Validation of an automated PhenoCycler-Fusion slide staining protocol on the Parhelia Spatial Station™

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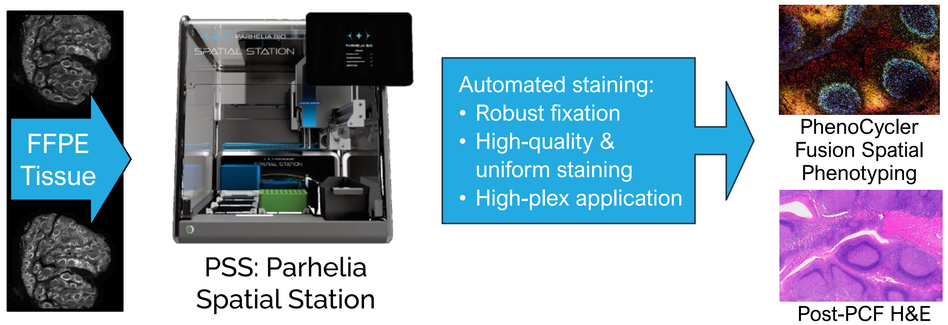
Abstract

Background: Spatial biology encompasses a dynamic and growing menu of assays for interrogating the tissue microenvironment at single-cell resolution. Despite these techniques' increasing multiplexing and multiomic capabilities, the multistep sample processing and staining workflows are mainly accomplished manually.

Experiment and Data: Using the Parhelia Spatial Station (PSS), we automated the staining protocol for PhenoCycler Fusion (PCF), a popular spatial biology proteomics assay.

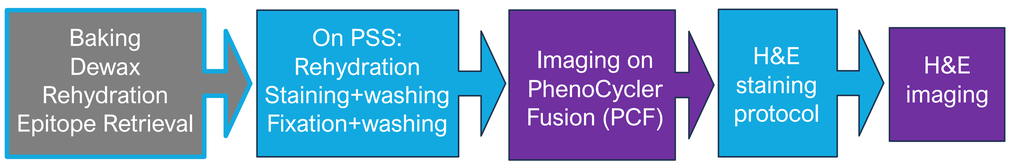
- Robust fixation and equivalent staining of two tonsil specimens on the same slide demonstrate the reliability and staining uniformity of the automated method, respectively.
- Parallel manual and automated multiplexed staining of tonsil samples yield equivalent staining quality.
- Automated staining of a high-plex panel reveals high-quality spatial topology of tonsillar structure, lymphocyte, and myeloid cell contexture.
- Automation of the hematoxylin and eosin (H&E) staining protocol after PCF imaging permits multiplexed imaging and morphology visualization on the same specimen.

Conclusion: These results validate the automation of the PCF staining workflow, enabling assay standardization for immunological and other discovery and translational spatial biology applications.



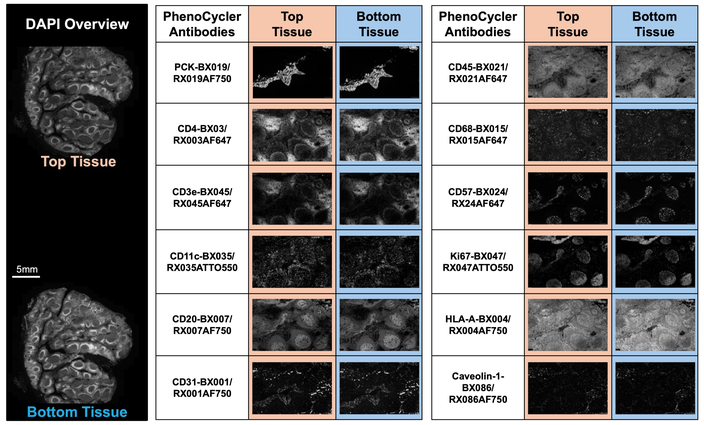
Experimental Setup

The manual processing and staining protocol for preparing FFPE samples for PCF imaging was recently published, and the methodology described here reflects that procedure unless otherwise noted.[2] The method utilizes Copley jars for buffer incubations and washes and the application of parafilm coverings during the long antibody staining step. Each step (~60 steps) requires the user's full attention and careful execution; noncompliance or timing differences can impact the reproducibility of the study.



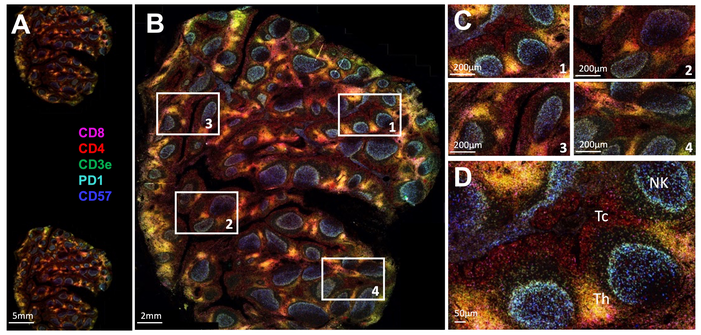
Automated staining on the PSS involves assembling a capillary reaction chamber by applying a removable Parhelia CoverPad to each slide. Up to 12 slide assemblies can be loaded into an S12 slide chamber and placed into the PSS with other reagents. The CoverPads are removed before PCF Flowcell assembly and imaging on the PCF instrument.

