

# Cellular decomposition and metabolic profiling of the tumor microenvironment tumor-stroma interface using high-dimensional spatial proteomics and image analytics (Conference Abstract 2961)

Arutha Kulasinghe<sup>1</sup>, Felicia Roland<sup>2</sup>, Michelle Poulin<sup>2</sup>, Ritu Mihani<sup>2</sup>, Katherine Hales<sup>3</sup>, Daniel Winkowski<sup>3</sup>

(1) Frazer Institute, University of Queensland (2) Akoya Biosciences, a Quanterix Company, Marlborough, MA, (3) Visiopharm Corp., Broomfield, CO.



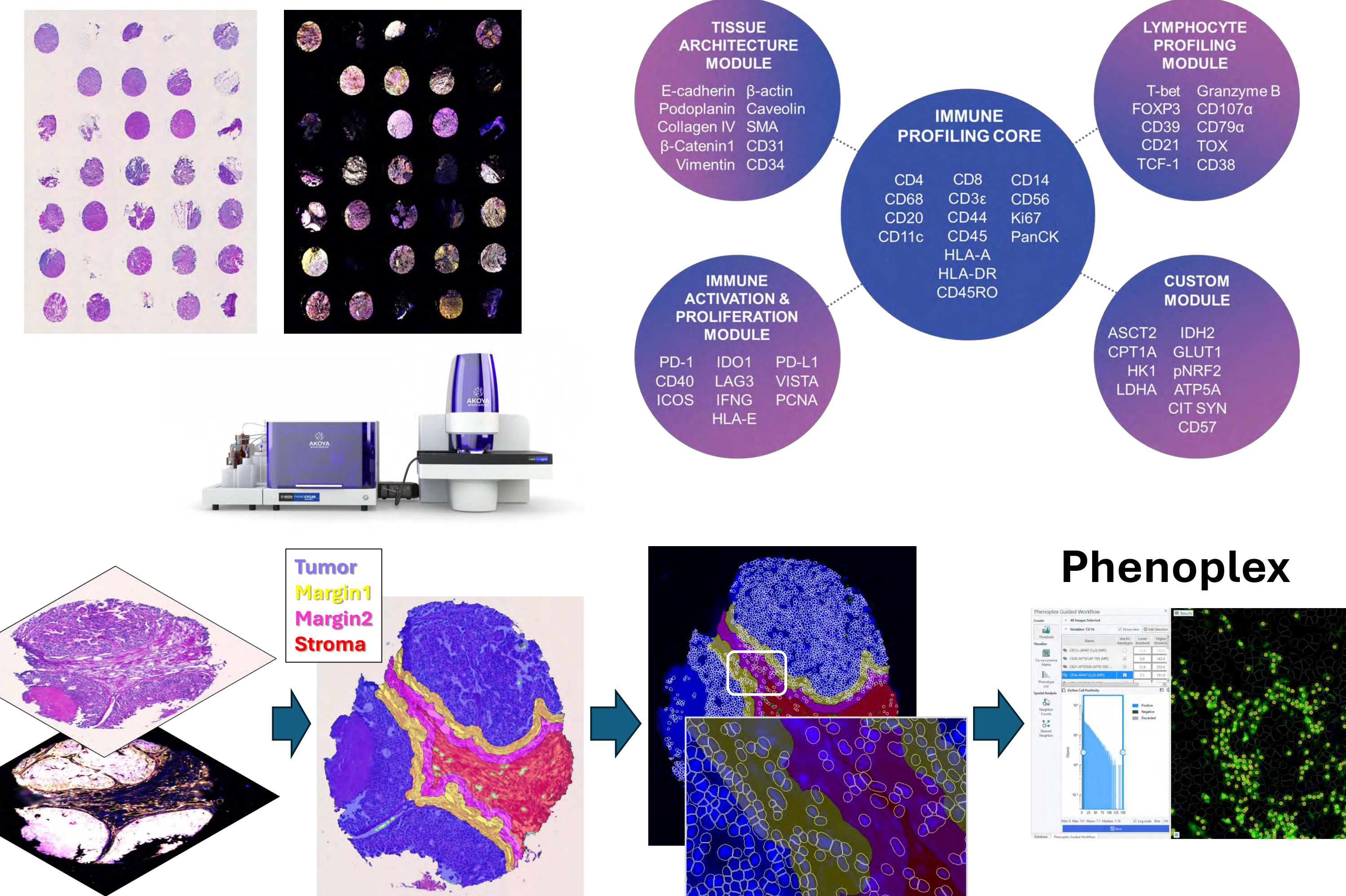
## Background

High-dimensional spatial profiling of the tumour microenvironment (TME) represents a critical frontier in understanding the biological determinants of therapy response and resistance across solid cancers. Current single-marker approaches, such as standard immunohistochemistry, are inadequate to capture the cellular complexity, spatial organisation, and functional heterogeneity that collectively govern tumour behaviour and immune evasion. There is therefore a pressing need to deploy comprehensive, discovery-based spatial proteomic platforms capable of simultaneously resolving multiple cellular and molecular programmes within intact tissue architecture.

In this study, we sought to address this gap by comprehensively characterising the TME in triple negative breast cancer (TNBC), one of the most aggressive and immunologically complex breast cancer subtypes. We applied a 68-plex spatial proteomic panel encompassing structural, tumour-immune, functional, and metabolic activity markers and the PhenoCyler-Fusion 2.0 workflow (Quanterix) to map the full biological landscape of the TNBC microenvironment at high resolution. Advanced image analytics (Visiopharm) were employed to precisely delineate tumour, stromal, and tumour-stromal interface regions, enabling compartment-specific analysis of cellular composition, spatial organisation, and protein expression. The study profiled a tissue microarray (TMA) comprising single-core biopsies from a clinically annotated cohort of TNBC patients, providing a spatially resolved, multi-dimensional portrait of the TME across this patient population.

