Cytobank | BECKMAN COULTER Life Sciences

QUICK START GUIDE

How to start analyzing data on the Cytobank platform

Thank you for your interest in the free trial for the Cytobank platform!

The cloud-based Cytobank data analysis platform is an ideal solution for immunologists and cytometrists who need to quickly gain insights from large datasets while having confidence in their results, reducing subjectivity of their analysis and eliminating error-prone and time-consuming steps. It offers fully integrated and quality-tested tools for advanced cytometry data analysis and statistical evaluation, providing comprehensive workflow coverage and automation.

This quick start guide covers some basic functions and the initial steps of a typical data analysis workflow to help build your confidence when using the platform.

STEP 1: Logging in

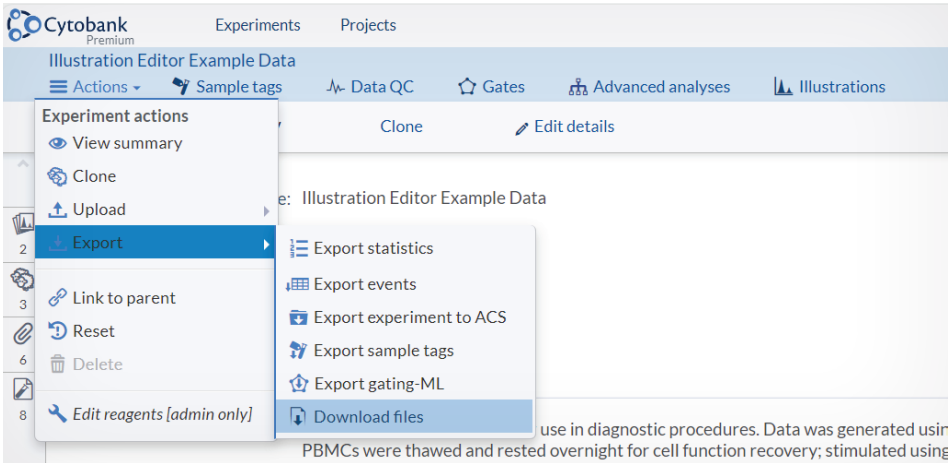
Please navigate to the **Cytobank login page** and access the platform with your credentials.

STEP 2: Open experiment

To access the example dataset, please click on the link to **this experiment**.

STEP 3: Download tutorial dataset

To download data files from within the experiment, navigate to **Actions > Export > Download files:**



A zip file containing all FCS files you have selected will be created and an email with the link to download will be sent to your inbox. Follow the link to download the zip file, unzip it and place the FCS files in a convenient location.

YOU ARE NOW READY TO START YOUR CYTOBANK JOURNEY.

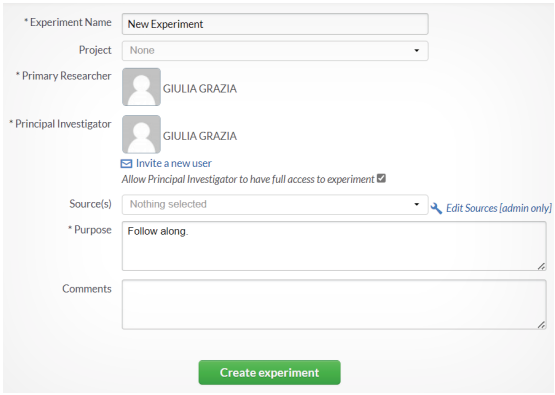
STEP 4: Creating an experiment

To create a new experiment, simply navigate to the Experiment Manager page by clicking on the Cytobank logo on the top left of your screen.

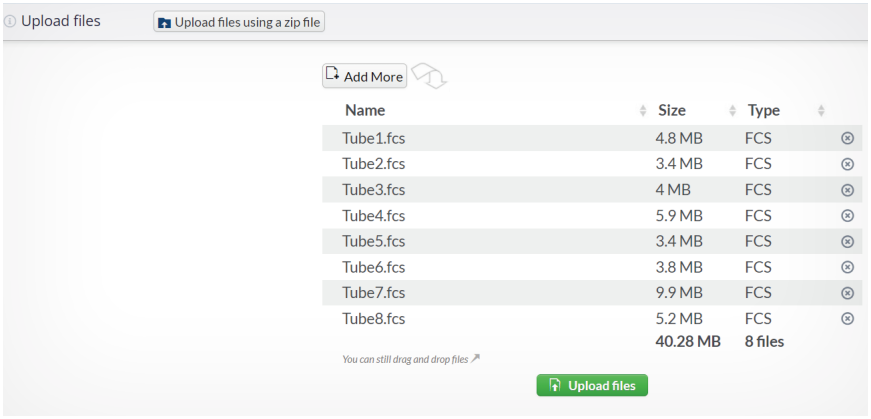
Clicking on the Cytobank logo will take you back to the Experiment Manager from anywhere in the app.

Click on the green button **New experiment**:

Give the experiment a name, a purpose and click on **Create experiment** at the bottom of the page.

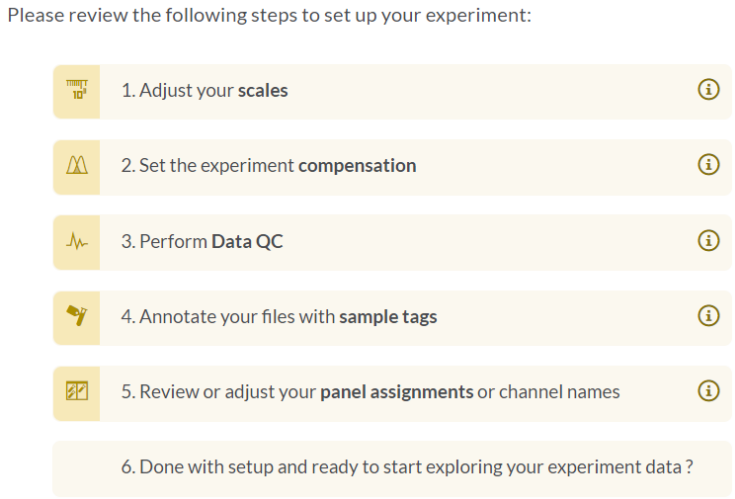


Select the FCS files you have previously downloaded and drag and drop them in the grey area of the screen or click the green button on top left and navigate to the correct location on your computer.



