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# Raman spectroscopy for glioma diagnosis toward precision neuro-oncology: A systematic review

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ABSTRACT

*Introduction:* Gliomas are the most common primary malignant tumors of the central nervous system and remain difficult to manage because of diffuse infiltration and molecular heterogeneity. As glioma classification increasingly relies on molecular biomarkers, interest has grown in label-free technologies capable of providing real-time biochemical information during surgery. Although Raman spectroscopy has emerged as a promising optical approach, its diagnostic performance and translational readiness remain incompletely defined.

*Methods:* This systematic review was registered in PROSPERO and conducted according to PRISMA 2020 guidelines. PubMed, Embase, Scopus, and Web of Science were searched for studies published between 2015 and 2025 evaluating Raman-based technologies for glioma diagnosis. Two reviewers independently performed study selection, data extraction, and risk-of-bias assessment using QUADAS-2. Owing to methodological heterogeneity, findings were synthesized using a SWiM-guided narrative approach.

*Results:* Forty-six studies met the eligibility criteria. Across conventional Raman spectroscopy, surface-enhanced Raman spectroscopy, stimulated Raman histology, stimulated Raman scattering, and multimodal platforms, Raman-based technologies consistently demonstrated strong performance for tissue discrimination, tumor margin assessment, molecular characterization, and intraoperative tissue evaluation. Recent studies increasingly integrated artificial intelligence for automated tissue classification and molecular prediction. However, substantial heterogeneity in acquisition protocols, analytical workflows, and validation strategies, together with predominantly retrospective single-center studies and limited external validation, reduced confidence in clinical generalizability. QUADAS-2 identified the greatest risk of bias in the patient-selection and index-test domains.

*Conclusions:* Raman spectroscopy is evolving from an optical imaging technique into a molecular decision-support platform for precision neuro-oncology. Future clinical translation will depend on methodological standardization, prospective multicenter validation, explainable artificial intelligence, and integration into routine neurosurgical workflows.

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## 1. Introduction

Gliomas are the most common primary malignant tumors of the central nervous system and remain associated with substantial morbidity and poor survival despite advances in neurosurgery, radiotherapy, and systemic therapies (Miller et al., 2021; Salari et al., 2023; Price et al., 2024). Their highly infiltrative growth pattern allows tumor cells to extend beyond radiologically apparent boundaries, limiting complete surgical resection and contributing to disease recurrence. Consequently, achieving maximal safe resection while preserving neurological function remains one of the principal challenges in contemporary neuro-oncology (Gerritsen et al., 2022; Aabedi et al., 2022; Jiang et al., 2023; Sulangi et al., 2024).

Accurate histopathological and molecular characterization is essential for contemporary glioma classification, prognostic stratification, and therapeutic decision-making. The current World Health Organization (WHO) classification increasingly incorporates molecular biomarkers, including isocitrate dehydrogenase (IDH) mutations, 1p/19q codeletion, MGMT promoter methylation, and other genomic alterations that define biologically distinct tumor entities and guide patient management (Komori et al., 2022; Śledzińska et al., 2022; Massaad et al., 2022; Wang et al., 2023). However, these data depend on tissue sampling, specialized laboratory processing, and molecular testing, limiting the availability of biologically relevant information during surgery when critical therapeutic decisions are made.

Although advances in magnetic resonance imaging (MRI), fluorescence-guided surgery, positron emission tomography (PET), neuronavigation, intraoperative ultrasound, and intraoperative MRI have substantially improved surgical planning and intraoperative guidance, these approaches primarily provide anatomical or metabolic information and may underestimate diffuse microscopic tumor infiltration (Li et al., 2016; Wen et al., 2023; Karschnia et al., 2023; Tang et al., 2025). This limitation has stimulated growing interest in technologies capable of providing real-time molecular characterization of tissue during surgery.

Raman spectroscopy has emerged as a promising label-free optical technique that generates biochemical fingerprints from endogenous cellular molecules without extensive sample preparation or exogenous contrast agents, enabling rapid discrimination between neoplastic and non-neoplastic tissues (Stupak et al., 2025). Recent technological advances, including fiber-optic probes, stimulated Raman scattering, stimulated Raman histology, and machine-learning-assisted analytical approaches, have considerably expanded its potential applications from tissue characterization to intraoperative tumor identification, margin assessment, molecular classification, and real-time surgical decision support (Iturrioz-Rodríguez et al., 2022; Jin et al., 2022; Herta et al., 2022; Vrazhnov et al., 2023; Ember et al., 2024; Liu et al., 2024). More recently, machine-learning-enabled Raman spectroscopy has been integrated with fluorescence-guided surgery to refine indocyanine green-defined glioblastoma boundaries, illustrating the potential of Raman-derived molecular information to complement established intraoperative guidance modalities (Han et al., 2026).

Recent reviews have highlighted the expanding role of Raman spectroscopy in glioma diagnosis, although with different scopes (Liu et al., 2024). Some have provided broad narrative overviews of Raman technologies and their applications in glioma detection, histological and molecular classification (Liu et al., 2024), whereas others have focused on specific modalities such as SERS (Yang et al., 2024) or SRH in defined clinical settings. Recent systematic reviews and meta-analyses have primarily quantified diagnostic performance for tumor–normal tissue discrimination or selected intraoperative tasks (Krzemińska et al., 2025; Trevisi et al., 2025). These studies establish the diagnostic potential of Raman-based approaches, but differences in scope, modality, analytical unit, and clinical endpoint leave methodological robustness, validation maturity, and translational readiness less consistently integrated within a single evidence synthesis.

Against this background, the present systematic review provides an integrated and critically appraised synthesis of 46 studies spanning conventional Raman spectroscopy, SERS, SRH, SRS, and multimodal platforms. Beyond diagnostic performance, we jointly examine methodological heterogeneity, analytical unit, QUADAS-2 risk of bias, machine-learning validation practices, clinical applications, and translational readiness. By distinguishing proof of analytical discrimination from evidence of patient-level generalizability and clinical utility, this review positions current Raman-based approaches along the translational pathway from experimental spectroscopy to AI-assisted molecular diagnostics and intraoperative decision support, while identifying the methodological and clinical priorities required for broader implementation in precision neuro-oncology.

## 2. Methods

The methodology of this review was established a priori in a previously published, peer-reviewed protocol (Vrancianu et al., 2026), prospectively registered in PROSPERO (CRD420251025922). This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement (Page et al., 2021), the PRISMA-P recommendations for systematic review protocols (Moher et al., 2016), and the Synthesis Without Meta-analysis (SWiM) reporting guideline (Campbell et al., 2020). The PRISMA 2020 checklist and PRISMA 2020 for Abstracts checklist are provided in Supplementary Table S1A and Supplementary Table S1B, respectively.

### 2.1. Eligibility criteria

The selection criteria are described below according to Patients, Intervention, Comparison and Outcomes (PICO) statements.

#### 2.1.1. Patients

**Inclusion criteria:** Studies were eligible if they included adults (≥18 years) with suspected or histologically confirmed glioma undergoing diagnostic evaluation, surgical resection, or image-guided tissue sampling using Raman spectroscopy. Mixed populations were included when glioma-specific diagnostic data were reported separately or represented most of the study cohort.

**Exclusion:** Studies involving pediatric populations, non-human subjects, cell lines, animal models, or lacking extractable glioma-specific data were excluded. Studies evaluating only multiclass tumor discrimination without an appropriate non-tumor comparator were also excluded. When participant age or eligibility criteria were unclear, corresponding authors were contacted. Eligibility was determined according to the pre-registered protocol if clarification was unavailable.

**Intervention:** The use of Raman spectroscopy, either alone or combined with machine learning algorithms, as a diagnostic approach for the detection and characterization of glioma.

**Comparison:** Where available, Raman spectroscopy was compared with histopathology, frozen section analysis, magnetic resonance imaging, fluorescence-guided surgery, positron emission tomography, molecular diagnostic methods, or other established reference standards. Studies without a direct clinical comparator were eligible if diagnostic performance was evaluated against histopathological or molecular reference standards.

**Outcomes:** The primary outcome was the diagnostic performance of Raman spectroscopy, including sensitivity, specificity, accuracy, area under the receiver operating characteristic curve (AUC), positive predictive value, negative predictive value, diagnostic odds ratio, and likelihood ratios, where reported. Secondary outcomes included intraoperative tissue characterization, tumor margin assessment, molecular stratification, real-time surgical decision support, and factors influencing clinical translation. Diagnostic estimates were interpreted according to study design, validation strategy, unit of analysis, and

reference standard.

#### 2.1.2. Information sources

Electronic searches were conducted in PubMed, Embase, Scopus, and Web of Science. The initial search was performed in June 2025 and included English-language studies published between January 2015 and June 2025. In accordance with the registered protocol (PROSPERO), the search was updated before data extraction to identify newly published eligible studies.

#### 2.1.3. Search strategy

The search strategy combined controlled vocabulary (MeSH, where applicable) and free-text terms related to glioma and Raman spectroscopy. The principal concepts included glioma (e.g., glioma, glioblastoma, astrocytoma, diffuse glioma, brain neoplasms) and Raman spectroscopy (e.g., Raman spectroscopy, Raman imaging, Raman scattering, Raman spectroscopic analysis), which were combined using Boolean operators ("OR" within concepts and "AND" between concepts). The search strategy was adapted for each database. The complete search strategies for all databases are provided in the Supplementary Material.

### 2.2. Study records

#### 2.2.1. Data management

Search results retrieved from PubMed, Embase, Scopus, and Web of Science were imported into Zotero (Corporation for Digital Scholarship, Vienna, VA, USA) for reference management. Duplicate records were removed using the software's deduplication function, followed by manual verification where necessary. The final de-duplicated library was used for title, abstract, and full-text screening.

#### 2.2.2. Selection Process

Study selection was performed independently by two reviewers in two stages: title and abstract screening followed by full-text assessment. Eligibility was determined according to the predefined inclusion and exclusion criteria specified in the registered PROSPERO protocol. Studies were excluded if they involved non-human models, cell lines, pediatric populations, non-glioma pathologies, non-Raman optical techniques, conference abstracts without full-text publication, reviews, editorials, preprints, retracted articles, or insufficient data to evaluate diagnostic performance. Disagreements were resolved through discussion and, when necessary, consultation with a third reviewer. Corresponding authors were contacted when additional information was required to determine eligibility. When demographic information or eligibility criteria were insufficiently reported, corresponding authors were contacted whenever feasible. Clarifications received during the predefined assessment period were incorporated into the eligibility assessment; when clarification was unavailable, eligibility was determined from the reported information according to the registered protocol. Reasons for full-text exclusion were documented and are presented in the PRISMA 2020 flow diagram.

#### 2.2.3. Data collection process

Data extraction was performed independently by two reviewers using a standardized Microsoft Excel form. Extracted variables included study characteristics, patient demographics, glioma subtype, Raman modality, acquisition parameters, analytical and machine-learning approaches, validation strategy, reference standards, and reported diagnostic outcomes. Data relevant to clinical translation, including intraoperative applicability, tumor margin assessment, molecular classification, external validation, and workflow integration, were also collected where available. Discrepancies were resolved through discussion and, when necessary, consultation with a third reviewer. Corresponding authors were contacted when essential data were incomplete or unclear.

