How to use the Tercen plugin for FlowJo.

Prerequisites

Some things you need to check before you start.

- You will need a full license of FlowJo installed on your computer.
- Make sure you have an open connection to the internet.

Install the Tercen plugin.

Step 1: Download the Tercen plugin folder and extract its contents.

Download the Tercen plugin (tercen.zip) from FlowJo Exchange.

Open the downloaded folder and extract the files. There will be two files.

Tercen_v1.0.0.jar

(N.B. Your Tercen plugin file may have different version numbers).

Tercen_Install_Instruction.pdf

This PDF contains installation instructions.

Step 2: Add the Tercen plugin file to your FlowJo plugin folder

To install the Tercen plugin, move or copy the Tercen plugin file (e.g. Tercen_v1.0.0.jar) to the FlowJo plugins folder.

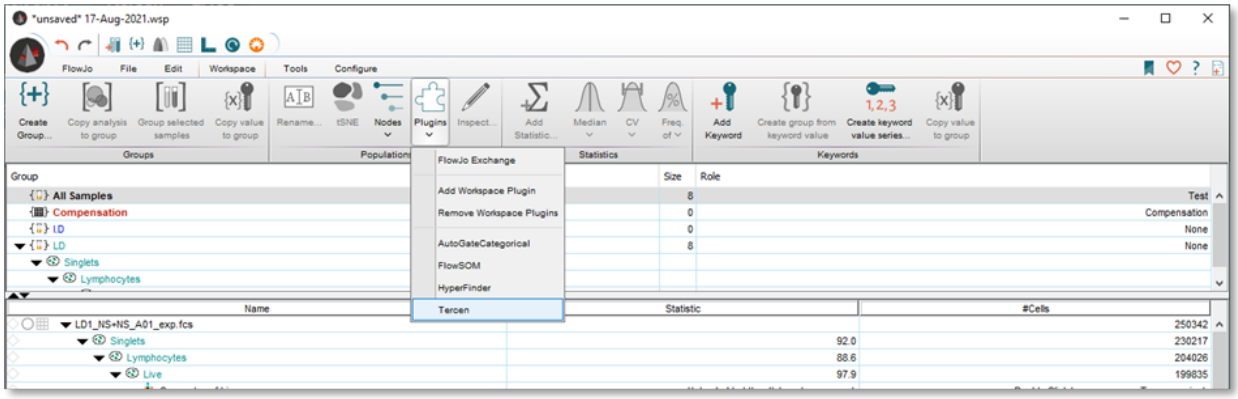
The location of the plugin folder differs depending on your operation system.

Refer to the [FlowJo Plugins Documentation] to set up or locate your plugins folder.

Step 3: Restart FlowJo

Close and re-open FlowJo to activate the plugin.

The Tercen plugin will now be listed in the Plugins drop-down menu.



