Published in final edited form as:
J Phys Chem C Nanomater Interfaces. 2009 January 1; 113(39): 16839–16842. doi:10.1021/jp907377h.

LSPR Imaging: Simultaneous Single Nanoparticle Spectroscopy and Diffusional Dynamics

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Abstract

A wide-field localized surface plasmon resonance (LSPR) imaging method using a liquid crystal tunable filter (LCTF) is used to measure the scattering spectra of multiple Ag nanoparticles in parallel. This method provides the ability to characterize moving Ag nanoparticles by measuring the scattering spectra of the particles while simultaneously tracking their motion. Consequently, single particle diffusion coefficients can be determined. As an example, several single Ag nanoprisms are tracked, the LSPR scattering spectrum of each moving particle is obtained, and the single particle diffusion coefficient is determined from its trajectory. Coupling diffusion information with spectral information in real time is a significant advance and addresses many scientific problems, both fundamental and biological, such as cell membrane protein diffusion, functional plasmonic distributions, and nanoparticle growth mechanisms.

Introduction

Spatial tracking of single particles has been of interest to the biological community due to the new information it provides on the organization of cell membranes, particle movement on cell surfaces, and the effects of the external cell environment.[1–7] Single particle tracking is used to determine the diffusion coefficients of individual particles, allowing for modes of motion inside cells to be characterized. For example, single particle tracking has revealed that diffusion in the cell membrane is not limited to Brownian motion, but instead includes directed, confined, tethered, and anomalous modalities.[1] Although the majority of particle tracking experiments have been performed with either fluorophores or quantum dots, noble metal nanoparticles have substantial promise as single particle tags because they are not susceptible to blinking or photobleaching.[7]

Single noble metal nanoparticles have proven to be attractive labels for bioanalysis due to the localized surface plasmon resonance (LSPR), the phenomenon responsible for the absorption and scattering spectra of the nanoparticles. The spectral position of the LSPR is dependent on the size, shape, and local dielectric environment of the nanoparticle.[8,9] Therefore, nanoparticle scattering spectra provide valuable information on the structure and surroundings of the nanoparticle. Because the analysis of single nanoparticles removes effects from ensemble averaging, researchers have focused on the characterization and sensing capabilities of single nanoparticles.[7,10–14] Additionally, particle tracking experiments with plasmonic nanoparticles can address other fundamental scientific problems

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such as nanoparticle growth mechanisms,[15] local ordering of liquid crystals,[16] and functional plasmonic distributions.

An ideal particle tracking experiment with plasmonic materials would yield information regarding both the diffusion properties and scattering spectra of multiple nanoparticles in real time. Typical methods of measuring single nanoparticle LSPR spectra require the nanoparticle to be isolated in a narrow field of view determined by the slit width of the spectrometer.[11–13] This method is inefficient for collecting multiple single nanoparticle spectra and limits experiments to particles that are immobile. Recently, Louit et al. acquired spectra of metal nanoparticles diffusing in living cells by using a translation stage to compensate for the motion of the nanoparticles; however, particle diffusion coefficients could not be measured.[17] Dual measurements of the LSPR spectra and diffusion coefficients of metal nanoparticles have been reported, but the two measurements were not correlated in real time.[7] The experiments reported here using wide-field LSPR imaging determine both the diffusion coefficients and scattering spectra of moving single Ag nanoparticles simultaneously, correlating both measurements.

Experimental Methods

Nanoparticle Synthesis

Ag colloids were synthesized by reducing Ag^+ by citrate according to the procedure developed by Lee and Meisel.[18] Briefly, 90 mg of $AgNO_3$ was dissolved in 500 mL of H_2O in a 1 L flask and brought to a boil. A 1% sodium citrate solution was added and the solution was boiled for 30 min. The resulting opaque, light brown-gray solution was removed from heat and was diluted with ~100 mL H_2O.

