Reichert™
TECHNOLOGIES
Life Sciences

The NEW
SR7500DC System

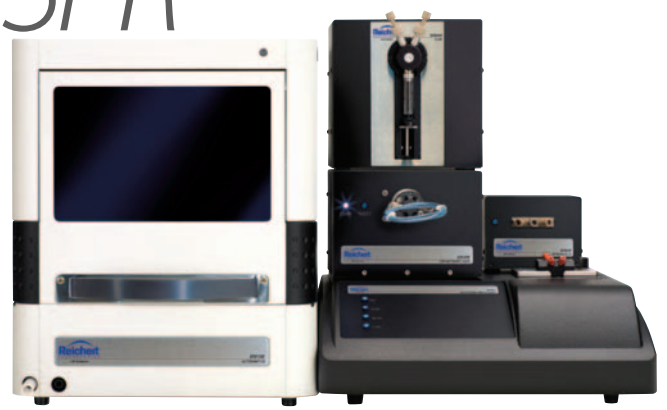
BlackMagic

The Next Generation of Surface Plasmon Resonance

New from Reichert SPR

SR7500DC Dual Channel Surface Plasmon Resonance System

Pushing the limits of detection and sensitivity in label-free interaction analysis



Explore Critical Interactions with *Black Magic* the New SR7500DC SPR System

Proteins
Nucleic Acids
Lipids
Carbohydrates
Small Molecules/Drugs
Whole Cells
Bacteria/Viruses
Polymers

The new **SR7500DC Dual Channel Surface Plasmon Resonance (SPR) System** provides the diverse interests of academia and industry with one of the most sensitive and flexible SPR platforms in today's marketplace. This reliable label-free system is used to characterize a broad range of molecular interactions that are important in numerous scientific disciplines. These interactions include those occurring with and between the major classes of biological macromolecules along with those involving small molecules and drugs. Quantitative information on such interactions is critical to research efforts in pharmaceuticals, drug discovery, antibody screening, protein structure/function, gene regulation and systems biology. This new SPR system is used to generate high quality data with outstanding precision for:

- Rigorous kinetics analysis (association/on and dissociation/off rates)
- Affinity measurements ranging from extremely weak (1 mM) to extremely strong (1 pM) interactions
- Precise determination of thermodynamic parameters (ΔH, ΔS)
- Accurate concentration analysis

Extreme Low Noise Extreme Low Drift Extreme Value

Reichert's new low noise, component-based SPR system provides outstanding flexibility and exceptional sensitivity when seeking high quality data for interactions of interest. The new **SR7500DC System** pushes detection limits and sensitivity to new lower limits, expanding the boundaries of traditional biomolecular interaction analysis. The system offers superior performance with the following key features:

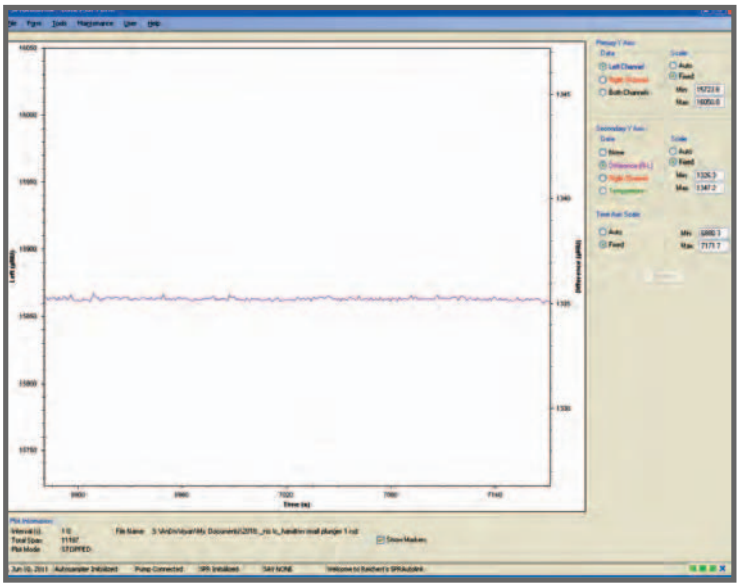
- High sample capacity (up to 768 samples)
- Temperature control from 10°C below ambient to 70°C
- Fast data sampling rates (up to 10 Hz)
- Broad refractive index range (1.32 to 1.52)
- Sophisticated, intuitive software with 21 CFR part 11 controls
- Minimal maintenance requirements
- Low life cycle costs
- Ultimate flexibility
- Extremely low noise (0.05 μRIU)
- Extremely low drift (0.01 μRIU/min)

