

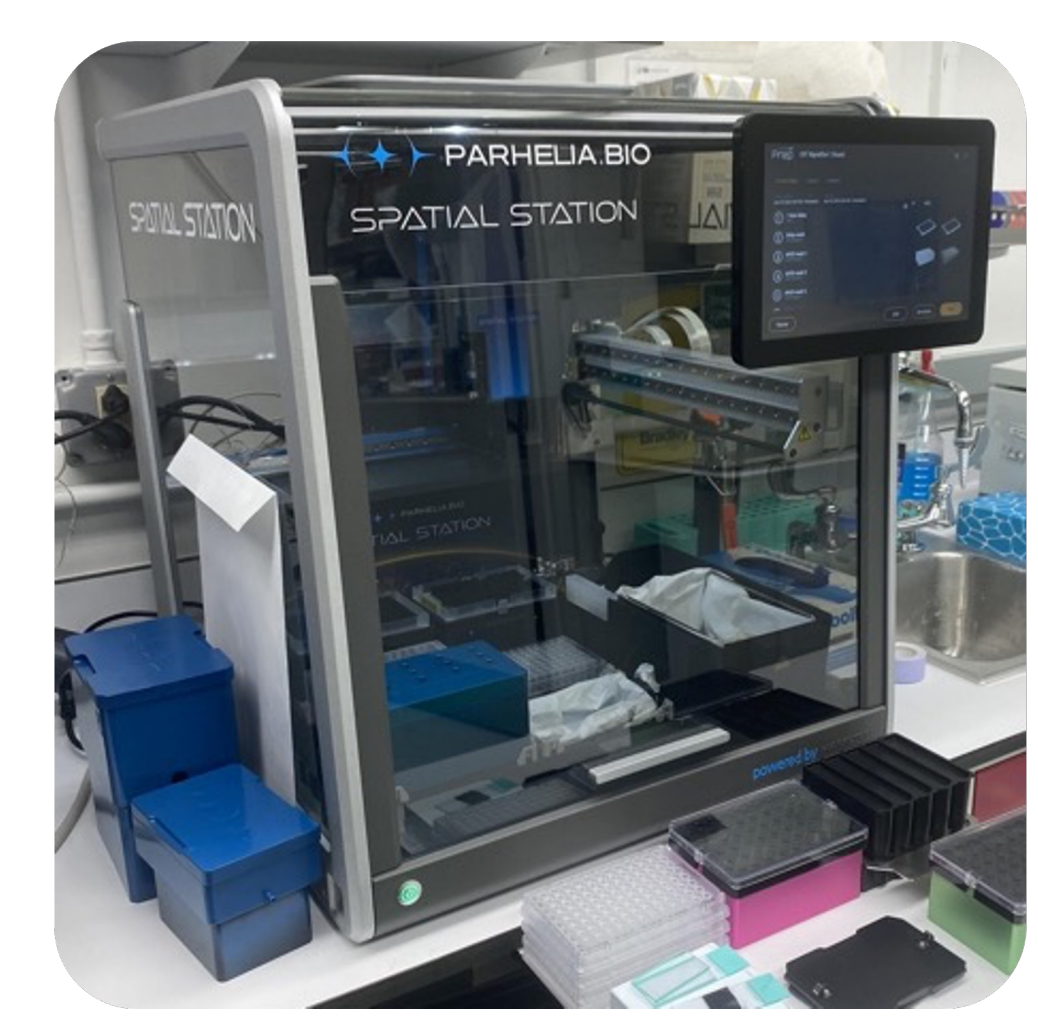
Background

- In recent years, an increasing number of multiplex immunofluorescence (MxIF) technologies have emerged to detect 40+ protein markers in tissues at the single-cell level. These allow for deep characterization of the tumor state and microenvironment, providing insights into tumor behavior, response to therapy, and disease outcome.
- Spatial proteomics** was named the method of the year in 2024, recognizing its transformative potential in revealing the organization of complex tissues and advancing biological research. However, manual handling and tissue processing limit the clinical impact of this cutting-edge technology.
- The workflow of current MxIF methods is complex, comprising multiple staining, fixation, and washing steps. Many protocols apply cyclic staining and signal removal, often without signal amplification, and use sequential imaging, which results in less robust assays and low throughput. One option to increase throughput is to stain and image multiple slides in parallel.
