

FACSFortessa™: User Checklist

Startup Procedure

- Turn on Fluidic Cart, check whether sheath tank is full and waste tank is empty.
- Start the instrument (green button at the right site).
- Start the computer, choose Operator (Welcome#1).
- Start Diva Software, log in with personal account password
- Check whether instrument connects to the computer (yellow filled circle on the lower right turns green).
- Let lasers warm up and stabilize for ~20-30 min.
- Prior to measurement prime the system:
Remove the tube from the SIP. Put the aspirator arm to the side, press Prime button. Wait until the Standby button turns orange again. Repeat 2x.

Data Acquisition

- Create new experiment by choosing one of the following options:
Folder Icon *or*
Right-click on previous experiment → Duplicate experiment without data.
- Delete Detectors/Colors you do not need.
Cytometer window → Parameters → Click on parameters → Delete
Note: This is important to reduce file size!
- Create Plots in the worksheet as needed. Start with FSC – SSC plot.
FSC and SSC usually in linear scale, exceptions are small particles e.g. bacteria.
- Start with control samples to set Gates and to adjust Voltages as needed.
Cytometer window → Parameters → PMTV
Note: Don't change PMTVs after having set up controls and set up compensation!
- Create further plots, gates and a hierarchy in the worksheet as needed.
- Record at least 10.000 cells to have a statistically reliable read-out.

- ☑ When Y or X axis in plots are cut off, click in Plot Inspector Window → select Bi-exponential Display
- ☑ Right-click in plot
 - ✓ Show population (displays only pre-gated populations).
 - ✓ Show population hierachy.
 - ✓ Create statistic view.

Shutdown Procedure

- ☑ Export your experiment *.XML or *.fcs files onto external harddrive (D:) Right-click on experiment → Export → Export Experiment *or* FCS files *or* Experimental template.
- ☑ Follow cleaning procedure strictly: 5 min Clean, 5 min Rinse, 5 min H₂O on HI.
- ☑ Close all open experiments.
- ☑ Let system stay in H₂O, LO and Standby.
- ☑ Log-out of DIVA Software or quit when no one else is coming after you.
- ☑ In case you used UV laser, start BD Coherent Connection Software, choose configuration “without UV laser”, close window.
- ☑ Switch off LSRFortessa when no one else is coming after you.
- ☑ Report your measurement in the LogBook next to the FACSVerse.
- ☑ For analysis (take *.fcs files) use FlowJo (M1007).
- ☑ For help, contact FlowKon staff (-3949 in M1010 or -2187 in M1024) or via flowkon@uni-konstanz.de.

Leaving the Room

- ☑ Check whether instrument and PC are turned off in case no one else is coming afterwards.
- ☑ Clean tables and working place.