NOTES & HINTS for using SONY MA900

July 2021

Before MA900 power on

- Check sheath tank
  - Fill with sheath fluid to the marked line, if needed
  - Ensure that pressure valve is closed (stopper ring to side)
- Check dH2O tank level
  - If dH2O level is under the marked line, add entire 1L bottle of sterile dH2O
  - Place the empty 1L bottle on the left side of the sink for recycling
- Add 500mL bleach to waste tank
  - Return the empty bottle to the right side of the sink for refilling
- Turn on house air supply
- Wipe sort chamber surfaces with damp KimWipe to eliminate static electricity
  - Deflection plates should be cleaned and dried

Instrument Calibration

- Sort chip
  - Can rescan within 24h of first use
- **Three main procedures:**
  - *1. Fluidics Check*
    - Load Sort chip

      Verify that sample line seals against the inserted sort chip: the white T-adaptor of the sample line will fully insert into position. If the white adaptor is moving in and out of position, check for pressure stability issues: house air is on, sheath tank lid is fully closed and sealed, all tank lids are closed, tank connector fittings are tightly closed, wait for air pressure to stabilize, etc. Reload chip after making adjustments to eliminate pressure leaks.
    - Debubble sheath filter and verify that sample line is dripping

      Follow the on screen instructions and read the status on the MA900 display. The sheath filter debubble step should be repeated until the droplet stream is stable and centred.
  - *2. Chip Alignment*
    - Using Sony Automated Setup beads
    - Look for two sharp peaks in histogram image
  - *3. Sort Calibration*
    - Using Sony Automated Setup beads
    - 3 stages: droplet calibration, side stream calibration, sort delay
    - Can leave the MA900 to complete calibration at this step

      This step takes the most time (up to 20 minutes). It is common for the sort calibration to repeat calibration stages while searching for the optimal droplet drive and droplet clock settings. The sort calibration will timeout after 45min and a troubleshooting message will appear.
- Choose only 488nm laser for calibration
- For 100 micron chip only, choose between standard vs targeted mode (larger/fragile cells)
- Disinfect the sample line between the calibration and sample run
  - 8 mL FACS Clean (1/10 buffered bleach) automated rinse ~10min
  - 10 mL sterile dH2O automated rinse ~10min

Between Users

- Disinfect the sample line using the automated <Bleach rinse> and <dH2O rinse> (~10 min each)
- Disinfect the sort chamber, collection tube holder, sample tube holder, BSC, and desk for the next user with EtOH or H2O2 wipes
- Chip Alignment and Sort Calibration settings will hold for the next user UNLESS the droplet stream or system pressure is interrupted, which interrupts the droplet breakoff:
  - Droplet stream is turned off (to troubleshoot a clog)
  - System pressure is turned off/interrupted to open & refill sheath tank
  - System pressure is interrupted to empty waste tank
  - Any tanks are removed from wet cart
  - MA900 software is shutdown/exited
- If any of the above conditions are met and sorting needs to be continued, the calibration with Sony Automatic Setup beads should be redone.
  - May need to redo only the sort calibration or redo both chip alignment and sort calibration steps.
  - Either follow the on screen prompts OR enter the MA900 software and choose the chip alignment and/or sort calibration icons in the ribbon toolbar menu.
- To avoid redoing the calibration, wait to change tank fluids until the new user logs back into the MA900 software and re-establishes the droplet breakoff monitoring.
  - This can only be done by one new user.

Backup Files

- Save any files to the “Public” folder on desktop and then to the LTRI server or email.
  - Stray files on all other folders will be moved to Public folder without notice.
  - Public folder is not secure or backed up, and will be purged periodically without notice.
- Print PDFs sample workspaces for plot images and sorting info, stats
- FCS files (3.0 & 3.1) can be exported from Experiment list
  - FCS files do not include gating or any sorting info (gates, stats, etc)
  - FCS files do include compensation matrix, assigned keywords
- Offline analysis is possible
- All experiment and FSC data files are stored in a database on the CPU
  - Database will be backed up every month by the facility
  - Database will be purged periodically without notice

Shut down (Daily last user)