- Automating these assays on reliable auto-stainers like Parhelia Spatial Station (PSS) or Leica BondRx, combined with optimized imaging on slide scanners like Zeiss Axioscanner, would increase the accessibility of these technologies for basic and translational research.

Goals

- Here, we report using **Parhelia Spatial Station** for the automation of different spatial proteomic applications to better serve the research community by:
- saving time and reagents and thus decreasing cost
 - assuring uniform staining, increasing reproducibility and throughput

Parhelia Spatial Station is a universal platform capable of end-to-end sample prep automation



Parhelia Spatial Station at SpITR

Benchtop flexibility:

- State-of-the-art robotics
- Temperature control, 0–100°C
- Open chemistries
- Flexible throughput

Capillary Laminar Flow Technology:

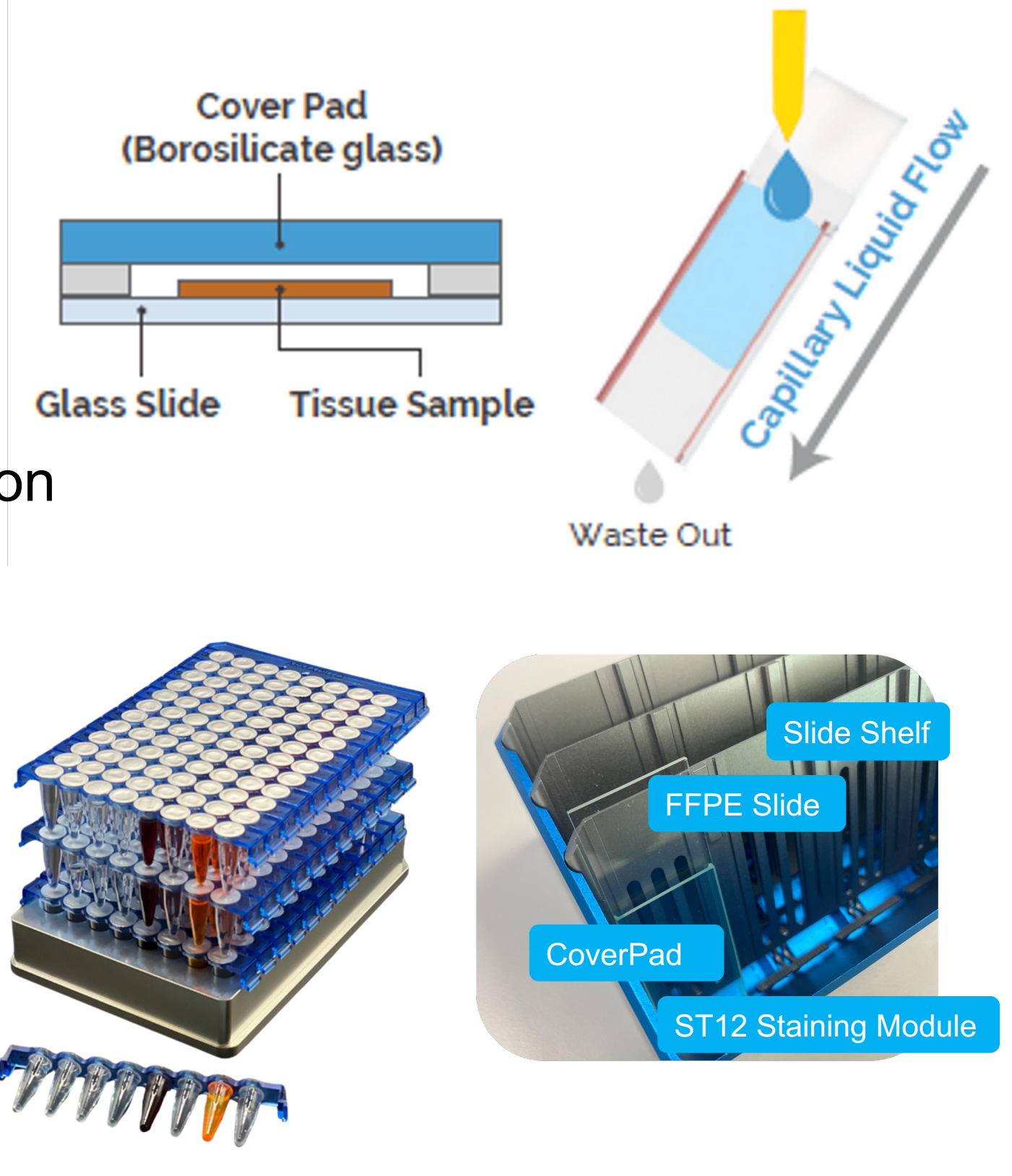
- Gently and uniformly applies and exchanges staining reagents without edge or bubble artifacts
- Protects specimens from dehydration, shear, and delamination
- Reduces reagent usage by 50%
- Improves S/N up to 10%

