

**AUTOLAB**

Surface

*Plasmon*

Resonance

*Instruments*

THE INSTRUMENTS

THE POSSIBILITIES

THE SOFTWARE

**DEDICATED  
TO RESEARCH**

# - International presence -

ISO 9001:2000 *certified,*

Eco Chemie

has been setting

the *benchmark* in

research instrumentation

Founded in 1986 and since 1999 a member of the Metrohm group of companies, Eco Chemie designs and manufactures state of the art Autolab series of instrumentation and software for biochemistry, electrochemistry and electrochemical characterization of materials.

Recently Eco Chemie updated the biosensor product line based on the principle of surface plasmon resonance, a phenomenon that occurs when p-polarized light hits a gold layer at the interface of media of different refractive index; the glass of a sensor disk with high refractive index and a buffer solution with low refractive index.

An evanescent wave is generated when the light reaches the gold layer and the energy is absorbed by the free electrons in the gold layer, generating resonance plasmons and causing a reduction in the intensity of the reflected laser light.

The surface plasmon resonance (SPR) technique has become a standard tool in life sciences and pharmaceutical research laboratories. Combined with an Autolab potentiostat/galvanostat, the SPR instruments have the unique possibility of simultaneous electrochemical and SPR measurements, usually referred to as ESPR.

The most commonly used techniques in combination with surface plasmon resonance are:

- Cyclic voltammetry
- Chrono-amperometry
- Impedance spectroscopy
- Stripping voltammetry

SPR can provide:

- Quantitative information
  - Total concentration of a biomolecule in solution
- Determination of reaction kinetics
- Affinity constants for molecular interactions
- Discrimination between active and non-active molecules
- Qualitative tool for ligand screening
- Complex assembly studies
- Investigation of steric/allosteric effects
- Biocompatibility with coatings
- Interaction of a molecule with a coating



With our background and knowledge in electrochemistry and biochemistry and our worldwide distributor network, our mission is to serve the research community worldwide by supplying state of the art instruments, software and accessories.

The Autolab SPR instruments serve a wide range of research applications:

- Biotechnology
- Conducting polymers and membranes
- Enzymes
- Ion channels
- Membrane proteins
- Nanotechnology
- Polymerisation
- Sensor development

# - Unique possibilities -

Autolab ESPRIT allows  
 the user to *combine*  
 SPR measurements with  
*electrochemical* measurements



## AUTOLAB ESPRIT

The surface plasmon resonance technique helps the scientist to analyse bi-molecular interactions in real time, without labeling requirements.

The study and characterization of molecular interactions are essential to explore the structure-function relationships of biomolecules and to aid our understanding of biological systems in life sciences.

Combining electrochemistry with SPR makes the Autolab ESPRIT perfect for biosensor and DNA/RNA or protein chip research.

The double channel ESPRIT is designed with an open cuvette system, giving the user access to the cuvette as well as the gold disk.

The first channel is used to measure the specific binding of the sample, the second channel is used to compensate for non-specific binding, matrix and temperature effects.

The integrated high throughput autosampler provides the possibility to measure multiple samples automatically without attendance, it also provides the option to recover precious samples or unknown interacted molecules.

ESPRIT comes with powerful data acquisition software, which includes automation tools for instrument control.

All recorded data is evaluated in a separate kinetic evaluation package, the raw data can also be exported into any third party file formats. An additional software component to comply with GLP guidelines is available.

### Application areas

- Chemical sensors
- Affinity sensors
- Immunosensors

### Fundamental prerequisites

- Efficient and stable immobilization
- Complete retention of the biological properties of the sample under study
- Accessibility of biomolecules

### Key features

- Automated high throughput
- Open cuvette system with reference channel
- Large dynamic refractive index range

# - Compact SPR -

*Entry level*

single channel

*SPR instrument with*

*ESPR capabilities*



## AUTOLAB SPRINGLE

SPRINGLE is the latest addition to the Autolab family of instruments. It is a single channel surface plasmon resonance instrument, based on the optical principle of the ESPRIT.

The compact design makes SPRINGLE an affordable alternative for electrochemists who want to acquire an SPR signal during electrochemical measurements or for biochemists who want to get acquainted with the SPR technique in general.

