Highway1™ Instructions for Use

STEMCELL Technologies Inc. recently acquired Cellular Highways Ltd., including Highway1™, from TTP Group Ltd.

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1 Highway1™ Cell Sorting System Overview

1.1 Highway1™ Instrument

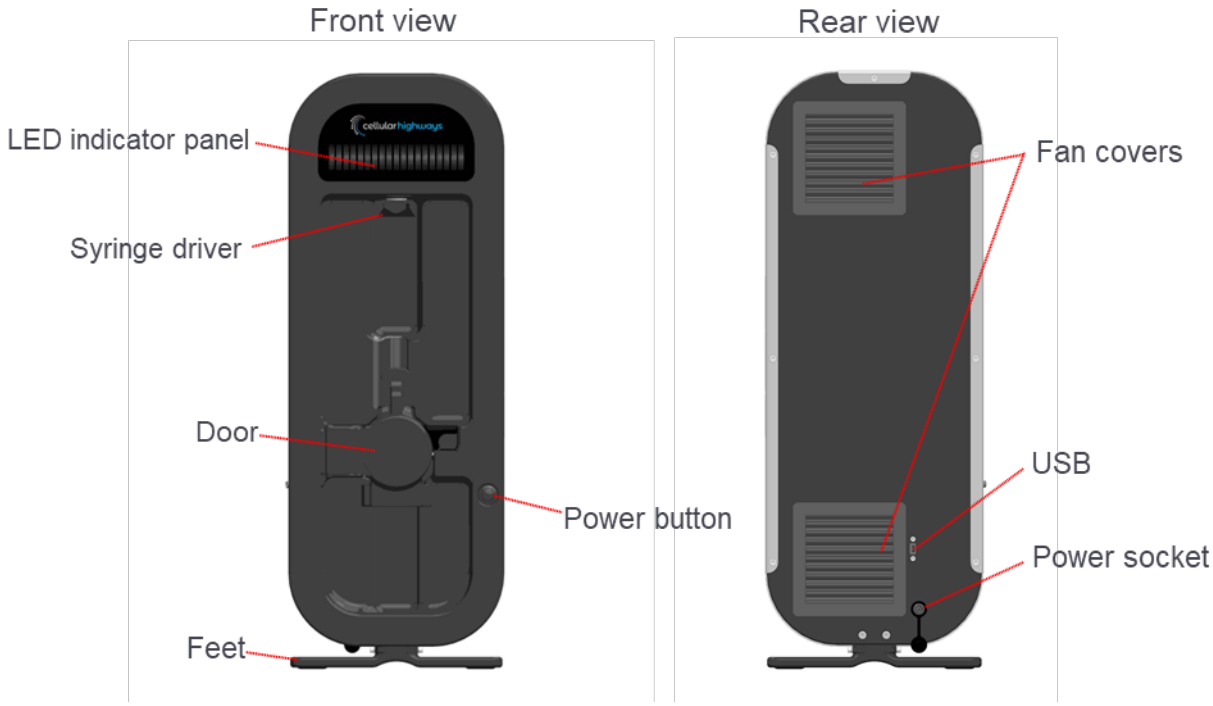

Figure 1-1 Highway1™ Instrument

1.2 Highway1™ Cartridge and Cartridge Loader

The front and rear views of the Highway1™ Cartridge and Cartridge Loader are shown below.

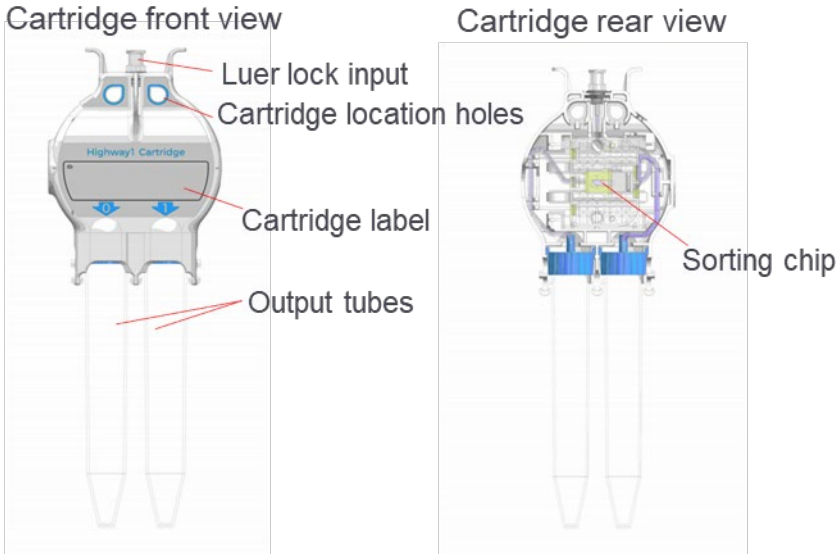

Figure 1-2 Highway1™ Cartridge

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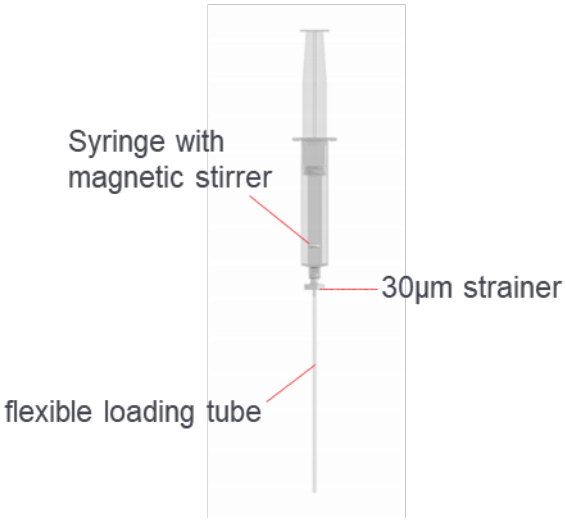

Figure 1-3 Highway1™ Cartridge Loader

1.3 Highway1™ Cold Pack Kit

The Highway1™ Cold Pack Kit includes an input syringe cold pack and an output tube cold pack. It can be used with the Highway1™ Cartridge to provide optional sample cooling during sorting.

Cold packs should be stored at -20°C and will maintain the sample at a temperature of 4 - 10°C for up to the duration of a full run. Cold packs are magnetically affixed to the sample input syringe and output tubes as needed.

Note: The input syringe cold pack is only compatible with a 20 mL syringe.

Figure 1-4 Highway1™ Cold Packs

1.4 HighwayR Software

Highway1™ is controlled by the HighwayR software, which is pre-loaded onto the host personal computer (PC) supplied by Cellular Highways. The operation and use of the software is described further in section 3.

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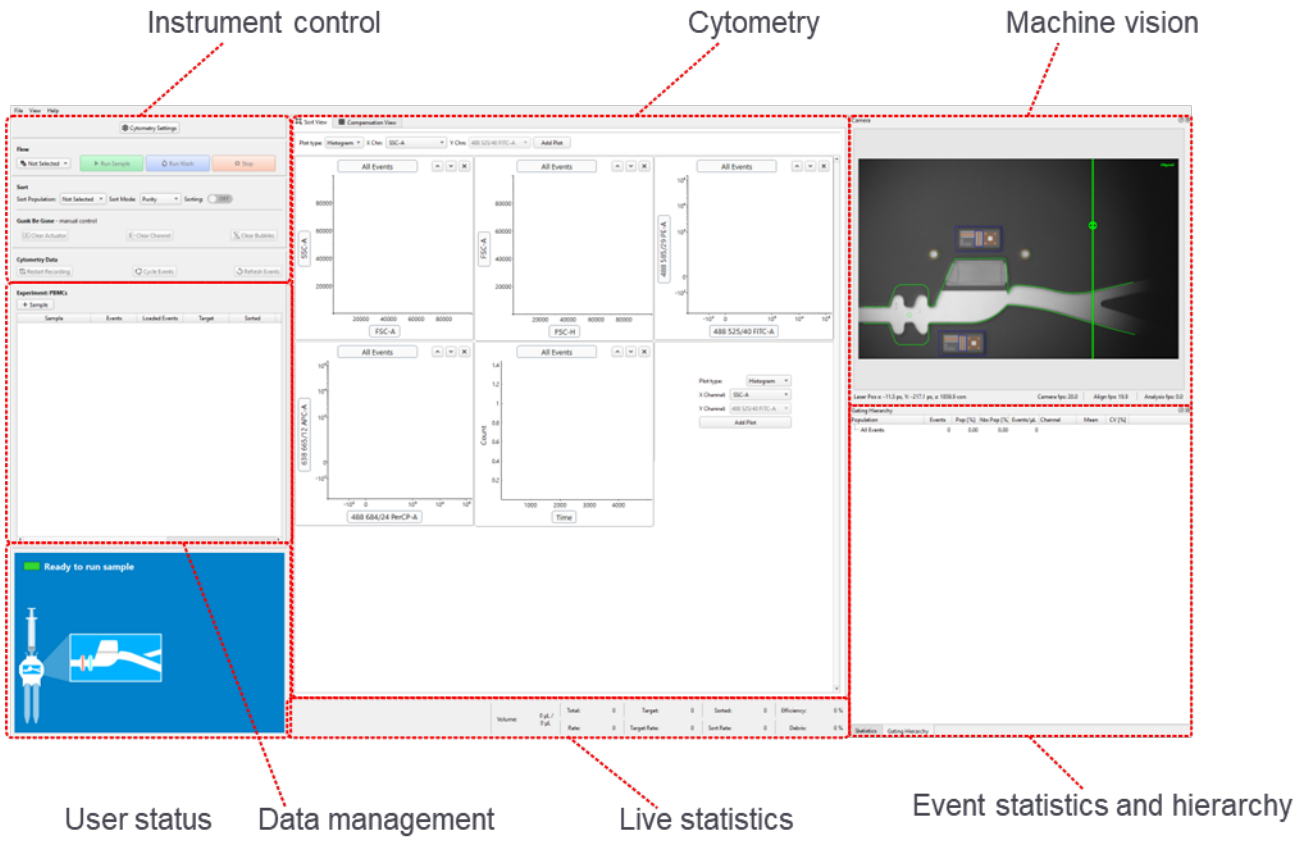

