

CyTOF Software 6.7

Contents

Introduction	2
About This Software	2
System Requirements	2
Software Upgrades on Instrument Workstations	2
Improvements and Recommendations	3
General	3
Improvements	3
Recommendations	3
Tissue Imaging Mode	4
Improvements	4
Recommendations	5
Cell Suspension Mode	6
Improvements	6
Recommendations	9
Install CyTOF Software 6.7 for Stand-Alone Processing Workstations	10
Download and Install Software	10
Access to CyTOF Software 6.7 for Windows Non-Administrator Users	17

Introduction

CyTOF® Software 6.7 offers support for imaging and suspension-based sample analysis to Hyperion™ and Helios™ users. The software has the built-in capability to switch sample introduction modes, providing users the flexibility to configure the hardware as required.

This document summarizes changes implemented since CyTOF Software 6.5.358 and provides usage recommendations.

About This Software

System Requirements

CyTOF Software 6.7 is a 64-bit application intended for operation on Windows® 7 Pro 64-bit computer systems (PN 104042 or 104043) with administrator privileges.

This software offers instrument control and data acquisition support for the following systems:

- Hyperion Imaging System: Hyperion Tissue Imager (HTI) coupled with the Helios, a CyTOF system
- Helios, including the AS-5 Autosampler

IMPORTANT CyTOF Software 6.7 is **NOT** compatible with CyTOF, CyTOF 2, or CyTOF C5 upgrade instruments.

Software Upgrades on Instrument Workstations

Users upgrading from CyTOF Software 6.5.358 and earlier builds of 6.7 (such as 6.7.516) should contact technical support to request a software upgrade.

Improvements and Recommendations

General

Improvements

CyTOF Software 6.7 is 64-bit software, an upgrade from earlier 32-bit versions, allowing full access to computer resources resulting in improved performance under intensive data acquisition and data processing conditions.

Recommendations

- To ensure access to sufficient computer resources, do not concurrently acquire data and process data.
- Do not use parentheses or the following unsupported characters in channel labels. These characters will interfere with FCS processing and file export with MCD Viewer 1.0.
 - < (less than)
 - > (greater than)
 - : (colon)
 - " (double quote)
 - / (forward slash)
 - \ (backslash)
 - | (vertical bar or pipe)
 - ? (question mark)
 - * (asterisk)
- Always use the Windows default font size. If the default font size is changed, a “Windows UI unhandled exception” error is displayed.
- Occasionally a message box may be displayed behind the main software window causing the software to appear non-responsive. This can occur during plasma start-up, plasma shutdown, automatic tuning, manual tuning, or while creating panoramas. To close the message box, click the Fluidigm icon in the Windows task bar (all associated windows and boxes are displayed), and then click the message box. After the message box is closed, access to the software is granted.

Tissue Imaging Mode

Improvements

Tuning and Hardware Configuration

- In the Tuning window Results tab, after tuning is complete, values for all instrument parameters, including those not optimized as part of the selected subcalibrations, are displayed.
- In the Tuning window, a message is displayed if region of interest (ROI) ablation completes before the tuning protocol is finished. (The recommended ROI size for automatic tuning is 100 x 1,000 μm .)
- In the HTI Camera window, initial slide coordinates are displayed in the status bar.
- Administrator mode only: Fixed a video time-out error that occurred if the HTI Settings window was accessed (**Control Panel > HTI > Settings...**) during panorama creation.

Data Acquisition

- Improved stability to support longer acquisition times:
 - Fixed a *System.OutOfMemory* exception reported in the HTI Acquisition log that interrupted acquisition. This error no longer occurs.
 - Fixed a memory leak that occurred if the integrated MCD Viewer (now Live Ion Image) remained open during acquisition. Live Ion Image can now remain open during acquisition.
 - The Rainplot window can be detached during acquisition. In v6.7.516, detaching the Rainplot window during acquisition caused the software to report errors or to become unresponsive.
 - Fixed a camera time-out error that interrupted acquisition when the camera was on. The camera can now stay on during acquisition.
- Sample ablation is stopped if plasma is lost during acquisition.
- In the HTI Acquisition window:
 - Acquisition of partially acquired ROIs can be resumed. To resume acquisition, right-click on the sample row and click Resume Acquisition.
 - Templates are applied to multiple ROIs more quickly (a progress bar is displayed). In earlier versions, the software would appear nonresponsive for several minutes.
 - ROIs can be added only when the dimensions are valid. Under Add ROI, the Add button is only enabled when valid width and height values are entered.
 - Fixed a *Target power is out of range* error that occurred under specific conditions when different ablation frequencies were applied to ROIs in the same batch.

- Batch acquisition can now be stopped during detector voltage (DV) optimization or frequency change.
- Data Acquisition Settings: An unhandled exception no longer occurs when Z or ablation energy is ramped.

Data Processing

- Text to TIFF Converter is removed. This functionality is now provided by the stand-alone MCD Viewer 1.0 software application.

