FOx BIOSYSTEMS
Breaking limits in bioanalysis

APPLICATION NOTE

Versatile and fast IgG screening with the Protein A sensor probe

Application note 7 | Version 1.0 | Kris Ver Donck, Filip Delport, Laura Marin, Emma Buchet, Kim Stevens

Summary

FOx BIOSYSTEMS' fiber-optic surface plasmon resonance technology (FO-SPR) is a robust tool that harnesses the power of SPR in an easy-to-use dip-in fiber-optic configuration. This document demonstrates the potential of FO-SPR to perform rapid, label-free determination of antibody concentration in complex samples using the WHITE FOx Protein A pre-functionalized sensor probe. Within a few minutes, you can get accurate information on target concentration, and a short second step of a few minutes also allows potency screening with IgG-specific antigens.

We discuss how the FO-SPR label-free assay can provide a performant alternative to conventional methods for characterizing antibodies in crude samples, with minimal processing. This allows for simple protocols and easy experimental setup, while avoiding sample clogging or contamination.

In the example described in this application note, we use the Protein A sensor probe to quantify human IgG in cell culture supernatants. Concentrations from as low as 0.10 µg/ml up to 100 µg/ml of human IgG are easily detected and measured label-free by FO-SPR through direct binding of IgG to the sensor probe. These sensor probes have the added advantage that they can be regenerated and reused multiple times, with near-complete recovery of signal. Hence, reducing the equipment, time, and costs needed to perform powerful and functional quantification or potency screens.

Introduction

FO-SPR: An attractive alternative to conventional SPR

Surface plasmon resonance (SPR) is increasingly used as a powerful biosensing technology and offers real-time measurement that can be used with and without labels. SPR exploits electromagnetic waves to measure local refractive index changes due to suspended analytes and immobilized biomolecules on the sensor surface. The most commonly used SPR biosensors are sensitive, reliable, and accurate. However, they typically use gold layer sensors inside microfluidics and a complex optical path with prisms and are often unsuitable for quick testing due to their size and cost.

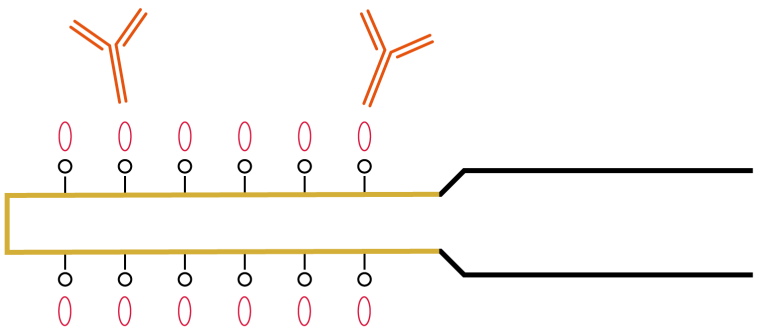


Figure 1: Representation of a pre-immobilized sensor probe with Fc bound IgG

Fiber-optic surface plasmon resonance (FO-SPR) offers a convenient alternative to conventional SPR biosensors. Here, the gold layer is applied externally to fiber optic SPR sensor probes which can be dipped into the sample of interest. This approach capitalizes on the speed and performance of SPR in an easy-to-use dip-in protocol. The fluidics-free setup means that samples can be analyzed with minimal processing and without the risk of clogging or contamination associated with microfluidics-based SPR systems.

In this application note, we use an IgG concentration screening assay to demonstrate the WHITE FOx Protein A pre-functionalized sensor probe: a generic surface for quantifying IgG which is ideal for screening IgG libraries for product yield and/or binding potency. The benefits of using this sensor probe include high sensitivity, speed, reproducibility, and efficiency. Using the Protein A sensor probe requires minimal sample manipulation and minimal volumes of reagents to deliver precise results. Additionally, each sensor probe can measure a different sample or a different concentration in the same assay setup.

