
- ✓ Presence of liquid / inflammation

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Study design

For the respiratory medicine field, the **similarities between pig and human lungs** give the porcine model particular potential for advancing translational medicine.

Judge, E. P., Hughes, J. L., Egan, J. J., Maguire, M., Molloy, E. L., & O'Dea, S. (2014). Anatomy and bronchoscopy of the porcine lung. A model for translational respiratory medicine. American journal of respiratory cell and molecular biology, 51(3), 334-343.

In BH PET/CT, the patients were instructed to **hold** their **breath** in the maximal inspiration position during the scout scan, for **10 s** of the CT scan, and for as long as possible during the PET scan.

Kawano, T., Ohtake, E., & Inoue, T. (2008). Deep-inspiration breath-hold PET/CT of lung cancer: maximum standardized uptake value analysis of 108 patients. Journal of Nuclear Medicine, 49(8), 1223.

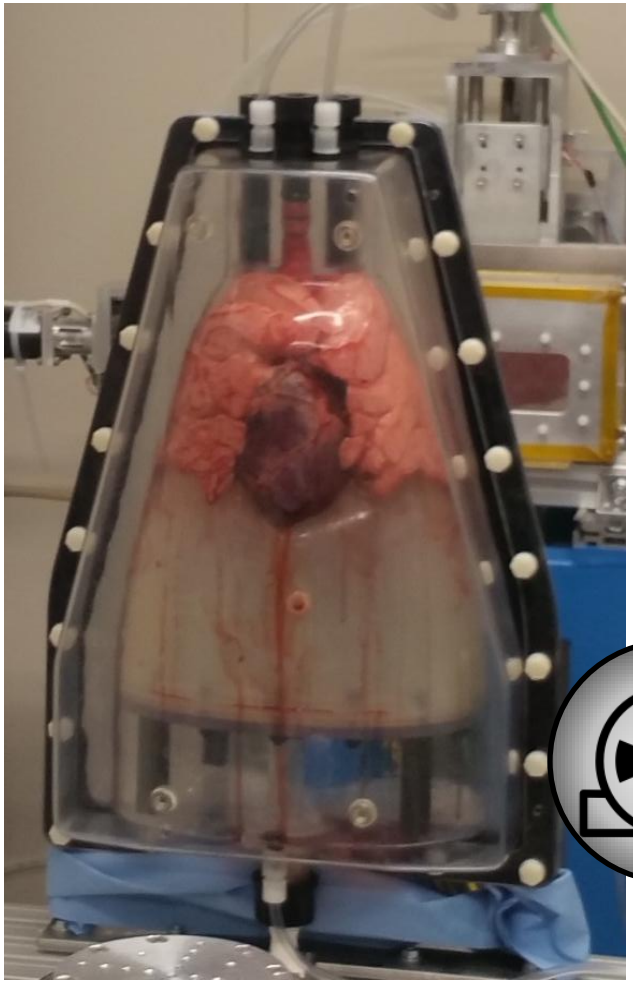
Lung nodules are usually about 0.2 inch (**5 millimeters**) to 1.2 inches (30 millimeters) in size. Human alveoli ~200 µm in size.

https://www.mayoclinic.org/diseases-conditions/lung-cancer/expert-answers/lung-nodules/faq-20058445

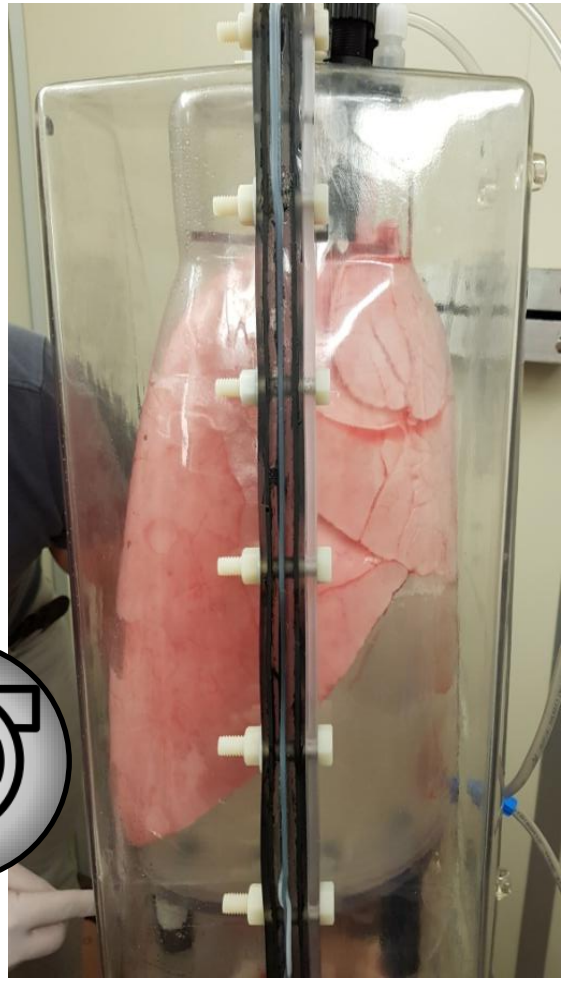
Detectors that can operate at a clinical relevant dose level have larger pixel sizes >~100 µm.
Is free propagation based phase contrast CT beneficial at this conditions?

