Core Equipment ID:

1.1. BPS4120**:** 2AAS400110223 (Autosampler), 2IAFC03651223 (CytPix)

1.2. BPS 5115: 2AAS400150525 (Autosampler), 2IAFC05221224 (CytPix)

Description: Attune CytPix and CytKick Max autosampler

Room: BPS 4120 and BPS 5115

Champion: Soo Hyun Ahn

1. Purpose

Standardize the process of control, maintenance, and ownership of the Attune CytPix flow cytometers located in BPS Building Room 4120 and 5115.

2. Attune CytPix Capabilities

2.1. The Attune CytPix flow cytometer is a benchtop flow cytometer with acoustic focusing and brightfield imaging capabilities. It is equipped with four lasers (violet 405nm, blue 488nm, yellow-green 561nm, and red 637nm) and 14 detection channels for fluorescence and two detection channels for scatter (SSC [side scatter]; 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.

2.2. The Attune CytKick Max autosampler can be used with 96-well, 384-well, microfuge tubes, and custom plates.

3. Attune Cytometric Software Capabilities

3.1. Attune Cytometric Software controls the Attune CytPix flow cytometer system in order to acquire data and analyze results. The software is utilized to "design and perform experiments, define independent instrument settings and optimize data collection, carry out instrument performance checks and track instrument performance, acquire and record data, and manage and process recorded data". For further information, refer to the Attune Cytometric Software User Guide.

4. Reason for Issue

4.1. Maintain a document that describes the Standard Operating Procedures (SOPs) that allow for the standard safe and optimal use of the Attune CytPix flow cytometer within the MSU Flow Cytometry Core Facility.

5. Process Description

Allow Core Facility Users to properly and effectively use the Attune CytPix conventional flow cytometer. The process description details the standard use of the Attune CytPix conventional flow cytometer. The

controlled standard must be maintained and adhere to proper and approved research and regulatory qualitative conditions.

5.1. SOP: ACP.4120.001 and ACP.5115.001 for Attune CytPix flow cytometer, authored by Soo Hyun Ahn, created on 09/15/2025, issued on 09/30/2025.

5.2. SOP: ACP.4120.001 and ACP.5115.001 apply to any User and / or Trainer of the Attune CytPix.

6. Responsibilities

All Users are responsible for obtaining proper approval and training before the use of the Attune CytPix flow cytometer. All Users are responsible for the proper use, according to defined protocol, when using the Attune CytPix flow cytometer

6.1. **New Users** need an Attune Cytometric Software 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.

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

6.3. **All Users** must complete the appropriate D2L modules and complete the required training for independence usage of the instrument.

6.4. **All Users** are required to complete a Core Policy and Biosafety form for each cell type to be analyzed prior to use of the scheduling use of instrumentation in the facility.

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

6.6. Only covered samples may enter Room 4120 and 5115. 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 16). 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.

6.7. All samples must be filtered just prior to running on the Attune CytPix.

6.8. Immediately after use and daily cleaning (See Section 15), the Attune CytPix flow cytometer must be appropriately shut down (see Section 16).

7. Equipment Safety Issues

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:

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

7.2. 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).

7.2.1. 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.

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

7.4. **Decontamination of work surfaces:** External surfaces in front of the Attune CytPix can be cleaned with 70% Ethanol, 10% Bleach, or wiped down with Sani-Cloth Plus germicidal wipes (or equivalent).

7.5. **Decontamination of Attune CytPix post-operation:** Following the Cleaning and/or Shutdown procedures (Section 16) will result in appropriate daily decontamination of the flow cytometer between uses. More extensive cleaning/decontamination may be performed every 90 days (See Sections 18 & 19)

7.6. BSL-2 samples are required to be fixed for 4120 Attune. Please consult Core Staff for discussion of exemptions.

7.7. **Radioactively labeled samples are prohibited.**

7.8. 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.

8. Spill control:

8.1. Samples must be brought to the facility in a standard spill control box that will contain any multiple tube or plate spill. All tubes and plates should be capped to maintain containment of samples. Seal multi-well plates with plate sealer or parafilm.

8.2. Report spills to the Core Facility staff.

8.3. In the event of a spill for BSL-2 samples, immediately cover the spill with absorbent paper towel and saturate the towel with 10% bleach. Allow it to soak for a minimum of 10 minutes. Wearing appropriate nitrile gloves, place the wet towel in a biohazard waste receptacle after contact. Cover the spill area with 10% bleach again, allow the area to soak briefly and wipe up the area with 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.

