FACSCanto II Checklist **16.3.2018 Laura Kummola**

A. BEFORE TURNING ON THE INSTRUMENT

1. Check the last logbook entry for anything that could affect your run.
2. Check fluid levels in the solution cubitainers, replenish or change Sheath or Shutdown solutions if needed.

B. BASIC STARTUP PROCEDURE

1. Turn on the computer. The password is BDIS. Wait until the computer has finished loading.
2. Turn on the FACSCanto II (green button on the left side).
3. Open up the FACSDiva software and login to your group user account.
4. Whenever a CST mismatch window appears, click *use CST settings*.
5. If you changed or replenished solutions, select *Cytometer -> Cleaning modes -> Prime after tank refill* ant check the appropriate boxes.
6. From the toolbar, select *Cytometer -> Fluidics Startup*.

CST check (once in 24 hours)

7. Before proceeding to the CST check, wait until lasers have finished warming up (indicated by a ready status).
8. From the toolbar, select *Cytometer -> CST*.
9. Prepare the CST-beads: add one drop of beads (shake the bottle well, do not vortex) into 350 µl of PBS. You can (and should) now vortex the beads in the tube.
10. Check that the bead bottle number matches the lot number on the screen. Click run and insert the tube when prompted. Close the doors.
11. Very often the systems now gives a fluidics error message. Remove tube completely, wait for SIT flush, click run again and reinsert tube.
12. Verify that the CST report is adequate. If failed or passed with warnings, cleaning the flow cell is likely to help.
13. Proceed to creating your experiment (**section C or D**).

C. SETTING UP A NEW EXPERIMENT, SAVING TEMPLATES AND APPLICATION SETTINGS

Go to section D if you are opening up a template (and possibly using already saved application settings).

1. In the *Browser* menu, create a new experiment (*brown folder icon* on the *Browser* icon bar).
2. Create specimens *(syringe icon)* and tubes under specimens *(tube icon).*
3. Activate any tube (the arrow next to it turns green) in the *Browser* menu and select *Cytometer window* from the *Browser* icon bar (*red laser icon*) if not yet open. Choose Parameters tab and delete any detectors you are not using. ***Tip! Check FSC H box for doublet discrimination option.***
4. Open your plots, histograms and statistical views in the *Global worksheet*. Statistical view can be added by accessing the mouse right button menu.
5. From the toolbar, select *Experiment -> Experiment layout*. Add labels to your fluorochromes. Select the amount of cells you want to collect (Acquisition tab).
6. Set the detector voltages: activate any tube in the *Browser* and run an unstained sample on acquire (use *Acquisition dashboard)*. Run an unstained sample to set the negative population, and a stained sample to check that the positive population is on scale. Adjust the voltages in the Parameters tab in the *Cytometer window*. Stop acquisition and unload the tube when you are happy with your voltages. ***Tip! Right-click Cytometer settings under your experiment name and choose Application settings -> Create Worksheet to access target box worksheet.***
7. You can now draw your gates of interest.
8. If you want to set a stopping or storage gate, go to *Experiment -> Experiment layout* and select the Global Worksheet column. Choose "Global sheet 1" from the drop-down menu. Now you can choose the stopping gate. Do this when you have decided on final gating. You can always come back after compensation to finish it.
9. You can now give correct labels for your plots in the *Global Worksheet* by clicking the axis labels.
10. You can save application settings and experiment template at this point. To save experiment template, right-click your experiment name in the *Browser* menu. *Select Export -> Experiment Template* and do as prompted in the appearing window. To save application settings, right-click *Cytometer settings* under your experiment name in the *Browser.* Select *Application Settings -> Save*. Name the settings.
11. Proceed to running compensation samples if needed (see **section E**), or just recording your sample data (**section F**), if no more adjustments are needed.

- *What do you want to have in your template? Certain tubes with names? A compensation matrix that cannot be updated? Saving the template before adding these saves you the time and effort of editing and deleting them the next time.*

D. USING TEMPLATES AND APPLICATION SETTINGS

If you already have set up your experiment previously and want to reuse its settings, these are the best options.

