REICHERT® 4SPR

Software User's Guide

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TECHNOLOGIES
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Introduction

Congratulations on the purchase of your new Reichert4SPR Surface Plasmon Resonance (SPR) instrument! This powerful instrument will enable you to characterize biomolecular interactions and obtain critical kinetic information. This easy to use software for both data acquisition and analysis will help you get started quickly.

This Software User's guide is designed as a training and reference manual. We recommend that you read it carefully prior to running experiments and follow the instructions provided herein.

Please retain this manual for future reference and to share with other users. Additional copies can be obtained from your authorized Reichert® dealer or from the Reichert Customer Service Department at:

Tel:716-686-4500
Fax: 716-686-4555
Email: reichertspr.lifesciences@ametek.com

Features and Functions

The Reichert4SPR software is used to setup and run experiments, and for data analysis.

The Reichert4SPR four channel Surface Plasmon Resonance system is physically composed of three modules - the Pump, the Autosampler and the SPR Instrument

The Reichert4SPR software guides you through the natural workflow of your experiments, from pH scouting to immobilization, through method development and scale up. Each experiment's workflow is determined by the selection of a couple or capture strategy for your target. The software lets you easily setup and organize experiments with a family tree approach. You can quickly build on the results of your last experiment - only modifying the relevant parameters in a Child experiment. Analysis is intuitive and easy, too.

To run experiments, users must supply information about sample names and concentrations, association and dissociation times, regeneration names and times, along with number of replicates, dilution factor and flow rate for the different steps in the SPR workflow. This information will automatically be used to determine sample placement in trays and to create a run sequence to provide automatic control.

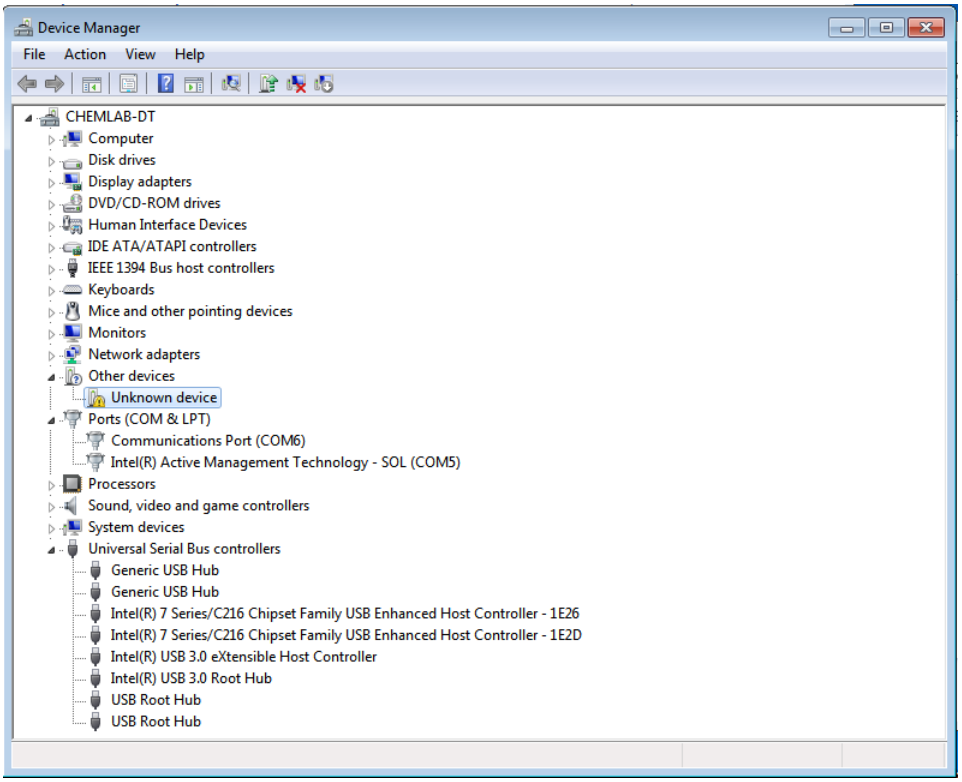
Context sensitive **Help** can be found on each page in the software where there is a question mark icon. Selecting this icon will take you to the relevant section of the Help documents.

Programs

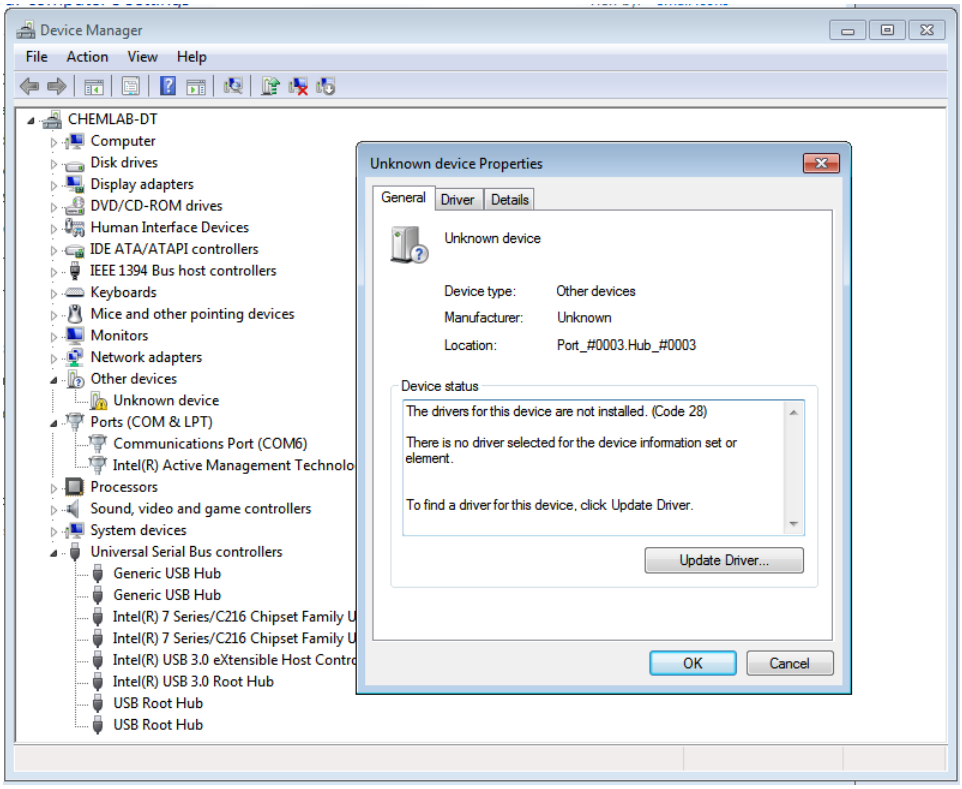
Installation of Reichert4SPR Software

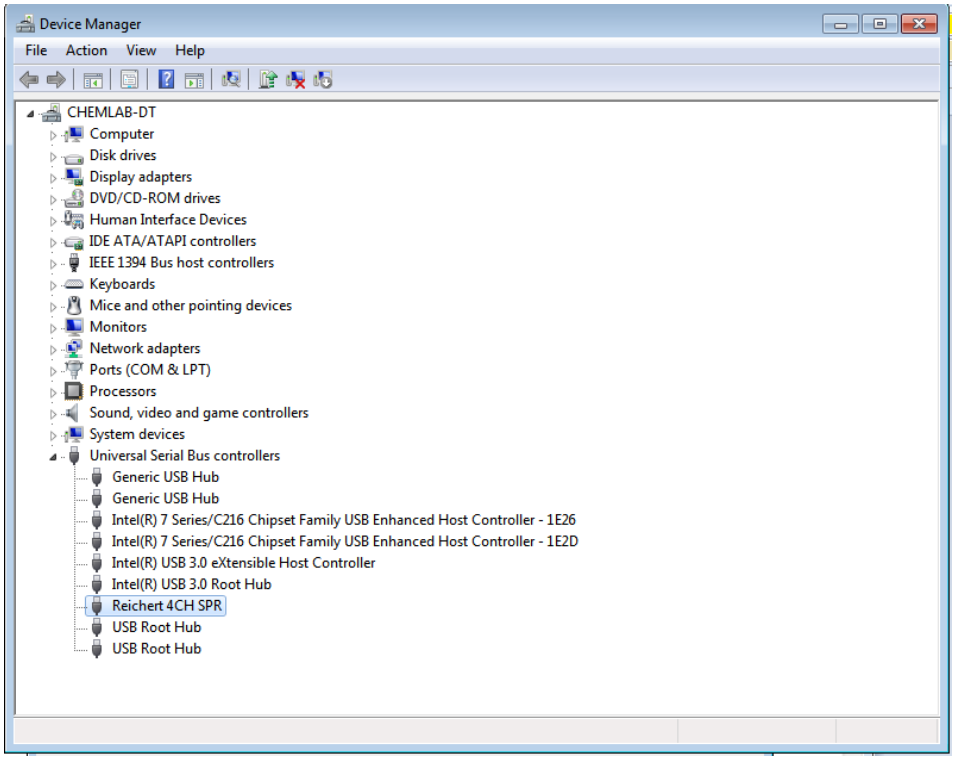
Prior to installation:

1) A certified Windows USB driver from Reichert must be installed.
   a) Be sure that the Reichert4SPR instrument is connected to the PC with a USB cable and that the SPR instrument is powered up.
   b) On the PC, go to the Device Manager utility of the operating system.

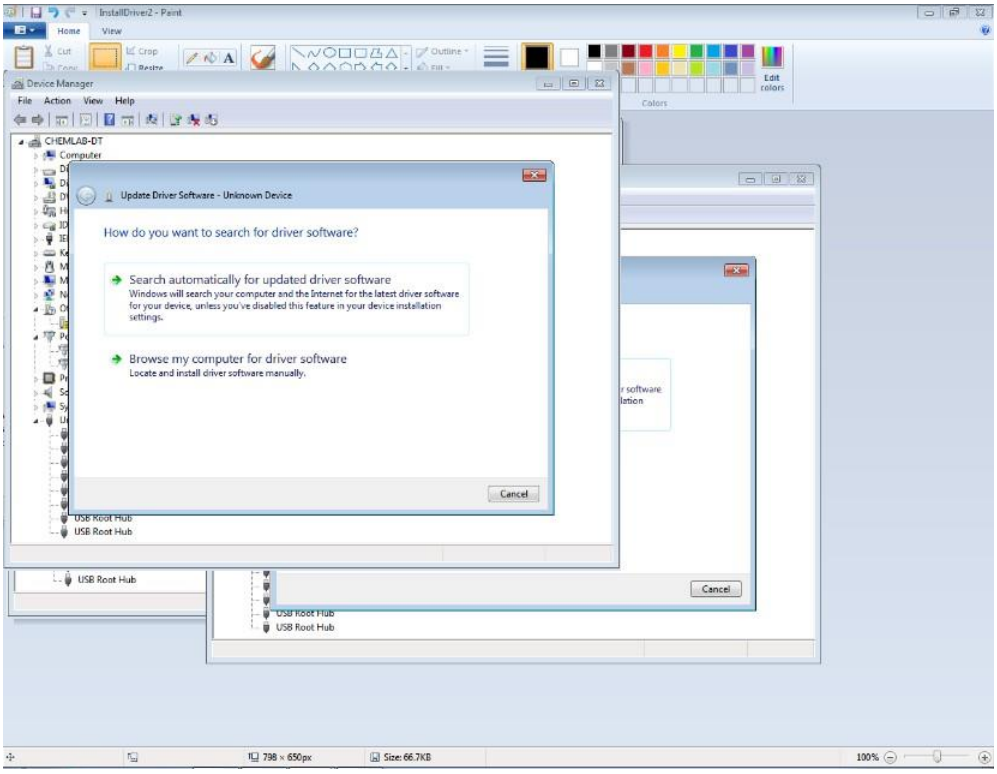


   c) Go to Other Devices and right click on the entry “Unknown Device” and click the Properties command in the popup menu. If the device does not appear, unplug the USB cable from the SPR instrument and plug it back in. It should then appear.

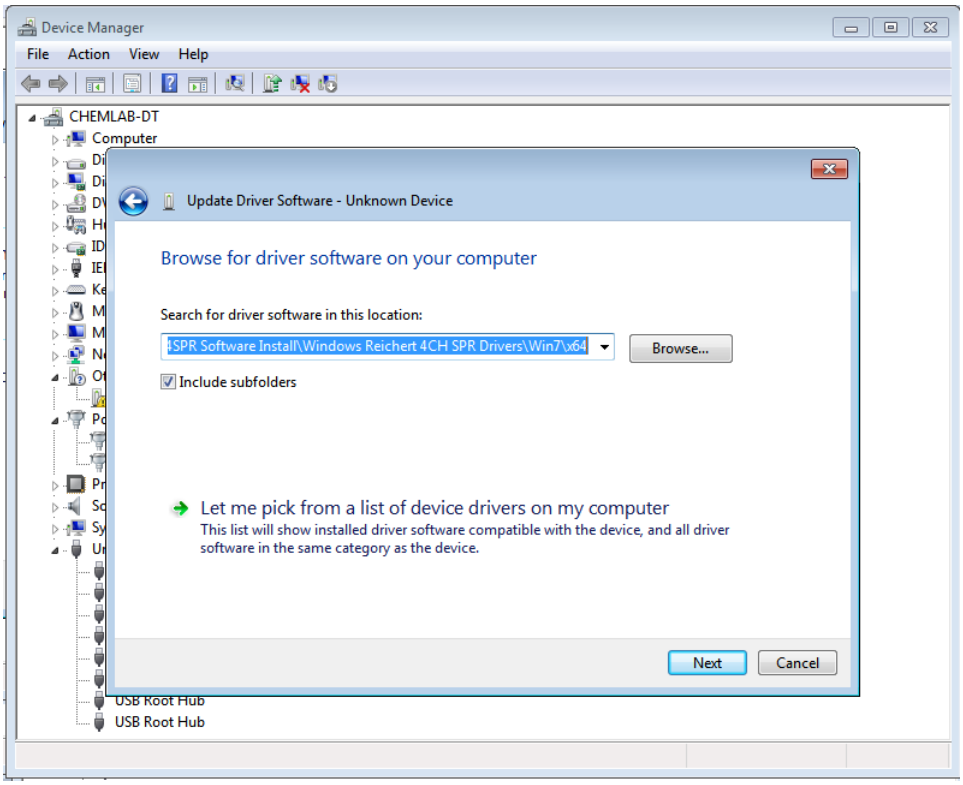


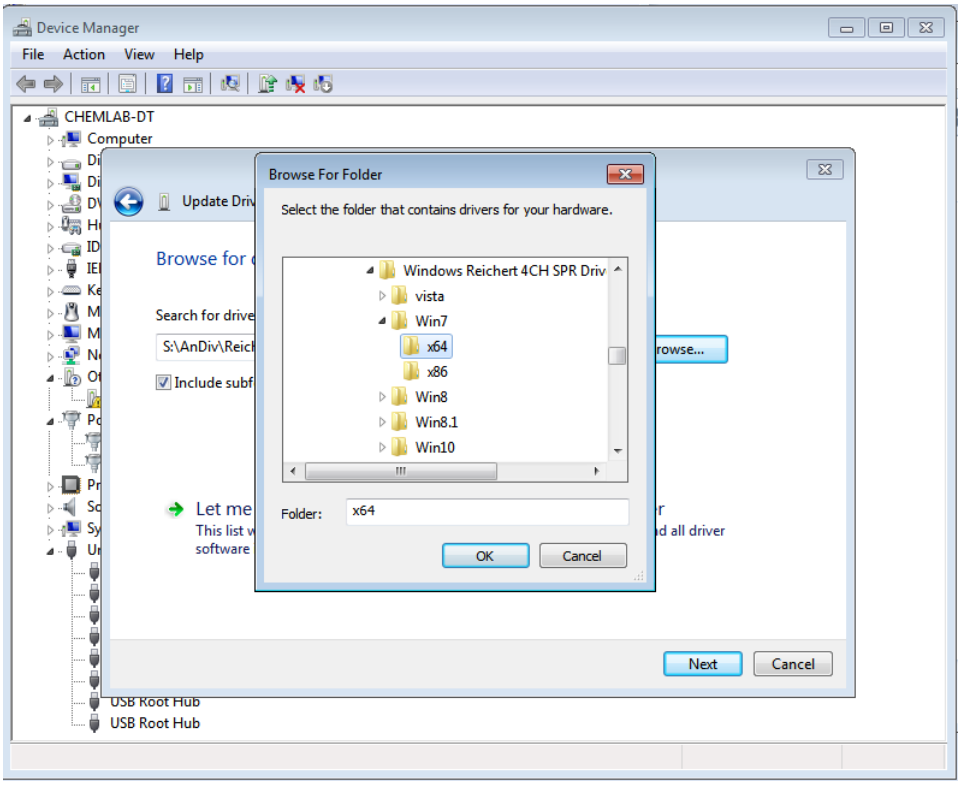


d) Next, select Driver, then Update Driver.

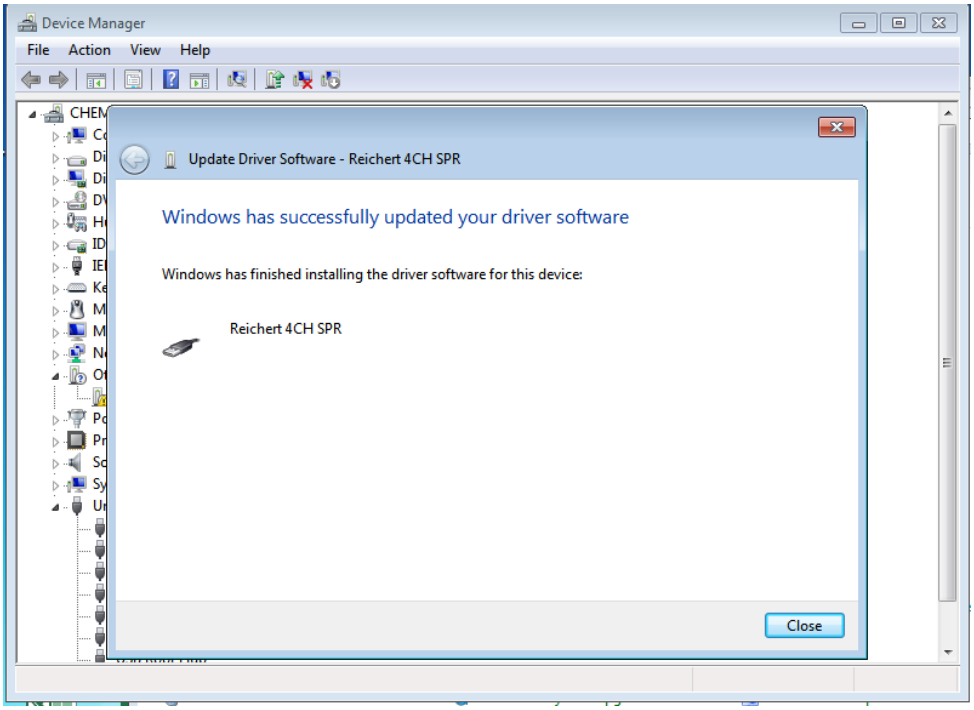


e) Select “Browse my Computer for Driver Software” and direct the browser to the “Windows Reichert 4CH SPR Drivers” folder on the Reichert4SPR flash drive that was provided with your instrument. Choose the appropriate version of Windows - 32 or 64 bit operating system.


Device Manager
File Action View Help
CHEMLAB-DT
Computer
Update Driver Software - Unknown Device
Browse for driver software on your computer
Search for driver software in this location:
4SPR Software Install\Windows Reichert 4CH SPR Drivers\Win7\x64
Browse...
Include subfolders
Let me pick from a list of device drivers on my computer
This list will show installed driver software compatible with the device, and all driver software in the same category as the device.
Next
Cancel
USB Root Hub
USB Root Hub



A message will come up OK, then Next and you will see “Windows has successfully updated your driver” and then you Close

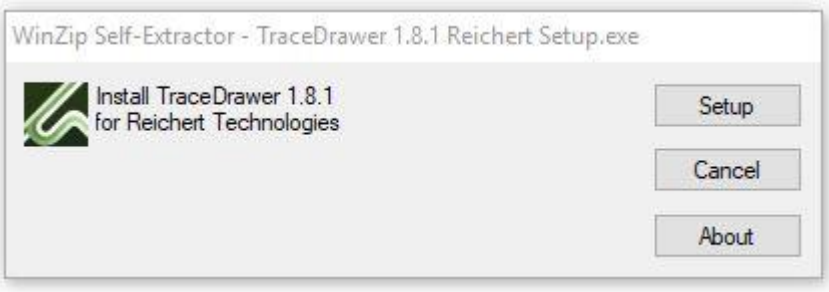


2) Region/Language setting. The software requires that the operating system is set to US/ US English. To verify or change the settings go to Control Panel -> Region and Language -> Format -> English (United States). Also, be sure to click Additional Settings -> Numbers -> Reset in case the English default settings were changed.
3) Go to the folder: Reichert4SPR Software Install\SPRAutolink_Install and copy the setup_xxx.exe file to the install computer's hard drive.

IMPORTANT NOTE: You will need administrative rights to install the software.

Program installation:

1) To install the Reichert4SPR program, right click on the Setup.exe file and choose Run as Administrator. You will be guided through the setup.
2) Select Next. Make sure you allow the program time to install completely.
3) When Setup is complete, select Finish.
4) You will then see the following popup box to Setup the data analysis package:



5) Select Setup to Install the data analysis package. Note: when you first open Tracedrawer you will need to enter your License key(s) (Choose Tools – License key) exactly as provided including the < and > signs.

6) When the installation is complete, separate icons for the Reichert4SPR software and the data analysis software (Tracedrawer) will automatically be added to your desktop.

Connecting the Pump via the MOXA Connector

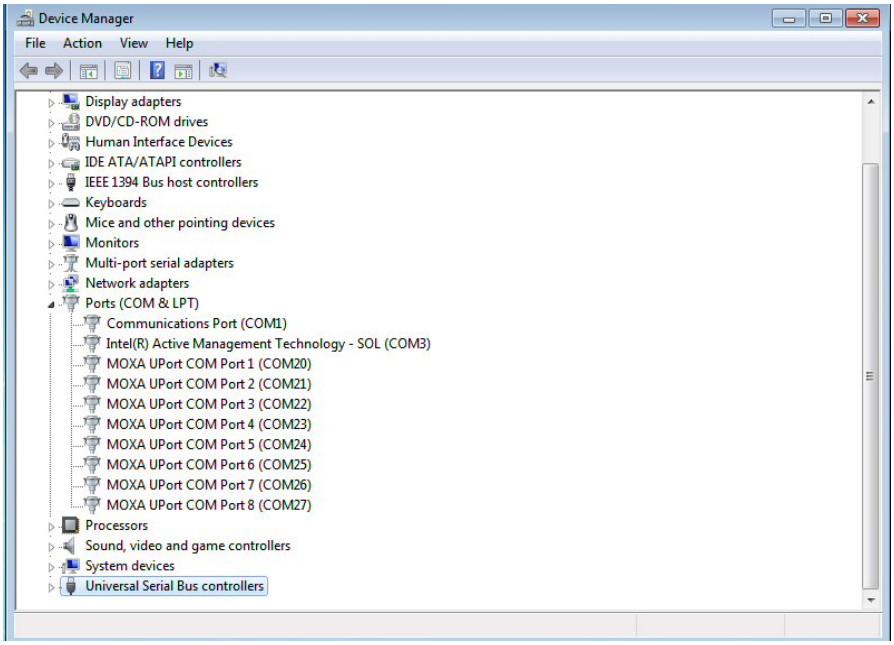
Reichert4SPR 4Channel Version 2.0.2 control software is designed to run the Syringe Pump as an external RS232 hardware device. Getting Reichert4SPR 4Channel Version 2.0.2 control software up and running with the Syringe Pump as an external RS232 hardware device involves connecting the Syringe Pump, installing and configuring the MOXA RS-232 USB to Serial Adapter (UPort 1110), and automapping the device to the PC/MOXA (UPort 1110) RS232 serial port as described below.

Connecting the Hardware

1) Connect serial connector of Syringe Pump to the MOXA RS-232 USB to Serial Adapter (UPort 1110) with an RS232 serial cable.
2) Connect the MOXA RS-232 USB to Serial Adapter (UPort 1110) USB connector to an available USB port on the PC with a USB cable.
3) Connect SPR USB to an available USB port on the PC with a USB cable.
4) Power up all hardware including the SPR.
5) Note: The use of the MOXA USB switch (UPort 207) is optional if the PC is equipped with two available USB connectors.

Installing and Configuring the MOXA Windows Driver

1) Run “driv_win_uport1p_v1.9_build_16020210_whql.exe” (UPort 1110) on Windows as administrator to install the MOXA RS-232 USB to Serial Adapter (UPort 1110) Windows Driver Ver1.9 using the default install options.
2) Run the “UPort Configuration Utility” located in the Windows Start Menu under MOXA UPort Windows Driver as administrator to set the starting COMM port to be port 20 by checking the “Enable Fixed-base COM Mode” checkbox and choosing “COM20” in the “Select a beginning COM number” in the drop down box (ignore in use warnings). Press OK.
3) Verify that the MOXA RS-232 USB to Serial Adapter (UPort 1110) is configured using port COM20 in Windows Device Manager. There must be at least one MOXA UPort COM Port device listed under Ports (COM & LPT) starting at COM 20 or greater.



Starting Reichert4SPR 4Channel Version 2.0.2 Control Software and Location of Associated Files

After installation, Reichert4SPR 4Channel Version 2.0.2 control software can be started from the desktop icon “Reichert4SPR” or from the Start Menu | All Programs | Reichert | Reichert4SPR. Database, log and rsd data files can be found in the C:\Reichert\ Reichert4SPR 4 Channel\ folders.

Automapping External Serial Syringe Pump Device to a Serial COMM port in Reichert4SPR 4Channel Control Software

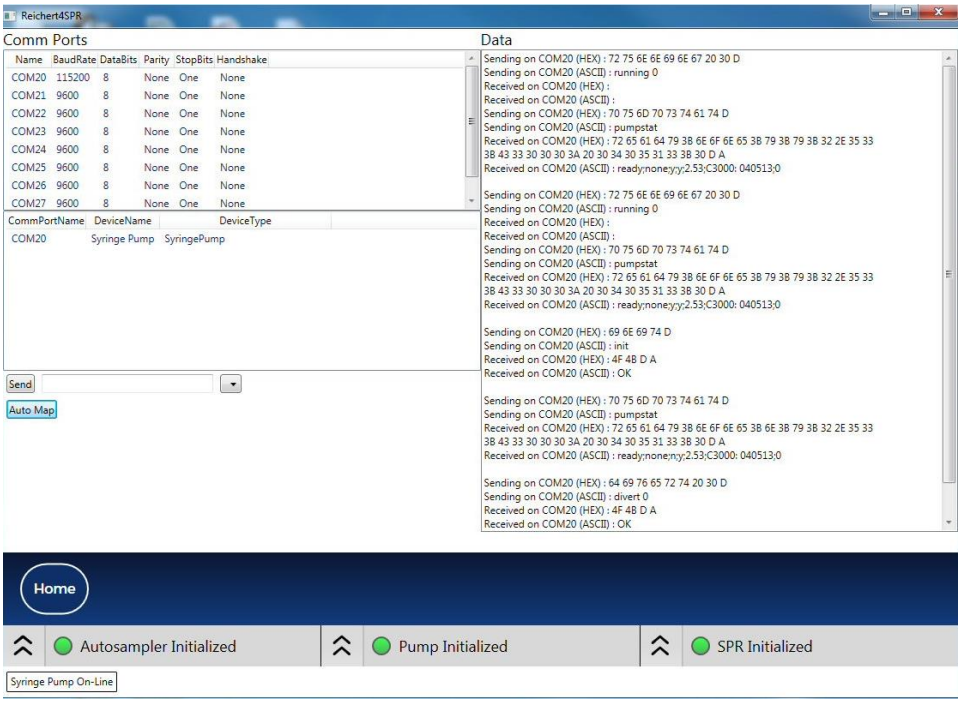
Power up all external hardware devices (Syringe Pump) and the SPR. Start Reichert4SPR 4Channel control software and automapping of the external RS232 serial Syringe Pump to a serial communication port (PC/MOXA) will automatically start. The automapping feature automatically assigns the Syringe Pump to an RS232 serial COMM port relieving the user of manually performing this configuration task.

