

# Pluto Code–Enabled Centrifuge-Free Automation: Toward a Universal Standard for Gentle Wash

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## Overview

Pluto Code enables centrifuge-free automation by delivering Curiox's C-FREE™ gentle wash technology as executable software for liquid-handling platforms.

- Centrifugation is a major bottleneck in automated cell-based assays
- Manual spin steps introduce variability, cell loss, and poor automation compatibility
- Pluto Code executes C-FREE centrifuge-free wash physics without added hardware
- Runs on users' existing liquid-handling platforms
- Supports walk-away automation with consistent assay performance

## Introduction

Automated sample preparation remains limited by centrifugation steps that are difficult to translate across liquid-handling systems.

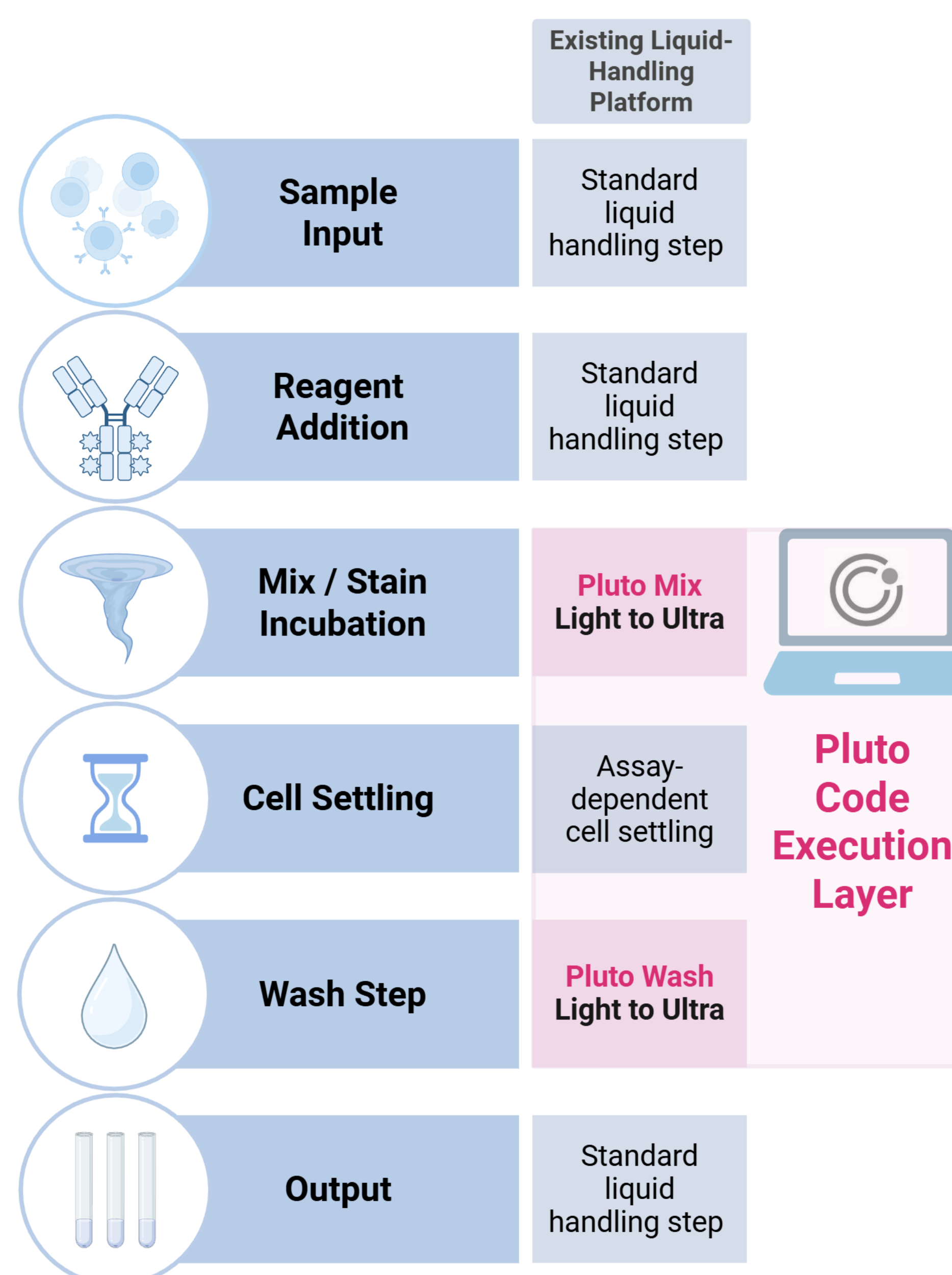
- Centrifuge-based workflows introduce operator dependency and inconsistent cell retention
- Spin steps disrupt automation, increasing hands-on time and assay variability
- C-FREE technology replaces centrifugation with controlled aspiration–dispense wash physics
- Pluto Code exposes this behavior as validated, platform-agnostic software
- This decouples gentle wash performance from instrument design, enabling standardized automation across platforms



