

THE OCTAVE SYSTEM

A New Automated Instrument for the Analysis of Molecular Interactions

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ABSTRACT

Surface plasmon resonance (SPR) technology is a powerful, label-free method for the analysis of molecular interactions. Prolinx[®], Inc. has developed a new automated instrument, the Octave Molecular Interaction Analysis System, that allows this powerful analytical platform to become ubiquitous in life science research and drug discovery laboratories. This technological breakthrough is the result of combining the miniaturized Texas Instruments Spreeta[™] 2000 SPR sensor with the Prolinx Versalinx[™] Chemical Affinity Tools. These technologies have made possible the development of an instrument of moderate cost that incorporates eight independent sensors operating in parallel. The sensor surfaces can be readily and efficiently modified with molecular targets, and exhibit low non-specific binding. Samples are introduced to the sensors from standard microwell plates using an integrated liquid handling robot. This new instrument will significantly increase the throughput of SPR-based molecular interaction analysis in basic biological science and drug discovery applications.

INTRODUCTION

The recent availability of complete genome sequences has revolutionized the way in which basic biological science is now being and will be performed in the future. Newly coined terms such as “proteomics”, “cellomics” and “metabolomics” reflect the fundamental shift in biological research from the characterization of isolated molecules or cells to the analysis and understanding of biological systems as integrated and interactive networks. A key to the successful realization of the analysis of complete biological systems is the development of powerful technologies that will enable the interrogation of complex assemblies of molecules with sufficient throughput to match the scope of the endeavor. This paper will describe the design and development of a novel instrument for the analysis of molecular interactions with increased throughput and ease-of-use. The instrument is made possible through the combination of two enabling technologies: the Spreeta 2000 sensor, a miniaturized, solid-state device for the high-sensitivity measurement of refractive index changes near a solid surface using surface plasmon resonance; and the Versalinx Chemical Affinity Tools, a novel technology for the robust and reproducible immobilization of active biomolecular species on solid surfaces.

MOLECULAR INTERACTION ANALYSIS

Biological processes are governed by the temporal and spatial interactions between molecules. Basic parameters which characterize these interactions include reaction stoichiometry, concentrations of interacting species, equilibrium (affinity) constants, kinetic (rate) constants, and specificity of interaction as functions of temperature and solution composition (pH, ionic strength). The *in vitro* determination of these parameters for a system of interest can provide important insight into the molecular basis of fundamental metabolic processes, supply essential information for the diagnosis of disease and help identify promising therapeutic candidates. Hence, molecular interaction analysis plays an important role in basic biological science as well as medicine. Table I summarizes the diversity of recently published applications of molecular interaction analysis.

Table I. Diversity of recently published applications of molecular interaction analysis [information from (1)].

- Drug discovery (lead identification, target validation)
- Ligand fishing
- Comparative binding specificity
- Mutation studies, structure-function relationships
- Cell signaling
- Replication, transcription, regulation
- Multi-molecular complexes
- Immune regulation
- Immunoassays
- Vaccine development
- Chromatographic process development

SURFACE PLASMON RESONANCE

Surface plasmon resonance (SPR) is a label-free optical detection technology that has proven extremely useful in the analysis of molecular interactions for over a decade. The technology provides a real-time method for measuring the interaction(s) between two or more molecules, one of which is tethered to a solid surface (2). Molecules used in such studies to date include: proteins, peptides, nucleic acids, carbohydrates, lipids and low molecular weight substances (e.g., hormones, pharmaceuticals) (3). Indeed, interactions between immobilized cells and ligands to cell surface receptors have been studied (3).

A surface plasmon is the oscillation of free electrons which is present at the surface of a conductor such as a metal. Surface plasmon resonance occurs under conditions of total internal reflection in a metal film present at the boundary between two substances of different refractive indices, such as water and glass. An incident monochromatic light beam in the first medium creates an evanescent wave at the point of reflection that crosses a short distance beyond the boundary. The evanescent wave couples with the surface plasmons in the metal at a particular angle of incidence that depends on the refractive index of the second medium. Energy is absorbed, with the result that the intensity of the reflected light is attenuated relative to the incident light. Thus, measurement of reflected light intensity as a function of angle of incidence can be used to monitor changes in the refractive index of the medium near the metal surface (4).

