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NEW TECHNOLOGIES USING FLUORESCENCE

Abstract. Intraoperative fluorescence is a technique used to improve the accuracy of surgical interventions [1] in various fields of medicine. In recent years, near-infrared fluorescence imaging technology, as an optical imaging method, has become one of the most popular intraoperative navigation technologies and is widely used in various surgical procedures, such as sentinel lymph node imaging, tumor resection, angiography, and intraoperative anatomy [2].

Keywords: near-infrared fluorescent probe, NIR-I and NIR-II imaging technology, near-infrared fluorescent probe for selective detection of severe hypoxia

Relevance of the study

Modern surgery requires new methods for visualization of blood vessels, anatomical structures, pathological processes, etc. The use of a near-infrared fluorescent probe helps to provide effective and complication-free treatment.

Fluorescence is the phenomenon where a substance absorbs light and emits it at a higher energy [3]. In medicine, this method has found widespread application in the diagnosis, visualization, and treatment of diseases.

In 1948, physician and scientist Robert Bollard first used fluorescent techniques to detect and study tumors. He used intravenous administration of a fluorescent protein into a patient. He then used a special lighting device to examine tumor tissue, marking the first step in using fluorescent methods to diagnose cancer.

In modern medicine, fluorescence is used in many fields, for example:

– cardiology (detection of heart defects, visualization of blood flow in vessels);

– neurosurgery (assessment of bypass patency, assessment of venous drainage abnormalities in cavernoma surgery and neurooncology, treatment of aneurysms and arteriovenous malformations (AVMs), detection and removal of tumors) [4];

– ophthalmology (detection of atypical vessels, study of retinal blood flow, detection of hemorrhages and neoplasms, detection of inflammation and edema) [5];

– oncology (possibility of complete removal of tumors, diagnosis of spread of metastases);

– urology (identification of renal vessels, removal of renal cortical tumors, kidney transplantation).

Currently, the near-infrared fluorescence navigation system mainly uses the first near-infrared (NIR) region (NIR-I, 760~900 nm) window for imaging, and is widely used in microsurgery such as cancer resection and vascular anastomosis [2]. Compared with the NIR-I window, the second near-infrared window (NIR-II, 900~1700 nm) has optical properties such as greater penetration depth, higher signal-to-background ratio, and lower biological autofluorescence.

Intraoperative navigation systems based on NIR-I fluorescence imaging technology are widely used in tumor removal surgeries to visualize small lesions, lesion boundaries, key nerves or conduits. However, its background noise is large and its ability to image deep tissues is poor. If the fluorescence imaging band is expanded to the NIR-II window, the accuracy of fluorescence navigation surgery will be further improved and the intraoperative damage will be reduced.

Professor Tian Jie's team used the ICG-IRDye800CW probe to perform NIR-II fluorescence navigation surgery, which can accurately display the boundary between hepatocellular carcinoma and liver parenchyma, thus accurately detecting hepatocellular carcinoma in patients with liver cirrhosis during surgery, preserving liver parenchyma, and reducing the risk of postoperative complications.

The imaging effect during surgery shows that NIR-II fluorescence navigation is sufficient for rapid and accurate segmentation of the lesion area during surgery (Fig. 1).

NIR-II imaging technology is also used in glioma resection surgery [2]. Compared with the NIR-I window, the NIR-II imaging technology can display more deep blood vessels and small blood vessels (Fig. 2), helps reduce intraoperative blood vessel damage, and can improve the rate of complete tumor resection and the postoperative survival rate of patients without compromising neurological function. The detection rate and complete resection rate of NIR-II imaging technology for brain gliomas reached 100%, which is much higher than that of the previous NIR-I window and white light imaging.

