SOP-E010

Check Laser Delay on CyAn

Objective: To check the delay settings on the violet and red lasers, on the Beckman-Coulter Cyan flow cytometer.

Procedure:
1. **For the 405 laser (violet) = laser2:**
2. Make a protocol (or open an already set protocol) which includes a single parameter histogram of *violet1-lin.*
3. Also set up a dot plot with *violet1-lin* on the x-axis, and *violet1-area* on the y-axis. Label this plot "delay laser 2".
4. Set the trigger to Forward Scatter (FS).
5. Set the acquisition to "cycle" mode.
6. Have the beads going at about 100/second.
7. While the beads are aquiring, do **Ctrl-Alt-F1** at the same time to bring up the laser delay settings window.
   a. Laser 2 delay = the 405nm (violet) laser
   b. Laser 3 delay = the 647nm (red) laser

8. Change the delay setting on the laser 2 button, by using the up/down arrows.
9. Watch the signal on the dot plot. You want the signal to go from the lower left to the upper right, as high and as tight as you can get it. Alternatevely, can look at the mean of the single parameter histogram of the violet1 signal.
10. To check if the delay is OK. Change the trigger signal from the FS signal to the violet1 signal, and back again to FS. The peak should be at the same place no matter which signal you are triggering on. If it is not at the same place, you need to adjust as above.
11. **For the 647 laser (red) = laser3:**
12. Make a protocol (or open an already set protocol) which includes a single parameter histogram of *APC-lin*
13. Also set up a dot plot with *APC-lin* on the x-axis, and *APC-area* on the y-axis. Label this plot "delay laser 3".
14. Follow the same steps as for laser 2 delay above, but this time change the laser delay setting on the laser 3 button.
15. To check if the delay is OK. Change the trigger signal from the FS signal to the APC-lin signal, and back again to FS. The peak should be at the same place no matter which signal you are triggering on. If it is not at the same place, you need to adjust as above.

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Verified by:_____Date:_____   _____

                         Print Name   Sign Name