



## Hints

The FCS standard is published and instrument manufactures are encouraged to follow the standard. The standard is designed to be shared between people such that they will get the same answers from whatever analysis program is used. This is particularly important from the standpoint of a publication record (i.e., defending the publications should a question arise).

Each manufacturer varies on how “strictly” they follow the standard, so keywords produced on different manufacturers’ instruments may be different or not used. Ultimately, the user will need to get a list of keywords from their instrument manufacturers, but FlowJo has provided a list of most frequently used keywords here: <http://www.flowjo.com/v76/en/fcskeys.html>

Common keywords:

\$BTIM - start time  
 \$ETIM - end time  
 \$CYT - cytometer used  
 \$DATE - date of acquisition  
 \$DATATYPE - I (Integer) or F (floating point)  
 \$FIL - the standard file name (may be different then the file name given on the Operating System)  
 \$PnR - the range of parameter n  
 \$PnN - parameter name of n; typically gives the channel of the instrument  
 \$PnS - stain name of parameter n; typically gives the stain used (e.g. CD3, CD8, ect.)  
 \$PnV - the voltage setting of parameter n  
 \$PnE - the log amplification of parameter n; typically 0,0 from digital instruments  
 \$PnG - the gain used of parameter n  
 \$SPILL - the compensation spillover values  
 Sample text box.

## Links and References

1. Murphy RF and Chused TM. **The proposal of a flow cytometric data file standard.** Cytometry. 1984; 5(5):553-555.
2. No authors provided. **Data file standard for flow cytometry. Data File Standards Committee of the Society for Analytical Cytometry.** Cytometry. 1990; 11(3):323-332.
3. Seamer LC, Bagwell CB, Barden L, Redelman D, Salzman GC, Wood JC and Murphy RF. **Proposed new data file standard for flow cytometry, version FCS3.0.** Cytometry. 1997; 28(2):118-122
4. Spilden J, Moore W, Parks D, Goldberg M, Bray C, Bierre P, Gorombey P, Hyun B, Hubbard M, Lange S, Lefebvre R, Leif R, Novo D, Ostruszka L, Treister A, Wood J, Murphy RF, Roederer M, Sauder D, Zigon R and Brinkman RR. **Data file standard for flow cytometry, version 3.1.** Cytometry A. 2010; 77(1):97-100.



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## Keywords of the Flow Cytometric Standard

The Flow Cytometric Standard (FCS) provides substantial information regarding information of the .fcs data file beyond the fluorescence values recorded at the time of acquisition. The .fcs files exported from the instrument contain numerous keywords, many with values that can be entered by the user during aquisition. Utilizing the metadata within the file can provide an easy and straightforward way to organize data, which in turn, will lead to quicker analysis, streamlined batching and better presentation of the results. In this technical document, the numerous methods to view, edit and present the metadata of the FCS file within FlowJo are covered.

## Introduction

The FCS standard has been through many iterations, with the most recent release of the FCS 3.1 standard coming in 2010 (1-4). Each FCS file contains the fluorescence values for every parameter recorded at the time of acquisition. However, there are numerous other values stored as part of the FCS standard as well. These values are called keywords and are found in the header of the file. The FCS file can be opened in a text editing program and the keywords and their values can easily be viewed. For example, the first line of the file will indicate the FCS version number of the file like FCS2.0 or FCS3.0. Some keywords are given values automatically (e.g.: \$CYT which is the cytometer used, \$BTIM is the start time, \$ETIM is the end time, \$DATE is the date the file was acquired and \$FIL is the file name) and some are blank (requiring user input). A more comprehensive list of “important” keywords is provided at the end of this document.

Flow Cytometrists are encouraged to enter in the values of keywords during acquisition in order to ensure that the values are “locked” into the data file when exported. To maintain the integrity of the standard, these values within the FCS file can not be easily edited after export from the acquisition instrument. However, FlowJo does provide the ability to edit most of the keywords, enter values for existing keywords and also create new keywords. These keyword additions or alterations are only stored within the workspace in which they occurred, so the original FCS file is left unchanged, once again ensuring the integrity of the standard.

Some primary uses of keywords are to organize data analysis in the FlowJo workspace through the use of unique groups and to provide values to reference exported data in the FlowJo Table Editor and Layout Editor.



# Key Words

## Procedure

Clicking on Sample Properties provides the list of keywords for each file and their associated value.

