

# The MTZ FACS Facility

The MTZ FACS Facility offers state-of-the-art

- FACS Analysis on the BD LSR II
- FACS Cell Sorting on the BD FACSArial

The facility runs as a user-operated facility. Therefore, certain rules have to be followed:

For technical assistance please get in touch with Manuela Guenther (manuela.guenther@tu-dresden.de) or Susann Hoefner (susann.hoefner@tu-dresden.de). She can also be booked via our online booking system (free of charge).

**ONLY USERS WITH SUFFICIENT TRAINING WILL BE ALLOWED TO USE THE FACS FACILITY! ABSOLUTELY NO EXCEPTIONS !!!**

## Operating Rules

**The machines are to be left in the condition you find them e.g. cleaned and ready to use. At the end of your session it is your responsibility to ensure that the machine is ready for the next user. If you find the machine in an unfit state, report this before you start so that the previous user can be notified.**

The first user of the day on the **FACSArial** runs the Fluidics start up (usually done by the FACS operator), see instruction posted next to the ARIAll.

After your sort run FACS Clean for 5 minutes, run FACS Rinse for 5 minutes and then Aqua dest. for 5 minutes at the highest flow rate. If another user will start within 2 hours of your finishing, keep the stream running at the lowest flow rate and leave the ARIAll with lid open.

Before the last user starts the fluidics shutdown, stop the stream and **wait another 30 seconds** for the nozzle to dry before you take it out and put the closed nozzle into the machine.

**Shutdown with Aqua dest. instead of cleaning solution (see instruction posted next to the ARIAll). Empty the waste (sinks in nearby labs), refill the PBS every evening, refill the cleaning tank!**

It is not possible to fill the sheath tank while the stream is on! There is always a little supply of PBS in the FACS room or in Room A.10.057.

Before analyzing or sorting all solutions and cell suspensions must be filtered through a nylon sieve (cell strainer) with a maximum pore size of 40 µm. Removal of clumps is essential!

**Human cells can only be sorted or analyzed if they comply with S1/L1 rules**, i.e. if they are tested free of HBV, HCV, HIV. etc (show proof). There is no aerosol management system. Thus, infectious particles may be released into the environment. Any negligence of this rule will result in permanent loss of permission to use sorter and analyzer, and may have other consequences.

Do not change nozzles or perform other demanding **replacement/repair techniques** on the machines without consulting one of the FACS experts! You will be shown during your orientation what you can modify/touch. For anything else ask for help. Inappropriate adjustments can easily lead to the need to call a service engineer and will result in downtime of the machine.

Ensure your samples for sorting are **sterile** even if you are not carrying out a sterile sort. This reduces risks of contamination for all users. To prevent the necessity of lengthy sterilisation of the machine we ask users to carry out sorting under good aseptic conditions.

Leave the **working space clean** and free of clutter, spilled buffer etc.

After your analysis run the cleaning procedure posted next to the **LSRII**. If you are the last user of the day switch the analyzer off after cleaning.

A dilution of Accudrop beads for **setting drop delay on the FACSArial** is kept in a tube on a clearly labeled shelf in cold room A.10.039. Please return the solution to this shelf after use. If this solution is low when you finish with it, notify lab personnel in room A.10.060.

## **General Rules**

An online booking system is available allowing you to reserve your FACS time. Please see: <https://cgc.med.tu-dresden.de/phpScheduleIt/> and generate your user account.

On-site training sessions performed by BD personnel will be scheduled, and announced in advance for reservations. Such training sessions are obligatory for the use of the FACSaria (the sorter).

The LSRII analyzer may be used after sufficient training by one of the experienced users which has been obtained and has been acknowledged in writing by that expert.

Regular hours are from 08:00 to 18:00 Monday through Friday. Only highly experienced users should use the machines at off-hours as there will be no expert around helping in case of problems. Regular users will only be allowed to book for regular hours.

We reserve the right to deny booking in case of insufficient training or insufficient experience for off-hours.

A personalized entry system into the room and a log-in procedure at the FACS computers will be required and will allow us to track the use and potential abuse of the machines. Damage other than the usual wear-and-tear inflicted by a user must be paid for by that user or that users' institution.

