Standard Operating Procedure

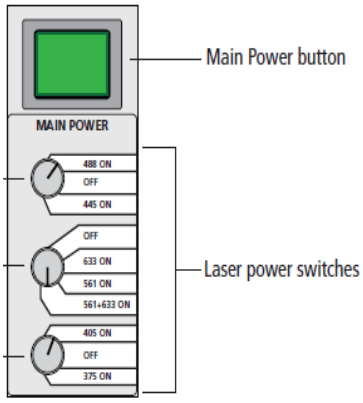
Workflow BD FACS Aria III Cell Sorter

Last change: 25.02.2020

Please note:

If images acquired on IMCES instruments are used in publications we would be grateful if you could mention the facility in the Acknowledgements.

Starting Up the System



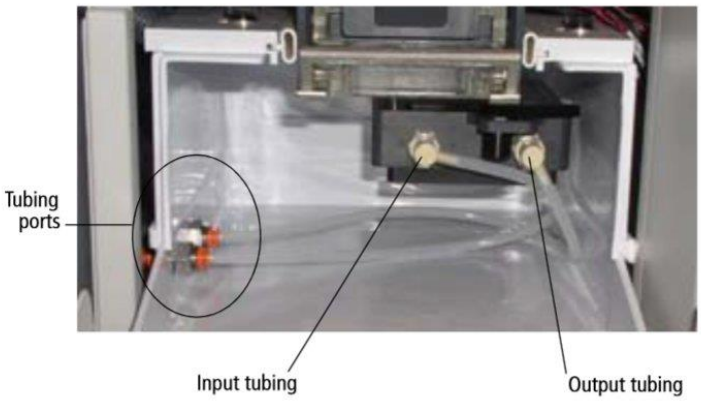
- Start up the computer, start Windows and log in as Operator
- Turn on the cytometer main power
- Start BD FACSDiva software with your Group-name
- Turn on the Laser power (for best performance wait 30` for laser stabilization)
- Check fluid levels in the Cytometer window

Fluidics Startup

- Go to the task bar and choose “cytometer”, “fluidics startup”
  (If there is air in the sheath filter: take a syringe, plug it into the wheel, screw it open and suck up liquids and air until there is no air anymore, close the screw and)
- Follow the program guide (but take off the closed loop nozzle from the flow cell)

Special setup

- Switch on both switches of the water bath-temperature unit
  - Press the power button of the temperature unit
  - Make sure there is enough distilled water in the chamber
  - Make sure it´s set up for your desired temperature
  - Connect input and output tubing to the tube holder
  - Press the power button



- For **S2 sorting** switch on the air pressure system under the table.
  - Check the life-time (if only red light is left - tell the staff)
  - Make sure there is not more than 20% of pressure applied

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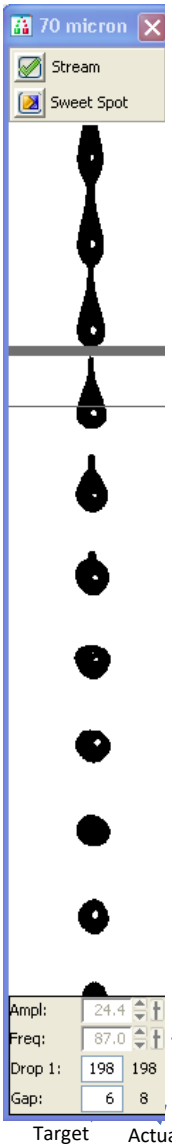
- Make sure the deflection plates are clean (important for sorting!)
- Verify that the appropriate sort setup is selected. (Go to Cytometer -> View Configuration ; choose the appropriate nozzle setup in the new window; press ->set configuration->OK and close the window)
- Turn on the stream
- Optimize the breakoff
  - with the Amplitude (ONLY)
  - Break off point needs to be in the upper third
  - Max. 5 satellite drops
  - Watch out for the correct gap-size (nozzle size dependent)

Nozzle	Cell size	**Gap**
70µm	<12µm	**6-7**
85µm	>12µm	**8-10**
100µm	≤15µm	**10-12**



- Now transfer “drop 1” and “gap” actual-value in the stream window to the white target-value field left beside it.
- Turn **on** the Sweet Spot

- Prepare a tube with one Drop of CS&T beads with ~400µl PBS
- Use Experiment ->New experiment -> CS&T test tube and run as a sample
  - OR Perform CST system setup in Cytometer ->CST(this will take 15 Minutes)
    - Take care to setup the correct CST lot number
    - Run the performance check

Adjust drop delay (every experiment)

- Use 1000µl PBS with 1 drop of "Accu Drop Beads" from the fridge
- Go to task bar and use "new experiment" choose "Accu Drop delay"
- Klick on "+" before the "specimen", "tube",
- Activate the little arrow on the left side (tube pointer) of the "tube_001" so it turns green
- doublecklick on "sort layout"
- **Close the cover of the machine**
- Load the tube and acquire
- Adjust the "threshold rate" with the "flow rate" in the acquisition dashboard.
- If you need to increase the "flow rate" higher than 5 please dilute the accudrops!

