フローサイトメーターとセルソーターの活用法（実習・英語/日本語）
Cytometry and Fluorescence Activated Cell Sorter (Practice in English/Japanese)

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フローサイトメーターは個々の細胞の分子の発現を半定量的に測定できる装置である。装置は解析専用のアナライザーと解析分取が可能なセルソーターの 2 機種があり、分子の発現の同定には蛍光色素で認識された抗体がよく用いられる。更に標識抗体以外の蛍光物質を測定する事も試みられている。

今回は実習として、機器更正用ビーズを用いて、FACS にて検出する

●実習内容：

機器更正用ビーズを用いて、FACS にて検出します。アナライザーの BD LSRFortessaX-20、BD FACSCanto II とセルソーターの BD FACSAria Fuison の 3 機種を用いて解析を行います。

Flow cytometer is a device that can semi-quantitatively measure the expression of individual cell molecules. There are two types of devices, an analyzer and a cell sorter. Antibodies recognized by fluorescent dyes are often used to identify the expression of molecules. It has also been attempted to measure fluorescent substances other than labeled antibodies.

As a practical training, we detected with FACS using beads for flow cytometer setup.

＊FACS (fluorescence activated cell sorter) is a trademark of Becton, Dickinson and company.

Practice content:

Beads for flow cytometer setup are detected with FACS.

We will analyze using three instrument, analyzer BD LSRFortessaX-20 and BD FACSCanto II, and cell sorter BD FACSAria Fusion.

【Practice】 Time schedule

1． 17:00 – 18:20 **Practice**（細胞工学実験室2、4）

Explanation of instrument

Data acquisition and analysis

Practice will be done in 3 groups and 2 instruments in turn.

Group A； LSRFortessa （17:00–17:40） → AriaFusion （17:40–18:20）

Group B； AriaFusion （17:00–17:40） → LSRFortessa (17:40–18:20)

Group C； AriaFusion (17:00–17:40) → Canto Ⅱ(17:40–18:20)

The aim for today's practice is to learn about the settings and analysis for FACS.

We want you to know that FACS is a useful and easy instrument.

■Practice

Samples to measure；

Samples are prepared in the tubes as shown below.

Adjust the voltage/compensation for settings.

Analyze the percentage of PE positive Beads.

Tube NO.	1	2	3	4	5
label	Unlabeled	Unlabeled FITC	Unlabeled PE	Unlabeled APC	Unlabeled FITC PE APC
Unlabeled	○	○	○	○	○
FITC		○			○
PE			○		○
APC				○	○

BD LSRFortessa™ X-20
Quick Reference Guide

Ver1.0 Diva8.0.1

BD Biosciences

Section1：Start up

[1] System

① Verify sheath and waste tank. Fill the sheath or empty the waste, if needed.

② Turn on the computer.

③ Click Admin icon. Password: BDIS#1

④ If there is sheath supply system (FFSS option), turn on the FFSS.

⑤ Turn on the main power of the instrument.

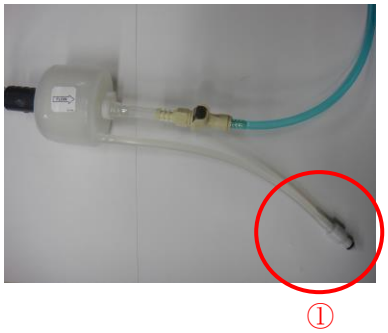
⑥ After 2 minutes, open the FACSDiva software: right click > open.
Log in ID: administrator no password

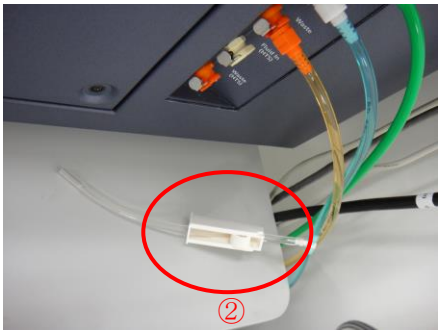
⑦ After connected, select the “Use CST Settings” in the CST mismatch window.

[2] Remove air bubble

① Remove air bubble from sheath filter.

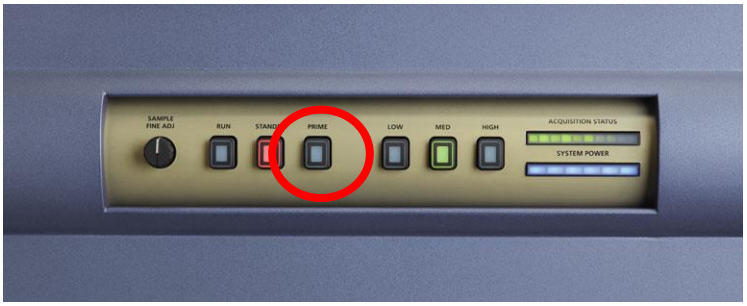
② Remove air bubble from sheath line using the pinch cock roller on the right side of instrument.





③ Remove DI water tube from sample injection port (SIP)

④ Press “PRIME” button on the instrument.



⑥ After “PRIME” is done, repeat “PRIME” twice again.

⑦ Install a tube with 2ml of FACSFlow to the SIP, Press “RUN” and Flow Rate “High” button.

⑧ After 5 minutes, press “STANBY” button.

⑨ Remove the tube with FACSFlow from the SIP, install a tube with 2ml of DI water.

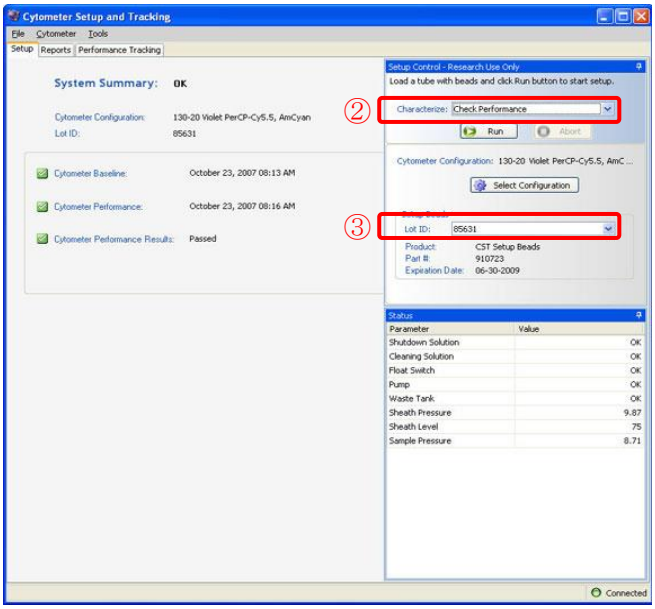
Section2：Check Performance

Before setting up an experiment, you must first run a performance check. A performance check ensures that the cytometer is performing consistently over time. It generates default cytometer settings that places each PMT within an optimal range.

