SARTORIUS

User Guide

Octet® SPR Discovery Software

User Guide

Octet® SPR Discovery User Guide

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Chapter 1:

Octet® SF3 System

Introduction

The Octet® SF3 system from Sartorius utilizes surface plasmon resonance (SPR) to enable real-time, label-free analysis of a wide-range of a wide variety of biomolecular interactions, from small molecule fragments to biologics. The system is intended for research and development activities.

Thanks to the unique OneStep® function, the Octet® SF3 system provides high quality kinetics and affinity measurements in a single injection in a matter of minutes.

The Octet® SF3 system provides high assay sensitivity over a wide dynamic range. Octet® SPR Discovery software enables rapid assay development thanks to its wizard-based methods while allowing experienced users true flexibility in assay design and optimization through custom methods and manual run modes.

To aid in optimal assay development a range of sensor chip surface chemistries are available.

Figure 1-1: Octet® SF3 System

Octet® SPR Discovery user guide

The Octet® SPR Discovery user guide guides you through installation, the components of the system, operation of the Octet® SPR Discovery software for creating and editing methods and performing assays. The user guide also includes a description of the surface plasmon resonance principle, safety instructions, system specifications and system maintenance.

Associated documentation

- Octet® SPR Chemistry User Guide - provides an in-depth look at the surface plasmon resonance principle and describes the available sensor chip surface chemistries and their applications.
- Octet® SPR Analysis User Guide - provides an in-depth look at how to analyze and evaluate Octet® SPR Discovery data.

Further documents describing the system and its uses in more detail are available from Sartorius.

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For support issues, contact your Authorized Distributor.

Chapter 2:

Safety and Specifications

Safety Information

The Octet® SF3 system has been designed and tested in accordance with IEC Publication 61010-1; Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, IEC61010-1, 3rd ed., UL 61010-1, 3rd ed., CSA No. 61010.1, 3rd ed., and EN61010-1:2010.

WARNING! *The user must adhere to the following warnings to ensure safe operation and to maintain the apparatus in a safe condition.*

- The system has been designed for indoor use only.
- An electrical earth ground is required to operate the system safely and per tested and approved conditions.
- The Mains fuse is user-serviceable at the back of the Octet® SF3 system. Fuse replacement must be performed with the AC power cord removed. The system requires two fuses of type T8AL, 250V.
- System installation should be at an altitude of < 2,000 m (6562 ft).
- The Octet® SF3 system is designed for use in Cat II electrical installations and for use in pollution degree 2 environments.
- Ensure the system is configured for the correct voltage and mains cordage prior to turning on the unit.
- Disconnect the system from all voltage sources before it is opened for any adjustment, replacement, maintenance, or repair.
- Do not perform any adjustments, maintenance, or repairs of the opened system while under voltage. If this is unavoidable, maintenance must be carried out only by a skilled person who is aware of potential hazards.
- Whenever it is likely that the system has been impaired, it should be rendered inoperative and secured against unintended operation by disconnecting the power cord.

WARNING! *The Octet® SF3 system has no operator serviceable parts other than cleaning of the equipment and replacements of operator replaceable fuses. Service other than cleaning of the equipment and replacement of operator replaceable fuses shall be performed by authorized Sartorius personnel only. Only trained personnel authorized to service the equipment regarding safety procedures and the product acceptance test procedures can verify the safe condition of repaired equipment.*

Contact the factory or local Octet® SF3 authorized representative if any of the following conditions occur:

- System shows visible damage.
- System fails to perform intended function.
- System exposed to unfavorable conditions, such as a dusty environment, greater than 85% humidity, or prolonged storage.
- System subjected to severe transportation stresses.

WARNING! *Do not operate the Octet® SF3 system in any other way than described in the user guide. Failure to comply may expose you to hazards that can lead to personal injury and may cause damage to the equipment.*

WARNING! *Octet® SF3 systems should only be installed, relocated, and/or moved by trained Sartorius personnel. To obtain more information, please contact Sartorius technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Sartorius is not responsible for personal injury or damages caused by unqualified personnel installing, relocating and/or moving an Octet® SF3 system.*

Warning messages

The following symbols provide information on safe system operation and proper use.

Table 1: Safety Symbols

Symbol	Definition
	**Caution!** Failure to follow instructions may result in bodily injury.
	**Caution!** Pinch point potential. Keep hands away from moving parts, especially the syringes.
	**Important!** Failure to follow instructions may cause damage to the system.
	**Important!** Failure to follow instructions may cause the system to perform poorly.
	Electric Shock Warning: An electric shock hazard exists.
	Static Sensitive Device: The device is sensitive to static electricity.
	Syringe Warning: Improperly handled syringes can break and result in sharp glass. Syringe needles are a puncture wound hazard.
	Gloves: Wear gloves during the indicated procedure.

Specifications

Table 2-1: Technical Specifications for Octet® SF3 Instrument

Description	Specification
Baseline Properties	
Refractive index range	1.33 - 1.40
Baseline noise	Typically < 0.025 RU (RMS)
Baseline drift	Typically < 0.3 RU/min
Molecular weight cut-off	No lower limit for organic molecules
Variable data rate	1, 2, 5, 10, 20 and 40 Hz
Affinity range	fM to mM
Working Ranges	
Association rate constant (k_a)	10^2 - 10^9 $M^{-1}s^{-1}$
Dissociation rate constant (k_d)	10^{-6} - 2.5 s^{-1}
Global affinity constant (K_D)	10^{-3} - 10^{-12} M
Concentration	Sample concentration >1 pM
Sample Acquisition	
Unattended running time	>72 hours with no built in run-time limit
Sample capacity	Any 2 sample racks plus 2 reagent racks
Sample racks	96 vial, deep well and PCR formats, 384-well microplates, custom high volume
System & sample temperature control	4 - 40 °C (max 15 °C below ambient)
Buffer line selection	Automatic switching between 3 independent lines
Inline buffer degasser	Yes
Flow Cell and Injections	
Number of flow channels	3
Flow path	1, 1-2, 1-2-3, 3, 3-2, 3-2-1
Flow channel volume	<90 nL
Channel-channel dead volume	<20 nL
Injection volume	2 - 700 µL
Flow rate	0.1 - 200 µL/min

Table 2-1: Technical Specifications for Octet® SF3 Instrument

Description	Specification
Injection rise and fall time	< 0.75 second @ 25 µL/min
Gradient injections	OneStep® (including in High-Throughput Mode), NeXtStep™
Simultaneous injections	Yes
Software	
Octet® SPR Analysis Hit Selection	Yes
Instrument	
Width x height x depth	61 cm x 61 cm x 51 cm (24" x 24" x 20")
Weight	66 kg (145 lb)

NOTE: *Specifications do not include external PC or laptop.*

Wetted materials

- Stainless Steel (type 316)
- Silicone Rubber
- Teflon™
- Tefzel®
- PEEK®

Computer Requirements

Compatibility

Octet® SPR Discovery and Octet® SPR Analysis software is compatible with 32- and 64-bit versions of Microsoft Windows 10. Refer to Microsoft's website for the minimum system requirements for these operating systems. Beginning with version 5.0 of the Octet® SPR Discovery and Octet® SPR Analysis software, Microsoft Windows 7 is no longer supported.

Table 2-2: Compatibility

Description	Specification
Operating System	Microsoft® Windows® 10 32-bit or 64-bit, version 1607 'anniversary update' or newer
Processor	1.5 GHz or faster
RAM	4 GB
Hard Disk Space	500 MB

Recommendations

For the best software experience, the following are recommended:

- Widescreen monitor screen resolution of 1920 x 1080
- Microsoft® Windows® 10, 64-bit edition

Computer connection

Figure 2-2: Computer Connection

Installation Requirements

Power cord

The Octet® SF3 system requires an approved power cord for operation.

IMPORTANT: *Use the power cord provided by Sartorius. Contact your Sartorius representative if you have questions regarding power cords.*

Uninterruptable power supply (battery backup)

NOTE: *To prevent data loss or power failure issues, the Octet® SF3 system requires an Uninterruptable Power Supply (UPS) for use and must be provided by the customer.*

The Octet® SF3 instrument and computer must be connected to the main power supply via an uninterruptable power supply (UPS, also known as a battery backup). Power line glitches and brief outages will cause the system to restart, and any running method will be aborted. The UPS should have a minimum capacity of 1.5 kVA. Note that during extended power outages, the UPS will eventually be depleted and system power will be lost.

Internet connection

It is highly recommended that the Octet® SF3 system be installed in a location which has internet access. Remote troubleshooting can be significantly enhanced in the rare event the system malfunctions. Also, software updates can be downloaded as they become available.

Performance Characteristics

Mass transport

Analyte contained in the sample stream entering the flow cell is delivered (mass transported) to the sensing surface via convection and diffusion. Minimizing the dimensions of the flow cell increases the mass transport coefficient by increasing the flow velocity and lowering the height of the non-stirred boundary layer that exists at the sensing surface. These measures to improve mass transport increase the rate at which molecules are introduced to the reactive surface. To address this issue each flow cell has a total volume of <125 nL with a height of ~25 µm.

Dispersion

It is important that the sample entering the flow cell not mix appreciably with buffer present in the channels. Rapid transition from buffer to sample is required to minimize the effects of changing buffer composition on the actual kinetics curves and to provide accurate measurements. In addition, the mixing of sample and buffer changes the actual initial concentration of the analyte injected into the system, which will alter concentrations or kinetics measurements. Octet® SF3 instrument has been designed to run the mixed liquid segment of the loaded sample to waste before directing the main bulk of the sample through the flow cells. In addition to this "run-to-waste" feature the Octet® SF3 system has been designed with low dead volumes. These features ensure high quality sample injections suitable to state-of-the-art real-time biomolecular interaction analysis.

Temperature

Both the sample racks and the analysis module have separate thermoelectric temperature control systems allowing the temperature of these two regions to be set independently. The temperature in the analysis module may be varied from 4°C to 40°C: thermal equilibration occurs at a rate of 5°C change per hour. The temperature of the sample rack can also be varied from 4°C to 40°C with a temperature gradient of less than 2°C across the sample rack.

IMPORTANT: *Changing the system thermal set point will require sensor chip normalization at the new set point temperature.*

Referencing

The S-shaped flow path implemented in Octet® SF3 instrument contains no embedded valves, thereby minimizing inter-channel dead volume. Waste port selection determines the flow path followed by sample and buffer. Only one waste port can be open at any time and all others are then dead ended. Reduction of the dead volume greatly improves reference curve subtraction. Large inter-channel dead volumes give marked dispersion, which in turn introduces interferences that cannot be subtracted by the double referencing technique. Furthermore, alignment between injection start and stop points is also improved by reducing dead volume.

The double referencing technique was first pioneered by David Myszka[1] as a means of eliminating interferences from the raw data obtained using SPR techniques. The technique is a two-step process.

In the first step, the user introduces sample over two surfaces simultaneously. One surface has immobilized ligand, the second surface does not. The majority of the response seen on the second surface is non-specific binding of analyte to the surface. Subtracting the response obtained on this reference surface from that obtained on the experimental surface at each time point removes the contribution of non-specific binding from the response.

1. Myszka, D. G. Improving Biosensor Analysis. *J. Mol. Recognit.* 1999, 12, 279-284.

Examination of the response curves following reference curve subtraction may reveal the presence of small distortions. These distortions are generally due to effects of slight temperature variations or bulk refractive index differences. When present, these distortions must be removed by the second step in double referencing. This second step requires the user to introduce a buffer "sample" over the surface. Any response observed is not due to surface binding but rather to the aforementioned temperature or bulk refractive index variations. Subtracting the response obtained for the blank sample from the corrected response obtained for the experimental surface in the first step of the double reference procedure removes the contribution of non-interaction related distortions from the response. Double referencing has proven very effective for most applications.

Chapter 3:

System Overview

Octet® SF3 System

The Octet® SF3 system is a fully automated, three channel, surface plasmon resonance-based biosensing system in a flow injection analysis configuration. The system is designed for high-end functional analysis of affinity interactions. The main system components are shown in Figure 3-1.

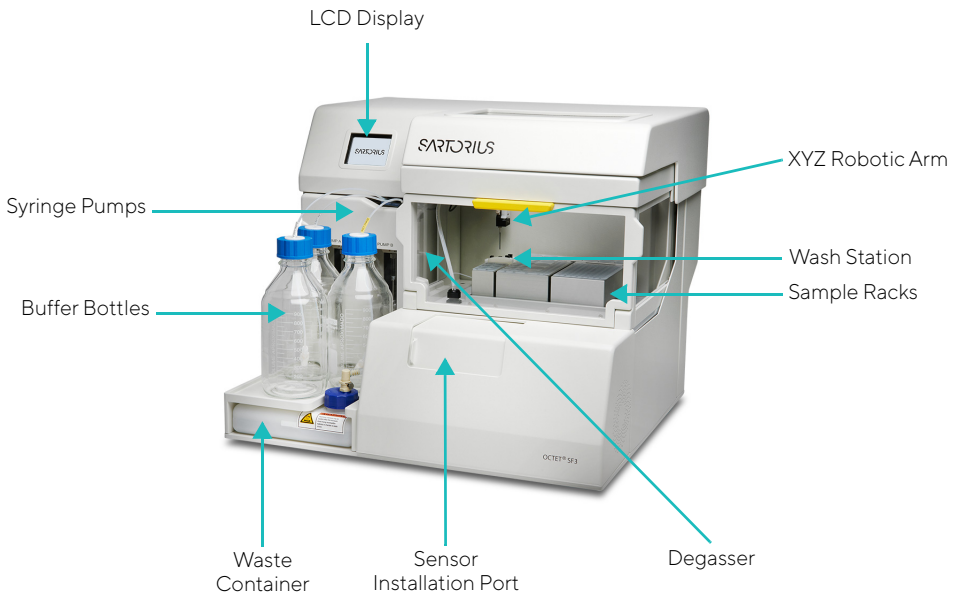

Figure 3-1: System Components

The Octet® SF3 instrument gathers information by optical interrogation of analyte samples exposed to a solid phase pre-functionalized with an affinity ligand. This information reveals a great deal about the analyte-ligand interaction including the analyte concentration, affinity, kinetics, stoichiometry, and specificity. The system is ideal for rapid optimization of interaction conditions. The Octet® SF3 instrument is a versatile system with a high degree of sophistication with user-friendly Octet® SPR Discovery software, which enables wizard-based methods and rapid method editing for experienced users. Together these allow the system to be programmed for unattended operation.

The Octet® SF3 system has three flow channels:

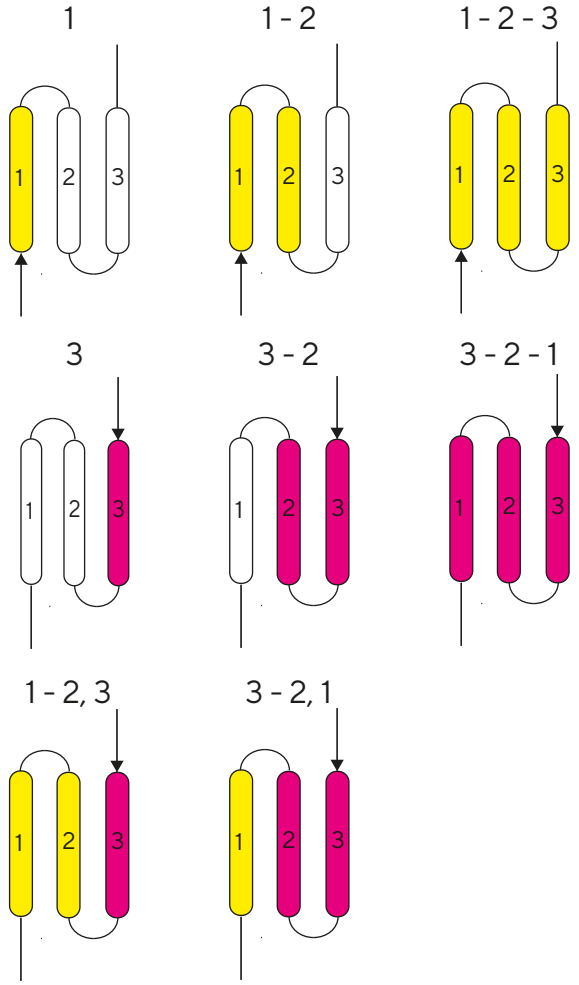

Figure 3-2: Flow Channels

Typically, one channel acts as a reference for the other two working channels, enabling real-time reference curve subtraction. The fluidic system is designed to give sub-second transition from buffer to sample within nanoliter volume flow cells, making accurate measurement of extremely rapid interactions possible. The system is designed to provide a continuous flow of liquid through the flow cell(s) while an analysis is in progress. Two internal sample loops and a dual lumen sample aspiration probe provide a high degree of flexibility.

Bottles containing buffer or water are placed upon the buffer tray and a buffer selector valve determines which buffer flows through the system and over the sensor chip surface.

The waste container is conveniently located underneath the buffer tray and allows both easy collection and emptying of waste.

Reagents and samples to be used during assays are placed into the sample racks - contained within the sample chamber — in either in glass vials or compatible microplates (N.B., a rack adapter is required to accommodate microplates).

A variety of sensor chip surface chemistries with different surface coatings are available from Sartorius to aid assay development for a wide range of applications.

Sensor Chip Cassette

Sensor Chip

The sensor chip is a glass chip coated with a semitransparent gold film. Chips are supplied with a variety of surface chemistries for specific attachment of the affinity ligand (see Octet® SPR Chemistry User Guide). The ligand is usually immobilized in-situ but ex-situ immobilization can be carried out. Additionally, bound analyte may be regenerated ex-situ in order to collect small volumes of highly concentrated materials for further study, by mass spectrometry, for example.

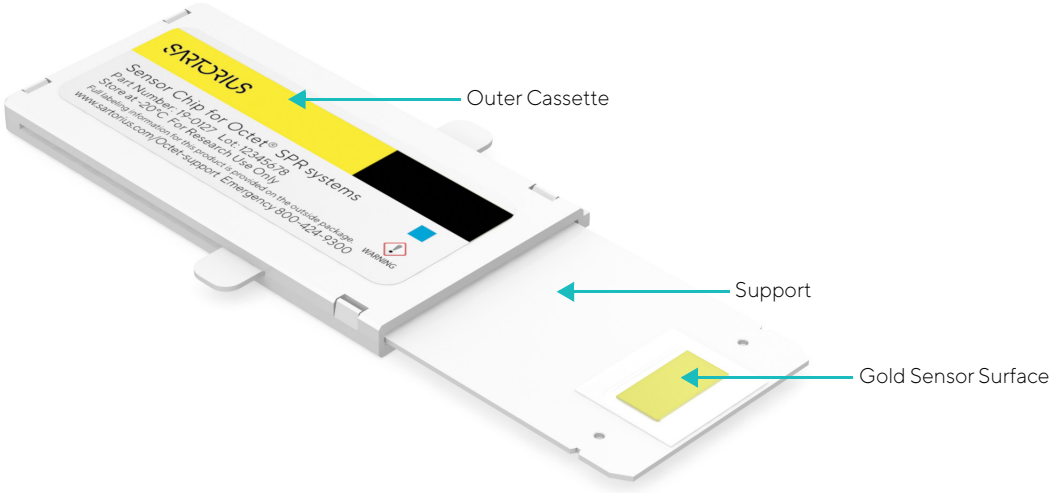

Figure 3-3: Octet® SPR Sensor Chip and Protective Cassette

The sensor chip is supplied within a protective outer cassette that shields the chemistry from direct handling as any physical contact with the surface can adversely affect sensor chip performance. The cassette is designed to fit into an installation port that is located inside the slide door on the front of the system.

Sensor chips are provided individually packed and are sealed in a nitrogen atmosphere. Therefore, it is recommended that sensor chips are used as soon as possible after opening the package.

Sensor chips in unopened packages may be stored for up to one year at the temperature indicated on the packaging and each package is marked with an expiration date.

Sensor chip installation port

The sensor chip cassette is placed into the sensor installation port located inside the slide door on the front of the system. Following the instructions on **Instrument Setup: Replace Sensor Chip**, the sensor chip is inserted into the system by depressing the tabs on the support all the way down. The sensor chip surface is automatically sandwiched

between the optical detector and the flow cell block. Pressure is applied to ensure a leak proof seal and a good optical interface. It is important not to place objects into the installation port that might hinder sensor chip installation. The port door should be closed during normal operation.

Figure 3-4: Sensor Chip Installation Port

Fluid Handling

Sample pumps

The Octet® SF3 instrument possesses two sample loops. The sample probe can aspirate sample into one or both loops depending on the desired injection type. The pumps have a distribution valve which allows flexibility in flow routing. Internal routing valves direct sample from the loops to the flow cell. Sample loaded into loop 1 enters the flow cell through the loop 1 input, contacting sensing channel 1 first, and sample in loop 2 enters the flow cell through the loop 2 input, contacting sensing channel 3 first. The flow direction in the flow cell is controlled by a stream selector valve, where only one of 4 waste exit ports may be open at any time.

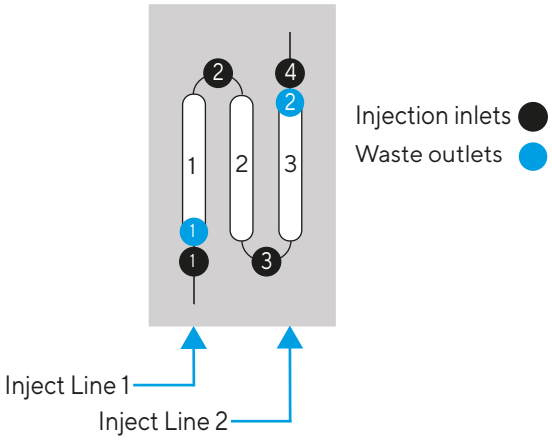

Figure 3-5: Inject Inlets and Waste Outlets

CAUTION:
Do not use flammable liquids with the Octet® SF3 system.

Not intended to be used with biohazardous substances.

Ensure the maximum fluid pressure in normal use does not exceed 65 PSIA.

Buffer Tray

The buffer tray located on the left-hand side of the system and is designed to hold up to two one-liter bottles and one half liter bottle with standard threaded screw caps. All liquids should be 0.2 µm filtered prior to use to prolong the life of the inlet filters.

CAUTION: *The buffer bottle is glass and may pose a potential cut hazard if broken. Use only the buffer bottle supplied with the Octet® SF3 system.*

Degasser

Degassing of buffers is not required as the Octet® SF3 system contains an in-line degasser that continuously eliminates dissolved gas from the running buffer, thereby reducing interference from air spikes. Buffers should always be filtered through filters with a 0.2 µm cutoff.

Waste Container

The waste container is located underneath the buffer tray and holds two liters (2 L) of waste solutions. Before starting use of the Octet® SF3 system it is important that the waste container is emptied, and the waste tube fittings are checked to ensure they are tight. The waste container should be washed periodically in a 0.8% sodium hypochlorite solution to prevent microbial growth.

Figure 3-6: Waste Container

CAUTION *All liquids used in the system will eventually empty into the waste container. It is the user's responsibility to ensure the reagents used do not interact to create harmful vapors or liquids.*

IMPORTANT:
The waste container holds 2 L of fluid but should be emptied each morning or at the end of a method in order to prevent the container from overfilling beyond the liquid level line.

Use only the factory approved waste container provided with the system. Any damage caused to the system from the use of a non-approved waste container may not be covered under warranty.

CAUTION: *Sodium hypochlorite should not be placed into the waste container before commencing an analysis as there is a risk that acids will react with hypochlorite to release toxic chlorine gas. Use adequate ventilation and caution. It is the user's responsibility not to use decontamination and cleaning agents that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained within.*

CAUTION: *After the Waste Container has been removed from the system, the addition of neutralizing hypochlorite to the waste should be performed under a fume hood and the waste should be handled with suitable protective gloves, eye protection and clothing. The specified volume of sodium hypochlorite could potentially release a maximum of* 4.0×10^{-4} *moles of chlorine gas (equivalent to 0.014g). Use adequate ventilation and caution.*

Sample Chamber

The temperature-controlled sample chamber contains the following components:

1. Sample probe
2. Autosampler XYZ-calibration station
3. Wash station
4. Sample Rack mixed format
5. Sample Rack 96-well
6. Front Reagent Rack
7. Rear Reagent Rack

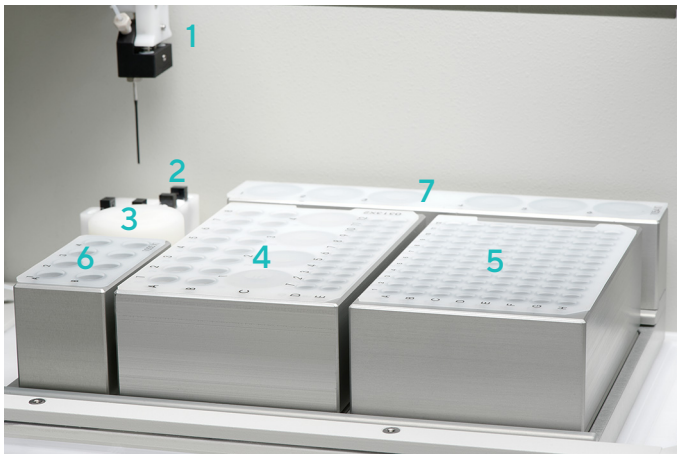

Figure 3-7: Sample Chamber Components

Sample Racks

The sample chamber contains two fixed-position reagent racks and can accommodate two interchangeable sample racks as shown in Figure 3-7. The system is supplied with two vial handling racks but can also accommodate standard microtiter plate formats. The formats and sample volumes are given below. Only factory-approved plates may be used in the system.

Table 3-1: Reagent Racks

Reagent Rack	Number of Wells	Well Volume	Dead Volume
Front	8	2.0 mL	200 µL
Rear	6	7.5 mL	750 µL

Table 3-2: Sample Racks

Sample Rack	Number of Wells	Well Volume	Dead Volume
96-Well	96	900 µL	30 µL
Mixed Format	44 (16, 4, 24)	2.0 mL, 7.5 mL, 900 µL	200, 750 and 30 µL, respectively
P-96-450R	96	500 µL	20 µL
P-DW-11-C	96	1.09 mL	20 µL
PCR-96-FS-C	96	200 µL	20 µL
P-96-280U	96	280 µL	20 µL
P-96-300CW	96	300 µL	20 µL

Table 3-2: Sample Racks

Sample Rack	Number of Wells	Well Volume	Dead Volume
P-384-120SQ	384	120 µL	10 µL
P-384-240SQ	384	240 µL	10 µL

IMPORTANT: *When placing vials in a rack ensure correct vial orientation as incorrect orientation will result in damage to the sample probe.*

Note that the top of the vial lies below the top surface of the rack. This allows a sealing mat to be used to seal each sample position and prevent evaporation.

Figure 3-8: Correct Vial Orientation

When installing a rack, the lip on the rack base will slide into the slot on the rack holder located furthest from the user (Figure 3-9). The user should push the rack toward the back of the system and then slide the rack down. The rack will automatically lock into position. Racks are designed to prevent incorrect docking.

IMPORTANT: *Care must be taken when configuring the installed rack in the Octet® SPR Discovery software as incorrect selection will result in damage to the sample probe. See "Define Sample Racks" on page 45 for further information.*

Figure 3-9: Installation of Sample Rack

To remove a rack, the user should slide the rack backward against the spring tension and lift upward to release the holding pins.

Evaporation of sample is reduced by using silicone septa. Septa must be used with all plate formats for best assay performance. The probe pierces the septum, which reseals after the probe is withdrawn. Any droplets on the probe are removed as the probe is withdrawn from the septum. Carry-over is not possible with this system. Samples may be chilled while held in the sample racks by setting the rack temperature. A thermoelectric cooler brings the racks to the set point temperature. The racks are installed as shown above. The septum must be firmly installed into the rack before installing in the system (Figure 3-10). A loose septum can catch on the autosampler probe and cause an error.

Figure 3-10: Installing Septa

XYZ Autosampler

The XYZ autosampler aspirates samples into the system for analysis. To avoid personal injury the robotic arm automatically deactivates when the sample chamber door is open. The autosampler is capable of self-calibration. In addition, the injection probe senses the bottom out position thereby avoiding damage to the probe. Damage to an injection needle, or probe, is a common failure mode in many other autosampler designs that results in expensive downtime and replacement parts.

CAUTION: *Make sure the autosampler is in the home position and the system is in an idle state before accessing the sample chamber.*

Sample Probe

The sample probe aspirates sample into one or both sample loops. Each line is connected to the probe via a low dead volume standard fitting. The probe possesses sensors that relay positional information to the system thereby allowing self-calibration. The probe is stainless steel with a Teflon™ coating on both the inside and outside surfaces.

Sample Rack Temperature Control

The temperature of the sample rack can be varied from 4 °C to 40 °C with a temperature gradient of less than 2 °C across the rack. The actual temperature the samples experience may vary depending on the rack type, vials, or plates chosen. This is acceptable as sample refrigeration does not require precise temperature control. If the rack temperature is set close to the lower limit, *e.g.* 5 °C, condensation may cause water accumulation when the air is humid. The water will drain to the waster container but it may also accumulate in the wells with the potential to contaminate samples. Therefore, it is best to avoid condensation by maintaining the rack temperature above the condensation point. After 24 hours of system inactivity, the rack temperature will automatically be raised to 15 °C to minimize condensation build up when the system is not in use.

Wash Station

At the wash station, the probe inserts into a tubular cavity and dispenses liquid — the “overflow fountain”. As the probe is immersed in this port, the liquid overflow washes the inside and outside of the probe. Droplets in the tip of the probe are removed by pulling the probe through the wash station cover.

Some basic washing procedures are an integral part of the basic injection mechanism but additional washes may be added if the user desires. When running samples, purging the probe between injections is recommended to further minimize risk of cross-contamination.

Analysis Module

Flow routing

All eight possible injection flow paths are shown in the following Figure 3-11.

Note that it is not possible to inject into channel 2 without first entering channel 1 or channel 3. The total volume that must be displaced when injecting over all three channels is <150 nL. Therefore, at an injection flow rate of 50 µL/min, the entire volume of the flow cell is swept out >5 times per second. This can be considered instantaneous delivery over all three surfaces as the time delay between channels is negligible. This eliminates the need to x-normalize the data set.

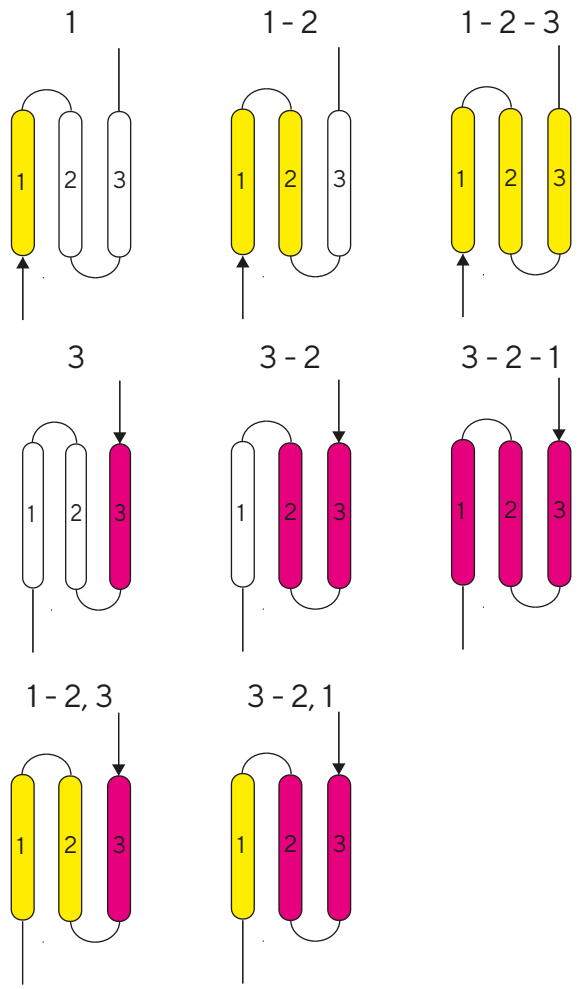

Figure 3-11: Eight Possible Injection Flow Paths for Injection on Octet® SF3 System (Sample A: Yellow, Sample B: Magenta)

The last two injection flow paths may be used simultaneously by using parallel injections. However, parallel injections cannot be done with fast rise and fall times. Parallel injections should only be used for immobilizations and, possibly, experiments to determine affinity constant values but should not be used for kinetics experiments.

The Octet® SF3 system uses a unique 'run-to-waste' pre-injection technique to bring sample to the flow cell surface without contacting the sensing regions. Once the dispersed sample at the beginning of the sample plug has exited to waste, the waste selector valve changes the exit port, causing sample to instantaneously arrive on the desired sensing channels at full concentration. Using this method rise times and fall times of <0.75 seconds are possible, thereby enabling fast binding events to be measured.

Analysis module temperature control

The temperature in the analysis module may be varied from 4 °C to 40 °C (maximum of 15 °C below ambient). It is necessary to control the temperature accurately because the SPR response varies significantly with temperature. A 1°C change causes a change in response of approximately 100 RU. The LCD display will indicate when the temperature set point has been reached; however, the baseline response should be examined until the desired stability is achieved. Alternatively, allow 1 hour per 5 °C change in set point for thermal equilibration. The long-term baseline drift of the system is 0.3 RU/min implying that the temperature at the sensing surface does not change by more than 0.003 °C per minute. This is readily achieved by a combination of thermoelectric control, thermal insulation, and heat sinking.

LCD Display

The LCD display provides at-a-glance information about the status of the system. An example of this display is shown in Figure 3-12. The main portion of the display is used to show the current operation in progress and an estimate of the time remaining to complete this operation.

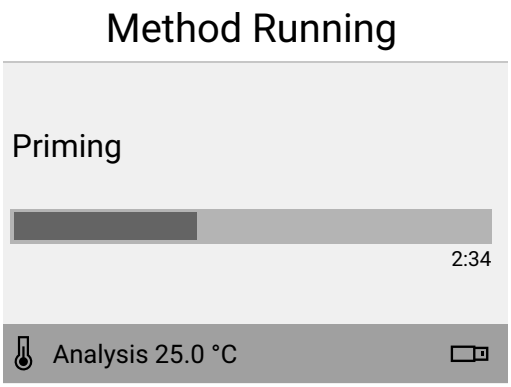

Figure 3-12: LCD Display

Chapter 4:

Common Operations

Buffer Loading

A wide variety of buffers may be chosen as running buffer but the most commonly used are HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffered saline (HBS) and Tris (tris (hydroxymethyl) aminomethane) buffered saline (TBS) buffers. We recommend HBS-EP+ buffer. Amine containing buffers, such as Tris or glycine, are best avoided until ligand immobilization has been completed if using the amine coupling method. Other buffers such as carbonate, phosphate, borate, etc., may be used, but these buffers have a tendency to precipitate more than organic buffers. It is advisable to filter all buffers through a < 0.2 µm filter. Degassing is not necessary as Octet® SF3 instrument is equipped with an in-line degasser. The buffer intake lines also possess filters to prevent possible blockages from particulates.

Water Line

The Octet® SF3 instrument contains a dedicated water line in order to minimize the risk of buffer precipitation and potential blockage formation Therefore, it is recommended that when not in use, the system is placed into deionized water via the water line, which can be achieved two ways:

1. Using the Instrument Setup page, choose **Switch Line** and select **Water Line** for the new line. Ensure that Prime into new line is checked and click **Start**.
2. Via use of the Switch Line tool at the end of a method (See "Switch Line" on page 117 for further information).

NOTE: *After 72 hours of inactivity, the system will automatically switch to the water line.*

After switching lines, the system will enter Hibernation. When in Hibernate, the system maintains the system temperature and constant buffer flow. At 4-hour intervals, a purge is performed in order to cause buffer flow through all wetted surfaces and channels. The hibernate routine will usually dissolve precipitates before they become blockages. This cycle is repeated indefinitely until the Hibernate routine is aborted.

It is important to note that all lines should be 'wet' for example if buffer line B isn't being used it must have water or another buffer attached to it and cannot be left dry in the air.

IMPORTANT: *If the system has been in Hibernate for more than 3 hours, the user should run a Prime routine with buffer before commencing a new analysis. At the end of running a long method, it is recommended that the method is set to switch line to the water line while the system hibernates.*

Sensor Chip Install/Uninstall

Installation of the chip is directed from the software through prompts. Go to the Instrument Setup tab then select **Replace Sensor Chip** from the tree control on the left (Figure 4-1). Follow the prompts as directed. It is not recommended to remove the sensor chip from the cassette. It is possible to store and reuse sensor chips (see Octet® SPR Chemistry user guide).

IMPORTANT: *The surface chemistry on the sensor chip can be damaged if touched.*

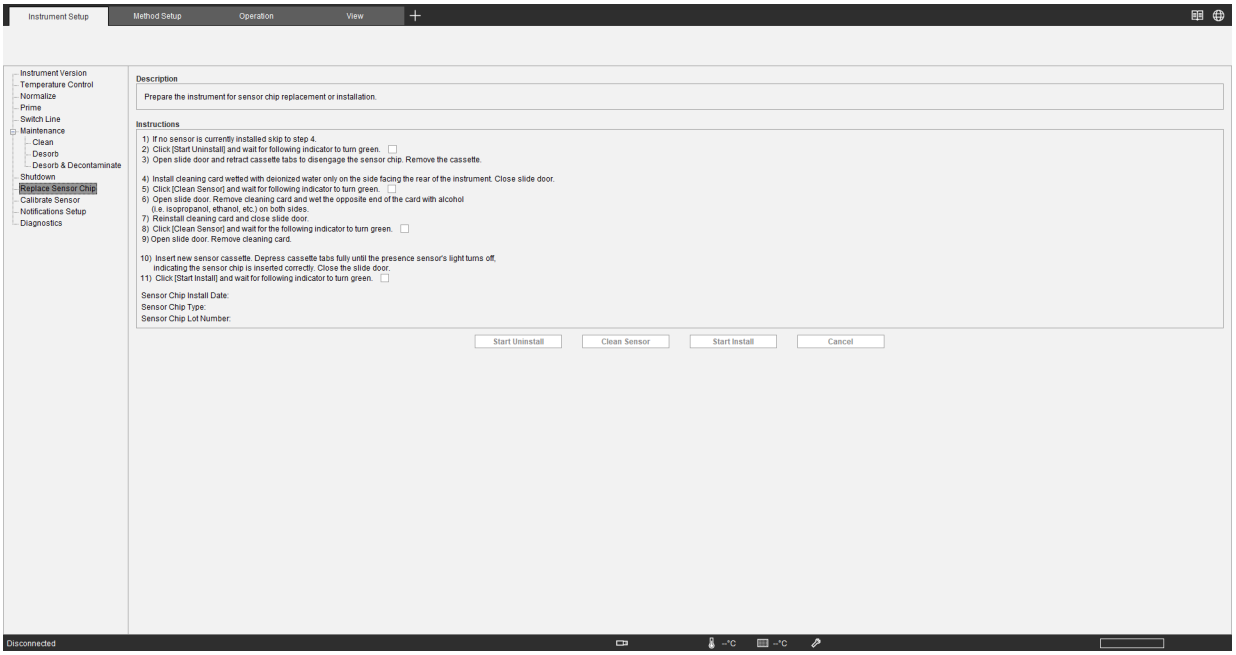

Figure 4-1: Instrument Setup Tab

When installing a new sensor chip, the dialog in Figure 4-2 will appear. Select the chip chemistry type (or maintenance sensor chip) from the drop down list or enter a custom chip type. The chip serial number should also be entered to allow accurate tracking of sensor chip usage. This information will be recorded with any subsequent data files recorded with the system. The data is retained in the system until the chip is uninstalled.

