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.

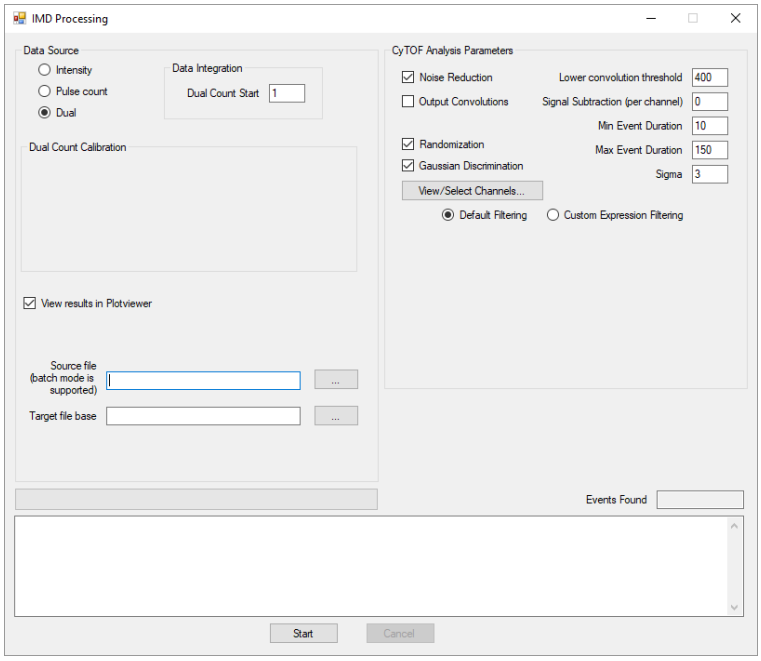
NOTE Data from excluded channels will remain in the reprocessed FCS file, but it will be excluded from event identification when the data is reprocessed.

Apply Custom Channel Filtering

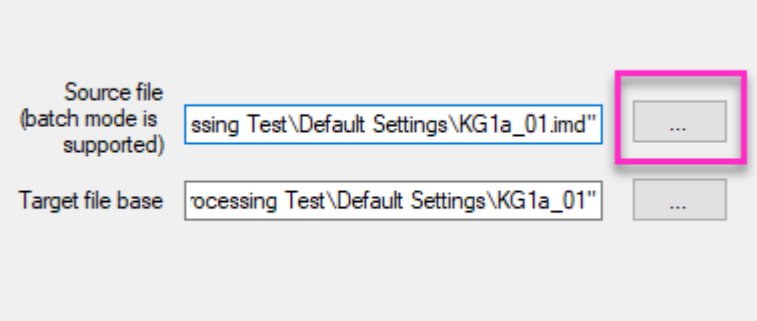
To generate a new FCS file with channel filtering

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**.

