Flow Cytometer FACSCalibur and FACSVantage flow sorter
Standard Operating Procedures

FACS Facility
Building 977 – 106

January 10, 2011

Please Note: These procedures will be updated as needed; with the current version always posted in the FACS facility, on the instrumentation. Users need to be ensuring that they always use the current, posted version.

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1.0 Research Description

See the appropriate section of users BUA

1.1 Work Overview

Cell flow instruments and the technical support and expertise will be available to the LBNL and external research community. These instruments include a Becton-Dickinson (BD) FACSVantage-SE high speed cell sorter, and a BD FACSCalibur bench top flow cytometer. The FACSVantage-SE will sort or isolate subpopulations of cells from a variety of sources including cells with specific reporters, stem cells, or primary tissues (blood or other human or mouse tissues or tumors).

All researchers wishing to have samples processed on these instruments will transport their FACS-ready samples to the FACS facility in 977-106 following EH&S guidelines for containment and safety. The samples will not be stored or incubated on site, but immediately processed on the cytometer/sorter by a well-trained manager, Michelle Scott (977-118 ext4281).

To maintain a record for EH&S, we require that each user fill out a form delineating what biological material is being analyzed and to disclose any potential biohazards. This request form (Appendix A) has been developed in collaboration with EH&S as a record of material exposure, and will be kept on file. Requests that entail unknown biohazards are referred to EH&S for review. No radioactive material is allowed in the facility. Once FACS users have gone through extensive, certified FACS training, they may be able to process their own samples.

1.2 Flow cytometer and Sorter (FACS) Analysis Summary

The Becton-Dickinson (BD) FACSVantage SE flow cytometer sorter can sort/isolate or measure subpopulations of cells from a variety of sources including cells with specific reporters, stem cells, or primary tissues (blood or other human or mouse tissues or tumors). Only RG 1 and 2 materials will be processed.

The FACS systems are located in a BSL2 lab in 977-106. Users must ensure proper protective clothing is worn at all times. While in 977-106, closed toe shoes and safety glasses are required at all times. When working with the sorters, or handling or sorting all biological specimens: closed toe shoes, safety glasses, lab coat, and appropriate gloves are required at all times.

All researchers wishing to use the FACS facility must ensure that they have taken all required EH&S training and that the FACS Standard Operating Procedure document, method of sample transport to the FACs facility, and trained users have been documented in their PIs BUA, before scheduling an experiment on the Oracle calendar. When scheduling, it is required that users give details of the samples that will be sorted under the “details” tab, listing any potential hazards.

All researchers wishing to have samples processed on these instruments will transport their FACS-ready samples to the FACS facility in 977-106 following EH&S guidelines for containment and safety as outlined in their respective PIs BUAs. The samples will not be stored or incubated

on site, but immediately processed on the cytometer/sorter. Researchers, who have not yet been trained, will schedule processing with the FACS manager, Michelle Scott. Researchers, who have passed extensive FACS training, will be certified by the FACS manager in the Certified User Log. They will then have their instrument login activated so that they can process their own samples.

Users are required to fully document and sign-in in the Certified User Log before each experiment. If this requirement is not met, users will lose FACS privileges. A computer log will automatically be generated with each FACS run and checked against the Certified User Log.

To maintain a record for EH&S, before each run each user is required to sign in to the user log and disclose any potential biohazards. This log has been developed in collaboration with EH&S as a record of material exposure, and will be kept on file. If the potential biohazards of a sample are not well understood, the user is to confer with EH&S prior to sample processing. No radioactive material is allowed in the facility.

Only trained and authorized users as documented in the Certified User Log-Book are allowed to operate the FACS systems.

The manager and any trained and authorized users will at all times follow the FACS Standard Operating Procedures (SOP) for 1) system operation and 2) running a sample for each of the instruments in the facility. The SOPs are maintained and available in the FACS facility with the current master copies posted next to each instrument. Information in the SOPs may change and all users are expected to familiarize themselves with any changes.

1.3 How to Use the BSL2 FACS Facility

(1) The appropriate red "warning sections" on protective measures are at the beginning of each SOP. Users are required to follow these and all BSL2 safety procedures when they are in the FACS facility.

(2) A calendaring step has been added to the summary which requires calendaring in ORACLE. In the "details" section of the entry, the user describes the sample in enough detail that potential biohazards are described.

(3) The Certified User Log has been divided into an excel file with 6 tabs. The first 3 tabs (1 per instrument) is the running list where the user signs in for a run (columns were added for the user to describe the sample and potential biohazards, and to put their signature). The last 3 tabs (1 per instrument) is completed by Sarah and is the list of Authorized/trained users (columns were added for the user and trainer to put their signatures).

(4) Prior to Training:
- (a) Each user checks that they are have completed all EH&S training courses to handle RG2 level samples (BSL2 safety procedures are always followed in the facility).
- (b) The user puts a copy of these Standard Operating Procedures in their PIs BUA adding a statement of how they will safely transport their samples from the PIs laboratory to the FACS facility.
- (c) If they are trained, they add their name as a "FACS VANTAGE (or FACS Calibur) user" on their PIs BUA (next time BUA is renewed - until then it is listed in the Certified Log in 106).

(5) When a user wants to run:
(a) They calendar their run (describing the potential hazards in "details")
(b) If they will need an Operator for their run, they notify Sarah of their run by email (at least a week in advance).
(c) The user brings his samples and all needed materials to the facility (gloves, tubes, pipettes etc); the user signs in fully on the Log sheet.
(d) The user verifies that the instrument was left in the correct shutdown state; they may want to run a cleaning cycle before they start, just to be sure it is ready.
(e) The instrument is operated, shut down and cleaned, and the samples are processed according to the current, authorized SOP (copy on instrument).
(f) The user signs out with the time of completion on the Log.
(g) The user discards all biohazardous waste in the appropriate receptacle (and logs the contents on the sheet affixed to the can), discards all other waste and thoroughly cleans up the bench area (using a solution of 10% household bleach and then 70% ethanol).
(h) Wash hands at the sink before gathering up items and leaving the facility.
(i) Please let Sarah know if any problems were encountered.

Remember for users to be allowed access to the facility, it is critical that they practice stringent BSL2 procedures, follow the SOPs, and thoroughly clean up after themselves.

2.0 Responsibilities

- Michelle Scott is the FACS facility manager and is responsible for the operation and maintenance of the FACS systems, while Damir Sudar provides oversight.

- FACS users will be responsible for keeping all required EH&S training updated and using only the authorized, current FACS SOPs posted in the FACS facility. They are also responsible for adding the following to their PIs BUA: (1) the FACS Flow Cytometer Sorter (FACS) Calibur and Scanner Standard Operating Procedures document, (2) documentation of sample preparation and transport to the FACS facility, (3) FACS users names and training level.

3.0 Determination of Hazards and Controls

3.1 Hazards

The main hazards associated with use of this equipment involves: biological materials, lasers and pressure.

Biological Materials:

The projects carried out in the FACS facility (977-106) involve the use of both Risk Group 1 (RG1) and RG2 biological agents-materials; and will all be carried out using standard Biosafety Level 2 (BL2) work practice controls. Personnel handling human tissues, human blood, and human cells will assume that bloodborne pathogens could be present and will handle these materials as if they are potentially infectious using “universal precautions.” Personnel handling primary human tissues, cells and viruses will be very limited and are noted in the body of their PIs BUA. The transfer of any biological

materials to the FACS facility will occur using BSL2 procedures, including sealed, leak-proof, secondary containment, gloves, eye protection and lab coat. Clean gloves will be used to open the doors between the labs.

Lasers: The instruments contain embedded lasers to which the class 1 laser protocol applies as approved by LBNL's Laser Safety Officer (LSO). Under the class 1 embedded laser protocol, only approved and licensed vendor technicians are allowed open beam alignment and repair activities. LBNL personnel will NOT perform these activities at any time but will provide oversight when vendor service personnel performs these activities. The FACS facility manager will be present at such time.

Pressure: The FACS Vantage utilizes a 100 um nozzle to generate pressures up to 20-21 psig. Injury may occur from the rupture of high pressure lines. Additional, materials being sorted can easily be aerosolized and become a inhalation hazard.

3.2 Controls

As mentioned previously, aspects of this project are conducted in a manual fashion in the laboratories listed, and are therefore authorized by the individual PI's BUAs.

3.2.1 Administrative Controls

Training: Training of all personnel using the FACS will be documented and covered under the BUA issued to their PI. Users are responsible for ensuring that their EH&S training and FACS training is complete and documented in their PIs BUA. Certified training provided by FACS staff on the FACS instrument of choice will be required before login rights are activated and independent access is allowed.

Work Practices:

- Standard BL2 practices will be used. All human or murine cell lines will be handled under BL2 containment.
- Hand washing will be required.
- Use of personal protective equipment (gloves, lab coats and safety glasses) is required.
- Surface disinfection (10% solution of household bleach), will be performed before and after each run.
- Agents being removed from these instruments will be either:

  (1) Handled as biohazardous waste (biohazard waste container and log in 977-109),

  (2) Decontaminated by flushing/spraying with a 10% solution of household bleach or

  (3) Contained in closed, leak-proof containers for storage, centrifugation, or other subsequent processing.
- The FACs instrumentation/tubing is disinfected by flushing with a 10% solution of household bleach. Liquid biological waste is mixed with

household bleach to a 10% concentration and held for at least 15 min prior to drain disposal.

- All of the solid biological waste (both RG 1 & 2) is handled as biohazardous waste and placed into red biohazard bags, which are then taken off site and treated by LBNL's licensed medical waste contractor. Bleach and quaternary ammonium compounds are effective surface disinfectants against HIV-1 and all cell lines used in this project.
- After work is completed, all work surfaces are sprayed with a 10% solution of household bleach and then 70% ethanol.

3.2.2 Engineer Controls:

Biosafety Cabinets:

BL2 containment within a biosafety cabinet or the ACCS unit in 977-116 will be used for all work involving the growth, treatment, transfection and harvesting of human or murine cell lines.

FACS High Efficiency Particulate Air (HEPA) Filtered Exhaust:

The FACS utilizes a stand-alone HEPA filtering exhaust unit to capture any aerosols generated by the FACS machine. To ensure the unit is properly operating, the settings on the unit must be checked prior to sampling. This unit is also included in the Preventative Maintenance (PM) program and must be HEPA tested and certified for use before it can be placed back into service.