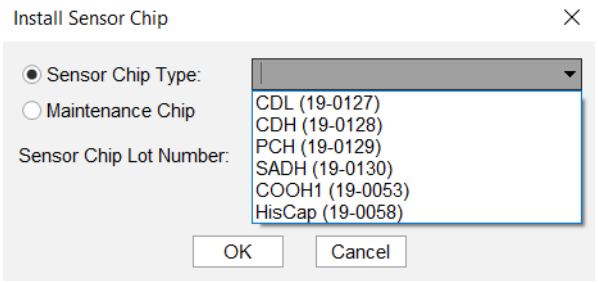

Figure 4-2: Install Sensor Chip Dialog

Open the sensor chip docking door by sliding to the right (Figure 4-3 left), install the sensor chip and depress the cassette tabs all the way down until the cassette tab bottoms out. When correctly installed, the optical sensor light that detects the sensor chip should turn off (Figure 4-3 right). After installing the sensor chip, close the sensor chip docking door by sliding it to the left (Figure 4-3 bottom).

Figure 4-3: (Left) Open sensor chip docking door with no sensor chip inserted. (Right) Sensor chip correctly installed with tabs pushed all the way down and the optical sensor light turned off. (Bottom) Closed sensor chip docking door after sensor chip installation.

IMPORTANT: *For best performance of the sensor chip, installation should be performed when the analysis chamber temperature is above 20 °C. Uninstallation should be performed when the analysis chamber temperature is below 30 °C. The control software will automatically enforce these constraints. After installation/uninstallation, the analysis temperature can be adjusted as desired.*

Cleaning Card

Cleaning cards are supplied to enable cleaning of the fluidic and optical interfaces before installing a new sensor chip. The cleaning card does not have an active SPR surface and is instead composed of an absorbent pad which is used to clean the SPR detector and the flow cell.

Run the replace sensor chip program by choosing **Instrument Setup > Replace Sensor Chip**.

A new cleaning card should be used for each sensor chip installation.

Loading Samples

When possible, samples should be centrifuged to remove particulate matter. If this is not possible, prepare samples in filtered buffer.

Load samples into the appropriate plate or rack on the bench before attaching the septum. Avoid trapping air bubbles in the bottom of wells. The septum may be pressed into position by hand or a manual press may be purchased for this purpose. It is best to load sample dilutions in order to avoid confusion. Random sampling can be chosen when running a method therefore, making it unnecessary to randomize samples in the racks.

Choose the large vial holder rack or the reagent racks for solutions required in large quantities. Refer to "Sample Racks" on page 19 for details on rack installation.

After installing the rack, select **Operation > Configure Racks** and choose the correct rack. Then **select Instrument Setup > Temperature Control** and set the **Sample Rack Temperature** as shown in Figure 4-4.

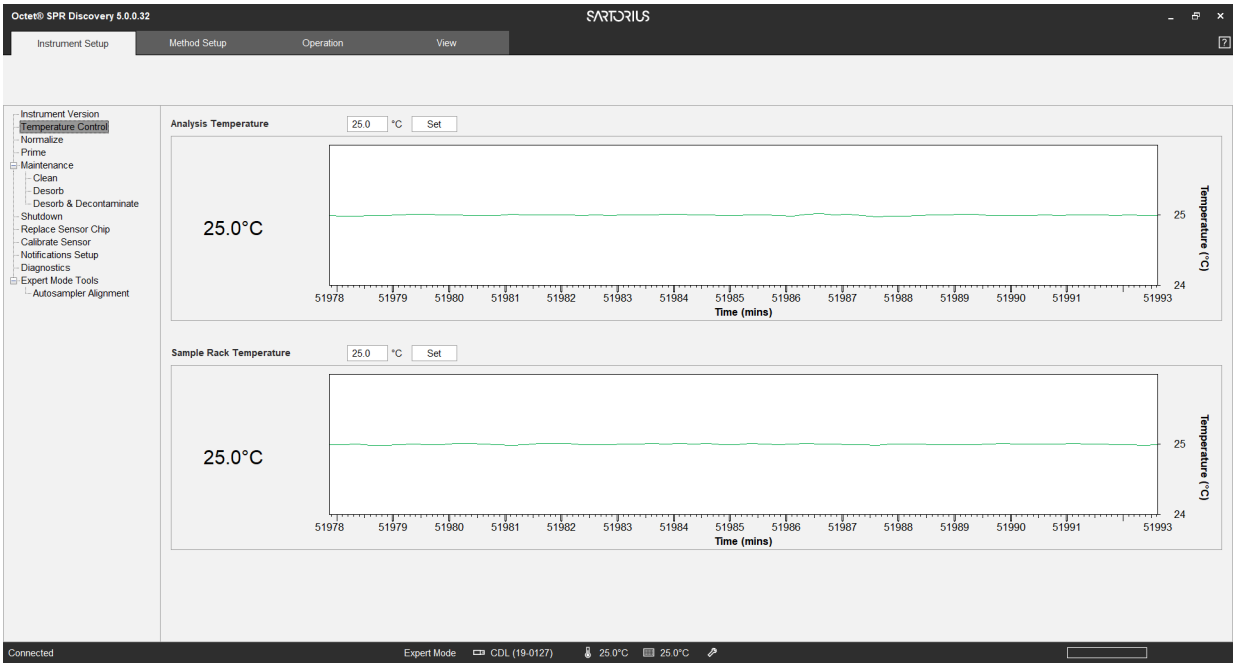

Figure 4-4: Instrument Setup Temperature Control Page

Usually, it is advisable to cool the samples below ambient to prevent denaturation of the samples while they sit in the rack. This is particularly important when a large number of samples are to be analyzed as samples may be in the rack for several days. Note that it is possible to set the system temperature from this page. Remember that re-setting the system temperature will require waiting at least 2 hours for complete temperature equilibration. The optical system should then be normalized before any analysis. Usually only thermodynamic analyses require frequent changes in temperature. All other assays are either performed at the standard 25 °C or at physiological temperature (*i.e.,* 37 °C). It is possible to track the system temperature while running response curves in the Operation page by checking the **temperature box** on the top right side of the response curve plot.

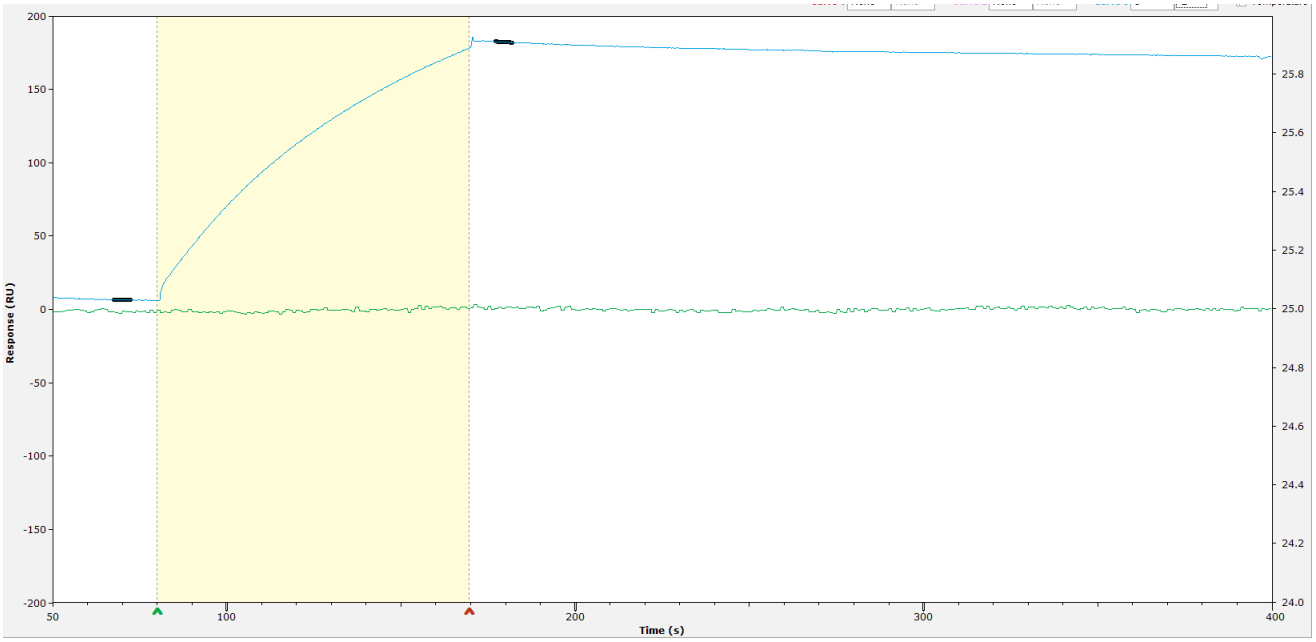

Figure 4-5: Response Curve Plot - Temperature Box

The temperature plot appears in green on the plot with the temperature scale on the right side of the plot.

The system temperature is the core temperature inside the insulated compartment containing the fluidics components and sensor. It is recommended that the user leaves the Octet® SF3 system in Hibernate mode rather than turning it off as this maintains the temperature, and when required, the user can resume normal operation without a long thermal equilibration period.

IMPORTANT: *If the system has been in Hibernate for more than 3 hours, the user should run a Prime routine with buffer before commencing a new analysis. At the end of running a long method, it is recommended that the method is set to switch line to the water line while the system hibernates.*

Normalization

The system is automatically normalized in air during SPR sensor chip installation. Therefore, it is recommended that the normalization method is run upon docking of a sensor chip in order to adjust the detectors response to any differences between individual sensor chips. It is recommended to run the normalization procedure prior to any immobilization.

Normalization is also required after any temperature change.

100% DMSO is recommended for normalization. There is also a high-viscosity normalization option which is slower but enables the use of non-DMSO solutions such as 70% glycerol as the normalization solution.

The normalization program can be found in the Octet® SPR Discovery software under **Instrument Setup > Normalization**.

To perform normalization in DMSO, drag a vial from an appropriate position in the rack into the position box (R2E1 shown in Figure 4-6). Add 606 µL of DMSO to an appropriate sized vial and add to the rack, ensuring that the position matches that chosen. Click **Start** to perform normalization in DMSO.

Ensure the **High Viscosity** box is checked when using non-DMSO normalizing solution such as 70% Glycerol. This will cause the normalization solution to be loaded and injected at a slower rate.

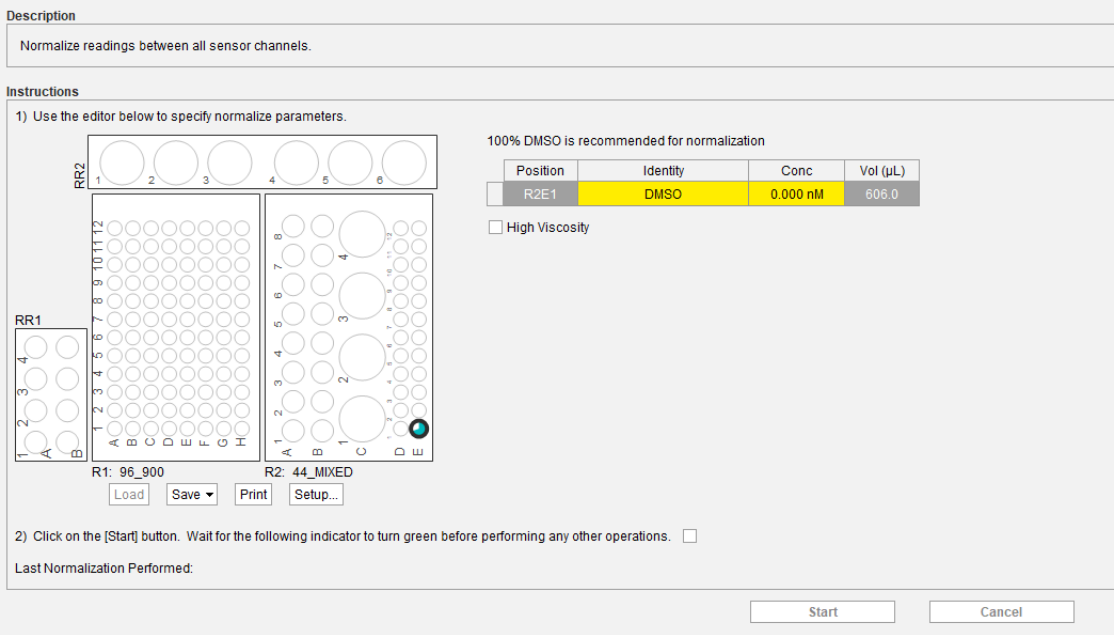

Figure 4-6: Instrument Setup Normalization Page

Chapter 5:

Software Overview

Overview

The control software is divided into four main pages which are selected by their tabs at the top of the window:

- Instrument Setup
- Method Setup
- Operation
- View

The software opens as default to the Operation section.

Each section is described briefly in the following sections.

Instrument Setup

The Instrument Setup page is the interface through which the user can control the analysis and rack temperature, install a new sensor chip, normalize sensor chips, and perform required maintenance procedures. Some of these procedures also appear in the Method Setup page and Operation page. When a particular procedure is selected, directions for that procedure appear on the panel. These prompts are brief and easy to follow.

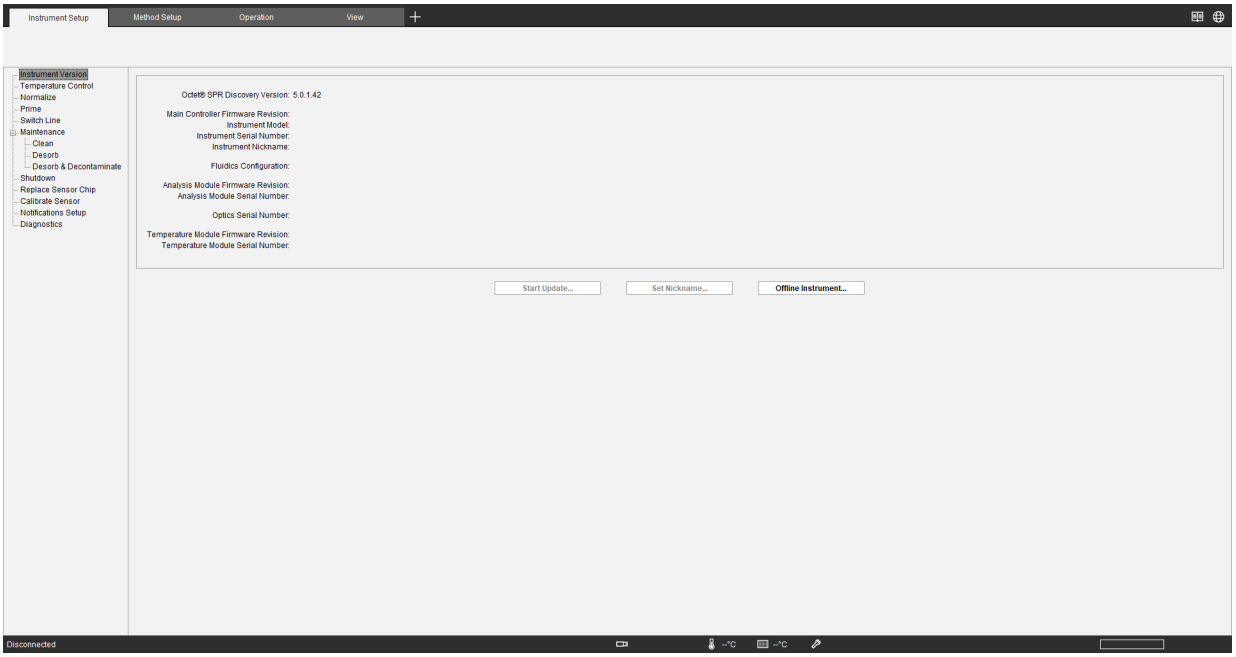

Figure 5-1: Instrument Setup Page

Method Setup

The Method Setup page allows the user to select from a number of pre-written methods that guide the user through method setup and also provides the flexibility to write, modify and view bespoke methods. The intuitive Octet® SPR Discovery software enables the user to construct even the most complex methods quickly.

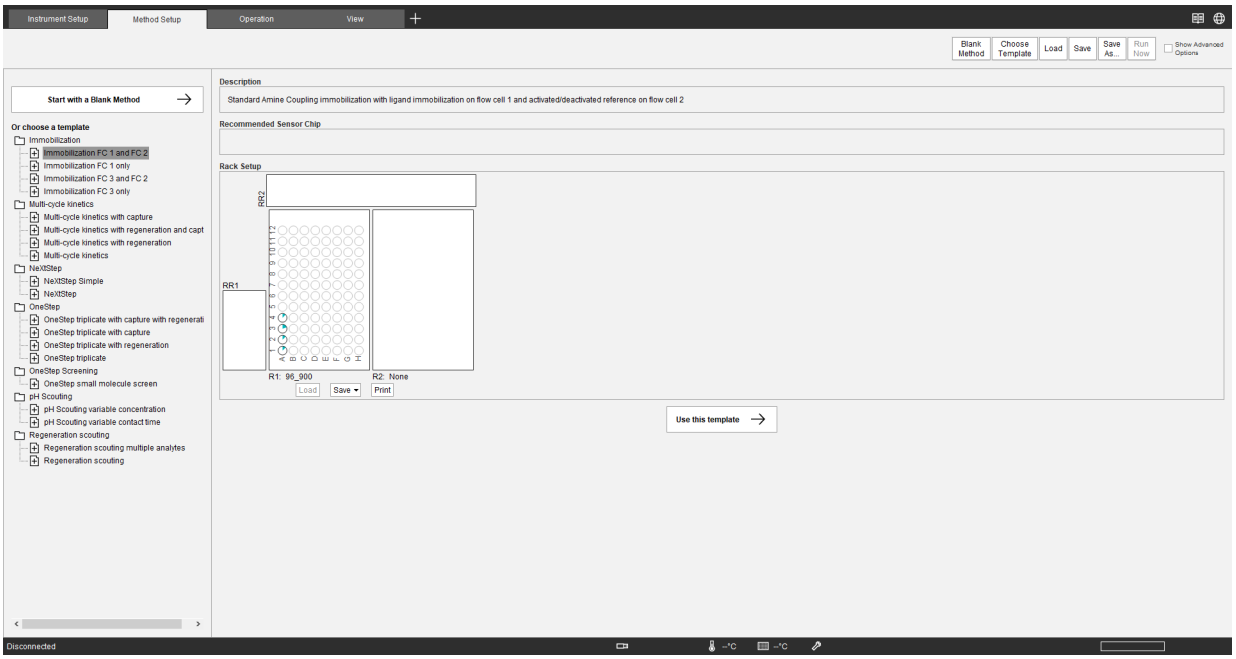

Figure 5-2: Method Setup Page

The Method Setup environment consists of a toolbar where system commands can be selected and then dropped into a method time strip. Methods can be constructed, checked for errors, saved, edited, and reloaded.

It is possible to open multiple method windows and SPR result files simultaneously by clicking on the plus sign and selecting the desired function.

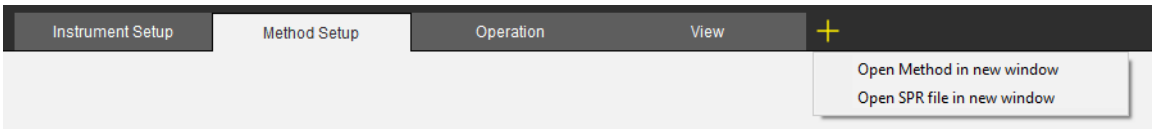

Figure 5-3: Opening Multiple Method Windows and Results Files

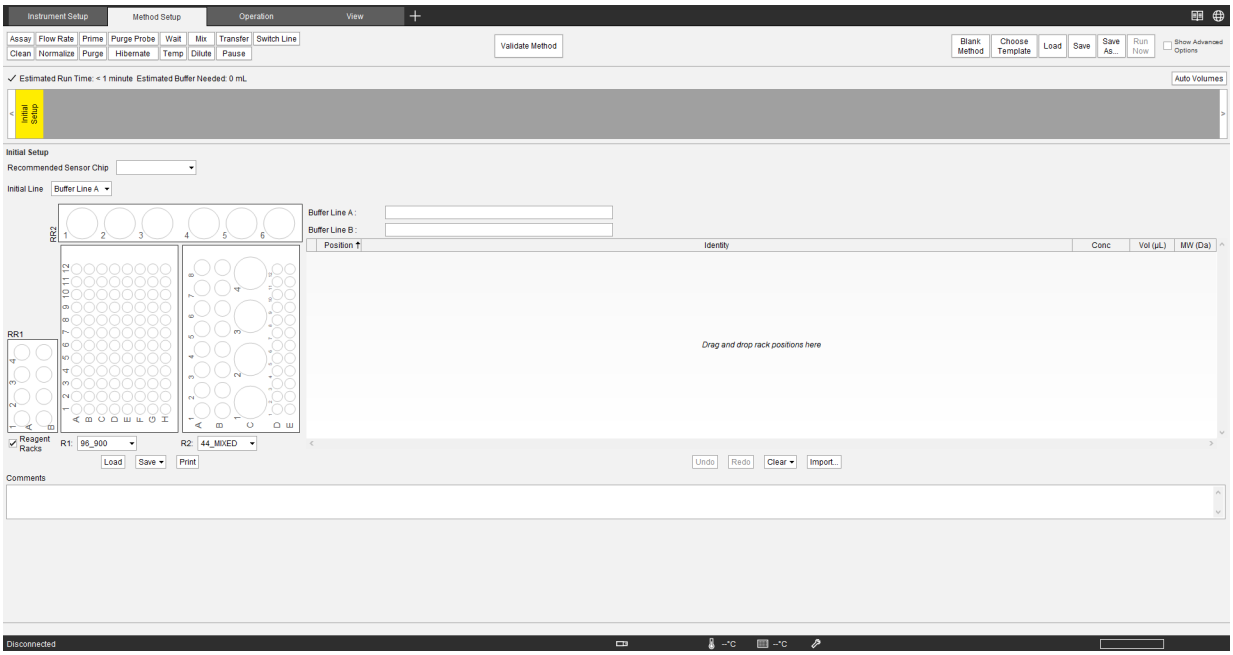

Figure 5-4: Selecting System Commands

Operation

The Operation page is the main user interface. There are two operation modes, Manual mode and Automated mode. In Manual mode, the user can perform basic operations such as dilutions or transfers as desired, or start a new response curve. While recording a manual response curve, samples may be injected in real time. The use of Manual mode allows the user to easily investigate, develop and optimize assay conditions in real time before constructing a method. In Automated mode, methods can be loaded and executed.

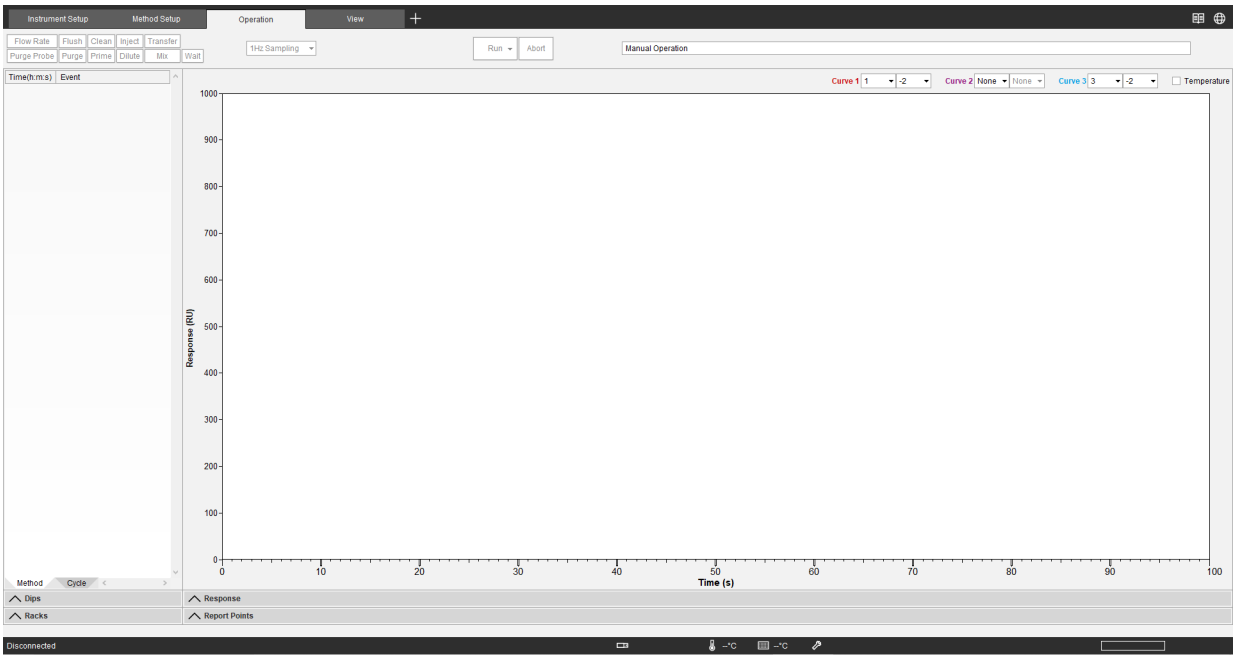

Figure 5-5: Operation Page

The Operation page contains a wealth of information when all panels are displayed simultaneously. It is advisable to minimize panels not in use, leaving only a single response curve plot and the event log. The Event Log displays the history of all events during that Method or specific recording cycle (selectable by the tab at the bottom of the Event Log).

The Dips panel shows the three SPR minima being tracked in order to generate the three channels of data in the response plots. Each dip shifts to the right (higher SPR angles) when the mass increases on the corresponding sensing surface. This shift is plotted as a function of time on the time plots. When operating the system the Dips plot is updated in real time and it may be used to check the quality of the SPR signal. The dips shown are an example of how the SPR signal should appear. The sample racks are shown in the bottom left panel.

Notice that each plot has an associated channel selection drop-down list. This allows the user to decide which plot to display. For example, the plot above shows channels 1 and 3 with channel 2 subtracted while the bottom plot shows the non-subtracted channels 1, 2, and 3. Each plot can display up to three curves, identified by the color of the text next to the channel selection drop-down list.

The bottom right table contains the report points for the response curves. The table will display the channels corresponding to the visible curves in both graphs. Report points may be added manually or pre-programmed and provide a means of reporting the response at any time during a response curve. This enables accurate determination of the response as at least 5 points must be included in the calculation. It is easy to hide any of these information panels by simply clicking the associated tab.

To enter Automated mode, click on the **Run** button, and choose the option to **Load and Run** a method. A new prompt will appear with additional options. Manual mode commands are disabled when a method is loaded or running. The software will automatically revert back to manual mode when a method is complete.

In Manual mode, commands are executed as the user selects them but further commands may be queued for performance after the current command. Commands such as Inject are only available in manual mode if a response curve is being recorded. Other commands such as dilute are not available when a response curve is being recorded. See the next chapter for a full description of Manual mode.

View

The View page allows the user to open previously saved data sets. The View page shares many functions in common with the Operation page, including all response curve related functions such as Add Report Point, Add Comment, etc. If a data file contains multiple assays, then each assay can be selected from the Assay drop-down list. If an assay contains more than one cycle then each cycle is opened separately by selecting from the Cycle drop-down list.

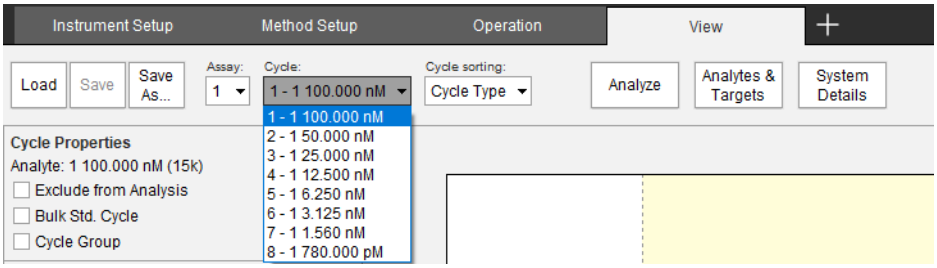

Figure 5-6: Selecting a Cycle

The graphs, event log, and report points are immediately updated with the data from the selected cycle. When selected, the Dips graph will display a representative dip signal recorded at the beginning of the selected cycle. An additional dip signals is saved at the midpoint of each injection, and a final dip signal is recorded at the end of the cycle.

By default, cycles are sorted into the order they were listed when the method was written. Since it is possible to set up an assay to run its cycles in random or reverse order, the Cycle Sorting dropdown may be changed to list the cycles in the order they were recorded by the system.

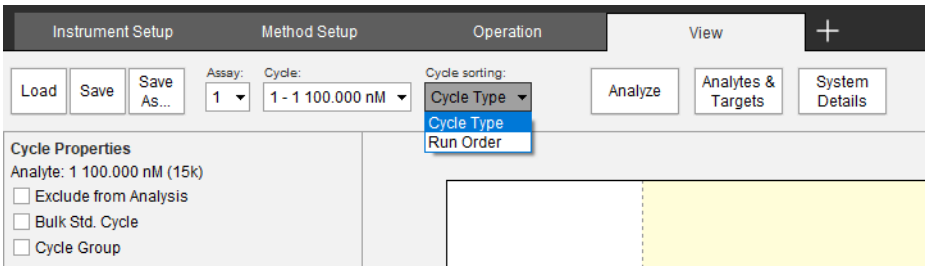

Figure 5-7: Cycle Sorting Selection

It is not possible to simultaneously view data for more than one cycle in the view page (although it is possible to open the same data set simultaneously in a separate window by clicking on the plus sign and selecting open SPR file in new window). The Octet® SPR Analysis software provided with the Octet® SF3 system is tailored for viewing and analyz-

ing multiple cycles. If a particular cycle appears to be an outlier, the 'Exclude Cycle from Analysis' box in Cycle Properties may be checked, and that particular cycle will not be included when the assay data is exported to Octet® SPR Analysis software.

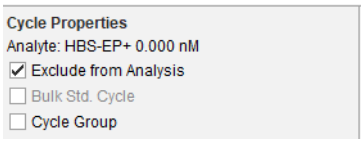

Figure 5-8: Cycle Properties

To easily group subsets of assay cycles, simply click on the **Cycle Group** box. Five different cycle groupings are allowed and each group is designated by a different color. Each click on the Cycle Group box will rotate through the different group colors. A specific Cycle Group can be exported by selecting the appropriate option in the Octet® SPR Analysis export dialog.

Changes made to a file including adding report points, comments, excluding a cycle, etc. can be saved back to the original file by clicking the **Save** button. Alternately, use the Save As button to choose a new file name and retain the original file without changes. Note that a file currently being recorded on the Operation page may be loaded simultaneously into the View page in order to view other cycles. However, in this case any changes made to the file from the View page will NOT be saved.

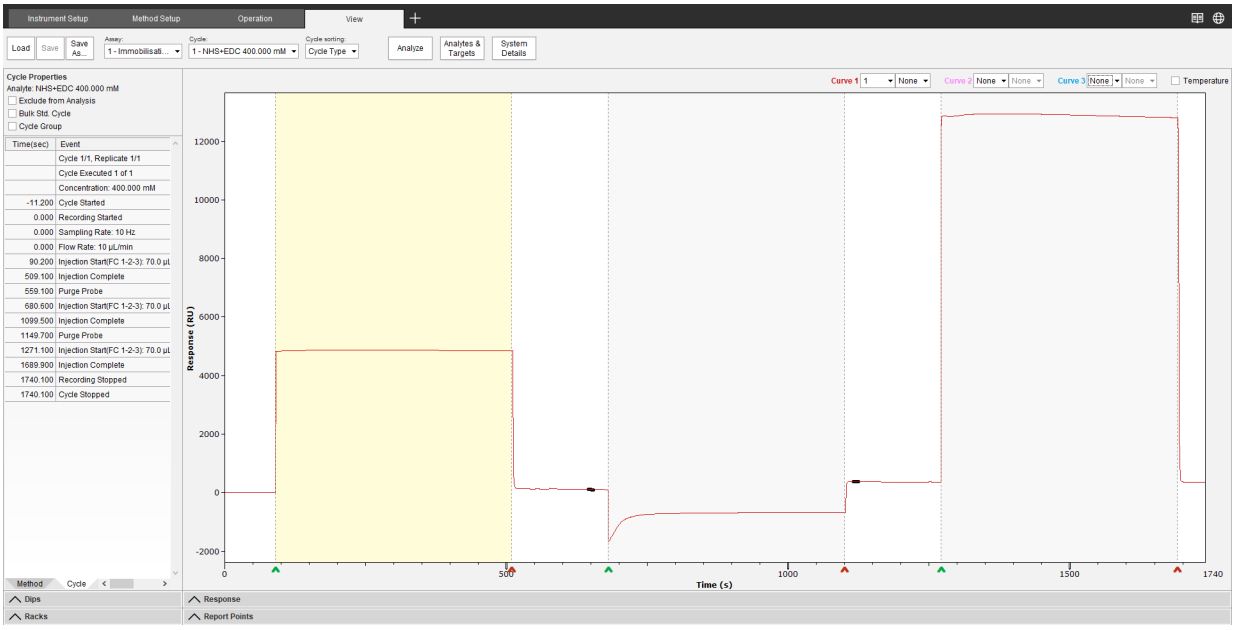

Figure 5-9: View Page

Graph Features

The graphs on both the Operation and View page share some common features that can be accessed by right-clicking on the graph.

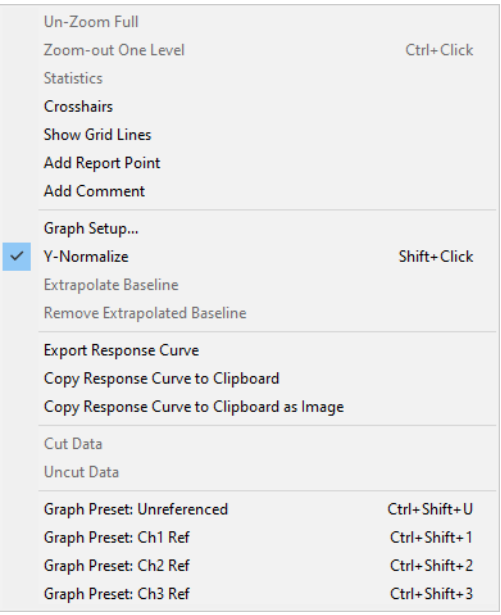

Figure 5-10: Graph Right-Click Menu

Zoom In/Out

To zoom in on a section of the response graph, click and hold the left mouse button. Dragging the mouse will draw a box on the graph indicating the zoom region. Release the left mouse button to zoom in. To zoom out, right-click on the graph and select one of the two un-zoom options: **Un-Zoom Full** or **Zoom-out One Level**. The latter option is only available if zoom-in has been performed more than once.

A shortcut for zooming out by levels is available by holding down the Control key while left-clicking once on the graph.

Crosshairs

The crosshairs tool allows the user to track all events and report points. When the crosshairs are active on the response curve plot, clicking on a report point in the report point table or an event in the event log will shift the crosshairs to that time point on the plot. The crosshairs can then be dragged to another location on the graph in order to assess changes in response levels.

It is advisable to restrict the number of injections in a response cycle to keep event and report point tracking manageable. When multiple dip signals have been recorded in a cycle, the crosshairs can be used to update the dip graph. The recorded dip signal that is closest in time to the crosshairs position will be shown on the graph.

Y-Normalization can also be performed by holding down the Shift key while left-clicking on the graph.

Adding report points in real time

To add a report point, right-click on the graph and choose the **Add Report Point** option from the menu. Note that if the crosshairs are currently enabled, the report point will be added at the time indicated by the crosshairs. If the crosshairs are disabled, the report point will be added at the time corresponding to the location of the right-click. Adding a report point is a more rigorous means of finding the response at any location as it includes the average over a number of points.

Enter the number of points to average, baseline selection, and comments in the pop-up dialog. This new report point will be added to the report point table. Note that this report point was not designated as a Baseline and will therefore, be relative to a previous report point that was designated as a baseline. If no such report point exists then one may be added, otherwise only the absolute response will be calculated. Report points can be deleted from the report point table via a right-click mouse menu. These functions are also available in the View page. It is recommended to save a backup copy of all files before making any modification in the View page.

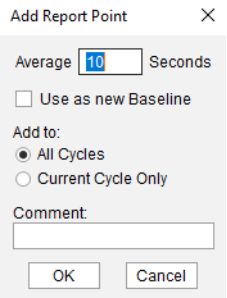

Figure 5-11: Adding a Report Point

Comments

To add a comment to a response curve, right-click on the graph and choose the **Add Comment** option. The text entered in the pop-up window will be inserted into the cycle event log in italics. As with report points, the time at which the comment is inserted depends on whether or not the crosshairs are enabled. If the crosshairs are disabled, the comment is inserted at the time corresponding to the location of the original right-click.

Cut data

Although extremely unlikely, air spikes can occur occasionally. It is recommend that any cycles with significant air spikes be omitted from data analysis. These spikes can occur over several seconds and cause large baseline shifts, thereby affecting resultant data quality, or they may be short and cause no baseline shift. In the latter case the data quality may be sufficient for analysis if the spike is removed. The Cut Data tool in the drop-down mouse menu can be used to remove these spikes. Zoom into the data region to be cut and then select **Cut Data** from the menu. The data points that have been removed will be replaced with data points that lie on a line connecting the segments on either side of the cut region. On occasion, spikes may appear in the response curve plot. These spikes are usually due to rapid bulk refractive index changes in the flow cells when exposed to low or high refractive index solution. The slight difference in sample arrival between the two sensing channels causes an offset in the reference curve subtraction that appears as a spike.

Any solution that has a bulk refractive index that is significantly different from the running buffer will cause this type of spike. It is highly recommended that the refractive index of the sample buffer be matched with that of the running buffer wherever possible. This is best achieved by preparing the sample in the running buffer.

Allowing an unclosed micro-centrifuge tube, filled with such a sample, to sit at room temperature for a few minutes will allow sufficient evaporation to cause an offset in bulk refractive index between the injected sample and the running buffer. It is advisable to fasten the septum on all racks immediately after loading samples to minimize evaporative losses that cause bulk refractive index changes.

Extrapolate baseline

This command allows the user to estimate the effect of a drifting baseline on a subsequent injection. For example, in the initial capture shown in Figure 5-12, a very low level of drift is occurring after the antibody has been captured. If a large degree of drift does occur after the capture step then it is usually difficult to determine the true dissociation rate constant for the second analyte interaction (usually the binding partner to the captured molecule). In addition, the readout panel will show a new response reading designated Curve 1-Base — this is the difference between the actual curve and the extrapolated baseline at the position of the crosshair. This gives an estimate of the relative binding response for the second injection.

