

# Methylene Blue–Based Near-Infrared Fluorescence Imaging for Breast Cancer Visualization in Resected Human Tissues

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## Abstract

Breast-conserving surgery is facing the challenge of objective tumor margin identification intraoperatively. Near-infrared fluorescence imaging would be an ideal approach to visualize tumor margins during surgeries. In this preliminary study, the feasibility of methylene blue–based near-infrared fluorescence imaging technique for breast cancer detection was assessed in resected human breast specimens after breast cancer surgeries. Thirty patients with breast cancer scheduled for surgical treatment were enrolled, including 10 patients with preoperative chemotherapy and 20 patients without. Each of them received an injection of 1 mg/kg methylene blue intravenously 3 hours before the surgery. Then, a home-developed methylene blue–specific near-infrared fluorescence imaging system was employed to image the resected breast tissues and identify the tumor by the fluorescence contrast. Specimens were taken for pathological examinations as the reference. There were no severe adverse events attributable to methylene blue. Of 20 patients, who did not receive preoperative chemotherapy, 16 exhibited fluorescent contrast on their resected tissues (signal-to-background ratio:  $1.94 \pm 0.71$ ). In contrast, tumors were identified in 3 of 10 specimens from patients who underwent preoperative chemotherapy (signal-to-background ratio:  $1.63 \pm 0.38$ ). A total of 35 tissues were sampled from 30 specimens. Besides 30 tumor samples, 5 more suspicious samples with fluorescence signal were confirmed to be benign hemorrhagic tissues. Therefore, a sensitivity of 0.63 and a positive predictive value of 0.79 were achieved by the methylene blue fluorescence imaging strategy. Here, we demonstrate the feasibility of using methylene blue fluorescence imaging to identify breast cancer. Preoperative chemotherapy had an impact on imaging effect, which may reduce the detection rate. After all, methylene blue fluorescence imaging has great potential to be used into breast-conserving surgery for tumor-positive margins detection, but further clinical trial study is needed (<http://www.chictr.org.cn/> Clinical Trial Registry ID: ChiCTR1800015400, Near-infrared fluorescence imaging applied in breast cancer identification with methylene blue).

## Keywords

near-infrared fluorescence imaging, breast cancer surgery, methylene blue, fluorescence contrast agent, intraoperative imaging

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## Abbreviations

BCS, breast-conserving surgery; BMI, body mass index; DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; FDA, Food and Drug Administration; H&E, hematoxylin and eosin; Her2/NEU, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ICG, indocyanine green; MB, methylene blue; MB-FI, methylene blue fluorescence imaging; MRI, magnetic resonance imaging; MRM, modified radical mastectomy; MUC, mucinous adenocarcinoma; NIR, near-infrared; NIR-FI, near-infrared fluorescence imaging; PR, progesterone receptor; ROI, region of interest; SBR, signal-to-background ratio; SLN, sentinel lymph node.

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## Introduction

Breast cancer is 1 of the 3 most common cancers worldwide and is especially a common malignancy in women.<sup>1</sup> Cancer statistics from 2018 estimate that breast cancer accounted for approximately 30% of all cancer cases and 14% of cancer deaths among women.<sup>2</sup> Common surgical treatments for solid breast tumors include mastectomy and breast conservation therapy.<sup>3</sup> In the era of precision medicine, breast-conserving surgery (BCS) leads to minimal trauma and is followed by rapid recovery, which is a standard option for patients. For BCS, one of the most important elements to improve survival and reduce the need for subsequent adjuvant therapies is the acquisition of negative cutting edge.<sup>4</sup> However, removal of tumors with negative margins is challenging in BCS due to the lack of a convenient and objective method to assist breast cancer identification, intraoperatively. It has been reported that 11% to 40% BCS patients had to perform re-excision due to inadequate margins.<sup>5-7</sup> A real-time intraoperative and breast cancer-specific imaging approach is highly desirable to minimize the rates of re-excision, unnecessary radical resections, and possible overtreatment.<sup>4,8</sup>

Currently, tumor detection methods during surgery rely on visual palpation, intraoperative ultrasound, and frozen section analysis.<sup>9</sup> Although useful, these methods are experience dependent, time-consuming, and constrained by sample errors.<sup>10</sup> Therefore, an intraoperative imaging method as an adjunct to them can be both helpful to surgeons in recognizing breast tumors during resection and to pathologists in locating suspicious lesions in resected tissues for rapid pathological examination.<sup>11</sup>

