

Review

Surface-enhanced Raman spectroscopy for glioma: from molecular profiling to intraoperative navigation

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Abstract

High-grade gliomas, especially glioblastoma, are the most malignant primary tumors of the central nervous system and have a poor prognosis due to their highly invasive growth. The precise detection and delineation of tumor margins are still significant challenges in present diagnosis and surgery. Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive spectroscopic technique that leverages plasmonic enhancement, and thus it has been employed to perform molecular-level diagnosis of glioma. Although SERS has been used to some extent in the diagnosis of gliomas, an integrated, multifaceted platform that combines molecular detection, surgical guidance, and artificial intelligence (AI)-assisted analysis for gliomas has not been built yet. Here, we present the general contents of gliomas based on the combination of SERS-enabled liquid biopsy, molecular profiling, intraoperative navigation, and AI for spectral analysis. On this basis, we examine the main translation problems that have arisen in SERS, such as blood-brain barrier crossing, substrate consistency, AI explainability, and safety, and put forward plans for the clinical application of SERS in precision neuro-oncology. Collectively, these advances highlight the considerable potential of SERS to improve glioma diagnosis, intraoperative guidance, and precision neuro-oncology.

Keywords: glioma; surface-enhanced Raman spectroscopy (SERS); liquid biopsy; intraoperative navigation; artificial intelligence (AI)

1. Introduction

Although there have been many achievements in neuroimaging and molecular pathology, the precise diagnosis and accurate demarcation of glioma margins are still problems to be solved in clinical practice [1-3]. The highly invasive and heterogeneous nature of gliomas often leads to indistinct tumor boundaries, which significantly compromises the completeness of surgical resection and ultimately affects patient prognosis [4-9]. Therefore, the current problem is that there is a lack of analysis methods that can provide timely molecular information for diagnosis and other decisions during surgery.

Currently, the main way to assess gliomas before surgery is by means of magnetic resonance imaging (MRI) and histopathological examination; both are still required in actual clinical work [10,11]. In addition to the traditional structure of MRI, other high-end imaging methods are also applied, such as magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), and perfusion-weighted imaging

(PWI), which can provide more details on tumor metabolic state, the organization of white matter tracts, and vascular characteristics [12-15]. The above methods have greatly advanced the diagnosis of gliomas radiologically, but they are unable to directly reveal molecular changes. Histopathological biopsy can provide definitive diagnostic information, but it is invasive and may not fully represent the marked spatial heterogeneity of gliomas due to sampling only a small part of the tumor [16,17]. Positron emission tomography (PET) offers additional metabolic insight, yet its routine use is constrained by limited spatial resolution, high cost, and restricted accessibility [18,19]. In short, these limitations underscore the need to integrate molecular information with traditional imaging to help us better diagnose and treat patients [20,21].

At present, the main treatments for gliomas are surgical resection, radiotherapy, and chemotherapy [22-24]. Glioblastoma is relatively diffuse, and it is easy

for the cells to spread along white matter pathways and perivascular spaces in the surrounding brain tissue [25]. Consequently, the true tumor boundary is often indistinct during surgery, making complete resection difficult while increasing the risk of postoperative neurological deficits if excessive tissue is removed. In recent years, in an attempt to increase the precision of tumor localization before and during neurosurgery, many kinds of imaging guidance have begun to be used, including intraoperative magnetic resonance imaging (iMRI), intraoperative ultrasound and 5-aminolevulinic acid (5-ALA) fluorescence imaging [26–28]. Each mode of imaging has its own advantages and disadvantages. Although iMRI can compensate for intraoperative brain shift, update tumor residual status, and improve extent of resection (EOR), it is equipment-intensive and may extend the time for surgery. Intraoperative ultrasound has the advantage of real-time imaging, but it is subject to the skill of the operator and has weak tissue contrast. In addition, infiltrative tumor margins and many low-grade gliomas have reduced the diagnostic accuracy of 5-ALA-guided fluorescence because there is a weak or no fluorescence signal. Therefore, other kinds of technologies that can provide timely molecular information to help clarify the boundary of gliomas in surgery more precisely need to be developed.

Surface-enhanced Raman spectroscopy (SERS) has become a powerful analytical platform for ultrasensitive and multiplexed molecular detection, relying on plasmon-mediated signal amplification at nanostructured interfaces [29–31]. By means of the localized surface plasmon resonance (LSPR) effect of noble metal or semiconductor nanostructures [32,33], SERS can increase the Raman signal of molecules by several orders of magnitude. Many kinds of substances in the body, such as metabolites, proteins, nucleic acids, and extracellular vesicles, can be recognized specifically by SERS through the production of different vibrational fingerprints for each biomolecule [34–36]. Importantly, it is particularly attractive for intraoperative applications because it is less susceptible to photobleaching compared to fluorescence-based techniques and allows for real-time detection.

With the development of nanomaterials and data-driven analysis technology in recent years, some progress has also been achieved in the application of SERS to glioma research [37,38]. These advances have allowed for not only sensitive biomarker detection in complex biological matrices but also real-time tumor margin identification and intraoperative guidance. Recent advances in artificial intelligence (AI) have substantially expanded the analytical capability of SERS by enabling the extraction of diagnostically

relevant information from complex, high-dimensional spectral datasets. Although many reviews have summarized the application of SERS in cancer diagnosis or pointed out developments in SERS technology, relatively few have specifically investigated its role at all stages of glioma diagnosis and treatment [39–41]. With the continuous development of nanotechnology, spectroscopy, and data-driven analytical methods, more and more attention is being paid to how these developments can be integrated into the clinical workflow for glioma treatment and how to address existing technical and translation problems. To date, there is no particular review article that has covered all the new developments in SERS-based liquid biopsy, molecular profiling, intraoperative guidance, AI-assisted spectral interpretation, and clinical application of gliomas from all these areas comprehensively.

Recently, some progress has been made in SERS for molecular diagnosis and surgical treatment of gliomas. This paper will briefly introduce the current situation of SERS in glioma research, such as its basic enhancement mechanism, the development of substrates, and typical applications in biomarker detection and tissue identification. We further critically evaluate the key barriers limiting clinical implementation and outline future directions for translating SERS from laboratory research to real-world neuro-oncology practice. This work aims to provide a systematic framework for the development of next-generation SERS platforms in precision neuro-oncology.

2. Mechanistic Basis of SERS Enhancement

To enhance the weak Raman signal, SERS has been applied to achieve sensitive molecular analysis by way of substrate mediation [42–44]. Signal amplification is generally the result of the combined effects of electromagnetic (EM) and chemical enhancement (CE) [45–47]. Therefore, in order to design a SERS platform for the detection of glioma biomarkers and guide surgery reasonably, we first need to know how the two mechanisms work together.

EM enhancement originates primarily from LSPR, which generates intense electromagnetic fields at nanoscale junctions known as hotspots. Molecules located within these hotspots will have Raman intensities that are several orders of magnitude higher [48,49]. Given that it can be applied to all kinds of molecules, EM enhancement is used as the main way in most biological SERS systems. However, the spatial distribution and stability of hotspots are still one of the main reasons for variations in signal reproducibility in complex biological samples [50,51]. Although there are

defects mentioned above, EM-based substrates can also be used to detect all kinds of glioma-associated molecular targets, such as metabolites, proteins, and nucleic acids [52-54].

Unlike EM enhancement, CE is confined to molecules in direct contact with the substrate and arises from interfacial electronic interactions, predominantly charge-transfer processes [55-57]. Chemisorption can modify the molecular electronic structure and alter the molecular polarizability, thereby influencing Raman scattering intensity [58,59]. Although the enhancement factor of CE is generally lower than that of EM, it offers the advantage of being applicable to different systems based on semiconductors or hybrid materials, and the charge-transfer pathway can be deliberately tuned to increase some biomolecular signals. Such substrate-dependent selectivity is particularly valuable for distinguishing glioma tissues and analyzing complex biological fluids, where chemically similar components often coexist.

Hybrid substrates, particularly metal-semiconductor systems, provide a representative example of the synergy [60,61]. In such systems, the generation of strong electromagnetic fields in plasmonic metals and charge-transfer interactions in semiconductors lead to combined EM and CE enhancement [62,63]. The dual mechanism not only increases overall signal intensity but also can

modulate spectral features, thereby improving molecular discrimination.

Table 1. Relationship between SERS enhancement mechanisms and representative glioma applications.

Enhancement mechanism	Main feature	Typical glioma application	Current challenge
Electromagnetic enhancement (EM)	Strong signal amplification generated by plasmonic hotspots	Label-free tissue analysis, tumor margin delineation, single-cell profiling	Signal variability caused by hotspot heterogeneity
Chemical enhancement (CE)	Charge-transfer-mediated signal enhancement with molecular selectivity	Metabolite sensing, biomarker analysis in biofluids	Relatively low enhancement factor
Hybrid enhancement	Electromagnetic amplification and chemical selectivity	Molecular classification, multiplex biomarker detection	Complex substrate fabrication and optimization

As shown in Table 1, the applications of each enhancement effect in glioma are presented. While EM enhancement ensures high sensitivity across a broad range of analytes, CE contributes to selective signal amplification for specific molecular species [64,65]. Thus, the balance between these mechanisms directly impacts the accuracy and reliability of SERS-based detection.

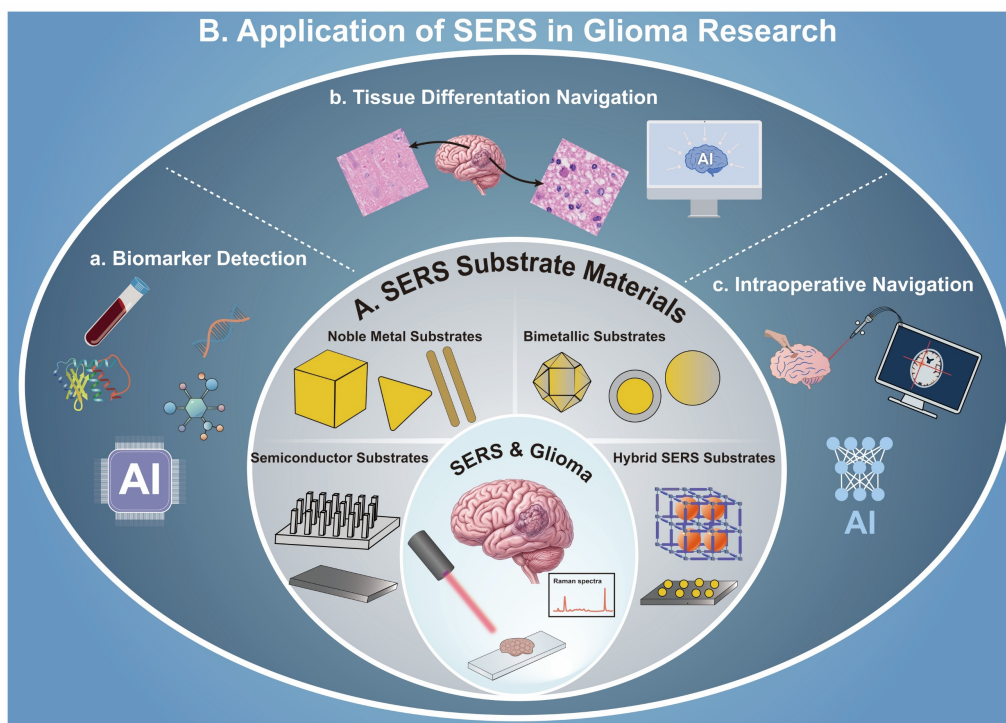


Figure 1. Overview of SERS strategies for glioma research: (A) SERS substrate materials and (B) applications of SERS in glioma research, encompassing (a) biomarker detection, (b) tissue differentiation and navigation, and (c) intraoperative navigation, with artificial intelligence (AI)-assisted spectral analysis and data interpretation facilitating SERS-based glioma diagnosis and clinical translation.

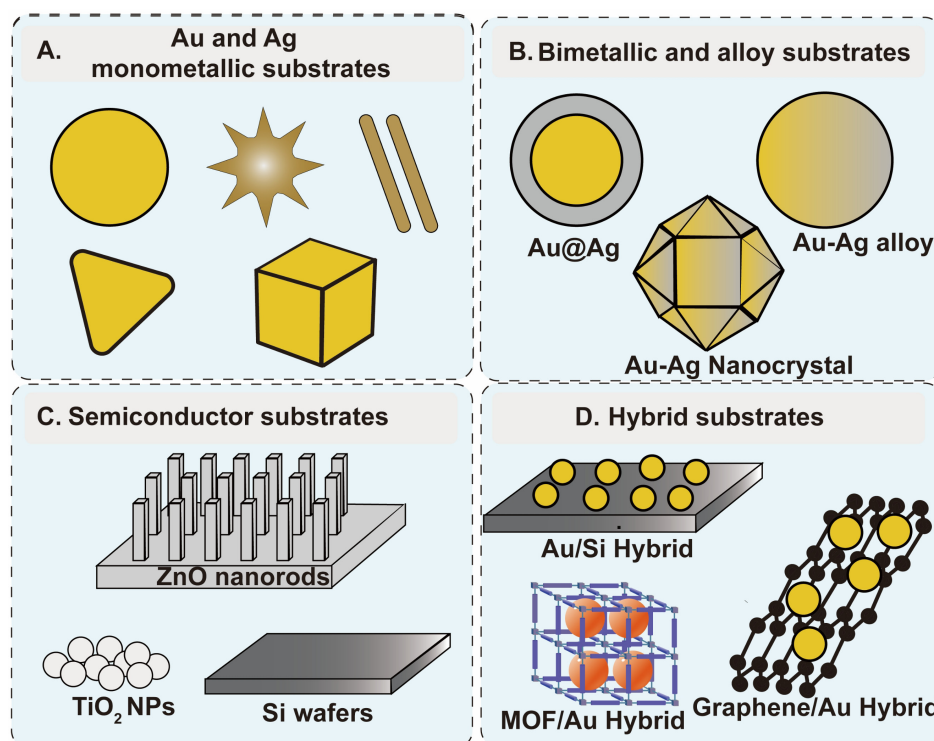


Figure 2. Overview of SERS substrate materials: (A) Au and Ag monometallic substrates, (B) bimetallic and alloy substrates, (C) semiconductor substrates, and (D) hybrid substrates.