#### 2.2.4. Data items

Data were extracted according to predefined categories based on the PICO framework. General study characteristics included publication year, country, study design, clinical setting, and sample size. Patient-related variables comprised demographic characteristics, glioma subtype, WHO classification, tumor location, eligibility criteria, and reference standards. Intervention-related variables included the Raman modality, sample type, acquisition parameters, analytical and machine-learning methods, validation strategy, and unit of analysis. Comparator methods and diagnostic reference standards were recorded where applicable. Outcome variables included diagnostic performance measures, molecular classification, tumor margin assessment, intraoperative applicability, external validation, and indicators of clinical translation.

### 2.3. Risk of bias assessment

The methodological quality of the included studies was assessed independently by two reviewers using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. The four QUADAS-2 domains (patient selection, index test, reference standard, and flow and timing) were evaluated for risk of bias and concerns regarding applicability. Disagreements were resolved through discussion and, when necessary, consultation with a third reviewer. Summary risk-of-bias visualizations were generated using the robvis package (McGuinness and Higgins, 2020). Study-specific methodological limitations identified during QUADAS-2 assessment were considered when interpreting diagnostic performance and the overall strength of the evidence.

### 2.4. Data synthesis

Evidence was synthesized using a structured narrative approach in accordance with the Synthesis Without Meta-analysis (SWiM) reporting guideline (Campbell et al., 2020). Quantitative pooling was considered inappropriate because the included studies addressed clinically and methodologically distinct diagnostic questions across conventional Raman spectroscopy, SERS, SRH, SRS, and multimodal platforms, with substantial differences in tissue preparation, acquisition protocols, analytical pipelines, validation strategies, and reference standards. Importantly, diagnostic performance was reported at non-equivalent analytical levels, including patient-, sample-, spectrum-, pixel-, image-, and patch-level analyses, precluding the assumption of a common unit of analysis. Outcome definitions and reporting were also inconsistent, with studies variously reporting sensitivity, specificity, accuracy, AUC, or other diagnostic measures for different clinical endpoints, including tumor detection, margin assessment, grading, and molecular classification. Pooling these heterogeneous estimates could therefore have generated summary measures with limited clinical interpretability and potentially misleading precision. Accordingly, a structured SWiM-guided narrative synthesis was considered more appropriate for preserving differences in clinical context, analytical design, and evidential maturity.

Formal GRADE assessment was not undertaken because the included studies addressed heterogeneous diagnostic questions and clinical endpoints using non-equivalent analytical units and validation frameworks, preventing formulation of sufficiently comparable outcome-specific bodies of evidence for meaningful certainty ratings. Instead, certainty and clinical applicability were interpreted qualitatively by integrating QUADAS-2 findings, validation strategy, analytical unit, study design, and consistency of findings across studies.

## 3. Results

### 3.1. Study selection

The study selection process is summarized in Fig. 1 according to the PRISMA 2020 guidelines. A total of 1540 records were identified

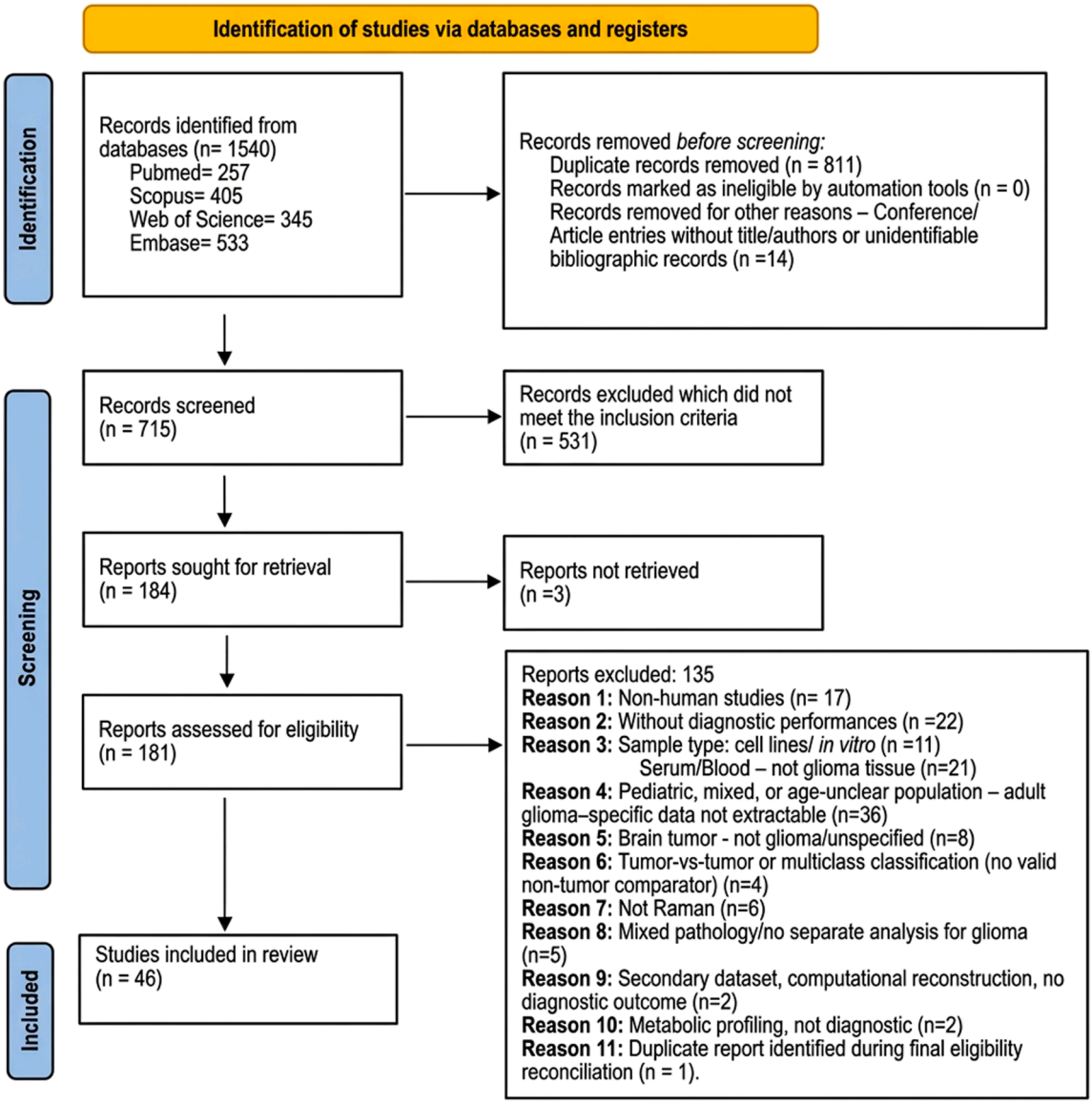



**Fig. 1.** PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram of study selection (Page et al., 2021).

through searches of PubMed, Scopus, Web of Science, and Embase. After removal of duplicate and ineligible records, 715 titles and abstracts were screened, of which 184 articles were selected for full-text evaluation. Three reports could not be retrieved, leaving 181 reports assessed for eligibility. Ultimately, 46 studies fulfilled the predefined inclusion criteria and were included in the qualitative synthesis.

The most frequent reasons for full-text exclusion were the absence of diagnostic performance data, inclusion of pediatric or mixed-age populations without separately extractable adult glioma data, non-glioma pathologies, non-human or in vitro studies, and investigations using serum, blood, cell lines, or other sample types not representative of human glioma tissue. Additional exclusions comprised studies focused exclusively on computational reconstruction, metabolic profiling without diagnostic outcomes, or non-Raman analytical techniques, as detailed in Fig. 1.

### 3.2. Characteristics of included studies

The principal characteristics of the 46 studies included in the qualitative synthesis are summarized in Table S2 (Supplementary Table S2). Published between 2015 and 2025, the included studies illustrate the rapid evolution of Raman-based glioma diagnostics from proof-of-concept spectroscopic investigations toward artificial intelligence (AI)-assisted platforms supporting intraoperative tissue characterization, molecular stratification, and real-time clinical decision-making (Livermore et al., 2020; Ember et al., 2024; Reinecke et al., 2024; Liu et al., 2024; Yin et al., 2025). Most studies originated from Europe, North America, and China.

The included cohorts comprised low- and high-grade gliomas, astrocytomas, oligodendrogliomas, and glioblastomas, with sample sizes ranging from fewer than 20 patients to more than 200 participants. Histopathology served as the reference standard in most studies, whereas more recent investigations increasingly incorporated WHO-integrated molecular biomarkers, including IDH mutation status, MGMT promoter methylation, DNA methylation subclasses, and other clinically relevant genomic alterations (Livermore et al., 2019; Sciortino et al., 2021; Lita et al., 2024; Liu et al., 2024; Yin et al., 2025).

Conventional Raman spectroscopy was the most frequently investigated modality, while recent studies expanded the field through SERS, stimulated Raman histology (SRH), stimulated Raman scattering (SRS), and multimodal Raman platforms. Tissue analysis included intraoperative *in vivo* assessment, fresh *ex vivo* tissue, frozen sections, and

formalin-fixed paraffin-embedded specimens. Recent technological advances also demonstrated the feasibility of handheld fiber-optic probes and real-time SRH platforms for intraoperative tissue characterization (Jermyn et al., 2017; Pekmezci et al., 2021; Straehle et al., 2022; Ember et al., 2024; Reinecke et al., 2024; Leblond et al., 2025).

Analytical approaches evolved from conventional statistical methods to machine-learning and deep-learning models, although substantial heterogeneity persisted in validation strategies and analytical units, with diagnostic performance reported at the patient-, sample-, spectrum-, image-, pixel-, or patch-level. Despite increasing use of independent test cohorts, prospective multicenter studies and external validation remained uncommon (Ember et al., 2024; Reinecke et al., 2025; Leblond et al., 2025).

Overall, heterogeneity in study design, acquisition protocols, analytical pipelines, validation methodologies, and outcome reporting precluded quantitative pooling and supported the use of a structured SWiM-guided narrative synthesis.

### 3.3. Sources of methodological heterogeneity

The included studies exhibited substantial methodological heterogeneity across nearly all stages of the diagnostic workflow, including Raman technology, clinical setting, tissue preparation, analytical methodology, validation strategy, outcome reporting, and study scale. Importantly, this variability extended beyond technical aspects to differences in diagnostic objectives, molecular endpoints, analytical units, and intended clinical applications. The principal sources of heterogeneity identified across the included studies are summarized in Table 1 and should be considered when interpreting diagnostic performance and translational potential.

Importantly, these sources of heterogeneity were clinically consequential rather than purely statistical, because diagnostic estimates derived from distinct clinical tasks and non-equivalent analytical units could not be assumed to represent a common underlying diagnostic effect. Quantitative pooling could therefore obscure clinically relevant differences between studies and overstate the precision and generalizability of the available evidence.

#### 3.3.1. Raman Modalities and Acquisition Strategies

The technological landscape of Raman-based glioma diagnostics evolved substantially during the study period. Earlier investigations primarily evaluated conventional spontaneous Raman spectroscopy for tissue discrimination and intraoperative guidance (Kast et al., 2015; Jermyn et al., 2015; Jermyn et al., 2016; Lu et al., 2016; Broadbent et al., 2018; Livermore et al., 2020; Livermore et al., 2019), whereas more recent studies increasingly adopted stimulated Raman histology (SRH), stimulated Raman scattering (SRS), surface-enhanced Raman spectroscopy (SERS), and multimodal optical platforms for rapid histological assessment, molecular characterization, and biomarker prediction (Straehle et al., 2022; Reinecke et al., 2022; Reinecke et al., 2024; Lita et al., 2024; Yin et al., 2025). Several studies also combined Raman spectroscopy with complementary optical techniques, including autofluorescence, diffuse reflectance spectroscopy, CARS, TPEF, and SHG (Galli et al., 2017; Jermyn et al., 2017; Ospanov et al., 2023; Uckermann et al., 2024).

