

Advances in surface-enhanced Raman scattering bioprobes for cancer imaging

Jie Lin | Ozioma Udochukwu Akakuru | Aiguo Wu

Cixi Institute of Biomedical Engineering, International Cooperation Base of Biomedical Materials Technology and Application, Chinese Academy of Science (CAS) Key Laboratory of Magnetic Materials and Devices, Zhejiang Engineering Research Center for Biomedical Materials, Ningbo Institute of Materials Technology and Engineering, CAS, Ningbo, China

Correspondence
Cixi Institute of Biomedical Engineering, International Cooperation Base of Biomedical Materials Technology and Application, Chinese Academy of Science (CAS) Key Laboratory of Magnetic Materials and Devices, Zhejiang Engineering Research Center for Biomedical Materials, Ningbo Institute of Materials Technology and Engineering, CAS, 1219 Zhongguan West Road, Ningbo, China.
Email: aiguo@nimte.ac.cn

Abstract
Surface-enhanced Raman scattering (SERS) technology with feature of high sensitivity, selective enhancement, in situ detection, nondestructive, label free, and fingerprint spectrum has been widely utilized in various practical applications, especially for bioanalysis. Cancer diagnosis and therapy based on SERS bioimaging has been reported as a promising approach in recent decades, and various types of material-based SERS bioprobes are rapidly designed continuously. SERS bioimaging has been successfully employed in cancer screening, diagnosis, and componential analysis in both in vitro and in vivo cancer tissue samples, which simultaneously provides visual morphology and biochemical information. Precise tumor tissue excision, real-time monitoring of cellular uptake process, and noninvasive cell tracking and labeling are realized due to the effective Raman image bioprobes, satisfying the requirements of precision medicine. Here, an overview of SERS bioprobes based on noble metals, metal oxides, and composite materials is presented by highlighting their respective, unique advantages and recent achievements in cancer imaging realm. This review also offers a distinctive perspective on designing multifunctional micro–nanoparticles with remarkable SERS activity, and proposing novel strategies for establishing high-performance sensors and imaging bioprobes derived from the deep insight of enhancement mechanism for different types of SERS substrates.

KEYWORDS
bioimaging, bioprobe, cancer diagnosis, cancer screening, SERS

Abbreviations: 2NAT, 2-naphthalenethiol; 3D, three-dimensional; 4-MBA, 4-mercaptobenzoic acid; 4BPT, biphenyl-4-thiol; AB, antibody; AR, Alizarin red; AuNRs, Au nanorods; AuNSs, Au nanostars; B-TiO₂, black TiO₂; CM, chemical enhancement mechanism; DNBA-N₃, (2-nitrobenzoic acid)-N₃; EF, enhancement factor; FA, folic acid; HAuNP, Au nanoparticles; PA, poly-L-arginine hydrochloride; PD, polydopamine; PEG, polyethylene glycol; PICT, photon-induced charge transfer; PMA, polyisobutylenemaleic polymer; SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance


1 | INTRODUCTION

1.1 | Surface-enhanced Raman scattering

Since 1976, surface-enhanced Raman scattering (SERS), an effective analytical technique, has been utilized in material science, analytical chemistry, trace detections, catalytic reactions, chemical sensors, food safety, biochemistry, and so forth by leveraging characteristic vibrational fingerprint spectrum and in situ detection analysis. Recently, cancer is one of the leading diseases that threaten human life and health, and early screening and diagnosis are the most effective means for its prevention and treatment. Raman signal with good anti-interference ability, high detection sensitivity, and selective enhancement gives SERS substrates unique advantages for componential analysis in complex systems, which provides great application potential in biological analysis and biosensing, especially for cancer precision medicine. SERS bioimaging has been recognized as a feasible approach in microscopic delineation of tumor components, and offers meaningful information in diagnostic oncology based on its ultrasensitive, appreciable accuracy, high efficiency, and fingerprint analysis Raman signal. Tissue autofluorescence and photobleaching of fluorescence-based imaging bioprobes substantially hinder the further application of fluorescence imaging in clinical medicine, which positions SERS bioimaging technology as a promising complementary imaging modality utilized in diagnostic oncology. The biomolecular sensing capability of high-precision SERS bioimaging is largely dependent on the surface physicochemical property of materials. Therefore, designing and constructing high-performance bioprobes is key for the application of SERS bioimaging in the fields of optical medical imaging and image-guided clinical setting, suggesting that more attention should be given to the development of novel SERS substrates with commendable enhancement characteristics.

