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COVID-19 detection using SERS technique

Abstract

Using Surface-enhanced Raman spectroscopy (SERS) can be a more promising choice for obtaining a faster detection of COVID-19 than the PCR. PCR protocol amplifies the single segment of DNA to 100 billion copies after 40 doubling cycles to provide sufficient intensities of fluorescent signals for virus detection, which takes hours. In this paper, we proposed that the enhanced signals of SERS, which can identify single nucleotide polymorphisms and unique gene sequences, can provide a more economic and accurate detection that does not require the long time needed for DNA amplification of COVID-19. Our proposed diagnostic tool is based on technology of single molecule SERS which checks if the Raman shift of the sample signal matches with the recorded one of COVID-19. In addition, its portability property provides the allowance of its usage in places other than labs and healthcare facilities. Therefore, the superiority of using SERS over fluorescence is manifested in the acquirement of a few-minute detection of coronavirus by the action of a SERS laser rather than the long and expensive measures taken in PCR.

Keywords: SERS, COVID-19, single molecule, DNA, RNA, PCR, raman

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Caroline Magdy,[1] Fatma Issam,[1] Pierre Amir,[1] Sandra Adel,[1] SherifH. ElGohary[1,2]

[1]Healthcare Engineering and Management Department, Cairo University Faculty of Engineering, Egypt

[2]Department of Biomedical Engineering and Systems, Cairo University, Egypt

Correspondence: SherifH. ElGohary, Department of Biomedical Engineering and Systems, Faculty of Engineering, Cairo University, 12613, Cairo, Egypt, Email sh.elgohary@eng1.cu.edu.eg

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Introduction

Acquiring and expanding the knowledge of any biological molecule has been the most sought after by researchers, scientists, and experimenters. Going through this endeavour prompts the invention, study, and utilization of tools and techniques that assist in comprehending the biological molecules of anything. Advances in technology caused the accomplishment of Raman spectroscopy, one of the most effective tools used in detecting materials. Raman spectroscopy is a scientific technique that detects, examines, and analyses the chemical composition of various substances through interaction of light with matter. It is able to present qualitative and quantitative information about the sample's chemical composition, polymorphism, phase, crystallinity, stress/strain, and contamination & impurity. Its mechanism is profoundly based on the Raman principle that was originally named after and discovered by the Indian Scientist C.V Raman, who won the Nobel prize after the revelation of the Raman effect. Over the past decades, Raman spectroscopy has been prominently exploited better in biological applications, where it is able to detect and analyze DNA and RNA molecules. Generally, there are four main types of Raman spectroscopy, but the most feasible in biological field is the surface enhanced Raman spectroscopy (SERS). SERS was notable in biosensing and was proven to detect several viruses like avian influenza A virus (AIV) and echovirus. SERS is expected to cause a revolutionary breakthrough in the virology field if it is able to detect today's notorious and deadly virus: COVID-19. COVID-19 is under Coronaviruses (CoV), which is a large group of analogous single-stranded RNA viruses that result in respiratory infections in humans. Basically, COVID-19 originates from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The family of CoV have similar structures that mainly include the capsid (a viral protein shell) that envelopes the viral nucleic acid, single stranded RNA with positive polarity (+ssRNA). CoVs consist of four proteins: Spike/surface glycoprotein (S), membrane protein (M), envelope protein(E), and nucleocapsid protein (N). What differentiate COVID-19 from other CoVs is the regions of S-protein in its amino acid sequence. Thus, the viral genome is crucial in the process of its detection. In this paper, we will use the single molecule SERS in detecting COVID-19 instead of the PCR technique.

Methodology

Basic interaction with light

Absorption and emission

Spectroscopy is the science that analyses the optical spectrum emitted by a probed matter due to its interaction with light. The origin of this spectrum is related to the molecular composition and the energy levels within, involving electronic energy levels and vibrational ones contained in them. Excitation takes place when the molecule absorbs a photon whose energy sufficiently equals a difference between two energy levels. An electronic absorption is when absorbed photon is in the UV or visible light range (i.e. excitation from S0 to S1) and its spectrum gives information about the electronic energy levels of this molecule. The vibrational absorption is when absorbed photon is in the IR or far IR range (i.e. excitation from $\nu=0$ to $\nu=1$) and its spectrum provides insights of the vibrational energy levels. Spontaneous Emission is when the molecule naturally relaxes and emits a photon whose energy equals the difference between energies of the excited and the relaxed states. Generally, a Radiative Transition happens when the state of the molecule changes due to its interaction with a photon. Non-Radiative Transition occurs among vibrational levels only, and results from the tendency of the molecule to re-distribute its energy to ensure its relaxation.