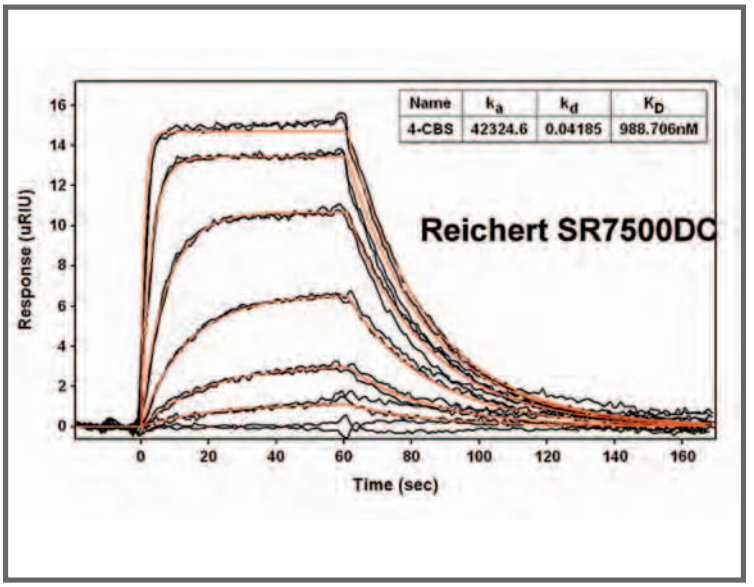
Reichert SPR Less Noise, Higher Quality and Precision

The **SR7500DC System** offers high precision in determining kinetics and affinities for a variety of biomolecular interactions. The system generates real-time data that provides invaluable insight into the dynamics of biomolecular interactions that regulate numerous biological processes. With its enhanced sensitivity, the SR7500DC System is ideal for:

- Cost-effective biomolecular interaction analysis
- Monitoring binding of low molecular weight compounds (< 100 Da)
- Identifying potential drug targets and therapeutics
- Antibody characterization

Extremely low noise (0.05 μRIU) and low drift (0.01 μRIU/min) baseline





The binding of 4-carboxybenzenesulfonamide to immobilized carbonic anhydrase II

Software

Autolink, Reichert's SPR System Software

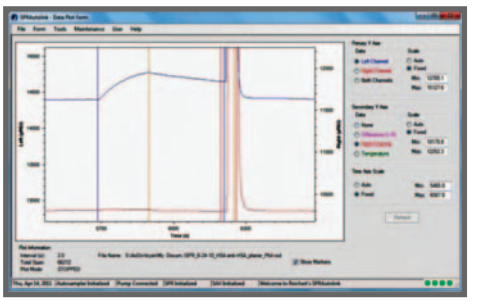
- Integrated sophisticated control of the Reichert SPR System. **Autolink** is intuitive, powerful, and easy to use.
- Reichert's **Autolink** integrates the three components of the SR7500DC SPR System – the injector, the pump, and the SPR spectrometer.
- Fully programmable, intuitive, step-by-step control of the pump, semi-automatic or fully automatic injectors and the SPR spectrometer.
- Drag and drop methods to set up multi-sample run tables.
- **Autolink** also allows each component to be controlled individually without constraints – the ultimate flexibility for real-time method development.

Autolink is Validated Software with 21 CFR Part 11 Technical Controls

Protecting the security and integrity of electronic records (ER) is essential for compliance. This includes ensuring the reliability and trustworthiness of ER used to support critical decisions. Features in Reichert's **Autolink** software include:

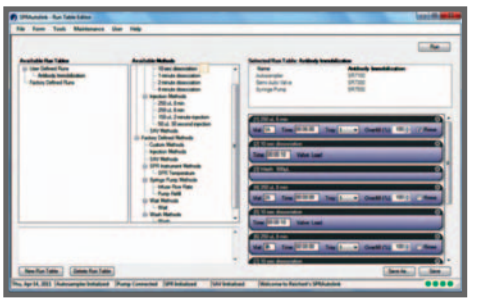
- Data Security and Integrity - Access control along with file encrypted checksums
- User Authorization Levels - Administrator, factory, and user levels set access rights to software functions
- Record Tracking - Experiment details, events, and user identities are logged in a date and timed stamped secure file
- Data can be exported both manually and automatically in a variety of formats including tab dilineated text files, and Scrubber® data analysis files

The software has been developed in accordance with an accepted development model to ensure adequate validation.