① Click “CST” from Cytometer menu.

② After connected, select the “Check Performance” from Characterize.

③ Check the setup beads (CST beads) Lot ID. The lot ID number is written on the vial.



④ Dilute CST beads: 1 drop in 0.35ml FACSFlow (sheath fluid).

⑤ Install the CST beads tube to the sample injection port.

⑥ Press Flow rate “LOW” and press “RUN” button on the instrument panel.

⑦ Click Run > OK.

⑧ After completed, press “STANBY”, and click “View Report”.

Verify that the cytometer performance passed.
If any parameters did not pass, perform the clean flow cell.

⑨ Select File > Exit to close the CST window and connect back to the BD FACSDiva interface.

⑩ Select the “Use CST Settings” in the CST mismatch window.

Sction3: Set up and analysis

[1] Create a new experiment

① Click the folder and experiment icon on the Browser toolbar.

② Click the plus sign (+) of the specimen.

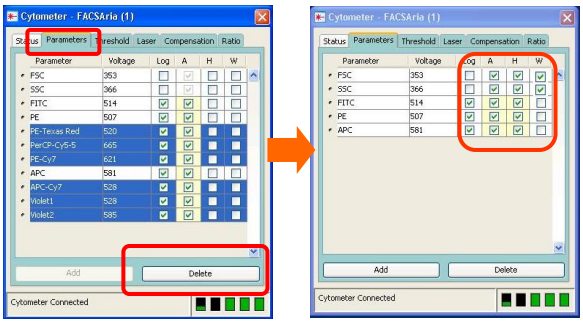
③ Click the “acquisition pointer” of tube 001.

④ Check of parameters in the cytometer window > parameter tab.

⑤ Delete unnecessary fluorescence parameters.

⑥ Check the “H(Height)” and “W(Width)”, if needed.

⑦ Create dot plots or histogram plots on the worksheet:
ex) FSC-A vs SSC-A, and each fluorescence.



[2] Adjustment of voltage in FSC,SSC and fluorescence parameter

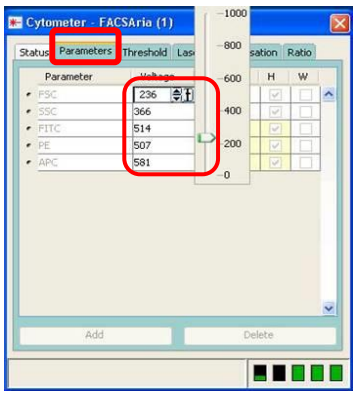
① Install a negative sample tube, press “RUN” button on the instrument, and click “Acquire Data” in the Acquisition Dashboard Window.

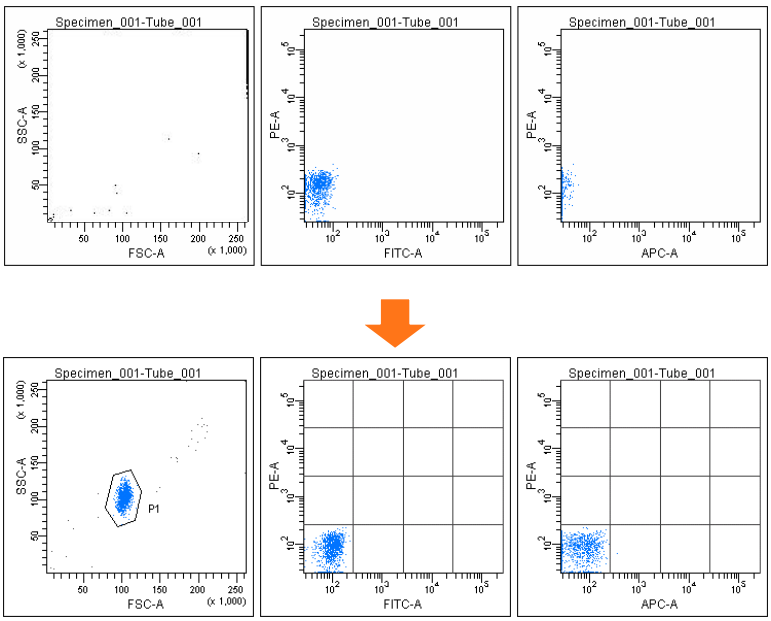
② Adjust the FSC and SSC voltages, in order to display the target population on the plot.

③ Adjust “Threshold” or “Area Scaling Factor”, if needed (refer to the Appendix).

④ Adjust the fluorescence voltage, and set up to the optimal negative position.

⑤ After setting up, click “Stop Acquiring” and press “STANBY” button on the instrument.





[3] Compensation

When two or more fluorochromes are being used, it may be necessary to compensate a spectral overlap of fluorescence.

You can select manual or auto compensation in FACS Diva software.

Manual Compensation

① Prepare the objective fluorescent plots for your experiment.

② Run and acquire the single stained sample > Stop Acquiring.

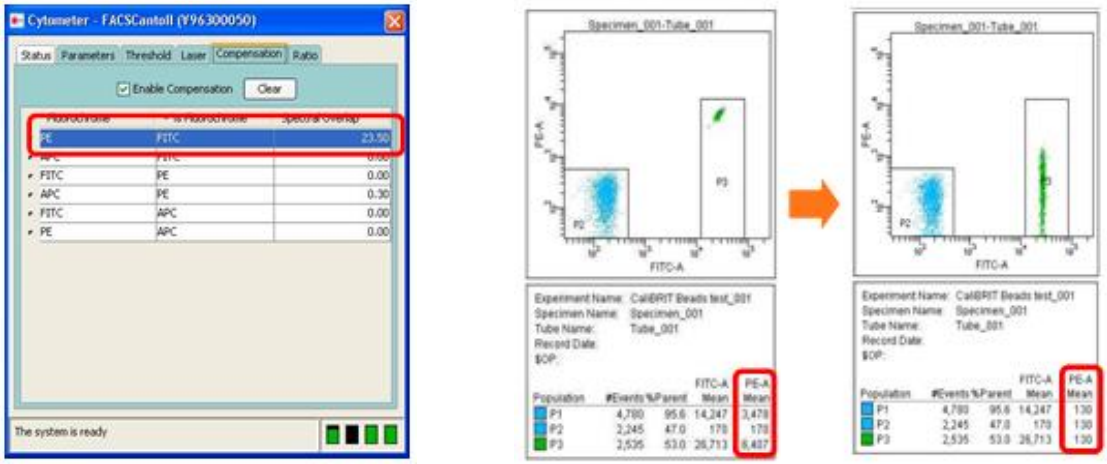
③ Create the gates to negative and positive population.

④ Right click on the plot > create statistics

⑤ Verify the mean value of negative and positive population in the statistics window.

When both mean values differ, adjust spectral overlap value from Compensation tab in the Cytometer window.

To apply suitable compensation, adjust the mean value of a negative and a positive to the same value.