To enable electrochemical measurements, the Autolab SPRINGLE can be connected to any of our Autolab potentiostat/galvanostat instruments. The SPR data can be used as a reference or to complement results of electrochemical experiments.

For example, during electropolymerization, SPR can be used to monitor the growth and properties of the polymer layer on the surface.

SPRINGLE uses disposable pipette tips to aspirate and dispense samples, allowing fast and clean sample changing.

The inject sequence is semi-automatic: the only manual action is placing a sample vial under the pipette tip, the sample is aspirated and injected in the cuvette when measurement starts. It is also possible to manually inject a sample using a pipettor.

The open cuvette system allows quick access, easy electrochemistry and does not have any clogging or congestion problems, the open system also allows multiple sample additions.

### Application areas

- Chemical sensors
- Affinity sensors
- Immunosensors

### Fundamental prerequisites

- Efficient and stable immobilization
- Complete retention of the biological properties of the sample under study
- Accessibility of biomolecules

### Key features

- Affordable compact instrument
- Open cuvette system
- Large dynamic refractive index range
- Single channel

# - Maximum flexibility -

## THE SPR PRINCIPLE

Surface plasmon resonance occurs when p-polarized light hits a gold layer at a certain angle under conditions of total internal reflection. An evanescent wave is generated and the energy is absorbed by the free electrons in the gold layer, generating resonance plasmons and causing a reduction in the intensity of the reflected laser light.

Since molecules are immobilized on the gold surface, the refractive index changes, altering the angle at which the plasmon effect occurs.

### Optical unit

The Autolab SPR instruments use a scanning mirror to alter the angle of incidence of the laser light. The mirror scans 4 degrees at a certain offset angle, with a frequency of 76 Hz.

The minimum in reflection intensity, known as SPR dip, is detected automatically and plotted as a relative SPR angle in the software. The offset angle can be manually controlled with a spindle, providing a large dynamic range.

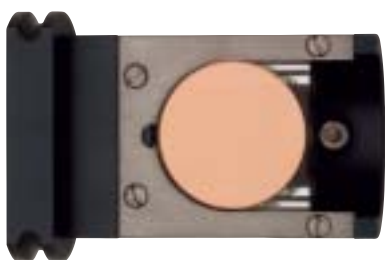
The high scan frequency results in low noise measurements. The laser spot has an area of 2 mm<sup>2</sup> on the sensor surface. Measured data will be an average of all events in that area.

This has the advantage that any inhomogeneity in, or defects of any of the additional layers on top of the gold surface will be eliminated.

### Substrates

Eco Chemie provides standard sensor disks: bare gold substrates made of BK-7 glass with 48 nm of gold deposited on a thin layer of titanium.

The low cost sensor disks are placed on a retractable slider with a hemicylinder (also BK-7). To increase compatibility, a special slider for measurements on Biacore sensorchips is available.



The range in refractive indices of the sample solution under study can be extended by using different types of glass hemicylinders and substrates.

### Cuvette

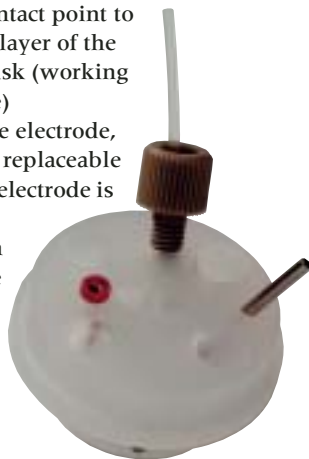
Except for the diameter of the cuvette and the geometry of the needle, the following parameters can be controlled by the user:

- Mixing volume
- Speed or frequency of mixing
- Distance of the pipette tip/needle to the sensor surface
- Volume of the solution in the cuvette
- Viscosity of the solution
- Temperature of the solution

This makes the instrument very suitable for fundamental research. It is also possible to use custom-built cuvettes and/or cuvettes of different materials.

The ESPRIT and SPRINGLE have a unique electrochemical cuvette that allows measurements on a 3-electrode system;

- Fixed contact point to the gold layer of the sensor disk (working electrode)
- Reference electrode, which is replaceable
- Counter electrode is a fixed Platinum electrode



### Liquid handling unit

Sample handling can be done manually, semi-automatic or fully automatic. In the Autolab ESPRIT, the two channels of the cuvette use independent syringe pumps to assure reproducible experimental conditions.

