Highway1 Instructions for Use

H1-0031v2.10

Table of Contents

1 Highway1 Cell Sorting System Overview

1.1 Highway1 Instrument

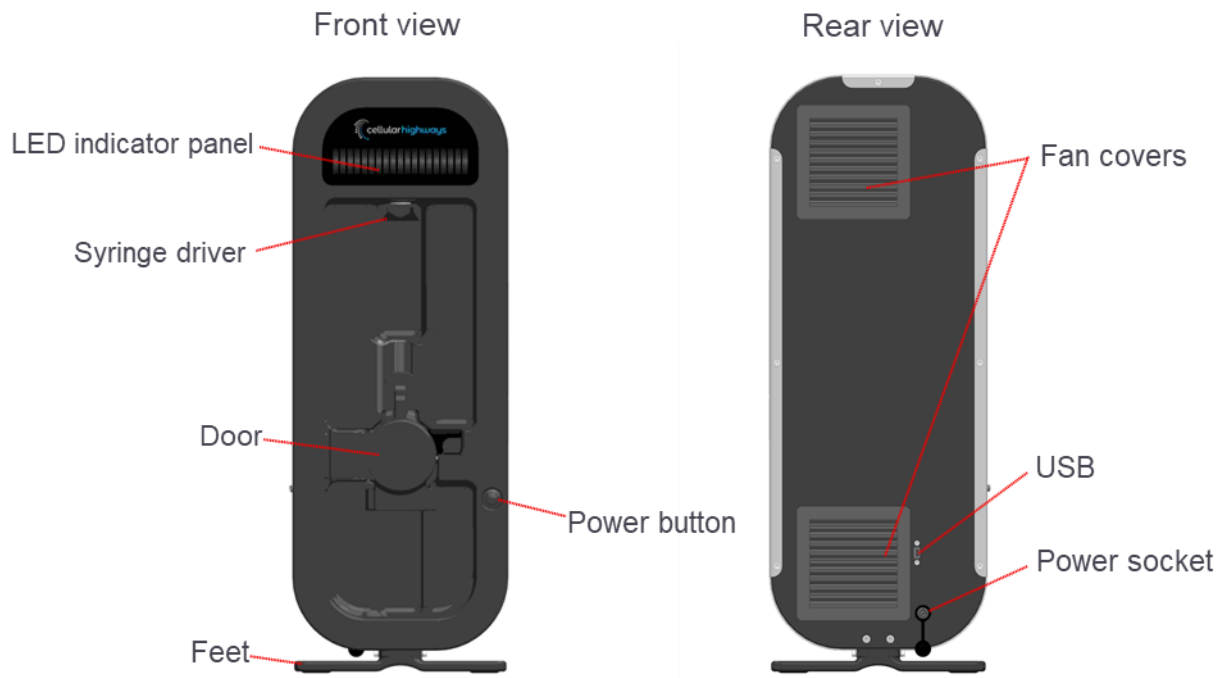


Figure 1-1 Highway1 Instrument

1.2 Highway1 RUO Cartridge and Cartridge Loader

The front and rear views of the RUO cartridge and cartridge loader are shown below.

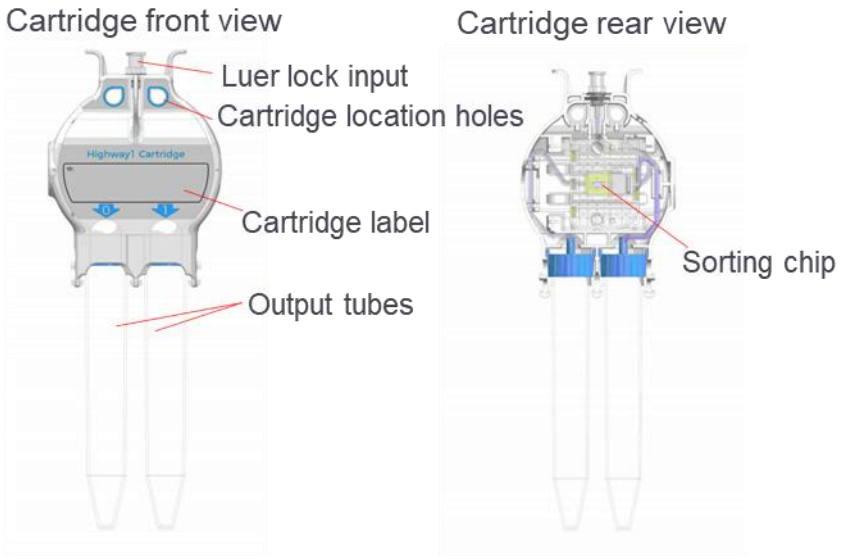


Figure 1-2 Highway1 RUO Cartridge

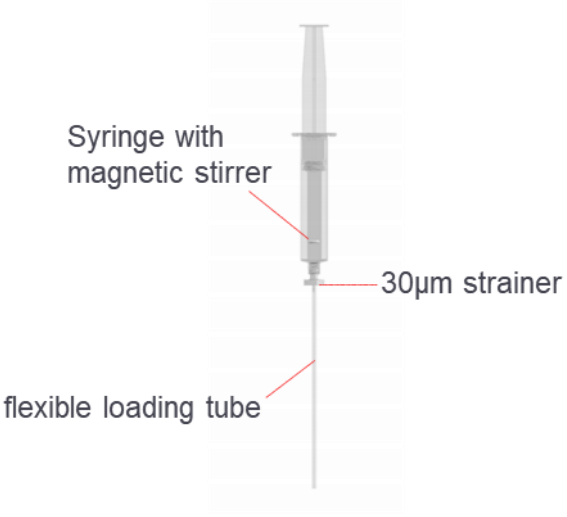


Figure 1-3 Highway1 Cartridge Loader

1.3 Cold Packs

Cold packs 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: Cooling blocks are only compatible with a 20mL syringe

