

MICROFLUIDIC INTEGRATION OF PLASMONIC APPLICATIONS FOR HIGHLY SENSITIVE BIOANALYSIS

C.Y. Xiao^{1,3†}, Z. Cao^{2†}, Z.F. Huang¹, Z. Xu³, J.X. Fu^{1*} and L. Yobas^{2*}

¹ Department of Physics, Hong Kong Baptist University, Kowloon Tong, HONG KONG

² Department of Electronic and Computer Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, HONG KONG

³ Institute of Optoelectronic Technology, Beijing Jiaotong University, Beijing, P.R.CHINA

ABSTRACT

We integrate plasmonic applications such as surface enhanced Raman spectroscopy (SERS) and metal enhanced fluorescence (MEF) with micro-patterned substrates and microfluidic device using localized oblique angle deposition. Plasmonic nanostructures such as Ag nanorods in microcavities and microwells exhibit high sensitivity for SERS and MEF, respectively. Further, a MEF enhancement factor of 6 is observed for the optical detection of amino acid peaks in capillary electrophoresis (CE).

KEYWORDS: Plasmonics, Microfluidics, Localized Oblique Angle Deposition

INTRODUCTION

Surface enhanced Raman spectroscopy (SERS) and metal enhanced fluorescence (MEF) have emerged as powerful techniques for sensitive bio-detection, owing to the plasmonic properties of Au or Ag nanostructures. [1, 2] Usually, these plasmonic applications are conducted on planar substrates, while lacking the control and manipulation of analytes. Microfluidics thus becomes a proper platform to be integrated with plasmonic applications. Previously, we have deposited Ag nanorods into pre-patterned microcavities and revealed that they are reasonably good SERS substrates, and could be easily integrated into a microfluidic processing system. [3] Here, we present our recent results on capillary electrophoresis (CE) integrated with Ag nanorods and the MEF detection of the amino acid peaks in separation.

MECHANISM AND PRINCIPAL

When a beam of light incidents onto Au or Ag nanostructures, free electrons inside them absorb the light energy and oscillate collectively. Consequently, a strongly intensified localized electric field is produced around nanostructures, resulting in the great enhancement of Raman or fluorescence signal of the analytes nearby. [4, 5] In this work, we employ localized oblique angle deposition (LOAD) technique to directly load Ag nanorods, therefore plasmonic structures, into the micro-patterned substrates and microfluidic devices.

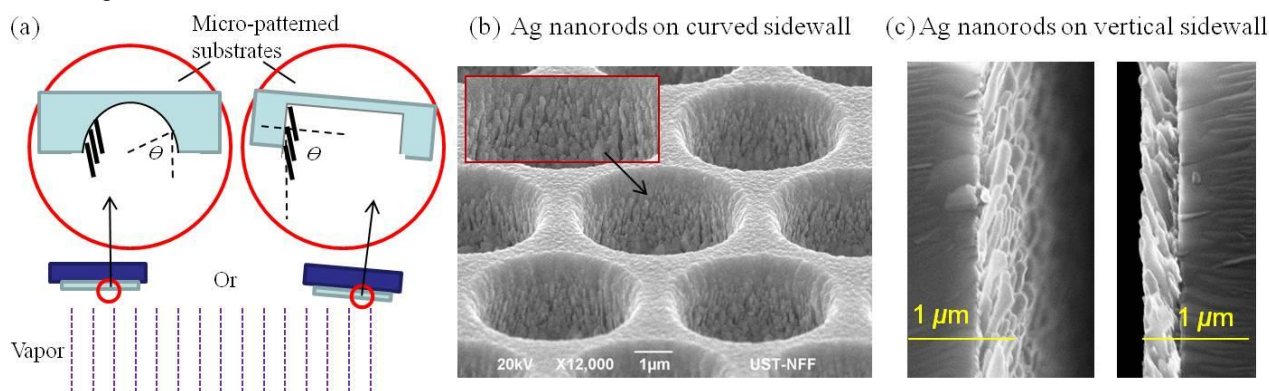


Figure 1: (a) Diagram of Localized Oblique Angle Deposition (LOAD); (b) SEM tilted image of Ag nanorods deposited on the curved sidewalls in micro-cavities and (c) SEM cross sectional images of Ag nanorods deposited on the vertical sidewalls in a CE channel by LOAD.