1.2 | Various materials for SERS

Noble metal materials based on Ag and Au are widely employed as SERS sensing platforms due to their ultrahigh SERS sensitivity, which is derived from the electromagnetic field enhancement mechanism between analytes and substrates. Surface plasmon resonance (SPR) occurs in the interface of plasmonic structures under incident wavelength, producing strong local electromagnetic field, magnifying analyte molecular Raman scattering cross section, and inducing huge SERS enhancement. SPR effect of noble material is effectively boosted by modulating material morphology, shape, and size, which shifts plasmon oscillation frequency to match well with the illuminated wavelength, resulting in ultrahigh SERS enhancement factor (EF). “Hot spots” can be created in the gap of dimer or trimer metal nanoparticles, and huge local electromagnetic field will be generated in the gap region. Therefore, this nanoparticle–nanoparticle junction gap is served as a perfect oscillator resonance cavity to bring out ultrahigh SERS EF (up to $10^9$) to satisfy single-molecule detection. Remarkable SERS activity endows noble metals with a significant advance in SERS bioimaging for cancer cells, which is targeted by the magnified Raman signal molecule.

In the past 10 years, SERS research has attracted great interests in exploring novel materials with the capabilities of high spectrum uniformity, good chemical stability, excellent anti-interference ability, and good biocompatibility. Recently, investigators report that metal oxide nanostructures serve as competitive SERS substrates due to their intriguing spectral properties, such as high stability and reproducibility, especially in tumor detection and imaging. The chemical mechanism responsible for the SERS enhancement performance of metal oxide materials is still poorly understood, leading to relatively low SERS EF and impeding their practical applications. Chemical enhancement mechanism (CM) is much more complex than electromagnetic mechanism, due to which it is not well understood so far. Generally, the essence of chemical mechanism is based on interfacial interaction between probe molecules and metal oxide substrates. The Raman scattering cross section of probe molecules is largely changed by interfacial interaction, which amplifies molecular polarization tensor, and ultimately results in significant SERS enhancement. Three theoretical models well demonstrate CM. First, interfacial charge transfer process occurs between probe molecules and SERS substrates under excitation wavelength, thus boosting molecular Raman scattering signal. Besides, a chemical bond is formed between the analyte and substrate, which magnifies molecular electronic state density and leads to a nonresonant enhanced SERS effect. In addition, surface complex is established between chemisorbed molecules and material substrates, and new energy levels can effectively resonate with the laser illumination as a consequence, thus producing strong SERS effect.

Based on the above analysis, several effective strategies have been proposed to boost SERS EF of metal oxide nano-materials and improve their SERS detection sensitivity and bioimaging performance. Introducing numerous surface defect states on metal oxide SERS substrates can promote interfacial charge transfer process efficiency due to the additional springboard energy levels, leading to metal-comparable SERS enhancement. Chemical bonds
(S–Ti, S–Cu, and S–Zn) have been deeply studied in metal oxide molecule SERS systems, which play a crucial role in generating stronger molecule–substrate interaction and largely affects electronic structure-related electron transition such as electronic work function-assisted interfacial electron transfer process. Amorphous metal oxide nanomaterials favor the development of a stable charge transfer surface complex caused by metastable electronic states and high electrostatic potential, endowing significant electron transition efficiency and remarkable SERS activity. These strategies suggest that regulating surface defect, crystalline state, and chemical bonding are effective ways to promote metal oxide SERS enhancement. The significant superiority of metal oxide SERS substrate is the capability of identifying a specific molecule with many other molecules in a complex environment. Selective SERS enhancement of metal oxides originates from photon-induced charge transfer (PICT) process based on Herzberg–Teller selection rule, making metal oxides an ideal set of biomedical sensors in cancer imaging and detection.

