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Title: Using micro-CT to map the small airways

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Body: Introduction: The loss or narrowing of small airways(<2mm) leads to an increase in peripheral resistance and is a major site of airway disease in COPD. Quantitative studies of the three dimensional architecture and organisation of the lung are required to assess this. Micro-CT permits direct examination of the small airways and microvasculature. However, most studies to date have used osmium or other contrasting agents precluding the use of historical samples. Aim: To use micro-CT to identify and measure the small airways in paraffin embedded tissue without contrasting agents. Methods: Tissue from the distal airways was fixed and embedded in paraffin wax. It was scanned with a Nikon/Metris HMX XCT scanner at the μ -VIS X-ray imaging centre, University of Southampton to obtain CT data with a scan time of four hours. An acquired voxel resolution of 7.9 μ m allowed examination of airway lumen along its length and a 3D volume reconstruction of a branching small airways network created using VG Studio Max (v2.1) and Aviso 7 image processing software. Results: Lung tissue was clearly distinguishable from the airspaces in scans permitting a clear 3D reconstruction of all airways and blood vessels in the sample. Airway cross section measurement was possible down to the respiratory bronchioles at <0.15mm. This will permit the estimation of potential volumetric air flows from the alveoli to the small airways. Conclusion: We have shown that it is possible to use micro-CT to analyse and reconstruct small airways structure in human tissue down to the respiratory bronchioles with a resolution of 7.9 μ m. Using formalin fixed and paraffin embedded tissue without any contrasting agents can provide robust 3D structural data from archival material.