Core Equipment ID: U0286

Description: Cytek Aurora

Room: IQ Building, Rm 2521

Champion: Matthew Bernard

1.0 Purpose

Standardize the process of control, maintenance, and ownership of the Cytek Aurora spectral flow cytometer located in IQ Building Room 2521.

1.1 Cytek Aurora Capabilities

The Aurora spectral flow cytometer is an air-cooled, multi-laser, compact benchtop flow cytometer. It is equipped with five lasers (UV 355nm, violet 405nm, blue 488nm, yellow-green 561nm, and red 640nm) and up to 64 detection channels for fluorescence and up to three detection channels for scatter (violet SSC [side scatter]; blue SSC/FSC [forward scatter]). Solid-state lasers transmit light through a flow cell where particles in suspension are focused, single file for interrogation by the laser. Proprietary, high-sensitivity, 16-channel semiconductor detector arrays are equipped to capture the emission spectra of dyes that emit in the 400 to 900-nm wavelength range. The resulting fluorescence and scatter are then collected and converted into electronic signals. On-board electronics convert these signals into digital data that can be acquired and recorded on the workstation. The Aurora is also equipped with a 96-well auto-sampler.

1.2 SpectroFlo v2.2 Software Capabilities

Cytek Aurora software (SpectroFlo) controls the Cytek Aurora spectral flow cytometer system in order to acquire data and analyze results. SpectroFlo enables spectral unmixing, an important concept to understand how data is generated and analyzed using the Aurora flow cytometer. Spectral unmixing is used to identify the fluorescence signal for each fluorophore used in a given experiment.

SpectroFlo Software provides the following features:

a. **QC & Setup** - Daily QC ensures that the instrument is in optimal condition for use. Run SpectroFlo QC beads daily to assess system performance and allow the software to adjust settings for day-to-day variation. Levey-Jennings reports keep track of trends in system performance.

b. **Setup** - Allows creation of Reference Controls.

c. **Acquisition** - The Acquisition workspace allows you to create experiments to acquire and analyze data. Experiments can be created through a guided wizard or created from previously saved templates.

d. **Analysis** - Here, FCS files can either be unmixed or compensated using virtual filters.

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e. **Library** - The Library allows you to store experiment templates, worksheet templates, user settings, fluorescent tags, SpectroFlo QC bead information, and label information.

f. **Preferences** - Software preferences can be changed to customize the software. Default plot sizes, fonts, gate colors, print layouts, statistics box table option, and more can all be changed in the Preferences.

g. **Users** - The Users workspace contains user management options and administrative controls.

2.0 Reason for Issue

Maintain a document that describes the Standard Operating Procedures that allow for the standard safe and optimal use of the Cytek Aurora spectral flow cytometer within the Pharmacology and Toxicology Core Facilities.

3.0 Process Description

Allow Core Facility Users within the Pharmacology and Toxicology Department to properly and effectively use the Cytek Aurora spectral flow cytometer. The process description details the standard use of the Cytek Aurora spectral flow cytometer. The controlled standard must maintain and adhere to proper and approved research and regulatory qualitative conditions.

3.1 SOP: U0286.2521.001 for Cytek Aurora flow cytometer, authored by Matthew Bernard, created on 03/31/2020, issued on 04/15/2020.

3.2 SOP: U0286.2521 applies to any User and / or Trainer of the Cytek Aurora.

3.3 **Responsibilities:** All Users are responsible for obtaining the proper approval and training before the use of the Cytek Aurora flow cytometer. All Users are responsible for the proper use, according to defined protocol, when using the Cytek Aurora flow cytometer

a. **New Users** need a SpectroFlo user account created for equipment access, before initial use. New accounts are authorized and created by the Equipment Champion and / or the Core Facility Director/Manager. A new account may be created during the initial training and prior to equipment use approval.

b. **All Users are expected to have completed EHS training programs Bloodborne Pathogens and Biosafety Principles, as required for respective research projects.**

c. **All Users must fill out Appendix I – Biosafety Questionnaire prior to use of the instrumentation in the facility.**

d. **All Users** must schedule equipment using the iLab Solutions portal.

e. **Approved Users** must record all equipment use in the Equipment Usage Logbook (**Appendix II**) post-use on the same day as the recorded use. The Logbook is located on the desk next to the Cytek Aurora flow cytometer. Within the Logbook on the current log sheet, Users must record the following: Date, PI, Name, and Error Messages, as appropriate (**Appendix II**).

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f. Only covered samples may enter Room 2521. Samples must be brought to facility in a standard **spill control box/leak-proof secondary container** that will contain any multiple tube or plate spill, per EHS standards (see Section 4.11c). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm. Spill control boxes must be labeled with Biohazard identification for BSL-2 samples.

g. **All samples must be filtered just prior to running on the Cytek Aurora.**

h. Immediately after use and daily cleaning (see Section 4.3a), the Cytek Aurora spectral flow cytometer must be appropriately shut down (see Section 4.9).

