12 January 2024

Issue 11

Research Flow Cytometry Facility

Quarterly Newsletter

Cincinnati Children's changing the outcome together | Research Flow Cytometry Facility

Information Provided by your CCHMC RFCF

OMIPs: How They Can Help

If you are making a new panel or having problems distinguishing populations because of the fluor choice, an OMIP may help make a better panel.

Optimized Multicolor Immunofluorescence Panel (OMIP) is a publication reporting designed and optimized multicolor panels for flow cytometry, fluorescence microscopy, image cytometry and other polychromatic fluorescence-based methods. OMIPs are peer reviewed and published in *Cytometry Part A.* OMIPs reduce the time it takes to optimize panels and provide a starting point for novel panels. OMIPs comprise 5 or more colors, have been thoroughly optimized, and include only publicly available reagents. A published OMIP will include a narrative, reagent table, a stained figure example which may be a gating tree, and detail differences from similar OMIPs. Each OMIP has "supportive information" in an online portion containing panel development strategy, references to related panels, exact staining protocol, instrument configuration, reagent information, and titration for each component. It may also include sample data and analysis files, FMO control information, and biological controls. An OMIP is dynamic, as it can be updated with improved reagents or fluorochrome options. The updates are kept in a separate section to not distort the original publication.

The International Society for Advancement of Cytometry (ISAC) has put together an OMIP database that can be found on the ISAC website under publications. The OMIP database can be searched by OMIP number, reagent (marker, clone, fluorochrome, catalog number), sample type, or cell population. Some panel design programs such as upgraded FluoroFinder can guide you through using an OMIP as part of your panel design process. How to use OMIPs can be viewed in a YouTube video by Aja Rieger.

Transporting Samples

Best practice for transport of all samples within the CCHMC research facility requires 3 elements.

1. **Sealed or capped primary tube (s)**.
2. A sealed leak proof secondary container with a biohazard symbol on it if it is BSL2 or above.
3. Absorbent between the primary and secondary containers to absorb the entire contents.

For transport of specimens on ice, place ice inside the hard-walled secondary container or place the secondary container in an ice bucket.

Please follow these guidelines set by the CCHMC Biosafety Committee.

New Sorter & Updated A5

The RFCF is replacing the FACSAriaII with a new BD S6SE sorter in January. This instrument is like the current S6 but also has spectral capabilities. The A5 analytical cytometer will be upgraded to have spectral capabilities allowing easy transition of panels to the S6SE sorter.

Analyzer Reservations

The RFCF is tracking reservations. Users making reservations and not using them for 5 or more hours per month without cancellation will be contacted. The user's rights can be revoked to ensure better usage by the entire community. We recommend creating a calendar invite on Stratocore as a reminder of scheduled reservations.

Analyzer Shutdown Procedure

After using an analyzer, clean it appropriately. Please DO NOT overfill the cleaning tubes. Keep the solution in the tube below the gray collar on the sample injection tube. Leave the Fortessas and A5 instruments in standby with H_2O on the SIP. Check the sheath tank and refill to the weld line. If the waste container is full, add bleach and a note for the time bleach was added. Put on a new waste container.

Filter Samples PLEASE!

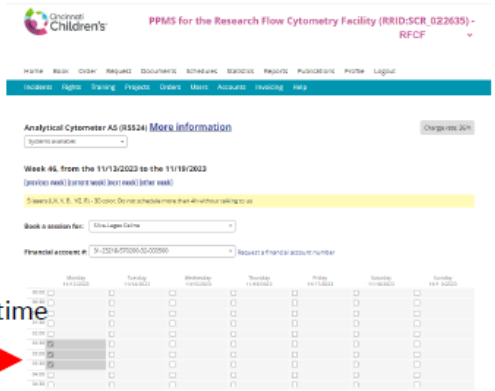
The RFCF instruments have been experiencing a lot of downtime due to clogs. This occurs when samples are not filtered or the concentration of the cells is too high. PLEASE! Filter samples right before coming to the facility. If samples have been sitting and waiting to be run, filter them again. Check the concentration of your cells and for sorting have them at 10-20x10^6cells/mL.

