The Four Key Concepts of Surface Plasmon Resonance and SPR with Affinité Instruments

Surface Plasmon Resonance, or SPR, is a bioanalytical technique that allows for the study of biological and chemical interactions in real time, and without labelling the analyte. SPR allows observations to be made concerning binding rates and binding levels between molecules so that the specificity, kinetics and affinity of the interaction can be determined.

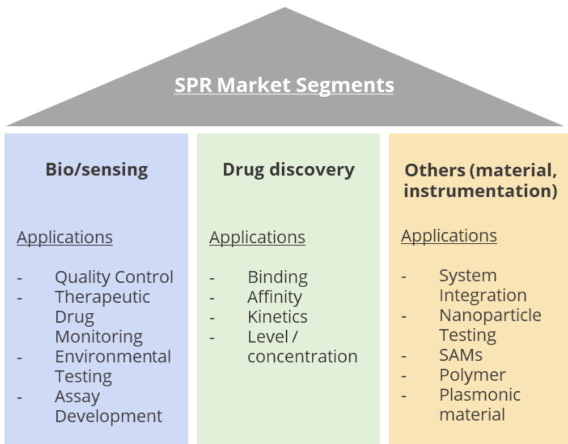

Figure 1 – SPR market segments.

Mostly known for applications in drug discovery, the SPR market has two other rapidly expanding segments, bio/sensing and other applications (Figure 1), driven by increase demand for accessible SPR instrumentation to tackle arising issues in industries such as biotech, environmental testing and food and beverage. A few key established and emerging applications include:

- screening and developing new pharmaceuticals and new bio-therapeutics
- fundamental research such as discovering and characterizing protein function and disease mechanisms
- developing new diagnostic assays
- sensing molecular and chemical targets for food safety and environmental applications
- quality control in bioprocess monitoring
- characterizing new surface chemistries to lower nonspecific binding in biosensing
- testing plasmonic materials for enhanced sensing properties

The objective of this technical note is to provide new and existing users a basic understanding of SPR as an analytical tool in the broader SPR market. The focus is on four key concepts to better understand why Affinité Instruments' SPR innovations can help researchers in various areas accelerate their discoveries.

The key concepts are:

1. Principle of SPR effect
2. SPR instrument configuration and implications
3. Classes of SPR sensors and considerations
4. SPR sensor functionalization for selectivity

Concept 1. Principle of SPR effect

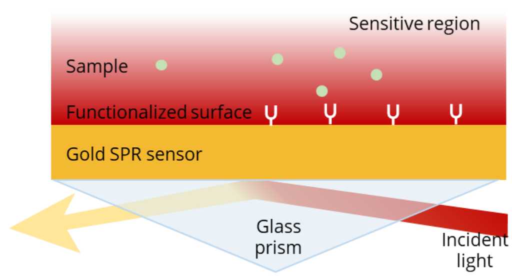

Figure 2 – Schematic of an SPR sensor. The SPR effect generated by the reflected incident light produces a sensitive region responsive to refractive index changes.

A typical SPR sensor is a thin metallic film, usually gold (Au) or silver (Ag) coated on a glass prism from which light is reflected to generate the SPR effect (Figure 2). The sample solution containing targets such as biomolecules or chemicals sits on top of the SPR sensor. The sensitive region is generated by the SPR effect induced by a coupling effect between the incident light in the glass prism and the free electrons of the sensor. The sensitive region extends about 200 nm out-of-plane of the sensor up into the sample solution. This probed region is highly responsive to refractive index (RI) changes. These RI changes occur in binding events, chemical concentration gradients, and polymers changing conformation, among others.