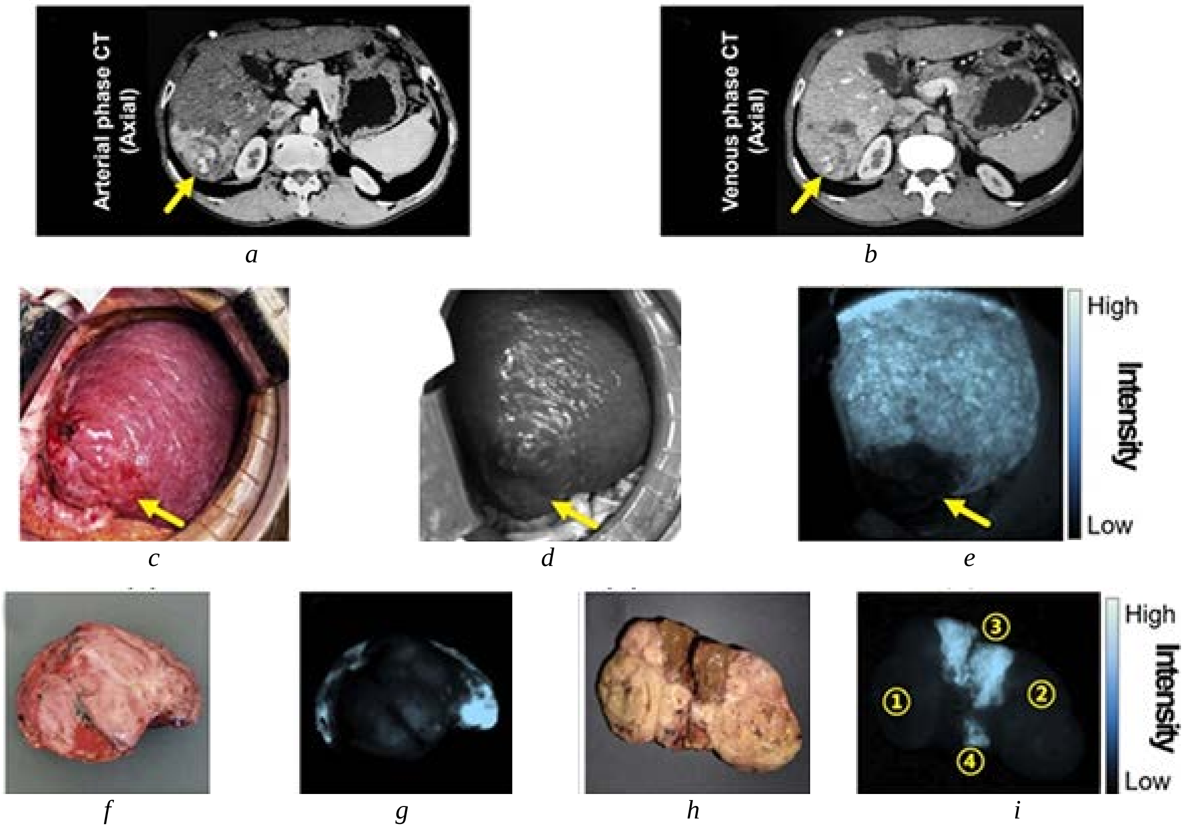

Fig. 1. Intraoperative fluorescence imaging and pathological findings in hepatectomy, (*a*, *b*) enhanced CT findings showed a lesion in the right lobe of the liver, (*c*, *d*, *e*) show in vivo images before resection of the lesion, (*c*) was acquired in the visible light spectrum, while (*d*) was observed in white light using the NIR-II camera, (*e*) NIR-II fluorescence was detected in the liver parenchyma and the lesion was visualized with high contrast, (*f*, *g*) the fluorescent edge on the resected lesion contributed to achieving complete resection, (*h*, *i*) demonstrates the distribution of NIR-II fluorescence on the lesion ex vivo [2]

