



INTELLIGENT INTRAOPERATIVE HISTOLOGY IN NEURO-ONCOLOGICAL SURGERY: STIMULATED RAMAN IMAGING, FOUNDATION MODELS, AND REAL-TIME MOLECULAR CLASSIFICATION

Mukhammadov Nuriddin Askarovich

Specialized scientific and practical center for Neurosurgery and Neurorehabilitation at the Samarkand State Medical University

Abstract

Background. Intraoperative neuropathological diagnosis influences extent of resection, biopsy adequacy, tissue allocation, and therapeutic strategy, yet frozen sections and smear preparations are limited by processing time, artifacts, sampling error, and dependence on subspecialty expertise. Stimulated Raman histology combined with artificial intelligence provides rapid, label-free visualization of fresh tissue and may support tumor classification, infiltration mapping, and molecular prediction.

Materials and methods. A structured narrative review integrated prospective diagnostic studies, multicenter cohorts, technical investigations, artificial-intelligence validation studies, rapid sequencing workflows, and consensus standards available through July 2026. Evidence was organized by optical principles, tissue acquisition, diagnostic accuracy, molecular inference, margin assessment, uncertainty calibration, workflow integration, safety, equity, and regulatory translation. A multicenter comparative study is proposed for adults undergoing resection or stereotactic biopsy of primary or metastatic central nervous system tumors.

Results. Stimulated Raman histology with deep learning has achieved near-real-time classification of brain tumors, differentiation of lymphoma from glioblastoma and metastasis, molecular screening of diffuse gliomas, and quantification of microscopic infiltration. Foundation-model systems can analyze fresh specimens within seconds, whereas nanopore sequencing and ultra-rapid molecular assays may provide integrated diagnoses during surgery. The proposed primary endpoint is patient-level diagnostic concordance with final integrated neuropathological diagnosis combined with a relevant change in surgical management. Secondary endpoints include turnaround time, margin sensitivity, nondiagnostic sampling, calibration, subgroup performance, operative duration, neurological morbidity, tissue preservation, and cost-effectiveness.

Conclusion. Intelligent intraoperative histology may transform neurosurgical pathology from episodic consultation into continuous decision support. Prospective external validation, explicit uncertainty, human oversight, and standardized reporting remain essential.

Keywords: brain tumor surgery, stimulated Raman histology, artificial intelligence, foundation model, intraoperative diagnosis, molecular classification, glioma infiltration, digital neuropathology.

ИНТЕЛЛЕКТУАЛЬНАЯ ИНТРАОПЕРАЦИОННАЯ ГИСТОЛОГИЯ В НЕЙРООНКОЛОГИЧЕСКОЙ ХИРУРГИИ: СТИМУЛИРОВАННАЯ РАМАНОВСКАЯ ВИЗУАЛИЗАЦИЯ, ФУНДАМЕНТАЛЬНЫЕ МОДЕЛИ И МОЛЕКУЛЯРНАЯ КЛАССИФИКАЦИЯ В РЕАЛЬНОМ ВРЕМЕНИ

Мухаммадов Нуриддин Аскарлович

Специализированный научно-практический центр нейрохирургии и нейрореабилитации при Самаркандском Медицинском Университете

Аннотация

Актуальность. Интраоперационная нейропатологическая диагностика влияет на объем резекции, достаточность биопсии, распределение ткани и лечебную тактику, однако замороженные срезы и мазки ограничены временем подготовки, артефактами, ошибками выборки и зависимостью от узкоспециализированной экспертизы. Стимулированная рамановская гистология в сочетании с искусственным интеллектом обеспечивает быстрое безметочное исследование свежей ткани и может поддерживать классификацию опухоли, картирование инфильтрации и молекулярное прогнозирование.

Материалы и методы. Структурированный нарративный обзор объединил проспективные диагностические исследования, многоцентровые когорты, технические работы, валидационные исследования искусственного интеллекта, ускоренные секвенирующие технологии и консенсусные стандарты, доступные до июля 2026 года. Доказательства систематизированы по оптическим принципам, получению ткани, диагностической точности, молекулярному прогнозированию, оценке краев, калибровке неопределенности, интеграции в рабочий процесс, безопасности, равноправию и регуляторному внедрению. Предложено многоцентровое сравнительное исследование взрослых пациентов, которым выполняется резекция или стереотаксическая биопсия первичной либо метастатической опухоли центральной нервной системы.

Результаты. Стимулированная рамановская гистология с глубоким обучением обеспечила почти мгновенную классификацию опухолей мозга, дифференциацию лимфомы от глиобластомы и метастаза, молекулярный скрининг диффузных глиом и количественную оценку микроскопической инфильтрации. Системы фундаментальных моделей анализируют свежие образцы за секунды, тогда как нанопоровое секвенирование и сверхбыстрые молекулярные тесты могут формировать интегрированный диагноз во время операции. Первичная конечная точка включает согласованность с окончательным интегрированным диагнозом и клинически значимое изменение хирургической тактики. Вторичные показатели включают время ответа, чувствительность краев, недиагностические образцы, калибровку, результаты подгрупп, продолжительность операции, неврологические осложнения, сохранение ткани и экономическую эффективность.

Закключение. Интеллектуальная интраоперационная гистология способна преобразовать нейрохирургическую патологию из эпизодической консультации в непрерывную поддержку решений. Необходимы внешняя валидация, явное представление неопределенности, человеческий контроль и стандартизированная отчетность обязательно в независимых центрах с длительным контролем клинических исходов пациентов.

Ключевые слова: хирургия опухолей головного мозга, стимулированная рамановская гистология, искусственный интеллект, фундаментальная модель, интраоперационная диагностика, молекулярная классификация, инфильтрация глиомы, цифровая нейропатология.