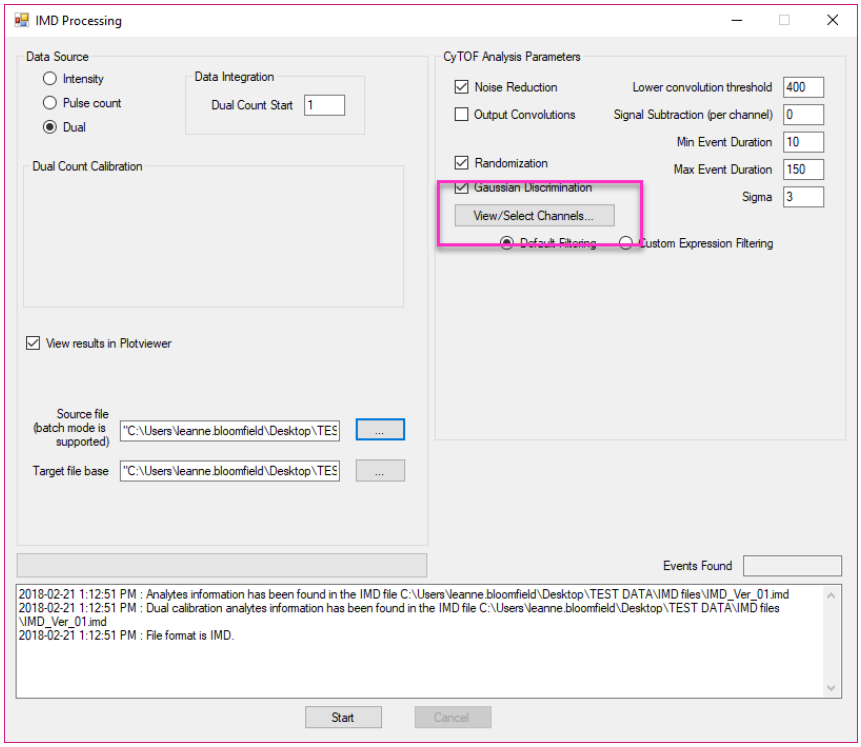


3 Next to Source file, click the browse button, and locate an IMD file to process.

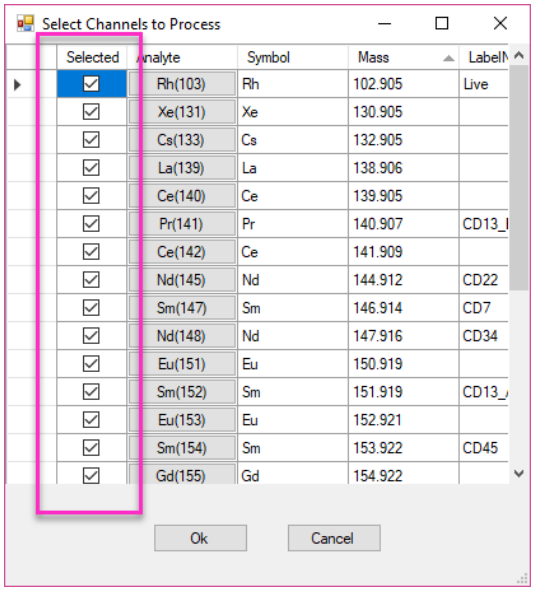


NOTE The Target file base textbox is automatically populated with the file path and name of the output FCS file. The default name for the output FCS file is the IMD file name appended with *_cells_found*; for example, a source file named *SourceFile.imd* would result in an FCS file named *SourceFile_cells_found.fcs*.

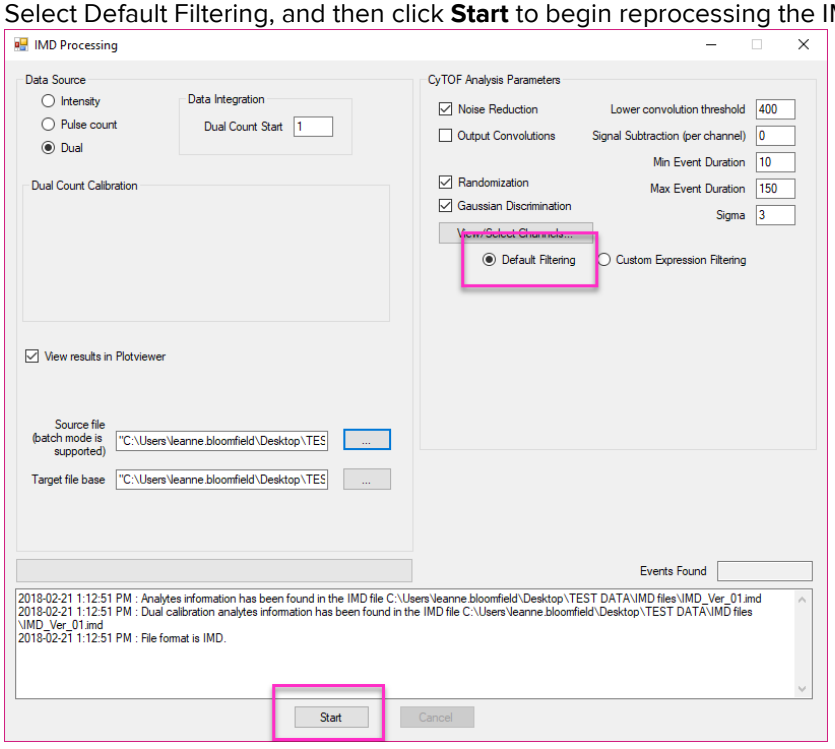
4 Under CyTOF Analysis Parameters, click **View/Select Channels...**



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



6 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.

7 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 acquistion 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.

