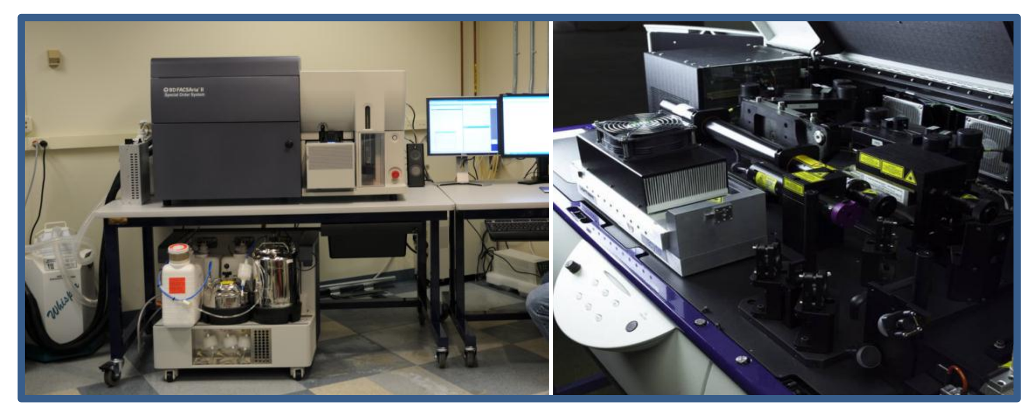

University of Massachusetts Medical School

Flow Cytometry Core Facility

BD FACSAria II Self-Sorting Training Program

Prepared by Marc Barnard

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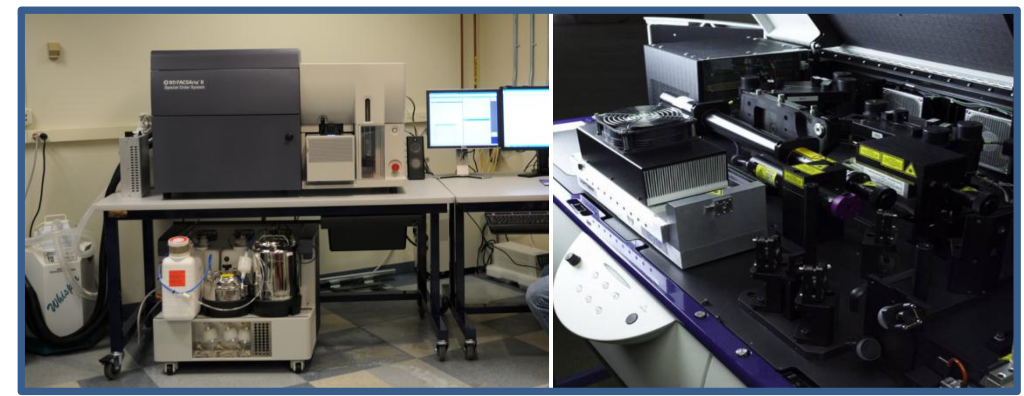


Introduction

Please drop by the main lab (S5-322) or call the core at (6-3276) to discuss the training program. LSR training and use is a pre-requisite. Candidates should be familiar with the FACSDiva program and have done their preliminary analysis work on an LSR. This program is intended for laboratories and individuals who have a need to sort regularly after normal core facility hours, 8 AM – 5 PM Monday-Friday. Sorts performed during normal working hours will be done by core personnel.

You will be asked to provide the cells for parts 2-3 of the individual training outlined on the next page, which can be actual sorts. These should be scheduled not more than 2 weeks apart. Training time required may vary from the description below.

FACSAria training program overview:

1) A 2 hour introduction to the FACSAria II with a practice bead sort. This will include stopping the stream, removing the nozzle, sonicating and reinserting it, restarting the stream and returning to the original drop position. This is an "Introduction to fixing a clog". Please review the training manual prior to this session. Your lab will just be charged for the time spent on the Aria at our normal sort rate for all phases of this program.

2) An actual sort during the work day where you provide the cells and we guide you through the setup. Post-sort instrument cleaning and shut-down will be performed.

3) An actual sort during the work day where you provide the cells and you do all the setup. A core faculty member will be present for questions and guidance.

4) A 1 hour review of what to do in case of a clog and performing the auto-delay in case the stream comes back in a different spot. The function of CST bead settings will also be reviewed.

5) Following this program you should do 1 or 2 independent self sorts during workday hours when core personnel are available. You will then be cleared to self sort after hours.

Starting up the system

During the work week the core will have the Aria ready for use. You will need to log on, start the stream and examine the breakoff image and values to confirm it is ready.