## Methods

CoRegistration → Map Tissue → Segment Cells → Phenotype



## Results – Compositional differences between Primary and Metastatic TNBC

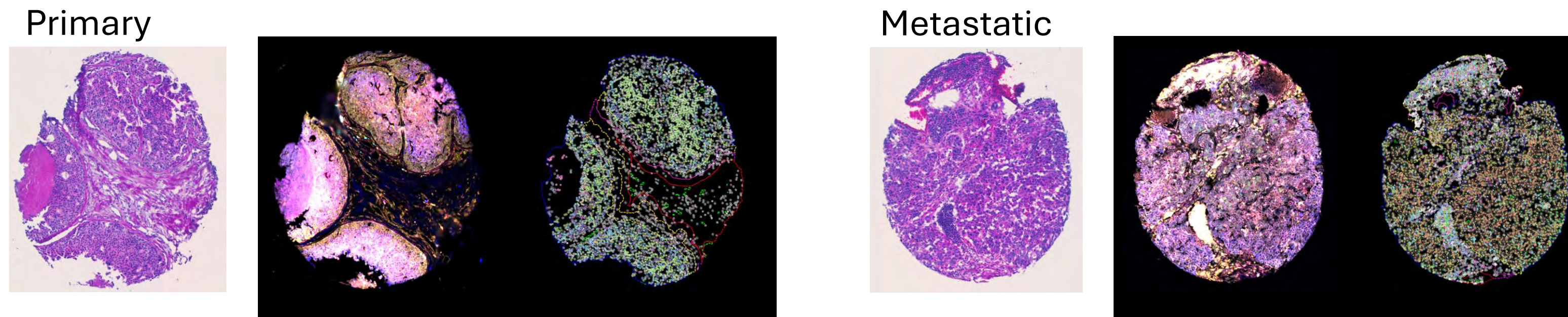


Figure 1. H&E and representative mIF cores of the Tissue Microarray

Total	22894	22662	4045	34243	27712	37471	12276	20454	10376	6984	16242	4949	2090	6693	42749	
BCLXL-AF750 (AF 750) (mIF)	22894	762	2217	1559	4507	2146	314	2771	1246	5195	1508	5987	8701	210	2890	18370
CD38-AF750 (AF 750) (mIF)	782	22662	1405	12873	9385	1897	156	4464	2371	221	1417	12464	422	826	874	
CD21-ATTO550 (ATTO 550) (mIF)	2217	1405	4045	620	1537	1248	214	112	216	2084	607	2649	3725	4	1010	2801
CD3e-AF647 (Cy5) (mIF)	1559	12873	620	34243	19974	28722	172	9389	9441	178	3715	17998	1199	653	1656	
CD4-AF647 (Cy5) (mIF)	4007	620	1537	18974	27712	20971	395	9336	4432	1518	4263	3275	17118	1581	2254	4578
CD45-ATTO550 (ATTO 550) (mIF)	2146	1897	1248	28722	20917	17471	306	12473	8432	173	4542	4292	21916	1370	1000	1779
CD56-AF647 (Cy5) (mIF)	314	156	214	172	395	306	1276	162	96	263	149	268	1051	18	157	935
CD68-AF647 (Cy5) (mIF)	2771	4464	112	8589	9536	12473	192	20454	3724	300	3969	3076	11291	983	652	1900
CD8-ATTO550 (ATTO 550) (mIF)	1048	2371	216	9461	4422	8422	96	3724	10376	178	1031	1472	5447	811	384	1218
E-cadherin-AF647 (Cy5) (mIF)	5195	221	294	178	1518	173	283	200	178	470	4246	5624	34	1402	8328	
FOXP3-AF647 (Cy5) (mIF)	1908	1417	607	3755	4885	4542	149	3609	1031	470	6984	1993	4455	170	842	1454
GRB7-AF750 (AF 750) (mIF)	5987	2112	2648	2539	3275	4002	248	3078	1472	6546	1953	16342	9532	337	1737	11806
GLUT1-AF750 (AF 750) (mIF)	8701	12464	1725	17956	12118	27916	1051	11201	5947	5924	4455	49449	906	1901	16333	
GranzymeB-ATTO550 (ATTO 550) (mIF)	210	422	6	1199	1983	1376	16	983	811	170	337	906	2090	47	512	
IKB1-ATTO550 (ATTO 550) (mIF)	2930	826	1010	853	2254	1000	197	652	384	1462	942	1737	3587	47	8693	5218
PanCK-AF750 (AF 750) (mIF)	18370	874	2890	1056	4378	1779	935	1900	1218	9328	1454	11906	16333	512	5218	42749

Figure 2. Co-Localization Matrix shows cellular co-expression of biomarkers across the TME making it easy to validate accurate thresholding and build confidence in the results.

