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Panel mixing automation on the Parhelia Spatial Station™ improves immunophenotyping reproducibility with uncompromised data quality

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Abstract

Spatial biology and flow cytometry are time-tested hallmarks of cellular biomarker discovery, immune profiling, and clinical practice. Advances in fluorescent probe chemistry, laser optics, detection methods, and bioinformatics enable a renaissance in both techniques, paving the way for comprehensive monitoring of the biochemical central dogma through complex multiomics analysis.

The growing complexity of highly multiplexed immune profiling experiments involving dozens of antibodies and oligonucleotides creates a challenge for researchers to design and prepare appropriate panels (or cocktails) and associated controls.

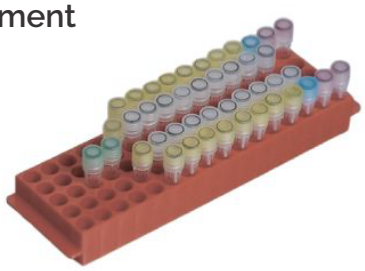
While precise panel design is essential for successful immune profiling by spatial biology[1] and flow cytometry,[2] experiments, manual preparation involves increased systematic errors, inter-operator variability, and researcher hands-on time. Such drawbacks risk repeating experiments or overlooking significant biomarker differences between study groups, costing significant time and wasting expensive reagents.

Advances in large immunophenotyping panel designs demand purpose-built, automated sample preparation workflows that seamlessly integrate with the specimen-to-data pipeline.[2] Here, we demonstrate the reproducibility of automated panel mixing on the Parhelia Spatial Station using a blue dye quality control experiment and immunophenotyping by flow cytometry.

Challenges of Manual Panel Mixing

Inventory Management

Handling multiple antibodies raises the risk of cross-contaminating stock vials, using the wrong antibody, or excessive temperature variation.[2]

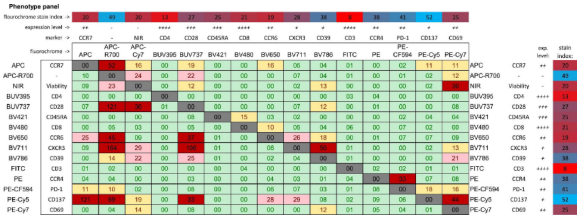


Labor-Intensive

Manual pipetting, capping and de-capping tubes, and mixing antibodies is tedious and time-consuming, taking ~3 hours of attention for a 25-probe panel.[2]

Optimization

Larger panels demand additional FMO controls and optimization (e.g., to address spectral overlap and compensation), requiring further manual processing.[3]



Complexity

Generating the correct antibody concentrations in larger panels or using oligo-tagged antibodies (e.g., CITE-Seq) is error-prone, typically requiring a ~25% volume overage in the cocktail.[4]

Solution: Automated Panel Mixing

Generate fully automated custom panels with the Parhelia Spatial Station. The P54 module securely holds up to 54 uncapped antibody vials with a silicone membrane. For each antibody, a fresh pipette tip pierces the silicone membrane prior to aspiration, avoiding cross-contamination. The entire P54 module can be further sealed with a lid and stored according to the antibody manufacturer's specifications. Furthermore, the system accommodates large plex panels by deploying multiple P54 modules.

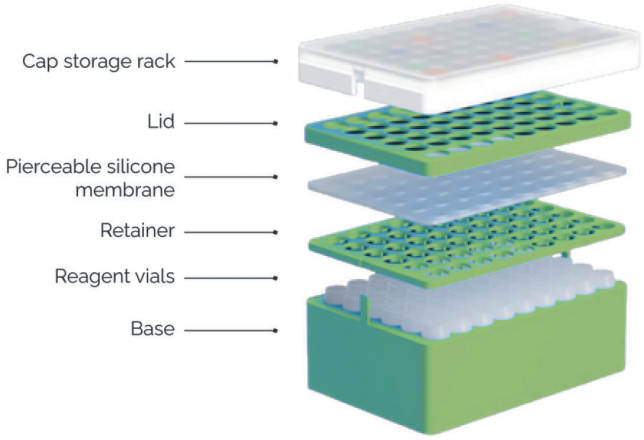


Figure 1. The P54 module enables the pipetting and storage of up to 54 antibody vials.

