

CyTOF Software 6.7: Reprocess IMD Files without Headers

Introduction

During acquisition, CyTOF® Software 6.7 writes integrated mass data to an IMD (.imd) file and automatically processes this file to create an FCS (.fcs) file for data analysis. The IMD file header containing the name and label of each channel acquired is written to the IMD file at the end of file processing. The header is not written to the IMD file if file processing is prematurely interrupted.

When an IMD file with a missing header is selected for reprocessing, the following warning message is displayed:

Source file *<location of file>*: header is empty

An IMD file without a header must be manually reprocessed to create an FCS file. This document describes how to use CyTOF Software 6.7 to reprocess an IMD file without a header to generate an FCS file.

Common Causes of Interruption

The most common cause of an interruption in acquisition file processing is insufficient storage space on the hard drive of the instrument workstation. Refer to [Estimate Storage Requirements](#) to determine the storage capacity required. Verify that the storage capacity of the workstation is adequate before starting an experiment.

Additional causes of file processing interruption:

- Closing CyTOF software
- Restarting the computer
- A power outage

How to Reprocess an IMD File and Generate an FCS File

This procedure describes the steps to reprocess an IMD file with the acquisition template to write the missing header content and generate an FCS file.

Files can be reprocessed on an acquisition workstation or a stand-alone data processing workstation.

To reprocess an IMD file and generate an FCS file

- 1 If you are processing files on an acquisition workstation, proceed to Step 4.
If you are reprocessing files on a data processing workstation, copy the following files from the acquisition workstation:
 - LunaTuning.conf (located in C:\ProgramData\Fluidigm\CytoF\conf)
 - Acquisition template from the time of the acquisition (.tem file)
 - The IMD file for reprocessing

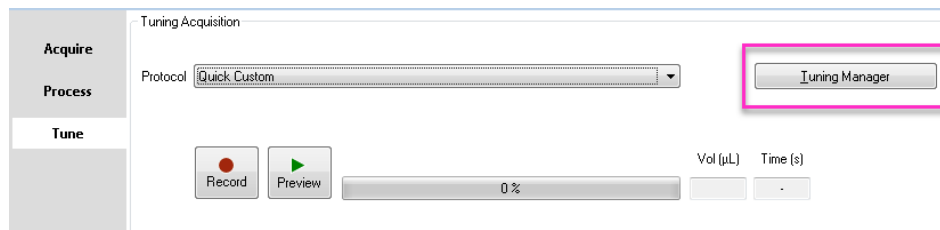
NOTE Record the date and time of the tuning run completed before the acquisition.

- 2 Close CyTOF Software 6.7 if it is open.
- 3 Copy the instrument workstation files to the data processing workstation.
 - a On the data processing workstation, browse to C:\ProgramData\Fluidigm\CytoF\conf, and then paste **LunaTuning.conf**.
 - b Paste the acquisition template (.tem file) and the IMD file to a directory of your choice.
- 4 Open CyTOF Software 6.7.

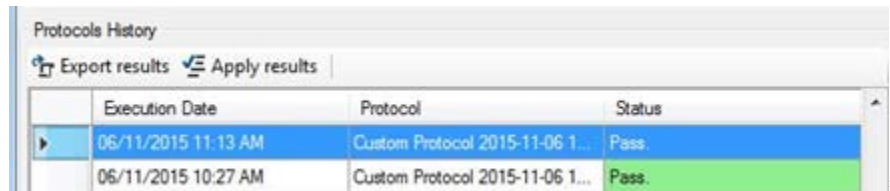
IMPORTANT If you installed an earlier version of CyTOF Software 6.7 for evaluation, confirm that you are using the released version of the software. To confirm, open the software, click **About**, and check that the software version is 6.7.1014. If an earlier version of CyTOF Software is installed on your acquisition workstation, contact your local field application scientist to upgrade the software. To upgrade the software on a data processing station, download the software and release notes from the [Fluidigm portal](#). Follow the instructions in the release notes.