Figure 1-5 HighwayR software

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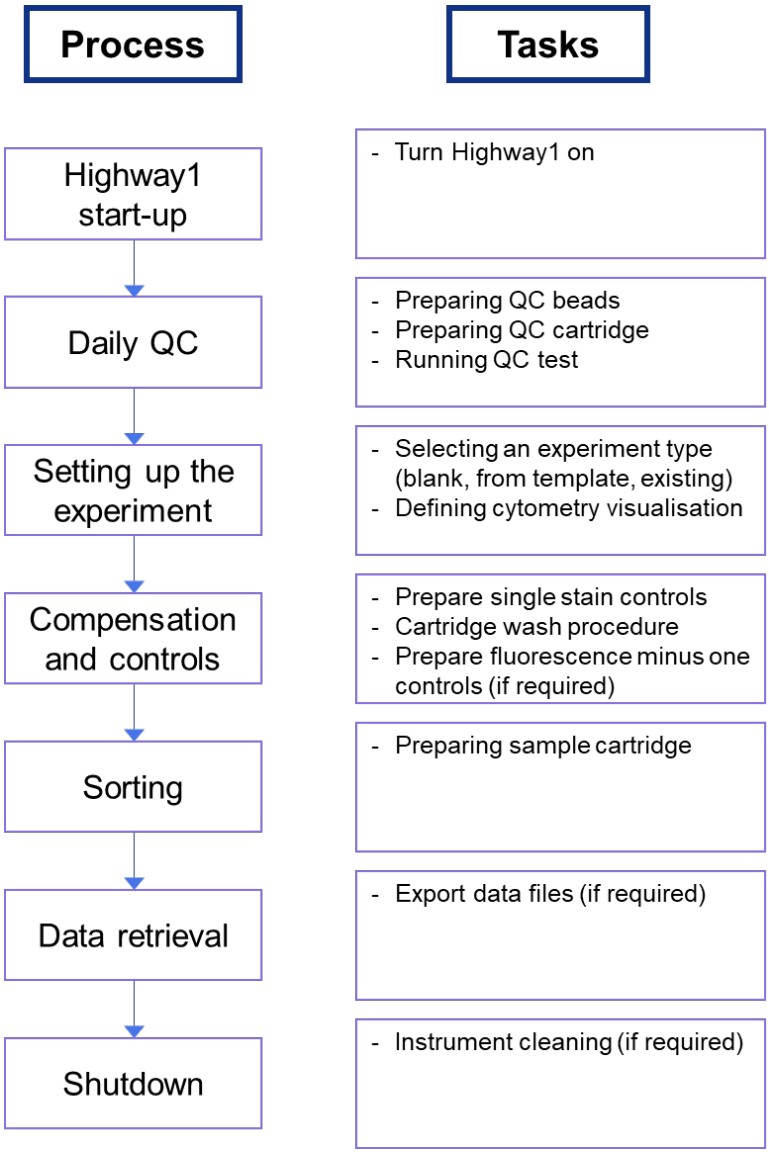
1.5 Generic Workflow Diagram



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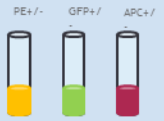
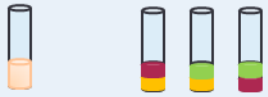
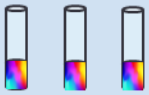
1.6 Types of Controls Used in Cell Sorting

Dilute full stain	• Used to set voltages; is essential
 Single colour controls (SCCs)	• Used for compensation (if required) • Each sample has single colour and negative • Can be beads or cells
 Unstained Fluorescence Minus Ones (FMOs)	• Used for gating • FMOs needed where gating is not clear (e.g. smear population, rare population or new sample) • Unstained is essential
 Fully stained samples	• For sorting!

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2 Materials

These are the materials referenced within this document. To order items as required, contact your STEMCELL sales representative.

Catalog #	Product Name	Size
100-2310	Highway1™	1 Unit
100-2314	Highway1™ Syringe Adapter 3 mL	1/Pack
100-2316	Highway1™ 3 mL Syringe	25/Pack
100-2317	Highway1™ 3 mL Syringe Blunt Needle	100/Pack
100-2318	Highway1™ 3 mL Syringe Strainer	25/Pack
100-2319	Highway1™ Fan Filter	5/Pack
100-2348	Highway1™ Daily QC Beads Kit	1 Kit
100-2349	Highway1™ Cold Pack Kit	1 Kit
100-2320	Highway1™ Cartridge Rack	1/Pack
100-2312	Highway1™ Cartridge	25/Pack
100-2313	Highway1™ Cartridge Loader	25/Pack
100-2321	Highway1™ Optics Wipes	30/Pack

Notes:

Only the syringes listed above and included in the Highway1™ Cartridge Loader are validated for use with Highway1™. The Highway1™ Cartridge Loader 20 mL syringe contains a magnetic stirrer, which is crucial for maintaining a homogenous sample and good sort performance during long sorts.

Highway1™ Cartridges are intended to be used once and then discarded. If cartridges are re-used, their performance and reliability may be adversely affected. If a cartridge is stored containing a salt solution (e.g. PBS), it will degrade and will no longer function correctly. Cartridges can be stored containing clean, filtered water if necessary.

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3 Operating Highway1™

3.1 Start-up Procedure

1. Turn on Highway1™ using the power button.
2. Highway1™ warm-up will be initiated; this requires up to 30 minutes.
3. Switch on the PC.
4. On the desktop, double-click the HighwayR icon. The software user interface (UI) will initialize.
5. Select an experiment or create a new one.
6. Once loaded, check the user status panel at the bottom left of the UI to make sure the Highway1™ and computer are connected.

   A red warning will say *Instrument disconnected* if there is a connection problem.
7. Once the warm-up is complete, the user status will state *Instrument Ready*.

3.2 Highway1™ Buffer Recommendation

The recommended cell sorting buffer is D-PBS/PBS (no calcium or magnesium) + 0.1% Poloxamer 188 + 24 units/mL benzonase and 0.5 mM $MgCl_2$. The recommended cell sorting buffer should be made fresh each day it will be used; any excess buffer should be discarded.

Tips to help with sample preparation:

- Filter the medium through a 0.2 µm sterilizing filter.
- If running the sample chilled, keep medium chilled.
- If running the sample at room temperature (15 - 25°C), keep medium at room temperature.

3.3 Highway1™ Cartridge Loading Procedure

The Highway1™ Cartridge is loaded with the following instructions. The cell suspension is drawn into a syringe through an in-line 30 µm strainer. This syringe is then placed on the cartridge, which is then closed to airborne microbes.

1. Unwrap Highway1™ Cartridge (consistent with aseptic handling technique, e.g., in a biosafety hood).
2. Place the cartridge upright in the Highway1™ Cartridge Rack.
3. Open the sample for loading (consistent with aseptic handling technique).
4. Unwrap the Highway1™ Cartridge Loader (consistent with aseptic handling technique).
5. Using the syringe, draw the sample into the Cartridge Loader, up to a maximum volume of 22 mL in the nominally 20 mL syringe.

   Overfilling the syringe may prevent the sorting cartridge from fitting onto the instrument.
6. With the end of the Cartridge Loader tube still in the sample, invert the syringe without detaching it from the loader tube.
7. By pushing on the syringe, expunge the air bubble so that no more than ~0.5 mL of air remains in the syringe.
8. Detach the syringe from the Cartridge Loader.

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9. Place the syringe on the cartridge luer lock and apply very gentle torque with the fingertips to seal.

   Do not apply any pressure on the plunger once the cartridge has been loaded. Too much pressure can cause the sample to split at the cartridge and contaminate the sort population.

   DO NOT OVERTIGHTEN THE LUER LOCK! A luer lock needs only very gentle pressure to seal as can be applied with the fingertips. Overtightening risks cracking the port.

The cartridge is now ready for sorting. If using a biosafety hood, the cartridge can now be taken out of the hood.

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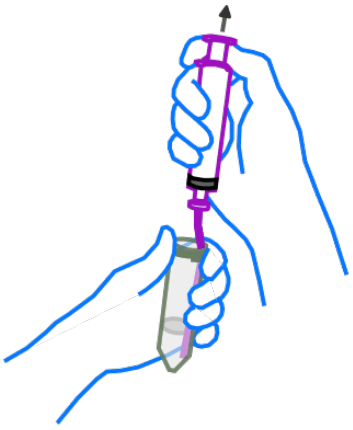
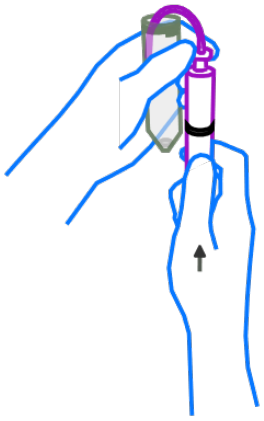
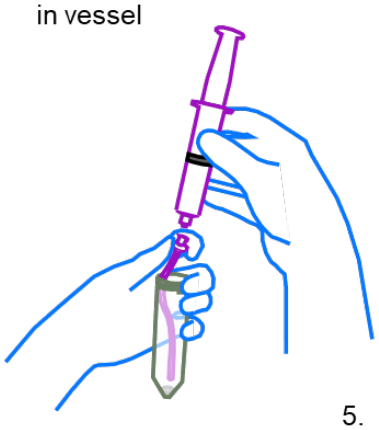
1. Load sample into cartridge loader
   - Dip filler tube into vessel and aspirate by pulling syringe plunger



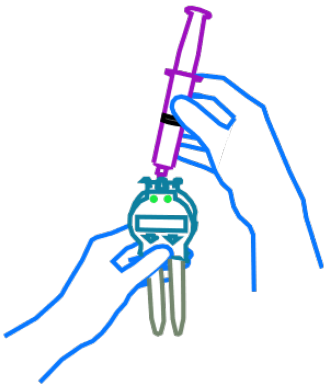
2. Expel bubble
   - Invert syringe and hold vertical, while keeping filler tube in vessel
   - gently expel bubble out of syringe



3. Detach filler tube and strainer
   - Unscrew luer syringe
   - Hold strainer and syringe upright so that no sample is spilt
   - Place input vessel in rack, leaving the filler tube and strainer in vessel



4. Attach syringe onto cartridge
   - Pick up cartridge and screw on syringe
   - Do not over-tighten luer!



5. Place cartridge on instrument
   - hold cartridge by the ring-pull while inserting on instrument

Figure 3-1 Schematic for loading and inserting a cartridge

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3.4 Highway1™ Cartridge Re-use

3.4.1 Recommendations

The Highway1™ cartridge is a single-use disposable. For sterility and optimal sort results, a new cartridge must be used.

In the event a cartridge is re-used, the sterility assurance is voided on first use, and it is up to the user to determine the fitness for their intended process.

There is always a risk of carryover if a cartridge is re-used.

Cartridges can be re-used for daily QC and sort set-up. For optimal data acquisition, these cartridges should be washed out with water prior to running. If QC fails when re-using a cartridge, refer to section 3.5.2, step 9.

