

Same-slide multiplex immunofluorescence and brightfield histological staining as a new research tool for fast and comprehensive pathology assessment of the tumor microenvironment



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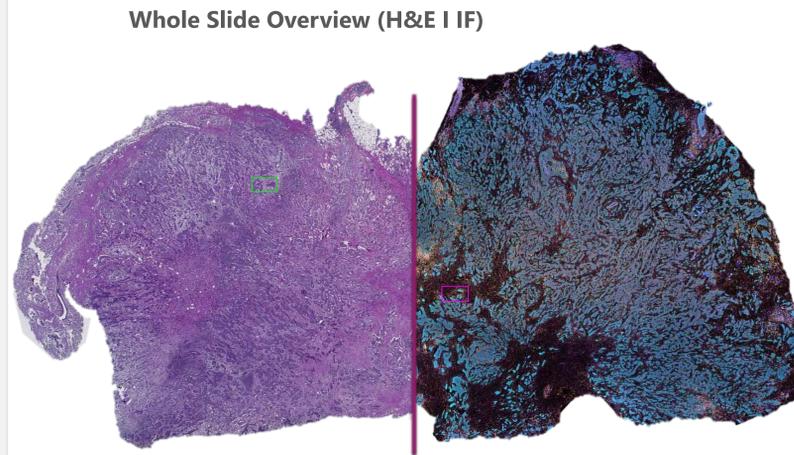
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Introduction:

Innovative and efficient translational research tools enabling a better understanding of the tumor and its microenvironment are critical for the development of digital pathology. Current immunohistochemistry (IHC) methods limit the depth of information from a single tissue sample to a single target in the case of chromogenic staining, or to sample morphology and general cell identification in the case of hematoxylin and eosin (H&E). Accurate phenotyping of each cell must be performed with a single section, as serial sections may not contain the same cells, especially small immune cells such as T cells. Multiplex immunofluorescence (mIF) methods have been established to provide insights into a wide number of markers of interest and their spatial context in a single sample. Here, we demonstrate a new research approach combining multiplexed detection of protein markers with standard H&E pathology review in tumor samples, in a streamlined, single-day sample-to-answer workflow.

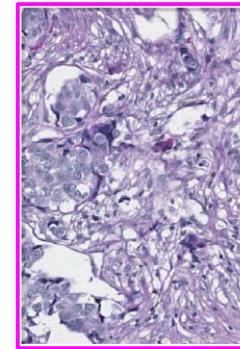
Whole Slide H&E and ISP Fusion: Tumor Assessment and Phenotyping



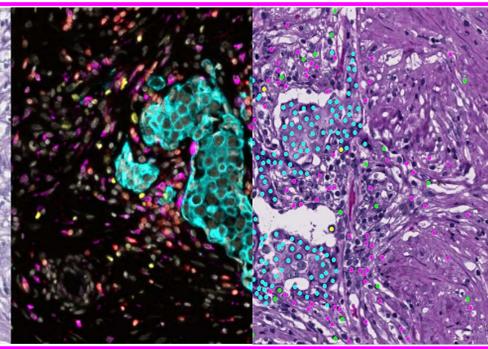
UltiMapper I/O reagents were used to stain a deidentified FFPE breast cancer tissue sample with 9 markers. After imaging, the same slide was stained with hematoxylin and eosin. Immunofluorescence and brightfield images were co-registered and fused using Ultivue's proprietary software. The resulting image allows for a traditional morphology assessment in parallel with deep phenotyping on the same tissue section.

Tumor distribution and immune infiltration can be assessed from the H&E, whereas cell subpopulation can be identified using the fluorescence data. Quantitative information can be obtained using image analysis and segmentation. Immune cell characterization can then be visualized on the fused image.

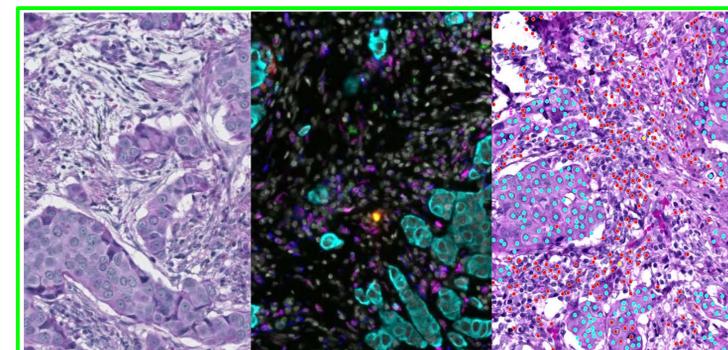
Morphology Assessment (H&E)



IF / ISP-H&E Fusion and Cell Type Identification



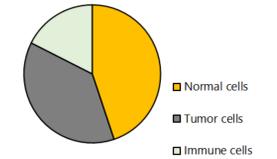
CK FoxP3 CD8 GrzB CD68 Tumor FoxP3+ Cells Cytotoxic Cells Macrophages



