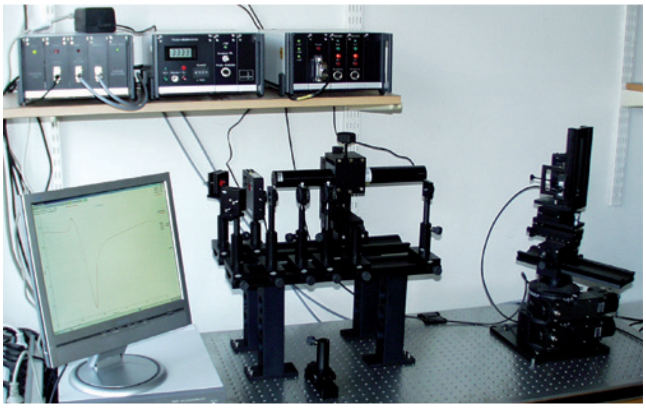
Surface Plasmon Spectrometer RT-08

Introduction

Surface Plasmon Spectroscopy (SPS) has established itself as a very powerful technology for labelfree interaction analysis on surfaces in real-time. It utilises changes in surface sensitive propagation of evanescent waves due to adsorption of molecules.

The RT-08 Surface Plasmon Spectrometer is the result of decade long development of optical evanescent wave spectroscopy set-ups within the group of Prof. Wolfgang Knoll (Material Science) at the Max-Planck-Institute for Polymer Research in Mainz, Germany.

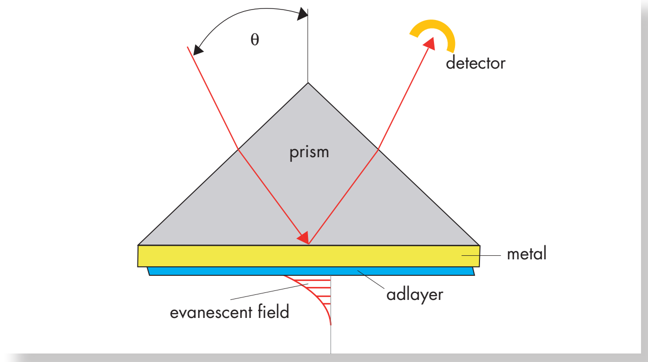
Designed originally for basic research the RT-08 Spectrometer combines easy handling and robustness with a maximum in flexibility. The use of high quality components guarantees highest reliability. A novel arrangement of the optical components makes the alignment of the spectrometer fast and simple. The open and modular system allows easy implementation of extensions for microscopy, electrochemistry, fluorescence detection or QCM-D.



SPR Technology

Surface Plasmons are surface electromagnetic waves that propagate parallel along a metal/dielectric interface. These plasmons are very sensitive to even small changes at this boundary as for example induced by adsorption of molecules to this surface. Plasmon excitation requires p-polarized light (polarization occurs parallel to the plane of incidence), most setups operate with a low power visible laser and polarisers. Most practical applications utilise the Kretschmann configuration. A thin metal film is evaporated on the base of a prism and the evanescent wave propagates through the metal film.

The major components, light source, sample holder and detector are mounted on a precise goniometer stage for precise control of incoming and reflected light beam. In case of plasmon resonance a dip in the reflected spectrum is observed.

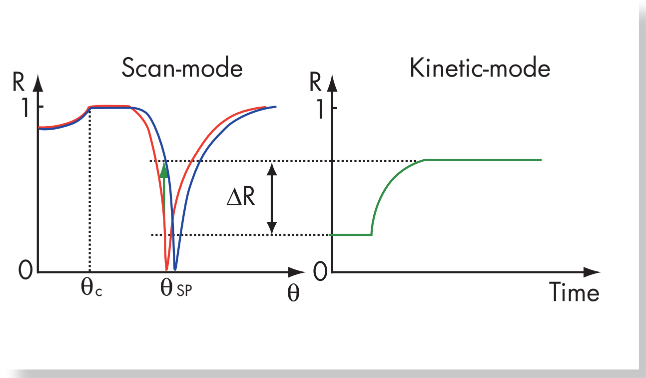


Kretschmann configuration

If an additional adlayer of a dielectric compound is attached to the metal layer, the resonance frequency, i.e. the angle at which the surface plamon is detected shifts to larger angles. In the so-called "scan mode", again, angular scans are performed to obtain surface plasmon spectra, from which the optical thickness of the dielectric adlayer can be obtained by model-fit calculations.