Data Acquisition window

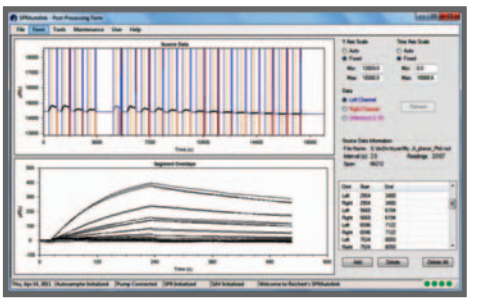
Data from the left channel, right channel, and difference channel can be viewed together or separately in real-time. Both the response (Y axis) and time (X axis) axis may be auto-scaled or set to user-defined limits. The data window can also be expanded over a defined region to allow close examination of the SPR response curves. In addition, this window continuously displays the status of each system component, read time interval, flow rate, temperature, file name and injection/dissociation markers.



Programming a run table

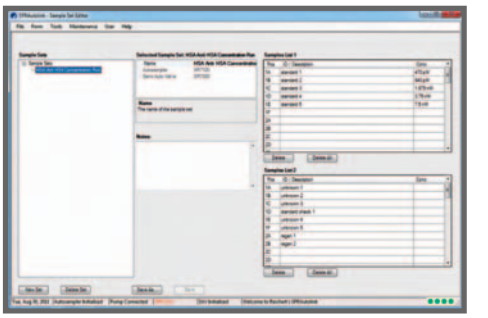
A typical run table injection sequence

Methods are dragged and dropped from the "Available Methods" section (center) to the Run Table Section. In this sequence step 1 is a pump refill and step 2 is a 1 minute wait for baseline stabilization. The third step is a 2 minute 150 µL sample injection from vial 1A followed by a 2 minute dissociation period. The final two steps are a 100 µL injection of regeneration solution and a one minute dissociation. The checked "Rinse" box in the next to last step washes the sample loop and needle with buffer prior to upcoming sample injections. Run table sequences are easily copied and pasted to quickly program run tables with many injections from 96 and 384 well plates or 48 vial sample trays.



Autolink post process window

SPR sensorgrams are extracted and aligned to the same zero start time and zero response. The aligned sensorgrams are then saved for analysis using Scrubber® or the curve fitting program of choice.

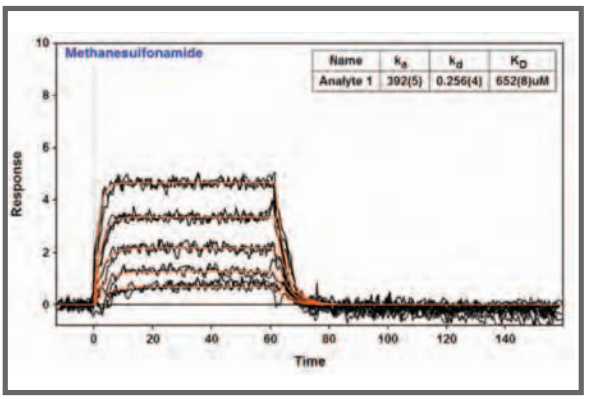


Sample set editor

The window allows the labeling of each injection with a user-defined description and concentration. This sample information is then displayed within the run table for each injection and ultimately carries over to the data analysis program.

Small Molecule Analysis

Carbonic anhydrase II (CAII) is an enzyme that catalyzes the reversible hydration of carbon dioxide to form bicarbonate with the release of a proton. CAII activity is strongly inhibited by a variety of aromatic and heterocyclic sulfonamides. In this example, carbonic anhydrase II is amine coupled to a carboxymethyl dextran surface and then the binding of a very low molecular weight inhibitor (95 Da), methanesulfonamide, is followed over a series of concentrations. The red lines show the global fit to a simple bimolecular binding model and the calculated association rate constant, dissociation rate constant and equilibrium dissociation constant values for this interaction are shown in the inset. This experiment illustrates the remarkable sensitivity and performance of the SR7500DC System.



The SR7120 Autosampler

Reichert's autosampler provides the user with flexibility in terms of sample holder options, tray cooling, sample aspiration speeds, and loop volume choices. The autosampler allows for the seamless execution of an experimental protocol over 24 hrs and can precisely inject samples of varying compositions. Features include:

- Sample cooling to 4°C
- Ability to inject from two sample trays, which can be 48 vial sample trays, 96 or 384 well plates, and/or large volume 12 vial formats
- Push/Pull sample aspiration enables precise pick-up of viscous samples and eliminates outgassing
- Accommodates a wide selection of sample loop volumes

Flow Cells

Specialized Collection

Reichert offers a standard flow cell with each instrument. This flow cell is used to perform molecular interaction studies and has low channel volume resulting in extremely fast solution exchange dynamics. In addition to a standard flow cell, Reichert offers specialized cells that provide the ability to couple SPR measurements with other valuable analytical techniques. They open up new avenues of investigation for specialized applications.