When the Syringe Pump is successfully found and auto mapped to a serial port the device will be initialized and designated as On-Line (“Syringe Pump On-Line”) in the external device status bar at the bottom of Reichert4SPR 4Channel control software.

The pump will Initialize and then flow can be started from Direct Control.

If the Syringe Pump is not found or not mapped to a serial port it will be indicated in red as “Syringe Pump Off-Line” in the external device status bar at the bottom of Reichert4SPR 4Channel control software.

Automapping can be performed by the user at any time by clicking on the Manual Serial Device Control / Diagnostics button at the bottom of the Home screen and clicking on the Auto Map button.



Manual Serial Control/Diagnostics

Manual Serial Device Control & Diagnostics can be accessed on the Home screen (far right at bottom) and is an advanced feature that can be used to view hardware configuration that is completed automatically by the Automap feature. In addition, the utility can be used to send and view responses of low level commands sent to the hardware devices (advanced).

Troubleshooting

In the case where the Syringe Pump does not come online after automapping.

1) Verify all hardware is connected as described in “Connecting the Hardware”.
2) Verify the MOXA Windows driver is installed and configured correctly as described in “Installing and Configuring the MOXA Windows driver”.
3) Recycle power to all hardware including the MOXA USB Switch (UPort 207) if used.
4) Disconnect and reconnect MOXA RS-232 USB to Serial Adapter (UPort 1110) to all hardware including the PC.
5) Disconnect and reconnect the SPR USB connector to all hardware including the PC.
6) Restart Reichert4SPR 4Channel control software to restart automapping.

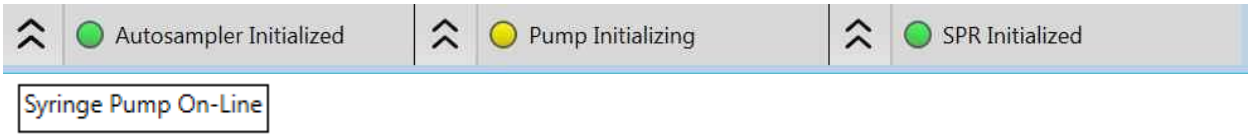
Reichert4SPR Software Revisions

You will be provided the latest version available on your date of purchase. The software version is shown on the upper right corner of the Home page.

Instructions for Use

Startup

Open the Reichert4SPR software. At the bottom of the screen, the SPR and Autosampler lights should turn green. The pump will initially try to connect and the message just below Autosampler Initialized will be syringe pump off-line and to the right of that, Mapping ports. When it completes connecting you will see the Syringe Pump On-Line box just below the Autosampler Initialize box. The pump light will be yellow until Initialization (syringe on pump moves to top of stroke) is complete at which time the light will change to green.



HOME PAGE

The page is organized to move the user logically through the steps of instrument preparation (Prime & Baseline Check), experimental run (Experiments), and cleaning (Clean). This page is also used as the starting point for Opening Data Files and implementing User Access Control. Note: accessing Service Manual Entry and Manual Serial Control/Diagnostics is generally for Factory use only. Each section is described below.

Prime

Prime is used at startup (or prior to cleaning and shutting down) to flush the pump and all the tubing with buffer (or water for cleaning).

Choosing Prime from the Home page, you will be asked to select Buffer 1 or Buffer 2 or Cancel. You will need to perform a Prime anytime you are changing from one buffer to another or from water to buffer or buffer to water. Be sure to fill the wash bottle on the autosampler with

the same buffer (or water) you are using to flush the system. Keep in mind that whichever Buffer you last ran a Prime for (Buffer 1 or Buffer 2) will be carried through onto the Experiments pages.

Run time for Prime is approximately 14 minutes and it can be Paused or Stopped at any time. Flow rate is increased from the default of 25 μL/min to 250 μL/min. It takes about 5-7 pump refills to changeover to a new buffer (or water) and these are automatically programmed. Over the course of the Prime, each entry and exit port tubing to the flow cell is flushed with buffer (or water) and periodic autosampler washes are performed (these are all also preprogrammed).

Once Prime, is complete, the flow rate will return to 25 μL/min. Perform a detector scan and check that all four SPR minima are visible and have similar depths. To perform a detector scan, click the double caret (⌃⌃) next to SPR Initialized on the bottom right and then click on the Detector Scan button. This verifies that the sensor chip is docked correctly, fluid flow is appropriate, and all sensing systems are functioning appropriately. You should see a response similar to the following:

Detector Scan

Baseline Check

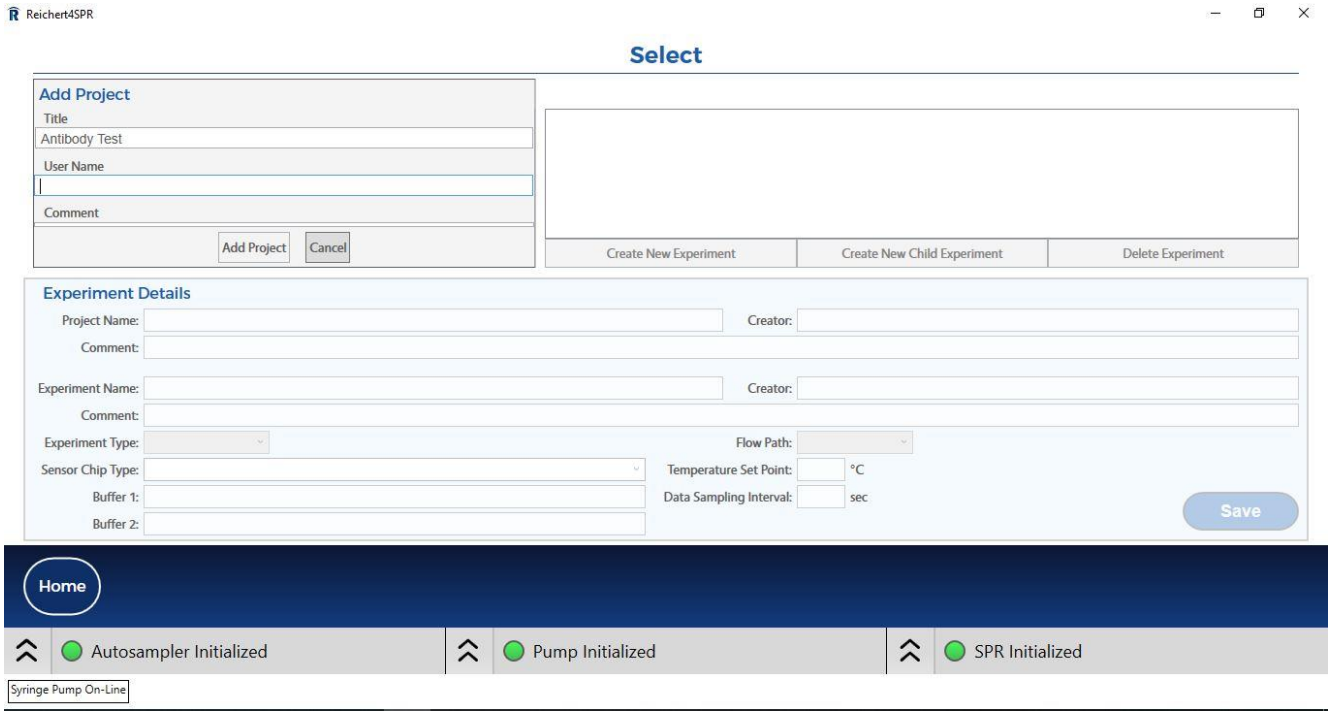
After Prime is complete, you will want to check that you have a stable baseline. Clicking the Baseline Check button will allow you to monitor the baseline on all 4 channels together, or on just channels 1 and 2, or on just channels 3 and 4. You can type in a flow rate or choose from the dropdown list. You can also choose the sampling interval (0.1 to 10 seconds) and the length of time you want to collect baseline for (hh:mm:ss). The baseline check can be stopped before it has completed or repeated as necessary if the baseline is not yet stable.

Experiments

The Experiment section has hierarchy designed to keep various projects and experiments organized, and to enable quick follow-on studies. The top level of the hierarchy is the Project. Under the Project, there are individual Experiments. Variants of those Experiments are called “Child” experiments that are attached to the parent experiment and show visually as a sub-experiment. Creating a Child experiment allows you to quickly follow-on from one experiment to the next by pulling in all the settings and information from the parent experiment. This enables you to quickly modify only the relevant settings before you start another experiment.

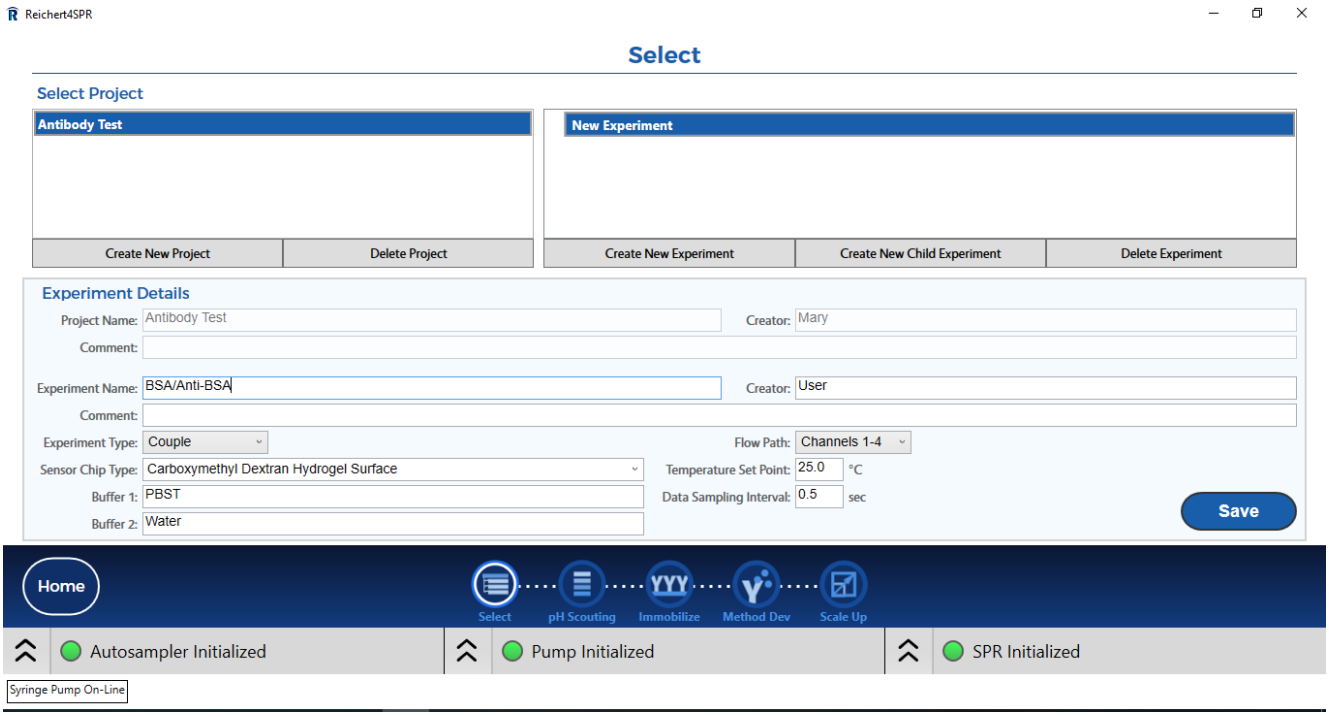
To create a new project on the Select page, click Create a Project. In the Add Project dialog, enter a Title, User Name and comments (if needed) for the new project, then click Add Project (or you can Cancel).

Select page - New Project



Next, choose the Create New Experiment button to add a new experiment in that project.

Select page - Create New Experiment



When you select an Experiment, you will see information about the selected Project and Experiment in the fields at the bottom. The “New Experiment” default name can be edited, as can the creator and comments fields for the experiment.

The correct Experiment Type must be set for the new experiment. When a “Couple” experiment type is selected, it will open a five-step workflow diagram at the bottom (see above picture). Choice of “Capture” experiment type will open a three-step workflow (pH scouting and immobilization are not needed). Typically, one will move through the steps sequentially. However, sequential progression is not required, as there are times where deviations from a standard workflow are required to meet experimental needs. You can select any step in the process and jump to the appropriate screen.

The Sensor Chip Type can either be selected from its dropdown box or manually entered.

The Flow Path selector enables you to choose the appropriate combination of channels for your experiment. You can choose to flow over channels 1-2 or channels 3-4 (useful for using one chip for 2 experiments) or you can flow over Channels 1-4 if you want to attach target to channels 1-3 and use channel 4 as the reference. Enter names for Buffers 1 and 2 in the appropriate fields and choose a temperature set point for the experiment (10°C below

ambient to 70°C) and the desired data sampling interval (0.1 seconds to 10 seconds in Hardware mode). Press the Save button to record your changes.

Once an experiment is selected, you can create related experiments easily by creating a Child experiment, which copies all experiment parameters and then allows new changes by the user. Click the “Create New Child Experiment” button to create a Child based on the currently selected experiment. You will see the new Child experiment added to the experiment tree, below your previously selected experiment.

If you want to Delete an Experiment you can do so on the Project page. If you Delete a Child Experiment, just that experiment will be deleted. However, if you Delete a parent Experiment, the individual Experiment and all its Child Experiments will also be deleted. Also, if you choose to Delete a Project, the Project and all the Experiments under it will all be deleted.

COUPLE EXPERIMENT

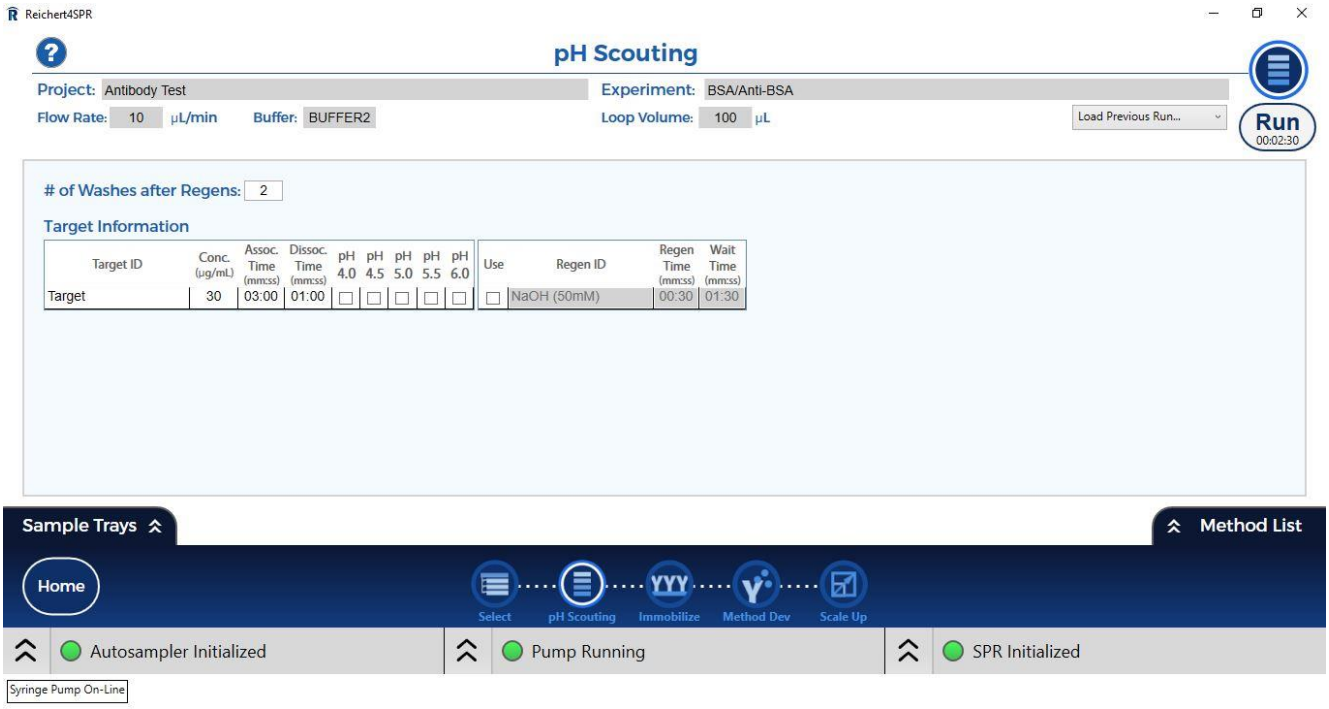
This experiment type is chosen when you want the target to form a covalent bond to the sensor chip surface. The target can be attached via either a primary amine or thiol group.

pH Scouting

From the Workflow buttons at the bottom, select pH Scouting. You only need to perform pH scouting if you do not know the proper pH to use for coupling. Generally, you will want to dissolve the target in buffer that has a pH that is 1 to 2 units below the pI of the protein being coupled. Common diluents are 10 mM sodium acetate at pH values ranging from 4 to 6.

Note that solutions will flow over Channel 1 only at a flow rate of 10 μL/min. Also, if you need to do a regeneration after the target injection, you can program the number of washes needed to clean out the loop before the next target injection.

pH Scouting



Information needed to Run pH Scouting

1) The Project, Experiment, Flow Rate, Buffer and Loop Volume information at the top will automatically be populated and cannot be edited on this page.
2) The number of Washes after Regens can be varied in whole numbers from 0 to 6.
3) The Target ID and Concentration (µg/mL) should be entered by the user.
4) The suggested Assoc. and Dissoc times can be edited by the user if needed.
5) The pH values to be tested should be checked.
6) If a Regen solution is needed, the box should be checked and the Regen ID, Assoc. and Dissoc. times should be edited as needed.
7) The Run button in the upper right corner will indicate the estimated run time.

Load previous run

Located just to the left of the Run button (see above), this drop down displays a listing of all previous pH scouting runs created in this Project/Experiment (in the order they were run). Selecting one from the list will open the file to view the data.

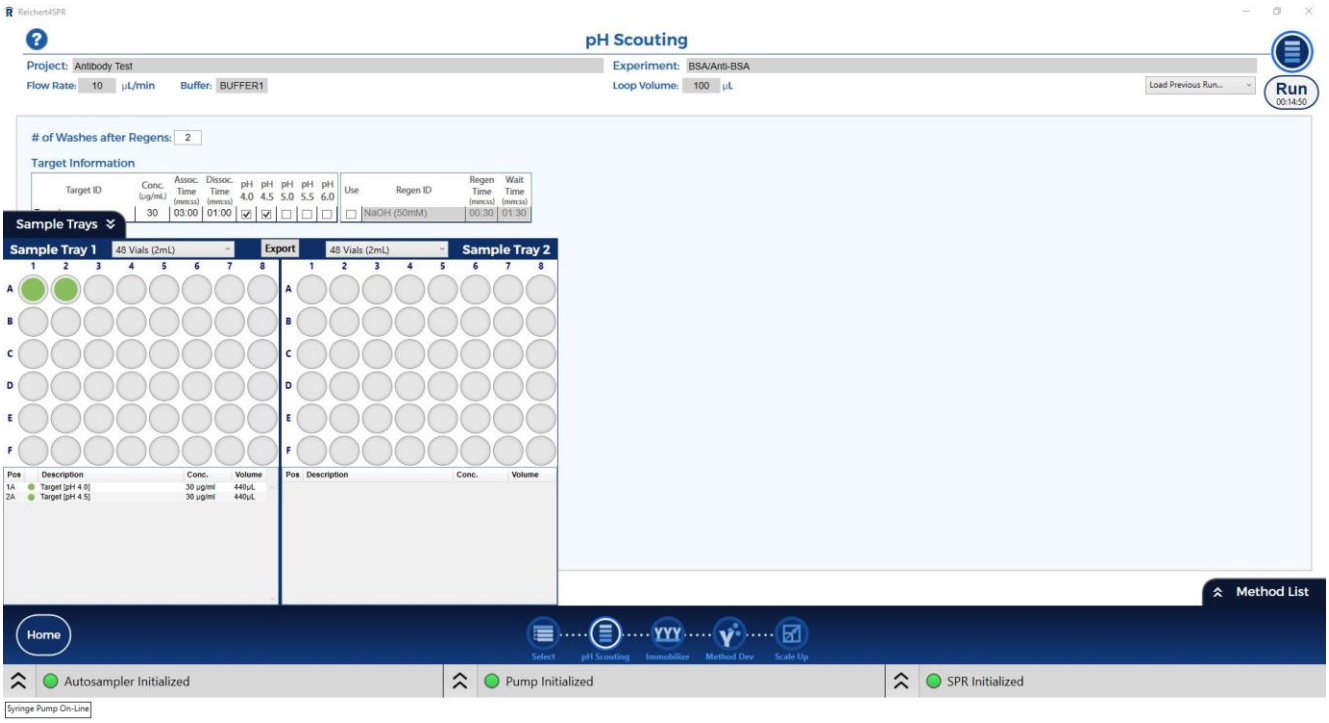
Help

The question mark in the upper left part of the page is the Help for this section (each page has its own Help (?)).

Sample Trays

Clicking the Sample Trays tab on the bottom left of the screen will bring up an image of the sample trays and will indicate where samples should be placed (see diagram). The dropdown lists at the top allow you to select the type of tray(s) you plan to use. The position, description, concentration and volume needed in each vial will be listed below the sample tray picture: If you would like to manually rearrange the samples in the trays, simply click and drag samples from one position to another in either tray. Keep in mind, however, that if you change anything about the samples being injected (eg. How many injections or the association or dissociation times), the sample positions in the tray will revert back to their original places.

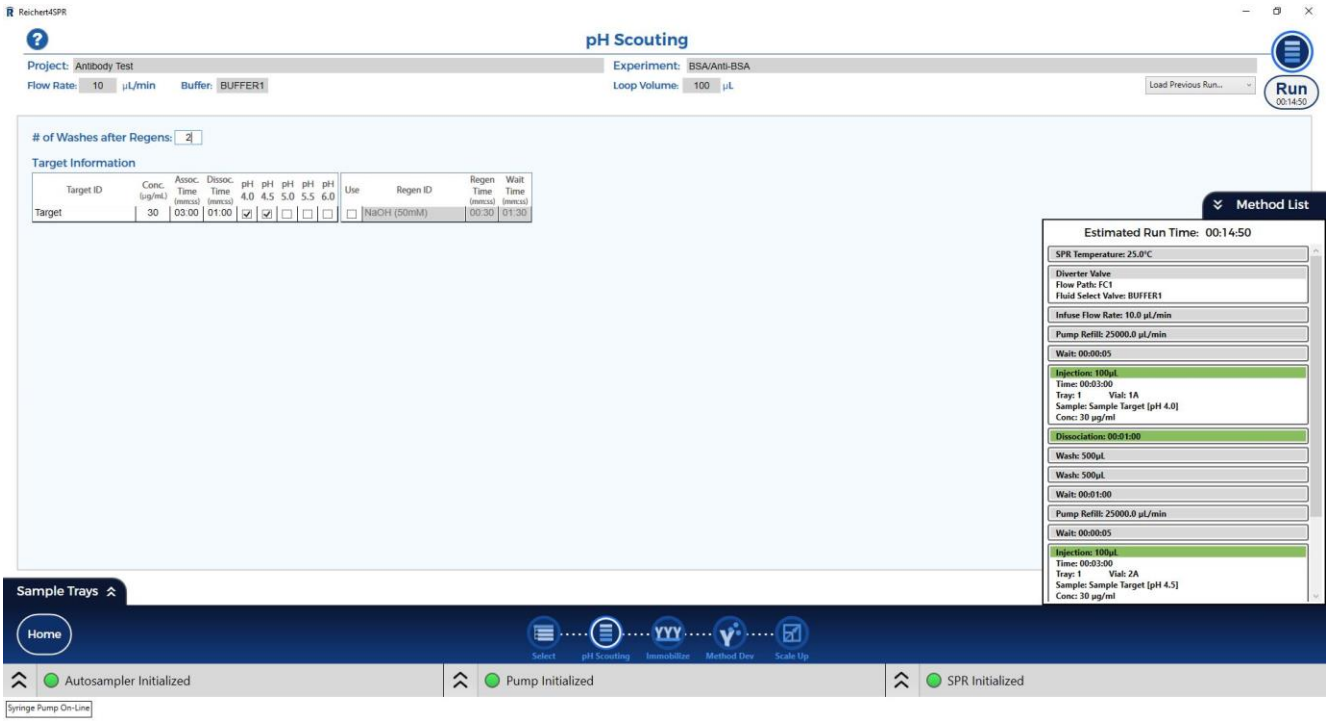
Sample Trays



Method List

Clicking the Method List tab will bring up a list showing the set of steps that will be executed by the instrument for the experiment and the total estimated run time. This is provided for informational purposes only. The method list is automatically created based on the sample information entered on the page.

