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# Ultrastructural characterization of colloidal metal films for bioanalytical applications by scanning force microscopy

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Colloidal metal films (CMFs) are prepared by the attachment of silver, gold, or platinum (or other metal) particles to a glass slide modified by silanization with 3-mercaptopropyl silane. The covalent attachment of the metal particles occurs through the metal-sulfur bond. In these samples the local electromagnetic field is enhanced near the surface of the CMF due to excitation of plasmon resonances. This phenomenon can be used for a variety of analytical applications. Because the optical properties are strongly dependent on the morphology of the film, its structural characterization becomes of great importance. To further characterize CMFs we have used scanning force microscopy (SFM). Initial studies revealed lateral dimensions of the particles as well as the particle density. Height measurements were made using the three-dimensional topographic image of the surface yielded by SFM, and were used to evaluate the selective deposition of a silica spacer layer onto the metal particles. Comparative SFM measurements in water and air using different imaging modes were performed to investigate the mechanical stability of CMFs against the forces exerted by the scanning tip. © 1996 American Vacuum Society.

## I. INTRODUCTION

Corrugated silver and gold surfaces exhibit surface enhancement of the electromagnetic fields.<sup>1</sup> This effect is the basis for surface enhanced Raman scattering spectroscopy (SERS) and surface-enhanced fluorescence (SEF) (for reviews, see Refs. 2 and 3). Both phenomena have great potential for analytical applications and this has stimulated the development of new substrates for the enhancement of the optical signals. Recently, colloidal metal films (CMFs) were introduced as new substrates for surface-enhanced spectroscopies.<sup>4,5</sup> They consist of small (10–100 nm) metal particles covalently bound to a derivatized substrate. Bonding is accomplished through different functional groups such as thiol, pyridyl, carboxy, and amino, which have a high affinity for the metal. In comparison to conventional substrates, e.g., vacuum deposited metal islands, the shape and the size of the metal structures can be more readily controlled. Unlike colloidal solutions, the CMFs do not undergo uncontrolled aggregation and can be easily studied in different chemical environments. The particle density at the surface as well as the aggregation of the colloids as a prerequisite for an enhancement can be controlled.<sup>6</sup> We are interested in applications of these substrates for the development of more sensitive optical detection methods.

The magnitude and distance dependence of the enhancement phenomena are strongly affected by surface topography. Because the strongest enhancement of the electromag-

netic field occurs in the space between particles,<sup>4</sup> selective adsorption of molecules in this space is critical for maximum SERS and SEF. Molecule adsorption directly on the metal particles of CMFs results in strongest Raman enhancement, whereas adsorption between the particles maximizes fluorescence enhancement.<sup>4</sup> In the case of molecules directly adsorbed onto the metal particles, SEF is influenced by quenching processes. Optimal positioning of analytes as well as minimization of fluorescence quenching can be achieved by surface modification of CMFs. One way of doing this is the selective deposition of a silica spacer layer on metal particles or in between them.

Microscopical characterization of CMFs using scanning electron microscopy (SEM) revealed the distribution of the colloidal particles on the surface as well as the lateral dimensions.<sup>4</sup> To monitor surface roughness and heights of adsorbed particles we used the scanning force microscope (SFM), which yields a the three-dimensional (3D) topography of the investigated surface.<sup>7</sup> Using this approach we characterized CMF modification by silanization. Moreover, we exploited the ability of the SFM to make measurements on CMF specimens in air and in solution.

## II. MATERIALS AND METHODS

### A. Colloidal metal films

The preparation of CMFs is described elsewhere.<sup>4</sup> Briefly, a glass slide was derivatized with (3-mercaptopropyl) trimethoxysilane (MPS), followed by immersion into the previously prepared aqueous colloidal suspension with different concentrations of Ag particles. CMFs were formed as result

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of adsorption of Ag particles on the derivatized glass surface [cf. Fig. 4(a)]. Silver colloids were prepared by citrate reduction.<sup>8</sup> Colloidal particles from the same batch were used for CMF preparation. CMFs were modified by deposition of silica as a spacer layer [cf. Figs. 4(b) and 4(c)]. This spacer layer was prepared using a standard silanization reaction by hydrolysis of tetraethoxysilane (TEOS) as described by Anderson *et al.*<sup>9</sup>

It was found that CMFs are most stable while stored in colloidal solution. Air drying was performed after rinsing the samples in distilled water. Electron microscopy was performed using a JEOL 1200EX scanning transmission electron microscope. For scanning electron microscopy a platinum/palladium (80:20) film of approximately 10 nm thickness was deposited on the CMF specimens using a Polaron E5 100 sputter coater. For transmission electron microscopy colloidal particles were adsorbed onto Formvar coated copper grids.

