



# Magnetically Enhanced Liquid SERS for Ultrasensitive Analysis of Bacterial and SARS-CoV-2 Biomarkers

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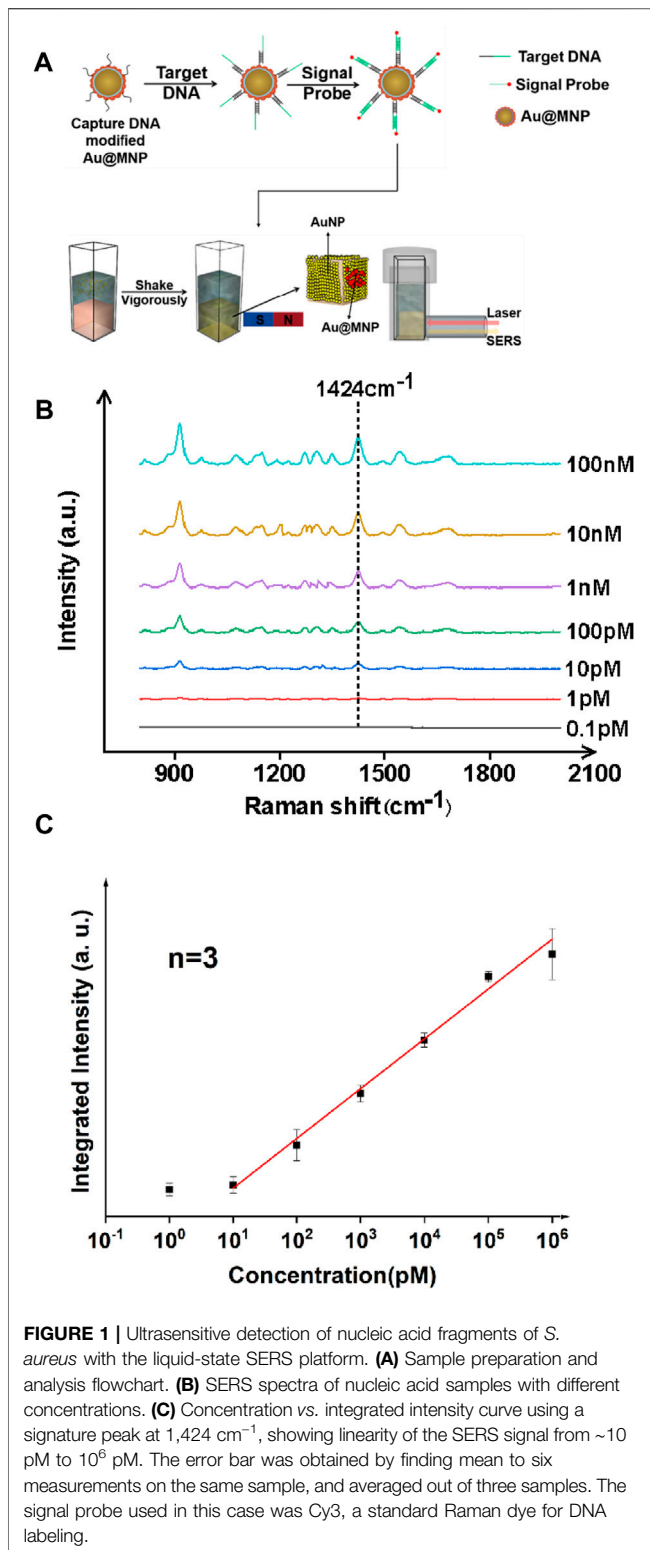
In this work, it is shown that surface-enhanced Raman scattering (SERS) measurements can be performed using liquid platforms to perform bioanalysis at sub-pM concentrations. Using magnetic enrichment with gold-coated magnetic nanoparticles, the high sensitivity was verified with nucleic acid and protein targets. The former was performed with a DNA fragment associated with the bacteria *Staphylococcus aureus*, and the latter using IgG antibody, a biomarker for COVID-19 screening. It is anticipated that this work will inspire studies on ultrasensitive SERS analyzers suitable for large-scale applications, which is particularly important for *in vitro* diagnostics and environmental studies.