#### 3.3.2. Clinical Setting and Tissue Preparation

Clinical implementation also varied considerably. Studies evaluated Raman spectroscopy using intraoperative handheld probes, fresh *ex vivo* tissue, frozen sections, cryosections, and formalin-fixed paraffin-embedded specimens (Jermyn et al., 2015; Desroches et al., 2018; Broadbent et al., 2018; Livermore et al., 2020; Riva et al., 2021; Herta et al., 2022; Ember et al., 2024;Klein et al., 2024; Lita et al., 2024). Diagnostic applications included tumor detection, infiltrative margin

**Table 1**
Major sources of methodological heterogeneity across Raman-based glioma diagnostic studies.

| Domain | Variability observed across included studies | Potential implications | Representative references |
|---|---|---|---|
| Raman modality | Conventional Raman spectroscopy, SERS, SRH, SRS, multimodal Raman platforms | Differences in biochemical information content, spatial resolution, intraoperative applicability, and clinical deployment potential | Kast et al., 2015; Lu et al., 2016; Sun et al., 2019; Reinecke et al., 2024; Yin et al., 2025 |
| Clinical settings | *In vivo* intraoperative measurements, *ex vivo* fresh tissue analysis, laboratory-based investigations | Differences in tissue handling, workflow integration, and translational relevance | Jermyn et al., 2015; Jermyn et al., 2017; Livermore et al., 2020; Herta et al., 2022; Ember et al., 2024 |
| Tissue preparation | Fresh tissue, frozen sections, cryosections, FFPE samples, archived specimens | Variability in tissue preservation, lipid integrity, water content, molecular composition, and spectral quality | Uckermann et al., 2018; Livermore et al., 2019; Sciortino et al., 2021; Lita et al., 2024; Reinecke et al., 2024 |
| Clinical objective | Tumor detection, margin assessment, infiltrative tumor identification, grading, molecular classification, intraoperative diagnosis | Studies frequently addressed different diagnostic questions despite reporting similar performance metrics | Jermyn et al., 2016; Pekmezci et al., 2021; Stables et al., 2016; Liu et al., 2024; Reinecke et al., 2024 |
| Analytical unit | Patient-level, sample-level, spectrum-level, pixel-level, image-level, patch-level analyses | Differences in effective sample size and clinical interpretability of diagnostic estimates | Livermore et al., 2019; Sciortino et al., 2021; Reinecke et al., 2022; Zhang et al., 2023; Reinecke et al., 2025 |
| Molecular endpoint | Histology only, IDH status, 1p/19q codeletion, MGMT promoter methylation, TERT promoter mutation, EGFR alterations, chromosome 7/10 signatures, CDKN2A/B deletion, DNA methylation subtypes, metabolic biomarkers | Different relevance for molecular stratification, precision oncology, patient risk classification, and personalized clinical decision-making | Uckermann et al., 2018; Livermore et al., 2019 ; Lita et al., 2024; Liu et al., 2024; Yin et al., 2025 |
| Machine-learning strategy | Conventional machine learning, deep learning, convolutional and transformer-based neural networks, explainable AI, multimodal learning | Differences in feature extraction, model complexity, interpretability, computational requirements, and risk of overfitting | Kast et al., 2015; Livermore et al., 2019 ; Reinecke et al., 2022; Liu et al., 2024; Li et al., 2025 |
| Validation strategy | Internal cross-validation, training–test splits, independent testing cohorts, external multicenter validation | Major differences in model robustness, clinical generalizability, reproducibility, and implementation readiness | Livermore et al., 2019; Sciortino et al., 2021; Ember et al., 2024; Reinecke et al., 2024; Reinecke et al., 2025 |
| Reference standard | Histopathology, neuropathologist assessment, molecular classification, comparator algorithms | Variability in outcome verification and benchmarking procedures | Jermyn et al., 2016; Uckermann et al., 2018; Pekmezci et al., 2021; Lita et al., 2024; Liu et al., 2024 |
| Outcome reporting | Sensitivity, specificity, accuracy, AUC, PPV, NPV, DOR, inconsistent reporting of contingency data | Reduced comparability and limited eligibility for quantitative synthesis | Jermyn et al., 2015; Livermore et al., 2020; Sciortino et al., 2021; Liu et al., 2024; Reinecke et al., 2025 |
| Study scale | Small single-center cohorts to multicenter external validation studies | Differences in statistical power, reproducibility, and translational readiness | Uckermann et al., 2018; Sciortino et al., 2021; Ember et al., 2024; Reinecke et al., 2024; Reinecke et al., 2025 |

assessment, grading, molecular classification, and intraoperative decision support.

#### 3.3.3. Analytical Approaches and Machine Learning

Analytical methods evolved from conventional multivariate statistics (PCA, LDA, QDA, SVM) (Kast et al., 2015; Jermyn et al., 2015; Stables et al., 2016; Uckermann et al., 2018; Livermore et al., 2019) toward ensemble-learning algorithms and deep-learning architectures, including convolutional neural networks, transformer-based models, DBCNet, and dedicated SRH classifiers (Sciortino et al., 2021; Riva et al., 2021; Li et al., 2023; Reinecke et al., 2022; Liu et al., 2024; Reinecke et al., 2024; Li et al., 2025; He et al., 2025; Yin et al., 2025).

#### 3.3.4. Validation Strategies and Units of Analysis

Validation methodology represented a major source of heterogeneity. Diagnostic performance was reported at the patient-, sample-, spectrum-, image-, pixel-, or patch-level using internal cross-validation or independent test cohorts, whereas external multicenter validation remained uncommon and was largely limited to recent SRH studies (Reinecke et al., 2025). Consistent with these findings, QUADAS-2 identified the patient-selection and index-test domains as the principal sources of methodological concern, supporting cautious interpretation of reported diagnostic performance.

### 3.4. Diagnostic Performance Across Raman Modalities

Diagnostic performance varied across Raman modalities but was consistently favorable for glioma detection, tissue classification, intraoperative guidance, and molecular characterization. Many studies reported accuracies exceeding 85%, with sensitivities and specificities frequently above 90% for differentiating glioma from non-neoplastic brain tissue. More recent investigations further expanded these applications toward AI-assisted molecular classification and digital pathology. Nevertheless, direct comparison between studies is limited by differences in Raman platforms, tissue preparation, analytical methods, validation strategies, and units of analysis.

#### 3.4.1. Conventional Raman Spectroscopy

Conventional Raman spectroscopy provides the largest body of evidence supporting glioma diagnosis. Early intraoperative studies using handheld probes demonstrated accurate discrimination between normal brain, infiltrative tumor, and dense tumor tissue, with reported accuracies around 90–92% and AUC values up to 0.96 (Jermyn et al., 2015; Jermyn et al., 2016). Desroches et al., 2015 further demonstrated the feasibility of intraoperative Raman-based tissue characterization, reporting 87% accuracy, 84% sensitivity, and 89% specificity for distinguishing necrotic from viable brain tissue in a cohort of 10 glioma patients. Comparable findings were subsequently reported using optical biopsy systems and multicenter glioblastoma cohorts (Desroches et al., 2018; Ember et al., 2024), while Herta et al. (2022) demonstrated improved detection of infiltrative peritumoral tissue compared with 5-ALA fluorescence. *Ex vivo* investigations likewise achieved excellent diagnostic performance, including accuracies above 97% and biopsy-level sensitivities and specificities approaching 100% (Broadbent et al., 2018; Livermore et al., 2020).

#### 3.4.2. Stimulated Raman histology and stimulated Raman scattering

SRH and SRS represent the principal advances enabling label-free digital histopathology. Following the proof-of-concept study by Lu et al. (2016), clinical investigations demonstrated high agreement with conventional neuropathology and strong diagnostic performance for tumor classification, margin assessment, and molecular prediction (Straehle et al., 2022; Pekmezci et al., 2021; Reinecke et al., 2024). The strongest evidence currently derives from recent multicenter validation, in which Reinecke et al. (2025) reported balanced accuracies exceeding 95% using external validation cohorts.

#### 3.4.3. Surface-Enhanced Raman Spectroscopy

Surface-enhanced Raman spectroscopy (SERS) extends the diagnostic capabilities of Raman technology beyond tissue discrimination by enabling highly sensitive molecular and metabolic characterization. Through signal amplification generated by nanostructured substrates, SERS facilitates the detection of subtle biochemical alterations associated with glioma biology and supports biomarker-driven diagnostics.

Representative studies demonstrated particularly encouraging results. Sun et al. (2019) reported sensitivities and specificities approaching 100% for glioma discrimination using SERS-derived metabolic fingerprints combined with machine-learning analysis. More recently, Yin et al., 2025 developed an AI-assisted SERS platform based on glutathione and hydrogen peroxide profiling that predicted IDH1 mutation status with accuracies exceeding 94%, highlighting the potential of SERS for intraoperative molecular stratification and precision neuro-oncology.

Although these findings illustrate the remarkable analytical sensitivity of SERS, most available studies remain limited by relatively small patient cohorts, highly controlled experimental settings, and scarce external validation. Consequently, further prospective clinical evaluation is required before these promising molecular applications can be translated into routine practice.

#### 3.4.4. Comparative synthesis of diagnostic performance

The evidence demonstrates complementary strengths across Raman modalities. Conventional Raman spectroscopy currently provides the most mature evidence for tissue discrimination, SRH/SRS support rapid intraoperative digital pathology, whereas SERS shows particular promise for molecular and metabolic characterization. However, comparisons between studies should be interpreted cautiously because reported diagnostic metrics frequently derive from different study designs, analytical units, and validation strategies. Greater methodological standardization, patient-level analyses, and prospective multicenter validation will therefore be required before routine clinical implementation.

### 3.5. Clinical Applications of Raman Spectroscopy in Glioma

The included studies demonstrate that Raman-based technologies are no longer limited to tumor detection but increasingly support multiple aspects of glioma diagnosis and surgical management. Five principal clinical applications emerged across the literature: tissue discrimination, tumor margin assessment, histopathological classification, molecular characterization, and intraoperative decision support. These applications differ considerably in their level of clinical maturity, supporting evidence, and barriers to implementation (Table 2).

#### 3.5.1. Tumor Detection and Tissue Classification

Tumor detection remains the most extensively investigated clinical application of Raman spectroscopy and currently represents the strongest body of evidence. Across conventional Raman spectroscopy, SRH, SRS, and handheld intraoperative systems, studies consistently demonstrated high diagnostic performance for distinguishing glioma from non-neoplastic brain tissue.

The feasibility of intraoperative Raman-guided tissue identification was established by Jermyn et al. (2015, 2016, 2017), and subsequently confirmed in independent cohorts using different acquisition platforms (Broadbent et al., 2018; Livermore et al., 2020; Ospanov et al., 2023; Ember et al., 2024). These studies indicate that Raman-derived biochemical fingerprints can reproducibly distinguish tumor from normal brain across different clinical settings and acquisition strategies.

