Correlative microfocus computed tomography and fluorescence microscopy of fixed human lung tissue

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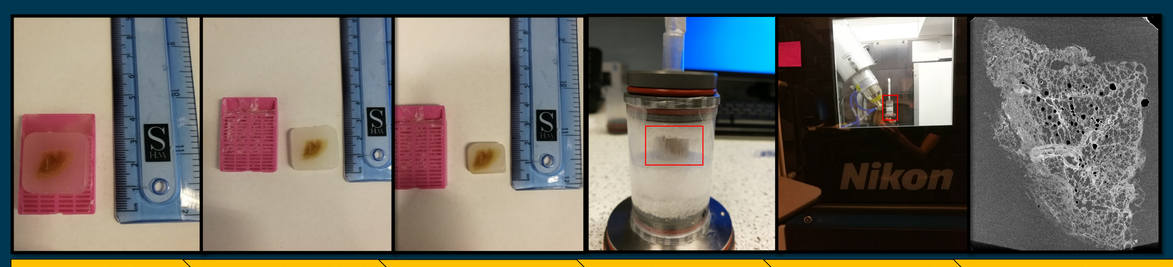
1. Background

- Microfocus computed tomography (µCT) is a non-destructive technique that provides the 3D micro-structure of human lung tissue in a resolution that is compatible with histology.
- Immunofluorescence provides high contrast images that identifies specific features (e.g. cytokeratin) on the same lung tissue in 2D.
- Staining serial tissue sections and correlating the fluorescence to the µCT could produce a method to segment features by thresholding.

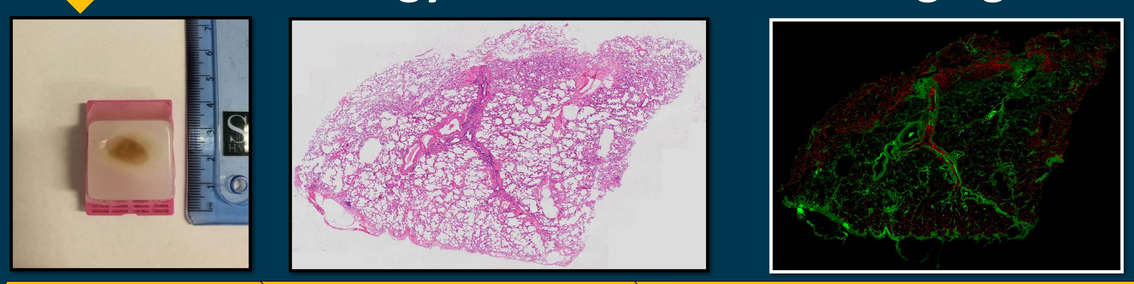
2. Methods

Sample preparation and µCT imaging



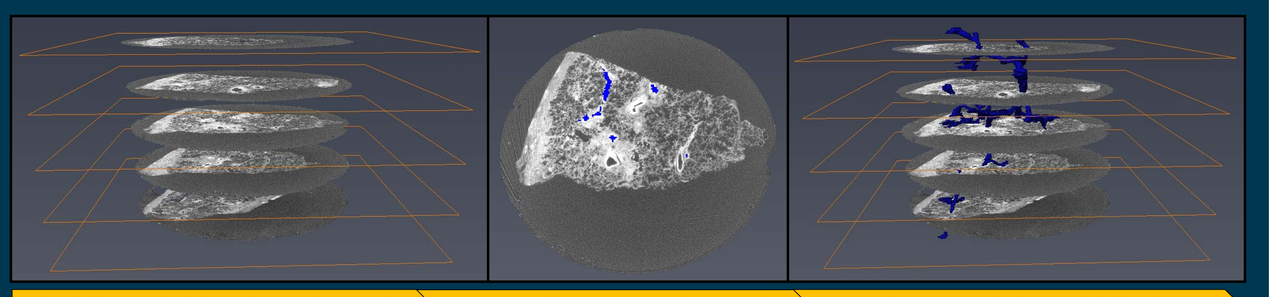
Formalin fixed paraffin embedded lung tissue → Wax tissue block is removed from the cassette → Excess wax is trimmed back around the tissue → Tissue block secured upright in holder → Tissue scanned at 8.5 µm resolution → Example of reconstructed µCT 'slice' of lung

