Flow Cytometry and Confocal Microscopy Core- IRBLleida Performance Standards

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1. Objectives

1. To offer technical assistance and advice regarding new applications, experimental design, sample adquisition and data analysis to all research groups in need.
2. To design protocols of analysis.
3. Data processing and adquisition on demand.
4. Basic Training for Core users, and also theoretical and practical lessons in different Universitat de Lleida degrees.

2. Facilities and Equipment

The Flow Cytometry and Confocal Microscopy Core is divided in two different locations, one for the Flow Cytometry Facility and the other for the Confocal Microscopy Facility. The first one is located in the IRBLleida Biomedicina 2 building next to the Hospital Arnau de Vilanova Facilities in B4.14 laboratory in the 4th floor. The Confocal Microscopy Facility is located in the basement of Biomedicin 1 building in lab -1.1.

The Flow Cytometry facility is owner of an analytical FACS-Canto II Flow cytometer (Becton Dickinson) with 3 lasers and with 4-2-2 configuration, enabling up to 8 color panels (refer to technical specifications in the annexes). This flow cytometer is equipped with a computer with FacsDiva v. 6.1.1 analysis software installed. The facility also has aditional software packages for different applications such as Flowlogic (Inivai Technologies) for offline analysis, ModFit LT for cell cycle determinations, FCAP Array for CBA analysis (BD) and FlowCytomix Pro for FlowCytomix (eBiosciences) analysis.

The facility also includes a magnetic cell sorter **AutoMACS Pro Separator** (Miltenyi Biotec) which enables the serparationa and enrichment of different cell types using the MACS® technique.

Other equipment available are: 4ºC fridge, -20ºC freezer, vortex, micropipets, color and black and white printers, computer for data analysis post-acquisition.

The Confocal Microscopy Facility has a 4 laser (405/488/543/633 nm) Olympus FV500 confocal microscope with 4 objectives (10x, 20x, 40x and 60x). As adquisition and analysis software, this microscopy has the Fluoview 500 software installed. Recently, the facility has incorporated two new confocal microscopes: the FV1000 spectral confocal microscope and the FV10i benchtop confocal microscopy. FV1000 spectral confocal sysetm is equiped with an multichannel argon laser (457/488/515 nm), a 543 nm He-Ne laser, a 633 nm He-Ne laser and a 405 nm

violet diode. It is also equiped with a 4x, 10x, 20x, 40x (immersion oil) and 60x (immersion oil). This system has been designed to perform in vivo studies in a time-lapse mode since it is equiped with an incubation chamber with a controlled temperature and atmosphere. FV10i benchtop confocal microscope is equiped with 4 diode lasers (405/473/559/635 nm) and two objectives (10x i 60x).

Also available in the facility is a Zeiss Axio Observer Z1 fluorescence microscope for time-lapse in vivo studies and it is equiped with different excitationa filters within a wide range of wavelengths (see attached technical specifications in the Annex section).

The facility also contains an Olympus IX81 fluorescence microscope and a Zeiss Axioskop 2 brightfield/epifluorescence microscope with led excitation and two cameras: a color one for brightfield and a monochromatic one for fluorescence.

3. Samples

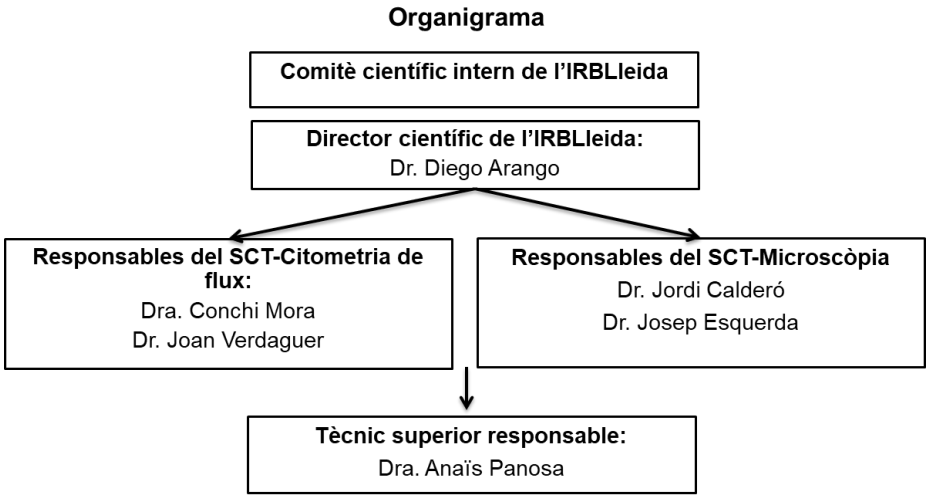
The Flow cytometry and Confocal Microscopy Core recieves samples from different origins (cell lines, mouse, rat primary cultures, whole blood, serum, human and animal plasma) potentially containing human pathogens, thus, it is of great relevance to maintain staff and user biosafety. For this reason, it is critical to know the sample origin and wether there are potentially infectious agents in order to maintain efective biosafety measures. To address these needs, the Flow Cytometer and Confocal Microscopy Core has developed a **Use Regulation** and a **Biosafety Questionnaire** that must be filled before starting any activity in the facility by the principal investigator (PI). In the same way, PI also must fill in the **Registration Questionnaire** in order to maintain the registration list updated.