### Fully automatic

The autosampler is controlled by the liquid handling tool in the software. Samples are taken from the microliter plate and injected in the cuvette. The automatic control allows the user to run measurements continuously.

The software has predefined rack-codes for different kinds of microliter plates, but it is also possible to define custom-made sample holders.

### Semi-automatic control

The sample is aspirated in the needle/pipette tip or pipetted in the cuvette manually.

Other liquid handling functionality like dispensing, draining, washing, mixing of sample and measurement is performed automatically.

### Manual control

After the sample is pipetted into the cuvette, all following steps like mixing, draining, washing, starting of measurement, rinsing etc. are controlled manually from the software with a simple mouse click.

### Autolab Spincoater

The Autolab Spincoater is an affordable tabletop spin coating system that can be used to coat uniform layers of for example polymers or metal oxides on a substrate.

The system has a bowl with a diameter of 13.5 cm. Rotation speed can be controlled manually from 100 - 10,000 rpm with an accuracy of 2 rpm in steps of 100 rpm.



#### SPECIFICATIONS AUTOLAB SPINCOATER

- |                             |                                   |
|-----------------------------|-----------------------------------|
| ● Standard sample size      | 25 mm Ø disk                      |
| ● Motor speed range         | 100 - 10,000 RPM                  |
| ● Manual speed setting      | 100 - 10,000 RPM in 100 RPM steps |
| ● Acceleration/deceleration | 4,000 RPM/s                       |

# - Easy to use -

The *sophisticated*

control and data

acquisition *software*

makes complex

tasks *easy*

## THE AUTOLAB SPR SOFTWARE

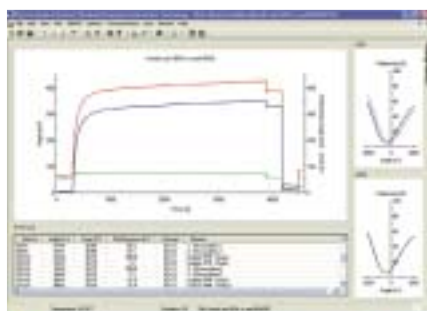
The Autolab SPR software package contains a program for data acquisition and a separate program for kinetic evaluation. Both programs operate under Windows 2000 and Windows XP.

### Data acquisition

The data acquisition software contains all necessary tools to control the instrument and to measure and store data of interaction measurements.

After stabilizing the sensor surface, phase one in interaction measurements is to make the sensor surface biospecific by immobilizing a ligand, the next phase is the measurement of analytes on the biospecific surface, and finally the sensor surface is regenerated.

All four measurement phases (baseline, association and regeneration) are plotted in the 'Data Presentation' window and are controlled through measurement sequences.

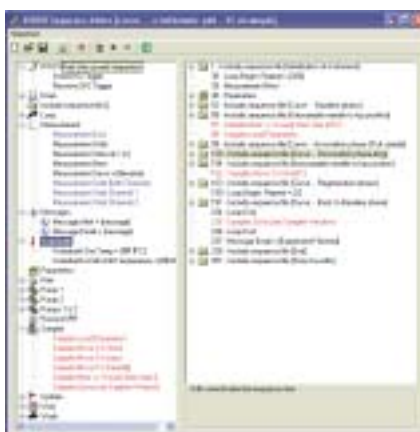


### Sequencer

A sequence is a list of commands that is used to automate the control of the instrument.

The Autolab SPR software comes with a complete set of sequences for all kinds of measurements. It is also possible to create new sequences or to edit existing ones in the 'Sequencer' window.

The sequencer, combined with the autosampler functionality of the Esprit, allows you to operate the instrument 24 hours per day, 7 days per week.



Multiple sample injections are executed by the sequencer based on sample positions indicated in the 'Auto-sampler Control' window.

In this window, sample positions, identification and incubation times can be specified.

### Sample recovery

It is possible to add samples during measurement, to perform duplicate measurements on each sample and to perform sample recovery.



### Data Presentation

The 'Data Presentation' window shows all measured data; the baseline, the association, dissociation and regeneration phase. The temperature of the slider is also recorded at the same interval, it can be used to compensate for temperature effects.

