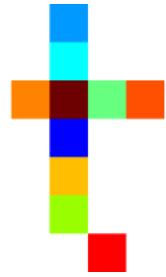




LEVEL^{UP} WORKSHOP I

Part B

0205 - Plot Channels vs. Channels (NxN)



In this lesson, we will create Channel vs. Channel (i.e., NxN) plots based on raw FCS data and on-the-fly data transformations (histogram) on Tercen.

- 1 Click on your copy of the Workshop II Tercen project.

The screenshot shows the Tercen software interface. At the top, there is a navigation bar with icons for home, user profile, and search. Below the navigation bar, the URL 'LevelUpWorkshopsTeam > Workshop II - Myrt' is displayed. The main area has a header 'LevelUpWorkshopsTeam' with tabs for 'Project' (selected) and 'Activities'. Under the 'Project' tab, a section titled 'Workshop II - Myrt' shows a message 'No description provided.' Below this, there is a list of files and folders:

- New data set
- New workflow
- New file
- Upload file
- Upload workflow
- Project

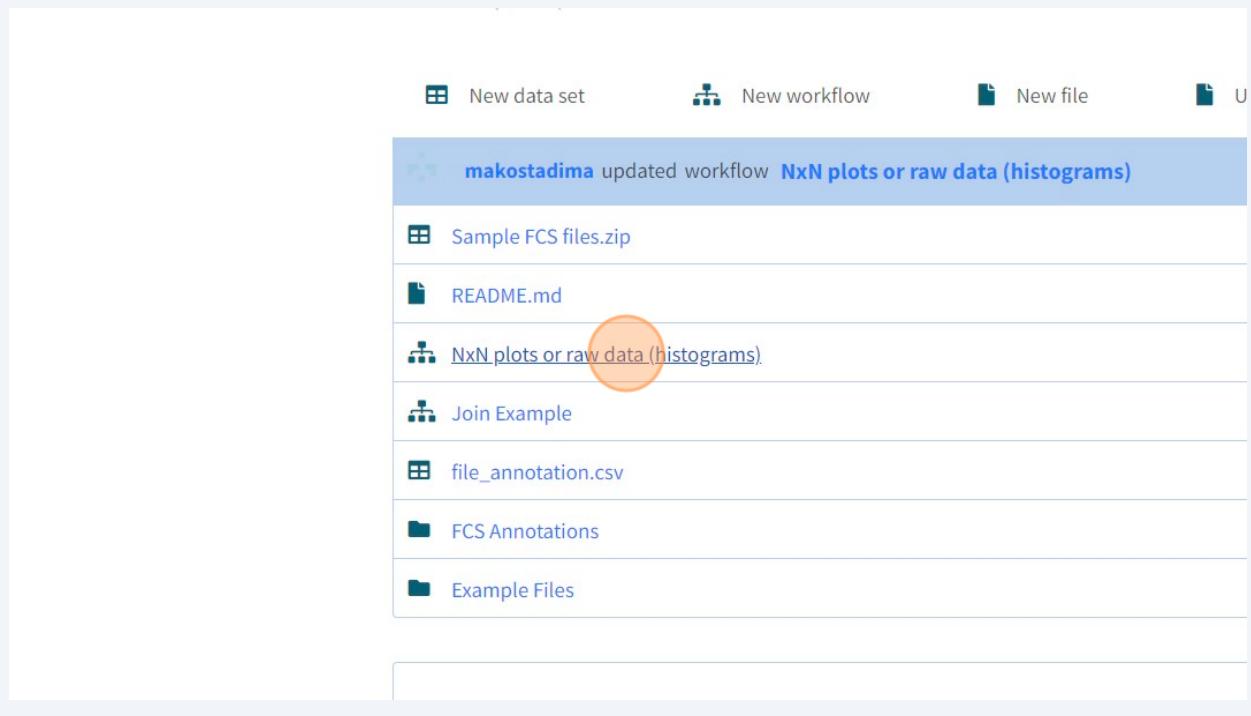
A blue banner at the top of the file list says 'makostadima updated workflow NxN plots or raw data (histograms)'. The file list includes:

- Sample FCS files.zip
- README.md
- NxN plots or raw data (histograms)
- Join Example
- file_annotation.csv
- FCS Annotations
- Example Files

- 2 For the purposes of this lesson, we will use an already created workflow called "NxN plots on raw data (histograms)".

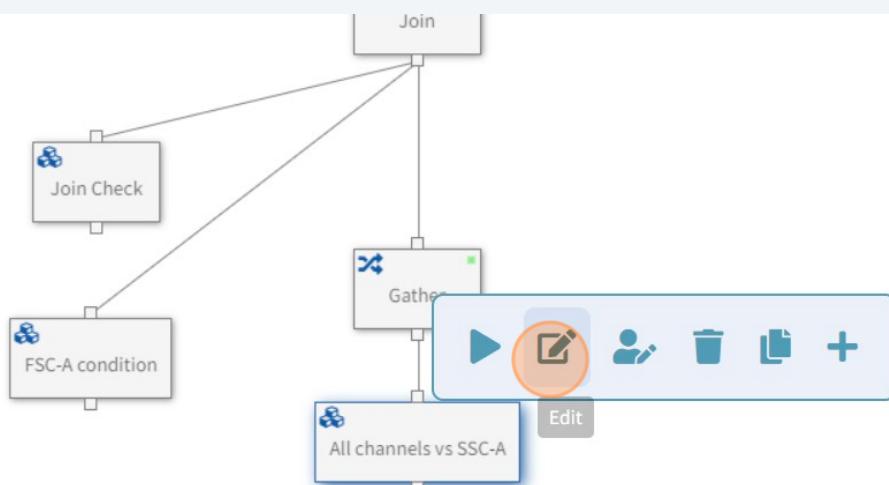
This workflow contains all the steps you've already seen and created in the previous lessons of the day.

- 3 Click "NxN plots on raw data (histograms)"



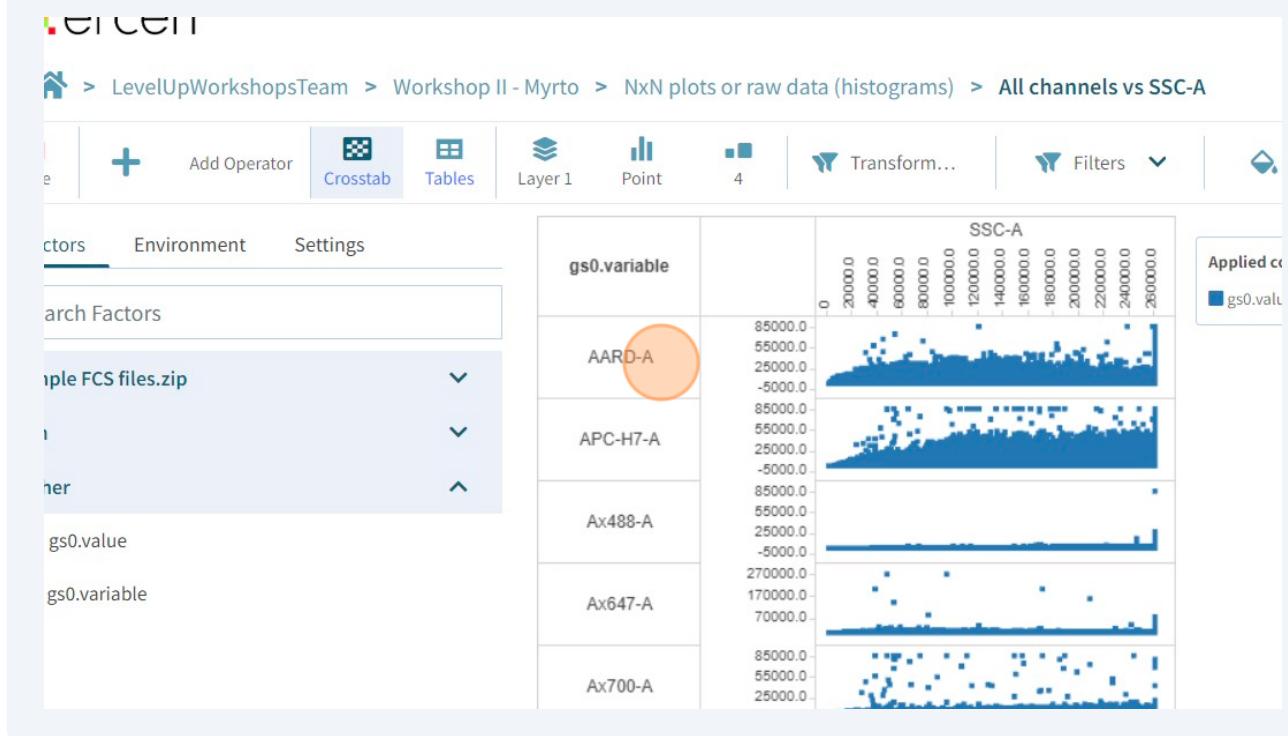
- 4 Now, you will review the last plot you generated in the guide '0203 - Gather'.

Click the 'All channels vs SSC-A' step and select **Edit**.



5

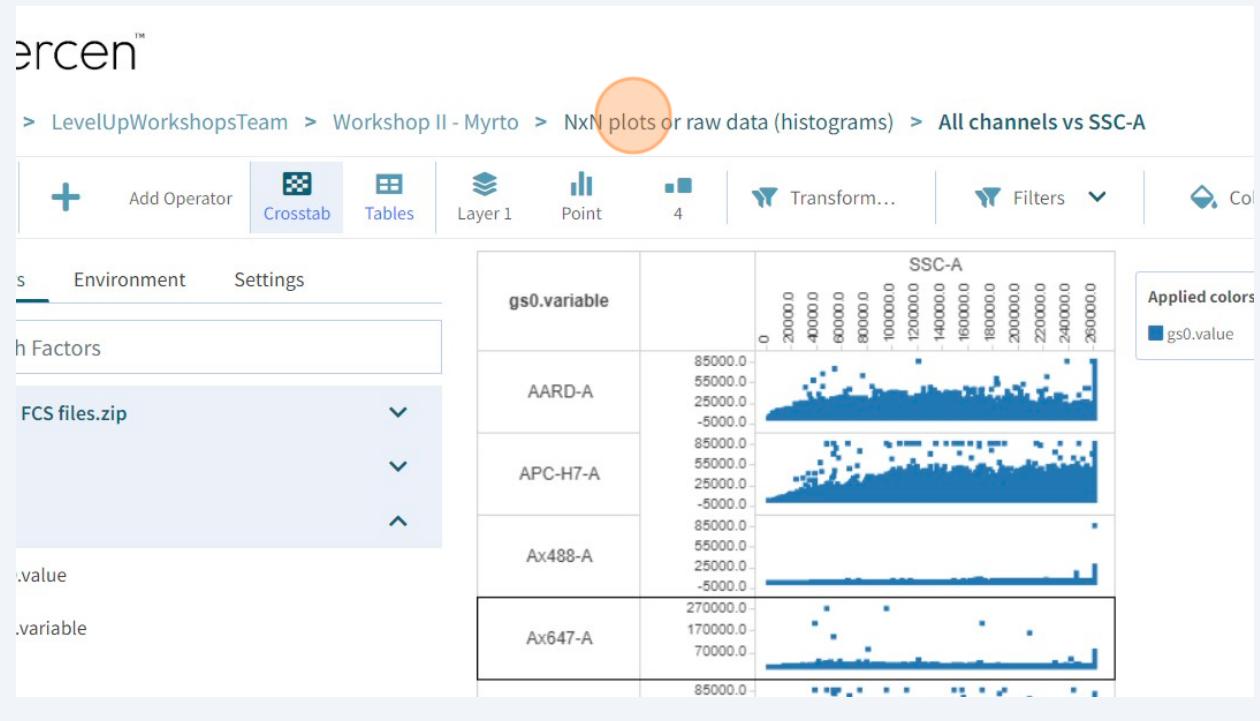
The marker names on the rows represent the colors used but are not very informative (unless one remembers the colors per marker used).



6 Go back to the workflow to fix this.

Save your workflow if needed. Is the disk icon orange? Then click on it to save your workflow.

Click "NxN plots on raw data (histograms)" on the breadcrumb to go back to the workflow.





The data table we will add is automatically generated by the FCS operator when we read our data onto Tercen and is stored under the folder 'FCS Annotations.' It contains two columns, **channel_name** and **channel_description**.

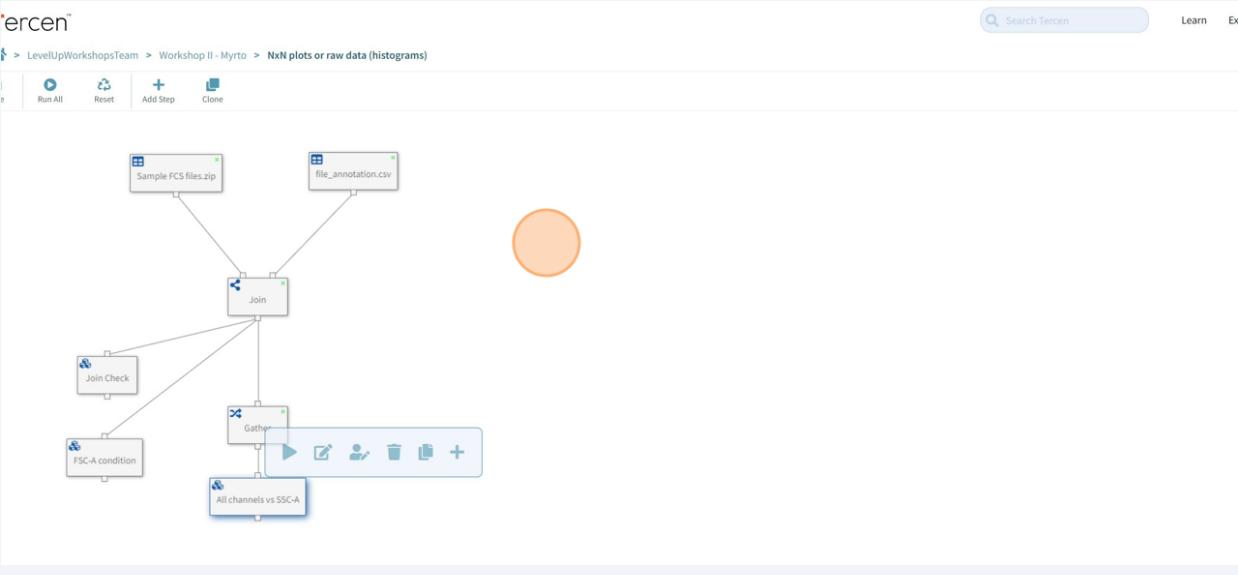
We will use the **channel_name** column to join the names of the channels to their description, which represent the names of the markers assayed.

The screenshot shows a data table with 13 rows. The first row is a header with columns for 'channel_name' and 'channel_description'. The 'channel_name' column contains values like 'FSC-A', 'FSC-H', 'SSC-A', etc., and the 'channel_description' column contains values like 'FSC-A', 'FSC-H', 'SSC-A', etc. A yellow box highlights the 'channel_name' column, and another yellow box highlights the 'channel_description' column. Arrows point from these boxes to the respective column headers in the table.

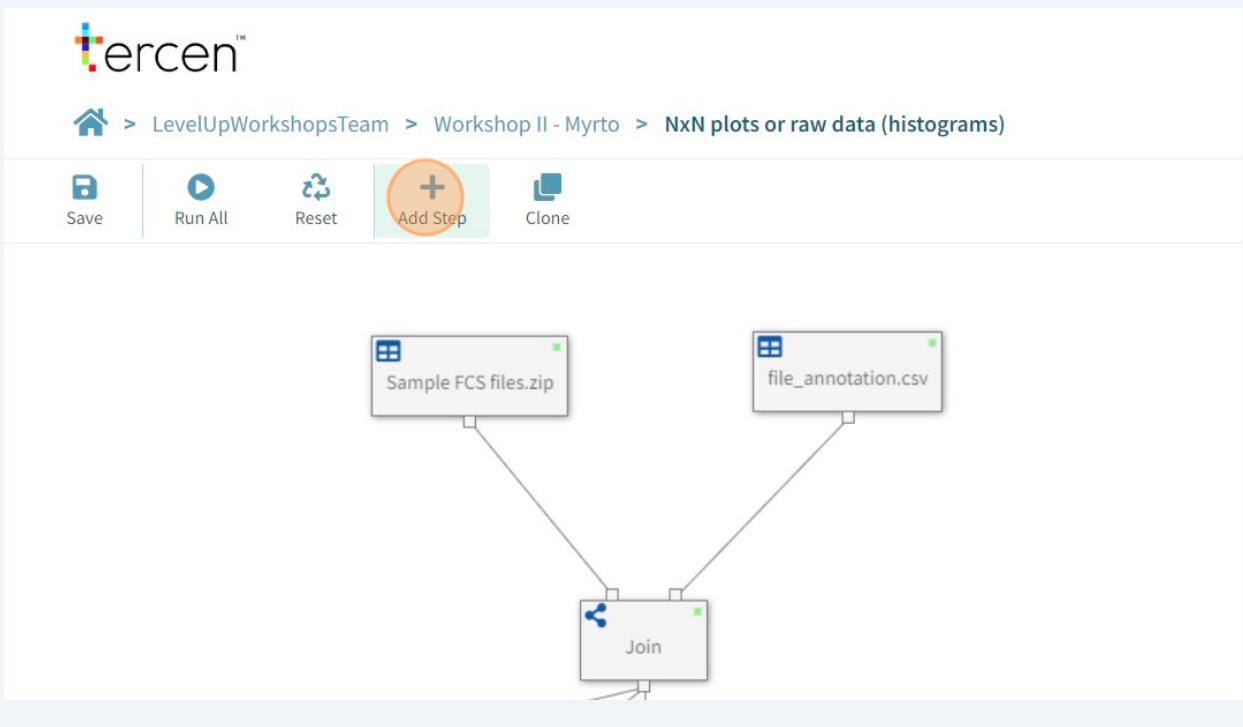
Column containing the colors used		Column containing the markers they represent
1	channel_name (character)	channel_description (character)
1	FSC-A	FSC-A
2	FSC-H	FSC-H
3	SSC-A	SSC-A
4	AARD-A	Dead
5	APC-H7-A	HLA-DR
6	Ax488-A	p-ERK1_2
7	Ax647-A	Blank
8	Ax700-A	CD3
9	PE-A	Perforin

7

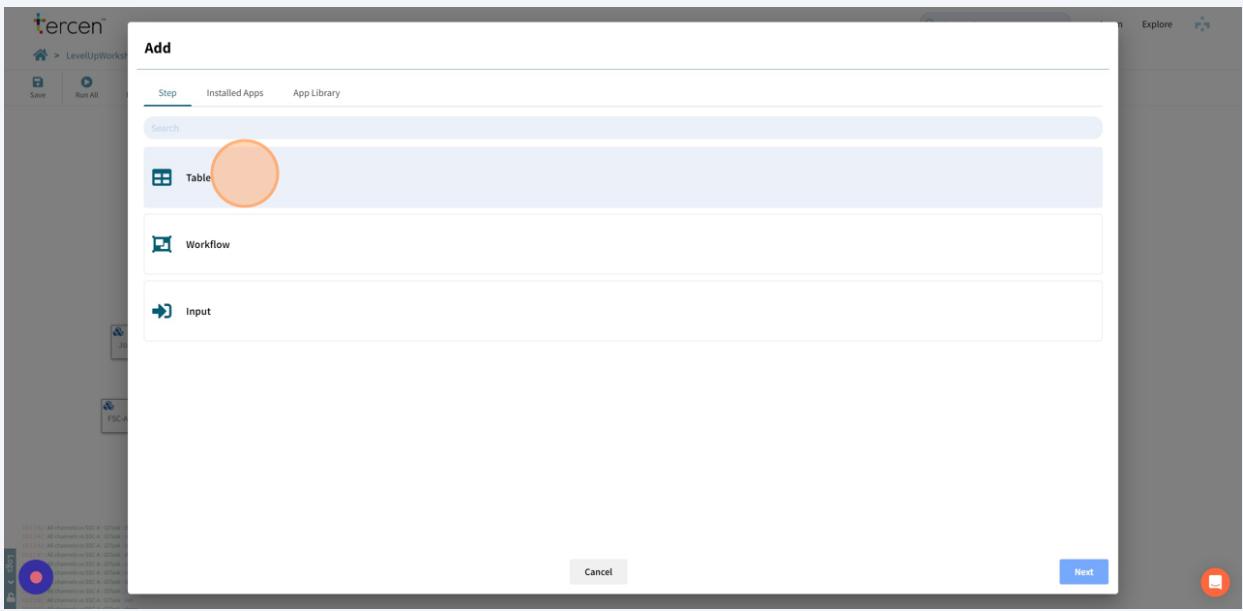
Click anywhere on the white area to remove any local toolbars.



- 8 Click on 'Add Step' from the global toolbar to add a new data table.

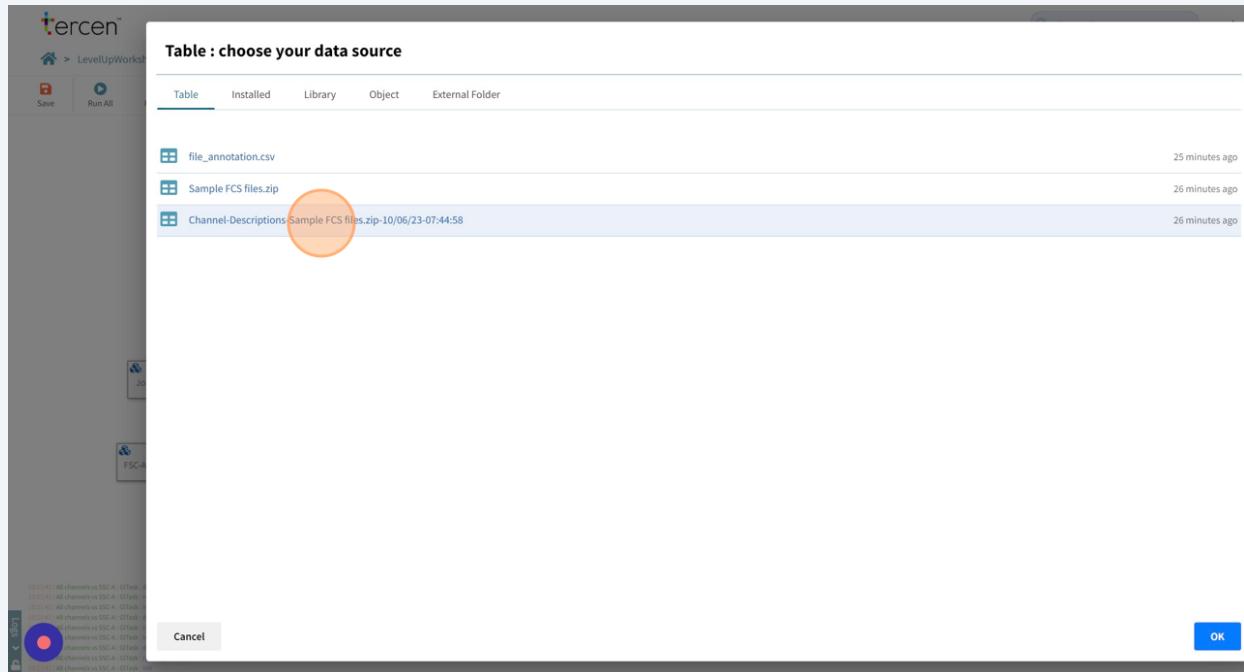


- 9 Click "Table"

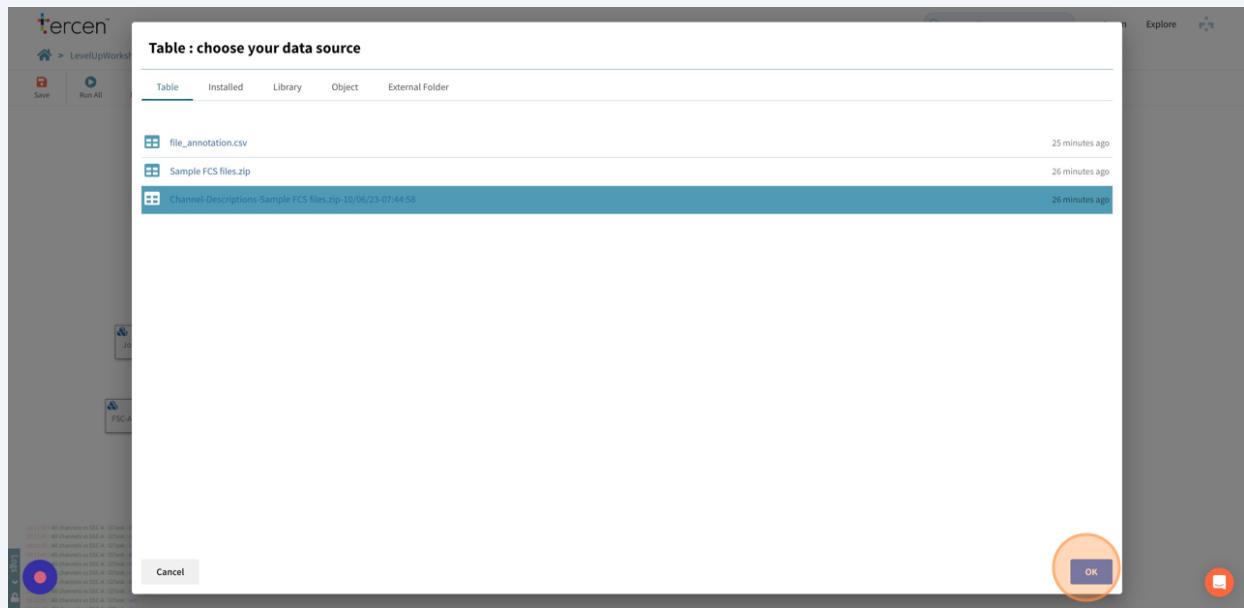


10 Click the file with the prefix 'Channel-Descriptions-Sample FCS files.zip'.

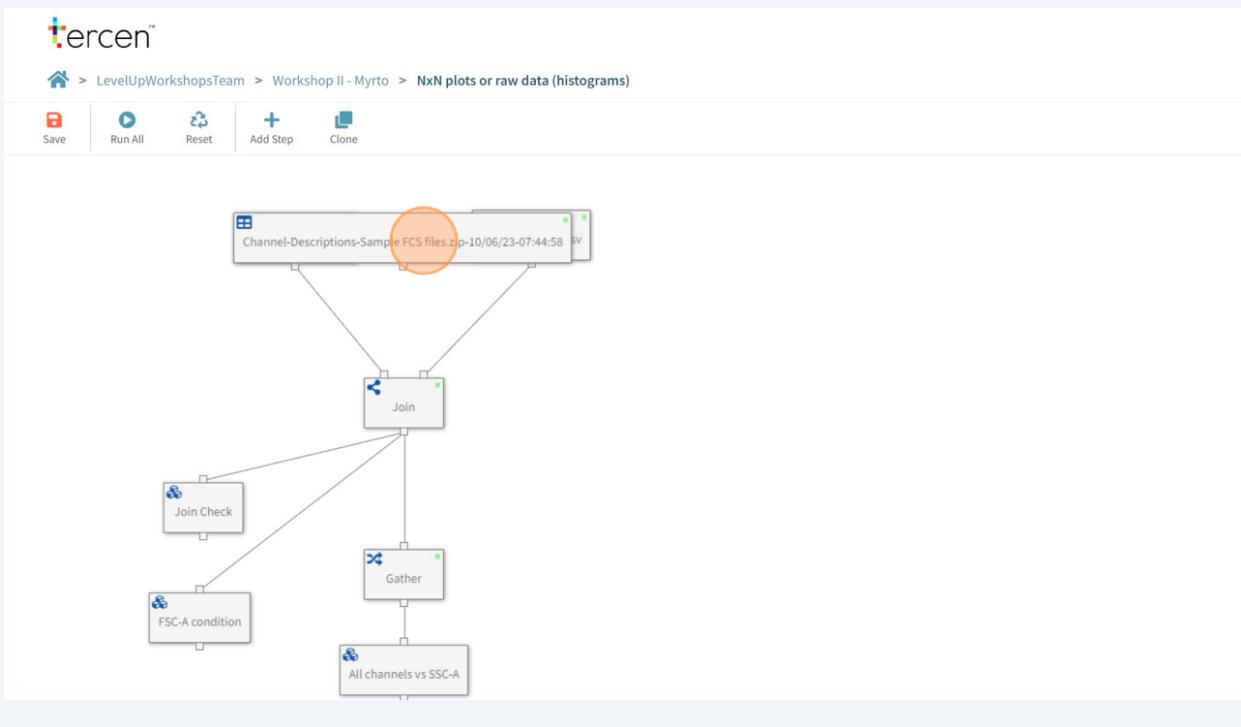
Note that the timestamp suffix will be different for each one of you.



11 Click "OK"

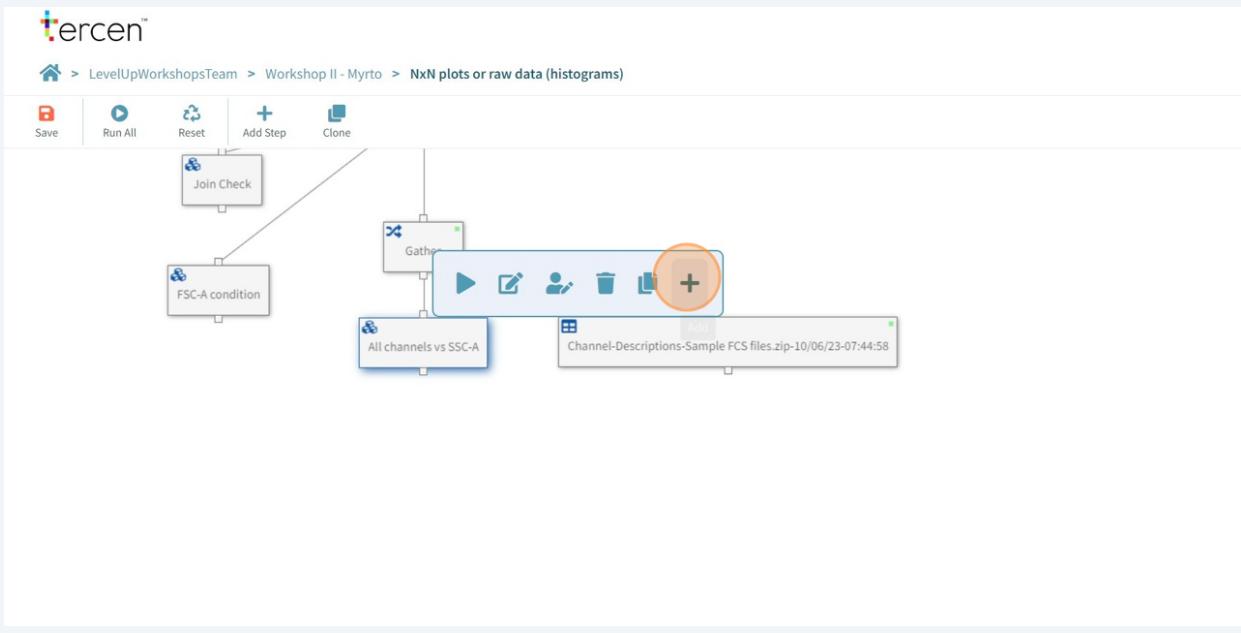


12 Click and drag the table to place it in an empty area of the workflow.



13 Let's do the join.

Click the **All channels vs SSC-A** data step to bring up the local toolbar and click "Add".

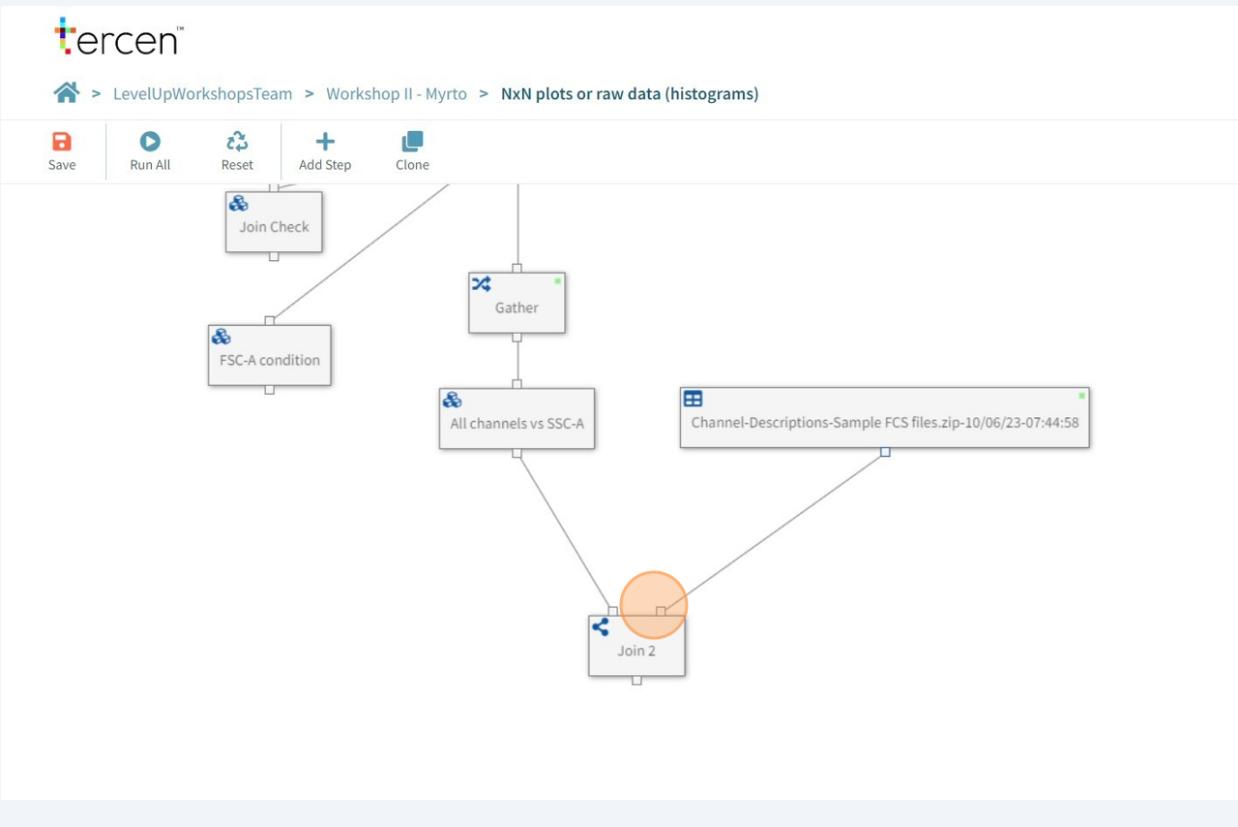


14 Select "Join (left table)".

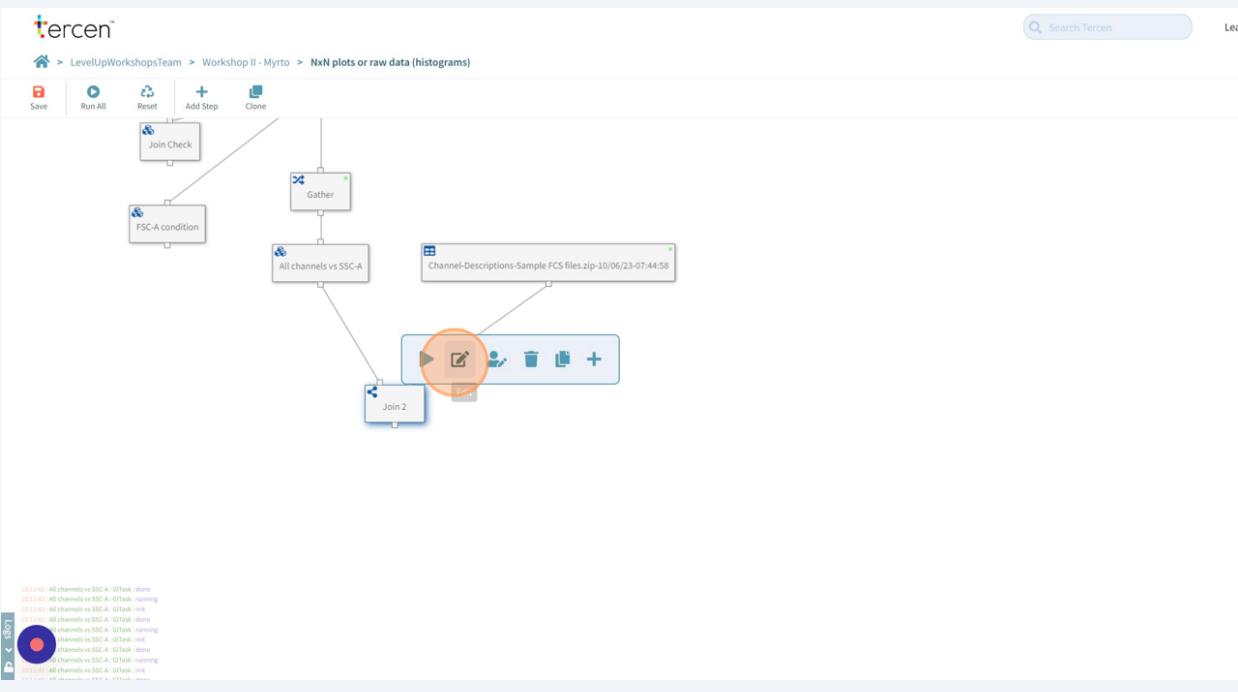
The screenshot shows the tercen™ platform interface. At the top, there's a navigation bar with the tercen™ logo, a home icon, and the text "LevelUpWorkshop". Below the navigation bar is a toolbar with "Save" and "Run All" buttons. The main area is titled "Add" and has tabs for "Step", "Operator", "Operator Library", "Installed Apps", and "App Library". The "Step" tab is currently selected. A search bar labeled "Search" is present. Below the search bar is a list of five data steps:

- Data step data**: Perform computation on user defined projection
- Multi data step data**: Perform computation on user defined projection
- Join leftTable**: Join two data sets (This step is highlighted with an orange circle)
- Join rightTable**: Join two data sets
- Gather table**: Convert data from a wide format to a long format

- 15** Link the **Join 2** step with the table using the bottom node of the table and the free top node of the Join 2 step.



- 16** Click on the **Join 2** step and from the local toolbar select **Edit**.



17 Select **gs0.variable (character)** from the left hand side table

Join keys

[] Select all

FSC-A (numeric)
 FSC-H (numeric)
 SSC-A (numeric)
 AARD-A (numeric)
 APC-H7-A (numeric)
 Ax488-A (numeric)
 Ax647-A (numeric)
 Ax700-A (numeric)
 PE-A (numeric)
 PE-Cy5-A (numeric)
 PE-Cy7-A (numeric)
 PE-TxRed-A (numeric)
 PacBlue-A (numeric)
 Time (numeric)
 fileId (numeric)
 event_id (numeric)
 gs0.value (numeric)
 gs0.variable (character)

[] Select all

channel_name (character)
 channel_description (character)
 channel_id (numeric)
 rowId (numeric)
 tableId (character)

Back Save & Run Step

18 And **channel_name (character)** on the right-hand side. These two represent the keys (name of colors used) on which the join will be performed.

Join keys

[**gs0.variable**] Select all

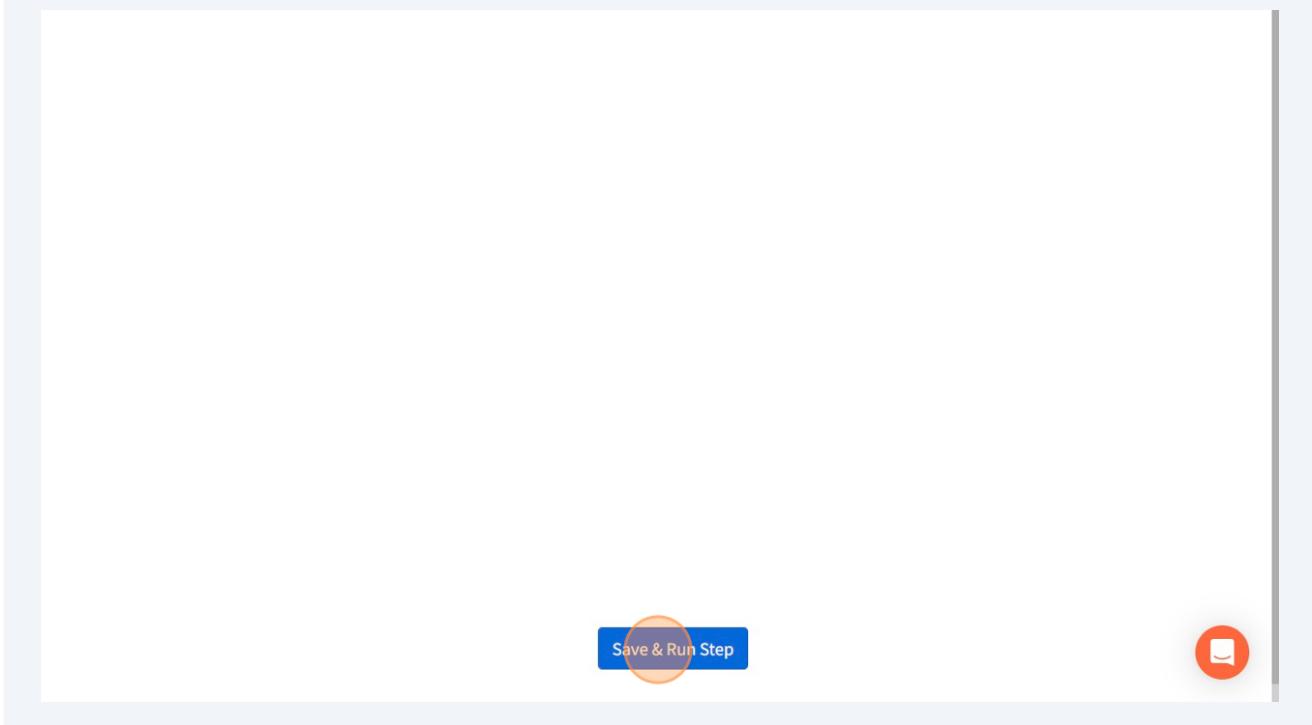
FSC-A (numeric)
 FSC-H (numeric)
 SSC-A (numeric)
 AARD-A (numeric)
 APC-H7-A (numeric)
 Ax488-A (numeric)
 Ax647-A (numeric)
 Ax700-A (numeric)
 PE-A (numeric)
 PE-Cy5-A (numeric)
 PE-Cy7-A (numeric)
 PE-TxRed-A (numeric)
 PacBlue-A (numeric)
 Time (numeric)
 fileId (numeric)
 event_id (numeric)

[] Select all

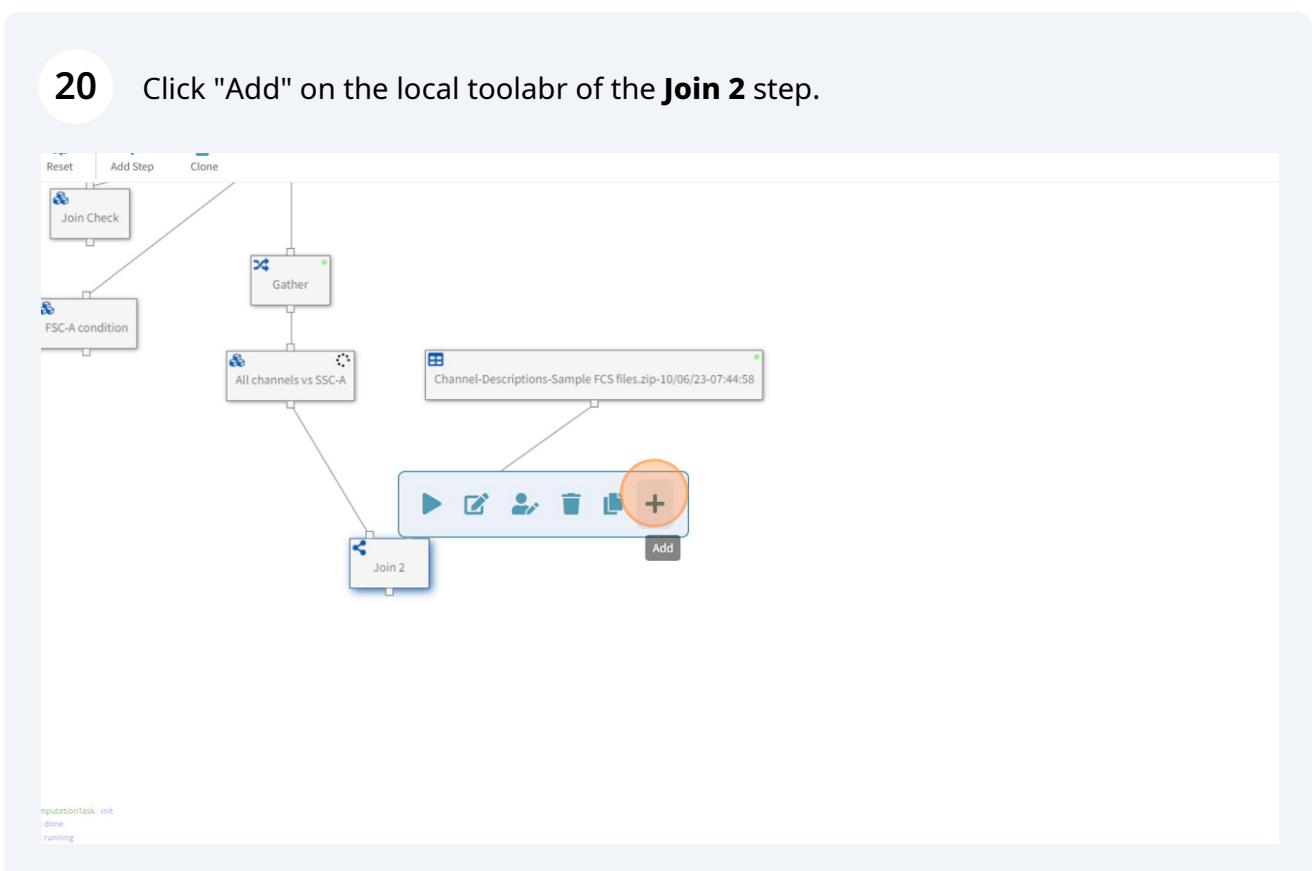
channel_name (character)
 channel_description (character)
 channel_id (numeric)
 rowId (numeric)
 tableId (character)

Back Save & Run Step

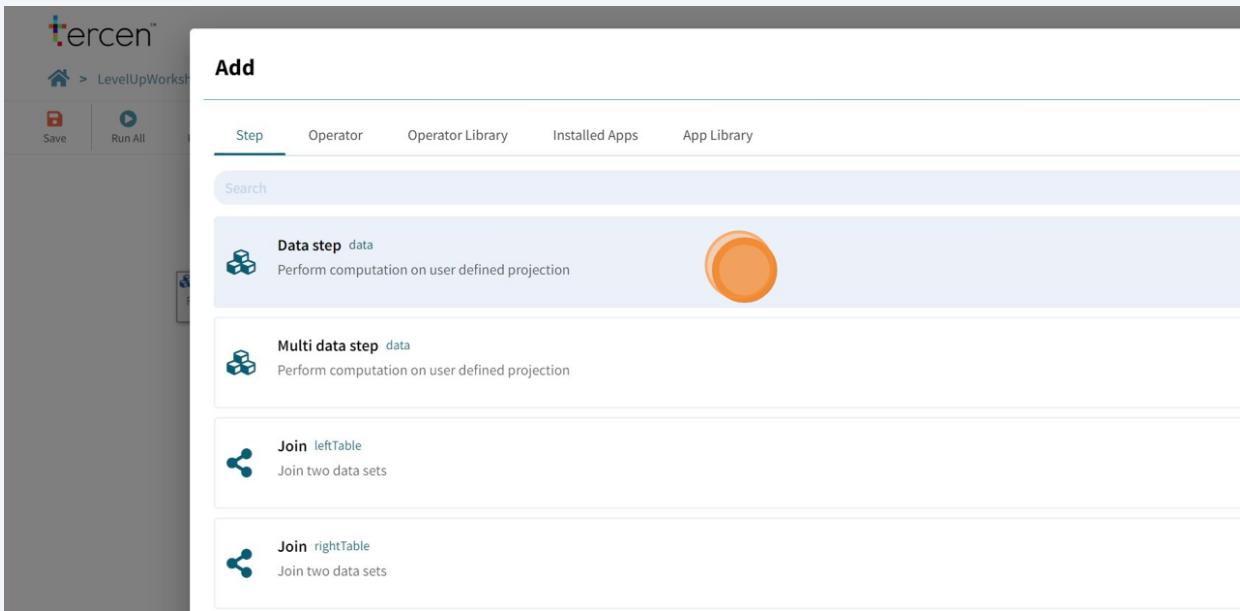
19 Click "Save & Run Step"



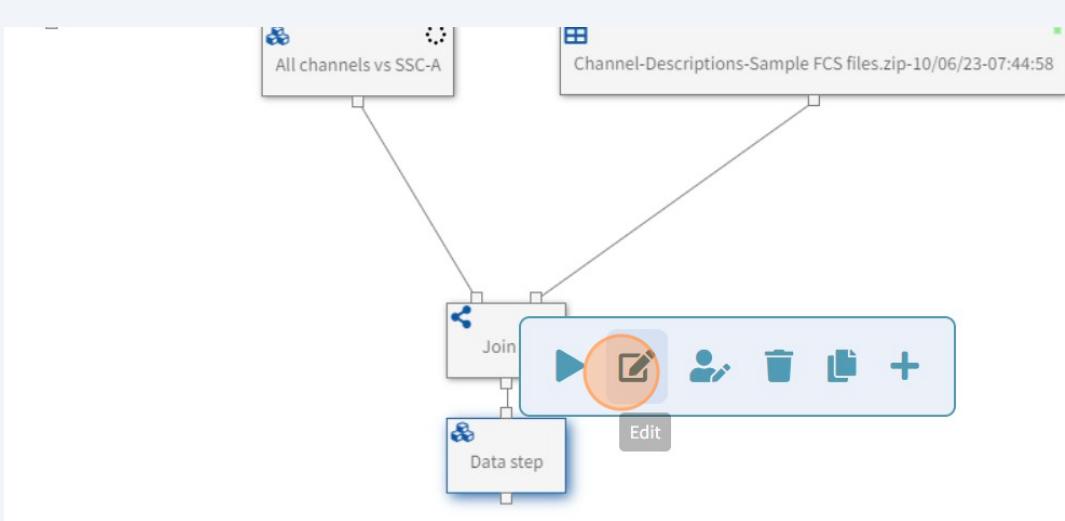
20 Click "Add" on the local toolbar of the **Join 2** step.



21 Select "Data step"

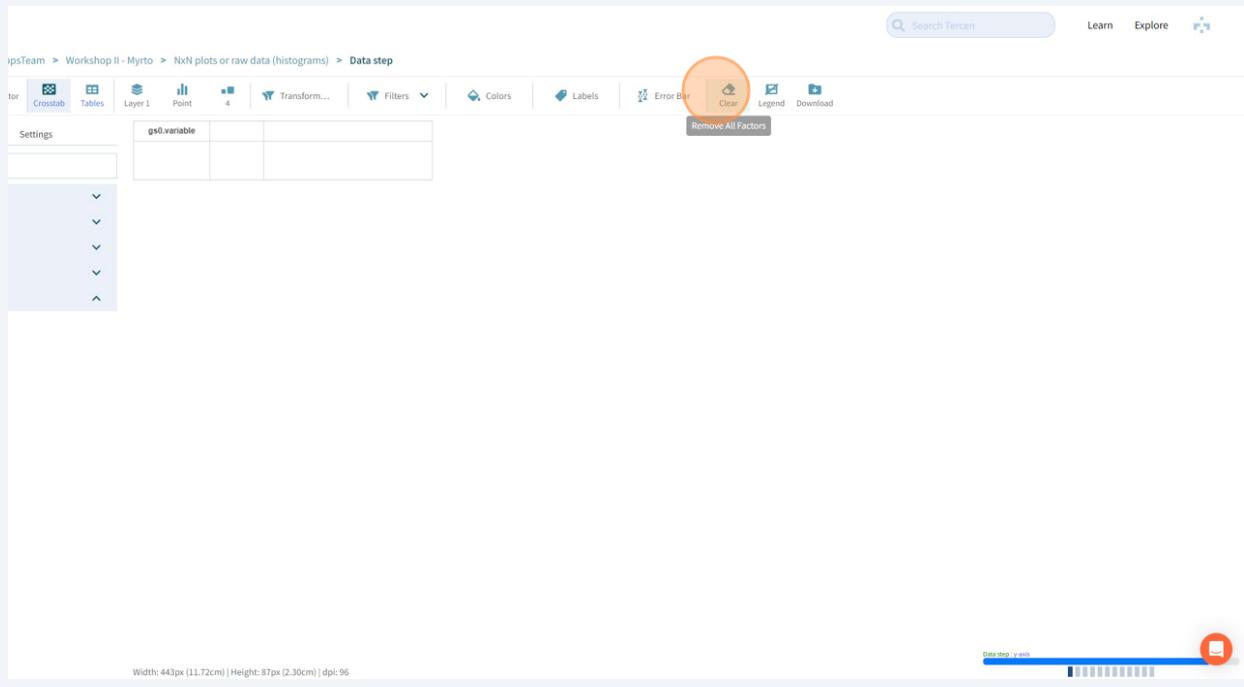


22 Click "Edit"



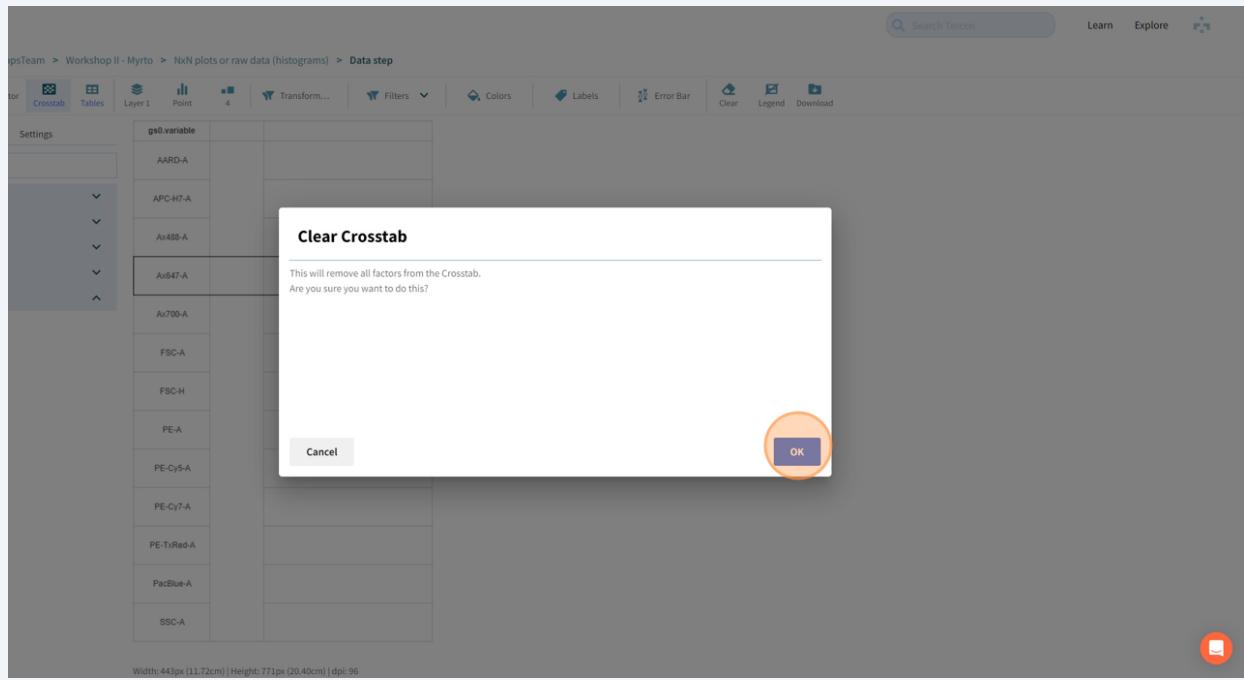
23

Clear any pre-defined data projections by clicking the "Clear" button on the toolbar.



24

Click "OK"



25 Expand the "Join 2" factors by clicking on it on the Factors list.

Search Factors

Sample FCS files.zip ▼

Join ▼

Gather ▼

All channels vs SSC-A ▼

Join 2 ▲

26 Drag and drop the **js1.channel_description** factor to Row.

Search Factors

Sample FCS files.zip ▼

Join ▼

Gather ▼

All channels vs SSC-A ▼

Join 2 ▲

■ js1.channel_name

■ js1.channel_description

□ js1.channel_id

□ js1.rowId

■ js1.tableId

27

Again, drag and drop the **js1.channel_description** factor to Column this time.

Search Tercen

Save Add Operator Crosstab Tables Layer 1 Point 4 Transform... Filters Colors Labels Error Bar Clear Legend Download

Factors Environment Settings

js1.channel_d

js1.channel_c

js1.channel_i

js1.channel_name
js1.channel_description
js1.channel_id
js1.rowid
js1.tableid

Width: 410px (10.85cm) | Height: 280px (7.41cm) | dpi: 96

Data step : measurement

28

Adjust the height of rows & columns, as desired.

Search Tercen

Save Add Operator Crosstab Tables Layer 1 Point 4 Transform... Filters Colors Labels Error Bar Clear Legend Download

Factors Environment Settings

js1.channel_d

js1.channel_c

js1.channel_i

js1.channel_name
js1.channel_description
js1.channel_id
js1.rowid
js1.tableid

Width: 410px (10.85cm) | Height: 280px (7.41cm) | dpi: 96

29

We will now set up a filter based on the names of the markers to filter down the markers for which the NxN plot will be generated.

Drag and drop the **js1.channel_description** factor to the Filters area.

The screenshot shows the Tercen software interface with the following details:

- Toolbar:** Save, Add Operator, Crosstab, Tables, Layer 1, Point, 4, Transform..., Filters, Colors, Labels, Error Bar, Clear, Legend, Download.
- Data Step Editor:** Factors, Environment, Settings.
- Search Factors:** Sample FCS files.zip, Join, js0.filename, js0.Condition, js0.rowid, js0.tableid.
- Join:** All channels vs SSC-A, Join 2, js1.channel_name, js1.channel_description (highlighted by a green box), js1.channel_id, js1.rowid, js1.tableid.
- Filters Area:** Shows 'Applied filters: Keep only Control sample', 'Applied label: event_id', and 'Applied colors:'.
- Bottom Status:** Width: 258px (6.83cm) | Height: 188.5px (4.99cm) | dpi: 96.

30

On the popup window, we will build a boolean filter to select a subset of the available markers. For our example, we will choose CD3, CD4 and CD8.

We start building the filter by clicking on the magnifying glass to select the desired marker from the list.

The screenshot shows the 'Filter settings' dialog box with the following details:

- Title:** Filter settings.
- Name:** js1.channel_description.
- Operator:** AND (checkbox checked).
- Condition:** js1.channel_description equals NaN.
- Buttons:** Cancel, OK.

31 Click "CD3"

ls	v	Nan
1	Previous	Next
#	js1.channel_description	
	(character)	
1	Blank	
2	CD3	○
3	CD38	
4	CD4	
5	CD8	
6	Dead	
7	FSC-A	

32 Click "OK"

CD4
CD8
Dead
FSC-A
FSC-H
HLA-DR
IFN σ

OK

33 Now, on to expanding the filter to add the other two markers.

Select 'OR'.