Materials and methods

Tools and reagents

To perform the procedures using the Protein A sensor probe you will need:

- WHITE FOx instrument with FOx-SPR acquisition software and WHITE FOx data processing tool
- Protein A sensor probes for label-free quantification (FOx BIOSYSTEMS product nr: 30.0006)
- Microsoft Excel, Tracedrawer, or other data calculation software for data handling and viewing
- PCR tube strips or 96-well PCR plate
- Micropipettes from 10 to 1000 µl with disposable tips
- LoBind Eppendorf tubes for protein solutions
- A microtube mixer and mini-centrifuge to remove protein aggregates

Buffer/Reagent	Concentration
Human IgG1	2.64 mg/ml
PBS: Phosphate buffer saline, pH 7.4	10 mM
Tween-20	Dissolve to 1 mg/ml stock
Glycine, pH 1.5	50 mM

Table 1: Reagents and buffers. All solutions were prepared with deionized water purified by a Milli-Q Plus system.

How does FO-SPR work

FOx BIOSYSTEMS turned an optical fiber into a mass-sensitive sensor using the well-established surface plasmon resonance (SPR) principle for biomolecular interaction analysis.

The SPR effect is achieved by coupling a white light source to the fiber optic sensor probe. In this consumable sensor probe, the light interacts with a gold layer and senses the refractive index up to 200 nm away from the outer surface. At the end of the sensor probe, light is reflected back through the bifurcated fiber to a spectrometer. The resonance condition is monitored by tracking the wavelength at which the least light is reflected.

The fiber-optic surface plasmon resonance (FO-SPR) sensor is coated with bioreceptor molecules which can bind to the target molecules of interest. This binding changes the refractive index, resulting in a wavelength shift that produces a sensor signal as shown in Figure 2. The sensor probe is simply dipped into the liquid sample to measure biomolecular interactions directly. Figure 4 shows an IgG sensorgram resulting from a complete assay procedure including antibody binding and dissociation.

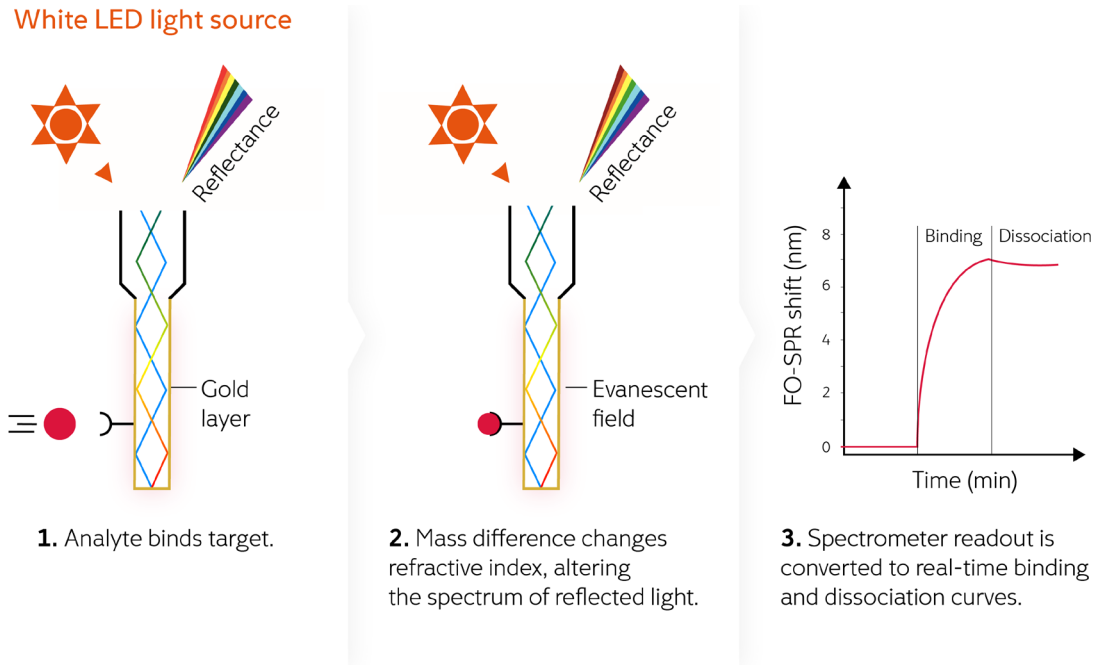


Figure 2: The FO-SPR principle

FO-SPR has been incorporated into a benchtop instrument: WHITE FOx by FOx BIOSYSTEMS.