To sum up, the mechanisms of SERS enhancement can help us develop more reasonable substrate engineering strategies in the next part. It is necessary to better control the distribution of hotspots and improve the reproducibility and customization of molecular interactions. On the basis of reasonably regulating the above two enhancement mechanisms, new SERS substrates for glioma have been developed.

3. SERS Substrates for Glioma Applications

The performance of SERS in glioma research fundamentally depends on the design of the substrate material. Substrate design affects not only signal enhancement but also reproducibility, biocompatibility, and ultimately, clinical applications [66,67]. As shown in Figure 2, the commonly used SERS substrate materials are mainly noble metals, which are combined with other materials (e.g., semiconductors, graphene and metal-organic frameworks (MOFs)) to form composite materials, in order to further enhance the performance of the substrate in biosensing and improve biocompatibility [68-72].

3.1 Hotspot Engineering for Signal Enhancement

The essence of SERS lies in the spatial confinement of electromagnetic fields into localized

hotspots, which govern the overall signal amplification. These hotspots typically form in regions of strong plasmon coupling, such as nano-gaps, sharp tips, and interparticle junctions [73-75]. In recent years, researchers have conducted a series of studies in the biological field by designing specific hotspot regions using nanomaterials with different morphologies, including nanostars, nanorods, and nanoflowers [76-78]. For example, Au nanoflowers modified on the SERS substrate constructed from two-dimensional red phosphorus nanosheets enable enhanced detection of tumor markers; multi-branched Au nanostars provide superior stability and enhancement for the detection of biomolecules [79,80].

In addition to SERS substrates with different morphologies, constructing nanostructures with controllable interparticle gaps at smaller scales (such as sub-2 nm) has a stronger enhancement effect. For example, sub-2 nm quasi-two-dimensional plasma nano-SERS substrate composed of a nanoparticle-on-mirror (NPoM) geometry and microplate-on-foil (MPoF) nanogaps can achieve in situ spatiotemporal detection with sub-nanoscale resolution [81]. Furthermore, the development of ordered nanoparticle assemblies and hierarchical architectures significantly amplifies plasmonic coupling and enhances hotspot density [82-84]. These structural innovations enable the ultrasensitive detection of low-abundance biomarkers, including circulating tumor

DNA, specialized metabolites, and exosomes [85-87]. As an illustration, a study reported a three-dimensional layered gold nanostructure SERS substrate formed by the self-assembly of head-cluster-shaped gold nanocolumns and DNA-modified gold nanoparticles, which can be used for label-free, ultra-sensitive detection of microRNAs [88]. Although the SERS substrate was developed for the detection of biomarkers in urine or serum, its excellent Raman enhancement effect provided a solid technical foundation for the detection of trace or ultra-trace biomarkers associated with gliomas in biological matrices [89-91]. However, the actual clinical applications of noble metal nanomaterials are not yet mature, and there are problems with long-term stability in the body, inconsistent signals, etc.

3.2 Hybrid and Multifunctional SERS Platforms

In recent years, hybrid SERS substrates have begun to be applied for the detection of biomarkers in that they can combine the various attributes of individual materials [92,93]. These systems can combine multiple functional components to achieve synergistic amplification and expanded analytical capabilities [94,95]. Metal-semiconductor heterostructures combine the strong electromagnetic enhancement effects of plasmonic metals with the chemical enhancement resulting from interfacial charge transfer interactions, thereby enhancing the amplification effect of the substrate material and meeting the requirements for high-sensitivity detection [96,97]. For example, by integrating inorganic perovskite-coated gold nanoparticles (Au NPs) onto nanocages to form a specific core-shell structure, which was then used as a SERS substrate, highly sensitive detection of neuron-specific enolases can be achieved [98,99].

Moreover, carbon-based materials, such as graphene and carbon nanotubes, are employed to enhance molecular adsorption and to inhibit fluorescence background, resulting in a significant improvement of signal-to-noise ratios in complex biological samples [100-102]. For example, Au NPs with graphene oxide (AuGO) can be used as a SERS substrate for ultra-trace detection of tumor markers [103]. Simultaneously, porous materials such as MOFs provide a tactical advantage in terms of selective enrichment and size-selective sieving of the analytes [104,105]. This is particularly beneficial for isolating low-abundance glioma markers from the protein-rich environment of cerebrospinal fluid. For instance, Au NPs-modified MIL-125(Ti) framework (Au/MIL-125(Ti)) can be assembled as a robust platform for rapid and non-invasive detection [106].

The above literature provides a brief description of common SERS substrate materials and their applications in the biological field. However, due to differences in their properties, the SERS substrate materials can be applied in various ways in glioma research. Surface plasmon substrates (such as Au nanostars, Au nanoflowers, and NPoM structures) typically provide the highest signal enhancement and are therefore more suitable for detecting low-abundance biomarkers, including ctDNA and microRNA. Hybrid substrates composed of graphene, MOFs, or semiconductors exhibit higher molecular selectivity and are particularly well-suited for metabolite and protein analysis in complex biological samples. For glioma tissue detection and intraoperative navigation, SERS substrate materials must demonstrate excellent uniformity and reproducibility. Consequently, ordered surface plasmon arrays and multifunctional nanoplateforms are more suitable. In general, the development of SERS substrate engineering has been shifting from simple material selection to application-oriented and multifunctional design strategies. Precise control of the architecture of hotspots can improve biocompatibility and the combination of multiple functions has significantly broadened the prospects of SERS in glioma research.

3.3 Biocompatibility and In Vivo Applicability

Biocompatibility of SERS substrates becomes a major consideration for applications with living systems, particularly for intraoperative navigation and in vivo sensing [107,108]. This is necessary since the central nervous system is immune-privileged and very sensitive to exogenous nanomaterials. To overcome these translational bottlenecks, surface modification and functionalization strategies have been extensively researched.

Biocompatibility, specific recognition ability and stability of biological samples can be greatly enhanced by modifying SERS substrates with polydopamine and surface molecularly imprinted polymers [109,110]. Polymer coatings such as polyethylene glycol (PEG) are commonly employed to prevent non-specific protein adsorption and to prolong the systemic circulation time in vivo [111]. In addition, biomimetic strategies such as cell membrane coating have provided a powerful tool to facilitate immune evasion and active transport across the blood-brain barrier (BBB) [112,113]. For instance, previous studies have reported the utilization of SERS nanomaterials coated with cell membranes derived from glioblastoma for the treatment of gliomas. This coating with autologous cell membranes enhances the ability of the material to cross the BBB, thereby improving the penetration of

SERS nanoprobe into tumor regions to some extent [114].

Furthermore, in addition to the approaches mentioned above, SERS nanoprobe can be designed to specifically recognize tumor-associated biomarkers using biological recognition elements such as antibodies, peptides, and aptamers as targeting ligands, thereby achieving the goal of preferential enrichment of SERS nanoprobe within tumor tissue [115–117]. To illustrate, the biocompatibility and targeting specificity of the substrates can be effectively enhanced by functionalized $\text{Fe}_3\text{O}_4/\text{Au}$ nanosatellite structures and Au/Ag nanorod SERS substrates with specific nucleic acid aptamers and bovine serum albumin (BSA) [118]. The above methods improve the biocompatibility of nanomaterials by modifying their surface properties, thereby ensuring that real-time molecular imaging and precise guidance can be performed during neurosurgical procedures.

4. SERS Strategies for Glioma: From Molecular Detection to Surgical Guidance

With the development of nano-synthesis and spectroscopic analysis technology, SERS has also evolved from a characterization method into an applied technology, such as SERS for glioma diagnosis and intraoperative guidance [119,120]. At present, not only can SERS detect particular categories of

biomolecules, but it is also possible to simultaneously identify many different types of biomolecules for molecular profiling, tissue differentiation and surgical navigation. With the help of AI, spectral analysis, feature extraction and database construction can be carried out on the obtained molecular spectra for clinical application (Figure 3).

4.1 SERS for Molecular Profiling and Liquid Biopsy

Molecular detection using SERS plays a key role in glioma research, particularly in the fields of liquid biopsy and early diagnosis. Compared to traditional tissue biopsies, SERS offers a rapid, ultra-sensitive, and minimally invasive method for detecting tumor-associated molecular biomarkers in biological fluids [121].

4.1.1 Metabolic Profiling of Glioma Microenvironment

Metabolic reprogramming is one of the hallmark features of gliomas, including pH, glutathione (GSH), hydrogen peroxide (H_2O_2), and hydrogen sulfide (H_2S), which reflect the redox state and metabolic activity of tumor cells [122–125]. Due to its fingerprint-like spectra and high sensitivity, SERS can be used to detect the above metabolic molecules and provide information related to the occurrence and development of gliomas [126].

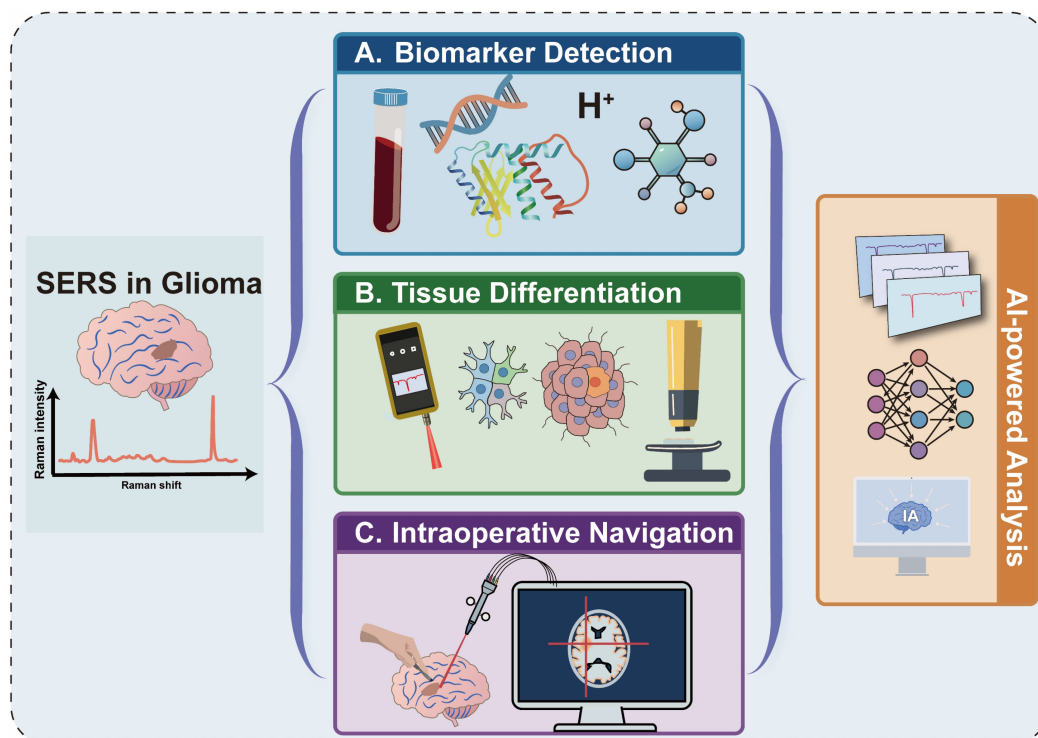


Figure 3. Overview of SERS-based approaches for glioma diagnosis and surgery: (A) biomarker detection, (B) tissue differentiation, and (C) intraoperative navigation assisted by AI-powered spectral analysis.

