Anal. Chem., **77** (8), 2393 -2399, 2005. 10.1021/ac048156v S0003-2700(04)08156-9

Double-Wavelength Technique for Surface Plasmon Resonance Measurements: Basic Concept and Applications for Single Sensors and Two-Dimensional Sensor Arrays

Alexander Zybin,*† Christian Grunwald,‡ Vladimir M. Mirsky,§ Jürgen Kuhlmann,‡ Otto S. Wolfbeis,§ and Kay Niemax†

ISAS - Institute for Analytical Sciences, Bunsen-Kirchhoff-Str. 11, D-44139 Dortmund, Germany, Max-Planck-Institute for Molecular Physiology, Otto-Hahn-Str. 11, D-44202 Dortmund, Germany, and Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, D-93040 Regensburg, Germany

Received for review December 14, 2004. Accepted February 9, 2005.

Abstract:

A new technique for on-line monitoring of analyte binding to sensor surfaces by surface plasmon resonance (SPR) detection is described. It is based on differential measurements using two wavelengths provided by two diode lasers. The technique is as simple and robust as the conventional SPR detection measuring the reflected radiation at fixed incidence angle, but it has the advantage of being nonsensitive to variations of the resonance width and providing essentially higher signal/noise ratios. The paper presents the first four channel prototype system for parallel 2D-monitoring at four different spots. One channel is always used as a reference to compensate temperature fluctuations and nonspecific adsorptions. Calibration with sucrose solutions revealed an absolute sensitivity of $\Delta n \sim 5 \times 10^{-6}$. The new technique is tested with a biotin-streptavidin binding and with hybridization/denaturation of DNA. Biotin binding to a streptavidin monolayer is detected with a signal/noise ratio of about 5, which demonstrates the high potential of the new technique for applications in drug discovery. Applications to gene analysis are tested with short oligonucleotides of the sequences used for genotyping human hepatitis C viruses. A selective response to complementary oligonucleotides is observed. The high reproducibility in subsequent cycles of hybridization/denaturation (by formamide or by heating) points out potential applications of the technique in medical diagnostics, food industry, genomics, and proteomics too.