3.4 Equipment Safety Issues

a. **Safety Issues** – The Core Facility operates at up to BSL-2 plus. Biosafety level and limitations for this facility are restricted to WHO and NIH risk groups defined as:

Risk Group 1 – Agents that are not associated with disease in healthy adult humans (no or low individual or community risk)

Risk Group 2 – Agents that are associated with disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).

Examples of risk groups 1 and 2 which may be analyzed include: 1) Plasma or serum from non-primate animals; 2) cell supernatants from cell lines of ATCC origin and those tested negative for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), and Epstein-Barr virus (EBV); 3) primary human serum or plasma if tested for HIV, HBV, HCV, and EBV; 4) Supernatants from primary human cells if tested for HIV, HBV, and HCV; 5) Supernatants from genetically modified cell lines using third generation lentivirus systems.

Research involving BSL-3 or BSL-4 requirements are not supported, which includes WHO and NIH risk groups 3 and 4.

b. **Decontamination of Cytek Aurora post-operation:**
Following the Shutdown procedures (Section 4.9) will result in appropriate daily decontamination of the flow cytometer between uses. More extensive cleaning/decontamination may be performed monthly or prior to service by Cytek (see Section 4.3d).

c. **Decontamination of work surfaces:**

d. External surfaces in front of the Cytek Aurora can be cleaned with Envirocide (or equivalent) or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent).

e. **BSL-2 samples are required to be fixed.** Please consult Core Staff for discussion of exemptions.

f. **Radioactively labeled samples are prohibited.**

g. **Under normal operating conditions, the Cytek Aurora cytometer does not create aerosols.**

h. **All samples exposed to or infected with bacterial or viral agents must be approved by EH&S on a case-by-case basis.** A related HURON Click must be submitted and approved by EH&S prior to scheduling analysis.

i. **Spill control:**
Samples must be brought to the facility in a standard spill control box that will contain any multiple tube or plate spill (see Section 4.11c). All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm.

Report spills to the Core Facility staff.

In the event of a spill for BSL-2 samples, the spill should 1st be covered with absorbent paper towel, which will then be saturated with 10% bleach and allowed to soak a minimum of 10 minutes. The wet towel should be placed in a biohazard waste receptacle after contact. The spill area will then be covered with 10% bleach, allowed to soak briefly, and then wiped up with an absorbent towel. After cleaning the spill, dispose of the absorbent material and gloves into a biohazard waste container. Squeeze bottles of 10% bleach are made fresh daily for spill control.

Report spills to the Core Facility staff.

j. Never place anything on top of the Cytek Aurora, including tube racks or kimwipes.

k. Ensure that the Cytek Aurora waste tank is filled with enough bleach to result in 10% bleach solution following use. Pour bleach waste down the sink after a minimum of 20 minutes contact time following shutdown and flush with additional water.

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3.5 Laboratory Conditions

a. IQ 2521 is a BSL-2 research lab with negative air pressure air flow. The lab door must be closed at all times. The room contains a sink for hand washing, germicidal soap, emergency eye wash station, and spill control kit/equipment.

b. **Signage:** Current BSL-2 and Chemical safety signs having laboratory practices and emergency contact information will be found at the door of Rm 2521.

c. **Access:** Access is limited to people with permission to run samples on the Cytek Aurora, which has been booked through the iLabs web portal. Only individuals involved in training exercises, running samples on the Luminex, Accuri C6, or other instrumentation in the room, or retrieving data should be in Rm 2521.

d. **PPE Requirements:** Standard PPE must be used at all times, which includes gloved hands, long-sleeve lab coat over full and coverage shirt and pants, and full coverage shoes with intact soles.

e. All samples will be handled with BSL-2 precautions, including proper handling, storage, transportation, disposal, and decontamination according to the MSU Biosafety Manual and BBP Exposure Control Plan.

f. **Exposure Control Plan:** Please refer to the Exposure Control Plan available on the MSU EHS website for instructions regarding what to do in the event of an exposure. The MSU Exposure Response Procedure is posted in Rm 2521.

i **Eye/Mucous Membrane Exposure:** Flush immediately at nearest eyewash station for 15 minutes.

Wounds/Needlesticks: Wash the area immediately, use warm water and sudsing soap to scrub the area for 15 minutes.

ii Notify your supervisor immediately if he/she is available.

iii Print Authorization to Invoice MSU Form to take to care facility. https://www.hr.msu.edu/benefits/workers-comp/documents/InvoiceMSU.pdf

iv Report to a Lansing Urgent Care facility for post-exposure follow-up as soon as possible.
https://www.lansingurgentcare.com/

v Be prepared to provide information about the agent or cells involved in the accident. Additionally, route of exposure, dose/concentration, unusual characteristics of the agent, animal infection, cell line, and PI contact information.