Click on upload files. A progress bar will show you the status of the upload.

When done, you will be directed to the Experiment Summary page, where you will be presented with the next steps to take in order to curate your data.

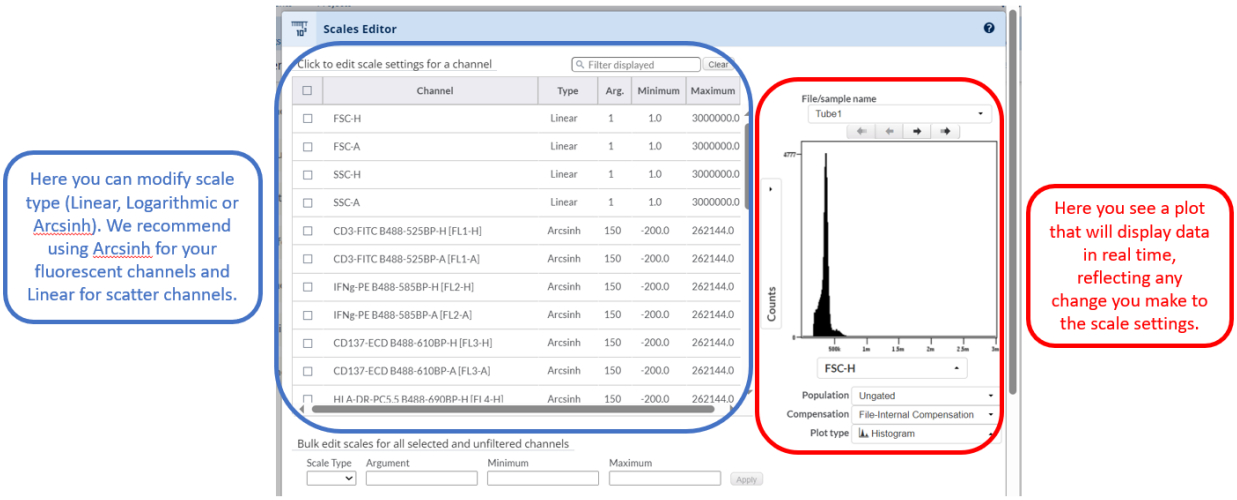


STEP 5: Scaling your data

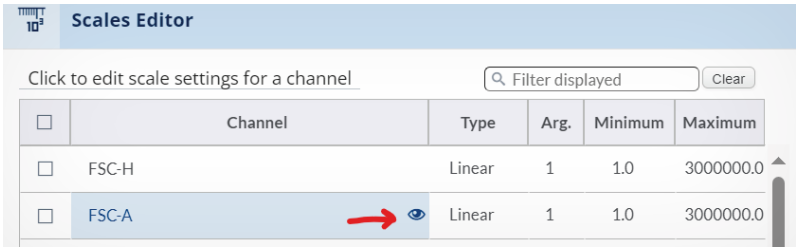
As a first and most important step, we need to adjust the scaling for our dataset. To do so, navigate to the **Scales Editor** from the top blue navbar.

There is only one version of scales in a Cytobank experiment at any given time. If the scales within an experiment change, then all saved illustrations that depend on those scales will also change.

The screen you are looking at now should be similar to this:

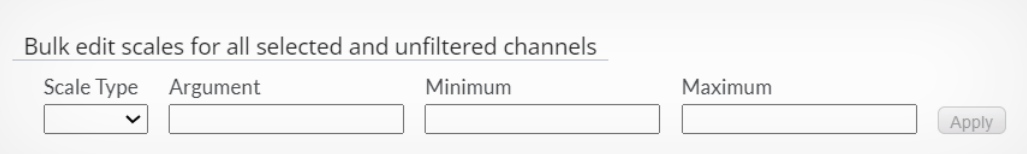


To change what is displayed in the dot plot/histogram on the right, you can modify directly from the dot plot axis, or hover over a channel of interest and click on the eye icon that will appear:



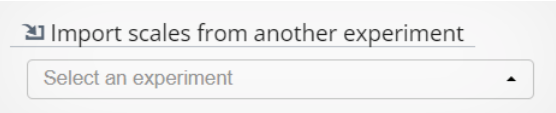
In our example experiment, please set the maximum for all the scatter channels at 1000000 just by typing the number in the Maximum column and watch the plot updating.

You can bulk adjust by multi-selecting several channels and using Bulk Edit at the bottom of the table and clicking on Apply:

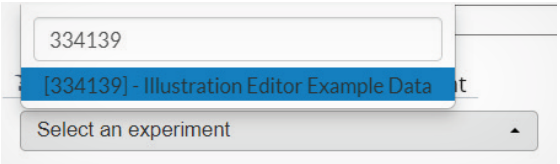


We recommend you take a careful look at all the scale settings for all the channels of your experiment as the first step before doing any other manipulation of the data in order to have the best results.

To facilitate the work, you can also import scale settings from another experiment.
Since we are using a demo dataset, you can easily import scaling from the reference experiment in this setting. To do so, access the function from the bottom of the page:



and type 334139 in the search box to select the reference experiment:



Click on **Import scales** to import the scale settings of the reference experiment into your new experiment.

Well done! Now you know how to modify/set the scales for any of your experiments in the Cytobank platform.