Auto Compensation

① Click the Experiment menu > Compensation Setup > Create Compensation Controls

② Click OK in the Create Compensation Controls dialog.

③ Click the (+) of compensation controls specimen, and click the acquisition pointer of unstained control tube.

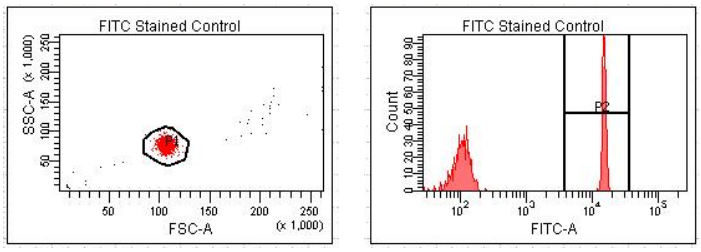
⑤ Run and acquire the negative sample and set the P1 gate to the target population.

⑥ Click “Record Data”.

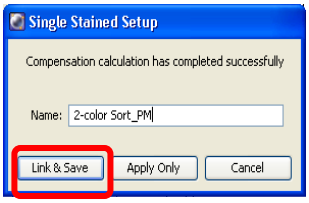
⑦ Right click on the P1 gate, and select “Apply to All Compensation Controls”.

⑧ Record data for each of the single color control tubes similarly.

⑨ Select each compensation worksheet tab and verify that the snap-to interval gate is encompassing the positive population. Adjust the gate, if needed.



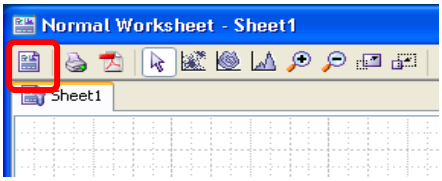
⑩ Click the Experiment menu >Compensation Setup > Calculate Compensation.
If the calculation is successful, a dialog appears.



⑪ Click the “Link & Save”.

⑫ Click the acquisition pointer of tube 001.

⑬ Click the sheet icon and it changes from Sheet to Global Work Sheet.



[4] Record sample data

① Run and acquire the stained sample.

② Set record number from the “Event to Record”, if needed.

③ Click the “Record Data”.

④ Click the “Next Tube”, if you have next sample.

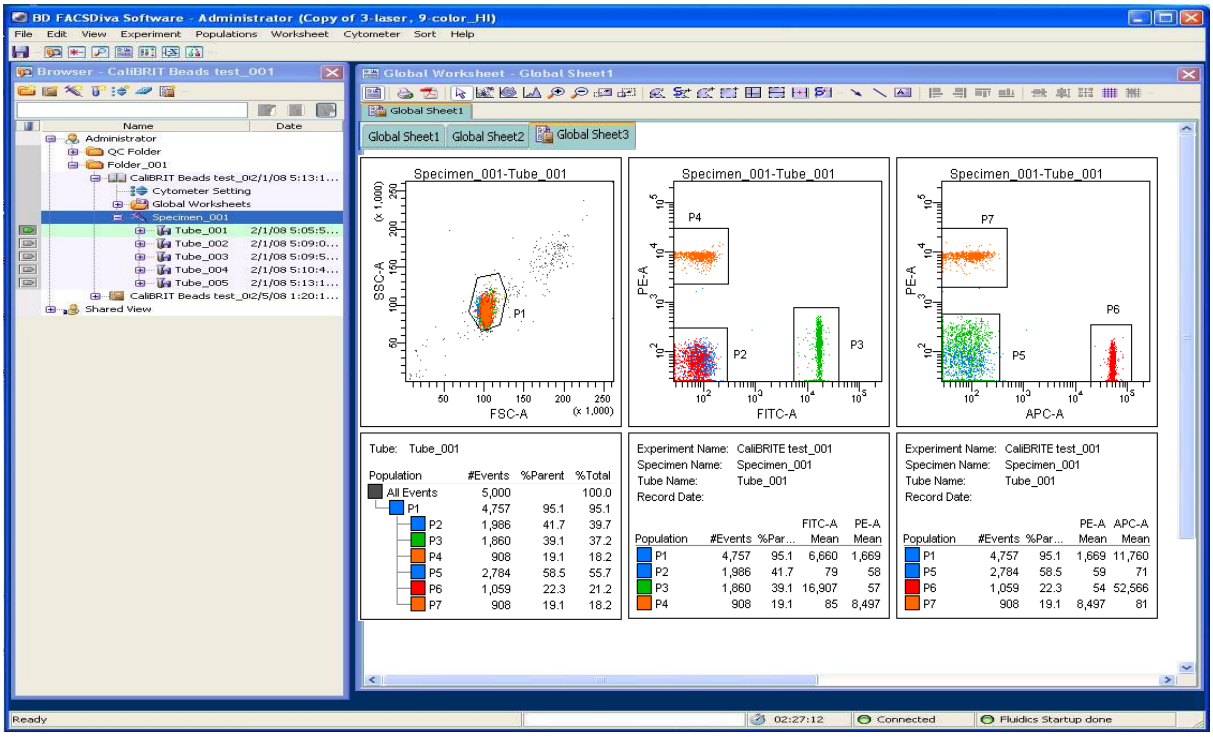
[5] Data analysis

① Right click the FSC,SSC plot, and click the “Show Population Hierarchy”.
Then, the gate hierarchy window is displayed on the sheet.

② Create the gate to target population.

③ If “Show Population” is used, only a target population can be displayed on a plot.

④ Right click the objective fluorescence plot> Create Statistics View.

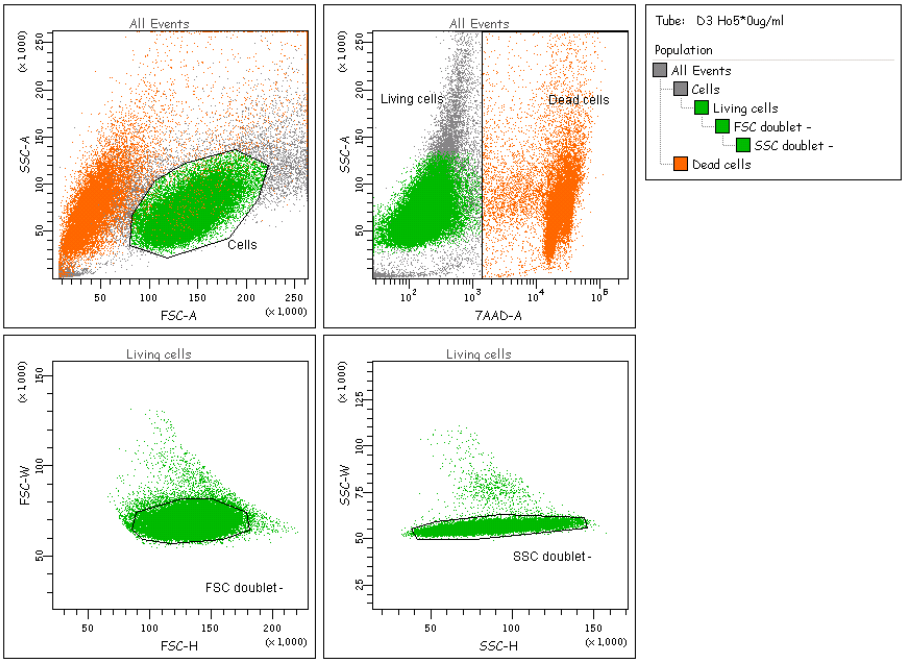


[6] Appendix

1. Removal of dead and doublet cells

Removal of dead and doublet cells are recommended for analysis or sorting of high purity.