improved classification of lung pathologies: spatial resolution <0.2 mm and acquisition time <10 s (and low dose)

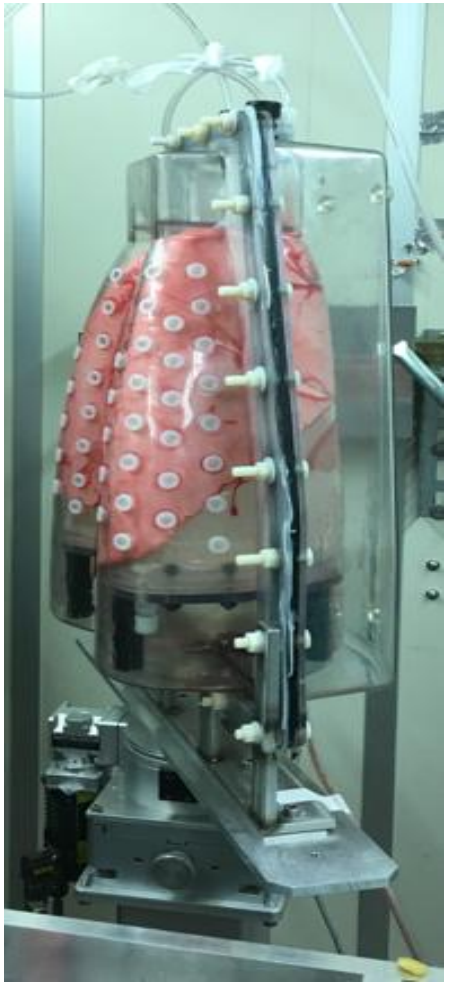
Phantom and specimens



artiCHEST with uninflated lung



after applying negative pressure



alternative cover with injection ports

<u>artificial lung nodules:</u>

mixture of agarose gel 3% and iodine (0.5 and 1% respectively), (previously described to generate 80 HU);