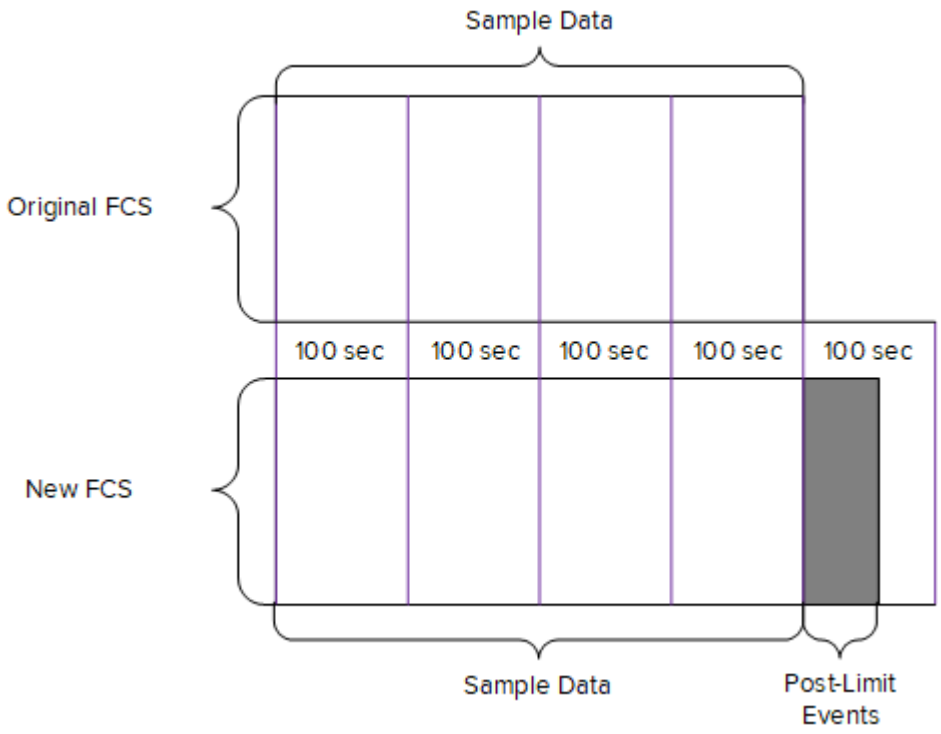


Figure 1 An example comparison of an original FCS file to a new channel-filtered FCS file segmented into 100 second time intervals. The grey area represents the events acquired after the event limit was reached. This data will also be normalized if >50 beads events are detected.

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.

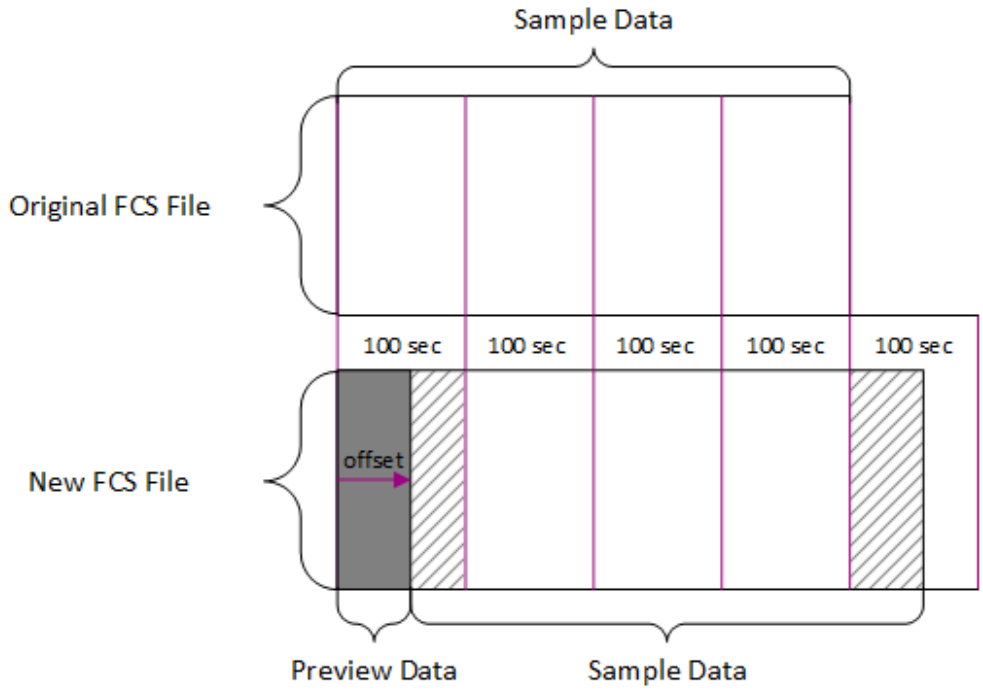


Figure 2 An example comparison of an original FCS file to a new channel-filtered FCS file segmented into 100 second time intervals. Shaded areas indicate acquisition data that may be excluded due to insufficient bead events (<50).

For technical support visit fluidigm.com/support

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | support.northamerica@fluidigm.com **Latin America** +1 650 266 6100 | techsupportlatam@fluidigm.com

Europe/Middle East/Africa/Russia +44 1223 859941 | support.europe@fluidigm.com **China (excluding Hong Kong)** +86 21 3255 8368 | techsupportchina@fluidigm.com

Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com

For Research Use Only. Not for use in diagnostic procedures.