Unlike conventional imaging modalities, Raman spectroscopy directly interrogates tissue biochemistry, allowing identification of molecular alterations associated with glioma without the need for exogenous contrast agents. Consequently, tissue discrimination currently represents the most clinically mature application of Raman

**Table 2**
Clinical maturity and translational readiness of Raman-based applications in glioma.

| Raman-based approach | Main applications | Representative diagnostic performance | Advantages | Limitations | References |
|---|---|---|---|---|---|
| Conventional Raman spectroscopy | Tumor detection; tissue classification; margin assessment | Up to 92% accuracy, 93% sensitivity, 91% specificity in intraoperative studies; > 97% accuracy reported *ex vivo* | Label-free biochemical information; minimal preparation; handheld/ fiber-optic implementation | Weak spontaneous signal; point-based sampling; acquisition and preprocessing variability | Jermyn et al., 2015; Jermyn et al., 2016; Broadbent et al., 2018; Livermore et al., 2020 |
| SERS | Tissue discrimination; metabolic and molecular profiling | 96% sensitivity, 100% specificity, 97.9% accuracy for glioma discrimination; > 94% accuracy for IDH1 prediction | High sensitivity; signal amplification; multiplex biomarker detection | Requires substrates/probes; small cohorts; limited external validation | Sun et al., 2019; Yin et al., 2025 |
| SRH | Histopathological classification; margin assessment; intraoperative digital pathology | 87.7% diagnostic accuracy in early clinical implementation; > 95% balanced accuracy in recent externally validated classification tasks | Rapid histology-like imaging; label-free; fresh tissue; AI-compatible | Specialized instrumentation; sampling effects; workflow and regulatory requirements | Straehle et al., 2022; Reinecke et al., 2024; Reinecke et al., 2025 |
| SRS | Chemical imaging; classification; grading | High diagnostic performance across individual studies; estimates not directly comparable across heterogeneous tasks | Rapid chemical imaging; high spatial resolution; biochemical and morphological information | Complex instrumentation; heterogeneous protocols; limited multicenter validation | Lu et al., 2016; Reinecke et al., 2022 |
| Multimodal Raman platforms | Tumor detection; margin assessment; intraoperative guidance | High performance in individual studies, but no representative cross-platform estimate | Complementary biochemical and structural information; integrated intraoperative assessment | Greater technical complexity; limited standardization and cross-platform validation | Galli et al., 2017; Jermyn et al., 2017; Ospanov et al., 2023; Uckermann et al., 2024 |

spectroscopy and provides the foundation for many of the more advanced applications that have subsequently emerged.

Importantly, reported performance reflects heterogeneous analytical contexts, ranging from point spectra and *ex vivo* tissue measurements to handheld intraoperative probes and image-based platforms. These approaches should not be considered interchangeable, as acquisition geometry, tissue preparation, analytical unit, and validation design directly influence both diagnostic performance and clinical applicability.

### 3.5.2. Tumor Margin Assessment

Detection of infiltrative tumor beyond the visible surgical margin represents one of the most clinically relevant applications identified in this review. Because diffuse infiltration is a defining characteristic of glioma and a major determinant of recurrence, technologies capable of identifying residual tumor during surgery could substantially improve surgical guidance.

Several studies demonstrated that Raman spectroscopy detects infiltrative disease extending beyond radiological or visual tumor boundaries. Jermyn et al. (2016) reported detection of invasive tumor beyond MRI-defined margins, while Desroches et al. (2018) demonstrated accurate tissue characterization using an optical biopsy needle. Herta et al. (2022) further showed that Raman spectroscopy identified infiltrative tumor not consistently detected by 5-ALA fluorescence, suggesting a complementary role during fluorescence-guided surgery. Similar observations were reported for SRH-based approaches, which successfully identified infiltrative tumor during intraoperative assessment (Pekmezci et al., 2021; Reinecke et al., 2024). Uckermann et al., 2020 demonstrated that combining CARS and two-photon excited fluorescence imaging with texture-based classification enabled discrimination between neoplastic and non-neoplastic brain tissue, achieving 96% sensitivity and 100% specificity in a mixed cohort of gliomas and brain metastases. Complementing these approaches, Li and Wang, 2024 developed a sparse spectral unmixing model to estimate glioma and normal tissue contributions within heterogeneous surgical margins. The model correctly classified 104 of 112 glioma-positive margin spectra (92.9%), although this finding does not establish diagnostic accuracy across independently validated positive and negative margins.

The clinical relevance of improved margin detection, however, extends beyond identifying residual tumor. Extent of resection remains an important prognostic determinant, but its benefit must be balanced against neurological risk and interpreted in the context of tumor biology (Karschnia et al., 2024 ; Mendoza Mireles et al., 2026). Raman-based margin assessment may therefore be most valuable not for indiscriminately extending resection, but for more precisely defining infiltrative disease within the limits of maximal safe resection, particularly in eloquent regions where oncological benefit must be balanced against preservation of neurological function.

From a workflow perspective, SRH may facilitate iterative margin assessment during surgery. Rapid analysis and sequential sampling can provide intraoperative feedback on residual tumor-cell density at resection margins while substantially reducing diagnostic turnaround compared with conventional frozen sections (Faraj et al., 2026).

Although these findings are encouraging, relatively few studies evaluated clinically meaningful endpoints such as extent of resection, progression-free survival, or overall survival. Consequently, margin assessment should currently be considered a promising but still evolving clinical application requiring prospective outcome-driven validation.

### 3.5.3. Histopathological Classification and Glioma Grading

Beyond binary tissue discrimination, Raman spectroscopy has increasingly been investigated for histopathological classification and glioma grading. Several studies demonstrated discrimination between low-grade and high-grade gliomas as well as classification of histological subtypes (Stables et al., 2016; Zhang et al., 2023; Li et al., 2025; Reinecke et al., 2024).

The introduction of SRH and SRS substantially expanded these capabilities by combining label-free virtual histology with artificial intelligence, enabling simultaneous preservation of tissue architecture and automated image interpretation (Reinecke et al., 2024; Reinecke et al., 2025). These developments increasingly position Raman imaging as a rapid adjunct to conventional neuropathology.

However, morphology-based grading alone has diminishing diagnostic autonomy within contemporary WHO classification, in which histological findings are integrated with molecular alterations. The translational value of Raman-based grading will therefore increasingly depend on whether rapid optical classification can complement, rather than merely reproduce, conventional histopathology.

Nevertheless, grading represents a substantially more complex diagnostic task than tumor-versus-normal discrimination. Performance remains more dependent on cohort composition, histological diversity, and validation strategy, indicating that additional multicenter validation is required before routine implementation.

#### 3.5.4. Molecular Characterization

Molecular characterization represents one of the fastest developing areas of Raman research in glioma. As the WHO classification increasingly relies on integrated molecular diagnostics, several groups have investigated whether Raman-derived biochemical signatures can predict clinically relevant molecular alterations.

Early work demonstrated discrimination of IDH-mutant gliomas (Uckermann et al., 2018), while subsequent studies confirmed the feasibility of machine-learning-assisted prediction of IDH status (Livermore et al., 2019; Sciortino et al., 2021; Liu et al., 2024; Yin et al., 2025). More recent investigations extended these applications to DNA methylation subclasses (Lita et al., 2024) and multiple WHO-integrated biomarkers, including 1p/19q codeletion, MGMT promoter methylation, TERT promoter mutation, EGFR alterations, chromosome 7/10 signatures, and CDKN2A/B deletion (Liu et al., 2024). Complementary SERS-based approaches further demonstrated rapid intraoperative prediction of IDH1 mutation through metabolic profiling (Yin et al., 2025).

The potential clinical value differs fundamentally from conventional tissue classification: rapid prediction of IDH, 1p/19q, MGMT, or other molecular features could provide information before completion of standard molecular testing and potentially during surgery itself. However, Raman-derived predictions represent surrogate biochemical signatures rather than direct measurements of the underlying genomic alteration and therefore require validation against standardized molecular reference methods in independent cohorts.

More broadly, intraoperative molecular characterization could shift surgical guidance from purely anatomical toward biologically informed tumor boundaries. Because the benefit of more extensive resection may vary with molecular and clinical characteristics, rapid molecular information could ultimately contribute to individualized surgical strategies based on tumor biology and patient-specific risk (Hou et al., 2025 ; Kueckelhaus et al., 2026). This potential link between Raman-based molecular prediction and precision glioma surgery remains compelling but has not yet been demonstrated in prospective outcome-driven studies.

Despite these advances, molecular characterization remains an emerging application. Most studies were retrospective, involved relatively small cohorts, and lacked independent external validation. Accordingly, current evidence primarily supports proof-of-concept rather than immediate clinical implementation.

#### 3.5.5. Intraoperative Decision Support and Digital Pathology

Among all applications identified in this review, intraoperative decision support demonstrates the greatest degree of translational maturity. The development of SRH and SRS transformed Raman spectroscopy from a point-based analytical technique into a digital pathology platform capable of generating histology-like images within minutes, without staining or conventional tissue processing.

Following the initial demonstration by Lu et al. (2016), successive studies progressively incorporated deep-learning algorithms into SRH workflows, achieving rapid intraoperative classification while maintaining high agreement with conventional neuropathology (Straehle et al., 2022; Reinecke et al., 2022; Reinecke et al., 2024; Reinecke et al., 2025). These systems illustrate the convergence of optical imaging, computational pathology, and artificial intelligence toward real-time surgical decision support.

However, successful clinical implementation will ultimately depend not only on diagnostic accuracy but also on workflow integration, acquisition speed, regulatory approval, operator training, and prospective multicenter validation. These factors currently represent the principal barriers to widespread adoption despite the encouraging diagnostic performance reported to date.

The principal advantage of these platforms is therefore not simply automated image classification, but compression of the diagnostic workflow: optical acquisition, image generation, and computational interpretation can occur directly from fresh tissue without fixation, sectioning, staining, and digitization. Their clinical value should consequently be assessed in terms of diagnostic turnaround time and impact on intraoperative decisions, in addition to conventional accuracy metrics.

Accordingly, Raman-based technologies are unlikely to replace established intraoperative modalities, but may function most effectively within multimodal workflows integrating anatomical, functional, optical, and molecular information. Emerging combinations of Raman, fluorescence, and AI-based interpretation may ultimately provide unified intraoperative decision support rather than parallel, modality-specific outputs (Faraj et al., 2026).

To facilitate comparison across Raman-based diagnostic approaches, their principal clinical applications, representative diagnostic performance, main advantages and limitations, and current translational maturity are summarized in Table 2.

Overall, the available evidence demonstrates that Raman spectroscopy has evolved into a versatile diagnostic platform addressing multiple clinically relevant questions in glioma management. While tissue discrimination currently represents the most mature application, rapid progress is being made toward intraoperative digital pathology and molecular characterization. At the same time, the degree of clinical readiness differs substantially across applications (Table 2), emphasizing that the translational value of Raman spectroscopy should be interpreted according to the specific clinical question rather than as a single, uniform diagnostic technology.

### 3.6. Machine Learning and Validation Maturity

The rapid development of Raman-based glioma diagnostics has been paralleled by major advances in computational analysis. Across the included studies, analytical approaches evolved from conventional multivariate statistics toward increasingly sophisticated machine-learning and deep-learning models. Although this evolution has substantially improved diagnostic performance, it has also increased the importance of robust validation, reproducibility, and model generalizability.