Pluto Code

## Methods

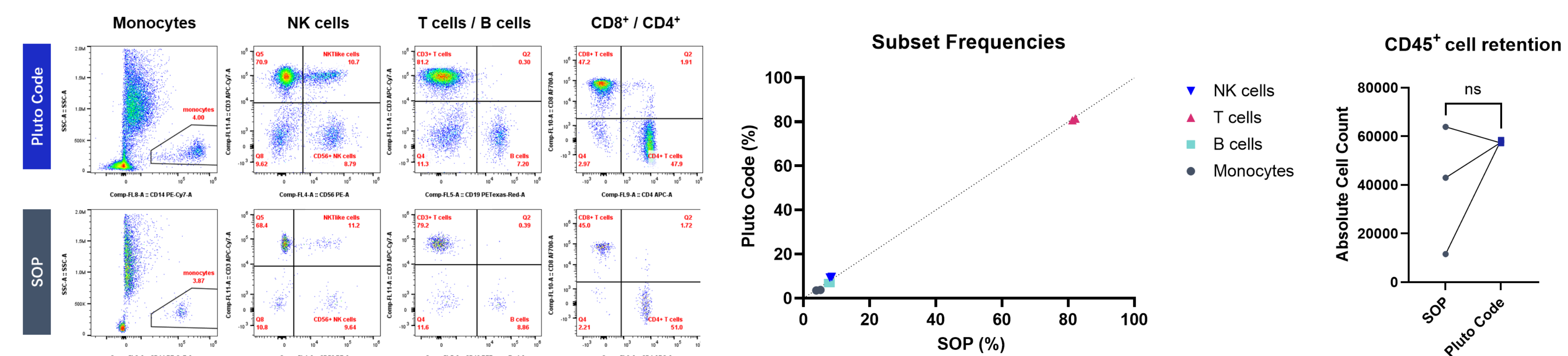
Pluto Code implements C-FREE gentle wash physics as executable mix and wash primitives within standard liquid-handling workflows.



**Figure 1. Pluto Code–enabled execution of C-FREE centrifuge-free washing within standard liquid-handling workflows.** Pluto Code implements C-FREE gentle wash behavior as executable Pluto Mix and Pluto Wash primitives that run natively within a liquid handler's method editor. User-authored steps (sample input, reagent addition, output) are combined with code-defined mixing, assay-dependent cell settling, and tunable wash stringency (Light to Ultra), enabling centrifuge-free, on-deck sample preparation while preserving workflow structure across liquid-handling platforms. Created in <https://BioRender.com>.

