BD FACSAria™ Fusion

Standard Operating Procedure

Last change: 6. 8. 2019

Basic principles

- Only registered and instructed users are allowed to operate the BD FACSAria™ Fusion cell sorter.

- When acquiring biohazardous samples, follow universal precautions at all times.
- When acquiring biohazardous samples, the *Aerosol Management System (AMS)* should always be used.
- When removing collection tubes, be aware that the outside of the tube is potentially contaminated. Use alcohol swabs or bleach to wipe the outsides of tubes.

- When working with the FACSAria Fusion the *biosafety cabinet (BSC)* has always to be used.
- When working inside the BSC use suitable protective gloves.

- A 12,000-volt potential exists between the *deflection plates* when they are on. Contact with the charged plates results in serious electrical shock. To prevent shock, turn off the plate voltage before cleaning on or around the deflection plates. Do not touch the deflection plates when the plate voltage is on. The plates remain energized even when the sort block door is open. To prevent arcing (sparking), make sure the plates are completely dry before you turn the plate voltage back on.

Before starting up the System, check the following components:

- External air supply.
  Open the valve of the external air supply. The pressure should be in the range of 6,6 – 6,9 Bar (95 - 100 PSI).
- Room temperature.
  The room temperature should be in the rang of 17,5° to 22,5° and remain constant over the duration of your experiment – if needed, use the air conditioner.
- If you want your samples to be cooled, switch on the water bath and the thermostat and check the preset temperature.
  ! Cooling down the water bath from 18°C to 5°C takes up to 45 min.
- Fluid level in the sheath tank and waste container.
  Refill the sheath tank up to the upper weld line on the inside of the tank and empty the waste container, if needed.
- Sheath filter.
  If there is an air bubble in the filter, let the air escape during the startup procedure - see Fluidics Startup.
- Fluid level in ethanol shutdown tank. If needed, refill the tank with 70-percent ethanol.
  Mix 2,5 l EtOH (1 canister) with 1,05 l dH2O.
- Deflection plates.
  If there is a salt buildup on the deflection plates, clean the plates before the Stream adjustment.

Bold text indicates software elements in the FACSDiva software such as windows, menus, buttons and tabs that are used to complete tasks.
Italics text indicates terms used in the BD FACSAria Fusion User's Guide and in the -User's Guide Addendum.

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FACSAria Fusion Startup

- Turn on the main power of the cell sorter (Fig. 1 - Power switch).

Fig. 1 *Power Panel*

! If the cytometer was just shut down, wait until the system is completely depressurized (stops hissing) before you switch on the main power again.

- Turn on the blower of the BSC by pressing the blower button on the cabinet controls panel (Fig. 2 - Cabinet Controls Panel).

Fig. 2 Cabinet Controls Panel

! The BSC should be switched on at least 20 min before your start with your experiment.

- Check the differential pressure gauge, the differential pressure inside the BSC vs outside the BSC should be 50 +/- 20% inch WC (water column).
- Open the front window of the BSC, ensure that the lower edge of the front window is at the save work access level, which is marked by white on red arrows located on both sides of the BSC frame.
- With the left button you can switch on and off the interior illumination of the BSC. The interior illumination will not work when the front window of the BSC is closed.

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- Turn on the workstation.
- Log in as workstation „Operator“.

- Start the BD FACSDiva software.

- Log in with your FACSDiVa account.
- Turn on the lasers you need for your experiment at the laser power panel (Fig. 3) and turn off the lasers you don't need. Please note that the lasers need up to 30 min for stabilization.

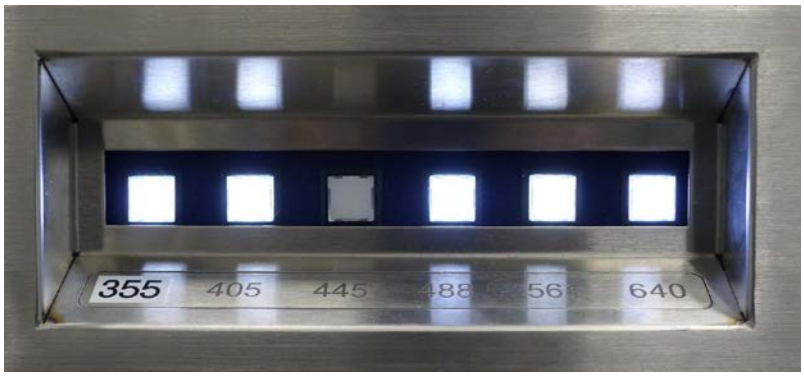

Fig. 3 *Laser Power Panel*

! This cytometer is not equipped with a 445 nm laser.

