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Flow Cytometry Sample Preparation Is Faster and More Accurate with I.DOT Liquid Handler

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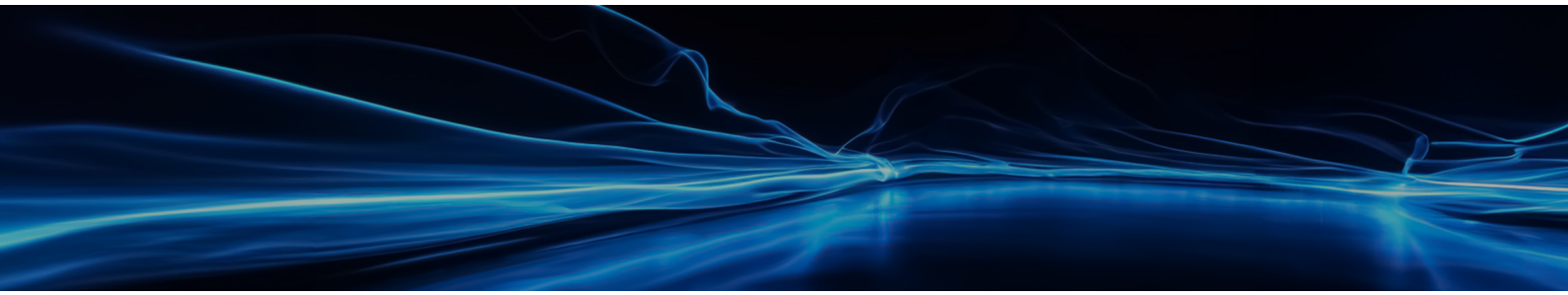

Abstract

Flow cytometry has become much more powerful in recent years. Scientists can now analyze over 40 markers on thousands of cells every second, thanks to new technology. However, this complexity creates a challenge: manually adding antibodies is slow, tedious, and error-prone.

This study explores the use of an automated antibody dispensing system to solve this problem. The system would improve efficiency and data quality by precisely dispensing the colored (fluorophore-conjugated) antibodies and allowing for more control samples.

The researchers compared different liquid handling systems for dispensing these colored antibodies. They focused on how fast the system worked, how much liquid was wasted, and how reliable it was. The researchers find that the I.DOT Liquid Handler is the best option for dispensing antibodies, reducing analysis time, improving accuracy, and enhancing data quality in their flow cytometry workflows. Incredibly, the I.DOT could do this in just 20 minutes, including all the necessary controls. These controls are crucial for ensuring the accuracy of the data.

Overall, using an automated dispensing system like the I.DOT can significantly speed up flow cytometry experiments, reduce errors, and improve data quality. This technology can also be easily integrated into a fully automated workflow for sample preparation.

Introduction

Our Body's Defense Turned Research Powerhouse: The Versatility of Antibodies
Antibodies, also known as immunoglobulins (Ig), are amazing Y-shaped proteins produced by our immune system's B cells in response to foreign invaders like bacteria or viruses. These highly specific molecules, like tiny lock-and-key pairs, bind to unique targets on the invader, called antigens. This binding flags the antigen for destruction by other immune cells.

But beyond their natural role, scientists have harnessed the power of antibodies for various research applications. Techniques like Western Blot, immunoprecipitation, and flow cytometry all rely on antibodies to detect specific proteins or cells within a sample.

The development of different antibody types, like primary and secondary antibodies, as well as highly targeted monoclonal antibodies, has further expanded their usefulness.

Today, antibodies are indispensable tools in medicine, research, and diagnostics, helping us diagnose diseases, understand biological processes, and even develop new therapies.

Flow cytometry has long been a powerful tool in cell analysis, allowing scientists to delve into the intricate details of cellular processes. However, with the increasing complexity of experiments and the need to analyze multiple parameters simultaneously, a bottleneck has emerged: the manual mixing of antibodies. This time-consuming and error-prone process has hindered the efficiency and accuracy of flow cytometry analyses.

