General remarks

Only power users having passed the sorter training at the Cytometry Facility are allowed to operate the Melody cell sorter.

Questions / reporting technical problems:

Feedback form

Emergencies:

044 635 05 12
044 635 02 17
044 635 53 36

Biosafety Note

The Cytometry lab is a BL2 laboratory. It is strictly forbidden to eat, drink or bring food into the lab. When using the sorter you are allowed to wear gloves all the time, however you ALWAYS have to spray them with 70% Ethanol before you touch the computer (mouse/keyboard). Instrument and computer must be left so they are safe to be touched without gloves by the next user!

Task for the 1st user of the day

Ø The first user of the day is responsible to empty the waste tank of the previous day. The liquid has been decontaminated with bleach overnight and is safe to empty directly into the sink.

Starting up the sorter and automatic stream and Drop delay setups

Ø Turn on the red switches of both multi-outlet power strips.

Ø Start the local and remote computers, set the switch board to “remote” and login with your core domain login on the remote computer. The connection to the local computer will be established automatically. (In case of failure establish the connection via the shortcut for the remote desktop on the desktop or switch the switch board to “local” and use the “Admin” account on the local computer, password “BDIS#1”).

Ø Start the Chorus software and login with the account “user” and password “MyMelody#1”.

Ø Turn on the Biosafety Cabinet and open front window to access FACSMelody.

Ø Turn on the main power switch located in the front of the instrument (when turned on a red light lights up).

Ø Change the air and fluidics lines from the EtOH tank to the PBS tank and make sure to spray each connector with EtOH before re-attaching it. Use the attached filter for both tanks.
Note: Make sure that the lid of the sheath tank is properly closed and the tank is pressurized (if the lid is not properly sealed air will leak out and Fluidics Startup will fail).

Ø When the instrument connects click on “Run Extended Fluidics Startup” in the displayed menu and follow the instructions on screen. When completed click “Close”.

Ø Click “Continue” and on the next menu click “Skip”.

Ø Check if the deflection plates are clean and dry.

Ø Remove the closed-loop nozzle and insert the sort nozzle (100 um).
Do not press continue before you have installed the sort nozzle!

Ø Once the sort nozzle is inserted click “Continue”. This will start the automatic stream setup. You can click on the “Stream” button on the left-hand bottom panel to open the stream camera window and check on it.

- Ø Press the “Skip” button when asked to “Run Cytometer Setup”.
- Ø On the next menu press “Run Drop Delay” and follow the on screen instructions (AccuDrop suspension should be in a concentration that will give an event rate between 1500-4000 evts/sec.; if too high or too low the Drop delay setup will fail and an error message with further instructions will appear on screen).
- Ø Press “Continue” once Drop Delay is completed successfully.

Creating a new experiment

- Ø Create a new experiment by clicking on the “New Experiment” button or, alternatively, duplicate a previous experiment without data. Rename your experiment! It should contain your name and the date so you can find it again later.
- Ø Choose the desired sample chamber temperature in the “Sample Temperature” drop-down menu.
- Ø Select the channels that you are using for this experiment and, if desired, insert the labels for each channel. Note that fluorochromes cannot be changed while the sample is running or after data has been recorded.

Note: fluorochrome names displayed for each channel are examples of what can be measured in a channel. Please check the optical configuration of the instrument to determine in which channel your fluorochromes are best measured. If desired, users can add the name of a target fluorochrome by clicking on the plus button behind each channel if it is missing from the list.

Tip: hover over any of the colored rectangles in the Fluorochrome selection menu for laser and filter information.

*Important: Channels with a * have no predefined compensation applied, all other channel tags without * have reference compensation values automatically applied from the Chorus reference library. This can lead to strong incorrect compensation if you do not run your own compensation controls. Check carefully what makes more sense in the context of your experiment.*

- Ø Melody has internal spectral references for automatic fluorescence spill-over compensation. If you wish to run your own compensation controls and overwrite the internal ones you can do so from inside the “2-View Data” tab by clicking on the “Update Compensation” button and following the on-screen instructions.

***Warning:** **Never** update compensation standards via Cytometer>Update Compensation Standards software option since this will overwrite the internal spectral references for all users.*

Setting up the sort

- Ø On the top right-hand menu click on “2-View Data” tab to setup your plots and gating strategy.
- Ø Set flow rate to 1, install the sample tube on the loading port and click the “Load Sample” button.
- Ø Adjust the flow rate, optimize FSC, SSC and channel PMTs if needed. Record data and verify if your gating hierarchy is correct.
- Ø On the top right-hand menu click on “3-Set Up Sort” tab and select the device type (tube, plate or slide), the volume of the sort device and the sort mode from the drop-down menus.
- Ø Tube sorting: For each tube select the initial volume of buffer that it contains; select the desired number of cells to be sorted in each tube (the software automatically calculates the maximum amount of cells that can be sorted depending on the initial buffer volume). Select a tube and select the population to be sorted from the population hierarchy menu. Repeat for the other tube if performing a 2-way sort.