Recommendations

Tuning and Hardware Configuration

- To ensure that the Hyperion Tissue Imager properly initializes each time it is turned on, wait until the POWER and UPPER COVERS status LEDs on the Hyperion Tissue Imager are solid green before starting CyTOF Software 6.7.
- The first time the software is started after turning the Hyperion Tissue Imager on, the system log will display the following message during initialization: *Version is not available and serial number is not available*. This means that the laser does not have power. To power the laser and synchronize it with the software:

- 1 Turn the plasma on: On the Toolbar, in the **Plasma** group, click **Start**.
- 2 Verify that the laser is synched with the software and the status is correctly displayed: Open the **HTI Status Panel** (Toolbar > **HTI Status**) and confirm the following statuses are displayed:

Laser Warmup State	Warmed up
Laser State	ON

NOTE If the laser is not on and warmed up, turn the laser on: On the Toolbar, in the **Laser** group, click **Start**.

- Do not stop a tuning protocol that includes Pre-XY or XY calibration during XY ramping. Wait until the the System Log indicates that XY ramping is complete. If tuning is stopped before XY ramping is complete, the stage will remain in the last position. To restore former XY calibration settings, close and reopen CyTOF Software.

Data Acquisition

- Limit acquisition time to a maximum duration of 60 consecutive hours and a maximum time of 2 hours per ROI.
- For optimal panorama navigation, limit panorama dimensions to 4,000 x 4,000 μm .
- For optimal image quality, limit ROI dimensions to 1,000 x 1,000 μm .

- The estimated acquisition time displayed in the HTI Acquisition window for a 200 x 200 μm ROI is overestimated by at least 20%. Greater overestimations are observed with larger ROIs.
- ROIs are acquired in the order that they are added to the Acquisition table, not grouped by panorama. Although new ROIs can be added to the Acquisition table during acquisition, an acquisition template cannot be applied and the ROI will not be acquired. To be acquired, ROIs must be added to the Acquisition table before acquisition is started.
- To save the acquisition log from the HTI Acquisition window, select the log content (click and drag to highlight) and click Save.
- Slide coordinates, displayed in the Camera status bar, are not updated after XY Home is clicked. To update the slide coordinates, use the Slide Move controls.
- The Acquisition window (Acquisition button on the toolbar), that provided single acquisition functionality in versions 6.7.516 and earlier is no longer available. All data acquisition is now performed from the HTI Acquisition window.

User Settings (Email Notifications)

- To receive email notifications by webmail accounts, configure webmail settings to SMTP. Open Authentication (OAuth) 2.0 protocol is not supported.
- If email notifications are configured, the SMTP password must be re-entered one time only following the software upgrade due to encryption upgrades. Go to User Settings and re-enter the SMTP password. If the password is not re-entered, the following error will be displayed when CyTOF Software attempts to send a notification email when a batch completes: *"Sending Email..." Key not valid for use in specified state.*

Cell Suspension Mode

Improvements

Tuning and Hardware Configuration

- Previous X and Y values from the last successful tune are restored if the tuning protocol is canceled during XY calibration.
- A minimum intensity threshold is applied to exclude low-intensity signal during mass calibration to prevent incorrect mass assignment.
- Updated the pass/fail criteria applied to tuning results.
- A new iterative approach to determine baseline calibration reduces variability between instruments.

- Fixed: Tuning protocols that include DV calibration are run with a Turn B range based on the actual current value.
- Optimized parameters from tuning results of any combination of selected subcalibrations can be applied to the instrument (**Tuning Manager > Protocols History > Apply Results**).
- The COM port for Sample Loader is now recognized. (This was an issue with version 6.7.516 and later.)

Data Acquisition

- MPR, TOF, and Rain Plot windows are improved for ease of use:
 - You can now make adjustments to data display on MPR and TOF windows when the Rainplot window is opened. For example, switch between Intensity-Pulses, Duals, change Y-axis scale, units, and min/max on the fly during acquisition and post-acquisition.
 - TOF window: By default, after selecting an analyte, the X-axis scale zooms to display the TOF window for the selected analyte only.
- The Sample Loader now stops immediately after sample acquisition completes instead of after FCS processing. The user can use this time to prepare the Sample Loader for the next sample by manually running deionized water:
 - 1 After the **Sample Introduction** status changes to **OFF**, replace the sample tube with deionized water.
 - 2 Manually turn the Sample Loader on: Under **Sample Introduction**, click **ON**.
- In **Experiment Manager**, under **Advanced** settings, the default template (default.tem) is configured to preserve the IMD files. Before acquisition, consider the following to determine hard drive space requirements:
 - The FCS file requires approximately 12 bytes per channel per event.
 - The temporary IMD file requires 300 kb per channel per second. For example, an FCS file containing 50,000 cell events and 17 channels requires 10 MB of disk space. Therefore, 5 minutes of data collection will result in a 1.5 GB IMD file.
 - Two temporary files are created during acquisition and deleted after completing the FCS conversion. One file requires 5–6 bytes per channel per event and the other requires 12–14 bytes per channel per event.
- Additional elements have been added to the Experiment Manager Element Table:
 - Arsenic: As(75)
 - Selenium: Se(76), Se(77)
 - Bromine: Br(79)
 - Krypton: Kr(82), Kr(83)

