

SOP#:	Date Issued: 8/03	Date Revised: 7/08
TITLE:	INSPIRE™ ImageStream®X MkII System	
SCOPE:	Research Personnel	
RESPONSIBILITY:	BORC staff	
PURPOSE:	To outline the proper procedures for use and maintenance of the INSPIRE™ ImageStream®X MkII System.	

1 PURPOSE

This SOP explains how to apply **INSPIRE™ ImageStream®X MkII System** in biomedical research.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE CONDUCTING YOUR EXPERIMENT

3.1 Sample Preparation Guide

3.1.1. Choice of Cell Type: The particle size should be less than 120 μm using 20x magnification, 60 μm using 40x, and 40 μm using 60x.

3.1.2. Final sample concentration and volume: At least 1 million cells in 50 μL (2×10^7 cells/mL) in PBS/2%FBS in a 1.5 mL siliconized microcentrifuge tube. Will run ~400 cells per second on low speed

3.1.3. Protocols: In general, any established labeling protocol used for flow cytometry will work with the ImageStream (see *Current Protocols in Cytometry* for general labeling techniques). Staining on ice in the presence of 0.1% Na-azide when possible will reduce non-specific capping of antibody. Use siliconized polypropylene tubes when possible.

3.1.4. Choice of Fluorochromes: Choose fluorochromes that are excited by the lasers in your ImageStream (**405, 488, 642nm** are most common). Use the chart on p.3 or look online at the Luminex spectral viewer that will help you plan which dyes will work the best. <https://www.luminexcorp.com/imaging-flow-cytometry-support/>

3.1.5. Compensation: Have a sample of cells each labeled with a single-color for each fluorochrome used (i.e. FITC only cells, PE only cells, etc.).

- 3.1.6. Cell Aggregation:** Minimize aggregation problems by straining the sample through a 70um nylon mesh strainer, or by using an anti-clumping buffer such as EDTA or Accumax prior to fixation.
- 3.1.7. Fixation:** If fixation is desired, fix cells with 1% PFA on ice for 20 min.
- 3.1.8. Number of samples:** No more than 30 total for feasibility experiments. Please limit the samples to the following; Positive and Negative ***biologic controls***, ***compensation controls***, and ***experiment samples***.
- 3.1.9. Brightness of Stain and Stain Balancing:** Quantifying the location and distribution of signals in an image is a demanding task that requires optimized labeling. Below are a few suggestions to help design the experiment:

		Excitation Laser (nm)										
Ch	Band (nm)	375***	405	488	561	592	642	730	785	Used	Ch	
1	435-480 (457/45)	375 & 405*** Ch1/Ch9 BF or *DAPI, BV421, AF350, Hoechst, PB							BRIGHTFIELD			1
2	480-560 (528/65)	*BV510, PacOrange, QD525,		FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, SpectrumGreen, LysoTrackerGreen, MitoTrackerGreen,							2	
3	560-595 (577/35)	*QD565, QD585, eFluor565		PE, PKH26, Cy3, DSRed, CellMask/CellTracker/SY TOX Orange	PE, AF546, DyLight550, PKH26, DSRed, Cy3, SpectmOrange						3	
4	595-642 (610/30)	Ch4/Ch10 BF or *QD625, eFluor625, BV605		Ch4/Ch10 BF or PE- TexRed*, ECD*, PE- AF610*, RFP, QD625*, eFluor625*	Ch4/Ch10 BF or AF568*, DyLight594*, PE-TxRed*, ECD*, PE- AF610*, RFP, mCherry*						4	
5	642-745 (702/85)	*QD705, eFluor700, BV711		PE-Cy5*, PE-AF647*, 7AAD*, PI* PerCP*, PerCP-Cy5.5*, eFluor650*, FuraRedto, Draq5*, LDS751*,	PE-Cy5*, PE- AF647*, 7AAD*, PerCP-Cy5.5*						5	
6	745-785 (765/40)	*QD800, BV786		PE-Cy7*, PE-AF750*,	PE-Cy7*, PE- AF750*,				SSC		6	
7	435-505 (457/45)	375 No 405*** *DAPI, BV421, AF405, Hoechst, PacBlue, CascadeBlue, eFluor450, DyLight405, CFP, LIVE/DEAD Violet AF350										7
8	505-570 (537/65)	*BV510, PacOrange, CascadeYellow, AF430,	*BV510, PacOrange, CascadeYellow, AF430, eFluor525, QD525								8	
9	570-595 (582/25)	Ch1/Ch9 BF or *QD565, QD585	Ch1/Ch9 BF or *QD565, QD585, eFluor565								9	
10	595-642 (610/30)	Ch4/Ch10 BF or *QD625, eFluor625, BV605	Ch4/Ch10 BF or *QD625, eFluor625, BV605		Ch4/Ch10 BF or TexRed*, AF594*, DyLight594*, mCherry*						10	
11	642-745 (702/85)	*QD705, eFluor700, BV711	*QD705, eFluor700, BV711		APC, AF647, AF660, Cy5, DyLight649, DRAQ5*	AF647, AF660, AF680, APC, Cy5, DyLight649, DyLight680, Draq5* PE- AF647*, PE-Cy5*, PerCP*, PerCP-Cy5.5*					11	
12	745-785 (765/40)	*QD800, BV786	*QD800, BV786		APC-Cy7, APC-AF750, APC-H7, APC- eFluor750	APC-Cy7, APC-AF750, APC-H7, APC- eFluor750, Cy7, AF750, DyLight750, PE-Cy7*, PE-AF750*	AF750, Cy7, DyLight750	SSC			12	

