Standardization and Calibration of Flow Cytometry Using Synthetic Cells with Multi-level Forward and Side Scatter

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INTRODUCTION

Cytometers vary in hardware components and measurement principles, resulting in different scaling and sensitivity for scatter data acquisition. However, while there are multi-level reference materials for fluorescence that are widely adopted and commercially available, no such standard exists for simultaneous forward (FSC) and side scatter (SSC) detection, making it particularly challenging to standardize scatter measurements across instruments.[1] To date, the mainstream method is to simply measure patient samples in a trial-and-error fashion and manually adjust settings until results agree.[2] This is not only time consuming, but it poses a biohazard risk, increases sample variability, and is also impractical for rare or costly samples.

METHODOLOGY

Slingshot Biosciences® breakthrough technology creates synthetic cells that match the optical, fluorescence and biochemical features of any cell type, even rare disease types.

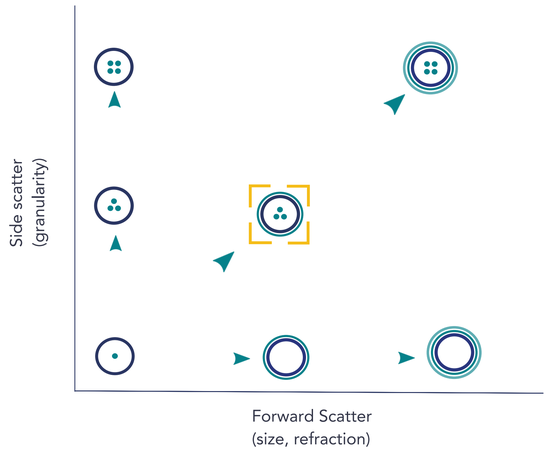

Figure 1. With Slingshot Biosciences® proprietary technology, orthogonal control of granularity and optical properties of cell mimics allows independent tuning of their FSC and SSC in a broad and biologically relevant range.

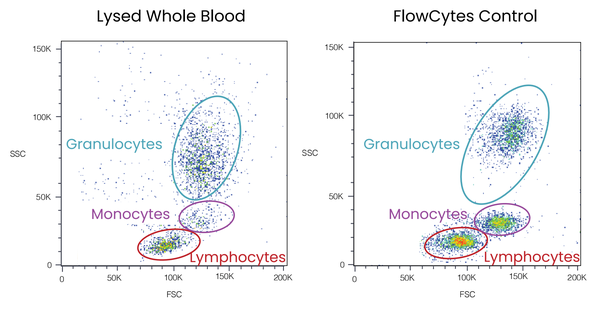

Figure 2. FlowCytes® are hydrogel-based synthetic cells optically resembling white blood cells. FlowCytes® are nearly indistinguishable between live cells when analyzed in a flow cytometer.

RESULTS

We developed a collection of nine inert, stable hydrogels that produces a discrete 3x3 signal matrix (ScatterGrid™, Slingshot Biosciences, Inc.) that are used as reference scatter signals (data acquired with multiple flow cytometers) for biological cells of interest in flow cytometry. The FSC and SSC range of these hydrogels covers most clinically relevant events, including but not limited to WBCs, bone marrow cells, and frequently used cell lines (examples shown: CAR-T, THP-1).

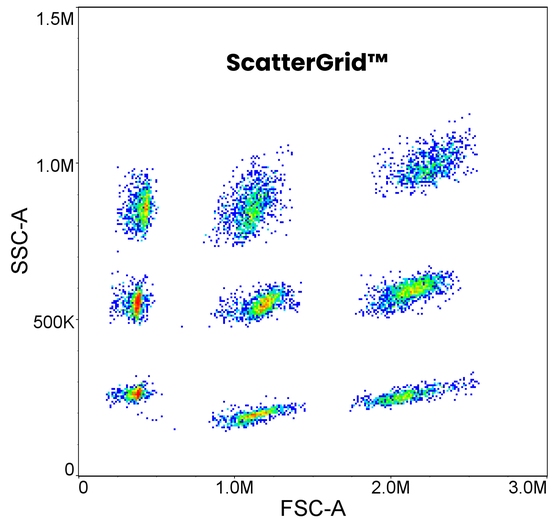

Figure 3. Flow cytometry scatter profile of ScatterGrid™, a collection of nine unique cell mimic populations, producing a 3x3 FSC/SSC signal on a Cytek® Aurora flow cytometer.

