Sony ID7000™

Acquisition and Data analysis in Standardization Mode

Version 1.0

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Part A: Startup and QC

1. Fluidics
   a. Empty the waste tank (follow your institution's guidelines for waste disposal).
   b. Fill the sheath tank.

2. Instrument power
   a. Before turning on the instrument, make sure that there is no rack or plate placed on the autosampler. If so, remove it first and then turn on the instrument. Otherwise, an error message appears.
   b. Turn on the two power switches on the left side of the ID7000.
   c. Press the power button on the front of the instrument. When the unit is turned on, the *Power* indicator lamp is lit green.
   d. Double click on the ID desktop icon to launch the ID7000 software.
      - **Note**: Two versions of the software are available, each with a different icon (and). When acquiring data using the instrument, launch the version without the "A". Use the version with the "A" when analyzing previously acquired data. The analysis version does not attempt to connect to the instrument.
   e. Log in with your username and password.

3. Priming
   At the start of each day, the software will automatically prompt the user to initiate priming. Confirm the initiation of priming by clicking *Start*.
   - If more than 8 hours have passed since the last priming, the priming wizard appears. Re-prime the instrument.
   - To perform priming at any other time, launch the *Priming* wizard by clicking on *Priming* on the *Cytometer* tab.

4. Daily QC (mandatory)
   Once priming is complete, perform daily QC. Daily QC is required for instrument operation and should be run every day. It takes approximately 12 minutes. It is performed using *Sony Align Check* beads.
   a. In the *QC* tab → *Daily QC*, click on *Start Daily QC*.
   b. Follow the instructions in the *Daily QC* wizard and click *Start*. **Ensure that the beads are well vortexed beforehand and that the bead lot number on the bead bottle matches the number you enter into the wizard.**

5. Performance QC (monthly)
   Performance QC checks the instrument's fluorescence detection performance and takes approximately 5 minutes. It is performed using *Sony 8 peak* beads. Although it is not required for instrument operation, it is recommended to run it regularly when tracking the performance of the instrument is important, such as during longitudinal studies. The recommendation is to run it once a month as a minimum.
   a. In the *QC* tab → *Performance QC*, click on *Start Performance QC*.

b. Follow the instructions in the *Performance QC* wizard. **Ensure that the beads are well vortexed beforehand and that the bead lot number on the bead bottle matches the number you enter into the wizard.**

Note: You can also perform *Daily* and *Performance QC* together by going to the *QC* tab → *Daily QC* and clicking on *Start Daily and Performance QC*.

6. Flow rate calibration (monthly)
   Flow rate calibration adjusts the fluidics pressure to achieve the optimum sheath fluid flow rate. While not mandatory, we recommend it be run once a month for best results. It is performed using *Sony Align Check* beads.
   Note: When time is used as the stop condition for data acquisition, flow rate calibration is mandatory.
   a. Ensure that no rack or plate is loaded.
   b. Launch the *Flow Rate Calibration* wizard by clicking on *Flow Rate* on the *Cytometer* tab.
   c. Follow the instructions in the *Flow Rate Calibration* wizard.

7. Optics alignment (weekly)
   Optics alignment adjusts the laser excitation position for the flow cell. Perform this procedure on a weekly basis. The software will automatically prompt you to run it directly after priming if more than 7 days have elapsed since the last optical alignment. It is performed using *Sony Align Check* beads.
   a. Ensure that no rack or plate is loaded.
   b. Proceed with the alignment procedure when prompted. **Note:** If it has been longer than a week since the instrument was last used, it is recommended to cancel the automatic *Optics Alignment* prompt and perform a *Flow Cell Purge* and *Decontamination → Bleach Cleaning and Rinse* from the *Cytometer* tab, in that order, before proceeding to *Optics Alignment* as described below.
   c. If the automatic prompt is cancelled, or to perform the alignment at any other time, launch the *Optics Alignment* wizard by clicking on *Optics Alignment* on the *Cytometer* tab.
   d. Follow the instructions in the *Optics Alignment* wizard.

Warning! *The Optics Alignment* wizard and *Daily QC* wizard windows look almost identical and use the same beads; however, they are two very different processes. *Optics Alignment* is prompted automatically and performed as described above, whereas *Daily QC* does not appear automatically and must be triggered by users every day the machine is in use. Always verify which wizard is about to run by checking its name. *Optics Alignment* is **not** the same as *Daily QC* and even when *Optics Alignment* is performed, *Daily QC* must still be completed afterward.

Note: *Flow Rate Calibration*, *Optics Alignment* and *Daily QC* are performed using *Sony Align Check* beads. To perform all procedures together, prepare double the usual amount of beads (i.e. add 4 drops of beads to 900 µl DI water or PBS) and proceed in that order (1. *Flow Rate Calibration*, 2. *Optics Alignment* and 3. *Daily QC*).

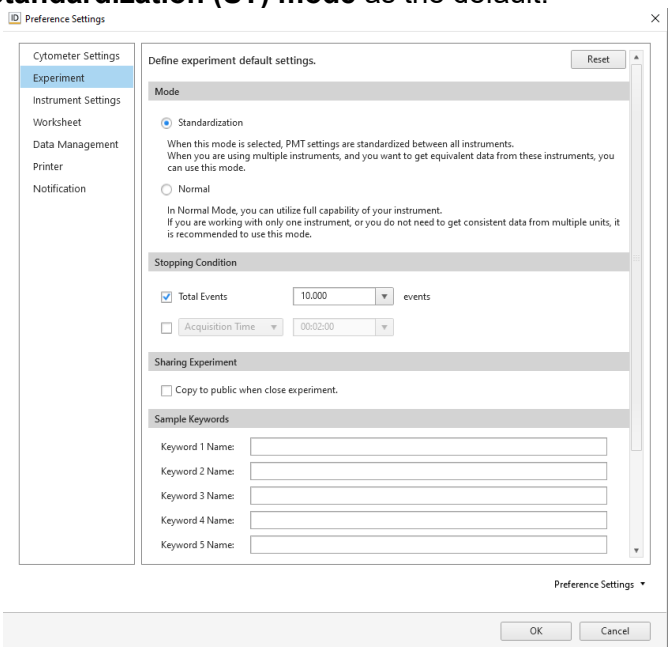
Part B: Users and User Preferences

In *Preference Settings*, each user can set up default settings based on their preferences.

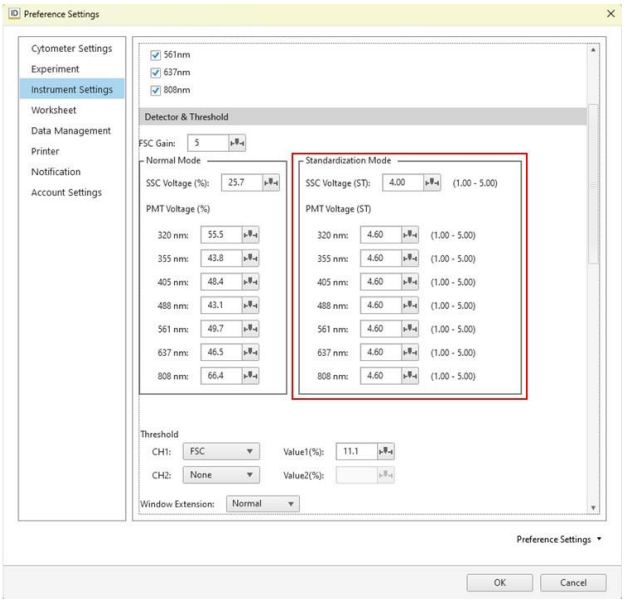
1. Click on the *File* menu at the top left of the screen.
2. In the *Information* tab click on *Preference Settings*.
3. Click on each category in the list to customize the settings.

The following are recommended settings:

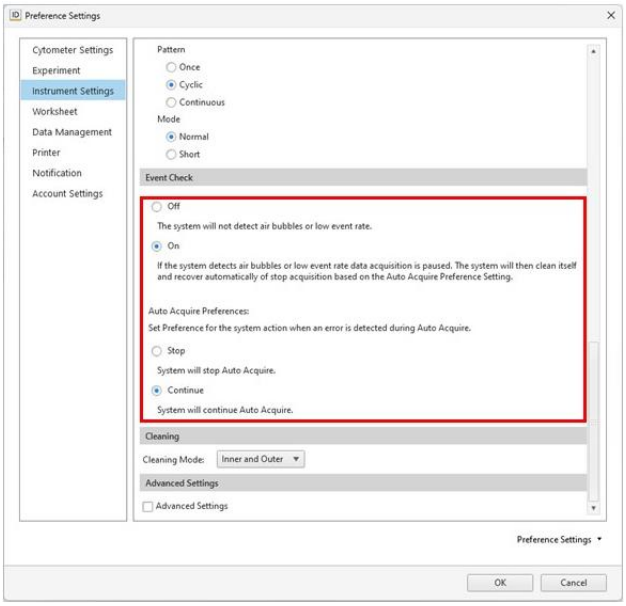
- Experiment Settings
  a. Select the **Standardization (ST) mode** as the default.



- Instrument Settings
  a. Tick all the lasers of your ID7000 system. **Note:** You must still ensure that all lasers are ticked at the start of each experiment to have them ON during measurement.
  b. In the *Detector & Threshold* settings in *Standardization* mode, set the *SSC Voltage* value to 4 and the *PMT Voltage* values to 4.6 in each of the detector arrays.
  This PMT Voltage value was internally tested at Sony Biotechnology to accommodate commonly used fluorochromes stained on compensation beads in spectral flow cytometry within the measurement window (MFI below 10^6). For single stained controls on cell, the optimal values may vary. Please carefully adjust them according to the species and starting material.



c. Activate *Event Check* and set *Auto Acquire Preferences* to *Continue* to take full advantage of automation using the ID7000. **Note:** For acquisition of extracellular vesicles or small particles, it is recommended to have *Event Check Off*.



d. Fine-tune the rest of the settings to your preferences. To save and finish, click on the *OK* button at the bottom.

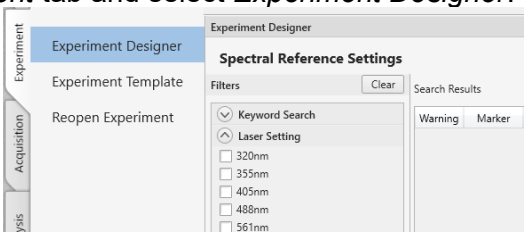
4. This step is optional but recommended for the settings above to ensure consistency between users.
   - Click the downward arrow beside *Preference Settings* at the bottom of the *Preference Settings* window. *Export* the settings to *Desktop*.
   - To import the settings into another account to share the same preference settings, click the downward arrow beside *Preference Settings* at the bottom of the *Preference Settings* window, and select *Import*. Then choose the exported settings file from the *Desktop*.

Part C: Data Acquisition

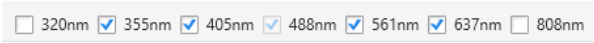
Standardization mode:
Always ensure the *Standardization* mode is selected as the default mode under *Experiment* in *Preference Settings*, as described above. In *Standardization* mode, the output of each channel is standardized so that the SSC and fluorescence detection sensitivities are the same over time within one instrument as well as between multiple instruments. When the standardized (ST) settings are the same in the software on multiple instruments, the sensitivity of the instruments is roughly aligned.

In practice, because the balance between all laser channels is maintained in *Standardization* mode, the controls and samples do not need to be acquired on the same PMT gain settings. Reference controls generated using PMT voltage settings A can be used to unmix fully stained samples recorded at PMT voltage settings B.

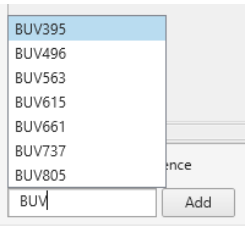
1. Starting a new experiment from scratch
   1.1. Single colour controls
      1.1.1. Create experiment
      a. Go to the *Experiment* tab and select *Experiment Designer*.



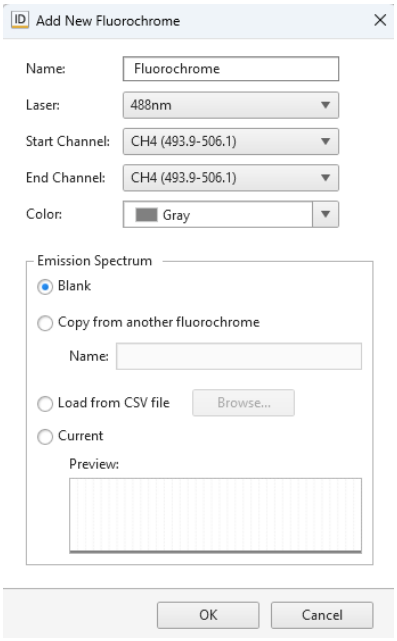
      b. Check the boxes at the bottom of the screen to ensure that all lasers of your ID7000 are ON.



      c. Add your fluorochromes to the experiment. Fluorochromes should be added in the same order as they are loaded in the sample rack or plate, but they can be rearranged on the next screen if needed. In the box at the bottom of the screen, enter the first few letters of the fluorochrome you want to add, then select it from the list. Click the *Add* button to add it. Repeat this process for each fluorochrome.