8.4. Report spills to the Core Facility staff.

8.5. Never place anything on top of the Attune CytPix, including tube racks or Kimwipes.

8.5.1. Ensure that the Attune CytPix 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.

9. Laboratory Conditions

- BPS 4120 and 5115 are BSL-2 research labs 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.
- **Signage:** Current BSL-2 and Chemical safety signs having laboratory practices and emergency contact information can be found at the door of Rm 4120 and 5115.
- **Access:** Access is limited to people with permission to run samples on the Attune CytPix following booking on iLab web portal. Only individuals involved in training exercises, running samples on the CQ1, SONY MA900, or other instrumentation in the room, or retrieving data should be in BPS Rm 4120 or 5115.
- **PPE Requirements:** Standard PPE must be always used, which includes gloved hands, long-sleeved lab coat over full coverage shirt and pants, and full coverage shoes with intact soles.
- 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.
- **Exposure Control Plan:** Please refer to the Exposure Control Plan available on the MSU EH&S website for instructions regarding what to do in the event of exposure. The MSU Exposure Response Procedure is posted in Rm 4120 and 5115.
- **Eye/Mucous Membrane Exposure:** Flush immediately at the 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.
- Notify your supervisor immediately if he/she is available.
- Print Authorization to Invoice MSU Form to take to care facility. These are also available in the room. **https://www.hr.msu.edu/benefits/workers-comp/documents/InvoiceMSU.pdf**
- Report to a Lansing Urgent Care facility for post-exposure follow-up as soon as possible. **https://www.lansingurgentcare.com/**
- 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.
- Follow up by completing the Report of Claimed Occupational Injury or Illness Form with your supervisor within 24 hours.

- Sample handling and decontamination within BPS Rm 4120 and Rm 5115 are covered in Section 7.

- All tubes, pipettes, plates, etc. that represent a biological hazard must be removed by the user and returned to their lab. Waste bins are available for non-hazardous waste. The nitrile gloves should be discarded in the biohazard bins. A biological waste container for waste generated during a biohazard cleanup is available in the lab. No needles or sharps are permitted in the Core Facility.

- Eating, drinking, or use of personal care products is 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. 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.

10. 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.

Contact Information

1. Soo Hyun Ahn: Core Manager, Office, BPS Building, Rm 4198, (517)-802-8074
2. Environmental Health & Safety: (517)-355-1053
3. Occupational Health (University Physician's Office): (517)-353-8933
4. MSU Police: (517)-355-2221

11. Procedure: Attune CytPix Flow Cytometer Use

11.1. Quality Measures

11.1.1. Daily: When in use, run Attune Performance Tracking beads daily. If an instrument fails this check, see the User's Guide for subsequent recommended procedures.

11.1.2. After service, major troubleshooting, or when a new bead lot has been imported: a Baseline performance test must be performed. Once the baseline calculation is complete, run the Sanitize SIP prior to sample acquisition (see Section 12).

11.2. Startup

11.2.1. Check fluid levels in all bottles/cubitainers/etc., ensuring that the Sheath (Focusing Fluid), wash, and shutdown solution are full.

11.2.2. Ensure that large waste 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.

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

11.2.4. Click on the Attune Software desktop icon to start the software. This software runs the Attune CytPix flow cytometer.

11.2.5. Log in to the Attune software under the appropriate lab profile.

11.2.6. Verify proper shutdown of instrument by the Status indicator lights displaying multicolor. If indicator lights are amber, refer to the Attune CytPix Flow Cytometer User Guide for troubleshooting.

11.2.7. Select the Startup function on the Instrument ribbon tab, and follow the instructions provided through the Startup dialog.

11.2.8. Lower the tube lifter and remove the tube from the SIP. Open the CytKick, remove the shutdown plate, and close the CytKick door. Startup will proceed.

11.2.9. Once Startup is complete, check the status bar to ensure that the instrument is idle. If indicators are not green, refer to Attune CytPix Flow Cytometer User Guide for troubleshooting. Proceed with Daily QC/Performance Tracking (Section 11.3).

11.3. Daily QC/Performance Tracking

11.3.1. Run Attune performance tracking beads daily

11.3.2. In the Main Menu workspace, select Performance Test.

11.3.3. Check instrument configuration by lifting the lid on the Attune Cytpix. Make sure all filters are in appropriate orientation, and none are present in the holder.

11.3.4. Verify the bead lot number on the Attune Performance Tracking beads matches the lot number under baseline information.

11.3.5. Load a 12 x 75-mm tube of Attune performance tracking beads (3 drops in 2.0 mL focusing fluid/sheath) on the SIP.

11.3.6. Select Run Performance test to begin the Daily QC run.

11.3.7. After the completion of the Daily QC run, a results table is generated. The table includes the following sections:

11.3.7.1. The channels are color coded to laser color and include scatter (FFSC and SSC), blue, red, violet, yellow, and green.