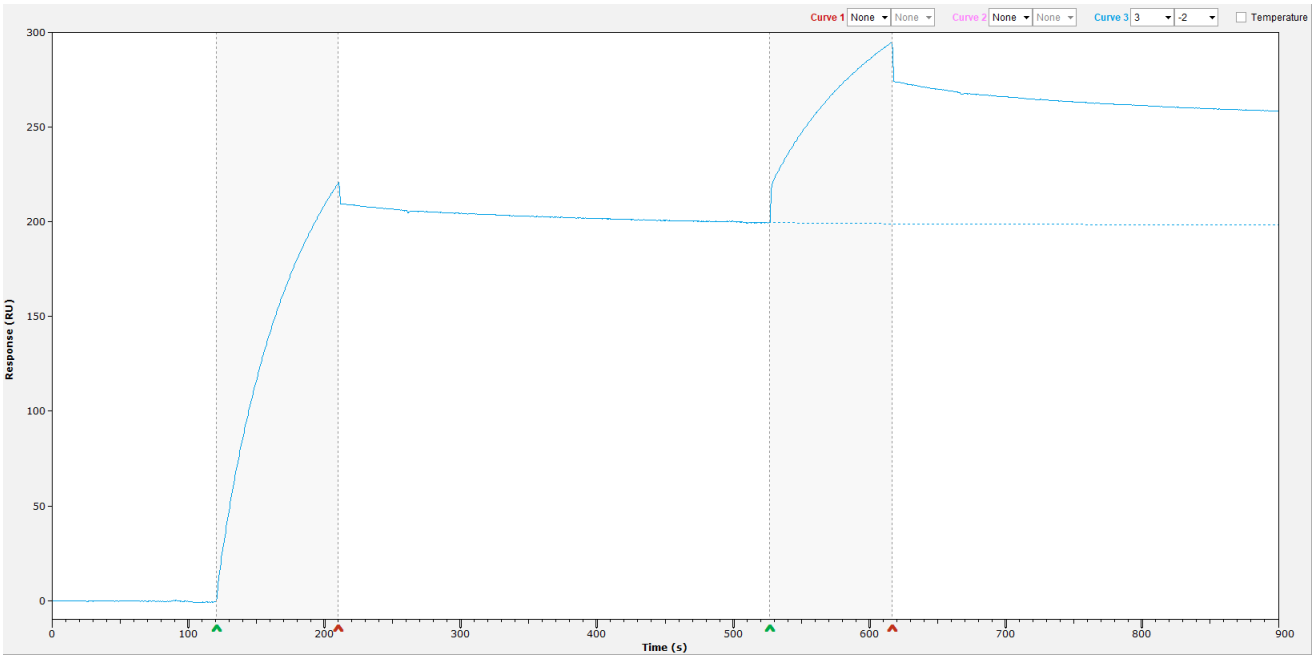

Figure 5-12: Extrapolated Baseline

Graph setup

By default, when not zoomed-in, the response graphs will automatically scale the x- and y-axes to show the entire response curve. Alternate behavior can be configured through the Graph Setup pop-up as shown in Figure 5-13. For the x-axis, the 'Most Recent' option will show a fixed x-axis window size that will move to show the most recent data as

it is being recorded. (This option is not particularly useful for the View page). Using a fixed y-axis may be useful, for example, to prevent the auto scale from masking the effect of a small analyte injection that is followed by a large bulk shift due to a regeneration injection.

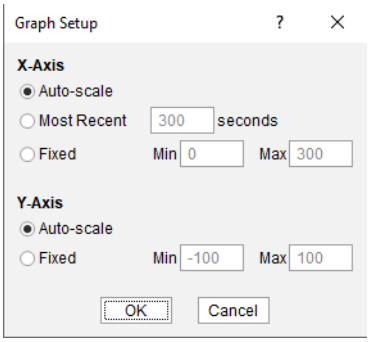

Figure 5-13: Graph Setup

Y-normalization

When two raw response curves are plotted on the same plot then there is usually an offset in response related to slight differences in the initial baseline position. This offset is inconsequential and is eliminated by simply overlaying the two curves at some point. The control software is programmed to perform this Y- Normalization 10 seconds after a response curve is initiated. However, this may be performed manually by right-clicking on the graph at the desired Y-Normalization time and then selecting **Y-Normalize** from the context menu. An existing Y-Normalization may be reversed in a similar manner. Y-Normalization can also be performed by holding down the **Shift** key while left-clicking on the graph.

Export response curve

This command enables the data set to be saved in .CSV file format, which may be edited in other programs such as Microsoft Excel. If a zoom-in has been performed on the graph, then only the data between the x-axis limits of the graph will be exported. Otherwise the entire response curve is exported.

Copy response curve to clipboard

This command enables the data set to be pasted into spreadsheets such as Microsoft Excel, GraphPad Prism, etc. As with the export function, if a zoom-in has been performed on the graph, then only the data between the x-axis limits of the graph will be copied. Otherwise the entire response curve is copied.

Copy response curve to clipboard as image

This command copies the current graph display to the clipboard as an image for pasting into word processing or presentation software. The graph image will include the x- and y-axes, and it will use the currently selected zoom settings.

Graph presets

These commands will quickly change the display to a commonly used view.

Table 5-1: Graph Presets

Graph Preset	Curve 1		Curve 2		Curve 3	
	Channel Displayed	Reference Applied	Channel Displayed	Reference Applied	Channel Displayed	Reference Applied
Unreferenced	Channel 1	None	Channel 2	None	Channel 3	None
Channel 1 Reference	Channel 2	Channel 1	None	None	Channel 3	Channel 1
Channel 2 Reference	Channel 1	Channel 2	None	None	Channel 3	Channel 2
Channel 3 Reference	Channel 1	Channel 3	None	None	Channel 2	Channel 3

For example, the Raw preset will automatically change the graph to display Ch1 response in curve 1, Ch2 response in curve 2, and Ch3 response in curve 3. The Ch1 Ref preset will change the graph to display Ch2-Ch1 in curve 1, no curve 2 plot, and Ch3-Ch1 in curve 3.

Curve Colors

To change the color of the plotted response curve, left-click on the associated text label (*e.g.* "Curve 1"). A color choice pop-up will appear and any color may be selected. To change the curve color back to the default, hold down the **Ctrl** key while clicking on the text label.

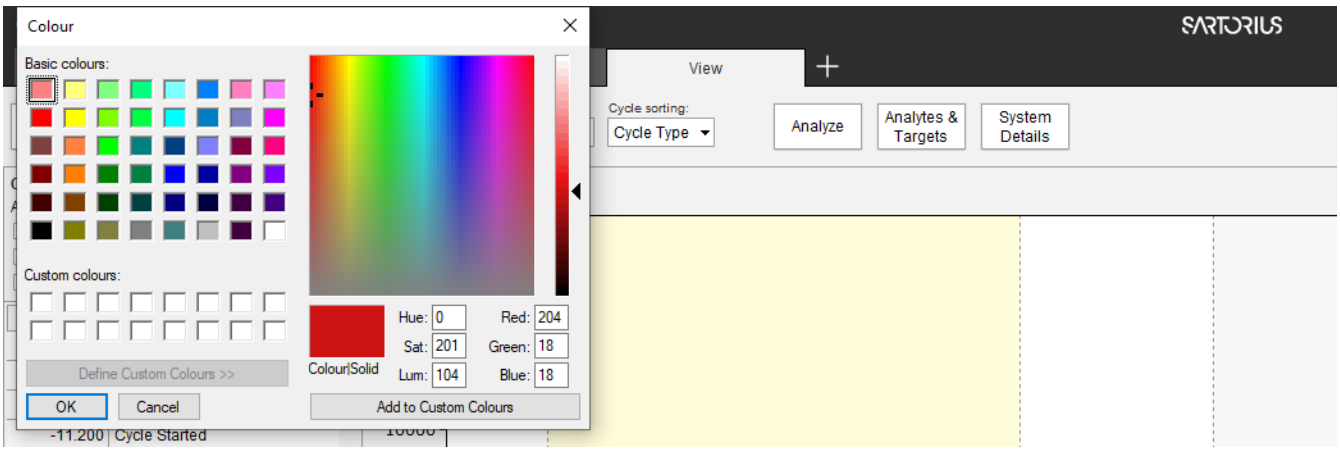

Figure 5-14: Changing Response Curve Color

Chapter 6:

Manual Mode

Manual Mode Walk-Through

In Manual mode, commands are performed in the sequence they are selected, and a single response curve cycle is produced in each session. In contrast to Automated mode, it is not possible to configure multiple cycles although commands can be queued to be performed within the same cycle. The cycle is terminated by manually stopping the cycle session.

The stages in a typical Manual mode cycle are given in the following example.

The system defaults to showing channels 1, and 3 with channel two reference subtracted. Where desired this can easily be modified via the dropdown selection boxes.

Prime the System

Select **Prime** from the Operation page.

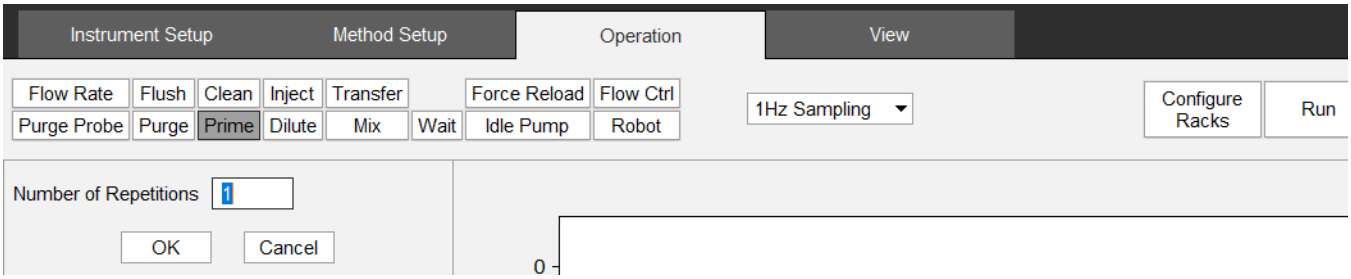

Figure 6-1: Priming the System from the Operation Page

Prime equilibrates all flow channels (including loops) with fresh buffer. It is important that fresh buffer fully displaces the previous liquid so it is advisable to run Prime at least three times before use. Where a new sensor chip has been docked, it is advised to prime the system three times before performing any work in manual mode. If a Prime routine has been run within the previous few hours and the buffer will not be changed for the experiment, it is not necessary to prime more than once.

Define Sample Racks

Load all samples to be analyzed in the experimental session into the appropriate rack. Install the rack in the system and close the sample door.

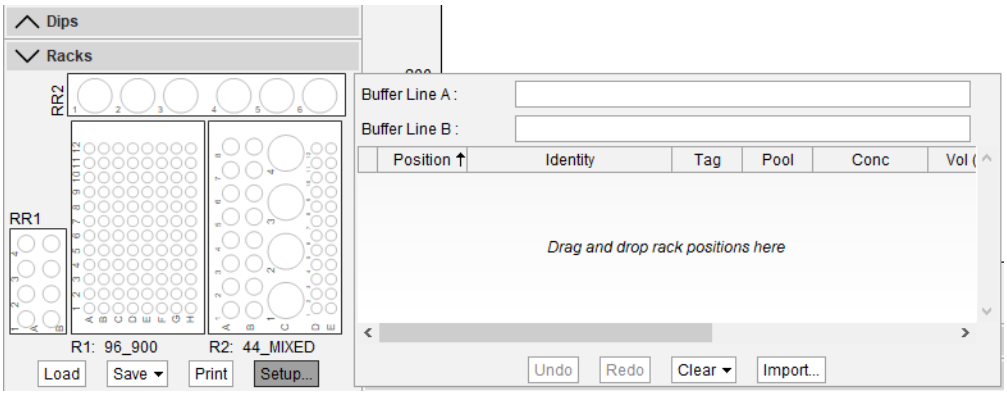

Figure 6-2: Racks Drop Down Display in Operation Page

To configure the inserted racks, click **Configure Racks** and select the desired racks and positions. To configure the samples, click the **Setup** button in the Rack dropdown to display the rack setup table (Figure 6-3). A series of samples may be selected by clicking on the first and last in the series while holding down the **Shift** key. If samples are not in order on the rack, they may be selected individually while holding down the **Ctrl** key. If the information to be entered into a particular column is the same for each sample, highlight the entire column by clicking on the column heading, type the information once, and press **Enter** on the keyboard. The text will be entered into all highlighted cells. Positions with samples are shown in teal. If an unwanted position was added unintentionally, left-click the button to the left of that row on the table and press **Delete** on the keyboard or right-click and select **Delete row(s)**.

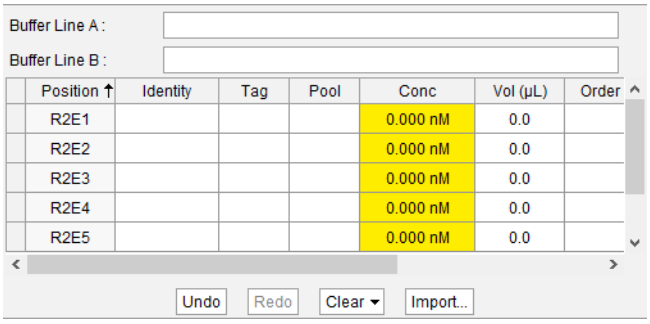

Figure 6-3: Rack Table

Usually analyte sample dilutions are placed in the sample rack in sequence so a simple shortcut that avoids typing each concentration individually can be used. For example, if the first (highest) concentration is 200 nM, type this into the first cell in the column (Figure 6-5).

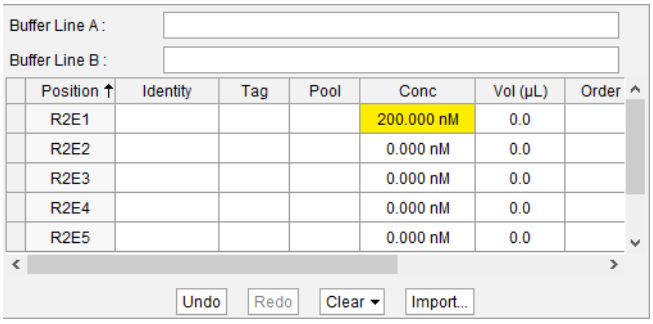

Figure 6-4: Entering Concentration in Rack Table

Select all cells to be filled (including the top concentration), place the mouse pointer over the top concentration cell, and right-click to open an option menu. Select the appropriate dilution form and the selected cells will automatically be filled (Figure 6-5).

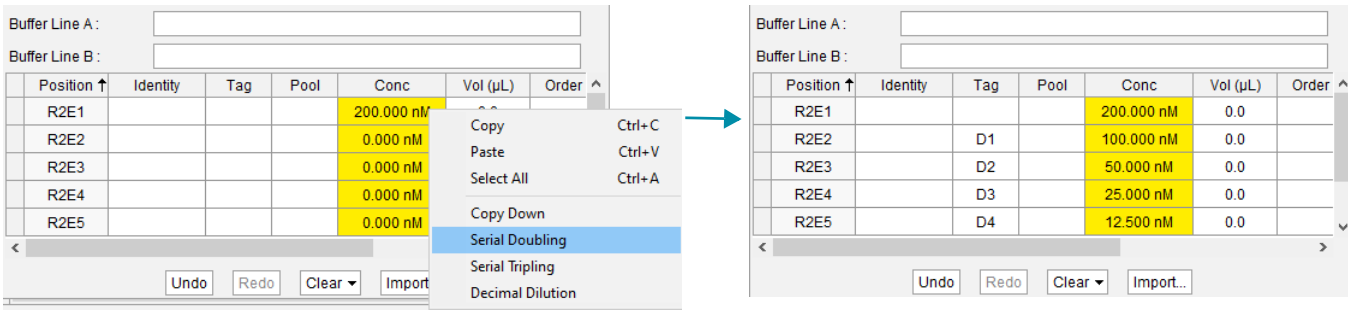

Figure 6-5: Selecting Dilution Form in Rack Table

When the desired reagents are inserted, click **Setup** to shut the display or click the **Save** button to save the sample rack configuration for future use. Use a file naming convention that permits discrimination between configurations. As it is common to repeat experiments using a single rack configuration, saving rack configurations avoids re-entering information. The configuration can be re-loaded by clicking the **Load** button and selecting the appropriate file. When complete, the rack setup table may be minimized by clicking on the **Setup** button a second time.

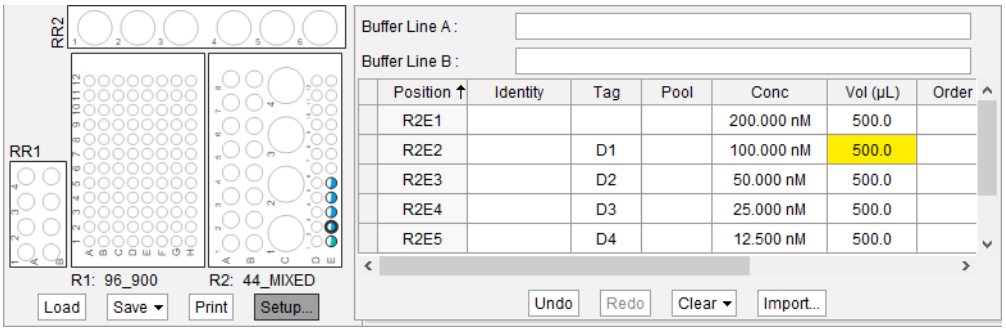

Figure 6-6: Minimizing Rack Setup Table

TIP: *If there are many samples, it is best to configure the rack in the software before loading samples. This allows the rack configuration to be used as a guide when loading samples. This simple tip greatly reduces the chances of error during plate loading. Click the* ***Print*** *button to print out a diagram of the racks and a table of all sample information. Each row in the table can then be checked off as that sample is added to the designated rack position. Install the rack in the system. Open the Operations page and load the previously saved rack definition from the Rack folder.*

Choose a Data Collection Rate

The Data Rate is changed in the Operation page from the dropdown menu. The default data rate is 1 Hz (*i.e.* one point per second) but it is possible to increase this data rate to help monitor very fast events. A maximum data rate of 40 Hz (40 points per second) is supported. Note that the baseline noise will be higher at the higher data rate. Weak interactions with affinity constants in the mM range will require a high data collection rate because the initial association phase will transition to a steady state very quickly. In addition, the dissociation phase may be very short. It is necessary to collect data at a rate that is considerably faster than the event itself to resolve these types of rapid events.

Figure 6-7 shows the results of a simulation that models a typical weak affinity interaction curve characterized by a rapid transition to a steady state and a rapid dissociation phase. Medium to high affinity interactions should be recorded at the default data rate in order to obtain the lowest baseline noise level.

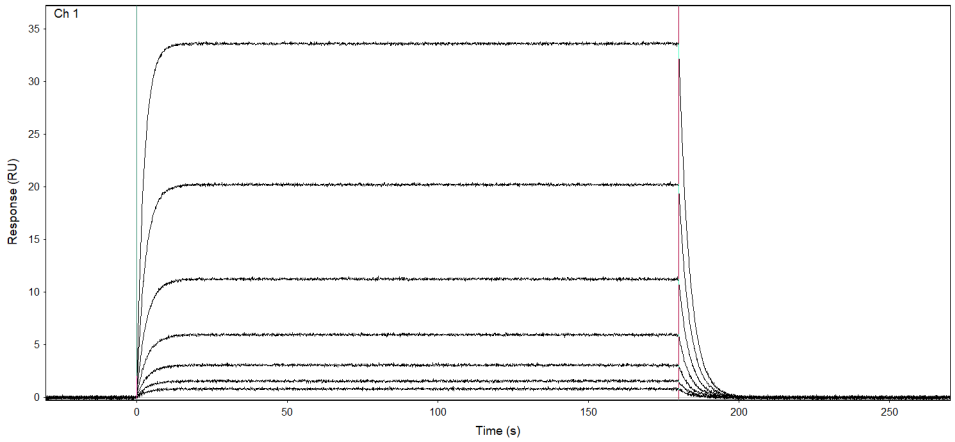

Figure 6-7: Example of a Weak Affinity Interaction Curve

Start a Response Curve

Click the **Run** button and select **Start Manual Recording** to start recording a new response curve. A prompt to enter a flow rate and a file name for the resulting data file will appear (Figure 6-8). Select the appropriate directory. The result files are saved as *.spr files. A flow rate editor will then appear on screen.

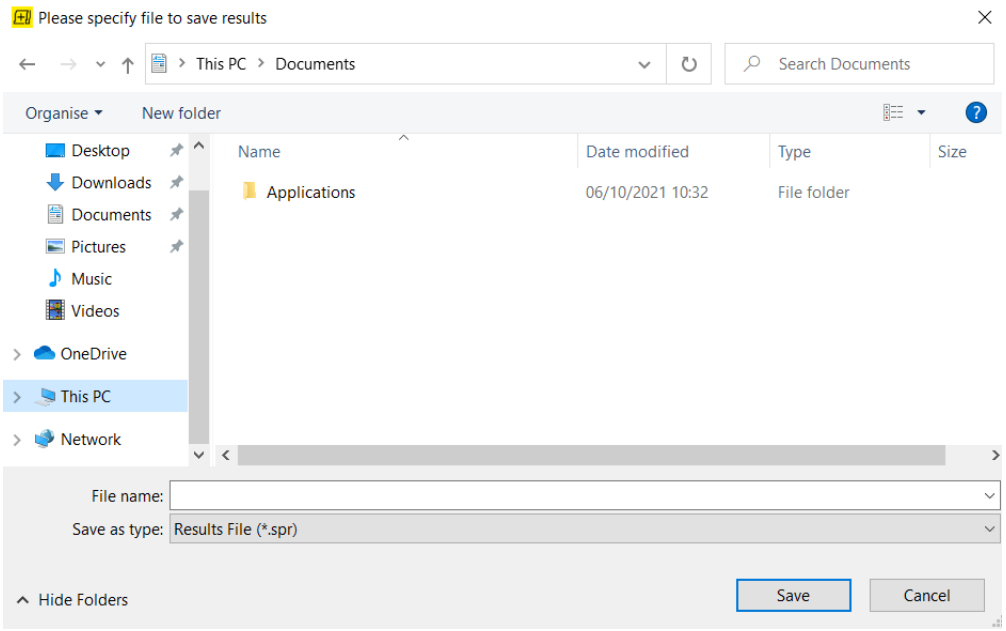

Figure 6-8: Entering Results File Name

Set a Flow Rate

Establish a flow rate by typing in an appropriate flow rate and pressing **Enter** on the keyboard (Figure 6-9). Usually a flow rate between 30 and 50 µL/min is recommended for kinetic injections. Very low flow rates might be used if long contact times are required such as in solid phase affinity analysis when the affinity is high. It is good practice to match the continuous buffer flow rate with the analyte injection flow rate. Non-matching flow rates may be used with non-critical injections such as regeneration injections. The flow rate may be changed in increments of 5 µL/ min starting at 5 µL/min with an upper limit of 150 µL/min. It is possible to change the flow rate used during manual mode when an injection is not being performed.

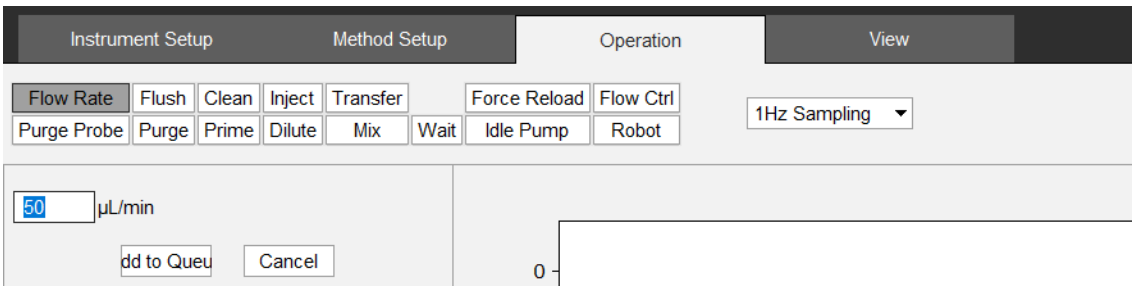

Figure 6-9: Entering Flow Rate

Perform an Injection

Click the **Inject** button in the tool bar. A graphic of the racks appears on the left and an empty table on the right (Figure 6-10). Click and drag the sample to be injected from the racks and drop it onto the table. The default flow path is across all channels and therefore, after setting the desired injection(s) it is important that the flow path is checked to ensure that injections only occur across user-defined channels. The default setting is common association and common dissociation, although where desired these may be deselected to allow individual association, dissociation and flow rates for each sample.

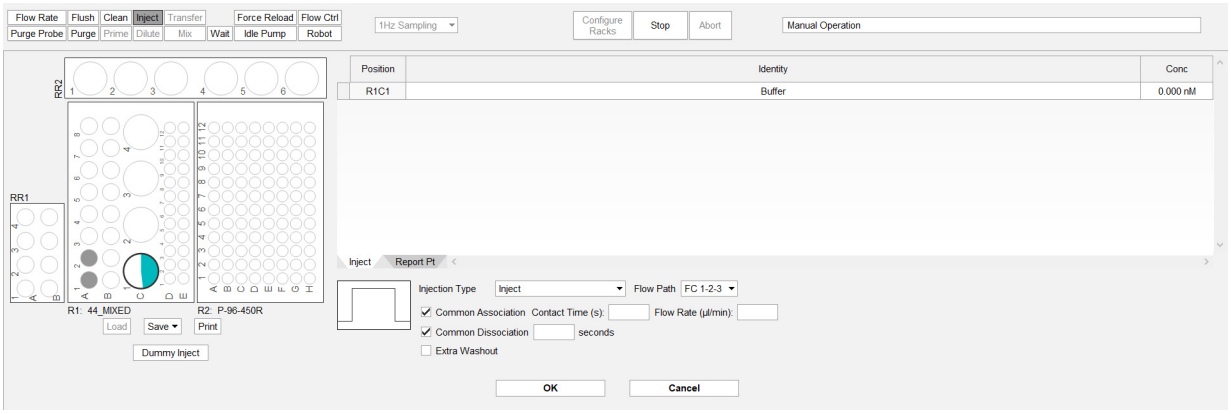

Figure 6-10: Sample Table

If more than one sample is entered into the sample table, each sample will be run consecutively as defined by the respective parameters entered in each row of the sample table. All these injections will be reported in a single response curve (*i.e.* cycle 1).

Add Report Points

Report points associated with an injection may be pre-configured or added manually as the injection proceeds. Pre-configured report points are added by selecting the **Report Point** tab at the bottom of the injection table (Figure 6-11). The injection table will reconfigure with new parameters as shown in the figure below. See "Report Point" on page 116 for a complete description of report points. In this example, a report point will be added at 20 seconds before the injection and another report point at 20 seconds before the end of the injection. If Set Baseline is checked, then the relative response between these two report points will be reported in the report point table along with the other parameters. A five-second average is designated for these report points; the responses for these report points will be an average of five points including the response at the designated report point time and two response points to either side of this time point. Usually five points is sufficient but occasionally it is useful to use a larger number of points. Report points are very useful in concentration assays as the average response values may be plotted as a function of analyte concentration in order to generate analyte calibration curves.

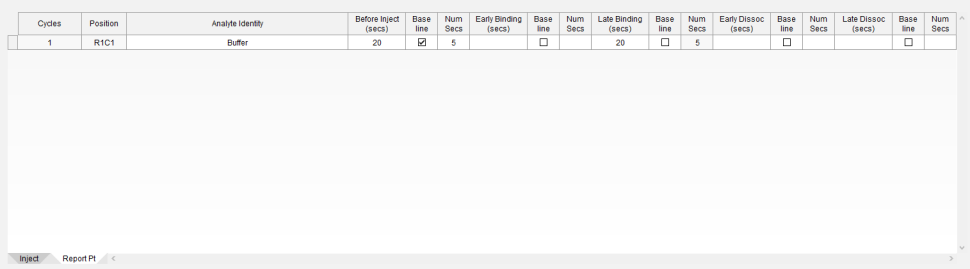

Figure 6-11: Report Pt Tab

Click **OK** when injection configuration is completed. The autosampler will begin loading and injecting the sample. When the sample injection starts, a green arrow will mark the injection start point and the graph background will be highlighted for the duration of the injection. The end of the injection is marked with a red arrow and the background color returns to white. The report point folder (located at the bottom of the screen) will contain any report points pre-set in the Report Point tab. It is often preferable to simply view only two response curves that have been reference curve subtracted, thereby minimizing the report point table to two rows per report point.

Stop Response Curve

In Manual mode the user must stop the response curve. While there is no fixed limit on the amount of time a manual response curve can be recorded, it is recommended that a single manual response curve be kept under 24 hours at a data rate of 1 Hz. It is possible to perform a large number of injections during this time. Where possible, it is best to restrict the number of injections to allow easy tracking of events. Always use a method, with multiple cycles, if many samples must be injected.

Abort

If something unexpected happens during an assay, for example an incorrect sample was found to be loaded into the rack; it is possible to abort that particular cycle by simply clicking the **Abort** button on the toolbar. The Abort command allows any command to be aborted. It will also allow a method to be aborted if running in Automated mode. The system will return to its default state, running continuous flow buffer.

Additional Commands

Additional functions such as Flush, Clean and Purge can be performed whilst in Manual mode. Chapter 10 “Function Reference” on page 98 for further details on each of these features.

Chapter 7:

Automated Mode

Method Setup

In the method Setup Editor, the user has access to multiple template methods that are pre-written to aid in rapid assay development and performance.

The following templates are included in the Octet® SPR Discovery software:

- **Immobilization -** Standard amine coupling with activation/deactivation of a reference flow cell
- **Multi-Cycle Kinetics -** To determine kinetics and affinity using multiple concentrations of analyte
- **NeXtStep™ -** To determine the behavior of an analyte in the presence of a competitor
- **OneStep® -** To determine kinetics and affinity using a single concentration of analyte
- **OneStep® Screening -** High-throughput screening of up to 768 analytes
- **pH Scouting -** Assessment of ligand pre-concentration at the sensor surface
- **Regeneration Scouting -** Determination of the optimum regeneration solution

Once selected, each of these templates is fully modifiable and the user also has the ability to develop new methods from a blank template.

Editor Introduction

In order to understand how to modify pre-written templates or prepare new methods it is important that the information contained in response curves is key to writing methods. The response curve (plot of sensor response versus time) is used as the basic building block. The response curve is a record of the changing environment at the sensing surface during sampling. A typical response curve is shown in Figure 7-1. The key elements include the steady baseline before the injection of sample, an association phase during the injection of analyte, followed by a dissociation phase, and finally a regeneration phase where the surface is exposed to conditions that disrupt the affinity complexes at the surface leaving free ligand. The response curve responds to affinity complex formation but also responds to other events such as bulk refractive index changes.

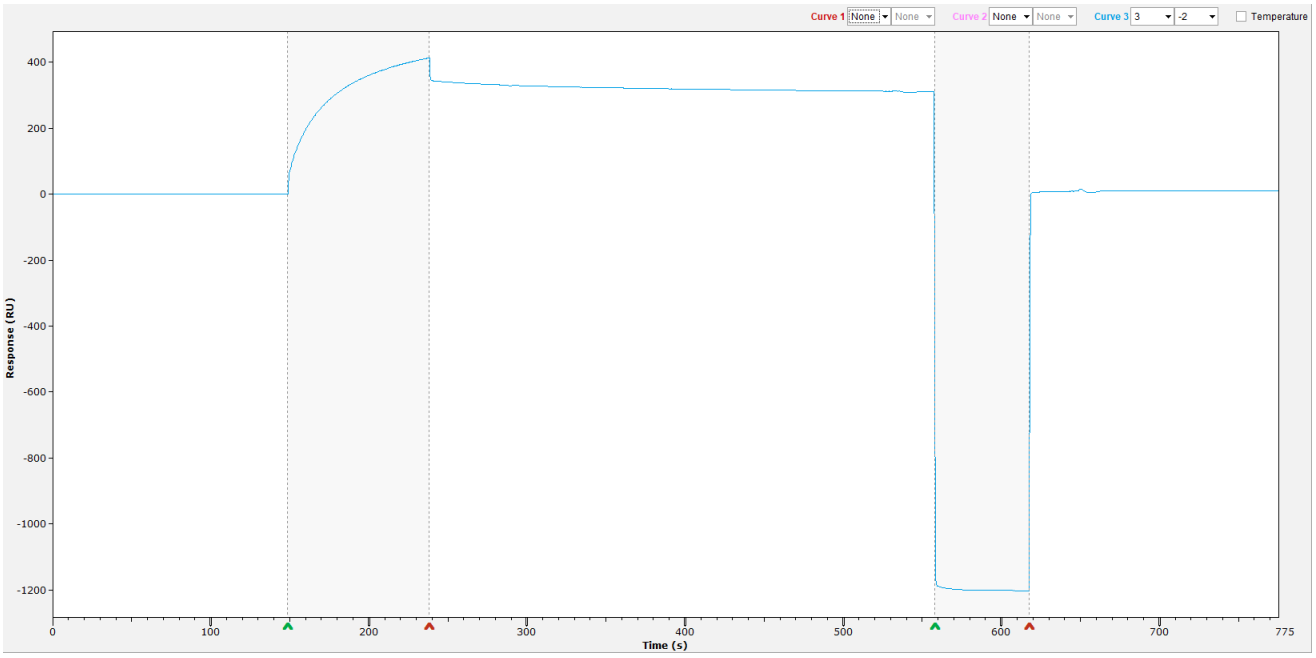

Figure 7-1: Typical Response Curve

In a typical multi-cycle kinetic assay it is necessary to record a response curve for multiple different concentrations of the analyte sample. To do this the basic response curve is repeated for each individual analyte sample concentration. In a very simple assay, the user might decide to run five different analyte concentrations giving five response curve cycles in the assay. (In the context of the assay editor, one cycle refers to all steps taken in preparation for the injection, the injection itself, and all steps relating to that injection that take place after it.) In order to repeat this kinetic assay at different temperatures another layer of complexity must be added to the programming. To simplify setting up an assay of this type, the Octet® SPR Discovery software has two programming levels to allow the user to program the system with minimal expertise. These two levels are the Cycle Editor and a higher level Method Editor as shown in Figure 7-2. These editors are time line bars into which commands are dropped from the toolbars. The method is then executed in the sequence defined in the Method Editor. The Parameter Editor will reconfigure and present a different configuration screen for each type of command, opened by clicking on that command in the editor. For example, the Parameter Editor in Figure 7-2 is for the first injection in the assay cycle (shown in green).

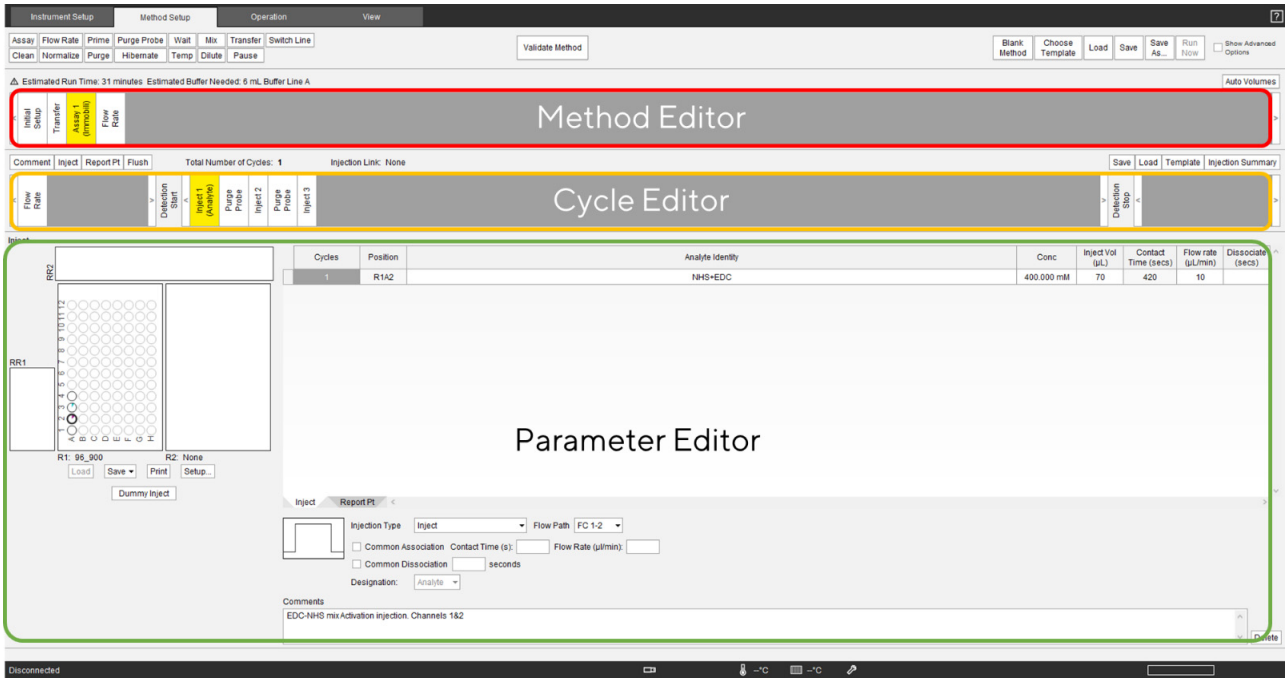

Figure 7-2: Method, Cycle and Parameter Editors

Why Use a Method?

A method is the best option to simplify analysis of a large number of samples. Methods allow the user to save sets of related response curves. In Manual mode, analysis of multiple samples results in overloaded response curves with too many injections that are difficult to decipher and evaluate. It is also impossible to repeat a manual response curve so as to preserve identical timing of injections between replicates. In a method, all events are executed at predetermined times allowing excellent reproducibility between replicate response curve cycles.

Using the Method Editor

Adding tools

The tools available for use in a method are shown as buttons at the top left of the Method Setup page. To add a tool to the method, simply click and drag the button to the desired location (Figure 7-3). A transparent button image will follow the mouse and when moved to a valid location, a black bar will indicate where the tool will be inserted when the mouse button is released. Tools may not be inserted before the 'Initial Setup' tool. Additional tools are available within the assay tool. For a complete description of each tool, refer to Chapter 10 “Function Reference” on page 98.

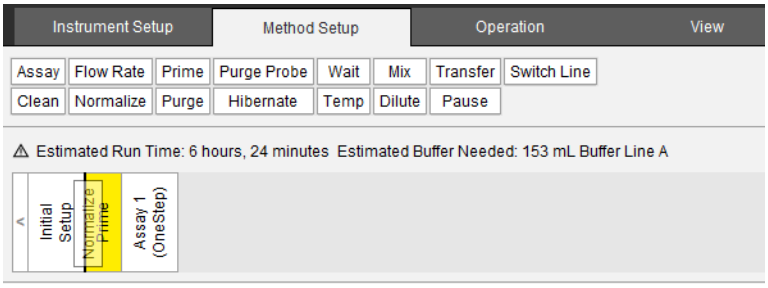

Figure 7-3: Adding a Tool

Moving and copying tools

After a tool has been added to a method, it can be easily moved to a new location by simply clicking and dragging it (Figure 7-4). Again, a black bar will appear indicating where the tool will be moved when the mouse button is released. The tool will retain all of its configured parameters when moved.

Similarly, an existing tool can be copied using the same method as moving a tool, except that the **Ctrl** key should be held down while dragging the tool. All of the configured parameters are copied along with the tool. This can be useful, for example, if an assay is to be repeated at different analysis temperatures.