4 PROCEDURES

4.1 Preparing the System

4.1.1 Power on the System and Log into the Software

- 1) Press the green power button inside the front door of the ImageStreamX MkII to turn on the instrument and start the computers.
- 2) Log on with the user name (Amnis) and password (is100).

- 3) Launch the INSPIRE software by double-clicking the INSPIRE icon on the desktop.

4.1.2 Set the Image Display Properties

Automatically Set the Image Display Properties

- 1) Click on the wand next to the analysis area tools and select the Display Settings Wizard.
- 2) Select the channels to set and click Finish. The wizard adjusts the display for a channel with greater than 75 Raw Max Pixel values using the Min and Max pixels from the images being collected. If there are no positive events in a channel the default settings (35-750) are used. The manual settings may be used for channels with rare events since there may not be enough data for the wizard.

Manually Set the Image Display Properties

- 1) Click on the Display Settings tool to open the window.
- 2) Select the channel by clicking on the channel name.
- 3) To change the channel name, type a new name. To change the channel color, click on the color box.
- 4) To set the display mapping, adjust the right and left green bars in the graph. You will adjust the Display Intensity settings on the graph (the Y Axis) to the Pixel Intensity (the X axis). The range of pixel intensities is 0-4095 counts. The display range is 0-255. The pixel intensities shown in gray are gathered from the images coming through in the specific channel and updates with every 10 images. Updates to the adjustments can be visualized in the image gallery. At each intensity on the X Axis of the graph, the gray histogram shows the number of pixels in the image. This histogram provides you with a general sense of the range of pixel intensities in the image. The dotted green line maps the pixel intensities to the display intensities, which are in the 0-255 range.
- 5) Click and drag the vertical green line on the left side (crossing the X Axis at 0) to manually set the display pixel intensity to 0 for all intensities that appear to the left of that line. This removes background noise from the image.
- 6) Click and drag the vertical green line on the right side to set the display pixel intensity to 255 for all intensities that appear to the right of that line. If a non-linear mapping occurs by moving the yellow crosshair, click Set Linear Curve to return to a linear transformation.

4.1.3 Prepare the ImageStream®X MkII System

- 1) Fill the rinse bottle with deionized water and the sheath bottle with PBS. Ensure the SpeedBead® reagent is loaded on the bead port and is well mixed. The beads are automatically mixed while the instrument is in use. If the instrument has been idle for a long period, remove the bead vial and vortex. Choose the appropriate sheath fluid using the Acceptable Sheath Fluid chart.1.

Acceptable Sheath Fluid

Sample Solution	Sheath Fluid	Acceptable
PBS	PBS	Yes
PBS	Water	Yes*
PBS/Surfactant	PBS	Yes
PBS/Surfactant	Water	No
Water	PBS	Yes
Water	Water	Yes
Water/Surfactant	PBS	No
Water/Surfactant	Water	Yes

*Cells in PBS run with water sheath will swell.

- 2) Empty the waste tank and add fresh bleach.
 - a. Push on the quick-disconnect buttons to remove the tubing from the Waste tank.
 - b. Empty the Waste tank.
 - c. Add 160 mL of bleach to the Waste tank.

