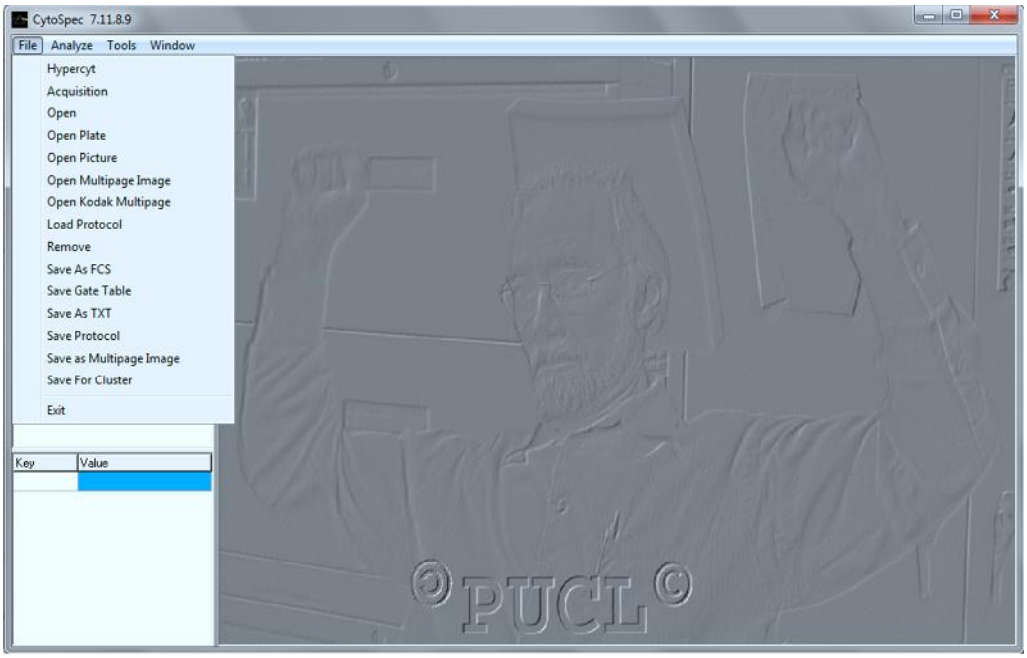
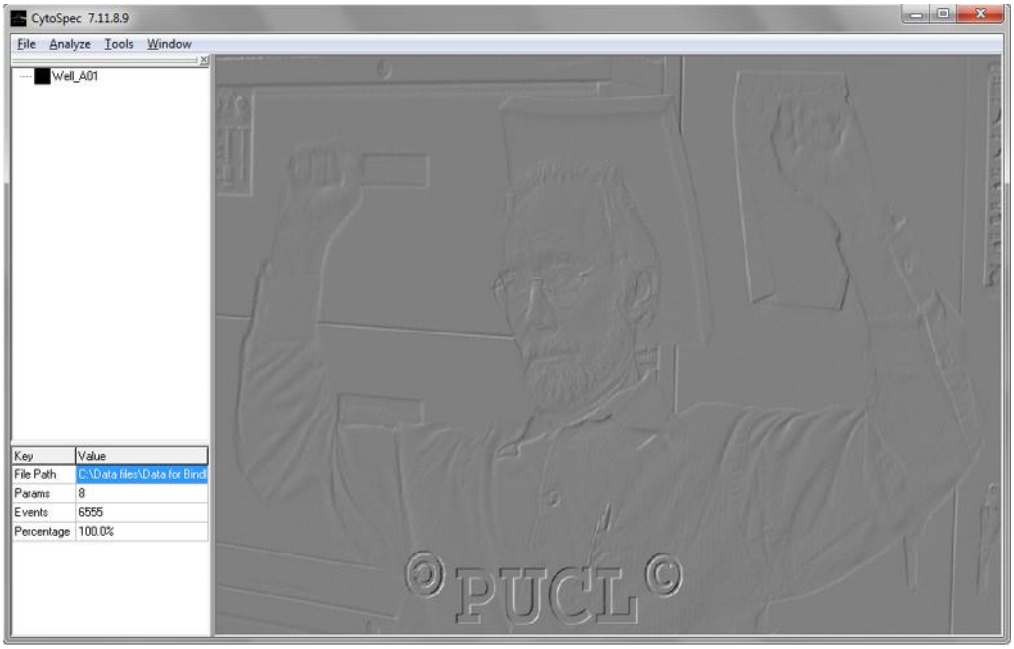
Purdue University Cytometry Laboratories Software Tutorial

