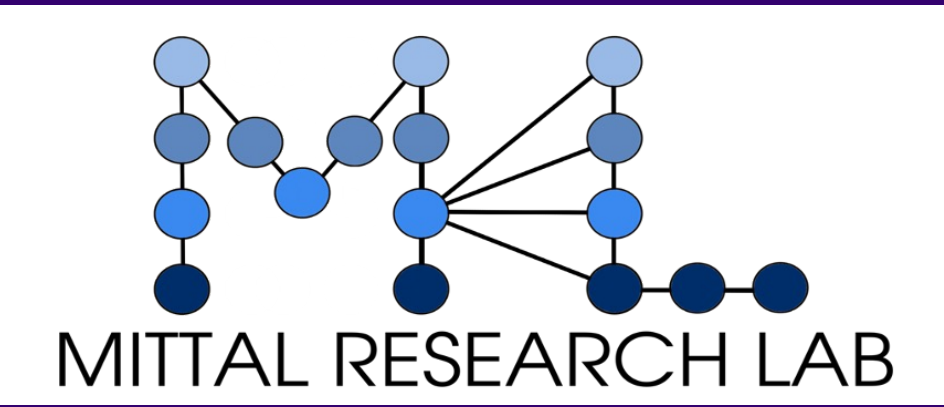


A Standardized end-to-end Computational Pipeline using Multiplexed Immunofluorescence Imaging: Prognostic Implications in Triple-Negative Breast Cancer Post-Neoadjuvant Chemotherapy