#### 3.6.1. Evolution of Bioinformatic Approaches

Analytical methods evolved from conventional statistical techniques, including principal component analysis (PCA), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), clustering methods, and logistic regression (Kast et al., 2015; Jermyn et al., 2015; Stables et al., 2016; Uckermann et al., 2018), toward machine-learning approaches such as support vector machines, random forests, ensemble learning, and gradient boosting (Livermore et al., 2020; Livermore et al., 2019; Sciortino et al., 2021; Riva et al., 2021). Li et al., 2023 applied an entropy-weighted fuzzy-rough nearest-neighbor (EFRNN) algorithm to Raman peak characteristics, including position, intensity, and half-width, achieving 86.99% accuracy, 87.21% sensitivity, and 86.49% specificity in distinguishing glioma from normal brain tissue. More specifically, Li et al., 2025 combined Raman peak-ratio feature enhancement with adaptive stacking ensemble learning to distinguish normal brain tissue, low-grade gliomas, and high-grade gliomas, reporting an overall classification accuracy of 82.1%. An early application of artificial neural networks was reported by Jermyn et al., 2016, who demonstrated improved robustness to operating-room light artifacts during *in vivo* Raman-based brain cancer classification, achieving 90% accuracy compared with 71% for boosted trees when artifact-affected measurements were included. More recent studies increasingly adopted deep-learning architectures, including convolutional neural networks, ResNet variants, transformer-based models, DBCNet, SRH-specific classifiers, and multimodal AI frameworks (Reinecke et al., 2022; Liu et al., 2024; Reinecke et al., 2024; Li et al., 2025; He et al., 2025; Yin et al., 2025). Li and Chen, 2025 further demonstrated the potential of combining deep-learning-based spectral feature extraction with ensemble classification, integrating Raman and

fluorescence information derived from a miniature Raman spectrometer. Their approach achieved 91.9% accuracy, 96.7% sensitivity, and 80.8% specificity in distinguishing glioma from normal brain tissue.

#### 3.6.2. Validation remains the principal limitation

Despite increasing algorithmic complexity, internal validation predominated across the literature, including leave-one-out, leave-one-patient-out, k-fold cross-validation, bootstrap resampling, and internal train–test splits. Validation strategies varied substantially, and preprocessing, feature selection, and model optimization were frequently performed within the same datasets used for model development, increasing the potential for overfitting. Consistent with these observations, QUADAS-2 identified the index-test domain as the principal source of methodological concern.

#### 3.6.3. Analytical level influences reported performance

A major source of variability concerned the analytical unit used for model evaluation. While some studies reported patient-level performance, many assessed individual spectra, pixels, or image patches generated from relatively small patient cohorts (Livermore et al., 2019; Sciortino et al., 2021; Zhang et al., 2023; Reinecke et al., 2022; Reinecke et al., 2024; Reinecke et al., 2025).

Although spectrum- and patch-level analyses are appropriate for algorithm development, observations originating from the same patient are not statistically independent. Consequently, exceptionally high diagnostic performance reported at the spectrum or image level should not be considered directly equivalent to patient-level clinical performance. This distinction is essential when comparing studies and assessing their translational relevance.

#### 3.6.4. Methodological Risks and Best Practices for Machine-Learning Validation

Machine-learning validation introduces specific sources of bias in Raman diagnostic studies because multiple spectra, pixels, or image patches are frequently derived from the same patient or specimen. Random measurement-level splitting can therefore place correlated observations in both training and test sets, causing data leakage and potentially inflating diagnostic performance. Partitioning should instead occur at the highest biologically independent level, with all measurements from the same patient or specimen retained within a single partition (Bradshaw et al., 2023; Fu et al., 2025). This distinction is clinically relevant, as per-spectrum analyses remain predominant in Raman–AI studies and show substantially greater between-study heterogeneity than per-patient estimates (Halimi et al., 2026).

Class imbalance represents an additional source of bias, particularly in small datasets, where aggregate accuracy may conceal poor discrimination of underrepresented diagnostic or molecular subgroups (Leng et al., 2024). Stratified patient-level or group-aware cross-validation can reduce sampling bias while preserving biological independence; preprocessing, feature selection, and hyperparameter optimization should remain confined to the training/validation workflow, leaving the test set untouched until final evaluation (Bradshaw et al., 2023; Lilek et al., 2025). Performance should consequently be reported using class-specific metrics alongside aggregate measures.

Patient-level independence, however, does not establish transportability across clinical settings. Validation within a single data source may remain optimistic when models encounter shifts in patient populations, instruments, acquisition protocols, or institutions. Source-level or internal–external validation can more directly assess robustness to such heterogeneity, followed ultimately by genuinely independent, preferably prospective multicenter validation (Leinonen et al., 2024; Efthimiou et al., 2024). External evaluation should assess not only discrimination but also calibration and, where appropriate, clinical utility before claims of generalizability are made (Santos and Amorim-Lopes, 2025).

Finally, reproducibility requires transparent reporting of instrumentation, acquisition parameters, preprocessing, dataset partitioning, model development, and the composition and respective roles of training, validation, test, and external datasets, consistent with STARD-AI recommendations (Sounderajah et al., 2025). Together, these safeguards are essential to distinguish high internal classification performance from evidence of clinically transferable Raman–ML models.

#### 3.6.5. External Validation and clinical translation

Independent external validation remains uncommon. Most studies were developed and tested within single institutions using individual acquisition platforms and relatively homogeneous patient populations. Given that Raman spectra are influenced by instrumentation, acquisition protocols, tissue preparation, and patient characteristics, the generalizability of these models remains uncertain.

Only a limited number of studies incorporated independent testing cohorts or multicenter validation. Among these, the work of Reinecke et al. (2025) represents one of the most mature validation frameworks currently available, demonstrating robust performance across external cohorts. Similarly, recent prospective intraoperative studies further illustrate the transition from proof-of-concept algorithms toward clinically deployable Raman-based diagnostic systems (Ember et al., 2024).

Overall, the literature demonstrates rapid progress in machine-learning-assisted Raman diagnostics, but validation frameworks have evolved more slowly than analytical methodologies. Future studies should prioritize patient-level evaluation, standardized validation protocols, transparent reporting, and multicenter external validation to establish the reproducibility required for routine clinical implementation.

### 3.7. Risk of bias assessment

The methodological quality of the included studies was assessed using the QUADAS-2 tool across four domains: patient selection, index test, reference standard, and flow and timing (Fig. 2). Overall, the evidence base showed moderate methodological quality. Methodological concerns were concentrated in the patient-selection and index-test domains, whereas the reference-standard and flow-and-timing domains generally demonstrated a lower risk of bias.

#### 3.7.1. Overall risk-of-bias profile

The distribution of QUADAS-2 judgments is presented in Fig. 2. Most studies were judged as having either low risk of bias or some concerns across individual domains, although the overall risk of bias remained moderate because relatively few investigations fulfilled all methodological quality criteria. More recent studies, particularly those evaluating SRH-based platforms, generally demonstrated more rigorous study design and validation procedures than earlier proof-of-concept investigations.

#### 3.7.2. Patient selection

The patient-selection domain represented one of the principal sources of methodological concern. Many studies were based on relatively small, single-center cohorts and retrospective recruitment, while eligibility criteria and sampling procedures were not always fully described. In addition, substantial variability in tumor subtype, grade, recurrence status, and control populations limited comparability across studies and may have influenced reported diagnostic performance.

#### 3.7.3. Index test domain

The index-test domain showed the highest frequency of high-risk or unclear judgments. This primarily reflected heterogeneity in spectral preprocessing, feature selection, machine-learning implementation, and validation methodology. Several studies optimized analytical pipelines using the same datasets employed for model development, increasing the potential for overfitting and optimistic performance estimates. The predominance of internal validation and the limited availability of

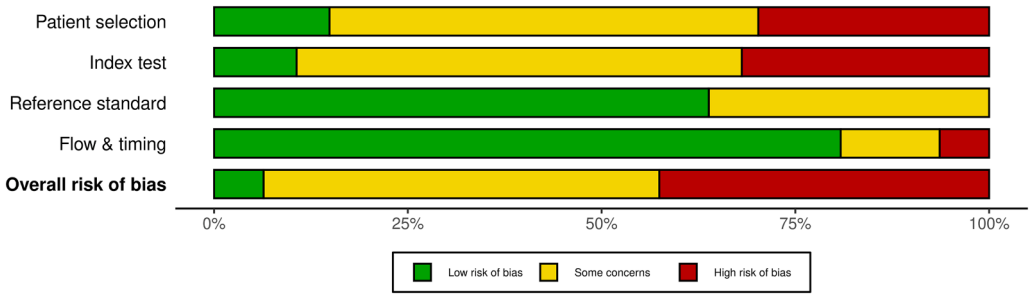



**Fig. 2.** Summary QUADAS-2 risk-of-bias assessment of included studies. Bars indicate the percentage of studies rated as low risk of bias (green), some concerns (yellow), or high risk of bias (red) across the four QUADAS-2 domains and the overall risk-of-bias judgment. The highest frequency of methodological concerns was observed in the patient-selection and index-test domains.

independent external cohorts further reduced confidence in the generalizability of published models.

#### *3.7.4. Reference standard and flow-and-timing*

In contrast, the reference-standard domain demonstrated consistently favorable assessments. Histopathological examination served as the reference standard in nearly all studies, frequently complemented by molecular characterization according to contemporary WHO classification criteria. Similarly, the flow-and-timing domain generally showed low risk of bias because Raman measurements were typically performed on tissue that subsequently underwent histopathological evaluation.

These favorable QUADAS-2 judgments should nevertheless be interpreted cautiously. Histopathology remains the appropriate clinical reference standard but is not an error-free ground truth for highly spatially resolved Raman measurements. At infiltrative margins, low and heterogeneous tumor-cell density increases susceptibility to sampling error, while histopathological interpretation may itself show observer-dependent variability. Pekmezci et al. (2021), for example, reported only moderate interobserver agreement for H&E-based assessment of glioma margins, with greater agreement for immunohistochemistry, and used consensus scoring to reduce individual interpretative bias. More broadly, biopsies obtained from glioma infiltration zones may contain mixtures of tumor and non-neoplastic tissue, making the assigned pathological label dependent on the precise region examined. This is particularly relevant because misclassified Raman biopsies have been shown to encompass such heterogeneous infiltration borders (Galli et al., 2019).

The degree of spatial correspondence between the Raman measurement and the reference specimen is therefore critical, as even small sampling offsets may result in the index test and histopathology interrogating biologically different tissue. This limitation varies substantially across study designs. Reinecke et al. (2024), for example, acknowledged that differences between specimens used for Raman-based assessment and pathological review could contribute to discordant classifications through tissue heterogeneity and sampling effects. Conversely, some prospective studies implemented rigorous site-matched validation: Herta et al. (2022) obtained biopsies immediately after Raman interrogation from the same neuronavigation-defined location, with blinded neuropathological assessment, while Ember et al. (2024) paired 976 in situ measurements with colocalized tissue specimens and histopathological estimates of tumor-cell burden. Such designs substantially reduce, but cannot completely eliminate, reference-standard uncertainty arising from microscopic heterogeneity.

