Chromogenic Multiplex: The Future of Clinical Biomarkers

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Background

Methods for multiplex immunoassays are advancing rapidly, and **simultaneous profiling of many biomarkers in a single tissue sample,** providing data-rich images, is now a routine research tool. However, there is still a large gap between informative research-based panels and clinically deployable technologies. This project aims to bridge that gap and offer potential for **instant benefits** in clinical settings through the development of clinically compatible multiplex chromogenic immunohistochemistry (mIHC) assays. These multicoloured chromogenic assays make economical use of scarce biopsy tissue and enable rapid and highly detailed histopathological characterisation, supporting **faster diagnostic turnarounds and new highly accurate biomarker tests.**

In collaboration with pathologists at the Queen Elizabeth University Hospital, we have designed three clinically relevant mIHC panels to address key diagnostic challenges and ensure translational relevance.

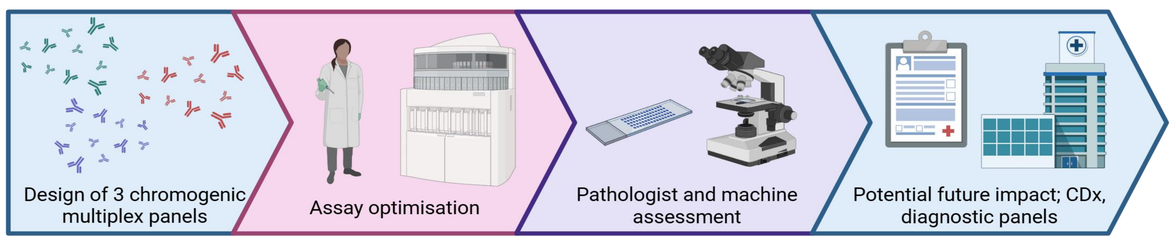

Figure 1. Flowchart demonstrating the pathway of assay development to potential clinical implementation and patient impact. Panels are as follows: NSCLC subtyping, immunotherapy prediction, and unknown malignancy subtyping.

Pipeline

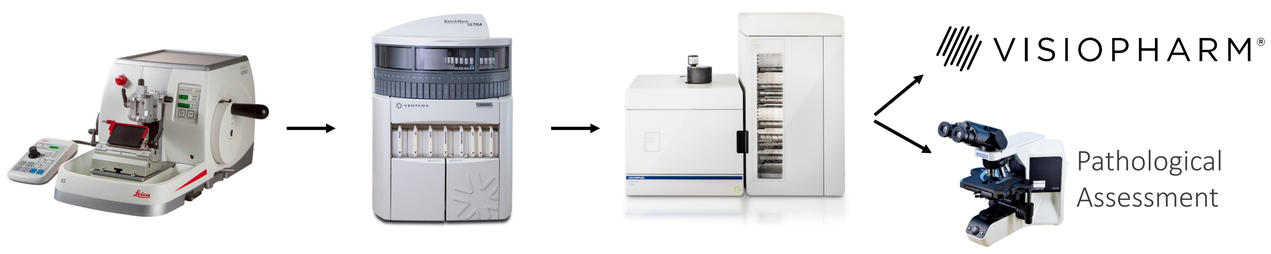

Figure 2. Flowchart demonstrating the protocol conducted for chromogenic multiplex assay. From left to right: automated microtome, Roche Discovery Ultra, Olympus VS200, Visiopharm/pathological assessment.

Assays are being developed and optimised on the Ventana Discovery Ultra Autostainer platform using a range of tissues, including large tissue microarray case series. Antibodies are first optimised using the gold standard DAB IHC for antibody sensitivity and specificity.