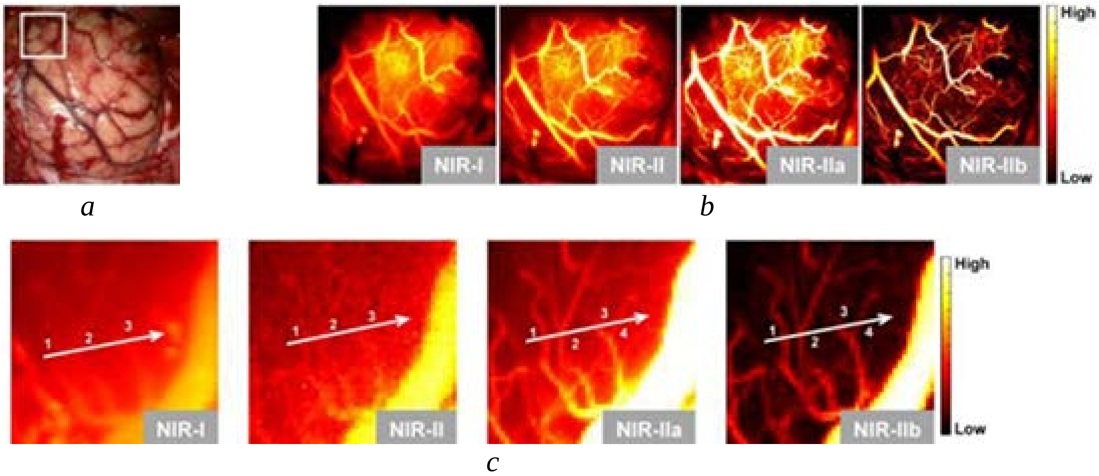

Fig. 2. Comparison of fluorescence images of the brain with different near-infrared windows, (*a*, *b*) Image of blood vessels of the brain in white light and different near-infrared windows, (*c*) (*a*) fluorescence image of blood vessels in each near-infrared window in the middle white area. Three veins can be distinguished in the NIR-I and NIR-II images, and four veins can be distinguished in the NIR-IIa and NIR-IIb images [2]

Recently, Professor Tian Jie's team combined NIR-II imaging technology with deep convolutional neural networks to provide real-time pathological diagnosis of gliomas in situ during surgery and assist neurosurgeons in confirming the best resection area.

Fluorescence-guided surgery has proven to be a widely innovative technique applicable in many fields of surgery [6]. An important step has been widespread implementation, with increasing evidence of patient safety and surgical efficiency. The potential indications for its use are diverse and can even be combined in selected cases.

NIR fluorescence using target-specific imaging probes has gained interest over the past decade as a promising and effective method for visualization of malignant targets. Potent targets should have a strong specific expression on target tissue relative to (healthy) surrounding tissue, with their expression minimally influenced by neoadjuvant treatments.

Hypoxia, a state of low oxygen concentration, plays a crucial role in various diseases like ischemia and cancer [7]. Fluorescent probes are utilized for its detection, however, existing near-infrared (NIR) probes lack specificity for severe hypoxia (around 1% oxygen). This research investigated JSiR as a scaffold for a new NIR probe capable of selectively detecting severe hypoxia. This study led by Yasuteru Urano, a team of scientists designed, synthesized, and evaluated T-azoJSiR640, a novel near-infrared fluorescent probe for severe hypoxia detection. The synthesized probe, T-azoJSiR640, demonstrated its ability to detect severe hypoxia both in vivo and in vitro, making it a promising tool for disease diagnosis and monitoring. Unlike existing probes, T-azoJSiR640 selectively visualizes severe hypoxia (around 1% oxygen or less) in both live cells and the ischemic liver of a mouse model in vivo. This makes T-azoJSiR640 a potentially valuable tool for analyzing, monitoring, and understanding various hypoxia-related diseases, such as cancer, where severe hypoxia activates processes that contribute to tumor progression.

Fan Chunhai's team from Shanghai Jiao Tong University, Shanghai University, and East China University of Science and Technology jointly developed a novel framework fluorescent nucleic acid probe for fluorescence imaging of the second near-infrared region (1000-1700 nm, NIR-II) window and surgical navigation in living animals [8].

This study introduces novel fluorescent DNA framework (FDF) dots for near-single-cell-level cancer imaging [9]. Utilizing second near-infrared window (NIR-II) emission and a unique structural design, FDFs achieve high sensitivity, deep tissue penetration (up to 1 cm), and tumor specificity. In mouse models, the probes demonstrated the ability to detect as few as 40 tumor cells, high tumor-to-normal tissue ratios, and long-term imaging capability (over 11 days). NIR-II image-guided surgery using FDFs enabled the successful resection of breast cancer metastases as small as 53 µm.