Note: The final volume of waste when full will be approximately 1600 mL. Therefore, the final bleach concentration for a full waste tank will be 10% bleach. Luminex® recommends that the waste be emptied every day and fresh bleach added before Startup.
- 3) Click Startup. This script fills the system with sheath and flushes out all of the old sheath or rinse that was in the system. The sample line is prepared by loading 50 µL of air into the uptake line. Beads are loaded into the bead pump from the 15 mL conical tube. The Initializing Fluidics window opens, showing the progress bar.

Note: The Run ASSIST after initialization box is checked by default to run ASSIST after the fluidics are ready. Uncheck the box if you wish to start ASSIST later. NOTE: a. b. c. NOTE: 3.
- 4) Click Start All Calibrations and Tests in the calibration window if ASSIST was not run in the previous step.
- 5) Center the core stream images (if necessary) by laterally moving the objective under Focus and Centering. Adjust centering by pressing the right or left arrows.

- 6) The event rate should be 800-1000 events per second. 5. 6.
- 7) When the calibrations and tests have passed the ASSIST, status light will change to green. Close the Calibrations window.
- 8) Instrument calibrations may also be run individually by selecting a particular procedure under Calibrations or Tests. Next to each calibration or test button is a green or red rectangle. If the procedure fails, it turns red. If a procedure fails, repeat it. If it fails twice, call Luminex Technical Support.

NOTE:

If adjustments are made to the instrument in order to pass an individual ASSIST calibration or test, it is important to re-run all calibrations and tests in order to record the current settings after adjustments.

4.1.4 Run ASSIST

1) Run ASSIST Calibrations and Tests

- a. Click Start All Calibrations and Tests to run all standard calibrations and tests.
- b. Optional: Click Run Beads to begin running beads without starting calibrations or tests.
- b. To run one calibration or test, click on an individual calibration or test and click Run.
- d. To stop a calibration or test, click Stop or Stop All if Start All was chosen.
- e. A calibration or test will be flagged red if it fails.

2) View Calibration or Test Results

View the latest result of a specific calibration or test using one of two methods:

- a. Click on the button to the right of the test or calibration you want to view the results of.
- b. Click on the History button and choose the routine and date you want to view.

3) Create a Calibration or Test Report

ASSIST calibration and test results are stored in a database that can be reported. To create a report for all calibrations and tests, complete the following steps:

- a. Click the Generate Report button. The Generate Report window opens.
- b. (Optional) Set a custom header for the report.
- c. Set a Date Range for the report using the radio buttons and the date drop-down menu.
- d. Click Generate. The custom report generates.

4.2 Running the Assay

4.2.1 Load and Run the Sample

- 1) In the file menu, choose Load Default Template.

NOTE: Application-specific instrument settings can be saved in a template and used to facilitate instrument setup, but Luminex® recommends that you verify the appropriateness of the settings for the specific experimental run. The default template has a set of Raw Max Pixel scatterplots for all channels helpful for setting laser powers.

- 2) Press Load and load an aliquot of the brightest sample in the experiment that fluoresces with each fluorochrome used. It is critical that you run this sample first to establish the instrument settings.

NOTE: DO NOT change laser settings for the experiment once established on this sample.

- 3) When prompted, place sample vial with 20-200 μ L into the sample loader.
- 4) In the Magnification & EDF window, choose the objective under Magnification (optional).
- 5) (Optional) Select the EDF checkbox if you want extended depth of field (EDF) collection.
- 6) (Optional) Select the High Gain checkbox if you want High Gain (HG) collection.
- 7) Click on the wavelength button to turn on each laser used in the experiment.
- 8) Set the laser powers so there is no saturation, and each fluorochrome has Raw Max Pixel Intensities between 100 and 4000 counts, as measured in scatterplots or histograms of the appropriate channels. Click the Set Intensity but-ton when finished.
- 9) Click a channel name to open the Channel Selection window.
- 10) (Optional) Click Display Settings to set the image display properties.
- 11) Select the box next to the channel name to enable collection of the specified channel. When you have selected all channels to be collected, click OK.
- 12) Select Brightfield channels. Default is Ch1 for a 6 channel system; Ch1 and 9 for a 12 channel system. Click Set Intensity.
- 13) Create graphs to gate on cells of interest.

