



Advanced Standardization with FACSTruQ™ Technology

Enabling Reproducible Flow Cytometry Measurements Across
Instruments, Operators, and Time



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Abstract

Flow cytometry relies on measured fluorescence signals to characterize underlying biological properties of cells. Yet day-to-day, instrument-to-instrument, and site-to-site variability can compromise data comparability. Changing detector settings can further alter reported fluorescence intensity even when the sample is unchanged, forcing users to sacrifice sensitivity, reacquire samples, maintain multiple gating templates, or apply post-acquisition normalization.

FACSTruQ™ Technology addresses these challenges within the BD FACSDiscover™ Platform's measurement architecture. An onboard multicolor LED pulser characterizes detector response across all settings, BD FACSDiscover™ Setup Beads assess system responsivity, and Detector Setting Independent (DSI) scaling places biological signals on a common scale independent of detector settings. The result is standardized, comparable data driven by biology, not the instrument, the operator, or the day.



Standardization in Flow Cytometry: Why It Matters

Flow cytometry has become an essential tool for biological discovery, translational research, and clinical applications. As assays become increasingly complex and studies span multiple operators, instruments, sites, and time points, the ability to generate reproducible data has become more important than ever.

Yet achieving comparable flow cytometry results remains challenging. Even when the same biological sample is analyzed, measured signals reflect contributions from both the biological specimen and the instrument used to acquire it, and therefore can be influenced by instrument design, detector settings, assay execution, and data processing approaches. As a result, differences observed in the data may not always reflect differences in biology.

Effective standardization should enable users to focus on biological interpretation rather than instrument variability. This principle guided the development of FACSTruQ™ Technology, a standardization architecture built directly into the BD FACSDiscover™ Platform. Specifically, FACSTruQ™ Technology characterizes the response of the instrument — the complete signal path comprising laser excitation, optics, filters, detectors, and signal-processing electronics — and monitors that response over time. BD SpectralFX™ Technology uses this instrument characterization to support spectral unmixing. Together, these technologies reduce the dependence of biological measurements on detector settings and instrument-specific response, thereby improving the consistency, comparability, and interpretability of flow cytometry data.

The Problem

The primary objective of flow cytometry is to generate measurements that support meaningful biological interpretation. To this end, conclusions drawn from these measurements should reflect the underlying biology rather than the fluorescent labels, detector settings, instrument configuration, or platform used to generate the data.

Achieving this level of comparability remains challenging. Flow cytometry measurements can vary across time, operators, instruments, and sites, even when nominally identical samples and assay procedures are used. These challenges have become more significant as modern flow cytometers have increased in complexity and as the number, diversity, and brightness of available fluorescent reagents have expanded.

The Root Cause

The flexibility and customizability that make flow cytometry broadly useful also create substantial opportunities for measurement variability.

Observed flow cytometry data may be influenced by:

- » Sample collection, preparation, storage, and handling
- » Reagent selection, fluorophore choice, and panel design
- » Staining procedures and operator technique
- » Detector settings and acquisition templates
- » Optical configuration and instrument design
- » Alignment and manufacturing tolerances between instruments
- » Changes in instrument performance over time
- » Data processing, unmixing, compensation, gating, and analysis methods

Consequently, a measured fluorescence intensity reported in arbitrary instrument units reflects contributions from both the biological specimen and the measurement system, making it difficult to determine whether observed differences arise from the sample or from the measurement system itself. Effective standardization should enable users to focus on biological interpretation rather than instrument variability.

