Palo Alto Veterans Institute for Research (PAVIR) FACS

The Palo Alto Veterans Institute for Research (PAVIR) FACS Core helps researchers who require extensive characterization or high purity isolation of cells using fluorescent tagging of markers from cells of various tissues. The PAVIR FACS Core is supported in part by the Palo Alto Veterans Institute for Research and the National Institute of Health. We accommodate a number of research disciplines including immunology, stem cell biology, pathology, cardiology and much more. Our core exists to provide Veterans Administration (VA) affiliated researchers flow cytometry and cell sorting services and to teach researchers appropriate and important techniques in flow cytometry. Users may have their samples run with the assistance of the core or may obtain training so that they can run the flow cytometers and sorters independently. The PAVIR FACS Cytometry Core also seeks to bring the flow cytometry users at the VA together and foster collaboration between many of the diverse disciplines present here at the VA in Palo Alto and Stanford University.

Researchers who want to use the PAVIR FACS Core will have to contact Brandon Carter. After users have demonstrated proficiency in the use of the Flow Cytometers, users will be granted access to the PAVIR-BookMyLab calendar scheduling system. PI's are responsible for supplying the core with basic supplies in accordance to the usage on the machines. Normal hours are **Monday through Friday 7:30AM to 4:00PM with first sorts targeted for 9:00AM**. With time and experience, users may obtain independent user status allowing them to work in the facility after normal business hours.

User Information

User Information

Access

The Flow Cytometers and Cell Sorters are available to VA Investigator laboratory personnel and their affiliates who have VA funding, been cleared by the WOC process, or who have checked in with the police and obtained a temporary badge and escort. Users sign up for time on a calendar available through gmail. In order to gain access to the calendar, new users must contact Brandon Carter. Prior to independent use of the instrumentation, extensive training on the software and procedures for proper start up/sorting/shut down must be scheduled with Brandon and usually requires up to three or more training sessions.

Cost to use core facilities:

For the remainder of this fiscal year, there is no direct cost or hourly usage rate to individual users or labs. The facility is supported in its entirety by the VA and PAVIR except for the cost of supplies which is split among the laboratories proportional to their usage, which averages out to $2-3/hr. Because of this, VA lab researchers are the sole users unless approved on a case by case basis through PAVIR.

New Users Information:

Please complete the following items to obtain access to the VA Flow Cytometry Core and start using the flow cytometers:

1. Obtain WOC clearance and verify your lab has VA funding.
2. Complete the VA Flow Cytometry Core Registration Sheet
3. Read the Rules and Regulations of the VA Flow Cytometry Core, sign, and return the sheet the Brandon Carter via email or in person to the VA Flow Cytometry Core located in Building 101 Room B4-115.
4. Complete BD Flow Cytometry E-Course and self evaluation tests. (approx. 1.5-2hrs)
5. Familiarize yourself with the SOPs for the **start-up and shut down procedures** of the Flow Cytometers and Cell Sorters.
6. Schedule a series of training sessions with Brandon to get orientated on the LSRs or Aria II and III sorters. Trainings are limited to 2 people at one time. Upon registering for the FACS Facility (Step 2 above), you will be assigned a BookMyLab user name and password and, together, you and Brandon will schedule training sessions. Trainings will occur at a mutually agreed upon time. Typically, trainings consist of an introduction with beads taking about 2 hours, a second 2 hour session run with your samples of choice, and additional sessions as deemed necessary. Sessions may take more than two weeks to schedule so plan accordingly!

7. Note that experience and proficiency with the instruments and their software are required to be extended sorting and analysis privileges beyond normal business hours. New users should plan to schedule **five** unassisted startup/sample-processing/shutdown sessions during normal business hours to prove competence before they will be allowed to use the flow cytometers after normal business hours, on weekends and/or on holidays. These privileges are granted using the BookMyLab software package.

Policies and Procedures

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General Policies

The VA Palo Alto Flow Cytometry Core is a shared facility that has the capacity to run BSL-2 samples. All users will be required to wear personal protective equipment (PPE): gloves, lab coats, and close toed shoes while using the machines. No food/drink is allowed in the Flow Cytometry Core Facility. Gloves will be provided by the core to users in the facility.

