B890: Three-Dimensional (3D) Spatial Reconstruction of Murine FFPE tissues with an Ultrahigh-plex Antibody Panel for Deep Phenotyping of Tissue Form and Function

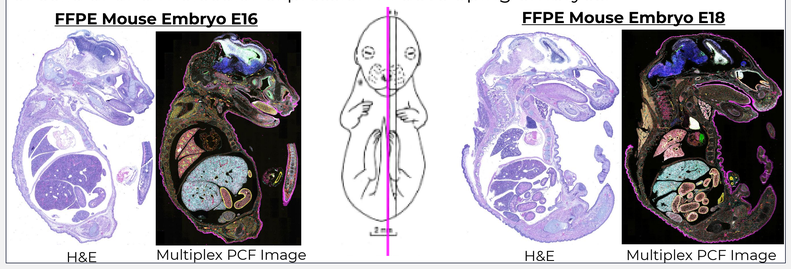
Tsz H. Tam[1], Bassem Ben Cheikh[1], Nadezhda Nikulina[1], Dmytro Klymyshyn[1], Ning Ma[1], James DeRosa[1], Niyati Jhaveri[1]

[1]Akoya Biosciences, Inc., Marlborough, Massachusetts, USA

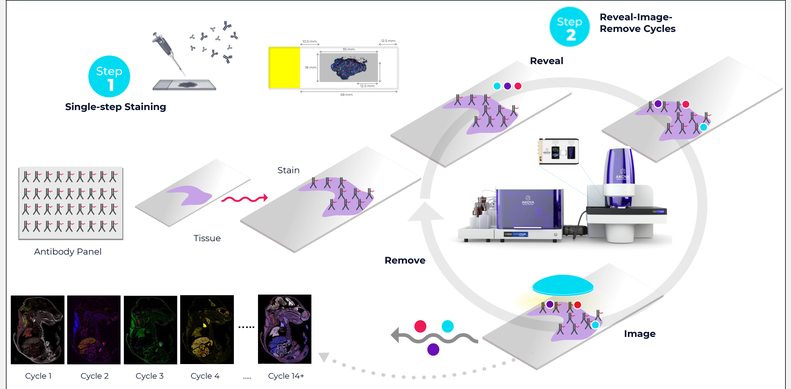
1. Murine Embryo Model in Developmental Biology

The murine embryo model is one of the most common animal models for studying developmental biology. Embryonic development is a highly coordinated process with spatial and temporal expression of proteins for regional specialization and tissue-specific functions. Application of spatial proteomics to developing embryos enables us to understand cellular organization and interactions within tissues along with regulatory mechanisms at the single-cell level.

In this study, we applied a 27-plex antibody panel, specifically designed for analysis of murine embryos at different stages (**E16** and **E18**) of development using the PhenoCycler® -Fusion (PCF) 2.0 Platform. This panel was developed by selecting biomarkers for immune cell lineages, immune activation, proliferation, structural and neuronal function. The panel developed as part of this study can be used to conduct in-depth research on various aspects of organogenesis and to study the spatio-temporal dynamics of cellular and molecular expression in developing embryos.



2. The PhenoCycler-Fusion 2.0 Technology



The PhenoCycler®-Fusion (PCF) 2.0 technology is the fastest whole-slide spatial biology platform that enables simultaneous detection of 100+ biomarkers by combining fully automated fluidics and iterative imaging of oligo-conjugated antibodies. Leveraging the high-plex, single-cell resolution and throughput of PCF, our study was able to identify the **spatio-temporal changes** in different developmental stages of the entire murine embryo (E16 and E18).

The 27-plex panel for murine embryo is shown below:

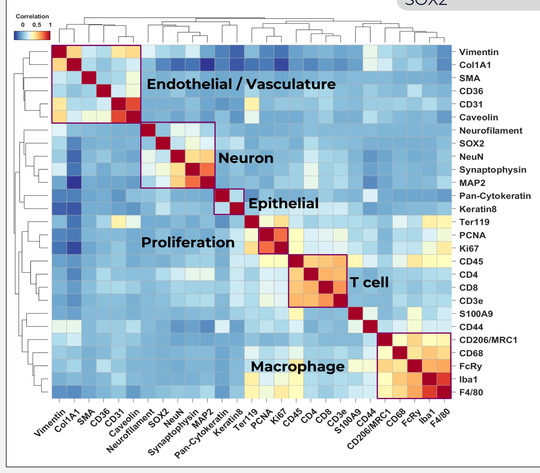
Immuno-Phenotyping Markers
CD45, CD44, CD3ε, CD8, CD4, Ter119

Structural Markers:
Vimentin, Col1A1, CD36, CD31, Caveolin-1, Keratin8, Pan-Cytokeratin, αSMA

