Coated Silica Microparticles

Visual Observations of SERRS from Single Silver-Coated Silica Microparticles within Optical Tweezers**

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Surface-enhanced resonance Raman scattering (SERRS) can be up to around 10^{14} times more efficient than Raman spectroscopy thereby enabling the detection of single molecules.[1–4] Although other techniques such as fluorescence have similar sensitivities, SERRS peaks are narrow, distinct from each other, and very sensitive to molecular structure. In a related field, optical tweezers use the extremely high gradient in the electric field that is produced near the waist of a tightly focused laser beam to trap or manipulate micron-sized organic, inorganic and biological particles. The apparatus required for both SERRS and optical tweezers are similar and comprise a laser light source focused into a sample by using a high magnification microscope objective. A particle in the vicinity of the optical tweezers trap can also be imaged by using the same objective lens making an optical-tweezers setup ideal for the collection of Raman scattering from the trapped particle. By combining optical tweezers and SERRS, we propose a common platform capable of the manipulation and recognition of specific particles and their interactions at the single microparticle level.

Key to the SERRS enhancement is the ability to appropriately coat the surface of the microparticle with metal such that it supports the optical excitation of a surface plasmon. One reason SERRS has not been more widely investigated within optical tweezers is that it is difficult to trap metallic particles.[5] Herein, we show for the first time that introducing a SERRS active particle to the trapping beam of an optical tweezers system creates intense SERRS emission that can be seen on a video camera. The complete SERRS spectrum can be recorded in 100 ms or less.

In previous work, SERRS spectra have been recorded from trapped silver clusters coated with rhodamine or phenylalanine.[6] However, even for integration times of 5 seconds, many particles had to cluster together to give a measurable signal. Here we report what we believe to be the first observation of SERRS from dye absorbed on a single silica microbead partially coated in silver and suspended in solution near the focus of an optical-tweezers trap. Our experimental configuration comprised a laser source at 532 nm brought to a focus in the sample plane by using a $\times 100$ microscope objective. Appropriate filters attenuated the backscattered laser light whilst allowing both white light imaging with a CCD camera and spectral analysis of the wavelength shifted light with a fiber-coupled spectrometer.

The mechanism for optical trapping**:** Normally optical tweezers rely upon the refraction of light by a transparent object to draw the object to the beam focus. However, for metallic particles, the additional scattering of light from their surface means that they are repelled from the trapping laser. Although it is possible to devise annular[7] or scanning[8] beams to trap metal particles, the associated complexity makes the approach restrictive. We use silica spheres partially coated with silver and with an azo dye (3,5-dimethoxy-4-(6′-azobenzotriazolyl)-phenylamine) that has previously been shown to adsorb strongly onto a silver surface.[9,10] Figure 1 shows transmission electron micrographs of the plain silica spheres and of silica spheres that have been partially coated in silver. The images show that the coated particles have a rough surface with a silver thickness varying up to about 100 nm. The particles resemble the SERRS-active microbeads of Lendl and co-workers.[11,12] and we have obtained strong

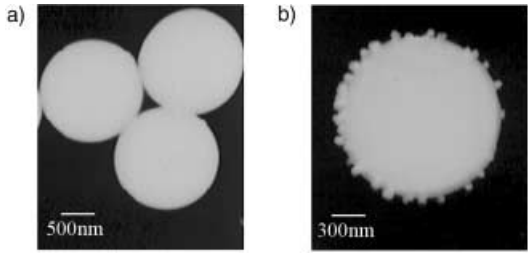

Figure 1. Transmission electron micrographs of a) plain silica microparticles and b) a partially silver-coated silica microparticle.

SERRS spectra from our beads in our standard Raman instrument. However, we also observed that the partial silver coating allowed many of these particles to be trapped in three dimensions within a conventional optical-tweezers setup without recourse to annular or scanning beams.

