Spatially Resolved Whole Transcriptome Profiling of Fibroblastic Foci in Idiopathic Pulmonary Fibrosis

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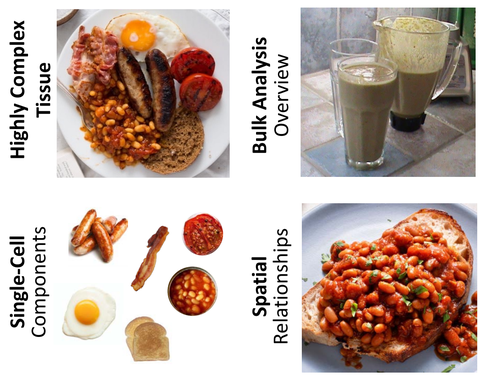
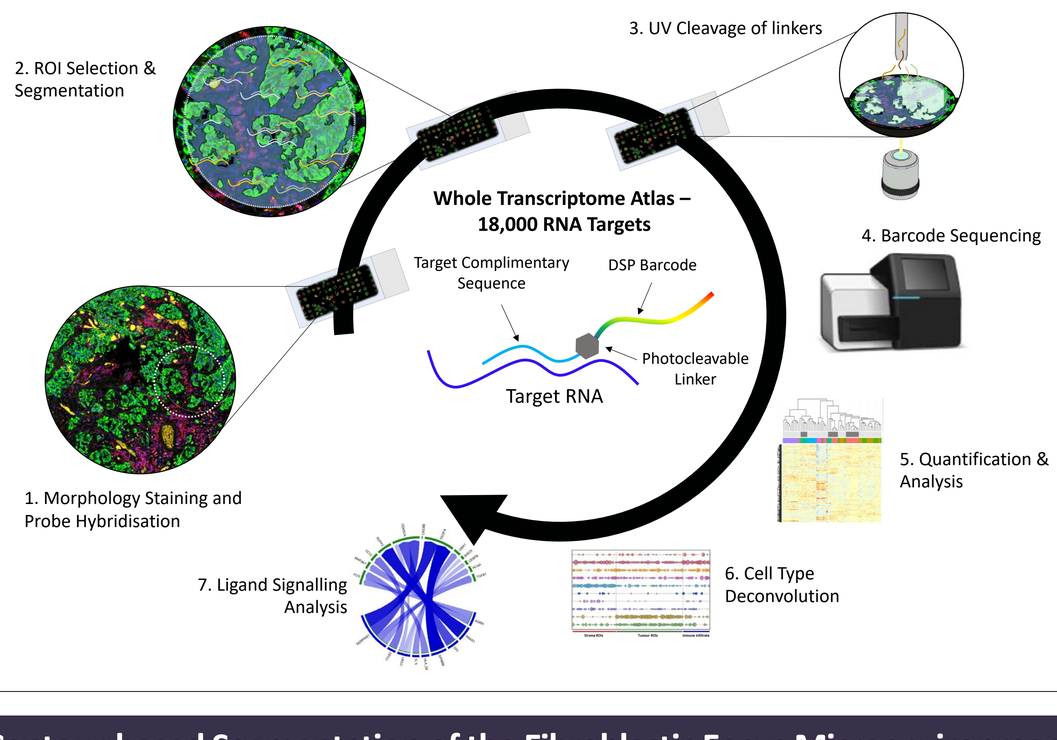
1. Whole Transcriptome Digital Spatial Profiling

Spatial Analysis - Tissues are not homogenous but are made of multiple discrete compartments. Bulk sequencing methods take a homogenized snapshot, resulting in a huge loss of complexity. Single-cell sequencing methods allow for the cataloguing of individual components within a tissue. However, it is only with spatial techniques that the relationships between these components can be analysed. GeoMx Digital Spatial Profiling (DSP) enables quantitative, non-destructive, whole transcriptome (WT) profiling in a single FFPE tissue section.