Figure 1-4 Highway1 Cold Packs

1.4 HighwayR Software

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

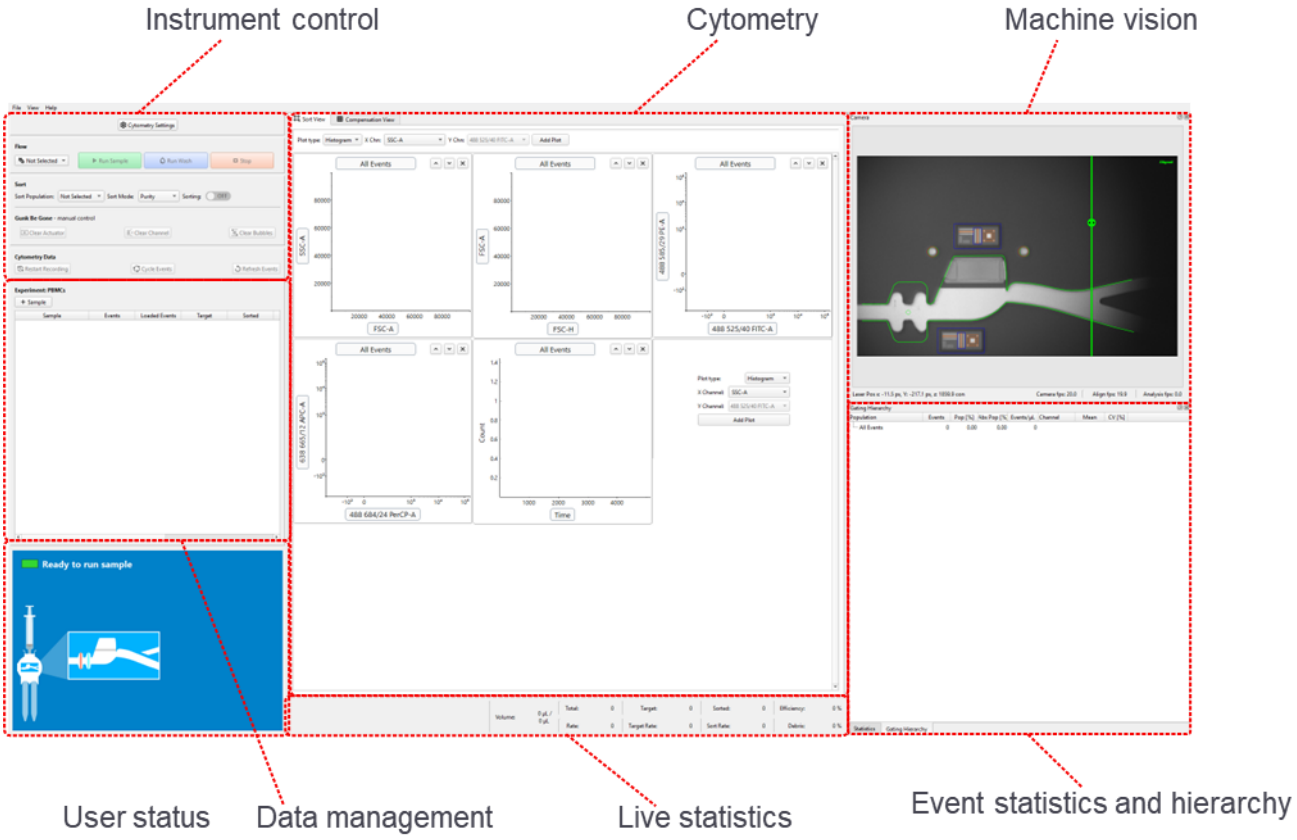
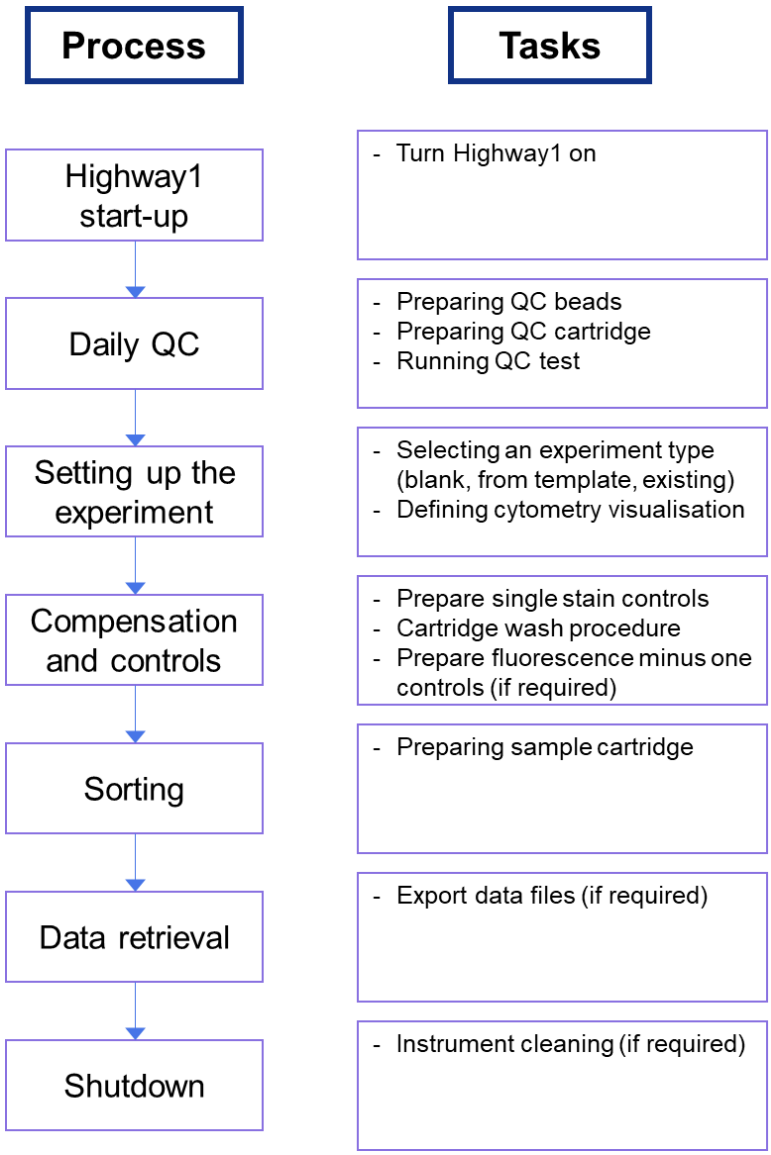
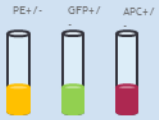
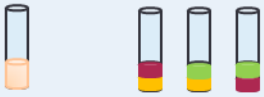


Figure 1-5 HighwayR software.