3.2.3 Personal Protective Equipment:

All personnel working in 977-106 will don the following personal protective equipment:

- Closed toe shoes and safety glasses are required in 977-106. When working with the sorters, or handling or sorting all biological specimens: closed toe shoes, safety glasses, lab coat, and appropriate gloves are required at all times.

- Double gloves will be worn at all times when handling unfixed primary cells, lentiviral preparations, transfected cells, or the combined transfection reagent.

4.0 FACSCalibur Operating Procedures

4.1 System Check Protocol

Only trained and authorized users, as documented in the Certified User Log, are allowed to operate the FACS Calibur .

Researchers are responsible for ensuring that all EH&S training and FACS BUA paperwork in their PIs laboratory is in order, before scheduling an experiment.

Users are required to calendar and sign in before each experiment.
If these requirements are not met, users will lose FACS privileges.

The FACS systems are located in a BSL2 lab. Users must ensure proper protective clothing (e.g., closed toe shoes lab coat, disposable gloves, and safety glasses) is worn at all times.

Users are responsible for transporting their samples to the FACS following EH&S guidelines for containment and safety. They must document this method, their FACS training and FACS SOPs in their PIs BUA.
Secondary containment in a sealed container (that won't leak if dropped) is critical.

All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

FACSCalibur SOP
Turn on/off and system check protocol

TURN ON:

- FACS Flow unit.
- Main power switch on cytometer.
- Macintosh workstation computer.

To Log-on to the computer:
Mac: Username: <your login>
Password: <your password>
The FACS CellQuest Pro software can be launched at this point.

START UP DECONTAMINATION

All work surfaces should be decontamined with an appropriate disinfectant prior to initiating any work and after the machines have been place in operation.

The Stream:

- Take the water tube off of the tube holder.
- Push button STAND-BY.
- Push button PRIME (wait till returns to STANDBY), repeat.

QC:

- Prepare tubes of CaliBrite beads according to kit insert.
- Push button RUN and LOW.
- Guide the CaliBrite beads tube onto the SIP (Sample Insertion Port) until a firm seal is made. Swing tube support arm under tube.
- Follow FACScomp instructions on monitor.
- Use the BD FACS Comp software to calibrate and QC the system.

CellQuest Pro SOFTWARE:

- If necessary, re-launch the software.

FACSCalibur SHUT-DOWN:

- Remove sample tube.
- Install a tube of 10% bleach.
- Set the system to RUN, set flow to HIGH, run for 3 minutes.
- Put the system to STAND-BY and remove the bleach.
- Install a tube of dH_2O.
- Set the system to RUN, set flow to HIGH, run for 3 minutes.
- Put the system in STAND-BY (leave tube of H_2O on SIP)
- Turn of Computer Power.
- Turn off the main power switch.
- Turn off the FACS Flow unit.

Shut Down Decontamination:

All work surfaces must be decontaminated with appropriate disinfectant at the end of each day's run.

Mac:

- Quit the CellQuest Pro software.
- Shut down the computer.

4.2 Running a Sample

Only trained and authorized users as documented in the Certified User Log are allowed to operate the FACSCalibur

Researchers are responsible for ensuring that all EH&S training and FACS BUA paperwork in their PIs laboratory is in order, before scheduling an experiment.

Users are required to calendar and sign in before each experiment.
If these requirements are not met, users will lose FACS privileges.

The FACS systems are located in a BSL2 lab. Users must ensure proper protective clothing (e.g., closed toe shoes lab coat, disposable gloves, and safety glasses) is worn at all times.

Users are responsible for transporting their samples to the FACS following EH&S guidelines for containment and safety. They must document this method, their FACS training and FACS SOPs in their PIs BUA.
Secondary containment in a sealed container (that won't leak if dropped) is critical.

All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

FACSCalibur SOP
Running a Sample

All samples to be run on the BD FACSCalibur must be in a single cell suspension. Run cells through a 40um filter prior to arriving at the sorter to achieve this if they are clumping together.
Only 5ml polystyrene round-bottom Falcon tubes can be inserted on the up-take port. (Cat # 352054).

- Uncap sample tube and place cap on an alcohol pad.
- Make sure the system is set to Stand-By.
- Carefully slide sample tube onto the SIP (Sample Insertion Port) and lift the tube up over the pressure seal.
- Move the bottom arm beneath the tube. If necessary, adjust the height of the base by turning it.
- Set the system to Run set flow to LOW or MED or HIGH.
- Once events are seen on the plots in the worksheet, you can make adjustments to voltages, compensation and threshold values.
- When all settings are as you want them, click 'Record'.
- When recording has finished, remove tube and set to STANDBY.
- Gates can be set on the populations of interest in the software.
- At the end of the run, turn the dial to Stand-By, cap & remove all collection tubes.
- Proceed with the shut-down procedure.

5.0 FACVantage Operating Procedures

5.1 System Check Protocol

Only trained and authorized users, as documented in the Certified User Log, are allowed to operate the FACS Vantage.

Researchers are responsible for ensuring that all EH&S training and FACS BUA paperwork in their PIs laboratory is in order, before scheduling an experiment.

Users are required to calendar and sign in before each experiment.
If these requirements are not met, users will lose FACS privileges.

The FACS systems are located in a BSL2 lab. Users must ensure proper protective clothing (e.g., closed toe shoes lab coat, disposable gloves, and safety glasses) is worn at all times.

Users are responsible for transporting their samples to the FACS following EH&S guidelines for containment and safety. They must document this method, their FACS training and FACS SOPs in their PIs BUA.
Secondary containment in a sealed container (that won't leak if dropped) is critical.

All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

FACSVANTAGE SOP

Turn on/off and system check protocol

Starting Up the Instrument
Follow these instructions to start up the instrument for operation in digital mode.

1. Open the vacuum source and air supply.
2. Verify that the sheath container is full and the waste container is empty. If needed, empty the waste.
3. Turn on the cytometer main power switch.(Lasers turn on automatically)
4. Switch on the digital control switch, if necessary.
5. The switch must be On to operate in digital mode.
6. Turn on the computer main power switch and start up the BD FACSDiva workstation.
7. After logging on to Windows, launch BD FACSDiva software by doubleclicking the shortcut on the desktop. Verify that the instrument is connected by checking the Instrument window in the software. The message Instrument Connected appears after the cytometer connects to the workstation (this can take several minutes).

To Log-on to the computer:
PC: Ctrl / Alt / Delete
Username: Administrator
Password: BDIS
Log on to: CC-S171-FACS VANTAGE (This computer)
The FACS DIVA software can be launched at this point.
Username: Administrator
Password: (no password needed)

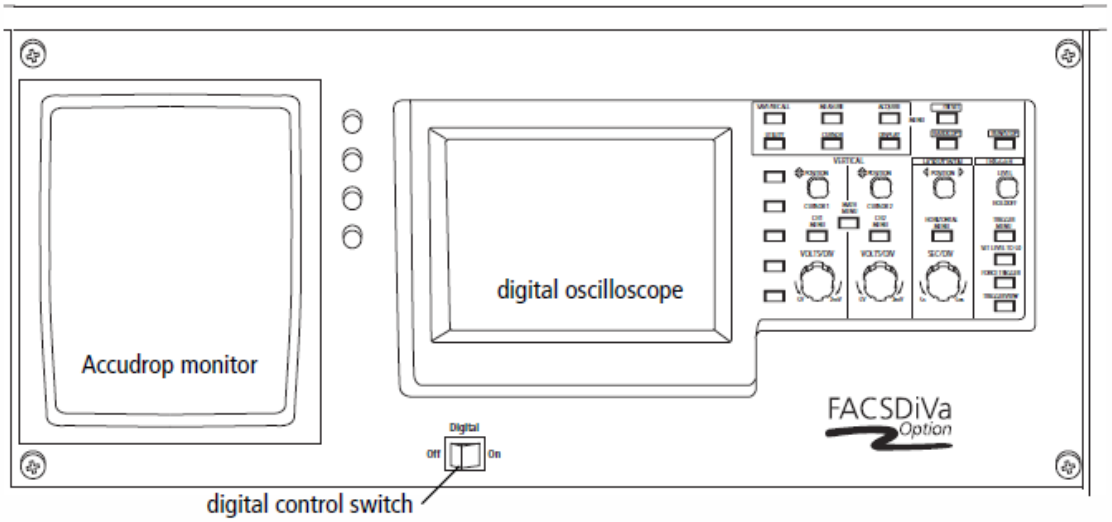
8. If the Instrument Disconnected message remains, refer to the troubleshooting suggestions in the software manual.

- All work surfaces should be decontamined with an appropriate disinfectant prior to initiating any work and after the machines have been place in operation.
- (OPTIONAL) Aerosol Containment Unit with the power button on the front. The main power switch at the back on the bottom of the unit should always be on.
- **The magnahelic gauge needle should be around 2. If not, the system power level should be changed to bring the indicator to the right level. Verify that the magnahelic gauge indicates between the two black marks.**

Check that the lights on the front of the unit indicate that the filter is still good and that it does not need to be replaced.

