Abstract number: 209

Fully automated *in situ* detection of protein-protein interactions in tissue

Authors
Hampus Elofsson, Linda Arngården, Olof Hahne, Tanja Büsgen, Lena Beckman, Agata Zieba-Wicher

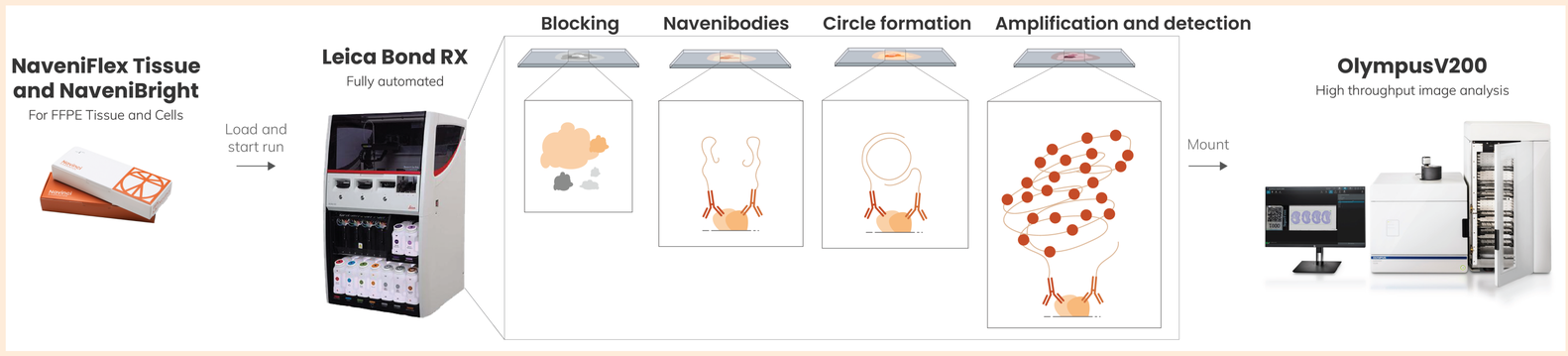
Navinci Diagnostics, Research and Development, Uppsala, Sweden. contact@navinci.se

Background

Detecting protein expression is an important yet unidimensional approach to understanding tissue microenvironment, cell function and communication. Revealing protein-protein interactions (PPIs) enhances our understanding of the tissue milieu since PPIs are essential for the performance of biological processes such as cancer-relevant signaling cascades, immune checkpoint activation, response to drug treatments, protein degradation, etc. The Naveni® in situ proximity ligation technology can detect proteins located within an interaction range (<40 nm) by means of antibodies conjugated to oligonucleotides that create amplified fluorescent or chromogenic signal if the proximity requirement is fulfilled. Since this method does not compromise the structural integrity of tissues, the communication between proteins can be studied in their native environment. Here we present the automation of the Naveni® technology on Leica Bond RX.

Technology and Methods

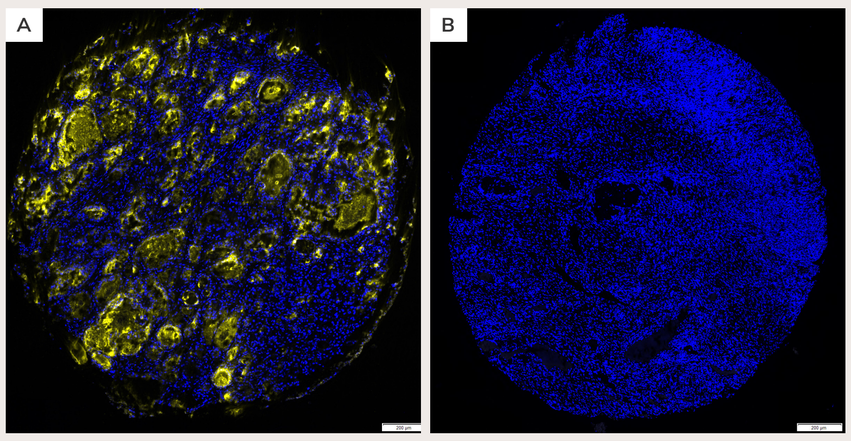
Human FFPE tissue sections or microarray slides were pre-baked at 60°C for 45 minutes and loaded onto a Leica Bond RX together with either NaveniFlex™ Tissue or NaveniBright™ reagents optimized for automated use. In the Leica Bond instrument, the slides were deparaffinized using a standard protocol, followed by antigen retrieval with ER2 for 40 minutes at 100°C. The instrument was then programmed to perform the Naveni® workflow, i.e., blocking, primary antibody and Navenibody incubation, ligation and rolling circle amplification followed by detection with either chromogenic or fluorescence readout. Detection was performed on an Olympus scanner VS200.