1.5 Generic workflow diagram



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!

2 Materials

These are the materials referenced within this document. Items can be ordered as required.

Product code	Product Name	Pack Size
A10000-1001	Highway1 Instrument	1
A10000-1029	Highway1 Syringe Adapter 3mL	1
A10000-1073	3mL Syringe	25
A10000-1076	3mL Syringe Blunt Needle	100
A10000-1077	3mL Syringe Strainer	25
A10000-1078	Highway1 Fan Filter	5
A10000-1079	Daily QC Beads Kit	1
A10000-1081	Highway1 Cold Pack Kit	1
A10000-1082	Highway1 Cartridge Rack	1
A10000-1083	Highway1 Cartridge	25
A10000-1084	Highway1 Cartridge Loader	25
A10000-1101	Highway1 Optics Wipes	30

Notes:

Only the syringes listed above and included in the Highway1 Cartridge Loader are validated for use with Highway1. The Highway1 Cartridge Loader 20mL 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, the performance and reliability may be adversely affected. If a cartridge is stored containing salt solution (e.g. PBS), it will degrade and will no longer function correctly. Cartridges can be stored containing clean, filtered water if necessary.

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 initialise.
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 states, *Instrument Ready*.

3.2 Highway buffer recommendation

The recommended cell sorting buffer is DPBS/PBS (no Calcium or Magnesium) + 0.1% Poloxamer 188+

24 units/mL benzonase and 0.5mM $MgCl_2$.

Tips to help with sample preparation:

- Filter medium through 0.2um sterilising filter.
- If running chilled, keep medium chilled.
- If running at room temperature, keep medium at room temperature.

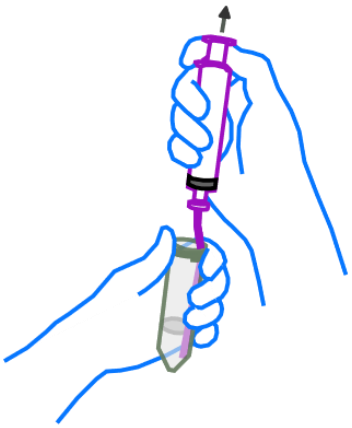
3.3 Highway1 Cartridge loading procedure

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

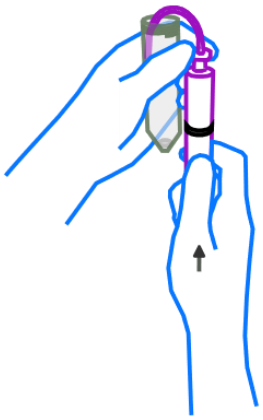
1. Unwrap Highway1 cartridge (consistent with aseptic handling techniques, e.g., in a biosafety hood).
2. Place the cartridge upright in the Highway1 Cartridge Rack.
3. Open the sample for loading (consistent with aseptic handling techniques).
4. Unwrap the Highway1 cartridge loader (consistent with aseptic handling techniques).
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.
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 around 0.5 mL of air remains in the syringe.
8. Detach the syringe from the cartridge loader.
9. Place the syringe on the cartridge luer lock and apply very gentle torque with the fingertips to seal.
   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.

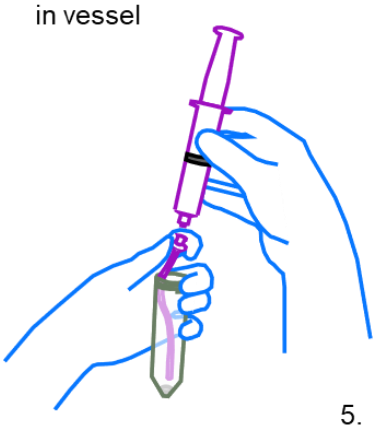
1. Load sample into cartridge loader
- Dip filler tube into vessel and aspirate by pulling syringe plunger



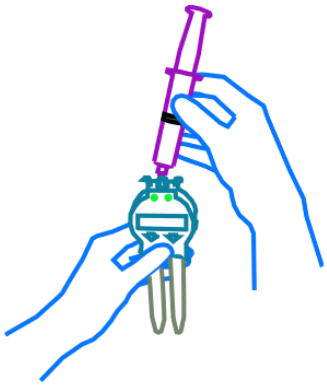
2. Expel bubble
- Invert syringe and hold vertical, while keeping filler tube in vessel
- gently expel bubble out of syringe



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

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.

Cartridges can be reused for daily QC and sort set-up. These cartridges should be washed out with water prior to running for optimal data acquisition.

3.4.2 Washout Protocol

1. Fill a 3mL syringe with water.
2. Label syringe as wash syringe and attach to set-up cartridge.
3. Insert cartridge into Highway1 and allow to align.
4. Select run wash.
5. Run wash until a minimum of 0.5 mL 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 the 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.

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

1. Vortex the Daily QC bead bottle for 10 seconds, then add 2 drops of beads to 2 mL of deionised water in a 15mL 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 3mL syringes.
3. Remove the blunt needle then attach the syringe to a Highway1 cartridge.
4. Discard needle by the appropriate sharps disposal route

