User Guidelines FCCF:

In General

An introductory training and brief formal orientation is mandatory for all users before beginning use of the facility. Training is provided for use of the analyzers and the Sony MA900 cell sorter. AriaIII, Aria Fusion and FACSDiscover cell sorters are in general only run by core employees and all experiments must be discussed with and scheduled with a core facility employee in advance. We do not permit access to the instruments for users who were not trained directly by core staff.

It is imperative that you plan your experiments carefully. The Core is always available to consult on protocols and procedures prior to implementation and we strongly recommend you come to us before starting any new experiment to avoid any expensive mistakes. This includes confirming that the fluorescent labels and their combinations can be analyzed on the instrumentation we have available in the Core.

Any non-routine multicolor/multifluorochrome experiment (i.e. not familiar to you/you have not done before) you wish to do should have a pilot experiment done beforehand. In our experience, although our instrumentation has multicolor capability of up to 50 colors, the antibody titering and instrument set-up and compensation or unmixing for multicolor applications is not trivial and may take several pilot runs to work out. It is imperative that you discuss any complex multicolor experiment with a core employee prior to planning and/or ordering supplies so as to avoid any problems or misunderstandings. This is even more important if you wish to utilize the Cell sorter multi-color capabilities. Good communication with the cell sorter operator is also essential for the success of your experiments.

Your first visit

We therefore recommend the following procedure. First you make an appointment with the core facility where we discuss the overall goals of your work and how to customize your experiments. When you have prepared your test samples according to the recommendations made

during the initial meeting with the core facility, we will introduce you to the instruments making a basic acquisition setup with beads. With this data we then will show you the possibilities of data analysis located at the core facility, thereby discussing any future recommendations for your experiments.

This does also mean that NO SAMPLES for CELL SORTING will be accepted and NO ACCESS to the analyzers will be given without prior consultation with a core employee.

It is important to have already a filled out the registration form that can be found under following link in the intranet: https://btc-community.uni-bonn.de/registration/

Biosafety

Flow cytometry core facilities are multi-user facilities where many different samples from various sources that may contain known or unknown human pathogens are investigated. The safety of facility personnel and the user is of ultimate concern.

Fees

	Training and Development	Core Facility Users from University:
Analysis	40€/person	15 €/h (Canto, MACSQuant, Luminex)18 €/h (Fortessa) 22€/h (Sony ID7000)

Image Stream Analysis	40€/person	40 €/h 80 €/h (assisted analysis)
Cellsorting	80€/person	80 €/h (assisted) 40€/h (unassisted)

Definitions

Core Facility Partners significantly contribute to the structure of the Core Facility, the development of techniques and the increase in knowledge in the field of Cytometry and related Sciences for the benefit of joint scientific projects. Expenses of the project are included in peer-reviewed funding or related cooperative projects, like for example the SFB 704 initiative.

Core Facility Users are members of the University of Bonn or Associates and have to contribute to the running costs of the Core Facility by internal cost allocation. Beside the access to the instrumentation of the core facility, they are given priority for training, service and assistance in design and interpretation of experiments by the Core Facility members as compared to other users.

All other users are all other users

Acknowledgement

Any formal presentations or publications resulting from work performed in the Core should be acknowledged and a reprint should be provided. Some instruments have been purchased from grants or other sources which also deserve acknowledgement. The following statement

is suggested: “We would like to acknowledge the assistance of the Flow Cytometry Core Facility at the Institute of Experimental Immunology, Medical Faculty at the University of Bonn.”

For any details on the organisation or the design of future projects please contact Andreas Dolf

Technical Considerations

Users can make an arrangement with the core facility so that they can prepare their samples in the work areas of the facility. Core employees are available for advice on sample preparation and some reagents are available for testing.

All data must be collected on the hard drive. Data can be transferred to the BTC server, cloud storage or in exceptions to an USB device. Users get automatically an account on the data server of the core facility for convenience. The Core is not responsible for lost data, so please make sure that you have saved your data properly. Data that are older than 3 month will be removed without warning.

Data analysis is available on two work stations located at the Core. There are two Windows computer, where you can use Flowjo software, Sony ID7000 Software and IDEAS for ImageStream Data analysis.

All samples run on the FACSCAntos or LSR Fortessas of the Core Facility must be in 12 X 75mm polystyrene tubes only from Sarstedt. (Ordering number: 55.1579.002) In general, cell densities should be 1×10^6 to 5×10^6/ml for the Canto, LSRFortessa orID7000 1×10^6 to 10×10^6/ml for the AriaIII and AriaFusion, and each tube should have a volume of 300 µl to 1 ml.

ALWAYS bring NEGATIVE AND POSITIVE CONTROLS. To make appropriate conclusions about your samples the proper controls are necessary. Negative controls: Cells only (No stain). Specificity controls: If an indirect antibody staining method is employed, it is important to include a control in which cells have been stained with only the secondary antibody. This will indicate if there is “non-specific staining” or Fc region binding from the secondary antibody. Compensation controls: In experiments which require simultaneous staining using two

or more fluorochromes, it is necessary to prepare controls that have been stained with each dye separately. These single color controls are required for adjusting cross-over signals between dyes or detectors and are crucial for multicolor immunophenotyping. Single stain controls can be performed on compensation particles e.g. from BD, Thermo Fisher, Biolegend, Slingshot or other vendors.

Samples which are suspected of having aggregates (cell clumps) must be filtered (this is for analyzers and sorters). Fourty micron nylon mesh is available at the Core. Falcon brand cell strainers for 50ml conical tubes (Falcon #35 2340 – 40um and Falcon#35 2350 – 70um) and filter top caps found on Falcon brand polystyrene 12×75 mm tubes (Falcon #35 2235) can also be used. We also recommend samples should be kept on ice or chilled and in dark until ready to run on the machine.

PLEASE SHOW UP ON TIME. You should give at least 24 hours notice if you need to cancel an appointment. If you are late for an appointment and run into the next users appointment you must stop acquiring your samples and finish at a later time. In particular if you are going to be late for a sorting appointment more than one hour then it is your obligation to check to see if your sort can still be done because other sorts may be scheduled on the same day. If there are other sorts scheduled then they will have priority.

Responsibility

All users are responsible for daily maintenance of the machine. After use, every user should follow the instrument specific Shut-down and maintenance procedure trained by the core facility staff at the introduction. Users that do not follow the rules, for example running unfiltered cells that clump and clog the machine, leaving the machine on, not doing End-of-Run maintenance and making sure that the waste is emptied, and the sheath tank is filled, etc. will be identified and excluded from using the instruments. These instruments are common equipment and must be treated with respect so that we may operate them for a long time without incurring additional expense due to misuse.