**Keywords:** SERS, COVID-19, SARS-CoV-2, bacteria, analysis

## INTRODUCTION

Surface-enhanced Raman scattering (SERS) spectroscopy holds promises for ultrasensitive detection of analytes in complex environments (Ding et al., 2016; Langer et al., 2020). This is of significance for two reasons: First, disease-related biomarkers are often present at concentrations lower than the limit of detection (LoD) of existing techniques (Wu et al., 2019). Examples of such samples include circulating tumor biomarkers (e.g., microRNA and cells), proteins, exosomes, bacteria pathogens, and SARS-CoV-2 biomarkers in blood (Mattioli et al., 2020; Jiang et al., 2020a; Sitjar et al., 2021). Second, SERS is capable of detecting single molecules (Bell et al., 2020; Zheng et al., 2015; Kneipp et al., 2006), and as a vibrational spectroscopic tool, the Raman spectra can provide molecular fingerprints of the sample that is highly specific (Zheng et al., 2013; Zheng et al., 2015; Ye et al., 2019; Langer et al., 2020; Mattioli et al., 2020), the signal of which is significantly enhanced when performed in ultraviolet (Renard et al., 2019; Chen et al., 2021). The dual sensitivity/selectivity of SERS is desirable for several applications, particularly *in vitro* diagnostics and environmental monitoring (Long and Gooding, 2016; Liu et al., 2017; Wei et al., 2017; Zhang et al., 2018; Chen et al., 2020a).

One goal for SERS is ultrasensitive quantification of rare species (e.g., sub-pM) using techniques that are both accessible and reproducible (Joseph et al., 2012). Conventional SERS measurements typically require substrates made with, for example, electron beam lithography, self-assembled nanoparticle arrays on solid support, or simply mixing analytes with nanoparticle suspensions (Ciallam et al., 2012; Thai et al., 2012). Among them, nanofabrication produces well-defined substrates but is challenging to manufacture at large scale (Ying et al., 2008). Colloidal suspensions are attractive for their straightforward production, but the resulting organization of the substrate can be poor due to the random structures formed (Banholzer et al., 2008).



Self-assembled super-lattice arrays on solid surfaces are promising for their reasonably good organization and easy fabrication (Thai et al., 2012; Ye et al., 2019). Issues remain in developing SERS-based detection to address real-world problems,