11.3.7.2. The parameters generated for each channel include PMTV (photomultiplier tube voltage), delta PMTV, target MFI (target median fluorescence intensity), measured MFI, robust %CV (robust percent coefficient of variation), quantum efficiency (Qr), background, linearity, area scaling factor (ASF), laser delay, and Pass/Fail Status

11.3.8. Pass/Fail Criteria - The pass/fail criteria are specified by the instrument.

12. Baseline Calculations

Baseline performance test must be performed **after service, major troubleshooting, or when a new bead lot has been imported.** Once the baseline calculation is complete, run the Sanitize SIP prior to sample acquisition.

12.1.1. In the Main Menu workspace, select Performance Test. Select Reset Baseline underneath the Run performance test button.

12.1.2. In the Select a bead lot file dropdown menu, select the appropriate bead lot #. See Section 12.2 on how to import a new bead lot file.

12.1.3. Check instrument configuration by lifting the lid on the Attune CytPix. Make sure all filters are in appropriate orientation, and none are present in the holder.

12.1.4. Verify the bead lot number on the Attune Performance Tracking beads matches the lot number under baseline information.

12.1.5. Load a new 12 x 75-mm tube of Attune performance tracking beads (4-8 drops in 3.0 mL focusing fluid) on the SIP.

12.1.6. Select Run Baseline. Once complete, Baseline Test Results will be displayed.

12.2. Importing a New Bead Lot File:

12.2.1. Visit the Attune™ Performance Tracking Beads (Cat. 4449754) web page located within the ThermoFisher website.

12.2.2. Under the Documents section of the webpage, download and save the bead lot file that matches the beads you will be using for baseline and Performance Test. **Note: Download the file for the Attune Acoustic Focusing Cytometer, not Attune NxT.** The bead lot file number is the first six digits printed on the label. Disregard the alpha numeric characters. The bead lot data file is a *.csv format.

12.2.3. On the Attune CytPix desktop, save the bead lot file to Desktop.

12.2.4. In the Attune cytometric software, under performance test, select “Get New Lot#” to navigate and select the bead lot data file. It will populate the drop-down list and copy to the correct destination.

12.2.5. Navigate to Performance Test and run a baseline using the new bead lot file.

13. Acquisition

Please refer to the Attune CytPix Software User’s Guide for detailed instructions regarding sample acquisition. The User’s Guide can be found on the Desktop of Attune Cytometer.

13.1. Attune Cytometric Software saves flow cytometry data in the FCS 3.1 format.

13.2. New and saved experiments can be created or accessed in the Experiment Explorer tab of the Main Menu and Attune Desktop.

13.3. Select *New Experiment* in the Main Menu.

13.4. The New Experiment window opens. Specify a name for the experiment and/or type in a description. Select experiment type, tubes or plates. If plates are selected, select the appropriate plate type. Select the default workspace and instrument settings or load in from user files saved on the desktop. The number of samples and groups may be entered for the experiment. Once complete, select OK to create the new experiment.

13.5. It is recommended that the file naming designation should use the following format: PI Name, Experiment Description, and the current Date.

13.6. In the Main application workspace, select the instrument settings panel to select the appropriate scatter and fluorescent channels, adjust voltages, and assign fluorophores/labels to appropriate channels. Minimally, select the H and W checkboxes to select Height and Width for FSC and SSC to enable doublet discrimination.

14. Voltage/Instrument Settings Optimization

14.1. Before recording data for a sample, instrument settings should be optimized. Optimizing instrument settings involves fine-tuning the PMT voltages, compensation, and threshold settings for each dye and sample used in the experiment, which allows you to adjust the positions of populations of interest on scale for scatter and fluorescence parameters.

14.2. Instrument setting optimization can be optimized using a fully stained sample. NOTE: It may be necessary for the user to prepare an additional sample for gain optimization.

14.3. The voltage for the PMT detector (channels, SSC) and a diode detector (FSC only) can be adjusted manually by entering desired voltage or using the slider bar. Adjustments made will automatically refresh the data in the workspace after a few seconds. If this does not occur, click on Clear button. If entering a voltage value manually, the enter key must be pressed to save changes and refresh data in the workspace.

14.4. Adjust FSC and SSC voltages to place target population on scale.

14.5. If fluorescence is off-scale on any detectors (usually due to use of fluorescent proteins, cellular dyes, or viability dyes) reduce gains on the specific detector that shows off scale events.

14.6. **NOTE:** The Attune CytPix Software is setup with optimized voltages for each fluorescence detector (ThermoFisher Assay Settings) to provide the highest resolution of the detector with dim fluorescence. Therefore, voltage settings generally only need to be decreased to bring populations on scale. Increasing voltages when assessing cellular populations does not typically increase resolution but will also increase background signal.