Note: Any required follow up visits must also take place at Lansing Urgent Care. The location in Frandor is open 24 hours.

vi Follow up by completing the Report of Claimed Occupational Injury or Illness Form with your supervisor within 24 hours.

g. Sample handling and decontamination within IQ Rm 2521 is covered in Section 3.4. All tubes, pipettes, plates, etc. that represent a biological hazard must be removed by the user and returned to their lab. Waste containers are available for non-hazardous waste. A biological waste container for waste generated during a biohazard cleanup is available in the lab. **No needles are permitted in the Core Facility.**

h. **Eating, drinking, or use of personal care products are prohibited in the facility.** Use of personal electronics will not be allowed if that use interferes with proper operation of the instrumentation in the facility. Those operating flow instrumentation in the facility must remove gloves and wash their hands before using any personal electronic device. Sani-Cloth Plus germicidal disposable wipes are available for wiping **keyboards and personal electronic devices if cross-contamination accidentally occurs.**

i. **Medical:** Users of the facility should have all current vaccinations, including those for HepB. Anyone who may be immune-compromised should visit Occupational Health before working in the facility.

3.6 Contact Information

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a. **Matthew Bernard: Core Director,** Office, IQ Building, Rm 2315 (517)355-4076; (585)703-5008 (cell)

b. **Environmental Health & Safety:** 355-1053

c. **Occupational Health (University Physician's Office):** 353-8933

d. **MSU Police:** 355-2221

3.7 Quality Measures

a. **Daily:** When in use, run SpectroFlo QC beads daily. If an instrument fails this check, see the User's Guide for subsequent recommended procedures.

4.0 Procedure: Cytek Aurora Flow Cytometer Use

4.1 Startup

a. Check fluid levels in all bottles/cubitainers/etc., ensuring that the Sheath (NERL2 or MilliQ water) is full.

b. Add an appropriate amount of bleach to the Waste tank (4L bottle or 20L cubitainer), as appropriate, to reach ~10% final bleach concentration.

c. Gently remove the current tube of ddH_2O (discard), and place a tube containing at least 2 mL of fresh ddH_2O on the SIP (sample injection port). **Tube is required for SIT (sample injection tubing) calibration during startup. Make sure that a tube is present before turning on instrument or logging into the software.**

d. Turn on the cytometer and the workstation.

e. Log into the computer. Sign in under User Account Name and password, as appropriate.

f. Click on the SpectroFlo desktop icon to start the software. This software runs the Cytek Aurora flow cytometer.

g. Check the indicator status to ensure that the instrument is Connected and Sheath and Waste indicators display a green checkmark. If indicators are not green, refer to Aurora Users Guide for troubleshooting.

h. Allow approximately 30 minutes of laser warm-up time before continuing to QC.

i. **Run water for at least 5 minutes on high prior to running QC.**

4.2 Daily QC

a. Run SpectroFlo QC beads daily.

b. Load a 12 x 75-mm tube of SpectroFlo QC beads (1 drop in 0.3 mL sheath: NERL2, PBS, or DI water) onto the SIP.

c. In the QC & Setup workspace, select Daily QC.

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d. Select the appropriate bead lot from the Bead Lot menu. Each time you open a new lot number of SpectroFlo QC beads you must import the bead lot ID into the Library so it is accessible when you run QC.

e. Select Start to begin the Daily QC run.

f. After the completion of the Daily QC run, a QC report is generated. The report includes the following sections:

  i The header section contains the name of the instrument, date of the Daily QC run, user who ran the Daily QC, instrument configuration, instrument serial number, SpectroFlo QC bead lot and expiration date, and Pass/Fail status of the run.

  ii The results section contains the gain, gain change, median fluorescent intensity of the daily QC bead, %rCV, and a pass/fail indicator for each detector channel. The center wavelength of the detector is shown in parentheses next to the detector name.

  iii The Laser Settings section contains the laser delays for all non-primary lasers, and area scaling factors for all lasers and the FSC detector.

  iv Pass/Fail Criteria - The pass/fail criteria are the following:

    1 %rCV must not exceed 6% for the FSC channel.

    2 %rCV must not exceed 8% for the SSC channel.

    3 %rCV must not exceed 6% for the V3 channel.

    4 %rCV must not exceed 6% for the B3 channel.

    5 %rCV must not exceed 6% for the R3 channel.

    6 Delta gain for all channels must not exceed 100 from the last Daily QC run performed.

4.3 Maintenance

a. **Clean the instrument after each use (up to multiple times daily).**

  i In the Cytometer tab, select Fluidics Shutdown.

  ii Follow the steps in section 4.9 for the Fluidics Shutdown.

  iii **When the shutdown is complete, turn the instrument off if you are the last person to use it for the day. If you have confirmed that the instrument will be used after you, you may leave the instrument on.**

b. **Clean the SIT, as appropriate.** A sample line backflush is performed whenever a tube is removed from the SIP after sample acquisition. If the sample line exhibits signs of carryover or becomes clogged after completing an experiment with a sticky dye such as propidium iodine, acridine orange, or thiazole orange, the sample line should be manually backflushed. To manually clean the SIT:

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i In the Cytometer tab or the Experiment Acquisition Settings or Acquisition workspace, select SIT Flush.

ii If carryover or a clog persists, place a tube of 10% bleach on the SIP and acquire at a High flow rate for 5 minutes. Afterwards, acquire a tube of DI water at a High flow rate for 5 minutes.

c. **Remove air bubbles from the flow cell, as appropriate.** Perform this procedure if the FSC and SSC signals appear abnormal. Air bubbles may be trapped in the flow cell, disrupting the sample flow.