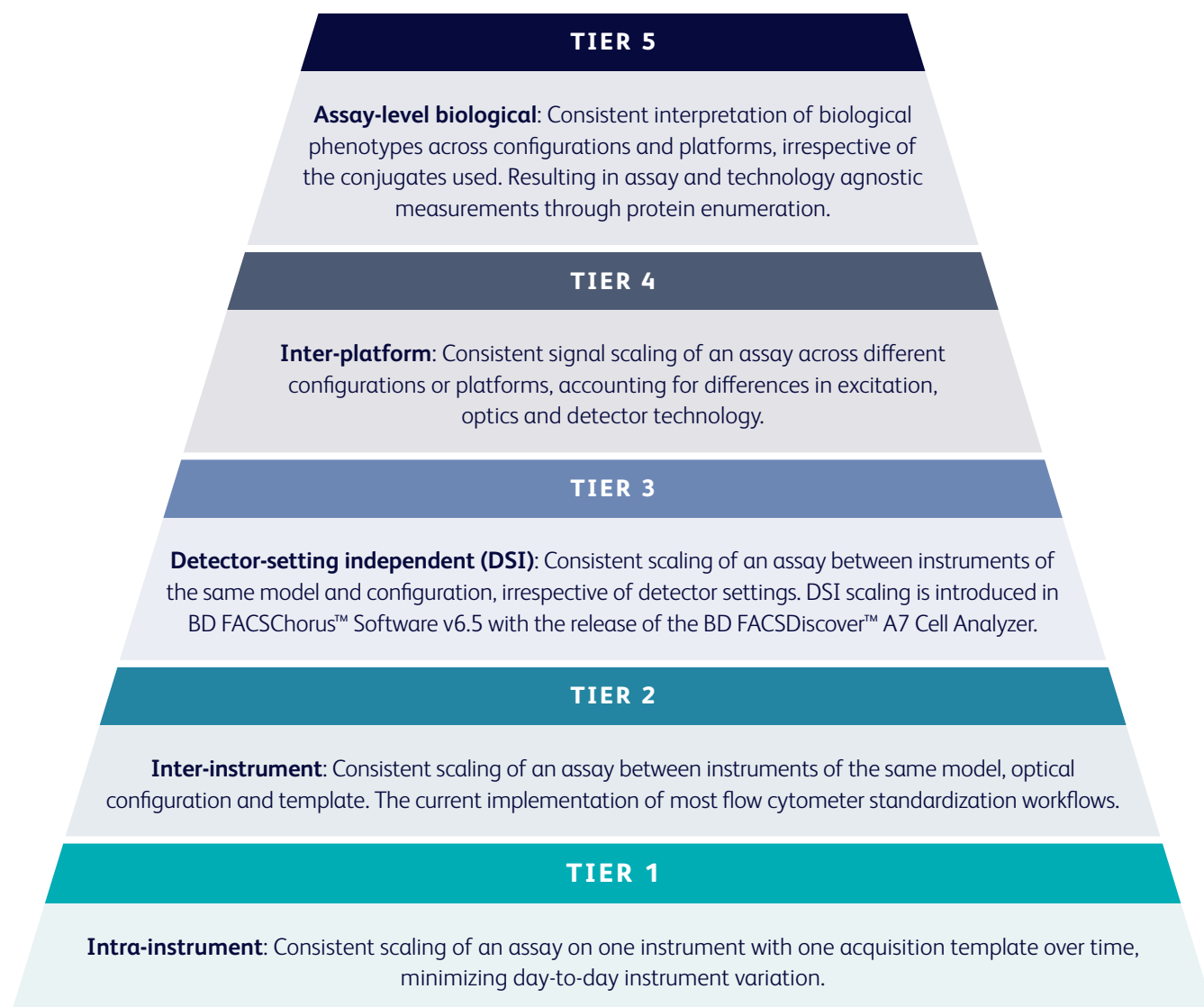
Waters' Vision for Flow Cytometry Standardization

Standardization should reflect underlying biology rather than instrument-specific behaviors. Measurements should remain reproducible across instruments, operators, and sites while preserving the flexibility required for modern assay development.

FACSTruQ™ Technology was designed around this philosophy, embedding standardization within the architecture of the BD FACSDiscover™ Platform. Synergistic technologies, including BD SpectralFX™ Technology, leverage this system characterization framework to further enhance data quality and analysis.