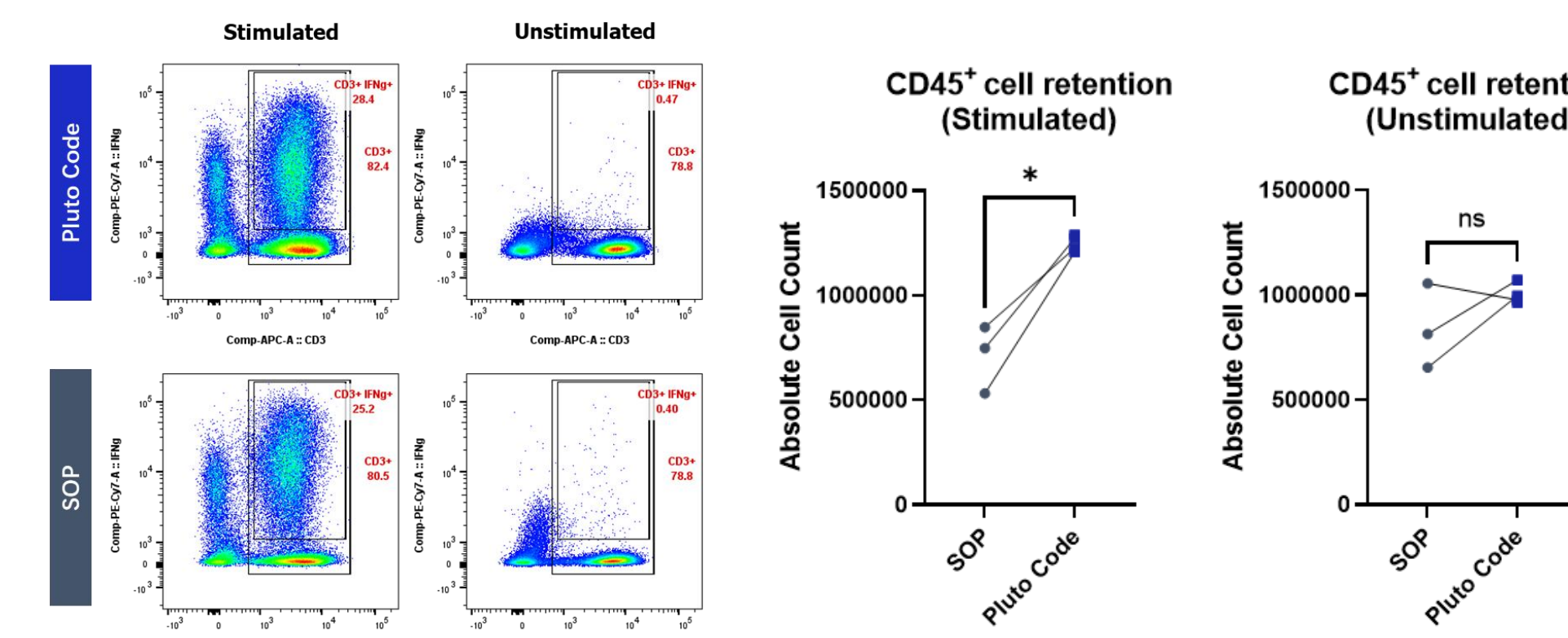
## Results

### Comparable Whole Blood Immunophenotyping without Centrifugation on an Opentrons Platform



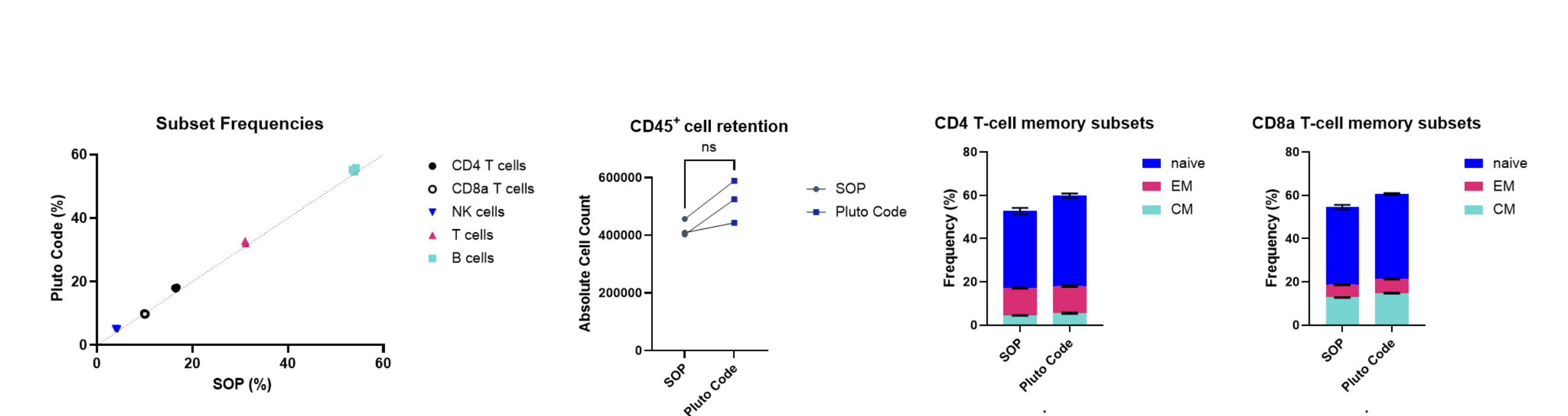
**Figure 2.** Whole blood immunophenotyping following Pluto Code or centrifuge-based SOP preparation on an Opentrons OT-2 platform. Representative gating plots, immune subset frequencies, and CD45<sup>+</sup> cell retention are shown. Correlation plot includes the line of identity. Statistical analysis by paired Student's t-test; no significant differences observed.