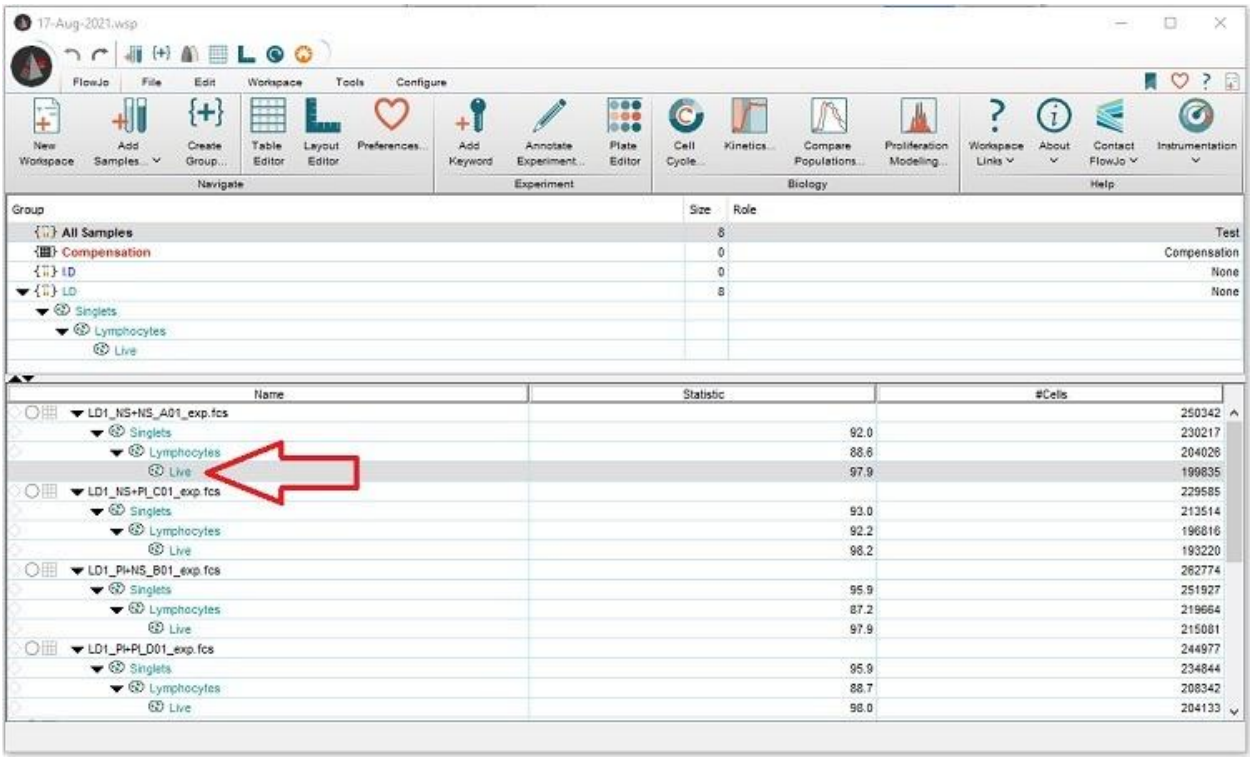
Upload from FlowJo to Tercen

Step 1: Select a population.

N.B. This guide does not attempt to show you how to use FlowJo software and we recommend you consult the FlowJo website for user manuals and tutorials.

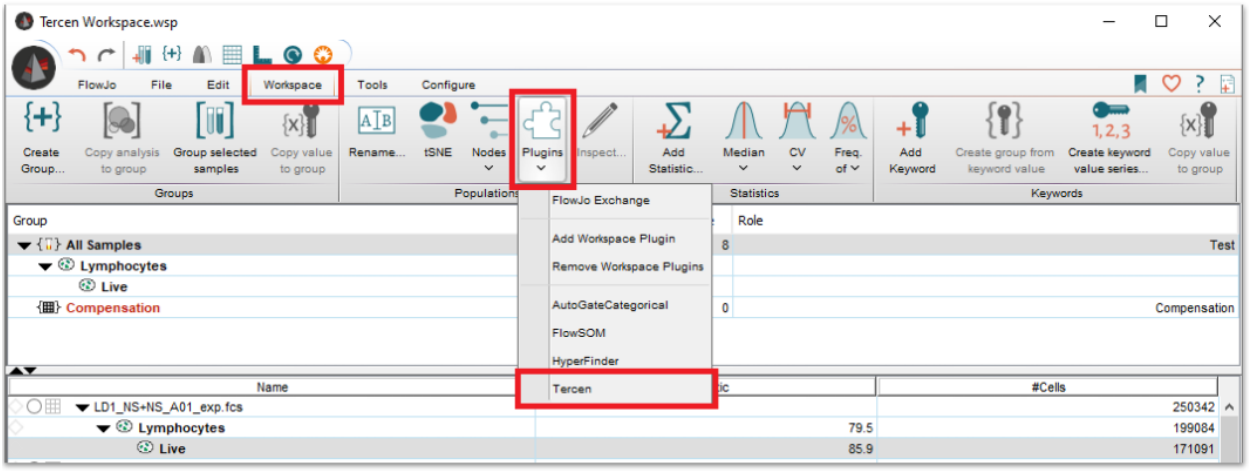
We recommend you QC and clean your data with FlowJo tools before uploading to Tercen. Removing abnormal data points, debris, doublets and dead cells in advance will speed up the uploading process and help you get a more accurate analysis.

After performing your preferred cleaning and gating strategy in FlowJo select a population of your interest by clicking on it with your mouse.