Besides the scan-mode which yields static information about the generated adlayer at the metal surface, the SPR technique can be used also to obtain kinetic information of the dielectric adlayer formation in-situ. In this case, the reflectivity is measured as a function of time at a distinct angle of incidence. Typically the angle chosen is at the low slope of the decreasing reflectivity within the surface plasmon resonance peak. Binding of a dielectric compound to the interface causes the resonance angle of the surface plasmons to shift to higher angles due to the formation of the dielectric adlayer. This shift causes the reflectivity at the low slope of the resonance peak to increase over time until a constant value of the reflectivity is reached, i.e. formation of the adlayer is completed.

RT-08 Spectrometer



Principle of Scan- and Kinetic-mode SPR

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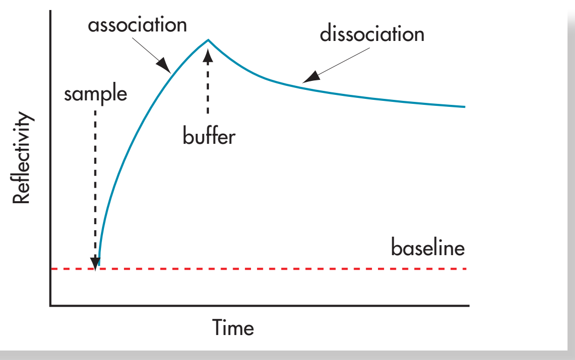
The RT-08 spectrometer is a flexible yet powerful bread-board based system. Plasmon excitation is achieved with a standard 632 nm HeNe laser, a sensitive silicon detector senses the reflected light. Polarisers allow easy selection of p- or s-polarised light, the measurement cell is specifically optimised for small sample volume, a peristaltic pump can be offered as an option. A preconfigured Windows based computer system controls the spectrometer. The WASPLAS software has been constantly improved for SPR applications since 1997. Careful component selection and optimisation of the setup guarantees low sensitivity measurements down to 10 pg/mm^2 for label-free protein adsorption. The optional PMT fluorescence detection enhances the sensitivity down to the atto-molar level, corresponding to a molecular surface concentration of only 10 fluorescence labelled proteins [mm^2 min]

The system comes in a light tight box with a small footprint of only 100 x 60 cm^2. System options like Lock-In detection, PMT Fluorescence and Temperature Control can easily be upgraded in the field.

Applications

Protein Interaction

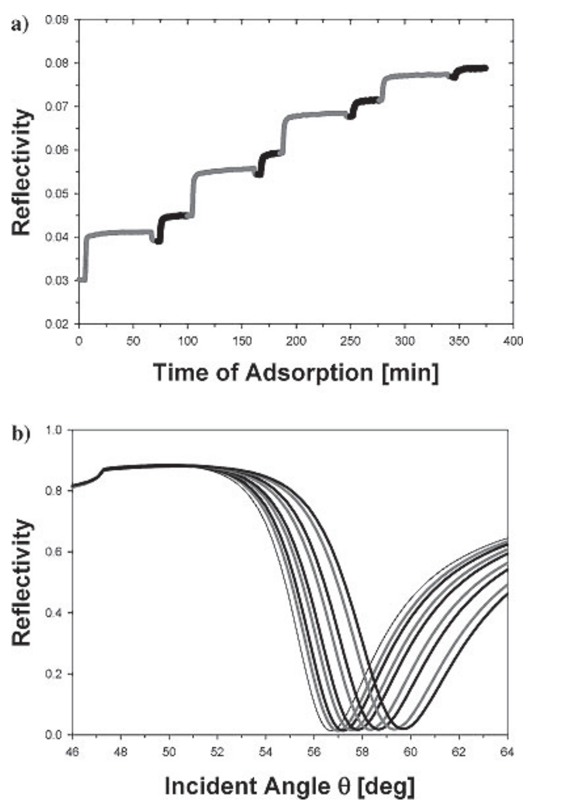
SPR spectroscopy today is the most accepted technology for label-free monitoring of molecular interactions in real-time. A sensor surface with an immobilised reaction partner is stabilised with buffer solution. Upon injection of a sample solution binding of the molecules is monitored using the kinetic-mode SPR (association step). Flushing back with buffer solution causes a certain percentage of sample molecules to desorb from the surface (dissociation step). Monitoring and modelling these reaction steps gives important information on the binding specificity, concentration, affinity and kinetics of the molecular interaction process.



