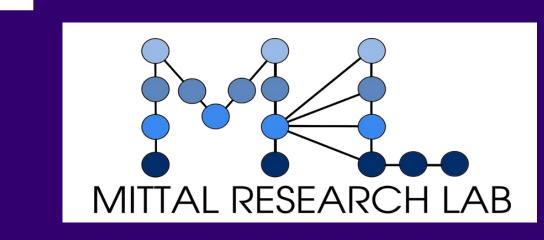
A Standardized end-to-end Computational Pipeline using Multiplexed Immunofluorescence Imaging: Prognostic Implications in Triple-Negative Breast Cancer Post-Neoadjuvant Chemotherapy

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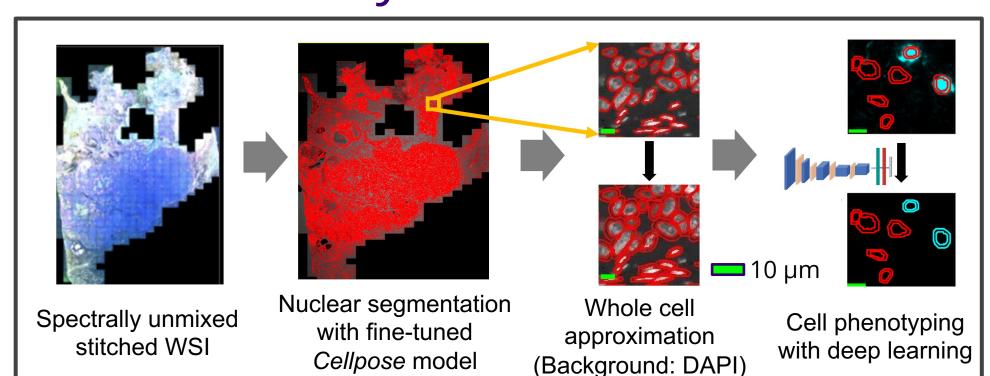
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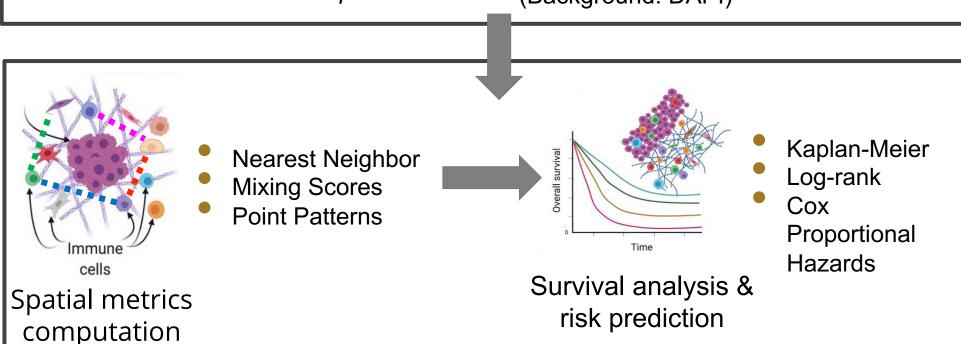


Background

- 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





mIF Dataset

n=43 TNBC patient cohort that underwent neoadjuvant chemotherapy

mIF biomarker panel allows spatial location of neutrophils & T-cells

Clinical Collaborators



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References

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- 2. Parra, E. R. et al. Immune cellular patterns of distribution affect outcomes of patients with non-small
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Cell Phenotyping Method **Cell Phenotype Classifier Training** Biomarker channel & Cohen's Kappa Multilayer perception CD8 + vs -) annotation phenotyped by MLF (concordance) between annotators ____10 μm Overturn incorrect MLP Biomarker (e.g., CD8) grayscale intensity feature matrix phenotype predictions intensity signal cell crops connected layer VGG19 model for mIF cell Base VGG19 mode pre-trained Thousands of training phenotype classification on mIF data thousands of phenotype annotations with cell fluorescence stain 2 µm predictions by MLP **Cell Phenotype Classifier Inference** Cell phenotype classification bounding boxes Efficient parallel for each biomarker Batched for man

