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# Synchrotron-based pulmonary imaging: current and future challenges in interdisciplinary data analysis

LINXS Workshop on Biomedical Imaging, 19.10.2022

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# Introduction

I. Introduction

II. Methods & Instrumentation

III. Results: *In vivo* imaging

IV. Results: Fixed samples

V. Challenges & Outlook

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# I. Introduction

- Lungs represent the integrative part of the mammalian respiratory system
- Lung diseases: one of the leading causes of morbidity and mortality worldwide [1,2]
- Problems of lung diseases are multifaceted:
  - Typically diagnosed at an already progressed state
  - Pathophysiology not entirely understood
  - Link between macroscopic observations and microscopic processes is unknown
- Preclinical research in combination with synchrotron-based lung imaging → crucial [3] (clinical CT/MRI/ultrasound → all limited by spatial resolution)
- Need for high-resolution imaging techniques:

→ fundamental for understanding a variety of lung diseases

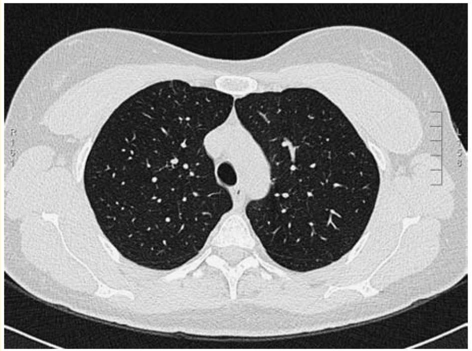
→ develop efficient (causal) therapies for lung diseases

[1] S. M. May & J. T. C. Li, Allergy Asthma Proc. **36**(1), 4, 2015.
[2] Statistik Schweiz, “Sterblichkeit, Todesursachen”, http://www.bfs.admin.ch/.
[3] “Advanced High-Resolution Tomography in Regenerative Medicine”, Springer 2019, eds Dr. A. Cedola & Dr. A. Giuliani)

# I. State-of-the-art and beyond

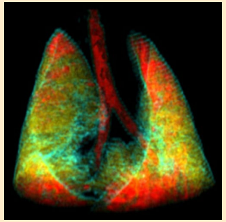
## Clinical diagnostics / Imaging

- Pulmonary function testing (PFT)
- Ultrasonography, MRI
- Gold standard (Imaging): HRCT [4]
- Radiology 2.0: CAD & Artificial Intelligence

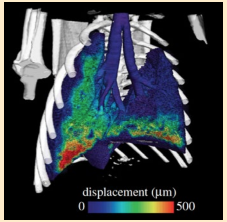


## Preclinical (Animal models)

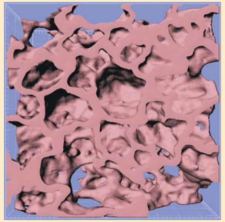
2D lung imaging (*in vivo*) [5]



3D lung imaging (*in vivo*) [6]



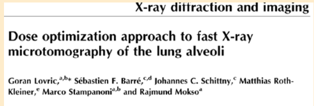

2D combined with PIV (*in vivo*) [7]



High-resolution 3D (*ex vivo*, fixed) [8]

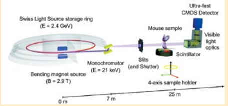
## Our contribution

- Image acquisition → doi.org/n3p | doi.org/bp54

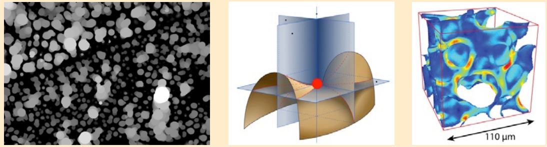

X-ray diffraction and imaging

Dose optimization approach to fast X-ray microtomography of the lung alveoli

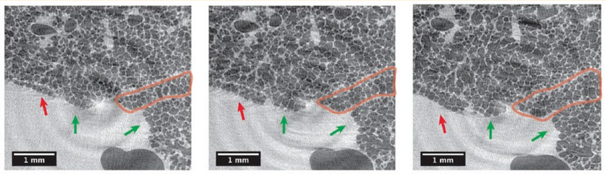
Goran Lovric, Sébastien F. Barré, Johannes C. Schittny, Matthias Roth-Kleiner, Marco Stampanoni and Rajmund Mokso



