Short Visit Grant 3633
Scientific Report: Investigation of biomolecular recognition on noble metal nanoparticles by micro spectroscopy

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Purpose of the visit

The optical properties of noble metal nanoparticles depend on the excitation of localized surface plasmons (LSPs) by electromagnetic irradiation. The position and intensity of the occurring plasmon band is influenced by particle specific features such as size, shape and material/composition. Important for biosensoric applications are changes in the local refractive index of the surrounding medium, which can also cause a detectable plasmon band shift.

Our research studies focus on the investigation of biomolecular recognition of antigen-antibody-interaction and DNA hybridization on the single particle level. Therefore one species of biomolecules are immobilized on the surface of plasmonic active nanoparticles and the spectral information is detected by micro spectroscopy. When target molecules (antigen or complementary oligonucleotide) binds to the functionalized particle surface, a resulting spectral shift is observed.

An important question in this field aims at the cause of sensitivity (that means extend of band shift upon refractive index change at the particle surface). Therefore, different particle types as well as (bio) chemical systems are compared in order to elucidate the mechanisms.

The main aim of the visit at the hosting institution is to compare the sensitivity for detecting DNA hybridization on the Jena bottom-up synthesized nanoparticles with that for the Graz top-down fabricated nanostructures. Therefore, capture DNA molecules will be bound onto the top-down structures prior to incubation with target DNA. Binding will result in band shift, which will be detected and compared to the shift previously measured with the same DNA hybridization system using bottom-up particles.

Experiments and main results

Sensing DNA hybridization

In the first part of the visit at the host institution, the sensitivity for detecting DNA hybridization of chemically synthesized gold nanoparticles should be compared to electron beam lithography fabricated gold nanoparticles. Therefore, gold nanoparticles with three different shapes - rings, split rings and discs - were prepared using electron beam lithography (EBL) and thermal evaporation in combination with a lift-off process. The principle of this top-down technique is shown in figure 1.

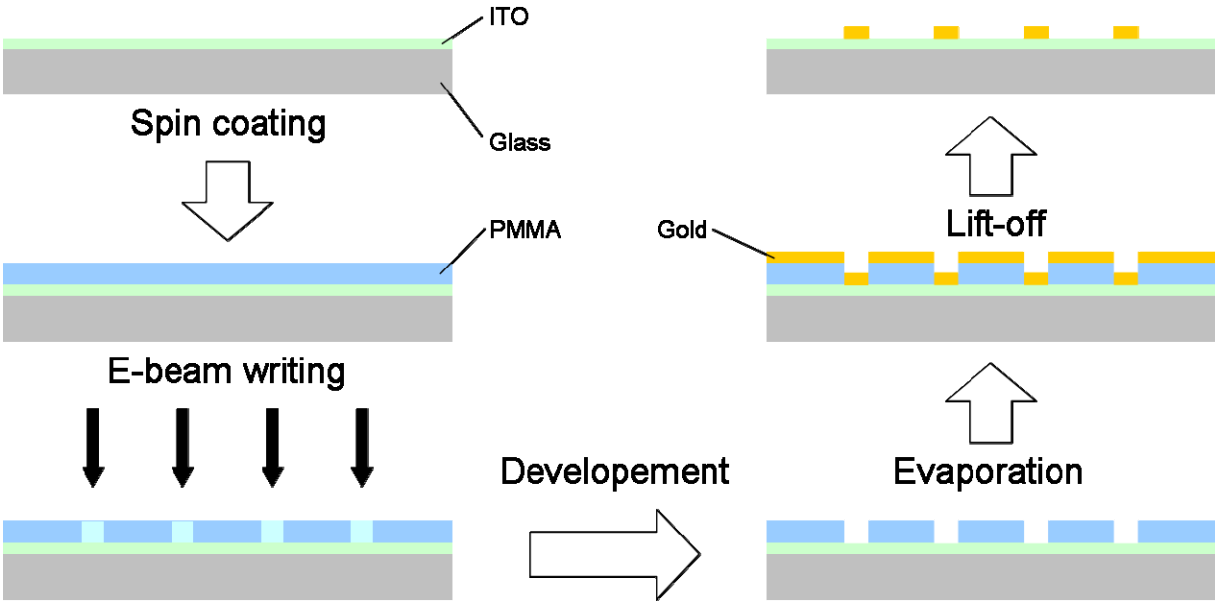
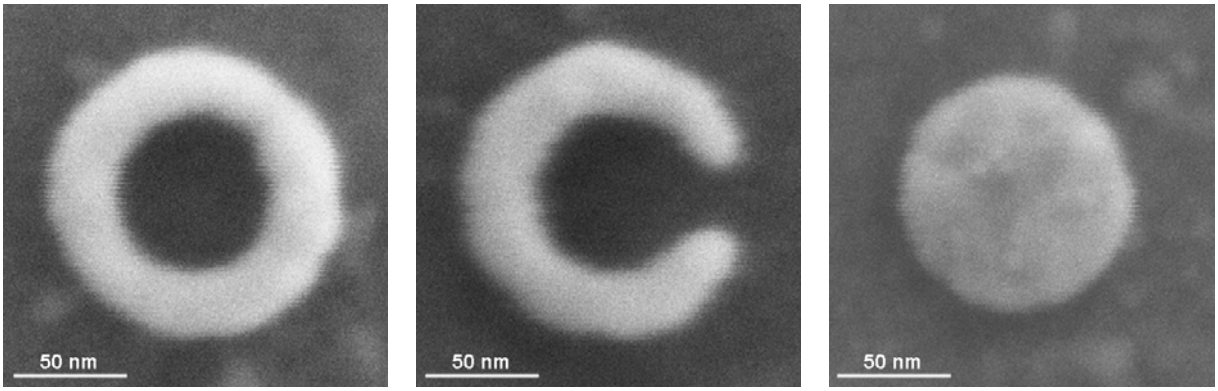