Data Processing

- The FCS normalization algorithm now uses the *median* signal intensity from filtered EQ™ Four Element Calibration Beads events to normalize sample data. Version 6.7.516 and earlier uses the *mean* signal intensity, which doesn't account for the presence of non-singlet bead events and may result in a reduction of the normalization factor. To determine whether existing normalized data is affected by this issue, compare the mean EQ bead intensity value of the ¹⁴⁰Ce mass channel in the normalized files to the global standard value of the bead passport used for normalization:
 - 2,313 dual counts for EQ-P13H2302 passport, or
 - 2,565 dual counts for EQ-P13H2302_ver2 passport

If the mean ¹⁴⁰Ce EQ bead intensity is not within 5% of the global standard value, or the beads have been removed from the file, it is recommended to normalize the raw FCS file using the median signal intensity from filtered bead events: **FCS Processing > Normalization > Median bead intensity**.

NOTE Acquired samples containing beads that were resuspended and diluted according to the product insert are likely unaffected. To confirm, gate on bead populations.

- In FCS Processing, the Normalization Passport now contains the most current beads passport only (EQ-P13H2302_ver2). It is recommended to reprocess data originally processed using the first version passport.
- Debarcoder supports FCS files created with Cytobank, FlowJo®, and MATLAB®. To debarcode MATLAB-normalized files, first concatenate the files in FlowJo/Cytobank.
- In FCS Processing under Normalization, Remove Beads removes all bead events from all files. Previously, during batch processing, bead events were removed from the first six FCS files processed.
- Fixed: The Time axis of debarcoded output files is correctly scaled in FlowJo version 7.6.5. In earlier versions, the Time axis scale was not displayed.
 - FlowJo 10 users must manually configure data scaling preferences in FlowJo. For instructions, go to: <http://docs.flowjo.com/d2/workspaces-and-samples/flowjo-and-your-cytometer/ws-instrumentation/>
- FCS files with greater than 10 million events are properly written. Events shown in the Full Statistics window and Plot Viewer match the events recorded in the Acquisition window.
- In IMD Processing, under CyTOF Analysis Parameters, lower convolution intensity threshold is now set to 400 to align with the default template lower convolution threshold setting. In earlier software versions, the lower convolution threshold setting was 200.
- On stand-alone data processing workstations only: FCS Processing and Debarcoder now support non-English Windows Regional Settings.

Recommendations

Tuning and Hardware Configuration

- To apply new parameter setting defaults, all tuning protocols created with earlier versions of CyTOF software must be recreated.
- If a new tuning protocol is loaded in Tuning Manager (Tuning tab > Tuning Manager) confirm that the Protocol displayed on the Tuning tab is updated before tuning is started.
- Do not immediately stop a tuning protocol that includes Pre-XY or XY calibration. Wait until the the System Log displays one of the following messages: a) Starting Pre-XY Calibration or b) Starting Masses Calibration.

If tuning is stopped before either message is displayed, the XY calibration settings are set to 0 instead of being restored.

To restore former XY calibration settings:

- 1 Open Tuning Manager.
- 2 Under Protocols History, click the last successful tuning results, and click Apply Results.

Data Acquisition

- Autosampler batch acquisition cannot be stopped after a sample plan is restarted. Do not restart a sample plan. Create a new sample plan to ensure that the batch can be stopped if needed.

Data Processing

- When processing FCS files, normalize the data before concatenating the files.
- In FCS Processing, the minimum number of beads per time interval is no longer a user specified parameter. It is now set to a minimum of 50 beads per time interval at all times.
- FCS processing fails if the input file contains analyte labels with hyphens between a pair of brackets.
 - In Experiment Manager, in the acquisition template Element Table, use underscores instead of hyphens when entering targets and labels. For example, enter CD45[HI_30] instead of CD45[HI-30]. To edit existing files that contain labels with hyphens, convert the FCS file to text, open the text file in Notepad, search and replace all hyphens with underscores, and then save the file. Use this file to convert to FCS.
 - Use square brackets instead of parentheses within an analyte label. Parentheses are automatically converted to underscores by the software. For example, CD45(HI_30) will be converted to CD45_HI_30_.

- The software version in the FCS header displays the version of the component used to process the FCS file instead of the CyTOF software version. For example:
 - The version is changed to 1.0.0 after Randomizing, Normalizing, or Clustering from the FCS Analysis.
 - The version is changed to 1.7.0 after Debarcoding.
 - The version is changed to 1.0.0 after converting from TXT to FCS from the FCS Analysis.
- In acquisition templates, do not include duplicate channels or isobars, for example, 176Lu and 176Yb, otherwise the names assigned to these channels in FSC output files from reprocessed IMD files, will be incorrect. For example, initial processing of an IMD file results in the correct channel name assignment, but subsequent reprocessing results in both channels receiving the same name (176Lu).