         - **Note**: If your fluorochrome is not present in the database, type the name of the fluorochrome, click *Add*, and then enter the fluorochrome information when prompted.
            - [Name]: Enter the name of the fluorochrome
            - [Laser]: Select the excitation laser from the drop-down menu.
            - [Start Channel] / [End Channel]: Select the range of valid detectors

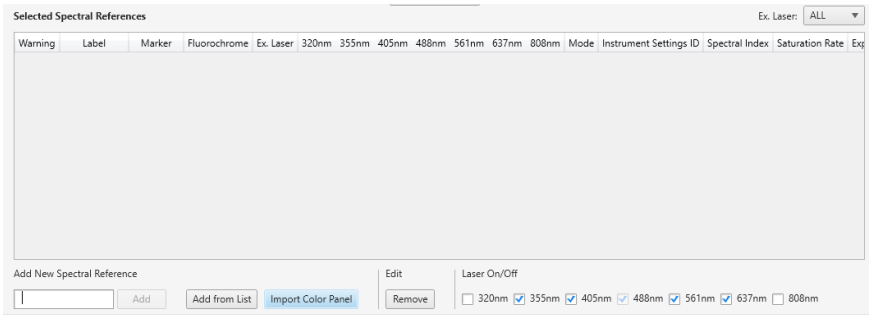


d. Add marker names in the *Marker* field next to each fluorochrome name. Type the first few letters of the marker name you want to add, then double-click on it from the list. If it is not on the list, type the entire marker name and press [Enter] to add it.

e. (Optional) Add a *Label* (panel name, fixation methods etc) to each fluorochrome. This makes it easier to search the spectral references once they are saved to the *Library* for future reuse (Label example: 25c T cell panel – only the 25 fluorochromes with this label will appear when searching the library for that label name). A label can also be added later, when saving spectral references to the *Library* (see section 1.1.4).

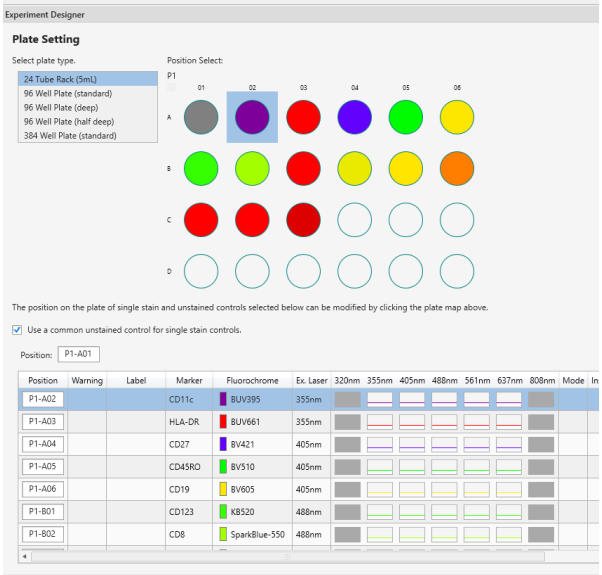
<u>How to import colour panels from CSV files (alternative method for adding colours to an experiment):</u>

- First export a panel from a previously run experiment to ensure that the spreadsheet is prepared in a compatible format.
  - In a previous experiment, go to the *Unmixing Settings* window, click on the downward arrow beside *Color Panel Settings*, and click *Export*. This will give you a template to build your panel on.
- Use this as a template to input your antibody and fluorochrome information, adding new rows as necessary. Use the list of the fluorochrome names at the bottom of the sheet as a reference.
  Note: Enter the fluorochromes in the spreadsheet in the exact order you want them to appear on the plate layout to minimize the need for rearrangement in step g below.
- In the *Experiment Designer*, instead of performing the procedures described in 1.1.1 c-d, click *Import Color Panel*, select a file and click *Import* to import your colour panel.



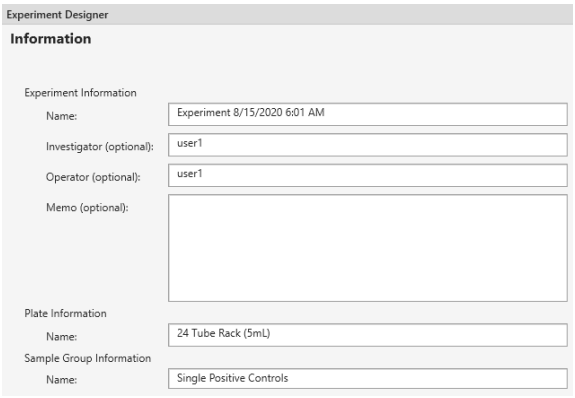
f. When done, click on the *Next* button (lower right corner of the screen).

g. Select the plate type (24-tube rack, 96/384-well plates) you are using. The *Experiment Designer* automatically places a common unstained control in position A01. Uncheck the box *"Use a common unstained control for single stain controls"* (lower left) if you do not have a separate unstained control. The single colour controls are arranged in the same order in which you added them to the experiment, from position A02 onwards. To change the order, type new positions into the boxes under *Position*. To move the unstained control, type a new position into the box. When done, click the *Next* button (lower right corner).

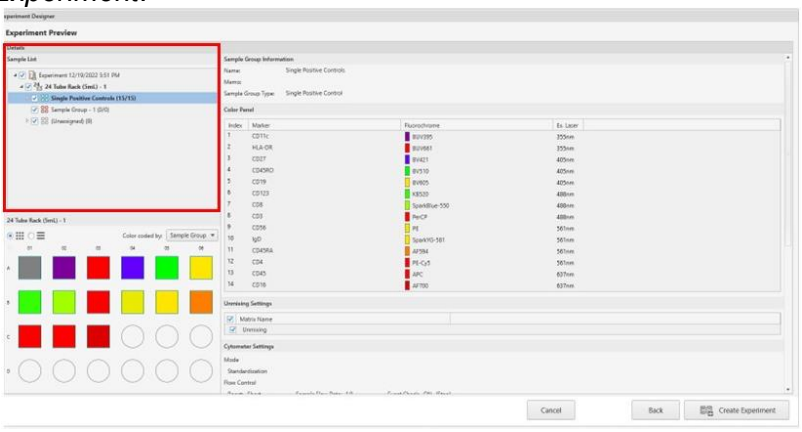


h. Modify the default *Acquisition Settings* as needed. *Acquisition Offset Time* of 3-5 seconds recommended. Other settings such as *Low Dead Volume* mode and *Agitation* may be modified as appropriate.

i. Change the default experiment *Name* and enter other information as needed.



j. Review the experiment details. Go back if you need to make changes. Otherwise, make sure all groups are selected in the *Sample List* and click on *Create Experiment*.



1.1.2. Preview and acquire unstained control and single stain controls

a. After placing the tube rack or the plates in the auto-sampler stage, go to the *Status* panel (upper left part of the screen) and click on the *Load* button.

b. Go to the rack/plate map (lower left). Ensure that the current position (black arrow) is on the desired sample. To change the current position, right click on the new well and click *Set Current Position* in the quick pick menu.

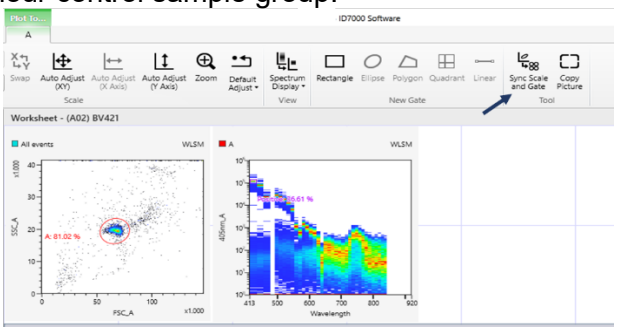
c. Go to the *Status* panel (upper left) and click the *Instrument* button to open the *Instrument Settings*. Ensure that the PMT voltages for all detector arrays are set to the value of 4.6. **Note**: This PMT gain is recommended for compensation beads used for single colour controls; if other materials (e.g. cells) are used for controls, adjust the gain as needed.

d. Select the well or tube with your unstained control, or one of the other controls if you do not have an unstained, and preview it by clicking on the *Preview* button.

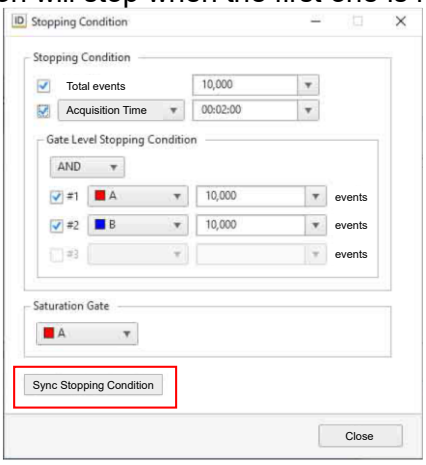
Note: *Preview* allows event visualization only. No events are recorded.

e. Adjust the axes scaling as needed so the bead population is clearly visible within the plot (see section 1.2 step e for instructions on axes scaling). Click the pre-drawn *Gate A* and adjust its size and shape by dragging the drawing handles to gate the main population of the unstained control (e.g. the main bead population). Recommended beads for minimal autofluorescence are *UltraComp eBeads Spectral Unmixing* beads from *Thermo Fisher Scientific* or *SpectraComp* beads from *Slingshot Biosciences*.

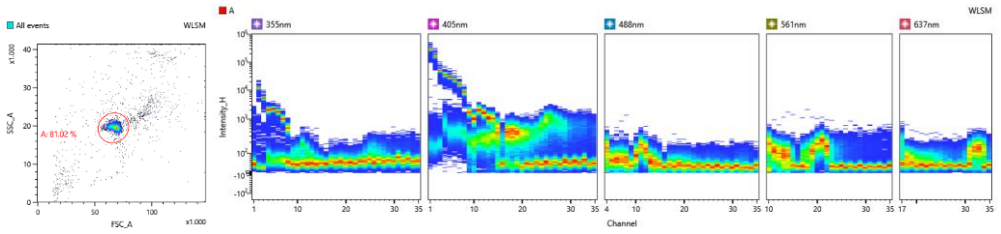
f. Click on the whole plot and then *Sync Scale and Gate* in the ribbon above to apply the scale and gate settings defined on your bead population across the entire single colour control sample group.



g. Next to the *Instrument* button, click on the *Stopping Condition* button. Set *Gate A* as your *Saturation Gate*, and define a *Stopping Condition* (e.g. total events, or gated events, or acquisition time) for your single colour controls. Click *Sync Stopping Condition* to apply these settings across the entire single colour control sample group. **Note:** If you select more than one type of stopping condition, the acquisition will stop when the first one is reached.



h. Preview your brightest fluorochromes (e.g., BV421, PE, BB515, PE-Dazzle 594) and ensure that they are not going off-scale (example below for BV421: in the spectral ribbon plot, no events are above the detection limit of 10^6). Monitor the *Saturation Rate* on the *Status* panel during preview and ensure it never exceeds 2-3%.



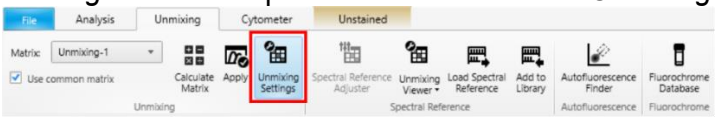
i. You are now set to record all your single colour controls. Click on the *Auto Acquire* button  to initiate acquisition.

j. Once auto-acquisition is complete, refine the positive (and if relevant, negative) gates for each fluorochrome and obtain clean spectra for each single colour control and if present, for the unstained control.

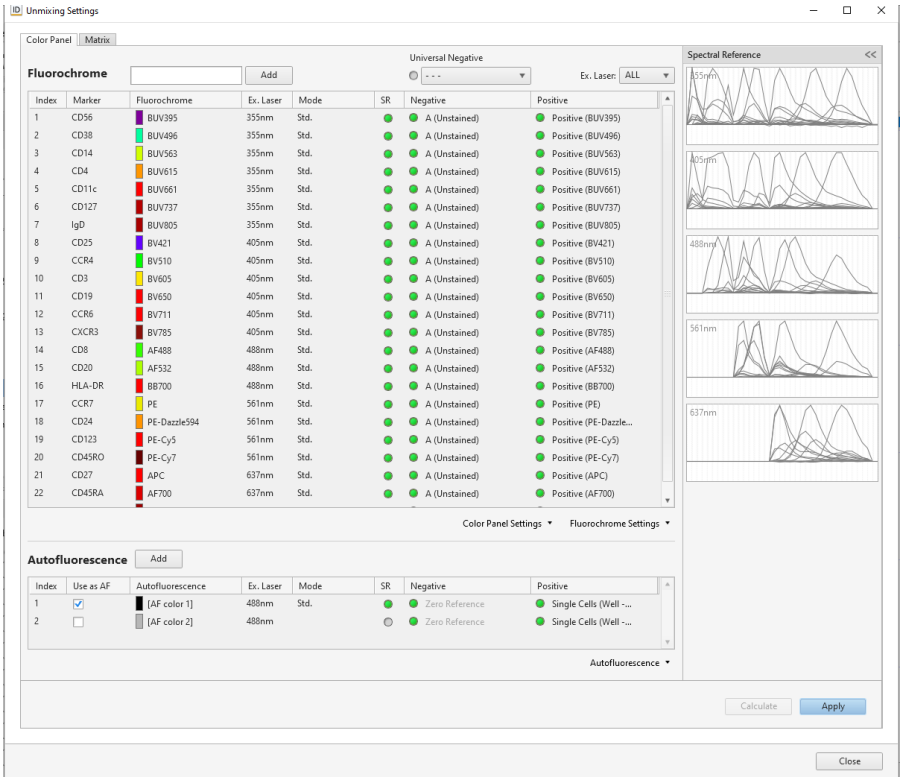
- Note: If you are using a common unstained control, the negative gate is to be created within the unstained control (gate A on the FSC/SSC plot is by default the negative gate), while if you are using internal negatives (unstained beads within each single colour control), the positive and negative gates should be adjusted within each single colour control.

