How to Quantify a Protein in Different Crude Samples in One Run using the XelPleX

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Crude sample means the sample existing in a natural state and unaltered by any process. Characterizing the molecular affinities in the native medium makes it possible to understand the biological mechanisms at the molecular scales. Our systems (XelPleX) allow the analysis of the molecular binding in a complex media and generate valuable data such as determination of real-time interactions and kinetics. Indeed, the injection of complex samples without dilution, such as serum or plasma, is no longer an issue for diagnostic research or drug screening.

The aim of this article is to demonstrate the capability of the Surface Plasmon Resonance imaging technology (SPRi) to detect and quantify a biomarker as an Acquired Immune Deficiency Syndrome (AIDS) diagnosis tool.

Introduction

Surface Plasmon Resonance (SPR) is an optical detection process that can occur when a polarized light hits a prism covered by a thin metal layer. Under certain conditions (wavelength, polarization and incidence angle) free electrons at the surface of the biochip absorb incident light photons and convert them into surface plasmon waves. Perturbations at the surface of the biochip, such as an interaction between probes immobilized on the chip and targets, induce a modification of resonance condition which can be measured. HORIBA Scientific Surface Plasmon Resonance imaging (SPRi) method consists in evaluating the angle where the variation of reflectivity will be the greatest when there will be an interaction and fixing this angle to monitor kinetics (reflectivity versus time). SPRi system XelPleX use a CCD camera that allows visualizing an area where many spots (several probes) can be immobilized, and then monitoring the reflectivity of these many spots (up to 400 spots in HORIBA Scientific systems) at the same time.Probes are immobilized on the SPRi-Biochip and targets run through the flow-cell above it. The SPRi-Biochip is illuminated by a source light beam whose angle can change. A CCD camera displays the images of the biochip on the screen, allowing monitoring of interactions on many spots in the same time (multiplex format) (Figure 1). SPRi technology is a good alternative to the use of Enzyme-Linked Immunosorbent Assay (ELISA) in the protein concentration determination. Unlike ELISA which needs labelling and which is time consuming, SPRi is label-free, real-time and requires only few minutes to screen a huge panel of samples.

The biomarker studied during this project is a protein and we will call it “Protein Y”. It is a component of the Human Immunodeficiency Virus (HIV) structure. This protein is commonly used on the AIDS diagnosis, in combination with other immunological tests. Till now, ELISA is the standard method for Protein Y based diagnosis. Nevertheless, this method shows some limitations in terms of rapidity and high throughput which are more and more required in pharmaceutical research area.

Moreover, crude samples can also be immobilized on the sensorchip for rapid and high throughput quantification which can be required in pharmaceutical research area as well. The immobilized biological molecules can be quantified in different crude supernatants with only a single injection of the target.

This article shows how we can detect and quantify a biomarker in different supernatants with only a single injection using the SPRi technology and it proves the potential of the high throughput concentration analysis in crude samples.

Experiment set-up

Figure 2 shows the assay set-up. During the printing phase, we used the microfluidic immobilization method, which generates higher spot homogeneity and immobili-

