



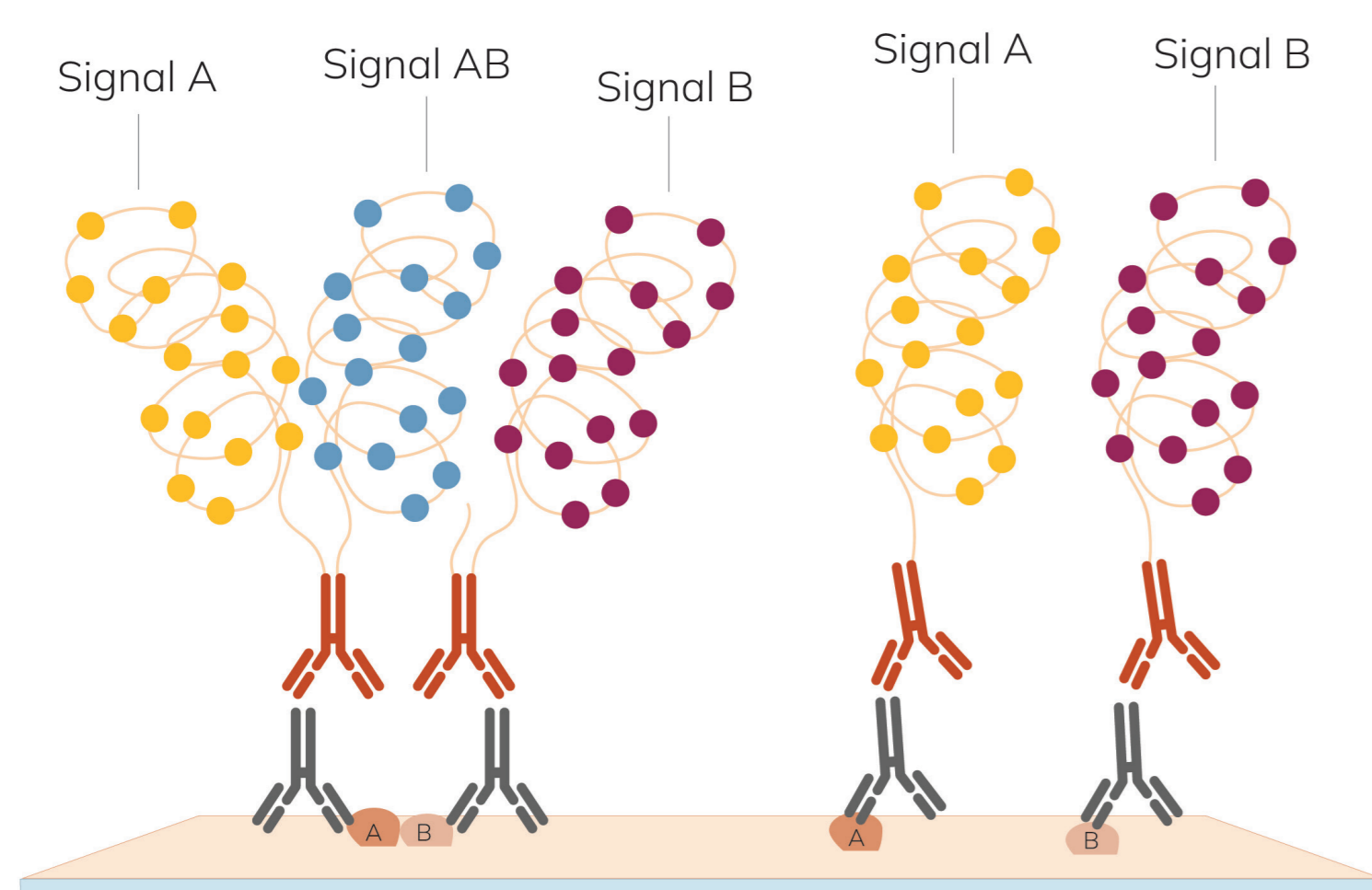
Naveni TriFlex Cell: An emerging method for simultaneous detection of free proteins and their interactions