Install CyTOF Software 6.7 for Stand-Alone Processing Workstations

Install CyTOF Software 6.7 to access improved data processing algorithms for **cell suspension data** acquired with CyTOF Software version 6.5.358 and newer.

- Normalize and concatenate FCS files within the FCS Processing window.
- Convert IMD files to FCS files within the IMD processing window.
- Debarcode barcoded FCS files within the Debarcoder window.
- Convert FCS files to .txt files within the Convert FCS to Text window.

IMPORTANT CyTOF Software 6.7 must be installed by a Windows administrator. After the software is installed, to grant access to Windows non-administrators see [Access to CyTOF Software 6.7 for Windows Non-Administrator Users](#).

Download and Install Software

Installation and configuration files are downloaded from the Fluidigm web portal. To access the files, a user account is required. If you do not have a user account, [click here](#) to create one.

To download and install CyTOF Software 6.7 for data processing

- 1 Log in to the installation computer with a Windows administrator account.
- 2 [Click here](#) to download files from the Fluidigm web portal.

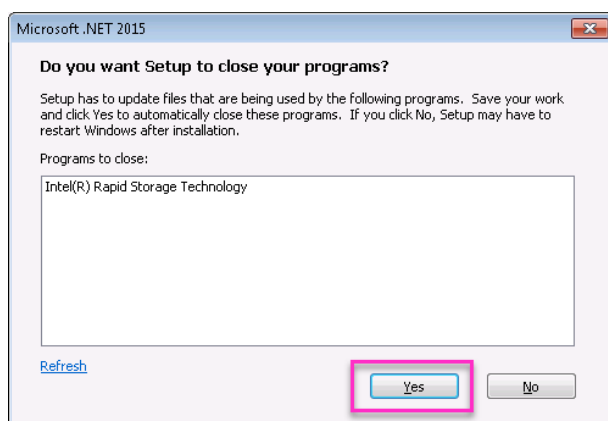
NOTE A user account is required to download the files. If you do not have an account, [click here](#) to create one. Accounts are typically activated within 24 hours. Log in after 24

hours to verify account activation. If assistance is required, contact your local Field Application Scientist.

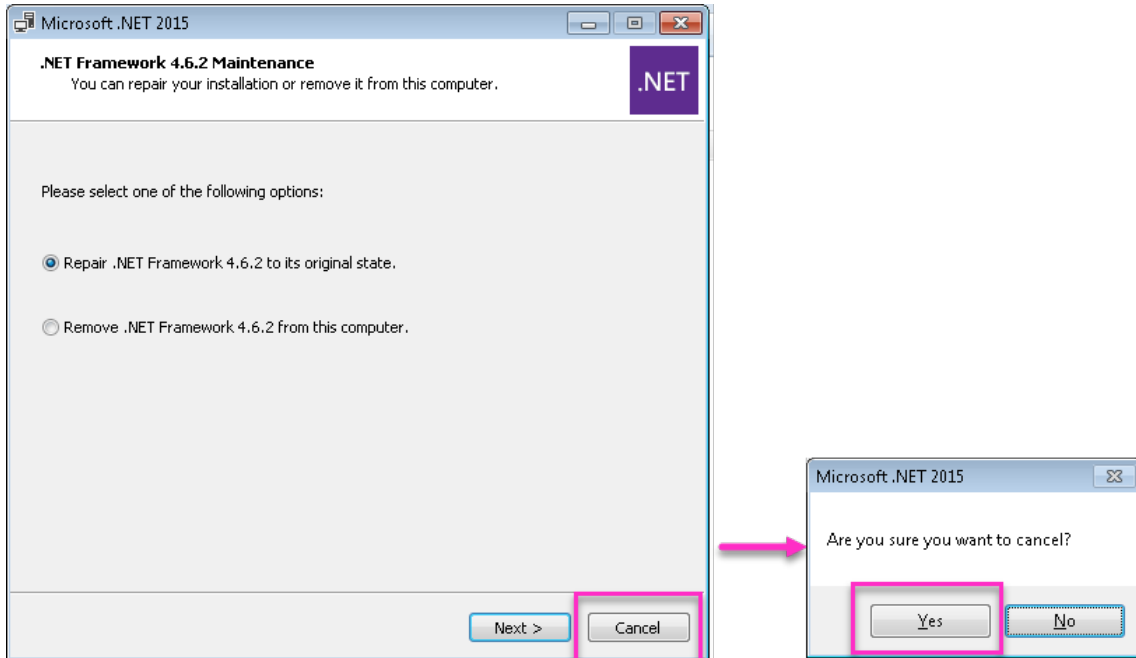
- 3 To log in to the Fluidigm web portal, type your email address and password, and then click **Login**.
- 4 Click **Data Processing Software & Documents > Helios > 6.7 Software Package for Stand-alone Workstations**. The download will start automatically.
- 5 After the download is complete, browse to C:\Users\[*username here*]\Downloads and locate 6_7_StandAloneProcessingPackage.zip. Copy this file to the Windows desktop.
- 6 To extract the zip file contents, right-click **6_7_StandAloneProcessingPackage.zip**, and click **Extract All...**