The Digital switch should be **ON**.
Digital Electronics Module
The digital electronics, oscilloscope, BD FACS™ Accudrop monitor, and digital

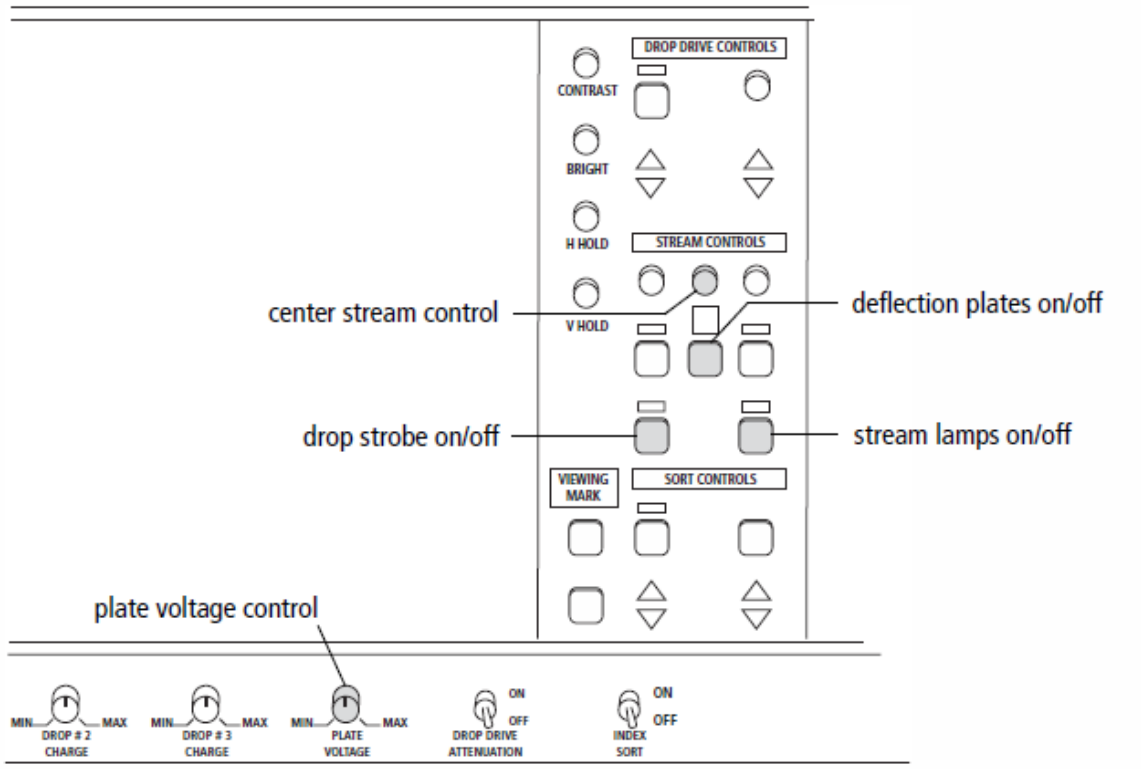
control switch are housed within the digital electronics module (Figure 1-2). The electronics are adjusted by your field service engineer during installation and do not require user maintenance.



Digital Operation
During operation in digital mode, analog settings for gain, threshold, compensation, and the event rate are displayed on the analog oscilloscope. Because threshold and gain are controlled differently depending on the electronic mode, these settings can differ from those displayed by the digital electronics. For an accurate event count, monitor the Acquisition Dashboard in BD FACSDiva software, rather than the analog oscilloscope.

NOTICE In digital mode, most controls on the instrument control panel are inactive (see Figure 1-4). Equivalent controls can now be found in BD FACSDiva software.

Figure 1-4 Instrument controls (shaded) active in digital mode



The STREAM:

- Fill Sheath Tank with PBS, empty Waste Tank.
- Disconnect pressure line and feed line from ETOH tank and connect them to Sheath Tank.
- Turn on the pressure switch. (Behind the tube holder)
- With the 100 um nozzle, the sheath pressure should be ~10-11 PSI.
- The Sample Differential should be ~ 1.2-1.5 PSI for running samples.
- Turn the dial to FILL then STAND-BY.
- Allow stream to run for at least 15 minutes to flush ETOH out of system.
- Set the dial to RUN. It will drip from the up-take tubing.

To flush the system: **(Only if necessary)**

- Place a cup or plastic holder beneath the syringe. (Next to tube holder)
- Turn the dial to STAND-BY
- Hold the syringe plunger in.
- Rotate the stopcock 180° from pointing to the instrument to pointing to yourself.
- Sheath will pour out from beneath the stopcock. This flushes the system of cleaning solutions such as ethanol or bleach and also bubbles.
- Do this for ~30 seconds. The return the stopcock to original position. (pointing towards instrument.)
- To flush the nozzle, put the dial to FILL, press the NOZZLE FLUSH button.
- For more forceful flushing of the nozzle, put the dial to STAND-BY.

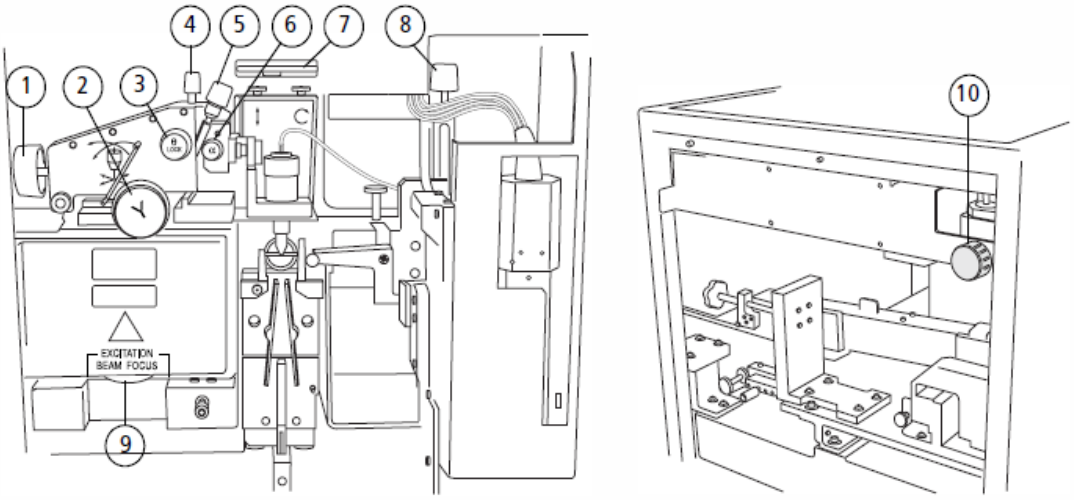
- Rotate the stopcock 90° to point down.
- The syringe will fill. Keep thumb on the plunger. When full, turn the dial to FILL.
- Push the plunger in.
- Repeat several times, by turning the dial to STAND-BY to fill the syringe and then to FILL< and pushing on the plunger.
- With the plunger in the pushed in position, rotate the stopcock back to face the instrument.

ALIGNMENT: (Usually only when necessary)

- Turn dial to STAND-BY.
- Guide a tube of rainbow beads onto the SIP (Sample Injection Port), keeping a drop of sheath at the end of the tubing so that no air comes between the tubing and the bead solution.
- Dial to RUN.
- To speed the beads through, temporarily increase Differential Pressure to 2.0.
- Adjust the Sample Differential to get the beads going through at desired rate of 1000 Threshold Events/Second.

Optimizing Signals from the Primary Laser

The following controls will be used to optimize signals from the primary laser beam.



1	X control—moves nozzle right and left
2	Y control—moves nozzle away from and toward you
3	Theta lock—prevents theta control from moving
4	Z control—moves nozzle up and down in an arc
5	Theta control—moves nozzle along an arc so stream moves right and left
6	Alpha control—moves nozzle along an arc so stream moves away from and toward you
7	Fluorescence channel height adjustment wheel—raises and lowers fluorescence objective lens
8	FSC obscuration bar vertical adjustment—moves FSC obscuration bar up and down
9	Excitation Beam Focus wheel—moves beam focus lens to adjust laser beam focal point on the sample stream
10	Fluorescence Focus control knob—moves objective lens to adjust focal point of the fluorescence image (access through the upper side door)

- For 488 laser alignment, you are moving the sort block. Use the X dial, the large dial in the front near the Y dial, the large dial inside the side door, at the top near the left, and the Fluorescence Channel wheel.
- Laser alignment only requires access to the externally accessible knobs on the turning towers. **No open beam alignment is allowed by LBNL personnel.** If proper alignment cannot be achieved using the alignment knobs, call Michelle Scott ext. 4281 .

Setting Up the Experiment

The steps in this section show you how to set up an experiment for instrument optimization. If you have already created a similar experiment, you can reuse it by saving it as an experiment template. Refer to the *BD FACSDiva Software Reference Manual* for information.

1 Choose Instrument > Instrument Configuration and verify the current configuration.

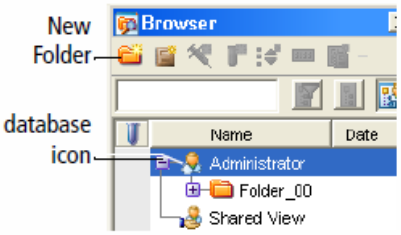
Make sure the configuration lists the parameters to be measured and that the channels correspond to the optical bench configuration.

⚠ For accurate data results, the instrument optics must match the current instrument configuration.

2 Click the corresponding buttons in the Workspace toolbar to display the Browser ( ), Instrument ( ), Inspector ( ), Worksheet ( ), and Acquisition Dashboard ( ) windows, as needed.

3 (Optional) Create a folder for instrument QC.

In the Browser, select the icon for your database and click the New Folder button in the Browser toolbar.



Rename the folder with your name. Alternatively, name the folder *Instrument QC* or create an Instrument QC folder inside another folder. Refer to the *BD FACSDiva Software Reference Manual* for ideas on how to organize experiments.

☑ **Tip** To place an experiment inside a folder, select the folder before you create the experiment.

4 Click the corresponding button(s) to create an experiment, specimen, and tube; rename the experiment with an appropriate name.

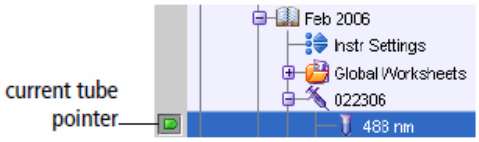
For example, use the current month and year, *Instrument Opt*, or the operator's initials followed by an appropriate identifier.

5 Rename the new specimen with today's date; rename the first tube *488 nm*.

This tube will be used to optimize signals from the first laser.

6 Click to set the current tube pointer next to the *488 nm* tube.

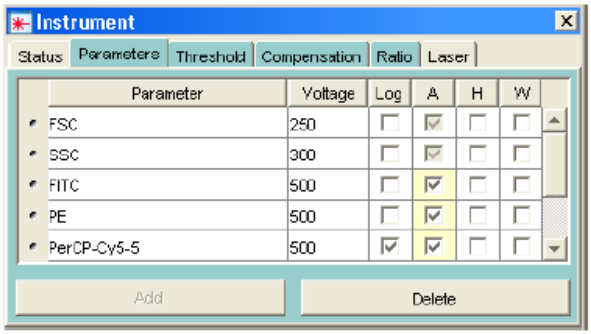
Your experiment should look similar to the following:



7 Edit the experiment-level instrument settings.

☑ **Tip** Save space in the database by collecting data for only those parameters that will be used in the experiment.