Five Tiers of Flow Cytometry Standardization

Standardization of flow cytometry signal can be considered as a progression through five tiers. Each successive tier builds upon the previous one while widening the scope over which measurements remain comparable. The most comprehensive tier requires consideration of the complete measurement system, from instrument performance and reagent properties to panel design, assay execution, data processing and biological interpretation.

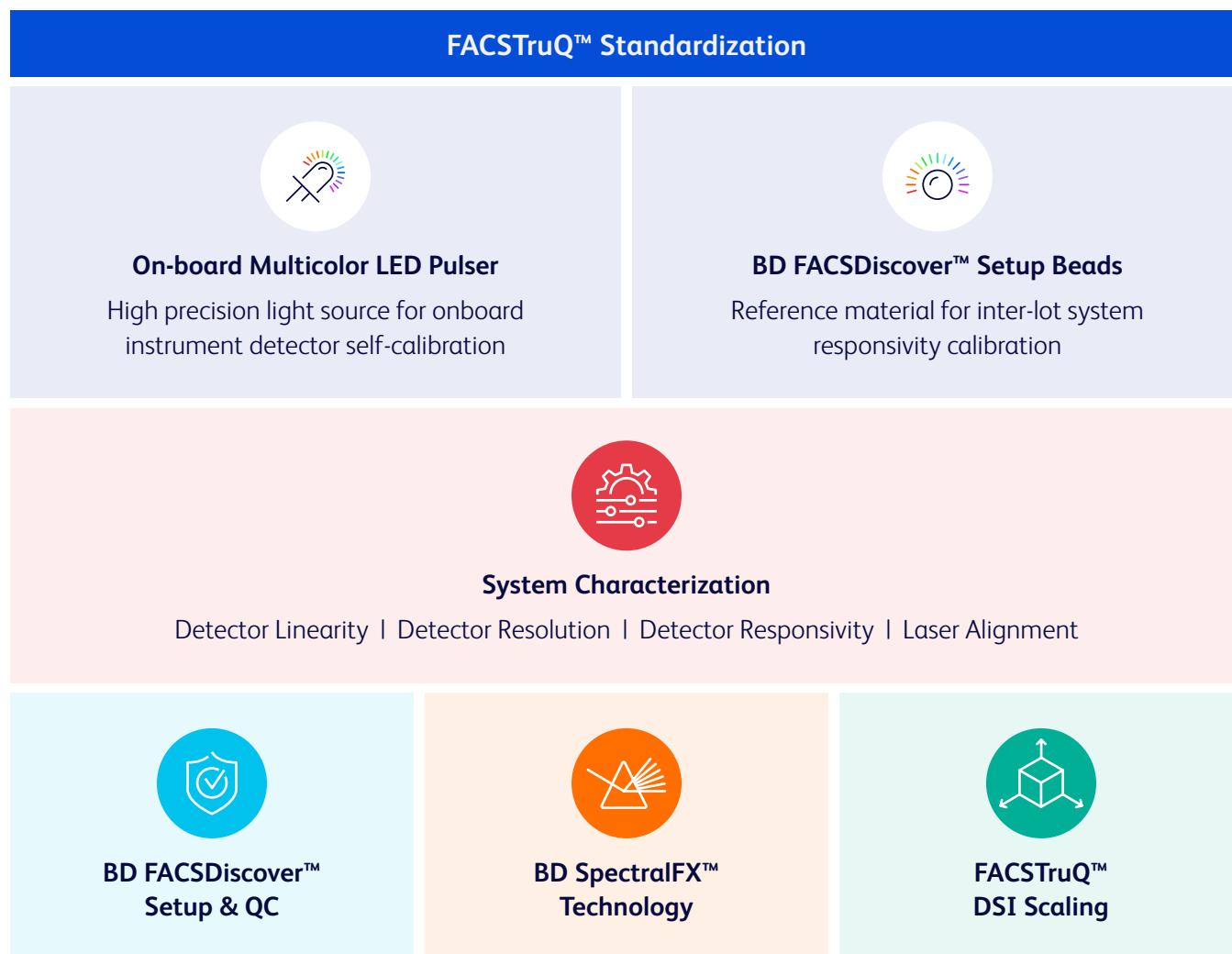


FACSTruQ™ Technology: Standardization by Design

FACSTruQ™ Technology is an integrated instrument-characterization and calibration framework built into the BD FACSDiscover™ Platform. Its purpose is to characterize the response of the measurement system, monitor that response over time, and apply the resulting information to support consistent scaling, instrument setup, quality control and spectral unmixing.