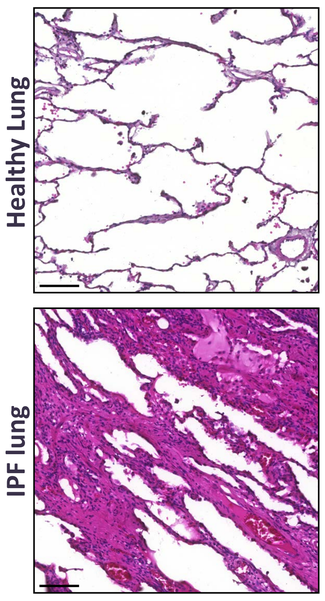


2. Idiopathic Pulmonary Fibrosis

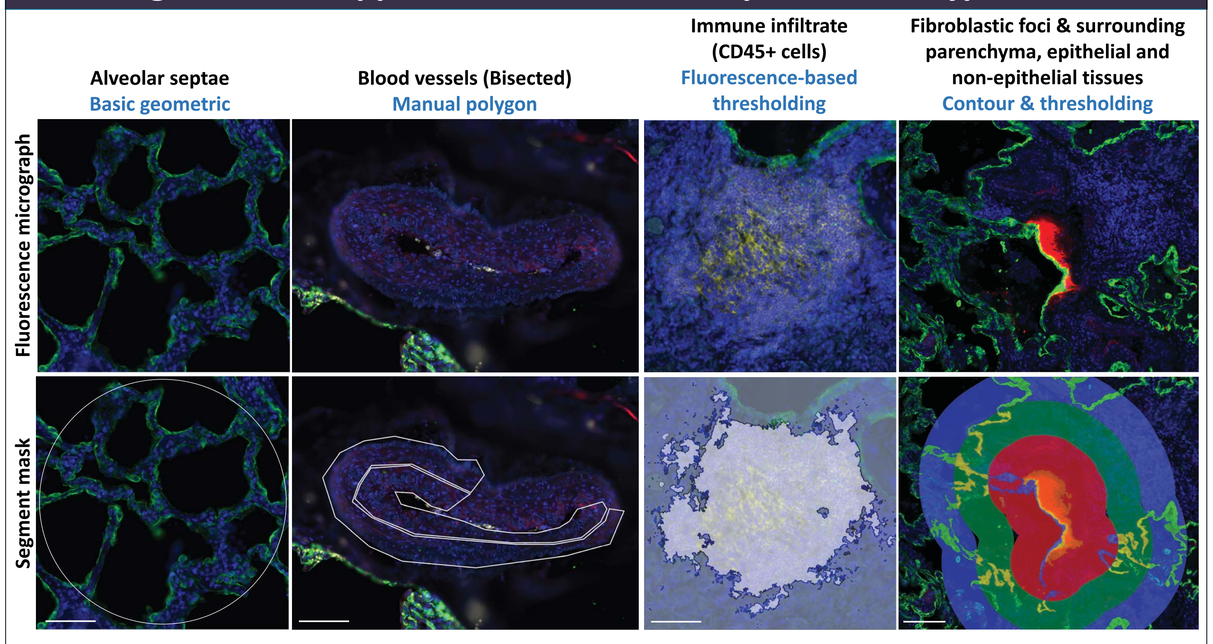
Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive lung disease, with an average life expectancy of 3-5 years following diagnosis.

A defining histopathological feature of IPF is localised tissue heterogeneity within the lung. Afflicted tissues present with discrete regions of fibrosis (fibroblastic foci) within the otherwise normal-appearing lung parenchyma. Regions surrounding fibroblastic foci exhibit aberrant cellular profiles and tissue architecture, while discrete aggregates of infiltrating immune cells are present close to (but often distinct from) foci.

Assaying IPF tissues with WT-DSP analysis enables these discrete regions to be profiled and analysed in the context of their native spatial niche, yielding more informative and biologically relevant transcriptomic data on the pathobiology of the disease than could be achieved with bulk methodologies.