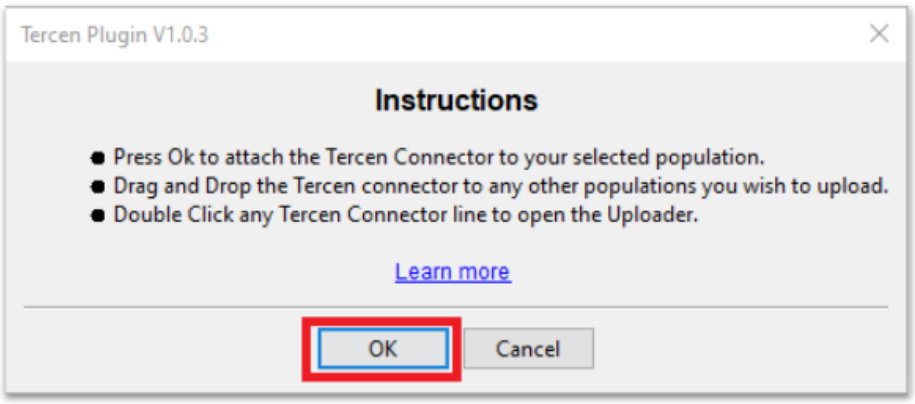


Step2: Apply the Tercen plugin to the selected population.

From the **Workspace** tab press the **Plugin** button and choose **Tercen** from the dropdown menu.



A popup with Instructions on how to use the Tercen plugin will appear.



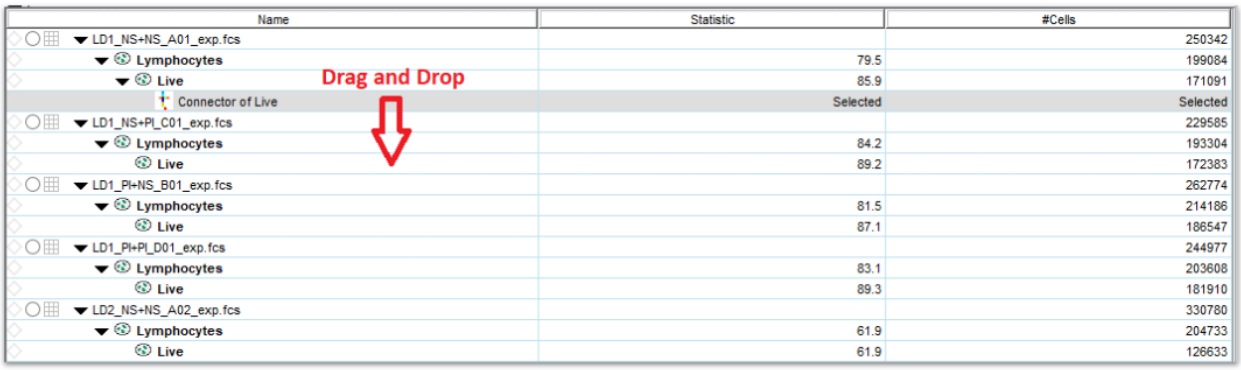
Press **OK** to continue.

A Tercen connector will now be created for your chosen population.

Step3: Copy the Tercen connector to more populations.

Tercen can upload multiple populations or raw data-files simultaneously. The connector must be applied to each selection individually.

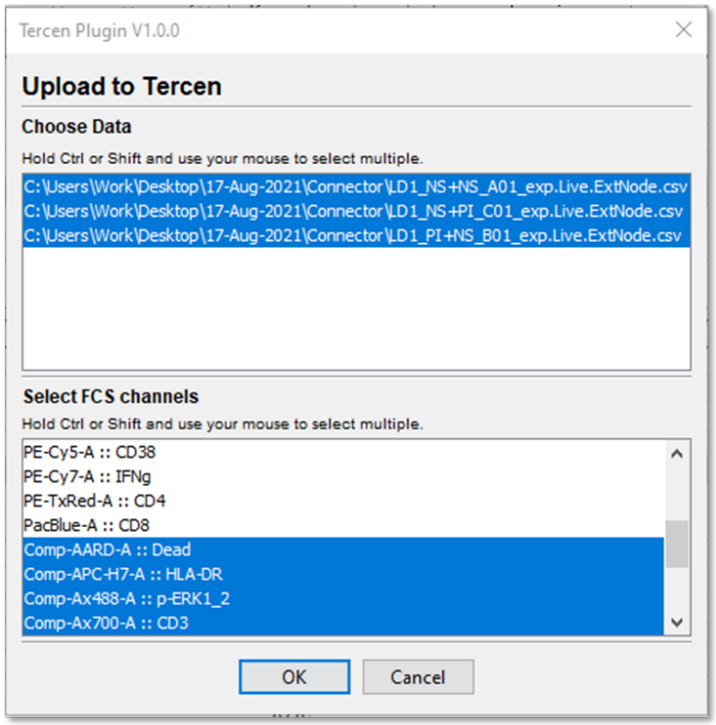
The connector can be applied to other populations individually by using **drag and drop** with your mouse or to all samples by using the **copy analysis to group** function in FlowJo.



