CASE STUDY

Improved workflows for advanced T-cell immunophenotyping

Immunophenotyping is an essential technique for subclassifying diseases, monitoring treatment responses, diagnosing hematologic malignancies, and detecting rare cell populations. Cell phenotyping uses surface markers to identify and distinguish immune cells while tracking developmental stages and physiological functions. Obtaining accurate and precise immune profiling data is critical for the successful interpretation and application of this biological data. This requires accurate and consistent methods, processes, and testing with minimal batch-to-batch variation.

Fluorescence-activated cell sorting (FACS) is a flow cytometry method that offers a fast way to record enormous amounts of data based on fluorescent signals from immune cells of interest. FACS is one of the traditional cell analysis approaches for immunophenotyping that allows the isolation of specific cell populations for further analysis. However, in recent years, image cytometry has provided several opportunities for the advancement of immune cell profiling data acquisition and processing times.

In this case study, we explore how Mr. Julian Reading, Senior Manager of Flow Cytometry at the Allen Institute of Immunology in Seattle, has been using image cytometry to complement a larger flow cytometry workflow.

Optimized workflow for human PBMC multi-omic immunosurveillance studies

Mr. Reading has spent the last five years building a team of highly trained specialists at the Allen Institute. In the first three years, the team used multiple large (25-marker) flow panels for overall immune surveillance to identify patterns for every subset of T cells, B cells, monocytes, and similar cell types. This exploratory work helped determine a focused subset of important markers for further monitoring and experimentation.

While flow cytometry is a valuable tool when working with large flow panels, instruments are usually located in a central core facility that may not be easily accessible to everyone. Flow cytometry also requires a dedicated trained operator and time-consuming maintenance including daily start-up and shutdown cycles. Flow cytometers are also handled by more than one operator and maintained at different levels. The consequences of these inherent site-to-site variances, such as the cleanliness of lines, impact the reliability and overall quality of results. However, if you focus on fewer markers, you can save the complex flow cytometry hardware for more complex experiments.

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With this in mind, Mr. Reading's team developed a robust workflow for profiling human PBMCs with high-dimensional flow cytometry, 10X Genomics single-cell RNA-seq, and the Cellaca® PLX image cytometry system (Figure 1). Automated fluorescence-based cell counting was maintained for the entire duration of these studies using the Cellaca PLX system with batch control for PBMC samples. They found that the optimized human immunosurveillance workflow and protocols reduced batch effects and hazardous liquid waste while providing robust data outputs and increasing throughput.

Discussing the difference between flow cytometry and the Cellaca PLX system, Mr. Reading describes flow cytometry as having a high dimension and low throughput, while the Cellaca PLX has a low dimension and high throughput. He goes on to further explain that the best utilization for flow cytometry using many markers is the initial screening and then the Cellaca PLX can help to analyze multiple samples once you've narrowed down which markers are important for your study.

