# STANDARD OPERATING PROCEDURE (SOP)

## BD LSRFortessa - Operation

| Document No: | CAF-SOP-003-V5 | Revision Date: | 15.06.2026 |
|---|---|---|---|

## Policy, purpose and responsibilities

- The goal of this SOP is to ensure a proper operation of the specified instrument.
- Following theoretical and practical introduction courses are mandatory prerequisite to use the instrument.
- The users must accept and sign the usage policies from the CAF before any instrument usage.
- Please immediately report any technical problems or questions to:

cellanalyticsfacility@unifr.ch

or

Sarah.cattin@unifr.ch

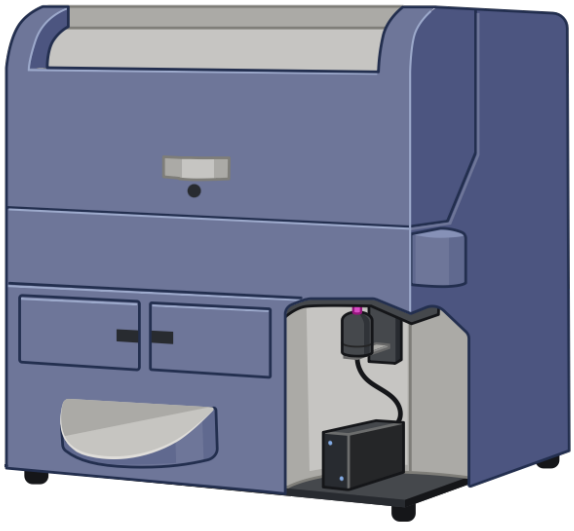
026/300.85.79

- Instrument is deeply cleaned and performance are checked by the CAF each Monday. QC information and follow-up in time are available in the bigdata server, in the CAF folder, open to all users to consult.

## Instrument procedure

### I. General information for the LSRFortessa

- The BD LSRFortessa instrument is a conventional flow cytometer equipped with 3 lasers and 11 fluorescent detectors channels (plus FSC and SSC). Samples are loaded manually on the instrument.
- The instrument is running with FACSFlow (PBS) as sheath fluid.
- In general, the maximum recommended sample concentration for analysis is $1 \times 10^6$ cells/ml.
- Data acquisition rate is up to 40'000 events/sec, but you might see a spread of the data starting at 10'000 event/sec in certain cases.
- Expected flow rate during acquisition are (**after closing the arm, that might aspirate quickly 50ul of your sample**):
  - *Low: 12 ul/min*
  - *Medium: 35 ul/min*
  - *High: 70 ul/min*



# II. Instrument Start-Up

- First user of the day: Start-Up
  - Check the level of FACSFlow and replace with a new box if needed (lift to check).
  - Empty the waste in the red containers.
  - Press the green button on the right side of the instrument to switch it on.
  - Perform 3x "Prime" on the instrument with a DI water tube attached.
    - Bubble should appear in the tube, otherwise the instrument is clogged!
  - Change DI water tube after priming! It will be contaminated.
  - Click on RUN HIGH and let water aspirating
    - If one of the buttons is orange, this means that you have a pressure issue and no samples will be aspirated.
  - Allow the laser to warm-up for min 20min minimum.
  - Turn on the computer (behind the screen), log into windows:
    - Session= .\BDAdmin, Password = BDIS#1$$
  - Start FACSDiva Software and login:
    - Session = USERS, Password = LSRFortessa#1
  - Wait for the software to connect with the cytometer.
  - Accept "Use CS&T Settings" if prompted.
- Following users:
  - Login into the FACSDIVA software:
    - Session USERS, Password = LSRFortessa#1
  - If the instrument is running while you need to change a solution:
    - switch off the supply system chariot (avoid bipping)
    - Change the needed tank
    - Restart the Supply System chariot.