Skylab kits:

- Single-use, 96-well format
- One strip of 8 reagents per sample
- Dewax/HIER
- Dewax/H&E

Automate any assay:

- Spatial -omics/multi-omics
- Immunohistochemistry
- Custom protocols & optimization



Methods

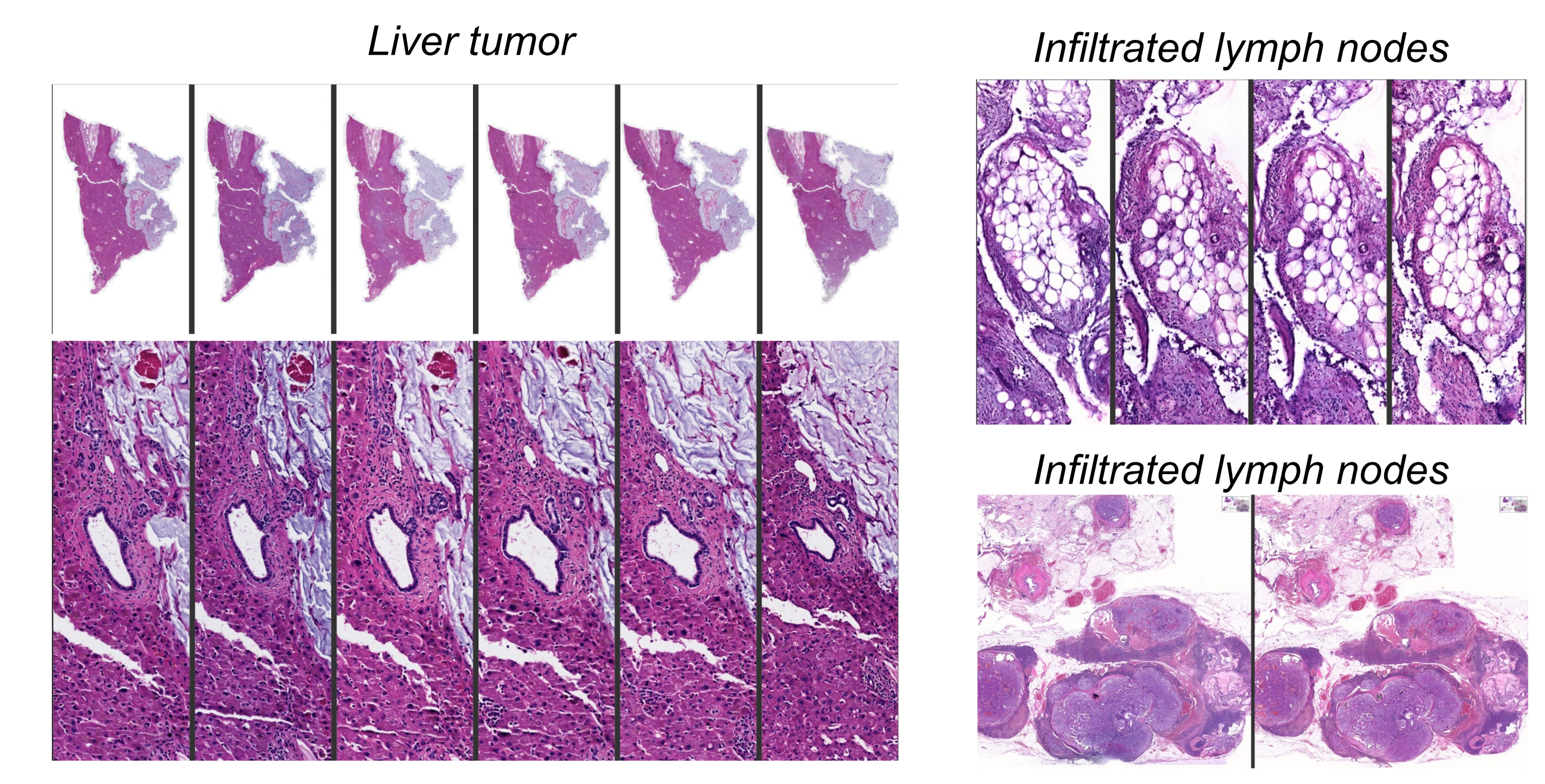
- H&E staining**
 - For classical pathology annotations using Skylab kit
 - After multiplex staining
- Dewax and Antigen retrieval**
 - Flexible protocol for temperature, duration, antigen retrieval solution (HIER)
 - Samples can be processed with different antigen retrieval solutions and their combinations
- Application of image-ready cover tiles for multi-cycle assays**
 - Ultivue, SignalStar: preserves the tissue, decreases cycle time
 - CODEX technology
 - Testing custom-conjugated antibodies
 - Parallel imaging on slide-scanner to increase throughput**