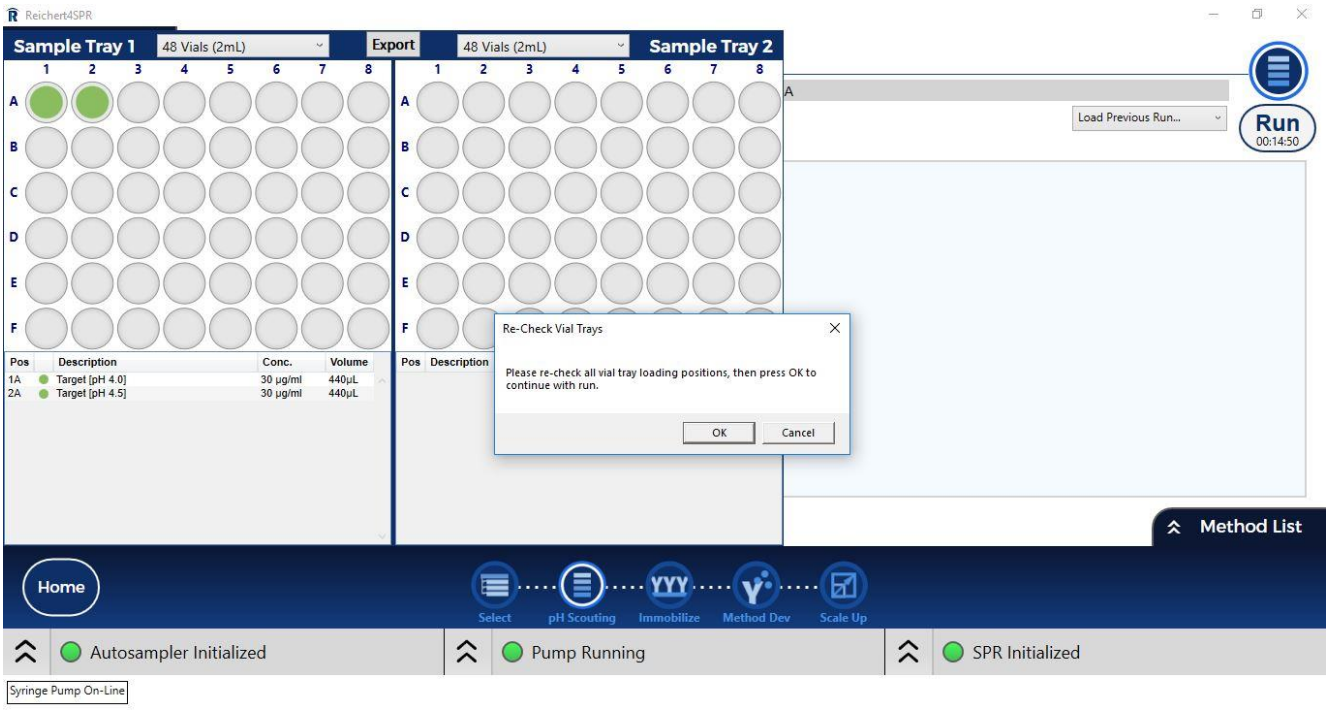
Method List



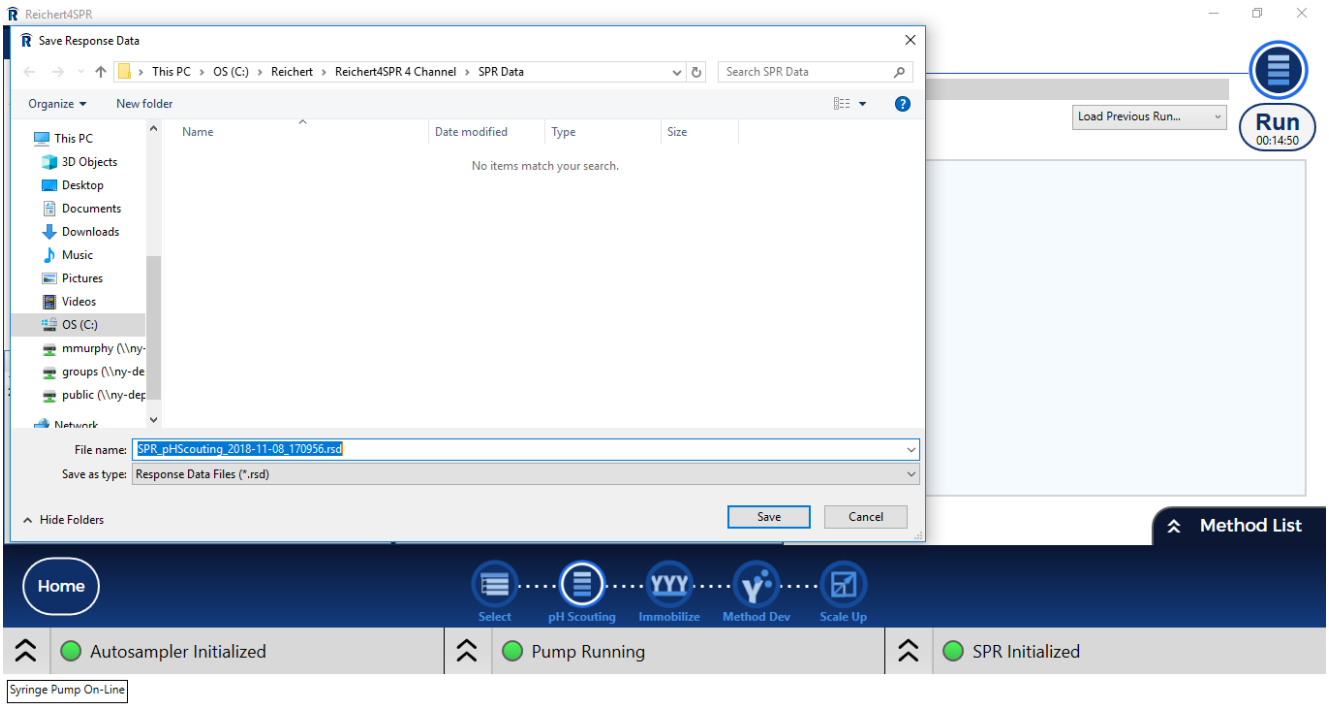
Run

Clicking the Run button will change the Pump flow rate to whatever the setting is on the current page. The Sample Tray will pop up for you to confirm where the samples are in the tray (you can adjust if needed). If you say OK, you will then be asked for the file name - default name is SPR, then step in project, then date and time – you can change the name or add additional information to the default name. Once you hit Save, the steps shown in the method list will be run.

Tray Check



Save Response Data

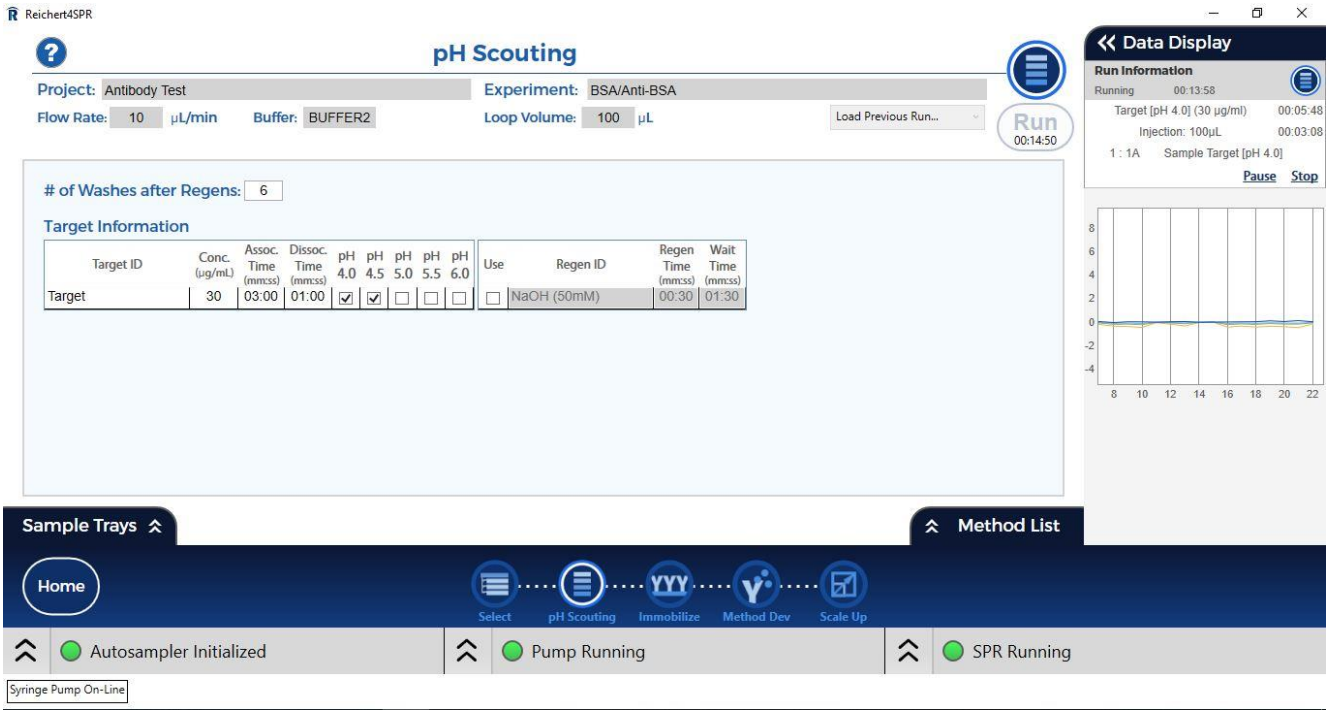


Data Display

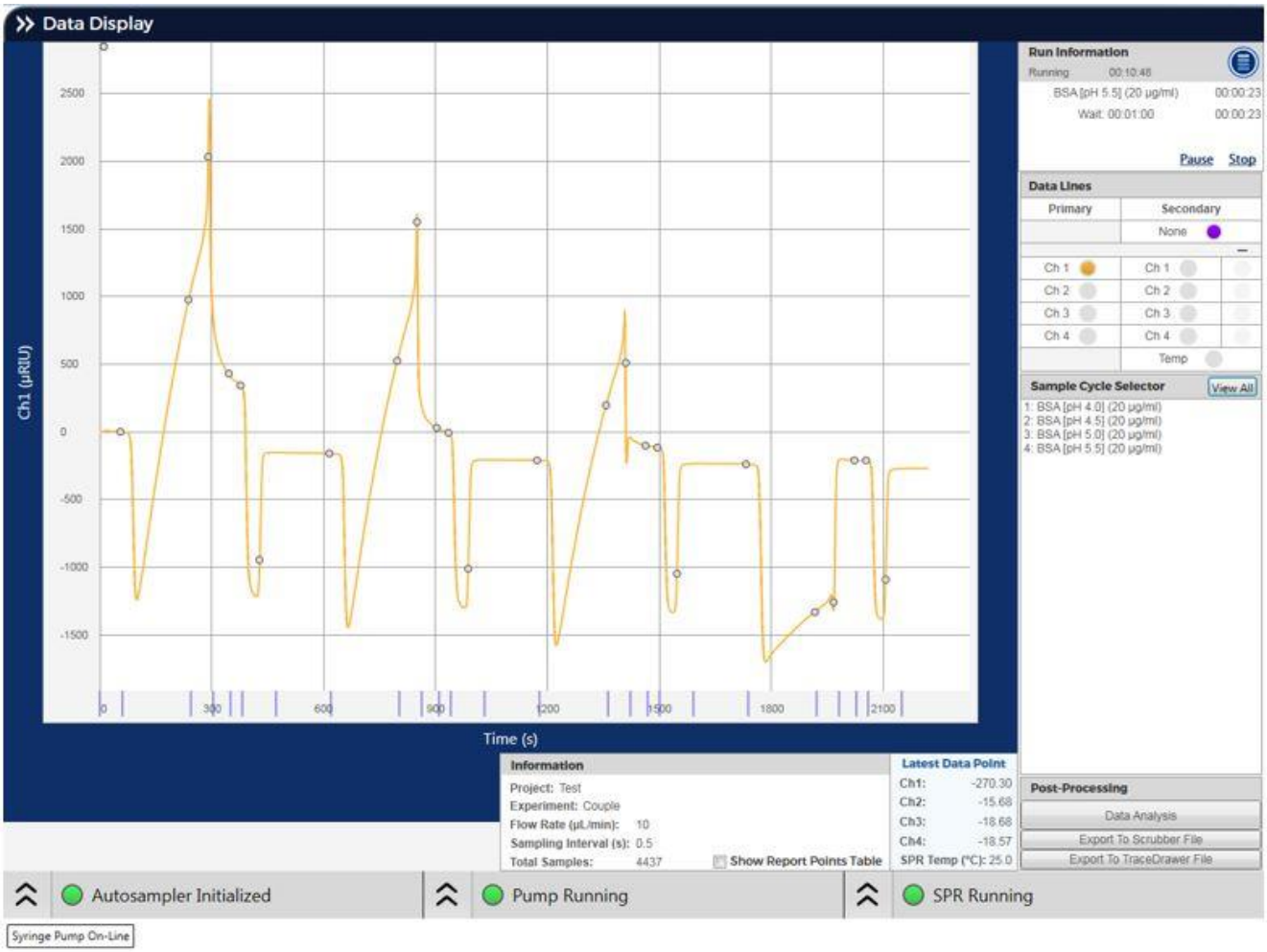
Once an experiment is running, an information panel will appear on the right side of the application. The Run Information box will display the estimated time for the complete run along with the estimated time for the current step. Clicking Pause will pause the run at the end of the current step. Data collection will continue, but experiment steps will not proceed until Resume is clicked. Clicking Stop will stop the experiment run. It will take a little time to reset the hardware and stop the data collection. If you select Stop, then select Run again on the same page, you can Append to the current file. If you leave the page and come back, Append is no longer active.

A small plot of the data for the current injection cycle is visible any time experiment data is loaded.

Run Information Sidebar



Clicking the Data Display tab will open an enlarged view of the experiment data. If an experiment is currently running, the data plot will show the current injection cycle. Selecting a cycle in the Sample Cycle Selector list will display the data for that cycle. Clicking the “View All” button will display the data for the entire run.

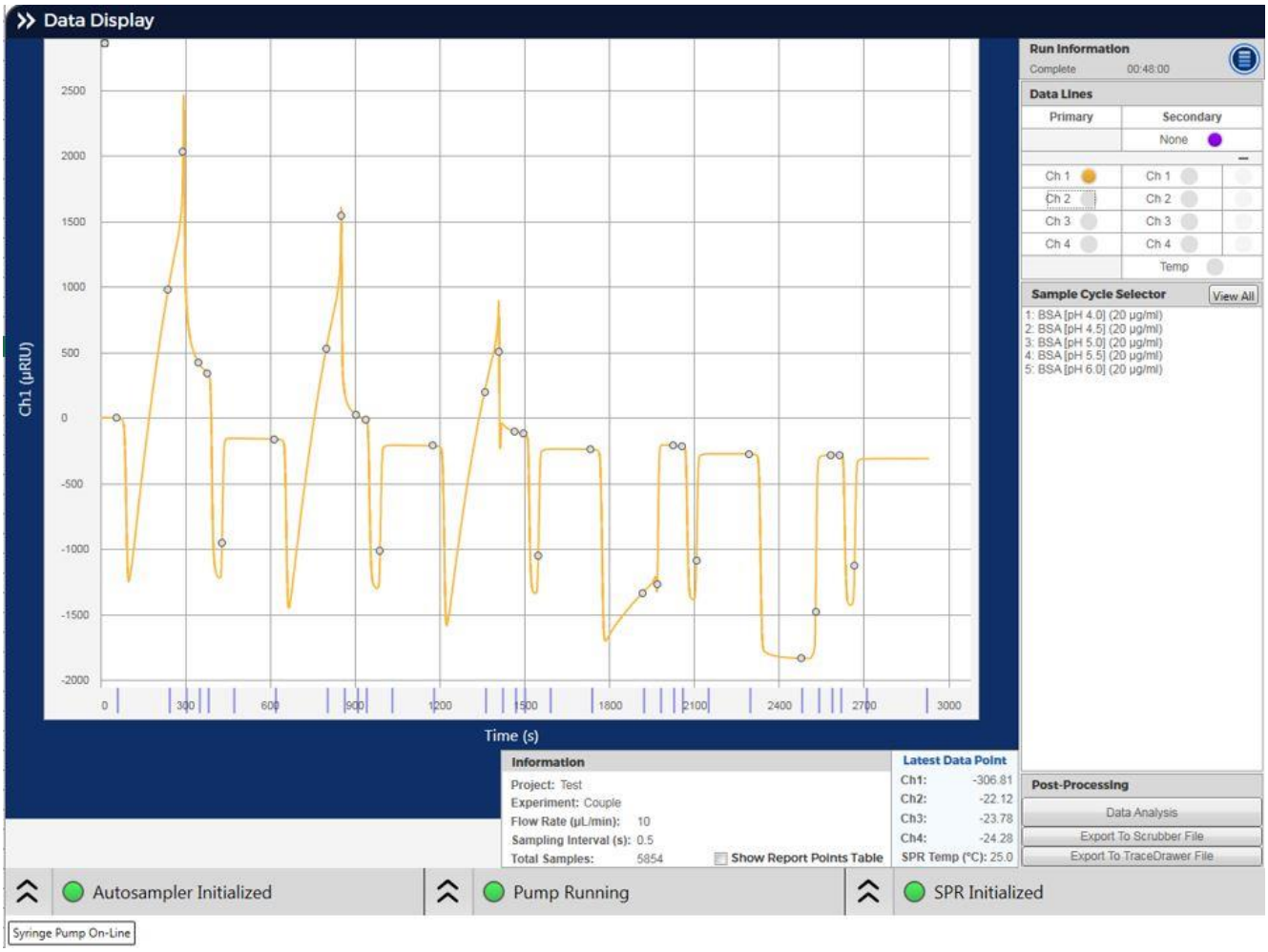


You can zoom in on the run data by doing a left-click and then dragging a box around the portion you would like to enlarge. If you right-click and drag, you can slide the data in the graph window (this works best when the run is stopped). Left-click and drag on a Y-Axis to adjust the visible range of that axis. Double-clicking on the graph will return to the full view of the selected injection cycle.

The Data Lines section allows you to select which channels' you want to be visible in the graph. Click the colored buttons in the Primary section to enable or disable viewing of data for Channels 1-4. The Secondary section allow you to add a secondary Y-Axis to view the temperature or channel difference responses. Click the Temp button to show Temperature and you will see the Temperature axis appear on the right side of the graph (+/- 2 degrees C). Selecting a secondary channel will display that channel on the right axis and will enable the second column of (-) channel buttons. Selecting a channel in the (-) column will display the difference between the two selected secondary channels on the right axis.

Below is an example of a pH scouting experiment using BSA as the target. Note that solutions only flow over Channel 1. You need to look at the slope of the rise to determine which is the optimum pH. In the example shown below, BSA was diluted in 10 mM sodium acetate at pH values of 4, 4.5, 5, 5.5 and 6. Note that the slope of the rise is significantly less at pH 5.5 and 6 compared to lower pH values. So, pH 5 or below would be preferred as the immobilization buffer.

Example of View All for pH Scouting at End of Run



Report Points

Pre-defined Report Points are automatically created during execution of the experiment for all experiments containing injections. They can be used to quickly see calculated difference amounts and are most commonly used to assess how much target has been immobilized.

The report points mark results at critical points and are shown as circles on the sensorgram data plot. Checking the **Show Report Points Table** checkbox at the bottom of the data plot screen will display a table of all report points.

The following definitions describe the report point data:

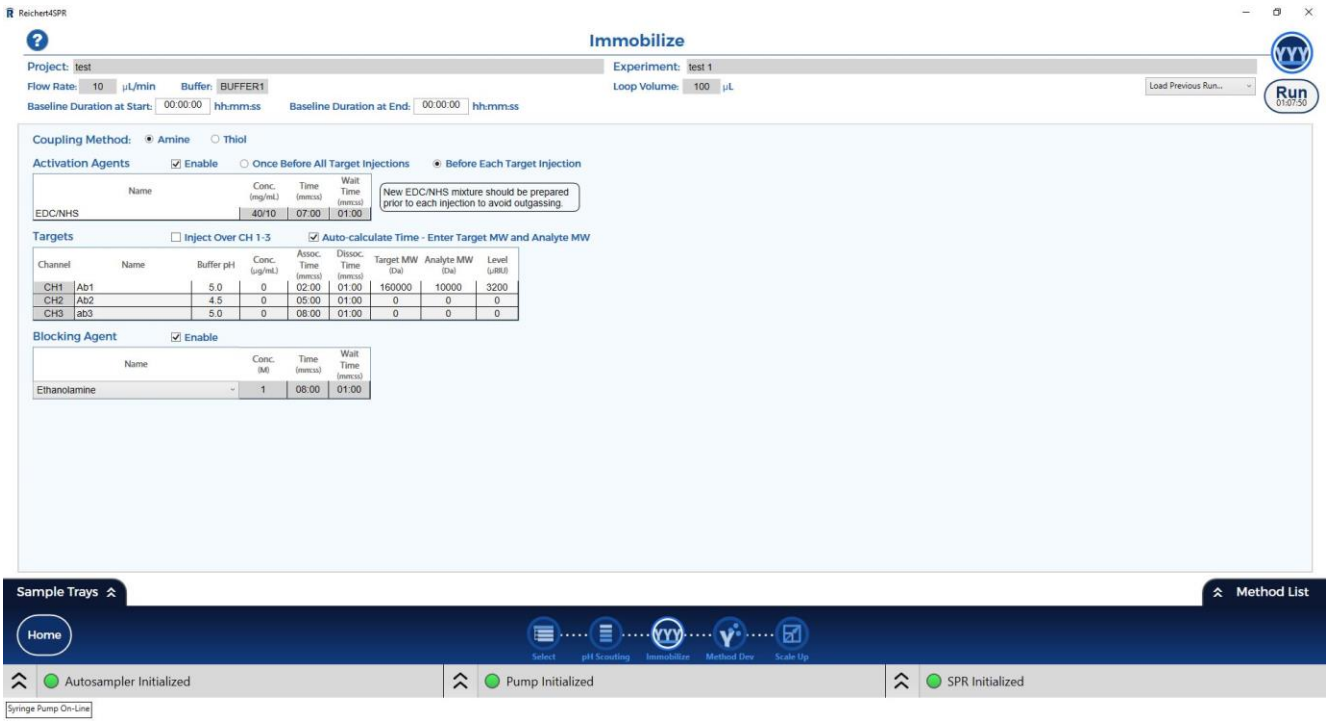
FC - The flow cell data plot curve containing the report point.
Type - The type of report point.
Cycle - The injection cycle associated with the report point.
Time(sec) - The time at which the report point is reported.
FlowRate(µL/min) - The syringe pump infuse flow rate used during this injection cycle.
FC Flow Path - Flow cell flow path during this injection.
Position - Position of report point.
Window(sec) - The time window for which some report point calculations are done.
Response - The µRIU or pixel response at the report point.
AvgResp - The µRIU or pixel response calculated as the mean value over the time window.
RelResp - Relative response difference from the report point to the baseline.
StdDev - Standard deviation of data points in the time window.
Sample - The sample associated with this report point's injection.
Conc - Sample concentration.

Immobilization

Select the next step in the Workflow, Immobilization: Once you know the proper pH to use for coupling, you can attach your targets to the sensor chip via either a primary amine or thiol group.

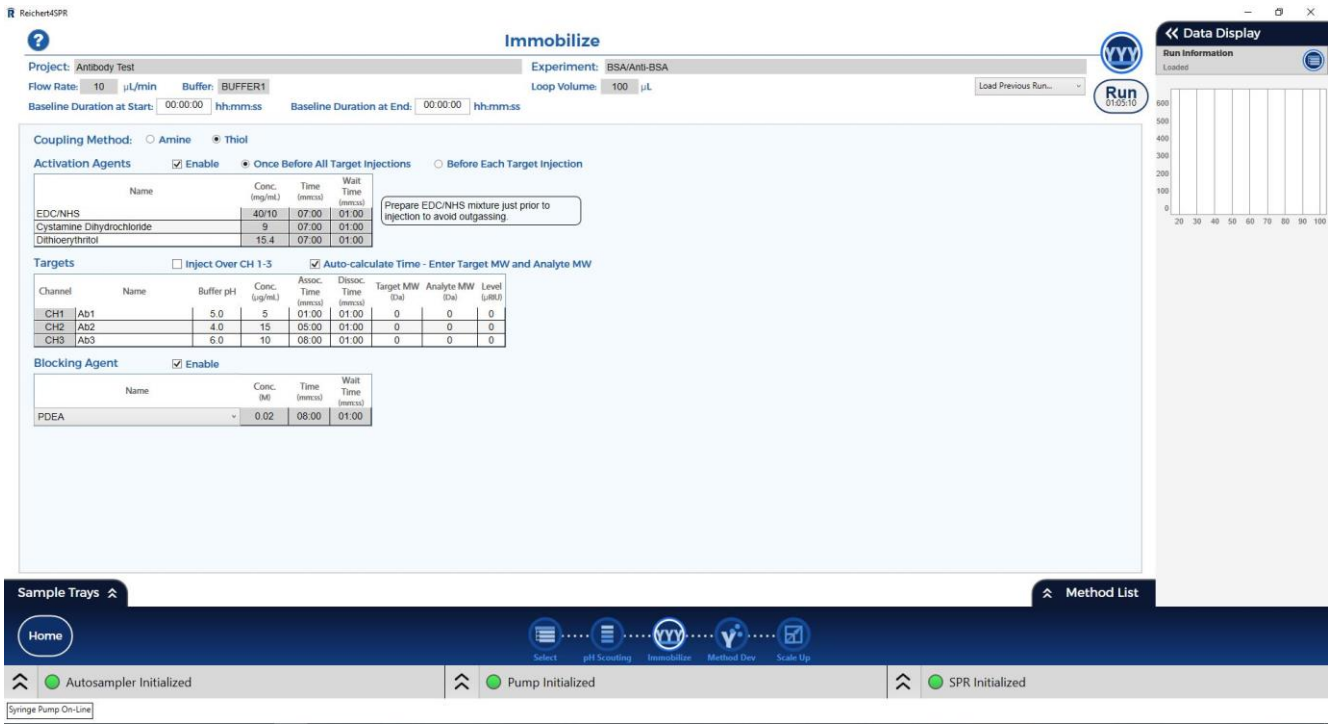
The first step in amine coupling is to inject a mix of EDC and NHS (40 mg EDC and 10 mg NHS per mL of water). You can inject the EDC/NHS over all 4 channels at the same time or channel 1 then 2 then 3-4 depending on which box you select next to Activation Agents. After the EDC/NHS linker is attached to the surface, a protein with a primary amine can be coupled to the surface via amide bond formation. When injecting Target, you can either inject over 1, 2 and/or 3 individually or over 1-3 at the same time to save on sample. Sites on the sensor chip surface that are not filled by target are then blocked by flowing 1 M ethanolamine HCl pH 8.5 over the sensor chip. Ethanolamine is injected over all active channels unless the Blocking Agents box is unchecked.

Immobilize via Amine Coupling



The first step in thiol coupling is also to inject a mix of EDC and NHS (40 mg EDC and 10 mg NHS per mL of water). After the EDC/NHS linker is attached to the surface, 40 mM cystamine dihydrochloride and then 0.1 M dithioerythritol are also injected. Next, the protein with an available thiol is coupled to the surface in acetate buffer. Sites on the sensor chip surface that are not filled by target are then blocked by flowing 20 mM PDEA mixed with 1 M NaCl over the sensor chip. Options for injecting EDC/NHS and target are as listed above for amine coupling.

Couple via Thiol Coupling



Setup of screen:

1) The Project and Experiment information at the top, Run button, Load previous run selector, and Help serve the same function as described for pH scouting. Flow rate of 10 μL/min and buffer (Buffer1 or Buffer 2 depending on what you chose for Prime) are automatically filled in.
2) You can collect baseline for a set period of time (hh:mm:ss) before and/or after the experiment starts.
3) Choice of the Coupling Method – Amine or Thiol – will populate the rest of the page with the required chemicals and will indicate the proper concentrations for the Activation agents and Blocking Agent sections.
4) If you check the box next to Activation Agents that says Once Before All Target injections, the activation agents will flow over all channels once before target is injected. This choice is needed if you plan on injecting Target over channels 1-3 with one injection. If you check the box Before Each Target Injection, each channel will be activated separately before each target is injected. For this choice, a note pops up saying you should replace the EDC/NHS each time to prevent outgassing.
5) In the Target section, if you check the box Inject Over CH 1-3, only the first box will be visible and will list Ch123.
6) Also in the Target section, if you check the box Auto-calculate Time and also fill in the Target and Analyte MWs in daltons, a suggested immobilization level (μRIU) will be calculated and an association time will be automatically filled in.
7) If you uncheck the Activation Agents section or the Blocking agents section, then it will be skipped.
8) Organization of Sample Trays, Method list and Data Display are the same as for pH Scouting.
9) Data display, report points and Save work the same way here as in pH scouting

Run

For Immobilization, EDC/NHS is either flowed over all four channels at once or channels 1 and 2 individually then 3 and 4. Thiol coupling is handled similarly except that cystamine dihydrochloride and dithioerythritol are also injected after the EDC/NHS. You can flow either different targets or varying concentrations of the same target over the surface separately or use just one concentration and time for all 3 sample channels (1-3) at the same time. In this example, EDC/NHS is flowed over all the channels at the same time and concentrations and contact times are varied for each of the separately injected targets for an amine coupling experiment.

