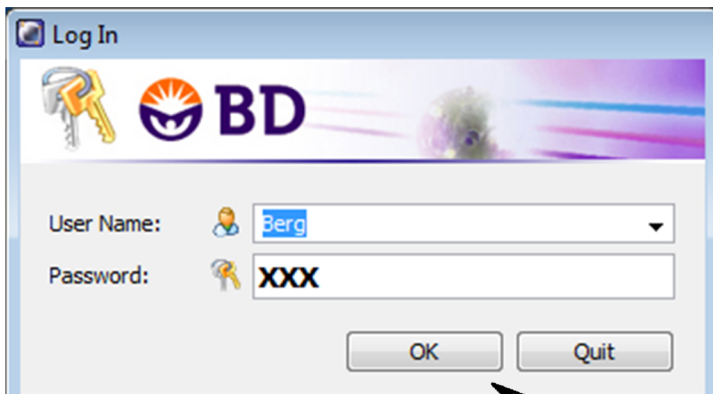


DiVa Software Guide for Acquiring Data

Login to computer using Healthcare or Iowa domain credentials (Healthcare ID or HawkID)

Double click on the FACSDiVa icon



Click on pull-down menu and choose principal investigator

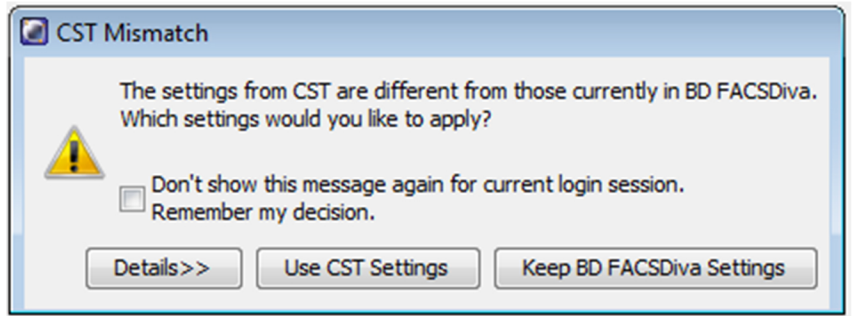


Password is principal investigator's first name (lower case)

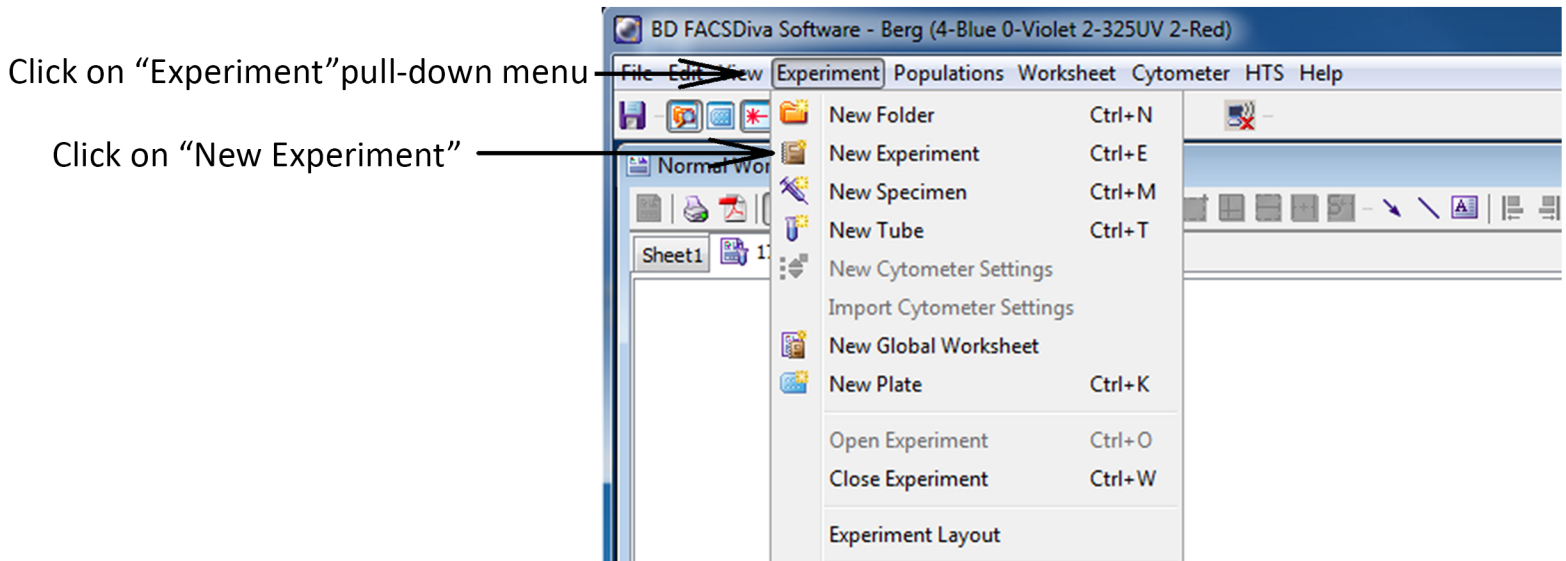


Click on "OK" or hit the "enter" key



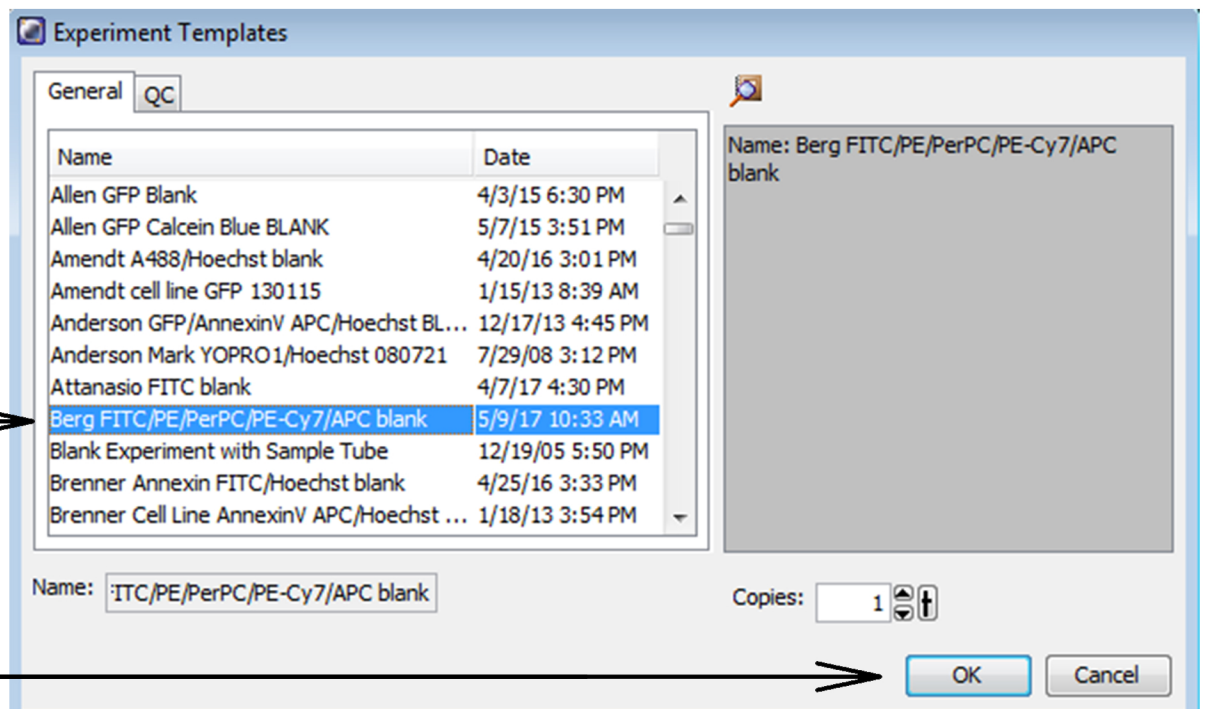


If prompted, click on "Use CST Settings"



Click on "Experiment" pull-down menu

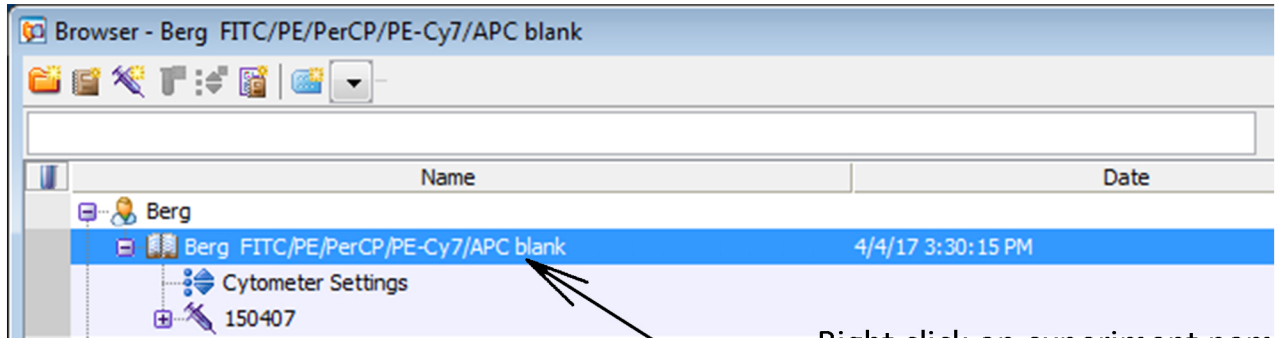
Click on "New Experiment"