NOTE A folder named 6_7_StandAloneProcessingPackage is created on the desktop. This folder contains:

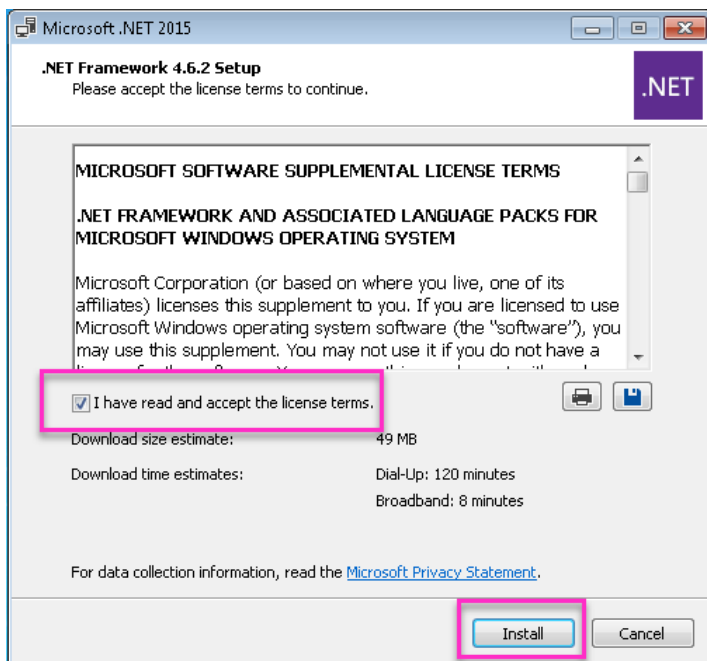
- CyTOF_6_7_1014.exe
 - conf_StandAloneProcessing folder:
 - Conf folder (CyTOF Software configuration files for stand-alone processing stations)
 - Microsoft .NET Framework 4.6.2 (NDP462-KB3151800-x86-x64-AllOS-ENU.exe)
 - Visual C++ x64 2013 Redistributable Package (vc redistrib_x64.exe)
 - README.txt
- 7 Install Microsoft .NET Framework 4.6.2.
 - a Open the **conf_StandAloneProcessing** folder and double-click **NDP462-KB3151800-x86-x64-AllOS-ENU.exe** to start the installer.
 - b If the following message is displayed, click **Yes** to close programs.



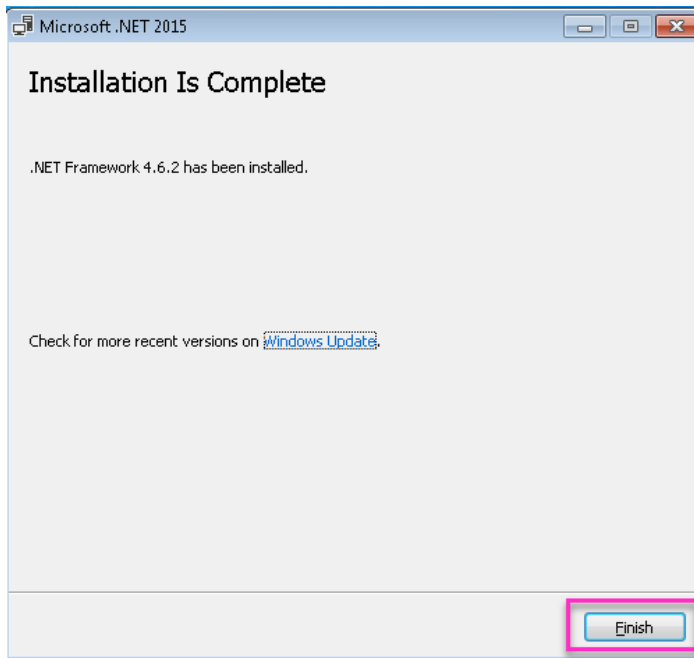
- c If the installer displays the following screen, then Microsoft .NET Framework 2015 is already installed. Click **Cancel** to exit the installer.