1.1.3. Calculate unmixing and obtain spectral references

a. Go to the *Unmixing* tab at the top of the screen and click *Unmixing Settings*.

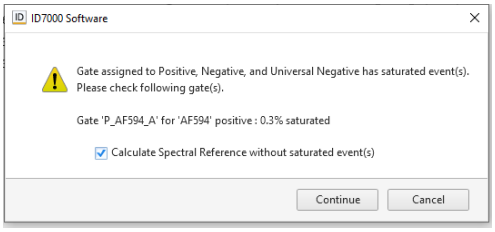


The *Unmixing Settings* window can also be launched by clicking on the wrench icon at the top right corner of the software screen.

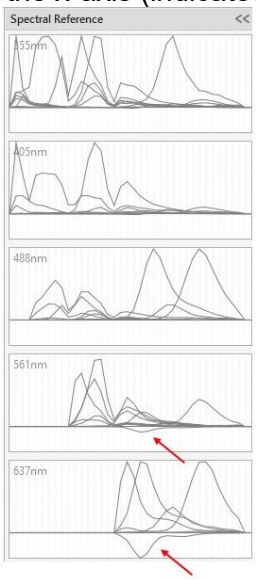


b. Ensure that all positive and negative populations for each fluorochrome are correctly selected. If any are incorrect or missing, click the well where the relevant control was recorded and select the correct gate for the positive or negative population from the dropdown menu within the *Unmixing Settings* window. Confirm that all gates for all fluorochromes are correctly assigned.

c. Press *Calculate* at the bottom of the *Unmixing Settings* window. If you see a warning about saturated events, always tick the box "*Calculate Spectral Reference without saturated event(s)*".



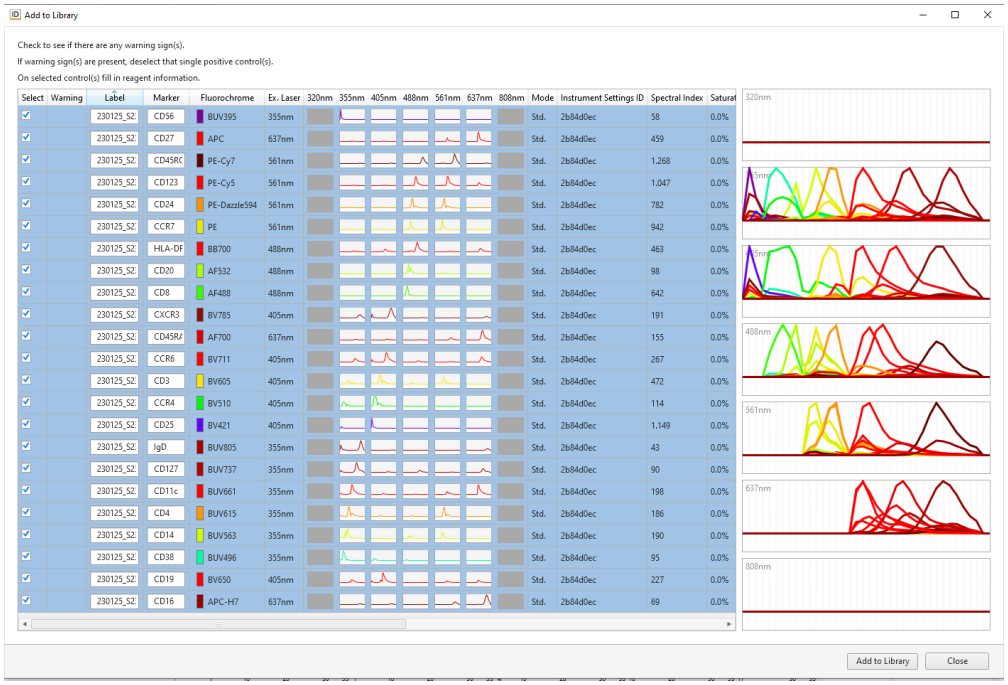
d. Click *Apply* when prompted to apply these settings to other sample groups.
e. You have now generated a spectral reference (SR) for each one of your fluorochromes.
   - If a warning sign appears in the *SR* field of the *Unmixing Settings* window, hover over it to see a pop-up describing the error.
   - If it states the SR was calculated using saturated events, press *Calculate* again and ensure the box is ticked as described above.
   - If the error states the positive control is too dim, go back to the relevant single colour control and adjust the positive gate by dragging it to the desired position to select the brightest events possible on the spectral ribbon plot. Press *Calculate* in the *Unmixing Settings* window once again. The warning sign on the *SR* column should disappear if the discrimination between positive and negative signals has improved. If the warning sign persists, re-record the control by staining with an increased amount of the antibody, or if this is not possible, try increasing the PMT voltage settings for that control.
   - Ensure that none of the calculated spectral references dip below the x-axis. The example below illustrates an incorrect spectral reference, where part of the spectrum dips below the x-axis (indicated by arrows).



1.1.4. Add the spectral references to the library

a. Click on the downward arrow beside the *Fluorochrome Settings* (bottom right in the *Unmixing Settings* window).
b. Click *Add to Library* to open the *Add to Library* tool, or click the *Add to Library* button within the *Unmixing* tab.

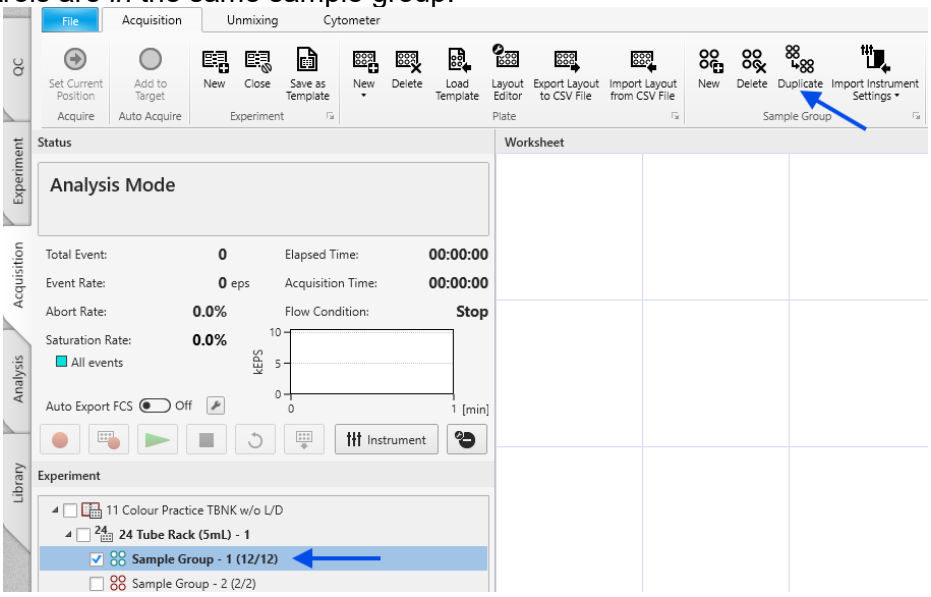
c. If you want to add an identification label at this stage, select all fluorochromes by clicking any fluorochrome and pressing [Ctrl] + [A].



d. Enter a unique label to identify this set of reference spectra into the *Label* field, then press [Enter] to apply the label text to all selected fluorochromes.
e. Click the *Add to Library* button (lower right) to finish.
   - Note: If a warning sign appears for any fluorochrome, ensure that the positive and negative gates are correctly assigned and resolve the issue before adding the reference control files to the *Library*.

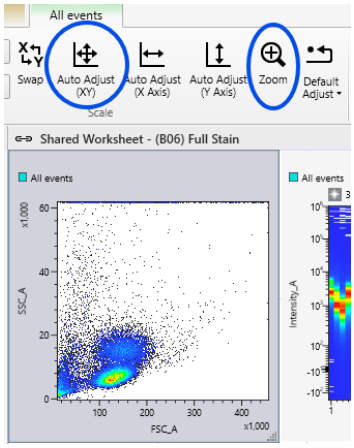
1.2. Unstained and fully stained samples – One Max or All Max method

a. Go to the rack/plate map. Select all sample wells to be acquired (click the wells while holding [Ctrl] on the keyboard), then right-click the selection, navigate to *Move to Sample Group*, and select the desired sample group if it already exists. If you wish to create a new sample group, click an existing sample group and then select *Duplicate* from the *Acquisition* tab at the top of the screen. In either case, ensure that the fully stained samples and their corresponding unstained controls are in the same sample group.



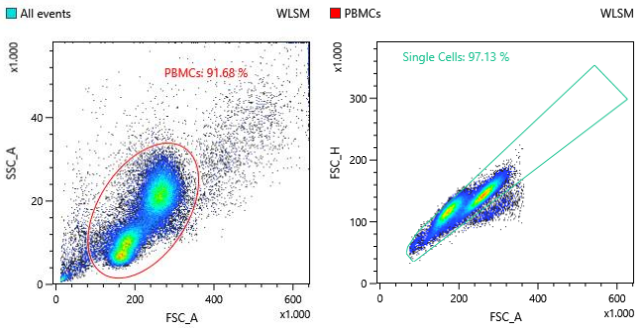
Sample groups:
Sample groups can be considered as separate entities within a single experiment. All samples within the same sample group share the same instrument settings and unmixing settings. This means that you can auto-acquire samples using different voltage and other instrument settings, or analyze samples stained with different colour panels, by placing them into separate sample groups within the same experiment.

b. Go to the *Instrument Settings* window and click the *Detector & Threshold* tab. Leave this window open while performing the following steps.
c. Right-click on the well to be previewed and click *Set Current Position* in the quick pick menu.
d. Go to the *Status* panel and click on the *Preview* button  .
e. Once events begin to appear, click the FSC/SSC plot, then click the *Auto Adjust (XY)* button on the top ribbon to automatically adjust the scaling to your sample. Adjust the scaling further as needed using the *Zoom* function and/or by dragging the axes manually.

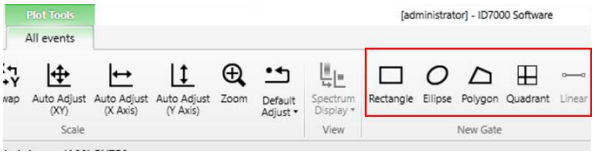


- Zoom: Click the plot to which you want to apply zoom. Click the *Zoom* button shown above to activate the zoom function, then drag the mouse pointer around the area of the plot you want to enlarge.
- Rescaling axes: There are two axis cursor types. Place the mouse cursor over the axes and drag it when it changes to one of the scaling cursors below.
  - ⟷ is used for expansion/compression of decades around 0.
  - ⊣⊢ is used for adjustment of axes minimum/maximum values.

f. If necessary, adjust the FSC gain and SSC voltage settings on the *Detector & Threshold* tab to properly position the cell population on the plot. **Note**: The FSC and SSC on the ID7000 have a wide dynamic range, so in most cases the cells can be visualized properly by scaling and zooming, without the need to alter the FSC gain or SSC voltages.
g. Click on the *Restart* button  as needed to refresh the data.
h. Draw *Gate A* for your cell population (example: PBMC gate below), then double-click on the gate to open a subplot and draw *Gate B* for your singlets (e.g., on a FSC-H vs FSC-A plot as shown below).

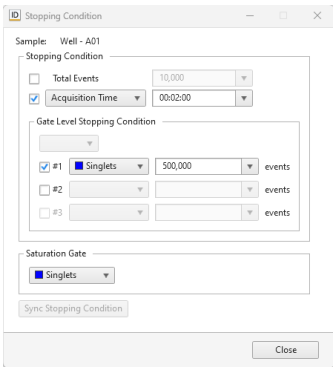


- To draw gates, click on a plot to which you want to add a gate. The *Plot Tools* will appear in the menu bar at the top.

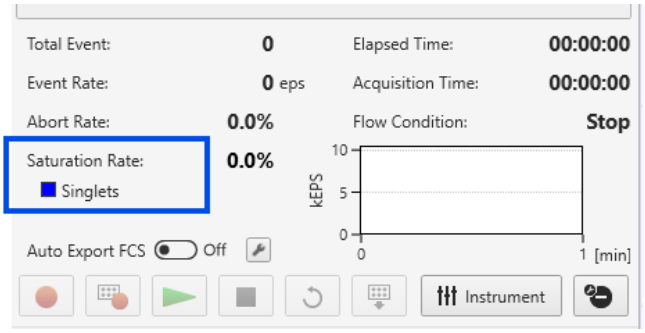


- Select the desired gate type from the toolbar. Draw the gates as follows based on the selected gate type:
  - [Rectangle] and [Ellipse] gates: Click and drag the mouse to define the shape and size of the gate.
  - [Polygon] gate: Click each vertex sequentially to outline the gate. Double-click to close and finalize the shape.
  - [Quadrant] gate: Click once to set the centre point of the quadrant.
  - [Linear] gate: This gate is used for histograms. Click at the lower boundary of the gate and drag the mouse to the upper boundary to define the range.