Parhelia Stainworks™ software facilitates the customization of any mixing workflow. Design panels on your computer and export them as an Excel file for use on the Spatial Station. The instrument's capacitive liquid sensing capabilities enable precise inventory tracking, ensuring that complete panels are produced with high accuracy.

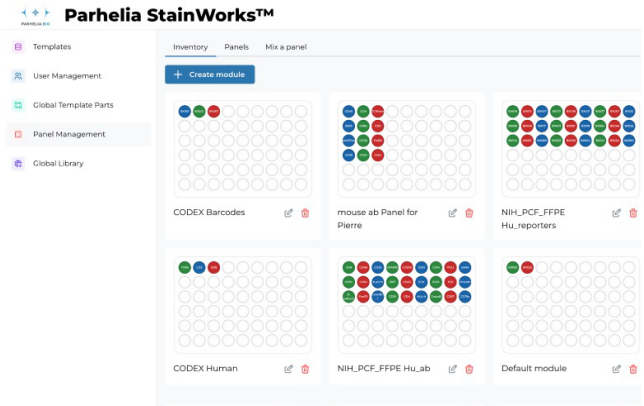


Figure 2. The StainWorks software interface enables easy panel design and inventory management.

Capacitive liquid sensing and on-board mixing enable hands-free execution of:

- Antibody cocktails
- Fluorescence minus one controls (FMOs)
- Cyclic spatial reporter plates (e.g., CODEX)
- Single stains (compensation controls)

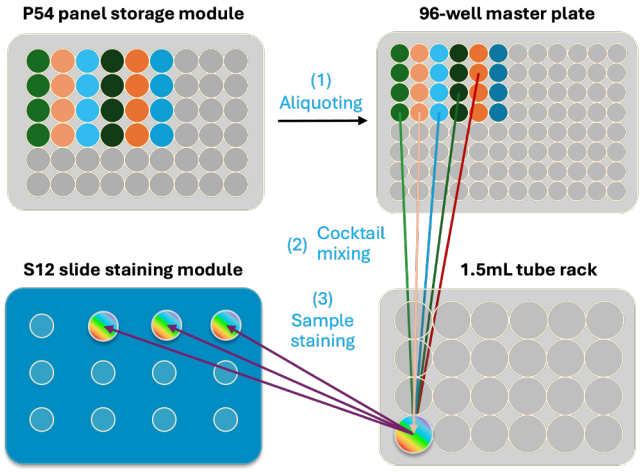


Figure 3. Panel mixing workflow. (1) Necessary volumes of antibodies stored in the P54 module are aliquoted into a master plate. (2) Cocktail mixing is performed in 1.5 mL tubes based on input from the StainWorks software interface. (3) Samples for flow cytometry or spatial biology (pictured) are subsequently stained with the mixed cocktails.

Results

Colorimetric quality control (QC) test

To test the Parhelia Spatial Station's (PSS) pipetting consistency, the 620 nm absorbance of a blue dye (BioTek Instruments) was measured using a plate reader over several weeks. Manual and automated pipetting of 2.5 µL and 5.0 µL volumes were aliquoted to 100 µL of PBS and compared by mean, standard deviation, and coefficient of variation (CV). The blue dye stock was replaced approximately halfway through the evaluation (**Figure 4**). At either 2.5 or 5.0 µL pipetting volume, the PSS performed as consistently as manual pipetting with the added benefit of walkaway autonomy.

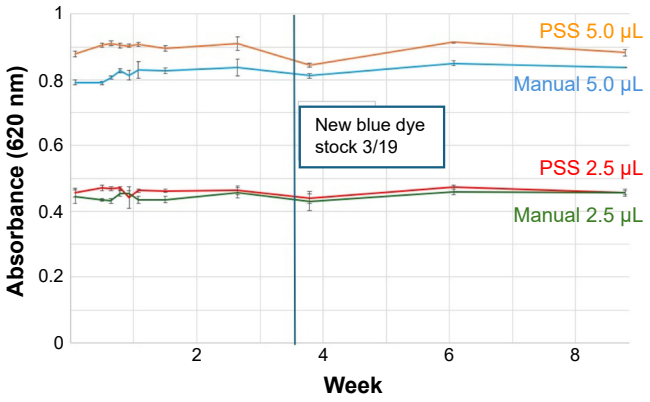


Figure 4. Absorbance vs time plot for automated (PSS) and manually pipetted blue dye tests. Error bars denote standard deviations for five technical replicates. *Data provided courtesy of the Flow and Immune Analysis Shared Resource Laboratory, Roswell Park Comprehensive Cancer Center.*