3.5.1 Performing the Daily QC test.

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

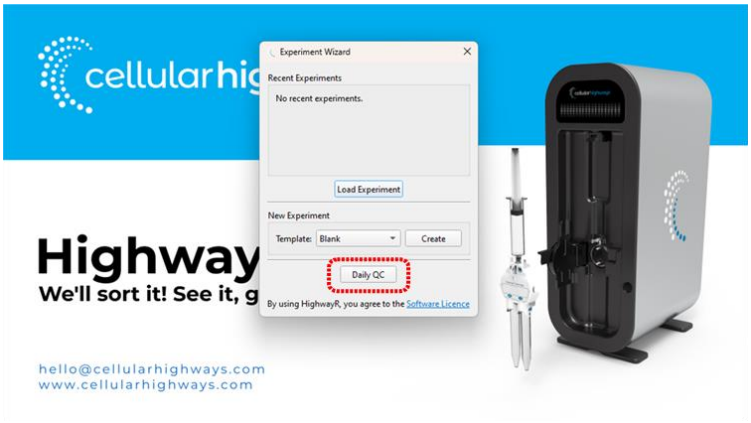


Figure 3-2 Start up window

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

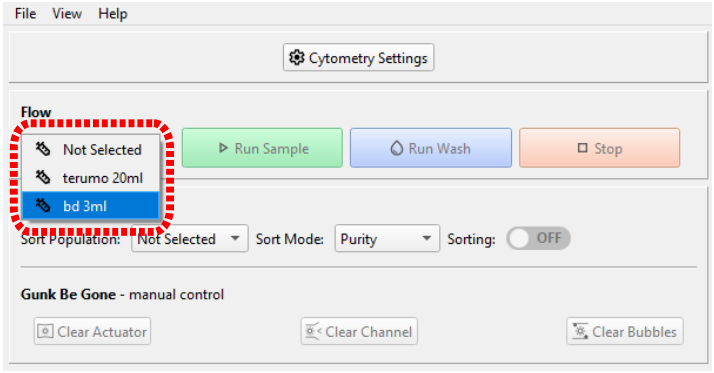


Figure 3-3 Instrument Control panel

3. Install the 3 mL adapter into Highway1.
4. Open the door and insert the prepared cartridge.
5. Close the door (ensure 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.

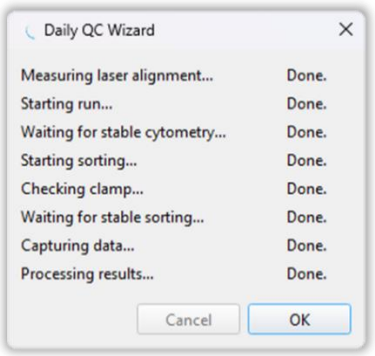


Figure 3-4 Daily QC Wizard

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.

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

4 Performing a sort

The first step to perform a sort is to prepare an experiment in the software in which the cytometry visualisation 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 stain compensation controls, fluorescence minus one 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 2mL (at 50,000/mL) of fully stained cells.
2. Adjust gains then restart recording and acquire data.
3. Run wash procedure (3.4.2), then run next sample, for example single colour control (SCC) or fluorescence minus one (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 the Highway1,

1. Load experiment,
2. Create new experiment blank experiment or from template.

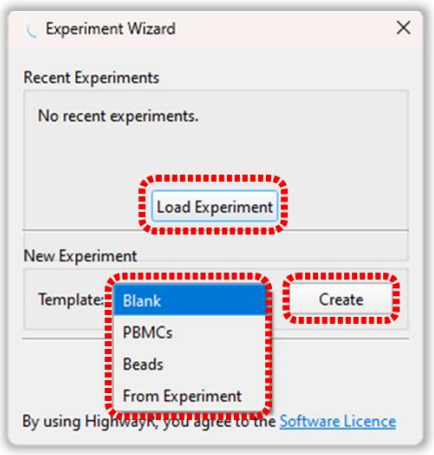


Figure 4-2 Experiment Wizard

Three template options are provided for new experiments 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 10µm 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 visualisation

To enable the user to visualise the cytometry data, the user can display event parameters (for example FSC-A) in either 1D or 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 creating a new experiment from any of the standard templates, the default plots are created.

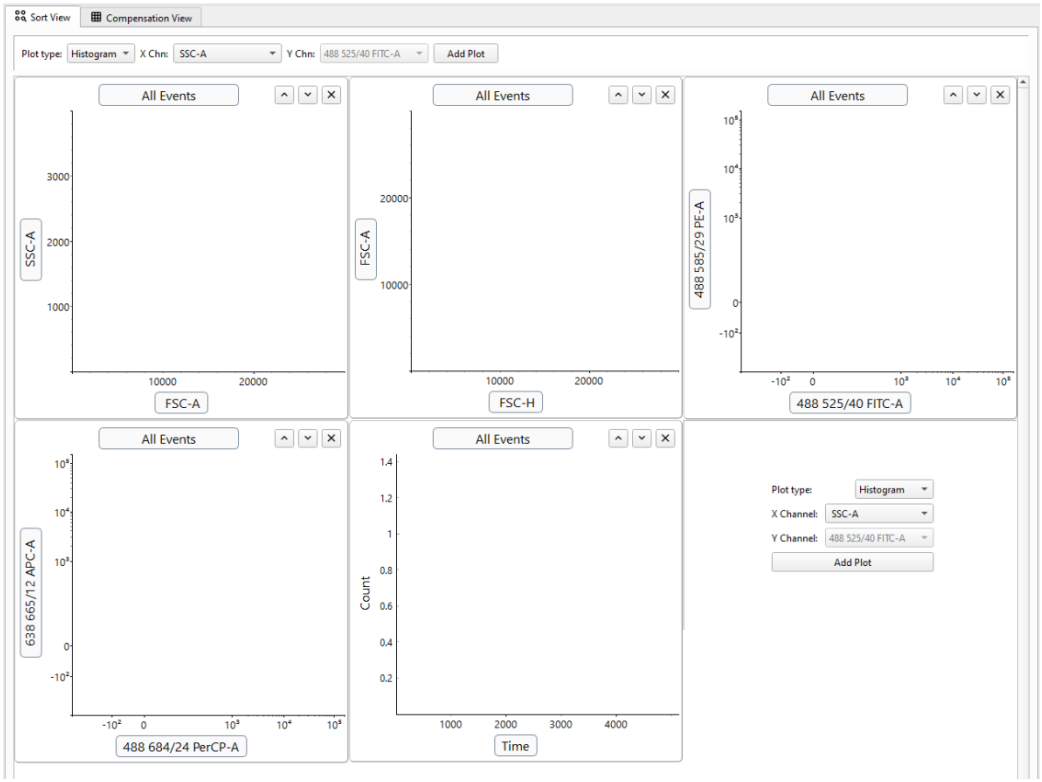


Figure 4-3 Default Plot layout and gates for PBMCs template.

The following operations can be performed to change the visualisation 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.

