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Diagnostics

Tuberculosis determination using SERS and chemometric methods

Raju Botta[a,*], Pongpan Chindaudom[a], Pitak Eiamchai[a], Mati Horprathum[a], Saksorn Limwichean[a], Chanunthorn Chananonnawathorn[a], Viyapol Patthanasettakul[a], Benjawan Kaewseekhao[b], Kiatichai Faksri[b], Noppadon Nuntawong[a]

[a] National Electronics and Computer Technology Center (NECTEC), Phahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani, Thailand
[b] Department of Microbiology and Research and Diagnostic Center for Emerging Infectious Diseases (RCEID), Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

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ABSTRACT

Nanostructures have been multiplying the advantages of Raman spectroscopy and further amplify the advantages of Raman spectroscopy is a continuous effort focused on the appropriate design of nanostructures. Herein, we designed different shapes of plasmonic nanostructures such as Vertical, Zig Zag, Slant nanorods and Spherical nanoparticles employing the DC magnetron sputtering system as SERS-active substrates for ultra-sensitive detection of target molecules. The fabricated plasmonic nanostructures sensitivity and uniformity were exploited by reference dye analyte. These nanostructures were utilized in the label free detection of infectious disease, Tuberculosis (TB). For the first time, TB detection from serum samples using SERS has been demonstrated. Various multivariate statistical methods such as principal component analysis, support vector machine, decision tree and random forest were developed and tested their ability to discriminate the healthy and active TB samples. The results demonstrate the performance of the SERS spectra, chemometric methods and potential of the method in clinical diagnosis.

1. Introduction

In the past few decades nanostructures have attracted many attentions of researchers because of the characteristic and superior optical properties against the bulk materials. These are used in the wide range of fields such as clinical diagnostics [1,2], solar cells [3], drug delivery [4] and forensic sciences [5]. The prominent properties of the nanostructures are size dependent. There are different nanostructures such as nanoparticles, nanowires, nanorods, nanopores, nanoribbons, nanotubes, nanocubes and nanoscaffolds. Nanostructure exhibits the tunability of localized surface plasmon resonance (LSPR) which plays a crucial role in the Surface Enhanced Raman Scattering (SERS). SERS is the enhancement of the Raman signal by several orders of magnitude when molecules adsorbed on or in close proximity to the nanostructure surface. The basic inherent property of small scattering cross-section of Raman spectroscopy has been overcome by the discovery of SERS in 1974 (Fleischmann et al. [6]). Since, then it has drawn many attentions of the researchers throughout the globe due to sensitivity and selectivity of the technique [7]. SERS can detect the molecules at ultra-low concentrations and even single molecule level [8,9]. The large enhancement of the Raman signal can be explained by electromagnetic enhancement (EM) and chemical enhancement (CE) mechanisms. The EM enhancement is due to the optical properties of metal nanoparticles and the contribution is up to 10^8-10^{10} [10] and can also go to 10^{14} [11] at the hot-spots or junctions between the nanostructures. The CE is the charge transfer between the metal nanostructures and adsorbed species and the contribution is as high as $\sim 10^2$ [12]. Because of the large enhancement and fingerprint of the molecule it has many applications viz. analytical chemistry [13], art and archaeology [14], biological sciences [15,16], forensic sciences [17] and clinical diagnostics [18–20].

Various methods have been employed to get SERS-active substrates such as colloidal metal nanoparticles [21,22], lithography [23], laser ablation [24] and sputtering techniques [8,17]. Colloidal nanoparticle and laser ablation have the possibility of nanoparticle contamination. Even though, lithography is an efficient method to get uniform signal but lacks the abundant hot-spots on the substrates. Sputtering method is an efficient method to fabricate the SERS-active substrates and has the advantage of capable of producing contaminated free nanostructures. Most of the SERS studies signal intensities on the substrates compared with one laser excitation wavelength and some studies on comparison between the two or more different substrates [25–27] with one or two

* Corresponding author.
E-mail address: raju.botta@nectec.or.th (R. Botta).

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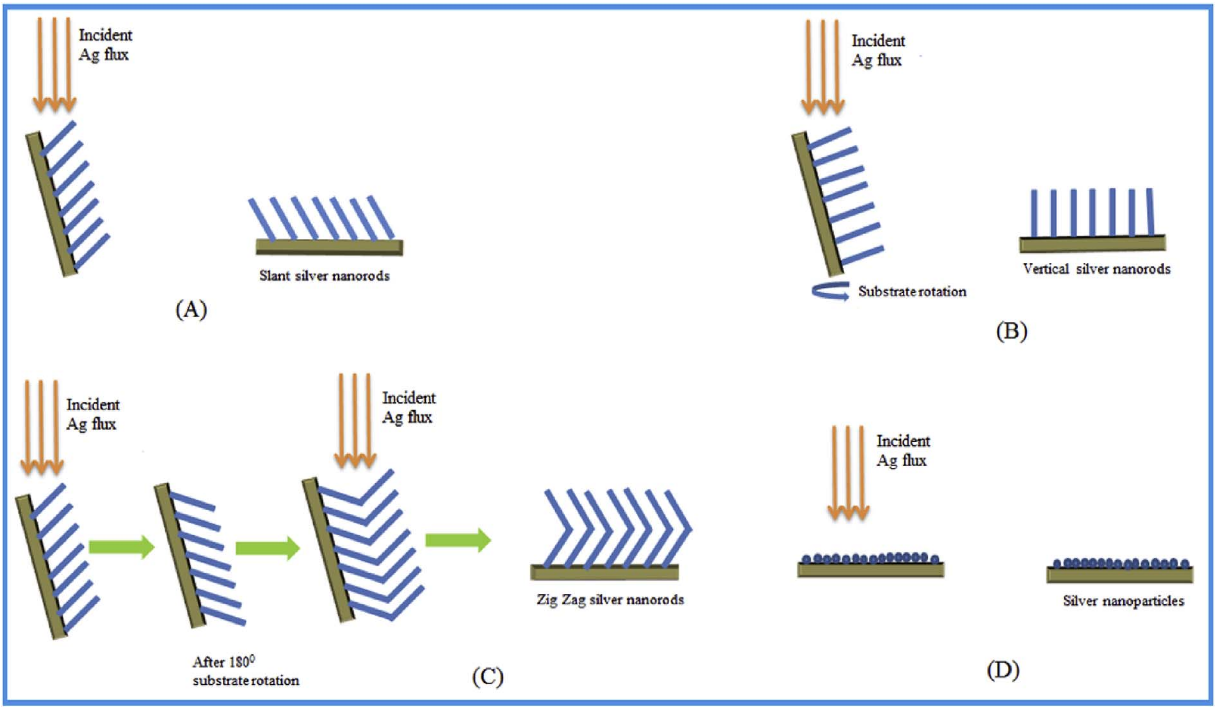