4. Organization

The Flow Cytometry and Confocal Microscopy Core is an organized structure which depends directly on the IRBLleida Scientific Director.

4.1 Organization Chart and Staff

The daily workgroup in the Flow Cytometry and Confocal Micrsoscopy Core is composed of a Senior Technician with full time job and the Service Managers with part time dedication. Decision making are done in internal meetings between Service managers, the Scientific Director, and also the IRBLleida Internal Scientific.



The main duties of Flow Cytometry and Confocal Microscopy managers are:

- To promote Flow Cytometry and Confocal Microscopy Facilities activities among researchers.
- To ascertain that Flow Cytometry and Confocal Microscopy use regulations are being followed.
- To call and assist in Flow Cytometry and Confocal Microscopy Core internal meetings.

The main duties of the Senior technician are the following:

- To be aware of Core Use Regulation compliance.
- To always atend users in need.
- To apply Standard Operation Procedures for each instrument in the facility.
- To advice, assist and train users.
- To perform administrative and management tasks of the facility.
- To attend to Core internal meetings

4.2 Users

On the one hand, facility users are IRBLleida researchers: PhD students (with PI permission) and PIs. Also researchers from HUAV and UdL are potential users of the core. All of them must fill in the **Registration Form** before starting their activities in the facility.

On the other hand, researchers from other Research Institutes or Private enterprises might be users if they request it puroposely.

5. Use Regulations

5.1 Ethical aspects

Ethical aspects, quality and viability of each research project must be verified by the Clinical Research Ethical Comitee (CEIC) following the current legislations:

- Llei 14/2007 d'investigació biomèdica (14/2007 Biomedical Research Law)
- European Council Agreement for human rights and dignity protection, considering biology and medicine applications, which become effective in Spain the 1st of January 2000.
- Additional protocol to the Human Rights and Biomedicine Convention, relative to biomedical research (2005).
- European Council Recommendation for research using biological materials of human origin from 15 march 2006.
- Guia de bona pràctica en la investigació en ciències de la salut. Institut Català de la Salut, 2003
- *Biosafety in Microbiological and Biomedical Laboratories*; US Department of Health and Human Services, 4th Edition.

5.2 Biosafety Aspects

In order to start using the Flow Cytometry and Confocal Microscopy Core it is compulsory to fill in the **Biosafety Questionnaire**. This questionnaire must be filled and signed by the lab manager who is requesting the analysis, always before starting experiments or developing a project. Every questionnaire will be filed for granting that no information is subsequently modified.

5.3 Flow Cytometry and Confocal Microscopy Users's Rights and Responsabilities

Before starting new experiments, user must follow these steps:

- To speak with the senior technician of the facility and explain the planed experiments in order for him to evaluate them and plan its execution.
- Every scientific project must be aproved by CEIC.
- To fill the **Registration For**m in order to be registered as a user of the facility.
- To read the **Use Regulation** document of the Flow Cytometry and Confocal Microscopy Core and accept its conditions.

- To read and fill in the **Biosafety Questionnaire**.
- To attend a basic training course to learn how to use the flow cytometer and all the different microscopes in the facility. This course will be held by Facility's staff and will be compulsory. Anyone in need to use instruments of the facility must have done the course and must be authorized by the staff when they consider the user is capable enough to use the instruments on their own.

5.4 Flow Cytometry and Confocal Microscopy Staff's Rights and Responsabilities

- Facility staff is responsible for daily instrument maintenance and Use Regulation compliance
- Facility staff will consider each new user and will decide if he/she is skilled enough to perform experiments by him/herself.
- Facility staff reserve the right to not to perform any experiment if they consider that facility guidelines haven't been followed properly.