While antibodies are crucial for scientific breakthroughs, traditional methods for using them can be time-consuming, labor-intensive, and expensive. This can hinder the reproducibility of experiments.

To overcome this challenge, our customers, researchers at VIB Flow Core Ghent, have delved into the realm of automation. By utilizing automated liquid handlers to dispense antibodies, they have significantly improved the throughput and analysis of high-parameter flow cytometry experiments. Their groundbreaking research not only demonstrates the effectiveness of liquid handling automation in streamlining workflows but also highlights the potential for increased accuracy and reproducibility in cell analysis studies.

This study compares various liquid handlers for dispensing fluorochrome-labeled antibodies in flow cytometry staining and recommends that other flow cytometry researchers also use the one that they found to work best.

Advantages of Using the I.DOT for Antibody Dispensing

- Antibodies can be dispensed instantly
- Eliminates pipetting inaccuracy and dispenses precisely and accurately
- Significant reduction in human labor
- Low dead volume
- Reliable and reproducible results
- Eliminates risk of cross-contamination

Liquid handling automation benefitted the experiments in three major ways:

1. Reduced time: The I.DOT prepared a complex test involving 25 markers in under 20 minutes.
2. Improved accuracy: Automation minimizes errors in dispensing the precise antibody amounts.
3. Enhanced data quality: Easier addition of control samples allows for better validation of the test results.

Automating Antibody Staining Mix Preparation with the I.DOT Liquid Handler

This section describes the use of the I.DOT S for preparing antibody staining mixes. The I.DOT Assay Studio software was used to control the instrument and define the dispensing parameters.

I.DOT Setup and Liquid Class Creation

A critical step involved creating a "liquid class" for the specific antibodies being used. This involved defining the dispensing pressure based on the antibody properties and verifying the dispensed volume through a drop-counting procedure. Acceptable performance meant dispensing within an 8% range of the target volume (i.e., between 92 and 108 droplets for a 220 nL target). This procedure was repeated for various antibodies.

To maintain antibody suspension in the source plate (where antibodies are stored), sealing foil or a warm water bath were used to maintain humidity. The I.DOT software or manual calculations determined the exact amount of antibody needed for each staining mix. This information was then translated into a comma-separated values (CSV) file.

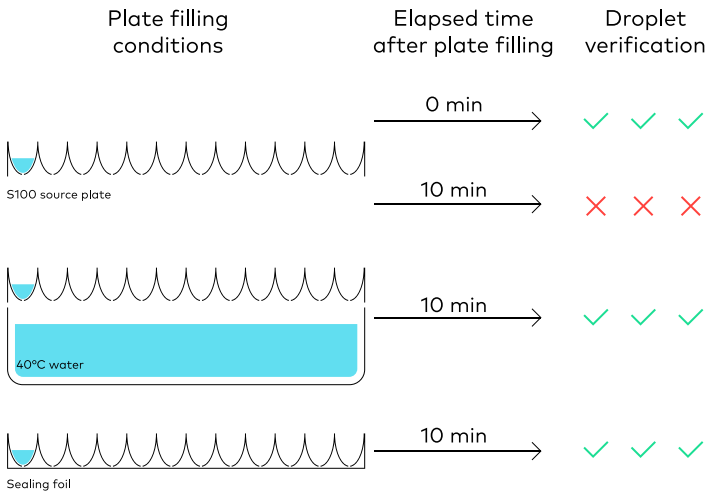

Figure 1. *To maintain antibody suspension, there are two effective methods. One approach involves maintaining a humid environment. This can be achieved by simply creating a sealed chamber around the source plate, or by placing it on top of a reservoir filled with warm water (40°C). Alternatively, sealing the individual source wells with foil can also maintain antibody suspension. Both methods ensure proper dispensing by the I.DOT system, even if there's a 10-minute delay between preparing the source plate and initiating the dispensing process.*

The I.DOT system has built-in error detection. It can identify wells with no dispensed droplets and automatically generate a new command list to complete the dispensing after refilling or replacing the problematic well. Leftover antibody volume can be retrieved due to the contact-free dispensing nature of the I.DOT. However, caution is advised to avoid contaminating the stock solution with other antibodies. Therefore, calculating the exact volume and adding a minimal dead volume is recommended.

