3D mapping of blood vessel networks and cells in COPD and non-COPD lung tissue samples using micro-computed tomography and immunofluorescence

M.J. Lawson[1], O.L. Katsamenis[2], M. Olding[3], O. Larkin[3], B. Smit[3], I. Haig[3], A. Alzetani[1], P. Schneider[4], P. Lackie[1], J. Warner[1]

[1]Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK [2]µ-VIS X-ray Imaging Centre, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, UK [3]Nikon X-Tek Systems Ltd., Tring, UK [4]Bioengineering Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton, UK

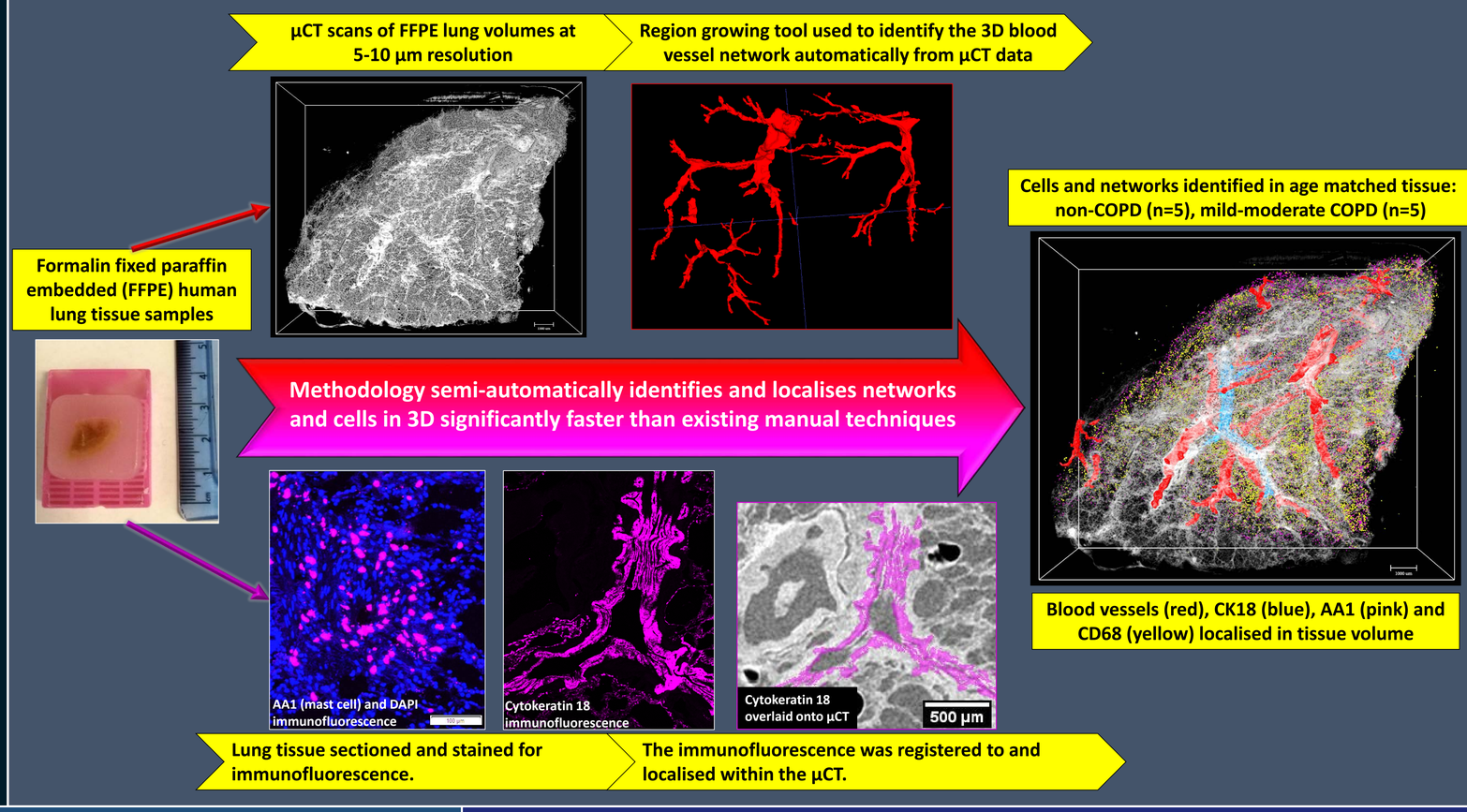
1. Background

- Micro-Computed Tomography (µCT) is a non-destructive X-ray imaging technique used to visualise the 3D micro-structure of human lung tissue, this was combined with immunofluorescence to identify specific cells within a 3D volume
- Microscopic changes in airways, vasculature networks and infiltrating cells are known to be features of lung diseases such as COPD but have not been quantified in 3D