Reproducibility of immunophenotyping by flow cytometry

Ten samples of human peripheral blood mononuclear cells (PBMCs) were split and stained using a panel prepared manually or automated via the PSS. Samples were injected into a Becton Dickinson Fortessa flow cytometer. Gating strategies (**Figure 5**) were executed identically on each of the manually and automatically prepared samples to identify cell subpopulations, which were reported as percentages of the parent population. The CVs of the manual:PSS ratio of parent subpopulations (**Figure 6**) for singlets, PBMCs, and cell populations (26 classifications total) were <25% except for three cell subtypes, which were <40%.

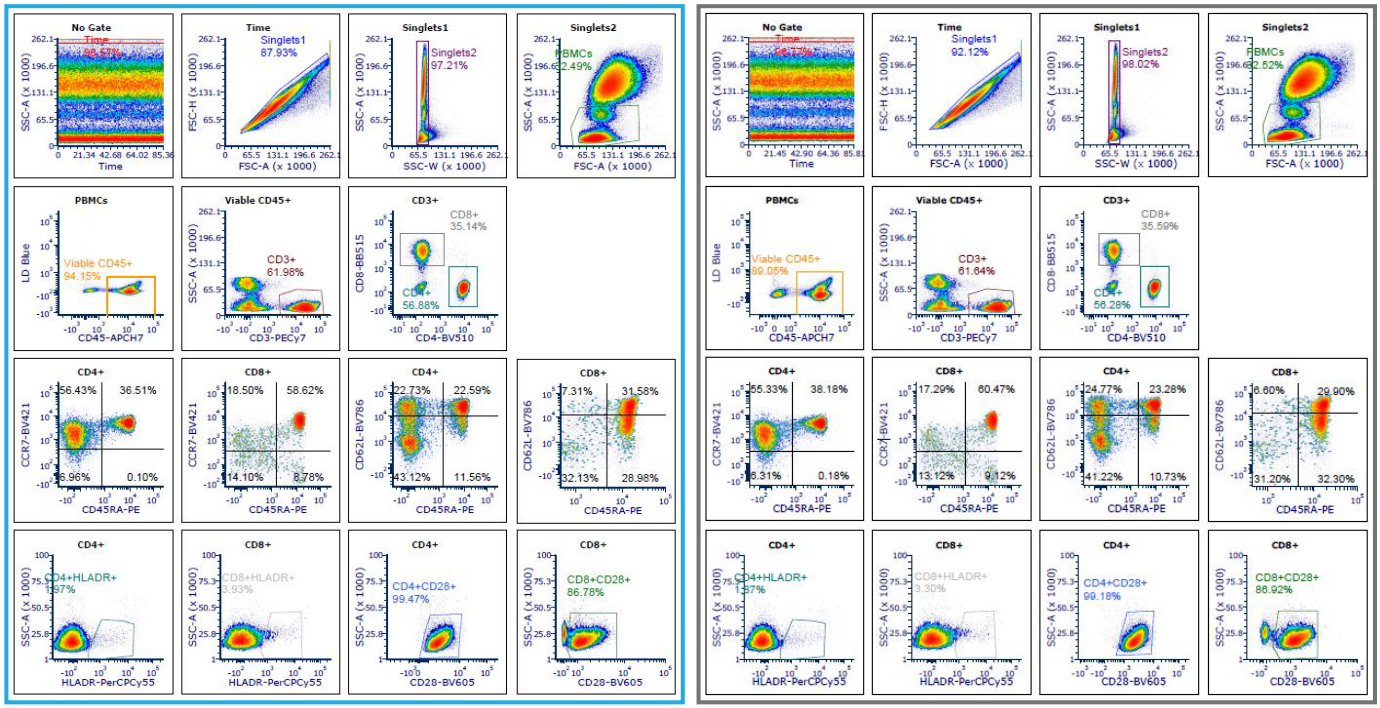


Figure 5. Flow cytometry gating strategy corresponding to (blue outline) automated panel mixing using the PSS or (gray outline) manual panel mixing of one representative identical human PBMC sample. *Data provided courtesy of the Flow and Immune Analysis Shared Resource Laboratory, Roswell Park Comprehensive Cancer Center.*