## B. Scanning force microscopy

SFM data were collected using a BioScope [Digital Instruments (DI), Santa Barbara, CA]. Images were obtained in the topographic mode, such that the feedback maintains the cantilever deflection/oscillation amplitude constant for the contact/tapping modes, respectively. The force exerted by the scanning tip was minimized by scanning with the lowest (contact mode) or highest (tapping mode) possible setpoint. In air data were collected using the tapping mode with silicon NanoProbe tips (DI), while in water standard pyramidal shaped  $\text{Si}_3\text{N}_4$  tips (DI) were used for the tapping and contact modes. The standard BioScope fluid cell was used without the O ring. Scanning was performed using a J scanner with a 100  $\mu\text{m}$  lateral scan range. Image processing and analysis were carried out using the NanoScope software and NIH-Image 1.49 (NIH, Bethesda, MD). Estimation of lateral dimensions of a topographic feature was done at the full width at half-maximum height.<sup>10</sup> For particle density estimation all particle centers in randomly chosen squares of 4  $\mu\text{m}$  size were counted and averaged.

## III. RESULTS

### A. Imaging of CMFs in water

CMF specimens before and after modification by deposition of silica were imaged in water. In contact mode imaging, the stability was generally low; only occasional scan lines revealed round particles covering the surface. Usually, the first scan was noisy (we believe this was due to transport of material by the tip), whereas the following scans resulted in surfaces without particles. By using tapping mode imaging, the stability could be increased for larger scan sizes (>10  $\mu\text{m}$ ), resulting in images of the modified surface in the first scan (Fig. 1). Despite a number of noisy scan lines, the submonolayer surface coverage by round particles was visible (e.g., a circled region). Other surface features included rodlike structures and clusters with an increased height compared to surrounding areas. Determination of particle dimen-

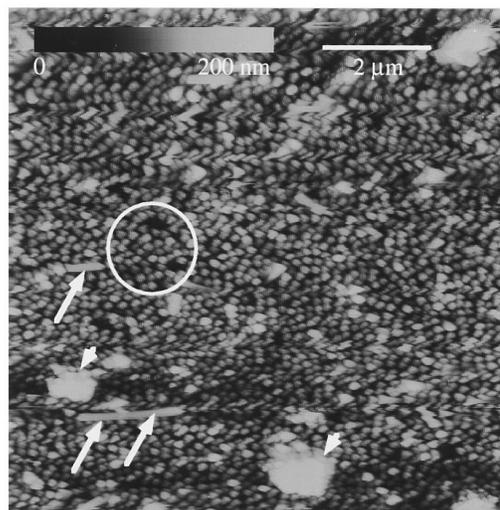


Fig. 1. Tapping mode scanning force microscopy of colloidal metal films in water. The height is brightness coded according to the gray-scale bar. A submonolayer of round particles is visible (e.g., circled region). Other observed features involve rodlike structures (arrows) and clusters of material exhibiting a greater height than the surrounding area (arrowheads).

sions was hampered by the instability of the images, but heights in the range of 70–100 nm could be measured. The unmodified surface yielded only occasional images of particles during noisy SFM measurements.

### B. Unmodified air-dried colloidal metal films

Scanning force microscopy revealed a flat surface covered by a submonolayer of round particles [Fig. 2(a)], confirming previous SEM studies of similar specimens.<sup>4</sup> The particles appeared to be regularly distributed and only occasionally were clusters visible. The particle density was about  $23/\mu\text{m}^2$ . A narrow distribution of particle height and width was found. Typical dimensions were  $\sim 100$  nm (height) and  $\sim 175$  nm (width) [inset, Fig. 2(a)]. Regions without metal particles exhibited a surface roughness in the range of a few nm.

In addition to the round particles, rodlike structures were occasionally found (arrows in Fig. 2); these latter had lengths of up to several  $\mu\text{m}$  and widths and heights comparable to the colloidal particles. In some cases, these struc-

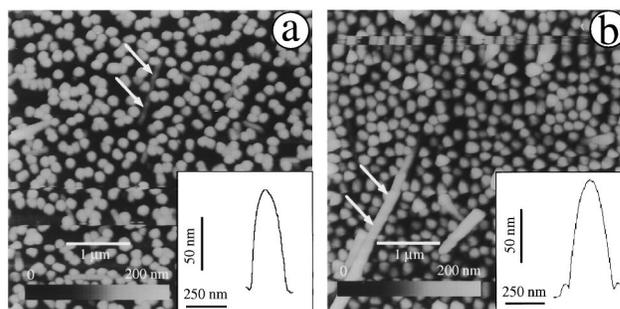


Fig. 2. Scanning force microscopy of colloidal metal films. Images were collected in tapping mode in air. Arrows mark rodlike structures. The insets show a cross section of one particle. (a) Unmodified specimen. (b) Specimen after surface modification by silica deposition.

