# DETECTION OF PROTEINS BY SPRI METHOD

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**Abstract**

SPRi (Surface Plasmon Resonance Imaging) biosensors are used for detection in real time with advantage of processing large number parallel measurements. Our goal is fast detection of low concentrations proteins. A low concentration testing of proteins in urea or blood serum is needed for clinical practice.

This article shows the problems of measuring proteins in real samples of body fluids. The original biochip detecting albumin (HSA - Human serum albumin) was implemented and tested both by standard tests, and by the patient samples.

**Keywords:** optical measurements, surface plasmon resonance, albumin

## 1. INTRODUCTION

In our industrialized society there is growing number of traffic accident and injuries. When body is injured, it is shutting down organs, even the kidney. In the case of late drug treatment, kidney will be damaged. In the case of early treatment the drugs will destroy liver.

Doctors have very narrow window of opportunity for decision and that's why they need to know state of the kidneys in every moment of treatment. The state of the kidneys can be determined from concentration of protein (albumin) in urine, that's why doctors need reliable, fast and mostly sensitive immune biochemistry method.

One of the methods with great potential is the method based on optical excitation of surface plasmons. Surface plasmons are collective oscillations of noble metal electrons. The excitation condition of the surface plasmon resonance (SPR) is very sensitive to changes of refractive index (the order of $10^{-5}$) of the inspected medium [1]. We are able to use functionalized surface of noble metal to measure biochemical interaction [2].

In our case, we detect proteins bonded to the metal-dielectric interface. Surface plasmon resonance imaging (SPRi) biosensors can be advantageously used for ultrasensitive label free and real time detection and can detect multiple agents at the same time [3]. Our research has been focused on the investigation of sensitivity increase and usage of real patients samples.

Examination of urinary protein remains one of the basic examinations in nephrology. It has an irreplaceable role both in early diagnosis of kidney diseases and in monitoring their activities. Current diagnosis is based on examination of total protein and albumin in urine.

Albumin in urine is an important marker pointing to the generalized vascular hyperpermeability. The persistent presence of albumin in urine, known as microalbuminuria reflects generalized pathological processes that damage the vascular system and indicate the development of diseases such as diabetes of I-st and II-nd type, hypertension and cardiovascular disease. For these reasons we have focused on methods detecting albumin by SPRi.

## 2. EXPERIMENTS

### 2.1. SPR Description

The basic principle of SPR is well described in many scientific papers [4]. For the SPR phenomenon generation we need a metal - dielectric interface, while certain conditions are provided for real and imaginary part of the metal permittivity. The intensity of reflected light is function of incidence angle. A beam of linearly p-polarized light falls to the prism-metal layer interface. If so called resonance condition is realized then an evanescent wave occurs at the metallic layer - outside environment interface. This evanescent wave excites surface electrons that utilize energy of the incidence beam. When incidence angle changes, while other parameters (environment refraction index and wavelength) remain constant, there is a change in intensity of reflected light intensity. It is possible to find a marked minimum as a consequence of resonance phenomenon. During the real measurement (of proteins) the incidence angle etc. parameters remain fixed, and we measure the intensity of reflected light that depends on optical parameters of dielectric environment under the metallic (golden) layer (influenced by the measured substance and measured proteins) using a CCD camera.

### 2.2. Experimental Arrangement

We have used the Horriba Company SPRi-Lab+ equipment. A beam that goes through a polarizer comes out of the light source. Then it falls onto a mirror that is controlled by software algorithms and sets-up a beam angle of incidence on a biochip. The biochip is placed in a chamber, through which the analysed substance flows. The radiation reflected by the biochip is detected by a CCD camera and the resulting signal is processed by the SPRi-View software [5].

Albumin in urine was set at University Hospital of Ostrava by the BN ProSpec Siemens analyser. This equipment is used for determination of protein concentration using specific antibodies. Albumin in urine was determined by nephelometry.

### 2.3. The Biochip

The most important element of the device is biochip. A biochip consists of an optical prism with thin vapour deposited golden layer (approx. 40 nm, Fig. 1). The golden layer has surface chemicals to bond biochemical substances deposited on it. Proteins and nucleic acids can be deposited on thus chemically processed layer as needed. This configuration also allows fast change of the biochip and thus detected substances. Detection takes place by bond of, for example, looked protein from analysed substance to its “opposite piece” immobilized on the biochip surface by a specific biochemical bond.

