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Modifications of the PAMONO-sensor help to size and quantify low number of individual biological and non-biological nano-particles.

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Introduction

In the series of works, we demonstrated that plasmon assisted microscopy of nano-objects (PAMONO) technique can be successfully employed for sizing and quantification of viruses, microvesicles and charged non-biological particles. The PAMONO-sensor allows for label-free and specific detection of **individual biological nano-particles.** In order to achieve homogeneous illumination and enlarged focused image area on the sensor slide, the PAMONO-sensor configuration was modified. These modifications are discussed in the present work. In future, applied modifications will help to analyze samples with low concentration of target nano-particles and also help to gain biochemical information about nano-vesicles of interest.

Aims of studies

1) Verify whether dependency between the value of light intensity step and size of nanoparticles remains linear for modified PAMONO-sensor.

2) Confirm the ability of the PAMONO-sensor to detect simultaneously 800nm and 200nm silica particles (they may serve as optical models of bacteria and viruses).

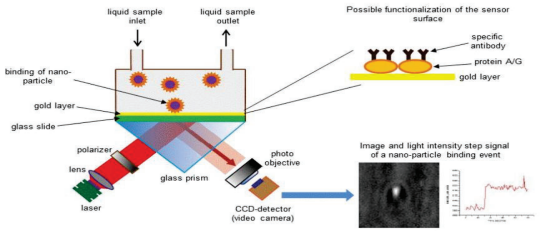
3) Verify whether formation the layers of biological molecules: Cys-conjugated protein A/G and antibodies remains detectable after applied sensor modifications.

4) Define possible duration time for the analysis of nano-particle samples using one sensor chip without its replacement.

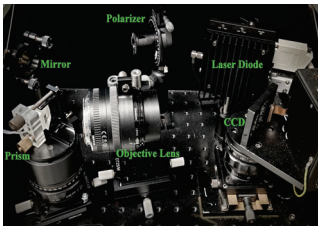
Detection of single particles, not particle layers

Classical SPR: The signal appears as a result of the resonance curve shift after formation of the biomolecule layer onto sensor surface.

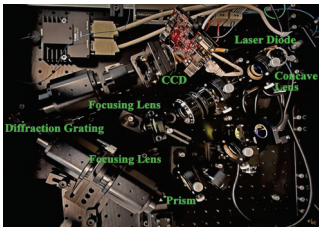
PAMONO-sensor: Particle binding signal appears as a bright spot on a gray backround. Secondary concentric plasmonic waves help to visualize a signal.



Standard and modified PAMONO-sensor



Standard PAMONO-sensor



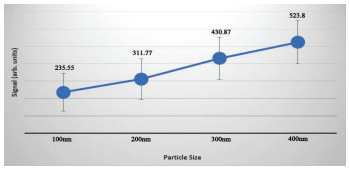
Modified PAMONO-sensor

In the modified PAMONO-sensor setup, a concave lens was employed to obtain homogeneous illumination of the gold sensor chip surface. Further, the enlargement of the image focused area for the modified PAMONO - sensor was achieved via mounting of a diffraction grating.

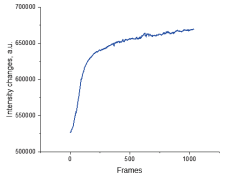
Results

It was important to verify whether key characteristics of the PAMONO-sensor demonstrated in our previous works (Gurevich, 2011), (Shpacovitch, 2015) (Shpacovitch, 2017) remain unchanged after modifications of the instrument's optical scheme. Thus, we proved that the modified PAMONO-sensor still demonstrates linear dependency between the value of intensity step and the size of analized nanoparticles. Further, we also show that modified PAMONO-sensor retains the traits of classical SPR sensors and helps to monitor the coating of sensor surface with different proteins such as Cys-conjugated protein A/G and antibodies, for example.

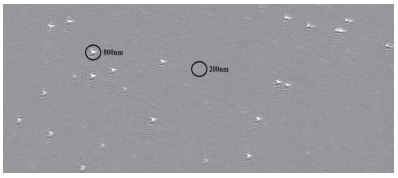
1. Dependency between the value of intensity step and the size of nanoparticles (200nm polystyren particles were used; modified PAMONO-sensor).

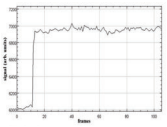


2. Monitoring the adhesion of Cys-protein A/G to the gold sensor surface of the modified PAMONO-sensor.

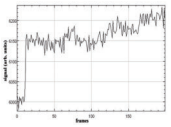


3. Simultaneous detection of 800nm and 200nm silica particles using modified PAMONO-sensor.





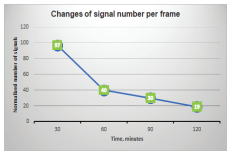
Step signal of an 800nm particle. Signal value is around 900 a.u.



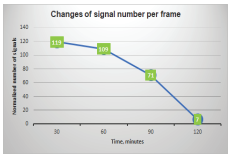
Step signal of a 200nm particle. Signal value is around 150 a.u.

4. Changes the number of signals appeared per recorded image during sample analysis (200nm polystyrene particles were used in experiments performed on standard and modified PAMONO-sensors).

Standard PAMONO-sensor



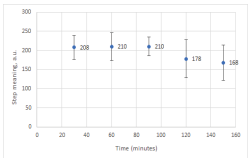
Modified PAMONO-sensor



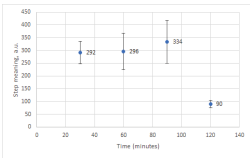
The received data were normalized according to the image area and also the concentration of the nano-particles used for analysis.

5. Changes the value of intensity step signal monitored during sample analysis (200nm polystyrene particles were used in experiments performed on standard and modified PAMONO-sensors).

Standard PAMONO-sensor



Modified PAMONO-sensor



Perspectives:

1. Define possible duration time for the analysis of samples containing biological nano-particles.
2. Confirm the power of the modified PAMONO-sensor for sizing and quantification of nano-particles in low concentrated samples

References:

1) Shpacovitch V. et al., ***Analytica Chimica Acta*** (2018) 1005 pp 1-15
2) Shpacovitch V. et al., ***Sensors*** (2017) 17, 244; 10.3390/s17020244
3) Shpacovitch V. et al., ***Analyt. Biochemistry*** (2015) 486 pp 62-69.
4) Gurevich E. et al., ***Sensors and Actuators B*** (2011) 160 pp 1210-1215.

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