Photothermal Correlation Spectroscopy of Gold Nanoparticles in Solution†

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We demonstrate correlation analysis of the photothermal signal from gold nanoparticles diffusing in solution. Our experimental setup is primarily optimized for large detection volumes and therefore has a relatively low numerical aperture (0.25). This requirement limits light collection and detection efficiency, but we can nevertheless detect particles as small as 20 nm in diameter in 100 µs. Photothermal correlation spectroscopy (PCS) in water and in water:glycerol mixtures gives access to characteristic diffusion times of some tens of milliseconds. Diffusion is enhanced for high pump intensities, due to the increased temperature around the particle. The photothermal signal at the detector arises from the interference of the scattered probe beam and a reference field. In the forward configuration, the reference is the transmitted probe beam itself. In the backward configuration, the reference wave is a reflection by a nearby interface. It has an optical phase difference depending on the distance of the particle to the reflecting interface. The backward signal also includes the directly modulated scattering signal. Potential applications of photothermal correlation in biological labeling are demonstrated on bacteriophage virus particles carrying 20 nm gold labels.

Introduction

The unique properties of gold and silver nanoparticles make them attractive objects for fundamental and applied research,1–16 as probes in nanophotonics,17–19 and biological labeling and sensing.20–30 Compared to organic dyes and semiconductor nanocrystals, gold nanoparticles are free from photoinduced bleaching or blinking, facilitating continuous observation of the same particle over long times. The absorption spectrum of a nanoparticle depends on its size, shape, and local environment,12–16 a property exploited in multicolor labeling schemes,15,21 but the low photoluminescence quantum yield makes detection of single particles difficult. Dark-field scattering by single metal particles is difficult for diameters less than 40 nm, because their scattering cross-section scales with squared volume.12 As interferometric signals scale as the scattered field instead of the scattered intensity, they provide access to smaller sizes.31 This approach was recently implemented for photothermal detection of single metal nanoparticles in two different ways: photothermal interference contrast22,32,33 (PIC) and photothermal heterodyne imaging34–37 (PHI). In photothermal detection, a nanoparticle is illuminated with a pump beam, usually at a wavelength close to the particle’s surface plasmon resonance. The absorbed energy is dissipated as heat to the surrounding environment. The detected signal arises from scattering of a second beam, the probe, by the local thermally induced variation of refraction index. The variation in scattered intensity is proportional to the particle’s absorption cross-section, which scales with the volume, and allows for observation of smaller objects. For instance, using PHI with high numerical aperture optics (NA = 1.4), Berciaud et al. detected individual gold particles with diameters down to 1.4 nm (only 67 gold atoms) with a signal-to-noise ratio SNR > 10, an integration time of 10 ms and a temperature rise on the particle surface of only about 2 K.34 This was achieved on an immobilized sample, and the same method was used for single-particle tracking in biological samples,27 providing two-dimensional trajectories of membrane receptors labeled with 5 nm-gold in live cells at video rates.

For diffusion faster than video rate, one can employ correlation analysis of the fluctuations of the photothermal signal, in analogy to the widely known technique of fluorescence correlation spectroscopy.38–41 In fluctuation spectroscopy, the observation volume is reduced to dimensions small enough that the diffusion-induced statistical number fluctuations of molecules (or particles) lead to measurable signal fluctuations. The large absorption cross-sections of gold nanoparticles (3–4 orders of magnitude higher than that of common fluorescent labels31) make them appealing for fluctuation spectroscopy. The strength of the photothermal signal and the absence of saturation at high pump powers (unless heating damage occurs) compensates for the absence of photoluminescence.

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The modulated signal power (S_{PHD}) is proportional to the scattered field (\tilde{E}_\text{sc}(t)) at a distance \text{of one particle radius}. In the above estimate, the temperature elevation around the particle decreases to half its value at the surface at a distance of one particle radius. In the above estimate, the constant probe power (see Supporting Information A), one only heating by the pump is considered; the probe contribution is neglected. Assuming equal powers for probe (633 nm) and pump (514 nm), the fraction of the temperature rise due to the probe is about 4\% for particles less than 60 nm in diameter and about 13\% for 80 nm. In our experiments, the power of the probe light is at least 1 order of magnitude smaller than the power of the heating light. Thus the influence of the probe light on the temperature rise is less than 1\%.