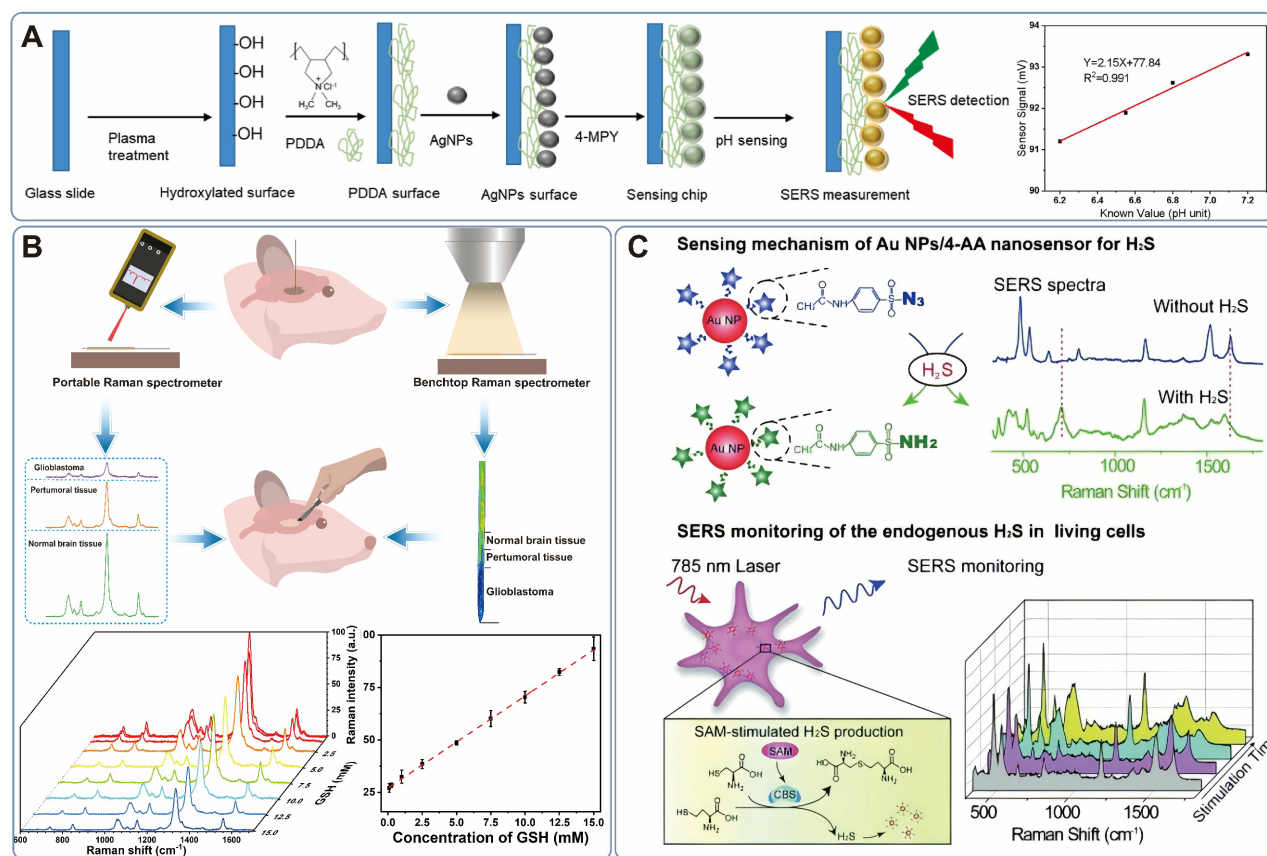


Figure 4. (A) Schematic diagram of the preparation of a pH-responsive SERS sensing chip and standard curve for microelectrode pH detection. Reproduced with permission from Ref. [127]. Copyright 2022, Elsevier. (B) Schematic diagram of the preparation and application of DTNB@Au@Ag microneedles for deep GBM tissue differentiation and Raman spectra and linearity plots of GSH measured using DTNB@Au@Ag microneedles. Reproduced with permission from Ref. [128]. Copyright 2024, American Chemical Society. (C) SERS nanosensors for monitoring endogenous H₂S in living glioma cells. Reproduced with permission from Ref. [129]. Copyright 2015, Wiley-VCH.

For instance, a ratio-type pH-responsive SERS sensor chip was developed using silver nanoparticles (Ag NPs) modified with pH-sensitive reporter molecules (such as 4-mercaptopyridine (4-MPY)) [127]. This SERS chip was able to accurately identify tumor margins in nude mouse models, enabling maximal tumor resection and thereby improving patient prognosis (Figure 4A). Beyond pH sensing, the high concentration of GSH in GBM has been leveraged to develop active diagnostic tools. GSH-responsive SERS microneedle (DTNB@Au@Ag) as a novel diagnostic tool can be used for the rapid identification of GBM tissue (Figure 4B). Evaluations in orthotopic tumor models and ex vivo patient samples confirmed that the microneedles could accurately distinguish malignant regions from healthy brain tissue, providing a real-time molecular solution for precision surgery [128].

Furthermore, Li *et al.* designed an azide-modified Au NPs sensor for the specific detection of H₂S [129]. By leveraging the H₂S-mediated reduction of azides to amines to induce a distinct spectral shift, the sensor achieved a response time within 1 min, enabling the real-time monitoring of endogenous H₂S flux within GBM cells (Figure 4C). Despite the high sensitivity of

metabolic sensors, the specific detection of individual metabolites can be confounded by the spectral overlap inherent in complex biological matrices.

4.1.2 Proteomic Biomarker and Nucleic Acids Detection

Metabolic molecules provide limited effective information due to their relatively simple structures. Therefore, in order to detect metabolic small molecules, SERS is widely used to analyze proteins and nucleic acids associated with glioma, thereby providing more direct information about tumor genotype and phenotype.

Compared to antibodies, aptamers exhibit greater stability and lower nonspecific binding. Consequently, SERS sensors constructed using aptamers have gradually been adopted for biomarker detection. For example, a pulse electro-deposited WO₃ film modified with methylene blue-labeled VEGF aptamers can be used to detect angiogenic biomarkers in serum [130]. This platform detects VEGF by exploiting fluctuations in the Raman signal caused by conformational changes, with a limit of detection (LOD) as low as 8.7 pg/mL, providing a reliable method for monitoring angiogenic biomarkers in

serum (Figures 5A and 5B). In another study, Liu *et al.* developed a site-specific, antibody-functionalized Ag@Au NIs LSPR biosensor for the label-free detection of MCT4, which is a key biomarker for GBM progression (Figure 5C). By exploiting the synergistic plasmonic enhancement between Ag NPs and Au nanodots, the sensor achieved ultrasensitive quantification of CD63 and MCT4 with detection limits as low as 0.38 ng/mL and 1.4 ng/mL, respectively, establishing a new platform for metabolism-related protein profiling in liquid biopsies [131].

Circulating nucleic acids are an important research subject in cancer detection, providing crucial

information for tumor development and progression [132–134]. To harness the information for glioma screening, dual-signal amplification platforms have been engineered by integrating SERS with catalytic hairpin assembly (CHA) and magnetic enrichment strategies (Figure 6A) [135]. This method employs Au-Ag nanorods as an enhancement substrate and magnetic beads as capture carriers, thereby enabling ultra-high-sensitivity simultaneous detection of glioma-associated ctDNA (IDH1 R132H, BRAF V600E) in mouse serum with attomolar sensitivity, representing a significant leap in the early diagnosis of glioma (Figure 6B).

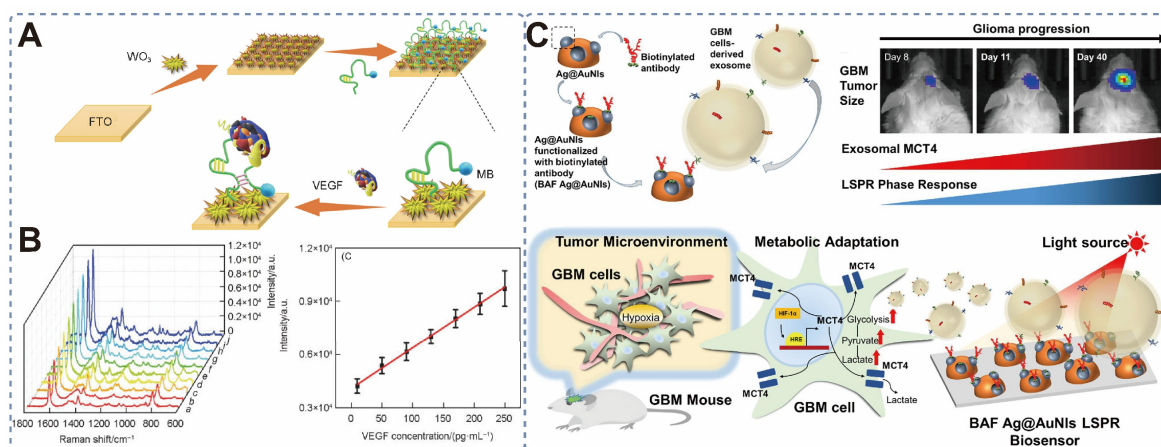


Figure 5. (A) Construction of SERS aptasensor based on WO₃ film for the determination of VEGF. (B) Raman spectra and linearity plots of VEGF at different concentrations detected using SERS aptamer sensors. Reproduced with permission from Ref. [130]. Copyright 2021, Springer-Nature. (C) Schematic illustration of the liquid biopsy of glioblastoma by the detection of MCT4 in blood-derived exosomes using a BAF Ag@Au NIs LSPR biosensor. Reproduced with permission from Ref. [131]. Copyright 2022, Elsevier.

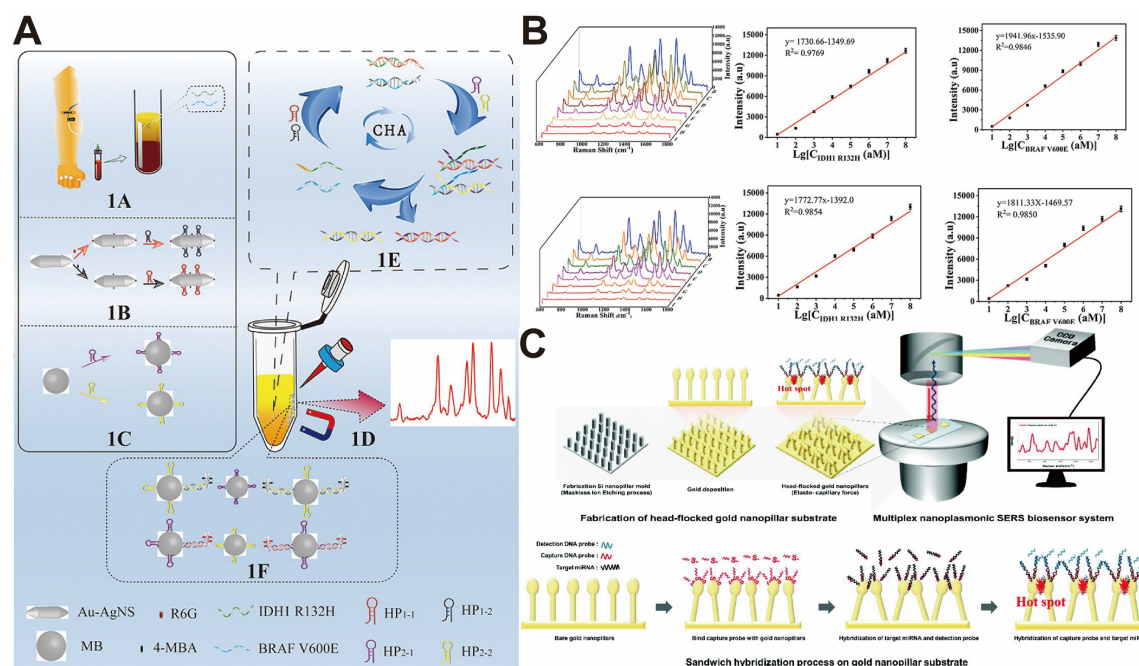


Figure 6. (A) Schematic diagram of SERS-assisted detection of glioma-associated ctDNA based on dual-signal amplification. (B) SERS spectra and corresponding linear calibration curves of IDH1 R132H and BRAF V600E at different concentrations in PBS buffer and serum. Reproduced with permission from Ref. [135]. Copyright 2023, Taylor & Francis Group, LLC. (C) Schematic of the multiplex SERS nanoplasmonic biosensor using head-flocked gold nanopillars for the label-free detection of miRNA. Reproduced with permission from Ref. [138]. Copyright 2019, The Royal Society of Chemistry.

Micro-RNAs (miRNAs) are highly stable in human body fluids and exhibit significantly abnormal expression levels during tumorigenesis or metastasis, making them ideal non-invasive biomarkers for gliomas [136,137]. To analyze glioma-associated miRNAs, researchers prepared a head-flocked gold nanopillar SERS substrate, utilized liquid-induced aggregation to form high-density signal hotspots, and combined this with nucleic acid sandwich hybridization technology to achieve label-free, multiplex detection of glioma-associated miR-10b, miR-21, and miR-373 (Figure 6C). This method is sensitive to femtomolar concentrations, can distinguish single-base mismatches, and has excellent stability of the substrate in serum [138]. Hence, the method is suitable for non-invasive screening, metastasis assessment, and prognosis evaluation of gliomas and other tumors, offering a novel detection strategy for liquid biopsy in cancer. The development of the above sensing methods demonstrates the immense potential of SERS in the molecular diagnosis of gliomas. However, there are still some problems that need to be addressed in the next stage of clinical application of SERS detection, such as weak signals and interference in complex biological environments.

4.1.3 Extracellular Vesicles (EVs)

Extracellular vesicles (EVs) are membrane-bound vesicles that exist in the cells of all types of organisms, including prokaryotes, higher eukaryotes, and plants [139]. The goal of EVs is to pass information to other cells and alter the function of the recipient cells. EV-mediated signals can be carried by all types of biomolecules, such as proteins, lipids, nucleic acids, and carbohydrates [140]. This specific kind of enclosure is protective, and it can carry a large number of messenger molecules to different places far from the starting point of the vesicle. Therefore, through the analysis of SERS-tagged extracellular vesicles from glioma, we can obtain some useful information for the diagnosis or intraoperative navigation of glioma.

Since the cell-free vesicles still have membrane proteins that can recognize their origin cells, we can use these surface markers to select for them and then study how they change in the development and progression of gliomas. Based on the above idea, Zhang and others have developed the GEMPAC nanodiagnostic platform to detect brain-specific proteins (ATP1B2 and EAAT2) and stem cell markers (CD24 and CD44) in extracellular vesicles simultaneously by means of multiplex SERS [141]. Rather than providing a single static measurement, this platform enables longitudinal monitoring of tumor evolution and treatment-induced drug resistance, offering a practical strategy for evaluating

therapeutic efficacy over the course of disease management.