### Improved IFN $\gamma$ T-Cell Retention in PBMC Intracellular Staining using Platform-Agnostic Pluto Code Wash Execution



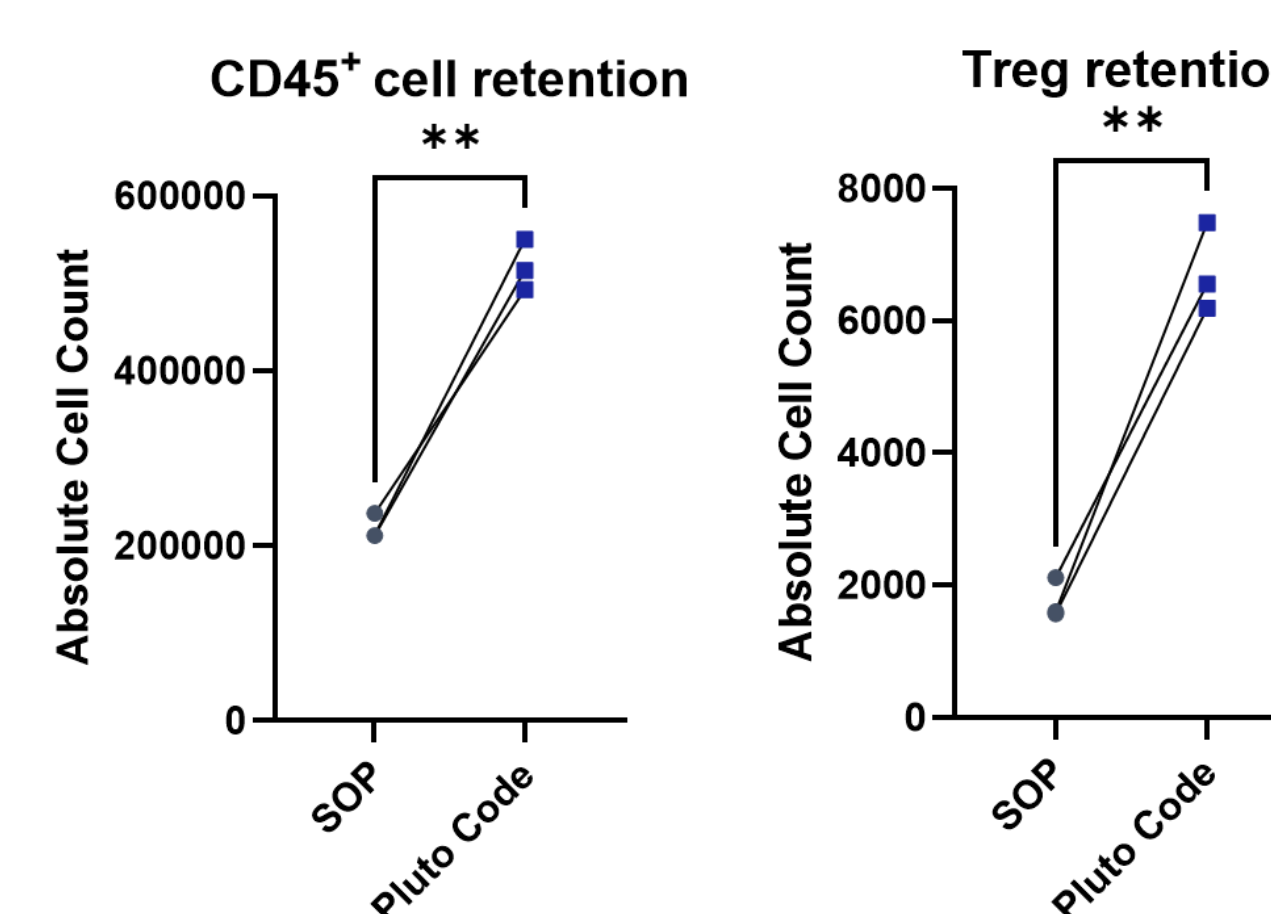
**Figure 3.** IFN $\gamma$  intracellular staining of stimulated and unstimulated PBMCs prepared by Pluto Code or centrifuge-based SOP on an Opentrons OT-2 platform. Representative gating plots and CD45<sup>+</sup> and IFN $\gamma$  T-cell retention are shown. Statistical analysis by paired Student's t-test.