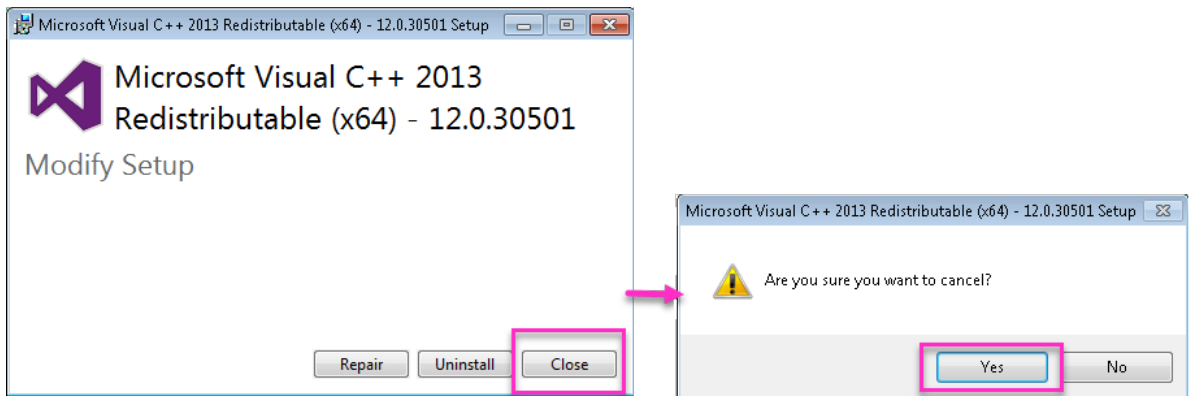
- d Select **I have read and accept the license terms**, and then click **Install**.



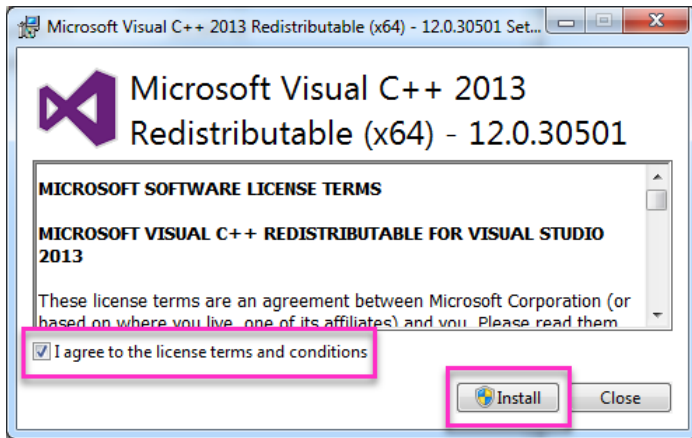
- e Click **Finish**.



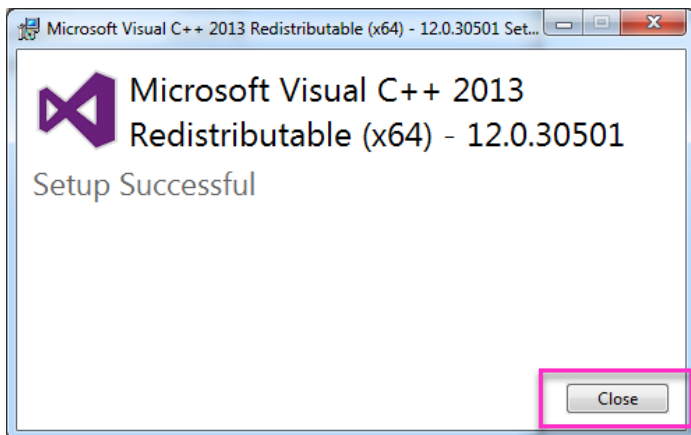
- 8 Install Visual C++ x64 2013 Redistributable Package.
 - a Open the **conf_StandAloneProcessing** folder and double-click **vcredist_x64.exe** to start the installer.
 - b If the installer displays the following screen, Visual C++ 2013 Redistributable (x64) is already installed. Click **Cancel** to exit the installer.



- c Select **I agree to the license terms and conditions** and then click **Install**.

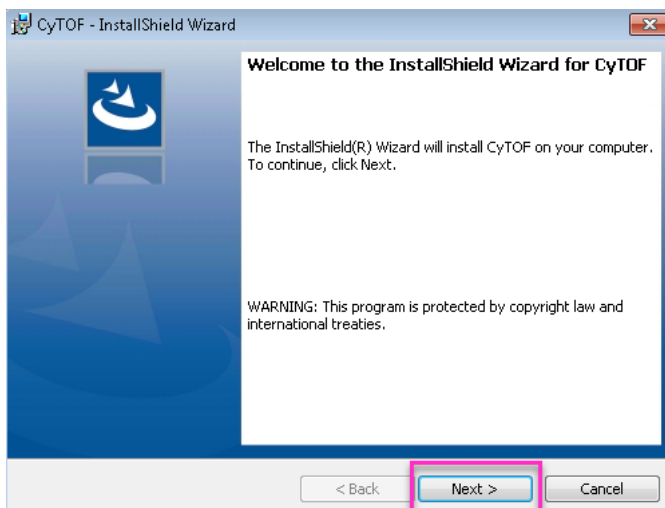


- 9 Click **Close**.

