

A workflow for combined detection of protein interactions and cell types for translational studies

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Background and Aim

Multiplexed imaging approaches are increasingly used in discovery and translational research, but complex instrumentation, data analysis and high costs per sample can limit clinical implementation. Here, we demonstrate a cost and time efficient workflow combining a low plex immunofluorescence panel with in situ proximity ligation assay (isPLA) to detect direct protein-protein interactions between neighboring cells within a single tissue section.

As a proof of concept with relevance for clinical adaptation, the immunotherapy axis involving programmed cell death protein 1 (PD-1) and its ligand PD-L1 was investigated in germinal centers (GCs) of tonsil tissue and tertiary lymphoid structures (TLSs) in bladder cancer tissue from a patient treated with immunotherapy.

Method

The workflow was automated using a poly-horseradish peroxidase (HRP) mediated tyramide signal amplification (TSA) kit, with an isPLA kit from Navinci Diagnostics integrated as the first staining cycle. Chromogenic detection in the isPLA kit was replaced with TSA, enabling combined detection of protein phenotypes and protein-protein interactions within a single tissue section.

The panel included five protein markers (CD45, CD3, CD8a, PanCK, Nectin-4) and the PD-1/PD-L1 interaction. Automation on the autostainer Spatial Station from Parhelia Bio reduced hands-on processing time by approximately 25 hours compared with manual staining. The workflow was evaluated in tonsil and bladder cancer tissue.

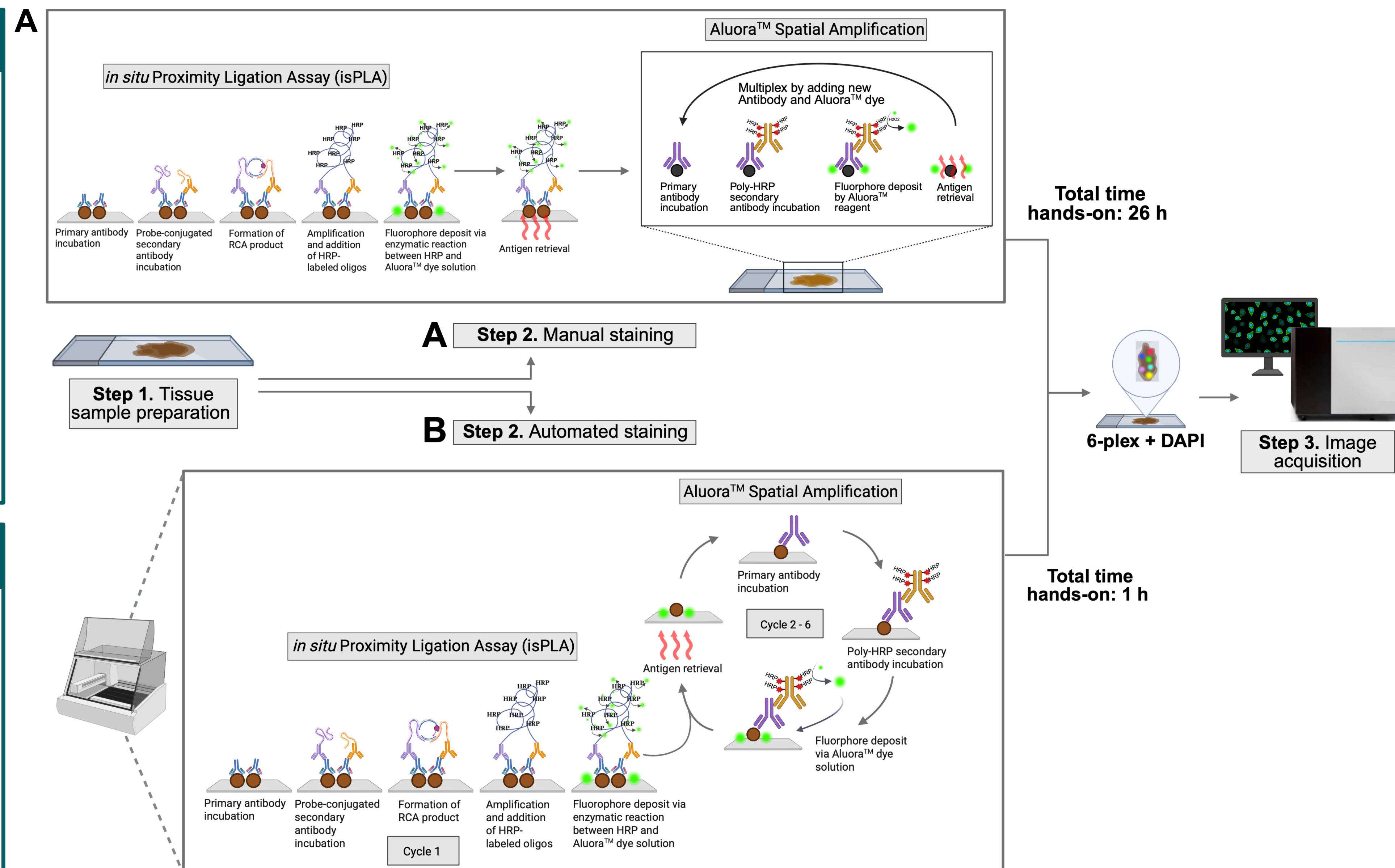
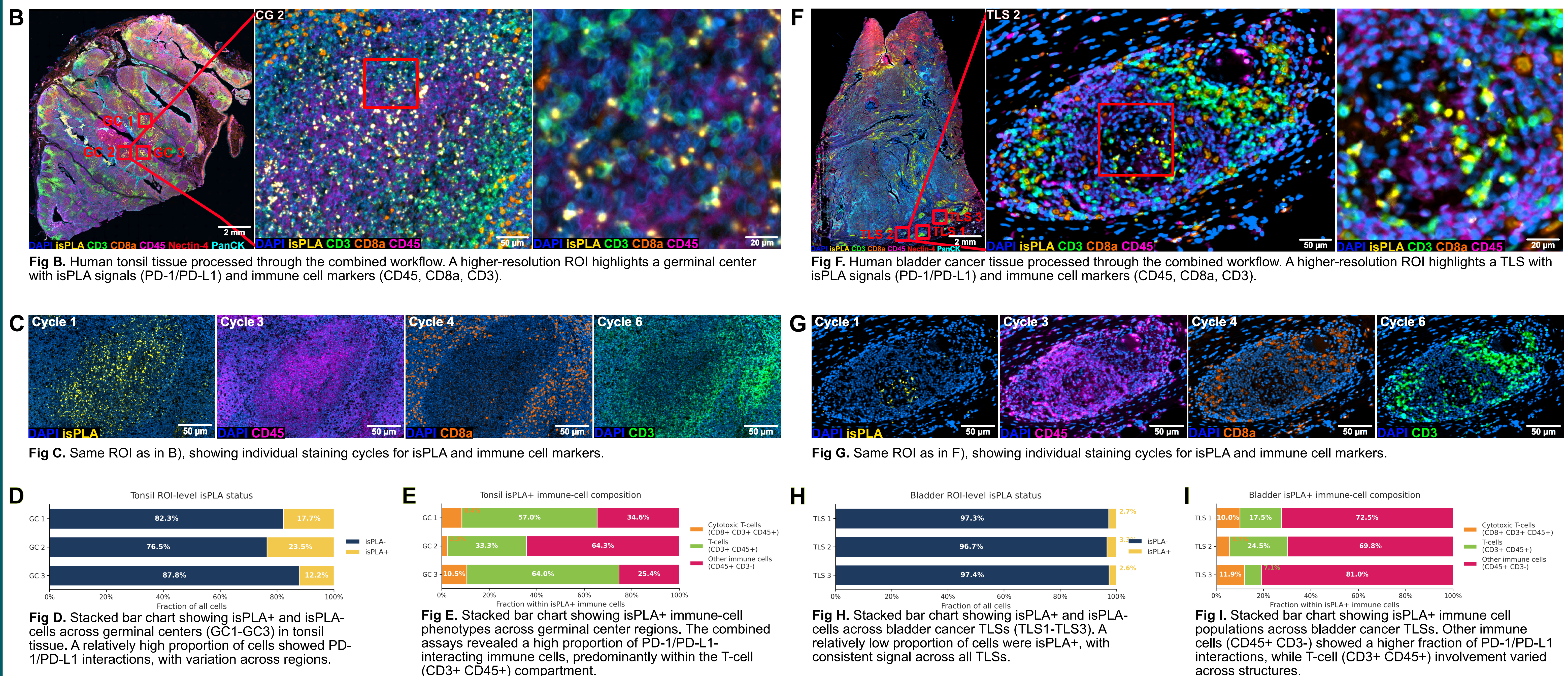