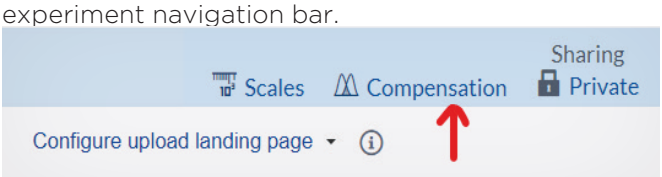
STEP 6: Check experiment compensation

If you are working with flow cytometry data (like we are with our example dataset), and you have compensated your data at the instrument, **you can skip this step**: the platform will automatically read the file-internal compensation for all the FCS files of your experiment.

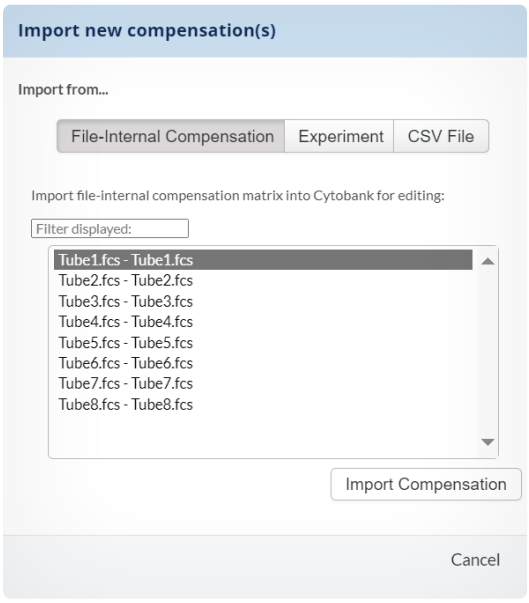
Please note that at this point, the compensation in the Cytobank platform is an Experiment-Wide Compensation: you cannot modify the compensation on a per file basis. In case this is required for your workflow, you can use the Kaluza-Cytobank plugin to generate new FCS files. Follow **this link** to learn more or contact Cytobank support at **support_cytobank@beckman.com** .

In the Cytobank platform, there are many ways to create and modify a new compensation matrix. The compensation matrix can also be imported from the file-internal compensation, an existing experiment, or a CSV file. It can also be exported as a CSV file. To learn more, please refer to **this article**.

If you are following along with the example dataset and you still want to look at the compensation matrix, you can access the **Compensation** page by clicking the **Compensation** tab at the top right corner on the experiment navigation bar.

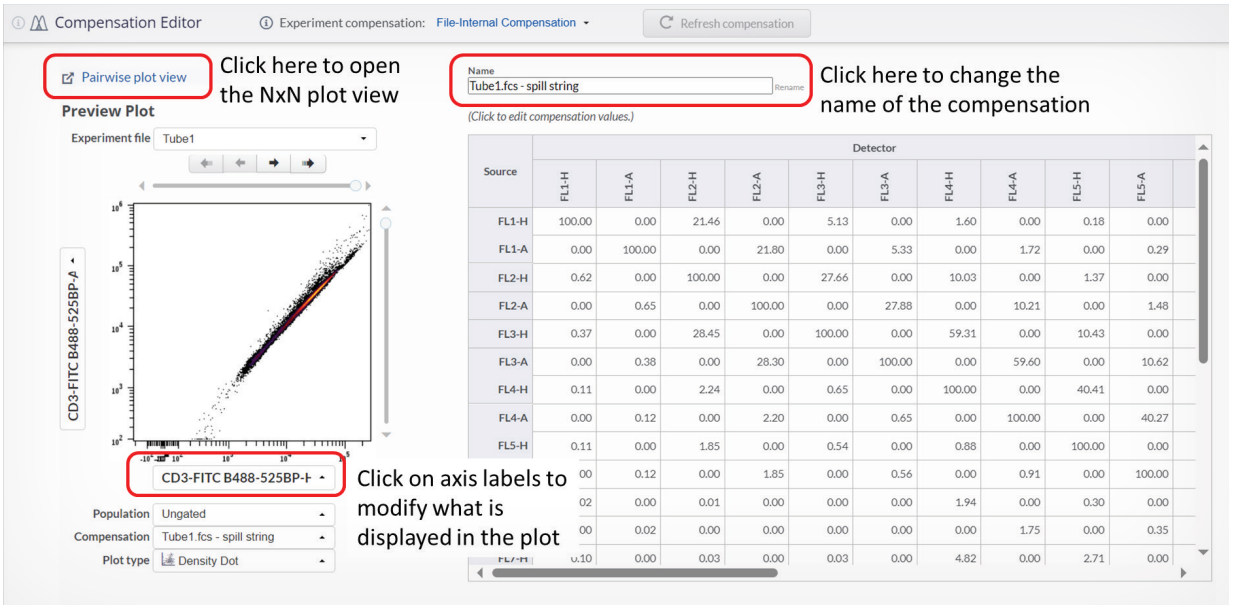


Then click on the import option and select Import from **File-Internal Compensation**, then select one of the files from our experiment and click on **Import Compensation** at the bottom.

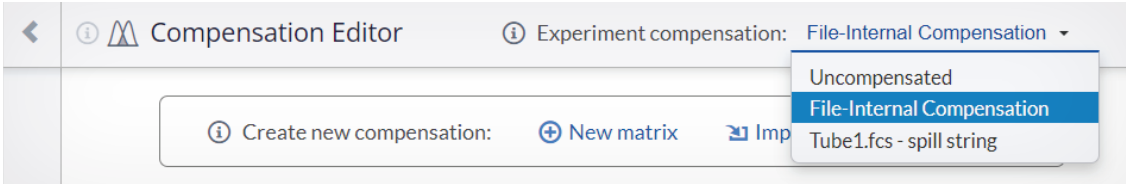


You will now see an editable compensation matrix and a preview plot with sliders that allow you to easily modify the applied compensation values.

Click on Pairwise plot view link on top left to see all the NxN plots and make several changes at once.