Injected at 40°C via 1ml syringe and 28 G cannula 0.025 to 0.2 ml

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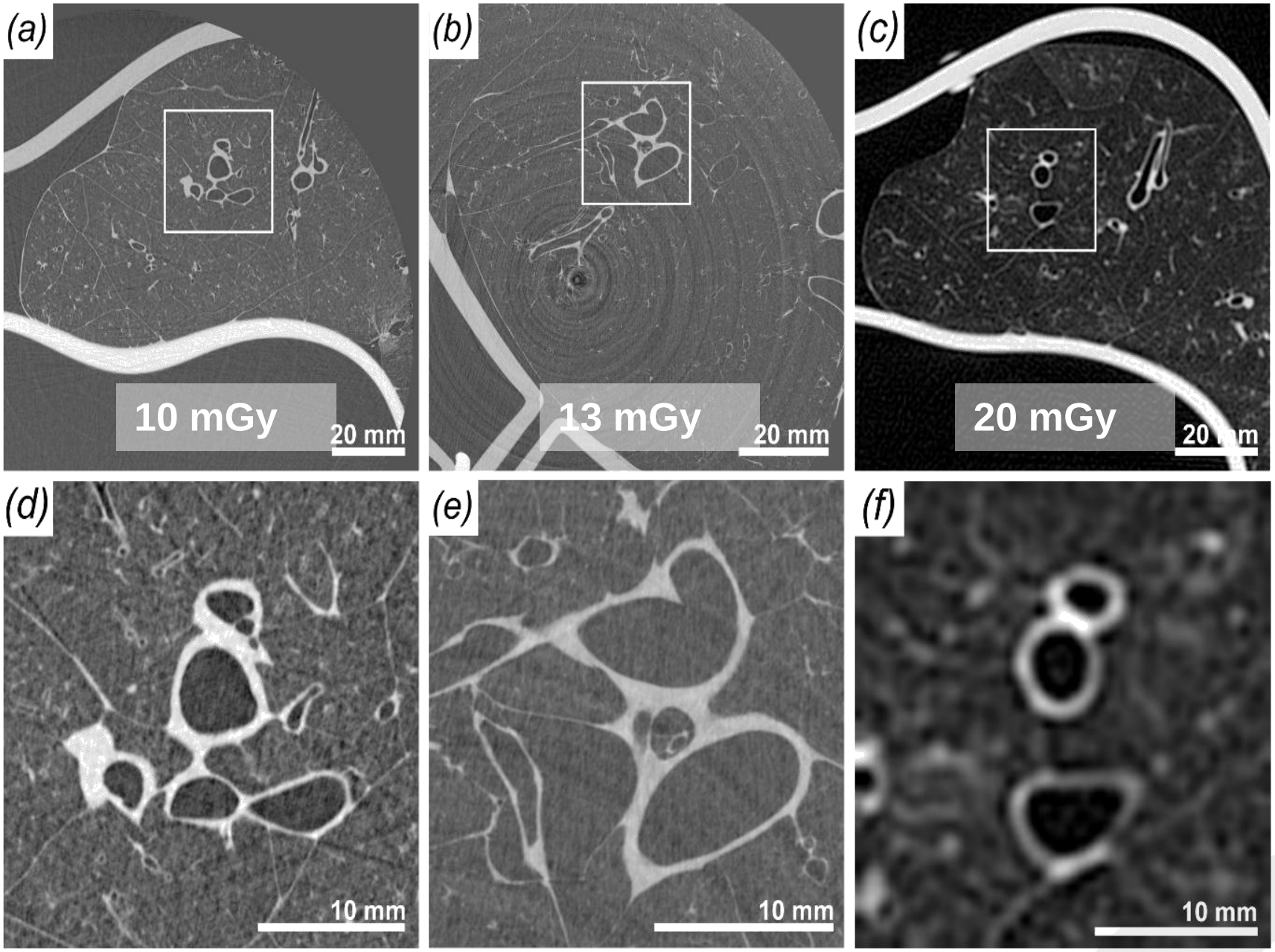
setup

	1st experiment	2nd experiment	3rd experiment	clinical HRCT
#	6	10	9	1/3/2
Energy	40 keV			120 kVp
SDD	2.6 m	10.7 m	11.0 m	-
effective pixel size	89 µm	67 µm	64 µm	450 µm
Projections	8100		3600	787
FOV	158 x 158 x 4 mm^3			346 x 346 x 354 mm^3
Acquisition time		3 min	10 s	37 s

XCounter Flite FX2 photon counting detector (Direct Conversion, Danderyd, Sweden) [CdTe-CMOS] / 19 keV low energy threshold, pixel-size 100x100 μm^2, 45 fps

Wagner, W. L., Wuennemann, F., Pacilé, S., Albers, J., Arfelli, F., Dreossi, D., ... & Dullin, C. (2018). Towards synchrotron phase-contrast lung imaging in patients–a proof-of-concept study on porcine lungs in a human-scale chest phantom. Journal of synchrotron radiation, 25(6), 1827-1832.