Uniform staining: 2 specimens on same slide



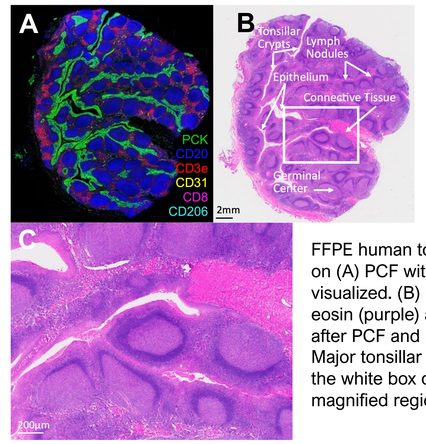
Automated PSS method yields uniform staining quality for both tissues of a doubly mounted sample. DAPI-stained overview (left) for commercially sourced FFPE tonsil tissue sections mounted on the same slide containing two specimens (labeled top and bottom). Images of each individual antibody staining corresponding to similar regions of the top and bottom tissue are indicated. Qualitatively, the staining is equivalent for both tissues.

High-quality staining with 27-plex panel



Whole-slide imaging of the automated staining protocol reveals lymphocyte contexture. PCF imaging of FFPE human tonsil tissues autostained with the PSS and imaged on PCF. (A) overall slide with two tonsil specimens, (B) top tonsil with white boxes indicating (C) four magnified regions labeled 1–4, and (D) Region 1 with spatial compartmentalization of cytotoxic CD8+ T cells (Tc), helper CD4+ T cells (Th) and natural killer cells (NK) indicated.

Automated H&E morphology staining after PCF imaging



Hematoxylin and eosin (H&E) morphology staining automation on the PSS after PCF imaging.

FFPE human tonsil tissue was imaged on (A) PCF with a subset of markers visualized. (B) Hematoxylin (pink) and eosin (purple) autostaining on the PSS after PCF and removal of the Flowcell. Major tonsillar features are indicated; the white box denotes the (C) magnified region.

Automation reduces hands-on time

Spatial Station Automation

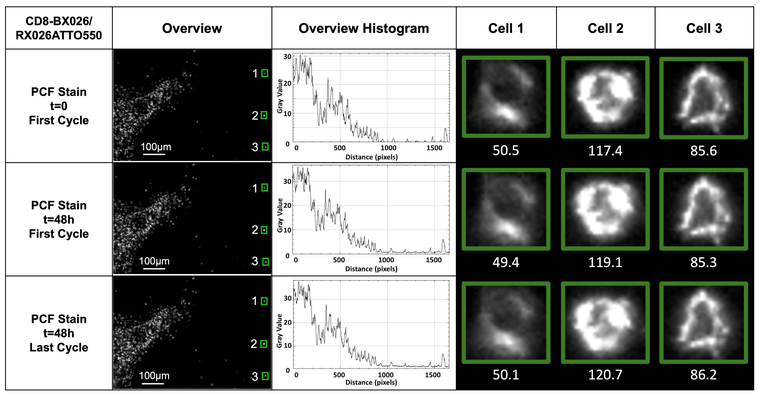
Step	Average Manual time	Possibility of human errors	Observation and outcome
1	20-30 mins	Low to none	Superior results, repeatable, and reproduceable.
2	20-30 mins	Very low	Superior outcome, repeatable, and reproduceable.
3	20-30 mins	Low to none	Consistent, high accuracy, and reproducibility.
4	10 mins	Very low	Consistent, high accuracy, and reproducibility.

Manual Protocol

Step	Average Manual time	Possibility of human errors	Observation and outcome
1	2-3 hours	High	Inconsistent between researchers/labs.
2	16 hours	Very High	Varies between researchers.
3	1 hour	High	Varies between researchers.
4	1 hour	High	Varies between researchers.

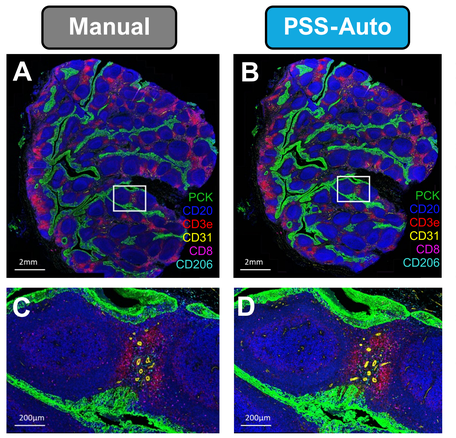
Time savings and reductions in error potential associated with automating the PCF staining protocol.

Uncompromised fixation demonstrates reliability



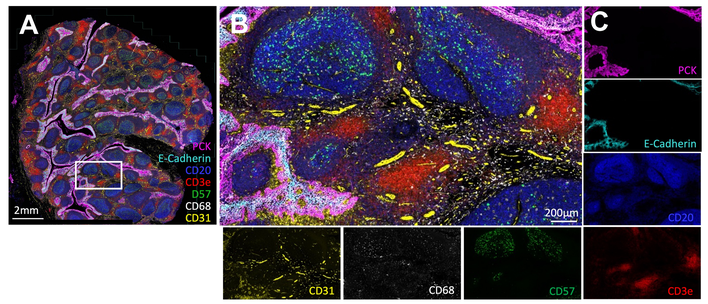
Fixation quality of PSS-Auto samples verifies method robustness. The antibody used was CD8-BX026/RX026ATTO550, Ratio 1:200, Exposure time: 250 ms.

Equivalent staining quality



Automated staining on the PSS yields at least equivalent staining as a manual protocol. The staining protocol was executed (A) manually or (B) automatically on the PSS and subsequently imaged on PCF. Similar regions for magnification are indicated with white boxes and displayed for samples stained (C) manually or (D) automatically on the PSS. PCK = Pan-Cytokeratin.

High-quality staining with a 27-plex panel



Whole-slide imaging of automated high-plex panel staining. (A) PCF imaging of FFPE human tonsil tissues (bottom tonsil shown) with a subset of markers visualized. The white box indicates the (B) magnified region displaying the seven markers, visualized individually in (C).

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