Cooling of samples and sort collection tubes

- If needed, samples can be cooled. For cooling of the sample injection chamber go to the cytometer menue, select temperature and choose the required temperature. For cooling of the sort collection tubes use the collection tube holders with ports for recirculating water and connect this ports with the input and output tubing.
  - Additonal Informations:
    BD FACSAria Fusion User's Guide, Page 345, Temperature Control Option.

Fluidics Startup

- Select **Fluidics Startup** from the BD FACSDiva **Cytometer** Menue *(*Abb. 4).

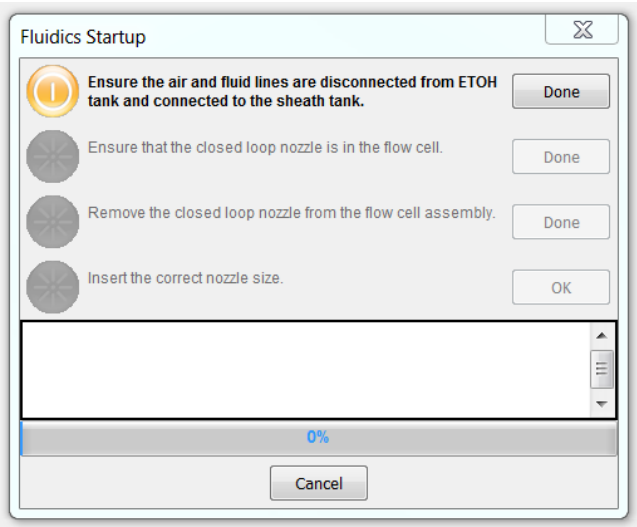

Fig. 4 *Fluidics Startup Panel*

- Follow the instructions from the **Fluidics startup** panel.

- Sheath filter.
  By now if you have detected an air bubble in the filter, you should remove the air bubble. To do this, open the filters vent cap, screw it open, let the air bubble escape and close the vent cap.
  ! The cytometer is under pressure. The filters vent cap will come off if you open the vent cap more than 1/4 turn.

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Stream adjustment

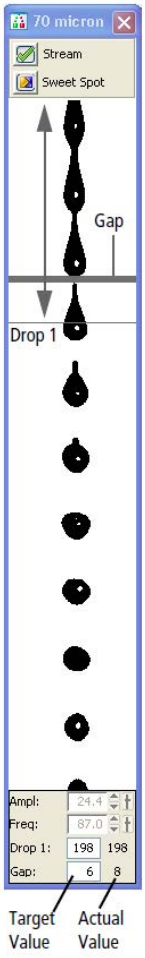

Fig. 5 *Breakoff Window*

- Verify that the appropriate *sort setup* is selected.
  If this is not the case go to: **Cytometer - View Configurations** and select the needed configuration. Enter **Set Configuration** and than **OK** to activate the configuration.
- Switch on the stream with the **Stream** button.
- Open the *sort block door* and verify that the stream flows smoothly from the nozzle to the center of the *waste aspirator*.
- Close the sort block door.
- Let the system run and wait at least 5 min until the *droplet breakoff point* has stabilized.
- Adjust the amplitude to determine the *breakoff point* in the upper third of the window and the *Gap* size matches the gap values from Tab. 1.
- There should be not more than five satellite drops.
- Enter the *Actual Values* for *Drop1* and *Gap* into the corresponding *Target Value* fieldes.
- Switch on the **Sweet Spot,** the *Target Values* will than be attained by the cytometer.

