Interface Processes

Use of the surface plasmon resonance technique for specific detection of single biological nano-objects

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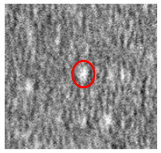
Introduction

Here we present a novel **label-free** analytical method for specific detection of **single** biological nano-objects. Modified SPRi technique (SPR nanoscopy) allows visualization of nano-object binding in the **real-time.** SPRi is also useful for determination of low nano-particle concentrations in aqueous solutions.

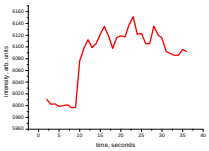
The method is based on the detection of single particles bound to the functionalized sensor surface. Functionalization could be performed with target-specific antibodies to detect biological nano-particles.

This method offers a broad range of biomedical applications, especially in the research field of interface processes (movement of nano-particles onto re-constracted bi-lipid membranes; fusion of biological nano-particles with lipid membranes; etc.)

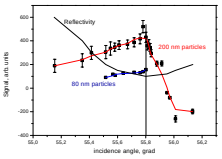
Analytical characteristics of SPR nanoscopy



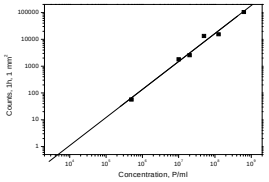
Binding image of inactivated influenza A virus



Intensity step after particle binding



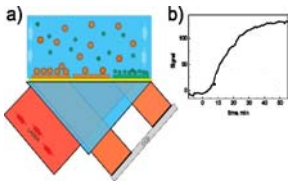
Signal dependency on the incidence angle

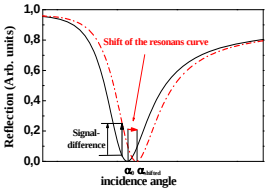


Measurements of particle concentration (200nm particles were used as a model)

Detection of individual particles instead of particle layers

Classical SPRi

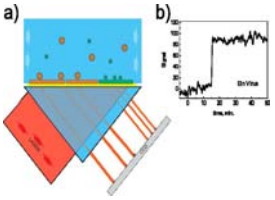


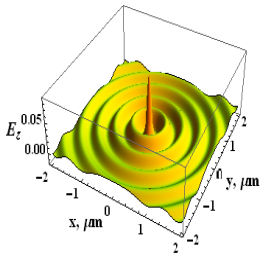


Classical SPR

The signal appears as a result of the shift of the resonance curve (after formation of the biomolecule layer onto sensor surface)

SPRi Nanoscopy

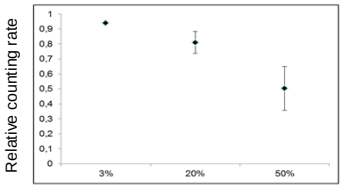




SPR nanoscopy

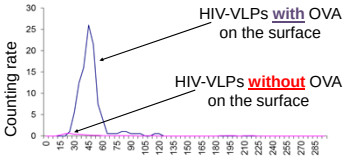
It is possible to visualize a signal because the appearance of concentric plasmon waves surrounding bound particle.

Results of the current study



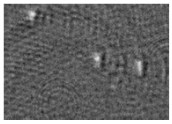
Concentration of serum (FCS) in buffer, in %

SPR nanoscopy is applicable for analysis of samples containing serum.

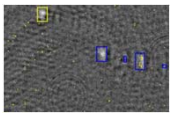


Intensity, arb. units

SPR nanoscopy specificaly detects the binding of bionano-particles to sensor surface (antibody against ovalbumin (OVA) and HIV virus-like particles were used).



Before analysis



Analysis of the sequence of recorded images

SPR nanoscopy needs the development of new software for data analysis. Our colleagues from TU Dortmund already created a program, which successfuly analyses images of 200nm particles.

Outlook

✓ To develop a sensor for the simultaneous detection of different biological nano-particles in one probe ("array format").

✓ To re-constract bi-lipid membrane onto sensor surface for further investigation of the fusion of biological nano-particles with re-constracted membrane. This issue is especially important for the biology of extracellular vesicles and drug-delivery particles.

✓ Online monitoring of liquid media for the presence of target nano-particles in minor concentrations.

References

E.L. Gurevich, V. V. Temchura, K. Überla, A. Zybin. Analytical features of particle counting sensor based on plasmon assisted microscopy of nano objects. Sensors and Actuators; B160, (**2011**)1210-1215.

A. Zybin, Y. A. Kuritsyn, E. L. Gurevich, V. V. Temchura, K. Überla, K. Niemax. Surface plasmon resonance for detection of dielectric nanoparticles and viruses. Plasmonics; 5, (**2010**) 31–35.

P4-02

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Modified surface plasmon resonance imaging (SPRi) technique was reported to be a novel method for the detection of the binding of single nano-particles to sensor's surface [1, 2]. However, bio-analytical features of this SPRi method and specificity of performed detection require further examination. In current study, we demonstrated that modified SPRi technique allows detection and visualization of single inactivated influenza viral particles and HIV-VLPs. The detection was performed in buffers without serum as well as in buffers containing different concentrations of serum (up to 50%). We also showed specificity the binding of biological nano-particles to the functionalized sensor's surface. Furthermore, we investigated the dependence of particle binding rate on the density of antibodies onto the biosensor surface and demonstrated the applicability of modified SPRi technique for the determination of particle concentrations in buffers. During this study we also developed new algorithms and software for the data processing and analysis. Together, our findings open new horizon for SPRi technique in such research areas as viral biology and biology of extracellular vesicles (exosomes and microvesicles).

[1] A. Zybin et al. (2010) Real-time detection of single immobilized nanoparticles by surface plasmon resonance imaging. Plasmonics; 5: 31-35.

[2] EL. Gurevich et al. (2011) Analytical features of particle counting sensor based on plasmon assisted microscopy of nano objects. Sensors and Actuators; 160: 1210-1215.