3.4.2 Washout Protocol for the QC Bead Cartridge

1. Fill a 3 mL syringe with water.
2. Label syringe as **wash syringe** and attach to the cartridge.
3. Insert the cartridge into Highway1™ and allow to align.
4. Select *Run Wash*.
5. Run wash until a minimum of 0.5 mL of the sort buffer has passed through the cartridge and the event rate is negligible compared to the expected event rate of the next sample.
6. Stop the wash. The cartridge is now ready for the new sample.

3.5 Daily QC Procedure

The performance of Highway1™ should be confirmed prior to use by running the Daily QC test. This checks both cytometry and sorting functions on a separate cartridge before sorting cells on a new sterile cartridge. The Daily QC Beads must be prepared ahead of the Daily QC test.

3.5.1 Preparing Daily QC Beads

To prepare the Daily QC Beads in a 3 mL syringe for running on Highway1™:

1. Vortex the Daily QC Beads bottle for 10 seconds, then add 2 drops of beads to 2 mL of deionized water in a 15 mL Falcon® tube.
2. Draw up the solution in a 3 mL syringe using a blunt needle. There is no need to expel air from the 3 mL syringes.
3. Remove the blunt needle, then attach the syringe to a Highway1™ Cartridge.
4. Discard needle by the appropriate sharps disposal route.

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3.5.2 Performing the Daily QC Test

1. Open HighwayR and select *Daily QC* as shown below.

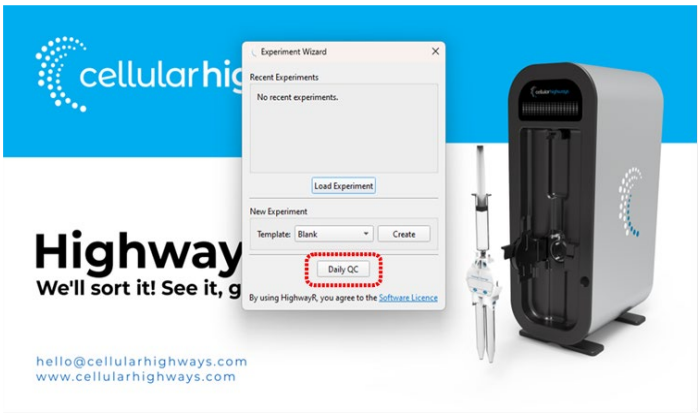

Figure 3-2 Start up window

2. Select the 3 mL syringe from *Flow* dropdown menu.

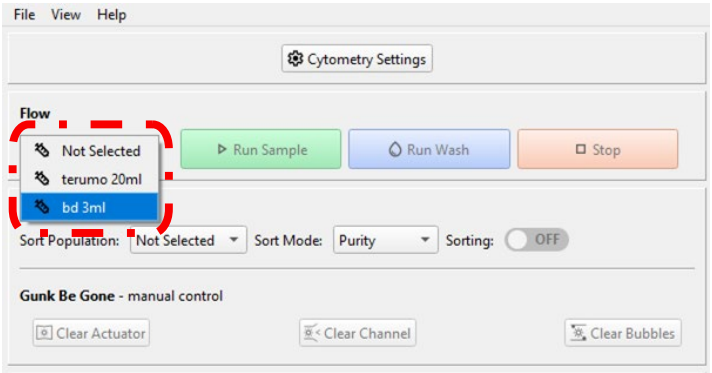

Figure 3-3 Instrument Control panel

3. Install the 3 mL adapter into Highway1™.
4. Open the door and insert the prepared cartridge.
5. Close the door, ensuring the latch engages.
    - Highway1™ will now start auto-alignment.
6. Wait until the status box states *Ready to Run Sample*.
7. Select *Start QC*.
    - The QC will go through several steps: checking laser alignment, checking cytometry acquisition, starting sorting, and checking the stability of sorting.

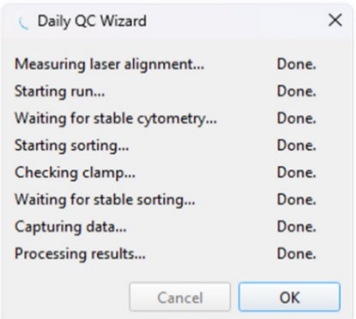

Figure 3-4 Daily QC Wizard

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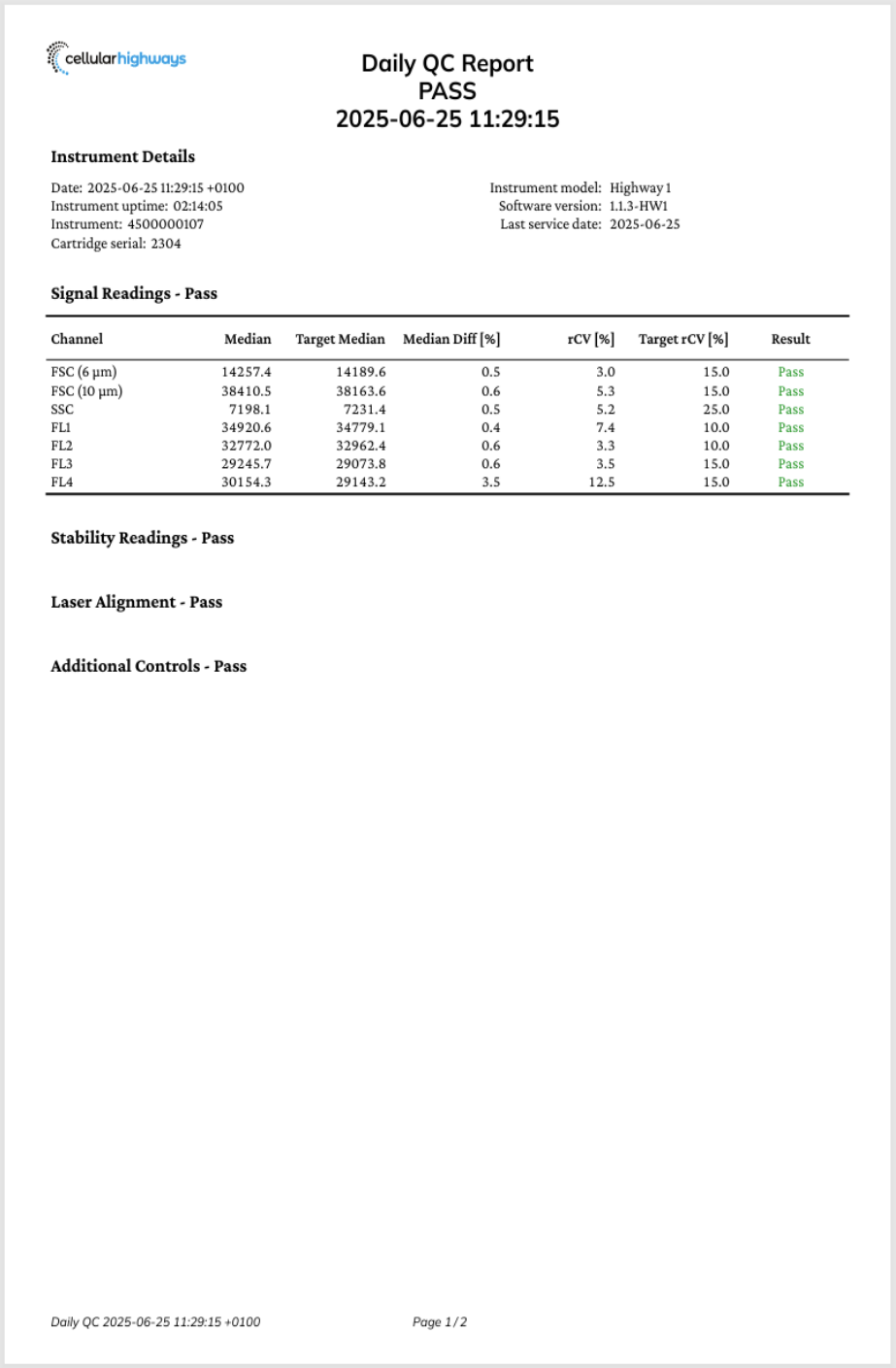
8. On completion, a PDF report will be generated, with either a pass or fail result, as shown in Figure 3-5. The test criteria and test results are reported in detail to allow remote debugging by Cellular Highways support in case of a failure.
9. If a failure is observed on the Daily QC test, inspect the report, and repeat the Daily QC once with a new cartridge. If the error recurs, contact Cellular Highways support (section 9).

Note: The Daily QC procedure tests both cytometry and the sorting process. This is the recommended test to make sure that Highway1™ is working correctly before running a real sample, ensuring that the sample will be sorted properly.



cellularhighways

Daily QC Report
PASS
2025-06-25 11:29:15

Instrument Details

Date: 2025-06-25 11:29:15 +0100
Instrument uptime: 02:14:05
Instrument: 4500000107
Cartridge serial: 2304

Instrument model: Highway 1
Software version: 1.1.3-HW1
Last service date: 2025-06-25

Signal Readings - Pass

Channel	Median	Target Median	Median Diff [%]	rCV [%]	Target rCV [%]	Result
FSC (6 µm)	14257.4	14189.6	0.5	3.0	15.0	Pass
FSC (10 µm)	38410.5	38163.6	0.6	5.3	15.0	Pass
SSC	7198.1	7231.4	0.5	5.2	25.0	Pass
FL1	34920.6	34779.1	0.4	7.4	10.0	Pass
FL2	32772.0	32962.4	0.6	3.3	10.0	Pass
FL3	29245.7	29073.8	0.6	3.5	15.0	Pass
FL4	30154.3	29143.2	3.5	12.5	15.0	Pass

Stability Readings - Pass

Laser Alignment - Pass

Additional Controls - Pass

Daily QC 2025-06-25 11:29:15 +0100 *Page 1 / 2*

Figure 3-5 Daily QC report example

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4 Performing a Sort

The first step in performing a sort is to prepare an experiment in the software in which the cytometry visualization and the instrument settings have been defined to allow proper identification of the target population.

We recommend that a diluted aliquot of the fully stained sample should be run first on Highway1™ to set voltages prior to any controls.

Once the experiment workspace has been defined and the appropriate controls (either single color control [SCC], fluorescence minus one [FMO] controls, or both) have been acquired, the sample of interest will be loaded, and the sort initiated. The sort will then run to completion.

4.1 Experiment Set-up Workflow Overview

1. Run the first sample. This is typically a diluted sample of at least 2 mL (at 50,000/mL) of fully stained cells.
2. Adjust gains then restart recording and acquire data.
3. Run wash procedure (section 3.4.2), then run next sample — for example, an SCC or FMO.
4. Repeat step 3 for each subsequent sample.
5. Once set-up is complete, move onto new sort cartridge and run sample.

