

User guidelines for the Core Facility Flow Cytometry PKZI (CFFC)

Before using our equipment, make sure that you are familiar with our user guidelines and billings structure. By signing these guidelines, you confirm that you have read and understood all chapters and will act as intended within this document. Any misuse can lead to an exclusion from the user list and so to the denial of all granted permission in the Core Facility Flow Cytometry (CFFC).

These guidelines contain of following chapters:

1. General Remarks

2. Scheduling

- LSRFortessa
- Aria
- ImageStream

3. Responsibilities

- Starting procedure
- Sample preparation
- Cleaning

4. Data management

5. Billing procedure and Fees

- Special arrangements

6. Biosafety issues

7. Training

8. Acknowledgement

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1. General Remarks

The Core Facility is located on the 3rd floor of the Paul-Klein Center for Immune Intervention, University Medical Center of the Johannes Gutenberg University Mainz, Langenbeckstraße 1, 55131 Mainz and is open around the clock for trained PKZI users. Support by the Core Facility Management will be provided during common working hours Mon-Fri.

The instruments of the Core Facility Flow Cytometry (CFFC) are for general use. They were expensive, so treat them with care, in order to maintain the instruments in a good condition. The cell instruments are operated by Kristian Schütze-trained users only.

Requirements for working on that system are:

- attend the lecture "Basics of Flow Cytometry"
- participate **either** in the regular practical course "Flow Cytometry in Practice" (offered twice a year, register by e-mail to CFFC@uni-mainz.de or a personal introduction in between this two courses
- Read the "Betriebsanweisung" for the operated instrument

A previous appointment for experimental discussion, i.e. sample preparation, staining panel, analysis, before having the first appointment on any of the instruments in order to avoid misunderstandings, time consuming and cost intensive experimental mistakes is mandatory.

2. Scheduling

There is an online booking calendar on the intranet webpage of the FZI/PKZI Core Facility Flow Cytometry (CFFC), where available instrument time is visible. Only the CFFC staff can give the users permission to book the analyser. If an operator is needed to perform an analysis, please inform the CFFC staff at least 1 week in advance.

An appointment for sorting cells can be reserved by sending a completely filled sort request sheet via e-mail to CFFC@uni-mainz.de. The form can be downloaded from the CFFC webpage. Within this request sheet, in order to book a convenient time slot, you will be asked for some important information about your experimental setup, i.e. type of sample, volume and cell number, number of provided controls (experimental and technical), biosafety, staining panel, etc. If the number of cells or samples exceeds the information given, we cannot guarantee sorting all of them. If it is less than announced, the whole time booked will be charged.

If additional time is required on an analyzer, please ask the next user for permission. In the worst case, you have to suspend your measurement and wait until the instrument is available again.

The computers connected to the instruments are not for analyzing your data. Use our data analysis workstation, which you can book in the same way as the analyzer. Following programs are available on CFFC Workstations:

- BD DiVa (offline version of the acquisition software on BD systems)
- FlowJo 10 (standard data analysis software in flow cytometry)
- IDEAS (Image Stream analysis software)

Cancellations

Cancellations within **36 hours or less prior** to the sort or analysis appointment will be billed if that time slot cannot be filled by any other user.

3. Billing procedure and Fees

Actual billing rates per hour are:

Instrument	FZI Member operator free (per hour)
BD Aria III, Cell sorter	35€
ImageStream	15€
BD LSRFortessa, Analyzer	25€
ImageStream	25€

If an operator is needed to use the instrument the fee adds up 30€ per hour. We charge for every 30 minutes started. You will be charged quarterly for the entire duration of all bookings made in the online calendar, irrespective if you really have used the instrument or not.

If you clogged the instrument or left it dirty, an unclogging/cleaning fee of up to 70€ will be charged if the problem can be solved by staff within 30 minutes. If a service technician is needed further costs will be charged.

You will be charged for the entire duration of all bookings made in the online calendar irrespective if you use the instrument.

Every newcomer will have a limited contingent of free training time (up to 4 h) to get familiar with instruments and software. For pilot studies if you need to set up new multicolour panels or try to isolate rare or fragile cell types, you can get up to 2 free trials to establish and adjust your protocols.

All the fees will be charged by Doreen Nothmann, coordinator of the FZI.

4. Responsibilities

It is in your responsibility to clean the fluidics as described in the displayed information sheet before and after starting your measurement and tidying up the working place before you leave. If you are the last user of the day, switch the instrument completely off and shut the PC down.