As FO-SPR is fully automated and can run measurements individually or with 4 parallel FO sensors, it shows great potential to quickly detect and quantify antibodies in crude samples obtained from cell culture bioreactors. The dip-in capability also means that it is simple to transfer the sensor probes bound to IgG into separate media containing specific antigens for potency assays.

The Protein A sensor probe

As depicted in Figure 1, the Protein A prefunctionalized sensor probe will capture IgG in a sample by binding to the Fc domain. The binding of IgG can be evaluated by the real-time measurement of the SPR shift (the wavelength shift from pre-binding to a given binding time point) through the FO-SPR technology. Assays, including IgG concentration, and antigen association and dissociation, can be performed on the protein A sensor probe. The resulting data file in standard open .csv format can be processed by the 'FOx BIOSYSTEMS Data Processor' to present detailed graphs that focus on target association and the respective SPR shift as shown in Figure 3. The wavelength shift data can be used to set up calibration curves, correlating SPR shift with concentration.

The data output file also is compatible with our SPR evaluation software suite 'Tracedrawer', which enables the swift kinetic analysis of real-time binding data, as well as calibration curve fitting.

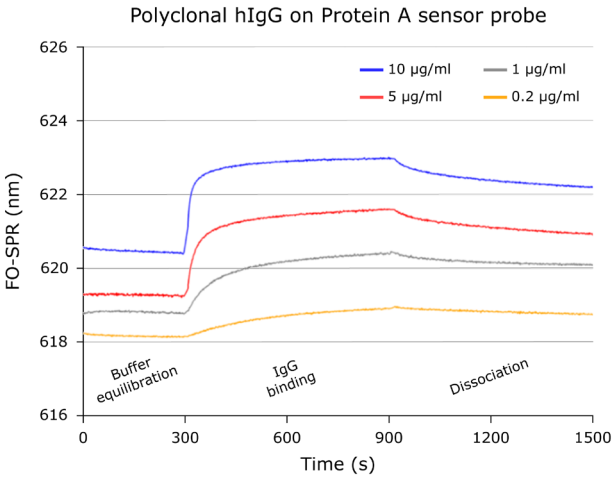


Figure 3. A sensorgram illustrating the responses generated by 4 different concentrations of hIgG (µg/ml) on a Protein A sensor surface including buffer equilibration (baseline) and optional extended association and dissociation steps.

Assay setup

Gold-layered fiber optic sensor probes with a pre-immobilized Protein A surface should be rehydrated with assay buffer. This ensures the native state of the Protein A capture molecule that is covalently bound to the sensor probe surface.

IgG binding

In the binding step, the target IgG antibody is directly coupled with its Fc-domain to the Protein A molecule. This takes about 5 minutes but can be sped up significantly for screening purposes.

Regeneration setup

Using the pre-functionalized sensor probes, it is also possible to regenerate and "reset" the sensor probes over 40 times. The regeneration sequence strips the IgG from the Protein A binding site which can be regenerated with a simple Glycine/HCl buffer mix at a low pH. This strips off the IgG, leaving the binding site free for the next sample, as shown in Figure 4. The function of the Protein A sensor is preserved for a next binding step. The Protein A binding function on the FO-SPR sensor probes shows high reproducibility after regeneration within a run, enabling the testing of multiple samples, concentrations, or replicates of the same sample on a single sensor probe.