Always use a syringe strainer when using samples containing cells.

Figure 4-1 Load cells into 3 mL syringe with strainer and blunt needle.

4.2 Setting Up an Experiment

There are two options to run an experiment on Highway1™ (Figure 4-2): a user can load an experiment or create a new experiment.

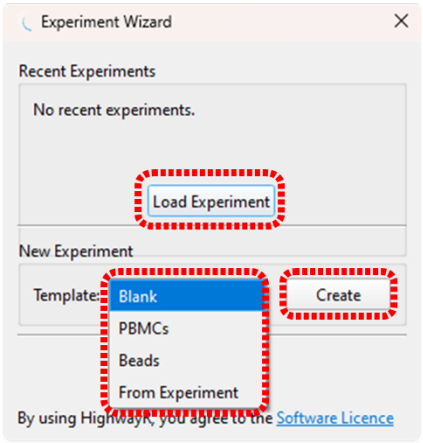

Figure 4-2 Experiment Wizard

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Four options are provided for new experiments. These options include starting an experiment from a blank layout or from three template options that set plot ranges appropriately:

- PBMCs — ranges appropriate to cells found in blood, such as B cells and T cells.
- Beads — ranges appropriate to polystyrene microspheres up to around 10 µm diameter.
- From Experiment — ranges, gains, plots, gate names, and gating hierarchy will be copied from an existing experiment file as selected.

If a new experiment is selected, file explorer will launch and prompt the user to name the experiment file.

4.3 Cytometry Data Visualization

To visualize the cytometry data, the user can display event parameters (for example FSC-A) in either one-dimensional (1D) or two-dimentional (2D) histograms. Subsets of data can be further interrogated by using a hierarchical gating structure.
This section will outline how to create, modify, and delete these plots to allow the user to set up the experiment as they require.
When a user creates a new experiment from any of the standard templates, the default plots are created.

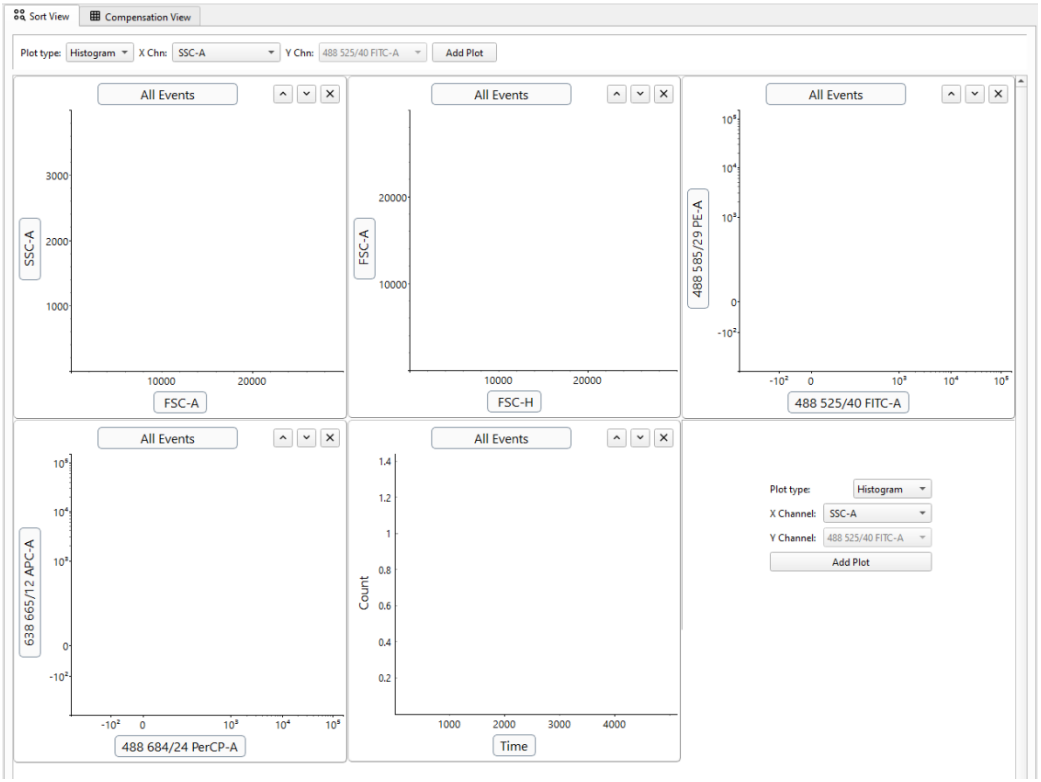

Figure 4-3 Default Plot layout and gates for PBMCs template

The following operations can be performed to change the visualization of the cytometry data:

a) Add or remove plots.
b) Change the ordering of the plots.
c) Change the axis parameters.
d) Change the scaling of the axis.
e) Change the parameter labels.

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4.3.1 Add or Remove Plots

To add a plot, at either the top or bottom of the *Sort View*, select Plot type (Density = 2D histogram; Histogram = 1D histogram) and the X and Y parameters. Then, press *Add Plot.*

Figure 4-4 Top Add plot menu

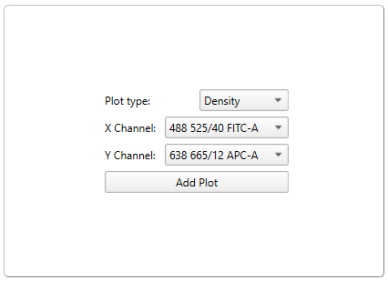
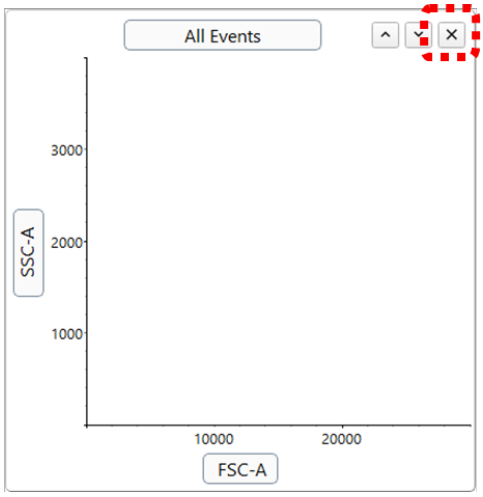


Figure 4-5 Bottom Add plot menu

To remove a plot, press the X in the top right corner of the plot to be deleted (this may also change the gating structure).



4.3.2 Graph Ordering

To change the ordering of the plot on the screen, press the up and down arrows in the top right corner.

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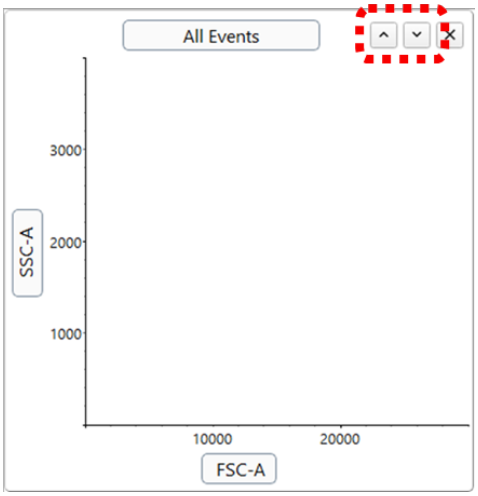
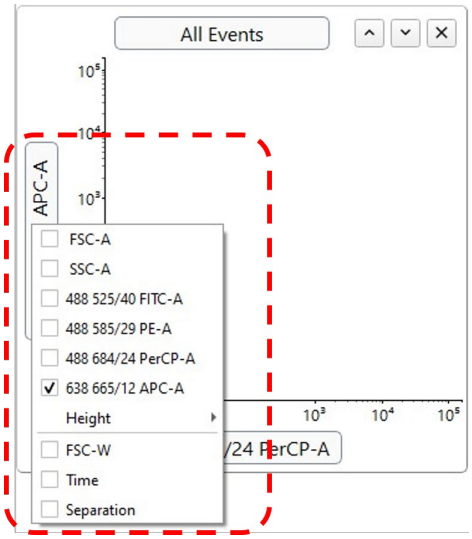


4.3.3 Axis Parameters

To change graph axis parameters, click on the axis label and select the chosen parameter from the dropdown menu.



4.3.4 Axis Scaling

The scaling of a plot can be changed by changing the values in *Cytometry Settings* > *Plot Ranges*; defaults are provided to ease usage. Access Cytometry Settings from the Instrument Control area (Figure 1-5).

The type of scaling can also be changed by right-clicking on a plot, selecting either x-scale or y-scale, and selecting one of the scaling options: linear, log, or logicle.

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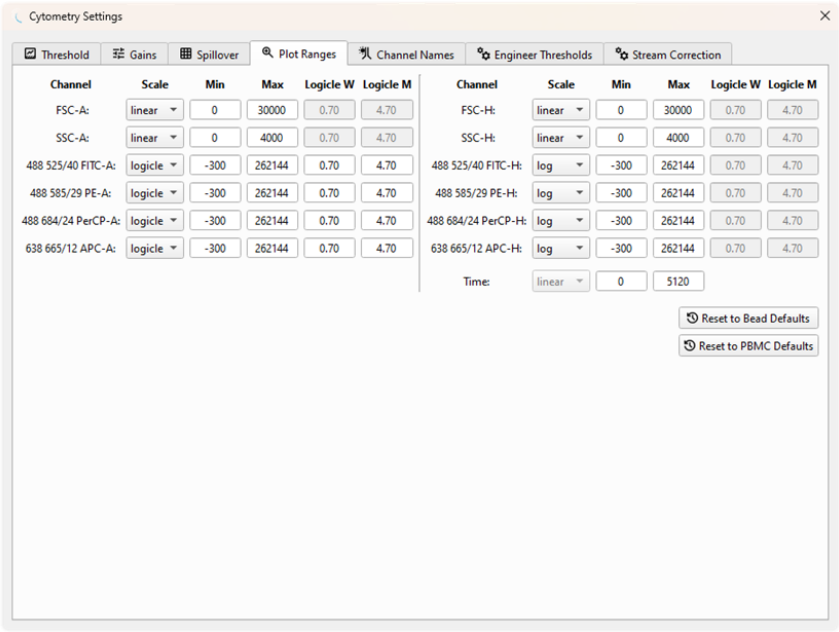
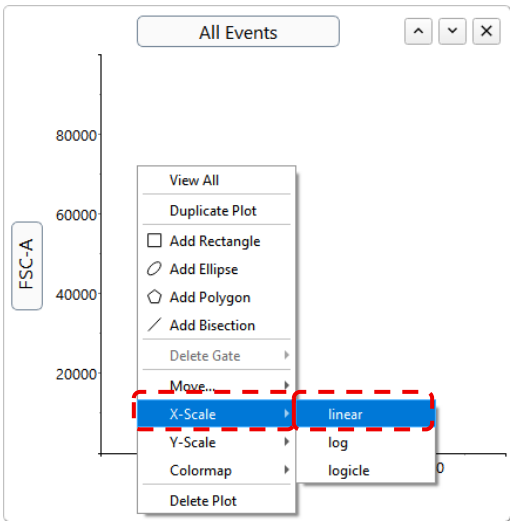