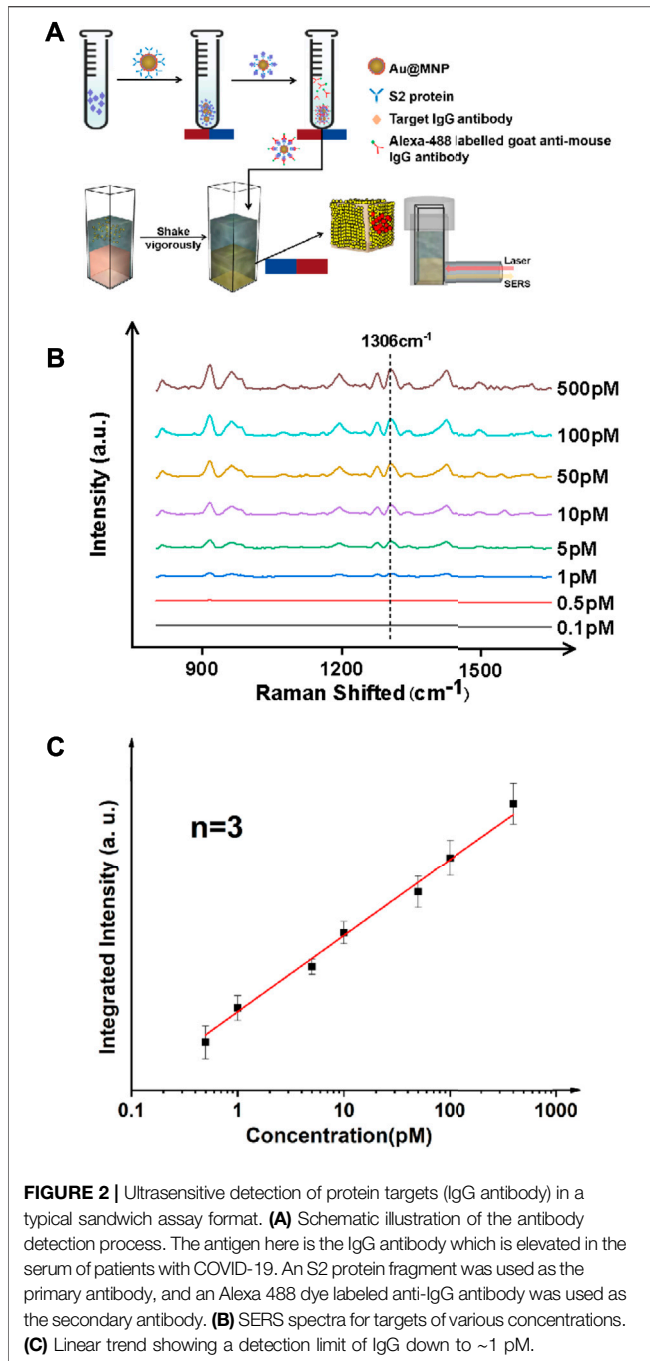
where factors such as sensitivity, specificity, cost of the instrument, and reproducibility of the assay should all be addressed to meet production requirements and analytical standards (Fan et al., 2020). Of interest is recent studies showing that well-defined SERS substrates can be produced directly in the liquid-state *via* self-assembling gold nanoparticles between the phases of immiscible solvents (Ma et al., 2016; Tian et al., 2018; Du et al., 2019; Su et al., 2019). This is attractive as gold colloids can be obtained easily, and it provided a simple and reproducible method to perform SERS at minimal cost. This was suitable for large-scale applications which predominantly rely on solution-based measurements.

In this work, we demonstrate that the limit of detection (LoD) of liquid interfacial SERS can be improved by three orders of magnitude to allow pM-level (parts per trillion) immunosensing. We show that using surface-modified, gold-coated magnetic nanoparticles (Au@MNPs), nucleic acid and protein targets with concentration lower than that of pM could be quantified. Protein assays were performed using IgG antibodies, a biomarker elevated in patients infected with SARS-CoV-2 viruses (Yao et al., 2020). The results were compared with paper-based lateral flow assays which are currently used for COVID-19 antibody screening (Chan et al., 2020). The experimental results were supported with a simple electromagnetic (EM) simulation, showing significantly enhanced SERS signals in the gap formed between the Au@MNP and the substrate. The current work demonstrated that magnetically enhanced liquid SERS could be used to detect analytes of ultralow abundance, which is pertinent for clinical and environmental applications, such as *in vitro* diagnostics and water quality monitoring (Beveridge et al., 2011).

## RESULTS AND DISCUSSIONS

### Analysis of *Staphylococcus aureus* Nucleic Acid

The synthesis and characterization of gold-coated magnetic nanoparticles is discussed in detail in the supporting information. In short, Au@MNPs were prepared *via* typical solution-based approaches *via* electrostatic adsorption, and then coated with DNA to form well-organized self-assembled monolayers on the surface. The performance of magnetically enhanced liquid SERS was first tested for a nucleic acid target. In this work, *Staphylococcus aureus* (*S. aureus*) was chosen, as it played a critical role in cross-infections among hospitalized patients (Pazos-Perez et al., 2016). A three-fragment assay was performed (Figure 1A), in which single-stranded DNA (surface strand, 20 bases) was attached onto Au@MNPs *via* gold-thiol bonding using freeze-induced surface modifications (Liu and Liu, 2019). The Au@MNPs were then coated with polyethylene glycol to reduce nonspecific adsorptions, after which target strands (*S. aureus* DNA fragments, 40 bases) were added. The sticky end of the target DNA was complementary to the signal probe strand labeled with Cy3 (20 bases), a standard dye for SERS analysis. In this way, thermodynamically stable, double-stranded surface geometry can be formed when the target strand was present.



