Biosensors based on surface plasmon resonance (SPR) register binding of analytes to the receptor molecules which are immobilized on the sensor surface. This measurement technology belongs to refractometry: changes of surface concentration are detected by measuring changes of the refractive index. An important task in the improvement of this technique is a separation of signals corresponding to the changes in the surface layer from undesired contributions caused by bulk phase, for example due to fluctuations of temperature, concentrations of solutes, pressure. We suggested here a new measurement technology for this topic: a penetration difference self-referencing surface plasmon resonance (PDSR-SPR). The wavelength of the incident light influence strongly the penetration depth of the corresponding evanescent wave. This dependence was exploited for compensation of the contributions of the bulk refractive index using differential SPR measurements at two wavelengths with different penetration depths. A performance of the PDSR-SPR was optimized by computer simulation and then evaluated in experimental measurements. Effects of temperature and ionic strength were tested. About 20 times suppression of the bulk phase contribution was achieved. The new approach was applied for immunosensing and for detection of streptavidin - biotin binding.

\[
\begin{align*}
\text{Signal}_1 &= \alpha_1 d + \beta_1 n \\
\text{Signal}_2 &= \alpha_2 d + \beta_2 n
\end{align*}
\]  

\[
\text{Output Signal} = \frac{\alpha_1}{\beta_2} \text{Signal}_1 - \frac{\alpha_2}{\beta_1} \text{Signal}_2 = d(\alpha_1 - \frac{\alpha_2}{\beta_1} \epsilon)
\]

Fig. 1. One of possible realizations of PDSR-SPR.

Keywords: SPR, surface plasmon resonance, evanescent wave, refractometry