**NEYROONKOLOGIK JARROHLIKDA INTELLEKTUAL INTRAOPERATSION
GISTOLOGIYA: STIMULLANGAN RAMAN TASVIRLASH, FUNDAMENTAL
MODELLAR VA REAL VAQT REJIMIDAGI MOLEKULAR TASNIFLASH**

Muhammadov Nuriddin Asqarovich

Samarqand davlat tibbiyot universiteti huzuridagi Neyroxirurgiya va neyroreabilitatsiya
ixtisoslashtirilgan ilmiy-amaliy markazi

Annotatsiya

Dolzarblik. Intraoperatsion neyropatologik diagnostika rezeksiya hajmi, biopsiya yetarliligi, to'qimani taqsimlash va davolash taktikasiga ta'sir qiladi, biroq muzlatilgan kesmalar hamda surtma preparatlar tayyorlash vaqti, artefaktlar, namuna olish xatolari va tor ixtisoslashgan ekspertizaga bog'liqlik bilan cheklanadi. Stimullangan Raman gistologiyasi sun'iy intellekt bilan

birgalikda yangi to'qimani tez, yorliqsiz ko'rish imkonini beradi hamda o'smani tasniflash, infiltratsiyani xaritalash va molekulyar prognozlashni qo'llab-quvvatlashi mumkin.

Material va usullar. Strukturaviy narrativ sharh 2026-yil iyuligacha mavjud bo'lgan prospektiv diagnostik tadqiqotlar, ko'p markazli kohortlar, texnik izlanishlar, sun'iy intellekt validatsiyasi, tezkor sekvenslash ish jarayonlari va konsensus standartlarini birlashtirdi. Dalillar optik tamoyillar, to'qima olish, diagnostik aniqlik, molekulyar xulosa, rezeksiya chegaralari, noaniqlik kalibratsiyasi, operatsion integratsiya, xavfsizlik, tenglik va regulyator joriy etish bo'yicha tizimlashtirildi. Birlamchi yoki metastatik markaziy asab tizimi o'smalari rezeksiyasi yoxud stereotaksik biopsiyasi bajariladigan kattalar uchun ko'p markazli qiyosiy tadqiqot taklif etildi.

Natijalar. Stimullangan Raman gistologiyasi va chuqur o'rganish miya o'smalarini deyarli real vaqtda tasniflash, limfomani glioblastoma hamda metastazdan farqlash, diffuz gliomalarni molekulyar skrining qilish va mikroskopik infiltratsiyani miqdoriy baholash imkonini berdi. Fundamental modellar yangi namunalarni soniyalar ichida tahlil qiladi, nanopora sekvenslash va o'ta tez molekulyar testlar esa operatsiya vaqtda integrallashgan tashxisni shakllantirishi mumkin. Birlamchi yakuniy nuqta yakuniy integrallashgan neyropatologik tashxis bilan bemor darajasidagi muvofiqlik va jarrohlik taktikasining klinik ahamiyatli o'zgarishidir. Ikkilamchi ko'rsatkichlar javob vaqti, chegara sezgirligi, nodiagnostik namunalalar, kalibratsiya, kichik guruhlar samaradorligi, operatsiya davomiyligi, nevrologik asoratlar, to'qimani saqlash va iqtisodiy samaradorlikni qamrab oladi.

Xulosa. Intellektual intraoperatsion gistologiya neyroxirurgik patologiyani epizodik maslahatdan uzluksiz qarorlarni qo'llab-quvvatlash tizimiga aylantirishi mumkin. Mustaqil tashqi validatsiya, aniq noaniqlik, inson nazorati va standartlashtirilgan hisobot, uzoq muddatli kuzatuv zarur.

Kalit so'zlar: bosh miya o'smasi jarrohligi, stimullangan Raman gistologiyasi, sun'iy intellekt, fundamental model, intraoperatsion diagnostika, molekulyar tasniflash, glioma infiltratsiyasi, raqamli neyropatologiya.

Introduction. Modern neuro-oncological surgery is performed at the intersection of anatomical preservation, cytoreduction, molecular diagnosis, and individualized oncological planning. The surgeon must determine whether tissue represents a diffuse glioma, metastasis, lymphoma, meningioma, ependymoma, treatment-related change, inflammatory lesion, or normal brain while simultaneously deciding whether additional resection is anatomically safe and biologically meaningful. These decisions are time-sensitive because the operative field changes continuously, brain shift reduces the accuracy of preoperative neuronavigation, and the opportunity to acquire diagnostically representative tissue may disappear after coagulation, aspiration, or completion of resection. Traditional intraoperative neuropathology relies on frozen sections, squash preparations, cytological smears, or rapid hematoxylin-and-eosin staining. These methods have supported neurosurgical decision-making for decades, but they require tissue transport, laboratory processing, technical personnel, and interpretation by an experienced neuropathologist. Freezing artifacts, tissue distortion, cautery injury, hemorrhage, necrosis, low cellularity, and very small biopsy specimens may reduce diagnostic confidence. The method also consumes or alters part of the specimen that might otherwise be used for molecular testing, research biobanking, organoid development, or clinical-trial eligibility assessment [1, 2, 3]. The 2021 World Health Organization classification of central nervous system tumors transformed neuropathological diagnosis from a predominantly morphology-based system into an integrated framework in which histological appearance is combined with mutations, copy-number alterations, gene fusions, chromosomal losses, and DNA methylation profiles. Adult diffuse gliomas are now classified principally through IDH mutation status and 1p/19q codeletion, whereas several pediatric and circumscribed tumors are defined by characteristic molecular alterations. A histologically plausible diagnosis may therefore remain incomplete or even incorrect until molecular information is available [3, 6, 10].