Amine Coupling View All

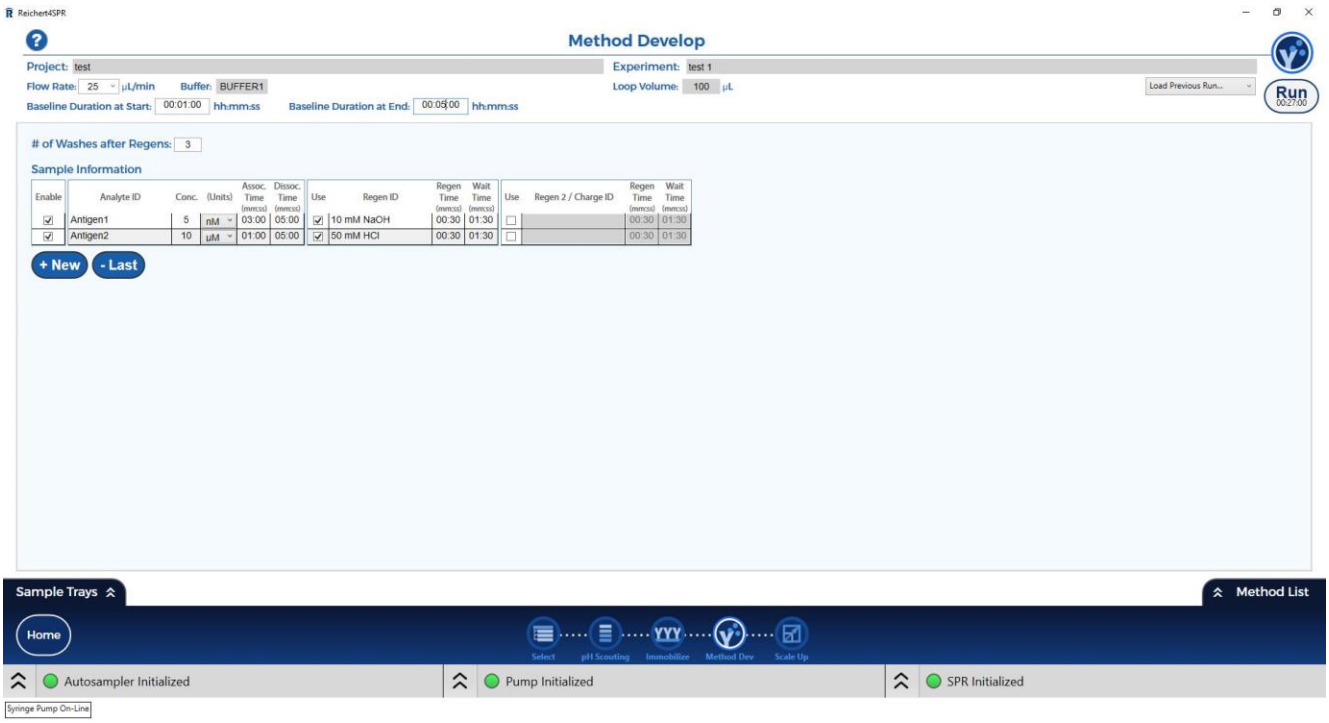
Method Develop

The next step is to work out experimental details by performing test injections. The method development section makes it possible to quickly run tests with maximum flexibility and ease. A Method Development sequence can be used to inject sample (with or without Regeneration) and then see if the injection returns to baseline. If the sample returns to baseline, then the injection should be repeated. The user could also run a blank, then select Data Analysis and get an estimate of the kinetics. Users can just program one or two regeneration injections to remove loosely bound target after immobilization.

Setup of screen:

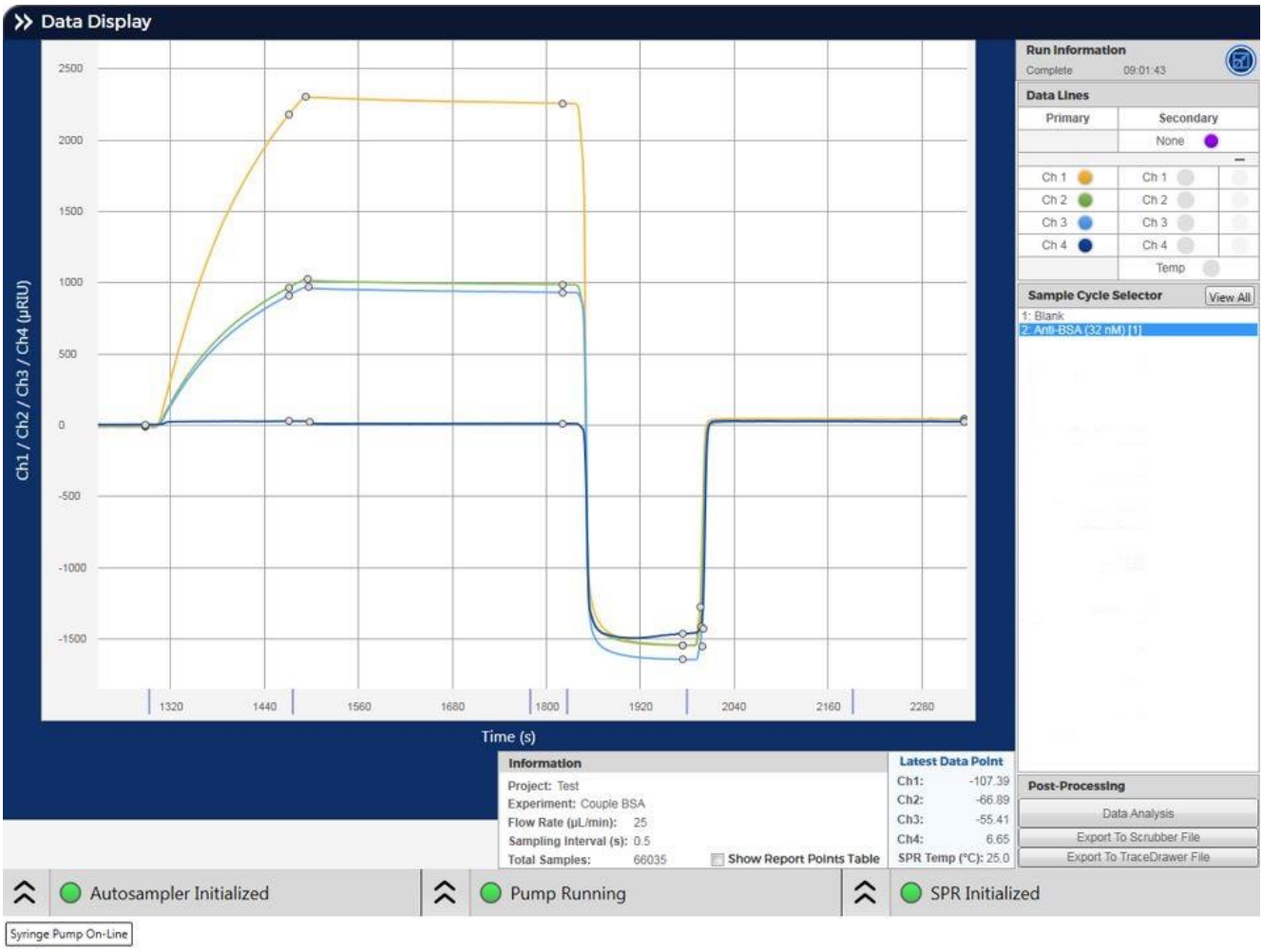
1) The Project information at the top, Run button, Load previous run selector, and Help serve the same function as described for pH scouting.
2) You can collect baseline for a set period of time (hh:mm:ss) before and/or after the experiment starts.
3) You can change flow rate, Analyte, concentration, Analyte Association and Dissociation times and Regeneration conditions (up to 2 separate regenerations can be completed per analyte injection). You can also type in the number of Washes you want after each Regen.
4) Organization of Sample Trays, Method list and Data Display are the same as for pH Scouting.
5) Data display, report points and Save work the same way as in pH scouting.
6) Selecting +New will add one additional line in the Sample Information list. Selecting -Last will remove the last line from the Sample information list.

Method Develop



An example sample cycle could look as follows – in this case Anti-BSA binds to immobilized BSA and the surface is regenerated with 20 mM HCl.

One sample cycle



Scale Up

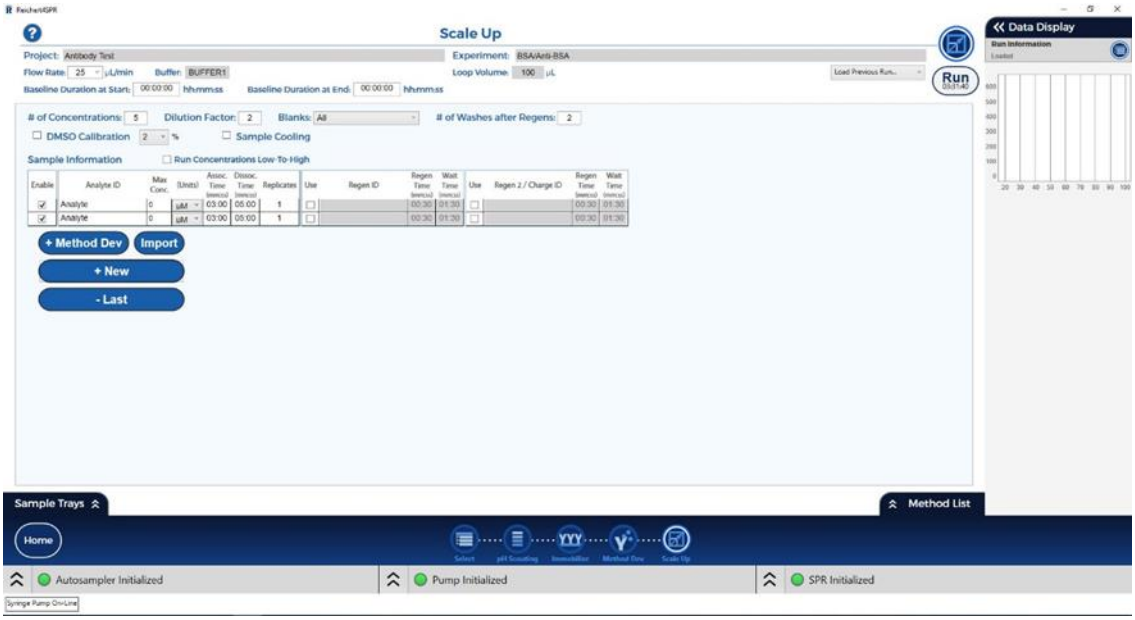
Once conditions have been worked out for running your samples, the experiment can be scaled up. The Scale Up screen has been designed to enable quick set-up and minimize data entry requirements. Additionally, there is the option to import a sample set from Excel.

Setup of screen:

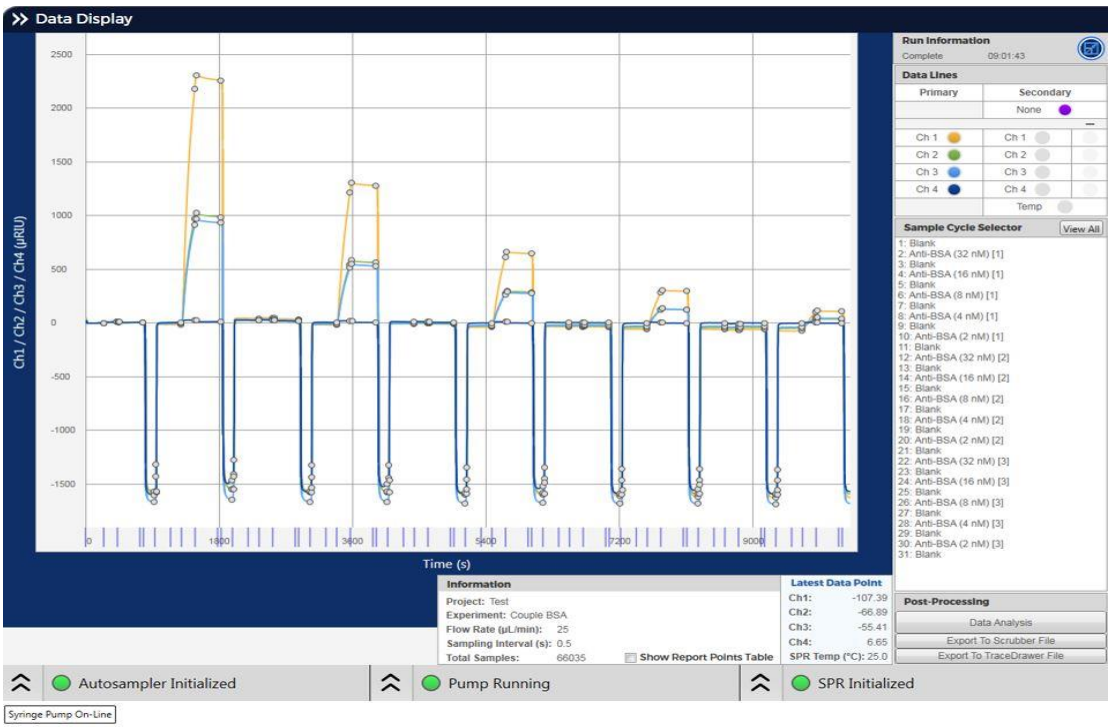
1) The Project information at the top, Run button, Load previous run selector, and Help serve the same function as described for pH scouting.
2) Select or enter the desired flow rate.

3) You can collect baseline at the beginning or end of the experiment for a set time.
4) For convenience, global settings for the experiment are at the top of the page. These include: number of concentrations of each analyte, Dilution factor and blank placement. These settings are used for all analytes in the experiment.
5) If DMSO correction is required, the box should be checked and % DMSO in running buffer chosen. DMSO correction is generally used whenever samples are dissolved in 100% DMSO and matching to the running buffer to correct for sample excluded volume is needed.
6) If the samples need to be refrigerated at 4 degrees C, the Sample cooling box should be checked (cooling will stay on until you turn it off or until the program is closed).
7) Analytes will be injected from High to Low concentration by default. If you prefer to run the samples from Low to High concentration, check the “Low to High” box.
8) You need to fill in the analyte ID, stock analyte concentration, Association and Dissociation times and Regeneration conditions, up to 2 regeneration injections in a row can be programmed, check or uncheck first and/or second box. You can also choose the number of Washes after each regeneration in whole numbers from 0 to 6. If you are doing replicates, the replicate injections are repeated at the end of the original concentration sequence. Enter the number of replicates in the box that precedes the regeneration boxes.
9) If you want to add in the last line from the Method Develop screen, select +Method Dev, if you want to add another line, select +New. If you have added lines, an additional box -Last will also be available to select.
10) Alternatively, to handle a large number of analytes, you can select Import and import the sample information from Excel. A template can be found on the flash drive that came with your instrument in the folder: C:\Reichert\Reichert4SPR 4 Channel
11) Organization of Sample Trays, Method list and Data Display are the same as for pH Scouting.
12) Data display, report points and Save work the same way as in pH scouting.

Scale Up



Selected Results



CAPTURE EXPERIMENT

Method Development Capture

Types of Capture Experiments:

Check boxes for four types of capture experiments are available for selection. The experiment types are Antibody capture, capture of a His-tagged protein over a Ni-NTA surface, capture of a biotinylated protein over Streptavidin or NeutrAvidin and Sandwich assay. Experimental setup will automatically adjust based on the choice. Generally, a protein is captured over a surface, then an analyte is injected and then both are removed using regeneration solution(s). The capture protein concentration does not vary, only the analyte concentration. Because capture via a Streptavidin or NeutrAvidin tag is stable, only the initial capture is needed.

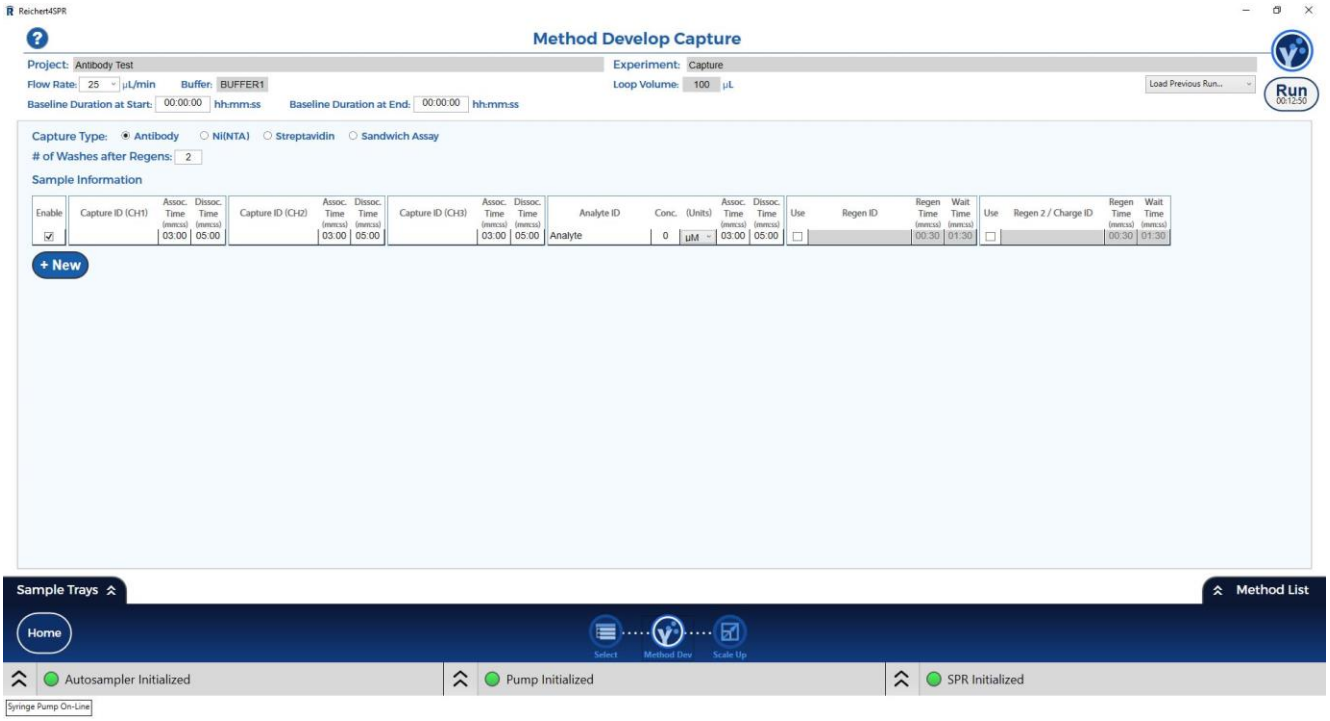
Method Develop provides a quick way to do a few test injections prior to scale up.

Setup of screen:

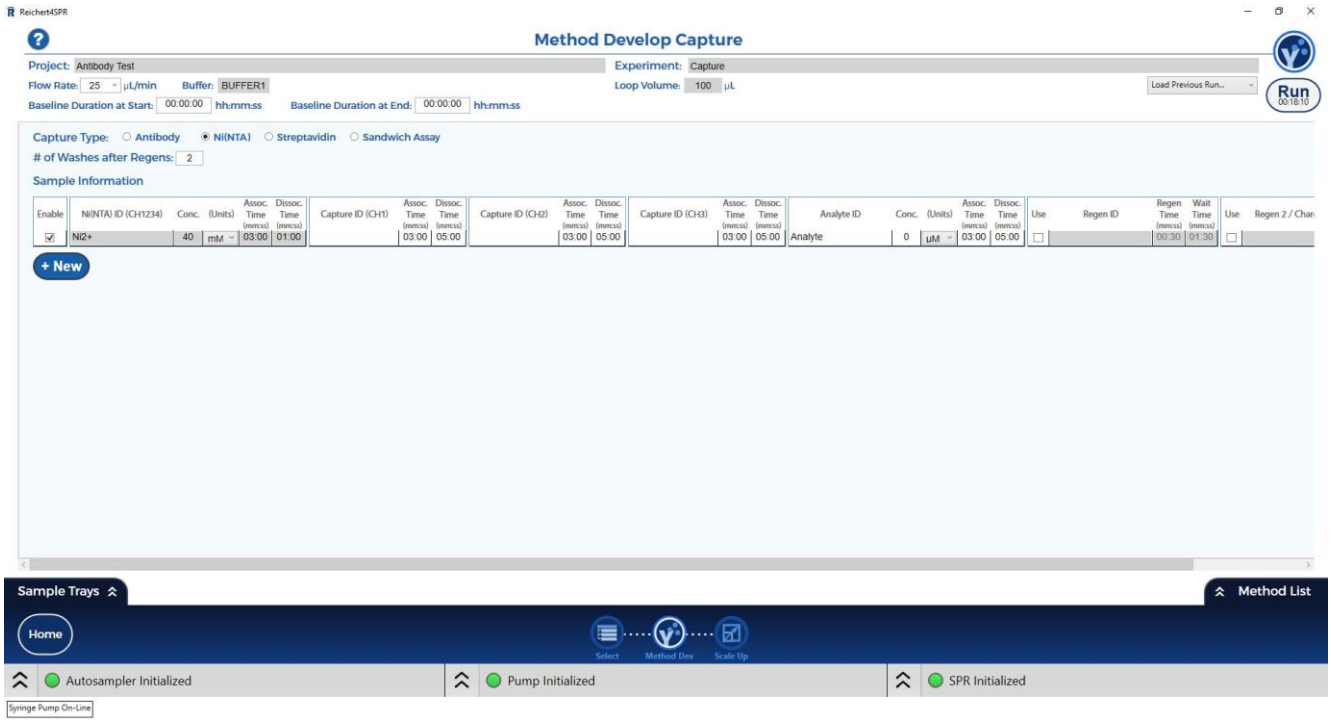
1) The Project information at the top, Run button, Load previous run selector, and Help serve the same function as described for pH scouting.
2) Select or enter the desired flow rate.
3) You can collect baseline at the beginning or end of the experiment for a set time.
4) Select the Capture type and the # of Washes after Regens (if needed).
5) For each sample, enter the capture agent name and its Association and Dissociation times, the analyte and its concentration, and the analyte association and dissociation times. Regeneration conditions (up to 2 separate regenerations can be done) should also be entered.
6) Organization of Sample Trays, Method list and Data Display are the same as for pH Scouting.
7) Data display, report points and Save work the same way as in pH scouting.

Method Develop Capture

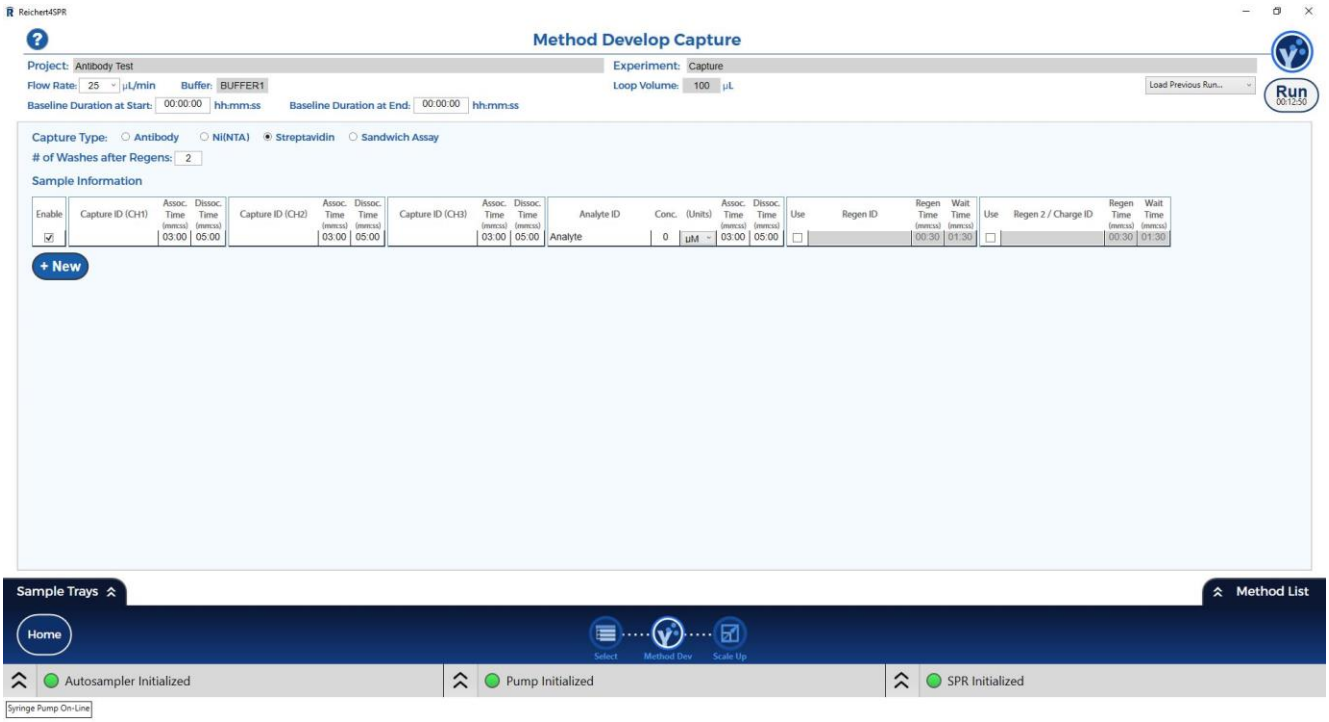
Antibody



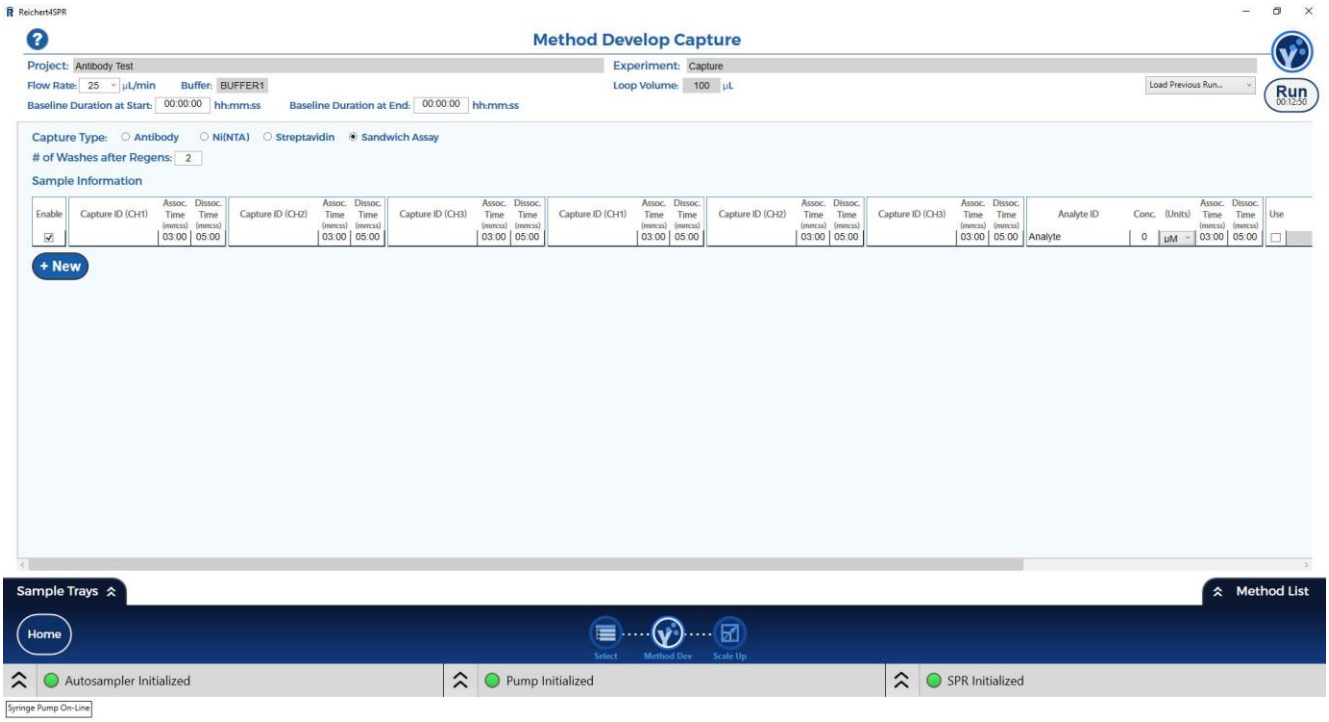
NiNTA



Streptavidin (or NeutrAvidin)

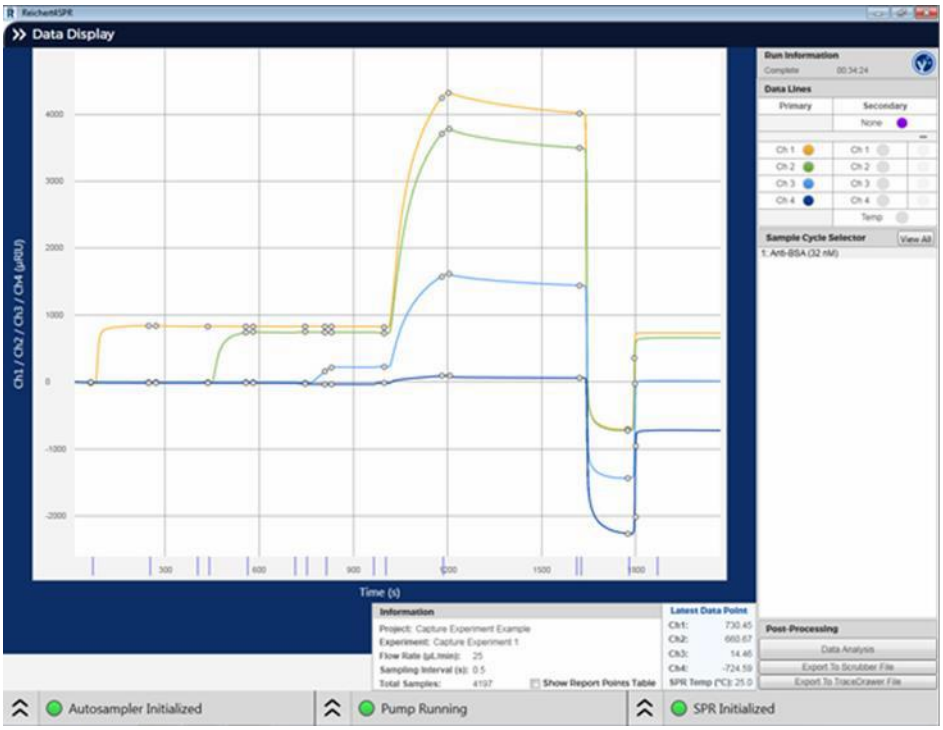


Sandwich Assay



An example is the capture of biotinylated BSA over a Neutravidin surface at 3 different concentrations on 3 different channels, followed by injection of Anti-BSA and regeneration with HCl.