Noble metal and metal oxide SERS-active materials exhibit unique advantages and performances, and combining the superiority of these two materials will realize an expansive development foreground in the field of SERS bioimaging. For this purpose, metal–metal oxide heterostructures have been successfully designed, and the reported EF strongly depended on both electromagnetic field and chemical mechanisms. In hybrid SERS platforms, huge SPR-induced electromagnetic field can be obtained by closely packed metal nanoparticles; the gap distance between metal nanoparticles is adjusted by the supported metal oxide material. Besides, additional photon-induced interfacial charge transfer process is introduced in the metal–metal oxide SERS heterostructures, by four possible charge transfer pathways: (1) metal oxide-to-molecule-to-metal; (2) metal-to-metal oxide-to-molecule; (3) metal-to-molecule-to-metal oxide; and (4) molecule-to-metal oxide-to-metal. The boosted electromagnetic field and charge transfer process synergistically endows metal–metal oxide hybrid substrates with higher SERS enhancement. The reliable hybrid SERS platforms combined by metal and nonmetal materials exhibit multifunctional detection characteristics, possessing great application potential for SERS bioimaging in cancer cell diagnosis and therapy.

In this paper, we present an overview on recent developments in the fundamentals of SERS mechanism for different types of micro–nanomaterials and their unique advantages in biomedicine. Recent research trends shed light on the successful application of SERS bioimaging in cancer screening, diagnosis, and componential analysis based on noble metals, metal oxides, and heterostructural materials. The reported SERS bioimaging probes have demonstrated significant advantages in terms of selective enhancement, considerable accuracy, high sensitivity, and provided straightforward biochemical information for cancer tissue samples in vitro and in vivo. Moreover, high-performance SERS mapping probes have been established in the cancer therapeutic realm, such as noninvasive cell tracking and labeling, microimage-assisted tumor tissue excision, and cellular uptake process monitoring, consequently playing a vital role in the visualization and diagnosis of cancer. We hope that the present scientific work not only offers a comprehensive introduction of SERS bioimaging as an efficient and reliable approach in precision medicine, but also opens a new viewpoint for developing high-performance SERS bioimaging platforms that can be applied as biosensors with the feature of good biocompatibility, strong signal anti-interference ability, remarkable sensitivity, and selective SERS enhancement.

2 ADVANTAGES OF SERS BIOIMAGING TECHNOLOGY IN CANCER DIAGNOSIS

Although traditional medical imaging methods (magnetic resonance imaging, computerized tomography imaging, etc.) can effectively image and distinguish carcinomas and normal tissues, the harmful radioactive irradiation of computed tomography is unavoidable and cannot satisfy the analysis in cellular level. In recent years, fluorescent-based nanoprobes have been widely used in targeting and imaging of cancer cells due to the capabilities of high sensitivity and microscopic visualization. However, the disadvantages of photobleaching, inferior photostability, and inevitable autofluorescence seriously limit its further application in cancer imaging. Hence, SERS-based technology with the feature of fingerprint spectrum, high spectrum stability, and multiple-labeling serves as a complementary approach to fluorescence imaging modality. Herein, serval significant scientific works are reviewed to provide a unique perspective on SERS bioimaging approach applied to cancer diagnosis and treatment.

3 SERS BIOPROBES FOR CANCER DIAGNOSIS

3.1 Noble metal-based SERS bioprobes

3.1.1 Cancer labelling

Zhang and co-workers reported that Au nanoparticle-based SERS bioprobes exhibited outstanding targeting
FIGURE 1 (A1–A3) Dark-field images of three cancer cells co-incubated with HAuNP–DNBA–FA bioprobe for 1 h, respectively. (B1–B3) SERS bioimaging of the selected region in the dark-field images (SERS mapping is acquired from 1332 cm\(^{-1}\) Raman peak). (C1–C3) The Raman signals are corresponding to the specific points in middle SERS images. Reproduced (Adapted) with permission.\(^6^0\) Copyright 2017, American Chemical Society (ACS)

