



**APC MICROBIOME INSTITUTE FLOW CYTOMETRY PLATFORM
USER LICENSE AGREEMENT**

Title: FLOW CYTOMETRY PLATFORM USER LICENSE AGREEMENT

Effective Date:

Approvals (Signature and Date):

Author Panagiota Stamou

Technical Authority (Signature)

Date:

1 User-license Agreement APC Flow Cytometry Platform

1.1 Preface

The APC microbiome flow cytometry Platform is available for every researcher working at APC and UCC as well as anybody from outside of the college. We offer professional and dedicated services for all our users, including training, fully operated sorts, panel designs, and seminars-presentations on flow cytometry for groups or individuals. We ensure equal access to state of the art FACS analysis and cell sorting instrumentation. The FACS facility houses UCC's professionally supervised shared category 2 biosafety level cell sorter in a Biosafety Cabinet. We work closely with Health and Safety to ensure a smooth process of risk assessment evaluation in order to accommodate as many as possible category 2 samples on the cell sorter.

Research gets ever faster, more demanding and complex. We can't do magic, but we can help you to meet your milestones quicker and with a positive input-output relation. APC microbiome flow cytometry Platform offer scientific and technical expertise on our respective analytic platforms and we will always protect the IP of our users.

As core facility we aim to stay in touch with the work of each individual user. We offer guidance and help, not only on technical aspects, but also on scientific questions whenever possible. We encourage all users to acknowledge the flow cytometry Platform on any papers that were generated with the help of instrumentation of the flow cytometry Platform or credit authorship to members of staff that have significantly helped the science published.

Contact: Dr. Panagiota Stamou
E-mail: panagiota.stamou@ucc.ie
Phone: +35321490 1395

Web:



1.2 Available services and instruments

The FACS core facility provides a number of services to each registered user as defined in the following list:

Service	Definition/Description
<p>Training of Msc/Bsc students</p>	<p>As most MSc/BSc students start in the lab at the same time, they will be asked to attend a general seminar on FACS and our main analyzer software FlowJo. Students will be taught the principles and limitations of FACS.</p> <p>As most MSc/BSc students are usually repeating existing protocols and rarely have to create new panels, their training will be simplified and reduced to:</p> <ul style="list-style-type: none"> • Starting the instrument/switching the instrument off + cleaning the instrument • Waste disposal • Running analyses from preexisting templates including compensation calculation • Exporting and deleting data • Data safety and integrity <p>If a MSc/BSc student has to establish new staining panels or will predominantly work with FACS, the training will be the same as for PhD students, Postdocs, or technicians.</p> <p>Training comes at no extra cost other than the runtime of the analyser.</p>
<p>Training of PhD students/PostDocs/Technicians</p>	<p>Training for research technicians / PhD students / Postdocs will be 1:1 or 2-3:1 sessions and require a simple stained sample supplied by the user. This will most optimally be a 4-5 color panel if the user is thinking of performing multi-color FACS analysis. A meeting prior to training is mandatory and will be requested by facility staff.</p> <p>The training will last min. 2 h and will include all necessary steps to establish and perform FACS analyses. Trainees are expected to have a basic knowledge of FACS analysis.</p>

	<p>If you don't have a fundamental knowledge of FACS, a separate meeting with a seminar on the technology will be scheduled prior to the training.</p> <p>The training covers:</p> <ul style="list-style-type: none"> • Starting the instrument/switching the instrument off + cleaning the instrument, waste disposal • Performing CST Instrument performance check • Creating new experiments and templates • Compensation theory and PMT optimization • Doublet exclusion and sensible gating strategies • Exporting and deleting data • Data safety and integrity • Possible data analysis strategies <p>Training comes at no extra cost other than the runtime of the analyser.</p>
<p>Experimental planning and panel design</p>	<p>We provide assistance in planning any experiments involving FACS analysis and cell sorting, including dye choice and additional preparation steps that would be beneficial for conducting experiments or cell sorts. We will advise on downstream processing of the samples according to our experience and experimental proficiency. We strongly suggest involving us at an early stage of your experimental planning in order to provide you with our recommendations gained from years of FACS analysis and cell sorting. Meetings can be requested at any time with the staff of the facility.</p>
<p>Panel optimization</p>	<p>If you encounter difficulties with your current panel, we will analyze your panel (dyes and markers) together with you and try to identify improvements for future experiments.</p> <p>Please make an appointment with the head of the Flow cytometry Platform, Lab to ensure that ample time is allocated for</p>