Figure 4-6 Plot Ranges window



4.3.5 Parameter Labels

To change the channel names, navigate from the Instrument Control area (Figure 1-5) to: *Cytometry Settings > Channel Names.*

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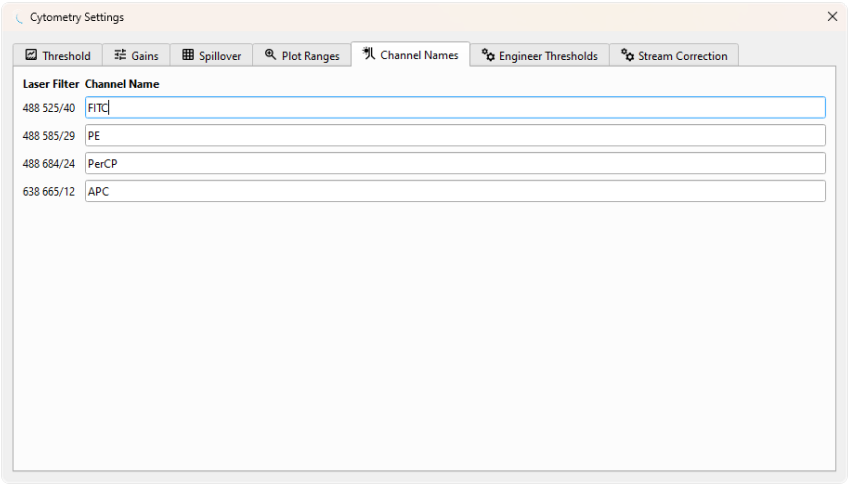

Figure 4-7 Channel Names window

4.4 Acquiring Samples

- Press + *Sample* to add a new sample. Rename the sample by double-clicking on sample name and entering the required text.

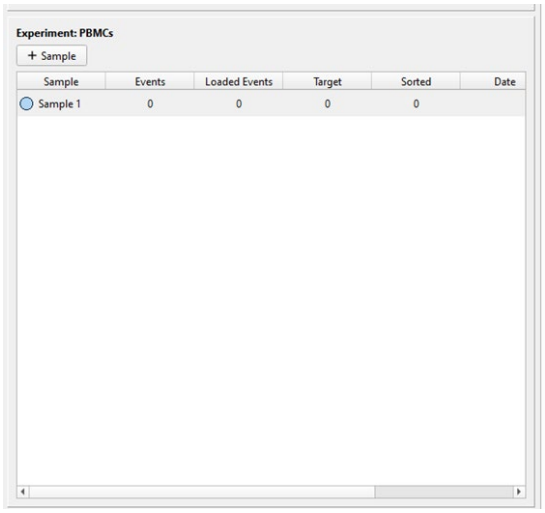

Figure 4-8 Sample view

- In *Flow*, select chosen syringe from the dropdown menu. Ensure the correct adapter is in the instrument.

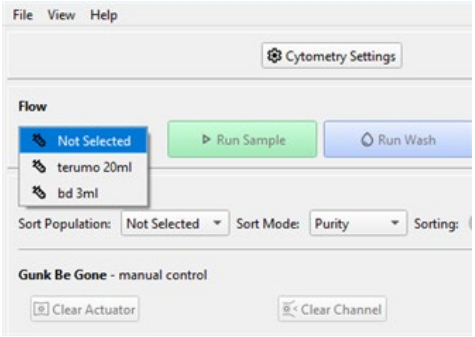

Figure 4-9 Syringe selection

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- Load syringe with a small amount of fully stained sample, attach it to the cartridge, insert it into Highway1™, and close the door.
- Wait until the status bar says *Cartridge Aligned*.
- Click Run Sample, then wait for status bar to say *Acquisition Stable*.

Figure 4-10 Flow control panel.

- Click *Restart Recording* to clear event data acquired during the stabilization stage.

4.4.1 User Threshold

Once data acquisition is stable, the user threshold can be checked. The user threshold is used to remove background noise from being included in cytometry plots and has been optimized. If adjustment is considered necessary, we recommend doing so with caution.

Note: We do not recommend gating out large volumes of debris with the threshold to improve efficiency; this will most likely negatively impact sort performance.

4.4.2 Stream Correction (Optional)

To ensure Highway1™ is sorting optimally, the stream correction plot may be checked within the cytometry settings when unexpected populations are observed, such as in Figure 4-11.

Stream correction is an automatic feature in the Highway1™ Cell Sorting System that allows the display of conventional cytometry data to the user.

To check and/or update the stream correction settings, follow these steps:

1. Begin acquiring sample.
2. Open Cytometry Settings, and navigate to the Engineer Thresholds tab (Figure 4-12).
3. Adjust the stream correction gate (dotted line) so that it diagonally divides the two populations, or press the *Estimate stream split* button, which recalculates the stream correction gate and all stream correction factors automatically.

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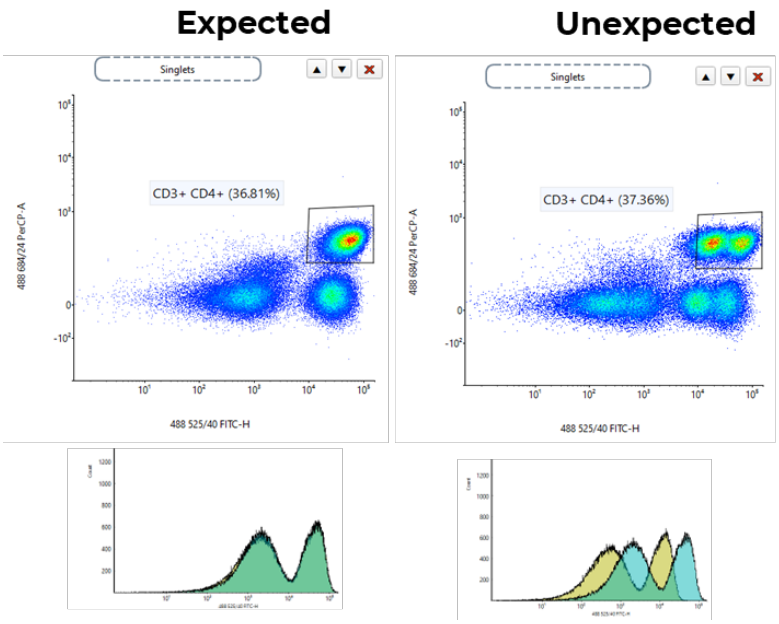

Figure 4-11 Expected vs unexpected cytometry. The unexpected cytometry is caused by incorrect stream correction.

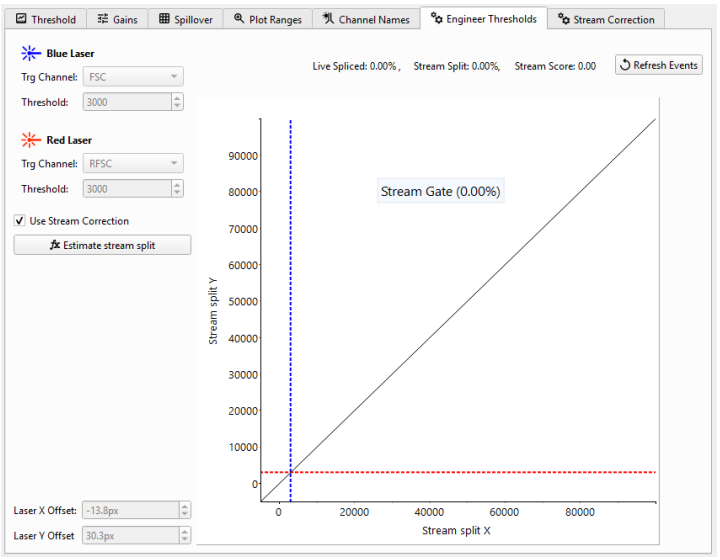

Figure 4-12 Engineering Thresholds window (Stream Gate)

4.4.3 Adjusting Forward and Side Scatter

To improve visualization of the FSC (Forward Scatter) and SSC (Side Scatter), go to *Cytometry Settings* > *Plot Ranges*, as described in section 4.3.4 above.

4.4.4 Setting Gains

To set gains go to C*ytometry Settings* > *Gains.* Alter detector voltages so that populations are well-resolved on the cytometry plots.

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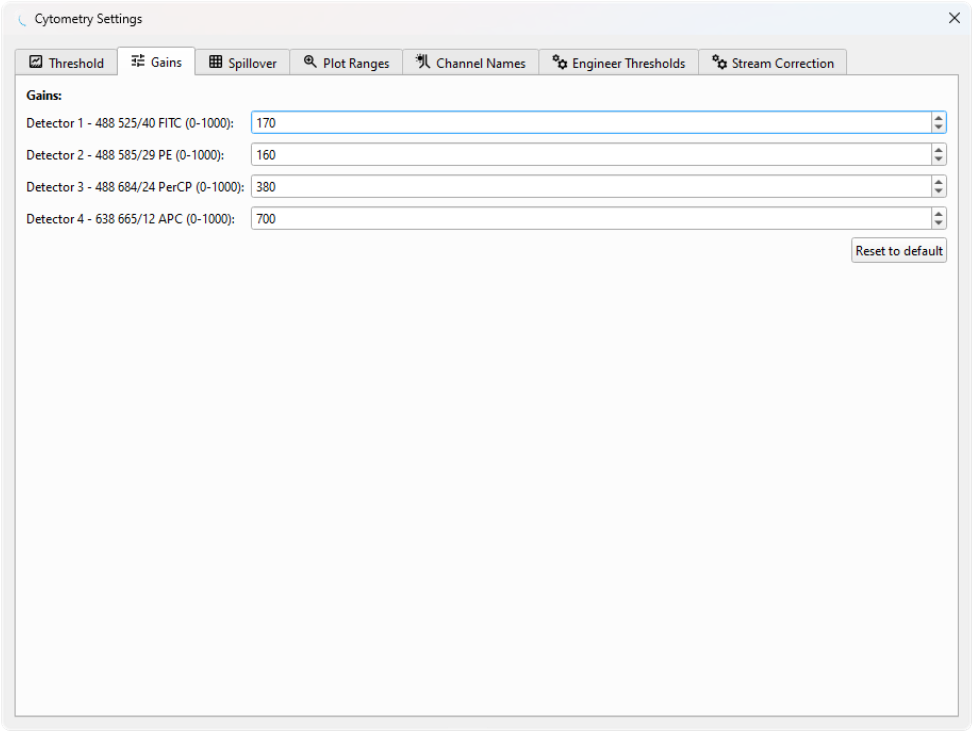

Figure 4-13 Gains window

4.4.5 Compensation

1. Once the voltages have been set with the fully stained control, click on the *Compensation View* tab to see if any compensation is required.
2. To compensate, first prepare your compensation samples (see note below). Run each control and record at least 2000 positive and at least 2000 negative events. Name each sample with the fluorochrome being used. Make sure to wash between each control to prevent sample cross-contamination.
3. Once the samples have been acquired, go to: *Cytometry Settings* > *Spillover* > *Auto Compensate* to automatically compensate each sample.
4. Alternatively, the compensation values can be altered manually in the table.
5. To clear, select Clear Spillover.