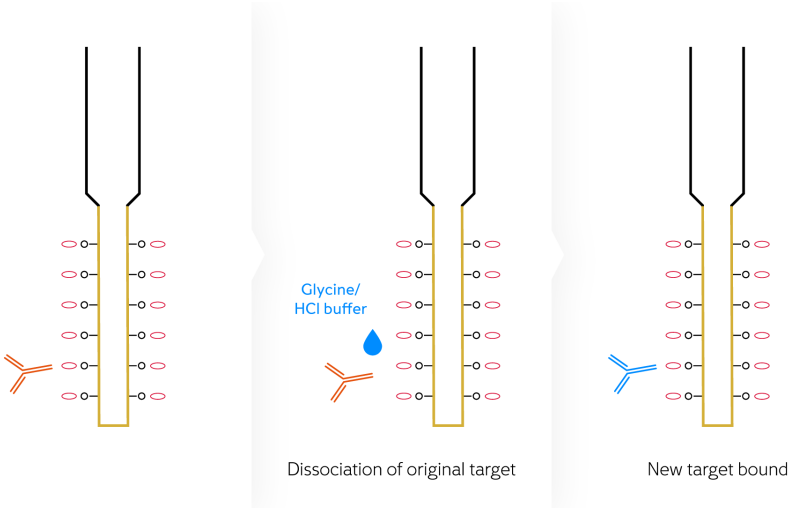


Figure 4. Regenerating Protein A sensor probes using a glycine/HCl buffer to measure a new target.

Results

Robust and fast sensor probe regeneration

Using a regeneration step, the antibody being measured can be stripped from the sensor probes and a new sample can be measured using the same sensor probe. The results are shown in Figure 5, and demonstrate that even after multiple regeneration cycles, the data collected is accurate with low variability. The inter-run Coefficient of Variation (CV) on IgG quantitation was well under 10%, even down to less than 2% for most samples, when using the same batch of sensor probe and reagents/buffers.