	your questions and that all necessary data is present at the meeting.
Cell sorting	Cell sorting is fully serviced and performed by facility staff only. We offer regular and sterile Cat2 cell sorting using our FACS ARIA FUSION sorter. For all sorts we require risk assessments (biosafety questionnaire) to be provided, but we offer help with this process as we have a good working relationship with Health and Safety. Terms of service for cell sorting can be taken from Flow Cytometry Platform Manager.
Lectures on FACS analysis	The Flow Cytometry Platform provides along a FACS seminar covering topics on flow cytometry. We strongly encourage students or researchers new to cytometry to attend this seminar. Smaller lectures on FACS analysis can be requested through the facility for research groups or departmental meetings. We are happy to incorporate/address specific questions that should be covered during the talk.
Lectures on cell sorting	As cell sorting has different challenges compared to regular FACS analysis we provide a seminar on the basics of cell sorting. This includes how to prepare samples for cell sorting, caveats to surpass, and what can be expected as a result from different sorting setups and samples. Seminars on cell sorting can be requested through the facility for research groups or departmental meetings. We are happy to incorporate/address specific questions that should be covered during the talk.

1.3 Available instruments

The APC Flow Cytometry Platform provides access to high-end FACS analyzers (BD FACS Calibur, Accuri, Celesta), a cell Imager (GE Healthcare In-Cell Analyser 1000) and one category 2 biosafety cell sorter within Biosafety Cabinet (BD Aria Fusion). All instruments are described in detail on the APC intranet page with specifications of excitation and emission for each parameter. The following list summarizes the main features of the instruments available:



Instrument	Description/Availability
<p>Becton Dickinson FACS Calibur FACS analyzer</p>	<p>Software: Cell Quest</p> <p>Excitation lasers: 488nm, 635nm</p> <p>Emission Detection 4 colors, Standard set installed: FL1 530 nm (FITC), FL2 585 nm (PE/PI), FL3 >670 nm (PerCP) FL4 and 661 nm (APC) with FL4 option.</p> <p>Service: Instrument is cleaned thoroughly every month.</p> <p>Specialty: This instrument can be used for up to 4 fluorescent parameters plus FSC, SSC.</p> <p>Availability: Instrument can be booked on line at: apc.uccfacscalibur@ucc.ie via outlook calendar after user training. The instrument is user operated.</p>
<p>Becton Dickinson ACCURI FACS analyzer</p>	<p>Software: BD Accuri C6 Plus Software</p> <p>Excitation lasers: 488 nm, 640 nm</p> <p>Emission Detection 4 colors, Standard set installed:</p> <ul style="list-style-type: none"> • FL1 533/30 nm (eg, FITC/GFP) • FL2 585/40 nm (eg, PE/PI) • FL3 >670 nm (eg, PerCP, PerCP-Cy5.5, PE-Cy™7) • FL4 675/25 nm (eg, APC) <p>Service: Instrument is cleaned thoroughly every month.</p> <p>Specialty: This instrument can be used for up to 4 fluorescent parameters plus FSC, SSC.</p> <p>Availability: Instrument can be booked via booking calendar after user training. The instrument is user operated.</p>