# III. Acquisition workflow

- Add a folder with your name (first time only).
- Create an experiment:
  - Add a new experiment or rename a duplicate from a previous experiment
  - Open your experiment (double-click to open the book )
  - Give a name, starting by your initials and date (ex: SC_20260310)
  - Add a specimen (little syringe icon)

- In the **specimen,** click on the tube until it's fully selected (green arrow)


- In the **cytometer tab**:
  - Delete all non-needed parameters of the cytometer settings.
  - Put FSC – SSC also in H and W (no log scale for these 2 parameters)
  - Select the needed parameter using the drop-down menu

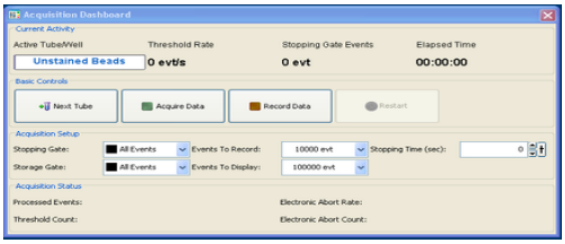
- In the **global worksheet page**:
  - Every new experiment will automatically provide you with one global worksheet.
  - Draw all needed graphs and prepare the worksheet.
  - Right click on the first graph and select "population hierarchy" to see your gating strategy, rename your gates and change the colors of the populations.
  - Right click on your graphs to select population to be shown on the graphs.
  - On the graph inspector, you can put your X and Y axis in biexponential for a better visualization of the negative population.
  - All tubes will have the same gates and strategy.
  - ***Important:*** *compensation are done in Normal Worksheet. After matrix calculation, click back on the icon of the global worksheet to go back, before any recording.*

- In the **tube explorer, under the label tab**:
  - Enter all the names of your colors + antibodies

- Set up your settings:
  - Make sure you have filtered and vortexed your samples well.
    - *When analyzing large cells, filter sample with 0.45µm filter directly before analysis.*
  - Place your sample tube on the SIT arm, **quickly close**, and press "**Run**" on the instrument.
    - *Do not switch in between samples on "Standby" because this will clog the sample needle! Instead remove the tube from the instrument and leave the sample arm open (backflush dripping to avoid carry over and clogs between samples*).
    - ***NEVER put the instrument in STANDBY without cleaning it.***
    - ***NEVER put on STANDBY while running a sample.***
  - Activate the tube in Diva software and press "Acquire".
    - *Maximum event rate is 10'000 events/sec.*

- Adjust the voltage for FSC & SSC to observe your cells on scale (no recording).
- Check you unstained sample for auto fluorescent signal, then all the color that you will use (or the full stained control directly)
- ***If needed (signal out of scale):*** Optimize the gains for the fluorescence channels, lowering as little as possible the corresponding channel.
  - *CS&T PMT voltage settings are minimum voltages for optimal resolution.*
- **Everything during the settings is done without recording. Once voltages are fixed, don't touch them again!**

- Start your acquisition:
  - Start to record your single stains (all references controls tubes):
    - **Compensate later:** just record your single staining as normal tube with correct naming
    - **Compensate first**:
      - go to "Experiment → Compensation Setup" and "create compensation controls"
      - Verify that all your fluorochromes are listed (only keep generic).
      - If recording an unstained sample, "include separate unstained control". If using beads or a mix of beads and cells, don't tick.
      - Tubes will be created for each control. Record each single stain.
      - Put the gate on the positive peak. If unstained is in the same tube, add a second gate on the unstained for each tube.
      - go to "Experiment → Compensation Setup → Calculate compensation
      - "Link&Save the matrix", then go back on the global worksheet and select your sample.
    - Set your gates and the cell number you would like to measure in the acquisition dashboard:
      - *Event to record = your target number*
      - *Stopping gate = in which population you want the target number*
      - *Storage gate = keep all event to record everything*




    - Load the correct sample and click on "acquire" on the software. Once you see the cels, and press "Record". Use the "low" speed to avoid big CVs.

***Important Notes:***

- *The cytometer is always aspirating the sample, even if you don't "acquire" on the software. Replace your sample with a water tube while you are thinking or waiting, otherwise you will lose your sample.*
- *When the arm is open, the cytometer is aspirating high speed! Be aware to rapidly put your sample down to avoid losing it.*

# IV. Data exportation

Note: Data will be deleted each Monday, be certain to have exported yours on the bigdata server!!