Nozzle size	Flow rate events
70nm	1000-3000
85nm	1000-2000
100nm	600-1500

- Switch on "voltage" and the "test sort" –button
- Activate the **near-left** side-stream
- Use the silver screw at the sorting chamber in the machine to get the stream-dots (most important in this case is the middle stream dot and the near left one.) as bright as possible
- Activate "optical filter" button;
    - to make sure to fit the near-left side stream in the filter box just deactivate and activate the optical filter
- **Close the cover of the machine**
- start the sort (**cancel !** warning window)
- Click on "auto delay"
- Use "start run"-button
    - => ! the result should be a parabolic curve with its maximum in the middle !
- Unload the tube
- Don´t save a copy of the sort report

Adjust the stream

- Put two or four tubes in the tube holder; adjust the sidestreams with the voltage sliders roughly
- Open machine, open sorting chamber;
- Turn **"voltage" on** again
- BE CAREFUL NOW! **DON´T TOUCH** THE DEFLECTION PLATES!
- Choose "test sort", close "waste drawer" and
- If the streams don´t hit the tubes properly adjust them with the voltage sliders.
- Turn off "test sort", "voltage", "waste drawer", close the chamber and the machine

Setting Up an experiment with compensation

- Create a new experiment and use e.g. the Doublet Discrimination analysis template.
- Select “cytometer settings” and tick height and width for the FSC and SSC parameters in the cytometer window.

- Delete all colors you do not need!

- Select “Experiment” > “Compensation Setup” > “Create Compensation Controls” > “OK”
- Select “compensation control”; double-click on “unstained”
- Load the unstained control tube.
- Set voltages for FSC and SSC appropriate to your cell type
- Adjust a gate around the population of interest, right click and “apply to all compensation controls”
- Load all tubes in the unstained control to setup the voltages properly.
- Start again with the unstained sample and “record data”
- Now record the data for each tube correctly.
- Check your single staining and adjust the bar around your brightest positive population if not done by machine properly after recording.
- Finish the compensation
- “Select Experiment” > “Compensation Setup” > “Calculate Compensation”
- If everything was correct choose “apply” or “**link and save**” to save your compensation to the panel
- Check your settings in “cytometer” “compensation “ if values are taken over

- You might want to perform (Fluorescence minus one) “ FMO-controls” to confirm that your compensation is correct

Sorting without additional compensation

- Create a new experiment and apply e.g. the Doublet Discrimination analysis template or use an existing experiment.
- Select “cytometer settings” and tick height and width for the FSC and SSC parameters in the cytometer window.
- Delete colors you do not need
- Create a “new tube” and apply a “sort layout” if not there jet
- Populate the global worksheet with plots (dot plot / density plot / histogram)
- Set all your gates of interest with your unstained cells
- Setting up you gating strategy:
    - gates for all tubes in your experiment on a global worksheet
    - gates for specific, single tubes in tube specific worksheet
- acquire some data
    - You might want to perform (Fluorescence minus one) FMO-controls to confirm that your manual settings are correct
- To create further tubes with the same settings just use the “net tube” button beside the “load” button
- Choose which population should go to which tube in the sort layout
    - ☝ big cells and populations in the middle, the smaller ones in the outer tubes
- If you apply stopping criteria do this for your smallest population
- Install the collection tubes and check the side steams
- If necessary change collection tubes and **fill in some sample buffer** (1 sorting droplet is ~1nl)
- “Load” your sample, “record”, “sort”
- **Don´t cancel** the warning window
- Monitor the stream during sorting.
- Stop the sort and save a copy of the sort report if needed
- Take away the tube holder when you are finished

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➢ **If there is a user after you :**

Standby

- Run filtered **FACS rinse** ~5 minutes FR11 (Flow Rate 11)
- Run filtered **FACS clean** for ~5 minutes FR11
- Run filtered **dH_2O** ~5 minutes FR11
- Logout (yes…let the stream keep on running)
- Open the cover of the machine

➢ **If you are the last user:**

Shutting down the system

- Run filtered **FACS rinse** for ~2 minutes FR11 (Flow Rate 11)
- Run filtered **FACS clean** ~2 minutes FR11
- Run filtered **dH_2O** ~2 minutes FR11
- Turn **off** the stream
- Change to the closed loop nozzle
    - Then put a full tube of FACS Rinse in to the loading port
    - Go to the task bar and choose: “cytometer”, “cleaning modes”, “clean flow cell”
    - Put a full tube of FACS Clean in to the loading port
    - Go to the task bar and choose: “cytometer”, “cleaning modes”, “clean flow cell”
    - Put a full tube of dH_2O in to the loading port
    - Go to the task bar and choose: “cytometer”, “cleaning modes”, “clean flow cell” twice
- Go to “cytometer” in the task bar and **perform a “fluidics shutdown” on Friday**
    - Follow the wizard
    - The wizard asks for “Cleaning fluidics”. This is **dH_2O**
- Turn off the Laser power
- Turn off the cytometer main power
- Shut down the computer
- Shut down the cooling unit
- Switch back the connectors from EtOH to PBS tank

Fill out the users-template in the folder.