<p>Becton Dickinson Celesta FACS analyzer</p>	<p>Software: BD DiVa 8.0.1.1</p> <p>Excitation lasers: 405nm, 488nm, 640nm</p> <p>Emission parameters:</p> <p>Service: Instrument cleaned thoroughly every second Monday, CST run prior to user bookings in the morning by staff.</p> <p>Availability: Instrument can be booked via booking calendar after user training. The instrument is user operated.</p> <table border="1" data-bbox="1128 430 1485 1176"> <thead> <tr> <th colspan="2">Blue/Violet/Red</th> </tr> <tr> <th>Lasers</th> <th>Fluorochromes</th> </tr> </thead> <tbody> <tr> <td rowspan="5">405 nm</td> <td>BD Horizon BV421, V450, Pacific Blue</td> </tr> <tr> <td>BD Horizon BV510, V500</td> </tr> <tr> <td>BD Horizon BV605</td> </tr> <tr> <td>BD Horizon BV650</td> </tr> <tr> <td>BD Horizon BV786</td> </tr> <tr> <td rowspan="3">488 nm</td> <td>BD Horizon BB515, FITC, Alexa Fluor® 488</td> </tr> <tr> <td>PE</td> </tr> <tr> <td>BD Horizon PE-CF594, PI</td> </tr> <tr> <td rowspan="3">640 nm</td> <td>PerCP, PerCP-Cy5.5, 7-AAD</td> </tr> <tr> <td>APC, Alexa Fluor® 647</td> </tr> <tr> <td>BD Horizon™ APC-R700, Alexa Fluor® 700</td> </tr> <tr> <td></td> <td>APC-H7</td> </tr> </tbody> </table>	Blue/Violet/Red		Lasers	Fluorochromes	405 nm	BD Horizon BV421, V450, Pacific Blue	BD Horizon BV510, V500	BD Horizon BV605	BD Horizon BV650	BD Horizon BV786	488 nm	BD Horizon BB515, FITC, Alexa Fluor® 488	PE	BD Horizon PE-CF594, PI	640 nm	PerCP, PerCP-Cy5.5, 7-AAD	APC, Alexa Fluor® 647	BD Horizon™ APC-R700, Alexa Fluor® 700		APC-H7
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<p>Becton Dickinson Aria Fusion cell sorter with Biosafety Cabinet</p>	<p>Software: BD DiVa 8.0 Excitation lasers: 488nm, 561nm, 640 nm Emission parameters:</p> <p>488 nm¹ SSC FITC, BD Horizon Brilliant™ Blue 515, Alexa Fluor® 488 PE BD Horizon™ PE-CF594, PE-Texas Red®, PI PerCP, PerCP-Cy™5.5 PE-Cy™7</p> <p>561 nm² PE, DsRed BD Horizon PE-CF594, PE-Texas Red®, mCherry, PI PE-Cy™5, PE-Cy5.5 PE-Cy7</p> <p>640 nm³ APC, Alexa Fluor® 647 Alexa Fluor® 700, BD Horizon™ APC-R700 APC-Cy7, APC-H7</p> <p>Service: The instrument is strictly run by facility staff only. Bookings can be made after contact with the Flow Cytometry Platform Lab manager via email at panagiota.stamou@ucc.ie. We provide sorting service for Cat1 and Cat2 samples with a possibility to prepare “sterile” runs with prior notice</p> <p>Availability: We are available for every user on a first come, first served basis. The instrument can be booked via email to the facility staff. The instrument is facility operated.</p>
<p>GE Healthcare In-Cell Analyser 1000</p>	<p>Automated cellular and subcellular imaging system for fast , automated multiwavelength imaging and analysis in fixed and live cells</p> <p>Illumination:Epifluorescence, 100 W xenon lamp, transmitted light source, CCD camera, Focusing: Confocal laser detection, Piezo positioner, focus time < 400 ms Plate formats: ompatible with 96well plates reader Availability: Instrument can be used after training. The instrument is user operated.</p>



1.4 APC Flow Platform Access Charging Plan:

The use of equipment and services offered by the facility will be free of charge for all APC-affiliated users. Services offered include: assisted acquisition, FACS sorting, practical and theoretical training, experimental design and data analysis. APC users are required to provide their own stock of SHEATH FLUID.

For UCC (non-APC affiliated) and Industry clients, there will be an access fee structure (charges per hour) for the operation of the Flow Cytometer and Cell Sorter platforms. In addition, a charging structure will be in place for accessing the assisted acquisition, FACS sorting, practical and theoretical training, experimental design and data analysis services offered by the Flow Cytometry Facility. The platform is in the process of implementing iLabs Operations Management Software which will facilitate streamlined storeroom management, sample processing, equipment reservation management, usage tracking, billing and invoicing, reporting, lab requisitioning, and spend tracking tools. Requisition and invoice integration into the UCC Agresso system is in progress.