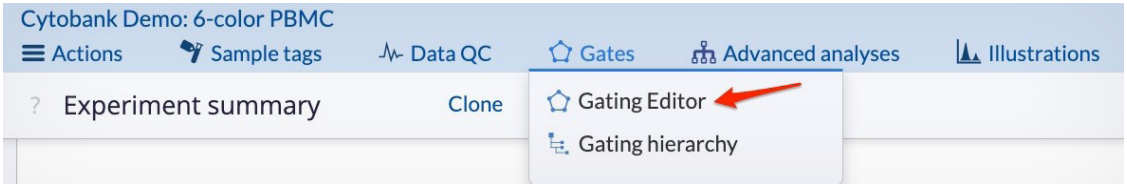
The compensation applied to the experiment is always displayed on the top grey bar. The platform defaults to the File Internal Compensation; should you wish to apply a different one, make your selection by clicking on the dropdown window and choosing the one you prefer.



So now, let's do some gating!

STEP 7: Gating

From any experiment, click on **Gates>Gating Editor** in the top blue navigation bar.

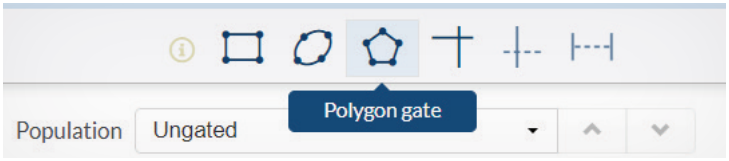


The Gating Editor has a big dotplot that allows you to create gates. In the gating interface you will see on the left of the screen a list with all the gates of your experiment, while on the right you will be able to see your population hierarchy.

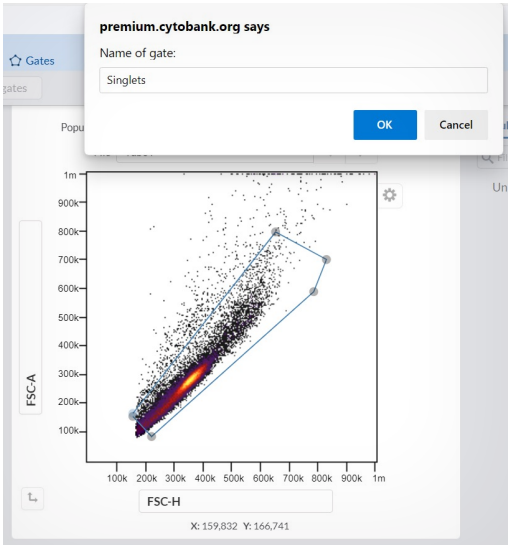
If you want to learn more about the difference between gates and populations in the platform, please **refer to this article**.

You can change what is displayed in the plot by clicking on the axis labels and selecting the channels you want to be presented to you.

To gate your data, select the gate shape from the top,

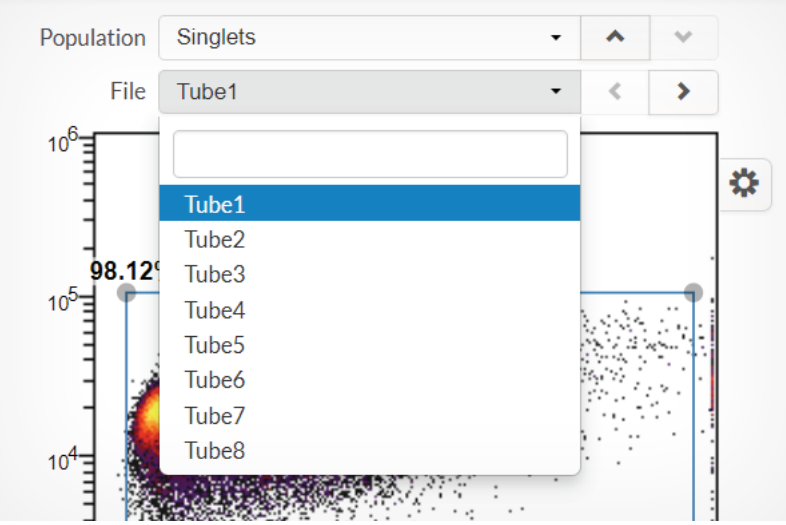


then draw your gate on the plot and give it a unique name and click on OK.

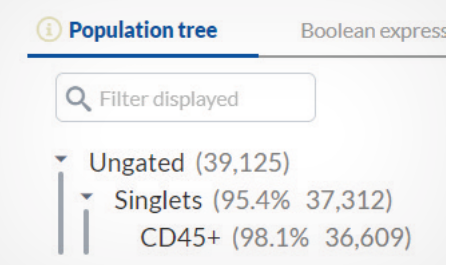


To move one level down in the hierarchy, double click inside the gate and modify the X and Y axes according to your preferred gating strategy.

The input population for the plot is displayed on top of the dotplot, in the Population row, while in the File row you will be able to select which FCS file is displayed in the plot. You can move among populations and files using the arrows or choosing from the dropdown menu that is displayed when clicking on the file or population name.



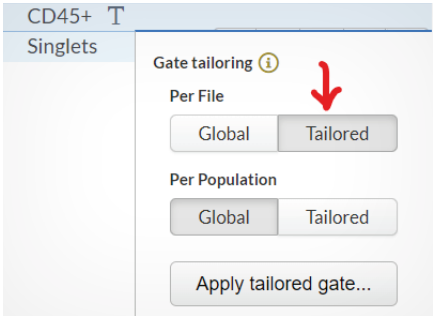
On the right side you will be able to see your population tree with the statistics corresponding to the file that is displayed on the dotplot.



Keep in mind that by default all the gates created in the platform are global, meaning that they are applied to all the FCS files present in an experiment.

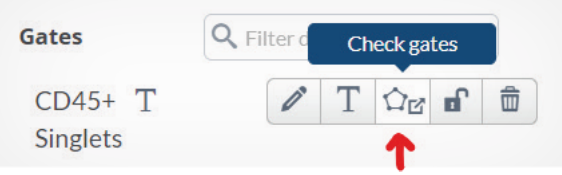
Of course, you might want to be able to set the position of a gate on a per file basis. To do so, you will have to "Tailor" the gate. To access this function, select the gate of interest from the list of gates on the left of the screen and click on the T icon.

