

Automatic spatial refinement for fast and precise acquisition of single particle spectra



A.Henkel, C. Sönnichsen, Johannes Gutenberg University Mainz , Germany (April 2014)

Application Note

Introduction

Plasmonic noble metal nanoparticles are good sensors for refractive index changes in their immediate environment. Illuminating the nanoparticles with white light leads to a collective oscillation of the conduction electrons, called the plasmon. Because of this phenomenon, light corresponding to the resonance frequency is scattered strongly. The resonance frequency depends on several factors including size, material, shape, and refractive index of the local environment. We can monitor the resonance position of a single particle as an indicator for changes in the local environment, such as binding of small particles or molecules [2-5].

As binding normally occurs on a short time scale, and most proteins only produce small shifts, it is important to build a setup with maximal time and spectral resolution. Here we present our home-build setup and data concerning the quality at detecting small shifts.

Experimental Setup

We have made a major improvement compared to common methods for acquiring spectra of single plasmonic particles by using an EMCCD camera.

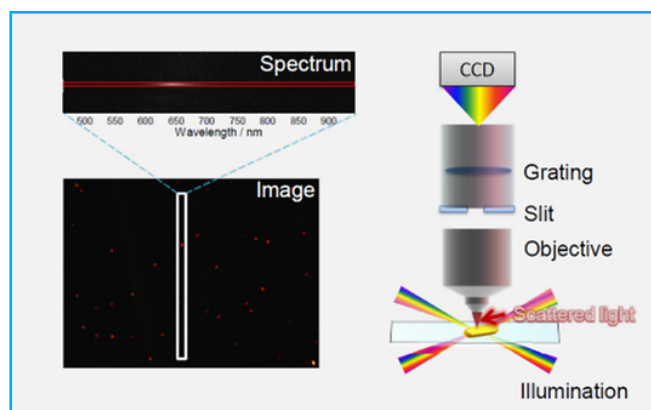


Figure 1: Experimental setup for time resolved spectral measurement of noble metal nanoparticles.

Figure 1 shows the experimental scheme. The core of our device consists of an inverted transmission microscope equipped with a XY-piezo stage and a Z-piezo. For illumination, we use a halogene white light source under dark-field illumination. Light scattered from single nanoparticles is collected by an objective and dispersed through a spectrometer. As detector we use

an EMCCD camera (Andor iXon3 DU888-EC-BV) and a Consumer DSLR camera. First, an image is acquired using the DSLR camera. Particle positions are registered and recalculated in stage positions. Then the particles are moved to the slit of the spectrometer in a serial fashion. Before the final spectrum is recorded each particle position is optimized in X-, Y-, and Z-direction so that the spectrum has maximum intensity. For this refinement we record one image stack while moving perpendicular to the slit and center the particle in the slit. Then – having found the optimal X- and Y-position - we record another image stack while moving in Z-direction to focus the particle. For the refinement we use very small exposure times around 10ms and high EM-gain around 100 to be able to identify the optimum positions reliable. For the final spectrum acquisition we use exposure times around 100ms to 500ms and lower EM-gain around 50 depending on the particle size investigated.

This measurement scheme allows for the collection of roughly 15 single particle spectra per minute including full position optimization.

Example

To demonstrate the potential of this spectroscope for fast and precise spectral acquisition, we measured the repeatability of the resonance wavelength for a set of single particles. Here, the standard deviation of the resonance wavelength of these spectra displays the precision in repetitive spectrum acquisition.

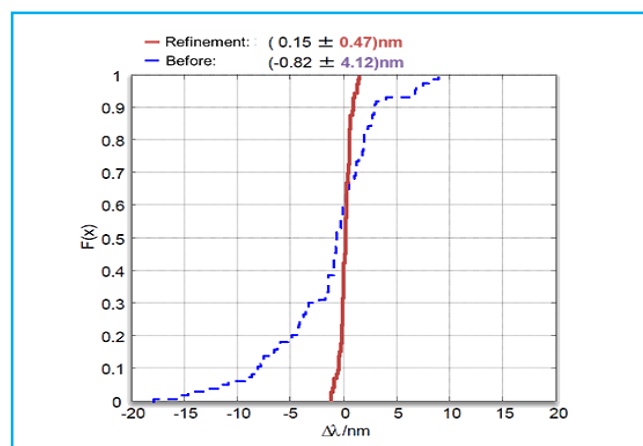


Figure 2: Repeatability of the spectral position for two consecutive measurements. The standard deviation is improved by one order of magnitude using the refinement method.

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Conclusion

The high sensitivity of the Andor iXon3 888 EMCCD camera in combination with a piezo stage and registered particle positions allows for improving the spectral precision to 0.5nm for repetitive spectrum acquisition as well as for improving the speed of acquisition of plasmonic resonance spectra up to about 15 particle/minute. These improvements allow us to observe interesting processes that were previously not resolvable, like the detection of a set of proteins to aptamer-functionalized sensors at nanomolar concentrations.[5]

References

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