1.4 User duties and facility background services

The APC Flow Cytometry Platform is open for every registered user for self-operated analysis in accordance with UCC Health and Safety and Security policy. Lone working rules must be followed at all times. FACS Sortings are performed solely by Flow Cytometry Platform staff during the core working hours (9.30am-6.00pm), but other times can be arranged in agreement with staff.

We kindly ask every user to follow the below stated rules and policies, in order to keep the Flow Cytometry Platform a clean and reliable working space and useful analysis platform:

Personal Protective Equipment (PPE):

- PPE must be worn at all times while handling and analyzing samples.
- PPE comprises of gloves and lab coats. If your sample requires glasses to be worn you have to bring them with you and inform the facility and other researchers.
- Gloves must be disposed into the autoclaving bags.

Working with the cytometers:

- Only registered researchers or students that have been trained by a member of facility staff, have signed the user licence agreement and instrument SOP may operate the instrumentation. Registered and trained researchers and students are allowed to operate the flow platform instrument unsupervised only after approval by the Flow platform manager.
- For supervised operation of the analyser machines bookings need to be made only after an agreed appointment with the facility staff.
- Users are asked to leave the analyzer and the surrounding equipment (pipets, buffers, filters, etc.) in the same clean state as they have found it. If users find the analysis station untidy, please approach the facility staff to ensure the previous user can be contacted and informed about his or her failure to clean.
- Users are expected to follow the general start-up, cleaning, and shut-down procedure present at each analyzer in writing and described in detail in the facilities “analyzer manual” and taught during the training session.
- All samples with biological safety category 2 or higher that have no approval from Health and Safety for live measurements must be fixed prior to entering the facility and running the analysis.
- All biological or contaminated waste must be disposed of into the “biohazard disposal bins” provided by the facility.
- Exchanging sheath and waste canisters is part of operating a facility owned analyzer. Every user is expected to refresh sheath fluid if it is running empty and to exchange waste tanks either when they are 50% full or when they shut down the instrument as last user. Waste treatment is described during the introduction and a SOP plus all necessary reagents can be found at the APC Flow cytometry Lab.
- Users should treat the analyzers with care when operating the system and loading samples. If you are in doubt of your operating procedures, please contact the staff and you will be shown proper use and procedure.
- Users are asked to export and delete their experiment data from the Flow cytometer analyser equipment computer harddrive immediately after recording their



experiments. Users can check the integrity of their data transfer before deleting the experiment from the database, but no more than a month must pass between recording and deletion. The **analysers computer drive will be cleaned every 1st Monday of a new month** regardless of the nature of the data.

- Bookings of the instruments are not to be changed within a one working day period prior to the actual appointment. Booking durations are set within one working day period and can only be changed or modified in agreement with the Flow cytometry platform manager. Failure to show up for a booked appointment without cancelling will result in full charge for the duration of the booking.
- The Flow cytometry platform will be open for help for user operated facs analysis at any time if we are not busy otherwise. The facility will not run your samples as part of the analyzer booking and we don't offer staining services. All samples must be stained by the user in his/her own lab.

Cell sorting:

- Cell sortings are done in close cooperation with Flow cytometry platform staff. All information on the panel and the sample numbers/conditions must be made available to the facility staff prior to the sort.
- Every sorter user should come and meet with Flow cytometry platform staff prior to the planned sort in order to discuss sample size, volume, nozzle setup, anticipated amount of cells, the required controls and sample timing.
- The facility expects users to be on time for their sorting appointment (+/- 10min). Sorting will start as booked in the calendar. If the sort requires more time and the Flow cytometry platform staff can manage to provide this extension, the booking will be amended accordingly.
- All samples will be filtered at the facility through a 30-100um mesh filter prior to loading on the cytometer
- The facility allows 30min increments for sorts and adds 1h for startup and setup onto the actual sort time. If a sterile setup is required, the facility will run a long sterilization protocol with an extra time of 45min.
- Collection tubes are offered by the facility, but they are untreated. If the user wishes to treat his tubes, he or she must come before hand to pick up the tubes. We recommend overnight coating with sterile FCS at 4°C on a tumbler or rotator.
- Cell numbers obtained from the sorter depend on the quality of the material handed to the facility.
- The facility reserves the right to stop a sort when 1) the booked time is over, 2) the facility core working hours (9:30-18:00 GMT) have passed and no late sort has been agreed on, 3) the quality of the sample is too bad to proceed with, or 4) if the facility obtains knowledge that the sample is not compliant with the associated risk assessment. The latter will result in action from the Flow cytometry platform management in compliance with Health and Safety to prevent such an incident from happening again.
- The facility will not count your sorted cells, but we make very sure that the instrument met all criteria that the number of expected cells sorted is about 80-85% of the software predicted amount (this is an approximation by experience and relatively reliable).



