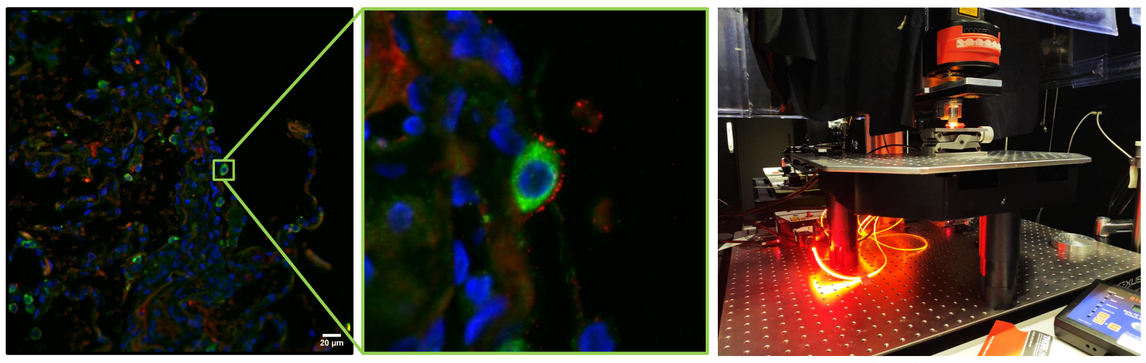
JUNO: Joint Utilization of Novel imaging technology for AT2 Organoids

Noah Heldt[1,2,*], Julia Rückoldt[3,4,*], Robert Schönherr[2,5], Lara-Jasmin Schröder[4,6], Mario Pieper[2,5], Stephanie Schubert[6], Christopher Werlein[6], Mareike Knoll[6], Lavinia Neubert[4,6], Peter König[2,5], Jan Christopher Kamp[3,4,*], Gereon Hüttmann[1,2,*]

[1]Institute of Biomedical Optics, University of Lübeck; [2]Airway Research Center North (ARCN), German Center for Lung Research (DZL); [3]Department of Respiratory Medicine and Infectious Diseases, Hannover Medical School; [4]Biomedical Research in Endstage and Obstructive Lung Disease Hanover (BREATH), German Center for Lung Research (DZL); [5]Institute of Anatomy, University of Lübeck; [6]Institute of Pathology, Hannover Medical School; *Contributed equally

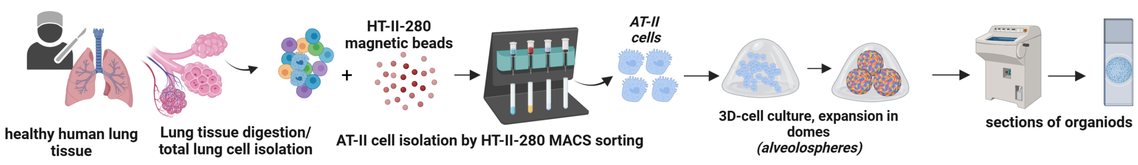
Introduction

- AT2 organoids needed to investigate fibrosis pathogenesis
- Exhibit stem cell-like properties
- Display substantial morphological variability
- Cellular organization must be characterized for stimulation experiments
- Requires high-resolution, label-free, and non-invasive imaging



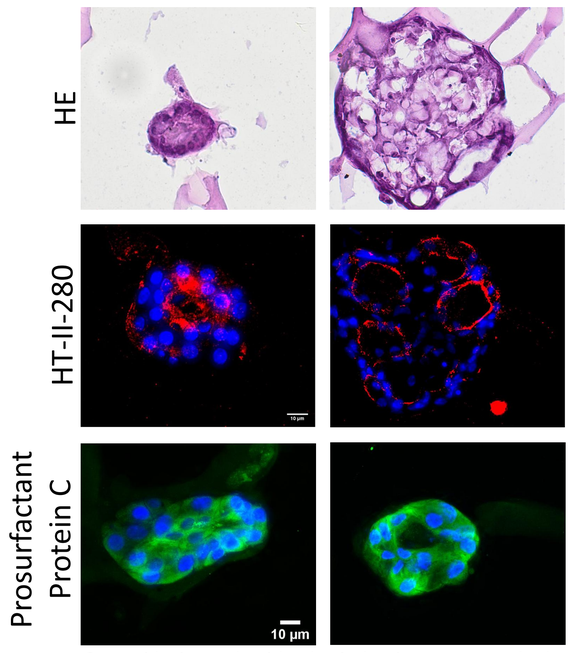
Fluorescence microscopy of the AT2 cells (left) and the DOCT System used for imaging the AT2 organoids (right).

AT2 Organoids



Workflow to cultivate and analyse primary human AT2 cells as organoids. Figure created with BioRender.

