Micro-CT analysis of human lung presenting
Idiopathic Pulmonary Fibrosis

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Aims
Idiopathic Pulmonary Fibrosis (IPF) is a fibrotic lung disease, characterized by progressive heterozygous fibrosis leading to architectural deformation of the parenchyma. Patients have a restrictive decline of pulmonary function with a survival prognosis of 50% 3–5 years after diagnosis with no adequate therapy available yet. Because diagnosis is very complex we want to use micro-ct as a bridge between radiological and histological data and gain knowledge on the impact of this disorder on the airways and alveolar tissue.

Method
When endstage IPF patients underwent a lung transplantation, the explant lung was inflated up to total lung capacity at 30 cm H₂O pressure and frozen at -192°C. The frozen lung was cut in 2cm slices with a bandsaw and cylinders of 1.5 cm were pinched with a hammer drill. As control, an unused donor lung has been processed in the same manner.

These frozen samples were fixed with glutaraldehyde-aceton, dehydrated with ethanol and hexamethyldisalazane followed by air drying. Tissue was scanned with a resolution of 8.4 µm in the SkyScan 1072 scanner at 40kV 250mA without filtration. Raw data was reconstructed with the SkyScan Nrecon software. Analysis was performed with SkyScan CTan software

Results
From one explant IPF lung 2 regions were selected (Fig.1 B and C), which represent an early and late stage of architectural deformation in end stage IPF. These regions were compared to control (Fig.1 A).

Fig. 1Reconstructed micro-CT image of A) Control, B) IPF early stage and C) IPF late stage
An increase of total tissue per volume was found for both IPF in early stage and late stage compared to control (Fig 2). Surprisingly there was almost no difference between IPF early stage and IPF late stage sample in total tissue. When specifically looking at alveolar tissue volume, almost no decrease was found in the control. In early stage IPF however there was a slight decrease, which was even more pronounced in late stage IPF.

**Fig. 2 Representation of Total Tissue or Alveolar Tissue over Total Volume**

**Fig. 3 Distribution curves of A) Structure Thickness and B) Structure Separation**
The distribution of the structure thickness of tissue (Fig 3A) showed a similar curve for Control (average of 80µm) and early stage IPF (average of 102 µm). For late stage IPF the curve has shifted to the right (average of 151 µm). Tissue from early stage IPF (average of 218 µm) showed an increased distribution of tissue with small structure separation, while the distribution curve from late stage IPF (average of 551µm) spread more towards higher separation with values higher than 900µm when compared to control (average of 328µm) (Fig 3B).

**Conclusion**

As expected on basis of histology we found an increase of tissue volume in samples with early and late stage IPF. Endstage IPF is heterozygous in its manifestation and shows different stages of fibrosis spread over the parenchyma. In early stages of fibrosis there is an increase of tissue with a slight decrease of alveoli. Due to neighboring fibrosis, alveoli in an early stage of IPF may be compressed resulting in a lower structure separation distribution compared to control. Fibrosis starts to form as is visible by looking at structure thickness.

In late stage fibrosis the parenchyma is severely deformed, resulting in decreased alveolar tissue. This is also measured by increase of structure thickness and an increase of structure separation as the alveoli are presumably disappeared by collapse and fibrosis with traction on the airways.

These preliminary data showed the possibility to use micro-CT imaging as a crossover between histology and conventional radiology. Being able to assess our samples in 3 dimensions brings more information of the ultrastructure of the airways and parenchyma.