

A circular inset image in the top left corner showing a tissue sample with multiplexed immunofluorescence. The image displays a dense network of cells with various colors (red, green, blue, yellow) highlighting different cellular components and markers.

# Getting TiME on your side

## Defining the future of precision medicine with advanced multiplexed immunofluorescence

Kirsteen Maclean PhD

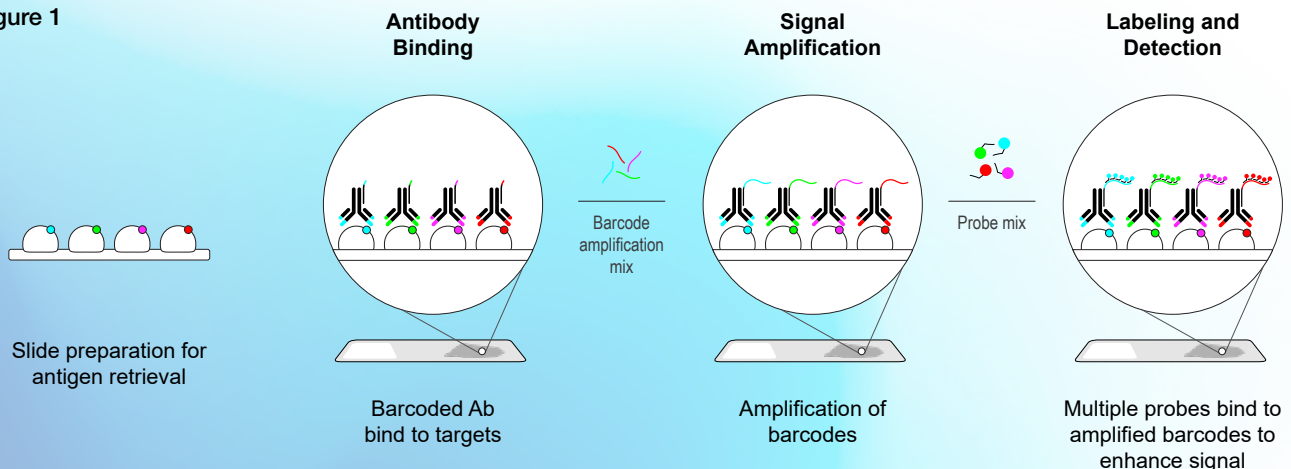
Immunotherapy has transformed the treatment of metastatic and recurrent solid tumors. Advances in technology in the past few years have created unprecedented opportunities to identify biomarkers of disease processes, especially by using multi-omics technologies and datasets to derive valid and useful signatures of disease. Despite these advances, today only a minority of patients respond to immunotherapies. Prediction of response to therapies such as checkpoint inhibitors that rely on activation of endogenous immune responses has been shown to be especially difficult due to complex and heterogeneous immune escape mechanisms in each patient. Increasing evidence suggests that measurement of robust biomarkers through spatial analysis of the tissue will be key to enable rational patient selection for an improved clinical trial process and design precise combination therapies.

The urgency to discover and implement new biomarkers lays bare the need to integrate a variety of advanced tools to probe the dynamic nature of events happening in the tumor microenvironment (TME). A further challenge is the minute and limiting amount of precious tissue available from most biopsies. Finally, multidisciplinary collaboration between translational scientists, pathologists, and computer image analysis software engineers will be required to create new biomarker development strategies.

To that end, multiplex immunohistochemistry/immunofluorescence (mIHC/IF) can provide critical insight into cellular composition, cellular functions, and cell-cell interactions in normal and diseased tissues. Importantly, recent studies using mIHC/IF to identify specific types of immune cells in the TME have emphasized the need for digital pathology tools to visualize and understand the scope of each tumor's heterogeneity, immune environment, and contextual relationships. Understanding relationships between spatial quantification of different immune and tumor cells measured by mIHC/IF and mutational drivers of the individual cancer measured by DNA or RNA sequencing will likely require advanced AI-driven digital tools to comprehend the high-dimensional complexity of the spatial and immunological heterogeneity in the patient's tissue. We anticipate that identifying the treatment, including combination and advanced therapies, most likely to benefit each patient will require comparison of their biopsy profile with a large atlas of reference data.