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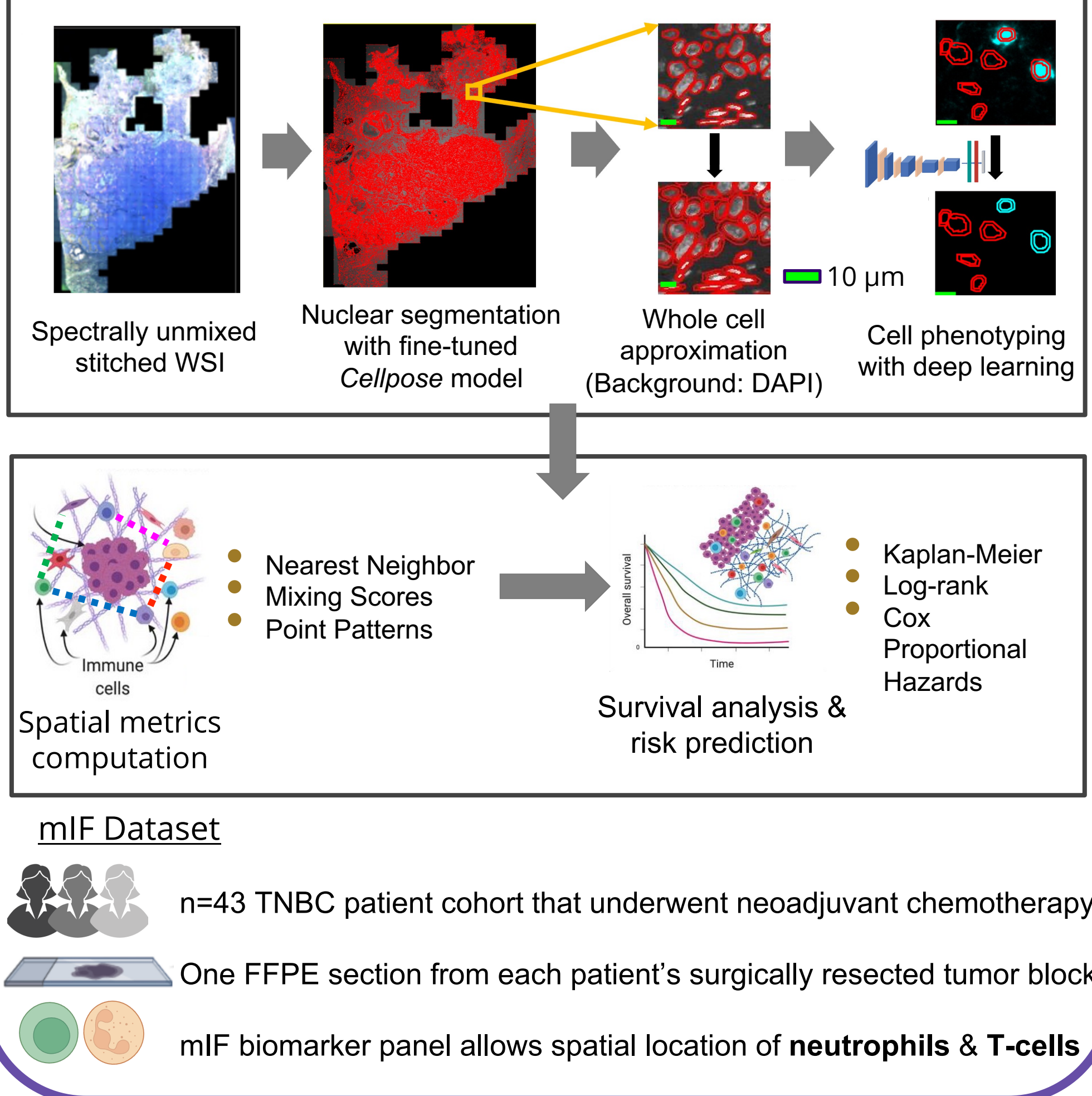
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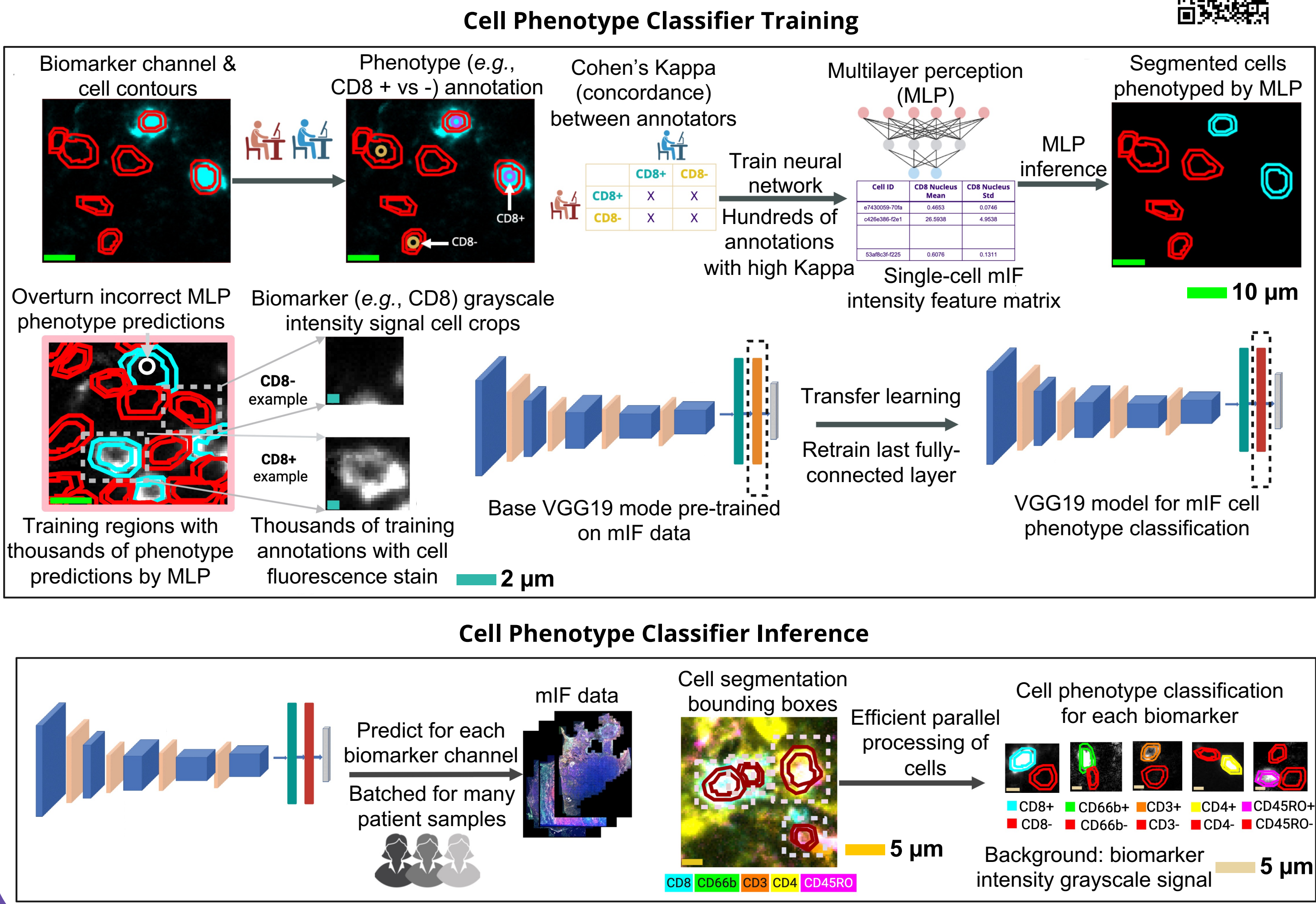
Background