Molecular spectrum phenomena

Fluorescence

Fluorescence takes place when the molecule is excited and experiences an electronic transition (S0 to S1). The electron, then, undergoes non-radiative transitions in the sub-structure of S1 until it reaches $\nu=0$. After that, it goes through a radiative transition and reaches a certain vibrational energy level in S0 and a photon is emitted whose energy is equal to this transition. We obtain a spectrum of the fluorescence phenomenon, as the intensities of fluorescent photons are recorded against their wavelength, which reveals the electronic and vibrational structures of the molecule. The peak of the fluorescence spectrum is located at a longer wavelength than that of the absorption spectrum as the fluorescent photons are lower in energy than the absorbed ones (Figure 1).

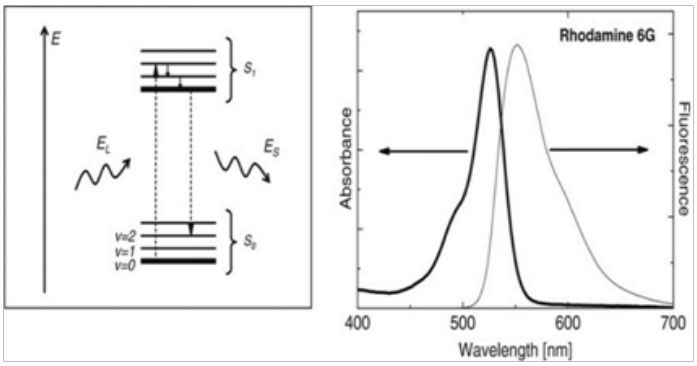

Figure 1 Simplified jablonski diagram of fluorescence. Absorption and Fluorescence Spectra of Rhodamine 6G Dye.[1]

Scattering process

Elastic and inelastic scattering

Elastic scattering (Rayleigh Scattering) is when the incident photon is absorbed and scattered without any transfer in energy between the molecule and the photon, and the excited atom returns to its initial vibrational level. This scattering type does not give any significant information about the internal structure of the molecule. Inelastic scattering is when the scattered photon is higher or lower in energy than the incident photon. The excited atom does not return to its initial vibrational level, and a transfer of energy between the incident photon and the molecule occurs which gives information about its inner composition.

Raman scattering and fluorescence

Raman Scattering (Inelastic Scattering) is like fluorescence, as in both the absorption of a photon occurs and is followed by the emission of a scattered or a fluorescent photon but there are major differences between them. Firstly, absorption and emission in scattering are instantaneous, unlike fluorescence which includes the intermediate step of the non-radiative transitions in S1. Secondly, the energy needed for excitation in scattering may not be equal to a difference in the energies of two electronic levels, and the photon absorbed results in the vibration of the atom to a virtual energy level, whereas in fluorescence, the transition must be electronic. In result, the energy required for scattering is less than that required for fluorescence so as to ensure that the vibrating atom does not reach the next electronic energy level.

Raman scattering

Stokes and anti-stokes

If the atom is in the ground vibrational state v=0, then is excited to a virtual energy level and relaxes to v=1, Stokes Raman Scattering occurs, and the scattered photon has less energy than the incident photon. In contrast, if the atom is already excited in the vibrational level v=1, then is excited again to a virtual energy level and relaxes to v=0, Anti-Stokes Raman Scattering takes place.

Raman shift

A resulting Raman spectrum can be obtained in which the intensity of the scattered photons is recorded against the Raman Shift. The Raman shift is defined as the energy lost by the photons during Raman scattering and is positive for the Stokes process in which the photon loses energy and negative for the anti-Stocks where the photon gains energy, and it is expressed in wave-numbers.