- 5 Apply the calibration results from the tuning run that was performed before the IMD file was acquired:

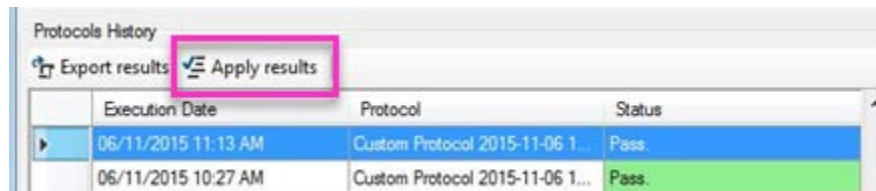
- a Click the **Tune** tab and then click **Tuning Manager**.



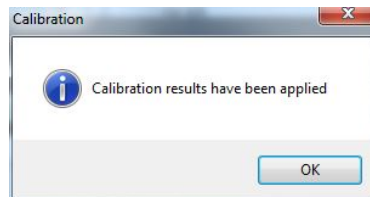
- b Under Protocols History, locate and click the tuning run performed before the start of the sample acquisition to highlight the row.



- c Click **Apply results**.

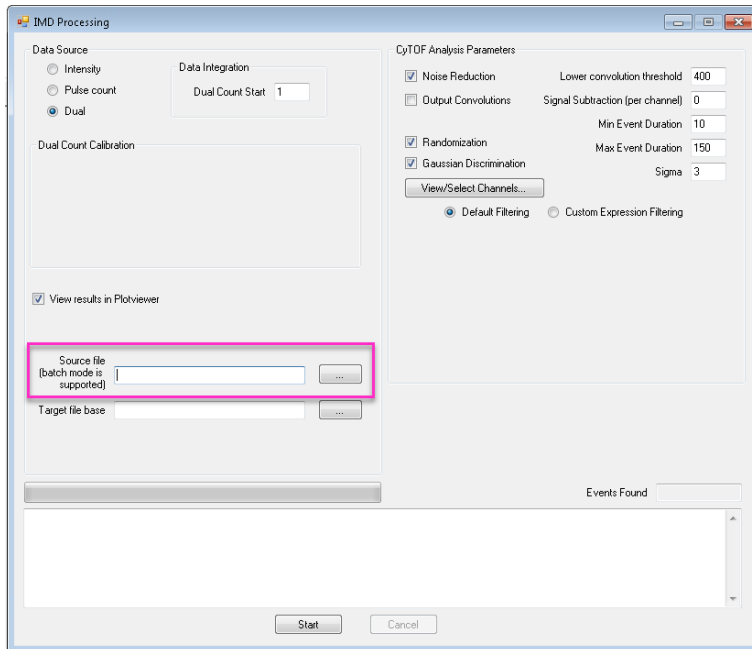


- d Click **OK** when you see a confirmation message stating that calibration results have been applied.