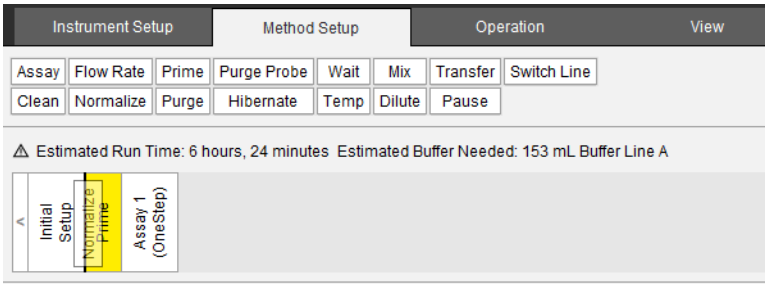

Figure 7-4: Moving a Tool

Removing tools from a method

There are three ways to delete a tool from a method:

1. Click and drag the tool off of the method timeline.
2. Click on the tool, which activates it for parameter editing. The active tool will be highlighted in yellow. At the bottom right corner of the Cycle Editor, there is a delete button which can be used to remove the tool.
3. Click on the active tool a second time. A different border will appear around the active button. Press the **Delete** key on the keyboard to remove the tool from the method.

Tips

Clicking on the **Save** button will generate a prompt to input a file name and location for the method file. Once saved, any changes will overwrite the existing method file if the save button is pressed again. To create a copy of an existing method, Load the file, and then choose the **Save As**... option to specify a new file name.

The current file name, if any, is displayed along the bottom of the main software window.

To re-use only the initial rack setup from an existing method file, click the **Load** button under the racks on the Initial Setup tool. Change the drop down list for Files of Type from *.rack to *. method and choose the desired method file.

When recording a results file, a copy of the method used to generate those results is automatically embedded in the SPR file. If the original method file becomes lost or is otherwise unavailable, it can be opened from the SPR file by clicking the **Load** button, and changing the drop down list for Files of Type from *.protocol to *.spr. For a manual response curve, only the initial rack setup can be loaded from the SPR file.

Assay Writing and Modification Example

The details of the assay described here are not inherently important but are useful in explaining how methods are constructed and modified. The basic outline of the experiment is as follows: The Octet® SF3 system will complete a kinetic analysis of a Fab fragment binding to an immobilized antigen. The antigen will be immobilized by amine coupling. The kinetic analysis will be repeated at two different surface capacities for quality control.

The method must direct the system to immobilize the antigen onto two sensing channels at different concentrations to fulfill the experimental requirements. Serial doubling dilutions of the Fab sample covering the nM concentration range (the affinity for this interaction is known to be in the nM range) must then be prepared and analyzed in duplicate in random order.

Therefore, the assay will consist of three main steps:

1. Amine coupling of the antigen
2. Preparation of Fab dilutions
3. Kinetic analysis

It is best practice to prepare individual methods for each step. In this way the user can inspect the results of one method before executing the next one. If all three were combined into a single method then poor results in the first method would result in failure of the remaining steps and unnecessary consumption of valuable reagents. If each step has been successfully performed in the past, then the three separate methods may be combined into a single method.

Define the initial sample rack setup

Where a predefined template is not used, loading a black method will cause Method Editor to appear which will always have an Initial Setup tool already present. Click on the tool and the following screen will appear.

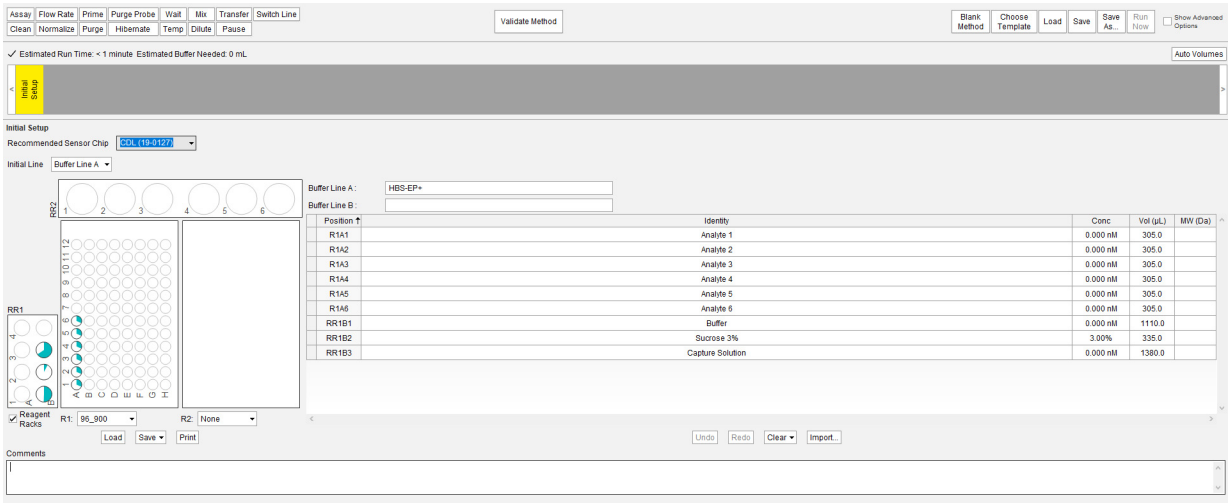

Figure 7-5: Rack Setup Tool

Load a blank method from the method setup page. The following reagents are required for immobilization:

Table 7-1: Immobilization Reagents

Identity	Concentration	Volume (µL)
EDC	400 mM	140
NHS	100 mM	100
Ligand	10 µg/mL	200
Ethanolamine	1 M	135

Using the mouse, click and drag the rack positions **A1, A2, A3** and **A4** from the racks on the left onto the table to the right. It is possible to select all four positions at once by using the **Shift** key to select a continuous block of positions. This also allows selection of multiple, random positions where required. Configure the identity, concentration, and initial volume for each sample as shown in the table above. Vials with liquid appear teal (Figure 7-6).

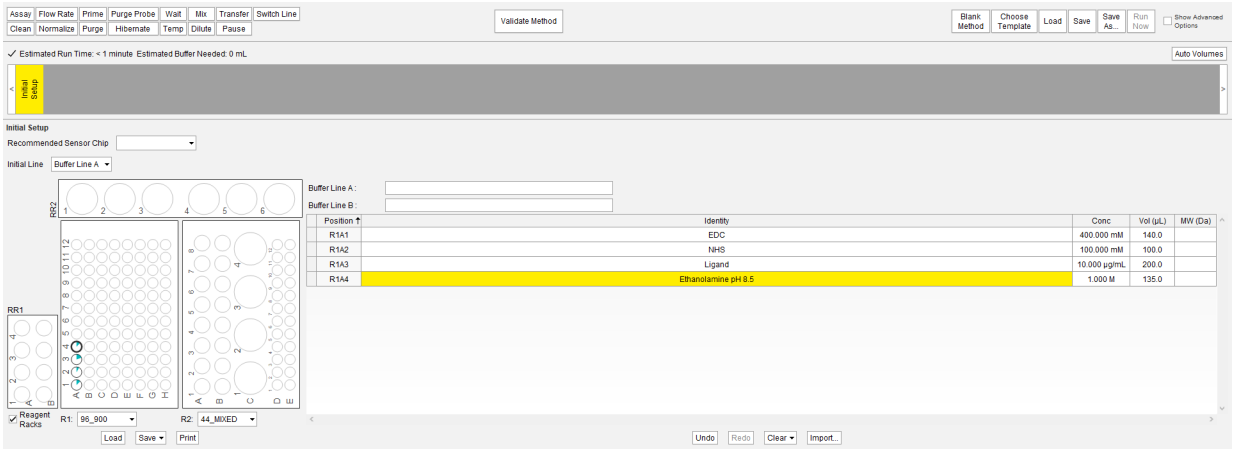

Figure 7-6: Configuring Samples

Immobilization assay

We will construct an assay to perform the immobilization. The term assay is used in a broad sense to include applications that are not strictly assays such as an immobilization.

Select the **Transfer** tool from the tool bar and drop it onto the Method Editor. Repeat for the Assay Tool (Figure 7-7). From the assay template pop-up, **empty** should be selected from the drop down selection panel. Finally, select the **Flow Rate** tool and drop it onto the Cycle Editor and click **Match Analyte Injection Flow Rate**.

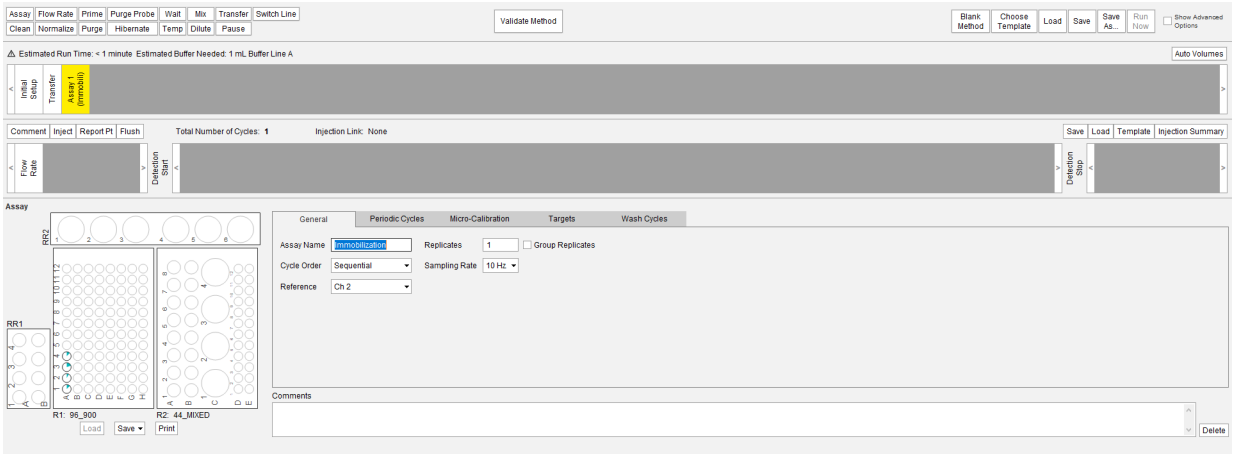

Figure 7-7: Adding Tools to the Method Editor

The Cycle Editor will appear below the Method Editor strip. The Cycle Editor is divided into three segments. The left hand segment contains all operations to be executed before a response curve recording is initiated and the right hand segment includes all operations to be performed after the response curve recording. The center segment contains all operations to be performed during the course of the response curve (*i.e.* cycle). In the Cycle Editor all operations required to complete the immobilization are defined. A second toolbar appears above this time strip for tools that cannot be added to the method time strip (Comment, Inject, Report Pt and Flush).

Many of the tools in the top tool bar may be placed within either the Method Editor or Cycle Editor strip, *e.g.* the user can drag a Flow Rate tool from the top toolbar (background color in both time strips turns yellow). In contrast, a tool such as Inject, from the Cycle Editor strip toolbar, can only be dropped into the center region of the assay time strip thereby restricting all injections to periods when the system is recording a response curve.

In Figure 7-8, all tools (*i.e.* commands) required for completing the immobilization have been added to the Method Editor and Cycle Editor in preparation for configuring the parameters for each command.

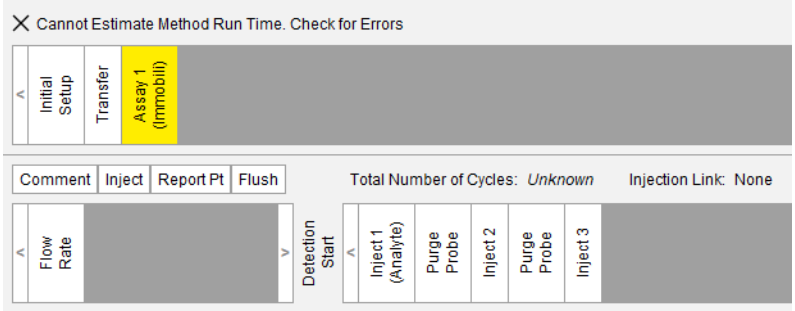

Figure 7-8: Immobilization Tools in Method and Cycle Editors

The amine coupling method requires that the EDC and NHS solutions be mixed in a 1:1 ratio and then injected. Select the **Transfer** tool in the Method Editor and drag **EDC (position R1A1)** onto the blank table on the right side. This populates the "from position", and the "to position" is completed by dragging the **NHS (position R1A2)** into the empty cell. To complete the transfer of EDC into NHS in position R1A2, enter a volume of **100 µL** and mix count of **5** (Figure 7-9). Where a different EDC:NHS ratio than the standard 1:1 is desired the user may either adjust the volume ratios or simply add an additional empty vial and mix the desired ratio.

Figure 7-9: Amine Coupling Method Setup

At this point, 200 µL of the EDC:NHS solution is located at R1A2. The mixed liquid will appear purple on the sample rack image.

In the next step, the activation injection must be configured. Click on **Assay** in the Method Editor and enter the assay name "Immobilization". Select the **Inject** tool from the Cycle Editor and drop it into the middle section of the Cycle Editor. The EDC:NHS mixture is added to the table by simply clicking and dragging the vial in position R1A2 to the right side table (Figure 7-10). An appropriate flow rate, flow path (here, FC1 and FC2 have been selected) and contact time can then be entered (see the Octet® SPR Chemistry User Guide for further information).

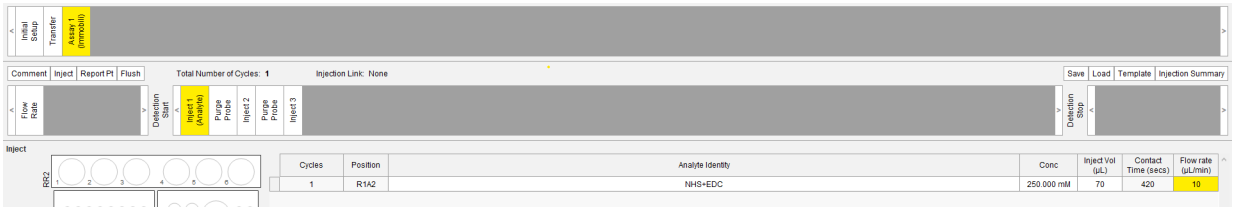

Figure 7-10: Activation Injection Configuration

It can be useful to determine how much material was immobilized once the final assay is performed. This can be done by adding report points manually after the data has been recorded or these report points may be pre-programmed as described below. To obtain an accurate estimate, a baseline report point should be added before any injection is performed and another report point added after the final (*i.e.* third injection) injection is complete. The baseline report point can be appended to the first injection by selecting the **Report Point** tab. Configure the report point as shown in Figure 7-11.

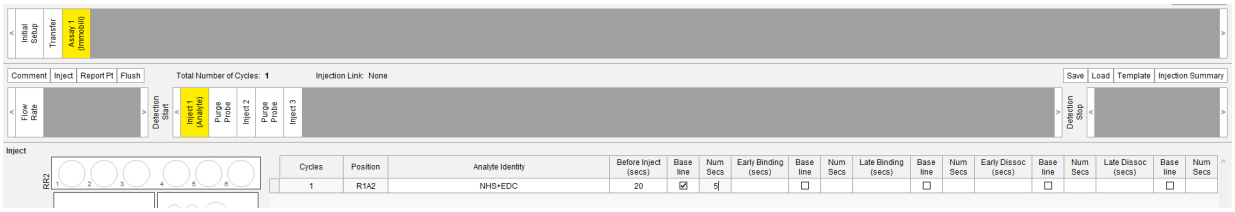

Figure 7-11: Adding Report Points

The parameters entered will place the report point, which has been designated as a baseline, on the response curve 20 seconds before this injection starts. The value for the report point will be the average of the report point and four points to either side of that point for a total of nine points. The remaining cells remain blank as there is no need of a report point directly after this first injection.

Once the activation solution is injected, the antigen sample is injected over only the active flow cells but not the reference flow cell. Select the **Inject** tool from the Cycle Editor and drop it to the right side of Inject 1 (Figure 7-12).

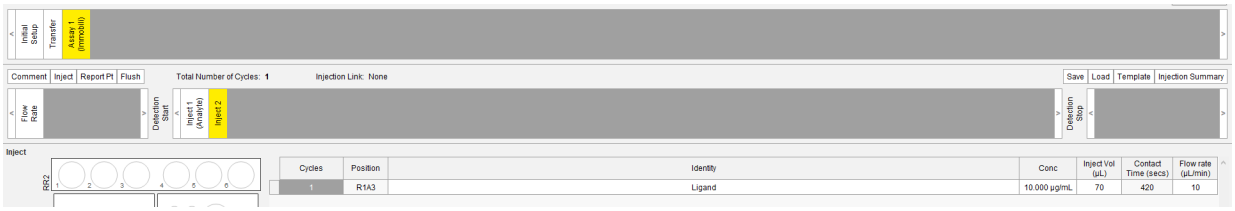

Figure 7-12: Antigen Sample Injection Setup

The ligand is added to the table by simply clicking and dragging the vial in position **R1A3** to the right-side table. An appropriate flow rate and contact time can then be entered (see the Octet® SPR Chemistry User Guide for further information). It is important to note that the inject tool has a default Flow Path of 1-2-3 and therefore, it is critical that the correct Flow Path is chosen (Here, FC1 has been selected).

Where both flow cells are to be immobilized with the same ligand, the secondary injection can be injected over both flow cells. If a lower antigen level is required on one of the flow cells, the contact time of the ligand can be reduced with one flow cell relative to the other using the Switch Inject command as shown in Figure 7-13.

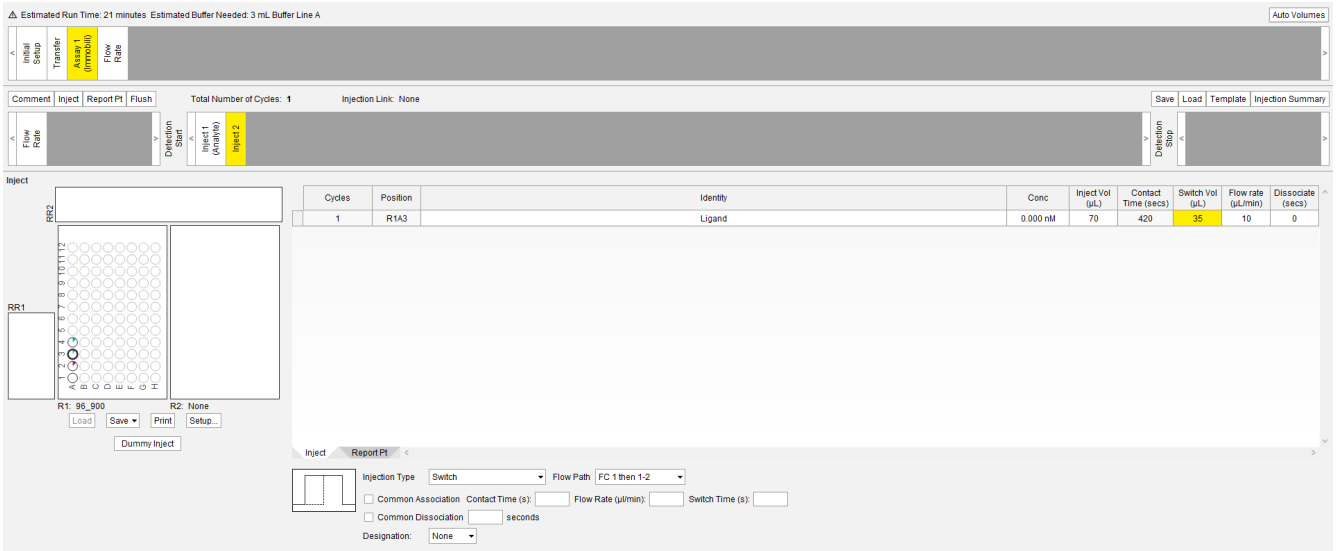

Figure 7-13: Switch Inject Command

An injection of 70 µL has been included in the method using Switch Inject - the first 35 µL will flow over channel 1 only while the remainder will flow over channels 1 and 2. This difference in contact time results in a greater amount of ligand being immobilized onto channel 1. In this type of experiment, channel 3 should be used as the reference channel.

The third and final injection in amine coupling is the Ethanolamine capping step where residual active groups are chemically substituted with unreactive hydroxyl groups. As previously, select the **Inject** tool from the Cycle Editor and drop it to the right side of Inject 2 and configure the injection as shown in Figure 7-14.

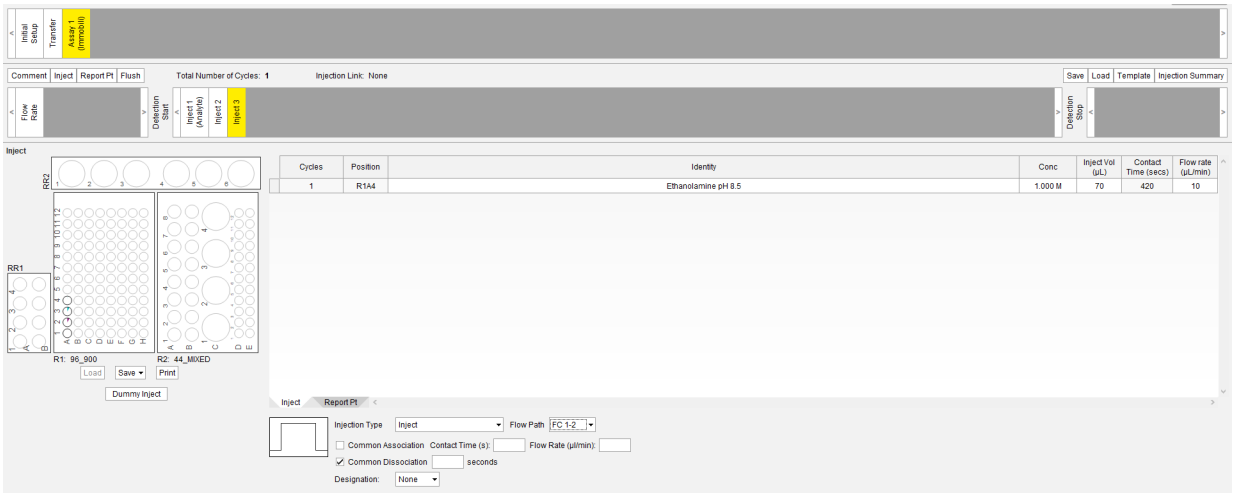

Figure 7-14: Ethanolamine Capping Setup

It is important to note that the Inject tool has a default Flow Path of 1-2-3 and therefore, it is critical that the correct Flow Path is chosen. As FC1 and FC2 were activated in Inject 1, it is critical that they are both included in the final Ethanolamine Inject 3 (Here, FC1 and FC2 have been selected).

In this final injection, a report point must be included after completion of the immobilization process. This report point will be relative to the baseline report point added before the first injection and will allow calculation of how much antigen has been immobilized. Configure the report point as shown in Figure 7-15.

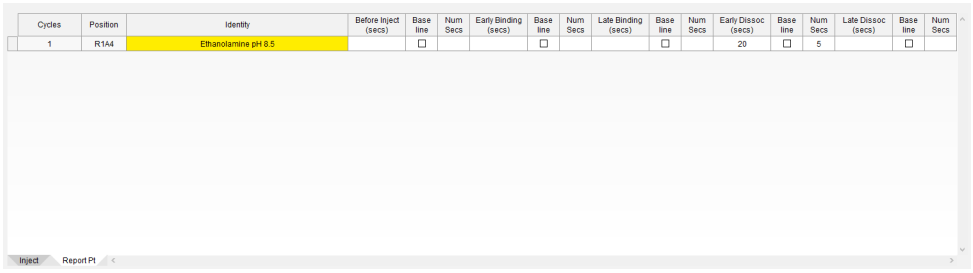

Figure 7-15: Report Point for Ethanolamine Inject 3

Save the assay using the **Save** option on the right side of the Assay time strip. Save the method using the **Save** command on the top right side of the page. Saving an assay separately allows that assay to be loaded into any other method.

Auto Volumes

The Octet® SPR Discovery software has the feature Auto Volumes, which is located at the top right side of the method setup page. Auto Volumes allows the user to construct the method as detailed above without having to input sample volumes at the start of method writing. As the required volumes are dependent upon several factors such as flow rate, number of replicates and association/dissociation time, the final volumes may not be known at the start.

To use the Auto Volumes feature, the user can simply click the button and the software will calculate the minimum volumes required, which includes the dead volumes of the vials or plates used. Care must be taken to ensure that the final volumes reflect the desired user outcome, especially when a transfer command is used.

Validate Method

It is good practice to use the Validate Method tool after writing a new method file. This tool will check that settings in the method are within the proper ranges for the system. Invalid entries are flagged for correction. Validation will also simulate volume dispensing and consumption from the sample racks, and present warnings for any rack positions that may become empty during the method. The volume tracking is a convenience function. Volume tracking errors will not prevent a method from being executed on the system. Also, validation is performed automatically when a method is loaded for execution on the Operation page. Only errors due to invalid parameters are flagged at that point.

TIP: *In early attempts at method construction, it is advisable to substitute all samples with a 3% sucrose in running buffer solution to test methods and ensure that the sequencing is as intended before running real samples.*

Dilution preparation

The next two steps in the example analysis are Fab dilution preparation and kinetic analysis. It is recommended that the user creates a blank method and from the assay template pop-up, **empty** should be selected from the drop-down selection panel. Select the **Initial Rack Setup** tool in the Method Editor strip and transfer vial positions **R1A1 to R1A6** to the right-side table. Drop a **Dilute** command from the toolbar onto the Method Editor strip and configure as shown in Figure 7-16.

In an experimental setup, a working stock of Fab (200 nM) would be loaded into position R1A1 and 5 empty vials positioned in R1A2 - R1A6. Load a working dilution of the Fab (*e.g.* 200 nM) into R1A1 and place 5 empty vials from **R1A2 to R1A6**.

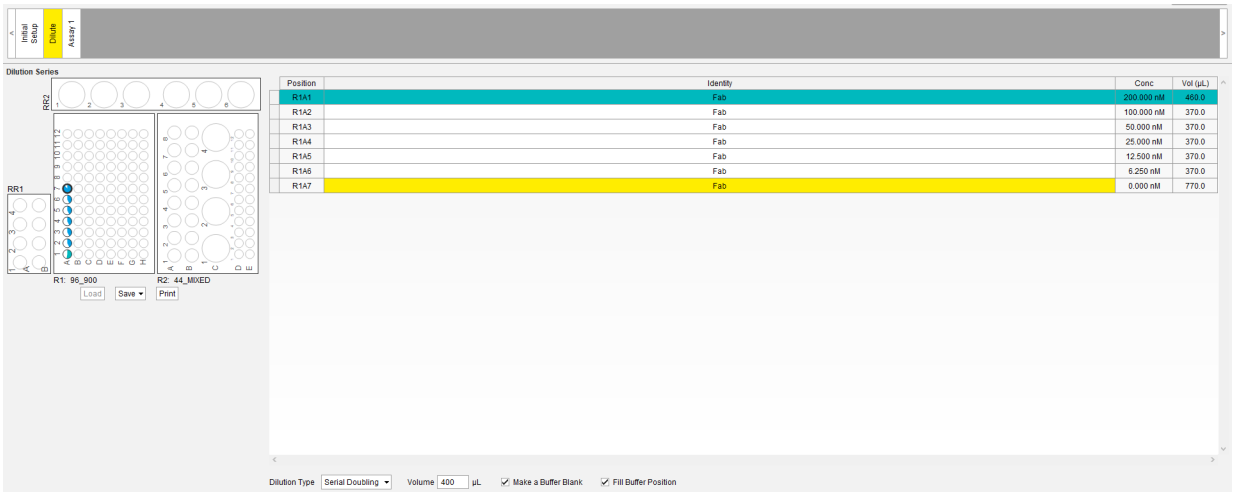

Figure 7-16: Dilute Command

This tool will prepare the 5 serial doubling dilutions from a single working dilution using the running buffer as diluent.

It is also possible for the system to prepare a buffer blank by including an extra blank vial and checking the **Make a Buffer Blank** box near the bottom of the screen.

Kinetic analysis

Drag a new **Assay** tool into the Method Editor strip and select **Multi-cycle kinetics with regeneration** and configure the kinetic assay as shown in Figure 7-17. In this example, a set flow rate command was added before the start of response curve. A wait command will instruct the system to wait a specified time before commencing the first injection. This is useful to establish a reliable baseline before running a kinetic injection. The Fab injection is then configured as shown below. Make sure the Designation drop down list shows Analyte for this injection. This tag will facilitate model fitting to this segment of the response curve using Octet® SPR Analysis software.

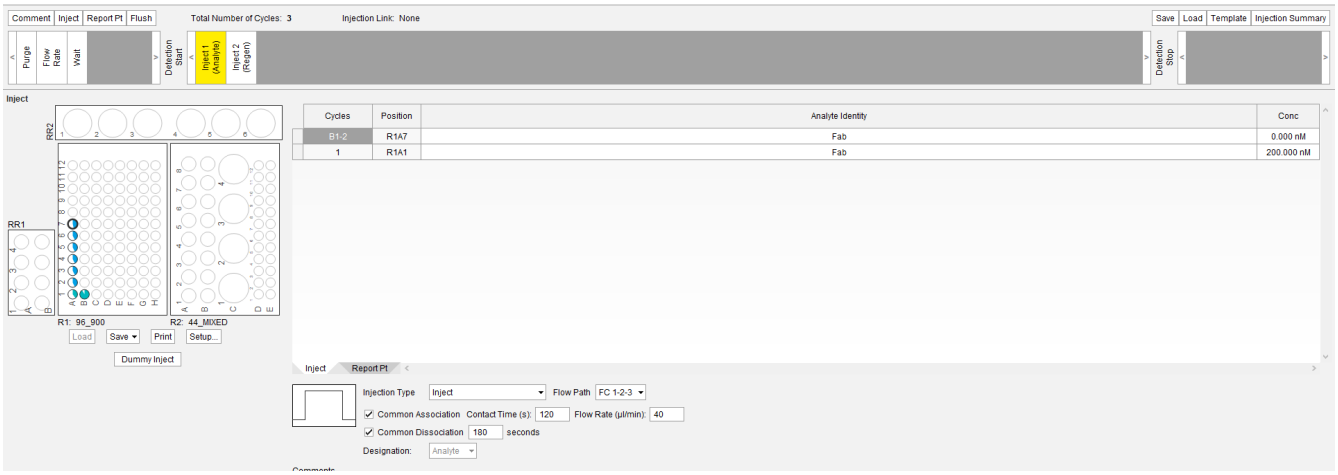

Figure 7-17: Kinetic Analysis Setup

Add the first Fab sample to the injection table and enter the appropriate parameters as shown in Figure 7-18. Next, configure the second injection under Inject 2 (regeneration). This will inject a solution that dissociates the interaction complexes that have formed on the surface, leaving the immobilized antigen available for binding the Fab sample in the next cycle. Add the regeneration solution to the rack and configure the Initial Rack Set Up. Then configure the regeneration injection as shown.

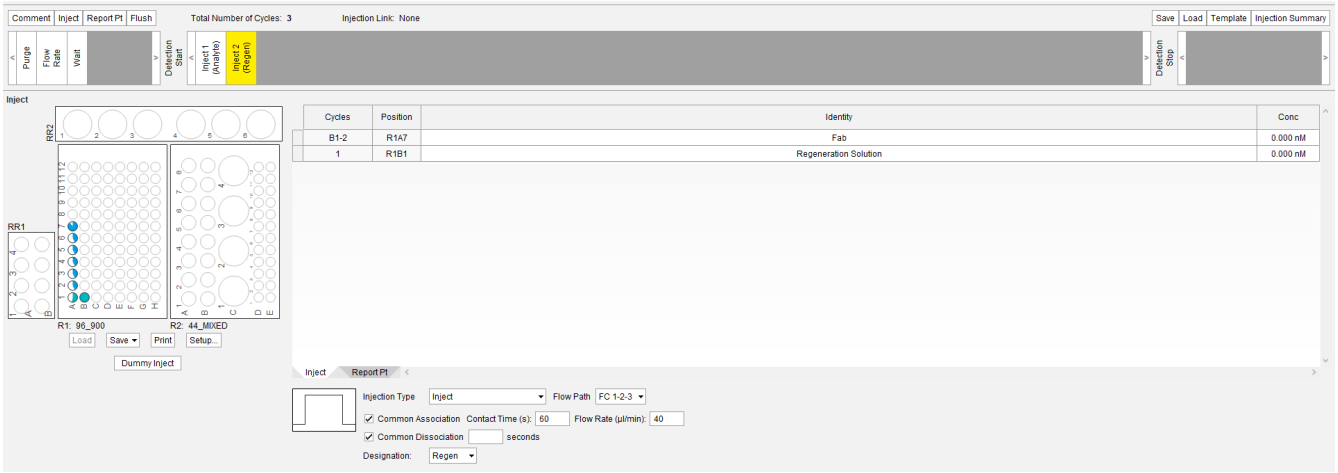

Figure 7-18: Regeneration Injection Configuration

Iteration of assay cycles

The previous steps have defined an assay cycle that will produce a single response curve containing two injections (injection 1 = analyte, injection 2 = regeneration). However, the assay as constructed will only perform this cycle for the first Fab sample contained in R1A1. The assay cycle can be made to repeat itself for each of the Fab dilutions by returning to the first injection in the assay cycle and dragging the other Fab dilutions into the sample table of that injection, and entering appropriate parameters as shown in Figure 7-19.

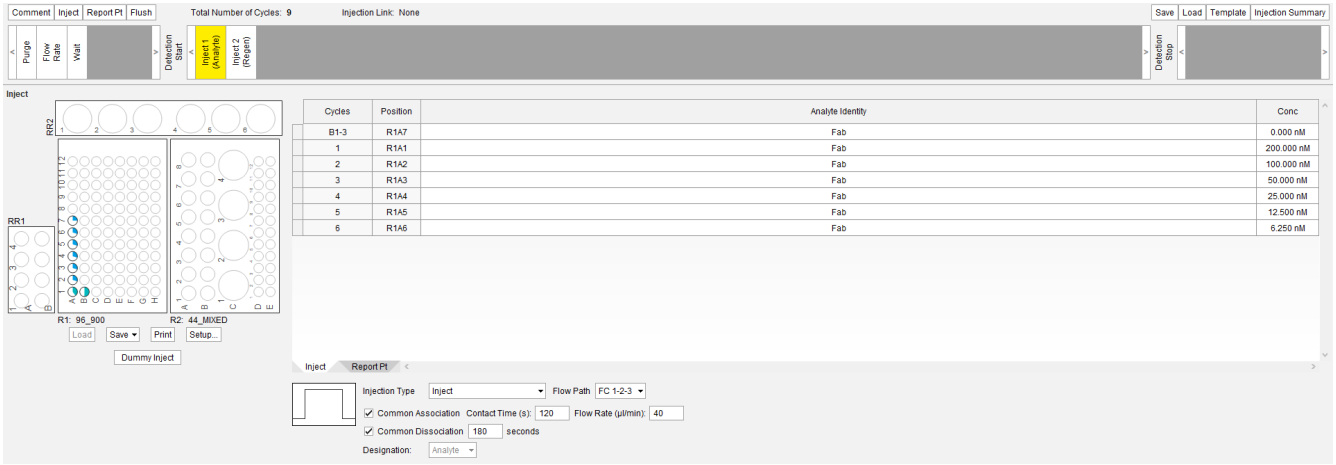

Figure 7-19: Iteration of Assay Cycles

The software will automatically assume that the entire cycle (injection 1 = analyte, injection 2 = regeneration) must be repeated for each Fab sample added to this injection table. This is indicated by the different cycle numbers for each sample on the first column of the injection table. This assay will repeat the same response curve six times but each time a new cycle is started a different Fab sample is selected for the first injection in that cycle.

Sample replicates may also be run. To avoid any artifacts caused by concentration bias, cycles should be run in random order. Select the **Random** option to run cycles in random order when the method is executed. Select these options from the Assay tool as shown in Figure 7-20.

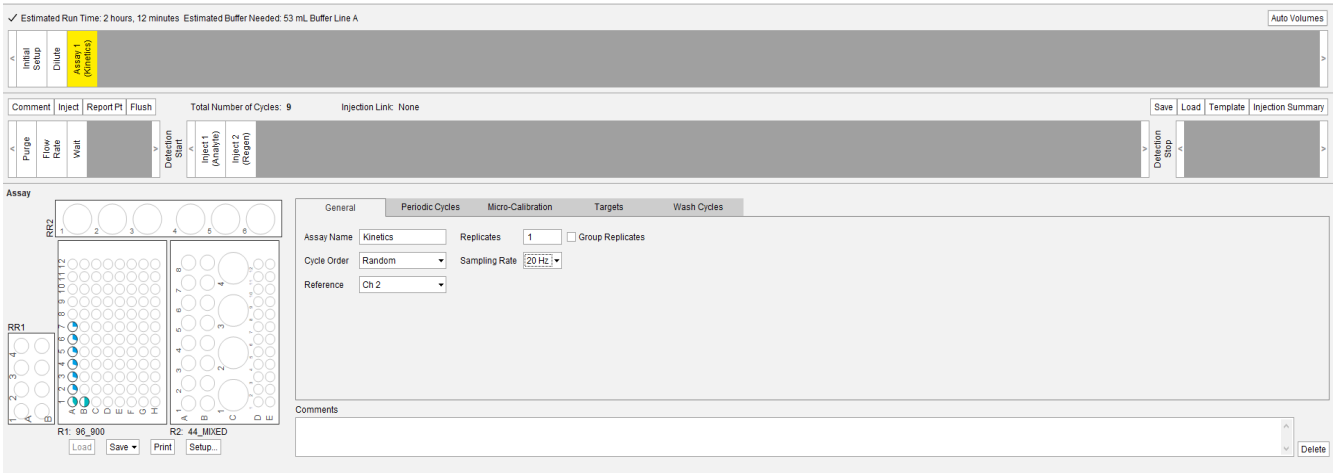

Figure 7-20: Random Option

The sample table will continue to show samples in order with respect to cycle number because randomization does not occur until the method is executed. Report points can be added to each of these injections by using the Report Point tab located at the bottom of the injection table. It is also possible to add report points that are not linked to the start, or the end, of an injection by dropping the Report Point tool (located in the assay time strip tool bar) onto the appropriate position on the assay time strip and configuring.

If a single sample is to be used repeatedly over an assay, it is best to fill a large vial with sufficient volume to complete the assay.

Switch line and hibernate

At the end of running a method, it is recommended that the user adds a switch line command to the Method Editor and selects the water line while the system hibernates. Where a switch line command is not included, the system will automatically hibernate after a 1 hour period of inactivity. This hibernation is in the same buffer line as used during the method. After 72 hours, the system will automatically switch to the water line.

Figure 7-21: Switch Line Command

Save the kinetic assay and the overall method as described earlier. Validate the method before executing it. Open the Operation page, Load the method, and select **Run**. The method will now execute. The event log will indicate all past and present events. All system commands are deactivated in the Operation page during method execution. All graphing functions will remain available. It is convenient to study previously saved data sets in the View page while a method is executing in the Operation page.

Common Association and Dissociation Parameters

Dissociation

In the prior example, the box for Common Association Contact Time and Common Dissociation are checked, which allows the association and dissociation times of analyte injections to be simply defined. Regeneration injections are also definable for common association and dissociation times but are independent of analyte injections. In most kinetic assays, the association and dissociation times are constant but where required, independent association and/or dissociation times can be entered easily.

For example, in a high affinity assay it may be necessary to perform a single long dissociation phase for the highest concentration analyte and this is achieved by unchecking the **Common Dissociation** box as shown in Figure 7-22.

