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Cell Segmentation-Free Analysis of Multiplexed Images with Unbiased Spatial Analytics and Explainable AI for Predicting Disease Outcomes

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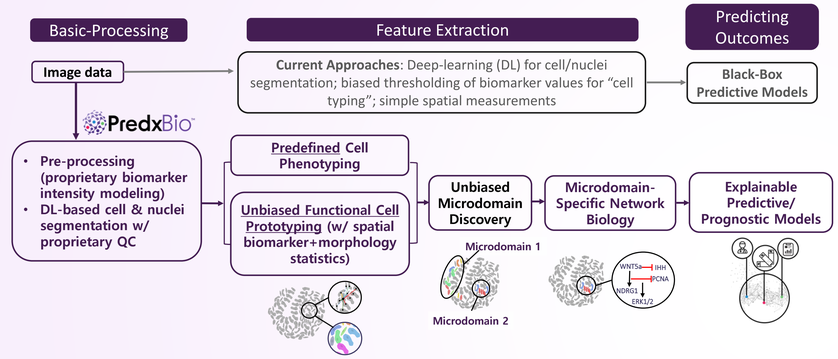
Summary

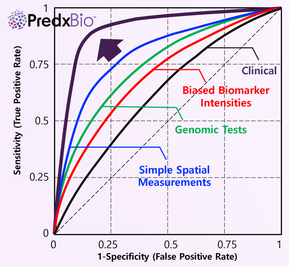
Background: The spatial intratumor heterogeneity (ITH) is widely acknowledged as driving therapeutic response and providing fuel for drug resistance [1,2]. Currently, patient selection for immunotherapy is driven mostly by PD-1/PD-L1 based IHC tests and mutational analysis. These oversimplified approaches fail to predict the risk of recurrence, therapeutic response and drug resistance with high accuracy. We hypothesize that functional responses of heterogeneous non-random spatial arrangements of tumor, stromal and immune cells in the tumor microenvironment are determined by distinct combinations of their internal states and spatial interactions within neighborhoods. This approach discovers functional collections of neighboring pixels highly predictive of disease progression and leads to tumor promoting and tumor restraining microdomains, as organizational units of spatial ITH, and microdomain-specific network biology predictive of disease outcomes [3-6]. Deriving the spatial networks within each microdomain with unbiased spatial analytics and the underlying network biology through explainable AI, is key to understanding tumor initiation, tumor progression, and response to therapy.

Problem: There has been an explosion of spatial imaging technologies using immunofluorescence and/or mass spectrometry for intact tissues measuring protein expressions, DNA and RNA probes. The first step in the current approaches for extracting high-value knowledge from these multiplexed datasets is to segment cells accurately. Despite decades of research, this step remains elusive due to imaging artifacts leading to low-quality cell segmentation. Those artifacts may lead to incorrect cell phenotypes, incomplete cell phenotype atlases, and missing rare cell, fusion cell and/or transition cell types.

Solution: To address these issues, we present a cell segmentation-free approach on a challenging, low-resolution hyperplexed imaging mass cytometry dataset of triple negative breast cancer [7]. This approach discovers functional collections of neighboring pixels highly predictive of disease progression and leads to tumor promoting and tumor restraining microdomains and microdomain-specific network biology predictive of disease outcomes

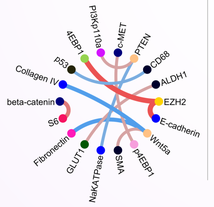
Unbiased Spatial Analytics and Explainable AI [3-6]

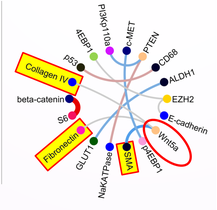




93% Accuracy in predicting CRC progression

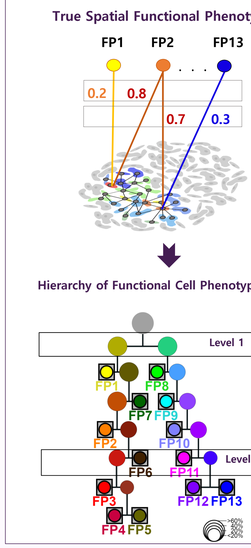
400+ patient cohort
5-year risk-recurrence