- dead cells plot: SSC-A vs reagent ex) PI, 7AAD, DAPI
- doublet cells plots: FSC-W vs FSC-H and SSC-W vs SSC-H

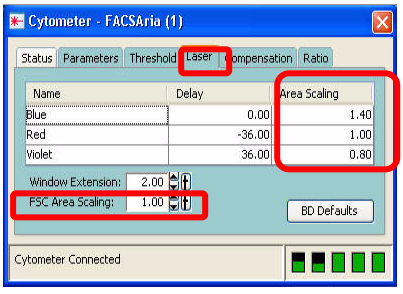


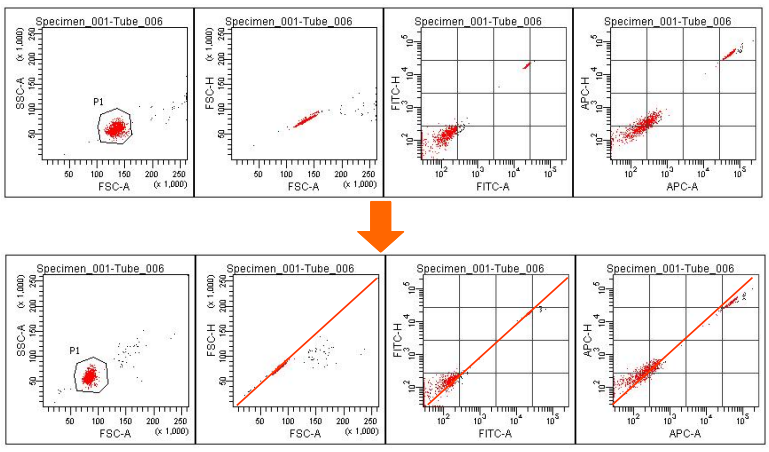
2.Threshold: Threshold tab > Default value: FSC 5000

Threshold is cutting the debris or electric noise in the lower-left of the FSC-A vs SSC-A plot. If your sample has a lot of debris, you can remove it by increasing threshold value in the threshold tab in Cytometer window.

3. Area Scaling Factor: Laser tab> Area Scaling

The required area scaling factor changes based on sheath pressure and sample size. The area scaling factors should be verified for each experiment performed on the cytometer. Correct Width data is obtained by arranging the dynamic range of Area and Hight data.





4. Data Export: right click experiment> export

Export FCS files:

FCS file is raw data. FCS 2.0 or FCS 3.0 (recommended) can be analyzed in another software application.

Export Experiments:

This is all experiment data (cytometer settings, plots, gates, statistics, and FCS files).

Save as data backup.

5. Duplicate of experiment: right click experiment> duplicate without data

Experiment data (cytometer settings, plots, gates, statistics) can be copy without tube data.

* The data of Laser tab (Area scaling, Laser Delay) is not copied.

Section4：Shutdown

① Install 3ml FACSClean tube to SIP.
② Press “RUN”, shift the support arm to the side and run for 1 minute.
③ Return the support arm to the center and run for 5 minutes. FlowRate is “High”.
④ Install 3ml FACSRinse tube to SIP.
⑤ Shift the support arm to the side and run for 1 minute.
⑥ Return the support arm to the center and run for 5 minutes.
⑦ Press “STANBY”.
⑧ Click “File” menu> “Quit”.
⑨ Turn off the main power, shutdown PC.
⑩ Install 1ml DI water tube to SIP.
⑪ Turn off the FFSS.　Fill the sheath and empty the waste, if needed.

Section 5: HTS option

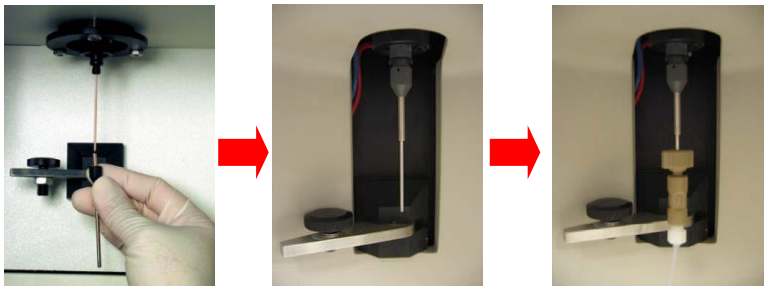
When using HTS, it's recommended to do Section1(Startup), Section2(Check performance), and Section3(Set up and analysis) manually using tubes.

[1] HTS start up

① Change the tube/plate switch on the machine (right side) to the plate mark.

② Exchange the tube protector of the SIP for HTS.

③ Connect the sample coupler to the SIP.

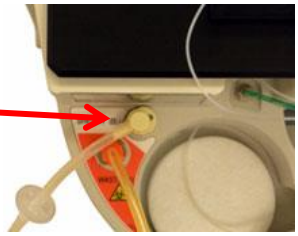


④ Remove air bubble from sheath line.

1. Remove the sheath line from HTS.
2. Connect the air removal tool to sheath line.
3. Remove air bubble using pinch cock roller.
4. Connect the sheath line to HTS.

2

1

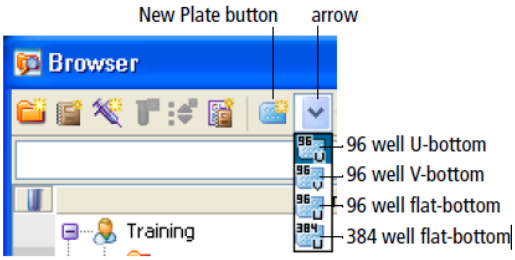


3

⑤ Prime the fluidics for HTS

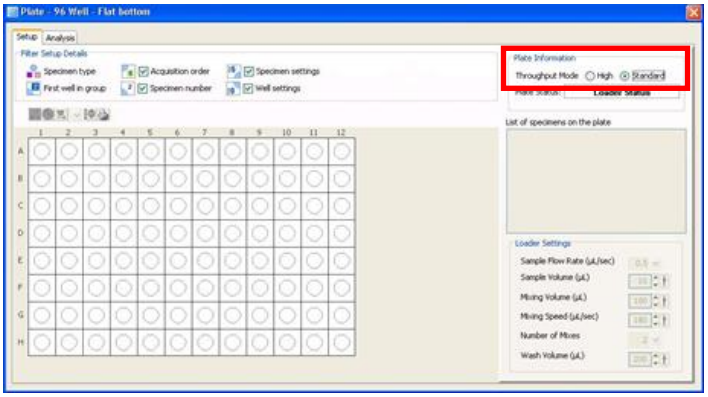
1. Put the cytometer is in RUN mode.
2. Select HTS menu > Prime on the software.
   Repeat twice for a total of three primes.
3. Click OK to close the completion dialog.
4. Verify that there are no air bubbles in the green tubing
5. Put the cytometer in STNDBY mode.

