

# Silver nanorod arrays can distinguish virus strains

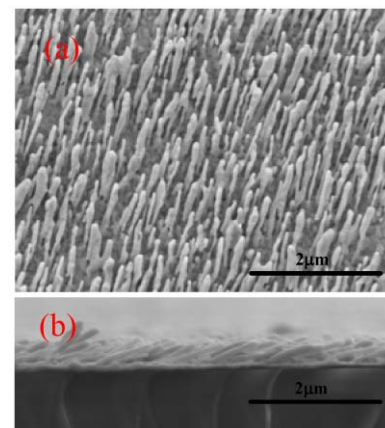
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*Substrates for sensitive surface-enhanced Raman spectroscopy are made from nanorod arrays prepared by oblique angle deposition.*

There is a critical need for a rapid and reliable means of diagnosing many infectious diseases, including viruses. The current state of the art for viral diagnostics involves one of three methods: immunofluorescent testing of isolated viral preparations; or an enzyme-linked immunosorbent assay, a method that uses antibodies linked to an enzyme whose activity can be used for quantitative determination of the antigen with which it reacts; or polymerase chain reaction, a method of amplifying fragments of genetic material so that they can be detected. These viral diagnostic methods are cumbersome, time-consuming, and have limited sensitivity. Nanotechnology-based biosensors, however, may be able to provide direct, rapid, and sensitive detection of infectious agents.

Among the biosensing methods being explored in virus research, surface-enhanced Raman spectroscopy (SERS) has received interest over the past few years due to its ability to detect even single molecules<sup>1,2</sup> at the same time as providing structural and quantitative information about the analytes. It has been used to detect bacteria<sup>3</sup> and viruses using either direct spectroscopic characterization or reporter-molecule-sandwich assemblies.<sup>4</sup> This remarkable analytical sensitivity has, however, for the most part not translated into the development of practical in situ diagnostic probes.<sup>5,6</sup> The technology has lacked a robust, simple, and reproducible procedure for preparing SERS-active substrates. Many different substrate fabrication methods, such as laser ablation,<sup>7</sup> chemical abrasion,<sup>8</sup> and deposition of metal colloid hydrosols,<sup>9,10</sup> have been developed, but none solves the problem completely.

We recently showed that a silver nanorod array fabricated using oblique-angle deposition (OAD) acts as an extremely sensitive SERS substrate, with enhancement factors greater than  $10^8$ .<sup>11,12</sup> OAD is a simple physical-vapor-deposition technique that overcomes some of the difficulties and disadvantages of the previously



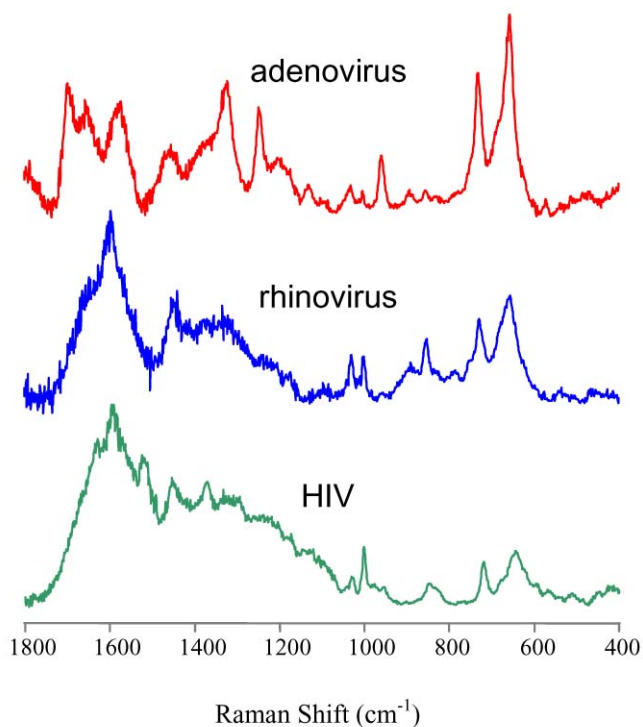
**Figure 1.** Two scanning electron micrographs (SEMs) of silver nanorod arrays show (a) a top view of the density of the rods, and (b) a cross-sectional view of the uniform slant of the rods.

mentioned methods. For the OAD method, we tilted the substrate such that the vapor arrives at close to the grazing angle. This process results in the preferential growth of nanorods on the substrate in the direction of deposition, due to a shadowing effect. Figure 1 shows scanning electron microscope (SEM) images of the optimal SERS substrates that we grew. The overall length of the nanorods was  $868 \pm 95$  nm, while the diameter of the nanorods was  $99 \pm 29$  nm. We calculated the density of the nanorods to be  $13.3 \pm 0.5$  rods/ $\mu\text{m}^2$  and the average tilt angle  $71.3 \pm 4.0^\circ$ .

