

BD FACSCANTO II

QUICK GUIDE: START UP, QUALITY CONTROL, CLEANING AND SHUT DOWN

PROCEDURE

1.0 Start Up

- 1.1. Check that the sheath cube is full, the waste tank is empty, and the FACS Flow Cart is on. Add 250 ml of bleach to the empty waste tank. If the sheath cube is low, remove the probe, replace the cube, replace the probe, and press "Restart" on the FACS Flow Cart.



- 1.2. Turn on the computer. Log in to Windows as the Operator. The password is "BDIS". Double-click the DIVA icon to open the instrument acquisition software. Log in to the software.



- 1.3. Turn on the instrument system power by pressing the green button on the left side of the instrument.

Green Power Button



1.4. Ensure that the plate coupler is attached to the SIP and the sliding doors are closed. On the main tool bar select Cytometer > Fluidics Start Up and follow the prompts from the software.



Plate coupler: SIP

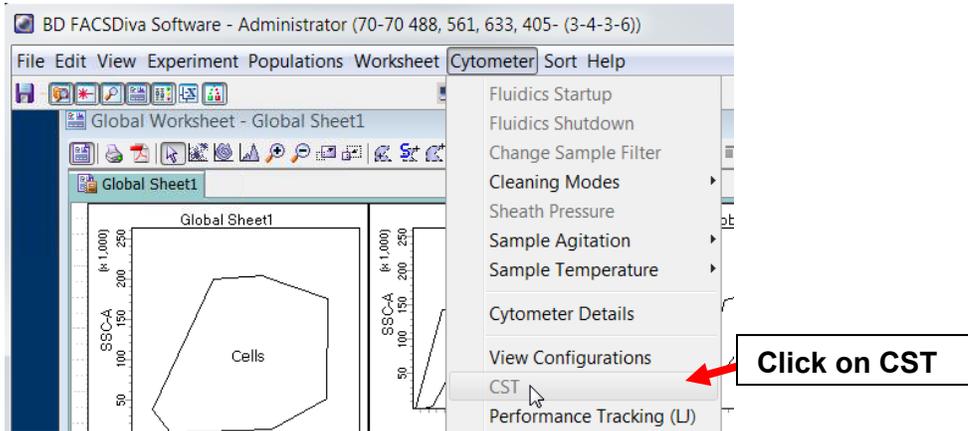
2.0 QualityControl

- NOTE: QC is run every weekday morning by Core staff, you need to run the QC only if Core Staff have not run it for the day. If you run the QC and if CST fails please contact Core staff via email or a note on the white board.**

2.1. Open the CST experiment template from the Flow Lab folder. Disconnect the plate coupler and place the CST beads on the SIP. Click "Acquire Data" on the Acquisition Dashboard and verify that events are accumulating on the plot.

The screenshot shows the BD FACSDiva software interface. The main window displays a 'Global Worksheet' with five plots arranged in a grid. The plots are titled 'Global Sheet1' and show various parameters on the x and y axes, including BSSC-A vs PSC-A, BV/BB-A vs DAPI-A, PerCp-Cy5-S-A vs FITC-A, PE-Cy7-A vs PE-A, and APC-Cy7-A vs APC-A. The Inspector window on the right shows the experiment name 'CST_803', owner 'Administrator', and modified date '4/7/20 2:14:05 PM'. The Browser window on the right shows a list of folders and files, including 'CST_803' which is highlighted.

- 2.2. Continue acquiring data for long enough to verify that two scatter populations and three fluorescent populations are discernable. Click “Stop Acquisition” and remove the CST beads.
- 2.3. On the main toolbar select Cytometer > CST and wait until the cytometer connects to the CST software.



- 2.4. Ensure that the correct CST bead lot number is selected then click “Run”. Review the Performance Tracking Report, and make a note of any problems or errors on the whiteboard.