- Quantitative analysis tools → doi.org/cddm

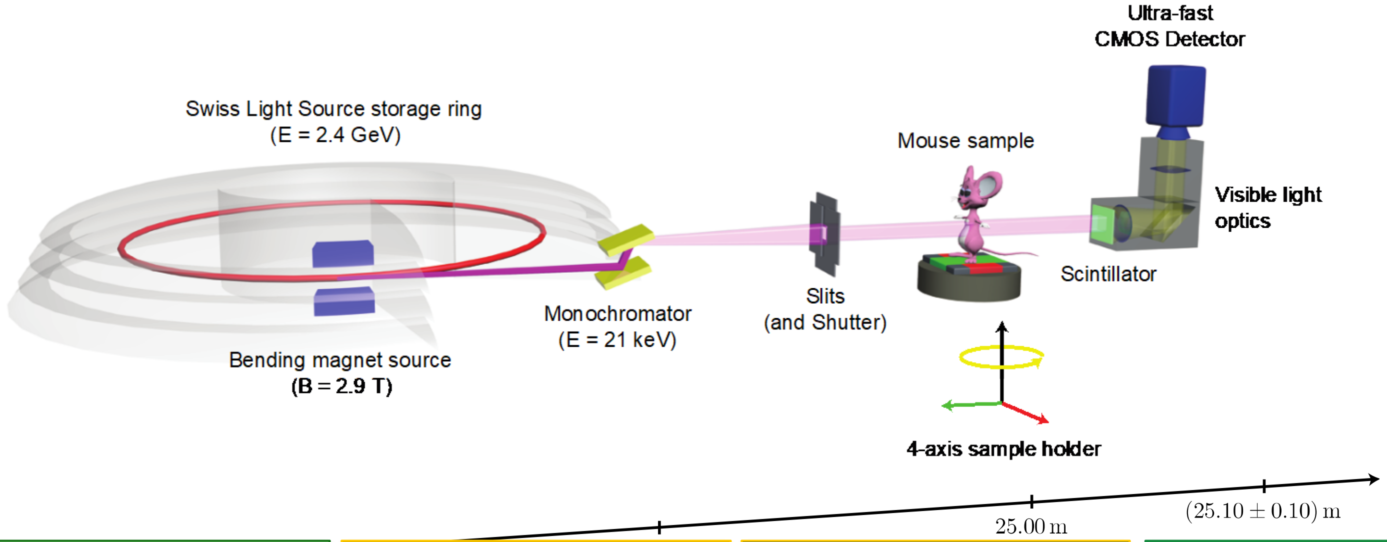



- Tomographic *in vivo* microscopy → doi.org/cdr2




[4] J. A. Verschakelen & W. de Wever, *Computed Tomography of the Lung: A Pattern Approach* (Springer, 2007).
[5] R. A. Lewis, N. Yagi, M. J. Kitchen *et al.*, Phys. Med. Biol. **50**, 5031 (2005).
[6] S. Bayat, L. Porra, H. Suhonen *et al.*, Eur. J. Radiol. **68**, S78 (2008).
[7] S. Dubsky, S. B. Hooper, K. K. W. Siu *et al.*, J. R. Soc. Interface **9**, 2213 (2012).
[8] J. C. Schittny, S. I. Mund, M. Stampanoni, Am. J. Physiol. Lung Cell Mol. Physiol. **294**, L246 (2008).