The technology is built around two complementary foundations:

1. An onboard multicolor LED pulser, which characterizes the detector system.
2. BD FACSDiscover™ Setup Beads, which characterize complete-system responsivity.





Foundation 1: Onboard Multicolor LED Pulser

The onboard multicolor LED pulser enables automated characterization of the detector system within each BD FACSDiscover™ Instrument.

By introducing controlled optical signals directly into the detector system, the LED pulser characterizes detector response across a range of detector settings and self-calibrates. This characterization establishes the relationship between detector settings, input signal, and measured output.

The resulting information can be used to assess:

- » Detector response across available settings
- » Linearity of measured signal
- » Detector resolution
- » Stability of detector performance over time
- » Relationships between detector settings and reported signal intensity

This characterization supports both BD SpectralFX™ Technology unmixing and DSI scaling.

For BD SpectralFX™ Technology, system characterization provides information about the behavior of individual detectors and how that behavior changes under different acquisition conditions. This enables the unmixing process to account for characteristics of the measurement system rather than treating the instrument as an idealized or static detector array.

For DSI scaling, the detector response characterization provides the information required to translate signals acquired using different detector settings onto a consistent output scale.



Foundation 2: BD FACSDiscover™ Setup Beads

BD FACSDiscover™ Setup Beads extend the detector-level characterization provided by the onboard LED pulser to the complete optical measurement system.

The beads are assigned values for each detector and calibrated between manufacturing lots. Their measured signals therefore provide a reference for quantifying instrument responsivity across the optical paths used for biological measurements.

This measurement captures contributions from components that are not fully evaluated by an internally generated LED signal, including:

- » Laser excitation
- » Optical alignment
- » Light collection efficiency
- » Filter and optical-path transmission
- » Detector responsivity
- » Instrument-specific hardware tolerances

By quantifying complete-system responsivity, the Setup Beads support correction for differences between instruments and changes in instrument performance over time.

This information provides a foundation for consistent experiment-template transfer, inter-instrument standardization, and DSI scaling.

Daily Setup and Quality Control

BD FACSCorus™ Software Daily Setup and Quality Control combines measurements from the onboard multicolor LED pulser and the BD FACSDiscover™ Setup Beads.

The LED pulser characterizes detector behavior, while the Setup Beads assess the response of the complete optical system. Together, these measurements provide a more comprehensive assessment than either component could provide independently.

Daily Setup and Quality Control can be used to:

- » Verify that detector and optical-system performance remain within established limits
- » Monitor instrument stability over time
- » Assess the instrument limit of resolution
- » Relevant day-to-day variation
- » Support consistent assay scaling
- » Maintain comparability of longitudinal and inter-instrument data

Rather than treating quality control as a simple pass-or-fail check, FACSTruQ™ Technology uses instrument-characterization data as an active component of assay standardization.

Why This Matters

Traditional standardization approaches often rely on external procedures, fixed acquisition settings, and operator-managed workflows. While these approaches can improve consistency, they frequently require users to maintain rigid experimental conditions to preserve comparability. FACSTruQ™ Technology takes a different approach. By characterizing both detector behavior and complete-system responsivity, it provides information that can be used directly by downstream measurement functions. This creates a connected standardization architecture in which quality control, signal scaling, detector settings, and spectral unmixing are informed by a common characterization of the instrument, making standardization a system capability rather than a workflow burden.

The result is greater consistency without requiring every measurement condition to remain fixed indefinitely.

