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

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References


DETECTION AND QUANTIFICATION OF NANOOBJECTS IN LIVING SYSTEMS

P4-01
THG microscopy for high-resolution in vivo imaging of nanomaterials

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The in vivo imaging of the localization and behavior of nanomaterials in cells or tissues is largely restricted to fluorescence microscopy that offers high spatial resolution and signal-to-noise ratio. However, this requires either inherently fluorescent nanomaterials, e.g., quantum dots (QDs), or the attachment of fluorescent labels that, in turn, might alter the properties of the nanomaterial. To address this problem, we assessed the potential of Third Harmonic Generation (THG) microscopy for in vivo imaging of non-fluorescent nanomaterials.

THG microscopy is based on optical effects induced by specific inherent physical properties of a specimen. Recently, we demonstrated that THG microscopy allows high-resolution label-free 4D visualization of cellular and tissue structures, in intact muscle of live mice.

Initially, we defined the multi-photon settings necessary to induce signals from anatase TiO₂ (NM-101) and Ag nanoparticles (NM-300) as well as from carboxyl QDs (Invitrogen) as fluorescent control particles and characterized the spatial resolution of the microscope system under the imaging conditions used (0.9 μm in x;y; excitation light 1275 nm, detection 417-477 nm).

Performing THG microscopy of live RAW264.7 macrophages 2 h upon incubation with TiO₂ nanoparticles clearly revealed their localization in intracellular vesicles as well as dynamic movement of these vesicles. By using THG microscopy on skeletal muscle tissue of live mice, we were able to detect TiO₂ as well as Ag nanoparticles in the blood stream immediately after systemic injection. Ag nanoparticles were formed to form stable associations with microvessel walls. Additionally, THG provided exact information about the localization of carboxyl-QDs translocated to skeletal muscle tissue 1 h after systemic administration, as verified by fluorescence microscopy.

Taken together, THG microscopy appears to be a suitable tool for high-resolution 4D imaging of nanomaterials in vivo.

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 percentages 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).