NOTE: Luminex recommends the following graphs:

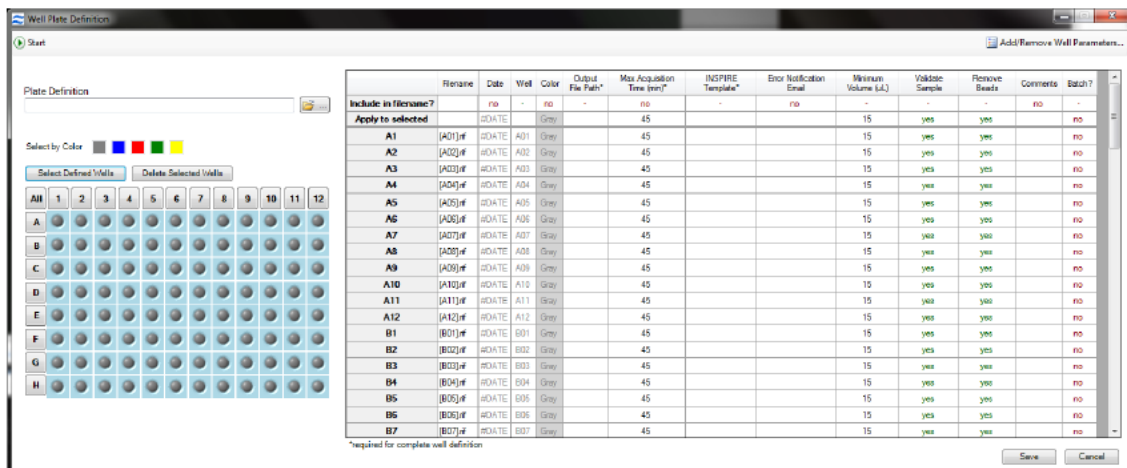
- Scatterplot of Area versus Aspect Ratio of Brightfield — gate on cells and eliminate debris.
 - Scatterplots or Histograms of Intensity — for channels used in the experiment. I
 - Scatterplots of Raw Max Pixel — observe any saturation.
- 14) Use the following features (available in any channel) to identify objects for inclusion in or exclusion from the acquir-ing data file:
 - Area: The number of pixels in an image reported in square microns.

- Aspect Ratio: The Minor Axis divided by the Major Axis is a measure of how round or oblong an object is. See below for the definitions for Major and Minor Axis.
 - Background Mean: The average pixel intensity of the background pixels.
 - Gradient RMS: The average slope spanning three pixels in an image. This feature measures image contrast or focus quality. |
 - Intensity: The integrated intensity of the entire object image; the sum of all pixel intensities in an image, back-ground subtracted.
 - Major Axis: The longest dimension of an ellipse of best fit.
 - Mean Pixel: The average pixel intensity in an image, background subtracted.
 - Minor Axis: The shortest dimension of an ellipse of best fit.
 - Object Number: The serial number of an object.
 - Raw Centroid X: The center of the object in the X dimension of the frame.
 - Raw Centroid Y: The center of the object in the Y dimension of the frame.
 - Raw Max Pixel: The intensity value of the brightest pixel in an image (no background subtraction).
 - Raw Min Pixel: The intensity value of the dimmest pixel in an image (no background subtraction).
 - Time: The object's time value in seconds.
 - Uncompensated Intensity: The integrated intensity of the entire object image; the sum of all pixel intensities in an image, background subtracted.
- NOTE: See the IDEAS® User Manual for more details on features and graphing.