Performance With Flexibility

Traditional standardization workflows frequently require assay settings to remain unchanged. This can preserve comparability, but it limits the ability to optimize an assay when samples, reagents, or experimental requirements change. Furthermore, it limits reproducibility of assays to those replicating assays without access to the original templates.

FACSTruQ™ Technology is designed to separate assay standardization from rigid dependence on a single detector setting. Researchers can adjust detector settings to improve population resolution or accommodate changes in assay signal while retaining a consistent scale for biological interpretation and high-quality unmixing of signals.

This enables flexibility in acquisition without necessarily sacrificing continuity with previously acquired data or deprecating the unmixing capabilities.



Detector Setting Independent Scaling: Flexibility Without Losing Standardization

The Challenge

Researchers may need to adjust detector settings during assay development or after a study has already begun.

A detector setting that was appropriate for an initial sample may later prove unsuitable because:

- » A population is dimmer or brighter than expected
- » A new reagent lot has a different signal intensity
- » A marker is outside the optimal measurement range
- » Additional populations or experimental conditions are introduced
- » The original acquisition template does not provide sufficient resolution

In a traditional workflow, changing a detector setting also changes the reported fluorescence intensity. Measurements acquired before and after the setting change may therefore no longer be directly comparable.

The Root Cause

The fluorescence intensity reported by a traditional flow cytometer reflects both the signal originating from the measured particle and the response of the detector system.

Increasing a detector setting generally increases the reported intensity of a positive event, even though the amount of fluorophore associated with that event has not changed. Conversely, reducing the detector setting lowers the reported intensity without any corresponding change in the sample.

The measured value therefore combines:

1. Biological information, including fluorophore abundance and fluorescence emission
2. Instrument response, including the detector setting and associated signal amplification

Because traditional Mean Fluorescence Intensity (MFI) values confound these two contributions, detector settings become embedded within the apparent biological measurement.

What Is DSI Scaling?

Detector Setting Independent scaling is designed to remove the effect of detector settings from the reported fluorescence-intensity scale.

The instrument characterizes how each detector responds across its available settings. This characterization is then used to transform acquired measurements onto a common reference scale that is independent of the sample being acquired.

As a result, changing a detector setting alters how effectively the population is resolved, but does not proportionally reposition the population on the standardized signal scale.