The protocol developed by Jin et al. was utilized for the synthesis of Ag nanoprisms.[19] In this synthesis, 0.1 mM $AgNO_3$ was stirred in the presence of sodium citrate (0.3 mM) on ice, followed by the addition of cold $NaBH_4$ (1 mL, 50 mM). To stabilize the particles, 1 mL 5 mM bis(*p*-sulfonatophenyl)-phenylphosphine dihydrate dipotassium (BSPP) was added drop wise. The resulting solution was stirred strongly on ice for 1 h, then stirred at a reduced speed for 3–4 h. The yellow solution was irradiated for 4–5 h with a 550 nm bandpass filter to yield a green solution.

Sample Preparation

To immobilize the particles, ~5 μL of the nanoparticle solution (either the Ag colloids or the Ag nanoprisms) were drop coated onto an 18 mm, no. 2 glass cover slip (Fisher Scientific) and dried with pressurized air. To characterize the nanoparticles' movement in solution, 10μL of the Ag nanoprism solution was added to ~100 μL of aqueous 66.67% glucose solution. A minute amount of the nanoprism-glucose solution mixture was sandwiched between a 22 mm, no. 1 glass cover slip (Fisher Scientific) and an 18 mm, no. 2 glass cover slip. To ensure a closed system, the edges were sealed with clear nail polish (Revlon ® Extra Life No Chip Top Coat).

Single Nanoparticle LSPR Imaging and Spectroscopy

Single nanoparticle spectroscopy was performed on an inverted microscope (Nikon Eclipse Ti-U) equipped with a dark-field condenser (Nikon, NA = 0.80 – 0.95) to illuminate the Ag nanoparticles and a variable numerical aperture 100x oil-immersion objective (Nikon, NA = 0.5–1.3, set to NA =0.5) to collect only the resonant Rayleigh scattered light from the nanoparticles. The scattered light from multiple nanoparticles was collected by the objective and sent through a liquid crystal tunable filter (LCTF, CRi VariSpec), which has a continuously tunable transmission from 400 nm to 720 nm with a spectral bandwidth of 7

nm, to a LN_2-cooled CCD detector (Princeton Instruments Spec-10 400B). A wide-field intensity image was obtained from light scattered by multiple nanoparticles at the specified wavelength. The wide-field LSPR imaging experiment apparatus with a wide-field intensity image of Ag nanoprisms at 535 nm is depicted in Figure 1.

For both the immobilized and moving nanoparticle experiments, the LCTF was scanned from 400 nm to 720 nm with 1 nm increments at a fixed time interval (integration time 1 s) and a series of images were collected where each frame has associated wavelength and time information. In the case of the immobilized nanoparticles, the intensity of the scattering was integrated as a function of wavelength to construct single nanoparticle spectra. For the diffusional dynamics study of Ag nanoprisms moving in a viscous 66.7% aqueous glucose solution, this wide-field method allowed us to not only determine the intensity of scattered light at the wavelength transmitted through the LCTF (generating an LSPR spectrum of a single nanoparticle) but also to simultaneously determine the location of each particle at a given time (single particle trajectory). The trajectories were obtained by marking the x-y centroid position of each nanoparticle at each intensity image frame over time, that is, at each wavelength. The mean-square displacement was calculated from the single particle trajectory.

Results and Discussion

Because the LSPR imaging experiment is wide-field, it is capable of acquiring the LSPR scattering spectra of hundreds of single nanoparticles in parallel. However, to ensure the LCTF wide-field imaging method yields spectra consistent with the conventional spectrometer grating method, the scattering spectra of several nanoparticles were obtained using both methods and compared. Figure 2 presents the scattering spectra of the same single nanoparticle obtained using both methods. Since the LCTF is a linear polarizer, the spectrum obtained using the LCTF is the sum of both parallel and perpendicular polarizations. The results in Figure 2 demonstrate that the LCTF wide-field imaging technique is an effective method for acquiring single nanoparticle spectra.