Step 4: Upload the connected data to Tercen

To upload data, **double-click** on any of the Tercen connectors.

A popup window will appear to allow you to refine your selected data..



New screen shot required

The **upper window** contains all files or populations which currently have a connector. The plugin will default to uploading all connected samples. Use your mouse to select or deselect populations as required.

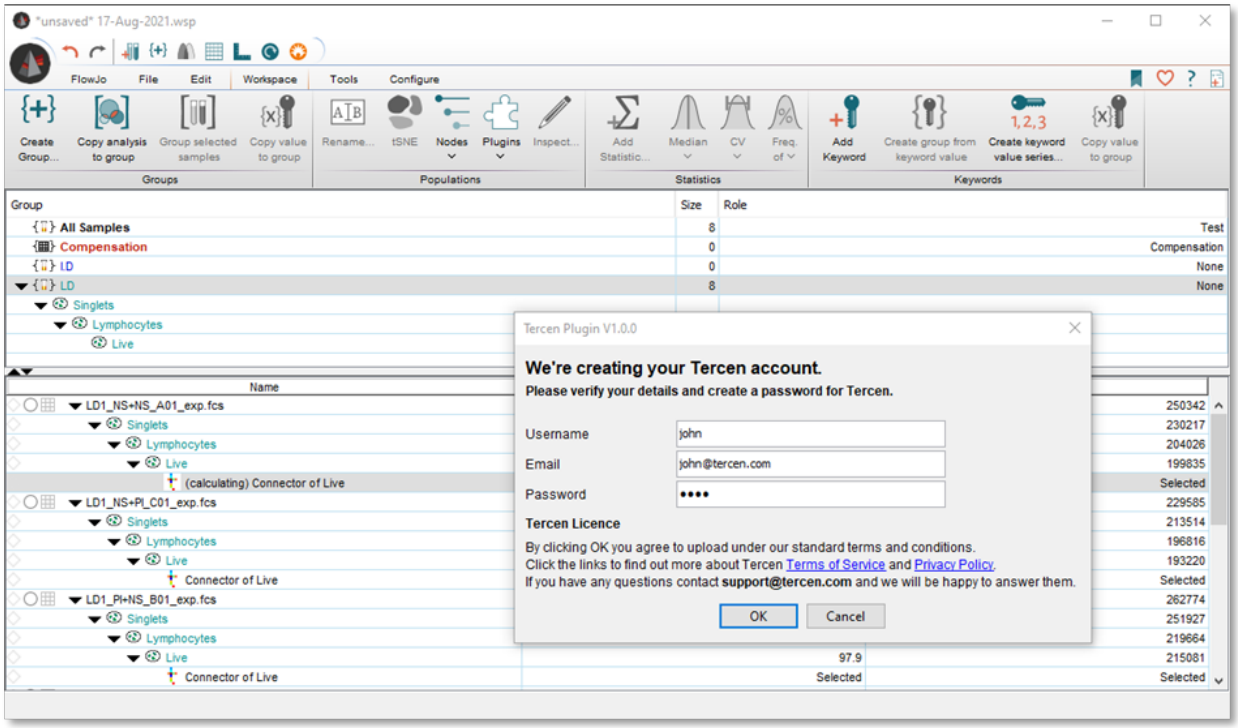
The **lower window** contains the parameters (channels) which will be grouped for analysis. Compensated parameters are automatically selected by default. Use your mouse to select or deselect channels as required.

When you are happy with your selection click **OK** to initiate the upload.

An upload progress bar will appear in a popup window.

Making a Tercen Account.

If you are new to Tercen the plugin will create an account for you. You will be able to use this Tercen account from any internet browser.