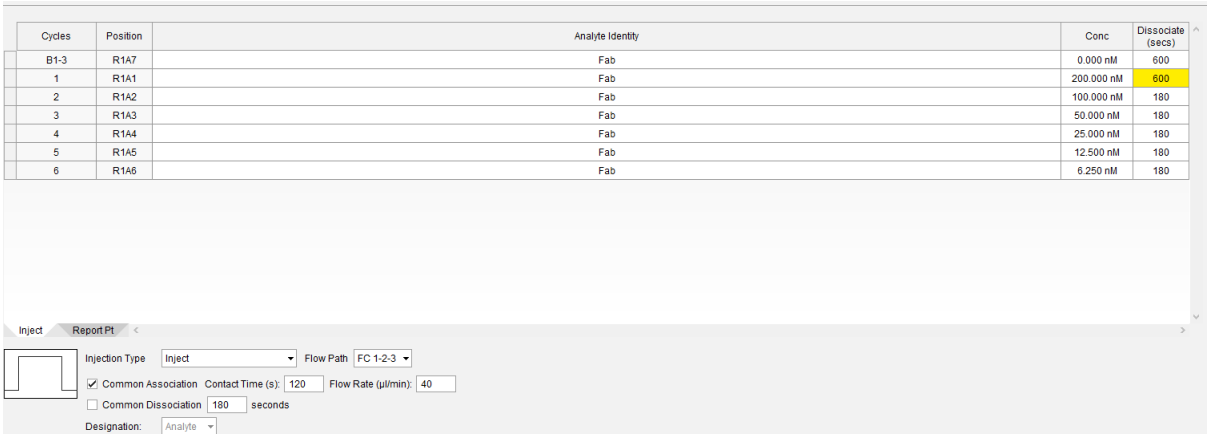

Figure 7-22: Uncheck Common Dissociation for High Affinity Assays

An additional column appears at the right side for dissociation and an independent time can be entered for the highest concentration (600 s vs 180 s for the other samples).

It is important to note that the dissociation time for blanks should match the longest dissociation time in the table (600 seconds), otherwise buffer subtraction in data analysis is not possible.

Variable flow rate

By unchecking the Common Association Contact Time box it is possible to vary all parameters in the inject sample table from one cycle to the next. For example, to confirm the absence of mass transport limitation the single sample (fixed concentration) can be injected at different flow rates (10–100 µL). If all sensorgrams have the same shape then mass transport limitation is not an issue. However, if the sensorgrams show increased binding as a function of flow rate then mass transport limitation is an issue. In this type of test it is usually desirable to keep the contact time constant. This can be done by increasing the injection volume to offset the additional consumption caused by increasing the flow rate. A typical injection is shown in Figure 7-23. In this injection, the sample position is constant from cycle to cycle but the flow rate and injection volume change but a constant contact time of 120 seconds is maintained.

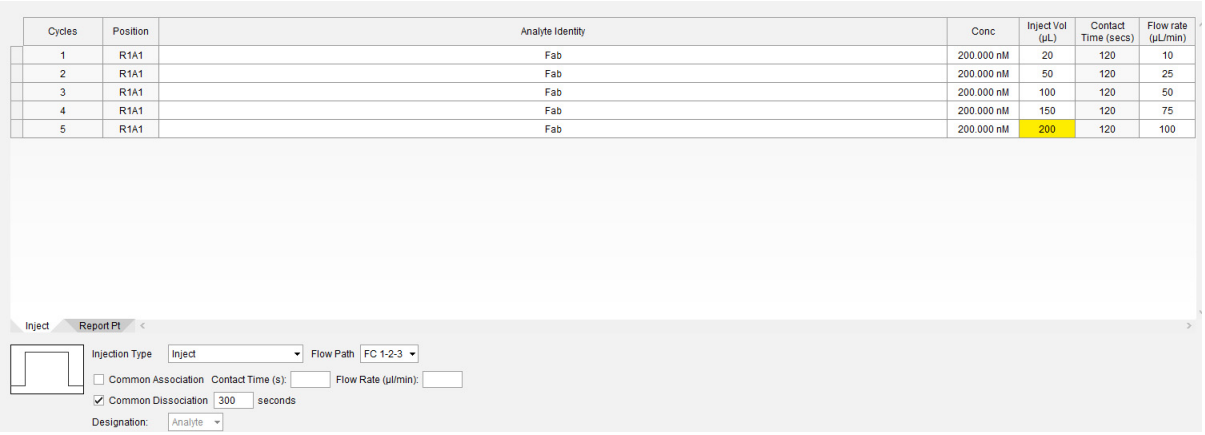

Figure 7-23: Variable Flow Rate and Injection Volume

Variable contact time

It is possible to perform a test for conformational shifting of affinity complexes by comparison of the dissociation phase curves for experiments with different contact times. A change in contact time may be programmed by fixing the volume and changing the flow rate but it is preferable to fix the flow rate and change the volume. The Octet® SPR Analysis software contains an option to align injections by their ending time. This facilitates comparison of the dissociation phase curves, which is the objective of this conformational shift test.

To change the flow rate, click on the **Flow Rate** column header and enter the desired flow rate. The contact time is then varied by altering the injection volume (Figure 7-24).

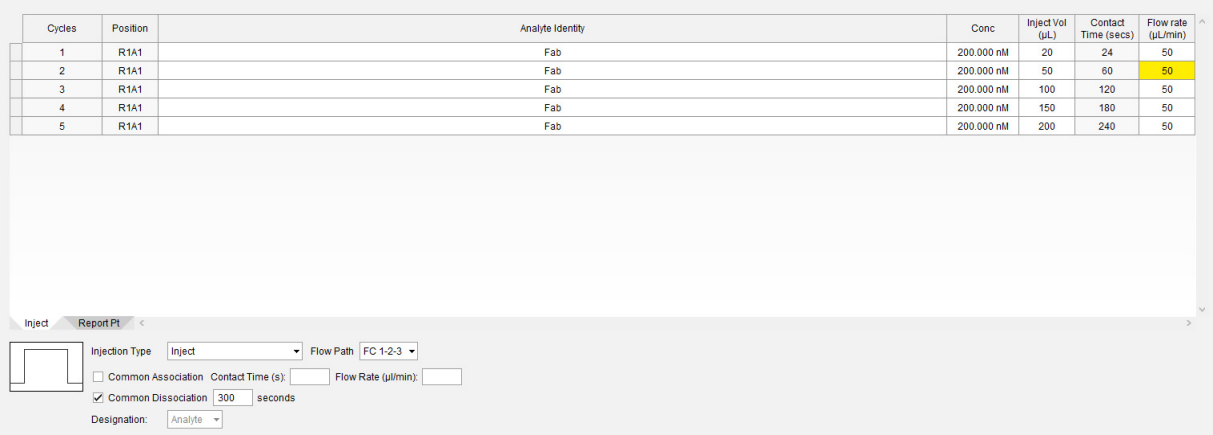

Figure 7-24: Variable Contact Time

Chapter 8:

Advanced Method Options

Injection Linking

As explained in the sample method detailed previously, it is possible to run multiple assay cycles by adding more than one sample to an injection table. However, this is only allowed for one injection of any assay cycle.

In the case of more complex sample injection sequencing the software offers three different assay formats:

- Interleave
- Permute
- Multiplex

In order to differentiate between these different assay formats, a worked example is shown:

Assay Setup

Table 8-1: Example Assay

Sensor Chip	CDL
Capture Ligand immobilized	Protein A
Ligand	Five different IgG1 antibodies (Ab)
Analyte	Five different antigens (Ag)

Therefore, the following possible antibody and antigen permutations are possible:

Table 8-2: Ab/Ag Permutations

Ab 1	Ab 2	Ab 3	Ab 4	Ab 5
Ag 1	Ag 1	Ag 1	Ag 1	Ag 1
Ag 2	Ag 2	Ag 2	Ag 2	Ag 2
Ag 3	Ag 3	Ag 3	Ag 3	Ag 3
Ag 4	Ag 4	Ag 4	Ag 4	Ag 4
Ag 5	Ag 5	Ag 5	Ag 5	Ag 5

An example sample rack setup is shown in Figure 8-1.

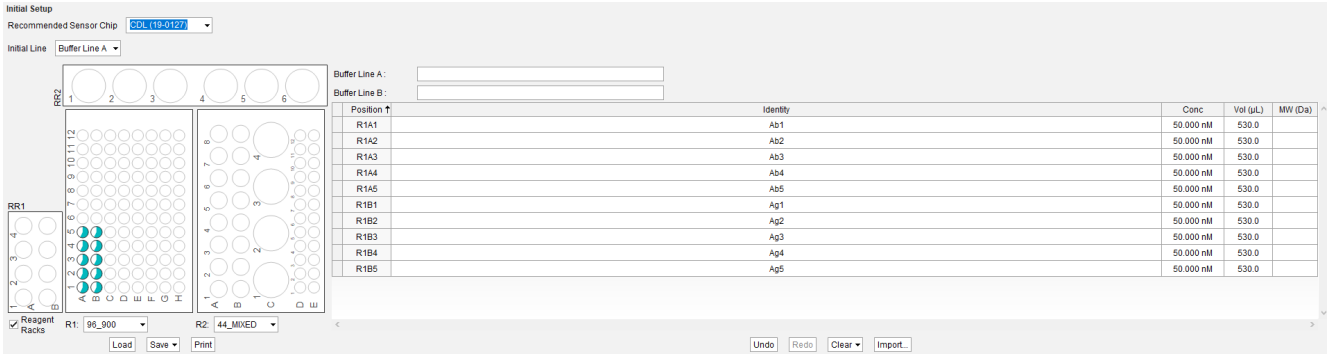

Figure 8-1: Sample Rack Setup Example

A standard kinetics setup is chosen from the assay template selection box and a second injection created.

Interleave link

The Interleave option allows more than one injection in a cycle to contain multiple samples.

With this option, each injection step in the link must contain the same number of rows. Samples from adjacent rows are paired off in repeating cycles. In the simplest case, a method to run a pair per cycle is constructed, *e.g.* antibody 1 against antigen 1, antibody 2 against antigen 2, etc.

The interleave option may be used to link the two injections as follows: To begin, set up each injection as normal. Drop in Ab1-Ab5 for the first injection, and drop in Ag1-Ag5 for the second injection. The injection tables for each injection are shown in Figure 8-2.

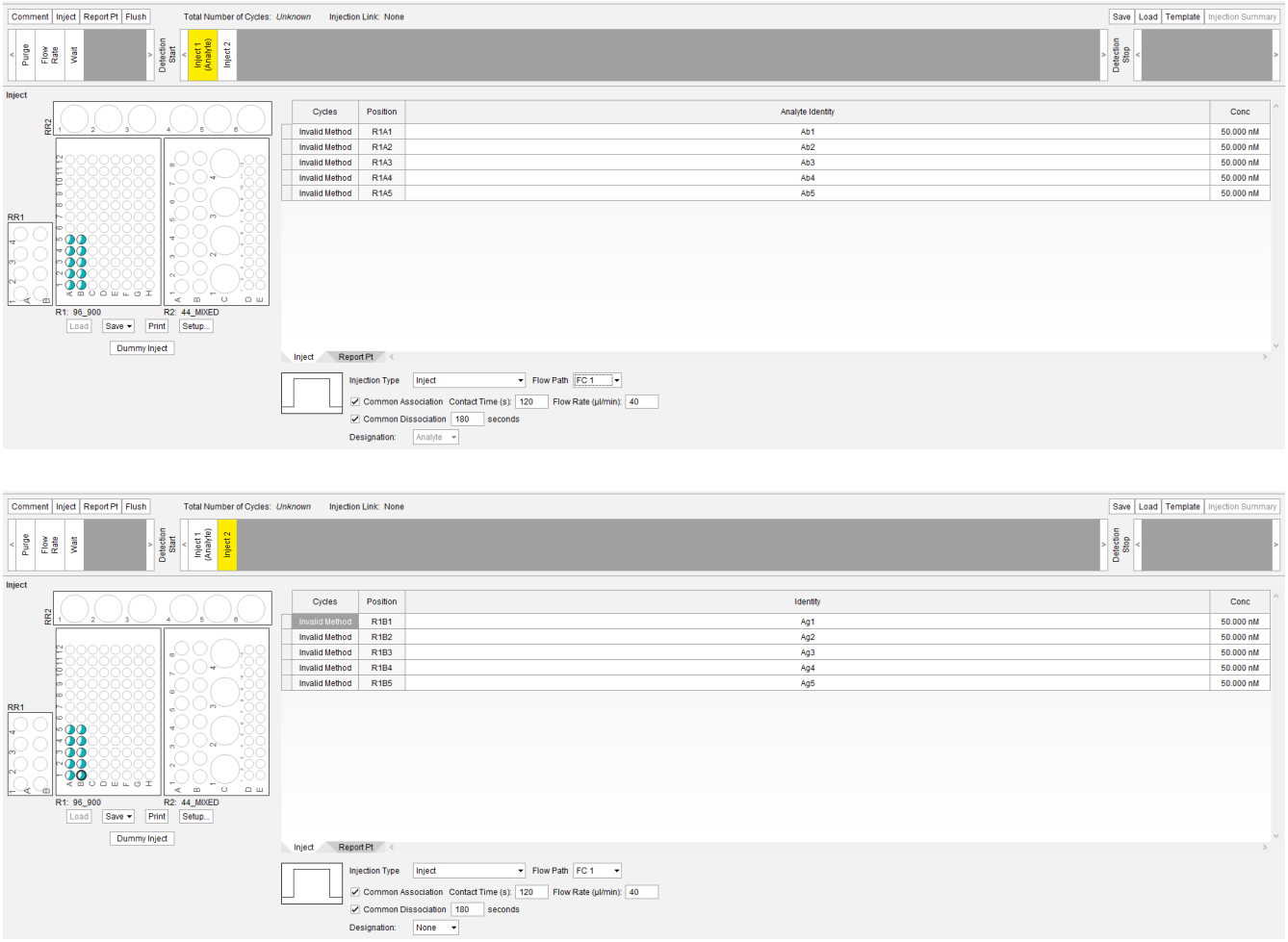

Figure 8-2: Injection Tables for Both Injections

The invalid method message will persist until the injection link is created.

To create the Interleave link, in the Cycle Editor bar, first left-click on one of the injections to link so that it is high-lighted in yellow.

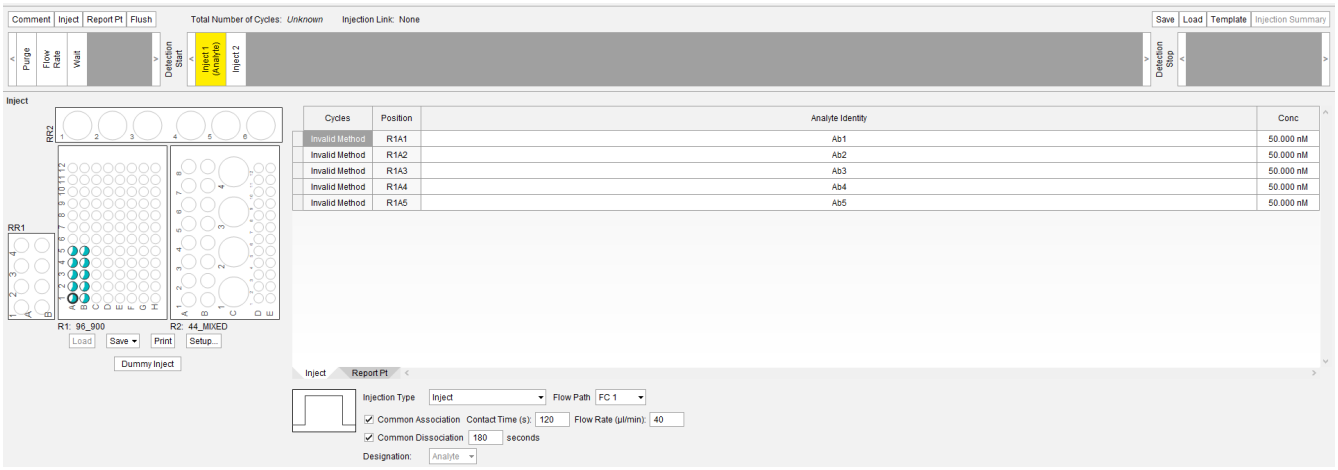

Figure 8-3: Selecting an Injection to Link

Then right-click on the other injection to open the link options and click **Add Interleave Link**.

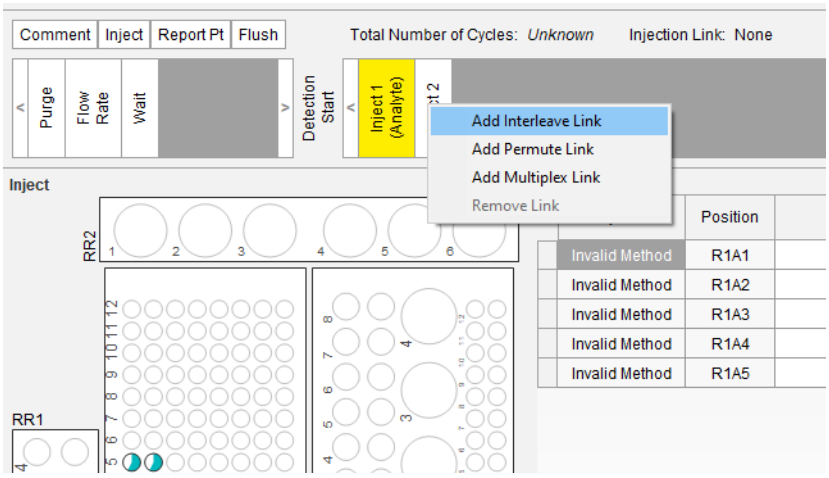

Figure 8-4: Selecting Add Interleave Link

The linked injections will become highlighted in orange, and the total number of cycles for the assay is updated.

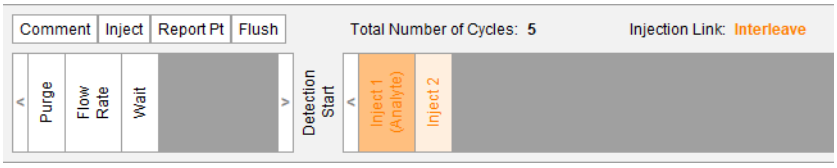

Figure 8-5: Linked Injections

The five assay cycles are summarized in Table 8-3:

Table 8-3: Assay Cycles

Cycle	Inject 1	Inject 2
1	Ab 1	Ag 1
2	Ab 2	Ag 2
3	Ab 3	Ag 3
4	Ab 4	Ag 4
5	Ab 5	Ag 5

Additional injections may be added to the link by selecting **Interleave** from the right-click menu, but remember that each Interleaved injection must have the same number of rows. To remove an injection from the link, select **Remove Link** from the right-click menu.

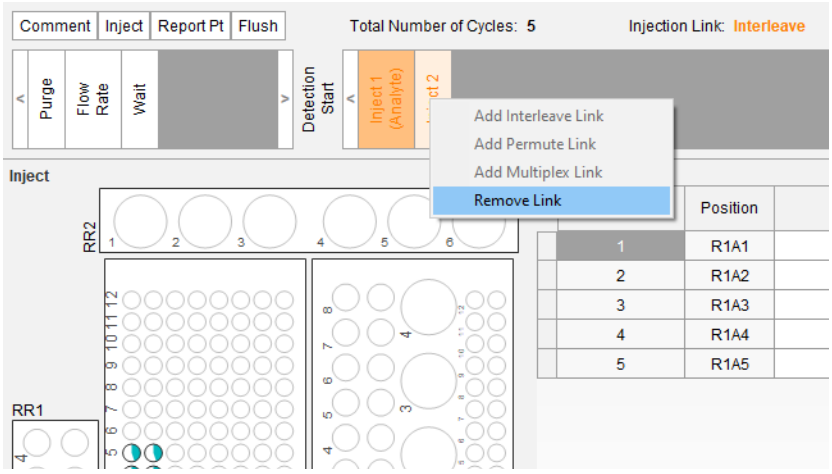

Figure 8-6: Removing Link

Permute link

Permute will run all possible combinations between the two sample tables.

The Permute option is similar to Interleave in that it allows more than one injection in a cycle to contain multiple samples but rather than pairing off matching rows in each injection permute runs all possible combinations between the two sample tables. It is not necessary in Permute link that the number of rows in each injection table are the same.

For example, there are 25 combinations of the five antibodies and the five antigens in the two injection tables discussed previously.

Antibody 1 will be run against all five antigens over the first five cycles; antibody 2 will be run against all five antigens over cycles 6-10, and so on for a total of 25 cycles. To use the Permute setup, injections can be configured as for Interleave but **Permute** is selected from the pop-up menu. The injections will appear on the assay editor as shown in Figure 8-7.

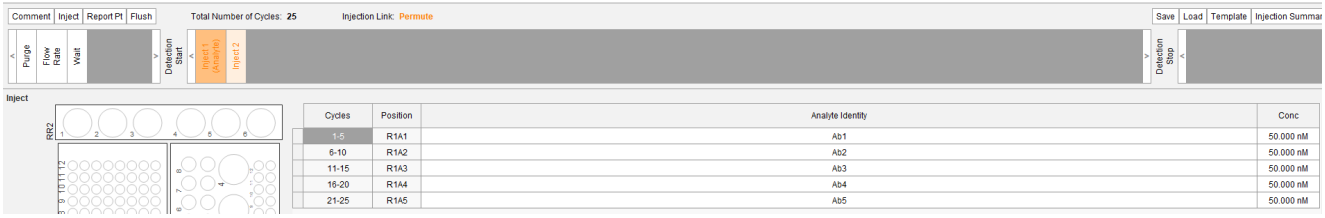

Figure 8-7: Permute Injection Link

Note that the Permute table reads in order but the 25 combinations may be run randomly by selecting **Random** in the Cycle Order dropdown in the General tab of the Method Editor.

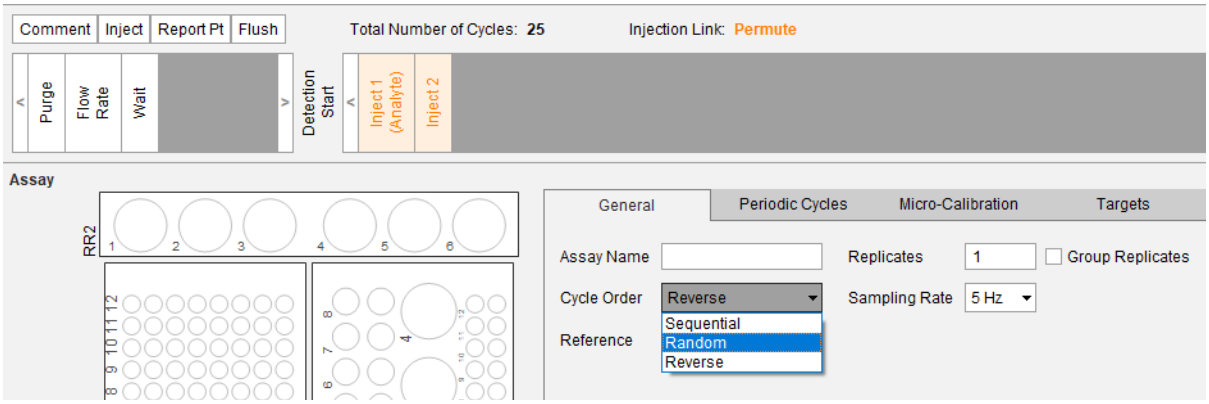

Figure 8-8: Selecting Random

The 25 assay cycles are summarized in Table 8-4:

Table 8-4: Assay Cycles

Cycle	Inject 1	Inject 2
1	Ab 1	Ag 1
2	Ab 1	Ag 2
3	Ab 1	Ag 3
4	Ab 1	Ag 4
5	Ab 1	Ag 5
6	Ab 2	Ag 1

Table 8-4: Assay Cycles

Cycle	Inject 1	Inject 2
7	Ab 2	Ag 2
8	Ab 2	Ag 3
9	Ab 2	Ag 4
10	Ab 2	Ag 5
...	...	...
25	Ab 5	Ag 5

Additional injections may be added to the link by selecting **Permute** from the right-click menu. Unlike Interleave, the number of rows for each injection step do not have to match. But remember that the total number of cycles for the assay will be the product of the number of rows in each step. The total number of cycles can become large very quickly!

Multiplex link

The Multiplex injection link was designed to accommodate a specific assay format and uses a combination of the Interleave and Permute ideas.

The typical use case for the Multiplex link is for an antibody screening assay, where a large number of antibodies need to be screened against a small number of analytes. Throughput can be improved by capturing one antibody on channel 1 via a discrete injection and a second antibody on channel 3 via a second discrete injection. The analyte injection follows the capture injections and is active over all three channels, resulting in data for two interaction pairs and a reference in a single cycle. The multiplex link works by *interleaving* any injections in the link designated as capture, and *permuting* those rows with any other injections in the link.

Consider the following example: We have six antibodies (Ab1-Ab6) to assess against two antigens (Ag1 and Ag2). To build Multiplex Link assay, three injections need to be designated as shown below and assigned as:

- **Inject 1** is designated as Capture for channel 1, a discrete Ch1 injection
- **Inject 2** is designated as Capture for channel 3, a discrete Ch3 injection
- **Inject 3** is designated as Analyte, an analyte injection that passes over channels 1-2-3

Ab1-Ab3 are then added to the Inject 1 table. Ab4-Ab6 are added to the Inject 2 table. Ag1 and Ag2 are added to the Inject 3 table.

To create the multiplex link, in the Cycle Editor bar, first left-click on one of the **injection 1**, so that it is highlighted in yellow.

Then right-click on **injection 2** to open the link options and left-click **Add Multiplex Link**.

To complete the multiplex link, select **injection 3** by left-clicking and then right-click on **injection 3** to open the link options and left-click **Add Multiplex Link**.

All three injections are then linked with the Multiplex link as indicated in Figure 8-9.

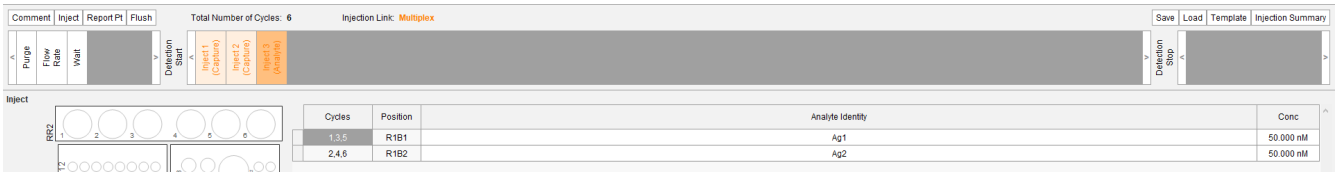

Figure 8-9: Multiplex Injection Link

The assay will have six total cycles, injected according to Table 8-5:

Table 8-5: Assay Cycles

Cycle	Inject 1 (Capture Ch1)	Inject 2 (Capture Ch3)	Inject 3 (Analyte Ch1-2-3)
1	Ab 1	Ab 4	Ag1
2	Ab 1	Ab 4	Ag2
3	Ab 2	Ab 5	Ag1
4	Ab 2	Ab 5	Ag2
5	Ab 3	Ab 6	Ag1
6	Ab 3	Ab 6	Ag2

In this manner, only six total cycles are required compared to twelve cycles if all six antibodies were placed in one Capture injection and simply Permuted against the two Analytes.

Special Assay Cycles

A common scenario in assay design is to include blank cycles for referencing, and positive control cycles. Cycle ordering can be explicitly defined by arranging the samples in order on the Inject table. However this can be tedious if these special cycles should occur at fixed intervals. The Octet® Discovery software includes a mechanism to simplify the configuration of these special cycles. These cycles are configured on the Assay tool editor, under the Periodic Cycles tab. An example of the configuration screen is shown below.

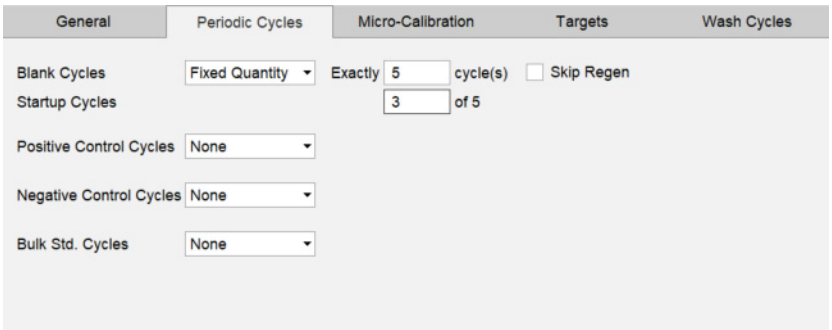

Figure 8-10: Configuring Special Cycles in the Periodic Cycles Tab

For each of the special cycle types, the cycles can be inserted into the assay format in a fixed quantity, or in a periodic manner. If a fixed quantity is selected, those cycles will be evenly distributed throughout the assay. If periodic insertion is specified, then the number of special cycles to be run will depend on the number of regular cycles in the assay.

Blank cycles can be further separated into startup cycles, which are only be performed at the start of the assay and not included in subsequent blank cycles.

As an example, consider an assay that has been configured to run 10 different samples in the Analyte injection. This will result in 10 regular cycles.

If blank cycles are configured to run Periodic for every 2 cycles, the resulting assay will run according to the schedule in Table 8-6:

Table 8-6: Assay Cycles

Cycle Number	Cycle Description
1	Buffer 0.000 nM
2	Ag1 50.000 nM
3	Ag2 50.000 nM
4	Buffer 0.000 nM
5	Ag3 50.000 nM
6	Ag4 50.000 nM
7	Buffer 0.000 nM
8	Ag5 50.000 nM
9	Ag6 50.000 nM
10	Buffer 0.000 nM
11	Ag7 50.000 nM
12	Ag8 50.000 nM
13	Buffer 0.000 nM
14	Ag9 50.000 nM
15	Ag10 50.000 nM

Startup cycles provide an option to run a fixed quantity of blanks as the first cycles in an assay. This is often done in order to establish system stability prior to recording the first "live" cycle. Note that because blank cycles run at the beginning of a regular cycle period, the number of startup cycles is always at least 1. In the previous example, if Startup Cycles are specified as 5, only four more cycles would be added, bringing the total assay cycle count to 19 as shown in Table 8-7:

Table 8-7: Assay Cycles

Cycle Number	Cycle Description
1	Buffer 0.000 nM
2	Buffer 0.000 nM
3	Buffer 0.000 nM
4	Buffer 0.000 nM
5	Buffer 0.000 nM
6	Ag1 50.000 nM
7	Ag2 50.000 nM
8	Buffer 0.000 nM
9	Ag3 50.000 nM
10	Ag4 50.000 nM
11	Buffer 0.000 nM
12	Ag5 50.000 nM
13	Ag6 50.000 nM
14	Buffer 0.000 nM
15	Ag7 50.000 nM
16	Ag8 50.000 nM
17	Buffer 0.000 nM
18	Ag9 50.000 nM
19	Ag10 50.000 nM

If a fixed quantity of blanks is specified, then the startup cycles would deduct from the desired quantity. For example, setting a fixed quantity of five blanks and the startup cycles to the same number would result in all of the blank cycles occurring at the beginning of the assay.

Table 8-8: Assay Cycles

Cycle Number	Cycle Description
1	Buffer 0.000 nM

Table 8-8: Assay Cycles

Cycle Number	Cycle Description
2	Buffer 0.000 nM
3	Buffer 0.000 nM
4	Buffer 0.000 nM
5	Buffer 0.000 nM
6	Ag1 50.000 nM
...	...
17	Ag10 50.000 nM

Changing the assay run order to random or reverse will not change the arrangement of the special cycles, only the order of the regular cycles.

Once the special cycle frequency has been configured, new rows will appear on the injection table for all injections in the assay. Specify a rack position which will be used for each occurrence of the special cycle. The injection parameters such as volume and flow rate will copy from the first regular cycle row in the table. If a large number of blanks or controls need to be recorded, it may be necessary to use a pooled rack position to achieve enough available volume for all of the injections.

Cycles	Pos	Analyte Identity	Conc	Vol (µL)	Order	Inject Vol (µL)	Flow rate (µL/min)	Dissociate (secs)
B1	R2C1		0.000 nM	6750.0				
PC1: 1	R2C2		0.000 nM	6750.0				
1	R1A1		0.000 nM	870.0				
2	R1A2		0.000 nM	870.0				
3	R1A3		0.000 nM	870.0				
4	R1A4		0.000 nM	870.0				
5	R1A5		0.000 nM	870.0				

Figure 8-11: Special Cycle Injection Rows

OneStep® Injection

The Octet® SF3 system can perform advanced injection types called OneStep® which are based on Taylor Dispersion theory.

OneStep® injections always require a bulk standard cycle to be recorded in order to accurately profile the dispersion loop under assay conditions. Enable bulk standard cycles by selecting this option on the Assay editor. It is recommended to use 3% sucrose in buffer as the sample for the bulk standard cycles.

Each type of injection is included by selecting the **Inject** tool from the Inject time-strip bar in the cycle editor. These injections can also be set up in manual mode on the Operation page. Select the sample(s) to be injected from the racks and drop it onto the table on the right.

To configure a standard OneStep® injection, with sigmoidal profile, select **OneStep** as the injection type (Figure 8-12).

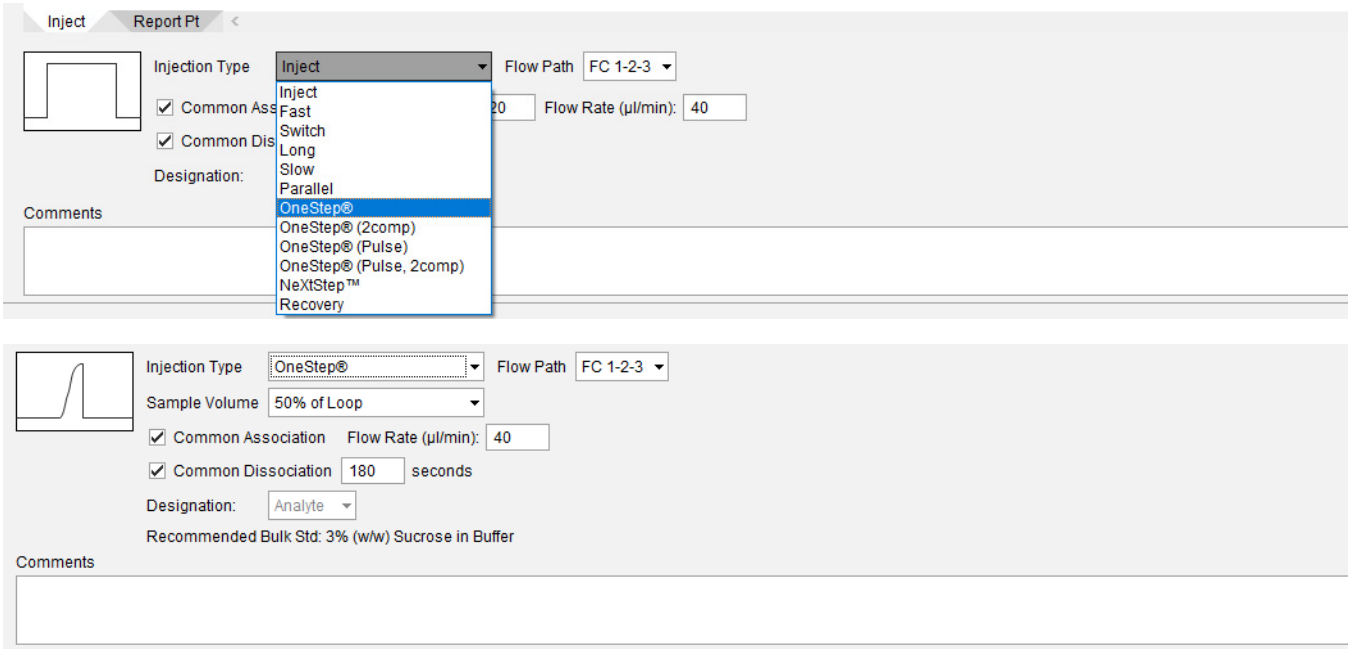

Figure 8-12: Selecting OneStep® Injection Type

Select the desired flow rate for the OneStep® injection. The volume of sample consumed is configurable as a percentage of the dispersion loop volume. The smallest percentage (50%) will result in the fastest injection, and the largest percentage (200%) will result in a longer plateau at full concentration. The sensorgrams in Figure 8-13 show the difference in using the two options.

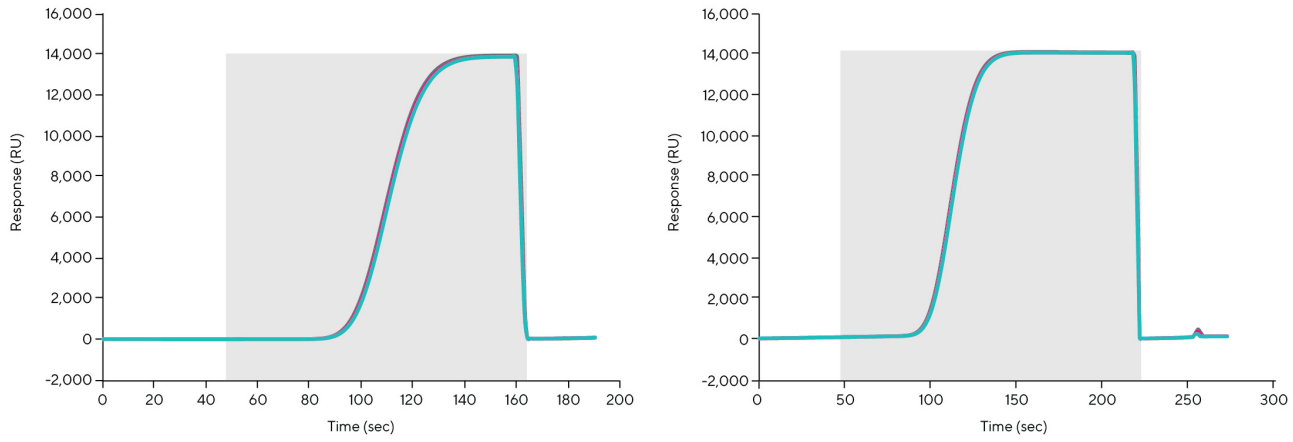

Figure 8-13: 50% (Left) and 100% (Right) Sample Volume Consumed

OneStep® (Two-component)

Two-component OneStep® injections are very similar to standard sigmoidal OneStep® injections; however, instead of dispersing sample into the running buffer, the dispersion loop is preloaded with an alternate buffer which the sample then disperses into. The setup page for a two-component OneStep® injection is shown in Figure 8-14. The only new option is an additional column for specifying a rack position to use for the dispersion loop preload. By default, the columns for Identity, Concentration, and remaining volume will display the information for the Inject Position. The Display drop down list can be used to switch the display to show the Identity, Concentration and remaining volume for the Preload Position. Sample information is always retained for both components when an SPR file is recorded.