abilities to specify cancer cell lines based on SERS bioimaging technology.\(^6^0\) The SERS bioprobe was synthesized with hollow Au nanoparticles (HAuNP), Raman signal molecule ((2-nitrobenzoic acid)–N\(_3\) [DNBA–N\(_3\)]), and folic acid (FA). DNBA–N\(_3\) molecule adsorbed on HAuNP via disulfide chemical bond exhibited strong SERS signals derived from SPR-produced electromagnetic field. FA was simply and effectively conjugated to HAuNP–DNBA–N\(_3\) through copper-free reaction, and HAuNP–DNBA–N\(_3\)–FA bioprobe displayed good spectral stability and biocompatibility. The designed SERS bioprobe (HAuNP–DNBA–N\(_3\)–FA) was directly utilized as an imaging agent to diagnose different cancer cells with excellent spatial resolution. The cancer labeling ability of this SERS bioprobe is obviously improved by its folate component, and the corresponding SERS images of three cancer cells are shown in Figure 1; KB cancer cells render stronger Raman signal and more intense SERS image compared to other two cancer cells under 633 nm laser illumination. The colorful SERS mapping was measured with the Raman peak of DNBA–N\(_3\) signal molecule at 1332 cm\(^{-1}\), illustrating that more bioprobases internalized into FA-positive cancer cells (KB) than that of FA-negative cells (Hela and A549). FA plays a vital role in promoting cellular uptake ability as SERS bioprobases are click functionalized to the surface of cancer cell, and folate-targeted SERS bioprobases exhibit selective imaging and diagnosis feature to FA-expressed cancer lines. It was demonstrated that SERS bioimaging method provides distinguishable colorful images for FR-positive and FR-negative cancers cells, implying that the designed noble metal-based SERS bioprobe exhibited a significant cancer cellular targeting ability and can serve as a high-spatial-resolution imaging agent for tumor therapy.

3.1.2 Three-dimensional imaging

Complex three-dimensional (3D) cell imaging is seriously hindered by spectral limitations, such as photobleaching, insufficient laser penetration, chemical degradation, and lack of complementary methods to describe 3D cell structures. Aberasturi et al. reported novel SERS bioprobases that served as a promising tool in analyzing 3D complex cell structure via SERS bioimaging.\(^6^1\) Au nanorods (AuNRs) and Au nanostars (AuNSs)-based SERS bioprobases were developed by coating with various Raman signal molecules such as 2-naphthalenethiol (2NAT), biphenyl-4-thiol (4BPT), polyisobutylene-alt-maleic polymer (PMA), and poly-L-arginine hydrochloride (PA). These two bioprobases exhibit high EF (10\(^6\)) due to SPR-induced electromagnetic field, endowing them with huge potential in 3D cell SERS bioimaging. In order to study 3D cell imaging ability based on SERS approach, processed dermal fibroblast (rHDF) cells were co-incubated with the above two SERS bioprobases. AuNS–4BPT@PMA–PA and AuNR–2NAT@PMA–PA nanoparticles were labeled with rHDF cell layers, and unlabeled rHDF cell layers were subsequently deposited on a CaF\(_2\) glass supporting base. AuNS-labeled cells were on the top, and AuNR-labeled cells on the bottom, which are separated by six layers of unlabeled rHDF cell. The corresponding X–Z SERS mapping was acquired from Raman vibration modes (1282, 1381, and 322 cm\(^{-1}\) for 4BPT, 2NAT, and CaF\(_2\), respectively) in a region of 1.4 mm x-axis and 400 \(\mu\)m z-axis. As shown in Figure 2, the two nanoparticles-labeled cell layers are clearly distinguished; red and blue SERS mapping regions are responsible for AuNR- and AuNS-labeled cells, respectively. The unlabeled cell layer can be roughly defined by the partial vertical section in Figure 2A, whereas the maximum peak intensities in xz-SERS map (Figure 2B–D) demonstrate most nanoparticles uptake, and the thickness of unlabeled cell layer was determined by the distance between the above two maximum peaks (approximately 26–34 \(\mu\)m). The novel 3D SERS bioimaging technology not only provides a proof-of-concept study for recognizing complex cell models, but also offers a very significant microimaging method in the clinical field of precise tumor tissue excision. Based on the ultrasensitive
SERS activity, noble metal-based SERS platforms have been widely reported as good cancer and other diseases' imaging bioprobes.\textsuperscript{62–65}

