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Powerful background reduction in fluorescent tissue stains with an improved proximity-based technology for detection of protein-protein interactions

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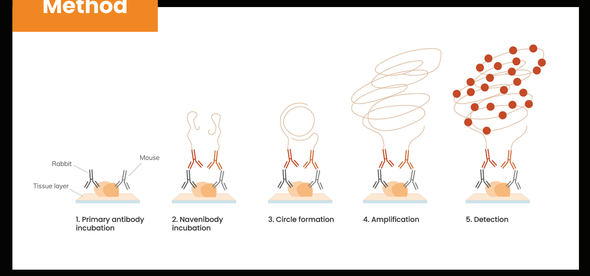
Introduction

Immunofluorescent staining of tissues via *in situ* proximity ligation assays is a well-established tool for highly sensitive detection of protein-protein interactions (PPI), post-translational modifications (PTM), and their localization. Standard immunofluorescence (IF) and *in situ* proximity ligation assays suffer from background signal originating from unspecific binding of fluorescently labeled detection reagents to certain types of cells (cell-specific background). As a result, it can be difficult to distinguish true biologically relevant signal from background signal. To address this problem, we developed NaveniFlex Tissue, a proximity ligation-based method which is highly optimized for tissue use. In contrast to previously available technologies, it can generate and visualize signal that would otherwise be obscured by background, thereby significantly increasing detection sensitivity.

Method



Conclusion

NaveniFlex Tissue enables the specific and sensitive fluorescent detection of PPIs and PTMs in FFPE and fixed frozen tissues originating from either human or mouse. It outperforms traditional proximity-based methods by efficiently reducing cell-specific background. This improves visualization of the signal and signal-to-noise ratios in various healthy and diseased tissues.

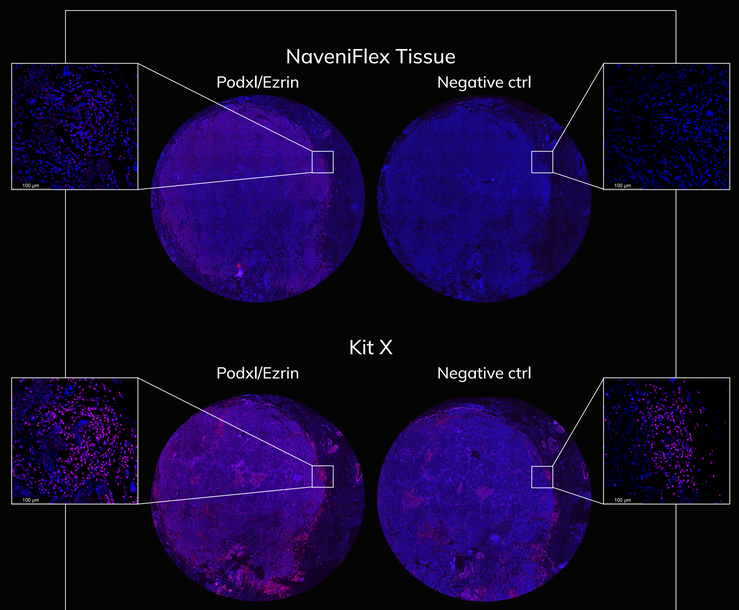


Fig. 1. Comparison of the ability of NaveniFlex Tissue and a commercial *in situ* proximity ligation kit X to detect Podocalyxin/ Ezrin in human breast cancer FFPE tissue. Kit X produced similar staining patterns in both the positive Podocalyxin/ Ezrin staining and the technical negative control. The high background in both images obscures any specific interaction signal. In contrast, NaveniFlex Tissue visualizes the interactions in the positive staining clearly (discrete red specks) and leaves the technical negative control blank.

Fig. 2. Comparison of the ability of NaveniFlex Tissue and a commercial *in situ* proximity ligation kit X to detect Podocalyxin/ Ezrin in the glomeruli of healthy human kidney FFPE tissue. Specificity is increased and background is decreased when using NaveniFlex Tissue vs kit X.

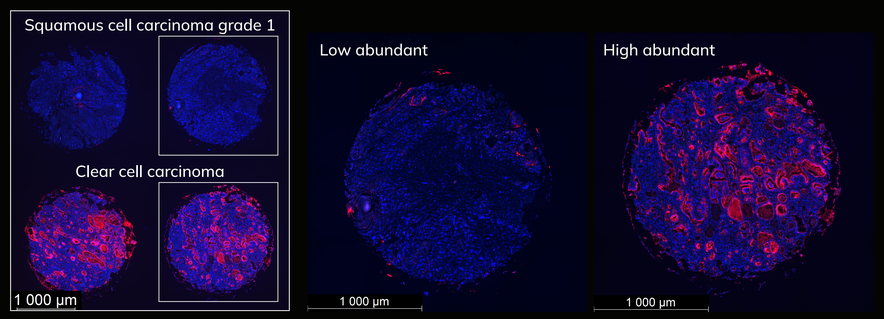


Fig. 3. Mucin/Mesothelin interaction in low and high abundance cores in an ovarian cancer TMA. The binding of Mesothelin to Mucin-16 contributes to the metastasis of ovarian cancer to the peritoneum, which is a sign of malignant progression. Therefore, the presence of the interaction may have prognostic significance in determining patient relapse-free survival. NaveniFlex Tissue successfully stains both sparse (grade 1 squamous cell carcinoma) and highly abundant (clear cell carcinoma) interactions (see zoom-ins).

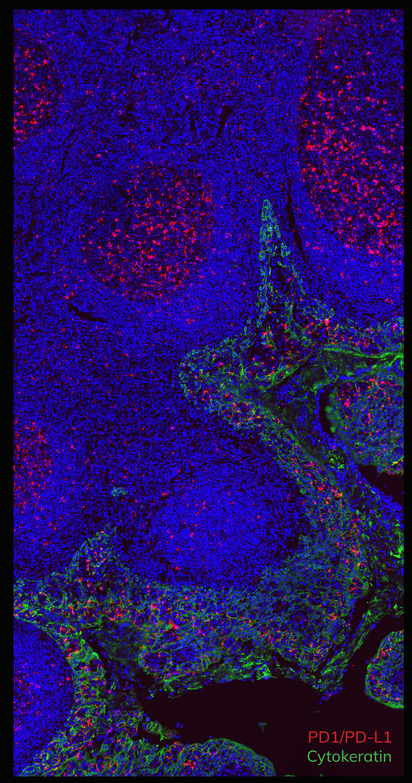


Fig. 4. PD1/PD-L1 interaction detected in human FFPE tonsil with NaveniFlex Tissue. The PD1/PD-L1 interaction (red) has important implications in immunooncology and has been notoriously difficult to detect with older proximity ligation methods. NaveniFlex Tissue, however, detects this prognostically important interaction. IF cytokeratin co-staining in green.

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