Near-infrared (NIR) fluorescence imaging (FI) has been reported for intraoperative applications because of its ability to identify tumor margins, sentinel lymph nodes (SLNs), and vital structures (blood vessels, nerves, and so on) in real time with no ionizing radiation.<sup>12-16</sup> At present, only indocyanine green (ICG) and methylene blue (MB) are approved by the Food and Drug Administration<sup>17</sup> as clinically available NIR fluorescence dyes. Indocyanine green-based NIR-FI is proven to be very successful in SLN detection in breast cancer surgeries.<sup>14,18-20</sup> However, it is seldom applicable for breast cancer imaging, which hinders the clinical impact of NIR-FI.

Different from ICG with excitation and emission wavelength of 780 nm and 820 nm, respectively, MB can be excited

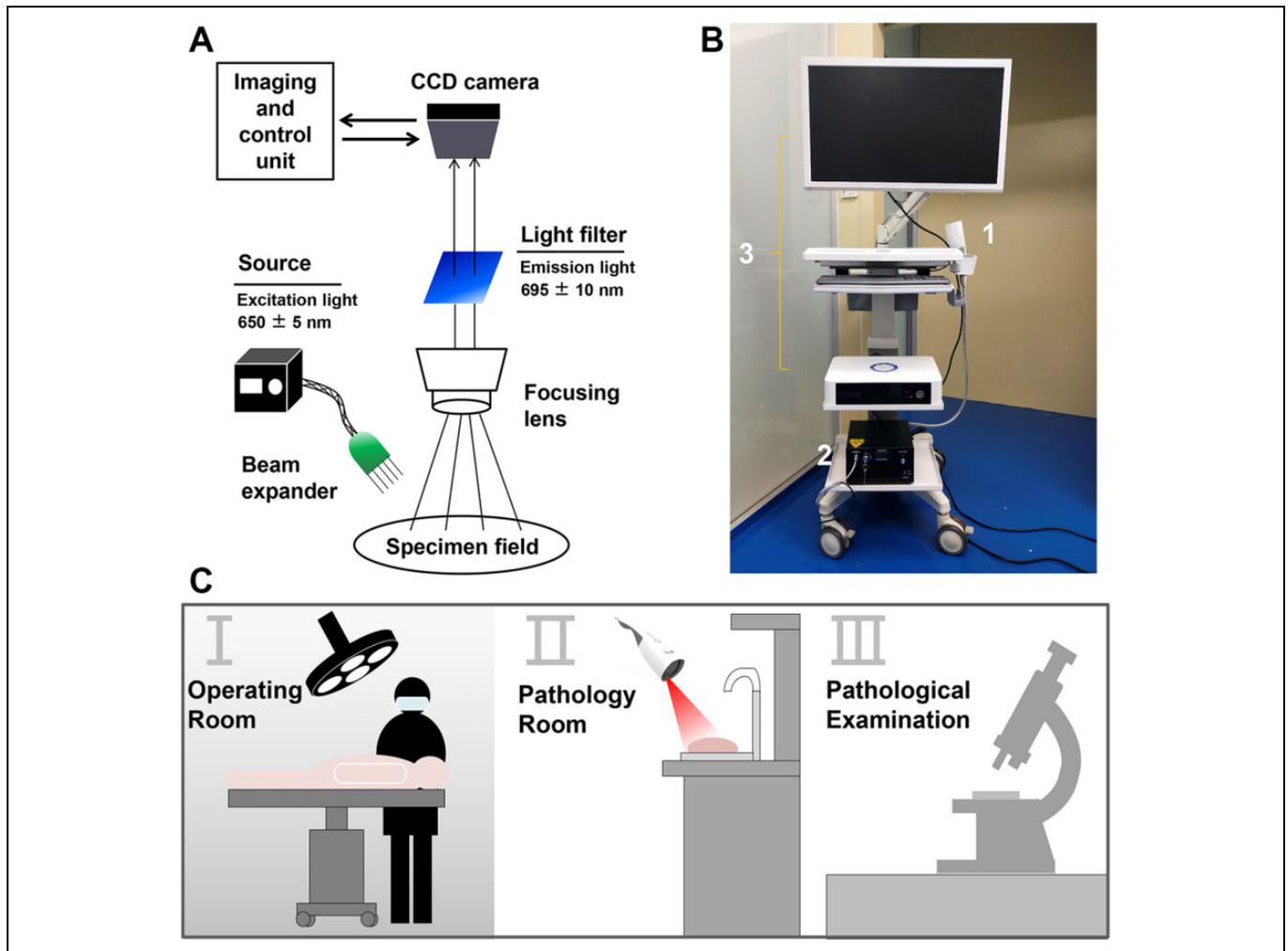
by 665 nm light, and its peak emission is in 686 nm. Several groups have demonstrated the feasibility and benefits of using MB-based NIR-FI for tumor lesion detection during surgeries, such as the identification of the solitary fibrous tumor in pancreas,<sup>21</sup> the parathyroid adenomas,<sup>22,23</sup> and the paraganglioma.<sup>24</sup> There was only 1 study performed by Tummers *et al* in the Netherlands using MB-FI for breast cancer identification,<sup>25</sup> in which 20 of 24 patients with breast cancer showed effective fluorescent signal in tumors. This opened a new door of using intraoperative MB-FI for breast cancer surgeons and pathologists to achieve more precise and faster surgical treatment.

