3877: Deep Ultra High-Plex Spatial Phenotyping of Human Cancer Tissues

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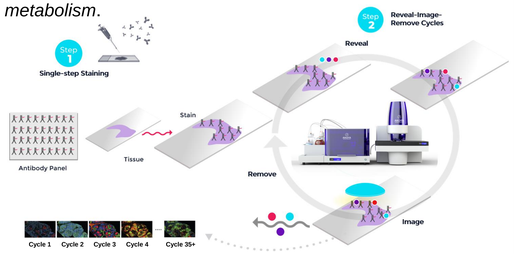
1. Rapid and Deep Spatial Phenotyping

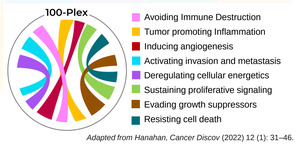
Single-cell spatial phenotyping has transformed cancer research and is poised to play formative roles in the development of effective therapeutic strategies. Here, we present ultrahigh-plex single-cell spatial phenotyping of whole-slide human FFPE tissues with 100+ protein biomarkers encompassing *immune cell lineage*, *activation states*, *immune checkpoints*, *tissue structure*, *apoptosis*, *DNA damage* & *metabolism*.



Deployment of our 103-plex deep spatial phenotyping panel occurs with rapid turn-around time on the PhenoCycler™-Fusion, an automated spatial biology platform for ultrahigh-plex imaging. The PhenoCycler (formerly CODEX®) integrates seamlessly with the Fusion for end-to-end spatial imaging at high resolution and scale.

2. Design and Development of 103-plex Panel

Immune Cell Profiling Core	*Advanced Lymphocyte Module*	*Advanced Myeloid Module*	*Structural Module*	*Metabolism*	*Stress & Death*	*Advanced Immune Module*
CD4	CD107a	CD163	E-cadherin	ASCT2	PARP	HLA-DPB1
CD68	CD21	CD11b	CD31	Na/K ATPase	BAK	LEF1
CD20	Pax5	MPO	Histone H3	HK1	BCL-XL	GAL9
CD11c	FOXP3	CD141	β-actin	LDHA	BAX	CD138
CD8	Gran B	iNOS	Podoplanin	G6PD	BAD	MC Tryptase
HLA-DR	CD38	*Immune Activation Module*	SMA	IDH2	Cyt. c	OX40
Ki67	CD39	PD-1	Vimentin	GLUT1	LC3B	ZAP70
CD45RO	CD79α	PD-L1	Collagen IV	pNRF2	Beclin-1	CD7
PanCK	TIGIT	ICOS	CD34	ATPA5	H2AX	C1Qα
CD3ε	*Tissue Specific/ Custom*	TIM3	β-catenin	SDHA	*Proliferation*	CCR6
CD44	TP63	LAG3	MMP-9	Cit. Syn	pRPS6	CD15
CD45	S100A4	IDO1	ZEB1	CPT1A	PCNA	AXL
HLA-A	TFAM	CD40			Cyc D1	CD227
CD14	GP100	HLA-E				
CD56	LaminB1	IFNG				
CD57	CK17	CD69				
CD19	CK19					
CD2	GATA3					
CD1a	SOX2					
	EpCAM					
	Caveolin					

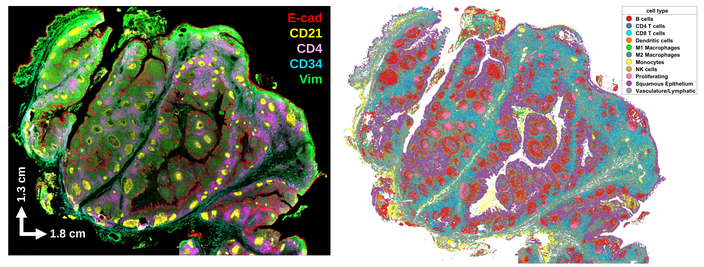


Adapted from Hanahan, Cancer Discov (2022) 12 (1): 31–46.