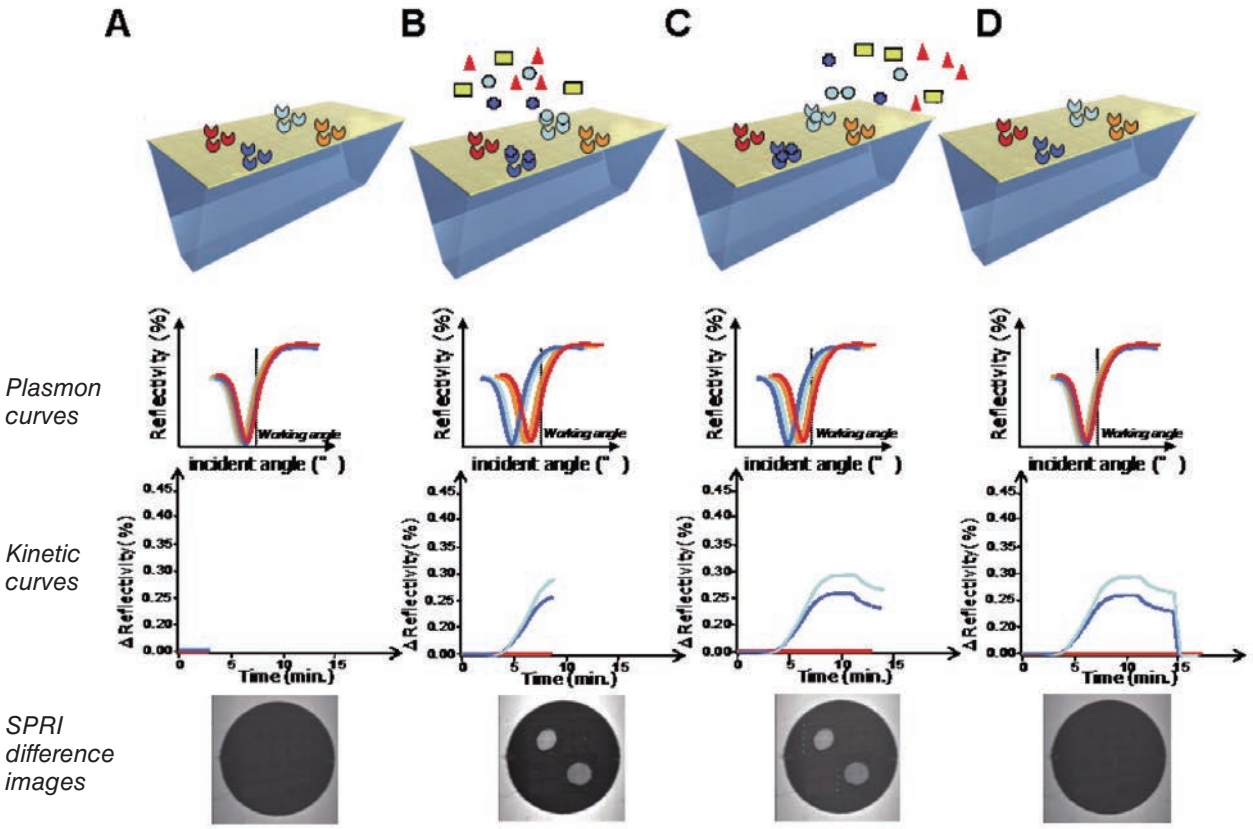


Figure 1 Spotting Monitoring of biomolecular interaction

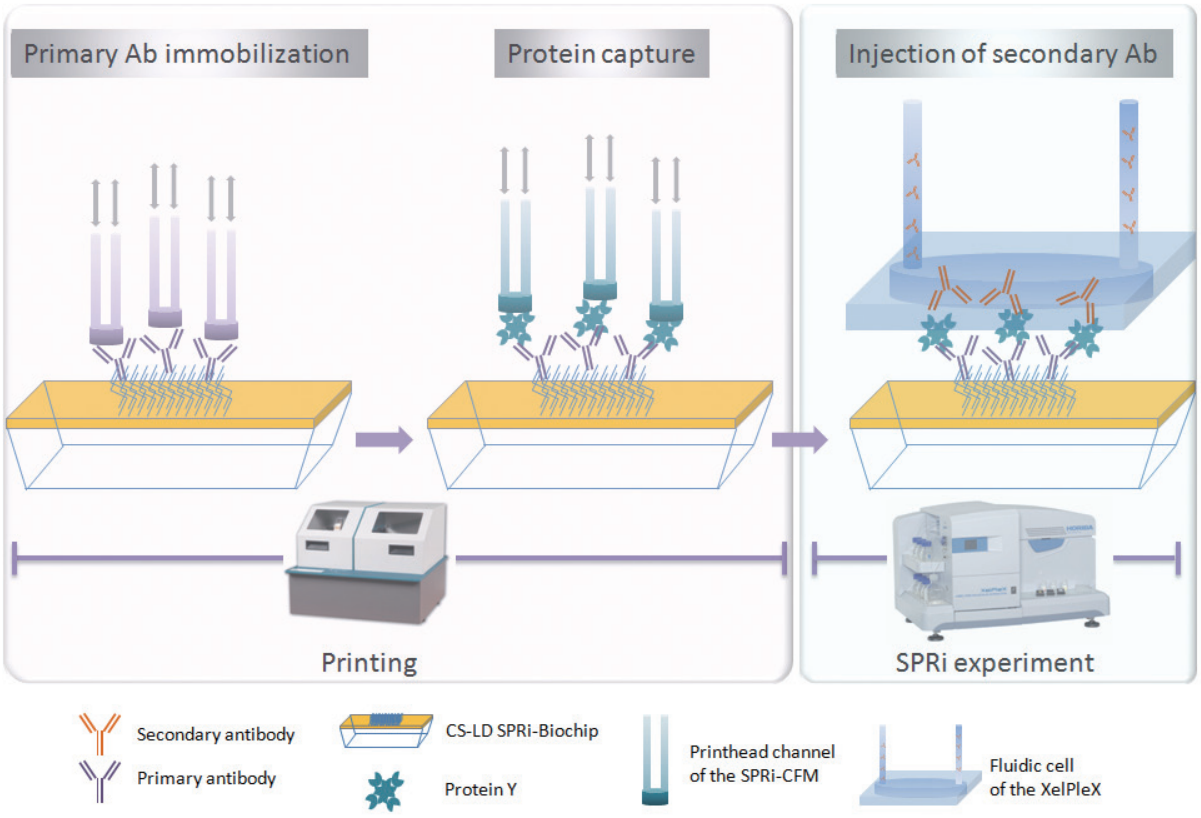


Figure 2 Assay set-up

zation levels.
During the SPRi experiment we used XelPleX system which allows the monitoring of the interaction in label-free way and in multiplex format.

The printing was performed in two steps:

Step1: Immobilization of antibodies (anti-protein Y, and the negative control anti-ovalbumine (anti-ova))

Step2: Capture of the purified proteins

Systems used:

The SPRi Continuous Flow Microspotter™ (SPRi-CFM) uses flow deposition to immobilize up to 48 molecules in a single run (up to 144 spots per chip).The SPRi-CFM uses flow to print biomolecules on a surface. Flow deposition allows samples to be cycled over the surface and captured from solution, leading to higher biomolecule density, better spot uniformity and improved assay sensitivity. The print head uses a three-dimensional network of microchannel that allows for the flow of solutions over a substrate area. The print head provides a seal to the SPRi-Biochip or the SPRi-Slide and confines the solution to the area of the individual spot, completely eliminating background signal and the possibility of cross-contamination. Each spot interface is linked to an inlet that allows fluid to circulate across the spotted area. The samples are drawn from the inlet wells to the outlet wells. The solution can be cycled back and forth (bi-directionally) between the inlet and outlet wells until the desired deposition level is achieved.

XelPleX is the high-performance and fully automated instrument for the analysis of label-free molecular interactions in a multiplex format. The array-based format of the sensor chips allows you to monitor up to several hundred interactions simultaneously and to accelerate your research. The optimized fluidic system is designed to give you full kinetic profiles within minutes and helps you make the right decisions quickly and confidently. XelPleX combines the power of multiplexing (measuring multiple interactions simultaneously) and the sensitivity of Surface Plasmon Resonance, resulting high-resolution kinetic profiles. The revolutionary fluidics system and the integrated autosampler are compatible with a broad range of applications, such as ligand fishing in complex fluids, cell studies, small molecule detection, protein screening and DNA analysis. The imaging configuration of XelPleX dramatically enhances the throughput of conventional SPR. Ligand molecules are immobilized in an array format onto the sensor chip surface and are screened against the interacting partner in the sample solution. Up to several hundred molecular interactions can be analyzed simultaneously, saving you time and money.