- When you book time on the cell sorter, the facility will provide the following services or items as part of the service:
 - Operator sample setup and sort procedure
 - Full operator surveillance of your sort
 - Pipeting equipment and cell strainers prior to the sort
 - Data transfer and back up.
 - Printout of your sort data and an instrument based assumption of the total number of sorted cells (no guarantee, it is an estimation!)

IT infrastructure and data transfer:

- Users are asked to export and delete their experimental data from the Flow cytometer equipment computer drives immediately after recording their experiments. Users can check the integrity of their data transfer before deleting the experiment from the database, but no more than 1 month must pass between recording and deletion. Flow cytometer equipment computer drives will be cleaned every first Monday of a new month regardless of the nature of the data.
- The facility takes no responsibility for the maintenance and physical integrity/storage of any user's data. We strongly encourage users to back up their data immediately after their analysis on their own groups secure server and to follow the above stated guidelines for data deletion and transfer at all times.
- The FACS data analysis software Diva8.0.1.1, will be available to a dedicated analysis computer in the APC Flow cytometry platform. The FACS data analysis software FlowJo is also provided and three FlowJo software dongles are available for the users to perform FACS data analysis on their computers. In order to get any of the Available FlowJo Dongles a user has to sign on the APC Flow cytometry Platform FlowJo Logbook their name, the Dongle number, the Date, the time they received the dongle and the time they return it. Under no circumstances any of FlowJo software Dongles is allowed to be transferred outside APC premises. A user will need to sign the return of FlowJo Dongles on APC Flow cytometry Platform FlowJo Logbook in the presence of Facility staff member. If the user fails to sign the return of a FlowJo Dongle then the user will be charged for the cost and purchase of a new FlowJo software Dongle.
- Basic introductions into FlowJo are available by the facility.

1.4.1 Cancellation policies

Cancellations of registered bookings can be made respecting the following simple and fair criterias:

Analysis bookings (FACS analysers): All analysis bookings can be cancelled one working day before the actual appointment (Example: Booking for Thursday at 14:00 can be cancelled free of charge until Wednesday 13:59). Please accept that facility staff must be informed by email when you amend or cancel a booking with less than 1 working day. Failure to show up for a booked appointment without cancelling will result in full charge for the duration of the booking.



Cell sorting: Cell sorting appointments can be cancelled seven working days before the actual appointment (Example: Booking On Thursday 16th October at 14:00 can be cancelled free of charge until Tuesday 7th October 13:59). Failure to show up for a booked appointment without cancelling will result in full charge for the duration of the booking.

1.4.2 Failure to provide a compliant sample or “no show”

Failure to provide a compliant sample for analysis or cell sorting: A non-compliant sample is a sample that does not match the criteria of the Risk Assessment or a sample that is improperly prepared and would, if processed as is, damage the instrument. If such a sample is presented to the facility, we will not process it and treat the appointment as a “no-show” event.

No-show: A no-show is a missed appointment or a cancellation on the same day (eg less than 24h). If several such appointments occur the facility reserves the right to apply measures.

1.4.3 Measures taken by failure to follow facility rules

Type of failure	1st Penalty	Repeat penalty
Measuring without booking	Meeting with user to discuss non-compliance	Account blocked until issue resolved with PI
Unannounced amending of booking within the 1 working day period	Meeting with user to discuss non-compliance	Account blocked until issue resolved with PI
Leaving analysis station without cleaning the instrument or the table	Meeting with user to discuss non-compliance	Account blocked until issue resolved with PI
Failure to follow waste disposal rules	Meeting with user to discuss non-compliance	Meeting with supervisor to discuss non-compliance
Misuse of facility equipment, removal of facility owned goods, damaging facility equipment without notifying the facility	Charge of repair costs if applicable, account blocked until issue resolved with PI	Account deleted and expulsion from facility. All other facilities will be informed of this step to raise awareness of problem with user.

