CyTOF Software v7.0

Introduction

CyTOF® Software v7.0 offers support for imaging and cell suspension-based sample analysis to Hyperion™ Imaging System and Helios™ system users. The software has the built-in capability to switch sample introduction modes, providing users the flexibility to configure hardware as required.

This cumulative release note summarizes the changes included in the latest CyTOF Software v7.0 release (build 7.0.8493) and the previous release (build 7.0.5189).

About This Software

System Requirements

CyTOF Software v7.0 is a 64-bit application intended for operation on Windows® 10 Pro and Windows 7 Pro 64-bit computer systems (Fluidigm 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 coupled with Helios or a CyTOF 2-to-Helios upgrade instrument

- Helios and CyTOF 2-to-Helios upgrade instruments, including the AS-5 Autosampler

IMPORTANT CyTOF Software v7.0 is **NOT** compatible with CyTOF, CyTOF 2, or CyTOF C5 upgrade instruments.

Software Upgrades on Acquisition Computers

For Hyperion Imaging Systems

Software upgrades for the Hyperion Imaging System must be performed by Fluidigm personnel. Contact Technical Support at techsupport.fluidigm.com to schedule a software upgrade.

NOTE Stage calibration is performed as part of the CyTOF Software v7.0 update. This step supports new software features that rely on accurate stage calibration, such as image coregistration and fiducials. As a result, the X/Y coordinates of ablations performed using CyTOF Software v6.7 or earlier will be offset accordingly.

For Helios or CyTOF 2-to-Helios Upgrade Instruments

Software upgrades for a Helios or CyTOF 2-to-Helios upgrade instrument can be performed by customers. Download the CyTOF Software v7.0 installer at fluidigm.com/software.

Features and Improvements (Build 7.0.8493)

Imaging Mode

- **Fixes.**

  - Resolved a timing issue that caused the following error: **Error in acquisition (Trigger Generator is not Armed. Trigger Generator is not warmed up.)**

  - Updated the error message displayed when an email notification cannot be sent.

- **Updated Help content.**

  - New topic: Optimize Ablation Energy for Acquisition

  - Revised topics: Create Fiducials (image updated to show ablation on plain glass) and Recalibrate the Reference Energy (The area recommended to complete this calibration is increased.)

Cell Suspension Mode

No changes to Cell Suspension mode in this release.

Features and Improvements (Build 7.0.5189)

Imaging Mode

- **New user interface.** A new user interface with docking windows provides a highly configurable workspace.

- **Offline batch creation.** Install CyTOF Software v7.0 on a personal computer to prepare batches offline. This is especially convenient for tissue microarray experiments that may require the selection of several regions of interest (ROIs), for working with annotated images, and for support of offsite users.

- **Improved workflow to define ROIs and panoramas.** New tools enable you to precisely define ROIs and panoramas and adjust the positions and dimensions.

  - Copy-and-paste functionality provides easier and faster selection of multiple ROIs directly on the sample image.

  - Import a list of ROI coordinates and dimensions from a CSV file.

    NOTE To create a compatible CSV file, download the CSV template from the Fluidigm website, modify the template in Notepad or Excel®, and save as a new CSV file or follow the instructions in the **Draw or Import ROIs** help topic.

- **Image coregistration.** For improved accuracy of ablation when using sample images, use the instrument camera to coregister an imported sample image with the physical sample inserted in the Hyperion Tissue Imager.

- **Fiducial creation.** Burn fiducials on a sample slide before removing the slide from the Hyperion Tissue Imager. Coregister the fiducials to calibrate the slide position before resuming acquisition of ROIs later.

- **Improved detector voltage optimization routine.** The new detector voltage algorithm supports 10x higher ^{131}Xe signal.

- **High-redundancy auto focus.** Auto focusing routine uses a 9-point pattern for redundancy results in more reliable and effective focusing.

- **Support for larger ROIs.** CyTOF Software v7.0 adjusts the stage height during ablation to compensate for slide topography and periodically optimizes the detector voltage. These improvements result in uniform ablation of ROIs >1 mm^2.