Figure 1: Fabrication of gold nanostructures by electron beam lithography and thermal evaporation with subsequent lift-off. The ITO-coated glass substrate ensures the electric conductivity required for electron beam exposure.

Different shapes like rings, split rings and discs have been produced by this technique. The particles were arranged in an 11 x 11 array with 5 µm distance between each particle allowing single particle measurement. Lateral particle dimensions were measured with SEM for controlling the quality of EBL (see figure 2).

The nanostructured particles were optically characterized by single particle scattering spectrsocopy. Therefore a Nikon Eclipse TE2000-S inverted microscope in combination with a self-made prism spectrometer was used. The illumination of the nanostructures was realized by a multimode optical fiber coupled slide projector lamp with a defined incidence of light (15 degrees to the sample surface). The scattered light from the particle son the top side is collected by a 40x microscope objective (NA = 0,75) and analyzed by a spectrometer with a Andor iXonEM 885 CCD camera. The optical setup is shown in figure 3.



(a) (b) (c)

Figure 2: *SEM image of selected gold nanoparticles fabricated on ITO-doped glass substrate by electron beam lithography (EBL). (a) Rings, (b) split rings (with different gap sizes) and (c) discs were prepared and used for biosensing of DNA hybridization events by single particle scattering spectroscopy.*