### 3.2 | Metal oxide SERS bioprobes

#### 3.2.1 | Cancer components imaging

Recently, the SERS activity of metal oxide–based SERS substrates is largely boosted by the effective strategies, such as constructing surface defect states, and surface amorphization. Moreover, the morphology design of metal oxide materials can greatly improve interfacial photon-induced charge process and bring out remarkable SERS activity. Excellent biocompatibility, selective SERS enhancement, low cost, and good anti-interference ability endow metal oxide nanostructures with great potential to be reliable platforms utilized in biosensing and bioimaging.\textsuperscript{66} Haldavnekar et al. reported that a ZnO-based quantum SERS bioprobe with the feature of remarkable SERS activity, label free, and good crystallinity is successfully utilized in distinguishing cancer and normal cells based on Raman spectral fingerprint information analysis of proteins, lipids, DNA, and RNA in single-cell level.\textsuperscript{67} The ultrahigh SERS EF ($1.37 \times 10^6$) and nanomolar limit of detection for target molecules are derived from the synergistic effects of (1) quantum corner-induced electromagnetic fields (SPR); (2) surface defects-promoted PICT process; and (3) target molecular intrinsic Raman resonance enhancement. Raman signals of different components in signal cancer cell were determined by the cellular uptake of ZnO quantum SERS bioprobes, which were clearly observed on the TEM images in Figure 3A. Besides, the ZnO SERS bioprobe exhibited good SERS spectral uniformity and reproducibility in various cancer cells (Figure 3B). In addition, ZnO bioprobes-based SERS bioimaging approach owns the ability of visually discriminating different components (DNA, RNA, proteins, and lipids) of tumor and normal cells, which is of great significance in early screening of cancer and precise resection of tumor margin (Figure 3C). The scientific work revealed that a nonmetal bioprobe with metal-comparable SERS activity can be an important analytical tool for cancer tissue samples in vitro and in vivo.

#### 3.2.2 | Cancer cell imaging

Wu and co-workers developed novel black TiO$_2$ (B-TiO$_2$) nanoparticles with crystal–amorphous core–shell nanostructure that exhibited outstanding SERS activity.\textsuperscript{51} The high-performance bioprobe composed of B-TiO$_2$ nanoparticles, Alizarin red (AR) reporter molecule, polydopamine (PD) layer, polyethylene glycol (PEG) molecule, and antibody (AB) is shown in Figure 4A. The EF of B-TiO$_2$ could reach $4.3 \times 10^5$ for probe molecules under visible and near-infrared laser illumination due to the synergistic contribution of the promising crystal–amorphous interfacial structure. Abundant excitons existed in crystal core and
served as charge source, thus providing sufficient electron for participating in PICT process. The unique crystal–amorphous heterojunction promoted interfacial exciton separation and induced charge injected into the amorphous shell. Significantly, relatively low Fermi level and high electronic density of states of the amorphous shell are benefits of the vibronic coupling effect between B-TiO$_2$ nanoparticles and the target molecule, strongly magnifying molecular Raman scattering cross section. B-TiO$_2$–AR–PD–PEG–AB SERS bioprobes with the advantages of good biocompatibility, high detection sensitivity, and excellent spectral uniformity exhibited great potential in biosensing and bioimaging fields. MCF-7 and drug-resistant MCF-7 (MCF-7/ADR) breast cancer cells are hard to be distinguished by morphologic method, but these cell types could be effectively differentiated by the B-TiO$_2$ SERS bioprobe through Raman signals and SERS bioimaging (Figures 4B and 4C). The B-TiO$_2$–AR–PD–PEG–AB SERS bioprobe is capable of quickly targeting MCF-7/ADR cancer cells due to its AB protein component, making the bioprobe effectively absorbed on the surface of specific cancer cells. Ultrahigh-resolution SERS bioimaging technology and ultrasensitive SERS spectra can quickly and accurately recognize MCF-7/ADR cancer cells, satisfying the requirement for early cancer screening and precision medicine.