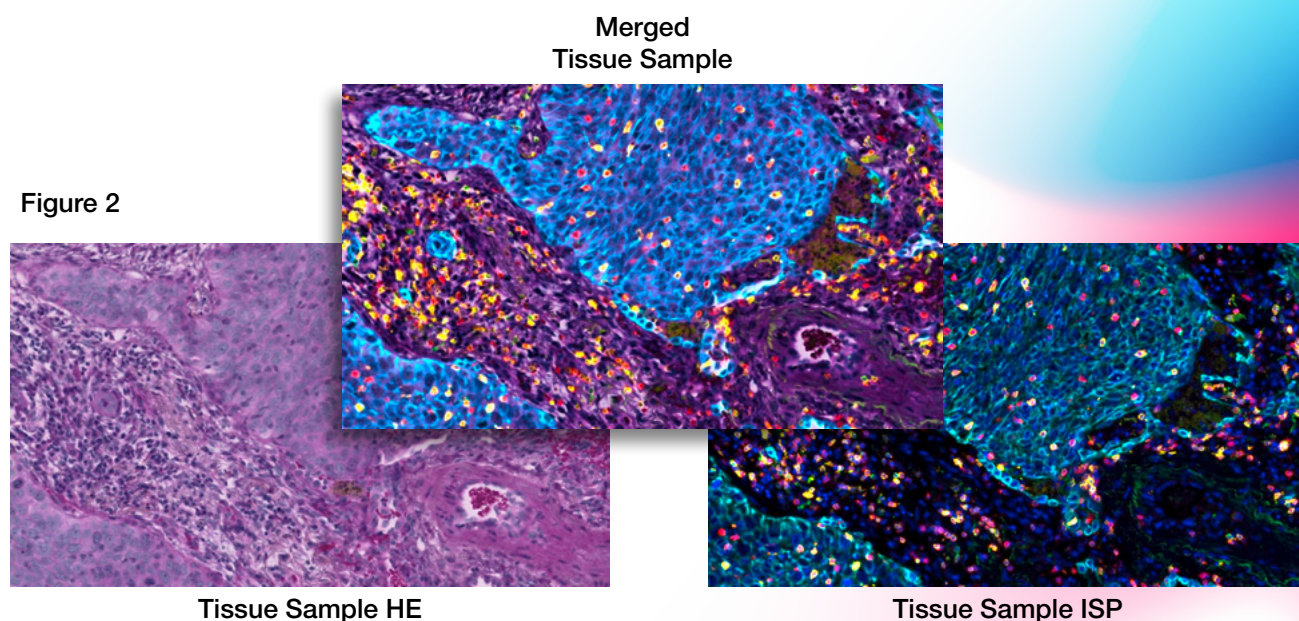
Unfortunately, many current mIHC/IF techniques involve long staining and assay times, require dedicated and/or captive instrumentation and entail tedious assay optimization, hindering their establishment as routine methods. Moreover, given the crucial importance of hematoxylin and eosin (H&E)-stained tissue as a foundation of the patient's cancer diagnosis, we hypothesize that an ability to overlay the multiplex marker data directly on the H&E-stained slide used to establish the patient's diagnosis will be an essential need of the pathologist. Here, we demonstrate the use of the InSituPlex® (ISP) a novel, robust mIF assay technology reliant on conjugating DNA barcodes to antibodies and using sequence-specific DNA-DNA hybridization to label antibodies for spatial profiling of immuno-oncology targets in FFPE tumor tissue. Importantly, the process is automated on standard robotic autostainers in a workflow that mimics traditional immunohistochemistry (IHC). Multiplex detection is achieved through standard fluorescent slide scanners and data can be analyzed with a wide variety of software.

**Figure 1**



Using DNA barcodes to amplify weak signals, ISP generates a higher signal-to-noise ratio over other techniques, ensuring a sensitive and robust detection of any marker that can be detected by traditional IHC. (Figure 1)

One key attribute of InSituPlex's unique approach, nucleic acid hybridization-based signal amplification and detection, means the tissue only needs to undergo mild antigen retrieval conditions during the staining process. Importantly, precise co-registration of multiplex images with the same H&E-stained tissue section used for pathologic diagnosis allows the pathologist to directly correlate the appearance of every cell in the tissue section with its marker profile. (Figure 2)



Another important feature of the technology is the rapid time to results. Preoptimized 4-plex panels (and an updated 8-plex panel) representing key Immuno-oncology targets can be run on automated slide stainers, reducing overall assay run time from days to around five hours. Similarly, assay development times are reduced from months (using traditional immunofluorescence methods) to only 2-3 weeks, enabling researchers to progress from marker discovery to optimized assay in just a few weeks.

I am hopeful that technology advancements afforded by multiplexed immunofluorescence and InSituPlex® technology can integrate with the data-driven and knowledge-based approaches for an improved biomarker identification that has the potential to advance the performance of translational applications, and especially how we improve patient selection and design clinical trials to be more successful in the future.

A large, hollow, black-outlined arrow pointing to the right, positioned to the left of the main title.

# Profiling Cancer Biology

## Talk to us

With every solution that we offer, Ultivue is setting the new standard for mIF solutions. Get in touch with our team of experts and find out how we can help you reduce assay development time, assess cell phenotypes and understand spatial relationships across the whole slide.