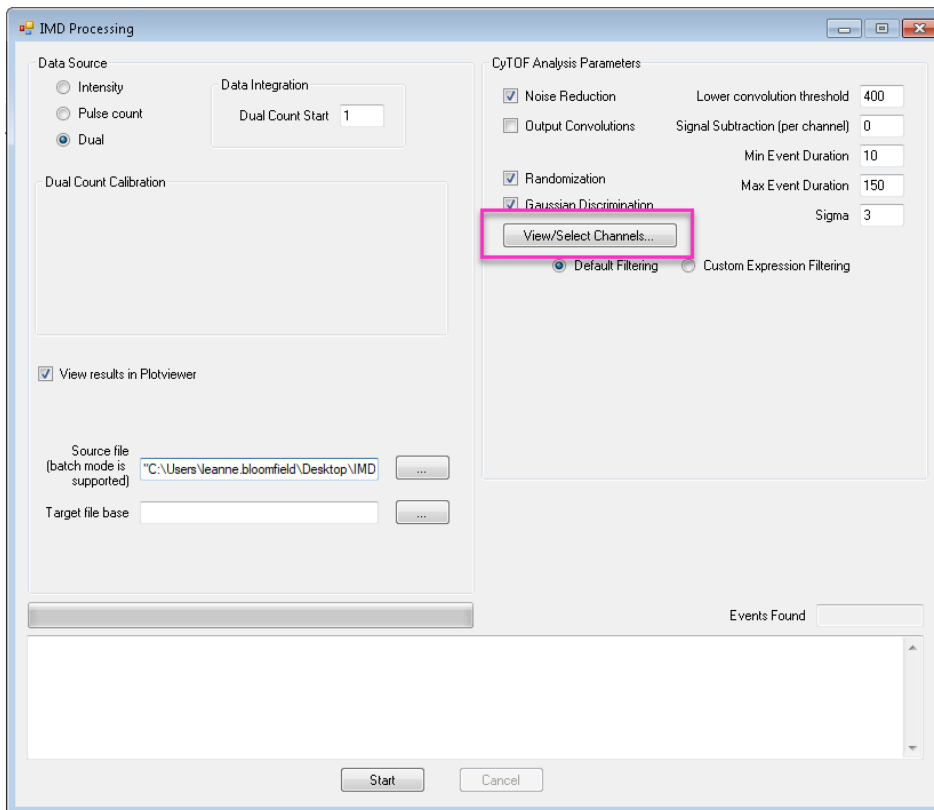
- 6 To open the IMD Processing window, click the **Process** tab, and then click **IMD Processing**.

- 7 Next to Source File, click the browse button  to select an IMD file.

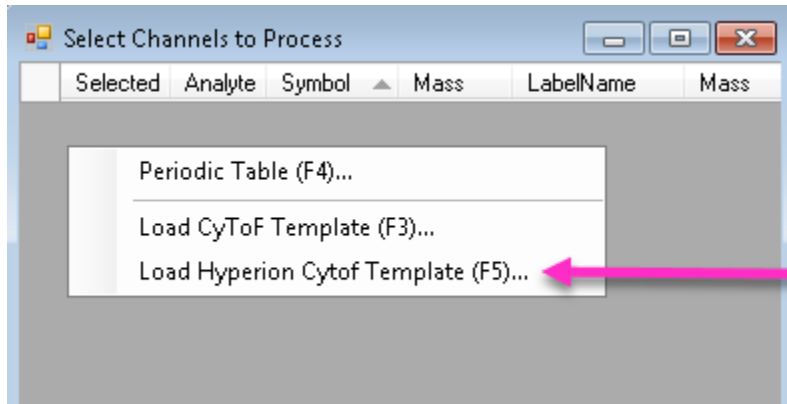


NOTE Batch mode for IMD reprocessing is not supported.

- 8 Click **View/Select Channels**.



- 9 Right-click in the Select Channels to Process window and click **Load Hyperion CyTOF Template (F5)...**

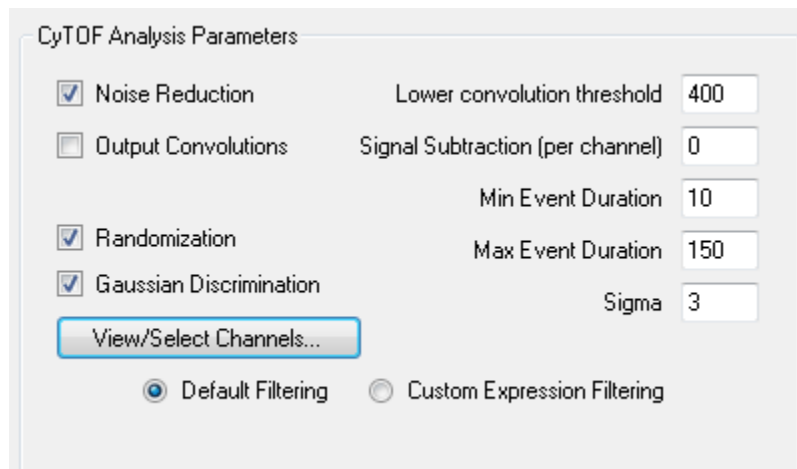


NOTE In CyTOF Software 6.7, the Hyperion™ CyTOF Template is the correct template for use with Helios acquired data. **Do not load the CyTOF template.**

- 10 Locate the template used for acquisition, click to select it, and then click **Open**.