Then click on **Per File > Tailored**



You are now able to move the gate only for the file that is displayed on the plot.

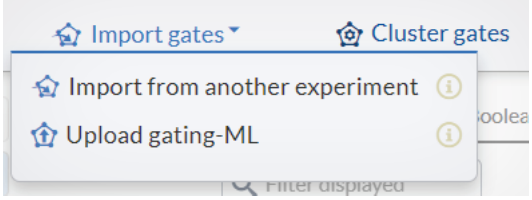
It often happens that you have many files in one single experiment and you want a quick way to check the position of a gate on all of them at a glance. To do so, select your gate of interest from the gate list on the left and click on the third icon, Check gates



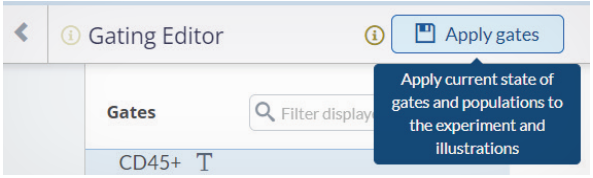
You will be directed to a new page where you will be able to gate on all the FCS files of your experiment.

In the platform, you also have the option to tailor a gate per population. Please read **this article** to learn more.

Importantly, just as we have seen for scales, the Cytobank platform allows you to import gates from another Cytobank experiment or upload a Gating-ML: you can do so by accessing the Import gates functionality from the top bar.



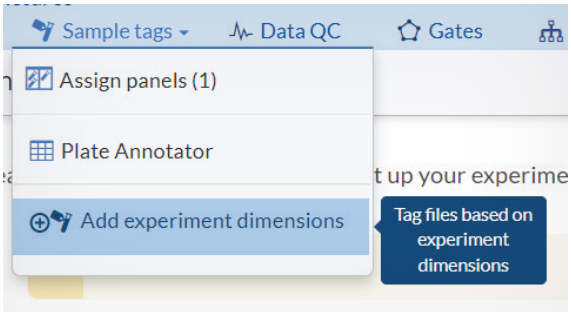
Please remember: before leaving the Gating Editor page you must apply your gates for them to become visible in other pages of the app. To do so, simply click on **Apply gates** button on top left.



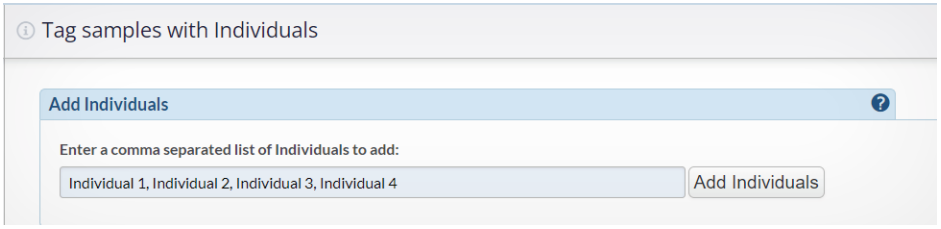
STEP 8: Assign Sample Tags

Sample Tags are the way you can bring the information about experimental variables (metadata) into a Cytobank experiment. Experimental dimensions/variables are, for example, type of treatments for each sample, or healthy donors vs patients, or timepoints for sample collection. Annotating your FCS files with Sample Tags will allow you to build illustrations faster, as it enables you to work one level above the single FCS file. To learn more read this article.

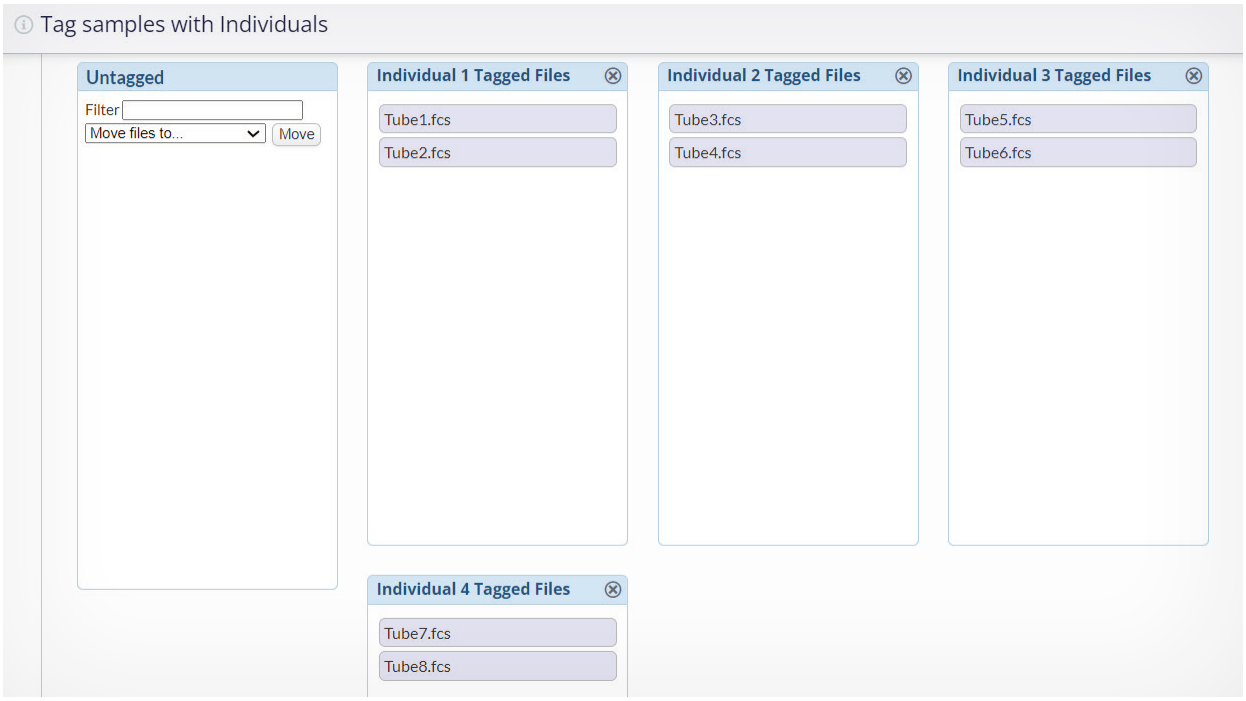
To add Sample Tags to our example experiment, access the Sample Tags function on the top blue navigation bar and click on Add experiment dimensions.



