HIGHLY MULTIPLEXED IMMUNO-FLUORESCENCE IMAGE DATA ANALYSIS FOR PROSTATE CANCER

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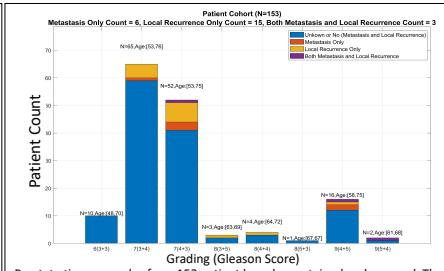
Abstract

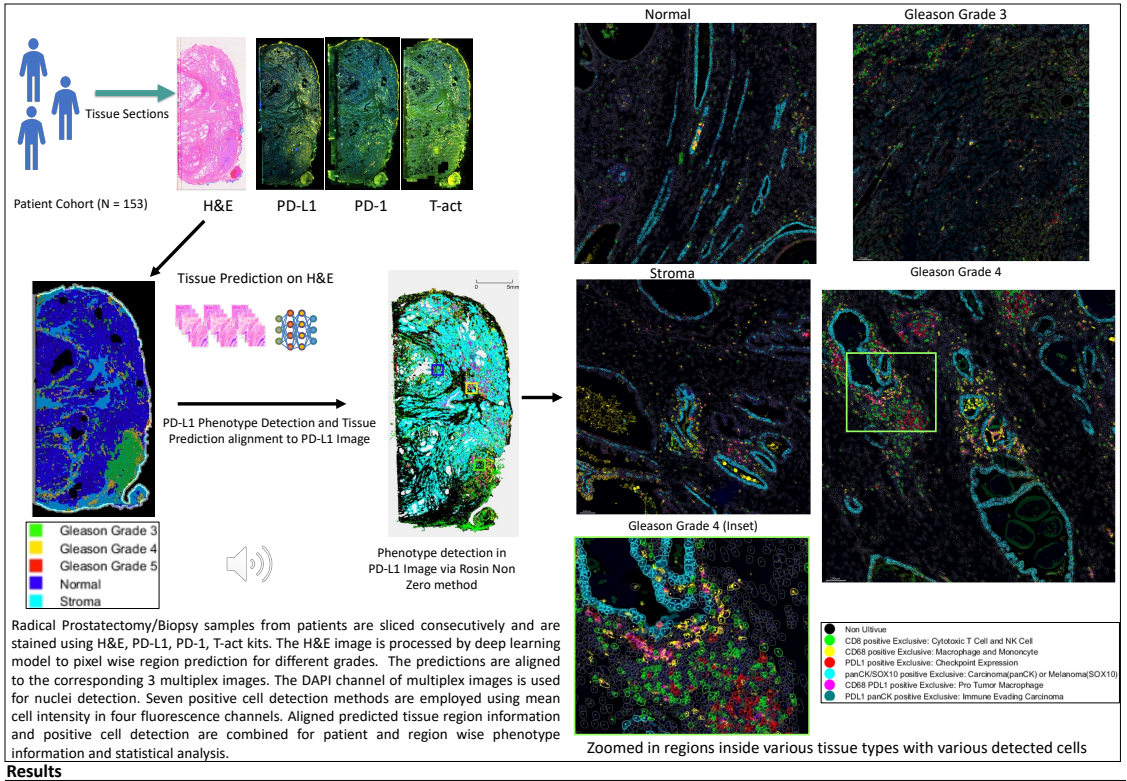
- Prostate Cancer (PCa) is estimated to cause >10% of all cancer deaths in men in the US.
- Quantification of immune and tumour cell distributions and interactions provides key insights into the architecture and evolution of tumour microenvironments (TME).
- Multiplexed immune-fluorescence (mIF) imaging enables a comprehensive spatiotemporal investigation of TMEs throughout the disease course.
- We have developed an automated image processing pipeline to quantify immuno-phenotypes in prostate TMEs, incorporating machine learning methods to analyse histopathological tissue whole slide images (WSI).

Patient Cohort



Prostate tissue samples from 153 patient have been stained and scanned. These patients have assigned Gleason scores by pathologist. We also indicate Local recurrence and Metastasis status for these patients.