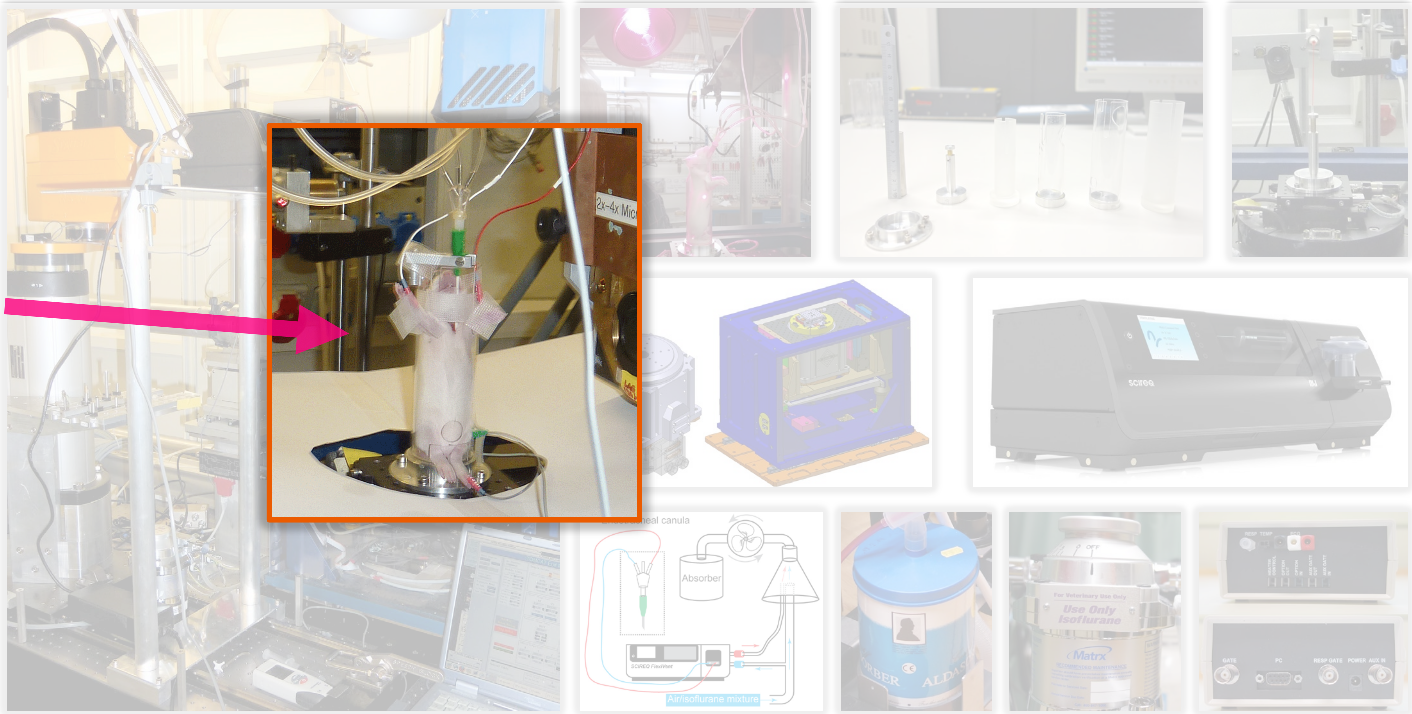
# II. Experimental setup




| Samples | Optics | Acquisition | Contrast |
|---|---|---|---|
| ▪ Rats (7-14 days old) and adult mice<br>▪ *Post mortem* & *in vivo*<br>▪ Isoflurane anesthesia | **Pixel sizes**: a.) 1.1 x 1.1 $\mu m^2$<br>b.) 2.9 x 2.9 $\mu m^2$<br>**FOV**: a.) 5.8 x 2.7 $mm^2$<br>b.) 2.2 x 2.2 $mm^2$ | **Projections**: 350–450<br>**Exposure times**: 1–3 ms<br>**Total scan time**: 1-2 min | Propagation-based phase contrast |


[9] G. Lovric, S.F. Barré, J.C. Schittny *et al.*, J. Appl. Crystallogr. **46** (4), 856 (2013) . → **doi.org/n3p**
[10] G. Lovric, R. Mokso, C.M. Schlepütz, M. Stampanoni, Phys. Medica **32**, 1771 (2016) . → **doi.org/bp54**

# II. *In vivo* endstation




[9] G. Lovric, S.F. Barré, J.C. Schittny *et al.*, J. Appl. Crystallogr. **46** (4), 856 (2013) . → **doi.org/n3p**
[10] G. Lovric, R. Mokso, C.M. Schlepütz, M. Stampanoni, Phys. Medica **32**, 1771 (2016) . → **doi.org/bp54**

# II. Propagation-based based phase contrast

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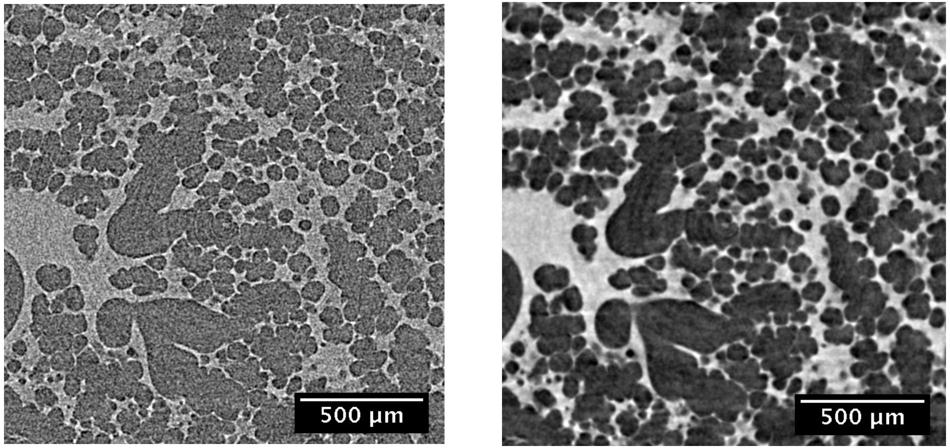
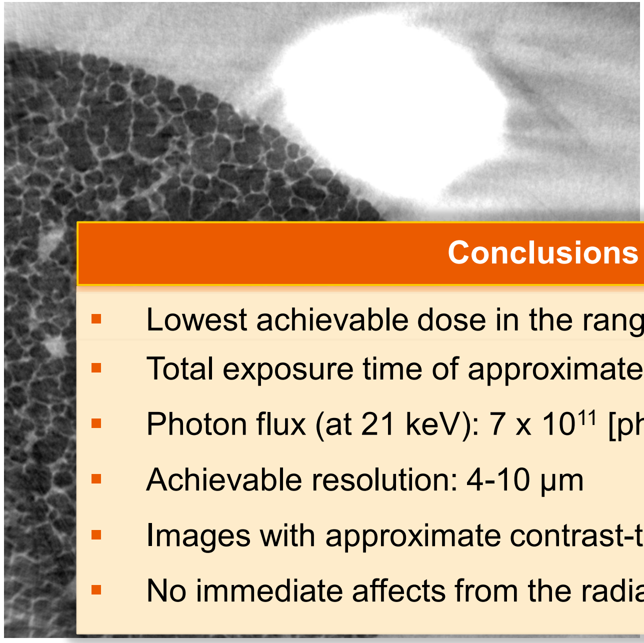
## Absorption vs. phase contrast