The screenshot shows the 'Filter settings' dialog box. The 'Name' field is set to 'js1.channel_description'. The operator selection area has 'AND' checked and 'OR' unchecked. A red circle highlights the 'OR' checkbox. Below the operators is a search bar with 'js1.channel_description' and 'equals' selected, with 'CD3' as the value. The background shows the Tercen interface with a 'Factors' tab active, displaying a list of joined datasets: js0.filename, js0.Condition, js0.rowId, and js0.tableId.

34 Click the '+' button.

The screenshot shows the 'Filter settings' dialog box after clicking the '+' button. The 'Name' field is still 'js1.channel_description'. The operator selection area now shows 'OR' checked and 'AND' unchecked. A red circle highlights the '+' button. The main search bar shows 'js1.channel_description' with 'equals' selected and 'CD3' as the value. The background shows the Tercen interface with a 'Factors' tab active, displaying a list of joined datasets: js0.filename, js0.Condition, js0.rowId, and js0.tableId.

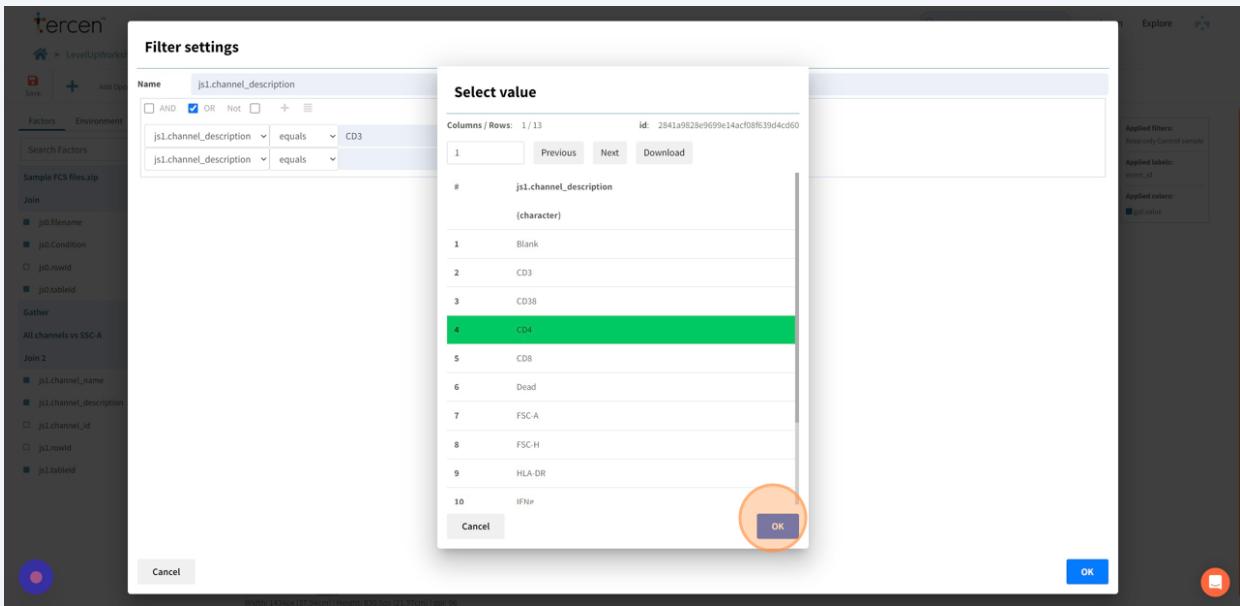
35 Change the default factor to **js1.channel_description** from the dropdown menu.

The screenshot shows the 'Filter settings' dialog in the Tercen software. The 'Name' field is set to 'js1.channel_description'. Below it, there are filter options: AND, OR (which is checked), Not, and logic operators (+, -, =). Two filter conditions are present: 'js1.channel_description equals CD3' and 'js1.channel_id equals NaN'. A dropdown menu titled 'js1.channel_id' is open, listing various channel names. The 'CD3' entry is highlighted and circled in orange.

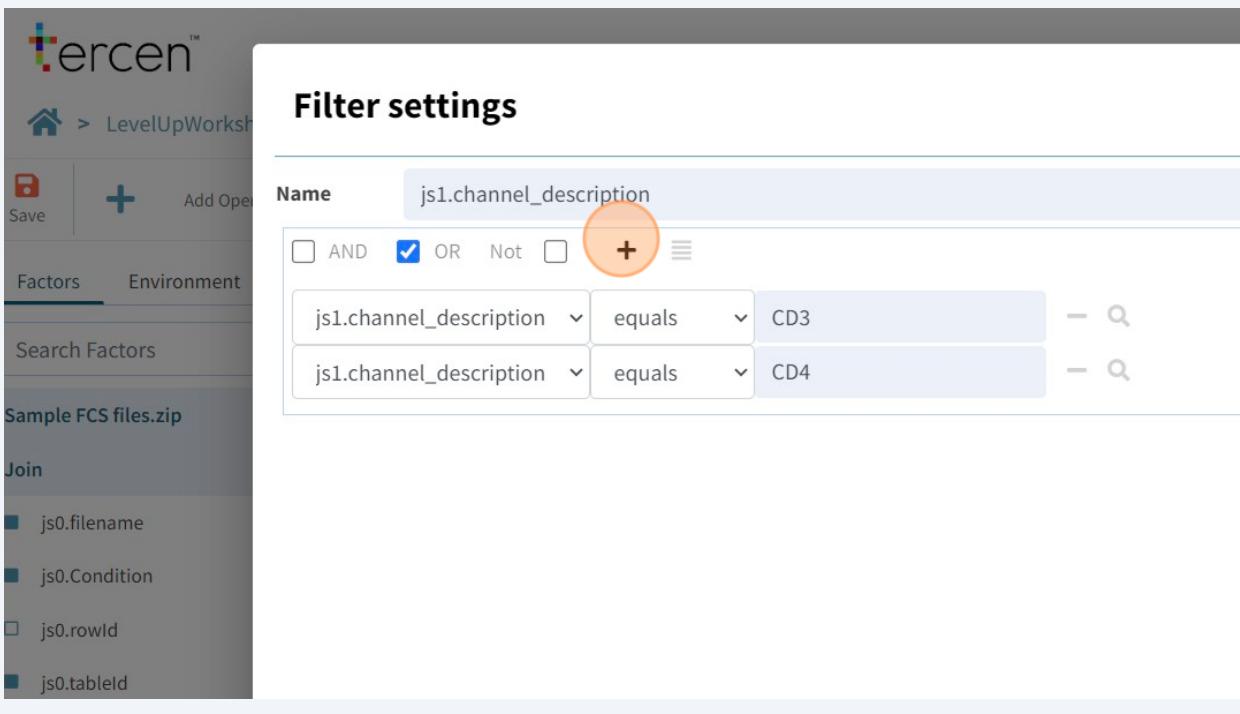
36 Click on the magnifying glass again.

The screenshot shows the 'Filter settings' dialog in the Tercen software. The 'Name' field is set to 'js1.channel_description'. Below it, there are filter options: AND, OR (which is checked), Not, and logic operators (+, -, =). Two filter conditions are present: 'js1.channel_description equals CD3' and 'js1.channel_description equals NaN'. A dropdown menu titled 'js1.channel_description' is open, listing various channel names. The magnifying glass icon next to the second entry 'js1.channel_description' is highlighted and circled in orange.

37 Select "CD4" and then click 'OK'.

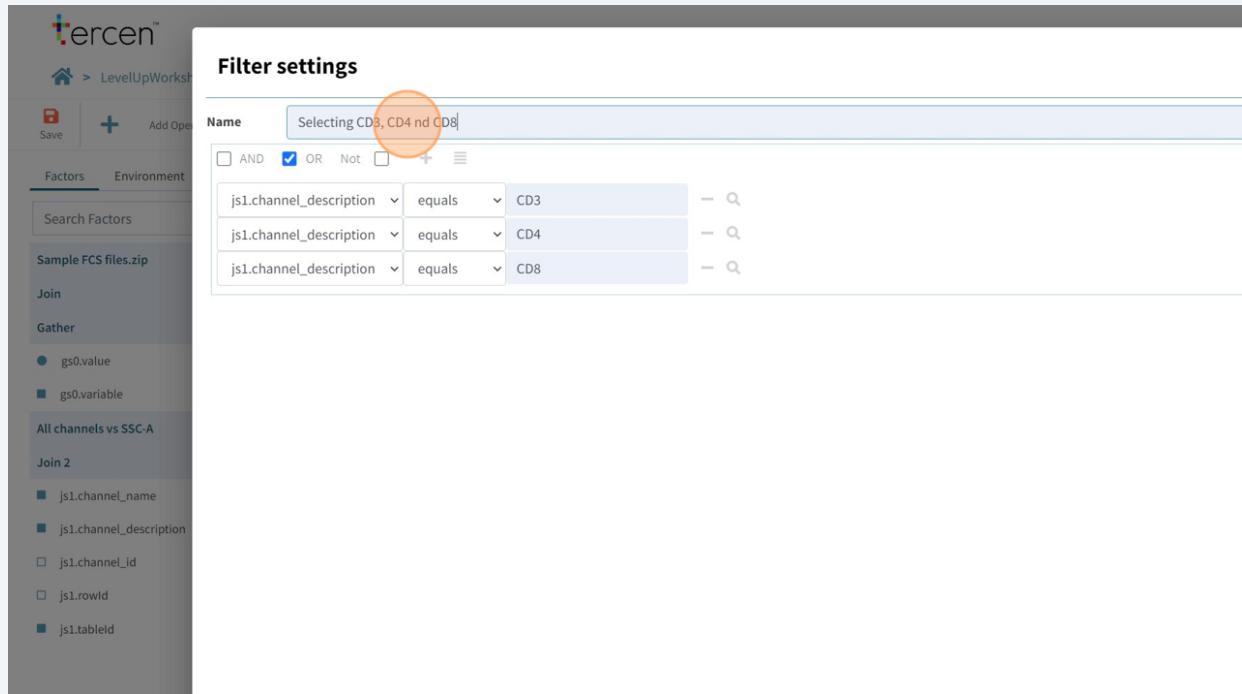


38 Repeat these steps to add the "CD8" marker to the filter.

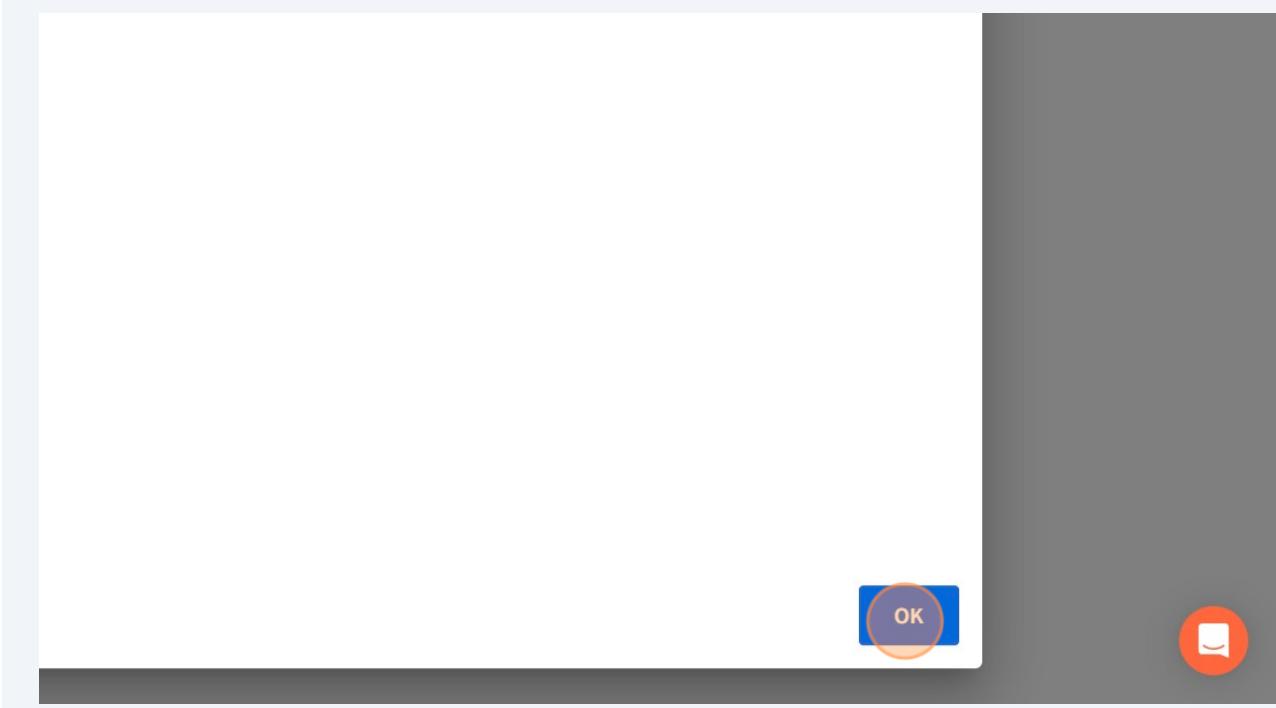


39 Once you have added all desired markers to the filter, we will edit its name.

Type "Selecting CD3, CD4 and CD8" in the relevant text box.



40 Click "OK"



41

Notice how the row & columns now contain only the three selected markers.

The screenshot shows a software interface with a toolbar at the top. The 'Crosstab' tab is selected. Below the toolbar is a table with three columns and three rows. The columns are labeled 'js1.channel_e' (empty), 'CD3', 'CD4', and 'CD8'. The rows are labeled 'js1.channel_d' (empty), 'CD3', 'CD4', and 'CD8'. On the left side of the table, there is a vertical list of markers: CD3, CD4, and CD8. To the right of the table, there is a large empty area.

js1.channel_e	CD3	CD4	CD8
js1.channel_d			
CD3			
CD4			
CD8			

42

Click "Gather"

The screenshot shows a software interface with a toolbar at the top. The 'Crosstab' tab is selected. Below the toolbar is a sidebar titled 'Factors' with tabs for 'Environment' and 'Settings'. A search bar says 'Search Factors'. Below it is a list of files: 'Sample FCS files.zip' (highlighted with an orange circle), 'Join', 'Gather' (highlighted with an orange circle), 'All channels vs SSC-A', 'Join 2', and a list of checkboxes: 'js1.channel_name', 'js1.channel_description', 'js1.channel_id' (unchecked), and 'js1.rowId'. To the right of the sidebar is a table with three columns and three rows. The columns are labeled 'js1.channel_e' (empty), 'CD3', 'CD4', and 'CD8'. The rows are labeled 'js1.channel_d' (empty), 'CD3', 'CD4', and 'CD8'. The 'Gather' item in the sidebar is highlighted with an orange circle.

js1.channel_e	CD3	CD4	CD8
js1.channel_d			
CD3			
CD4			
CD8			

43

Drag and drop the **gs0.value** factor to the Y-axis to project the marker measurements.

The screenshot shows the Tercen software interface. On the left, under the 'Factors' tab, there is a search bar and a list of factors. The 'gs0.value' factor is highlighted with an orange circle. Below it is 'gs0.variable'. Under 'Join 2', there are three items: 'js1.channel_name', 'js1.channel_description', and 'js1.channel_id'. To the right is a data grid with columns 'js1.channel_d' and 'CD3'. The 'js1.channel_d' column has rows for 'CD3', 'CD4', and 'CD8'. The 'CD3' row contains a single data point.

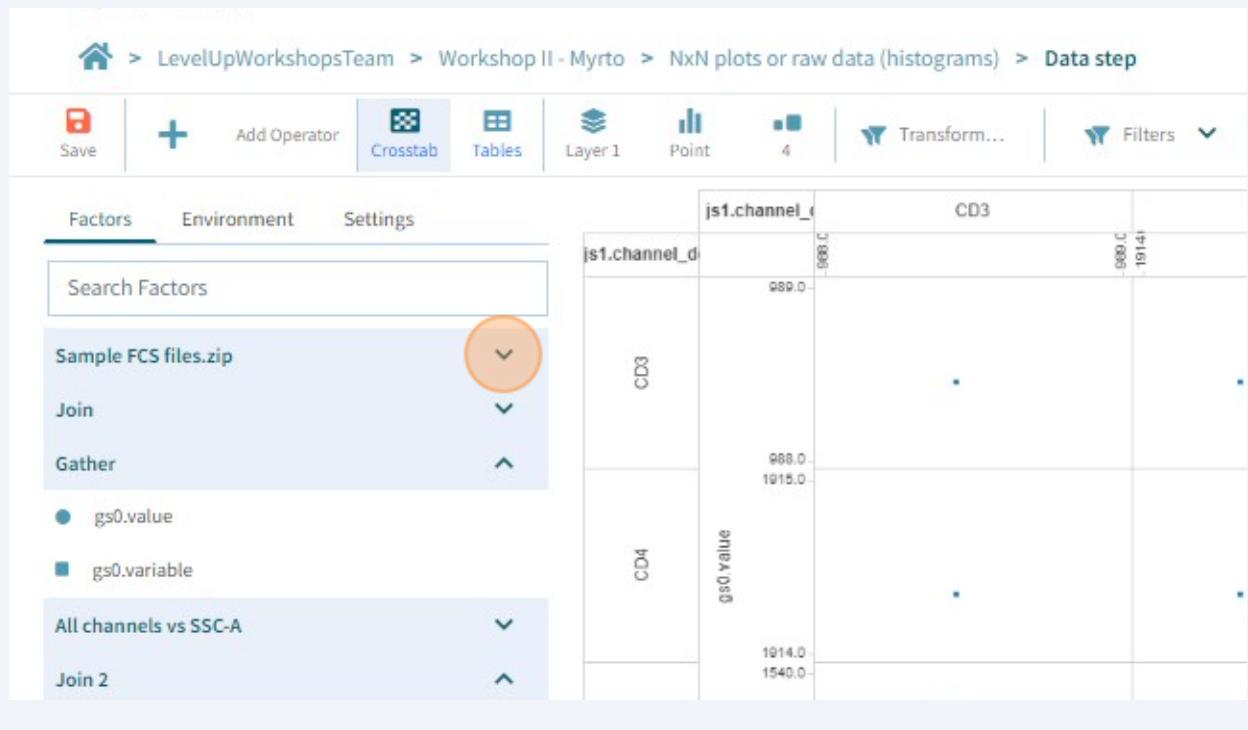
js1.channel_d	CD3
CD3	
CD4	
CD8	



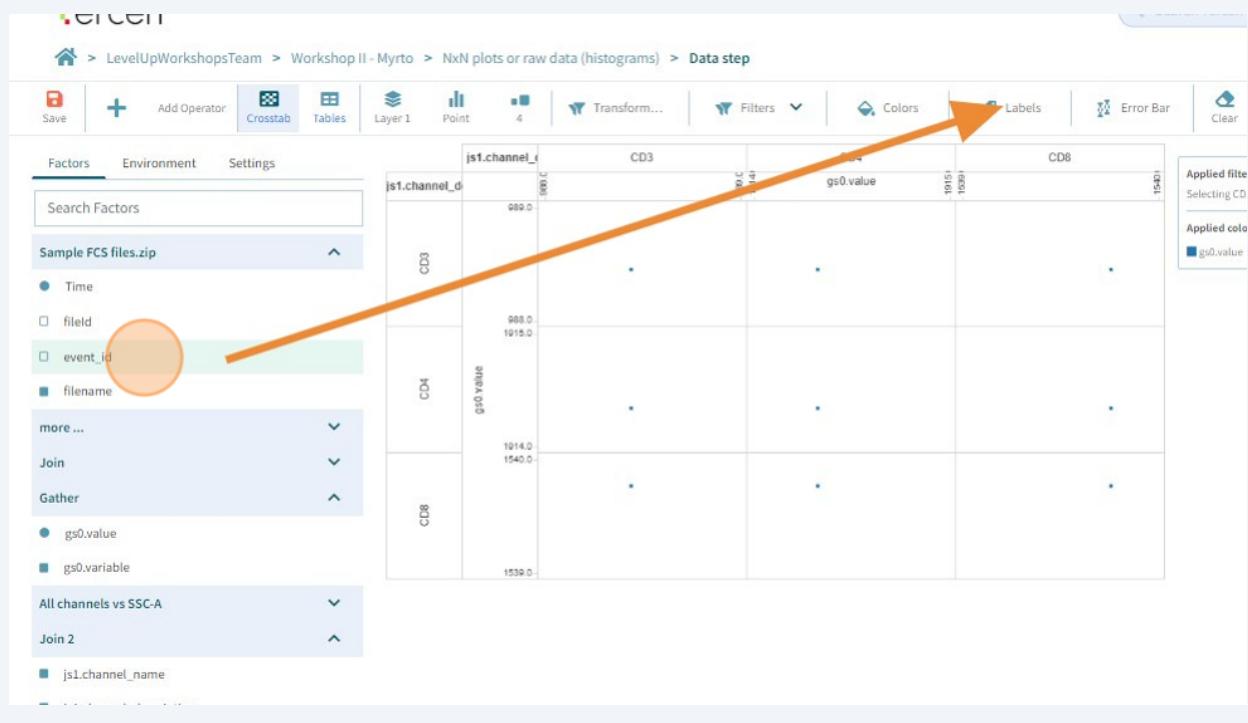
Note that for pairwise plots, like the NxN plots we are building, Tercen also adds the **gs0.value** to the X-axis.

You will also notice only one data point appears in each data cell. We require one last action to complete the pairwise plot.

- 44 Click 'Sample FCS files.zip' to expand the factors listed under it.



- 45 Drag the **event_id** factor and drop it on 'Labels'.

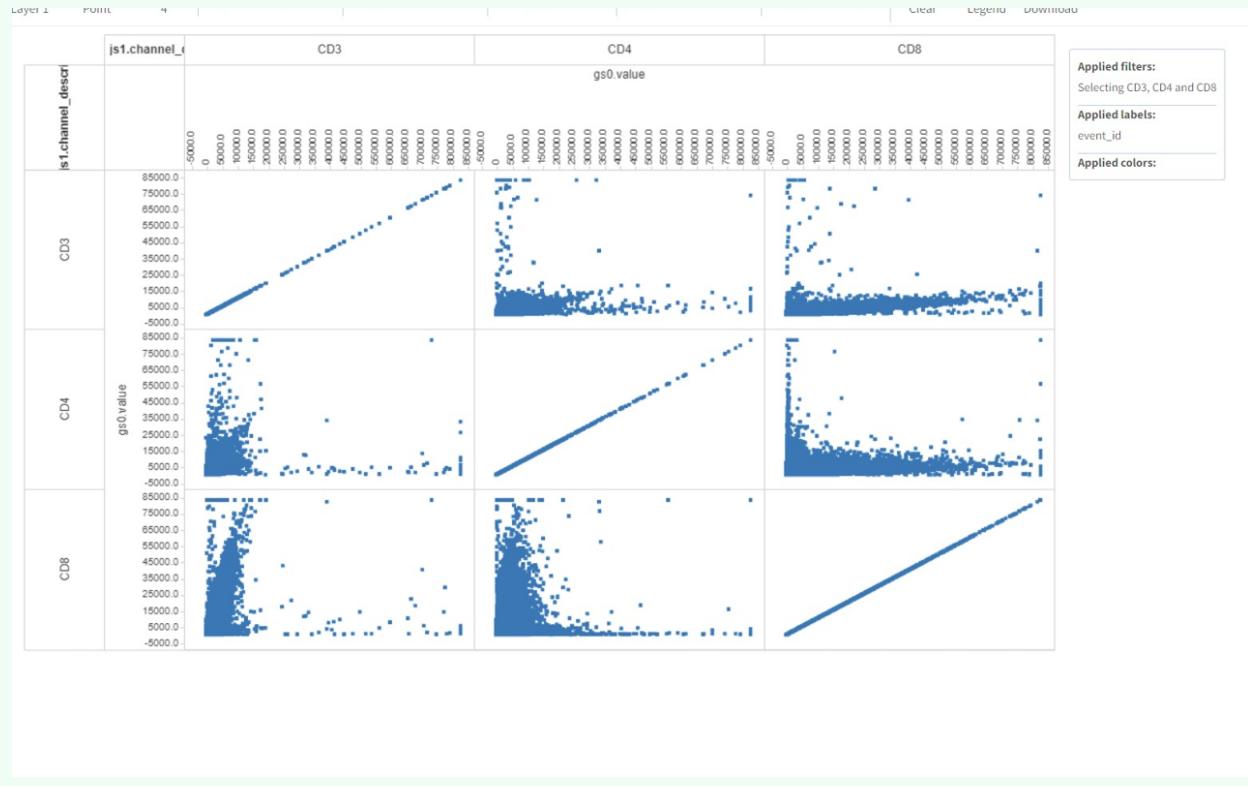




You have just created a pairwise plot in Tercen. Every dot is an event from the uploaded .FCS file(s).

It is far more useful though to create a summary of the data rather than looking at individual data points, where in many cases one will be falling on top of the other when they have similar values.

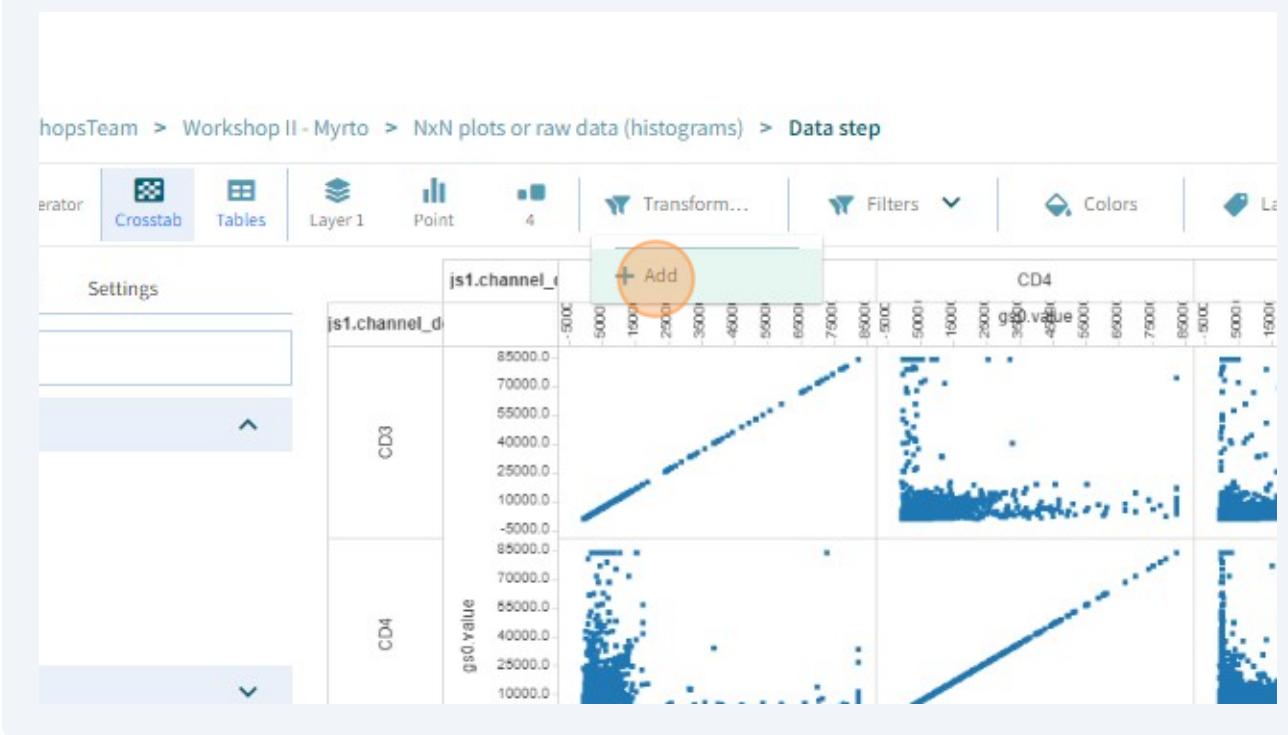
We will therefore display histograms, which provide a count summary on an interval scale and help illustrate the major features of the data distribution.



46

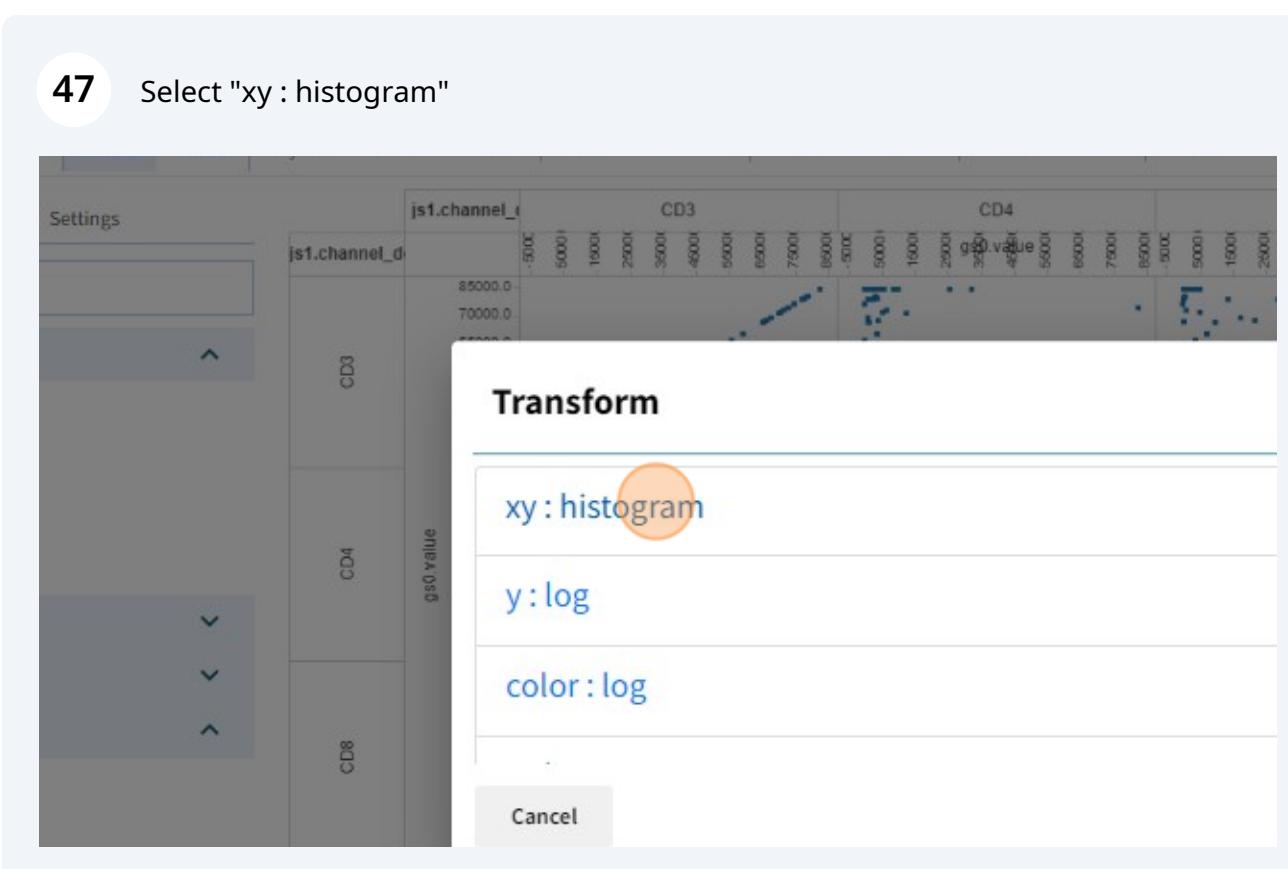
To transform the values visualized on the fly Tercen includes a "Transformation" tab on the toolbar.

Mouse over "Transform" and click "Add".

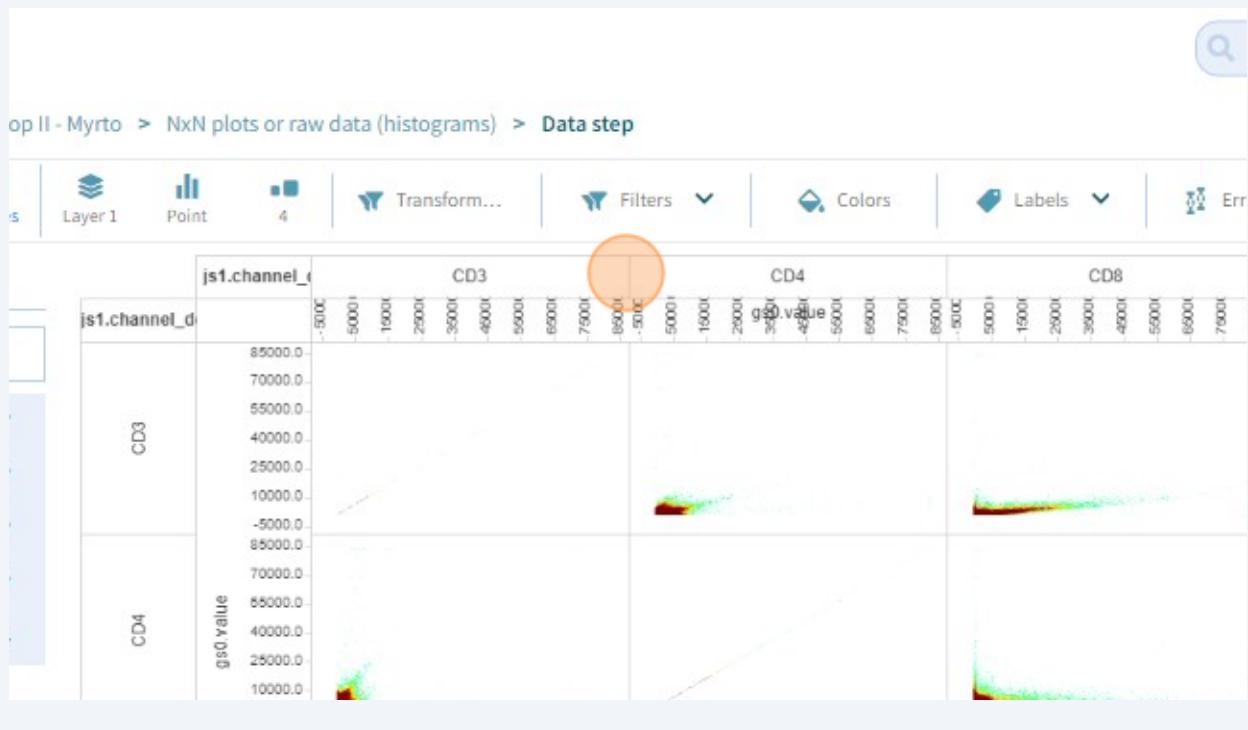


47

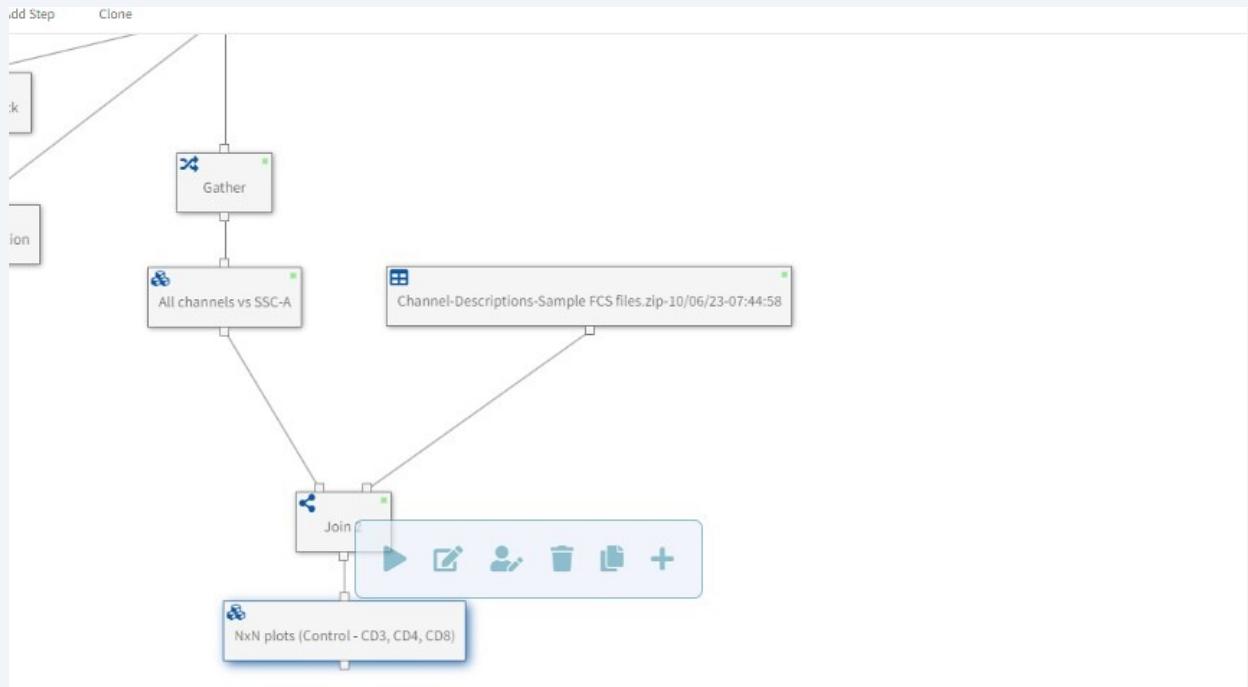
Select "xy : histogram"



48 Adjust the height of the X-axis as desired.

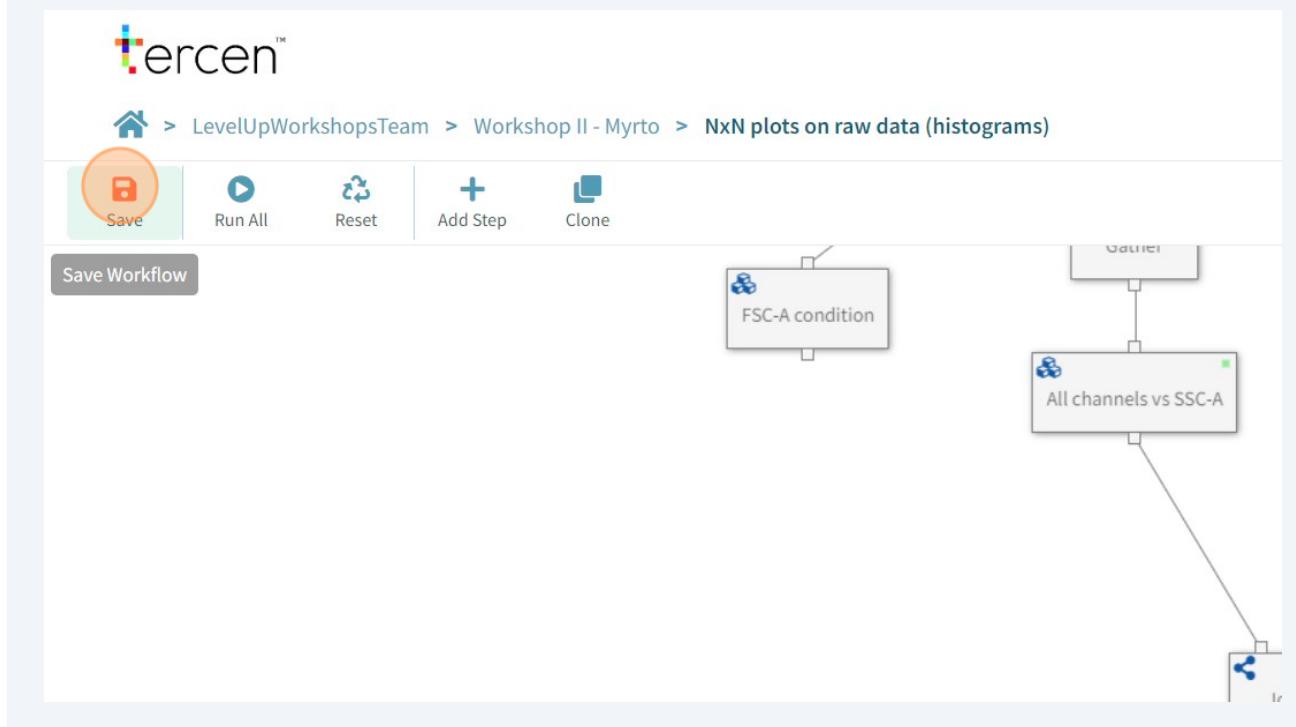


49 Go up to the workflow and rename the Data Step to "NxN plots (Control - CD3, CD4, CD8)"



50

Do not forget to save your progress by clicking on the Save button on the leftmost side of the toolbar.

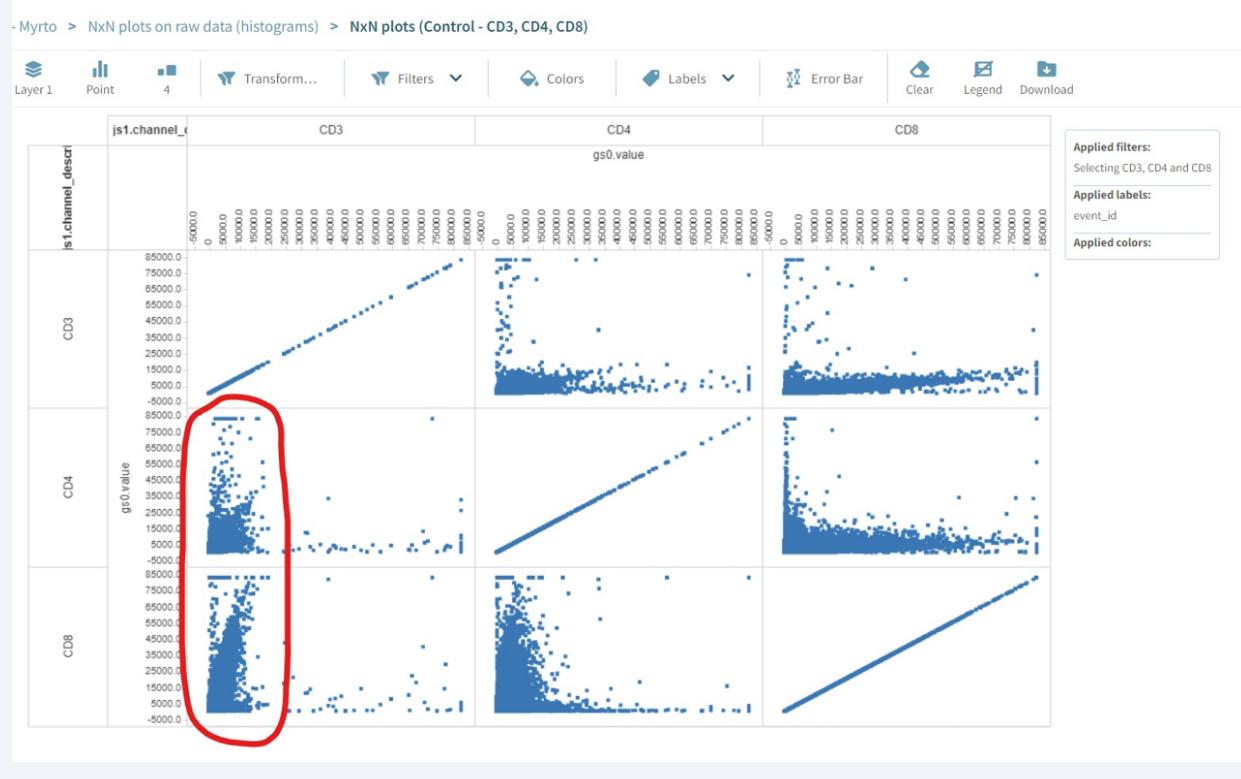


51

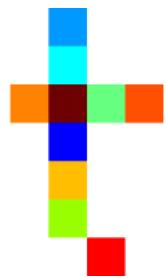
You have now created a pairwise plot of three channels against three channels. The raw FCS data is shown.

The raw data has negative values, and the data points are squashed on the axis.

To make this more useful, we will need to further transform the data. Stay tuned!



0206 - Transform Data



In this lesson, we will perform data transformation using the logic operator in Tercen.

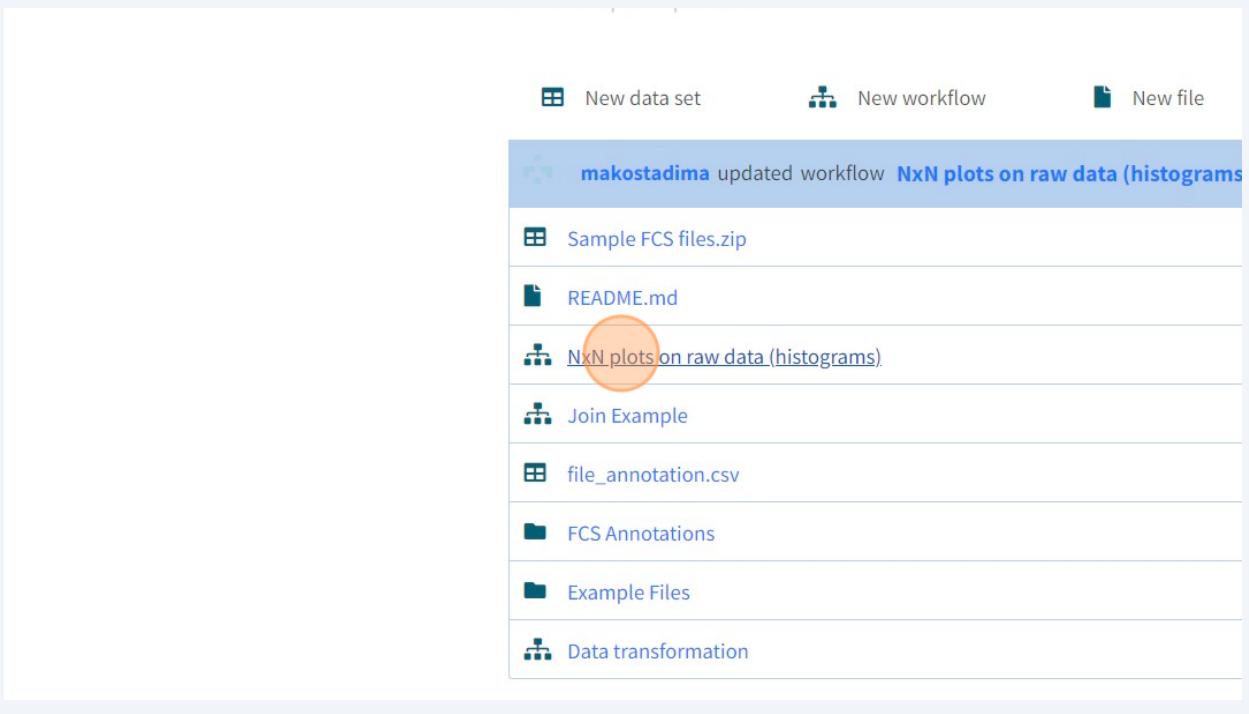
- 1 Click on your copy of the Workshop II Tercen project.

The screenshot shows the Tercen platform interface. At the top, there is a navigation bar with a home icon, the text 'LevelUpWorkshopsTeam > Workshop II - Myrto', and a search bar. Below the navigation bar, there is a header with a purple square icon and the text 'LevelUpWorkshopsTeam'. Underneath the header, there are two tabs: 'Project' (which is selected) and 'Activities'. The main content area displays a project titled 'Workshop II - Myrto' with the subtext 'No description provided.' Below the project title, there are three buttons: 'New data set' (with a data icon), 'New workflow' (with a workflow icon), and 'New file' (with a file icon). A blue banner at the bottom of the page indicates that 'makostadima updated workflow NxN plots on raw data (histograms)'.



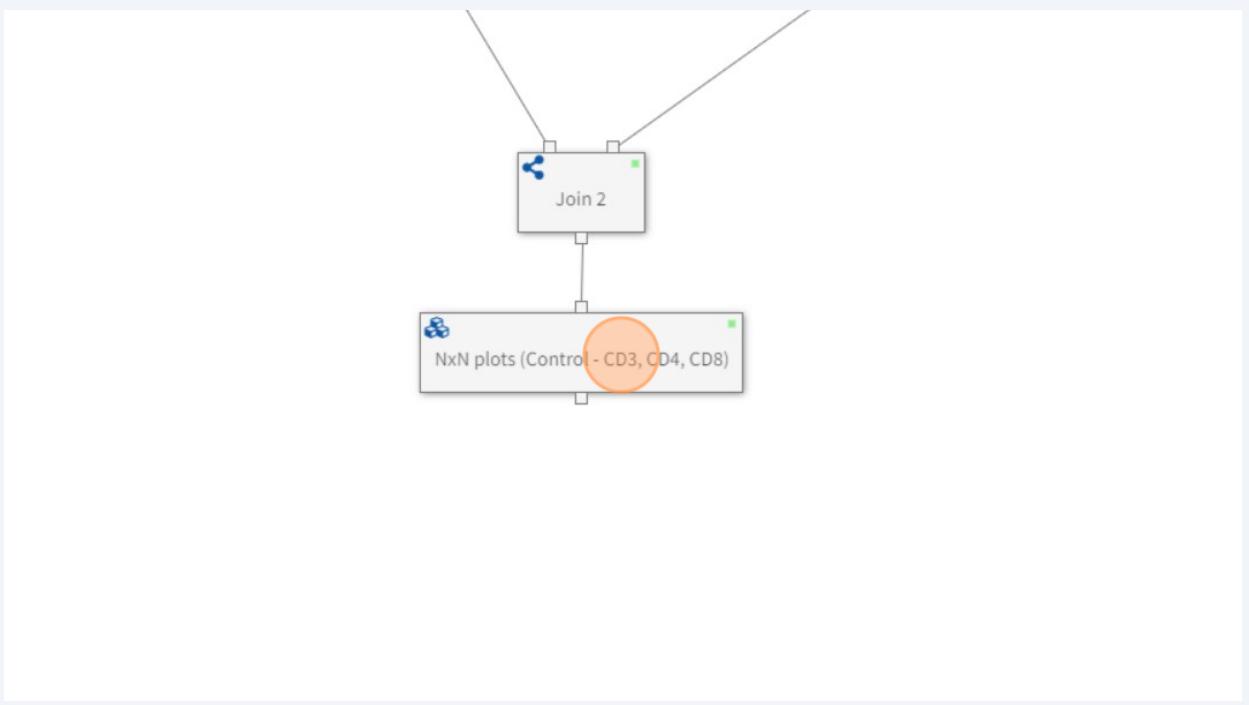
For this lesson we will continue working on the workflow from the previous guide, called **NxN plots on raw data (histograms)**.

- 2 Click "NxN plots on raw data (histograms)".

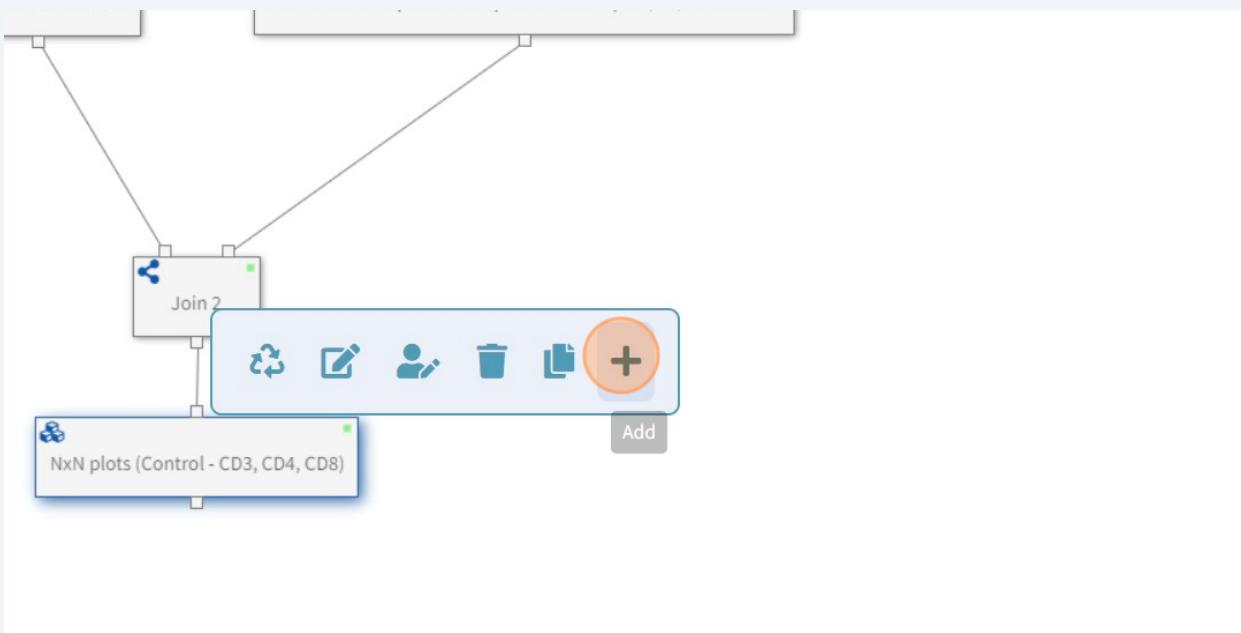


- 3 Navigate down to the last data step created, called **NxN plots (Control - CD3, CD4, CD8)**.

Click on this step so that the local toolbar appears over it.



4 Select **Add**.



5 Select "Data step".

Add

Step Operator Operator Library Installed Apps App Library

Search

Data step data

Perform computation on user defined projection



Multi data step data

Perform computation on user defined projection



Join leftTable

Join two data sets

- 6 Clear the preselected factor on the Columns by clicking on the 'x' on the top left corner when you mouse over, as shown below.

The screenshot shows the Tercen software interface with the following details:

- Top Bar:** Shows the Tercen logo, navigation path: Home > LevelUpWorkshopsTeam > Workshop II - Myrto > NxN plots on raw data (histograms) > Data step.
- Toolbar:** Includes Save, Add Operator, Crosstab (selected), Tables, Layer 1, Point, 4, Transform..., Filters, and a search bar.
- Left Panel:** Factors section with a search bar and a list of factors:
 - Sample FCS files.zip
 - Join
 - Gather (highlighted with an orange circle)
 - All channels vs SSC-A
 - Join 2
 - NxN plots (Control - CD3, CD4, CD8)
- Right Panel:** A grid for creating NxN plots. The first row contains 'js1.channel_d' and 'CD3'. The second row contains 'CD4'. The third row contains 'CD8'.

- 7 Click to expand the factors under the "Gather" step.

The screenshot shows the Tercen software interface with the following details:

- Top Bar:** Shows the Tercen logo, navigation path: Home > LevelUpWorkshopsTeam > Workshop II - Myrto > NxN plots on raw data (histograms) > Data step.
- Toolbar:** Includes Save, Add Operator, Crosstab (selected), Tables, Layer 1, Point, 4, Transform..., Filters, and a search bar.
- Left Panel:** Factors section with a search bar and a list of factors:
 - Sample FCS files.zip
 - Join
 - Gather (highlighted with an orange circle)
 - All channels vs SSC-A
 - Join 2
 - NxN plots (Control - CD3, CD4, CD8)
- Right Panel:** A grid for creating NxN plots. The first row contains 'js1.channel_d' and 'CD3'. The second row contains 'CD4'. The third row contains 'CD8'.

- 8 Drag and drop the **gs0.value** factor to Y-axis.

The screenshot shows the 'Factors' tab of a data step interface. On the left, there's a search bar and a list of factors. The 'Sample FCS files.zip' factor is expanded, showing its contents. Under the 'Gather' section, 'gs0.value' is selected and highlighted with an orange circle. To the right, a table titled 'js1.channel_d' lists categories: CD3, CD4, and CD8. Each category has a corresponding row in the table.

- 9 Expand the "Sample FCS files.zip" factors by clicking on it on the Factors list.

The screenshot shows the 'Factors' tab of a data step interface. The 'Sample FCS files.zip' factor is expanded, showing its contents. The entry 'gs0.value' is highlighted with an orange circle. To the right, a table titled 'js1.channel_d' lists categories: CD3, CD4, and CD8. Each category has a corresponding row in the table.

10 Drag and drop the **event_id** factor to Column.

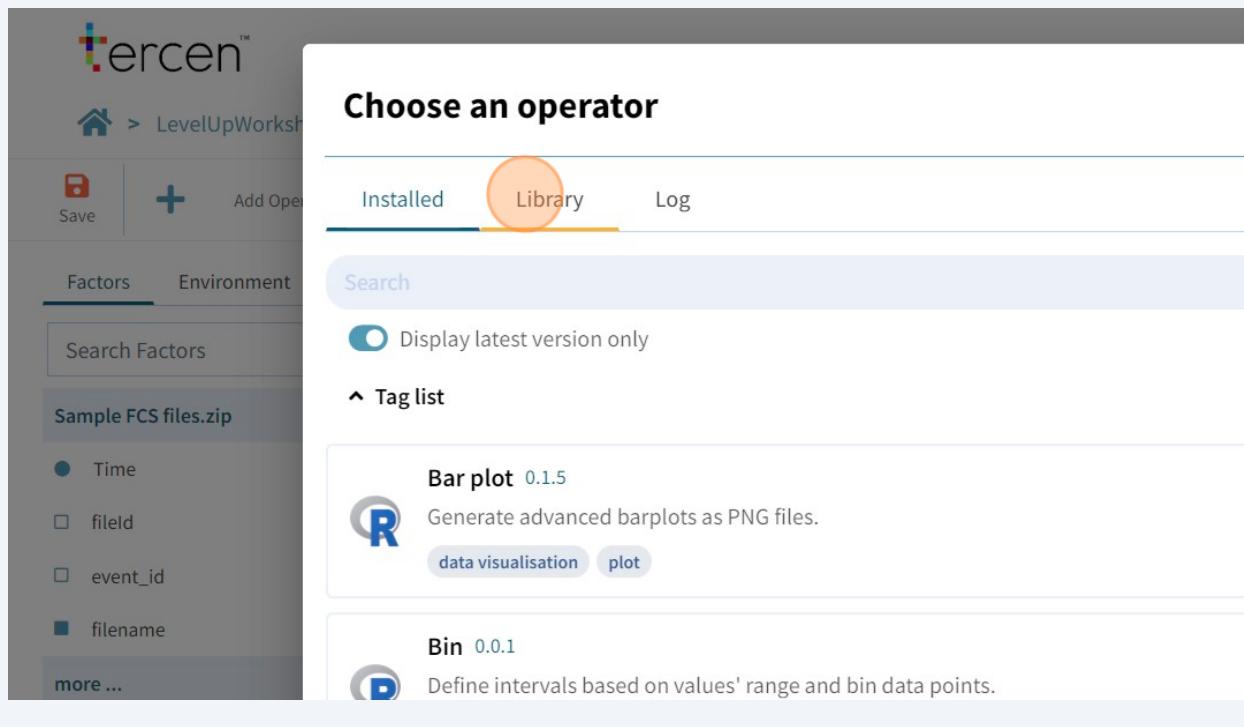
The screenshot shows the tercen software interface. On the left, the 'Factors' tab is selected. A list of factors includes 'Time', 'fileId', 'event_id' (highlighted with an orange circle), and 'filename'. Below this is a 'more ...' section with 'Join' and 'Gather' options. The 'Gather' section shows 'gs0.value' and 'gs0.variable'. To the right is a table with columns 'js1.channel_d' and 'gs0.value'.