5.5 Rates

The Flow Cytometry and Confocal Microscopy Core sets each year rates for the Flow Cytometer Analyser. These rates can change depending if the it is an IRBLleida, HUAV, and UdL user or if it is an external user (companies, other research institutes, etc...). It may also vary depending if it is an assissted hour of use with the technician or it is an selfservice hour.
Rates are the following:

Preu/hora	Usuaris interns		Usuaris Externs
	Sense operador	Amb operador	Amb operador
Disseny experimental, consultoria	-	20€	35€
Adquisició de mostres FACS CantoII	12€	20€	35€
Anàlisi de dades	-	20€	35€

Usuaris interns: membres del IRBLleida, UdL o HUAV
Usuaris externs: altres institucions o empreses
*Es poden aplicar reduccions en les tarifes en funció de la facturació.
IVA no inclòs

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At the end of the week, Flow Cytometry and Confocal microscopy Core staff will count how many hours has the cytometer been used (using the **Use Form**) in order to calculate the amount of money each group will have to pay.
Each semester, a bill will be emitted and sent to PIs, which will correspond to services rendered to each group

6. Services and Applications

Applications already available in the **Flow Cytometry Core** are:

1. Immunophonotyping: Population analysis using from one to 8 different colors. Surface and intracellular antigen expression analysis.
2. Apoptosis Studies (TUNEL assays, annexin-V assays).
3. CBA (Becton Dickinson Cytometric bead array to calculate protein concentration) and FlowCytomix (from eBiosciences)
4. PhosFlow: Single cell cellular activiation level analysis of multiple proteins. It allows to combine surface and intracellular markers to study immunophenotype and intracellular signaling.
5. Oxidative stress studies.
6. Cell cycle analysis.

Facility staff is opened to develop new techniques and applications if the user requires them.
Services and applications of **Confocal Microscopy Core** are:

- Olympus Confocal microscopes (FV10i, FV500 i FV1000)
    1. Tridimensional analysis of biological samples: cells and tissue immunostaining, etc...
    2. Tridimensional analysis of up to three color colocalization.
    3. Molecular interaction studies and cellular processes: FRET, FARP
    4. *In vivo* time-lapse studies (FV1000).
    5. Spectral unmixing (FV1000).

- Zeiss Time-lapse fluorescence microscope
    6. Live cell studies: time-lapse, intracellular ion measurement, 4D studies
    7. Cell *in vivo* analysis and in real time using markers and/or fluorescent fusion proteins (GFP) and derivates

8. Colocalization studies, internalization and intracellular trafiking
9. Physiological analysis of calcium response

7. Service request

After being registered as a user in the Flow cytometry and Confocal Microscopy Core and filled in all documents (User registration, Use Regulation, Biosafety questionnaire) each time the user wants to use the facility must do the following:

- To book in advance by using the booking application available in the IRBLleida intranet.
- It is not advisable to book the same day cause it may be no availability.
- Experiments must be prepared carefully, trying to adjust booked hours with real using time. In thay way we will avoid interferences with other people work plan.
- It is essential to arrive on time.
- Booking request cancellation must be done at least 24 h in advance in order to let other users book that time.
- Is user responsability to be sure that all results are copied in a data storage device. FACSDiva Database will be deleted from server every three months and anually for confocal microscopes computers.
- Every incidence or error occured in an instrument during usage must be communicated immediately to the facility staff.

8. Annexes

Documents mentioned in the text:

- FACS-CantoII Flow cytometer Technical specifications
- FV500, FV1000 and FV10i confocal microscopes and Zeiss Axio Observer Z1 fluorescence microscope technical specifications
- Use Regulation of Flow Cytometry Core
- Use Regulation of Confocal Microscopy Core
- Biosafety Questionnaire

FACS-Canto II Flow cytometer: 3 lasers: 4-2-2 combination (8 colors)

- 405 nm Violet Laser: 30 mW
- 488 nm Blue Laser: 20 mW
- 633 nm Red laser: 17 mW

Canto II 488 nm Blue Laser			
Detector	**LP (mirrors)**	**Band Pass (Filters)**	**Fluorochromes**
A	735	780/60	PE-Cy7
B	655	670 LP	PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy5.5, 7AAD
C	610	-	No PMT
D	556	585/42	PE, PI, EB
E	520	530/30	FITC, Alexa 488, GFP
F	none	488/10	SSC
G	-	-	-
H	-	-	-

Canto II 633 nm Red Laser			
Detector	**LP (mirrors)**	**Band Pass (Filters)**	**Fluorochromes**
A	735	789/60	APC-Cy7
B	685	-	No PMT
C	-	660/20	APC, Cy5

Canto II 405 nm Violet Laser			
Detector	**LP (mirrors)**	**Band Pass (Filters)**	**Fluorochromes**
A	502	510/50	AmCyan
B	-	450/50	Pacific Blue
C	-	-	-

Digital signal processing system allowing height, area and width pulse determinations. Compensation can be done offline and post-acquisition.