The relationship of the surface temperature increase \( \Delta T_{\text{surf}} \) with the integration time of the photothermal signal for particles of different diameters with a fixed SNR is shown in Figure 2. The same SNR can be achieved either with a longer integration time and a lower temperature rise or a shorter integration and higher temperature. Since the SNR is proportional to \( \text{NA}^2 \) for the constant probe power (see Supporting Information A), one has to increase either the temperature rise or the integration time for a lower NA (see Figure 2), if one wishes to recover a comparable SNR.

In our PCS experiments, the heating and probe beams are focused into overlapping volumes of the colloidal solution (colloid) in which the nanoparticles diffuse. We consider separately two possible experimental configurations.

(i) **Forward Mode.** Because the two interfering fields propagate in the same direction, the phase mismatch between the reference and the scattered fields is weak. Residual phase mismatches can arise from spatial mode mismatch (including the Gouy phase

\[
\Delta T_{\text{surf}} = \frac{P_{\text{abs}}}{4\pi\kappa R}
\]
We fitted the experimental ACFs with the usual FCS expression for a two-dimensional Brownian diffusion, 

$$G(\tau) = 1 + \frac{1}{N} \left(1 + \frac{1}{\tau_d} \right)^{-1}$$  \hspace{1cm} (5)$$

where \( N \) is the average number of particles in the detection volume and \( \tau_d \) is the diffusion time of the particle across the detection volume. The approximation of 2-D diffusion is valid in our setup because of the reduced numerical aperture. The axial extent of the detection volume is much larger than its diameter.\(^{45}\) However, as discussed below, the conventional expressions of Brownian diffusion to PCS only apply at low enough heating power, because heating around the particle may change the local viscosity and the diffusion rate. This change was evaluated through numerical simulations of a model with a position-dependent diffusion coefficient, which will be reported elsewhere.

(ii) Backward Mode. In the backward mode, the photothermal signal arising from interference with the reflection has a random phase, and averages out in the diffusion perpendicular to the interface. In the correlation function, this signal contributes a fast component corresponding to diffusion within a half-wavelength, but does not contribute to the slow component corresponding to diffusion across the much larger focal volume. An additional signal arises from time-modulated scattering by the particle (i.e., the difference in scattering strength between hot and cold particle-environment system). This signal can be seen as the interference of the modulated part of the scattered field, \( E_{sc,p}^{\text{BW}}(t) \), with the static part of the scattered field, \( E_{sc,p}^{\text{BW}} \). Being obviously free from any phase mismatch, this signal has a fixed sign, provides an average photothermal signal, and contributes a slow component when the particle diffuses in the focal volume.

Accordingly, the backward (BW) photothermal signal can be expressed as the sum of two terms, \( S_{\text{PHD,BW}}^\text{BW} = 2\text{Re}[E_{sc,p}^{\text{BW}} E_{sc,p}^{\text{BW}}(t)] + 2\text{Re}[E_{sc,p}^{\text{BW}} E_{ic,p}^{\text{BW}}(t)] \). The distance between the diffusing particle and the optical interface introduces a phase mismatch between the scattered \( E_{sc,p}^{\text{BW}}(t) \) and the reflected \( E_{ic,p}^{\text{BW}} \) fields, causing the second term in \( S_{\text{PHD,BW}}^\text{BW} \) to change sign upon diffusion along the optical axis (see Figure 1). Therefore, for large enough particles, or for an out-of-focus reflection, the photothermal signal arises mainly from the first term of \( S_{\text{PHD,BW}}^\text{BW} \), and the photothermal bursts then present a dominant sign. The correlation function of the total photothermal signal contains two contributions, one from the first term corresponding to diffusive motion across the beam and a second, fast component from the second term in \( S_{\text{PHD,BW}}^\text{BW} \) corresponding to diffusion across the interference fringes (the cross-term averages out). This situation is completely analogous to that of FCS on a mirror,\(^{48}\) with a different “effective fringe visibility” \( V = n_{a,f} \), given by the relative contributions \( r \) and \( a \) of the reflected \( E_{ic,p}^{\text{BW}} \) and scattered \( E_{sc,p}^{\text{BW}} \) fields (note that this effective visibility strongly depends on particle size and reflection intensity).