The purpose of our work was to pursue this direction and verify the feasibility of using MB and our home-developed MB-specific NIR-FI system for breast cancer detection in resected human tissues during mastectomy and BCS.

## Materials and Methods

### Fluorescence Imaging System

In this study, the MB-FI system used to detect breast tumors was developed by the CAS Key Laboratory of Molecular Imaging. The system was designed to detect the MB emission wavelength (Figure 1A). Because the excitation and the emission wavelengths of MB are relatively close, in order to minimize the light pollution of the excitation light to the imaging, we chose the upper edge of MB peak excitation spectrum to be 650 nm and the lower edge of MB peak emission spectrum to be 695 nm as the parameters of the system. The system mainly included 3 components (Figure 1B): (1) Image acquisition: the NIR-fluorescence signal was detected using a lens (FL-CC0814A-2M; Ricoh Co, Ltd, Tokyo, Japan), through the light filter (narrow band pass filter,  $695 \pm 10$  nm), then transmitted to a charge-coupled device camera (MVC1300MF-M00; Beijing Microview Science and Technology Co, Ltd, Beijing, China). (2) Illumination design: a 650-nm NIR excitation light source was used (L-650/5WF; Zhongshan You Sheng Photoelectric Technology Co, Ltd, Zhongshan, Guangdong, China) to provide the source light. (3) Imaging and control component: process the signal and obtain the images by using the imaging software designed by the Key Laboratory of Molecular Imaging, Beijing, China. Real-time imaging data were recorded in video rate. However, the system, as a prototype



**Figure 1.** System and experiment workflow. (A) Methylene blue–fluorescence imaging (MB-FI) structure and principle, (B) MB-FI system, and (C) Experiment workflow. MB-FI was performed on resected breast tissues in the pathology room.

equipment, was unable to display white light and fluorescence images simultaneously.

### Patients

Patients were enrolled according to the hospitalization order. Thirty female patients (age 32–68 years, median age 53 years) diagnosed with breast cancer who were scheduled for resection surgery were enrolled in this study between March 2018 and August 2018. The clinical trial was registered at the Chinese Clinical Trial Registry (<http://www.chictr.org.cn/>, ChiCTR1800015400) and approved by institutional review board of the hospital (20171105). All patients provided written informed consent to be examined in this study. Both patients with and without preoperative chemotherapy were enrolled, and the preoperative chemotherapy was taxotere–epirubicin–cyclophosphamide. Magnetic resonance imaging (1.5 T EXCITE HD, GE. Repetition time = 6.1 milliseconds, echo time = 2.9 milliseconds, inversion time = 13 milliseconds, and field of view = 36 × 36 cm) was applied to patients for clinical examination with the contrast agent

Gd-DPTA. Breast tumors of enrolled patients included invasive ductal carcinoma (IDC) in 22 patients, mixed carcinoma in 4 patients, mucinous adenocarcinoma (MUC) in 1 patient, and ductal carcinoma *in situ* (DCIS) in 3 patients. Patients with a history of severe food or drug allergy, diabetes, or serious organ diseases such as those of the heart and lung, abnormal liver and kidney function, pregnancy, or lactation were excluded from the study.