### 3.3 Hybrid SERS bioprobes

#### 3.3.1 Multiplex cancer imaging

Constructing metal oxide–noble metal hybrid SERS substrates is an effective strategy to improve detection sensitivity, increase spectrum uniformity, avoid aggregation, and reduce biotoxicity, exhibiting multifunctional characteristics. Fernández et al. designed a heteroassembly of Au–SiO$_2$ Janus particles with excellent SERS activity based on the hybrid Au core–satellite model, producing electromagnetic-related hot spots and generating huge EF ($4.9 \times 10^6$). The Au–SiO$_2$ hybrid SERS substrate is adsorbed with 4-mercaptobenzoic acid (4-MBA) and rhodamine B (RhB) target molecules, and the two
Raman-active molecules can be separately enhanced by different laser excitation colors. Raman signal of RhB molecule derived from molecular intrinsic resonance (550 nm) was acquired at 532 nm wavelength. Besides, relatively weak SERS signal of 4MBA molecule was collected on hybrid substrate under 785 nm wavelength. The mixed SERS spectrum of 4MBA and RhB molecules was simultaneously acquired by 633 nm laser due to the localized SPRs of Au–SiO₂ hybrid SERS substrate, enabling obvious wavelength-selective SERS enhancement behavior of the hybrid bioprobe. Double target molecules-labeled SERS bioprobes own unique advantages in cancer cell imaging due to the independent spectral fingerprints in complex biological environment. Hybrid SERS bioprobe has been as a powerful tool for imaging J744 macrophage cell in vitro (Figure 5A), and Raman signals of the SERS bioprobe internalized by cells still maintain distinguishable SERS activity under different illuminations (Figure 5B–D). Hybrid SERS bioprobes are co-incubated with a single cell for 1 h, and SERS bioimaging of the same cell can be successfully achieved as the excited laser switching to another color wavelength, allowing fingerprint, multiplex, and realizing color-dependent SERS bioimaging in single cell. This hybrid SERS nanostructure utilized in wavelength-sensitive bioimaging endows SERS bioprobe visual, explicit, and spatially defined fingerprint information, occupying an extremely important position in cancer diagnosis.

3.3.2 Near-infrared light-induced cancer imaging

SERS substrates combined with different types of materials exhibit multifunctional characteristics in cancer diagnosis and therapy, overcoming the weakness of material-induced biotoxicity and breaking through near-infrared excitation wavelength-limited SERS activity. SERS bioimaging technique has the capability of reaching single-cancer-cell resolution and acquiring precise tumor location for early cancer screening without harmful ionizing radiation. Hybrid SERS platform has been a promising diagnosis system due to the advantages of ultrahigh detection sensitivity (single-molecule level) and excellent biological compatibility. Zhu and co-authors developed novel and tunable core–shell hybrid SERS sensor (Au–SiO₂–WO₃) that was successfully utilized in high-resolution cancer imaging under near-infrared laser illumination. The Au shell provided sufficient EF for cancer SERS bioimaging based on the SPR mechanism. WO₃ with good sensing activities, excellent biocompatibility properties, and favorable antibacterial characteristic makes a contribution to
FIGURE 5  (A) Optical image of J744 macrophage cell line without co-incubation with SERS bioprobe. (B–D) Raman signal of hybrid bioprobe and J774 co-incubated with SERS bioprobe, respectively (left); optical image and SERS bioimaging (right) simultaneously acquired in single cell co-incubated with hybrid bioprobe; SERS bioimaging was collected and analyzed from 4-MBA molecule (1078 cm$^{-1}$) and RhB molecule (1617 cm$^{-1}$), which was illuminated by different laser wavelengths, 532 (B), 785 (C), and 633 nm (D). Raman signals in panels B–D are magnified by approximately 14, 4, and 20, respectively. Scale bar: 20 $\mu$m. Reproduced (Adapted) with permission. Copyright 2015, American Chemical Society (ACS)

