

Attomole (amol) myoglobin Raman detection from plasmonic nanostructures

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Received 6 October 2007; received in revised form 14 December 2007; accepted 27 December 2007

Available online 20 January 2008

Abstract

We have demonstrated the fabrication of nano-structures using electro-plating and electron beam lithography techniques to obtain a pattern of gold nanograin aggregate structures of diameter in the range between 80 and 100 nm with interstitial gap of 10–30 nm. The nanostructure based SERS substrate enables one to have better control and reproducibility on generation of plasmon polaritons. Using calculation, we have shown that Raman spectra are derived for the myoglobin concentration down up to attomole. These results are obtained using drop coating deposition Raman (DCDR) method in which solution of interest is microdeposited (2 μ L) on SERS substrate.

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Keywords: Surface plasmon; Surface-enhanced Raman scattering (SERS); Myoglobin

1. Introduction

In the recent years, there have been much interests in the field of plasmonics to control the interaction of electromagnetic waves with matter. Surface-enhanced Raman scattering (SERS) is a phenomenon resulting in strongly increased Raman signals when molecules are attached to nanometer-sized metallic structures [1]. Many early SERS substrates used a random roughening of the surface so only small uncontrolled areas of the total metal surface would have the correct geometry for Raman enhancement. When the laser interacts with sharp metallic surface, the increment in localized electric field causes an enhancement in Raman signal. Among the most remarkable effects associated with such local fields there is giant Raman scattering that allowed for the detection of single molecules on colloidal metal fractal clusters [2,3]. Most traditional way of prepar-

ing SERS surface is the chemical way; preparing Ag colloids and attaching the protein/peptides to these colloids [4,5]. Recently, efforts are also being made to fabricate the reproducible nanostructured SERS substrate [6]. In the past, few attempts have been made to fabricate the periodic nanostructures using e-beam lithography and nanosphere lithography techniques [7,8]. Kahl et al. [7] fabricated his SERS device based on Ag metal, which illustrates Raman enhancement for an organic compound (thiobenzene) but because of the inherent properties of Ag, the device durability as a SERS substrate is limited as the Ag surface oxides in air. In this letter, we report the fabrication of plasmonic gold agglomerated SERS substrate by electro-plating and e-beam lithography techniques in order to detect the myoglobin protein of low concentration. Here we present a novel device for generation of PP's made of array of gold nanoaggregates on gold-chromium base plated Si wafer. The presence of gold nanoaggregates and high resolution e-beam patterning down up to 10 nm of interspatial distance between two

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nanoaggregates are the major fabrication novelty of this present work. Various SERS measurements were carried out for myoglobin deposited on nanostructure using DCDR method [7] by varying concentration and by changing the position on nanostructure. To be noticed, the dried hydrated protein using DCDR is capable of detecting the biomolecules with shorter integration times [9]. Gold nano-grain-aggregates show advantages over silver nanograin-aggregates, having similar dielectric constant in NIR, because of its chemical inactivity. NIR laser (830 nm) excitation light source is used because the fluorescence background of Raman signal can be strongly quenched. Raman effect in this case contributes total enhancement from SERS substrate. Moreover, NIR Raman can show an increase in intensity of factor 10^2 – 10^3 with respect to fluorescence intensity in a given time as the Raman photon vibrational relaxation time is much shorter than the electronic relaxation time [2]. Here we shall show that it could be possible to detect very small amount of molecules by exciting plasmons on interaction of light and gold nanoaggregates.

2. Experimental

2.1. Device fabrication

Devices are fabricated onto an Au–Cr base-plated silicon wafer following a high resolution fabrication techniques, using electro-plating and e-beam lithography, shown in Fig. 1. Several patterns of nano-holes were designed, using a “Crestec CABL-9000C” e-beam lithography system, on a layer of electronic-resist spun onto the

wafer. The nanograins of gold were grown inside the nano-holes by using gold-plating growing. Afterward, the resist film was removed. In this way, nano-structures of gold were obtained. The nanograin size and the mutual distance between two nanoaggregates are 80 nm diameter and 10–30 nm gap, respectively.