[2] Perform HTS

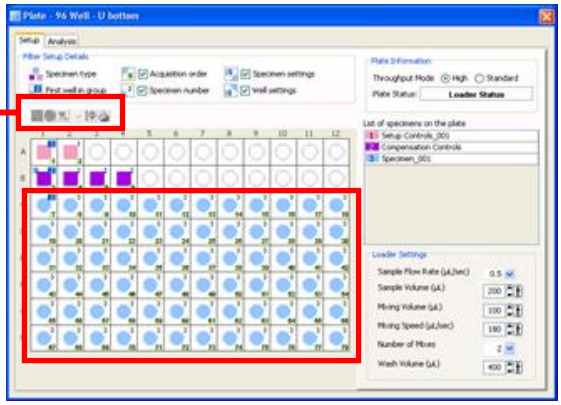


① Set up the plate view

1. Open the objective experiment
2. Select plate type of the new plate button on the browser window
3. The Plate view is displayed.
4. Select “High” or “Standard” mode from the plate information.

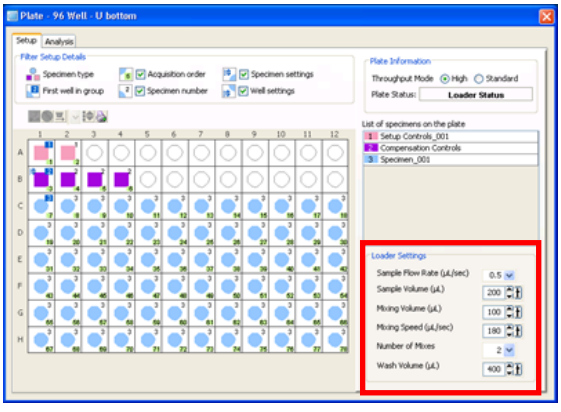


5. Designate a measurement position using a Specimen icon.



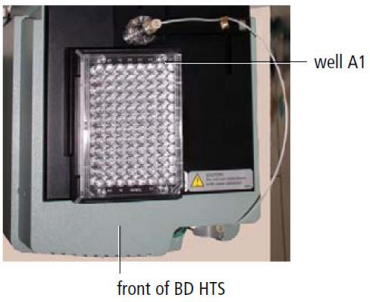
6. Set up the Loader Settings for each well.

Setting	Standard Mode			High Throughput Mode		
	Default	Range	Res	Default	Range	Res
Sample Flow Rate (µL/sec)	1	0.5–3.0	0.5	1	0.5–3.0	0.5
Sample Volume (µL)	10	2–200	1	3	2–10	1
Mixing Volume (µL)[a]	100	5–100	1	50	5–100	1
Mixing Speed (µL/sec)	180	25–250	1	200	25–250	1
Number of Mixes (cycles)	2	0–5	1	2	0–5	1
Wash Volume (µL)	400	200–800	1	200	200–800	1



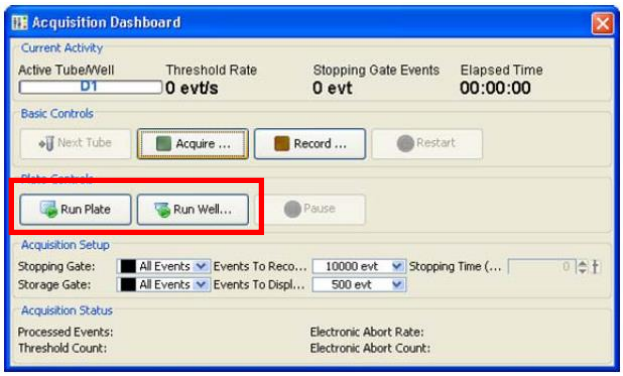
② Install a plate on the HTS

1. Remove the HTS safety cover.
2. Place the plate on the plate holder, well A1 is over the inscribed A1 on the plate holder.
3. Replace the HTS safety cover.



③ Measurement

1. Check the Event to Record for each well.
2. Press RUN button on the instrument.
3. Click RUN Plate or RUN Well on the dashboard

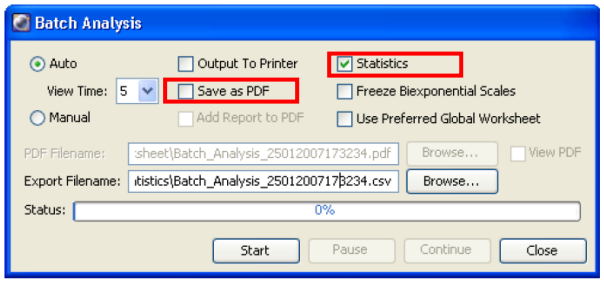


④ Batch Analysis (To export PDF or statistics data)

In the Plate window, click and drag across wells to select them, then right-click and select Batch Analysis.

Select the check box in the Batch Analysis dialog as shown in the figure.

Click Start to begin the batch analysis.



[3] HTS shut down

① Prepare a cleaning plate

Fill the wells of a 96-well plate according to the following table.

Wells	Solution	Volume (µL)
A1–A4	BD FACSClean[a]	200
B1–B4	DI water	200

② Run a cleaning plate

1. Install the cleaning plate on the HTS unit.
2. Select HTS > Clean.
3. In the Plate Templates dialog, select the Daily Clean - 96 well U-bottom template and click OK.

   The plate layout changes to show the cleaning template and a confirmation dialog is displayed.
4. Set the fluidics mode to RUN.
5. Click OK to begin the cleaning cycle.

   The cleaning procedure can take up to 15 minutes.
6. Click OK when the completion message appears.
7. Set the fluidics mode to STNDBY.

③ After HTS clean, remove the sample coupler from SIP. And replace the tube protector.

④ Perform the SIP cleaning. Refer to Section4:Shutdown.

⑤ Select File > Quit to exit BD FACSDiva software.

⑥ Turn off the computer.

⑦ Turn off the instrument.

⑧ Install 1ml DI water tube to SIP.

⑨ Change the tube/plate switch on the machine (right side) to the tube mark.

⑩ Turn off the FFSS. Fill the sheath and empty the waste, if needed.