Conventional comprehensive molecular testing usually requires days or weeks. This delay is acceptable for definitive postoperative reporting but limits intraoperative use. The surgeon may

need to know immediately whether a lesion is lymphoma, for which extensive resection may be unnecessary; a metastasis requiring maximal local removal and sufficient tissue for systemic profiling; an infiltrative glioma requiring function-preserving margin assessment; or a molecularly defined low-grade tumor in which aggressive resection may have distinct prognostic implications [4, 7, 14]. Stimulated Raman histology is a label-free optical method that generates microscopic contrast from the intrinsic vibrational properties of tissue molecules. Two synchronized laser beams are tuned to molecular vibrational frequencies, allowing rapid visualization of lipid-rich myelin, protein-rich tumor cells, nuclei, vascular structures, necrosis, and tissue architecture without fixation, sectioning, staining, or chemical labeling. Fresh tissue can be placed directly into a reusable or disposable imaging chamber and visualized near the operating room [3, 6, 7]. The resulting images resemble conventional histology but also preserve biochemical information that may be difficult to appreciate in standard frozen sections. Because the specimen is not consumed by staining, it can subsequently undergo permanent histopathology, immunohistochemistry, sequencing, methylation analysis, or biobanking. Stimulated Raman histology therefore functions as both an imaging platform and a tissue-preservation strategy [11, 12, 20]. Artificial intelligence has accelerated the clinical relevance of this technology. A deep neural network can analyze thousands of image fields, distinguish diagnostic from nondiagnostic regions, quantify cellularity, identify histological patterns, and provide class probabilities within an operative timeframe. The first large prospective work in this area demonstrated near-real-time classification of brain tumors using stimulated Raman histology and deep neural networks. Subsequent systems expanded from morphological classification to diffuse-glioma recurrence detection, molecular screening, stereotactic-biopsy assessment, lymphoma differentiation, infiltration quantification, and spinal-tumor classification [3, 6, 15, 18].

DeepGlioma combined stimulated Raman histology with multimodal deep learning and genomic information to predict the molecular alterations defining adult-type diffuse gliomas. In a prospective international cohort, it predicted IDH mutation, 1p/19q codeletion, and ATRX alteration with a mean molecular classification accuracy of approximately 93%, producing results in less than 90 seconds [2, 3, 10, 18]. FastGlioma subsequently introduced a visual foundation-model approach trained through self-supervision on approximately four million optical images. The system provided an infiltration score from fresh, unprocessed tissue within seconds and achieved an average area under the receiver-operating-characteristic curve of approximately 92% in a prospective international cohort of 220 patients. Its performance remained high across participating centers, patient groups, and diffuse-glioma molecular subtypes [5, 6, 10]. These advances indicate that intraoperative pathology may evolve from a single binary consultation into continuous, spatially distributed decision support. Instead of sending one specimen from the tumor center, surgeons could sample multiple margins, verify diagnostic adequacy during stereotactic biopsy, identify regions with high tumor-cell burden for molecular testing, and determine whether ambiguous tissue contains viable tumor or reactive brain. The central translational question is no longer whether artificial intelligence can recognize optical tumor patterns. It is whether these predictions improve surgical decisions, reduce nondiagnostic procedures, preserve neurological function, shorten operative delays, improve tissue allocation, and remain reliable across hospitals, tumor entities, demographic groups, scanners, software versions, and changing pathological classifications. The purpose of this article is to evaluate stimulated Raman histology, Raman spectroscopy, artificial intelligence, foundation models, and rapid molecular testing as components of an intelligent intraoperative neuropathology ecosystem. A prospective multicenter comparative study is proposed to connect diagnostic performance with patient-centered and surgical outcomes rather than evaluating algorithmic accuracy in isolation.

Materials and Methods. A structured narrative review was conducted using evidence available through July 2026. Eligible sources included prospective diagnostic trials, multicenter cohort studies, blinded comparisons, stimulated Raman histology investigations, in situ Raman-spectroscopy studies, deep-learning and foundation-model studies, rapid nanopore-sequencing workflows, ultra-rapid molecular assays, and reporting standards relevant to artificial-intelligence diagnostic systems. Evidence was organized across ten domains: optical imaging principle, tissue

acquisition and preservation, morphological classification, molecular prediction, infiltration and margin assessment, biopsy adequacy, uncertainty quantification, human-machine integration, external validity, and clinical implementation. Studies were prioritized when the optical or molecular test was performed on fresh human central nervous system tissue and compared with a final integrated neuropathological diagnosis. Retrospective computational studies were included when they provided relevant evidence about model architecture, domain shift, calibration, or rare tumor classification. Studies based exclusively on conventional postoperative whole-slide images were used only when they informed the development of foundation models or molecular prediction. The reference standard was the final integrated neuropathological diagnosis established from formalin-fixed paraffin-embedded histology, immunohistochemistry, targeted sequencing, copy-number analysis, fusion testing, and DNA methylation classification when clinically indicated. For margin assessment, the reference standard consisted of permanent histology combined with tumor-specific immunohistochemical or molecular markers.