i In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select SIT Flush.

ii Alternatively, perform a Clean Flow Cell with water.

d. **Decontaminate the fluidic lines approximately monthly or before Cytek service.** Decontaminate the fluidics system monthly by running the Long Clean fluidic mode. Run the Long Clean just prior to service calls and if you run high volumes of unwashed samples or samples stained with propidium iodide, acridine orange, or thiazole orange. **Do not run bleach or detergent through the sheath filter. This can damage the filter, resulting in paper fragments that could clog the flow cell.**

i In the Cytometer tab, from either the QC & Setup or Acquisition workspace, select Long Clean.

ii Follow the instructions that appear. Prepare the appropriate cleaning tubes and fluidic tanks.

iii Empty the waste tank. Replace the sheath filter with the sheath filter bypass (long clean tubing) assembly (see Aurora User's Guide for depiction).

iv Detach the sheath tank and replace it with a tank containing a 10% bleach solution.

v Install a tube containing 3 mL of a 10% bleach solution on the SIP.

vi Proceed with the Long Clean in the software.

vii Once the bleach cleaning cycle is complete, reattach the sheath tank.

viii Remove the tube of 10% bleach from the SIP and replace with a tube of 3 mL of DI water.

ix Proceed with the Long Clean in the software.

x When prompted, remove the long clean tubing assembly and re-install the sheath filter.

e. Record Maintenance in the Accuri Equipment log, as appropriate (**Appendix III**).

4.4 Instrument Setup – Reference Controls

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Reference Controls must be acquired and recorded to ensure accurate spectral unmixing of the data. References are obtained by acquiring particles stained with individual fluorescent tags. Either beads or cells can be used as single-stained controls for acquiring references. You can select whether to create new Reference Controls or update Reference Controls already stored in the Library.

Please refer to the Cytek Aurora User's Guide for detailed instructions regarding setting up reference controls.

a. Select *New Reference Controls* from the Reference Controls tab in the QC & Setup workspace. A wizard opens allowing you to create new Reference Controls.

b. Select fluorescent tags. The left pane displays the fluorescent tag groups found in the Library. From the expanded list of fluorescent tags, select the fluorescent tags used in the experiment. Once selected, the fluorescent tags appear in the selection pane on the right side of the Define Fluorescent Tags window. You can select fluorescent tags by dragging and dropping, double-clicking, or using the *Add* button. Multiple tags can be chosen at one time. Confirm the tags selected, and then click *Next*.

c. Define the control type for the fluorescent tags, as well as the unstained controls. Once the controls have been defined, select *Next*. If any of the fluorescent tag controls lack a negative population and are of the same type as the unstained control, check the Universal Negative checkbox at the right.

d. Enter labels associated with the fluorescent tag for identification and tracking, as appropriate.

e. If necessary, adjust gain settings. See Section 4.7 for gain optimization.

NOTE: Be sure to save your specific Assay Settings as a new Assay Settings file, preferably with a file name with lab PI and date in the title.

f. Place the appropriate sample on the SIP and click *Start* to view the data.

g. Select *Next* when you are satisfied with the gain settings. Proceed to running controls.

h. Place a tube of the appropriate single-stained particles on the SIP. Click *Record* to begin acquiring. Make sure to follow the order listed in the left-hand panel.

i. During acquisition obtain spectral information by moving the polygon gate on the FSC-A vs SSC-A plot to include the population of interest. Hold down the Ctrl key while adjusting the gate to move the polygon gates for all the fluorescent tags at once.

j. Adjust the positive gate on the histogram. The software automatically displays the emission spectrum of the positive particle in the spectrum plot. SpectroFlo software sets the default gate on the peak emission channel. The gate can be selected manually. It is best to set the gate on the brightest emission as this can make distinguishing the positive and negative population easier. Readjust the positive and/or negative gate on the histogram, if necessary.

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k. Select *Save* to save the Reference Controls to the Library.

4.5 Acquisition

SpectroFlo software saves flow cytometry data in the FCS 3.1 format. Data is saved in both raw and unmixed formats. Raw data contains all the fluorescence information from each detector. Each detector channel is designated by its excitation laser and position in the array. For example, B3 is the third channel of the blue laser detector array. Unmixed data has been spectrally deconvolved based on a set of fluorescent tags and their corresponding Reference Controls. Fluorescence information in unmixed data is classified according to the reference spectra.

The Acquisition workspace provides the necessary tools that allow you to lay out an experiment worksheet. An experiment is a set of tubes, instrument settings, acquisition criteria (stopping rule), fluorescent tags, labels, and worksheets designed for the acquisition of samples.

New and saved experiments can be created or accessed in the Experiments tab of the Acquisition workspace.