2.2. Characterization technique

Micro-confocal Raman measurements (Renishaw) were excited by 830 nm diode laser (power = 6 mW and accumulation time = 100 s) in backscattering geometry with resolution of about 1.3 cm^{-1} . Myoglobin, dissolved in water with different concentration, of 2 μl volume was deposited, following the DCDR method, on the nanostructures [9]. To be noted, Zhang et al. carried out many measurements for the aqueous samples and their corresponding DCDR samples, and observed that the spectra resemble each other. Moreover, after each deposition, it was ensured through optical image that the ring (inner diameter-3.5 mm and width-0.08 mm) of adsorbed protein remains on the nanograin-aggregates array, shown in Fig. 2. The Raman measurements have been carefully performed at two positions; (1) on nanograin aggregate ‘a’, (2) on No nanostructures ‘b’, by putting attention that at both position protein concentration remains equivalent.

3. Results and discussions

Raman spectroscopy is a spectroscopic technique which enables one to provide the structural and chemical information of the protein. Myoglobin (Mb) protein, as

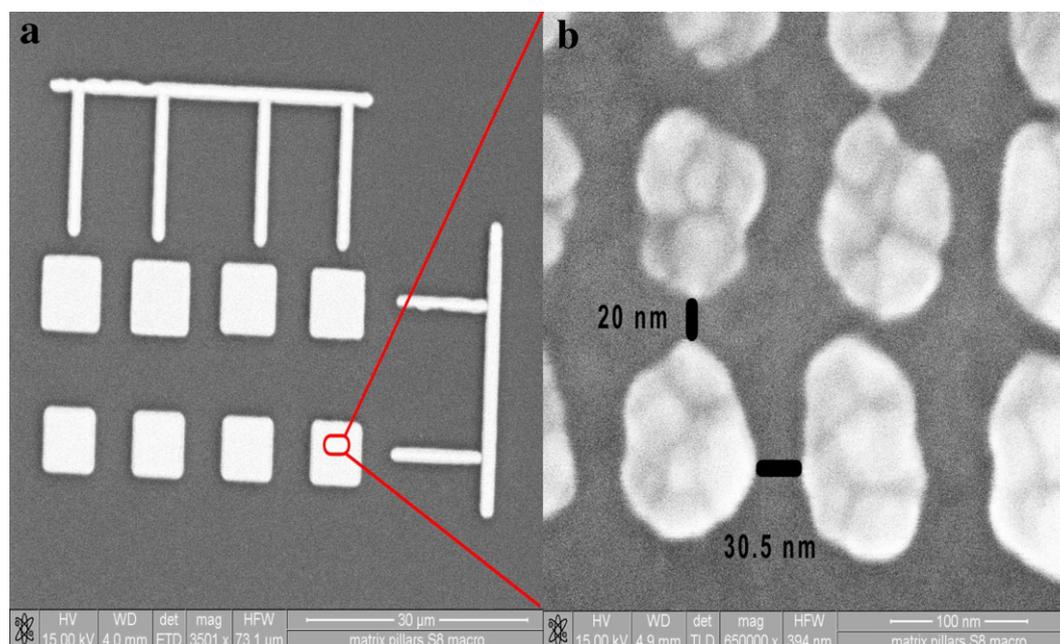


Fig. 1. (a) SEM image of nanostructures pattern fabricated on gold–chromium base plate using electro-plating and e-beam lithography techniques, (b) zoom of one of the gold nanostructures, showing gold nanograins aggregate array.