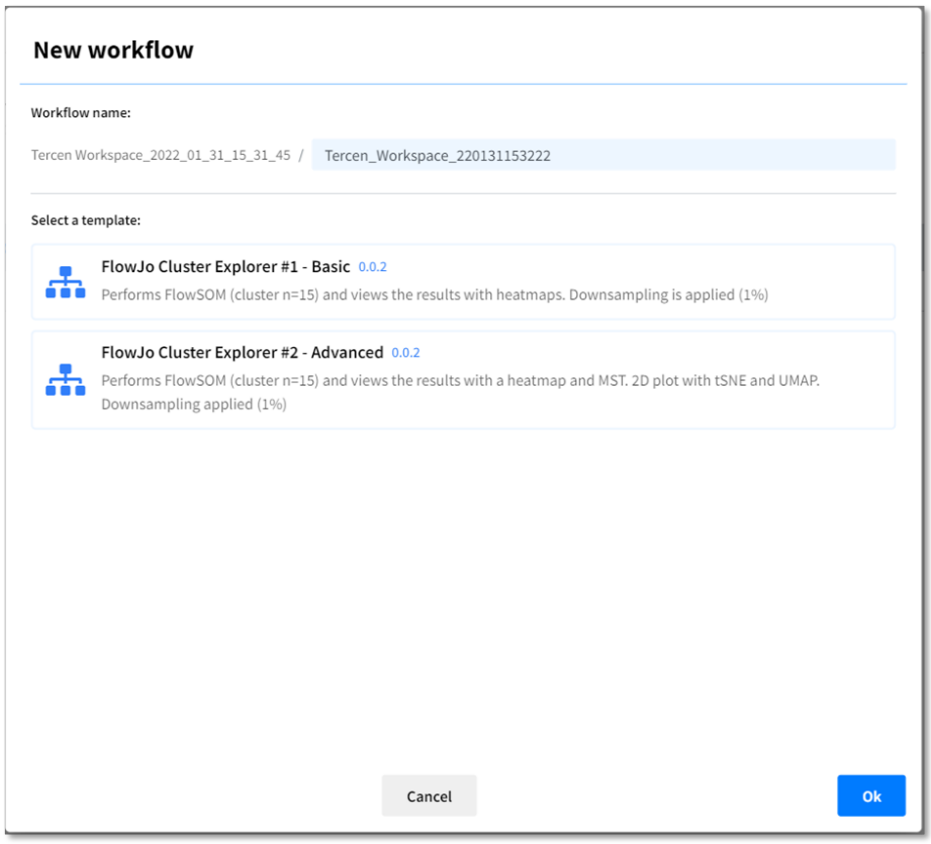
Tercen will default to your FlowJo software license details if they are available.

Follow the instructions on screen to set a password and choose a username.

You may also read the Tercen Terms of Service and Privacy Policy to learn our terms and conditions for using our product and the commitments we make on how we look after your data.

Step 5: Select an analysis workflow template

When the data upload is successful Tercen will open a page in your browser.



Tercen will create a default name for your workflow but you can change it here.

Next there are some pre-made template workflows for you to choose from. These templates are designed for immunophenotyping. They explore the characteristics of a high-dimensional flow cytometry dataset using clustering and dimensionality reduction tools.

There are two templates available.

- **The FlowJo Cluster Explorer #1 - Basic**

  This workflow contains steps for a standard clustering analysis using self-organizing maps (FlowSOM) and has visualizations of the clustering results in a heatmap format.

- **The FlowJo Cluster Explorer #2 - Advanced**
  In addition to the clustering steps described above, this workflow contains steps which perform dimensionality reduction, using tSNE and UMAP. It includes visualizations of

their results. It also presents an additional heatmap, created using MEMscore values, that allows the evaluation of the characteristics of each flowSOM cluster.

When you have selected an analysis template press **OK**.