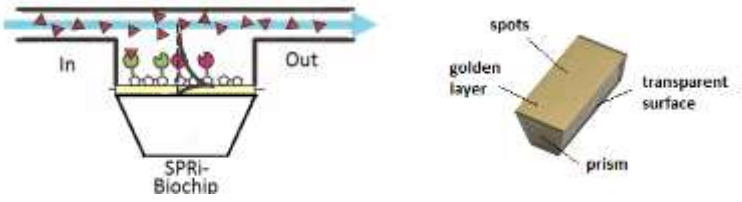


**Fig. 1** The biochip

For transport of analysed substance through the biochip chamber we use a degasser, peristaltic pump and injection valve. The experiment runs as follows: The biochip chamber is constantly washed by a puffer and speed of 50 µl/min by the pump (**Fig. 1**). During measurement we transport an exact amount of analysed substance (a solution containing sought substance, typically 200 µl) into the chamber. As soon as the analysed substance goes through the SPRi chamber, the puffer starts to flow through it again. Reflectivities of individual spots are recorded in time for the whole period of experiment.

To prepare the biochip for determination of albumin (HSA) in urine we have used the monoclonal antibody anti-HSA, clone AL-01. Using the SPRi-Arrayer it was spotted to the surface of CS functionalized biochip together with the mouse antibody IgG, as a negative control. We have chosen a chessboard arrangement using 18 spots of the anti-HSA antibody and 18 spots of IgG mouse antibody. The biochip for determination of albumin in urine is shown in **Fig. 2**.

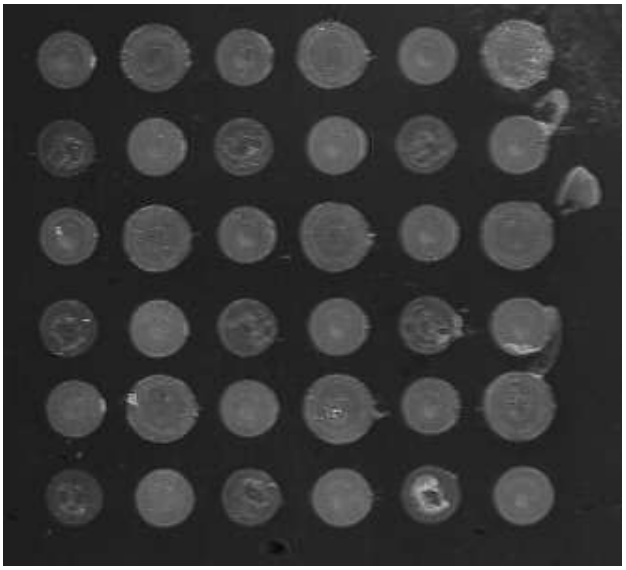

**Fig. 2** Biochip for determination of albumin in urine. The first spot in the left corner is of mouse IgG, on the right of it in the line is a spot of anti-HSA

### 2.4. The Experimental Part

In order to prepare the biochip we have first performed its testing on Siemens standards. To determine the amount of albumin in urine we have used the standard with albumine concentration of 42.9 $g.l^{-1}$. This standard solution was diluted by a physiological solution to six different calibration samples with the concentration range of 1 $mg.l^{-1}$ to 500 $mg.l^{-1}$. The measurement results are shown on the **Fig. 3**. We have performed a measurement for very low concentrations, in order to find capabilities of the used biochip. The results show that the biochip is responsible to detect concentrations around 0.1 mg/l (see **Fig. 4**).

After measuring the calibration relationships we have measured patient urine samples (altogether 61 samples obtained from the University Hospital of Ostrava) that were mostly diluted in the ratio of 1:10. During the measurement itself we have used an undiluted sample first. Unfortunately during the passage through SPRi chamber there was a problem with smooth flow of patient urine samples that contained especially significant amounts of salts. Therefore first we diluted the samples in the ratio of 1:10 and in some cases even 1:100.

Analysis was not possible during the time when urine was going through SPR chamber due to completely different viscosity of urine and used puffer. There was a biochemical reaction that was very well recorded after the urine went through. This process is quite apparent on **Fig. 5**. The urine is flowing up to the time of 400 s, and the analysis was possible between the times of 600 to 650 s.