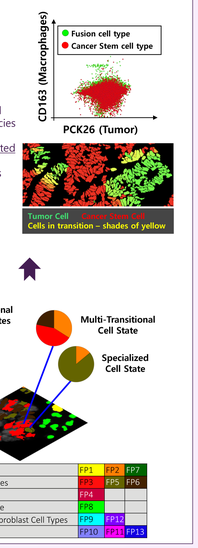


Unlike the Biased Intensity Thresholding Approaches

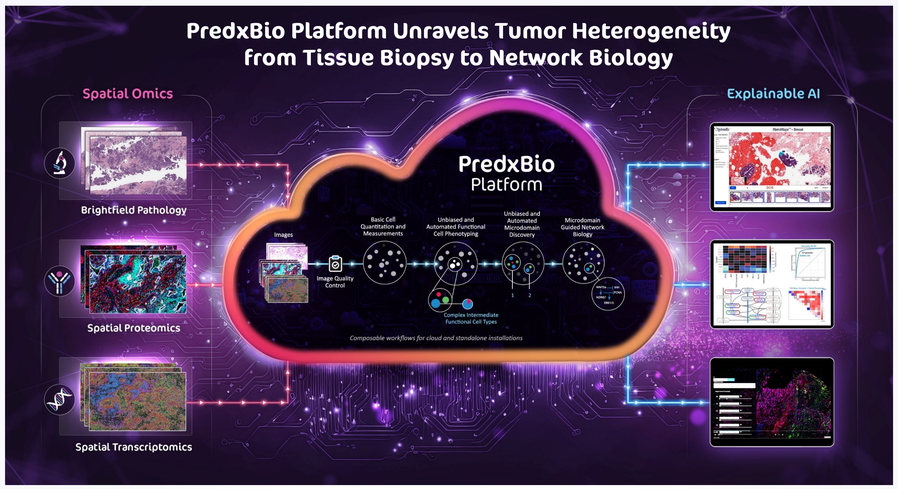
Unbiased and Automated Functional Cell Phenotyping Discovers Transitional, Multi-Transitional Cell States and Fusion Cell Types Critical to Disease Progression



- Heterogeneity in biomarker expression causes subjective and biased thresholding methods to fail
- Transition cell states emerge with statistical and spatial dependencies
- Unbiased and automated functional cell phenotyping identifies fusion cell types and transitional cell states



Tumor Cell Types	FP1	FP2	FP7
Cancer Stem Cell Types	FP3	FP5	FP6
Fusion Cell Type	FP4		
Macrophage Cell Type	FP8		
Cancer Associated Fibroblast Cell Types	FP9	FP12	
Immune Cell Types	FP10	FP11	FP13



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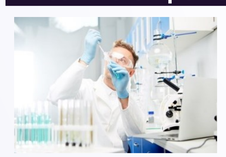


Accelerated Drug Discovery

Optimized Clinical Trials

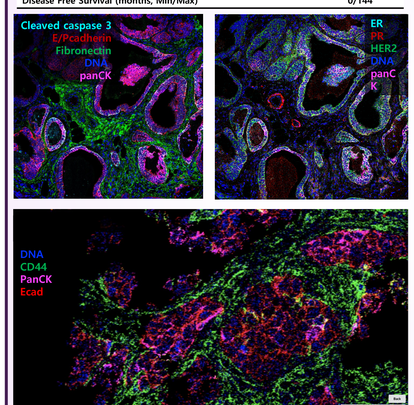
Advanced Companion Diagnostics

Personalized Therapeutic Options

Bending the survival curve for immunotherapy cancer patients with spatially intelligent biology

Cell Segmentation-Free Analysis of Low-Resolution Hyperplexed Imaging Mass Cytometry Dataset of Triple Negative Breast Cancer