- **The VA Palo Alto FACS Core is a shared facility that has the capacity to run BSL-2 samples.** All users will be required to wear personal protective equipment (PPE): gloves, lab coats, and close toed shoes while using the machines. No food/drink is allowed in the Flow Cytometry Core Facility. Gloves will be provided by the core to users in the facility.
- **Users that do not have a VA badge will not be allow to run samples on the PAVIR FACS Facility Cell Sorters or Analyzers.** All users of the FACS core must go through proper VA WOC clearance procedures and have a badge before they may access the core facility.
- **Users that are found allowing unauthorized users access to the facility and the flow cytometers will face a ban from the machines.** Users of the PAVIR FACS Facility must be affiliated with a Principal Investigator with a VA or PAVIR appointment or a collaboration with the VA to utilize the Flow Cytometry Core.
- **Cancellations:** Users may cancel without being charged for time on the machines 24 hours before their reservation. It is the user's responsibility to remove their time from the calendar and **notify the user before them (via email/phone) with confirmation that the next user received notice**, if they are to cancel their reservation. If users do not adhere to this rule, they are subject to charge for the time the machine is left on without supervision. This rule is in place due to machines being repeatedly left on overnight.
- **User's using human samples must follow BSL-2 guidelines and universal precautions for running human samples on the machines.** Please alert those around you that you are running BSL-2 samples.
- **Policies and procedures differ from those used at the Stanford FACS Cores.** Please be sure to follow directions (Posted SOPs near machines) for proper shut down procedures between users or if you are the last user. Check the calendar after your time on the instrument to verify that the person after you is still using the machine.
- **Changes to the calendar within 24 hours of your scheduled time are not allowed unless there are special extenuating circumstances.** VA Palo Alto FACS Core machines are heavily used by multiple labs and last minute changes allow for machine misuse and user miscommunication, as well as booking time that could be used by other labs.
- **Flash Drives are prohibited on any of the Cell Sorter or Analyzer computers as of January 1, 2019.** Data may be transferred on to a data storage server housed elsewhere on the VA. Access to this drive will be restricted to registered users of this facility and their PIs.
- **Data stored on the instrument and on the data storage server will be held locally for three months with the oldest month purged monthly.** You, the user, are responsible for obtaining and storing your data.
- **Try to adhere to your scheduled time:** If you are running late, let the scheduled user after you know!
- **Never leave the instruments in the FACS core unattended.** The facility is a BSL-2 laboratory and samples are treated as such. Note that cells can aggregate and sediment with time and can clog the machine or disrupt the stream creating changes in drop delay. All result in poor sort efficiency. Be responsible and take care of your samples and the machines.
- **Clean up after you use the VA Core Flow Cytometers.** Follow documented instrument cleaning procedures appropriate to your sample type. Remove used tubes and caps and paper towels and care for spills appropriately.
- **Do not book more than 2 machines per for individual user during prime time hours (between 12pm-5pm).** One may NOT monitor more than two instruments at one time. After hours and weekend users may book more than two machines if they have been approved for such access and have a second trained individual who will be assisting them.
- **Clinical Samples:** Please contact the VA Flow Core if you have a specimen that you want to run. Currently we have only two machines that are capable of sorting live human samples and human cell cultured samples (The Aria 2u (Captain America) and The Aria 3.2 (Captain Marvel)) that are not virally infected. At this time, the VA flow cytometry core does not have the capacity to run infected human samples from patients with diseases such as CMV, HIV, HCV.

Instrument Operating Procedure Forms:

LSR II and LSR Fortessa start-up SOP

LSR II and LSR Fortessa shutdown SOP

Aria Sorter start-up SOP

Aria Sorter and LSR Shutdown SOP

CST, AMO, and Nozzle Swap SOP

Aria Nozzle ideal breakoff and troubleshooting Images

Flow Cytometry Core User Registration Sheet

LSRII and LSRFortessa Training Guide

Aria Cell Sorter Training Guide

Flow Cytometer Information

Flow Cytometer Information

- Availability: 24 hours, 7 days a week for verified users.
- Normal Working Hours with Assistance and Flow Cytometer Set Up: Monday- Friday 7:30am-4pm.

Brandon is accessible by phone from 7:30AM to 9PM, Monday through Thursday, and Friday 7:30AM to 4PM unless on PTO. Calls after 9PM or on the weekends will be sent to voice mail. I will address voice mails during off hours as able but most often not until the next business day.

