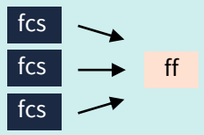
Cytometry analysis with FLOWSOM: : CHEAT SHEET

To update an old FlowSOM object to a new one, use UpdateFlowSOM(fsom)

Basics

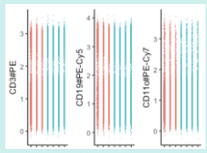
PREPARING DATA

While FlowSOM has some integrated preprocessing functions, it is often easiest to prepare preprocessed files upfront: compensate, transform, normalize …



AggregateFlowFrames(fileNames, cTotal)
Combine multiple fcs files for training. Uses random sampling without repetition.

PlotFileScatters(files, channels, groups, …)
Makes a 2D scatter plot for each subset of interest.



TRAINING A FLOWSOM MODEL

FlowSOM(input, **scale = FALSE**, colsToUse = NULL, xdim = 10, ydim = 10, nClus = 10, seed = NULL, …)

input: flowFrame, flowSet, matrix, file or directory path

scale: Logical, if TRUE, every channel undergoes a z-scale transfomation to mean 0 and standard deviation 1

colsToUse: Channels, markers or ids. If NULL, all columns are used

xdim, ydim: xdim * ydim = number of clusters

nClus: Number of metaclusters

seed: For exact reproducibility

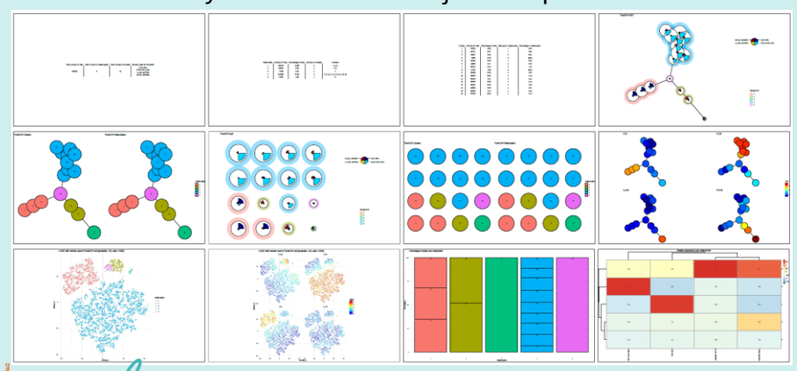
Extra parameters can be found in the FlowSOM documentation.

EXPLORATIVE FUNCTIONS

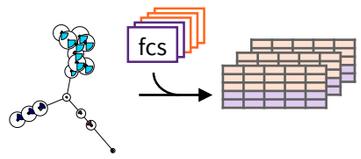
print(fsom)
Prints a short fsom summary.

GetClusters(fsom) – **GetMetaclusters**(fsom)
Gets the (meta-)cluster label for each cell in the fsom object.

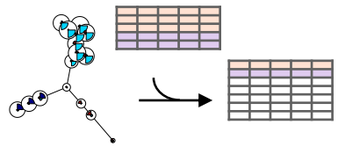
FlowSOMmary(fsom, …)
Plots a summary of the FlowSOM object to a pdf file.



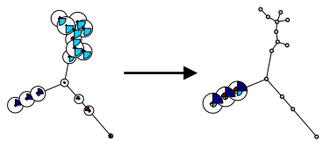
Comparing multiple files



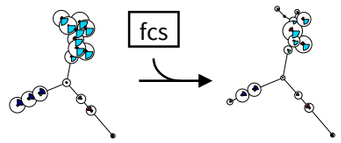
GetFeatures(fsom, files, level = c("clusters", "metaclusters"), type = "counts", MFI, positive_cutoffs, filenames, silent)
Extracts the features specified in type ("counts", "percentages", "MFIs" and/or percentages_positive) from clusters and/or metaclusters.