Starting procedure (LSR Fortessa)

At any step, you may ask CFFC staff, if you need help.

When you arrive, first start with checking for any leakage, potential contaminations, breaks, etc. If you face any problems, please inform the CFFC staff immediately via phone, e-mail or directly, or else you will be held responsible and will be excluded from using the instruments. Second step should be checking the waste and flow levels and if necessary emptying/refilling them.

Log into the "cleaning" account. Perform the quality control by running 1 minute the bead mix in the "cleaning". Enter your name as sample name and check if the instrument is working properly before your flow session. Record your cleaning

Start up and cleaning the instrument (manually):

- 2 minutes with Decon 90
- 2 minutes Incubation (standby)
- 2 minutes with ddH₂O

Sample preparation

Directly before loading every sample has to be filtrated through a at least 70 µm mesh or finer.

Cleaning

After finishing your measurement, repeat the above-mentioned cleaning (see Starting procedure). Tidy up and clean the working space, export your data and delete the data in your experiment in the DiVa software. If any small waste bag is full, prepare it for autoclaving. Ensure that the instrument is working.

5. Data management

The computers connected to the instruments are for data acquisition only and not for data storage. Especially the databases of our BD machines are quite sensitive to size. Hence, we regularly clean up the database without any announcement. The responsibility for data created in the CFFC lies with the user. We recommend exporting your data immediately. We have therefore connected a network drive U: on our file server. It is available for all registered users in the PKZI. Data on this file server will be deleted automatically after three months. Data saved anywhere on the computer will be deleted on an irregular basis.

6. Biosafety Issues

The CFFC is **not** allowed to sort samples beyond biosafety level 1 (S1).

The CFFC can accept human samples from tested patients only if you provide a PI or physician signed datasheet that the sample is negative for HIV and Hepatitis (A, B, C). If you want to sort human material, please contact the CFFC staff prior to analysis. Human cell lines have to be tested regularly for contaminations and authenticity. 10 µl of every human sort sample has to be provided for storage.

To process S2 material, you might use our S2 laboratory after approval by the representative for genetic engineering (GVO-Beauftragter). 10 µl of every sample has to be provided for storage. It is forbidden to analyze unfixed human material on our analyzers. If you want to do this kind of experiment please contact the CFFC staff in advance.

7. Training

As already mentioned, we are offering twice a year one lecture and one practical course, namely

- Basics of Flow Cytometry (mandatory)
- Data analysis in Flow Cytometry
- Flow Cytometry in Practice
- additional personal introduction to the analyser
- additional personal introduction to the sorter
- additional personal introduction to the ImageStream

To apply for any of the trainings, send an e-mail to CFFC@uni-mainz.de. In urgent cases, you may ask for a personal introduction. To do so, send an e-mail to CFFC@uni-mainz.de. The lectures do not require any registration.

8. Acknowledgment

For publishing data generated by the CFFC services (e.g., using FACS machines, analysis tools, bench space etc.), we ask you to include an acknowledgement in the form of "Support by the FZI/PKZI Core Facility Flow Cytometry is gratefully acknowledged." Failure to do so may affect the future use of the CFFC services. We would very much appreciate a copy (PDF) of the published article/doctoral thesis and a quick note when you have concluded your project. Thank you for your cooperation!

9. Closing remarks

Please follow these rules to prevent unnecessary downtimes and expenses due to misuse. Deliberate infringement or careless behaviour will lead to a forbiddance of using our instruments.

We want to cover all the needs of our users, including new (uncommon) methods and new instruments. Feel free to ask or discuss everything related with us.

CFFC Manager:

Kristian Schütze

CFFC@uni-mainz.de

Tel.: 06131/17 6179

Head of CFFC:

Prof. Dr. Tobias Bopp

CFFC@uni-mainz.de

Agreement

I have read the above terms of use and agree to comply with them. I understand that a violation of these terms of use may result in the withdrawal of permission to use the Core Facility Flow Cytometry (CFFC) instruments and/or facilities.

Date:	
Printed name of user:	
Signature of user:	
Printed name of group leader*:	
Signature of group leader:	
Please fill in group name and billing address:	
FZI-member (yes/no)	
Please specify if the user is paid by a grant (for accounting purposes only):	

* The group leader's name and signature are required for billing authorization purposes.