Results

1. Hematoxylin and Eosin staining

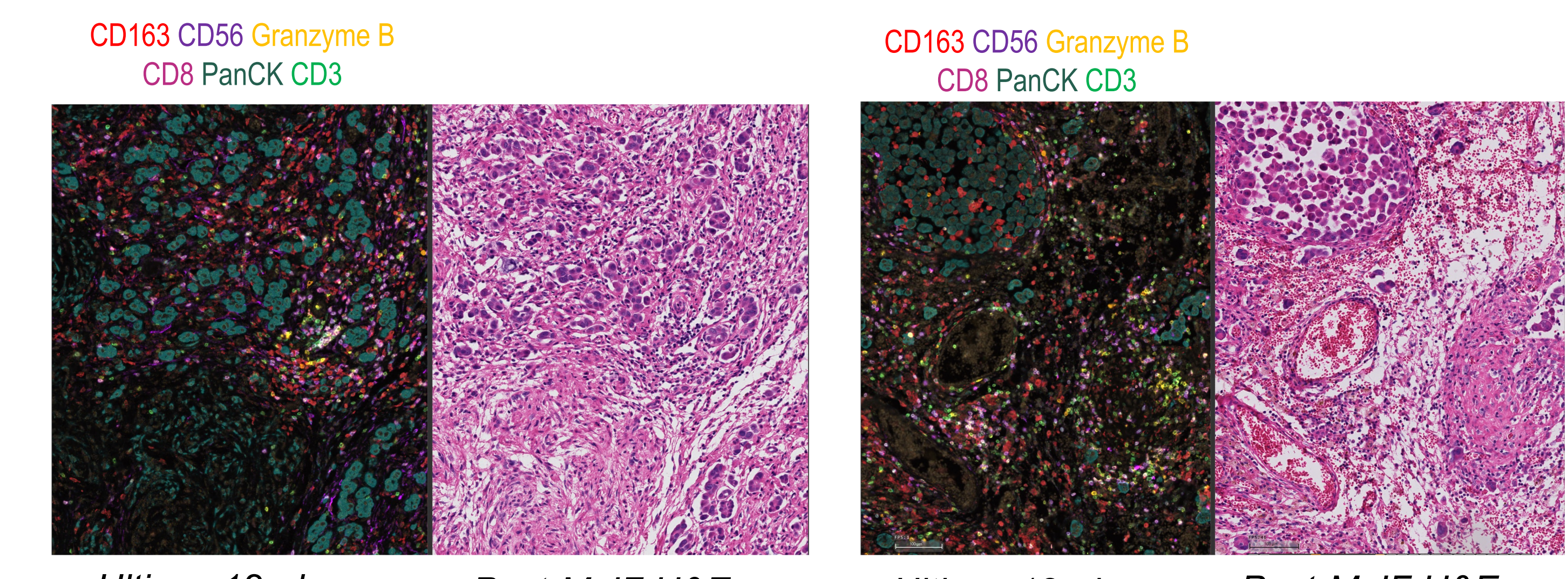
H&E staining is a fundamental technique in histology and pathology used to visualize cellular and tissue structures. It aids in the diagnosis of diseases and understanding biological processes. It has been used for more than a century worldwide. Even if the staining looks simple, a variety of reagents and protocols are available.