4.2.2 Define the Plate

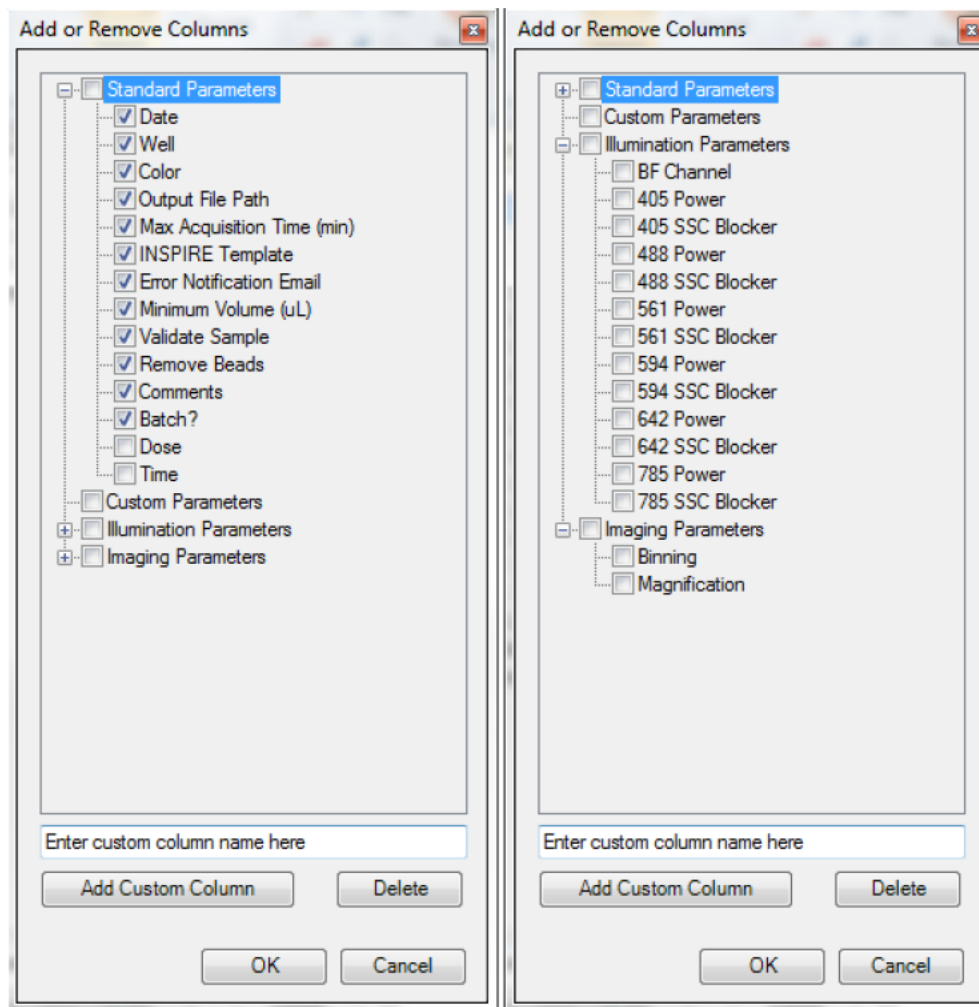
The steps required to create a Well Plate Definition (.def) and run a plate are outlined below:

- 1) Choose Define Plate from the Auto sampler menu to open the Well Plate Definition window.



- 2) Create a new definition. Alternately, click the folder icon to browse for a previously saved definition to edit.
- 3) Name the plate definition.

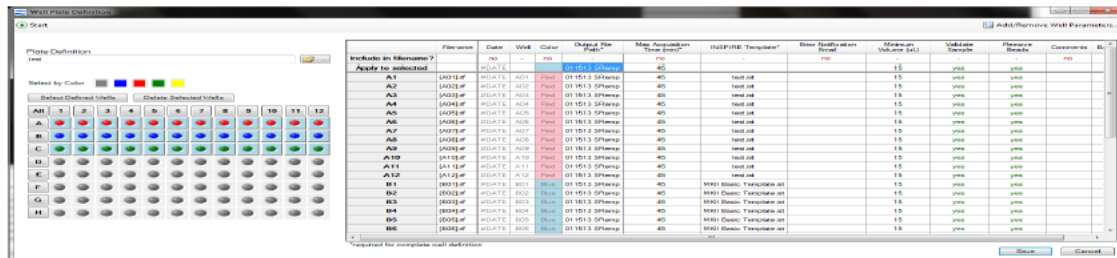
NOTE: At a minimum, each well requires an Output File Path, Max Acquisition Time, and Template File in order to be considered "defined". Other parameters can be added to the definition in the next step.
- 4) Choose the parameters you would like to use. Click Add/Remove Well Parameters to choose the parameters you want to report for the wells.



- 5) Define the wells. Select wells to define by clicking a) individually (or Ctrl click / shift click for multi-select), b) rows or columns, c) color, d) the 'Select Defined' button or e) All. In this example column 1 is selected.

NOTE: Selecting and defining wells with shared parameters first and then refining the definition for sets of wells makes it easier to organize the definition.

- 6) You can edit values for some of the Custom and many of the Standard parameters. You can do this for all selected wells or for individual wells.
- 7) For example, if you want to collect with Max Acquisition Time 10 minutes for the selected wells, type 10 in the 'Apply to selected' box below the Max Acquisition Time heading. If you want to only apply this to a single well, type this value in the box corresponding to that well.