BD FACSCanto™ II Flowcytometer
Quick Reference Guide

Ver1.2

BD Biosciences

Section1：Start up

【1】System

WindowsXP　(BD FACSDiva software Ver.4.0 - 6.1.3)

1. Turn on the main power.
2. After 2 minutes, turn on the computer.
3. Log in ID: Administrator　Password: BDIS
4. Open the FACSDiva software: right click > open.
5. Log in ID: administrator　no password
6. After connected, select the “Use CST Settings” in the CST mismatch window.

Windows7　(BD FACSDiva software Ver7.0 -)

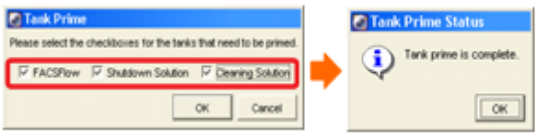
1. Turn on the computer.
3. Click Admin icon.　Password: BDIS#1
4. Turn on the main power.
5. Open the FACSDiva software: right click > open.
6. Log in ID: administrator　no password
7. After connected, select the “Use CST Settings” in the CST mismatch window.

【2】Fluidics start up

⑦ Verify fluid levels in the cytometer window. Replenish fluids or empty the waste, ifneeded.

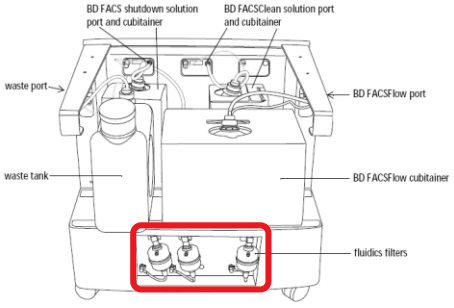
⑧ Prime the fluidics system.

Select Cytometer > Cleaning Modes > Prime After Tank Refill.



Check the all tanks, then click “OK”.

⑨ Remove the air bubble from each fluid filters.



⑩ Remove a DI water tube from sample injection tube.

⑪ Click Cytometer menu > Fluidics Startup > OK

After Fluidics Startup, message “Fluidics Startup Complete. The System is Ready” is displayed > click OK.

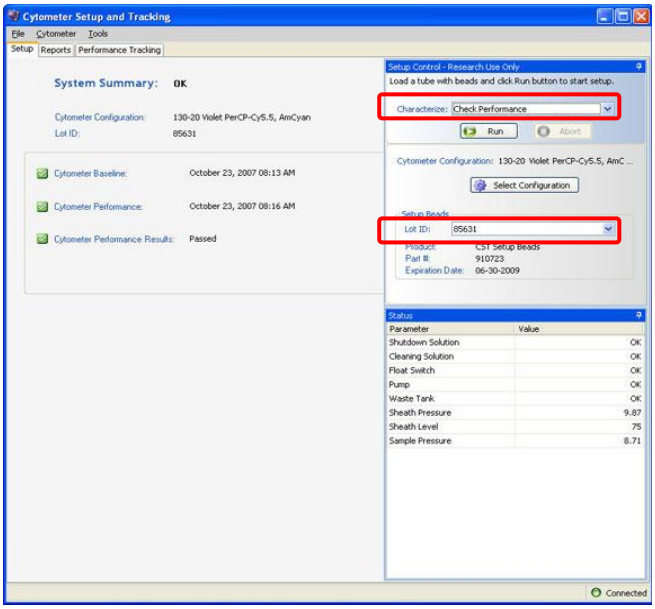
Section2：Check Performance

Before setting up an experiment, you must first run a performance check. A performance check ensures that the cytometer is performing consistently over time. It generates default cytometer settings that places each PMT within an optimal range.

① Click “CST” from Cytometer menu.

② After connected, select the “Check Performance” from Characterize.

③ Check the setup beads (CST beads) Lot ID. The lot ID number is located on the CST beads vial.



④ Create of CST beads: 1 drop in 0.35ml FACSFlow(sheath fluid).

⑤ Install the beads tube to the sample injection tube.

⑥ Click Run> OK.

⑦ After complete the performance check, click View Report.

Verify that the cytometer performance passed.
If any parameters did not pass, perform the clean flow cell.

⑧Select File > Exit to close the CST window and connect back to the BD FACSDiva interface.

⑨Select the “Use CST Settings” in the CST mismatch window.

Sction3: Set up and analysis

【1】Create a new experiment

① Click the folder and experiment icon on the Browser toolbar.

② Click the plus sign (+) of the specimen.

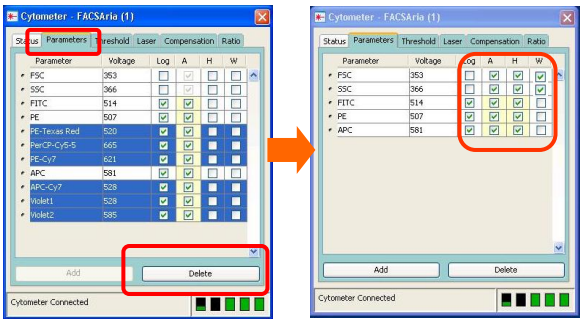
③ Click the “acquisition pointer” of tube 001.

④ Check of parameters in the cytometer window > parameter tab.

⑤ Delete unnecessary fluorescence parameters.

⑥ Check the “H(Height)” and “W(Width)”, if needed.

⑦ Create the dot plots or histogram plots:
FSC-A vs SSC-A, and each fluorescence.



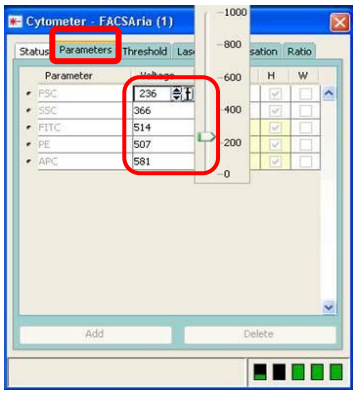
【2】 Adjustment of voltage in FSC,SSC and fluorescence parameter

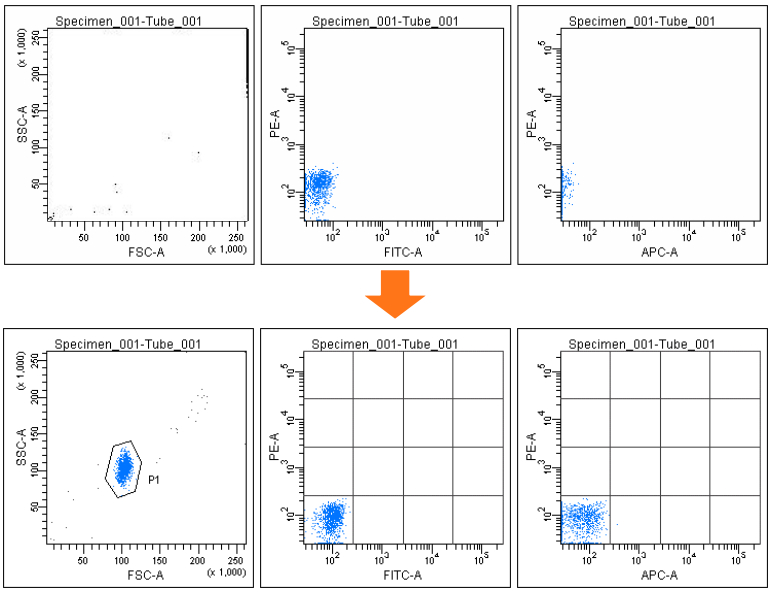
① Install a negative sample tube, and click “Acquire Data” in the Acquisition Dashboard Window.