Turn on the cytometer main power (switch on left side)

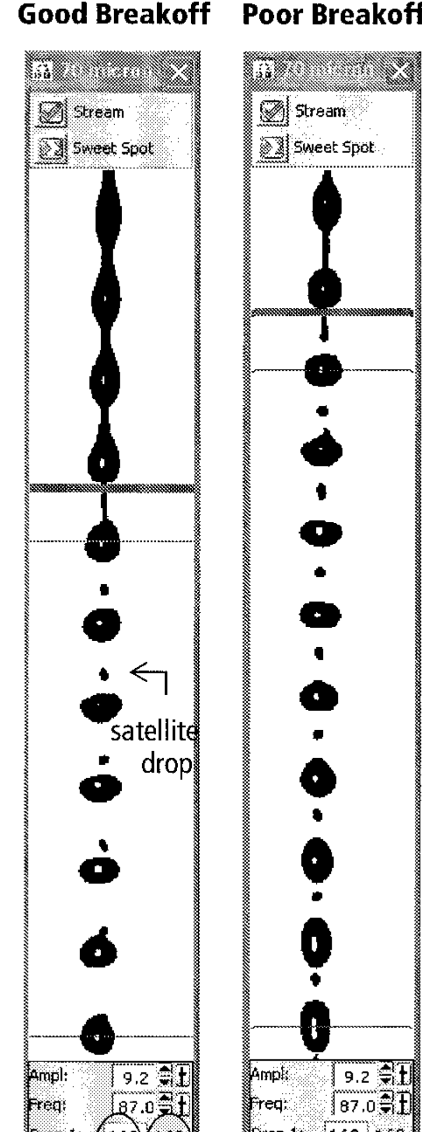
Turn on the computer if needed, log on with your UMass ID

Open the "Coherent Connection" software

Open FACSDiva, log in with your unique password which we will set up for you

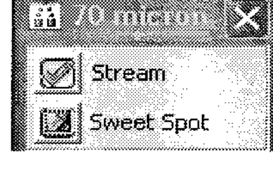
Wait for FACSDiva to connect to the cytometer and "Use CST settings" when the CST mismatch dialog box appears

Start the stream*, one nozzle (85 µm) only will be used for self-sorting in the core which is suitable for most cell types. You should use the recorded drop breakoff parameters (amplitude, frequency, drop 1 and gap) which are written in the log book and are stored in FACSDiva



You may need to adjust the amplitude to move drop 1 close to the recorded spot. Engage the "sweet spot"

Verify that the small satellite drops are merging with the large drops.



The satellite drops should merge into the large drops in 6 or fewer satellites.

NEVER run a sample which has NOT been filtered through our 37 µm mesh or BD tubes # 352235

All Figures courtesy of Becton Dickinson

Cleaning and Shutting down the system

You must clean the instrument as detailed below at the end of your sort. Always Filter 10% Bleach and Coulter Clenz before running it on the Aria. It is good practice to run water or PBS between critical samples and prior to cleaning for shut-down.

You do NOT need to refill the sheath and empty the waste tanks.

Initial cleaning:

1) **Run Filtered bleach on flow rate 11 (high) for 4 minutes**
2) **Run Filtered Coulter Clenz (Blue) on flow rate 11 (high) for 4 minutes**
3) **Run Filtered water on flow rate 11 for 4 minutes**

Clean the flow cell

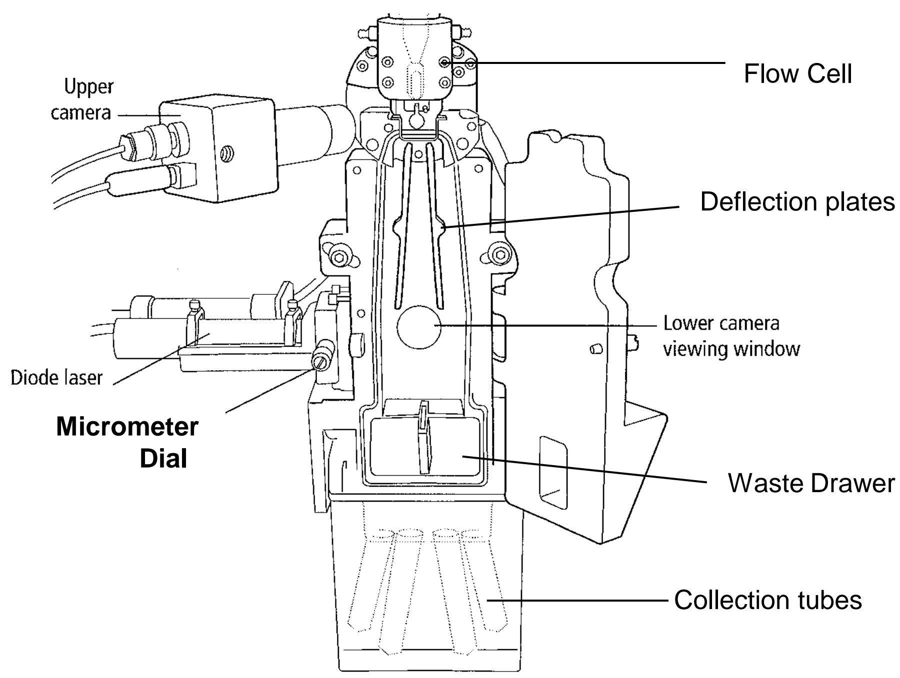
1) **Turn off stream**
2) **Remove nozzle !! DO NOT TOUCH O-RING !! and install the closed-loop nozzle (in holder, attached by tubing). Sonicate nozzle.**
3) **Select Cytometer>Cleaning Modes>Clean Flow Cell**
4) **When prompted, install a tube containing aprox. 3 mL filtered distilled water then click OK. The cytometer loads the tube and fills the flow cell with DI water.**
5) **Click OK when the completion dialog appears.**