An additional consideration applies to molecular classification. Molecular labels established at the patient or specimen level may not necessarily represent the precise tissue volume interrogated spectroscopically when molecular testing and Raman acquisition are performed on adjacent or otherwise non-identical samples. For example, Uckermann et al., 2018 determined IDH1 status by DNA sequencing at the specimen level, whereas Raman spectra were acquired from selected tumor regions guided by histological assessment of an adjacent section. Given the intratumoral heterogeneity of gliomas, this distinction is important when evaluating Raman-based prediction of molecular subtypes and biomarkers.

Overall, the QUADAS-2 assessment indicates that study design and analytical methodology remain major sources of bias in the current literature. However, favorable reference-standard ratings should not be interpreted as evidence of uniformly error-free diagnostic verification, particularly at infiltrative margins or in molecular-classification studies. Histopathological sampling, observer variability, spatial correspondence between index and reference tests, and site-level molecular verification should therefore be considered alongside cohort selection, prospective study design, standardized analytical pipelines, and independent multicenter validation when interpreting the reported diagnostic performance.

### *3.8. Translational readiness and strength of evidence*

Overall, the available literature indicates that Raman-based technologies have progressed beyond exploratory proof-of-concept studies and now demonstrate reproducible diagnostic performance across several clinically relevant applications. However, translational readiness differs substantially according to the intended clinical application, the maturity of validation strategies, and the quality of the supporting evidence.

#### *3.8.1. Applications Supported by the Strongest Evidence*

The strongest evidence currently supports tumor detection, tissue discrimination, and intraoperative tissue characterization. These applications have been evaluated across multiple Raman modalities, independent research groups, and different clinical settings, consistently demonstrating accurate differentiation between glioma and non-neoplastic brain tissue (Jermyn et al., 2015; Broadbent et al., 2018; Livermore et al., 2020; Ember et al., 2024). Similarly, SRH- and SRS-based platforms have shown increasing potential for rapid intraoperative diagnosis and digital pathology, with recent studies demonstrating diagnostic performance approaching conventional neuropathological assessment (Lu et al., 2016; Straehle et al., 2022; Reinecke et al., 2024).

Tumor margin assessment also represents a promising translational application. Several studies reported improved detection of infiltrative tumor beyond radiological or fluorescence-defined margins, although evidence linking these findings to improved surgical or survival outcomes remains limited (Jermyn et al., 2016; Herta et al., 2022; Pekmezci et al., 2021; Reinecke et al., 2024).

#### *3.8.2. Emerging Applications*

Molecular characterization and AI-assisted precision neuro-oncology represent rapidly expanding areas of research. Recent studies demonstrated encouraging results for predicting IDH status, DNA methylation

subtypes, and other WHO-integrated molecular biomarkers directly from Raman-derived data (Livermore et al., 2019; Lita et al., 2024; Liu et al., 2024; Yin et al., 2025). These findings suggest that Raman spectroscopy may evolve from a tissue-identification technique toward a platform supporting integrated molecular diagnostics.

Nevertheless, evidence supporting these applications remains less mature than that available for tissue discrimination. Most studies were retrospective, included relatively small cohorts, and lacked independent external validation, indicating that these applications should currently be regarded as promising rather than clinically established.

#### 3.8.3. Remaining barriers to clinical implementation

Across the included studies, the principal barriers to clinical translation were methodological rather than technological. Considerable variability in acquisition protocols, analytical pipelines, validation strategies, and outcome reporting limited direct comparison between studies. Furthermore, most investigations relied on internal validation, whereas external multicenter validation remained uncommon.

Additional challenges include the predominance of spectrum- or image-level analyses over patient-level evaluation, together with limited evidence regarding workflow integration, regulatory implementation, and clinical effectiveness in routine neurosurgical practice.

#### 3.8.4. Overall Strength of Evidence

Taken together, the current evidence consistently supports the diagnostic potential of Raman-based technologies for glioma, particularly for tissue discrimination and intraoperative assessment. In contrast, applications involving molecular characterization and precision neuro-oncology remain supported primarily by early translational studies.

Overall, the principal limitation of the current evidence is not the absence of diagnostic capability, but the limited availability of standardized prospective studies with independent multicenter validation. Future progress will therefore depend on harmonized acquisition protocols, patient-level validation, transparent reporting, and prospective clinical evaluation to establish the reproducibility and clinical utility required for routine implementation.

## 4. Discussion

### 4.1. Biological rationale and principal findings

This systematic review synthesized evidence from 46 studies evaluating conventional Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), stimulated Raman histology (SRH), stimulated Raman scattering (SRS), and multimodal Raman platforms for glioma diagnosis.

A distinctive contribution of the present review is therefore the evaluation of Raman-based glioma diagnostics not solely according to reported diagnostic accuracy, but across the broader pathway from analytical performance to validation maturity and clinical translation. By integrating clinical application, methodological quality, analytical unit, validation strategy, and translational readiness, our synthesis identifies where apparently strong diagnostic evidence is supported by clinically robust validation and where important evidence gaps remain.

The evidence shows that Raman-based technologies have progressed beyond experimental spectroscopic discrimination toward clinically oriented applications encompassing intraoperative tissue characterization, histopathological assessment, tumor-margin delineation, molecular stratification, and precision neuro-oncology.

A principal finding is that high discriminatory performance has been demonstrated across multiple Raman modalities, whereas evidence of clinical generalizability remains substantially less mature. This distinction is important: strong performance across technically diverse platforms supports the presence of robust biochemical differences between glioma and non-neoplastic tissue, but does not by itself establish transportability across patients, institutions, instruments, or clinical workflows. Recent meta-analyses similarly reported high pooled diagnostic performance for glioma–normal tissue discrimination while identifying substantial between-study heterogeneity and a persistent need for *in vivo* and clinically representative validation (Krzemińska et al., 2025; Trevisi et al., 2025). Particularly important sources of variability include tissue and control definitions, acquisition conditions, spectral preprocessing, machine-learning pipelines, validation strategies, and outcome reporting. Thus, the central translational challenge is increasingly the conversion of analytical discrimination into reproducible patient-level clinical performance.

The biological basis for this discrimination is compelling. Unlike conventional neuroimaging, Raman spectroscopy interrogates the molecular composition of tissue directly, capturing differences in lipids, proteins, nucleic acids and other biochemical constituents that accompany malignant transformation and remodeling of the tumor microenvironment. Multiple studies included in this review identified diagnostically informative spectral alterations within these biochemical domains (Uckermann et al., 2018; Livermore et al., 2019; Sciortino et al., 2021; Liu et al., 2023; Yin et al., 2025). Notably, Ge et al. (2025) linked Raman signatures across biomarker, cellular, and tissue levels, showing that glioma-associated tissue spectra reflected alterations in lipid-related components and RNA and shared spectral features with GABA and MMP-2. These observations are biologically consistent with the extensive metabolic reprogramming characteristic of glioma, which contributes not only to tumor growth but also to adaptation, invasion and therapeutic resistance (Abdul Rashid et al., 2022; Tu et al., 2026). Raman classification therefore has a plausible mechanistic substrate rather than relying solely on statistically separable spectral patterns.

The transition toward molecularly integrated glioma classification further expands this rationale. Alterations such as IDH mutations produce profound metabolic consequences and associated biochemical phenotypes that can be interrogated spectroscopically (Liu et al., 2023; Nassiri et al., 2024; Wang et al., 2025). At the same time, diffuse glioma infiltration creates a spatial continuum between tumor core, infiltrative margin and apparently non-neoplastic brain rather than a sharply defined biological boundary (Doroszko et al., 2025; Prathaban et al., 2023). This provides a mechanistic explanation for the encouraging performance reported for both molecular biomarker prediction and detection of infiltrative tumor beyond conventional macroscopic boundaries (Jermyn et al., 2016; Broadbent et al., 2018; Uckermann et al., 2018; Livermore et al., 2019; Pekmezci et al., 2021; Sciortino et al., 2021; Herta et al., 2022; Liu et al., 2024; Reinecke et al., 2024; Yin et al., 2025).

These findings place Raman-based technologies within the broader transition from precision medicine to precision neuro-oncology. Precision neuro-oncology applies patient-specific diagnostic and therapeutic principles to central nervous system tumors by integrating histopathological, molecular, imaging, and clinical information. This paradigm is particularly relevant to glioma, where molecular alterations increasingly define tumor entities and influence prognostic and therapeutic stratification (Komori et al., 2022; Śledzińska et al., 2022; Massaad et al., 2022; Wang et al., 2023). Within this context, the contribution of Raman spectroscopy extends beyond tissue discrimination: the included studies demonstrate its potential to characterize infiltrative tumor, predict clinically relevant molecular features, and provide biochemical information during surgery (Jermyn et al., 2016; Herta et al., 2022; Lita et al., 2024; Liu et al., 2024; Reinecke et al., 2024). This potential also extends to patient-specific biochemical phenotyping, as machine-learning-assisted Raman spectroscopy has discriminated glioma grades across tissue and serum while capturing glycosylation-related spectral changes and differences associated with IDH1 status (Quesnel et al., 2023).

Rather than replacing histopathology, molecular testing, or neuroimaging, Raman-based technologies may therefore serve as a

complementary source of real-time tissue information within multimodal precision-neuro-oncology workflows, as conceptually summarized in Fig. 3.

Importantly, these applications should not be considered equally mature. *Ex vivo* tissue discrimination and intraoperative tissue characterization currently have the strongest translational foundation, whereas molecular classification and biomarker prediction remain supported predominantly by retrospective or single-center datasets with comparatively limited independent validation. Margin assessment occupies an intermediate and particularly promising position because Raman spectroscopy may complement rather than replace established intraoperative guidance. This application is clinically relevant because residual infiltrative disease beyond contrast-enhancing boundaries may influence outcome; maximal resection of both contrast-enhancing and non–contrast-enhancing tumor has been associated with improved survival in molecularly characterized glioblastoma cohorts, a relationship further supported by recent meta-analytic evidence (Molinaro et al., 2020; Geens et al., 2025). Other optical approaches, including OCT, are likewise being investigated for intraoperative delineation of glioma margins, with volumetric OCT recently demonstrating three-dimensional visualization of peripheral and depth margins in a preclinical glioma model (Park et al., 2024). Supporting the feasibility of Raman-based margin assessment, Romanishkin et al. (2022) demonstrated that spontaneous Raman spectroscopy combined with dimensionality reduction and machine learning could discriminate malignant tissue encompassing the tumor center and infiltrative edge from normal-appearing brain based on biochemical spectral differences. Daoust et al. (2025) subsequently developed a large-field-of-view Raman imaging system that generated spatial cancer-likelihood maps from freshly resected glioblastoma specimens using a machine-learning model trained on *in vivo* Raman spectra. The underlying model achieved 90% sensitivity and 95% specificity, while specimen-level Raman maps showed spatial agreement with histopathological assessment, including heterogeneous tissue from the tumor periphery. Recent machine-learning-enabled Raman approaches have also been used to refine fluorescence-defined glioblastoma boundaries, illustrating the potential value of biochemical information in regions where conventional optical guidance becomes uncertain (Han et al., 2026). Such multimodal integration may represent a more realistic translational pathway than positioning Raman spectroscopy as a standalone diagnostic technology.