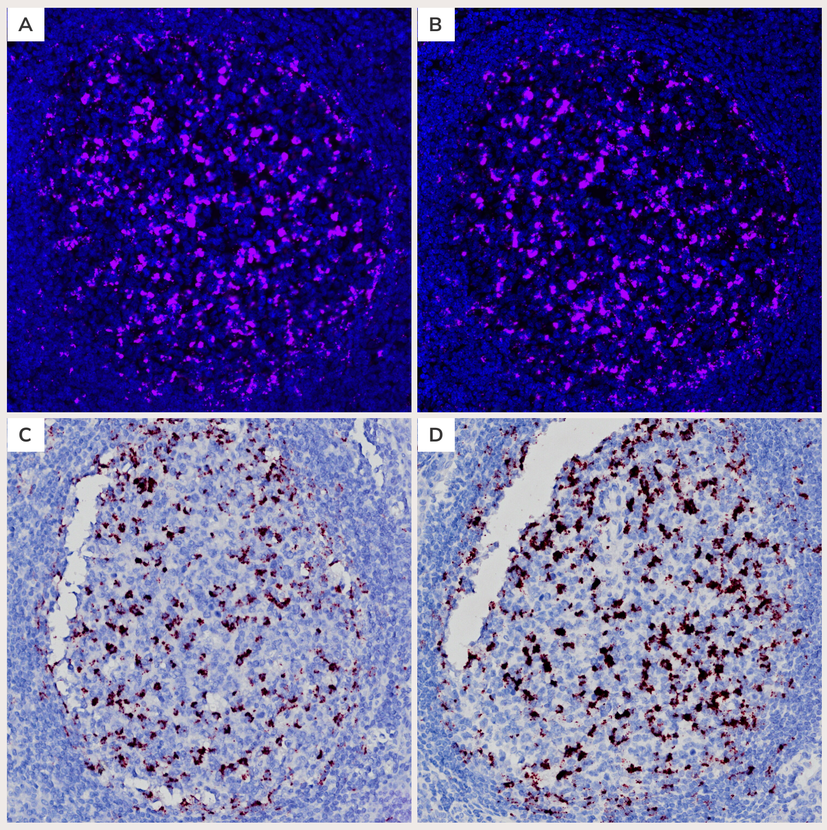
Schematic representation of the automated Naveni® workflow.

Results

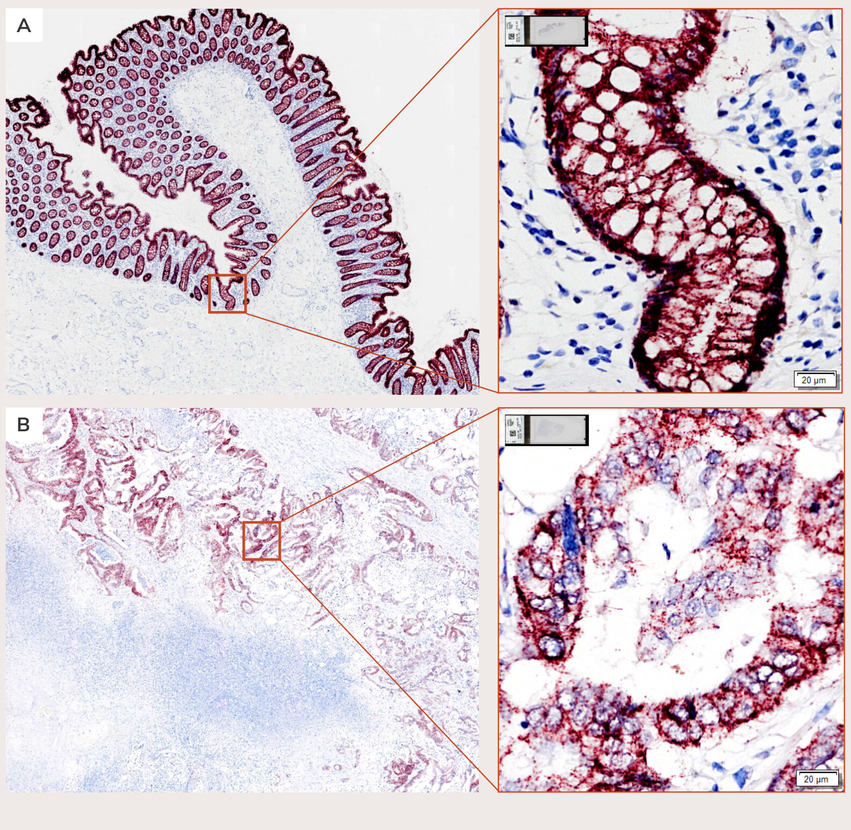
We developed a fully automated workflow for the Naveni® *in situ* proximity ligation technology for studying PPIs. The workflow allows for several slides to be stained in the Leica Bond RX instrument in a single run, and then scanned in an automated fashion. Three interactions playing key roles in different stages of cancer were investigated in the same run. PD1/PD-L1, E-cadherin/β-catenin and Mesothelin/Mucin interactions all showed differential staining in healthy vs cancer tissue. In addition, automated and manual staining produced congruent results.