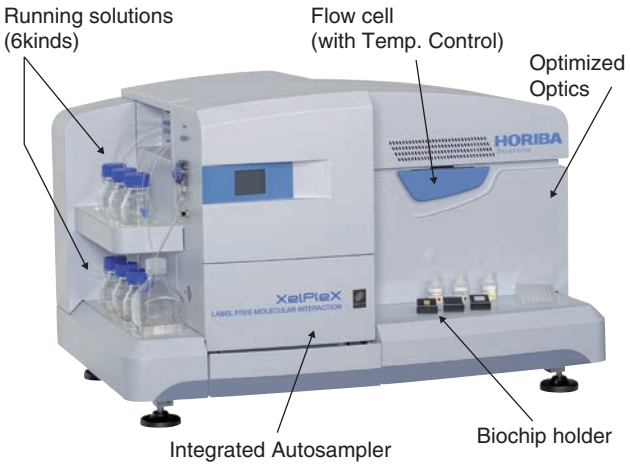


Figure 3 Instrument Configuration of XelPleX

Combining the SPRi-CFM printer and XelPleX allows for a highly multiplex platform, and made possible in a few minutes the quantification of the target in different throughout calibration curve. The standardization of such protocols will dramatically improve the existing research methods and become by the way a potential diagnostic tool.

Experiment 1: Proof of concept

The first test was performed to optimize the experimental conditions and provide a proof of concept. Anti-protein Y antibody, prepared at 1 µM in 10 mM sodium acetate at pH 4.0 and pH 5.0 was immobilized on the CS-LD surfaces using the SPRi-CFM system.

The Figure 4 shows the spotting map (right) and the corresponding flow-cell image and difference image during the interaction (left). We immobilized two different molecules in different experiment conditions:

* The anti-ova was immobilized pure and in conditioned medium.
* The anti-protein Y at two different pH 4.0 and pH 5.0.

Then, we captured the purified Ovalbumin and the protein Y and Ovalbumin in conditioned media. Finally the spotted biochip was inserted into XelPleX.

The difference image shows a specific interaction between the injected secondary antibody and the captured protein Y. The signal for the antibody immobilized at pH 5.0 was higher (data is not shown). This pH will be used for the quantification experiment.
In this preliminary test, we demonstrate the feasibility of the molecule capture from purified samples. Thanks to the multiplex approach, we tested several immobilization parameters. The optimized parameters will be used for the next experiment.

