

# Custom Channel Filtering with CyTOF Software v6.7

## Introduction

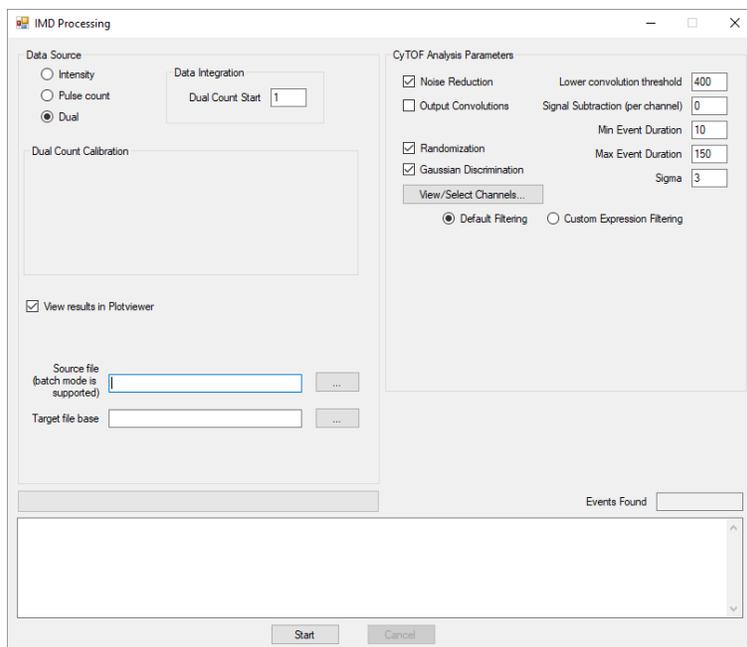
Channels with high signal, due to environmental contamination, poor antibody titration, or high cell concentration, can interfere with event detection and post-acquisition data processing. This document describes how to reprocess an IMD file with custom channel filtering to exclude high-signal channels from event identification and generate a new, unnormalized FCS file.

**NOTE** Data from excluded channels will remain in the reprocessed FCS file, but it will be excluded from event identification when the data is reprocessed.

## Apply Custom Channel Filtering

### To generate a new FCS file with channel filtering

- 1 Open CyTOF Software v6.7 and log in as an administrator.
- 2 On the Toolbar, click the **Process** tab and then click **IMD Processing**.



- 3 Next to Source file, click the browse button, and locate an IMD file to process.

Source file (batch mode is supported)

Target file base

**NOTE** The Target file base textbox is automatically populated with the file path and name of the output FCS file. The default name for the output FCS file is the IMD file name appended with *\_cells\_found*; for example, a source file named *SourceFile.imd* would result in an FCS file named *SourceFile\_cells\_found.fcs*.

- 4 Under CyTOF Analysis Parameters, click **View/Select Channels...**

IMD Processing

Data Source  
 Intensity  
 Pulse count  
 Dual

Data Integration  
Dual Count Start

Dual Count Calibration

View results in Plotviewer

Source file (batch mode is supported)

Target file base

Events Found

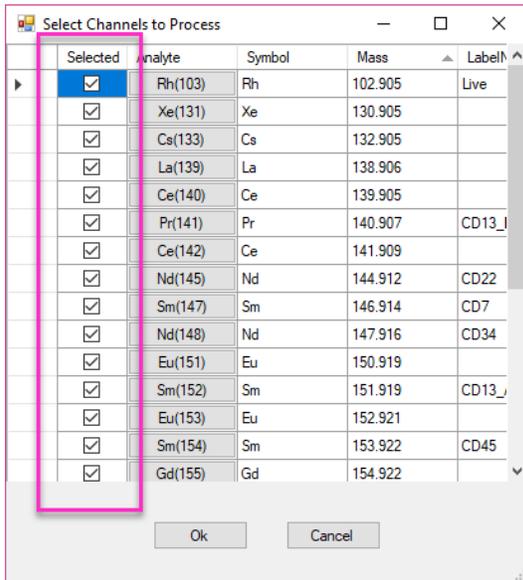
2018-02-21 1:12:51 PM : Analytes information has been found in the IMD file C:\Users\veanne.bloomfield\Desktop\TEST DATA\IMD files\IMD\_Ver\_01.imd  
2018-02-21 1:12:51 PM : Dual calibration analytes information has been found in the IMD file C:\Users\veanne.bloomfield\Desktop\TEST DATA\IMD files\IMD\_Ver\_01.imd  
2018-02-21 1:12:51 PM : File format is IMD.