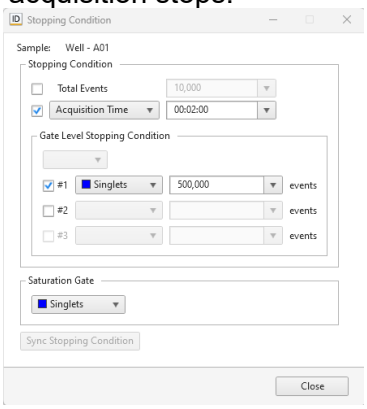
i. Open the *Stopping Condition* window and define the *Singlets* gate as your *Saturation Gate*:



- This will allow you to monitor the percentage of saturating singlet events (those above the detection limit in the spectral ribbon plot) by observing the *Saturation Rate* during preview.
- As a rule of thumb, keep the *Saturation Rate* value on singlets to ≤ 3% when performing PMT voltage adjustments. **Recommendation:** Be more conservative and maintain the saturation value ≤ 1%, as 3% saturation may cause highly expressed activation markers to go off-scale, resulting in loss of accurate intensity information for important events.



j. Additionally, select *Stopping Condition* and specify the time or number of events to be collected before data acquisition stops.



- **Note:** If you select more than one type of stopping condition, the acquisition will stop when the first one is reached. However, you can select multiple gate level stopping conditions and link them with either AND or OR from the drop-down menu. When AND is used, acquisition stops when the specified event numbers for all selected gates are satisfied, while when OR is used, acquisition stops when the specified event number for any one gate is met.

k. Now that your population of interest is defined and stopping conditions are set, it is time to perform the adjustment of the PMT voltage settings according to the method you want to employ in your study. Refer to sections 1.2.1 and 1.2.2 to adjust PMT voltage settings using One Max or All Max methods respectively, and return to the next step in this section when you are happy with the settings.

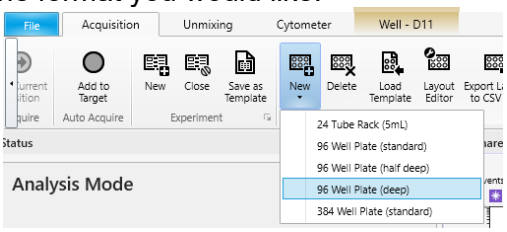
- **One Max** – if the final sample volume is low or cell numbers are very limited, One Max is the ideal method as voltage adjustments are quick to achieve and still give robust fluorescence intensity signals across your spectral panel.
- **All Max** – if the number of cells is not limiting, All Max allows you to maximize detection of fluorescence intensity for each individual detection deck and may give you even better resolution than One Max.

Both methods were internally tested at Sony Biotechnology to provide a robust and easy-to-use workflow in Standardization mode, ensuring optimal separation of cell populations and maximum output in fluorescence intensity. You are encouraged to determine which of the two methods works best for your specific applications, spectral panel or sample type (e.g., some tissues are more autofluorescent which can lead to more data spread in the UV/violet detector arrays). For more information on operation of the ID7000 in Standardization mode and the One Max / All Max methods, please consult the Sony ID7000 training slides provided by your local Field Application Scientist or reach out to your FAS directly.

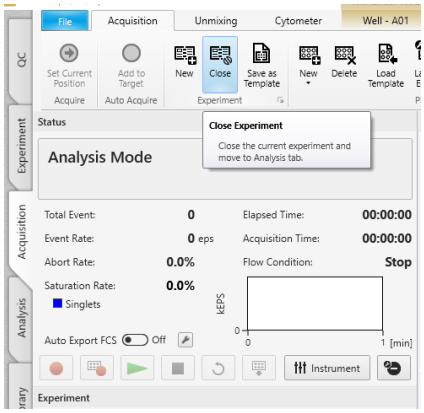
l. Once the PMT voltages are adjusted either by One Max or All Max method, you are now set to record your unstained and fully stained samples. Right-click on all wells to be acquired and select *Add to Auto Acquisition Target* from the quick pick

menu. The wells are now filled in with the corresponding sample group colour. Click on the *Auto Acquire* button  to initiate acquisition.

- If you need to add another plate/tube rack to the experiment, go to *New* within the *Plate* subsection of the *Acquisition* tab at the top and add a plate/tube rack in the format you would like.



m. Once acquisition is completed, close the experiment as shown below and it will be automatically moved to the *Analysis* tab in the software. For the data analysis workflow, proceed to Part D. **Note:** Analysis can be performed even while the experiment remains in the *Acquisition* tab of the software. The main advantage of closing it is that it allows you to analyze data while another experiment is open or acquiring in the *Acquisition* tab.



1.2.1. One Max method

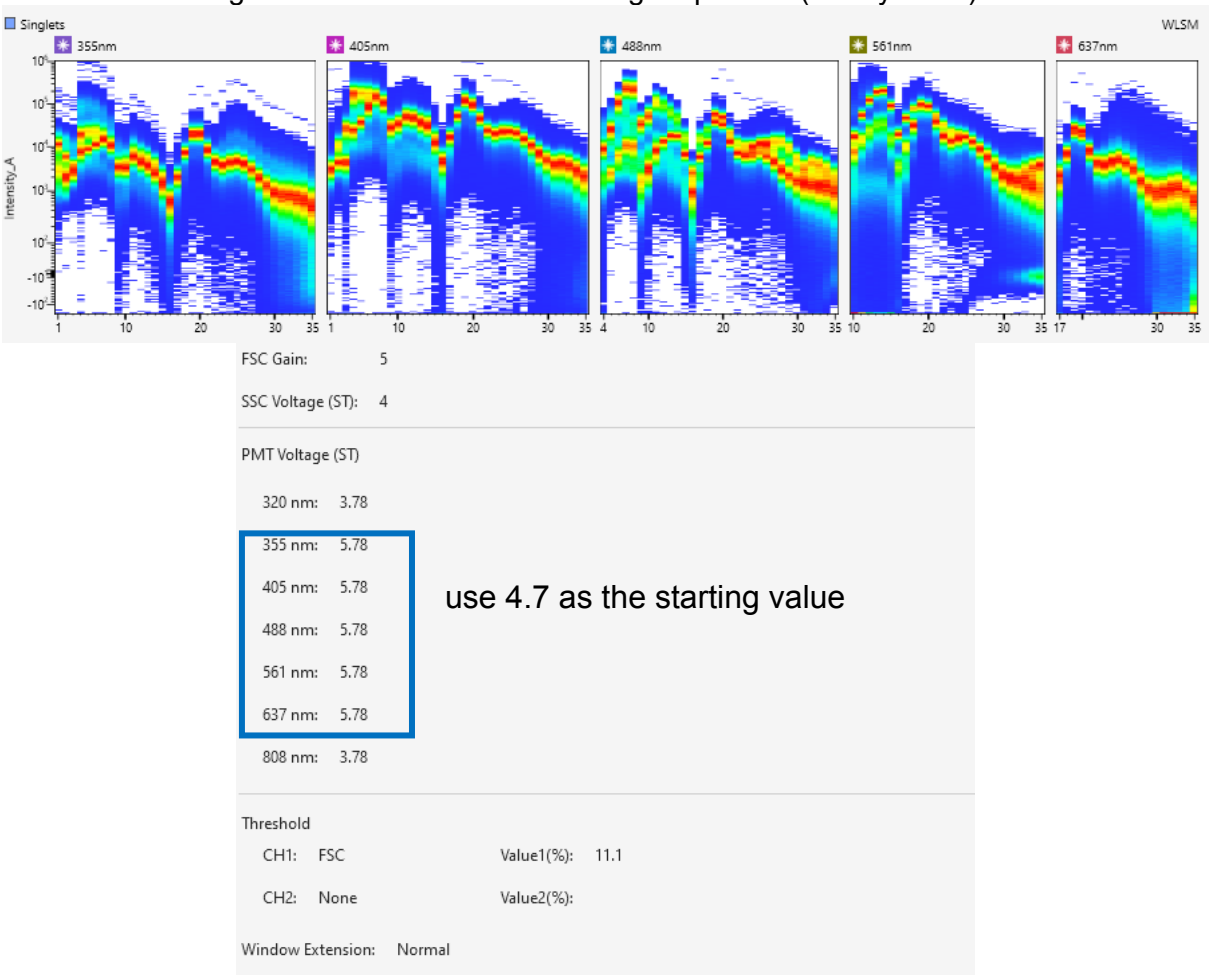
Goal: increase the PMT voltages across all decks in a synchronous way until the detection deck with the brightest signal reaches saturation, between 10^5 - 10^6 in MFI. Use the spectral ribbon plot and the *Saturation Rate* % to guide you while setting up the PMT voltages.

How to achieve One Max:

1) While previewing the fully stained sample with the *Instrument Settings Detector & Threshold* tab open, increase the PMT voltages from 4.6 (used for single colour controls) using the *Synchronous Voltage Adjustment* buttons until the brightest signal in your sample reaches the target range in MFI (between 10^5 - 10^6).
2) **Acquire both the fully stained and the unstained cell sample, which will be used to extract autofluorescence during analysis, using the same settings.**

- **Note:** A 1.00-unit change in ST mode corresponds to a full log increase in signal. Therefore, avoid making multiple 1.00-unit voltage adjustments using *Synchronous Voltage Adjustment*. The 0.10- and 0.01-unit steps are generally more appropriate.
- In the example below from a 5-laser ID7000, PMT voltages were increased to 5.78 using *Synchronous Voltage Adjustment*, as the brightest signal in this sample, found in the 561 nm detection deck, reached near saturation. One deck was therefore at its maximum (One Max), and all other detection decks were

consequently left at the same PMT voltage of 4.7. The saturation rate at the singlets level was below 3% during acquisition (ideally ≤ 1%).



1.2.2. All Max method

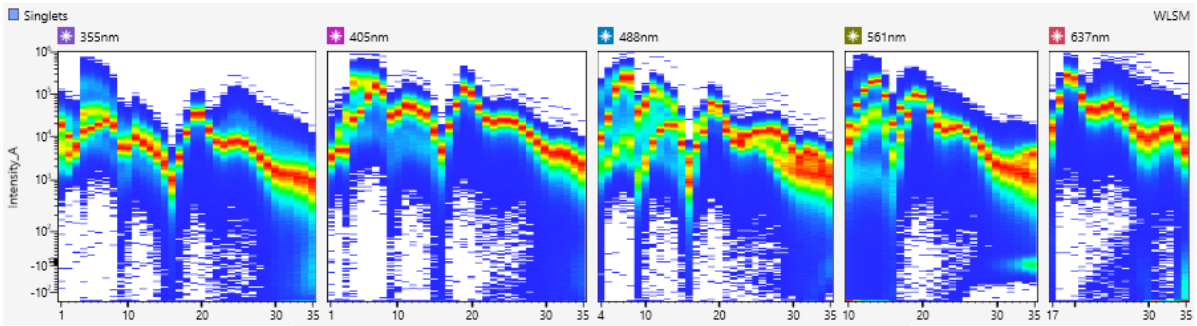
Goal: maximize the PMT voltages across all detection decks, meaning the brightest signal for each detection deck will be between 10^5 - 10^6 in MFI. Use the spectral ribbon plot and the *Saturation Rate* % to guide you while setting up the PMT voltages.

How to achieve All Max:

1) Perform the One Max method first (see previous section).
2) Once the One Max PMT settings are reached, leave the deck nearing saturation unchanged and perform individual PMT adjustments on the remaining detection decks by entering the desired voltage values manually and refreshing the data to visualize the spectral ribbon.

- Ensure that the brightest signal for each detection deck is between 10^5 - 10^6 in MFI.
- As described before, ensure that you have the saturation rate at the singlets level below 3% during preview of your sample (ideally ≤ 1%).
- Once you have settled on the maximized voltage settings across all decks (All Max), **acquire both the fully stained and the unstained cell sample, which will be used to extract autofluorescence during analysis, using the same settings.**

- In the same example as before, after increasing the ST PMT voltages to 4.7 by synchronous adjustment, the 561 nm deck was left at this value while further voltage adjustments were performed individually in the remaining detection decks:



FSC Gain: 5

SSC Voltage (ST): 4

PMT Voltage (ST)

