A LAB-ON-A-CHIP SYSTEM INTEGRATED WITH SUBWAVELENGTH PERIODIC PATTERNED METAL SURFACES FOR SERS-BASED MOLECULAR IDENTIFICATION BIOSENSING

M. Jin1, V. Pully2, L. Shui1, C. Otto2, A. Van den Berg1, and E.T. Carlen1*

1BIOS Lab on a Chip Group, 2Medical Cell BioPhysics Group, MESA+ Institute for Nanotechnology, and MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands

ABSTRACT

We present a new lab-on-a-chip (LOC) analysis platform that is integrated with nanotextured surfaces with periodically spaced subwavelength nanogroove and nanopiramid structures with precisely defined pitch λg and separation gaps of ~2 nm spacing that form high density arrays of hot-spot scattering sites ideally suited for surface enhanced Raman scattering (SERS) and Raman spectroscopy using small sample volumes. The simple fabrication technique requires conventional lithography, wet anisotropic etching and a bonded polymer microfluidic layer. Measured Raman enhancement factors of G~10^6 from rhodamine 6G (R6G) on patterned Au surfaces with λg=200 nm are consistent with numerical calculations.

KEYWORDS: Surface Enhanced Raman Spectroscopy, Plasmonics, Nanostructures, Lab-on-a-Chip

INTRODUCTION

Since the discovery of SERS on metal surfaces with nanoscale features [1], a new era for Raman spectroscopy has emerged for molecular identification in aqueous solutions at low sample concentrations, which provides a unique capability for the label-free detection and identification of a variety of different analytes. The large electromagnetic field enhancements created near the nanometer-scale metal structures when subjected to optical excitation are due to localized surface plasmon resonance on the nanostructure surface and most importantly in spaces between adjacent nanoparticles, called hot-spots [2]. Conventional SERS substrates (colloid solutions and solid surfaces) have reasonably well-controlled nanostructure dimensions, however, do not have precisely controlled nanometer scale spacing between adjacent nanostructures and cannot be easily aligned with the excitation polarization. We present new large area (~100 μm^2) nanotextured surfaces consisting of densely packed nanopirmids and nanogrooves with nearly atomically precise sizes and spacing. The new nanopyrmaid surfaces are ideally suited for SERS as the lateral and vertical dimensions can be varied to any dimension down to ~50 nm, with any suitable plasmonic layer, such as Ag or Au, and contain a large density of hot-spots located at the base of the closely spaced nanopymids. The advantages of the new nanopyrmaid surfaces presented in this work include: 1. High density periodic nanostructure arrays with nanometer-scale sizes and spaces that can be controlled with high precision, 2. SERS hot-spot dimensions of ~2 nm can be reproducibly fabricated in high density and precisely aligned to the excitation polarization, and 3. The SERS substrate can be easily integrated with microfluidic devices using conventional materials.

THEORY

Subwavelength periodic patterned surfaces have been shown to form standing surface plasmon polariton waves in narrow crevices with large localized electromagnetic field enhancements near the base of the crevice [3]. In all reported subwavelength grating or patterned surfaces, large localized electromagnetic field enhancements were reported near the base of the nanocrevices due to strong coupling between surface charges from the opposing sides of the nearest-neighbor structures. The electromagnetic Raman scattering enhancement can be approximated as G=|E(x,y,z,ω)/E(x,y,z,0)|^4, where E(x,y,z,ω) is the electric field of the incident excitation [4]. Nanopiramid surface cross-sections with λg=200 nm, 10 nm SiO2 layer, and 70 nm thick Au layer have been modeled using two-dimensional finite difference time domain (FDTD) calculations (Fullwave, Rsoft, Inc.) to determine the total electric E(x,y,z) and magnetic H(x,y,z) field distributions near the metal surface of the fabricated structures (Fig. 1).

Figure 1: (a) Triangular cross-section representing nanopiramid and nanogroove surfaces (b) 2D steady state FDTD simulation showing H enhancement at the nanocrevice base.

EXPERIMENTAL

The periodic nanopiramid arrays were constructed using a single lithography step, wet etching, metal deposition, and a bonded polymer microfluidic layer (Fig. 2). A masking layer was first patterned with electron-beam lithography on a silicon (100) wafer and the nanopiramds and nanogrooves are spontaneously formed with a wet anisotropic etching step.
that results in precisely controlled pitches $\lambda_0 = 2d_t \tan(54.7^\circ)$, which are defined by the silicon single crystal structure. The substrate surface is then covered with SiO$_2$ (10 nm) and Au (70 nm) layers. The microarray layer was fabricated from polydimethylsiloxane (PDMS) and compression bonded between a glass cap layer and the SERS substrate following oxygen plasma treatment of both surfaces.