In addition, nanophotonic-integrated microfluidic platforms have been developed for the label-free detection of EVs [142]. Through principal component analysis (PCA), the platform successfully distinguished tumor-derived vesicles from normal glial EVs with high accuracy (Figures 7A and 7B). Similarly, Mahshid *et al.* introduced a MoSERS microfluidic device integrating MoS₂-plasmonic nanocavity arrays [143]. This setup allowed for molecular profiling at the single-EV level, identifying key alterations, such as EGFRvIII mutations and MGMT expression, with 87% diagnostic accuracy in GBM patient blood samples (Figures 7C and 7D).

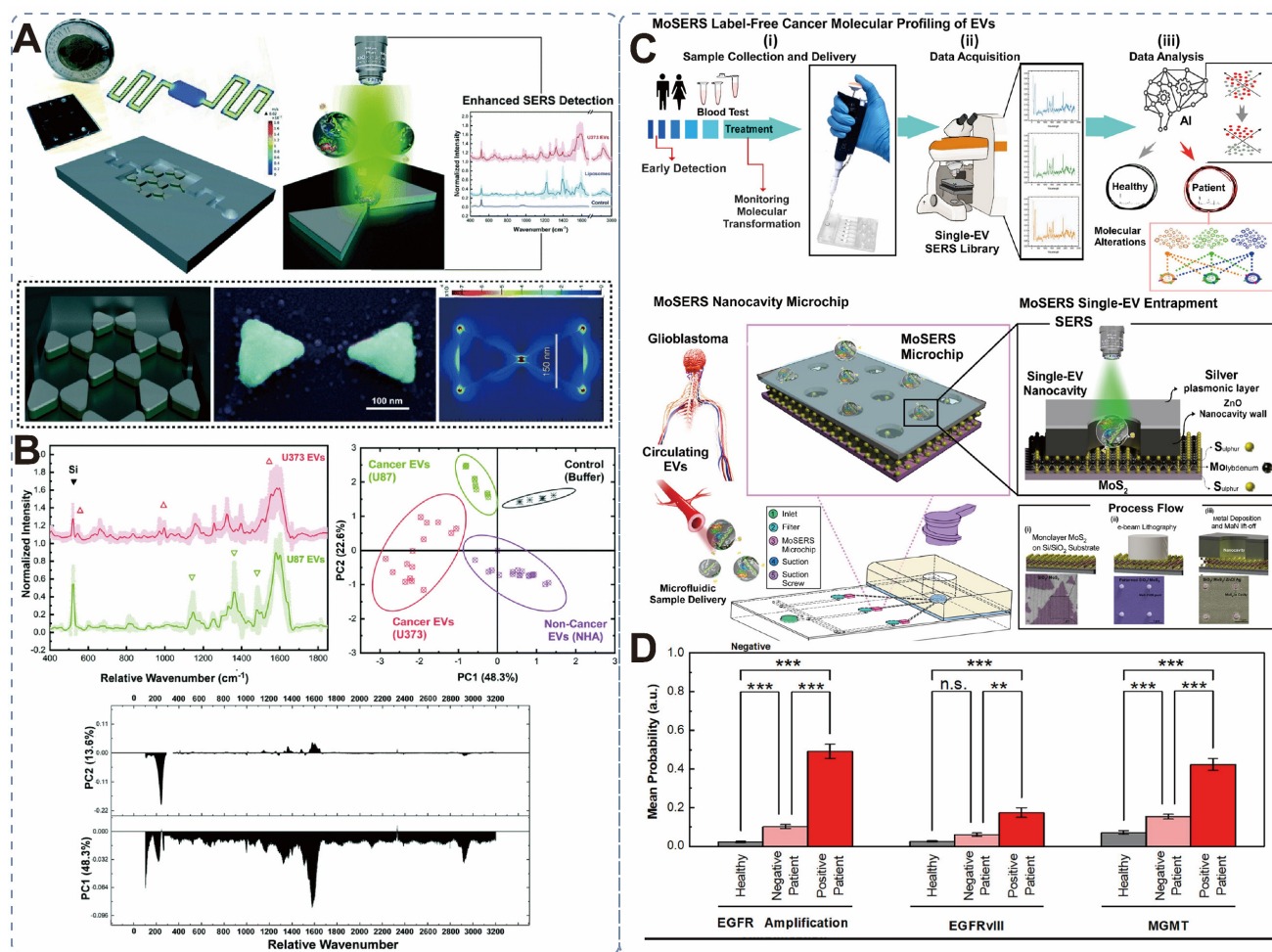
Furthermore, researchers have incorporated machine learning (ML) into SERS testing to analyze extracellular vesicle subtypes and predict tumor progression [144,145]. The machine learning-based SERS detection platform is capable of rapidly analyzing acquired SERS spectra, which significantly reduces the time required for data analysis during surgery and marks an important step forward in the transition of this technology from laboratory testing to clinical testing. For instance, self-functionalized Ni-Au/Pd superlattice sensors have been coupled with partial least squares discriminant analysis (PLS-DA) to achieve the direct detection of glioblastoma stem cell-derived EVs (GBM CSC-EVs) from serum without the need for prior isolation (Figure 8A). This platform achieved an amol-level sensitivity and 100% sensitivity in a limited cohort in clinical validation [146]. Furthermore, the characterization of circulating immune vesicles (CIVs) has been advanced through the use of femtosecond laser-engineered nickel quantum probes combined with artificial neural networks (ANN) (Figure 8B). This immunological analysis strategy achieved a sensitivity of 96.82% in glioblastoma diagnosis. By recognizing the specific expression of PD-L1 and CTLA-4, this platform can effectively distinguish glioblastoma from other malignant tumors of the central nervous system [147].

Although SERS technology has been used to detect various glioma-related biomarkers, the progress of clinical translation varies significantly among different types of biomarkers. CtDNA and EVs-based biomarkers show the greatest potential for clinical application, as both provide tumor-specific molecular information and have been validated in clinical studies of liquid biopsy. Exosomes have become a research hotspot due to their ability to cross the BBB and protect tumor-derived components from degradation. In contrast, metabolic biomarkers such as GSH and H₂S lack specificity for glioma and lack sufficient clinical validation data; therefore, they

remain primarily in the basic research stage at this stage. Protein biomarkers contain a wealth of biological information and can provide insights into tumor angiogenesis and tumor progression. However, their low specificity and susceptibility to endogenous degradation limit their clinical application. Therefore, future SERS-based clinical assays could develop protein biomarkers with high specificity for gliomas and employ a series of modifications to reduce the likelihood of degradation. The above experiments can be used in conjunction with the platform for exosome detection in liquid biopsies to improve the sensitivity of the detection. In addition, advances in AI have greatly facilitated the application of SERS in liquid biopsy for gliomas. AI-powered spectral analysis can

extract subtle molecular features from complex Raman data, thereby shortening diagnostic time. At the same time, AI-powered spectral analysis methods not only improve the accuracy of biomarker classification but also enable multiplex biomarker analysis and further facilitate the automated analysis of liquid biopsy data. The new SERS protocol will have good clinical value for the diagnosis of glioblastoma and changes over time.

Overall, SERS molecular analysis technology enables non-invasive, multiplex detection of glioma markers, but its clinical translation is still limited by the reproducibility of substrate materials and interference from complex substances in biological matrices.



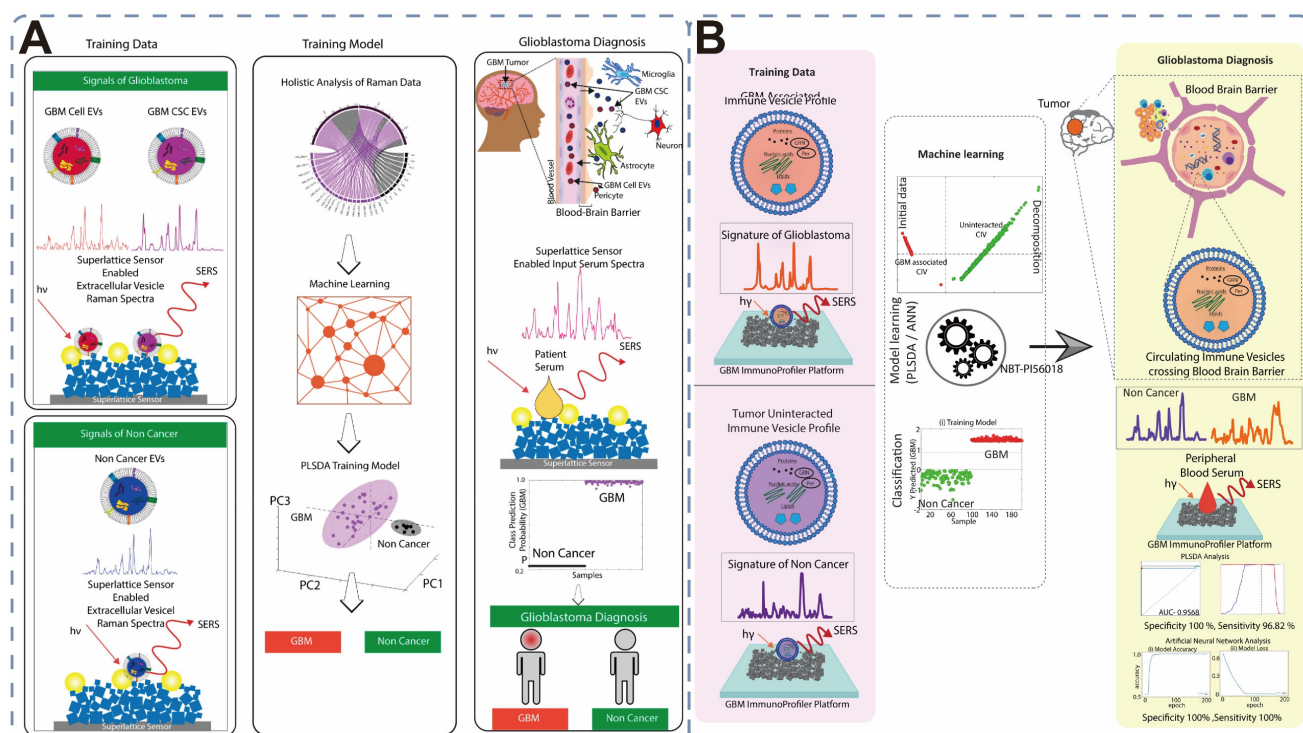


Figure 8. (A) Schematic illustration of a 3D superlattice sensor capturing extracellular vesicles and generating significantly enhanced SERS signals, combined with machine learning to distinguish between serum samples from healthy individuals and those with brain glioma. Reproduced with permission from Ref. [146]. Copyright 2023, American Chemical Society. (B) Schematic illustrating the utilization of SERS for the detection of extracellular vesicles associated with glioblastoma and the application of supervised machine learning classifiers for glioblastoma. Reproduced with permission from Ref. [147]. Copyright 2022, American Chemical Society.

4.2 Cellular-Level Analysis: Mechanistic Insights and Limitations

Liquid biopsy is generally used for the detection and analysis of biomarkers, while SERS can be used to examine individual cells or a small number of cells to obtain more information about the molecular condition of the tumor microenvironment by showing how nucleic acids, proteins, and lipids work together [148–150]. Single-cell and multicellular molecular profiling is essential to decipher intratumoral heterogeneity, identifying rare cell populations such as glioblastoma stem cells, and elucidating tumor development and progression.

4.2.1 Intracellular Molecular Mapping

The development of high-performance substrates is crucial for amplifying the Raman characteristic peaks of intracellular substances. A gold-coated black silicon (bSi/Au) SERS substrate was engineered, which can achieve an enhancement factor as high as 10^8 under 785 nm excitation (Figure 9A). This platform can successfully detect characteristic peaks of malignant transformation markers (e.g., nucleic acids, proteins, and lipids) in rat C6 glioma cells, providing a reliable solution for low-cost, scalable on-chip SERS

biosensing [151]. Floriana Burgio and colleagues prepared SERS probes by modulating the surface chemistry of Au NPs and conjugating Raman reporter molecules (1,4-BDT) and polyethylene glycol (PEG) with anti-EGFR antibodies in an optimized ratio [152]. Probes can accurately recognize glioblastoma cells and quickly and accurately distinguish between tumor cells and normal cells in vitro (Figure 9B).

4.2.2 Immunological Profiling in Peripheral Blood

Beyond direct tumor cell sensing, SERS excels in profiling tumor-associated immune cells. Glioblastoma-associated T cells (GBMAT/GSCAT) crossing the BBB can serve as systemic diagnostic proxies (Figure 10A) [153]. By integrating SERS with ML algorithms, the platform achieved a sensitivity of up to 100% and a specificity of 98.7% in a limited cohort for GBM diagnosis from patient blood, highlighting the potential for non-invasive immunological monitoring (Figure 10B).

Despite some progress in SERS detection for glioma cells, cellular-level SERS analysis still faces some inherent limitations, such as the heterogeneity of the intracellular environment, laser damage to cells, and the difficulty in obtaining individual cells during clinical surgery.

