How to FlowClean

Introduction

Modern flow cytometers allow hundreds of samples to be analyzed in a single day. Data quality control remains challenging and it is further complicated when large number of parameters are measured. In particular, fluorescence measurements for a sample over the collection time may not remain stable due to fluctuations in fluid dynamics. In order to address this issue, FlowClean was developed, an algorithm that automatically identifies and flags fluorescence anomalies in flow cytometry data so that these can be easily removed. The FlowClean algorithm is developed in R; here we present a FlowJo plugin that integrates FlowClean's functionality to FlowJo.
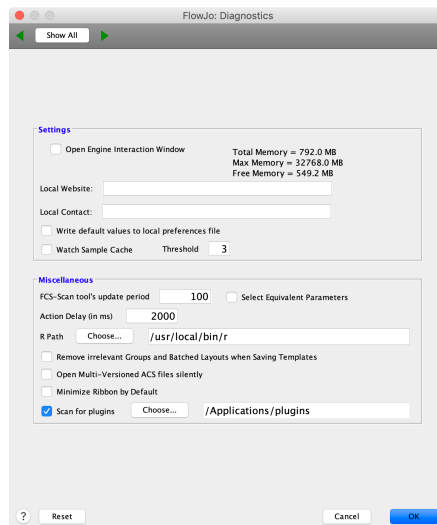
Learn more using the FlowClean plugin in this blog post.

Setting up the FlowClean Plugin

There are detailed instructions on how to set up plugins in our online documentation.

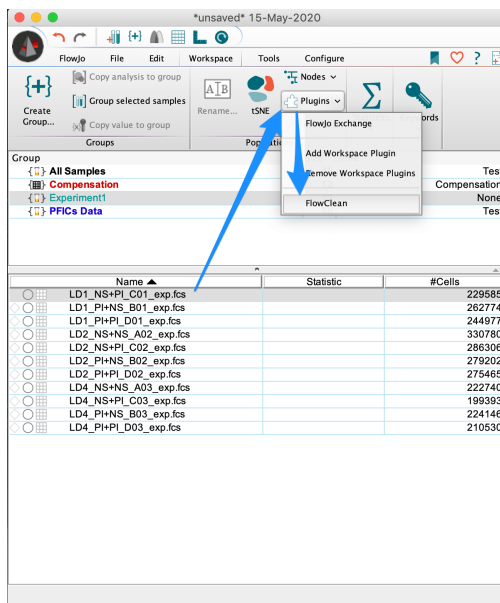The FlowClean plugin can be set up with the following steps:
1. Place the FlowClean JAR file into a "plugins" folder.
2. Ensure that the [R program] (https://cran.r-project.org/) is installed on the computer.
3. Go to the Preferences (heart) icon in FlowJo or SeqGeq and select Diagnostics.
4. Select the plugins folder.
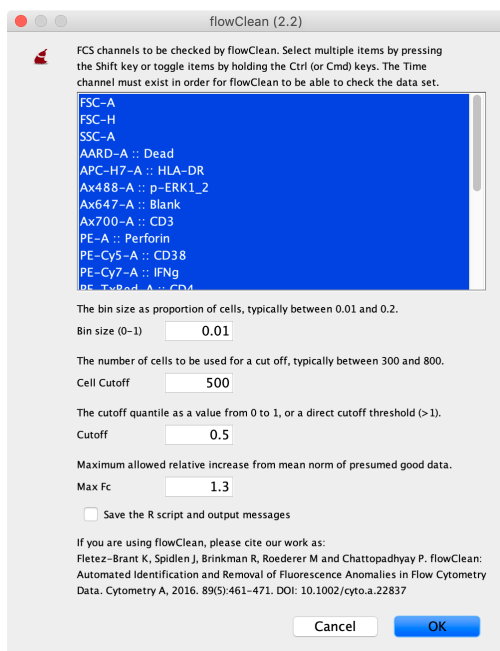5. Set the correct R path in the R Path field.

6. Restart the application.
7. When first running the FlowClean plugin it should automatically install its required R packages.