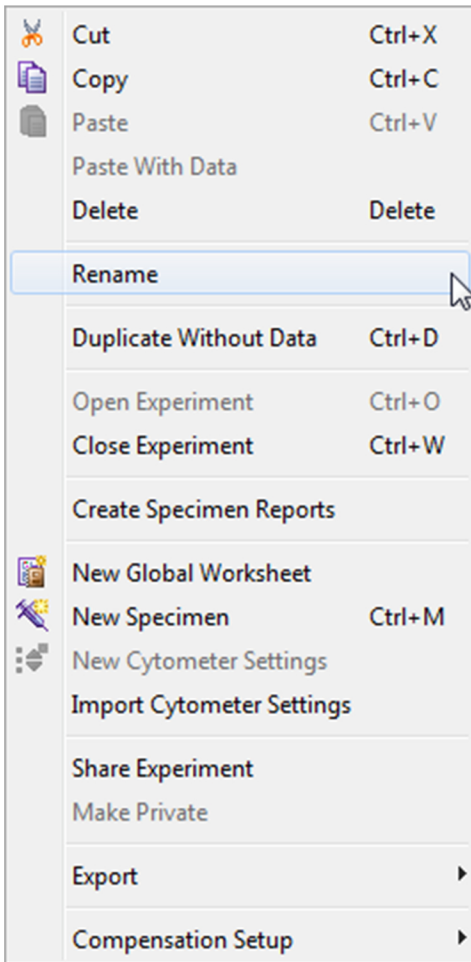
Choose appropriate template

Click "OK"

Find the Browser window

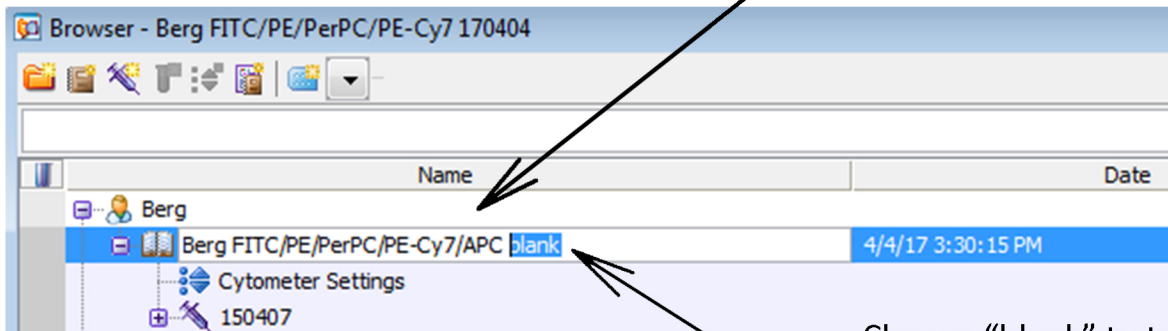


Right click on experiment name

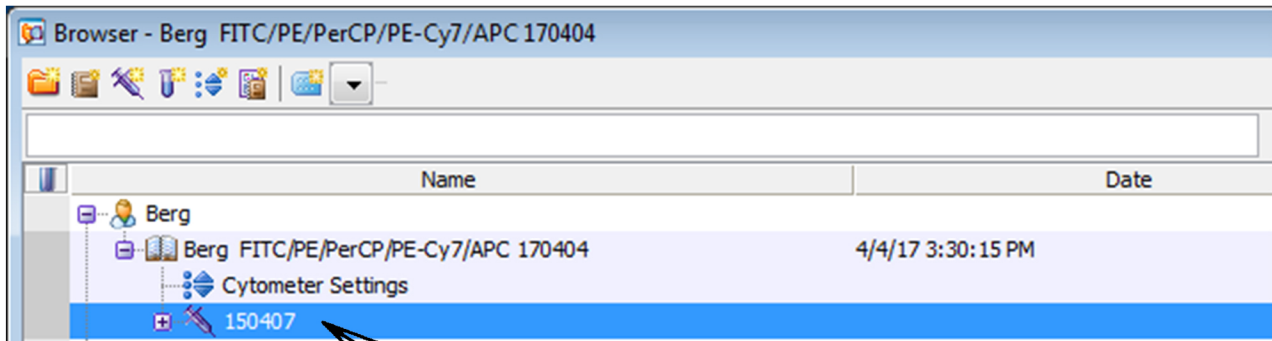


Click on "Rename"

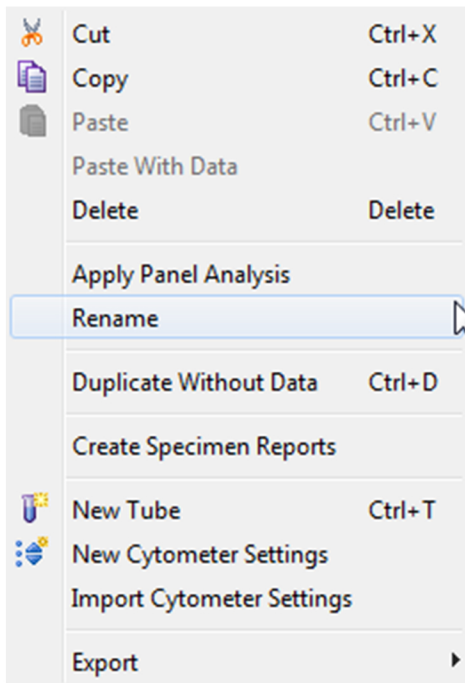
The general naming scheme for each experiment should include the PI's last name plus an experiment description plus the date, e.g. "Berg FITC/PE/PerCP/PE-Cy7/APC 170404"



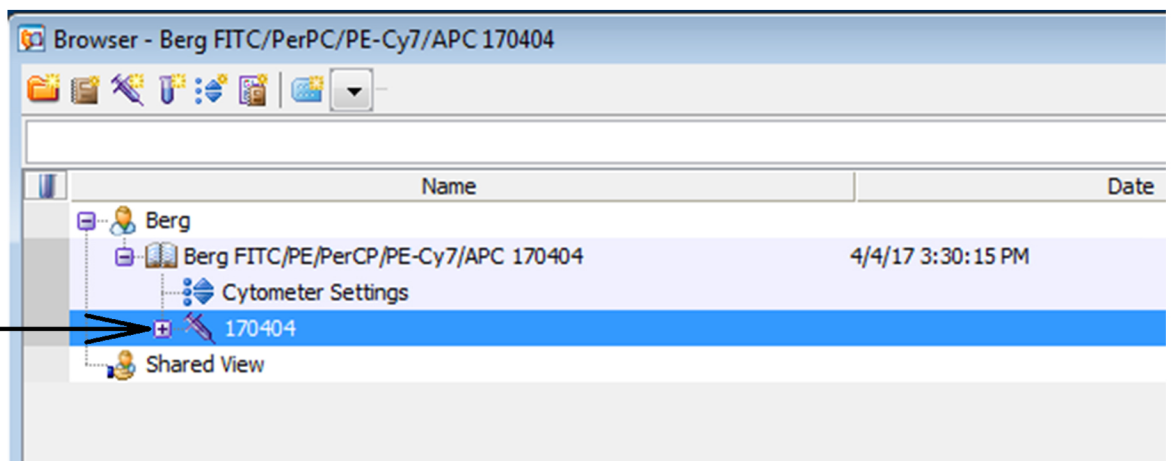
Change "blank" to today's date by yymmdd, e.g. 170404 is April 4, 2017



Right click on specimen name



Click on "Rename" and change to today's date by yymmdd, e.g. 170404 is April 4, 2017



Click on the "+" to expand the tree and expose the sample line

Data Acquisition Controls

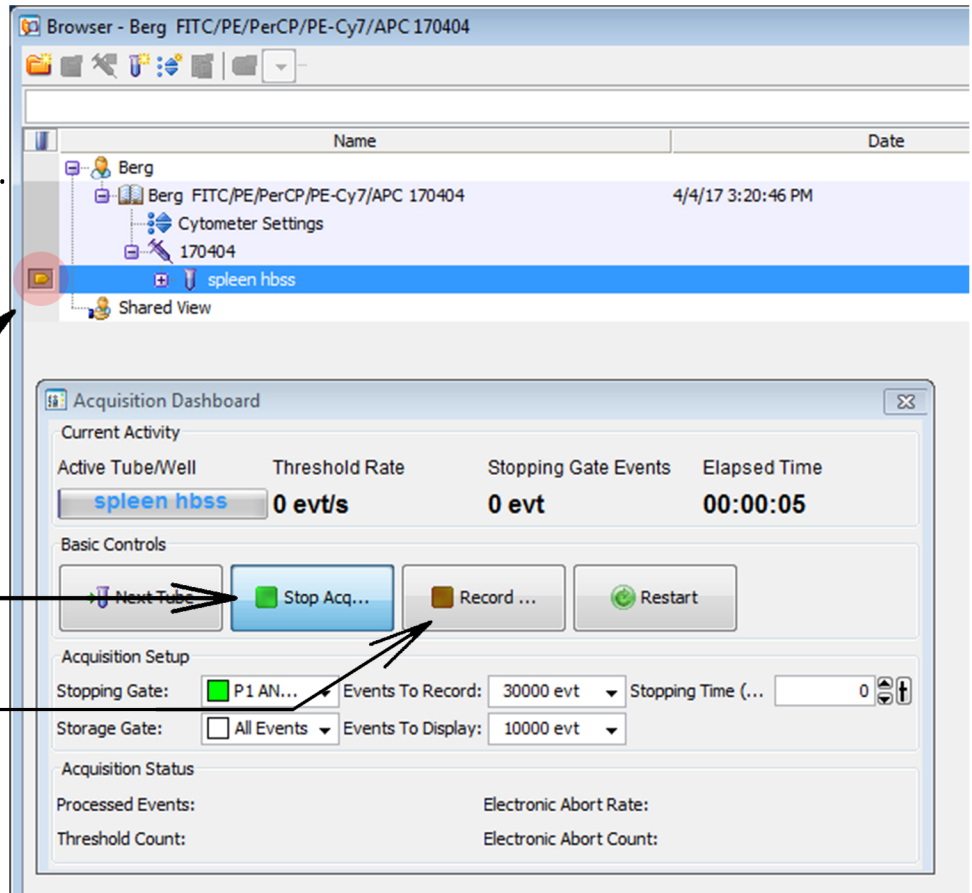
With a sample tube loaded on the instrument and the fluidics control on "run," events will appear in the plots on the computer monitor when the software is in acquisition mode. The record button saves a data file.