Please refer to the Cytek Aurora Software User's Guide for detailed instructions regarding sample acquisition.

a. Select *New* in the Acquisition Experiment menu.

b. The Create New Experiment wizard opens. Specify a name for the experiment and/or type in a description. **File naming designation should use the following format: PI Name, Experiment Description, and Date.**

c. Select the fluorescent tags used in the experiment from the Library pane on the left. You must select all fluorescent tags present in the experiment, as this will determine which Reference Controls are to be used during spectral unmixing.

d. Once all fluorescent tags have been chosen from the Library, confirm the list in the selection pane, and then click *Next*.

e. Select Reference Group if you are intending to unmix with controls acquired in this experiment. This creates a list of tubes for each fluorescent tag specified as part of the experiment.

NOTE: If you plan on unmixing the samples with Reference Controls only from the Library, steps 4.5e-i are not necessary.

NOTE: To mix and match references acquired in the experiment with Reference Controls stored in the Library, define the controls to acquire in the Reference Group, acquire the controls, then after selecting Unmix, select the remaining controls from the stored Reference Controls.

f. **IMPORTANT:** Define an unstained control by selecting its control type. The unstained control needs to be of the same type as the sample, as this will ensure accurate unmixing and autofluorescence quantitation.

g. Select the control type for the single-stained Reference Controls.

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h. Select the label that is conjugated to the fluorescent tag, as appropriate. Select *Save*.

i. Once the Reference Group has been created, entries for each of the references will be displayed. Each of the Reference Group tubes will have an icon (tube with the letter R) associated with it. Create sample groups and samples by selecting the *add Group or Tube* option in the upper left.

j. Add labels to the remaining sample tubes before continuing, as appropriate. They can be chosen from the label list, entered directly into the table, or copied and pasted. Labels can be applied to multiple cells selected at once.

k. Select *Next* when all tubes have been created and labeled.

l. Select the worksheet for the sample tubes.

m. Select the stopping gate, the number of events to collect, and the stopping time. Acquisition stops when the first stopping criterion is met.

n. Once worksheet and stopping criteria have been determined, click *Save and Open* to open the new experiment.

4.6 Unmixing Workflows

There are two data acquisition workflows available in SpectroFlo software:

•Live Unmixing

•Post-Acquisition Unmixing

When data is acquired with live unmixing, references are acquired as raw data either in the experiment as part of the Reference Group or previously acquired in the QC and Setup workspace as Reference Controls. References for all fluorochromes used in a given experiment must be present in the system in order for live unmixing of multicolor samples to occur. The live unmixing functionality allows you to visualize fully compensated data during acquisition. Multicolor samples can be acquired as raw data and unmixed post acquisition as well. This can be done either in the Acquisition workspace or in the Analysis workspace.

a. **To Perform Live Unmixing**:

i Create a new experiment with fluorescent tags defined.

ii Create a Reference Group in the experiment with the fluorescent tags, if there are any that have not been stored as Reference Controls.

iii Acquire all Reference Control tubes. If acquiring beads, collecting 5,000 singlet events is recommended. If acquiring cells, collecting 10,000 events is recommended.

NOTE: Avoid acquiring too many events in the Reference Group tubes. The more events you acquire, the longer it takes to compute the compensation matrix.

iv Select *Unmix* in the upper-left toolbar.

v The Unmixing wizard appears with rows corresponding to the defined fluorescent tags. Select *Use Control from Library* if unmixing with the unstained Reference Controls. Select the *From Library* checkbox if unmixing with fluorescent tag Reference Controls. This checkbox is only active if Reference Controls for those fluorescent tags have already been saved to the Library.

vi Use the Identify Positive/Negative Populations window to include the positive and negative populations for each fluorescent tag in the appropriate gate. Move the polygon gate in the FSC vs SSC plot to include the singlet population. Hold down CTRL to move all the polygon gates at once.

vii Move the interval gate on the spectrum plot on the right to select the channel that exhibits the brightest fluorescence intensity. This channel is the peak emission channel for the fluorescent tag.

viii Move the interval gate in the histogram for the peak channel labeled Positive to include the positively stained population. Move the interval gate in the histogram for the peak channel labeled Negative to include the negative population.

ix Select Live Unmixing.

b. **To Perform Post-Acquisition Unmixing:**
To perform post-acquisition unmixing in the Acquisition workspace, perform the same workflow as live unmixing EXCEPT the following:

i Acquire all Reference Control tubes and sample tubes prior to selecting the unmix button in the upper-left pane.

ii Select Unmix, Save & Open.

iii Experiments that have been unmixed post-acquisition can be found in the *Unmixed* tab of the *My Experiments* menu.

NOTE: If one of the controls is questionable, you can reacquire it, overwriting the original file, and then unmix it again.

4.7 Gain Optimization

a. Gains for all fluorescence-based detectors should be optimized based upon Cytek Assay Settings.

b. If fluorescence is off-scale on any detectors (usually due to use of fluorescent proteins, cellular dyes, or viability dyes) reduce gains proportionally across all detectors for each laser of the detectors that are off-scale only.

c. Collect single stained reference controls with these gain settings, making sure not to save over the most recent version of the Cytek Assay Settings (make sure to save a copy of Assay settings with your gain settings under a different name).

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4.8 Loader Operation

The new Automatic Micro-Sampling System (AMS), referred to as the Loader, is an optional sample loading accessory that delivers a standard 96-well plate to the cytometer for sample acquisition. The Loader plate stage houses a metal element that can cool/heat from 4°– 30°C, if you choose. A wash-station, located at the back of the plate stage is used for cleaning the mixing probe.