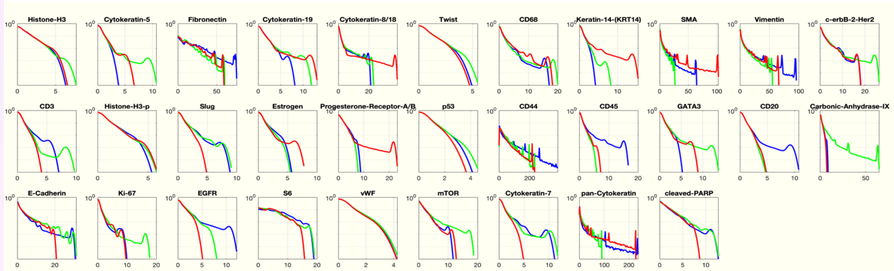
Triple Negative Breast Cancer	
Number	43
Age (years, Mean +/- SD)	62 +/- 17
Age (years, Min/Max)	26/90
Disease Free Survival (months, Mean +/- SD)	38 +/- 37
Disease Free Survival (months, Min/Max)	0/144



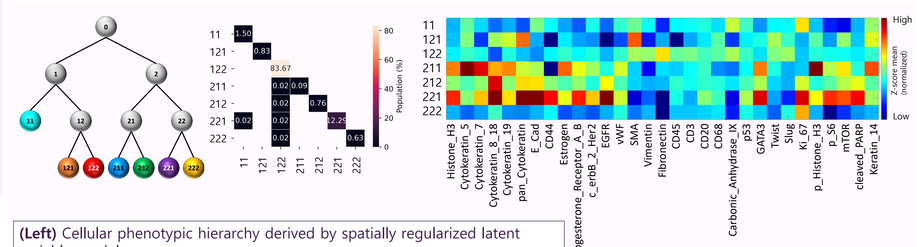
Antigen	Description
Cytokeratins (CK)	
CK5	Basal CK
CK7, CK8/18, CK14, CK19	Luminal CKs
AE1/AE3	Pan-CK
Adhesion Molecules	
E/P-Cadherin	Cell Adhesion
CD44	Cell Adhesion
Hormone Receptors	
ER	Estrogen Receptor
PR	Progesterone Receptor
RTK Signaling	
c-erB-Her2	RTK
EGFR	RTK
Endothelial	
vWF	Endothelial Cells
Mesenchymal Markers	
SMA	Myoepithelial
Vimentin	Myoepithelial
Fibronectin	Matrix Glycoprotein

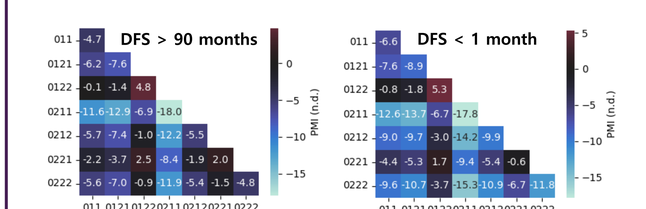
Antigen	Description
Immune Context	
CD45	Pan-Immune
CD3	T-Cells
CD20	B-Cells
CD68	Macrophages
Hypoxia and Cell Death	
Carbonic-Anhydras e-IX	Carbonic Anhydrase
Cleaved-PARP	Apoptosis
Nuclei	
Histone H3	Chromatin
Transcription Factors (TF)	
p53	Tumor Suppressor
GATA3	Luminal TF
Twist	EMT TF
Slug	EMT TF
Cell Growth and Division	
Ki67	Cell Proliferation
p-Histone H3	Mitosis
P-S6	Growth
P-mTOR	Growth

Triple Negative Breast Cancer Study: 43 patients, 31 Abs, imaging mass cytometry [7]



Biomarker histograms highlight the tissue heterogeneity in the TNBC patient cohort.
For illustration we show the sub-cohorts in red (DFS > 7 years), blue (DFS < 1 months) and green (19 < DSF < 39 months).





PMI maps are computed for each tissue sample to quantify the spatial co-occurrence of each pair in the phenotypic hierarchy in relation to a random background distribution to highlight the spatial tissue heterogeneity in the TNBC cohort.

(Left) Cellular phenotypic hierarchy derived by spatially regularized latent variable model.
(Middle) Lower triangular matrix depicting the number of cells with shared ownership probabilities between each pair of FPs.
(Right) Z-Score weighted signatures for the phenotypic hierarchy.

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Publications