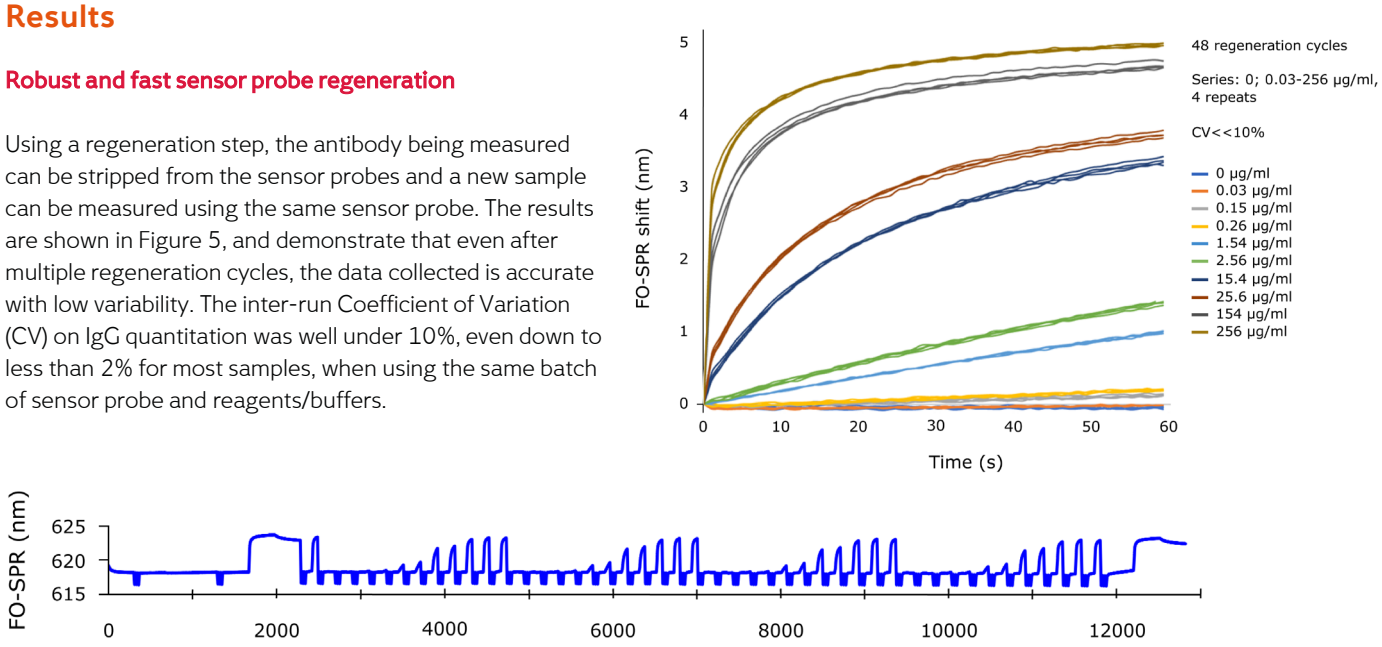


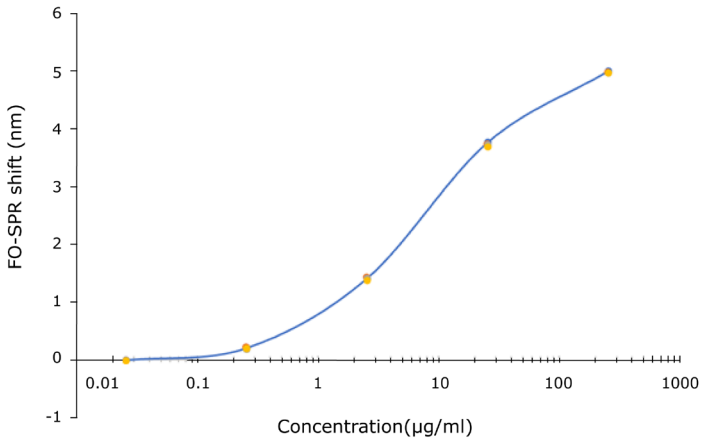
Figure 5: Results from a concentration screening including 48 regeneration cycles. The CV measured was less than 10%, generally presenting at around 2-5%. Screening cycles including regeneration used here were performed in less than 4 minutes per sample. Actual binding measurement for concentration determination took 60 seconds per sample.

Use case: Concentration screening

Concentration screening can be performed for multiple samples at once and using the same sensor probes. Regeneration cycles can be run over 40 times in one session, priming the sensor probe each time for a new sample.

Beginning with a short binding run and a baseline buffer equilibration step, two IgG concentration series were bound, optionally dissociated, and rebound to a regenerated sensor probe. Figure 6 shows that the sensor probes give a reliable readout, even when different concentrations ranging from 0.03 to 256 µg/ml are bound in subsequent measurements.

Figure 6 shows an example concentration curve that can be used to calculate IgG concentration from the FO-SPR shift.



µg/ml	Average (mm)	SD	CV%
256	5.08	0.09	1.8%
25.6	3.80	0.05	1.4%
2.56	1.44	0.02	1.4%
0.26	0.22	0.01	4.9%
0.03	0.02	0.02	

Figure 6: A typical concentration calibration curve showing the relationship between the FO-SPR shift and IgG concentration. Dots represent 3 individual replicate measurements for each concentration.

Use case: Potency screening

Next to IgG quantification, potency screening can be performed in just a few minutes. The IgG-bound sensor probes can be dipped into wells containing a fixed concentration of specific antigens and a kinetic binding assay can be carried out to determine the binding potency between IgG and antigen using the kinetic analysis suite.

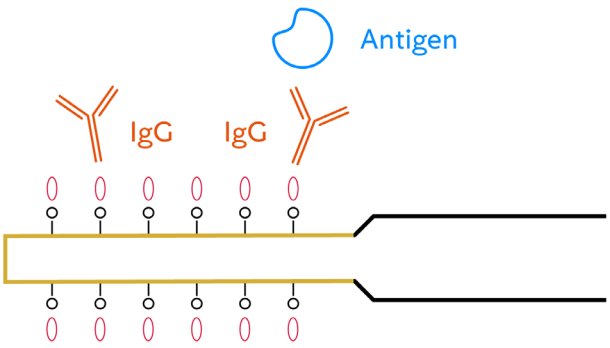


Figure 7. Antibody potency screening can be carried out on the same sensor probes as the concentration screening by simply dipping the antibody-hybridized sensor probes into wells containing specific antigens.