Loader settings can be adjusted for the duration and speed of probe mixes, the number of SIT flushes, and the stage temperature. Pre-defined Loader settings include the default settings for high throughput mode, standard mode, and low carryover mode.

a. Startup:

i Turn on the power to the Loader. The power switch is at the back of the Loader where the fluidic lines connect.

ii If there is a tube on the SIP, remove it.

iii Pull the shifter lever towards you to bring the mixer forward.

iv Set the Sample Delivery Mode in Acquisition Preferences to Plate.

v Before running your experiment, you must perform a SIT calibration to set the proper SIT depth within the well.

b. Calibrating the SIT:

i Use the *Calibrate SIT* feature to set the proper SIT depth within a plate well. You will need to perform a SIT calibration prior to running your first experiment of the day in plate mode; every time you switch from tube mode to plate mode; if you change plate types. The calibration value applies only to your individual user account. Once calibration is complete, the settings apply to all plates of the same type

ii Load a 96-well plate.

iii In the Cytometer tab, from either the QC & Setup or Acquisition module, select *Calibrate SIT*.

iv A dialog appears prompting you to enter the SIT Lift Distance. Enter the distance, and then click *Calibrate SIT*. The lift distance is the distance in millimeters from the tip of the SIT to bottom of the well.

v The recommended starting point for the SIT Lift Distance is 1.5 for U-bottom plates. Set the value higher for V-bottom plates. If you experience clogs, try setting the SIT LIFT Distance higher.

vi Check the depth of the sample line in the well. If it's satisfactory, click *OK*. If it needs to be raised or lowered, adjust the SIT Lift Distance and click *Calibrate SIT*.

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vii Repeat the calibration until the sample line depth is satisfactory. Click *OK.* Once SIT calibration is complete, you can run your experiment.

c. Setting up a plate:

i If necessary, select *Eject* from either the QC & Setup or Acquisition module to eject the stage, bringing it forward.

ii Load a plate on the plate stage so that position A1 is located in the front-left corner. Insert the edge of the plate behind the metal tabs at the front of the plate stage, and then press down on the back edge of the plate to secure it in the holder.

iii Select *Load* from the QC & Setup or Acquisition module to load the plate, followed by Start to begin acquisition. Or select *Start* to load the plate and begin acquisition.

iv When acquiring from a well, a boost is applied to deliver the sample to the flow cell as quickly as possible. This process, as well as mixing, results in the loss (consumption) of up to 35 µL of sample, as the sample is neither acquired nor recorded. We recommend a minimum volume of 150 µL of sample per well, keeping in mind that approximately 35 µL will be lost. This can be reduced further by selecting to skip the fluidics boost.

v **Group Hierarchy/Plate Display:** The Group and Tube hierarchy pane when using a Loader defaults to one group with one tube. It also provides an option to add a plate. You can view either a hierarchical list (List View), or once a plate is added, a graphical representation of the plate (Plate View).

vi **Loader Acquisition Controls:** The *Acquisition Control* pane allows you to start, stop, and pause acquisition, record data, and restart acquisition counters. To show, hide, or undock (float) this pane from the experiment panel, use the dock/undock and hide icons in the top-right corner.

vii **Loader Settings:** Several default Loader settings are available. You can select the Default, High Throughput, or Low Carryover Loader Setting, depending on your application. You can also create custom settings. Loader settings are enabled once a plate is added. Adjust the settings before acquisition.

viii **Experiments in Plate Mode:** The steps to create a new experiment when running in plate mode are the same as when running in Tube mode, except for the following tasks—creating groups and defining Loader settings—outlined below.

ix **Creating Groups When Using the Loader:** Before you can add groups when creating a new experiment, you must add a plate. To add groups when running in plate mode, click *Add Plate,* then select the plate type (96 U-, V-, or Flat-Bottom)

x A plate image appears on the right. Three icons at the far right allow you to add groups. Click in the plate image to select a well, or click and drag to select multiple wells corresponding to the wells in the group you wish to add, then

click the appropriate icon to the right of the plate to define the sample types in the group:

1. To add a group for samples, click +.
2. To add a group for reference controls, click R.
3. If you are intending to unmix with controls acquired in this experiment you must add a reference group. When you add a reference group you will be prompted to define the reference controls.
4. To add a group for cleaning well(s), click C. For example, you can add DI water to wells to rinse the SIT and clean the mixing probe to prevent carryover. **A 10% bleach C well should be included and run prior to running Fluidics Shutdown.**

xi Wells will be marked as sample (S), reference control (R), or cleaning (C).

xii When all groups have been defined, click *Next*.

xiii Defining Loader Settings: We recommend using the Default settings for most immunophenotyping applications. When running sticky samples, such as lyse, no wash (LNW) samples, we recommend adding cleaning wells between samples to thoroughly clean the mixing probe. For example, add two wells, one with 10% bleach and the other with DI water. At the end of a plate, consider adding a group of four wells, two with 300 µL of 10% bleach and two with 300 µL of DI water. Program a long mix (15 seconds at 1500 rpm) to thoroughly clean the mixing probe.

xiv Once the worksheet and stopping criteria have been defined, click *Save and Open* to open the new experiment.