The SERS spectra were measured for targets at various concentrations, as shown in **Figure 1B**. Analysis was performed in solution when incubating Au@MNPs with the target strands prior to applying the magnetic field. Quantification was performed using an excitation source of 785 nm continuous wave laser (power: 100 mW, integration time: 15 s) and signal recorded with a Raman fiber spectrometer. Using a signature peak of the Cy3 dye at  $1,424\text{ cm}^{-1}$ , which corresponds to the C-H in-plane deformation mode (Li et al., 2020a), a linear trend between the concentration of the nucleic acid and peak intensity was observed in the dynamic range between  $10\text{ pM}$  and  $10^6\text{ pM}$ , with a linear

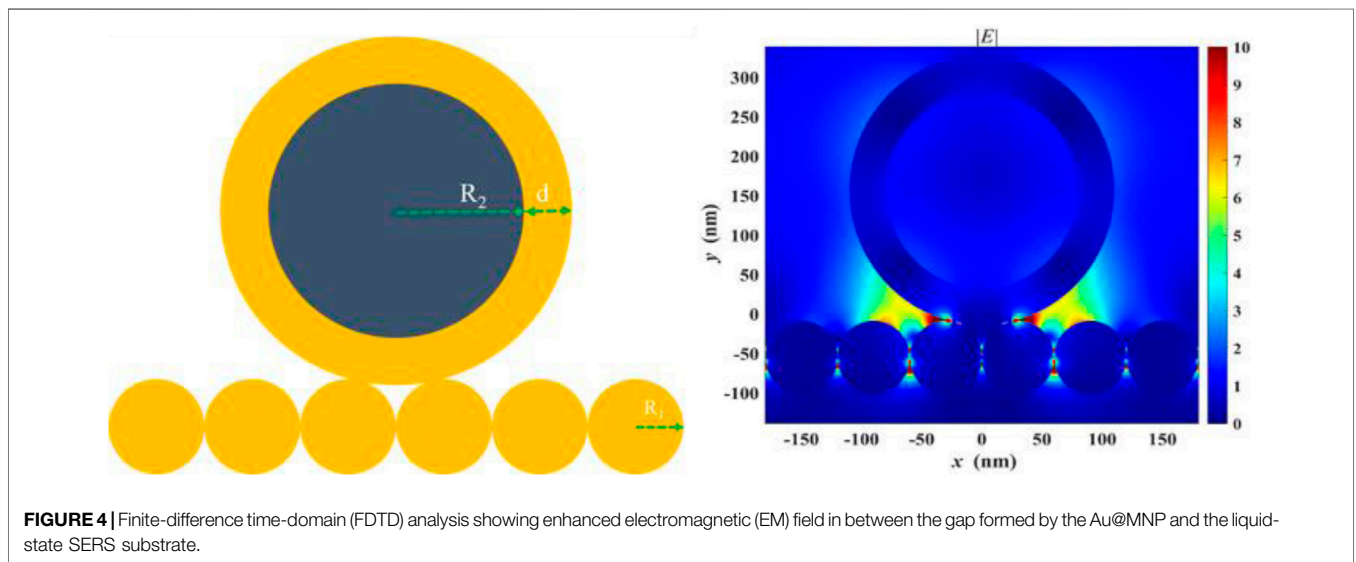
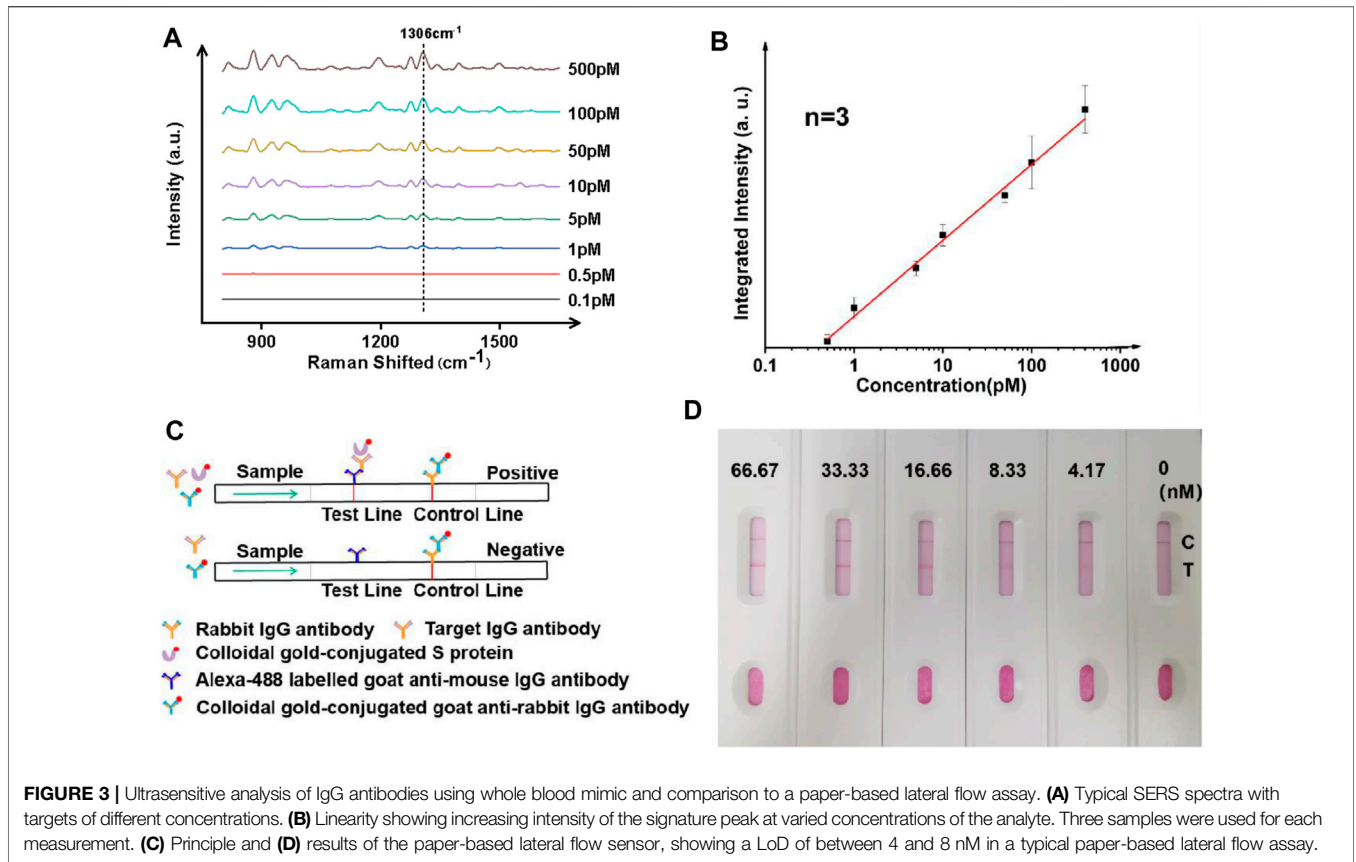
correlation coefficient of  $>0.99$ . A limit of detection was found to be  $\sim 8.5\text{ pM}$  using a signal-to-noise (S/N) ratio above three. The use of the liquid SERS substrate was important because it was found that the Raman signature was weak with only using Au@MNPs (**Supplementary Figure S1**). This also suggested that the liquid-state SERS substrate remained stable even when magnetic nanoparticles were attached to it.

