**Neurosurgical Advances**
Vol 1 No 1 June 2025
ISSN: XXXX-XXXX (Electronic)
Open Access: https://journal.medopenpress.com/navs

# Can intraoperative near-infrared fluorescence imaging improve tumor resection margins in glioblastoma surgery? A technical note review

**Ruchit Jain[1], Jeremiah Hilkiah Wijaya[2]**
[1]Department of Neurosurgery, Stanford University School of Medicine, Stanford, California, USA
[2]School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

**Article Info**

***Article history:***
Received 1 January 2025
Revised 25 April 2025
Accepted 25 June 2025

***Keyword:***
Glioblastoma, Near-Infrared Fluorescence Imaging, 5-Aminolevulinic Acid (5-ALA), Tumor Resection Margins, Intraoperative Imaging

**ABSTRACT**

Glioblastoma (GBM) is a highly aggressive brain tumor with limited treatment options, where achieving maximal safe resection is critical for improving patient outcomes. However, distinguishing tumor margins from surrounding healthy tissue intraoperatively remains challenging due to the infiltrative nature of GBM. Near-infrared (NIR) fluorescence imaging has emerged as a promising tool to enhance surgical precision by providing real-time visualization of tumor tissue during resection. This technical note evaluates the utility of intraoperative NIR fluorescence imaging in improving tumor resection margins in GBM. Current technical note review demonstrates that NIR fluorescence imaging significantly enhances the ability to achieve complete tumor resection while sparing normal brain tissue. This technique offers real-time guidance, improves accuracy, and integrates seamlessly with existing surgical workflows. Despite its promise, limitations such as variability in tumor metabolism and the inability to detect deeply infiltrative cells highlight the need for further refinements. Larger studies are warranted to validate these results and explore the long-term impact on survival. In conclusion, intraoperative NIR fluorescence imaging is a valuable adjunct in GBM surgery, offering the potential to improve resection margins and patient outcomes.



## INTRODUCTION

Glioblastoma (GBM) is the most aggressive and common primary malignant brain tumor in adults, with a dismal prognosis despite advances in surgical and adjuvant therapies.1 Achieving maximal safe resection of the tumor is critical for improving survival and quality of life, as residual tumor tissue often leads to rapid recurrence. However, distinguishing tumor margins from surrounding healthy brain tissue intraoperatively remains a significant challenge due to the infiltrative nature of GBM.2

Traditional methods such as neuronavigation, intraoperative ultrasound, and contrast-enhanced MRI have limitations, including reduced accuracy during surgery due to brain shift or incomplete visualization of infiltrative tumor cells.3 To address these challenges, near-infrared (NIR) fluorescence imaging has emerged as a promising adjunctive tool. This technique utilizes fluorescent agents, such as 5-aminolevulinic acid (5-ALA) or indocyanine green (ICG), which selectively accumulate in tumor tissues, enabling real-time visualization of tumor margins under NIR light. This technical note describes the application of intraoperative NIR fluorescence imaging in GBM surgery, focusing on its ability to improve tumor resection margins.

## TECHNICAL NOTE

Patients received oral 5-ALA at a dose of 20 mg/kg body weight 4–6 hours prior to surgery. Tumor cells metabolize 5-ALA into protoporphyrin IX (PpIX), a compound that emits red fluorescence under blue light (375–440 nm). Indocyanine green (ICG), an alternative agent for NIR fluorescence

(780–820 nm), was noted for its utility in vascular mapping and tumor demarcation, though 5-ALA remained the primary agent.

A standard craniotomy was performed to access the tumor site, guided by preoperative MRI data. The surgical approach prioritized minimizing damage to adjacent functional brain regions. Standard neurosurgical techniques ensured adequate exposure of the tumor mass while maintaining hemostasis and cortical integrity.

An operating microscope equipped with an integrated NIR fluorescence module (e.g., Zeiss Pentero or Leica M530 OHX) enabled real-time visualization of fluorescent tissue. Under blue light, PpIX-rich tumor regions emitted vivid red fluorescence, contrasting sharply with the dark background of normal parenchyma. This distinction allowed surgeons to identify tumor margins with enhanced accuracy, particularly in areas where visual or tactile cues were ambiguous.