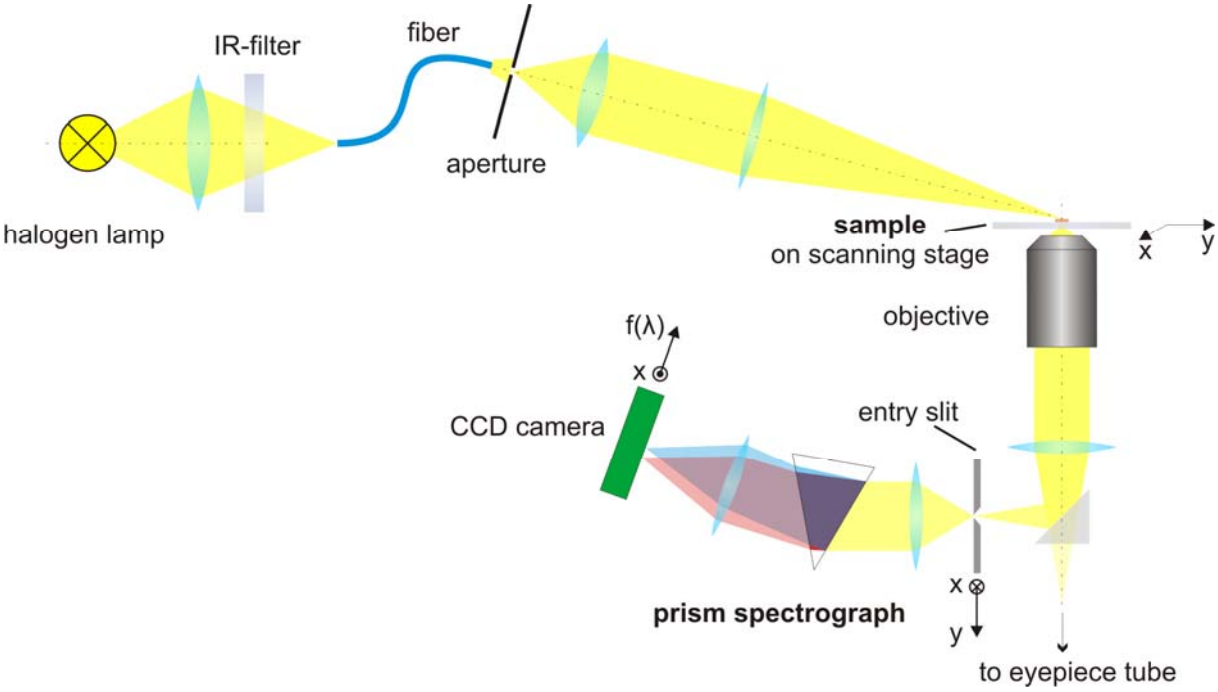


Figure 3: *Optical setup for single particle scattering spectroscopy. The setup allows the spectral analysis of all particles positioned in the entry slit for parallelized investigation of the optical properties.*

In order to investigate the changes in localized surface plasmon resonances due to local refractive index changes of the nanoparticle surrounding medium caused by attaching biomolecules, a capture oligonucleotide was immobilized on the EBL-structured nanoparticles. The attachment of oligonucleotides to the gold nanoparticles was realized by a thiol modification, which is known for their high affinity to gold surfaces (Csaki et al. 2001). The following step was the hybridization of target oligonucleotides with complementary base pair sequence to the immobilized capture oligonucleotides. After each step single particle scattering spectra were collected by the method decribed above, and a slightly red shift due to the refractive index changes (caused by the binding of the DNA layer on the particle surface) could be observed. Representative results for one of the three different particle shapes are shown in figure 4.

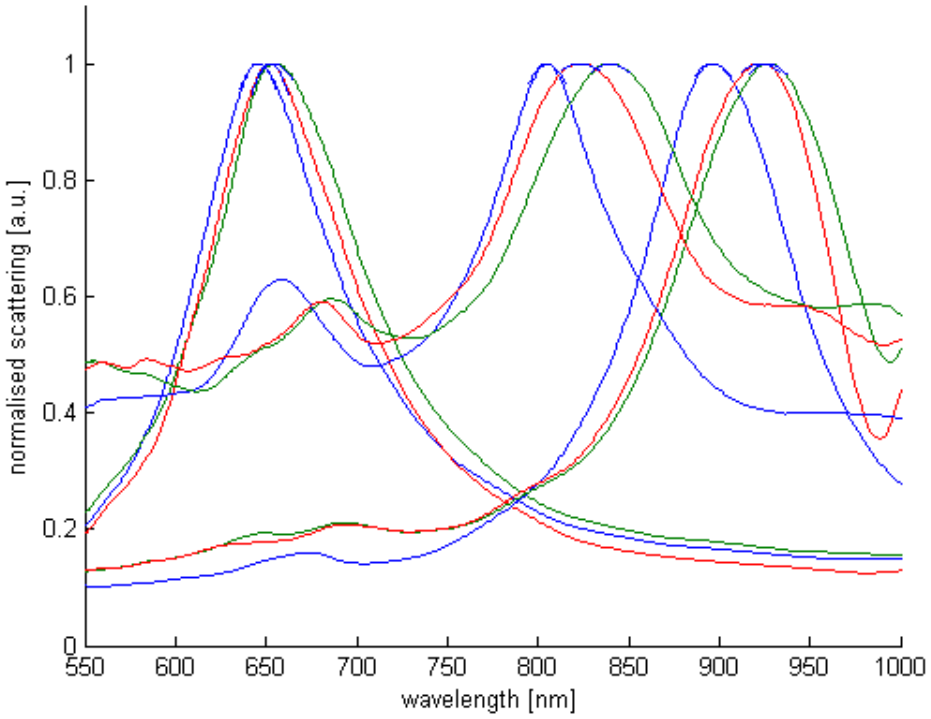


Figure 4: *Normalized scattering spectra of three different nanostructures (from left to right: disc, split ring and ring). Blue: clean nanostructure; red: DNA immobilization; green: DNA hybridization. The red shift upon DNA binding could be detected for all observed particle shapes.*

For all different particle shapes, the expected red shift upon binding of capture and target DNA was detected. The red shifting of the LSPR peak was caused by a higher refractive index of the particle surrounding medium (from n_{air} = 1,0 to n_{DNA} = 1,7-1,8). For comparing the sensitivity of the different nanoparticles upon binding DNA, the applied oligonucleotide concentration (1 µM) and incubation time (2 h) was constant for all particles. The averaged spectral shift for all measured disc, ring and split ring nanostructures are shown in figure 5.