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

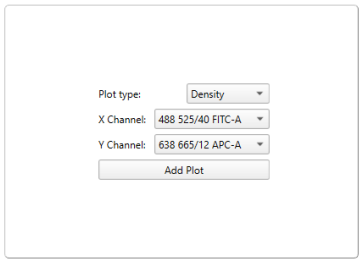
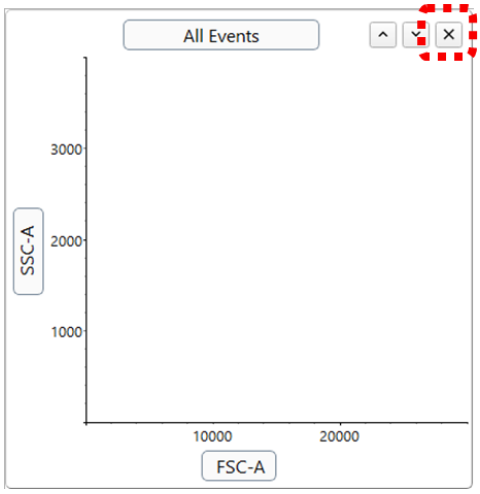


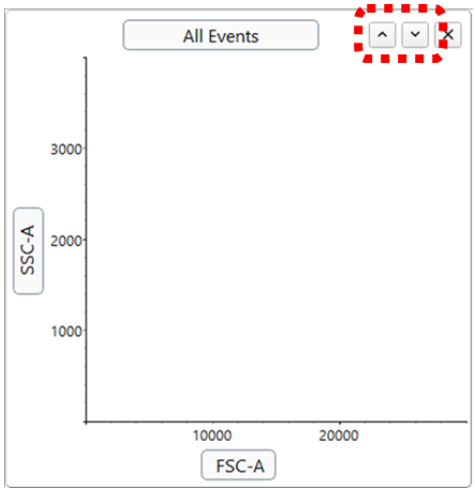
Figure 4-5 Bottom Add plot menu

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



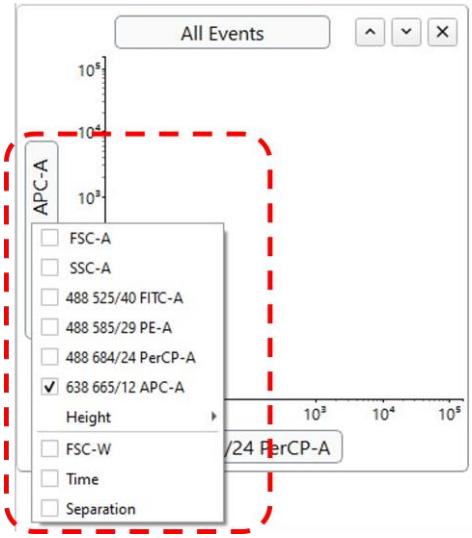
4.3.2 Graph ordering

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



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.

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.

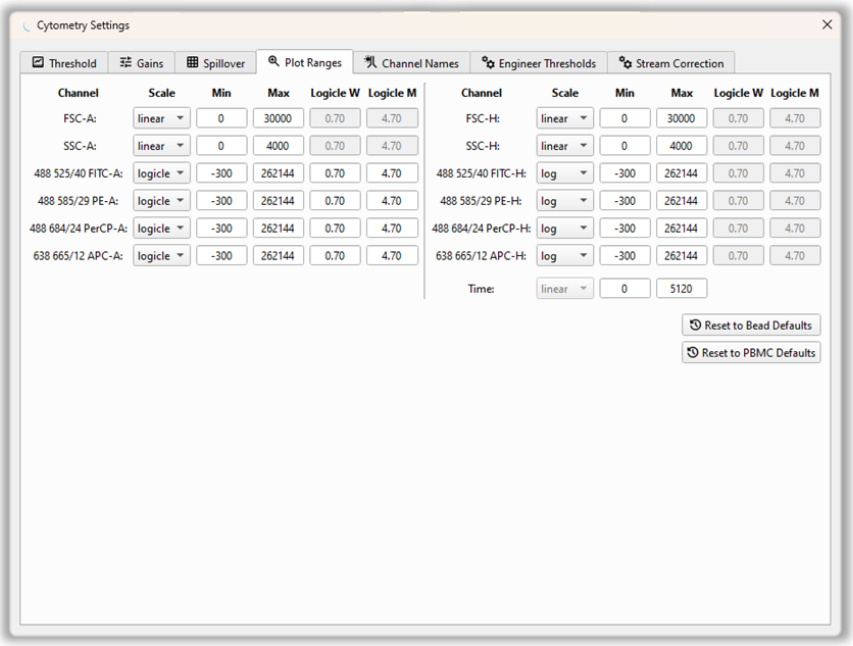
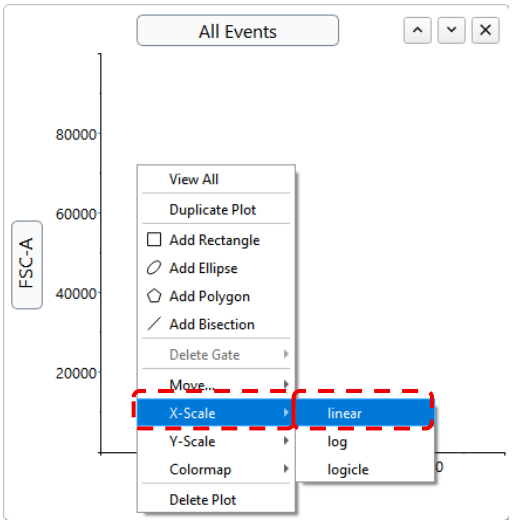


Figure 4-6 Plot Ranges window



4.3.5 Parameter labels

To change the channel names, navigate to: *Cytometry Settings* > *Channel Names.*

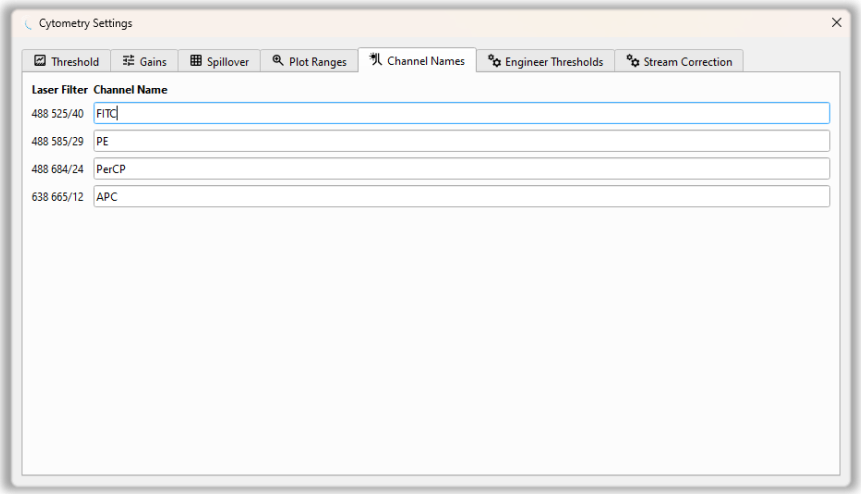


Figure 4-7 Channel Names window

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.