Raman spectrum

The Raman spectrum is incredibly significant in revealing the vibrational structure of the molecule as the Raman shift of a peak is equal to the vibrational energy corresponding to a specific vibrational transition. The Raman intensity varies from transition to transition and the intensity at the anti-Stocks side of the spectrum in less than that at the Stocks side, as in anti-Stocks, the atom must be previously excited at v=1 which can only happen through thermal excitation. According to Boltzmann factor, the majority of the atoms are normally distributed in the ground state with a small minority in the excited state, so the efficiency of the Stocks is greater than that of the anti- Stocks. In all cases, it can be concluded that the Raman spectrum provides a characteristic fingerprint for the molecule (Figure 2).[1–9]

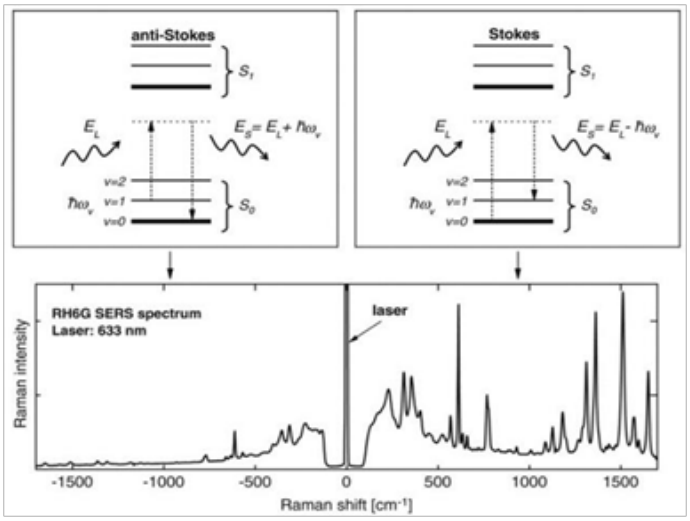

Figure 2 Simplified jablonski diagram of stokes and anti-stocks raman scattering. raman spectrum of rhodamine 6G Dye.[1]

Surface enhanced raman spectroscopy (SERS)

One method to amplify weak Raman signals is to utilize surface-enhanced Raman scattering (SERS). Surface enhanced Raman scattering (SERS) is a sensitive technique which offers orders of magnitude increases in Raman intensity, overcoming the traditional drawback of Raman scattering as the Raman signals are inherently weak, especially when using visible light excitation as a low number of scattered photons only are available for detection. The term surface enhanced Raman spectroscopy shows that it provides the same information that traditional Raman spectroscopy does, simply with a greatly enhanced signal. Enhancement factors can be as much as 10,[10,11] which are sufficient to detect even single molecule using Raman without the need for fluorescent labels. It enhances Raman scattering by molecules adsorbed on a plasmonic nanostructure rough metallic surface. Typical metals used are gold or silver, preparation of the surface can be through electrochemical roughening, metallic coating of a nanostructured substrate, or deposition of metallic nanoparticles (often in a colloidal form).

It is necessary to use a laser of wavelength suitable for the chosen

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SERS metal, but rather than this there are no major difficulties. SERS spectra sometimes differ from a normal Raman spectrum of the same material, so analysis of data must be considered. The choice of surface metal is also determined by the plasmon resonance frequency. Near infrared radiation (NIR) and visible are used to excite Raman modes. Silver (Ag) and gold (Au) are typical metals for SERS experiments because their plasmon resonance frequencies fall within these wavelength ranges, providing maximum enhancement for visible and NIR light. Copper's absorption spectrum also falls within the range suitable for SERS experiments. Platinum and palladium nanostructures also shows plasmon resonance within visible and NIR frequencies. But in general, Au and Ag are most often used as SERS substrates because they are air stable materials (Figure 3).

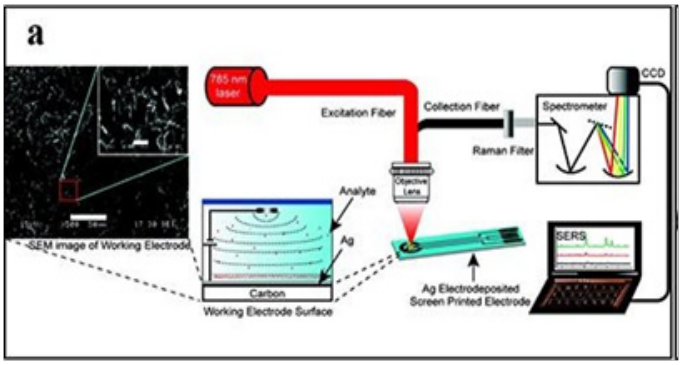

Figure 3 Surface enhanced raman spectroscopy.