Capture of Biotinlyated BSA over NeutrAvidin followed by binding to Anti-BSA



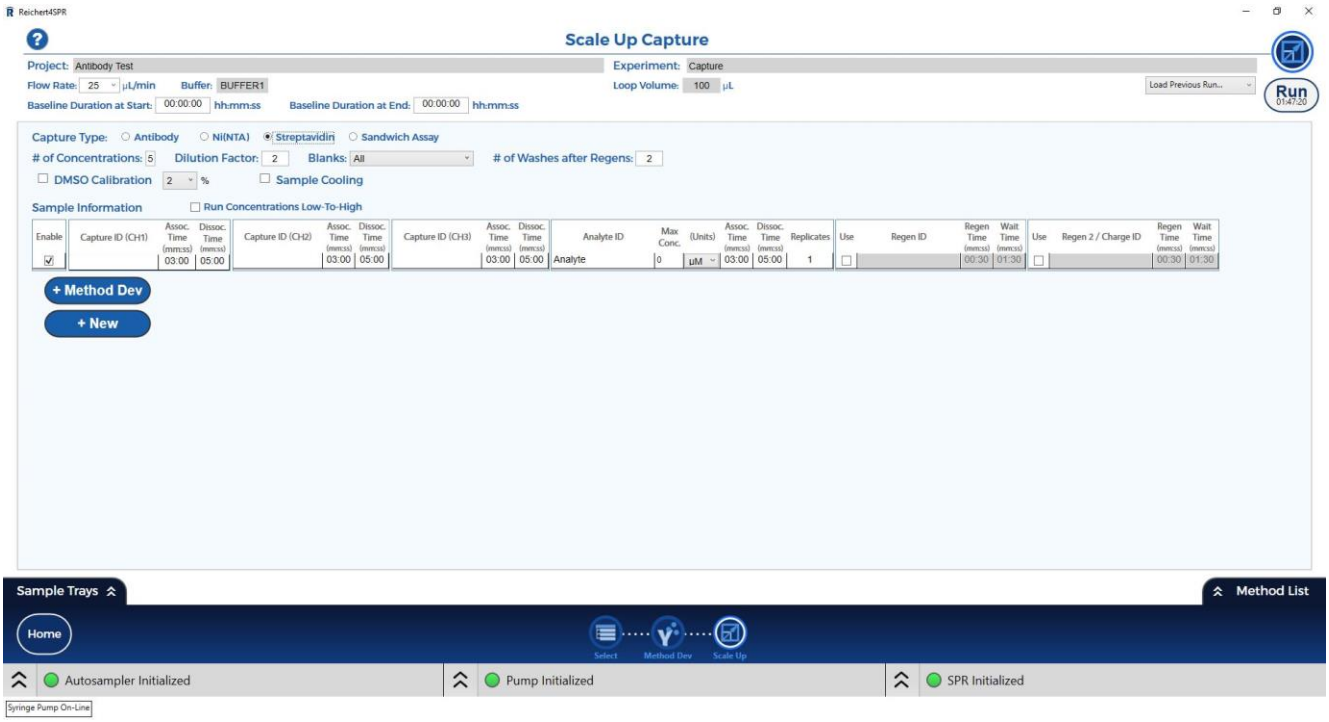
Scale Up Capture

Once conditions are worked out for running your samples, the experiment can be scaled up.

Setup of screen:

1) The Project information at the top, Run button, Load previous run selector, and Help serve the same function as described for pH scouting.
2) Select or enter the desired flow rate.
3) You can collect baseline for a set period of time (hh:mm:ss) before and/or after the experiment starts.
4) Find the experiment type and check the box next to it.
5) For convenience, global settings for the experiment are at the top of the page. These include: number of concentrations for each analyte, Dilution factor and blank placement. You can also choose the number of Washes after each regeneration (0 to 6 in whole integers). These settings are used for all analytes in the experiment.
6) The number of concentrations of analyte, Dilution factor and blank placement must be chosen.
7) If DMSO correction is needed, the box should be checked and % DMSO in running buffer chosen.
8) If the samples need to be refrigerated at 4 degrees C, the Sample cooling box should be checked (cooling will stay on until you turn it off or until the program is closed).
9) On this Scale Up page, you can edit the capture IDs and their association and dissociation times, the analyte IDs, analyte stock concentration, and number of replicates, analyte Association and Dissociation times and Regeneration conditions.
10) Analytes are injected High to Low unless you check the Low to High box. If you are doing replicates, they are repeated at the end of the original concentration sequence
11) Alternatively, to handle a large number of analytes, you can import the sample information from Excel. A template can be found on the flash drive that came with your instrument in the folder: C:\Reichert\Reichert4SPR 4 Channel
12) Organization of Sample Trays, Method list and Data Display are the same as for pH Scouting.
13) Data display, report points and Save work the same way as in pH scouting.
14) Selecting + Method Develop will add the last line from the method development page. Selecting + New will add a blank line. Once a line has been added,– Last will become available to remove the last line if no longer needed.

Scale Up Capture

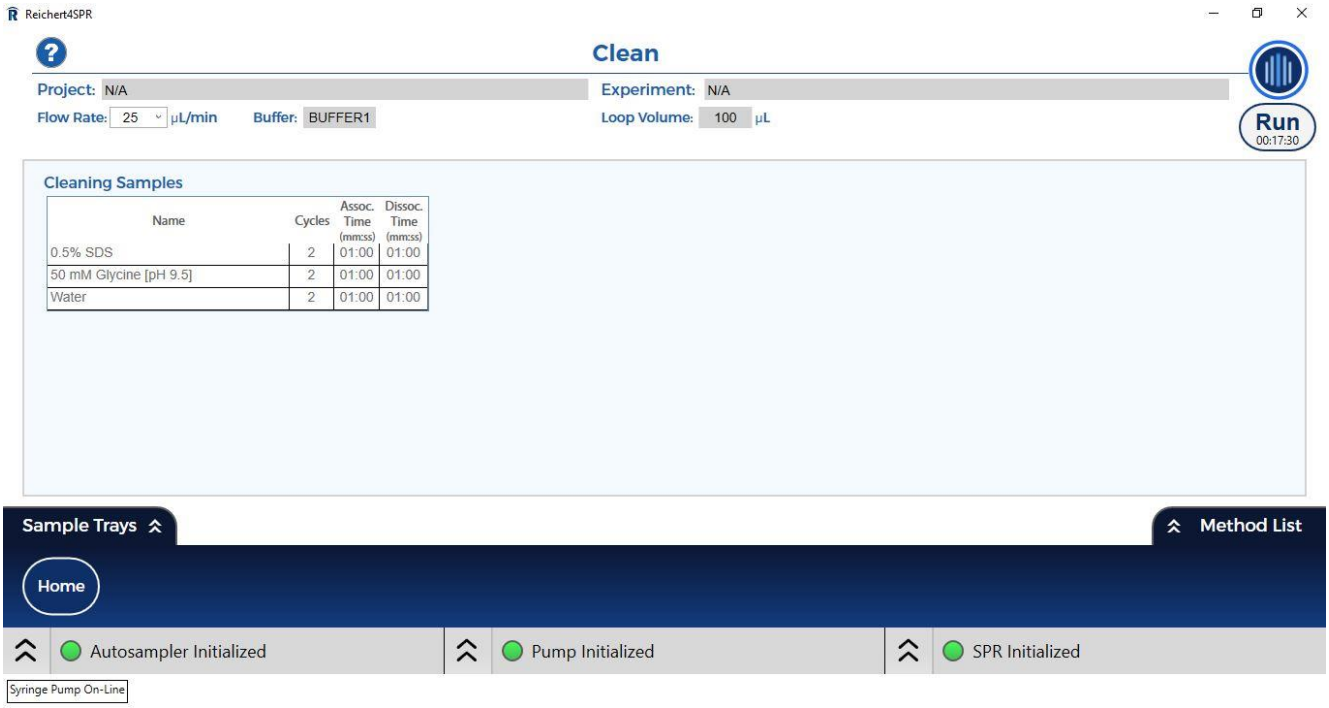


Clean

At the end of each week, it is suggested to clean the system with 0.5% SDS, then 50 mM glycine pH 9.5. These solutions remove adsorbed protein from the autosampler, all the tubing and the flow cell. In addition, the glycine solution will remove any residual SDS. Water is then injected to remove any residual cleaning solution.

Note: If your experiments are complete and you are preparing for Shutdown, you **must** first run Prime with water in both the solvent reservoir and autosampler wash bottle prior to running the Clean.

Choosing Clean on the Home page sends you to the following page:



Setup of screen:

1) The flow rate can be varied but the loop volume and cleaning solutions are preset.
2) You can open Sample Trays to see suggested locations for cleaning solutions, volumes and solution types.

3) You can also click and drag the vials to other locations in the tray if desired. The Run will take about 19 minutes.
4) The Method List will show each step in the run.

SHUT DOWN

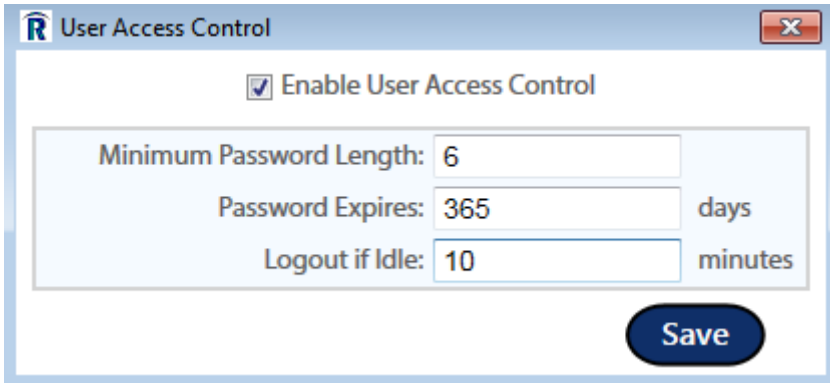
To shut down the instrument:

1) Run a Prime to water.
2) Run the Clean procedure.
3) Exit/close the software.
4) Power off the components (order is not important at shutdown).

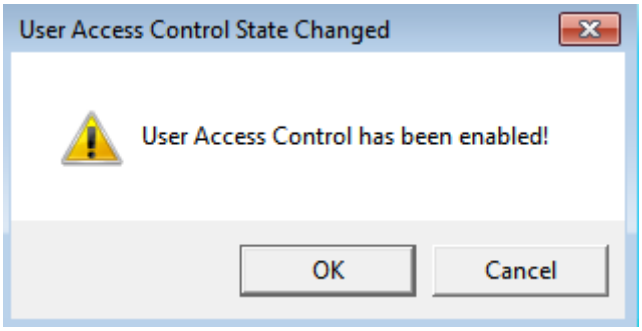
UAC (User Access Control)

User Access Control provides you with the capability to set up users and permissions for the instrument and track usage.

Choosing UAC on the Home page will open the following box. Note: values listed below are default suggestions and can be changed by the Admin user:



If you check Enable User Access Control and select Save, you will see the following message:



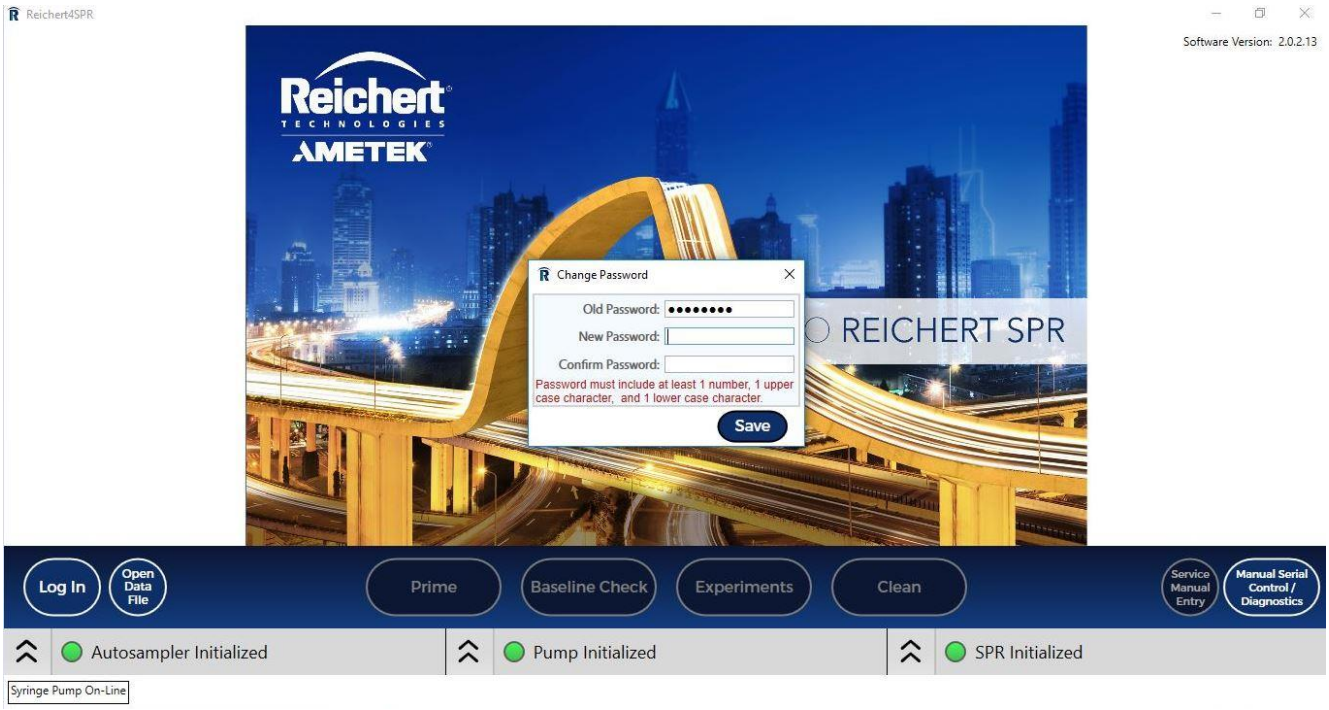
If you select Cancel, User Access Control will not be implemented. If you choose OK, all of the functions related to starting up the instrument and running experiments will be grayed out until you Log In.

Note: Only the Admin can turn off User Access Control once it is enabled.

The initial default login is: Username: Admin and Password: password.

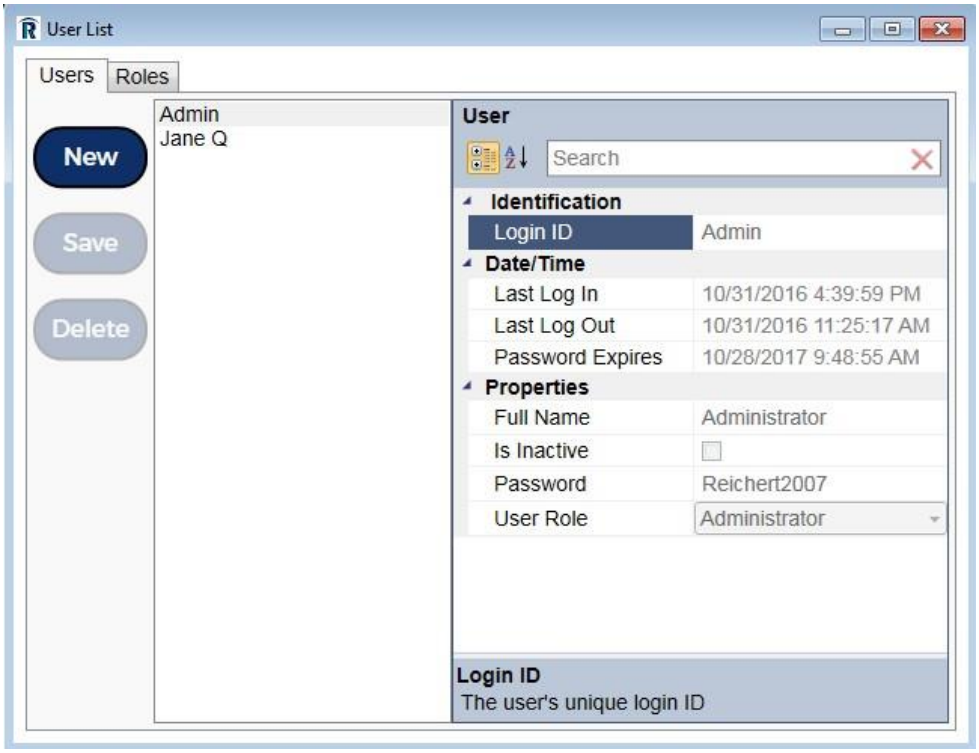
The first time when you click on Log in, you will be asked to change the password.

Your new password must be a combination of lowercase and capital letters and numbers and be at least 6 characters long. Once you click Save, you will be prompted to log in with your new password.

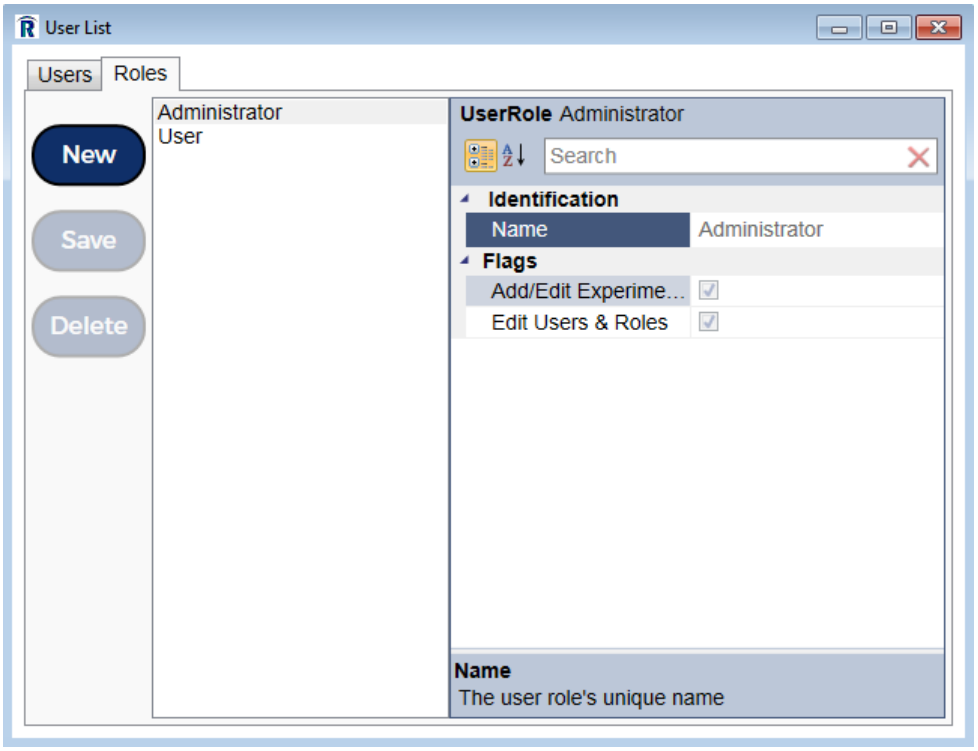


Once you are logged in as an administrator, additional choices will appear on the bottom right of the screen including User List and Change Password.

When you select Users List, on the Users tab, you can create, edit, and delete user accounts:

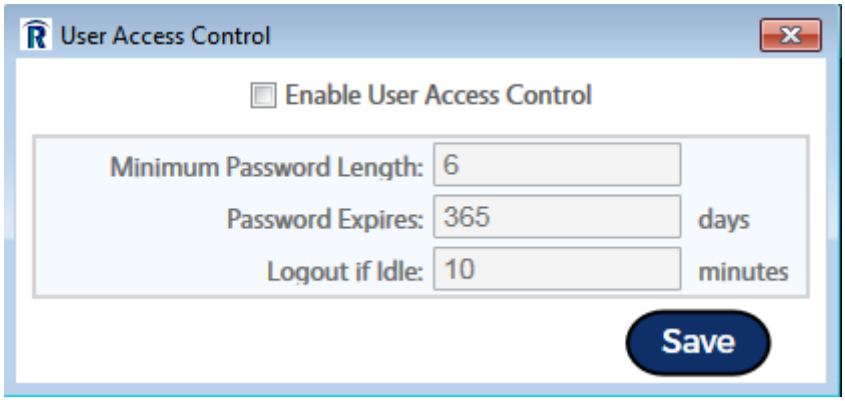


On the Roles tab, you can create, edit, and delete user roles.

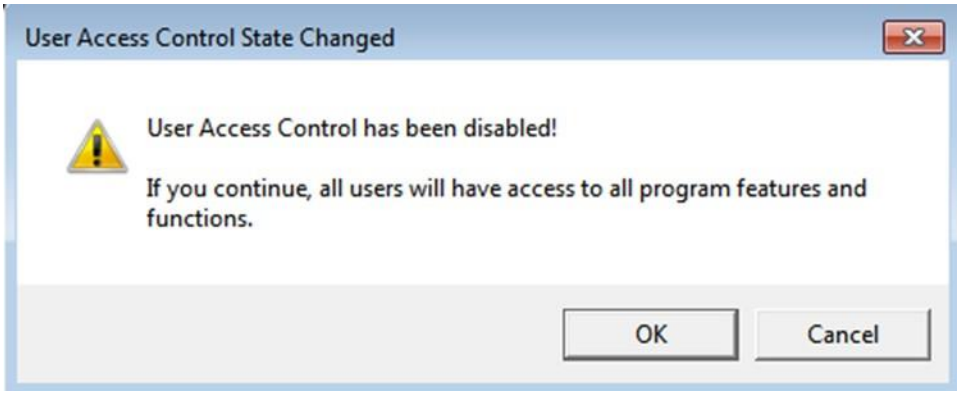


Even if you Log Out, User Access Control will be on when you next try to run an experiment.

Turning off UAC: UAC can only be turned off by the Administrator. To turn off UAC, select UAC, then uncheck the UAC box and select Save.



Once User Access Control is turned off, the following message will be seen.



Open Data File

This button allows you to open any data file collected with Reichert4SPR software (.rsd file). This is useful for opening files created on a different PC. For data files created on this PC you can either open files here or open each file through the Project/Experiment/Workflow page where it was created. Clicking the “Open Data File” button will open a Windows file dialog to allow the user to select a file to open.

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Service Manual Entry and

This selection is for Factory Use only.

Manual Serial Control/Diagnostics

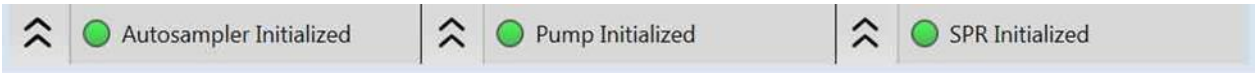
This selection is described on page 15 of this manual and for Factory use.

ADVANCED EXPERIMENTS

The Reichert4SPR software workflow has been designed to enable users to quickly and easily run the most common types of experiments. However, the software is flexible enough to enable a wide variety of experimental methods. This section describes additional capabilities and suggestions to take advantage of the flexibility required for advanced methods.

Hardware Direct Control

The direct control panels at the bottom of the screen enable users to adjust or change parameters outside of running a standard experiment. Clicking on the double-caret icon on either of the three status bars will open a pop-up panel where the user can edit hardware settings, send commands, and monitor hardware settings and communication. After making any changes in the upper hardware settings section, you must click Save Settings.



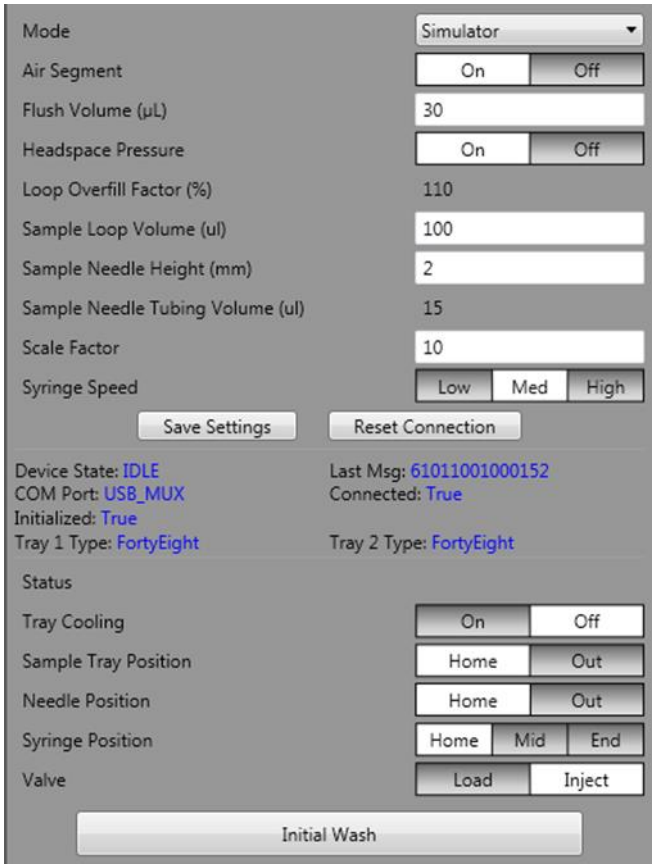
1) **Autosampler Direct Control** – shows all parameters used by the autosampler and allows the user to change parameters as shown below.
    - Mode (Simulator, Hardware) – Default is Hardware, choose Simulator if running on a computer not connected to an instrument (perhaps for training purposes, etc). **Note: You must click the "Reset Connection" button to apply the Mode change.**
    - Air segment (On, Off) – Default is On (it is not recommended to change this setting).
    - Flush Volume – cannot be adjusted and is set at 30 µl.
    - Headspace pressure (On, Off) – default is On. Headspace pressure is used to minimize risk of air bubble creation during sample pickup. It can be turned off, for example, when using well plates without covers.
    - Loop volume – default is 100 µl but acceptable range is 5-5000 µl.
    - Sample Needle Height – default is 2 mm, can be increased to 3 mm if needed.
    - Scale Factor – This setting fine tunes the speed of sample pickup. Valid entries are from 1 to 10, with 1 multiplying the speed by 0.1 and 10 multiplying the speed by 1. Default is 5. Recommend 5 or less if samples are stable and longer injections (> 50% of loop volume used) are done.
    - Syringe Speed – broader setting for how fast sample pickup is – recommended default is Low – you can adjust to Med if time is critical– can also do a fine adjust with Scale Factor

    Note: You must click "Save Settings" if any changes are made.

Direct Control buttons

- Tray cooling (On (4 degrees C), Off) – default is Off, turn On if samples need refrigerated storage.
- Sample tray position (Home, Out) – default is Home, you can choose Out for easier access or to view tray contents.
- Needle position (Home, Out) – default is Home, move to Out if you need to change needle or remove for cleaning.
- Syringe position (Home, mid, End) – default is Home, move to mid or End if you need to remove it.
- Valve (Inject, Load) - determines whether buffer flows through loop (Inject) or bypasses loop (Load) – default is Load.
- Initial Wash – washes loop with the fluid in the autosampler wash bottle.