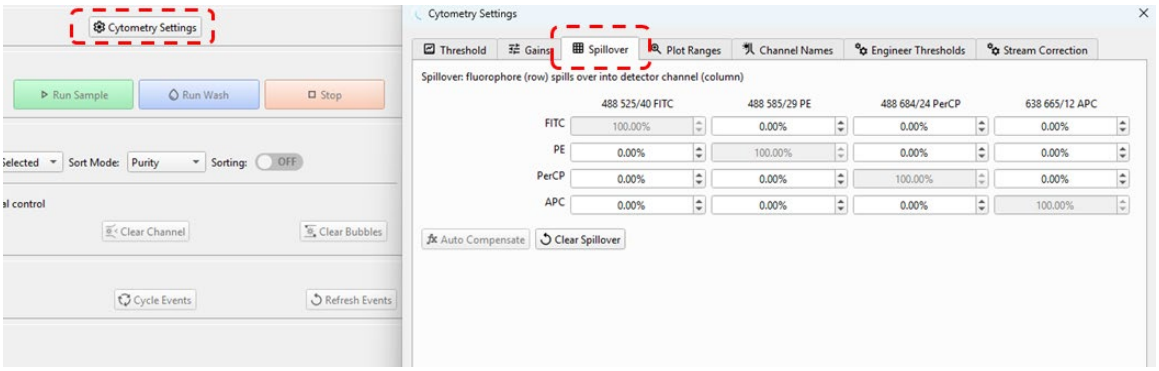

Figure 4-14 Spillover matrix

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Compensation can be done with either compensation beads, antibody-capture beads, or cells. It is possible to use all the usual methods of compensation that cytometrists employ:

- Compensation from the individual single-color controls

These controls may be fluorescent beads, antibody-capture beads, or stained cells. HighwayR's automatic compensation normally works well on all these sample types.

- Compensation from the mix of individual single-color controls

HighwayR's automatic compensation normally works well on the mix fluorescent beads or antibody-capture beads. This normally saves time, since the compensation can be done on a single sample with no washing.

- Manual compensation of the fully stained sample

This is typically performed when there is a strong expectation of what the compensated cytometry should look like.

4.4.6 Drawing Gates

1. To draw gates, right-click on a cytometry plot and select *Add Rectangle/Ellipse/Polygon/Bisection.*
2. To adjust a gate, select a handle so that it turns red and drag it to the desired location.
3. To move an entire gate, hover the mouse over the center of the gate until all the lines turn dotted and blue, then drag the gate.
4. To add another handle (polygons only), hover over one of the edges until it turns to a dashed blue line, then left click.
5. To delete a handle, right-click on the unwanted handle and select *Remove handle*.
6. To convert to a polygon (rectangles only), right-click on the gate and select *Convert to Polygon*.
7. To delete a gate, either right-click on the plot containing the gate and go to *Delete Gate* in the dropdown and select the desired gate. Or right-click on the gate and select *Delete Gate.*
8. To change gating hierarchy, click on the plot title (default: *ALL EVENTS*) at the top of a plot and select a gate from the dropdown menu.

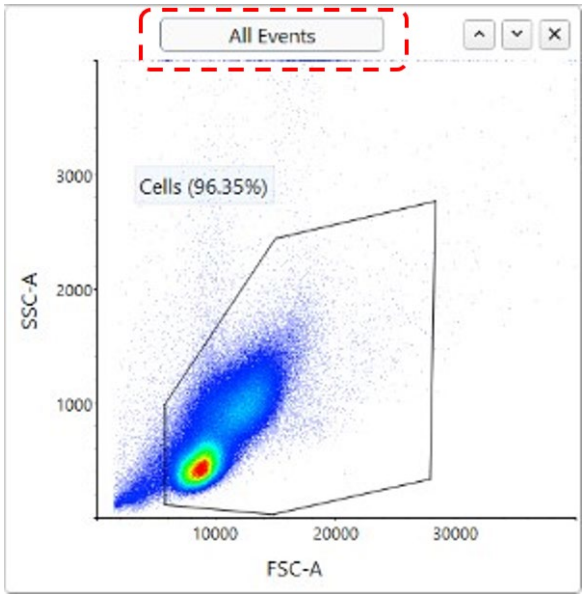

Figure 4-15 Plot Title

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4.4.7 Displaying Data

When running, HighwayR will show the most recent 500,000 events. In Cytometry Data, the current data displayed can be altered.

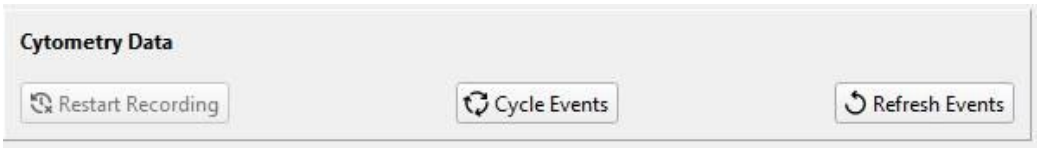

Figure 4-16 Cytometry Data Control Panel

- Restart Recording
  - This will clear all logged data from the run. The data will be deleted and will not be recoverable.
- Cycle Events
  - This allows the number of most recent events to be shown.
- Refresh Events
  - This will clear the data that is currently shown. The data will not be deleted and will be part of the full sample once the acquisition is finished.

4.5 Sorting

4.5.1 Selecting the Sort Population

1. Once compensation has been completed, return to *Sort View,* and set gates for fluorescence parameters. Fluorescence minus one (FMO) control samples may be required for this if there are not well-separated positive and negative populations.
2. Set gating strategy for sorting and select *Sort Population.*
3. Select the *Sort Mode.* There are three options: Enrichment, Purity, and High Purity.

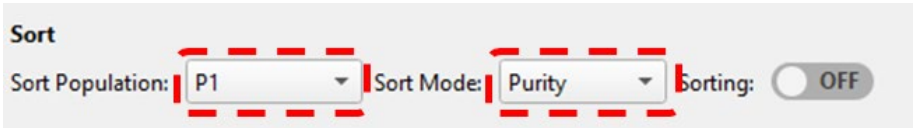

Figure 4-17 Sort Control Panel

4.5.2 Recommended Sorting Concentration

Table 1 below lists the recommended and maximum cell concentrations for the purity and enrichment modes, although slight adjustments may be required depending upon cell type (for example, larger cells may need to be more dilute). The maximum input volume of 22 mL runs in 85 minutes; for purity and enrichment modes, the maximum number of cells equates to 50 million and 185 million cells, respectively.

Table 1 Summary of Purity and Enrichment modes

	Purity mode	Enrichment mode
Recommended concentration	1 - 2 million/mL (4,000 - 8,000/s)	4 - 6 million/mL (18,000 - 26,000/s)
Maximum concentration	2.3 million/mL (10,000/s)	8.4 million/mL (37,000/s)

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4.5.3 Recommended Sort Rates

The maximum sort rate in all sort modes is 4,000/s. The minimum recommended sort rate in all modes is 40/s. The user may choose to sort below 40/s; however, there may be an impact on purity and recovery of the sample.

4.5.4 Running a Sort

1. Load a new cartridge with sample.
2. Place the cartridge in the instrument and close the door.
3. Optional: Magnetically attach the cold pack to keep the sample cool during the sort.
4. Wait until status bar states *Ready to run sample*.
5. Run sample. Wait until status bar states *Acquiring. Ready to sort*.
6. Switch Sorting to On.
7. When the status bar changes to Sorting, you can walk away from the instrument.
8. The status bar will show *Sorting complete* once sorting has finished.

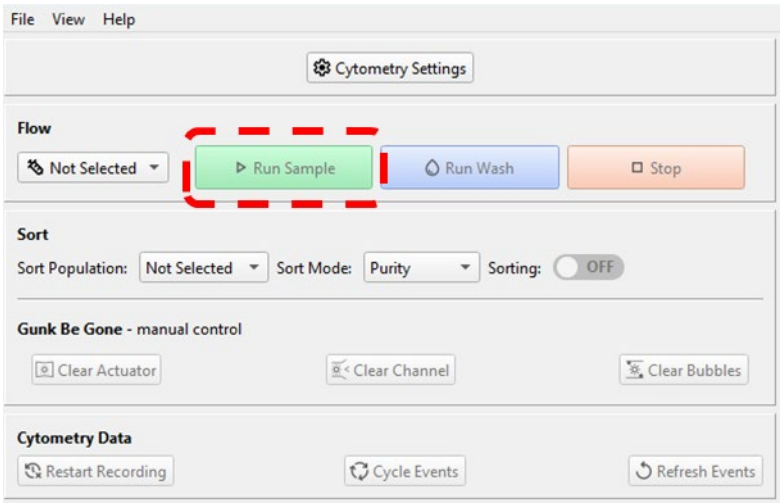

Figure 4-18 Instrument Control Panel

Note: When sorting is completed (or if the Stop button is pressed), the machine vision panel of HighwayR may still show stray particles passing through the chip. This can be ignored, as it is a small residual flow that does not reach the Sort 1 and Sort 0 outputs.

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5 Data Storage

Data from each sort operation (and all Daily QC data) is automatically saved on the Host PC.

Data is automatically organized in the Cellular Highways folder in the Documents. There will be two folders: Experiments and Daily QC.