Single-cell Metrics Investigated **Nearest Neighbor Distance** (Spatial) **Cell Density (Abundance)** Spectrally unmixed stitched mIF WSI CD8+ cell location CD8 channel signal NND G metric Cancer cell Phenotype Phenotype NND G distribution for a patient Median NND FROM A (e.g. CD8+ cvtotoxic T) cells TO CD8+ cell count = 9637 One channel at a time B (e.g., CD4+ helper T) cells Cell segmentation (DAPI signal) WSI analyzed area = 88 mm² (e.g., CD8) Cell CD8 phenotyping (CD8 signal **CD8+ cell density** = 9637/88 50 100 150 200 250 Automated pipeline developed = 110 cells/mm² Distance from A cell (r) in µm

Cell Phenotyping Model Accuracy

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

3 hold-out WSIs with ~50/50 split of biomarker +ve & -ve cells sampled Ground truth cell phenotype labels independently annotated by three researchers

■ CD8- ■ CD66b- ■ CD3- ■ CD4- ■ CD45RO-

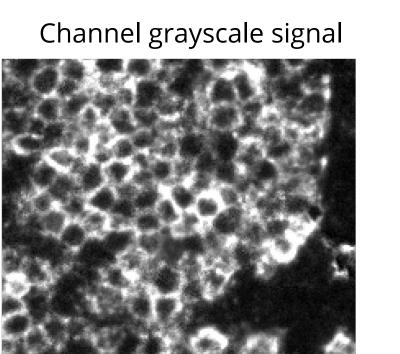
■5 μm

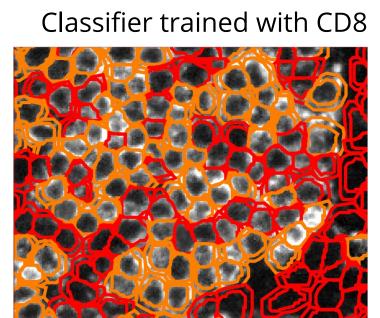
Background: biomarker

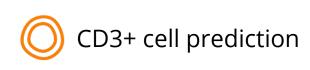
intensity grayscale signal

- Model was trained only on CD8 signal
- CD8 classifier has good transferability to other T cell markers, e.g., CD3 and CD45RO

