SOP-E012

MoFlo XDP Sort Set-Up

Purpose: to prepare the XDP for cell sorting

Procedure:

1. Follow the standard daily start-up procedure [SOP-P072].
2. Run and record the regular QC beads.
3. On the aXcess Control Panel screen, go to the Stream Configuration Screen, press the **Charge Plates** button the (left button underneath the area of the droplet camera) to turn them on. This turns on the drop drive and the strobe.
4. Refresh the plate HV one time by dragging the voltage all the way up and down, then set the plate voltage to 4000 V. (If you don't do this step you may not get any reflection of the streams.)
5. Let the drop drive warm up at least 15 minutes, one half hour is preferred.
6. On the IntelliSort Configuration Screen (Droplet Control Screen) press the **IntelliSort Camera Reset button** (located directly underneath the Nozzle Size selection, it shows a stream with two yellow double arrows). Do this before every sort set-up, before activating the "Brain". Do not give any other commands during this process.
7. Once the piezoelectric crystal has had adequate time to warm up, you need to **optimize the droplet formation.**
8. If needed the droplet camera can be raised or lowered to visualize the last attached drop. The control arrows for this are located along the right side of the droplet camera screen on the aXcess Control Panel Droplet Control Screen.
9. Start with the **amplitude** setting from the previous sort. If you don't know, or it has been turned off, start with a setting between 8 and 18 volts for the 70µm tip, at 60psi. See the Cytocalc Table on page E-1 of the manual for other nozzle/psi settings. (100µm tip amplitude ~30 volts at 25psi)
10. Find the shortest droplet breakoff point by adjusting the drop drive frequency. The frequency should be around 95,000 Hz (for the 70µmtip), (around 40,000 Hz for the 100µm tip). You may need to move the camera postion at this point.
11. Fine tune the amplitude adjustment to achieve a clean breakoff of the first satellite drop. You want a symmetric, thick neck on the last attached drop, and you want the first satellite drop to be elongated
12. Once you have established a good last attachéd drop it is time to **check the side streams**.
13. On the Stream Configuration Screen, you must select the stream number, then enable it by pressing the middle of the three buttons just underneath the stream deflection readout. The stream depicted will turn yellow when it is enabled. Continue this process until all streams you wish to use are enabled.

14. Press the Charge Plates button to turn on the charge plates. This is the left button of the three just under the stream deflection reading. It looks like plates with a lightning bolt between. Make sure the plate voltage is at 4000V, if doing a 4-way sort.
15. Press the test pattern button. This is the right most button of the three buttons under the deflection readout.
16. If the **streams are fanning, adjust the charge phase** on the Stream Configuration Screen. Find the worst setting, then go 180° in the opposite direction. So, if 30° is worst setting, then add 180° = 210°. Set the charge phase to 210°, should have nice clean side streams.(If fanning at 235°, then you want to be at 55°, if fanning at 160°, then want to be at 340°.)
17. To tighten up **the waste stream, adjust Defanning.**
18. Adjust the stream delfection to steer the streams into the test sort tubes.
19. When all looks good, press the Test Pattern button to turn it off.
20. Adjust the droplet camera so that the last attached drop is in the center of the droplet window and move the red marker line to a set point, such as just below the last attached drop.
21. If you wish to use the **IntelliSort (Brain)** function, turn it on now. This button is located just below the droplet window, it is the button which has a "brain" highlighted. **DO not bump the instrument, or make adjustments to the instrument while Intellisort is preparing**. This process takes about three minutes. You will know it has completed its set-up cycle, when the status reads "**maintain**", in the IntelliSort Status oscilloscope window (bottom left hand corner). When IntelliSort is active, the frequency, amplitude, and charge phase controls are disabled. (This is now the function of the "brain".)
    a. NOTE: Changes in the sheath fluid temperature and pressure will cause the last attached drop to drift from it's starting position. Your red marker will show this. This is normal. IntelliSort is controlling other parameters in order to maintain the correct number of attached drops.
    b. NOTE: If IntelliSort exits Maintain mode, it is usually due to some sort of fluidics issue. (i.e. ran out of fluid, loss of pressure, clog, bubbles)

