London Regional Flow Cytometry Facility

General Facility Information, Instrument Descriptions and User Fees

Flow cytometry is a technique for the high-throughput examination of microscopic particles in a fluid stream. Flow cytometers allow the simultaneous measurement of multiple physical and/or chemical properties of cells at the single-cell level, through the use of lasers and fluorescent probes. Flow cytometric cell sorters physically isolate purified populations of such cells with distinct measured characteristics for further experimental analyses.

The **London Regional Flow Cytometry Facility (LRFCF)** is the only research-based flow cytometry analysis and cell sorting facility in the London and area research community. Located at the Robarts Research Institute at Western University, the LRFCF is a **full-service multi-user core facility** that provides access to flow cytometric analyzers, cell sorting services, computers with dedicated flow cytometry analysis software, a flow cytometry reference library, and full-time technical expertise for experimental design and troubleshooting. The LRFCF equipment and services are available on a fee-for-use basis to any academic or corporate investigators within the London and area research community whose research programs depend on flow cytometry technologies.

Users access the LRFCF for a wide variety of flow cytometry techniques, including cell sorting, immunophenotyping, cell cycle analysis, cell proliferation, gene transfer (efficacy and tracking), cell death (i.e. apoptosis), cell kinetics, activation states, redox states, chromosome analysis, and cytokine detection.

LRFCF Services:

Cell sorting: Is performed by LRFCF staff, and is available by appointment only. Cells ranging in size from bacteria through to large mammalian cells, expressing from 1 to 13 compatible fluorophores, can be sorted into tubes (1 to 4 populations at a time), multiwell plates or slides, at various temperatures.

New user training program: A comprehensive program designed to ensure facility users have the background knowledge and tools to successfully design and properly implement experiments relying on flow cytometric data, using LRFCF cytometers. This training includes an introduction to flow cytometric theory, guidelines for designing and implementing multi-colour flow cytometry experiments, and extensive training for hands-on operation of any of the facility's analysis cytometers. Successful trainees are capable of independent cytometer operation and data analysis, and are granted 24/7 access to the facility's analysis cytometers and computers.

Operator-assisted data acquisition and analysis services: Offered to those users who wish to utilize our staff's flow cytometric expertise rather undergoing individual training. By appointment only, and based on operator availability. Our staff can implement experiments on any of the facility's analysis cytometers or cell sorters, depending on experimental requirements.

Consultation for experimental design and troubleshooting: Available with LRFCF staff (*appointments recommended),* for all users of the facility's cell sorter or analysis cytometers. Topics include instrument troubleshooting, experimental design and implementation. It is strongly recommended that users wishing to develop reagent staining panels for polychromatic flow cytometry experiments consult with LRFCF staff for optimum panel design and selection of proper controls AFTER beginning new user training, and BEFORE reagent purchase.

Location/Hours:

Robarts Research Institute, Rm 4260

The facility is staffed Monday through Friday, 9:00 to 4:30. If not sorting or training, the manager is generally available to answer quick questions. For detailed advice regarding cytometer operation, experimental design, etc., please contact flow@robarts.ca to make an appointment.

LRFCF Equipment:

Coming Soon:

Cytek Aurora Spectral Cytometer (Cytek): *Coming Soon* (early 2025)

Optical Platform: 5 laser spectral cytometer. Parameters include Forward Scatter, Side Scatter (including Small Particle detection) Five spatially separated lasers (UV, Violet, Blue, Yellow-Green, Red) coupled to 64 fluorescent channels over the full emission spectra allows simultaneous detection/resolution of upwards of 40 unique fluorophores with careful panel design and optimization.

Sample loading: Automated Sample Loader (ASL) permits high throughput sample processing from 96-well plates (normal and deep well), and 40 tube carosel with sample mixing.

Software: Cytek SpectroFlo® software (Windows 10)

Current Analysis Cytometers:

Available for independent use after user completes **Flow Cytometry Training Program** OR **Certification in Cytometer Operation.**

FACSymphony A1 (BD Biosciences): Robarts 4260 (*NEW* May 2024)

Optical Platform: A 19 parameter digital analysis cytometer. Parameters include Forward Scatter, Side Scatter, Small Particle Side Scatter, and 16 fluorescent parameters from four lasers with spatially separated beam paths.