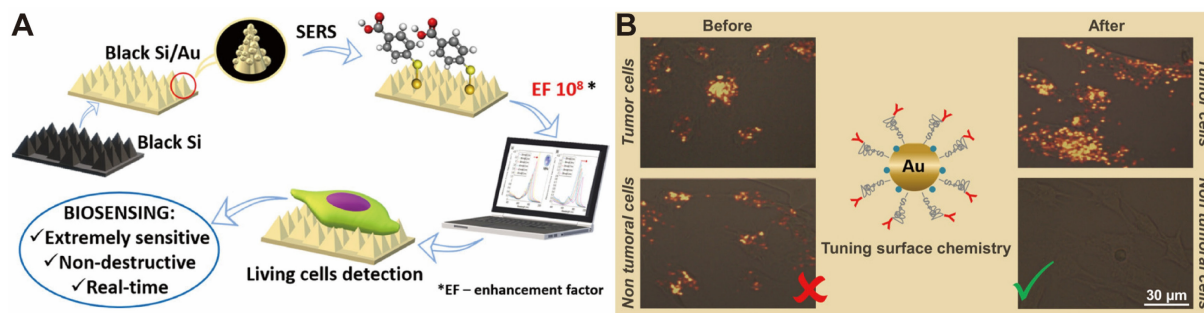


Figure 9. (A) Schematic illustration of SERS detection of trace amounts of small organic molecules in live C6 glioma cells from rats using a bSi substrate coated with a 25-50 nm Au layer. Reproduced with permission from Ref. [151]. Copyright 2020, American Chemical Society. (B) Schematic illustration of the specific detection of glioblastoma cells using SERS by modulating the surface chemistry of Au NPs. Reproduced with permission from Ref. [152]. Copyright 2020, American Chemical Society.

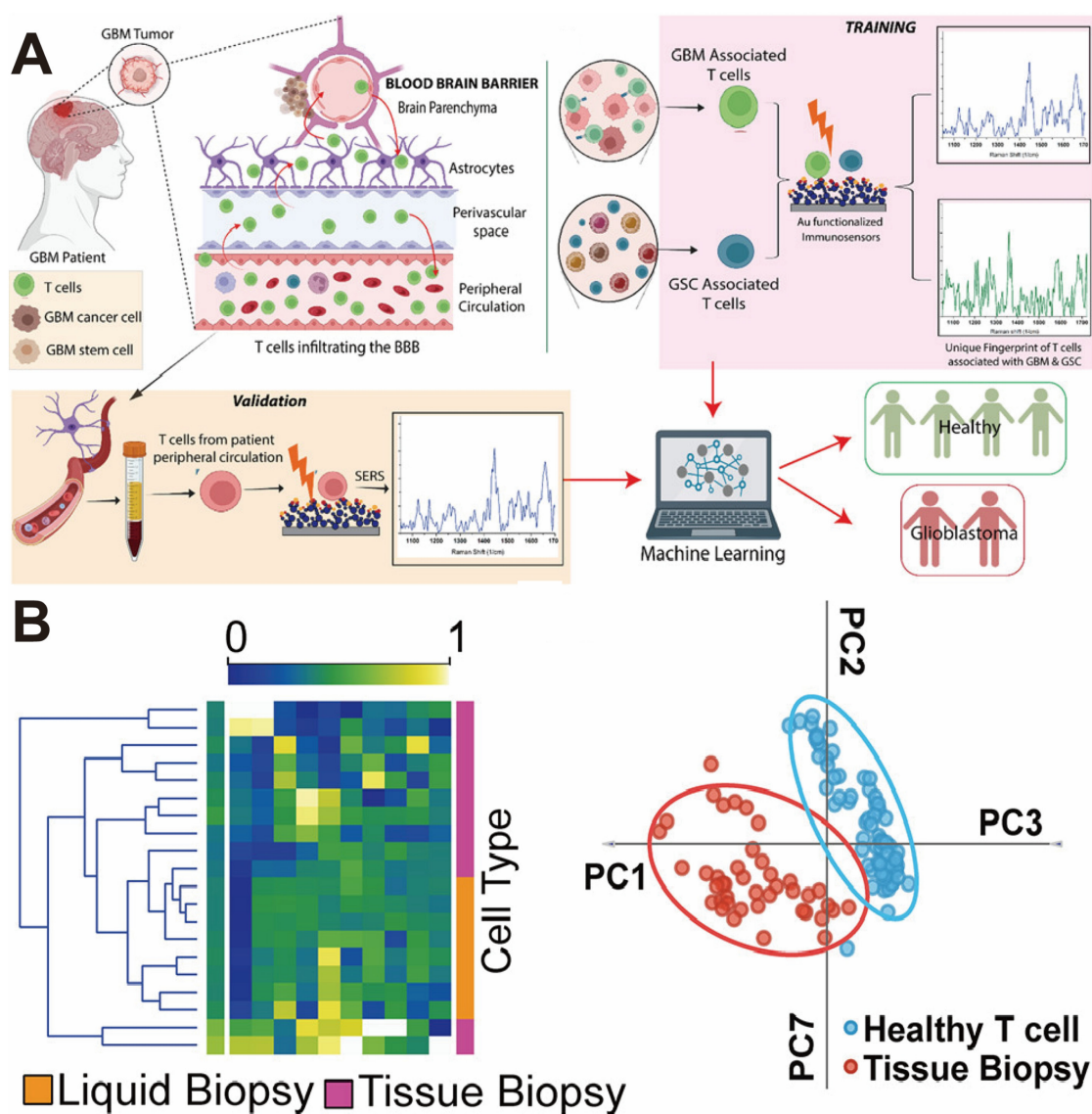


Figure 10. (A) Schematic illustration of SERS combined with machine learning for the diagnosis of glioblastoma using glioblastoma stem cell-associated T cells. (B) Comparison of SERS for GBM T-cell detection: PCA heatmaps of T cells from tissue biopsies and liquid biopsies, and PCA scatter plot showing differences between T cells from tissue biopsies and healthy T cells. Reproduced with permission from Ref. [153]. Copyright 2022, American Chemical Society.

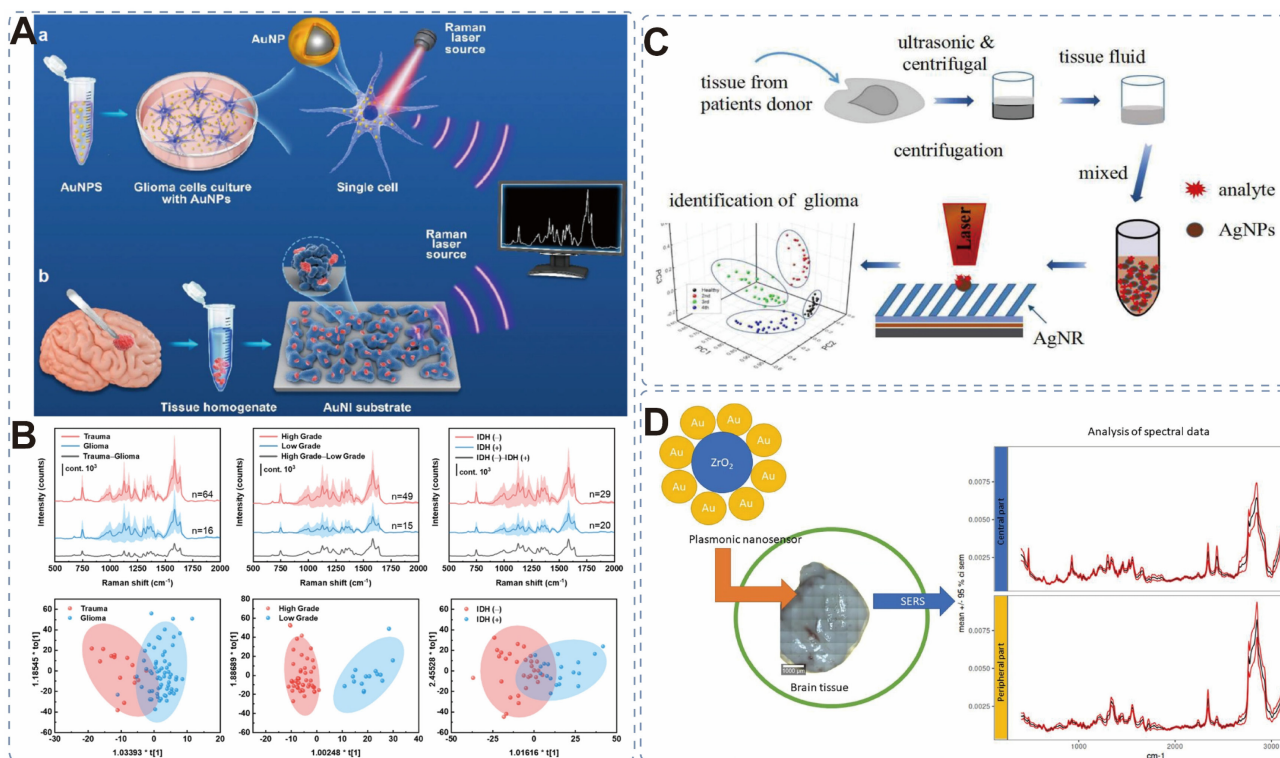


Figure 11. (A) Schematic diagram of the SERS detection process for glioma cells (a) and glioma tissue (b). (B) Average Raman spectra of different types of tissue samples and the corresponding OPLS-DA plots for the three groups. The shaded regions indicate the 90% confidence intervals. Reproduced with permission from Ref. [160]. Copyright 2024, Elsevier. (C) Schematic diagram of the processing of glioma tissue and the utilization of an Ag NPs@Ag NRs SERS substrate for tissue detection. Reproduced with permission from Ref. [161]. Copyright 2020, Elsevier. (D) Schematic diagram of Au@ZrO₂ plasmonic nanosensor combined with Raman spectroscopy for distinguishing brain tumor tissues. Reproduced with permission from Ref. [162]. Copyright 2024, Elsevier.

4.3 SERS for Intraoperative Tumor Margin Identification

Currently, SERS-based margin identification leverages two distinct yet complementary strategies: label-free intrinsic molecular fingerprinting and targeted functionalized nanoprobes.

4.3.1 Label-Free SERS Based on Molecular Fingerprinting

Label-free SERS utilizes the inherent vibrations of molecules to generate spectral fingerprints of biological tissues [154,155]. Compared to normal tissue, tumor tissue typically exhibits changes in its lipid, protein, and nucleic acid composition, resulting in spectral profiles that differ from those of normal tissue [156,157]. Based on the spectral differences between normal and tumor tissues, combined with statistical analysis methods such as principal component analysis (PCA) and support vector machines (SVM), effective differentiation can be achieved [158]. This method is simple to implement and avoids issues such as the biotoxicity and biocompatibility associated with exogenous probes, making it more suitable for clinical applications.

Label-free confocal Raman spectroscopy combined with ML was used to distinguish between stem cells (GSCs) and differentiated cells. By analyzing the subcellular structures (such as nuclei and mitochondria) of 1,146 cells, an accuracy rate of 91.7% was achieved [159]. This method effectively addresses the issue of tumor heterogeneity and provides a rapid means of distinguishing highly oncogenic cells during surgery. Building upon this, Wang *et al.* developed SiO₂@Au core-shell nanoprobes and Au NPs SERS substrates to perform SERS detection of gliomas at both the cellular and tissue levels (Figure 11A) [160]. At the cellular level, SVM algorithms precisely distinguish astrocytes from glioma cells. At the tissue level, OPLS-DA models enable differentiation between glioma and traumatic tissue, tumor grading, and IDH mutation identification, with 100% accuracy in a limited cohort (AUC > 0.99) (Figure 11B). The data obtained from the above study demonstrate that label-free SERS has certain potential for differentiating tumor tissues. However, this study is based on a small sample size and may not reflect its diagnostic performance in larger, more heterogeneous, multicenter clinical populations. The label-free approach provides a rapid strategy for real-time intraoperative diagnosis and molecular subtyping.

To further ensure reproducibility across different tumor grades, a SERS substrate consisting of Ag NPs modified with Ag nanorods (Ag NPs@Ag NRs) (with an enhancement factor of 1.37×10^9) enabled the differentiation between healthy brain tissue and brain glioma tissue of various grades (Figure 11C) [161]. By analyzing characteristic peaks at 653 cm^{-1} and 724 cm^{-1} in the SERS spectra and combining with PCA, the method can achieve rapid, label-free differentiation between healthy tissue and grades I-IV, providing an efficient method for the non-invasive and rapid grading diagnosis of brain gliomas. This approach eliminates the need for complex labeling procedures and rapidly acquires comprehensive molecular information about the tissue, making it potentially valuable for real-time intraoperative detection. Similarly, an Au@ZrO₂ plasmonic nanosensor can be used to distinguish tumor cores from peripheral regions intraoperatively (Figure 11D). The ZrO₂ coating effectively suppresses fluorescence interference while enhancing Raman signals, achieving an LOD of 10^{-7} mol/L for Rhodamine 6G [162]. Analysis of the 178 spectral sets from the nine patients showed good discrimination at a certain threshold, and thus a new tool for precision neurosurgery has been obtained.

Based on the above, label-free SERS is currently one of the ways that is closer to clinical application. Label-free SERS does not need to be added to the sample, so the preparation steps before surgery are simpler. Label-free SERS detection has its own problems as well. Compared with the nanoprobe-assisted method, it is relatively more dependent on spectral differentiation for diagnosis, and it is also easy to be affected by tissue heterogeneity and fluorescence. Therefore, going forward, more efforts will need to be made in the field of label-free SERS detection to standardize spectral acquisition, uniform pretreatment protocols and multi-centre clinical validation to improve the reproducibility of clinical applications.