Observation of SERRS from single particles: Translation of the sample stage allowed a variety of particles to be moved into the focused laser beam and micrometer capture range of the optical trap. As a dye-coated particle approached the laser focus, a bright flash of light was emitted from the particle edge with a spectrum corresponding to a SERRS emission. Interestingly particles with the weakest SERRS were the easiest to trap whilst those with the strongest SERRS were repelled. This is indicative of the amount of silver coating on the particle. In all cases once trapped, the SERRS from the particle ceased, which probably resulted from photobleaching of the dye in the intense laser beam.

When a second particle was brought close to a trapped, now SERRS-inactive particle, a flash of light was observed, this time from the second particle and a SERRS spectrum was recorded. The sequence of photographs in Figure 2 shows the second particle approaching the trapped particle, flashing briefly, and then moving away. Light flashes could be readily observed from numerous individual SERRS-active microspheres and a SERRS spectrum associated with each emission was recorded. Figure 3 shows the spectrum from a single

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SERRS active microparticle, compared to that from a colloidal suspension. Figure 4 shows the intensity of the spectral peak at 1369 cm^{-1} as a function of time, which suggests that the duration of these flashes are of the order of 200–300 ms. There are no appreciable changes in the relative intensities of the peaks in the spectrum between each 100 ms accumulation. Thus, the flashes are due to SERRS, which is sufficiently strong to be visualized on a standard (non-intensified) CCD camera.

The trapping beam is extremely tightly focused, giving both a small spot (< 1 μm) and an associated large numerical aperture (1.3). Hence, the excitation volume corresponding to the high light intensity covers a few cubic microns in the vicinity of the beam focus. However, due to the extremely high divergence of the beam, a significant level of illumination light extends over tens of microns. We observe that this light can be sufficient to excite SERRS emissions from a distance of 20 μm. Thus, by having a SERRS-active particle with close to the trapping beam, a longer-lived lower-intensity signal is observed on both the CCD camera and spectrometer. Figure 5 shows two such particles that have been excited by

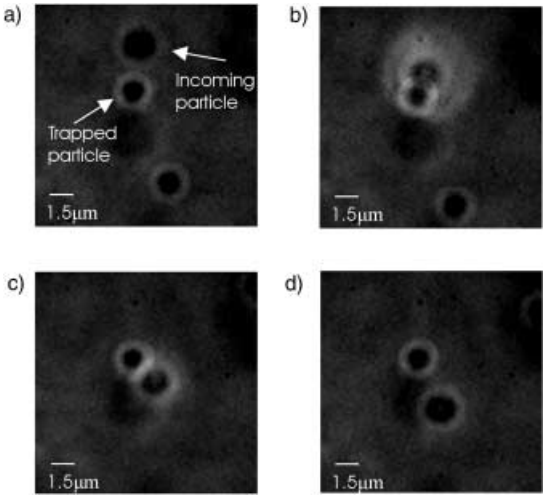

Figure 2. Sequence of images showing a) the approach of a dye coated particle to a trapped particle, b) its contact with a trapped particle and resulting SERRS emission, c) and d) followed by the movement of the particle away from the trap position.

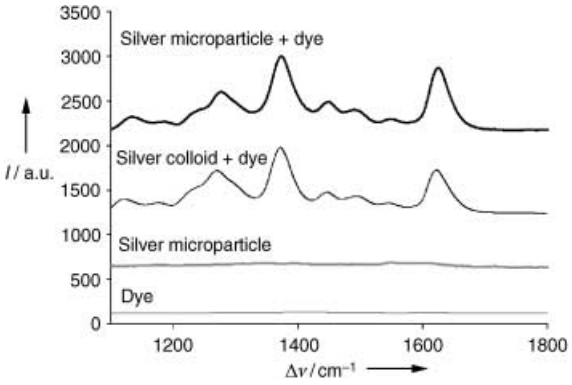

Figure 3. The Raman spectra from the pure azo dye, the plain silver-coated microparticles, the SERRS spectra from the dye adsorbed on aggregated silver colloid, and the dye adsorbed on the silver-coated microparticles. *I* = intensity, arbitrary units.