② Adjust the FSC and SSC voltages, in order to display a target population on the plot.

③ Adjust “Threshold” or “Area Scaling Factor”, if needed (refer to the Appendix).

④ Adjust the fluorescence voltage, and setting up to the optimal negative position.





⑤ After setting up a control position, load the positive sample.

⑥ It checks that the positive populations are displayed in fluorescence plot.

【3】Compensation

When two or more fluorochromes are being used, it may be necessary to compensate a spectral overlap of fluorescence.

You can select manual or auto compensation in FACS Diva software.

Manual Compensation

① Prepare the objective fluorescent plots for your experiment.

② Create the gates to negative and positive dots population by acquire the negative and single stained sample.

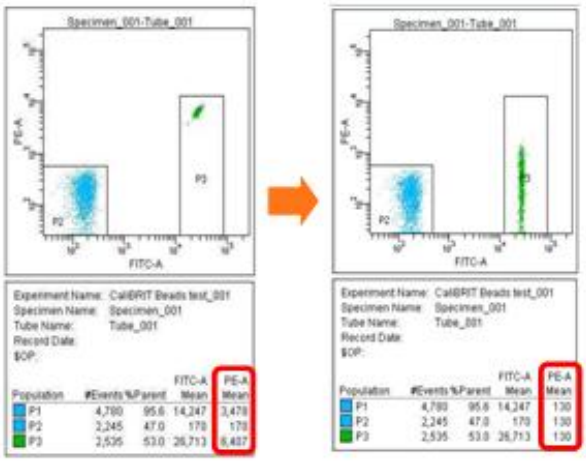
③ Right click on the plot > create statistics

④ Acquire the single stained sample > Stop Acquiring.

⑤ Verify the mean value of negative and positive population.

When both mean values differ, adjust spectral overlap value from Compensation tab in the Cytometer window.

Suitable compensation will be applied by adjusting the mean value of a negative and a positive to the same value.



Auto Compensation

① Click the Experiment menu > Compensation Setup > Create Compensation Controls

② Click OK in the Create Compensation Controls dialog.

③ Click the (+) of compensation controls specimen, and click the acquisition pointer of unstained control tube.

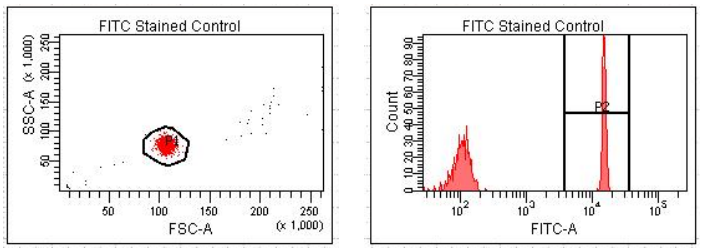
④ Acquire the negative sample and set the P1 gate to the target population.

⑤ Click “Record Data”.

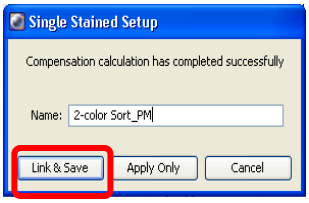
⑥ Right click on the P1 gate, and select “Apply to All Compensation Controls”.

⑦ Record data for each of the single color control tubes similarly.

⑧ Select each compensation worksheet tab and verify that the snap-to interval gate is encompassing the positive population. Adjust the gate, if needed.



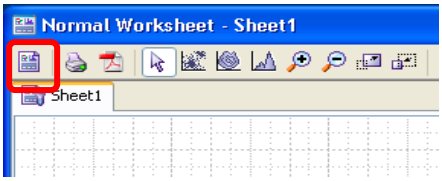
⑨ Click the Experiment menu >Compensation Setup > Calculate Compensation.
If the calculation is successful, a dialog appears.



⑩ Click the “Link & Save”.

⑪ Click the acquisition pointer of tube 001.

⑫ Click the sheet icon and it changes from Sheet to Global Work Sheet.



【4】Record sample data

① Acquire the sample.

② Set record number from the “Event to Record”, if needed.

③ Click the “Record Data”.