Standard Flow Cell

Reichert's new teflon body standard flow cell represents a significant improvement in flow cell performance. This gasketless flow cell features very small dead volumes, low channel volume with extremely fast rise and decay times on both channels.

The flow cell uses standard PEEK™ HPLC/FPLC fittings and is compatible with tubing sizes down to 0.0025" inner diameter (65 micrometers). The mounting mechanism for the flow cell has been completely redesigned.

The flow cell is placed over the sensor chip and locked into place with a mechanical lever. This ensures the flow cell is always mounted in a level position with consistent pressure.

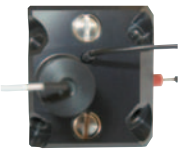
Quartz Window Flow Cell

The Reichert quartz window flow cell facilitates experiments combining SPR with photochemistry, imaging fluorescent labeled molecules on the sensor surface by direct excitation and surface plasmon field enhanced fluorescence spectroscopy. Surface plasmon field enhanced fluorescence spectroscopy is an extremely sensitive and effective tool for detecting and quantifying biomolecular binding. This technique depends on excitation of a fluorophore near the gold sensor surface of an evanescent field.

Resonance of p-polarized light with surface plasmons (oscillating electrons) in the gold layer produces the evanescent field.

Electrochemical Flow Cell

Reichert's electrochemistry flow cell utilizes a three-electrode design. Electrical contact is made between a platinum wire counter electrode, an Ag/AgCl reference electrode, and the working electrode, which is a standard Reichert SPR gold slide. The electrical leads from the electrodes are connected to a potentiostat to control the potential being applied to the gold slide surface. You can adjust the potential at the gold surface simultaneously with SPR data collection to carry out a variety of experiments including SPR/amperometry, SPR/pulse voltammetry and SPR/cyclic voltammetry. These experiments have been carried out to monitor polymer formation and for other novel applications.



MALDI Spectrometry Flow Cell

A novel flow cell for combining SPR with mass spectrometry (MS), is the matrix-assisted laser desorption and ionization (MALDI) flow cell which carries removable, miniaturized sensing pins that can be inserted into MALDI target plates for mass spectrometric detection of analytes on the sensor surface. This is especially important in a **ligand fishing** experiment where the aim is to identify the molecule(s) captured on the sensor surface. After verifying binding of the unknown species with SPR, these removable pins can be inserted into a modified MALDI target, where the ligand can be directly analyzed or subjected to a digestive treatment. Combined with the application of new hydrogel sensor surfaces, this flow cell allows measurements of higher sensitivity and better reproducibility with SPR-MS.

Extreme Sensitivity and the Ultimate in Computing Power

The sensitivity starts with image detection. The SR7500DC System uses two RL1210 Perkin Elmer Photodiode 1024 pixel arrays that feature 100,000:1 dynamic range and 0.4 picoAmp dark current. This all translates to extreme sensitivity.

On board computing power is via an Altera FPGA with a virtual softcore 32-bit processor. This equates to massively fast and furious pipeline processing. And the processor is field programmable for future upgrades. Illumination is via two 780 nm arrays, each with 66 LEDs. The twin LED banks illuminate an integrating sphere to provide two watts of fully homogenized light power. LED current is critically controlled to provide constant output power via an isolated feedback circuit.

The SR7500DC System features a new high speed USB interface. Data sampling rate is 0.5 to 10 Hz. An embedded peripheral hub controls the complete system including the syringe pump, autosampler and/or semi-automatic injection valve.

Sensor Chips

Reichert offers a wide variety of sensor chips at affordable prices so researchers can explore more interactions without higher running costs. The available sensor chips include:

Plain Gold Sensor Chip

The plain gold chip provides the opportunity to study surface formation and adsorption in real-time on bare Au and allows researchers to coat the chip with user-defined chemistries.