NOTE After the template is selected, the channels are displayed in the Select Channels to Process window. Verify that this is the correct template, and then click **OK**.

- 11 Under CyTOF Analysis Parameters, use the same parameters and settings from the acquisition for IMD processing. (Shown below are the default parameters and settings.)



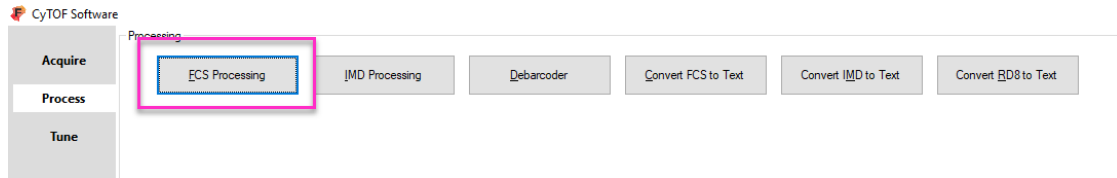
NOTE The default lower convolution threshold value for acquisition on Helios is 400. To verify the lower convolution threshold value used for your data, open the acquisition template in Experiment Manager and click **Advanced**.

- 12 Click **Start** to begin reprocessing the IMD file.
- 13 Review the resulting FCS file using Cytobank (or any other FCS data analysis program) to confirm that the FCS file was correctly created.

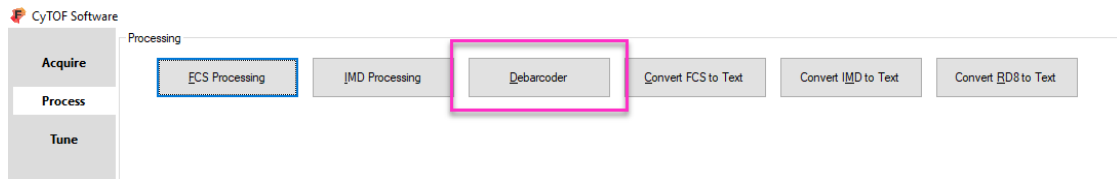
NOTE If the FCS file was not correctly generated or you are unable to repair the IMD file, contact your local field application scientist for assistance.

14 Perform additional processing tasks:

- To concatenate or normalize the data, click **Process > FCS Processing**.



- To debarcode the data, click **Process > Debarcoder**.



Appendix A: Data Acquisition Recommendations

Data Storage

Save data to the E: drive.

IMPORTANT Do not save data to the C: drive, an external hard drive, or a network drive.

Estimate Storage Requirements

Estimate adequate storage space requirements to prevent acquisition interruption (sample loss) and IMD header issues due to insufficient hard drive space. Consider the following when you estimate the storage space required:

- An 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 FCS conversion. One file requires 5–6 bytes per channel per event and the other requires 12–14 bytes per channel per event.

Computer Resources

- **Preserve as much free space on the C: drive as possible.** Do not install software that is not required for the instrument.
- **Allow system resources for data acquisition.** Do not run additional software or perform data processing, such as FCS processing or debarcoding, during acquisition. These actions reduce the system resources available for acquisition.
- **Do not rename data storage folders.** If you do so, an error will occur and an IMD file named “acquisition_<date of run – YYYYMMDD>_<#>” will be saved without a header.

Additional Recommendations

- Do not defragment the drives on the instrument workstation. Frequent defragmentation will decrease the lifespan of the drives.
- Do not map the E: drive to a network. This may compromise the read and write operations of the acquisition software.

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