The implementation of SPR as a detection technology for molecular interaction analysis is illustrated by the following simplified example which is depicted in Figure 1 (5, 6). A thin film of a conducting metal, typically gold, is deposited on the surface of a glass prism. A molecular recognition element, such as an antibody or other protein receptor, is immobilized in a molecularly thin layer on the surface of the metal film using any of a variety of methods. Monochromatic light is then directed onto the gold film by the prism. The gold film is brought in contact with a stream of flowing solution containing the (putative) binding partner(s) for the immobilized recognition element. As the binding partner interacts with the surface immobilized recognition element, the dielectric value (and thus refractive index) of the material on the metal surface changes. This change in refractive index causes a change in the angle of the incident light beam required for maximal coupling into the surface plasmons. The incident light beam is scanned through a variety of angles and the angle of minimum reflected intensity is measured. If this measurement is made and plotted as a function of time, the result is a curve that characterizes the binding or association process. If the solution with binding partner is now replaced with a solution that is devoid of the binding partner, bound analyte is released yielding a curve that characterizes this dissociation process. Kinetic and equilibrium constants characterizing the interaction can be mathematically extracted from this data based on given binding models.

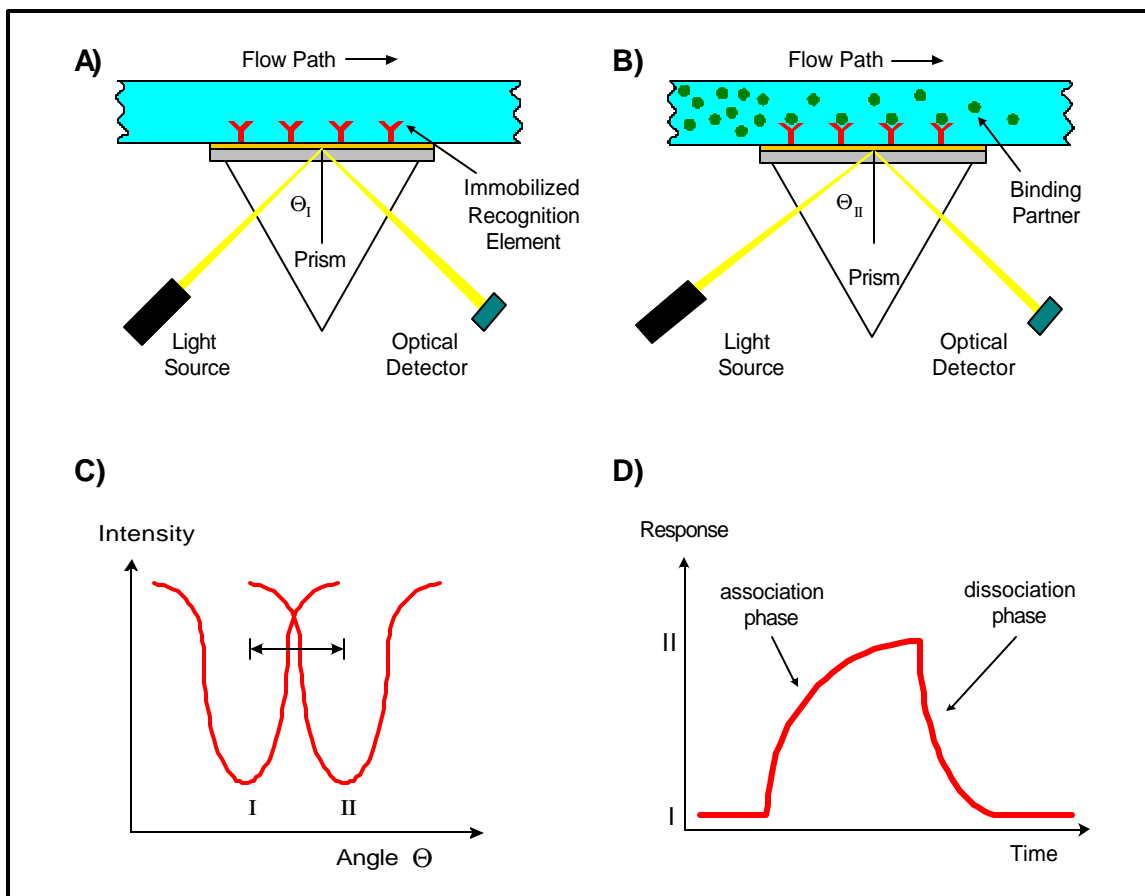


Figure 1. (A and B) One configuration for molecular interaction analysis using SPR detection as described in the text. The drawing in (A) represents the system in the absence of the binding partner for the recognition element; (B) represents the system in the presence of a saturating amount of the binding partner. (C) Raw SPR data. The red curves represent the dependence

of the reflected light intensity as a function of angle of incidence Θ . Position I is the angle of incidence for minimum reflected light intensity in the absence of binding partner. Position II is the angle of incidence for minimum reflected light intensity in the presence of a saturating amount of binding partner. (D) Plot of the angular position of the minimum of the curve with time. The association and dissociation phases are as described in the text. This curve is typically referred to as a “sensorgram”.