④ Click the “Next Tube”, if you have next sampl

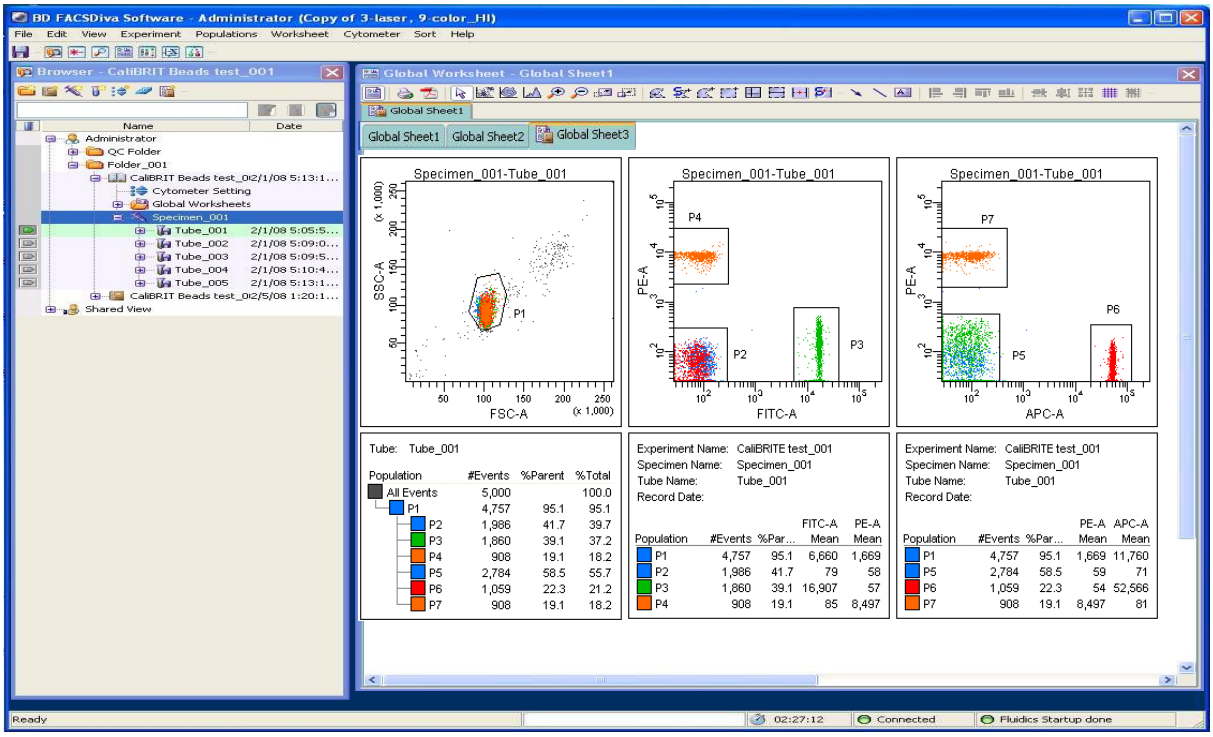
【5】Data analysis

① Right click the FSC,SSC plot, and click the “Show Population Hierarchy”.
Then, the gate hierarchy window is displayed on the sheet.

② Create the gate to target population.

③ If “Show Population” is used, only a target population can be displayed on a plot.

④ Right click the objective fluorescence plot> Create Statistics View.

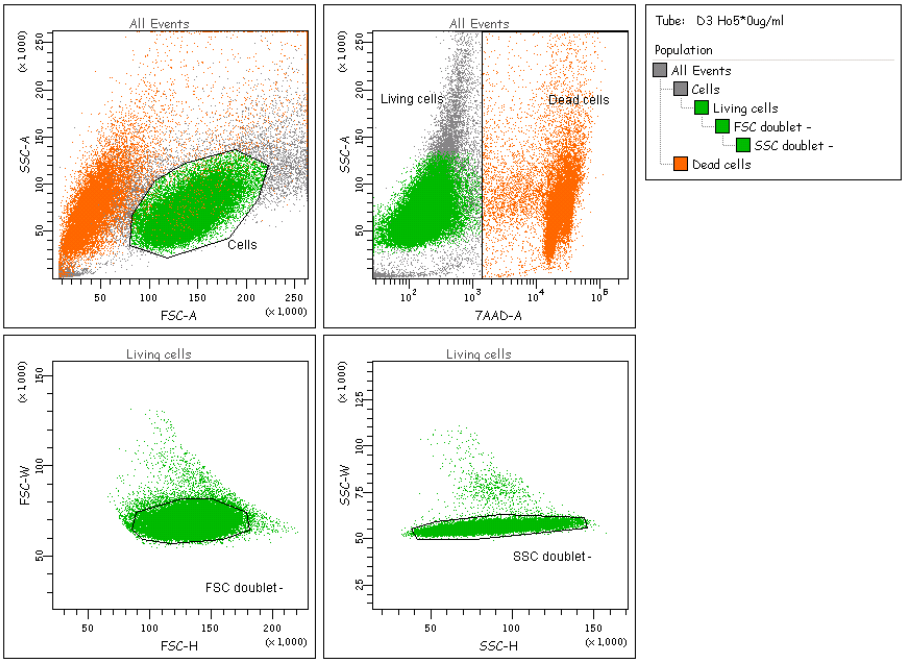


【6】Appendix

1. Removal of dead and doublet cells

Removal of dead and doublet cells are recommended for analysis or sorting of high purity.

- dead cells plot: SSC-A vs reagent ex) PI, 7AAD, DAPI
- doublet cells plots: FSC-W vs FSC-H and SSC-W vs SSC-H

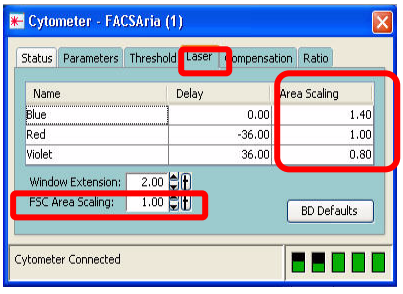


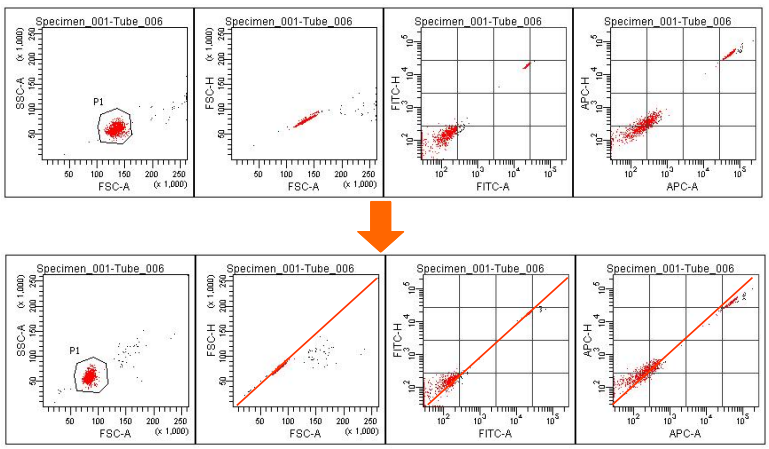
2.Threshold: Threshold tab > Default value: FSC 5000.

Threshold is cutting the debris or electric noise in the lower-left of the FSC-A vs SSC-A plot. If your sample has a lot of debris, you can remove it by increase threshold value in the threshold tab in Cytometer window.

3. Area Scaling Factor: Laser tab> Area Scaling

The required area scaling factor changes based on sheath pressure and particle size. The area scaling factors should be verified for each experiment performed on the cytometer. Correct Width data is obtained by arranging the dynamic range of Area and Hight data.





4. Data Export: right click experiment> export

Export FCS files:

FCS file is raw data. FCS 2.0 or FCS 3.0 can be analyzed in another software application.

Export Experiments:

This is all experiment data (cytometer settings, plots, gates, statistics, and FCS files).

5. Duplicate of experiment: right click experiment> duplicate without data

Experiment data (cytometer settings, plots, gates, statistics) can be copy without tube data.

* The data of Laser tab (Area scaling, Laser Delay) is not copied.

Section5：Shut down

① Install 2mL FACSClean tube in the sample injection tube.
② Flow Rate “High”, and click Acquire Data, wait for 5 minutes.
③ Remove FACSClean tube, and install 2mL DI water tube.
④ Click Acquire Data, wait for 5 minutes.
⑤ Remove DI water tube.
⑥ Click Fluidics Shutdown from Cytometer menu > OK.
⑦ After Fluidics Shutdown, Click Quit from File menu.
⑧ Turn off the main power and shut down the computer.
⑨ Install 2 mL DI water to the sample injection tube.
⑩ Empty the waste tank and refill the sheath tank, if needed.