The proposed original study is a prospective, international, multicenter, paired diagnostic-impact trial. Adults undergoing open resection or stereotactic biopsy of a suspected primary or metastatic central nervous system tumor would be consecutively enrolled. Pediatric cases could be evaluated within a separate predefined stratum because pediatric tumors differ substantially in molecular taxonomy, histomorphology, and prevalence. Each participant would contribute at least one diagnostic specimen. Resection cases would additionally contribute spatially registered samples from the tumor center, radiographic margin, surgeon-defined uncertain tissue, and final resection boundary when anatomically safe. The location of each sample would be recorded using neuronavigation and updated intraoperative imaging when available. Fresh tissue would undergo stimulated Raman histology without fixation or staining. The artificial-intelligence system would generate a predicted tumor class, tumor-versus-nontumor probability, infiltration score, molecular-screening result when applicable, image-quality score, and uncertainty estimate. The model would be required to abstain when image quality or confidence fell below a locked threshold. The comparator would be standard intraoperative neuropathological assessment using frozen section, smear, or squash preparation according to institutional practice. Both methods would be interpreted independently before unblinding. The operating surgeon would document the provisional surgical plan before receiving either result and record any change in resection strategy, biopsy trajectory, tissue allocation, or decision to terminate the procedure.

The primary endpoint would be a composite of patient-level concordance with the final integrated diagnosis and a prespecified clinically meaningful effect on surgical management. Diagnostic accuracy without any influence on care would be considered technically valuable but insufficient to establish clinical utility. Secondary endpoints would include sensitivity and specificity for tumor detection, accuracy of entity classification, accuracy of molecular screening, margin sensitivity, turnaround time, proportion of nondiagnostic biopsies, number of tissue samples required, operative delay, extent of resection, residual tumor volume, permanent neurological morbidity, reoperation for diagnosis, tissue available for molecular studies, calibration error, false-confidence rate, and cost per correct intraoperative diagnosis. The analysis would use paired comparisons because each case would undergo both intelligent optical assessment and standard pathology. Hierarchical mixed-effects models would account for clustering by patient, surgeon, neuropathologist, center, tumor entity, and imaging device. Prespecified subgroup analyses would examine age, sex, race or ethnicity where legally permissible, tumor type, primary versus recurrent disease, previous radiation, specimen size, blood contamination, necrosis, and center experience.

Table 1. Comparison of intraoperative neuropathological technologies

Technology	Tissue preparation	Typical information	Approximate workflow speed	Principal advantage	Principal limitation
Frozen section	Freezing, sectioning, staining	Morphology and provisional entity	Approximately 20–40 minutes	Familiar clinical standard	Artifacts, tissue consumption,

					specialist dependence
Cytological smear or squash preparation	Mechanical spreading and staining	Cellular morphology	Approximately 10–30 minutes	Useful for small biopsies	Distorted architecture and sampling bias
Stimulated Raman histology	Fresh unprocessed tissue	Label-free cellular and biochemical morphology	Seconds to several minutes	Rapid, nonconsumptive, bedside imaging	Specialized optical equipment
In situ Raman spectroscopy	Probe applied directly to tissue	Spectral tumor-versus-normal classification	Seconds	Assessment before resection	Limited architectural information
Ultra-rapid droplet digital PCR	Rapid extraction and mutation-specific amplification	Quantitative mutation burden	Approximately 15 minutes	Highly sensitive molecular quantification	Requires predefined mutation target
Rapid nanopore sequencing	Rapid DNA extraction and sequencing	Methylation class, copy number, mutations, fusions	Approximately 30 minutes to several hours	Broad molecular profiling	Greater laboratory and computational complexity
Integrated optical–molecular platform	SRH plus rapid targeted or genomic testing	Morphology, infiltration, molecular class	Intraoperative timeframe	Multimodal decision support	Cost, validation, workflow complexity

Results. Stimulated Raman scattering detects molecular vibrational contrast rather than transmitted-light staining. Imaging commonly targets spectral bands associated with lipid and protein content. Computational color mapping can then produce virtual histological images in which nuclei, cytoplasm, myelinated brain, necrosis, and vascular structures appear in a format familiar to pathologists. The method avoids formalin fixation, cryosectioning, and exogenous stains. It is particularly suitable for small stereotactic biopsies because the tissue remains available for subsequent molecular analysis. This is important when the diagnostic specimen measures only a few millimeters and must support histology, immunohistochemistry, sequencing, and clinical-trial testing [3, 6, 15, 18]. Near-real-time deep-learning classification was demonstrated in a prospective clinical workflow involving fresh neurosurgical tissue. The model classified common brain-tumor entities with an overall diagnostic accuracy reported at approximately 95%, while image acquisition and algorithmic prediction were completed within minutes [11, 12, 20]. The diagnostic value extends beyond common gliomas. Models have been developed for pediatric brain tumors, recurrent diffuse glioma, meningioma, metastasis, pituitary lesions, and other central nervous system neoplasms. The most relevant clinical advantage is rapid recognition of a treatment-changing category rather than exhaustive classification of every rare entity. For example, distinguishing primary central nervous system lymphoma from glioblastoma or metastasis may alter the operative objective. Lymphoma has traditionally been managed through biopsy and systemic therapy, whereas glioblastoma and solitary metastasis may justify maximal safe resection. A 2025 multicenter study

combined stimulated Raman histology with deep learning to differentiate lymphoma from common intracranial tumors during the operative period, addressing a diagnostic problem complicated by overlapping morphology and small biopsy size [7, 11, 12].

The model should not be interpreted as an autonomous replacement for neuropathological assessment. Corticosteroid exposure may reduce lymphoma cellularity, and treatment-related tissue may contain inflammation, necrosis, macrophages, and reactive astrocytes that mimic neoplasia. The safest use is as an adjunct that provides probabilities, highlights representative regions, and prompts additional sampling when uncertainty remains.