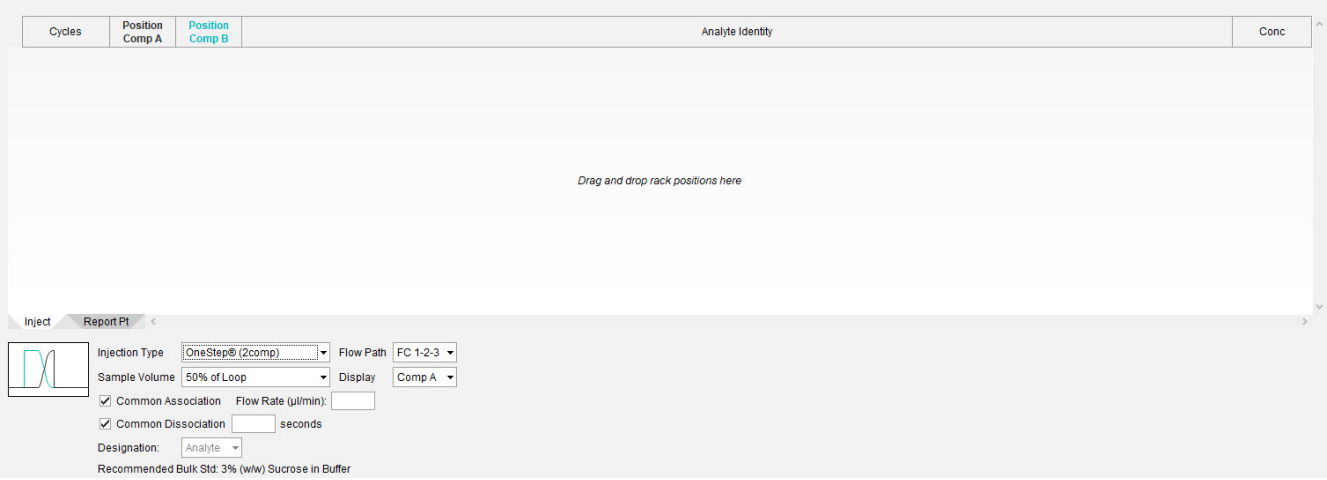

Figure 8-14: Two-component OneStep® Injection

OneStep® injections always require a bulk standard cycle to be recorded in order to accurately profile the dispersion loop under assay conditions. Enable bulk standard cycles by selecting this option on the Assay editor. It is recommended to use 3% sucrose in buffer as the sample for the bulk standard cycles.

NeXtStep™ Injection

NeXtStep™ is a proprietary gradient injection method and are well suited for assays which examine the behavior of an analyte in the presence of a competitor molecule.

Two samples are aspirated from the sample rack back- to-back and allowed to disperse into each other as they are loaded into the sample holding line. The resulting single volume mixture is then injected into the flow cell. The order of injection components is reversed such that the first component loaded (component A) is the last to enter the flow cell, and the second component loaded (component B) is the first to enter the flow cell. Thus from the perspective of the flow cell, the beginning of the injection is nearly 100% component B. As the injection progresses, the apparent concentration of component B decreases, and the concentration of component A increases as demonstrated in Figure 8-15.

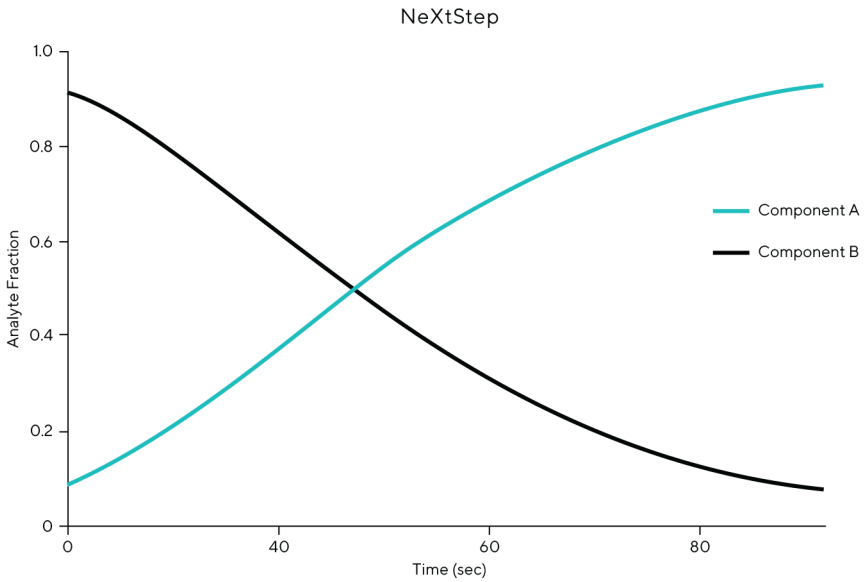

Figure 8-15: NeXtStep™ Injection Component Concentration Injection

The relative shape of the injection is determined by the volumetric ratio of the two samples loaded, and also the total volume of each component loaded. For example, a volume ratio A:B of 1:10 will, in general, produce an injection that remains substantially in component B for longer than component A is the dominant component. The effect can be exaggerated by increasing the total contact time (and thus actual volume loaded) for a given flow rate.

A typical assay format would have component B prepared as the competitor molecule in buffer. Component A would then be a mixture of the competitor molecule plus the analyte, where the concentration of competitor is equal to the concentration of competitor in component B. When injected as a NeXtStep™ injection, the concentration of the competitor remains constant throughout the entire injection, while the concentration of analyte increases in a gradient fashion as shown in Figure 8-16.

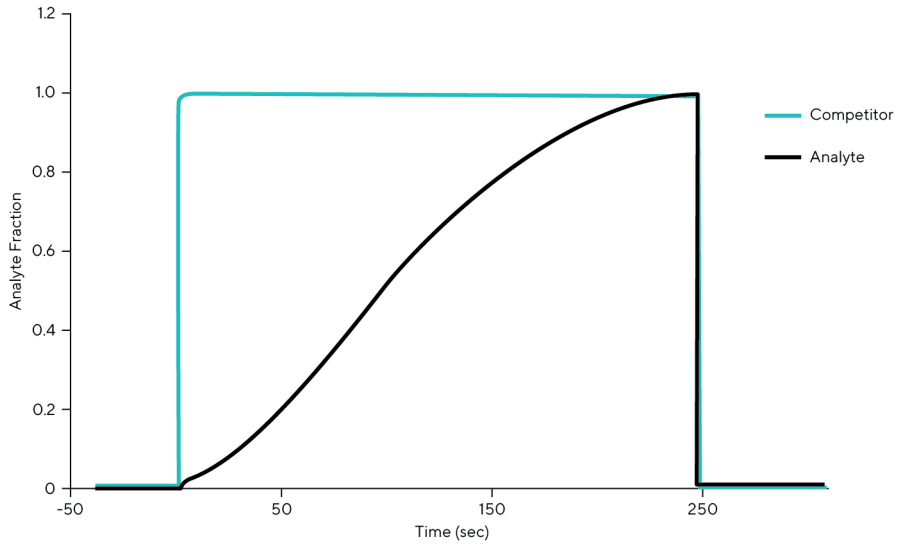

Figure 8-16: NeXtStep™ Injection Competitor and Analyte Injection

High-Throughput Injection Mode

The Octet® SF3 system contains an internal fluidic configuration which enables a high-throughput injection. High sample throughput is a key requirement of fragment screening applications. In a typical assay, only a single fragment injection is required per assay cycle.

In a conventional assay, the fragment injections are performed in sequential order, as demonstrated in Figure 8-17.

Load Sample 1	Inject Sample 1	Cleanup Injection 1	Load Sample 2	Inject Sample 2	Cleanup Injection 2	Load Sample 3	Inject Sample 3	Cleanup Injection 3

Figure 8-17: Fragment Injections

The fluidic architecture of the Octet® SF3 system allows the system to take advantage of what is otherwise dead time during the sample injection. The dual pump design allows one pump to perform the sample injection, and at the same time, the other pump is free to perform sample load of the next injection, as demonstrated by Figure 8-18.

Load Sample 1	Inject Sample 1	Cleanup Injection 1	Inject Sample 2	Cleanup Injection 2	Inject Sample 3	Cleanup Injection 3
	Load Sample 2		Load Sample 3			

Figure 8-18: Dual Pump Sample Injection Process

In High-Throughput mode, the Octet® SF3 system is able to reduce the assay cycle time by eliminating the time normally required for sample loading. In certain assays, this is a reduction in cycle time of up to 30%, and allows the Octet® SF3 system to complete a screen of two 384-well plates in under 24 hours.

High-Throughput mode requires a specific assay design. The simplest way to ensure the assay is compatible with high-throughput mode is to select the **OneStep® Screening** assay template when adding an assay step to the Method Editor.

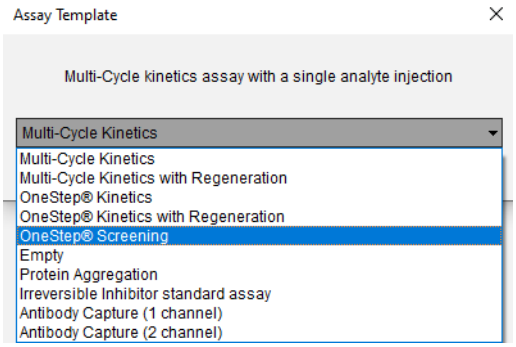

Figure 8-19: OneStep® Screening Assay Selection

When an assay meets the criteria for high-throughput mode, the option will appear at the top of the assay timeline as shown below. High-Throughput mode can be disabled by deselecting the checkbox. In that case, the assay will run in the conventional format. The validate method option may be used to compare assay run time estimates with and without High-Throughput mode.

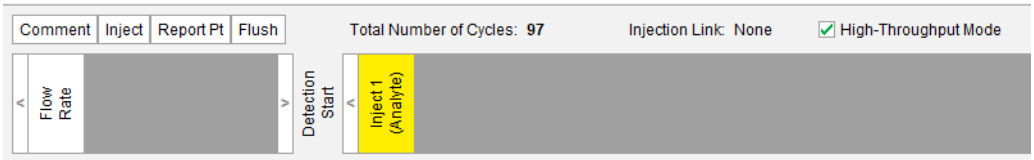

Figure 8-20: High-Throughput Mode Selected

High-Throughput mode is available for assays with a single injection and which do not include full washout operations such as a Purge. Standard OneStep® (sigmoidal shape) injections as well as FAST fixed concentration injections can utilize High-Throughput mode. FAST injections must use the 3-2-1 flow path, with all volumes and flow rates the same, and have injection contact time that exceeds the sample loading time.

Assay Micro-calibration

Assay micro-calibration is a feature that allows correct calibration of referencing errors due to large changes in sample refractive index (RI) occurring with high-capacity immobilization of biomolecules. This effect is commonly referred to as the 'excluded volume' effect because the volume displaced by an immobilized biomolecule is no longer sensitive to changes in RI and is thus excluded for biosensing. A flow channel with a large quantity of immobilized ligand will be relatively less sensitive to change in RI than an unmodified channel.

In a standard assay the refractive index of the samples is approximately constant and therefore, double reference subtraction is sufficient to correct any RI changes. However, solutions such as DMSO cause large changes in RI. 1% of DMSO gives a response of ~1,200 RU and therefore, any small variations in DMSO can have a large effect upon the expected response signal, which can be very low when assessing fragment and small molecule compounds.

These effects are typically removed by injecting a series of solvent correction standards, extrapolating a linear or polynomial standard curve representing the effect, and extrapolating the subtraction factor for the samples. Micro-calibration in the Octet® SF3 system is a dynamic procedure that produces the standard curve in a single cycle before an assay is run and then normalizes RI sensitivity for the three channels in real time. When an assay is recorded after micro-calibration, the resulting data is already calibrated for RI effects.

To set up micro-calibration for an assay, choose the **Micro-Calibration** tab in the assay editor, then click the drop-down box and select **High+Low**. This indicates that two standards will be used with RIs higher and lower than the running buffer baseline. Next, identify the vials that hold the "High" and "Low" RI standards in their respective fields. Finally, make sure that the correct **Reference Channel** is selected under the General tab. Choose the channel that will be used as a reference, preferably an unmodified channel.

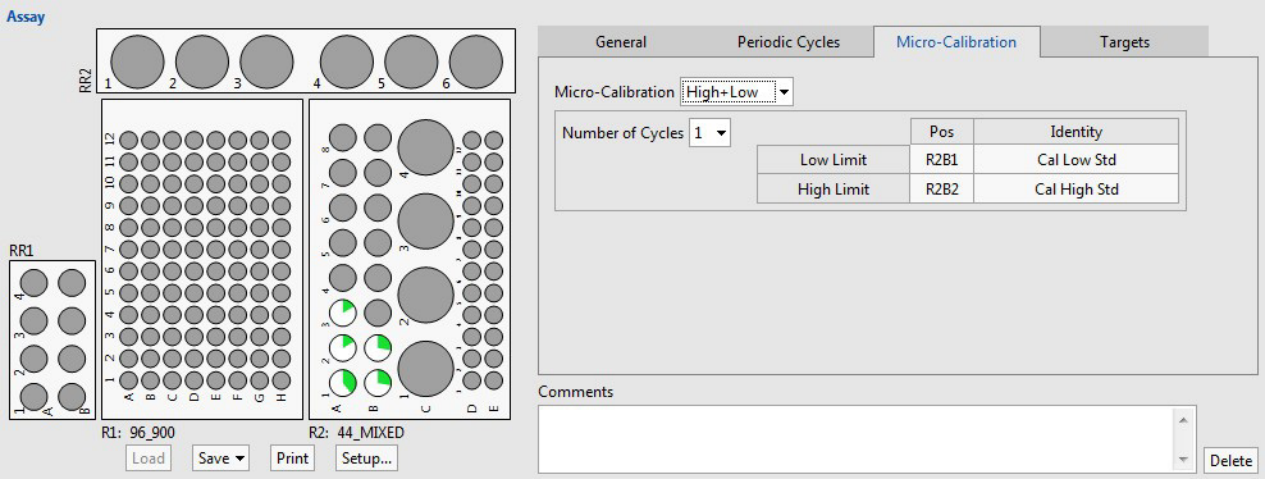

Figure 8-21: Micro-Calibration Setup

NOTE: *When preparing micro-calibration standards, it is important that the "High" and "Low" standards actually have higher and lower refractive indices, respectively, than the running buffer. If they do not, the software will report an error and halt the method.*

Changes in sample RI most often occur when a sample stock contains high RI substances such as DMSO or glycerol but can also result from the RI of the analyte. When the sample is diluted in a running buffer it is often difficult to perfectly compensate for these RI differences. Experiments in which high RI components are present in the running buffer will commonly require micro-calibration. Micro-calibration standards should be chosen to represent the range of expected error due to pipetting. For example, if the running buffer contains 3% DMSO and the sample buffer contains 3% DMSO, the calibration standards should contain a concentration of DMSO greater or less than 3%. For broad calibration, buffer calibration standards containing 2% DMSO and 4% DMSO could be used. For more precise calibration, the recommended standards should contain 2.6% DMSO and 3.75% DMSO (approximately -500 to +1500 RU around the percentage DMSO concentration).

Data that are recorded with micro-calibration are not permanently modified but are instead displayed in the software such that the calibration can be toggled on and off. When an assay conducted with micro-calibration is viewed in the software, a toggle button appears so the data can be viewed in its calibrated and un-calibrated forms.

Conditional Steps

Within an assay, some steps may be flagged as Conditional Steps. The steps are:

- Prime
- Purge
- Purge Probe
- Clean

The main utility of the Conditional Steps is to enable additional washout or purging for assay cycles that include particularly "sticky: analytes. See "Wash Function Comparison" on page 112 for a full description of each conditional step.

Conditional Steps can be enabled by first checking the **Show Advanced Options** checkbox at the top right of the Method Setup tab. Any of the above listed steps will now include a Conditional option within their setup page on the cycle editor. Conditional steps will be highlighted in purple on the assay time strip. In the example below, an additional conditional purge is included in the post-recording phase of the assay.

Figure 8-22: Conditional Steps

Within a particular assay cycle, the conditional steps will not run unless triggered. Conditional Steps can be forcibly triggered for a particular cycle by selecting the **Logic** tab on an Injection and checking the **Always Run Conditional Operations** box. For example, the assay below includes four cycles. The conditional purge at the end of the cycle will run in cycles 1 and 2, but not in cycles 3 and 4. Conditional Steps can also be triggered by the response levels of an injection. This type of trigger is configured by clicking on the **Injection Logic** button for the injection of interest.

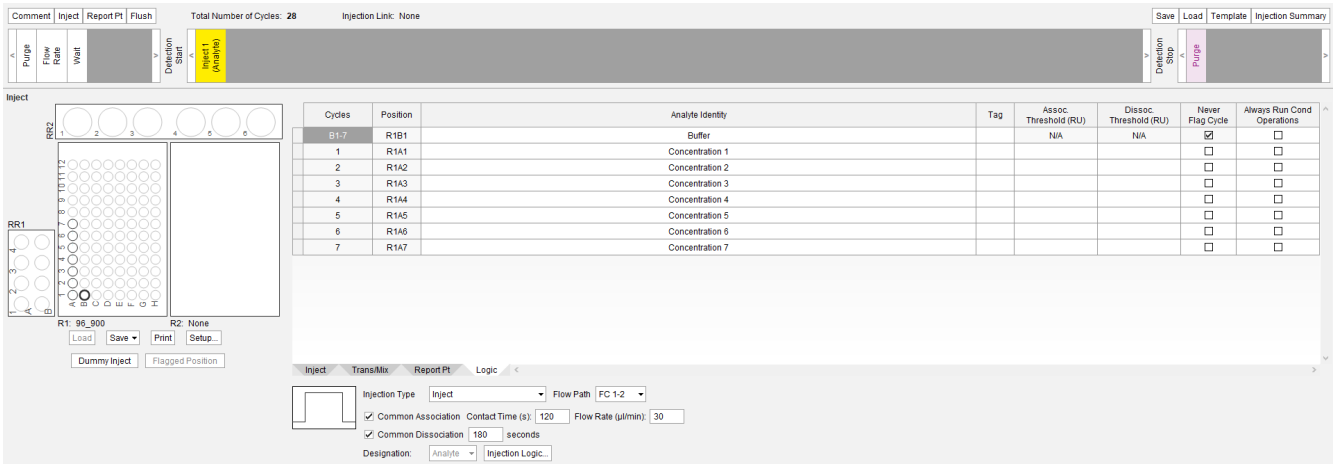

Figure 8-23: Triggering Conditional Steps

Sample Recovery

The Sample Recovery injection is a special type of injection for eluting the analyte from the sensor surface. The injected analyte is passed over the specified flow cell(s) and then returned via the sample probe to a new location in the sample racks.

The graph in Figure 8-24 demonstrates a recovery injection. An injection across a pre-immobilized flow cell captures approximately 175 RU of the analyte to the sensor chip surface. The captured analyte can then be recovered by a second injection containing a regeneration solution (recovery agent). As the regeneration solution is injected, an air bubble passes through the flow cell to prevent dispersion of the regeneration solution into the system buffer. Once the regeneration solution reaches the flow cell, the flow is paused for a configurable amount of time; which allows removal of the captured analyte from the sensor chip surface.

After pause period is completed, the recovered analyte is withdrawn from the flow cell along with the air bubble (thus the signal decrease at the beginning and end of the recovery injection, Figure 8-24). The recovered analyte is then dispensed to a configurable recovery position in the sample racks.

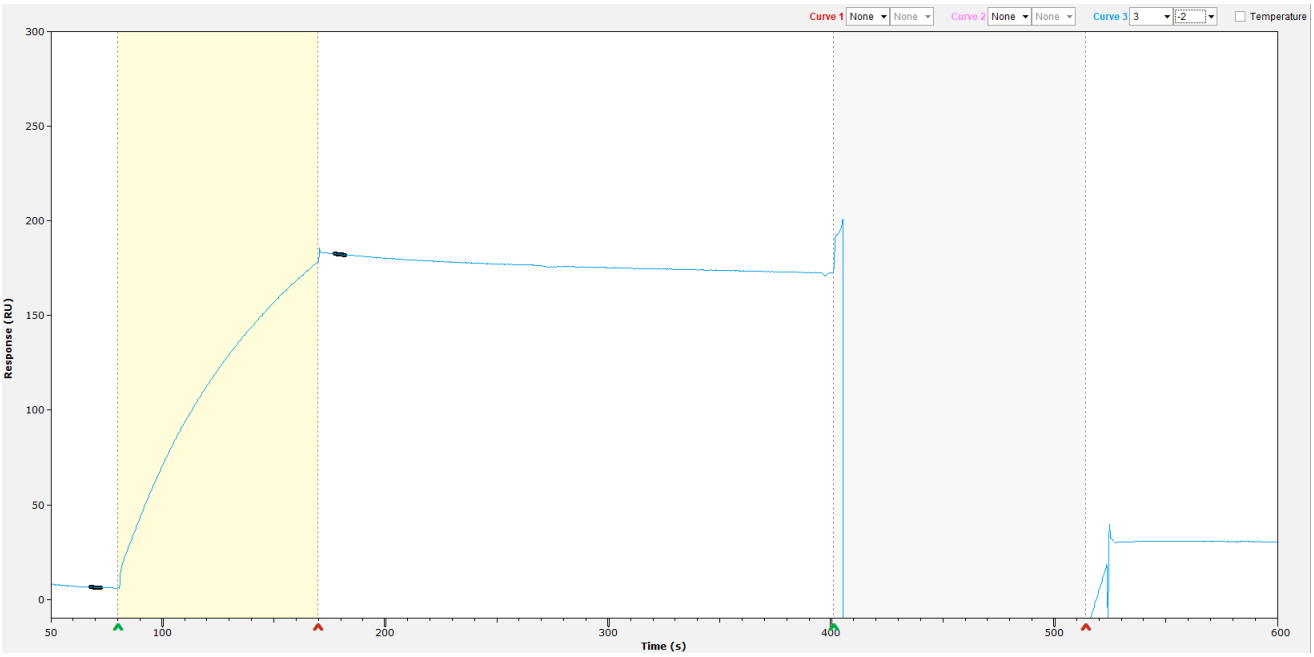

Figure 8-24: Recovery Injection

Useful Terms

Regeneration

The term regeneration refers to the reversal of the affinity complex formed during a binding interaction leaving only free ligand binding sites or capture molecule binding sites on the surface. Usually a surface is regenerated by exposure to extremes of pH, ionic strength, chaotrophic agents, temperature, or a combination of one or more of these. In practice it is often difficult to remove all affinity complexes without reducing the binding capacity of the immobilized ligand-ligand denaturation. If the loss in binding capacity after each regeneration cycle is relatively low (*e.g.* <3%) then it is possible to complete a set (*e.g.* 5-10) of binding interaction curves for kinetic and affinity analysis. It is also possible to compensate for the loss in capacity by normalizing the set of curves with respect to the change in binding capacity. For example a curve recorded on a surface that has experienced a 50% loss in capacity is simply multiplied by a factor of two. Use the multiplication factor button on the tool bar for this. It is also possible to simply fit a model where the change in capacity is simply a parameter to be fitted. This procedure is a simple approach but the resulting maximum binding response values should be checked to ensure that they make sense within the context of what is experimentally reasonable. If the immobilized ligand is not a protein but the analyte is then a protease may be used as an effective regeneration step by digesting all proteins at the sensing surface. It is common to have a significant drift in the baseline following regeneration. This is caused by conformational shifting of the surface chemistry at the surface on exposure to harsh conditions. The surface usually recovers to steady state within a few minutes. This effect can be dramatic when using hydrogels but the planar nature of the Octet® SF3 sensor chip surface chemistry greatly reduces such effects. Nevertheless, it is advisable to allow a few minutes for the baseline to recover before running another sample injection.

Ligand denaturation

The stability of proteins and other biomolecules varies greatly making it difficult to estimate the useful life of a surface functionalized with ligand. The immobilized ligand will typically be exposed to 25 °C, the standard operation temperature, for the duration of its useful life. In our experience small molecules (*e.g.* drugs, peptides) tend to have very high stability and a surface may remain useful for several weeks. In contrast, the binding activity of some monoclonal antibodies begins to degrade immediately on exposure to ambient conditions resulting in a progressive loss in activity.

These issues must be considered when estimating the number of sensors required for any particular application. Where possible it is advisable to choose the more stable biomolecule of the affinity pair of interest as the ligand in order to prolong the active life of the surface and reduce sensor consumption. However, this is not always possible as one may need to consider other parameters. For example, we may be interested in the kinetics of the interaction of a stable protein with a monoclonal antibody. Immobilizing the protein would be recommended to extend the useful life of the surface but the bivalency of the monoclonal antibody will interfere with kinetic analysis unless the monoclonal antibody is immobilized. (Note: When immobilized, the two active binding regions of the antibody behave as independent binding sites and a 1:1 binding model may be used as a reasonable approximation).

An alternative approach is affinity capture where a stable anchoring molecule is immobilized onto the surface and used to affinity capture the ligand which in turn binds the analyte. After regeneration only the covalently bound affinity capture molecule remains. Protein A, protein G, protein L anti-species polyclonal antibodies are all useful affinity capture molecules for antibody-antigen interaction studies. Other more generic ligands include the use of peptide tags and capture ligands, *e.g.* metal chelate-polyhistidine tags. It is possible to extend the useful life of a surface dramatically using these approaches. We have recorded several hundred binding interaction cycles for the interaction of immobilized protein A with various IgG molecules.

Chapter 9:

Other Features

Sample Table Import

When configuring the sample racks for a method, it is not always practical to enter sample information manually. Sample information can be copied and pasted directly into the rack table, but the well positions must first be placed on the table. It is also likely that existing sample information is not available in the same layout that the Octet® SPR Discovery software expects. In these cases it is beneficial to use the Sample Table import feature.

Activate the import wizard by clicking on the **Import** button on the Initial Rack Setup tool on the Method Setup page. The pop-up in Figure 9-1 appears.

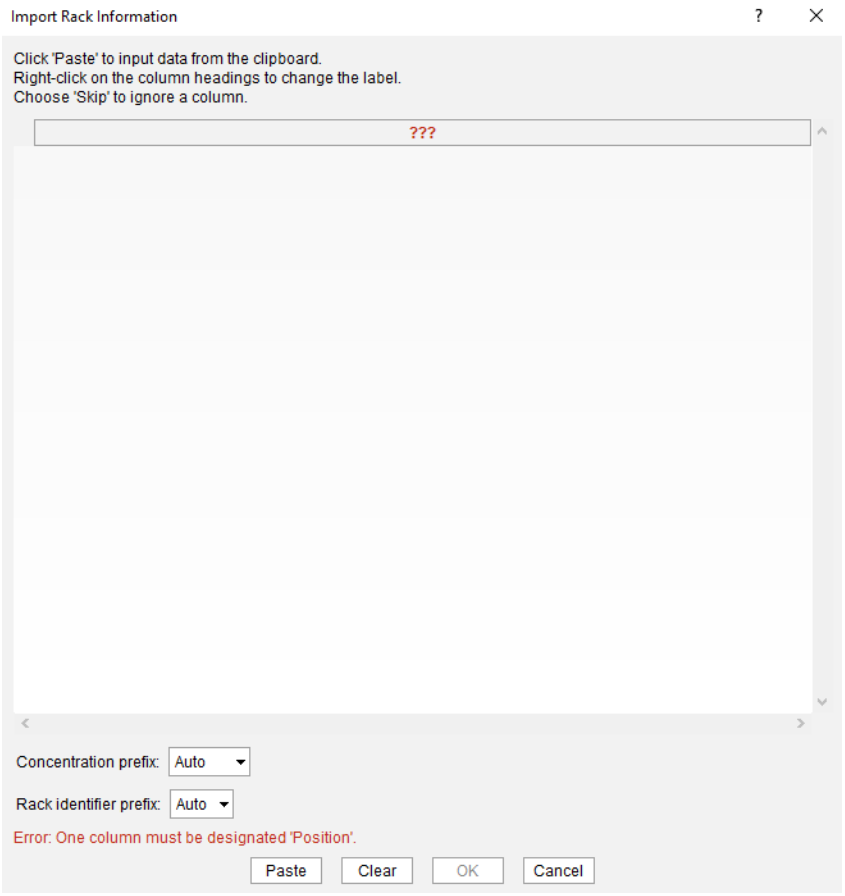

Figure 9-1: Import Wizard

As an example, suppose we have the following sample information in a Microsoft® Excel® spreadsheet:

	A	B	C
1	ID	Concentration	Position
2	Analyte A	1.00E-09	r1a6
3	Analyte B	1.50E-09	r1a8
4	Analyte C	2.50E-09	r1a10

Figure 9-2: Example Sample Spreadsheet

In this case, the columns are not in the same order that the Octet® SPR Discovery software uses, the concentrations are specified in molar units, and the rack positions are not capitalized. To import this data, select **rows 2-4** and **columns A-C**, then choose **copy** from the menu (or use the keyboard shortcut **Ctrl+C**). Switch back to the rack import pop-up and click the **Paste** button at the bottom of the screen. The result is shown in Figure 9-3:

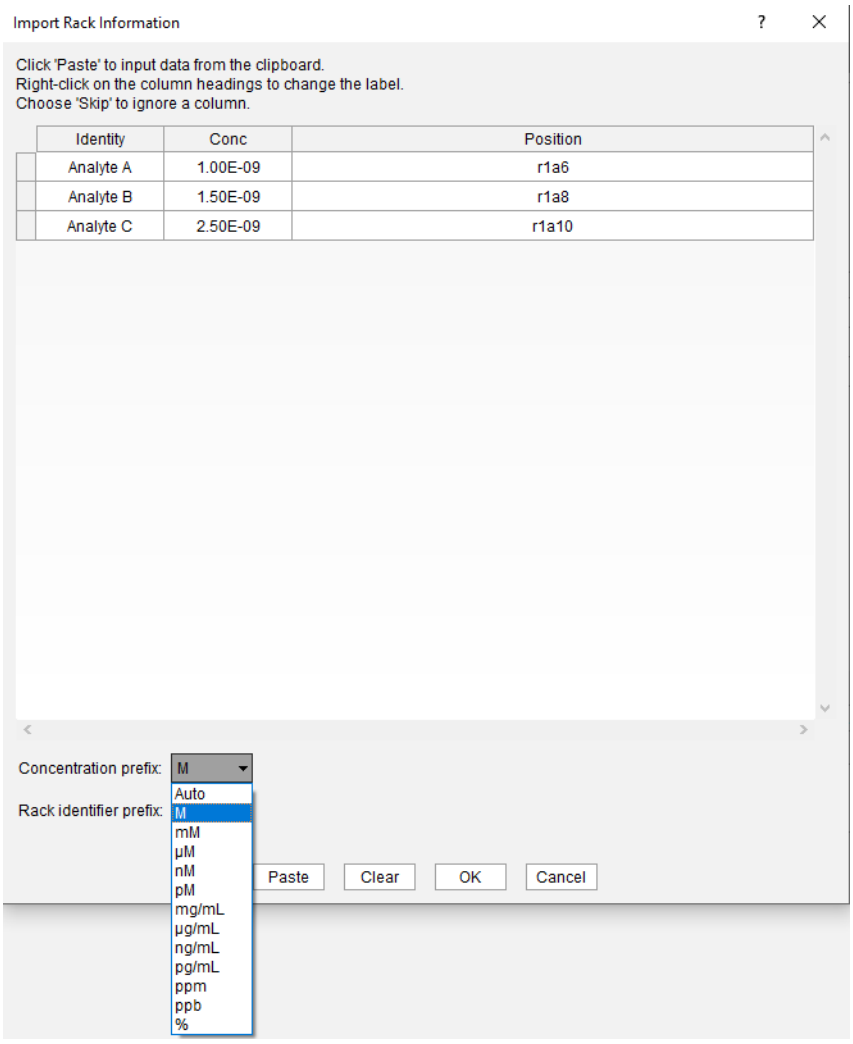

Figure 9-3: Example Sample Import

The software was able to automatically determine which column contains the sample position. Change the label for the other two columns by right clicking on the headings and selecting the appropriate column name. When the concentration prefix option is set to Auto, the software assumes that the concentration includes a trailing unit prefix, such as 1.0n or 2.5u. In this example, no unit prefix has been included so the option box should be changed to **M** for molar concentrations (Figure 9-3, dropdown). When all of the columns are named and no other conflicts are detected, click **OK** to complete the import (Figure 9-4). If there are extra columns in the Microsoft Excel sheet that do not have a matching field in the import wizard, simply choose the **Skip** option for that column and it will not be imported.

Position	Identity	Conc	Vol (µL)	MW (Da)
R1A6	Analyte A	1.000 nM	0.0	
R1A8	Analyte B	1.500 nM	0.0	
R1A10	Analyte C	2.500 nM	0.0	

Figure 9-4: Sample Import Default Concentration Units

To maintain a specific sample run order in an assay, include a number in the Order field indicating the run order. When samples are inserted into the injection table, they will be automatically sorted by the Order field.

For cases where the source data has the position information in a simple row/column format, use the Rack Identifier Prefix dropdown to choose a specific rack to place the samples into. The import wizard will then prepend the selected rack identifier. For example, the positions A1, A6, C8, C12 are pasted from the source data file. Selecting rack identifier prefix **R2** from the dropdown will change the positions to R2A1, R2A6, R2C8, R2C12. In the case where two sample racks are used, simply run the import wizard twice, selecting **R1** the first time, and **R2** the second time.

Email Notifications

The Octet® SPR Discovery software includes a notification feature which will send an email message to specified recipients when a running method is finished.

To complete the initial setup for email notifications, go to the Notifications Setup page under the Instrument Setup tab.

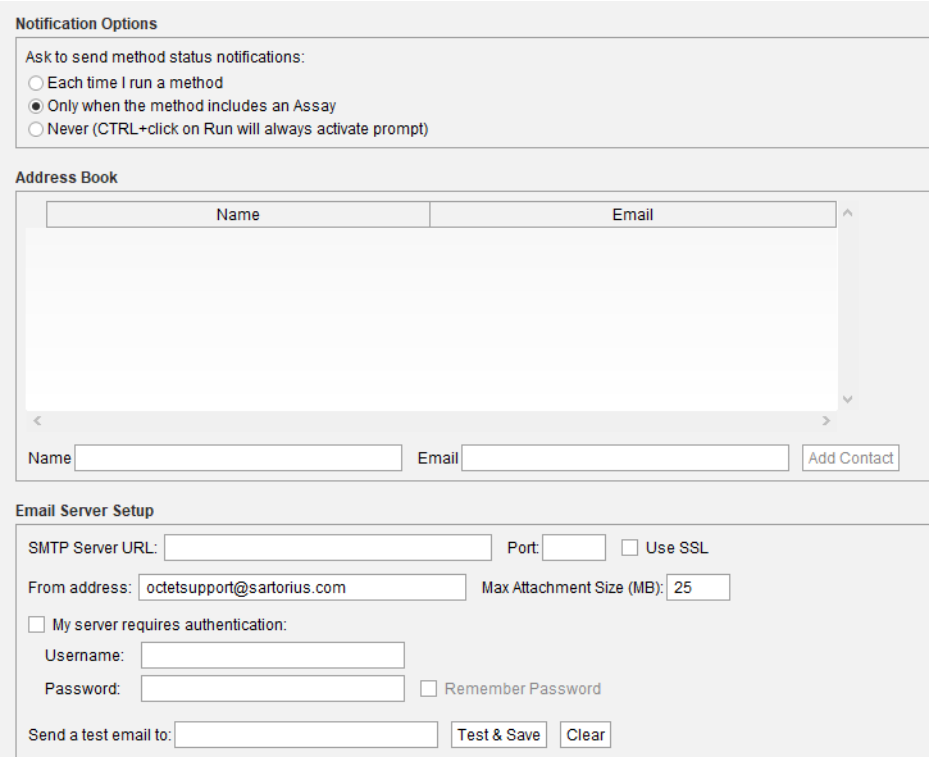

Figure 9-5: Notifications Setup

First, add contacts to the address book by entering a name and email address, then clicking **Add Contact**.

Complete the email server setup with the appropriate information for your email server. Contact your company's Information Services department if you are unsure of the details. Many companies will have an internal SMTP server that may be used. Ensure you are aware of any corporate policies regarding disclosure of company data before using the email notification system. If server authentication is required, we recommend setting up an account expressly for the Octet® SPR Discovery software to use. Do not use your personal or business email credentials. The email server settings will not be saved until a test email has been sent successfully.

Choose an option for when the software should prompt to send a method complete notification. By default, the prompt will appear any time a method which includes an assay is run. Note that holding the **CTRL** key while clicking on the **Run** button will always present the notification setup prompt, as shown in Figure 9-6.

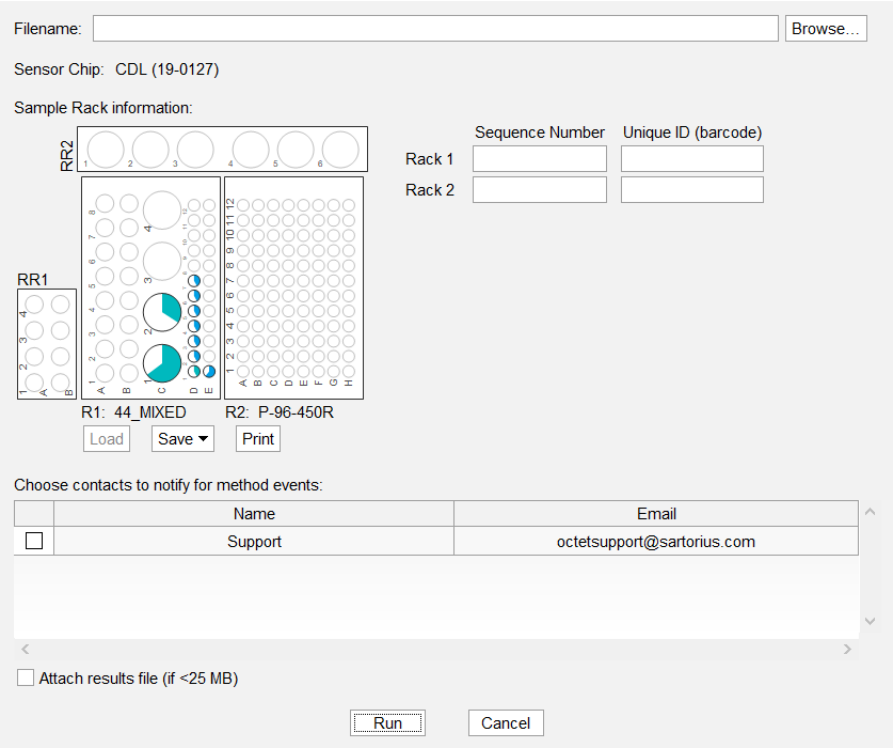

Figure 9-6: Notification Options

Select each recipient that should receive an email notification when the method is complete. If all the boxes are left unchecked, no email notification will be sent. If an*. spr file is recorded as part of the method (method includes at least one assay), then the "Attach results" option will appear. When checked, this option will send the *.spr file as an attachment to the notification email. Notification emails are sent upon successful completion of the method, or in case of an error.

Chapter 10:

Function Reference

Introduction

Information on the different functions built into the Octet® SF3 system is listed in the following sections. Many of the functions can be accessed in Manual Mode, but there may be some limitations on when the function can be used. A Method can be thought of in terms of two levels: the Top Level and the Assay Level. The Assay Level may be further divided into three regions: Pre-Recording, Recording, and Post-Recording. The levels where each function can be placed are given.