### Imaging Procedure

Methylene blue was purchased from Jumpcan Pharmaceutical Group Co, Ltd, Taixing, China. Patients were given MB through a peripheral vein 3 hours before surgery. To determine the appropriate experimental conditions, the first 3 experimental cases were administered with a dose of 0.5 mg/kg of MB intravenously to each, but this dosage failed. Subsequently, we increased the dose to 1 mg/kg of body weight. This dose led to a detectable fluorescence signal in tumor areas, and the following 30 patients enrolled in the study were administered according to this dose standard.

The trial was performed on *ex vivo* specimens from patients with breast cancer in the pathology room next to the operating room. After routine injection of MB, patients received surgical treatment, either modified radical mastectomy (MRM) or BCS. During the experimental process, the surgical procedure was not influenced by MB-FI (Figure 1C). After surgical resection, the MB-FI of breast surgical specimens was performed *ex vivo* in the pathology room, in order to detect breast tumors. Then, the sampled tissues were sent for pathological examination. The fluorescence images were displayed and recorded in video rate. Because of the limitation of the penetration depth by FI, the specimens were bisected longitudinally based on the locations of the breast masses. In this study, the signal-to-background ratio (SBR) threshold value was defined at 1.3, according to the imaging results and corresponding pathological examinations of first 6 cases. The identified breast masses, nondetectable masses by MB-FI and other suspicious tissues with fluorescence signal detected, were removed and set aside for pathological examinations. According to the standard pathological procedure with hematoxylin and eosin (H&E) staining analysis, all the sampled tissues were examined by pathologists.

### Statistical Analysis

Signal-to-background ratio was quantitatively determined based on the recorded images. To calculate the SBR, we delineated the region of interest (ROI) using the image-processing software ImageJ (National Institutes of Health, Bethesda, Maryland). Then, the mean brightness value of the ROI ( $B_{ROI}$ ) was calculated within a range of 0 to 255. Surrounding tissue brightness was calculated 3 times in different locations in the same way and averaged as the background brightness ( $B_{background}$ ). The SBR values were obtained by dividing  $B_{ROI}$  by  $B_{background}$  and was calculated 3 times and averaged to reduce calculation error.

Mann-Whitney  $U$  test was used to evaluate differences in the variables. Pearson  $\chi^2$  test or Fisher exact test (2 sided), if appropriate, was used to analyze the variable distribution differences between the 2 tumor groups (fluorescence signals detectable and nondetectable). Based on the pathological examination results of the sampled tissues, sensitivity and positive predictive rate were calculated, respectively. Data calculations, graphing, and analysis were performed using SPSS Version 25 (IBM Corporation, Armonk, New York) and GraphPad Prism Version 7 (GraphPad Software Corporation, San Diego, California).  $P$  values  $<.050$  were considered statistically significant.

## Results

### General

A total of 30 patients who met study criteria were included in this study, of which 10 patients had received preoperative chemotherapy and 20 patients did not. In nonpreoperative chemotherapy group, 16 of 20 resected breast tissues showed

detectable fluorescent signals in tumor areas using our MB-FI system. Of 10 tumors, 3 were detected in specimens of patients with preoperative chemotherapy (Figure 2A). Therefore, 19 of 30 resected breast tissues showed detectable fluorescent signals, while the other 11 were nondetectable. Patients' characteristics are counted in Table 1. During experiments, there were 5 extra suspicious tissues with fluorescence light spots in the nonpreoperative chemotherapy group (SBR:  $2.19 \pm 0.40$ ). Fluorescence intensity between tumors and suspicious tissues was calculated (Figure 2B). The mean SBR for tumors was  $1.94 \pm 0.71$  (range: 1.31-3.90) and  $1.63 \pm 0.38$  (range: 1.39-2.06) in the nonchemotherapy and chemotherapy group, respectively (Figure 2C). Representative white and fluorescence images of tumor in nonchemotherapy group, tumor in chemotherapy group, and suspicious tissue in nonchemotherapy group are shown in Figure 2D to F. After statistics, a total of 35 sampled tissues were collected, including 30 tumor tissues of every enrolled patients and 5 suspicious tissues.

