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

M.J. Lawson1, O.L. Katsamenis2, M. Olding3, O. Larkin3, B. Smit3, I. Haig3, A. Alzetani1, P. Schneider4, P. Lackie3, J. Warner1

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

- µCT slice with identified Blood vessel network
- µCT volume (3D)
- Micro-CT slice with identified Blood vessel network
- Methodology semi-automatically identifies and localises networks and cells in 3D significantly faster than existing manual techniques

3. Identifying and analysing 3D blood networks

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

<table>
<thead>
<tr>
<th>Lung tissue</th>
<th>Blood volume fraction of tissue (%)</th>
<th>Network length (mm/mm³)</th>
<th>Branch number</th>
<th>Mean lumen thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-COPD</td>
<td>2 ± 1.5</td>
<td>2.0 ± 0.8</td>
<td>175 ± 142</td>
<td>84 ± 17</td>
</tr>
<tr>
<td>Mild-moderate COPD</td>
<td>2 ± 0.8</td>
<td>2.2 ± 0.07</td>
<td>176 ± 148</td>
<td>162 ± 7.8</td>
</tr>
</tbody>
</table>

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

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

- Blood vessels (red), CK18 (blue), AA1 (pink) and CD68 (yellow) localised in tissue volume

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

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