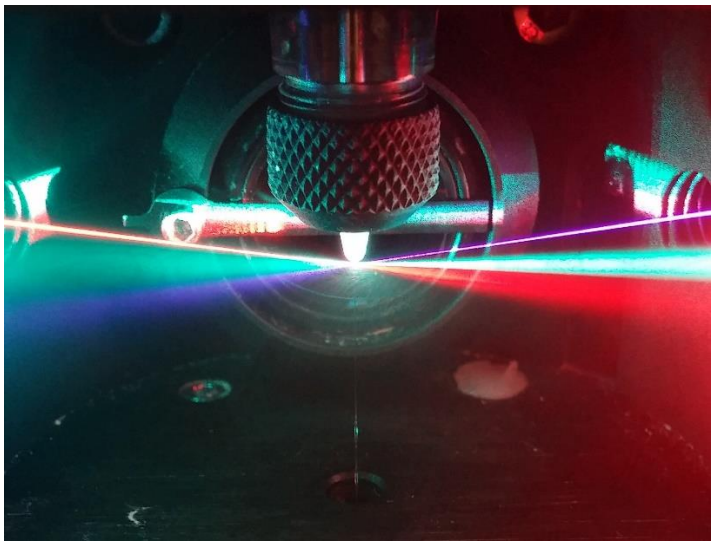

Cell Sorting Core Facility

Operator: Hao Zhang, M.S., M.D.
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Core Mission:
The facility was established in 1991 and has a long history of commitment and dedication in flow cytometry and cell sorting services. It offers state of the art instrumentation and expertise in flow cytometry and cell sorting services at Bloomberg School of Public Health. Our goal is to provide high quality and cost effective service to investigators at Johns Hopkins University.

Getting Started for New Users:
Potential users must discuss their protocols with Dr. Margolick or Mr. Hao Zhang (sorting) beforehand. A written, short description of the project, along with the title and number of the grant supporting the project should be filled in the User access form.

Service Charge:
The following rates will apply for sorting on two different sorters at the core facility:

Instruments	User from JHU (rate per hour)	User from Outside JHU (rate per hour)
Instrument setup	$60 (applies for each sort session)	$60 (applies for each sort session)
MoFlo Legacy	$120	$150
MoFlo XDP	$120	$150

Reservation for sorting service:

Procedure for online reservation on the sort calendars.

To obtain access to the web calendar and schedule appointments, e-mail Hao Zhang at haozhang@jhu.edu or Joseph Margolick at jmargol1@jhu.edu.

- Appointments must be canceled at least 48 hours in advance. Failure to do so prevents other potential clients from using that allotted time. If the appointment is not canceled in advance, the reserved time will be charged.

Instrumentation:

Currently the sorting lab has two functional MoFlo sorters: The MoFlo Legacy and MoFlo XDP. Both of the sorters can analyze and sort cells up to 20,000 cells/second with very low abort rate and test signals (electrical pulses as test events) up to 200,000 events/second. The MoFlos are stream in air by design and have extremely stable fluidics. This allows the sorters to separate large amount of specific cell populations at high speed, viability, purity, yield and recovery. Both sorters are equipped with Cyclone, a computer driven robotic arm that permits precise and rapid deposition of sorted cells from small to large into any format of test tubes, micro-well plates (6, 24, 48, 96, 384, 1536 wells), slides, slide based culture chambers and petri dishes. In addition, both sorters are capable of sorting 4 different cell populations simultaneously. The Legacy sorter has 4 lasers and 8 detectors. The XDP has 4 lasers and 18 detectors.

Laser configuration and choices of flourochrome detections:

MoFlo legacy:

The MoFlo Legacy can simultaneously analyze and sort up to 6 different colors by choosing different detection filters. The filters and detectors can be easily reconfigured and exchanged for color detections based on the experimental design. It has been in service since 2001. It still functions like new.