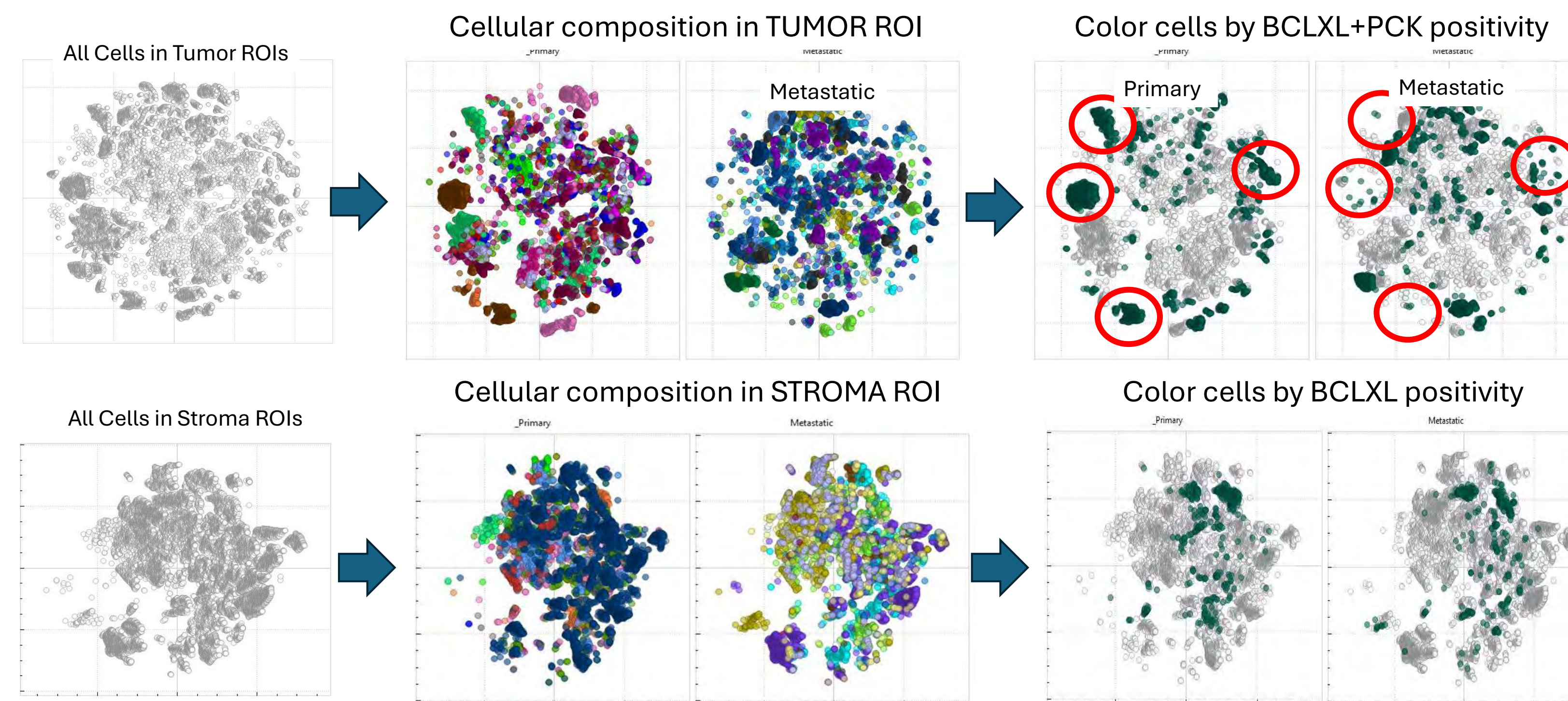


Figure 3. t-SNE maps of the cellular composition in the tumour (top) and stromal (bottom) regions between primary and metastatic TNBC