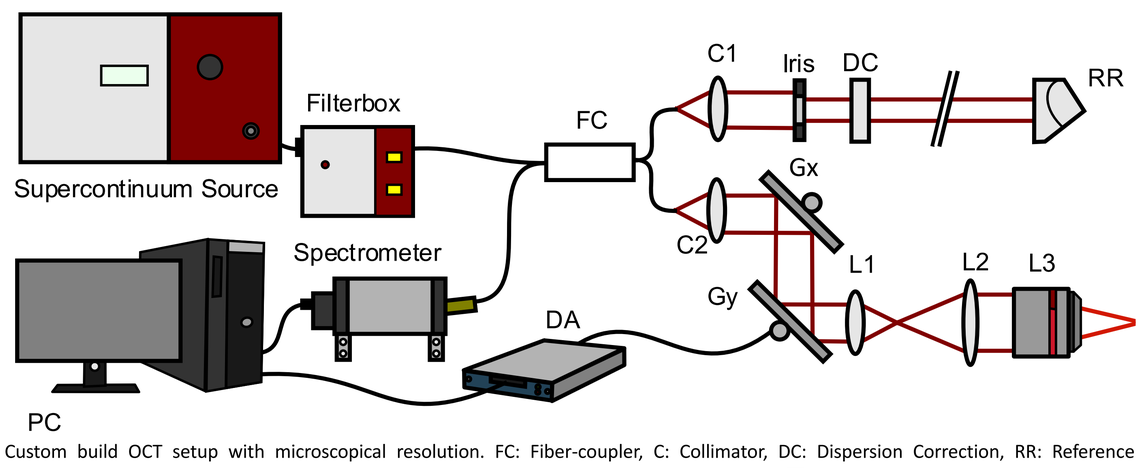
- Lung fibrosis may be developed due to AT2 cells transitioning into mesenchymal cells
- The process of epithelial-to-mesenchymal transition needs to be studied
- Investigating this requires assessment of stimulation induced changes
- Conventional microscopy approaches are insufficient or invasive



Organoids imaged with classical microscopy methods. HT-II-280 and proSP-C are expressed, confirming AT-2 cells in the organoids. See Poster 631.

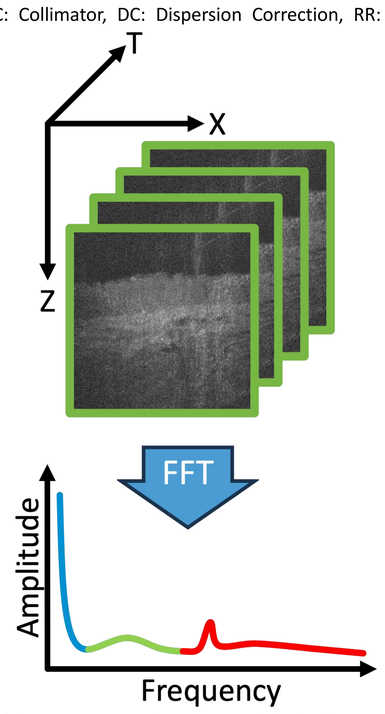
Dynamic OCT

- 1 µm nearly isotropic resolution
- Non-invasive imaging by detection of backscattered light
- Label-free functional contrasting analysis of tissue dynamics



Custom build OCT setup with microscopical resolution. FC: Fiber-coupler, C: Collimator, DC: Dispersion Correction, RR: Reference Reflector, G: Gyroscopic mirror, L: Lens, DA: Digital-Analog-converter

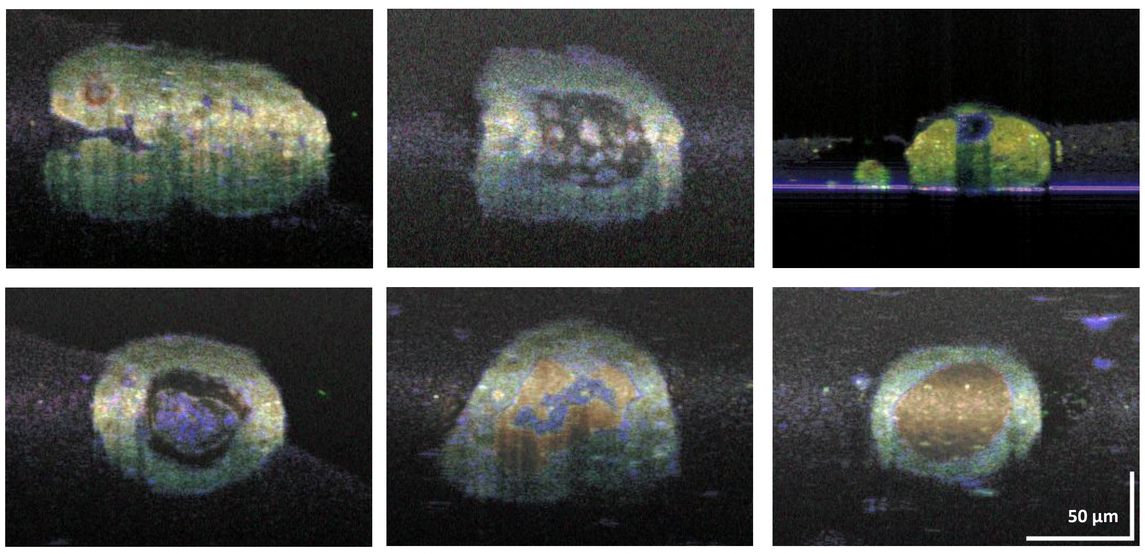
- Multiple frames (B-Scans) are acquired using a framerate of about 125 Hz
- Pixelwise Fourier transform yields frequency spectrum of the signal fluctuations in each pixel
- These spectra are binned into 3 channels to form an RGB image
- Binning is automated using the Neural Gas algorithm