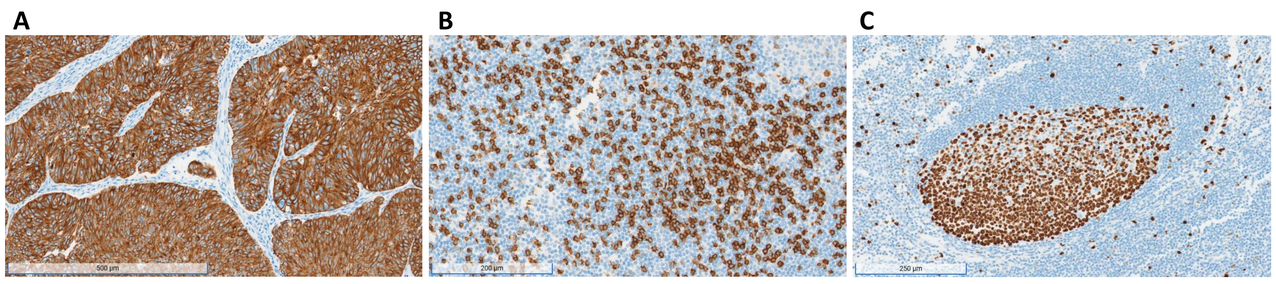

Figure 3. Optimised DABs using Roche primary antibodies. A) PanCK in SCC. B) CD8 in tonsil. C)KI67 in tonsil.

Each antibody will be evaluated with multiple chromogens (blue, purple, green, yellow, red, teal) to optimise chromogen intensity and comparison to DAB (Figure 4).

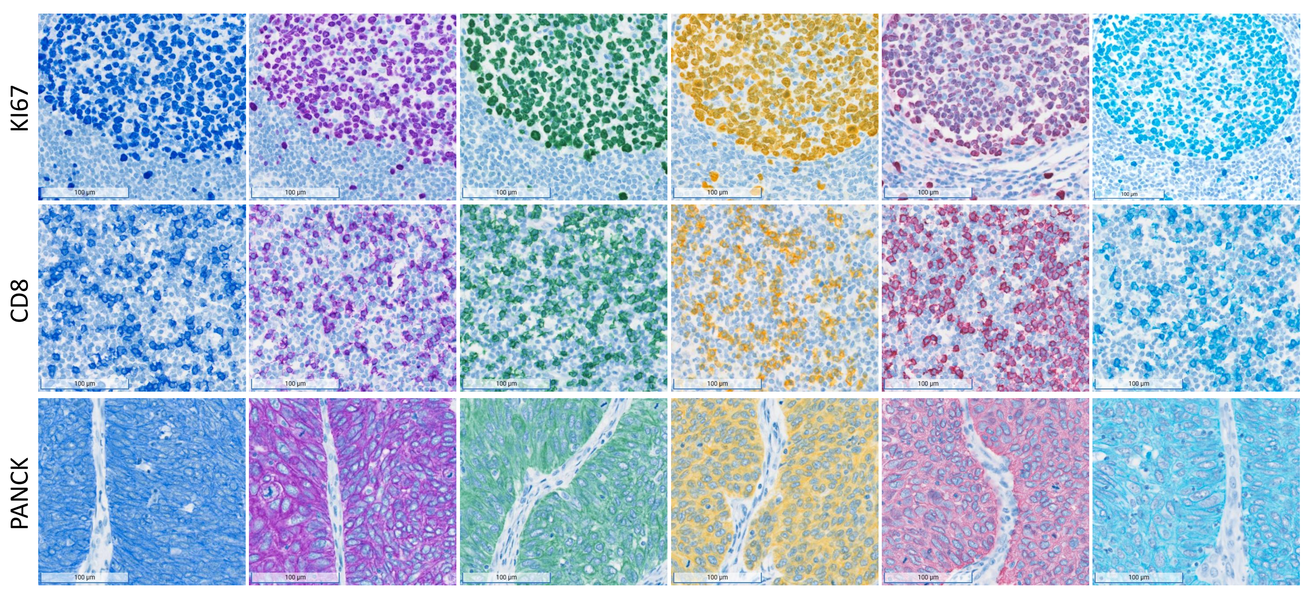

Figure 4. Examples of Ki67, PanCK and CD8 in different chromogens.

To achieve the best image quality, antibody-chromogen pairs will be explored, as will the order of antibody application in the autostainer platform (Figure 5).