## Results – TIL scoring

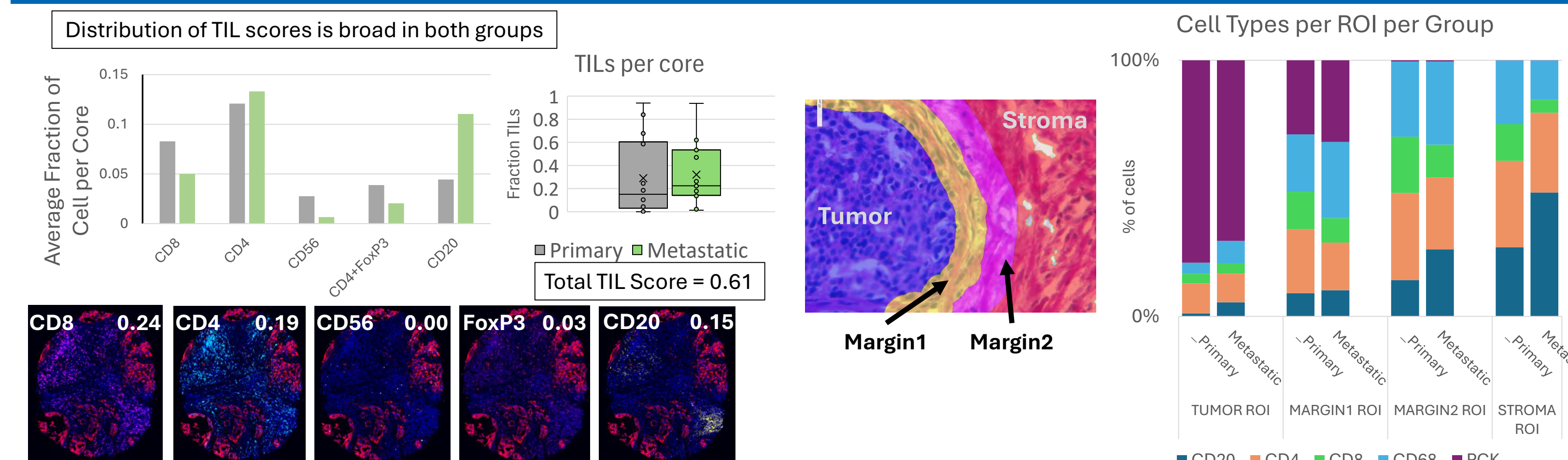
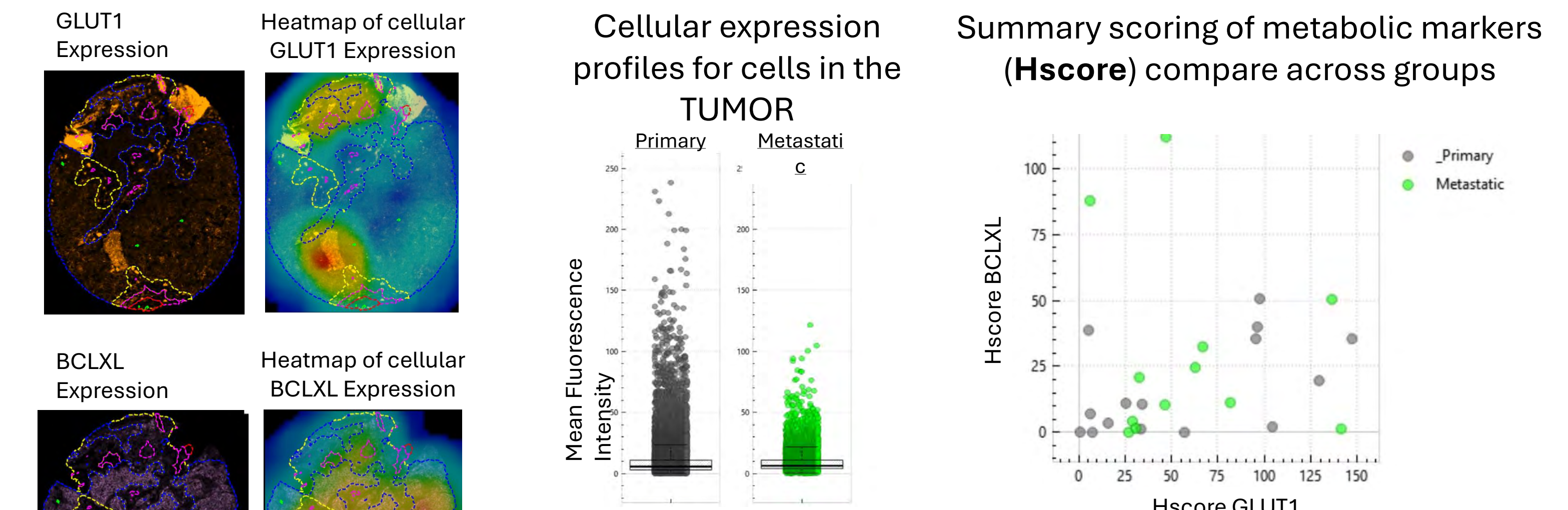


Figure 4. Immune cell scoring across each core for CD8, CD4, CD56, CD20 and across the tumour, stroma and margin regions.

## Results – Metabolic scoring



Sample-level Hscore GLUT1 and BCLXL are not correlated. Suggests that TNBC exhibits metabolic state heterogeneity.