THE VERSALINX CHEMICAL AFFINITY TOOLS

SPR-based molecular interaction analysis requires that a molecular recognition element be immobilized on the surface of the metal film employed for SPR. Therefore, an immobilization chemistry appropriate to the molecules being studied is a necessity. Key attributes of such a chemistry are:

- Efficient, easy to perform, reproducible and predictable
- Flexible enough to be applicable to a wide variety of molecular species
- Optimally presents the immobilized molecules to the incoming binding partners such that full and specific biological activity is retained
- Minimal non-specific binding of analytes to prevent loss of detection sensitivity and specificity

The Versalinx Chemical Affinity Tools are a novel system for the immobilization of biological macromolecules. They are based on the highly specific complex formation between two families of small molecules, the simplest representatives of which are phenylboronic acid (PBA) and salicylhydroxamic acid (SHA) (7,8). This interaction is depicted in Figure 2. The only byproduct of complex formation is an equivalent of water. The complex can be dissociated into its component parts either at extremes of pH or by using competitive binding reagents.

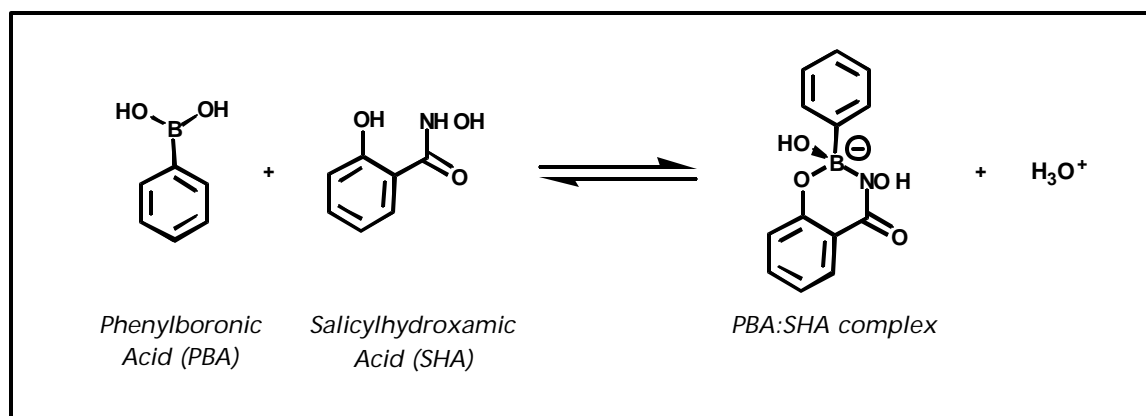


Figure 2. The Versalinx Chemical Affinity Tools are based on the specific interaction between phenylboronic acid (PBA) and salicylhydroxamic acid (SHA). These two molecules form a coordinate covalent complex under variety of conditions, the only byproduct of which is an equivalent of water. The complex can be reversed into its component parts under appropriate conditions.

Complex formation occurs readily in aqueous solution in the pH range 5 to 9. It forms in the presence of most buffer systems (borate being an exception); monovalent and divalent inorganic salts to 1.5 M; chaotropes such as urea and guanidine hydrochloride; organic solvents such as dimethyl sulfoxide and simple aliphatic alcohols; and detergents such as dodecyl sulfate. In addition, once the complex is formed, it is stable under an even greater range of solution conditions.

The Versalinx Chemical Affinity Tools are a series of reagents that enable the immobilization of biomolecules on solid surfaces by virtue of PBA:SHA complex formation. In general, the strategy for biomolecule immobilization is as follows. A solid surface is chemically derivatized with SHA using one of several chemical alternatives. The biomolecule to be immobilized is optimally conjugated with an appropriate PBA reagent. The PBA-conjugated biomolecule is contacted with the SHA-modified surface, and rapid immobilization due to PBA:SHA complex formation occurs. Excess PBA-conjugated biomolecule (if any) is removed by washing, and the surface is ready to use.