4.9 Shutdown

a. If using the Loader switch back to Tube mode in Preferences. Slide the shifter bar back and turn off the Loader.

b. In the Cytometer menu from either the QC & Setup or Acquisition workspace, select Fluidics Shutdown.

c. Place a tube containing 3 mL of 10% bleach on the SIP. Once loaded, the instrument begins drawing in the tube contents. This takes approximately 2 minutes.

d. When prompted, remove the tube and place a tube containing 3 mL of milliQ water on the SIP. Once loaded, the instrument begins drawing in the tube contents. This takes approximately 2 minutes.

e. When prompted, remove the tube and place a tube containing 3 mL of 50% Contrad on the SIP. Once loaded, the instrument begins drawing in the tube contents. This takes approximately 2 minutes.

f. When prompted, remove the tube and place a tube containing 3 mL of milliQ water on the SIP. Once loaded, the instrument begins drawing in the tube contents. This takes approximately 2 minutes.

g. Carefully replace the tube with a fresh tube of DI water on the SIP. Make sure the SIT is submerged in the DI water at the end of the Fluidics Shutdown procedure.

h. Turn off the cytometer and workstation.

i. Exit SpectroFlo software by clicking the X in the upper-right corner of the application window.

j. Empty the waste tank (4L bottle), as appropriate, containing ~10% bleach down the sink while wearing appropriate PPE (ie, lab coat, safety glasses, gloves) and rinse with DI water.

k. Clean keyboard, mouse, and work surfaces in front of the Cytek Aurora with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent).

4.10 Records

a. **Records of Use** – All Cytek Aurora system use must be recorded. Refer to 3.3e.

b. **Error Messages / System Issues –** All error messages and system issues must be relayed to the Equipment Champion and the Core Facility Director/Manager and appropriately recorded, refer to 3.3e, on the same day as equipment use.

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4.11 Resource Index

a. Cytek Aurora and SpectroFlo User Guide:
Cytek Aurora flow cytometer and SpectroFlo software literature and resources for the following items can be found with the Cytek Aurora flow cytometer in room 2521.

b. Cytek Technical Support is available to users in the U.S. and Canada by calling 1-877-922-9835.

Cytek Company Representatives:

Monica DeLay, MS, CYM(ASCP)CM
Technical Application Specialist
Cytek Biosciences
Ph: 513-470-6322
E-mail: mdelay@cytekbio.com

Christopher Fleming, PhD
Technical Application Specialist
Cytek Biosciences
Ph: 502-536-2002
E-mail: cfleming@cytekbio.com

Stephen Whitaker
Technical Sales Specialist
Cytek Biosciences
Ph: (314)313-3781
E-mail: swhitaker@cytekbio.com

c. Transport of Biological Materials:
For detailed information about the transport of biological materials, see the EHS recommended procedures available at:

https://ehs.msu.edu/lab-clinic/shipping/bio-transport-local-vehic.html

5.0 Competences, Authorization and Training

New Users must receive proper authorization from either the Equipment Champion and / or Core Facility Director/Manager before equipment use. A new User may contact the Equipment Champion or Core Facility Director/Manager to schedule training. Training includes SOP and flow cytometer familiarization and any additional required or specialized training. Once training is complete authorization may be issued and a system account and password may be set up. All Users are individually responsible for current SOP familiarization. All New Users must refer to 3.3a during new Cytek Aurora flow cytometer account creation.

6.0 SOP Performance and Equipment Review

The effectiveness of the SOP: U0286.2521.001 will be monitored by the Core Facility Director/Manager, Equipment Champion and All Users. Any procedural or qualitative deviations will be reflected within an updated SOP. Any Approved User should aptly report any procedural or qualitative issues and / or errors to the Core Facility Director/Manager or Equipment Champion. The Core Facility Director/Manager and Equipment Champion's name and contact information can be found on the Pharmacology and Toxicology Core Laboratory in iLabs. Updated SOPs will be published and Approved Users will be notified. SOP: U0286.2521.001 review will occur every two years.

7.0 Definitions

7.1 **SOP:** Standard Operating Procedure, which is a standard guide that officially standardizes the process of control, maintenance, and ownership of the Cytek Aurora flow cytometer. The SOP number stands for (xxx . xxx . xxx) equipment serial number . room number . SOP version number.

7.2 **Originator / Author:** The individual representing the Pharmacology and Toxicology Core Facilities that created SOP: U0286.2521.001

7.3 **Stakeholder:** Any individual that uses or performs the task of which is the subject of the SOP, including the Pharmacology and Toxicology Core Facilities Department.

7.4 **New User:** An individual who has not completed the requirements of section 3.3.

7.5 **Approved User:** An individual who uses the Cytek Aurora flow cytometer and has fulfilled section 3.3. This title may only be given by the Equipment Champion and / or the Core Facility Director/Manager.

