

# Index Sorting Plugin

This plugin was designed in order to give researchers the ability to automatically gate wells from .fcs data generated using the BD FACSAriaIII™, the BD FACSAria Fusion™, the BD FACSMelody™, the BD Influx System™, and the BD FACSymphony™ S6 cell sorters.

## Installation

As with all plugins you'll need to place the IndexSort .jar file into your FlowJo Plugins folder and restart FlowJo. In addition, this plugin has dependencies in R which should be installed using the following commands there:

```
source("https://bioconductor.org/biocLite.R")
biocLite("flowCore")
install.packages("tidyverse")
```

## Important Note about the BD FACSMelody™

In the case of the BD FACSMelody™ only, the .fcs data comes associated with a corresponding index sorting .csv file, which is used by the plugin to identify the sorted cells. Please navigate within the file system and select associated index .csv file when starting the plugin.

## How-To

Simply select the index sorted .fcs data file you'd like to use with the plugin, once prompted point the plugin to the associated .csv index file

if you sorted using the BD FACSMelody™ or directly proceed by pressing OK for all other data files.

Please note that the plugin will use the \$CYT keyword to automatically identify the cell sorter. In case this keyword is missing in the .fcs or the value has been changed, you can use the menu to manually select the cell sorter when starting the plugin.

After calculations are completed the plugin will generate derived parameters corresponding to the X and Y axes of your plate (IdxRow and IdxCol), and automatically return gates for each *row* of that sort, and sub-gates for each *column* of each row.

Additionally, the plugin generates an illustration of the plate currently displaying the number of cells per well and two important .csv files: (i) one with the Fluorescent Intensity per cell and (ii) one with the Median Fluorescent Intensity per well.

Dragging and dropping the plugin node from your sample into the Layout Editor will display an illustration of the plate. Selecting IdxCol and IdxRow in the graphical window allows users to visualize and explore Fluorescent Intensity across the sorted plate with the color mapping option.

## New in Version 1.1

- ✓ Improved illustration of the plate
  - added view of empty wells
  - added cell number in wells
  - reduced size of illustration
- ✓ Adapted for two new cell sorters
  - tested with BD FACSAriaIII

- added BD Influx System™
- added BD FACSymphony™ S6
- ✓ Automatic cell sorter recognition via \$CYT
- ✓ Or possibility to manually define cell sorter
- ✓ Updated prompting message
- ✓ New error messages for troubleshooting
- ✓ Several minor code improvements