- Export directly your FCS file:
  - Right click on your samples to export.
  - Export your data as FCS files (3.0 or 3.1; never as 2.0!) to the "data" folder on the user folder.
  - *Important: Open the destination folder and check that the size of each FCS file is > 0 kb. If files have 0 kb this may indicate a corrupted data export and you will need to repeat the export procedure!*
- Copy your data in your research group folder in the bigdata server within the CAF partition (on the desktop).

- To keep an experiment template:
  - Right click the experiment and choose "Duplicate without data".

- To keep settings for longitudinal studies:
  - Once your settings (voltages + panel) are set for a long-term experiment, you can save if as "application settings".
  - Give a name with you experiment + initials
  - Next time, when starting your experiment, right-click on the cytometer settings and apply the recorded acquisition settings.
  - For me information on this specific procedure, contact directly the CAF.

- After exportation, before closing DIVA Right-click on your experiment(s) and select "Delete".

# V. Instrument cleaning and shutdown

- Check on Open IRIS if somebody is booked after you: http://iris.science-it.ch
- In between users:
  - The instrument must be cleaned between every user. If you are running problematic samples, you might have to clean in between your samples to avoid clogging.
  - Set the aspiration speed on HIGH and pass each of the following solutions:
    - *Run FACSClean (bleach) for 1min with open arm and 5min with close arm*
    - *Run FACSRinse (soap) for 1min with open arm and 5min with close arm*
    - *Run Water for 1min with open arm and 5min with close arm*
  - Change the water tube and acquire (without recording) on any tube for 1min.
  - If more than 20events/sec are detected, repeat the cleaning procedure.
  - Put the instrument in STANDBY – LOW mode, keeping the water tube on the SIT arm to avoid salt crystallization.
  - Clean the work area with 70% EtOH, making sure it is ready for the next user.
  - Empty waste / Refill sheath tank if necessary
  - Log-Out of the user's session on DIVA.

- Last user of the day:
  - Follow the "in between users" cleaning procedure.
  - Close DIVA software for acquisition.
  - Shutdown the computer.
  - Switch Off the instrument (on the side, green button)
  - Clean the bench with 70% EtOH, making sure it is ready for the next user.
  - Empty waste / Refill sheath tank if necessary (put first in standby the instrument).

# VI. Optical configuration of the instrument

| *Laser* | *Channel Name* | *Filter* | *Main colors examples* |
|---|---|---|---|
| Violet (405nm) | Violet 450/50 | 450/50 | Spark Violet 423, V500, **V450**, PacBlue, AF405, **BV421, BV510**, VioBlue, *DAPI* , CFP, BFP, *LD/Zombie violet* |
| | Violet 610/20 | 610/20 | AF430, BV605, BV570, *LD/Zombie Yellow* |
| | Violet 710/50 | 710/50 | BV750, **BV711**, SB702 |
| Blue (488nm) | Blue 530/30 | 530/30 | **AF488**, AF514, GFP, **BB515**, FITC, YFP, CFSE, Spark blue 515, *LD/Zombie Green* |
| | Blue 575/26 | 575/26 | AF546, **PE**, *PI* |
| | Blue 610/20 | 610/20 | **RB613**, Pe-Vio615, *LD Red, PI* |
| | Blue 695/40 | 695/40 | BB700, PerCP, Pe-Cy5, Pe-Cy5.5, *7AAD, DRAQ7* , **RB670** |
| | Blue 780/60 | 780/60 | **Pe-Cy7**, Pe-Vio770, **RB744, RB780** |
| Red (640nm) | Red 670/30 | 670/30 | **AF647**, **APC**, APC-Cy5, APC-Cy5.5, AF680, RR688 |
| | Red 730/45 | 730/45 | **R718**, AF680, AF700, APC-Cy5.5, Spark NIR 685, *Zombie NIR* |
| | Red 780/60 | 780/60 | AF750, **APC-Cy7, APC-H7**, APC-Fire750, APC-Fire 810, *Zombie NIR* |

## SOP revision (annually at minimum)

| *Revision* | *Date* | *Description of Changes* | *Reviewed By* |
|---|---|---|---|
| *V1 – V4* | *2019 to 2024* | *Initial release and minor updates* | *Sarah Cattin* |
| *V5* | *15.06.2026* | *Structural changes* | *Loïc Chentout / Sarah Cattin* |
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