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If you want to sort S2 specimen, or human blood samples please follow the IMCES S2 working procedure!

Before sorting **S2**-cells please:

- Switch on the air pressure system under the table.
  - Check the filter- life-time (if only red light is left - tell the staff)
  - Make sure there is not more than 20% of pressure applied
- For your own safety make sure you touch your sample, as well as the Aria only with gloves.

After sorting either **human , or S2**-cells please:

- Run filtered **FACS rinse** for ~2 minutes FR11 (Flow Rate 11)
- Run filtered **FACS clean** ~2 minutes FR11
- Run filtered **dH_2O** ~2 minutes FR11

- Stop the stream
- Change to the closed loop nozzle
- Then put a **full** tube of FACS Rinse in to the loading port
- Go to the task bar and choose: “cytometer”, “cleaning modes”, “clean flow cell”
- Put a **full** tube of FACS **Clean at 40 °C!!!** in to the loading port
- Go to the task bar and choose: “cytometer”, “cleaning modes”, “clean flow cell”
- **Leave this hot solution in the flow cell for 2 minutes**
- Put a **full** tube of ddWater in to the loading port
- Go to the task bar and choose: “cytometer”, “cleaning modes”, “clean flow cell” twice

- Go to “cytometer” in the task bar and **perform a “fluidics shutdown”**
  - Follow the wizard
  - The wizard asks for “Cleaning fluidics”. This is **dH_2O**

- Turn off the Laser powers
- Turn off the cytometer main power
- Shut down the computer
- Turn off the cooling unit
- Switch back the connectors from EtOH to PBS tank

After your sort clean the Aria and your bench space and the computer table with 70% Ethanol!

Troubleshooting

Problem:	Precise Problem:	Possible solutions:
Stream; there is **no stream**	• There is no visible stream in the stream chamber	• Activate the stream in the stream window • Check if there is a nozzle in the flow cell • Check if the pressure tubing is connected to the PBS tank • Check if the blue PBS tube is connected to the blue tubed filter • Check if there is enough PBS in the tank
	• There is a stream in the stream chamber but not visible in the Software	• Clean the camera besides the stream
Stream; stram is **not looking as usual**	• Stream is vibrating	• Check if there is the tank vibrating against the blue tubed filter • Check if something is hitting along the blue tube
	• Stream is not forming droplets	• Make sure the right sort setup is chosen for the inserted nozzle • Check if the right nozzle is inserted • Make sure the blue tube is not connected to the ethanol tank
Accu Drops; there are **no stream dots**	• There are no visible side streams in the stream chamber	• Make sure you are sorting with PBS • Check if the voltage is swiched on • Activate the test sort button again • Make sure the side stream voltage sliders are >0
	• There is just a bright line in the side stream window	• The stream might be hitting the waste; adjust the chamber with the screw driver

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	• There are side streams visible in the stream chamber but not in the software	• Close the cover of the machine if its open • Use the silver screw to bring the stream laser in the focal plane
Accu Drops; the side streams **don´t look as usual**	• The center stream does not look as usual	• Use the silver screw to bring the stream laser in the focal plane • Use the 2nd 3rd 4th Drop values to tighten the center stream dot (every value should be half of the one before) • Change the Amplitute by +/- 1
	• You cannot get the center stream in the “optical filter”	• Use the center stream voltage slider to adjust • Move the camera behind the stream chamber with your hand
	• The side streams don´t look as usual	• Use the silver screw to bring the stream laser in the focal plane
	• There are more side streams than desired	• Make sure there are only the side streams activated you wanted (set all other sider stream voltage sliders to 0) • Change the 2nd 3rd 4th Drop values (every value should be half of the one before)
	• You cannot see the left stream	• Check if the optcal filter is activated; deactivate • Make sure the side stream voltage slider is not set to 0

Accu Drops; the Accu Drop Delay is **not woring**	• The machine is not measuring beads after loading the tube	• Make sure the cover of the machine is closed • Open an new Accu Drop Delay- template via the task bar • Check if the lasers are switched on
	• You cannot start the measurement	• Make sure the sorting is not paused • Check if you started the Auto Delay • Adjust the maximum threshold rate for your nozzle
	• The Accu Drop Delay is not coming to a result	• Check if you set up the near left side stream and not the far left
	• There is no curve appearing after measurement	• Just compare the result to a prior measurement with the same nozzle and the same Frequency
	• The curve is not parabolic	• Check if you are measuring Accu Drop Beads • Make sure the deflection plates are clean • Rerun the Accu Drop Delay