Authors

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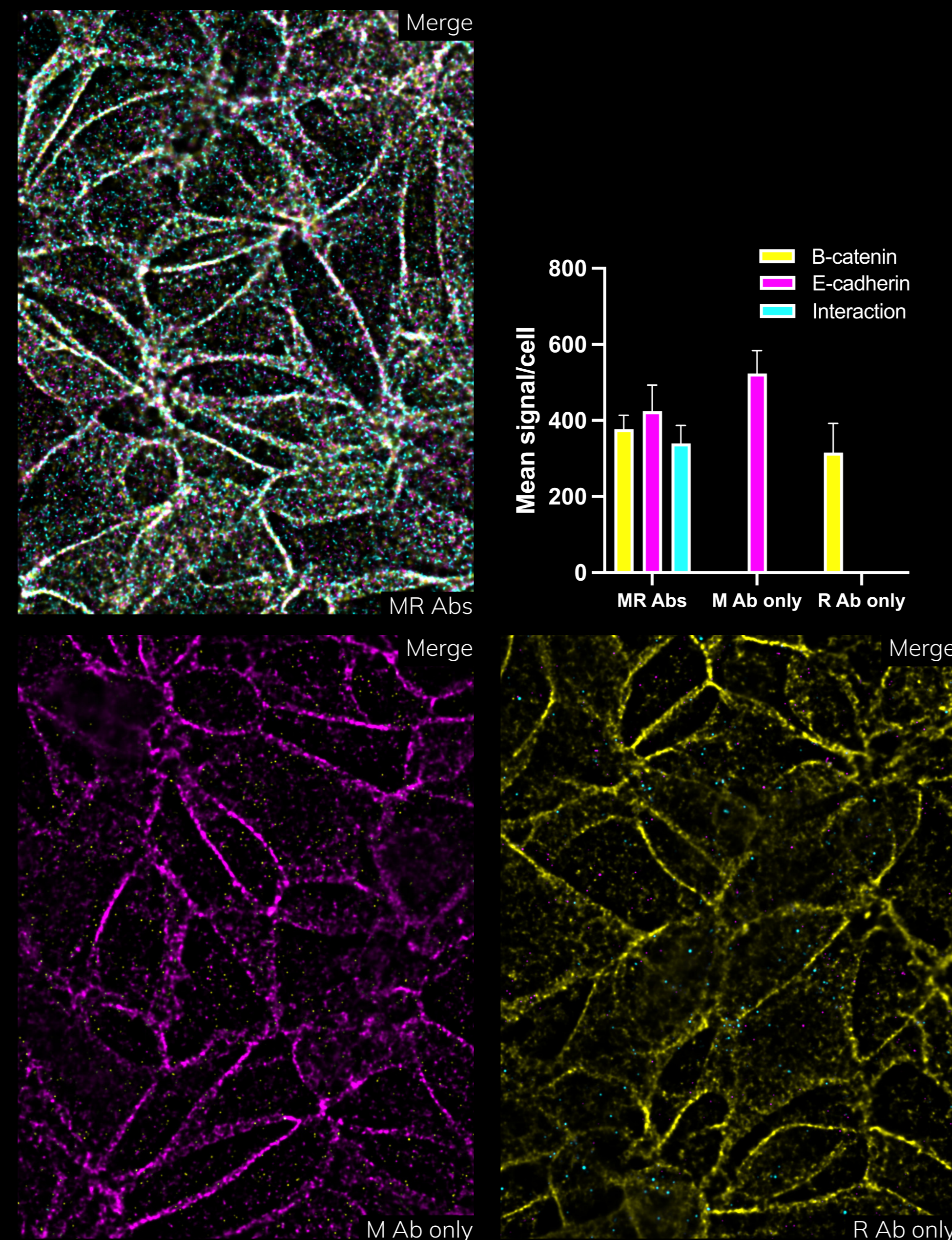
Introduction

Protein-protein interactions (PPIs) are essential for the performance of cellular functions and biological processes. The already challenging task of PPI identification can be taken a step further by simultaneously visualizing the pool of proteins which do not participate in the interaction. The possibility to take a snapshot of two proteins as they come together or remain unbound opens the door to studying their functional states and interplay in response to stimulation/ inhibition, cell division, protein degradation, and more.



Naveni™ TriFlex Cell can simultaneously detect two proteins as they interact or remain unbound

TriFlex is a proximity ligation-based method which detects total protein A (both unbound and interacting, yellow), total protein B (purple), and the AB interaction (blue). Only proteins located at <40 nm distance are recorded as interacting. The detected A, B and AB signals are amplified and generate fluorescent readout in three channels corresponding to each protein pool.

