**LSR II Basic Steps to Setting Up in DiVa 6.1.2**

1. Get new experiment from top of Browser window.
2. Set up Global Sheet with desired dot plots and histograms.
3. Choose Parameters

a. Highlight “Cytometer Settings” under Experiment name in Browser window.

b. In the Inspector window, delete parameters not in use.

1. Set up Automatic compensation

c. From Toolbar select Experiment Compensation Setup Create Compensation controls

d. Verify the parameters you are using with the controls you have

e. Click “OK”

1. Unstained control

f. Activate unstained control in Browser

g. Show Grid on all histograms in Unstained control worksheet.(Ctrl+A)

h. Place Unstained on instrument and run in LOW

i. From the Parameters tab in the Cytometer window Set FCS and SSC voltages until population of interest is centered in dot plot.

j. Set all FL# voltages until autofluorescence is within first grid quadrant.

k. Record data file.

1. Single stained controls

l. Click “Next Tube”

m. Run single stained tubes and record data files.

1. Run Auto Comp

n. From Toolbar select Experiment Compensation Setup Calculate Compensation

o. If successful, leave the numbers and click "Link and Save"

p. Click on the “page” icon to go back to your Global Sheet.

1. Get new Specimen

q. Activate the given tube

r. Check compensation by selecting Compensation in the Cytometer window.

1. Run Samples

s. Rename Specimen (Data file prefix)

t. Rename Tube (Data file suffix)

u. Set Labels

v. Set number of Stopping events to record.

w. Install tube on cytometer in Run

x. Click “Record”

y. Click “Next Tube” and repeat b-f

1. Export FCS

z. Right click on Experiment name and select ExportFCS Files

aa. Select 3.0

bb. Choose directory L:\FCS

cc. You can also export your Experiment if you would like to keep it backed up.

1. Transfer Data

dd. On the Desktop select “Shortcut to FCS **L** drive” and find your folder.

ee. You may use e-mail, burn a CD, or USB storage device to transfer data files.

ff. Delete Old Experiments and data files when data is safely on another computer.