Flow Cytometry Technologies

Integrating user-adjustable post-unmixing compensation into spectral workflows in Flowscape™ software

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Real-time refinement

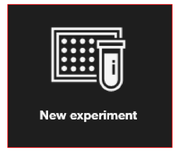
Coming to Invitrogen™ Flowscape™ software for the Invitrogen™ Attune™ Xenith™ Flow Cytometer and the Invitrogen™ Bigfoot™ Spectral Cell Sorter, this newly added feature enables users with additional control over their spectral protocols.

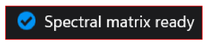
Fine-tune spectrally unmixed data with tools built directly into the existing spectral workflow. Adjust compensation in real time, save multiple matrices per protocol, and quickly apply them to evaluate compensated data across samples.

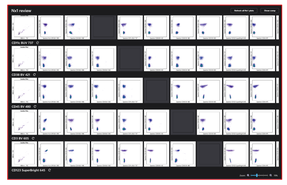
Enhanced spectral workflow

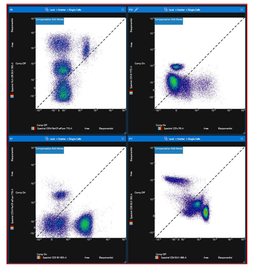
Existing workflow steps in **black**; new, optional, compensation workflow steps in **red**.

- Setup a spectral protocol
- Acquire controls
- Generate a spectral matrix
- Review Nx1 plots of single-color controls (SCCs)
- Refine compensation based off SCC data
- Record experimental samples
- Refine compensation based off experimental data
- Store multiple compensation matrices in Compensation Settings (CS) badges
- Apply or remove CS matrices to/from experimental samples
- Export data









Nx1 compensation tools

Adjust compensation within the matrix or directly on plots themselves.

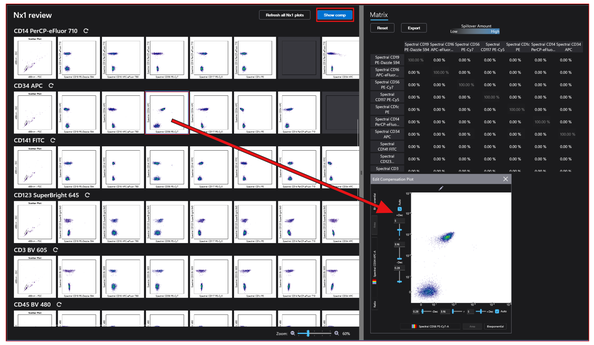


Figure 1. Nx1 Spectral Review tool with compensation enabled.
On the Nx1 Spectral Review screen, Nx1 plots are automatically created for all single-color controls in a protocol. Selecting “Show comp” enables the user-scalable Matrix panel where compensation values can be modified.

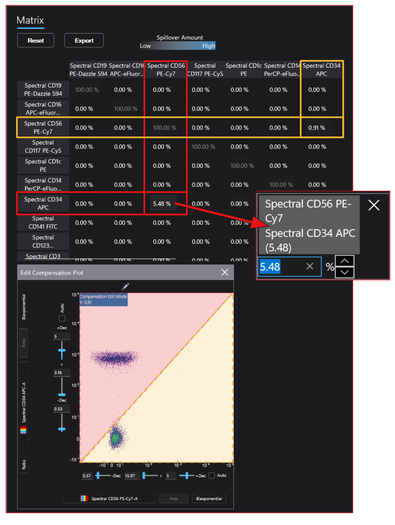


Figure 2. Interactive compensation options available to the user
Compensation values can be manually entered and modified in the matrix, or users can opt for on-plot compensation adjustment via click and drag interactions. Users can compensate X out of Y and Y out of X on a single plot; colors are overlaid and matched with corresponding matrix values for clarity.

Workspace compensation

Workspace compensation adjustments are updated live to give real-time feedback.

