CyTOF®2 Software Release notes

Version: 6.0.626
Date: January 20, 2013

Overview:

This document summarizes the changes included in software version 6.0.626 and provides installation instructions on the CyTOF2 Mass Cytometer. This version includes several major improvements and critical revisions to the previous version, including:

1. XY alignment speed and accuracy improvements
2. Simplified workflow for collection of large volume samples
3. Enhanced control of syringe pump and flow injection valve
4. FCS file concatenation tool now available
5. Two levels of operator access
6. Bead normalization tool now available
7. Autosampler sample handling improvements
8. IMD file saving for advanced users now available
9. Updated default values for Auto-tuning and Event Analysis
10. Editable ID and Description fields in Tuning Profiles tab

Detailed instructions for how to use each of these important revisions are found on the following pages.

Installation instructions:

1. Back up the current CyTOF2 Software (In case DVS needs to access in the future)
   a. Copy this folder: C:\Program Files (x86)\DVSSCIENCES\CyToF\
   b. Place in a desktop folder named ‘CyTOF2 Backup files’
2. Rename folder with appropriate version number. For example, if your current CyTOF2 version is 6.0.449, name the folder “CyTOF2 449”.
3. Go to the control panel and Uninstall your existing CyTOF2 software.
4. Download 6.0.626 Software by clicking here.
5. Double-click the cytof_setup_6.0.626.msi icon and follow the installation instructions.
6. Start the CyTOF2 software.

For any questions, please contact DVS support by email support@dvssciences.com or phone +1-855-387-2986.
Summary of changes:

1. XY alignment speed and accuracy improvements

A new Auto-tuning XY algorithm has been implemented that rapidly and accurately aligns the torch to the interface.

   a. Follow the User Manual for setting up Auto-tuning.

   b. In General Parameters, also select Pre Calibration XY Optimization and XY Optimization.

   c. Click Run in the Control Tab to start the full calibration.

   d. Calibration will start for all the parameters selected as described below and in the User Manual.

      i. The Pre Calibration XY Optimization step will find a coarse XY optimum. This is the first tuning step, followed by mass calibration.

      ii. After mass calibration, the XY Optimization further fine-tunes the optimal XY position. After XY calibration, Detector Voltage, Dual Calibration, Gas/Current Optimization and QC Report will follow.
2. Simplified workflow for collection of large volume samples

Samples with volumes greater than 500 µL (up to 5mL) can now be collected as a single FCS file. This is achieved by programming the acquisition time for longer than it takes for 500 µL to pass through the system and injecting sequential 500 µL aliquots into the dual loop system until data acquisition completes.

a. In the Acquisition Parameters box, input time required to collect the sample. This can be determined using the following formula:

\[
\text{Acquisition Time (s)} = \text{Volume (mL)} + \frac{\text{Acquisition Speed (mL/min)}}{60 \text{ (s/min)}}
\]

For example, for a 1 mL sample running at 0.045 mL/min will take 1333 seconds to acquire.

b. Split the sample into 500 µL aliquots and inject the first 500 µL when ready.

c. Click **Run** to begin acquisition.

d. Inject second aliquot during acquisition.

e. The instrument will automatically collect the second loop after completing collection of the first loop.

f. If acquisition requires more than two injections, the following prompt will appear after completing collection of the second loop.

![Prompt to load another sample](image)

 g. Inject the third aliquot only when the prompt appears, then click **OK**.

h. This process (f and g) is repeated until the entire sample is collected.

i. Once acquisition completes, a single FCS file is generated.
3. Enhanced control of syringe pump and flow injection valve

New controls have been added that enable syringe pump operation while plasma is off and for control of the valve that switches between sample loops. These controls are particularly helpful for checking nebulizer spray before starting an instrument, as well as for cleaning procedures after tuning, in between samples or experiments, and at the end of the day.

a. Additional Syringe Valve Controls:

b. Using the syringe pump controls to check nebulizer spray before plasma start:
   i. Remove Nebulizer from Nebulizer Port.
   ii. In Control Panel (Setup) > Analog Control, find Nebulizer Gas.
   iii. Click Set Actual Current Value. This will start the flow of Nebulizer Gas.
   iv. Ensure that the Carrier reservoir is filled with Deionized water. Once the Nebulizer Gas has dried all visible water residue from the Nebulizer, click to start the syringe pump.
   v. Observe the spray from the nebulizer. It should appear as a fine aerosol that leaves the nebulizer in an even, symmetrical pattern. If not, replace the nebulizer.
   vi. Click to stop the syringe pump.
c. Using the syringe pump controls for daily cleaning procedures

i. Cleaning after running Tuning solution

1. Inject 1mL of Washing Solution and click to switch the loop.
2. Wait 2-5 minutes to allow Washing Solution to run through.
3. Repeat steps 1-2 to clean the other loop.
4. Inject 1mL of DIW and click to switch the loop.
5. Wait 2-5 minutes to allow DIW to run through.
6. Repeat steps 4-5 to clean the other loop.
7. Check the status by clicking “Preview” in the control tab. This will display 10 snapshots of any ion signal traces that are detected.
8. Repeat for the other loop.