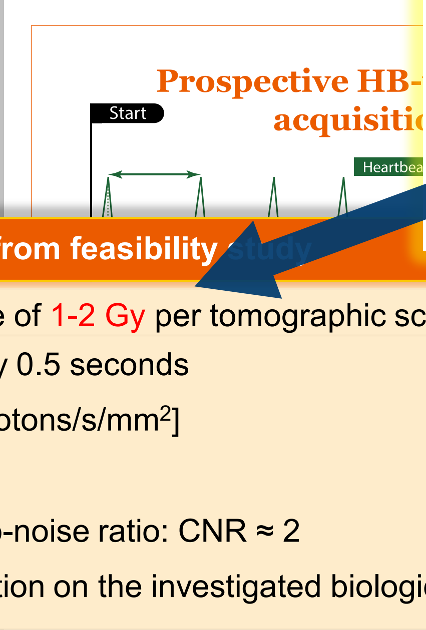


# III. *In vivo* pulmonary imaging at the µm-scale [11]

- 14d old rat, 600 tomographic projections, $t_{exp}$ = 3 ms, 2.9 x 2.9 µm$^2$ pixel
- 5x pressures: 15, 20, 25, 30, 35 cm$H_2O$

**High-numerical-aperture macroscope optics [12]**





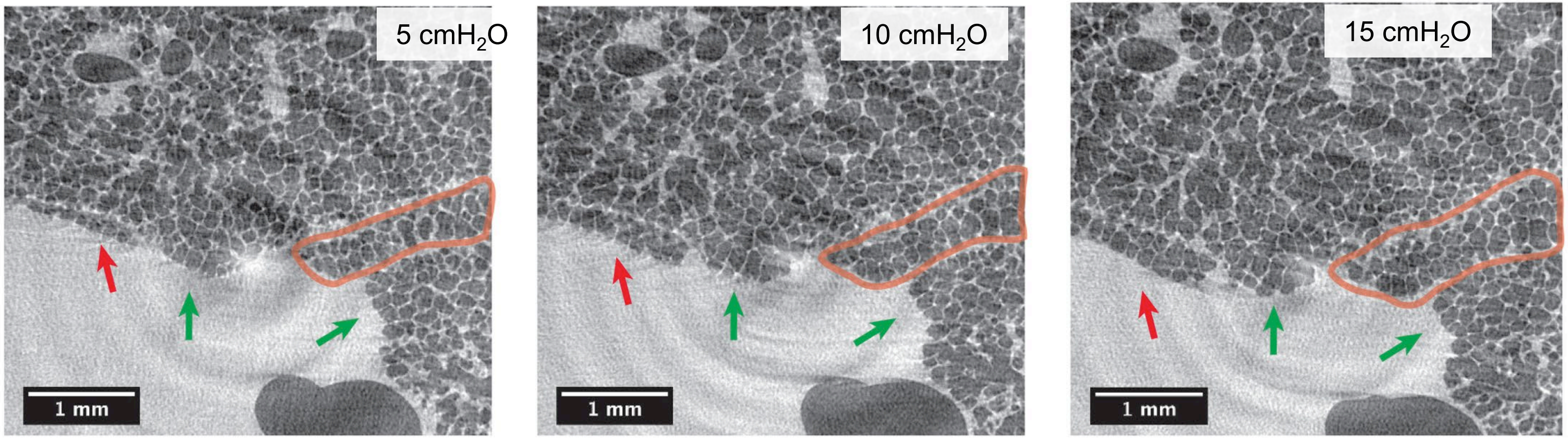

**Conclusions from feasibility study**

- Lowest achievable dose in the range of 1-2 Gy per tomographic scan
- Total exposure time of approximately 0.5 seconds
- Photon flux (at 21 keV): 7 x $10^{11}$ [photons/s/mm$^2$]
- Achievable resolution: 4-10 µm
- Images with approximate contrast-to-noise ratio: CNR ≈ 2
- No immediate affects from the radiation on the investigated biological samples [13]

[11] G. Lovric, R. Mokso, F. Arcadu *et al.*, Sci. Reports **7,** 12545 (2017). → **doi.org/cdr2**
[12] M. Bührer, M. Stampanoni, X. Rochet *et al.*, J. Synchrotron Radiat. **26**(4), 1161 (2019).
[13] Z.-Y. Hong, S.H. Eun, K. Park et al., J. Radiat. Res. 55 (4), 648 (2014).

# III. *In vivo* pulmonary imaging at the µm-scale

- Quasi-static inflation in 9d old rats
- Some regions can be matched completely, but not whole slice
- Asymmetric inflation (dependent on lung lobe)
- Heterogeneous distension pattern (no cyclic opening and closing)
- Green arrows: matching lung structures
- Red arrows: alveoli being "pushed" into the field of view from above/underneath

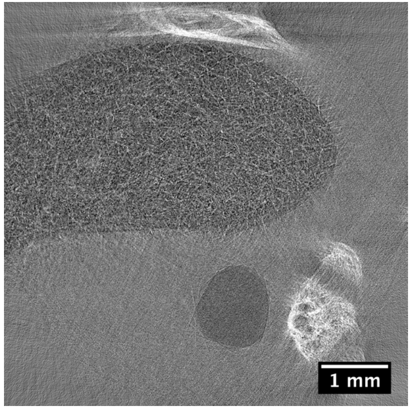



[11] G. Lovric, R. Mokso, F. Arcadu *et al.*, Sci. Reports **7,** 12545 (2017). → **doi.org/cdr2**