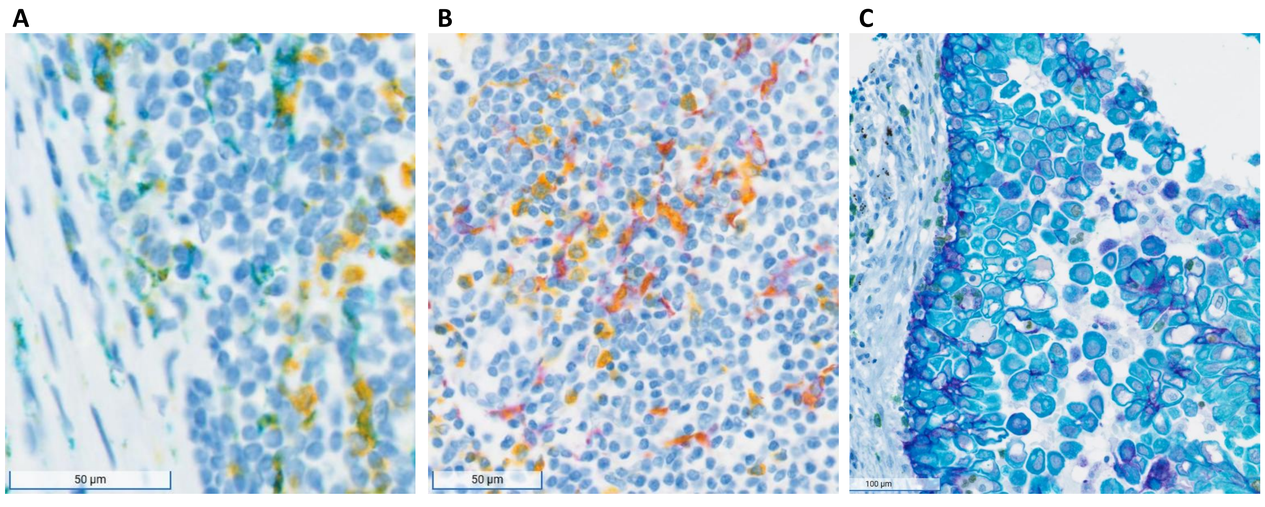

Figure 5. Example images of chromogenic duplex. A) Tonsil stained with teal for CD68 and yellow for CD163. B) Tonsil stained with purple for CD68 and yellow for CD163. C) Lung adenocarcinoma stained with purple for PDL1 and teal for panCK.

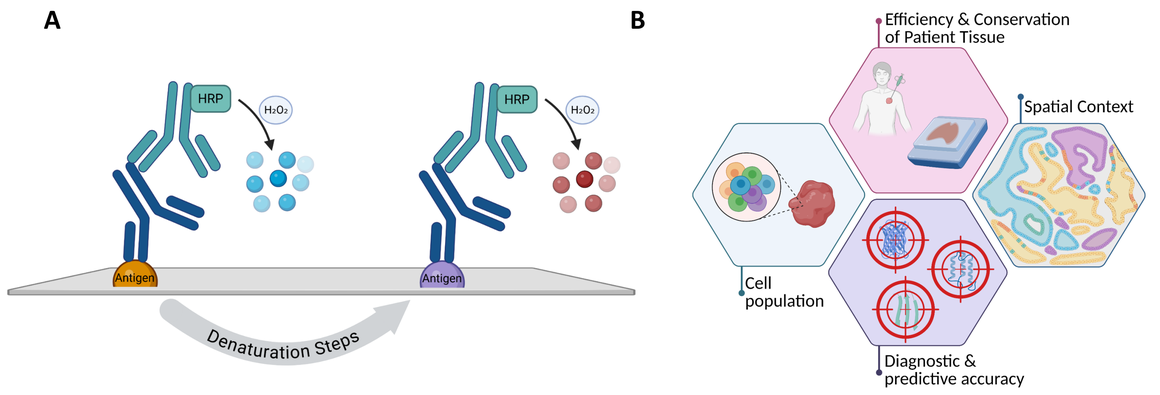

Figure 6. (A) Schematic demonstrating the process of chromogenic multiplexing. Primary antibody binds to specific antigen, secondary antibody conjugated to HRP binds the primary antibody, and catalyses deposition of tyramide-linked chromogen. Subsequent denaturation steps ensure minimal cross-talk/non-specific staining. (B) Overview of the advantages of chromogenic multiplexing.