As in FCS on a mirror, two fluctuation processes take place on different time-scales: the diffusion through the whole observation volume (slow fluctuations, fixed sign) and diffusion between the optical fringes of different brightness (fast fluctuations, random sign). We fitted the ACFs for backward mode detection with the model developed for FCS on a mirror (eq 6)\(^{46-48}\)

$$G(\tau) = 1 + \frac{1}{N} \left[ 1 + n_f \exp(-\tau/\tau_f) \right] \left(1 + \frac{1}{\tau_d} \right)^{-1}$$  \hspace{1cm} (6)$$

where \( N \) is the average number of particles in the detection volume, \( n_f \) is a constant related to the fringe visibility, \( \tau_f \) is a characteristic time for diffusion between the fringes, and \( \tau_d \) is the diffusion time across the transverse direction of the detection volume. The last term in eq 6 can be recognized as the term for 2-D Brownian diffusion (see eq 5).
Experimental Section

The experimental setup is a modification of the home-built photothermal microscope reported previously. Briefly, the heating beam is provided by the 514.5 nm line of an argon ion laser (Coherent Inc., Innova 310). The intensity of the heating beam is modulated by an acousto-optic modulator (AA Opto-Electronic, modulator AA.MT110/A1.5-VIS) and RF driver AA.MOD.110.D47, which is driven by a square wave at the modulation frequency $\Omega = 500$ kHz. The probe beam is provided by a Helium-Neon laser (Melles Griot, 25LHP991–230, $\lambda = 632.8$ nm). Both beams are expanded by telescopes, combined by a dichroic mirror (AHF/Chroma F43–525) and guided to the sample through a microscope objective (UPLSAPo10x, numerical aperture NA = 0.4, and working distance 3.1 mm). The heating laser and probe laser were focused into spots with a radius $\omega_0$ of 1.2–1.4 $\mu$m and a Rayleigh length of about 15 $\mu$m. The axial overlap of two foci is controlled by knife-edge profile scanning. The effective NA for the underfilled objective lens is calculated to be $N_{\text{eff}} = 0.25$. The detection volume is estimated to be 200 fl, which is larger, by more than 2 orders of magnitude, than the volumes typically used in FCS. This extended detection volume is advantageous for applications in high-throughput screening, as discussed previously. Slight variations in the detection volume of the backward configuration compared to the forward configuration are due to differences in the numerical aperture of the collection objectives.

In the backward mode (BW), the same objective is used for collection. The collected light passes through a cleanup interference filter (Semrock, MaxLine 632.8 nm) to block reflections from the heating beam. The filtered beam is focused onto an avalanche photodiode (APD; New Focus, 1801FS-AC-M). In forward mode (FW), a second objective (Mitutoyo MPlanApo20x, numerical aperture 0.28, working distance 30 mm) is used for collection. The light collected is cleaned from the heating beam by a dichroic mirror (AHF/Chroma F43–525) and an interference filter (Semrock, MaxLine 632.8 nm). Another avalanche photodiode detector (Femto, HCA-S-200M-SI) is used for the forward detection. The APD output is fed into a lock-in amplifier (Stanford Research Systems, SR844) tuned to the modulation frequency. The lock-in amplifier detects the signal with a time constant of 20–40 $\mu$s. The time traces of the photothermal signal were acquired for up to 400 s with a time-bin of 0.1 ms.

Samples of gold colloids with diameters of 20 and 80 nm (British Biocell International, EM.GC20 and EM.GC80) were prepared by dissolving an adequate volume of stock solution in ultrapure water or glycerol. The concentrations of the stock solutions of gold colloids are $7 \times 10^{11}$ and $1.1 \times 10^{10}$ particle/mL for 20 and 80 nm particle sizes respectively (according to the supplier’s specifications; the diluted suspensions of gold colloids are identified by the volume ratio $x:y$ of dilution). The measurements were performed in cells made from two glass coverslips and a rubber O-ring which was attached to the bottom coverslip. Prior to cell assembly, all parts were carefully cleaned to avoid colloid aggregation. The glass coverslips were sonicated in isopropanol (absolute grade) for 10 min, dried, and treated in a UV-ozone cleaner for another 20 min. Samples of bacteriophage virus with 20 nm gold colloids attached were prepared by genetically engineering M13 phages to display the biotin epitope on the coating proteins P3 (3–5 copies/phage) and P8 (2700 copies/phage). The engineered phages were then mixed with an excess quantity of gold colloids coated with streptavidin (British Biocell International, ArraySTP20). The concentration of viable phages was determined by titrations and