320 nm: 3.78

355 nm: 6

405 nm: 5.8

488 nm: 5.8

561 nm: 5.78

637 nm: 6.86

808 nm: 3.78

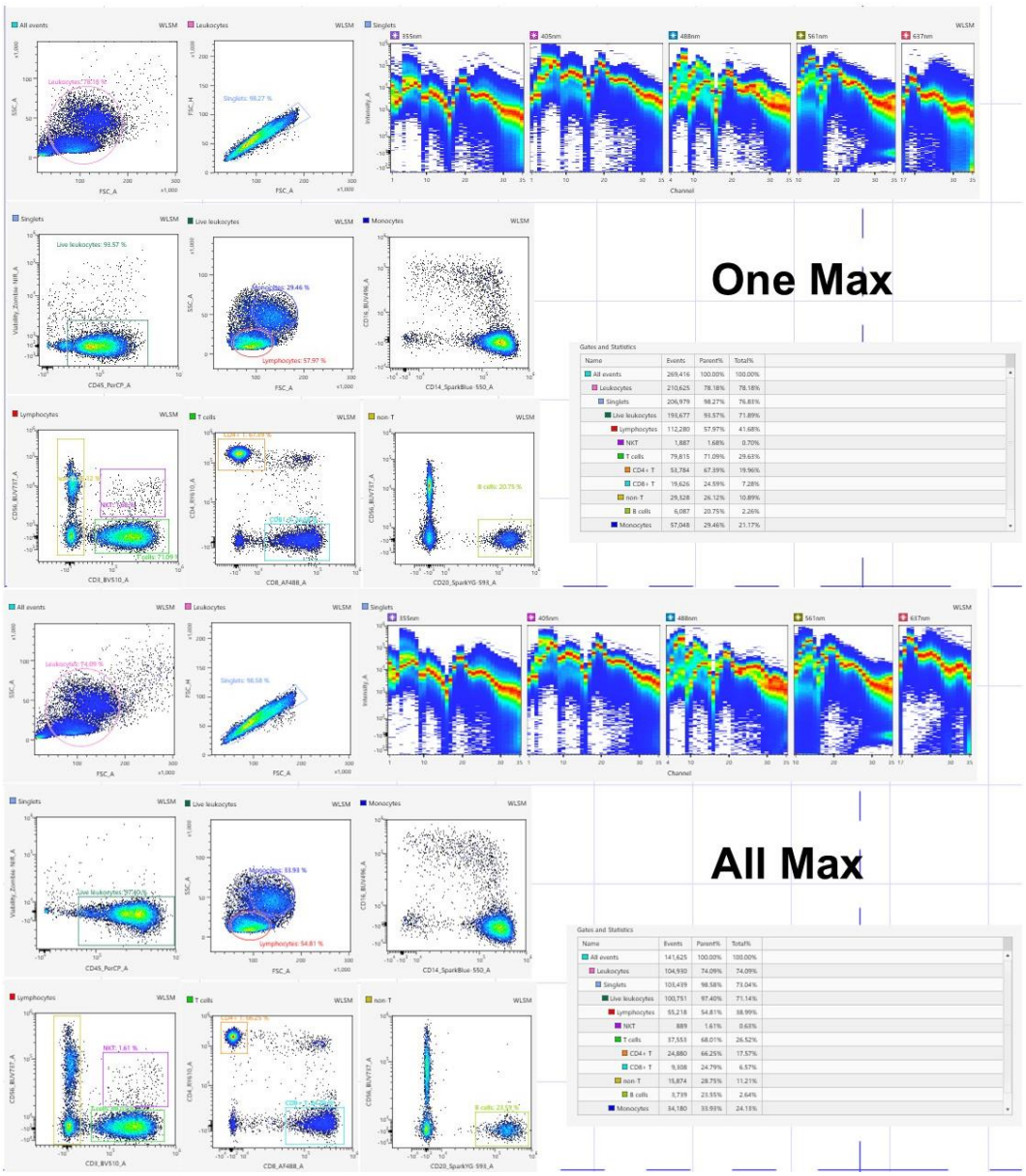
Threshold

CH1: FSC Value1(%): 11.1

CH2: None Value2(%):

Window Extension: Normal

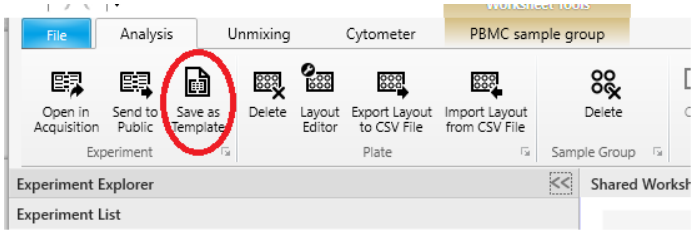
In the next figure the same sample, stained with a 9-colour human PBMC panel, was acquired using the One Max and All Max methods. The All Max method provided better separation of cell populations after unmixing, with minimal increase in data spread. Nevertheless, as previously noted and depending on sample volume/cell numbers, you should test both methods on your specific spectral panels and biological samples, as each will have its own optimal settings.



1.2.3. Saving experiments as template

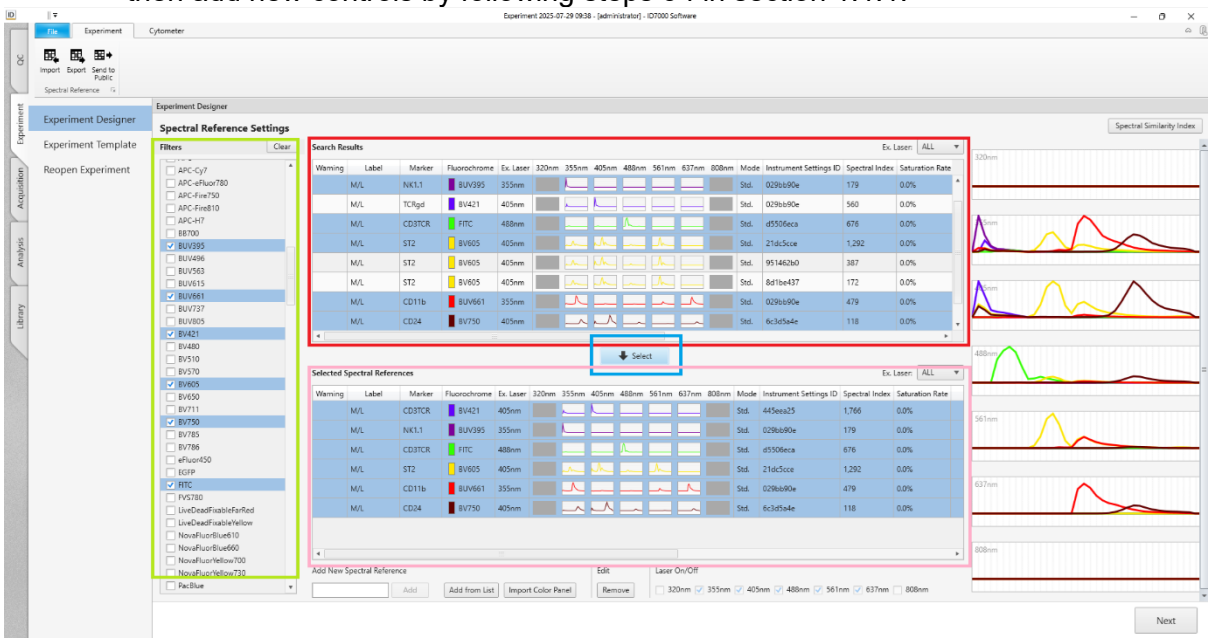
If you wish to run the same experiment using the same settings in the future, it is possible to save the experiment as template. A template will preserve unmixing settings, spectral references, instrument settings, stopping conditions and plate layout – everything except actual data.

- Select the experiments you want to save as template in *Experiment List*. Click the *Save as Template* button within the *Acquisition/Analysis* tab at the top of the screen.



- See Section 3 below on how to use templates to start a new experiment.
- Experiment templates can be saved as private or public templates to share with other users.

2. Starting a new experiment using spectral references saved in the *Library*
Instead of freshly recorded reference controls, previously recorded spectral references saved to the *Library* can be used to unmix newly acquired fully stained samples.
   a. Follow steps a-b of section 1.1.1 to start creating an experiment.
   b. Select previously recorded spectral reference controls from *Search Results* window (red box in the picture below). Add them to the panel by clicking on *Select* button (blue box). The controls to be used for the experiment are now added to the *Selected Spectral References* window (pink box). Click *Next*.
      - To refine your spectral reference search, use search filters in the *Filters* window to the left (green box). You can filter controls based on *Label*, *Laser Setting*, *Fluorochrome*, *Marker* etc.
      - If you wish to have a combination of previously acquired spectral references and freshly run controls in the experiment, simply perform the procedure above and then add new controls by following steps c-f in section 1.1.1.



Note: It is not recommended to use spectral references from the *Library* for tandem dyes as they degrade and may result in unmixing errors. For this reason, fresh reference controls for tandem dyes should be recorded regularly. If you want to reuse reference controls for tandem dyes, note the date of acquisition and how the controls were treated to determine if reuse is appropriate. It is not recommended to use spectral references with warnings (e.g., spectral index below 5 or acquired with saturated events).

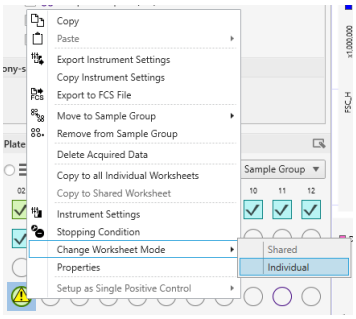
   c. Follow steps g-j in section 1.1.1 to finish creating an experiment. Proceed to sample acquisition as described in section 1.2.

3. Starting a new experiment from a template
Samples can be acquired using previously saved templates. Section 1.2.3 describes how to save an experiment as a template. In addition, all previously run experiments in the *Analysis* tab are available to be used as templates at any time.
   a. In the *Experiment* tab, select *Experiment Template*.
   b. In the *My Templates* or *Public Templates* section, select a previously saved template or a previously run experiment from *My Experiments*. Modify the experiment name in the *Experiment Information* window on the right-hand side, and click *Create Experiment* at the bottom.
   c. Proceed to sample acquisition as described in section 1.2.

4. Reopening a previously run experiment
   You can continue acquiring data in previously run experiments. This method preserves previously acquired data and all of the settings, and does not create a new experiment. The drawback is that, over time, if a large amount of data continues to be added to the same experiment, the resulting file may become very large.
   a. In the *Experiment* tab, select *Reopen Experiment*.
   b. Under your account, select the experiment in which you want to continue acquiring data. If desired, modify the experiment name in the *Experiment Information* window on the right-hand side, then click *Reopen in Acquisition* at the bottom.
   c. Proceed to sample acquisition as described in section 1.2.

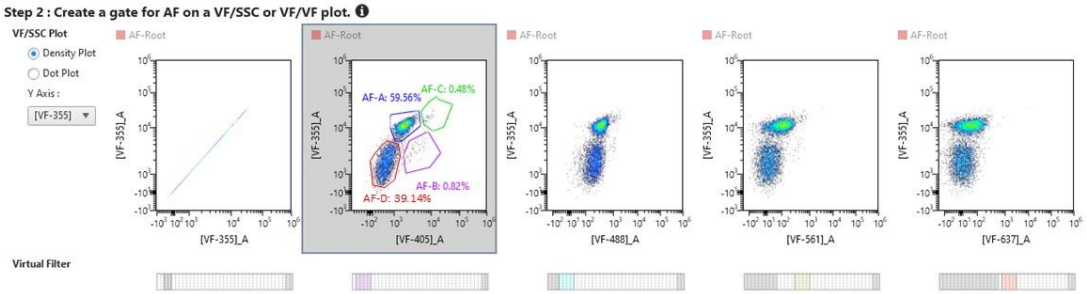
Part D: Data analysis

1. Autofluorescence extraction
   a. In the rack/plate map, select the tubes or wells containing unstained controls. Right-click the selection, navigate to *Change Worksheet Mode*, and select *Individual* to switch the worksheet to an individual worksheet.
      - This ensures that the plots and gates created for fully stained samples are independent from those for unstained controls.



> **Worksheet mode**:
> There are two types of worksheets: shared and individual. Wells with shared worksheets are depicted as circles, while those with individual worksheets are square-shaped. Wells with a shared worksheet within any one sample group share the same plots, gating strategy and stopping conditions. Those with individual worksheets have plots, gating strategies and stopping conditions independent of any other wells.

   b. Select an unstained sample well and click on the *Autofluorescence Finder* tool in the *Unmixing* tab at the top.
      - **Note:** Unmixing needs to be turned OFF to be able to open the *Autofluorescence Finder* tool.
   c. Follow the autofluorescence finder wizard to identify autofluorescent populations in your samples.
      Step 1: In the *Display Event* box on the right, select 50,000 or 100,000 events.
      Step 2: On the top left-hand plot, create a gate around the populations you will be analyzing. Refer to section 1.2 step h for instructions on how to draw gates. You can only create one gate called *AF-Root* gate here, so make sure to include all the cells of interest in your sample. Create the gate similar to your gating strategy in the stained samples (e.g., for T cell panels, create the gate around the lymphocytes)
      Step 3: In the middle row of plots, identify all cell populations that can be distinguished as individual populations and create a gate around each one as shown below.



      - These plots show the readout of fluorescence intensity from each laser deck. Use the drop-down menu on the left to set the Y axis to the laser that is most likely to induce autofluorescence, such as the 320 nm, 355 nm and 405 nm lasers. This

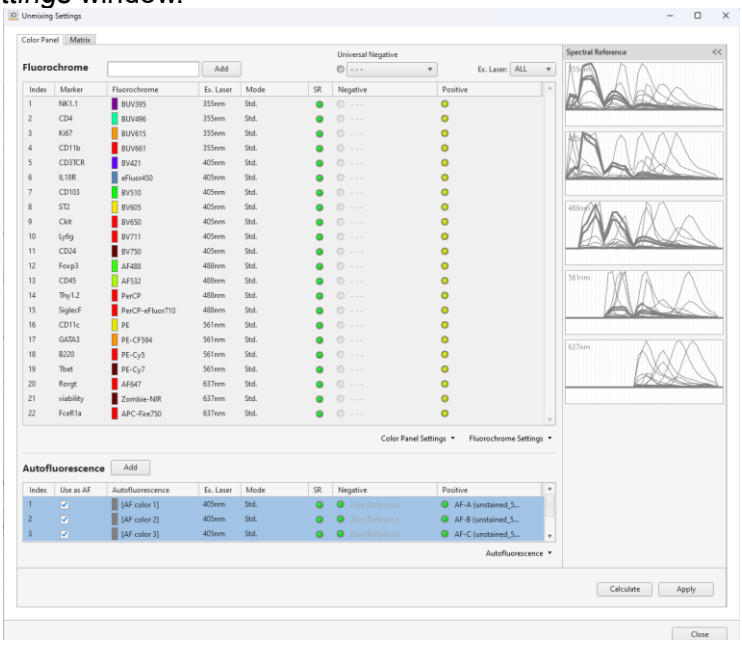
should allow you to identify all individual populations present in the sample. Ensure that you capture as many distinct populations as possible. You can identify up to 8 AF populations at any one time.