Carboxymethyl Dextran Sensor Chip

Hydrogel surfaces, particularly carboxymethyl dextran hydrogels, offer many advantages when used as a SPR sensor chip surface. Carboxymethyl dextran surfaces are very stable and resistant to non-specific binding of biomolecules. The dextran layer is in the form of a highly flexible non-cross-linked brush like structure extending 100 to 200 nm from the surface. The flexible nature of the dextran contributes to the accessibility of binding sites on an immobilized ligand. Biomolecules are easily coupled to the surface utilizing a variety of techniques similar to those used in affinity chromatography. Large amounts of protein, up to 50 ng/mm^3, can be immobilized on carboxymethyl hydrogel surfaces due to the 3-dimensional nature of the hydrogel layer. This is important for experiments where a low molecular weight analyte binds to a surface immobilized ligand.

Planar Polyethylene Glycol/ Carboxyl Sensor Chip

This surface consists of a mixed, self-assembled monolayer of alkanethiolates generated from the combination of polyethylene glycol-terminated alkanethiol (90%) and COOH-terminated alkanethiol (10%). The terminal polyethylene glycol chains minimize non-specific binding while the COOH groups provide a functional attachment site for immobilizing/capturing a molecule of interest. Amine coupling, utilizing EDC/NHS chemistry, is the most common ligand immobilization approach but thiol, aldehyde, and maleimide coupling are also possible with this surface using the appropriate cross-linking chemistry.

Nickel Nitrilotriacetic Acid Sensor Chip

This surface is used to capture histidine-tagged molecules such as recombinant proteins. The captured molecule is oriented well on the surface as the ligand is captured directly at the histidine tag via Ni^{2+}/NTA chelation. The surface is easily regenerated with an injection of imidazole or EDTA to remove the metal ions and the captured ligand.

Hydrophobic Planar Alkyl Sensor Chip

This surface is a self-assembled monolayer of long-chained alkanethiol groups directly attached

to gold. It is ideal for studying membrane-associated interactions. In addition, vesicles spontaneously adsorb to the surface forming a supported lipid monolayer. It is easily regenerated with an injection of a detergent such as CHAPS.

Streptavidin/NeutrAvidin Sensor Chip

This surface is used for the high affinity capture of biotinylated molecules such as proteins, peptides, and nucleic acids. The binding of streptavidin to biotin is one of the strongest non-covalent interactions known so the surface can be regenerated without having to recapture the ligand after each regeneration step. Minimal biotinylation of the ligand and subsequent capture on a Streptavidin/NeutrAvidin chip results in a more oriented arrangement of ligand molecules on the surface as compared to the more random arrangement from chemical immobilization such as amine coupling.

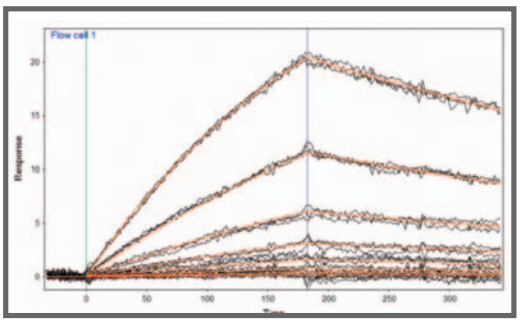
Protein A Sensor Chip

This surface is used for capturing certain antibodies. Protein A contains four high affinity binding sites capable of interacting with the Fc region from IgG of several species, including human and rabbit. Optimal binding occurs at pH 8.2, although binding is also effective at neutral or physiological conditions (pH 7.0 to 7.6).

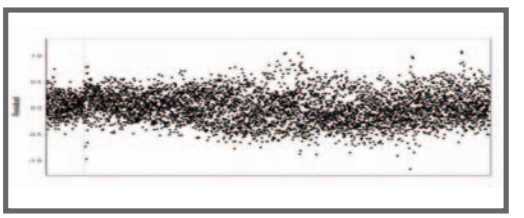
Applications

Low Immobilization Level Anti-HSA/HSA Data

In this application, a very low amount of anti-HSA (< 100 μRIU) is immobilized to a carboxylmethyl dextran surface via amine coupling. HSA is then injected over the immobilized antibody at different concentrations ranging from 7.5 to 0.45 nM. Each concentration is injected in duplicate to ensure reproducibility. The red lines show the global fit of the data to a simple bimolecular binding model.



This example shows that high quality SPR data can be obtained on the SR7500DC System despite having extremely low ligand immobilization levels. In fact, low ligand immobilization levels are often desired to prevent crowding on the surface and this application illustrates that the SR7500DC System can provide precise kinetic data for low responses with great confidence.