Stereotactic-biopsy adequacy. Stereotactic biopsy has a lower tissue burden than open resection and a greater risk of nondiagnostic sampling. The needle may traverse necrosis, reactive gliosis, edema, hemorrhage, or nonrepresentative tumor margin. Repeated sampling increases procedural time and may increase the risk of hemorrhage. Stimulated Raman histology can assess fresh biopsy cores immediately without exhausting the specimen. Artificial-intelligence classification can identify whether the sample contains tumor, viable cellular tissue, necrosis, or normal brain. A 2024 investigation applied this workflow to stereotactic biopsy specimens and combined tumor classification with molecular subtyping, demonstrating the feasibility of obtaining rapid information from very small samples [2, 3, 10, 18]. The clinical endpoint should not be only classification accuracy. A biopsy system has practical value when it reduces the proportion of nondiagnostic procedures, limits the number of needle trajectories, shortens anesthesia exposure, or ensures that sufficient viable tissue is reserved for genomic testing. A model that correctly recognizes a broad “tumor” category but misidentifies lymphoma as glioma may cause inappropriate tissue allocation or excessive sampling. Diagnostic hierarchy is therefore important. The first level should distinguish diagnostic from nondiagnostic tissue; the second should identify treatment-changing classes; the third may provide detailed tumor subtype and molecular predictions.

Molecular prediction from optical images. The integrated classification of diffuse glioma requires molecular information that cannot always be inferred from morphology alone. IDH-mutant astrocytoma, oligodendroglioma, and IDH-wild-type glioblastoma may share overlapping histological features, particularly in small or heterogeneous specimens. DeepGlioma was designed as a rapid molecular-screening system rather than a replacement for definitive genetic testing. It combined stimulated Raman histology with multimodal deep learning trained on public genomic data and prospective optical imaging. The system predicted the molecular alterations used to classify adult diffuse gliomas, including IDH mutation and 1p/19q codeletion, within less than 90 seconds [1, 3, 10, 13]. This type of prediction may influence intraoperative behavior. If a lesion is predicted to represent an IDH-mutant diffuse glioma, the surgeon may prioritize maximal function-preserving resection, collect spatially diverse tissue, and preserve samples for detailed molecular profiling. If the model indicates an IDH-wild-type aggressive tumor, the team may prioritize high-cellularity tissue for genomic testing and trial eligibility. However, molecular predictions derived from morphology are probabilistic. IDH mutation produces metabolic and histological consequences that may be visible indirectly, but optical imaging does not directly sequence the gene. 1p/19q codeletion, ATRX loss, TERT-promoter mutation, CDKN2A/B homozygous deletion, H3 alterations, and methylation classes may have variable morphological expression. The prediction should therefore be presented as a calibrated screening result. A high-confidence model may guide tissue allocation or provisional discussion, but definitive integrated diagnosis should remain dependent on validated molecular assays.

Foundation models and infiltration quantification. Conventional supervised models are trained on predefined labels and may perform poorly when confronted with rare tumors, new scanners, unfamiliar institutions, or changes in tissue preparation. Foundation models seek to overcome this limitation by learning general visual representations from very large, diverse datasets before adaptation to specific clinical tasks. FastGlioma was pretrained using self-supervision on approximately four million stimulated Raman microscopy images and more than 11,000 specimens. A smaller annotated dataset was then used to teach the system an ordinal infiltration scale ranging from normal brain to dense tumor [5, 6, 10]. The system can provide a normalized infiltration score rather than a binary tumor-versus-normal answer. This is clinically important because glioma

margins are continuous biological gradients. Sparse infiltrating tumor cells may be present beyond contrast enhancement, fluorescence, or visible tissue abnormality. In a prospective multicenter cohort of 220 patients, FastGlioma achieved an average area under the receiver-operating-characteristic curve of approximately 92% for distinguishing degrees of infiltration. The fast imaging mode acquired lower-resolution images at approximately tenfold higher speed with minimal reported performance loss [4, 7, 14, 19]. A head-to-head analysis indicated that the foundation-model approach detected infiltration more accurately than conventional image-guided and fluorescence-guided adjuncts in the studied cohort. These findings do not prove that removal of every model-positive margin improves survival. Infiltrative cells may extend into eloquent networks where further resection would cause unacceptable neurological injury. The correct clinical role is to inform a benefit–risk decision. A positive margin in noneloquent tissue may support further resection, while a positive margin adjacent to the corticospinal tract, language network, optic radiation, basal ganglia, or brainstem may instead guide targeted adjuvant therapy or postoperative surveillance.

Table 2. Potential artificial-intelligence outputs and associated clinical actions

Model output	Possible intraoperative interpretation	Potential surgical action	Required safeguard
High-confidence normal brain	No evidence of tumor in sampled tissue	Avoid unnecessary resection	Confirm correct spatial registration
Sparse infiltration	Low-density tumor cells at margin	Consider additional sampling or limited extension	Integrate functional anatomy and neurological risk
Dense tumor	High tumor-cell burden	Continue resection or reserve tissue for molecular studies	Exclude treatment-related mimic
Nondiagnostic tissue	Blood, necrosis, artifact, insufficient tissue	Obtain another sample	Avoid repeated unsafe trajectories
Probable lymphoma	Treatment-changing diagnostic category	Limit resection and preserve tissue for flow cytometry	Require neuropathologist confirmation
Probable metastasis	Non-glial malignant tumor	Pursue local control and systemic profiling	Correlate with clinical and radiological history
Predicted IDH mutation	Probable adult-type IDH-mutant glioma	Optimize function-preserving cytoreduction	Confirm with validated molecular testing
High uncertainty	Model outside reliable decision range	Defer to conventional pathology and additional testing	Mandatory abstention policy

In situ Raman spectroscopy. Stimulated Raman histology analyzes excised tissue. Handheld Raman-spectroscopy systems pursue a complementary objective: identifying tumor directly in the operative field before resection. A multicenter study evaluated an in situ Raman system in 67 patients undergoing surgery for glioblastoma, brain metastasis, or meningioma. The system collected 976 spectral measurements paired with histopathological reference samples and reported diagnostic accuracies of 91% for glioblastoma, 97% for metastasis, and 96% for meningioma [9, 14, 15]. The advantage of an in situ probe is immediate spatial assessment without removing tissue. The surgeon can interrogate a cavity margin, suspicious surface, or boundary between tumor and normal brain. The limitation is that a spectrum provides less architectural information than an optical histology image. Surface blood, irrigation fluid, cautery change, probe pressure, and tissue angle may alter spectral quality. The sampled volume is also limited, and a

single negative measurement does not exclude nearby infiltrating tumor. In situ spectroscopy and stimulated Raman histology may therefore be complementary rather than competing technologies.