Qualitative results



a,d) 2nd experiment, b,e) 1st experiment, c,f) clinical HRCT

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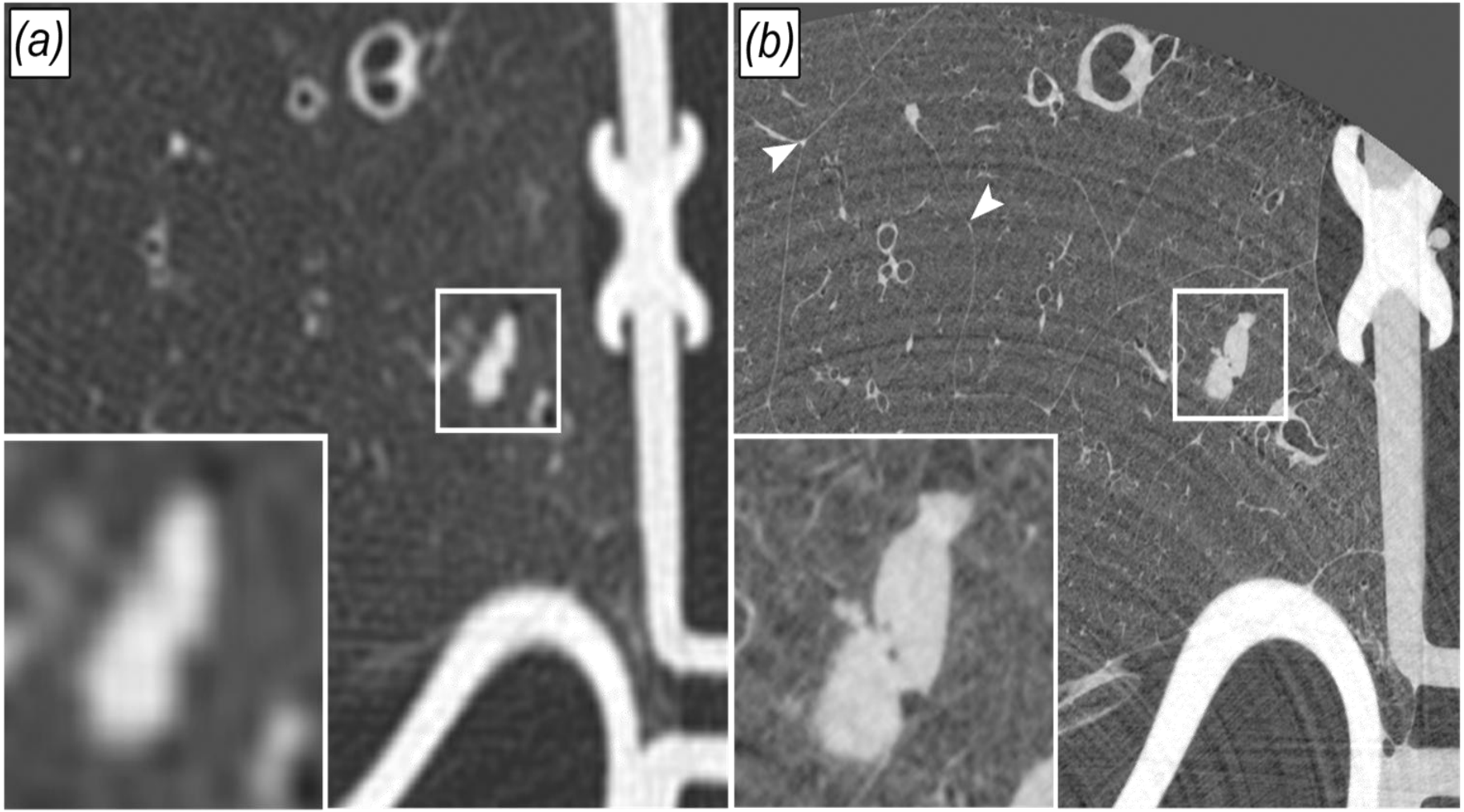
Characterization of nodules



a) clinical HRCT (0.45x0.45x0.9mm^3) nodule clearly visible, no characterization possible, b) SSD 2.6m ~13mGy, 0.09x0.09x0.09mm^3

✓ surface of nodule can be characterized

1st experiment, 182 times smaller voxel, only 40% of the entrance dose, better nodule characterization