Following flow cell cleaning:

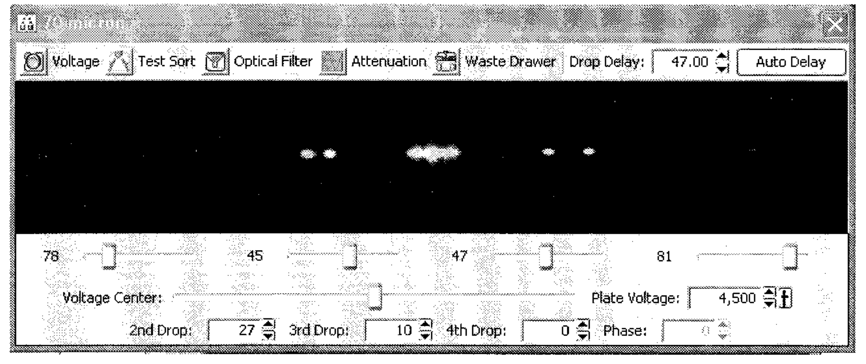
1) **Export FCS files and experiment if you have not already done so.**
2) **Close the "Coherent connection" software**
3) **Close FACSDiva**
4) **Shut down computer**
5) **Power off Aria**

Optimizing the side streams

1) **Click the Voltage button in the side stream window to turn on the deflection plates !!Warning 12,000 volts! Do NOT touch plates!!**
2) **Open the flow cell access door.**
3) **Click the Test Sort button and adjust the micrometer dial on the diode laser to better view the streams.**



Adjust the 2nd, 3rd, and 4th drop settings to tighten up the center stream
The stream below is not optimized, note fanning of center stream.