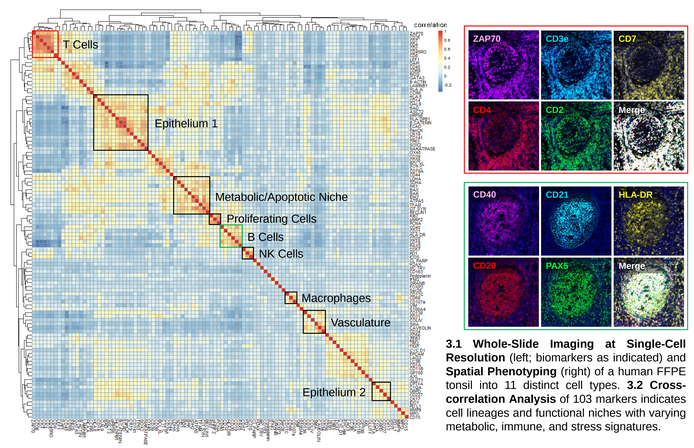
The design of the 103-plex antibody panel is based on the **hallmarks of cancer.** The panel includes markers for cell lineage, immune activation and checkpoints, cellular energetics, mediators of proliferation, metastasis and stress responses, and more. Each marker and each module has been carefully selected to reveal unique information on different pathways and, when multiplexed together, provides an integrated overview of the landscape of cancer progression

3. Largest Single-cell Spatial Phenotyping Dataset from a Single Sample

3.1 Deploying a 103-plex Antibody Panel in FFPE Tissue

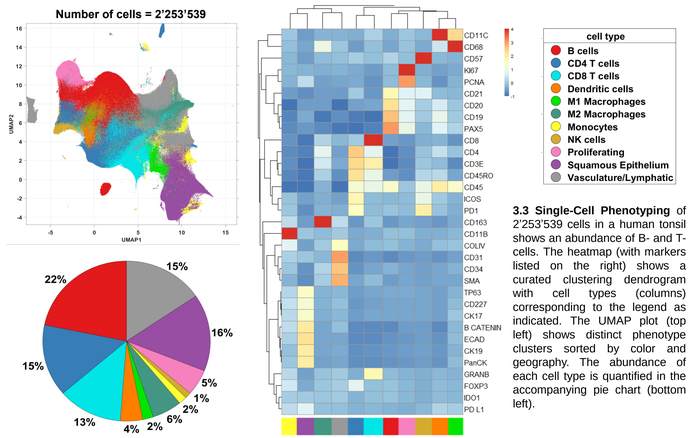


3.2 Cross-Correlational Analysis of 103 Markers Across the Entire Sample



3.1 Whole-Slide Imaging at Single-Cell Resolution (left; biomarkers as indicated) and **Spatial Phenotyping** (right) of a human FFPE tonsil into 11 distinct cell types. **3.2 Cross-correlation Analysis** of 103 markers indicates cell lineages and functional niches with varying metabolic, immune, and stress signatures.

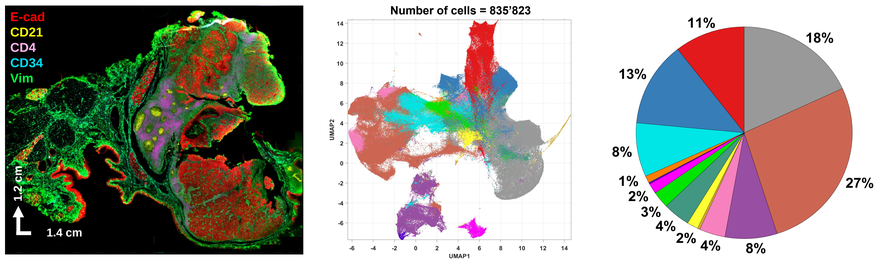
3.3 Single-Cell Phenotypic Characterization of 2.25 Million Cells



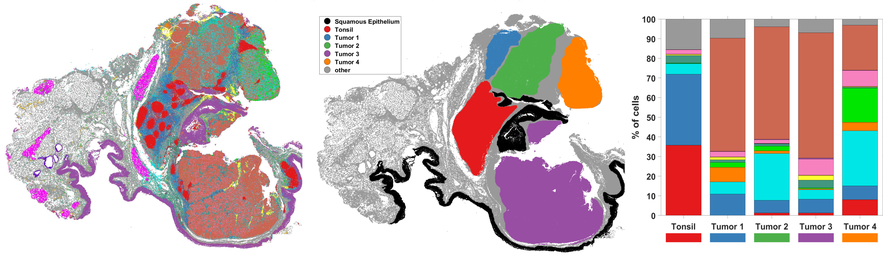
3.3 Single-Cell Phenotyping of 2'253'539 cells in a human tonsil shows an abundance of B- and T-cells. The heatmap (with markers listed on the right) shows a curated clustering dendrogram with cell types (columns) corresponding to the legend as indicated. The UMAP plot (top left) shows distinct phenotype clusters sorted by color and geography. The abundance of each cell type is quantified in the accompanying pie chart (bottom left).