Autosampler direct control



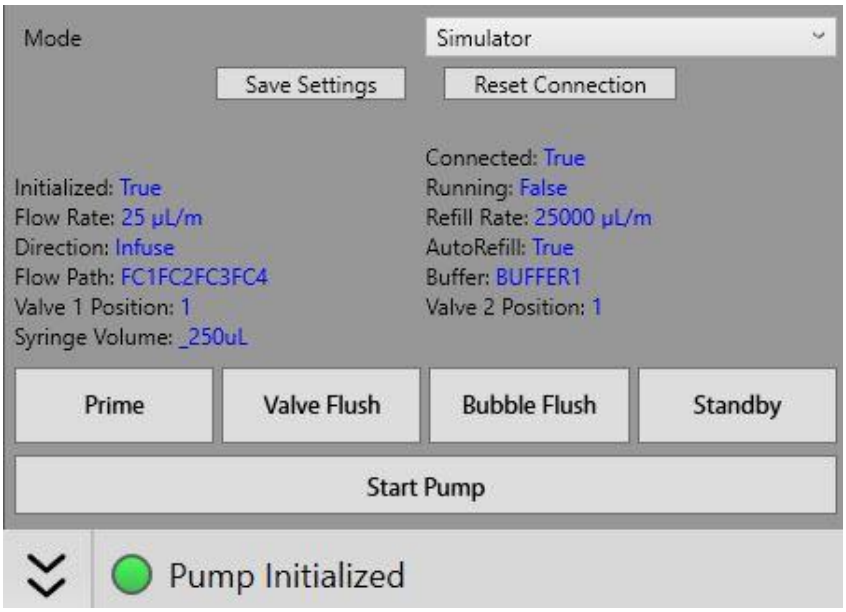
2) Pump Direct Control

- Mode (Simulator, Hardware) – Default is Hardware, choose Simulator if on a computer not connected to an instrument. Click “Save Settings” and “Reset Connection” after a change.

Direct Control buttons

- Prime system - Performs the same prime routine as the button on the home screen. Completes a flush of all the lines at 250µL/min after you choose Buffer 1 or 2. Also performs some Autosampler Washes and takes around 14 minutes to complete.
- Valve Flush – increases flow rate to 100 µL/min and flushes out all the channel combinations that are possible to use. Takes several minutes to run and returns to 25 µL/min when complete.
- Bubble Flush - increases flow rate to 250 µL/min for about 2 minutes to push out trapped air in the flow cell then returns to 25 µL/min when complete.
- Standby - sets flow path to FC1FC2FC3FC4 and flow rate to 5 µL/min. You can use this command to set flow over all 4 channels if Valve Flush was stopped prematurely.
- Start Pump – starts pump flowing at a rate of 25 µL/min

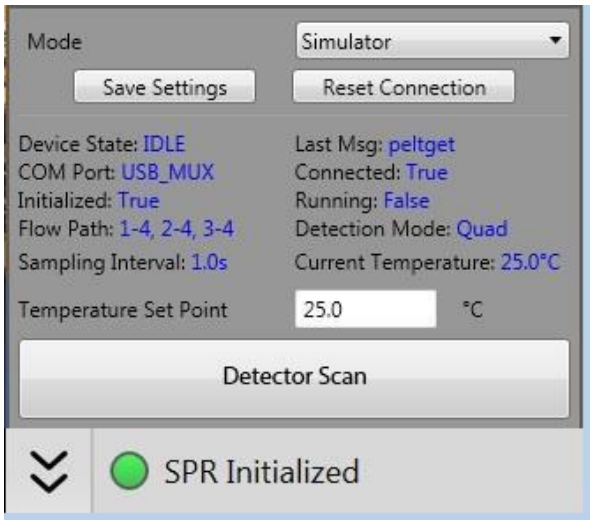
Pump Direct Control



3) SPR direct Control

- Mode (Simulator, Hardware) – Default is Hardware, choose Simulator if on a computer not connected to an instrument, Click “Save Settings” and “Reset Connection” after a change.
- Temperature Set Point – default is 25.0°C - can be changed over the range from 10°C below ambient to 70°C (will adjust Prism temperature beneath sensor chip).
- Detector Scan can be used to look at the SPR minima for all 4 channels – all four minima should be at about the same depth or there could be a bubble problem.

SPR Direct Control



Advanced Methods

Coupling to a single channel

There may be instances where a user wants to immobilize on a single channel – either for a special incubation, or to inject fresh EDC/NHS just prior to immobilization, etc.
Example – immobilizing on Ch 2 only:

1) Go to the immobilization page
2) uncheck the activate and block boxes.
3) Enter EDC/NHS into CH2 (others must be blank)
4) Run
5) Return to immobilization page
6) Enter the target in Ch2 (delete the EDC/NHS)
7) Run

The above can be repeated as necessary. The above procedure ensures that all injections are only over the channel of interest. When you are completing the last immobilization, be sure to check the block checkbox so the 1M ethanolamine HCl will flow over all four channels.

Incubation

Certain experiments require incubation of the target. In such cases, the procedure above can be used to precisely control conditions on each channel. The immobilization can be run any number of times with different reagents as needed. When incubation is complete, remember to block the surface to prevent non-specific binding.

Surface Conditioning

There are some specialty surfaces that require preconditioning. If your surface requires preconditioning, utilize the Method Development screen to inject the preconditioning chemistry over the surface prior to beginning the pH scouting or immobilization steps.

Coupling over all 4 channels

Use this process to prepare a streptavidin or NeutrAvidin surface. For such a preparation, one needs to flow EDC/NHS, then Streptavidin or NeutrAvidin and lastly 1M ethanolamine HCl over all four channels. The most efficient way to accomplish this is to use the Method Develop screen within a couple experiment.

1) Navigate to Method Develop screen
2) Enter EDC/NHS as the first sample and enter appropriate association and dissociation times
3) Enter coupling agent (use Streptavidin or NeutrAvidin) as the second sample and enter association and dissociation times
4) Enter Ethanolamine HCl as the third sample and enter appropriate association and dissociation times
5) Run

DATA ANALYSIS

Once an experiment is complete, the next step is data analysis. The Reichert4SPR software integrates the instrument control software with data analysis. To enter the data analysis module, click the Data Analysis button in the Data Plot. Note: you do not need to wait until the end of an experiment to begin analysis. You can analyze files during or at the end of Method Dev or Scale Up runs. The following provides an introduction to the most common data analysis needs. Detailed help instructions are available in the software to cover all aspects of the software in depth.

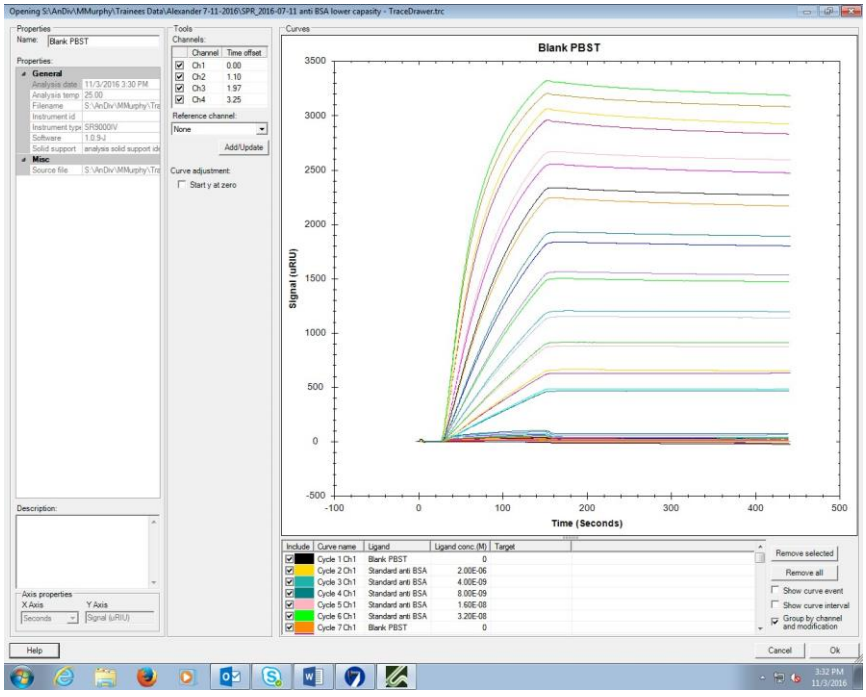
(Otherwise, you can choose to Export to either Scrubber or Tracedrawer which will save overlaid analyte, blank, and/or DMSO injections for future use in either program.)

When you choose Data Analysis on either the Method Develop or Scale Up screens, all injections that have been completed will be overlaid and the initial screen will look similar to the following.

The default name for the overlays will be the name of the first injection.

The initial screen provides a preliminary visualization.

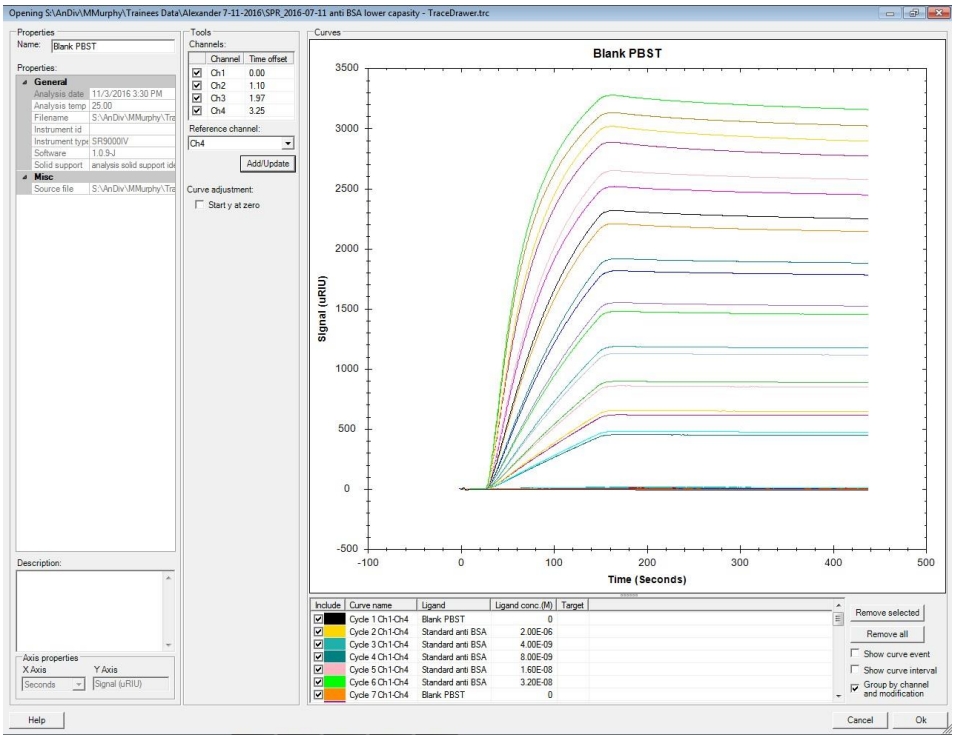
Initial screen when Data Analysis Button is chosen



It is at this time that reference subtraction is done.

Step 1: Select Remove All (the Curves area will be empty)
Step 2: Choose Reference Channel:4 (for example – if running only over Ch1-2 for example, then choose 2)
Optional: Choose Start Y at 0
Step 3: Select Add/Update. The Curves area will be filled in with the newly reference subtracted curves.

Plot of Reference subtracted Data

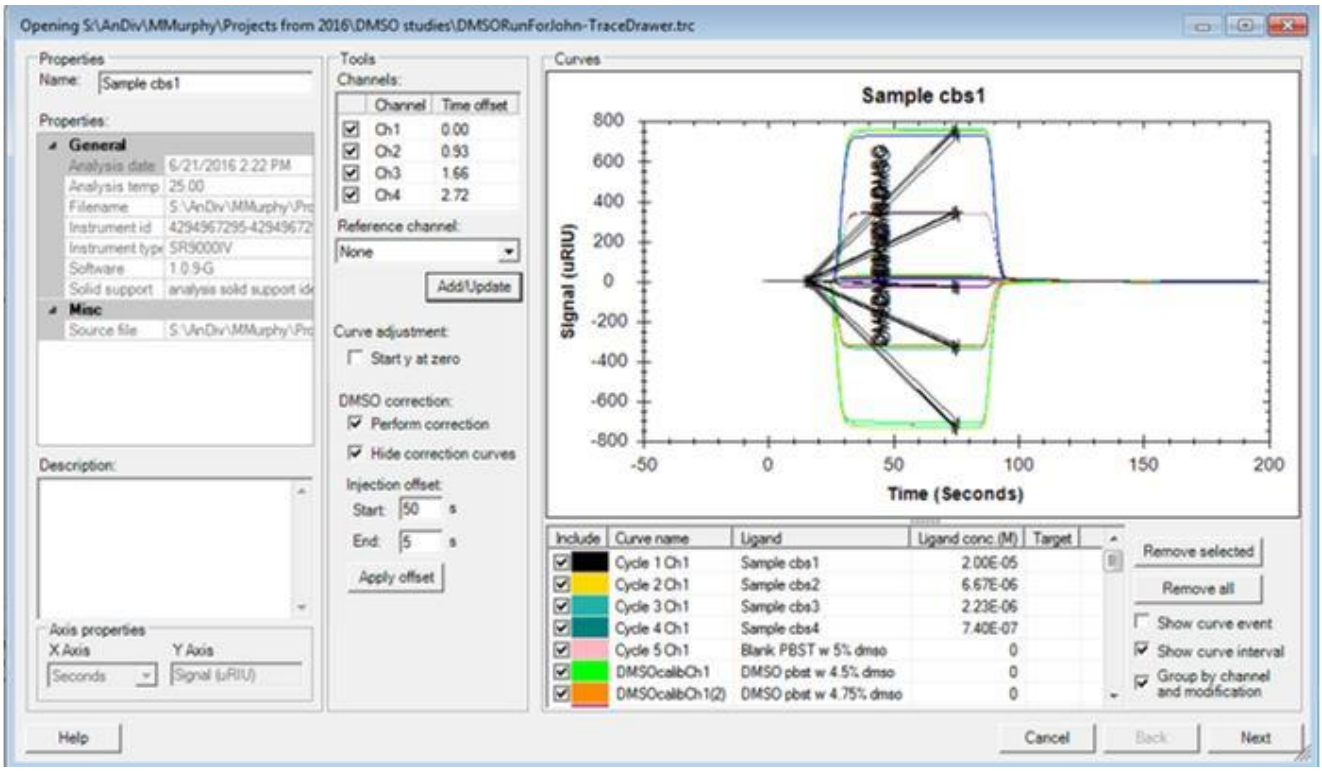


Note: The Time offsets will be automatically filled in. The values applied are based on the flow rate you used for your injections. This value should work well for the majority of applications. However, if you wish to change the value, you can do so by typing in a new value for Time offset in seconds (next to any of the four channels). If you are trying a different offset value, select Remove all, then, Add/Update to see if the new offset value improves the reference subtraction. Repeat as often as needed.

Once you select OK on the bottom right of this screen, the main data analysis program will open.

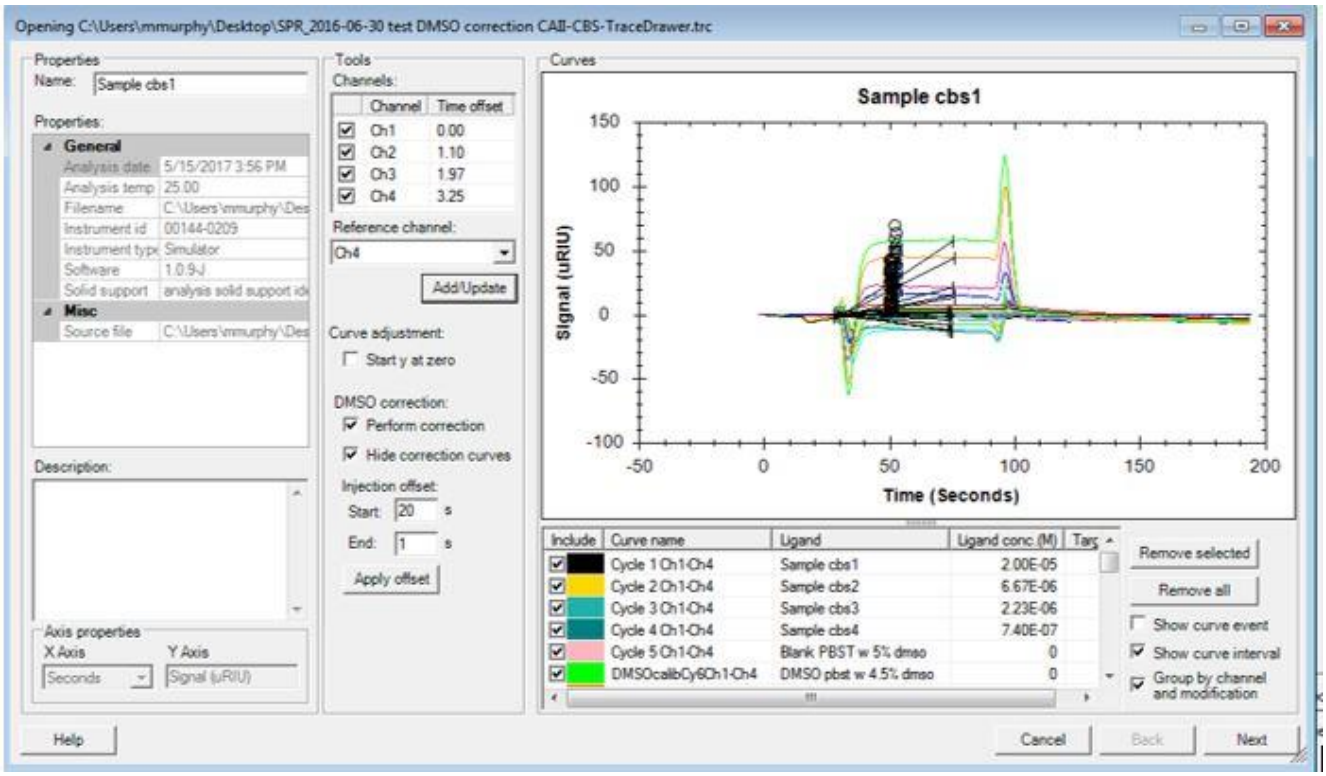
For samples needing DMSO correction:

Click on Data Analysis. The following window will open:

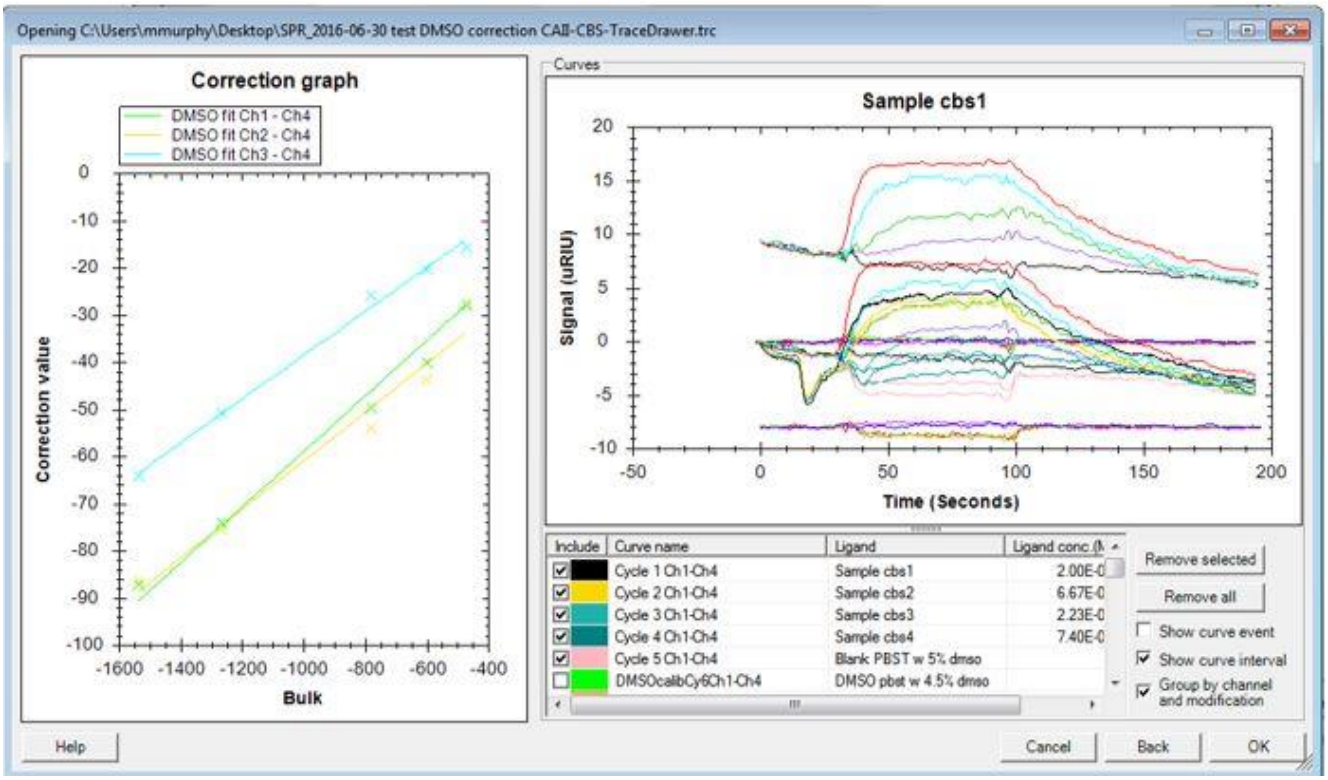


Step 1: Under DMSO correction, both boxes shoud be checked. The injection offset Start and End should be adjusted so that points are chosen where the baseline is flat before the DMSO injections and the max is chosen - you can check by clicking Apply Offset.

Step 2: Choose the reference channel, then Remove all and Add/Update. The screen will look similar to the following:



Step 3a: Select Next. The following Screen will be seen showing DMSO calibration for the individual channels on the left and corrected and uncorrected results on the right:



Step 3b: When you select OK, the overlays will be in Add Run in Tracedrawer, 1st showing without DMSO correction and then showing with DMSO correction results for each channel combination (1-4, 2-4, 3-4).

Organization of Main Data Analysis program

The left side is organized into 3 sections – Runs (bottom section), Overlays (top section) and Evaluations (middle section). This represents the three major steps in the analysis. The Runs section is the initial location of data imported into the analysis module. Curves/injections can be selected or hidden in this section to limit what is used in the overlay. Overlays is the section used to modify and align the data in preparation for the fit/analysis. The fitting and calculation of the objective results are completed in the Evaluations section.

The center section of the screen shows a plot of the selected data and contains various detailed tabs for manipulation or calculations.

The upper right section of the screen shows a list of the curves in the plot. In this section, curves are selected for manipulation in the various tabs, or can be hidden by unchecking the box. Once hidden, the curve is not used in the analysis.

When the data is first brought in (assuming the run utilized all four channels), you will see 3 thumbnails (data is grouped by channel) in the Runs section, and depending on which thumbnail is highlighted, you will see the enlarged picture on the middle of the screen and the corresponding individual injections listed to the right of the enlarged picture (this is consistent throughout). Naming convention for each thumbnail is: name of first injection- reference subtracted then ch 1-4, 2-4 or ch 3-4.

Next you should select New overlay then left click and drag one thumbnail (1-4, 2-4 or 3-4) from Add run to the New overlay thumbnail. In this section you will do some additional correction of the data including any alignment that is needed and blank subtraction.

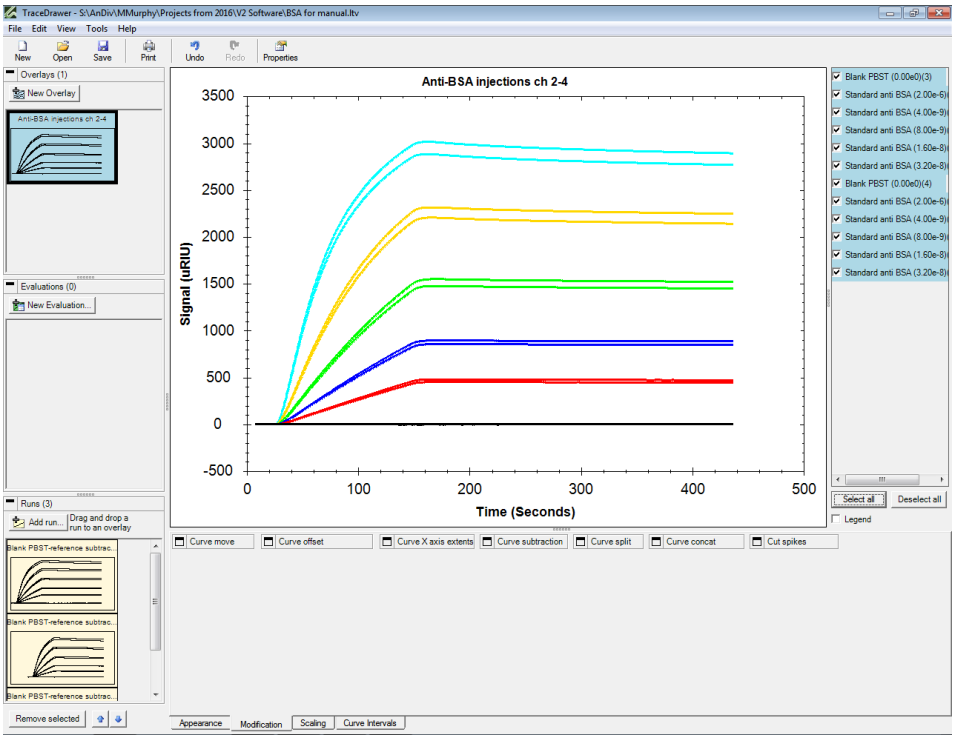
In the last step, the corrected overlays can be opened in Data evaluation so that the data can be fit to particular models. Throughout, the right section of the screen lists each of the injections with names and concentrations you provided during data acquisition.

Note: Detailed Help is available at any time by selecting Help- Help from the top menu.

New Overlay

Step 4: Select New overlay and drag and drop in the overlays from one of the channel combinations (1-4, 2-4 or 3-4) from the Add run section. **Be sure to highlight the New overlay you just added and on the list of injections on the right choose Select All.**

Plot of Data in Data Analysis program after transfer from Add run to New Overlay



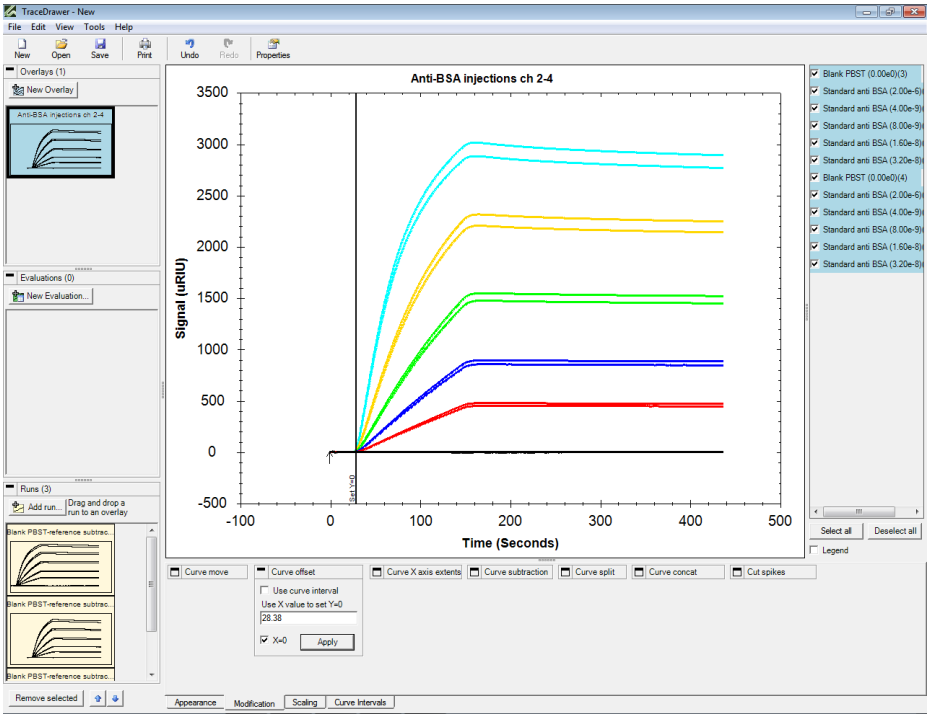
Appearance Tab

Optional: Change Name to include information about the channels (1-4, 2-4 or 3-4) and any other identification information that you will want carried into the data evaluation section. It is recommended to choose the Curve colors by concentration.