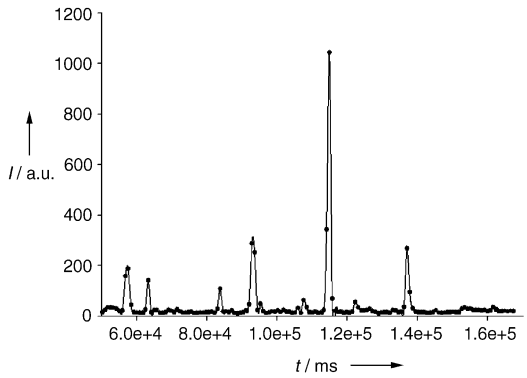

Figure 4. Intensity of the SERRS signal at 1369 cm^{-1} against time (*t*). Each point in the graph represents 100 ms.

the periphery of the beam. This lower-intensity SERRS was observed for a period of more than ten seconds.

The SERRS emission after touching occurs from a number of points on the particle surface to produce a speckled effect (Figure 5), which suggests that there are strongly enhanced localized optical fields on the particle surface. Markel et al.[13] have shown by theory and experiment that when clusters of silver colloid particles are excited by

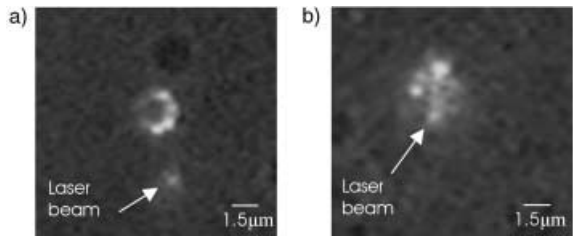

Figure 5. Two examples of the characteristic speckle pattern observed when a single dye-coated particle comes close to the edge of the laser beam. a) A long lived SERRS emission from a particle on the periphery of the beam, and b) a short lived SERRS emission from a particle near the trapping beam.

light tuned to excite a surface plasmon, the intensity distribution in the near-field is heterogeneous with most of the excitation concentrated in hot spots. Their field-intensity distribution forms a similar speckled pattern to that observed in our SERRS emission. Similarly, Hillenbrand and Keilmann[14] have used a scattering-type scanning near-field optical microscope to measure the optical near-field from gold nanoparticle clusters and found that some particles exhibited enhanced optical near fields, but the highest amplitudes occur in specific location within gaps between particles. With microparticles, it is probable that the local hot spots occur in particular types of topographical environment and are dependent on the coverage, thickness, and surface roughness of the coated particle.

In conclusion, we have detected strong and short-lived SERRS emission (< 100 ms) from single, partially silver-coated, dye-adsorbed microparticles within optical tweezers. Full spectra, recorded from single particles, clearly show that the emission is due to SERRS.

On the periphery of the trapping beam, where the excitation intensity is much lower, the SERRS emission is longer-lived but can still be observed with a standard CCD

camera. The SERRS emission is from individual spots on the surface of the particle rather than the particle as a whole.

The high sensitivity, the ease of molecular recognition and the relatively simple chemistry of SERRS can be combined with the ability to observe, trap, and subsequently manipulate particles. In future this will provide an effective platform on which biologically functionalized beads can be manipulated to give a localized probe, for example, to detect individual binding events.

Experimental Section

Silica beads of 1.5 μm diameter were lightly coated in silver by using Tollen's reagent. The configuration for optical trapping used an infinity-corrected Zeiss Plan-Neofluar oil-immersion objective lens with a numerical aperture of 1.3 and a magnification of $\times 100$. The trapping laser is a frequency doubled commercial Nd:YVO4 laser (100 mW, $\lambda = 532$ nm). Particles were viewed with a CCD camera protected by a filter to remove light of the laser frequency. The spectra were recorded on a S2000 Ocean Optics spectrometer. To confirm that the spectra recorded in the optical-tweezers setup was from the dye adsorbed on the beads, a SERRS spectrum from the dye adsorbed on silver colloid was acquired. Silver colloid (250 μL), water (250 μL), 1×10^{-7} M dye (10 μL) and 1M sodium chloride (10 μL) were mixed together and analysed in a cuvette using an acquisition time of 3 seconds.

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