Results and Discussion

We begin this section with results of photothermal correlation spectroscopy measured in backward mode for gold particles of 80 nm in diameter, diffusing freely in water. For convenience reasons, most measurements were done in this configuration, although the signal-to-noise ratio was much better in the forward mode. For 80 nm particles, the signal bursts are strong enough to be clearly discriminated against the noise. The effect of dilution, illustrated in Figure 3A, is an increase in contrast of the autocorrelation function. The shape of the autocorrelation curves is not affected by dilution (inset in Figure 3A), which confirms that particle–particle interaction or aggregation are negligible. Figure 3B shows the effect of dilution by glycerol, with a shift of the autocorrelation curves to longer times (inset) as the viscosity increases. The slower diffusion in the water-glycerol mixture reveals a short component at about 100 microseconds, which we attribute to diffusion through the interference pattern (see the Methods section). We hereafter discuss fits of eq 6 to the correlation curves of Figure 3. Figure 4 shows that the average numbers of particles in the detection volume obtained from the fits ($N_{\text{eff}}$) are in good agreement with bulk values ($N_{\text{bulk}}$), confirming that the bursts arise from individual particles. For the water:glycerol mixtures, it is possible to retrieve both diffusion times $\tau_1$ and $\tau_2$ (in pure water, $\tau_1$ falls below our time resolution). According to the model for FCS on a mirror, the ratio of the diffusion times $\tau_1$ and $\tau_2$ should not depend on the viscosity of the medium, but only on the beam waist $\omega_0$, the probe wavelength $\lambda$ and the refraction index of the medium $n$.

\[
\frac{\tau_d}{\tau_1} = 4\pi n \frac{\omega_0^2}{\lambda^2}
\]

Indeed, we verified that the values of $\tau_d/\tau_1$ are independent of the mixture composition and agree within experimental uncertainty with the value of 286 predicted with eq 7 (refractive index of glycerol $n_g = 1.47$, $\omega_0 = 1.4 \mu$m). The values calculated for the ratio of the diffusion times $\tau_d$ and $\tau_1$ fitted from the curves in Figure 3B are $320 \pm 37$, $306 \pm 25$, and $316 \pm 65$ for 1:1, 1:2, and 1:5 compositions of water:glycerol mixtures, respectively. This supports our interpretation of the fast signal fluctuations as diffusion in an interference pattern. The values fitted for the pre-exponential factor $n_1$ in eq 6 decrease with the fraction of glycerol in the mixture: 1.3, 1.1, and 0.75 for compositions 1:1, 1:2, and 1:5, respectively. As the fraction of glycerol increases, the index of refraction of the mixture should approach that of the bottom glass interface ($n_{\text{glass}} = 1.5$) and the reflected fraction of the probe beam decreases, thus leading to a decrease of the fringe visibility and $n_1$ (see the Methods section).

Increasing the heating intensity raises the particle’s temperature as well as that of its surroundings, which leads to a faster diffusion. This was verified experimentally by increasing the power of the heating laser and following the decrease in the diffusion times fitted from the respective autocorrelation curves (Figure 5). The decrease in the diffusion time $\tau_d$ for the suspensions in pure water is not as pronounced as that for the water:glycerol mixtures. The behavior of the diffusion times is well described by the fits in Figure 5, which assume heating-
induced changes of the liquid’s properties, in particular the viscosity \(\eta\) (see Supporting Information B), around each nanoparticle. The model predicts a decrease of the diffusion time by a factor of up to 7.2 (2.8) for water:glycerol (pure water) in the considered range of heating powers. In water:glycerol mixtures, a more accurate analysis should consider the local change of mixture composition via the Soret effect,51,52 neglected here.

In the forward mode, as no interference effect occurs, no short component appears in the correlation (Figure 6). The photothermal signal is much stronger. We could easily detect 20 nm diameter gold particles diffusing in water (highly diluted to 25 K already 20 nm away from the particle surface). The interference effect, which occurs in the backward mode, could be useful to study diffusion in confined geometries, like micro- and nanochannels, where interactions with the channel walls could interfere with diffusion dynamics or diffusion processes perpendicular to the main flow of the fluid. The fringe values predicted for the diffusion time of dimers (25.0 ms) and aggregates up to hexamers (28.4 ms) are significantly longer.53

The heating effect of the photothermal measurement on the medium properties was taken into account. The maximum temperature rise at the surface of a particle at the objective focus, estimated from eq 3 with a heating beam power of 52 mW (at 1.2 MW/cm², with \(\omega_p = 1.2 \mu m\)), was found to be \(\Delta T_{surf} = 51 K\). This temperature increase may appear too high for certain applications, but one should not forget that the present detection time is short (100 \(\mu\)s), that each particle stays only a fraction of a second in the focus, and that the temperature rise decreases to 25 K already 20 nm away from the particle surface.