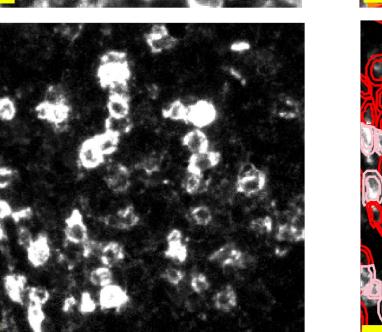
CD8 model for other markers

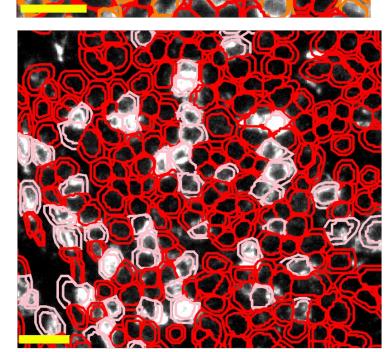






CD3- cell prediction

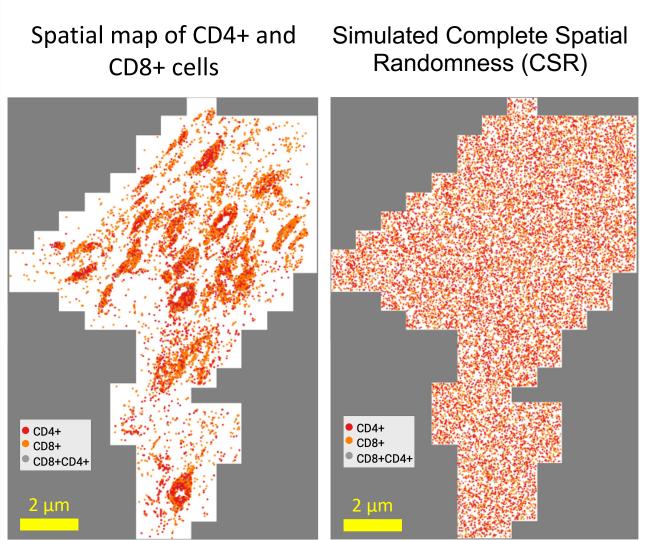


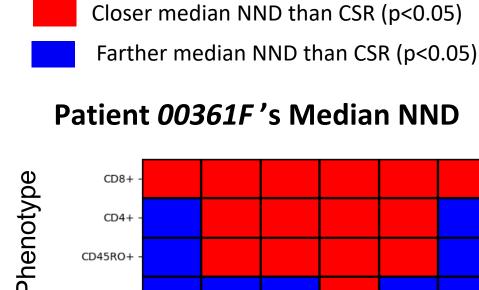


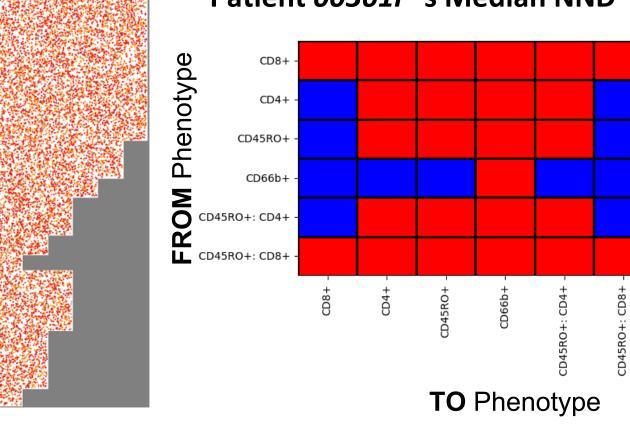
CD45RO+ cell prediction (C) CD45RO- cell prediction

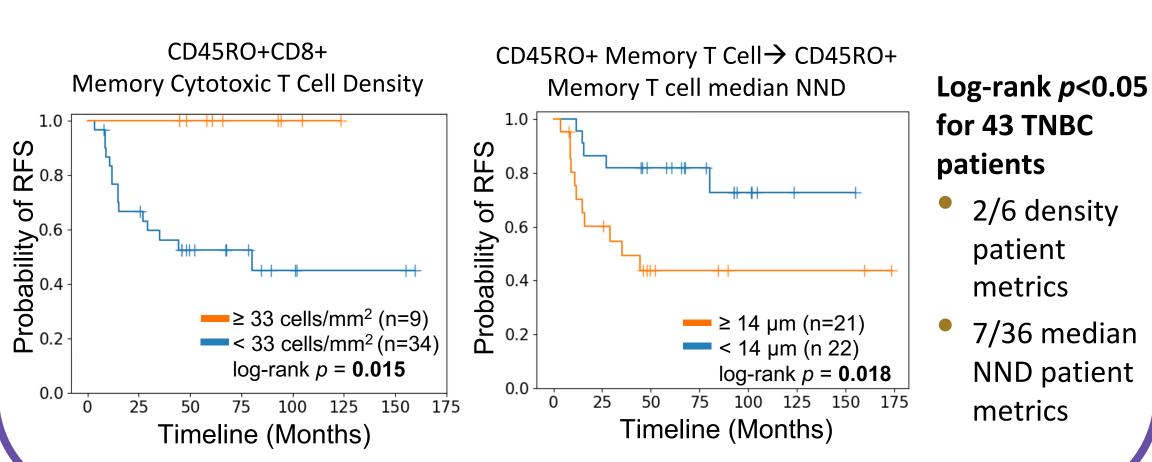


Spatial & Prognostic Insights









2/6 density patient metrics

^{*} 7/36 median NND patient metrics

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