The Versalinx approach to biomolecule immobilization has several powerful attributes for SPR-based molecular interaction analysis. First, it provides a single, universal SHA-modified surface that can be used to immobilize any PBA-conjugated biomolecule. Biomolecule conjugation with PBA is very flexible, as PBA derivatives are available for modifying amines (active ester), thiols (maleimide), oxidized carbohydrates (hydrazide), oligonucleotides (phosphoramidite), DNA (dUTP) and RNA (UTP). Analyses can thus be performed using immobilized proteins, carbohydrates, nucleic acids, etc. on a single type of sensor surface using the same immobilization chemistry. Additionally, SHA-modified surfaces typically show very little interaction with non-PBA labeled biomolecules, resulting in very low noise levels due to non-specific binding. Also, the sensor surface may be regenerated for subsequent analyses using the same immobilized recognition element by chemically removing the binding partner, or it may be stripped to the native SHA surface for reconstitution with the same or a different recognition molecule. In some cases, it may be possible to remove intact recognition element/binding partner complexes for further analysis (e.g., mass spectroscopy) using competitive reversal of the PBA:SHA complex.

It has been empirically observed that immobilization of biomolecules using Versalinx typically results in a higher retention of biological activity of the surface-bound species relative to alternative methods of surface immobilization.

THE SPREETA 2000 SENSOR

In 1996, Texas Instruments, Inc. demonstrated the first fully integrated miniature technology for refractive index sensing using surface plasmon resonance (9). The most recent implementation of this technology, trade-named Spreeta 2000, contains all of the optics and electronics necessary for the acquisition of SPR data in a highly miniaturized device. A drawing and a photograph of the device are shown in Figure 3. It consists of: a printed circuit board upon which are installed a light source (830 nm light emitting diode), a photodetector (128 pixel linear photodiode array), and a memory chip along with some electronic circuitry; an optical plastic "sail" that acts as a waveguide to focus light on the gold sensing surface (surface plasmon layer); and a mirror atop the optical sail to re-direct the reflected light to the photodetector. The short light path of the device results in excellent detection sensitivity. The card-edge connector allows the device to interface with state-of-the-art digital signal processing (DSP) electronics, allowing the high-speed collection of SPR curves in real-time. The resident memory chip (16

kilobit) can be utilized for storage of sensor identification information, calibration data, use history and the like. Additionally, the Spreeta 2000 has been designed so that multiple sensors can be aligned side-by-side on 9 mm centers.

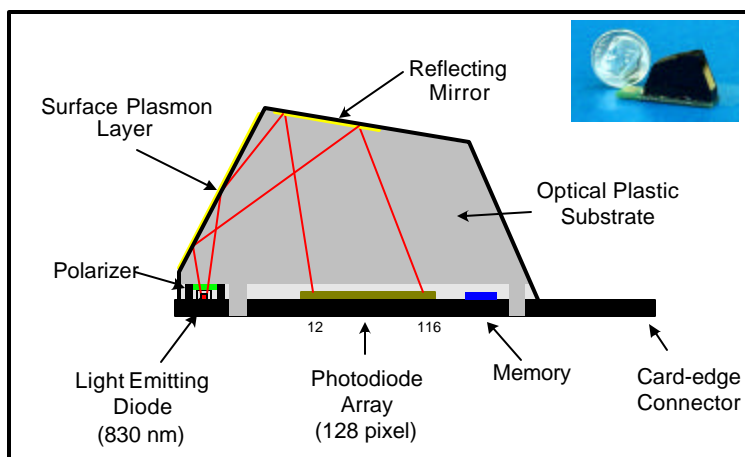


Figure 3. Drawing of the components of the Spreeta 2000 SPR sensor. The inset photograph provides an indication of the actual size of the device relative to a U.S. dime.

THE PROLINX OCTAVE SYSTEM

The Prolinx Versalinx Chemical Affinity Tools coupled with the Texas Instruments Spreeta 2000 enable the development of a unique instrument (the Octave System) for increased throughput molecular interaction analysis. The remainder of this paper will focus on the design of the Octave System and detail many of its unique features.