Rapid molecular testing. Optical artificial intelligence predicts molecular state indirectly. Rapid molecular assays can provide direct genetic or epigenetic evidence during surgery. Ultra-rapid droplet digital polymerase chain reaction was developed to quantify predefined tumor mutations from fresh tissue within approximately 15 minutes for a single specimen. The method can estimate very low tumor fractions and may be combined with stimulated Raman histology to connect morphology with mutation burden [10, 16, 17]. The principal advantage is analytical sensitivity. When a patient's tumor carries a known mutation, ultra-rapid testing may quantify tumor cells at a margin even when histological infiltration is sparse. The limitation is that the assay must target a predefined alteration. It is less useful when the tumor genotype is unknown or heterogeneous. Nanopore sequencing provides broader genomic and epigenomic information. MethyLYZR used sparse nanopore methylation data and a computationally efficient Bayesian framework to classify more than 200 brain tumors, with selected clinical samples producing classification results within approximately 15 minutes of sequencing [11, 18, 19]. Rapid-CNS² combines adaptive nanopore sequencing, real-time methylation classification, copy-number information, and later comprehensive molecular profiling. A 2025 prospective multicenter validation reported intraoperative methylation and copy-number results within approximately 30 minutes, followed by broader profiling within 24 hours [12, 18, 20]. These technologies suggest a tiered diagnostic workflow. Stimulated Raman histology can provide morphology and cellularity within seconds or minutes. Targeted polymerase chain reaction can confirm a specific mutation within minutes. Nanopore sequencing can provide a broader molecular classification within the operative period.

Table 3. Proposed multimodal intraoperative diagnostic workflow

Operative stage	Diagnostic question	Preferred technology	Expected output
First biopsy specimen	Is the tissue diagnostic?	SRH plus AI quality control	Tumor, normal brain, necrosis, blood, or nondiagnostic tissue
Early tumor sampling	What broad entity is present?	SRH classifier with neuropathologist review	Glioma, metastasis, lymphoma, meningioma, other
Molecular screening	Which integrated category is likely?	DeepGlioma or rapid targeted assay	IDH, 1p/19q, ATRX or other selected alterations
Margin assessment	Is viable tumor present?	FastGlioma, in situ Raman probe, or mutation quantification	Infiltration probability and tumor-cell burden
Definitive intraoperative molecular analysis	Is a molecularly defined diagnosis available?	Nanopore methylation and copy-number workflow	Provisional integrated diagnosis
Final postoperative diagnosis	What is the definitive entity and grade?	Formal histology, immunohistochemistry, sequencing, methylation	WHO-compatible integrated neuropathological diagnosis

Sampling error and histological ground truth. Artificial intelligence cannot correct an incorrectly sampled location. If the surgeon samples necrosis while viable tumor lies several millimeters away, a correct model may report necrosis without solving the diagnostic problem. Spatial registration is therefore as important as image classification. Every research specimen should be linked to neuronavigation coordinates, imaging phenotype, fluorescence status, surgeon impression, and final histopathology. Brain shift should be considered when interpreting late margin

samples. Intraoperative ultrasound or MRI may improve spatial correspondence. The reference standard itself contains uncertainty. Histological assessment of sparse infiltration varies among neuropathologists, and molecular assays have detection limits. A specimen may contain only a few tumor cells, making a negative immunohistochemical result unreliable. Consensus annotation by multiple neuropathologists, tumor-specific markers, and quantitative mutation testing can strengthen ground truth. Disagreements should not be concealed by forcing a single label. They should be modeled as reference-standard uncertainty.

Uncertainty calibration and model abstention. A clinical classifier must know when it is uncertain. A model that provides a confident but incorrect diagnosis can be more dangerous than one with slightly lower average accuracy that reliably abstains from unfamiliar cases. Sources of uncertainty include rare tumor entities, mixed tumors, prior radiotherapy, treatment-related necrosis, inflammation, low-cellularity specimens, image artifacts, scanner variation, and incomplete tissue coverage. Confidence may also be artificially high when a model encounters a pattern that resembles a common tumor but belongs to an unseen entity. Calibration measures whether predicted probabilities correspond to observed accuracy. If predictions assigned 90% confidence are correct only 70% of the time, the model is overconfident. Expected calibration error, Brier score, reliability diagrams, and class-specific confidence distributions should be reported alongside sensitivity and specificity [3, 10, 18, 27]. An abstention threshold should be locked before clinical deployment. Below that threshold, the system should report “insufficient confidence” rather than selecting the most likely class. The interface should also distinguish low biological confidence from poor image quality.

Human-machine workflow. Intelligent intraoperative histology should support a collaborative workflow involving the neurosurgeon, neuropathologist, operating-room staff, molecular laboratory, and data system. The surgeon provides clinical context, radiological localization, and the precise surgical question. The optical system generates a rapid image and automated prediction. The neuropathologist confirms whether the image is interpretable, evaluates morphology, and contextualizes the artificial-intelligence output. The surgeon then integrates this information with functional anatomy, operative goals, and procedural risk. Displaying only the predicted diagnosis is insufficient. The system should show representative image regions, confidence, alternative diagnoses, quality warnings, and whether the case resembles training data. For infiltration models, a spatial heat map and quantitative score are more useful than a binary label. The system should record every prediction, software version, image-acquisition parameter, user interaction, and final decision. This audit trail is necessary for quality assurance, regulatory surveillance, and investigation of diagnostic errors.