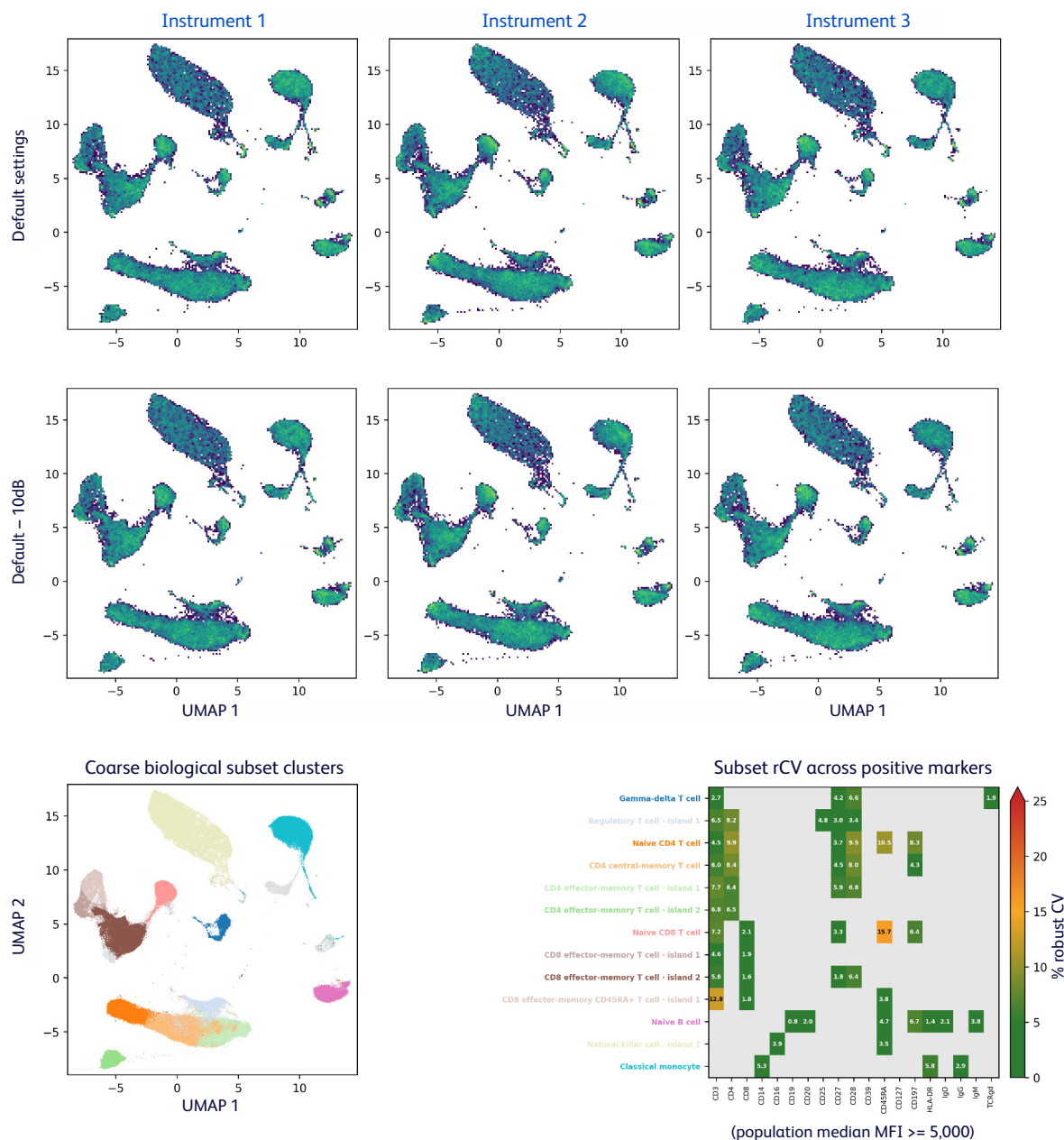
In practical terms:

- » Detector settings control the resolution and dynamic range of the measurement
- » DSI scaling provides the ability to control how the resulting signal is represented
- » Biological signal intensity remains consistently scaled across compatible detector settings

This creates a more natural relationship between acquisition settings and biological interpretation.

DETECTOR SETTING INDEPENDENT SCALING: FLEXIBILITY WITHOUT LOSING STANDARDIZATION (CONTINUED)

FIGURE 1. How DSI Improves the Consistency and Comparability of Biological Data. UMAP embeddings of PBMC populations stained with 20 markers across 3x BD FACSDiscover™ A7 Cell Analyzers at default settings (left top) and minus 10 dB (left bottom). UMAP colored by biological subset clusters (top right) and their respective MFI % rCV (bottom right).



DETECTOR SETTING INDEPENDENT SCALING: FLEXIBILITY WITHOUT LOSING STANDARDIZATION (CONTINUED)

Without DSI scaling, increasing a detector setting can make a population appear biologically brighter even when the sample has not changed. The instrument setting therefore influences both measurement quality and apparent signal magnitude.

Without DSI scaling, changes in detector settings can alter the apparent fluorescence intensity of a population even when the biology remains unchanged. Consequently, detector settings influence both measurement quality and perceived biological signal.

With DSI scaling, detector settings primarily affect measurement resolution while biological signals remain represented on a consistent scale. This helps separate biological interpretation from instrument response and supports more reliable comparison of results across experiments and instruments.

DSI therefore helps distinguish two concepts that have historically been coupled:

- » How well a population is resolved
- » Where that population is positioned on the fluorescence-intensity scale

Separating these concepts supports more reliable comparison of biological measurements.