- In the Instrument window, select the Parameters tab and delete any unnecessary parameters.
- Deselect the Log checkboxes for FITC and PE. (When aligning with beads, you might also need to deselect the checkbox for PerCP-Cy5.5.)



8 In the Acquisition Dashboard, set the Events to Record to 10,000 events and the Events to Display to 500 events.

☑ **Tip** Decreasing the number of displayed events will increase the data refresh rate.

9 Create the following plots on the global worksheet:

- FSC vs SSC and FSC vs FITC dot plots
- FSC, FITC, PE, and PerCP-Cy5.5 histograms

☑ **Tip** To easily create multiple plots, double-click a plot button to keep the button selected. Click multiple times within the worksheet to create the required number of plots. When you are finished, click any other button to undo the selection.

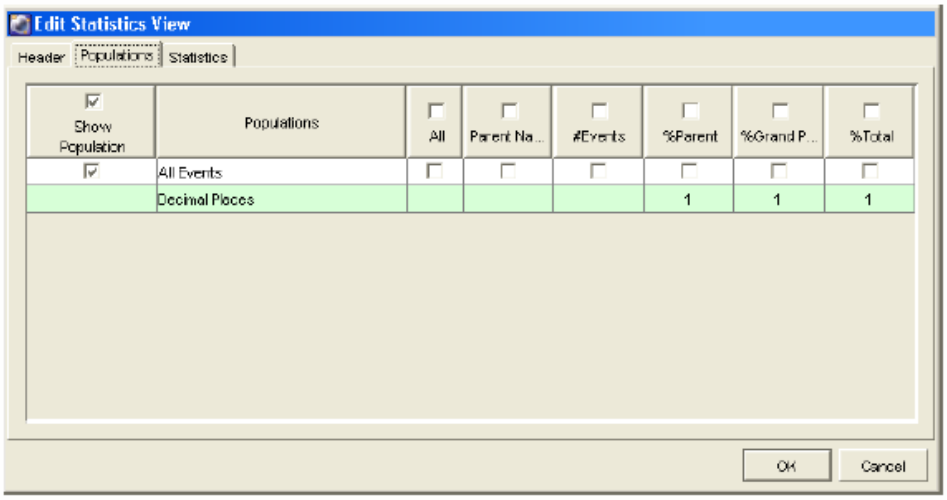
10 Resize the plots so that they fill 2/3 of the worksheet.

☑ **Tip** To resize multiple plots simultaneously, resize one plot and then press Ctrl-A to select all other plots. Click the Resize button to make all plots the same size as the first plot.

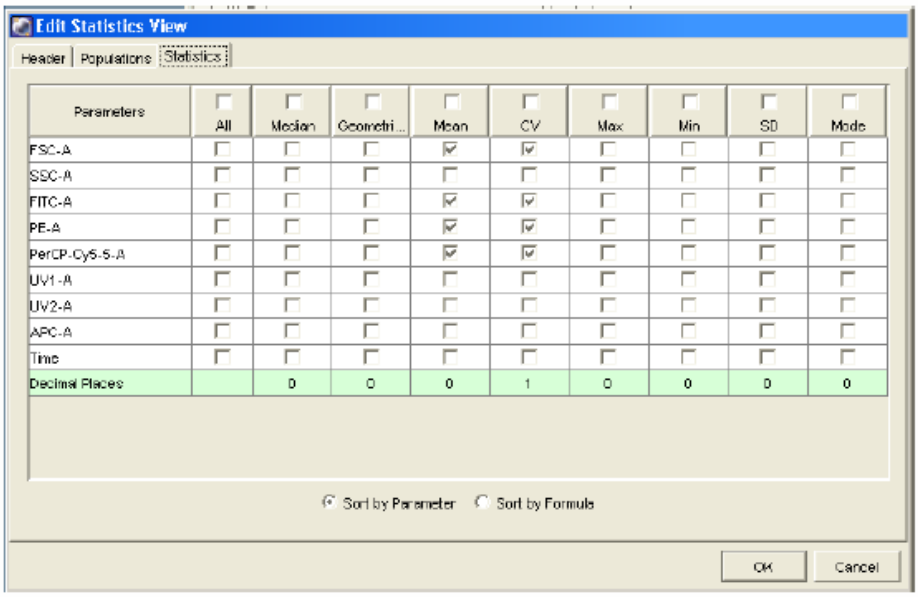
11 Right-click any plot on the worksheet and choose Create Statistics View.

12 Edit the statistics view as follows.

- Right-click the statistics view and choose Edit Statistics View.
- Select the Populations tab and deselect the checkboxes for #Events and %Parent.



- Set up the Statistics tab to display the mean and CV for FSC and each fluorescence channel detected by the 488-nm laser.

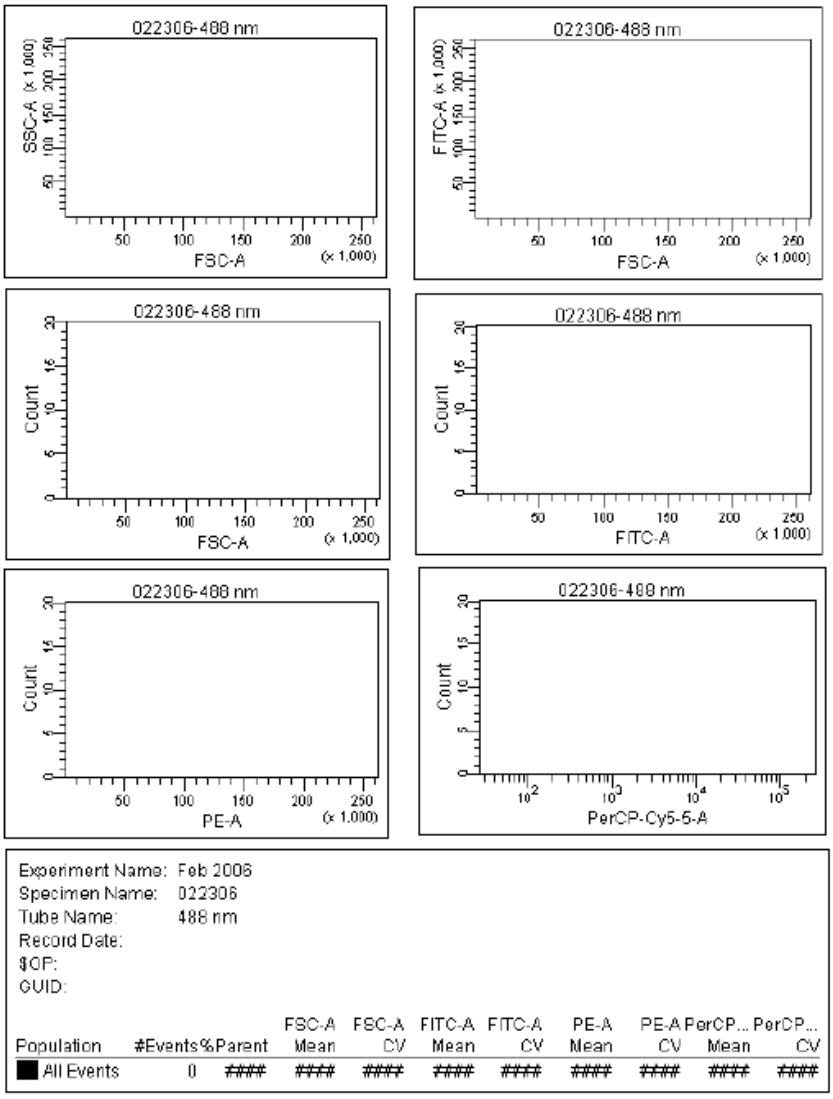


- Click OK.

13 Resize the statistics view to fill the remaining 1/3 of the worksheet.

Your worksheet should look similar to the example shown in Figure 2-1.

Figure 2-1 Instrument QC worksheet



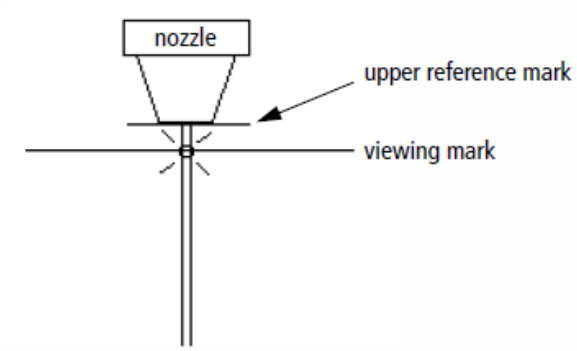
14 Select the 488 nm tube in the Browser; in the Inspector, choose *Global Sheet1* from the Global Sheet menu.

The specified worksheet will appear when you move the current tube pointer to the 488 nm tube.

1 Set or check the Z distance.

- Turn on the drop strobe.
- Use the camera vertical adjustment wheel to position the viewing mark at the laser intercept (Figure 2-2).
- If the laser intercept is not visible, adjust the Y control.
- Adjust the Z control to position the nozzle at the upper reference mark.

Figure 2-2 Setting the Z distance



2 Check the trajectory of the fluid stream.

The stream should be entering the center or front third of the stream aspirator. If necessary, adjust the Alpha and Theta controls to correctly position the fluid stream.

3 Install the alignment sample onto the cytometer; turn the Fluidics Control knob to Run.

4 Set the sample differential pressure to its standard level for this alignment procedure.

5 Verify that the current tube pointer is in front of the 488 nm tube in the Browser; click once on the pointer to begin acquisition.

Alternatively, click the Acquire Data button in the Acquisition Dashboard; events appear in the plots.

6 Maximize the FSC signal.

Adjust the Excitation Beam Focus wheel and Y control to obtain the highest FSC signal intensity. If the FSC signal is out of standard range, check the position of the FSC obscuration bar.

7 Maximize the FITC signal.

Adjust the X control, the Fluorescence Focus control knob and the Fluorescence channel height adjustment wheel to obtain the highest FITC signal intensity.

☑ **Tip** If you don't see any signal, increase the PMT voltage or turn on Log before adjusting the stream controls.