Then click on Tag files with Individuals, then add the list of four individuals separating it with a comma, and click on the Add Individuals button.

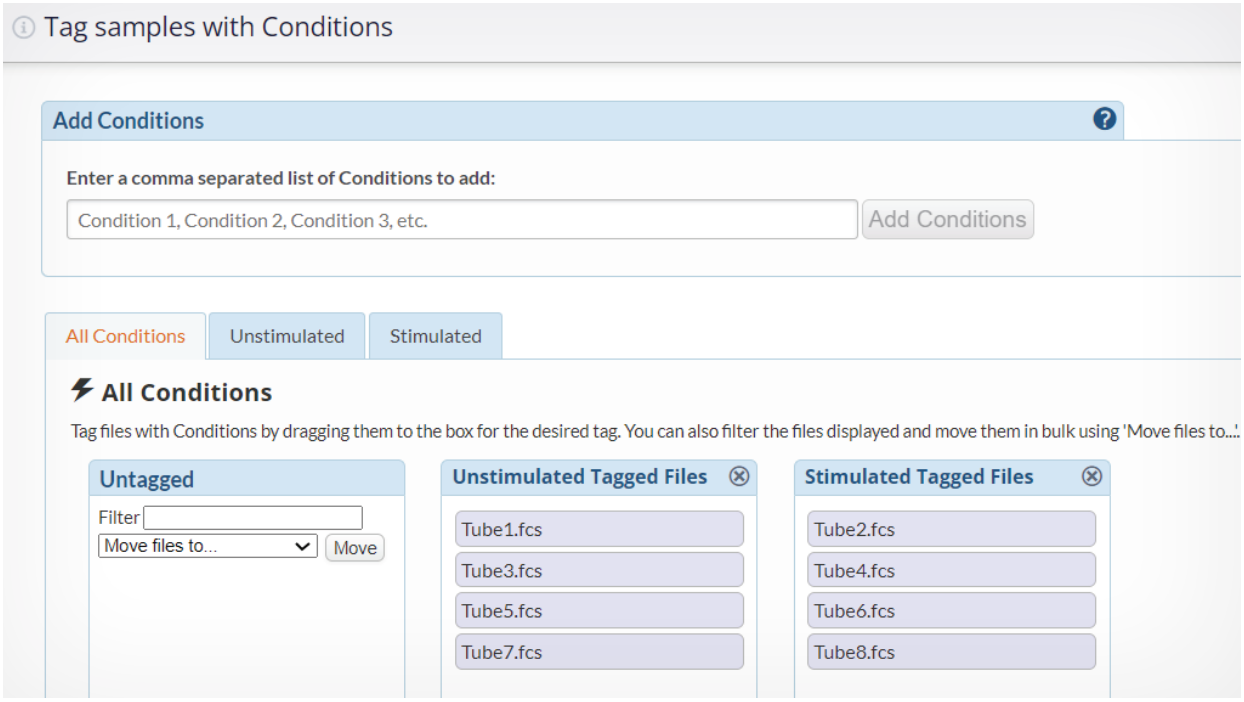


The platform will create 4 columns and you will be able to drag and drop each FCS file from the Untagged to the appropriate location (for our example experiment, follow the picture below).



If you chose a name that was even partially reflected in the FCS file name, the platform would have automatically assigned the samples.

Repeat the same steps to tag files with the below conditions:

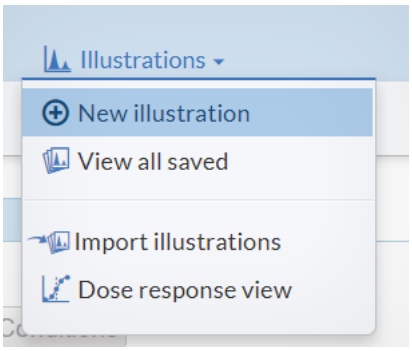


Remember that everything in the app is instantly saved: no need to search for a "save" button.

Now it's time to create an Illustration!