To characterize the distribution and heterogeneity of the LSPR λ_{max} of Ag colloids and Ag nanoprisms, histograms were constructed, as depicted in Figures 3a and 3b, respectively. The Ag colloids have an average size of 35 nm, composed of spheres, disks, rods, and other shapes, and the heterogeneity is observed from the plasmon distribution. Comparatively, the Ag nanoprisms have length edges of ~100 nm and are largely triangular shaped.[19] Although the Ag nanoprisms are relatively monodisperse, single nanoparticle studies have revealed variations in the LSPR spectra of single Ag nanoprisms,[13] signifying a varying structure in the Ag nanoprism sample, consistent with the plasmon distribution in Figure 3b. These histograms not only demonstrate the high-throughput capabilities of the wide-field method described here, but also characterize the plasmon distribution of different nanoparticle samples.

As demonstrated in Figure 3, our wide-field method is capable of measuring the LSPR spectra of ~10^2 immobilized nanoparticles simultaneously. For the analysis of the diffusional dynamics of moving nanoparticles, however, we focus on three Ag nanoprisms moving in a ~66.7% aqueous glucose solution. Figure 4a displays the LSPR scattering spectra of three moving individual nanoparticles which have λ_{max} of 524 nm, 627 nm, and 689 nm, representative of the plasmon distribution in Figure 3b. The two-dimensional trajectories of the same three individual nanoparticles are shown in Figure 4b. The mean square displacement ($<r^2>$), calculated from the nanoparticle trajectories, was plotted as a function of time lag, t, (Figure 4c) demonstrating a linear relationship. For Brownian diffusion in two dimensions, the relationship between $<r^2>$ and t is expected to be linear

with the slope equal to 4*D*, where *D* is the diffusion coefficient. Here, the motion of the three nanoparticles is fit with a linear regression to extract the diffusion coefficient for each nanoparticle. Particles that diffuse over larger distances within a certain time have higher diffusion coefficients. From the linear fit in Figure 4c, particles 1, 2, and 3 were determined to have diffusion coefficients of 1.33×10^{-10} cm^2/s, 8.75×10^{-11} cm^2/s, and 5.73×10^{-11} cm^2/s, respectively. Larger nanoparticles should diffuse at slower rates than smaller particles as predicted by the Stokes-Einstein relationship. Additionally, larger nanoparticles of similar shape generally scatter longer wavelengths of light than do smaller nanoparticles. As depicted in Figure 4a and 4c, our data are consistent with these trends.

The frictional coefficient for a sphere was used in the Stokes-Einstein relationship to estimate the sizes of the Ag nanoprisms from the measured diffusion coefficients and compare these results to the established Ag nanoprism size (~100 nm).[19] The glucose viscosity was obtained from models developed by Bui and Nguyen and was determined to be 91.8 mPa.s at T=22 °C.20 Using this value, the Ag nanoprism sizes were found to be 177 nm, 269 nm, and 410 nm for particles 1, 2, and 3, respectively. The sizes of the Ag nanoprisms were larger than expected by a factor of ~2–4. That is, the measured diffusion coefficients are a factor of ~2–4 times lower than expected given the established Ag nanoprism size.

There are three contributors to this discrepancy. First, these calculations were made using the frictional coefficient for a sphere, but the Ag nanoprisms are triangular. Second, there may be an error in the glucose solution concentration (and thus the viscosity) due to the temperatures necessary to prepare such highly concentrated glucose solutions. For example, if the concentration of the glucose solution is 10% greater than expected, the viscosity is 141.5 mPa.s. Therefore, particles 1, 2, and 3 are predicted to have sizes of 115 nm, 174 nm, and 266 nm, respectively, although these values are still larger than expected by a factor of ~2. Third, the diffusion coefficients were determined based on a two-dimensional trajectory, although the measurement is actually a two-dimension projection of a three-dimensional trajectory. Since the z-dimensional movement is not accounted for in these experiments, the particles *appeared* to move only in the x-y plane, giving rise to lower diffusion coefficients.

