LEAPS Meets Life Sciences Conference** (14.05.-19.05, Elba), **Bioimaging

Investigating human lung tissue by propagation-based phase-contrast X-ray tomography

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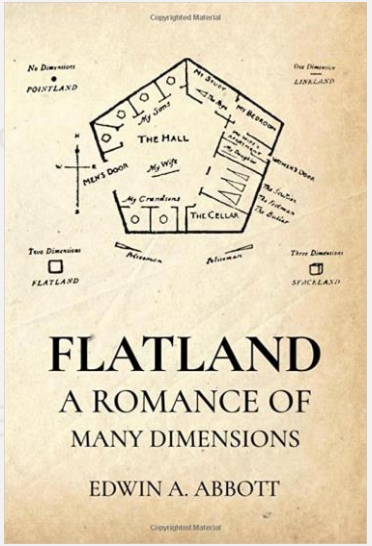
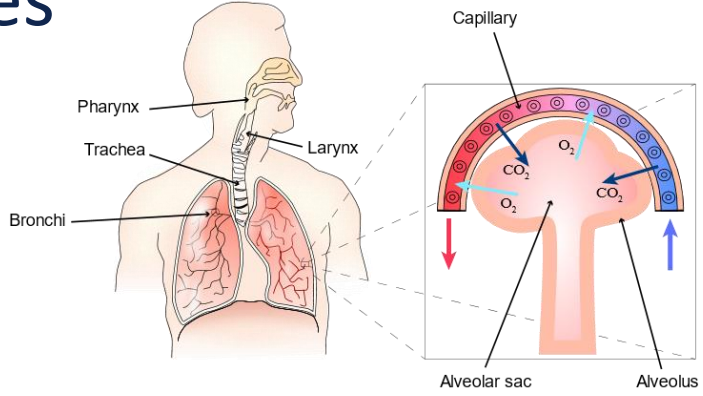
Conventional Histology vs. 3D Virtual Histology

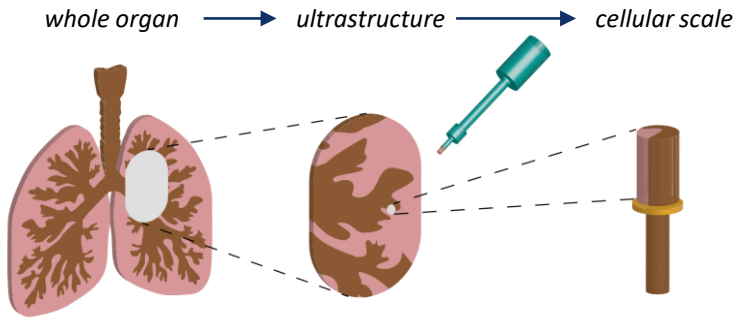


Marcello Malpighi (1628-1694)
https://en.wikipedia.org/wiki/Marcello_Malpighi

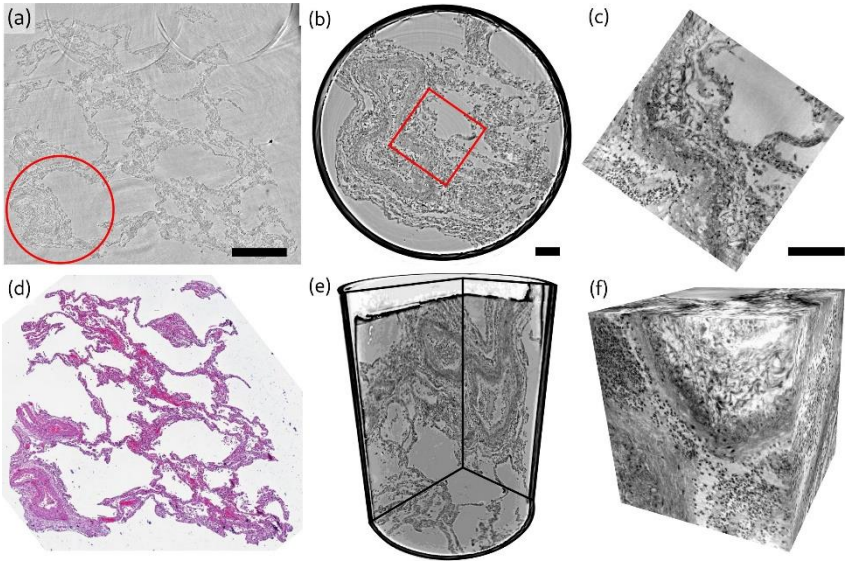
Human lung on multiple scales

- lung perfect example of how **function of organ** is **enabled by 3D structure**, here formed by **intricate and intertwined networks** of ventilation and vasculature
- **3D structure** of lung cytoarchitecture key to **physiology and pathophysiology**, i.e. on cellular and histological level
- **multiscale approach:** from whole organ to subcellular features





Imaging the Human Lung



Eckermann M, Frohn J, Reichardt M, et al. 3D virtual pathohistology of lung tissue from Covid-19 patients based on phase contrast X-ray tomography. *Elife*. 2020;9:e60408. Published 2020 Aug 20. doi:10.7554/eLife.60408

Aims & Objectives

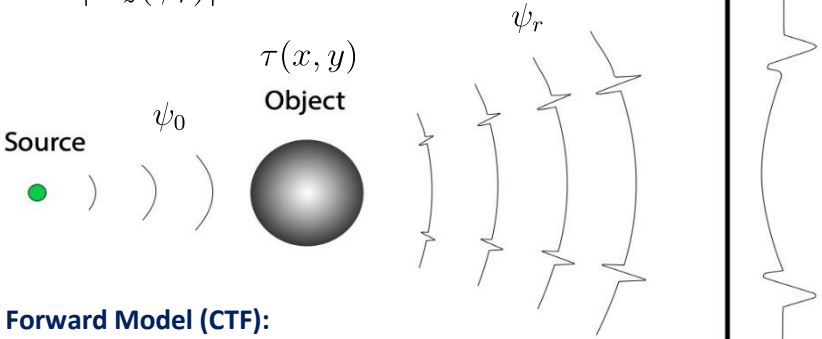
- exploiting **difference in electron density** detected by **phase shift** in soft tissue
- develop **optimized sample preparation** protocols
- physiological functions ⟷ pathological mechanisms
- shedding new light on **three-dimensional structure** of the lungs
- established techniques do lack in **resolution, non-destructivity or three-dimensionality**

	Resolution	Non-Destructivity	3D - Imaging
Histology	high	✗	✗*
MRI	low	✓	✓
Electron Microscopy	high	✗	✗*
Phase-Contrast CT	high	✓	✓