1. Cytospec

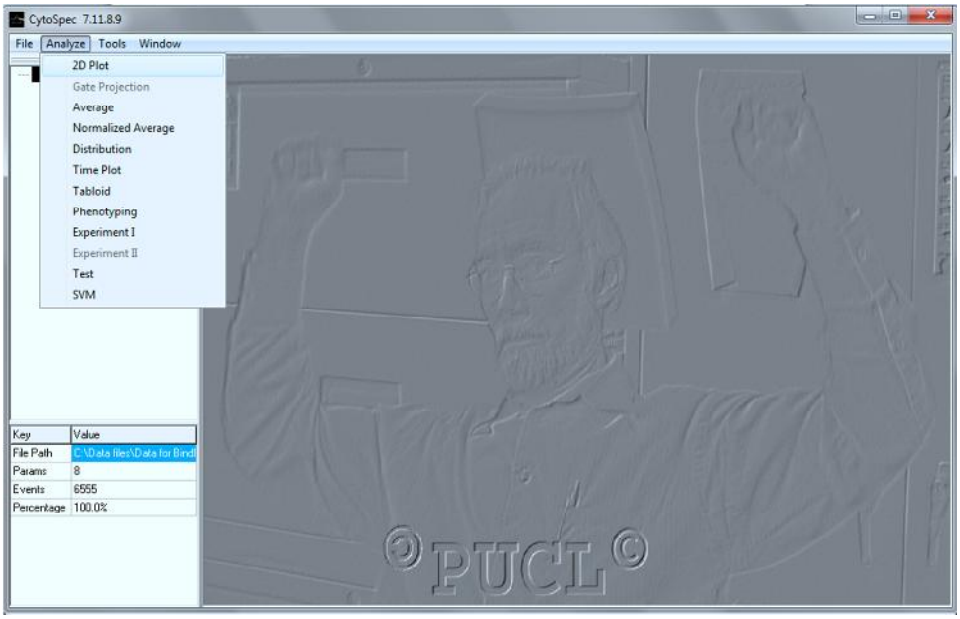
Run the exe file. Open a a list mode file. Click FILE, OPEN



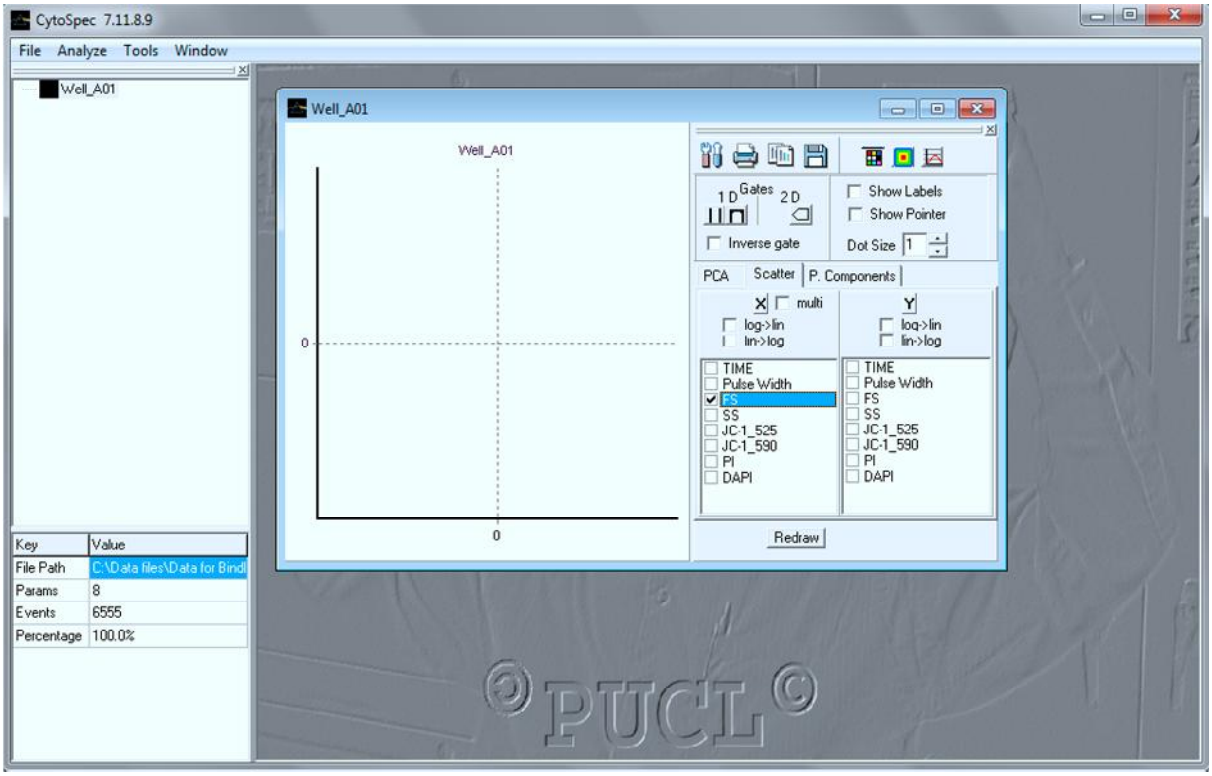
Once the file is opened you will see the following:



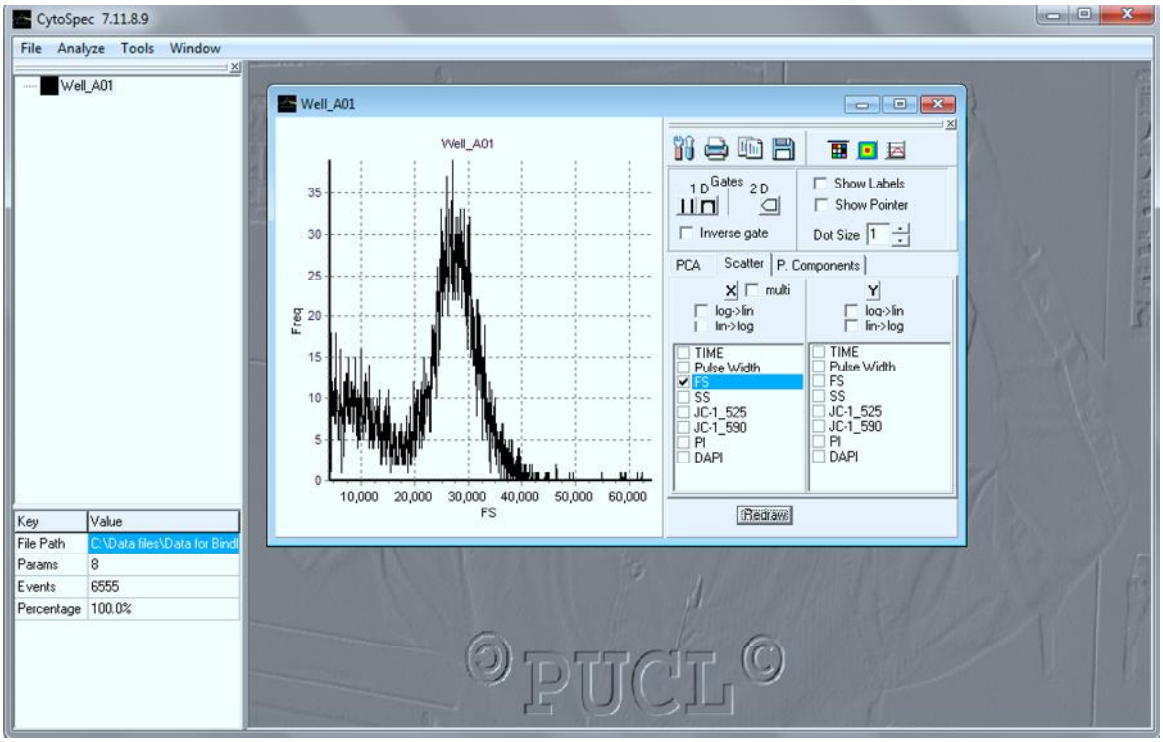
Highlight the file (there could be many listmode files in this list) and click ANALYZE ad follows:



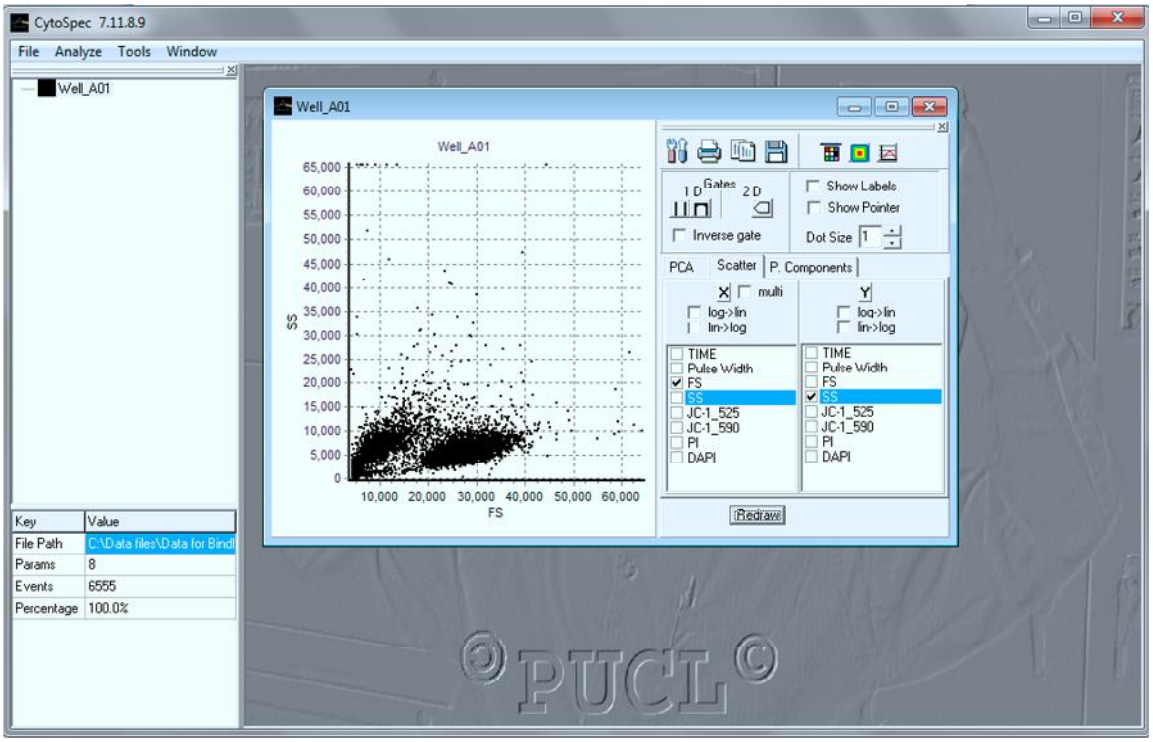
Select the 2Dplot:



You can now select any of the parameters identified for plotting. If you select one parameter, you get a histogram – you must click REDRAW for every time you change anything to redraw the plot.



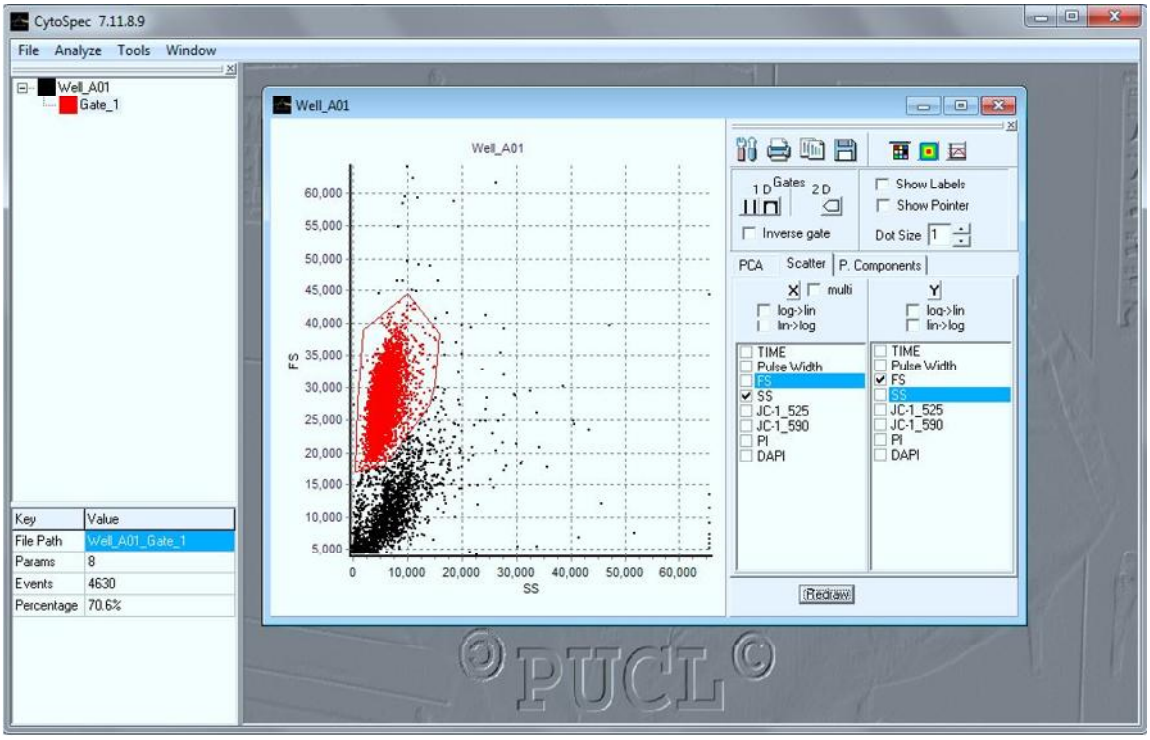
Or you can select 2 parameters this:



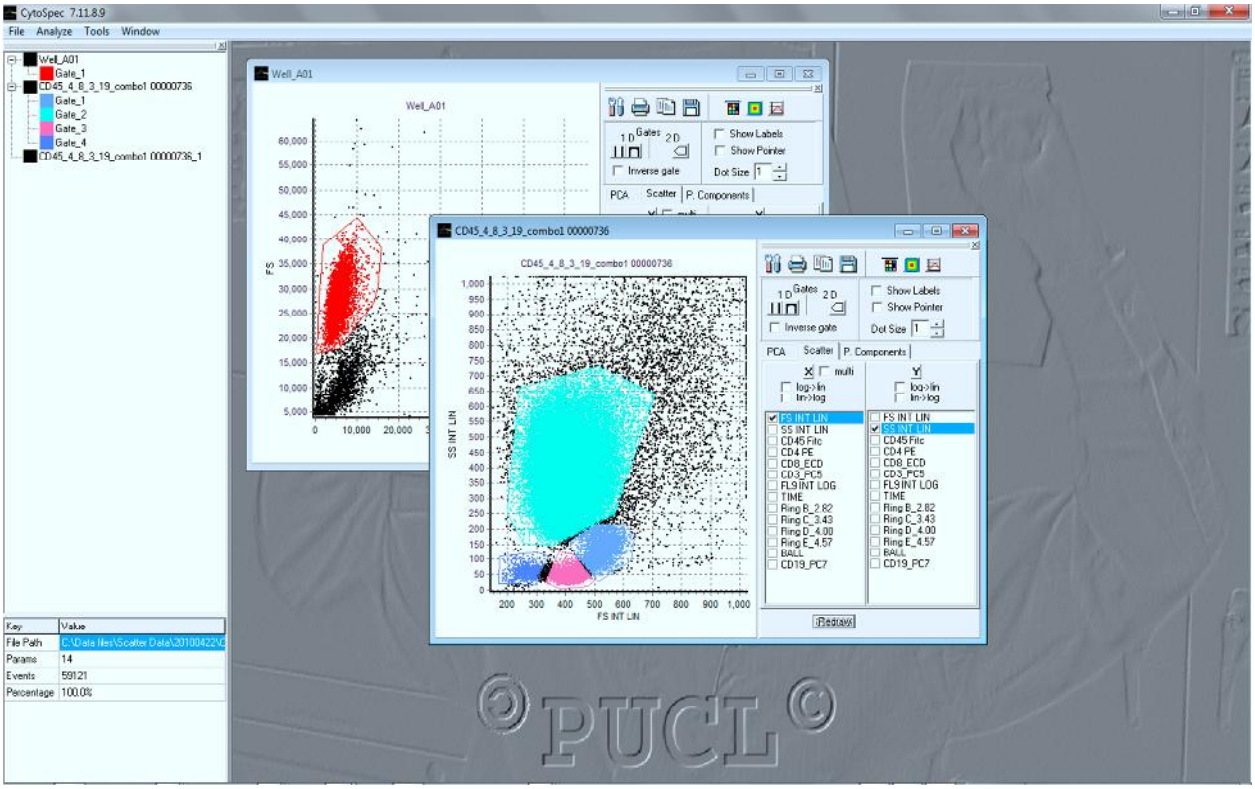
Now you can play:

Gating:

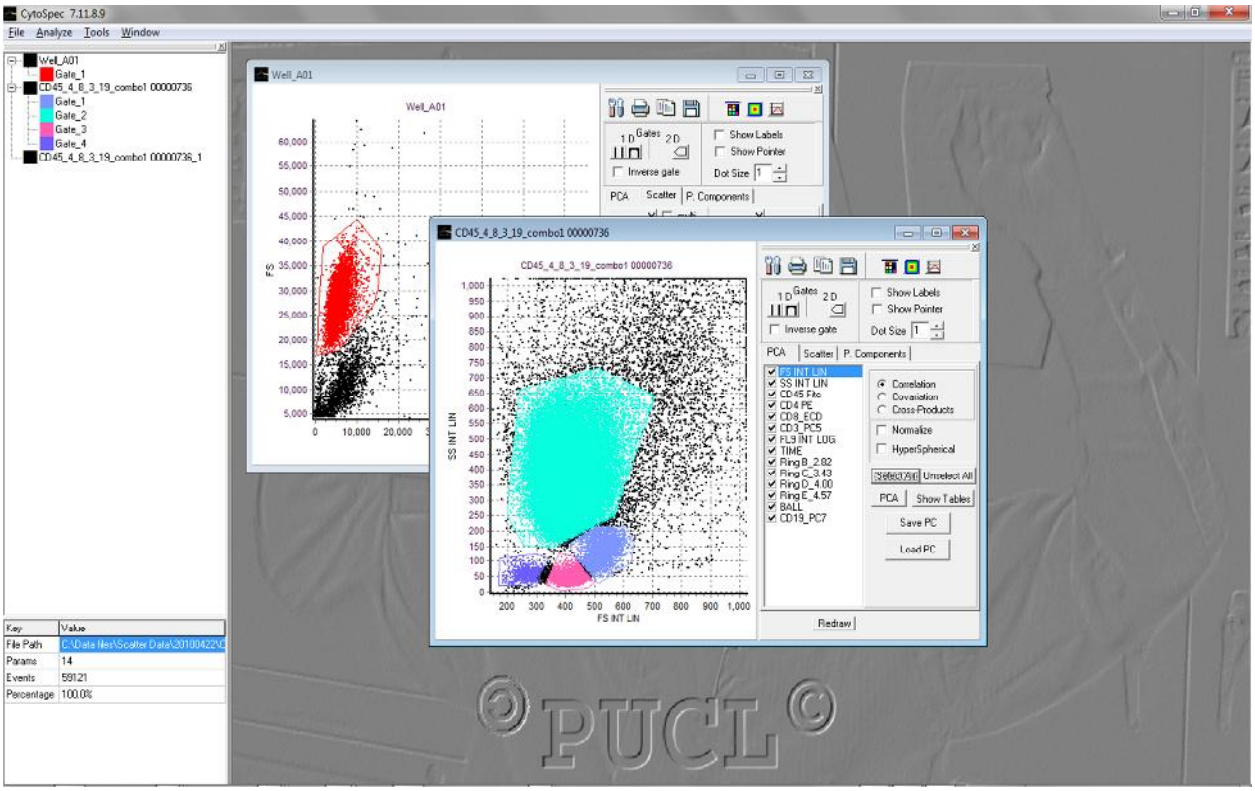
Fine the 2D box and click it – it will become slightly highlighted. Then move the mouse to a starting point on the histogram and click, and move around the gating area clicking the mouse at intervals to separate the desired area. When you are close to completing, RIGHT CLICK and it will close the area. This GATE can be moved around, expanded, reduced etc at will. The COLOR is automatically set, but you can go over to the left side where it will say something like GATE_1 – right click on the color and you will get a color box to change color. You can also RENAME the Gate_1 name to anything you want. The new GATE is now a fully analyzable file. You can click on it, select ANALYZE and it will be opened as a completely new set of data and you can do everything as above. You can also SAVE as a NEW LIST MODE file by selecting FILE, AVES AS FCS, or TXT or others.