Conclusion

In summary, a new wide-field LSPR imaging technique using an LCTF to image and track moving particles as a function of wavelength and time has been demonstrated. As a result, we report the first single particle tracking experiment that determines both LSPR scattering spectra and the diffusion coefficients of several single Ag nanoparticles simultaneously in real time. Although the diffusion coefficients were slightly lower than expected, the work described here is a proof-of-principle report and several explanations may account for the difference between the predicted and measured diffusion coefficients. Primarily, a small error in glucose concentration largely affects the viscosity and predicted nanoparticle size. A less viscous glucose solution will ensure a more accurate determination of solution viscosity, but the high viscosity medium was necessary due to current camera speed limitations. However, future experiments using a faster camera will be limited only by the switching time of the LCTF (50 ms), such that diffusion coefficients as high as 2.33×10^{-4} cm^2/s can be obtained, which are well within the biologically relevant regime.[7,21] In conclusion, this wide-field LSPR imaging technique is a high-throughput method capable of measuring scattering spectra of hundreds of single nanoparticles allowing the plasmon distribution of any noble metal nanoparticles to be characterized. It is anticipated that the wide-field LSPR imaging technique using an LCTF will be applicable to many fundamental scientific problems such as nanoparticle growth mechanisms, functional plasmonic distributions, and cell membrane organization.

Acknowledgments

This work was supported by the National Science Foundation (Grants EEC-0118025, CHE-0414554, BES-0507036, DMR-0520513, and CHE-0911145) and the National Cancer Institute (1 U54 CA119341-01).

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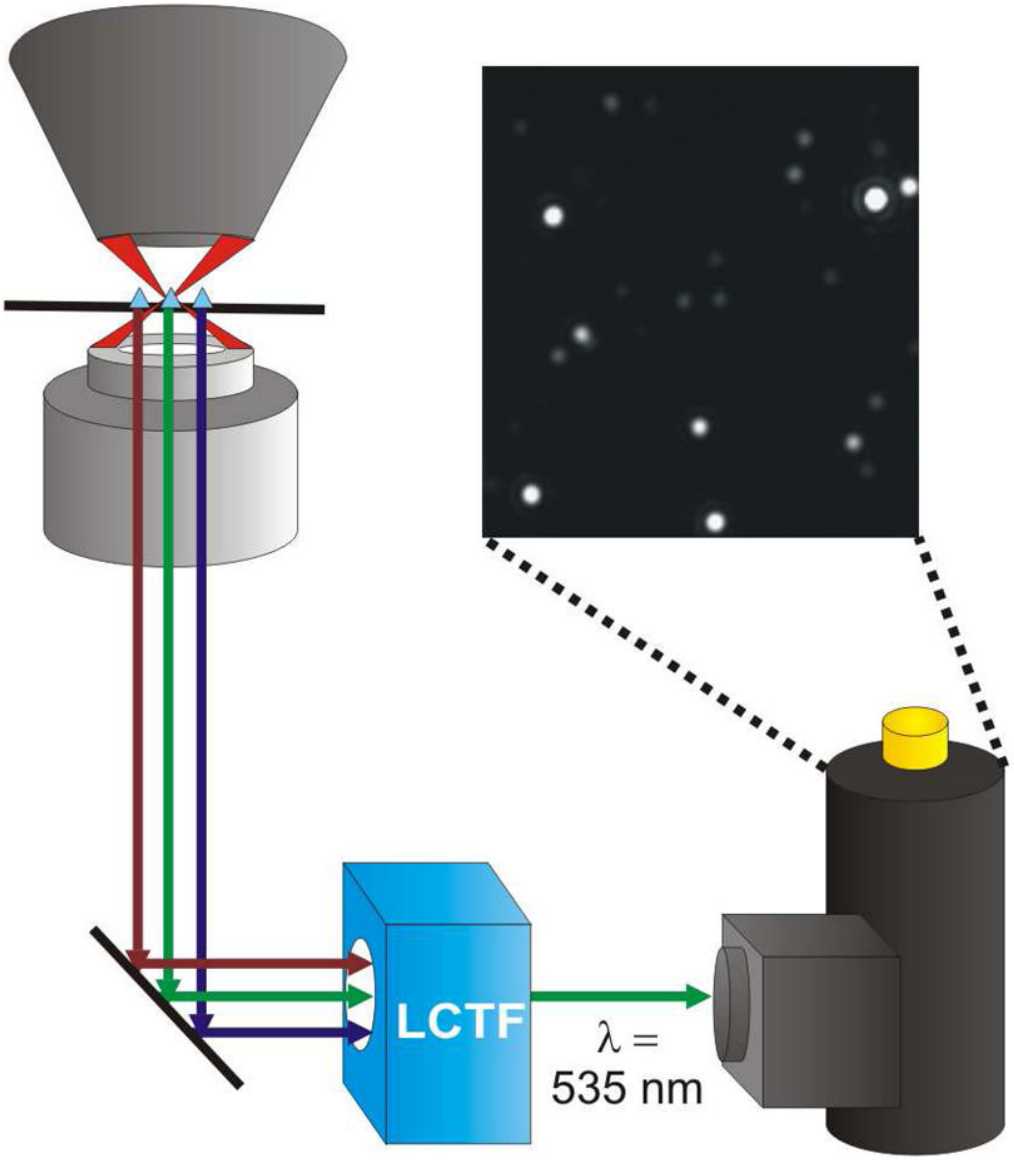