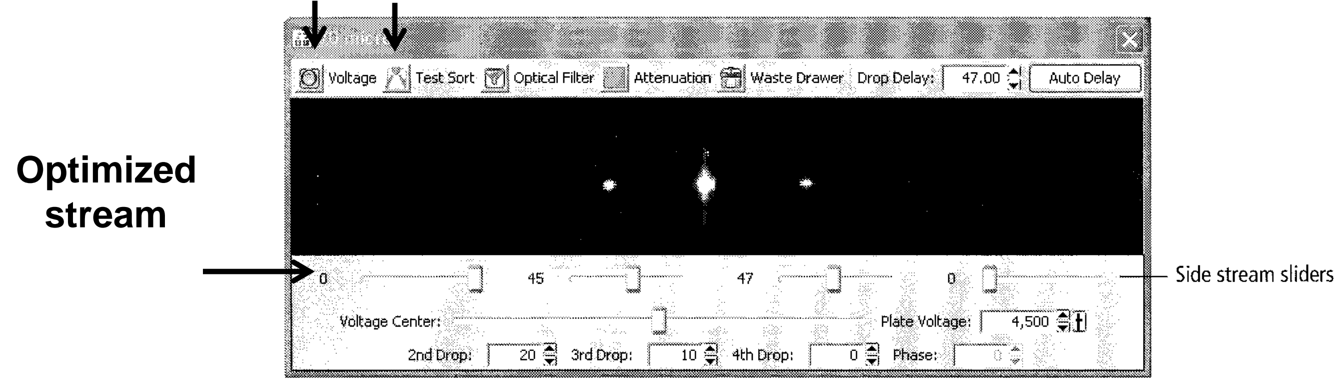
Side Stream Window

Typically , 2nd drop 10-20, 3rd drop 5-10, 4th drop 0-5

Aiming the streams

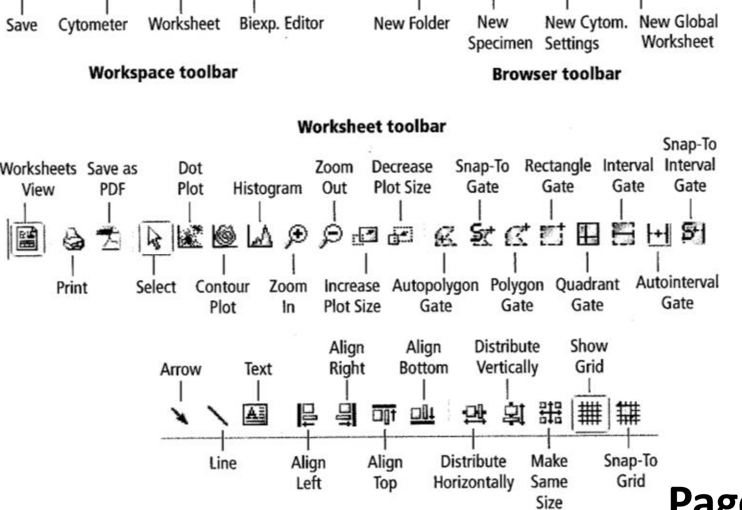
Once the center and side streams are optimized, and the sweet spot is on aim the streams for a 2-way (2 population) sort with the "Test Sort" on.

1) **Turn off the far right and far left streams by moving the stream sliders to the 0 position. Only 2 streams should be visible.**
2) **Place a tube holder (2 or 4 tube) on the bottom of the sort collection block.**
3) **Click the Waste Drawer button in the side stream window to open the drawer.**
4) **Open the sort block door, look to see where the test streams are aimed.**
5) **Adjust the left and right stream sliders as needed to aim streams into the test tubes.**
6) **Click the Waste Drawer button to close the drawer and click the voltage button to turn off the deflection plates.**
7) **Remove the tube holder and discard the wet tubes.**



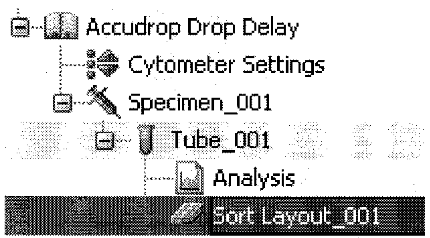
BD FACSDiva Software Toolbars

Refer to this toolbar guide when setting up for the 5 peak bead sort exercise



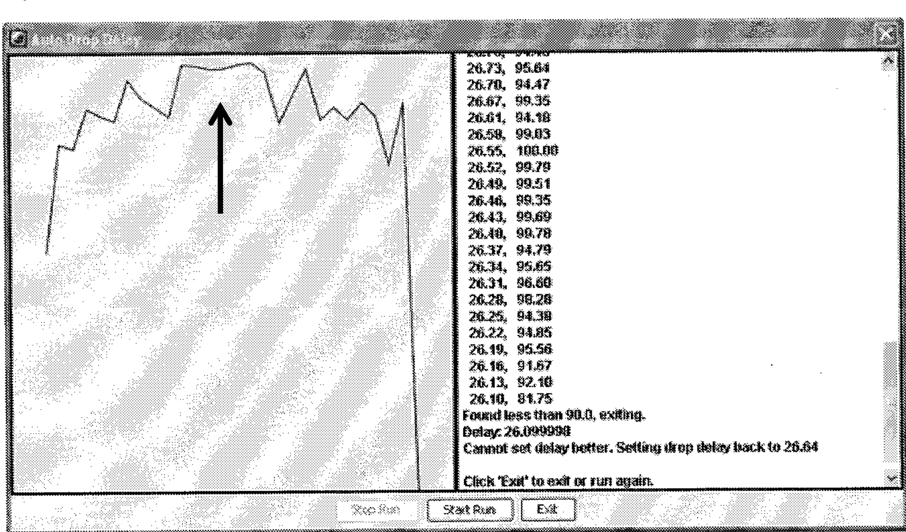
Setting the drop delay with Accudrop Beads

The Flow Core has made an “Accudrop delay” experiment. Please use this for setting drop delay. This is only necessary if Drop 1 cannot be brought back to within 10 (±5) of today’s dated target value listed in the log book.



1) **Open the Accudrop Delay experiment and expand Tube_001**
2) **Open the Sort Layout by double-clicking it**
3) **Close the sort block door and flow cell access door**
4) **Load a tube of Accudrop beads (in the sort room refrigerator)**
5) **Adjust the flow rate to achieve a flow rate of 800-1500 (85 µm nozzle)**
6) **Click sort in the sort layout**
7) **Click cancel in the Confirm dialog (waste drawer stays closed)**
8) **Click Auto Delay button in the side stream window**
9) **Click Start run in the Auto Delay dialog.**
10) **Monitor the auto delay window for progress. A message appears when the process is completed.**
11) **Click Exit in the Auto Drop Delay window to close it.**
12) **Unload the Accudrop tube. Cancel dialog box asking if you would like to save sort report.**

