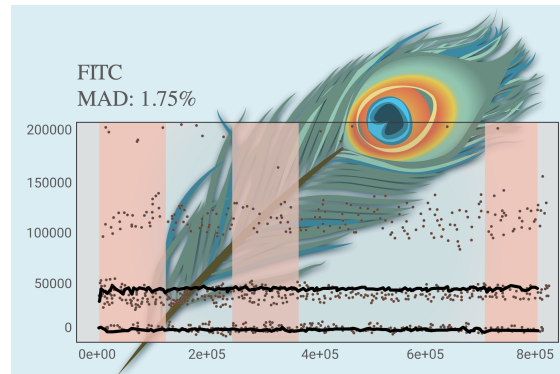


“How To PeacoQC”

The PeacoQC plugin for FlowJo !



The Peak Extraction and Cleaning Oriented Quality Control (PeacoQC) plugin performs quality control on cytometry data, evaluating the sample signal for regions of irregularity. This algorithm can help remove clogs and changes in flow rate, helping clean up the data. The algorithm identifies density peaks within each parameter and removes abnormal events based on their position in the isolation tree and the mean absolute deviation distance from the peaks. The plugin separates cells into two subpopulations marked “Good Events” and “Bad Events”. The Good Events can then be used for further analysis.

The PeacoQC algorithm has been developed and implemented as a R package by Annelies Emmaneel & the Seays lab at the University of Ghent. The source code is available here: <https://github.com/saeyslab/PeacoQC>.

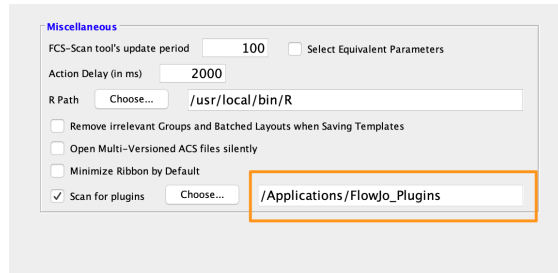
You may read more about PeacoQC in the research paper here:

Emmaneel A, Quintelier K, Sichien D, Rybakowska P, Marañón C, Alarcón-Riquelme ME, Van Isterdael G, Van Gassen S, Saey S, Saey Y. PeacoQC: Peak-based selection of high quality cytometry data. *Cytometry A*. 2022 Apr;101(4):325-338. doi: 10.1002/cyto.a.24501. Epub 2021 Oct 3. PMID: 34549881; PMCID: PMC9293479. (<https://onlinelibrary.wiley.com/doi/10.1002/cyto.a.24501>)

Watch our video demonstrating this plugin! <https://fjinstallers.s3.amazonaws.com/Plugins/PeacoQC.mp4>

Download and installation

1. Place the plugin .jar file in your Plugins folder, and direct FlowJo to that folder using the Diagnostics section of the Preferences.
2. Make sure you have R installed and the R path is specified in the R Path field of the Diagnostics section of the Preferences.



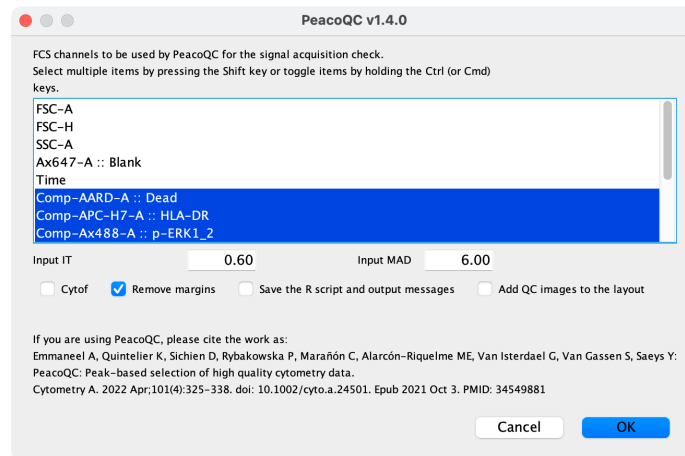
- Running the plugin for the first time will install the needed R packages to allow the calculation to run in the R environment. Sometimes these installations can fail and you may need to manually install the R packages. To install the required R packages, use the following commands in R:

```
install.packages(c('BiocManager', 'devtools', 'flowCore'))
BiocManager::install(c('ComplexHeatmap', 'PeacoQC'))
```

Note This plugin was tested in R versions 4.1 & 4.2 and PeacoQC R package version 1.6.0.