Setting	70 micron	85 micron	100 micron	130 micron
Sheath pressure	70	45	20	10
Amplitude	60	32	12	24
Frequency	87	47	30	12
Drop 1	150	150	150	150
Gap (upper limit)	6 (14)	7 (17)	10 (21)	12 (21)
Attenuation	Off	Off	Off	Off
Drop delay	47	30	27	16
Far left voltage	100	100	80	60
Left voltage	40	35	30	20
Right voltage	40	35	30	20
Far right voltage	100	100	80	60
Plate voltage	4500	4000	2500	2000
2nd drop	20	20	10	0
3rd drop	10	10	5	0
4th drop	0	0	0	0
Flow rate events	1000 – 3000	1000 – 2000	600 – 1500	500 – 1200

Tab. 1 *Typical Sort Setup Values*

Fig. 6 *Acquisition Dashboard*

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Drop delay **adjustment**

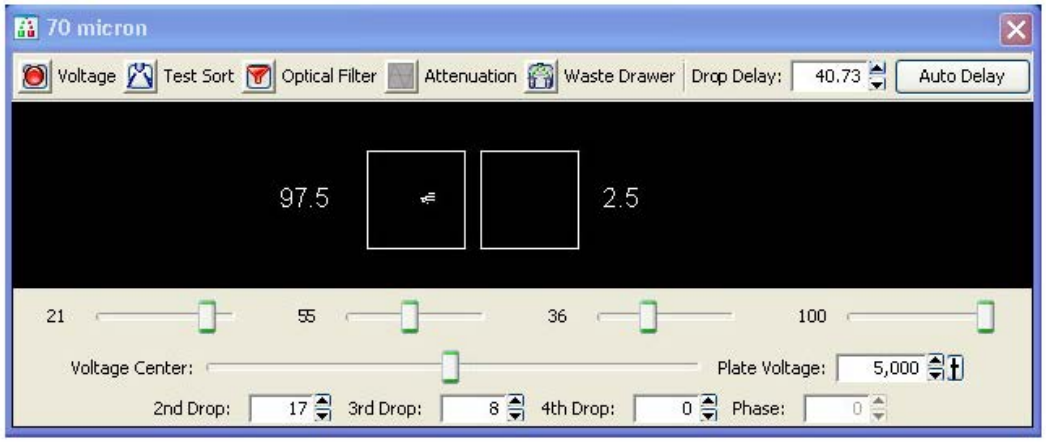

Fig. 7 *Side Stream Window with Optical Filter activated*

- Take one droplet *Accudrop beads* and dilute the droplet within 1 ml PBS. (Depending on nozzle diameter and real *Accudrop Beads* concentration it might be necessary to change the *Accudrop beads* concentration).
- Close the F*low cell access door*.
- Open an *Accudrop Drop Delay* Experiment.
- Activate a sample (click on the little arrow left from the tube, the arrow turns to green when the tube is activated).
- Open the **Sort Layout**.
- Set the **Flow Rate** to a low value in the **Acquisition Dashboard** (Fig. 6).
- Put the *Accudrop beads* onto the loading port and continue with **Load** in the **Acquisition Dashboard**.
- Set the **Flow Rate** to a value so that the **Threshold Rate** matches the Flow Rate events value from Tab. 1.
- Click on the **Voltage** and the **Test Sort** button in the *Side Stream Window* (Fig. 7).
- Activate the near left side stream and set the corresponding voltage value to around 25.
- To make sure that the near left stream is in the right position activate and deactivate the **Optical Filter**.
- Use the long screw left from the sort block to get the stream dots from the middle and the near left stream as bright as possible.
- Start **Sort** in the **Sort Layout**.
- When prompted click on **Cancel** in the *Confirm dialog* for the *waste drawer.*
- Click on **Auto Delay** in the *Side Stream Window* (Fig. 7).
- Auto delay estimation was well done when the result is a parabolic curve in the middle of the window.
- **Unload** the Accudrop beads ditution.

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Side Stream **adjustment**

- Verify if the appropriate tube settings are selected.
- If not, start the *Sort Alignment Software*.
- Select the appropriate device in the *Stream Aiming Device Setup* window.