11 You will then add the operator that performs the logic transformation.

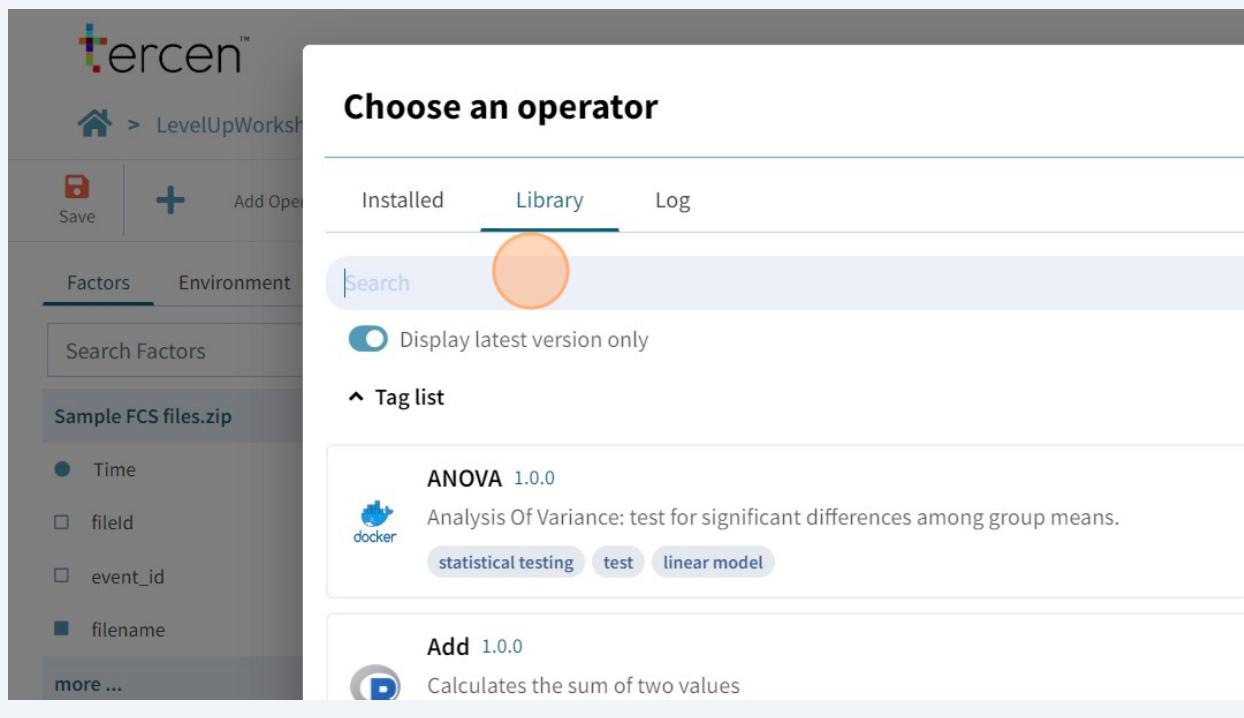
Click the '+' sign next to the Add Operator tab of the toolbar.

The screenshot shows the tercen software interface with the 'Add Operator' tab selected. The toolbar includes 'Save', 'Add Operator' (highlighted with an orange circle), 'Crosstab', 'Tables', 'Layer 1', 'Point', 'Transform...', and 'Filters'. Below is a table with columns 'event_id', 'js1.channel_d', 'CD3', 'CD4', and 'Y.value'. The 'CD3' column shows a histogram-like distribution of event_id values.

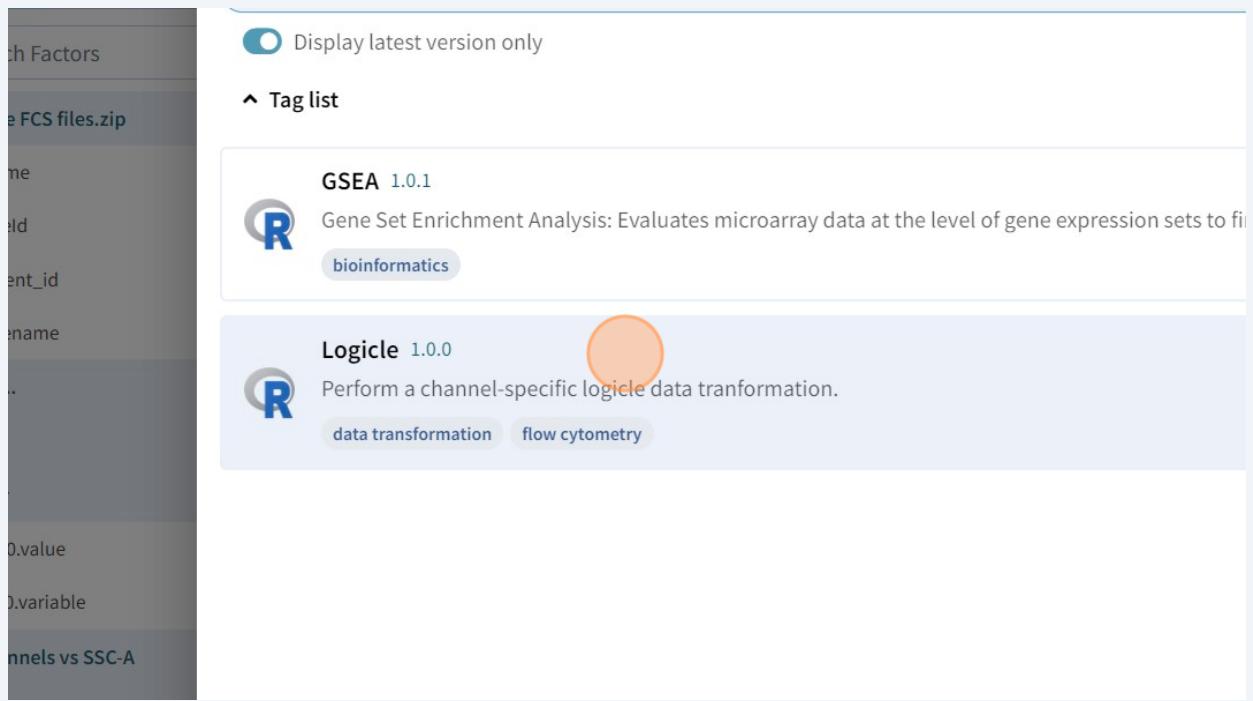
12 Click "Library".



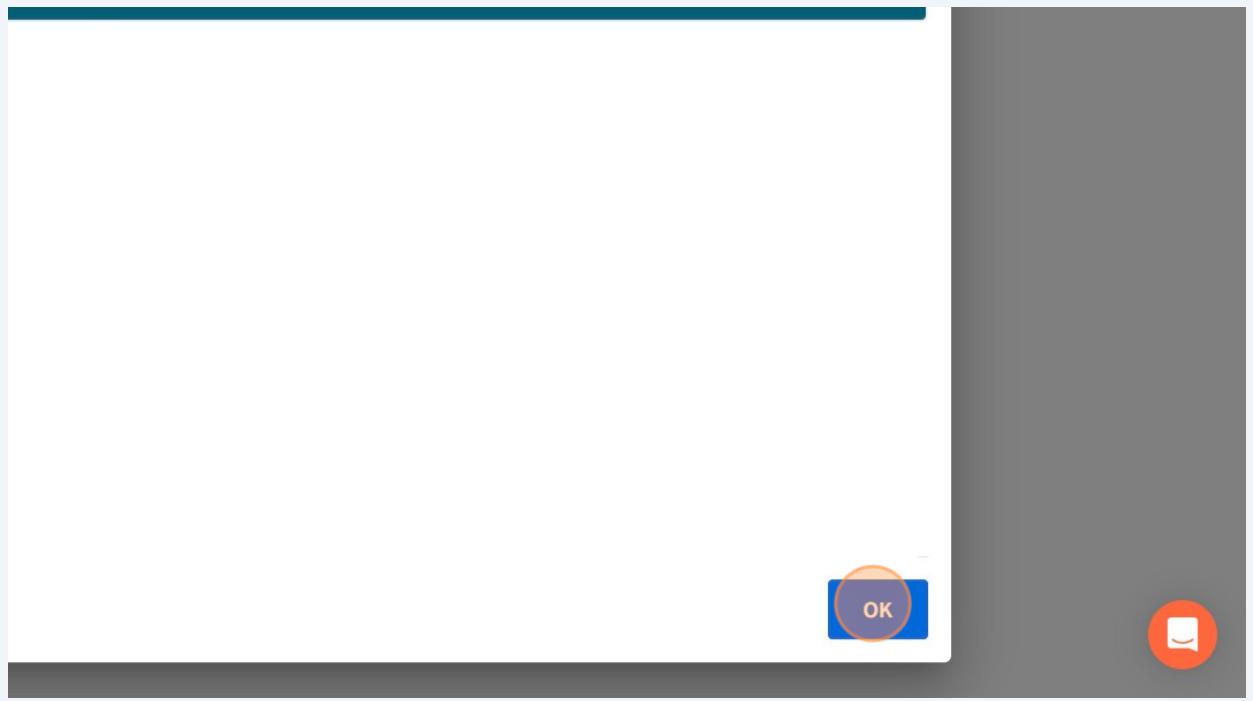
13 In the Search field type 'logic'.



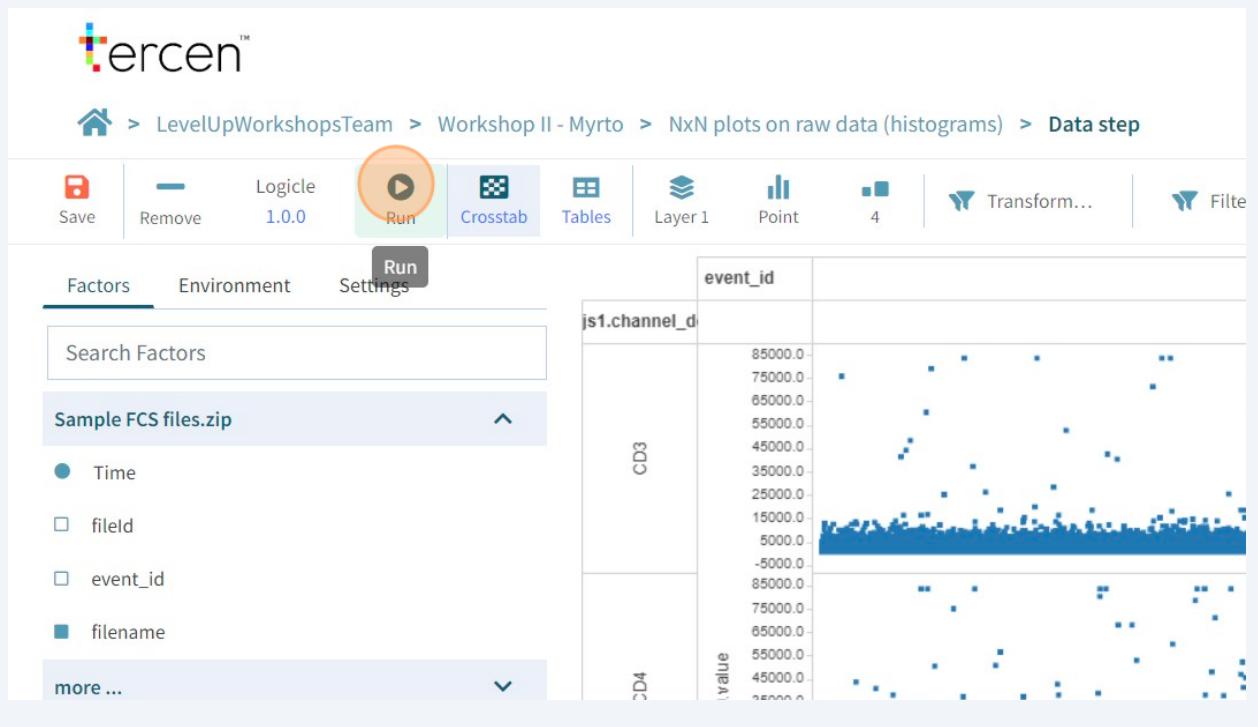
14 Select the **Logicle** operator.



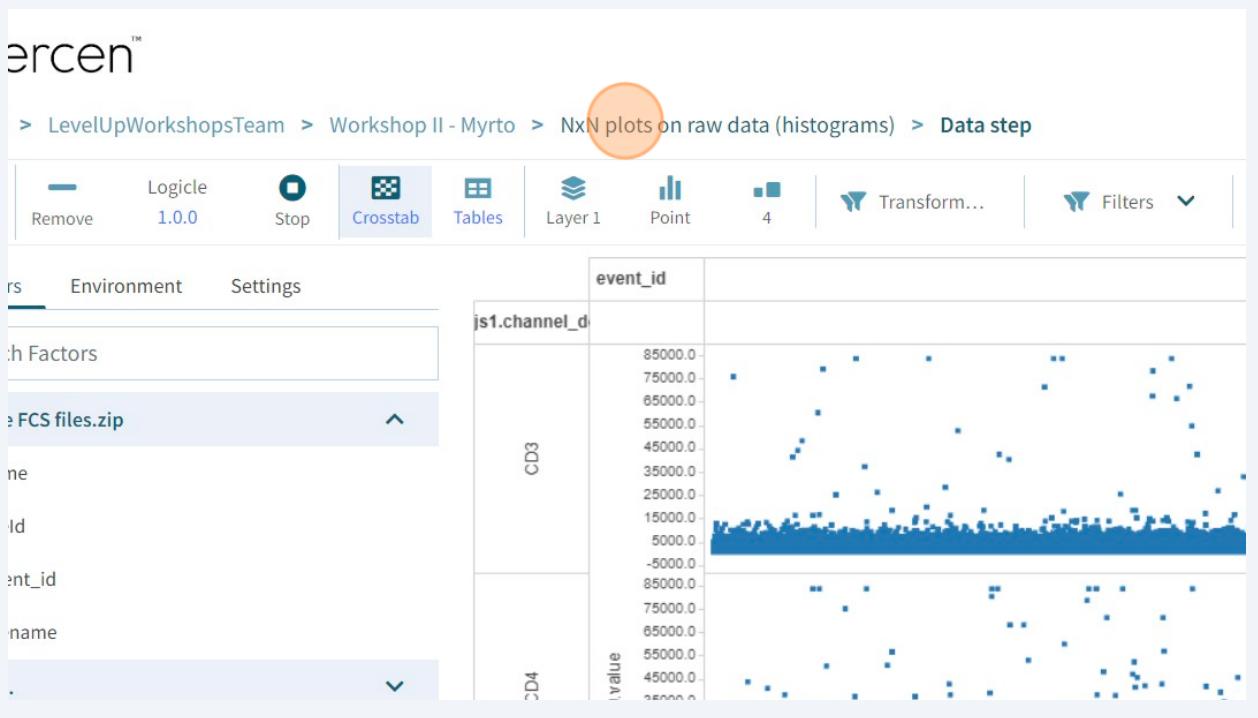
15 Click "OK"



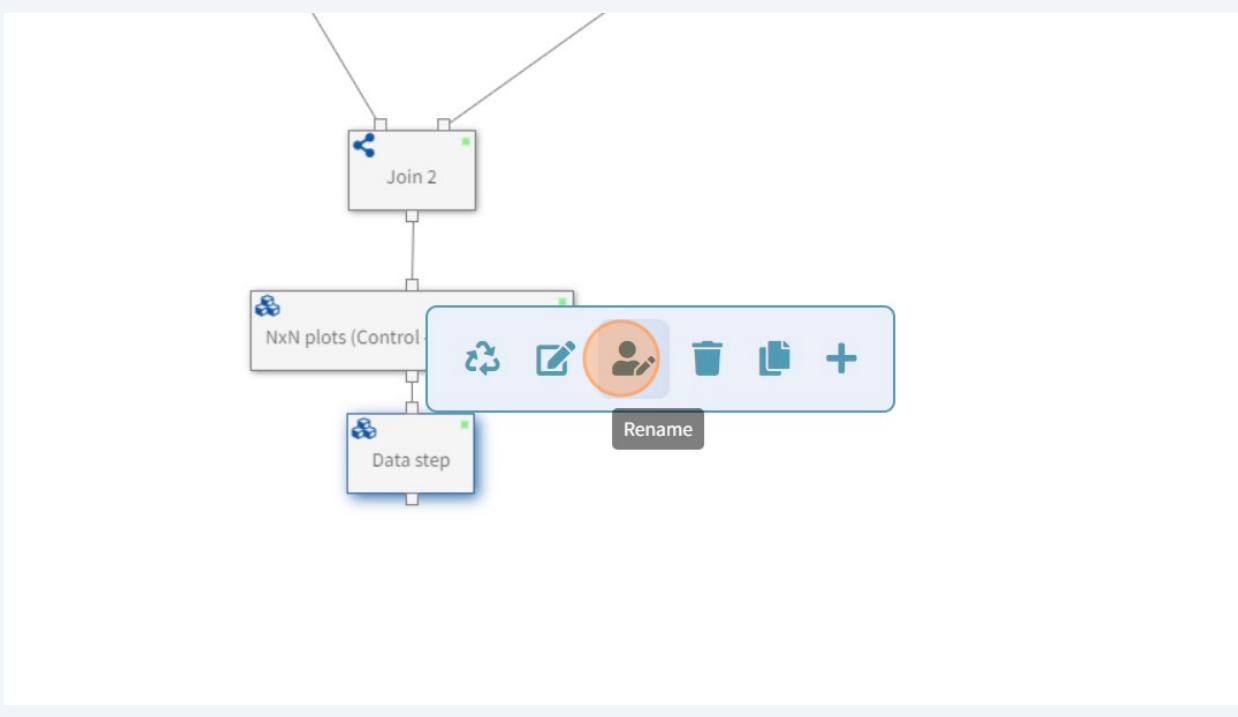
16 Click 'Run'.



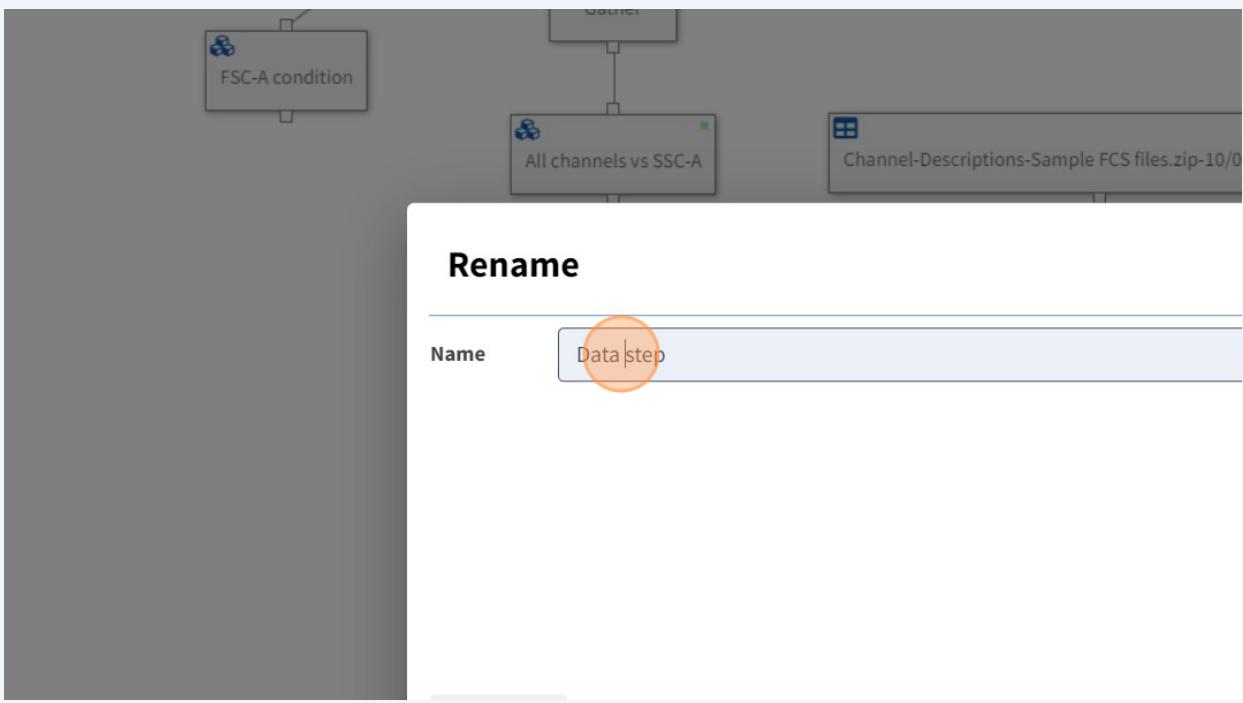
17 Click "NxN plots on raw data (histograms)" to return to the workflow.



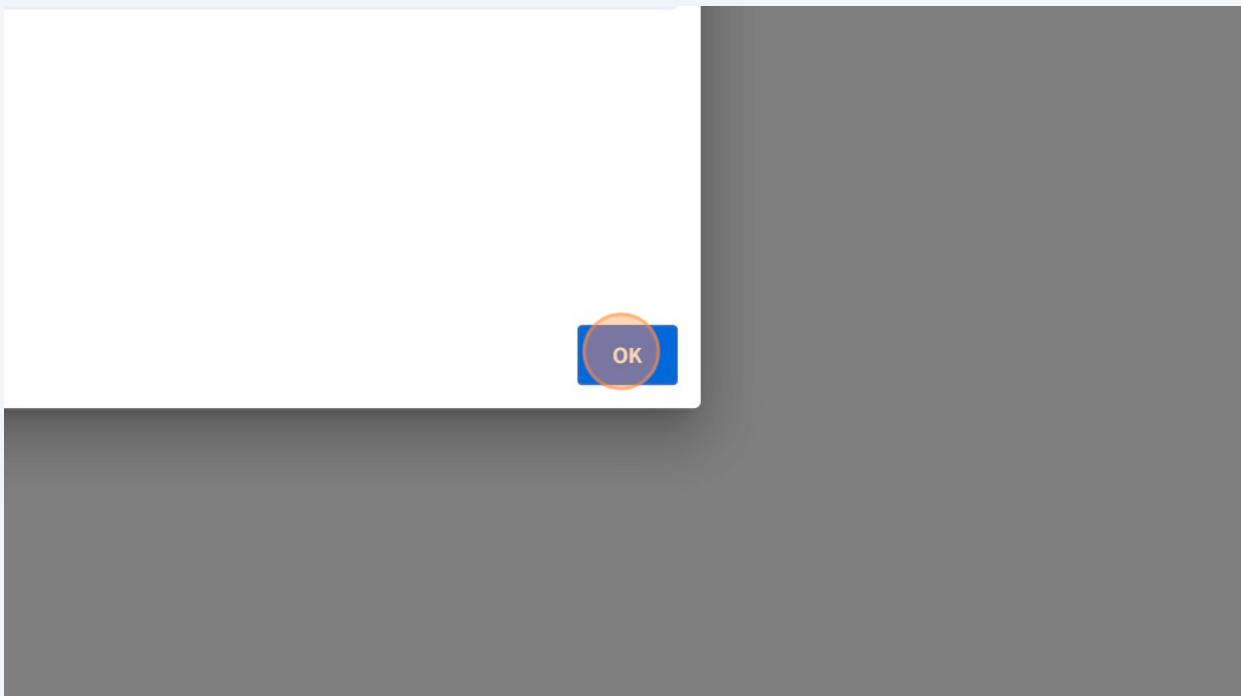
18 Click 'Rename'



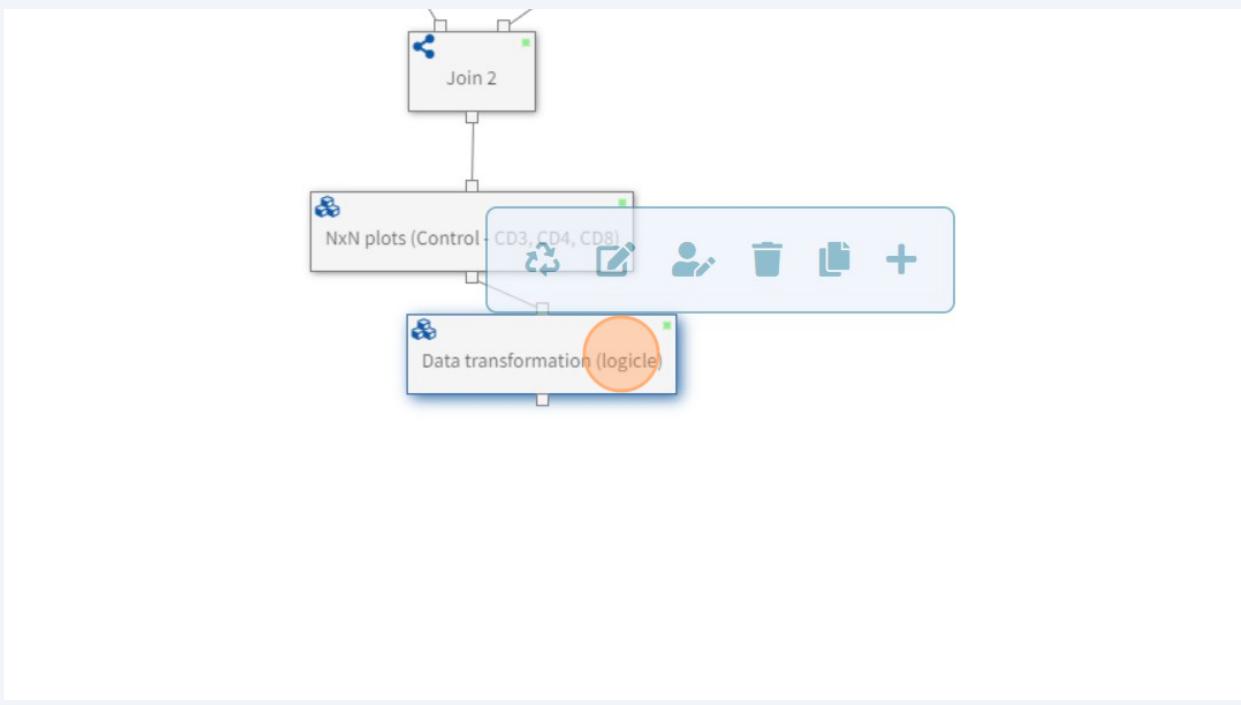
19 Type "Data transformation (logicle)" in the name text box.



20 Click "OK"



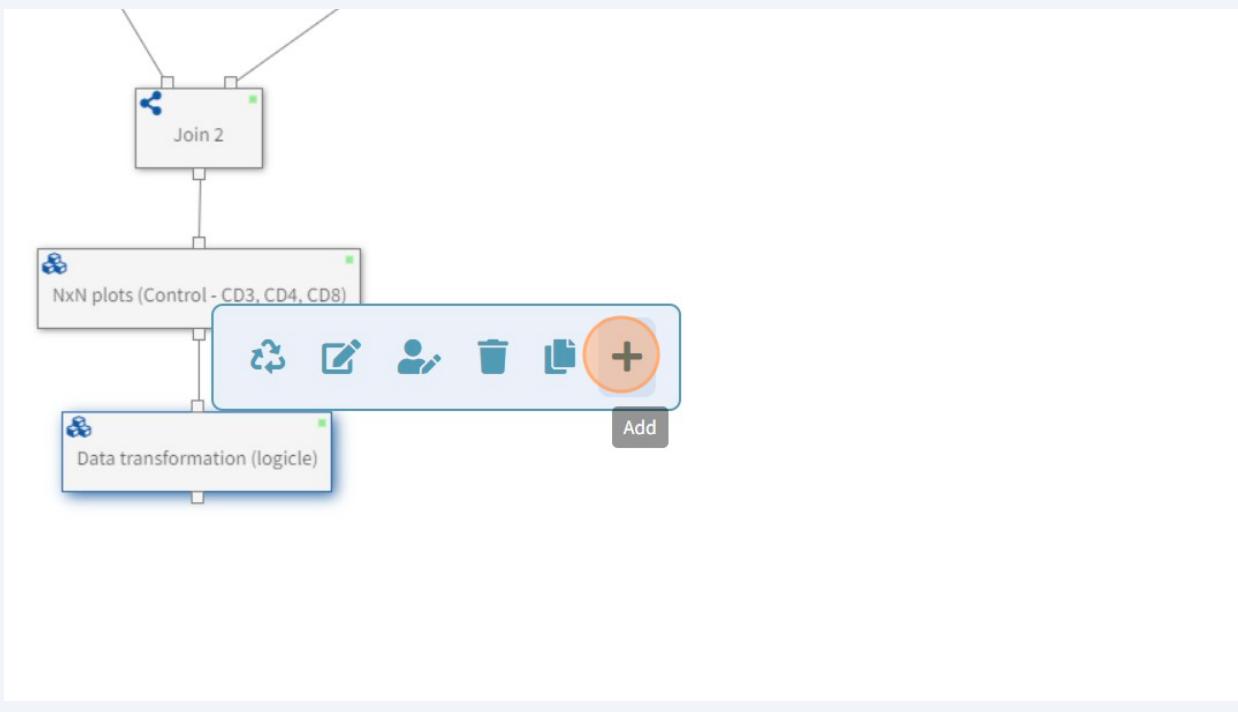
21 (OPTIONAL) If you need to order like me, drag and drop the data step to ensure it aligns perfectly with the previous one.



22

Now let us investigate how the logic transformation has modified our Channels vs Channels ($N \times N$) plots.

Click "Add".



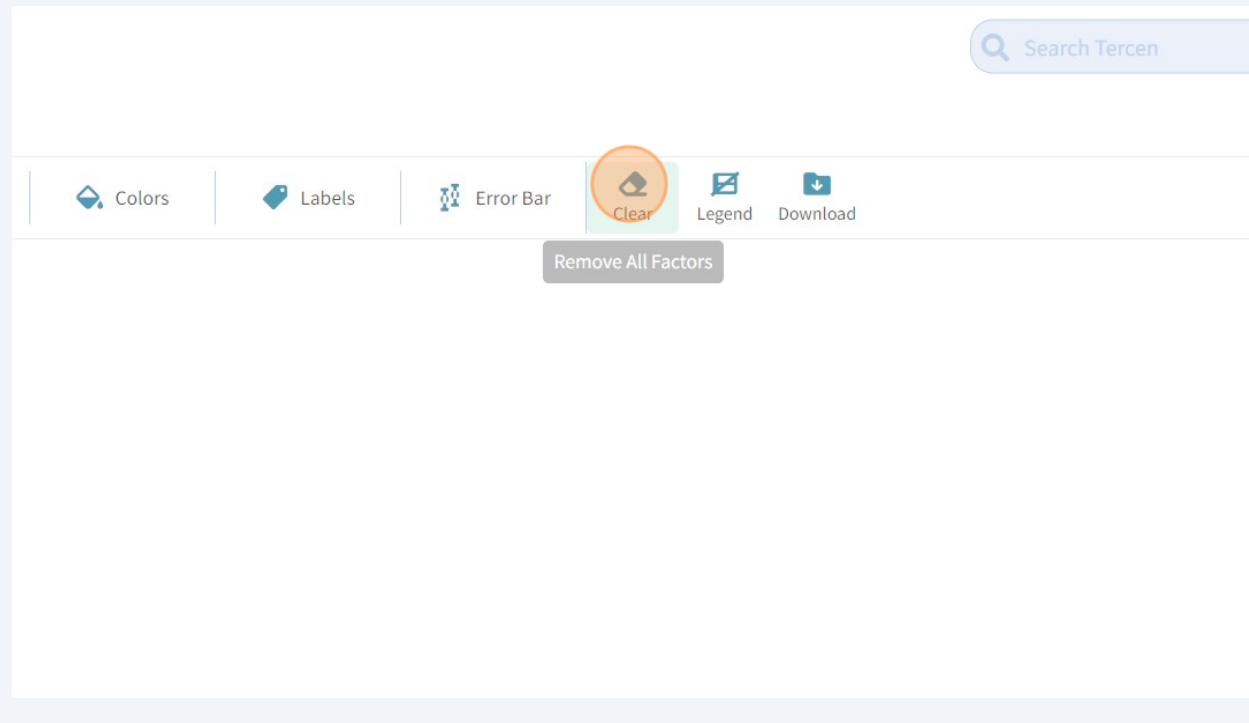
23

Select "Data step (data)"

Step	Operator	Operator Library	Installed Apps	App Library
Data step data				
Multi data step data				
Join leftTable				

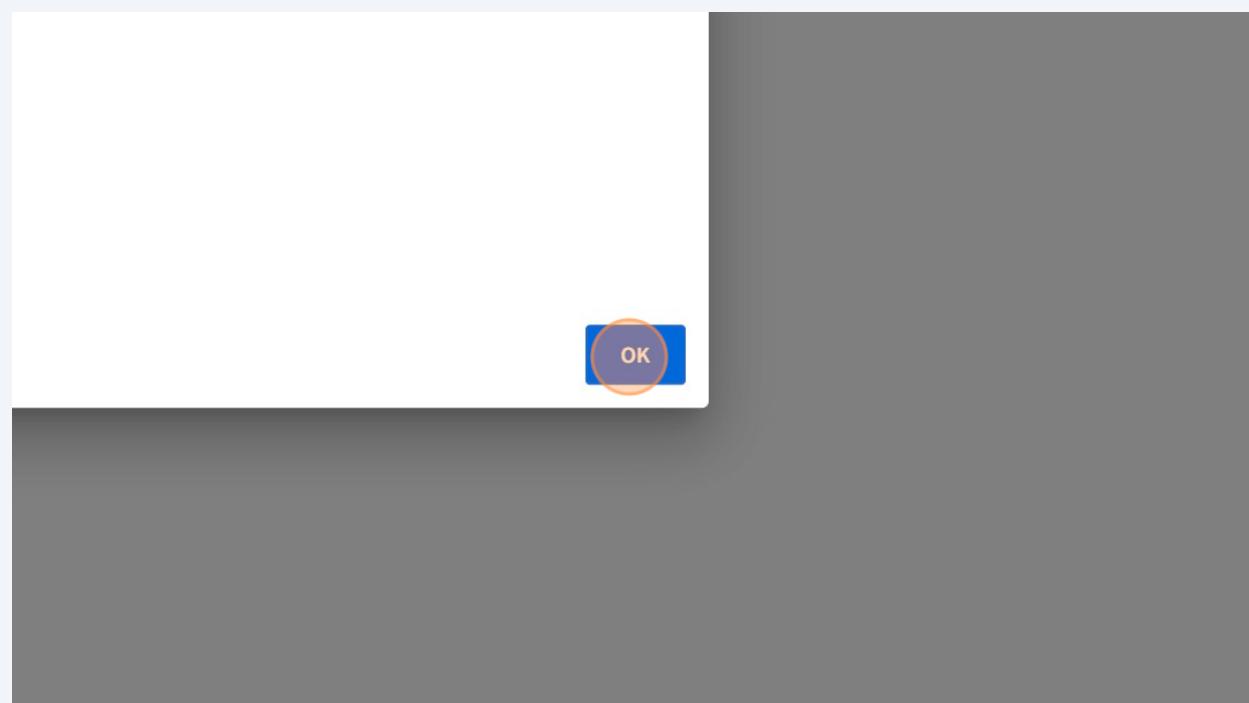
24

Clear any pre-defined data projections by clicking the "Clear" button on the toolbar.



25

Click "OK"



26 Drag and drop the **ds4..channel_description** factor to Row.

All channels vs SSC-A

Join 2

NxN plots (Control - CD3, CD4, CD8)

Data transformation (logicle)

- ds4.value
- ds4.event_id
- ds4.channel_description

27 Again, drag and drop the ds4..channel_description factor to Column this time.

All channels vs SSC-A

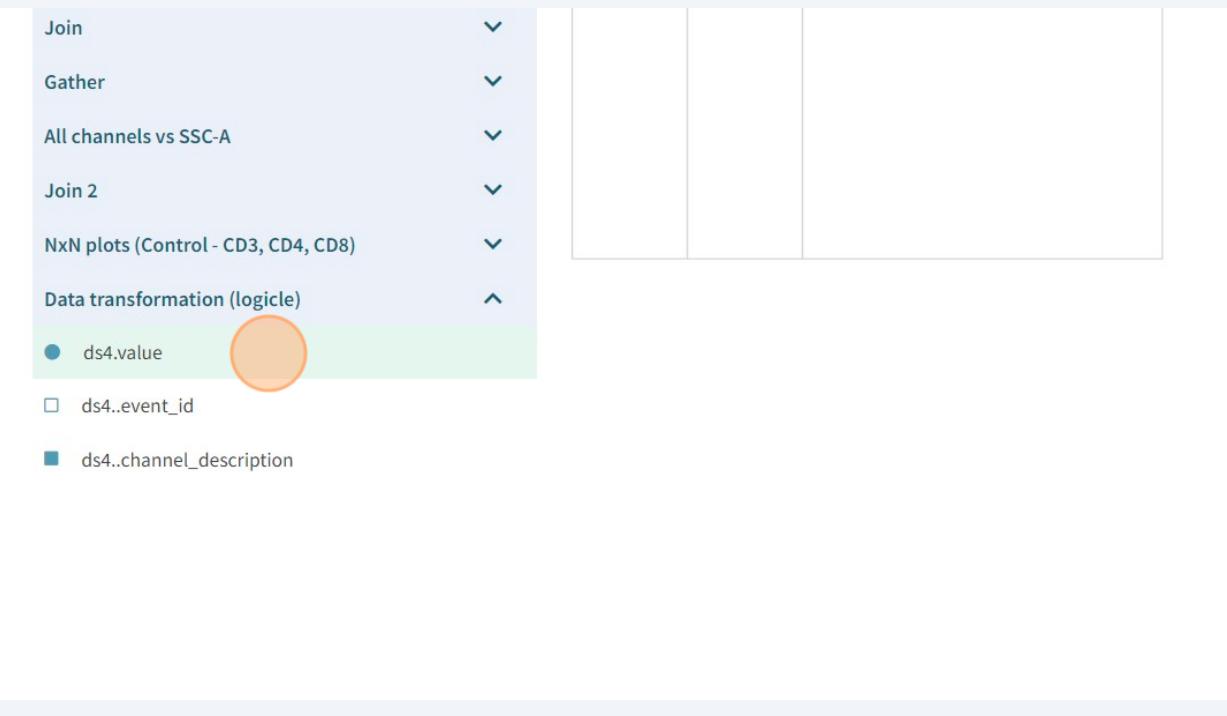
Join 2

NxN plots (Control - CD3, CD4, CD8)

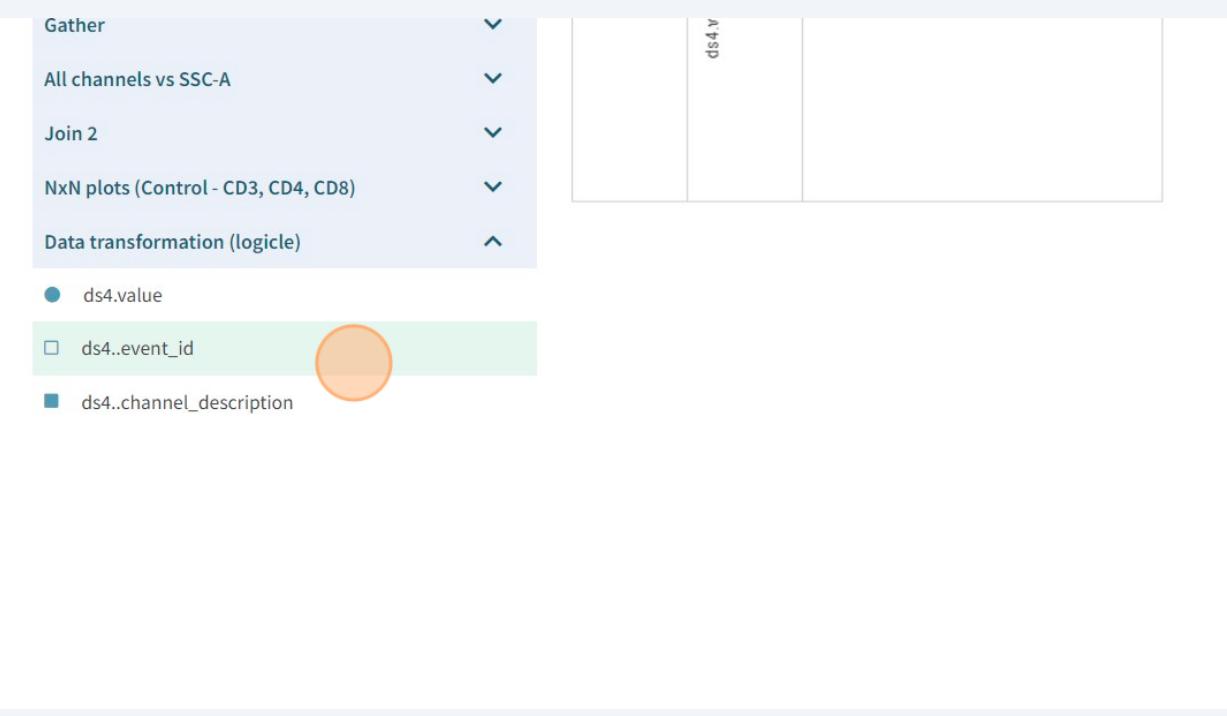
Data transformation (logicle)

- ds4.value
- ds4.event_id
- ds4.channel_description

28 Drag and drop the **ds4.value** factor to Y-axis.



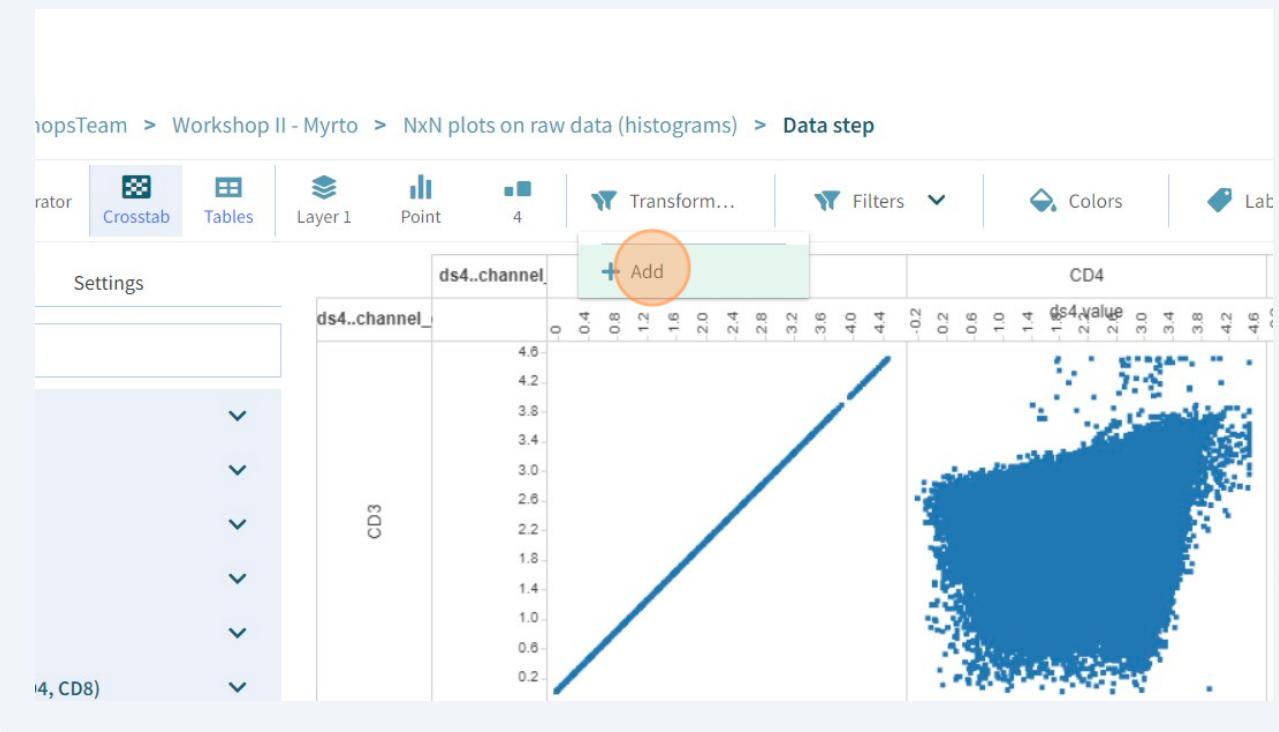
29 Drag and drop the **ds4..event_id** factor to Labels.



30

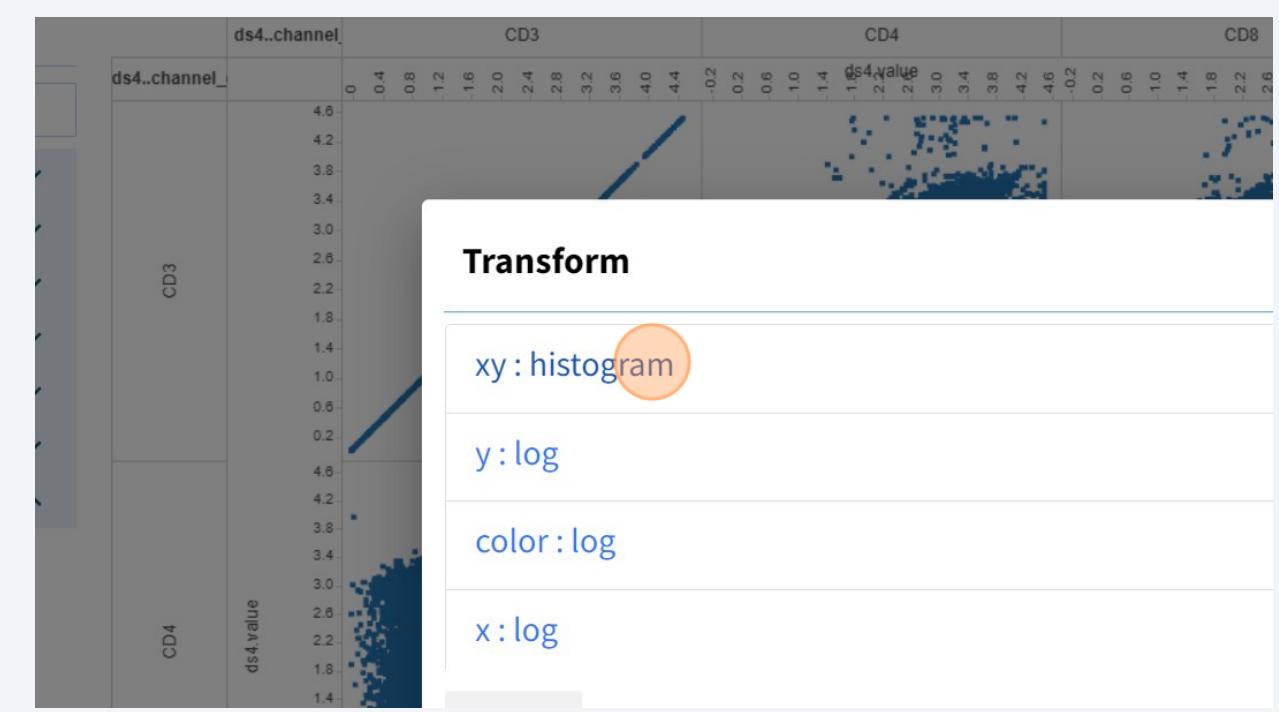
Again, we will add the histogram transformation to the data in order to visualize them.

Mouse over "Transform" and click "Add".



31

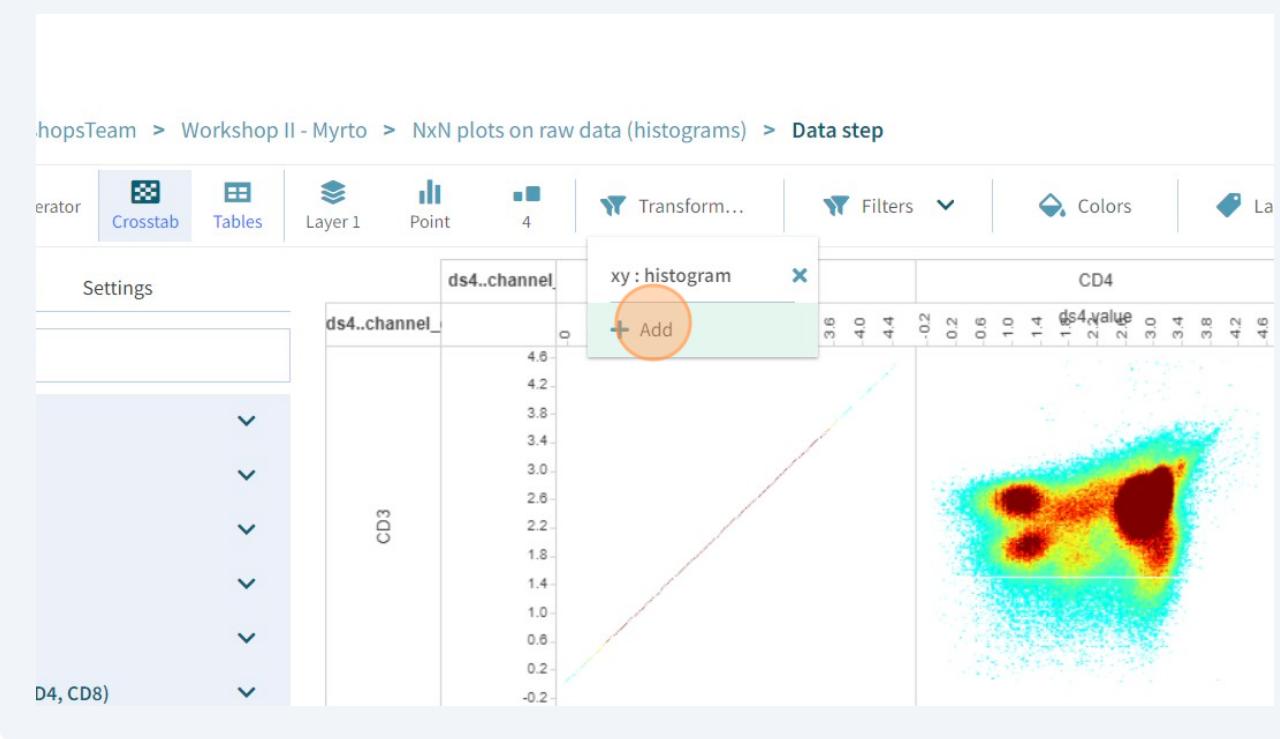
Select "xy : histogram"



32

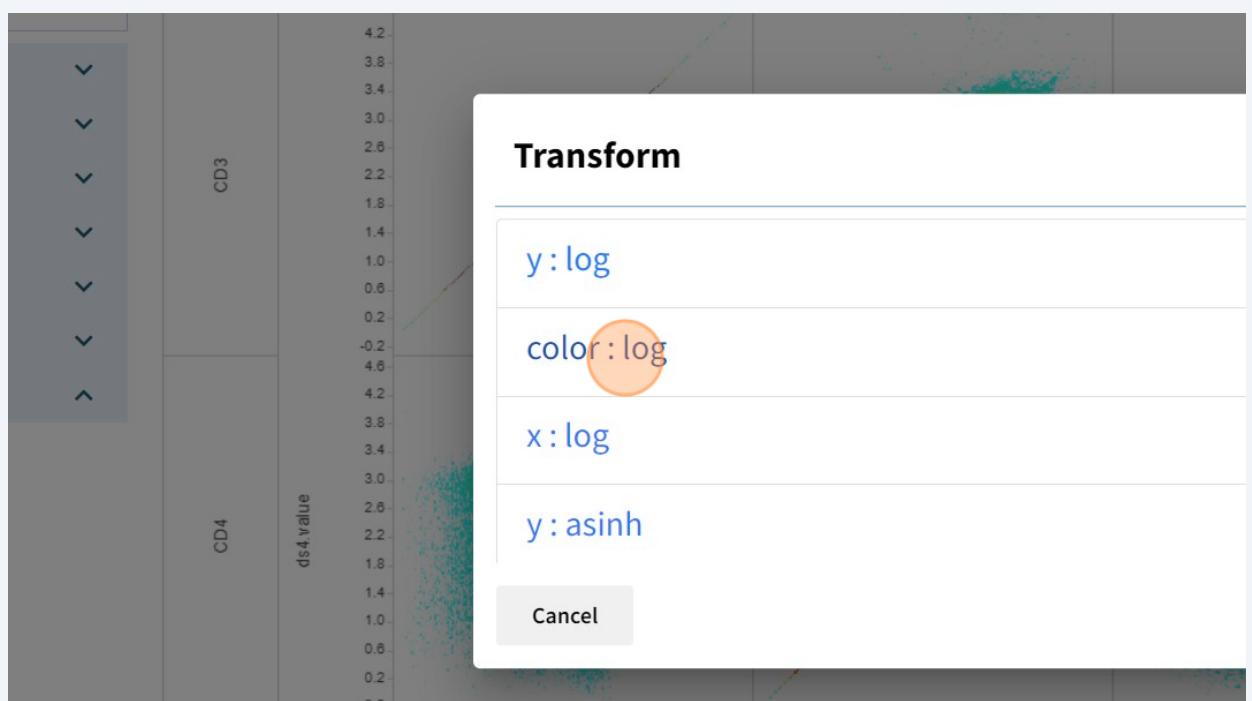
In this lesson, we will also see how we can transform the counts per bin (represented by the color).

Again, mouse over "Transform" and click "Add".

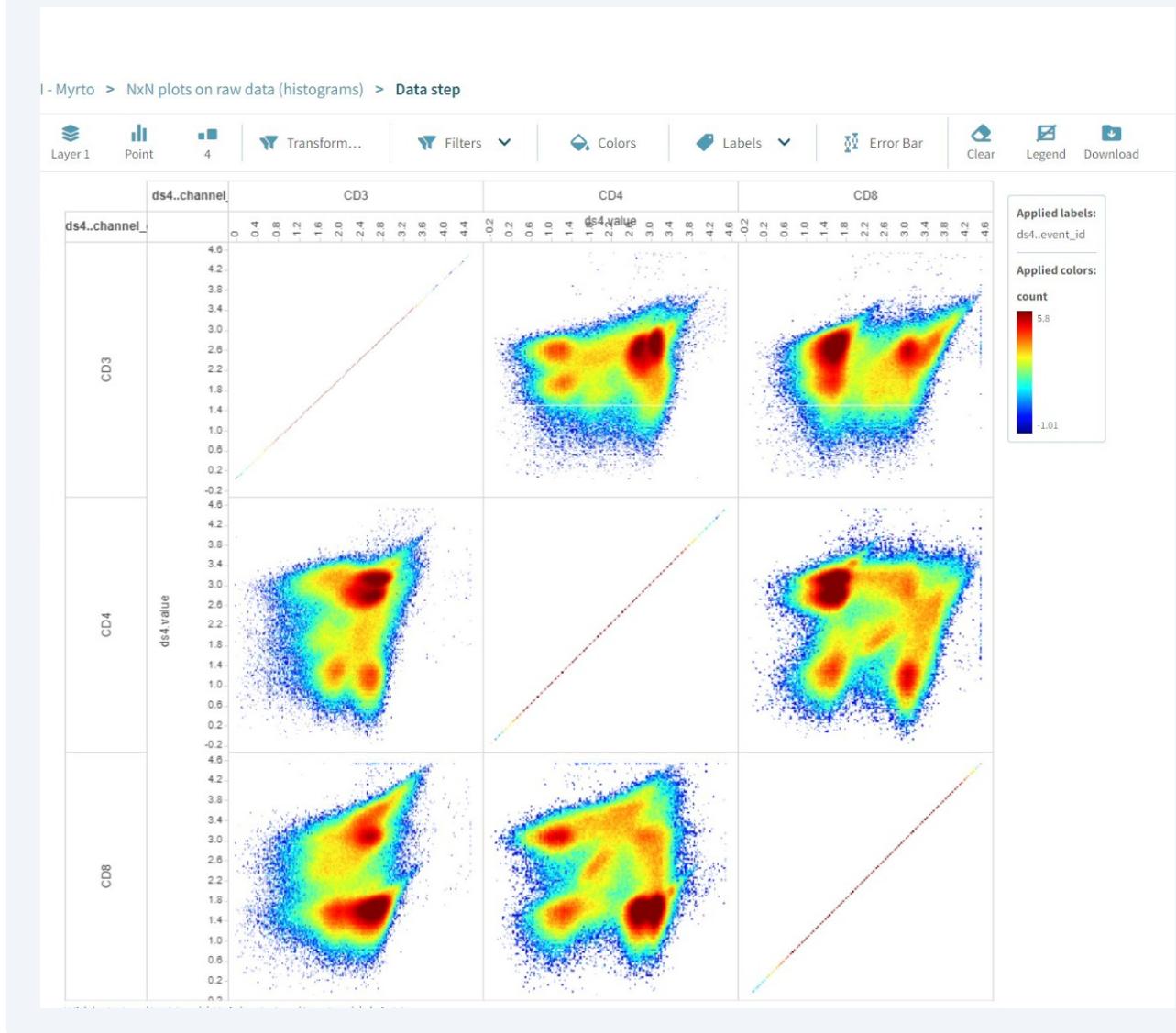


33

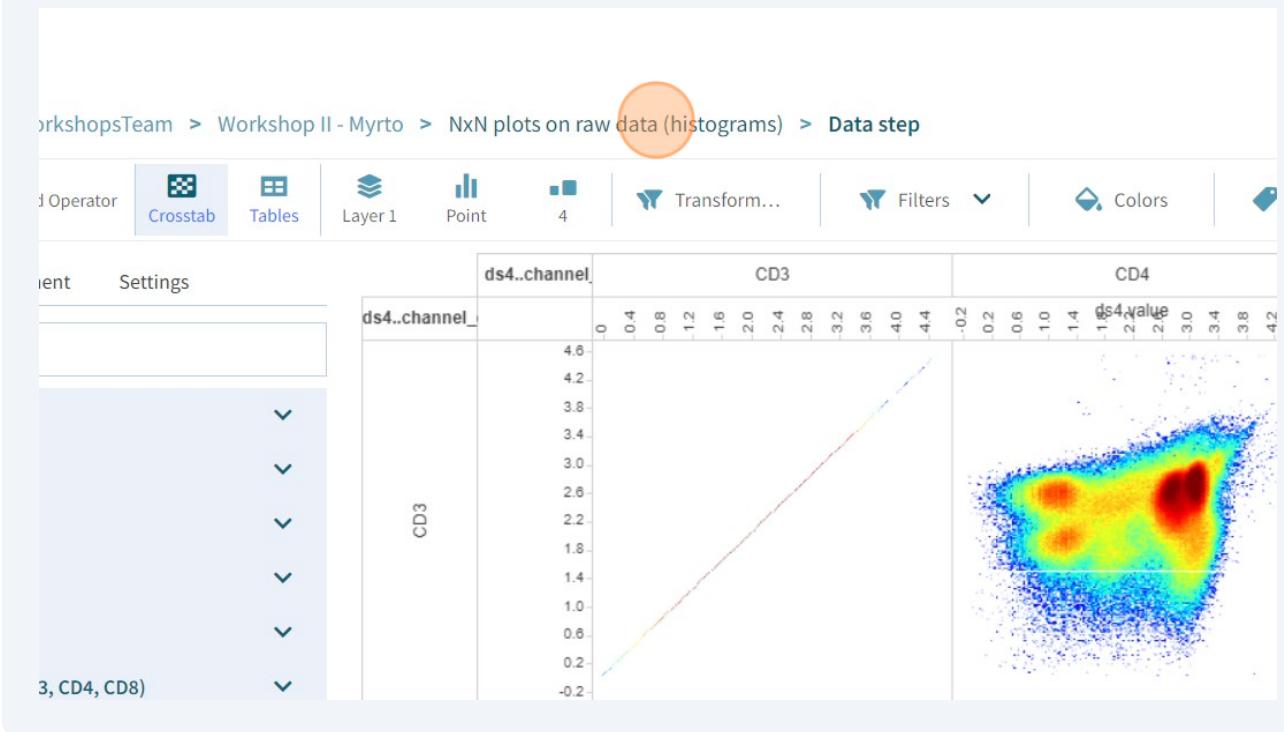
Select "color : log".