Fig. 1. Schematic diagrams depicting the major steps followed for the fabrication; (A) Slant silver nanorods, (B) Vertical silver nanorods, (C) Zig Zag silver nanorods and (D) silver nanoparticles.

wavelengths. To improve the SERS method further, it is necessary to fabricate the different plasmonic nanostructured SERS-substrates and study their signal influence or efficiency with different laser wavelengths.

TB is an infectious disease caused by *Mycobacterium Tuberculosis.* It affects mainly the lungs (pulmonary TB) and also the other parts of the body (extrapulmonary TB). TB is one of the leading causes of mortality and it was estimated by world health organization (WHO) that 10.4 M people are detected as active TB and about 1.4 M people died in 2015 [28]. It is more vulnerable in low and middle income countries. However, propagation of TB can be prevented by early diagnosis and treatments. Various methods have been used for diagnosis TB such as sputum smear microscopy (SSM), culture method, chest radiography, tuberculin skin testing etc. [29]. SSM is the front-line diagnostic technique for low-to-middle income countries where majorities (~80%) of the TB cases were found [30]. Poor sensitivity [31] and requirement of multiple sputum samples at different times are demerits of this method. Thus, SSM cannot be used for the reliable identification of early-stage infection. Even though, culture is the most sensitive method, it takes 12 weeks' time to get the results. In addition, it requires correct biosafety infrastructure. So, there is a very much interest to develop rapid and sensitive method to detect TB. Raman spectroscopy is an alternate nondestructive method in terms of its rapid and sensitive detection for TB sensing. In the previous literature, Raman spectroscopy has been used to detect TB from different samples like cerebrospinal fluid [32], 16S rRNA sequencing for the identification of M*ycobacterium tuberculosis complex* strains [29], tuberculosis and non-tuberculosis M*ycobacterial strains* extracted from lipophilic acid [33] and lab-on-chip SERS for identification of M*ycobacteria* [34]. For present study, serums were used as an alternative samples for the investigation of TB. The merit of this method is that many trace mycobacterial antigens are found in serum of TB-infected patients [35]. Further, developing the Raman spectroscope in clinical diagnosis, including diagnostic algorithms, various algorithms were reported. The algorithms like Linear discriminate analysis (LDA) [36–39], Principal Component Analysis (PCA) [40,41], Partial Least Squares (PLS) [42], Artificial Neural Networks (ANN) [43] and Support Vector Machines (SVM) [44], Ant colony optimization [45,46] have been successfully used in the analysis of Raman spectroscopy.

The present report discusses about fabrication of four different nanostructures (Vertical, Slant, Zig Zag nanorods and Spherical nanoparticle), sensing and chemometric methods. The SERS activities were comparatively exploited with all these four nanostructures. The important aspects in this report: (i) SERS performance studies using a reference dye as the analyte molecule with three different laser excitation wavelengths, (ii) applications of these nanostructures in biomolecule sensing and (iii) chemometric methods; such as PCA, SVM, decision tree analysis (DTA) and random forest (RF), are successfully applied to discriminate the healthy control subjects and TB-infected samples. To the best of our knowledge, this is the first attempt to use serum samples for the TB sensing.

2. Experimental techniques

SERS-active thin-film samples of vertical silver nanorod (AgNR), slant silver nanorod (SL AgNR), Zig Zag silver nanorod (ZZ AgNR) and silver nanoparticle (AgNP) structures were deposited on silicon wafers by a DC magnetron sputtering system. A 3-inch high-purity (99.99%) silver material (K.J. Lesker make) was used as a sputtering target. During the fabrications, a DC power supply at 0.4 A and 340 V was used to deposit the silver nanostructures, with deposition rate of ~1.0 nm/s, as calibrated by spectroscopic ellipsometer (J.A. Woollam; HTC-190).

2.1. Fabrication of nanorod and nanoparticle arrays

In our previous publication [17], it has been reported that at an oblique angle of 85°, nanorods with average lengths of 1000 nm produced intense Raman signal enhancement. Hence, same preparation conditions of 85° oblique angle and deposition time to get an average length of 1000 nm micron used to get nanorod structures. Schematic representation of fabrication procedure of different nanostructures was illustrated in Fig. 1. As shown in Fig. 1A and B, SL AgNR and AgNR structures were designed by placing the Si substrate with 85° oblique angle to the incident Ag flux and deposition time to get an average