- Virtual Filter (VF) is a visualization tool that selects a defined range of detectors, enabling us to view signals from a specific part of the detector array, analogous to applying an optical filter in conventional flow cytometry.
- At the bottom of each plot is a VF scroll bar, which indicates which part of the detector array is currently displayed. Scroll the VF left and right on each laser deck to view different detectors and rescale the axes as needed to achieve optimal separation of individual populations. Refer to section 1.2 step e for instructions on rescaling the axes.
- Once all gates are drawn, switch to *Dot Plot* visualization on the left. This assigns different colours to each gated population. Review all plots and change the Y axis to each laser deck in turn to ensure that no populations were missed.
- **Note**: In many cases the best separation of different AF populations can be achieved in the VF-405/VF-355 nm dot plot. However, the AF patterns can vary. Therefore, carefully check that no AF populations were missed, as described above.

d. Exit the wizard by clicking *OK*. If you see a warning about saturated events, check the box "*Calculate Spectral Reference without saturated event(s)*".

e. In the unstained sample worksheet, prepare a few subplots of the AF-Root gate and visualize parameters with high autofluorescence background on the x- and y- axes of each.
   - Fluorochromes excited by shorter wavelength lasers (320 nm, 355 nm, 405 nm, 488 nm) and emitting at <600 nm, as well as PE, are commonly affected by autofluorescence; therefore, plot all fluorochromes in your panel that meet these criteria.

f. Turn ON unmixing and adjust the scaling of the plots created in step e to avoid any biexponential scaling artifacts in the negative region (see section 1.2 step e for instructions on axes scaling). Open the *Unmixing Settings* window. AF parameters identified in the *Autofluorescence Finder* tool are now listed at the bottom of the *Unmixing Settings* window.



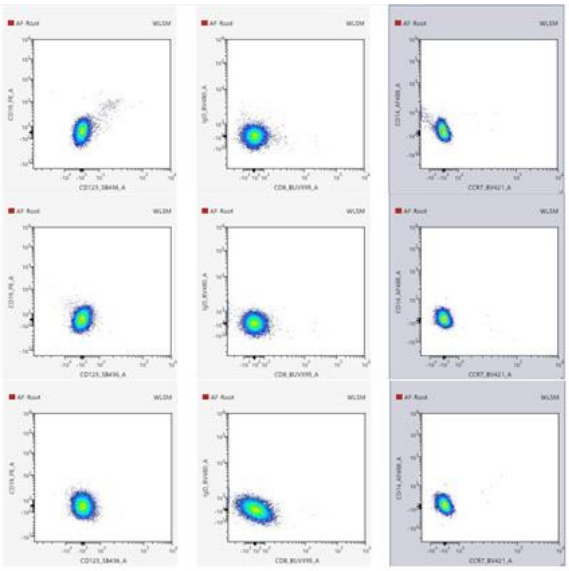
g. Untick all boxes in the *Use as AF* column of the *Autofluorescence* box and note the effect of this on the plots created in steps e-f. Now tick one box at a time to test how efficiently each AF parameter extracts autofluorescent signals during unmixing by observing the shape of the unstained cell populations on the plots. The goal here is

to identify one or more AF parameters that enable extraction of all autofluorescent events with minimal data spread, and to exclude any parameters that do not improve AF signal extraction.

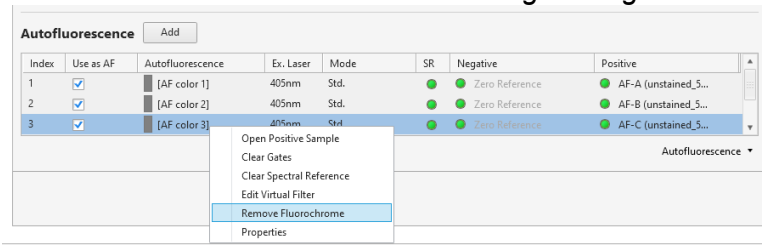
AF-1 only:
AF extraction not optimal

AF-1 + AF-2:
AF extraction good

AF-1 + AF-2 + AF-3:
No further improvement in AF extraction, data spread compared to AF-1 + AF-2

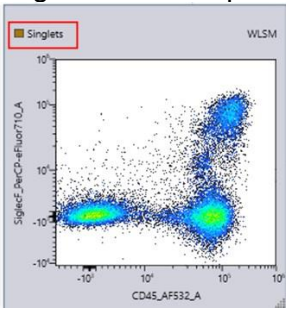


h. Remove unnecessary AF parameter(s), if any, by right-clicking on the parameter and selecting *Remove Fluorochrome*. Close the *Unmixing Settings* window.

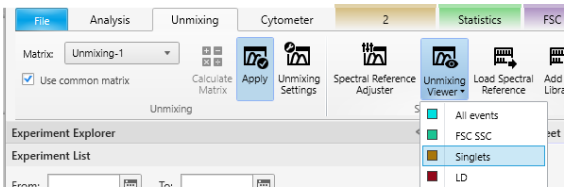


2. Unmixing QC

a. Perform hierarchical gating on your fully stained sample to include a cell gate, singlet gate, live/dead gate and if present, CD45+/- gate. You can also draw gates for the main cell subsets, e.g., lymphocyte gate, monocyte gate etc.
   - Refer to section 1.2 step e for instructions on how to rescale axes.
   - To create a child plot, double click on the gate. A plot will appear. Repeat the process to create hierarchical gating.
   - The parental plot will be indicated at the top left corner of each plot. In the example plot below, the plot originates from a parental gate *Singlets*.

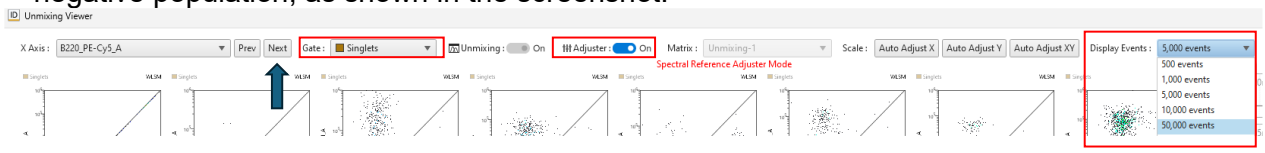


b. Go to the *Unmixing* tab, click on the downward arrow under the *Unmixing Viewer* tool and select the population you want to view. The *Unmixing Viewer* opens to display NxN plots of one fluorochrome against every other fluorochrome in the panel.
   - It is recommended to examine all singlets initially, without excluding any parameters.

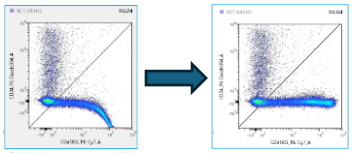


c. Use the *Auto Adjust XY* button at the top right of the *Unmixing Viewer* to automatically adjust the axes to your populations, then manually adjust all axes further so that the maximum fluorescence intensity displayed on all plots is 10^6. Refer to section 1.2 step e for instructions on rescaling axes.

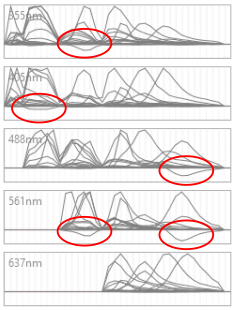
d. Turn ON the spectral reference *Adjuster* as shown on the next screenshot, then change *Display Events* on the right to 50,000 or 100,000 events. Assess whether there is an unmixing error on any of the plots, indicated by populations bending towards the negative fluorescence values ("banana shaped" populations). Correct the error by dragging the population up/down or left/right with your mouse until the mean fluorescence of the positive population is approximately aligned with that of the negative population, as shown in the screenshot.



Unmixing error:
A "banana-shaped" population bending towards the negative fluorescence value.

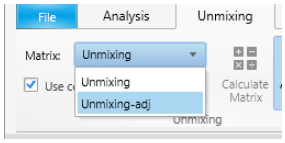


e. Click *Next* to go through each fluorochrome in turn until you have examined all NxN plot combinations, and correct any unmixing errors you can spot.
   - It is important to monitor the spectral references on the right-hand panel of the *Unmixing Viewer* so the spectra do not start to dip into the negative values as shown in the example below. Any corrections that result in negative spectra should not be performed. You can undo corrections by using the shortcut [Ctrl] + [Z] on your keyboard.



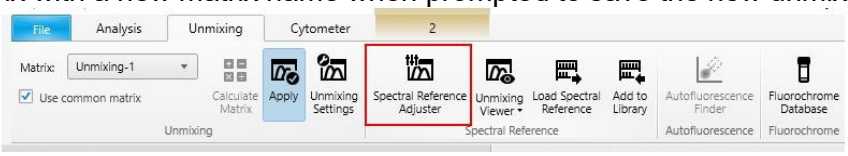
   - It is not recommended to correct any AF spectra.

f. Once all unmixing errors are corrected, switch the *Adjuster* OFF and you will be asked whether you want to save the adjusted unmixing matrix. Click *Save* and assign a new name to save it as a new matrix. You can now switch between the original and new matrices at any time by clicking the drop-down menu next to *Matrix* in the top left corner of the screen. You can also save a new matrix more than once by switching the *Adjuster* off at regular intervals during unmixing corrections and saving a new matrix every time.



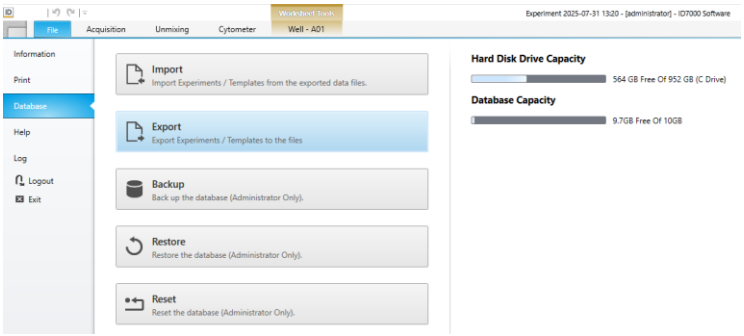
Note: It is recommended to keep the original unmixing matrix in case incorrect unmixing adjustments have been made.

g. Click *Close* (bottom right) to exit the *Unmixing Viewer*.
   - **Note:** To fine tune unmixing directly on the plots in worksheet, click on *Spectral Reference Adjuster* in the *Unmixing* tab. Adjust unmixing errors as described above and click *OK* to close the *Spectral Reference Adjuster* window. Save the matrix with a new matrix name when prompted to save the new unmixing matrix.

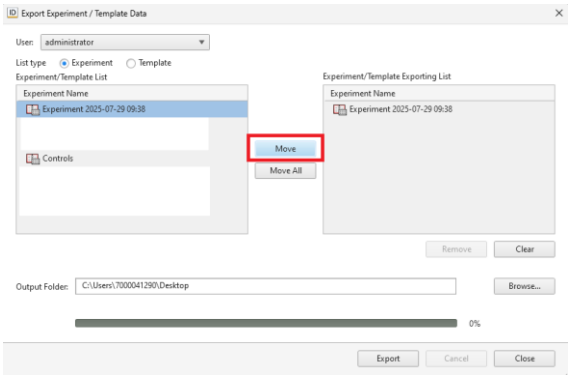


Part E: Data export

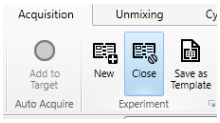
1. Exporting SONY experiment files (.exdat files)
   It is highly recommended to export and keep the original ID7000 experiment files, so you can go back and make any corrections if needed in the future. **In the event of experiment deletion or data loss from the ID7000 software, it is not possible to re-import FCS files into it, so please ensure to keep the .exdat files of your experiments as described below.**
   a. Go to *File* and find the *Database* tab.
   b. Click *Export*.



   c. When the data export window opens, select the experiment(s) you want to export and click on the *Move* button in the middle of the window.

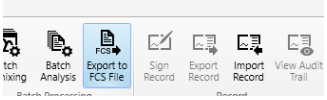


   Note: If your experiment is not listed on the left window, the experiment may still be open in the *Acquisition* tab. Go to the *Acquisition* tab, click *Close* and retry the export.

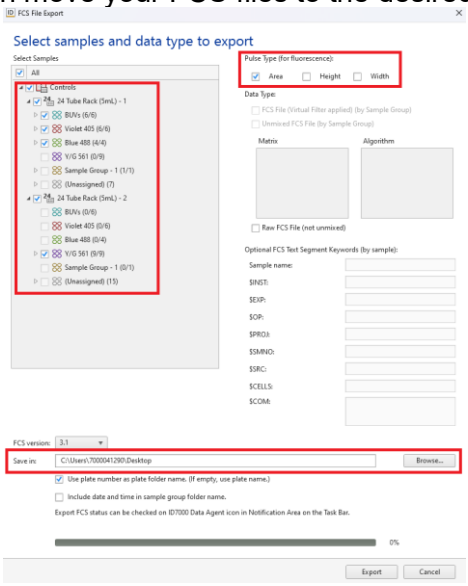


   d. Browse the location where you want to save your files and click *Export* to start exporting the data. It is recommended to export to *Desktop* in the first instance, then move your .exdat files to the desired location from there.
   e. Once the export is completed, close the window. The relevant .exdat files will be created in a new folder on the *Desktop*.

2. Exporting FCS files
   ID7000 data can also be exported in FCS format for analysis using third-party software. Note that, as mentioned above, **FCS files cannot be imported into the ID7000 software, so please keep the .exdat files even if you export FCS files.**
   a. Select an experiment in the *Analysis* tab. Click *Export to FCS File* in the menu at the top of the screen.

