

Core Equipment ID: U0286

Description: Cytex Aurora

Room: IQ Building, Rm 2521

Champion: Matthew Bernard

1.0 Purpose

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

1.1 Cytex 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 Software Capabilities

Cytex Aurora software (SpectroFlo) controls the Cytex 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.
- 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.
- h. **Autofluorescence Explorer** – This wizard provides users with the ability to explore multiple autofluorescent populations in a sample and simplify the unmixing process.

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 MSU Flow Cytometry Core Facility.

3.0 Process Description

Allow Core Facility Users 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: U0286.2521.002 amendment, authored by Matthew Bernard, on 2/16/2024, issued on 11/01/2024. Appendices I, II, and III have been removed, as Biosafety Questionnaire, User Log, and Equipment Maintenance log are no longer needed due to the online Biosafety Questionnaire, the iLab system scheduling, and the electronic Equipment Maintenance Log, respectively. New Autoloader procedures have been appropriately updated. Hazardous waste disposal procedure updated.
- 3.3 SOP: U0286.2521 applies to any User and / or Trainer of the Cytek Aurora.
- 3.4 **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 Staff. 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.**
 - a. **All Users are required to complete a Biosafety Questionnaire for each cell type to be analyzed prior to use of the scheduling use of instrumentation in the facility.**
 - c. **All Users** must schedule equipment using the iLab Solutions portal.
 - d. Only covered samples may enter Room 2521. Samples must be brought to the 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 6.11c). 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.

- e. **All samples must be filtered just prior to running on the Cytex Aurora.**
- f. Immediately after use and daily cleaning (see Section 4.3a), the Cytex Aurora spectral flow cytometer must be appropriately shut down (see Section 4.9).

3.5 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 Cytex Aurora post-operation:**
Following the Shutdown procedures (Section 6.9) 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 Cytex (see Section 6.3d).
- c. **Decontamination of work surfaces:**
External surfaces in front of the Cytex Aurora can be cleaned with 70% Ethanol or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent).
- d. **BSL-2 samples are required to be fixed.** Please consult Core Staff for discussion of exemptions.
- e. **Radioactively labeled samples are prohibited.**
- f. **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.
- g. **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.

- h. Never place anything on top of the Cytek Aurora, including tube racks or kimwipes.
- i. Ensure that the Cytek Aurora waste tank is not over filled. Once the waste carboy has reached the 'Full' line. Disconnect carboy and add bleach to 'Bleach' line to generate a 10% bleach solution (0.5% sodium hypochlorite). Exchange with an empty carboy and arrange with EH&S for pickup of the full, disinfected carboy.

3.6 Laboratory Conditions

- a. IQ 2521 is a BSL-2 research lab with negative air pressure air flow. The lab door must remain closed. 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 iLab 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 3.5. 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.

3.7 Contact Information

- a. **Matthew Bernard: Core Director**, Office, IQ Building, Rm 2315, (517) 355-4076
- b. **Daniel Vocelle: Assistant Director**, Office, BPS Building, Rm 4198, (517)-355-1536
- c. **Environmental Health & Safety:** 517-355-1053
- d. **Occupational Health (University Physician's Office):** 517-353-8933
- e. **MSU Police:** 517-355-2221

3.8 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: Cytex Aurora Flow Cytometer Use

4.1 Startup

- a. Check fluid levels in all bottles/cubitainers/etc., ensuring that the Sheath (MilliQ water) is full.
- b. Ensure that large carboy has not reached "Full" level. Swap carboys if waste is full and add appropriate amount of bleach to large carboy prior to EH&S pickup.
- c. **Make sure that a tube containing ~ 1 mL of milliQ water is present on the SIT (sample injection tubing) before turning on the instrument or logging into the software. The tube is required to be present for SIT calibration during the fluidics startup.**
- 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 Cytex 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: DI water) onto the SIP.
- c. In the QC & Setup workspace, select Daily QC.
- 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:
 - 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 Cytex 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 [Equipment Maintenance Log](#).

4.4 **Recording Library Reference Controls**

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 Cytex 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 6.6 for gain optimization.

NOTE: If you have optimized your assay, 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 profile by moving the polygon gate (P1) 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.
- 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 Cytex 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 6.5e-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.
- 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. It is not recommended to select a storage gate, as this will only record events within a particular gate, excluding all other events.

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

4.6 Gain Optimization

- a. Gains for all fluorescence-based detectors should be optimized based upon Cytex 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 Cytex Assay Settings (make sure to save a copy of Assay settings with your gain settings under a different name).

4.7 Unmixing Workflows

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.

To Perform Live Unmixing:

- . Create a new experiment with fluorescent tags defined.
 - i Create a Reference Group in the experiment with the fluorescent tags, if there are any that have not been stored as Reference Controls.
 - ii Acquire all Reference Control tubes. If acquiring beads, collecting 5,000 singlet events is recommended. If acquiring cells, collecting 10,000 events is recommended. The minimum required positive events for each reference control is 200.

NOTE: It is often helpful to record a similar number of events for Unstained sample as you would for fully stained samples. This will be helpful for Unmixing internal autofluorescence signals. Select *Unmix* in the upper-left toolbar.