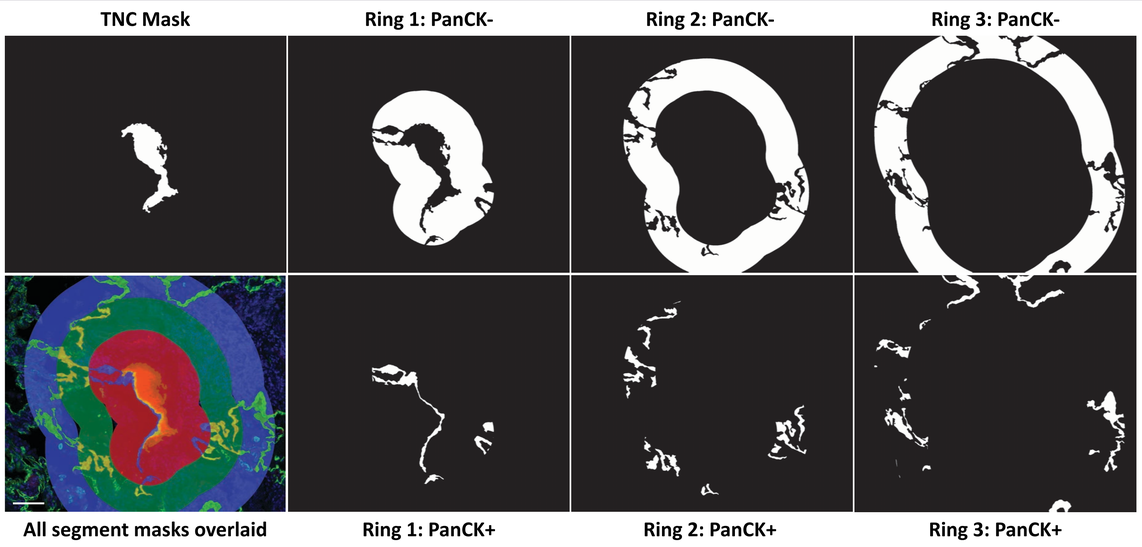
3. ROI Segmentation Approaches to Match the Spatial Phenotype of Tissues



Using the GeoMX-DSP platform, ROI segmentation methods were tailored to match the histological features of IPF tissue. To profile entire alveolar septae, where high spatial resolution segmentation is unnecessary, simple geometric shapes were utilised. To delineate the inner and outer regions of blood vessels, manual polygons were used to define segments. To selectively capture CD45+ (immune) cells within ROIs, tissues were immunostained with CD45-specific fluorescent probes, and staining intensity is used for signal-based thresholding. To segment fibroblastic foci and their surrounding tissues, a custom image processing algorithm (developed by MDC) was used to segment regions of the tissue based on protein expression (fluorescence signal) and distance from foci (further details panel 4).

DNA (nuclei), Pan-cytokeratin (epithelial cells), CD45+ (immune cells), TNC (fibroblastic foci). Scale bar = 100µm.

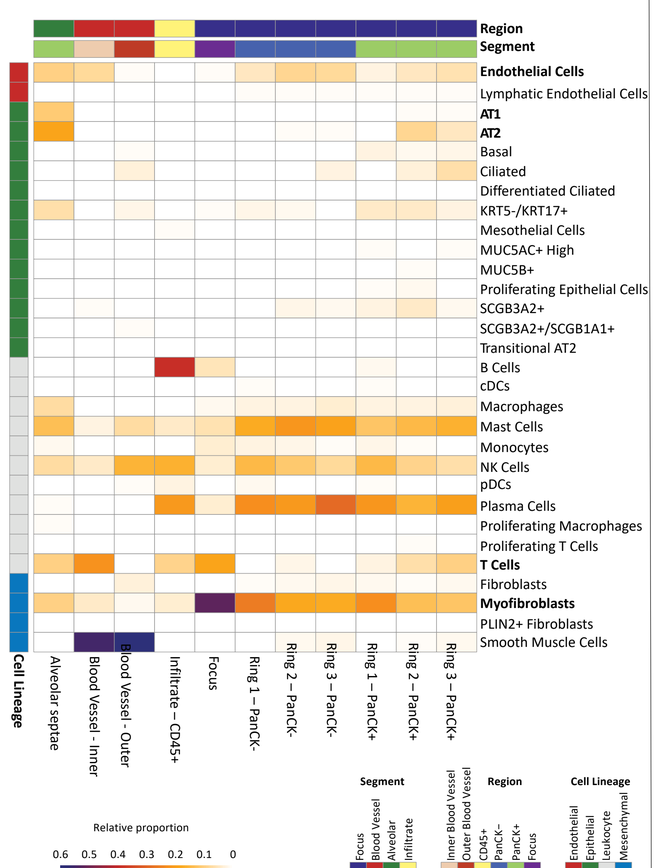
4. Contour-based Segmentation of the Fibroblastic Focus Microenvironment