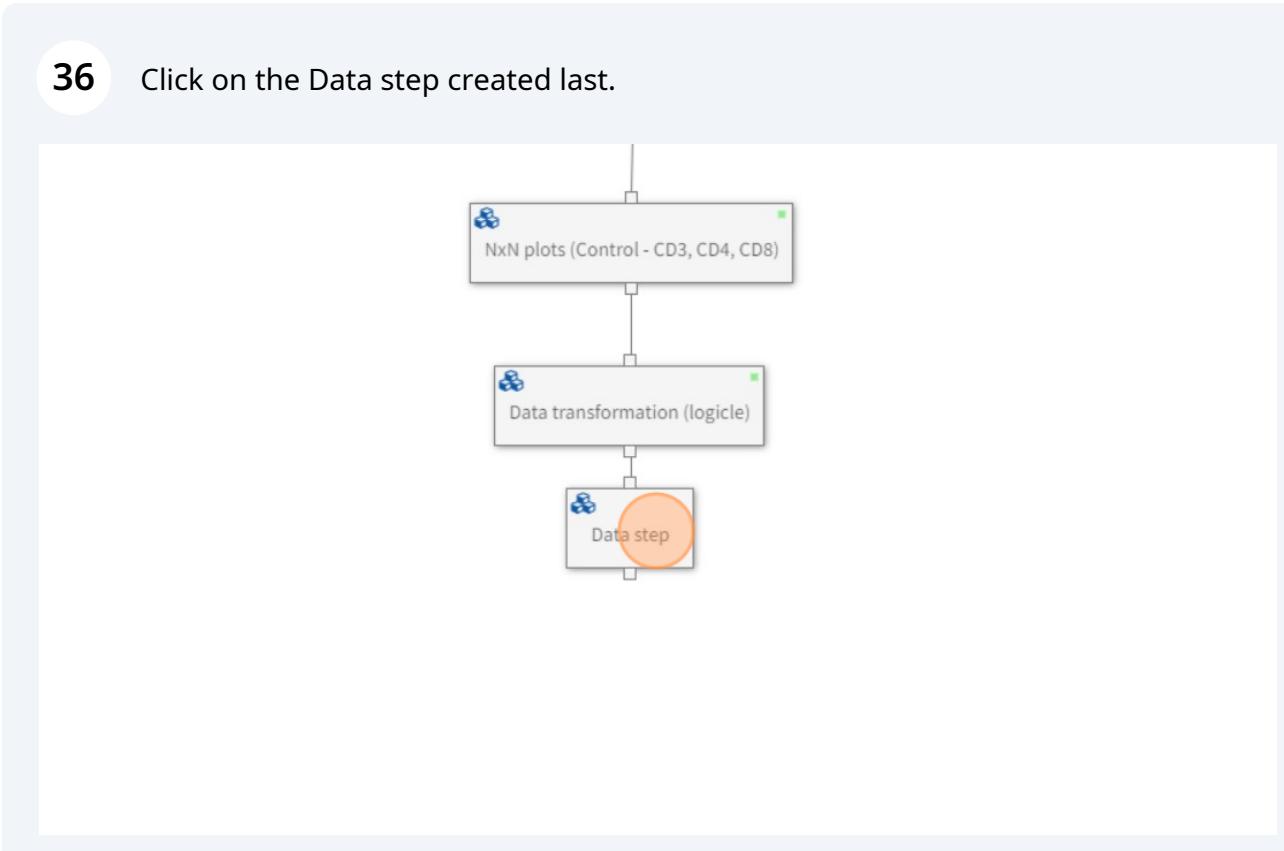
34 Observe how the colors have changed.



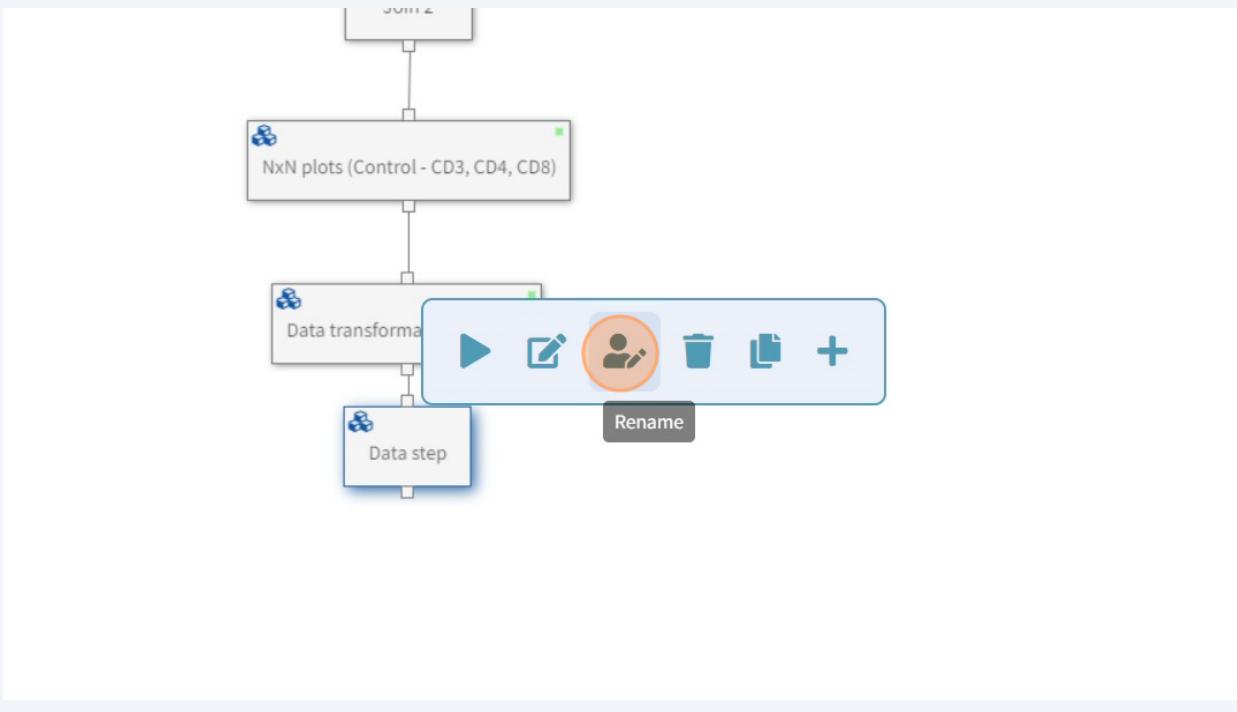
35 Navigate to the workflow by clicking "NxN plots on raw data (histograms)".



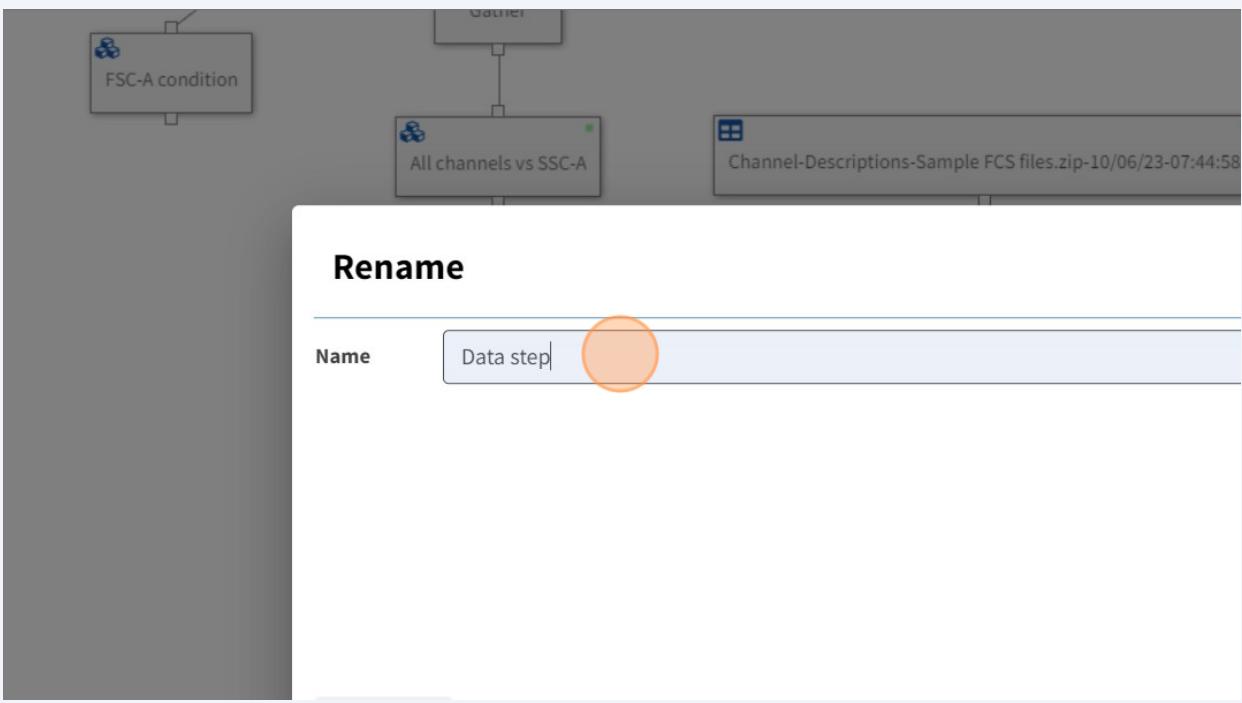
36 Click on the Data step created last.



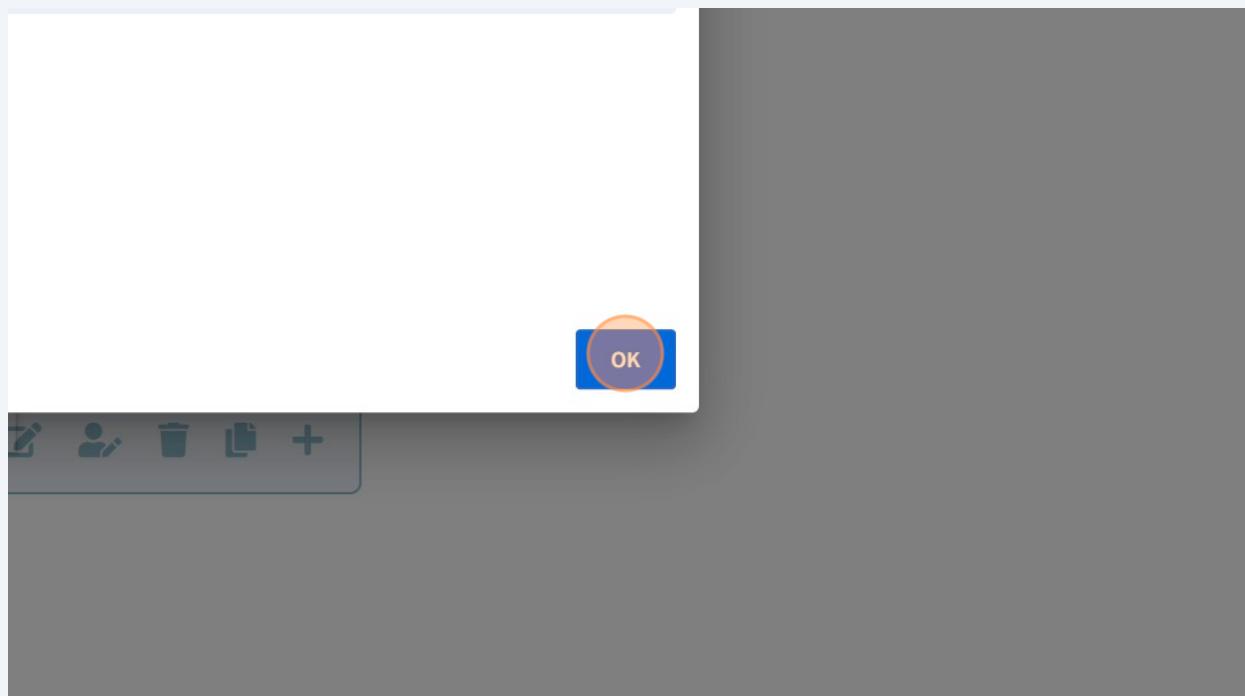
37 Select "Rename".



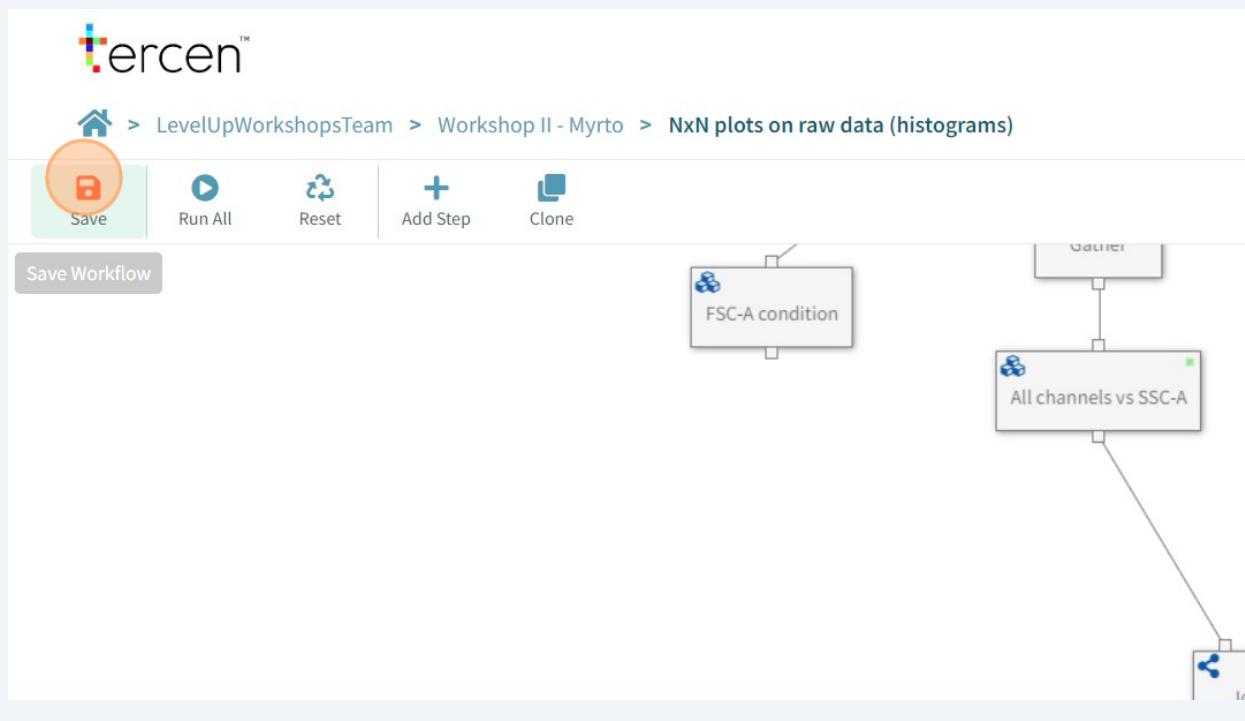
38 Type "NxN plots (logicle transformed CD3, CD4, CD8)" in the name text box.



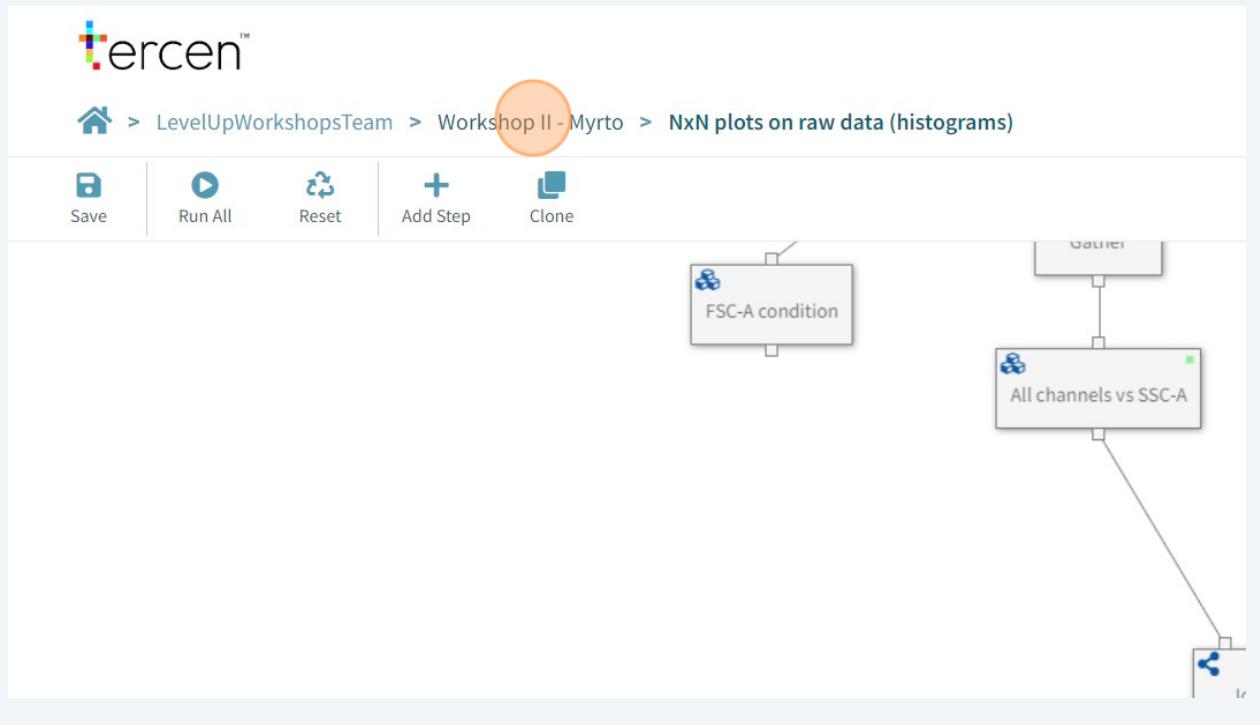
39 Click "OK"



40 Do not forget to save your progress by clicking the Save button on the leftmost side of the Global toolbar.



41 Click "Workshop II - Myrto".



42 Click "Rename"

The screenshot shows the tercen™ software interface. At the top, there are tabs for "Project" and "Activities", with "Project" being the active tab. Below the tabs, the title "Workshop II - Myrto" is displayed, followed by the message "No description provided.". A horizontal line of buttons includes: New data set, New workflow, New file, Upload file, Upload workflow, Project settings, and Clone project. The main area lists project activities in a table:

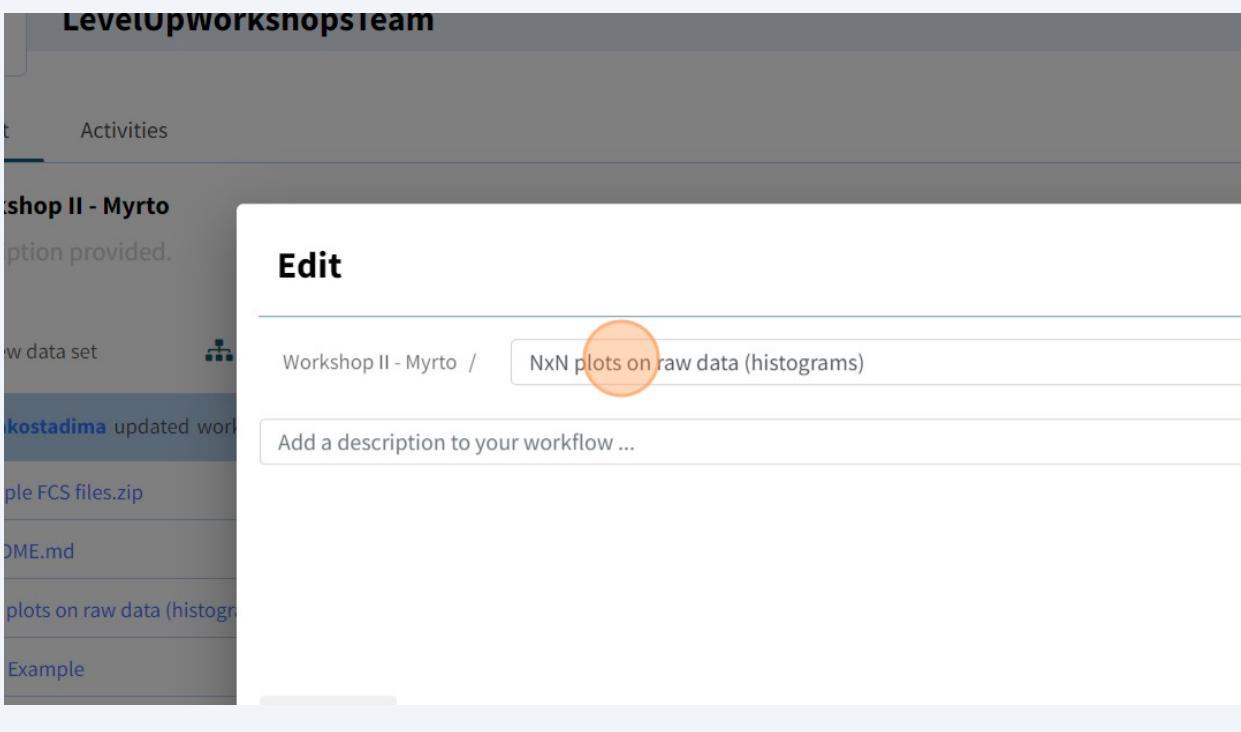
Activity	Last Updated
makostadima updated workflow NxN plots on raw data (histograms)	1 minutes ago
Sample FCS files.zip	2 days ago
README.md	2 days ago
NxN plots on raw data (histograms)	1 minutes ago
Join Example	2 days ago
file_annotation.csv	2 days ago
FCS Annotations	2 days ago
Example Files	2 days ago
Data transformation	2 hours ago

Below this table is another table with a single row:

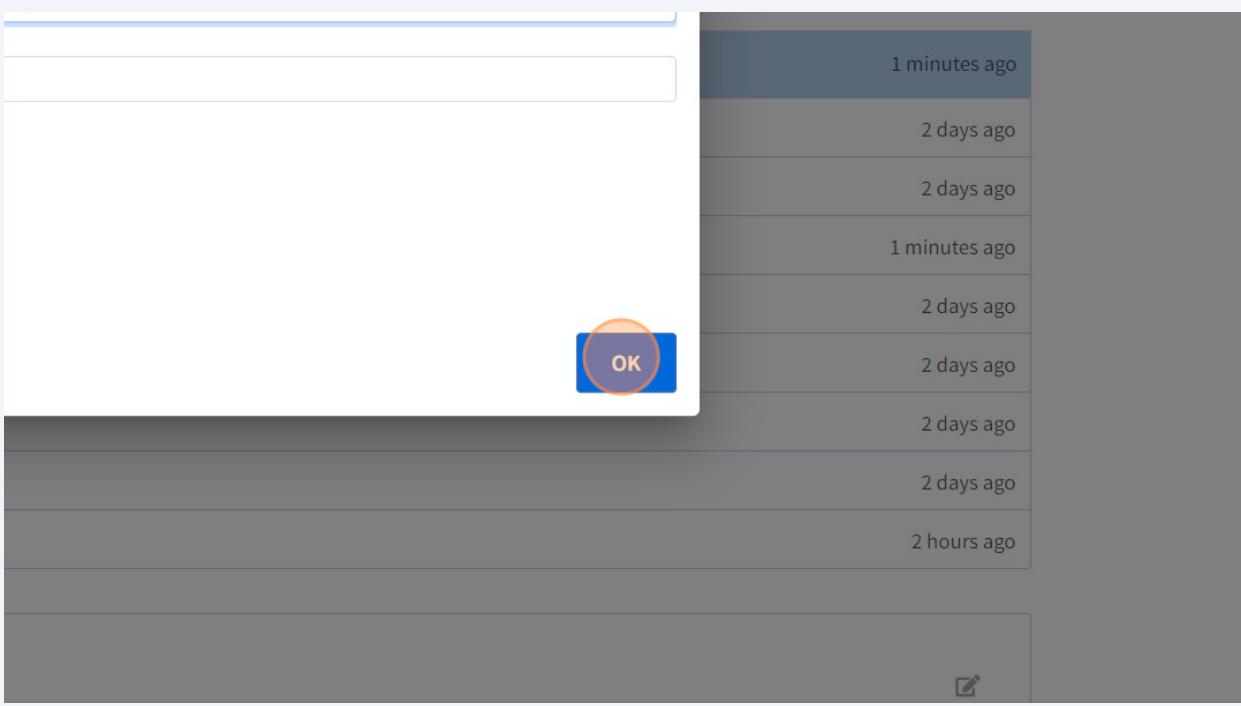
README.md	
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An orange circle highlights the "Rename" button in the second table.

- 43** Rename the workflow to "NxN plots (histograms)".



- 44** Click "OK"

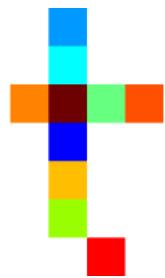


45

This guide has shown you how to transform your flow data.

Well done!

0207 - Clean Data



This lesson provides an introduction to automatic data quality control using the flowCut algorithm in Tercen.



Systematic changes throughout the data acquisition can occur because of bubbles, cell clumping, washing, laser malfunction, etc. Identifying and removing such spurious events is crucial for downstream data analysis.

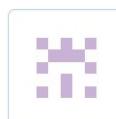
Several algorithms have been published to achieve that automatically, including flowAI, flowClean, flowCut, and PeacoQC.

This introduction will teach you to use [flowCut](#) in Tercen.

- 1 Click on your copy of the Workshop II Tercen project.



> LevelUpWorkshopsTeam > Workshop II - Myrto



LevelUpWorkshopsTeam

Project

Activities

Workshop II - Myrto

No description provided.

New data set

New workflow

New file



makostadima updated data Macrophages + Leishmania + oATP

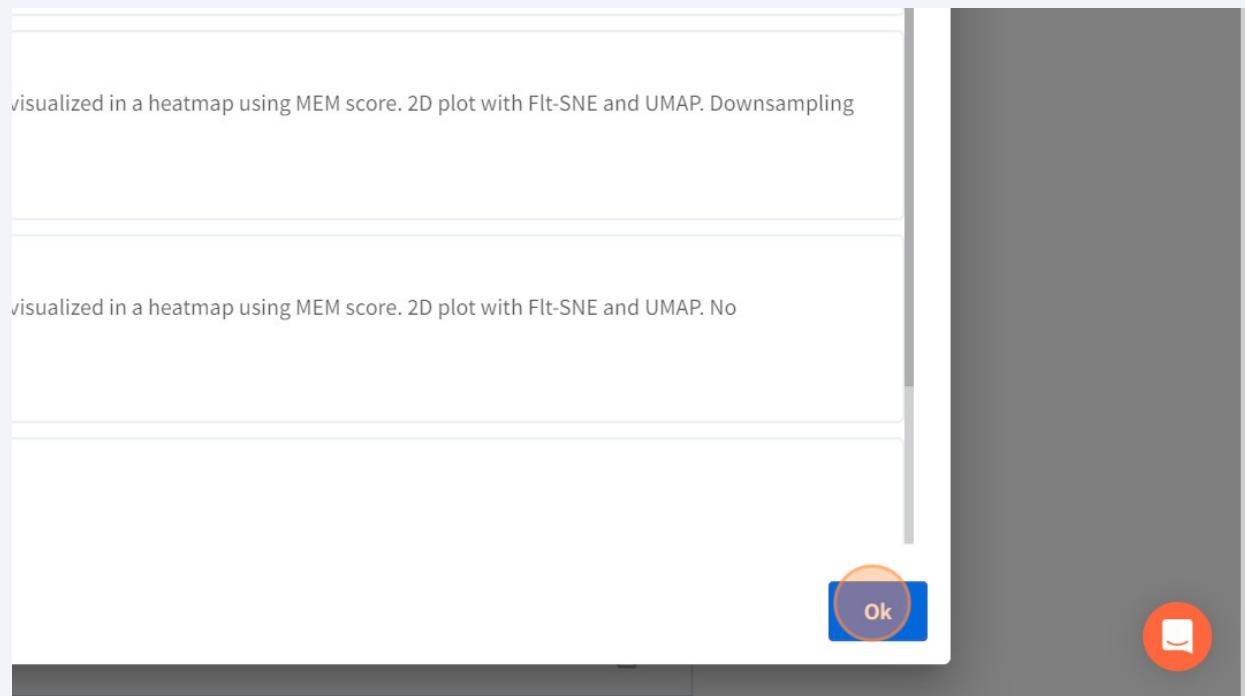
- 2 Click "New workflow".

The screenshot shows a web-based application interface for a project titled "LevelUpWorkshopsTeam". At the top, there are two tabs: "Project" (which is selected) and "Activities". Below the tabs, a section titled "Workshop II - Myrto" is displayed, with a note "No description provided.". Underneath this, there are several file-related buttons: "New data set", "New workflow" (which is highlighted with an orange circle), "New file", and "Upload file". A blue banner at the bottom of this section indicates that "makostadima updated data Macrophages + Leishmania + oATP.fcs". Below the banner, a list of files is shown: "Simple", "Sample FCS files.zip", "README.md", and "NxN plots (histograms)".

- 3 In the Workflow name text box type "Automatic data clean up".

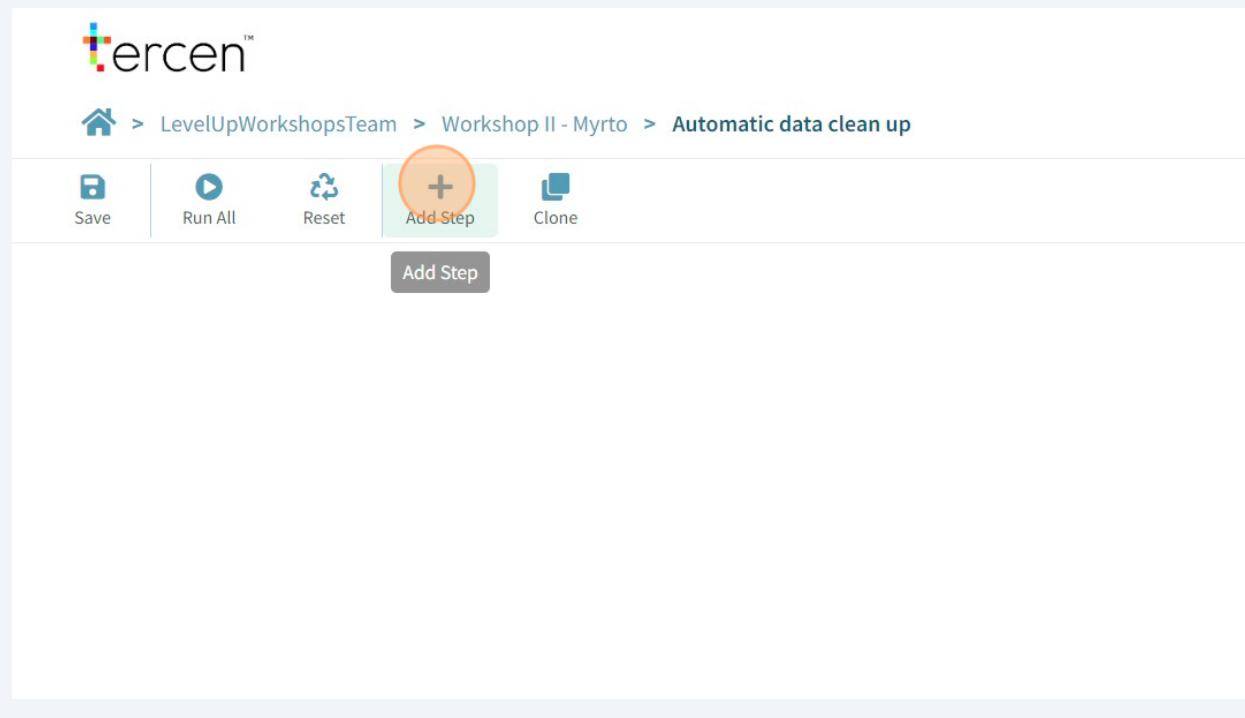
The screenshot shows a "New workflow" creation dialog. At the top, it says "New workflow". Below that, there is a "Workflow name:" label followed by a text input field containing "Workshop II - Myrto / Name your workflow ...". This input field is highlighted with an orange circle. Below the input field, there are two tabs: "Library" (which is selected) and "Installed". Further down, there is a "Search" bar, a checked checkbox for "Display latest version only", and a "Tag list" section. At the bottom, there is a card for "Get Started with Flow Cytometry 0.0.4" which describes the workflow as "Performs FlowSOM (cluster n=15) and views the results with a heatmap, 2D plot with UMAP".

4 Click "Ok".

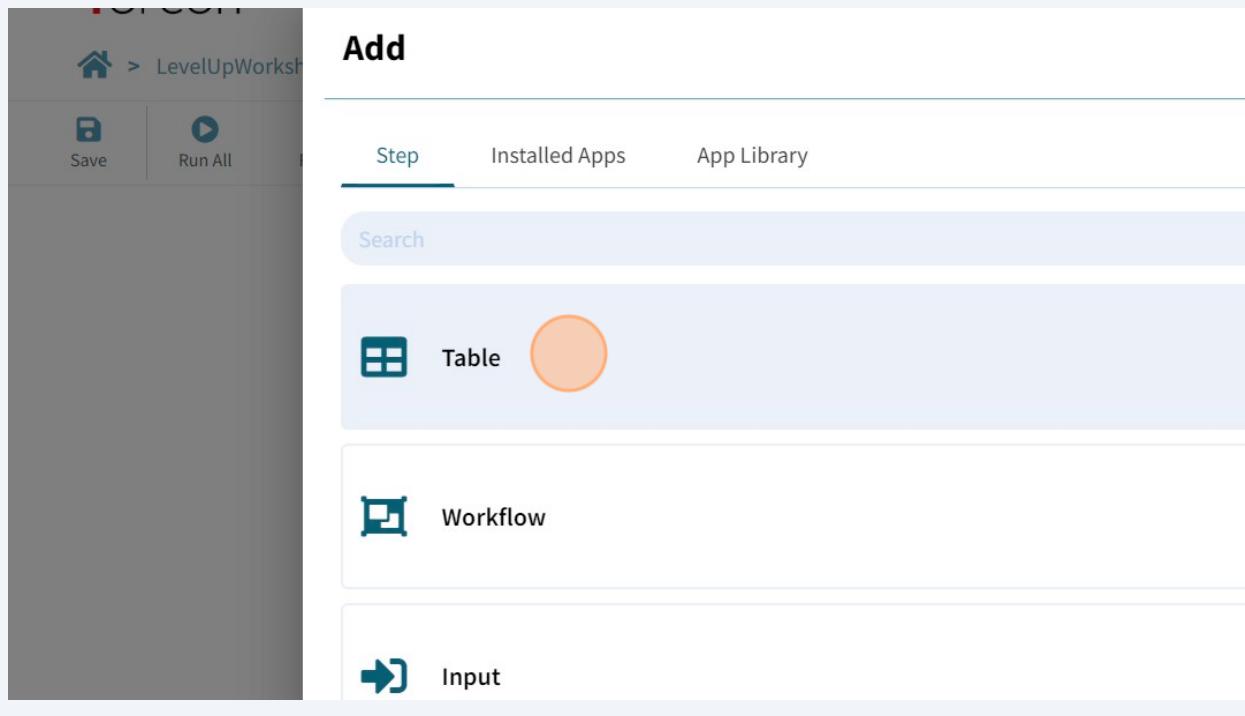


5 You will start by adding a data table to the empty workflow.

Click "Add Step".



6 Select "Table".

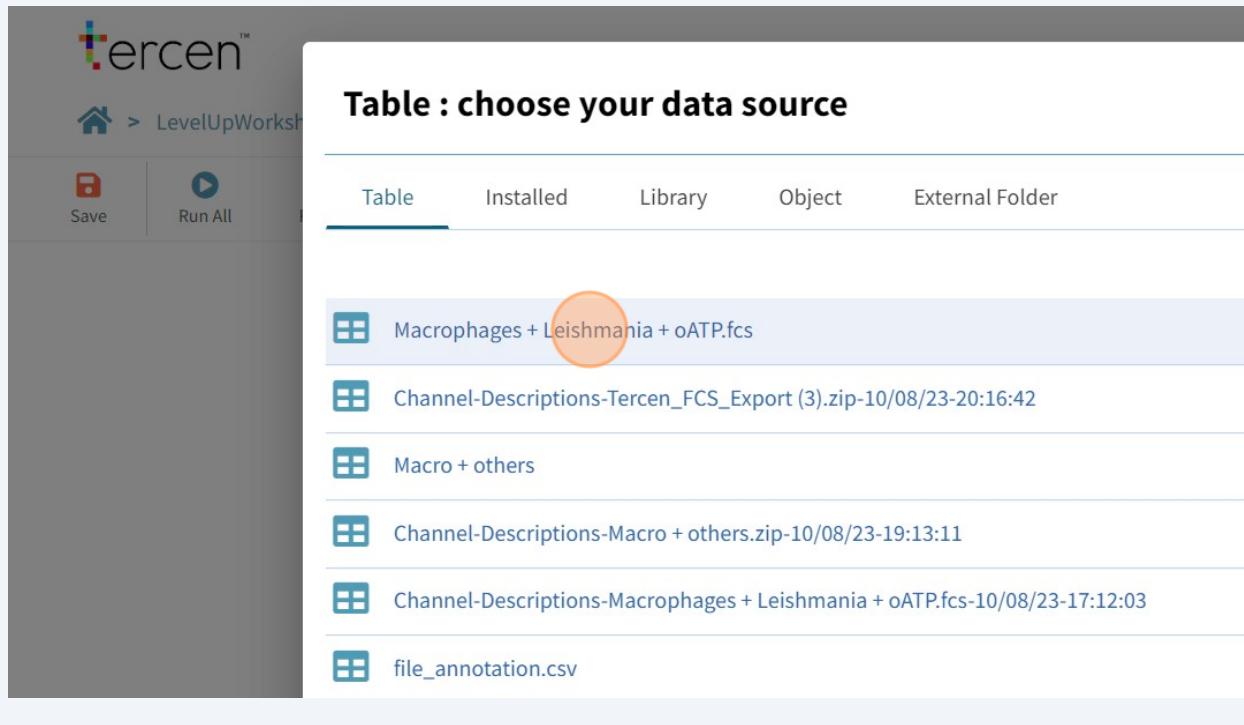


7

For this lesson, we will work with an FCS files that was generated to evaluate the effect of periodate-oxidized ATP (oATP) on parasite control in Leishmania-infected macrophages. The dataset was originally published [here](#) and can be accessed on [FlowRepository](#).

We have already read the data into Tercen, which is stored under the "Example Data" folder.

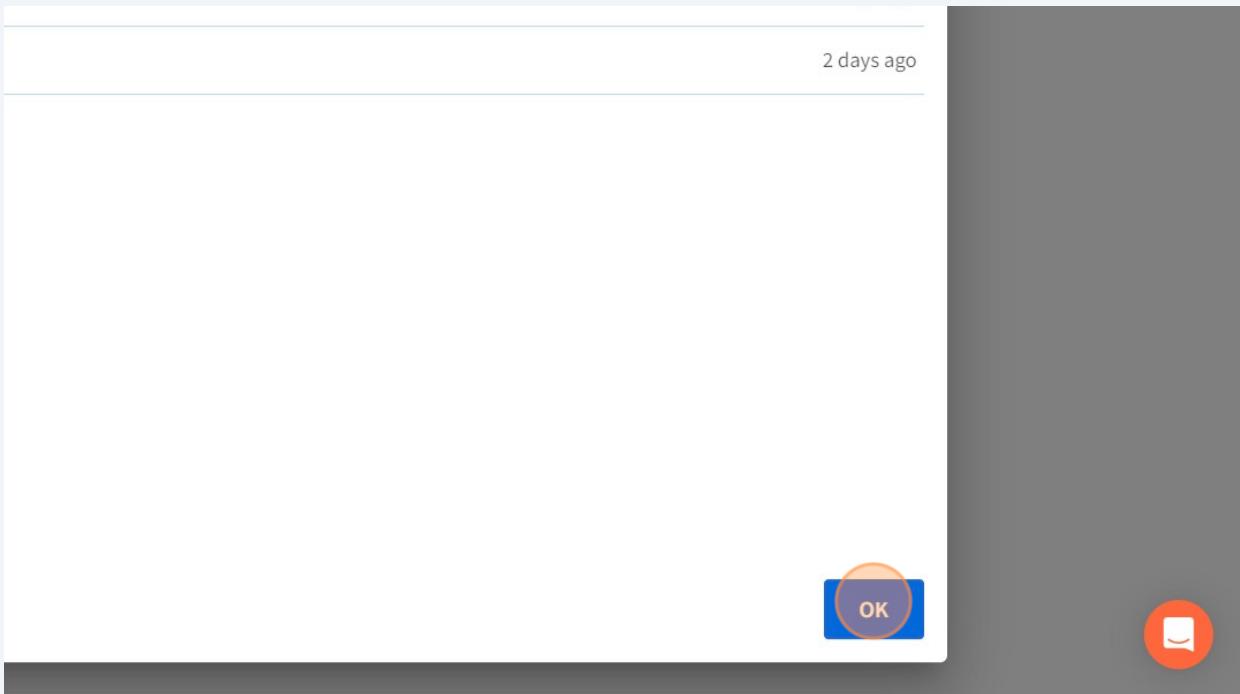
On the popup window, select "Macrophages + Leishmania + oATP.fcs"



The screenshot shows the Tercen software interface. On the left, there's a sidebar with a home icon, a 'Save' button, and a 'Run All' button. The main area has a title 'Table : choose your data source'. Below it is a table with five columns: 'Table' (which is underlined and bold), 'Installed', 'Library', 'Object', and 'External Folder'. The table contains six rows, each with a small icon and a file name:

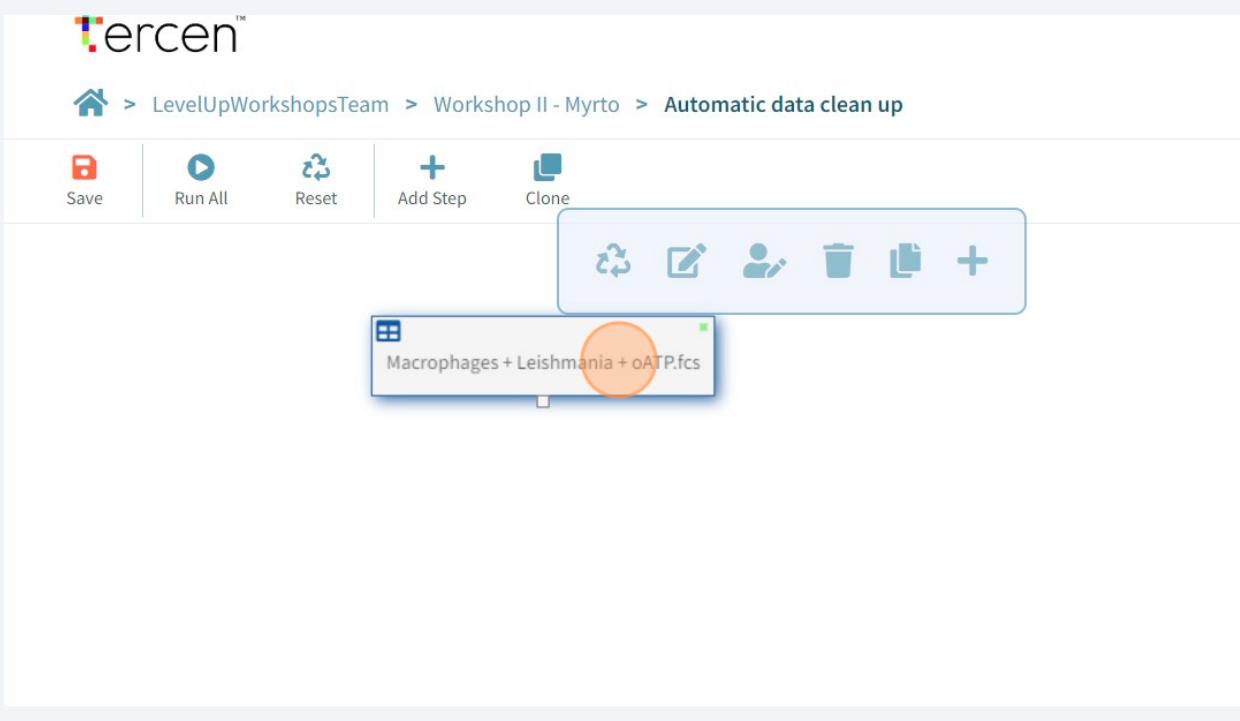
Table	Installed	Library	Object	External Folder
Macrophages + Leishmania + oATP.fcs				
Channel-Descriptions-Tercen_FCS_Export (3).zip-10/08/23-20:16:42				
Macro + others				
Channel-Descriptions-Macro + others.zip-10/08/23-19:13:11				
Channel-Descriptions-Macrophages + Leishmania + oATP.fcs-10/08/23-17:12:03				
file_annotation.csv				

8 Click "OK".

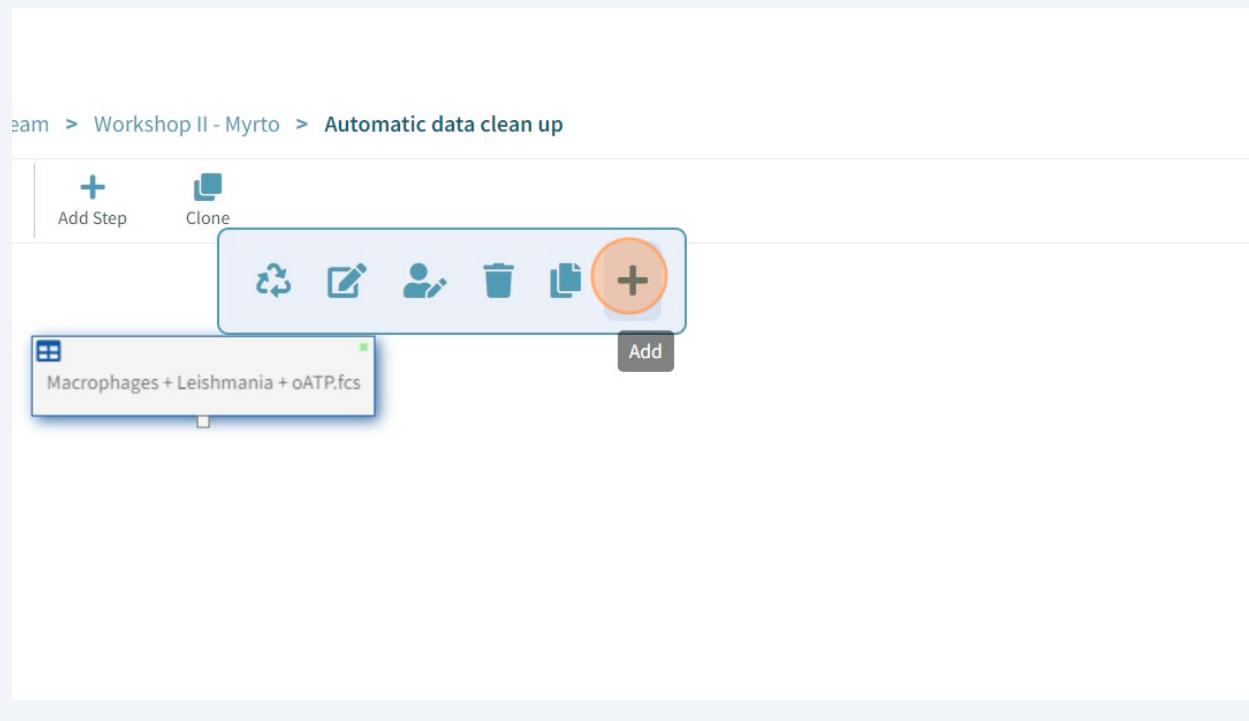


9 You will go on to convert the data into a long format using a **Gather** step.

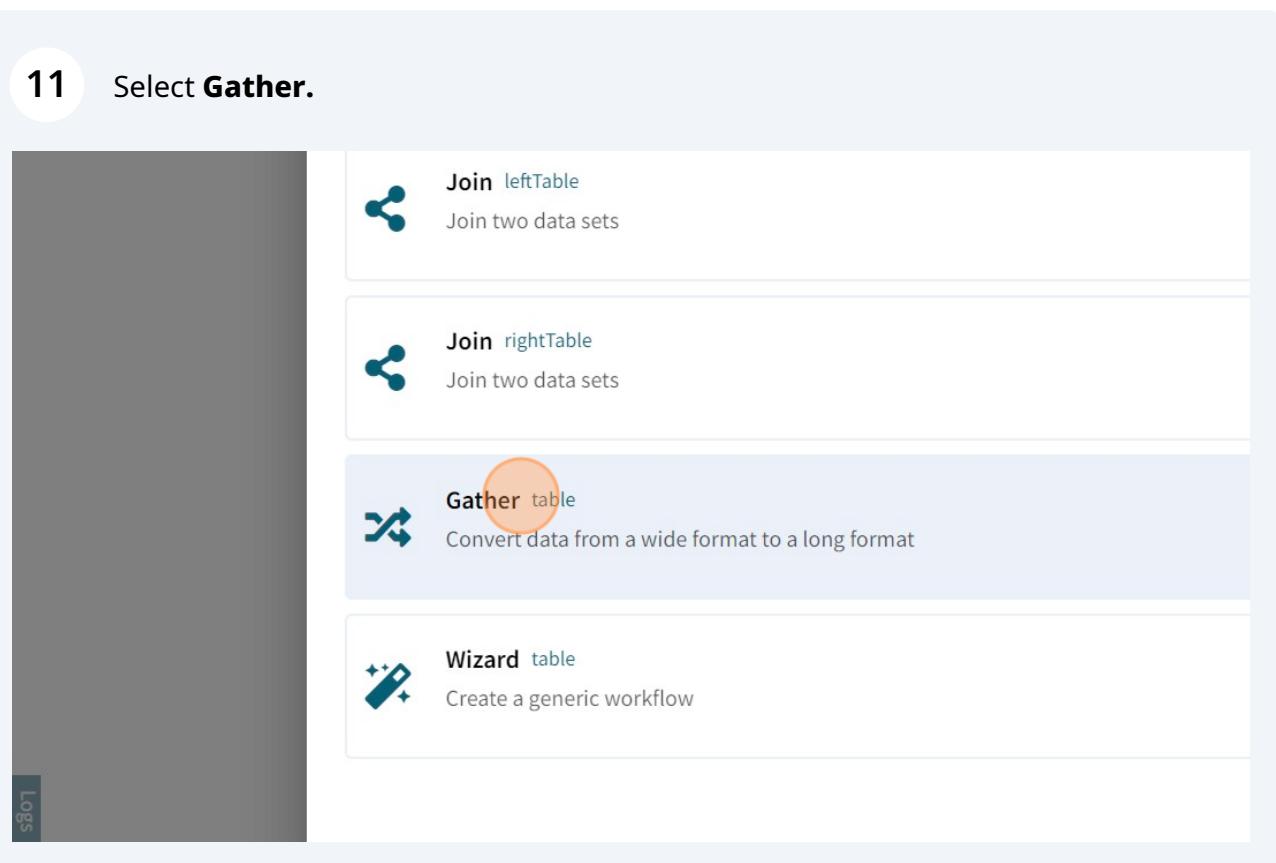
Click on this step so that the local toolbar appears over it.



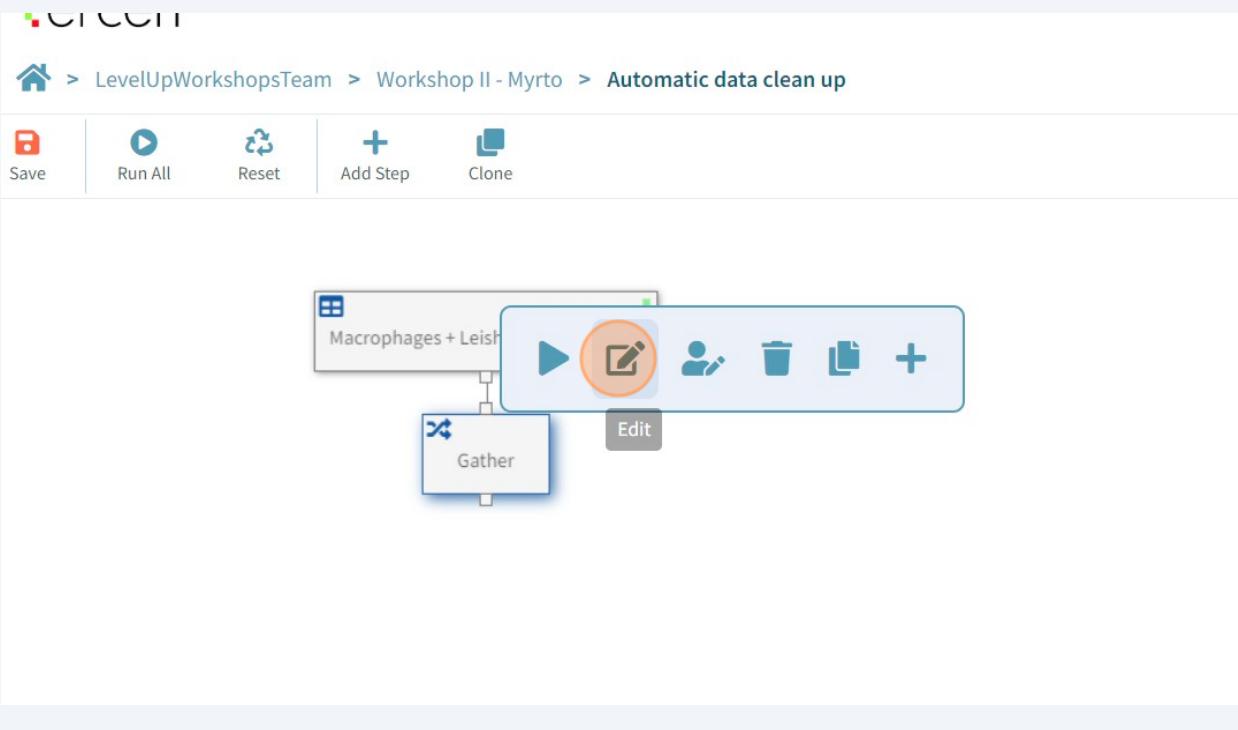
10 Select **Add**.



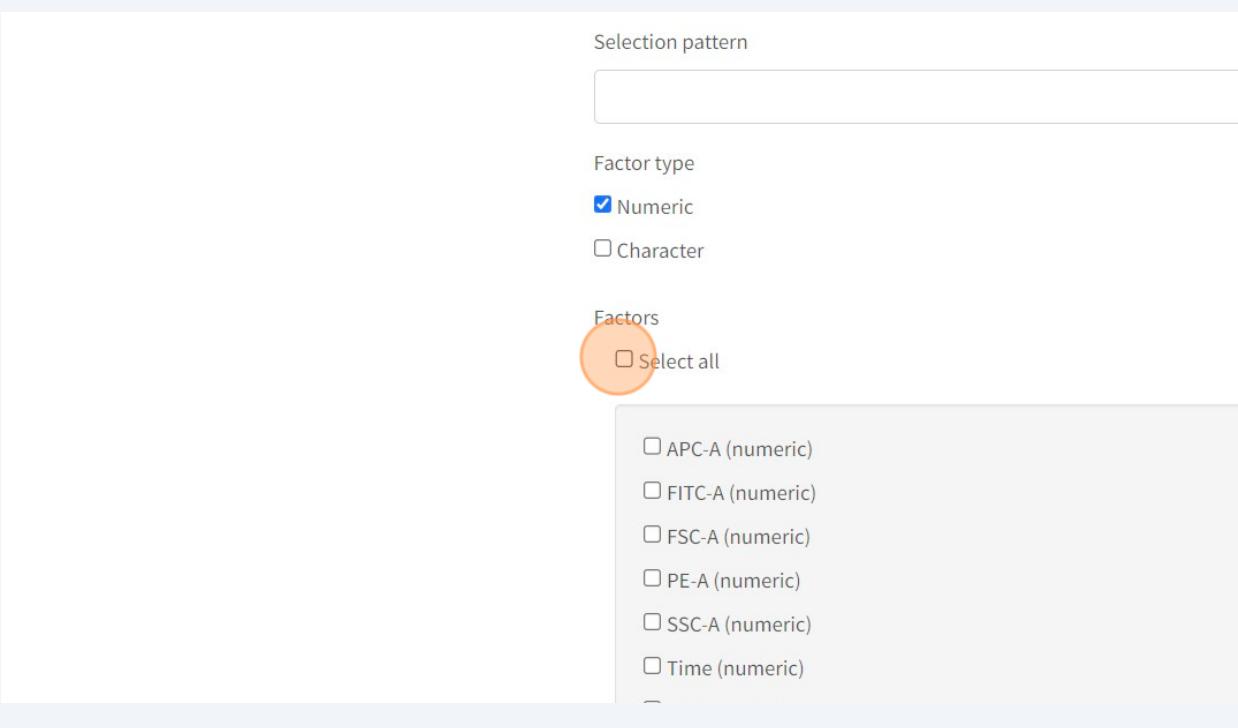
11 Select **Gather**.



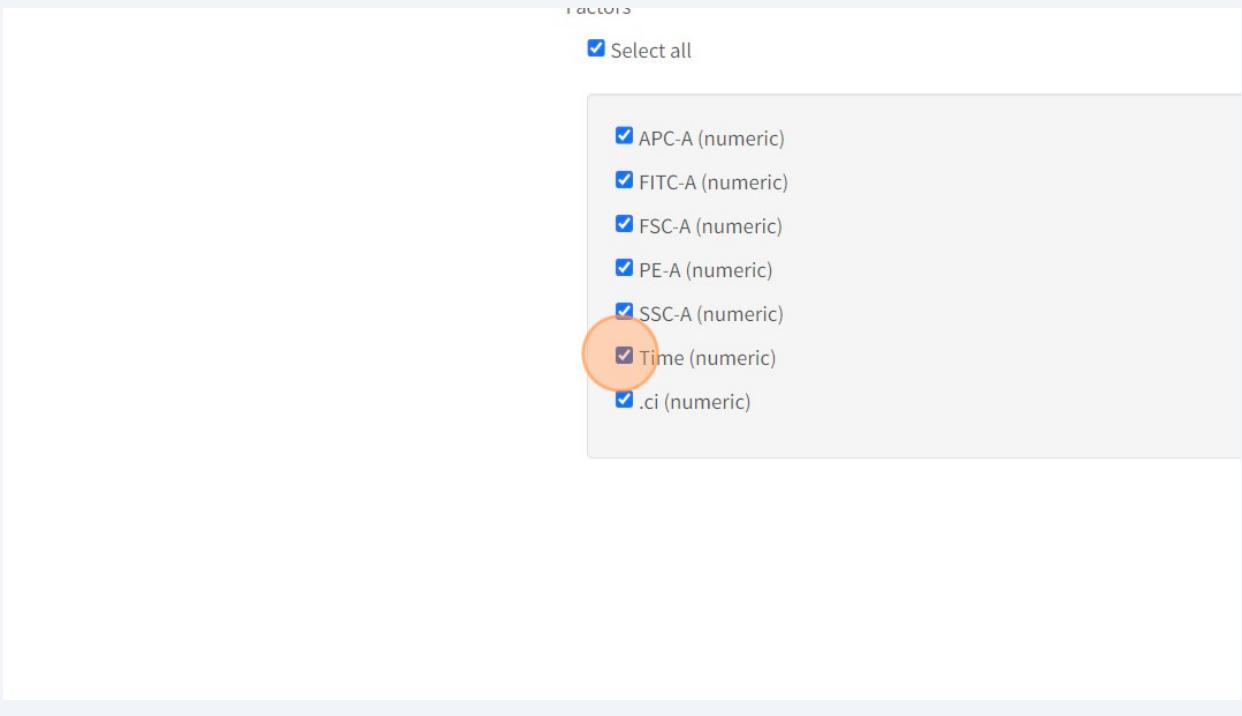
12 Click **Edit**.