During the intravenous injection of MB, 2 of the enrolled patients had mild vascular pain. No other serious adverse reactions were noted.

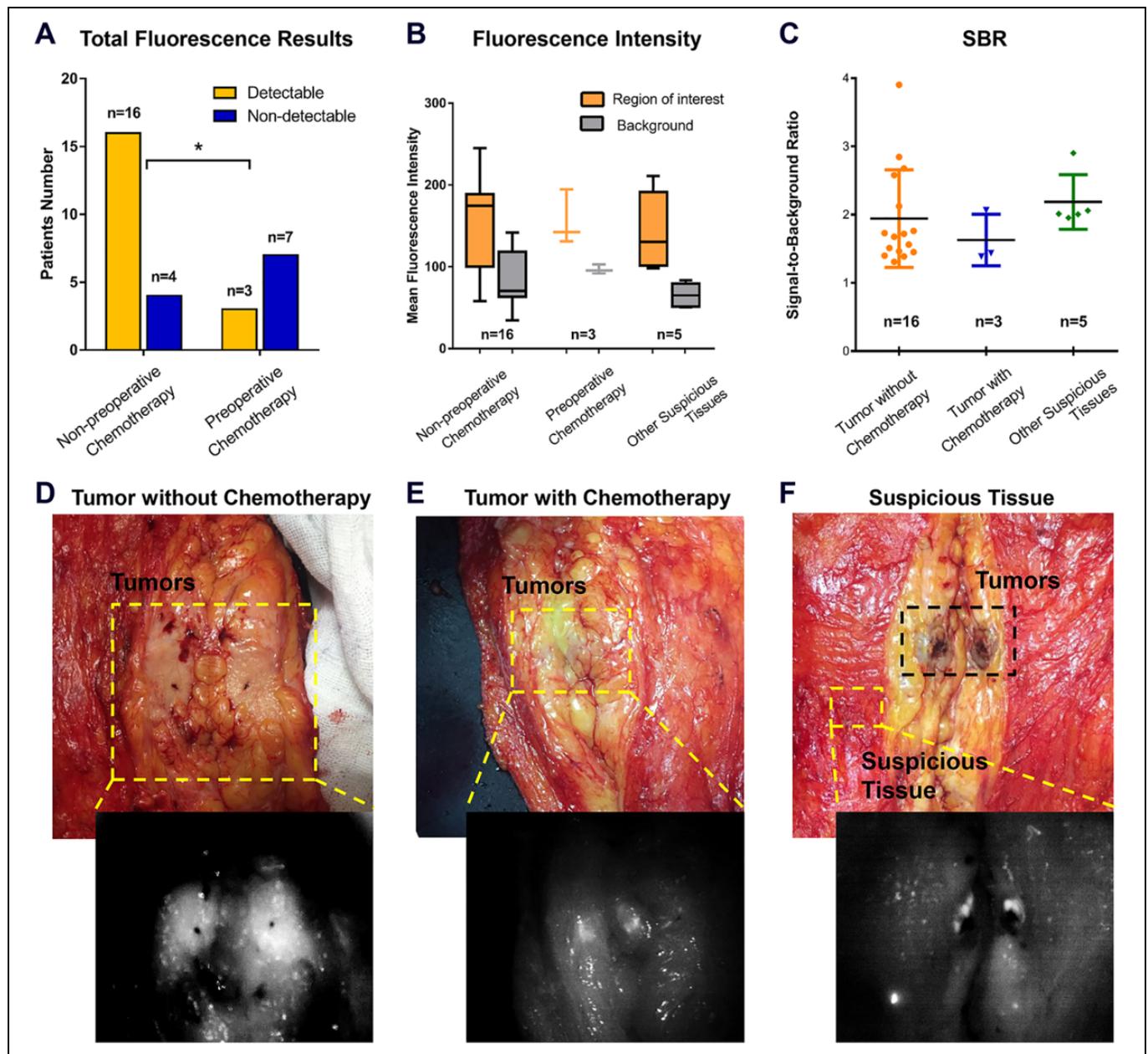
### Breast Tumor Imaging

Nineteen of 30 resected breast tissues showed detectable fluorescent signals in the tumor areas by our MB-FI system (Figure 1A and B), while the other 11 were nondetectable (Table 1). Statistical analysis revealed that preoperative chemotherapy influenced the imaging performance significantly (Table 1;  $P < .05$ ). For patients without preoperative chemotherapy, MB-FI visualized tumors in 16 of 20 tissues. One of the imaging results is shown in Figure 3A. According to the location of the tumor detected by palpation, the breast cancer specimen of this patient was longitudinally bisected *ex vivo* to expose the tumor to the surface. Fluorescence signals were detected in the tumor areas. Sliced tissue with tumor was demonstrated to be IDC. No fluorescence signals were detected in the necrotic tissue areas.