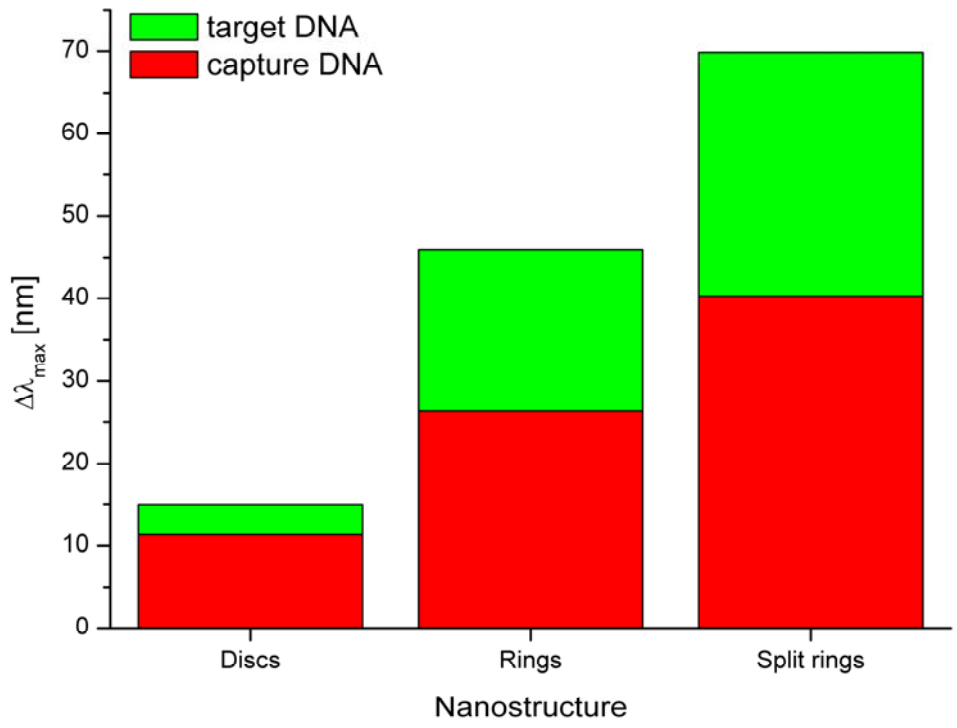


Figure 5: Averaged LSPR peak red shift after immobilization (red) of capture and hybridization (green) of target DNA. A large shift of the plasmon peak could be observed for the split rings nanostructures with about 30 nm for the hybridization step.

As seen in figure 5, the nanodiscs shown a small peak shift of about 3 nm, while rings (about 20 nm) and split rings (about 30 nm) shows large LSPR sensitivity upon binding of molecules to the particle surface. These results indicates, that nano split ring structures are predestinated for utilization in further (bio-)sensing experiments. A peak shift of about 30 nm for top-down structures is much higher than the peak shift observed for bottom-up structures in previous experiments (about 5 nm), so that subsequent studies will also be done with these EBL-fabricated nanoparticles.

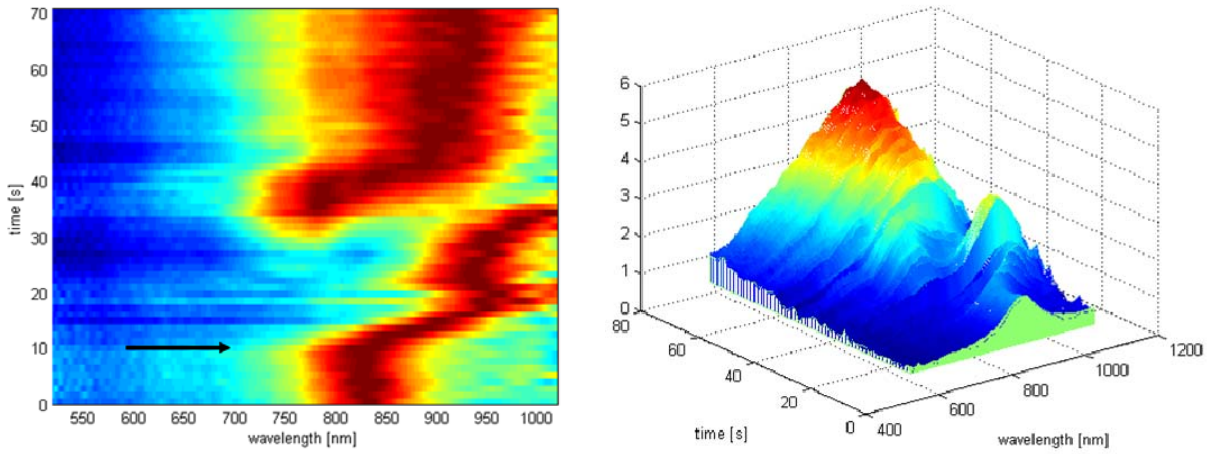
In an additional experiment the binding kinetics of DNA immobilization and hybridization should be determined by measuring in a fluid medium. In a first step, spectral properties of the nanostructures in water have been measured at the hosting group, but for determining the binding characteristics, a flow cell chamber for a continious flow of the fluid is required. A further cooperation will be helpful to find a solution for the online measurement.