Customer Use Cases

Customer Use Case 1: Changing Detector Settings During a Study



THE QUESTION:

“What happens if I discover halfway through a study that a detector setting needs to be changed?”

Traditional Workflow

In a traditional workflow, changing the detector setting changes the measured fluorescence intensity. Data acquired after the change may no longer align with data collected earlier in the study.

The researcher may need to:

- » Maintain a suboptimal setting for the remainder of the study
- » Repeat previously acquired samples
- » Apply a post-acquisition normalization
- » Create separate analysis templates
- » Accept reduced comparability between study phases

Each option introduces additional work, uncertainty, or potential loss of information.

DSI-Enabled Workflow

With DSI scaling, the detector setting can be adjusted to improve measurement quality while retaining a common standardized scale.

Previously acquired and newly acquired samples can remain directly comparable, provided that the measurements are made within the validated operating range of the DSI framework.

Impact on Data Comparability

DSI reduces the dependence of longitudinal measurements on a fixed acquisition setting. This supports more flexible study design and decreases the risk that an initially selected detector setting will limit the quality or comparability of later data.



Customer Use Case 2: Maintaining a Consistent Gating Template



THE QUESTION:

“How can I keep a gating template consistent when detector settings change?”

Why Gates Move With Traditional Detector Changes

Gates are commonly positioned using arbitrary intensity coordinates. When a detector setting changes, the location of positive and negative populations can shift on the light scatter or fluorescence scale even when the underlying biology is unchanged, resulting in the original gate no longer aligning with the population. The user must then reposition the gate or maintain separate templates for different detector settings. This introduces operator dependence and complicates assay transfer.

One of the first sets of detector settings to be adjusted and gates to be drawn when setting up an assay are the light scatter channels. Typically, events are triggered on light scatter and are used as an initial gate for a high-level separation of single events and/or major cell types e.g. lymphocytes, monocytes, granulocytes. Often these initial settings, triggering thresholds, and gates are highly subjective depending on the user.

How DSI Improves Gate Stability

DSI scaling maintains populations on a consistent fluorescence and light scatter intensity scales across compatible detector settings. Detector settings can therefore be adjusted to improve resolution without causing the same proportional movement of populations and gates.

A gating template developed under one detector condition can remain applicable under other validated detector conditions because the scale used for biological interpretation is preserved. In the case of light scatter this capability gives rise to global templates that can be used across assays for gating common cell types, such as lymphocytes, monocytes, granulocytes.

