Localized Surface Plasmon Resonance Imaging: Simultaneous Single Nanoparticle Spectroscopy and Diffusional Dynamics

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A wide-field localized surface plasmon resonance (LSPR) imaging method using a liquid crystal tunable filter 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.6,9 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 In addition, particle tracking experiments with plasmonic nanoparticles can address other fundamental scientific problems, 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 metallic 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 AgNO3 was dissolved in 500 mL of H2O 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 the heat and was diluted with ∼100 mL of H2O.

The protocol developed by Jin et al. was utilized for the synthesis of Ag nanoprisms.19 In this synthesis, 0.1 mM AgNO3 was stirred in the presence of sodium citrate (0.3 mM) on ice, followed by the addition of cold NaBH4 (1 mL, 50 mM). To stabilize the particles, 1 mL of 5 mM bis(p-sulfonatophenyl)-phenylphosphine dihydrate dipotassium (BSPP) was added dropwise. 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 coverslip (Fisher Scientific) and dried with pressurized air. To characterize the nanoparticles’ movement in solution, 10 µL of the Ag nanoprisms solution was added to ~100 µL of aqueous ~66.7% glucose solution. A minute amount of the nanoparticle-glucose solution mixture was sandwiched between a 22 mm, no. 1 glass coverslip (Fisher Scientific) and an 18 mm, no. 2 glass coverslip. 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 100× 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 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 to 720 nm with 1 nm increments at a fixed time interval (integration time 1 s), and a series of images were collected in which 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 not only to determine the intensity of scattered light at the wavelength transmitted through the LCTF (generating an LSPR spectrum of a single nanoparticle) but also simultaneously to 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 $\lambda_{\text{max}}$ of Ag colloids and Ag nanoprisms, histograms were constructed, as depicted in Figure 3a and b, 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.$^{19}$ 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 nanopram 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 nanoparticles...
individual nanoparticles which have \( \lambda_{\text{max}} \) of 524, 627, 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 \( \langle r^2 \rangle \), 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 \( \langle r^2 \rangle \) and \( t \) is expected to be linear with the slope equal to \( 4D \), 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 \( 1.33 \times 10^{-10} \), \( 8.75 \times 10^{-11} \), and \( 5.73 \times 10^{-11} \) cm\(^2\)/s, respectively. Larger nanoparticles should diffuse at slower rates than smaller particles, as predicted by the Stokes–Einstein relationship. In addition, larger nanoparticles of similar shape generally scatter longer wavelengths of light than do smaller nanoparticles. As depicted in Figure 4a and c, 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 to compare these results to the established Ag nanoprism size (\( \sim 100 \) nm). 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 nanoprisms sizes were found to be 177, 269, and 410 nm for particles 1, 2, and 3, respectively. The sizes of the Ag nanoprisms were larger than expected by a factor of \( \sim 2-4 \). That is, the measured diffusion coefficients are a factor of \( \sim 2-4 \) 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, 174, and 266 nm, respectively, although these values are still larger than expected by a factor of \( \sim 2 \). Third, the diffusion coefficients were determined on the basis of a two-dimensional trajectory, although the measurement is actually a two-dimensional 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 $2.33 \times 10^{-4} \text{ cm}^2/\text{s}$ 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.

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References and Notes

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