- *If you want your experiment to be updated by the latest CST to ensure data comparability, you must use application settings – not just copies and templates!*
- *Application settings include parameters, area scaling and PMTV. Templates and copies include all these, and also gates and compensation. Saved compensation matrices can never be updated so compensation must be run anew!*

1. Either copy an old experiment (right-click and *Duplicate without data*), or open up a previously saved template: in the toolbar menu, choose *Experiment -> New experiment*. Choose your template from the appearing list.
2. In the *Browser* menu, right-click *Cytometer settings* below your experiment name. Make sure to have run CST at this point (if not valid from yesterday). Select *Unlink from (the name of your previously saved compensation data)*, if applicable.
3. Right-click again *Cytometer settings* below your experiment name. Choose *Application settings -> Apply*, and choose previously saved settings from the list that appears. If asked about compensation, *Set to zero*. If eg. Area scaling has changed, choose *Overwrite*. The text *Cytometer settings* has now changed to *Application settings*.
4. If compensation is not needed, proceed to recording your sample data (**section F**).
5. Otherwise check that compensation in your samples is now zero: activate a sample, open the *Cytometer window (red laser icon)* and see Compensation tab. Diva is sometimes tricky here!
6. Run new compensation controls (see **section E**). Check that the new compensation is now applied to your samples.

- *Application settings can also be applied to a completely new experiment (see **section C**).*

E. COMPENSATION

Perform compensation whenever you have two or more fluorochromes with overlapping spectra in the same sample.

1. From the toolbar, select *Experiment -> Compensation setup -> Create compensation controls*. If you have only one marker per fluorochrome, delete everything that does not have the label "general". If you use beads, check the box that includes a separate unstained tube. If compensating with cells, leave it unchecked. ***Tip! If you need an empty channel, delete it here but leave it in Cytometer window, Parameters tab***.
2. Switch to *Normal Worksheet* (icon on the worksheet corner). Activate the first compensation sample in the *Browser* menu.
3. Load the correct single-stained sample. Set the gate to singlets and press *Record*. Collect preferably at least 10 000 events (can be set in the *Experiment layout* or one by one in the *Acquisition dashboard*). Unload sample.
4. Right-click the P1 gate and select *Apply to all*. Click the next sample either in the *Browser* or in the *Acquisition dashboard*. Load new sample and record. Repeat until you have run all the compensation tubes.
5. Verify correct positioning of the P2 gates. If you did not include a universal negative, create P3 gate on the negative peak by using the *Snap-to-gate icon*.
6. In the toolbar menu, select *Experiment -> Compensation setup -> Calculate compensation*. If you forget this, software will say nothing and you will not have any compensation! In the pop-up window, choose *link and save*. You can change the name if you wish.
7. Change back to *Global Worksheet*.
8. Now you can verify and fine tune correct gating by running (on acquire) an appropriate sample, if necessary. You must not change any voltage other than FSC or SSC from now on – it would make the compensation invalid.
9. Proceed to recording your data (**section F**).

F. RECORDING YOUR SAMPLE DATA AND EXPORTING FSC-FILES

- *Filtering your samples might be a good idea – it is always less annoying to filter them than to open a clogged system***.**
- *Instantly remove the tube from the SIT when you hear the system depressurize. Always let the system perform a SIT flush before inserting a new tube!*
- *Make sure that the SIT Flush box is checked on the Acquisition dashboard.*

1. Activate your first sample in the *Browser* and place the tube into the SIT. Click *Acquire.*
2. Set the flow rate so that you get enough events per second. Generally, lower flow rate is better, but with dilute samples it might take too long.
3. You can change the event number to be recorded in the *Experiment layout* or in the *Acquisition dashboard,* if needed*.* Press *Record*.
4. Move to the next sample by activating it in the *Browser* or by clicking *Next tube* in the *Acquisition dashboard*. Start recording.
5. After the last sample, insert a USB storage device and right-click your experiment in the *Browser*. Select *Export -> FSC Files*.
6. A window opens, click OK after checking that file format is FSC 3.0.
7. Choose where to save your files and click OK.

G. SHUTDOWN PROCEDURE

1. Optional: Clean flow cell (Cytometer -> Cleaning modes -> Clean flow cell) 2-3x with FACSClean and 2-3x with mqH_2O (or hot tap water).
2. Load a tube of FACS Clean and run on acquire for 5-10 minutes on high flow rate. Check the event rate! It is best to use a cleaning template with high FSC and SSC voltage to see small debris.
3. Optional: repeat with FACSRinse.
4. Repeat with mqH_2O.
5. From the toolbar, select *Cytometer -> Fluidics shutdown*.
6. Delete old experiments that are no longer needed in the database.
7. Turn off the FACSDiva, computer and FACSCanto II (from the big green button).
8. Wipe the SIT and clean any spilled fluids or salt buildup.
9. Empty the waste tank. Rinse away all foam. Pour some FACSClean on the bottom (around 100 ml is enough). Attach the tank back to the fluidics cart.
10. Fill in the logbook (both of them).