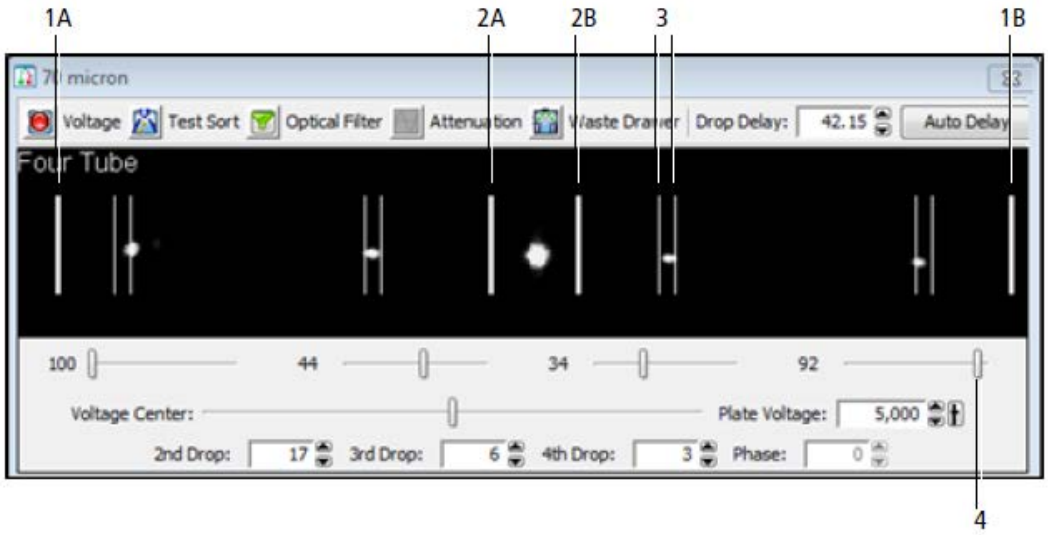

Fig. 8 *Side Stream Window (with reference lines for side streams)*

- Click on **Voltage** and **Test Sort** button in the Side Stream Window (Fig. 8) to show the side stream dots.
- Use the *voltage sliders* (4) to align the *side streams* to the center of each of the *collection device's reference lines* (3).

Setting up an experiment

Compensation

Running Samples

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Experiments with biohayzardous samples (Risk group 1 or 2)

Fig. 9 ***A**erosol **M**anagement **S**ystem Control Panel*

The *AMS* is incorporated into the *BSC* to rapidly evacuate aerosolized particles from the *sort collection chamber* during sorting or analysis. Equipped with a separate HEPA filter, the *AMS* operates independently of the *BSC*. The *AMS* will continue to operate if the *BSC* blower fails, providing an additional level of biosafety protection.

- While working with biohazardous samples the *AMS* should always be in use.
- Before you start your experiment, turn on the *AMS* with *air evacuation rate* low (Fig.9 **Low** button).
- In case of increased aerosol generation stop the acquisition of your sample and set the *AMS air evacuation rate* for at least 1 min with *aspirator drawer* in open position to high.
- Solve the problem with the increased aerosol generation.
- Return the *air evacuation rate* back to low.
- Continue with you experiment.

The air flow should be in the range of 14 ±1 CFM (cubic feet per minute) in Low position. In High position the air flow should be >26 CFM.

In case of a nozzle clog see *BD FACSAria Fusion User's Guide, Page 336, Responding to a Nozzle Clog During a Sort.*

After a sort experiment or before an extended interruption of an experiment

- Run a tube of freshly filtered *FACSRinse* for 5 min on **Flow Rate** 11.
- Run a tube of freshly filtered *FACSClean* for 5 min on **Flow Rate** 11.
- Run a tube of freshly filtered sterile dH_2O for 2 min on **Flow Rate** 11.

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After the last experiment of the day

Preparation for a Fluidics Shutdown

- Check the fluid level of the *ethanol tank* to ensure that the tank will not run empty during the *fluidics shutdown.*
- Check the fluid level of the *waste container* to ensure that the container will not fill up completly during the *fluidics shutdown*.
- Turn off the **stream**.
- Remove the *nozzle* from the *flow cell.*
- Install the *closed loop nozzle* into the *flow cell.*
- Load a tube of freshly filtered *FACSRinse* onto the *loading port.*
- Go to the Task bar and select **Cytometer - Cleaning Modes - Clean Flow Cell** and click **OK** in the *Confirm* window. Repeat this step 3 times.
- Do the same with a tube of freshly filtered *FACSClean.*
- Do the same once with a tube of freshly filtered steril dH_2O.