22. Once IntelliSort has stabilized in **Maintain** mode, it is time to **set up the Drop Delay**. IntelliSort must be enabled before you determine Drop Delay.
23. Run whatever beads you want at 100 eps
24. Gate the bead population on scatter and fluorescence.
25. Remove the Sort Receptacle from the Sort Chamber.
26. Select the **Sort** menu in Summit and select **Drop Delay Wizard**
27. It will prompt you to ensure the path of the CyCLONE is clear.
28. The CyCLONE will come out.
29. Place a clean microscope slide in the CyCLONE slide space.
    NOTE: the chamber door must be closed
30. The estimated drop delay value will be the same as the last time, you can start there. Otherwise check the chart on page E-1 for estimates.
31. **Set the plate voltage down to around 2800V. The delay wizard uses the Right 2 Stream**.
32. Enter the number of beads you want it to deposit per puddle. (We like 100.)
33. Click the edit button. The Right 2 Stream Sort Logic dialog box appears.
34. Select the appropriate gate logic for the beads you are running, and click **OK**.

35. Click **Next**.
36. Click **Run**.
37. The machine should begin depositing 10 puddles onto the slide. **NOTE: You will want the beads to be relatively concentrated for this process to be quick.**
38. When it is finished making puddles, remove the slide and view under a microscope. Determine which puddle contains the most beads. Count the number of beads in the puddles adjacent to the puddle with the most beads. Enter these values in the Drop Delay Wizard.
39. Click **OK**. Rerun this test until the difference between the number of beads in the puddles adjacent to the target is less than 3%.
40. Click **Finish**.
41. **Return the HV setting from 2800V back to 4000V.**
42. You are now ready to acquire data from your sample, set regions and gates from which to **set sort decisions**.
43. Click the **Sort** tab in Summit.
44. Click the menu icon in the **Sort Logic and Statistics** Panel.
45. Select **New Decision**.
46. You must select sort decisions for each stream you wish to sort.
47. Select **Sort Logic**
    a. Choose gate logic for sorting.
48. Select a **Sort Mode. We usually use purify.**
    a. **Enrich mode:** recovery most important, all positive events are sorted except hard coincidence events (you will take some of what you don't want in order to recover as much of what you do want)
    b. **Purify mode:** purity most important, all positives are sorted only when there are no negatives in the window of the drop envelope. (You will lose some of what you want in order to not get what you don't want) You can in this case collect the aborted events, and resort for greater recovery.
    c. **Single mode:** single cell mode, only one positive event must be contained in the drop envelope to pass the sort logic. If two positives, or a negative are in the window, it will fail, and the two positives will be aborted. Used for sorting sigle positive cells into wells. Also used for drop delay calculation.

49. Select a **Drop Envelope**. See page 8-23. in the manual for an explanation of the options. **We usually will use 1 drop**. I drop provides the best yield (fewest soft aborts) at high event rates when using the Purify Sort Mode. DO not use this value in Single Sort Mode.
50. Select an **Abort Stream**. See page 8-24 in the manual for explanation. We usually use **waste**.
51. **Begin your sort**.
52. Make sure you have a receptacle in place to catch your sort with appropriate media.
53. Get your sample going.
54. From the **Sort** Menu in Summit select **start** or press **F4.** Pressing F4 again will stop the sort.
55. To pause the sort, for instance your receptacle has become full and you want to change it to collect more sorted events, select **Pause** in the **Sort** menu.
56. To resume sorting from pause, go to **Sort** menu and select **Resume**.
57. Press **F4**, or in the **Sort** menu select **Stop** to stop the sort.

Created by: Kathy Ragheb, July 24, 2012
Atoned by:

Verified by:_____Date:_____ _____

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