- 6 detectors from 100 mW 405nm violet laser excitation
- 2 detectors from 100 mW 488nm blue laser excitation
- 5 detectors from 100 mW 561nm yellow-green laser excitation
- 3 detectors from 100 mW 640nm red laser excitation

Software: BD FACSDiVa 9 (Windows 10 OS)

CytoFLEX S (Beckman Coulter): Robarts 4260 (Installed Nov 2022)

Optical Platform: A 15 parameter digital analysis cytometer. Parameters include Forward Scatter, Side Scatter, and 13 fluorescent parameters from four lasers with spatially separated beam paths.

- 4 detectors from 80 mW 405nm violet laser excitation
- 2 detectors from 50 mW 488nm blue laser excitation
- 4 detectors from 30 mW 561nm yellow-green laser excitation
- 3 detectors from 50 mW 638nm red laser excitation

The CytoFLEX S is equipped with a deep well **plate loader**, allowing for high-throughput sampling in 96-well plates, in addition to loading individual tubes.

Software: Beckman Coulter CytExpert Software (Windows 10 OS)

Service: These cytometers are maintained on full service & maintenance agreements, including the recommended schedule of preventative maintenance inspections.

Legacy Cytometers:

Training no longer available on legacy cytometers. Available for use by currently trained users, as long as they remain in good working condition. They are no longer on service & maintenance agreements, and will reapired in case of breakdown. Users are encouraged to transition to Symphony A1 or CytoFLEX S as soon as possible.

LSR II SORP (BD Biosciences): Robarts 4260 – RETIRING SOON (exact date TBD)

Optical Platform: A 15 parameter digital analysis cytometer. Parameters include Forward Scatter, Side Scatter, and 13 fluorescent parameters from four lasers with spatially separated beam paths.

- 3 detectors from 50 mW 405nm violet laser excitation
- 2 detectors from 20 mW 488nm blue laser excitation

- 5 detectors from 50 mW 561nm yellow-green laser excitation
- 3 detectors from 40 mW 640nm red laser excitation

Installed: June 2010. **Software:** BD FACSDiVa 9 on Windows 10 OS (computer upgraded Jun 2021).

FACSCanto SORP (BD Biosciences): Robarts 4260 – RETIRING SOON (exact date TBD)

Optical Platform: A 12 parameter digital analysis cytometer. Parameters include Forward Scatter, Side Scatter, and 10 fluorescent parameters from four lasers with three spatially separated beam paths.

- 2 detectors from 200 mW 488nm blue laser excitation
- 4 detectors from 50 mW 561nm yellow-green laser excitation
- 4 detectors from 50 mW 405nm violet laser and 75 mW 642nm red laser (co-linear) excitation

Installed: June 2015. **Software:** FACSDiVa 9/Windows 10 OS (upgraded Jun 2021/ Nov 2022)

Flow Cytometric Cell Sorter: operated by facility staff, by appointment only.

FACSAria III:

Optical Platform: A 15 parameter digital cell sorter. Parameters include Forward Scatter, Side Scatter, and 13 fluorescent parameters from four lasers with spatially separated beam paths.

- 2 detectors from 20 mW 488nm blue laser excitation
- 5 detectors from 50 mW 561nm yellow-green laser excitation
- 3 detectors from 17 mW 633 nm red laser excitation
- 3 detectors from 30 mW 405 nm violet laser excitation

Sort Parameters:

- Up to four populations can be simultaneously sorted into microfuge tubes or 5mL round bottom tubes
- Up to two populations can be simultaneously sorted into 15mL tubes
- Using the Automated Cell Deposition Unit (ACDU), defined numbers of cells can be sorted into multiwell plates or onto microscope slides (ideal for single cell cloning).
- Sample cooling and heating available.

Installed: Dec 2010

Software: BD FACSDiVa 9 (Windows10 OS) upgraded May 2022.

Service: This cell sorter is maintained on full service & maintenance agreement, including semi-annual preventative maintenance inspections, and all repairs as needed.

Biosafety:

- The FACSAria III is housed within a Baker BioProtect IV biocontainment enclosure, to allow sorting under CL2 containment conditions.