- Plate and slide sorting: Select the correct plate/slide size; select the initial buffer volume per well; select the whole plate or a group of wells and add the sort gate from the population hierarchy menu; while having your wells still selected choose the number of events to be sorted into those selected wells.
  To eject the plate robot use the eject button, to retract change from plate to tube sort mode in the software.

- ***WARNING:*** *A great voltage potential exists between the deflection plates when they are on. Contact with the charged plates will result in serious electrical shock.* ***Do not touch the deflection plates when the plate voltage is on.*** *The plates remain energized even when the sort block door is opened. Red light on the left of the sort block indicates whether the plates are charged or not.*

Starting and monitoring the sort

BIOSAFETY NOTE: *If you are sorting samples that need to be deactivated before disposal (such as virus-infected or human cells), add 200 ml of 14% bleach to the empty waste container.*

- Open the sort collection chamber door and install the collection tubes, plate, or slide.
- On the top right-hand menu click on “4-Sort” tab. Load the sample or resume acquisition and click on the “Start sort” button.

Note: the software will automatically check and position correctly the side streams for the sort; the software also monitors the stream and corrects it automatically. The stream camera can be viewed by clicking on the “Stream” button on the bottom left-hand corner of the screen.

- Record a data file of the sort by clicking on “Start Recording” button.
- Monitor the sort by checking the sort status and sort population plots. Adjust the sample flow rate to optimize the sorting speed and/or the sort efficiency.
- Sorting continues until the required number of cells has been sorted. Acquisition stops and the drawer closes when sorting is complete. If the number of target events is set to the maximum calculated number of cells, sorting continues until one of these situations happen: you manually stop sorting by clicking the “Stop Sorting” or “Pause Sorting” button, the max. no. has been reached or the sample tube is empty.
- To pause the sorting, click the “Pause Sort” button. Sort counts are retained when you restart sorting by clicking the “Resume” button again. But not if you change/unload your sample tube in between!

After the sort

- Stop the sort and unload the sample tube.
- If desired perform a reanalysis and check the sorted populations for purity.
- Go to “5 – View reports” and export the sort report(s) as a PDF if necessary.
- Export your data as fcs files.
- Clean the instrument, i.e. the sample loading area and the collection tube area with an EtOH-soaked paper towel. Also clean up the work space.
- Load a tube of FACSClean and run it for 5 min at sample rate 100.
- Load a tube of FACSRinse and run it for 5 min at sample rate 100.
- Load a tube of fresh, sterile DI water and run it for 5 min at sample rate 100.

- Ø Check whether another user is scheduled after you in the EZ booking calendar. The stream should be switched off when the time between you and the next user is more than 30 min (open the stream camera window and click “Stop Stream”).
- Ø Close the remote desktop view and log out of your personal core domain account on the remote PC and leave the machine running for the next user.

Last user of the day

If you are the last user of the day, proceed to the fluidics shutdown procedure

- Ø Go to “Cytometer” side tab on the navigation bar.
- Ø On the Startup/Shutdown menu select the “Long-Term Shutdown” option.
- Ø Starting the procedure will stop the stream automatically.
- Ø Check that the EtOH tank is filled at least to the inner weld line. Refill if needed.
- Ø Change the air and fluidics lines from the PBS tank to the EtOH tank. Do not change the filter! Use the attached filter for both tanks. Leave the PBS sensor connected to the machine during the shutdown procedure and make sure that this tank is full enough not to have a yellow warning in the software, otherwise the shutdown procedure cannot be started.
- Ø Click “Ok” but ignore the instructions in steps 3 and 4.
- Ø When completed click “Close”.
- Ø Turn off the machine using the front button with the red light on (should go off once the instrument is not working).
- Ø Turn off the computer and the water bath (don’t forget to export your data before).
- Ø Turn off BOTH red switches of the multi-outlet power strips – this disconnects all devices from electricity and cuts the air pressure supply for the device. (Being constantly under pressure will harm it.)
- Ø Follow the waste handling SOP by the instrument to decontaminate the waste (Waste SOP also hanging next to the sink).
- Ø Clean the working area – don't leave tubes, gloves, waste etc. behind.

Data Export Policy

- Ø Export your data as FCS files to the “data” folder on our server (more info on our website under IT infrastructure) or to your institute server (if available). **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.
- Ø **Please note: Only data stored on the server are secured by a backup**.
  The local computer and Chrorus database have no backup.
  Locally saved data can be deleted without further notice.
- Ø You are allowed to keep experiments in the database if they are clearly labeled with date and name. Unnamed experiments which cannot be assigned to a specific user will be deleted **without further notice**.
- Ø Every user is responsible for securing their data directly after their measurement.
- Ø For safety reasons the use of USB sticks is blocked. Check “Data Management & Access” under “IT Infrastructure” on our website for more information.

Note: Any violation of these rules will result in penalty points (see SOP “Penalty points”)!