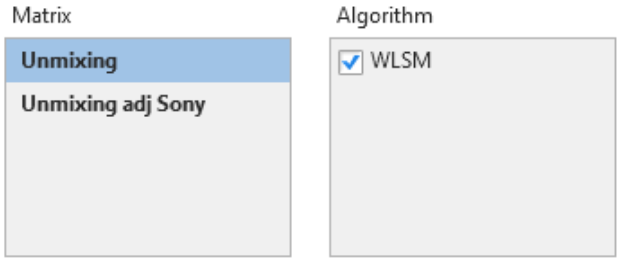


b. When the *FCS File Export* window opens, deselect the parameters you do not wish to include (e.g., Height, Width etc) and confirm that all sample groups you want to export are selected. Browse the location where you want to save your files and click *Export* to start exporting the data. As above, it is recommended to export to *Desktop* in the first instance, then move your FCS files to the desired location from there.

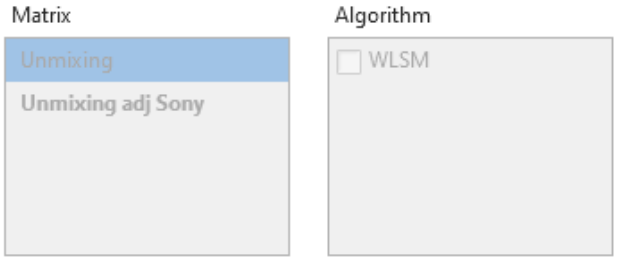


Note: If several unmixing matrices have been created, a separate FCS is generated for each unmixing matrix. To deselect the unwanted matrices, click on the specific sample group in the *Select Samples* window on the left, and untick the WLSM box.

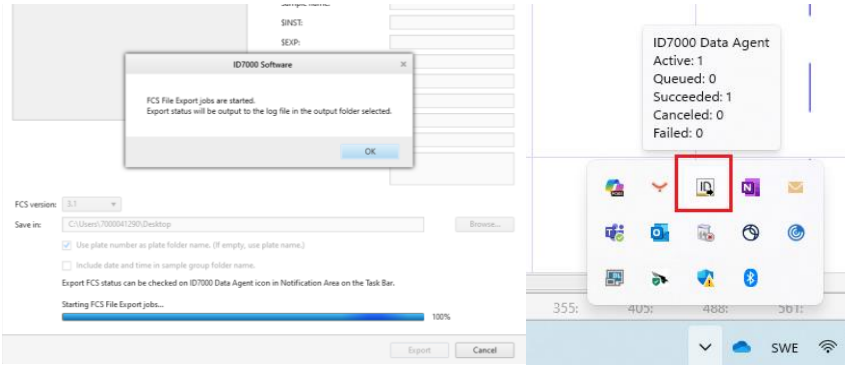
Before:



After:



c. The progress is shown by the blue bar at the bottom, and a message will pop up once the export has started. Click *OK*, then *Cancel* to close the window. The data export will continue in the background. The export status can be viewed by hovering the mouse over the *ID7000 Data Agent* icon in the task bar.


ID7000 Software
FCS File Export jobs are started.
Export status will be output to the log file in the output folder selected.
OK
FCS version:
Save in:
Export FCS status can be checked on ID7000 Data Agent icon in Notification Area on the Task Bar.
Starting FCS File Export jobs...
100%
Export
Cancel
ID7000 Data Agent
Active: 1
Queued: 0
Succeeded: 1
Canceled: 0
Failed: 0
SWE

Part F: Cleaning and Shutdown

1. Cleaning without hardware shutdown

   Decontamination can be performed if you want to clean the instrument between users without shutting down the hardware. This method can also be used for cleaning if there is a suspected clog in the sample line.

   a. In the *Cytometer* tab at the top of the screen, click on *Decontamination* and select the *Bleach Cleaning and Rinse* option.
      - Do not use the *Bleach Cleaning* option. Bleach can damage the instrument if it is not thoroughly removed. Bleach cleaning must be followed by water cleaning.

   b. Follow the instructions in the *Bleach Cleaning and Rinse* wizard.
      - See Appendix section 4) below for recommendations on bleach and rinse solutions.

      Note: Leave the software logged in between users as the air tank of the ID7000 unit is not pressurized while the software is logged out. If the system remains logged out for an extended period, the air tank pressure will gradually decrease and may fall below the lower limit, triggering a pressurization error. Therefore, to monitor and maintain proper pressurization of the instrument in between users, the software needs to remain logged in.

2. Cleaning and hardware shutdown

   a. In the *Cytometer* tab at the top of the screen, click on *Hardware Shutdown* and select the *Bleach Cleaning and Rinse* option.

   b. Follow the instructions in the *Bleach Cleaning and Rinse* wizard.
      - See Appendix section 4) below for recommendations on bleach and rinse solutions.

   c. Once the cleaning is complete, click *Shutdown*. The instrument will be turned off. Close the software and turn off the two power switches on the left side of the ID7000.

3. Automatic shutdown

   The instrument can be programmed to clean and shut down automatically once acquisition is completed.

   a. In the *Cytometer* tab click *Automatic Shutdown*. A confirmation window appears indicating that, once acquisition is completed, the instrument will automatically proceed to cleaning and shutdown. Click *OK*. The *Automatic Shutdown* button remains enabled.

   b. Set up your samples for acquisition as described previously. Make sure that all wells are added to auto-acquisition target (colour filled in). When ready, click on the *Auto Acquire* button  .

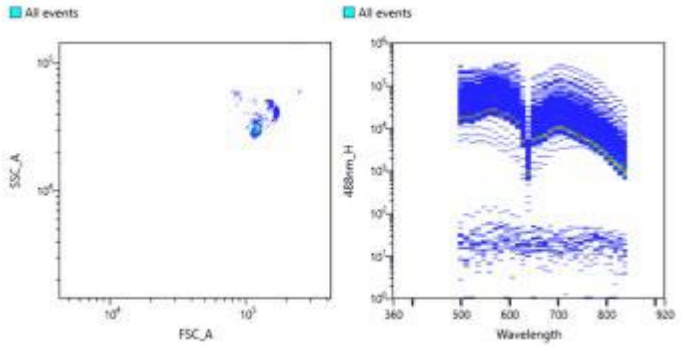
   c. A window appears prompting you to insert bleach and rinse solutions into the appropriate positions. Once this is done, press *OK*. You may leave the instrument at this point, as the remaining steps proceed automatically. Acquisition will begin and, once complete, the instrument will automatically proceed with cleaning and shutdown (including logging out of the software).

Part G: Troubleshooting

1. QC failure

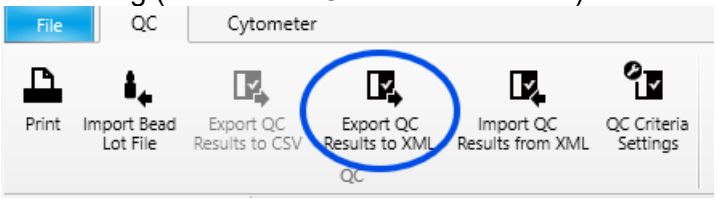
   If QC fails, perform the following:

   a. Check the beads:
   - Were there enough beads? Check if the tube is empty.
   - Was the correct concentration of beads prepared? **2 drops in 450 µL of PBS or MilliQ water, or multiples thereof**. If not sure, discard and prepare again.
   - Was the bottle containing the beads vortexed well before using? If not sure, discard and prepare again.
   - Was clean, fresh MilliQ water or PBS used for the preparation of the beads? If not or not sure, discard and prepare again.
   - Were the beads left exposed to the light for a longer time? If yes, discard them and open a new vial.
   - Was the correct bead lot selected in the QC wizard? If not, select the correct lot.
   - Are the correct beads placed in the correct slot? *Align Check* beads need to be placed in the first slot from the left, *8 peak* beads (if used) need to be placed in the middle slot. For reference, this is how the *Align Check* beads profile usually looks like:



   - If there is any doubt about the previous points, prepare new beads.

   b. Run a flow cell purge by clicking on *Flow Cell Purge* on the *Cytometer* tab.
   c. Run a cleaning cycle by clicking on *Decontamination* → *Bleach and Rinse Cleaning* on the *Cytometer* tab.
   d. Run optics alignment by clicking on *Optics Alignment* on the *Cytometer* tab.
   e. Run flow rate calibration by clicking on *Flow Rate* on the *Cytometer* tab.
   f. Run both *Daily and Performance QC* again.
   g. If the QC is still failing, click on the *QC* tab at the top of the screen, then on *Export QC Results to XML*. Send the resulting zipped folder to the Sony FAS Europe team for further troubleshooting (see Part H: Contact information).



2. Clogs

   If the event rate suddenly drops to 0 during acquisition and you suspect a clog in the system, perform the following:

   a. Run a flow cell purge by clicking on *Flow Cell Purge* on the *Cytometer* tab.
   b. If the problem persists, prime the fluidics system again by clicking on *Priming* on the *Cytometer* tab.

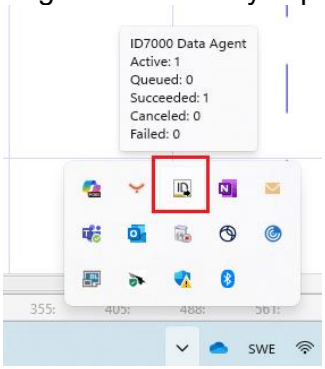
c. If the above do not resolve the issue, run a cleaning cycle by clicking on *Decontamination → Bleach and Rinse Cleaning* on the *Cytometer* tab. Follow the instructions in the *Bleach Cleaning and Rinse* wizard.
   - See Part G section 2 for recommendations on bleach and rinse solutions.

d. If the issue still persists, contact the Sony FAS Europe team for further troubleshooting (see Part H: Contact information).

3. FCS file export failure

If exporting FCS files fails, perform the following:

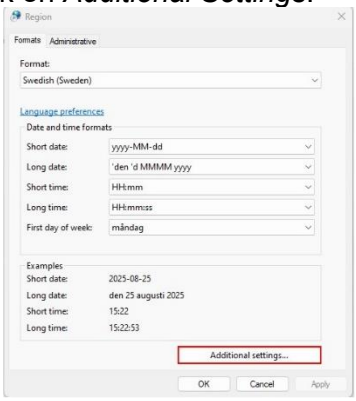
a. Click the *Show Hidden Icons* arrow in the *Windows* system tray on the task bar at the bottom right corner of the screen. Close the *ID7000 Data Agent* by right clicking on the small ID icon and selecting *Exit*. Then retry exporting.



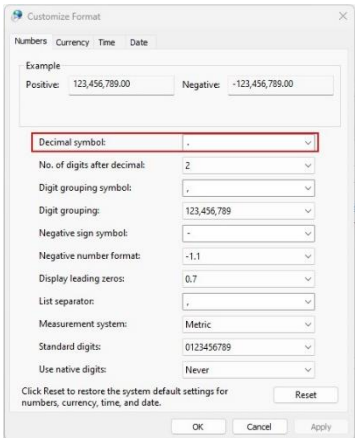
b. If a does not work, try to export from administrator account.

c. If neither of the above methods works, shorten the experiment name by right-clicking the experiment in the *Experiment List*, selecting *Properties*, and changing the experiment name to a short, text-only name. Additionally, choose an export destination folder with a short path name (e.g., C:/drive or *Desktop*). Then retry exporting.

d. If none of the above works, change the *Computer Settings* as below and retry exporting:
   - Navigate to *Control Panel → Clock and Region*
   - Select *Change Date, Time and Number Formats*.
   - In the *Region* window, click on *Additional Settings*.



   - In the *Customized Format* window, change the decimal symbol from "," to ".". Make sure the symbol is a dot.

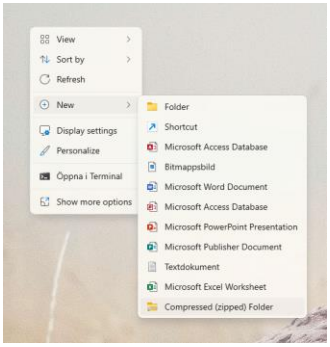


4. Log files

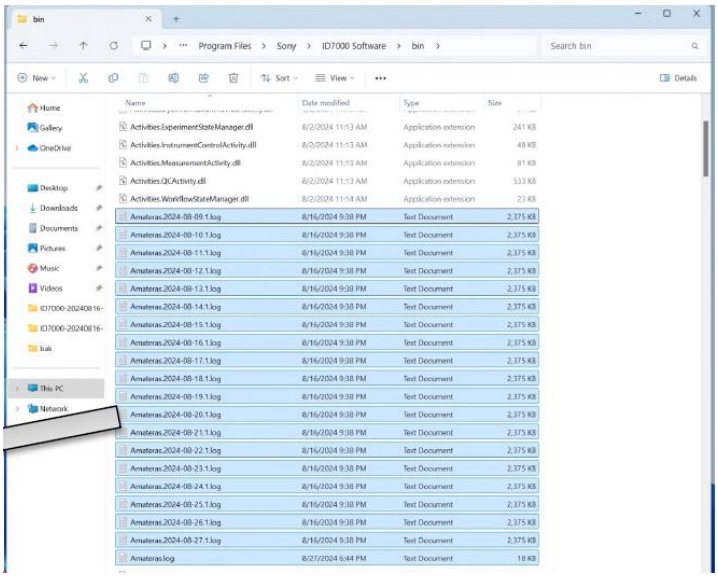
If any other issues arise with the ID7000, collect all the logs described below and send them to the Sony FAS Europe team (see Part H: Contact information).

i. Amateras logs

a. Create a compressed (zipped) folder on the *Desktop* and rename it to amateras.zip.



b. Go to C:\Program Files\Sony\ID7000 Software\bin, and copy the Amateras log file and all Amateras.yyyy-mm-dd.x. log files from this folder to amateras.zip on the *Desktop*.