We further verified the performance of the magnetically enhanced SERS with protein targets, as seen in **Figure 2**. The SARS-CoV-2 antibody, immunoglobulin G (IgG), was chosen as the target antigen. The IgG antibody binds strongly to the S spike protein fragments on the surface of SARS-Cov-2 virus, and is a standard biomarker for tracking and screening COVID-19 patients, typically using blood samples (Chen et al., 2020b; Yao et al., 2020). In practice, the concentration of IgG in serum is often below the LoD of many of the existing technologies, down to the pM range (Li et al., 2020b; Kohmer et al., 2020). This is challenging for early-stage/asymptomatic infections. Given the scale and severity of the COVID-19 pandemic with a possibility to prevail in the next few years given the many emerging mutants, such as Delta and Lamda variants (Kemp et al., 2021), it is of importance to develop reproducible methods to quantify IgG antibodies with high sensitivity (Deeks et al., 2020).

Au@MNPs were modified with a biotinylated PEG layer before being coated with a streptavidin-labeled, anti-IgG primary antibody, as shown in **Figure 3A**. Using a secondary antibody (Alexa-488 labeled goat anti-mouse antibody) to capture IgG, the assay was conducted in a standard sandwich format, and SERS measurements were performed following magnetic pull-down. The reason that Alexa 488 (instead of Cy3 used for DNA measurements) was used was due to the commercial availability of the dye as protein labels. As can be seen in **Figure 3, B-C**, with target enhancement, clean SERS spectra were obtained for target concentrations below 1 nM. Using a signature peak in Alexa 488, which occurred at  $1,306\text{ cm}^{-1}$ , corresponding to the amide stretch (Chen et al., 2011), a clear linearity between peak intensity and analyte concentration was seen between  $\sim 0.5$  and  $\sim 500\text{ pM}$ , a detection range inaccessible to current liquid-SERS measurements. In this case, an LoD was found to be  $\sim 0.37\text{ pM}$  using the S/N ratio of 3.

Since the majority of IgG tests for COVID-19 in clinical environments are performed with serum samples, the performance of the SERS analyzer was further studied using a commercially obtained whole blood mimic. As seen in **Figures 3A,B**, the performance of the magnetically enhanced SERS sensor was comparable to when using buffer-based systems, with a linear trend observed when concentration of the target was between  $\sim 0.5$  and  $\sim 500\text{ pM}$ , with a correlation coefficient over 0.98. An LoD of  $\sim 0.42\text{ pM}$  was determined using the S/N ratio of above three. The fact that the assay could be performed using whole blood mimic was promising, as the use of the PEG layer on the surface of nanoparticles could minimize nonspecific adsorption of serum proteins (Wang et al., 2012; Zheng et al., 2013; Jiang et al., 2020b).

The performance of the SERS analyzer was compared with the paper-based lateral flow assay (LFA) (**Figure 3C**). The paper-based LFA is a standard method for antibody screening and had comparable LoD with the enzyme-linked immunosorbent assay



(ELISA) but has the advantage of not relying on instruments. Herein, it was prepared using a standard nitrocellulose filter membrane (Quesada-González and Merkoçi, 2015), the testing line (T-line) was imprinted with anti-IgG antibody (goat anti-mouse), and the control line (C-line) was imprinted with rabbit IgG antibody (IBL, JP17312). To perform an IgG antibody test, a mixture containing three components was added: (1) gold

nanoparticle solution with surface modified with S protein, (2) gold nanoparticle colloidal with surface modified with goat anti-rabbit IgG antibody (Abcam, ab6702), and (3) the target antibody (IgG). A gradual increase of color was observed when the concentration of IgG was varied between 0 and 66 nM, with an LoD found to be in between 4 and 8 nM. The results indicated that the magnetic liquid-SERS analyzer has superior LoD than paper-based LFA.