Table 4. Proposed multicenter clinical-impact study

Component	Proposed specification
Population	Consecutive adults undergoing resection or stereotactic biopsy of suspected CNS tumors
Design	Prospective, multicenter, paired diagnostic-impact study
Index strategy	SRH with locked AI classifier, infiltration model, and uncertainty output
Comparator	Standard frozen section, smear, or squash preparation
Reference standard	Final integrated WHO-compatible neuropathological diagnosis
Primary endpoint	Diagnostic concordance plus clinically meaningful change in surgical management
Secondary diagnostic endpoints	Sensitivity, specificity, molecular accuracy, margin detection, calibration
Workflow endpoints	Turnaround time, operative delay, number of samples, tissue consumption
Surgical endpoints	Extent of resection, residual tumor, biopsy adequacy, reoperation for diagnosis
Safety endpoints	New neurological deficit, hemorrhage, additional unsafe sampling

Equity endpoints	Performance across demographic groups, centers, scanners, and tumor prevalence
Economic endpoints	Cost per diagnosis, operating-room time, pathology workload, avoided repeat surgery
External validation	Locked model evaluated in centers not involved in training
Originality	Direct linkage of AI accuracy with decisions, safety, tissue preservation, and patient outcomes

Discussion. Intelligent intraoperative histology represents a transition from visual pathology to computationally augmented surgical diagnosis. The most mature systems can now image fresh tissue, recognize common tumor entities, estimate glioma infiltration, and screen for molecular classes within a clinically relevant timeframe. The technological progress is substantial, but diagnostic performance should not be confused with clinical effectiveness. A model may achieve a high area under the curve without changing the operation. Conversely, a modest improvement in biopsy adequacy may prevent repeat surgery and produce meaningful patient benefit. Future trials should therefore be designed around decisions. Before receiving the result, the surgeon should state the intended action. After receiving the result, the surgeon should document whether the plan changed and why. The change should later be adjudicated as beneficial, neutral, or potentially harmful. A lymphoma classifier illustrates this principle. The important endpoint is not merely whether lymphoma was recognized. It is whether unnecessary resection was avoided, sufficient tissue was obtained for definitive analysis, corticosteroid-related diagnostic failure was recognized, and treatment began without delay. Margin assessment requires a different endpoint. A model-positive margin does not automatically justify additional tissue removal. The neurological value of the adjacent tissue may outweigh the oncological value of resection. Artificial intelligence should define the biological boundary, while the surgeon determines the safe functional boundary.

The concept of supramaximal or fluorescence-beyond resection becomes more precise when infiltration is quantified. Instead of classifying a cavity as either complete or incomplete, samples can be placed on a continuous axis of tumor-cell burden. This may support standardized studies relating residual microscopic disease to progression patterns and survival. The main risk is overresection driven by false-positive or clinically misinterpreted results. Reactive astrogliosis, macrophage-rich treatment effect, inflammation, and tissue compression may resemble infiltrative tumor. An algorithm trained mainly on untreated diffuse gliomas may not generalize to recurrent tumors after chemoradiation. Recurrent disease should therefore be validated separately. The clinical question is frequently viable tumor versus radiation necrosis, pseudoprogression, or inflammatory treatment response. These states may coexist within the same specimen, making a single class label biologically inadequate. Rare tumors also present a problem. A broad classifier trained on common entities may force an uncommon lesion into the nearest familiar category. Foundation models and zero-shot methods may improve representation, but rare-disease performance must be measured directly rather than inferred from overall accuracy. Class prevalence affects predictive value. A lymphoma classifier developed in a referral center with many lymphomas may produce more false-positive results when deployed in a general neurosurgical population. External validation must reproduce the intended clinical setting.

Domain shift can arise from scanner hardware, laser calibration, imaging chamber, specimen thickness, tissue compression, blood contamination, software preprocessing, and institutional sampling practice. A model should undergo quality-control testing after device servicing, software updates, or transfer to another center. Continuous learning creates regulatory complexity. Updating the model with new cases may improve performance but can alter behavior unpredictably. A clinically deployed model should have a versioned, locked configuration. Updates should undergo predefined validation before replacement of the previous version. Data governance is particularly important because optical images are linked to molecular and clinical information. Deidentification may be difficult when rare diagnoses, dates, and genomic profiles are combined. Institutions require clear policies for data ownership, algorithm training, commercial use, and cross-border sharing. Human oversight should not be treated as a temporary limitation that disappears when the model becomes sufficiently accurate. Neuropathologists contribute contextual reasoning, recognition of

unusual entities, assessment of specimen quality, integration of ancillary studies, and responsibility for the final diagnosis.