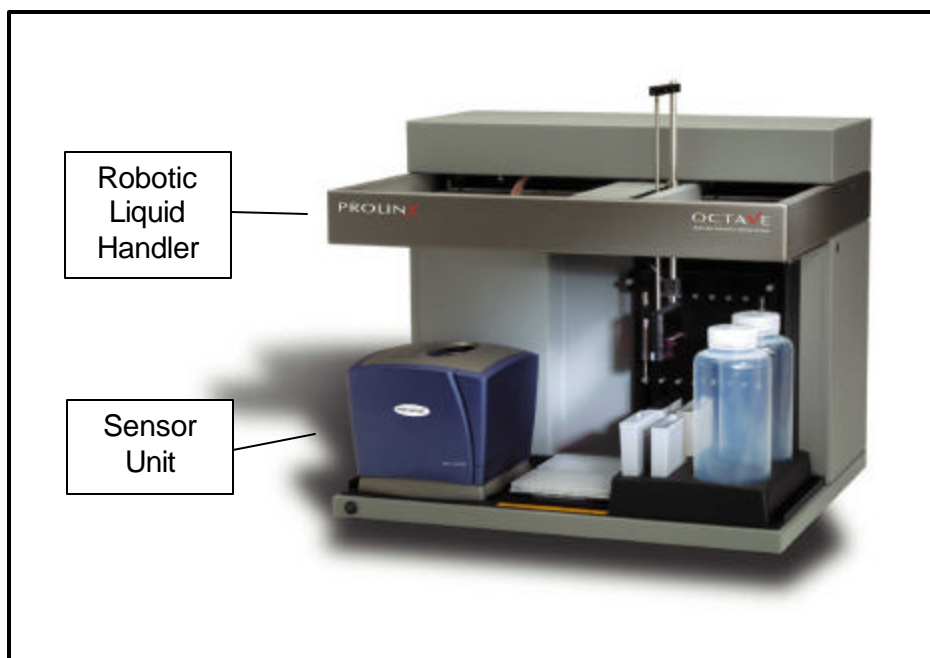
Sensor Surface Chemistry

Prolinx has developed proprietary Versalinx reagents that allow the incorporation of SHA on the surface of a gold film through the formation of a binary self-assembled monolayer (SAM), a well-characterized process (10,11). This SHA-SAM is designed to provide optimal immobilization of PBA-conjugated biomolecules as well as to exhibit extremely low non-specific binding. The molecularly thin, uniform SAM lessens the complicating effects of inefficient or obstructed mass transport during the association and dissociation processes. It also minimizes loss of SPR sensitivity due to its close proximity to the gold surface (SPR sensitivity decreases exponentially with distance from the metal film). Immobilization of PBA-conjugated recognition elements takes place rapidly (15 to 60 minutes). The density of immobilized biomolecule can be easily tuned by adjusting the quantity of input material. Degraded or spent surfaces can be stripped of immobilized species and reconstituted with fresh PBA-conjugate.

System Design

The small size, low mass and self-contained optics of the Spreeta 2000 sensor allow for several unique design opportunities for the Octave. As mentioned previously, the Spreeta 2000 sensor has been designed to allow multiple sensors to be aligned side-by-side on 9 mm centers. Such an alignment corresponds with the well-to-well spacing in industry standard 8 x 12 multi-well

sample plates commonly used in biological research. A large number of instruments are commercially available for manipulating samples in this multi-well format. Hence, the Octave combines an OEM Robotic Liquid Handler for manipulating samples stored in multi-well plates with a small, modular Sensor Unit containing eight linearly arrayed Spreeta 2000 sensors. Figure 4 provides an illustration of the Sensor Unit and Robotic Liquid Handler. Communication between the control computer and the Sensor Unit utilizes a USB interface. Communication with the Liquid Handler passes through the Sensor Unit to manage command sequencing and



timing more efficiently. Data can be acquired and stored from all eight sensors simultaneously.

Figure 4. Illustration of the Prolinx Octave System (minus control computer).

Sensor Unit

The Sensor Unit contains the following components: thermal block, agitator assembly, optical shutter, control electronics, and digital signal processing electronics.

The thermal block houses the eight Spreeta 2000 sensors. Each sensor is packaged in an individual plastic cartridge which is easily inserted into and removed from an electrical connector in the thermal block. The cartridge contains a slotted silicone gasket which sits atop the sensing surface of each Spreeta, and defines the area of the gold film on which the desired biomolecule is immobilized ($\sim 12.5 \text{ mm}^2$). The hinged top of the thermal block contains a replaceable plastic well liner, which mates with the silicone gaskets of the array of cartridges to provide wells for the samples to be analyzed. The wells will support volumes from approximately $20 \mu\text{L}$ to about $100 \mu\text{L}$. Cartridges with spent sensors are disposable. The well liner also contains sixteen “pre-conditioning” wells for thermal equilibration of samples and solutions prior to and during analysis. Figures 5 and 6 provide illustrations of the sensor cartridge and the thermal block.

