User Workflow MACS Quant$_{10}$ - UConn Health

1. Touch screen to power on
2. Log-in using Lab name- this must be typed (password left blank)
    a. first user: turn on Lasers (~5 min.) click power button > click Acquisition mode
    b. first user: perform a flush. Right click the drop and select flush (takes 16 min., not stoppable)
    -total ~20 minutes warm-up time from steps a. and b.
    c. not first user: perform a rinse (click the drop)
3. Setup scatter voltage, select fluorescent channels, and data scaling
    a. choose single tube rack or one well of Chill5/Chill96
    b. enter your name in *Project*
    c. enter something in *Description* text box (e.g.: scatter setting)
    d. leave the box checked next to *File Name* to get the default file name (uses sample description / date)
    e. Choose fluidics for tube/well: enter volume information/mix/fluid speed/wash mode (Fast)
    f. choose scaling (hlog, 5log, 4log, 3log, lin) for each detector in channels tab and set trigger parameter (usually FSC)- note: the Advanced button allows height and width to be collected
    g. Open an analysis window
    h. press *Play* [Note: you can right-click the Play button to *Pause* fluidics or *Skip* to next sample; click 1x on Pause button to resume Play.]
    i. adjust voltage settings$^{*as\ necessary}$ for all channels (gain) and threshold value (Trigger) for your cells and stains
    j. hit *Clear* button twice to refresh the display in plots
    k. when finished, hit stop, or run remaining volume
4. Compensation Multicolor (not compensating?- skip to 5)
    a. Choose scaling under *Channels* tab (hlog, 4log, 5log)
    b. In *Experiment* tab, choose rack (note- wells cannot be skipped)
    c. select compensation wells or tubes and *group* them (right click > group)
    d. In *Experiment* > under the *Settings* tab, choose the *Express* button then select *setup* and *compensation multicolor*
    e. Choose fluidics for rack/tubes/wells: enter volume information/mix/fluid speed/wash mode (Fast)
    f. Set S*ample IDs* individually for all compensation tubes/wells *(wells selected with orange outline)*- you must have a blank (unstained)
    g. Make sure project has your name
    h. Label *Description* text box as desired (e.g.: compensation, comp, setup…)
    i. Double check plate/rack by selecting *View > Experiment table*
    j. Press play

k. When prompted, set a live gate if you desire, then allow the rest of samples to run
l. When prompted, save settings file
m. Examine the compensation file and export compensation files as FCS if you want them (see 6 below)
n. Click on *File > New workspace* (no need to save anything else)

5. Setup experiment
    a. Choose/change Rack type (chill 96 or chill 5)
    b. If you compensated, open your saved instrument settings
    c. Deselect unused parameters in the settings tab
    d. Setup analysis window/analysis template/collect all or some of first tube (allows for stopping gate)
        i. Select one well/Tube
        ii. Label *Sample Description* enter your name in *Project*
        iii. Choose fluidics for tube/well: enter volume information/mix/fluid speed/Wash mode
        iv. Optional-enter stopping event number
        v. Label parameters under *Experiment>Annotation Tab* (any label you want)
        vi. Open analysis window/analysis template
        vii. Press play. You can pause the sample or stop the sample if you are finished
        viii. Create/adjust Gates and Plots
        ix. Save analysis template? Select ***A*** button and Save
    e. Choose the remaining wells/tubes (selected wells will have an orange outline)
    f. Label all the orange highlighted wells at once by typing in *Description* text box enter your name in *Project* or label each individually by highlighting in orange and tying in *Description*
    g. Choose fluidics for each tube/well or entire rack (orange): enter volume information/mix/fluid speed
    h. Choose Wash mode for each tube/well or entire rack (orange): Fast is default; choose screen, standard, or extended wash as desired (increasing time)
    i. Optional-enter stopping gate and stopping event number in *Experiment* tab > *Settings* tab
    j. Label parameters under *Experiment* > *Annotation Tab* (any label you want)
    k. Double check plate/rack by selecting *View > Experiment table*
    l. Check sheath and waste levels
    m. Press play – estimate how long samples will take, you may leave and come back

6. Export files and cleaning
    a. Find the “live” sample, right click, and select apply instrument settings, live will move to a blank space
    b. Export FCS: highlight the samples from the sample list that you want to export to FCS, right click and export *leave skip subpopulations checked or you will get an FCS file for each gate!
    c. Copy data files to external USB disk or burn CD/DVD (*File menu > Copy*)
    d. Run clean program: switch to the single tube rack in Experiment panel, add 1ml squirt bottle bleach to tube in single tube holder, right click drop icon and select clean (takes 10 min., not stoppable)