- Multiplexed immunofluorescence (mIF) is a technology for the spatial detection of multiple biomarkers on a single tissue section
- mIF is increasingly being utilized in clinical studies to reveal spatial metrics indicative of prognosis^{1,2}
- Current mIF image analysis solutions are expensive, lack customizability, and involve manual steps
- Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that accounts for 10-15% of cases and does not respond to targeted hormone therapy³
- We have developed an automated and standardized workflow for the integrated image analysis of mIF in scalable clinical studies
- We are using our mIF analysis pipeline to generate patient-level immune spatial metrics to indicate TNBC patient prognosis

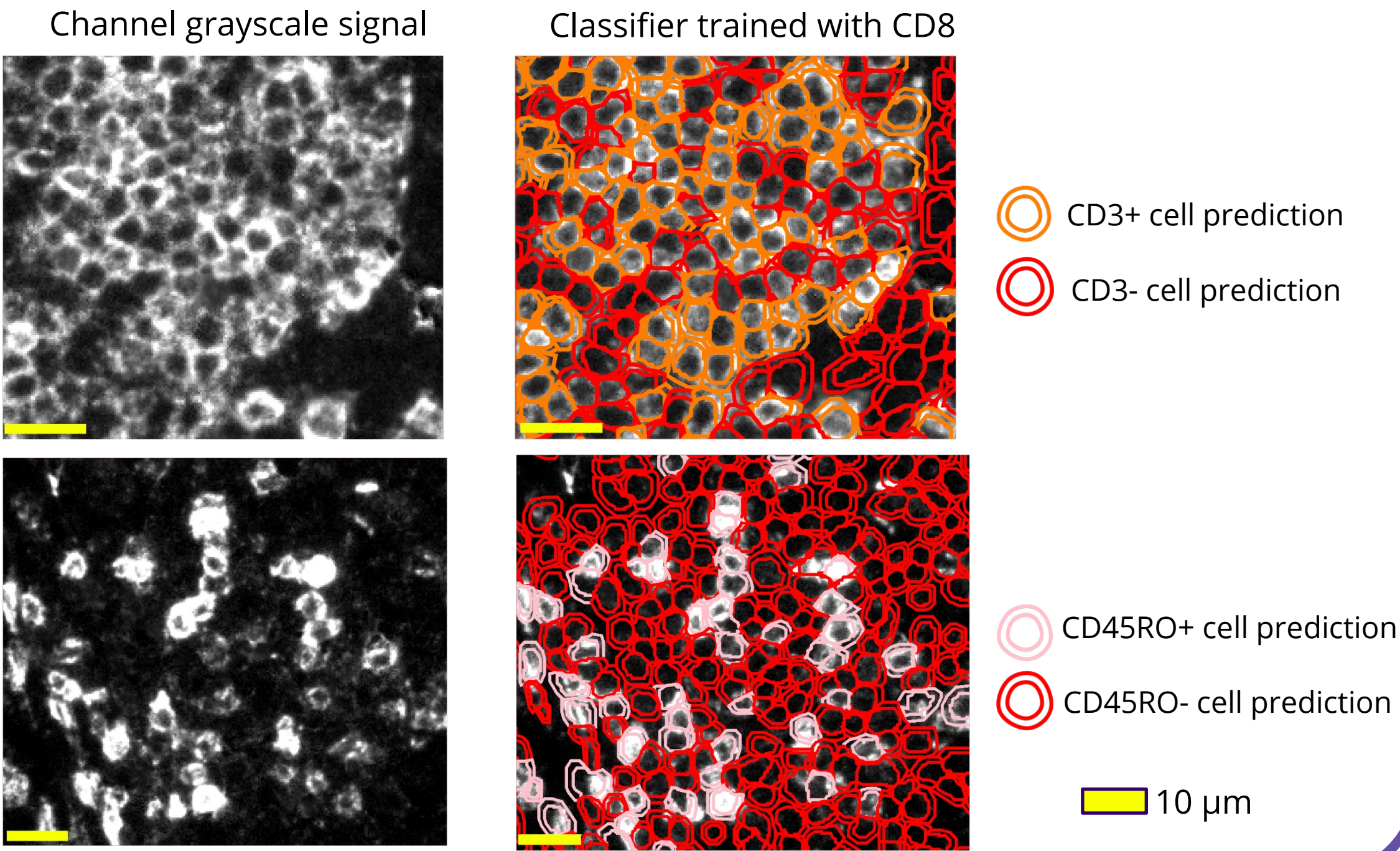