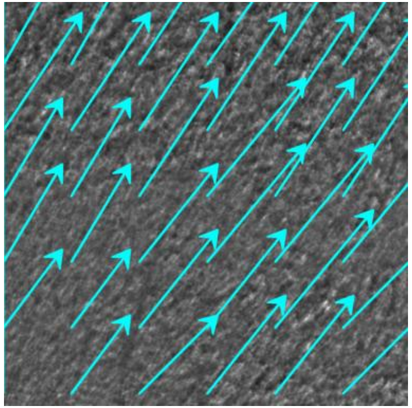
# III. *In vivo* pulmonary imaging at the µm-scale

**Why is it actually so challenging?**

Tomographic slice @ breath-hold




Radiography @ breath-hold



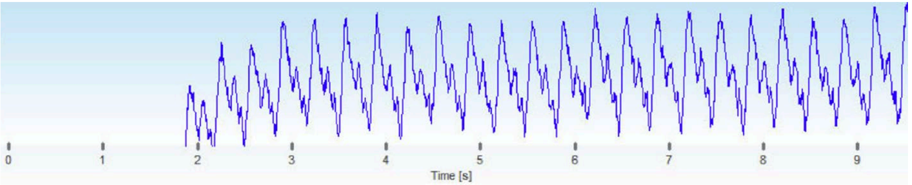
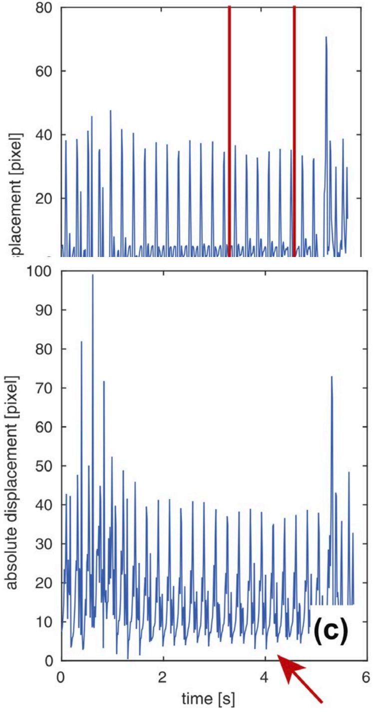



**Fig. 6.** Pressure oscillations measured with the FlexiVent small animal ventilator. The vertical scales ranges from 10.2 to 10.4 cm $H_2O$.

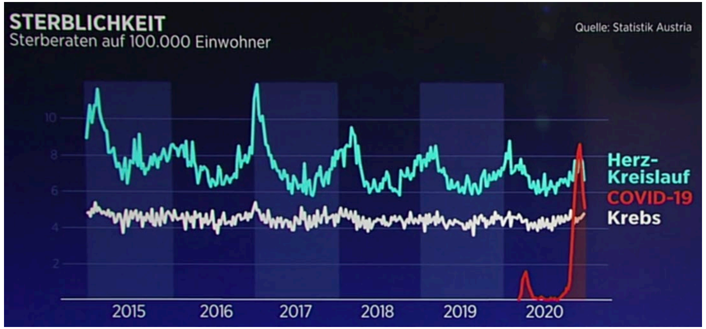



[10] G. Lovric, R. Mokso, C.M. Schlepütz, M. Stampanoni, Phys. Medica **32**, 1771 (2016) . → doi.org/bp54

# III. Collaboration with Grenoble/Uppsala University

**Application to the study of VILI/ARDS**

- Acute respiratory distress syndrome (ARDS) is a severe lung condition that necessitates advanced ventilator treatment in the intensive care units (ICU)
- ARDS is triggered by an underlying condition, e.g. sepsis, trauma or major surgery
- ICU: 150,000 patients/year in the European Union with mortality of about 40% [14,15] (Costs: 3000 EUR per patient and day)
- Although life-saving, ventilatory management is highly contributing to the bad outcome
- **COVID-19** complications may include pneumonia, ARDS, cardiovascular complications etc.
  → ventilator treatment (critical)





[14] S. S. Tan, J. Bakker, M. E. Hoogendoorn *et al.*, Value Heal. **22**(15), 81 (2012).
[15] G. F. Nieman, J. Satalin, M. Kollisch-Singule *et al.*, J. Appl. Physiol. **122**(6), 1516 (2017).

