Imaging Center Essen	Institut für Experimentelle Immunologie und Bildgebung	
Standard **O**perating **P**rocedure **Workflow BD FACS A1 Symphony** Please note: If images acquired on IMCES instruments are used in publications we would be grateful if you could mention the facility in the Acknowledgements.		Last change: 18.11.2025

Starting Up the System

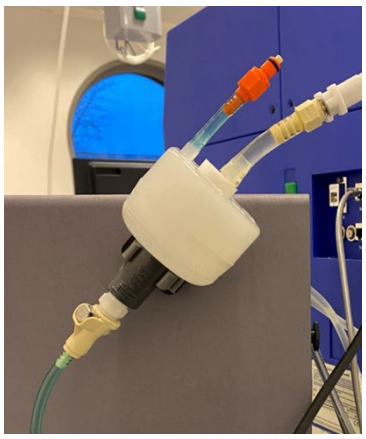
- Check the fill level of the FACS Flow tank and the waste tank (if necessary exchange FACS Flow tank and/or waste tank)

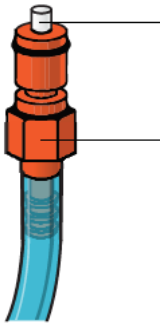
- Turn on the FACSFlow Supply System (under the table/Cytometer)
- Turn on the cytometer main power (big green button on the right side of the instrument)
- Start up the computer, start Windows and log in as Operator
- Start BD FACSDiva software and login with your Group-name
- Wait 20` for laser warmup and stabilization before you do CS&T and/or measure samples (you can do the “Instrument preparation” steps outlined below in that time)

Instrument Preparation

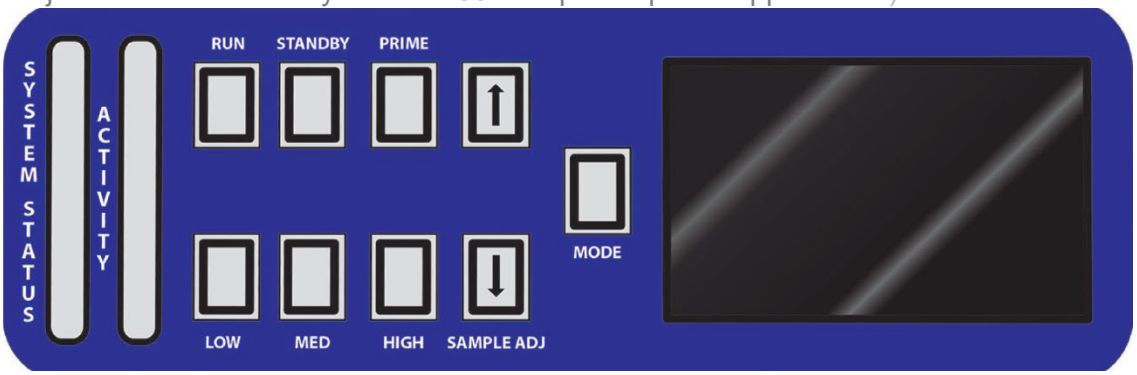
- Check the sheath filter for trapped air bubbles (If there is air in the sheath filter: gently tap the filter body with your fingers to dislodge the bubbles and force them to the top. Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.)

Sheath filter

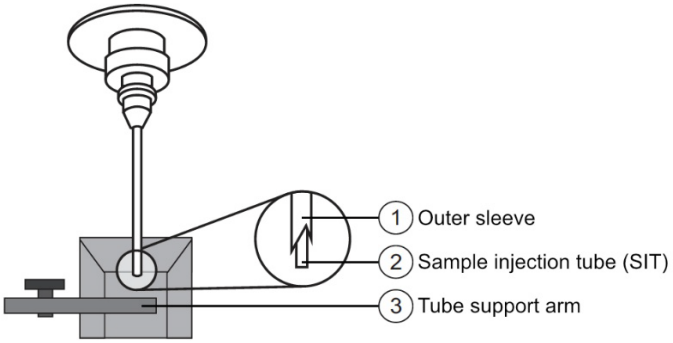




- Press the “PRIME” button on the control panel and wait for priming to finish, then discard tube with dH_2O
- Press the “RUN” button and choose flow rate “HIGH” on the control panel (the flow rate fine-adjustment should always be on 250 except for special applications)