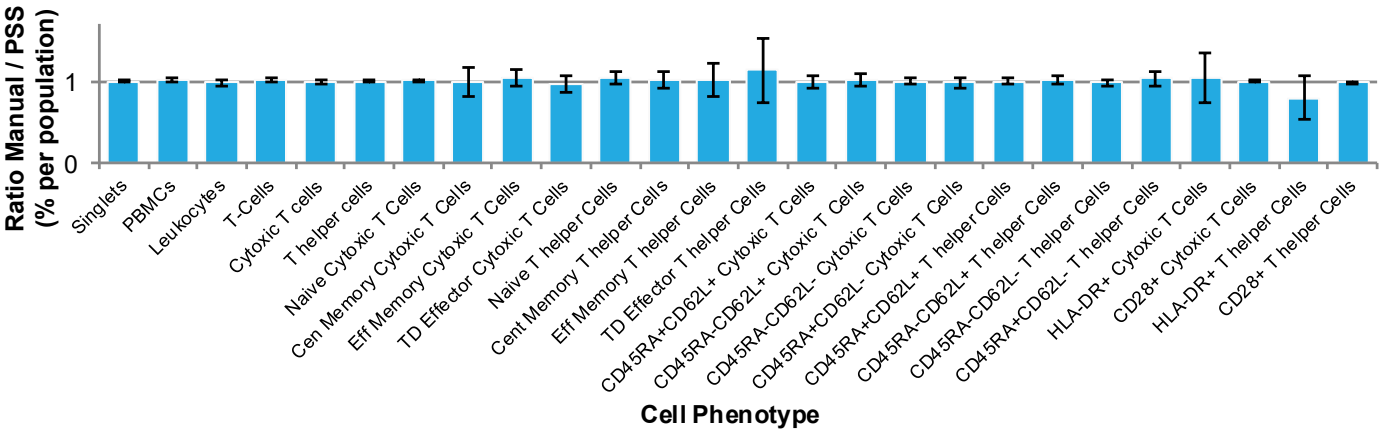


Figure 6. Mean ratios of manual-to-PSS panel mixing relative abundances of gated cell phenotypes. Error bars represent the standard deviation of 10 biological replicates. *Data provided courtesy of the Flow and Immune Analysis Shared Resource Laboratory, Roswell Park Comprehensive Cancer Center.*

Overall, automated panel mixing on the PSS provides a relatively hands-free, reproducible, and quality controlled mechanism for evaluating cell subpopulations by flow cytometry or spatial biology techniques. Capacitive liquid sensing and antibody inventorying facilitate flexibility and customization to ensure optimization of any protocol. Researchers can design panels with confidence by selecting the liquid type (i.e., viscosity), liquid volumes, pipetting speeds, and mixing dispensing to maximize results and minimize reagent volumes, saving time and cutting costs.

Conclusions: low assay variability with walkaway efficiency

Like astronauts performing experiments in space, researchers utilizing immune profiling techniques need a multifunctional habitat to execute protocols and safeguard their specimens. With the Parhelia Spatial Station, you can customize panel mixing, eliminate manual tube handling, and manage your antibody inventory with precise volume tracking and secure, uncapped storage, all in a matter of minutes. Designed to automate complicated sample preparation protocols, the Spatial Station seamlessly integrates your multiomics workflows into one turnkey benchtop workstation.

Edit protocols easily with an intuitive touchscreen

Track remaining reagent levels with precision liquid sensing

Integrate multiple assays with automated staining and a heating + cooling module

Run protocols unconstrained by waste capacity with waste management

Ensure compliance with safeguards like labware autodetection and a step-by-step Run Report

References

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