*slow, with distortions

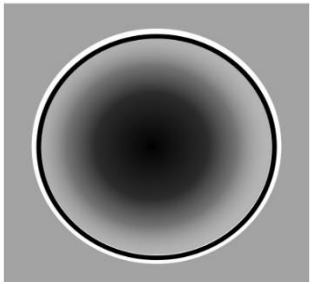
PROPAGATION-BASED X-RAY PHASE-CONTRAST CT

$$I = |D_z(\psi_r)|^2$$



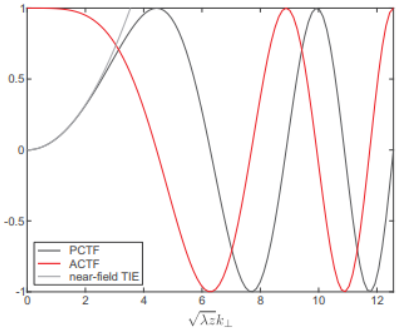
$$n(r) = 1 - \delta(r) + i\beta(r)$$

z12 >> z01



Forward Model (CTF):

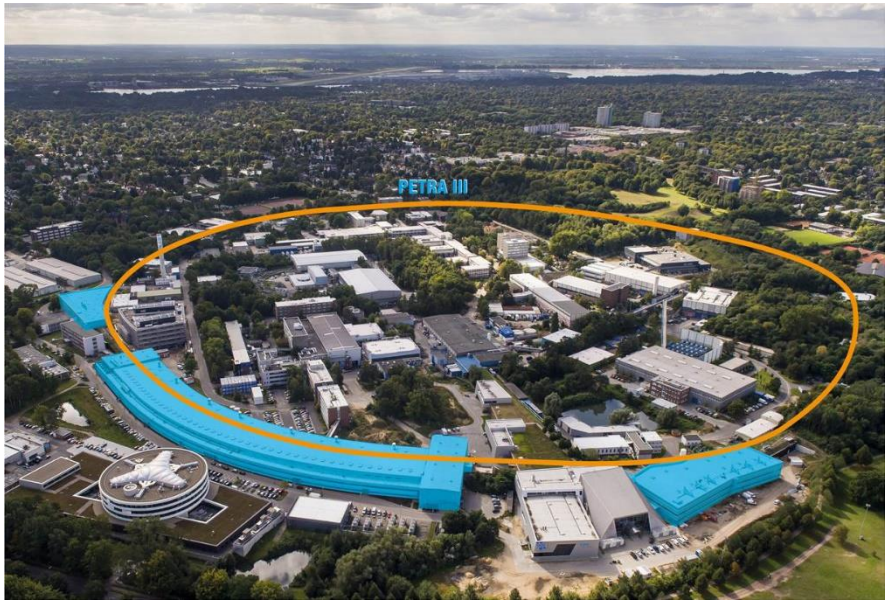
$$\frac{\hat{I}_\omega(\mathbf{k}_\perp, z)}{I_0} = 2\pi\delta_D(\mathbf{k}_\perp) + 2sin\frac{\lambda z\mathbf{k}_\perp^2}{4\pi}\hat{\phi}_\omega(\mathbf{k}_\perp) - 2cos\frac{\lambda z\mathbf{k}_\perp^2}{4\pi}\hat{\xi}_\omega(\mathbf{k}_\perp)$$

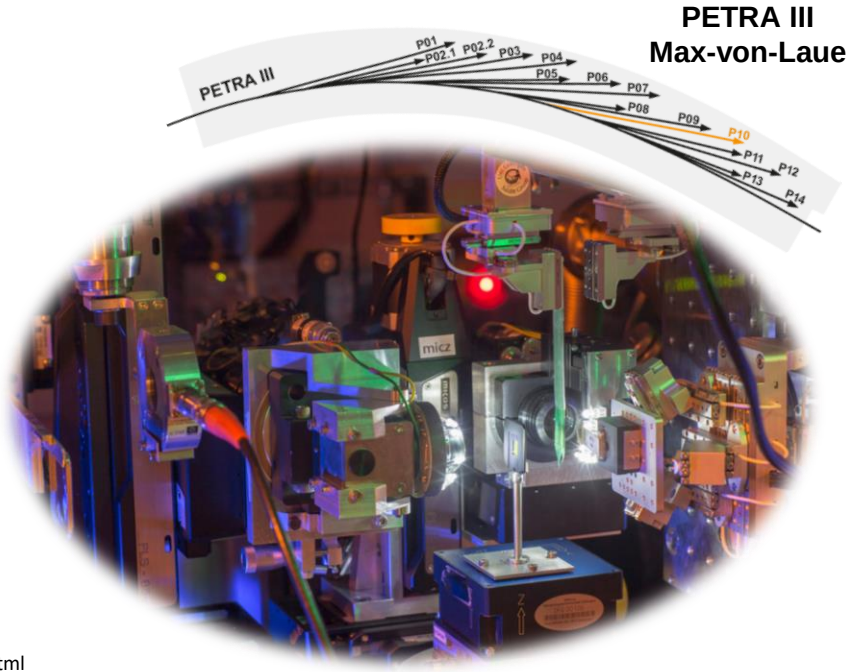


$$\phi(\boldsymbol{r}_\perp) = \mathcal{F}_\perp^{-1}\left\{\frac{\sum_{m=1}^{N}\left[\sin\chi_m + (\beta/\delta)\cos\chi_m\right]\mathcal{F}_\perp\left[I^{(\exp)}(\mathbf{r}_\perp, x) - 1\right]}{\sum_{m=1}^{N} 2\left[\sin\chi_m + (\beta/\delta)\cos\chi_m\right]^2 + \alpha(\mathbf{k}_\perp)}\right\}.$$

- **Fresnel fringes** connected to **phase of wave front** by **Laplacian**
- **oscillatory contrast in phase** and amplitude along propagation axis
- allows high-contrast imaging of soft tissue due to **high sensitivity to density alterations**
- **biological samples** especially suitable for being examined with phase contrast due to the significant **difference** in **refractive index** in **organs and tissue**
- **spatial coherence** prerequisite to observe Fresnel fringes