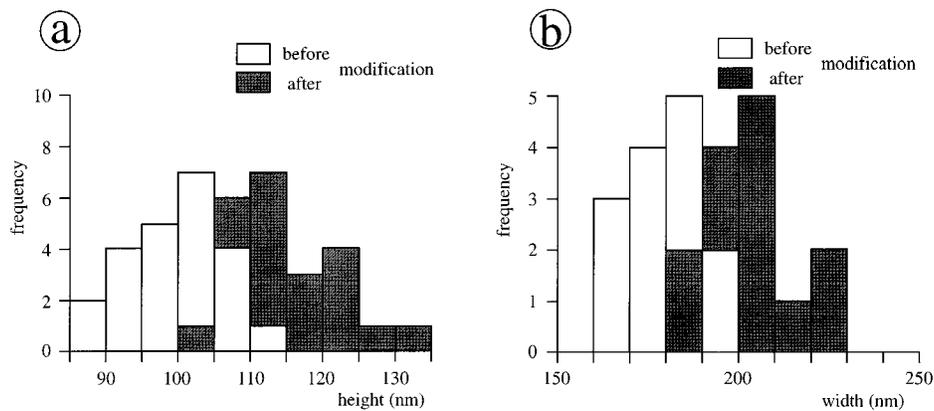


FIG. 3. Dimensions of an unmodified (white bars) colloidal metal particle and after silica modification (gray bars). In the overlapping regions the higher bars are shown in the background.

tures were observed to be partially covered by colloidal particles [Fig. 2(a)] as well as laying on top of the round particles [Fig. 2(b)].

### C. Modified CMFs in air

CMF specimens were imaged in air after deposition of silica. No apparent changes in contrast or stability were observed [Fig. 2(b)]. The surface coverage seemed to have increased, but determination of the particle density resulted in the same value as before the modification procedure ( $\sim 23$  particles/ $\mu\text{m}^2$ ). The distribution of the particles over the surface appeared slightly different from the unmodified sample: Fewer clusters of particles were found. An increase in the particle dimensions was observed [inset, Fig. 2(b)]. The height was  $115 \pm 8$  nm compared to  $100 \pm 7$  nm for the untreated particles [Fig. 3(a)], and the width reached  $203 \pm 11$  nm compared to  $179 \pm 10$  nm for the particles before modification [Fig. 3(b)].

## IV. DISCUSSION

The SFM was successfully used to image modified CMFs in water, revealing the metal particles bound to the substrate (Fig. 1). We initially visualized the particles before air drying, an event that could cause structural changes of the sample due to the high surface tension. However, no significant differences in the images of the hydrated sample compared to the air-dried samples were observed and we conclude that at the limit of resolution of this imaging method the CMFs are stable against air-drying effects.

Imaging in water was not possible in contact mode, probably due to the high shear forces exerted by the scanning tip that resulted in the removal of the particles from the surface. This supposition is supported by the nature of the instability (which resembles material transport by the tip) and the images of the subsequent scans, that revealed empty surfaces with improved imaging stability. We believe that the surface was covered with colloidal particles before scanning, as shown by occasional visible particles and by imaging the same sample using tapping mode in water (cf. Fig. 1) or air

dried [cf. Fig. 2(b)]. Further support comes from an observation made by simultaneous imaging with the inverted optical microscope that yielded “windows” in the brown surface layer after imaging by SFM. The size of these windows corresponded roughly to the scan size.

Scanning in water using tapping mode yielded different mechanical stability for the samples before and after surface modification. Unmodified samples were only occasionally stable, but samples with modified CMFs could be imaged with a reasonable amount of stability (Fig. 1). We assume this phenomenon of stabilization is due to increased substrate adsorption caused by the additional layer introduced by silica modification. The silica layer could stabilize the particles by coverage of the whole sample and “immobilizing” underlying material on the substrate surface [Fig. 4(c)], or at least by increasing the contact area between particle and substrate [Fig. 4(b)].