Histology and fluorescence imaging



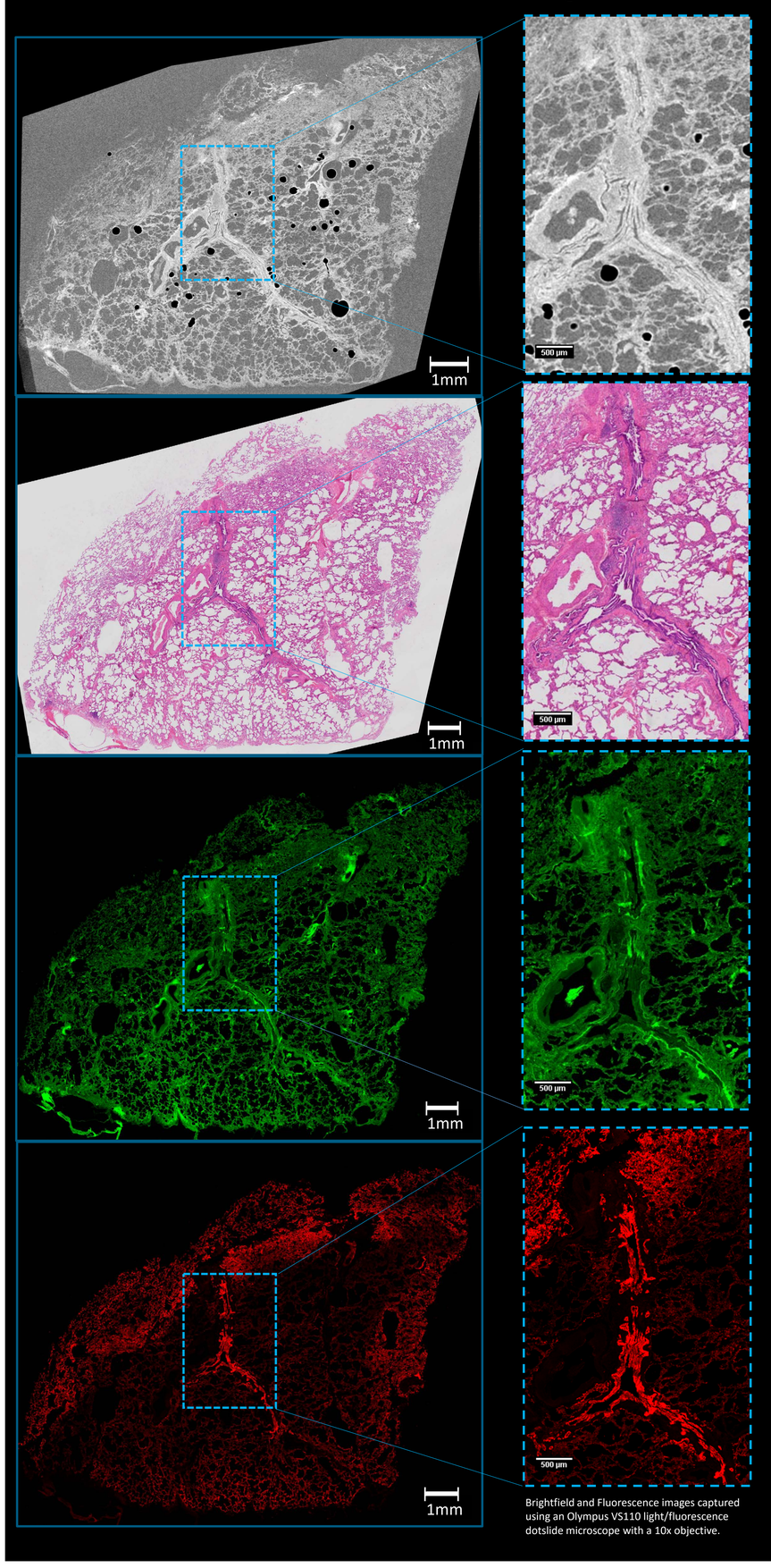
Tissue is re-embedded onto plastic cassette → Tissue is sectioned and stained with haematoxylin and eosin (H&E) OR… → Tissue sections stained for immunofluorescence and imaged at multiple wavelengths of light