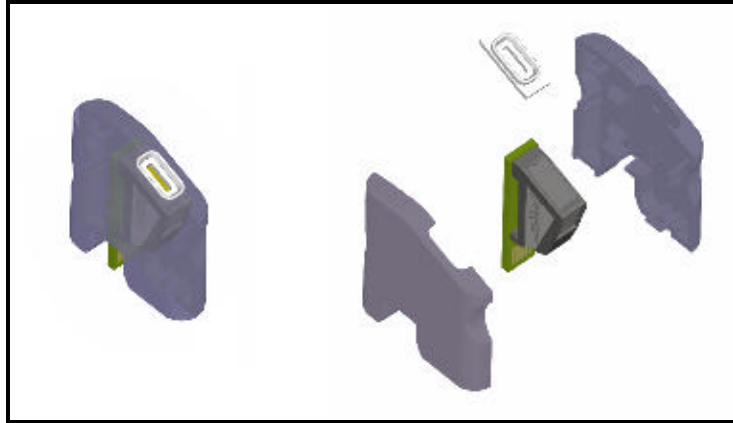


Figure 5. Assembled and exploded views of the sensor cartridge.

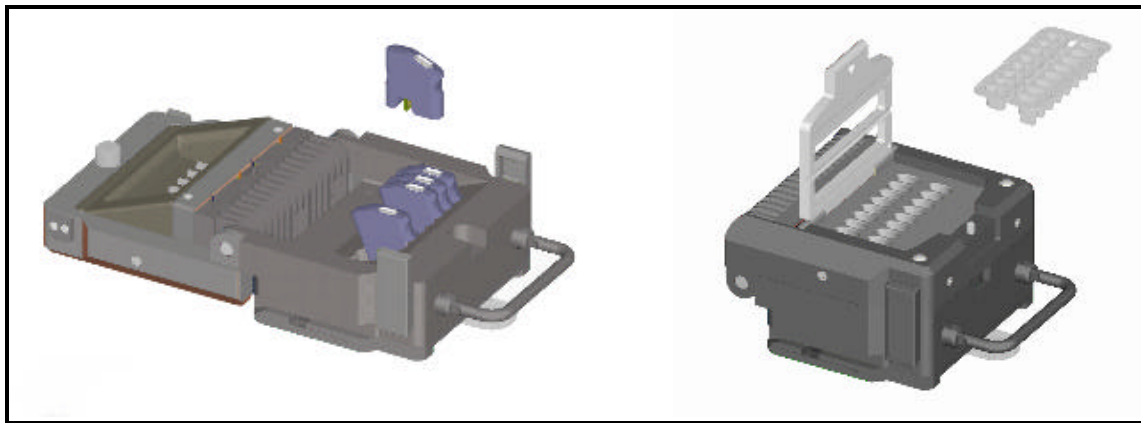


Figure 6. Thermal block views. The left-hand illustration shows the thermal block with its top open for access to the sensor cartridges. The right-hand illustration depicts the removable plastic well liner.

The thermal block provides highly accurate Peltier control of the sample temperature during analysis. This is critical as refractive index is very sensitive to temperature. Sample temperature is maintained to within $\pm 0.1^{\circ}\text{C}$ of the set-point over the temperature range 15°C to 40°C . Additionally, well-to-well temperature uniformity is $\leq \pm 0.2^{\circ}\text{C}$ over the same temperature range. The thermal block, cartridge and sensor materials as well as the surface chemistry are compatible with temperatures as high as 65°C .

The agitator assembly provides the means for efficient orbital sample mixing during analysis. Agitation speed is user-programmable from 150 to 1500 rpm, and the optimal agitation speed is determined by the user. The small radius of orbit (0.5 mm) and the shape of the sample wells minimizes vortexing during sample agitation. The thermal block slides into slots in the agitator platform and locks in place during use. The agitator has a magnetic homing capability that assures that the block returns to the same location following each analysis.

The optical shutter opens to allow transfer of samples into the sample and pre-conditioning wells, and closes during data acquisition to minimize background noise due to stray light.

The electronics for temperature and agitation control as well as for digital processing of the eight sensor signals reside primarily on two highly dense PC boards located in the base of the unit. The unit is designed to prevent damage to the electronics by accidental liquid spills.