In the experiment series in Figure 8 below, a 14% signal ratio of antigen to bound antibody was found, where 10% was expected. Moreover, 90% of denatured antibodies were identified correctly, while less than 80% correct identification was expected.

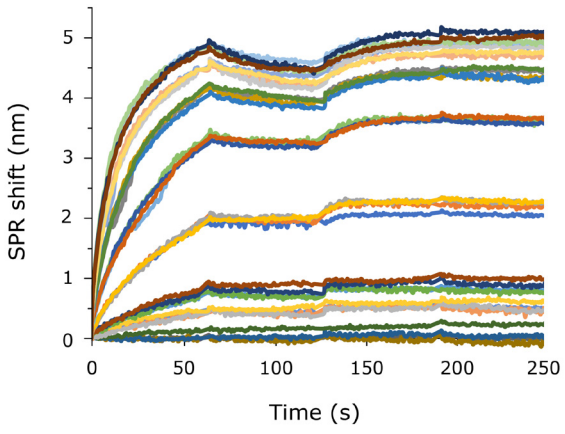


Figure 8: Example of an IgG potency screening with multiple concentrations of an IgG bound to the Protein A sensor probe, exposed to a fixed concentration of an antigen, in test cycles of less than 5 minutes including regeneration. Kinetic analysis of binding and dissociation of the antigen using the potency model can provide a potency ranking of the IgG's tested.

Discussion

Simple protocols

Conventional SPR configurations use microfluidics, which can be susceptible to cross-contamination, or even clogging, when measuring samples in complex matrices. The Protein A sensor probe **can work on whole samples or samples with minimal manipulation**, allowing you to start measuring your targets directly.

There is no need to include a functionalization step when running the assay, as the sensor probes are pre-functionalized. In addition, using WHITE FOx will simplify the assay, allowing the **entire experiment to be run in one machine with a simple setup**. Insert your reagents and targets of interest and simply click RUN.

The straightforward regeneration protocol means that each sensor probe can measure a different sample or concentration for over 40 samples, **reducing the amount of equipment and time needed** per setup.

Benefits of using WHITE FOx Protein A pre-functionalized sensor probes: Fast yet sensitive

When compared to using FO-SPR with a functionalization step, the pre-functionalized sensor probe setup can **accelerate the entire assay time up to 10x**, making it easy and fast to detect and quantify antibodies of interest. In the extended assay described in this app note, the binding time for IgG to the sensor probe is 5 minutes. When including the baseline, wash, and regeneration, this increases to 11 minutes. For screening purposes, the binding can even be set as short as 1 minute, and a complete cycle with regeneration takes only 2.5 minutes.

Speeding up the process does not necessarily mean a drop in sensitivity. The FOx Protein A pre-functionalized sensor probes have a **dynamic range of up to 3 log10**. Human IgG concentrations from as low as 0.10 µg/ml up to 100 µg/ml can be detected label-free.

Conclusions

When measuring antibodies in research samples, it is essential for tests to be sufficiently sensitive to detect and quantify them at relevant concentrations. In addition, the ability to deal with complex crude samples (such as samples from cell culture media) without the need for time-consuming purification procedures is highly advantageous. Furthermore, the dip-in sensor probes allow subsequent potency screening of the bound antibodies to test binding strength to specific antigens.

As presented here, the assay cycle time of 3 to 5 minutes is already substantially faster than conventional SPR, which typically has an assay cycle time of 10 minutes or more. With pre-functionalized sensor probes kept ready in the assay buffer, the assay time can be further reduced to less than 3 minutes per cycle.

This demonstrates that WHITE FOx is a robust tool for fast antibody screening and quantification, with multiple rounds being possible due to the regeneration potential of the sensor probes (as shown in Figure 6). With the additional possibility of potency screening (Figure 8), FO-SPR can be a valuable tool for antibody development.

Related application notes

Application note 2: Sensitive protein quantification in crude serum samples: comparison of FO-SPR and ELISA

Application note 4: Label-free protein quantification in bioproduction samples

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