All benchtop BD Flow Cytometers are located at the address below:

Palo Alto VA
3801 Miranda Ave, Palo Alto
Bldg.101, 4th Floor, Rm. B4-115

Contact for training and/or access to the flow cytometers
Primary Contact: Brandon Carter.
Phone: (650) 223-1657
Email: bcarter@pavir.org

Palo Alto Veterans Institute for Research
BookMyLab

LSRII (Nickname: Nightcrawler)

BD Serial # S/N H1014

The LSRII is a flow cytometer equipped with four lasers (405nm (violet), 488nm (blue), 532nm (yellow-green) and 633nm (red)) and can detect up to 16 fluorophores:

Common fluorophores used in this configuration:

Violet (405nm) Laser (6 detectors): Pacific Blue, AmCyan OR V500, BV605 or Qdot605, BV or Qdot655, BV710, BV786

Blue (488nm) Laser (4 detectors): FSC, SSC, FITC, PerCP or PerCPCy5.5

Yellow/Green (532nm) Laser (5 detectors): PE, PE-TexasRed, PE-Cy5, PE-Cy5.5, PECy7

Red (635nm) Laser (3 detectors): AF700, APC or AF647, and APC-Cy7

The LSRII has the hardware for automated data acquisition from 96 well plates as well (High Throughput Sampler).

Follow link to see detailed **LSRII (Nightcrawler)** lasers and filter configurations.

Follow link for assistance building an experimental panel for use on the LSRII (NightCrawler) using FluoroFinder: https://app.fluorofinder.com/pavir

LSRFortessa (Nickname: Storm)

BD Serial # H5B500011

The LSRFortessa is a flow cytometer equipped with five lasers (355nm (near UV), 405nm (violet), 488nm (blue), 532nm (yellow-green) and 640nm (red)) and can detect up to 18 fluorophores:

Common fluorophores used in this configuration:

UV (355nm) laser (3 detectors): BUV395, BUV450, BUV737

Violet (405 nm) laser (6 detectors): Pacific Blue, AmCyan or V500, BV or Qdot605, BV or Qdot655, BV710, BV786

Blue (488nm) laser (4 detectors): FSC, SSC, FITC, PerCP or PerCPCy5.5

Yellow/Green (532nm) laser (4 detectors): PE, PE-TexasRed, PE-Cy5.5, PE-Cy7

Red (635nm) laser (3 detectors): AF700, APC or AF647, and APC-Cy7

Storm enables sampling only from 5 mL FACS tubes.

Follow link to see detailed **LSRFortessa (Storm)** lasers and filter configurations.

Follow link for assistance building an experimental panel for use on the Fortessa (Storm) using FluoroFinder: https://app.fluorofinder.com/pavir

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Cell Sorter Instrument Information

Cell Sorter Instrument Information

- Availability: 24 hours, 7 days a week for verified users.
- Normal Working Hours with Assistance and Sorter Set up: Monday- Friday 8:30am-5pm.
- All benchtop BD FACS Aria Cell Sorters are located at the address below:

Palo Alto VA
3801 Miranda Ave, Palo Alto
Bldg.101, 4th Floor, Rm. B4-115

Contact for training and/or access to the FACS Aria Cell Sorters
Primary Brandon Carter
Phone: (650) 223-1657
Email: bcarter@pavir.org

Aria Sorter General Info:

Aria cell sorters are equipped with 3-5 lasers and typically use 70um or 100um nozzles for cell sorting, but the core has 85um and 130um nozzles available to those who request non-standard size nozzles. All Aria cell sorters can perform 1-way, 2- way, 3- way or 4-way sorts allowing up to 4 separate populations to be sorted simultaneously.

Aria IIu (Nickname: Captain America)

BD Serial # 99900023

The Aria IIu is a fluorescent activated cell sorter (FACS) flow cytometer with three lasers (405nm (violet), 488nm (blue), and 633nm (red)) and can detect up to 11 fluorophores:

Common fluorophores used in this configuration:

Violet (405nm) laser (3 detectors): Pacific Blue, AmCyan or V500, BV or Qdot605

Blue (488nm) laser (7 detectors): FSC, SSC, FITC, PE, PE-TxRed, PerCP or PerCPCy5.5 or PECy5 or PECy5.5, PECy7

Red (635nm) laser (3 detectors): APC, AF700, and APC-Cy7

The Aria IIu is also equipped to sort into 6, 12, 24, 28, and 96 well plates and is equipped with an aerosol evacuation unit for running BSL-2 samples

Follow link to see detailed **Aria 2u (Captain America)** lasers and filter configurations.