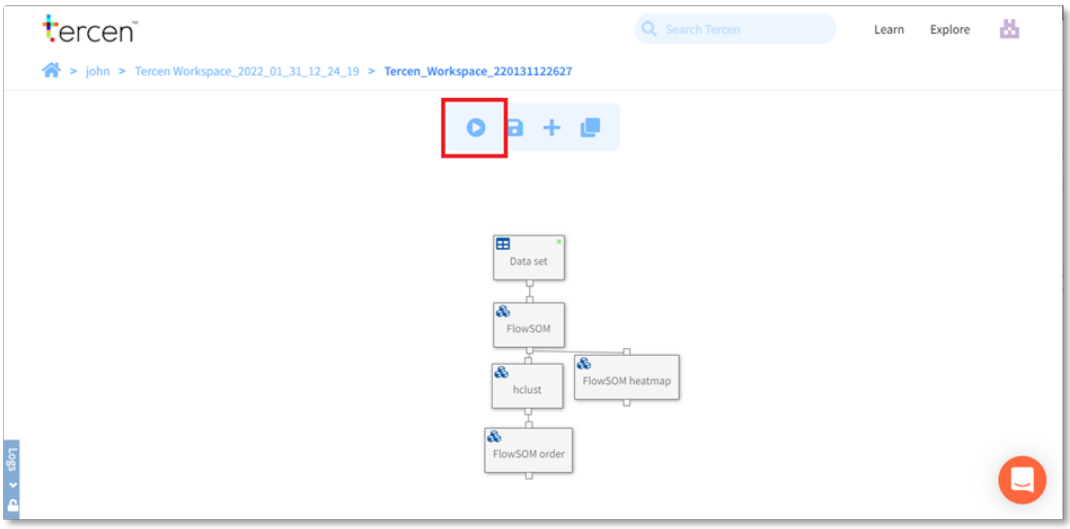
Tercen creates a unique project name using your FlowJo Workspace name. Projects are not overwritten if data is re-uploaded, a new project is created instead. This means you will be able to return to your original data analysis even if you make later modifications to gatings or population selections in FlowJo and choose to re-upload these.

Step 6: Run the analysis and get the result

The workflow canvas will open showing the steps of the template.

Your data is already loaded into the table step.

In the global task bar select **Run all Data Steps**

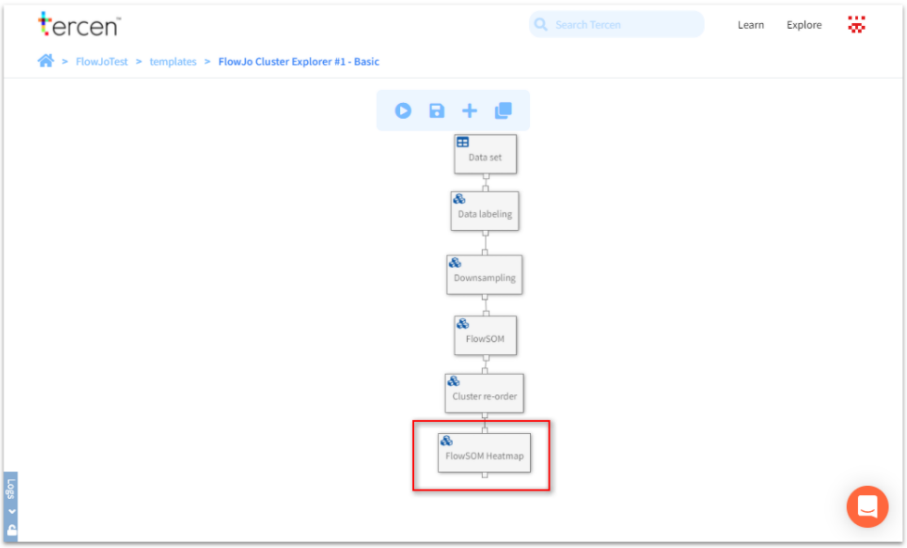


Tercen will now run all of the analysis steps in the template.

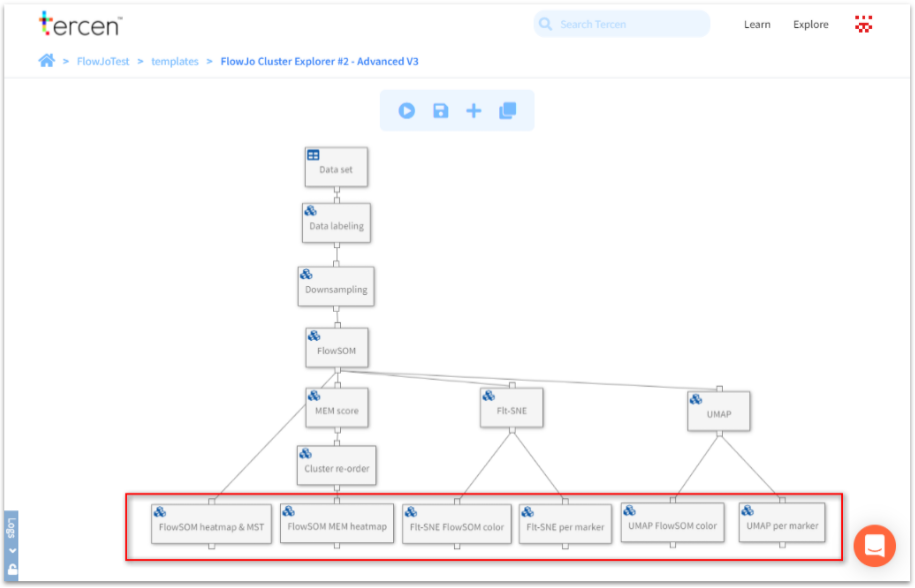
When the workflow is finished each step will have a green dot to say the analysis was successful.