Analysis Software: BD FACSDiva v. 6.1.1

Cell cycle analysis Software: Modfit LT™ 3.0

FCAPArray Software: CBA analysis (BD)

FlowCytomixPro Software: FlowCytomix analysis (eBiosciences)

Olympus FV500 Confocal Micrsocope Technical specifications:

Laser	Exitation	Emission filter	Examples
1	405	430-460	DAPI
2	488	505-525	GFP, FITC
3	543	560>	TRITC, Cy3
4	633	660>	Cy5

Motorized rotor for direct observation has 4 filters with equivalent excitation and emission properties.

Olympus FV1000 Spectral Confocal microscope technical specifications:

- Spectral Confocal Microscope with two detectors with adjustable width and a standard detector.
- It allows you to perform transmitted light, DIC and also epifluorescence.
- It comes with an Olympus IX81 inverted microscope with motorized plate.
- It si possible to perform in vivo analysis thanks to an incubation chamber with controlled temperature and atmosphere.
- Is suitable for performing Spectral unmixing and removing autofluorescence form very autofluorescent samples.

FV10i Confocal benchtop microscope technical specifications:

- Fully motorized Benchtop confocal microscope.
- 10x and 60x (oil) objectives
- 4 laser lines: 405/473/559/635 nm
- It allows you to adquire images from predefined regions.

Axio Observer Z1- Time-lapse Zeiss epifluorescent microscope technical specifications:

Zeiss reference	Excitation	Splitter	Emission	Use
Filter Set 49	G 365	FT 395	BP 445/50	DAPI
Filter Set 47HE	BP 436/25	FT 455	BP 480/40	CFP
Filter Set 38HE	BP 470/40	FT 495	BP 525/50	GFP
Filter Set 46HE	BP 500/25	FT 515	BP 535/30	YFP
Filter Set 43HE	BP 550/25	FT 570	BP 605/70	DsRed

USE REGULATION OF FLOW CYTOMETRY CORE

There are a few regulations user must follow in order to assure the good functioning of the facility.

- When a user decides to perform an experiment or a project it is compulsory to fill in the registration form (there is a booking sowftware in the intranet area in IRBLleida's website) and also the biosafety questionnaire.
- It is advisable to describe briefly the research project. With this information, the Core facility is able to write activity reports that will be used for funding and grant purposes.
- Bookings can be done by telephone or personally using the booking software in IRBLleida intranet.
- It is not recommended to book in the same day as it might be no availability.
- Experiments must be carefully prepared, trying to adjust booked hours with actual used hours. Behaving in such a way will avoid interfering in other user's work.
- It is essential to arrive on time. More than 15 minutes delay may involve losing the booked time and being billed for it. Booked time must be defined acording experiment complexity; if an experiment lasts more than the expected time and there is a user waiting for his turn, you must continue your experiment according to machine availability. On the other hand, if your experiment lasts less time than expected, total used time will be billed.
- In order to let the maximmum number of users to use the Flow cytometer, there will be a maximmum number of tubes for user and day depending on day's bookings. This maximmum number is 100 tubes/day.
- Booking request cancellation must be done at least 24h in advance, in order to let other users to book that time. If booking was not to be cancelled and investigator didn't appear on the schedulled time, booked hours will be billed.
- Is user's responsability to be sure to record his/her results in some data storage device. FACSDiva Database will be deleted from server every three months and anually for confocal microscopes computers. Users will be asked to delete all experiments in advance. All not deleted data will be erased.
- Every incidence or error during use of any instrument of the facility must be alerted to facility staff promptly.