The mechanism of LOAD originates from that of oblique angle deposition (OAD), where the tilted nanostructures are self-aligned on a flat substrate by shadowing effect and surface diffusion [6]. If the angle θ between the incident vapor of atoms and the normal of the substrate surface is larger than 75° , shorter nuclei on the surface will be shadowed by those taller. This shadowing effect results in the voids between the emerging nanorods as the deposition proceeds. Ag nanorods fabricated by OAD have shown significant enhancement for SERS and MEF. [1, 2]. Here, LOAD localizes the formation of nanorods on structured surfaces as described in Figure 1(a). As can be seen, the sidewalls of micro-patterned substrates become the depositing surface. To fulfill the requirement of the deposition angle θ , the substrate is either horizontally placed or tilted by a small angle for curved or vertical sidewalls. Figures 1(b) and 1(c) show scanning electron microscopy (SEM) images of Ag nanorods deposited by LOAD on the curved and vertical sidewalls, respectively. These Ag nanorods are expected to perform plasmonic applications in microfluidic environment.

EXPERIMENTAL

Experimental procedure for Ag nanorods deposited in microcavities and their SERS measurements is detailed in our previous work. [3] Briefly, microcavities with curved sidewalls were etched by a combination of isotropic dry etching and deep reactive ion etching (DRIE). The diameter and depth were $\sim 4.4 \mu\text{m}$ and $3 \mu\text{m}$, respectively. Ag film with a thickness of 500 nm and then Ag nanorods with a nominal thickness $1 \mu\text{m}$ were deposited onto sidewalls. The above structures were incubated with Rhodamine B (RhB) dissolved in methanol for 24 hours, subsequently rinsed with DI water, dried in air and then transferred to a Raman microscope (RM3000, Renishaw) for SERS probing.

Microwells and CE microchannels, both with vertical sidewalls, were lithographically etched in silicon wafers by DRIE to a depth of $18 \mu\text{m}$. For the former, the patterns were circles, whose diameter and spacing were $2 \mu\text{m}$ and $1 \mu\text{m}$, respectively. For the latter, as shown in Figure 2, the device was composed of a sample-injection region and a separation region, containing 20 parallel separation channels with $5 \mu\text{m}$ width, $5 \mu\text{m}$ spacing and 1 cm length. To avoid the voltage breakthrough, a layer of $1 \mu\text{m}$ silicon dioxide was thermally grown. Ag nanorods with a nominal thickness $2 \mu\text{m}$ were deposited with the deposition angle $\theta = 85^\circ$. For CE device, only a $2 \text{ mm} \times 1 \text{ mm}$ window at the rear of the separation channels was exposed to Ag vapor during the deposition.

For MEF characterization, a solution of $3 \mu\text{l}$ fluorescein isothiocyanate (FITC) with a concentration of 10^{-5} M was sandwiched between the microwell substrate and a piece of cover slip ($12 \text{ mm} \times 12 \text{ mm}$). The chip was examined by Luminescence Spectrometer LS 50 B (Perkin Elmer). CE devices were enclosed with polydimethylsiloxane (PDMS; Dow Corning 184) slab. Using borate (10 mM) as the running buffer, three FITC labeled amino acids (Sigma-Aldrich), Glycine (Gly), Serine (Ser), and Glutamine (Gln) with the same concentration of 1 mM ($\text{pH } 9.2$) were mixed at $1:1:1$ (v/v/v) ($\text{pH } 10.2$), diluted 100 times and pipetted into the sample-injection channel. The CE separation was monitored through an epifluorescence microscope (FN1; Nikon, Japan) equipped with a mercury lamp (100 W) as well as a filter cube (Ex/Em $492/520 \text{ nm}$). High-voltage power supply (Tianjin Dongwen Co. Ltd, China) was connected to reservoirs by platinum electrodes (Leego Precision Alloy, China) and controlled by Labview (National Instruments).