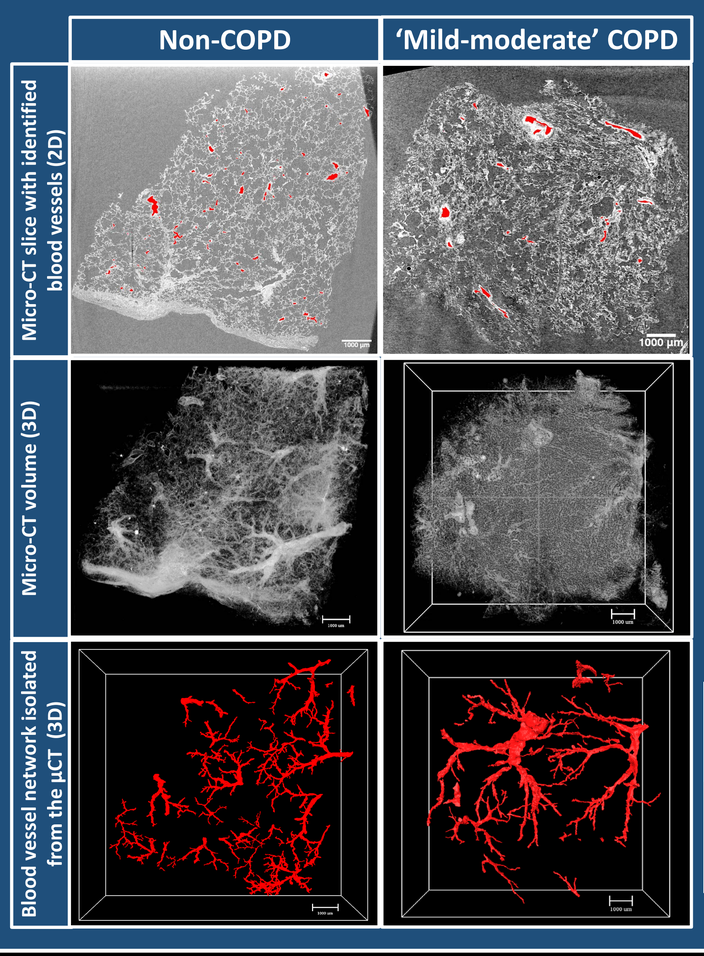
Aims

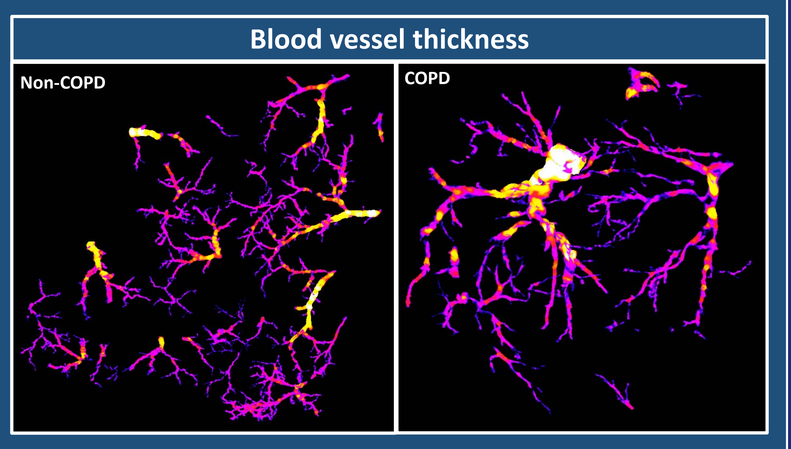
1. Visualise the 3D networks and cells types in human lung tissue by registering and segmenting immunofluorescence (IF) to µCT
2. Analyse the 3D networks of blood vessels in non-COPD and COPD lung tissue samples
3. Assess populations of specific cell types to quantify their relative location to blood vessels in 3D