Göttingen Instrument for Nano-Imaging with X-Rays (GINIX) at DESY



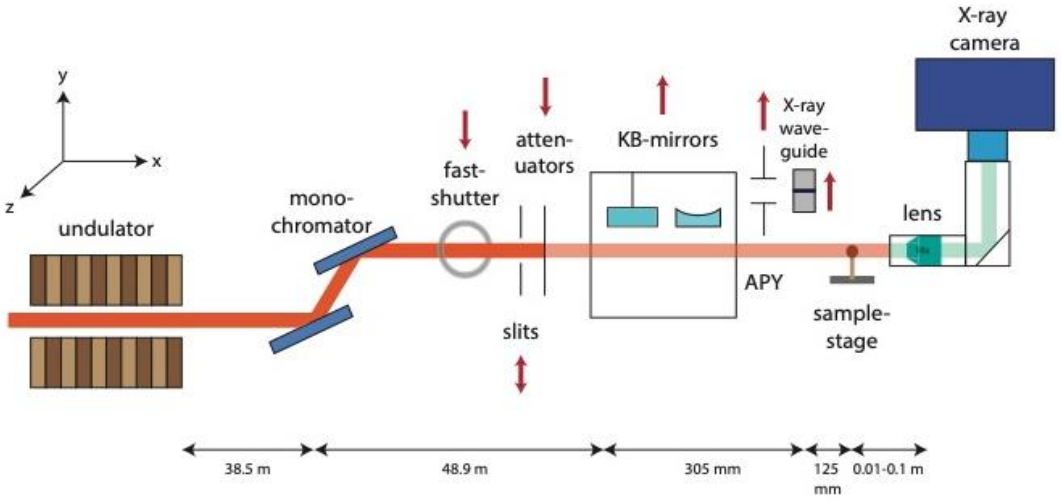


https://photon-science.desy.de/facilities/petra_iii/beamlines/p10_coherence_applications/index_eng.html

Experimental setup: Synchrotron-based imaging

Parallel beam geometry

FOV	1.6 mm x 1.4 mm
Pixel Size	650 nm
Regime	Direct Contrast
Exposure	0.035 s
Total Exposure	75 s (continuous)
Volumetric Flow Rate	3.75 x 10^7 µm^3 s^{-1}

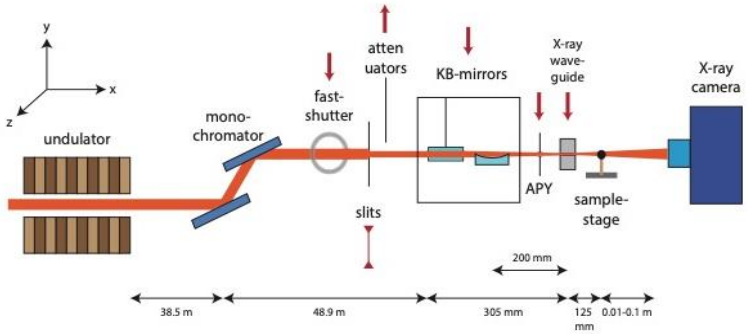


- **3 × 3 tomographic scans** of each sample at same height
- overlap to **merge volumes** after reconstruction

Experimental setup: Synchrotron-based imaging

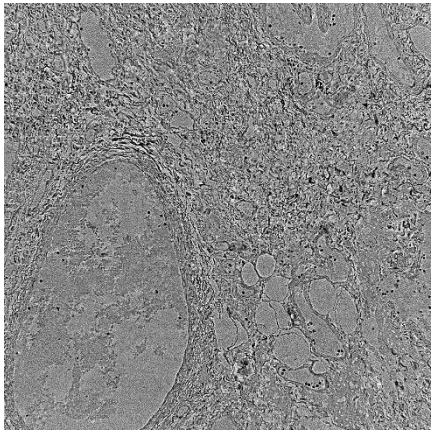
Cone beam geometry

FOV	0.4 mm x 0.35 mm
Pixel Size	167 nm
Regime	Holographic
Exposure	1 s
Total Exposure	42 min (start-stop)
Volumetric Flow Rate	1.93 x 10^4 µm^3 s^{-1}

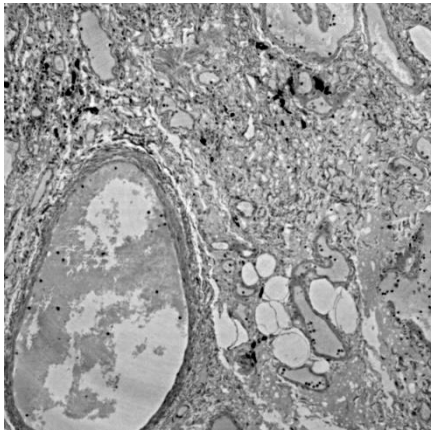


Phase Retrieval

- phase-retrieval performed from dark & empty corrected holograms
- **linearized single-step CTF-approach** (Cloetens et al., 1999) and **nonlinear Tikhonov regularization** (Huhn et al., 2022)