Follow link for assistance building an experimental panel for use on the Aria IIu (Captain America) using FluoroFinder: https://app.fluorofinder.com/pavir

Aria III.1 (Nickname: Falcon)

BD Serial # P28200111

The Aria III.1 is a fluorescent activated cell sorter (FACS) flow cytometer with three lasers (405nm (violet), 488nm (blue), and 633nm (red)) and can detect up to 12 fluorophores:

Common fluorophores used in this configuration:

Violet (405nm) laser (5 detectors): Pacific Blue, AmCyan or V500, BV605 or Qdot605, BV655 or Qdot655, BV710

Blue (488nm) laser (7 detectors): FSC, SSC, FITC, PE, PE-TexasRed, PerCP or PerCP-Cy5.5 or PE-Cy5 or PE-Cy5.5, PE-Cy7)

Red (635nm) laser (2 detectors): APC and APC-Cy7

The Aria III.1 is also equipped with a chiller unit for keep sorted samples at 4C while being sorted. At this time, the Aria III.1 (Falcon) can only handle BSL1 samples and is not equipped for plate sorting.

Follow link to see detailed **Aria 3.1 (Falcon)** lasers and filter configurations.

Follow link for assistance building an experimental panel for use on the Aria III.1 (Falcon) using FluoroFinder: https://app.fluorofinder.com/pavir

Aria III.2 (Nickname: Captain Marvel)

BD Serial # P64828200017

The AriaIII.2 is a fluorescent activated cell sorter (FACS) flow cytometer with three lasers (405nm (violet), 488nm (blue), and 633nm (red)) and can detect up to 13 fluorophores:

Common fluorophores used in this configuration:

Violet (405nm) laser (5 detectors): Pacific Blue, AmCyan or V500, BV605 or Qdot605, BV655 or Qdot655, BV710

Blue (488nm) laser (7 detectors): FSC, SSC, FITC, PE, PE-TexasRed, PerCP or PerCP-Cy5.5 or PE-Cy5 or PE-Cy5.5, PE-Cy7)

Red (635nm) laser (3 detectors): APC, AF700 and APC-Cy7

The Aria III is equipped with a chiller unit for keep sorted samples at 4C while being sorted. The Aria III.2 is also equipped to sort into 6, 12, 24, 28, and 96 well plates and is equipped with an aerosol evacuation unit for running BSL-2 samples.

Follow link to see detailed **Aria 3.2 (Captain Marvel)** lasers and filter configurations.

Follow link for assistance building an experimental panel for use on the Aria III.2 (Captain Marvel) using FluoroFinder: https://app.fluorofinder.com/pavir

Aria III.3 (Nickname: Spider-Man)

BD Serial # P64828200406

The AriaIII.3 is a fluorescent activated cell sorter (FACS) flow cytometer equipped with five lasers (355nm (near UV), 405nm (violet), 488nm (blue), 532nm (yellow-green) and 633nm (red)) and can detect up to 18 fluorophores:

Common fluorophores used in this configuration:

UV (355nm) laser (3 detectors): BUV395, BUV450, BUV737

Violet (405nm) laser (6 detectors): Pacific Blue, AmCyan or V500, BV605 or Qdot605, BV655 or Qdot655, BV710, BV786

Yellow/Green (532nm) laser (4 detectors): PE, PE-TexasRed or mCherry, PE-Cy5.5, PE-Cy7

Blue (488nm) laser (4 detectors): FSC, SSC, FITC, PerCP-Cy5.5 or PI

Red (635nm) laser (3 detectors): APC, AF700 and APC-Cy7

The Aria III is also equipped with a chiller unit for keep sorted samples at 4C while being sorted and is also equipped for plate sorting. At this time, the Aria III.3 (Spiderman) can only handle BSL1 samples.

Follow link to see detailed **Aria 3.3 (Spider-Man)** lasers and filter configurations.