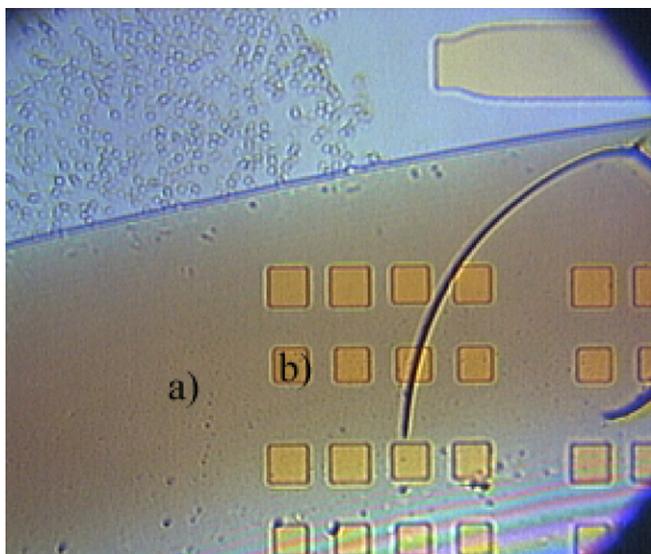


Fig. 2. Optical image of myoglobin DCD spot. In figure position (a) is referred to the place where no nanostructures are placed, and position (b) is the place where nanostructures have been developed.

in our case, of ~ 4.9 nm diameter and of almost spherical molecular structure contains one planar Fe-protoporphyrin prosthetic heme group implanted in polypeptide chains, containing very high amount of α -helix (75%). The major spectroscopic properties of myoglobin contribute from π - π^* transitions within these heme groups. The absorption band of the stretching vibrations of ring π -bands falls in the 1000 – 1700 cm^{-1} region. SERS spectra, carried out on gold nanoaggregates plasmonic nanostructure substrate, for Mb with fixed concentration are illustrated in Fig. 3a in 500 – 1800 cm^{-1} region. Fig. 3a shows the well known vibrational band for Mb centred at 1126 , 1373 , and 1560 cm^{-1} which are attributed to the C–N stretching,

[10] an oxidation marker band of heme iron [11] and C–C vibrational band, respectively (selected regions in Fig. 3a). A sharp peak around 520 cm^{-1} in Raman spectra arises from Si wafer over which the SERS substrate is fabricated. Raman protein spectra show some unique intense peaks at 760 , 1011 , 1365 and 1554 cm^{-1} , could be attributed to the different vibrational modes of tryptophan's (Trp) whereas the bands around 1005 and 1034 cm^{-1} could be relied to the phenylalanine (Phe) residues. Fig. 3a shows that with decrease in myoglobin concentration there is decrease in intensity which is obvious because higher number of Mb could be attached to the gold nanograins with the increase of protein concentration.

Raman spectra, performed at both position (a,b) of device Fig. 2 and on uncoated SERS nanostructures, are illustrated in Fig. 3b. The figure shows that the myoglobin Raman intensity reduces and the Si substrate contribution increases at position 'a' in comparison to position 'b'. The SERS substrate Raman spectrum (multiplied by five) is also shown in the same figure. The measurement has also been performed for myoglobin deposited on c-Si wafer. A faint peak of Phe residue is observed whereas rest of the protein Raman is covered by strong Si Raman band (not shown here). The intensity of Si band (520 cm^{-1}) for various measurements carried out for myoglobin on Si wafer, nanograin-aggregates, and on base plate without nanostructures is illustrated in inset of Fig. 3b.

Noticeably, protein Raman was not observed at positions farther away from ring. Here, all the calculations are being carried out for 7 μM – 120 μM protein (concentration of our protein). If, we suppose that all the protein is located on ring of DCD mark then the protein surface density will be approximate to the 15 – 270 pmol/mm^2 in the ring and if the proportion is $1/10$ or $1/3$, then the protein surface density would be 8 – 235 pmol/mm^2 or 3 – 100 pmol/mm^2 ,