8 Close the FL1 iris incrementally as you continue optimizing the FITC signal.

Continue adjusting the controls and closing the iris until the iris is completely closed.

9 With the iris closed, adjust the Y control and Excitation Beam Focus wheel for maximum FITC signal.

10 Compare the FITC signal intensity with the iris open and closed.

You should not lose more than half the maximum FITC signal intensity with the iris completely closed.

11 Open the FL1 iris and adjust the beam splitters for maximum fluorescence intensity.

On the appropriate plots, maximize the signal for SSC, PerCP-Cy5.5, and PE.

12 Adjust the obscuration bars for minimum FSC and SSC noise, if necessary.

13 Verify the trajectory of the fluid stream.

The stream should remain in the center or front third of the stream aspirator. If necessary, adjust the Alpha and Theta controls to correctly position the fluid stream. After adjusting the controls, repeat steps 6 through 12.

Verifying Area Scaling for the Primary Laser

BD FACSDiva software uses area as its default parameter. The area measurement provides a complete measurement of the voltage pulse, but it can be affected by how well the laser is focused and by the sheath pressure.

To ensure that the PMT works within its linear dynamic range, it is important to adjust the height and area measurements to the same magnitude. For accurate linearity, verify area scaling each time you optimize laser signal. Refer to the software user's guide for more information about area scaling.

1 With the current tube pointer next to the 488 nm tube in the Browser, click the Parameters tab in the Instrument window.

2 Select the height (H) checkbox for the FITC parameter.

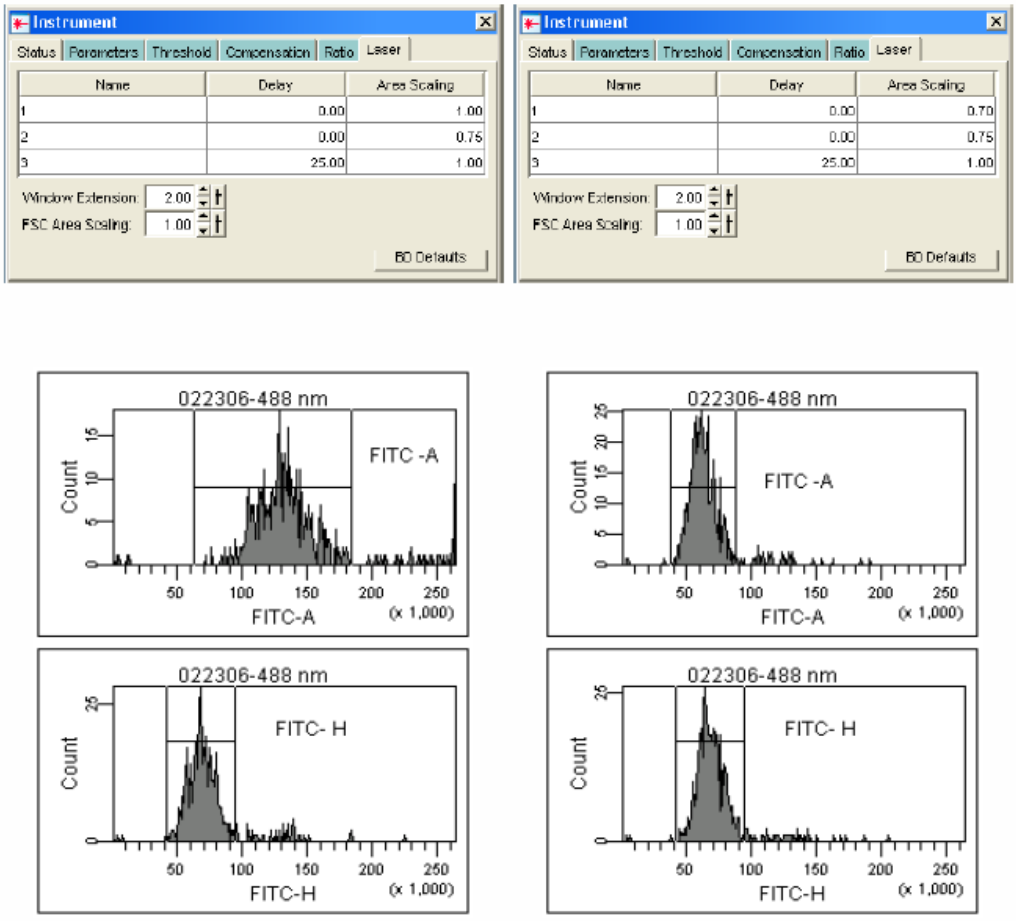
3 Change the axis on the PerCP-Cy5.5 histogram to FITC-H.

4 Click the Laser tab in the Instrument window.

5 Adjust area scaling for the first laser until the FITC-A intensity is similar to the FITC-H intensity.

See Figure 2-3 on page 36 for an example.

Figure 2-3 Primary laser area scaling before (left) and after (right) adjustment



6 Change the FITC-H histogram plot to PerCP-Cy5.5-A.

7 Deselect the checkbox for the FITC-H parameter.

- **(Only done by Michelle Scott)** Align the second and third lasers, one at a time by adjusting the specific alignment knobs for each laser. Be sure to change the oscilloscope parameters to be reflecting the correct detection channel. Again, adjust alignment until the events are showing as high & tight as possible

Laser Delay: Set by Michelle Scott

DIVA SOFTWARE:

- If necessary, re-launch the DIVA software.
- Make sure that Digital switch is ON.
- Change oscilloscope to dot plot.

Note: When the DIVA software is not connected, the stream window appears dark. When it is connected, you can see the illuminated stream window.

- Turn dial to RUN for the rainbow beads.
- Have the oscilloscope set to FSC v SSC.

- The events will show near the bottom of the screen until you click on 'ACQUIRE'. Then they will pop up to where you can see them.
- Note that on the computer screen in the DIVA software, the signal is in a 'normal position, however on the oscilloscope FSC and/or the SSC will be very high.
- Decrease SSC gain from 400 to 100. Decrease FSC gain from 800 to 100 or 200.
- In the BreakOff /Streams window, under the BreakOff tab, turn on the Drop Drive.
- BreakOff frequency is set at 22.3 Khz.
- Amplitude at about 3.0 volts. This may need to be slightly increased through the day.

When the Drop Drive is turned on, noise may appear in the oscilloscope window. If it looks like FSC noise, adjust the obscuration bar. If it looks like SSC noise, adjust the two silver dials directly above the optics block.

Michelle has marked the Droplet Screen to indicate the Last Connected Drop (LCD), Gap, and Drop #1. Drop #1 should be very close to Viewing Mark = 150. Adjust the Breakoff Amplitude and Breakoff Phase to place LCD and Drop#1 in the proper place

- Adjust the amplitude to optimize the last connected droplet and minimize the satellite droplets.
- Put the charging plates into position, angled out slightly at the bottom.
- Make sure the stream is going into the center of the waste collection trough.
- Turn the Plate Voltage to ON on the instrument panel.

Do **NOT** touch the charged plates when the Voltage is on

- Double check the stream is still going into the center of the waste collection trough. If not, adjust with the dial.
- Turn on the side streams in the BreakOff/Side stream window, using the Test Sort button.
- The side streams can be dragged in or out using numerical values under the Streams Tab.
- Check that side & center streams all look tight. It may be easier to see if the stream laser is dimmed slightly.
- Adjust 2nd/3rd & 4th drops if necessary to tighten the center stream.

ACCUDROP:

- Remove rainbow beads from the sample port.
- Set dial to RUN with no tube in place to rinse the up-take tubing.
- Open the ACCUDROP experiment in the Browser window.
- Load a tube of Accudrop beads.
- RUN.
- Turn off all external lights and get the room as dark as possible.
- Run the beads at about 4000 events/sec.
- Click Acquire on the software.
- In the sort window, set the precision to Initial.

- Put Accudrop filter in place by flipping the small silver bar on the side of the sort door.
- Adjust the Drop Delay to optimize the stream on the left.
- Set Sort Preciscion to Fine Tune
- Adjust drop delay again, focusing on the center stream. Try to get the center (waste) stream as dim as possible.

VANTAGE SHUT-DOWN:

- Remove sample tube.
- Turn off the plate voltage.
- Turn off the Drop Drive.
- With Fluidics switch in Standby, hold Nozzle Flush button and rotate Fluidics from Standby to Off.
- Dissconnect Seath Tank pressure line and feed line, Connect pressure and feed line to ETOH tank.
- Turn Fluidics switch to Standby, then to Run (no tube on sample port).
- Allow ETOH to run through system for 5 minutes.
- Put tube of ETOH on Sample Port
- Turn Fluidics switch to Off.
- Turn off the Drop Strobe and the Stream Lamps.
- Turn off the aerosol containment unit
- Decontaminate all work surfaces with an appropriate disinfectant.

PC:

- Quit the DIVA software.
- Shut down the computer.

MAIN Switches (Inside Door)
Main switch OFF
Computer switch OFF

5.2 Running a Sample

VANTAGE SOP
Running a Sample

Only trained and authorized users, as documented in the Certified User Log, are allowed to operate the FACS Vantage.

Researchers are responsible for ensuring that all EH&S training and FACS BUA paperwork in their PIs laboratory is in order, before scheduling an experiment.

Users are required to calendar and sign in before each experiment.
If these requirements are not met, users will lose FACS privileges.

The FACS systems are located in a BSL2 lab. Users must ensure proper protective clothing (e.g., closed toe shoes lab coat, disposable gloves, and safety glasses) is worn at all times.

Users are responsible for transporting their samples to the FACS following EH&S guidelines for containment and safety. They must document this method, their FACS training and FACS SOPs in their PIs BUA.
Secondary containment in a sealed container (that won't leak if dropped) is critical.

All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

FACSVantage SOP
Running a Sample

All samples to be run on the BD FACS Vantage must be in a single cell suspension. Run cells through a 40um filter prior to arriving at the sorter to achieve this if they are clumping together.
Only 5ml polystyrene round-bottom Falcon tubes can be inserted on the up-take port. (Cat # 352054).