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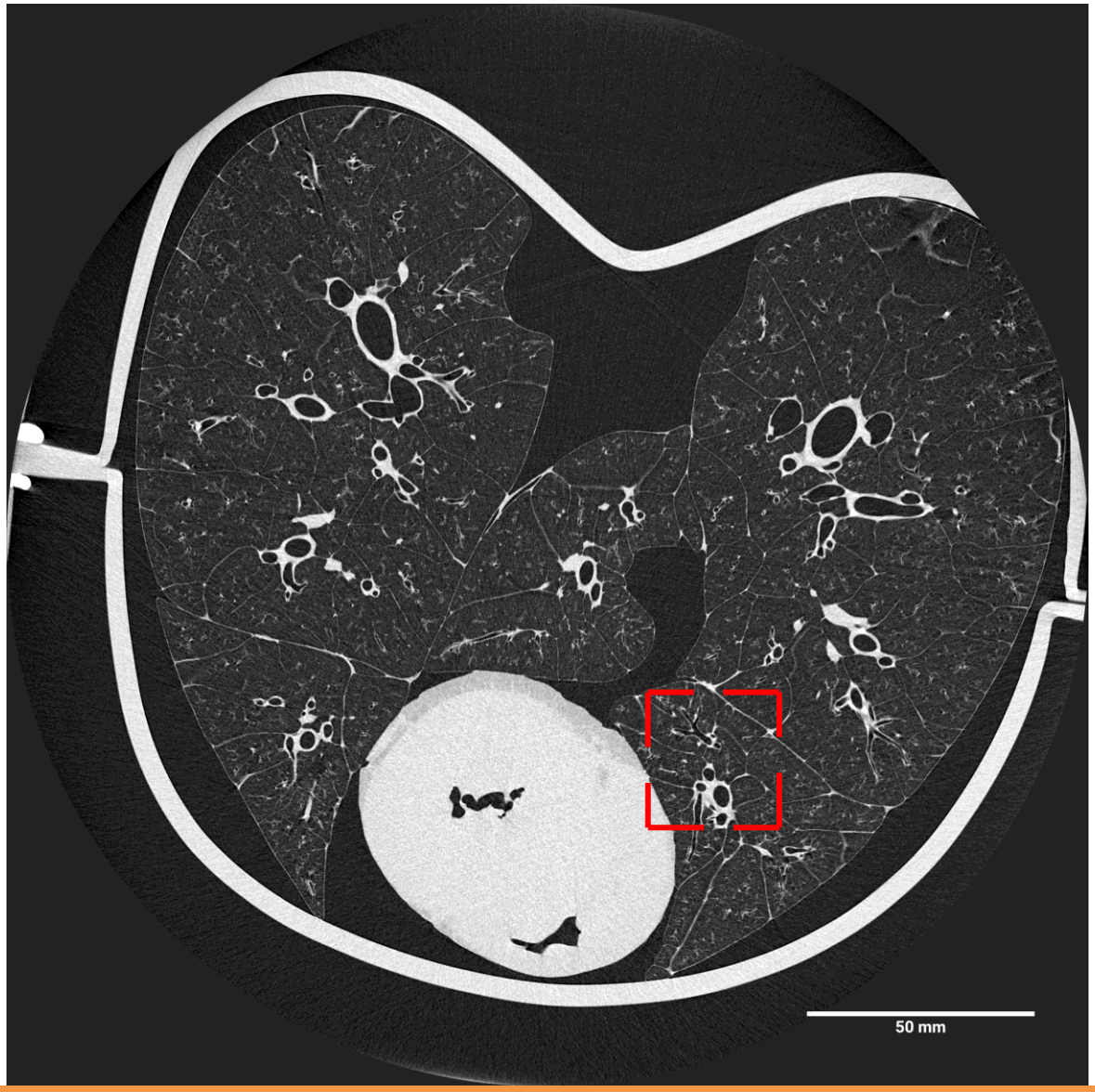
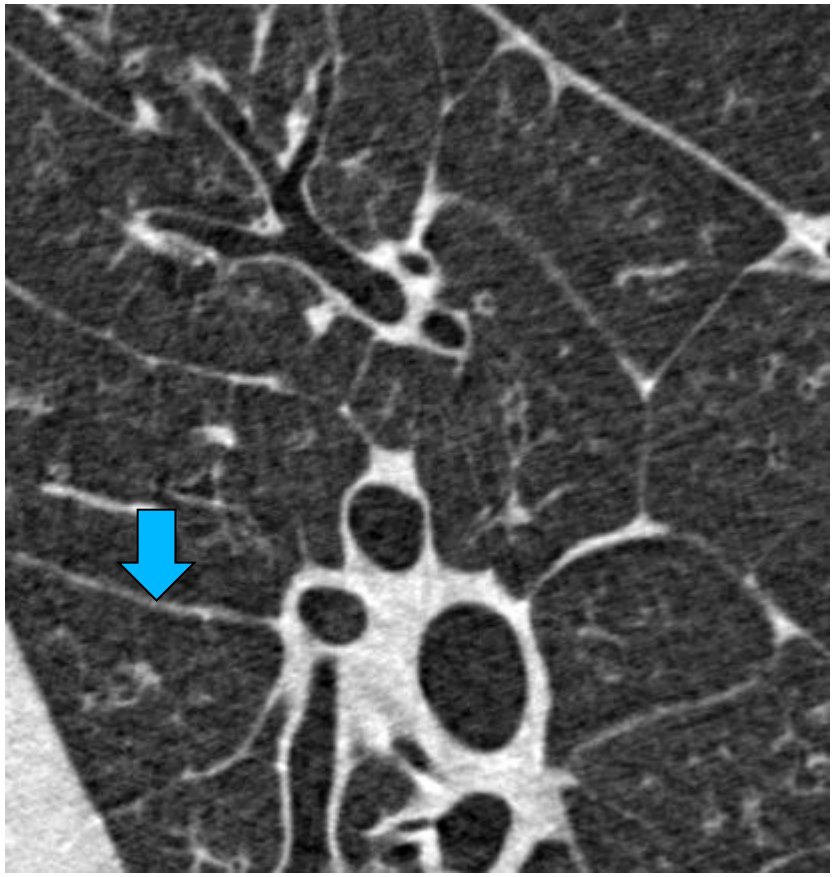
Latest results (last week)