Users can add markers to any data point on the plot and add comments on special events or specific areas of interest that are logged into an event recorder. The double channel ESPRIT instrument has the option to plot the data of both channels as well as the differential signal.

### Measurement integrity

The data acquisition software was developed to meet GLP guidelines and facilitates compliance with 21 CFR Part 11. The software security features include:

- Log in with user ID and password
- Multiple access levels
- Protected sequences for standard use
- Protection of data with electronic signatures
- Log and audit trails

### Kinetic evaluation

The SPR data collected by the data acquisition software can be analysed with the kinetic evaluation software.

With the kinetic evaluation software the following parameters can be calculated:

- Kinetic rate constants
- Affinity constants
- Thermodynamic parameters

The above parameters can be calculated using the multiple curves measured with different concentrations.

The actual curves can be fitted to different kinetic models.

SPECIFICATIONS	ESPRIT	SPRINGLE
<ul style="list-style-type: none"> <li>Measuring principle</li> <li>Transducer principle</li> <li>Liquid handling</li> <li>Parallel channels</li> <li>Fixed wavelength</li> </ul>	Surface plasmon resonance Scanning mirror Cuvette system 2 670 nm	Surface plasmon resonance Scanning mirror Cuvette system 1 670 nm
<ul style="list-style-type: none"> <li>Sample loading and injection</li> <li>Mixing</li> <li>Autosampler</li> <li>Pumps</li> <li>Flow rate range</li> <li>Sample volume</li> </ul>	Manual, semi-automatic and automatic control Continuous with autosampler needles Integrated Syringe and peristaltic 0.8 - 227 µl/s 20 - 150 µl	Manual and semi-automatic control Continuous with pipette tip n.a. Syringe and peristaltic 0.8 - 227 µl/s 20 - 150 µl
<ul style="list-style-type: none"> <li>Offset of SPR angle by spindle</li> <li>Dynamic range</li> <li>Angle resolution</li> </ul>	62° - 78° 4,000 m° < 0.05 m°	62° - 78° 4,000 m° < 0.05 m°
<ul style="list-style-type: none"> <li>Refractive index</li> <li>Refractive index resolution</li> <li>Minimum molecular weight</li> <li>Measuring frequency</li> <li>Time interval range</li> <li>Baseline noise</li> </ul>	1.26 - 1.38 (Standard BK7) 1.32 - 1.44 (Special N-BAF3) 1.40 - 1.52 (Special NSF15) < 2 × 10 <sup>-6</sup> 180 D 76 Hz 0.1 s - 300 s 0.1 m°	1.26 - 1.38 (Standard BK7) 1.32 - 1.44 (Special N-BAF3) 1.40 - 1.52 (Special NSF15) < 2 × 10 <sup>-6</sup> 180 D 76 Hz 0.1 s - 300 s 0.1 m°
<ul style="list-style-type: none"> <li>Sensor disk</li> </ul>	Gold coated glass disk (25 mm Ø)	Gold coated glass disk (25 mm Ø)
<ul style="list-style-type: none"> <li>Standard supplied cuvette</li> <li>Additional cuvette</li> <li>Biacore sensor chip adaptor</li> </ul>	Combined ESPR cuvette Optional for normal SPR Optional	Combined ESPR cuvette Optional for normal SPR Optional
<ul style="list-style-type: none"> <li>Weight</li> <li>Dimensions (W × D × H)</li> <li>Power requirements</li> </ul>	40 kg 60 × 51 × 45 cm <sup>3</sup> 170 W	23 kg 40 × 33 × 36 cm <sup>3</sup> 170 W



## ECO CHEMIE

KANAALWEG 29 G  
3526 KM  
UTRECHT

PO BOX  
85163  
3508 AD  
UTRECHT  
THE NETHERLANDS

TELEPHONE  
+ 31 30 289 31 54

TELEFAX  
+ 31 30 288 07 15

E-MAIL  
AUTOLAB@ECOCHÉMIE.NL

HOMEPAGE  
WWW.ECOCHÉMIE.NL



ISO 9001:2000

THE INSTRUMENTS

THE POSSIBILITIES

THE SOFTWARE

YOUR AUTOLAB DISTRIBUTOR