Click on the pointer to start acquisition

Clicking on either of these will toggle acquisition on and off.

When the pointer is yellow and "Acquire" box is green, the software is in acquisition mode.

To save data to a FCS (Flow Cytometry Standard) file, click on the "Record Data" button.



Puts instrument in acquisition mode (events show up on the computer monitor)

Creates next data file

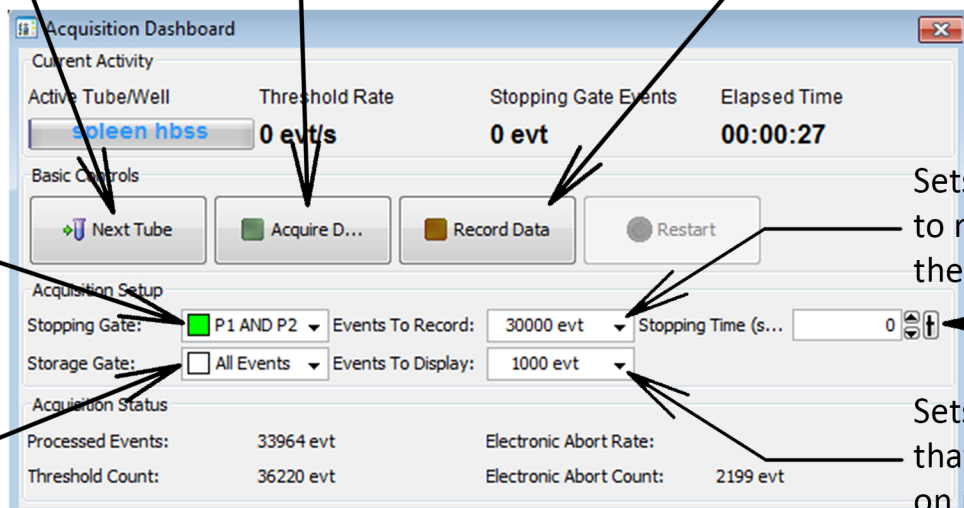
Records data file

Sets stop criteria

Sets number of events to record that satisfy the stop criteria

Always set the "Storage Gate" to "All Events"

Sets number of events that will be displayed on monitor



Adding or Deleting Channels or Changing Fluorochrome Name Assigned to a Channel

Click on "Cytometer Settings" under experiment name

Go to the inspector window

Click on "Parameters" tab

Click on parameter that needs changed

Choose new parameter from list of fluors

Click on the "Add" button to add another parameter, e.g. add an eighth color to a seven color experiment. To delete an unwanted parameter, click on the parameter name and then click the "Delete" button.

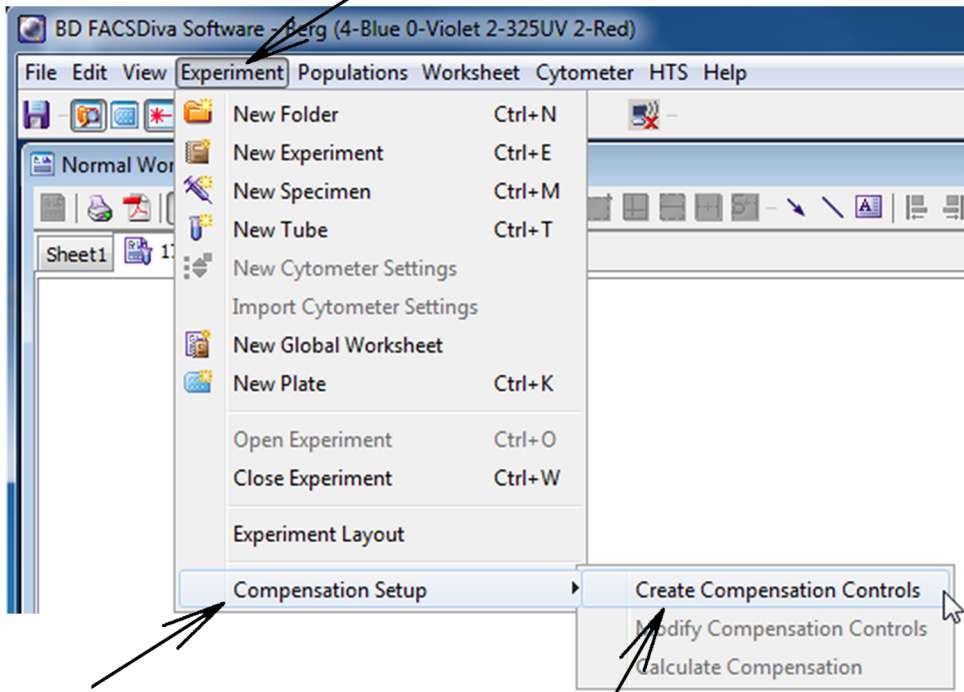
Be sure that "W" (pulse width) is checked for "FSC" (forward scatter)

Parameter	Voltage	Log	A	H	W
FSC	250	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
SSC	300	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
FITC	500	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PE	500	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PerCP	724	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PE-Cy7	662	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
APC	676	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Creating Compensation Controls

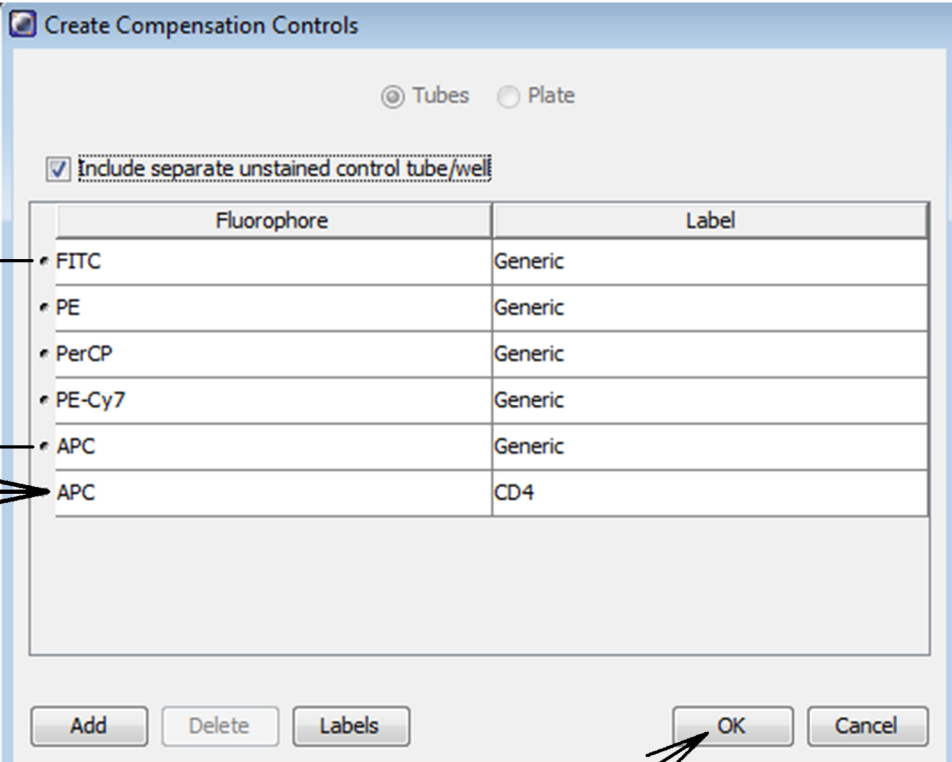
If your experiment requires compensation and the protocol you are using doesn't have compensation controls, use the following steps to create them.

Click on "Experiment" pull-down menu



Scroll down to "Compensation Setup"

Click on "Create Compensation Controls"

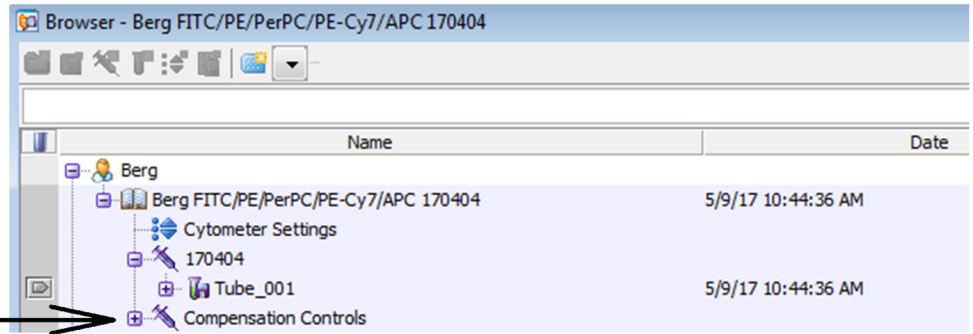


Fluors must match your stains exactly

Delete controls that have an antigen label
(unless using multiple comp controls of the same tandem fluorochrome)