4.3.2 Nanoprobe-Based SERS for Enhanced Sensitivity

Although label-free methods are simple to operate and can achieve high classification performance through small-sample testing combined with multivariate analysis, their analytical performance mainly depends on the inherent spectral differences between tissues. In complex clinical samples, factors such as weak Raman scattering cross-sections, fluorescence background, tissue heterogeneity, and overlapping characteristic peaks can reduce spectral discrimination and limit stability

across different patients and clinical centers. To overcome the problems of weak Raman signals and overlapping characteristic peaks in label-free detection, researchers used plasmonic nanoparticles composed of functionalized Raman reporter molecules and targeting ligands to detect tissues, thereby improving signal intensity and specificity.

For instance, a SERS probe was exploited to simultaneously detect two metabolites in gliomas, with a deep learning model (DBCNet) to analyze Raman spectra (Figure 12A) [163]. By constructing an orthogonal response SERS probe (Au-R12P), they were able to simultaneously quantify GSH and H₂O₂ (Figures 12B and 12C). Combined with DBCNet, they completed intraoperative IDH1 genotyping of gliomas within 7 min, achieving an area under the curve (AUC) of 0.985 in 31 patients, thus providing a rapid new method for intraoperative molecular subtyping, precise diagnosis, and treatment of gliomas (Figures 12D and 12E).

4.4 From Margin Detection to Surgical Navigation

Compared with many other solid tumors, the surgery of glioma faces several unique challenges. Gliomas exhibit highly infiltrative growth patterns, resulting in poorly defined tumor margins that are difficult to distinguish from normal brain tissue using conventional visual inspection or neuronavigation alone [164,165]. In addition, because the brain contains many functional areas and gliomas often occur near critical regions responsible for language, motor, and sensory functions, it is essential to preserve neural function as much as possible while removing as much of the tumor as possible during surgical resection [166,167]. Also, substantial brain shift during the operation can decrease the precision of preoperative MRI-based navigation [168,169]. These factors collectively limit the effectiveness of conventional intraoperative guidance techniques.

Conventional 5-ALA fluorescence navigation is often compromised by photobleaching and limited sensitivity in low-grade gliomas, while MRI is hindered by high costs and poor real-time feedback [170,171]. SERS technology addresses these bottlenecks through its high spatial resolution, photostability, and superior capacity for precise margin delineation. As shown in Table 2, compared to traditional intraoperative navigation methods for gliomas (e.g., MRI, 5-ALA fluorescence and histopathology), SERS has significant potential in terms of resolution, molecular specificity, and real-time intraoperative navigation.

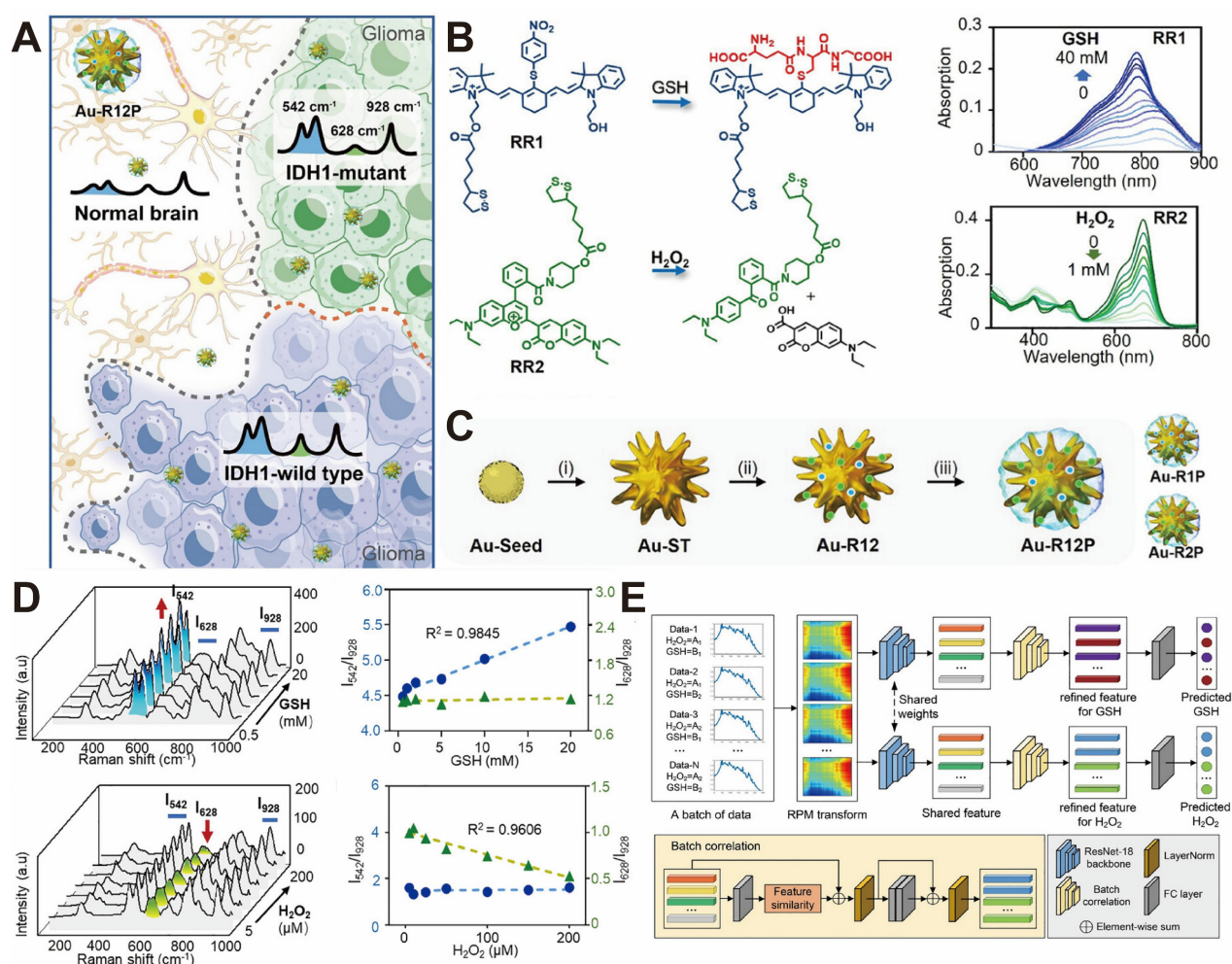


Figure 12. (A) Identifying IDH1 genotypes of gliomas by determining redox-associated metabolites. (B) Chemical structures of Raman molecular reporters RR1 and RR2; absorbance of RR1 (5×10^{-6} M, upper panel) and RR2 (1×10^{-5} M, lower panel) as a function of GSH and H₂O₂ concentration, respectively. (C) Synthetic procedure of Au-R12P. (D) Raman spectra of Au-R12P in PBS solution as a function of GSH concentration (0.5–20 mM) and H₂O₂ concentration (5–200 μM). (E) Schematic diagram of the proposed DBCNet for analyzing the Raman spectra of Au-R12P in the presence of redox-associated metabolites. Reproduced with permission from Ref. [163]. Copyright 2025, Wiley-VCH.

4.4.1 Metabolism-Guided Surgical Strategies

SERS-guided glioma surgical resection can be divided into two categories. One category directly uses SERS to detect normal and tumor tissues, and distinguishes tissues based on the differences in the characteristic peaks of metabolite molecules between the two types of tissues. The other category is based on the difference in the concentration of metabolites (such as extracellular acidosis) between normal and tumor tissues, and uses the response of specific SERS probes to metabolites to perform tissue resection.

For instance, Ag NPs as a substrate can be employed to differentiate between normal cells and glioblastoma cells based on the common characteristic peaks of two metabolites at 655 cm⁻¹ and 717 cm⁻¹ [172]. By combining neural networks with a multinomial regression model, this method can accurately delineate tumor boundaries during surgery, providing a new, rapid quantitative diagnostic method for the surgical resection of gliomas (Figure 13A).

To address chemical gradients, Duan *et al.* developed a pH-responsive SERS probe (AuS-IR7p), based on the spatial orientation-induced intramolecular energy transfer (SOIET) mechanism (Figure 13B) [173]. This probe circumvents the BBB, identifying acidic tumor margins within 5 s and enabling guided resection up to a depth of 2.2 mm, which significantly improved survival in rat models.

Similarly, a probe-free intelligent SERS platform was utilized for surgical boundary navigation [174]. The system utilizes extracellular acidosis during glioma cell metabolism as a characteristic biomarker of gliomas. Combined with a one-dimensional convolutional neural network deep learning model, it can rapidly extract tumor margin metabolites during surgery and generate high-resolution pH maps, thereby accurately defining the boundaries of acidic infiltration. Furthermore, animal experiments demonstrated that surgery guided by this sensing platform significantly prolonged the overall survival of glioma models (Figures 13C and 13D).

Table 2. Comparison of SERS with conventional intraoperative and diagnostic modalities for glioma.

Modality	Spatial Resolution	Sensitivity (%)	EOR	Intraoperative application	Key Limitations
MRI	~1-2 mm	80-95 (reported range)	Facilitates preoperative planning	Yes	Limited molecular information, brain shift
5-ALA fluorescence	~0.1-1mm	75-95 (HGG); 20-60 (LGG)	Increases gross total resection rates by approximately 15-25% compared with white-light surgery	Yes	Photobleaching, low sensitivity in LGG
Histopathology	1-10 μ m	90-99	Provides definitive diagnosis	No	Time-consuming, sampling bias
SERS	<1 μ m (subcellular)	Variable (85-99 in selected studies)	High accuracy for tumor margin; detection in preclinical and early translational studies	Yes	Reproducibility, standardization

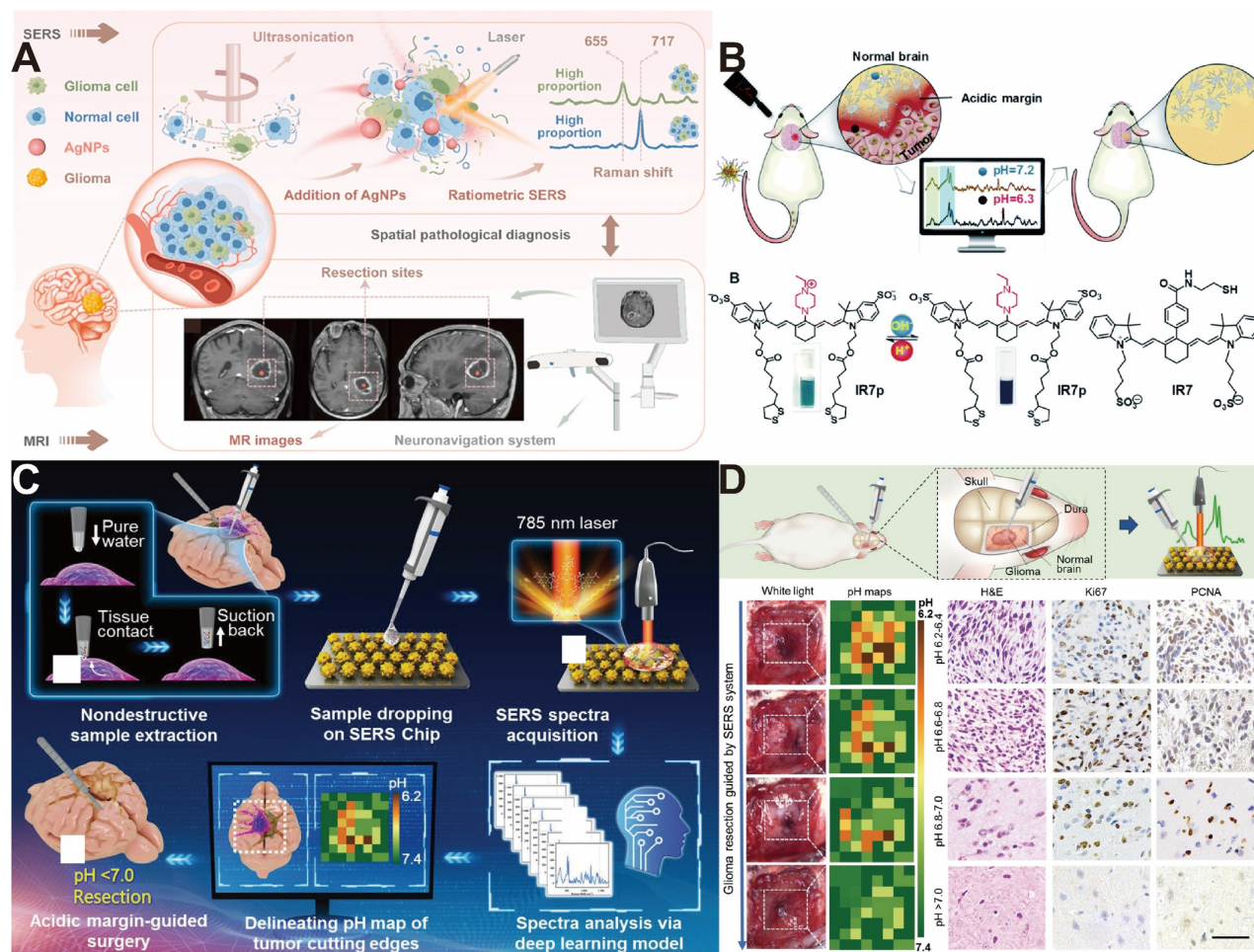


Figure 13. (A) Schematic illustration for the detection of glioma cell proportion with an Ag NPs-based SERS method. Reproduced with permission from Ref. [172]. Copyright 2024, Elsevier. (B) Design and synthesis of pH-responsive SERS probe and schematic illustration of its application in guiding the surgical resection of gliomas. Reproduced with permission from Ref. [173]. Copyright 2020, The Royal Society of Chemistry. (C) Schematic diagram illustrating the use of SERS navigation system to automatically analyze Raman spectra using deep learning models, map the pH distribution at the tumor margin, and resect acidic tissue with a pH below 7.0. (D) Schematic illustration of the surgical procedure for acid-edge-guided glioma resection in an animal model, along with representative H&E, Ki67, and PCNA staining images of resected tissue under different pH conditions. Reproduced with permission from Ref. [174]. Copyright 2022, Wiley-VCH.