Kinetic studies of silver enhancement

A goal of our research group is tuning the sensitivity of the nanoparticle sensor by silver enhancement. For particle solutions an increasing sensitivity for sensing nanoparticle-surface attached (bio-)molecules could be shown for gold-silver core-shell nanostructures with a thin shell thickness of about 2-4 nm [Stranik et al.; Steinbrück et al.]. Our aim was to investigate the change of the spectral properties of synthesized core-shell nanoparticles by single particle scattering spectroscopy. The shift of the LSPR maximum over time should be observed with the optical setup in the hosting group.

The measurement was done by fabrication of EBL-nanodiscs by the method described above. Silver deposition was realized by a silver enhancement kit, which contains two solutions. A silver salt solution and a reducing solution were mixed together in equal volumes and added on the immobilized nanoparticles. The reaction can be stopped by adding a high amount of water. For our experiments, the kit solutions were diluted in water to slow down the reaction by a low concentration of silver in the reaction mixture.

Scattering spectra of the EBL-nanostructures were collected before adding the silver salt solution for creating the silver shell. The reaction was started by adding the diluted silver kit solution and 100 spectra were taken each 1,2 seconds with exposure time of 1 second. The progress of the LSPR peak is shown in figure 6.



(a) (b)

Figure 6: (a) Normalized scattering spectra of gold nanodiscs during the silver enhancement process is shown. The black arrow indicates the injection time of the silver enhancement solution. (b) 3D graph of scattering spectra of gold nanodiscs during the silver deposition process.

In general, LSPR spectra of metal nanoparticles are very sensitive to the addition of a metallic shell around the particles. This effect was clearly seen in our experiments. Immediately after adding the silver enhancement solution, a change of the LSPR plasmon peak was observed,

which indicated the growth of a silver layer around the nanoparticle. The LSPR was gradually shifted from 830 nm to the longer wavelength. Approximately 25 seconds after adding the silver solution a new LPSR peak around 780 nm was formed. With the time, this plasmon peak also shifted to the longer wavelength until around 900 nm. There was no change of the profile of the scattering spectra and only the intensity was still slightly increasing after 40 seconds. We assume that the first peak is associated with the plasmon mode in the gold core and the second peak formed around 25 seconds after deposition is associated with the plasmon resonance of the thick silver shell. Currently, numerical simulations are carried out in order to confirm this prediction.

Future collaboration and projected publications

Future collaborations with the hosting institution are planned to further investigate reaction kinetics of biosensoric issues like DNA hybridization, DNA-protein- and also protein-protein-interactions like antigen recognition by specific antibodies and ligand-receptor-interactions. Tuning of nanosensor structures by silver deposition is another point of further experimental observations. For all these experiments the planning of a flow cell chamber is actually in progress and will be the focus for following visits at the hosting research group.
A publication in a field related journal is also projected. The topic will be the sensitivity comparison of different nanostructures build by the top-down and also bottom-up procedure. Silver enhancement of nanoparticles for creating high sensitive biosensors will lead to a further publication.

References

Csaki, A., R. Moeller, et al. (2001). "DNA monolayer on gold substrates characterized by nanoparticle labeling and scanning force microscopy." Nucleic Acids Research **29**(16): e81.

Steinbrück, A., et al. (2010). „Sensoric potential of gold-silver core-shell nanoparticles - Part 1: Experimental.“ Anal Bioanal Chem Submitted.

Stranik, O., et al. (2010). „Sensoric potential of gold-silver core-shell nanoparticles - Part 2: Theory.“ Anal Bioanal Chem Submitted.