Lasers	Detection Filters: Ranges	Commonly used Fluorochromes
488nm 200mW	530/30: 515nm-545nm	ALDH(green), FITC, GFP, YFP, Alexa488,CFSE, Rodamine123
	580/30: 565nm-595nm	PE,Td-Tomato, DsRed, PI
	630/30: 615nm-645nm	PE-TexasRed, Qdot 565,
	670/40: 650nm-690nm	PECy5,PerCP-Cy5.5, PE-Cy5.5, 7AAD, (Don't Use PerCP, it gets photo bleached on MoFlo)
	740L: 740nm-900nm	PECy7,
405nm 100mW	450/65M: 417nm-482nm	DyeCycle Violet (cell cycle), BV421, PacBlue, V450
	670/40: 650nm-690nm	DyeCycle Violet (Side Population), Qdot655,
532nm 100mW+ 642nm 100mW lasers combined	580/30: 565nm-595nm	RFPs (Td-Tomato, DsRed, Tag-RFP, TurboRFP), Cy3
	610/20: 600nm-630nm	mCherry, PE-CF594, TexasRed
	670/30: 655nm-685nm	APC, Alexa647, DRAQ5,Cy5
	740L: 740nm-900nm	APC-Cy7, APC-H7

MoFlo XDP:

The MoFlo XDP is the second generation of MoFlo sorter that is constructed in a modular fashion. It is also the first true 32-bit-high resolution 5-decade multi-channel digital system that enabled higher sensitivity and higher dynamic range for fluorescent detection. In other words, it has a real 5 decade log verses conventional 4 decade log. The XDP is equipped with IntelliSort II for beadless drop delay determination and accurate sort monitoring. It can simultaneously analyze and sort up to 16 different colors by choosing appropriate fluorescent dyes and detection filters. The filters and detectors can be easily reconfigured and exchanged based on the experimental design. The sample to be sorted and cells being collected can be maintained at a set temperature.

Lasers	PMTs	Detection Filters: Ranges	Commonly used Fluorochromes
488nm 200mW	FL1	514/30: 499nm-529nm	ALDH(green), FITC, GFP, YFP, Alexa488,CFSE, Rodamine123, Dioc2(3), JC-1
	FL2	575/25: 563nm-587nm	PE, Td-Tomato, DsRed, PI
	FL3	625/26: 612nm-638nm	PE-TexasRed, Qdot 565,
	FL4	670/30: 615nm-685nm	PECy5, 7AAD, (Don't Use PerCP, it gets photo bleached on MoFlo)
	FL5	720/13: 714nm-728nm	PE-Alexa700, PerCP-Cy5.5 , PE-Cy5.5
	FL6	740L: 740nm-900nm	PECy7,
405nm 50mW	FL7	448/59: 418nm-477nm	DyeCycle Violet (cell cycle), BV421, PacBlue, eFluor450, V450, CFP
	FL8	513/26: 500nm-526nm	BV510,
	FL9	576/21: 565nm-586nm	BV570
	FL10	615/24: 603nm-627nm	BV605, eFlour605NC, eFluor625
	FL11	664/22: 653nm-675nm	BV650, eFluor650,
	FL12	710/45: 680nm-725nm	BV711, Qdot705
	FL13	795/70: 760nm-830nm	BV785, Qdot800
561nm 100mW + 642nm 100mW lasers combined	FL14	610/30: 595nm-625nm	RFPs (Td-Tomato, DsRed, Tag-RFP, TurboRFP, mCherry), Cy3, PE-CF594, TexasRed (optional filter 580/30),
	FL15	671/28: 657nm-685nm	APC, Alexa647, DRAQ5,Cy5, mKate, Katushka, PECy5
	FL16	722/42: 701nm-743nm	PE-Alexa700, Alexa700
	FL17	795/68: 761nm-829nm	APC-Cy7, APC-H7

Policies for Facility Users

1. When in the lab, Good Laboratory Safety Practices must be observed at all times
    a. Proper laboratory attire is required.
    b. Food and drink are not allowed in the laboratory.
2. Users should arrive on time for the appointment. Service charges will start at the reserved time.

3. A fixed instrument set-up charge of $60 will be added to the sort time charges. This charge covers instrument start-up, calibration, cleaning, and shutdown.
4. Publications that include data obtained using the Cell Sorting Core Facility should recognize the contributions of cell sorting laboratory personnel by co-authorship or explicit acknowledgement.

The Cell Sorting Laboratory reserves the right to revoke use privileges from any user who consistently disregards the above policies.