The OAD technique offers a flexible and inexpensive method for fabricating integrated nanoproboscopes for high-sensitivity SERS applications. The SERS substrates produced by OAD have the advantages of large area, uniformity, and reproducibility. These novel substrates allow us to rapidly and cost-effectively develop SERS-based biosensors to detect extremely low levels of viruses.

We used our substrate to demonstrate that SERS can distinguish among different RNA viruses.<sup>13</sup> Figure 2 shows the SERS viral spectra of adenovirus, rhinovirus, and human immunodeficiency virus (HIV) after baseline correction. The spectra were

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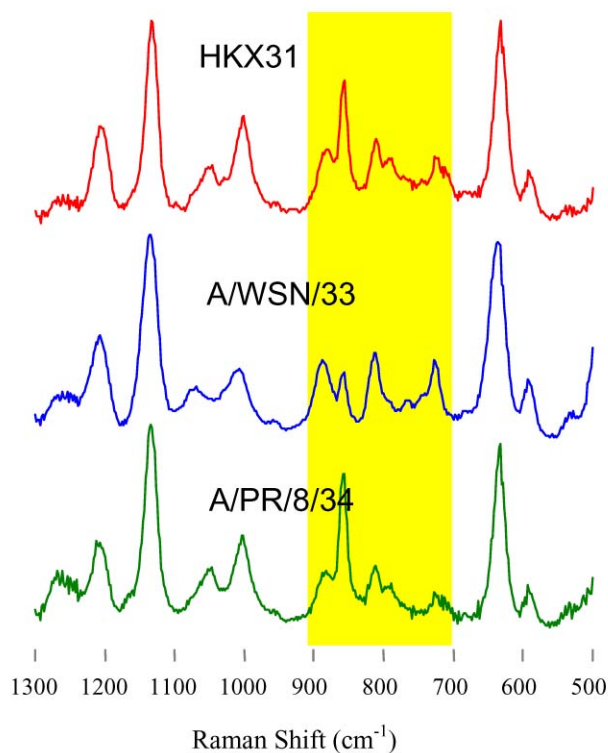


**Figure 2.** Spectra of different virus types were obtained using surface-enhanced Raman spectroscopy (SERS) performed with the viruses on Ag nanorod substrates.

collected within 1min at a virus volume smaller than 5 $\mu$ l. The spectra collected from multiple spots on the substrate were similar except for minor differences in relative bandwidths and intensities. These results demonstrate that SERS can be used to establish reproducible molecular fingerprints of several important human respiratory viruses, as well as HIV. These results highlight the potential of this detection method for important pathogenic viruses central to human health care.

We also analyzed the sensitivity of SERS to viral strain variations from a single pathogen. We obtained the SERS spectra for three influenza A strains: A/HKx31, A/WSN/33, and A/PR/8/34 (see Figure 3). We found that common features are sufficient to distinguish all the influenza types from the other viruses, yet detailed analysis also allows strains to be distinguished from each other.

In summary, we have developed an easily implemented nanofabrication method, OAD, to produce Ag nanorod array surfaces that allows us to obtain SERS signals with high sensitivity. The SERS enhancement factors can routinely approach  $10^8$ . Experiments on various virus samples show that Raman spectra of viruses can be used to rapidly and readily distinguish between viruses, and can serve as molecular fingerprints for classifying and identifying viruses. Compared with current state-of-



**Figure 3.** The SERS spectra of different strains of influenza virus, also obtained using the Ag nanorod substrates, were all recognizably influenza. The highlighted section allowed us to distinguish the different strains.

the-art virus detection, the speed, specificity, and relative ease of implementation of the SERS technique make it a highly promising alternative to current viral diagnostic tools and methodologies, and offers the possibility of designing new virus-detection schemes. By further tuning the structural parameters of the Ag nanorod arrays, we can develop optimal SERS substrates for detecting other chemical and biological molecules—such as explosives, toxins, or food contaminants—or for environmental monitors.

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