Example of a 360° scan of a healthy pig lung



Intralobular septum (smallest building block of the lung) clearly visible (arrow)

3rd experiment, the new sample stage allows to cover the entire cross-section in 18 s

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Summary part III

- ✓ We successfully performed phase contrast CT imaging in a human chest phantom at a clinical relevant dose level and a resolution of ~70µm (~600 times smaller voxel volume with 50% of the dose used for HRCT)
- ✓ We improved the characterization of artificial nodules
- ✓ With the new setup we can reach an acquisition in 10s (or less)

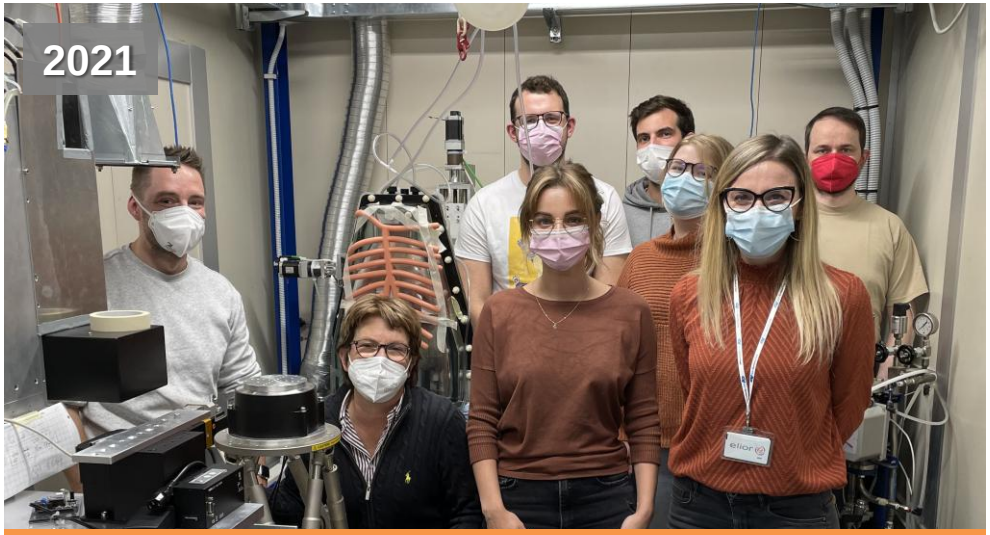
Limitations:

Only a few millimeter thin section can be imaged per rotation (however helical scanning is feasible)

Clinical CT for pretargeting might be needed

Acknowledgement

from left: Giuliana Tromba, Francesca Di Lillo, Christian Dullin, Anne Rothermel, Jonas Albers, Fulvia Arfelli, Felix Wünnemann, Willi Wagner



from left: Willi Wagner, Giuliana Tromba, Jonas Albers, Angelika Svetlove, Lorenzo Damico, Johanna Reiser, Elena Longo, Christian Dullin

Göttingen:
Prof. Frauke Alves
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Angelika Svetlove
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Francesca Di Lillo
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Prof. Marco Confalonieri
Prof. Fabrizio Zanconati
Maria Cova
Stefano Kette

and many more ….

Thank you for your attention!

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