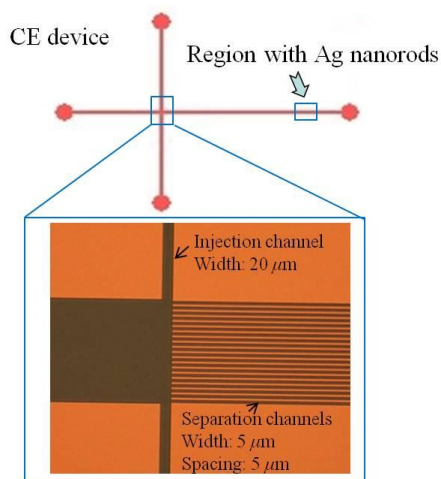


Figure 2: Top view of a CE device. The enlarged inset indicates the dimensions of injection and separation channels. The latter are 1 cm long. Only the rear part of separation channels (about 2 mm long) is coated with Ag nanorods.

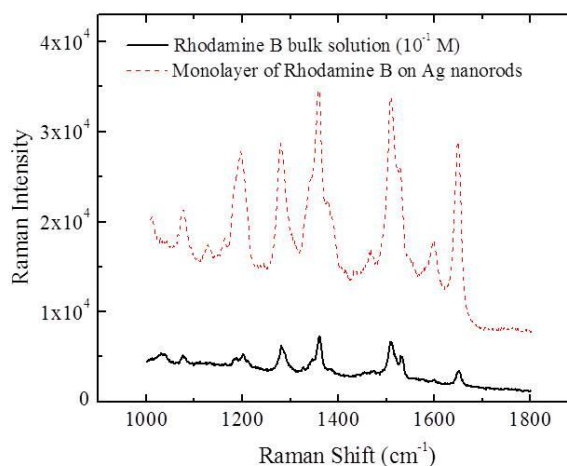


Figure 3: SERS measurements of Rhodamine B on Ag nanorods inside microcavities.

RESULTS AND DISCUSSION

SERS measurements performed on Ag nanorods in microcavities returned significantly increased signals as compared to the Raman signal of bulk RhB solution, as shown in Figure 3. The enhancement factors calculated are in the order of 10^5 . [3] Such SERS-active microcavities could be easily integrated with a sample processing microfluidics. Here, MEF enhancement is examined by measuring fluorescence spectra of $3 \mu\text{l}$ FITC solution sandwiched between microwells and a cover slip before and after deposition of Ag nanorods. The fluorescence intensity at the emission peak position (510 nm) is plotted in Figure 4(a). As can be seen, after Ag nanorods deposition, fluorescence intensity is greatly increased with an average enhancement factor of 8. Further, MEF detection of CE separation is performed by depositing Ag nanorods in the separation microchannels. Figure 4(b) is the electropherogram for the analysis of a mixture of three FITC labeled amino acids under 300 V/cm before and after entering the region coated with Ag nanorods. The peaks correspond to amino acids Gln, Gly, and Ser according to the order of their migration time (from fast to slow). As can be seen, the fluorescence intensity of FITC increases by 6 folds after the amino acids enter the region with Ag nanorods, as a result of MEF.