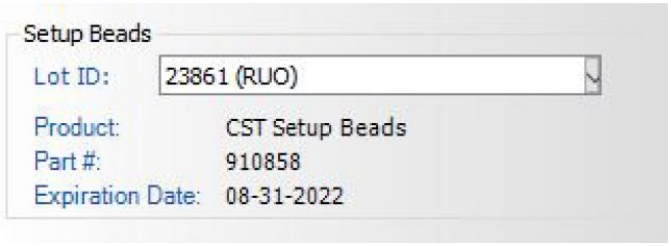


- Put a FACS tube with ≈ 4ml Detergent solution on the SIT and let it run for ca. 5 minutes at high flow rate. Leave the tube support arm for the first minute aside. Reuse and refill the existing tube if possible. Detergent tubes do not need to be replaced frequently like dH2O tubes!
- Put a (new) FACS tube with ≈ 4ml dH_2O on the SIT and let it run for ca. 5 minutes at high flow rate. Leave the tube support arm for the first minute aside.

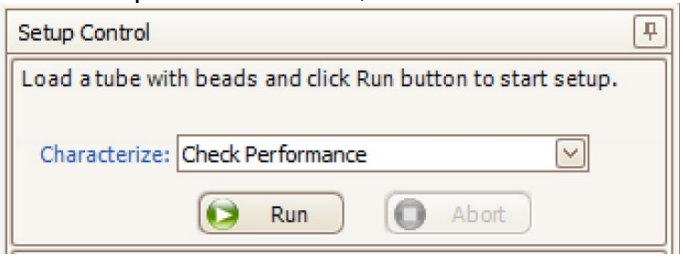


Running a performance check (CS&T)

- Running the performance check is **recommended before critical experiments, but unnecessary for routine use**. It is performed at least once per week by IMCES staff, which is enough in general.
- Prepare a FACS tube with CST Setup beads by mixing one drop of beads with 350 µl FACS Flow (before adding the drop of beads to the water, mix the bead vial by inverting the vial 4-5 times by hand, **do not vortex the bead vial!**)
- Select Cytometer > CST in the software menu
- Make sure that the window size of the CST-window is half the size of the screen
- Verify that the bead LOT information under setup beads matches the Cytometer Setup and Tracking bead lot



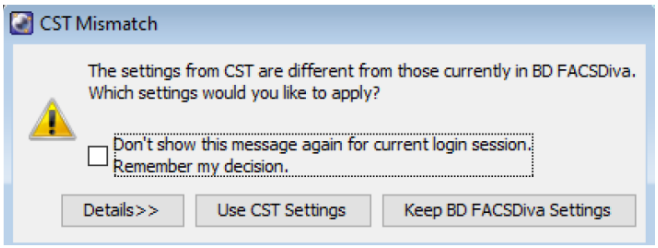
- In the Setup Control window, select Check Performance from the Characterize menu.



- Put the FACS tube with the CST Setup beads mix on the SIT, press RUN and LOW on the control panel
- Click Run in the Setup Control window.
- Once the performance check is complete, click View Report and verify that the cytometer performance passed.

- If there are warnings you can proceed (the IMCES personnel regularly check the CS&T performance checks carried out and will take care of any problems coming up.)
- If there is an error please call the IMCES personnel

- If the performance check passed (with warnings), select File > Exit to close the CS&T window and return to the BD FACSDiva Interface. The CST mismatch dialog opens.
- Click Use CST Settings.