14.7. If appropriate, adjust the Threshold to remove debris and background.

15. Compensation in the Attune CytPix Software

15.1. Fluorophores emit light over a range of wavelengths. Although optical filters limit the range of wavelengths measured by a given detector, when two or more fluorophores are used in an experiment, there is often an overlap between the wavelength ranges. Compensation is the mathematical method used to correct the overlap of one fluorophore's emission into another fluorophore's emission channel. The software calculates the compensation settings automatically as it guides you through the process.

15.2. If the experiment requires compensation, prepare the necessary compensation controls. Each fluorophore used for compensation requires single-stained positive controls (i.e., compensation beads or cells). Additionally, unstained samples (universal negative) or negative populations within each single-stained sample (internal negative) are required.

15.3. Ensure voltage optimization has been performed prior to performing compensation with single stained and unstained samples/controls, as appropriate. Once data has been recorded for these samples and compensation calculated, instrument settings for all fluorescent channels should not be changed.

15.4. **To Perform Compensation:** Select Compensation Setup button to launch the Compensation Dialog. Alternatively, double-click the Compensation file that is automatically created with the experiment folder.

15.5. **In tube mode,** double-clicking the Compensation file will open up the Compensation dialogue. Make sure that correct filters are selected. Run unstained sample and single color samples until all of the controls are recorded. For each fluor, the worksheet will show a dotplot and a histogram. Arrange the gate to highlight the cells, which will then automatically populate the histogram. Select the highest peak fluorescence using the bracket gate. Repeat for all fluors.

15.6. **In plate mode,** double-clicking the Compensation file will open up the Compensation dialogue where the user need to select the well for each filter. NOTE: if these wells are already selected and have put under a specific group, the well number will not show up. Close the dialogue, delete those files and then go back to the Compensation wizard. Post selecting the wells for each filter, close out of the wizard. In plate mode, the user can choose to run entire group of samples that are used for compensation. These options are available under the drop down menu under the Record button. Follow the same strategy post acquisition for gating the cells and highlighting the peak fluorescence using the bracket gate. Repeat for all fluors.

15.7. **Post acquisition, assess the spillover by clicking on the Compensation matrix located on the *Compensation* tab.**

16. Daily cleaning procedure between users

16.1. The cleaning protocol must be performed after each use (up to multiple times daily). Ensure that appropriate PPE (e.g., lab coat, safety glasses, gloves) is utilized. Clean keyboard, mouse, and work surfaces in front of the Attune CytPix with Envirocide (or equivalent) or with Sani-Cloth Plus germicidal wipes (or equivalent).

16.2. The following Cleaning procedure applies to both 4120 and 5115 Attune Cytpix:

Cleaning Procedure for CytPix Users (Tubes):

16.2.1. In the Instrument ribbon tab, select Unclog. When prompted, lower the tube lifter, place a clean and empty tube on the SIP, and raise the tube lifter. The instrument will begin a back flush operation to remove clogs.

16.2.2. Next, select Sanitize SIP. When prompted, lower the tube lifter, place a tube containing 3 mL of 1:3 dilution of Hellmanex III, and raise the tube lifter. Once loaded, the instrument will begin washing and sanitizing the SIP.

16.2.3. Next, select Sanitize SIP. When prompted, lower the tube lifter, place a tube containing 3 mL of a 10% Bleach solution, and raise the tube lifter. Once loaded, the instrument will begin washing and sanitizing the SIP.

16.2.4. **For 5115 BSL-2 Attune CytPix:** Allow the instrument to stand for a minimum of 10 minutes of contact time with 10% Bleach for decontamination.

16.2.5. Unload tube and select Rinse to run an extra rinse function due to the viscosity of Hellmanex. Lower the tube lifter once prompted and the instrument will begin rinsing the sample lines.

16.2.6. Gently clean the SIP with a Kimwipe. **For 5115 BLS-2, use a lint-free swab** (e.g., microfiber Puritan(R) PurSwab (R), or equivalent) to clean the SIP. Swabs should be disposed of into an appropriate biohazardous waste container.

16.2.7. Leave a tube with approximately 1 mL of MilliQ water on the SIP.

Cleaning Procedure for CytKick Users (Short Plates):

16.2.8. In the Instrument ribbon tab, select Unclog. When prompted, lower the tube lifter, place a clean and empty tube on the SIP, and raise the tube lifter. The instrument will begin a back flush operation to remove clogs.

16.2.9. Next, select the Sanitize SIP dropdown menu, and select Auto Sampler SIP (Standard). Once prompted, lower the tube lifter, place a tube containing 3 mL of a 1:3 dilution of Hellmanex III, and raise the tube lifter. Open the CytKick Max autoloader, place a 96-well shutdown plate with 250 µL MilliQ in well A2, and close the door. Once loaded, the instrument will begin washing and sanitizing the SIP.