4. Ultra High-Plex Single-Cell Spatial Phenotyping of Human FFPE Oropharyngeal Squamous Cell Carcinoma

4.1 Single-Cell Spatial Phenotyping of Human Oropharyngeal Squamous Cell Carcinoma reveals 14 Distinct Cell Types

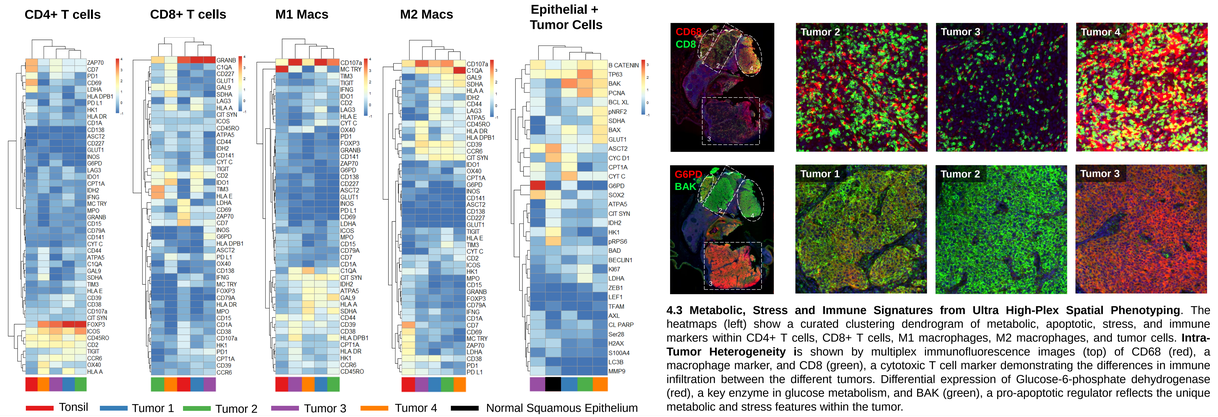


4.2 Whole-Slide Spatial Phenotyping reveals Complex Topography across the Tumor



4.1 Whole Slide Imaging at Single-Cell Resolution of a FFPE human head and neck tumor (left; biomarkers as indicated) and **Spatial Phenotyping** (right) of all 835'823 cells into 14 distinct cell types. The heatmap (far right) includes a curated clustering dendrogram with cell types corresponding to the legend as indicated below it. The UMAP plot and the pie chart show the abundance of distinct phenotype clusters sorted by color and geography. **4.2 Deep Spatial Phenotyping** shows the complex topography and disruption of normal tonsil architecture in tumor tissue (scatter plot; far left). Further analysis reveals 4 distinct tumors within the tissue (shown here in blue, purple, green and orange) with varying abundance of immune, proliferating, epithelial, and vascular cell types compared to the normal lymphoid area (red) and the normal squamous epithelium (black). The accompanying bar chart demonstrates the intra-tumor heterogeneity and highlights the importance of single-cell spatial phenotyping to understand the complexities and nuances of tumor progression.

4.3 The 103-Plex "Cancer Hallmark" Panel reveals Distinct Immune, Metabolic, and Stress Signatures reflecting Intra-tumor Heterogeneity



4.3 Metabolic, Stress and Immune Signatures from Ultra High-Plex Spatial Phenotyping. The heatmaps (left) show a curated clustering dendrogram of metabolic, apoptotic, stress, and immune markers within CD4+ T cells, CD8+ T cells, M1 macrophages, M2 macrophages, and tumor cells. **Intra-Tumor Heterogeneity** is shown by multiplex immunofluorescence images (top) of CD68 (red), a macrophage marker, and CD8 (green), a cytotoxic T cell marker demonstrating the differences in immune infiltration between the different tumors. Differential expression of Glucose-6-phosphate dehydrogenase (red), a key enzyme in glucose metabolism, and BAK (green), a pro-apoptotic regulator reflects the unique metabolic and stress features within the tumor.

5. The Need for Deep, Unbiased Spatial Analysis at Scale

This study demonstrates the power of rapid, deep single-cell spatial phenotyping enabled by the PhenoCycler-Fusion system. We present a meticulously designed, ultra high-plex antibody panel to decipher critical mechanistic insights into tumor biology. Whole-slide, single-cell spatial phenotyping uncovers the intricacies of intra-tumor heterogeneity and can aid in revealing mechanisms underlying clinical response and therapeutic resistance.