2. Materials and methods



3. Identifying and analysing 3D blood networks



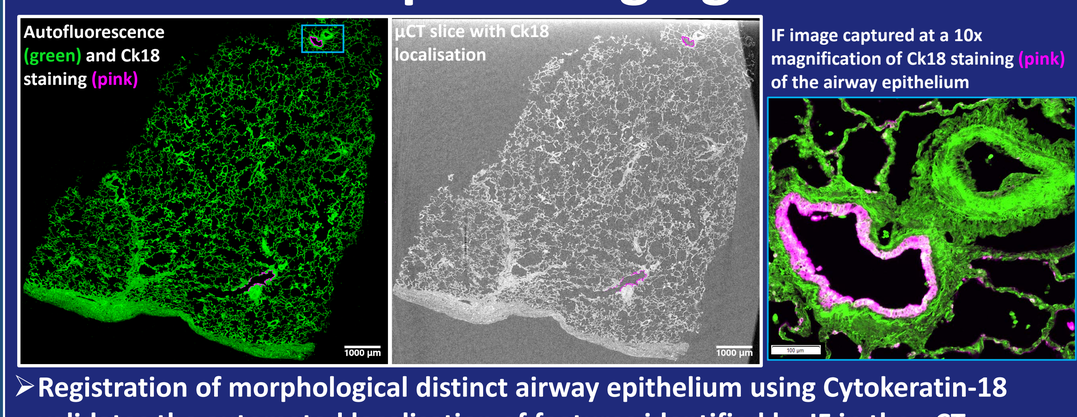


➢ Thickness maps as a proxy for lumen diameter of blood vessel networks (brighter colour=thicker vessel)

Table 1. Analysis of blood vessel networks from 5 non-COPD and 5 COPD patient samples, mean results with standard deviation reported.

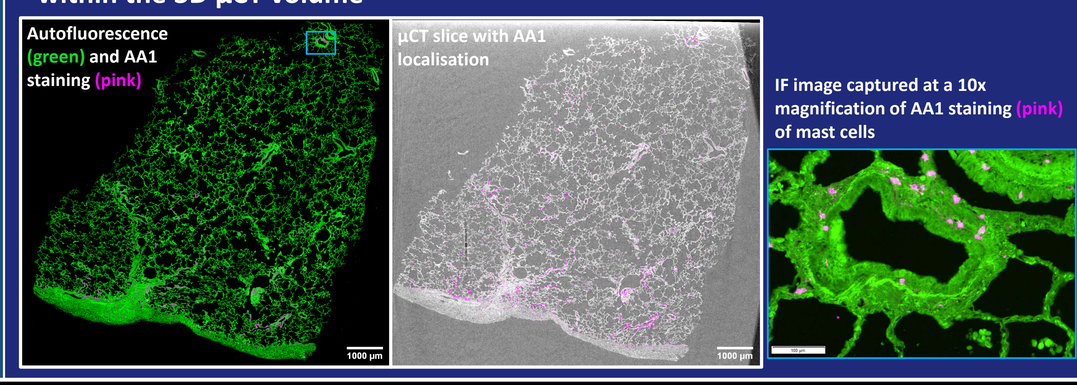
Lung tissue (n=5)	Blood volume fraction of tissue (%)	Network length (mm/mm³)	Branch number	Mean lumen thickness (µm)
Non-COPD	2 ± 1.5	2.0 ± 0.8	175 ± 142	84 ± 17
Mild-moderate COPD	2 ± 0.8	2.2 ± 0.07	176 ± 148	162 ± 7.8

