

Hints for Working with Diva FCS 3.0 files in FlowJo Workspaces

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Naming FCS Files in Diva Software

If you are using FlowJo for data analysis it is important that your file names do not exceed **28** characters. This is especially important for FlowJo versions 8 or lower. Also do not use non-alphanumeric characters (dashes, spaces, symbols etc). FlowJo will "lose the link" to your FCS files. This happens only in the context of moving the workspace from one computer to another, or from one web-server to another. It is best to use shorter file names when analyzing DiVa files in FlowJo - your analyses will be more robust over time. File names in DiVa are taken from the *speciman_tube* field of the DiVa Browser.

An alternative is to use the SAMPLE ID keyword field in DiVa (found in the Inspector window) to enter in more detailed sample info and keep the file name simpler.

The screenshot shows the 'Inspector - Tube_001' window in FlowJo. The 'Keywords' tab is selected, displaying a table of metadata for the specimen and tube. The 'SAMPLE ID' field is highlighted with a red box.

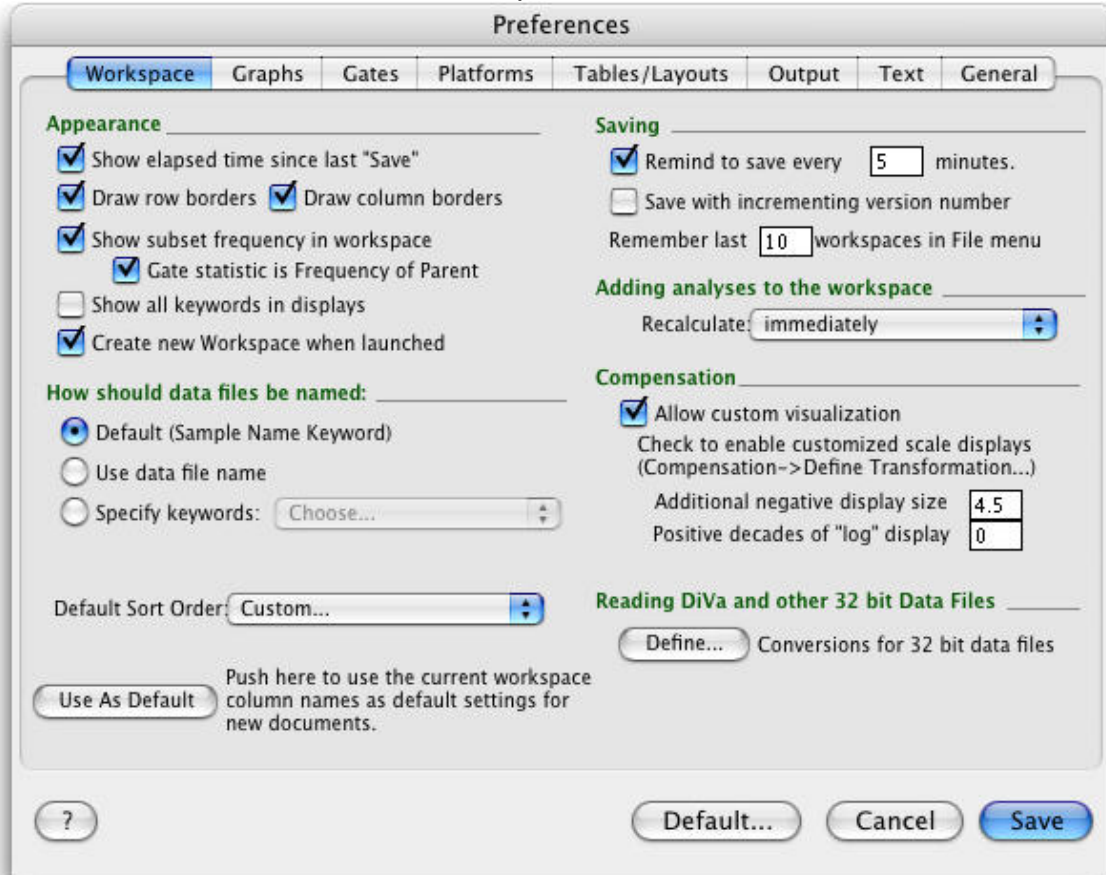
Name	Value	Suffix	Description
CST SETUP STATUS	SUCCESS		Cytometer Performance
CST BEADS LOT ID	45489		Lot ID information for CST
CYTOMETER CONFIG NAME	New Advanced		Cytometer configuration name
CYTOMETER CONFIG CREATE	05/29/2009 12:14:33 PM		Cytometer configuration
CST SETUP DATE	01/05/2010 11:34:17 AM		Date and time of cytometer
CST BASELINE DATE	10/21/2009 02:53:54 PM		Date and time of cytometer

Name	Value	Suffix	Description
CST SETUP STATUS			Cytometer Performance
CST BEADS LOT ID			Lot ID information for CST
CYTOMETER CONFIG NAME			Cytometer configuration name
CYTOMETER CONFIG CREATE			Cytometer configuration
CST SETUP DATE			Date and time of cytometer
CST BASELINE DATE			Date and time of cytometer

Name	Value	Suffix	Description
* SAMPLE ID			String used to identify a
* PATIENT ID			String used to identify a
* \$OP			User name of application
* \$INST			Institution where data was
* GUID			String value with a unique
* CST SETUP STATUS			Cytometer Performance
* CST BEADS LOT ID			Lot ID information for CST
* CYTOMETER CONFIG NAME			Cytometer configuration name

By default, if you enter in a SAMPLE ID keyword it will appear in the “Name” column in the FlowJo workspace window list. If no SAMPLE ID is entered the file name will be displayed. What appears in the “Name” column in the FlowJo workspace window list can be controlled in the Preferences dialog in FlowJo.