Staining of 6 different tissues with H&E Skylab kit: uniform dewax and staining with good cellular details, even in challenging fatty tissues



Images scanned on Zeiss Axioscanner 7

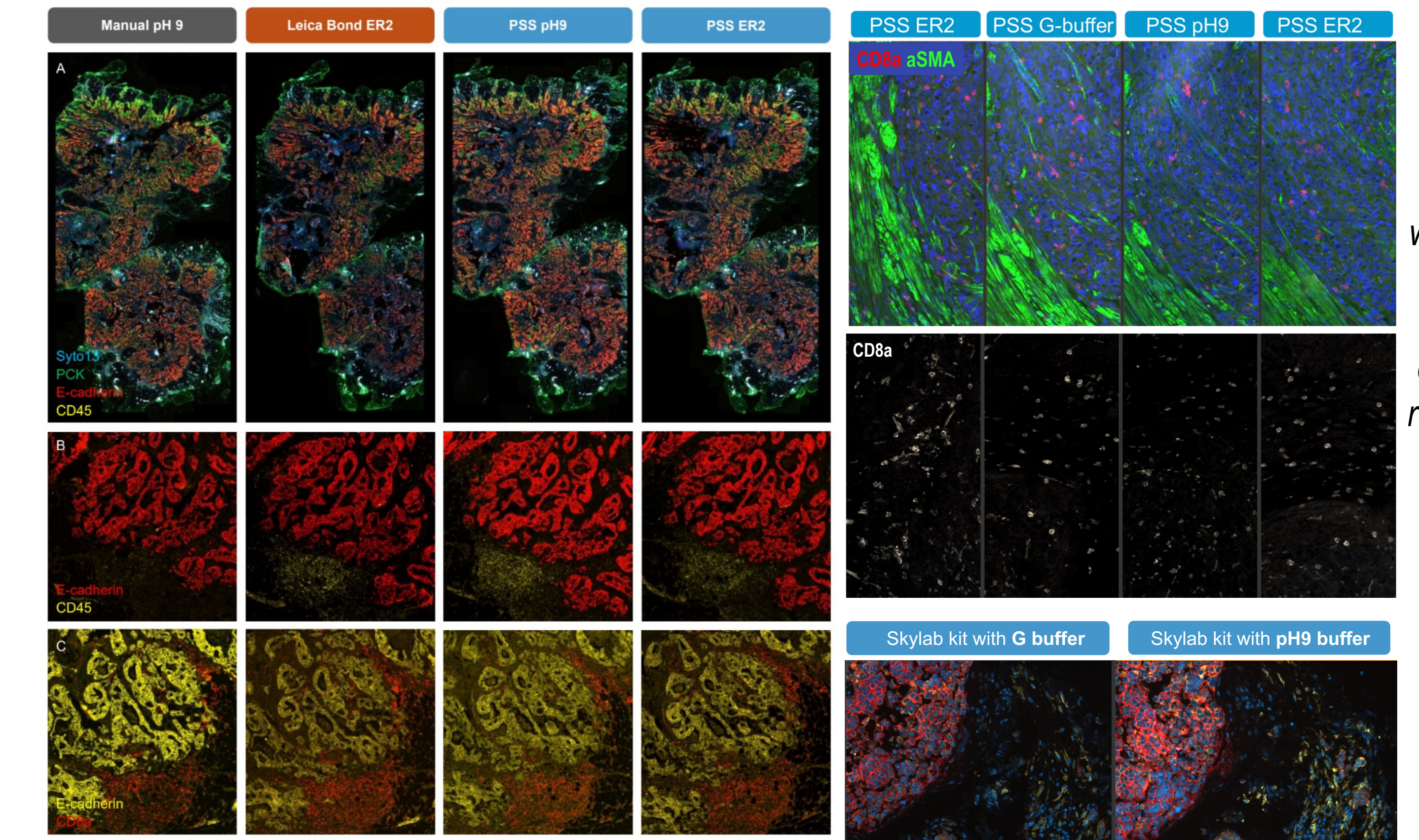
H&E staining after multiplex assays becomes a standard: after image registration, pathology annotations are done on brightfield images (different PSS protocol)