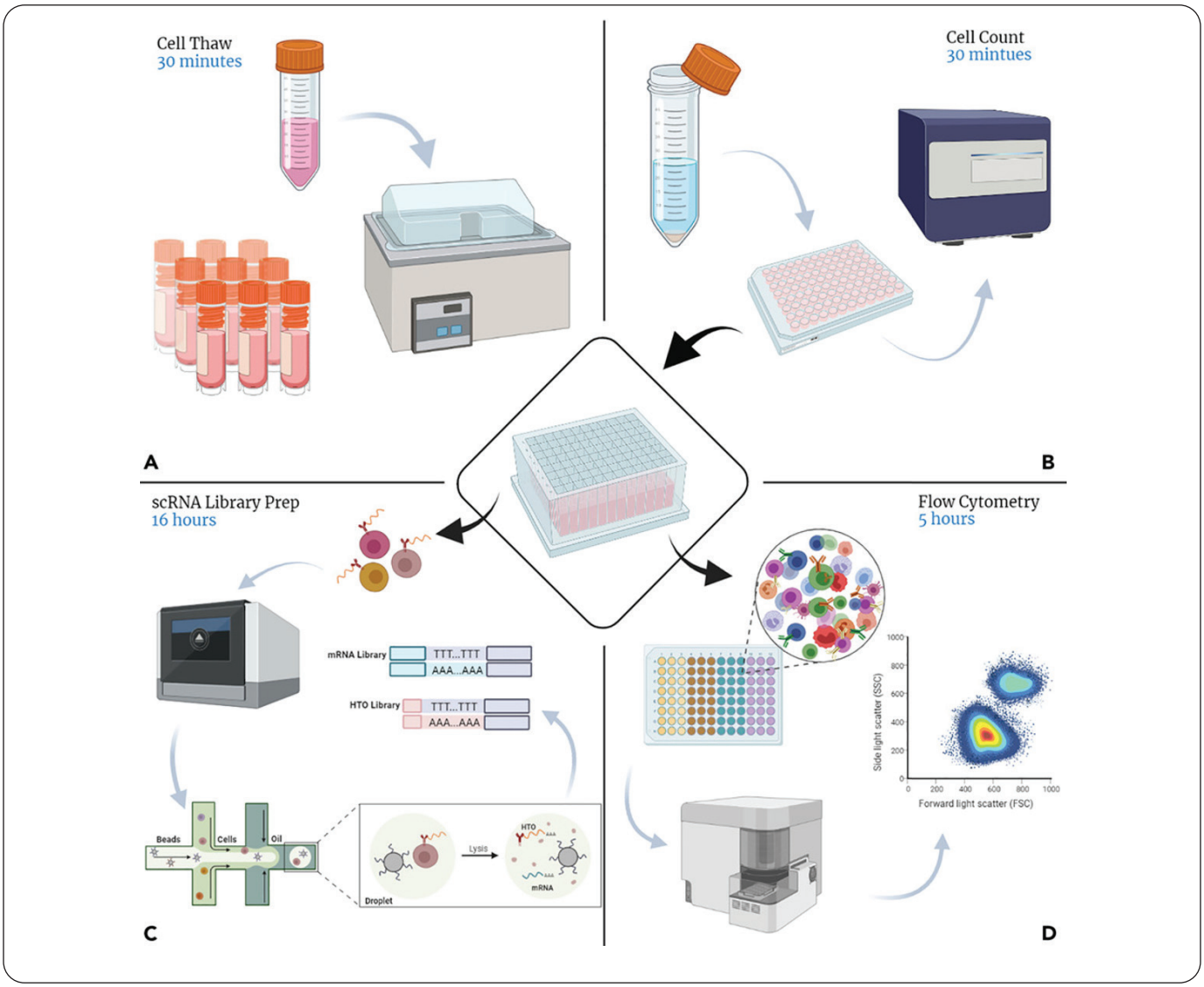

Figure 1: A robust pipeline for profiling of human PBMCs by both high-dimensional flow cytometry and single-cell RNA-seq. These protocols reduce batch effects, generate reproducible data, and increase throughput. Image credit: STAR Protoc. 2021 Dec 17; 2(4): 100900. Published online 2021 Nov 5. doi: 10.1016/j.xpro.2021.100900.

By adding a more focused immunophenotyping workflow using image cytometry, the group noted significant advantages when complementing a larger flow cytometry workflow including faster results with less sample usage. For example, Mr. Reading and his team gathered excellent results while comparing flow cytometry to the Cellaca PLX image cytometry system in the analysis of a wide range of new surface markers, for both high-frequency and low-frequency markers. Commenting on the work, Mr. Reading said: "The Cellaca PLX applies to the sort of immunophenotyping data that pushes past just T cells to look at some of those rarer cells."

Automated cell counting for simple and robust workflows

The Cellaca PLX image cytometry system offers automated cell counts, viability readouts, and multiplex analysis that is useful throughout all stages of development. The Cellaca PLX system can also be used for surface markers detection, such as CD3, CD4, CD8 for T cells, CD19 for B cells, CD56 for NK cells, and CD14 for monocytes for immunophenotyping. This system has the additional benefit of performing focused surface marker panels for a fraction of the cost of other methods. Also, since the Cellaca PLX system has no fluidics, it is easy to transfer assays from site to site with fewer opportunities for experimental variability.

The Cellaca PLX image cytometer, in addition to delivering precise multiparametric results more swiftly and affordably, offers a simpler alternative to traditional flow cytometry techniques. The Cellaca PLX system is simple and robust, even in untrained hands if you have a defined protocol. For instance, Mr. Reading recently had an intern working with him who operated the instrument within a week. "That is a pretty good endorsement," he enthused.

Generating high-quality longitudinal flow cytometry data from fixed human PBMCs opens the doorway for further research and the potential for adapting this method for other tissue types. More studies are needed to expand the capabilities of what can be achieved with quick, simple, and inexpensive alternatives for biomedical research while improving data quality.

Read the full study[1] to learn more about protocols to reduce batch effects, generate reproducible data, and increase throughput. The study describes:

- A robust workflow for the profiling of human PBMCs
- Streamline cell sample preparation procedures and protocols for flow cytometry and single-cell RNA-seq
- Batch effect reduction with a bridging cell control and commercial buffers
- Increased throughput with high speed and automation ready cell counting

Reference

1. Genge, P. C., Roll, C. R., Heubeck, A. T., Swanson, E., Kondza, N., Lord, C., Weiss, M., Hernandez, V., Phalen, C., Thomson, Z., Torgerson, T. R., Skene, P. J., Bumol, T. F., & Reading, J. (2021). Optimized workflow for human PBMC multiomic immunosurveillance studies. STAR protocols, 2(4), 100900. https://doi.org/10.1016/j.xpro.2021.100900

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