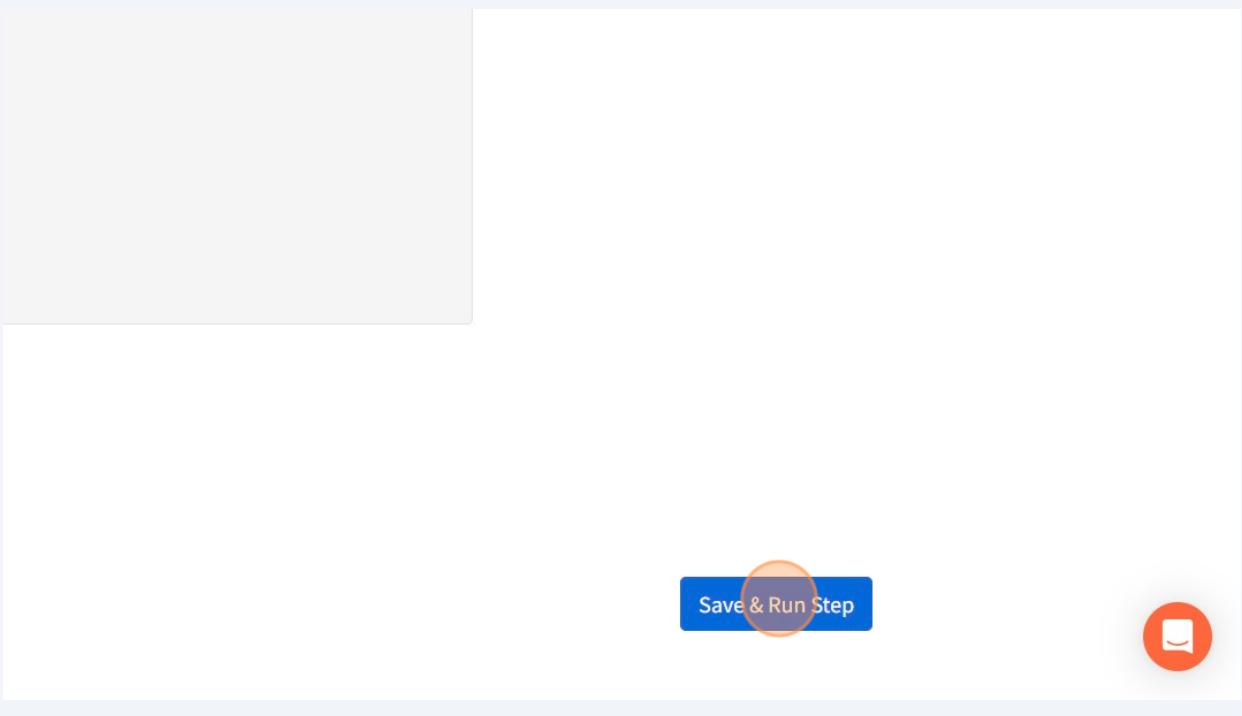
13 Click the "Select all" field.



- 14** Unselect the "Time (numeric)" & ".ci (numeric)" fields.

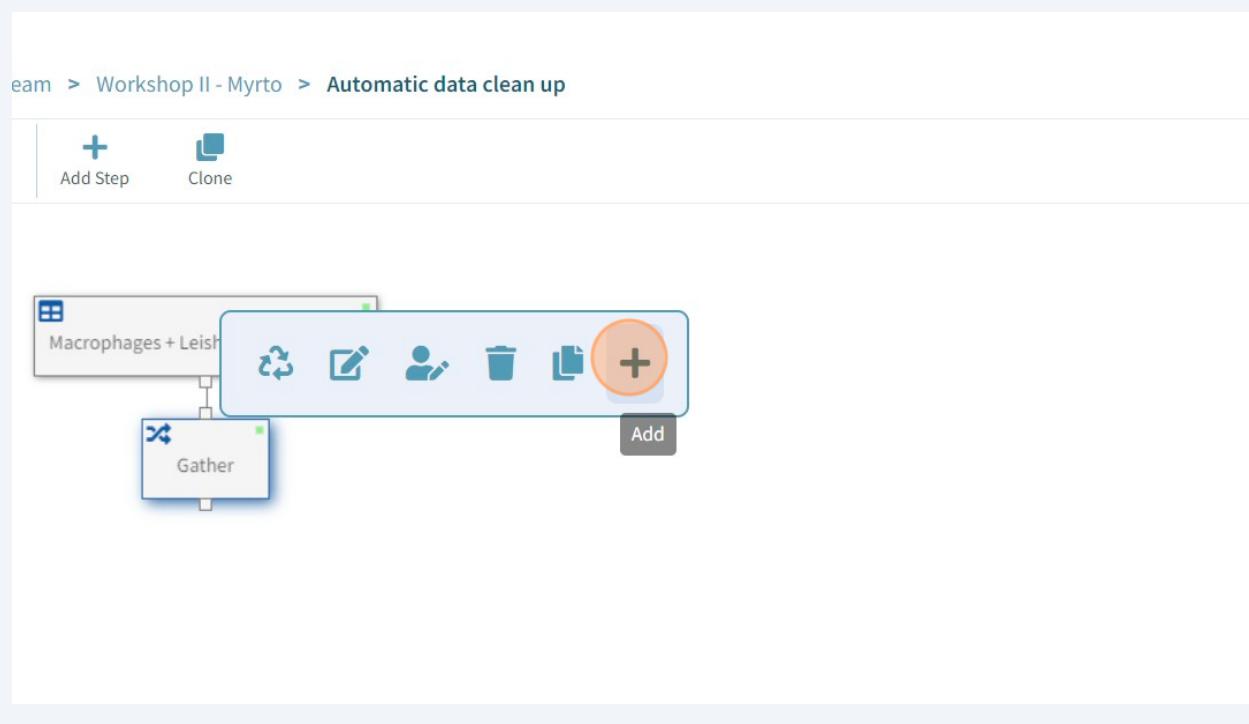


- 15** Click "Save & Run Step".



16 You will then proceed to transform the data using the logic operator.

Click **Add**.



17 Select "Data step".

The screenshot shows the 'Add' dialog box from the Tercen platform. The 'Step' tab is selected. The dialog has tabs for 'Step', 'Operator', 'Operator Library', 'Installed Apps', and 'App Library'. A search bar is present above the list of steps. The first item in the list is 'Data step' (data), which is highlighted with a red circle. Its description is 'Perform computation on user defined projection'. Below it are other options: 'Multi data step' (data) and 'Join leftTable'.

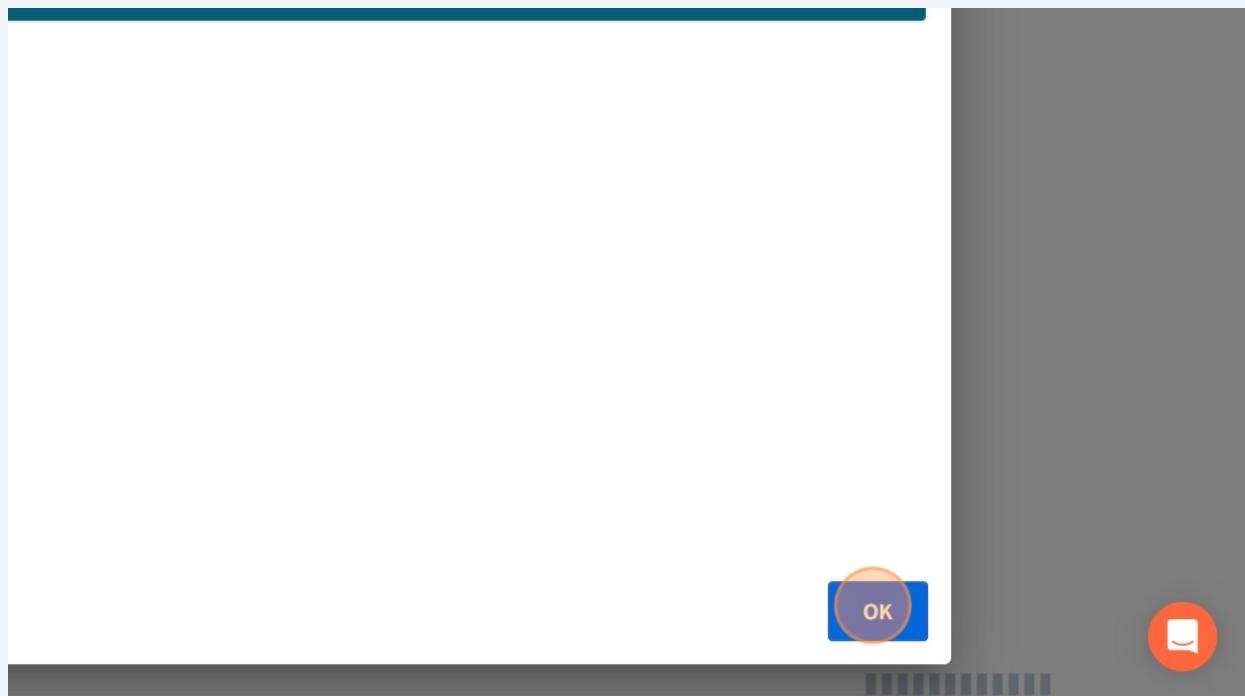
18 Click the '+' button to add the logicle operator.

The screenshot shows the tercen™ software interface. At the top, there's a navigation bar with 'tercen™' logo, followed by a breadcrumb trail: Home > LevelUpWorkshopsTeam > Workshop II - Myrto > Automatic data clean up > Data step. Below the navigation is a toolbar with icons for Save, Add Operator (highlighted with an orange circle), Crosstab, Tables, Layer 1, Point, Transform..., Filters, and Applied colors. Under the 'Factors' tab, there's a search bar and a dropdown menu showing 'Macrophages + Leishmania + oATP.fcs'. The dropdown menu lists several variables: Time (selected), fileid, event_id, and filename. A 'more ...' button is at the bottom of the list.

19 In the Library tab search for and select the Logicle operator.

The screenshot shows the 'Choose an operator' dialog box. The 'Library' tab is selected. A search bar contains the text 'logic'. Below it, there's a checkbox for 'Display latest version only' and a 'Tag list' button. Two operators are listed: 'GSEA 1.0.1' and 'Logicle 1.0.0'. The 'Logicle 1.0.0' entry is highlighted with an orange circle. It includes a brief description: 'Perform a channel-specific logicle data tranformation.' and categories: 'data transformation' and 'flow cytometry'. At the bottom of the dialog are 'Cancel' and 'OK' buttons.

20 Click "OK".

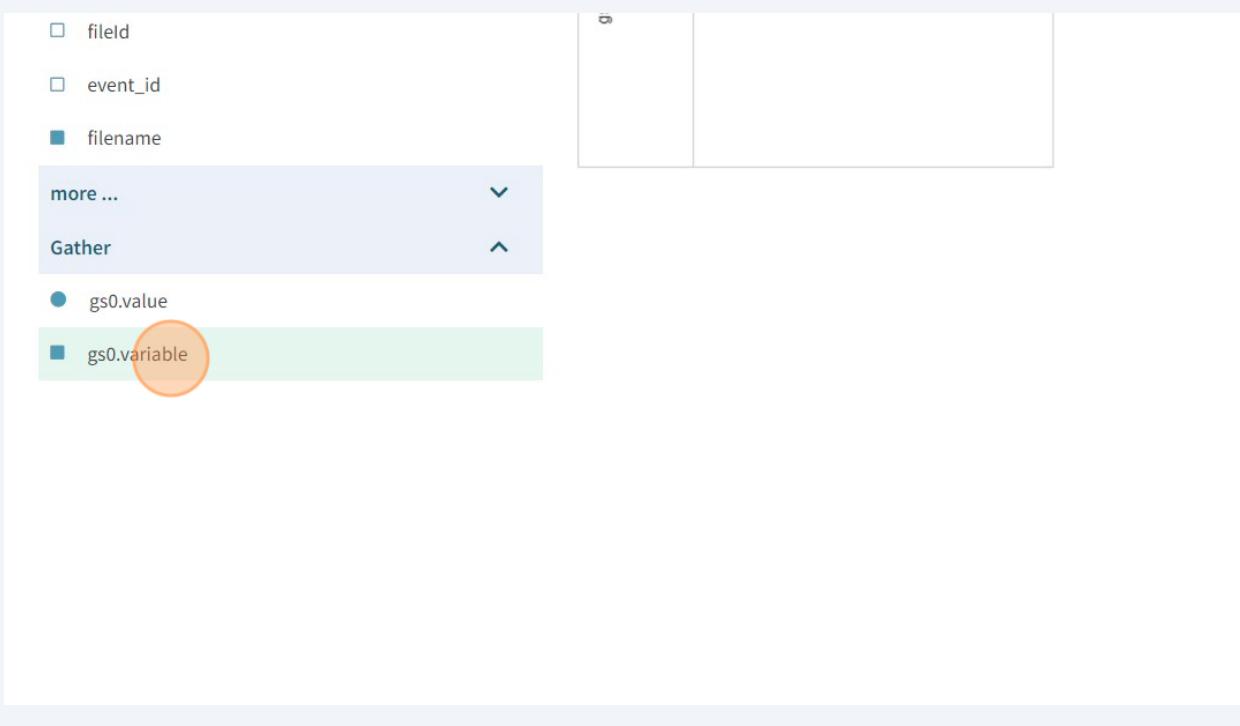


21 Let us start projecting the data in order to run the logic operator.

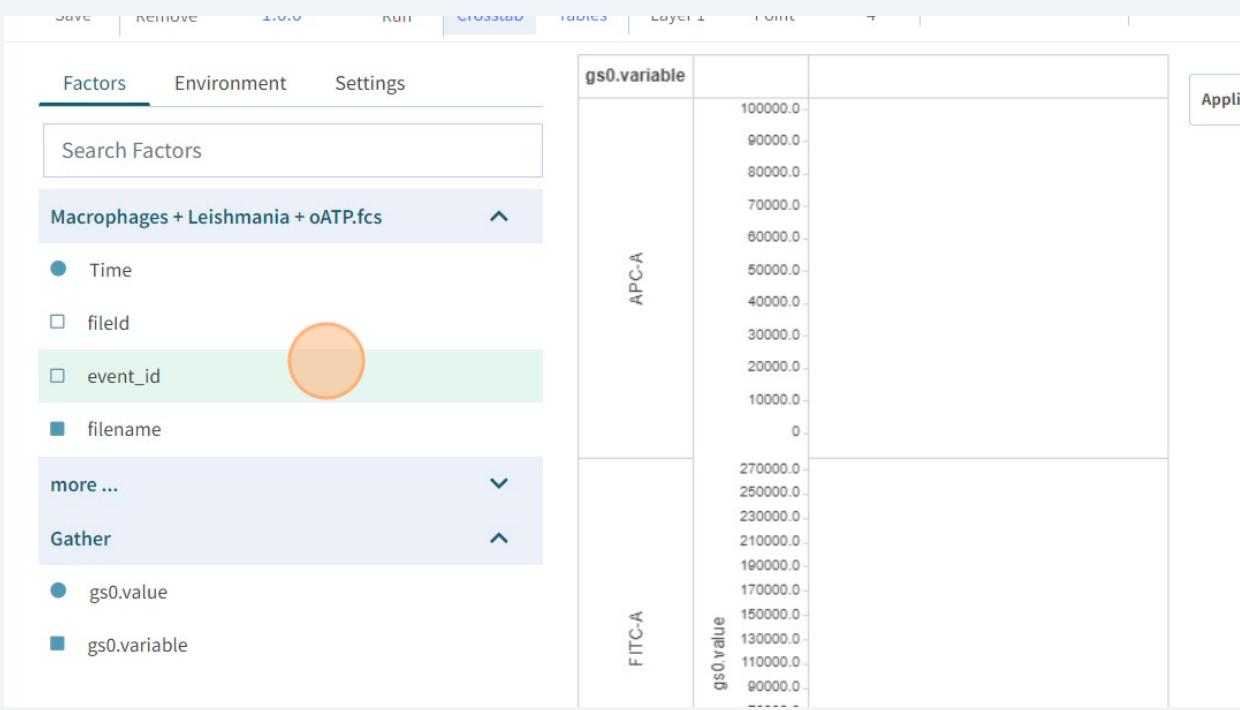
Drag the **gs0.value** factor and drop it in the Y-axis.

A screenshot of a data projection interface. On the left, there is a legend with four items: 'Time' (blue circle), 'fileid' (orange square), 'event_id' (yellow square), and 'filename' (green square). Below the legend is a 'more ...' button with a dropdown arrow. Underneath is a 'Gather' section with an upward arrow button. Inside the 'Gather' section, there are two items: 'gs0.value' (blue circle) which is highlighted with a red circle, and 'gs0.variable' (green square). The background of the interface is light gray.

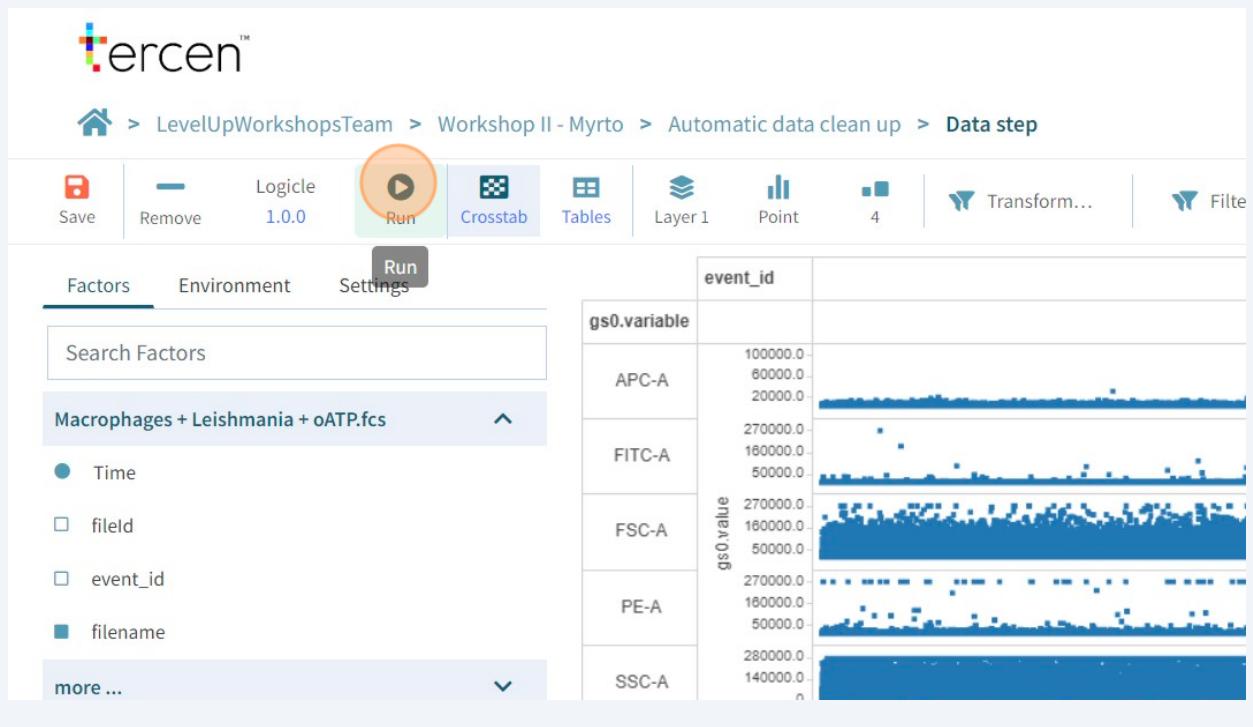
22 Drag the **gs0.variable** factor and drop it in the Row.



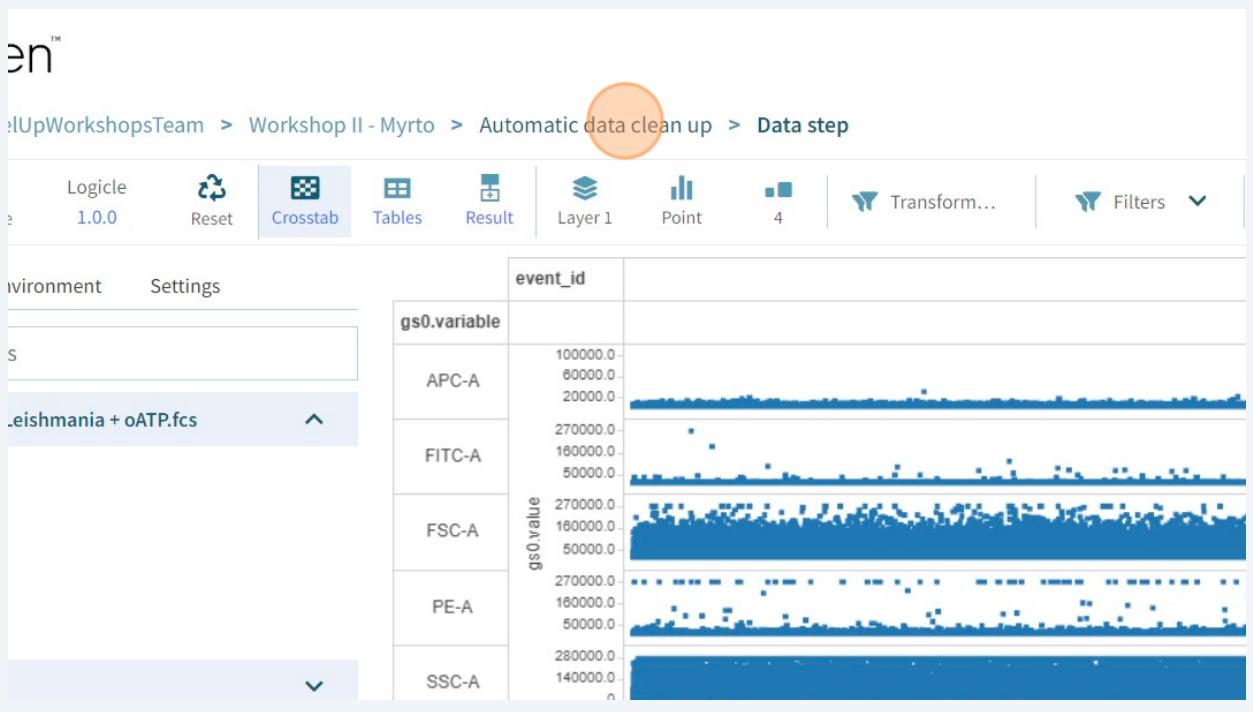
23 Drag the **event_id** factor and drop it in the Column.



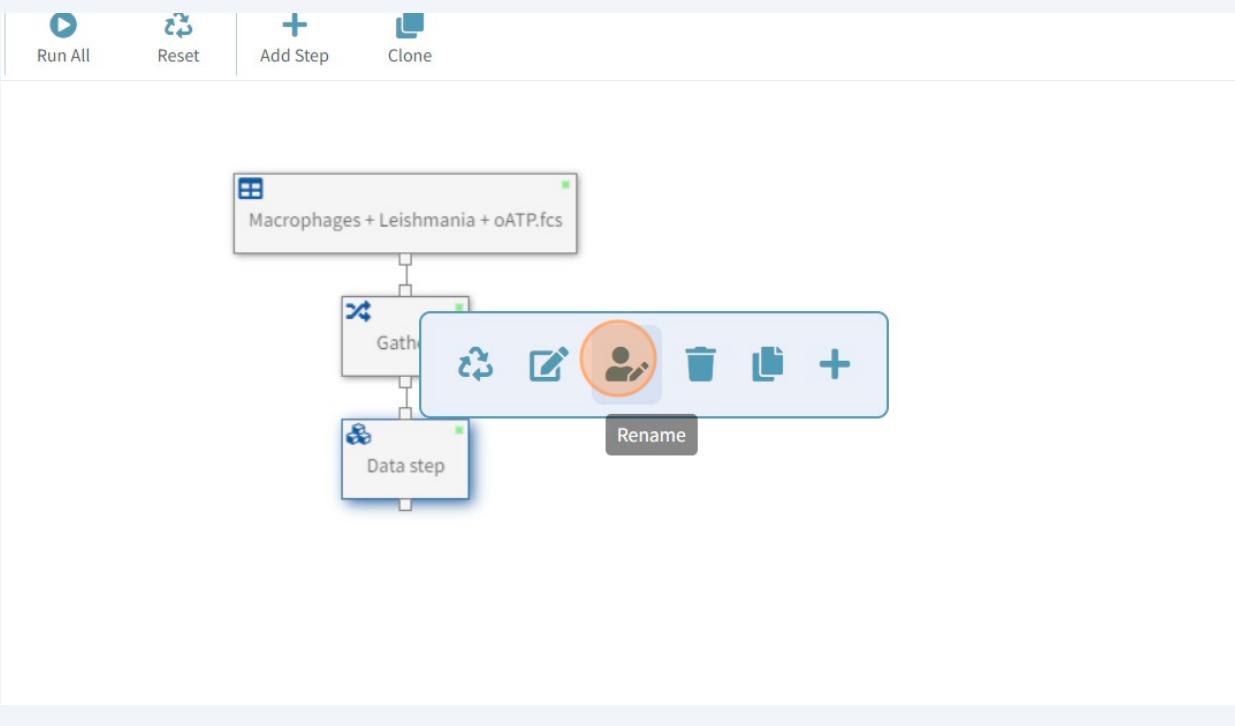
24 Click 'Run'.



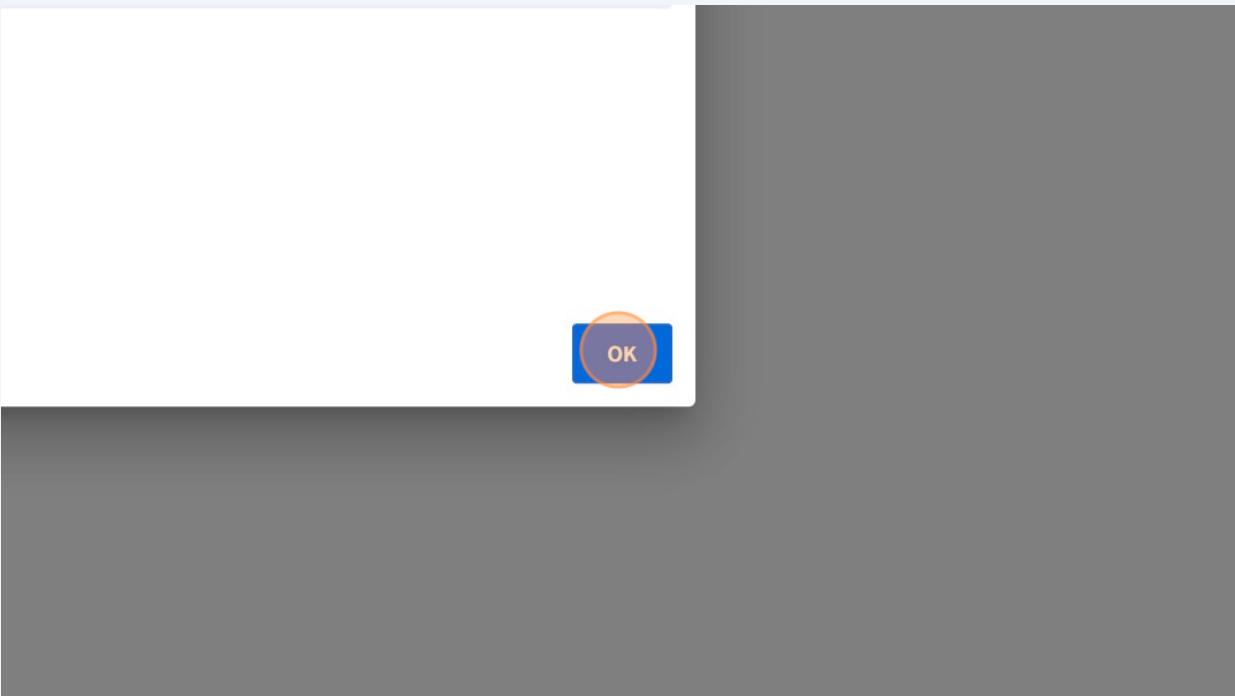
25 Click the "Automatic data clean up" link to return to the workflow.



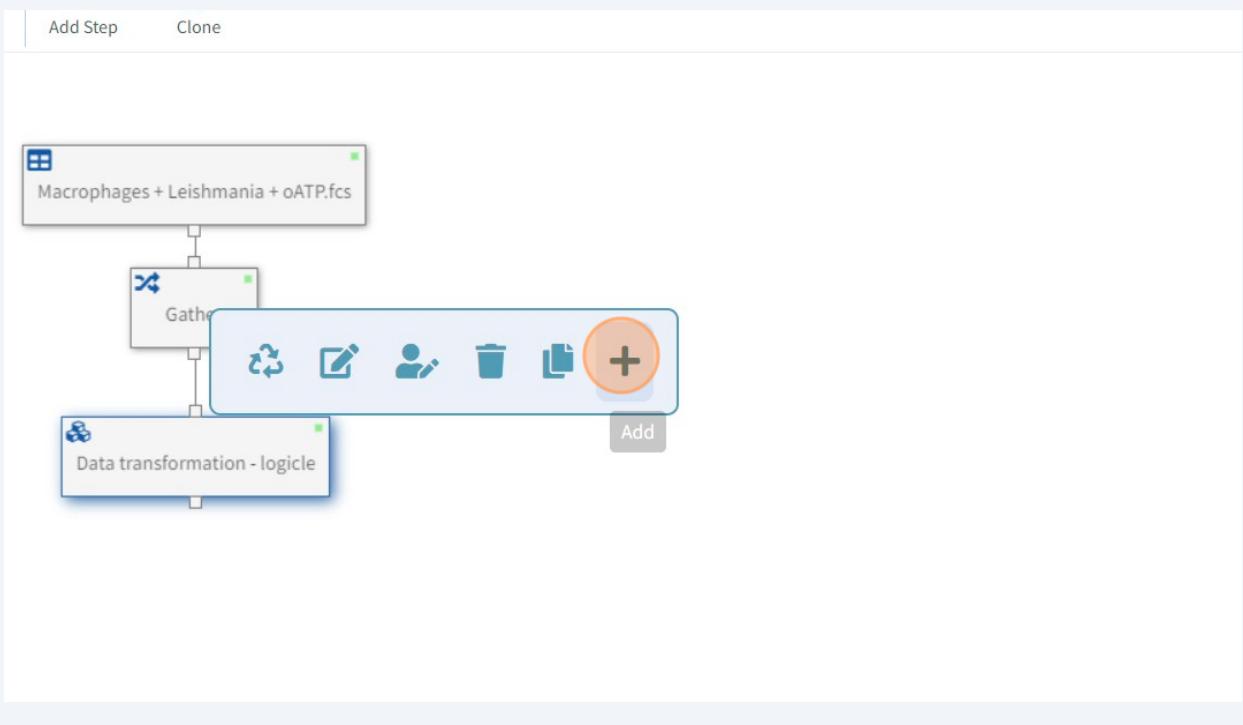
26 Rename the Data step to "Data transformation - logicle".



27 Click "OK".



28 Click 'Add'.



29 Select "Data step".

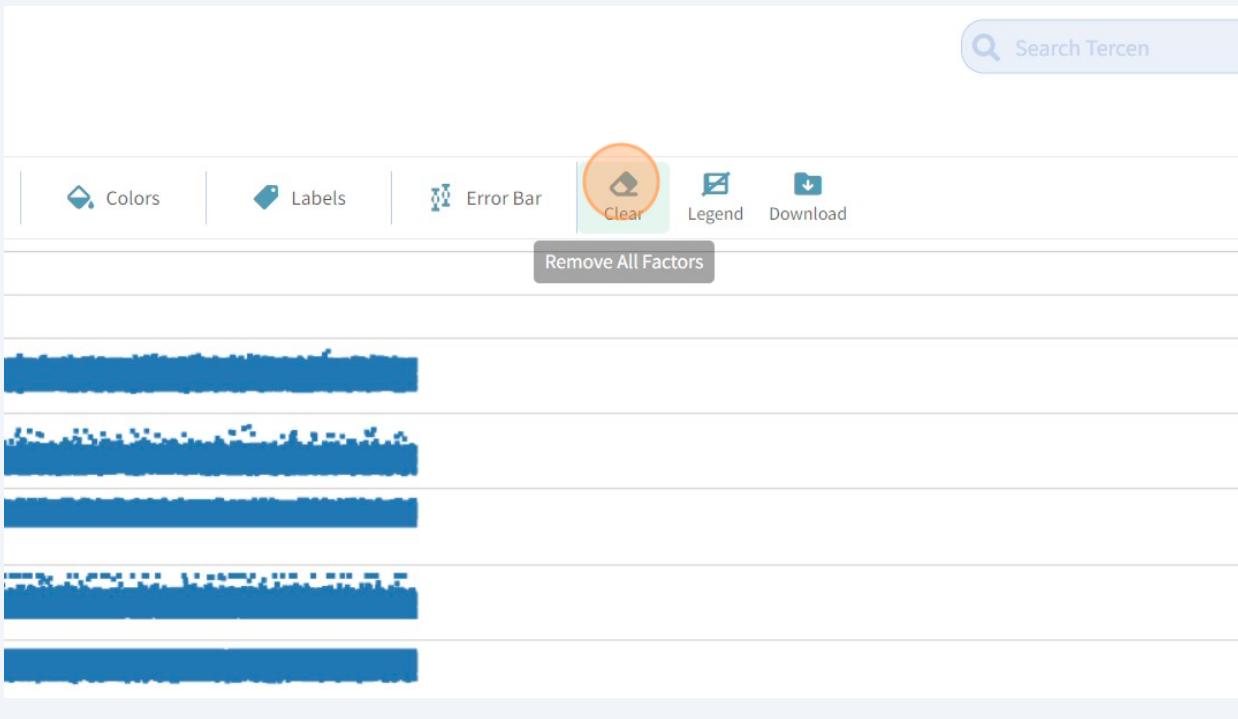
The screenshot shows an 'Add' dialog box with the title 'Add' at the top. Below the title, there is a navigation bar with five tabs: 'Step' (which is underlined), 'Operator', 'Operator Library', 'Installed Apps', and 'App Library'. A search bar is located below the tabs.

The main area displays three data step options:

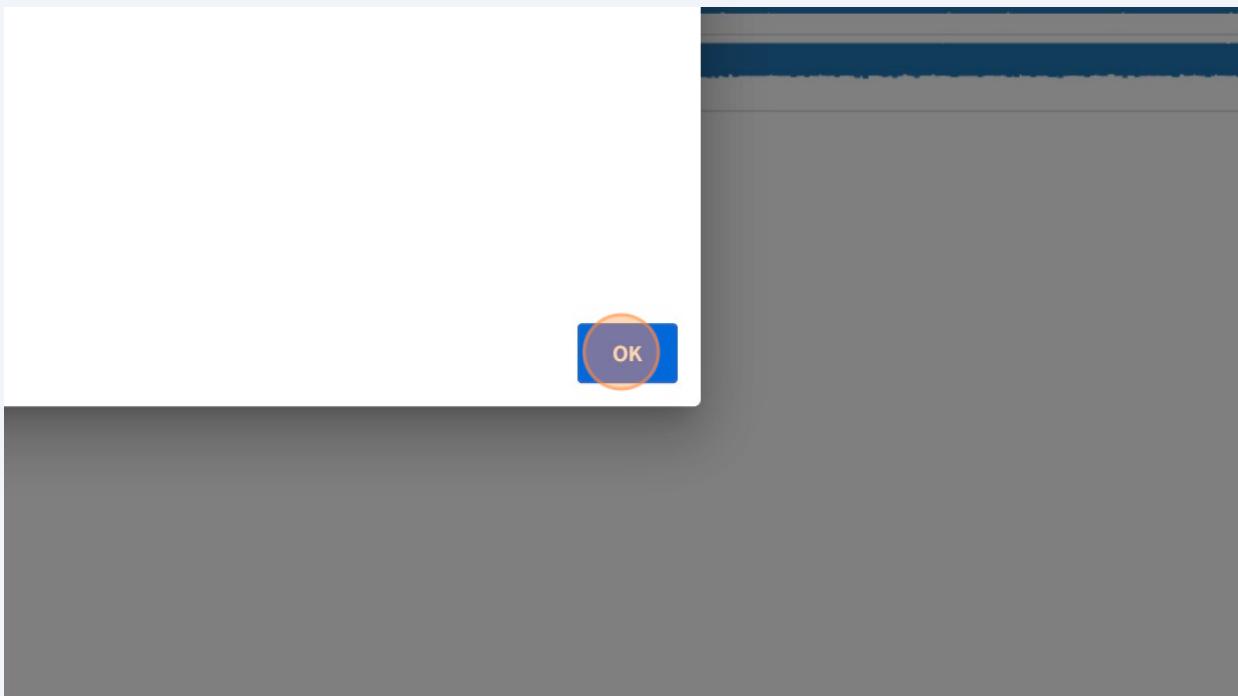
- Data step** data
Perform computation on user defined projection
- Multi data step** data
Perform computation on user defined projection
- Join** leftTable
Join two data sets

The first option, 'Data step', is highlighted with a red circle, indicating it has been selected.

- 30** Clear any preselected data projection using the 'Clear' button.



- 31** Click "OK".



32 Again, you will start by adding the relevant operator to the Data step.

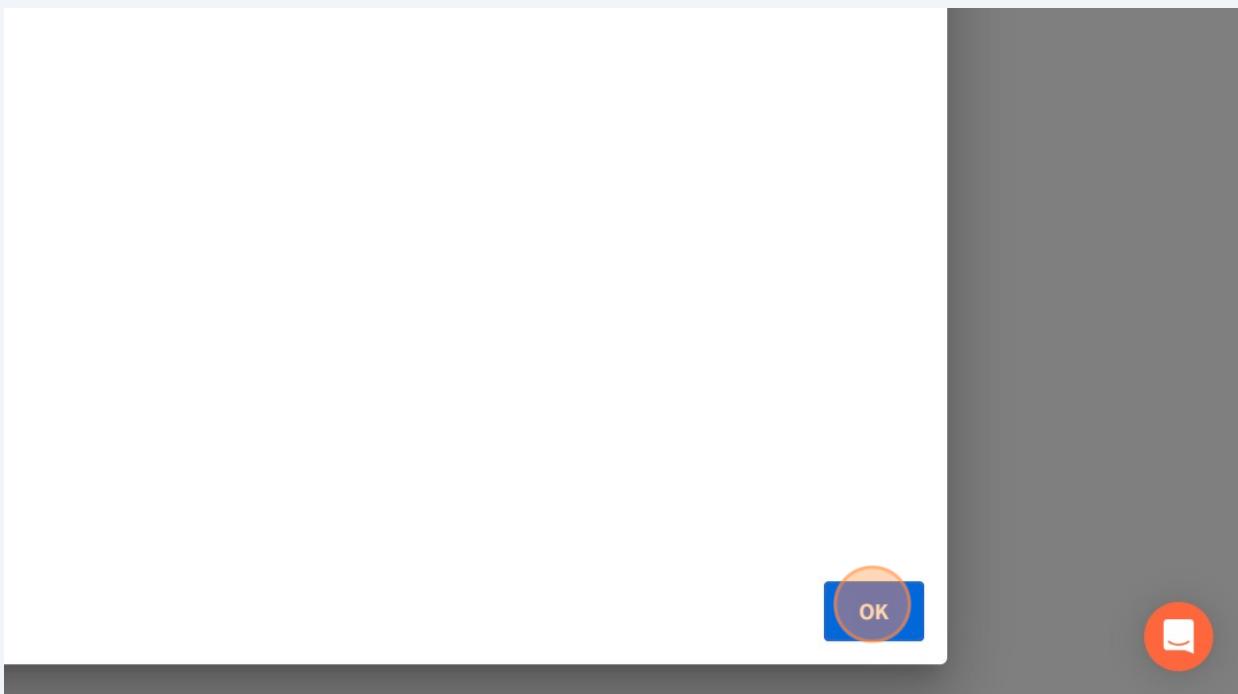
Click the '+' button.

The screenshot shows the tercen™ software interface. At the top, there is a navigation bar with icons for Home, Save, Add Operator (highlighted with an orange circle), Crosstab, Tables, Layer 1, Point, Transform..., and Filters. Below the navigation bar, there is a breadcrumb trail: Home > LevelUpWorkshopsTeam > Workshop II - Myrto > Automatic data clean up > Data step. On the left side, there is a sidebar with tabs for Factors (selected), Environment, and Settings. Under the Factors tab, there is a search bar labeled "Search Factors" containing the text "Macrophages + Leishmania + oATP.fcs". Below the search bar is a list of selected factors: Time (radio button selected), fileid, event_id, and filename. There is also a "more ..." button. To the right of the sidebar is a large empty table area.

33 In the Library tab search for and select "flowCut".

The screenshot shows the Library tab in the software interface. On the left, there is a sidebar with tabs for Environment, actors, and others. The main area has tabs for Installed, Library (selected), and Log. A search bar at the top contains the text "flowCut". Below the search bar is a toggle switch labeled "Display latest version only". Underneath the search bar, there is a section titled "Tag list" with a collapse arrow. A card for "flowCut 0.1.6" is displayed, featuring a docker icon, the text "Perform quality control of flow cytometry data using flowCut.", and tags for "flow cytometry" and "QC".

34 Click "OK".



35 Click on the operator's version, as shown below, to get directed to the operator's Github repo to get information on how to correctly project the data for this operator.

A screenshot of the Tercen software interface. At the top, there is a navigation bar with links: Home, LevelUpWorkshopsTeam, Workshop II - Myrto, Automatic data clean up, and Data step. Below the navigation bar is a toolbar with various icons: Save, Remove, flowCut 0.1.6 (which is highlighted with a red circle), Run, Crosstab (selected), Tables, Layer 1, Point, 4, Transform..., and Filter. The main area has tabs for Factors, Environment, and Settings, with the Factors tab selected. A search bar labeled 'Search Factors' contains the text 'Macrophages + Leishmania + oATP.fcs'. Below the search bar is a list of factors: Time (selected with a blue dot), fileid, event_id, and filename. At the bottom left is a 'more ...' button with a dropdown arrow.



Expected data projection:

Y-axis: the logicle-transformed values

Row: channels

Column: Time + event_id

flowCut operator

Description

The `flowCut` operator performs quality control on flow cytometry data.

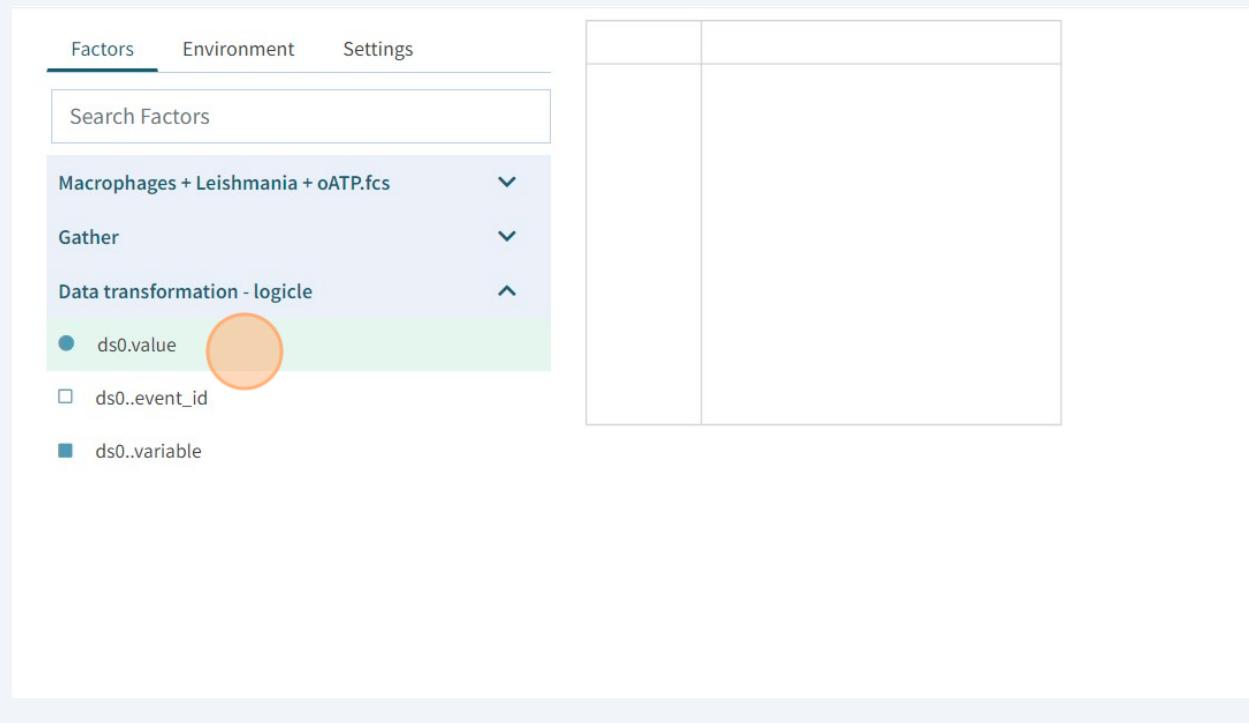
Usage

Input projection	.
<code>row</code>	represents the variables (e.g. channels, markers)
<code>col</code>	represents the observations (Use 'Time' on top of rowid.)
<code>y-axis</code>	measurement value

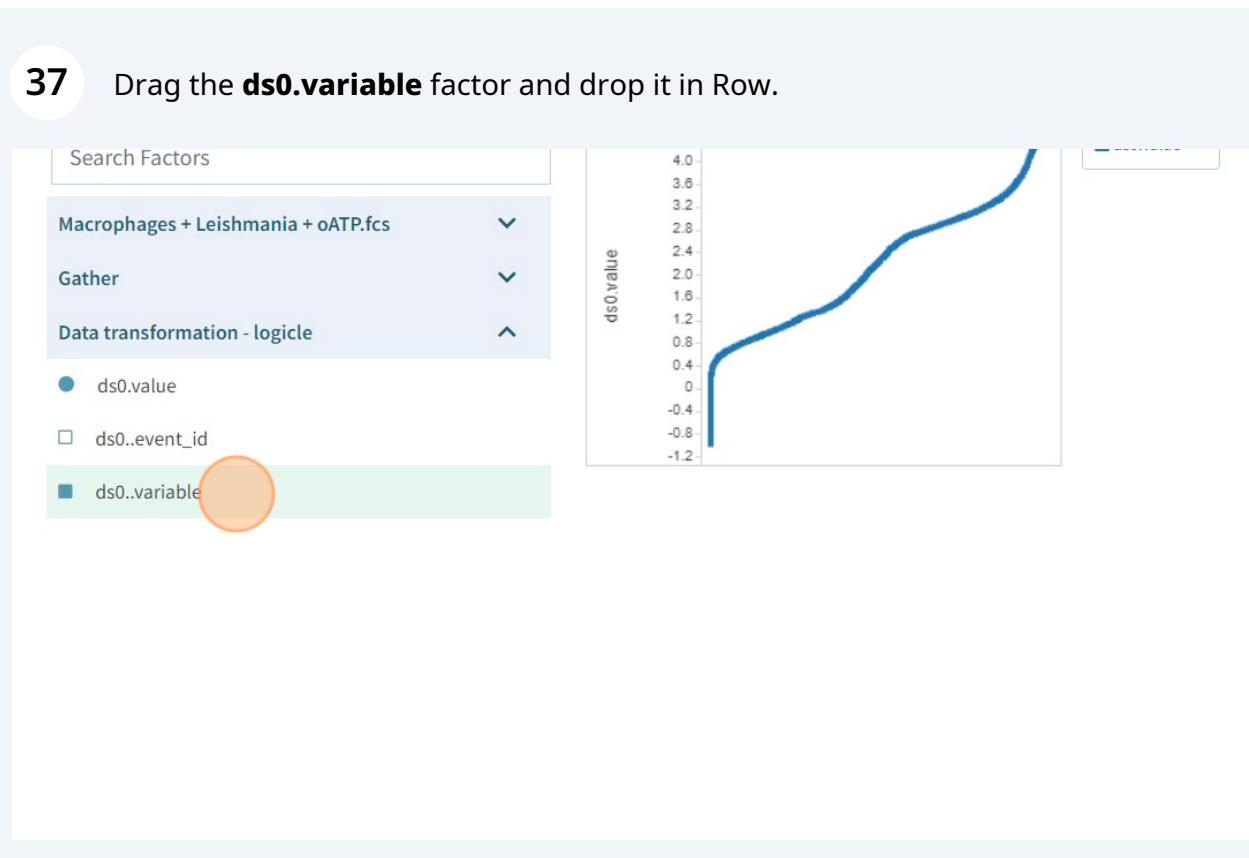


For this operator the order of the column factors is important. Place Time on top of event_id.

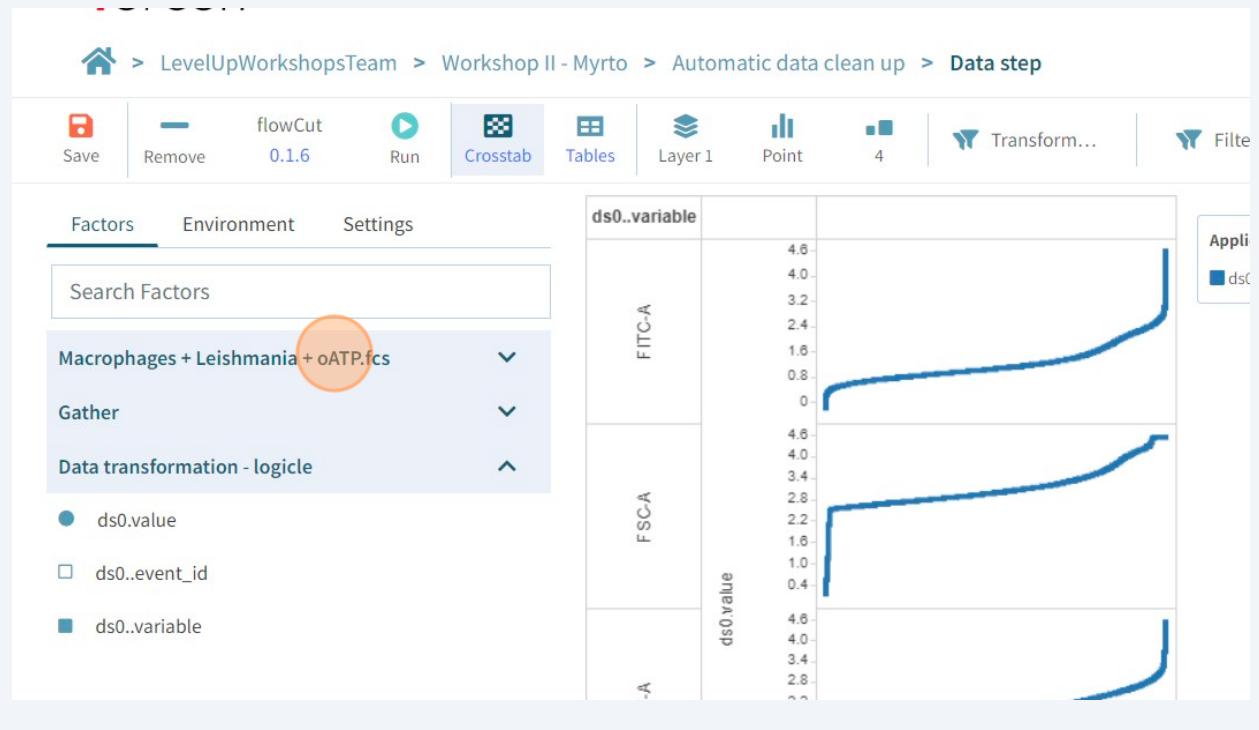
36 Drag the **ds0.value** factor and drop it in Y-axis.



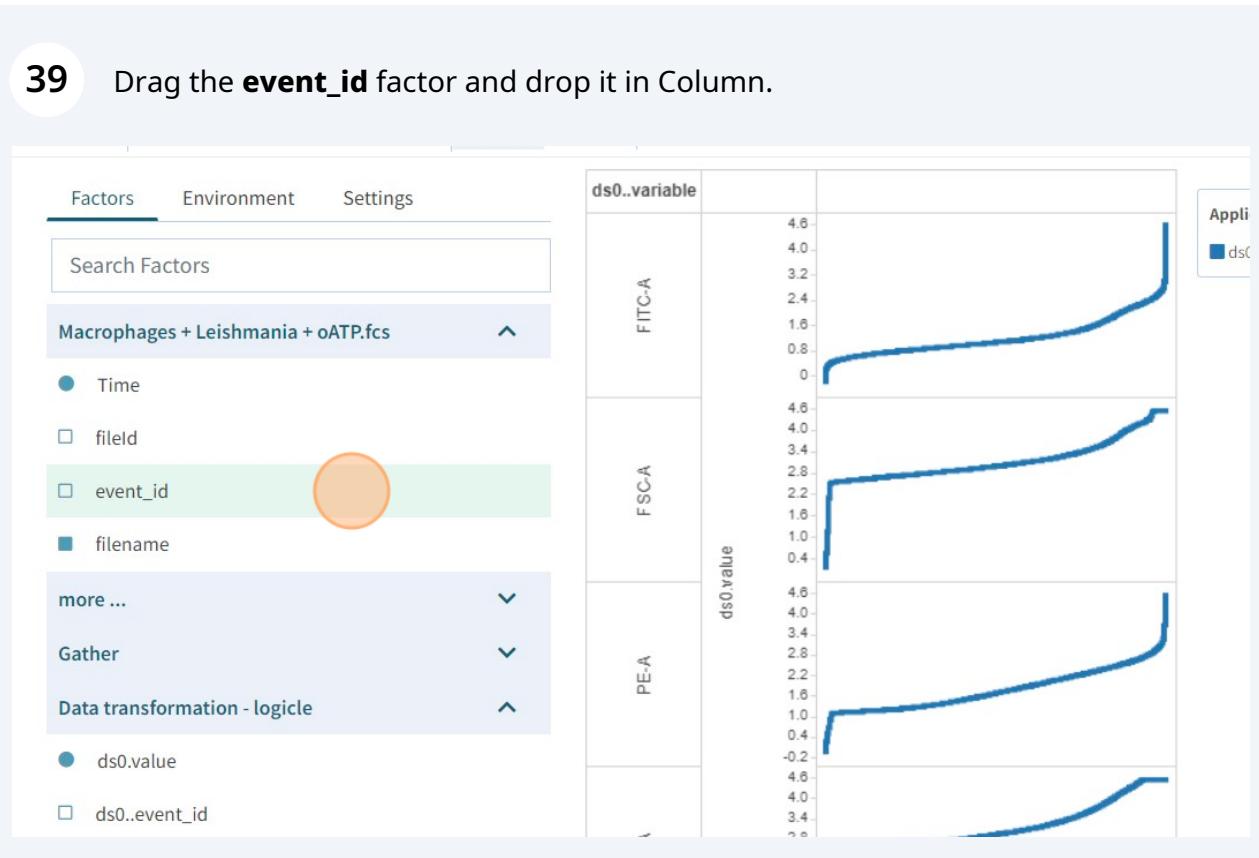
37 Drag the **ds0.variable** factor and drop it in Row.



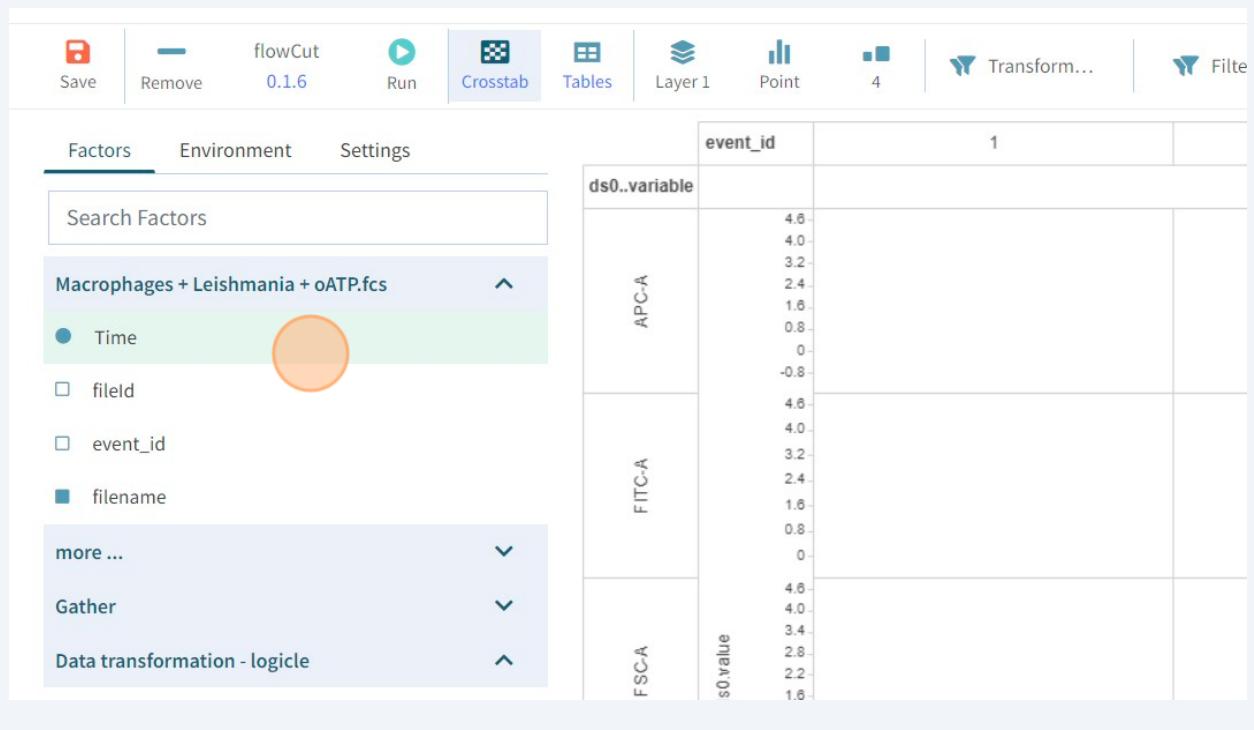
38 Expand the "Macrophages + Leishmania + oATP.fcs" factors list by clicking on it.



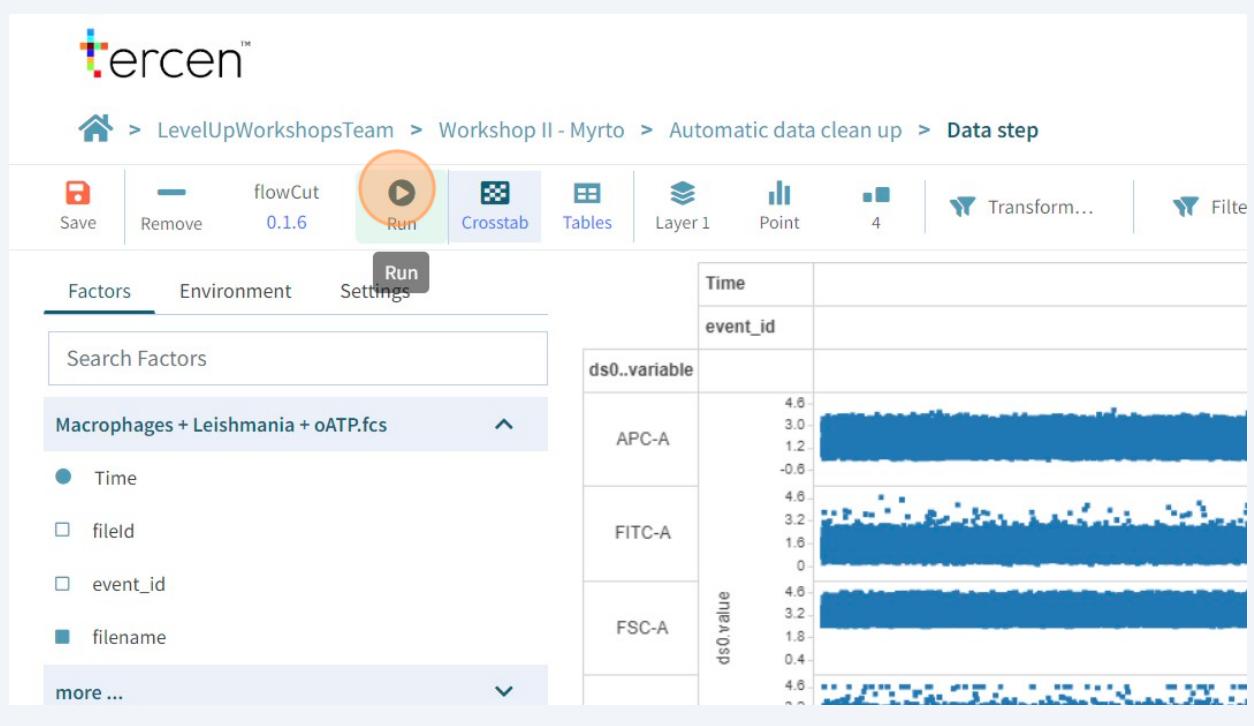
39 Drag the **event_id** factor and drop it in Column.