- Verify that the Whisper unit is turned on, that the filter life indicates 40% or more, and that the gauge is set to a level between the 2 black marks around the value 2. If any of these conditions cannot be achieved, stop and contact the facility manager. DO NOT CONTINUE!!!
- Uncap sample tube and place cap on an alcohol pad.
- Make sure the Vantage dial is set to Stand-By.
- Carefully slip the up-take tubing into the sample tube and lift the tube up towards the pressure seal.
- Move the bottom arm beneath the tube. If necessary, adjust the height of the base by turning it.

Close the doors and the flap of the sort chamber.

If the Vantage is being used for analysis only, (No sorting) there is no need to turn on the drop drive, voltage or to open the sort chamber doors.

- Set the dial to Run.
- Increase Sample Differential Pressure to 2psi to get the sample moving more quickly through the machine, then reduce Sample Differential Pressure to 1.5psi.
- Once events are seen on the plots in the worksheet, you can make adjustments to voltages, compensation and threshold values.
- When all settings are as you want them, click 'Record'.
- Set the dial on the Vantage to 'Standby' after the file has finished recording.
- Gates can be set on the populations of interest in the software.
- Choose 'Sort Layout' from the Browser window, or open a previously used 'Sort Layout'.
- Select the type of sort. (ie. 2-Tube/4-Tube/Standard slide etc).
- Turn on the plate voltage by pressing the Voltage button on the instrument control panel.

Do **NOT** touch the plates when the voltage button is pressed.

- Open the sort chamber door and place practice tubes or slide etc. in position.
- Turn on 'Test Sort'.
- In the software, click on the 'Streams' tab. Drag out the streams you wish to use by applying appropriate voltage to them.

Note: If only using 1 or 2 streams, use the 'left' & 'right' streams if using 3 or 4 streams, include the 'outer left' & 'outer right' streams. If using the plate-sorting option, use the 'outer left' stream only.

- Apply the appropriate charge values to each stream in order to get the streams to aim for the center of their respective collection vessels. To see this, stand up and look down over the tops of the collection tubes to see the streams, (this will appear as a thin white line, like a strand of hair).
- When the streams are correctly positioned, turn off the Test Sort.
- Remove the practice tubes or slide etc.
- Replace with actual collection vessels, containing the appropriate collection media, if applicable.

Close the sort chamber doors and the flap before running the sample.

- In the software, go to the Sort Layout window, for each stream, select a population to be sorted in that direction.
- Turn the dial on the sorter to 'Run'.
- Click 'Sort' on the sort layout window when you are ready to begin sorting.
- If collection tubes need to be changed during the sort, turn the dial back to 'Stand-By'. Open the chamber doors, replace tubes, close the chamber doors again. Then turn the dial back to 'Run' and resume sorting by clicking 'Sort' in the Sort Layout window.

Before retrieving any collection tubes from the sort chamber wait a period of 15 seconds to allow the aerosol containment unit to evacuate the space.

- At the end of the run, turn the dial to Stand-By, cap & remove all collection tubes.
- Proceed with the shut-down procedure.

6.0 FACSVantage Preventive Maintenance Procedures

Precautions

Only BD trained and certified service personnel are allowed to service the FACS Vantage. LBNL personnel are not allowed to service the instrument.

The FACS facility manager will make sure the BD service personnel works in a safe manner consistent with LBNL EH&S practices.

The FACS systems are located in a BSL2 lab. Users must ensure proper protective clothing (e.g., closed toe shoes lab coat, disposable gloves, and safety glasses) is worn at all times.

This equipment has been both surface and system decontaminated prior to releasing the equipment for PM maintenance, and after. The use of PPE during the PM service is required

All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

FACS Vantage Preventive Maintenance Procedure

Specific procedures for LBNL marked in RED.
Steps not applicable at LBNL have been crossed out and marked in RED.

Objective

To provide a procedure and checklist for preventive maintenance (PM) service call to be performed every six months. As part of the PM procedures, a number of safety checks will be performed to ensure the system meets the specifications for biological and laser safety as required by LBNL.

Purpose

The purpose of this program is to enhance the performance and improve the reliability of the FACS Vantage instrument base by performing routine checks, cleaning, and some parts replacements Estimate time to complete: 4 hr's.

Procedure

Prior to, and directly after, the PM, the Field Service Engineer (FSE) is directed to perform resolution and sensitivity performance checks with 2.49 μm Nile Red Beads, sterile Chicken Red Blood Cells (cRBCs), and CaliBRITE Beads, (use of Blue Beads and UV beads on an as required basis). The intent of these checks is to document the instrument's performance level prior to the PM, and any change to the performance brought about by the PM.

Pre PM

Prior to the site visit, contact the customer and discuss the instrument's operation and performance. Determine if the instrument is in need of minor repair, if so, order the appropriate parts. If the instrument is in need of major repair (your judgment), advise the customer of the need for a repair visit prior to the PM. A PM will increase reliability and may enhance performance, it is not intended to be a substitute for a repair visit. Order the FACS Vantage PM kit (p/n 343640).

Check the instrument's Field Change Notice (FCN) and Service Bulletin (SB) status. Order any parts required to bring the instrument up to current revision levels.

Upon arrival to site, align and operate the instrument and document (hardcopy) the performance level.

Sensitivity Check:

Maximum separation of bimodal cRBC peak in the FSC detector.

Dispose of remaining sample and any materials that have come in contact with sample into the LBNL-approved biohazard waste stream.

PPE such as closed toe shoes, lab coats, impervious disposable gloves, and safety glass will be worn when working with the FACS machines/samples. All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

Resolution Check:

Minimum %CVs with 2.49 pm Nile Red beads in FSC, SSC, FLI, FL2, and FL3 detectors, Blue and UV beads in at least one detector if required equipment present.

Fluidic/Pneumatic System

Reservoir Levels

With the system pressurized, take note of the sheath and waste levels indicated on the video monitor. Depressurize the system.

Check fluid levels in sheath and waste reservoirs, compare levels with the level indicated on the video screen. Calibrate if necessary.

Remove sheath filter and prefilter, discard.

343541 FILTER MILLIPORE (old Style)

343542 SALINE FILTER CA 2503 (new style)

PPE such as closed toe shoes, lab coats, impervious disposable gloves, and safety glass will be worn when working with the FACS machines/samples. All FACS waste will be considered biohazardous waste and must be properly placed in the correct waste stream.

Replace air filter

342832 FILTER ELEMENT-FOR REPLACEMENT ON 342831
342831 FILTER AIR 150PSI 1/8-NPT W/BOWL-GARD (listed for reference)

Monthly Cleaning

Caution: Perform this procedure only upon customer approval as bleach residues could cause customer applications problems.

Perform 10% Household Bleach / 90% Distilled Water (DI) (freshly made) system flush.

Empty waste reservoir and fill sheath reservoir approximately 1/4 full with DI water. Rinse the sheath reservoir thoroughly with DI water and remove all residual sheath solution. Empty fluid from sheath reservoir.

Fill the sheath reservoir approximately 1/4 full with bleach / DI solution, pressurize the system.

Manipulate the fluidics control knob between FILL, OFF, and STANDBY until air is purged from the fluidic system. After the air is removed, set the control knob to each setting for sixty seconds. Repeat this cycle four times. Once cycled, set the fluidics control knob to the RUN position and allow the instrument to operate in this condition for 1/2 hour. This will allow the bleach to neutralize any bacteria present in the system.

Pour small quantity (.25 L) of bleach / DI solution into the sink assembly to clean the vacuum drain.

Perform DI Water System Flush

Empty the sheath of bleach / DI solution. Fill the sheath reservoir 1/4 full with DI water. Rinse the inside of the sheath reservoir by shaking it thoroughly to remove all residual bleach / DI solution and empty. Fill the sheath reservoir approximately 1/4 full with DI water.

Manipulate the fluidic system control knob as in step (2.5.3) above, repeat this cycle twenty times to ensure all bleach / DI solution is purged from the fluidics system. Empty the sheath reservoir and fill it approximately 2/3 full with sheath fluid. Replace the sheath filter.

Inspect all pneumatic lines feeding into the instrument for leaks. Secure with ty-wraps as needed. Replace / redress any discolored / kinked tubing.

84-10003-00 MOUNT 2-WAY CABLE-TIE ADHESIVE BACKED

84-10008-00 TIE WRAP 5.6" long

84-10019-00 TY WRAP 14 5/8" long x 0.14" width

343690 TUBING SILASTIC-LAB.125"ID X.25" OD

Open the Fluidics Control Panel and inspect all fluidic pneumatic lines for leaks, deformation, or discoloration. Pay particular attention to the valve switching area. Replace / redress as required. (FV/FSP only)

343505 TUBING SILICONE 0.06 IN ID X 0.030 IN

343664 TUBING SILASTIC-LAB.062"ID X.125" OD

Check sheath, sample, and bias pressure regulators for proper output.

Sheath Regulator / +.15 to 30 psi* (60 psi for FVSE)
*Limited by the amount of pressure from the source, usually 22 psi. (80 psi for FVSE)

Sample Regulator / -14 to +18 psi**
** This regulator is capable of regulating vacuum, not applicable here.

Bias Regulator / 20 psi (Fixed)

Check cam actuated leaf springs for proper pinch function. (FV/FSP only)

Remove nozzle holder from the head drive, remove the nozzle tip and replace the nozzle holder o-ring. Reinstall nozzle tip onto nozzle holder and set aside.

343619 O-RING NOZZLE HOLDER

Remove and replace the three o-rings located between the sample tube (valve) clamp and the flow manifold. Apply a small amount of vacuum grease to each o-ring before replacement.

343625 O-RING SAMPLE AGITATOR (FV/FSP x2), (FVSE x3)

343623 O-RING – FACSTAR (FV/FSP only)

347306 LUBRICANTO-RING SUPER-O-LUBE 1/2OZ TUBE

Replace the sample line and sample filter at this time, if required. Leave these assemblies behind if not used. Inspect the sample head area for air leaks using DI water placed on top of the knurled nut when running the instrument in run with a sample of DI water in a tube. Any air bubbles forming at the top of the nut should be addressed by tightening the nut slightly until the bubbles no longer form.