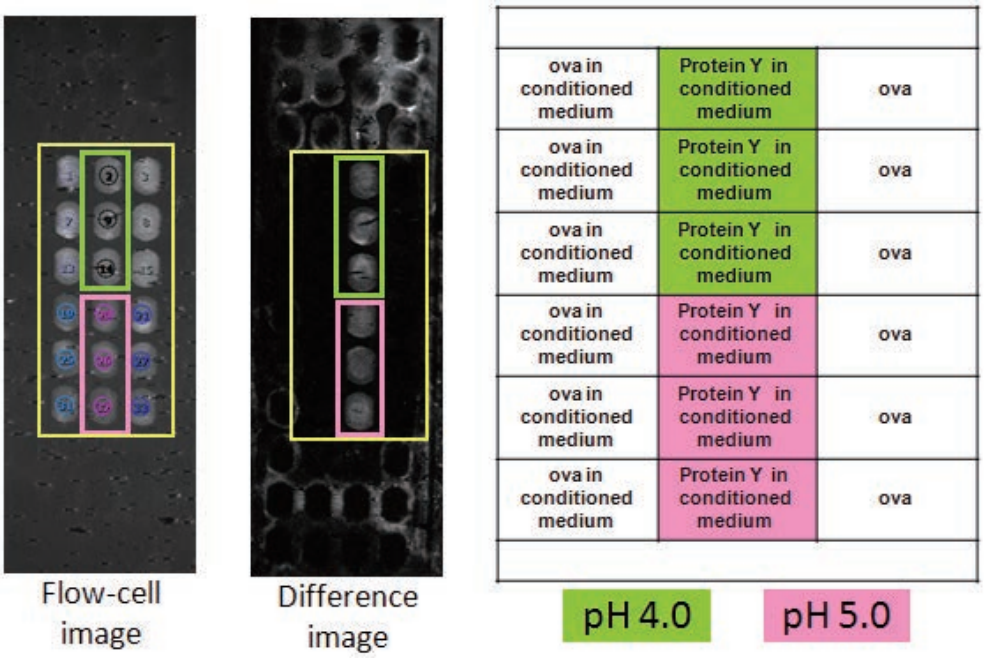


Figure 4 Spotting map and SPRi-biochip flow-cell and difference images

Experiment 2: Protein quantification

Printing protocol

The printing was performed in 2 steps (Figure 2)

Step 1: Primary antibodies immobilization

Primary antibodies directed against the Protein Y were immobilized on a CS-LD SPRi-Biochip , that is made of a self-assembled monolayer of polyoxyde ethylene glycol activated using an EDC/sulfo-NHS solution in preparation for amine coupling.

The capture antibody was prepared at 1 µM in 10 mM sodium acetate at pH 5.0 and immobilized on the activated surface of the SPRi-Biochip™ using the SPRi-CFM system.

Step 2: Protein capture

The Protein Y was captured at 10 ng/mL, 40 ng/mL, 120 ng/mL, 370 ng/mL, 1.1 µg/mL, 3.3 µg/mL and 10 µg/mL. The concentration range was chosen to cover the concentrations of the tested supernatants. The 5 supernatants tested were used without dilution.

A negative control (anti-Ovalbumin IgG) and a positive control (Protein Y spiked in crude medium) were also immobilized. Both purified Protein Y and anti-Ovalbumin IgG were diluted in 10 mM PBS pH 7.4. After the immobilization procedure, the CS-LD SPRi-Biochip was blocked using 1M ethanolamine. Figure 5 shows the printing pattern and the image of the CS-LD SPRi-Biochip.

	A	B	C	D
1	Supernatant1	Supernatant1	Supernatant1	Supernatant1
2	Supernatant2	Supernatant2	Supernatant2	Supernatant2
3	Supernatant3	Supernatant3	Supernatant3	Supernatant3
4	Supernatant4	Supernatant4	Supernatant4	Supernatant4
5	Supernatant5	Supernatant5	Supernatant5	Supernatant5
6	NC	NC	NC	NC
7	PC	PYC5	PYC3	PYC1
8	PC	PYC5	PYC3	PYC1
9	PC	PYC5	PYC3	PYC1
10	PYC7	PYC6	PYC4	PYC2
11	PYC7	PYC6	PYC4	PYC2
12	PYC7	PYC6	PYC4	PYC2

Figure 5 Printing pattern (left) and image of the CS-LD SPRi-Biochip (right)
(Masks superimposed on the right image). NC: Negative control, PC: Positive control, PY C1: PY at 10 ng/mL, PY C2: PY at 40 ng/mL, PY C3: PY at 120 ng/mL, PY C4: PY at 370 ng/mL, PY C5: PY at 1.1 µg/mL, PY C6: PY at 3.3 µg/mL, PY C7: PY at 10 µg/mL