Assay

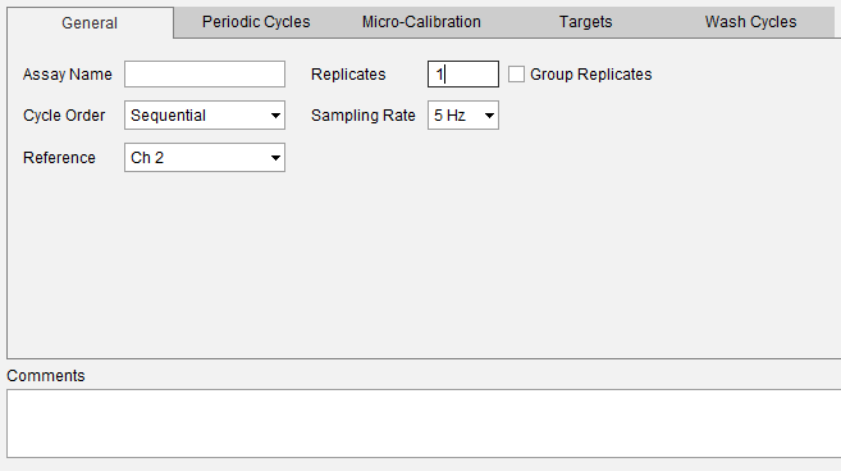

Figure 10-1: Assay Functions

Manual Mode Access: Not available

Method Mode Levels: Top Level

General Tab:

- **Assay Name:** Optional name for the assay. The first few characters will be displayed in the Method Timeline to easily distinguish different assays. The assay name will also be displayed in the Assay drop-down list on the View page.
- **Cycle Order:** Choose from Sequential, Random and Reverse. Sequential means the cycles will be executed as written in the method. Random means the cycles will be shuffled upon method execution. The randomization is done at run time on the system, and thus is likely to be different each time the method is executed. Reverse order executes the cycles in the opposite order of the Sequential option.
- **Reference:** Choose which of the three sensing channels will serve as the reference. This selection will be automatically passed through when exporting data for analysis.
- **Replicates:** Number of times to repeat each cycle.
- **Group Replicates:** When checked, this option causes all replicates of a unique cycle to be run back-to- back, even when cycle randomization is enabled. It is best practice to leave this option unchecked; however it may be useful for certain assay formats. This option has no effect when the Replicates option is set to 1.

- **Sampling rate:** Choose a data rate at which to record each cycle in the assay.

Periodic Cycles tab:

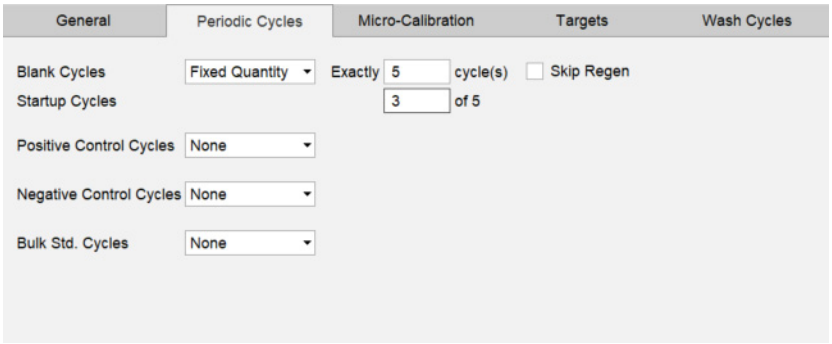

Figure 10-2: Periodic Cycles Tab

- **Blank Cycles:** Automate the insertion of blank cycles within the assay. Choose **Periodic** and enter a period for the blanks. The number of blanks run in the assay will depend on the number of regular cycles configured by the injections in the assay. Choose **Fixed Quantity** to insert a fixed number of blank cycles into the assay. Startup cycles are blank cycles that are recorded at the beginning of the assay. This is useful for establishing the sensor chip conditions prior to running real samples. Also choose whether to skip any injections labeled as Regeneration injections for the blank cycles.
- **Positive Control Cycles:** Automate the insertion of positive control cycles within the assay. Operation is similar to the Blank Cycles option. Choose the number of unique positive control compounds required in the assay (1 to 3). Each unique control will be run in a separate cycle.
- **Negative Control Cycles:** Automate the insertion of negative control cycles within the assay. Options are identical to those of the Positive Control.
- **Bulk Standard Cycles:** Automate the insertion of bulk standard cycles within the assay. Bulk standard cycles are required by OneStep® injections. Other analyte injection types do not require a bulk standard cycle. Choose a fixed quantity of bulk standard cycles to include in the assay. Typically only 1 or 2 bulk standard cycles are necessary. As with blanks, choose whether to skip the regeneration injection(s) for bulk standard cycles.

Micro-calibration tab:

- Automate the insertion of micro-calibration within the assay. Micro-calibration cycles are required during assays that may contain a large refractive index change, such as those containing DMSO. See the assay micro-calibration section for further information.

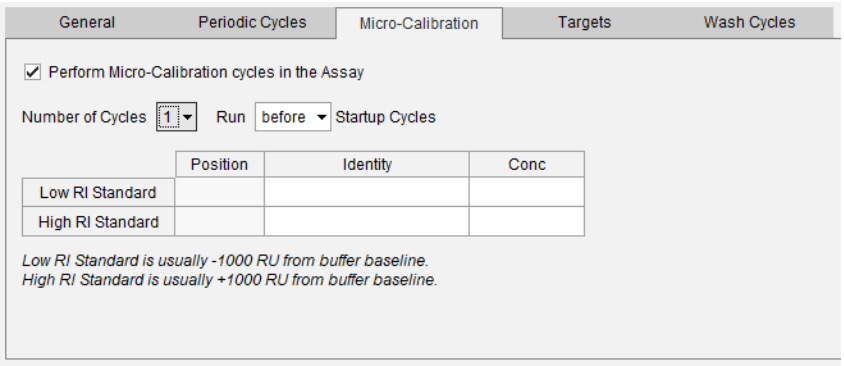

Figure 10-3: Micro-Calibration Tab

Targets tab:

- **Target Information:** Targets are the compounds immobilized to the sensor chip surface during the assay.
- **Target Information Source:** The option 'Fixed' is for assays where the immobilized target does not change during the assay. This information can also be entered on the View page after a data file has been recorded. The option 'Dynamic' should be used with assays where the immobilized target can be different in each assay cycle, such as with an antibody capture assay. The injection(s) designated as Capture injections will then automatically provide the per-cycle target information when analyzing results.
- **Target Information Table:** Enter target information manually or drag and drop samples from the Racks to automatically copy in names and molecular weights.

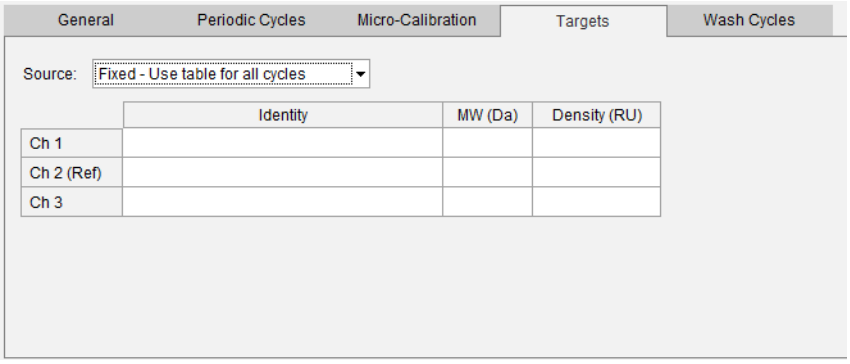

Figure 10-4: Targets Tab

Wash cycles:

- Automate the insertion of wash cycles within the assay. Wash cycles are required during assays that may contain 'sticky' analytes that may affect subsequent cycles, such as with small molecules. Options to select the frequency and flow path allow full flexibility as to what the wash cycle solution contacts during the method.

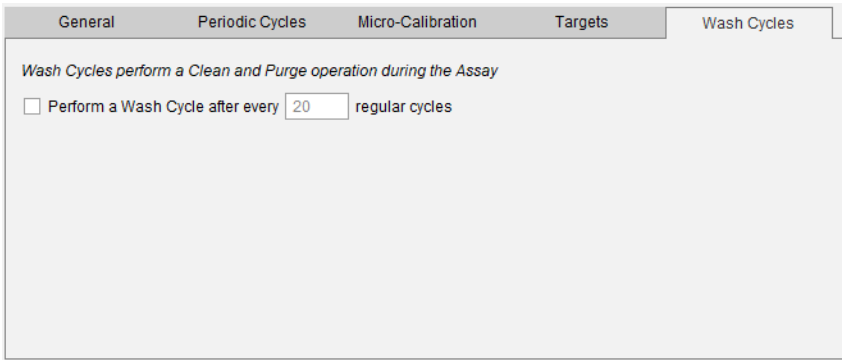

Figure 10-5: Wash Cycles Tab

Calibrate

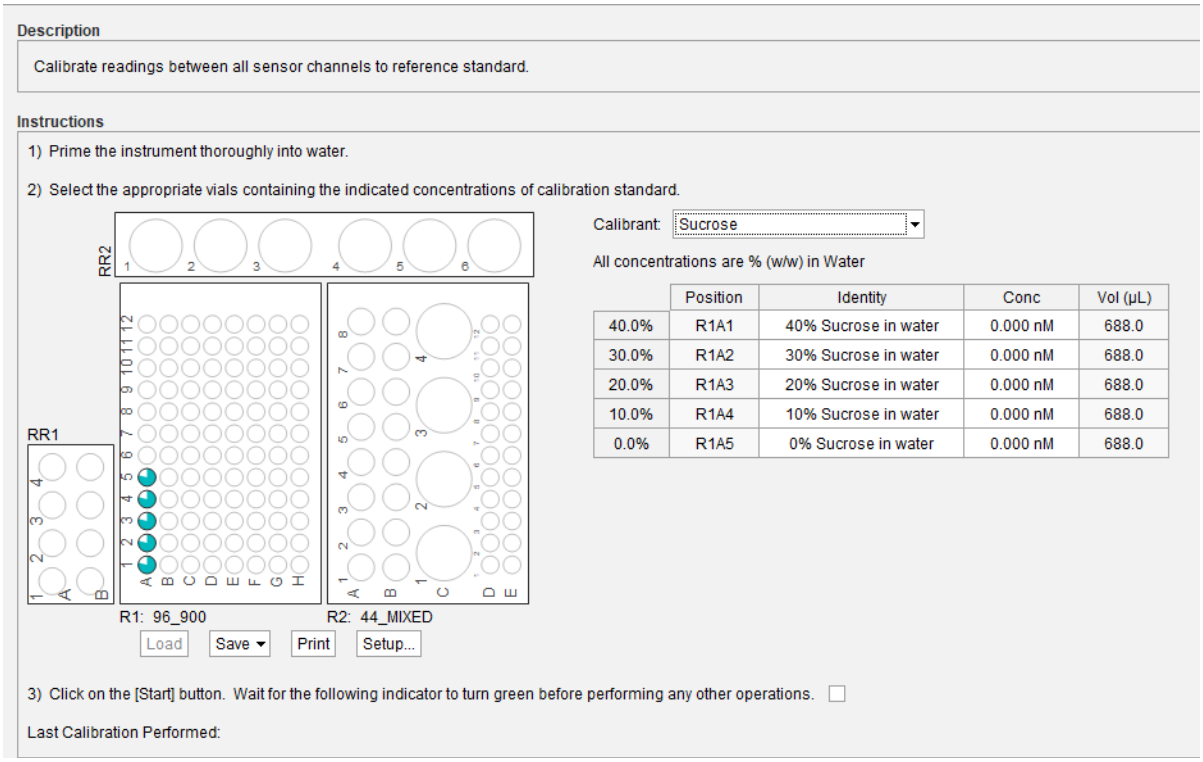

Figure 10-6: Calibrate Functions

Manual Mode Access: Instrument Setup page when not recording data.

Method Mode Levels: Top Level (Show Advanced Options enabled)

Options:

- **Positions:** Specify five rack positions containing 40%, 30%, 20%, 10%, and 0% sucrose or potassium carbonate (K_2CO_3) in deionized water (w/w).

Clean

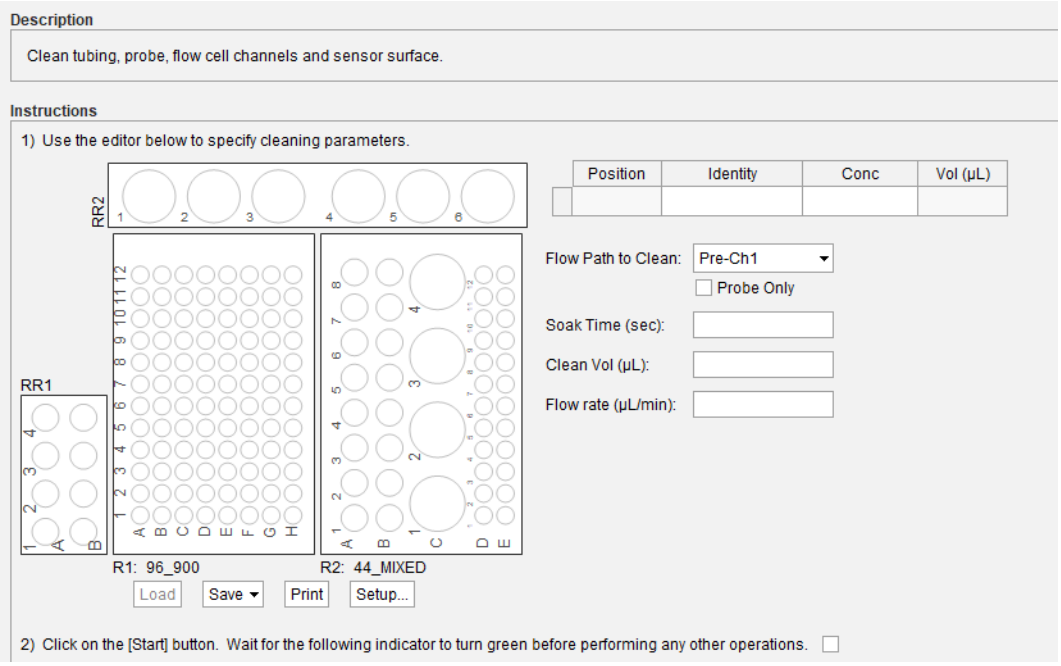

Figure 10-7: Clean Functions

Manual Mode Access: Instrument Setup page. The 'All' flow path option is not available during a manual recording.

Method Mode Levels: All levels. The 'All' flow path option cannot be selected when placed in the Recording section of the Assay Level.

Options:

- **Position:** Specify a rack position from which to load the cleaning solution by dragging from the rack graphic on the left. The identity and volume will be filled in automatically if sample information has been entered for that rack position.
- **Clean Volume:** Volume of cleaning solution to load into the system. This option is fixed by the system if the Flow Path is set to 'All.'
- **Flow Rate:** Rate at which the cleaning solution will be injected into the flow cell. This option is fixed by the system if the Flow Path is set to 'All' or the Probe Only option has been selected.
- **Flow Path to Clean:** Select from different flow paths over which to inject the cleaning solution. The 'All' option will cause the system to use a predefined cleaning routine that loads the cleaning solution simultaneously into both sample loops and then injects the cleaning solution through all possible flow paths.

- **Probe Only:** When checked, this option will load the cleaning solution into the sample loop corresponding to the selected path, but instead of dispensing through the flow cell, cleaning solution will be dispensed out the probe into the waste port.
- **Soak Time:** Number of seconds to allow the cleaning solution to soak in the sample loop(s).

Dilute

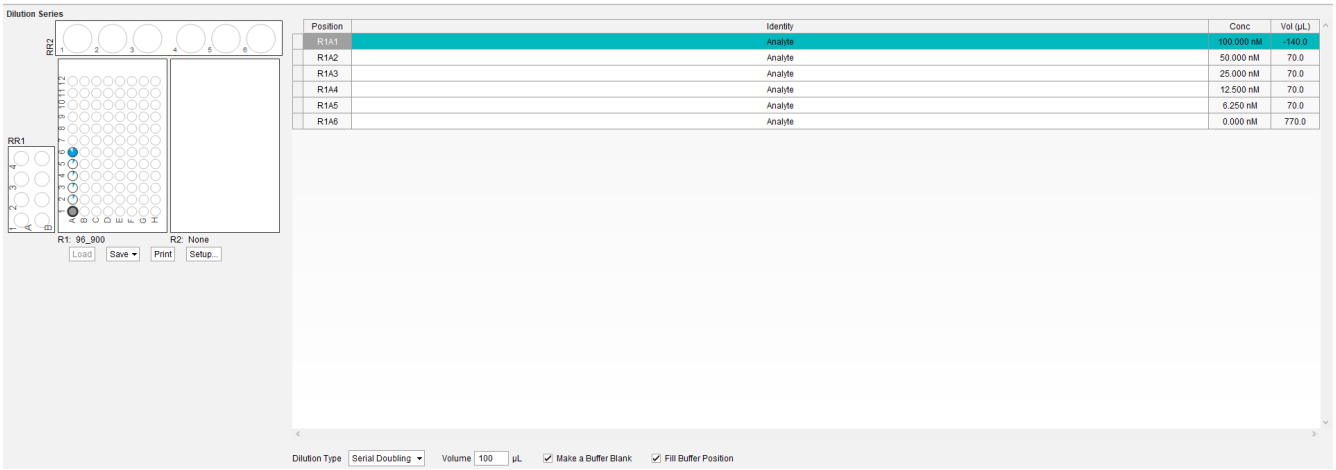

Figure 10-8: Dilute Functions

Manual Mode Access: Operation page when not recording data.

Method Mode Levels: Top Level

Options:

- **Position Table:** Specify rack positions for the dilution series by dragging from the rack graphic on the left. The first entry in the table will be highlighted in teal to indicate that it is the undiluted or stock concentration position. The identity and concentration will be filled in automatically if sample information has been entered for that rack position. The dilution series will be created in the remaining positions on the table from high to low concentration. The sample identity and concentration will be filled in automatically in both the table and the rack graphic. The last entry in the table will always be a buffer blank.
- **Dilution Type:** Choose from serial doubling, serial tripling or decimal (linear). The concentration column in the table will update automatically.
- **Final Volume:** Volume of each dilution to create. Note that the serial doubling and tripling dilutions are created by cascading from the previous concentration. Thus entering a final volume at or close to the vial size will result in a validation error as the vial will overflow during the dilution process. Note that vials have a dead volume which cannot be reliably aspirated (see Table 3-1 on page 19). The software automatically subtracts this dead volume and displays the 'available' sample volume. This is the reason for the discrepancy between the 100 µL setting and the vials indicating 70 µL available in the above example. Where a decrease in dead volume is desired, rack adapters are available to allow usage of multi-well plates.
- **Make a Buffer Blank:** The last position on the table may optionally be a buffer blank or simply the final concentration in the dilution series.

- **Fill Buffer Position:** This option is available when the 'Make a Buffer Blank' option is selected. The last position on the table is the buffer blank, which has concentration 0. In order to facilitate multiple blanks in an assay, this position can be filled to the smallest of 800 µL or the vial capacity. Unchecking this option will fill the final position to the same volume as the other diluted positions.

Flow Rate

Figure 10-9: Flow Rate Functions

Manual Mode Access: Operation Page at any time.

Method Mode Levels: All Levels.

Options:

- **Flow Rate:** Change the idle flow rate of the pump. '0' may be entered to stop the idle flow, but this should be used only momentarily as stagnant buffer may cause blockages in the system.
- **Match Analyte Injection Flow Rate:** This option is available when used within an assay. The flow rate will automatically be matched to the analyte injection's flow rate for the current cycle. This is useful for assays in which the analyte injection has a different flow rate each cycle.

Flush

Figure 10-10: Flush Functions

Manual Mode Access: Operation page at any time.

Method Mode Levels: Assay Level.

Options:

- **Flow Path:** Select which flow path to flush.
- **Number of repetitions:** The flush may be repeated multiple times for more thorough cleaning.

Hibernate

Figure 10-11: Hibernate Functions

Manual Mode Access: Not available. The system will automatically hibernate after a 1 hour period of inactivity.

Method Mode Levels: Top Level.

Options:

- **Indefinite or Fixed Time:** The indefinite option implies that the system will remain in hibernation until some other operation is executed such as changing the flow rate or running a method. The fixed time option is only available in Method Mode. The indefinite time option is only allowed when hibernate is the last action in the Method.
- **Hibernate Time:** When the Fixed Time option is selected, enter the time the system should remain in hibernation in minutes or hours. This option may be useful when changing temperatures during a method in order to allow a stabilization period.

Inject

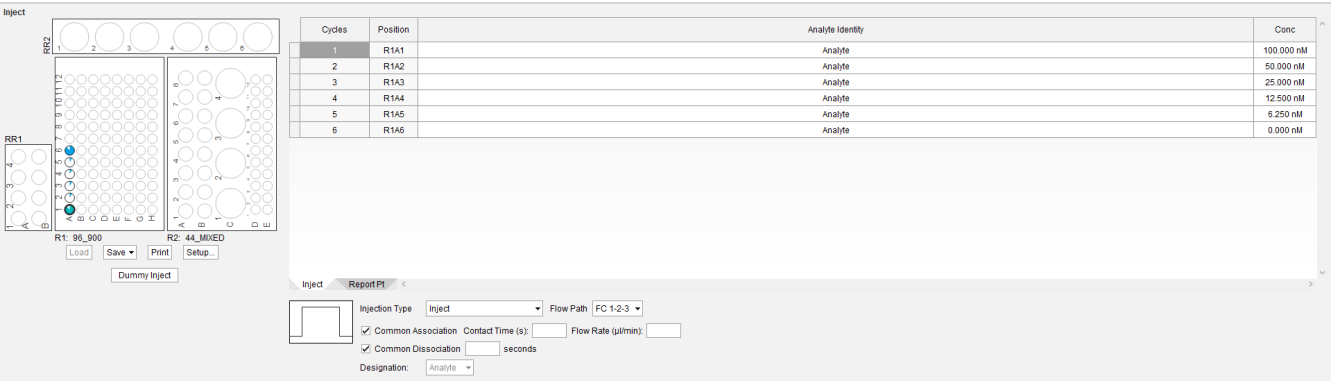

Figure 10-12: Inject Functions

Options:

- **Position Table:** Specify rack positions for the injection by dragging from the rack graphic on the left. The identity, concentration and volume will be filled in automatically if sample information has been entered for that rack position. The specified volume will be injected at the specified flow rate. Dissociation time is the amount of time the system will wait after completing the injection before performing post-injection housekeeping routines. The table may include additional columns depending on the injection type selected.
- **Injection Type:** Choose from the following options:
  - **Inject -** (regular injection): Reproducibly injects sample with low dispersion and fast rise/fall times.
  - **Fast -** Same as a regular injection but eliminates some of the preparative steps to enable a faster sample load while still providing good quality. Dispersion may be somewhat higher in this injection.
  - **Switch -** Similar to regular Inject but the flow path is changed during the injection after a specific volume of sample has been injected. This is useful when a variable contact time is required between different channels, for example, immobilizing different quantities of ligand on different sensing channels. When this injection type is selected, the Position Table will contain a new column for 'Switch Volume' which is the volume that will be injected before the flow path switches to the second position.
  - **Long -** Similar to Inject but a volume larger than the pump syringe may be configured. As such, the syringe pump will need to be reloaded during the injection and there may be a minor disturbance to the response at that time.
  - **Slow -** A slow inject drives the syringe pumps in a non-continuous, pulsed manner in order to achieve very low effective flow rates (0.1 µL/min to 2.5 µL/min). This allows long contact times while consuming a small volume of sample. This injection type is not suitable for kinetic injections.
  - **Parallel -** This type of injection will have slower buffer-to-sample and sample-to-buffer transitions and is not suitable for kinetic injections. Two rows in the Position Table are used to configure this injection. This enables two different samples to be injected simultaneously over different sensing channels and is useful for rapid ligand immobilization. There are only two possible flow path options for this type of injection. Note that the parameters for the second row in the injection pair are automatically matched to the first row; however, different Transfer and Mix parameters may be configured for each row.
  - **OneStep, OneStep(2-comp), OneStep(Pulse) and OneStep(Pulse, 2-comp) -** See “OneStep® Injection” on page 82 for more information.
  - **NeXtStep -** Rapid dispersion injection of two samples. The dispersion profile can be manipulated by specifying the volume ratio of the two samples. See “NeXtStep™ Injection” on page 84 for more information.
  - **Recovery -** Special injection type for eluting sample from the sensor chip surface. The injected sample is passed over the specified flow cell channel(s) and then returned via the sample probe to the Recovery Position. Use the Soak Time parameter to specify time the injected sample should remain in the flow cell before being extracted and returned to the Recovery position. The injection volume is also the volume that will be returned to the Recovery Position. See “Sample Recovery” on page 90 for more information.
- **Flow Path:** Choose the flow cell channels over which sample should be injected.
- **Designation:** In Method Mode this option is available to flag the injection as the Analyte, a Capture injection, or a Regeneration injection. Exactly one injection in an assay must be marked Analyte. More than one injection may be a Capture or Regen injection. Injection Designation is not available in Manual Mode.

Report points

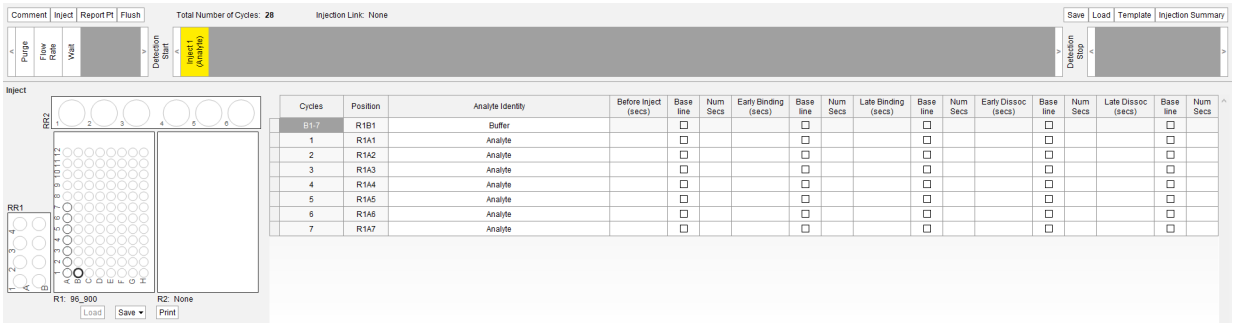

Figure 10-13: Report Point Functions

Options:

- **Before Inject:** Time offset to place the report point relative to before the start of the injection.
- **Baseline:** Indicates that the 'Before' report point sets a baseline for report points that follow it.
- **Num Secs:** Number of seconds of data to be averaged when calculating the report point.

- **Early Binding:** Time offset to place the report point relative to after the start of the injection.
- **Baseline:** Indicates that the 'Early Binding' report point sets a baseline for report points that follow it.
- **Num Secs:** Number of seconds of data to be averaged when calculating the report point.

- **Late Binding:** Time offset to place the report point relative to before the end of the injection.
- **Baseline:** Indicates that the 'Late Binding' report point sets a baseline for report points that follow it.
- **Num Secs:** Number of seconds of data to be averaged when calculating the report point.

- **Early Dissociation:** Time offset to place the report point relative to after the start of the dissociation phase.
- **Baseline:** Indicates that the 'Early Dissociation' report point sets a baseline for report points that follow it.
- **Num Secs:** Number of seconds of data to be averaged when calculating the report point.

- **Late Dissociation:** Time offset to place the report point relative to before the end of the dissociation phase.
- **Baseline:** Indicates that the 'Late Dissociation' report point sets a baseline for report points that follow it.
- **Num Secs:** Number of seconds of data to be averaged when calculating the report point.

Pause inject

During a Manual Mode Injection of type 'Inject' or 'Fast,' the Pause inject control will appear on the Operation page above the event log. During sample loading and pre-inject, the injection may still be aborted in the usual way, but the pause controls are not visible until sample enters the flow cell.

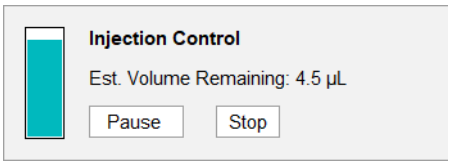

Figure 10-14: Pause Inject

While the injection is in progress, the display will update with the estimated remaining injection volume available. The injection can be stopped at any time, which is essentially the same as aborting the injection. The pause feature halts dispensing of sample to the flow cell, and continuous flow buffer is routed over all three sensing channels. The injection can then be resumed at any time with minimal delay. The pause control will disappear when either the injection is stopped or the entire sample has been injected.

Mix

Figure 10-15: Mix Functions

Manual Mode Access: Operation page when not recording data.

Method Level: All levels except Assay Recording.

Options:

- **Position Table:** Specify rack positions to be mixed by dragging from the rack graphic on the left.
- **Mix Count:** Number of times to repeat the mix operation at the specified rack position.

Normalize

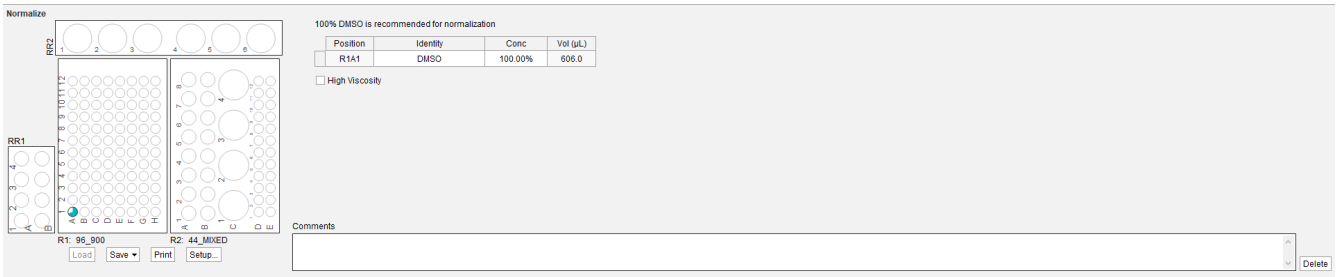

Figure 10-16: Normalize Functions

Manual Mode Access: Instrument Setup page when not recording data.

Method Level: Top Level.

Options:

- **Position:** Select a rack position by dragging from the rack graphic on the left.
- **High Viscosity:** Checking this box will cause the normalization solution to be loaded and injected at a slower rate. Useful when normalizing with viscous solutions such as glycerol.

Pause

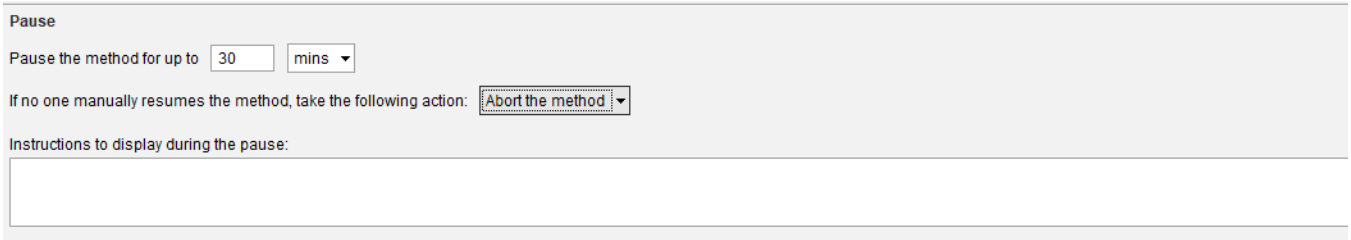

Figure 10-17: Pause Functions

Manual Mode Access: Not available

Method Level: Top Level

Options:

- **Pause Time:** Specify the time in minutes or hours that the method should pause.
- **Action:** The pause function normally requires interaction from the user to resume the method. If the method has not been resumed before the Pause Time has elapsed, choose to either abort the method or continue the method automatically.

- **Instructions:** The text entered here will be displayed as a prompt to the user when the pause step is encountered. If the software has been configured to send notification emails, the text will appear in the email as well.

Wash Function Comparison

The Octet® SF3 system includes several different wash functions to accommodate a variety of assay designs. Table 10-1 explains the differences between these functions.

Table 10-1: Octet® SF3 System Wash Functions

Function	Description	Run Time and Buffer Consumption	When to Use
Prime	Dispenses a large volume of buffer through all possible flow paths in the system, including sample loops, autosampler probe, flow cell, and all waste ports.	Longest Run Time (>3 minutes) Highest Buffer Consumption (>5 mL)	Use a 3x Prime when changing buffer solutions. 1x Prime recommended before running an assay.
Purge	Dispenses a moderate volume of buffer through all possible flow paths in the system, including sample loops, autosampler probe, flow cell, and all waste ports. Similar to a Prime, but faster and uses less buffer.	Medium Run Time (~1 minute) Moderate Buffer Consumption (2-3 mL)	1x Purge recommended at the start of each assay cycle, especially when sample carryover is a concern.
Purge Probe	Dispenses buffer through the selected sample loop and exiting via autosampler probe. Flow does not enter the flow cell or pass through the waste ports.	Fast Run Time (<15 seconds) Low to Moderate Buffer Consumption (500 µL)	Extra washout of the sample loop when a full Purge is not needed. Often used together with a Flush step.
Flush	Dispenses a small volume of buffer through the sample injection lines into the flow cell and exiting through one waste port. Counterflow of buffer from the other pump prevents any remaining sample from contacting the sensing surfaces.	Fast Run Time (<15 seconds) Very Low Buffer Consumption (25 µL)	Extra washout of the sample injection lines when a full Purge is not needed. Often used together with a Purge Probe.

Table 10-1: Octet® SF3 System Wash Functions

Function	Description	Run Time and Buffer Consumption	When to Use
Clean	Loads a cleaning solution from the sample racks and passes it through the system in a similar fashion to a regular Injection. Includes additional options not available with Injections, such as a soak time for holding the cleaning solution in the sample loop, and flow paths such that the cleaning solution is injected to the flow cell but prevented from contacting the sensing surfaces.	Configurable Run Time Moderate Buffer Consumption	For routine maintenance and cleaning of the system. Also useful in assays with compounds prone to sticking to tubing surfaces, where a buffer wash would be ineffective.

Prime

Figure 10-18: Prime Functions

Manual Mode Access: Instrument Setup page when not recording data.

Method Level: All Levels except Assay Recording.

Options:

- **Number of Repetitions:** Number of times to repeat the Prime function.

Purge

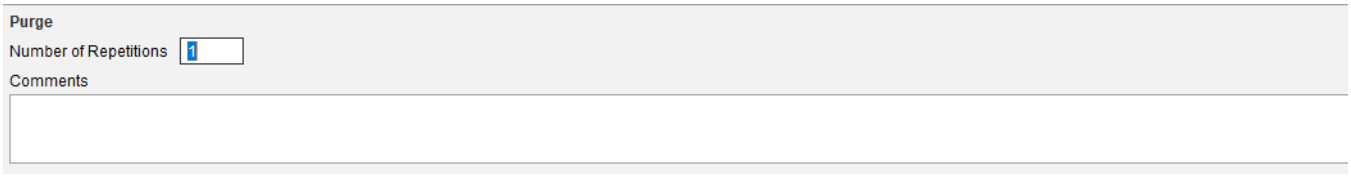

Figure 10-19: Purge Functions

Manual Mode Access: Operation page at any time.

Method Level: All Levels

Options:

- **Number of Repetitions:** Number of times to repeat the Purge function.

Purge Probe

Figure 10-20: Purge Probe Functions

Manual Mode Access: Operation page at any time.

Method Level: All Levels.

Options:

- **Loop(s) to Purge:** Select either or both of the sample loops for purging. Note that when both loops are selected and the system is recording data, the loops will be purged sequentially. If the system is not recording data, both loops will be purged simultaneously.
- **Number of Repetitions:** Number of times to repeat the Purge Probe function. Each repetition purges the selected loop(s) with one full syringe of buffer.

Initial Setup

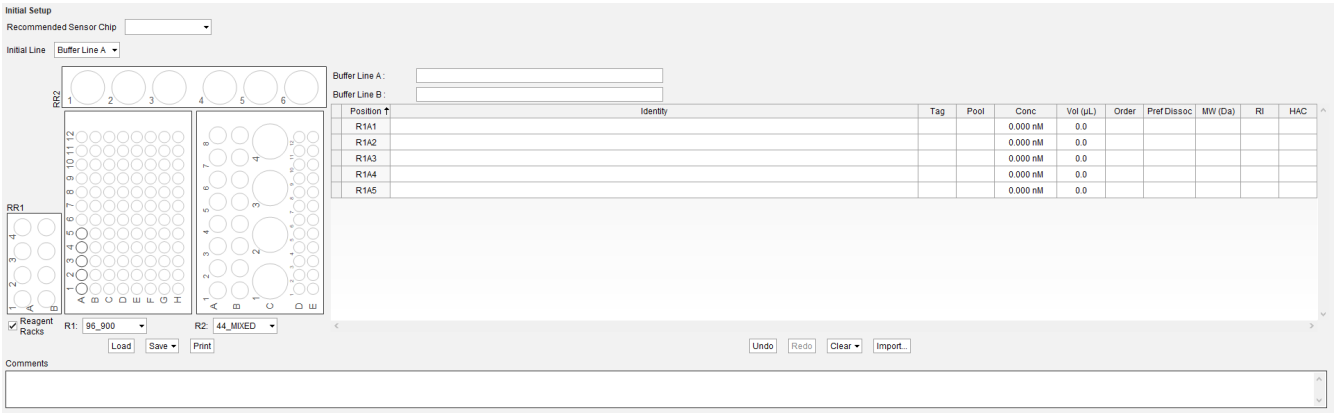

Figure 10-21: Rack Setup Functions

Manual Mode Access: Operation page, Racks tab.

Method Level: Top Level. Always part of a method. Cannot be deleted.

Options:

- **Recommended Sensor Chip:** This field is used for reference only as a memory aid to what the recommended sensor chip is for the current method.
- **Initial Line:** This allows the user to configure which buffer line the method will initiate from. This allows the user to start a method in one buffer and switch to another buffer or the water line as desired during running the method.
- **Buffer Line A and Buffer Line B:** These fields are mainly used for reference, especially if the method is to be used with a specific buffer. Also, when transferring buffer to an empty rack position, this buffer name is used as the sample identity.
- **Position Table:** To configure rack positions, drag from the rack graphic on the left. To select an entire column of data, click on the heading for that column. A range of grid cells may be selected by holding down the **Shift** key while making a selection. If multiple cells are selected and a new value is entered, that value will be automatically copied into all the selected cells. To delete a specific row, click on the box just to the left of the row. The row will be highlighted. Press the **Delete** key to remove this row.
- **Identity:** Name of the sample in the rack position.
- **Tag (Show Advanced Options must be checked):** Useful for differentiating samples which have the same identity, for example a dilution set. If the first character in the tag is the letter D, then the position will be marked in light blue on the rack.
- **Pool (Show Advanced Options must be checked):** Enter a number between 1 and 20 to identify rack positions which contain the same sample. When the identity or concentration of a position that belongs to a pool is changed, all positions that belong to the pool are updated. Pools can be used as an inject or transfer "from" position to simplify sample management when even a single large vial does not have enough volume to handle all of the desired operations.