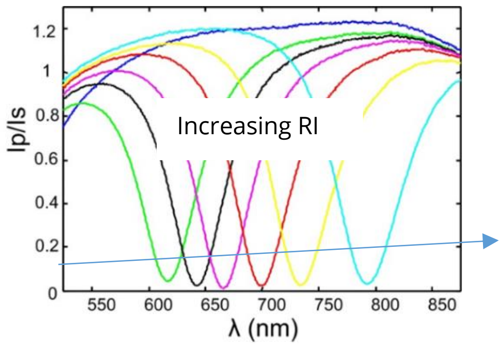

Figure 3 –Reflectance spectra (light polarisation ratio) of solutions with different RI on an Au SPR sensor in wavelength interrogation mode.

The SPR effect is measured in reflectance spectra via light reflected at the glass/Au interface. The spectral signal of SPR is characterized by a sharp drop in reflectance at either a specific wavelength or angle (Figure 3), depending on the interrogation mode. In the most common SPR sensors, the SPR effect occurs in the visible part of the spectrum.

The SPR effect is observed not only with thin film sensors but also with nanoparticles, nanohole arrays and other more exotic designs of SPR sensors. Despite other designs potentially having greater sensitivity than thin films, thin films remain the benchmark in commercial SPR for their advantages, including low-cost manufacturing scalability, high analytical performance reproducibility and instrumentation flexibility (easy to miniaturize and integrate compared to other methods).

Concept 2. SPR instrument configuration and implications

Briefly, SPR instrumentation is defined by the type of light source and detector used to interrogate the SPR sensor. Different classes of SPR sensors require specific types of instrumentation which will be discussed further under Concept 3. Here, the focus is on the instrumentation interrogation design and its implication for thin film SPR sensors.

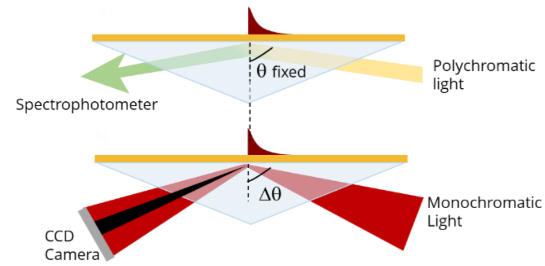

Figure 4 - Wavelength (a) and angular (b) interrogation.

The most popular SPR sensor configuration is the Kretschmann configuration. Here, the metallic thin film sits directly on the surface of a glass prism, as described previously. Light traveling in the glass prism to interrogate the SPR sensor can be performed in two ways: via wavelength interrogation (Figure 4 top) or angular interrogation (Figure 4 bottom).

For wavelength interrogation SPR (also known as fixed angle SPR; Figure 4 top), the incident light source is fixed and is polychromatic, such as a tungsten lamp or a broadband LED. As the light probes the SPR sensor, the spectrum of reflected light is acquired to identify the wavelength of the SPR band. Changes in RI of the sample solution are monitored by tracking the wavelength location of the SPR band over time – as binding occurs for instance.

For angular interrogation SPR (Figure 4 bottom), the light source is monochromatic, often a 633 nm HeNe laser. The incident light is swept at various angles to find the SPR band of the sensor. This method requires a high level of angular precision and alignment between the moving light source and the detector to track the optimal SPR band. Typically, the angle of the incident light is set near the minimum of the SPR band and held constant during an experiment. As the SPR band shifts, the changes in intensity of reflected light at that angle correlate with RI changes in the sample.

Table 1 - Comparison of SPR wavelength against angular interrogations.

Parameters	Wavelength	Angular
Robustness	X	
Miniaturization potential	X	
Sensitivity		X
Throughput	Low	High
Equipment cost	< $100,000	> $100,000
Maintenance / repair fees	$	$$$
User friendly	X	

As presented in table 1, SPR instrumentation based on wavelength interrogation offers a tremendous opportunity to access SPR spectroscopy by reducing the cost barrier affiliated to angular SPR device. The equipment cost of wavelength SPR systems are sometime cheaper than the annual maintenance cost of certain angular systems. In addition, the miniaturization potential and robustness of wavelength SPR devices might unlock new solutions beyond of lab settings such portable field testing device, point-of-care testing and system integration for process monitoring.