4.4.2 Targeted Nanoprobe-Guided Surgery in Animal Models

Modifying the surface of SERS substrate materials with functionalized antibodies or peptides

can specifically bind to tumor cells, enabling precise localization of tumor areas during surgery and significantly improving the contrast between tumors and normal tissues, thus facilitating more thorough tumor resection in animal models.

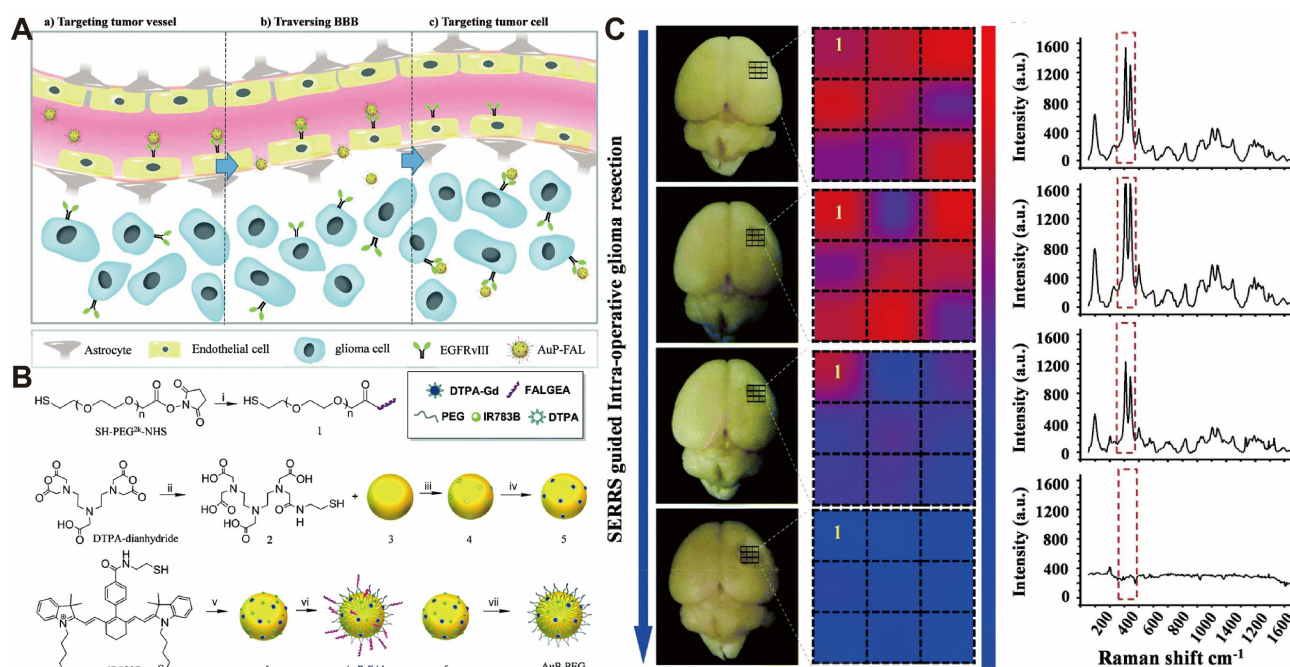


Figure 14. (A) Schematic diagram of AuP-FAL visualization of brain tumor margins by targeting EGFRvIII. (B) Synthesis of the nanoprobe. (i) FAL, (ii) 2-aminoethanethiol, (iii) H₂O, (iv) GdCl₃·6H₂O, (v) 5, (vi) 1, PEG-SH and (vii) PEG-SH. (C) AuP-FAL guiding intra-operative brain tumor resection by handheld Raman detector. Reproduced with permission from Ref. [175]. Copyright 2017, The Royal Society of Chemistry.

Qi *et al.* designed an EGFRvIII-targeting bimodal Au nanoprobe, AuP-FAL, with 60 nm gold nanospheres modified with MRI-enhanced Gd³⁺-DTPA, Raman reporter molecule IR783B, and targeting ligands FAL peptide and PEG (Figures 14A and 14B). In vitro validation showed that the probe had high targeting and uptake rates on EGFRvIII⁺ U87MG cells. In a nude mouse orthotopic glioma model, the tumor boundary could be delineated using preoperative MRI with a high target-to-background ratio, combined with intraoperative SERS imaging using a handheld Raman scanner for precise resection (Figure 14C). Postoperative verification showed no tumor residue and it could also overcome false positives of brain displacement [175]. This provides an efficient tool for image-guided surgery of EGFRvIII⁺ glioblastoma and has clinical translational potential.

4.4.3 Multimodal SERS Platforms

In addition to using the SERS method alone to guide surgical resection of gliomas, it can also be combined with other novel methods (such as fluorescence imaging, photothermal therapy, and photodynamic therapy) to simultaneously achieve diagnosis, intraoperative navigation, and treatment, showing broad prospects for the integrated development of diagnosis and treatment [176,177].

Xu *et al.* developed an integrated SPIT nanoprobe

based on Au NSs (Figure 15A). The Au NSs were coated with macrophage membranes overexpressing the SIRPα variant, enabling their accumulation in tumor regions through BBB disruption and targeting strategies [178]. This strategy enables precise identification of tumor margins and micrometastases during surgery and eliminates residual tumors postoperatively through the photothermal effect. Using this approach, tumor recurrence in mice was significantly suppressed, while the survival was prolonged. The combination of intraoperative detection and postoperative treatment provides a new approach to the comprehensive diagnosis and treatment of glioblastoma.

Furthermore, Au NRs with tumor cell membranes derived from glioblastoma patients (GBM-PDTCM) can create GBM-PDTCM@AuNRs (Figure 15B) [114]. Leveraging its homology, the probe efficiently crosses the BBB and precisely targets gliomas. It emits both fluorescence and SERS signals, enabling precise, complete resection via dual-modal navigation within 15 min. Additionally, it can destroy in situ tumors through photothermal therapy, extending the median survival of mice from 25 days to 52 days. This approach offers a new integrated surgical and non-surgical diagnostic and therapeutic strategy for glioblastoma at all stages.

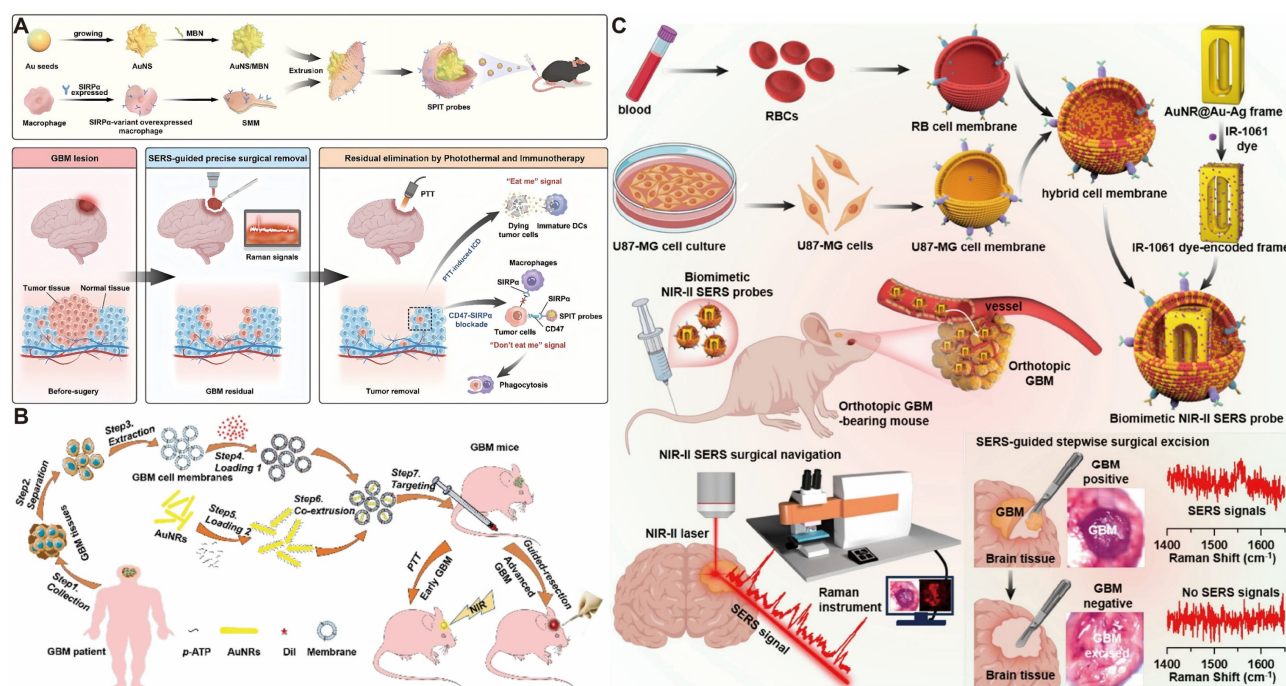


Figure 15. (A) Schematic diagram of SPIT probe preparation and SPIT probe for glioma therapy integrating SERS and PTT. Reproduced with permission from Ref. [178]. Copyright 2025, Wiley-VCH. (B) Schematic illustration of the preparation of GBM-PDTCM@AuNRs and their application in GBM therapy. Reproduced with permission from Ref. [114]. Copyright 2023, American Chemical Society. (C) Schematic illustration of the preparation and application of hybrid CM-biomimetic NIR-II SERS probe for intraoperative resection guidance of in situ glioblastoma. Reproduced with permission from Ref. [179]. Copyright 2025, American Chemical Society.

In addition, an AuNR@Au-Ag framework plasmonic structure was coated onto a mixed cell membrane of erythrocytes and U87-MG cells to construct a near-infrared II (NIR-II) SERS probe (Figure 15C) [179]. This probe can effectively cross the BBB and target glioblastoma in situ. Utilizing the low scattering and high signal-to-noise ratio advantages of the NIR-II window, the probe can identify intraoperative microlesions ($\approx 110 \mu\text{m}$) in real time, thereby achieving precise stepwise resection. This study is the first to apply NIR-II SERS to glioma navigation, providing a high-performance optical solution for precise brain tumor surgery.

As shown in Table 3, we analyzed and compared the detection targets mentioned in the aforementioned literature. Metabolites, nucleic acids/proteins and exosomes possess significant clinical potential for various stages of glioma management, including preoperative diagnosis, intraoperative navigation and postoperative monitoring. Among these, exosomes are recognized as the most promising detection targets for glioma biomarkers due to their easily accessible nature and high information density. On the other hand, SERS detection of a single cell is limited by the high precision of instruments and the practical difficulties in obtaining a single-cell sample in clinical applications, which limit its general use. Label-free SERS has great clinical potential for direct detection of glioma tissue at the tissue level with easy specimen

acquisition and simple sample preparation. For clinical applications of SERS-labeled probes, it is important to validate the biocompatibility and toxicity of the substrate materials. In summary, the SERS technology has extensive application prospects in the accurate surgical navigation of gliomas.

Overall, nanoprobe-guided SERS provides superior sensitivity and molecular specificity, whereas label-free SERS offers greater simplicity and easier clinical integration. Despite encouraging preclinical performance, most navigation platforms have only been validated in small animal models or limited patient cohorts. Before SERS technology can be employed in actual neurosurgery, many more prospective clinical studies and rigorous prospective clinical trials must be carried out to confirm its reproducibility and long-term biosafety.

4.5 Artificial Intelligence for SERS-Based Glioma Diagnosis

By means of combining artificial intelligence technology with SERS, all kinds of data related to tumors can be obtained from the analysis of complicated spectral information, thereby enabling highly sensitive detection of gliomas [180,181]. Unlike traditional spectral data analysis methods, AI-based data analysis models can rapidly identify and distinguish subtle spectral fluctuations that are complex and difficult to discern intuitively, thereby

providing accurate information about gliomas in a short period of time to assist physicians in developing surgical resection plans (Figure 16). However, the effectiveness of AI models is closely related to the quality of SERS data. Therefore, it is necessary to establish an analytical framework guided by real-world clinical problems.

4.5.1 Characteristics of SERS spectral data and implications for AI modeling

SERS spectra acquired from biological samples present challenges that are rarely encountered in conventional spectroscopic analyses, making the development of robust AI models considerably more difficult. One major limitation is the imbalance between the large number of spectral variables and the relatively small number of clinically annotated

samples, a situation commonly referred to as the “small n, large p” problem, which substantially increases the risk of model overfitting [182,183]. Changes in the substrate, fluctuations in the excitation environment, and different compositional structures of biological samples can affect the performance of the model due to spectral changes. Background interference and fluorescence can further obscure diagnostically relevant spectral features, placing greater demands on feature extraction and model robustness rather than on classification accuracy alone. Consequently, in order to conduct reliable AI-assisted SERS analysis, improvements need to be made in the machine-learning algorithms, standardization of data acquisition and strictness of spectral preprocessing, and also careful control over experimental protocols.