- **Concentration:** Concentrations can be entered with one of four concentration units: molar, weight per volume (mg/mL), percentage, and parts-per-million. Molar concentrations can be entered by using standard unit prefixes. For example, entering **100 m** will be saved as 100.00 mM. To enter micro-molar concentrations, type the letter **u**, and for molar concentrations, use a capital **M**. To enter a concentration in weight per volume, use a standard prefix with a **g** (for grams) at the end. For example, entering **2 mg** will be saved as 2.000 mg/mL, and entering **5** ug will be saved as 5.000 µg/ mL. To enter a percent concentration, simply append a % character to the concentration. To enter a concentration in ppm or ppb, simply append the characters 'ppm' or 'ppb.' Note that Octet® SPR Analysis software requires concentrations to be in molar units for analysis. Please enter a molecular weight for non-molar concentrations so that an automatic conversion can be performed when exporting to Octet® SPR Analysis software. To input a dilution series, start by entering the first concentration. Select the applicable cells in the concentration column, and then right-click on the first concentration. The pop-up menu has options for auto-filling in serial doubling, serial tripling, decimal dilutions, as well as copying the first value into all of the selected cells.
- **Volume:** Enter the volume of sample available in the rack position. The volume will be automatically limited to the maximum volume of the rack position.
- **Order (Show Advanced Options must be checked):** Enter a number here to indicate a specific sample run order to be used when configuring an injection.
- **MW:** Enter a molecular weight in Daltons for this sample. Molecular weight must be entered for samples that will be analyzed with OneStep® injections.
- **Pref Dissoc (Show Advanced Options must be checked):** Enter a preferred dissociation time in seconds for this sample. When this rack position is added to an Inject tool, the dissociation time will be pre-populated with this value for convenience.
- **Clear:** Removes entries from the Position Table, clearing the rack contents. Positions can be cleared on a per-rack basis, by selection, or all positions.
- **Save:** Store the rack setup as a *.rack file for use in other methods, or save as a *.pdf file for archiving or printing later.
- **Print:** Print an image of the sample racks and the sample information in table form. Useful for manually preparing sample racks in the lab.
- **Load:** Load a previously saved rack setup in order to minimize data entry. Tip: Rack setups can also be loaded from method files by changing the file type from *.rack to *.protocol in the load rack dialog.

Report Point

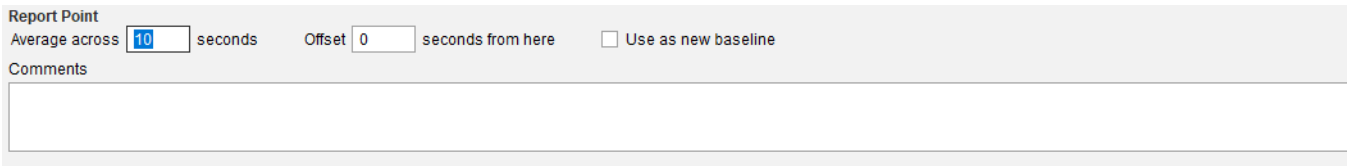

Figure 10-22: Report Point Functions

Manual Mode Access: Graph right-click menu in Operation and View pages.

Method Level: Assay Recording Level.

Options:

- **Number of seconds:** Number of seconds of data to be averaged when calculating the report point.
- **Offset:** The report point may be placed at an offset relative to its position in the assay. For example, if a Wait tool follows the Report Point, the offset may be used to place the report point within the Wait time instead of just before the Wait begins.
- **Use as new baseline:** Indicates that this Report Point sets a baseline for report points that follow it.

Switch Line

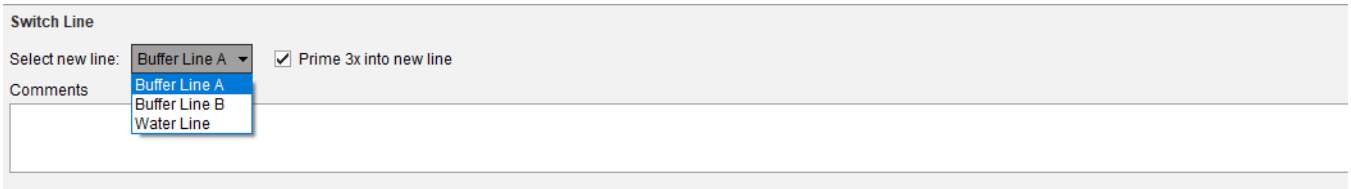

Figure 10-23: Switch Line

Manual Mode Access: Not available. The system will automatically switch lines to the water line after a 72-hour period of inactivity.

Method Mode Levels: Top Level.

Options:

- **Select new line:** Allows the user to switch to/from the current line to either of the other available lines, including Buffer Line A, Buffer Line B and Water Line.
- **Prime into new line:** Upon switching lines, the system may also be primed into the new line three times. It is recommended that this feature remains checked to ensure optimal line switching and system hydration in the new line.

Temperature Control

Figure 10-24: Temperature Control Functions

Manual Mode Access: Instrument Setup page.

Method Level: Top Level.

Options:

- **Temperature Controller:** Choose **Analysis Temperature** to set the temperature for Octet® SPR Discovery software. Choose **Sample Rack Temperature** to set the temperature of the sample racks.
- **Wait time:** Specify a wait time after changing the temperature setpoint before continuing with the next step in the method. Large changes in temperature require longer wait times for stabilization.

Transfer

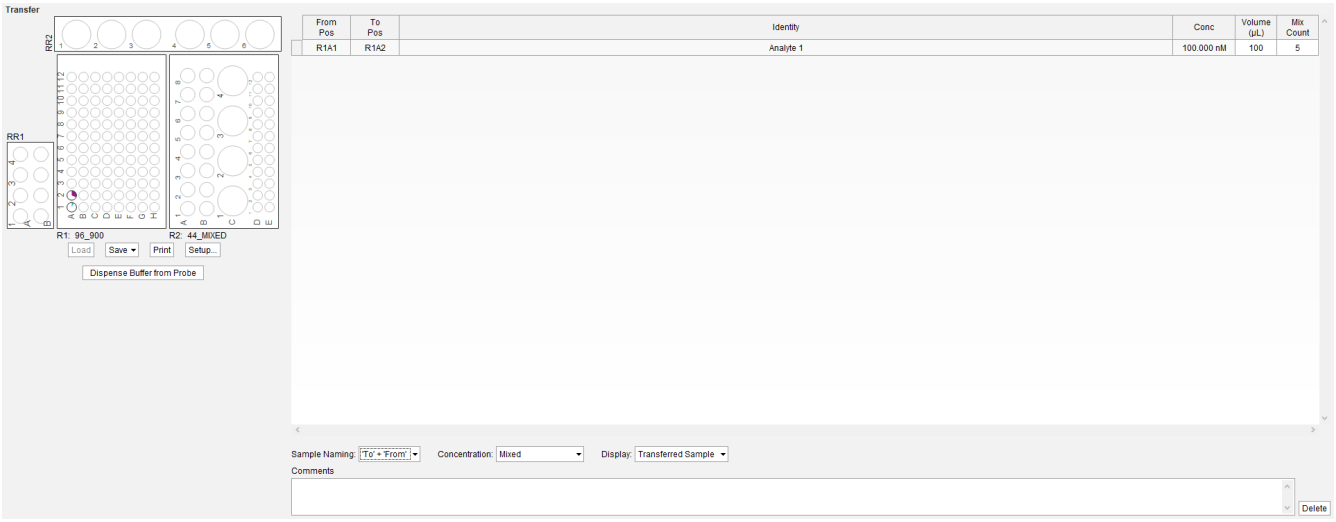

Figure 10-25: Transfer Functions

Manual Mode Access: Operation page when not recording.

Method Level: All Levels except Assay Recording.

Options:

- **Position Table:** Specify rack positions to be mixed by dragging from the rack graphic on the left.

NOTE: *Running buffer may be dispensed to a rack position by dragging from the* ***Dispense Buffer from Probe*** *button located below the rack graphic.*

- **Volume:** Volume to transfer between positions.
- **Mix Count:** Number of times to repeat the mix operation at the 'To' rack position. When transferring to an empty rack position, this value may be left blank (or zero). The mix count is appropriate for transfers that cause a mixing of two solutions.
- **Sample Naming:** Choose the resulting sample Identity for the 'To' position after the transfer is complete. Options are 'To' + 'From', 'To' only, and 'From' only.

- **Concentration:** Choose the resulting sample concentration for the 'To' position after the Transfer is complete. Options are a hybrid “Mixed” concentration, use the original concentration in the To position unchanged, use the original concentration in the ‘To’ position but dilute by the volume being transferred in, use the original concentration in the ‘From’ position unchanged, or use the original concentration in the ‘From’ position, but dilute by the volume originally in the ‘To’ position.
- **Display option:** For convenience when setting up a transfer, optionally change the displayed sample identity and concentration between “Transferred Sample” - which displays the Identity and Concentration of the ‘From’ position, or “Result of Transfer” - which displays the final Identity and Concentration in the ‘To’ position at the conclusion of the transfer.

Wait

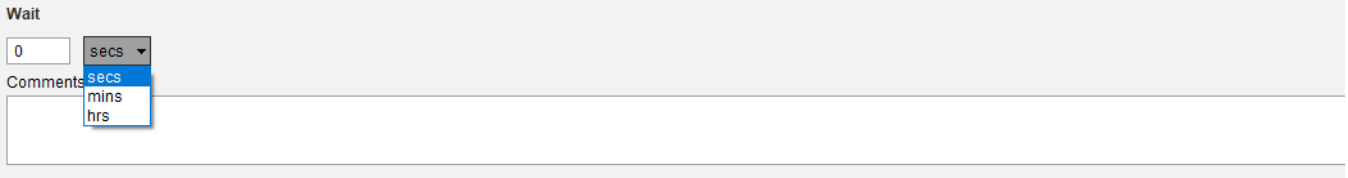

Figure 10-26: Wait Functions

Manual Mode Access: Operation page at any time.

Method Level: All Levels.

Options:

- **Wait time:** Enter a delay in hours, minutes or seconds. This function is useful in assays to allow the system to stabilize after a Purge before recording starts, or to establish a baseline while recording before performing the first injection of the cycle. Also useful for allowing the system to stabilize when the temperature is changed.

Chapter 11:

Evaluation of Response Curves using Octet® SPR Analysis Software

Introduction

The high-quality data recorded on the Octet® SF3 system is easily analyzed using Octet® SPR Analysis software. Its user-friendly interface guides you through a series of transformations that clean up data in seconds. In addition, Octet® SPR Analysis software enables fitting of interactions models to the data set for estimation of kinetic and affinity constants along with simple concentration analysis. Kinetic fitting models include the pseudo first-order binding model and the pseudo first-order binding model with mass transport (*i.e.* two-compartment model). Refer to the Octet® SPR Analysis Software User Guide for complete user instructions.

Please review the Octet® SPR Analysis software help file and software tutorials for detailed descriptions of data analysis using Octet SPR Analysis software.

Export Options

Several options are available when exporting data to Octet® SPR Analysis software. Some options are only available for assays with a specific analyte injection type.

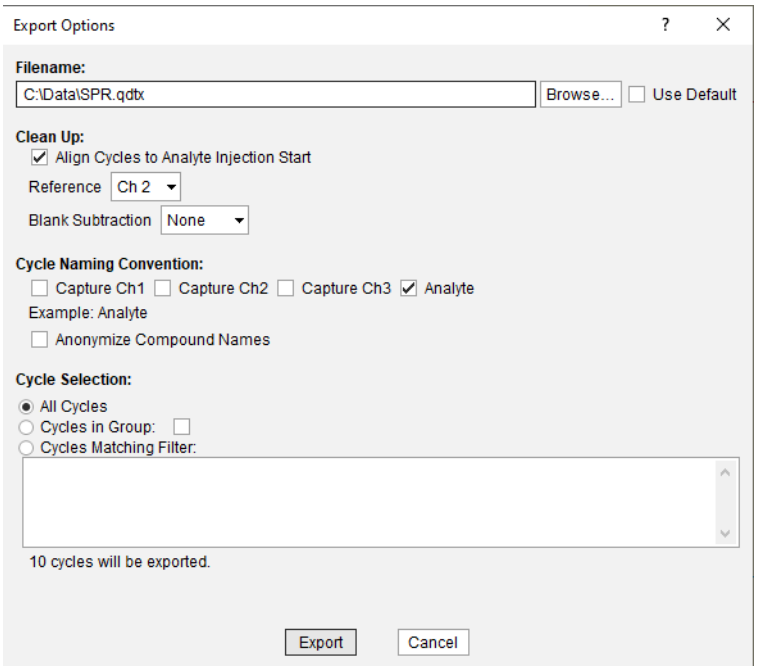

Figure 11-1: Export Options

Export options:

- **Filename -** By default, the software will place the exported .qdtx file in the same folder as the .spr file, and append a designator for the assay number being exported.
- **Align Cycles -** Shifts each cycle individually so that the Analyte Injection start time is zero for all cycles. This enables better reference curve subtraction.
- **Downsample -** Reduces the sample rate of the data set, if it was originally recorded at a rate greater than 1 Hz.

- **Reference -** Select the channel to use for reference curve subtraction.
- **Cycle Naming Convention -** Provides a way to export a combined name for the cycle analyte. Typically the name from the Analyte injection should be used. However, for antibody capture assays it may be more informative to name each cycle according to the name in the Capture injection.
- **Anonymize Compound Names -** Replace all compound names with a number. When enabled, the export will also create a text file with a mapping of anonymized number back to compound name.
- **Cycle Selection -** Choose **Cycles in Group** to export only the cycles in a specific cycle group (group color). Choose **Cycles Matching Filter** to selectively export cycles based on a text filter of the analyte name. Blank and Control cycles will always be exported. Enter multiple analyte names one per line, or separate with a comma. By default, the filter will attempt to match the beginning of the name entered. For example, entering only "furo" (without the quotes) will match the analyte name Furosemide. Begin a search term with "+" (plus sign) to match anywhere within the analyte name. For example, "+ose" will still match Furosemide. Begin a search term with "*" (asterisk) to match at the end of the analyte name. For example, "*mide" will match Furosemide. Finally, use "=" to require an exact match of the analyte name. For example, "=Furosemide". Analyte name filtering is case insensitive.
- **Data Corrections -** These options are available for OneStep® injections only. Enables the application of microcal corrections for improved data analysis.
- **Break into one cycle per injection -** Available only for manual mode curves with multiple injections. Breaks the single manual cycle into discrete cycles where each cycle contains one injection. Useful for subtracting blank injections when using manual mode.

Chapter 12:

Maintenance

System Maintenance

Regular maintenance of the Octet® SF3 system is critical to ensure consistent and reliable results. Day to day usage of the system can cause adsorption of material to the microfluidic tubing and potential microbial growth within the system. These can lead to blockage issues and potentially further larger issues and therefore, both user-maintenance and Sartorius-supplied preventative maintenance should be performed regularly. Sartorius Technical Support are able to discuss the service frequency most suitable to your situation.

Predefined maintenance programs can be found in the Octet® SPR Discovery software under **Instrument Setup: Maintenance**.

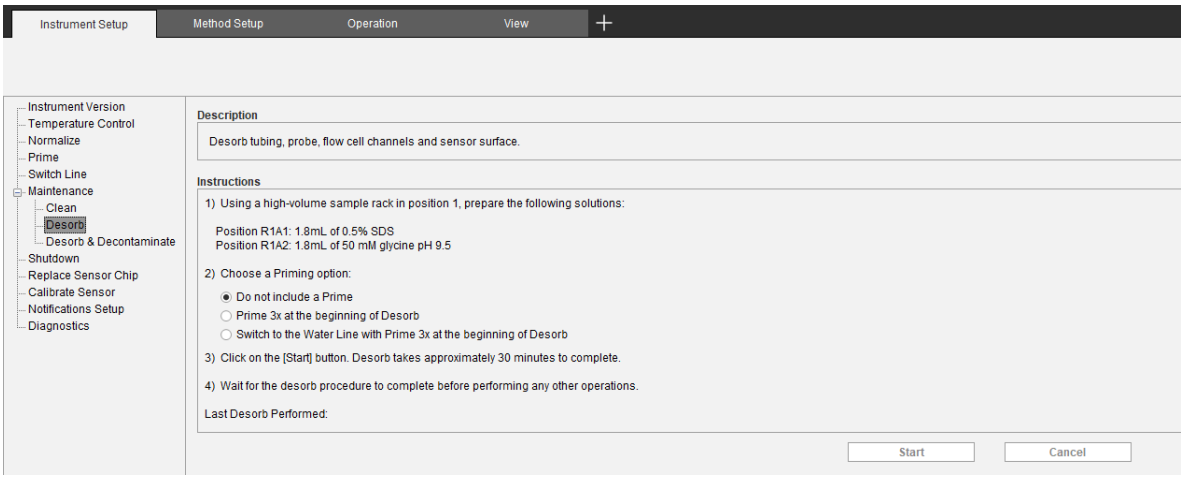

Figure 12-1: Instrument Setup Maintenance Screen

A maintenance kit containing all basic reagents and a designated maintenance sensor chip is available from Sartorius (Part Number: 19-0137) and it is recommended to use this kit in order to ensure optimum performance of the system.

Ensure that the maintenance chip is used when running the methods Desorb and Desorb and Decontaminate. The maintenance sensor chip surface should be inspected prior to use and if necessary, any contaminants removed using deionized water and dried by using oil-free compressed air or carefully blotting the corner of the sensor chip surface with a lint-free wipe.

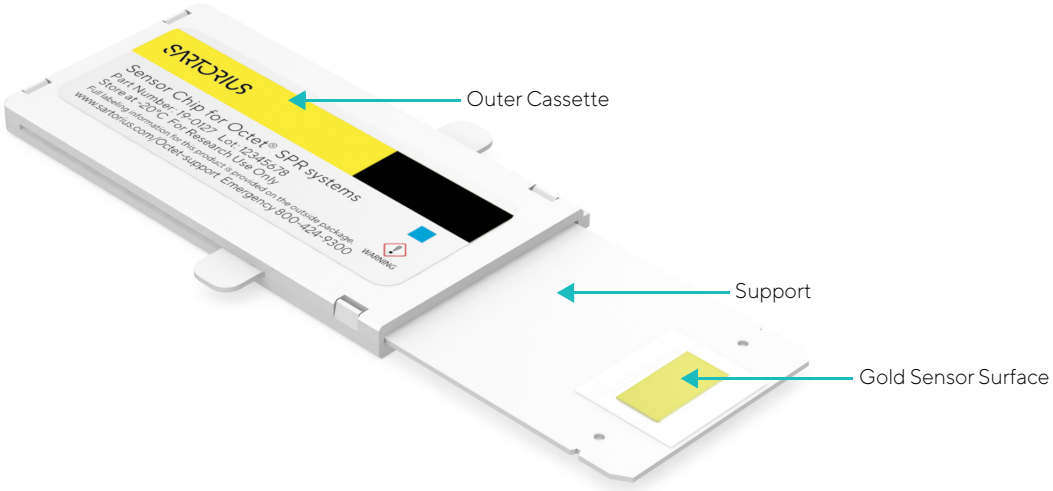

Figure 12-2: Octet® SPR Sensor Chip and Protective Cassette

IMPORTANT: *In order to prevent potential poor sensor chip docking and leakage, the plastic support of Octet® Sensor Chips must be completely dry before docking. The plastic support should be dried by using oil-free compressed air or carefully blotting the surface of the sensor chip support.*

System Care

General

It is recommended that, when idle, the system should only be running in deionized water as described in "Water Line" on page 26. It is best practice to switch to the water line at the end of a method, but where this is not possible, the Octet® SF3 system will enter hibernation mode after 72 hours and will use the water line to hydrate the system. Buffer solutions should be used only during experiments.

Be sure to refill deionized water or buffer solution at least every third day while system is on to prevent possible drying out of the micro fluidics.

If the system will be left idle for more than two weeks, it is recommended that all three buffer lines are placed into deionized water.

Do not turn off the system without following the Shutdown procedure.

All buffer solutions used in the system should be 0.2 µm filtered. (Alternatively, small volume samples can be centrifuged and supernatant recovered for injection). It is recommended that the Desorb method is performed after the assay in order to minimize any potential blockage issues.

Clean up any buffer spills quickly to prevent buffer salts from corroding system parts.

If the system is used with molecules that are 'sticky' such as serum or some small molecules it is recommended that the system is thoroughly cleaned post run using the desorb and decontaminate program.

At least one Preventative Maintenance (PM) service per year is recommended to keep the system running smoothly. Contact Sartorius Technical Support to inquire about PM service and service contracts. It is also recommended that the buffer filters are replaced every 6 months to maintain optimal performance (Part Number: 19-0109).

After a method

As described in "Water Line" on page 26, the system will automatically switch to the designated maintenance buffer after a period of 72 hours. Therefore, it is possible to leave the used sensor chip docked in the system for further use or remove it for storage. If the sensor chip is removed, it is advised that the maintenance sensor chip is docked and the system primed into deionized water 3 times.

The system can remain idle (hibernating) in deionized water for up to two weeks. When hibernating, periodically check that the source bottle has plenty of liquid as the system will continue to prime. If the downtime is anticipated to exceed 2 weeks, it is recommended that all three buffer lines are placed into deionized water.

Re-use of sensor chips

Sensor chips can tolerate up to 3 dockings before housing components can begin to degrade and potentially cause harm to the system fluidics.

Only perform cleaning routines with a Maintenance Sensor Chip. It is not recommended that a previously used sensor chip is used for maintenance purposes. A designated maintenance sensor chip is available from Sartorius (Part Number: 19-0136) and should be used for maintenance and cleaning procedures.

Before re-docking a sensor chip, gently rinse the gold surface for several seconds with clean deionized water from a squirt bottle. Shake off excess deionized water into waste and then dry the surface with oil-free compressed air or carefully blotting the corner of the sensor chip surface with a lint-free wipe.

IMPORTANT: *In order to prevent potential poor sensor chip docking and leakage, the plastic support of Octet® Sensor Chips must be completely dry before docking. The plastic support should be dried by using oil-free compressed air or carefully blotting the surface of the sensor chip support.*

Cleaning and Maintenance Schedule

It is important to not ignore the maintenance schedule reminders found in the information bar at the bottom of the screen. Hover the mouse over the maintenance symbol to see further information:

Table 12-1: Routine Cleaning and Maintenance Schedule

Symbol	Meaning
	Maintenance is up to date
	Maintenance is due soon
	Maintenance is overdue

IMPORTANT: *Clean up any buffer spills quickly to prevent buffer salts from corroding the system.*

Routine cleaning and maintenance should be performed using the following schedule:

Table 12-2: Routine Cleaning and Maintenance Schedule

Time	Requirements	Section
Daily	▪ Prepare and degas new buffers and/or deionized water for use if required ▪ Check that the waste container does not require emptying. Empty if required. ▪ If required, prime the system with the new buffers and deionized water by choosing **Instrument Setup: Prime**	N/A
Weekly	▪ Remove the pump cover and visually inspect all tubing, fittings, pumps and syringes to check for leaks and salt deposits. Any leaks at the tubing and fittings should be cleaned with deionized water. ▪ Run the desorb program by choosing **Instrument Setup: Maintenance: Desorb**	"Desorb" on page 128
Monthly	▪ Remove the pump cover and visually inspect all tubing, fittings, pumps and syringes to check for leaks and salt deposits. Any leaks at the tubing and fittings should be cleaned with deionized water. ▪ Run the desorb & decontaminate program by choosing **Instrument Setup: Maintenance: Desorb & Decontaminate**	"Desorb & Decontaminate" on page 129

The following user-controlled programs are also available for maintenance:

Table 12-3: Maintenance Programs

Program	Description	Section
Clean	Loads a user-defined cleaning solution from the sample racks and passes it through the system in a similar fashion to a regular Injection. Includes additional options not available with Injections, such as a soak time for holding the cleaning solution in the sample loop, and flow paths such that the cleaning solution is injected to the flow cell but prevented from contacting the sensing surfaces.	"Clean" on page 130
Decontaminate	Loads a sodium hypochlorite cleaning solution from the sample racks and passes it through the system in a similar fashion to a regular Injection. Contacts tubing, probe, flow cell channels and sensor chip surface.	N/A

Desorb

It is recommended to run the desorb maintenance program at least once a week to remove any adsorbed proteins or other materials from the system.

Reagents required (Octet® SPR maintenance kit):

Table 12-4: Octet® SPR Maintenance Kit Components

Item	Description
Desorb 1	0.5% (w/v) sodium dodecyl sulfate (SDS) - stored at room temperature
Desorb 2	50 mM glycine pH 9.5 - stored at 2 - 8 °C
Maintenance Chip	Glass surface sensor chip

Additional materials required:

- Distilled and 0.2 µm filtered deionized water
- Lint-free wipes
- 70% (v/v) ethanol in deionized water

Exterior of the machine

Use 70% ethanol to clean the external surfaces of the system, the rack area, and the probe and wash station. An ammonia-free cleaner should be used to clean the glass surfaces. Clean and dry these areas with a lint-free cloth. Ensure that no fluid remains after cleaning and drying.

Visually inspect all exterior tubing, fittings, pumps and syringes to check for leaks and salt deposits. Any leaks at the tubing and fittings should be cleaned with deionized water. Leakage at the pumps and syringes should be reported to Sartorius Technical Support.

Desorb

Run the desorb program by choosing **Instrument Setup: Maintenance: Desorb**. Place all tubes into deionized water prior to running desorb and ensure a maintenance sensor chip is docked.

NOTES:
Set rack and analysis temperature to 25 °C prior to performing desorb. This is due to the precipitation of Desorb Solution 1 at temperatures below 20 °C.

It is recommended that the system is primed into new running buffer at least 3 times prior to performing any assays.

Desorb & Decontaminate

It is recommended to run the desorb & decontaminate maintenance program at least once every 4 weeks to remove any adsorbed proteins, other materials and prevent microbial growth in the system.

Reagents required (Octet® SPR maintenance kit):

Table 12-5: Octet® SPR Maintenance Kit Components

Item	Description
Desorb 1	0.5% (w/v) sodium dodecyl sulfate (SDS) - stored at room temperature
Desorb 2	50 mM glycine pH 9.5 - stored at 2 - 8 °C
Maintenance Chip	Glass surface sensor chip

Additional materials required:

- Distilled and 0.2 µm filtered deionized water
- Lint-free wipes
- Sodium Hypochlorite (10 - 15%)
- 70% (v/v) ethanol in deionized water
- 50% DMSO

Exterior of the machine

Use 70% ethanol to clean the external surfaces of the system, the rack area, and the probe and wash station. An ammonia-free cleaner should be used to clean the glass surfaces. Clean and dry these areas with a lint-free cloth. Ensure that no fluid remains after cleaning and drying.

Visually inspect all exterior tubing, fittings, pumps and syringes to check for leaks and salt deposits. Any leaks at the tubing and fittings should be cleaned with deionized water. Leakage at the pumps and syringes should be reported to Sartorius Technical Support.

Desorb & Decontaminate

Run the desorb program by choosing **Instrument Setup: Maintenance: Desorb & Decontaminate**. Place all tubes into deionized water prior to running desorb and ensure a maintenance sensor chip is docked.

NOTES:
Set rack and analysis temperature to 25 °C prior to performing desorb. This is due to the precipitation of Desorb Solution 1 at temperatures below 20 °C.

It is recommended that the system is primed into deionized water at least 3 times prior to performing desorb and decontaminate.

Just prior to use, prepare a 0.7 - 1% sodium hypochlorite and 50% DMSO solution as follows:

Table 12-6: Sodium Hypochlorite Solution Prep

Item	Volume (mL)
Sodium Hypochlorite (10 - 15%)	0.2
Distilled and 0.2 µm filtered deionized water	2.8

Table 12-7: DMSO Solution Prep

Item	Volume (mL)
99% DMSO	1.5
Distilled and 0.2 µm filtered deionized water	1.5

NOTE: *It is recommended that the system is primed into new running buffer at least 3 times prior to performing any assays and allowed to equilibrate for at least 1 hour.*

Clean

It may be necessary to add a third clean cycle using additional cleaning solution such as 0.1 - 0.5 M NaOH or DMSO to remove specific contaminants.

Run the clean program by choosing **Instrument Setup: Clean**. Place all tubes into deionized water prior to running clean and ensure a maintenance sensor chip is docked.

Position: Specify a rack position from which to load the cleaning solution by dragging from the rack graphic on the left. The identity and volume will be filled in automatically if sample information has been entered for that rack position.

Clean Volume: Volume of cleaning solution to load into the system. This option is fixed by the system if the Flow Path is set to 'All.'

Flow Rate: Rate at which the cleaning solution will be injected into the flow cell. This option is fixed by the system if the Flow Path is set to 'All' or the Probe Only option has been selected.

Flow Path to Clean: Select from different flow paths over which to inject the cleaning solution. The 'All' option will cause the system to use a predefined cleaning routine that loads the cleaning solution simultaneously into both sample loops and then injects the cleaning solution through all possible flow paths. Probe Only: When checked, this option will load the cleaning solution into the sample loop corresponding to the selected path, but instead of dispensing through the flow cell, cleaning solution will be dispensed out the probe into the waste port.

Soak Time: Number of seconds to allow the cleaning solution to soak in the sample loop(s).

Re-use of maintenance sensor chip

When the maintenance sensor chip is not in use it should be stored separately in an airtight container. The maintenance sensor chip surface should be inspected prior to use and if necessary, any contaminants removed using deionized water and dried by using oil-free compressed air or carefully blotting the corner of the sensor chip surface with a lint-free wipe.

NOTE: *Only perform cleaning routines with a maintenance sensor chip.*

Sample Rack Seal Replacement and Cleaning

With the system idle, open the sample chamber door and remove the sample rack(s). To remove the rack seal, gently grasp a corner of the seal and lift up slowly until the seal lifts entirely away from the rack. Set the rack aside.

CAUTION: *Gloves and goggles should be worn when cleaning the sample rack seals.*

1. To clean the rack seal, hold it in a gloved hand with the bottom (unnumbered) side facing up. Using a spray bottle, coat the entire seal in a 0.7 - 1% Sodium Hypochlorite solution and allow it to soak for ten seconds.
2. Using another spray bottle, rinse the seal thoroughly with deionized water.
3. Finally, spray the seal with 70% ethanol and allow it to dry.

IMPORTANT: *The sample rack seal must sit flat across the sample rack. Failure to properly install the sample rack seal can cause the autosampler to function incorrectly.*

4. When the rack seal has dried, reinstall it on the sample rack by first setting it lightly over the rack. Make sure the number and letter designations line up. Grip the sample rack from the top with the fingers around the side so that the thumbs are directly above the seal as shown in Figure 12-3. Press each row with the thumbs firmly until it is in place. Once all rows have been sealed, view the sample rack from the side to ensure the seal is flat across the rack.
5. Reinstall the sample rack into the sample chamber and close the doors.

Figure 12-3: How to Grip the Sample Rack

Waste Container Removal and Cleaning

CAUTION: *Gloves should be worn when working with the waste container.*

1. To remove and empty the waste container, begin by setting the flow rate to **0 µL/min** from the Operation page of the software. Locate the waste tube coming from the sample chamber and connecting to the waste container (Figure 12-4).

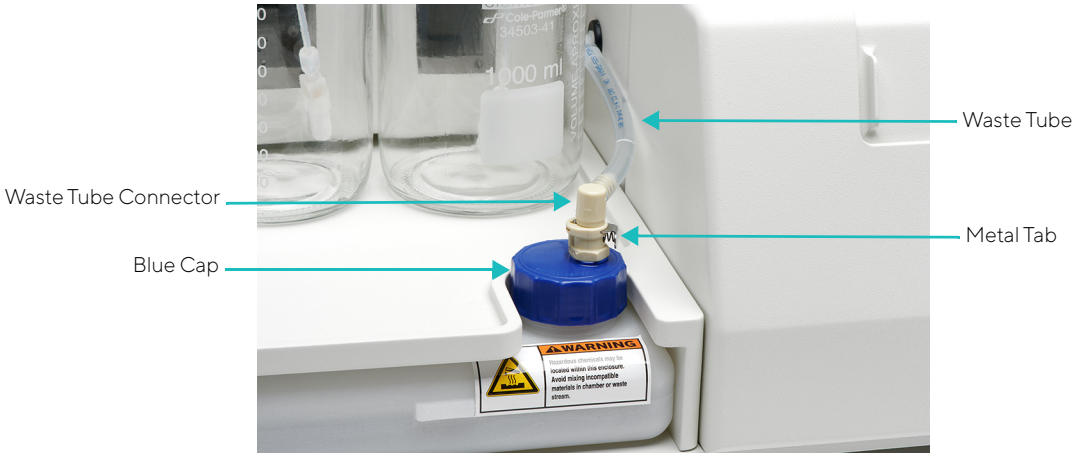

Figure 12-4: Waste Tube and Waste Container

2. Gently tap on the waste tube multiple times to ensure all fluid in the tube moves into the waste container. With one hand, disengage the tube lock by pressing on the metal tab. With the other hand gently pull the connector loose from the waste container (Figure 12-5).

Figure 12-5: Removing Waste Tube Connector

CAUTION: *The waste container is vented. Use caution when moving the waste container as waste fluid may splash out.*

Although the waste container holds a large volume, it must be emptied regularly. If the waste container is not regularly emptied, waste can spill out of the vent and onto the outside of the container and the bench top.

3. Slide the waste container out of the system by gently and firmly pulling it towards the front. Take the waste container to an appropriate waste disposal area. Unscrew the cap on the waste container. Dump the waste into an appropriate container.

CAUTION: *If particularly dangerous or infectious materials have been used with the system, the waste container should be rinsed with a bleach solution before being reinstalled into the system.*

4. Screw the cap back onto it. Slide the waste container back into position on the system underneath the buffer bottle. Reconnect the waste tube by pressing on the metal tab on top of the waste container with one hand while gently pushing the waste tube connector into place with the other hand.

IMPORTANT: *Use the control software to restore the previous idle flow rate.*

The waste container is now properly reinstalled and the system is ready to resume operations.

Decontamination Procedure

For basic decontamination of the sample loops and flow cell, use the decontaminate function in the system software (refer to the Instrument Setup tab).

For a more thorough decontamination of all fluidic lines, empty and clean the waste container as directed in the previous section.

1. Change the buffer contents to a 0.7%–1.0% Sodium Hypochlorite solution.
2. Use the software to perform the Prime function 10 times.
3. Change buffer to deionized water and use software to perform the Prime function 5 times.

4. Empty and clean the waste container and reinstall in the system.

CAUTION: *It is the user's responsibility not to use decontamination and cleaning agents that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained within.*

Hibernate - Short Term

It is highly recommended that the system is left to hibernate in 0.2 µm filtered deionized water when not in use. The Hibernate routine ensures that all flow paths are periodically activated to prevent precipitation of buffer salts. Hibernate is a suitable state for up to 2 weeks.

IMPORTANT: *Make sure there is enough liquid in the buffer bottle to support hibernation for the desired time period.*

Shutdown - Long Term

It is highly recommended that the system is left to hibernate in deionized water using the water line when not in use. The Hibernate routine ensures that all flow paths are periodically activated to prevent precipitation of buffer salts. Hibernate is a suitable state for up to 2 weeks. If the downtime is anticipated to exceed 2 weeks, replace the water bottle content with new deionized water and empty the waster container. It is also important to perform the Desorb and Desorb & Decontaminate maintenance schedules to ensure optimal performance.

Where system shutdown is required, follow the instructions in the Octet® SPR Discovery software **Instrument Setup: Shutdown**.

IMPORTANT: *Failure to follow the proper shutdown procedure may result in blockages that can cause the system to perform poorly or fail.*

Appendix A:

Troubleshooting

Troubleshooting

Symptom: Sudden system malfunction

Possible Cause	Remedy
Electronics failure	Contact support.
Software bug	Restart software and/or system. Contact support.

Symptom: Spikes in response curve

Possible Cause	Remedy
Buffer not degassed	Check degasser operation. Contact support.
Out of buffer	Refill buffer bottle.
Air leak into the system	Check fittings from buffer bottle to degasser and pumps. Contact support.
Electronics failure	Contact support.
Loose cable	Contact support.

Symptom: Spikes during injection

Possible Cause	Remedy
Not enough sample available for injection	Include more sample in the injection vial. Run method validation to assess sample volume requirements. Use a larger vial if necessary.
Air leak into the system	Check for correct installation of rack seals. Contact support.

Symptom: Poor SPR dips curve

Possible Cause	Remedy
Buffer in flow cell during normalization	Renormalize sensor chip and try again. If the problem persists, contact support.

Symptom: Liquid leak on bench or buffer bottle tray

Possible Cause	Remedy
Flow lines blocked or loose fitting	Contact support.
Waste container full	Empty waste container.

Symptom: Poor injection quality

Possible Cause	Remedy
Blockage in flow lines or valve	Contact support.

Symptom: Expected response curve not observed

Possible Cause	Remedy
Sensor chip chemistry not functional	The chemistry on the gold sensor chip may be faulty or expired. Install a new sensor chip and try again.
Hardware component failure	Contact support.
Flow cell blockage	Complete a Flowpath Test under Instrument Setup, Diagnostics and send results to support for analysis.
Docking problem	Reinstall sensor chip. Contact support if issues persist.

Symptom: Excessive baseline drift

Possible Cause	Remedy
System not thermally equilibrated	Wait for system to equilibrate before beginning experiments.
Incompatible buffer or solution	Consult Octet® SPR Chemistry User Guide or contact support.
Flow cell leak	Uninstall sensor chip and check for breakage. Reinstall sensor chip and try again. If the problem persists, contact support.
Chip not hydrated	Hydrate chip by priming the system at least three times in running buffer.

Symptom: Excessive baseline noise

Possible Cause	Remedy
Renormalization needed	Renormalize sensor chip and try again.
Buffer in flow cell during normalization	Renormalize sensor chip and try again. If the problem persists, contact support.
Chip not hydrated	Hydrate chip by priming the system at least three times in running buffer.

Symptom: High rise/fall times in response curves

Possible Cause	Remedy
Robot Z position out of calibration	Restart system. If the problem persists, contact support.
Blockage in waste tubing	Check waste tubing for any sign of damage or twisting. If the problem persists, contact support.

Symptom: Probe error

Possible Cause	Remedy
Probe caught on sample rack evaporative seal	Reattach rack seal properly. Straighten probe by hand if necessary.
Sample vial inserted upside down	Remove vial and replace in correct orientation.
Rack installed incorrectly	Install the rack that matches the running method.

Symptom: Unable to start recording/method

Possible Cause	Remedy
Maintenance Chip Installed	Cannot record with maintenance chip installed. Uninstall chip and install standard chip.
Water line running	Use the Switch Line function to change from the water line to buffer line A or B.

System Errors and Error Codes

Table A-1: Errors and Error Codes

Error	Remedy
System displays Error code (4 digit code)	Restart instrument. If the error is persistent, contact support.
Maintenance Warning/Maintenance Needed	Complete Desorb/Desorb & Decontaminate according to the regular schedule.
Flowcell Leak Detected	Uninstall chip and clean flowcell using cleaning cards. Reinstall chip. If issues persist, contact support.
Chip Not Detected	Remove sensor chip and reinstall.

Appendix B:

GPL Software Notice

GPL Software Notice

The Octet® SF3 system contains — in part — some free software licensed under the terms of the GNU General Public License (you may obtain copies at http://www.gnu.org/licenses/licenses.html). This license allows you to freely copy, modify, and redistribute that software. As part of the license terms, the source code for these components will be made available to you upon written request.

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