Accordingly, further increases in internally measured accuracy alone are unlikely to resolve the major barriers to clinical translation. The field now requires rigorous patient-level evaluation, prevention of data leakage between training and test sets, prospective and external multicenter validation, standardized acquisition and preprocessing pipelines, transparent reporting of machine-learning development, and assessment under the conditions in which the technology is intended to operate. Analytical performance, clinical validity and clinical utility should therefore be treated as distinct stages of evidence generation. A model that accurately separates spectra within a curated dataset is not necessarily capable of maintaining performance across instruments, centers,

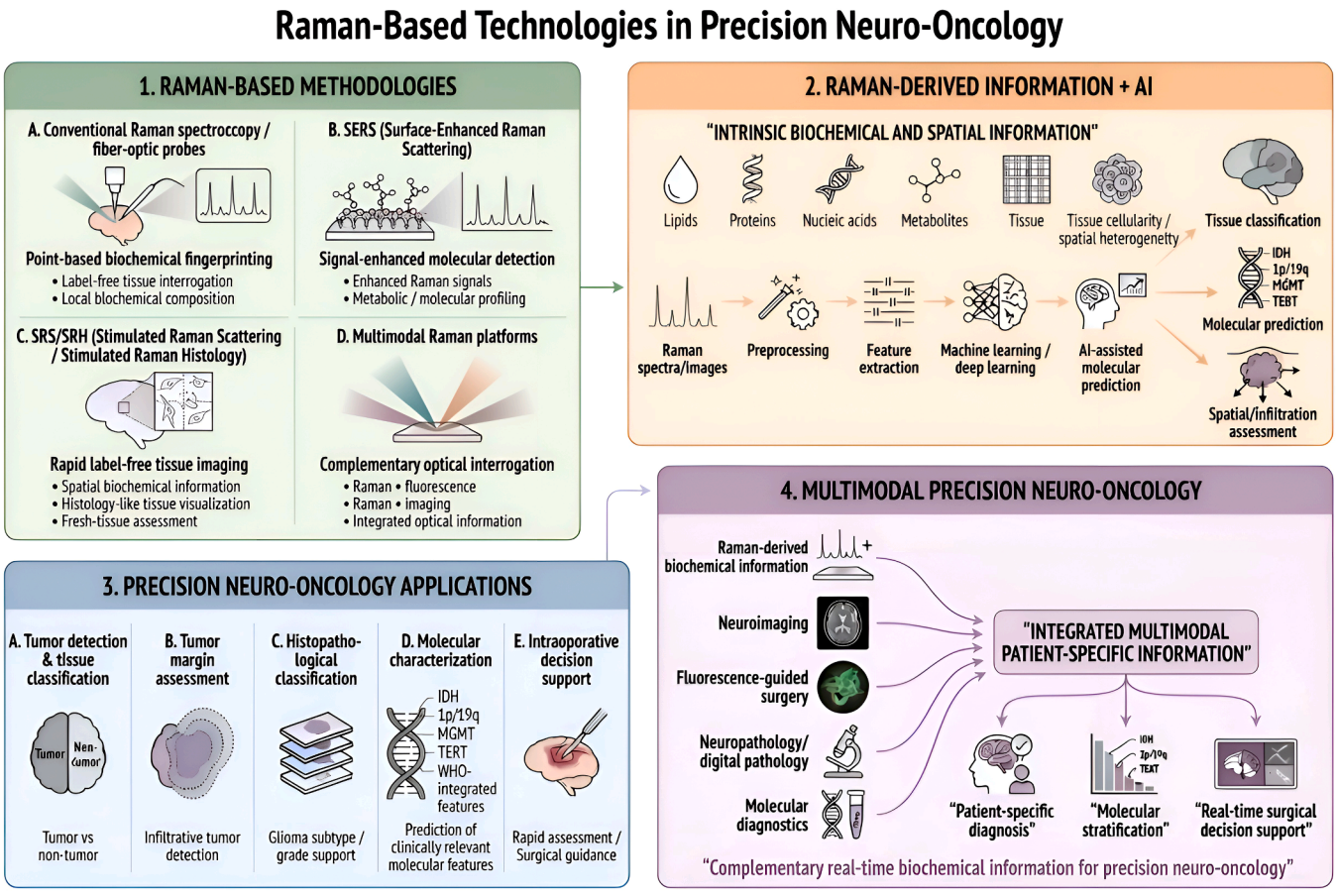



**Fig. 3.** Raman-based technologies within the precision neuro-oncology framework. Conventional Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), stimulated Raman scattering/stimulated Raman histology (SRS/SRH), and multimodal Raman platforms provide complementary biochemical and spatial tissue information that can be integrated with machine-learning and deep-learning approaches for tissue classification, infiltrative margin assessment, histopathological characterization, molecular prediction, and intraoperative decision support. Within precision neuro-oncology, Raman-derived information may complement neuroimaging, fluorescence-guided surgery, neuropathology/digital pathology, and molecular diagnostics, contributing to integrated patient-specific diagnostic and surgical decision-making.

tumor subtypes or real-time surgical conditions. These priorities underpin the translational roadmap summarized in Table 3.

### 4.2. From Intraoperative Raman Imaging to AI-Assisted Molecular Diagnostics

The principal clinical value of Raman spectroscopy lies in its potential to provide molecular information during surgery, addressing a persistent challenge in glioma management: distinguishing infiltrative tumor from functionally preserved brain while maximizing safe resection. Contemporary neurosurgery increasingly integrates neuronavigation, intraoperative MRI, ultrasound and fluorescence-guided surgery, yet these approaches primarily provide anatomical or indirect biological information and remain imperfect for detecting diffuse infiltration beyond radiologically or visually defined tumor boundaries (Bonosi et al., 2023; Sulangi et al., 2024; Perera Valdivia et al., 2024). Navigation accuracy is further compromised by intraoperative brain shift, progressively weakening the correspondence between preoperative imaging and the evolving surgical field (Mazzucchi et al., 2022; Rai et al., 2025).

By interrogating intrinsic tissue biochemistry rather than morphology alone, Raman spectroscopy can provide complementary information on tumor infiltration. For example, Bury and collaborators (Bury et al., 2020) demonstrated 94% accuracy and 98.8% sensitivity for Raman-based discrimination of fresh-frozen tumor from non-tumor brain tissue, although specificity was substantially lower and classification performance decreased when individual tumor types were distinguished. Multiple studies have demonstrated discrimination between tumor and non-tumor tissue together with assessment of cellularity, infiltration and molecular composition (Jermyn et al., 2015; Jermyn et al., 2016; Broadbent et al., 2018; Livermore et al., 2020; Pekmezci et al., 2021; Herta et al., 2022). Importantly, the emerging clinical paradigm is one of complementarity rather than replacement. Raman spectroscopy may increase sensitivity to infiltrative disease where conventional visual or fluorescence boundaries become uncertain, whereas established modalities can provide rapid macroscopic localization or greater specificity within the tumor core (Herta et al., 2022; Livermore et al., 2020). Han et al. (2026) extended this principle by integrating wide-field ICG fluorescence with machine-learning-assisted Raman spectroscopy in a "search--and-confirm" workflow, coupling rapid lesion localization with molecular interrogation of suspicious tissue. Such workflows may be more clinically realistic than expecting a single optical modality to satisfy localization, margin assessment and molecular characterization simultaneously.

Stimulated Raman histology (SRH) extends this concept from point-based molecular interrogation to rapid digital tissue assessment, generating histology-like images from fresh specimens and thereby linking vibrational spectroscopy with computational neuropathology (Lu et al., 2016; Straehle et al., 2022; Reinecke et al., 2022; Reinecke et al., 2024; Reinecke et al., 2025). However, morphological equivalence should not be assumed. Recent comparative evidence indicates that stimulated Raman approaches can reproduce several diagnostically relevant glioblastoma features, while conventional H&E remains superior for selected WHO-defining morphological criteria (Sippl et al., 2026). SRH should therefore currently be viewed as an intraoperative complement to, rather than a substitute for, conventional neuropathology.

The added value of combining Raman imaging with AI, rather than applying AI solely to conventional digital pathology, lies primarily in the nature and timing of the information available for computational analysis. Conventional digital pathology predominantly derives predictive information from tissue morphology after histological processing and staining, whereas Raman-based imaging exploits intrinsic vibrational contrast and can retain biochemical information from lipids, proteins, nucleic acids, and other tissue constituents while preserving spatial architecture (Lu et al., 2016; Lita et al., 2024). Recent multimodal AI approaches have substantially expanded the information obtainable from conventional histology; for example, virtual spatial proteomic profiles can now be inferred from routine H&E images, illustrating the increasing molecular reach of computational pathology (Valanarasu et al., 2026). However, such molecular information is computationally inferred from morphological patterns, whereas Raman spectroscopy directly interrogates tissue-associated vibrational signatures. In SRH workflows, this information can be acquired directly from fresh,

**Table 3**
Translational roadmap for implementing Raman-based diagnostics in precision neuro-oncology.

| Current challenge | **Why it matters** | **Priority for future research** | **Expected contribution to precision neuro-oncology** |
|---|---|---|---|
| Lack of standardized Raman acquisition and preprocessing protocols | Reduces reproducibility and limits comparison across institutions | Develop international consensus protocols for acquisition, preprocessing, quality control, and reporting | Harmonized multicenter datasets and reproducible diagnostic performance |
| Predominance of retrospective, single-center studies | Limits generalizability and evidence quality | Conduct prospective multicenter clinical validation studies with standardized protocols | Higher-level clinical evidence supporting routine implementation |
| Limited independent external validation | High reported accuracy may not translate across institutions or patient populations | Validate algorithms using geographically and technically independent cohorts | Robust, clinically generalizable diagnostic models |
| Reliance on spectrum-, pixel-, or patch-level analyses | May overestimate real-world diagnostic performance | Prioritize patient-level analyses and clinically meaningful outcome measures | More reliable estimates of clinical utility and diagnostic effectiveness |
| Incomplete integration with molecular diagnostics | Contemporary glioma classification increasingly depends on WHO-integrated molecular biomarkers | Integrate Raman spectroscopy with genomic, epigenomic, methylation, and digital pathology workflows | Rapid intraoperative molecular stratification supporting precision neuro-oncology |
| Limited explainability and uncertainty estimation in AI models | Restricts clinician confidence and regulatory acceptance | Develop explainable, uncertainty-aware, and clinically interpretable AI frameworks | Greater clinician trust, regulatory readiness, and safer clinical deployment |
| Workflow integration and regulatory implementation remain insufficiently evaluated | High diagnostic accuracy alone is insufficient for routine adoption | Assess workflow efficiency, turnaround time, cost-effectiveness, regulatory compliance, and implementation feasibility | Successful incorporation into routine neurosurgical and neuropathology practice |
| Limited multimodal integration | Individual modalities provide complementary but incomplete biological information | Develop multimodal platforms combining Raman spectroscopy with MRI, fluorescence guidance, digital pathology, and molecular profiling | Comprehensive intraoperative decision support enabling precision surgical oncology |

unprocessed tissue and converted into histology-like images suitable for automated interpretation within minutes, avoiding the fixation, sectioning, and staining steps required by conventional pathology (Reinecke et al., 2022; Reinecke et al., 2024). Importantly, AI can exploit these Raman-derived biochemical signatures not only for morphological classification but also for prediction of molecularly relevant features, including IDH status and other WHO-integrated biomarkers (Lita et al., 2024; Liu et al., 2024). The potential advantage is therefore not that Raman–AI is intrinsically superior to digital pathology–AI, but that it can add biochemical and molecularly informative contrast to rapid intraoperative tissue assessment. The two approaches should consequently be regarded as complementary, with conventional digital pathology retaining greater morphological familiarity and established diagnostic infrastructure, while Raman–AI offers a route toward rapid, label-free biochemical and molecular decision support.