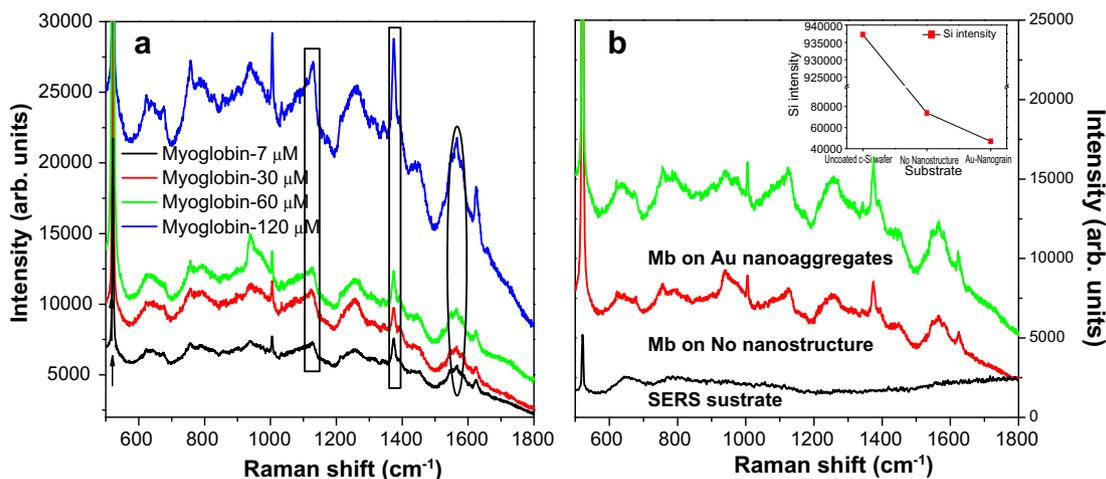


Fig. 3. (a) Raman spectra for myoglobin protein by varying the concentration but by keeping the laser spot centered at nanostructures, (b) Raman spectra for Mb covered nanostructures, Mb covered no nanostructures and no myoglobin on uncoated nanostructures ($\times 5$). In the inset of (b), the Si vibrational band intensity measured for DCD deposited substrates (c-Si wafer, no nanostructures, and Au nanostructures), keeping the measurement parameters same.

respectively. Realistically, the actual protein surface density could be around 5–10 pmol/mm² and 140–230 pmol/mm² for 7 μM and 120 μM protein, respectively. By assuming the size of one myoglobin molecule to be 20 nm², the minimum amount of myoglobin within the focal spot of laser (radius: 1 μm) could be, therefore, estimated around 10–240 attomole.

4. Conclusions

We have fabricated a controllable and reproducible SERS substrate comprised of gold nanograins aggregate array with interspatial distance between two gold nano-aggregates structures down up to 10 nm. Various measurements were performed on different positions of device in order to demonstrate the enhancement of Raman signal. By carrying out the calculation of the mole concentration as described by Zhang et al. [9] we are able to achieve the concentration in the order of attomole (10⁻¹⁸) of myoglobin protein with reproducible vibrational Raman spectra. This opens up broad applications in which the detected molecule allows for its identification in real time.

Acknowledgements

The financial support of “MIUR PRIN 2005039992” is acknowledged.

References

- [1] M. Moskovits, *Rev. Mod. Phys.* 57 (1985) 783.
- [2] K. Kneipp, Y. Wang, H. Kneipp, L.T. Perelman, I. Itzkan, R.R. Dasari, M.S. Feld, *Phys. Rev. Lett.* 78 (1997) 1667.
- [3] S. Nie, S.R. Emory, *Science* 275 (1997) 1102.
- [4] K. Kneipp, H. Kneipp, S. Abdali, R.W. Berg, H. Bohr, *Spectroscopy* 18 (2004) 433.
- [5] H. Xu, E.J. Bjerneld, M. Kall, L. Borjesson, *Phys. Rev. Lett.* 83 (1999) 4357.
- [6] N.M.B. Perney, J.J. Baumberg, M.E. Zoorob, M.D.B. Charlton, S. Mahnkopf, C.M. Netti, *Opt. Express* 14 (2006) 847.
- [7] M. Kahl, E. Voges, S. Kostrewa, C. Viets, W. Hill, *Sensor. actuator. B: Chem.* 51 (1998) 285.
- [8] Xiaoyu Zhang, Chanda Ranjit Yonzon, Richard P. Van Duyne, *J. Mater. Res.* 21 (2006) 1083.
- [9] D. Zhang, Y. Xie, M.F. Mrozek, C. Ortiz, V. Jo Davisoon, Dor Ben-Amotz, *Anal. Chem.* 75 (2003) 5703.
- [10] T.G. Spiro, *Adv. Protein Chem.* 27 (1985) 111.
- [11] H. Sato, H. Chiba, H. Tashiro, Y. Ozaki, *J. Biomed. Opt.* 6 (2001) 366.