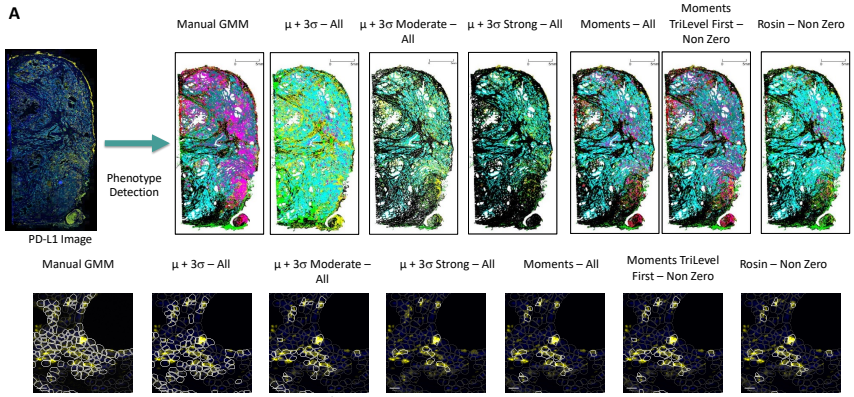
Method and Experiment Design

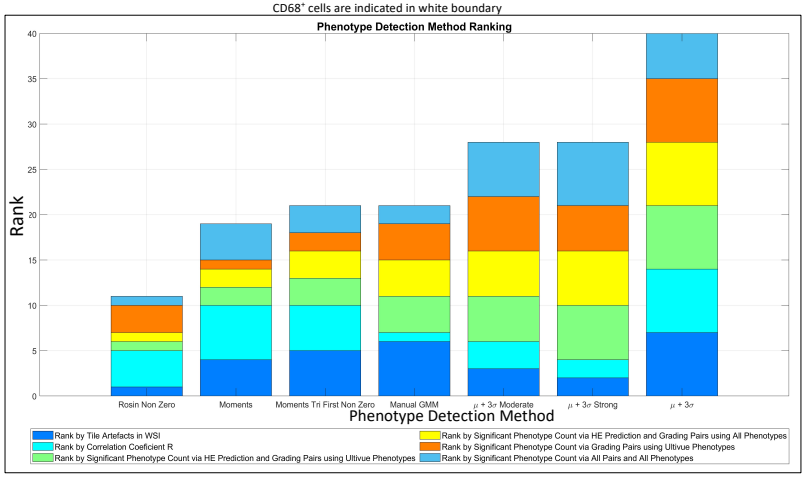


Radical Prostatectomy/Biopsy samples from patients are sliced consecutively and are stained using H&E, PD-L1, PD-1, T-act kits. The H&E image is processed by deep learning model to pixel wise region prediction for different grades. The predictions are aligned to the corresponding 3 multiplex images. The DAPI channel of multiplex images is used for nuclei detection. Seven positive cell detection methods are employed using mean cell intensity in four fluorescence channels. Aligned predicted tissue region information and positive cell detection are combined for patient and region wise phenotype information and statistical analysis.

Zoomed in regions inside various tissue types with various detected cells

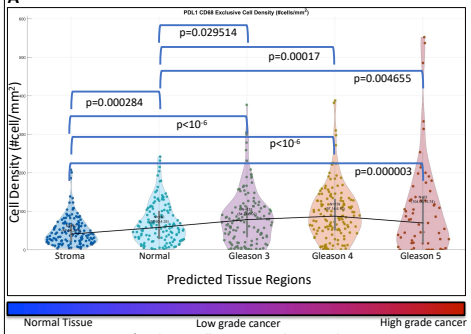
Manual and automated phenotype detection methods

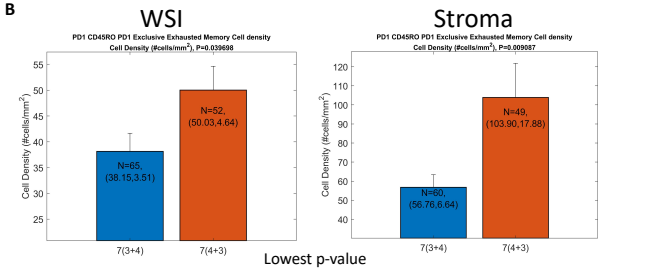




Defining most reasonable phenotype detection method: **A)** Example phenotype detection results using 7 detection methods. We also illustrate CD68⁺ Inclusive cell detections for these 7 method on the same region in a PD-L1 image. **B)** Ranking detection methods for reasonable phenotype detection method. Lower values of the rank is better. **Rosin Non Zero** is the best performing method.

Results





A) Increasing trend for CD68⁺ Exclusive cells within tissue from normal tissue to high grade cancer. We also indicate p-values for t-test across tissue regions. **B)** Exhausted Memory Cell Density (#cell/mm²) has p-value <0.05 for patients graded 7(3+4) vs patients graded 7(4+3) in both Whole Slide Image (WSI) region and "Stroma" region indicating tumour aggressiveness (4+3 vs 3+4). All results are via Rosin Non Zero method.

Summary and Future Work

Results show several statistically significant immuno-phenotypic differences (p<0.05) dependent on Gleason Score (GS) and TME. Patients graded GS 4+3 (N=52) have higher Exhausted Memory Cell (CD45RO⁺PD1⁺) density compared to GS 3+4 (N=65, p=0.003969), which is consistent with "Stroma"-only regions (p=0.009087, 4+3 (N=49), and 3+4 (N=60)), an indicator of tumour aggressiveness (4+3 vs 3+4). We also report a trend toward increasing Macrophages/Monocytes (CD68⁺) density (in WSI/tissue and within the panCK+ cell (tumour) neighbourhood) with higher grade cancer tissue indicating increasing immune response. Our results suggest that mIF imaging can highlight complex molecular relationships in PCa pathophysiology by comprehensively characterising TMEs. Our image processing pipeline leads to analyses quantifying cell-cell interactions and cell distributions, which together can elucidate the disease course of prostate and other cancers to identify biomarkers for diagnosis and targets for therapeutic intervention.