Images scanned on Zeiss Axioscanner 7

2. Dewax and antigen-retrieval

Dewaxing and HIER are essential components of all spatial proteomics and transcriptomics technologies when working with FFPE tissues. Retrieving epitopes is necessary to enable antibody binding. Antibodies function best under specific temperature and pH conditions. The PSS protocol is designed for flexibility, allowing comparison between different conditions and even combining high and low pH retrieval for optimal staining.



Liver cancer tissue stained with DAPI, aSMA and CD8A comparing four different antigen retrieval solutions

Colorectal cancer tissue stained with Syto 13, PanCK, E-cadherin and CD45 after dewax and HIER in four different conditions

Tumor tissue stained with DAPI, PanCK and EPCAM after using dewax and HIER Skylab kit

3. Application of image-ready coverpads for multi-cycle assays

Removing coverslips in multi-cycle assays can damage the tissue and increase the assay time. Image-ready CoverPads allow for cyclic staining and imaging without needing to remove the CoverPads, thus allowing for improved assays with better images in a shorter time. Image-ready CoverPads have been tested for 3-cycle Ultivue and 2-cycle SignalStar assays with good results.

Here, we compare cyclic image reveal of CODEX staining imaged on a Zeiss Axioscanner compared to imaging on Phenocycler Fusion (PCF). PCF images have background subtraction, unlike Zeiss images.