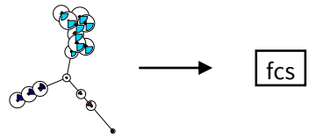
GroupStats(features, groups)
Calculates statistics between 2 groups based on the GetFeatures output. Medians, p-value, BH-correct p-value, -log10(p-value), fold changes and log10 fold changes will be returned. The groups should be a named list with per group a vector of file names, corresponding to the row names of the features.



FlowSOMSubset(fsom, ids)
Takes a subset from a FlowSOM object, with in "ids" the ids of which cells to keep.



NewData(fsom, input, madAllowed = 4, …)
Maps new data to an existing FlowSOM object.



SaveClustersToFCS(fsom, originalFiles, preprocessedFiles, selectionColumn, …)
Writes FlowSOM clustering results to the original FCS files.

Annotating clusters

QueryStarPlot(fsom, query, plot = TRUE, colorPalette, backgroundColors, …)
Identifies nodes in the tree that resemble a certain profile of "high" or "low" marker expressions.

QueryMultiple(fsom, cellTypes, plotFile, …)
Takes a named list of multiple cell types, where every item is a named vector with values "high"/"low" and where the names correspond to the markers or channels.

GetFlowJoLabels(files, wspFile, group = "All Samples", cellTypes, getData, …)
Reads a flowjo workspace file and returns a list with a matrix, containing gating results, and a vector with a label for each cell from a set of specified gates.

ManualVector(manualMatrix, cellTypes)
Summarises the gating matrix into one vector, only including the cell types of interest.

UpdateMetaclusters(fsom, newLabels, clusterAssignment, levelOrder)
Adapts the metacluster levels. Can be used to rename, split or merge the metaclusters, add a metacluster and/or reorder the levels of the metaclustering

Getter functions

GetClustersMFIs(fsom, …) – **GetMetaclustersMFIs**(fsom, …)
Gets the MFIs of (meta-)clusters.

NClusters(fsom) – **NMetaclusters**(fsom)
Gets the number of (meta-)clusters in the fsom object.

GetChannels(object, markers, exact = TRUE) – **GetMarkers**(object, channels, exact = TRUE)
Gets the channel- or marker names from the markers or channels respectively out of a flowFrame or fsom object.

GetClustersCVs(fsom) – **GetMetaclustersCVs**(fsom)
Gets CV values for all (meta-)clusters.

GetCounts(fsom, level = "metaclusters")
Gets counts of number of cells in (meta-)clusters.

GetPercentages(fsom, level = "metaclusters")
Gets percentages of number of cells in (meta-)clusters.

GetClusterPercentagesPositive(fsom, cutoffs, …) – **GetMetaclusterPercentagesPositive**(fsom, cutoffs, …)
Gets percentage-positive values for all (meta-)clusters.

TestOutliers(fsom, madAllowed = 4, fsomReference, plotFile)
Tests if any cells are further than expected from their cluster centers.

DaMBi
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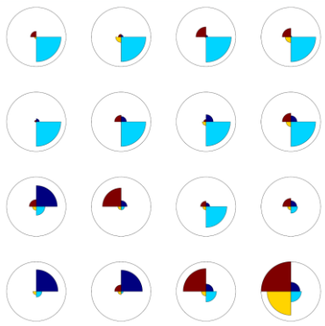
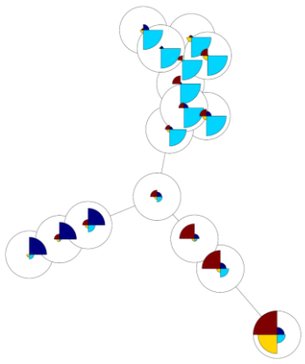
FlowSOM plotting

GRID VIEW AND MINIMUM SPANNING TREE (MST)

The grid structure is built during the SOM clustering. The data is presented cell by cell to the grid and is attached to the closest cluster center. Then this cluster center and the surrounding centers get updated. In the end the clusters close to each other will be similar.
view = “grid”