CyTOF Analysis Parameters

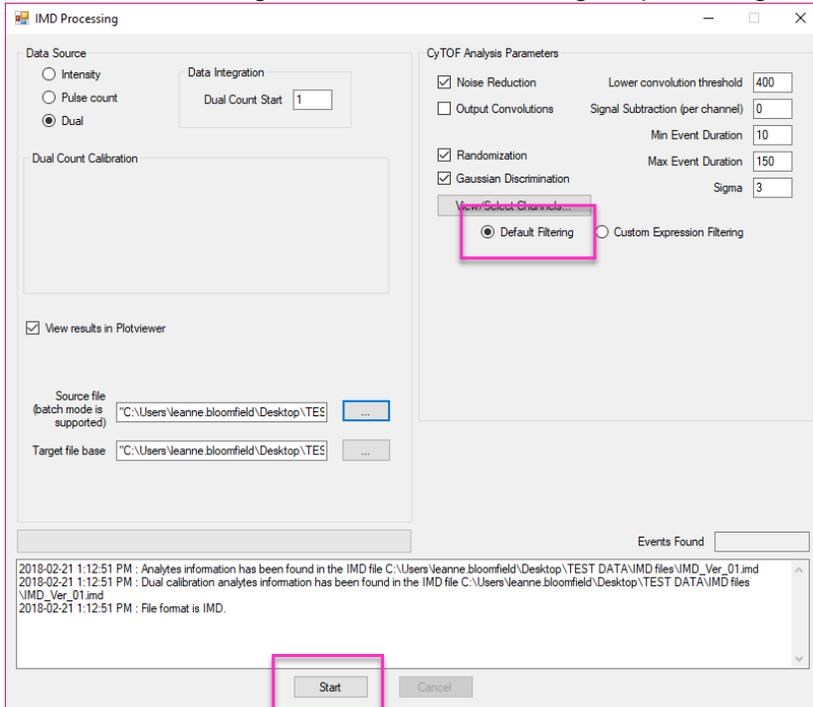
Noise Reduction Lower convolution threshold   
 Output Convolutions Signal Subtraction (per channel)   
 Randomization Min Event Duration   
 Gaussian Discrimination Max Event Duration   
 Sigma

Default Filtering  Custom Expression Filtering

- In the Select Channels to Process window, uncheck the high-signal channels to exclude them from cell identification, and click **OK**.



- Select Default Filtering, and then click **Start** to begin reprocessing the IMD file.



**NOTE** If the IMD file was previously processed, the following error message is displayed: *Target file base: Target FCS File: [file\_name].fcs already exists!* To reprocess the IMD, modify the Target file base file name and click Start.

- After the new FCS file is created, open FCS Processing to normalize the data.

**NOTE** Fewer events will be detected if the removal of the high-signal channels lowers the combined signal intensity below the event threshold.

# Considerations for Normalization

IMD files contain preview data and acquisition data. Also, when an acquisition event limit is used additional data is saved at the end of the IMD file.

The original FCS file, created at the time of acquisition, contains only acquisition data. However, when an IMD is reprocessed to generate a new FCS file, all data in the IMD file are included. This will affect the normalization of initial and/or final time intervals if the new FCS file is normalized with the same time interval as the original FCS file.

## If an Event Limit was Set

When >50 channels are acquired with an event limit, a delay between achieving the event limit and stopping acquisition occurs. The data acquired during this delay is stored in the IMD file, but it is not included in the original FCS file.

When the IMD file is reprocessed the additional data are appended to the end of the new FCS file. The delay, and therefore, the amount of data, increases with the number of channels acquired.