- <Shutdown rinse>
  - Bleach & water automated rinse ~20min
  - Followed by instrument & software shutdown
- <Ethanol rinse & Shutdown>
  - Done every 2 weeks on a Friday
  - Always use an empty 30mL tube, despite software prompts for an empty 15mL tube
- Disinfect all areas:
  - Sort chamber and deflection plates
  - Sample loader
  - BSC surfaces
  - Desk area
- Fill the sheath tank to the marked line
  - Best practice is to take the sheath tank to sink to refill to avoid spills.
  - Fill with sheath fluid from the 10L box or the filled 1L bottles in the fridge. Place the empty bottles/boxes by the sink for recycling.
- Empty the waste tank
  - Unclip the tank connector: always transport the tank with the lid attached
  - Rinse the waste tank and sink with water
  - Do not put bleach into empty tank (prevents corrosion of lid parts)
- Shutdown computer

SORTING Best Practices

- Sample prep
  - Sort sample buffer
    - PBS v HBSS
    - Requires 25mM HEPES
    - No more than 1% protein: creates excessive FSC/BSC noise events
  - Accumax
  - Culture media (Beware: phenol red emits in red wavelengths; can use phenol red free media if needed)
- Always add a viability dye
  - Cell impermeable DNA dyes: DAPI, Sytox dyes (Thermo), 7-AAD, PI
  - Fixable viability dyes: FVD eFluor (eBiosciences), Zombie (Biolegend)
- Sort mode: Normal v Semi-purity
- Verify side stream targeting into collection tube holders with a test sort of actual sample cells, then adjust side stream deflection in <Settings> as required
  - 4-way sorting far right, far left positions
  - 2-way 15mL tube sorting
- Post sort checks should be done
  - For rare sort populations, consider sorting a non-target population to do post sort check
- Monitor Droplet Breakoff Camera
  - Solid green: all good
  - Flashing green: trying to establish breakoff monitoring, can sort but not recommended, will time out
  - No color: no breakoff monitoring and cannot sort
  - Assess the Droplet Breakoff Camera image. If the droplet breakoff changes or degrades (even if the indicator remains solid green), consider using some clog troubleshooting steps.
- Clog troubleshooting
  - Unload sample
  - Chip debubble up to 10X
  - Load a tube of dH2O or blue FlowClean reagent, run at sample pressure setting 10
- Catastrophic clog (stream is deflected left or right, stream is hitting the deflection plates, no stream camera image)
  - Turn off stream: <Settings> <Advanced options> <System pressure off>
  - Run BSC AMS (aerosol management system) for 1min on HIGH to clear the sort chamber of biohazardous aerosols
  - Open the sort chamber: remove collection tube holder, clean any spills and dry the deflection plates from top to bottom
- Hints for visualizing data and drawing plots:
  - Consider axis scaling.
    - Log v Biexponential
    - Customize the range of the axis values. Choose plot <Right click> <Properties>, choose Maximum and Minimum axis values.
      In biexponential scaling, dim fluorescent signals are best resolved from negative signals using lower Minimum Values.

SORTING Best Practices (cont.)

- Hints for visualizing data and drawing plots:
  - Consider axis scaling.
    - Log v Biexponential
    - Customize the range of the axis values. Choose plot <Right click> <Properties>, choose Maximum and Minimum axis values.
      In biexponential scaling, dim fluorescent signals are best resolved from negative signals using lower Minimum Values.
  - Density plots v dot plots
  - Use dot plots for coloured backgating. Find your target cells in the parent population plots to ensure that the target cells are on scale and included in all relevant gates.
  - For rare populations, first plot view should be for fluorescence of target cells, not FSC v BSC
  - Gate shapes can be changed
    - Choose gate <Right click> <Convert to> Choose gate shape from drop down menu
    - For polygon gate, vertex can be edited: Choose gate, hover over gate line or vertex, <Right click> <Add vertex> <Remove vertex>
  - Parameter names can be edited within the EXPERIMENT under <Measurement settings>
- Interested in MFI Targeting? Setup of new multicolour expts? Other questions? ASK US!!

Also, check out the Sony MA900 sorting presentation video on Labroots YouTube channel:
Cell Sorting for Multiple Applications How to Optimize your Sort Setup
https://www.youtube.com/watch?v=rXltZ1qAg9U&t=2339s

Timestamps:

- 07:51 Setting up the MA900 – This is great to follow if you need reminding of the steps!
- 13:16 Fluorescent Detection
- 22:38 Why Does Compensation Matter
- 26:38 Switch from Tube Sort to Plate Sort
- 28:21 Plate Holders
- 31:37 Summary