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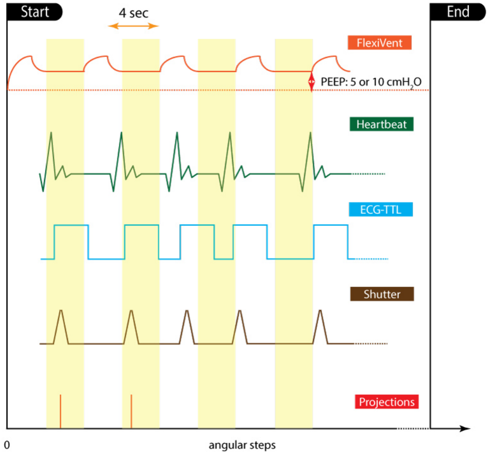
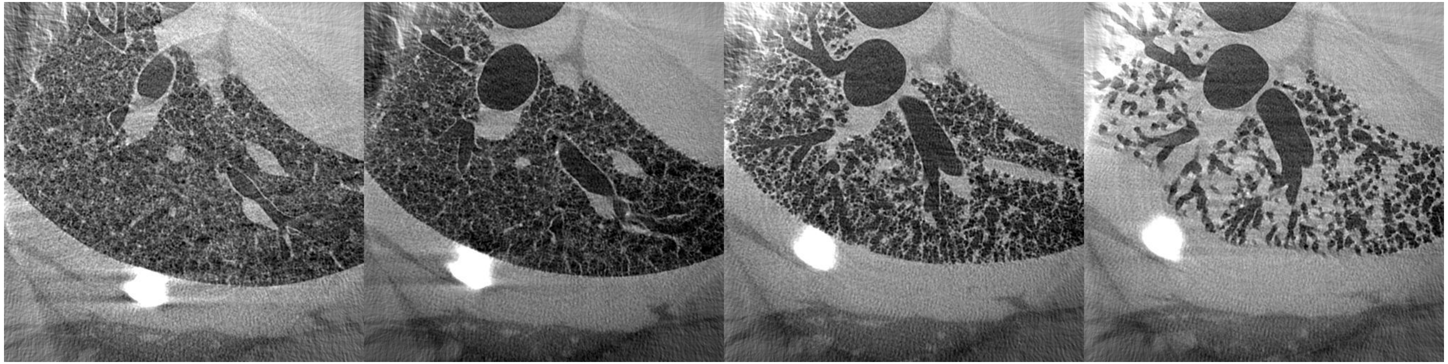
# III. Study of VILI/ARDS

- Developed new acquisition mode(s) & shutter design
- Study of gradual development of ventilator-induced lung injury (
  → tomographic slices (craniocaudal view)
- Very heterogeneous
- Images taken at end-expiration: PEEP 10 $cmH_2O$
- VILI associated with collapse/edema, but also hyper-expansionn



At baseline

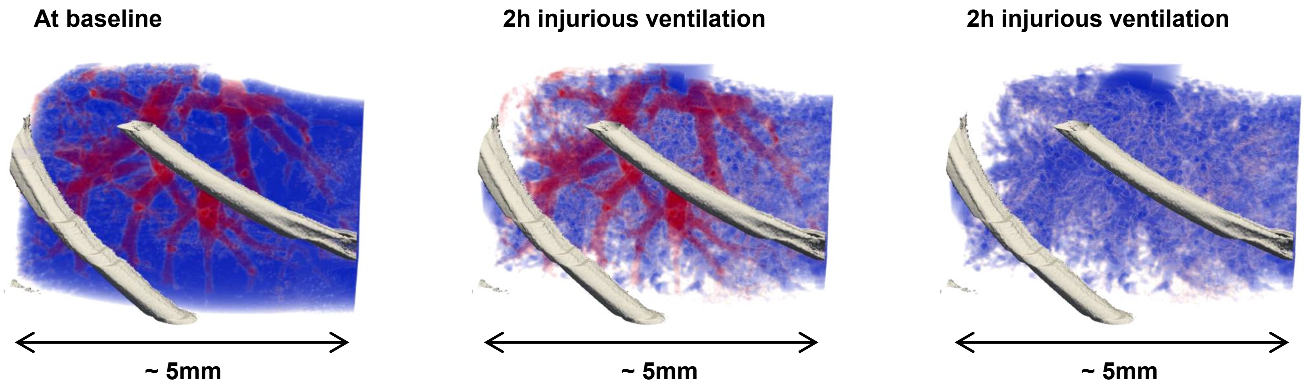
1 h injurious ventilation

2 h injurious ventilation

2.5 h injurious ventilation

# III. Study of VILI/ARDS

- 3D visualization of the lack of alveolar aeration + spatial distribution
- Peripheral regions appear more affected by VILI
- Regions closer to the hilus of the lung appear to be better aerated
- <u>Work in progress:</u> quantify aeration, displacement and recruitment at different rates of VILI
- <u>Challenge:</u> precise quantitative description of the volumes

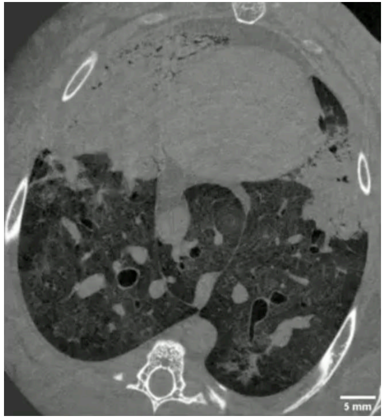


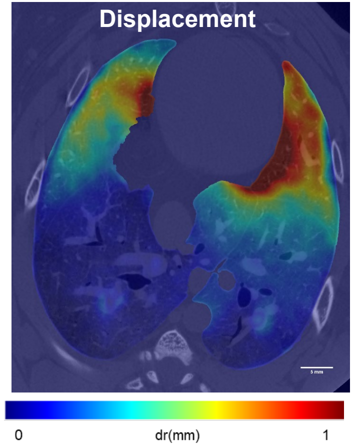

G. Lovric et al., In preparation (2022).

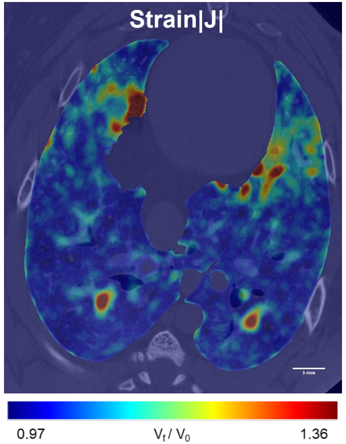
# III. *In vivo* VILI experiments @ ID-17 (ESRF)

