Background

Immuno-oncology and targeted molecular therapies have acquired a central role in the treatment of multiple cancers. Consequently, high-throughput biomarker analysis and tumor immunoprofiling have seen an increased demand. Multiplexed immuno-assays are a powerful tool to address these needs, yet time- and resource-consuming. With this study we show how microfluidics can improve those techniques by providing faster and better results.

Objectives

The goal of this study is to develop a fast and automated 6-plex assay plus counterstaining, with high-quality signal, staining uniformity across the tissue and performant elution.

Methods

Marker panel and materials

- **Tissue**: FFPE non-cancerous tonsil
- **Counterstaining**: DAPI

Tyramide Signal Amplifications (Opal® kit)

- **Detection**: Primary Ab → HRP enzyme → Tyramide fluorophore
- **Amplification**: HRP → Reactive tyramide-fluorophore
- **Elution**: Heating

Multiplexing workflow on LabSat™ automated stainer

1. **Tissue preparation (Deparaffinization)**
2. **Reagents/Buffer loading on device**
3. **LabSat™**
4. **Mounting & Coating**

Quantification tools

- **InForm™**
- **Spectral Unmixing**
- **Cell Segmentation**

Results

Single-plex optimization

Following a monoplex optimization, a bright and specific signal as well as a high signal-to-background ratio can be achieved with LabSat™ for each marker. The robust system along with automated protocols offer a high degree of consistency in results, proven by a reproducibility study on each marker.

Uniformity

LabSat™ technology enables fine control over reagent dispense on the tissue, which allows to reach only 6% lateral signal gradient over 1cm versus 31% for standard incubation. This is key for image processing purposes with digital pathology tools such as signal quantification.

Antibody strip-off

The elution step on LabSat™ consists of an active heating-cooling cycle after each marker detection, ensured by a heating element placed below the tissue slide. Elution assessments resulted in 99% removal of the signal for each antibody.

Preserved tissue morphology

Ultra-rapid incubation times limit the exposure of tissue to harsh conditions, which allows the tissue to be highly preserved, including nuclear morphology and epitope detection, even after repeated elution cycles.

Conclusions

LabSat™ autostainer enables 6-plex multistaining runs in a timely manner, opening the perspective of including tumor microenvironment screening in routine diagnostics. Moreover, the control over staining parameters provided by the microfluidic technology delivers high-quality results and very good reproducibility.