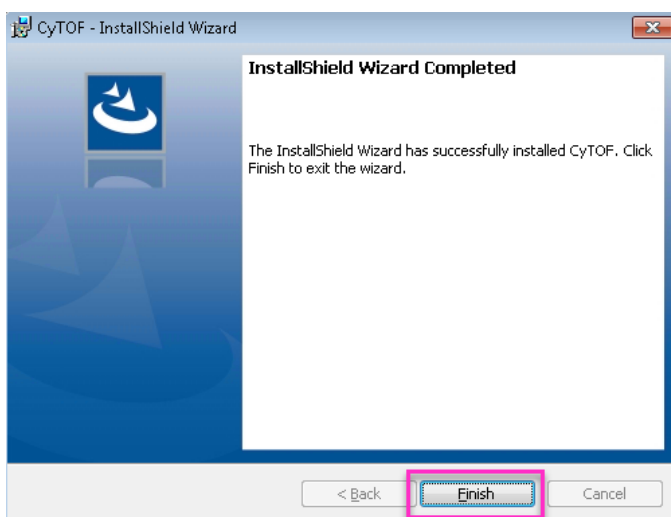


- 10 Install CyTOF software 6.7.

- a Double-click CyTOF_6_7_1014.exe to start the CyTOF installer.
- b Click **Next** to begin installation.



- c Click **Finish**.

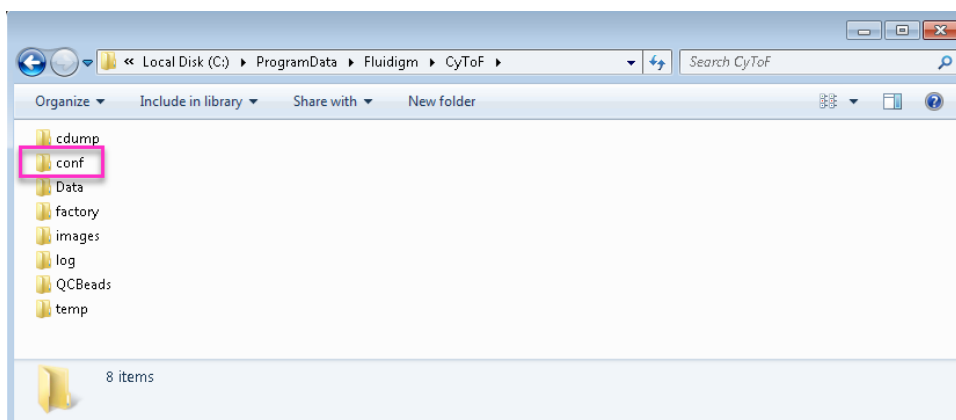


- d Click the red X to close the CyTOF Login dialog box.



- 11 Replace the installed **conf** folder with the stand-alone processing **conf** folder.

- a Open the **conf_StandAloneProcessing** folder and copy the **conf** folder.
- b In Windows Explorer, browse to **C: > ProgramData > Fluidigm > CyToF**.

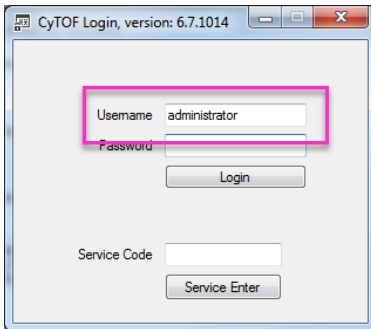


- c Paste the new **conf** folder to this location and overwrite the installed **conf** folder.

12 Start CyTOF software.

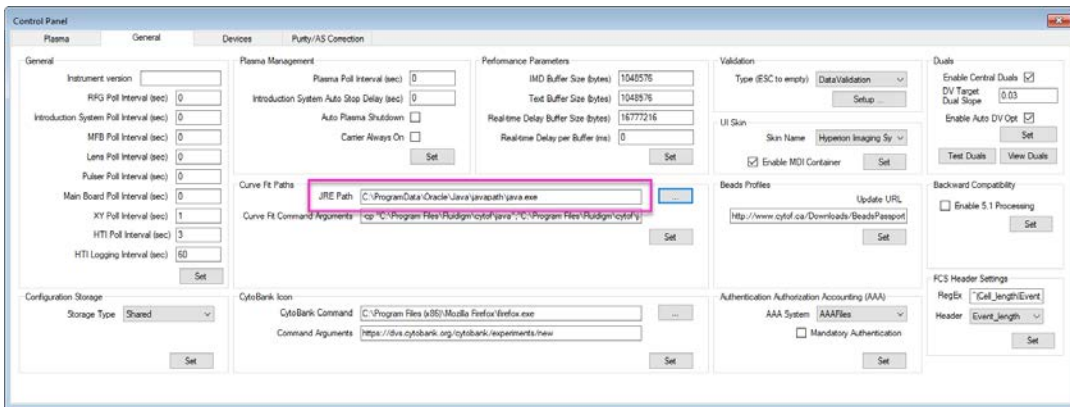
- a Double-click the desktop icon.
- b In the CyTOF **Login** dialog box, type administrator. Click **Login** (or press **Enter**).

NOTE Do not type a password. Initially, the administrator account does not have a set password. To set a password after log in, go to User Settings.