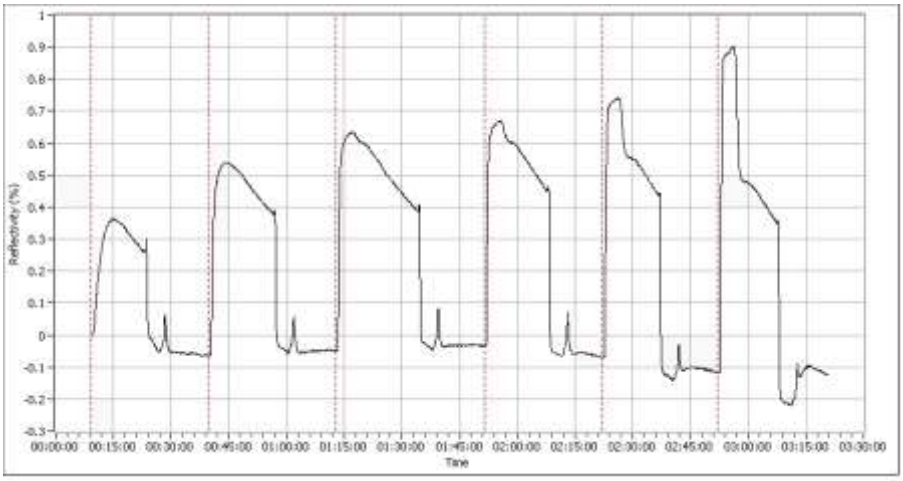

**Fig. 3** Calibration of the biochip for detection of albumin. The calibrations used were 1 mg/l, 10 mg/l, 50 mg/l, 100 mg/l, 250 mg/l and 500 mg/l

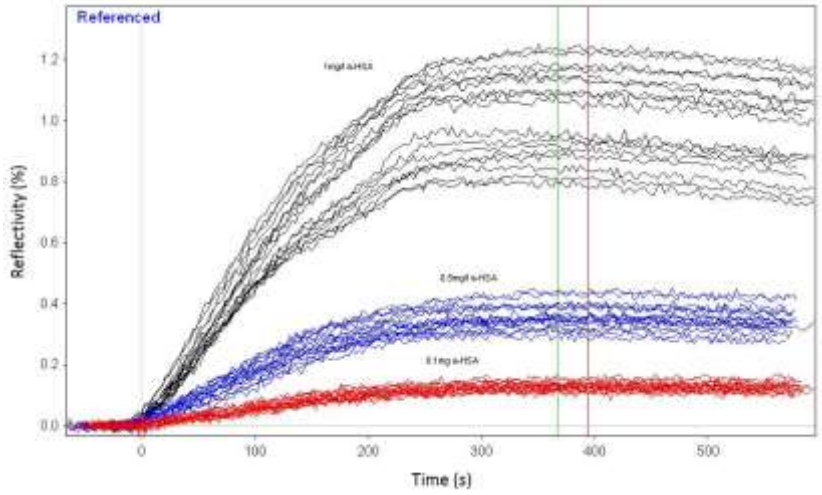


**Fig. 4** The measurement of low concentrations

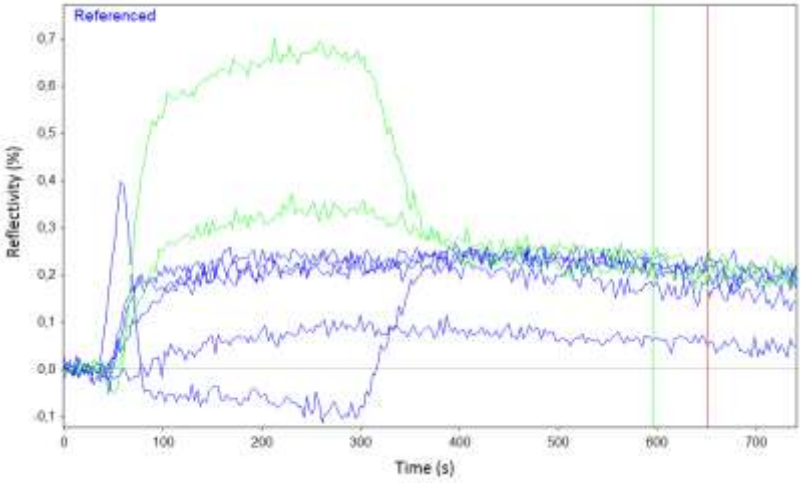


**Fig. 5** Real patient urine sample

## 3. CONCLUSION

We have proven on a series of measurements that the SPRi method can be used for detection of the amount of albumin in urine. A biochip was developed and tested first by albumin volume samples. In the second step the measurements of 61 patient samples were performed. The patient sample concentrations were from 3.6 to 1005 $mg.l^{-1}$. During comparisons using the Siemens BN ProSpec, it is possible to utilize results in the range of 21.0 to 1010 $mg.l^{-1}$ only. It was found that the SPRi method does not have satisfactory results for lower concentrations. This is predominantly due to the use of real urine samples.

## ACKNOWLEDGEMENTS

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