ABSTRACT
We report a microfluidic surface-enhanced Raman scattering (SERS) sensor for online monitoring the mixing and reaction between chemicals of low concentration. The SERS sensor is made up of a SERS-active substrate and a polydimethylsiloxane cap with microchannels for delivering solutions. The SERS substrate consists of noble-metal covered silicon nanopillar-forest patterns, which are fabricated based on an oxygen-plasma-treating-on-photoresist technique. Enhancement factor of the device is self-calibrated to be on the order of 5.2×10⁵, by utilizing the flat-metal areas beside the nanopillar-forest patterns as references. The mixing process of Crystal Violet and Rhodamine 6G trace solutions is characterized in the SERS sensor online and in real-time. Other kinetic features, including flow and diffusion, are also revealed.

KEYWORDS
Microfluidic surface-enhanced Raman scattering sensors; Chemical mixing and reaction; Silicon nanopillar forests; Oxygen-plasma-treating-on-photoresist technique.

1. INTRODUCTION
The manufacture procedure of pharmaceuticals [1], crystallization of drugs [2], and synthesis of polymers [3] involve processes of chemical mixing and reaction. A non-destructive analytical methodology, which is able to directly measure the processes of mixing and reaction, as well as the corresponding resultants, and at the mean time quickly provides feedback, can ensure final quality of products. Therefore, such an analytical approach is essential for fine control of the manufactory.

Since years ago near-infrared (NIR) spectroscopy was employed to monitor critical pharmaceutical process of raw materials [4,5], and it has become an on-line analytical tool with wide application fields. Compared with NIR spectroscopy, Raman spectroscopy possesses the features of fast response, simple or even no sample preparation, and non-destructive. It also offers a proportional relationship between signal intensity and the amount of material exhibiting vibrational shift, which is a critical measurement attribute for successful implementation as a process/product controlling technique. As a result, Raman spectroscopy has been employed as a tool of advantage for online monitoring chemical mixing and reaction processes [6]. However, the inherent feature of low sensitivity in Raman measurement sometimes hinders its implementations on analytes of low concentrations. Surface-enhanced Raman scattering (SERS) detection, which is applicable for minute quantity of analytes, paves the way of Raman spectroscopy to the realm of trace detection [7]. Recently, with advances in formation of novel materials, fabrication of innovative micro and nanostructures, and better understanding about the details of plasmonic functions, SERS is being increasingly used in diverse fields such as biomedical, agricultural and environmental analysis [8-10], in which the capability of trace and real-time detection is of great importance.

In this paper, a microfluidic SERS sensor with an enhancement factor (EF) on the order of 5.2×10⁵ is presented for online monitoring trace chemical mixing and reaction. In the sensor, different types of analytes can flow from different inlets to the same outlet through polydimethylsiloxane (PDMS) microchannels, which cover nanopillar-forest SERS-active substrate. With the help of mixing pillars in the central region of the microchannels, the fluids mix well with each other, which is proved by the SERS measurement results at different positions. Such a sensor combines the trace-detection capability of SERS effect with the advantages of online detection.

2. FABRICATION
The fabrication process for the SERS sensor is schematically depicted in Fig.1. To start the process, patterned silicon nanopillar forests are fabricated based on oxygen-plasma-treating-on-photoresist (OPTOP) technique (Fig.1 (a-1)-(a-6)) [11]. The detailed process are as follows: photoresist with a thickness of 1-2 microns is spin-coated on a silicon substrate (Fig.1 (a-1)); this is followed by photo-patterning of the photoresist layer, and simultaneously introduce a material distributed in random nanodots within the original pattern regions of the photoresist (Fig.1 (a-2)); subsequently, the nanodots of the material are employed as etching masks in deep reactive ion etching (DRIE) of silicon, leading to initial silicon nanopillar forests (Fig.1 (a-3)); after that, a spacer technique is adopted to adjust the dimensions of and spacing among the nanopillars, in the process of which silicon oxide is utilized as the sidewall material (Fig.1 (a-4)-(a-6)). After that, a noble-metal (e.g. Au) film is deposited and patterned on the nanopillar-forest and also some flat areas (Fig.1 (b))
to form a SERS-active substrate, where the flat-metal areas are used for self-calibration of EF (Fig.2 (a)).

At the same time, PDMS prepolymer and a curing agent are thoroughly mixed in a 10:1 weight ratio. Subsequently, the mixture is poured onto a silicon mold, on which the heights of the structures are 20 $\mu$m. The mixture is then cured in an oven for 60 minutes at a temperature of 60 $^\circ$C. After the PDMS is dried, it is peeled off from the substrate carefully, and thus a PDMS cap with microchannels and also mixing-pillars in the central region is achieved (Fig.2 (a)). Holes are then punched through on the PDMS cap at the endings of the microchannels, which will be utilized as inlets and outlets. After a short-time of oxygen-plasma treatment (e.g. 5 to 10 seconds) on the front surfaces of the open SERS-active substrate as well as the PDMS cap to make the surfaces adhesive, the two layers are bonded together thus to form a micro device. To reduce the flexibility of the PDMS layer and thus to reach a stronger bonding, the device is further baked at 120 $^\circ$C for few hours (Fig.1 (c)). Finally, plastic or metallic tubes are inserted into the inlets and outlet to finally achieve the microfluidic SERS sensor.