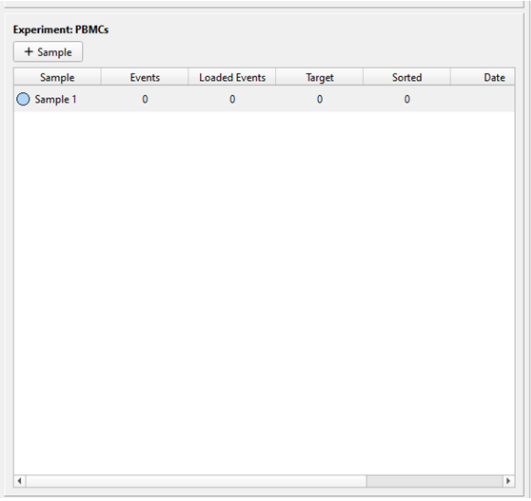


Figure 4-8 Sample view

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

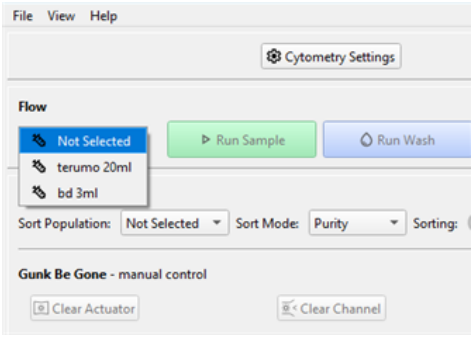


Figure 4-9 Syringe selection

- Load syringe with a small amount of fully stained sample and attach to setup cartridge.
- 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 stabilisation 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 optimised. 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 the Highway1 is sorting optimally, the stream correction plot may be checked within the cytometry settings.

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.
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.

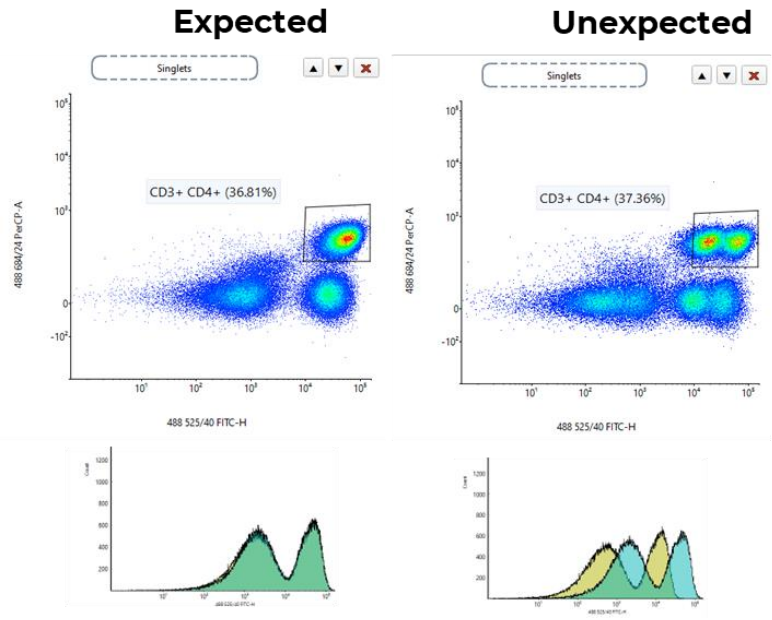


Figure 4-11 Expected vs unexpected cytometry. The unexpected is caused by incorrect stream correction

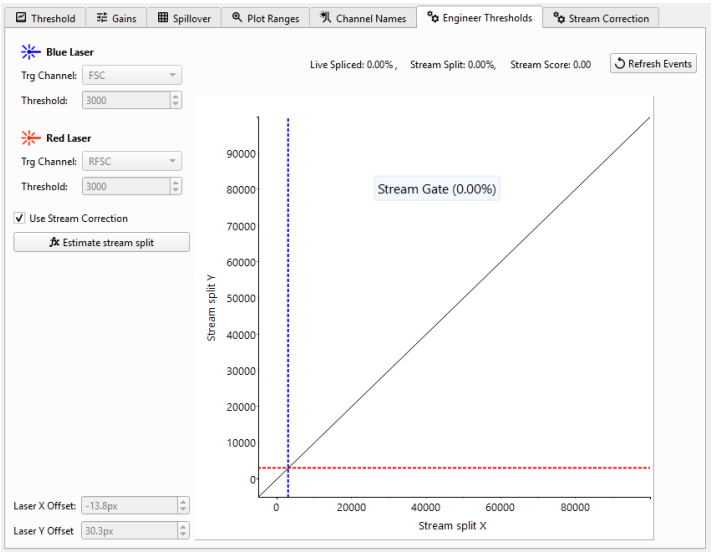


Figure 4-12 Engineering Thresholds window (Stream Gate)