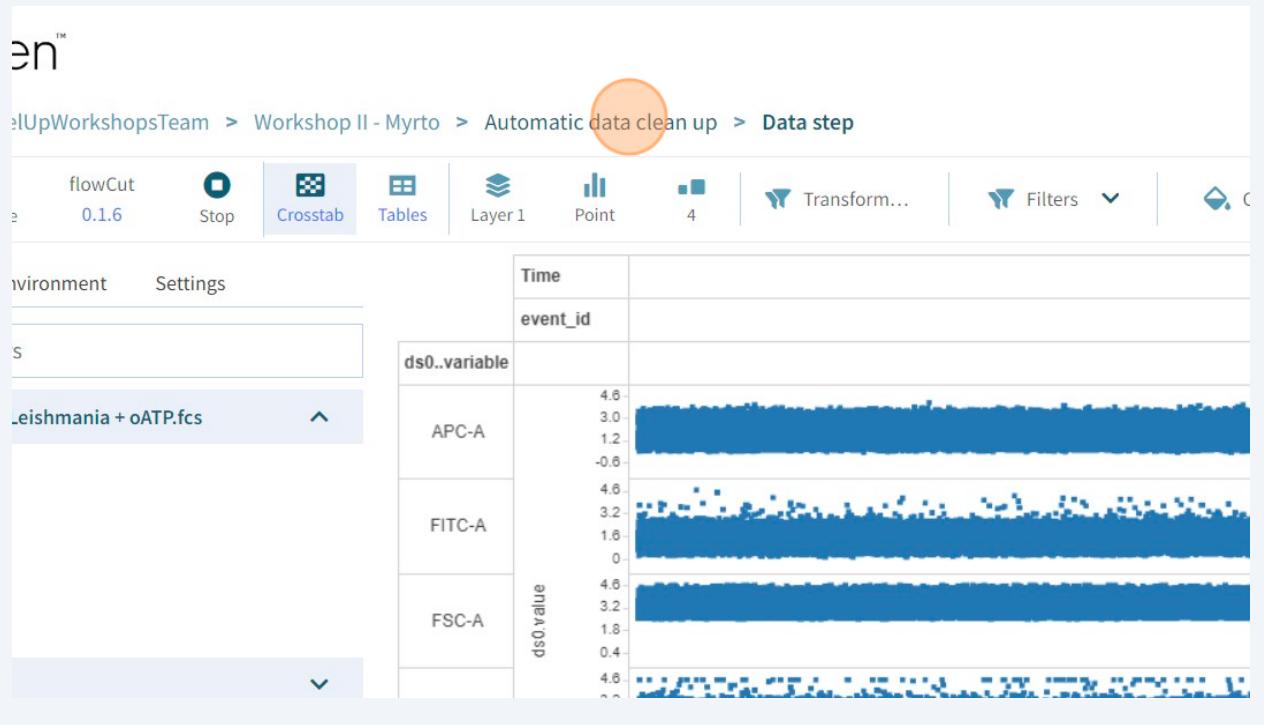
40 Drag the **Time** factor and drop it in Column *ABOVE* the event_id factor.



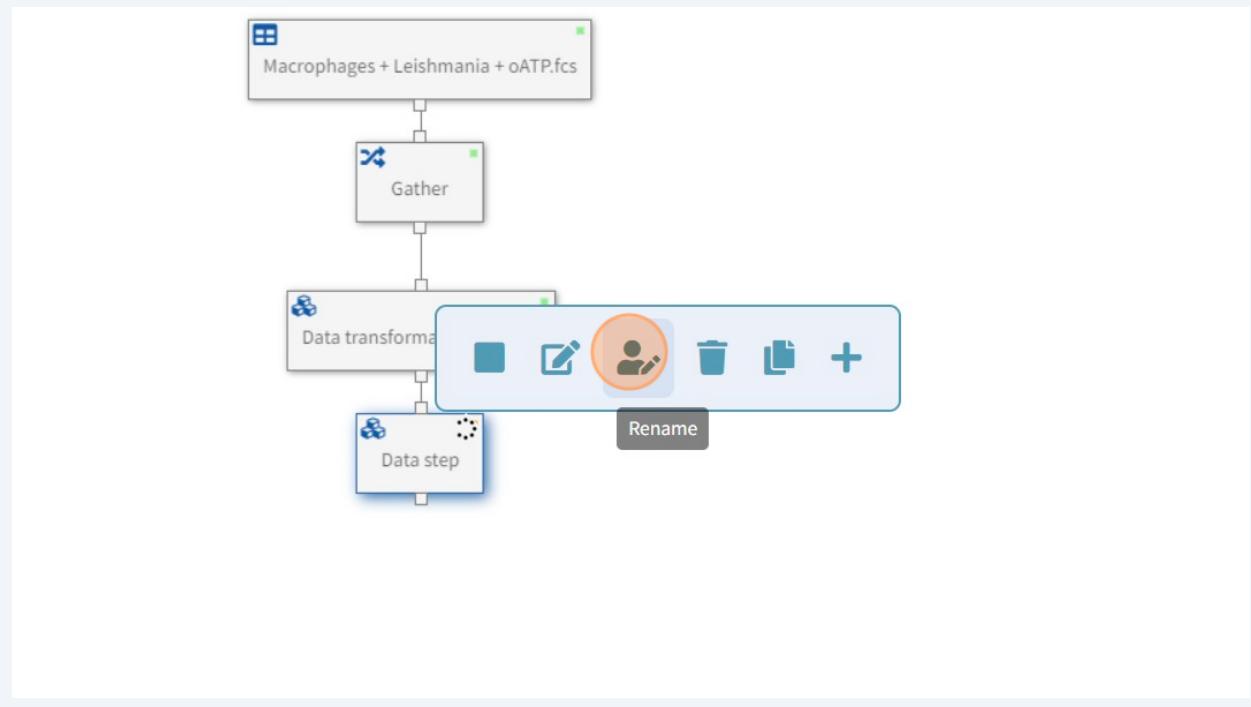
41 Click 'Run' to start the computation.



- 42** Click the "Automatic data clean up" link to go back to the workflow.



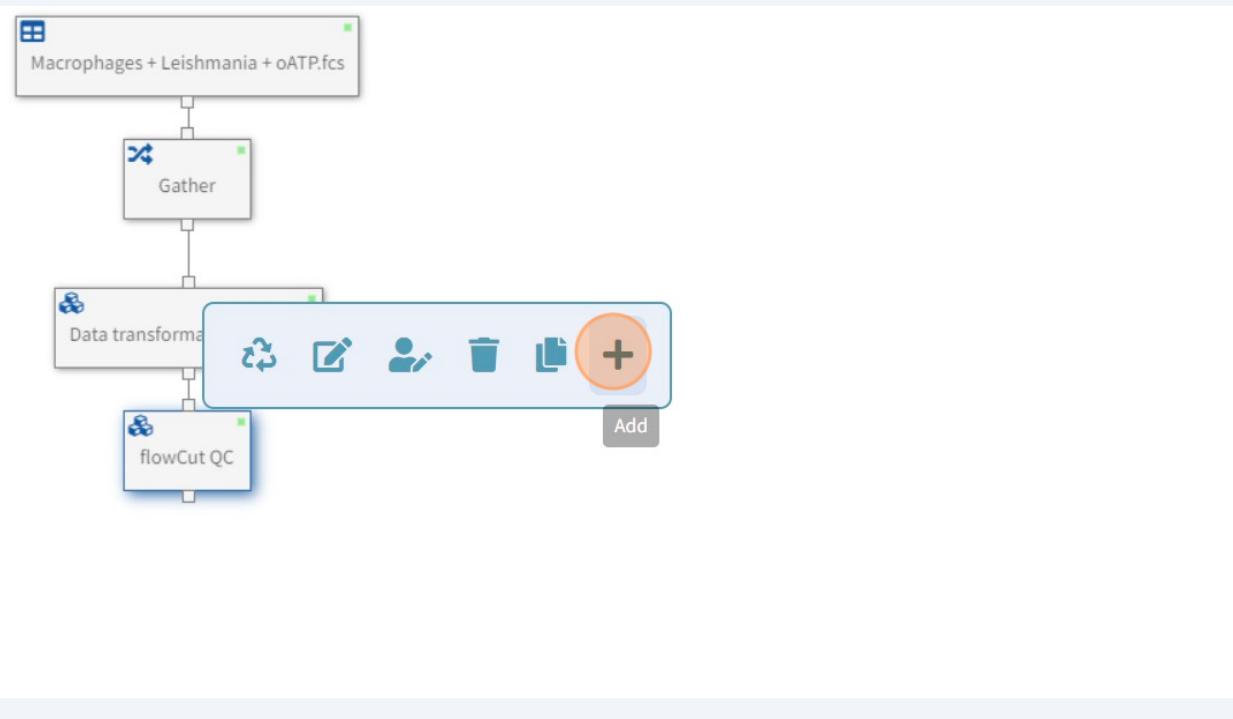
- 43** Rename the Data step to "flowCut QC".



44 Finally, you will build a data step to check the results of flowCut.

For every event, the algorithm returns a quality control flag that can be pass or fail and is stored in the **QC_flag** factor.

Click 'Add'.



45 Select "Data step".

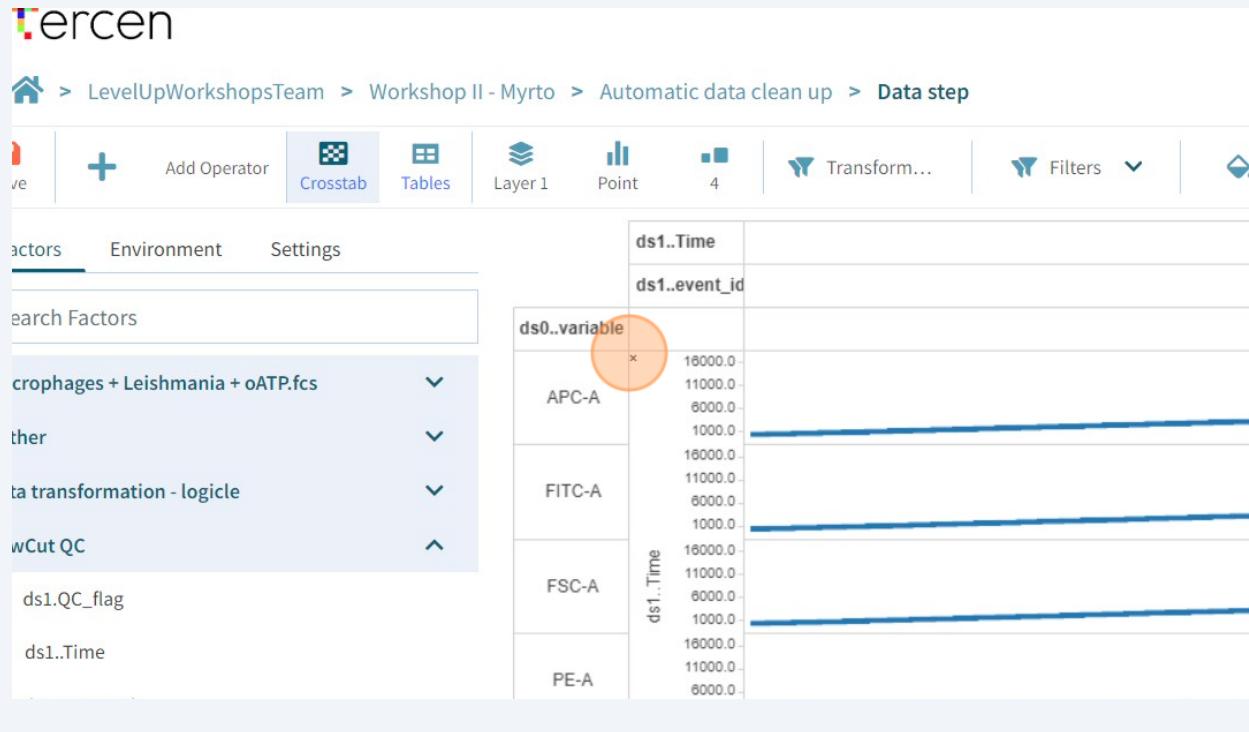
Step	Operator	Operator Library	Installed Apps	App Library
<input type="text" value="Data step"/>				

Data step data
Perform computation on user defined projection

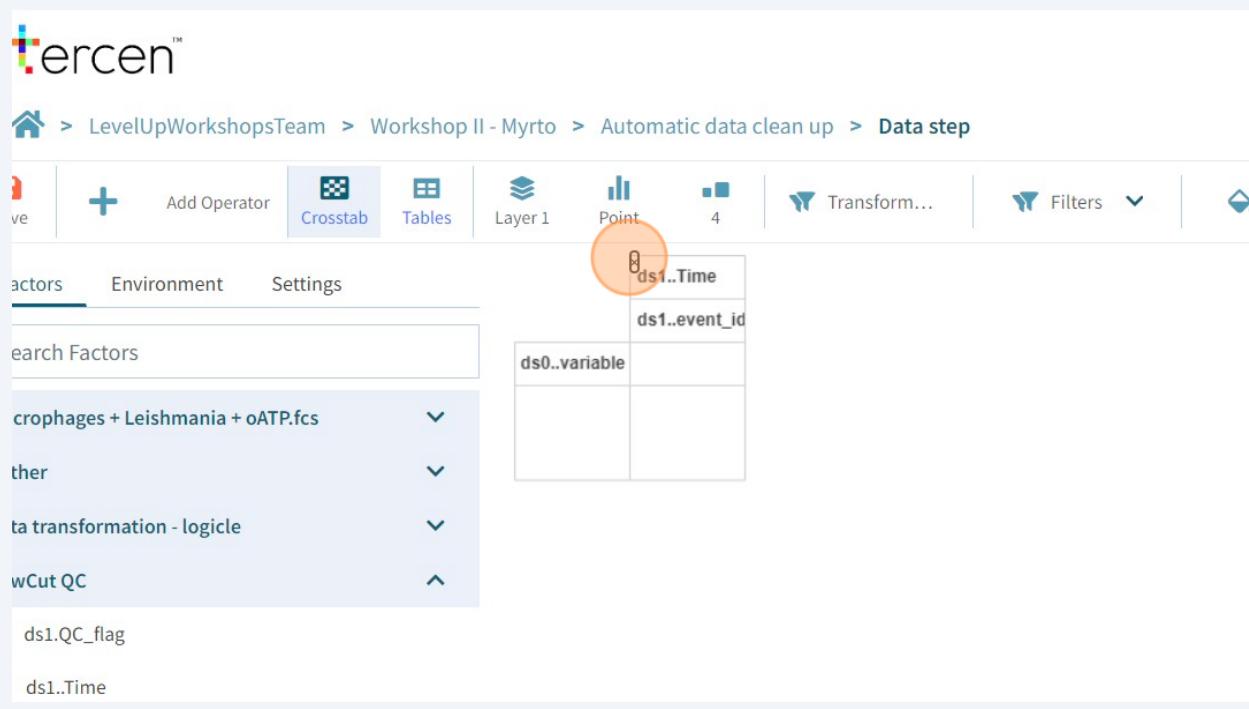
Multi data step data
Perform computation on user defined projection

Join leftTable
Join two data sets

- 46 Clear the **ds1..Time** factor from the Y-Axis by clicking the "x" button when you mouse over it.



- 47 Do the same thing for Column.



- 48** Click the "Data transformation - logicle" data step to expand its factors list.

The screenshot shows the Cytobank software interface. At the top, there are several icons: Save, Add Operator, Crosstab (highlighted in blue), Tables, Layer 1, Point, 4, Transform..., and Filters. Below these are three tabs: Factors (selected), Environment, and Settings. A search bar labeled 'Search Factors' is present. A list of data steps is shown: 'Macrophages + Leishmania + oATP.fcs' (with a dropdown arrow), 'Gather' (with a dropdown arrow), 'Data transformation - logicle' (highlighted with an orange circle), and 'flowCut QC' (with a dropdown arrow). Under 'Data transformation - logicle', there are three items: 'ds1.QC_flag' (checkbox), 'ds1..Time' (radio button), and 'ds1..event_id' (checkbox). To the right, there is a table with columns 'ds1..event_id' and 'ds0..variable'. The 'ds0..variable' column contains five entries: APC-A, FITC-A, FSC-A, PE-A, and SSC-A.

ds1..event_id	ds0..variable
	APC-A
	FITC-A
	FSC-A
	PE-A
	SSC-A

- 49** Drag the **ds0.value** and drop it in the Y-axis.

This screenshot shows the same Cytobank interface as the previous one, but the 'Data transformation - logicle' data step is now expanded. The 'ds0.value' item is highlighted with an orange circle and is being dragged over the Y-axis area. The rest of the interface and the table on the right remain the same as in the previous screenshot.

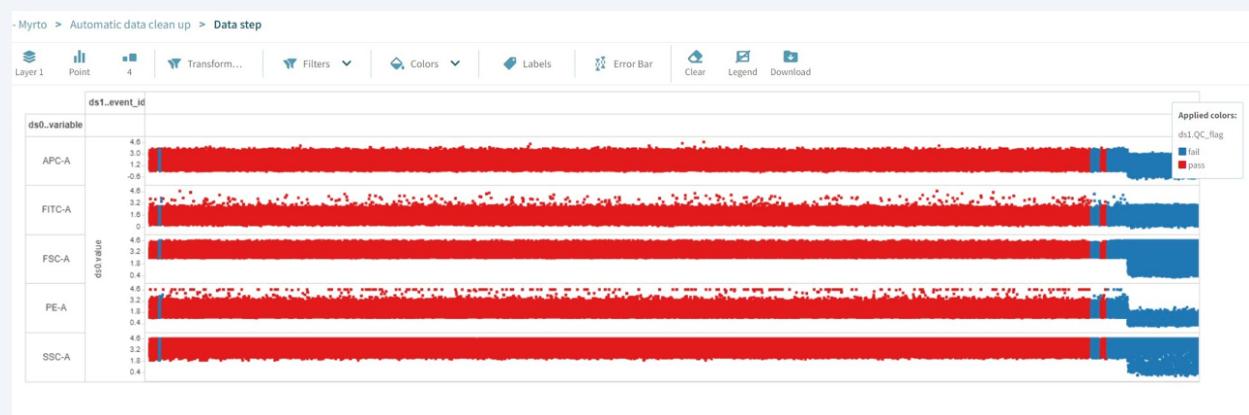
ds1..event_id	ds0..variable
	APC-A
	FITC-A
	FSC-A
	PE-A
	SSC-A

50 Let us know the color of every event based on the QC flag reported by flowCut.

Drag the **ds1.QC_flag** factor and drop it in Colors.

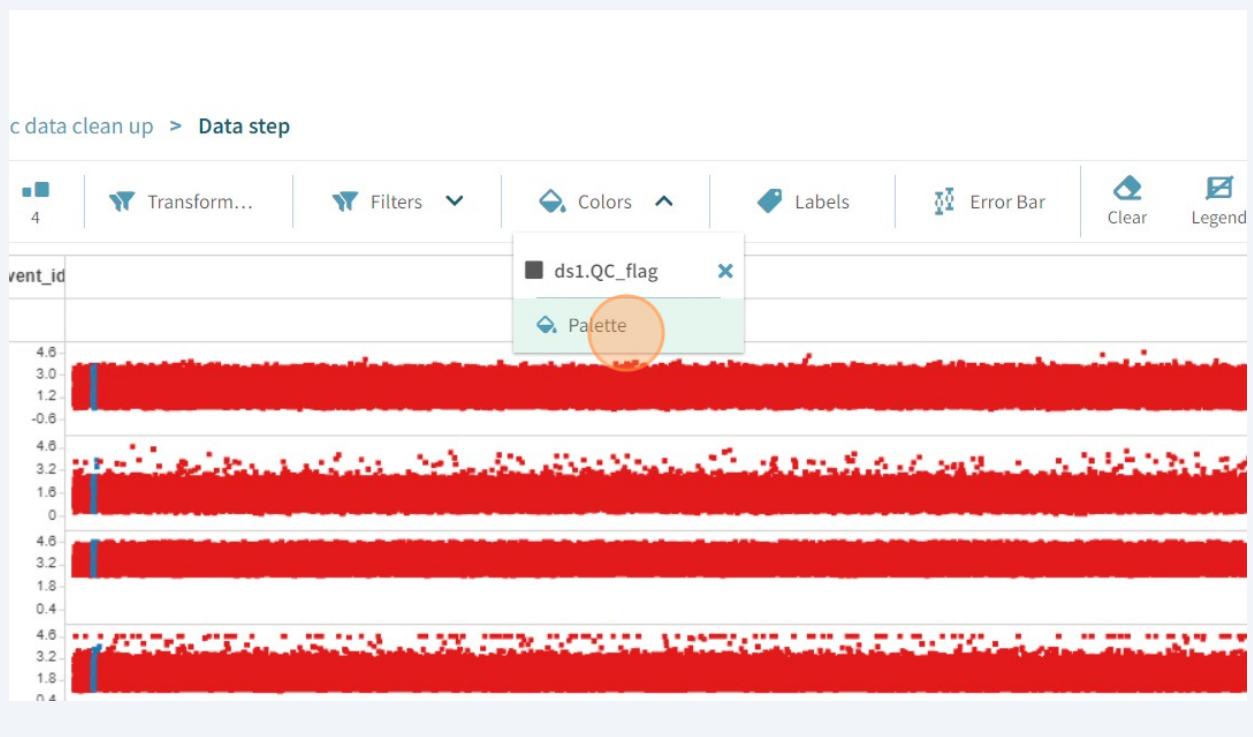
The screenshot shows the 'Gather' panel in the Myrto software. Under 'Data transformation - logic', there is a dropdown menu. The option 'ds1.QC_flag' is highlighted with an orange circle. Other options in the dropdown include 'ds0.value', 'ds0.event_id', 'ds0.variable', 'ds1..Time', and 'ds1..event_id'.

51 Notice how the data points have changed color depending on the flowCut result. Each event has been marked with a pass or fail flag.

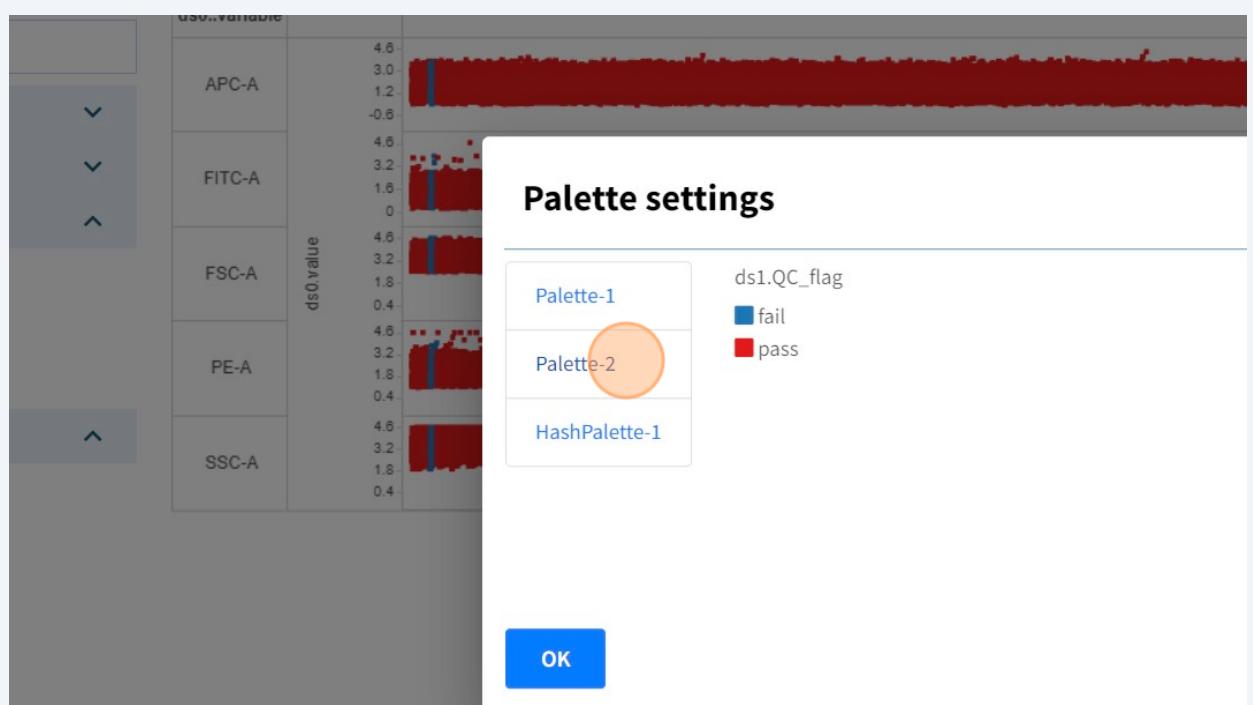


52 Unfortunately, the default choice of colors (red for pass and blue for fail) is not intuitive in this case. Let us change those.

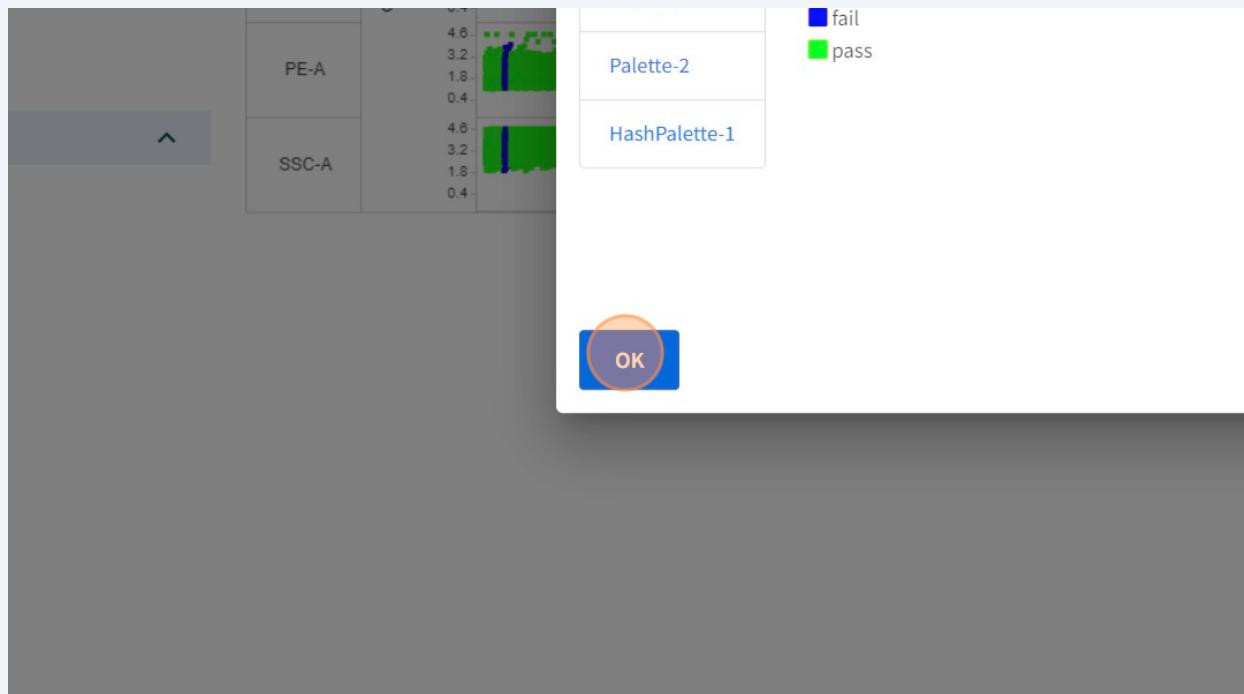
- 53** Mouse over the Colors button and click 'Palette'.



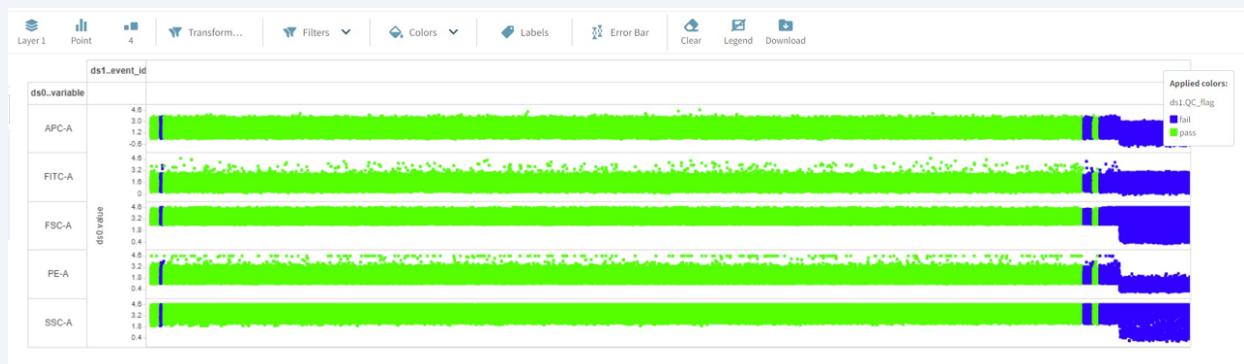
- 54** Select "Palette-2".



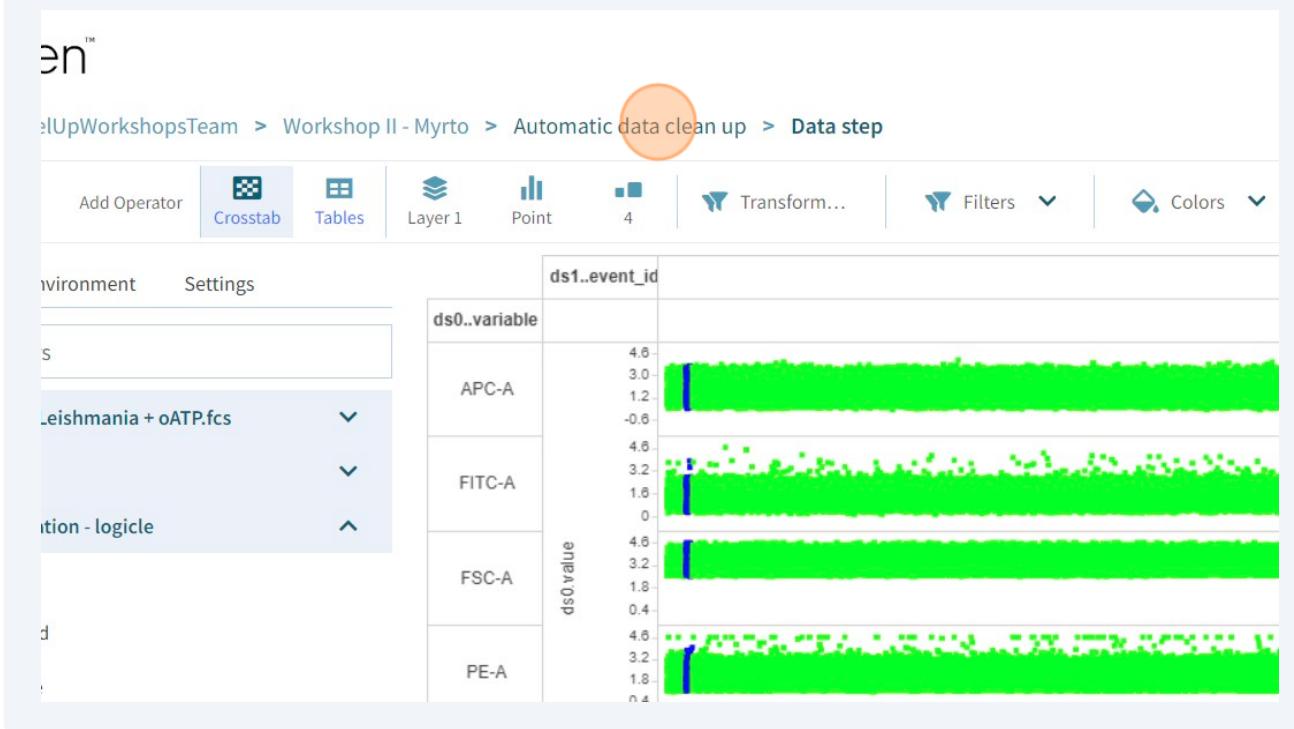
55 Click "OK".



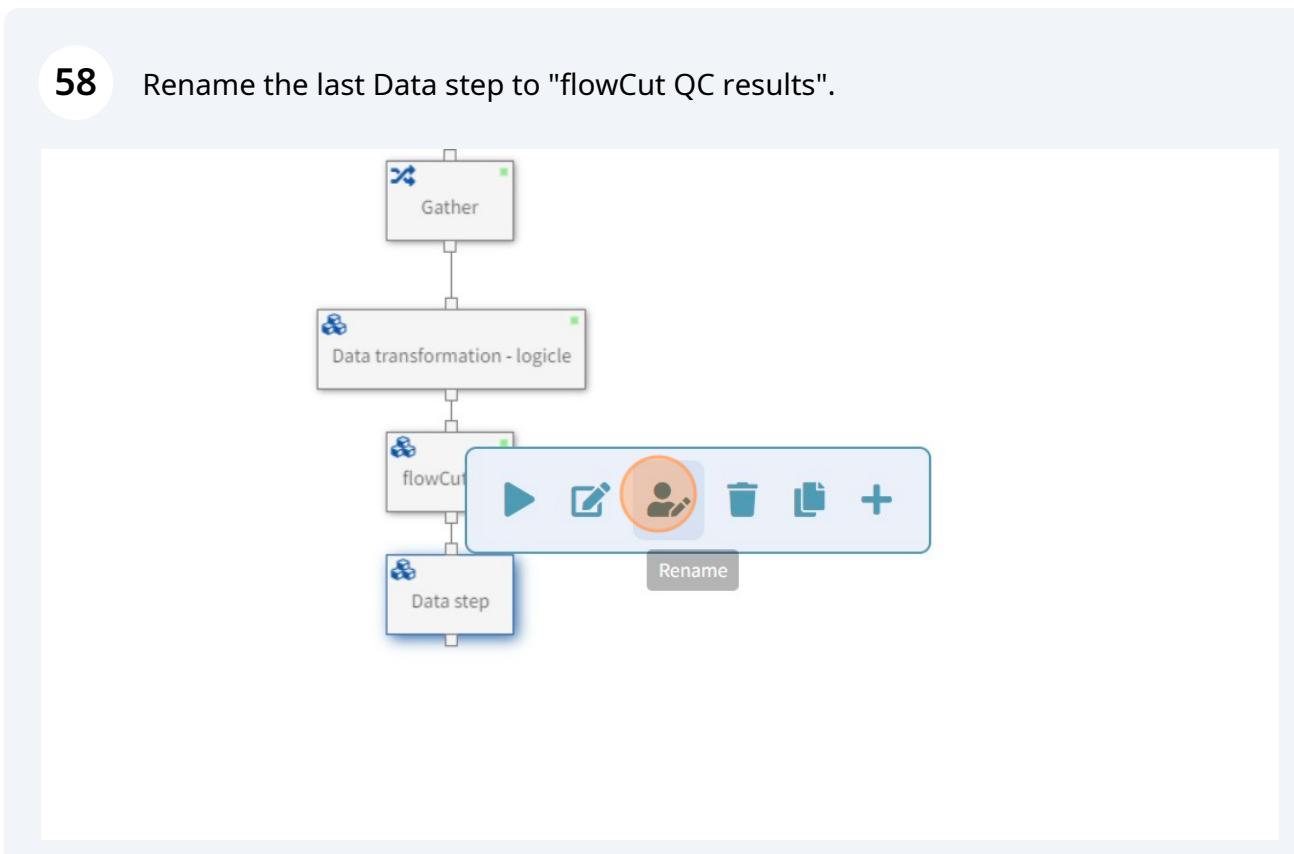
56 Now it is much better!



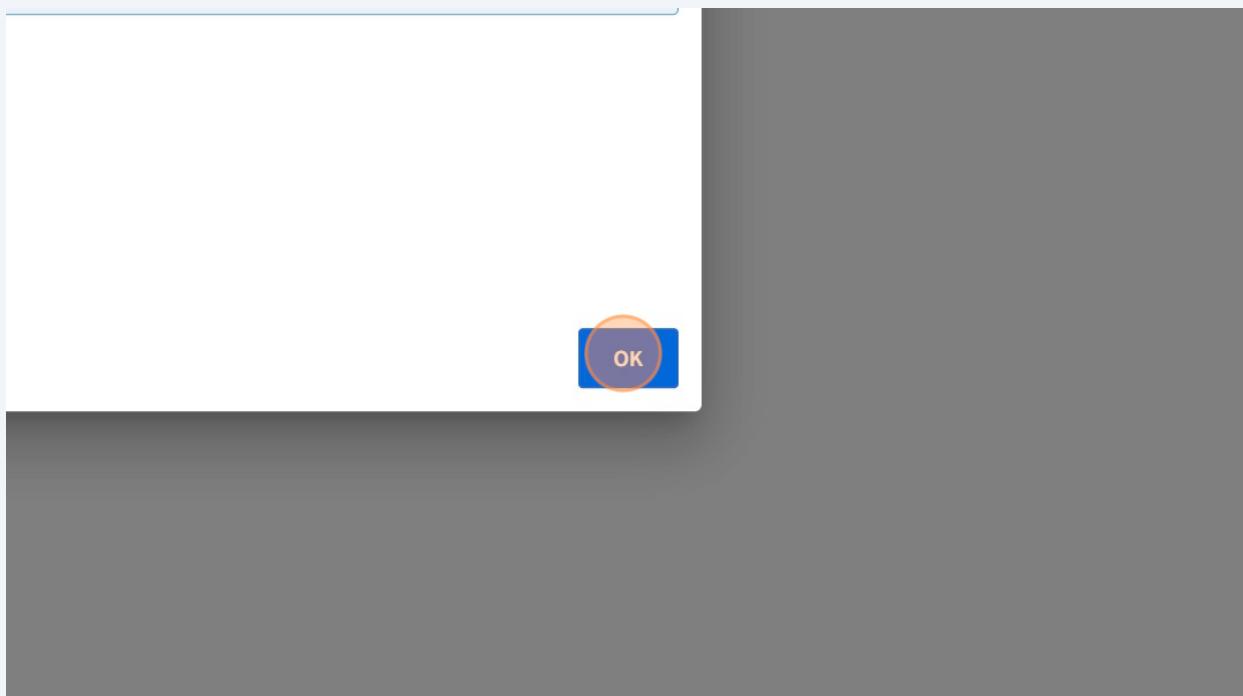
- 57** Click the "Automatic data clean up" link to return to the workflow.



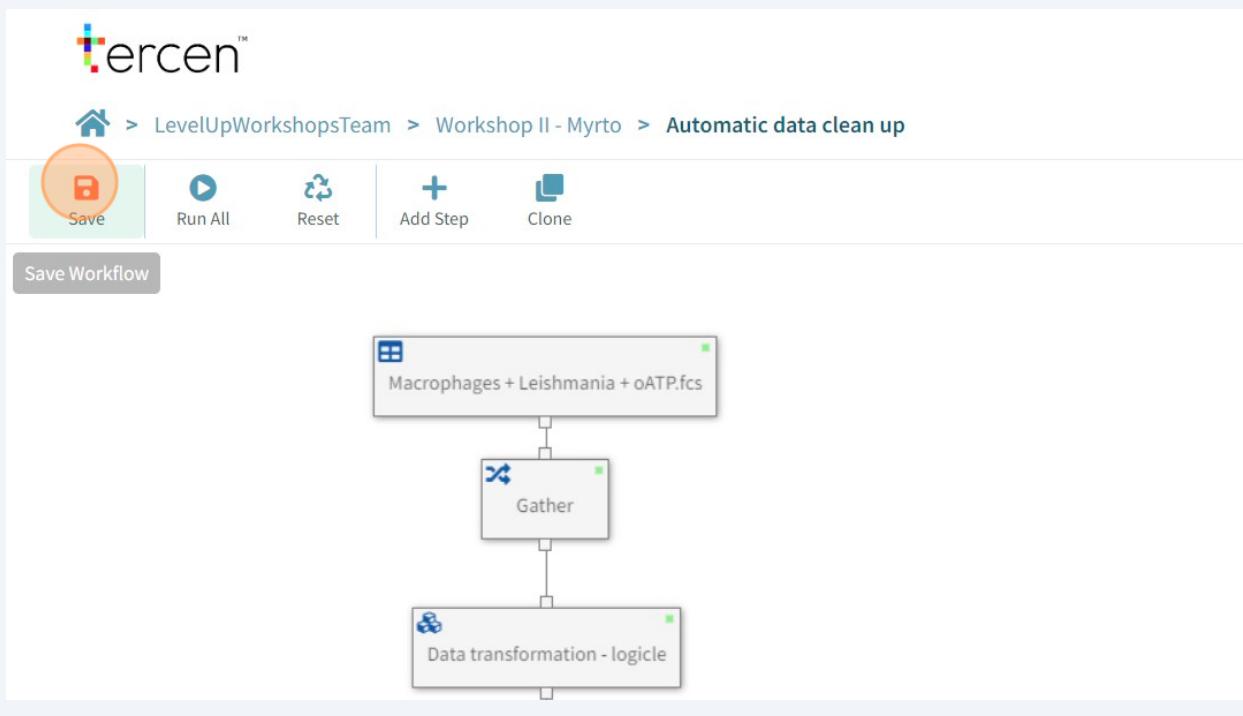
- 58** Rename the last Data step to "flowCut QC results".



59 Click "OK".



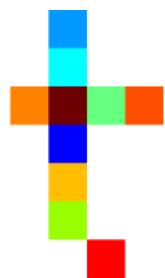
60 Do not forget to save your progress by clicking the Save button on the leftmost side of the Global toolbar.



61

This guide has shown you how to automatically clean your FCS files.

0208 - Downsample Data



- 1 Navigate to the top level of "Workshop II"

tercen™

Home > LevelUpWorkshopsTeam > Workshop II

LevelUpWorkshopsTeam

Project Activities

Workshop II

No description provided.

New data set New workflow New file Upload file

faris.naji updated workflow **downsample**

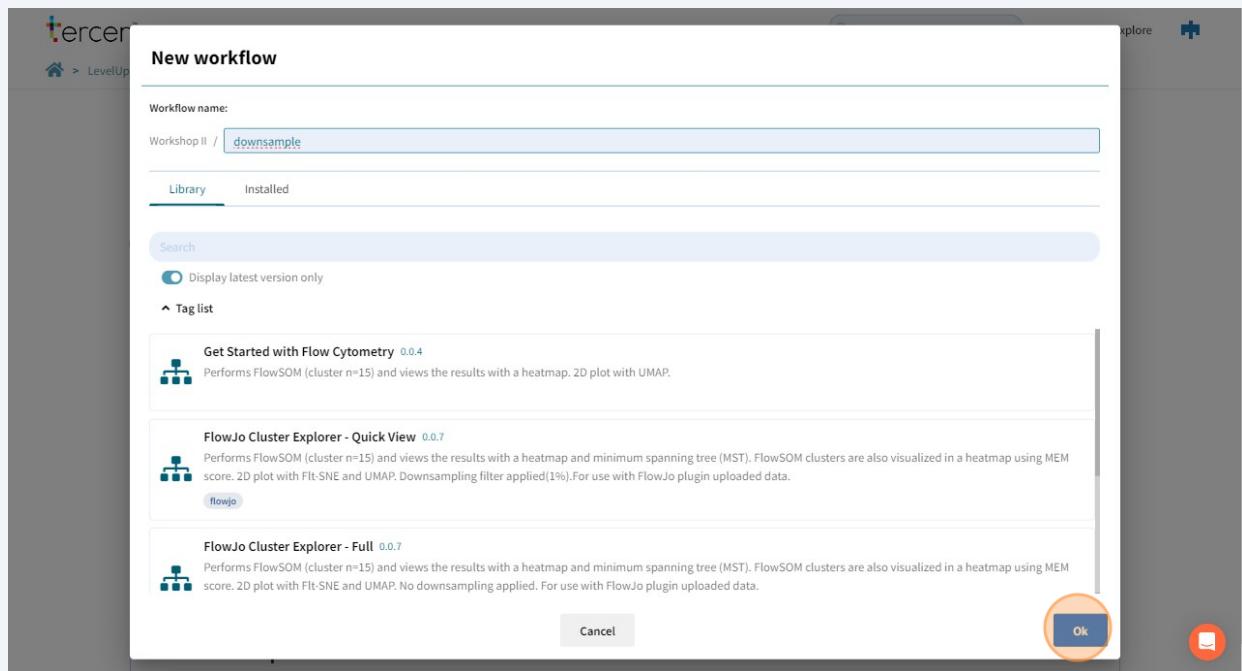
2 Click "New workflow"

The screenshot shows a web-based application interface for a project named "LevelUpWorkshopsTeam". At the top, there's a navigation bar with tabs for "Project" and "Activities". Below the navigation bar, the title "Workshop II" is displayed with a lock icon, followed by the note "No description provided.". A horizontal toolbar contains several icons: "New data set", "New workflow" (which is highlighted with an orange circle), "New file", and "Upload file". Below the toolbar, a blue header bar shows a profile icon and the text "faris.naji updated workflow downsample". The main content area lists several items: "README.md", "FCS Annotations", "Example Files", and "Example Data".

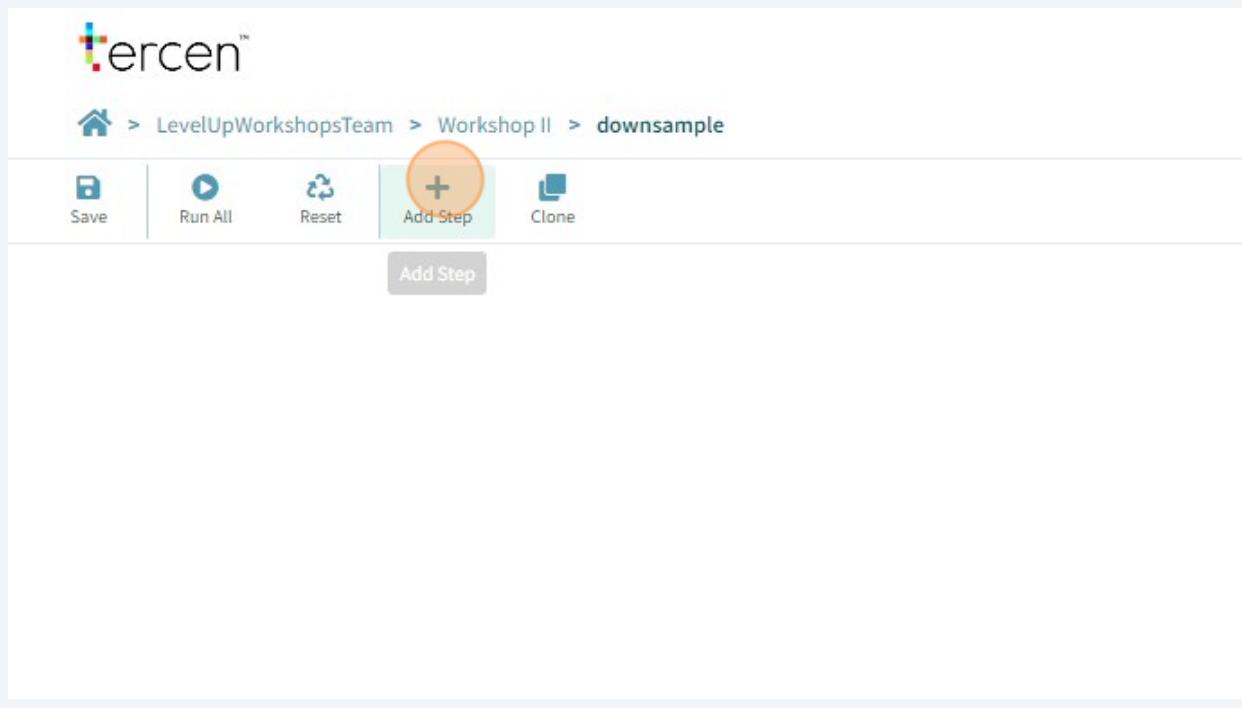
3 Give the name "downsample"

The screenshot shows a modal dialog titled "New workflow". It has a "Workflow name:" input field containing "Workshop II / Name your workflow ...". Below the input field are two tabs: "Library" (which is selected) and "Installed". At the bottom of the dialog is a "Search" input field.

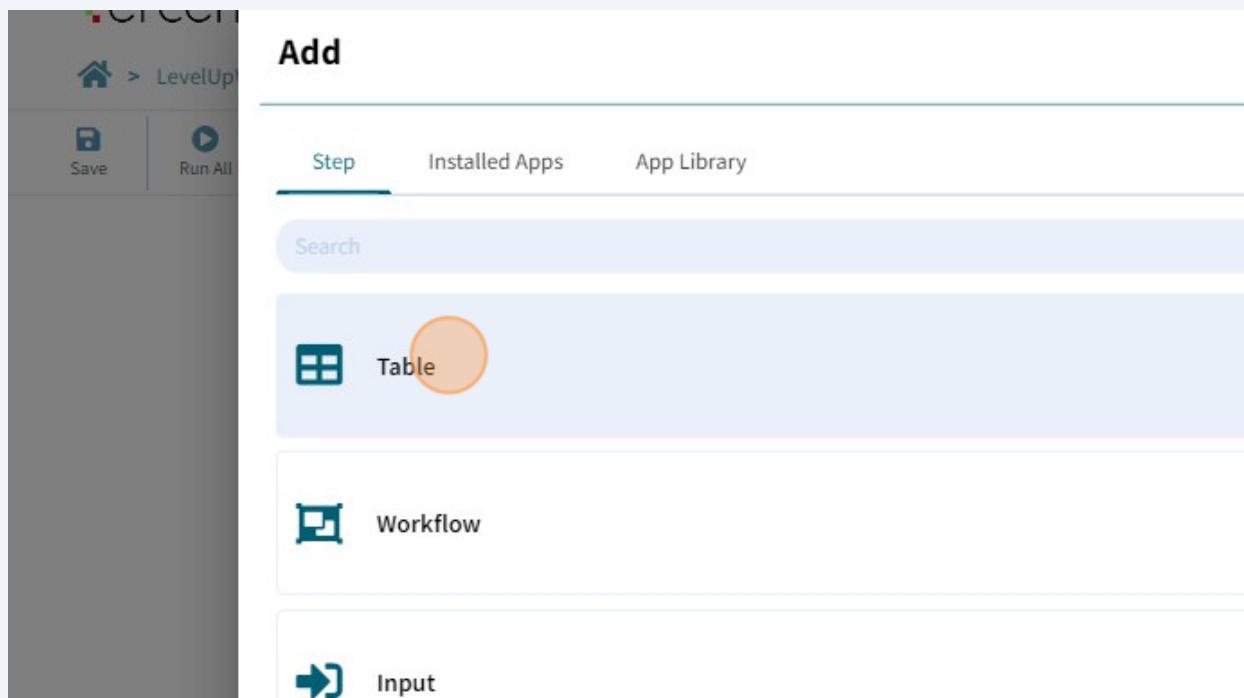
4 Click "Ok"



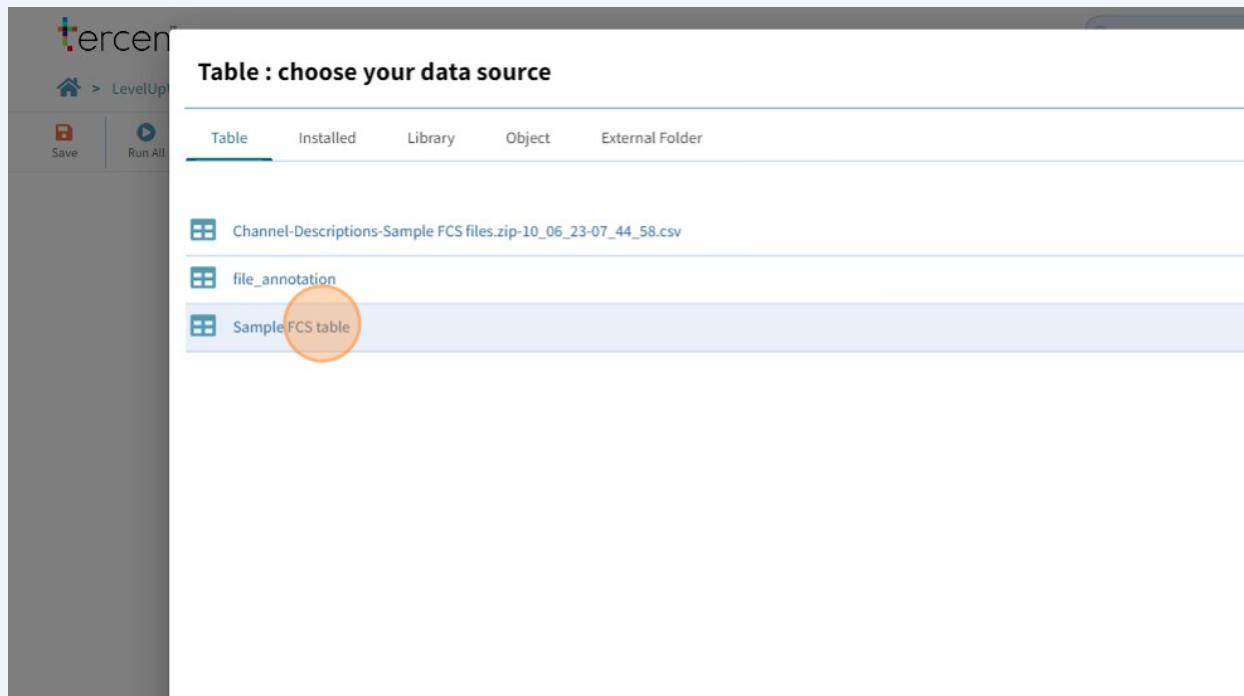
5 We will begin to create a workflow from scratch. Click on the **Add Step** icon



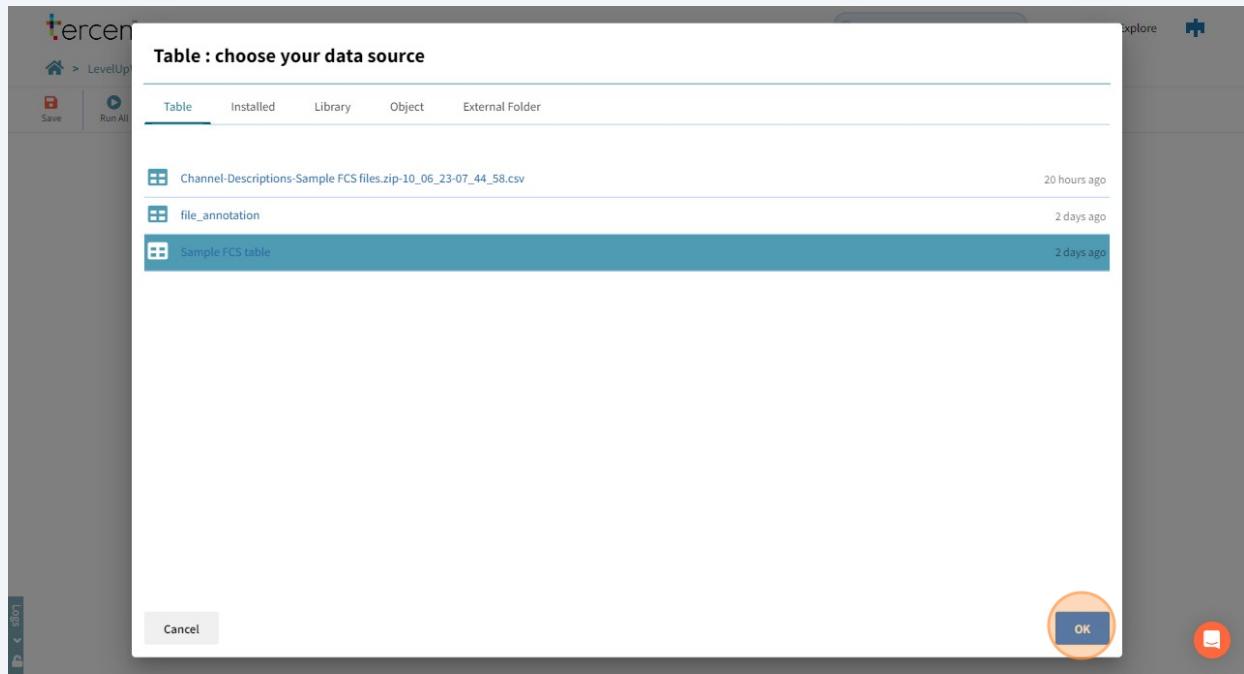
6 Select Table by Click on it.