Resection focused on systematically removing fluorescent-positive tissue, with continuous intraoperative feedback refining the resection boundaries. Surgeons prioritized complete excision of high-fluorescence regions while sparing non-fluorescent brain tissue. In borderline zones, fluorescence intensity guided decisions, balancing maximal tumor removal with preservation of neurological function.

Following initial resection, the cavity was re-examined under fluorescence to detect residual tumor. Absence of fluorescence signaled complete resection, while persistent glowing areas prompted further targeted excision. This step aimed to minimize residual tumor burden, a critical factor in delaying GBM recurrence.

**Table 1. Current Published Literature on Intraoperative NIR Fluorescence Imaging.**

| Study/Source | Objective | Key Findings | Limitations/Challenges |
|---|---|---|---|
| Kalisvaart et al 2022[4] | To explore the use of NIR fluorescence imaging for optimizing intraoperative tumor identification in gastrointestinal stromal tumors (GISTs). | ICG-based NIR imaging improved tumor visualization and aided in precise resection during surgery. | Limited to tumors with sufficient vascularization; requires further validation in larger patient cohorts. |
| Handgraaf et al 2014[5] | To review emerging modalities like NIR fluorescence imaging and freehand nuclear imaging using tumor-specific contrast agents. | NIR fluorescence imaging enhances tumor detection and margin delineation; tumor-specific agents improve specificity. | Dependence on contrast agent availability; variability in tumor uptake. |
| Mieog et al 2010[6] | To validate a novel handheld NIRF camera system equipped with a 690 | The handheld NIRF camera effectively identified tumor margins and guided | Requires integration into existing surgical workflows; cost and accessibility of the |

| | | | |
|---|---|---|---|
| | nm laser for detecting and guiding tumor resection. | resection, demonstrating high sensitivity and specificity. | technology may limit adoption. |
| Oosterom et al 2022[7] | To engineer "click-on" fluorescence detectors that transform robotic surgical instruments into molecular sensing devices. | The click-on detectors enabled real-time fluorescence-guided surgery using robotic platforms, enhancing precision and adaptability. | Limited to robotic systems; potential technical challenges in widespread implementation. |
| Pepper et al 2024[8] | To determine the safety and efficacy of repeated oral 5-ALA as a radiosensitizer in glioblastoma treatment. | 5-ALA fluorescence-guided surgery improved gross total resection (GTR) rates and demonstrated safety in repeated applications; ongoing trials aim to confirm long-term benefits. | Variability in tumor metabolism affects fluorescence intensity; infiltrative cells may remain undetected. |

## RESULTS AND DISCUSSION

Intraoperative near-infrared (NIR) fluorescence imaging has emerged as a transformative tool in GBM surgery, offering real-time visualization of tumor margins. This advancement is particularly significant given the aggressive and infiltrative nature of GBM, which often complicates complete resection. By enhancing the ability to distinguish between tumor and normal brain tissue, NIR fluorescence imaging provides surgeons with a critical advantage in achieving maximal safe resection.9,10 From a physiological perspective, this technology leverages the selective uptake and metabolism of fluorescent agents, such as 5-aminolevulinic acid (5-ALA), by tumor cells.11 The resulting fluorescence highlights areas of high metabolic activity, which are characteristic of GBM cells, thereby guiding surgical decisions in real time.

Compared to traditional methods that rely on preoperative imaging or visual and tactile cues during surgery, NIR fluorescence offers several distinct advantages. One key benefit is real-time guidance, which allows surgeons to dynamically adjust their approach based on the fluorescence patterns observed during the procedure.12,13 This adaptability reduces the likelihood of leaving behind residual tumor tissue, which is a common cause of recurrence in GBM patients. Furthermore, the improved accuracy of tumor delineation minimizes damage to surrounding healthy brain tissue, preserving neurological function and improving postoperative quality of life. These advantages underscore the potential of NIR fluorescence imaging to address longstanding challenges in GBM surgery, where precision is paramount.