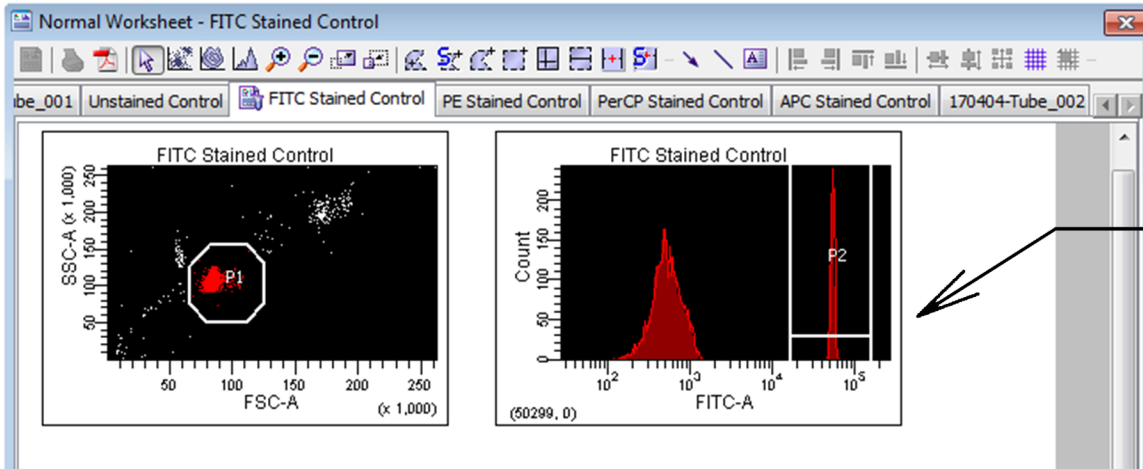
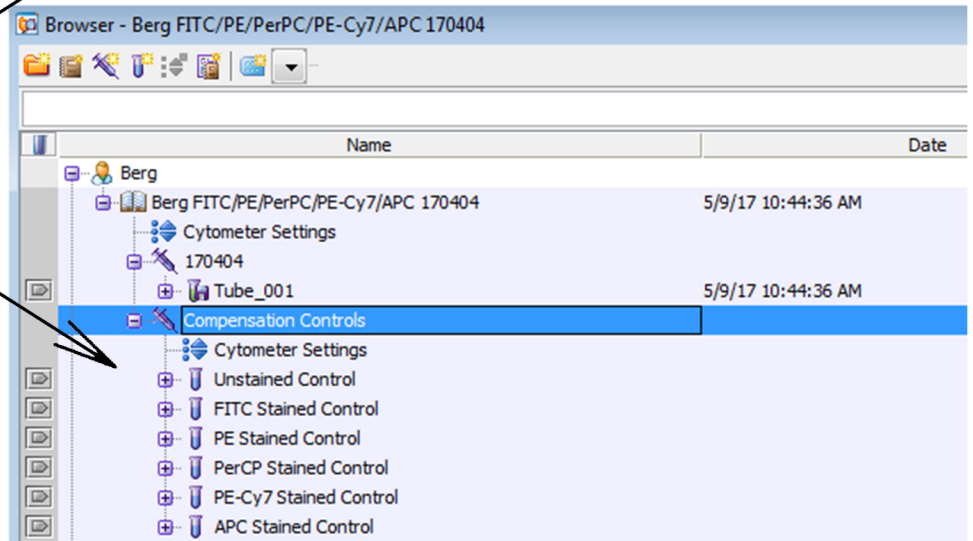
Fluorophore	Label
FITC	Generic
PE	Generic
PerCP	Generic
PE-Cy7	Generic
APC	Generic
APC	CD4

Click on "OK"

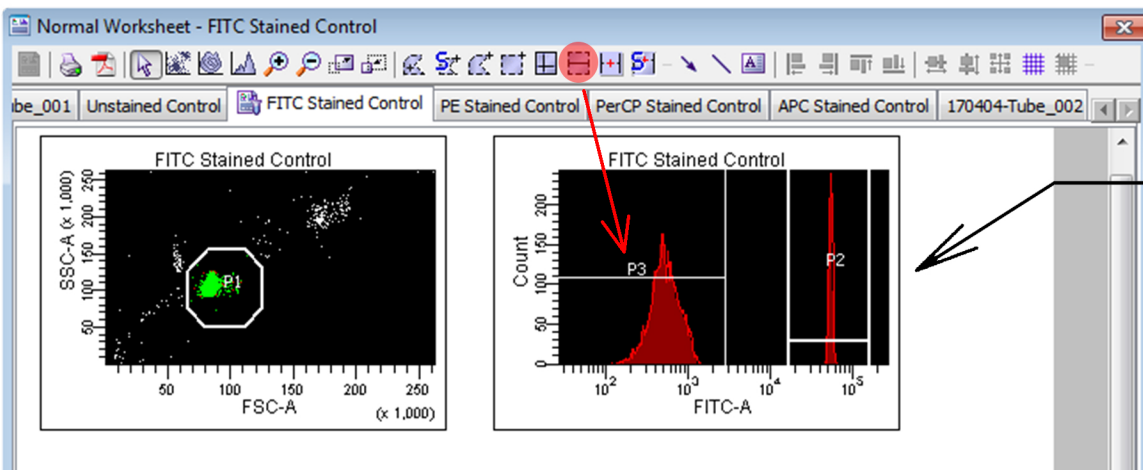


Compensation controls will appear here

Click on the "+" to reveal the individual comp controls

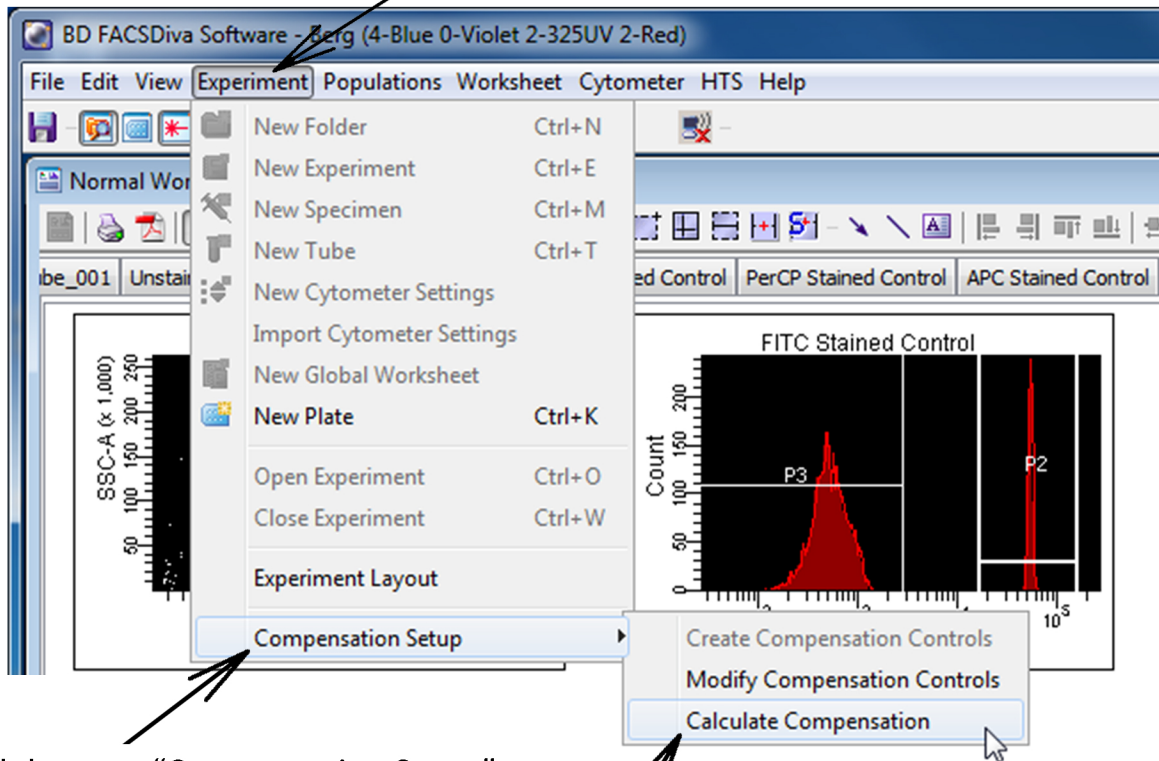


After running comp tubes, center P2 gate over the positive population for each comp tube



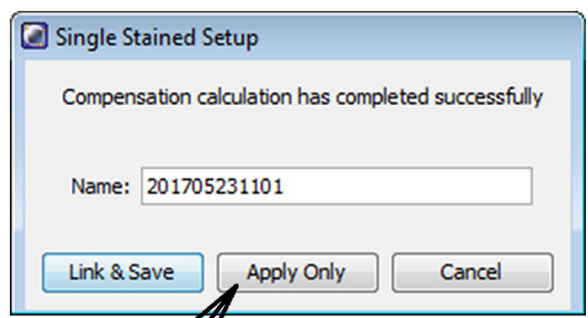
If using beads, add a P3 gate around the negative population for each comp tube

Click on "Experiment" pull-down menu



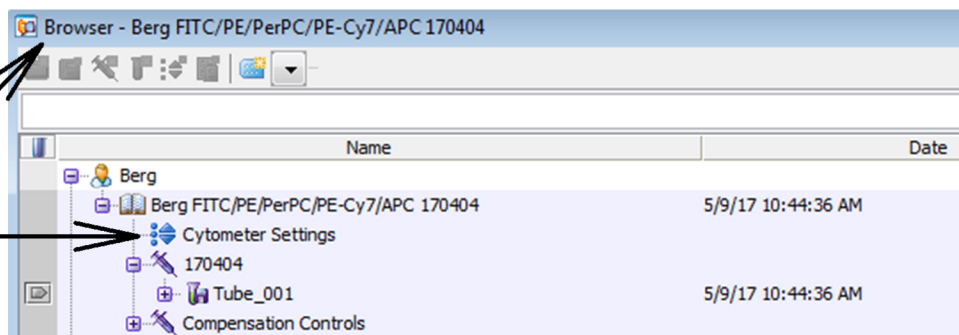
Scroll down to "Compensation Setup"

Click on "Calculate Compensation"