Fluidics Shutdown

- Go to the Task bar and select **Cytometer - Fluidics Shutdown.**
- Follow the instructions in *the Fluidics Shutdown panel (Fig. 10).*

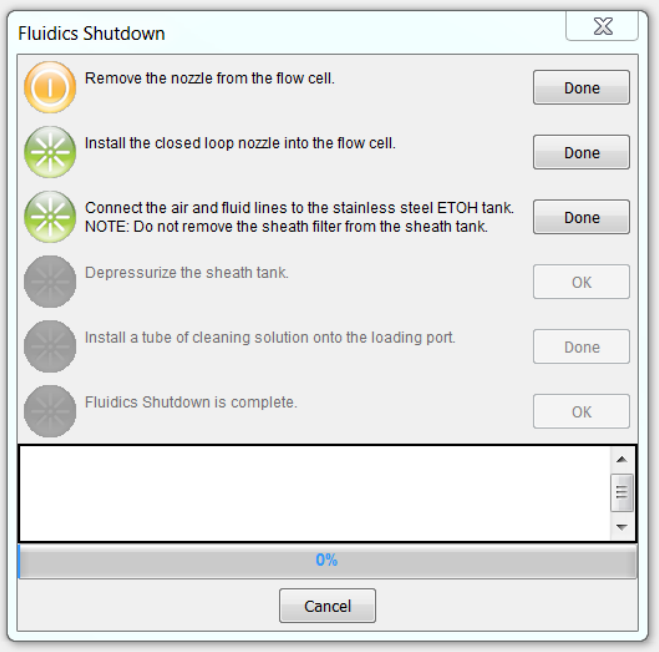

Fig. 10 *Fluidics Shutdown panel*

Use freshly filtered sterile dH2O as cleaning solution.

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After the Fluidics Shutdown

- Turn off the lasers in the *Laser Power Panel* (Fig. 3).
- Release the pressure from the s*heath tank* and the *ethanol tank.*
- Reconnect the air and fluid lines from the *ethanol tank* to the *sheath tank*.
- Export your experiment data.
- If another nozzle than the 70 µm nozzle was used in the experiment, set the configuration back to settings for the 70 mm nozzle.
- Close the *FACSDiva* software.
- Switch off the workstation.
- Switch off the main power of the cell sorter (Fig. 1 - Power switch).
- Close the valve of the external air supply.
- If the water bath and the thermostat was in use, switch them off.
- Close the front window of the *BSC*.
- Fill up the *sheath tank*.
- Replace the *waste container* with an empty one.
- If you have not worked with hazardous samples than let the blower of the BSC run for 15 min.

- If you have worked with hazardous samples, risk group 2 than:
  - Let the blower of the *BSC* run at least for 12 more hours.
  - The subsequent mentioned parts and surfaces should first be disinfected with Kohrsolin extra.

- The following surfaces and parts should be inspected and cleaned when necessary:
  - Inside the *sort chamber* including the used *tube holders*.
  - Inside the *sort block* including *deflection plates*.
  - *Sample loading port*.
  - Collection devices.
- To keep the system free from salt buildup, wipe down all cytometer surfaces that have been exposed to sheath fluid. Clean surfaces with a cloth dampened with Kohrsolin extra, followed by DI water.
- Clean up the laboratory table an the surrounding and put all your waste into the stainless steel waste container.
- Enter your experiment in the logbook.

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Alternative Fluidics Shutdown procedure

(with dH_2O, daily except the last working day of the week)

- Do the same as described in Preparation for a *Fluidics Shutdown* (page 8) but use the dH_2O tank instead of the *ethanol tank*.
- Continue with the **Cytometer - Fluidics Shutdown** procedure (with dH_2O).

Alternative Fluidics Shutdown procedure

(with ethanol as sheat fluid, daily except the last working day of the week)

- Turn off the stream.
- Remove the *nozzle* from the *flow cell*.
- Let the ethanol run for 5 min.
- Insert the *closed loop nozzle* into the *flow cell*.
- Put a tube of freshly filtered sterile dH2O onto the *loading port*.
- Go to **Cytometer - Cleaning Modes - Clean Flow Cell** and click **OK** in the *Confirm window*.

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Standard **O**perating **P**rocedure

Data Management

To save memory (and disk space for permanent storage), it is recommended that you save only parameters that are being used and not more events as you need for a profound statistical evaluation.

While there is no impact on data collection or cytometer performance, responsiveness can decline as more plots, statistics, gates, and events are displayed for each tube. To improve system response time, limit the number of plots displayed in the viewable area of the Worksheet window**.**

To prevent data loss, you should export your data to an external electronic storage medium after you have finished your experiment. (bevorzugter Speicherort auf UKE-Datenserver)

The BD database will be backed up regularly but restoring the database is time consuming and you cannot access the database backup at any time.