Applications

Typical Enthalpy Application:

Thermodynamic Investigation of an Enzyme-Inhibitor Pair

Figure 1 presents the temperature-dependent response curves of 4-CBS binding to CAII at 20°C (blue lines) and 35°C (red lines). The results indicate that temperature has a drastic effect on the profile of the response curves. Specifically, the association and dissociation rate constants increase with temperature. To quantify the change in rates, the data at each temperature was fit to a simple bimolecular model using Scrubber® (Biologic Software) to determine the rate constants and the equilibrium dissociation constants.

Figure 2 presents a van't Hoff analysis of the data by plotting ln K_D versus 1/T. The data fits fairly well to a linear regression model ($r^2 = 0.989$), thus the thermodynamic parameter ΔH can be determined directly from the non-integrated form of the van't Hoff equation. In this case, the slope is ΔH/R. Thus, ΔH is determined to be -6.0 kcal/mol for this inhibitor-enzyme pair.

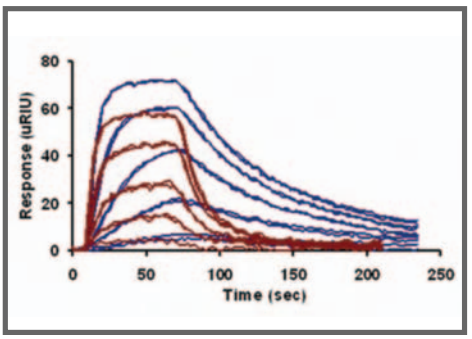


Figure 1: Temperature-dependent response curves of 4-CBS binding to CAII at 20°C (blue lines) and 35°C (red lines).

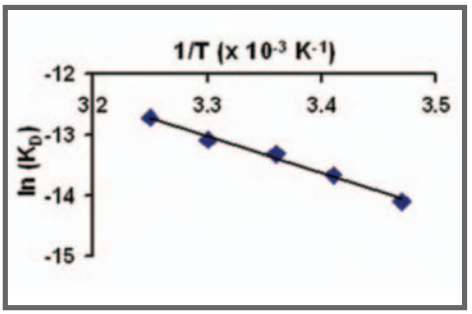
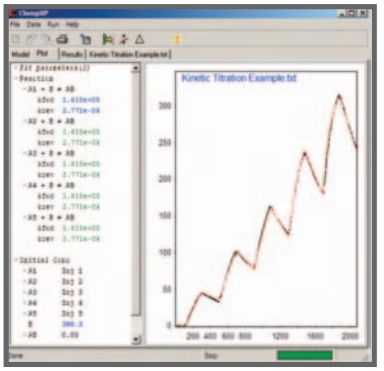


Figure 2: van't Hoff plot of the Thermodynamic Data from 4-CBS binding to CAII.

Kinetic Titration

Identifying a suitable regeneration solution can sometimes be a bottleneck when carrying out a traditional SPR experiment. In this case, a kinetic titration approach is desired where analyte is injected over the surface at increasing concentration in a single cycle without regenerating the surface between each concentration injection. This kinetic titration



approach reduces the amount of time spent on assay development as it eliminates the need to optimize regeneration conditions. This approach also allows for the analysis of molecular interactions that were previously difficult to characterize due to problems in finding an appropriate regeneration solution. Reichert's

Applications

Capture SPR Analysis Using Reichert Carboxymethyl Dextran (CMD500k) Sensor Chips

Figure 1 (right) presents data from a capture experiment. Initially, about 2,000 μRIU of goat anti-mouse IgG Fc was amine coupled to the CMD500k surface. For each series of injections, a constant concentration of monoclonal anti-HSA IgG (50 μg/mL) is captured over the surface, and then varying concentrations of HSA are injected. Both anti-HSA and HSA are then removed during each regeneration cycle.