On the below plots, the “Without Compensation” plots display spectrally unmixed data only, while the “With Compensation” plots display spectrally unmixed data with user-adjustable compensation applied. Plot scaling and gates were held constant between both plots, only compensation was toggled on and off for demonstration purposes.

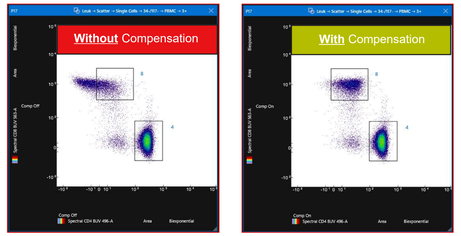


Figure 3. Spectrally unmixed experimental data displayed with and without additional compensation applied – Correcting for poor controls and unmixing errors
The above example shows unmixing error due to the use of poor-quality controls. This error was rectified by applying user-adjustable compensation directly to experimental data in the workspace.

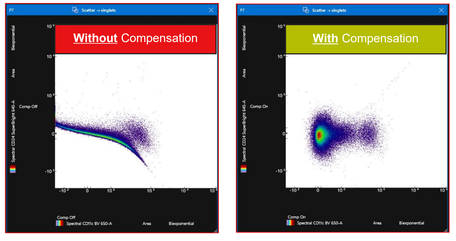


Figure 4. Spectrally unmixed experimental data displayed with and without additional compensation applied – Highly similar fluorochromes
The above example exhibits unmixing difficulties due to highly similar fluors (SuperBright645 and BrilliantViolet650 have a 0.96 similarity score) that was rectified by applying user-adjustable compensation directly in the workspace.

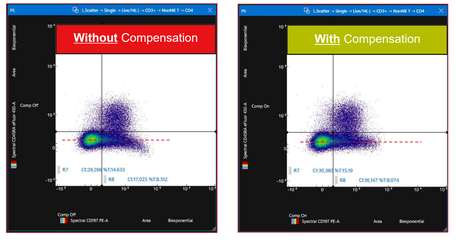


Figure 5. Spectrally unmixed experimental data displayed with and without additional compensation applied – Minor visual adjustments to experimental data
The above plots display a minor compensation adjustment to spectrally unmixed data to slightly enhance the resolution of this memory T-cell population.

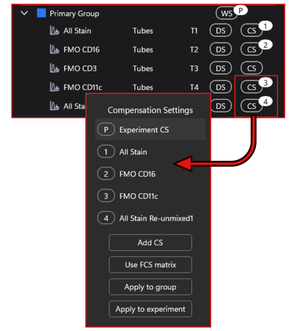
Compensation Settings management

Figure 6. Compensation Settings (CS) badges
Compensation Settings (CS) badges are used to organize, manage, and quickly apply matrices to samples in your protocol.

Multiple matrices can be present in a single protocol.

When a CS badge is applied to a sample, the data will automatically refresh and replay with new compensation settings applied.

CS badges can quickly be applied to individual samples, groups, or to the entire experiment.



Best practices to reduce reliance on SW tools

Spectral unmixing performance and perceived panel resolution can be impacted by multiple factors including, but not limited to:

- Instrument setup and QC
- Panel design and complexity
- Quality of single-color controls
- Control gating
- Channel voltages
- Protocol gating strategy
- Use of FMOs

Materials

The following fluorophores were used in data generated for this poster: HLA-DR BUV395 (Thermo Fisher (TF)), CD4 BUV496 (BD/Waters), CD8 BUV563 (BD/Waters), CD11c (TF), CD38 BV421 (TF), CD45RA eF450 (TF), CD45 BV480 (TF), CD3 BV605 (BD/Waters), CD123 & CD24 BV650 (TF), CD11c SuperBright645 (TF), CD141 FITC (TF), CD13 PerCP-eF710 (TF), CD197 PE (TF), CD117 PE-Cy5 (TF), CD56 PE-Cy7 (TF), CD19 PE-Dazzle594 (TF), CD34 APC (TF), CD116 APC-eF480 (TF).

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