ii. Cleaning after running Beads

1. Inject 1mL of DIW and click to switch the loop.
2. Wait 2-5 minutes to allow DIW to run through.
3. Click “Re-Preview” to check for residual beads.
4. Repeat steps 1 -3 to clean the other loop.
5. If the beads are persistent in the loops, inject 500uL of Washing Solution and click to switch the loop.
6. Wait 2-5 minutes to allow Washing Solution to run through.
7. Repeat steps 5-6 to clean the other loop.
8. Flush the Washing Solution out by injecting 1mL of DIW and clicking to switch the loop.
9. Repeat to flush the other loop.
10. Wait 2 minutes to allow DIW to run through.
11. Click “Preview” to check status for both of the loops before proceeding.

iii. Cleaning between samples

1. Inject 1-3mL of DIW and click to switch the loop.
2. Wait 2-5 minutes to allow DIW to run through.
3. Repeat steps 1-2 to clean the other loop.
4. Check background signal using “Preview”.
   a. If background signal has returned to baseline, proceed to the next sample.
   b. If background signal is high, inject 1 mL of Washing Solution and click to switch the loop.
   c. Inject another 1mL to clean the second loop.
   d. Wait at least one minute for washing solution to run through.
   
   e. Inject 1mL DIW and click to flush through. Repeat for the other loop.
   f. Check in “Preview” before proceeding to the next sample.

iv. Cleaning between experiments or at the end of the day

   1. Inject 1mL of Washing Solution and click to switch the loop.
   2. Wait 2-5 minutes to allow Washing Solution to run through.
   3. Repeat steps 1-2 to clean the other loop.
   4. Inject 1mL of DIW and click to switch the loop.
   5. Wait 2-5 minutes to allow Washing Solution to run through.
   6. Repeat steps 4-5 to clean the other loop.
   7. Click “Preview” to check both loops.
   8. Repeat if background signal has not returned to baseline.
4. FCS file concatenation

Multiple FCS files from the same sample can now be concatenated into one file. Concatenation should be done after normalization, if applicable.

a. Open FCS Analysis from the menu bar.
b. Select the FCS files to be concatenated; Ctrl-select multiple files.

c. Check Concatenate .

Note: Only concatenate files that have the same analytes and labels in the channel selection.
d. If Normalization Beads are selected, click the drop-down menu and use the Esc key to clear the selection.

e. Click Start to start the FCS concatenation.
f. The concatenated file will appear in the folder specified in Target FCS file, with "_0" as a suffix to the file name.
5. Two levels of operator control

Two levels of CYTOF2 operator control, User and Administrator, are now available. Without logging into the software, User access allows Auto-Tuning and sample acquisition. Logging in as administrator provides additional access to manual tuning, and various settings for auto-tuning and regular optimization of the instrument for consistent performance, along with access to details for troubleshooting purposes. To access administrator mode for the first time:

a. Open CyTOF Software.

b. Close the Status Panel.

c. Click About in the menu bar.

d. Click Login and enter “administrator” for the user name.

e. Leave password blank and click Login.

f. Create administrator password by clicking on Users Settings from the menu bar.

g. Under Personal settings, edit personal information and change the password.
h. Administrators also have the ability to manage users and other administrators. To manage user settings: Under **User Management**, create and edit user or administrator accounts for different levels of access.
6. Bead normalization tool

This tool normalizes sample intensity data against signal drift using information from EQ Four Element Beads spiked into the sample. For detailed instructions on the normalization procedure, see the Bead Normalization User Guide (UG13-02; download from the link on the first page of this document). To normalize files:

a. Open FCS Analysis from the menu bar.

b. Select the FCS file(s) to be normalized.

Note: Batch mode is also available. The normalized files will be saved in the same folder with a suffix (“_1”) appended to the file name.

c. Under Normalization, select the lot of Normalization Beads that were used in the experiment.

Note: Each lot of beads has a unique bead passport file generated at DVS that contains its expected metal intensities. At present, since there is only one lot of beads (P13H2302), select ‘EQ-P13H2302’ from the dropdown. For future lots of beads, load their passports using the appropriate Update button, then select the loaded passport in the dropdown.
d. Leave ‘Remove Beads from Results’ unchecked.
e. Click **Start**. The normalized data will be written to the destination folder.

7. **Autosampler sample handling improvements**
   In previous software versions, approximately 140 μL of sample was retained in the uptake probe for samples with volumes larger than 400 μL. Autosampler sample handling parameters in SW v6.0.626 eliminate sample retention by the probe, maximizing sampling efficiency.

8. **IMD file saving for advanced users is available**
   For Advanced users, IMD files can now be saved. Users that prefer to save the IMD files need to perform regular file transfer and defragmentation of the computer drives for efficient operation of the CYTOF2 computer.

9. **Updated default values for Auto-Tuning and Event Analysis**
   Default values for new Auto-Tuning profiles have been corrected (unless default profiles have already been set). Access to tuning ranges of Detector voltage, Gases, and Current are open to Administrators.

   Default Maximum Event Duration (Event Length) values in Acquisition > Analysis have been updated to 150.

10. **Editable ID and Description fields in Tuning Profiles tab**
    Calibrations in the Tuning Profiles can now be named and a detailed description can be entered. The default ID and description include timestamps of when the calibration is created.