DIVA Quick Reference Guide: Creating Compensation Controls

Before starting compensation, set the voltages for all your fluorochromes. Make sure all stains are on scale before collecting data files for compensation. FSC and SSC voltages can be changed after compensation, voltages for all fluorochromes must remain the same for all tubes when acquiring for compensation and cannot be changed afterwards.

If you find that you need to change a voltage during compensation, you have to start over and collect ALL tubes under the same set of voltages.

In order to utilize the DIVA compensation software you must have the following controls:

1. Unstained Cells or Beads.
2. Single stained Cells or Beads for each fluorochrome in the experiment.

To Create Compensation Controls

1. Make sure all colors are added in the Parameter Tab of the Cytometer Window.
2. Click on *Experiment* in the Tool Bar.
3. Select Compensation Setup.
4. Click on *Create Compensation Controls*
5. A dialogue box will appear.
6. Verify that all of the labels read “generic”.
7. Click OK.
8. A specimen will be added to your experiment labelled “Compensation Controls” along with single stained control tubes and corresponding Normal worksheets.
9. Acquire your controls.
10. Use your unstained sample to gate on your population of interest.
11. Once you know where you want your FSC/SSC gate to be, right click on the gate and choose the “*apply to all compensation controls*”.
12. Now this gate will be applied to all of your normal worksheet tabs for the Compensation Tubes.
13. Once all compensation controls have been acquired click on *Experiment* in the Tool Bar.
14. Select Compensation and *Calculate Compensation.*
15. When the dialogue box appears, rename the compensation with the date and your name (or PI).
16. Link and Save the compensation to your experiment.
17. Notice that you now have a small chain link connected to the cytometer setting portion of your experiment.
18. To view your compensation overlap values, click on any tube in your sample group (not

a tube under the Compensation Control specimen as these will all read 0.00). Click on the *Compensation* tab in the Cytometer Window.