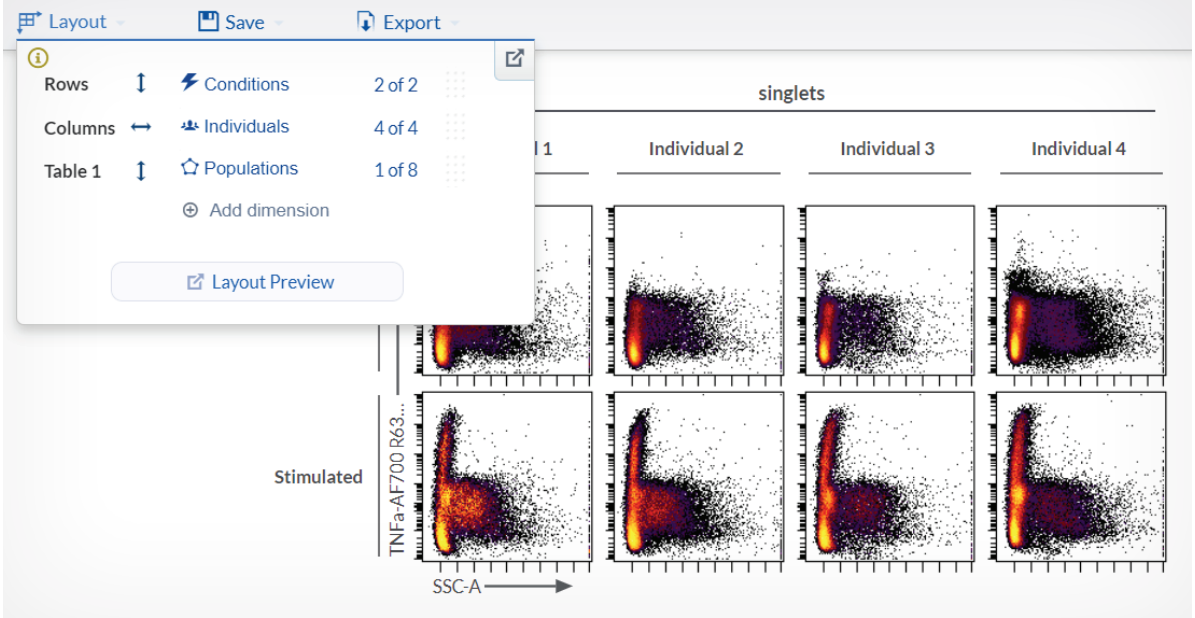
STEP 9: Create an Illustration

To inspect your data, leverage the Illustration Editor to quickly build meaningful figures based on the Sample Tags. In the Illustration Editor, Sample Tags become variables for configuring an Illustration; these variables can be toggled on or off and rearranged dynamically to build and modify a plot layout.
To create an Illustration, access the Illustration Editor by clicking on Illustration > New Illustration in the blue navigation bar on top.



Give it a name and click on OK.

To modify what is displayed, click on Layout on top left and change the Rows, Columns and Table 1 to follow this picture:

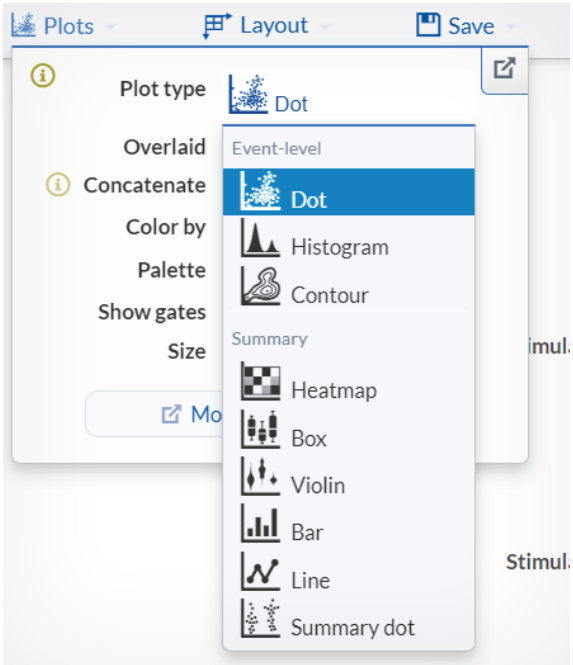


The axis labels are clickable, and you can click on them to open the dropdown menu to pick which channels to display.

To learn more on how to modify Illustrations, please **read this article.** In the Cytobank platform, we aim at making everything as intuitive as possible, therefore you should be able to easily learn how to use the Layout menu and the Plot menu to build your desired figure. Nevertheless, while exploring the Illustration Editor functionalities, please refer to the **Cytobank support website** to access a comprehensive list of tutorial articles and videos to help you.

STEP 10: Create Summary plots

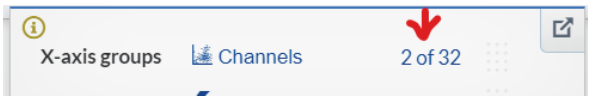
In the Cytobank platform, you can access both event-level as well as summary plots. To switch between one and the other representation, access the Plot menu on the top navigation bar.



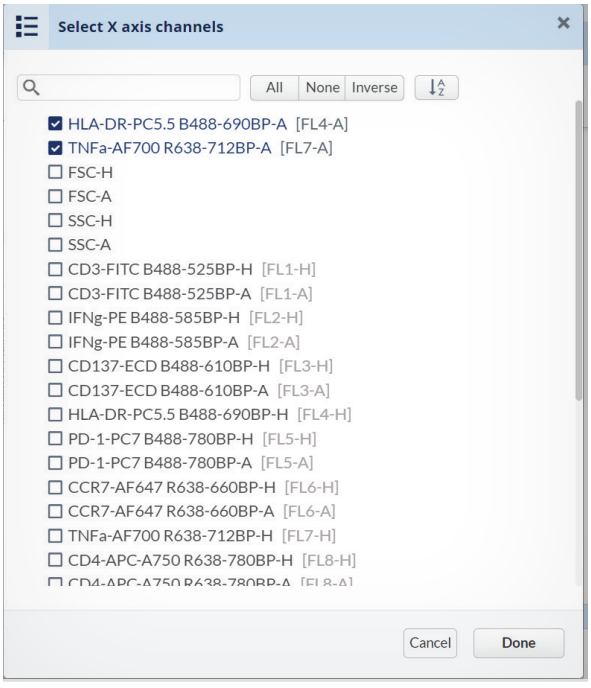
Modify the Illustration you have created in the previous paragraph to display Box plots instead of Dot plots by clicking on “Box” from the dropdown menu.