4. Correlative immunofluorescence and µCT imaging

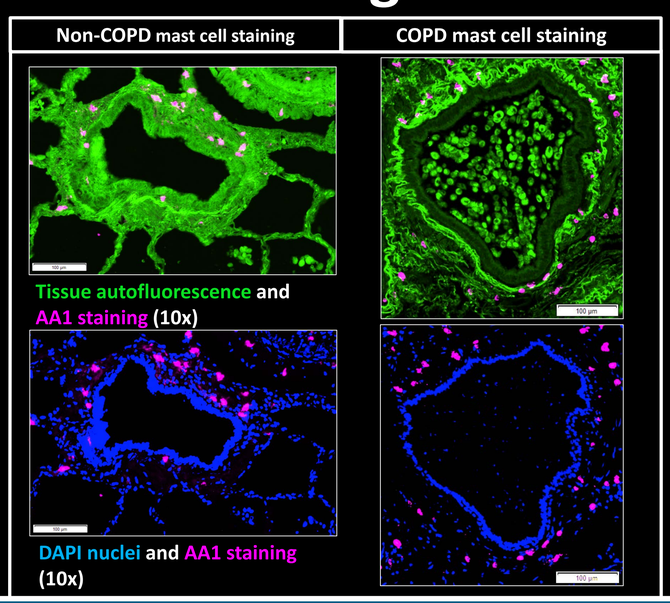


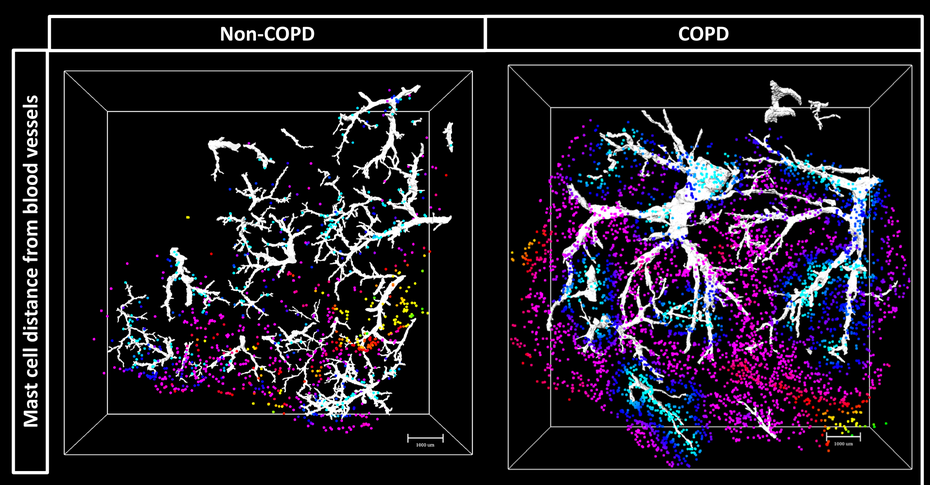
➢ Registration of morphological distinct airway epithelium using Cytokeratin-18 validates the automated localisation of features identified by IF in the µCT

➢ Correlative 2D imaging with immunofluorescence enables localisation of cell types within the 3D µCT volume



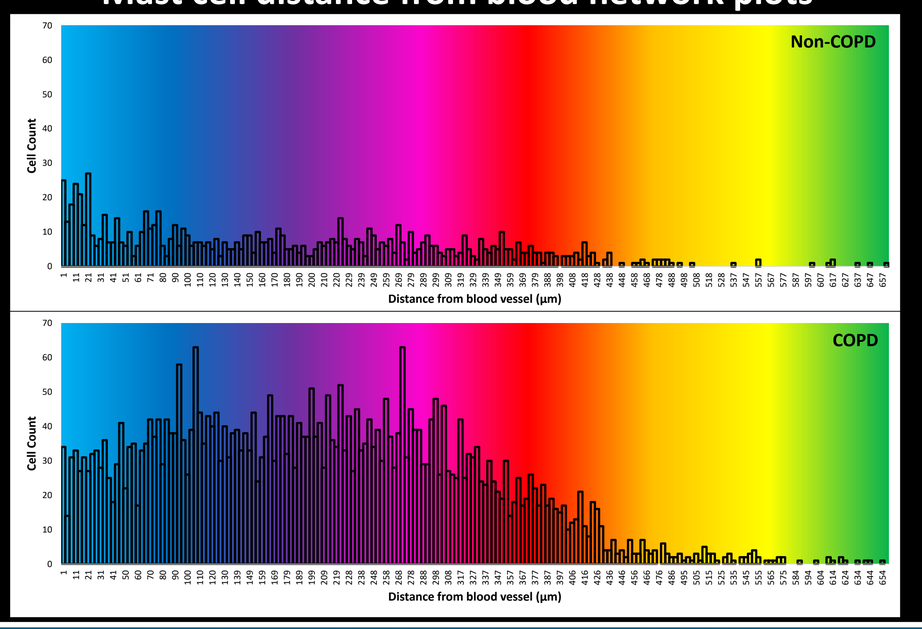
5. Combining 3D network information with cellular staining





➢ More mast cells appear to be present in COPD tissue but distribution in relation to blood vessel network is similar to non-COPD

Mast cell distance from blood network plots



6. Conclusions

➢ Micro-CT combined with immunofluorescence was successfully used to identify blood vessels, Ck18, macrophages and mast cells within the three-dimensional lung volume in both non-COPD and 'mild-moderate' COPD lung tissue samples

➢ Analysis of the blood vessels suggests little difference between these networks in healthy and COPD lungs in 3D however there is great variability between samples in each group

➢ Mast cells are widely distributed as individual cells throughout the tissue with a trend towards more mast cells being identified in COPD tissue

➢ The distribution of mast cells in relation to blood vessels did not change between COPD and non-COPD with majority of mast cells located within 400 µm of the nearest blood vessel in the 3D volume

Acknowledgments

ML is supported by a MRC-CASE studentship with Nikon Metrology. We would like to thank the staff and students of the Biomedical Imaging Unit, the Histochemistry Research Unit, the µ-VIS X-ray imaging centre and the IRIDIS High Performance Computing Facility at the University of Southampton and also Nikon X-Tek Systems Ltd., Tring UK for their support over the course of this project.