Dates to Note (all in S6.125)

January 19, 9-10am: High Parameter (HP) meeting. Sandra Andorf, PhD: Overview of high-parameter flow cytometry data analysis steps.
January 24, 1-2pm: ORVCA meeting. Cytek: Assay Optimization.
February 2, 9-10am: HP meeting. Ty Troutman, PhD: Sort on BigFoot and readout by RNAseq.
February 16, 9-10am: HP meeting. Alzbeta Godarova, PhD: Substracting (or not) an extra autofluorescent population in fetal lungs from non-human primates.
February 28, 1-2pm: ORVCA meeting. React4life: Microfluidic chip.
March 1, 9-10am: HP meeting. Sherry Thornton, PhD & Noel Gibson: Panel development for analysis of macrophages, monocyte, and T cell populations in systemic juvenile idiopathic arthritis.

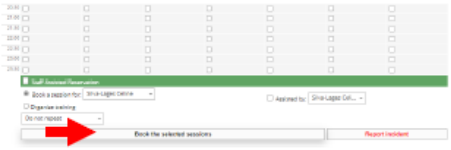
How to create a calendar invite on Stratocore

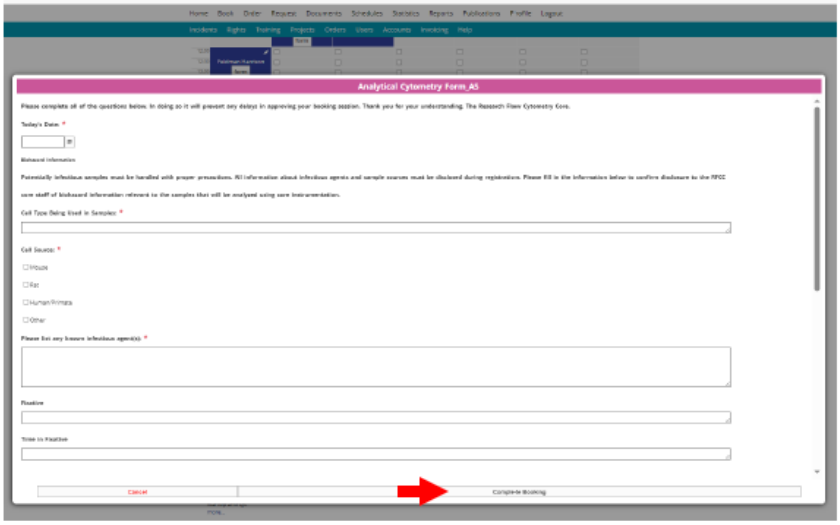
1) Create your new appointment



1) Select time

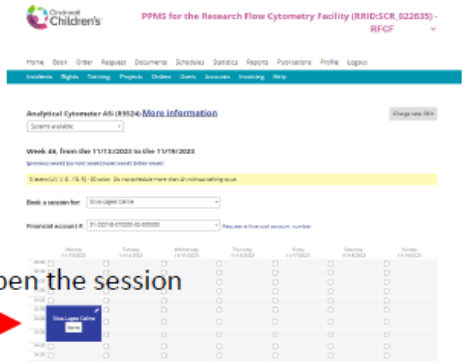
2) Book the session



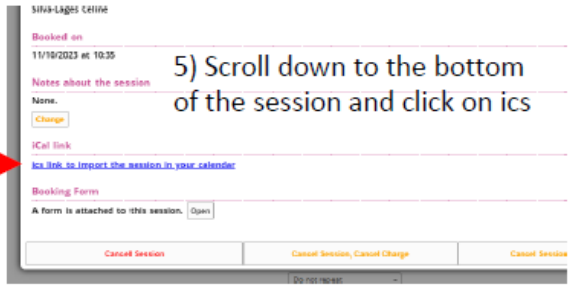


3) Complete the form and complete the booking

2) Download the appointment on Outlook

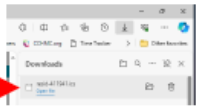


4) Open the session



5) Scroll down to the bottom of the session and click on ics

6) Open the Downloads and click on the .ics



7) Save and Close the created appointment