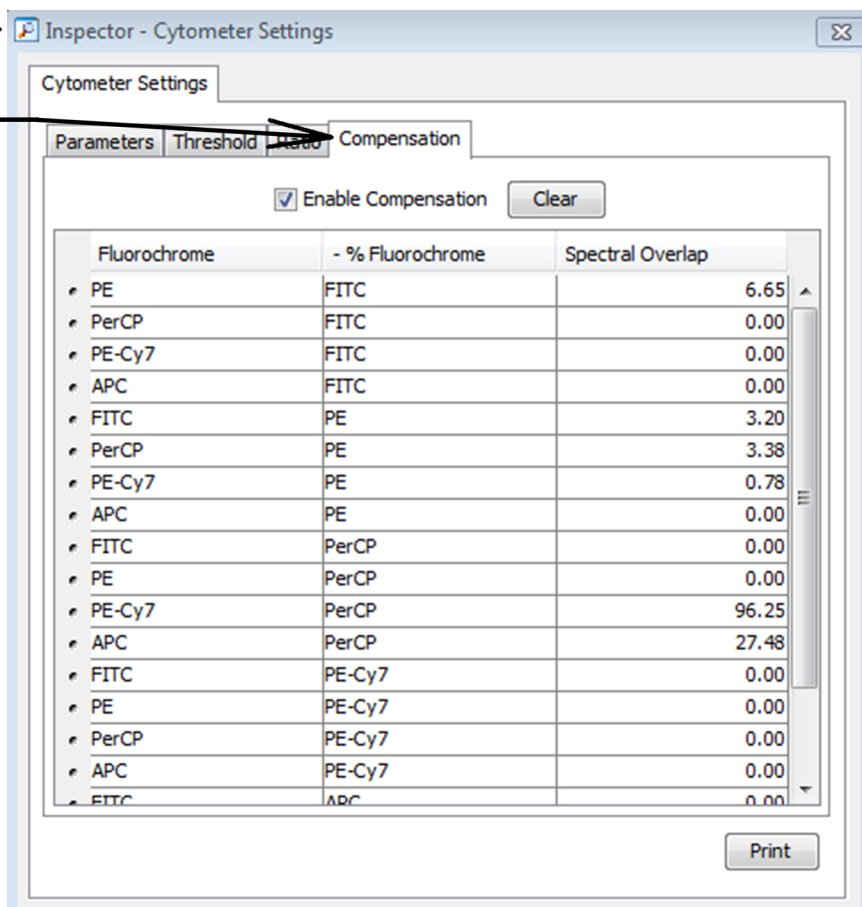


Click on "Apply Only"

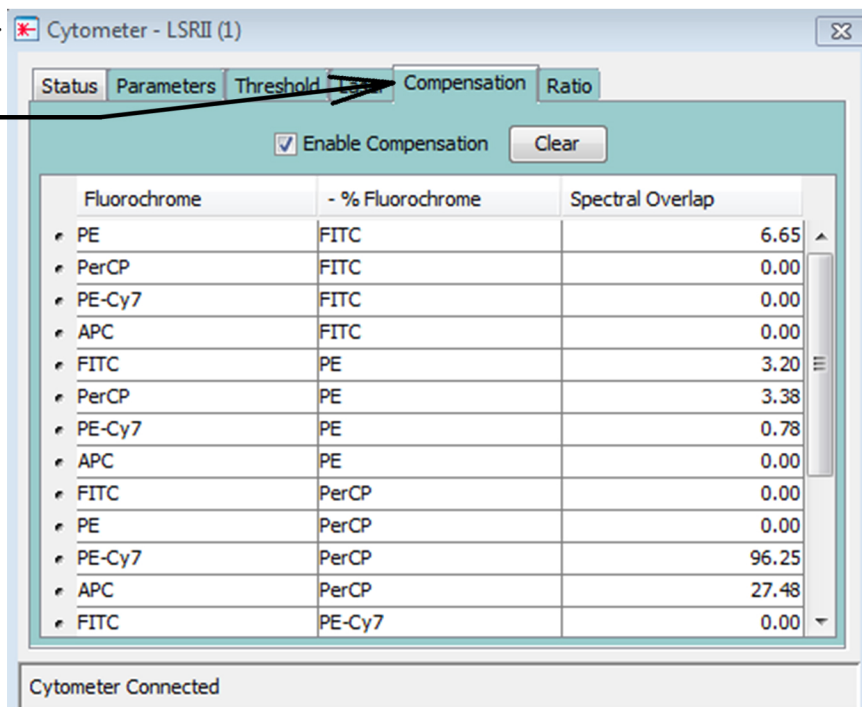
To view compensation values, find the Browser window and click on "Cytometer Settings"



Then find the Inspector window and click on the "Compensation" tab



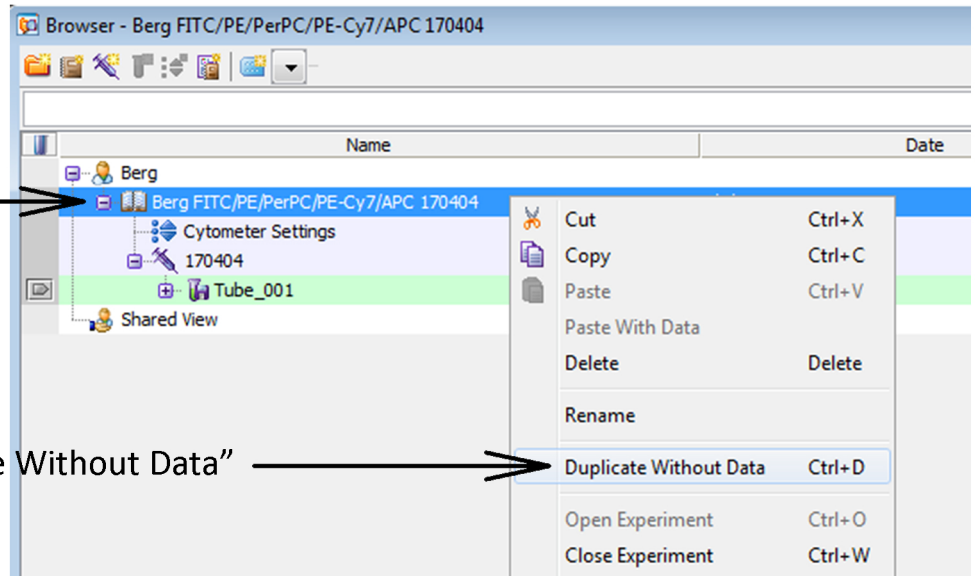
Or find the Cytometer window and click on the "Compensation" tab



Creating a Template

Right click on the experiment name

Click on "Duplicate Without Data"

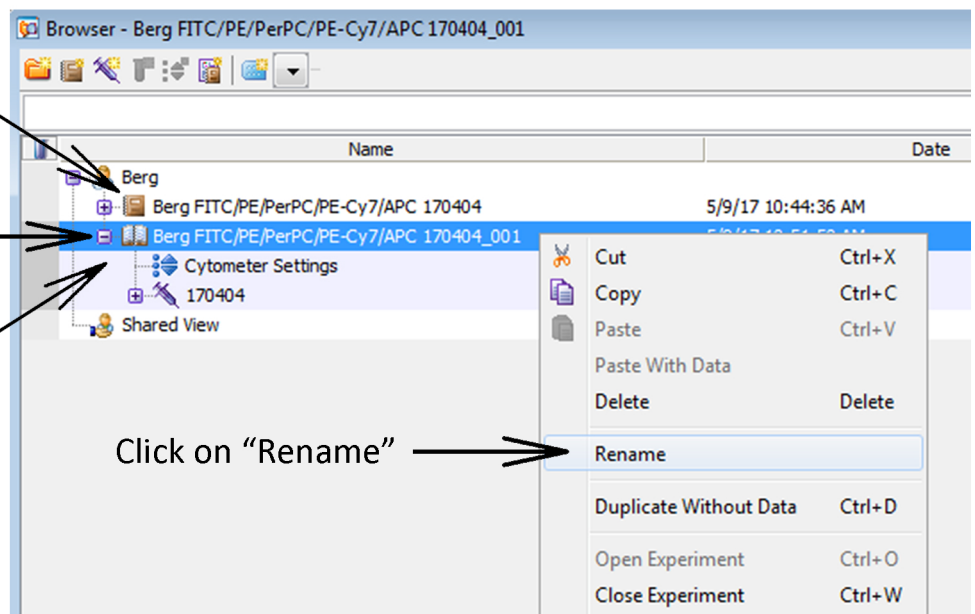


Original experiment

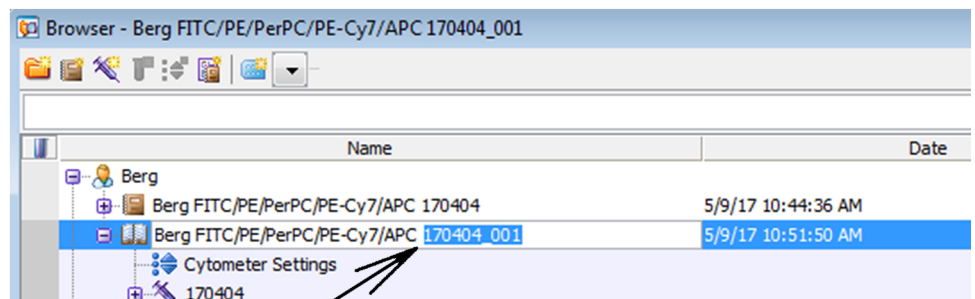
Duplicated experiment

Right click on duplicated experiment name

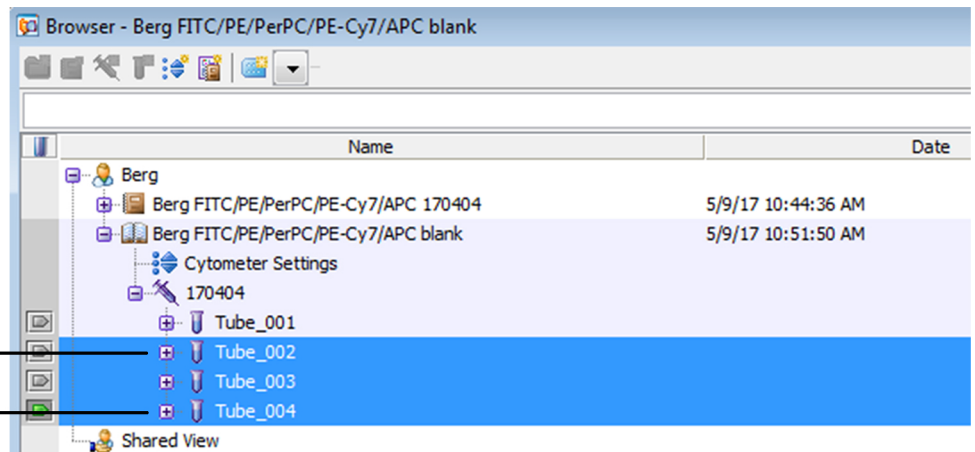
Click on "Rename"



