MACSQuant Analyzer Quick Guide

* **NOTE: Optical bench requires a 30 minute warm-up. Please complete “Start Up” steps 1-5 to initialize warm-up and begin cleaning.**

**Start Up**

1. Turn on Power switch on Right side of instrument.
2. Touch the monitor to wake up instrument.
3. Log in under your PI.
4. To initialize warm-up, click the C:\Documents and Settings\boydm\Local Settings\Temporary Internet Files\Content.IE5\A4RLAY20\MC900441501[1].png icon in the upper right corner and select “Acquisition Mode”.
5. Run “Clean” Program (#1 below).

**Cleaning Programs**

1. Clean: Decontamination at start up.
   1. Right-click, or press and **HOLD** the 🌢 icon at the bottom of the screen and select “Clean.”
   2. This mode cleans with 10% bleach and takes 10 minutes.
2. Rinse: Between plates.
   1. Click the status bar 🌢 icon at the bottom of the screen.
   2. This mode flushes aggregates and takes about 5 minutes.
3. Flush: Clearing clogs and monthly maintenance.
   1. Right-click, or press and **HOLD** the 🌢 icon at the bottom of the screen and select “Flush.”
   2. This mode incubates the flow cell and takes 16 minutes.

**PMT Calibration**

1. Retrieve the MACSQuant Calibration Beads from the core refrigerator and vortex.
2. Click the barcode  icon and present the vial barcode to the reader when it begins to flash.
3. It has read the barcode when the reader flashes green.
4. Follow the on-screen prompts to proceed.
   1. Place a 5mL tube in the single tube holder.
   2. Place one drop of beads into the tube and press “OK.”
5. MACSQuant will automatically mix and dilute beads, open template and begin analysis.
6. Once Analysis is complete, make sure all parameters have “Passed.”
7. Turn off the orange highlighted A icon at the top of the screen to exit analysis mode.

**Acquisition Settings**

1. Go to **File > New Workspace** (or **File > Open** to open existing workspace).
2. In the Experiment Tab, select appropriate rack (ie. Chill 96-Well Rack).
3. A rack template should pop up in the middle of the screen. Click on the wells you want to analyze.
4. Wells Highlighted with an orange ring will be programmed using the Experiment tab.
5. Set your Sample ID for each well, row or column by highlighting the desired wells.
6. Select all wells and set Flow Rate.
   1. Low = 25uL/min for high cell densities or sensitive measurements.
   2. Medium = 50uL/min.
   3. High = 100uL/min for low densities and quick analysis.
7. Well A1 will be used to set parameters, it is wise to set this well to run in LOW.
8. Always check “Mix Sample”
9. Mode is always standard
10. Uptake Volume is the amount to be analyzed (cannot be greater or equal to Sample Volume).
11. Sample Volume is the total volume in the well (if this is overestimated, Instrument may take up air during mixing).
12. Annotations Tab: Set labels of Antibodies and fluorophores.
13. Settings Tab: Check “Events” and set number desired **only if** you want the instrument to stop and move on to the next sample once it has reached this number of events. (10.000=10,000)

**Define Layout**

1. On the top of the screen, click on the icon that looks like a dot plot.
2. Select a layout suitable for your analysis.
3. Change axis on plots and set a FSC/SSC “P1” gate.
4. Set other plots to P1 by selecting “Live/P1” from the pull down at the top of the plot.

**Plate Acquisition**

1. Once you have your desired wells configured and your plots displayed, click on the C:\Documents and Settings\boydm\Local Settings\Temporary Internet Files\Content.IE5\A4RLAY20\MC900432684[1].png icon in the bottom right hand corner to begin plate acquisition.
   * NOTE: If you need to stop the acquisition, press the C:\Documents and Settings\boydm\Local Settings\Temporary Internet Files\Content.IE5\3PJAX3QF\MC900432688[1].png icon in the bottom right hand corner. In order to resume this plate, you may want to delete wells from the template that have already been run or the instrument will attempt to reacquire them.
2. During the first well, your unstained sample, click on the Channels tab and adjust FSC/SSC until your cells are centered in the dot plot and within P1.
3. Then adjust other applicable colored parameters so that your cells are within the first grid decade, below 1e0.
4. Once you have the parameters set, press the “Clear” button on the bottom center of the screen to refresh the data file.
5. Continue to run the remainder of the plate.

**Data Retrieval**

1. Place a flash drive into the USB hub to the left of the MACSQuant.
2. Select **File > Copy**
3. In the window that pops up, select “Data” from the left sidebar.
4. Find your folder, select all of the files (Ctrl+A) and “Copy.”
5. “Eject” when you are finished.

**Shut Down**

1. To initialize fluidic wash and shut down, click the C:\Documents and Settings\boydm\Local Settings\Temporary Internet Files\Content.IE5\A4RLAY20\MC900441501[1].png icon in the upper right corner and select “Instrument off.”