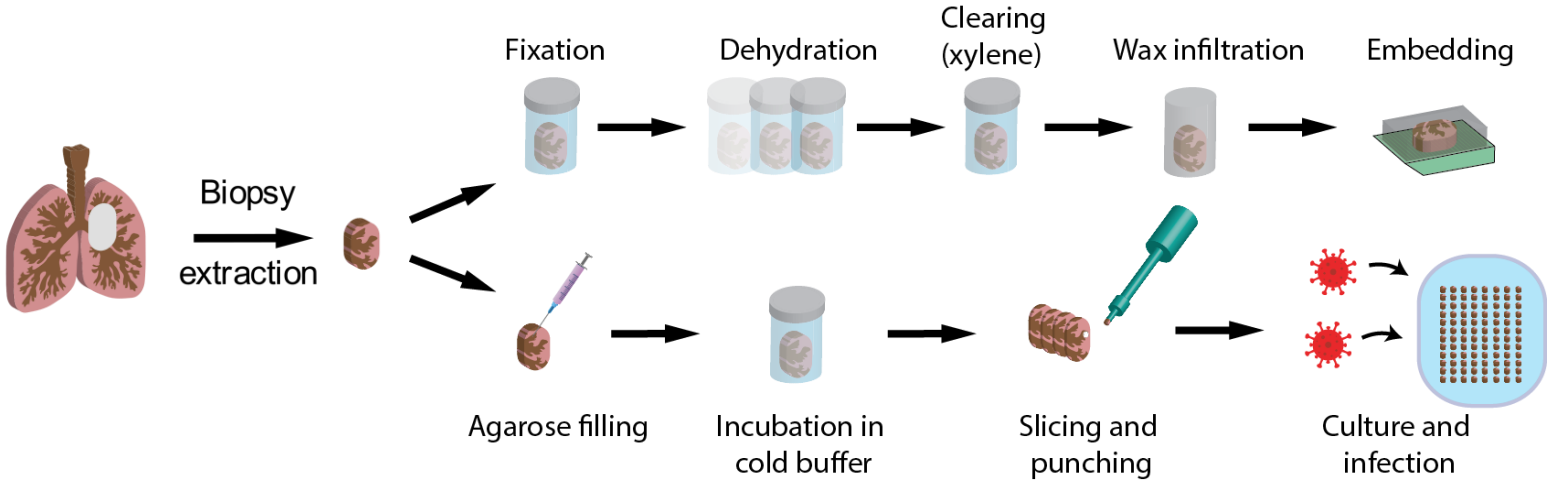
No phase retrieval



CTF-based phase retrieval

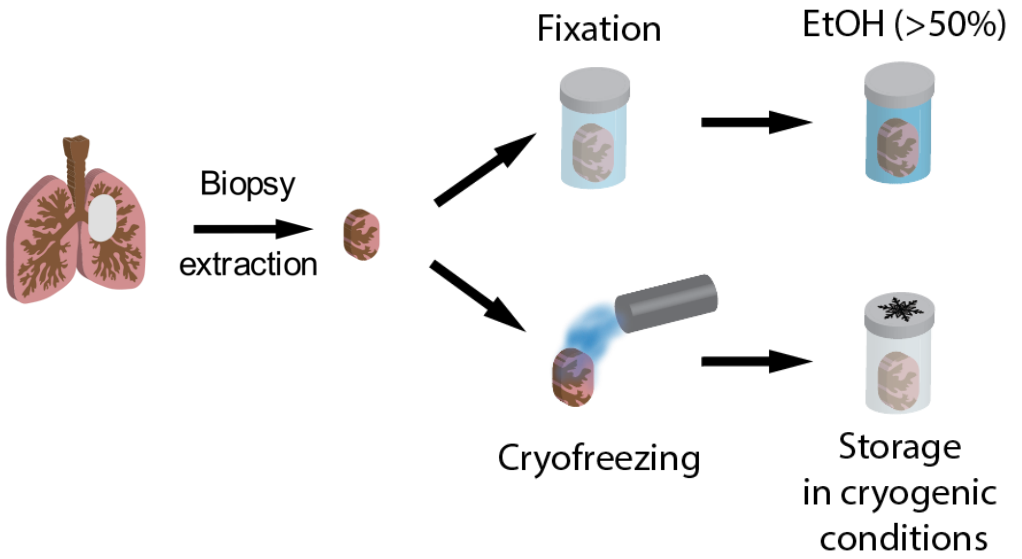
Sample Preparation

Paraffin embedding



Precision cut lung slices

Sample Preparation

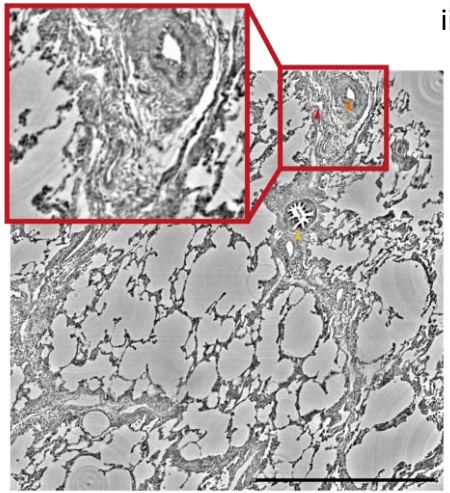


Fixation in EtOH

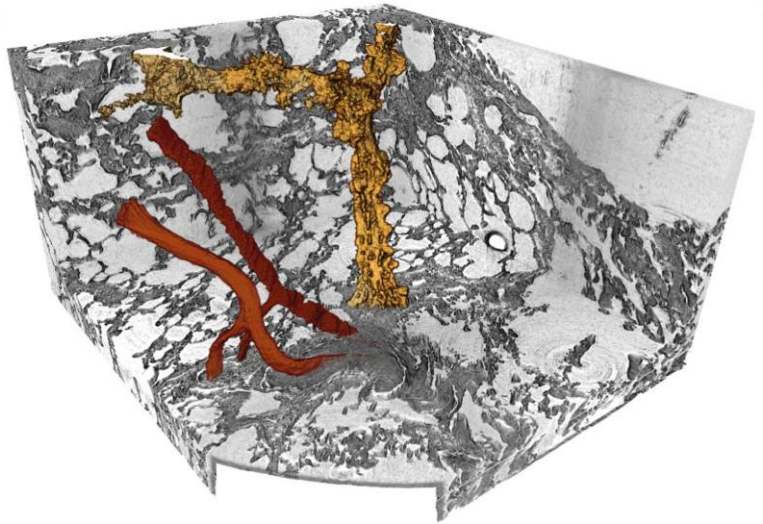
Cryogenic fixation

Multiscale Synchrotron Imaging of Pulmonary Disease

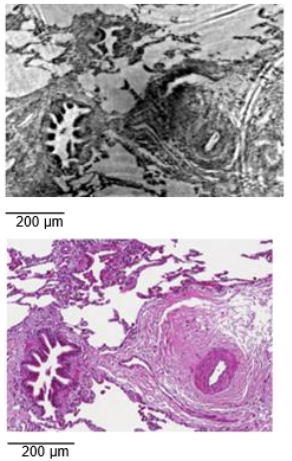
i)



ii)



iii)

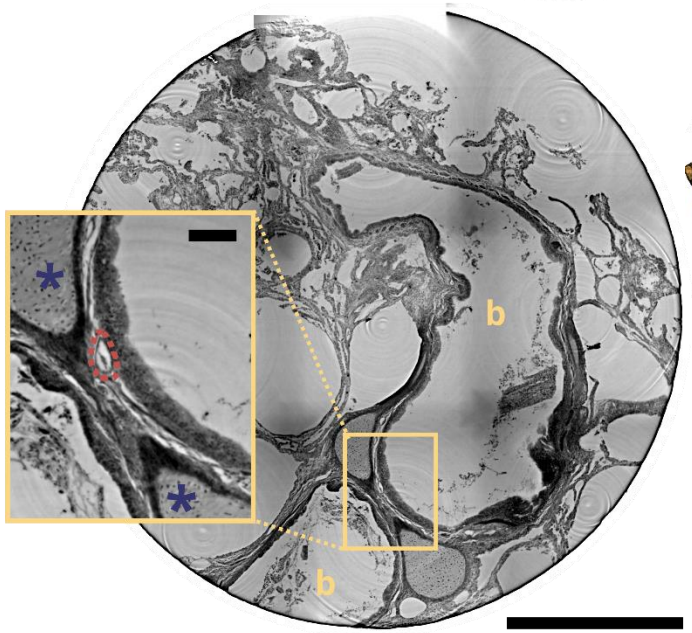


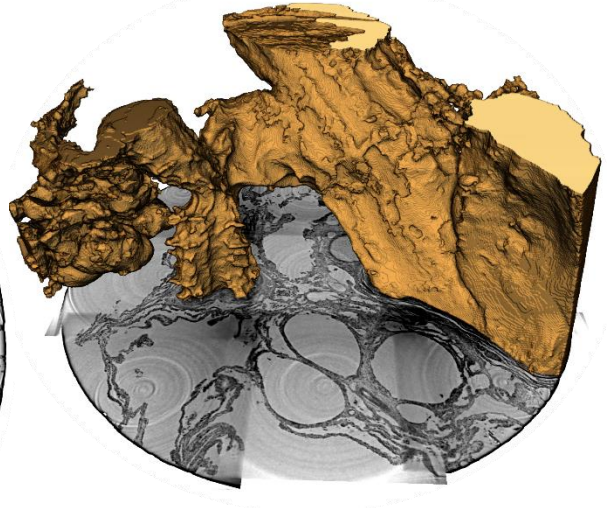
i. slice of **human lung sample** with **bronchovascular bundle** from patients with **alveolar capillary dysplasia** (ACD). Nine volumes (each ≈ 1.6 mm^3) **merged** to 3x3 tomogram (**FOV: ≈2.5 x 2.5 mm**, scale bar: 1 mm)