Scheduling:

- Sorts are scheduled during regular working hours, generally 10am-4pm, based on operator availability
- For sort availability, contact flow@robarts.ca for an introductory email and invitation to view our scheduling calendar. At http://fbs.robarts.ca, login and select the FACSAria III schedule to find a time when the sorter/sort operator are free. Email flow@robarts.ca with desired appointment times and required forms.
- Note that during peak times of use, sorts may need to be booked up to 3-4 weeks in advance

Other Equipment:

- **1 iMac analysis workstation with FlowJo X** flow cytometry data analysis software. Microsoft Office (including Microsoft Word, PowerPoint and Excel) aids in data analysis and publishing.

User Fees*

Service	Description	Fees	
		Academic	Corporate
Cell Sorting (min 1 hour / appointment)	FACSAria III (4 laser, 13 colour:	$120 / hr	$240 / hr
User training *:	FACSymphony A1 or CytoFLEX Includes online training resources, first cytometer demonstration, and FlowJo introduction.	$150 / person	$250 / person
	Additional sessions charged at hourly cytometer rate. (training no longer available for FACSCanto, LSR II)	+ 2-5 sessions, ~ 3hr each	+ 2-5 sessions, ~ 3hr each
FACSCanto SORP: (Appointments rounded up to nearest 15 minutes)	Unassisted	$50 / hr	$100 / hr
	Assisted	$90 / hr	$180 / hr
FACSymphony A1 / CytoFLEX S / LSR II: (Appointments rounded up to nearest 15 minutes)	Unassisted	$65 / hr	$130 / hr
	Assisted	$105 / hr	$210 / hr
Cytek Aurora: Coming Spring 2025	Unassisted	tbd	tbd
	Assisted	tbd	tbd
Data Analysis:	Assisted	$90 / hr	$180 / hr
Expert advice, assistance & experimental design:	Experiments using LRFCF cytometers	no charge	no charge
	Experiments using off-site cytometers	$90 / hr	$180 / hr

**Fees have been in effect since November 1, 2013, and are subject to change to maintain a balanced facility budget.*

Billing:

Western speed codes or invoice request must be provided when booking each appointment. Charges will be itemized per appointment (by lab and user) on fee statements. Billing summaries outlining flow cytometry charges will be submitted through the Mustang Market requisition system on a quarterly basis.

The London Regional Flow Cytometry Facility is currently funded by user fees, the Schulich School of Medicine and Dentistry at The University of Western Ontario.

For further information, or to schedule an appointment, please call 519 931-5777 x24042 or email flow@robarts.ca

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Flow Cytometry Training – Program Description

- The flow cytometry training program for the London Regional Flow Cytometry Facility (LRFCF) is designed to allow individuals to achieve independence in safely operating our IN HOUSE analysis cytometers (Beckman Coulter CytoFLEX S or BD Biosciences FACSymphony A1) for acquisition of high-quality flow cytometric data.
- This training program is NOT intended for users operating cytometers housed outside of the LRFCF.
- Training or certification by staff of the facility is mandatory for access to, and use of, all LRFCF cytometers.
- Untrained or user-trained persons are not allowed to independently operate LRFCF cytometers.

Participation in the full training program is mandatory for those with no flow cytometric experience on the facility's instruments, and includes both theory and application of flow cytometry techniques. For those with previous moderate or extensive experience ON THE CYTOMETER OF INTEREST, a certification session may be available, focused on ensuring competency with software & hardware operation, and instruction in LRFCF-specific procedures.

Full Training Program

The full training program consists of approximately 5-6 sessions, and is mandatory for all new users who have limited or no background in flow cytometry.

Pre-training videos and self-directed learning:

Prior to hands-on training, each user will be given a comprehensive list of online learning resources to begin their flow cytometric education, beginning with the **'Molecular Probes Tutorial Series'** published by ThermoFisher. These are well-designed introductory tutorials about flow cytometry and fluorescence, and are an excellent place to start learning about flow cytometry.

Mandatory videos:	Optional (but recommended) videos:
• **Introduction to Flow Cytometry:** https://youtu.be/sfWWxFBltpQ • **Analyzing Flow Cytometry Data:** https://youtu.be/ccR5snuCE80	• **Introduction to Fluorescence:** https://youtu.be/SGFlr1jFNBM • **Anatomy of Fluorescence Spectra:** https://youtu.be/oVxpaUfTuXI • **Overview of Filters and Light Sources:** https://youtu.be/xJGmARfBasU

Next,users will be referred to the Flow Cytometry Training Webinars with Derek Davies, a Flow Cytometry Educator at Francis Crick Flow Cytometry Core (London, UK).