Follow link for assistance building an experimental panel for use on the Aria III.3 (Spiderman) using FluoroFinder: https://app.fluorofinder.com/pavir

Contact Information and Links

Contact Information

Brandon Carter
Palo Alto Veterans Institute for Research (PAVIR) FACS Core Director

VA Flow Core Physical Address:
Palo Alto Veterans Institute for Research
Veterans Affairs Palo Alto Healthcare System
3801 Miranda Ave, Bldg 101, Room B4-115

Email: bcarter@pavir.org

PAVIR Phone: (650) 223-1657

To get on the PAVIR FACS core mailing list, please sign up at:
https://uit.stanford.edu/service/mailinglists/tools and enter *"va_facsusers"* at the subscriber page, or send email to the Flow Core Director Brandon Carter.

To schedule time on the PAVIR FACS Core instruments, please follow the link below:

Important Links

VAPAHCS – Research
https://www.paloalto.va.gov/researchpa.asp

Palo Alto Veterans Institute for Research Links
http://pavir.org

BD Spectral Viewer
http://www.bdbiosciences.com/us/s/spectrumviewer

Biolegend Fluorescence Spectra Analyzer
http://www.biolegend.com/spectraanalyzer

eBioscience FluorPlan Spectra Viewer
http://www.ebioscience.com/resources/fluorplan-spectra-viewer.htm

International Society for the Advancement of Flow Cytometry
http://isac-net.org/

BD Flow Cytometry Introductory Course
http://www.bdbiosciences.com/us/support/s/itf_launch

Compensation tutorial and Info:
http://www.drmr.com/compensation/

Fluorofinder Panel Builder for PAVIR:
https://app.fluorofinder.com/pavir

Acknowledgements and Grants

Acknowledgements and Grants

Please cite in your acknowledgments section the contribution of the Palo Alto VA Flow Cytometry Core in your publications/presentations! You can help by adding or modifying the following statement for your publication, presentation, or grant:

Flow cytometry and/or Fluorescent Activated Cell Sorting (FACS) was done with instruments in the Palo Alto Veterans Institute for Research (PAVIR) FACS Core, which is supported by the US Department of Veterans

Affairs (VA), Palo Alto Veterans Institute for Research (PAVIR), and the and the National Institutes of Health (NIH).

For other VA guidance on publications, please check VHA HANDBOOK 1200.19.

Please send us a reference to the published paper and we will add it to our list publications below.

Thank you for using and acknowledging the PAVIR FACS Core in your work!

Grant Application Description of the PAVIR FACS Facility

List of recent publications that have utilized the PAVIR FACS Core

Zeng R, Bscheider M, Lahl K, Lee M, Butcher EC (2016) Generation and transcriptional programming of intestinal dendritic cells: essential role of retinoic acid. Mucosal Immunol. 9(1):183-93.

Liu L, Cheung TH, Charville GW, Rando TA (2015) Isolation of skeletal muscle stem cells by fluorescence-activated cell sorting. Nat Protoc, 10: 1612-1624.

Rodgers JT, King KY, Brett JO, Cromie MJ, Charville GW, Maguire KK, Brunson C, Mastey N, Liu L, Tsai CR, Goodell MA, Rando TA (2014) mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). Nature, 510: 393-396.

Lee M, Kiefel H, LaJevic MD, Macauley MS, Kawashima H, O'Hara E, Pan J, Paulson JC, Butcher EC (2014) Transcriptional programs of lymphoid tissue capillary and high endothelium reveal control mechanisms for lymphocyte homing. Nat Immunol. (10):982-95.

Watchmaker PB, Lahl K, Lee M, Baumjohann D, Morton J, Kim SJ, Zeng R, Dent AK, Ansel M, Diamond B, Hadeiba H, Butcher EC (2014) Transcriptional and functional profiling of human intestinal dendritic cells reveals conserved specialization and a role for Bcl-6 and Blimp-1 in terminal subset differentiation. Nat Immunol. 2014 15(1): 98–108.

Sen A, Rott L, Phan N, Mukherjee G, Greenberg HB (2014) Rotavirus NSP1 protein inhibits interferon-mediated STAT1 activation. J Virol. 88(1):41-53.

Liu L, Cheung TH, Charville GW, Hurgo BM, Leavitt T, Shih J, Brunet A, Rando TA (2013) Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. Cell Rep, 4: 189-20.