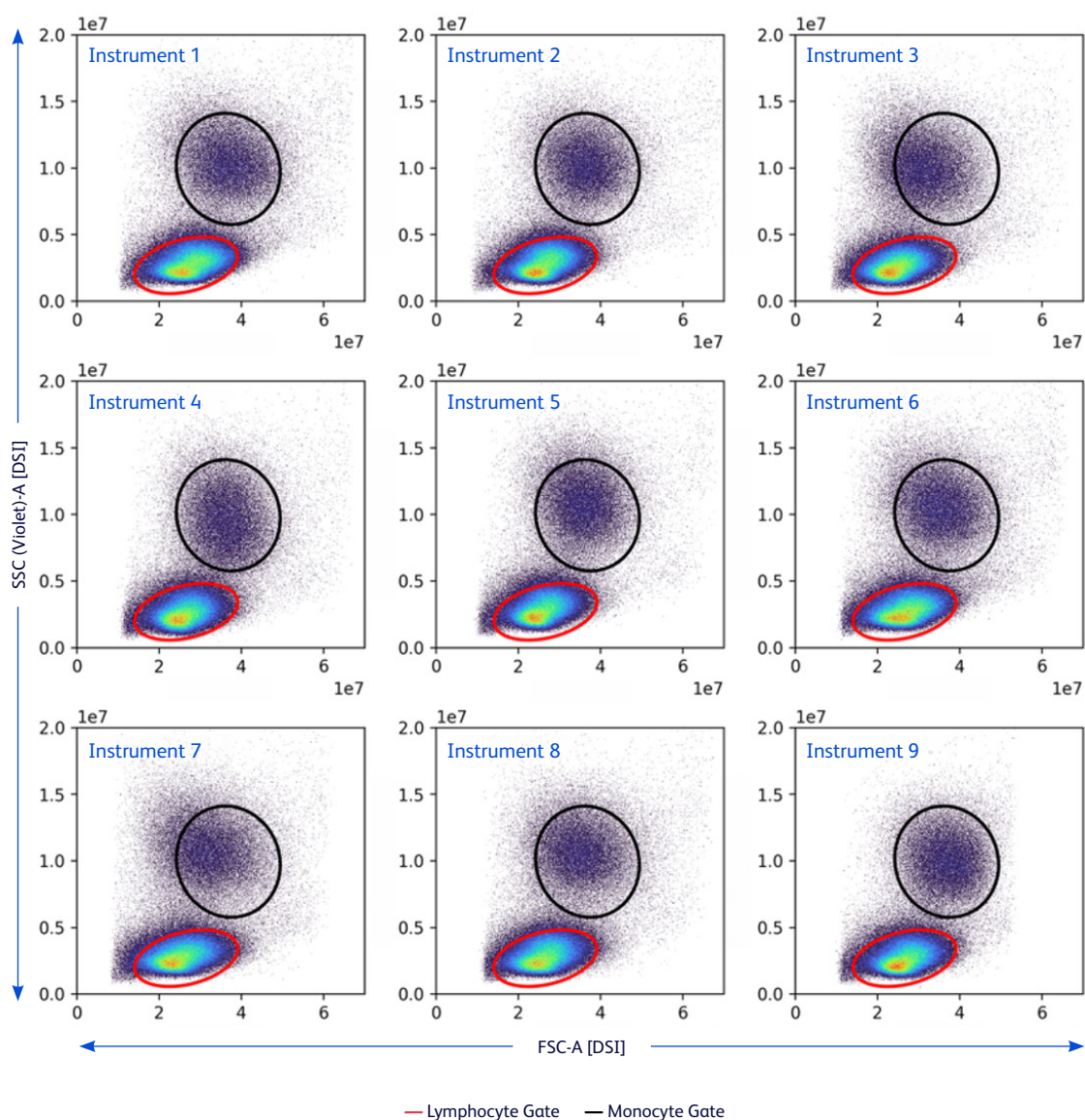
Benefits for Assay Transfer and Routine Use

More stable gating templates can provide several practical benefits:

- » Reduced manual gate adjustment
- » Lower operator-to-operator variability
- » Simplified assay transfer between instruments
- » Greater consistency across longitudinal studies
- » Improved compatibility with automated analysis
- » Reduced dependence on a single acquisition template
- » More reliable application of predefined assay criteria

FIGURE 1. Universal Light Scatter Gating of DSI Scaled Data on BD FACSDiscover™ A7 Cell Analyzers.

FSC vs SSC (Violet) DSI scaled data of PBMC population acquired on 9 BD FACSDiscover™ A7 Cell Analyzers with identical gating strategy.



Key Takeaways

- » Keeping an assay consistent and reliable — across days, instruments, operators, and sites — remains one of the most significant challenges in flow cytometry.
- » FACSTruQ™ Technology transforms standardization from a workflow challenge into a built-in system capability.
- » FACSTruQ™ Technology combines onboard detector characterization, calibrated setup beads, and integrated quality control to provide a foundation for reproducible measurements across instruments and over time.
- » DSI scaling extends this capability by separating biological signal interpretation from detector settings while preserving measurement flexibility.
- » FACSTruQ™ Technology, combining next-generation quality control with DSI scaling, enables more reproducible data, simplified workflows, and greater focus on biology rather than instrument adjustment for comparability.





Additional technical background regarding the implementation of DSI scaling is available in the dedicated BD FACSDiscover™ A7 Cell Analyzer white paper:



BD flow cytometers are Class 1 Laser Products. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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