Then modify the Layout menu to have Channels in the x-axis groups, and Conditions in the Subgroups, as in the following picture:

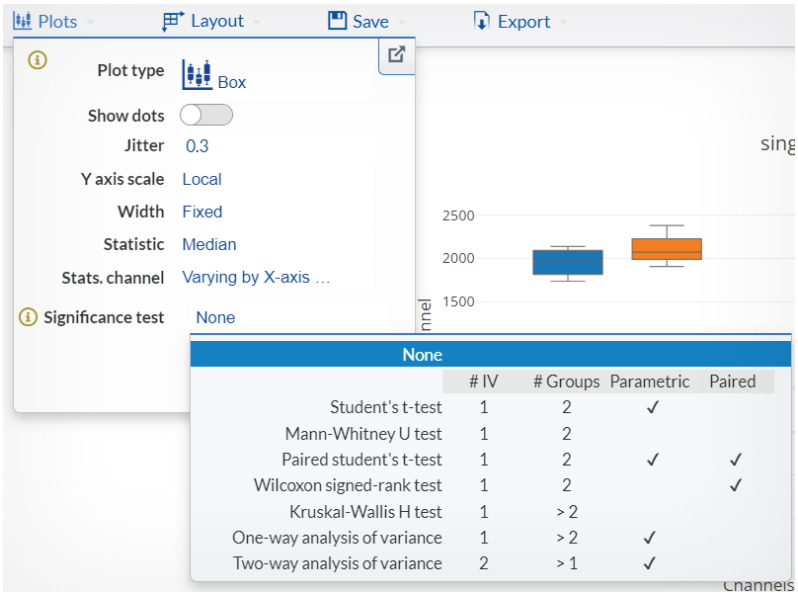
To display relevant channels, click on the X-axis groups row, where the numbers are displayed,



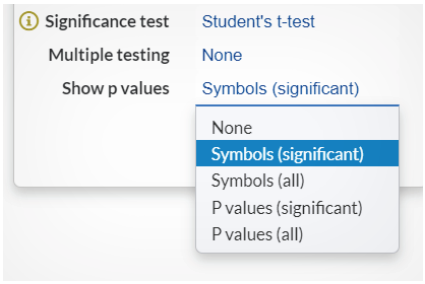
and you should be presented with a screen like this, where you can make your selection of the channels to be included in the figure:



In the Cytobank platform, besides using summary plots to comprehensively evaluate your data, you can also run some statistical tests. To do so, access the functionality via the Plots menu on the top blue bar and click on the Significance test menu row to be presented with a table of what is available in the platform.

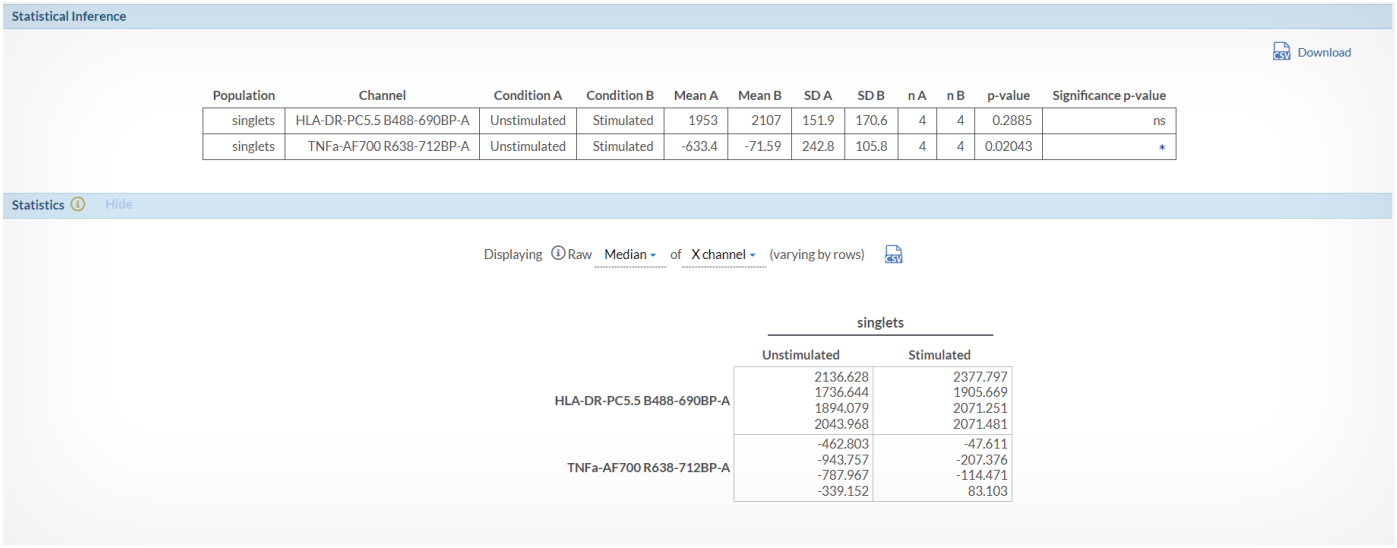


Upon selection, you will be able to access further customization tools:



If you have followed along with the demo dataset, you should now see a figure like this:

By scrolling to the bottom of the page, you are able to access other relevant data, like table of statistics and raw values:



Statistical Inference

Download

Population	Channel	Condition A	Condition B	Mean A	Mean B	SD A	SD B	n A	n B	p-value	Significance p-value
singlets	HLA-DR-PC5.5 B488-690BP-A	Unstimulated	Stimulated	1953	2107	151.9	170.6	4	4	0.2885	ns
singlets	TNFa-AF700 R638-712BP-A	Unstimulated	Stimulated	-633.4	-71.59	242.8	105.8	4	4	0.02043	*

Statistics Hide

Displaying Raw Median of X channel (varying by rows)

	singlets	
	Unstimulated	Stimulated
HLA-DR-PC5.5 B488-690BP-A	2136.628 1736.644 1894.079 2043.968	2377.797 1905.669 2071.251 2071.481
TNFa-AF700 R638-712BP-A	-462.803 -943.757 -787.967 -339.152	-47.611 -207.376 -114.471 83.103

Both the Illustration as well as Statistics can be exported for your further reference.

Congratulations!

You have reached the end of this quick start guide and you should now be all set to start using your data in the Cytobank platform.

Please refer to the search function of the **Cytobank support website** for access to an extensive collection of articles that will guide you through any step of data analysis using the Cytobank platform.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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