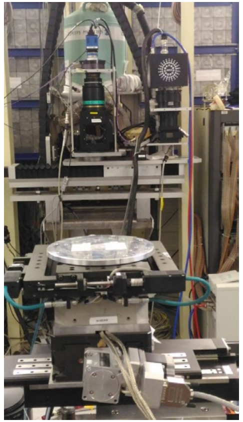
## A yet different acquisition mode

- Synchronization of mechanical ventilation heartbeat (by triggering with R-peak)
  → followed by a retrospective gated acquisition protocol
- Scan parameters: 22.6 µm (pixel size) // 52 keV (X-ray energy) // 10 ms exposure time // 1.5 m sample-to-detector distance
- Cyclic recruitment/de-recruitment in an ARDS rabbit animal model



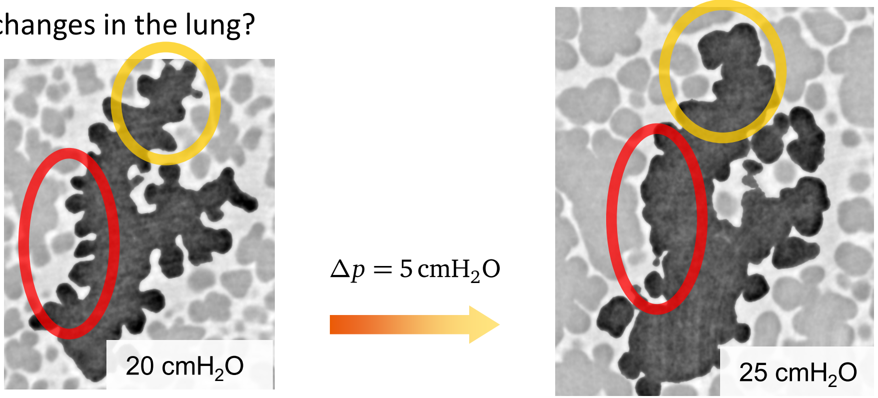



[16] L. Fardin, L. Broche, G. Lovric *et al.*, Sci. Reports **11,** 4236 (2021). → doi.org/fxh5

# IV. Challenges in microscopic lung imaging

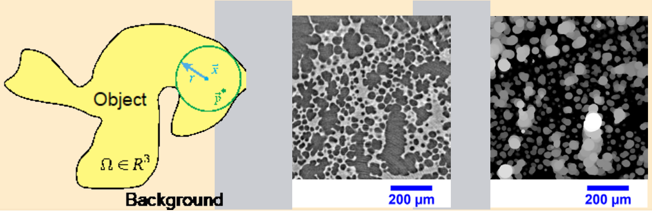
- How to detect non-linear and regional changes in the lung?
- How to quantify them?




## Air volume thickness map analysis

- Diameter of the largest sphere containing point p:

$$\tau(\vec{p}) = 2 \times \max(\{r \mid \vec{p} \in \mathrm{sph}(\vec{x}, r) \subseteq \Omega, \vec{x} \in \Omega\})$$

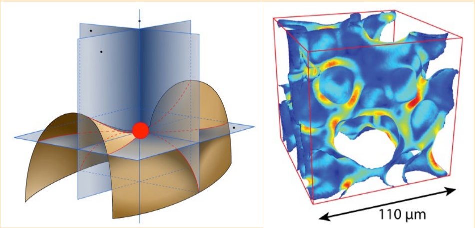



## Curvature analysis

- Mean (H) and Gaussian (K) curvatures from principal curvatures: $\kappa_1, \kappa_2$

$$H = \frac{\kappa_1 + \kappa_2}{2}$$

$$K = \kappa_1 \cdot \kappa_2$$



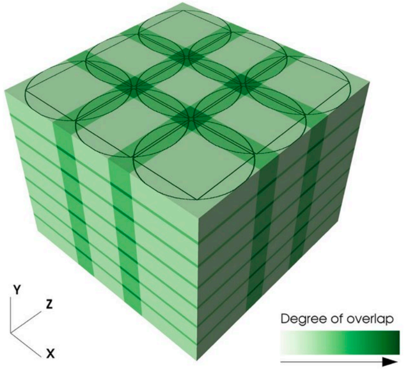


[17] G. Lovric, I. Vogiatzis Oikonomidis, R. Mokso *et al.*, PLOS ONE 12(9), e0183979 (2017). → **doi.org/cddm**

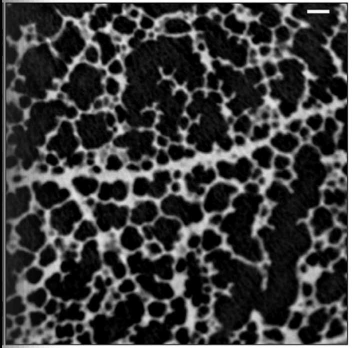
# IV. Full-volume reconstruction of an intact post-mortem juvenile rat lung