Concept 3. Classes of SPR sensors and considerations

Thin-film SPR sensors, 50 nm in thickness, belong to one of the two large classes of sensors, the second being nanoparticles (i.e. particles smaller than 100 nm). SPR using nanoparticles is often referred as localized SPR (LSPR) because the free electron oscillation is confined within the boundaries of the nanoparticle. In contrast, the free electrons on a thin-film SPR sensor propagate along the film axis over a few microns. Each class of SPR sensor is defined by its specific SPR excitation mode, SPR evanescent wave characteristics, manufacturing process and analytical performance (Table 2).

Table 2 – Overview of key differences between thin-film SPR and nanoparticle LSPR.

Parameters	Thin-film SPR	Nanoparticle SPR	Considerations
Excitation mode	Total internal reflection (TIR)	Transmission	- TIR: Light never crosses sample path. Direct detection in complex/opaque media possible. Little to no sample preparation. - Transmission: Light must cross sample path. Limited to clear samples. Requires sample preparation.
Sensor configuration	Thin film on glass	Nanoparticle suspension in solution or immobilized on glass	- Thin-film sensor on glass: easy to prepare in large scale. Highly repeatable manufacturing process. Easy to functionalize by spin coating or electro-polymerization. - Nanoparticles, in solution: high risk of precipitation. Poor compatibility with complex media. Better for interactions with biomolecules (more degrees of freedom in movement). - Nanoparticles, immobilized: multi-step process, increasing variability. Fragile due to immobilization.
Evanescent wave penetration depth	200-300 nm	20-30 nm	- Longer penetration depth: greater sensitivity to RI variation; greater flexibility for detection of biomolecules farther from the glass surface using strategies such as nanoparticle coupling, thicker polymer films or complex sandwich assays. - Shorter penetration depth: greater sensitivity to RI changes close to the sensor surface, limited sensing strategies.
Evanescent wave propagation	Microns	Nanometers (localized)	- Propagation over microns: uniform SPR signal obtained as the average of multiple events on an area of a few microns square. - Localized propagation: variable SPR signal from multiple nanoparticles, each with a different SPR response based on nanoparticle size, shape and spatial distribution.

Sensitivity to RI change (nm/RIU)	2000-3000	500-600	- High sensitivity: better limit of detection. Highly responsive to biomolecules. - Lower sensitivity: worse limit of detection. Lower sensitivity to bulk RI variation (e.g., buffer)
Diffusion	Planar	Radial	- Planar: the vast majority of SPR kinetics reported in the literature are based on planar diffusion. - Radial: different kinetic profile than planar. Fewer references in scientific publications.

A key consideration when comparing both classes of SPR sensors to consider is the large body of literature based on the planar diffusion of thin-film SPR sensors. Some recent studies with nanoparticle sensors report different kinetic data due to radial diffusion which may result in less accurate kinetics.

Concept 4. SPR sensor functionalization for selectivity

Figure 5 – General description of surface functionalization.

The SPR effect is intrinsically responsive to RI changes and indiscriminate of the source of the change. Most applications investigate a specific response such as binding of a biomolecule or a chemical compound to a target. Two complementary strategies make SPR sensors specific: increase specificity and reduce non-specificity. Increasing specificity involves using a recognition element such as an antibody or a DNA probe and leveraging their high affinity or selectivity for a specific target. Reducing non-specificity involves decreasing interactions with molecules other than the target. To optimize this balance, researchers use various surface chemistry (e.g., polymer such as PEG, self-assembled monomer layers, hydrogel), biofunctionalization elements (e.g., antibodies, DNA, enzyme) and buffer conditions.