Our results show that forward-scattering is clearly more appropriate for high-throughput detection in solution. However, the interference effect, which occurs in the backward mode, could be useful to study diffusion in confined geometries, like micro- and nanochannels, where interactions with the channel walls could interfere with diffusion dynamics or diffusion processes perpendicular to the main flow of the fluid. The fringe
patterning of the detection volume enables addressing diffusion processes on length scales of a half-wavelength. Such a concept was demonstrated in studies of protein diffusion in tubular structures within cells using the technique of FCS on a mirror.\(^5\)

Finally, we present PCS measurements in forward mode for bacteriophage viruses labeled with 20 nm gold particles. The photothermal bursts of these objects are much longer than those of the free gold particles. A M13 bacteriophage is a rod-like shaped virus with a length of approximately 800 nm and a width of 8 nm. As a consequence, the autocorrelation curves reveal longer diffusion times for the phages than for the free streptavidin-coated 20 nm gold particles (by approximately 1 order of magnitude, see Figure 7). The diffusion time obtained for the streptavidin-coated 20 nm gold particles is 21.3 ± 0.8 ms, slightly longer than that of noncoated particles (15.7 ms), but significantly shorter than the estimated time for clusters.\(^6\) The correlation shape for gold-labeled phages clearly differs from that of the free particles, which suggests that the diffusion dynamics of these large objects is more complex. This may be related to their high aspect ratio and to the local heterogeneity of the temperature profile around the virus, as its length is comparable to the transverse dimensions of the detection volume. Currently, we are using the phages expressing biotin to the coat proteins P8 (cP8), that form the cylindrical body of the phages, and the coat proteins P3 (cP3), that are in the phages’ extremities, to attach the streptavidin-coated gold particles. Some limited phage aggregation may take place because of possible multiple labeling.

Another issue that deserves closer attention is the effect of heating on the viability of the phages. The heating powers used for detection of these samples correspond to a surface temperature rise of up to 51 K for 20 nm gold labels. However, this temperature decreases as the inverse distance from the particle’s center. Furthermore, the residence times of the detected objects in the observation volume range from some tens to hundreds of milliseconds, which might be sufficiently short to avoid substantial heating damage for many biological species showing no aggregation.\(^7\) Heating damage can always be minimized by sacrificing detection bandwidth or by reducing the heating power, and compensating the lower signal with an increase in the probe power.

Conclusions

We have presented the combination of photothermal microscopy and correlation spectroscopy. This method was applied to characterize the diffusion of gold nanoparticles in liquid solution. Two principal modes of operation were considered, backward and forward detection. In the backward mode, we observed axial striation of the detection volume by interference between the back-scattered light and the interface reflection; this effect gives rise to an additional short-time component in the diffusional correlation function and can be understood quantitatively by a model developed earlier to describe FCS on a mirror. We verified experimentally that increasing the heating power leads to shorter diffusion times, as it is expected from the larger temperature change around the particle.

In the forward mode, the interference effect is eliminated and the signal intensity increases. This allowed us to detect gold particles as small as 20 nm in diameter and to perform photothermal correlation spectroscopy with a time-resolution of 100 microseconds. The size limit of 20 nm could be improved with high-numerical aperture optics, however our experimental setup was designed for a compromise between the detection efficiency and the size of the observation volume.\(^8\) An extended detection volume is advantageous for applications in high-throughput screening because it enhances the capacity of processing “large” sample volumes. Being free from bleaching and blinking, gold nanoparticles are attractive labels for screening applications with single-object sensitivity. This screening method opens the way to multistage sorting steps, and to the rejection of false negatives to arbitrarily low levels. Finally, we show how photothermal correlation can be applied to detect gold-labeled bacteriophage viruses and study their diffusion.

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Supporting Information Available: (A) Calculations of the photothermal signal-to-noise ratio and numerical aperture. (B) Diffusion in water and water:glycerol mixtures and influence of the heating. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

Gold Nanoparticles in Solution


(42) The criterion defined in ref. 37 for the low frequency regime is \( \Omega \ll \Omega_c \), with \( \Omega_c = 2D(2\pi n/\lambda)^2 \). Using this expression we estimate \( \Omega_c \approx 8 \) MHz; our experiments were performed at a modulation frequency \( \Omega = 500 \) kHz, so we expect a better signal-to-noise ratio in the forward configuration relative to the backward configuration.


(53) The variation of the estimated diffusion time for the clusters is not monotonic with the cluster size due to the interplay between two counteracting effects: the increase in the hydrodynamic radius of the cluster, which makes diffusion slower and the higher absorbed power that increases the temperature rise around the object and, thus, accelerates diffusion. Note that a further complication, coupling between the plasmon resonances of the aggregated particles, was neglected in these estimates.