16.2.10. Next, select the Sanitize SIP dropdown menu, and select Auto Sampler SIP (Standard). Once prompted, lower the tube lifter, place a tube containing 3 mL of a 10% Bleach solution, and raise the tube lifter. Open the CytKick, place a 96-well shutdown plate with 250 µL MilliQ in well A2, and close the door. Once loaded, the instrument will begin washing and sanitizing the SIP.

16.2.11. **For 5115 BSL-2 Attune CytPix:** Allow the instrument to stand for a minimum 10 minutes contact time with 10% bleach for decontamination.

16.2.12. After unloading the tube, select Rinse to run an extra rinse function due to the viscosity of Hellmanex. Lower the tube lifter once prompted and the instrument will begin rinsing the sample lines.

16.2.13. Gently clean the SIP with a Kimwipe. **For 5115 BSL-2, use a lint-free swab** (e.g., microfiber Puritan(R) PurSwab (R), or equivalent) to clean the SIP. Swabs should be disposed of into an appropriate biohazardous waste container.

16.2.14. Leave a tube with approximately 1 mL of MilliQ water on the SIP.

Cleaning Procedure for CytKick Users (Deep Well Plates):

16.2.15. In the Instrument ribbon tab, select Unclog. When prompted, lower the tube lifter, place a clean and empty tube on the SIP, and raise the tube lifter. The instrument will begin a back flush operation to remove clogs.

16.2.16. Next, select the Sanitize SIP dropdown menu, and select Auto Sampler SIP (Deep Well). Once prompted, lower the tube lifter, place a tube containing 3 mL of a 1:3 dilution of Hellamenx, and raise the tube lifter. Open the CytKick, place a 96-well shutdown plate with 1.5 mL of a 1:3 dilution of Hellmanex III in well A1, and 2 mL of MilliQ in well A2,

and close the door. Once loaded, the instrument will begin washing and sanitizing the SIP.

16.2.17. Next, select the Sanitize SIP dropdown menu, and select Auto Sampler SIP (Deep Well). Once prompted, lower the tube lifter, place a tube containing 3 mL of a 10% Bleach solution, and raise the tube lifter. Open the CytKick, place a 96-well shutdown plate with 1.5 mL of 10% Bleach solution in well A1, and 2 mL of MilliQ in well A2, and close the door. Once loaded, the instrument will begin washing and sanitizing the SIP.

16.2.18. **For 5115 BSL-2 Attune CytPix:** Allow the instrument to stand for a minimum 10 minutes contact time with 10% bleach for decontamination.

16.2.19. Select Rinse to run an extra rinse function due to the viscosity of Hellmanex. Lower the tube lifter once prompted and the instrument will begin rinsing the sample lines.

16.2.20. Gently clean the SIP with a Kimwipe. For 5115 BLS-2, use a lint-free swab (e.g., microfiber Puritan(R) PurSwab (R), or equivalent) to clean the SIP. Swabs should be disposed of into an appropriate biohazardous waste container.

16.2.21. Leave a tube with approximately 1 mL of MilliQ water on the SIP.

17. Shutdown

17.1. The end-of-the-day user must perform Shutdown protocol. The following applies to both BPS 4120 and BPS 5115 unless otherwise stated specifically for each instrument. After Shutdown has been initiated, turn off the BSC (5115 BSL-2 Attune only).

Shutdown Procedure for CytKick Max Autosampler Users (Short Plates):

17.1.1. In the Instrument ribbon, select Unclog. When prompted, lower the tube lifter, place a clean empty tube, and raise the tube lifter. Once loaded, the instrument will begin a back flush into the tube. This takes approximately 3 minutes.

17.1.2. Next, select the Sanitize SIP dropdown menu, and select Auto Sampler SIP (Standard). Once prompted, lower the tube lifter, place a tube containing 3 mL of a 1:3 dilution of Hellmanex III, and raise the tube lifter. Open the CytKick, place a 96-well shutdown plate with 250 µL MilliQ in well A2, and close the door. Once loaded, the instrument will begin washing and sanitizing the SIP.

17.1.3. Select Shutdown– Thorough. When prompted, lower the tube lifter, place a tube containing 3 mL of 10% bleach solution, and raise the tube lifter. Open the CytKick, place an empty 96-well shutdown plate, and close the door. Once loaded, the instrument will begin the shutdown cycle, which takes approximately 3 hours to complete.

17.1.4. **For 5115 BSL-2 Attune CytPix only:** Swap out the waste with an empty waste container to complete Shutdown. As the instrument is undergoing Shutdown, add 180mL of bleach to the 1.8L waste bottle (for 10% bleach v/v) and leave the bottle in the BSC overnight. This will be disposed of 24 hours by the Core

Shutdown Procedure for CytKick Max Autosampler Users (Deep Well Plates):

17.1.5. In the Instrument ribbon, select Unclog. When prompted, lower the tube lifter, place a clean empty tube, and raise the tube lifter. Once loaded, the instrument will begin a back flush into the tube. This takes approximately 3 minutes.