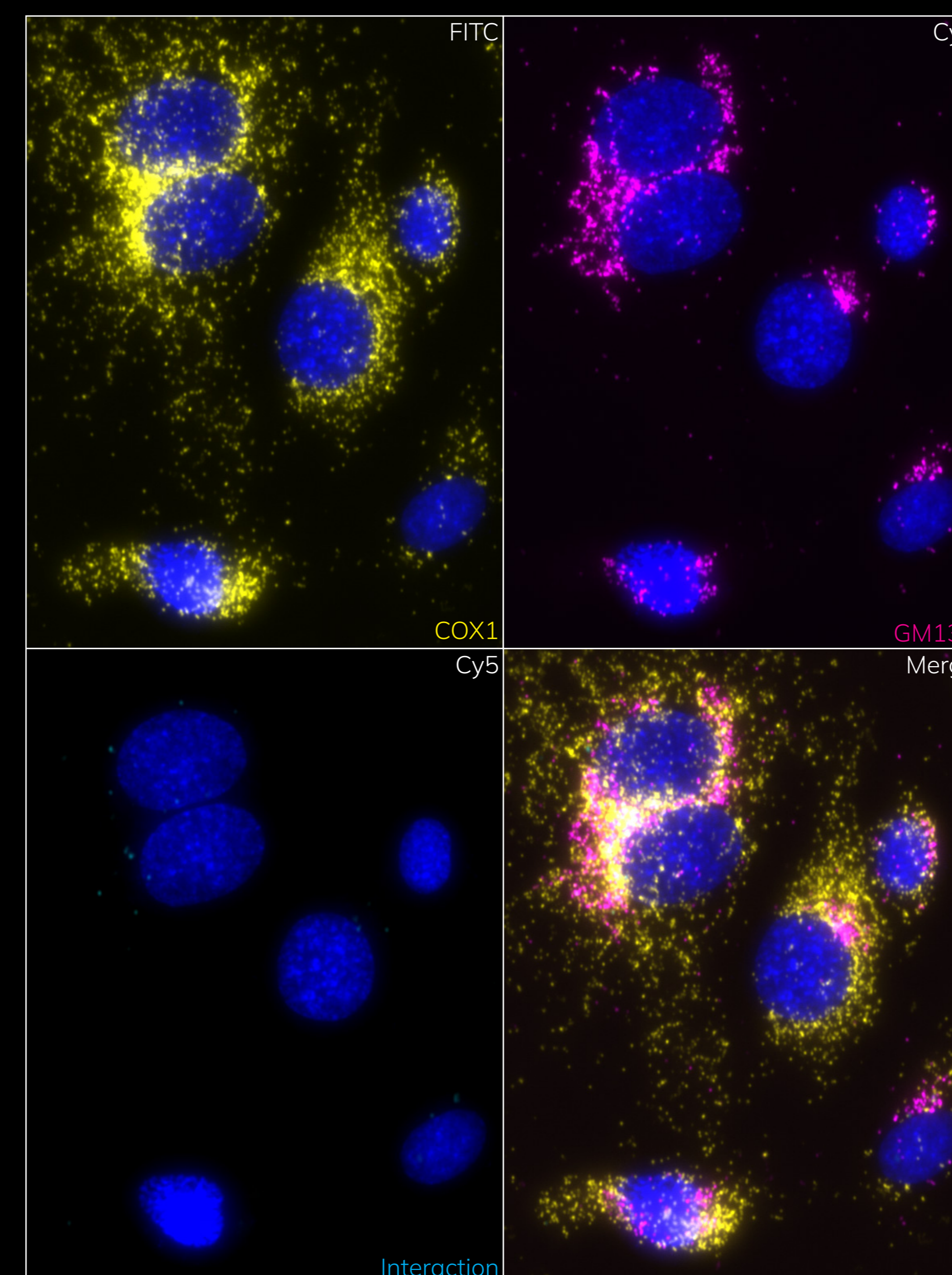


Simultaneous detection and quantification of total and interacting β -catenin and E-cadherin

E-cadherin (magenta, "protein A") and β -catenin (yellow, "protein B") are localized in the cell membrane and cytoplasm. E-cadherin/ β -catenin complexes (teal, "interaction AB") play an important role in tissue fibrosis, cancer progression and maintenance of epithelial integrity by participating in the formation of the adherens junctions. In each assay, different primary antibody combinations were used with all kit components to show specificity of detection. MR Abs = both primary antibodies (positive staining). M Ab only = E-cadherin only (technical control). R Ab only = β -catenin only (technical control). 40x magnification, deconvolved.

Co-occurrence of fluorescent signal is not a synonym for protein interaction

COX1 (yellow) is a mitochondrial marker that does not interact with GM130, a Golgi marker (magenta). The two proteins are localized in different cellular compartments, yet they can appear as partially co-localizing (white) in regions where signals are particularly dense (see Merge). However, TriFlex correctly detects no interaction (absence of teal signal, see split image in Cy5 channel). Due to the proximity restriction when detecting an interaction, TriFlex achieves higher resolution compared to what is possible with regular co-staining in widefield microscopy. 40x magnification.



Interplay between Histone H3 and Lamin B1 in different stages of the cell cycle

In mitotic cells, Lamin B1 signals (yellow) are diffuse due to nuclear envelope breakdown, whereas Histone H3 (magenta) remains around the metaphase plate. Lamin B1/Histone H3 interactions (teal) have disassociated.

In non-dividing cells, Lamin B1 is localized in the nuclear envelope, and multiple Lamin B1/Histone H3 interactions are observed in the nucleoplasm together with chromatin-bound Histone H3.

During the initial stages of oncosis, the dying cell's nucleus swells and the nucleoplasm contains fewer interactions along with unbound Lamin B1 and Histone H3.

Conclusion

Naveni TriFlex Cell is the first proximity-based technology that not only detects protein interplay, but also provides quantitative information on each interacting partner, individually and as part of the interaction. Subtle shifts in the protein states (bound vs unbound) can be observed and measured under various conditions, while the spatial information of their subcellular localization is retained.