Segmentation of tissue



µCT yields a stack of tissue images that together make a 3D volume → Immunofluorescence used to identify airway epithelium on each slice → The segmented airway epithelium is interpolated to produce a 3D network

3. Correlation of µCT and histology



Brightfield and Fluorescence images captured using an Olympus VS110 light/fluorescence dotslide microscope with a 10x objective.

µCT

- Reconstructed µCT slice represents tissue histology
- µCT used as reference to correct sectioning deformations

Histology

- H&E confirms lung microstructure seen by µCT
- Bigwarp plugin in ImageJ used to correlate histology to µCT

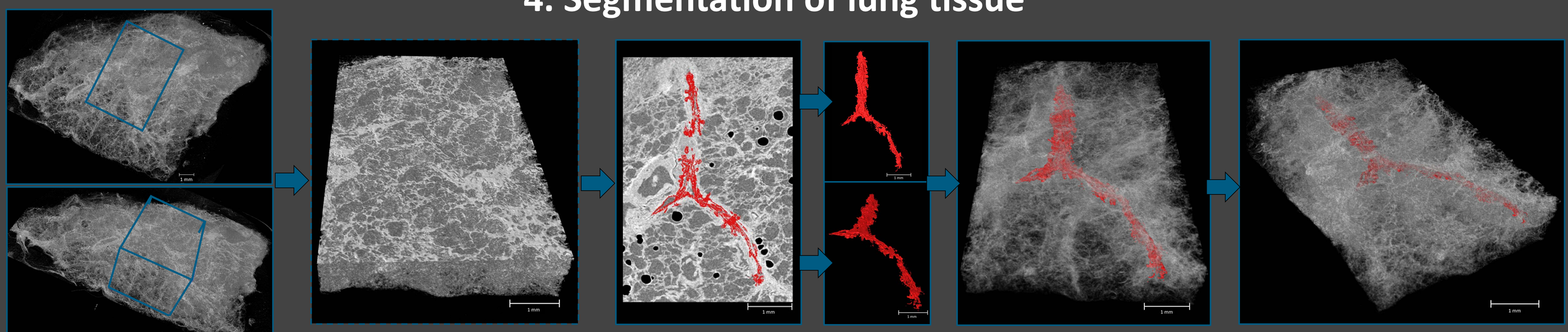
Autofluorescence

- Auto fluorescence used for correlation to the µCT
- High contrast images produced
- Provides structural information
- Correlates the µCT to the immunofluorescence

Immunofluorescence

- Cytokeratin 8/18 primary antibody (CY-90, Abcam, UK) staining
- Alexa Fluor® 647 secondary antibody (Fisher Scientific, UK)
- Airway epithelial cells specifically visualised
- High contrast allows the staining to be easily thresholded

4. Segmentation of lung tissue



- Whole lung 3D volume visualised in Amira 6.1.1 (FEI, USA)
- 3D volume around central airway taken as region of interest
- Visualised as 2D slices for segmentation
- Immunofluorescence is thresholded for staining only
- Thresholded image used to segment the epithelium on the µCT
- Segmentation between multiple fluorescence slices is interpolated
- 3D surface view of airway epithelium generated in Amira
- 3D epithelium localisation visualised in 3D lung volume
- Provides semi-automatic segmentation of features in the 3D dataset

5. Conclusions

- Lung volumes imaged by µCT can also be sectioned and imaged with immunofluorescence.
- Bigwarp plugin in ImageJ correlates immunofluorescence to the µCT.
- Immunofluorescence used for threshold based segmentation.
- Cytokeratin immunofluorescence used to localise & segment airway epithelium in 3D
- Interpolation between immunofluorescent sections is possible
- Potential to substantially speed up segmentation of tissue features

Acknowledgments

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