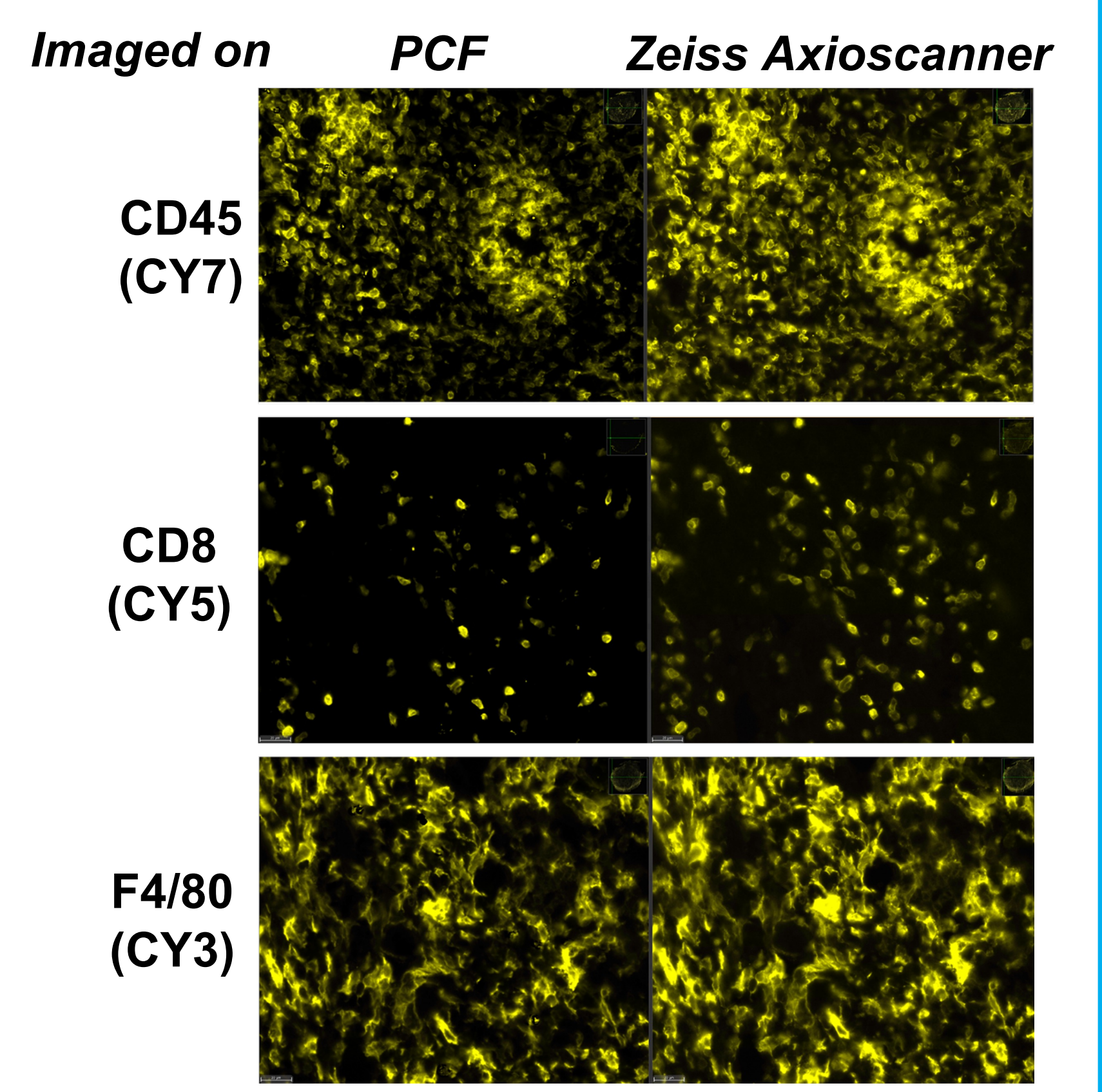
Mouse fresh frozen tissue was stained with 12 CODEX antibodies on PSS using Image-ready CoverPads.

Reporters were added using PSS reveal protocol and imaged on Axioscanner.

The reporter staining was repeated after imaging with another set of reporters.

Unlike on PCF, cycles can have 4 reporters on the FITC, CY3, CY5, and CY7 channels for tissues with little autofluorescence.

Multiple slides can be imaged in parallel compared to 2 slides on PCF.



Conclusions

- The Parhelia Spatial Station is a versatile platform capable of end-to-end sample prep automation.
- Multiple protocols are optimized on the Spatial Station for different spatial proteomics and transcriptome technologies.
- The platform is highly flexible to accommodate custom modifications on protocols for special needs.
- The Skylab kits (H&E and HIER) are ready-to-use reagents for different applications.
- The Image-ready CoverPads improve the quality of and save time for multi-cycle assays: Ultivue, SignalStar, CODEX technology

Learn more at:



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