## GLUT1 Expression across TME

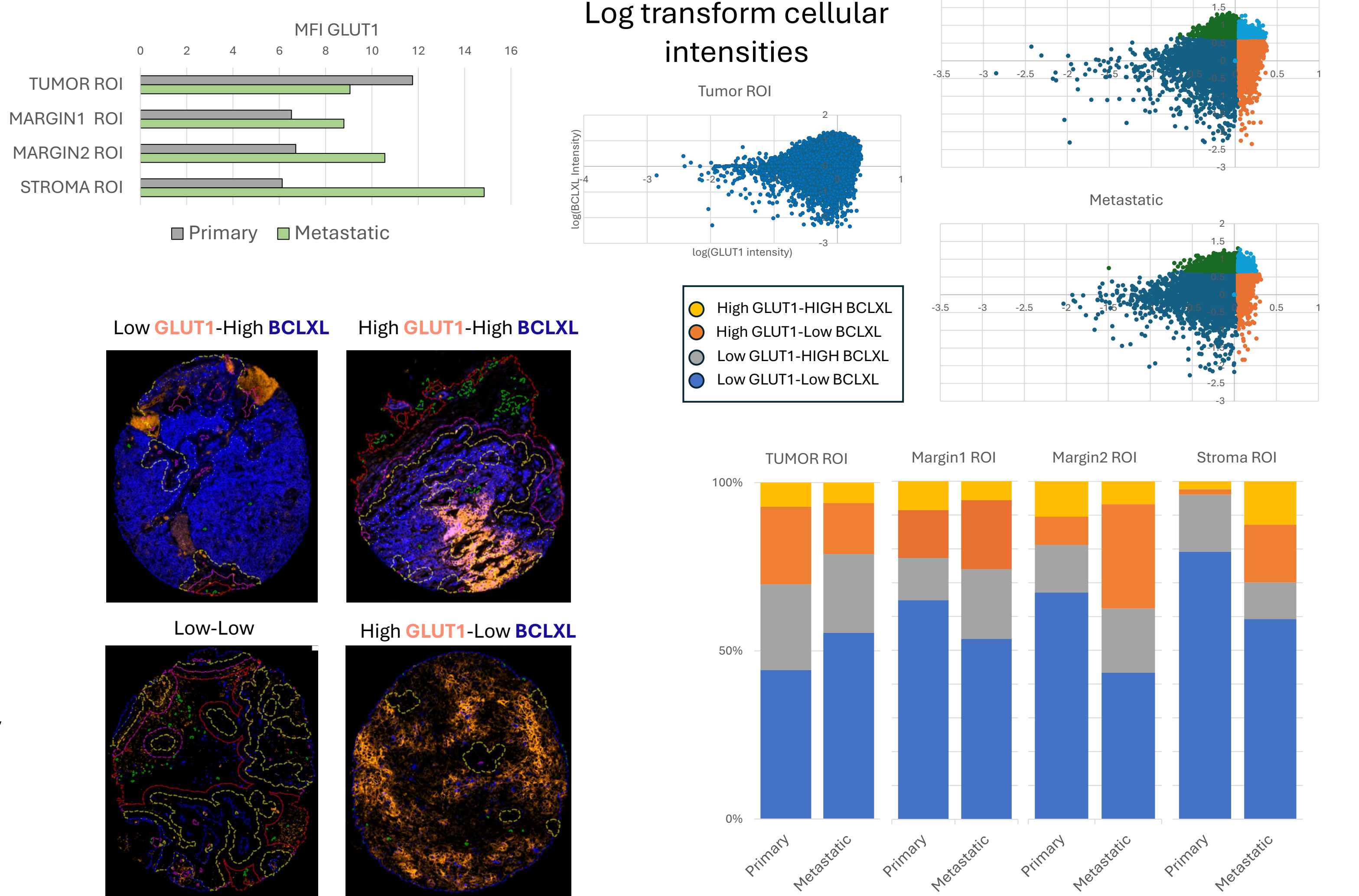


Figure 5. Framework for metabolic marker scoring across the TME landscape.

## Conclusions

This study highlights the framework for ultrahigh-plex discovery spatial proteomic profiling and image quantification in TNBC. Most notably, the modular analysis capability highlights how features of importance can be analysed (composition, TIL and metabolic scoring). Most notably, the metabolic scoring could be developed into a "H-score" enabling quantification of this signal to aid in functional cell phenotyping.