What is the mechanism of surface modification is an important question. Do the silica spacer layers cover the whole sample [Fig. 4(c)], or is there any specific adsorption by particles [Fig. 4(b)] and/or substrate? Compared to the particle before modification [Fig. 4(a)], a nonspecific covering should result in no height increase and an increase in sample

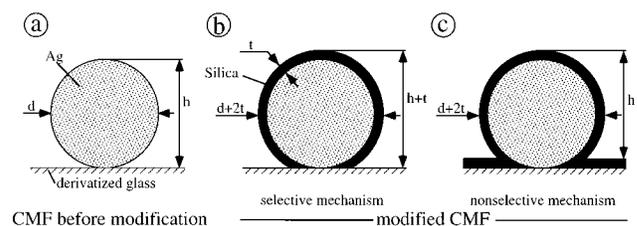


FIG. 4. Cross-sectional view through one particle with dimensions of  $h$  (height) and  $d$  (diameter), demonstrating dimensional changes that would result from selective and nonselective mechanisms of modification. (a) Unmodified silver particle on the derivatized glass surface. (b) Particle selectively modified with a silica layer (thickness  $t$ ); the width increased by  $2t$ , the height by  $t$ . Note the unmodified substrate. (c) Particle and substrate are modified with a silica layer, so the width is again  $d + (2t)$ , but there is no apparent height increase compared to the unmodified particle.

width of two times the thickness of the silica layer [Fig. 4(c)]. A similar broadening is the result of particle-specific modification but, in contrast to the nonspecific case, there should be an increase in the silica thickness height [Fig. 4(b)]. The lateral resolution of SFM is complicated by convolution of sample and tip geometry, resulting in a sample broadening approximated by the tip diameter. This convolution affects all measurements of similar dimensions in a similar manner; therefore, unless absolute widths are desired, it can be discounted. The SFM revealed  $179 \pm 10$  nm for the width of unmodified particles compared to  $203 \pm 11$  nm for the width of modified particles [Fig. 3(a)]. The broadening determined was  $\sim 24$  nm based on two layers [one on each side of the particle, cf. Figs. 4(b) and 4(c)], indicative of a silica layer thickness of about 12 nm. Silica modification increased the particle height from  $100 \pm 7$  nm (unmodified) to  $115 \pm 8$  nm (modified), resulting in an  $\sim 15$  nm difference. This height increase supports a mechanism of specific silica modification of the particles, seen in Fig. 4(b), in which the silica layer thickness should be about 15 nm. Support for the specific modification mechanism comes from the width measurements that resulted in a similar value ( $\sim 12$  nm) for the layer thickness.

The particle height observed by SFM of unmodified CMF surfaces correlates very well with the particle diameter obtained by transmission electron microscopy. This encourages the use of SFM height measurements for determination of absolute particle diameter. We also used SEM to image unmodified and modified CMFs. In order to obtain SEM images a thin conductive layer is deposited on the specimen surface to eliminate charging. Insofar as we could not precisely control the thickness of this layer, absolute measurements were difficult to perform. SEM yields a range of 10–22 nm for the thickness of the silica modification layer on silver particles, supporting the SFM measurements discussed above.

An interesting observation was the apparent difference in clustering of the particles between the unmodified and modified samples after air drying. In the unmodified sample, clusters formed by particles with distances (center to center) in the diameter range could be observed [Fig. 2(a)]. On the other hand, the modified sample exhibited only rare examples of such aggregation [Fig. 2(b)]. In solution, the particles exhibit a repulsion due to their negative charge.<sup>11</sup> This repulsion should result in CMF formation without aggrega-

tion. An explanation for the observed aggregation could be the high surface tension during the air drying, overcoming the repulsion between neighboring particles. The modification could stabilize the particles against this effect. This assumption is supported by the increased stability of the modified particle while SFM imaging in water (Fig. 1) compared to the unmodified samples. Because the aggregation increases the Raman enhancement,<sup>6</sup> further investigations are underway to elucidate the mechanism of this phenomenon.

## V. CONCLUSIONS

Colloidal metal films were characterized by scanning force microscopy. The result of a surface modification procedure was elucidated by monitoring particle dimension changes in the nanometer range. Modified CMFs were visualized in water, opening the way for investigation of CMFs without structural changes induced by air drying. Particle density and distribution were determined with air-dried CMFs. Aggregation of particles was pronounced in unmodified samples and abated somewhat in silica coated samples.

Substrate surface roughness of a few nm was observed between metal particles at the substrate. Therefore, the SFM can be used to monitor immobilization of macromolecules (e.g., proteins) on CMF surfaces.

## ACKNOWLEDGMENTS

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