1.4.4 Booking and fairness

We ask for some fairness from all our users in order for everybody to have a good working environment at the FACS facility. A quick overview of the requests within the document is listed for review below:



1. Everybody takes care to ensure that tidiness and waste removal is performed without supervision by facility staff.
2. PPE is worn without reminding, no food is consumed in the laboratory space, and doors are treated according to the “no gloves on door handles” rule.
3. Analysis bookings are not amended without prior notice and approval by the facility staff 1 working day before the actual experiment.
4. Facility provided tubes and tips are for facility use only and not to be carried into the users lab for staining procedures or other experiments
5. The computers and IT infrastructure is only used for data acquisition and analysis, but not for storage.
6. Every user deletes her experiments from the Flow cytometer equipment computers hard drive within a month. All experiments will be deleted without prior check on every 1st Monday of a new month by the facility. Failure to delete experiments will result in penalty.

1.4.5 Crediting the facility

The APC Flow Cytometry Platform is a non-profit resource aimed at providing its users with the highest possible support in flow cytometry and high-end equipment. The facility provides scientific support to everybody and we happily give away our valuable experience to our users, but we must be able to prove our worth and productivity to University College Cork College and APC microbiome Institute.

All new equipment must be grant financed and as a core facility, we compete with other core facilities across Ireland to secure funding for state of the art shared resources. It is therefore necessary that the facility and its staff are credited for its work, support, and help in a measurable and auditable fashion.

Acknowledgement: We kindly ask that the facility is by default credited in papers/posters with the following acknowledgement: “We thank APC Flow Cytometry Platform and their staff (names can be used if wanted) for support and instrumentation”. An acknowledgement is justified when facility instrumentation was used, the facility provided training to the authors of the study, and/or sorted during the normal working hours without providing further help other than setting up the experiment or helping with data interpretation.

Possible authorships if more sophisticated help was provided by the facility: We kindly ask users to consider any facility member for co-authorship on papers where the facility has provided in-depth help in designing, testing/setting-up a staining panel, and helped analyzing resulting data for experiments published with the paper or when the facility provided extensive and flexible service (sorting, measurements, analysis) which helped the respective study to conclude (eg. early/late hour and weekend sorts, heavy support with experimental setup, help on post-acquisition data analysis or interpretation).

We understand and accept that the final decision on the form of credit lies with the PI of the individual study. We ask users to consider that the facility aims to support all users, yet, we must make sure that our work and scientific support is acknowledged in order to attract



good staff and future funding. All of this will be again a benefit to the whole of the APC Flow Cytometry Platform users.

1.4.6 User Registration Form

The following form has to be completed in order to be registered as a user of the APC Flow Cytometry Platform. The form can be easily completed with the help of your departmental administration. The form must be presented to the manager of APC Flow Cytometry Platform prior to any use of the facility.

1) Please write in capital letters

New User Name: _____

User E-Mail: _____

Supervisor: _____

Supervisor E-Mail: _____

Department: _____

2) I have read, understood and will oblige to the above stated. I accept the terms of service.

3) I've read and will comply, where necessary and feasible, with the policy of acknowledging the work and support of the APC Flow Cytometry Platform and its staff.

Date:

User Signature:

Supervisor Signature



1.4.7 Check list

The following form accompanies the user registration form:

BSc/MSc Student	yes	/	no
Technician/PhD Student/Postdoc	yes	/	no
Fundamental knowledge of FACS	yes	/	no
Risk assessment present & provided to the facility upon registration	yes	/	no
FACS analysis required	yes	/	no
Cell sorting required	yes	/	no
Lone working allowed	yes	/	no
Capability to lift 20kg	yes	/	no
Will you follow all rules described before	yes	/	no
I understand that I have to save my own data and that the Facility waives all responsibility for keeping my data intact and stored on their instruments and IT infrastructure	yes	/	no
I will follow advice from facility staff and will follow commands of the facility staff when issued in case of any kind of emergency	yes	/	no
I understand that the facility head reserves the right to deny me access to the facility, if I fail to follow the rules and policies stated in this document or if security reasons require a temporary closure of the facility	yes	/	no

Date:

User Signature:

Facility Head Signature