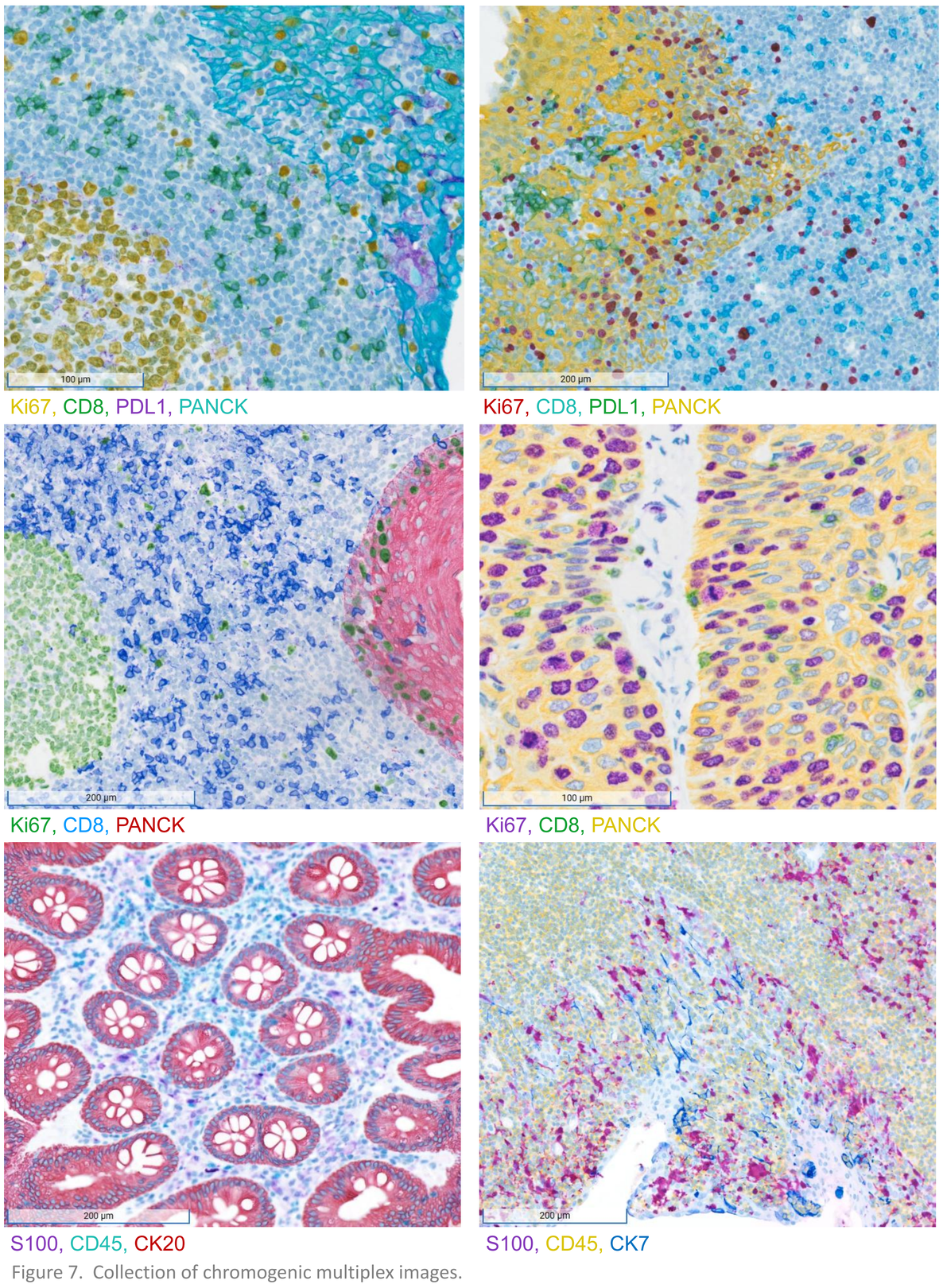

Figure 7. Collection of chromogenic multiplex images.

Ongoing Work

Image sets will be shared with NHSGGC pathologists using digital pathology software to gather feedback on preferred colour/antibody combinations, gauge enthusiasm, and identify areas for future development.

mIHC assays will be finalised for application to tissue microarray (TMA) cohorts.
(1)A non-small cell lung cancer diagnostic panel: TTF-1, p40, PD-L1, and panCK
(2)A pan-cancer immune engagement panel: panCK, CD8, Ki67, and PD-L1
(3)A poorly differentiated malignancy diagnostic panel: CK7, CK20, CD45, and S100

Images will be scanned and then passed through our quantitative Visiopharm pipelines to assess **machine-interpretability** of these multicoloured images.

Through the development of clinically relevant mIHC panels compatible with automated staining/routine workflows, this study will test demonstrate the feasibility of translating multiplex tissue profiling into clinical settings. These outcomes will support the **next generation of diagnostic tests for tumour classification and treatment selection.**

Acknowledgements

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