SPR biosensing applications are well documented, especially in the immunoassay format (Figure 5). Similarly to enzyme-linked immunosorbent assays (ELISA), SPR sensors can be functionalized with antibodies to detect and quantify specific ligand binding. SPR is faster (minutes to hours, compared to hours to days for ELISA) due to its real-time monitoring and label-free sensing capacity. In addition, SPR provides additional information such as kinetics and affinity.

In molecular sensing and chemical testing, the functionalization step is often critical, especially when a new test or assay is being developed. This development stage requires a large number of sensors to test different surface chemistry, conditions or compounds and sensing strategies such as direct detection, sandwich assay or competitive assay. Lower-cost sensors are advantageous.

Affinité Instruments' portable, 4-channel SPR (P4SPR)

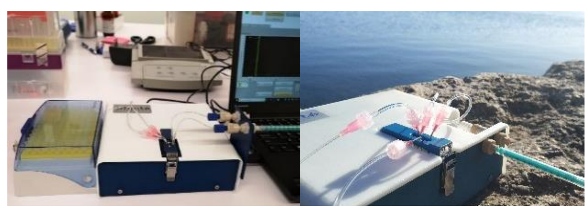

Figure 6 – P4SPR device in the lab (left) and in the field (right).

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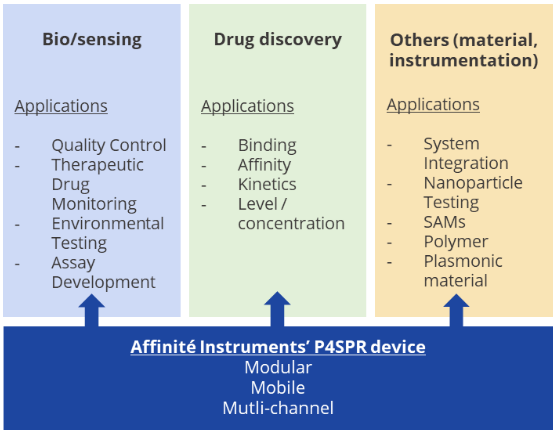

Figure 7 –P4SPR applications.

The P4SPR is a unique modular, mobile and multi-channel device designed to democratize SPR in drug discovery, bio/sensing and system integration. Simple and easy to use, the multi-purpose P4SPR provides rapid access to film-based SPR in the lab or in the field (Figure 6). Furthermore, the P4SPR open fluidic design and LabVIEW software are adaptable to combine with other analytical methods such as spectroscopy, electrochemistry and chromatography for an unparalleled flexibility to address needs across various SPR segments (Figure 7).

At the core of the P4SPR technology lies the benchmark thin-film SPR sensors delivering high quality SPR data. The Kretschmann configuration. SPR device enables direct detection in complex media. It requires little to no sample preparation, saving users the cost of purification kits and reagents. Additionally, the P4SPR takes advantage of the high sensitivity and larger sensitive region of thin-film SPR, thus broadening the range of applications for the technique.

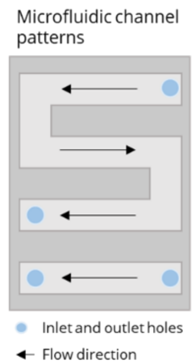

Figure 8 – P4SPR microfluidic cell design.

The multi-channel microfluidic design gives you more per test through simultaneous acquisition of triplicate measurements plus a reference for each chip (Figure 8). The 4-channel microfluidic system requires a minimum sample volume of 50 microliters in direct injection mode via micropipette. In addition to low volume injection, long contact time for slow kinetics are possible in the microfluidic chamber without any variation due to evaporation. Undesired intrinsic variations caused by temperature shifts and bulk refractive index changes are measured in real time in the reference channels for sample correction in post-processing analysis. This unique feature improves data quality to deliver readily analyzed, reliable and robust information with every test and assay.

About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built on deep knowledge accrued throughout more than a decade of research in SPR. The commercialization of promising innovations is spearheaded by a leadership team experienced in business, science and engineering.