The Octave can acquire raw SPR data from the Spreeta 2000 sensors at up to 400 curves per second. The curves are averaged on-the-fly and the minimum of the averaged curve calculated by the DSP electronics before being sent to the host computer. Figure 7 shows sample SPR data from the Spreeta 2000 sensor taken using the Octave Sensor Unit. The sample is pure water; the sensor was initialized in air to provide the background blank. The plotted curve represents the average of 200 individual scans acquired in one second. Baseline noise was determined by acquiring averaged SPR curves every second for 600 seconds and calculating the position of the minimum of each curve using a first moment of resonance below baseline algorithm (12). The plot of the minimum versus time was analyzed using a sliding 60 second window to calculate the RMS noise. The trace shown is a plot of the calculated noise at 5 second intervals, plotted at the endpoint of the time window. The typical average noise value is $<1 \times 10^{-7}$ refractive index units (RIU).

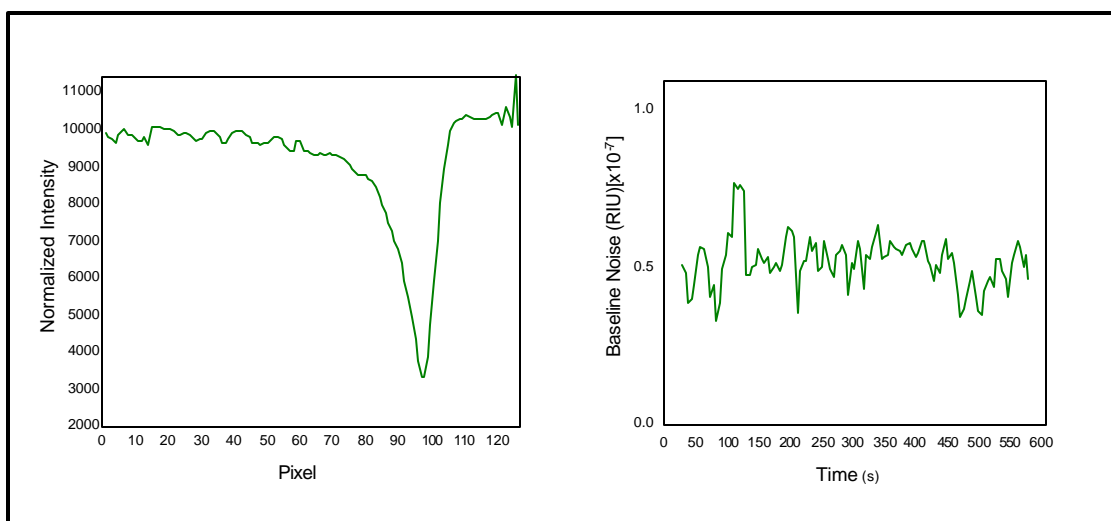


Figure 7. Typical SPR data (left-hand plot) and baseline noise (right-hand plot) for a typical Spreeta 2000 sensor.

Robotic Liquid Handler

Liquid handling on the Octave system is performed using a modified CAVRO MSP9000, a rugged and reliable OEM instrument for liquid handling in multi-well plate format. The footprint of the robot (minus computer) is 22 inches wide by 19 inches deep by 20 inches high, so that the Octave occupies a relatively small amount of valuable bench space. The deck of the robot contains the Sensor Unit, two multi-well sample plates, a wash station for the liquid handling probes, up to three solution stations, a wash buffer bottle and a waste bottle. Liquids are transferred by three-dimensional translation of the liquid handling head. The head has eight dual-needle probes. One needle of each probe is connected to an 8-channel syringe pump for precision transfer of sample solutions, regeneration buffer, and the like among the multi-well plates, sensor wells, pre-conditioning wells and solution stations. Additionally, liquid from the wash buffer bottle is delivered through these needles. Liquid transfer and wash buffer delivery is controlled by a solenoid valve on each syringe. The other needle is used to aspirate liquids to

waste using a diaphragm pump, primarily during probe washing cycles. All transfers by the liquid head are performed in a row of eight at a time using single set of transfer parameters.

Software

Two software packages for the Octave system are currently under development: Instrument Control/Data Acquisition and Data Analysis/Modeling. They are designed to assist novice users in setting up and performing experiments and in analyzing the resulting data in the context of several common interaction models, as well as to provide expert users with sufficient flexibility to create their own unique methods and analyze data according to non-standard models.