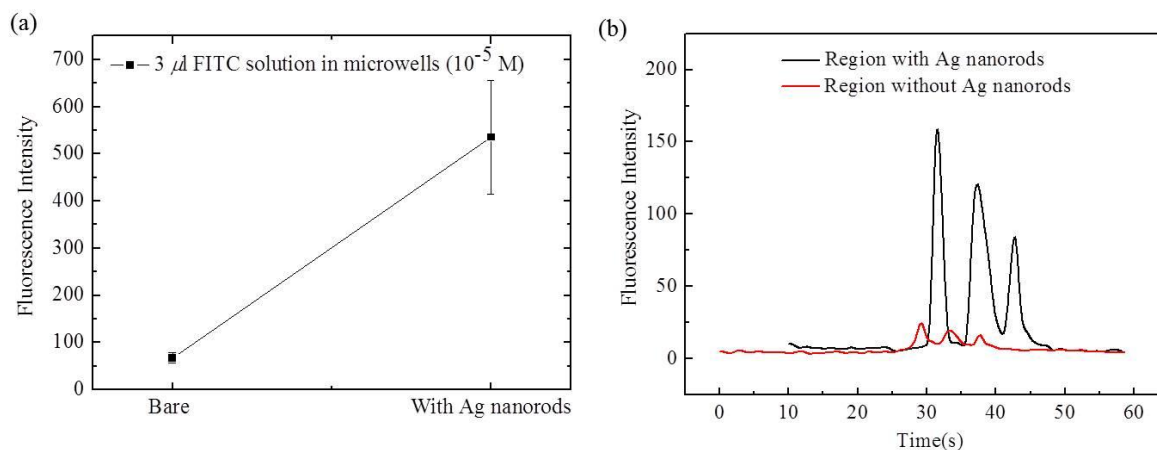


Figure 4: (a) MEF measurements of FITC solutions on Ag nanorods inside microwells. (b) Electropherogram for the analysis of a mixture of three FITC-labeled amino acids under 300 V/cm before and after entering the region coated with Ag nanorods. The peaks correspond to amino acids Gln, Gly, and Ser according to the order of their migration time (from fast to slow).

CONCLUSION

In summary, we have demonstrated SERS and MEF activity in microcavities and microwells coated with Ag nanorods by the LOAD technique. Furthermore, MEF has been applied to a micro-CE system where the optical detection is enhanced by the Ag nanorods decorating the separation channels. It is believed that the integration of plasmonic applications with microfluidic systems is likely to produce more versatile and highly sensitive bioanalysis.

ACKNOWLEDGEMENTS

The authors thank the financial support from the HKBU Faculty Research Grant FRG1/11-12/036 and the HKUST Research Project Competition Grant RPC11EG09.

REFERENCES

- [1] Tripp, R.A., Dluhy, R.A., Zhao, Y., "Novel nanostructures for SERS biosensing," *Nano Today*, vol. 3, pp. 31-37, Jun.-Aug. 2008
- [2] Abdulhalim, I., et al., "Surface-enhanced fluorescence from metal sculptured thin films with application to biosensing in water," *Applied Physics Letters*, volum 94, pp. 063106, Feb. 2009.
- [3] J Fu, Z.Cao. L.Yobas, "Localized oblique-angle deposition: Ag nanorods on microstructured surfaces and their SERS characteristics," *Nanotechnology*, vol. 22, pp. 505302, Nov. 2011.
- [4] Lakowicz, J.R., "Radiative decay engineering 5: metal-enhanced fluorescence and plasmon emission," *Analytical Biochemistry*, vol. 337, pp. 171-194, Feb. 2005.
- [5] Kneipp, K., et al., "Surface-enhanced Raman scattering and biophysics," *Journal Of Physics-Condensed Matter*, vol. 14, pp. R597-R624. Apr. 2002.
- [6] Robbie, K. and M.J. Brett, "Sculptured thin films and glancing angle deposition: Growth mechanics and applications," *Journal of Vacuum Science & Technology a-Vacuum Surfaces and Films*, vol. 15, pp. 1460-1465. May 1997.

CONTACT

*: Corresponding authors:

Junxue Fu, Tel: +852-34115157; phyjxfu@hkbu.edu.hk

Levent Yobas, Tel: +852-23587068; eelyobas@ust.hk

†: Equal contribution