4.4.3 Adjusting Forward and Side Scatter

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

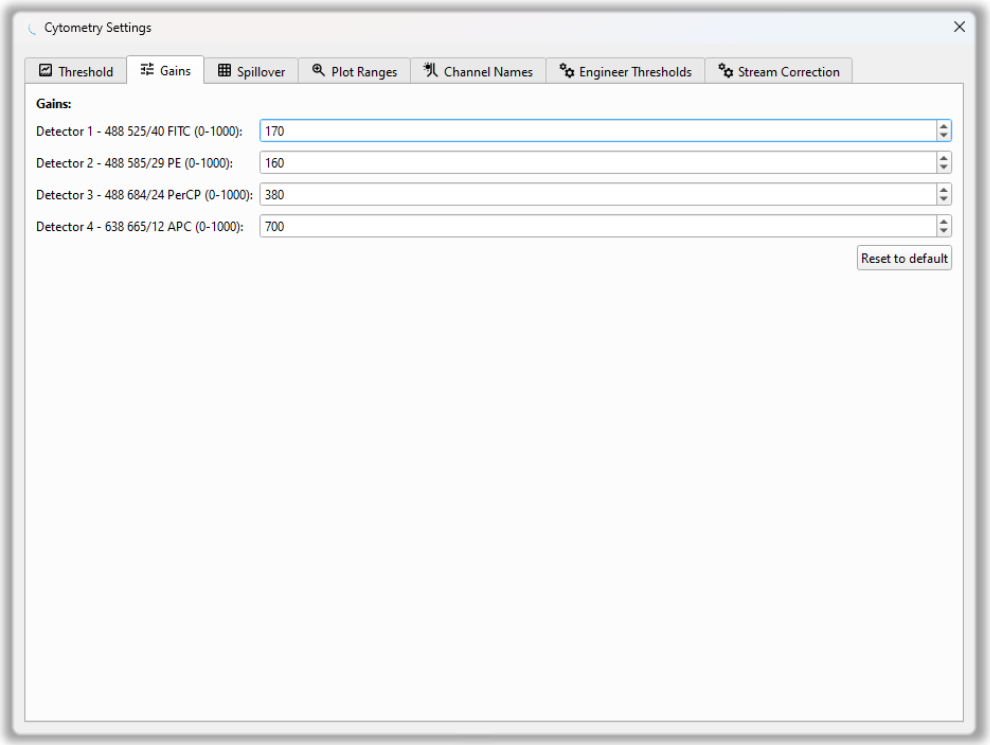


Figure 4-13 Gains window

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). Flow 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 spillover.
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.

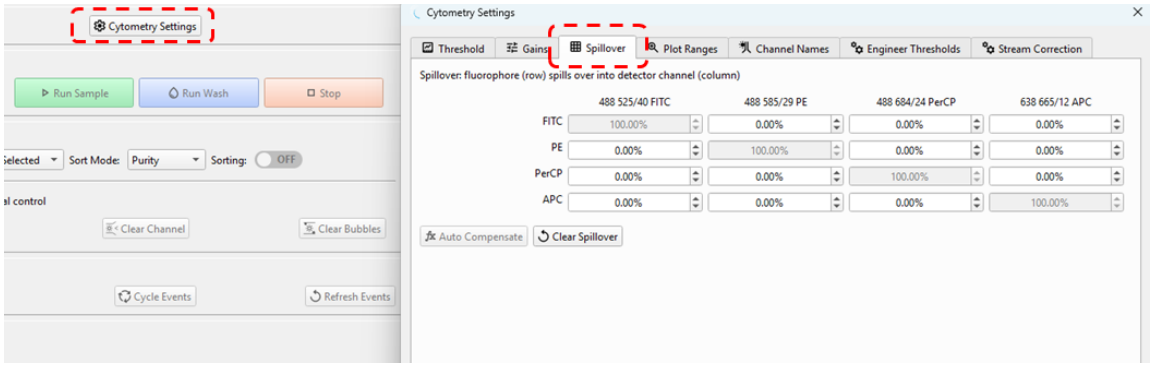


Figure 4-14 Spillover matrix

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-colour 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-colour 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.

- Compensation of the fully stained sample, by eye.
  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 centre 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.

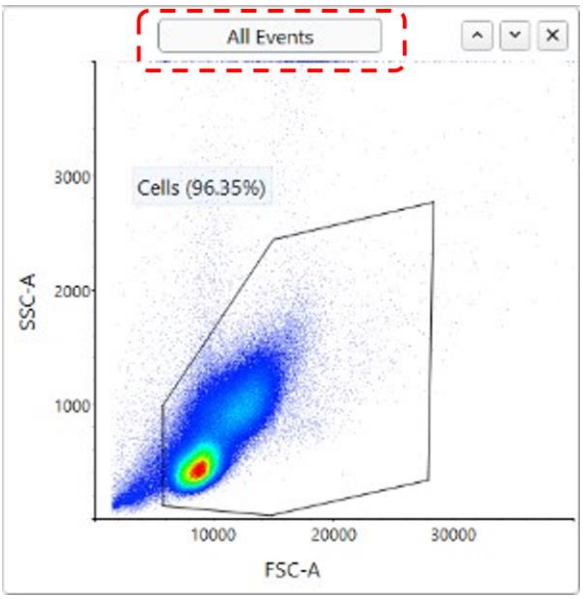


Figure 4-15 Plot Title

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.

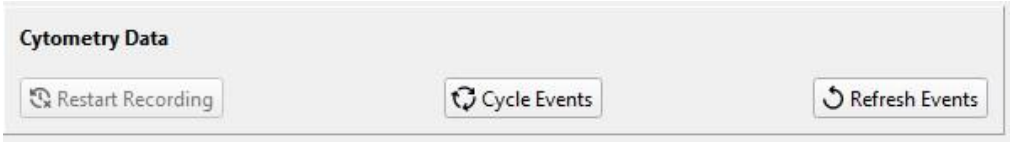


Figure 4-16 Cytometry Data Control Panel

- 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 fluorescent 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.

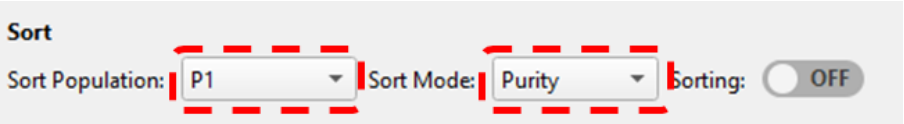


Figure 4-17 Sort Control Panel

4.5.2 Recommended sorting concentration

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

Table 1 Summary of Purity and Enrichment modes

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

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 sample.

4.5.4 Running a sort

1. Load a new cartridge with sample
2. Place in the instrument and close door.
3. (Optionally) 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 say *Sorting complete* once sorting has finished.