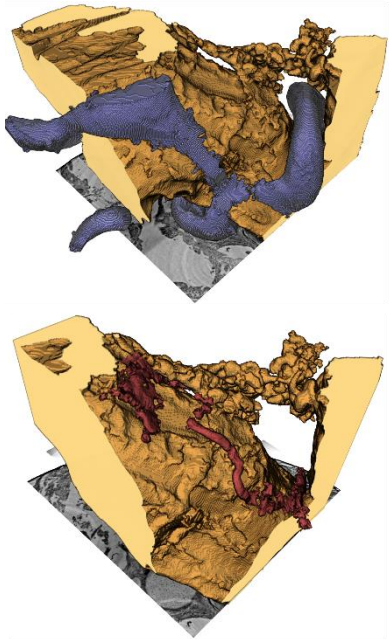
ii. segmented **bronchi** (yellow) and **vasculature** (**artery**: orange; **vein**: red)

iii. **correlation** of reconstructed images with **H&E stained histological slices** at region of interest

Multiscale Synchrotron Imaging of Pulmonary Disease

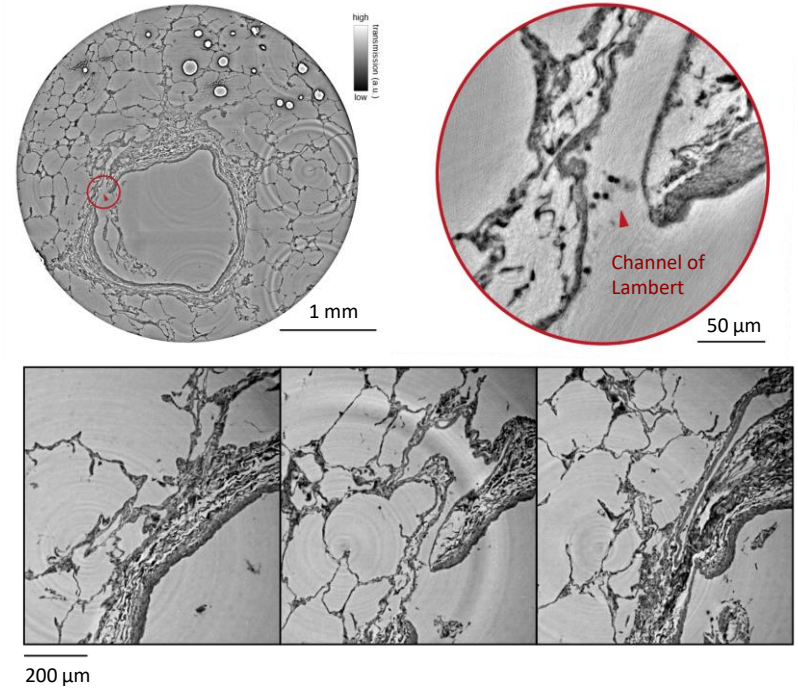


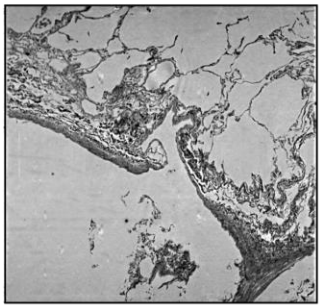


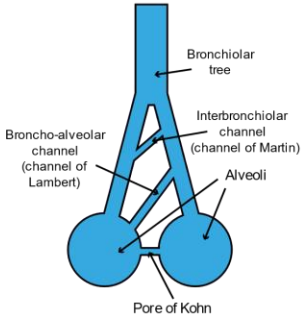


- bronchus/secondary bronchi
- cartilage
- vein/artery

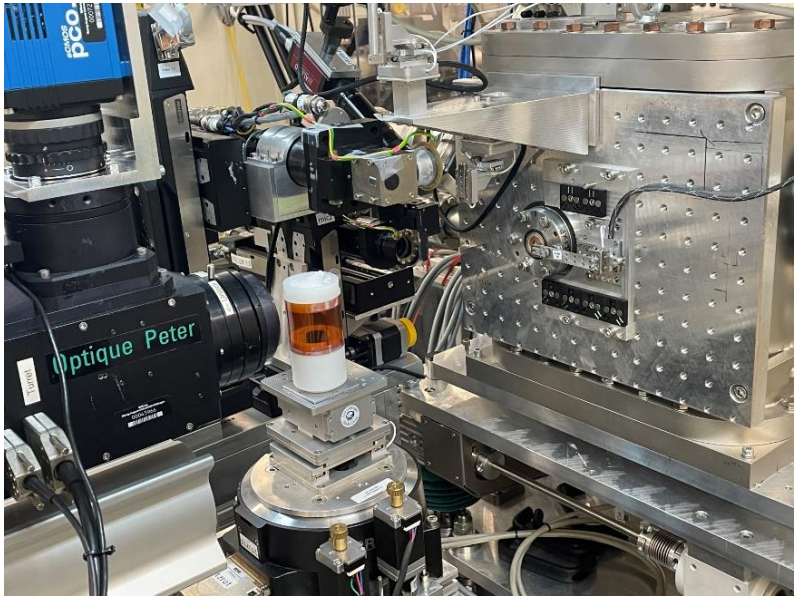
Multiscale Synchrotron Imaging of Pulmonary Disease