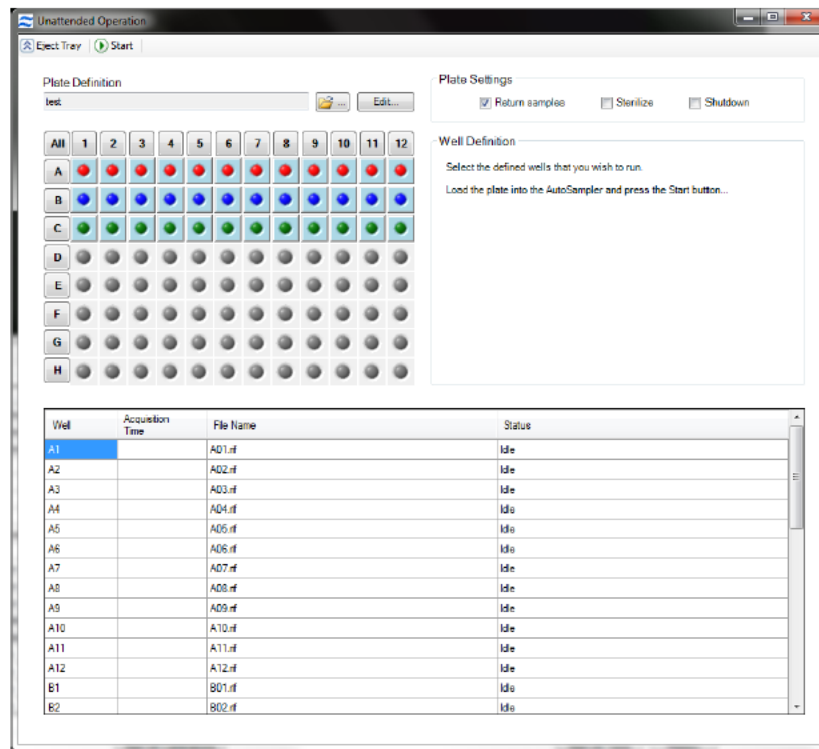


8. Highly recommended - select Error notification Email from the list of standard parameters and type in the user email address in the 'Apply to Selected' box.
9. When done, click Save.

NOTE: A warning may be displayed if there are undefined or partially defined wells. Select Yes to return to plate definition or No to continue.

4.2.3 Start the AutoSampler

- 1) Click Start to run the plate. The Auto Sampler Unattended Operation window opens with the Plate Definition you just saved.
 - If you wish to choose a different Plate Definition, browse for it by clicking on the folder icon. I
 - If you want to edit the Plate Definition, click Edit and you will be taken to the Well Plate Definition window.



2) Check or uncheck the boxes Return samples, Sterilize, Shutdown.

NOTE: Note that these boxes may be checked or unchecked while the plate is running and the operation will apply after the current sample is finished.

3) Select the wells to run (they will appear in the list).

4) Click Eject Tray to extend the plate nest.

5) Add at a minimum 75 μ L samples to the 96 well plate, cover with Sigma-Aldrich X-Pierce Film (XP-100, Cat # 2722502) to minimize evaporation and allow the membrane piercing probe to access the sample. Place your plate on the tray with well A1 positioned at the upper left corner. Luminex® recommends using round bottom (U-bottom) plates to facilitate mixing. The user has the option to use deep well plates accommodating up to 2 mL.

6) Click Start to begin.

7) The Status column will be updated for each well as it is run. For each sample, the instrument performs the following in sequence: 1) Mixing and resuspension of sample, 2) Load, 3) Validation (flow speed CV, focus, bright field intensity, object rate), 4) Data Acquisition, 5) Result (success or error).

4.2.4 Collect and Save Data Files

Once the sample is running and the ImageStream®X MkII is properly set up, you are ready to acquire the data as a raw image file (.rif) and/or an FCS file. The .rif contains uncompensated pixel data along with instrument settings and ASSIST information in a modified TIFF format. The file includes only those objects defined by the population selected in the acquisition section.

- 1) Enter the file name in the Filename field.
- 2) Enter the sequential number in the Seq # field.

NOTE: The sequential number is appended to the file name, followed by the .rif or .fcs extension. The sequence number increases by 1 with each successive data acquisition. Files collected with BF off will be appended with "noBF," and files collected with EDF enabled will be appended with "EDF" in the file names. File names must be 256 or fewer characters in length, including the path and file extension. In addition, file names cannot contain the following characters: \,/,*,<, >, or |.