This computational layer extends Raman analysis beyond tumor classification toward clinically relevant molecular and phenotypic characterization. Machine-learning approaches have been applied to features including IDH status, DNA methylation subtypes and integrated tumor classification (Kast et al., 2015; Sciortino et al., 2021; Liu et al., 2024; Lita et al., 2024; Yin et al., 2025; Reinecke et al., 2024). Recent proof-of-concept evidence further suggests that Raman–AI may capture biologically distinct tumor phenotypes beyond established molecular classes. Wurm et al. (2026) combined confocal Raman spectroscopy with deep learning to distinguish the pediatric SF188 glioblastoma phenotype from adult-derived GBM cell lines, achieving 83.6% accuracy and an AUC of 0.855 while identifying differences across lipid-, protein- and nucleic-acid-associated spectral features. However, because the pediatric group was represented by a single cell line, these findings primarily demonstrate phenotype-level feasibility rather than generalizable pediatric–adult classification. More recent SRH studies extend this trajectory towards rapid quantification of glioma infiltration using multicenter foundation models and automated differential diagnosis between glioblastoma and CNS lymphoma (Kondepudi et al., 2025; Scheffler et al., 2025). Preliminary results from the SIGNAL framework further suggest that AI-assisted SRH could infer multiple molecular features, including IDH, 1p/19q and ATRX status, alongside integrated glioma classification (Goff et al., 2026). The potential clinical value is therefore not merely faster image classification, but compression of morphological and molecular information into the intraoperative time window. Nevertheless, molecular predictions remain inferential and require prospective validation against reference-standard molecular testing before they can meaningfully influence treatment decisions.

This distinction exposes an important evidence gap. Increasing algorithmic complexity and internally measured accuracy should not be conflated with increasing clinical maturity. Many studies remain retrospective and depend on spectrum-, pixel- or patch-level analyses and predominantly internal validation, creating potential dependence between training and test observations and limiting assessment of performance in genuinely unseen patients. For AI-assisted Raman diagnostics, the effective unit of validation must ultimately be the patient and, for clinical translation, the external clinical setting rather than the individual spectrum or image patch. Patient-level separation, independent external cohorts, multicenter and multi-instrument evaluation, prospective workflow testing and transparent reporting of model development are therefore more informative indicators of translational readiness than incremental improvements in internally validated accuracy (Guo et al., 2026; Vershinina et al., 2025; Zhang et al., 2026).

The likely translational endpoint is consequently not a standalone Raman classifier, but an integrated intraoperative decision-support ecosystem. Raman probes could provide targeted biochemical interrogation, SRH rapid digital tissue assessment, fluorescence and conventional imaging spatial guidance, and AI computational integration of morphological, spectral and molecular information. The critical question is therefore shifting from whether Raman spectroscopy can distinguish glioma tissue to whether Raman-derived information adds reproducible clinical value beyond existing workflows, for example, by identifying otherwise occult infiltration, reducing diagnostic turnaround time, improving margin decisions or providing actionable molecular information while surgery is still underway.

### 4.3. Translational challenges and future perspectives

Despite substantial technological progress, the evidence synthesized in this review indicates that the major barriers to clinical translation increasingly lie beyond proof of diagnostic feasibility. Across conventional Raman spectroscopy, SERS, SRH, SRS and multimodal platforms, discrimination of glioma from non-neoplastic tissue has been repeatedly demonstrated. However, substantial heterogeneity persists in instrumentation, acquisition conditions, tissue handling, spectral preprocessing, machine-learning pipelines, validation strategies, outcome definitions and reporting practices. Studies reporting apparently similar diagnostic performance may therefore represent fundamentally different analytical settings, limiting reproducibility, cross-study comparison and regulatory evaluation. The next phase of development requires convergence around standardized analytical and reporting practices rather than continued proliferation of independently optimized pipelines.

This requirement is particularly important for machine-learning-assisted Raman diagnostics. Model performance can vary substantially according to validation strategy, and inappropriate partitioning of correlated observations may produce optimistic estimates when spectra, pixels or patches from the same patient contribute to both model development and evaluation (Lilek et al., 2025). This issue extends beyond Raman spectroscopy: evidence from other AI-based medical diagnostic fields demonstrates the importance of patient-level separation, multisource validation and genuinely independent external testing for estimating model transportability (Leinonen et al., 2024; Mäenpää and Korja, 2024). Recent reporting standards such as STARD-AI further emphasize transparent description of datasets, reference standards, model evaluation and human–AI interaction in diagnostic accuracy studies (Sounderajah et al., 2025). For Raman-based AI, the relevant benchmark should therefore shift from performance on held-out spectra to performance in unseen patients, instruments and clinical environments.

Three research priorities follow from these limitations. First, analytical standardization is required across Raman acquisition, tissue handling, preprocessing, quality control, dataset partitioning and reporting, while retaining sufficient flexibility for modality-specific differences. Second, clinical validation should progress from internal retrospective evaluation to prospective, patient-level, multicenter studies incorporating independent external cohorts, multi-instrument testing and predefined clinically meaningful endpoints. Third, clinical utility should be evaluated for the most mature platforms through prospective workflow studies asking whether Raman-derived information changes intraoperative decisions, reduces diagnostic turnaround time, improves detection of infiltrative disease or provides actionable information beyond neuropathology and established surgical guidance. These priorities define a sequential translational pathway from analytical reproducibility to clinical validity and ultimately clinical utility, rather than treating diagnostic accuracy as the final endpoint.

Artificial intelligence will remain central to this progression, but greater algorithmic complexity cannot compensate for inadequate study design or unrepresentative data. Explainable AI, uncertainty-aware learning, multimodal computational pathology and foundation models may improve robustness and facilitate integration of spectral, morphological and molecular information (Saueressig et al., 2025; Saueressig et al., 2026; Zhao et al., 2025; Zhang et al., 2026). However, uncertainty estimates require calibration in independent populations, explainability does not itself establish biological validity, and foundation models remain dependent on the diversity and quality of their training data. The principal question is therefore not whether

increasingly complex models can extract additional information from Raman data, but whether that information remains reliable when transferred across patients, centers, instruments and clinical contexts.

A broader conceptual transition is consequently emerging from standalone spectroscopy towards integrated intraoperative diagnostic ecosystems. Raman probes could provide targeted biochemical interrogation, SRH rapid digital tissue assessment, conventional imaging and fluorescence spatial guidance, and AI-assisted integration with molecular neuropathology. Such multimodal systems align with the transition of neuro-oncology towards WHO-integrated molecular classification and precision medicine, but they also raise a higher evidentiary threshold: Raman-derived information must demonstrate incremental clinical value beyond the modalities already available, rather than merely reproduce information obtainable through established diagnostic pathways. Future studies should therefore compare Raman-enabled workflows against clinically relevant standards of care and quantify their added diagnostic, temporal or decision-making value.

Several limitations of this systematic review should also be acknowledged. Although the registered protocol anticipated quantitative meta-analysis, marked heterogeneity across Raman modalities, analytical units, study designs and reported outcomes precluded statistically and clinically meaningful pooling. The evidence was therefore synthesized using a structured SWiM-guided narrative approach. Similarly, the planned GRADE assessment and formal evaluation of publication bias were not undertaken because the available studies lacked sufficient methodological and outcome comparability for reliable application of these methods. These protocol deviations should be considered when interpreting the review, particularly because narrative synthesis cannot provide a pooled estimate of diagnostic performance or formally quantify small-study effects. Nevertheless, this approach allowed systematic examination of methodological heterogeneity, risk of bias and translational readiness across otherwise poorly comparable technologies.

Overall, the field has reached a point at which further gains in internally measured diagnostic accuracy are unlikely, by themselves, to determine clinical adoption. Progress will depend on reproducible acquisition and analysis, rigorous patient-level and external validation, interoperability across instruments and institutions, prospective workflow evaluation and demonstration of measurable clinical benefit. Meeting these requirements would establish whether Raman spectroscopy can progress from a promising diagnostic technology to a reliable component of precision neuro-oncology.

## 5. Conclusions

Raman spectroscopy has emerged as a promising label-free technology for glioma diagnostics, providing molecular tissue characterization that complements conventional histopathology and neuroimaging. Across the studies included in this review, Raman-based approaches consistently demonstrated strong performance for tumor detection, infiltrative margin assessment, molecular profiling, and intraoperative tissue evaluation, reflecting the evolution of the field toward AI-assisted molecular diagnostics.

The available evidence indicates that the value of Raman spectroscopy extends beyond tissue classification by capturing biologically relevant metabolic signatures that support contemporary molecular classification and precision neuro-oncology. However, clinical implementation remains constrained by methodological heterogeneity, limited external validation, and the lack of standardized acquisition and analytical workflows.

Accordingly, the immediate priorities for the field are to establish standardized and reproducible Raman workflows, validate them prospectively at the patient level across independent centers, and determine their added clinical value within integrated intraoperative diagnostic pathways. Progress along this sequence, from standardization to external validation and demonstration of clinical utility, will be critical for translating Raman spectroscopy from promising diagnostic performance into routine precision neuro-oncology.

### Critical View

In our view, Raman spectroscopy has reached a pivotal stage in its evolution. Across conventional Raman spectroscopy, SERS, SRH, SRS, and multimodal platforms, diagnostic feasibility has been consistently demonstrated. The key question is therefore no longer whether Raman spectroscopy can distinguish glioma from non-neoplastic tissue, but whether it can be translated into standardized, reproducible, and clinically deployable molecular diagnostic systems.

A major limitation of the current literature is the tendency to compare diagnostic performance across fundamentally different analytical settings. Patient-, sample-, spectrum-, pixel-, and patch-level analyses are frequently interpreted together despite representing different levels of clinical evidence, while substantial heterogeneity in acquisition protocols, preprocessing pipelines, validation strategies, and outcome reporting limits reproducibility and meaningful comparison between studies. Consequently, future progress will depend more on methodological standardization, external validation, and transparent reporting than on incremental improvements in classification accuracy.

Unlike previous reviews, which primarily summarized individual Raman modalities or specific diagnostic applications, this systematic review provides a comprehensive translational evaluation of the field. We synthesized evidence across conventional Raman spectroscopy, SERS, SRH, SRS, and multimodal platforms, critically integrating diagnostic performance with methodological quality, risk of bias, validation maturity, sources of heterogeneity, artificial intelligence, intraoperative applications, molecular classification, and barriers to clinical implementation. This broad scope enables a more balanced assessment of the current evidence and identifies the principal factors that determine translational readiness for precision neuro-oncology.

Overall, we believe that the field has entered a translational rather than technological phase of development. Future success will depend less on achieving marginal gains in diagnostic accuracy than on demonstrating reproducibility, interoperability, prospective multicenter validation, and clinically meaningful benefit. If these challenges are addressed, Raman spectroscopy has the potential to become an integral component of precision neuro-oncology rather than simply another optical adjunct.

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The authors declare that they have no known competing financial

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.critrevonc.2026.105675.

## Data availability

The data supporting the findings of this systematic review were extracted from previously published studies. The extracted study characteristics and reported diagnostic performance metrics are provided in Supplementary Table S2.

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