The role of the neuropathologist may shift from manual examination of every image field to supervision of computational outputs, evaluation of uncertain cases, quality assurance, and integration of molecular data. This could extend specialist expertise to hospitals without continuous on-site neuropathology. Telepathology and cloud-based artificial intelligence may improve access, but dependence on internet connectivity and remote servers creates failure points. Local processing is preferable for time-critical functions, with secure transfer used for expert consultation and model monitoring. Economic evaluation should include more than the purchase price of the optical device. Relevant costs include disposable chambers, maintenance, laser calibration, computational hardware, software licensing, staff training, pathology time, operating-room delay, and molecular testing. Potential savings include reduced frozen-section workload, fewer nondiagnostic biopsies, fewer repeat operations, shorter anesthesia time, improved tissue allocation, and avoidance of unnecessary extensive resection. Cost-effectiveness will vary by surgical volume and pathology infrastructure. Equity analysis is essential. Models trained predominantly in high-income academic centers may not represent tumor prevalence, patient demographics, tissue handling, or treatment patterns in other regions. A system that requires expensive proprietary hardware may widen diagnostic inequality. Conversely, a standardized optical platform with local artificial-intelligence analysis could bring rapid neuropathological support to hospitals without on-site subspecialists. This benefit will depend on affordability, technical support, multilingual interfaces, and open validation. Artificial intelligence may also influence clinical-trial recruitment. Rapid identification of IDH mutation, BRAF alteration, H3 mutation, fusion-driven tumors, or other molecular groups could allow immediate tissue allocation and early screening. However, treatment enrollment must rely on validated confirmatory testing.

The integration of stimulated Raman histology and rapid nanopore sequencing is especially promising. Optical imaging can identify the most cellular part of the specimen, reducing the risk that molecular testing is performed on necrotic or low-tumor tissue. Sequencing can then confirm the molecular class that optical artificial intelligence predicted indirectly.

Ultra-rapid droplet digital polymerase chain reaction may provide quantitative margin assessment when a known mutation is available. A patient-specific mutation can act as a molecular barcode, allowing a margin to be evaluated for low-level tumor burden. The ideal system may therefore be modular. A rapid optical classifier provides immediate morphology. A foundation model quantifies infiltration. A targeted assay confirms a critical mutation. Nanopore sequencing provides integrated molecular classification when the operative timeframe permits. These components should not overwhelm the surgical workflow. The system must answer a defined question at each stage rather than produce an unfiltered collection of probabilities and genomic findings. The proposed multicenter study links diagnostic accuracy to changes in management, neurological safety, tissue preservation, and cost. A paired design permits direct comparison with standard pathology in the same patient. Blinded interpretation is necessary during the validation phase. If the neuropathologist knows the artificial-intelligence result, their interpretation may be biased toward the model. If the model is trained using diagnoses from the same institution and patients, information leakage may exaggerate performance.

Training, validation, and test sets should be separated at the patient and institution levels. Multiple images from one patient must not be divided across training and testing. External validation should include institutions with different patient populations and workflows. Calibration and selective prediction should be prespecified. Cases below the confidence threshold should be classified as abstentions, not errors hidden through retrospective exclusion. The abstention rate itself is a clinically important outcome. Model fairness cannot be assessed solely through equal overall accuracy. Sensitivity for treatment-changing diagnoses, false-negative margin rates, calibration, and abstention should be examined across subgroups. Long-term outcomes remain uncertain. No current evidence proves that use of intelligent intraoperative histology improves progression-free survival or overall survival. Such endpoints require large samples and prolonged follow-up and are influenced by molecular subtype, treatment, age, performance status, and extent of resection. Earlier

outcomes are more appropriate for initial trials: diagnostic concordance, biopsy adequacy, operative decisions, residual tumor volume, neurological morbidity, tissue preservation, and reoperation.

Several limitations of the present review should be acknowledged. The technologies differ in optical hardware, image processing, model architecture, tumor taxonomy, reference standards, and clinical objectives. Published studies frequently originate from highly specialized centers. Some studies used simulated rather than live intraoperative workflows. High diagnostic accuracy under retrospective conditions may decrease when the system is exposed to motion, time pressure, incomplete specimens, device failure, and unfamiliar users. The rapid evolution of WHO classification creates another challenge. Models trained under one taxonomy may require reannotation and revalidation when diagnostic categories change. A system should retain lower-level outputs, such as morphology and molecular markers, rather than only a final label. Despite these limitations, the field has progressed from proof-of-concept optical imaging to prospective multicenter artificial-intelligence validation. FastGlioma, DeepGlioma, rapid lymphoma classifiers, in situ Raman probes, ultra-rapid polymerase chain reaction, MethyLYZR, and Rapid-CNS² demonstrate complementary routes toward intraoperative integrated diagnosis. The next scientific step is not another isolated accuracy study. It is a pragmatic trial showing that the combined system improves decisions without increasing neurological harm.

Conclusion. Intelligent intraoperative histology has the potential to reshape neuro-oncological surgery by providing rapid, label-free, and computationally augmented analysis of fresh tissue. Stimulated Raman histology can visualize cellular and biochemical architecture without fixation or staining, while deep-learning and foundation-model systems can classify tumor entities, estimate microscopic infiltration, and screen for molecular alterations. Recent prospective studies demonstrate that artificial-intelligence systems can analyze specimens within seconds or minutes and maintain high diagnostic performance across multiple centers. Complementary technologies, including ultra-rapid droplet digital polymerase chain reaction and nanopore sequencing, can provide direct molecular evidence during the operative period. The principal clinical value lies not in replacing the neuropathologist but in creating a continuous human–machine diagnostic workflow. The neurosurgeon defines the operative question, the optical system analyzes fresh tissue, artificial intelligence quantifies probability and uncertainty, the neuropathologist interprets the findings, and rapid molecular testing confirms critical alterations. Clinical adoption requires evidence that the technology improves biopsy adequacy, tissue allocation, resection decisions, operative efficiency, and patient outcomes. Diagnostic accuracy alone is insufficient. Future studies should use prospective paired designs, final integrated neuropathology as the reference standard, locked external validation, explicit abstention thresholds, calibration analysis, subgroup evaluation, and detailed documentation of how results alter surgical management. Patient safety must remain central. A model-positive margin does not mandate resection when the tissue supports language, movement, vision, memory, or behavior. Artificial intelligence should identify biological risk, while neurosurgical judgment defines the acceptable functional boundary.

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