17.1.6. Next, select the Sanitize SIP dropdown menu, and select Auto Sampler SIP (Deep Well). Once prompted, lower the tube lifter, place a tube containing 3 mL of a 1:3 dilution of Hellamenx, and raise the tube lifter. Open the CytKick, place a 96-well shutdown plate with 1.5 mL of a 1:3 dilution of Hellmanex III in well A1, and 2 mL of MilliQ in well A2, and close the door. Once loaded, the instrument will begin washing and sanitizing the SIP.

17.1.7. Select Shutdown – Thorough. When prompted, lower the tube lifter, place a tube containing 3 mL of 10% bleach solution, and raise the tube lifter. Open the CytKick, place an empty 96 – deep well shutdown plate, and close the door. Once loaded, the instrument will begin the shutdown cycle, which takes 3 hours to complete.

17.1.8. **For 5115 BSL-2 Attune CytPix only:** Swap out the waste with an empty waste container to complete Shutdown. As the instrument is undergoing Shutdown, add 180 mL of bleach to 1.8 L waste bottle (for 10% bleach v/v) and leave the bottle in the BSC overnight. This will be disposed of 24 hours by the Core.

17.1.9. Logout of Attune Software and exit by clicking the X in the upper-right corner of the application window.

17.1.10. In both BPS Rm 4120 and Rm 5115, 20L waste tanks are available for biohazardous waste collection.

17.1.11. For waste tanks in 4120, the waste generated from the flow cytometer and autosampler are automatically discarded into the 20L waste tank. Once the liquid level reaches the "Full" line, top it up with 100% bleach to the "Bleach" line to ensure 10% bleach solution. Schedule pick up with EH&S. Replace the carboy with a new one.

17.1.12. For waste tanks in 5115, the users will fill this tank with biohazardous waste already decontaminated with 10% bleach. The 1.8L waste bottles located on the flow cytometer will be filled with 100% bleach (180 mL) to ensure final bleach concentration is 10%. After 24 hours, these bottles will be emptied into the 20L waste tanks. Once these waste tanks are full, schedule pick up with EH&S.

17.1.13. **For 5115 BSL-2 Attune Cytpix:** At the end of the day, replace all tubes with clean ones. 5 mL polystyrene tubes used for Unclog will be capped and disposed of in the biohazard bins. These tubes will contain sheath fluid with possible unfixed materials that may be dislodged from the tubing. The liquid resulting from the 5 mL tubes used for Sanitize SIP containing 1:3 dilution of Hellmanex III will be discarded into an appropriate hazardous waste container for EH&S collection. The empty tube can be capped and discarded in a biohazard waste container. The bleach solution in the 5mL tubes used for Sanitize SIP containing 10% bleach will be dumped into the bleach waste container. The tube itself can be discarded in the conventional waste bin.

17.1.14. Shutdown plate: spray down the shutdown plate with 70% ethanol and let it dry in the hood.

Shutdown Procedure for CytPix Users (Tubes):

17.1.15. In the Instrument ribbon, select Unclog. When prompted, lower the tube lifter, place a clean empty tube, and raise the tube lifter. Once loaded, the instrument will begin a back flush into the tube. This will take approximately 3 minutes.

17.1.16. Next, select Sanitize SIP. When prompted, lower the tube lifter, place a tube containing 3 mL of 1:3 dilution of Hellmanex III, and raise the tube lifter. Once loaded, the instrument will begin washing and sanitizing the SIP.

17.1.17. Select Shutdown– Thorough. When prompted, lower the tube lifter, place a tube containing 3 mL of 10% bleach solution, and raise the tube lifter. Open the CytKick, place an empty 96-well shutdown plate, and close the door. Once loaded, the instrument will begin the shutdown cycle, which takes 3 hours to complete.

17.1.18. **For 5115 BSL-2 Attune CytPix only:** Swap out the waste with an empty waste container to complete Shutdown. As the instrument is undergoing Shutdown, add 180mL of bleach to the 1.8L waste bottle (for 10% bleach v/v) under the BSC and leave the bottle in the BSC overnight. This will be disposed of by the Core after 24 hours.

18. Maintenance

- Record all Maintenance in the **Equipment Maintenance Log**.
- Weekly: Perform a Power Cycle
- Approximately every 90 days: Decontaminate the external fluidics supply, flow cytometer, and autosampler approximately every 90 days (See Section 18.3 and 18.4). Decontaminate the fluidics system by running the decontamination script in Attune Software. External fluidics supply must be decontaminated after decontamination of the flow cytometer and autosampler. Once EFS decontamination has been completed, replace focusing fluid filters.

18.1. Records

18.1.1. 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.

