Helios Instrument Control Software
v6.5.358

System Requirements
This software version is intended for installation on Helios™, CyTOF® 2-to-Helios Upgrade instruments, and on Windows® 7 Pro 64-bit stand-alone computers for data analysis. It must NOT be installed on CyTOF, CyTOF 2, or CyTOF C5 Upgrade instruments.

Summary of Changes
This document summarizes the changes included in the Helios instrument control software version 6.5.358 for the Helios mass cytometer. This version provides:

• Support for automated sample acquisition in event mode using the AS-5 Autosampler.
• Support for the acquisition of single samples and tuning using the AS-5 Autosampler.
• Improved tuning ease-of-use by adding a pass/fail criteria for the tuning results.
• Additional workflow enhancements.

For more information, refer to the Helios, a CyTOF System User Guide (PN 400250).

Details of Changes

Graphical User Interface that Supports the AS-5 Autosampler

• The Experiment Manager window contains an enhanced Sample Plan that allows users to set up and record data for their 96-well plate samples.
• Acquisition settings such as Sample Volume (µL), Stop at Event or Stop at Time, Pre-Wash timings for water wash and acid wash, Add Volume, and Agitation Cycles can all be adjusted in the Sample Plan within the Sample Table, and may be applied to all samples in the Plate Setup using the right-click drop-down menu.

• Fill down is available in the Sample Table right-click drop-down menu.

• The Add Volume feature allows users to add a specified volume of deionized water (DIW) from the carrier reservoir to dilute and resuspend samples.

• The Sample grouping function allows users to determine sample acquisition order in the Sample Plan. Samples within the same group are acquired alphanumerically (I-A01, I-A02 ... I-A12).

• Ungrouped samples are run alphanumerically on the plate (I-A01, I-A02 ... I-A12) after all grouped samples are acquired.

• Improved functionality in the sample uptake sequence allows each sample to be completely acquired before the subsequent sample is picked up by the sample probe to avoid extended sitting time in the sample loop.

**Autosampler Single-Sample Control**

The control bar on the lower panel of the Helios software can be used to control single-sample loading and tuning procedures in AS-5 Autosampler mode.

• Users can manually load autosampler wells with samples and adjust parameters in the Settings window. Users can also choose to set time for pre-washing. This is particularly useful for tuning the instrument with tuning solution.

• The Load button initiates sample loading into the sample loop. During sample loading, the Well ID and Vol fields turn yellow. The Well ID and Vol fields turn blue after the sample has been loaded.

• During instrument tuning procedures, users begin tuning by choosing the appropriate tuning protocol (Full Protocol, Quick Protocol, or Custom Protocol) in the Current Protocols window and clicking **Record** in the Tune tab after tuning solution has been loaded.

• To run single samples without using the Sample Plan in Experiment Manager, users acquire data by clicking **Record** in the Acquire tab after the sample has been loaded.

• The Purge button within the Control bar discards contents from the idle sample loop and flushes the loop with Carrier Fluid.

• The Settings window enables users to adjust Tray Type, Agitation Cycle, and Acquisition Delay for the single-sample acquisition. The Return Plate button returns the plate to the sample tray holder. The Reload Pumps button reloads the syringe pumps to eliminate air bubbles.
**Enhanced Instrument Tuning**

- Improved tuning procedures provide users with pass/fail indications for Mass Resolution, Dual Slopes, R2 (of the Dual Slopes), Oxide Ratio, Dual Counts, and %RSDs within the Results tab of the Tuning Manager. Green indicates a pass and red indicates a fail. A failed tuning result means that tuning must be repeated after instrument adjustments, such as cleaning of parts.

- In the Tuning window, you may click **Preview** to visualize data prior to clicking **Record**.

  **NOTE** Customers should contact Fluidigm Support to generate new tuning protocols with updated pass/fail parameters in the Tune tab.

**Additional Workflow Enhancements**

- **Automatic Detector Voltage Optimization:** An optional feature to automatically optimize detector voltage before each acquisition is available in this software release. When this feature is enabled, the detector voltage is optimized using the intensity to pulse ratio for $^{131}$Xe. The resulting algorithm provides detector voltage adjustments when the background signal for $^{131}$Xe is lower than 400,000 dual counts. Technical support guidance is advisable prior to enabling this capability.

- The **Carrier Always On** checkbox, previously in Control Panel > Devices > Plasma Management, is removed from user interface in this version and this functionality is active by default. Users can click Sample Introduction ON and OFF to check nebulizer spray when plasma is off. Sample introduction will be automatically shut off when the software is closed.

- **Automated COM port detection for the Sample Loader:** Users do not require manual COM port selection when installing the Sample Loader.

- The Status Panel shows the correct values for the Nebulizer and makeup gases on Helios instruments when MFBHelios is selected from the Devices tab in the Control Panel.

**Known Limitations**

- In the Experiment Manager window, elements with identical masses may be selected. To avoid issues in downstream analysis, design acquisition templates to contain channels with a unique mass.

- In the Experiment Manager window, solution mode templates cannot be applied to Autosampler Plans.

- In the Experiment Manager window, the templates applied to a Sample Plan when using the Sample Loader must have the same type of acquisition mode (i.e., event mode or solution mode).
In the Experiment Manager window, using AS-5 Autosampler mode, a warning symbol may appear beside the Limit column when a new plan is created. To avoid this error, choose **DVS-T96-250UL** from the Plate drop-down menu and then choose the **DVS-T96-500UL** plate. This will also clear a validation error that may occur in conjunction with the warning symbol when exiting the Experiment Manager window.

In the Autosampler Single Sample control window, when a sample is loaded and the Helios software is closed, the software does not remember the status of the loaded sample upon restarting. Do not close the software before beginning acquisition when a sample has been loaded.

If the Tuning Manager <Results tab is open during tuning, when tuning is complete, the results for Gases/Current will not be updated until the window is refreshed by closing and reopening the Tuning Manager.