Modification Tab

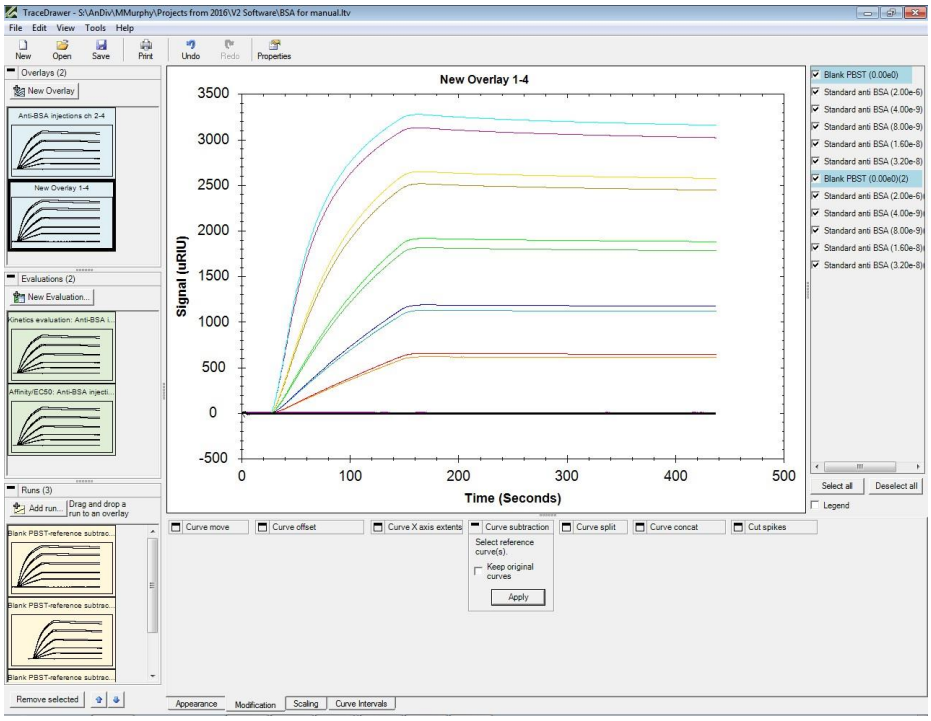
Step 5: First ensure that all your overlays are properly zeroed and start at zero by selecting Curve offset, placing the X=0 cursor on the baseline just before the association curve starts to go up and select the box X=0 and Apply.

Zeroing of all curves

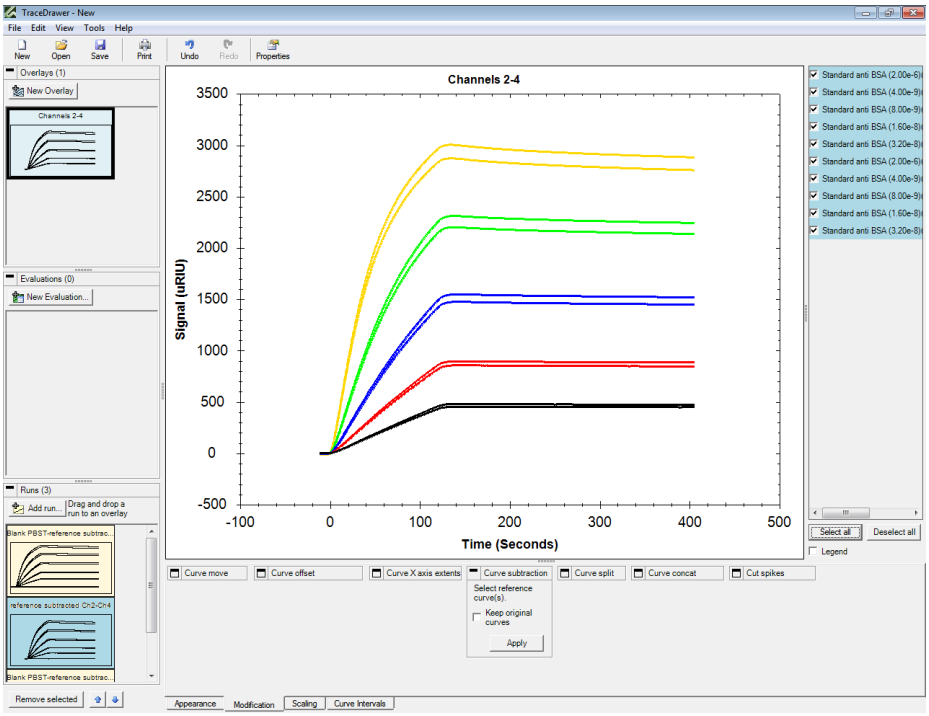


Step 6: The next step is to select curve subtraction. Select all your Blank injections (hold down Ctrl and highlight each Blank injection), then Apply. They will be subtracted from the sample injections and automatically removed.

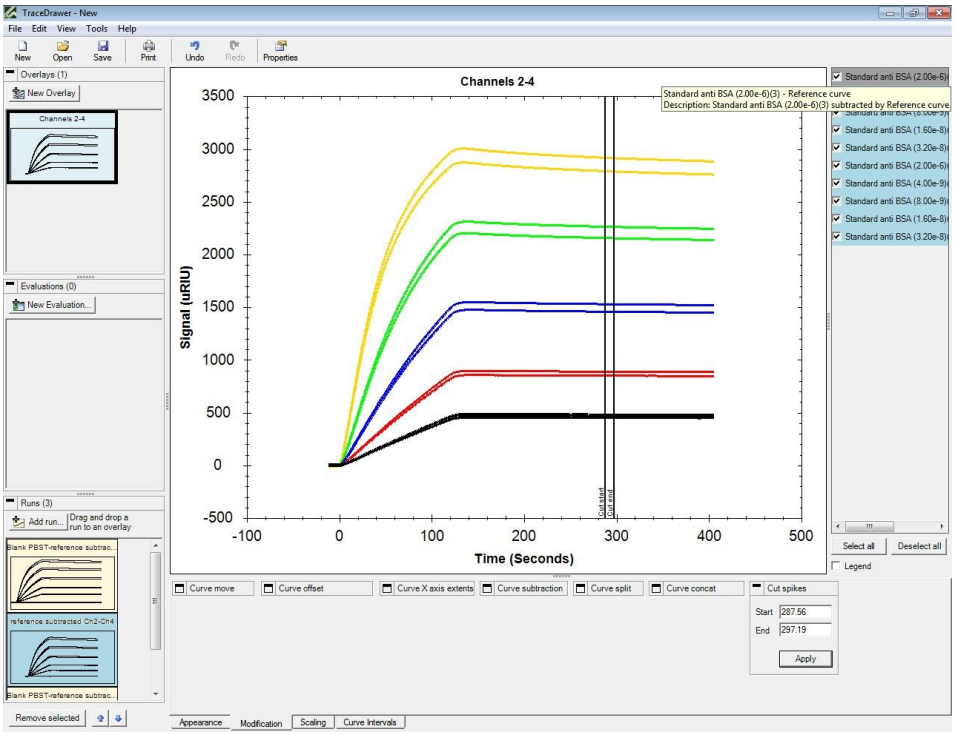
Highlighted Blanks for Curve Subtraction



Blank subtracted curves



Optional: Go to Cut spikes, highlight the injection with a spike you want to remove (you can also Select all) and move the Cut start and Cut end markers to bracket the spike, then select Apply. Select the minus sign (-) when complete.

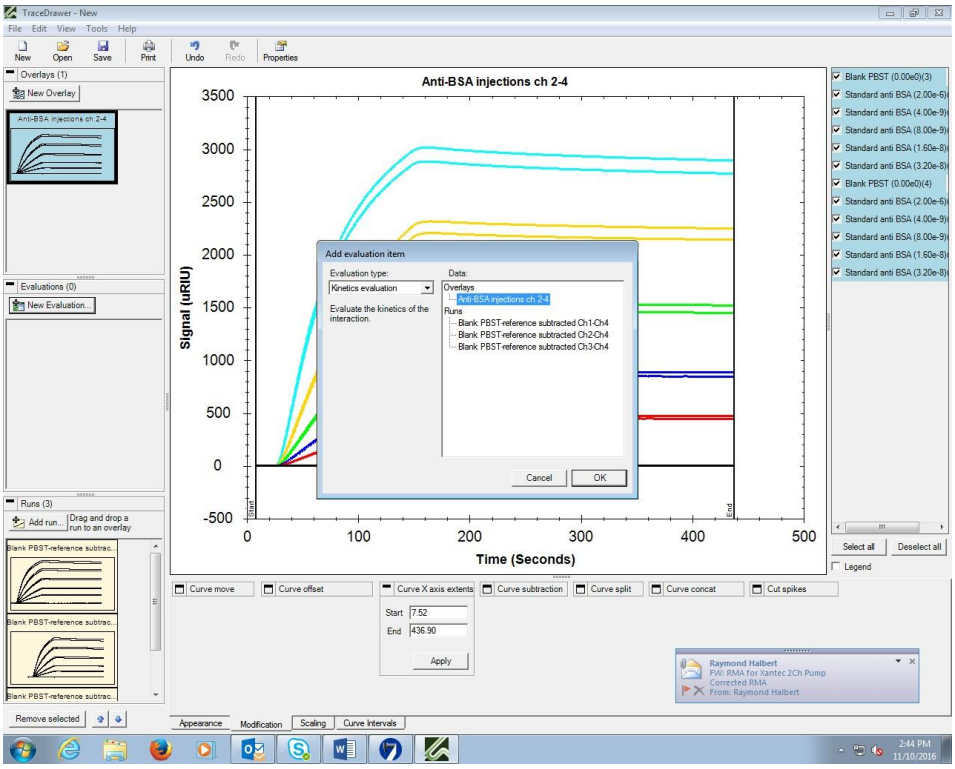


Optional: Also, you can go to Curve X axis extents where you can choose the start and end points for the data before analysis. This is useful if you want to remove part of the data prior to data analysis, for instance if the sample returned to baseline sooner at the end of the Dissociation step than what you programmed for when acquiring data.

New Evaluation

Step 7: Select New evaluation on the middle left of the screen, a box with the list of Runs and Overlays you have added to the screen so far will be displayed along with a drop down of Evaluation Type. The Evaluation type is chosen from the drop down list – choose only Kinetics evaluation, Affinity EC50 or Summary. Once you choose the Type, then highlight the Overlay you want to do data analysis on and Select OK. At this point, the Overlay has been reference subtracted, zeroed and blank subtracted and any spikes have been removed. This step is where the parameters k_a, k_d and k_D can be calculated.

Choose fit type and which set of overlays to fit



Kinetics evaluation

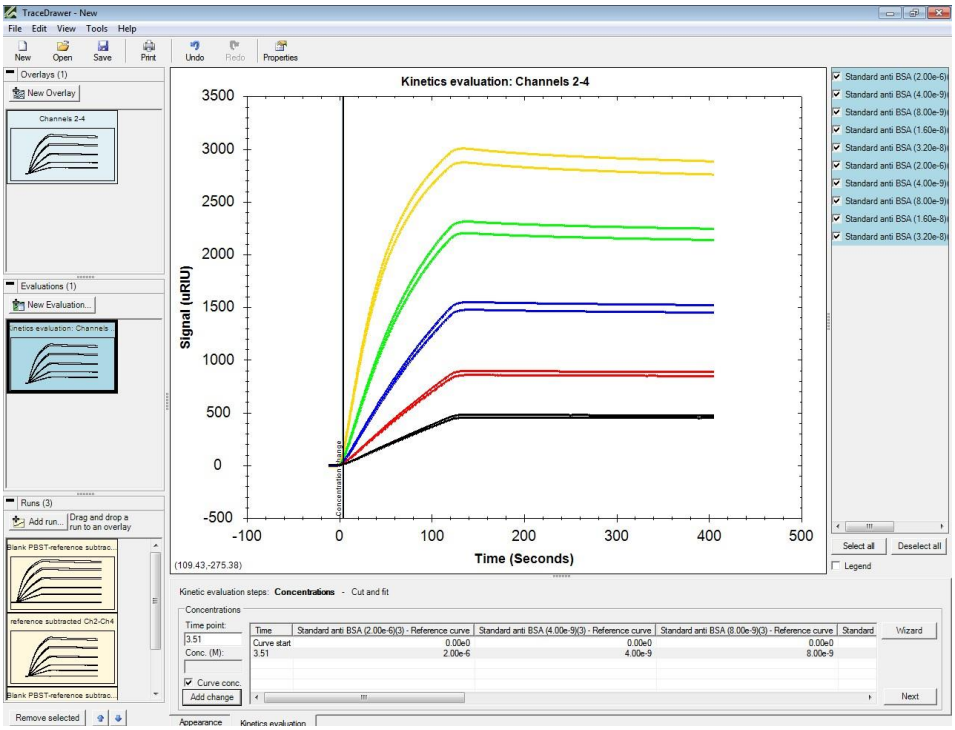
Step 8: Once the Overlay you selected opens, a large picture of the Overlays will be seen and the listing of each injection to the right will be highlighted. The name will be Kinetics evaluation: and whatever you named the thumbnail in the New overlay section. It is recommended to choose the Curve colors by concentration under Appearance since it will not be carried through from the prior section.

Step 9: The program needs to have times set for the start of the association and start of the dissociation and to know the concentration for each injection that makes up the Overlays being evaluated.

.

1) Move the Concentration Change marker to just before where the Association curve starts to go up and select Add change.

Move marker to the start of the association

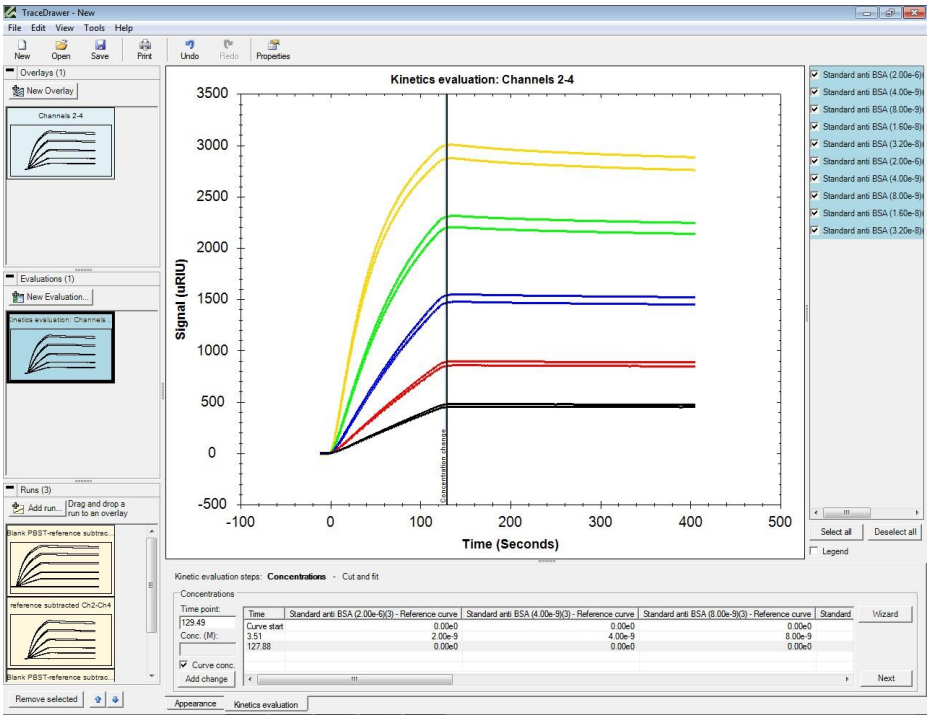


In the table at the bottom, curve start will automatically be filled in and the row that was just added will be highlighted. The concentrations used when you set up the experiments will automatically be populated when you place the concentration start at the start of the association and select Add change.

Note: You can also highlight those injections where you need to correct the concentration and make the change here - left click in each box after the time and fill in the concentration in M (molar). Note: Shorthand concentrations of n for nanomolar, u for micromolar, etc. are also acceptable.

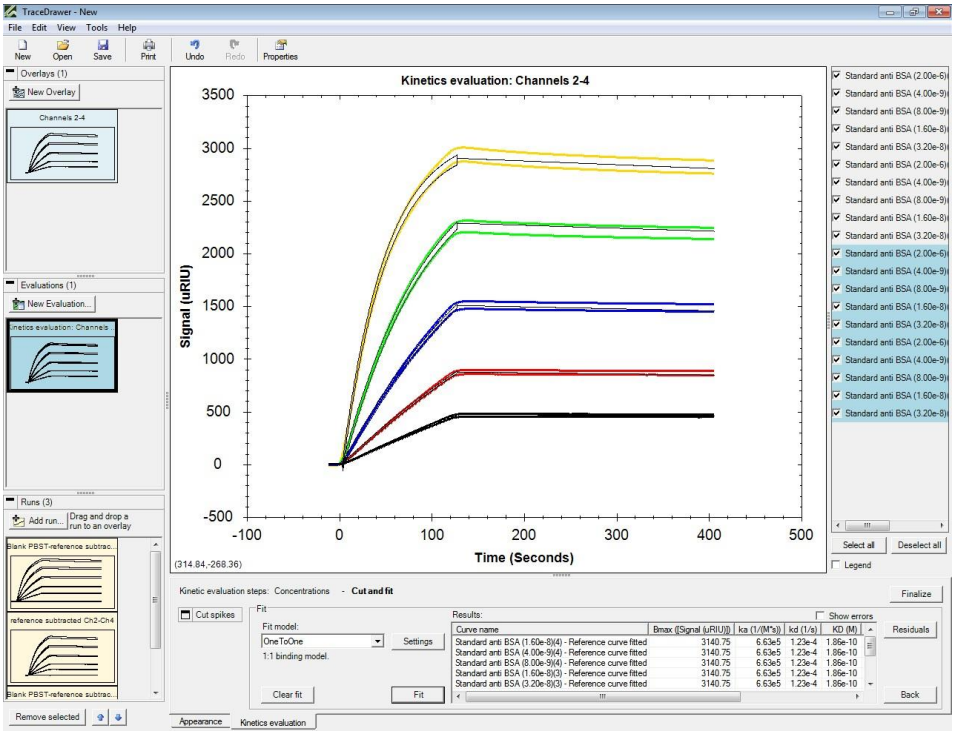
2) Move the Concentration Change marker to where the Dissociation starts and select Add Change. Then Next.

Move marker to start of dissociation



3) Choose a Fit Model to use (the default is One to One – different models are available from a dropdown list). Select Fit. See Fit values under Results when fit is complete - see the fitted points on the graph.

Data (arranged in colors by concentration) and data fit (black lines)



4) The calculated kinetic values are shown in the box at the bottom. Note: If you have a bulk shift, you can click on Settings and set the BI Fit type to Local.

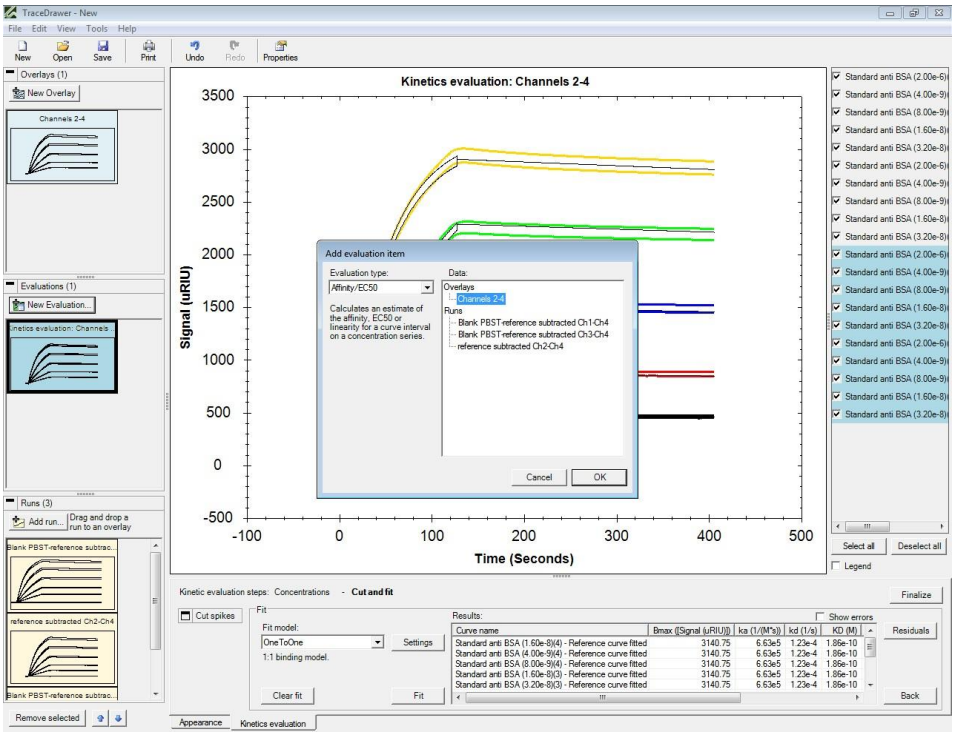
5) For detailed information on all the available models and for settings and descriptions, select Help, then Help again, in the upper left corner of the screen.

Affinity/ EC50

This fit type is used to obtain equilibrium Data or to get an Isotherm for any data set.

Select New Evaluation and choose Evaluation type: Affinity/EC50. Highlight the Overlay you are interested in fitting and select OK

Choose Affinity evaluation

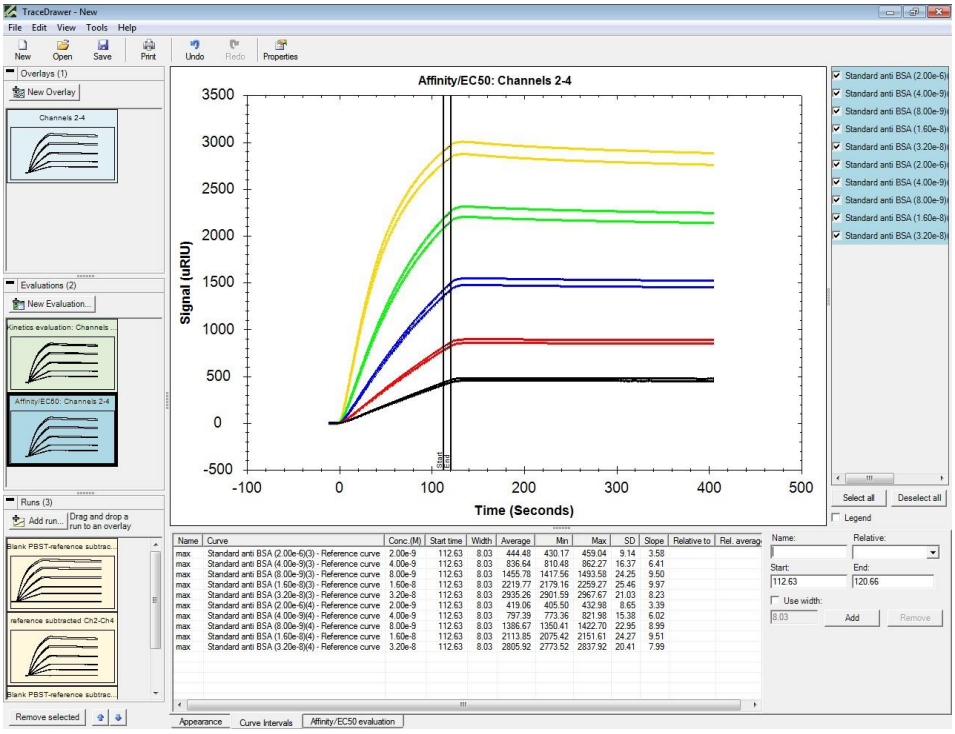


Be sure all of the injections to be fit are highlighted (or Select All) and all of the boxes are checked. Be sure the Concentration (Conc (M)) is filled in for all the injections. If it is not filled in, change the concentrations in the Conc (M) column. Note: Shorthand concentrations of n for nanomolar, u for micromolar, etc. are also acceptable

In the Appearance tab – change Name, axis name or colors as needed (see previous comments about the Appearance tab)

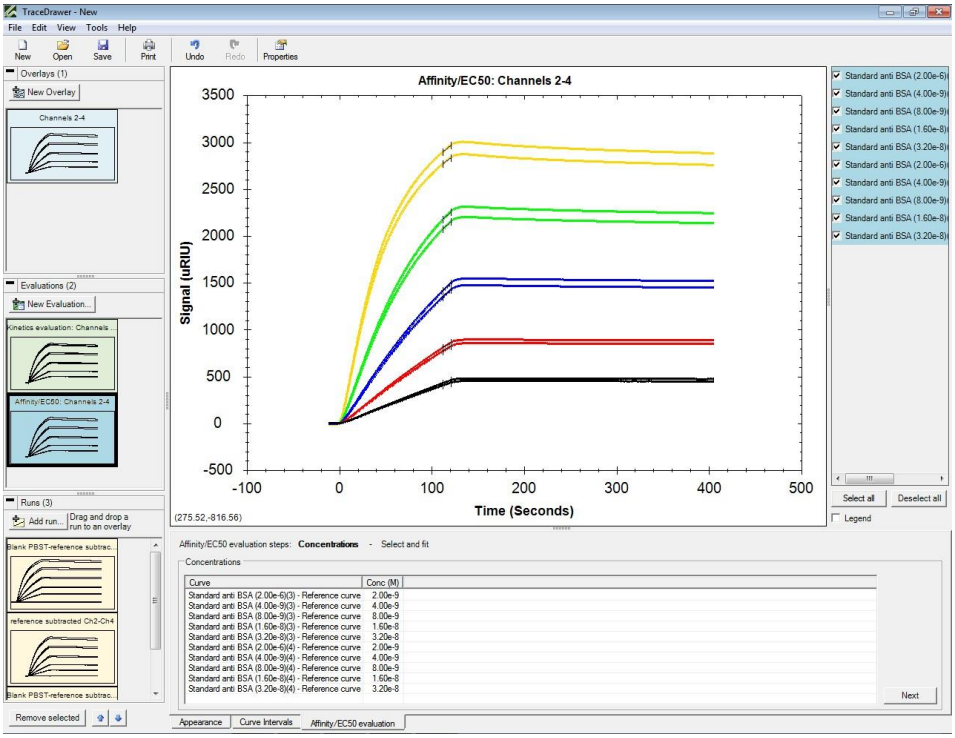
In the Curve Intervals tab – Select Start and End points near the end of the Association part of the injection and Name the Interval. To do so, click and drag the Start and End lines seen on the screen. Then, select Add.