- **Faster panorama creation.** A 1 cm^2 panorama is completed 40% faster than with earlier versions of CyTOF Software, and >40% faster for smaller panoramas.

- **Automated calibration of reference energy.** Calibration of reference energy to 0 dB for ablation of tuning film is now automated.

- **Preventive maintenance reminder.** A reminder is displayed when it is time to schedule preventive maintenance. You can choose to automatically notify the Fluidigm Service Department by email or snooze the reminder.

- **Live ion images in color.** View multicolor ion images to assess sample and data quality in real time or post acquisition.
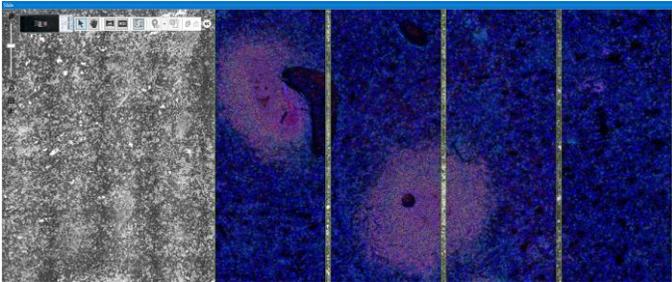
Cell Suspension Mode

- **Support for Maxpar® Direct™ Immune Profiling Assay™.** Acquire data compatible with Maxpar Pathsetter™ software from samples prepared with the Maxpar Direct Immune Profiling Assay using the panel-specific acquisition template: Maxpar Direct Immune Profiling Assay.tem.

  Maxpar Direct Immune Profiling Assay.tem is a noneditable acquisition template designed to record channels for the Maxpar Direct Immune Profiling Assay and channels to monitor background. Panel-specific channels are locked and cannot be deleted or modified, but unlocked channels (to monitor background) can be deleted, and new channels can be added.

Use Save As in Experiment Manager to edit unlocked channels and add new channels to the Maxpar Direct Immune Profiling Assay.tem template. The panel-specific channels remain locked in the derived template.

Mass ▲	Element	Target	Label
89	Y	CD45	89Y_CD45
103	Rh	Live_Dead	103Rh_Liv...
120	Sn		120Sn
127	I		127I
131	Xe		131Xe
133	Cs		133Cs
138	Ba		138Ba
140	Ce	Bead	140Ce_Bead
141	Pr	CD196_CC...	141Pr_CD1...
142	Ce	Bead	142Ce_Bead
143	Nd	CD123 IL-3R	143Nd CD...

Gray channels are Maxpar Direct Immune Profiling Assay channels. They are locked and cannot be deleted or modified.

White channels are unlocked and can be deleted or modified. Use Save As to modify the Maxpar Direct Immune Profiling Assay.tem template with a new name.

NOTE

- When Maxpar Direct Immune Profiling Assay.tem is saved as a new template, the Advanced acquisition parameters are unlocked. Do not modify the Advanced settings. If modified, the FCS file will be incompatible with Maxpar Pathsetter.

- In acquisition templates, use underscores instead of spaces in channel labels. When FCS metadata is written, spaces are automatically replaced with underscores, causing a mismatch between the FCS metadata and the channel labels. They must match in order to create new templates with additional locked channels with Maxpar Pathsetter in Method Develop mode.

- **No Event Processing mode in Experiment Manager is available to CyTOF Administrators.** Create an acquisition template with No Event Processing selected to prevent automatically processing IMD data into FCS files. This reduces the time required for each acquisition and allows all IMD files to be manually processed into FCS files at a later time or on a different computer. Before acquisition, verify that there is sufficient storage space for the IMD file. See the **Data Storage Requirements** help topic for more information.

- **Low disk space warning.** A low disk space warning is displayed when <500 GB of free space is available on the E: drive.

- **Notification of duplicate mass channels in acquisition templates.** A notification displays when mass channels with duplicate mass numbers are included in the same acquisition template. Such duplication increases the time required to process data.

- **Confirmation prompt is displayed when plasma is stopped.** To prevent accidentally stopping plasma, a prompt is displayed to confirm plasma shutdown.

- **Resolved issues**

  Tuning

  - Quick Protocol is named Instrument Check Protocol.