regulate plasmonic-related performance of the Au shell. Porous SiO$_2$ nanoparticles served as intermediate interface to bind the Au shell and WO$_3$ core, and reduce the toxicity of the SERS bioprobe. 4-MBA target molecule adsorbed on the shelled Au–SiO$_2$–WO$_3$ composite SERS bioprobe exhibited more intense Raman signal than that of seeded Au–SiO$_2$–WO$_3$ substrate, which was attributed to the improved SPR via monodispersed WO$_3$ cores and thinner SiO$_2$ shells (Figure 6A–C). Through analyzing 4-MBA Raman signal, SERS bioimaging was potentially utilized in labeling cancer cells via 2D mapping illuminated by near-infrared laser as shown in Figure 6D–F. Three-dimensional SERS bioimaging is able to visually identify the location of bioprobe phagocytized in MDA-MB 231 breast cancer cells, which is not well defined by optical microscopy (Figures 6G and 6H). As SERS bioproses attached or phagocytized by cancer cells through cancer ligands (antibody), precise location of specific cancer cells can be easily achieved, such as MDA-MB 231 breast cancer cells (Figure 6I). Moreover, the 4-MBA signal molecules played an important role in cancer cell SERS bioimaging (Figure 6J). The study demonstrated that the SERS bioprobe with low cytotoxicity and high SERS sensitivity under near-infrared light can be a promising SERS bioimaging candidate for in vivo biosensing at clinical stage. High-performance SERS bioimaging method really opens up a practical path for diagnosing clinic diseases, and the presented cases of SERS imaging bioproses utilized in cancer diagnosis are summarized in Table 1.

4  | SUMMARY AND OUTLOOK

This review presents a big picture of nanomaterials-based SERS bioproses as a powerful diagnostic tool in biosensing and bioimaging fields, which benefit from the characteristic of in situ, high sensitivity, nondestructive, and fingerprint spectra. The reported noble metal-based SERS bioproses have demonstrated significant advantages in detection sensitivity and ultrahigh EF, which is in favor of providing microimaging information for trace biological components. Metal oxide–based SERS bioproses exhibit encouraging spectral stability, good biocompatibility, excellent anti-interference ability, and selective enhancement character, endowing these bioproses with an important analytical and imaging tool in complex biological systems. Hybrid SERS bioproses not only serve as a multifunctional theranostic nanopatorm for visual and high spatially defined SERS bioimaging of cancer cell, but also offer potential opportunities for cancer therapy. Based on the abovementioned SERS substrates, SERS bioimaging approach has been widely utilized in noninvasive cancer cell tracking, cell labeling, precise cancer cell localization, type identification of tumor cells, and cancer cell
**FIGURE 6** (A–C) SERS signals (1078 and 1589 cm$^{-1}$) of 4-MBA target molecule adsorbed on Au–SiO$_2$–WO$_3$ core–shell composite, Au seeds–SiO$_2$–WO$_3$ composite, and intrinsic 4-MBA Raman spectra under 830 nm laser illumination, respectively. (D) Optical microscopy image of Au–SiO$_2$–WO$_3$ core–shell composite SERS bioprobe. (E and F) SERS bioimaging of 4-MBA–Au–SiO$_2$–WO$_3$ core–shell composite bioprobe (4-MBA as Raman signal molecule) on a silicon wafer under 785 and 830 nm excitation wavelengths, respectively. (G and H) Three-dimensional coherent anti-Stokes Raman scattering (CARS) imaging of the SERS bioprobe phagocytized in MDA-MB 231 breast cancer cells. (I and J) CARS imaging of breast cancer cells performed by Au–SiO$_2$–WO$_3$ core–shell composite with and without 4MB target molecule, respectively. Reproduced (Adapted) with permission.© Copyright 2019, Wiley