Shang W and colleagues developed a phage-based NIR fluorescent nanoprobe called PLSWT7-DMI, which can specifically bind to BCa cells and was shown to be non-toxic in animal experiments [10]. Clinical studies further confirmed the purity, efficacy, and safety of PLSWT7-DMI, and no toxic reactions were observed. Twenty-two patients with suspected non-invasive bladder cancer were enrolled in the study. While inserting a probe into the bladder, the researchers used an endoscope that could be switched to NIR fluorescence mode to obtain a complete image of the bladder mucosa and perform histopathological examination of the suspected lesion. The results showed that the fluorescence intensity of tumor tissue was 5.1 times higher than that of normal tissue. The sensitivity and specificity of the probe reached 91.2% and 90%, respectively, which can effectively distinguish common small satellite tumors, carcinoma in situ and benign suspicious mucosa from cancerous tissues. This is the first time this method has been applied to humans, and no side effects have been observed. The research team believes that PLSWT7-DMI-based NIR fluorescence endoscopy is a safe and effective diagnostic method that helps completely remove tumors, thereby reducing the risk of recurrence.

For patients with end-stage renal disease (ESRD), kidney transplantation is the gold standard treatment for the disease. Zhao et al. developed a multifunctional NIR activity probe suitable for kidney transplantation. Due to the strong aggregation-induced emission (AIE) effect, the probe has significant fluorescence intensity in the second near-infrared window and performs several functions such as structural imaging of the kidneys, evaluation of renal function, and prevention of vascular complications of the ureter. In addition, the long circulation time allows for monitoring and evaluation of the entire kidney transplantation process.

Conclusions

Near-infrared fluorescence imaging technology is well compatible with most surgical procedures [2]. Performing non-contact real-time observation and implementing near-infrared fluorescence visualization, allowing the surgeon to consider both the normal white light field of view and the fluorescence field of view with fluorescent marker information. However, unlike the NIR-I window, the NIR-II window has a larger range with a visible light band.

Fluorescence imaging improves the quality of surgery by allowing surgeons to more accurately distinguish and localize the specific location and extent of removal of malignant tumors, helping to reduce disease recurrence and improve patient outcomes[10]. It also reduces the risk of intraoperative injuries and prevents iatrogenic injuries that may occur during surgeries.

Literature

1. Як розпізнати та лікувати пухлини головного мозку: повний гід для пацієнтів. URL: https://neurooncology.com.ua (дата звернення: вкажіть дату, коли ви переглядали сторінку).
2. NI, H.-W., & QIAN, J. (2023). Clinical research progress on the fluorescence imaging in the second near-infrared window. *Journal of Infrared and Millimeter Waves*, *42*(6), 895.
3. Флуоресцентні методи в медицині. URL: https://www.0532.ua (дата звернення: вкажіть дату, коли ви переглядали сторінку).
4. Переваги флуоресценції в судинній нейрохірургії. URL: https://alt.ua/blog/perevagi-fluorestsentsiyi-v-sudinnij-nejrohirurgiyi (дата звернення: вкажіть дату, коли ви переглядали сторінку).
5. Holobrodska, A. V. (2024). *Decisio support system for diagnosing and treating ophthalmological pathologies* (Bachelor's qualification work). Odesa Polytech. Nat. Univ.
6. Sutton, P. A., van Dam, M. A., Cahill, R. A., Mieog, S., Polom, K., Vahrmeijer, A. L., & van der Vorst, J. (2023). Fluorescence-guided surgery: comprehensive review. *BJS Open*, *7*(3), zrad049. https://doi.org/10.1093/bjsopen/zrad049
7. Kasai, T., Fujita, K., Komatsu, T., Ueno, T., Kojima, R., Hanaoka, Y., & Urano, Y. (2025). Development of a near-infrared fluorescent probe for the selective detection of severe hypoxia. *RSC Chemical Biology*, *6*, 449-456.
8. Shanghai Jiao Tong University. (2024). *Shanghai Jiao Tong University has made significant progress in near-infrared fluorescent nucleic acid probes and surgical navigation*. Department of Chemical Biology, School of Chemistry and Chemical Engineering.
9. Liu, X., Shi, B., Gao, Y., et al. (2025). Ultrabright near-infrared fluorescent DNA frameworks for near-single-cell cancer imaging. *Nature Photonics*, *19*, 79–88. https://doi.org/10.1038/s41566-024-01543-7.
10. Zhang, W., Nurdebek, D., Zebibulla, A., Bingzhang, Q., & Rexiati, M. (2024). Research progress of near-infrared fluorescence imaging in urological surgery. *Progress in Clinical Medicine*, *14*(4), 478-483. https://doi.org/10.12677/acm.2024.1441047.