Single-molecule surface enhanced raman spectroscopy(SM-SERS)

SM-SERS allows the detection of one molecule within a crystal or a cell through optical excitation. Single- molecule spectroscopy (SMS) can image, down to nanometers, at subwavelength scales. SM-SERS was achieved due to the presence of hot spots, which are regions with highly concentrated plasmon emitting to provide a significant electromagnetic coupling effect among particles, usually the gap junctions between metallic nanostructures. More essential is the distribution of hot spots, as they should be uniform enough to produce a reproducible SERS signal. Figure 4 Shows a diagram of a typical single molecule SERS experiment performed in a colloidal solution of silver or gold. Spectra are excited by a cw Ti: sapphire laser operating at 830 nm. A microscope attachment is used for laser excitation and to collect the scattered light of the Raman. The analyte is added to a solution of small colloidal clusters of silver or gold. Nanocluster concentration ratios and target molecules of at least 10 make it unlikely that more than one analyte molecule can attach to the same colloidal cluster, preventing the formation of target molecule aggregates on the surface.

Mechanism

The main enhancement mechanisms for SERS include chemical enhancement (CM) and electromagnetic enhancement (EM), with the EM playing a predominant role in enhancement. The total SERS enhancement factor is the product of the chemical and electromagnetic enhancement mechanisms.

Electromagnetic enhancement (EM)

Generally, it is now agreed that the dominant contributor to most SERS processes is the electromagnetic enhancement mechanism. When the incident light strikes the surface, localized surface plasmons are excited. Free Surface plasmons can be set to oscillate collectively by light with a suitable frequency, causes a redistribution and non-focusing of density of the photon energy at certain areas across the surface. This light concentration occurs in the gaps or sharp features of plasmonic materials. Based on this, SERS substrates usually support plasmonic fields and enhance the Raman signal with great sensitivity. The unique advantages of SERS include the enormous multiplexing capacity for simultaneous target detection due to the narrow width of the vibrational Raman bands, quantification based on a specific SERS fingerprint of the corresponding labels, conformation and structural studies of the targets. More importantly, only a single laser excitation is necessary to excite the Raman reporters for multiplex detection compared with the multiple wavelengths necessary for multiplex fluorescence signal. Owing to the high sensitivity, less interference from the environment and amplified fingerprint of the SERS signals. The SERS effect is so recognizable because the field enhancement occurs twice. First, the field enhancement magnifies the intensity of incident light, which will excite the Raman modes of the molecule, therefore increasing the signal of the Raman scattering. Second, The Raman signal is then magnified by the surface due to the same mechanism that excited the incident light, resulting in increasing the total output.

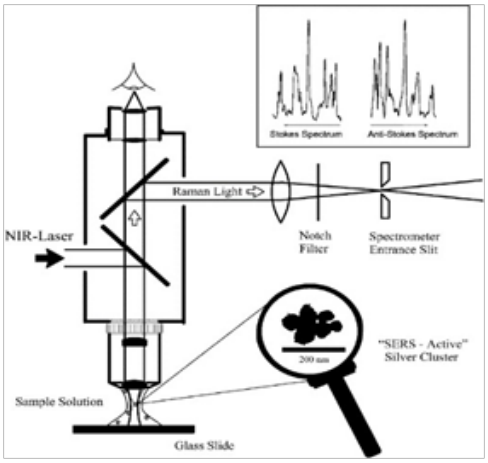

Figure 4 Schematic diagram of a typical single-molecule SERS experiment, copyright 2002 institute of physics publishing, Ltd.

Chemical enhancement (CM)

The other mechanism involved in signal enhancement is chemical enhancement, which primarily involves charge transfer mechanisms between the adsorbed molecule and the surface of the metal, where the excitation wavelength is resonant with the molecule and metal charge transfer electronic states. It is believed to arise from the formation of active Raman intermediates between the molecule and the metal surface which give rise to enhanced Raman Scattering as they are able to couple resonantly with the excitation light (Figure 5).

Results and discussion

Selective fluorescence signal is a key method in molecular biology. It is used in real-time PCR, which is the standard method for sequence-specific DNA detection and quantification. PCR is used to reproduce and amplify selected sections of DNA or RNA. PCR done in test tubes, it takes only a few hours. The experimental PCR protocol was as

follows: an initial 50 min at 30°C and 2 min at 68°C for RT, followed by 40 cycles of 45 s of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of extension at 68°C. The detection limit is 400 genome equivalents per ml of serum. This cycle is repeated about 40 times in a machine termed a thermal cycler that repeats the heating-cooling cycles, with the amount of each DNA sequence doubling each time the heating-cooling cycle is completed. What initially was a single segment of DNA can be amplified to about 100 billion copies after 40 doubling cycles. The fluorescence dye need amplified DNA to produce fluorescent signals with high intensity which tracks its quantity within the sample after each cycle. When the quantity goes over a particular level of fluorescence, this confirms that the virus is present While SERS is using ramen scattering technique that needs single molecule only by selecting the SERS laser on it which gives us a high intensity signals that can be analyzed within minutes as it doesn't need any amplification of the RNA or DNA.[12–23]