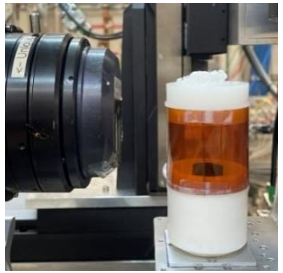


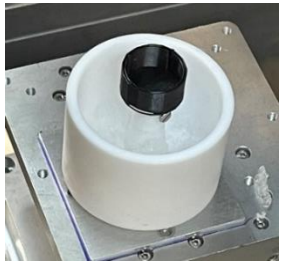


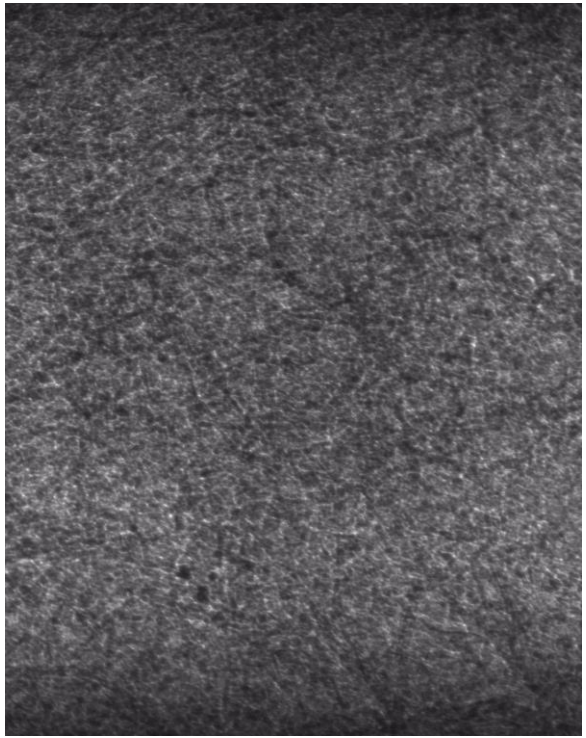
Cryo Lung Imaging - Setup



	Cryo-Sample
Energy	13.8 keV
Regime	Holographic
z_{12}	49 mm

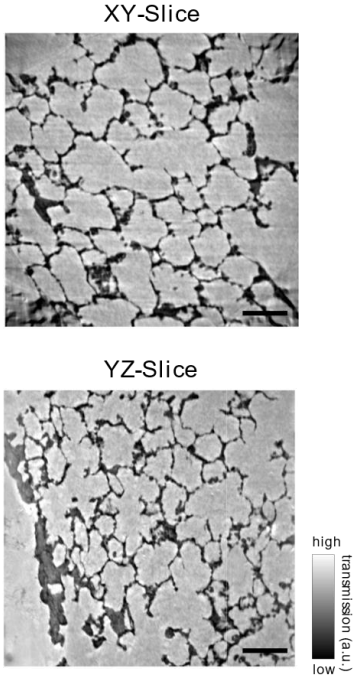


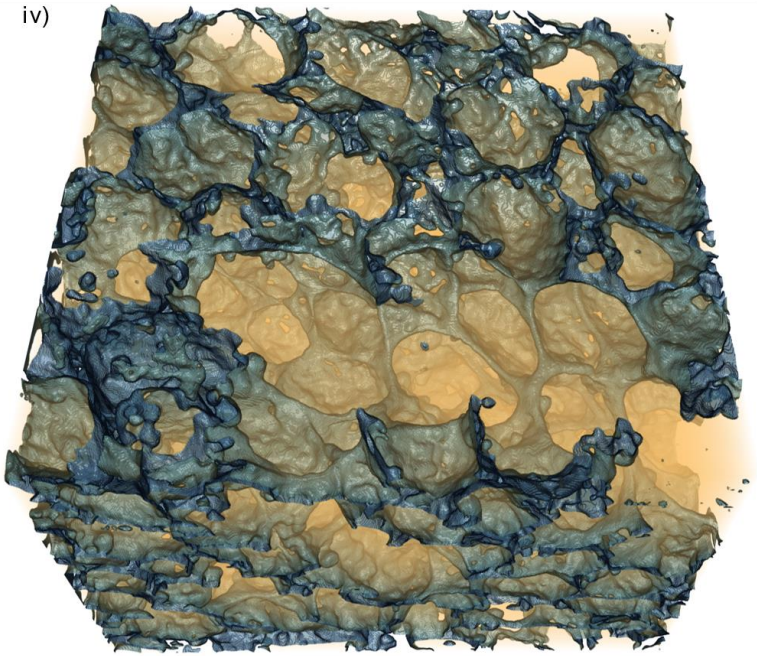




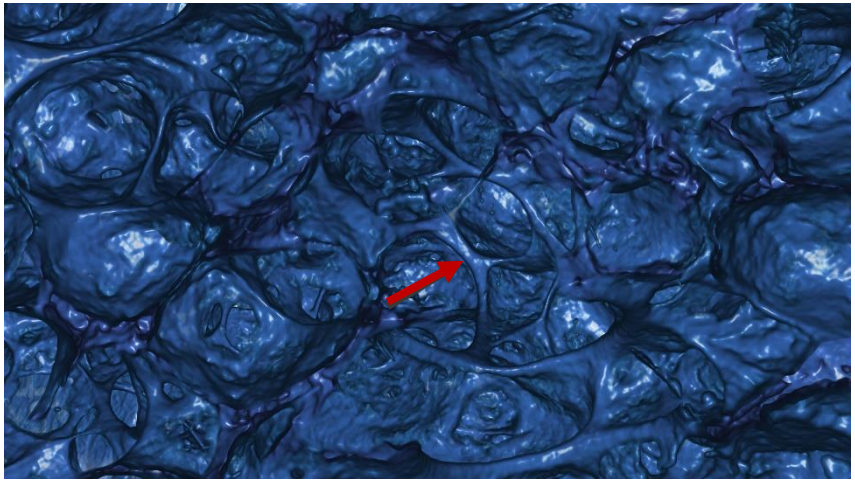
Cryo Lung Imaging

- constant temperature **below -20°C** during scan
- nevertheless, some **shrinkage** of tissue resulting in few moving artifacts
- general **proof of concept**, further investigations planned