Table 3. Comparison of SERS strategies for glioma detection and diagnosis.

Strategy	Target	Development Status	Advantages	Limitations	Ref
Metabolite sensing	pH, GSH, H ₂ S	Preclinical	Rapid response	Low specificity	127-129
Nucleic acid/protein	ctDNA, VEGF	Early clinical validation	High specificity	Complex preparation	130,131, 135,138
Exosomes	EVs	Early clinical validation	Non-invasive	Standardization needed	141-143; 146, 147
Cellular analysis	Glioma cells	Research stage	Mechanistic insight	Poor clinical translation	151-153
Label-free tissue SERS	Tissue fingerprint	Advanced translational stage	No labeling	Lower sensitivity	159-161
Nanoprobe-based imaging	Tumor markers	Preclinical	High contrast	Biosafety concerns	163
SERS-guided surgery	Tumor margin	Preclinical	Real-time guidance	Insufficient clinical evidence	172-175; 114,178,179

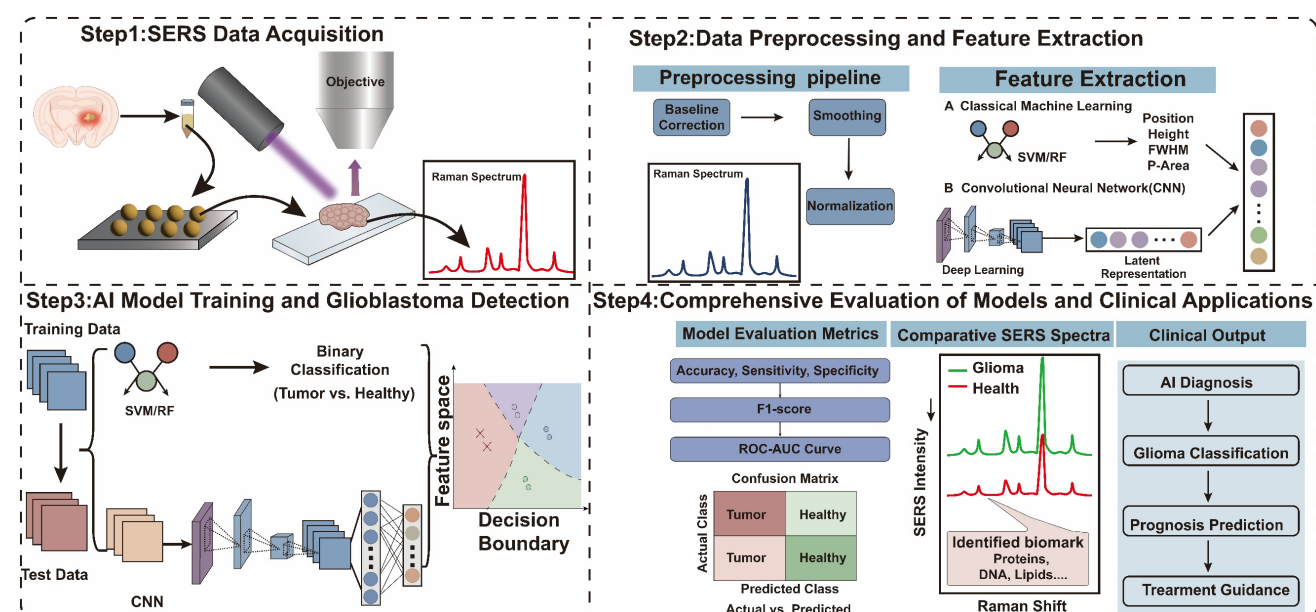


Figure 16. SERS-based artificial intelligence framework for glioblastoma detection: from data acquisition to clinical decision support.

4.5.2 Evolution of AI methodologies for SERS analysis

With the development of artificial intelligence technology over time, many studies have since been conducted to apply it to SERS-based analysis of gliomas, and deep learning (DL) methods have begun to replace traditional machine learning techniques [184,185]. Early studies primarily relied on classifiers such as principal component analysis-support vector machines (PCA-SVM) and partial least squares discriminant analysis (PLS-DA), where model performance depended heavily on feature extraction and dimensionality reduction before classification [186]. Due to the above reasons, the traditional machine learning model can still be employed for diagnosis with a small number of data samples. For example, Wang *et al.* combined SERS with multiple machine-learning algorithms to differentiate glioma from traumatic brain tissue and determine IDH mutation status, achieving classification accuracies above 97% and area under the curve (AUC) values exceeding 0.99 [160]. However, the above methods are often unable to capture the intricate, non-linear connections among the many pieces of information in high-dimensional spectra.

Recently, more research has begun to be conducted on how to use DL architectures, especially CNN and ResNet-based models, to solve the above problems [187]. By learning hierarchical feature representations directly from raw spectra, these networks reduce dependence on manual feature engineering while improving robustness to spectral variation and noise [188,189]. With the development of such technology, SERS is now being employed to recognize various types of molecules and investigate extracellular vesicles in glioblastoma. Jalali and others have developed a CNN-based framework for the analysis of single extracellular vesicle SERS spectra [143]. Based on this, it has been found that the genes of glioblastoma are altered in clinical samples with an accuracy of 87%, and at a very low frequency of 1.23%, mutant vesicles have also been identified. From the above results, it can be concluded that DL will be used to construct a high-sensitivity, label-free liquid biopsy assay.

Despite the above favorable developments, some problems have not been solved and thus cannot be applied to clinical work. Most of the reported models have only been trained and tested on data from a single hospital with a small number of cases, so it is unknown whether they can be reproduced and generalized to other groups of patients, instruments and sample-processing methods. In order to solve the problems mentioned above, more multi-centre datasets and independent external validation need to

be obtained. In addition to the traditional machine learning method, new few-shot learning and domain adaptation approaches will also be employed to address the problem of small datasets and improve the consistency of performance in different clinical centres. In conjunction with standardization of spectral acquisition and preprocessing protocols, it is hoped that the new technology will be used in many clinics in the near future.

A key challenge currently facing the use of data models is the limited availability of data due to the difficulty in obtaining clinical samples. Therefore, in addition to traditional ML models that require extensive clinical data analysis, the development of emerging methods, such as those based on learning from small sample sizes, should be pursued to improve model accuracy when clinical samples are scarce. Furthermore, explainable artificial intelligence (XAI) frameworks are expected to play an increasingly important role. This approach improves model transparency and enhances clinical credibility by establishing links between spectral features and biologically meaningful molecular biomarkers. Leveraging the characteristics of Raman spectroscopy data, a standardized database can be constructed to further enhance cross-platform generalization capabilities and advance the standardization of molecular diagnostics.

Furthermore, the multimodal data fusion strategies involving SERS with histopathology, MRI, genomics and clinical information are anticipated to offer a more complete characterization of glioma biology and to improve the accuracy of diagnosis. Together, these developments will enable the transition of AI-assisted SERS systems from proof-of-concept studies to clinically deployable decision support tools.

Generally, AI and SERS have been used together to improve the analysis of large amounts of spectral data and enhance the accuracy of diagnostic information extraction for gliomas. According to the progress of algorithm development, construction of a standard spectral database, an interpretable analytical model and strict validation in many clinical centres will be conducted, and further development will be carried out. Due to the reasons listed above, a stable SERS-AI platform that can be used in the clinical application of neuro-oncology accurately will be built.

5. Challenges and Future Perspectives

5.1 Critical Challenges for Clinical Translation

SERS has shown some promise for the diagnosis and surgical guidance of glioma so far, but many problems have yet to be solved. One of the most critical

challenges is that SERS nanoprobe generally exhibit limited ability to efficiently cross the BBB. Most of the platforms have reported so far that it is passive accumulation due to an increase in permeability and retention (EPR) effect or local disruption of the BBB caused by glioma progression, and active and selective transport across an intact BBB has not yet been achieved. This limitation is particularly relevant in early-stage gliomas, where BBB integrity is largely preserved, and nanoprobe uptake is consequently restricted.

The other critical question is that the SERS substrates are not reproducible. Even a small change in the shape of the nanostructure or in the distribution of hot spots will result in a relatively large shift of the spectrum and thus reduce the accuracy of quantification and interlaboratory consistency. The AI-assisted SERS system also has the problem that it is not working well due to a small amount of low-quality spectral data. Many deep learning models are not easy to interpret, and thus they have not been widely used in serious cases of medical treatment. Another translation issue is that we do not know how safe plasmonic nanomaterials are in the long run. Extensive research has been conducted on the biodistribution, pharmacokinetics, metabolism, and excretion of these materials, as well as stringent regulatory approvals; only then will they be widely used in the clinical treatment of gliomas.

Importantly, future progress is unlikely to rely solely on increasingly complex deep learning architectures. Instead, standardized SERS data acquisition and high-quality multicenter datasets are expected to contribute more substantially to clinical performance than algorithmic complexity alone.

5.2 Future Perspectives: Toward Precision Neuro-oncology

5.2.1 Technological Advances for SERS-Based Glioma Research

As nanotechnology, spectral analysis techniques and AI algorithms continue to advance, SERS has great potential in glioma research applications. Despite the rapid development of SERS for glioma diagnosis and intraoperative navigation, there are still several challenges to be solved for its extensive clinical application. Future work may be directed to the development of smart nanoprobe capable of crossing the BBB and accumulating specifically in invasive tumor regions. Furthermore, combining SERS with NIR-II imaging may enhance tissue penetration depth and minimize background noise, enabling real-time imaging of infiltrative tumor borders during molecularly guided surgery.

Another key direction is to improve the reproducibility of the substrate. The future SERS substrate will move away from the dependence on randomly generated hotspots and utilize photolithographically engineered surface plasmon arrays, DNA origami-assisted assembly of nanoparticles, and microfabrication techniques to realize highly uniform electromagnetic enhancement and batch-to-batch consistency. Such approaches could allow for large-scale production and regulatory approval.

Biosafety in the long term is also a prerequisite for clinical translation. Future studies should focus on short-term toxicity studies and systematic studies of biodistribution, metabolism, clearance pathways and chronic biological effects of nanoparticles. Long-term safety concerns can be reduced by using biodegradable plasmonic materials and nanostructures that are cleared by the kidneys.

There are issues such as the substrate materials for current Raman analysis, and thus no particular quantitative standards have been established yet, except for multiplexing. Future strategies, including internal standards, ratio-based SERS and AI-assisted spectral calibration, may improve the analytical accuracy and reproducibility, thus facilitating the clinical applications.

5.2.2 Emerging Applications and Clinical Translation in Precision Neuro-oncology

Beyond technological improvements, emerging interdisciplinary approaches are expected to further expand the applications of SERS in precision neuro-oncology. In particular, advances in single-cell analysis and AI are expected to further broaden the capabilities of SERS. The combination of single-cell analysis with high-resolution SERS imaging will allow us to characterize the heterogeneity of gliomas at the cellular level, thus providing new insights into glioma evolution, treatment resistance and tumor microenvironment interactions. Moreover, the combination of SERS with AI may enable the development of digital pathology platforms for automatic tumor classification, molecular subtype recognition, and intraoperative decision making.

The integration of multi-omics liquid biopsy with SERS is likely to broaden the scope of molecular characterization in glioma by enabling the concurrent analysis of circulating tumor DNA (ctDNA), microRNAs, proteins, metabolites, and extracellular vesicles. Thus, one of the reasons for the good spectral resolution of Raman spectroscopy has been exploited. Raman bands are generally only about 10-20 cm^{-1} wide, much narrower than fluorescence emission bands, and thus many molecular signals can be

identified in one measurement. Therefore, SERS is suitable for multiplex biomarker detection, and it will be used to study the molecular heterogeneity of gliomas in more detail.

New combinations of neurotechnology and SERS will be developed in the future. By means of SERS and electroencephalography (EEG) coupled with neural recording platforms, changes in both molecules and electricity in the brain can be observed simultaneously. All the above many-format approaches will likely help improve the environment for early warning of all kinds of diseases and advance the construction of intelligent diagnostic workflows and other related technologies.

Although there have been some good developments, there are currently no FDA-approved SERS products for the treatment of gliomas, and most studies are still in the preclinical stage. Nevertheless, tissue-based diagnostics and liquid biopsy platforms are generally considered the most promising candidates for near-term clinical translation. Further advances are required to translate SERS from a promising research tool to a practical clinical technology for precision neuro-oncology, including substrate standardization, large-scale clinical validation, regulatory approval pathways and interpretable artificial intelligence.

In conclusion, although SERS still faces some problems in glioma research, the application potential of SERS in precision diagnosis, intraoperative navigation and personalized treatment will be further realized with continuous technological development. With further technological validation and clinical studies, SERS is expected to be a key tool for glioma diagnosis and therapy through interdisciplinary collaboration and expanded clinical research.

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Authorship contributions

Min Zhang: Investigation and Writing. **Ya-jun Luo:** Investigation and Figures. **Ya-nan Liu:** Investigation. **Ji-sen Li:** Proofreading and Supervision.

Artificial intelligence (AI) usage statement

During the writing of the paper, AI tools (ChatGPT) were used solely for language polishing and grammar checking to enhance the academic quality of the paper. AI tools were not used for drafting the manuscript, analyzing data, creating figures and tables, or drawing conclusions. All content, including the interpretation of literature,

presentation of data, and revision of the final draft, was independently completed, reviewed, and approved by the author. The author assumes full responsibility for the accuracy, originality, and completeness of this study.

Competing Interests

The authors have declared that no competing interest exists.

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