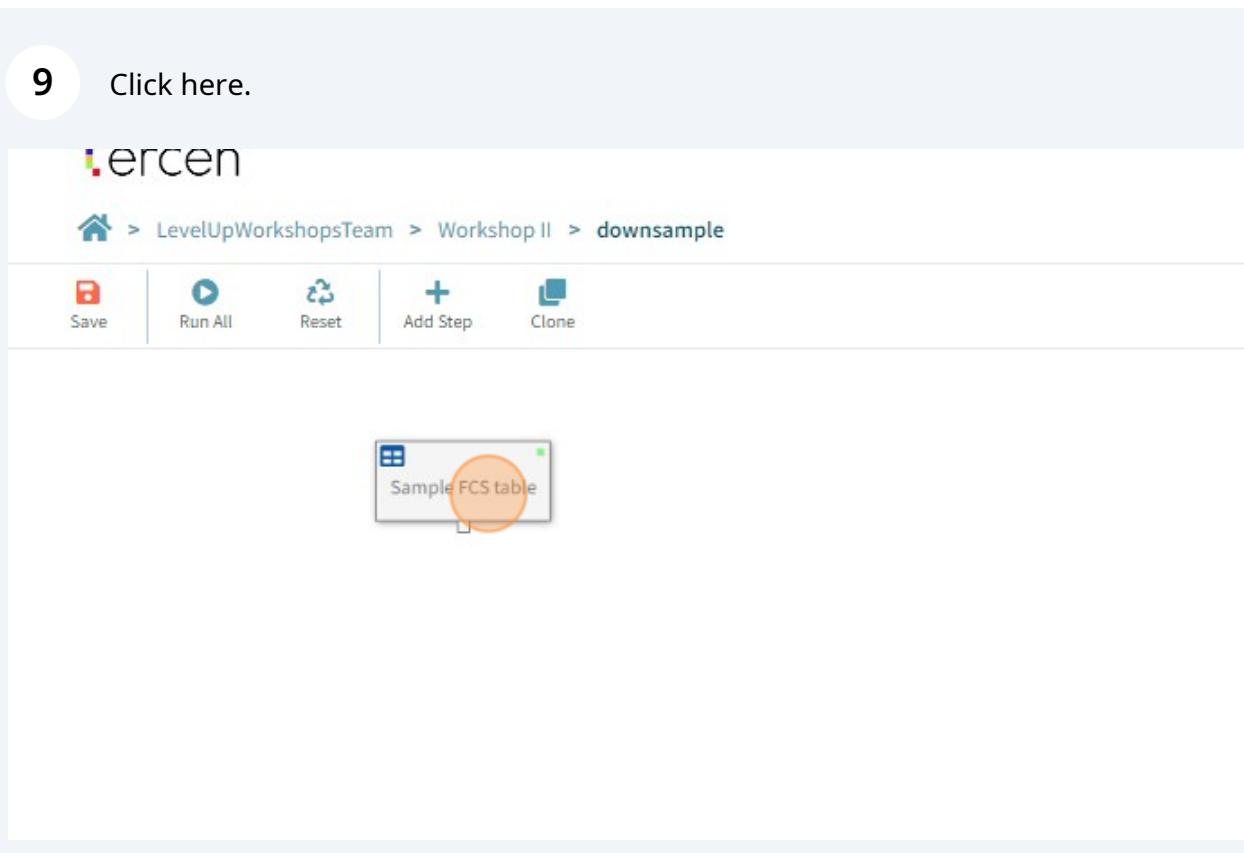
7 Click "Sample FCS table"



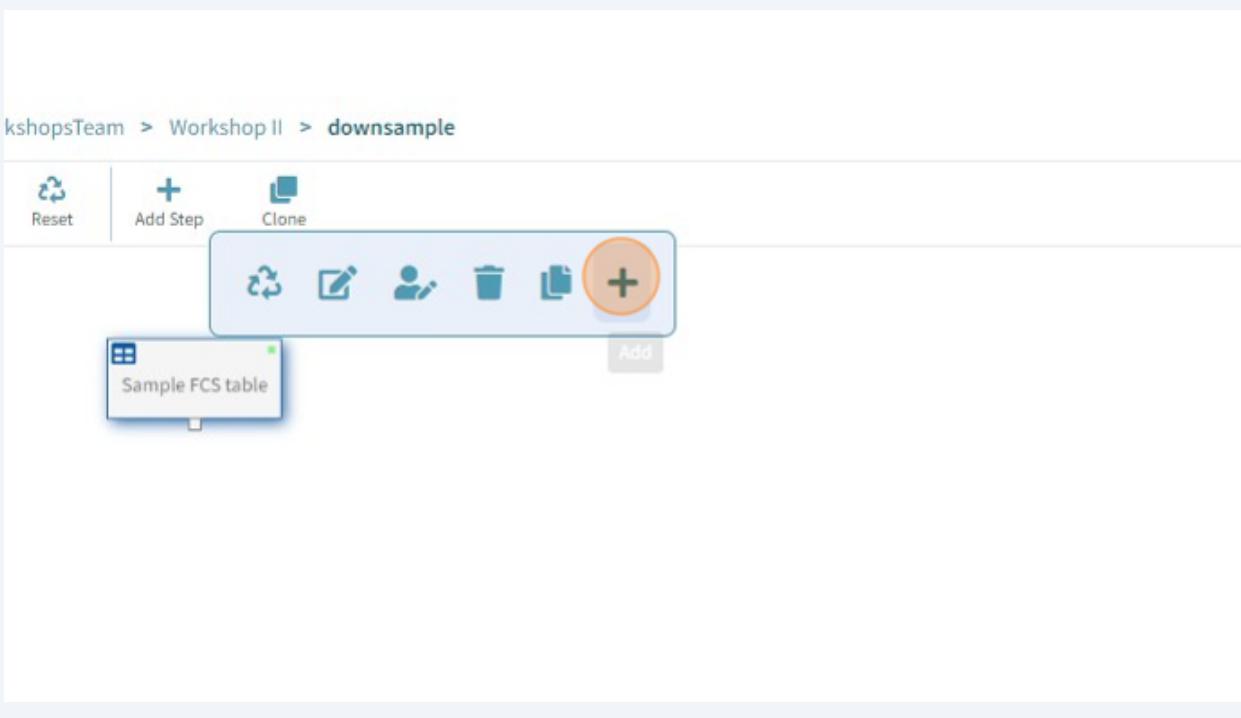
8 Click "OK"



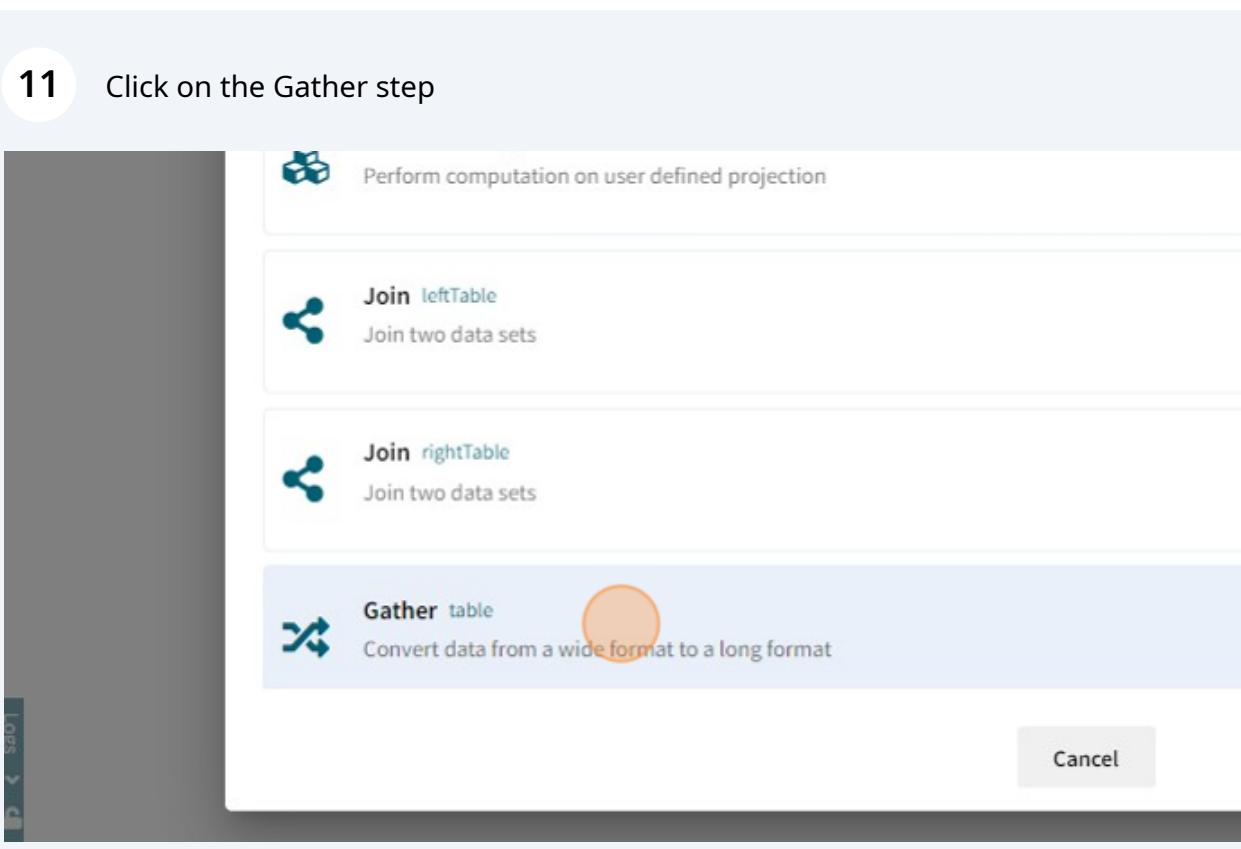
9 Click here.



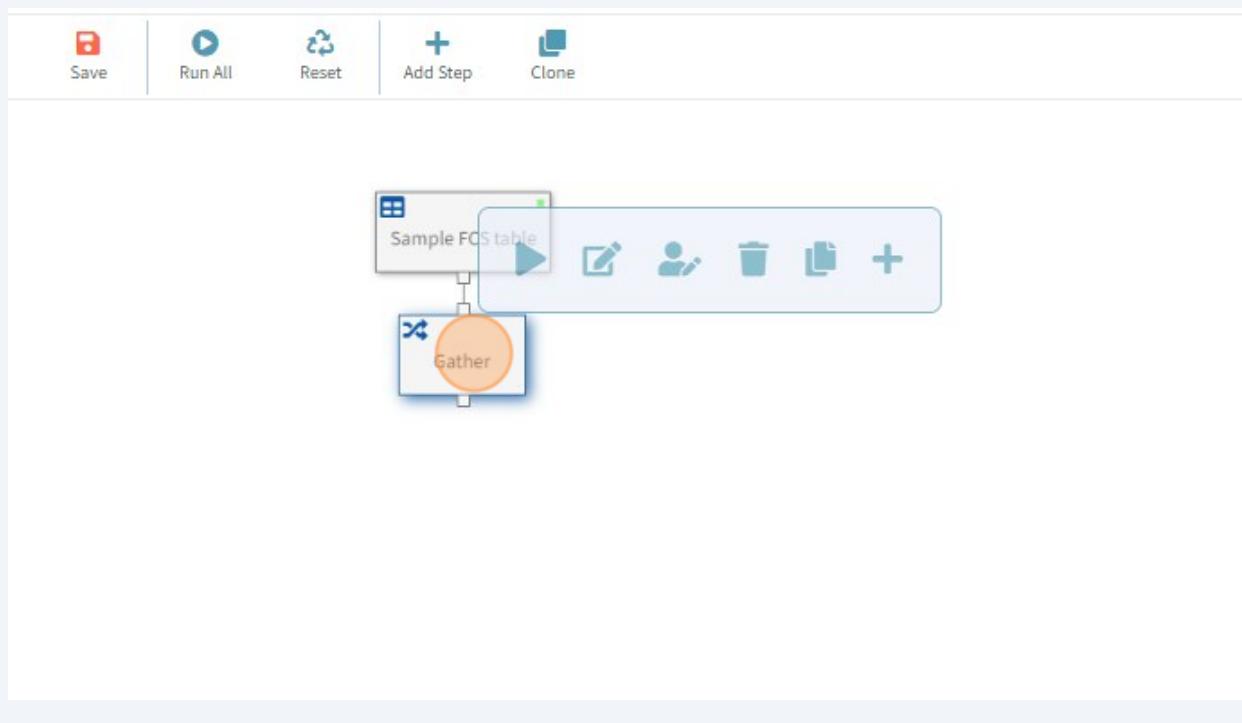
10 Click here.



11 Click on the Gather step



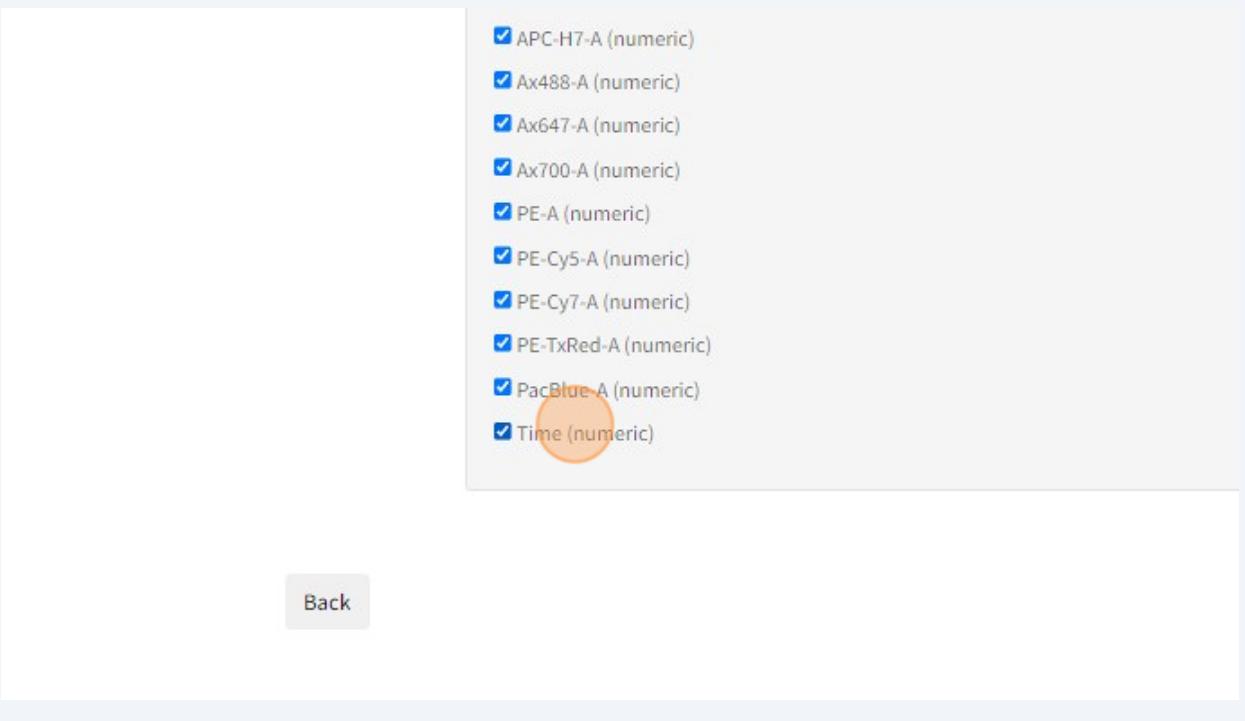
12 Double-click here.



13 Click "Select all"

The screenshot shows a 'Selection pattern' dialog box. At the top, it says 'Selection pattern' and has a large empty text input field. Below that, 'Factor type' is listed with two options: 'Numeric' (which is checked) and 'Character' (which is unchecked). Under 'Factors', there is a section titled 'Select all' which contains a checkbox that is also highlighted with an orange circle. Below this section, a list of factors is shown with individual checkboxes next to each: 'FSC-A (numeric)', 'FSC-H (numeric)', 'SSC-A (numeric)', 'AARD-A (numeric)', 'APC-H7-A (numeric)', and 'Ax488-A (numeric)'.

14 Click "Time (numeric)"

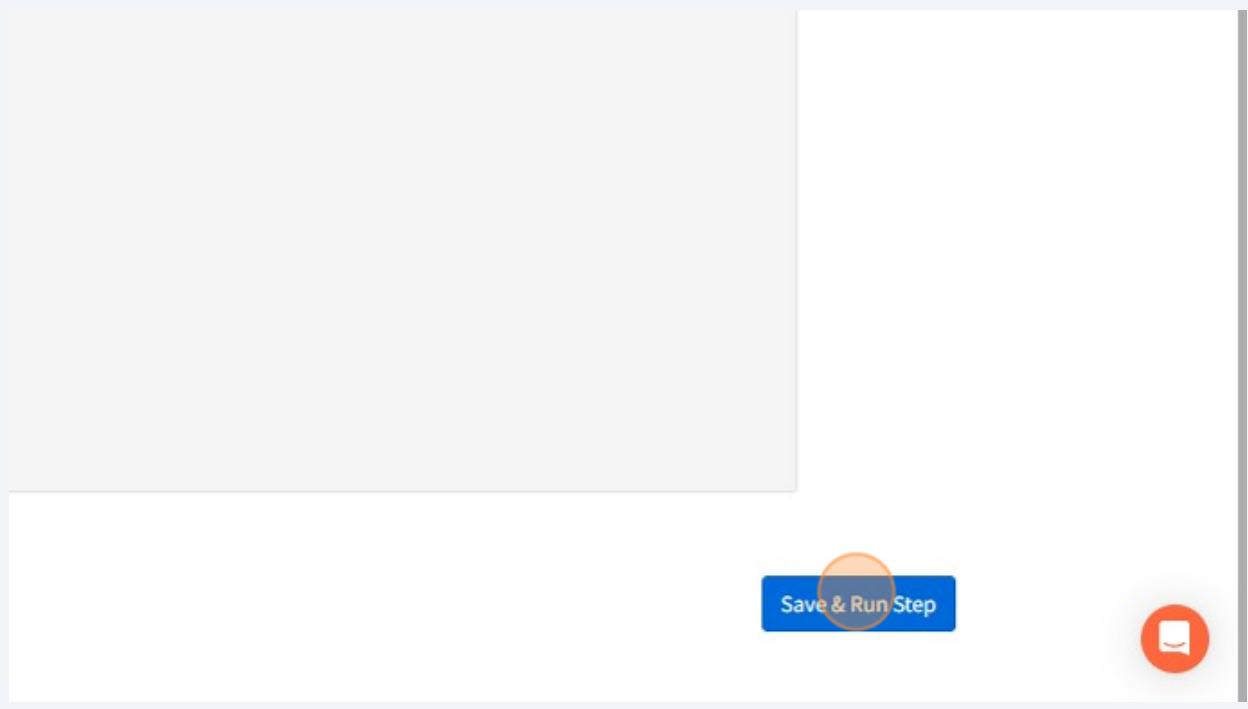


Back

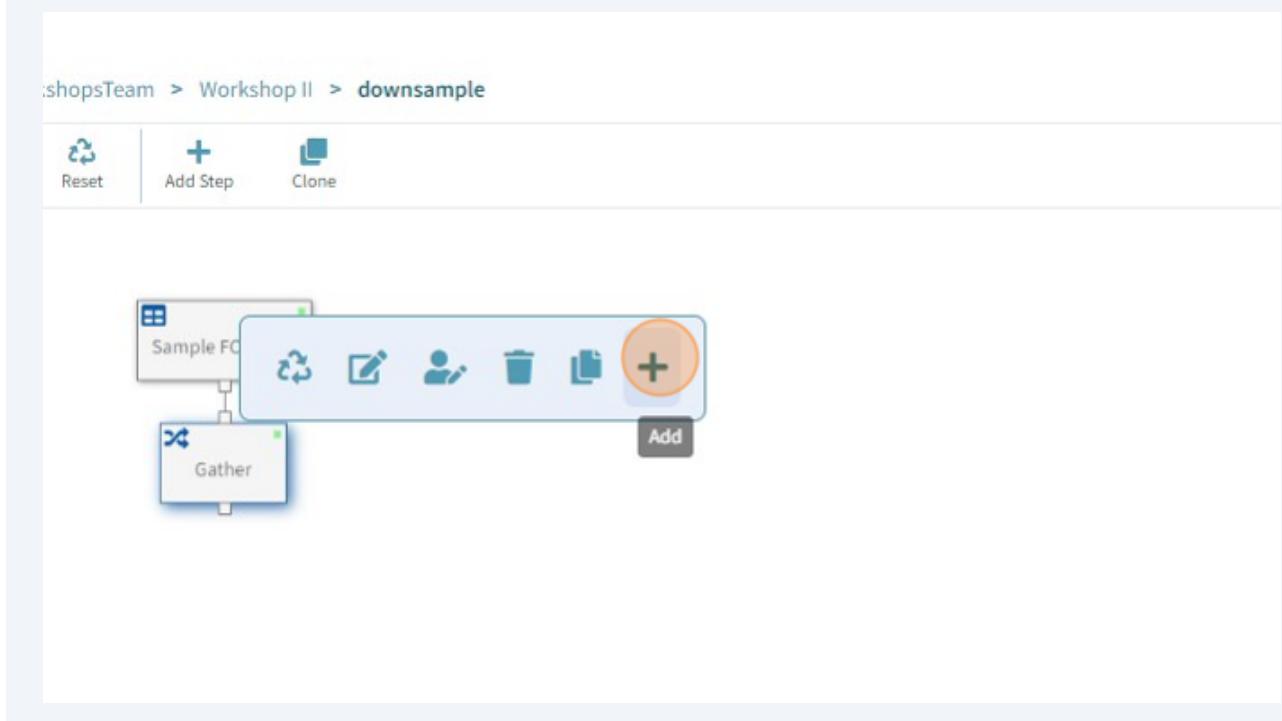


The Time channel needs to be unchecked since we do not need this to be gathered with the other channels. It represents a different concept. The other channels represent measurements on the biological cells. The time channel represents an annotation of the event.

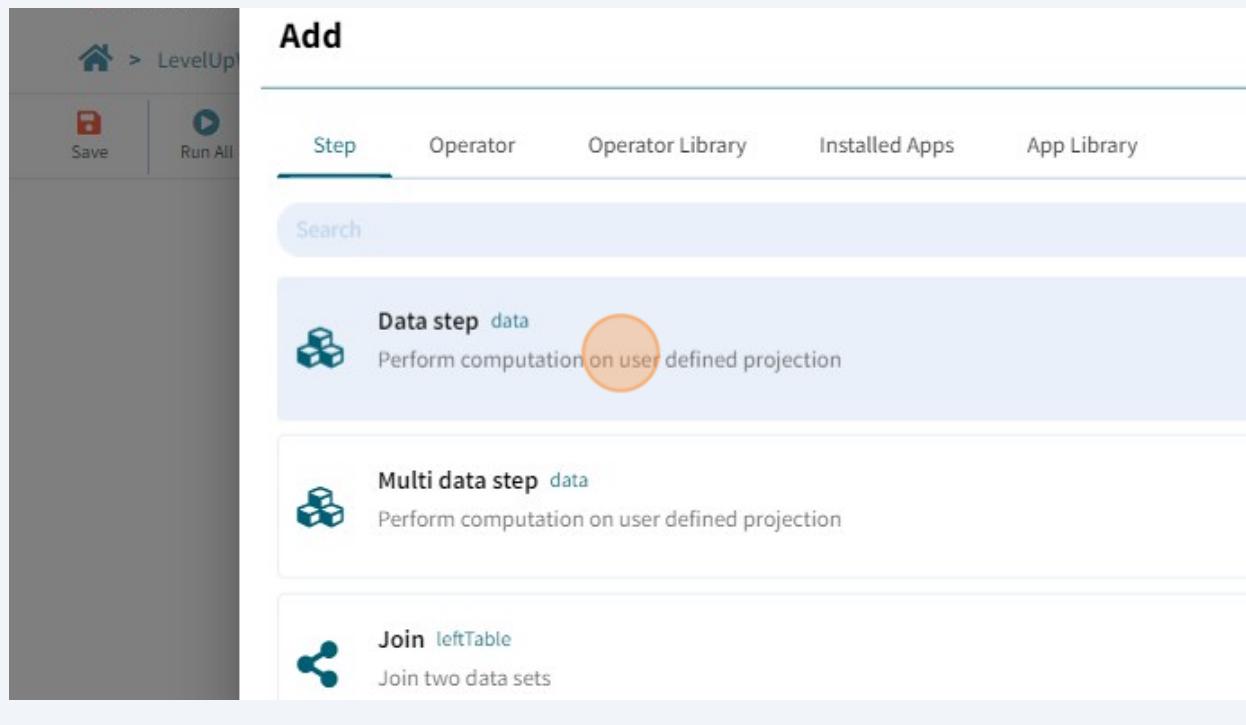
15 Click "Save & Run Step"



16 Click here.



17 Click on "Data step"

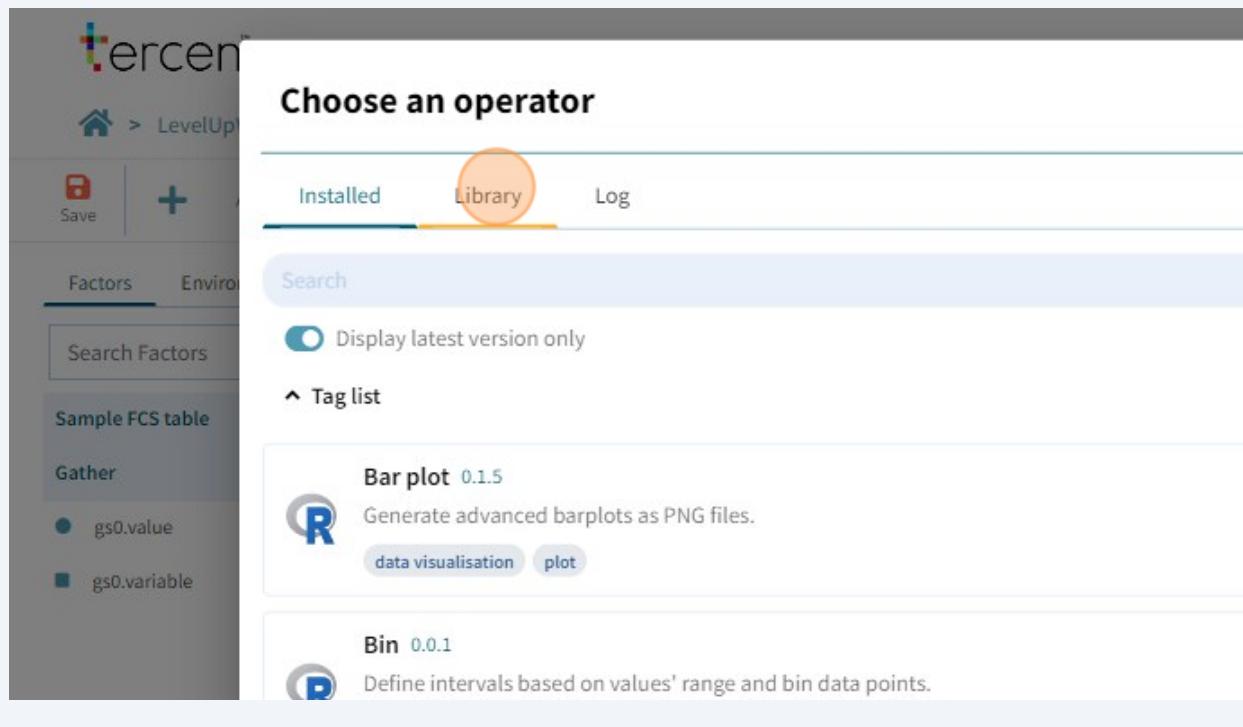


18 Click the large + icon.

This allows us to add an operator.

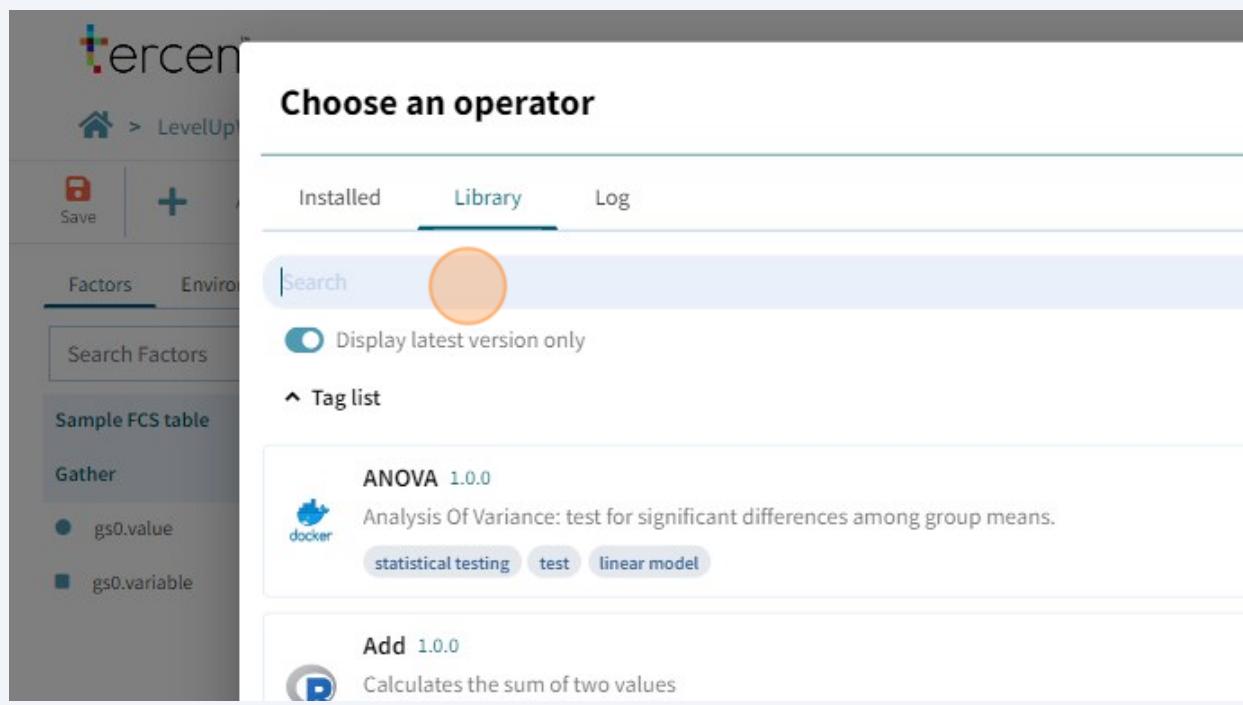
The screenshot shows the 'Data step' configuration screen. At the top, there are buttons for 'Save', 'Add Operator' (which is highlighted with an orange circle), 'Crosstab', 'Tables', 'Layer 1', 'Point', 'Transform...', 'Filters', and a dropdown menu. Below these are tabs for 'Factors', 'Environment', and 'Settings', with 'Factors' selected. A search bar labeled 'Search Factors' is present. A dropdown menu labeled 'Sample FCS table' is open, showing 'Gather' and two items: 'gs0.value' and 'gs0.variable'. To the right is a large, empty table grid.

19 Click "Library"

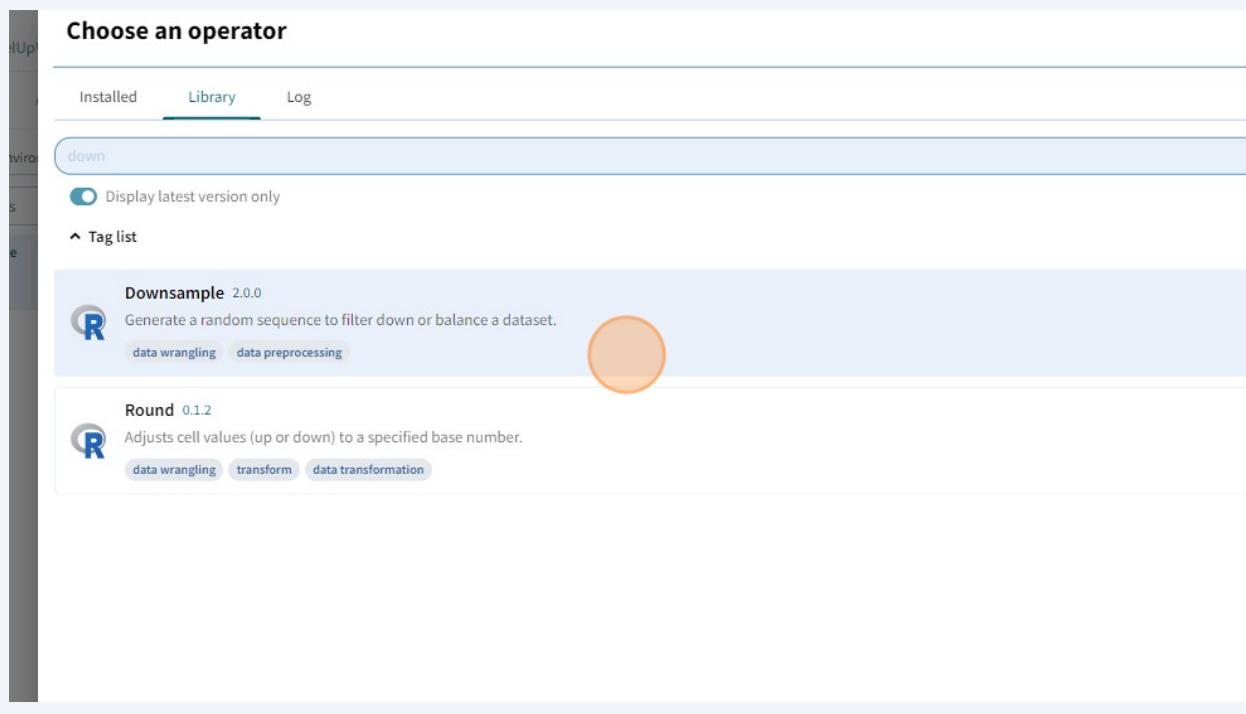


20 Click the "Search" field.

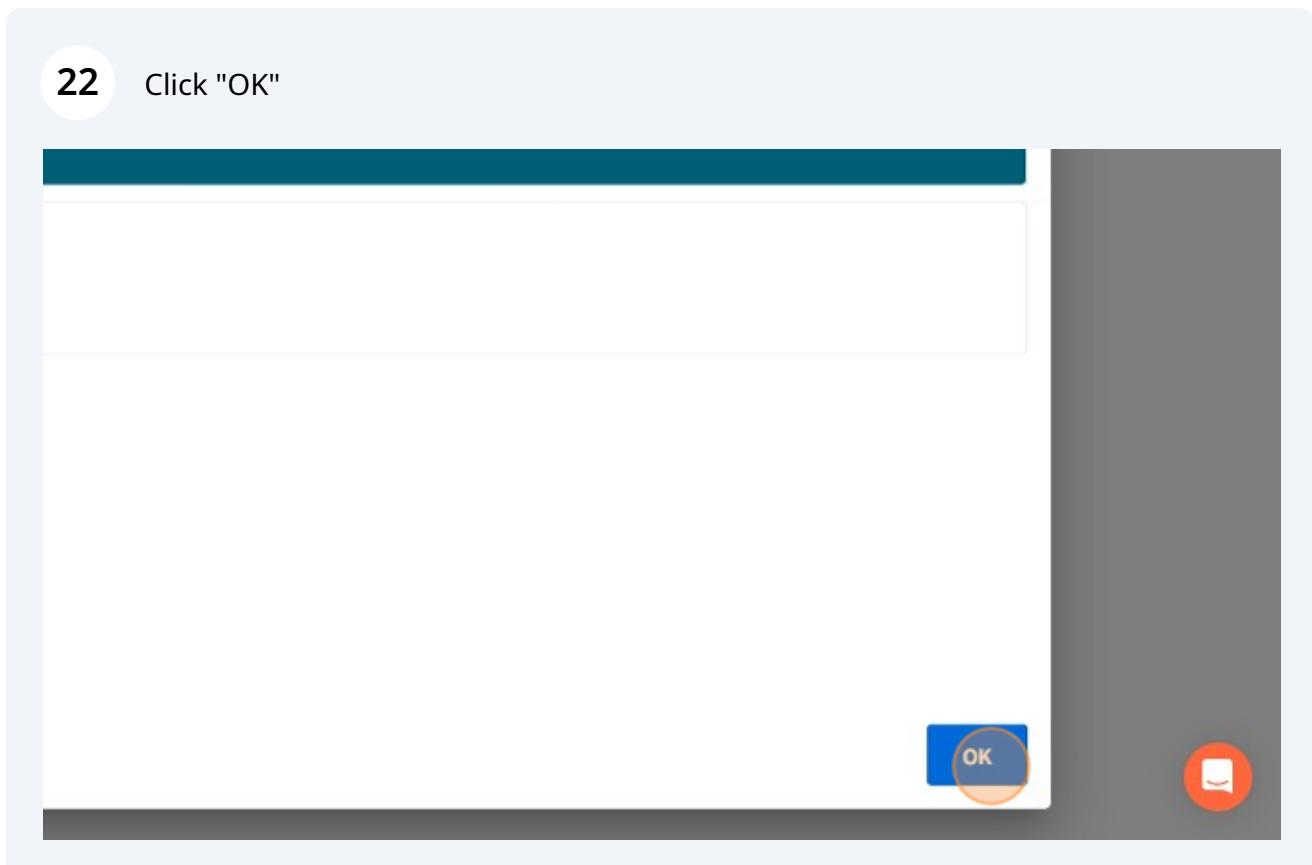
and type "down" this will return a list of operators with the word "down" in it.



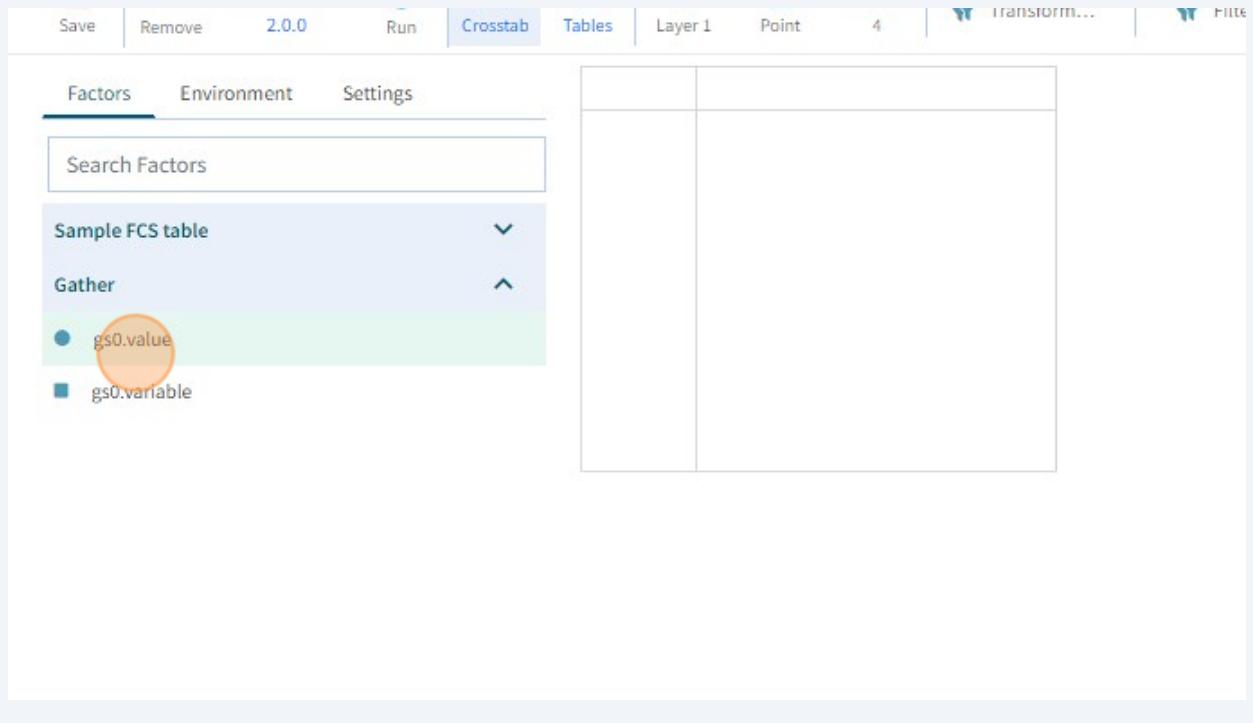
21 Select the "Downsample" operator



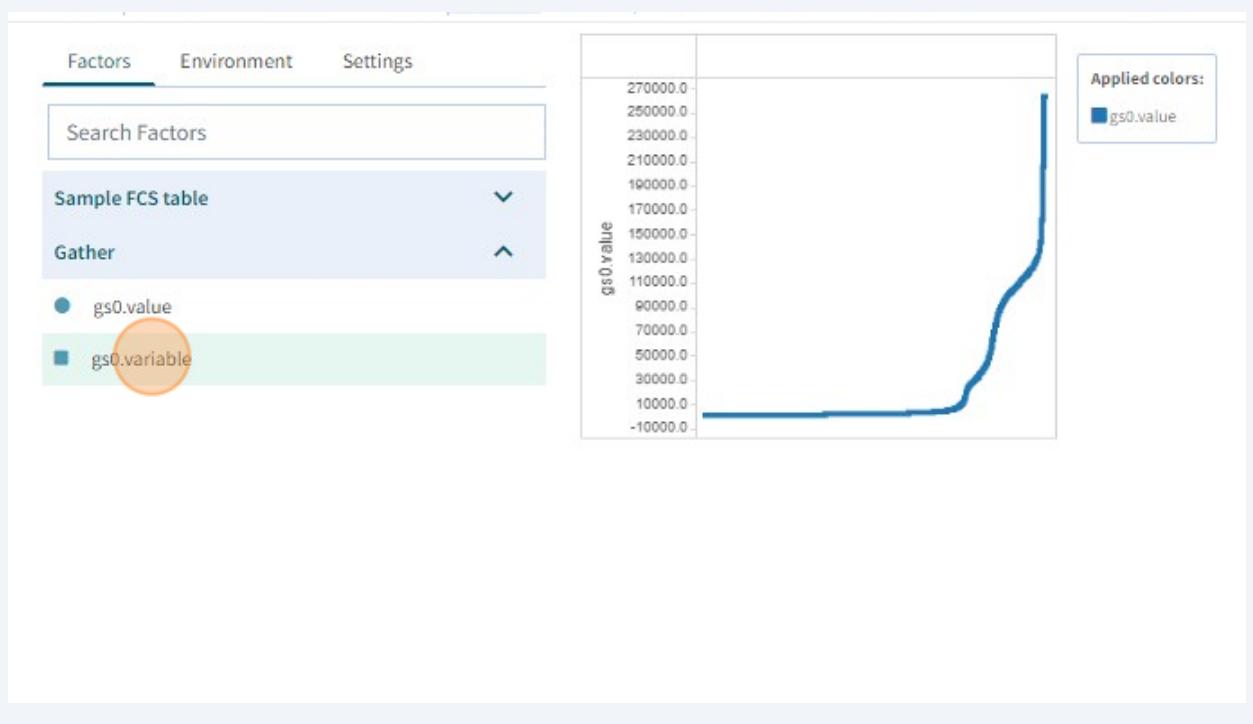
22 Click "OK"



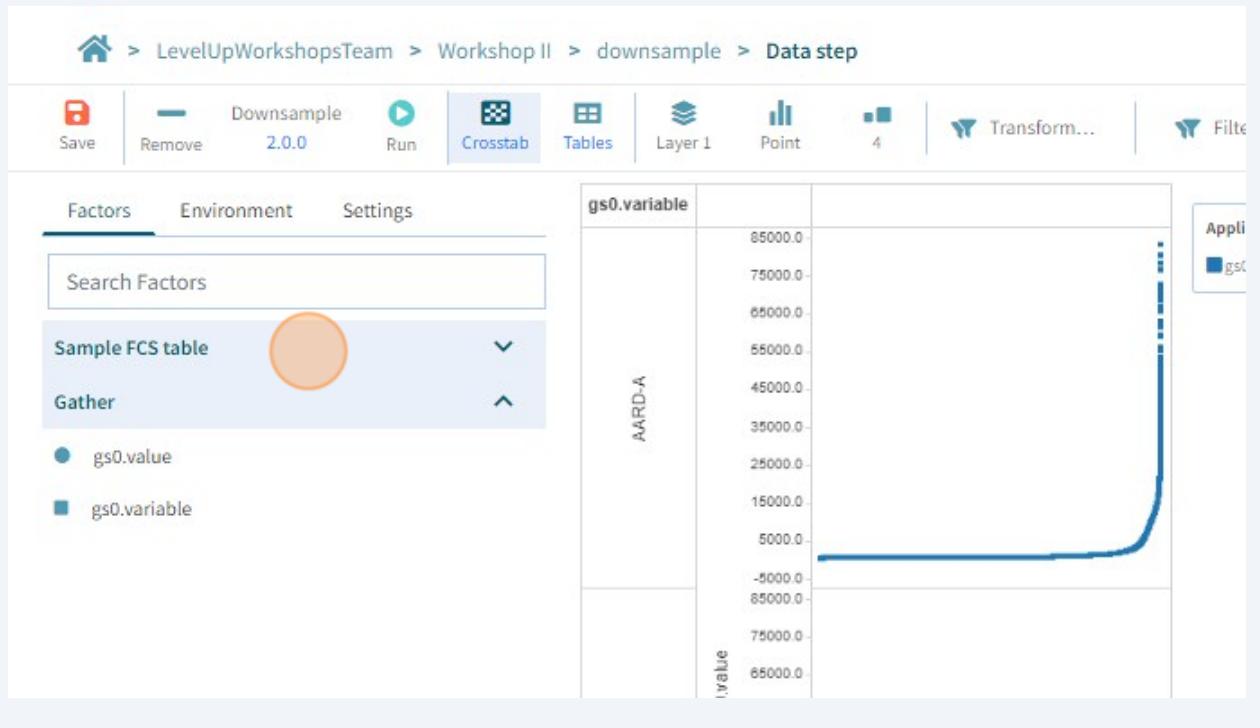
23 Drag **gs0.value** and drop onto the Y-Axis.



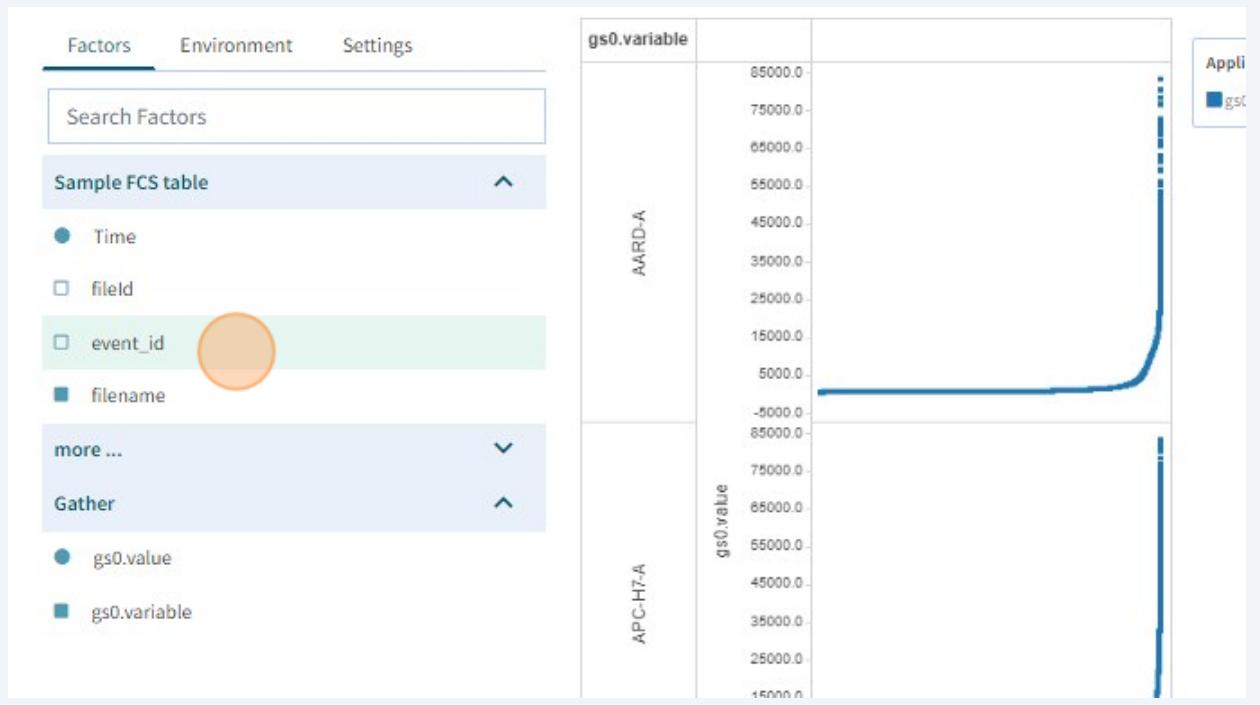
24 Drag **gs0.variable** and drop on the Rows



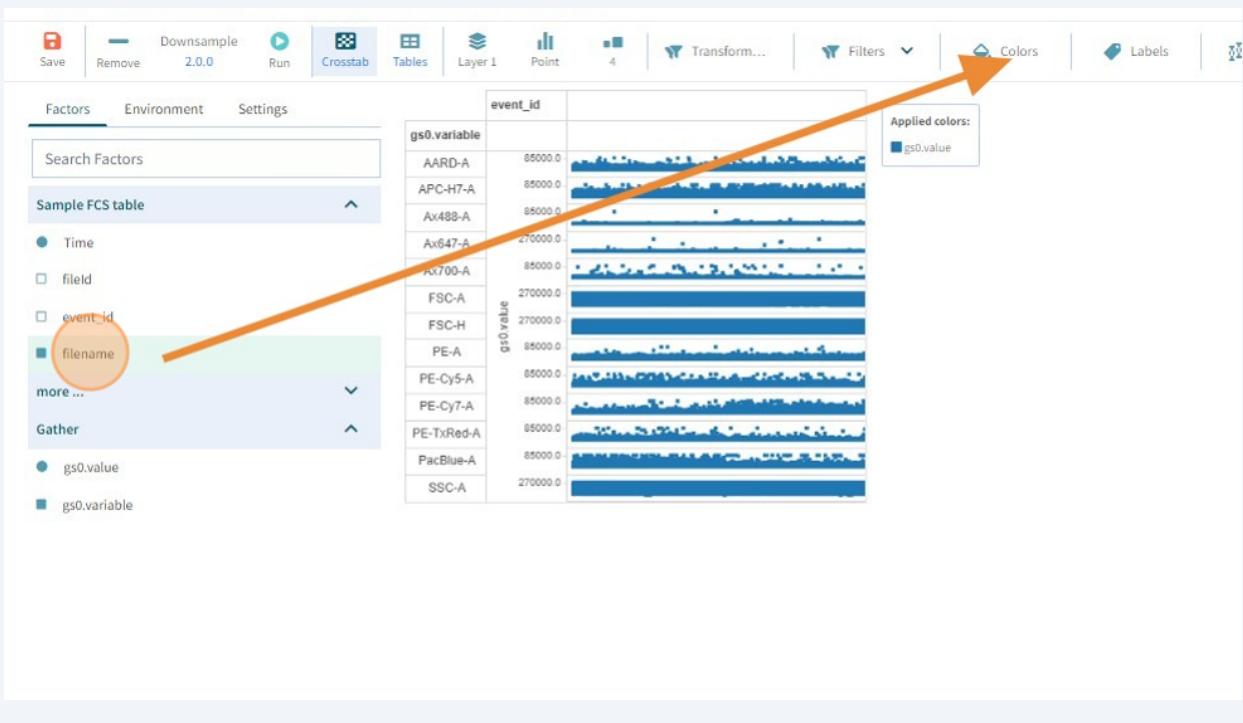
25 Click "Sample FCS table"



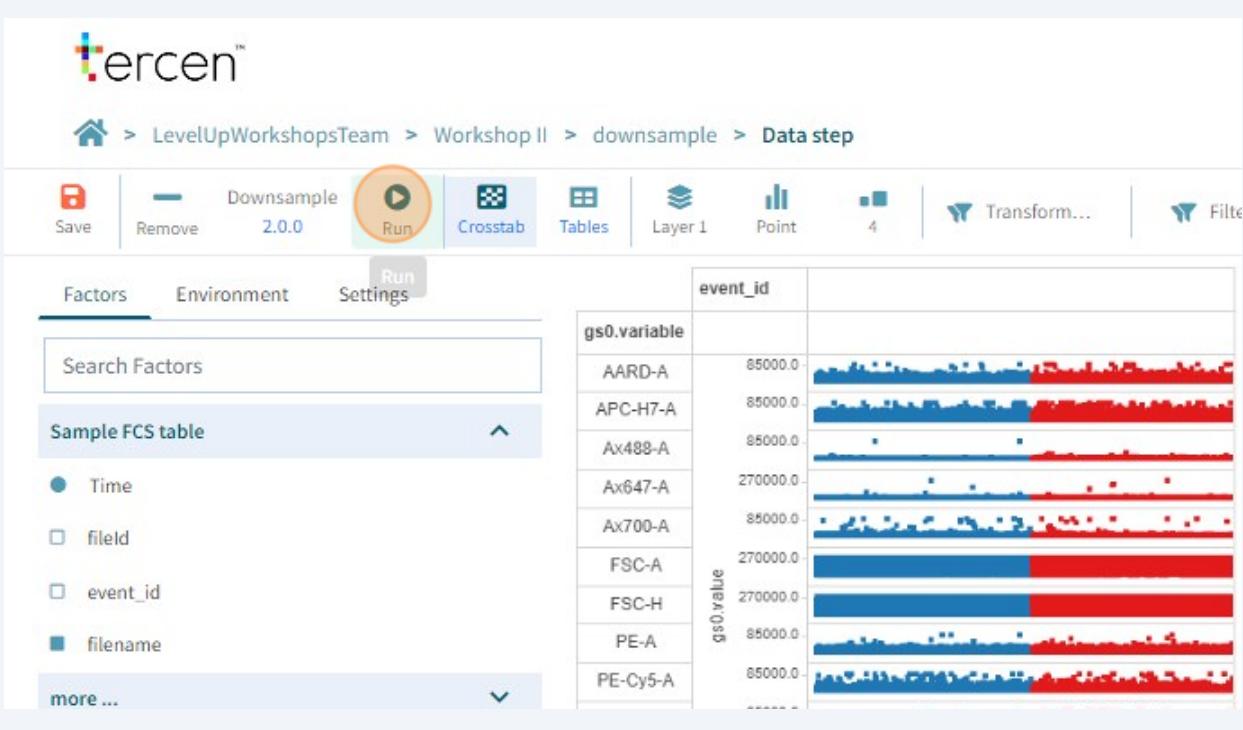
26 Drag **event_id** and drop onto Columns



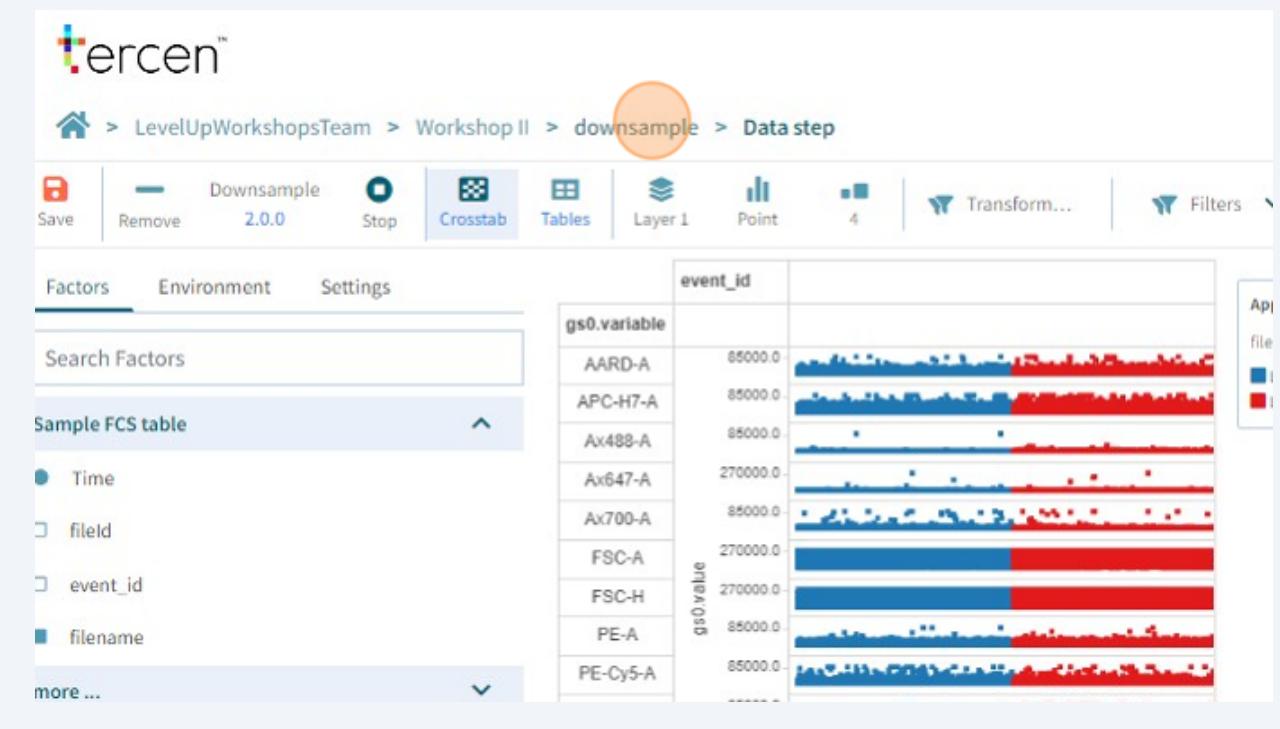
27 Drag "filename" and drop it into Colors



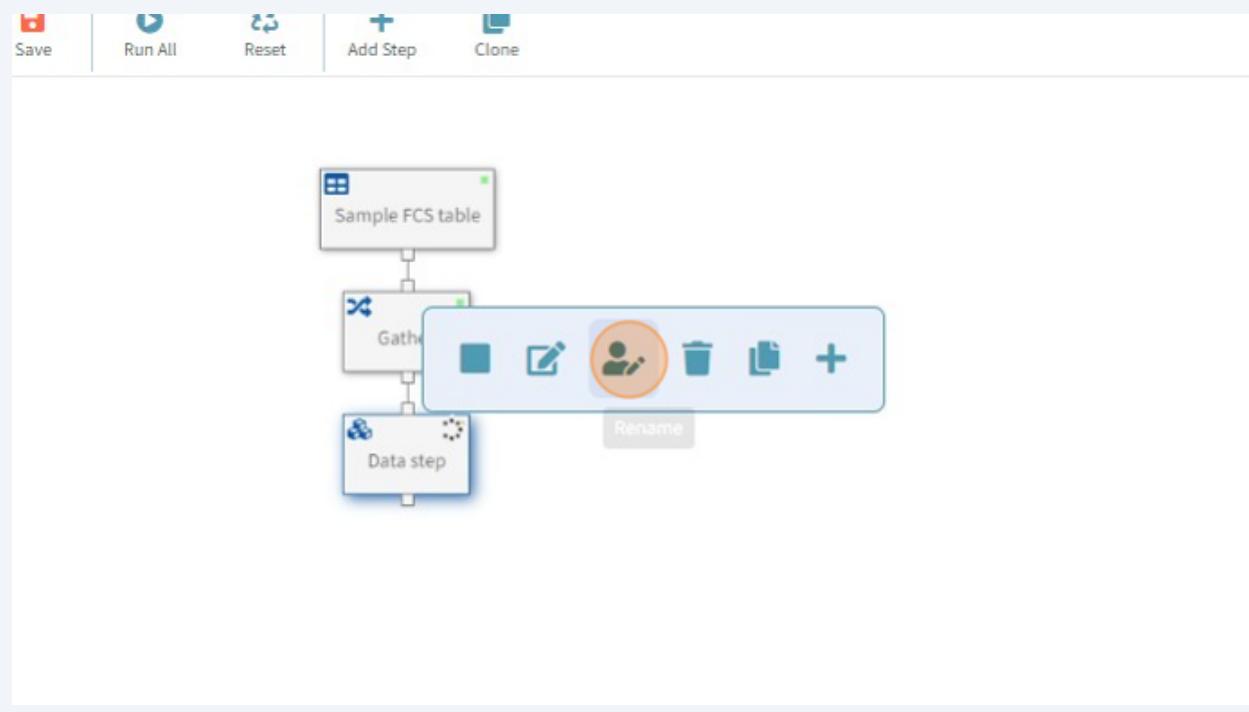
28 Click on the Run icon.



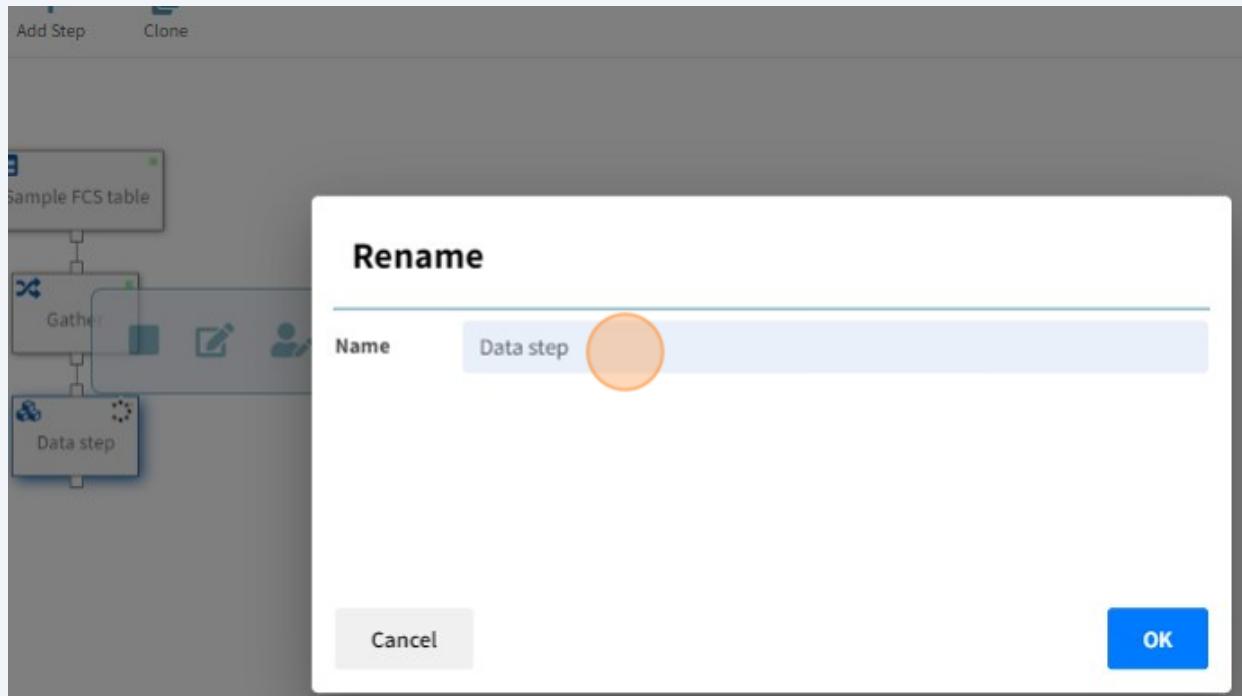
29 Navigate to workflow level by clicking on "downsample" in the breadcrumb.