SPR curve protein binding

Film Formation: Layer-by-layer Assembly (LbL)

Layer-by-layer deposition was and still is one of the most common applications for SPR spectroscopy. In scan-mode SPR will give information about the film thickness on the metal substrate. LbL of polyelectrolyte multilayers (PEM) is a versatile technique used to fabricate multilayer thin films with tailored properties. First reported in 1991 by Decher et al. polyelectrolyte multilayers find more applications as substrates or coatings. Recent findings demonstrate that different multilayer architectures can promote protein and cell adsorption.



a) Kinetic-mode SPR scans of a layer-by-layer process demonstrated by the successive deposition of a dendrimer and modified Au nanocrystals. The grey curves indicate the dendrimer addition and the black curves the addition of modified Au nanocrystals, respectively.

b) Scan-mode SPR spectroscopy after the consecutive deposition of dendrimer/Au nanocrystals up to 4 bilayers.

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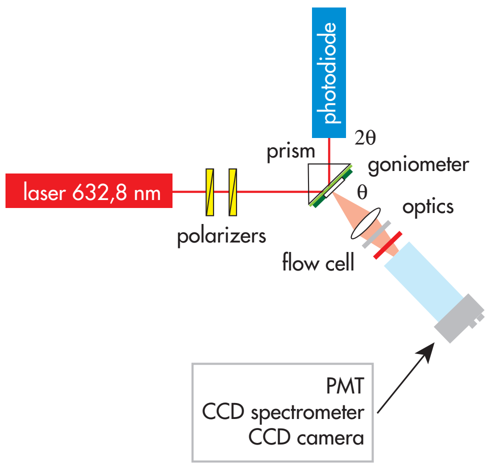
Options

- **Temperature Control**
  While the vast majority of SPR measurements is done at room temperature some applications might require working at different temperatures or controlling the temperature precisely at a set value. Our RT-Temp thermostated cell allows convenient operation at temperatures ranging from 15-50 °C. High precision is guaranteed by a computer controlled Peltier element.

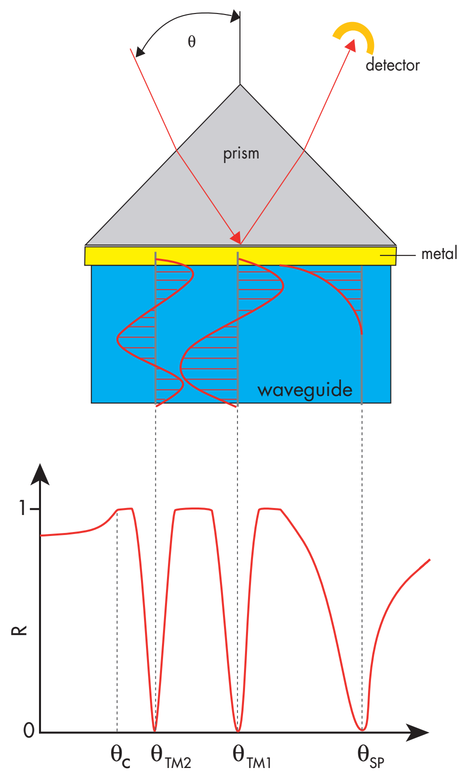
- **Lock-In Amplifier**
  For performing measurements with a maximum in signal-to-noise ratio and straylight rejection we offer our RT-LockIn accessory for the RT-08 spectrometer. In comparison to the standard layout without Lock-in, the S/N ratio is improved by a factor of 2-3. The RT-LockIn option comes complete incl. amplifier and chopper. The RT-08 is already preconfigured, therefore installation is easy and straightforward and can be done in the field.