Figure 1.
Wide-field LSPR imaging experimental set-up. The Ag nanoparticles are illuminated by a high NA dark-field condenser. The light scattered by the nanoparticles is collected by the objective and sent through an LCTF, where only the specified wavelength of light is transmitted (535 nm shown here) to the CCD. An intensity image of the 535 nm scattered light from all the particles is shown.

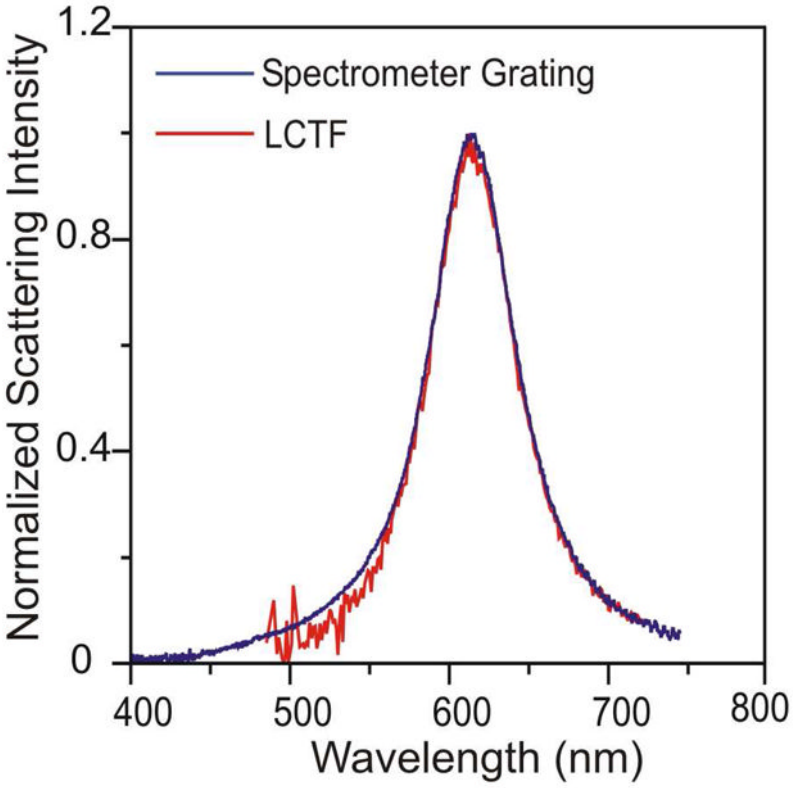

Figure 2.
Comparison of two scattering spectra of the same nanoparticle obtained by two different methods. The scattering spectrum in blue was acquired using the spectrometer grating by isolating the nanoparticle in the spectrometer slit. The scattering spectrum in red was acquired using the wide-field imaging technique with an LCTF and is the sum of both the parallel and perpendicular polarizations. The spectrum obtained using the LCTF is in good agreement with the traditional spectrometer grating method.