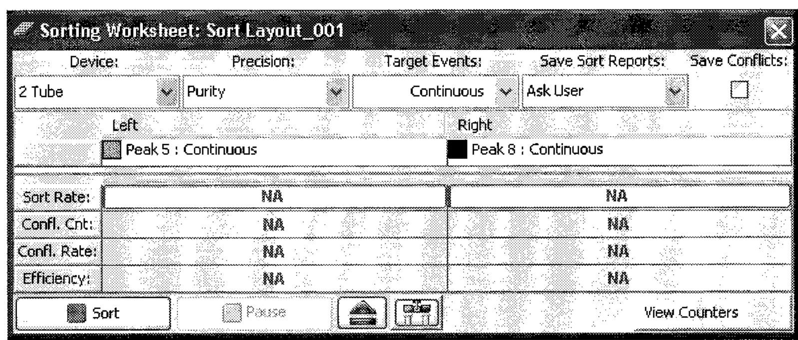
Figure 4-1 Auto Drop Delay window



Performing a sort using 5-peak beads

See the next page for example setup

1) Create a new experiment, use the new experiment button in the toolbar
2) Create a new specimen and re-name it: 5-peak bead sort_001
3) With specimen tube highlighted go to the Parameters tab in Cytometer controls and delete all but FSC, SSC, FITC and APC
4) Click the height (H) box for FSC, this is necessary for detecting singlets
5) In the Workspace create dot plots of FSC-A vs. SSC-A, FSC-A vs. FSC-H, and FITC-A vs. APC-A. Create a histogram of FITC-A. See examples on the next page. Regions will be made after the beads are collected.
6) Set the flow rate to 2 and load the 5 peak bead sample. NOTE: Always filter biological samples prior to running on the sorter!!
7) While acquiring, make adjustments to the PMT voltages in the parameters field of Cytometer controls so the beads appear similar to the example on the next page. Click record and collect 10,000 total events
8) Create regions on the dot plots as in the example on the next page
9) Click New Sort Layout on the browser toolbar, a new Sort layout appears and the Sort Layout icon appears under the Sorting Worksheet in the Browser
10) In the Sort Layout select *2 tube* in the device menu, select *purity* from the precision menu, select the left sort field and add one population to be sorted and select the right sort field and add the second population to be sorted
11) Open the sort collection chamber door and install clean collection tubes with 1 ml of PBS (collection media) in them
12) Load the 5 peak beads, start acquisition, click Sort on the Sort layout and click OK in the confirm dialog box
13) Stop the sort when 20,000 beads of each population have been collected.
14) Remove the sample and collection tubes. Save the sort report when the dialog box appears
15) Create new tubes (5-peak bead sort_002 and _003) and record each of the 2 collection tubes (purity checks) to confirm sort performance



EXAMPLE SET-UP:

5-peak sort exercise Set-up for a "2-way" sort of peaks 1 and 5

Workspace →

Sort layout →

Browser

Cytometer Controls

Acquisition dashboard

Inspector

Example Sort report

Sort Report

File

Experiment : 6 Peak Sort for Aria training
Specimen : Specimen_001
Tube : Global Sheet1
Sort Layout : Sort Layout_001
Application : FACSDiva Version 6.1.3

Sort Report

Report Date : 2013.01.15 at 14:20:37
Device : 2 Tube
User ID : Administrator
Cytometer : 5B-3R-3V-2UV-5YG,ACDU,AMO,TCO (FACSAriaII) (P69500116)

Sort Settings

Sort Setup	85 micron	Phase Mask	0
Frequency	44.2	Single Cell	Off
Amplitude	11.9	Sweet Spot	On
Phase	0.00	First Drop	220
Drop Delay	24.21	Target Gap	9
Attenuation	Off	Plates Voltage	3,200
Precision	Purity	Voltage Centering	0
Yield Mask	32	Sheath Pressure	45.00
Purity Mask	32		

Side Stream Voltage (%)

Far Left	Left	Right	Far Right
0.00	52.00	58.00	0.00

Neighboring Drop Charge (%)

2nd	3rd	4th
15.00	5.00	3.00

Acquisition Counters

Threshold Count	428308
Processed Events Count(evt)	14926
Electronic Aborts Count(evt)	1826
Sort Elapsed Time(hh:mm:ss)	00:09:24

Sort Counters

	Left	Right
Sort Rate(evt/s)	50	48
Conflicts Count(evt)	6610	6528
Conflicts Rate(evt/s)	11	11
Efficiency(%)	81	80