- **PMT Fluorescence Detection**
  Surface plasmon (field enhanced) fluorescence spectroscopy (SFPS) uses the greatly enhanced electromagnetic field of a surface plasmon mode for the excitation of surface confined fluorophores. The general setup of a SFPS is schematically shown below. The ability to simultaneously monitor the interfacial refractive index changes and the fluorescence signals in real time offers a huge potential for applications of SFPS in surface immunoreaction detection. The RT-PMT fluorescence option comes standard with a photomultiplier tube for maximum sensitivity.
  It can be ordered together with the system or later upgraded in the field. The RT-08 comes with all necessary connectors which makes the upgrade very simple.
  Alternatively to the single element PMT we can offer CCD spectrometers for wavelength resolved measurement or scientific grade CCD cameras for fluorescence imaging on request.

- **Optical Waveguide Fluorescence Spectroscopy**
  Optical waveguide (field-enhanced) fluorescence spectroscopy (OWFS) is a technique similar to SPFS. If the thickness of a dielectric layer on a metal- (e.g., gold or silver) coated substrate is sufficiently large, optical waveguide modes are observed in addition to the surface plasmon resonance (when a substrate with a high refractive index is used).
  By use of the different waveguide modes measured in reflectivity for p- (TM-) and s- (TE-) polarization, respectively, one can calculate/simulate various properties of the waveguide structure. Additionally key parameters of the guided modes can be extracted for example, the angular dependence of the optical fields as one sweeps through the various resonances, the field distribution within the waveguide structure, the optical intensity integrated for each mode across the slab waveguide, etc.



Schematic drawing of PMT fluorescence detection with SFPS



Principle of Optical Waveguide Fluorescence Spectrometer

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Software

The control of the spectrometer is done via WASPLAS software. The origin of WASPLAS dates back to 1997. Until then it was continuously developed by incorporating the feedback and requirements from the 40+ spectrometers installed worldwide. Today WASPLAS is one of the key factors ensuring reliable and robust operation of the spectrometer.

Three different modes of operation are includes with WASPLAS:

- Scan Mode
  Takes the complete angular scan. The scan acquisition time is typically in the range of a few minutes.
- Kinetic Mode
  Monitors the reflectivity as a function of time at a fixed angle of incidence with a time resolution <0,1 s.
- Tracking Mode
  Monitors the shift of the plasmon resonance dip in real time with a time resolution <4 s.

Operation at arbitrary angles of incidence and control of s- and p-polarization of the incident light enables anisotropy measurements and separation of optical constants n and d.

WINSPALL software computes the reflectivity of optical multilayer systems. It is based on the Fresnel equations and the matrix formalism. The typical result is usually the optical thickness [nm] and/or surface coverage [ng/cm^2]

Specifications	
HeNe Laser	632 nm, <10 mW, class 3B
Goniometer	2 cycle with electronic control unit
Coupling Angle	0.005°
Accuracy	< 30 arcsec
Repeatability	< 2 arcsec
Time Resolution	<4s tracking mode <0.1s kinetic mode
Si Photodiode	active area 200 mm^2 , dark current typ. 5 nA
Polarizer	Glan-Thompson Prism, extinction >10^6
Sample Volume	~100 µl
Flow Cell Volume	20 µl Volume down to 2 µl on request
Limit of Detection for Protein Adsorption	appr. 10 pg per mm^2 label free or at attomolar level (10^{-18}) labelled
Dimensions	100 cm x 60 cm x 60cm
Weight	100 kg
Operating Voltage	110/220V / 50/60Hz
Computer	Midi Tower, 17" TFT-display, AMD Athlon 64 processor 3500+ 2.2 GHz, Windows XP

Ordering Information	
RT-08	Complete turnkey SPR spectrometer
RT-LockIn	Lock-In option for RT-08 spectrometer
RT-PMT	PMT fluorescence option for RT-08 spectrometer
RT-Temp	Temperature control, 15 - 50 °C for RT-08 spectrometer
RT-Au	Gold substrates, 5 pcs.
RT-SiO2	SiO_2 substrates, 5 pcs.
RT-C1	Flow cell
RT-C2	$LaSFN_9$ prism

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