Usage

To run the PeacoQC plugin on your FCS file, select the sample of interest within the workspace. Do not select a subpopulation. Go to the Workspace tab and select the PeacoQC option from within the Plugins drop-down menu. This will bring up a dialog where researchers can choose which parameters will be used in the analysis. The algorithm author suggesting performing the algorithm on compensate parameters. Time will always be included if present.



Input IT This value represents the gain limit used in the Isolation Tree (IT). Lowering this limit will cause the algorithm to remove more outliers.

Input MAD The Mean Absolute Deviation within each of the channels. Lowering the MAD will cause the algorithm to remove more cells.

Cytof Select this option to remove cells with zero expression.

Remove Margins Select this option to remove cells at the margins (near the graph edges).

Add QC Images to Layout This option will add the images generated by PeacoQC to the layout after the algorithm finishes.

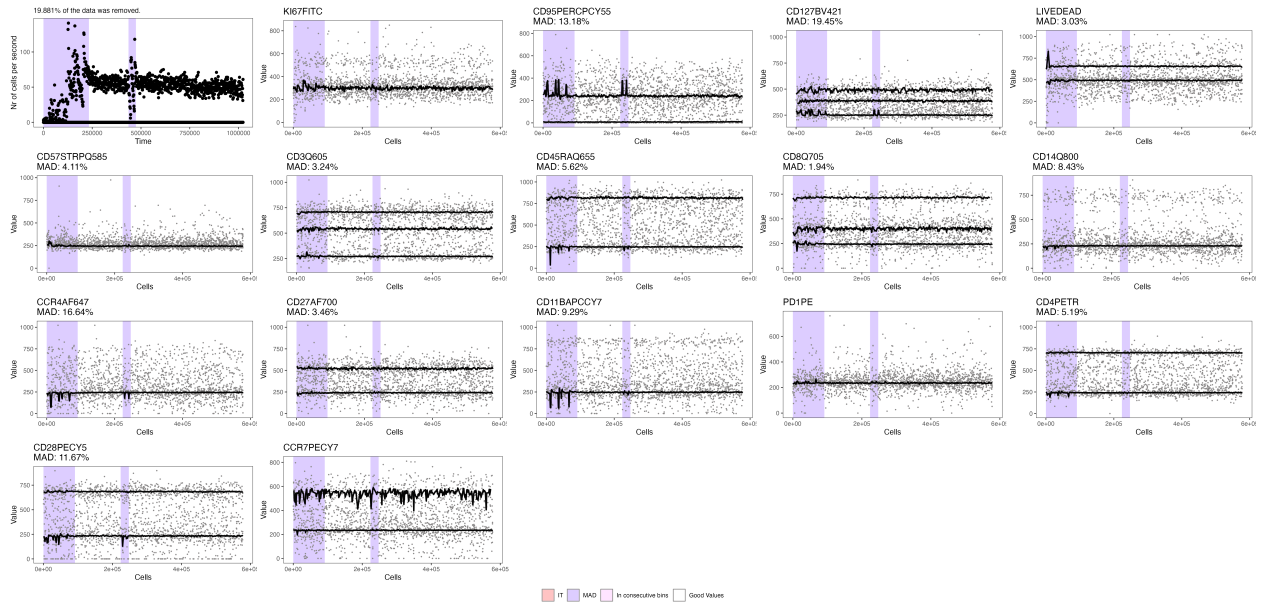


Figure 1 - The image returned by the PeacoQC algorithm showing the areas where cells were removed (in purple). The first plot shows the flowrate over time and the rest of the plots so the signal intensity of the cells over the acquisition.

Leave us your feedback

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