The substrate surface is then covered with SiO$_2$ (10 nm) and Au (70 nm) layers. The microarray layer was fabricated from polydimethylsiloxane (PDMS) and compression bonded between a glass cap layer and the SERS substrate following oxygen plasma treatment of both surfaces.

RESULTS AND DISCUSSION

Figures 3a-3b show high resolution scanning electron microscopy images of representative patterned two-dimensional surfaces at different magnifications. One-dimensional nanogroove surfaces have been realized (Fig. 3c). A high-resolution transmission electron microscopy image of a silicon nanocrevase with separation between adjacent nanostructures in the ~2 nm range (Fig. 3d). The integrated LOC-SERS microarray is shown in Fig. 3e and the inset shows an atomic force microscopy (AFM) image of the nanotextured region after gold coating (70 nm).

Raman spectra of physically adsorbed R6G on flat and patterned nanopyramid surfaces coated with Au layer, diluted in deionized water (dH$_2$O) solutions of varying concentrations, have been measured. We used Au surfaces due to its stability. A 10 µL aliquot of each solution was placed in the center of the nanopyramid surface and immediately covered with glass cap. Each sample was incubated for 15 minutes at room temperature prior to a measurement. The sample was then placed under the objective of the confocal Raman microscope and horizontal nanocrevices were aligned perpendicular to the excitation polarization. The acquisition time for all measurements is 30 seconds. Figure 4 shows representative measurements of R6G (in dH$_2$O) on flat and nanopyramid Au surfaces. Figure 4a shows the Raman spectra for a 5 µM R6G sample solution; the lower trace shows the measured sample response from a flat Au surface, which consists of the deposited Au film on a flat SiO$_2$/Si surface using identical measurement conditions. It should be noted that most previous studies were conducted under surface enhanced resonance Raman conditions with an excitation wavelength near the peak absorption wavelength of R6G (~532 nm). Raman spectra for a 100 nM R6G concentration in dH$_2$O (Fig. 4b) include the 934, 1011, 1124, 1266, 1312, 1382, 1525, 1599, and 1636 cm$^{-1}$ modes, where all modes have been previously reported [5-7], except for the 1011 cm$^{-1}$ and 1525 cm$^{-1}$ modes; the 1525 cm$^{-1}$ mode has not been previously reported under SERS or resonant SERS conditions and is associated with vibration of the xanthene ring and NHC$_2$H$_5$ end groups [8]. The 1636 cm$^{-1}$ mode has not been previously assigned and is likely related to the 1650 cm$^{-1}$ xanthene ring mode. For R6G sample concentrations ranging from 1 µM to 100 µM, the measured spectra are very consistent and repeatable with respect to location and time. The enhancement factor has been estimated by comparing the peak heights of vibration spectra on the nanopyramid array surfaces with peak heights from reference measurements in solution while maintaining the same laser power (135 µW), microscope, spectrometer configuration, and R6G sample.
In order to compare the peak heights, a larger R6G concentration is used for the normal Raman reference measurement. The resulting enhancement factor can be roughly estimated as $EF \approx S_{SERS} [C_R]/S_R [C_{SERS}]^{-1}$, where $S_{SERS}$ and $[C_{SERS}]$ are the intensity amplitude and R6G concentration from the SERS substrate, respectively, and $S_R$ and $[C_R]$ are the intensity and R6G concentration from the normal Raman reference measurement, respectively. Due to the pre-resonance activity of R6G and low required laser power, enhancement factors at all measured vibration modes was not possible, however, an $EF \sim 10^6$ has been measured for the $\nu=1310$ cm$^{-1}$ mode and we consider this to be a lower bound for the Raman scattering enhancement.

**CONCLUSION**

In conclusion, large area nanotextured surfaces with periodically spaced subwavelength nanogroove and nanopyramid structures with precisely defined $\lambda_g$ and adjacent nanostructure spacing that form high density arrays of scattering sites ideal for surface enhanced Raman scattering (SERS) have been presented. The simple fabrication technique requires a single lithography step and wet anisotropic etching. Measured Raman enhancement factors in dH$_2$O of $G \sim 10^6$ for R6G on Au surfaces with $\lambda_g=200$ nm are consistent with numerical calculations of the electromagnetic field enhancement. The surface enhanced Raman scattering substrates presented here represent an important step forward in forming high-density arrays of nanostructures with large electromagnetic field enhancements, or hot-spots with high precision and reproducibility. Our SERS substrate integrated microarray system will be very convenient for molecular identification biosensing.

**ACKNOWLEDGEMENTS**

We thank Johan G. Bomer for technique assistance and discussions and Mark Smithers for making the high resolution SEM images.

**REFERENCES**


**CONTACT**

*E.T. Carlen, tel: +31 53489 5399; e.t.carlen@utwente.nl*