The Instrument Control/Data Acquisition software contains the Users, Sample Table, Methods, Sensors and Reports screens. The Users screen lists all authorized users of the instrument, along with any permissions or privileges assigned to them (e.g., access to other users' methods, ability to modify instrument parameters). The Sample Table screen provides a graphical and tabular interface for the user to input sample information such as source, location in sample plate, The Methods screen lists all of the methods available to the logged-in user and allows creation of new or editing of existing methods. Method creation and editing utilize a graphical interface, with icons representing fundamental hardware processes that are added to a method using "point-and-click" functionality. Certain hardware processes (e.g., "transfer sample", "start acquisition", "wash probe") have user-selectable parameters (e.g., volume, from position/to position, data acquisition period). Methods and sequences are run from this screen as well. When a method is running, the active process is highlighted on screen, and if acquisition is occurring, the data is graphically displayed (both SPR curves as well as calculated curve minimum versus time). The user can select how many channels of data are displayed simultaneously. Data is stored in an SQL-compatible data base, and can optionally be exported as Microsoft Excel spreadsheets or text files. The Sensors screen details information about the eight sensor positions (e.g., sensor installed, sensor initialized, sensor serial number, sensor use history). It also maintains a data base of all sensors that have been used in the instrument. The Reports screen provides printable listings of methods, sensors and users.

Experienced users have access to the Instrument screen in the Instrument Control/Data Acquisition software. This allows the user to set various instrument parameters prior to running a method (e.g., data acquisition rate, number of SPR curves averaged per data point, LED brightness).

The Data Analysis/Modeling software is designed to assist users in selecting potential models of the interaction under study, fit the acquired data to the models, and assess the "goodness-of-fit" to each model. Depending on the experimental setup, users can obtain rate constants, equilibrium constants and component concentrations using first or second order interaction models. The user can select from non-linear least squares (13) and global analysis (14) curve-fitting routines from which to extract the parameters of interest. Residuals for each curve fit are plotted to assist the user in qualitatively assessing the "goodness-of-fit". Experienced users can import more complex interaction models if desired.

SUMMARY

The Prolinx Octave System combines a novel miniaturized SPR-based sensor with reliable and easy-to-use surface chemistry to provide a high-throughput automated instrument for molecular interaction analysis.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance received from Nicholas Pavlenko, Chuck Fontana, Nancy Neel and Rich Filice of CAVRO Scientific Instruments, Inc. in modifying the existing MSP 9000 robot for the Octave system. Additionally, the authors thank Dr. Tim Chinowsky and Dr. Charlie Campbell, both of the University of Washington, for their continuing insightful suggestions during the development of the instrument.

REFERENCES

1. Rich, R. L. and Myszka, D.G. (2000) *Curr. Opin. Biotechnol.* **11**, 54-61.
2. Schuck, P. (1997) *Annu. Rev. Biophys. Biomol. Struct.* **26**, 541-566.
3. Myszka, D.G. (1999) *J. Mol. Recognit.* **12**, 390-48.
4. Liedberg, B., Nylander, C. and Lundstroem, I. (1983) *Lab. Sensors and Actuators* **4**, 299-304.
5. Nice, E.C. and Catimel, B. (1999) *BioEssays* **21**, 339-352.
6. Salamon, Z., Tollin, G. and Macleod, H.A. (1999) United States Patent No. 5,991,488.
7. Stolowitz, Ahlem, C., Hughes, K., Kaiser, R., Kesicki, E., Li, G., Lund, K., Torkelson, S. and Wiley, J. (2001) *Bioconjugate Chem.* **12**, 229–239 .
8. Wiley, J., Hughes, K., Kaiser, R., Kesicki, E., Lund, K. and Stolowitz, M. (2001) *Bioconjugate Chem.* **12**, 240-250 .
9. Melendez, J., Carr, R., Bartholomew, D.U., Kukanskis, K., Elkind, J., Yee, S. Furlong, C. and Woodbury, R. (1996) *Sens. Actuators B* **35**, 1-5.
10. Prime, K.L. and Whitesides, G.M. (1991) *Science* **252**, 1164-1167.
11. Lahiri, J., Isaacs, L., Tien, J and Whitesides, G.M. (1999) *Anal. Chem.* **71**, 777-790.
12. Chinowsky, T.M., Jung, L.S. and Yee, S.S. (1999) *Sens. Actuators B* **54**, 89-97.
13. O'Shannessy, D.J., Brigham-Burke, M., Soneson, K.K., Hensley, P. and Brooks, I. (1993) *Anal. Biochem.* **212**, 457-468.
14. Beechem, J.M. (1992) *Meth. Enzymol.* **210**, 37-54.

TRADEMARKS

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