7.6 **Champion:** An individual whose direct expertise with the Cytek Aurora instrument has been recognized by the Pharmacology and Toxicology Core Facility Committee. This title may only be awarded by the Pharmacology and Toxicology Core Facility Committee.

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8.0 Approvals

The below signatures and dates are required for full SOP approval and implementation.

This SOP was written/authorized by:

Dr. Matthew Bernard *Matthew Bernard* 4/13/20

This SOP was reviewed by:

Dr. Daniel Vocelle *Daniel Vocelle* 4/13/2020

Issue Date: 4/13/20

Appendix I

MSU Flow Cytometry Biosafety Questionnaire

The MSU Flow Cytometry core facility is now operating under BSL-2 laboratory conditions. This questionnaire serves to gather information important information that will help us render effective core facility services. Part I provides information about the Principal Investigator, each of the independently funded research projects, and the researchers associated with each project. Part II will identify the samples to be analyzed.

Part I

Principal Investigator:
Department: College:
Office Location (building/room):
Office Phone:
E-mail:

The following questions are designed to ID individual grants or projects.

Funding agency:
Project Title:
Grant # or project #:
Account # to be charged for services rendered:
Business Office Address:

Please ID the instrument samples will be analyzed on: Select an instrument

Identify researchers working on this project:

Part II – The Samples

List the type of samples (e.g., animal, human, plant, bacteria) and sources (e.g., spleen, bone marrow, cultured cells):

Has the research protocol used to generate these samples been reviewed by the appropriate Animal (IACUC, please provide AUF #) or Human use Committees (please provide IRB identification and/or EH&S BMR ID#/CLICK#)?

Biosafety level required:

Will the samples be fixed prior to flow cytometric analysis or sorting? ☐Yes ☐No
If yes, describe the fixation protocol:

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<u>Required for BSL-2 samples:</u>

Were tissue/blood donors screened for the following pathogens: HIV, SIV, HepB, HepC, HepD, Herpesvirus simiae, HTLV-1, HTLV-2, LCMV, SARS, Mycobacteria tuberculosis, Mycobacterium bovis, Neisseria meningitides?

☐Yes: (List pathogen and the test results)
☐No: Unknown

Does the sample contain any other known infectious agents, if so please describe?

Has the infectious agent been inactivated? If so, please describe the method:

What precautions does the facility need to employ to safely handle these samples?

<u>Required for Genetically modified samples:</u>

Identify the method of cell transformation. If a virus was used, please identify it:

Were the cells genetically engineered? ☐Yes ☐No

If yes, how were they genetically altered?

What precautions should be taken with these cells?

Once completed and reviewed by the PI, please sign and date the form.

Researcher (Print)	Researcher (Signature)	Date
PI (Print)	PI (Signature)	Date

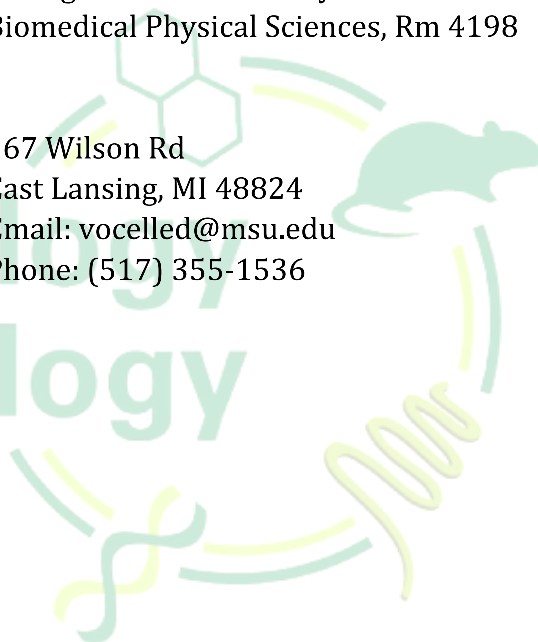
Return the completed form to your appropriate core manager:

Matthew Bernard, Ph.D.
Assistant Professor, Pharmacology & Toxicology
Director, MSU Flow Cytometry Core
Michigan State University
IQ Building, Rm 2315

775 Woodlot Dr
East Lansing, MI 48824
Email: mbernard@msu.edu
Phone: 517-355-4076

Daniel Vocelle, Ph.D.
Core Manager, MSU Flow Cytometry Core
Michigan State University
Biomedical Physical Sciences, Rm 4198

567 Wilson Rd
East Lansing, MI 48824
Email: vocelled@msu.edu
Phone: (517) 355-1536



Appendix II

Cytek Aurora User Log

Please record the following information after each use.

Date	Start Time	End Time	User	PI Name	Shutdown Run?	Turned Off?	Comments / Actions

Please contact Matt Bernard at 517-355-4076 or mbernard@msu.edu with any issues.

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Appendix III

Cytek Aurora Equipment Maintenance Log

Please record the following information after each use.

Date	Time	Initials	Decon Fluidics - Long Clean (Monthly)	Preventative Maintenance (Bi-annually)	Comments / Actions

Please contact Matt Bernard at 517-355-4076 or mbernard@msu.edu with any issues

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