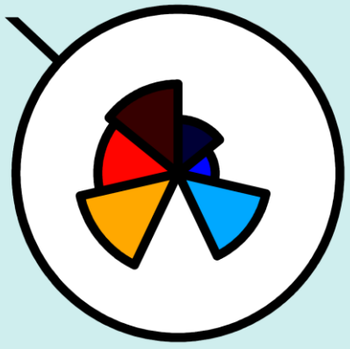


MST connects the nodes of a graph in such a way that the sum of the weights of the branches is minimal. By doing this, nodes will get connected to the ones they are the most similar too, taking the multi-dimensional topology of the data in account. Similarity inferences can only be made on close nodes.
view = “MST”



FLOWSOM STARCHART PRESENTATION

The height of each part indicates the expression of that marker: if the part reaches the border of the circle, the cluster's median intensity value is the highest of all FlowSOM clusters.



The size of the node indicates the relative number of cells assigned to each node.

Plotting FlowSOM objects

GENERAL PLOTTING FUNCTIONS

PlotStars(fsom, markers = fsommapcolsUsed, colorPalette, list_instead_of_ggarrange = FALSE, …)
Shows MFI expression of multiple markers.

PlotNumbers(fsom, level = “clusters”, …)
Shows (meta-)cluster numbers.

PlotMarker(fsom, marker, refMarkers = fsommapcolsUsed, colorPalette, lim, textColor, textSize, …)
Shows the median value of every cluster for this marker.

PlotSD(fsom, marker, …)
Shows the standard deviation of every cluster for this marker.

PLOTTING WITH EXTERNAL INFORMATION ON CLUSTER LEVEL

PlotLabels(fsom, labels, textColor, textSize, …)
Shows a label for every cluster.

PlotVariable(fsom, variable, variableName, colorPalette, lim, …)
Shows a variable for every cluster.

PLOTTING WITH EXTERNAL INFORMATION ON CELL LEVEL

PlotPies(fsom, cellTypes, colorPalette, …)
Shows the percentage of cells belonging to each cell type.

For most of these plotting functions, there is also an “Add…”-function available that adds the layer to an existing plot.
PlotStars() ⟷ PlotFlowSOM() %>% AddStars()

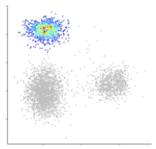
PARAMETERS AVAILABLE IN THE ABOVE FlowSOM PLOTTING FUNCTIONS

view:	‘MST’ (default), ‘grid’ or coordinates
nodeSizes:	Node sizes. Default = fsommappctgs
maxNodeSize:	Maximum node size. Default = 1
refNodeSize:	Reference for node size against which node sizes will be scaled. Default = max(nodeSizes).

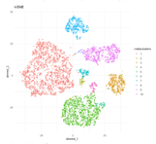
$$nodeSizes_{scaled} = \sqrt{\frac{nodeSizes}{refNodeSize}} * maxNodeSize$$

equalNodeSize:	Logical, should nodes be equal sized or not. If TRUE, nodesizes will be equal to maxNodeSize. Default = FALSE
backgroundValues:	Values used for background coloring
backgroundColors:	Colors used for background coloring
backgroundLim:	Limits for numerical background values
title:	Title for plot

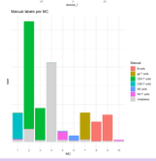
Other plotting functions



Plot2DScatters(fsom, channelpairs, clusters, metaclusters, …)
Shows a scatterplot of (meta-)clusters of interest.



PlotDimRed(fsom, colsToUse = fsommapcolsUsed, colorBy = “metaclusters”, cTotal, dimred = Rtsne::Rtsne, extractLayout = function(dimred){dimred$Y}, label = TRUE, returnLayout = FALSE, seed, title, …)
Makes a dimensionality reduction plot colored by (meta-)clusters or marker.



PlotManualBars(fsom, fcs, manualVector)
Plots the manual labels per FlowSOM (meta-)cluster in a barplot.