343501 LINE ASSEMBLY SAMPLE

343647 FILTER SAMPLE SPARE

Replace the following spare parts as required

343626 O-RING SAMPLE HEAD SM

343618 O-RING FOR QUICK DISCONNECT

343543 FILTER SINK FACS VANTAGE

Clean the outside of the sheath and waste tanks and inspect the inside of tanks for corrosion. Replace tank O-rings

Optical System

Remove and clean all fluorescence optical elements, reinstall.

Note: Inspect band pass and dichroic filters for fingerprints and clean if dirty using methanol and lens paper. These are coated lens so use as little pressure as possible to avoid damaging the coating

Collection Filters

Beam Splitters

Field Lens

Inspect primary (1, 2), secondary (5, 6) and, if applicable, tertiary (7, 8) input prisms. Use dust chaser to remove any particulate. If prisms appear contaminated, clean with reagent grade methanol.

Inspect beam steering prisms (3, 4), use dust chaser to remove any particulate. If further cleaning is necessary, leave prisms mounted and utilize an orange stick with lens paper wrap to clean with reagent grade methanol.

Inspect beam focusing lens. Use dust chaser to remove any particulate. Clean all elements if necessary.

Clean fluorescence objective face with cotton swab and DI water.

Clean Stream Viewing Mirror.

Remove and clean the FSC collection lens. Replace the two sets screws securing the collection lens and front iris.

74-30101-01 SCR 2-56X1/16 SOC ST CP C (x2)

Clean the two exposed optical surfaces mounted on the inside of the camera-viewing door (FV only)

Clean the Accudrop camera optic if installed (FVSE only).

Mechanical

For the majority of mechanical checks listed below, the FSE is directed to attempt repair by making adjustments or mechanical repair. Assembly drawings of these mechanical subsystems are attached in order to assist in any on-site repair.

Slides – Rack and Pinion Type

Check for smooth positive action, no rough spots, with minimum backlash.

Fluorescence objective focus slide

Beam steering slides and rotational controls (qty 2)

Beam focus slide

Fluorescence Obscuration Holder Assembly

Check fluorescence obscuration adjustments for smooth and full range movement of both vertical and rotation controls. Vacuum grease and included hardware should be used to repair any problems detected.

Forward Scatter Channel

Check FSC obscuration adjustments for smooth and full range movement of both vertical and rotational controls. Use replacement hardware to repair any problems detected.

Check for proper retraction of FSC front collection barrel.

Check front and rear iris function.

Head (Stream) Adjust

Check X, Y, and Z controls for smooth movement and adequate slide clearance.

Check pitch controls for proper operation. (FV/FSP only)

Wipe down the entire deflection illumination area, to include the deflection plates, with DI water.

Ventilation

The stand-alone HEPA unit must be check to ensure it is operating properly as specified by the manufacturer. Hoses will be checked for leaks or damage and will be replaced as necessary. When necessary, the HEPA filter will be replaced and disposed of as biohazardous waste. The HEPA will then be leak tested and certified before the FACS unit is placed back in service.

Laser(s)

If open beam alignment is likely necessary (i.e. there are indications that any of the lasers are significantly out of alignment, LBNL personnel will alert the Lab's Laser Safety Officer (LSO) beforehand and arrange for a Temporary Work Authorization (TWA) for the duration of the open beam work. All controls and procedures in the TWA will be followed without exception.

Check and record output of all lasers at full power and primary operating wavelength. Check for proper mode.

Primary diode laser: 488nm, 200mW

Red diode laser: 644nm, 40mW

Violet diode laser: 405nm, 100mW

Note: All Coherent output couplers are of a wedged shape design. This will cause the beam path to shift if the optic is rotated in relation to its holder. After the coupler has been removed from the laser, and previous to removing the optic from its holder, place a mark on the side wall of the optic and a corresponding mark on the orientation, minimizing alignment changes.

Power down the laser(s).

Note: Performed only for customers having a Laser Contract

Realign and peak all lasers. Check and record output of all lasers at full power and primary operating wavelength. For lasers displaying improper mode, check again at this time for improvement.

Check and record on the FSR the tube current and aperture setting at the standard operating wavelength and power.

Electronics

Measure and record on the FSR the line voltage, should be 120 VAC ± 10%. Check the Main AC Power Cord for excessive wear or fraying. Replace the power cord if necessary (#04-10547-00).

04-10907-00 CABLE ASSY, POWER CORD (for 120 volts) (listed for reference)

04-10908-00 CABLE ASSY, POWER COR-INT (for 240 volts) (listed for reference)

Check +5V Digital, +15V and –15 Analog low voltage power supplies on the Log Amp PCB by mounting the board on an extender board and probing the edge connectors. Check the +15V Digital on the Deflection Illumination Board.

Log Amp PCB:

Analog Ground: Pin 42

Digital Ground: Pin 1

+5V Digital: Pin 93 (Acceptable +5.0 to 5.10)

+15V Analog: Pin 39 (Acceptable +15.0 to 15.3)

-15V Analog: Pin 37 (Acceptable -15.0 to -15.3)

Deflection Illumination PCB:

+5V Digital: U4, Pin 16 (Acceptable +5.0 to 5.10)

Card Cage

Check for operation and function of user adjustments.

P1 – P8 Log Offset – will shift a population approximately one log decade. (Card 14, 15)

Dead Time Adjust – lengthens intensified region on pulse display, normally CCW, minimum dead time length, (use CellQuest on the FVSE).

Dual Laser Delay Adjust – controls the time delay of second laser signals relative to the first, , (use CellQuest on the FVSE).

Test Pulse Rate – controls the frequency at which test signals are synchronously generated in the eight detector channels. (Card 8)

Test Pulse Amplitude (six Controls) – controls the amplitude of the eight detector test pulse generators.

Test Signal Noise – controls the level of noise superimposed on top of the two test populations. (Card 8).

Drop Sync Switch – synchronizes test signal generation with the drop dire generator. (Card 2 FVSE; Card 6 FV/FSP)

Main Control Panel

Check for proper operation of the following controls (For DiVa option use appropriate controls):

Left, right, and center stream controls

Phase control

Amplitude control

Deflection power

Droplet strobe switch

Drop drive frequency control

Stream illumination switch

Log Amp Check

Perform for each fluorescence parameter. Position a test signal at channel 80 in linear gain. Set gain to log and record channel number (x). Reset to a linear gain and position the test signal at channel 800. Set gain to log and record channel number (y). For field purpose of this procedure is to identify log amplifiers that have been tampered with and are therefore grossly out of calibration. This test is not intended to check calibration with the accuracy of that accomplished in manufacturing with test fixtures.

Computer

Clean CRT screen(s)

Clean printer(s) and verify proper operation utilizing self test diagnostics.

Verify read/write operation of hard disk drive(s).

Check for proper keyboard function.

Clean the ball on mechanical mice.

Overall Cleaning and check of biosafety provisions.

With a DI soaked towel or commercial cleaner, wipe down all exterior surfaces.

Verify that the Aerosol Management Option (AMO) is operating within specifications using the AMO Field Service Kit.

Check the filter integrity gage located on the Whisper vacuum pump. If integrity reads 40% or below, direct LBNL personnel to order a replacement filter kit, part no. 333030.

If a new filter is needed, LBNL personnel will order a new filter kit.

LBNL personnel will schedule the Lab's contractor (currently TSS) to verify the proper functioning of the filter kit.

LBNL personnel will install the new filter kit and dispose of the used kit in the biohazard waste.

Post PM

Power up the instrument, realign optical system.

Run and document performance checks as in Pre PM.

During sort check, run sort profile, determine drop delay offset, if any adjust offset on Sort Control PCB if needed, follow the general guidelines below.

Run 2 um Nile red beads and acquire histograms for primary laser. Record the results on the FSR

If present, run UV alignment beads for secondary or tertiary laser alignment of UV laser. Record the results on the FSR

If present, run Blue beads for secondary or tertiary laser alignment of red laser. Record the results on the FSR

Run CRBCs and acquire a histogram for FSC. Record the ratio of the two peaks on the FSR.

Using Calibrite beads if available or another bead mixture perform a sort at the pressure most often used by the customer. Attach a print out of the results with the FSR and record the purity on the FSR.

End Of Procedure

Appendix A: LBNL – Life Science Divison Flow Facility

FACS User Log

Date	Time	Users Name	Users Signature	PI	e-mail	Sample Description	Potential Hazards	EH&S Training Com-pleted	BUA Com-pleted	FACS Training Level

Appendix B: **FSE Tools/Supplies Required**

1) 342868 Chicken Red Blood Cells (cRBCs)
2) 347240 2.49 μm Nile Red Beads
3) 347241 6.0 μm Blue Beads
4) 344669 2.5 μm UV Beads
5) 340486 Three Color CaliBRITE Beads
6) 340487 APC CaliBRITE Beads
7) 349523 DNA QC Kit
8) 343675 Falcon 12 X 75 Tubes
9) Extender Board
10) Laser Power Meter
11) Air Pressure Gauge
12) Digital Volt Meter

Customer Supplies Required

1) DI Water (Double Glass Distilled)
2) Bleach (Clorox)
3) Vortex or Sonication Bath
4) Reagent Grade Methanol
5) Paper Towls
6) Rubber Gloves
7) Safety Glasses
8) Lab Coat
9) Floppy (and Optional Removable Media) Drive Head Cleaning Kit
10) CRT Cleaner
11) Commercial Cleaner

PM Kit FS/FSP & FV/FVSE (p/n 343640) PartsList

Material 343640 D 0003 Alt. Usage 1	
PM KIT FS/FSP & FV/FVSE	Valid 12/21/1999
Reqd qty 1.000 Base quant 1.000 EA	