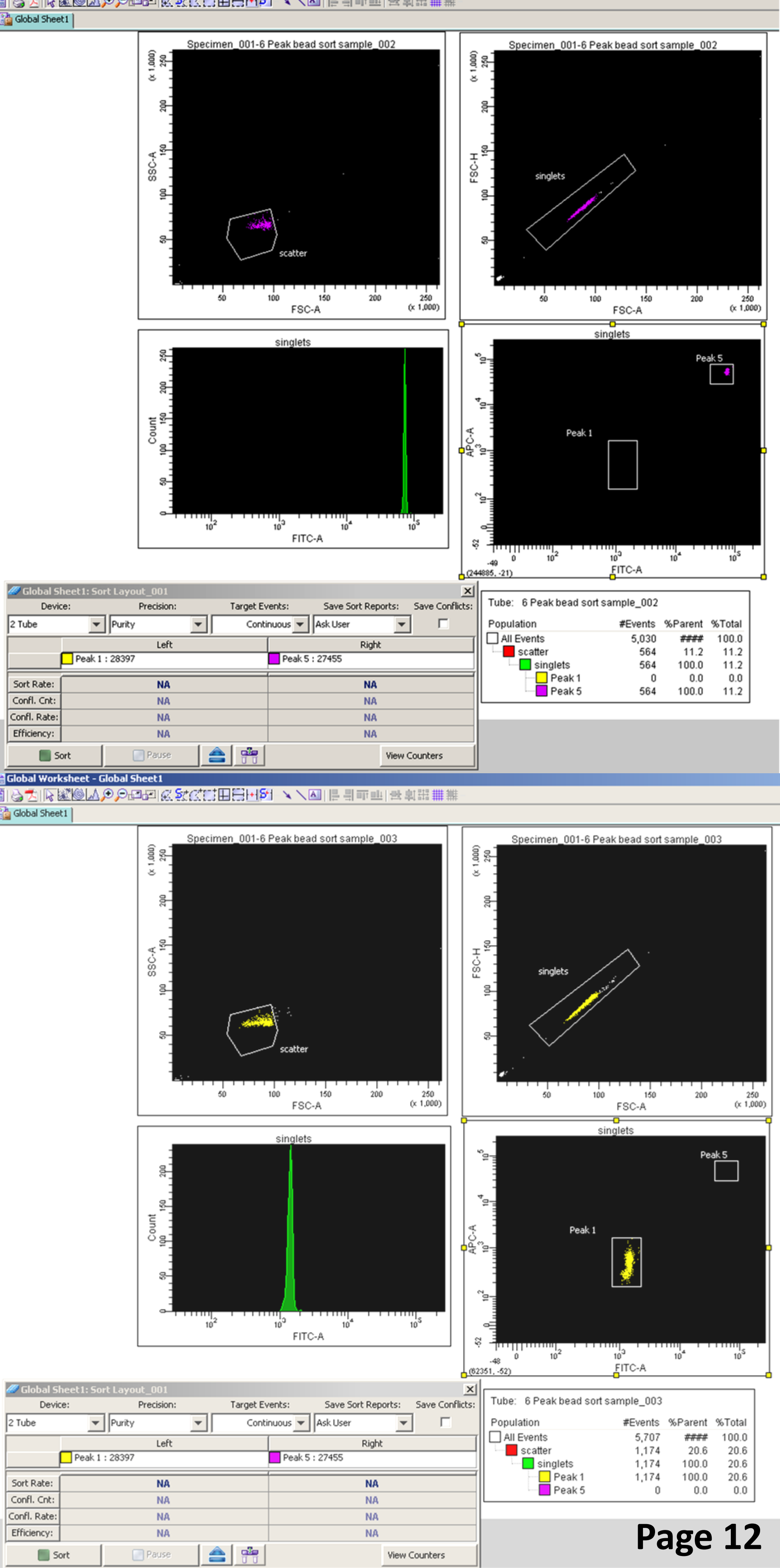
Sort Layout

Left	Right
Peak 2 : 28397	Peak 6 : 27455

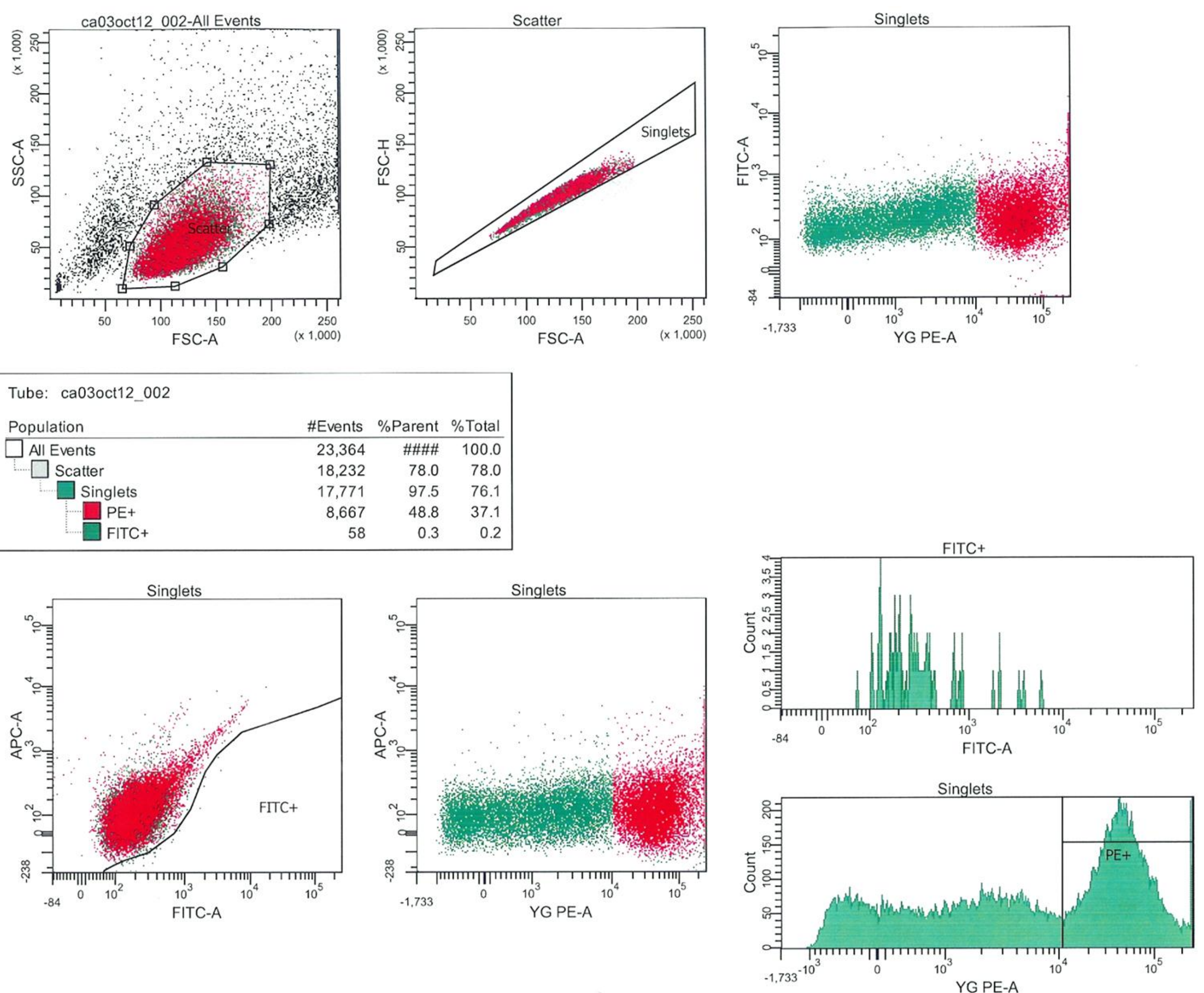
Example

Post-sort
Peak 1
“Purity check”

Post-sort
Peak 5
“Purity check”



Example sort setup for a cell line
Sorting for RFP positive cells



Tube: ca03oct12_002

Population	#Events	%Parent	%Total
All Events	23,364	####	100.0
Scatter	18,232	78.0	78.0
Singlets	17,771	97.5	76.1
PE+	8,667	48.8	37.1
FITC+	58	0.3	0.2

Task Bullet List with “Clog Fix” by Susanne Pechhold

- Turn on computer
- Turn on instrument
- Log on to Windows and FACSDiva, start Coherent Connection
- Make a new folder in FACSDiva browser
- Make an Experiment (or duplicate a former experiment without data then cut and paste into your new folder)
- Make sure the 85 µm nozzle is in, Start stream
- Wash 5 min Filtered 10% Bleach and 5 min Filtered water
- Check stream and drop breakoff, change amplitude to bring drop 1 near target value.
- Test side streams and adjust drop charge to optimize (no center strean fanning). Change Drop1 target if necessary.
- Turn on Sweet Spot
- Perform Accudrop drop delay
- Filter all Biological samples or you WILL clog. Use provided 37 µm mesh squares or BD cell strainer tubes PN 352235 (sterile)

CLOG!!

Okay, so you did everything right and it clogged. It happens. Maybe you ran it too fast.

- Remove the nozzle and Sonicate it !! DO NOT TOUCH O-RING !!
- Dry with compressed air
- Inspect it under the dissection scope
- Carefully re-insert the nozzle and start stream
- If it still looks bad, partial clog, repeat the steps above

FACS Aria Sort Check List by Glenn Paradise

Did you:

1) Put the FACS Aria cover down.

2) Turn the stream on if it is off. Give it time to stabilize.

3) Make sure you can see drops (adjust amplitude until you can see drops).

4) Make sure the sweet spot is engaged.

5) Make sure the drop 1 # and the gap # are correct.

6) Change the flow rate to 2 before you load the sample.

7) Filter the sample just before loading. You will not be allowed to use the self sorting service if repeated clogging of the sorter occurs.

8) Adjust your side stream sliders to the correct position for your collection tubes.