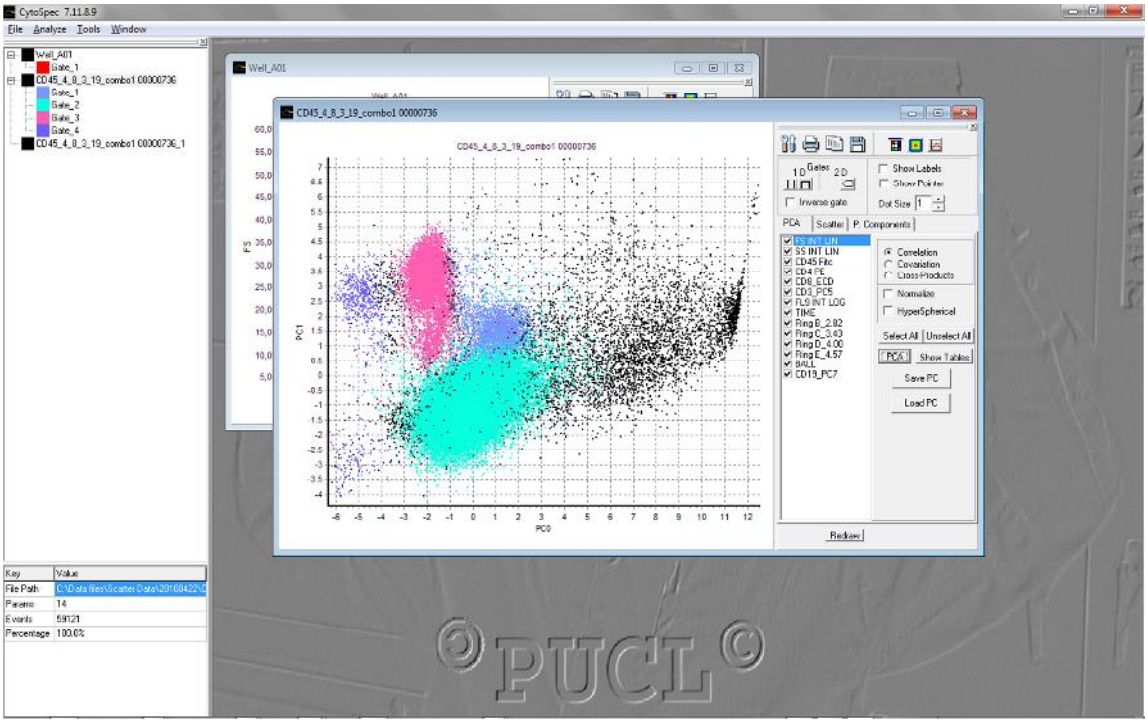
Multiple Gating and PCA



PCA – Now you can select PCA – and you can SELECT ALL if you want to do PCA on all parameters, or you can click whatever boxes you want.