### Preserved Murine Immune Subset Frequencies Following Pluto Code Preparation



**Figure 4.** Surface-stained murine splenocytes prepared by Pluto Code or centrifuge-based SOP on an Opentrons OT-2 platform. Correlation of immune subset frequencies includes the line of identity. CD45<sup>+</sup> cell retention and CD4 and CD8a T-cell memory subset composition are shown. Statistical analysis by paired Student's t-test; no significant differences observed.

### Improved Leukocyte and Regulatory T-Cell Retention in Murine Foxp3 Staining



**Figure 5.** Murine splenocytes stained intracellularly for Foxp3 following preparation by Pluto Code or centrifuge-based SOP on an Opentrons OT-2 platform. CD45<sup>+</sup> leukocyte retention and regulatory T-cell retention are shown. Statistical analysis by paired Student's t-test.

## Key Results

**Centrifuge-free surface and intracellular staining executed on Opentrons OT-2**  
 Pluto Code **preserved** immune subset frequencies and cell retention across whole blood, PBMC, and murine splenocyte workflows.

**Improved performance in sensitive intracellular assays**  
 PBMC IFN $\gamma$  and murine Foxp3 staining showed **increased** target cell retention, consistent with gentler handling during fixation and permeabilization.

**Centrifuge-free wash behavior across automation platforms**  
**Platform-agnostic** wash behavior demonstrated on Opentrons OT-2 and Flex, with additional validation on Hamilton STAR and VANTAGE systems.

Related Hamilton STAR PBMC workflows are presented in a companion poster by an invited speaker (Poster #1044-E, Session E), describing implementation of Pluto technology for fully automated, centrifuge-free flow cytometry sample preparation on a Hamilton STAR system.

## Discussion

**Pluto Code enables centrifuge-free automation across multiple assay types while maintaining or improving assay performance.**

- Lysed whole blood surface staining on Opentrons OT-2 showed comparable CD45<sup>+</sup> cell retention and immune subset frequencies relative to centrifuge-based SOP workflows.
- PBMC intracellular cytokine staining (IFN $\gamma$ ) showed higher retention of IFN $\gamma$ <sup>+</sup> CD3<sup>+</sup> T cells, consistent with gentler handling during fixation and permeabilization.
- Murine splenocyte surface staining preserved T, B, and NK cell subset frequencies, while murine Foxp3 intracellular staining demonstrated improved CD45<sup>+</sup> leukocyte and regulatory T-cell retention.

Together, these results demonstrate that Pluto Code reduces sample loss while preserving biological readouts across assay complexity.

## Conclusion

**Pluto Code enables platform-agnostic, centrifuge-free automation across diverse liquid-handling systems.**

- Executes Curiox's C-FREE gentle wash behavior as software-defined mix and wash primitives within native liquid-handler methods, eliminating the need for centrifugation.
- Delivers comparable or improved cell retention and assay performance across surface and intracellular staining workflows.
- Demonstrates platform portability across Opentrons OT-2, Opentrons Flex, and Hamilton STAR and VANTAGE systems, enabling centrifuge-free automation without disrupting existing automation logic.