9) Make sure you are using the purity precision mode.

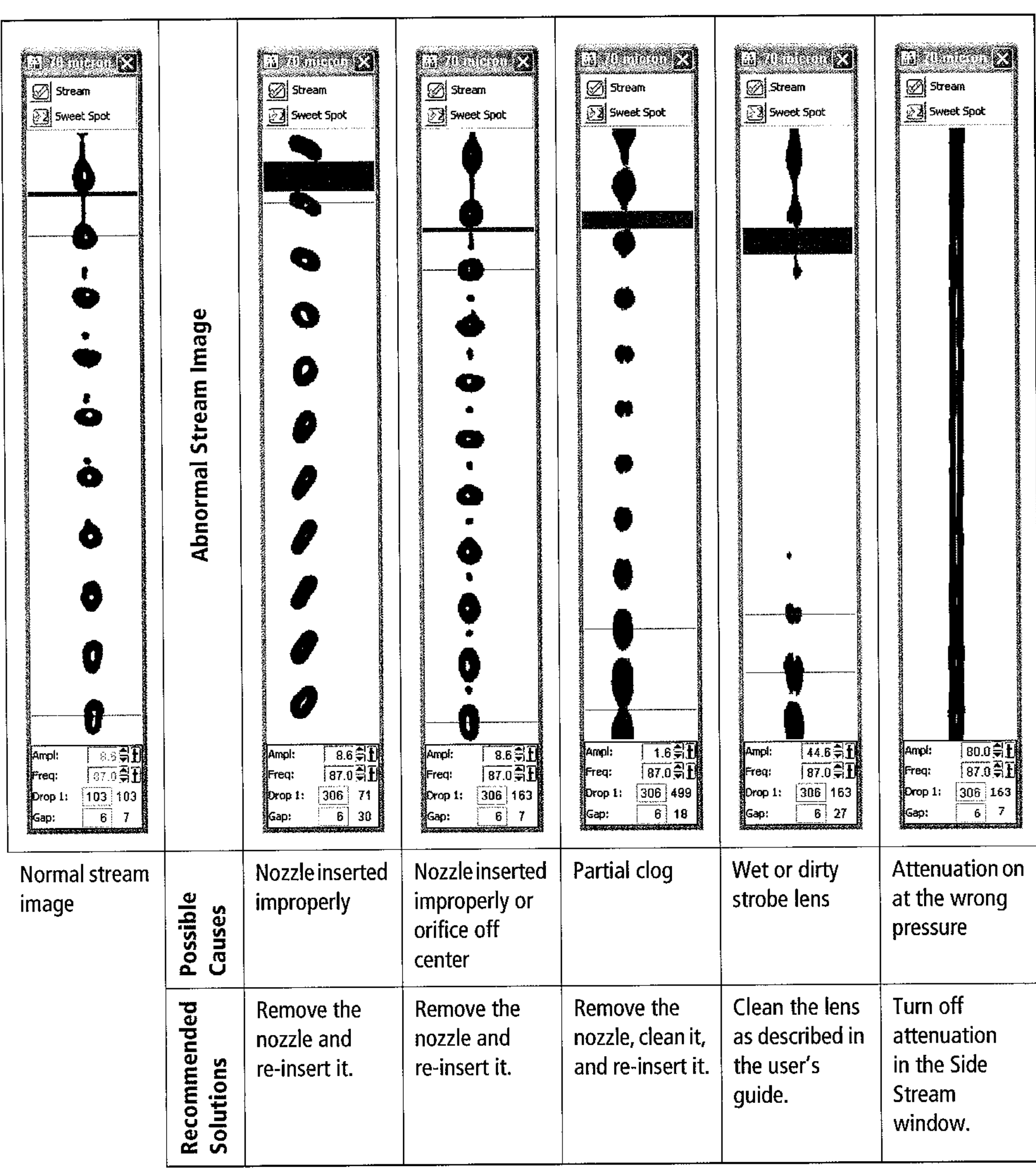
10) Make sure your gates are set properly and they define the population to sort correctly. Caution, do not put a gate to close to a population of cells you do not want, this will decrease the purity of the sorted cells.

11) Load your collection tubes. Use left and right first followed by far left and far right.

12) Give the sort command!

13) Adjust the flow rate up to no greater than 6 or until your efficiency is at the lowest acceptable value (~72%).

Troubleshooting the Breakoff



Normal stream image						
	Abnormal Stream Image					
	Possible Causes	Nozzle inserted improperly	Nozzle inserted improperly or orifice off center	Partial clog	Wet or dirty strobe lens	Attenuation on at the wrong pressure
	Recommended Solutions	Remove the nozzle and re-insert it.	Remove the nozzle and re-insert it.	Remove the nozzle, clean it, and re-insert it.	Clean the lens as described in the user's guide.	Turn off attenuation in the Side Stream window.