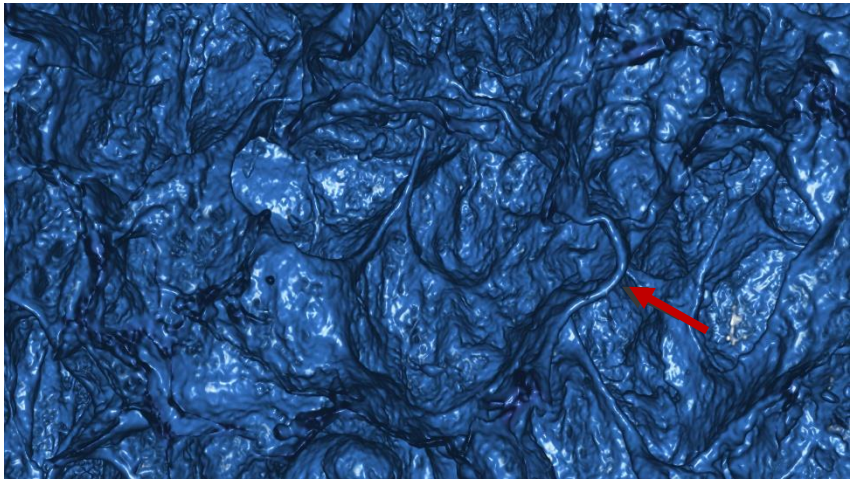




Rendering of alveolar structures

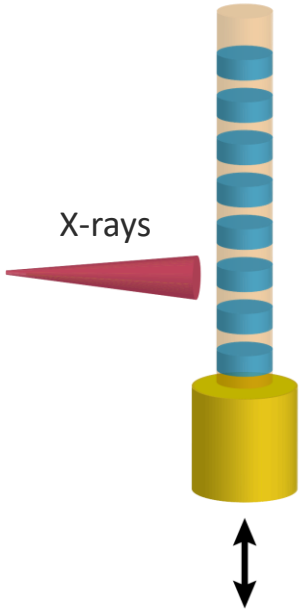


Alveolar sacs of human lung from a **young** patient



Alveolar sacs of human lung from an **old** patient

High-Throughput Lung Screening

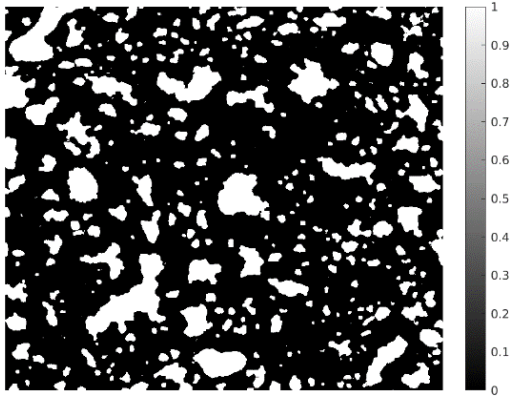


Automated
Phase Retrieval

&
Reconstruction

Image Analysis

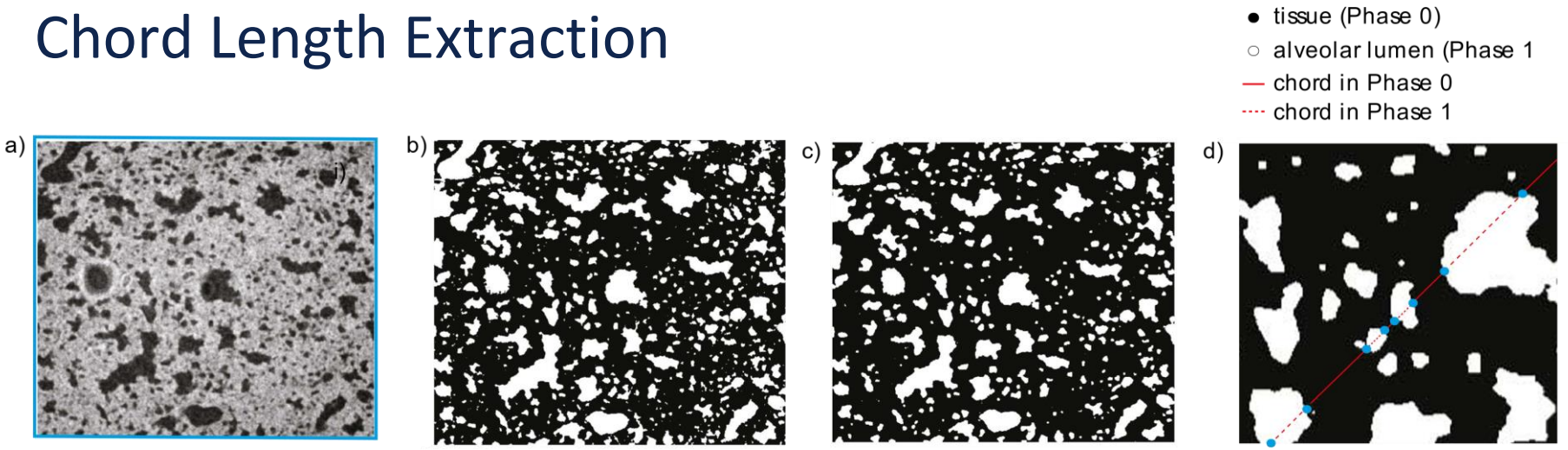
&
Feature
Extraction



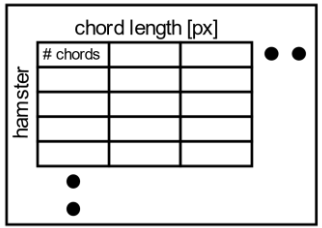
Feature Examples:

- shape (e.g. curvature, sphericity)
- compactness
- connectivity/uniformity
- **chord length distribution**
- optimal transport methods