Proliferation Markers
Ki67, PCNA

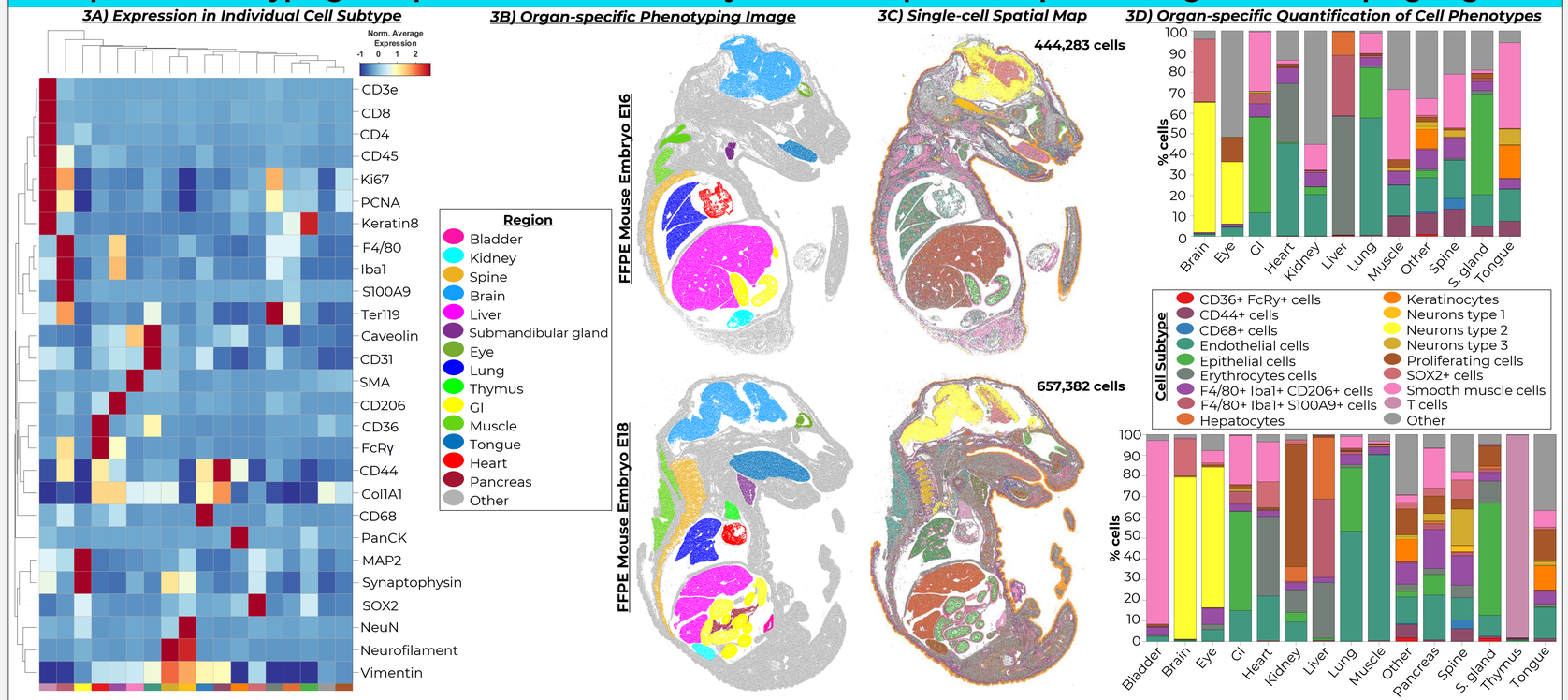
Myeloid Markers:
F4/80, CD68, Iba1, CD206, S100A9, FcRγ