The data in the Experiments folder is structured as follows:

1. All the samples within one experiment are saved in a folder named with the experiment name and accompanied by a .hwe file of the same name.
2. Each time a new acquisition or sort is initiated, a new sample will start recording in HighwayR's native format (.hwr).
3. Once the run is complete the sample will automatically be exported as a standard FCS file (.fcs). The acquired data is subsampled automatically to reduce the file size (500,000 events default).

The Daily QC folder contains one experiment folder (Daily QC), a sample file (Daily QC.hwe), and all the Daily QC Reports.

Highway1™ data files

There are several files saved in each run. The following are primarily intended for the user:

- HWE file
  - <Experiment name>.hwe
  - Used to re-open an experiment on HighwayR software.
- HWR file
  - <Sample name>.hwr
  - HighwayR's native data file containing the full cytometry data.
- FCS file
  - <Sample name>.fcs file
  - The data file required to load a sample in a range of analysis software, for example, FlowJo.
- Sort Report
  - Sort Report <Date> <Time>.pdf
  - Sort report summary, including sort counts and flow cytometry plots.

Other files are intended for Cellular Highways Support and troubleshooting.

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6 Shutdown

1. Close HighwayR software.
2. Switch off Highway1™:
   a. Press the power button once; the lights on Highway1™ will then flash blue.
   b. Press the power button a second time; the lights on Highway1™ will flash yellow, and Highway1™ will shut down within 10 seconds.
3. Shut down the PC.

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7 Instrument Cleaning

Cleaning of the Highway1™ external surfaces may be required to decontaminate the instrument before entry into controlled lab areas or after use with biological material as part of standard lab practices. This procedure is intended to be used for these purposes.

Cleaning of the Highway1™ optical window glass (behind the door) should be conducted only when required, such as when dust or liquid or stains are observed on the window surface. Any attempts to clean the optical window glass of a new Highway1™ are likely to reduce cleanliness. Experience and a careful approach are necessary to obtain a good result and avoid damage to the optical surface.

If cleaning is required, only the following materials are approved for use with Highway1™:

Purpose	Material
External Highway1™surfaces	Standard biology disinfectant (e.g. 70% ethanol solution)
Optical window glass	Highway1™ Optics Wipes
Gloves	Powder-free and oil-free gloves

7.1 External Surface Cleaning Procedure

1. Wear gloves and select suitable lab disinfectant (if in doubt, contact Cellular Highways).
2. Unplug power supply and insert attached blanking plug into power socket.
3. Unplug USB cable.
4. Close Highway1™ door.
5. Spray lab disinfectant over each Highway1™ surface, avoiding overspray onto the optical window behind the door and avoiding direct spray into the back fan ducts.
6. Wipe down each Highway1™ surface with lab wipes, avoiding contact with the optical window behind the door.

7.2 Optical Window Cleaning Procedure

This procedure should not be performed routinely; it is best not to touch the optical window unless it is contaminated. This should only be performed if necessary to improve cytometry measurements. Contact Cellular Highways support if in doubt.

1. Put on a new pair of gloves (powder-free and oil-free).
2. Open the Highway1™ door and inspect the optical window.
3. If there are any particles, care should be taken with the first wipe to avoid scratching the surface.
    - Using a Highway1™ Optics Wipe, apply light pressure and wipe from the top of the window to the bottom in one continuous motion, minimizing the contact with the surrounding metal, as this may introduce more contamination.
4. Rotate the wipe to the next unused section and repeat the above step, avoiding using the same section of the wipe more than once.
5. Continue to repeat the above step until the window appears free of dust or stains.
6. As a last step, draw an unused section of the wipe slowly with light pressure from the top of the window to the bottom, aiming for the motion to take 10 - 20 seconds (to minimize streaks from solvent residue).
7. If this process is not effective, contact Cellular Highways for further advice.

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8 Troubleshooting

If an issue occurs, first try the following quick solutions. If the issue cannot be resolved with the troubleshooting guide below, contact Cellular Highways Support (section 9).

8.1 Startup

Issue	Solution
Instrument or computer not turning on	Check all cables are plugged in and switched on at the mains.
Instrument not connecting to the computer	1. Check that all cables are connected. 2. Close the HighwayR software and open it again. 3. Restart the instrument and host PC. 4. Shut down the instrument and host PC; turn off both at mains switch, turn on, and try again.
Lasers not turning on/ Laser error reported	1. Close HighwayR. 2. Restart the instrument. 3. Reopen HighwayR.
High chip resistance error reported	Change to a new cartridge.
Alignment error reported	1. Make sure door is closed properly until it clicks. 2. Reseat cartridge and close door. 3. Try a new cartridge.
Flow rate outside range error reported	1. Check that the correct syringe is selected and that the corresponding adapter is installed. 2. Remove cartridge and check for bubbles in syringe. Purge any bubbles present. 3. Replace cartridge.
Pressure instability or blockage error reported	1. Check for clumps in the sample. 2. Run again and manually activate Clear Channel. See whether gunk is shifted and whether run proceeds without issues. 3. Run wash. 4. If an old syringe, replace with new syringe. 5. Change cartridge.
Leak error reported	1. Check whether there really is a leak — is the cartridge wet or has any liquid dropped from the bottom of the cartridge? 2. Check that the correct syringe is selected and that the corresponding adaptor is installed. (Incorrect syringe or adapter may be falsely reported as a leak.) 3. Change cartridge.

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8.2 Acquisition

Issue	Solution
Cytometry unstable error reported: ▪ Splice error ▪ Laser delay instability ▪ Stream correction error	1. Press Clear Channel once. If this does not work after 10 seconds, press Clear Actuator once. 2. Stop run, then restart run and sorting. 3. Wash cartridge with 1 mL H_20 or PBS. 4. Change cartridge.
No events seen	1. Are bubbles trapped in the laser pockets on the live machine vision panel? If any bubbles are observed, press Clear Bubbles. 2. Run Daily QC Beads through Highway1™ at recommended concentration.
Outgassing of bubbles in laser pockets	1. Avoid introducing bubbles greater than 0.5 mL when loading sample into syringe. 2. Press Clear Bubbles. 3. Avoid changing temperature in sample: a. Keep buffer and sample at room temperature prior to sorting if running sample at room temperature. b. Keep buffer and sample on ice prior to sorting if running sample cooled with cold packs. Note: Cold packs can only be used with a 20 mL syringe.
Event rate too low	Concentrate the sample by pelleting the cells and resuspending in a lower final volume of the sorting buffer.
Event rate too high	Dilute sample.
High gunk level	Try to reduce sample debris and clumping by: ▪ Running samples with cold packs attached. ▪ Filtering reagents through 0.02 µm filter. ▪ Filtering cells/beads prior to running. ▪ Using recommended buffer. Note: If none of these options work and there is still too much aggregation occurring during sorting, it is recommended to improve sample preparation before sorting on Highway1™. Otherwise, results will be less than optimal.

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8.3 Sorting

Issue	Solution
Low sort fidelity error reported	1. Ensure target rate is greater than 40 events per second. 2. Press Clear Channel once. If this does not work after 10 seconds, press Clear Actuator once. 3. Stop run, then restart run and sorting. 4. Wash cartridge with 1 mL H_20 or PBS. 5. Change cartridge.
Low efficiency	1. Ensure most appropriate sort mode is selected. 2. Increase user threshold to ignore debris from sort decisions. 3. Reduce cell concentration to reduce number of coincidence-based aborts. Note: Purity mode rejects coincidence events. At the recommended maximum purity mode event rate of 10,000 cells/s, efficiency should be around 70 - 80% due to about 20 - 30% coincidence events. In enrichment mode, however, efficiency should be close to 100%, provided that the maximum sustained sort rate is not exceeded (i.e. target rate below 4000/s).
High focussing error rate	1. Verify that cells are larger than 5 µm in diameter. 2. Try to reduce sample debris and clumping by: a. Using recommended buffer. b. Filtering reagents through 0.02 µm filter to remove debris from buffers. c. Filtering cells through a 30 µm filter prior to sorting. d. Running cells in a 20 mL syringe (minimum volume 3 mL) with the magnetic stirring disc rather than a 3 mL syringe to maintain single-cell suspension. e. Diluting sample.
Target rate greater than 4000/s	Dilute sample so that target rate is lower than 4000 events per second.

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Issue	Solution
Low yield caused by high rate of actuator fouling	1. Try to reduce actuator fouling by: a. Using recommended buffer. b. Filtering reagents through 0.02 µm filter to remove debris from buffers. c. Filtering cells through a 30 µm filter prior to sorting. d. Running cells in a 20 mL syringe (min volume 3 mL) with the magnetic stirring disc rather than a 3 mL syringe to maintain single-cell suspension. e. Diluting sample. 2. Change cartridge.
Actuation position instability	1. Press Clear Channel once to clear any upstream blockages. 2. Stop run, then restart run and sorting. 3. Change cartridge.
Low actuation image presence	1. Ensure target rate is greater than 40 events per second. 2. Press Clear Channel once. If this does not work after 10 seconds, press Clear Actuator once. 3. Stop run, then restart run and sorting. 4. Wash cartridge with 1 mL H_20 or PBS. 5. Change cartridge.

8.4 Daily QC

Issue	Solution
Fluorescence or scatter parameters are failing	1. Prepare fresh sample of Daily QC Beads and rerun. 2. Wait 10 minutes and rerun. 3. Try a new cartridge.
Fluorescence or scatter parameters passing but sorting fails	Try a new cartridge.

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9 Contact Cellular Highways Support

For Cellular Highways support, contact support@cellularhighways.com. In the email, include a screenshot, log file, or Daily QC report showing the issue, if applicable.