18.2. Instrument Power Cycle (Weekly)

18.2.1. The Attune Flow Cytometer, Autosampler, and desktop should be power cycled once a week or if Attune software is slow and unresponsive.

18.2.2. Power off the Autosampler by pressing the switch located on the back of the CytKick Max autosampler.

18.2.3. Power off the Flow Cytometer by pressing the switch located on the back of the flow cytometer.

18.2.4. Restart the desktop PC.

18.2.5. Approximately 30 seconds later, or once computer has powered back on, turn on the Autosampler first, by pressing the switch located on the back of the autosampler. Once the autosampler is turned back on, it will cycle through ejection of the plate, closing of the autosampler door. Wait for the autosampler to complete the cycle.

18.2.6. Power on the Flow Cytometer by pressing the switch located in the back of the instrument. Wait approximately 30 seconds to 1 minute before opening Attune Software.

19. Flow Cytometer & Autosampler Decontamination:

19.1. The decontamination script is broken into 3 parts/phases and will take approximately 45 minutes to complete.

19.2. **Before you begin for Attune 4120:** Replace the 1.8 L flow cytometer focusing fluid sheath and waste bottles with the original unmodified lids. This is to ensure the external fluidics supply is disconnected. **For Attune 5115**, the lids are not modified because there is no external fluidics supply cart, so the same ones can be used.

19.3. **Phase 1 (follow the prompts)**

19.3.1. In the Instrument Ribbon tab, select the Decontamination System button.

19.3.2. Empty and rinse the focusing fluid bottle from both the autosampler and the cytometer with MilliQ water. Fill each with 500 mL of 10% bleach.

19.3.3. Empty and rinse the shutdown fluid bottle and fill with 100 mL of 10% bleach.

19.3.4. Fill the wash fluid bottle with 100 mL of wash solution.

19.3.5. Place an empty tube on the SIP and place an empty 96 well plate in the autosampler.

19.3.6. Reconnect all fluidics and select next to begin Phase 1 of the decontamination script.

19.4. **Phase 2:**

19.4.1. Once Phase 1 is complete, rinse both focusing fluid bottles (autosampler and cytometer) with MilliQ water and fill each with 500 mL of MilliQ water.

19.4.2. Rinse the shutdown and wash bottles and fill each with 100 mL of MilliQ water.

19.4.3. Remove the tube on the SIP, empty the contents, and place back on the SIP. Select next to begin Phase 2 of the decontamination script.

19.5. **Phase 3:**

19.5.1. Once Phase 2 is complete, rinse the shutdown, wash, both focusing fluid (autosampler and cytometer) bottles with MilliQ water, and refill with the appropriate solutions. Reconnect all fluidics.

19.5.2. Lower the tube lifter and remove the tube on the SIP. Remove the 96 well plate in the autosampler. Select next to begin Phase 3 of the decontamination script.

19.5.3. **For 5115 BSL-2 Attune:** Replace the focusing fluid filters located within the cytometer. Refer to pages 18-19 in the Attune Flow Cytometry Maintenance and Troubleshooting Guide. IMPORTANT: flush each focusing fluid filter with 100 mL of milliQ water or focusing fluid prior to installation. Make sure to flush the liquid in the direction of the arrow marked on the filter (direction of liquid flow) This will ensure that any fibers that are present from the manufacturing process are removed prior to installation and will not become lodged in the system.

19.5.4. **For 4120 Attune:** Move to complete EFS decontamination (Section 19)

19.5.5. Run 3 startups, 2 de-bubbles, and 2 rinse functions to saturate the filters and remove air from the system.

19.5.6. Run performance tracking to ensure that the instrument is functioning properly.

20. External Fluidics Supply Decontamination (Perform only on 4120 Attune)

20.1. Following decontamination of the flow cytometer, perform decontamination of EFS as soon as possible (i.e. Same day, the day after).

20.2. Focusing Fluidics Decontamination:

20.2.1. Power off the Attune CytKick Max (autosampler), then the Attune CytPix.

20.2.2. Remove the 1.8 L flow cytometer focusing fluid bottle, dispose of the remaining sheath, and reconnect the fluid bottle and sensor line. Do not place the modified lid back onto the bottle. Instead, place it into the 2L glass beaker or an Erlenmyer flask that is stored for this purpose in the cabinet. This is to ensure bleach solution will not be pumped into the 1.8 L focusing fluid sheath bottle.

20.2.3. Prepare 2 L of a 10% bleach solution and pour it in an empty focusing fluid cubitainer. Place the cubitainer umbilical (cap with sensor cable and fluidics line) from the original focusing fluid sheath cubetainer onto the 10% bleach cubetainer.

20.2.4. Place the cubitainer containing 10% bleach onto the EFS cart and re-connect the fluidic and electric connections/cables.