The complex histological features of fibroblastic foci and their surrounding tissues was deconvolved with a bespoke ROI segmentation method. Fibroblastic foci were first identified through immunostaining with a tenascin C (TNC) specific antibody. The TNC mask was then sequentially dilated to create concentric masks expanding radially from the foci ("rings"). Concentric ring masks were subsequently segmented based on pan-cytokeratin (PanCK) immunostaining to segregate the epithelial and non-epithelial regions for each ring. This approach allowed WT data to be analysed in the context of proximity to foci. Scale bar = 90µm.

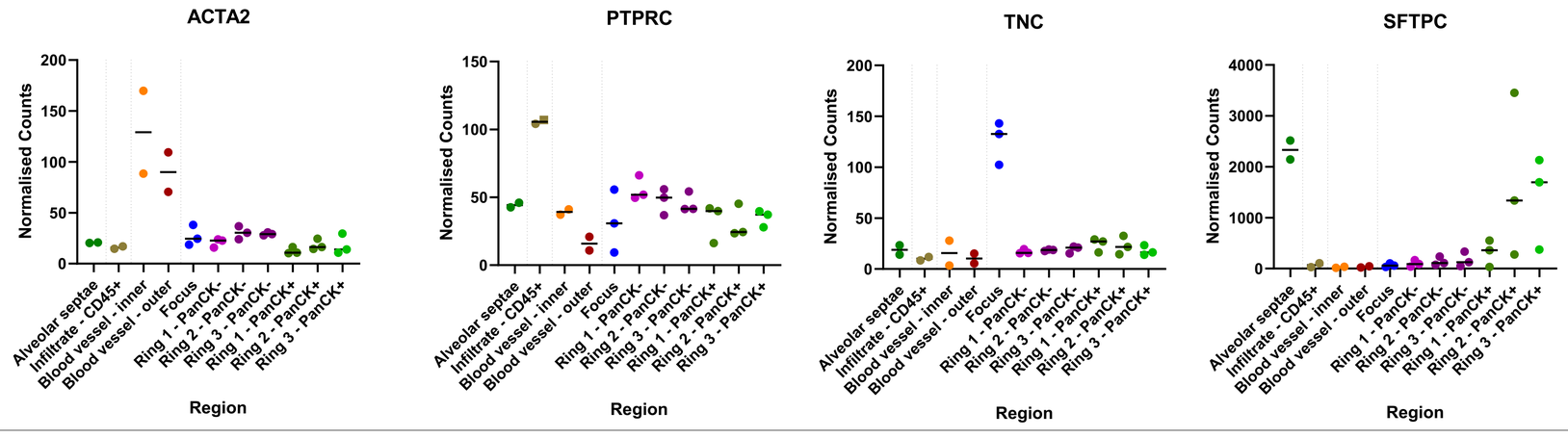
5. Spatial cellular and gene expression signatures identified in IPF tissues

Analysis of whole transcriptome profiling data revealed expression of individual genes displays segment-specific enrichment. Expression of SFTPC (surfactant protein C) displays a proximity-to-focus dependent relationship in epithelial tissues.



A publicly available single-cell RNA-seq dataset was used to generate a transcriptional profile of the different cell types present in lung tissue. This was then used to estimate the proportion of each cell type within each segment.

Cell type deconvolution identified several cell types that display a proximity-to-focus dependent relationship in focus and concentric ring segments, such as T-cells (panCK+ segments only) and myofibroblasts. In non-focus ROIs, segment-specific enrichment of key cell types validated the approach, e.g. the enrichment of endothelial cells in the inner (luminal) vs outer (distal) halves of blood vessels and alveolar type I & II (AT1 & AT2) in the alveolar septae.



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