The bottom steps of the workflow contain the visualizations.

If you picked the Basic workflow, double-click on **FloSOM Heatmap**. It shows you the marker expression level and pattern in each cluster as well as the size of the cluster within the sample.



For the Advanced workflow we recommend you open the **Fit-SNE per marker** and **UMAP per marker** to see an overview of marker expression level and pattern. Then open the **FlowSOM MEM Heatmap** to see a scoring of the clusters based on marker enrichment. Then right-click on the **FlowSom Heatmap & MST** step and select **Run** to further explore the characteristics of the clusters.



Further Information

Understanding the pre-made workflows

Workflows contain computation and visualization steps. The former are used to perform analytical processes and the latter to explore the results of the computation steps through an interactive display.

1. **Computation Steps:**

Common to Basic and Advanced

- **Data labeling:** Tagging each event with a random number between 0 and 100.
- **Downsampling:** Reducing the amount of events for the analysis (optional, used to speed up the analysis).
- **FlowSOM:** Clustering flow cytometry data using Self-Organizing Maps (SOMs).
- **Cluster re-order:** Reorganizing the computed FlowSOM clusters in hierarchical order.

Advanced

- **MEM score:** Calculating enrichment scores to assess the enrichment of each marker within cell populations.
- **FIt-SNE:** a statistical technique for dimensionality reduction
- **UMAP**: a statistical technique for dimensionality reduction

NB: Some algorithms (for example UMAP) can take a while to process large amounts of data. Depending on how large your data set is, you might need to leave the system running for a while.

When all boxes have a green dot you can explore your results. The following data steps are the result viewers and the short explanation of what you would expect from those views.
Double-click a data step to see the visualization it has made.

Visualization steps:

Basic

- **FlowSOM heatmap:** FlowSOM generated clusters visualized in a heatmap format colored by the channels (markers) expression level. The clusters are organized in hierarchical order.

Advanced

- **FlowSOM heatmap and MST :** In addition to the heatmap, FlowSOM output is visualized in the minimum spanning tree (MST) that depicts population abundances and channel (marker) expressions.
- **FlowSOM MEM heatmap:** FlowSOM generated clusters visualized in a heatmap format using the values calculated by MEM score and colored by the score of the channels (markers).The clusters are organized in hierarchical order.
- **Flt-SNE FlowSOM color:** Visualization of Flt-SNE results in a 2D plot format per sample, colored by FlowSOM cluster.
- **Flt-SNE per marker:** Visualization of Flt-SNE results in a 2D plot format per channel (marker) and sample.
- **UMAP FlowSOM color:** Visualization of UMAP results in a 2D plot format per sample, colored by FlowSOM cluster.
- **UMAP per marker:** Visualization of UMAP results in a 2D plot format per channel (marker) and sample.

Note: To view FlowSOM MST, select the Operator view tab on the left side of the screen.

Adjusting the downsampling algorithm

We have added a downsampling step to the workflows. This allows you to get a quick overview of your data and check visualizations.