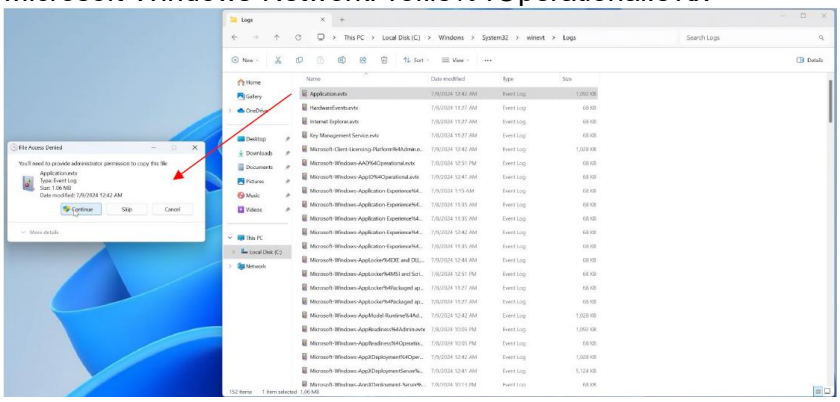
c. Double click amateras.zip to confirm that all logs have been copied.

ii. Windows Event logs

a. Go to C:\Windows\System32\winevt\Logs. Copy the following 5 files from the folder to the *Desktop*.

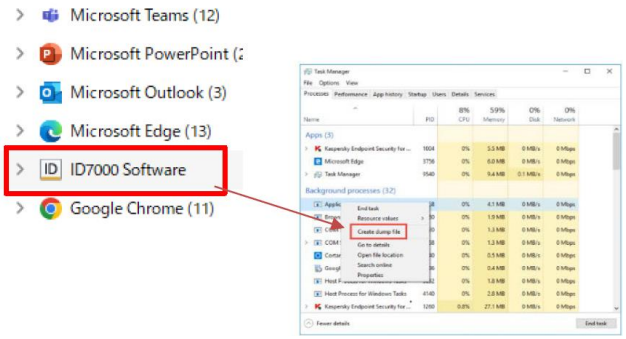
- Application.evtx

- Security.evtx
- Setup.evtx
- System.evtx
- Microsoft-Windows-NetworkProfile%4Operational.evtx

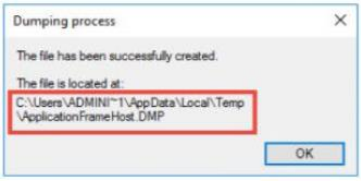


iii. Dump files

a. Create a compressed (zipped) folder on the *Desktop* and rename it to dump.zip.
b. Go to C:\Program Files\Sony\ID7000 Software\bin. Find the *Dump* folder and copy its contents to dump.zip on *Desktop*.
c. If a *Dump* folder does not exist, it means it was not created at the time of the issue. In this case, obtain the logs using *Task Manager* as follows:
   - Open the *Task Manager* on your PC
   - Select the *Processes* tab
   - Right-click the process you wish to create the dump file of (i.e. ID7000 software)
   - Select *Create Memory Dump File*



   - A dialog will appear with the location of the saved dump file. Go to the file location and copy the file to dump.zip on *Desktop*.



Part H: Contact information

If you have any further questions regarding the ID7000, or need help with troubleshooting as above, the Sony FAS Europe team is happy to help you. Please send your queries to our centralized support helpdesk at SBTE.helpdesk@sony.com.

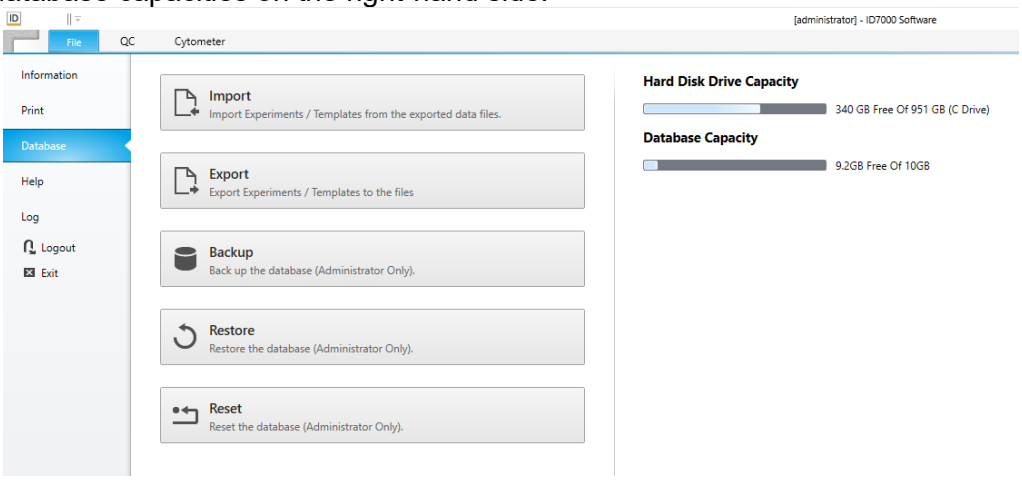
Appendix

1) Database backup, restoration and reset

As the hard disk and the software database fill up over time, the reduced capacity can affect the performance of the software. It is therefore highly recommended that you create regular database backups in addition to exporting .exdat files as described in the previous section. This allows deletion of old experiments and spectral references from the software, freeing up database and hard disk space.

The Backup, Restore and Reset functions are only available when logged in as administrator.

1. Backup
   a. Go to *File* and click on the *Database* tab. You can view the hard disk drive and database capacities on the right-hand side.



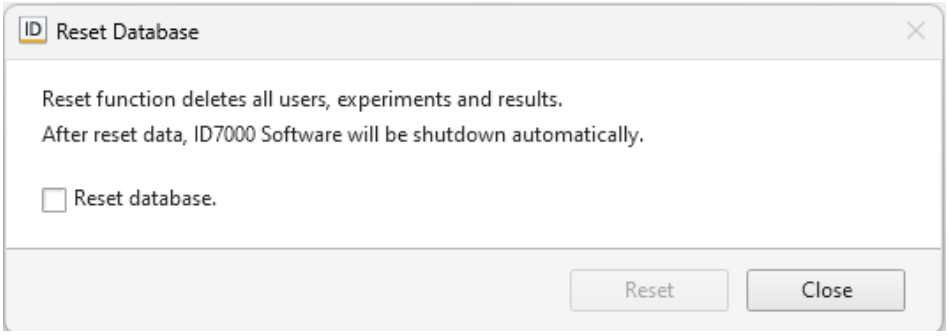
   b. Click *Backup*.
   c. Browse to the backup device location. This can be a cloud location or an external hard drive; however, if backing up to an external drive, ensure that the device is NTFS formatted. Depending on the size of the database, the backup file may be very large so the backup location should have sufficient space (at least 2 TB is recommended).
   d. Click on *Backup* to start the process. A progress bar is displayed. If the database is large, the backup may take several hours.
   e. Once the backup process is completed, a confirmation message appears. Click *OK*.
   f. Click *Close* to close the dialog. You have now created a complete copy of everything in the ID7000 software, including user profiles, experimental data, library and everything else, exactly as they are at that point in time.

2. Restore
   a. To restore a previously created backup, go to *File* and click on the *Database* tab, and then select *Restore*.
   b. Browse to the backup file location, then click *Restore* and wait for the process to complete (depending on the size of the backup file, this may take a considerable amount of time).

   Warning! Upon restoring a previous database backup, the ID7000 software reverts to its exact state at the time the backup was created. **This means that any experiments, user profiles, and account passwords created after that point will be overwritten and replaced with those contained in the backup file.**

3. Reset

The *Reset* function wipes the slate clean and deletes all data and users from the ID7000 software. When pressing *Reset*, the following pop-up appears:

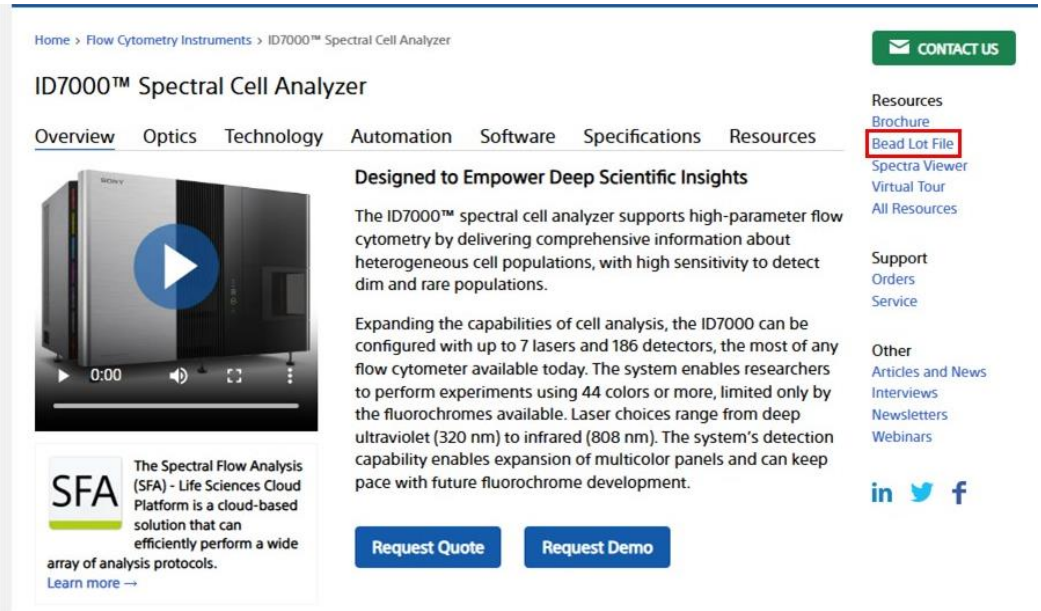


Only tick the box and proceed if you are certain you wish to wipe everything from the software and start from the beginning.

2) Importing bead lot files

If you cannot find the bead lot file in the dropdown menu of the QC wizards, you can download and import it manually.

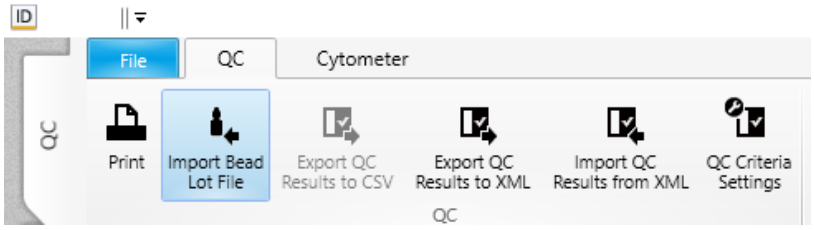
a. Go to *ID7000 Spectral Cell Analyzer* page on the *Sony Biotechnology* website (ID7000 Spectral Cell Analyzer - Overview - Sony Biotechnology). Click on *Bead Lot File*.



b. Click on *Download Bead Lot File* to download the zip file containing bead lot files.

c. Extract all files from the downloaded zip file.

d. Click on *Import Bead Lot File* button in the *QC* tab. Select the specific bead lot files you want to import from the extracted files.



3) Long term shutdown

1. If using MilliQ water as the sheath fluid
   Shut down the instrument using normal procedures described in Part F sections 2 or 3.

2. If using PBS as the sheath fluid
   If you do not plan to use the instrument for one week or longer, perform the following procedure to replace the PBS in the fluidics system with MilliQ water to prevent PBS from precipitating and causing clogging. This process will take approximately 40 minutes.
   a. Click on *Long Term Storage* button in the *Cytometer* tab of the ribbon to launch the *Long Term Storage* wizard.

   b. Click *Start*.
   c. Discard the sheath fluid from the sheath tank and refill it with MilliQ water. Empty the waste tank. Click *Next*.
   d. Once the process is completed, shut down the instrument as described in section 1.2.
   e. When you return from the long-term shutdown, you will be prompted to complete *Priming* from *Long Term Storage*. This will take approximately 15 minutes. Click *Start*.
   f. Discard the remaining MilliQ water from the sheath tank and refill it with PBS. Empty the waste tank. Click *Next*.
   g. Once completed, the ID7000 is ready for use.

4) Maintenance

- Always perform the hardware shutdown with *Bleach Cleaning and Rinse*.
  - Ensure to have good quality sodium hypochlorite as your bleach solution.
  - Sodium hypochlorite should be between 1-3% concentration and prepared fresh weekly.
  - Rinse solution should be MilliQ water.
- Exchange the waste tank air filter (red tubing) on the external waste tank once a month. To do this:
  - Grasp the plastic connector. Using one hand, press and hold the quick release O-ring, then use your other hand to pull out the short length of red tube.
  - Exert gentle force while holding the O-ring if the tube feels difficult to pull out from the connector.
  - Release the O-ring. Insert the replacement air filter tube into the connector and push it firmly into place.
  - Write the date on the white plastic part of the filter to keep track of the waste filter exchanges.
- Clean the sheath tank on a weekly basis. Discard any remaining unused sheath fluid from the sheath tank, rinse the tank with 500 mL of sheath fluid, and then refill the tank with fresh sheath fluid.
- Open the external door on the right-hand side of the ID7000 instrument – inside it will look as shown below.

- Swap the green tubing attached to the *Sheath* connector port with the green tubing attached to the *Sheath Wash* connector port (both indicated below by the blue rectangle) every 2-3 weeks. Swapping them around at regular intervals ensures the sample probe wash remains efficient and prevents accumulation of large air bubbles in the system.