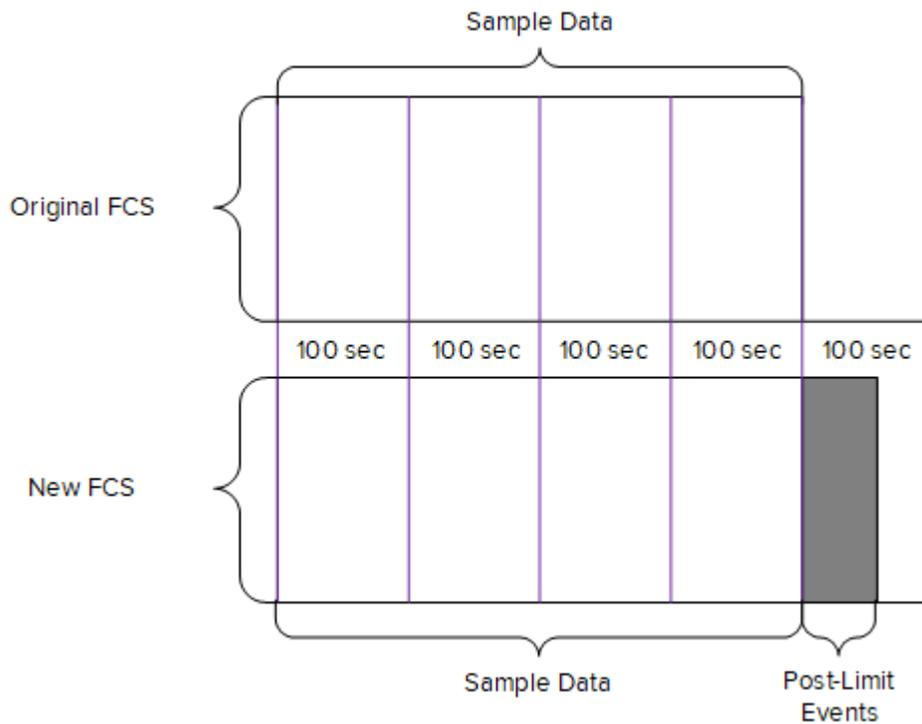


Figure 1 An example comparison of an original FCS file to a new channel-filtered FCS file segmented into 100 second time intervals. The grey area represents the events acquired after the event limit was reached. This data will also be normalized if >50 beads events are detected.

## If Preview was Used

If Preview was used to view signal before acquisition was started, the data is stored in the IMD file. The preview data will prepend the acquisition data in the new FCS file. If the new FCS file is normalized with the same time interval as the original FCS file created at the time of acquisition, the initial time interval(s) and final time interval may not contain enough events for normalization and will be excluded from the normalized file.

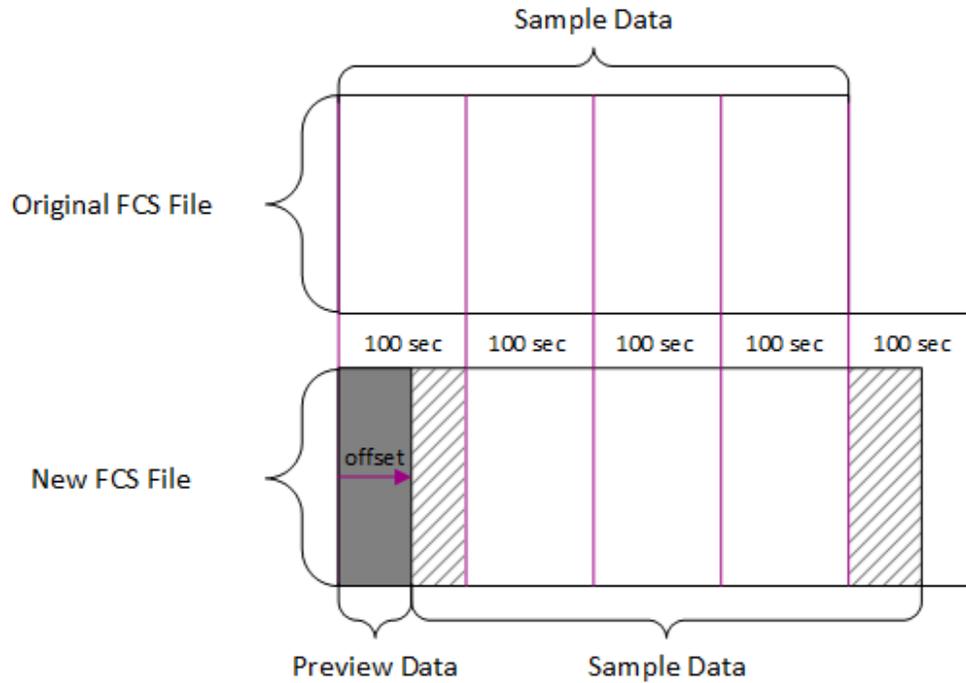


Figure 2 An example comparison of an original FCS file to a new channel-filtered FCS file segmented into 100 second time intervals. Shaded areas indicate acquisition data that may be excluded due to insufficient bead events (<50).

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