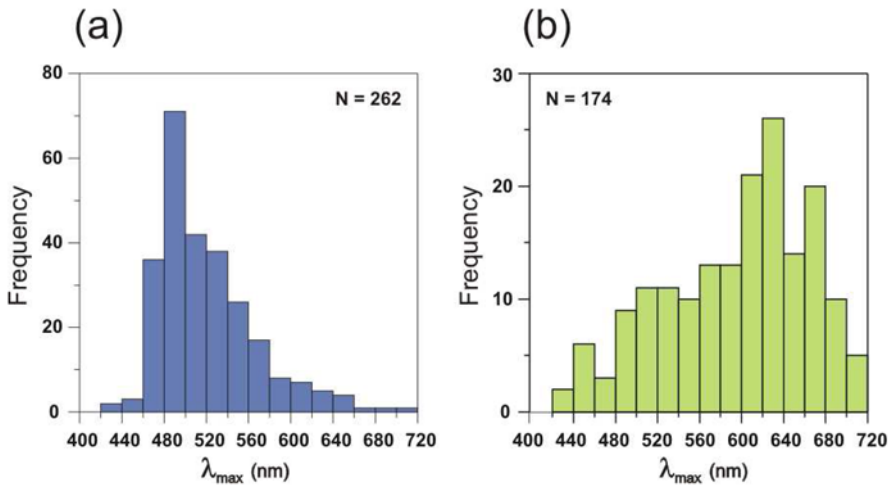

Figure 3.
Histograms illustrating the plasmon distribution of chemically-synthesized nanoparticles (a) Ag colloids, N=262. (b) Ag nanoprisms, N=174.

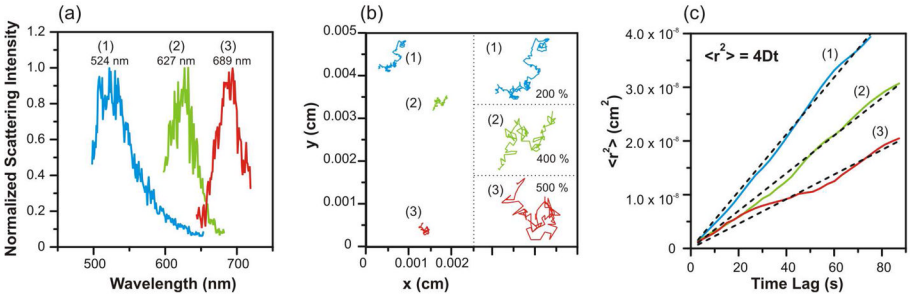

Figure 4.

Characterization of three single moving nanoparticles. Faster diffusing nanoparticles are correlated with shorter wavelength scattering spectra. (a) The normalized LSPR scattering spectra of particles 1, 2, and 3 with λ_{max} of 524 nm, 627 nm, and 689 nm, respectively. The scattering spectra were obtained while the particles were moving according to the trajectories in (b). (b) Two-dimensional trajectories of particles 1, 2, 3. The insets show a magnification of the trajectories at 200%, 400%, and 500% for particles 1, 2, and 3, respectively. (c) The mean square displacement, $<r^2>$, is plotted as a function of the time lag, *t*, for particles 1, 2, and 3 and fit with a linear regression.