Select curve interval for affinity evaluation – choose start and end points near end of association



Select the Affinity/EC50 evaluation tab and the curve intervals will be shown on each curve that was highlighted. Then**, select Next.**

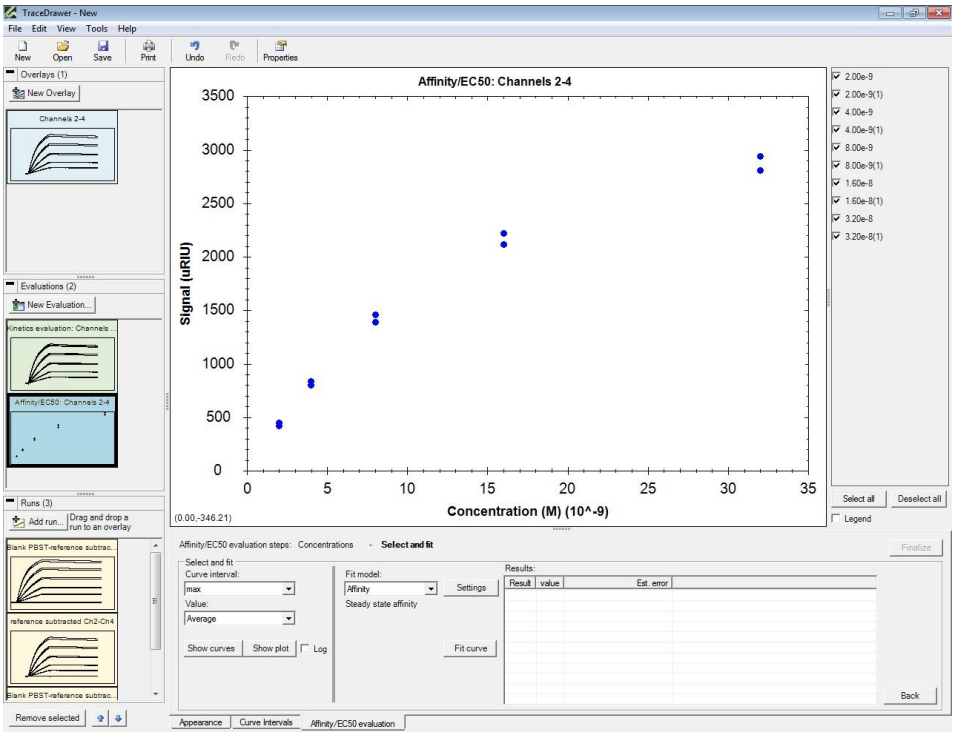
Curve interval and concentration are shown



You should see your points (from the curve intervals) plotted for each concentration.

In the Select and Fit box, choose – the curve interval you just named (in the example below it is called Max) and for Value choose Average (average should be the default and your curve interval should be filled in automatically).

Plot of Response at curve interval vs concentration

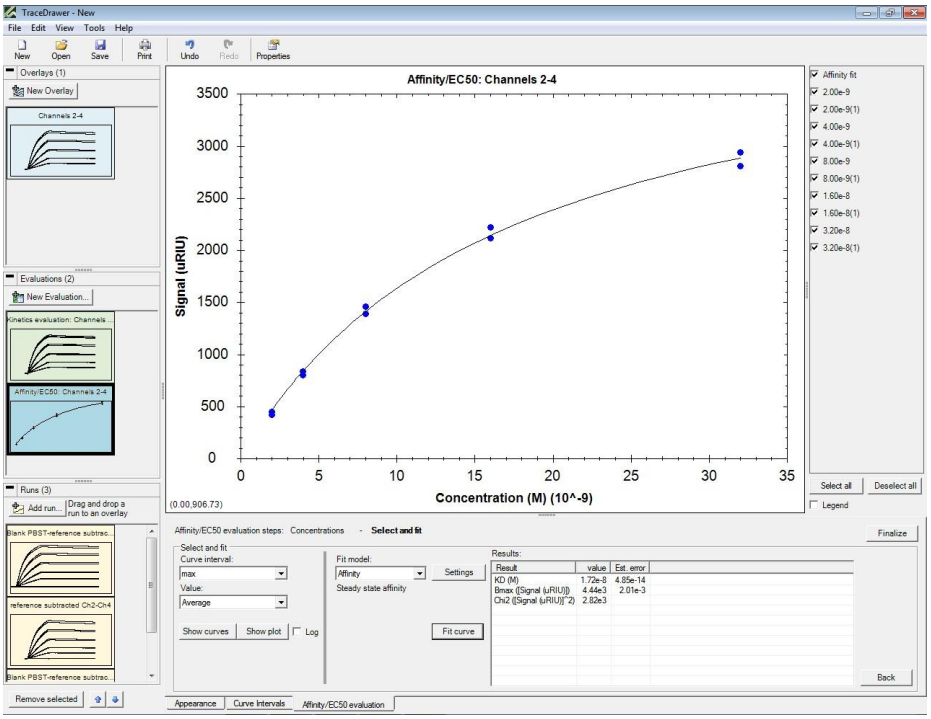


Choose your Fit model. The model called Affinity is for 1:1 binding. Other models can also be chosen from the dropdown list. Select Settings if you to want to change the initial guesses used for the model you have chosen. Then, select Fit curve.

Results will be shown in the Results box.

You can copy and paste the figure and values at bottom into other programs (eg. Excel, Powerpoint, Word, etc.)

Affinity plot also called Langmuir Isotherm

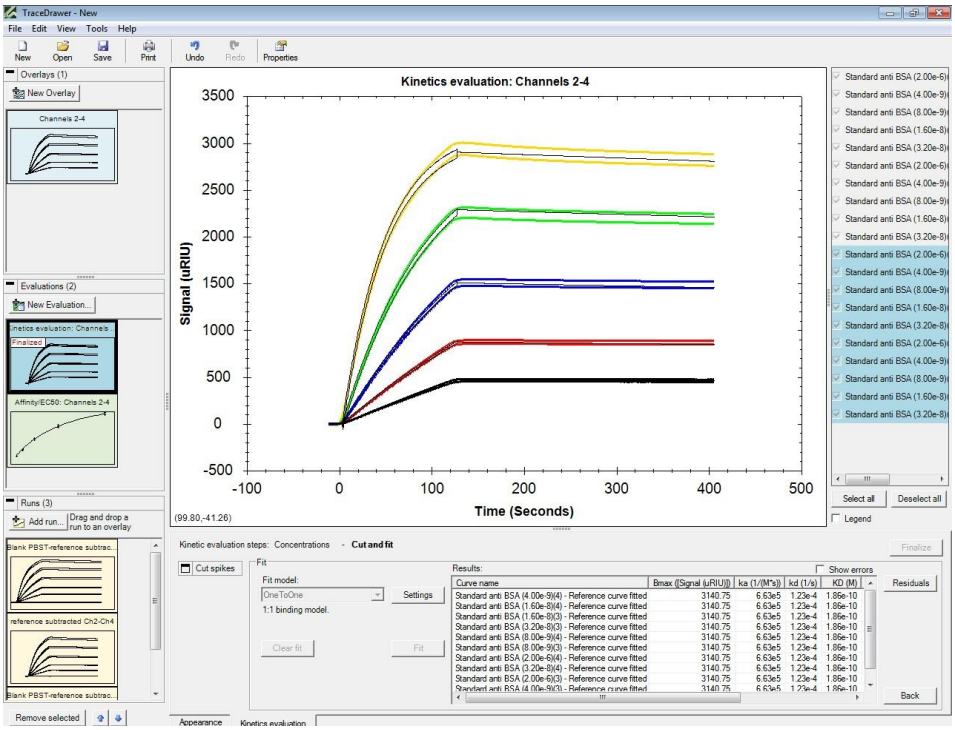


Summary Report

You can combine results from different experiments**.**

Open one of the TraceDrawer files that you have already analyzed and want to be part of the Summary Report. In the New Evaluation area, highlight the curve and select Finalize.

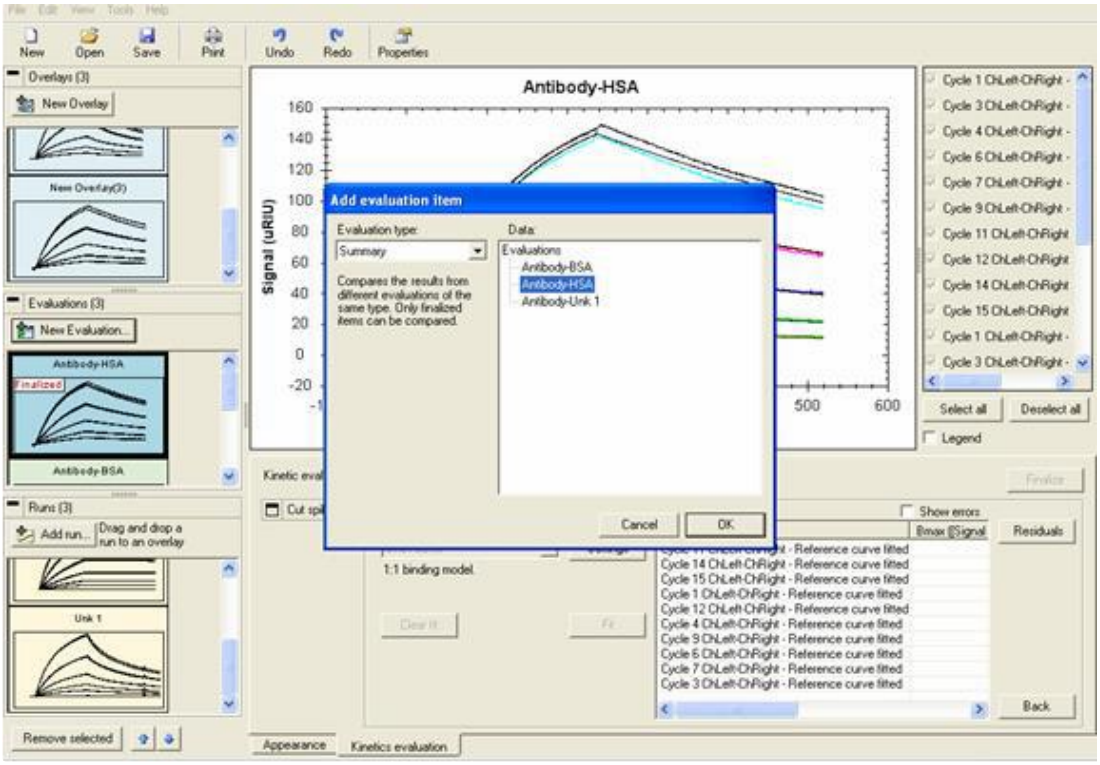
New Evaluation (middle area) on left showing results are Finalized



Next, go up to File and Select Open and Merge. Select another file that you want to be part of the Summary Report and the file will be added into the current file (be sure each overlay that will be part of the Summary is Finalized).

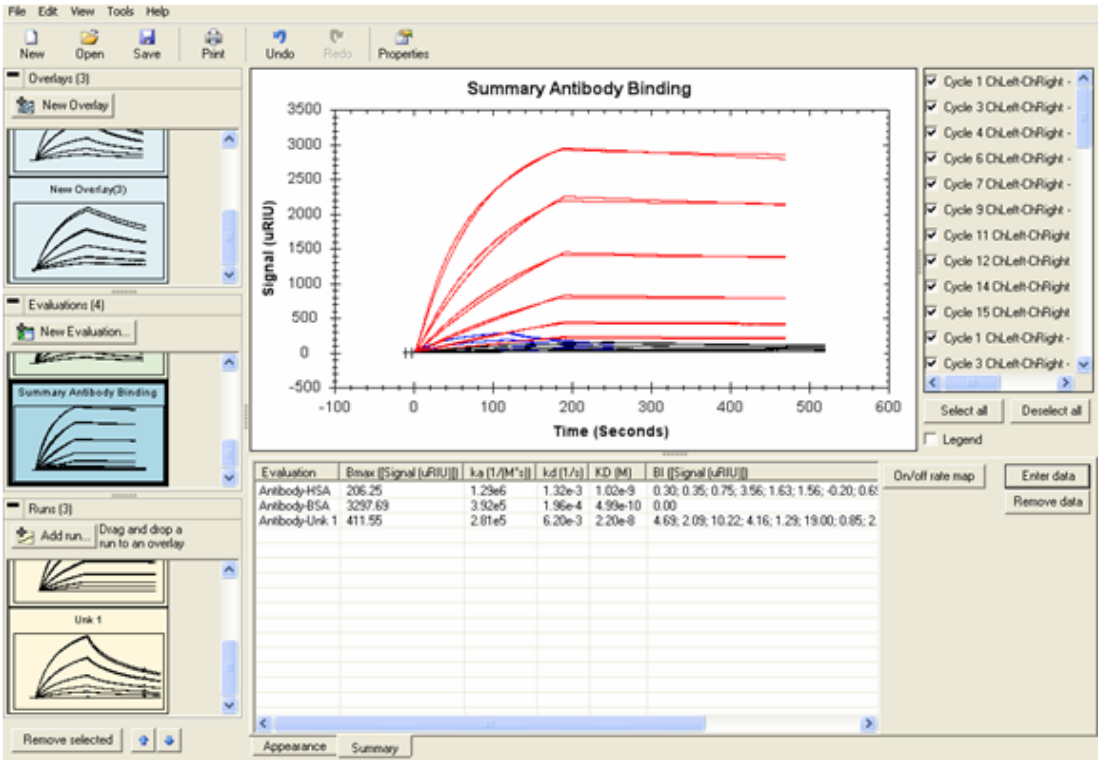
When all of the files that you want to be part of the Summary Report have been merged and Finalized, Select New Evaluation and Summary, then highlight one of the Evaluations and select OK. A thumbnail (in this case a Finalized Evaluation) will be created into which all of the other Finalized Evaluations can be selected and dragged into. **Note:** Be sure that the Summary is the last item in the Evaluations area so that it will be easy to click and drag each thumbnail into the Summary thumbnail.

Select Evaluation type as Summary



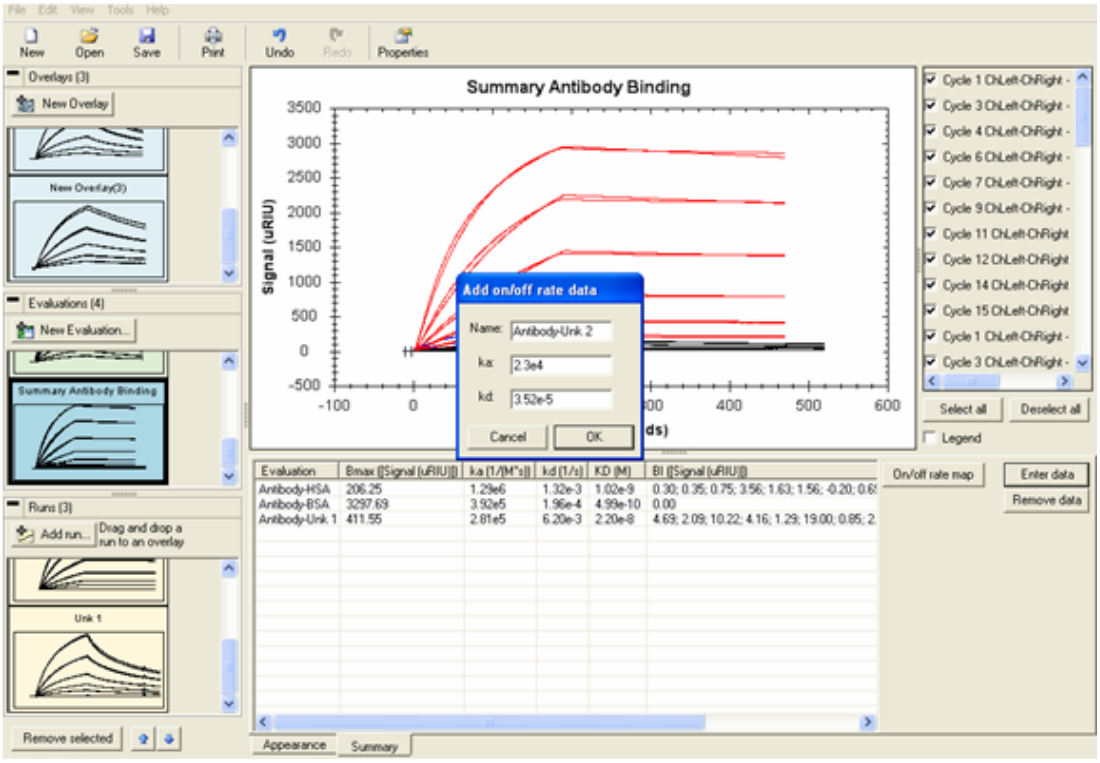
The Summary Report can be viewed as either curve overlays with kinetic results or as an On/off rate map. For the Summary Evaluation, be sure to choose the Name you want in the Appearance tab (you can also adjust the axis and the colors of the curves, if needed). When you Select Summary, the overlay plots of all the results you merged will be seen along with a table of the names and kinetic information from each merged Evaluation.

Curve overlays Summary View



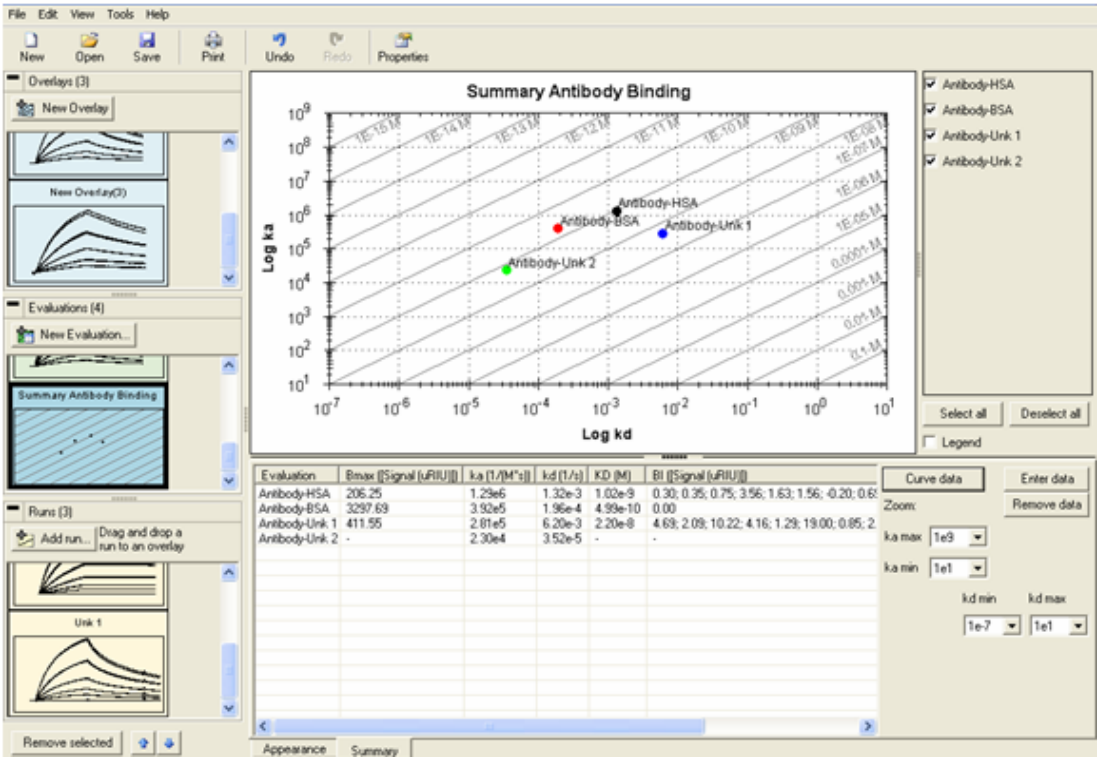
You can also enter additional results into the kinetics table manually, but the plots will not be seen. You will be prompted as shown. **Note**: Only manually added data can be deleted by choosing Remove data.

Manual Addition of Summary Results



The values added will be included in the Evaluation table and in the On/off rate map:

On/Off Rate Map for Finalized sample comparison



At the end, select Save. You can also Print the results. You can also copy and paste any figure or table created into other programs (eg. Excel, Powerpoint, Word, etc.)

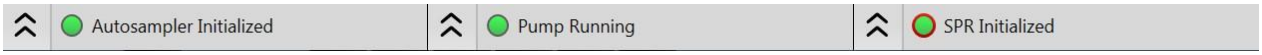
Troubleshooting

Direct Control Errors

These errors are found on the bottom of the main program:

A red light on the SPR Direct Control button indicates a sensing problem on one or more channels.

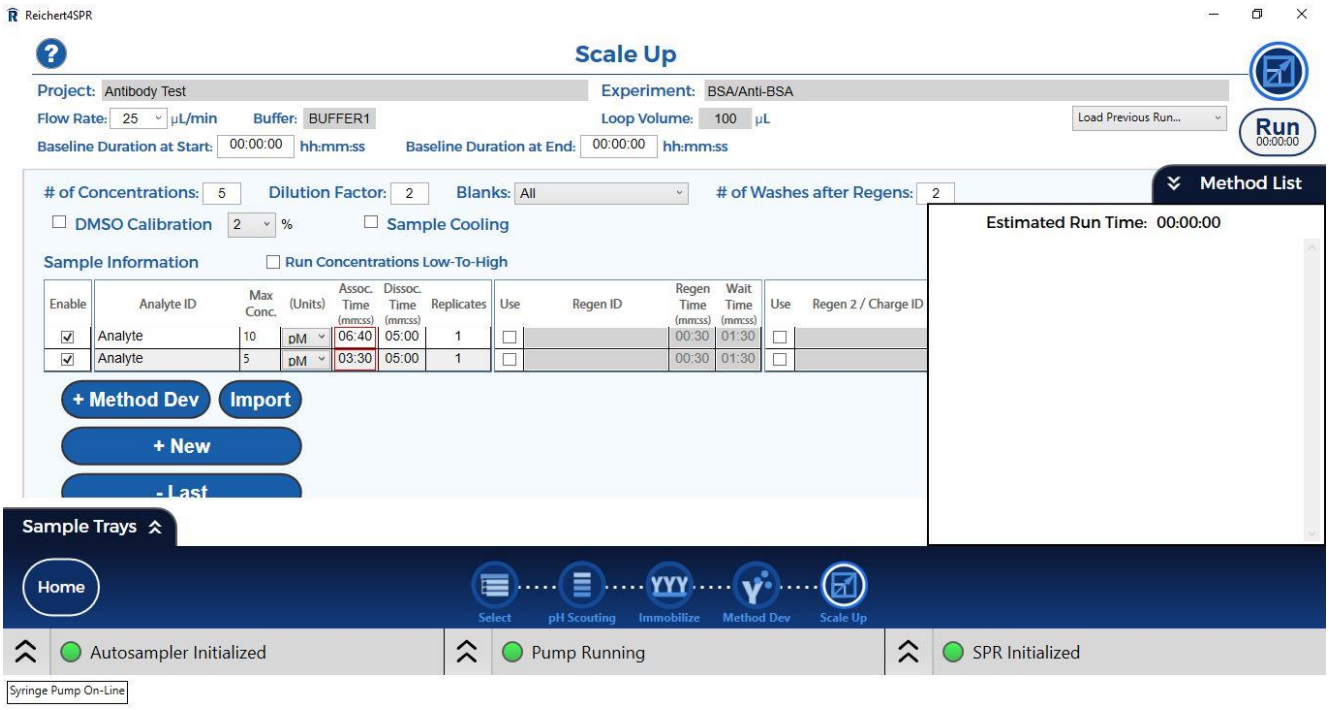
A red ring indicates errors have been recorded, but not viewed yet.



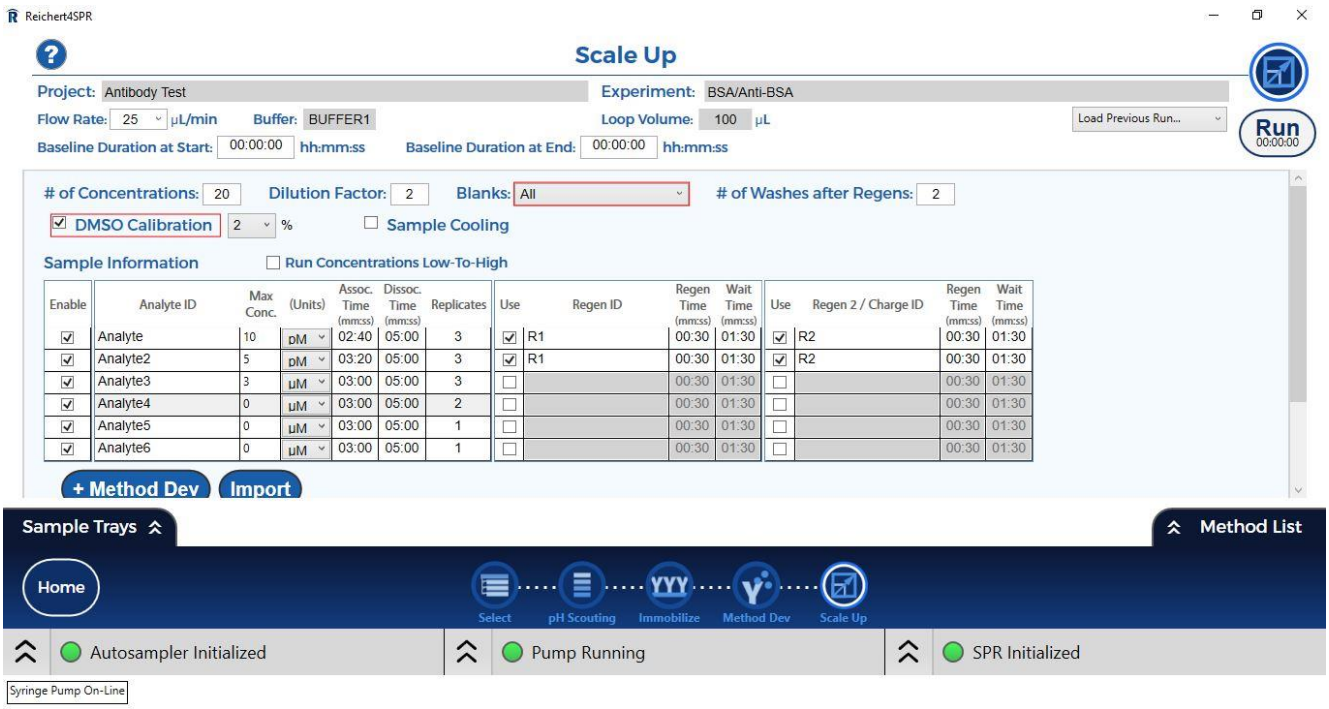
Clicking on the light will open a dialog displaying any errors.

Other Errors

1) If you enter an association time that would cause the injection to require a volume greater than 75% of the loop volume, the time in the Sample Information will have a red box around it, indicating an error. You will need to either decrease the flow rate, shorten the association time, or use a larger loop volume.



2) If you try to program more injections than what you have space for in your trays, you will see a red box around the Name, Blanks or DMSO depending on which boxes you have checked and values you have entered. You may need to do fewer concentrations, run fewer replicates, or use a different vial type to accommodate all your injections.


Reichert4SPR
Scale Up
Project: Antibody Test
Experiment: BSA/Anti-BSA
Flow Rate: 25 µL/min
Buffer: BUFFER1
Loop Volume: 100 µL
Load Previous Run...
Run
00:00:00
Baseline Duration at Start: 00:00:00 hh:mm:ss
Baseline Duration at End: 00:00:00 hh:mm:ss
# of Concentrations: 20
Dilution Factor: 2
Blanks: All
# of Washes after Regens: 2
DMSO Calibration 2 %
Sample Cooling
Sample Information
Run Concentrations Low-To-High
Enable | Analyte ID | Max Conc. | (Units) | Assoc. Time (mm:ss) | Dissoc. Time (mm:ss) | Replicates | Use | Regen ID | Regen Time (mm:ss) | Wait Time (mm:ss) | Use | Regen 2 / Charge ID | Regen Time (mm:ss) | Wait Time (mm:ss)
Analyte | 10 | pM | 02:40 | 05:00 | 3 | R1 | 00:30 | 01:30 | R2 | 00:30 | 01:30
Analyte2 | 5 | pM | 03:20 | 05:00 | 3 | R1 | 00:30 | 01:30 | R2 | 00:30 | 01:30
Analyte3 | 3 | µM | 03:00 | 05:00 | 3 | 00:30 | 01:30 | 00:30 | 01:30
Analyte4 | 0 | µM | 03:00 | 05:00 | 2 | 00:30 | 01:30 | 00:30 | 01:30
Analyte5 | 0 | µM | 03:00 | 05:00 | 1 | 00:30 | 01:30 | 00:30 | 01:30
Analyte6 | 0 | µM | 03:00 | 05:00 | 1 | 00:30 | 01:30 | 00:30 | 01:30
+ Method Dev
Import
Sample Trays
Method List
Home
Select
pH Scouting
Immobilize
Method Dev
Scale Up
Autosampler Initialized
Pump Running
SPR Initialized
Syringe Pump On-Line

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