**Study of full pathway from gas intake to gas exchange**

- Large biological samples: prone to degradation and motion during extended scan times.
- 63 individual volumes with 3x3x7 mosaic geometry in ~22 min
- Reconstructions achieved by various post-processing steps:
  - Dynamic flat-field correction
  - Explicit recording of tomographic angles
  - Non-rigid stitching
- <u>Full tomographic dataset</u>:
  → 9095 x 9106 x 7084 voxels
  → 1.2 TB
  → ~20 min for Thickmap calculation







[18] E. Borisova & G. Lovric, A. Miettinen *et al.*, Histochem. Cell Biol. **155**(2), 215 (2021). → **doi.org/dvsq**

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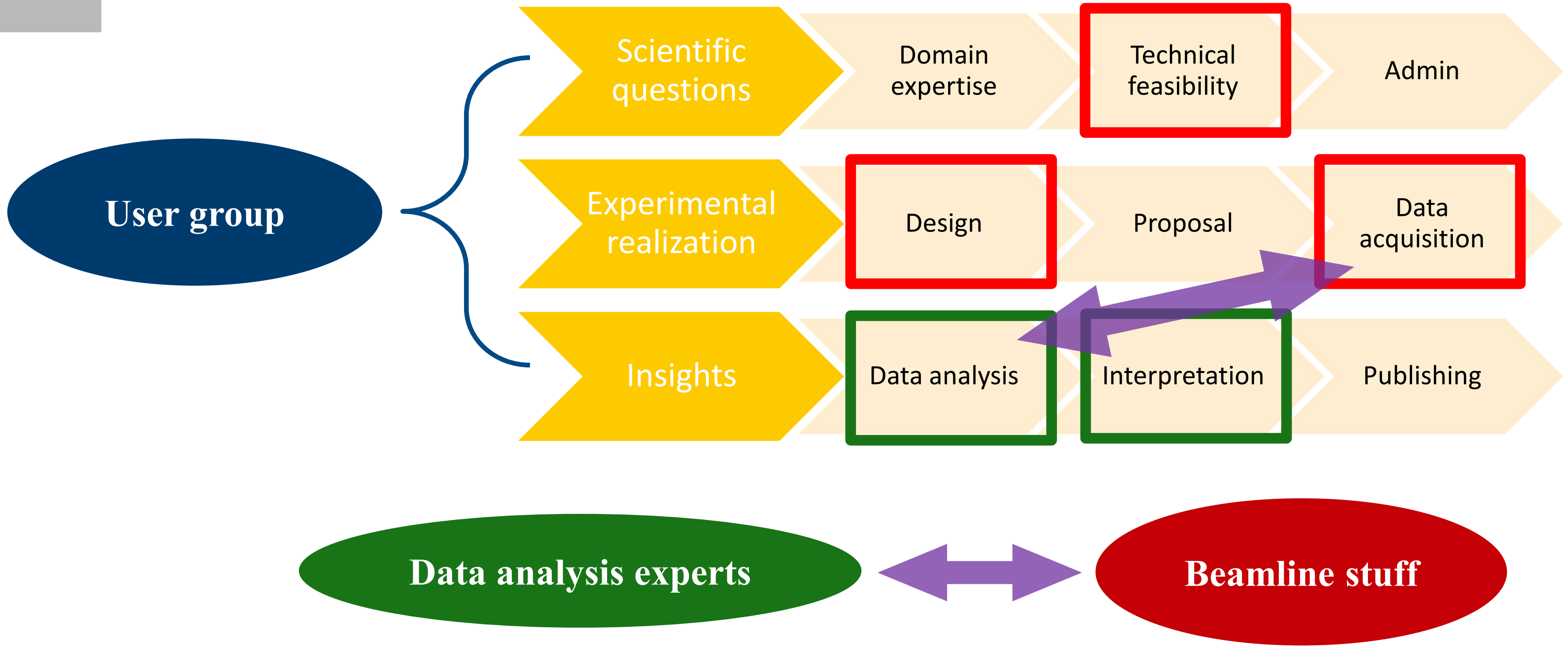
# IV. Pulmonary vascular biology

- „Pulmonary hypertension“ (PH): increased blood pressure within arteries of the lungs
- No cure for PH → prognosis is usually poor → mostly supportive treatment measures
- Micro-anatomy, including the vasculature, is highly complex
- Subsequent sectioning/staining → identify cellular and matrix contributors
- Intrapulmonary shunting occurs between pulmonary arteries and bronchial arteries

[19] C. Norvik *et al.*, Am. J. Physiol. Cell. Mol. Physiol. **318**(1), L65 (2020). → **doi.org/dqgr**

# Current and future challenges

**Lifecycle of (typical) synchrotron imaging experiments**



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# Conclusion & Outlook

– High-resolution in vivo (functional) imaging sets wide range of applications
  → preclinical models
– Airway and alveolar structure imaging down to 3µm pixel size  routinely achievable
– Interdisciplinary approach required for developing new techniques and experimental design
– Real-time Imaging (Tomography / Phase-retrieval / Visualization / Post-processing)
– High field-of-view / high-resolution imaging (TB-sizes datasets)
– "Low-Dose imaging", Multi-ROI, multi-scale & landmark-driven imaging