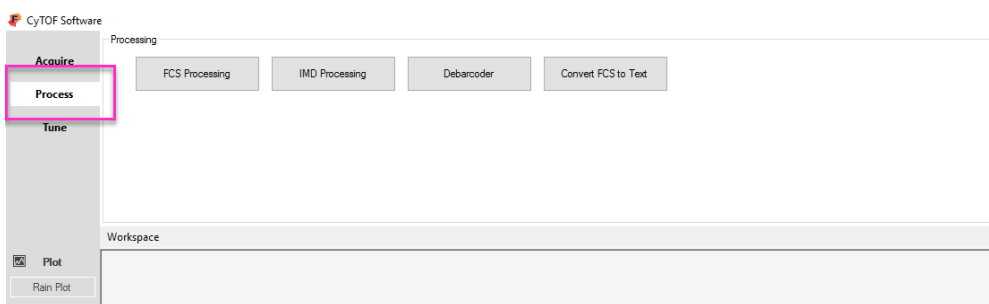


13 Verify that the Java path is correctly registered.

- a In the left navigation bar, under **System**, click **Control Panel** > **General**.
- b Under **Curve Fit Paths**, verify that the JRE (Java Runtime Environment) path is **C:\ProgramData\Oracle\Java\javapath\java.exe**. If this is not the JRE Path specified, manually type it in.



14 To access data processing tools, click the **Process** tab.



NOTE If a Gaussian discrimination error occurs during IMD Processing or Gaussian channels are missing from the resultant FCS file, then Java is not correctly configured. Go to Control Panel > General tab and double-check that the path is correctly typed. If the path is correctly typed and the issue persists, then Java is not installed. Download the latest Java installer here: <https://java.com/en/download/>

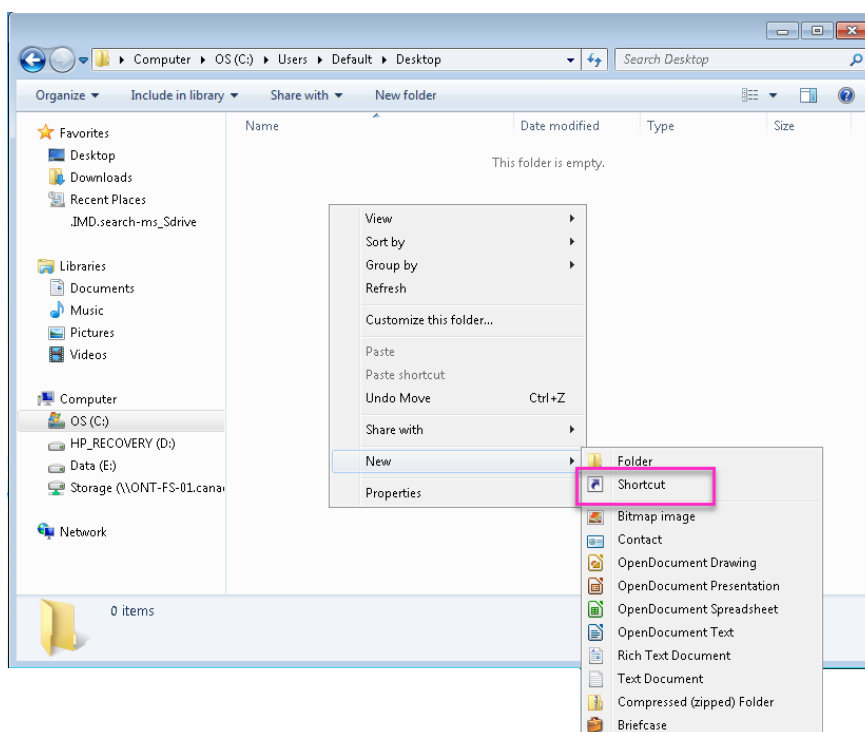
Access to CyTOF Software 6.7 for Windows Non-Administrator Users

To grant Windows non-administrator users access to CyTOF Software 6.7, a modified shortcut must be created using a Windows administrator account.

1 After CyTOF Software 6.7 is installed, open Windows Explorer and go to C:\Users\Default\Desktop.

NOTE Creating the shortcut on the Default desktop will place the shortcut by default on the desktop all new Windows accounts. Existing users must copy the shortcut to their desktop.

2 Right-click in the window and click **New > Shortcut**.



- 3** On the Create Shortcut dialog box, enter the following (replacing *<UserName>* with the name of a local Windows administrator account): **C:\Windows\System32\runas.exe /user:<UserName> /savecred "C:\Program Files\Fluidigm\CytoF\CytoF.exe"**
- 4** Click **Next**.
- 5** Name the shortcut **CytoF**.
- 6** Click **Finish**.
- 7** Log out of the administrator account, and then log in to the non-administrator account.
- 8** On the desktop, locate and double-click the CyTOF shortcut.

If this is an existing Windows account, copy the shortcut from C:\Users\Default\Desktop to the active desktop.

NOTE For first-time use only: For first-time use on each Windows non-administrator account requires the Windows administrator password.

For technical support visit fluidigm.com/support.

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