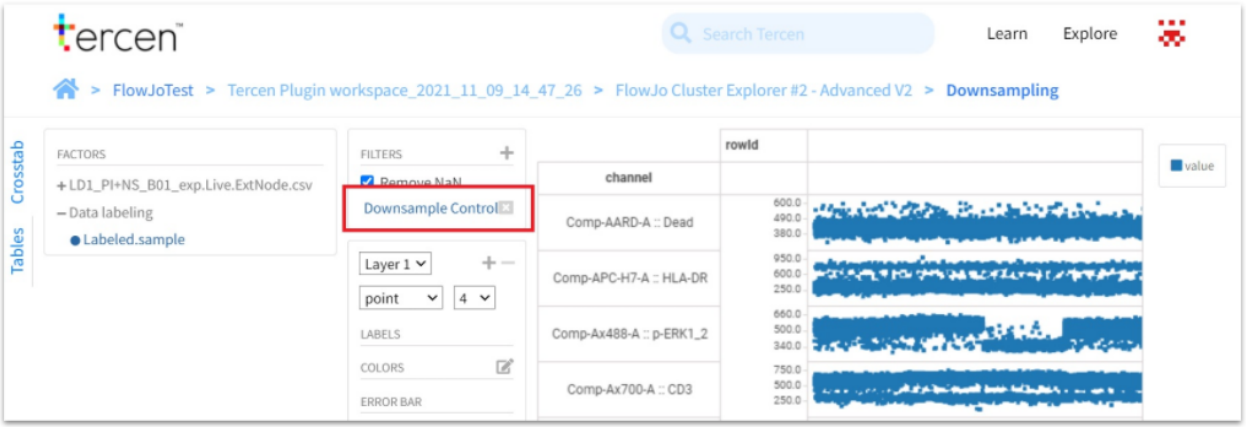
The method being used is called Uniform Downsampling and, by default it reduces the sample size to 1% of data.

To adjust the sample size follow these steps.

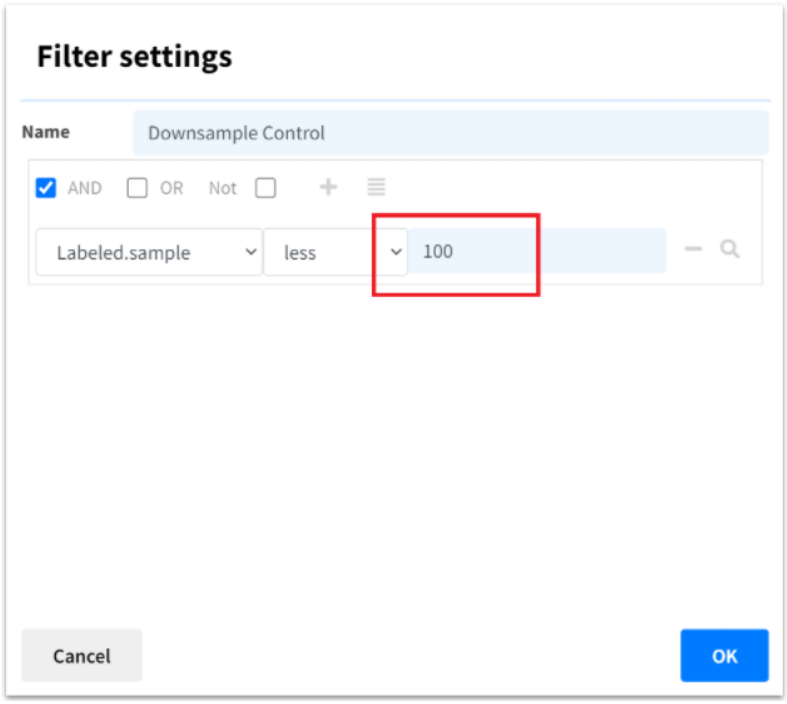
*Note: You will need to reset the Downsampling step if it has already run (i.e has a green dot). Right-click with your mouse and select **Reset** from the menu before continuing below.*

Double-Click on the Downsampling Data Step (or right-click and select **Open**) to open it.

Click on the **Downsample Control** Filter



Change the Filter Settings parameter to the percentage of your data (1-100%) you wish to be included in the calculations.



Press **OK**

Save your changes by pressing the orange save icon.

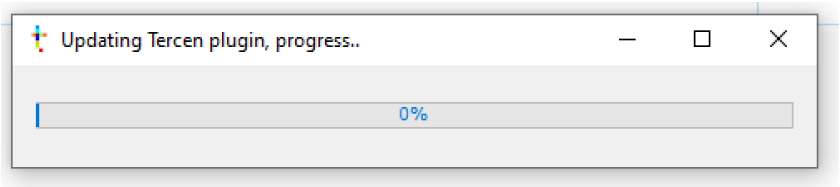
Return to your workflow.

Click the Play icon to run all steps.

The Tercen plug-in will stay up-to-date.

Before uploading data Tercen checks that you have the latest version of the plugin.

If we have released a new feature since you installed the plugin then Tercen will download a new one automatically and prompt you with a popup message to restart your FlowJo.



Useful Links

Tercen Learning Center
FlowJo Instructions on installing plugins
FlowJo Documentation
FlowJo software tutorials
Tercen Tip: How to find your FlowJo Plugins Folder