**Keyword Info**

SBEGINDATA: 3584  
 SBEGINTEXT: 0  
 SBTIM: 11:52:08  
 SBYTEORD: 4,3,2,1  
 SCYT: LSR11  
 SDATATYPE: F  
 SDATE: 10-OCT-2008  
 SENDDATA: 2231503  
 SENDTEXT: 0  
 SETIM: 11:52:32  
 \$FIL: B1 .fcs  
 \$INST:  
 \$MODE: L  
 \$NEXTDATA: 0  
 \$OP: Administrator

Edit the workspace columns to add additional keywords to the workspace. Double click a cell the edit the text.

Name	Statistic	#Cells	FITC_Alexa 488-A reagent	PE-A reagent	PE-Cy55-A reagent	PE-Cy7-A reagent	CD3 CD14 Live_Dead-A reagent
B1.fcs		37132	CD3	CD11c	CD3	CD45RO	7AAD
B2.fcs		46747	CD56	CD11c	CD3	CD45RO	7AAD
B3.fcs		76153	CD56	CD11c	CD3	CD45RO	7AAD
B4.fcs		68916	CD56	CD11c	CD3	CD45RO	7AAD
B5.fcs		71737	CD56	CD11c	CD3	CD45RO	7AAD
B6.fcs		22701	CD56	CD11c	CD3	CD45RO	7AAD
B7.fcs		54464	CD56	CD11c	CD3	CD45RO	7AAD
B8.fcs		39216	CD56	CD11c	CD3	CD45RO	7AAD
B9.fcs		30457	CD56	CD11c	CD3	CD45RO	7AAD

Add a new keyword to the workspace. Give it an unique name and this column will be created in the workspace. The values for this keyword in each sample can then be entered into the workspace.

**Add Keyword**

What is the name of the keyword to add?  
 New Keyword

Cancel OK

Name	Statistic	#Cells	New Keyword
B1.fcs		37132	made
B2.fcs		46747	in
B3.fcs		76153	FlowJo
B4.fcs		68916	
B5.fcs		71737	
B6.fcs		22701	
B7.fcs		54464	
B8.fcs		39216	
B9.fcs		30457	

**Create Group**

Appearance  
 Name:   
 Color: Group Name Style: bold  
 Role: Test Parameter Key:

Sample Inclusion Criteria  
 Live group  Synchronized  
 Include samples that use the following staining:  
 CD32, CD86, CD209, CD14, 7AAD, 2H2, CD3, CD19  
 CD56, CD11c, CD3, CD45RO, 7AAD, 2H2, CD4, CD8

Select:  Equals:  Choose...

More Choices Fewer Choices  Show all keywords in menus

With reference to samples in another group:  
 Only choose from samples in Group (No specified group)  
 Also include all

Apply Changes Close Create Group

Use the Group Dialog to organize subsets of samples in the same workspace.

Have different staining panels? If the keyword values for the parameters were entered, FlowJo provides those staining panels automatically! You can have several groups of different staining conditions all in the same workspace. Different gating strategies can then be performed on the different groups.

**Table Editor:** Add keywords to the Table Editor and the value of the keyword for each sample will be present in the resulting table!

Columns Plots

Add Keyword v

Add Parameter Keyword v

Keywords

Ancestry Subset Statistic For	Day	lymphs Freq. of Parent
A1.fcs	0	53.9%
A2.fcs	2	59.3%
A3.fcs	4	59.2%
A4.fcs	6	53.5%
A5.fcs	8	55.1%
A6.fcs	10	37.7%
A7.fcs	12	57.7%
A8.fcs	14	52.1%
A9.fcs	16	59.5%
B1.fcs	0	56.5%
B2.fcs	2	60.5%
B3.fcs	4	59.4%
B4.fcs	6	54.1%
B5.fcs	8	59.4%
B6.fcs	10	44.4%
B7.fcs	12	55.9%
B8.fcs	14	49.6%
B9.fcs	16	55.3%
Mean	8.00	54.6%
Standard Deviation	5.31	5.87%

**Layout Editor :** Insert Keywords into text boxes within the Layout Editor so the value is automatically updated when batched or used in a template.

Insert Keyword: \$Cells

For example, in the text box below, we selected the Patient ID keyword to be added. (FlowJo uses bracket code to indicate to the program what is being entered. However, the user is only required to select the keyword desired from the drop down menu interface shown above.)

The CD8+ Count for Patient <Key dataset="1" originalDataset="1" name="\$Cells"/> is: <Statistic DataSet="5" originalDataset="5" path="ForSc, OrthSc subset" name="Count"/>

The output looks like this and will iterate with sample selection and also batch!

The CD8+ Count for Patient ID 1001 is: 7626

Keywords can also be used as iterators and discriminators to set up sophisticated layouts for more advanced batching. (See the "Advanced Batching" Technical Note for more information.)

\$Cells ID 1001 w/ CREATOR