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10 Specification and Compliance Information

10.1 Technical Specifications

Cytometry	*Lasers*	488 nm (200 mW) blue 638 nm (180 mW) red
	Detection	4 SiPM (fluorescence) 3 Photodiodes (FSC, SSC, RFSC)
	Filters	488 nm laser detector 525/40 nm 488 nm laser detector 585/29 nm 488 nm laser detector 684/24 nm 638 nm laser detector 665/12 nm
	DSP	24 bit 80 MHz 200,000 events per second
	Scales	5-decade log scale Linear scale Logicle scale
	Peak Measurements	Height, Area, Width (FWHM)
	Sensitivity	MESF 50 (FITC) MESF 70 (PE)
Sorting	*Purity*	99%
	Yield	80%
	Sorting rate	4 kHz sustained deflection rate
	Automation	Actuation timing and amplitude Automated sort verification
Instrument	*Dimensions*	210 mm x 570 mm x 318 mm (W x H x D)
	Weight	20 kg
	Environmental temperature	18 to 25°C
	Power requirements	100 - 240 V, up to 250 W
Fluidics	*Cell sizes*	5 - 25 µm
	Output	2 x 15 mL centrifuge tubes
	Input	3 mL or 20 mL syringe. Maximum volume 22 mL
	Flow rate	Approximately 265 µl/min Flow rate varies to keep events at a fixed speed.
	Viability	Low hydrodynamic stress Peak energy dissipation rate 2 W/mL
PC and Software	*Operating System*	MS Windows 11 on Dell host
	Monitor	27” 1440 p
	Connection	Ethernet, Wi-Fi, USB ports

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	Data compatibility	FCS 3.1 export
	Language	English (UK) only

10.2 Name and Address of Manufacturer

Cellular Highways
Building 7100 Cambridge Research Park Beach Drive
Waterbeach
Cambridge
CB25 9TL
United Kingdom

10.3 Equipment Handling and Installation

- Equipment should only be handled and installed by trained Cellular Highways personnel. Contact Cellular Highways before moving the equipment.
- If an equipment move is approved by Cellular Highways, lifting should be done while keeping the weight close to the body and following manual handling best practice. A 2-person lift is recommended. Take care to avoid any drops or collisions, which may damage the sensitive equipment.
- Equipment requires a minimum gap of 50 mm behind the rear panel for adequate ventilation. Location of the equipment must be approved by Cellular Highways.
- Equipment should not be placed on the same bench as equipment that may generate vibrations (e.g. centrifuge, shaker). Location of the equipment must be approved by Cellular Highways.
- The supply disconnection device for this equipment is the mains plug. Access to the disconnection device should not be obstructed and it must be possible for the operator to easily disconnect if required.

10.4 Equipment Maintenance Details

- Change fan filters every 12 months. This will be done on the annual service.
- Do not replace mains power cords with any replacement not specified by the manufacturer.
- Do not open instrument covers.
- Only use accessories specified by the manufacturer.
- Do not move the instrument without first contacting Cellular Highways.

10.5 Environmental Information

- Indoor use only
- Altitude up to 2000 m
- 18 - 25°C ambient temperature range
- Relative humidity up to 60%
- Mains supply voltage fluctuations up to +/-10% of the nominal voltage
- The applicable pollution degree of the environment is pollution degree 2.

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10.6 Equipment Electrical Rating Information

- External AC-DC power supply rating information:
    - Input voltage range: 100 - 240 Vac
    - Input frequency: 50 - 60 Hz
    - Maximum power: 250 W
- Instrument rating information:
    - Input voltage: 24 Vdc
    - Maximum power: 250 W

10.7 Waste Electrical and Electronic Equipment (WEEE)

This equipment is classified as electrical and electronic equipment, which must be separated from other waste for disposal or recycling. Please contact Cellular Highways for disposal or recycling options, including a take-back service if a new unit has been purchased.

Before disposal or recycling, the unit must be fully decontaminated. The user must provide confirmation of decontamination to ensure the safety of all personnel who may contact the unit after it is returned to Cellular Highways.

10.8 FCC Compliance

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

Changes or modifications not expressly approved by Cellular Highways Ltd. could void the user's authority to operate the equipment.

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11 Safety

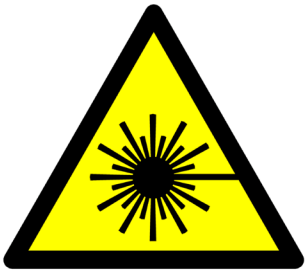
11.1 Intended Use

The Highway1™ Cell Sorting System is for Research Use Only. It is not sold as a medical device. Any clinical application of the cells (i.e. the use of cells in humans) is exclusively the responsibility of the user of the Highway1™ Cell Sorting System. This use is not intended by Cellular Highways. Therefore, the safety and regulatory compliance of any clinical application is exclusively the responsibility of the user.

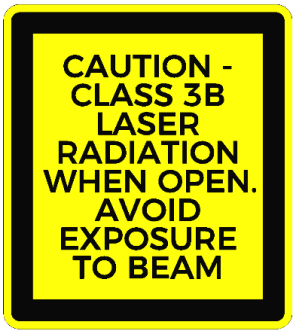
11.2 General Safety

- Do not operate Highway1™ without training from an authorized representative of Cellular Highways.
- Do not open the casework or alter Highway1™ in any way.
- If Highway1™ is used in a manner not specified within these instructions, the protection provided by the instrument may be impaired.
- Highway1™ is fitted with a glass panel in front of the LED display. Avoid impacts and loading that may damage the glass. In the event of breakage, be aware of the potential hazard due to handling glass shards. Take appropriate precautions in the clean-up process and ensure disposal into sharps waste.

11.3 Laser Safety



- The instrument contains class 3B lasers, which emit intense, coherent electromagnetic radiation that can cause irreparable damage to skin and eyes.
- 488 nm wavelength laser with maximum power of 200 mW
- 632 nm wavelength laser with maximum power of 180 mW
- Do not remove casework or alter the instrument in any way.
- Only authorized Cellular Highways service personnel are allowed to remove the casework to adjust any components.
- Caution – Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.
- Highway1™ has been classified as a class 1 laser product in accordance with BS EN / IEC 60825-1:2014.
- There are no apertures through which laser radiation in excess of class 1 limits is emitted when the Highway1™ instrument and cartridge are used according to instructions.
- The labels below have been affixed to the rear panel of Highway1™.



11.4 Biosafety — Cartridge Disposal

Cartridges can be disposed of via conventional biohazard disposal routes (including incineration and autoclaving).

11.5 Biosafety — Sample Containment

- The use of the Highway1™ cartridges with hazardous samples is entirely within the responsibility of the user. Risks must be assessed, and safety measures must be applied by the user.
- The following advice is provided to the user to aid the risk-assessment process:
  - Do not overtighten the syringe luer connection to the cartridge, as this may result in breakage of the luer fitting and leakage of sample.
  - Do not re-use the cartridge multiple times, as this may increase the likelihood of sample leakage.
  - Ensure the correct syringe type is selected in the Highway1™ software, as an incorrect selection may result in sample leakage.
  - Ensure that the cartridge is kept in the vertical position after use to minimize the risk of sample leakage.
  - Avoid overfilling the cartridge output tubes (for example by pre-filling with additional liquid) to minimize the risk of cartridge leakage.

11.6 Trapping Hazard

- Only trained users should operate the Highway1™ instrument.
- Avoid touching any moving parts during operation, as there is a risk of finger trapping and crushing.

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12 Sécurité

12.1 Utilisation prévue

Le système de tri cellulaire Highway1™ est destiné exclusivement à la recherche. Il n'est pas commercialisé comme dispositif médical. Toute utilisation dans un cadre clinique (c'est-à-dire leur utilisation chez l'humain) de cellules triées relève de la seule responsabilité de l'utilisateur du système de tri cellulaire Highway1™. L'utilisation dans le cadre clinique n'est pas prévue par Cellular Highways. Par conséquent, la sécurité et la conformité réglementaire de toute application clinique sont de la seule responsabilité de l'utilisateur.

12.2 Sécurité générale

- N'utilisez pas le système Highway1™ sans avoir reçu de formation auprès d'un représentant autorisé de Cellular Highways.
- N'ouvrez pas le boîtier et ne modifiez en aucune façon le Highway 1™.
- Si le Highway1™ est utilisé d'une manière non spécifiée dans ces instructions, les termes de garantie et/ou le contrat de service pourraient être compromis.
- L'appareil Highway1™ est équipé d'une vitre devant l'écran LED. Évitez les chocs et les charges susceptibles d'endommager la vitre. En cas de cassure de verre, soyez conscient du danger potentiel lié à la manipulation des éclats de verre. Prenez les précautions nécessaires lors du nettoyage et veillez à les jeter dans un conteneur approprié pour objets tranchants.

12.3 Sécurité laser

- Cet instrument contient des lasers de classe 3B, qui émettent un rayonnement électromagnétique intense et constant susceptible de causer des dommages irréparables à la peau et aux yeux.
- Laser de longueur d'onde 488 nm avec une puissance maximale de 200 mW.
- Laser de longueur d'onde 632 nm avec une puissance maximale de 180 mW.
- Ne retirez pas le boîtier et ne modifiez en aucune façon l'instrument.
- Seul le personnel de service agréé de Cellular Highways est autorisé à retirer le boîtier pour ajuster les pièces.
- Attention – L'utilisation de commandes ou de réglages, ou l'exécution de procédures autres que celles spécifiées ici, peut entraîner une exposition dangereuse aux rayonnements.
- Le Highway1™ a été classé comme produit laser de classe 1 conformément à la norme BS EN / IEC 60825-1:2014.
- Il n'existe aucune ouverture par laquelle un rayonnement laser dépassant les limites de la classe 1 est émis lorsque le Highway1™ et la cartouche sont utilisés conformément aux instructions.

- Les étiquettes ci-dessous ont été apposées sur le panneau arrière du produit Highway1™.

12.4 Biosécurité — Élimination des cartouches

- Les cartouches peuvent être éliminées par les voies d'élimination conventionnelles des déchets biologiques (y compris l'incinération et l'autoclavage).

12.5 Biosécurité — Confinement des échantillons

- L'utilisation des cartouches Highway1™ avec des échantillons dangereux relève entièrement de la responsabilité de l'utilisateur. Ce dernier doit évaluer les risques et appliquer les mesures de sécurité nécessaires.
- Les conseils suivants sont fournis à l'utilisateur pour faciliter le processus d'évaluation des risques:
  - Ne serrez pas trop le raccord Luer de la seringue à la cartouche, car cela pourrait entraîner la rupture du raccord Luer et une fuite de l'échantillon.
  - Ne réutilisez pas la cartouche plusieurs fois, car cela pourrait augmenter le risque de fuite d'échantillon.
  - Assurez-vous que le type de seringue approprié est sélectionné dans le logiciel Highway1™, car une sélection incorrecte peut entraîner une fuite d'échantillon.
  - Après utilisation, veillez à conserver la cartouche en position verticale afin de minimiser les risques de fuite d'échantillon.
  - Évitez de trop remplir les tubes de sortie de la cartouche (par exemple en les pré-remplissant de liquide supplémentaire) afin de minimiser le risque de fuite de la cartouche.

12.6 Risque de piégeage

- Seuls les utilisateurs formés sont autorisés à utiliser l'instrument Highway1™.
- Évitez de toucher les pièces mobiles pendant le fonctionnement, car il existe un risque de pincement et d'écrasement des doigts.

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