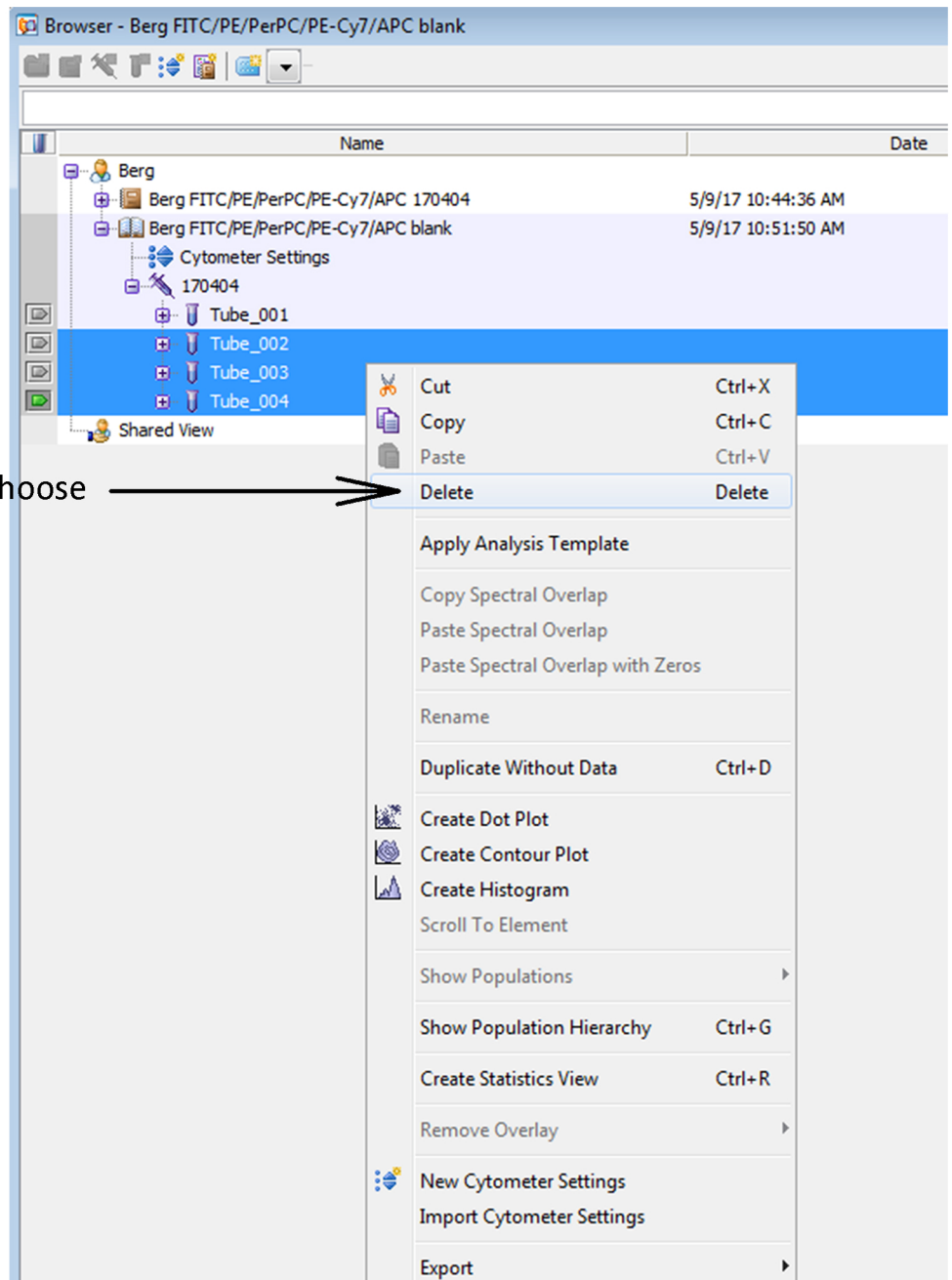
Remove the date information and replace it with the word "blank."



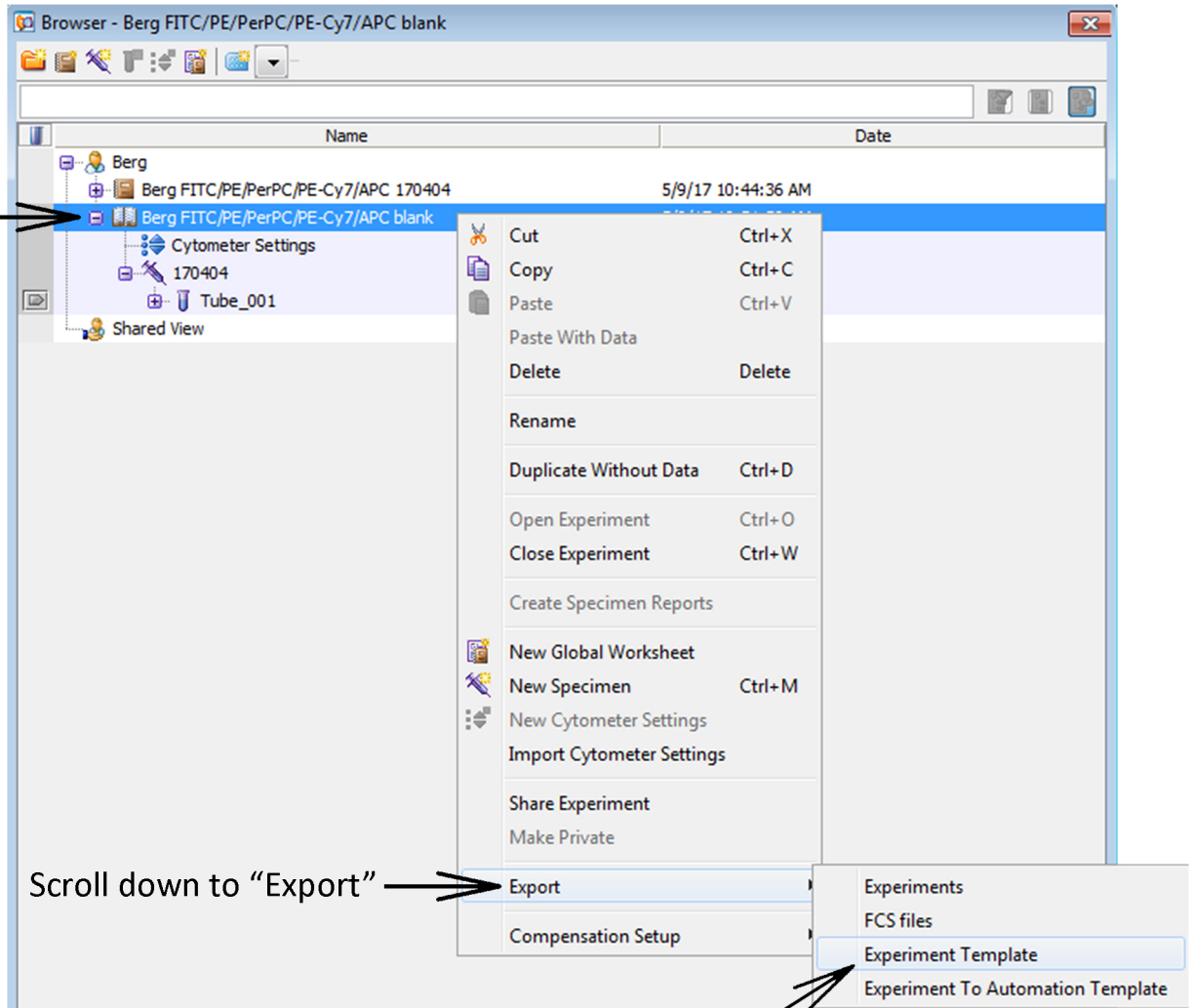
Hold down the shift key and highlight all of the tubes except the first the tube



Right click and choose "Delete"

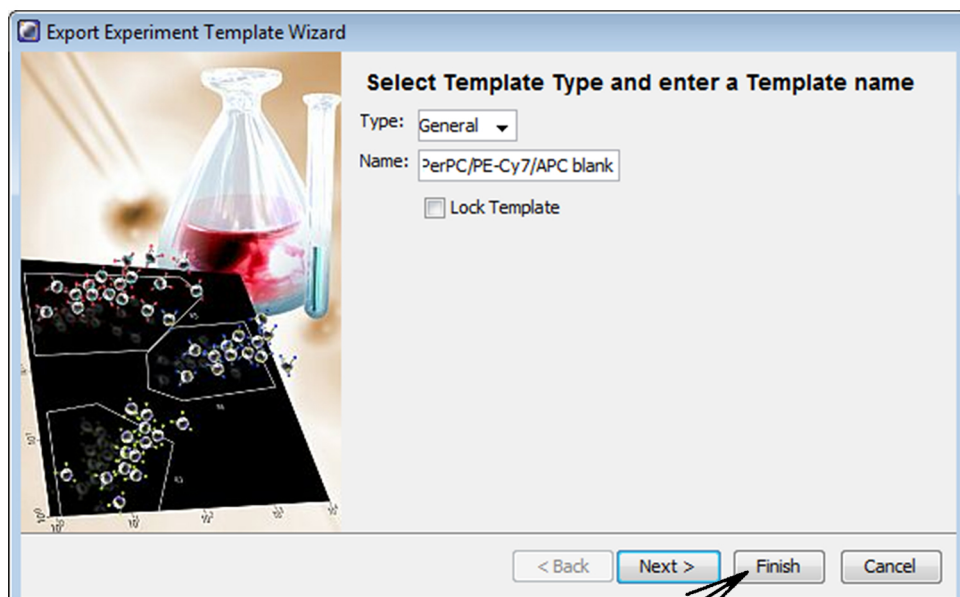


Right click on duplicated experiment



Scroll down to "Export"