Binning of the Fourier transformation yields the dynamic contrast.

Results

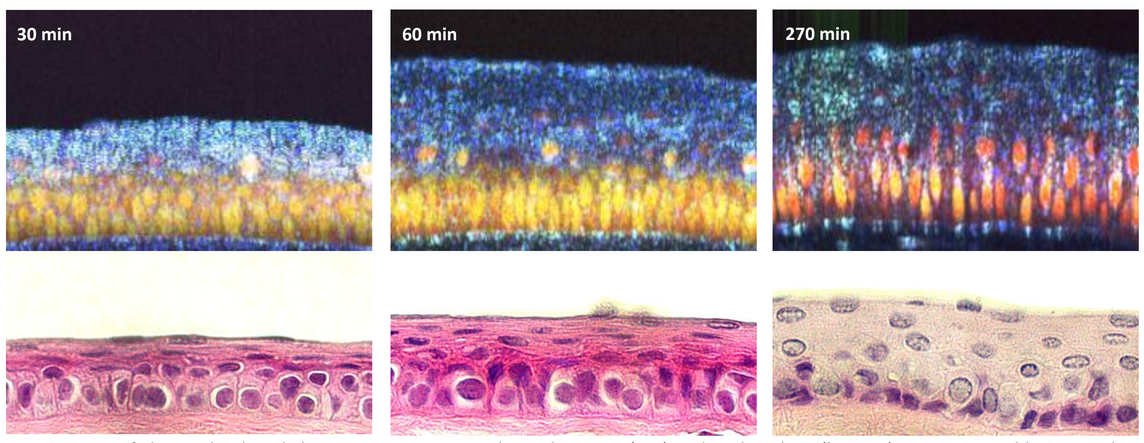
- Organoids from 3 patients were imaged
- 3D imaging of whole organoids was performed
- Vast morphological differences could be confirmed
- Organoids outgrowing the Matrigel were observed for the first time



Several AT2 organoids imaged with DOCT. Vastly different morphologies and activity levels can be observed.

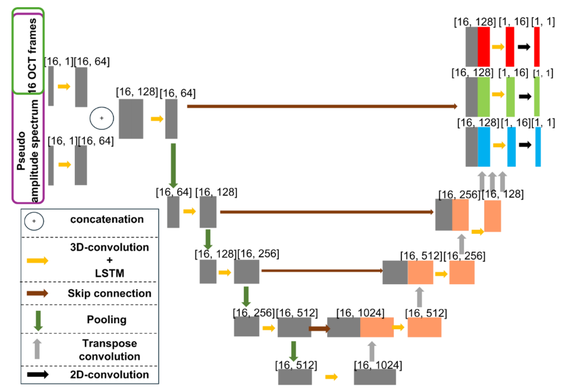
Outlook

- DOCT enables longitudinal studies
- Imaging across stimulation experiments to characterize effects
- Additional 2-Photon & Spinning-Disk Microscopy



Investigation of chemical induced changes in retina across hours by DOCT (top) and HE histology (bottom). DOCT is capable to visualize the same changes in a label-free and non-invasive manner.

- AI enhanced contrasting
- Predict full spectral information from short sequences
- Enables interleaved volumetric scanning
- Allows for faster acquisitions
- Cooperation with University of Tsukuba, Japan



Used 3D-Unet to predict enhanced DOCT contrast from undersampled sequences.

Conclusion

Dynamic OCT can capture the complex morphology of AT2 organoids. Moreover, DOCT is suitable for longitudinal studies, allowing to characterise effects in stimulation experiments.

E-Mail: noa.heldt@uni-luebeck.de, rueckoldt.julia@mh-hannover.de