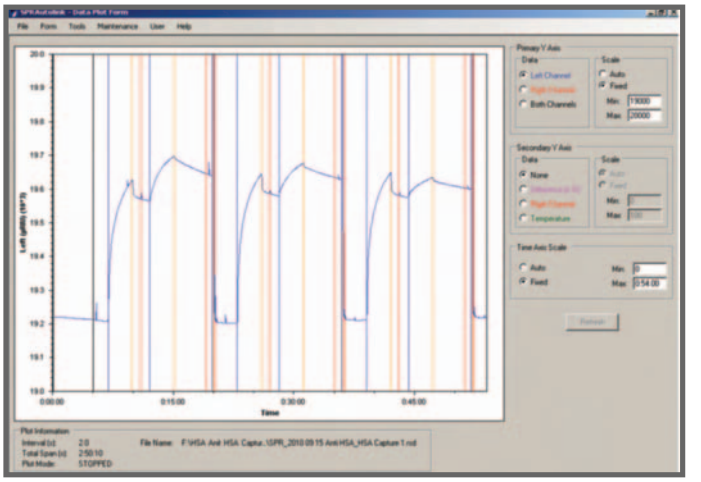
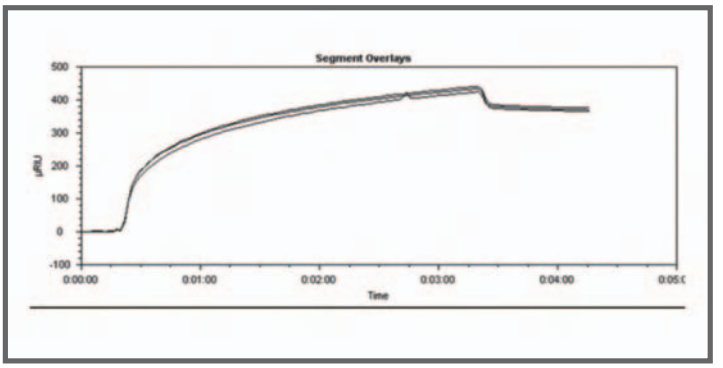
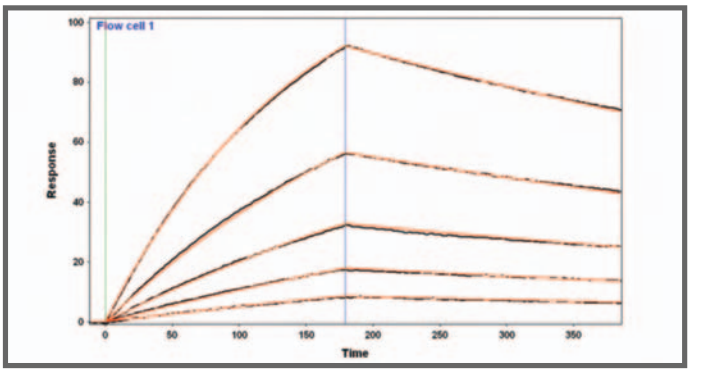


Figure 3 (below) shows the data and global kinetic fit of the HSA injections. HSA is injected at concentrations ranging from 1.25 to 20 nM. The red lines are the fit obtained in Scrubber® for binding to a 1:1 model. The equilibrium dissociation constant (K_D) obtained is 4.93 nM.

Figure 2 (below) shows the excellent reproducibility of the capture step and the chemical stability of the CMD500k surface. Even after multiple injection-regeneration cycles, the surface is stable and gives reproducible results.





SR7500DC System can collect kinetic titration data and analyze it to determine the binding kinetics of the interaction of interest. The figure (left) shows kinetic titration data from the anti-HSA/HSA assay. In this example, HSA is injected at successively higher concentrations ranging from 1.25 to 20 nM in a single cycle (no regeneration). The data is then fit to a kinetic titration model (red line is the fit to the data) and the kinetic rate constants are determined for the interaction.

Concentration Analysis

Figure 1 shows the calibration tab from Reichert's Autolink Software concentration module. The plot is the 175-second association response point vs. concentration for an antibody (immobilized ligand) / antigen (injected analyte) interaction.

Figure 2 shows response at 10 seconds vs. analyte concentration.

Concentration Analysis

SPR is a very precise and accurate method for determining the concentration of a biomolecule. Concentration measurements are based on the concept that as the concentration of an analyte increases, the rate of binding to an immobilized binding partner increases. Generally, a calibration curve is constructed by plotting responses at a time point in the sensorgram versus known concentrations.

Response points are generally chosen at later stages of the binding association or dissociation curves. The response at later stages in the association is typically exponentially increasing or decreasing if a point is chosen in the dissociation phase. These response versus concentration plots are non-linear. These plots are generally fit to a non-linear function such as a quadratic, 3rd order polynomial or a 4-parameter equation. Figure 1 is a calibration plot of the 175-second response point versus concentration. This data was fit to a 4-parameter function. Generally, choosing points in the non-linear later stages of a sensorgram provides better accuracy and reproducibility.

Response points chosen very early in the sensorgram - 2 to 10 seconds - represent the initial rate of binding. The initial rate is typically linear. The calibration plot of concentration versus initial rate or a time point in this region of the sensorgram is linear (see Figure 2 below).