Fig A. Schematic overview of workflow. The workflow consists of three main steps, (1) Tissue preparation, (2) Tissue staining using a manual bench workflow (A) or an automated workflow on the Parhelia Spatial Station (B) and (3) image acquisition on EVOS S1000. Both workflows used cyclic TSA based immunofluorescence, with each cycle detecting a protein marker or interaction using a unique fluorescent dye. Each cycle includes primary and HRP-conjugated secondary antibody staining, followed by HRP mediated TSA. HIER is performed between cycles to enable subsequent staining. The isPLA was integrated as the first cycle, using primary and probe-conjugated secondary antibodies followed by probe ligation and rolling circle amplification (RCA). HRP-labeled probes then bind the RCP and generate covalently bound Aluora dye signal at the site of interaction.

Results

The automated workflow was applied to tonsil and bladder cancer tissue under the same conditions, enabling detection of protein-protein interactions and cell phenotyping within GCs in tonsil tissue and TLSs in bladder cancer tissue. Although the marker panel was limited and not designed to investigate interactions between immune cell subsets in GCs and TLSs, the workflow enabled combined detection of both readouts within a single tissue section without protocol modification across tissue types.



Conclusion and Prospects

We developed an automated workflow for combined detection of multiple protein markers with optional protein-protein interaction detection using isPLA. The workflow reduced hands-on time and showed consistent performance across technical replicates and tissue types, with a cost of only a few hundred dollars per tissue slide. The flexible marker and interaction panel supports adaptation to different clinical questions and large-scale translational studies. Future work will evaluate the workflow in a larger patient cohort to assess its feasibility for clinical implementation.

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