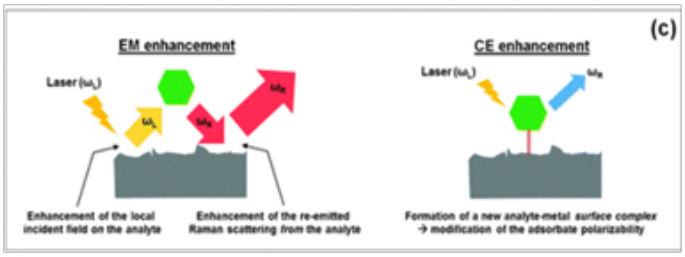

Figure 5 Electromagnetic and chemical enhancement.

Detection of DNA and RNA

SERS substrates are used to detect the presence of biomolecules with low concentration, the ability to identify the composition of a mixture on the nanoscale makes the use of SERS substrates beneficial. In particular, it is reasonable to say that SERS has enhanced biology. For example, the detection of proteins, DNA and RNA, observe targeting is achieved due to the unique advantage of SERS over fluorescence in biology, including multiplex detection, photostability, and especially the optimum contrast provided by the use of red to NIR excitation to reduce auto fluorescence from biological species such as blood, tissue, and cells (Figure 6).

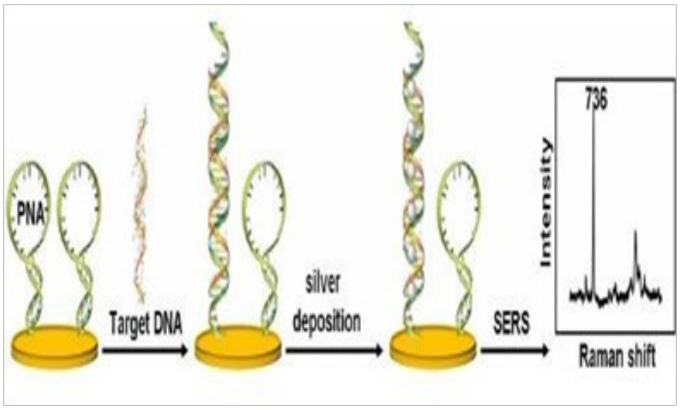

Figure 6 surface enhanced raman spectroscopy detection of DNA-silver metallization.

Oligonucleotide targeting

SERS used a combination of gold and silver nanoparticles and Raman-active dyes, such as Cy3, to target specific DNA and RNA sequences. Using this technique, Specific single nucleotide polymorphisms (SNP) can be identified. The gold nanoparticles facilitate the formation of a silver coating on the DNA or RNA dye-labeled regions, so that SERS can be performed. This has several potential applications: for example, gene sequences can be uniquely identified using this technique for HIV, Ebola, Hepatitis, and Bacillus Anthracis, so we can also use it to identify the coronavirus. Each spectrum was specific, which is advantageous over fluorescence detection; some fluorescent markers overlap and interact with markers of other gene. The advantage of this technique to identify gene sequences is the commercial availability of several Raman dyes which could lead to the development of non- overlapping gene detection probes. Such as antibodies, aptamers and DNA, SERS labels are more beneficial in ultrasensitive detection. The detection of protein, DNA and other components is based on SERS labels. Quantitative SERS for gene expression was also carried out using Raman multiplexers developed for alternative gene splicing. Screening based on SERS labels shows excellent promise in rapid multiplex detection and targeting as shown in Figure 7.

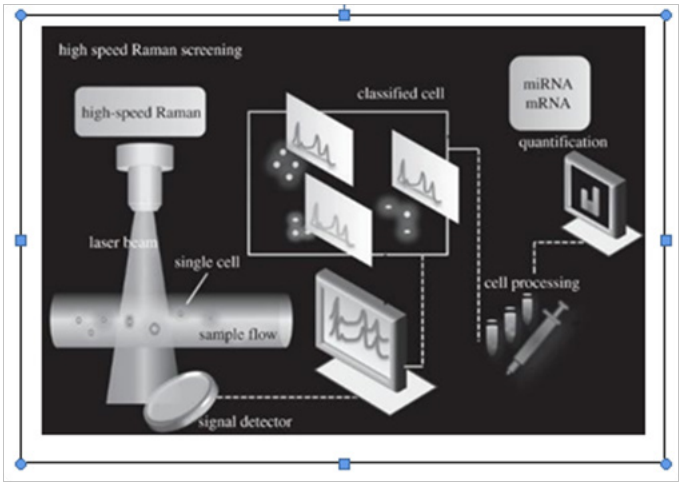

Figure 7 SERS-based high-speed Raman for simultaneous sorting of cells based on cellular fingerprint and SM quantification of targets.