NOTE: To collect a .fcs file at the same time as a .rif file, choose the .fcs option under the File menu.

- 3) Click the folder icon to select a folder where data acquisition files will be saved. You can also create a new folder to save acquisition files in.
- 4) Enter the number of cells you want to acquire in the Count field and select the population from the adjacent drop-down.
- 5) (Optional) To count one population but limit the collection to another population, click on the bracket to break the link and enable the Collect drop-down.
- 6) Click Acquire to begin acquiring a data file. The data file(s) are automatically saved in the selected folder once the desired number of objects are collected.
- 7) Click to prematurely stop acquisition. The system prompts you to either discard the acquired data or to save the collected data in a file. 1. 2. 3. 4. 5. 6. 7.
- 8) Click to pause acquisition while running or to resume acquisition when paused.
- 9) Once acquisition finishes, either load the next sample or return the remaining sample.

NOTE: If the next sample has no nuclear dye and follows a DNA intercalating dye-stained sample, load a solution of 10% bleach and then load PBS to ensure that residual dye does not stain the subsequent samples.

- 10) Change the file name for the next sample and continue collecting samples until you have acquired data for all the desired samples.
- 11) When finished running the experiment samples or after setting the template, run single color compensation con-trols with the same laser settings as the experimental samples, with the exception of the scatter laser 785, which turns off in compensation mode.

- 12) Click in the analysis tools section and choose Compensation to launch the Compensation Wizard.

NOTE: The Compensation Wizard sets the instrument for compensation controls that must be collected with bright-field and scatter (785 nm laser) OFF and every channel set to be collected. Keep all laser powers the same as for the experimental samples.

- 13) Follow the prompts in the Compensation Wizard to collect all compensation control files:
 - a. Click Load. Alternately, if a compensation control sample is already running, click Next.
 - b. Place the tube on the uptake port and click OK.
 - c. Click Next when sample is running.
 - d. Verify the channel for compensation.
 - e. If not all cells are positive, draw a region on the Uncompensated Intensity scatter plot to define the positive population. View the population in the Image Gallery and choose this population to collect.
 - f. Name the file and choose the path to save the data.
 - g. Click Collect File. The compensation coefficients are calculated. The compensation coefficients and an Intensity scatter plot using the coefficients displays.
 - h. Click OK on the Acquisition Complete popup window.
 - i. Click Load to continue with the next single color control sample or click Return (optional).
 - j. Repeat the previous steps for each compensation sample. For each sample, reset the Image gallery population to view All, then create an appropriate population for each sample.

The R1 gate can be moved for each sample. NOTE:

- k. Click Exit when done with the Compensation Wizard.
- 14) Save the coefficients to a compensation matrix file. The beginning template is restored and the saved matrix is used to compensate the Intensity feature.

NOTE: Other features are not compensated. The compensation can be cleared if desired from the compensation menu. Scatter plots can be made with the feature Uncompensated Intensity to compare with and without compensation.

- 15) Continue to collect experimental files.
- 16) Click Shutdown when done.

4.2.5 Collect a Data File Using the EDF Element

- 1) Set instrument settings for the experiment.
- 2) Check the EDFbox in the instrument control panel.
- 3) Adjust any regions being collected to accommodate using EDF.
- 4) The calibration kernels saved during the last EDF calibration will be appended to the file and the file name will be appended with "-EDF."

4.2.6 Collect a Data File Using High Gain

- 1) Set instrument settings for the experiment.
- 2) Select the High Gain box in the instrument control panel.
- 3) Adjust laser powers to optimize signal and minimize saturation.
- 4) Adjust any regions being collected to accommodate the increased sensitivity.
- 5) Use the Brightfield Set Intensity button to set the background mean intensity to ~800.
- 6) High Gain calibrations will automatically be applied to files collected with HG active.
- 7) Acquire samples as usual.

4.3 Remove Debris

Some samples have an abundance of small particulate debris. Debris can be eliminated from collection by gating or by using Squelch to reduce the sensitivity of object detection. As opposed to gating debris away from cells, squelching debris can prevent INSPIRE™ crashes related to overburdening the computer processor with an abnormally high event rate. Squelch should only be used if the rate of total objects per second reaches 4000. Squelch values range from 0 to 100. Increasing the value decreases object detection sensitivity.

