Standard Operating Procedures (SOPs): MACSQuant

General remarks

Following the introduction course and signing the Cell Analytics Facility policies are mandatory prerequisite to use the MACSQuant Analyzer.

Report any technical problems or questions to:

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I. Instrument Start-Up

- Tap the touchscreen monitor to activate the software
- Login under your own session (**First Name LAST NAME**), **no password**
- Click on the top right "start/stop" button and activate the "acquisition mode"
- Wait for the instrument to start
- Click on "View Hardware" and allow the blue laser to warm-up until at 37°C (30min)
- During this time:
    - **Check the waste level!!** If full, empty it in the red containers (not in the think) and put back 2 tabs "Virkon" in the glass container.
    - **Check the running buffer and the other buffer (color coded).** If you open the last pack of running buffer (meaning the last carton of 8 bottles, on top), please advice the facility staff.

II. Acquisition

- Create a new experiment or import a previous "workspace" template:
    - Select correct rack type
    - Insert file name and descriptions
    - Enter an uptake volume (max. 450ul at the time)
    - Enter a define cell number if needed (settings – customs)
- Open a new analysis window and prepare the worksheet, or import an analysis template.
- If you are working with the plate loader, design your experimental settings for each well.
- 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 tube on tube loader and press "play"

  The uptake volume will be acquired and lost. Choose small volume for settings.

  Each sample will be save.

  NEVER move the needle yourself; it's damaging the instrument.

  OPTIMAL EVENTS SPEED: 5'000-7'500 events/sec, NEVER MORE then 10'000
- Adjust the voltage for FSC & SSC to observe your cells on scale.

  Lower the voltage for fluorescence channels only if off scale due to high fluorescence intensity. Calibration voltage settings are minimum voltages for optimal resolution.
- For automated compensation refers to the Miltenyi worksheet manual next to the instrument.

- Set your gates and the cell number you would like to measure and press "Play". Use the "low" speed to avoid big CVs (25ul/sec).

III. Data Export

Note: All data will deleted from the MACSQuant database each month without notice!

- Data are automatically converted in FCS 3.1 files (originally .mqd files)
- Insert a USB key behind the instrument.
- Go under "file"-"copy", check that your USB key is the destination folder (copy to) select the folder of the day (or any template) that you want to copy on the USB, click on "Copy".
- Eject the USB key.
- Important: Before copying check that the size of each FCS file is > 0 kb. If files have 0 kb this may indicate a corrupted data conversion and you will need to do manually the conversion procedure.
- If you wish to keep your experiment settings as a template, go under "file" – "save" and choose the template you want to save.

IV. Cleaning and Shutdown

Note: The MACSQuant as a cleaning procedure during shutdown.

- The instrument must be cleaned between every user. If you are running problematic samples, you might have to clean in between your experiments to avoid clogging.
- Check on Open IRIS if somebody is booked after you: http://iris.science-it.ch

- *Last user of the day (or nobody within the next 3h)*
  - Click on the "start/stop" button top right
  - "Instrument off"
  - Clean the work area - don't leave used tubes, gloves, and etc. behind.

- *Between different users of the day:*
  - Right click on the "drop" button down right
  - Click on "Clean" (take 10min)
  - Click on "logout" on top right
  - Clean the work area - don't leave used tubes, gloves, and etc. behind.

- If you experiment a clot of the system (nothing is passing), perform a "Clean" (see up), Follow by a detergent wash:
  - Right click on the "drop" button down right
  - Click on "Flush" (take 18min), put a tube containing 1% Bleach