Portable device for coronavirus detection

The necessity or lack of something prompts us to think different, propose, develop, design, and enhance. Thus, innovation and development become the fruit of deficiency and urgency. The recent pandemic of COVID-19 is menacing and needs efficient, effective, and accurate measures and methods to be taken. As mentioned previously, although PCR is today's sole technique used for identifying SARS-CoV-2, the shortcomings of the technique are inevitable starting with its tendency to obstruct DNA amplification till its time-consuming and strenuous process. Accordingly, we visualize a promising diagnostic tool for detecting SARS-CoV-2 more efficiently. In accordance to what was previously discussed about SERS's distinguished ability in detecting a viral DNA or RNA, our proposed tool will be based on the rapid and highly sensitive technology of single molecule SERS. It would compare the resulting signal with the recorded one and examine the match to provide an accurate detection. The DNA sample, to be tested, after opening the device it is applied over the chip that contains gold or silver. It is exposed to the Laser that coming from the Device upwards to the chip and to the sample. The device offers portability and inexpensiveness, for it can be accessible,

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feasible, and utilized countless times by anyone to test for infection. The asset of a portable device permits testing for COVID-19 to be used widely in any community, for instance in rural areas, schools, and work facilities, by anyone. Furthermore, this handheld device enables users to maintain it easily. It also features data and analysis of the results on a touch screen with buttons for alternative transitions. This data is the Raman shift if it matches with the one saved on the Device therefore the patient tested positive otherwise he tested negative. Another feature in the design of our SERS based diagnostic device is an apparent USB slot to permit exchange of data between the device and any phone/computer. We foresee that this SERS based diagnostic device can emerge as a powerful, revolutionary tool in the detection of SARS-CoV-2 efficiently and accurately.

Conclusion

During this time, we pass through a pandemic that reap thousands of people's life every day; accordingly, the inefficiency and the long time taken by PCR tests to indicate the presence of the virus cannot be tolerated. Hence, we strive to use non-traditional technique that detect the virus in infected patients and show an alternative method based on recent advances in physics related to the interaction of light with matter. That alternative is based on the development of a new test technology for SARS-CoV-2 using single molecule surface-enhanced Raman spectroscopy (SM-SERS). Throughout the paper, we start our discussion about the light interaction then the surface enhanced Raman scattering (SERS) and its mechanism, as SERS is a technique that is very convenient in various fields especially in the medical field. Our discussion leads to the development of the SERS technique to detect only a single molecule, select a laser ray on a sample taken from a patient, use the silver and gold nanoparticles as dye to coat the DNA to produce signals, and finally analyze the signal to give the Raman fingerprint of COVID-19's DNA. This method that is implemented in our device, shown in Figure 8, would facilitate testing if the patient is carrying the virus or not within minutes, resulting in the decrease of the spread of the virus and increase of people's survival rates.

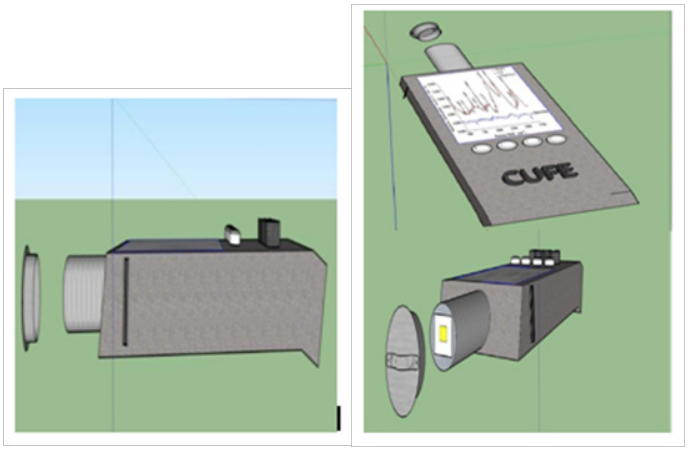

Figure 8 Our portable SERS device for coronavirus detection.

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None.

Conflict of interest

The authors declare, that there is no conflict of interest.

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