Cytometer Performance Report

Cytometer: FACSariaIII Cytometer Name: FACSariaIII Serial Number: P658919000001 Input Device: Manual Cytometer Configuration: 70-70 488, 561, 633, 405- (3-4-3-6)	User: Administrator Institution: N/A Software: BD FACSDiva 8.0 Date: 02/21/2020 09:06 AM Cytometer Baseline: 02/11/2020 11:18 AM P/F: Pass
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Setup Beads

Bead Product: C Part #: 910858
 ST Expiration Date: 11/30/2021
 Lot ID: 9
 30
 Bead Lot Information: A
 val

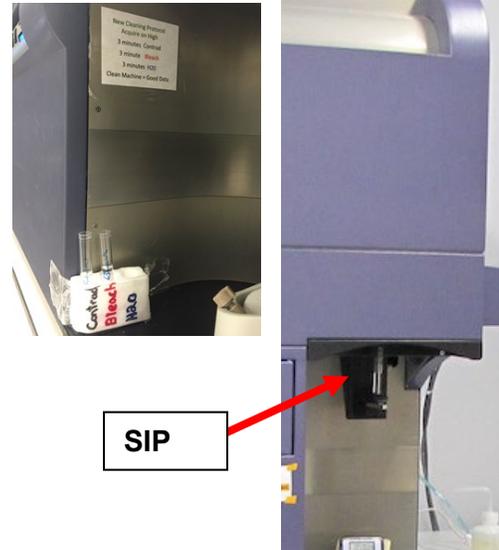
Detector Settings

Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead % Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
Blue	FSC	FSC	125000	124585	-1	3.38	124378	3.44
Blue	C	SSC	125000	125463	0	6.25	125987	6.46
Blue	B	FITC	5910	6173	4	3.86	128	13.37
Blue	A	PerCP-Cy5-5	14073	13832	-2	4.91	407	14.9
Red	C	APC	14043	13646	-3	3.3	462	7.44
Red	B	Alexa Fluor 700	8973	8637	-4	4.33	317	7.56
Red	A	APC-Cy7	8970	8605	-5	4.66	300	7.64
Violet	F	DAPI	2586	2572	-1	4.46	187	10.33
Violet	E	AmCyan	11594	11553	-1	4.05	276	8.21
Violet	D	BV605	18066	18210	0	5.19	407	23.97
Violet	C	BV650	19044	18915	-1	4.38	975	12.69
Violet	B	BV711	9781	9467	-4	5.36	409	12.22
Violet	A	BV786	14435	13928	-4	6.92	332	16.06
561 Yellow-Green	D	PE	13241	12836	-4	4.89	828	7.66
561 Yellow-Green	C	PE-Texas Red	10951	10481	-5	5.24	231	13.9
561 Yellow-Green	B	PE-Cy5	8512	8343	-2	6.06	271	16.77
561 Yellow-Green	A	PE-Cy7	7816	7408	-6	6.6	236	11.32

- 2.5. Remove the CST beads from the SIP.
- 2.6. Exit the CST software and allow the instrument to reconnect to DIVA.

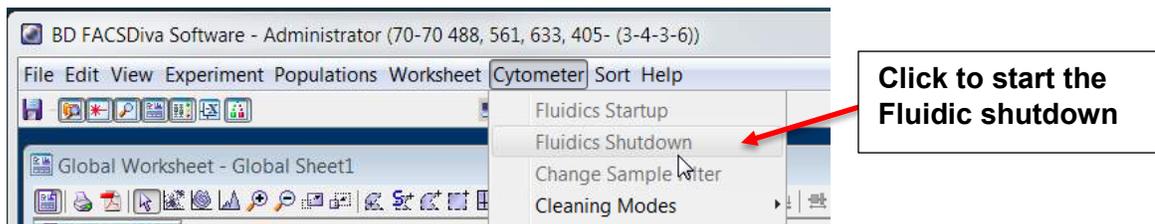
3.0 Cleaning

- 3.1 Place a tube with approximately 3 ml of Contrad on the SIP and leave the sample arm open. Press RUN and HIGH and leave it for one minute. Close the sample arm and continue running for one minute.
- 3.2 Repeat this procedure using a tube of 10% bleach.
- 3.3 Repeat this procedure using a tube of diH2O.
- 3.4 Leave the diH2O on the SIP and place the instrument in STANDBY.



4.0 Shut Down

- 4.1 Ensure that the plate coupler is attached to the SIP and the sliding doors are closed. On the main tool bar select Cytometer > Fluidics Shutdown and follow the prompts from the software.



- 4.2 Once shutdown is complete turn on fluidic cart power switch located on side of cart and close air valve on wall.
- 4.3 Turn off the instrument system power by pressing the green button on the left side of the instrument.
- 4.4 Exit DIVA and shut down the computer.

RELATED PROCEDURES

This handout is related to ACSF SOP IN003 Please see the full SOP for further information.

Handout: IN003-01

Version 1.1

2020-04-06