Fees are currently calculated at 10 € per hour (for medical school personnel) for the FACSariaII and for the LSRII. Fees will be charged based on the booked time period. The full fee of 10 €/h will be charged if cancellations of booked time periods are made less than 24 h in advance. Other German universities charge between 50 and 300 € per hour, and we may not be able to maintain our extremely low rate. This depends, for example, on the careful handling of the machines by every user! Fees will be regularly evaluated and follow a strict regimen of cost accounting – the funds will exclusively be used to run and maintain the FACS facility. Charge for colleagues from other research institutions (non medical faculty, non science faculty) currently is 40 € per hour, for commercial institutions it is 100 €/hour.

Data will be collected on the PCs connected to the FACS machines, but should not be analyzed on these computers. Data has to be transferred via our server onto your own computer. It is not allowed to use USB sticks, DVDs or CDs on any computer in the FACS room. Data will be deleted after a certain time period (to be announced) from the FACS computers. There is one additional computer in the FACS facility for analysis purposes, which runs the FACSDiva software (Room A.10.008). We expect this computer to be heavily used and not always available for your data analysis.

Thus, you will need your own software for analysis. We recommend FlowJo, which you can buy from Treestar Corp.; see <http://www.flowjo.com/flowjo.html>. It runs on either Mac or PC. You may alternatively buy the FACSDiva software from BD.

**In case of gross negligence, wasteful and other irresponsible behaviour, we will exclude such users from further use of the facility.**

The FACS facility runs under the supervision of the Institute of Physiological Chemistry (Prof. Dr. Rolf Jessberger). Andrea Stephan is responsible for the administration (billing, access cards, training) and you can contact her on number 6446 or via e-mail [andrea.stephan@tu-dresden.de](mailto:andrea.stephan@tu-dresden.de).

Experts who have received special training  
and may be contacted in case of technical problems are:

Dr. Glen Pearce, Inst. Phys. Chem.  
Dr. Carlos Ocana-Morgner, Inst. Phys. Chem.  
Dr. Karsten Kretschmer, CRTD  
Dr. Sonja Schallenberg, CRTD  
Manuela Guenther, technician, Inst. Phys. Chem.  
Susann Hoefner, technician, Inst. Phys. Chem.

## **Technical Specifications**

### **BD LSR II**

4 lasers: 633 nm (red), 488 nm (blue), 405 nm (violet), 355 nm (UV).  
12 fluorescence and 1 side scatter detector, 1 forward scatter diode detector.  
RFP, GFP, YFP enabled  
Software: B&D FACSDiva 6.1.2.  
20.000 particles/second acquisition mode

### **BD FACSArialI**

3 lasers: 633 nm (red), 488 nm (blue), 405 nm (violet).  
Auto-calibration and -compensation, automatic adjustment of drop-delay.  
10 fluorescence detectors and 1 side scatter detector, 1 forward scatter diode detector (12 parameters).  
Up to 4-way sorting (4 separate cell populations simultaneously).  
Up to 70.000 particle per second sortable (depending on parameters)  
Cell sorts into tissue culture plates (6-, 24-, 48-, 96- and 384-well) of defined numbers of events (from 1 cell until your well is full).  
Temperature-controlled collection device.  
3 nozzle sizes: 100 µm, 85 µm, 70 µm, which allow you to sort cells of different sizes.  
Software: B&D FACSDiva, 6.1.3.

**Additional computer for data analysis.** (Room A.10.008)

## **Data transfer instructions for the LSRII and ARIAI**

It is not allowed to use USB sticks, DVDs or CDs on any computer in the FACS room. Please use our new server.

Server details:  
IP Address: 10.91.33.240  
User name: bd-user  
Password: facs

The LSRII/ ARIAI computer is automatically connected to the server.  
Copy your exported data files to the server.  
Go to your own computer and log into the server using a ftp transfer program (e.g. the add-on application fireftp for the firefox browser) at the above IP address.  
Copy your files onto your computer.

Data is automatically deleted after 1 week.

Only users that confirm their agreement to these rules will be accepted.