Setting Up an experiment without compensation

- Create a new experiment or load an experiment template
- Select “cytometer settings” and tick height and width for the FSC and SSC parameters in the cytometer window.
- Delete colors you do not need, add colors as needed (avoid creating useless data!)
- Create and activate “sample tube”
- Populate the global worksheet with plots (dot plot / density plot / histogram) as needed (do not create useless plots, as this slows down the software!)
- Set all gain voltages according to your stained cells (use your corresponding brightest sample(s) to set voltages for each channel)
- Check with unstained cells that you get sufficient contrast between positive and negative controls
- Set all your gates of interest:
  - Gates for all tubes in your experiment on a “Global Worksheet” (recommended)
  - Gates for specific, single tubes in tube “Normal Worksheet” (not recommended unless you have a good reason to do that)
  - Acquire some data
  - You might want to perform (Fluorescence minus one) FMO-controls to confirm that your manual settings are correct
- To create further tubes with the same settings just use the “Next Tube” button beside the “Acquire Data” button
- If you apply stopping criteria do this for your smallest population
- Load your samples of interest, “Record Data”

Setting Up an experiment with compensation

- Create a new experiment
- Select “cytometer settings” and tick height and width for the FSC and SSC parameters in the cytometer window.
- Delete all colors you do not need!
- Select “Experiment” > “Compensation Setup” > “Create Compensation Controls” > “OK”
- In the database, select “Compensation Controls”; double-click on “Unstained”
- Set all gain voltages according to the procedure described in the previous section
- Load unstained cells and acquire some data

- Adjust a gate around the population of interest, right click and “apply to all compensation controls”
- Obtain compensation data by pressing “Record Data”
- Continue with each single-stain tube, adjusting the gate on fluorescence signal as required. Record the data for each tube correctly (pay attention to which tube is active at which time in software).
- Finish the compensation: “Select Experiment” > “Compensation Setup” > “Calculate Compensation”
- If everything was correct choose “apply” (use but do not keep) or “link and save” (use and keep for future sessions) to save your compensation to the panel
- Activate a sample tube, return to “Global Worksheet” mode, and check if values are kept in the “cytometer -> compensation“ window
- Not mandatory but good practice: Perform “Fluorescence minus one” (FMO) controls to confirm that your compensation is correct

Data Management

- Export all data after measurement to your group folder on *Data (D:) / Users*
- Export your samples as fcs-files for analysis
- Export your experiment as experiment template if you want to measure with the same settings again
- Delete your experiment within the FACSDiva-Software. **The Software is not for storing data!!** (too much data within the software will cause problems like crashes, running very slow or not at all, etc.)
- Keep the saved data on the Drive in your group folder as low as possible and save a backup somewhere else (best is to keep just the last experiment, the stored data is not backuped by the IMCES)

After you finished:

Check the booking schedule at the end of your measurement

- **If there is a user after you:**
  Standby

- Run Detergent solution ~5 minutes on flow rate HIGH. Reuse and refill the existing tube if possible. Detergent tubes do not need to be replaced frequently like dH_2O tubes!
- Run dH_2O ~1 minute on flow rate HIGH (Detergent and FACS Clean must not be mixed)
- Run FACS clean for ~5 minutes on flow rate HIGH. Reuse and refill the existing tube if possible. FACS clean tubes do not need to be replaced frequently like dH_2O tubes!
- Run dH_2O ~5 minutes on flow rate HIGH
- Press Standby
- leave a FACS tube with dH_2O on the SIT
- Logout from the FACSDiva software

- **If you are the last user:**
  Shutting down the system

- Run Detergent solution ~5 minutes on flow rate HIGH
- Run dH_2O ~1 minute on flow rate HIGH (Detergent and FACS Clean must not be mixed)
- Run FACS clean for ~5 minutes on flow rate HIGH
- Run dH_2O ~5 minutes on flow rate HIGH
- Press Standby
- leave a FACS tube with dH_2O on the SIT
- Logout from the FACSDiva software
- Shut down the computer
- Turn off the cytometer main power
- Shut down the FACSFlow Supply System

If you do not turn up for a booked appointment and are unable to cancel it in the booking system, please inform the IMCES staff (call or email imces-support@uk-essen). Especially if the device is booked before you, so that it can be ensured that the device is switched off.
Outside of regular working hours, it is your responsibility to ensure that the device is not left switched on unused. If in doubt, please come to IMCES and switch off the device.

If you want to measure Biosafety Level 2 specimen or human blood samples please follow the IMCES S2 working procedures!

Before measure BSL2 samples, please:

- Ensure that you are authorized to perform these experiments at IMCES.
- For your own safety make sure you touch your sample, as well as the Symphony, only with gloves.