Using the FlowClean plugin

Drop some samples (FCS files) into your FlowJo workspace. With a sample selected, navigate to the FlowClean menu item, which is located under Plugins on the Workspace ribbon as below.
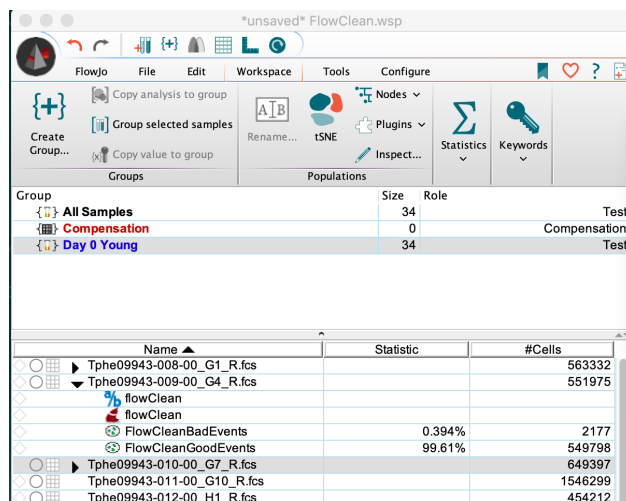

This will open a dialog box with FlowClean input parameters as shown in below. Select the FCS channels that you want FlowClean to inspect. The Time channel needs to be present in the FCS file in order for FlowClean to process the data; this channel will be used automatically even if you do not select it. Generally, selecting all fluorescence channels provides good results. All other parameters have reasonable values, but you change those if preferred. Please see the detailed FlowClean reference manual [1] and the paper [2] for details about these parameters and how they affect FlowClean's results. Run the module by pressing the OK button.
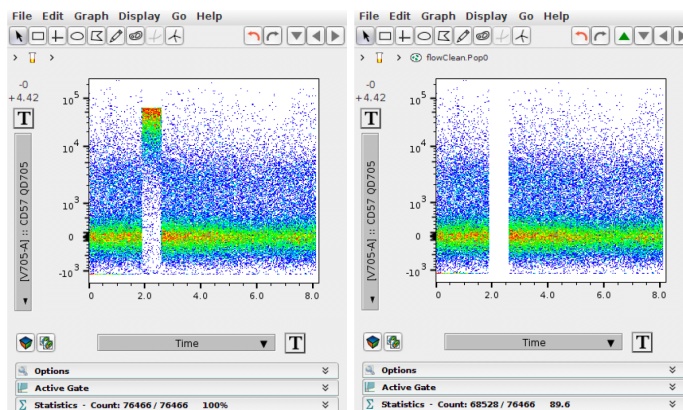
FLowClean Results

Once the algorithm completes its calculation, the results will appear as 1 or 2 different populations as shown below. There will be 2 populations if FlowClean identifies fluorescence anomalies in your data. The 2 populations represent events in sections with Fluorescence anomalies ("bad data") and events in sections without Fluorescence anomalies ("good data"). About 0.394% of events have been flagged as "bad" in the shown example below.

Inspection of FlowClean's Results

In order to manually validate FlowClean's results, we suggest plotting the Time vs. channels as shown in the example below. The ungated population of all events is shown on the left, the automatically gated population of events considered good is shown on the right. Normally, for each of the fluorescence channels, we would expect the fluorescence measurements for a sample to remain stable over the data collection time. However, fluctuations in dynamics and other factors may lead to instabilities and to the emergence of false populations.

When FlowClean identifies such anomalies, there will be one or more fluorescence channels showing a non-stable behavior when plotted against time. When inspected by eye, these fluctuations may or may not be as apparent as demonstrated in the provided example. In addition, although using FlowClean with the default parameter settings appears to work well for most researchers, you can use those types of plots in order to determine optimal settings and fine tune FlowClean specifically for your type of data.

Additional

Users can manually install packages in R with the following commands:

```r
install.packages("BiocManager")
BiocManager::install(c("flowClean","flowCore"))
```

Referencing FlowClean If you have used FlowClean and found it useful, the authors would appreciate if you would cite our work as: Fletez-Brant K, Spidlen J, Brinkman R, Roederer M and Chattopadhyay P. FlowClean: Automated Identication and Removal of Fluorescence Anomalies in Flow Cytometry Data. Cytometry A ... (PMID: 26990501).

References

[1] Fletez-Brant K. FlowClean Reference Manual, version 1.7.1, 2015. Available at https://www.bioconductor.org/packages/devel/bioc/manuals/flowClean/man/flowClean.pdf.

[2] Fletez-Brant K, Spidlen J, Brinkman R, Roederer M, and Chattopadhyay P. flowClean: Automated Identification and Removal of Fluorescence Anomalies in Flow Cytometry Data. Cytometry A (accepted), 2016:p 0.

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Please write to flowjo@bd.com or seqgeq@bd.com with any questions!