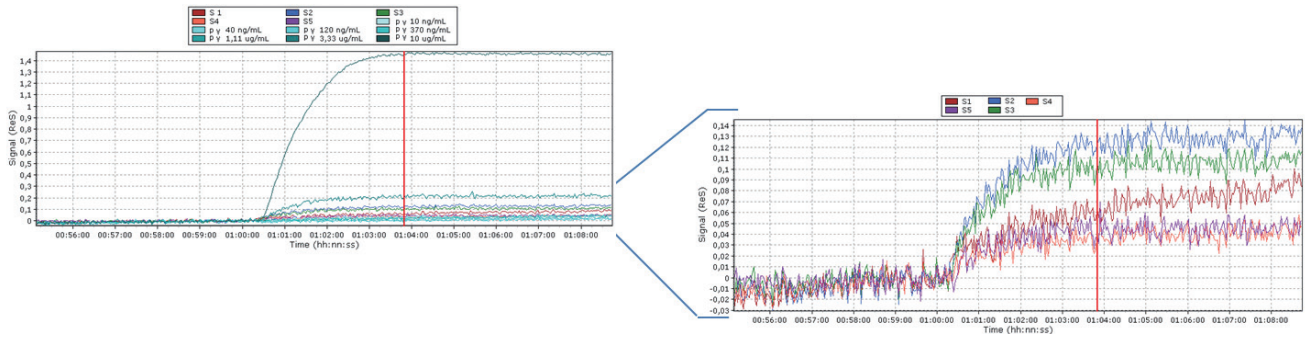
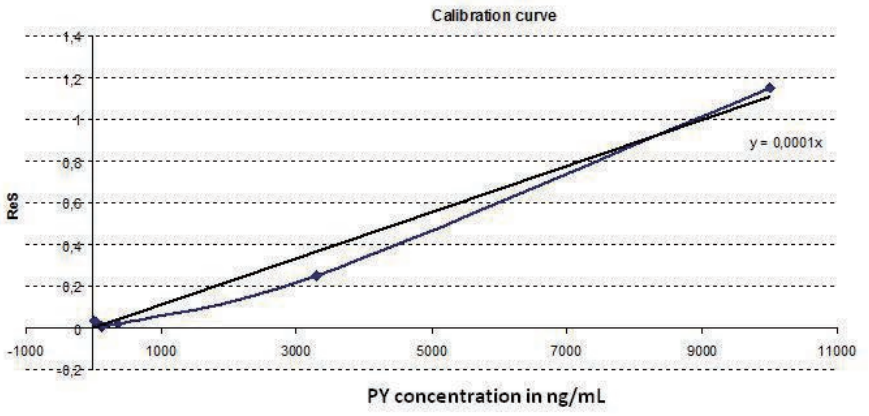


Figure 6 Averaged and reference-subtracted kinetics curves (left) and zoom on the curves corresponding to the 5 supernatants (right) after injection of the secondary antibody at 100 nM



Sample	PY concentration in µg/mL
Supernatant1	0.73
Supernatant2	1.36
Supernatant3	1.09
Supernatant4	0.44
Supernatant5	0.42

Figure 7 Calibration curve and Protein Y concentrations determined in the supernatants

SPRi experiment

The printed CS-LD SPRi-Biochip was then loaded into XelPleX, the interactions were monitored using software "EzSuite". The running buffer was 10 mM PBS pH 7.4 and the working temperature was set to 25°C. Then, 200 µL of the secondary antibody were injected into the fluidic system at a flow rate of 50 µL/min.

Results

After a blank injection (injection of the buffer), the secondary antibody was injected at 100 nM. Figure 6 shows the kinetic curves obtained for the purified Protein Y captured at known increasing concentrations and for the supernatants (left) and on the Protein Y captured from the five tested supernatants (zoom on the right).

As shown in Figure 6, the Protein Y (PY) was detected in all supernatants tested. A calibration curve was drawn using the responses obtained for each concentration of purified Protein Y immobilized (Figure 7). The curve was used to evaluate the concentration of the Protein Y in the different supernatants in a single biochip and with a single injection.

Conclusion

The multiplex feature and the flexible fluidic of the label-free interaction analysis XelPleX allows protein quantification in several crude samples simultaneously, enabling to save time and money. Through a single injection, we were able to determine the concentration of the Protein Y in 5 different supernatants using a calibration curve established on the same SPRi-Biochip. This demonstrates that the SPRi platform is a powerful tool for rapid and high throughput quantification applications. On a single biochip, 14 different conditions were tested and 48 sensorgrams were generated in less than 15 mins.

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