**TABLE 1** | Comparison of this work with typical SERS assay in the literature.

Assay type	Limit of detection	Scalability/accessibility	Time of operation	Instrument cost
Magnetic liquid SERS (this work)	$10^{-12}$ M	Excellent	< 1 min	Low < 10 k USD
Liquid SERS (Ma et al., 2016; Tian et al., 2018)	$10^{-9}$ M	Excellent	< 1 min	Low < 10 k USD
Nano star SERS (Indrasekara et al., 2014; Li et al., 2021)	$10^{-15}$ M	Good	~ 5 min	Varies
Single-molecule SERS (Yang et al., 2016; Li et al., 2021)	$10^{-18}$ M	Poor	~ 15 min	High Over 100 K USD

We attribute the excellent performance of the magnetically enhanced liquid state SERS to two reasons. First, sampling is improved when targets are enriched near the surface of the liquid-liquid interface of the plasmonic substrate. Second, magnetic enhancement leads to the formation of SERS hotspots between the substrate and the nanoparticles, in which gap-mode SERS can occur, as seen in **Figure 4**. In these scenarios, the EM enhancement factor can reach  $\sim 10^8$  times or higher, which corresponds to  $\sim 10^{12}$  time SERS enhancement, much higher than that of using the nanoparticle or substrate only. This is because

$$G_{SERS} \approx (|E_{local}(\omega_r)|)^4 / (|E_0(\omega_0)|)^4,$$

where  $G$  is the enhancement factor and  $E$  is the intensity of EM field at particular frequency ( $\omega$ ). The gap-mode system shows stronger enhancement than what can be achieved with the nanoparticles or substrate only, regardless of the magnetic core.2 Saying this, due to the limited scope of this work, a detailed analysis as to what extent each factor contribute to the final LoD is not done, but can be investigated in future studies. It is expected that with improved geometry of the Au@MNP, such as using star-shaped nanoparticles, the performance of the magnetic liquid SERS analyzer can be further improved. Another direction worth exploring is the effect of surface-enhanced resonant Raman scattering (SERRS) effects in this system. Resonant Raman can be important for its high-bond selectivity and capability for multiplexed detection (Faulds et al., 2005) This is particularly attractive when performed in ultraviolet waveband because DNAs and proteins absorb UV light strongly (Renard et al., 2019; Wu et al., 2021)

Since there are many studies in the literature focusing on ultrasensitive SERS measurements, a main advantage of this study is not to show the sensitivity is more superior than that of others but to demonstrate accessibility and high sensitivity can be achieved at the same time with ease with cost-effective instruments. In fact, pM sensitivity already answers to clinical demand, and the method is scalable at an industrial level. A comparison to several selected methods is shown in **Table 1**.

## CONCLUSIONS AND FUTURE PERSPECTIVE

This study can be summarized as follows: (1) magnetic enhancements significantly improve the sensitivity of liquid-state SERS by lowering the limit of detection (LoD) by at least three orders of magnitude to enable pM-level detection of biomolecules. (2) The LoD for nucleic acid targets was

determined by using DNA fragments associated with *Staphylococcus aureus*, a bacteria associated with cross-infections in hospitals. (3) Analysis for protein antibodies was determined with IgG antibodies used for COVID-19 screening, with a LoD of lower than 0.5 pM. The results were compared with paper-based LFA, showing significantly improved LoD of the magnetic liquid-SERS method. Due to the simple, reproducible process of substrate preparation and high performance of the assay toward both protein and nucleic acid targets, we expect this work to inspire further studies toward ultrasensitive SERS biosensors that could be scaled to mass production.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## AUTHOR CONTRIBUTIONS

ZJ, CZ, YY, and JJ performed experiments. HD performed simulation. EF, XC, and SH supervised the study. All authors contributed to the manuscript writing.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fbioe.2021.735711/full#supplementary-material>

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