The schematic structures of the entire sensor are shown in Fig. 2. It can be seen that there are the mixing pillars in the central region of the microchannels, and there on the silicon substrate beside the nanopillar-forest patterns are flat-metal areas, which will be applied for self-calibration for the EF of the SERS sensor. Photos of the microfluidic SERS sensor, the patterned nanopillar forests and the Au-covered nanopillars are illustrated in Fig. 3.

3. MEASUREMENT AND DISCUSSION

A Raman system employing He-Ne 632.8 nm excitation laser was employed to measure the Raman spectra of analytes. In the measurement, a 50× microscopy objective lens was used for focusing the laser onto the sample on the surface of the substrates. Meanwhile, the backscattering of Raman photo within the detection regions were also collected by this same objective lens. The acquisition time for each spectrum measurement was 10 seconds. The whole measurement procedure was monitored by a charge coupled device.

Crystal Violet (CV) and Rhodamine 6G (R6G) molecules were employed as the SERS probes, and their solutions with different concentrations were prepared.
Firstly, R6G solution with concentration of $2.1 \times 10^{-6}$ M was injected into one of the SERS sensors by micro-sample injectors. Five to ten minutes later (wait for the sinking of molecules onto the substrate), the laser was focused onto the nanopillar-forest regions, and subsequently, Raman spectra of the R6G solution were obtained. The average spectrum is shown as the black curve in Fig.4. Later on, R6G solution with concentration of $4.2 \times 10^{-2}$ M was injected into another SERS sensor with the same structures. The Raman spectra of this solution on the flat-metal areas were also acquired, and the average spectrum is shown as the red curve in Fig. 4. Consequently, the EF of the sensor was self-calibrated to be $5.2 \times 10^5$ by comparing spectral intensities of the two solutions at different regions (the intensity difference of ~26 times at 1360 and 1510 cm$^{-1}$ was employed for the calculation).

Online monitoring of chemical mixing was realized using CV and R6G solutions. In the measurement, the CV and R6G solutions with $2.1 \times 10^{-6}$ M in concentration were delivered simultaneously into another SERS sensor from two different inlets (as shown in Fig.2 (b)). The flow rate and quantity of these two solutions were carefully controlled to be almost the same. With help of the mixing-pillars, the fluids mixed with each other in the central region of the microchannels, and then outflow through the outlet tube. The Raman system was then employed for online collection of Raman signals.

On the sensor, Raman spectra of the individual solutions and their mixture were detected at Position A, B and C (as shown in Fig.2 (b)), respectively. The measurement results are demonstrated in Fig.5. According to Fig.5, the spectra at Position A and B coincide with those of CV and R6G, which were illustrated in Reference [12]. Meanwhile, the spectra at Position C are in good accordance with the average spectra measured at Position A and B, demonstrating that, because of the mixing-pillars in the channels, the two different solutions are very well mixed, with almost 1:1 mixing ratio.

Some other kinetic features of the fluids, including flow and diffusion, which are of great importance for understanding microfluidics, are also able to be reflected in such a device. In one of the SERS sensors, soon after the CV and R6G solutions were injected into, spectra of the solutions were monitored one after another at 12 different positions from Position A to B along the microchannels. The measurement interval was about 30 seconds, including ~20 seconds cost for changing investigation positions and re-setting up optical focusing. At the beginning of the measurement, namely at Position A, only CV molecular characteristic peaks were observed. While as each measurement cost a period of time, on the R6G injection side, namely at Position B, some CV characteristic peaks were also observed.
indicating diffusion of CV molecules during the Raman spectra detection. The Raman spectra of the solutions are demonstrated in Fig.6, in waterfall format.

Figure 6. Spectra from A to B along the microchannel, in waterfall chat. Some CV molecular characteristic peaks are also observed in the R6G injection side, indicating diffusion of CV molecules during the Raman spectra detection.

As mentioned above, the injected solutions can be well mixed within the microchennals, and the process can be finely demonstrated. If the delivered solutions can react or have reaction functions with each other and thus lead to new products, e.g. the reaction function in the manufactory processes of certain drugs, and the reaction function between antigen and antibody, etc., the SERS sensor may also be able to monitor trace chemical reactions online. As shown in Fig.2 (b), when two reactant solutions (the reactants do not react with the materials related to the sensor, e.g. PDMS, gold and silicon) with low concentrations are injected into inlets 1 and 2, their corresponding spectra could be detected at Position A and B, respectively. Then, after the mixing, spectra of their resultants might be obtained at Position C. Meanwhile, the intermediate states of the reaction could also be monitored along the channels from A/B to C positions.

4. CONCLUSIONS

A microfluidic SERS sensor with an enhancement factor on the order of $5.2 \times 10^5$ has been presented. It employs noble-metal covered silicon nanopillar forest as the SERS-active substrate and a PDMS microchannel structure as the cap. Kinetic features of fluids including flow, diffusion, mixing and even reaction of different analytes’ solutions within the sensor could be monitored, which makes the SERS sensor an important analytical tool for online trace monitoring. It is expected that the sensor may be used for minute quantity antigen/antibody reaction and minute quantity blood detection for blood-related diseases in the future.

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