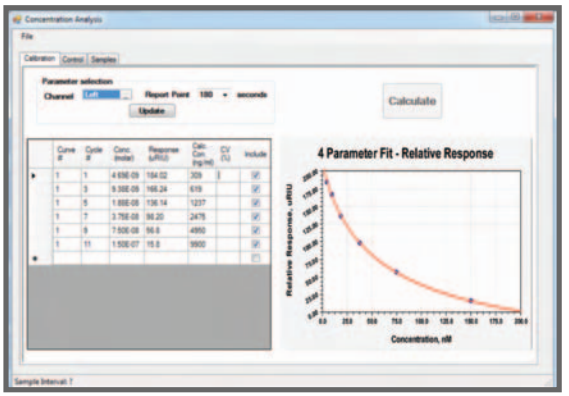


Figure 1

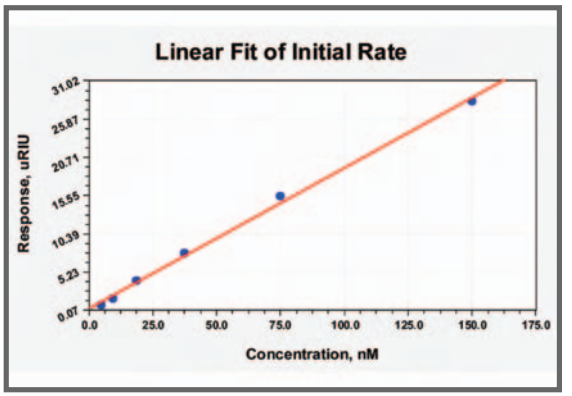


Figure 2

Fluidics Kit

Fluidics Kit

Our SPR system is designed to use low-cost, off-the-shelf HPLC fittings and tubing, allowing for quick and easy changeover. Reichert SPR systems are supplied with a fluidics kit that provides all the connectors and tubing needed to properly plumb the system, including spares. The primary contents of the kit include an assortment of tubing nuts and ferrules, luer adapters, a tubing cutter, a pair of forceps, and a selection of different inner diameter tubing spindles and sample volume loops. The SPR system fluidics can accommodate a wide range of sample compositions (from salt solution to cell lysates), varying viscosities, and particulate matter.

Specifications

Technical Information

Transducer Principle	Kretschmann prism, multiple angles from fixed light input
Measurement Channels	Two (either parallel or series fluid connection)
Sample Loading	Autosampler or semi-automatic injector, standard HLPC tubing and connectors, external syringe pump
Sample Capacity	Any combination of up to 2 trays can be used. Choose: 12 (10 mL) or 48 (2 mL) Vials; or 96-well (high or low) or 384-well Plates
Sample Storage	4°C or ambient temperature
Flow Cell Volume per Channel	0.18 µL
Flow Cell Surface Area per Channel	4.5 mm² (reference value)
Aspect Ratio	>25 (width/height)
Fluid Contact Materials	Teflon™, Acetal Copolymer, PEEK™, Kalrez™, ETFE (Tefzal™)
Sample Volume	1 to 5000 µL (depends on installed loop volume)
System Fluid Volume	(typically) 28 µL (0.01" I.D. tubing) or 7.5 µL (0.005" I.D. tubing)
Temperature Range	10°C below ambient to 70°C

Measurement Sensitivity

Refractive Index Resolution	$< 10^{-7}$ RIU (< 0.1 µRIU)
Refractive Index Range	1.33 to 1.42; up to 1.52 with higher RI glass (@780nm)
Analyte Concentration Range	1 mM to 1 pM
Minimum Molecular Weight Detection	< 100 Daltons
Baseline Noise	0.1 µRIU peak-to-peak, 0.05 µRIU RMS, @ 25 µL/min
Baseline Drift	< 0.01 µRIU/min

Typical Kinetic and Equilibrium Constant Ranges

Association Rate Constant	10^{3} to 10^{7} $M^{-1}s^{-1}$
Dissociation Rate Constant	10^{-1} to 10^{-5} s^{-1}
Equilibrium Dissociation Constant	0.1 mM to 1 pM

Electrical

AC Power Supply, standard international voltage range w/universal adapter from 100 to 240 V & 50 to 60 Hz

Regulatory

Compliance with the applicable sections of the European EMC Directive and IEC safety requirements for laboratory electrical equipment for measurement and control

Product Safety

Compliance with IEC 61010-1 (Low Voltage Directive) under a Category classification EMC and Safety: CE mark certification (Class A, Type II)

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