**TABLE 1** SERS imaging bioprobes utilized in cancer diagnosis

| Material (bioprobe)                  | Enhancement mechanism | Cancer cell lines          | Application                      | Reference |
|-------------------------------------|-----------------------|---------------------------|----------------------------------|-----------|
| Black TiO$_2$                       | CM                    | MCF-7 cells               | Cancer cell imaging              | 51        |
| Au nanoparticles                     | EM                    | KB, Hela and A549 cells   | Targeting cancer cell            | 60        |
| Au nanorods and Au nanostars        | EM                    | rHDF cells                | 3D cell imaging                  | 61        |
| Au nanoparticles                     | EM                    | HeLa cells                | Cervical cancer detection        | 63        |
| Quantum ZnO                         | CM                    | HeLa, MDA MB 231 cells    | Component analysis               | 67        |
| Au–SiO$_2$ composite                 | EM (dominant)         | J744 macrophage cells     | Cancer cell imaging              | 68        |
| Au–SiO$_2$–WO$_3$ core–shell composite | EM (dominant)     | MDA-MB 231 cells          | 3D cell imaging                  | 69        |
| Au nanorods                          | EM                    | HeLa and MCF-7 cells      | Component analysis               | 70        |
| Au–SiO$_2$ core–shell composite      | EM (dominant)         | Glioblastoma multiforme cells | Assisting tumor resection         | 71        |
| GO–Au composite                      | EM (dominant)         | HeLa and MCF-7 cells      | Biomarkers detection in cells    | 72        |

Abbreviations: CM, chemical enhancement mechanism; EM, electromagnetic field enhancement mechanism.
Although nanostructures-based SERS bioimaging technology holds strong promise in biosensing and bioanalysis, several critically scientific points should be solved to improve their full potential. First, developing homogenous material-based SERS bioimaging bioprobe and SERS bioimaging bioprobe with good spectral reproducibility and sensitivity simultaneously is the most urgent need under the present situation. Strategies have been proposed to modify noble metal materials with high spectral reproducibility by designing array structures. Surface amorphization, surface defect, and morphology control have been implemented to improve SERS sensitivity of metal oxide substrate. Second, SERS bioimaging of cancer cell is largely dependent on the labeled Raman signal molecule, which seriously hinders SERS bioprobe directly applied for in vivo bioimaging. Label-free SERS bioimaging for tumor tissue can be accomplished by designing high-performance metal oxide substrates based on the feature of fingerprint spectrum and selective enhancement Raman modes, satisfying the requirement of biosensing in vivo. Third, compared to single-labeled fluorescence spectra, multi-labeled bioprobes can be easily realized by SERS spectra via the modification of various signal molecules under different laser illuminations in situ. Multi-labeled SERS bioimaging bioprobe will become an important tool for discriminating tumor tissue margin and provide a creative possibility for identifying different types of cancer cells. Additionally, according to the reported scientific works, practical applications of SERS bioimaging is largely restricted to cellular level and rarely applied on tumor tissue due to the limitation of laser penetration depth. Exploring novel nanomaterial-based bioprobe with remarkable SERS activity under infrared wavelength and combining SERS bioprobe with endoscope are the key points to directly utilize SERS bioimaging on tumor tissues in vivo. It is believed that high-resolution SERS bioimaging technology can be an excellent analysis tool for investigating clinical cancer samples if the abovementioned scientific challenges can be successfully solved in the future.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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ORCID
Aiguo Wu https://orcid.org/0000-0001-7200-8923

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Ozioma Udochukwu Akakuru obtained his Master's Degree in Industrial Chemistry from the University of Benin, Nigeria in 2013. He then joined the Department of Chemistry, University of Calabar, Nigeria in 2014 as a lecturer. In 2017, he was awarded the CAS-TWAS President’s Fellowship for a Doctoral Degree at Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo, China. He has more than 35 publications in scientific journals of international repute. His research focuses on the synthesis and application of targeted nanoscaled biomaterials for image-guided cancer therapy.

Prof. Aiguo Wu received his PhD from the Chinese Academy of Sciences supervised by Prof. Erkang Wang and Prof. Zhuang Li in China in 2003. He stayed at the University of Marburg (Prof. Norbert Hampp group) in Germany during 2004–2005, Caltech (Prof. Ahmed Zewail group) in USA during 2005–2006, and Northwestern University (Prof. Gayle Woloschak group) in USA during 2006–2009. In 2009, he joined NIMTE, CAS as a PI. He has published over 210 peer-review papers, H-index = 50, four books, and seven book chapters and has been awarded 65 invention patents. His lab focuses on using nanoprobe for early diagnosis and therapy of diseases and so forth.

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