- iii 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.
- iv 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 for same sample type (beads or cells) at once.

- v 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.
- vi 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.
- vii Select *Live Unmixing*.

4.8 Autoloader Operation

The Automatic Micro-Sampling System (AMS), referred to as the Loader, is an optional sample loading accessory that delivers a standard 96-well plate or 40 tube rack to the cytometer for sample acquisition.

Loader settings can be adjusted for the duration and speed plate mixing, the number of SIT flushes, and optional sample return. Pre-defined Loader settings include the default settings for high throughput mode, standard mode, and low carryover mode.

a. Autoloader Setup:

- 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 move the SIP guide in place.
- iv In the Acquisition tab of the experimental setup, select plate type and *Add Plate*.
- v 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 10% bleach to clean the SIT. **A 10% bleach C well should be included and run prior to running Fluidics Shutdown.**

- vi Wells will be marked as sample (S), reference control (R), or cleaning (C).
 - vii When all groups have been defined, click *Next*.
 - viii Setup experiment with appropriate recording limits in the *Acquisition* tab and click *Next*.
 - ix **Defining Loader Settings:** Parameters including shaking speed, duration, frequency, etc. can be defined for the whole plate or individual groups or wells. 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 μ L of 10% bleach and two with 300 μ L of DI water.
 - x Once the worksheet and stopping criteria have been defined, click *Save and Open* to open the new experiment.
- b. **Acquiring a plate:**
- i If necessary, select *Eject* from 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 back of the plate stage, and then press down on the back edge of the plate to secure it in the holder, paying careful attention not to move the loader in the x- or y-plane.
 - iii Select *Load* from the Acquisition module to load the plate, followed by Start or Record to begin acquisition.
 - iv A dialog appears prompting you to *Calibrate SIT* height. Select the well designation and click *OK*. Ensure that the SIT drops in the center of the well and lifts enough after hitting the bottom to allow sample acquisition. The SIT lift distance can be set in the *Preferences>Cytometer* menu and is the distance in millimeters from the tip of the SIT to bottom of the well.
 - v When acquiring from a well, a boost is applied to deliver the sample to the flow cell as quickly as possible. This process results in the loss (consumption) of up to 35 μ L of sample, as the sample is neither acquired nor recorded. We recommend a minimum volume of 150 μ L of sample per well, keeping in mind that approximately 35 μ L will be lost. This can be reduced further by selecting to skip the fluidics boost.
 - vi **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).
 - vii **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.

- 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)

4.9 Shutdown

- a. If using the Loader switch back to Tube mode. Slide the shifter bar back and turn off the Loader.
- b. In the Cytometer menu, 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 30% 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.
- i. Exit SpectroFlo software by clicking the X in the upper-right corner of the application window.
- j. Replace the waste tank if full and add 10% bleach to the full carboy prior to pickup scheduling with EH&S. Ensure that appropriate PPE (e.g., lab coat, safety glasses, gloves) is utilized. Clean keyboard, mouse, and work surfaces in front of the Cytex Aurora with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent).

4.10 Records

- a. **Error Messages / System Issues** – All error messages and system issues must be relayed to the Equipment Champion and the Core Facility Staff and appropriately recorded in the [Instrument Maintenance Log](#) on the same day as equipment use.

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:

Joel Crespo, PhD	Gregory Kleedtke
Technical Application Specialist	Technical Sales Representative
Cytek Biosciences	Cytek Biosciences
Ph: 317-448-2338	Ph: 810-224-1277
E-mail: jcrespo@cytekbio.com	E-mail: gkleedtke@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 Competencies, Authorization and Training

New Users must receive proper authorization from either the Equipment Champion and / or Core Facility Staff before equipment use. A new User may contact the Equipment Champion or Core Facility Staff 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 Section 3.4 during new Cytek Aurora flow cytometer account creation.

6.0 SOP Performance and Equipment Review

The effectiveness of the SOP: U0286.2521 will be monitored by the Core Facility Staff, 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 Staff or Equipment Champion. The Core Facility Staff and Equipment Champion's name and contact information can be found on the Pharmacology and Toxicology Core Laboratory in iLab. Updated SOPs will be published and Approved Users will be notified. SOP: U0286.2521 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 MSU Flow Cytometry Core Facility that created SOP: U0286.2521
- 7.3 **Stakeholder:** Any individual that uses or performs the task of which is the subject of the SOP, including the MSU Flow Cytometry Core Facility Department.

- 7.4 **New User:** An individual who has not completed the requirements of Section 3.4.
- 7.5 **Approved User:** An individual who uses the Cytex Aurora flow cytometer and has fulfilled Section 3.4. This title may only be given by the Equipment Champion and / or the Core Facility Staff.
- 7.6 **Champion:** An individual whose direct expertise with the Cytex Aurora instrument has been recognized by the MSU Flow Cytometry Core Facility Staff. This title may only be awarded by the MSU Flow Cytometry Core Facility Staff.

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  01Nov2024

This SOP was reviewed by:

Dr. Daniel Vocelle  11/13/2024

Issue Date: November 13th, 2024