Overall Analysis Workflow



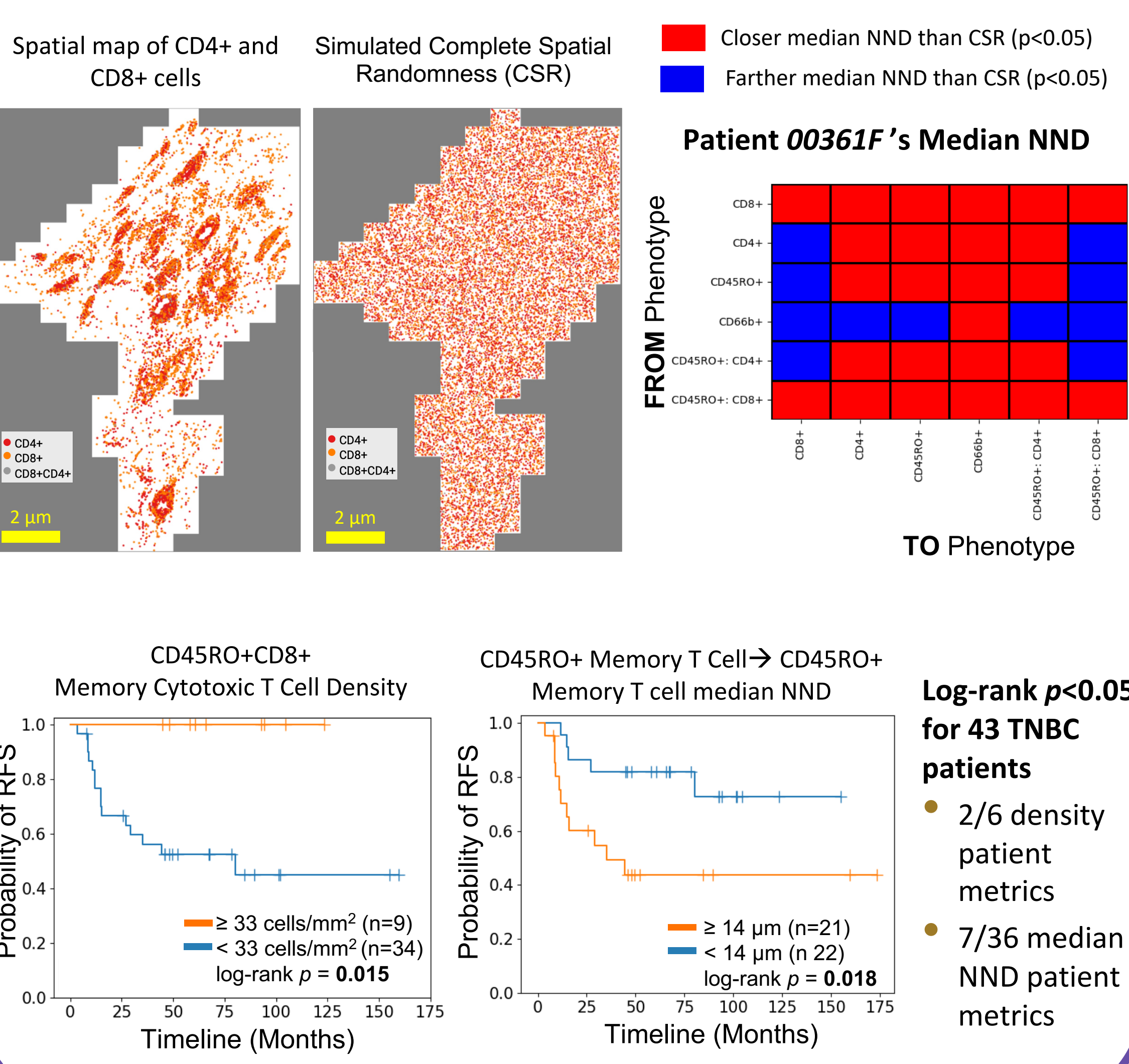
Cell Phenotyping Method



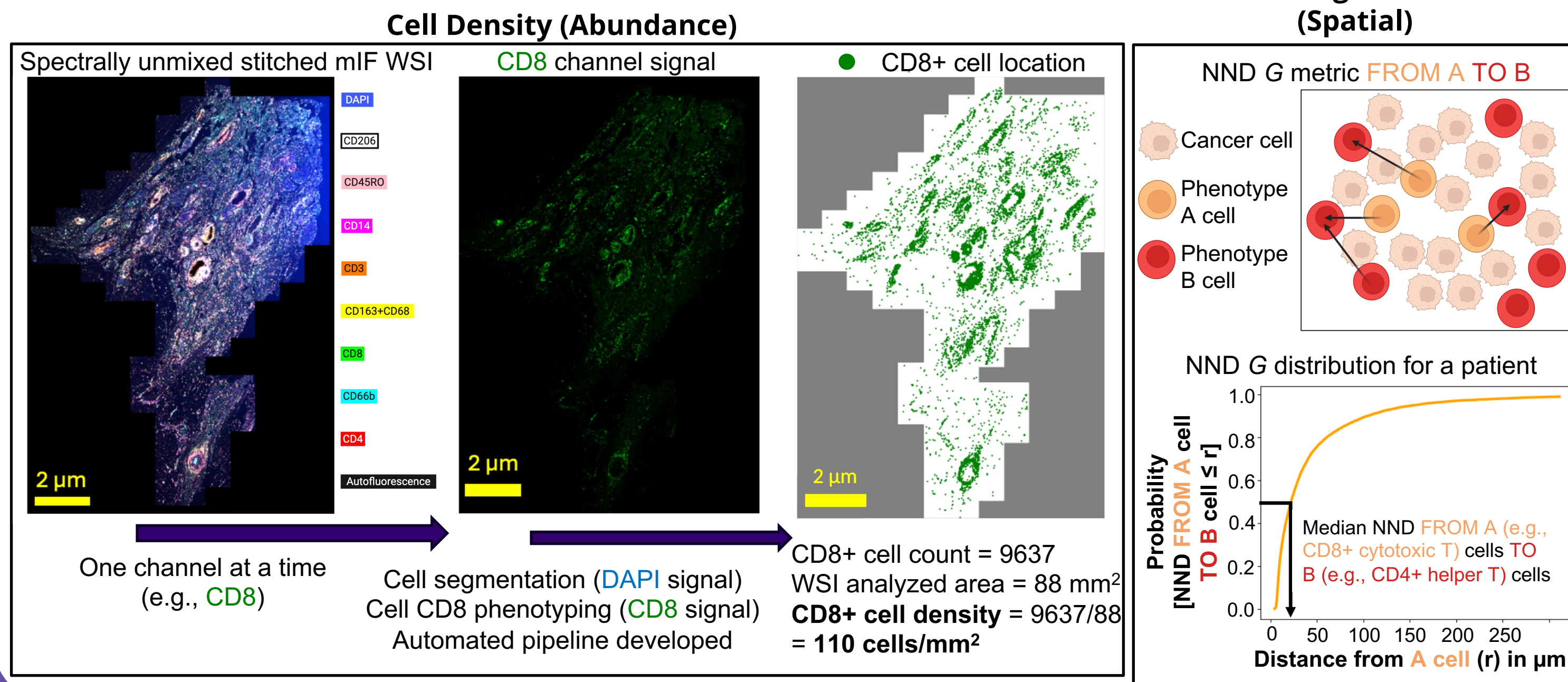
CD8 model for other markers



Spatial & Prognostic Insights



Single-cell Metrics Investigated



Cell Phenotyping Model Accuracy

Biomarker Channel	Num. WSIs	Num. Cells	Phenotyping Accuracy
CD8	3	1831	0.94
CD45RO	3	1859	0.97
CD3	3	1813	0.93

3 hold-out WSIs with ~50/50 split of biomarker +ve & -ve cells sampled
Ground truth cell phenotype labels independently annotated by three researchers
Model was trained only on CD8 signal
CD8 classifier has good transferability to other T cell markers, e.g., CD3 and CD45RO

Clinical Collaborators



References

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- Parra, E. R. et al. Immune cellular patterns of distribution affect outcomes of patients with non-small cell lung cancer. *Nature Communications* 14, 1–16 (2023).
- Zagami, P. et al. Triple Negative Breast Cancer: Pitfalls and Progress. *npj Breast Cancer* 8, 1–10 (2022).

CONCLUSIONS & FUTURE STEPS

- Developed robust pipeline for automated single-cell immunophenotyping of mIF data
- Discovered clinically prognostic single-cell patient-level metrics in TNBC
- Next step: Build cell phenotyping models with robust performance for other protein biomarkers and external datasets
- Next step: Explore other single-cell spatial metrics to discover clinical insights in TNBC
- Next step: Dimensional reduction to yield a handful of patient metrics for survival analysis