Go to FlowJo -> Preferences- “Workspace” tab



Look in the “How should data files be named:” section. The Default is “Sample Name Keyword”. This is equivalent to the “SAMPLE ID” keyword in DiVa. You can also select “Use Data File Name” or choose a specific keyword by selecting “Specify keywords.”

Adding Additional Sample Info in FlowJo

You can also use the annotation column in FlowJo to manually enter in additional sample information. In the workspace go to the “Edit...” column and select “Annotation” and click “add column”.

Copying and Pasting Keywords Into and From FlowJo

You can easily copy sample info and keywords from FlowJo into an excel spreadsheet or quickly add sample info and keywords into FlowJo from an excel spreadsheet. Please note that you cannot change parameter names in FlowJo but you can add or alter parameter labels. If the fluorochrome you are using is not listed you can indicate in the parameter label the fluorochrome you are using.

Starting with a FlowJo workspace:

1. Open your workspace. You don't need the data files for this task. If this is a "broken" workspace, if it lost link to source data after one or the other were moved, it will still work.
2. Make sure all pertinent keywords are showing in the workspace as columns.
3. Click Edit menu/Copy samples as text.
4. Go to your spreadsheet and paste the clipboard there. It should look like just like your workspace:

The image shows two windows side-by-side. The top window is a FlowJo workspace displaying a table of sample information. The bottom window is an Excel spreadsheet titled 'Workbook1' showing the same data pasted into a grid.

Edit...	Sort...	Name	Statistic	#Cells	FL1-H	\$COM
		Sample 1 (Control)		10000		
		Sample 2		10000	CD8.8	2 ug/ml
		Sample 3		10000	CD8	1 ug/ml
		Sample 4		10000	CD8	.5 ug/ml
		Sample 5		10000	CD8	.25 ug/ml
		Sample 6		10000	CD8	.125 ug/ml
		Sample 7		10000	CD8	.0625 ug/ml
		Sample 8		10000	CD8	.031 ug/ml

	A	B	C	D	E
1	Name	Statistic	#Cells	FL1-H	\$COM
2	Sample 1 (Control)		10000		
3	Sample 2		10000	CD8.8	2 ug/ml
4	Sample 3		10000	CD8	1 ug/ml
5	Sample 4		10000	CD8	.5 ug/ml
6	Sample 5		10000	CD8	.25 ug/ml
7	Sample 6		10000	CD8	.125 ug/ml
8	Sample 7		10000	CD8	.0625 ug/ml
9	Sample 8		10000	CD8	.031 ug/ml
10					
11					

Tip: if your "paste" ended up including gates like below try collapsing all of the gates. To do this quickly, hold down the [cmd] key while you collapse one of the gates. This will batch-collapse or batch-expand all gates.

Sort...	Name	Statistic	#Cells
▼	Sample 1 (Control)		10000
▼	⊗ FSC-H, SSC-H subset	71.3	7128
	⊗ RL1-H+	0.014	1
	⊗ RL1-H-	100	7127
▼	Sample 2		10000
▼	⊗ FSC-H, SSC-H subset	70.9	7093
	⊗ RL1-H+	28.6	2028
	⊗ RL1-H-	71.4	5065
▼	Sample 3		10000
▼	⊗ FSC-H, SSC-H subset	70.1	7010
	⊗ RL1-H+	32.5	2276
	⊗ RL1-H-	67.5	4734
▼	Sample 4		10000
▼	⊗ FSC-H, SSC-H subset	69.3	6928
	⊗ RL1-H+	27.5	1904
	⊗ RL1-H-	72.5	5024
▼	Sample 5		10000
▼	⊗ FSC-H, SSC-H subset	70.9	7087
	⊗ RL1-H+	33.1	2349
	⊗ RL1-H-	66.9	4738
▼	Sample 6		10000
▼	⊗ FSC-H, SSC-H subset	71.5	7151
	⊗ RL1-H+	30.7	2193
	⊗ RL1-H-	69.3	4958
▼	Sample 7		10000
▼	⊗ FSC-H, SSC-H subset	71.2	7120
	⊗ RL1-H+	32.1	2289
	⊗ RL1-H-	67.9	4831
▼	Sample 8		10000
▼	⊗ FSC-H, SSC-H subset	70.9	7091
	⊗ RL1-H+	32.3	2289
	⊗ RL1-H-	67.7	4802

From this point on, the instructions are identical with the next section (starting with a spreadsheet.)

Starting with a spreadsheet of keywords: (the keywords' names should be in one row and all their values should be in columns)

1. In your spreadsheet, select the keyword values, excluding the keyword name. You can select multiple columns at a time.
2. Go to "new" workspace. Double click the first cell corresponding to the same keyword in the new workspace - notice the cell appears as though you could type into it.

Tip: Keywords which are not modifiable will not be processed - those columns

will be ignored. These keywords include parameter names and scaling etc.

3. Use the keyboard shortcut **[cmd]+[v]** to paste the clipboard. All values for all cells should appear in the workspace.

Tip: if you pasted the wrong values in the wrong place, you can't undo, but you can paste over any existing values. So find the right cell and do the paste again. Also, if you need to quickly erase a whole column of wrong values, double-click any value in that column, erase it (backspace or delete) and hit **[cmd]+[e]**. This applies current value to the whole column.

Tip: the new transferred meta data only exists for the current workspace. If you want to make it part of the FCS files permanently, select all files in the workspace and choose the 'export' command from the Workspace menu. The new data set will include the keyword changes.