Dispensing Process and Error Handling

An additional 1 µL + 5% buffer volume was added to the calculated source volume to avoid running out of antibody during dispensing. The destination plate layout, a 96-well round-bottom U-well plate, was designed using the software or imported from a CSV file. Each well held a specific staining mix (full stain, controls, or single stains). Controls were created by omitting a specific antibody from the full stain mix (FMO or MMO).

For efficiency, the full stain mix was prepared as a master mix for multiple samples. This approach minimized dispensing time and inter-sample variability.

Materials and Methods

The researchers followed these methods for immunophenotyping of murine splenocytes and human PBMCs.

Mice

- Spleens were harvested and digested to create a cell suspension.
- Red blood cells were removed.
- Cells were stained with antibodies targeting specific molecules on their surface (surface markers) and a viability dye to distinguish live and dead cells.
- A flow cytometer was used to measure the fluorescence intensity of the stained cells, which allowed identification and quantification of different cell populations.

Humans

- Peripheral blood mononuclear cells (PBMCs) were isolated from blood using density gradient centrifugation.
- PBMCs were frozen for storage and thawed before use.
- Like the procedure for mice, cells were stained with antibodies and a viability dye, followed by analysis with a flow cytometer.

Results and Discussion

The researchers tested various devices, focusing on speed, wasted material, and accuracy. Three automated liquid handlers were evaluated for their suitability in dispensing fluorochrome-labeled antibodies for flow cytometry staining mixes:

- **Janus Mini Varispan (Revvity):** This system uses a 4-tip dispensing arm for aspirating and dispensing antibodies from various reservoirs.
- **Echo 650 (Beckman Coulter):** This acoustic dispenser rapidly and accurately dispenses small volumes using sound waves.
- **I.DOT (DISPENDIX):** This pressure-based non-contact dispenser utilizes an 8-channel head to dispense small volumes precisely from a source plate containing microfluidic pores.

The study found that using an automated dispenser significantly improves the ease and efficiency of including more controls in flow cytometry experiments. These controls are essential for validating the accuracy of the data analysis.

Specifically, the I.DOT Liquid Handler allowed the researchers to prepare a 25-marker panel in under 20 minutes, whereas manual preparation would take 3 hours. This enabled them to include all the necessary controls, including FMOs (fluorescence minus one) and MMOs (markers minus one) (***Fig. 2***).