Click on "Experiment Template"



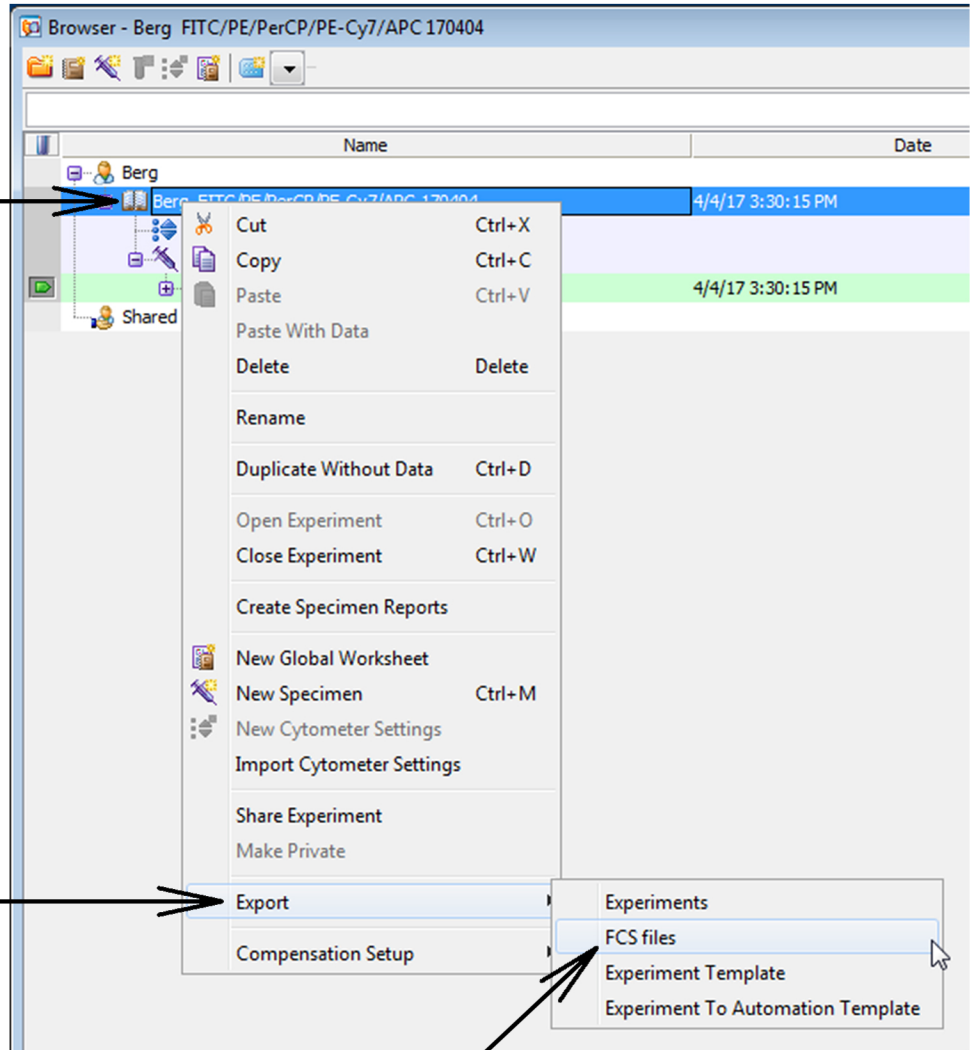
Click on "Finish"

Exporting Data

Right click on experiment name (acquisition must first be in "stop" mode).

Scroll down to "Export"

Click on "FCS files"



Leave default setting
(FCS3.0) or choose FCS3.1

Parameter	Parameter Type
FSC-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
FSC-W	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
SSC-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
FITC-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
PE-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
PerCP-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
APC-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
PE-Cy7-A	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None
Time	<input checked="" type="radio"/> Linear <input type="radio"/> Log <input type="radio"/> None

OK Cancel

Click "OK"

Set to "c:\export"

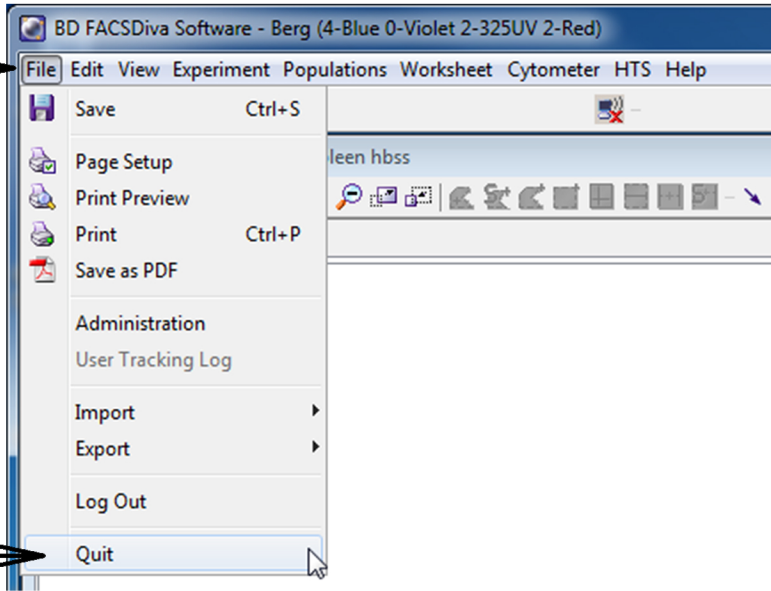
Click "Save"