MICROSCOPY FACILITY BOOKING RULES

- Systems cannot be booked with more than two weeks in advance. Thus, bookings will be opened 14 days in advance for the whole week (on Sunday at 10PM), we think this will help you to better planify your experiments.
- No bookings longer than 4h per user between 9 AM to 21 PM from monday to friday are allowed (except time-lapse experiments). Out of these office hours you can book more than 4 hours.
- Late cancellation (<24 hours in advance) will result in billing if the time slot is not reserved by another user. If the user don't show up one hour after the start of the booking, any user can use that slot.
- Every research group can only book maximmum 16 hours per week (four 4h-slots from 9 AM to 18PM). This restriction is released if 48h in advance there is a free slot. Example: The Experimental Neuromuscular Pathology group has already booked its 4 slots (16 hours: 4h for Sara, 4h for Sílvia, 4h for Pol and 4h for Jordi) but on wednesday they see that on friday afternoon nobody has booked the 14h to 18h slot. In this case, Sara can book it.
- Microscope systems in the Facility can ONLY be used in autoservice after an introduction session is received by the Facility Staff. To receive this training you should contact us.
- From now on, our Service will book FV1000 confocal microscope every wednesday from 10h to 14h for educational and maintenance purposes. This slot can also be available for those users who have an urgent experiment (such as a primary culture) and that couldn't manage to book a slot that week. If it was the case, you should contact us. If the Service doesn't need to use the slot, we will inform through this email list .

BIOSAFETY QUESTIONNAIRE

Flow Cytometry and Confocal Microscopy Core Laboratories 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 users is of ultimate concern. Information about the sample sources and potentially infectious agents is critical for effective biosafety mesures.

Consequently, this sample information form must be filled out completely and signed by the laboratory director who is requesting samples to be analysed before projects or experiments are started. The same biosafety questionnaire will be kept on file provided none of the information it contains has changed.

INVESTIGATOR AND PROJECT INFORMATION:

Investigador principal	_____
Telèfon	_____
E-mail	_____
Investigador (responsable de l'experiment)	_____
Telèfon	_____
E-mail	_____
Ubicació del laboratori	_____
Títol del projecte	_____
Entitat de finançament i número de projecte	_____

Summary or description of project: Provide details related to cells that will be analyzed. Limit to one paragraph.

Haga clic aquí para escribir texto.

List type of sample and source (i.e., mouse spleen cells, human peripheral blood mononuclear cells, cells from an animal en-grafted with human cells, etc…); for cell lines, describe cell origin an biosafety level.

Haga clic aquí para escribir texto.

SAMPLE INFORMATION:

What type of sample do you wish to analyze?

Species origin ☐ Human ☐ Mouse ☐ Rat

☐ Others (specify): Haga clic aquí para escribir texto.

Sample Type:

☐ Patient Indicate type of sample or tissue: Haga clic aquí para escribir texto.

Has it been tested for:

HIV: ☐ Yes ☐ No Results: ☐ Positive ☐ Negative

HBV: ☐ Yes ☐ No Results: ☐ Positive ☐ Negative

HCV: ☐ Yes ☐ No Results: ☐ Positive ☐ Negative

COVID-19: ☐ Yes ☐ No Results: ☐ Positive ☐ Negative

Other pathogens (specify): Haga clic aquí para escribir texto.

☐ Xenograft Cellular type of origin: Haga clic aquí para escribir texto.

☐ Cell Line: Name: Haga clic aquí para escribir texto.

Cell type: Haga clic aquí para escribir texto.

☐ Primary Culture: Tissue of origin: Haga clic aquí para escribir texto.

Others (specify): Haga clic aquí para escribir texto.

Indicate if cells have been transduced, transfected, infected, transformed or altered. ☐ Yes ☐ No

If yes, indicate which method it has been used:

☐ EBV, HTLV-1, Herpes samiri, adenovirus, retrovirus, retrovirus vectors, herpesvirus, CRISPR

☐ Others: Haga clic aquí para escribir texto.

Briefly describe the method: Haga clic aquí para escribir texto.

For how long have they been modified before analysis?

Haga clic aquí para escribir texto.

Have the cells been tested for mycoplasma before its analysis?

☐ Yes ☐ No

If yes, provide the date and results of the test since they have to be done the week before previous to send the samples to the facility.

Haga clic aquí para escribir texto.

Your experiment uses live or fixed cells?

☐ Viable

☐ Fixed Fixation method: EtOH ☐ MetOH ☐ PFA ☐

Others: Haga clic aquí para escribir texto.

Fixation time: Haga clic aquí para escribir texto.

Fixative concentration: Haga clic aquí para escribir texto.

Has the sample been in contact with CMR products (carcinogenic, mutagenic and/or toxic for reproduction)? Specify: Haga clic aquí para escribir texto.

I have read above questions carefully and certify the information provided to be correct.

Date: Haga clic aquí para escribir una fecha.

Signature (Laboratory Director, Principal investigator)