The ease of integrating NIR fluorescence imaging into existing surgical workflows is another factor contributing to its growing adoption. Modern surgical microscopes are increasingly equipped with NIR fluorescence modules, making this technology accessible to a wide range of healthcare facilities.14 This integration not only enhances the technical capabilities of surgeons but also aligns with the broader trend toward minimally invasive and precision-based surgical techniques. From a practical standpoint, the compatibility of NIR fluorescence with standard surgical equipment reduces the need for extensive training or additional infrastructure, facilitating its widespread implementation.15 This accessibility is particularly important in the context of GBM, where timely and effective treatment can significantly impact patient outcomes.

Despite its promise, NIR fluorescence imaging is not without limitations. A critical constraint is its reliance on the metabolic activity of tumor cells for the selective accumulation of fluorescent agents like 5-ALA. Variability in tumor metabolism among patients can affect the intensity and reliability of fluorescence, potentially leading to incomplete visualization of tumor margins.16 Additionally, GBM are known for their infiltrative growth pattern, with tumor cells often extending beyond the visibly fluorescent margin.17–19 This biological characteristic highlights the need for adjuvant therapies, such as radiation and chemotherapy, to target residual microscopic disease and prevent recurrence. These limitations underscore the importance of viewing NIR fluorescence imaging as a complementary tool rather than a standalone solution.

The ongoing refinement of NIR fluorescence imaging holds promise for addressing current limitations and expanding its applications in GBM surgery.20 Advances in fluorescent agents, such as the development of more specific and sensitive probes, could enhance the accuracy of tumor detection and reduce variability in fluorescence patterns. Moreover, combining NIR imaging with other intraoperative technologies, such as artificial intelligence-driven image analysis, may further improve the precision of tumor resection. From a physiological and clinical perspective, these innovations could lead to better stratification of patients based on tumor characteristics, enabling personalized treatment strategies. While challenges remain, the continued evolution of NIR fluorescence imaging represents a critical step forward in the quest to improve outcomes for patients with GBM, one of the most challenging cancers to treat.

## CONCLUSION

Intraoperative near-infrared fluorescence imaging is a valuable tool for improving tumor resection margins in GBM surgery. Current technical note demonstrates its potential to achieve gross total resection with minimal morbidity. While larger studies are needed to validate these findings, this technique holds promise for enhancing surgical outcomes and prolonging survival in patients with GBM. Future research should focus on optimizing fluorescent agents, refining imaging technologies, and integrating NIR fluorescence with other intraoperative modalities to further improve precision and safety.

## REFERENCES

1. Kanderi T, Munakomi S, Gupta V. Glioblastoma Multiforme. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2025 Jun 20]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK558954/
2. Patel V, Chavda V. Intraoperative glioblastoma surgery-current challenges and clinical trials: An update. Cancer Pathog Ther. 2024 Oct;2(4):256–67.
3. Dixon L, Lim A, Grech-Sollars M, Nandi D, Camp S. Intraoperative ultrasound in brain tumor surgery: A review and implementation guide. Neurosurg Rev. 2022 Aug;45(4):2503–15.
4. Kalisvaart GM, Meijer RPJ, Bijlstra OD, Galema HA, De Steur WO, Hartgrink HH, et al. Intraoperative Near-Infrared Fluorescence Imaging with Indocyanine Green for Identification of Gastrointestinal Stromal Tumors (GISTs), a Feasibility Study. Cancers. 2022 Mar 18;14(6):1572.
5. Handgraaf HJM, Verbeek FPR, Tummers QRJG, Boogerd LSF, Van De Velde CJH, Vahrmeijer AL, et al. Real-time near-infrared fluorescence guided surgery in

gynecologic oncology: A review of the current state of the art. Gynecologic Oncology. 2014 Dec;135(3):606–13.