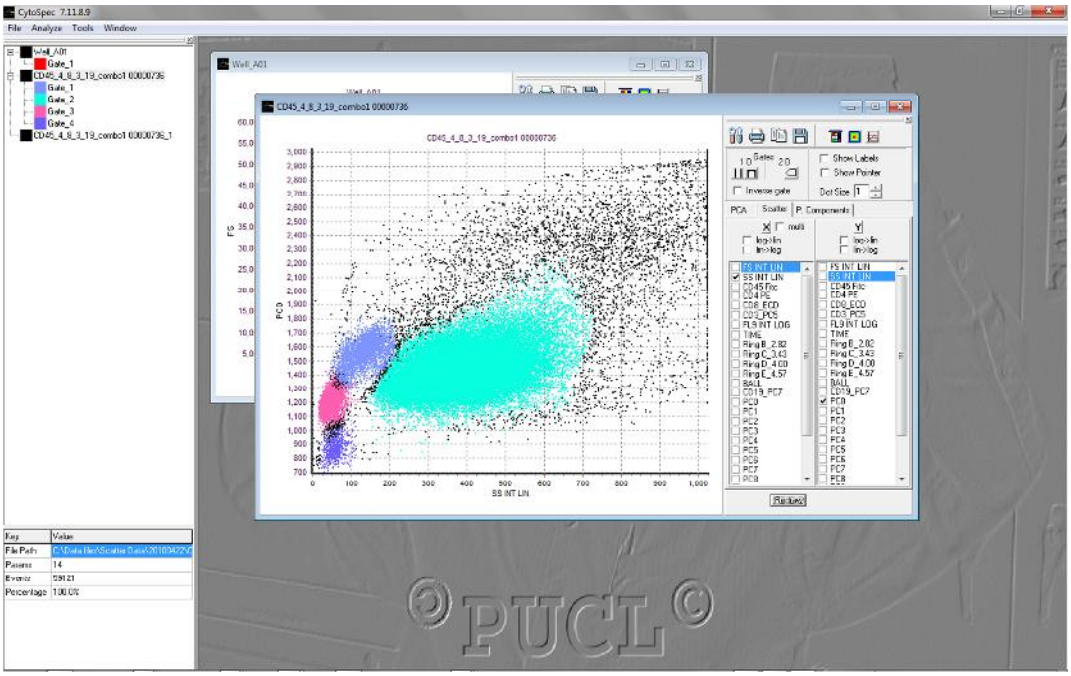


And then REDRAW



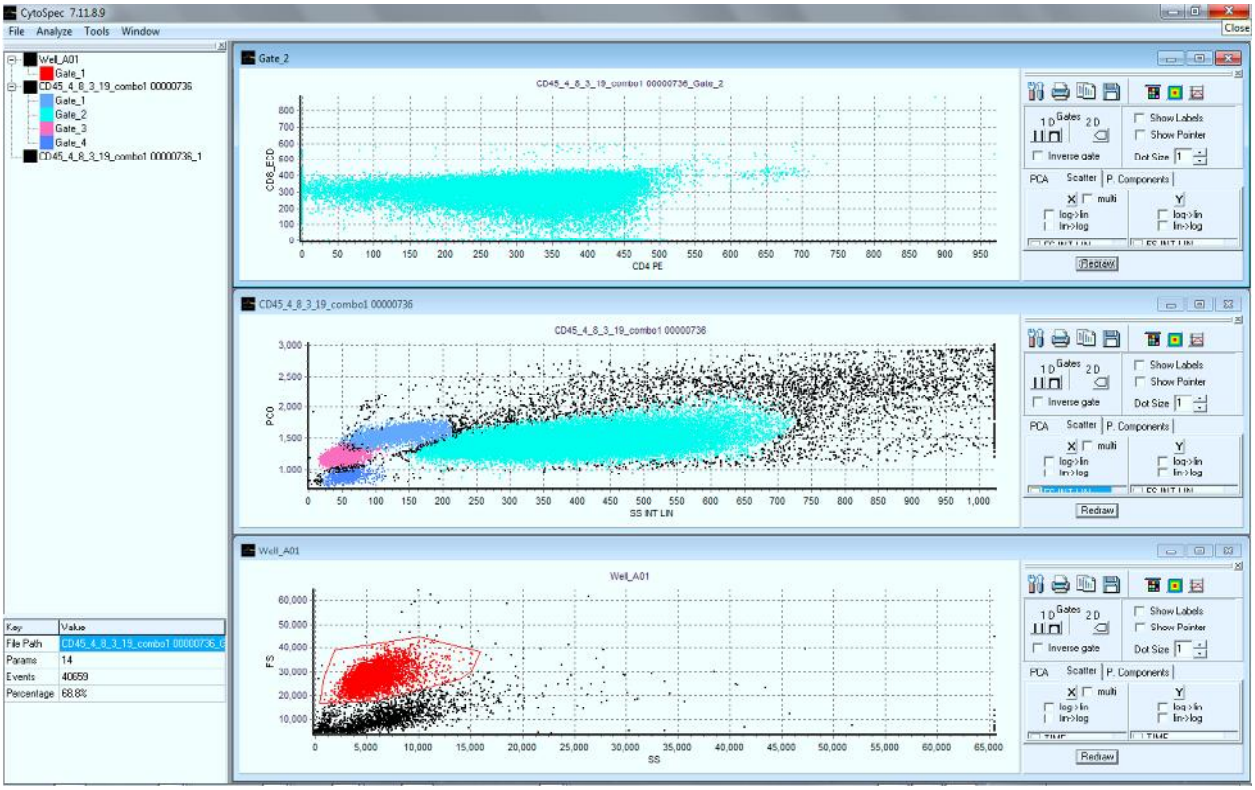
Now you have a PCA plot of PC) vs PC1

If you now click SCATTER again, you can actually plot PCA vs any parameter (Be careful here, as this is a complex area you are getting into!) and you can select a variety of methods for plotting.



Many options are present.

You can covert from lin to log for any channel, you can plot with multiple formats, and you can have any number of windows open, and do regular windows features such as TILE or CASCASE (see top line, WINDOW)


CytoSpec 7.11.8.9
File Analyze Tools Window
Close
Well_A01
Gate_1
CD45_4_8_3_19_combo1 00000736
Gate_1
Gate_2
Gate_3
Gate_4
CD45_4_8_3_19_combo1 00000736_1
Gate_2
CD45_4_8_3_19_combo1 00000736_Gate_2
CD8_ECD
CD4 PE
Show Labels
Show Pointer
Inverse gate
Dot Size
PCA
Scatter
P. Components
multi
log->lin
lin->log
Redraw
CD45_4_8_3_19_combo1 00000736
PCO
SS INT LIN
Well_A01
FS
SS
Key
Value
File Path
Params
14
Events
40659
Percentage
68.8%