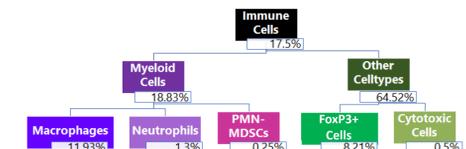
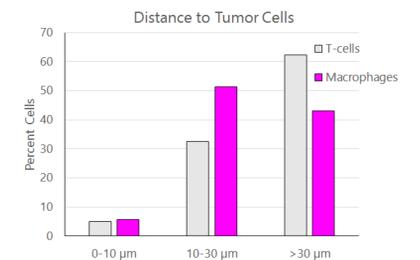
CK CD11b CD15 HLA-DR CD68 Tumor Myeloid cells PMN-MDSCs Macrophages

Whole Slide Image Analysis

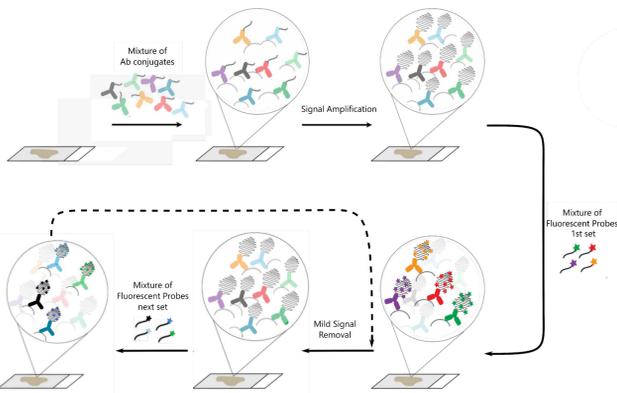
Morphology-based



Segmentation-based



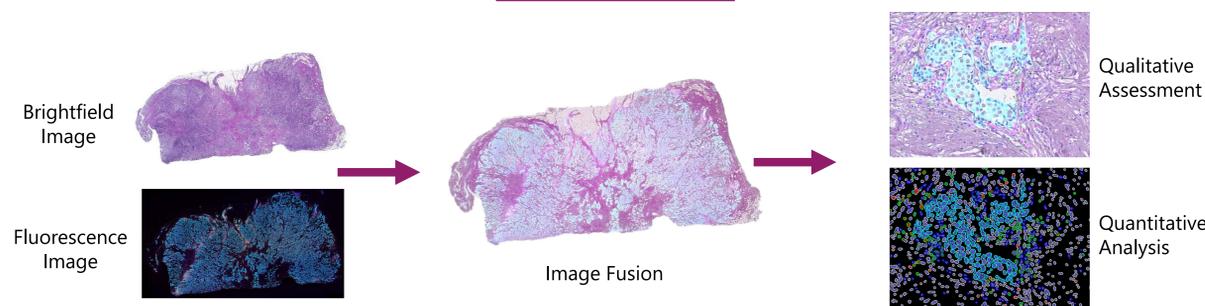
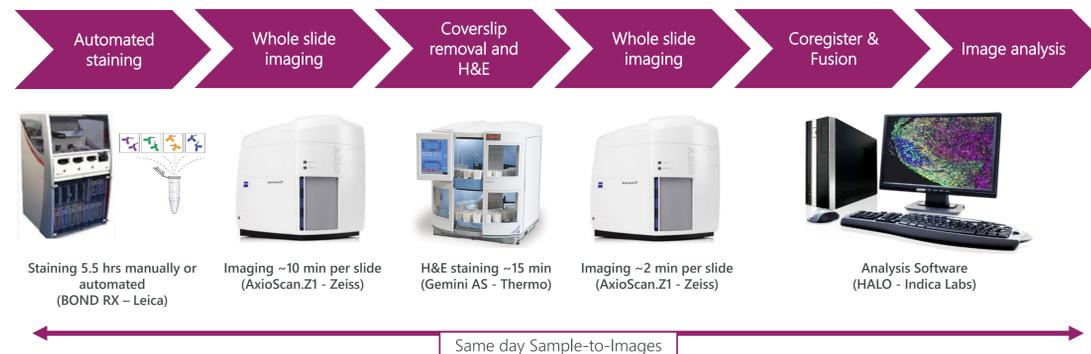
Methods:



Key Assay Features

- ▶ Easy staining protocol allows for a fast workflow
- ▶ Whole slide imaging capabilities for all markers
- ▶ Gentle signal removal preserves specimen morphology
- ▶ Workflow compatible with conventional imaging and software analysis platforms

ISP and H&E Fusion Workflow:



Conclusion:

The whole-slide H&E and ISP fusion workflow allows researchers to stack mIF images and H&E images from the same section into a single fused image available for tissue and cell analysis without the need for proprietary equipment or spectral unmixing.

This new workflow enables a quick and easy way to study high-plex, quantitative, cell-to-cell interactions the response of the immune system within the tumor microenvironment.

Combining the benefits of standard tissue morphology assessment with multiplexing allows researchers to benefit from fast qualitative assessment, deep phenotyping, and quantification of immune cells from the same section.

Key Technology Feature

Ultivue technology allows for fast and comprehensive analysis of the tumor microenvironment by combining novel mIF approaches with traditional staining methods on the same tissue section.

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