Neuronal Function Markers:
NeuN, MAP2, Synaptophysin, Neurofilament, SOX2



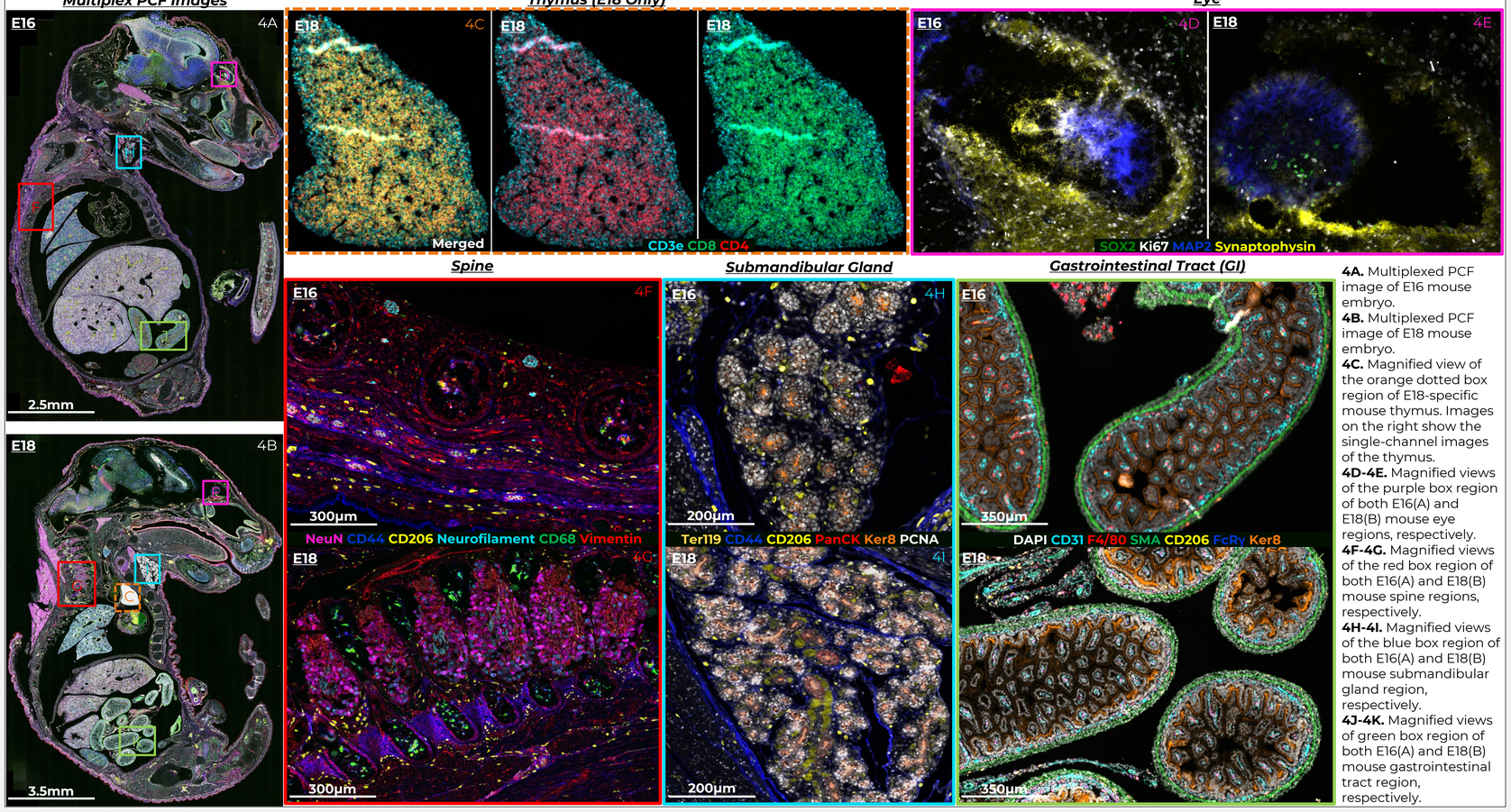
Marker-Correlation Matrix of 27 markers showing close association of markers for endothelial/vascular, neuronal, epithelial and immune subsets in distinct matrix domains.

3. Spatial Phenotyping Comparison of Murine Embryos Reveals Spatio-Temporal Changes in Developing Organs



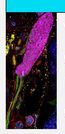
3A) Heatmap with unbiased Leiden clustering showing cellular phenotypes annotated based on the expression of immune cell lineage, immune activation, proliferation, structural and neuronal markers (see legend *Cell Subtype* on the right).
3B) Organ-specific Phenotyping Images of both stage E16 (top panel) and E18(bottom panel) embryos, highlighting individual regions represented by the corresponding organs.
3C) Single-Cell Spatial Maps (with accompanying bar charts **(3D)**) illustrate the difference in the percentage of cells and organization of the 18 distinct cellular phenotypes across 15 unique organ. Here, the organ submandibular gland is abbreviated as "S. gland".

4. Spatial Phenotyping of Murine Embryo Reveals High Degree of Cellular Heterogeneity Expressed Within Organs



4A. Multiplexed PCF image of E16 mouse embryo.
4B. Multiplexed PCF image of E18 mouse embryo.
4C. Magnified view of the orange dotted box region of E18-specific mouse thymus. Images on the right show the single-channel images of the thymus.
4D-4E. Magnified views of the purple box region of both E16(A) and E18(B) mouse eye regions, respectively.
4F-4G. Magnified views of the red box region of both E16(A) and E18(B) mouse spine regions, respectively.
4H-4I. Magnified views of the blue box region of both E16(A) and E18(B) mouse submandibular gland region, respectively.
4J-4K. Magnified views of green box region of both E16(A) and E18(B) mouse gastrointestinal tract region, respectively.

5. Value of Ultrahigh-Plex Spatial Phenotyping for Studying Murine Developmental Process



We present the application of a comprehensive high-plex panel including markers from immune cell lineages, immune activation, proliferation, structural and neuronal function in different developing stages of the murine embryos. Deployment of this panel allowed us to gain new insights of region-specific, spatial distribution in a highly coordinated and rapidly growing microenvironment. The PCF 2.0 platform is uniquely suited for parallel temporal comparison studies by leveraging high throughput, high resolution, ultrahigh-plex scale and unparalleled speed.

For Research Use Only