20.2.5. Power on the Attune CytKick Max, then the Attune CytPix. The empty 1.8 L focusing fluid sheath bottle will trigger the sensor and begin pumping the 10% bleach solution through the focusing fluid lines and into the empty 2 L collection glass beaker or Erlenmeyer flask.

20.2.6. Once it has finished pumping, power off the Attune CytKick Max, and then the Attune Cytpix. Wait for 20 minutes, allowing the fluid lines to soak in 10% bleach.

20.2.7. After 20 minutes, empty the 2 L collection beaker or Erlenmyer flask and repeat steps 19.2.5 - 19.2.6 to repeat the pumping of 10% bleach through fluidics line for decontamination of the focusing fluid lines.

20.2.8. Empty the 2 L collection beaker. There should be an empty cubitainer labeled milliQ Decon. Fill this with 2 L of milliQ water. Place the cubitainer umbilical (cap with sensor cable and fluidics line) from the 10% bleach cubinator to the milliQ cubitainor. Repeat steps 19.2.3 - 19.2.6 but with a cubetainer containing 2 L MilliQ water to rinse the fluid lines with water. A 20-minute wait time is not required.

20.2.9. Next, purge the focusing fluid lines with air using the following steps. This is to prevent milliQ water from diluting the system fluidics.

20.2.9.1. Reconnect the fluidics and sensor cable of the 1.8 L flow cytometer focusing fluid bottle. Place the modified focusing fluid bottle insert (focusing fluid cap with EFS tubing) in an empty 2L collection beaker/Erlenmyer flask and secure the tubing.

20.2.9.2. Remove the focusing fluid cubetainer on the EFS cart. Disconnect and remove the cubetainer umbilical (cap with sensor cable and fluidics line) from cubetainer.

20.2.9.3. Reconnect the cubetainer umbilical (cap with sensor cable and fluidics line) back into the EFS cart. Hold the umbilical with the float sensor down (hold it upright rather than upside down position when on EFS cart) to trigger pumping of the focusing fluid line into the 2.0L collection container. Repeat as necessary until the focusing fluid line is empty.

NOTE: Use 2 L beaker at all times because 1.8 L focusing fluid bottle has already been decontaiminated

20.3. Waste Fluidics Decontamination:

20.3.1. Power off the cytometer to begin decontamination of the EFS waste fluid line.

20.3.2. Remove the 1.8L waste bottle from the cytometer, empty the contents into a 20 L waste carboy, and fill to the marked line with a 10% bleach solution. Reconnect the waste bottle into the cytometer.

20.3.3. Power the cytometer on. The full 1.8 L bottle will trigger the EFS sensor to begin pumping the 10% bleach through the waste fluid lines and into the waste carboy on the EFS cart.

20.3.4. Refill the 1.8 L flow cytometer waste bottle with 10% bleach and allow for a second pumping of 10% bleach through the waste fluid lines and wait for 20 minutes for decontamination of the fluid lines.

20.3.5. Remove the 1.8L flow cytometer waste bottle, and fill to the marked line with MilliQ water. Allow MilliQ water to be pumped through the waste fluid lines and into the waste carboy on the EFS cart.

20.3.6. Remove the cytometer waste bottle and empty the contents. Reconnect all fluidics and sensor cables.

21. Resource Index

21.1. Attune CytPix User Guide: Attune CytPix flow cytometer and Attune Cytometric Software literature and resources for the following items can be found on the Desktop. Following are our ThermoFisher Representatives:

	Rene DuBois (She/Her) **Field Service Engineer II** **Thermo Fisher Scientific** **Remote – MI** **rene.dubois@thermofisher.com**

22. Transport of Biological Materials:

22.1. 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

23. Competencies, Authorization and Training

23.1. 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 6 during new Attune CytPix flow cytometer account creation.

24. SOP Performance and Equipment Review

24.1. The effectiveness of this SOP 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 review will occur every two years.

25. Definitions

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

25.2. **Author:** The individual representing the MSU Flow Cytometry Core Facility that created SOP: ACP.4120.001, ACP.5115.001

25.3. **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.

25.4. **New User:** An individual who has not completed the requirements of Section 6.

25.5. **Approved User:** An individual who uses the Attune CytPix flow cytometer and has fulfilled Section 6. This title may only be given by the Equipment Champion and / or the Core Facility Staff.

25.6. **Champion:** An individual whose direct expertise with the Attune CytPix 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.

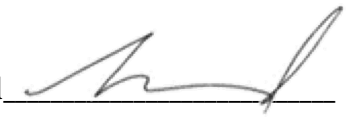
26. Approvals

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

26.2. This SOP was written/authorized by:

26.2.1.1.1. Dr. Soo Hyun Ahn Soo hyun ahn

26.3. This SOP was reviewed by:

26.3.1.1.1. Dr. Matthew Bernard 

26.4. Issue Date: October 30th 2025