Directory Path

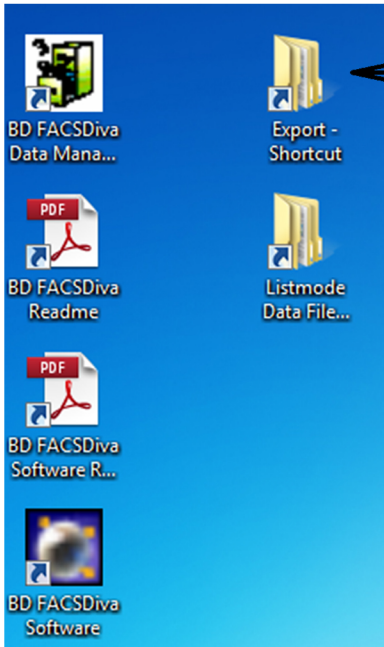
c:\export Browse

Save Details>> Cancel

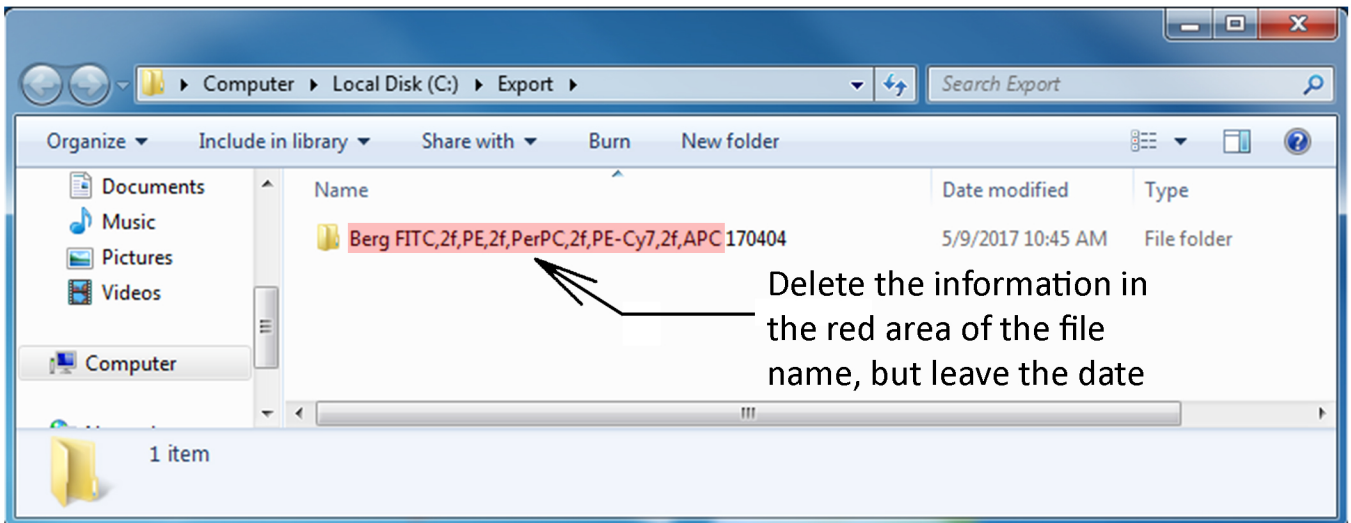
Click on the "File" pull-down menu



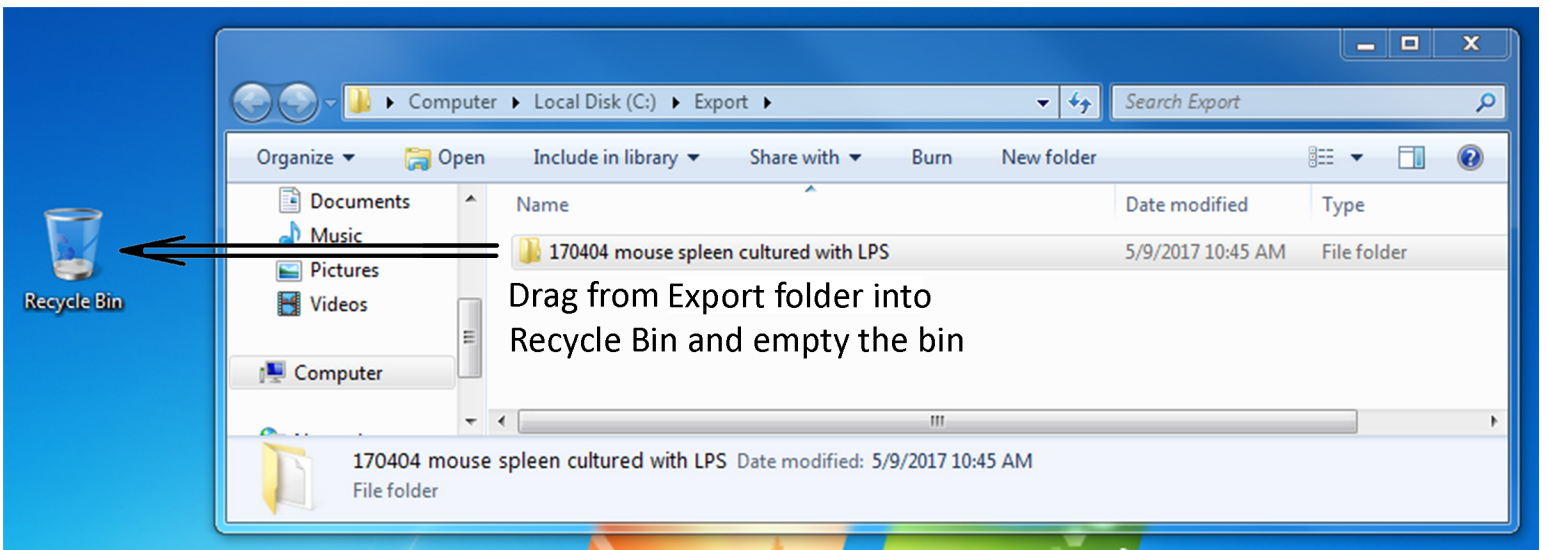
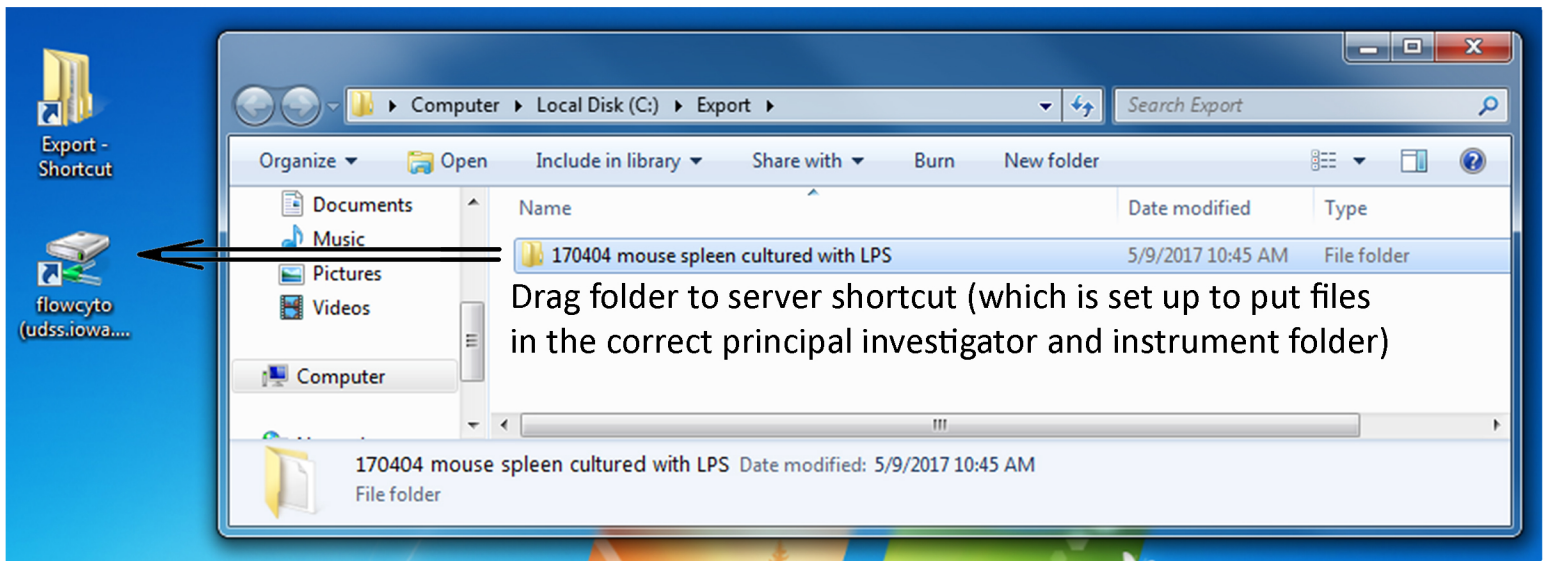
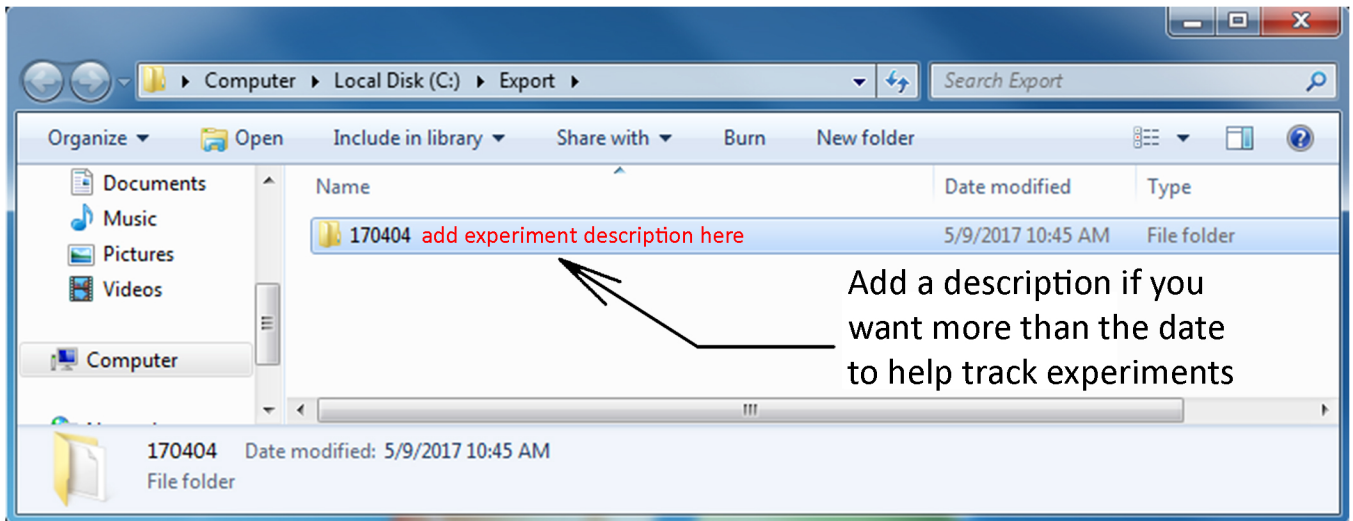
Scroll down to "Quit"

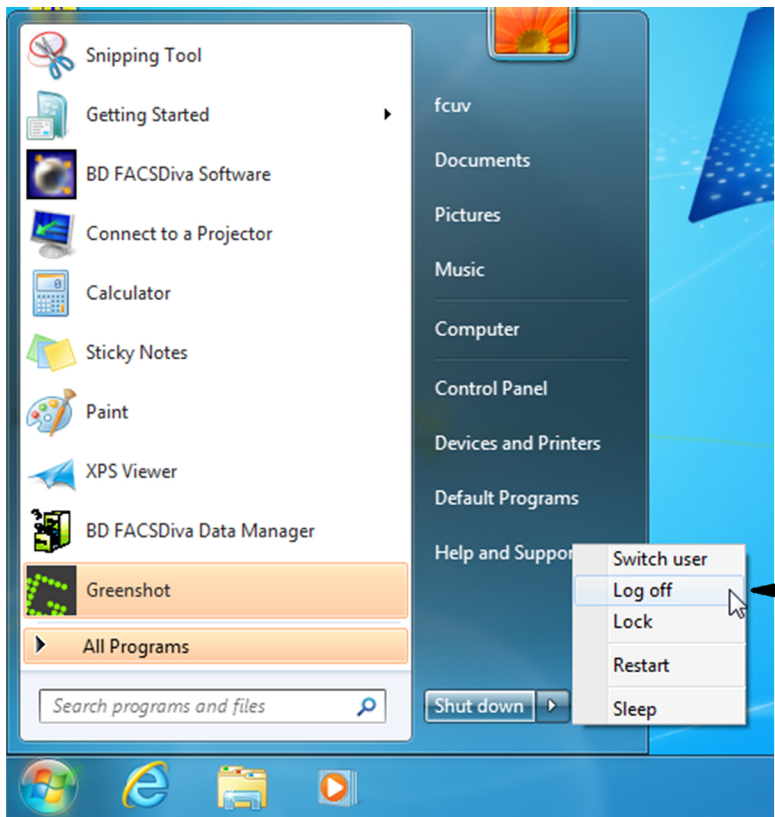


On the desktop, double click on the "Export Shortcut"



Delete the information in the red area of the file name, but leave the date





- Switch user
- Log off
- Lock
- Restart
- Sleep

Log off computer