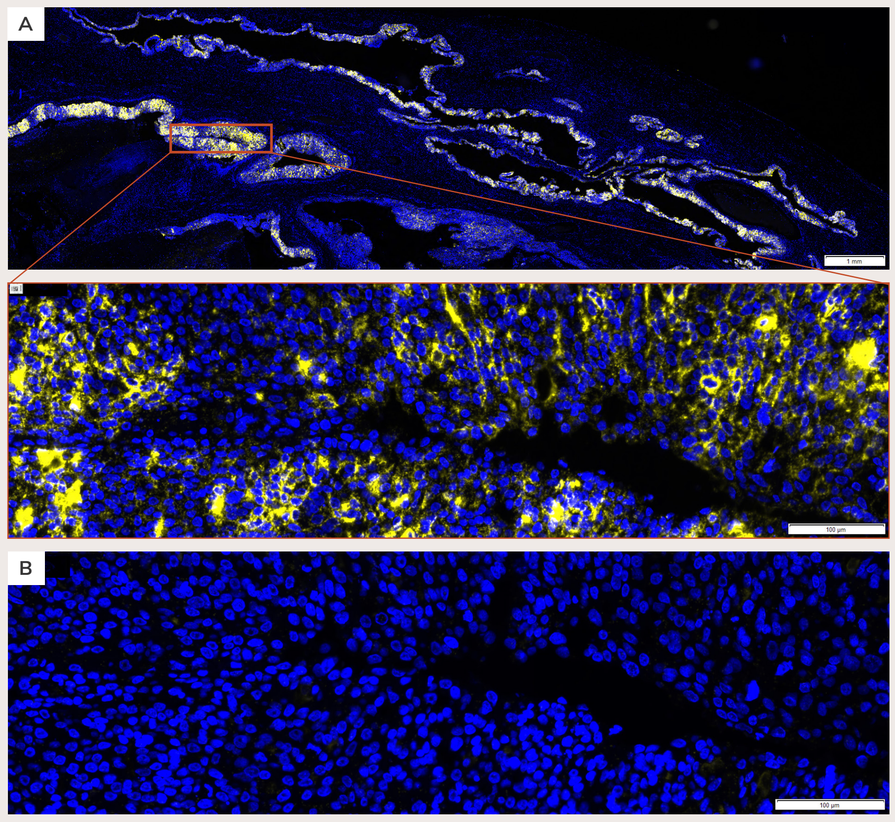
Automated detection of Mesothelin/ Mucin interaction in ovarian clear cell carcinoma **(A)** and normal ovarian tissue **(B)** with NaveniFlex™ Tissue Atto647N. Normal tissue is negative for the interaction.



Manual **(A,C)** and automated **(B, D)** detection of PD1/PD-L1 interactions in tonsil with Naveni® PD1/PD-L1 produce comparable results with both fluorescent (**A, B;** Atto647N) and brightfield **(C, D;** HRP) readout. Multiple interactions in the germinal center are observed.



Fully automated detection of E-cadherin/β-catenin interactions in healthy colon tissue **(A)** and in colon adenocarcinoma **(B)** with NaveniBright™ HRP. Disruptions of cell-cell contacts due to the diminished number of interactions are clearly visible in the cancer tissue.



Automated detection of Mesothelin/Mucin interaction in ovarian serous carcinoma (A and zoom-in below) and technical negative control **(B)** with NaveniFlex™ Tissue Atto647N. Image **A** shows a whole slide with multiple interactions in the cancer tissue, whereas image **B** is negative for interactions and background-free.

Conclusions

Proximity ligation is an ultrasensitive technology for observing PPIs in the context of a preserved tissue architecture, thus illuminating complex biological processes in a manner unattainable with standard immunostaining methods. To widely adopt it and unfold its potential as a diagnostic tool, the method must be robust and high-throughput. Applying the Naveni® technology to tissue slides in an automated manner via the Leica Bond fully automated stainer contributes to a shortened workflow, higher processivity, and reproducibility, and answers the demands on high throughput without compromising sensitivity. This makes proximity ligation technology a promising tool with a great potential for clinical and diagnostic applications.

Come find us at booth 919 for further discussions

Have more questions?
contact@navinci.se

Download the poster here:

Visit
www.navinci.se