- The **Load more solution** prompt is no longer erroneously displayed at the end of the plasma shutdown sequence.

- In earlier versions, the last run tuning protocol was still applied when a tuning protocol was selected after sorting by Description. The correct tuning protocol is now applied.

Debarcoder

- The Barcode Separation graph refreshes when changes are made to the debarcoding key.

- Changes are saved to a new barcoding key when SaveAs is used.

- Accuracy of the estimated barcode yield is improved.

FCS Processing

- The same time range is applied to an IMD reprocessed into an FCS file on an acquisition computer and on a processing computer.

- Data randomization is applied when IMD data is processed into FCS files. To prevent duplicate data randomization, the Randomization setting in the FCS Processing window is now off by default.

Acquire mode

- To prevent acquisition from being interrupted accidentally with a keystroke, the Stop button is no longer selected by default after acquisition is started.

- Temporary files are always saved to the E:\CyTOF folder.

- After switching from imaging to cell suspension mode, all hardware acquisition settings are restored to cell suspension settings.

General Improvements

- **Searchable, topic-based software help system.** Help content is now installed with CyTOF Software and accessed within the software. Content is organized by topic and employs a predictive text search feature that allows you to quickly find relevant information.

Recommendations

For All Computers

If Internet Explorer is the default browser, the browser settings may need to be modified to correctly display and search the CyTOF Software Help content. Modify Internet Explorer security settings to allow active content. To update the Internet Explorer settings:

1   Open Internet Explorer and click the Settings icon (upper right corner), and then click **Internet options**.

2   On the Internet options dialog box, click the **Advanced** tab.

3   Scroll down to **Security** and check **Allow active content to run in files on My Computer**. Click **OK**.

If this does not resolve the issue, set another browser as the default browser on the system.

For Acquisition Computers

- To reduce or eliminate the occurrence of acquisition interruption due to errors, such as API buffer overflow:

  - Preserve as much free space on the C: drive as possible. Do not install software that is not required for the instrument.

  - Do not acquire data to a network drive. To prevent the effect of network latency on performance, save data to the E: drive.

  - Preserve system resources for data acquisition. Do not run additional software or process data during acquisition. These actions reduce the system resources available for acquisition.

  - Disable or reschedule automatic processes. Windows Automatic Update, disk defragmentation, and virus scanning require computer resources. If a process is scheduled to run during acquisition, acquisition may fail due to insufficient computer resources.

- To ensure adequate disk space and prevent data loss and acquisition interruption, do not copy files to the hard drive while data is being acquired.

- The y-axis on the Masses Per Reading (MPR) plot is automatically scaled based on the channel with the highest signal. To zoom on lower-intensity channels, uncheck channels with high signal.

Imaging Mode Only

Data Acquisition

- Create a new MCD file for each sample slide. Do not acquire data from more than one slide to a single MCD file. File content such as the focal map and optical images are specific to an individual slide.

- For best performance, do not exceed a maximum ROI size of 1,500 x 1,500 μm.

- Data cannot be acquired to the C: drive. The Start acquisition button remains grayed out until the MCD file is transferred to the E: drive.

- Stand-alone ablation cannot be performed from Camera View. Ablation must be performed in Data Acquisition mode. Create a new MCD file and draw an ROI on the Slide Layout.

- Renaming acquisition templates is not supported. If an acquisition template is renamed the analyte list and acquisition settings are lost. To restore the analyte list and acquisition settings, restore the original template name.

- On rare occasions, if an unusually high signal is detected during instrument startup, the detector voltage is automatically set to –2,200 V. This causes detector voltage calibration to fail. If failure occurs, the correct detector voltage must be manually restored. To restore an optimal detector voltage:

    1 Log onto CyTOF Software as an administrator.

    2 Click **Instrument Control** > **Auto Tune**.

    3 In the **Tuning Protocol** table, click the last successful tuning, and then click the **Results** tab. Under **Instrument**, record the **Optimal Detector, V** value.

