SOP-E013

How to Determine MoFlo XDP Laser Delay

Change the nozzle tip, adjust the sheath and sample pressures, check for air in the sheath lines and nozzle assembly. Let the system stabilize.

Alignment should be done using SpectrAlign (or comparable) beads at a concentration of 10^6/ml. Create a Summit Protocol displaying FSc vs SSc do plot and linear Area histograms for all laser parameters. Display median and CV statistics.

Laser Alignment

1 Set system to trigger off FSc. Align 488 laser and optimize signal, CVs and median by adjusting sheath stream and laser line micrometers. Ensure threshold is low enough (0.5-1%) to establish 100-150 eps. After optimizing, mark medians (use bar regions) and note CV values for each parameter.
2 Repeat step 1 for any lasers in pinholes 2 and 3 changing the trigger sourse for each laser path being aligned. Again, after optimizing, mark medians and note CV values.

Note when changing triggering, populations may move or disappear from histograms depending on laser delay settings.

Setting Laser Delay

1 Press CTRL, ALT and F1. This will open a dialogue box where you can adjust the laser delay of path 2 and path 3. **Note** Ignore the top two input boxes and button.Laser delay box 1 displays values from pinholes 1 to 3 in nanoseconds (ie 3200 for 60 psi.) Laser delay box 2 displays values from pinholes 2 to 3 (ie 1600 for 60 psi). **RECORD THE CURRENT VALUES** – these can be re-entered when returning to the starting sheath pressure.

2 Gate the FSc vs SSc population to the parameter histograms

3 Place Summit in "cycle display mode" (cycle count ~ 100 events) and set the event rate to ~100eps.

4 While triggering on FSc increase the threashold until pinhole 1 populations (FL1, FL2, FL3 etc) begin to disappear. Lower the threshold so the population peaks return and can be easily viewed. As the threshold increases, the pulse width becomes smaller, and the median values decrease. If necessary, mark these new median values by creating new or moving bar regions.

5 Repeat stem 4 for the middle and bottom pinholes by changing the trigger source and adjusting the threshold so that the relavent populations are just visible. Increasing the threshold ensures that only the top of each pulse is being displayed. This will make determining the laser delay quicker and more precice in the following steps.

6 Return to trigger off of a pinhole 1 parameter (ie FSc).

7 Adjust the laser delay values to return to the highest median and CV values recorded for laser 2 and 3 parameters found when aligning the lasers by increasing (if the sheath pressure was lowered) or decreasing (if the sheath pressure was increased) the laser delay in nanoseconds. The most accurate method to do this is to adjust the relevant path's laser delay (using the laser delay input box arrows) until the median and CVs move as far as possible to the right (record laser delay value) and continue adjusting

the laser delay in the same direction until both the median and CV begin to degrade (record this value). Set the laser delay to the average of these two values. See the example below.

Tip (micron)	Sheath Pressure (psi)	Laser Delay (nanoseconds)			
		Lower delay	Upper delay	Laser 1 delay (pinhole 1&3)	Laser 2 delay (pinhole 2&3)
70	60	3430	3880	3655	1828
	50	3860	4060	3960	1980
	40	4290	4600	4445	2223
	30	4980	5280	5130	2565
	20	6060	6300	6180	3090
100	30	4880	5480	5180	2590
	20	6200	6430	6315	3158

Note These laser delay values were determined between pinholes 1 & 3. Laser delay 2 values are ½ laser 1 delay values but were not determined.

Typical Values:

Tip	PSI	Laser 1 (ns)	Laser 2 (ns)
70	60	3200	1600
80	40	4400	2200
100	30	5000	2500

8   Record values. Under most conditions these can be inputted when changing tip and sheath pressures without pre-determining the laser delays. Close laser delay adjustment window.

9   Lower trigger thresholds.

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