Optoplasmonic detection of single particles and molecules in motion

Asgari, N.

Summary

Detecting nanoscopic objects plays an important role in nanoscience in particular, in the rapidly growing field of nanobiology. The forebear to modern super-resolution microscopy for single molecule investigation, is fluorescence microscopy. Fluorescence as a contrast mechanism, however, brings several restrictions. These include (1) the use of the label itself, which may introduce artifacts to the interpretation, (2) the limited photoemission caused by photobleaching and photoblinking as well as (3) low bandwidth of the emission. Fluorescence-free alternatives are thus highly desirable to overcome these limitations. Optical detection of individual proteins with high bandwidth holds great promise for understanding important biological processes on the nanoscale. In this thesis, we investigate label-free optoplasmonic detection of single proteins and particles in motion. Analysing the data provide information about the hydrodynamic volume of the diffuser and interaction such as binding events.

Optoplasmonic bio-detection assays commonly probe the response of plasmonic nanostructures to changes in their dielectric environment. The accurate detection of nanoscale entities such as virus particles and proteins requires optimization of multiple experimental parameters. In **chapter 2** of this thesis, we introduce photothermal spectro-microscopy as a benchmarking tool for the characterization and optimization of optoplasmonic detection assays. We showed the photothermal signal-to-noise ratio (SNR) scales directly with the SNR found for average perturbations caused by small nanodroplets entering and exiting a nanorod's nearfield. In **chapter 3** we use immobilized gold nanorods as opto-plasmomic sensor, read out changes in its resonantly scattered field, and observe single proteins as they traverse the plasmonic near field of individual gold nanorods. We optimized experimental parameters of photothermal spectroscopy. We demonstrated the transient detection of single proteins with masses as low as 64 kDa traversing the sub-attoliter volumes spanned by plasmonic near fields during times as short as 100 ns and with a signal-to-noise ratio (SNR) exceeding 5.

From our experiments, we determined hydrodynamic radii that agree well with literature values and with complementary DLS measurements.

Rotational Brownian dynamics had a vast impact in fields ranging from physical chemistry to biology. Monitoring the rotational diffusion requires strong and anisotropic scatterers such as plasmonic nanoparticles. Gold nanorods (GNRs) are such strong, anisotropic plasmonic scatterers, which are chemically inert and very photostable at the low intensities required for scattering measurements. In **chapter 4** of this thesis, we have studied experimentally the rotational diffusion of individual GNRs as they diffuse through the confocal volume of the microscope. We studied the effect of the polarization configuration on the rotational correlation. We also investigate the change of tumbling rate due to hot Brownian rotation of the nanorods. Finally, we studied how tumbling of these particles changes in presence of very low concentrations of a polymer (PVA) in the solution. Enhancement of the tumbling time of GNRs is due to binding of polymer molecules to the rods. We found that this process is described by a Langmuir isotherm with evidence for heterogeneity of the binding sites. Therefore, detailed studies of rotationally diffusing plasmonic probes reveal specific adsorption sites, and local mobilities with high resolution in time and space.

Measuring the orientation dynamics of non-fluorescent molecules in real time with optical methods is still a challenge in nanoscience and biochemistry. In **chapter 5** of this thesis we examine the possibility to detect conformational changes of single proteins (which yields orientation changes of protein subdomain), by a so-called plasmonic goniometer. We have simplified the sensing components to a sensor GNR and freely diffusing plasmonic analytes and detected the rotational diffusion of analytes. Our detection method is based on monitoring the dark-field scattering of a large sensor GNR (40 nm in diameter, 112 nm in length) as smaller plasmonic analytes cross the near field of the sensor. We observe the rotational motion of single small analyte gold nanorods (around 5 nm in diameter and 15.5, 19.1 and 24.6 nm in length) in real time with a time resolution around 50 ns. Plasmonic coupling enhances the signal of the analyte gold nanorods, which are one order of magnitude smaller in volume (about $300\,\mathrm{nm}^3$) than those used in previous rotational diffusion experiments. Yet, as the gain in angle sensitivity from opto-plasmonic coupling compared to confocal measurements is modest, it seems unlikely that this improved sensitivity by itself will justify the efforts required to build reliable plasmonic goniometers.