GENERAL RULES 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 FACSAriaII and BD FACS Aria III

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

For technical assistance please get in touch with 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 **FACSAriaII and FACSAria III** runs the Fluidics start up (usually done by the FACS operator), see instruction posted next to the ARIAII.
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 30 minutes of your finishing, keep the stream running at the lowest flow rate and leave the ARIAII 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 ARIAII and ARIAIII).
Empty the waste (sinks in nearby labs), refill the PBS every evening, refill the cleaning tank before starting the Shutdown!
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.

GMOS can only be sorted or analyzed when the booking is labeled as followed
"Kurzfassung des Aktenzeichens des SMEKUL, Art der Zellen, GMO" (z.B. 1/91, Mauszellen, GMO)
"SMEKUL reference number, type of cells, GMOS" (e.g. 1/91, mouse cells, GMO)

Any negligence of this rule will result in permanent loss of permission to use sorter and analyzer, and may have other consequences.

Do not 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 asceptic 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 FACSAriaII** 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.

For individual on-site training please refer to Susann Höfner. She will give you the introductions to use the analyzer as well as the sorters.

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 15 € per hour (for personnel at the Faculty of Medicine) for the FACS AriaII, FACS AriaIII and for the LSRII. Fees will be charged based on the booked time period.
The full fee of 15 €/h will be charged if cancellations of booked time periods are made less than 7 days 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 Faculty of Medicine, non Faculty of Science) 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/CDs, external hard drives or mobile phones 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. We have 5 FlowJo dongles at hand which you can lend for a certain time period.

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 Heinze is responsible for the administration (billing, access cards, training) and you can contact her on number 6446 or via e-mail andrea.heinze@tu-dresden.de.

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

Susann Hoefner, Technician, Physiological Chemistry
Dr. Glen Pearce, Physiological Chemistry
Dr. Carlos Ocana-Morgner, Physiological Chemistry
Dr. Alexander Gerbaulet, Immunology

Individual trainings for LSR and ARIA

Training ARIA
1. Some appointments with own samples in which software and sorting procedure are explained, may also be done with highly experienced colleagues

2. At least two appointments for Startup, Drop Delay, Sidestreams and Shutdown
1. Appointment - everything is shown and explained and done once by the user.
2. Appointment - procedures are done without help, errors spotted and fixed by user.
If applicable, there will be a 3. appointment when there were major problems.

Training LSR II
At least two appointments with own samples. Getting to know the machine and the software. Setting up experiments and measuring the samples.

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 LSRFortessa

5 lasers: 640 nm (red), 561nm (yellow green) 488 nm (blue), 405 nm (violet), 355 nm (UV)
18 fluorescence and 1 side scatter detector, 1 forward scatter diode detector.BD High Throughput Sampler for 96-well plates and 384-well-plates

BD FACSAriaII
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: BD FACSDiva, 6.1.3.

BD FACSAriaIII
3 lasers: 635 nm (red), 488 nm (blue), 405 nm (violet)

Auto-calibration and -compensation, automatic adjustment of drop-delay.
11 fluorescence detectors and 1 side scatter detector, 1 forward scatter diode detector (13 parameters).
Up to 4-way sorting (4 separate cell populations simultaneously).
Up to 70.000 particle per second sortable (depending on parameters)
Temperature-controlled collection device.
3 nozzle sizes: 100
3 nozzle sizes: 100 µm, 85 µm, 70 µm, which allow you to sort cells of different sizes.
Software: BD FACSDiva, 6.1.3.

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

Data transfer instructions for the LSRII, ARIAII and ARIAIII

It is not allowed to use USB sticks, DVDs/CDs, external hard drives or mobile phones on any computer in the FACS room. Please use our server.

Server details:

LSRII and ARIAII
IP Address: 10.91.33.240
User name: bd-user
Password: facs

ARIAIII
IP Address: 10.91.50.136
User name: bd-user
Password: facs

The LSRII/ ARIAII/ ARIAIII 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.