- 4.3.1. Choose All in the image gallery.
- 4.3.2. Observe the relative proportion of cell to debris images appearing in the imaging area and the event rate (Total/Sec under Acquisition Status).
- 4.3.3. On the Advanced Setup -Acquisition tab, increase the Squelch value until the observed proportion of cells to debris increases in the imaging area.
- 4.3.4. Observe the Total/Sec event rate on the Setup tab under Acquisition Status. If it is still greater than 500, increase the Squelch value until you achieve the desired results.

4.4 Set ImageStream®X MkII Speed and Sensitivity

The optimal operating speed is set at the factory for each instrument and is approximately 60 mm/sec. This speed corresponds to the highest resolution setting with a pixel size of 0.5 μm at 40 X magnification. In order to collect images at higher speed, the rows on the camera can be binned. Image collection speed is inversely related to image resolution (sensitivity).

The table below shows the pixel resolution, relative sensitivity and approximate time to collect 10,000 objects at a concentration of 1×10^7 objects per ml at different magnifications and speed settings. In order to run at a higher speed, the rows on the camera are binned, this means the signal is collected in 1, 2 or 4 rows of the CCD at a time.

Objective	Speed	Bin	Pixel Resolution	Sensitivity	Core Diameter	Velocity mm/sec	Sample obj/ml	Cells/Sec	Time to 10,000
20x	Low	1x	1 x 1 μm^2	High	10 μm	55	$1 \times 10^{7\text{th}}$	200	54 sec
	Med	1x	1 x 1 μm^2	Med	10 μm	110	$1 \times 10^{7\text{th}}$	360	28 sec
	High	2x	1 x 2 μm^2	Low	10 μm	220	$1 \times 10^{7\text{th}}$	720	14 sec
40x	Low	1x	.5 x .5 μm^2	High	10 μm	55	$1 \times 10^{7\text{th}}$	200	50 sec
	Med	2x	.5 x 1 μm^2	Med	10 μm	110	$1 \times 10^{7\text{th}}$	360	29 sec
	High	4x	.5 x 2 μm^2	Low	10 μm	220	$1 \times 10^{7\text{th}}$	720	14 sec
60x	Low	1x	.3 x .3 μm^2	High	7 μm	40	$1 \times 10^{7\text{th}}$	60	160 sec
	Med	2x	.3 x .6 μm^2	Med	7 μm	55	$1 \times 10^{7\text{th}}$	75	135 sec
	High	4x	.3x1.2 μm^2	Low	7 μm	110	$1 \times 10^{7\text{th}}$	150	68 sec

4.5 Shut Down the System

This procedure sterilizes the ImageStream®X MkII system and leaves it with pumps empty and water in the fluidic lines. The instrument is left on with INSPIRE running.

4.5.1. Fill the Rinse, Cleanser, Sterilizer, and Debubbler bottles if necessary.

4.5.2 Empty the Waste bottle.

4.5.3 Remove any tubes from the uptake ports.

4.5.4 Click Shutdown.

NOTE: This procedure automatically turns off all illumination sources and rinses the entire fluidic system with deionized (DI) water, sterilizer, cleanser, de-bubbler, and then water again. The sterilizer is held in the system for ten minutes to ensure de-contamination. It takes about 45 minutes of unattended (walk-away) operation to complete.

5 MAINTENANCE

5.1 Daily Maintenance

5.1 Clean the Instrument

5.1.1 Clean spills on the exterior of the ImageStream®X MkII with a mild detergent.

5.1.2 Using gloves, clean the sample portal and sample elevator with a 10% bleach solution.

5.1.3 Dispose of waste using proper precautions and in accordance with local regulations.

5.2 Preventative Maintenance

The ImageStream®X MkII contains no serviceable parts. Only Luminex®-trained technicians are allowed to align the laser beams or otherwise repair or maintain the instrument. The instrument fluidic system is automatically sterilized after each day's use. This reduces the occurrence of clogging. Tubing and valves are replaced by Luminex service per-sonnel as part of routine preventative maintenance.

5.3 As Needed Maintenance (Replace the Bead Clamp)

The bead tube mixer is an essential part of proper operation and calibration of the ImageStream®X MkII. It assures that the SpeedBead® reagent is maintained at the proper concentration when being used by the system. On rare occasions, this clamp may break and need replacing. Replacement parts can be obtained by contacting your Luminex service representative or by email at support@luminexcorp.com. The part can be easily be installed following these instructions

6 REFERENCES

Refer to the manufacturer's manual for additional information.

END OF DOCUMENT