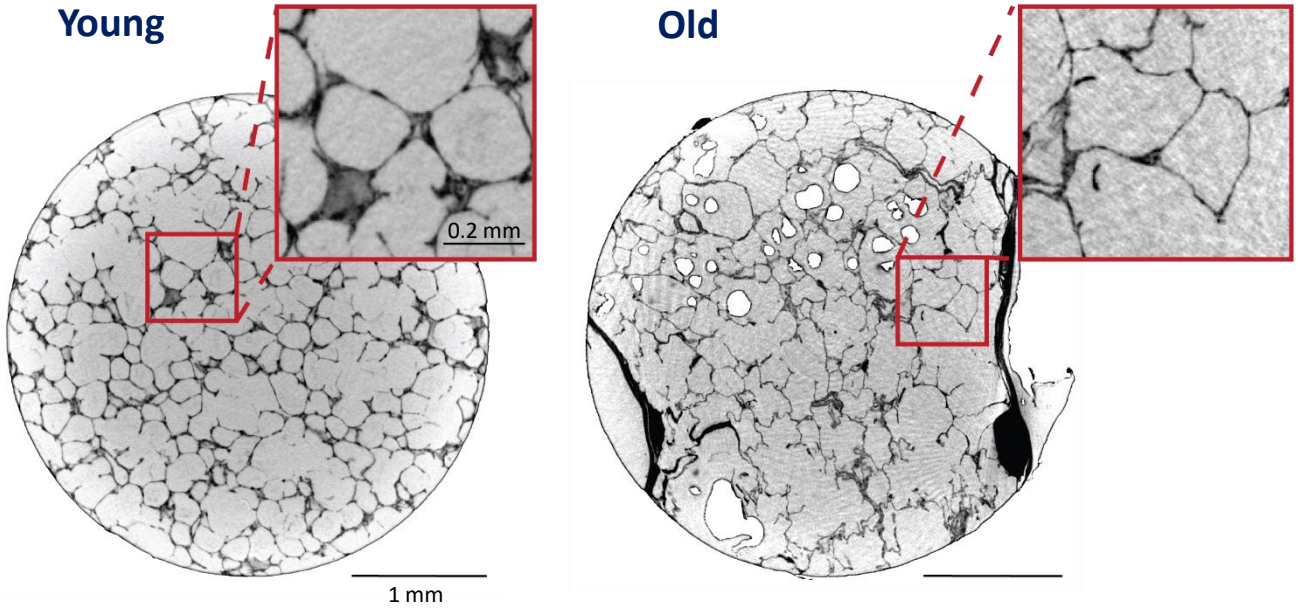
Chord Length Extraction

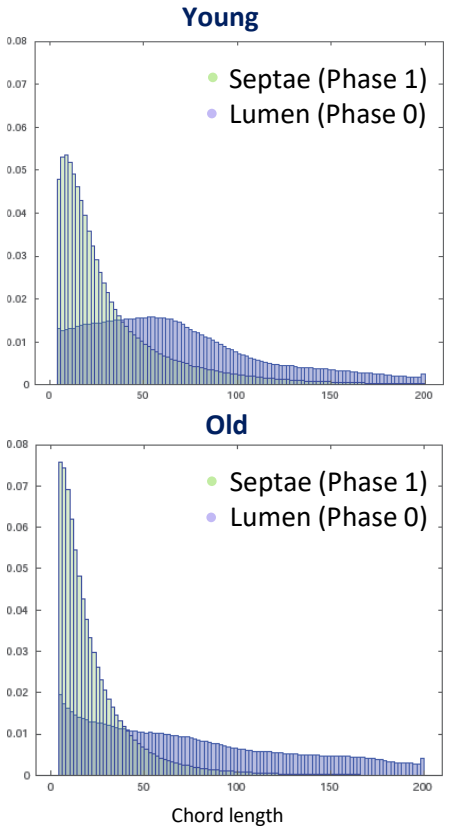


a) **cropping** volume to cube in all directions
b) binarization by **thresholding** (Otsu' method, summation with empirical value)
c) morphological operations:
Opening (dilation -> erosion): removes small objects from the foreground
Closing (erosion -> dilation): removes small holes in the foreground
d) introduce **chords** at **multiple angles** through entire volume



Feature Analysis – Chord Length Distribution



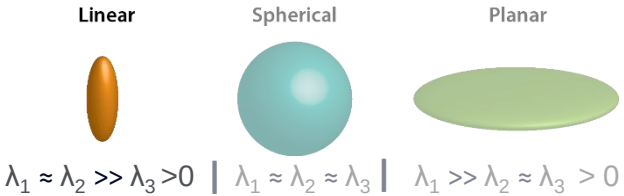


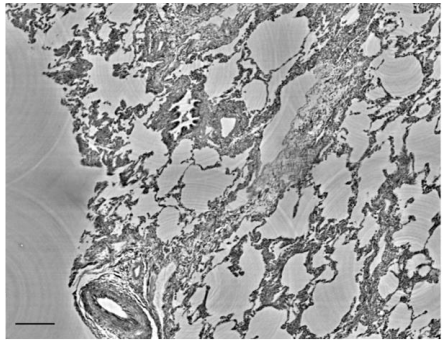
Shape Measure

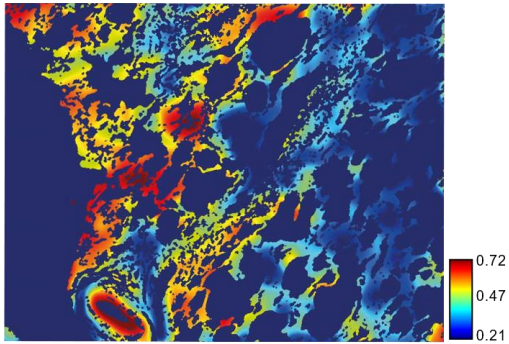
- **structure tensor** encodes **predominant orientation** and **degree of anisotropy**

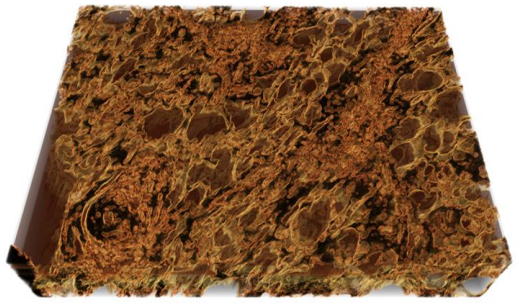
$$S = \sum_w \begin{pmatrix} I_x^2 & I_x I_y & I_x I_z \\ I_y I_x & I_y^2 & I_y I_z \\ I_z I_x & I_z I_y & I_z^2 \end{pmatrix}, I_a = \frac{\partial I}{\partial a}$$

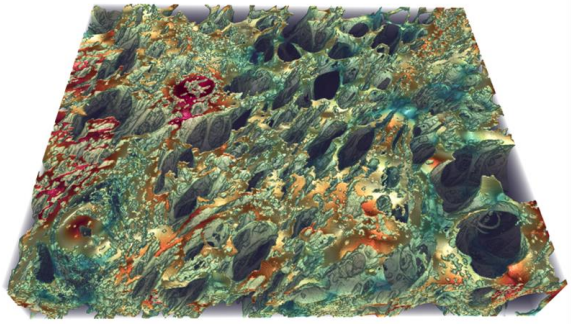
- used to find **dominant direction** by Eigendecomposition
- **eigenvalues** ≙ degree of anisotropy
  first eigenvector ≙ dominant direction
- **shape measures** as discriminative local feature [1]











[1] Patrick M. Jensen, Camilla Himmelstrup Trinderup, Anders B. Dahl, Vedrana Andersen Dahl: Zonohedral Approximation of Spherical Structuring Element for Volumetric Morphology. SCIA 2019: 128-139
[2] M. Reichardt et al. (2021) 3D virtual histopathology of cardiac tissue from Covid-19 patients based on phase-contrast X-ray tomography eLife 10:e71359 https://doi.org/10.7554/eLife.71359

Significance & Take-Home-Message

- poorly understood **3D structures** can be identified in **larger volume overview** and subsequently studied in **more detail** at higher resolution
- respective **physiological functions** of airways or vascular networks, and different **pathophysiologic mechanisms** can be elucidated
- **quantification** of 3D datasets by **morphometric tools** such as **shape measure analysis** and **chord length distribution** allows for **objective assessment** of tissue and can help **to identify structures** not easily detectable
- synchrotron data can be used to **validate laboratory protocols** and provide **ground truth for standardizing the method**

Outlook

- advancement in terms of **sample environment & preparation** such as cryogenic fixation and PCLS
- further development of **multiscale approach** to achieve highest resolution while maintaining a large field of view, whole organ imaging at BM18 in Grenoble
- upgrade to **PETRA IV** in next years**, higher brilliance and coherence** of X-ray beam (≈ ×100 # of photons)
- **multimodal acquisition** by combination of XPCT with e.g., FIB-SEM, SBEM and conventional histology
- **extension and automatization of quantitative analysis** of features of pathological indicators
- **standardization** of techniques and processes to improve repeatability, allowing higher throughput and translate the developed techniques to medical facilities

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Multiscale Bioimaging Cluster of Excellence (MBExC)

Institute for X-Ray Physics, University of Göttingen

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