6. Mieog JSD, Hutteman M, Van Der Vorst JR, Kuppen PJK, Que I, Dijkstra J, et al. Image-guided tumor resection using real-time near-infrared fluorescence in a syngeneic rat model of primary breast cancer. Breast Cancer Res Treat. 2011 Aug;128(3):679–89.
7. Van Oosterom MN, Van Leeuwen SI, Mazzone E, Dell'Oglio P, Buckle T, Van Beurden F, et al. Click-on fluorescence detectors: using robotic surgical instruments to characterize molecular tissue aspects. J Robotic Surg [Internet]. 2022 Apr 9 [cited 2025 Jun 20]; Available from: https://link.springer.com/10.1007/s11701-022-01382-0
8. Pepper NB, Eich HT, Müther M, Oertel M, Rehn S, Spille DC, et al. ALA-RDT in GBM: protocol of the phase I/II dose escalation trial of radiodynamic therapy with 5-Aminolevulinic acid in patients with recurrent glioblastoma. Radiat Oncol. 2024 Jan 22;19(1):11.
9. Mansour HM, Shah S, Aguilar TM, Abdul-Muqsith M, Gonzales-Portillo GS, Mehta AI. Enhancing Glioblastoma Resection with NIR Fluorescence Imaging: A Systematic Review. Cancers (Basel). 2024 Nov 27;16(23):3984.
10. Rauf SA, Ahmed R, Hussain T, Saad M, Shah HH, Jamalvi SA, et al. Fluorescence in neurosurgery: its therapeutic and diagnostic significance - a comprehensive review. Ann Med Surg (Lond). 2024 Jul;86(7):4255–61.
11. Traylor JI, Pernik MN, Sternisha AC, McBrayer SK, Abdullah KG. Molecular and Metabolic Mechanisms Underlying Selective 5-Aminolevulinic Acid-Induced Fluorescence in Gliomas. Cancers (Basel). 2021 Feb 2;13(3):580.
12. Meijer RPJ, van Manen L, Hartgrink HH, Burggraaf J, Gioux S, Vahrmeijer AL, et al. Quantitative dynamic near-infrared fluorescence imaging using indocyanine green for analysis of bowel perfusion after mesenteric resection. J Biomed Opt. 2021 Jun;26(6):060501.
13. Fan X, Yang J, Ni H, Xia Q, Liu X, Wu T, et al. Initial Experience of NIR-II Fluorescence Imaging-Guided Surgery in Foot and Ankle Surgery. Engineering. 2024 Sep;40:19–27.
14. Ma L, Fei B. Comprehensive review of surgical microscopes: technology development and medical applications. J Biomed Opt. 2021 Jan;26(1):010901.
15. Ullah Z, Roy S, Gu J, Ko Soe S, Jin J, Guo B. NIR-II Fluorescent Probes for Fluorescence-Imaging-Guided Tumor Surgery. Biosensors. 2024 May 30;14(6):282.
16. Mazurek M, Szczepanek D, Orzyłowska A, Rola R. Analysis of Factors Affecting 5-ALA Fluorescence Intensity in Visualizing Glial Tumor Cells-Literature Review. Int J Mol Sci. 2022 Jan 15;23(2):926.
17. Unger J, Hebisch C, Phipps JE, Lagarto JL, Kim H, Darrow MA, et al. Real-time diagnosis and visualization of tumor margins in excised breast specimens using fluorescence lifetime imaging and machine learning. Biomed Opt Express. 2020 Mar 1;11(3):1216–30.
18. Kravchenko Y, Sikora K, Wireko AA, Lyndin M. Fluorescence visualization for cancer DETECTION: EXPERIENCE and perspectives. Heliyon. 2024 Jan;10(2):e24390.
19. Zhou Y, Tao L, Qiu J, Xu J, Yang X, Zhang Y, et al. Tumor biomarkers for diagnosis, prognosis and targeted therapy. Sig Transduct Target Ther. 2024 May 20;9(1):132.
20. Cheng H, Xu H, Peng B, Huang X, Hu Y, Zheng C, et al. Illuminating the future of precision cancer surgery with fluorescence imaging and artificial intelligence convergence. npj Precis Onc. 2024 Sep 9;8(1):196.