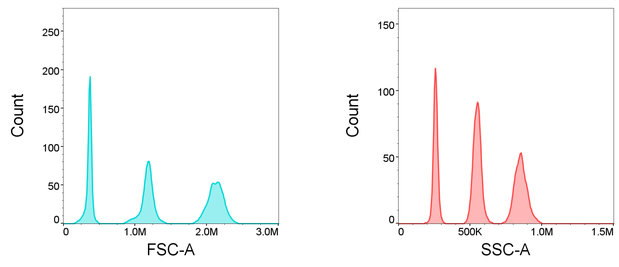

Figure 4. FSC (left) and SSC (right) histogram of select populations in ScatterGrid™, producing a multi-level FSC/SSC signal on a Cytek® Aurora flow cytometer.

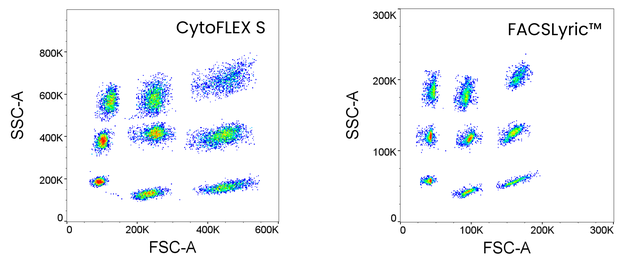

Figure 5. Flow cytometry scatter profile of ScatterGrid™, a collection of nine unique cell mimic populations, producing a 3x3 FSC/SSC signal on a Beckman Coulter® CytoFLEX S (left) and a BD® FACSLyric™ flow cytometer (right).

RESULTS

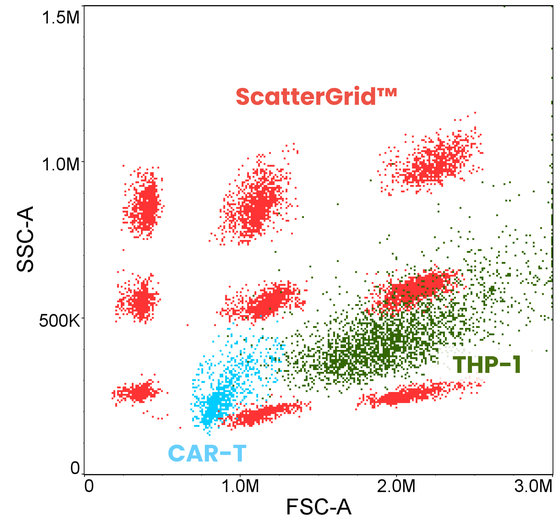

Figure 6. Flow cytometry scatter profile of ScatterGrid™, overlayed with CAR-T (blue) and THP-1 (green) cells on a Cytek® Aurora flow cytometer.

CONCLUSIONS

In this work, we demonstrated a collection of hydrogels that produces diverse FSC/SSC signals in conventional flow cytometry measurements. The stable and cell-mimicking optical properties of these hydrogels, along with the diverse yet distinct levels of FSC/SSC signals, creates great opportunities in the following use cases:

- **Standardization** of scatter measurements between cytometric instruments and lab sites. This standardization minimizes experimental development and validation in longitudinal scientific research and mass clinical studies.
- **Calibration** of cytometric instruments. The addition of diverse FSC/SSC levels can detect instrument linearity or scaling changes, valuable for instrument manufacturers and end users alike.
- **Metadata analysis and harmonization** of cytometric results across studies.

REFERENCES

[1] Wang, L., Hoffman, R. A. Standardization, calibration, and control in flow cytometry. *Current Protocols in Cytometry* **79**, (2017).

[2] Kalina, T., Flores-Montero, J., van der Velden, V. et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia* **26**, 1986–2010 (2012).