Lv Item	Component no. / Description		Quant	Un	Ict	Asm Ex.
1 0010	343647	C	1.000	EA	L	
	FILTER SAMPLE SPARE					
1 0020	343501	A	1.000	EA	L	
	LINE ASSEMBLY SAMPLE					
1 0030	343541	A	10.000	EA	L	
	FILTER MILLIPORE					
1 0040	59-10027-00	B	2.000	EA	L	
	FITTING, "T" 1/16 ID					
1 0050	59-10056-00	A	4.000	EA	L	
	FITTING ELBOW (L10-1)					
1 0060	74-30101-01		2.000	EA	L	
	SCR 2-56X1/16 SOC ST CP C					
1 0070	343664	B	50.000	IN	L	
	TUBING SILASTIC-LAB .062"ID X .125" OD					
1 0080	343690	B	50.000	IN	L	
	TUBING SILASTIC-LAB .125"ID X .25" OD					
1 0090	343505	A	50.000	IN	L	
	TUBING SILICONE 0.06 IN ID X 0.030 IN					
1 0100	343619	A	1.000	EA	L	
	O-RING NOZZLE HOLDER					
1 0110	343710	A	2.000	EA	L	
	CONN,FEM LUER TO 10-32					
1 0120	343568	A	2.000	EA	L	
	FIT, 1/8 MALE					
1 0130	343623	A	3.000	EA	L	
	O-RING - FACSTAR					
1 0140	343625	A	2.000	EA	L	
	O-RING SAMPLE AGITATOR					
1 0150	88-20138-00	A	3.000	EA	L	
	WASHER, RUBBER SEAL W/FILTER					
1 0160	343626	A	1.000	EA	L	
	O-RING SAMPLE HEAD SM					
1 0170	343618	A	5.000	EA	L	
	O-RING FOR QUICK DISCONNECT					
1 0180	343680	A	1.000	EA	L	
	STOPCOCK 3 WAY					
1 0190	343542	A	1.000	EA	L	
	SALINE FILTER CA 2503					

1 0200 343538 A 2.000 EA L
FLTR AIR HYDROPHOBIC WHITE 1-1/4 IN DIA
1 0210 343543 A 1.000 EA L
FILTER SINK FACS VANTAGE
1 0220 343546 A 1.000 EA L
FILTER VAC SHIELD 3INDIA/REPL 2-1/4INDIA

|Material 343640 D 0003 Alt. Usage 1 |
|PM KIT FS/FSP & FV/FVSE Valid 12/21/1999|
|Reqd qty 1.000 Base quant 1.000 EA |

Lv	Item	Component no. / Description	Quant	Un	Ict	Asm / Ex.
1	0230	343531 COUPLING INS 1/4 IN NPT	1.000	EA	L	A
1	0240	347306 LUBRICANTO-RING SUPER-O-LUBE 1/2OZ TUBE	1.000	EA	L	A
1	0250	347308 TISSUE LENS CLEANER 4" X6"50-SHT BOOKLET	1.000	EA	L	A
1	0260	99-30122-00 APPLICATOR, COTTON TIPPED	1.000	EA	L	B
1	0270	59-10084-00 ELBOW BARB FITTING 10-32	1.000	EA	L	A
1	0280	84-10008-00 TIE WRAP	10.000	EA	L	A
1	0290	84-10019-00 TY WRAP	5.000	EA	L	A
1	0300	88-20149-00 O-RING 3.475 X .275	2.000	EA	L	A
1	0310	84-10003-00 MOUNT 2-WAY CABLE-TIE ADHESIVE BACKED	3.000	EA	L	B
1	0320	59-10057-00 PORT TUBE (NYLON) - FACSTAR	4.000	EA	L	01
1	0330	59-10200-00 1/4 FLOW INSERT 1/4" HOSE	1.000	EA	L	A
1	0340	59-10198-00 1/4 COUPLING 1/4 HOSE & PANEL	1.000	EA	L	A
1	0350	59-10194-00 1/4"COUPLING 1/4" HOSE	1.000	EA	L	A
1	0360	59-10199-00 1/4 INSERT 1/4 HOSE PANEL	1.000	EA	L	A
1	0370	342832 FILTER ELEMENT-FOR REPLACEMENT ON 342831	2.000	EA	L	A

FACS Vantage

Preventive Maintenance Checklist

FLUIDIC/OPTICAL

( ) Check Sheath/Waste Lvl Detectos / Calibrate
( ) Replace Air Filter
( ) DI/Bleach Flush
( ) DI Flush/Chg Sheath Filter
( ) Check Tubing/Fittings
( ) Check Regulators
( ) Check Leaf Springs
( ) Replace Nozzle O-ring
( ) Replace 3 Sample Manifold O-rings
( ) Replace Sample Line/Filter
( ) Clean Fluor. Optics
( ) Inspect/Clean Input Prisms
( ) Inspect/Clean Steer. Prisms
( ) Inspect Beam Focus Lens
( ) Clean Fluor. Objective
( ) Clean Stream View Mirror
( ) Clean FSC Collec. Lens
( ) Clean Camera Optics

MECHANICAL/LASERS

( ) Check Fluor. Focus Slide
( ) Check Beam Steer. Slides
( ) Check Beam Steer. Rotate
( ) Check Beam Focus Slide
( ) Check Fluor. Obscuration
( ) Check FSC Obscuration
( ) Check FSC Iris (qty 2)
( ) Check Stream Adjust Assy.
( ) Clean Defl. & Illum. Area
( ) Check Laser(s) Power
( ) Check Laser Optics
( ) Check Tube(s) Voltage
( ) Check Laser(s) Power

ELECTRONIC/COMPUTER

( ) Check Line Voltage
( ) Measure DC Power Supplies
( ) Check PCB Card Controls
( ) Check PMT Power Supplies
( ) Check Main Control Panel
( ) Log Amp Check
( ) Clean CRT & Floppy Drive
( ) Clean/Check Printer
( ) Check Read/Write Floppy Drive
( ) Check Read/Write Hard Drive
( ) Check Keyboard

PERFORMANCE CHECK

( ) Sensitivity/Resolution
( ) Sorting
( ) Hard Copy Attached

History of Changes

Changes from original version to documented version p/n 33xxxx Rev. A

1. Provide sequential numbering to document.
2. Procedure: Remove 2.0µm beads, replace with 2.49µm Nile Red Beads.
3. Procedure: Added, “use Blue Beads and UV Beads as required”.
4. Pre PM: Changed PM Kit part number from p/n 12-00307-00 to p/n 343640.
5. Pre PM Resolution Check was:
   Minimum %CV with 2 micron green beads in FSC and FL2 detectors. Changed to:
   Minimum %CV with 2.49µm Nile Red beads in FSC, SSC, FL1, FL2 and FL3 detectors, Blue Beads and UV in at least one detector if required equipment present.
6. Fluidic/Pneumatic System, step 3: Add part number for new sheath filter (p/n 343542) and old sheath filter element (p/n 343541).
7. Fluidic/Pneumatic System, step 4: Remove obsolete air filter (p/n 41-10001-00) replace with Filter Element (p/n 342832) for use on the FVSE.
8. Perform DI water system flush B; was: as in step 6C above, should be: as in step 5C.

9. Fluidic/Pneumatic System, step 7: Ty Wrap 8 1/8” long x 0.14” width (p/n 84-10001-00) was removed from the PM kit, use Ty Wrap 5.6” long (p/n 84-10008-00) or Ty Wrap 14 5/8” long x 0.14” width (p/n 84-10019-00).
10. Fluidic/Pneumatic System, step 7: Tubing Silicone .125IDX.250 OD (p/n 80-10011-00) is obsolete, replaced with Tubing Silastic-Lab .125"ID X .25" OD (p/n 343690).
11. Fluidic/Pneumatic System, step 8: Tubing 1/16X1/32 (p/n 80-30005-00) is obsolete, replaced with Tubing Silicone 0.06 IN ID X 0.030 IN (p/n 343505).
12. Fluidic/Pneumatic System, step 8: Tubing Silicone.062IDX.125 OD (p/n 80-10005-00) was replaced in the kit by Tubing Silastic-Lab .062"ID X .125" OD (p/n 343664).
13. Fluidic/Pneumatic System, step 9: Updated to reflect FVSE pressure requirements.
14. Fluidic/Pneumatic System, step 11: O-Ring .146IDx.032W 70BN is obsolete, replaced with O-Ring Nozzle Holder (p/n 343619).
15. Fluidic/Pneumatic System, step 12: O-Ring Buna-N Parker 2-006 –FS (p/n 88-20058-00) is obsolete replaced with O-Ring Sample Agitator (p/n 343625).
16. Fluidic/Pneumatic System, step 12: O-Ring - FACStar (p/n 88-20056-00) changed part number, replaced with O-Ring - FACStar (p/n 343623).
17. Fluidic/Pneumatic System, step 13: Sample Line Assy, .010 (p/n 02-61358-00) changed part number, replaced with Line Assembly Sample (p/n 343501).
18. Fluidic/Pneumatic System, step 13: Assy, Sample Filter S/D/L ANL SENS (p/n 02-30726-00) changed part number, replaced with Filter Sample Spare (p/n 343647).
19. Mechanical, step 2, Fluorescence Obscuration Holder Assembly: Compression Spring (S.STL)-FS (p/n 78-10040-00) and Spring, Extension (p/n 78-10055-00) were removed from the kit.
20. Mechanical, step 3, Forward Scatter Channel: Spring Extension 3/4 IN (p/n 78-10006-00) and Spring (p/n 78-10008-00) were removed from the kit.
21. Electronics, step 1: Cable Assy, Power Cord (p/n 04-10547-00) is obsolete, replaced with Cable Assy, Power Cord (p/n 04-10907-00) for 120 volts. (p/n 04-10908-00) for 240 volts.
22. Electronics, step 3, Card Cage, B and c, Added “use CellQuest on the FVSE”
23. Electronics, step 5, updated for DiVa option.
24. Updated Tools/Supplies Required.
25. Remove (1.3.1.1 Maximum separation of unlabeled CaliBRITE Beads and cRBCs in all fluorescence detectors. PMTs set on unlabeled beads, compensation at %0.00.) as FSEs do not carry CaliBRITE Beads.
26. Monthly cleaning performed only upon customer approval.
27. Added (2.11. Inspect the sample head area for air leaks using DI water placed on top of the knurled nut when running the instrument in run with a sample of DI water in a tube. Any air bubbles forming at the top of the nut should be addressed by tightening the nut slightly until the bubbles no longer form.)
28. Added (2.13 Clean the outside of the sheath and waste tanks and inspect the inside of tanks for corrosion. Replace tank O-rings).

29. Note on 3.1
30. Added (3.9 Clean the Accudrop camera optic if installed (FVSE only).)
31. Added Note on 5.2.
32. Removed: (6.4 PMT Power supplies)
33. Removed: activities with floppy drive.
34. Added 9.2.1 to 9.2.5
35. Added LBNL-specific safety steps