    4 Close **Auto Tune** and open **Control Panel**. Click the **Analog Controls** tab.

    5 In the **Detector Voltage** row, double-click the **Actual Current Value** cell, and enter the Optimal Detector voltage recorded in Step 3. Click **Set**.

    6 Run **Auto Tune**. (Any combination of subcalibrations is fine.) If Auto Tune fails to optimize the detector voltage, return to **Control Panel** > **Analog Controls**, and increase the **Detector Voltage** by 50 V. Run **Auto Tune** again. Repeat until the detector voltage can be optimized using Auto Tune.

Performance and Compatibility

- MCD files containing large optical images (imported images and/or multiple panoramas) may affect software responsiveness.

    - Limit total panorama area to a maximum of 2×10^8 µm^2. For more information, see the Create Panorama help topic in CyTOF Software.

    - Downscale partial slide images with dimensions >10,000 x 10,000 µm or an equivalent number of pixels. For more information, see the Import an Image help topic in CyTOF Software.

- Software responsiveness may be affected after an image is imported if the hardware configuration includes two or more monitors or a very large monitor.

- MCD files acquired with CyTOF Software v6.7 must be updated for compatibility with v7.0. A software prompt is displayed. You can choose to preserve the original file for backward compatibility or update without preserving the original file.

    IMPORTANT Updating the file does not affect the integrity of the data.

    NOTE MCD files acquired with CyTOF Software v7.0 cannot be opened with earlier versions of CyTOF Software.

Image Import

- An image cannot be rotated after it is imported. Be sure to orient the image in the Import Slide Image window before clicking Apply.

- To avoid accidentally moving or resizing an imported image, lock the image in place. Click the lock icon in the upper right corner of the Slide Layout. When the lock icon is red, the image is locked. Click the icon again to unlock the image.
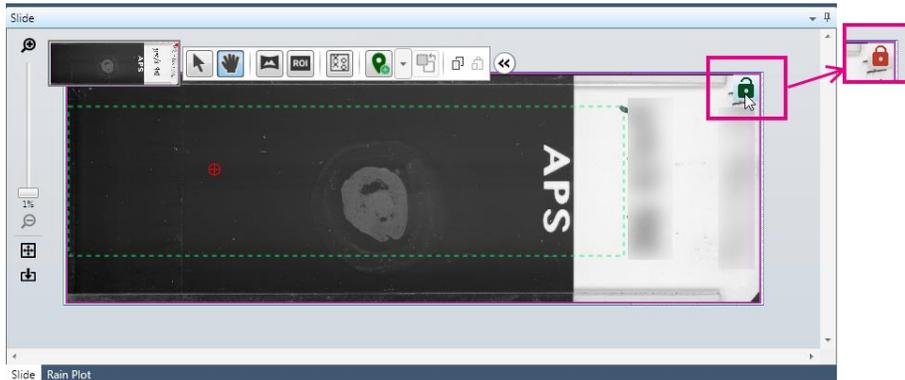
  NOTE An image is automatically locked after coregistration is applied.

Image Coregistration

- Coregistration can be applied after three coregistration pins are placed on an imported image. To activate the Apply Coregistration button, be sure to select objects such as the imported image and unablated ROIs for coregistration. For more information, see the Coregister an Image help topic in CyTOF Software.

Slide Layout

- Undo and redo functions are not available. A confirmation prompt is displayed when an object such as an unablated ROI, panorama, or imported image is deleted. When confirmed, objects are permanently deleted.

- ROIs are no longer grouped by panorama. Use an updated version of MCD™ Viewer (v1.0.560.2) to view and export data acquired with CyTOF Software v7.0.

  NOTE MCD Viewer v1.0.560.2 is also compatible with MCD files acquired with CyTOF Software v6.7. For more information, see MCD Viewer v1.0.560.2 Release Notes (400316-4).

Panoramas

- Panoramas may contain an empty tile if not enough computer resources are available during panorama creation. Do not perform resource-intensive tasks on the computer while creating panoramas. If this issue is observed, create another panorama over the missing tile.

- Before creating a panorama or acquiring data from an uneven surface such as hydrophobic barriers or a chamber slide, verify that focal pins are placed over flat areas. For more information, see the Move Pins on the Slide Layout help topic in CyTOF Software.