- **Mandatory video: Introduction to Flow Cytometry** https://youtu.be/EHqZ80GNYCE?si=Ig5_mHDe_GLNcqKu

Upon receipt of a completed New User Application form, the trainee will be provided with a more **comprehensive list of additional online training resources** to further expand their flow cytometric knowledge prior to beginning hands-on instruction, in both theory and hands-on software operation.

Hands on Cytometer Introduction (FACSymphony A1 or CytoFLEX S) (3.0 hours) *$150 per person*

In a small-group format of 1-4 people, trainees will be shown basic startup/shutdown, quality control, cleaning and troubleshooting procedures on their selected cytometer. They will also be shown how to operate the cytometer data acquisition software, and will learn the importance of proper controls and compensation. Users can opt to bring a simple experiment of no more than 3 fluorescent parameters (preferred) or a demonstration using 3 colours of fluorescently labelled beads. An instruction manual for cytometer operation will be provided prior to this first session, and must be brought to all subsequent sessions

One-On-One Instruction for Experiment Setup and Implementation (2-3 hours each, avg 2-4 sessions required) *– hourly instrument fee charged for each required experiment*

In these sessions, the flow operator and trainee will work together on-on-one to set up the cytometer and acquire the trainee's experimental samples. The trainee is expected to gain increasing independence in performing flow cytometry experiments over the course of these sessions, with staff only assisting as necessary. Time will be made for questions and discussion about experimental design and troubleshooting, if needed. The trainee must exhibit a **high level of proficiency and independence** in the maintenance and operation of the cytometer and software for the acquisition of sound data to exit this phase of the training program. Trainees are only granted access to the facility's equipment upon successful completion of this phase of the training program, which takes on average 2-4 sessions.

Cytometer Setup (Assist ~30-60 min each) *– hourly instrument fee charged for each required experiment*

Once independent access is granted, each trainee is encouraged to request assistance from the facility manager when they attempt new or more difficult protocols, that require changes in instrument setup. Staff will observe the experiment setup, offer advice for cytometer adjustments, and then leave the trainee to acquire their samples independently once a satisfactory experiment setup has been obtained.

Flow cytometry data analysis (1.0 hour)

Trainees are encouraged to independently explore training resources on the FlowJo website:
https://www.flowjo.com/learn/flowjo-university

One ~1 hour long hands-on session using FlowJo software for data analysis can be provided, upon requested, to analyze data from one of the hands-on training sessions. Additional follow-up may be provided when needed.

The Hands on Cytometer Introduction and FlowJo data analysis session are covered by the initial training fee. For all One-on-One and Setup assist instruction sessions, trainees are charged at the cytometer's standard hourly rate.

Note that the FACSymphony A1 and CytoFLEX S cytometers operate with different fluidics and software systems. Training is specific for ONE cytometer and software system. If the user wishes to be trained in BOTH systems, an additional New User Training Fee will be applied.

Certification in Cytometer Operation

Certification in cytometer operation consists of one session that is 3 hours in length, and is mandatory for all new users to the LRFCF who have moderate to extensive flow cytometry experience **on equipment similar to that of the LRFCF**. The user will be asked to demonstrate startup/shutdown, cleaning and troubleshooting procedures on the cytometer, as well as to perform a simple experiment of 2-4 colours, from cells the user prepares themselves. The user must demonstrate a high level of proficiency and independence in the operation of the cytometer and software for the acquisition of sound data. The session also includes an introduction to the facility, its resources, services and procedures. **The user will be billed the standard hourly rate for assisted cytometer operation.**

Recommendations for Training: timing guidelines

Please ensure that your own experimental samples are available for analysis for the one-on-one sessions within 1-2 weeks of starting training. Select cells that are similar to what you plan for experiments, but easy to procure and prepare. Cells used for training are designed to familiarize yourself with the instrument, and should NOT be cells from critically important experiments.

Completion of Training

Upon completion of training, the user will be issued a unique username and password to schedule independent appointments on analysis cytometers and data analysis computers. Trading or swapping of username and passwords is strictly prohibited. After-hours keycard access to Robarts and the LRFCF can be issued for graduate students, postdocs and Western staff, but not to undergraduate students or volunteers.