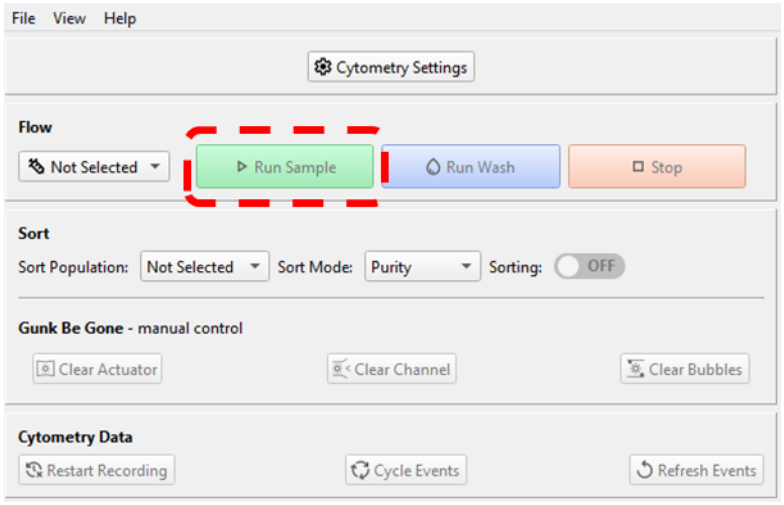


Figure 4-18 Instrument Control Panel

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 Sort1 and Sort 0 outputs.

5 Data storage

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

Data is automatically organised 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.

6 Shutdown

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

7 Instrument cleaning

Cleaning of the Highway1 external surfaces may be required to decontaminate the Highway1 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, A10000-1101
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 the specified cleanroom wipe (part number 10000-3257 supplied by Cellular Highways), apply light pressure and wipe from the top of the window to the bottom in one continuous motion, minimising the contact with the metal surround 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 minimise streaks from solvent residue).
7. If this process is not effective, then contact Cellular Highways for further advice.

8 Troubleshoot Guide

If an issue occurs, first try the following quick solutions. Otherwise, contact Cellular Highways Support (contact details below).

8.1 Startup

Issue	Solution
Instrument or computer not turning on	1. Check all cables are plugged in and switched on at the mains
Instrument not connecting to the computer	1. Check all cables are connected 2. Close the HighwayR software and open it again. 3. Restart instrument and host PC. 4. Shut down 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	1. 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 correct syringe is selected and 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 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 correct syringe is selected and corresponding adaptor is installed. (Incorrect syringe or adapter may be falsely reported as a leak.) 3. Change cartridge

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 1mL H20 or PBS. 4. Change cartridge.
No events seen	1. Are bubbles trapped in the laser pockets on the live machine vision panel? Press Clear Bubbles if any bubbles are seen 2. Run QC beads through Highway1 at recommended concentration
Outgassing of bubbles in laser pockets	1. Avoid introducing bubbles greater than 0.5mL 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 cooling blocks Note: cooling blocks can only be used with 20mL syringe
Event rate too low	1. Concentrate sample
Event rate too high	1. Dilute sample
High gunk level	1. Try to reduce sample debris and clumping by: ▪ Running samples with cooling blocks attached ▪ Filtering reagents through 0.02µm filter ▪ Filtering cells/beads prior to running. ▪ Use recommended buffer. Note: if none of these options work and there is still too much gunk, it is recommended to improve sample preparation before sorting on the Highway1. Otherwise less than optimal results will be achieved

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 1mL H20 or PBS. 5. Change cartridge.
Low efficiency	1. Ensure most appropriate sort mode 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 cells are larger than 5µm in diameter 2. Try to reduce sample debris and clumping by: a. Using recommended buffer b. Filtering reagents through 0.02µm filter to remove debris from buffers c. Filtering cells through a 30µm filter prior to sorting d. Running cells in a 20mL syringe (min volume 3mL) with the magnetic stirring disc rather than a 3mL syringe to maintain single cell suspension e. Diluting sample
Target rate greater than 4000/s	1. Dilute sample so that target rate is lower than 4000 events per second
Low yield caused by high rate of actuator fouling	1. Try to reduce actuator fouling by: a. Using recommended buffer. b. Filtering reagents through 0.02µm filter to remove debris from buffers. c. Filtering cells through a 30µm filter prior to sorting. d. Running cells in a 20mL syringe (min volume 3mL) with the magnetic stirring disc rather than a 3mL 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 1mL H20 or PBS 5. Change cartridge

8.4 Daily QC

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

9 Contact CHW Support

Email: support@cellularhighways.com, include screenshot, log file or Daily QC report showing the issue, if applicable

10 Specification and Compliance Information

10.1 Technical specification

Cytometry	*Lasers*	488nm (200mW) blue 638nm (180mW) red
	Detection	4 SiPM (fluorescence) 3 Photodiodes (FSC, SSC, RFSC)
	Filters	488 525/40 nm 488 585/29 nm 488 684/24 nm 638 665/12 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	4kHz 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 2W/ml
PC and Software	*Operating System*	MS Windows 11 on Dell host
	Monitor	27” 1440p
	Connection	Ethernet, Wi-Fi, USB ports
	Data compatibility	FSC 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

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 which 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 accessories specified by the manufacturer should be used.
- 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.

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: 250W
- Instrument rating information
  - Input voltage: 24 Vdc
  - Maximum power: 250W

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, and a certificate provided to ensure safety for all who may contact the unit.

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.

11 Safety

11.1 Intended Use

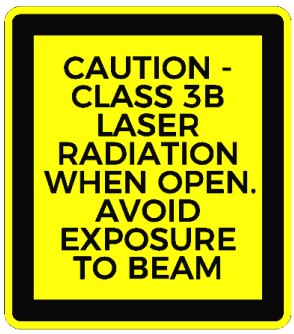
- The Highway1 Cell Sorting System is for Research Use Only. It is not sold as a medical device. Any clinical application of the cells (i.e. the use of cells in humans) is exclusively the responsibility of the user of the Highway1 Cell Sorting System. This use is not intended by Cellular Highways. Therefore,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

11.2 General safety

- Do not operate the Highway1 without training from an authorised representative of Cellular Highways.
- Do not open the casework or alter the Highway1 in any way.
- If the Highway1 is used in a manner not specified within these instructions, the protection provided by the instrument may be impaired.
- The Highway1 is fitted with a glass panel in front of the LED display. Avoid impacts and loading which may damage the glass. In the event of breakage, be aware of the potential hazard due to handling of 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.
- 488nm wavelength laser with maximum power of 200mW
- 632nm wavelength laser with maximum power of 180mW
- Do not remove casework or alter the instrument in any way.
- Only authorised 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.
- The 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 and cartridge are used according to instructions.
- The labels below have been affixed to the rear panel of the Highway1 product.



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 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 minimise the risk of sample leakage.
    - Avoid overfilling the cartridge output tubes (for example by pre-filling with additional liquid) to minimise 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.