- The minimum starting X coordinate for panoramas is 500. An error is displayed in the Panorama table if an X coordinate is <500.

- To export panoramic images, use MCD Viewer. High-resolution panoramas cannot be exported from CyTOF Software v7.0.

Hardware Configuration

- Power the Hyperion Tissue Imager off and on again if the following messages are shown in the System Log: **Integra not available** and **Energy Meter initialization failed**. This issue occurs after the USB cable is disconnected and reconnected when the Hyperion Tissue Imager is connected to a USB 3.0 port. To avoid this issue, connect the Hyperion Tissue Imager to a USB 2.0 port.

Tuning

- To determine the reference energy shift applied when the reference energy offset is recalibrated, record the original ablation energy range displayed in the Manual Tune window, and record the new range after the Eref adjustment is applied.

- Reference energy calibration: In the Manual Tune > Settings window, be sure the following checkboxes are not checked before starting reference energy calibration:
  - Save to TOF File
  - Save to Masses File
  - Save to Transients File

  An error may occur and reference energy calibration may not continue.

Cell Suspension Mode Only

- Acquisition templates (.tem) created with v7.0 are not compatible with earlier versions of CyTOF Software. To create a new acquisition template, export the element list from the v7.0 acquisition template to a CSV file, import the CSV file into an earlier version of software, and then enter the Advanced Settings into the new acquisition template.

  NOTE NoEventProcessing is not available in earlier versions of CyTOF Software.

- Acquisition templates (.tem) created with earlier versions of CyTOF Software are immediately upgraded when opened with v7.0 and are no longer backward compatible. For backward compatibility, create a copy of an acquisition template before opening it with v7.0.

- Reduce or eliminate the occurrence of an API buffer overflow while previewing data by following the general recommendations for all acquisition computers described in Recommendations for Acquisition Computers.

- When Java is updated, the installation path may change. If an incorrect Java path is defined in Control Panel > General > Curve Fit Paths, Gaussian parameters are not generated during FCS processing. To avoid this, turn off automatic updates for Java. If

the Java path in CyTOF Software is incorrect, enter **Java** in the Windows search bar to find the directory and update the path in CyTOF Software.

- When creating a Sample Plan with the AS-5 Autosampler configured, enter all values and settings, including an acquisition template before adding another sample. Otherwise, modified settings will revert to default values.

- **Close CyTOF Software after disconnecting USB cable for the Sample Loader**. Failure to close the CyTOF software while making changes to the hardware will result in problems with tuning during the daily set-up of Helios. The user will also have software errors.

For Non-Acquisition Computers

- **Windows 10 operating system only.** If a Microsoft .NET Framework installation message is displayed and CyTOF Software installation does not continue, exit the CyTOF Software installer and update Windows 10. After Windows is updated, start the CyTOF Software installer and follow the instructions.

- **For personal computers with Windows 7 operation system only.** If the message **.NET Framework 4.7 is not supported on this operating system.** is displayed during installation, exit the CyTOF Software installer, download and install Windows 7 Service Pack 1 (support.microsoft.com/en-ca/help/15090/windows-7-install-service-pack-1-sp1), and then start the CyTOF Software installer.

- **Adjust compatibility settings for small screens with Windows 10 and Windows 8.** Some elements of the software user interface may not display properly on a small screen such as a laptop screen. Adjust compatibility settings for small screens with Windows 10 and Windows 8 for optimal display.

  - Windows 10: Right-click the CyTOF Software desktop shortcut and click **Properties > Compatibility > Change high DPI settings**. Under Settings, check **Override high DPI scaling behavior** and then under **Scaling performed by**, click **System**. Click **OK**.

  - Windows 8: Right-click the CyTOF Software desktop shortcut and click **Properties**. Click the **Compatibility** tab, and under **Settings**, check **Disable display scaling on high DPI settings**. Click **OK**.

Revision History

Revision	Date	Description of change
[07]	06/2021	Include revision history table in document.
[06]	06/2021	Add an additional recommendation under Cell Suspension Mode Only, to close CyTOF Software after disconnecting USB cable for Sample loader.