30 Click here.



- 31** Click and gave it the name "downsample"



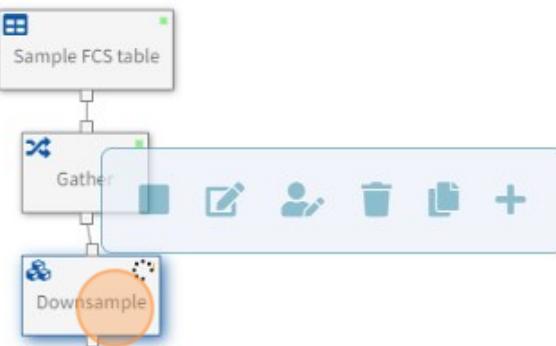
Tip!

You do not have to wait for the operator to finish executing to change the name of the Data Step

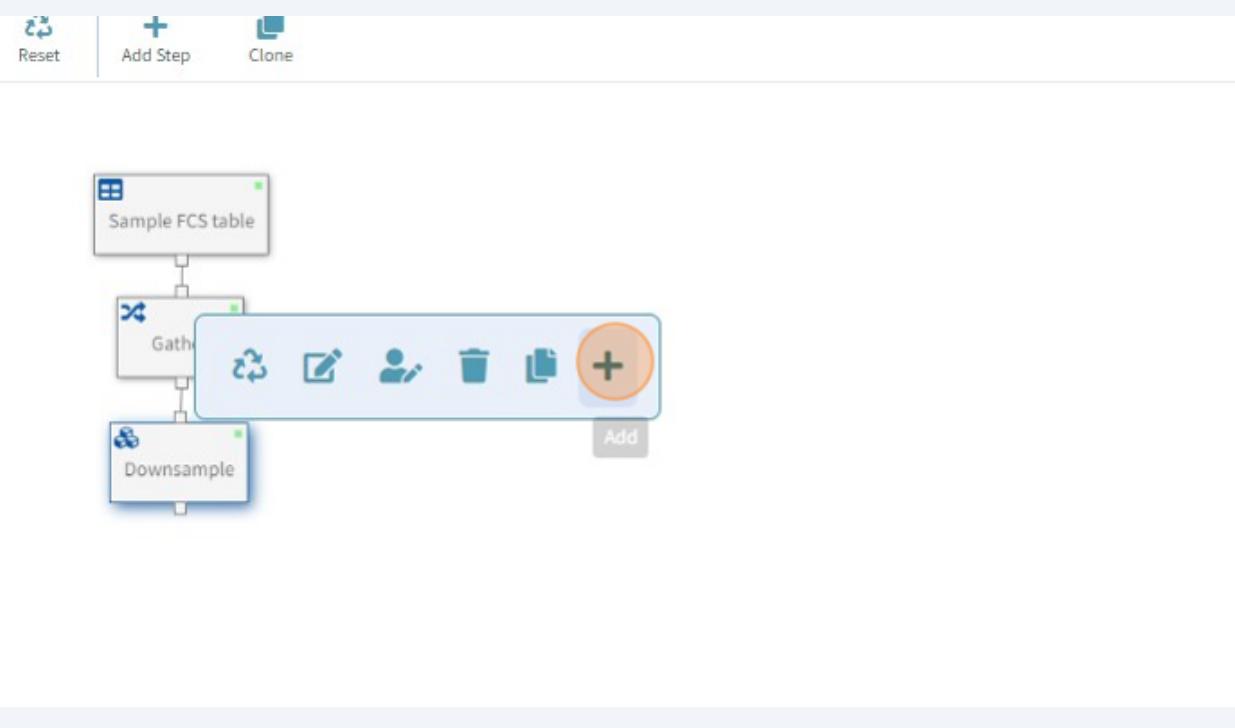
32 Let the downsample operator finish.

We will add another Data step after it.

Click on the step the toolbar will appear.



33 Click here.



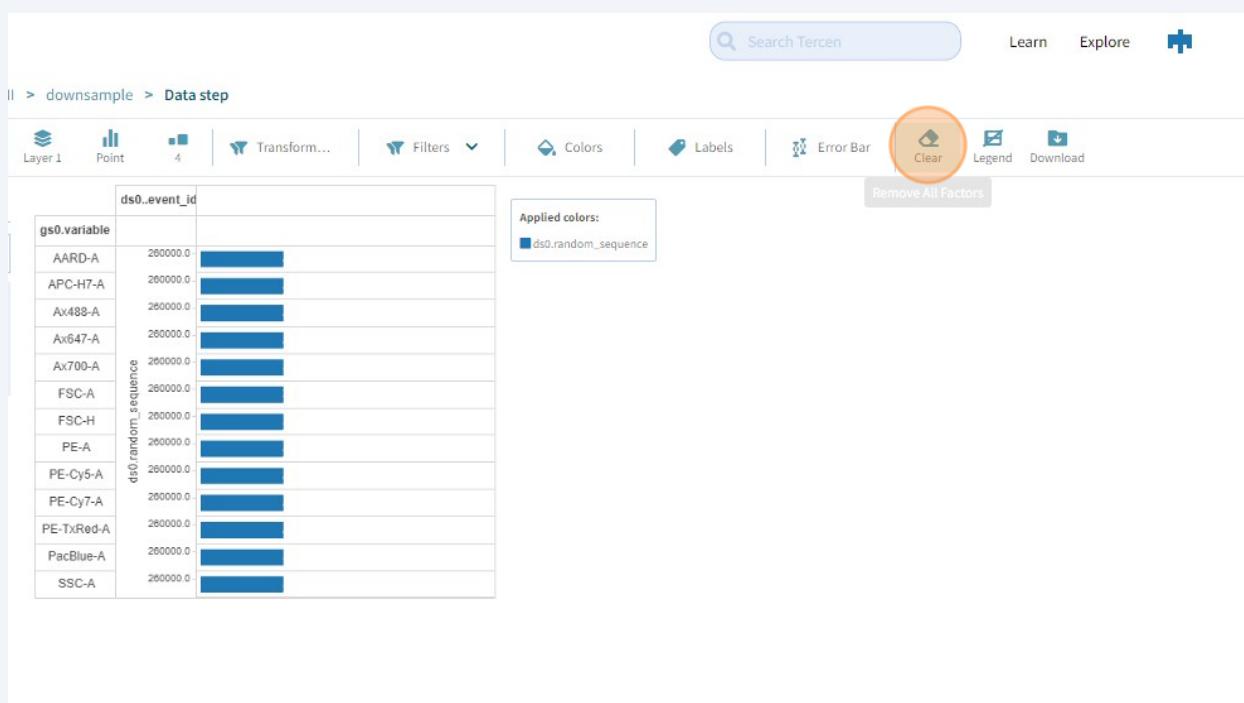
34 Select the Data step.

The screenshot shows the 'Step' tab selected in the navigation bar. Below the navigation bar is a search bar labeled 'Search'. Underneath the search bar is a list of three items:

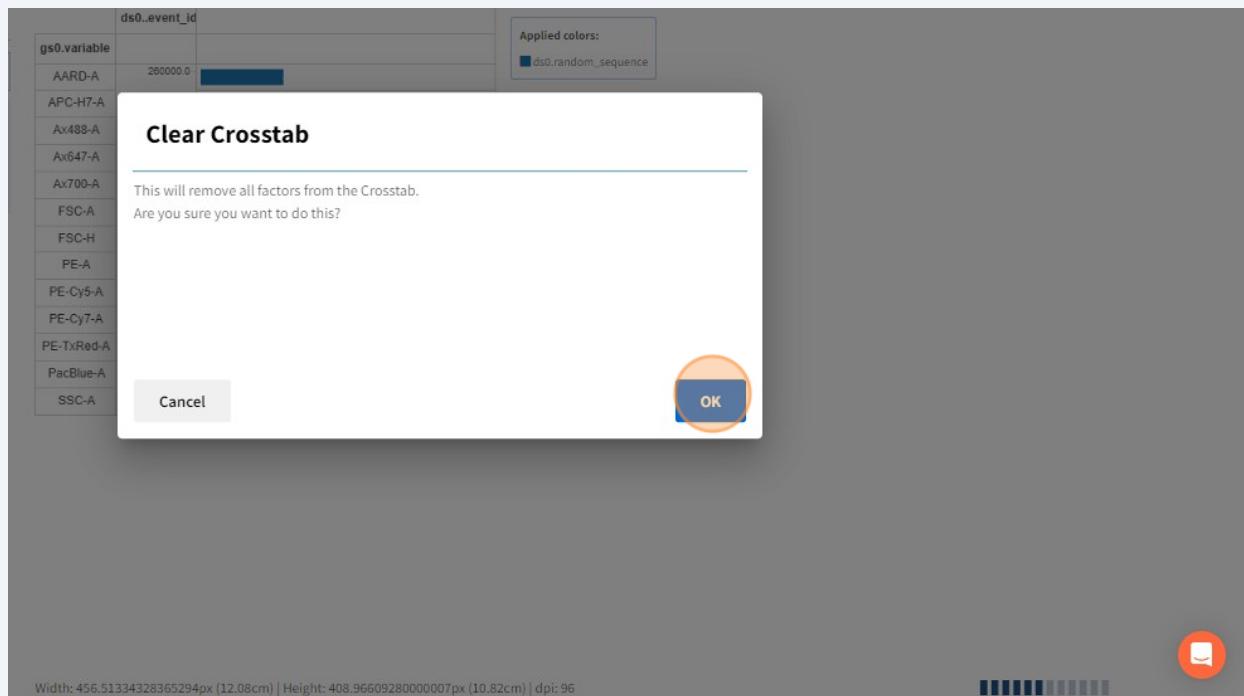
- Data step data**: Perform computation on user defined projection
- Multi data step data**: Perform computation on user defined projection
- Join leftTable**: Join two data sets

An orange circle highlights the first item, 'Data step data'.

35 Click here to clear the crosstab window.



36 Click "OK"



Did you notice new entries at the bottom of the Factors list?

The two important ones are:

ds0.random_sequence

ds0.random_percentage

These were computed by the downsampling operator.

37 Click "Gather" entry in the Factors list to expose the sub-list of factors.

The screenshot shows the FCS Studio interface with the 'Data Step' tab selected. The top navigation bar includes 'Save', 'Add Operator', 'Crosstab' (which is currently selected), 'Tables', 'Layer 1', 'Point', 'Transform...', 'Filters', and a dropdown menu. Below the navigation is a toolbar with 'Factors', 'Environment', and 'Settings' tabs, where 'Factors' is selected. A search bar labeled 'Search Factors' is present. The main panel displays a list of factors under 'Sample FCS table'. The 'Gather' entry is highlighted with an orange circle. Other entries include 'Downsample' (with up and down arrows), 'ds0.random_sequence', 'ds0.random_percentage', and 'ds0.event_id' (with a checkbox). The right side of the interface is a large, empty workspace.

38 Drag **gs0.value** and drop onto the X-axis.

This screenshot shows the same FCS Studio interface as the previous one, but with a different selection. The 'Crosstab' tab is now selected. The 'Gather' entry under 'Sample FCS table' is highlighted with an orange circle. The 'gs0.value' entry is also highlighted with an orange circle. Other entries in the 'Gather' list include 'gs0.variable'. The 'Downsample' section and other factor entries ('ds0.random_sequence', 'ds0.random_percentage', 'ds0.event_id') are visible below. The right side of the interface is a large, empty workspace.

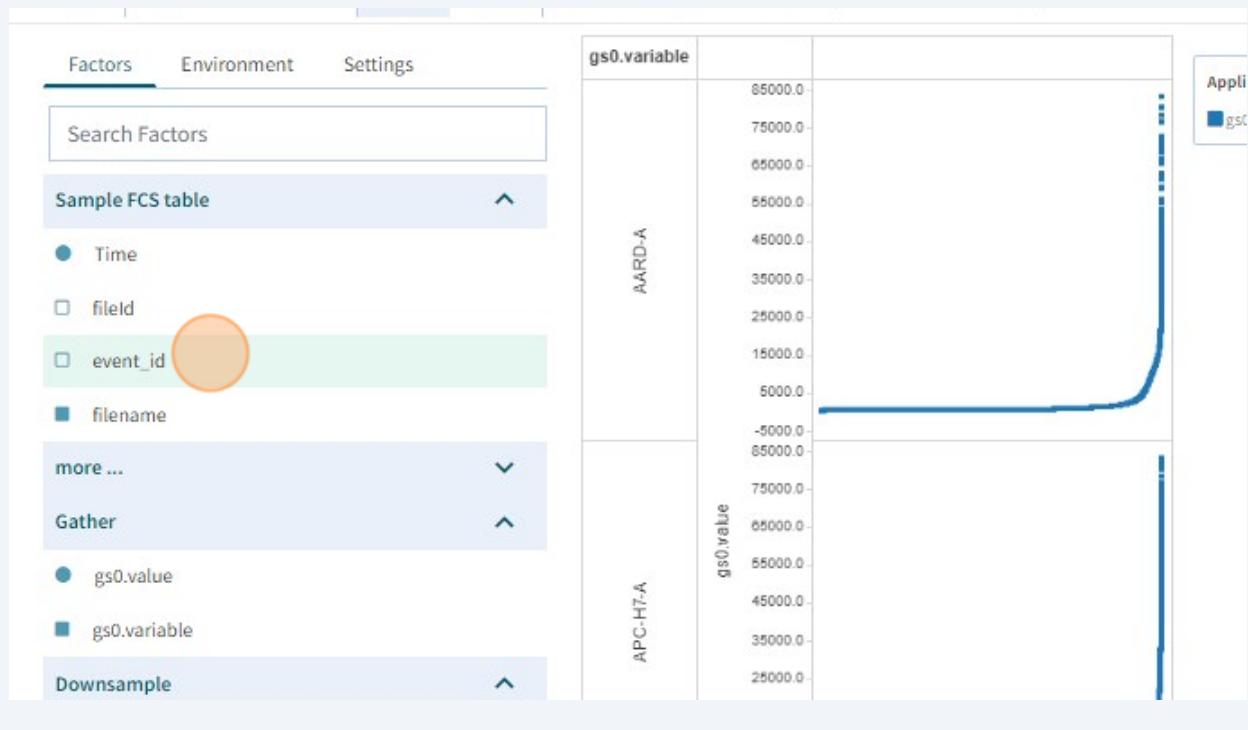
39 Drag the **gs0.variable** and drop onto the Rows.

The screenshot shows the FCS Studio interface with the 'Factors' tab selected. In the center, there's a search bar labeled 'Search Factors'. Below it, a dropdown menu is open, showing the entry 'Sample FCS table' followed by a list of options: 'Gather', 'Downsample', and three other items starting with 'ds0.'. The option 'gs0.variable' is highlighted with an orange circle. To the right, there's a large, empty rectangular area labeled 'gs0.value'.

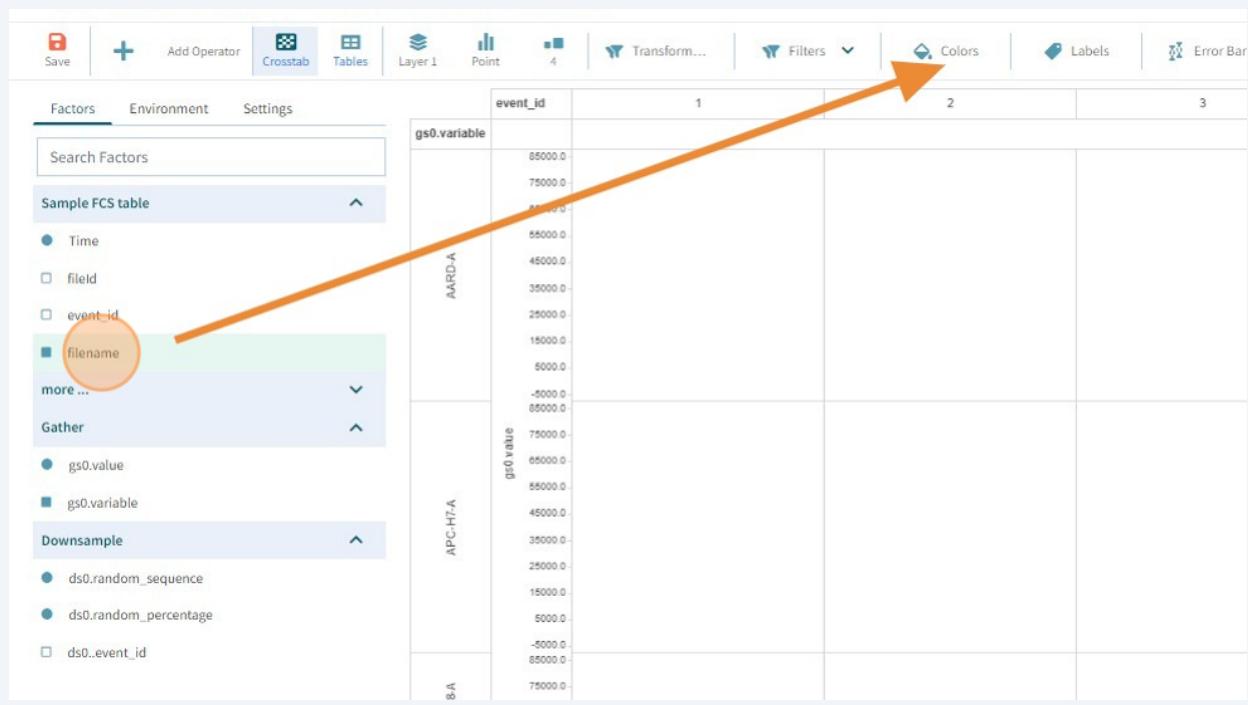
40 Click "Sample FCS table" entry to expose the factor sub-list

The screenshot shows the FCS Studio interface with the 'Factors' tab selected. The 'Sample FCS table' dropdown is now closed, and its previous selection, 'AARD-A', is visible in the list. A chart on the right displays a distribution of 'value' for 'gs0.variable'. The x-axis is labeled 'AARD-A' and the y-axis is labeled 'value'. The distribution is highly skewed, with most values falling between -5000.0 and 5000.0, and a few extreme outliers reaching up to 85000.0.

41 Drag **event_id** and drop onto the Columns.



42 Drag **filename** and drop into Colors.





Tip!

Resize the crosstab window to get an overview of all your data.

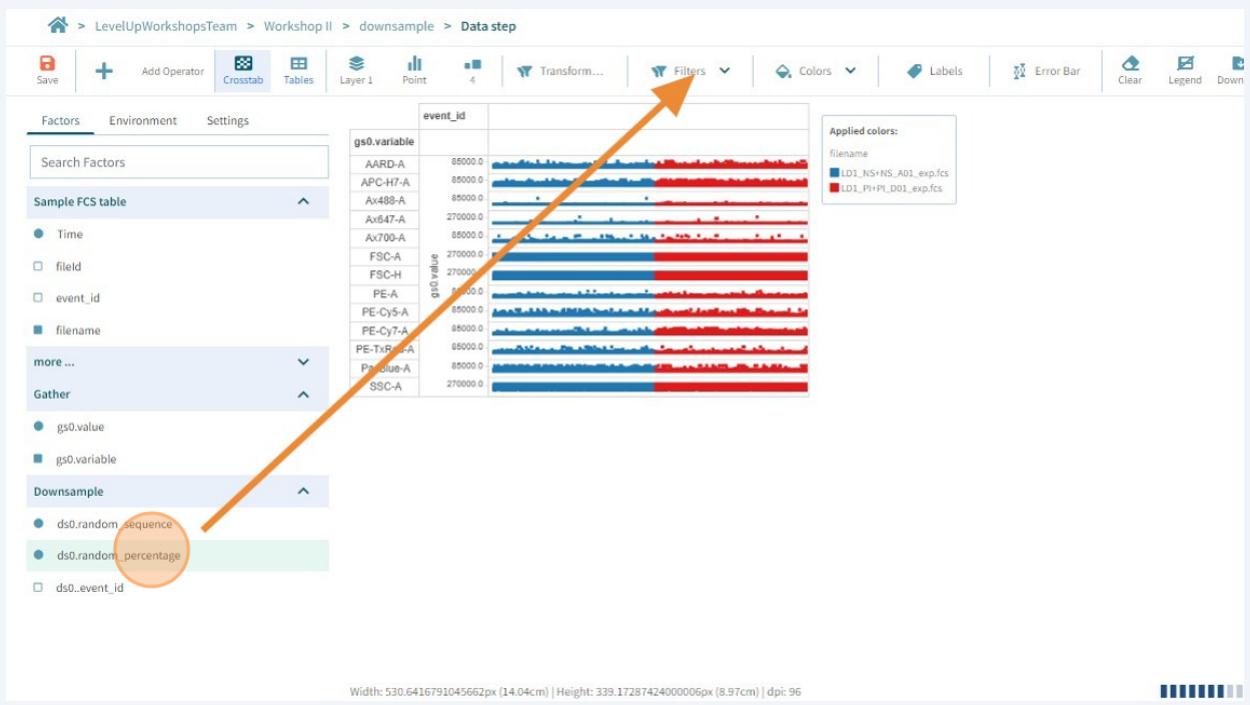
Place the mouse on the Row area, and press the CTRL key while using the wheely on your mouse to zoom in. This will squash the window vertically.

Place the mouse on the Column area, and press the CTRL key while using the wheely on your mouse to zoom in. This will squash the window horizontally.

This gives an overview of all the events and all the channels.

43

Drag **ds0.random_percentage** and drop onto Filters



44 Click this dropdown.

The screenshot shows the 'Filter settings' dialog with the following details:

- Name: ds0.random_percentage
- Operator: AND (selected)
- Condition: equals
- Value: NaN
- Dropdown menu open: A dropdown menu is open next to the 'equals' operator, with 'less' highlighted and circled in orange.

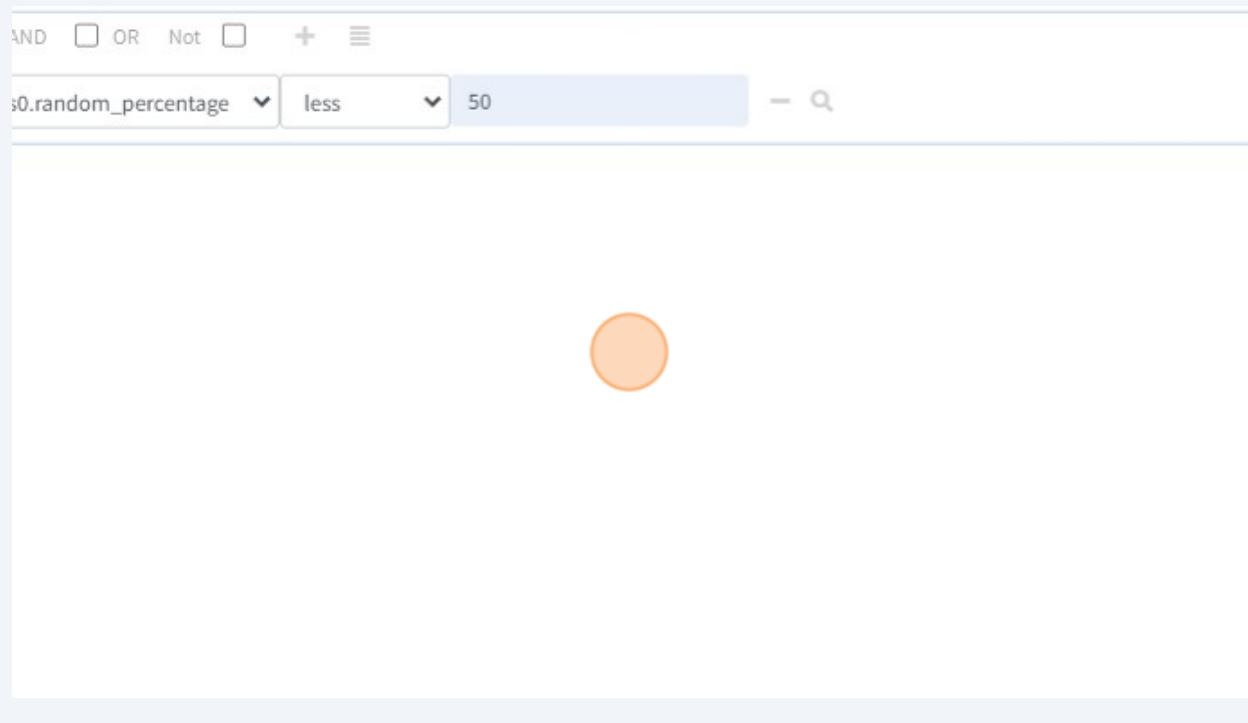
45 Select "less" and type "50"

The screenshot shows the 'Filter settings' dialog with the following details:

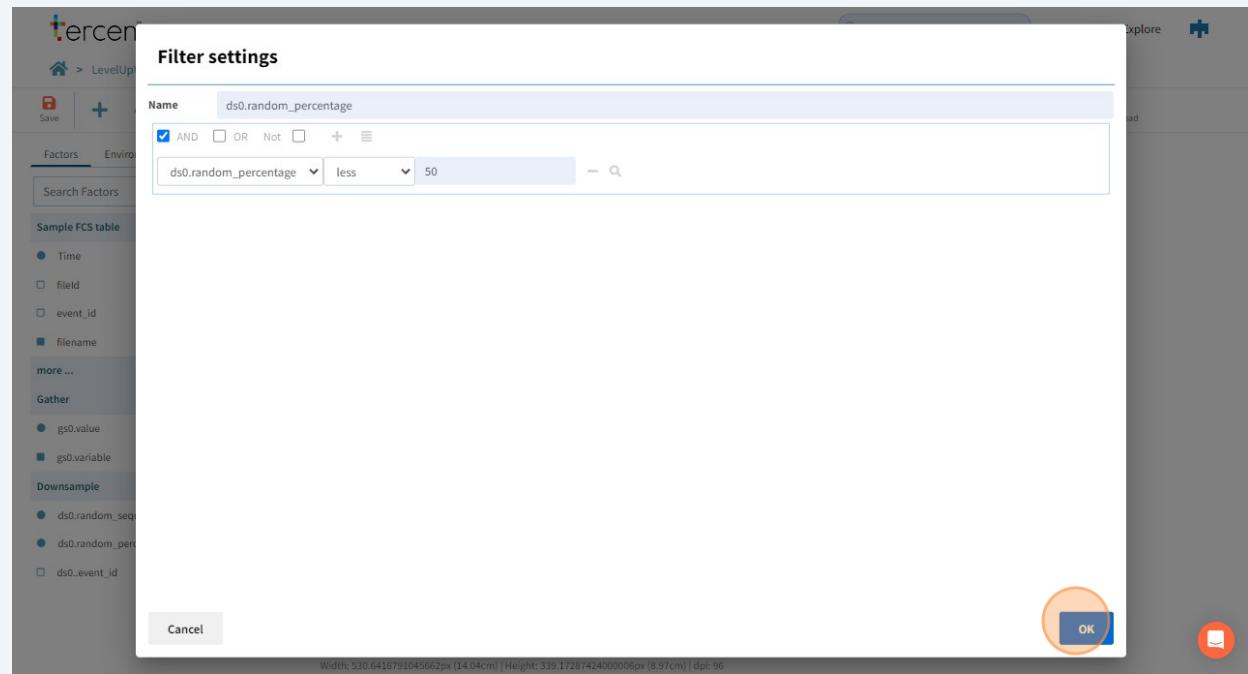
- Name: ds0.random_percentage
- Operator: AND (selected)
- Condition: less
- Value: NaN

A user has typed '50' into the value field, which is currently empty. The 'less' condition is highlighted and circled in orange.

46 Click here.



47 Click "OK"





You should now have half the amount of data as before.

The filter setting of 50 reduces the number of events by 50% in each file. There will be an equal number of events as it will use the smallest file as the reference.

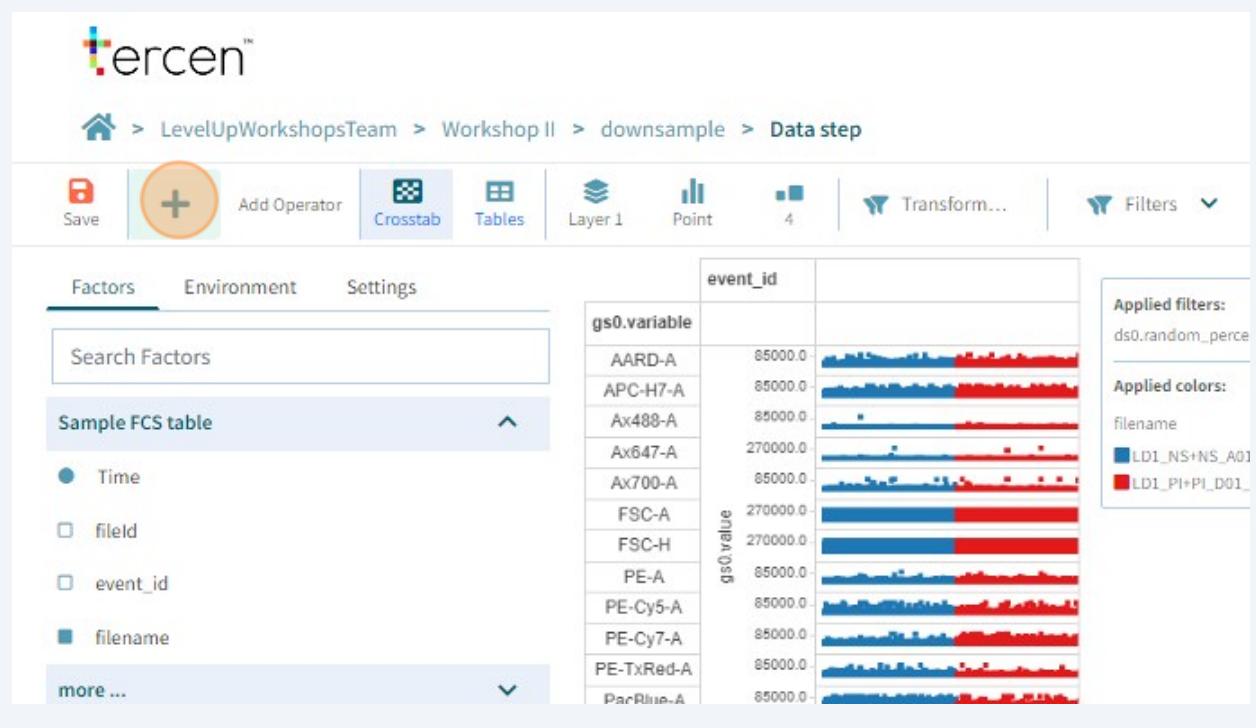


We will now export this reduced dataset as two FCS file.

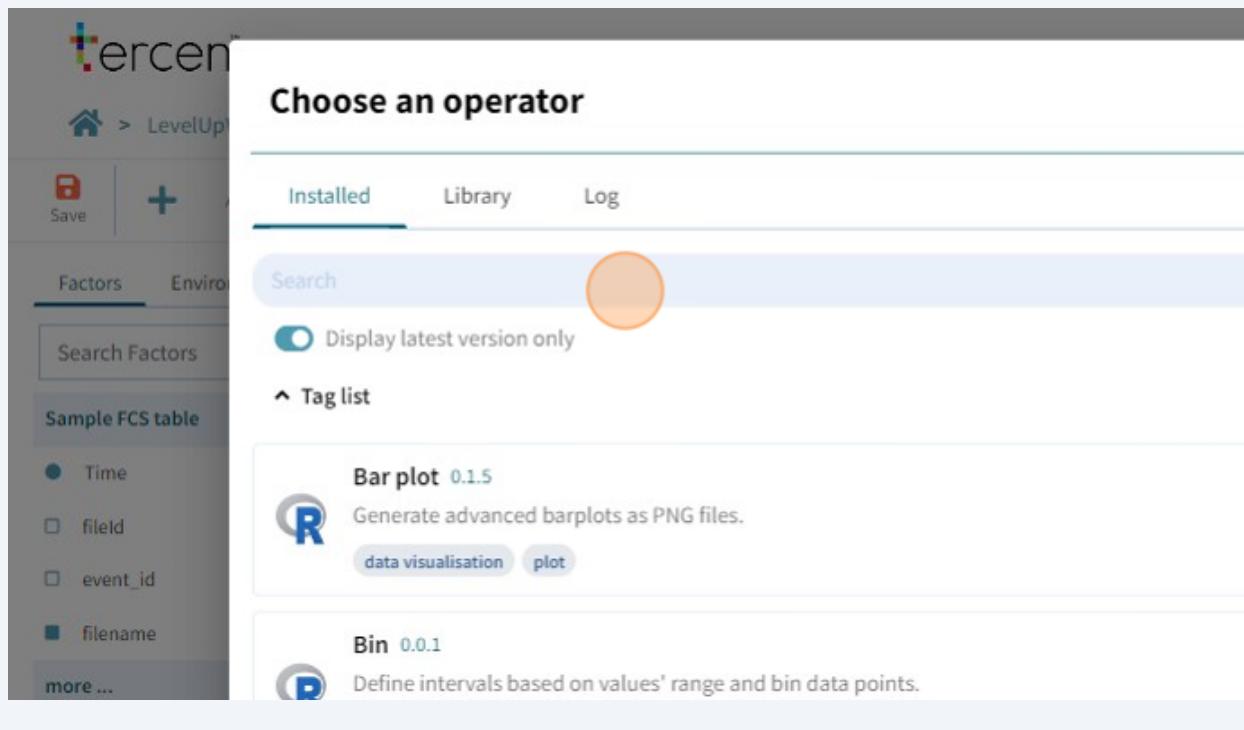
This is done by using the "Export FCS" operator in Tercen.



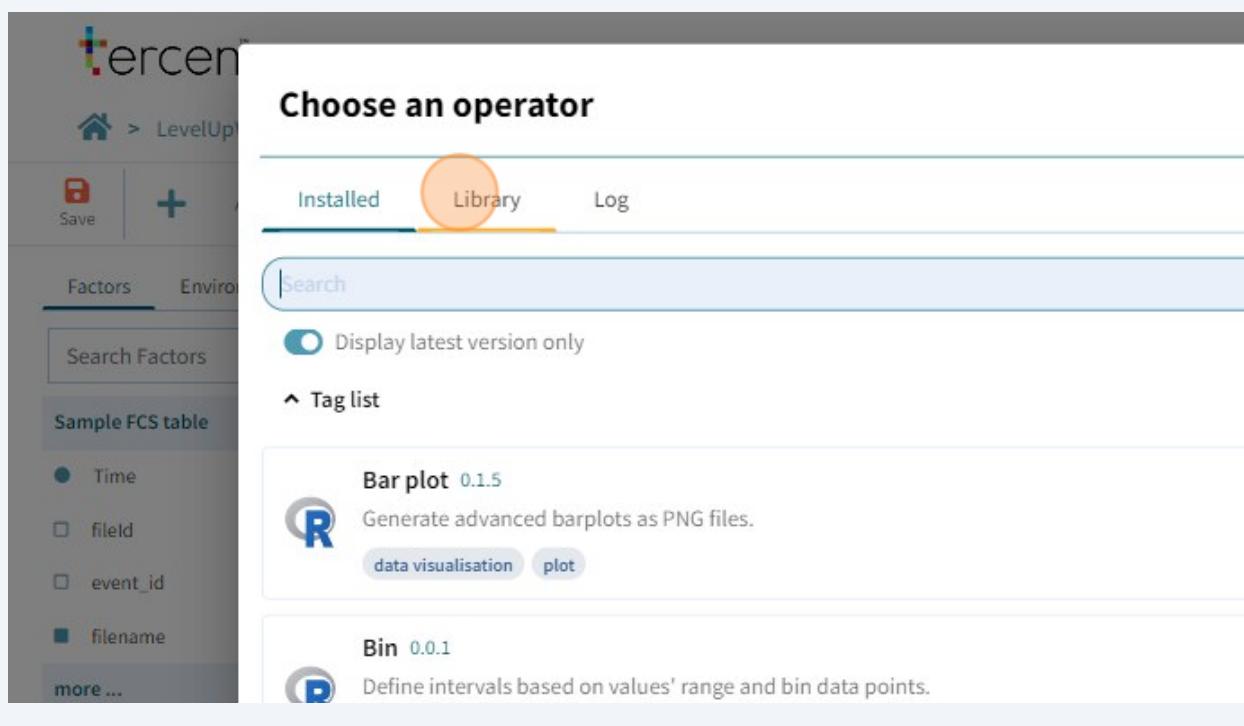
Click on the large "+" icon.



50 Click the "Search" field.



51 Click "Library"



52 Click the "Search" field and type "export"

The screenshot shows the tercen software interface. On the left, there's a sidebar with 'Factors' selected, showing options like Time, fileid, event_id, filename, and more. The main area is titled 'Choose an operator' with tabs for 'Installed', 'Library' (selected), and 'Log'. A search bar contains the text 'Search' with an orange circle highlighting it. Below the search bar is a checkbox for 'Display latest version only'. The results list includes:

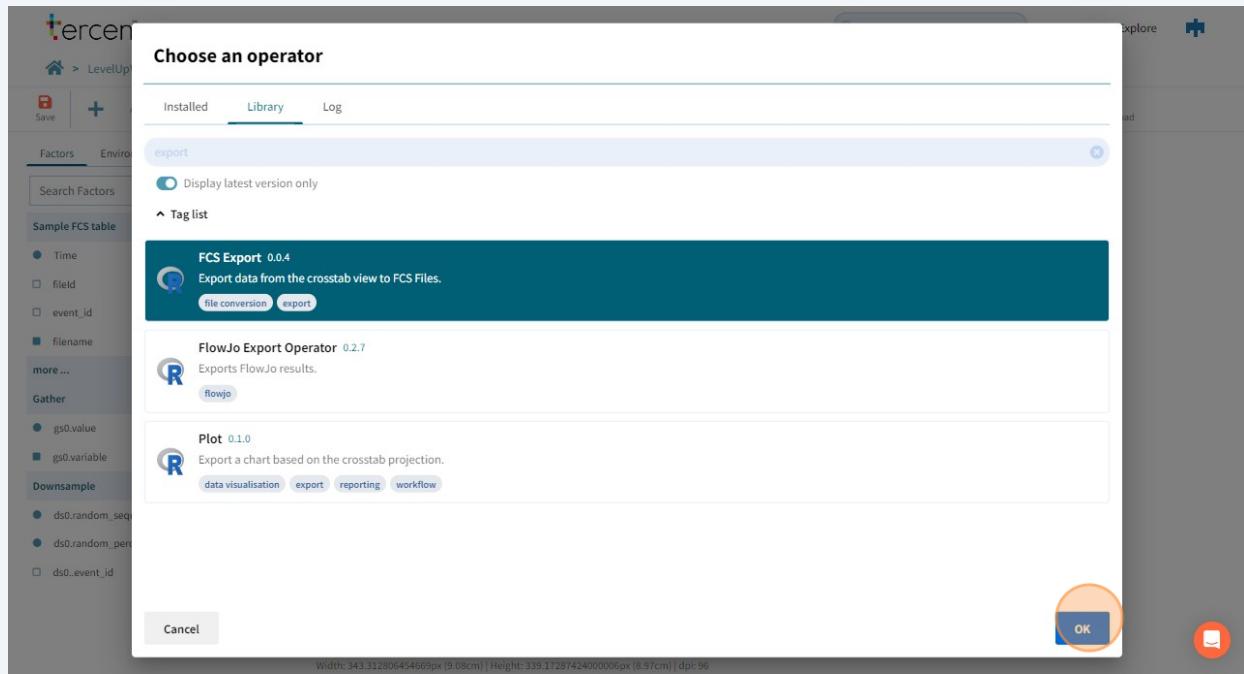
- ANOVA 1.0.0** (docker icon) - Analysis Of Variance: test for significant differences among group means. Tags: statistical testing, test, linear model.
- Add 1.0.0** (P icon) - Calculates the sum of two values.

53 Select the "FCS Export" operator

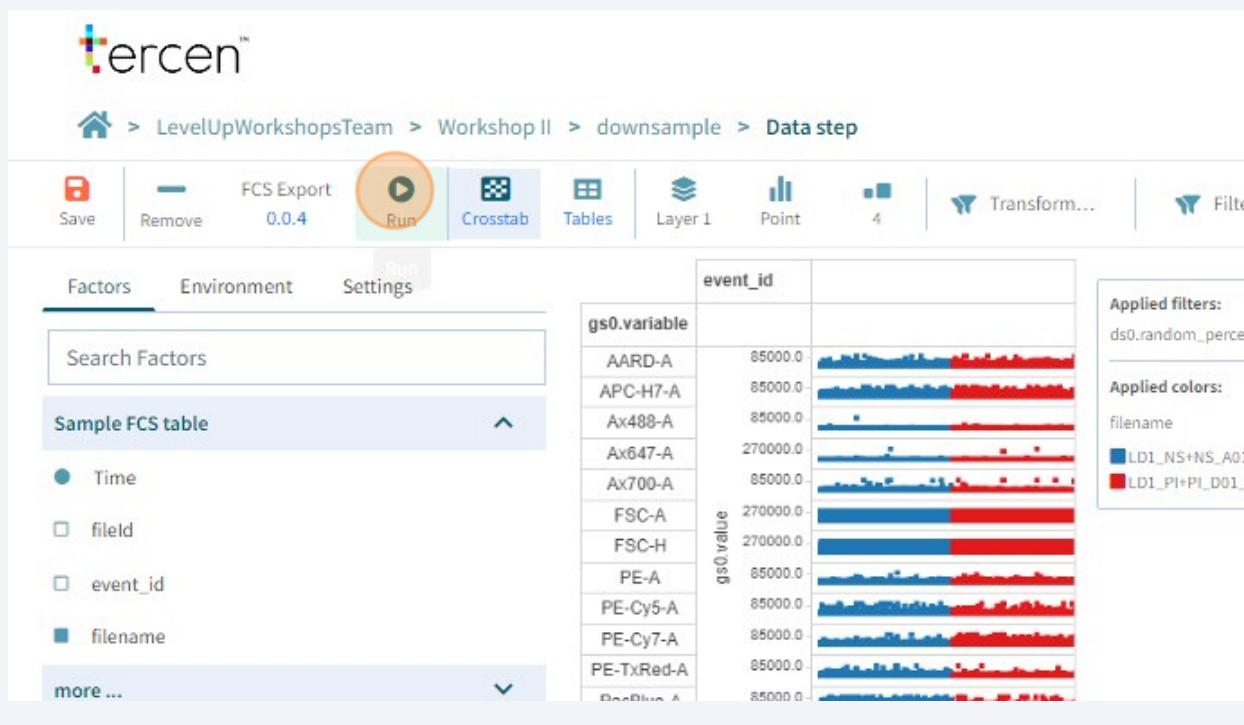
The screenshot shows the tercen software interface. The sidebar on the left has 'Factors' selected, showing Time, fileid, event_id, filename, and more. The main area shows the 'Library' tab selected with the search bar containing 'export'. An orange circle highlights the search term. Below the search bar is a checkbox for 'Display latest version only'. The results list includes:

- FCS Export 0.0.4** (R icon) - Export data from the crosstab view to FCS Files. Tags: file conversion, export.
- FlowJo Export Operator 0.2.7** (R icon) - Exports FlowJo results. Tags: flowjo.
- Plot 0.1.0** (P icon) - Export a chart based on the crosstab projection.

54 Click "OK"



55 Click the "Run" icon to execute the operator and generate the two FCS files.



56 A new pane appear with the zip of the two FCS files.

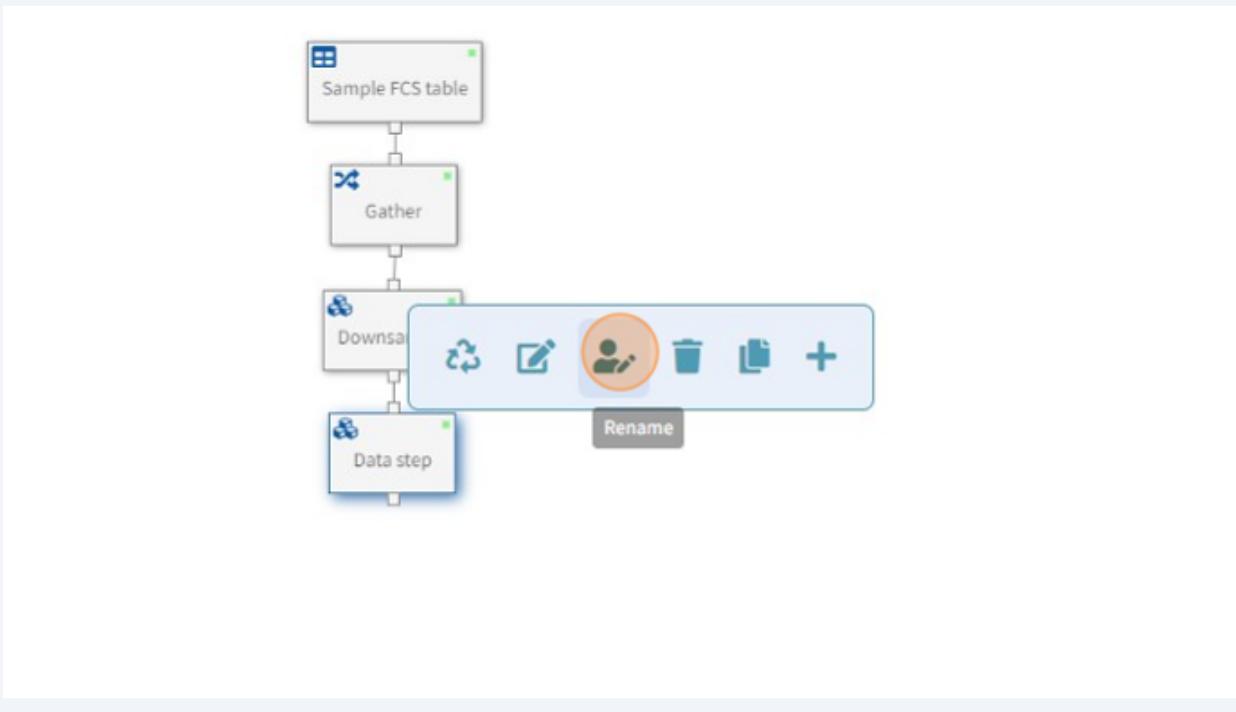
Click "Tercen_FCS_Export.zip" to download it to your drive.

The screenshot shows the Tercen software interface. At the top, there's a breadcrumb navigation: > LevelUpWorkshopsTeam > Workshop II > downsample > Data step. Below the breadcrumb, there are several tabs: Remove, FCS Export 0.0.4, Reset, Crosstab, Tables, and Result. The Result tab is currently selected. Underneath the tabs, there are two tabs: Environment and Settings, with Environment being the active one. On the left, there's a search bar labeled 'Search Factors' and a dropdown menu labeled 'FCS table'. On the right, there are two tabs: Files and Table, with Files being the active one. Under the Files tab, there is a list containing a single item: 'Tercen_FCS_Export.zip'. This item is circled in orange.

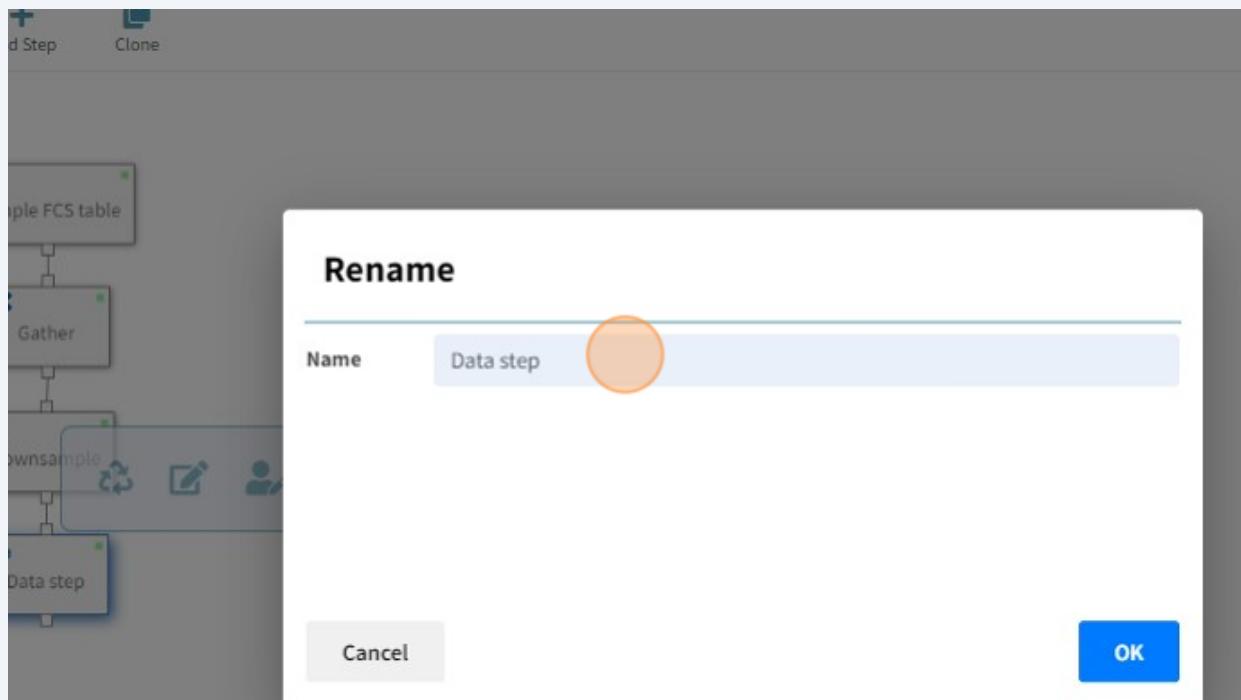
57 Navigate to the workflow level by clicking on "downsample" in the breadcrumb.

The screenshot shows the Tercen software interface. The breadcrumb navigation is identical to the previous screenshot: > LevelUpWorkshopsTeam > Workshop II > downsample > Data step. The tabs at the top are the same: Remove, FCS Export 0.0.4, Reset, Crosstab, Tables, and Result. The Result tab is selected. The sidebar on the left has tabs for 'Factors' (which is active), 'Environment', and 'Settings'. The main area on the right shows a list of factors: 'Time', 'fileId', 'event_id', 'filename', and 'more ...'. The 'downsample' part of the breadcrumb is circled in orange.

58 Click on the Rename icon

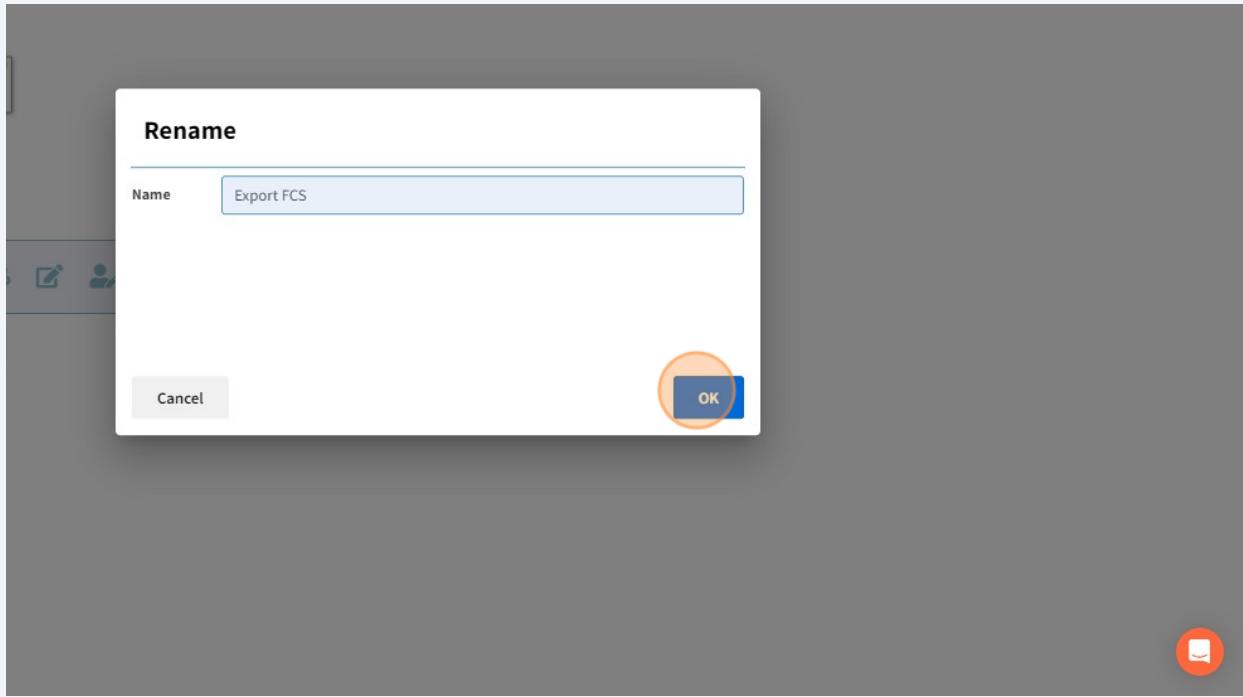


59 Type the text "Export FCS"

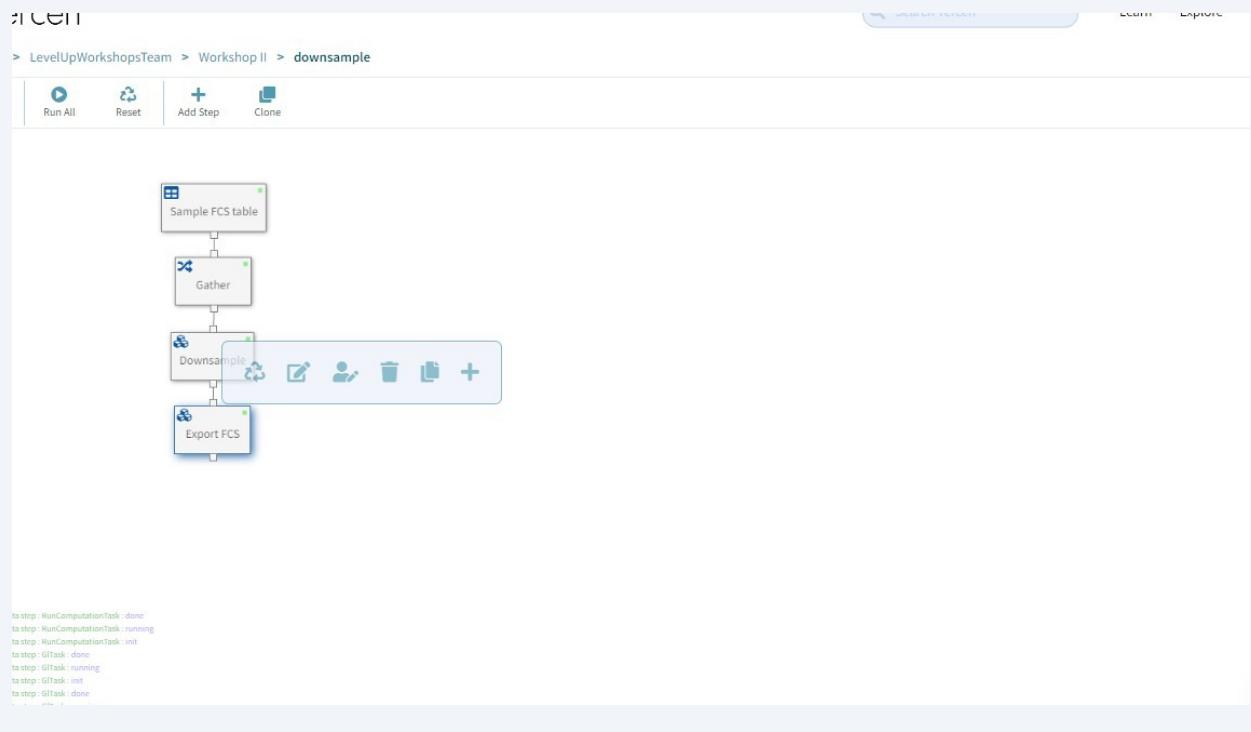


60 Type "xport FCS"

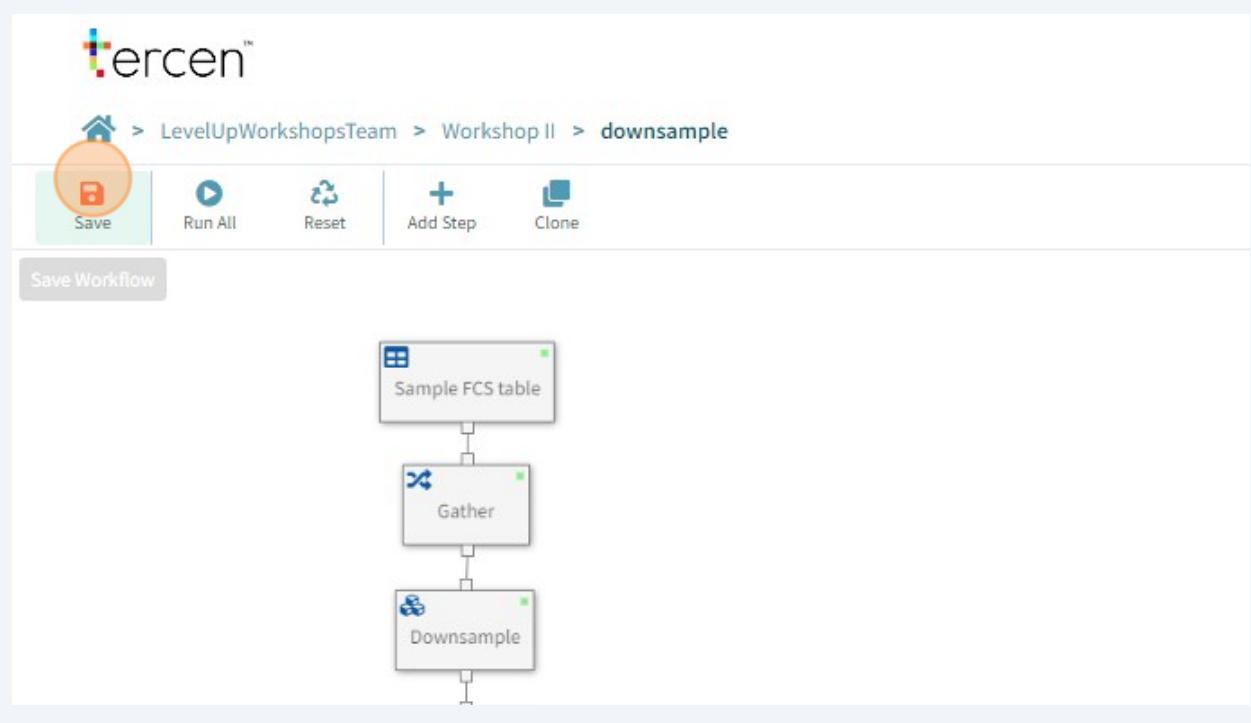
61 Click "OK"



62 Your workflow should look like this.



63 Click on the Save icon to save your workflow.



64

This guide has shown you how to downsample and export two FCS files.

Well done!