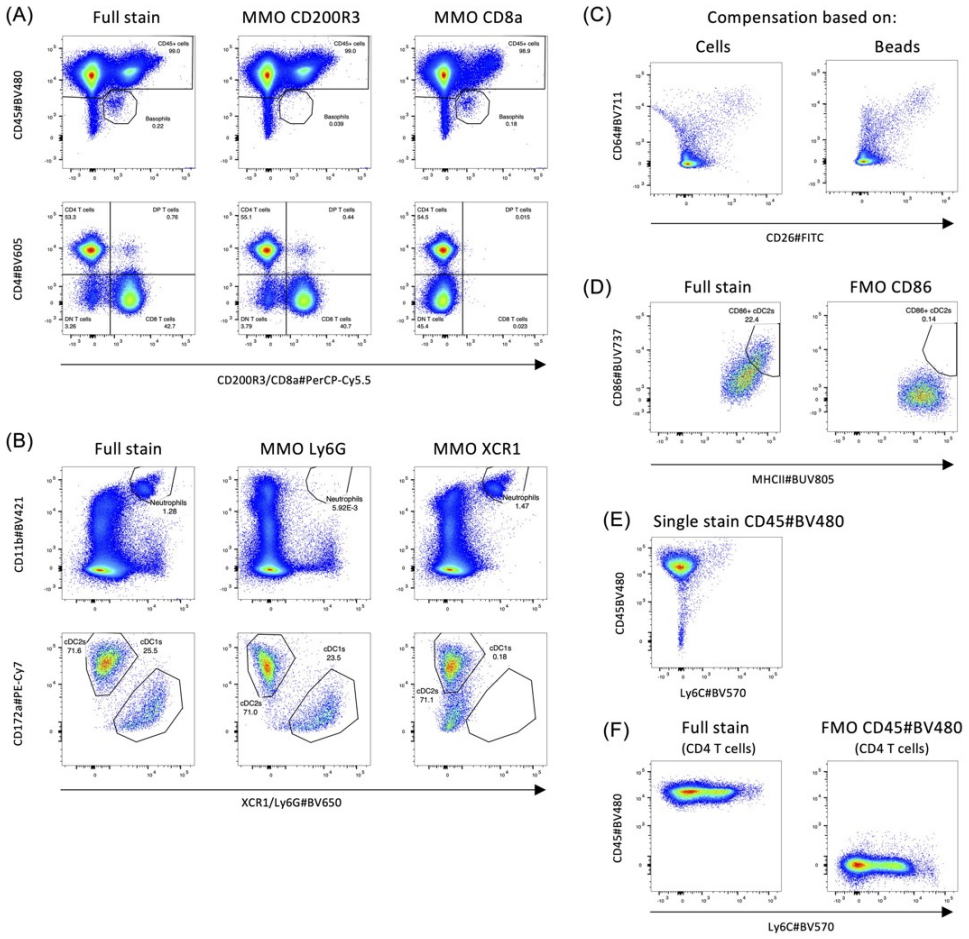

Figure 2. *Using a liquid handler to dispense antibodies makes it much easier to run tests that check the accuracy of the experiment. MMOs (markers minus one) controls are used to confirm that antibodies targeting different markers with the same fluorescent tag can be reliably distinguished (A,B). Having both single-stained cells and beads allows researchers to choose the best way to compensate for spectral overlap between different fluorophores (C). FMOs (fluorescence minus one) controls help differentiate true signals from background noise or data spread issues (D,E,F). Overall, the use of an automated dispenser like I.DOT Liquid Handler makes it easier to incorporate these essential controls, leading to more robust and informative flow cytometry experiments.*

FMOs help ensure the validity of the gating strategy by showing whether a signal is due to a specific antibody or background noise. MMOs are useful for differentiating between markers that use the same fluorochrome. Additionally, having both single stained cells and beads allows for better compensation, which corrects for spectral overlap between fluorochromes.

Overall, the I.DOT facilitates the use of more controls, leading to more reliable and informative flow cytometry data.

Table 1. *Summary of I.DOT's unique benefits related to antibody dispensing compared across the instruments tested in this study. The I.DOT is the only dispenser that performs true dispensing verification*

Feature	Manual	Janus	Echo	I.DOT
Technology	Pipette	Automated Liquid Handler	Acoustic Dispenser	Pressure-based (non-contact) dispenser
Instrument Cost	-	$$	$$$	$$
Dead Volume	0 µL	20 µL	2.5 µL (special plates needed)	1 µL
Precision	0.1 µL	0.5 µL	2.5 nL	8 nL
Verification	None	Bubble Detection	Source Volume Survey	Droplet Verification

Based on these unique benefits, they found the DISPENDIX I.DOT Non-Contact Dispenser to be the preferred solution for antibody dispensing.

Conclusion

This study suggests that automated antibody dispensing can significantly improve flow cytometry workflows.

The recommendation to fellow flow cytometry researchers to adopt automated liquid handlers echoes the sentiment of progress and innovation in the field. By embracing technology to optimize experimental processes, scientists can unlock new possibilities and push the boundaries of what is achievable in cell analysis. This study serves as a beacon of hope for the future of flow cytometry, paving the way for enhanced efficiency and accuracy in cell research.

References

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