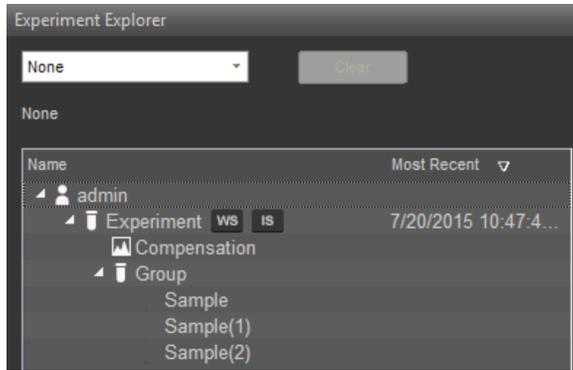# Setting up FlowJo to work with Attune NxT Files

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Revised:

On the Attune, there are three components to name: Experiment, Group, and Sample. Within the experiment, you can choose to create as many groups and samples as you wish.

To translate this information to the FCS file names, the FCS files will be named as "experiment_group_sample.fcs". However, you will find that when you put these files into FlowJo, you will only see the sample name. This protocol will show you how to get the group name to show in FlowJo.

Why might you want to have the group name? It depends on what information you put into the group name. Some people may use one group for controls and a second group for samples. Some people may use the groups to separate different cell lines or tissues within the same experiment. One scenario where knowing the group name is important is if you have multiple gentoypes or cell lines and multiple treatments:

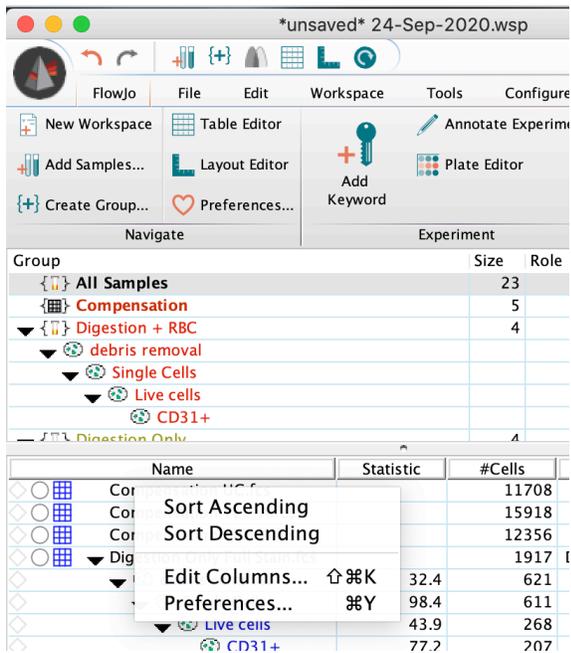|	Treatment 1	Treatment 2	Treatment 3	Treatment 4
WT				
KO				

In this scenario, there are 8 tubes. I may choose to label each sample WT treatment 1, WT treatment 2, etc. Or I can save time if I use the groups. I can label "group 1" as WT and within that group, make 4 samples for treatment 1-4. Then I can duplicate my WT group and change the group 2 name to KO. This is the fastest way to label samples, but when the files are moved to FlowJo, it is critical to know the group name and sample name.

Now if I move these files into FlowJo, I will only see the sample name (Treatment 1, Treatment 2, etc.) and not the group name (WT or KO). Therefore I will have two files for each treatment, and I will not be able to immediately tell which one is WT and which one is KO.
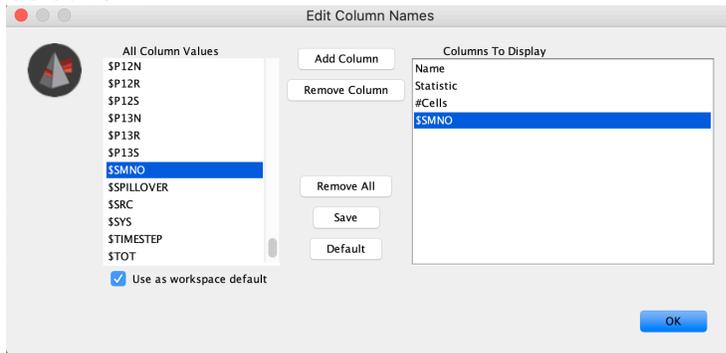
In FlowJo, the group name can be found with the "$SMNO" keyword. There are two ways to make this keyword show in the FlowJo workspace.

Option 1: Add a fourth column in the samples pane
1. Right click the column header of the samples pane (where it says Name, Statistic, #Cells) and select "edit columns"

2. Select the keyword "$SMNO" from the list on the left and select add to move it to the list on the right. Select OK

3. The group name is now added as the fourth column

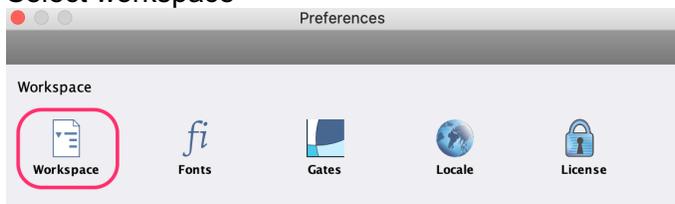| Name	Statistic	#Cells	$SMNO
Compensation UC.fcs		11708	Compensati...
Compensation YL1-A.fcs		15918	Compensati...
Compensation YL2-A.fcs		12356	Compensati...

Option 2: Add a keyword to the tube name

(If you are planning on using batch functions in FlowJo, this method will make the batch functions work as expected)

1. Select the preferences (heart icon)

2. Select workspace

3. Move the radio button selection to "keywork combination" and use the dropdown menu next to it to first select "$SMNO" and then "$FIL". The text below should now say "TUBE NAME + $SMNO + $FIL"

4. The name column in the sample pane should now contain both the group name and sample name for each file.