The 4 tissues with undetectable signals in nonpreoperative chemotherapy group comprised 1 case of MUC (Figure 3B) and 3 cases of IDC. In addition, in 1 specimen from a patient with mixed carcinoma including MUC, IDC, and invasive lobular carcinoma (ILC), fluorescence signals were detectable in all suspected tumor areas except the MUC area (Supplementary Figure S1). In contrast, only 3 of 10 breast tissues showed fluorescent contrast in the preoperative chemotherapy group. All the 10 patients in the preoperative chemotherapy group were diagnosed with IDC.

### Suspicious Tissue Imaging

In the nonpreoperative chemotherapy group, besides acquiring 20 malignant tissue samples inside breast tumor areas, 5 extra suspicious tissues around the tumor with high fluorescence signal were also sampled for the pathological examination (Figure 3A). The final pathological results confirmed that they



**Figure 2.** Fluorescence imaging statistics and representative images of tumors and suspicious tissue. A, Total fluorescence imaging results between groups with and without chemotherapy. B, Fluorescence intensity between tumors of nonpreoperative chemotherapy, tumors of preoperative chemotherapy, and suspicious tissues. C, Signal-to-background ratio (SBR) between 3 different groups, tumor without chemotherapy:  $1.94 \pm 0.71$ ; tumor with chemotherapy:  $1.63 \pm 0.38$ ; and other suspicious tissues:  $2.19 \pm 0.40$ . D, White light and fluorescence images of tumor (yellow frame) without chemotherapy. E, White light and fluorescence images of tumor (yellow frame) with chemotherapy. F, White light and fluorescence images of suspicious tissue (yellow frame).

were benign tissues with hemorrhage (Figure 3A-E). The mean SBR for these 5 suspicious tissues was  $2.19 \pm 0.40$  (range: 1.95-2.90).

### Sampled Tissue Statistics

During the study, 30 breast tumor tissues and 5 suspicious tissues were sampled. Pathological examination confirmed that the tumor tissues were malignant, while the 5 extra bright spots

were benign tissues. The statistical analysis of the 35 samples demonstrated that the sensitivity was 0.63 and the positive predictive value was 0.79 by using this MB-based NIR-FI strategies (Table 2).

### Discussion

In this study, we assessed the feasibility of breast tumor identification using MB-based NIR-FI technique on resected

**Table 1.** Patients Characteristics with 1 mg/kg MB.<sup>a</sup>

Characteristics	Detectable, N = 19	Nondetectable, N = 11	P Value
	Mean (Range)	Mean (Range)	
Age	55 (32-68)	53 (36-67)	.779
BMI	24 (18.8-34)	24 (18.7-28.3)	.698
Tumor size, mm	22 (10-50)	21 (15-35)	.895
Location of breast	N (%)	N (%)	.705
Left	10 (53)	5 (45)	
Right	9 (47)	6 (55)	
Clinical stage			.48
0	3 (16)	0 (0)	
1A	5 (26)	4 (36)	
2A	6 (32)	4 (36)	
2B	4 (21)	1 (9)	
3A	1 (5)	1 (9)	
3C	0 (0)	1 (9)	
Type of operation			.126
MRM	19 (100)	9 (82)	
BCS	0 (0)	2 (18)	
Histological type			.26
IDC	12 (63)	10 (91)	
IDC + ILC + MUC	1 (5)	0 (0)	
IDC + ILC	1 (5)	0 (0)	
MUC	0 (0)	1 (9)	
DCIS + IDC	2 (11)	0 (0)	
DCIS	3 (16)	0 (0)	
Receptor status			
ER positive	14 (74)	6 (55)	.284
PR positive	11 (58)	6 (55)	.858
Her2/NEU positive	15 (79)	7 (64)	.361
Triple negative	1 (5)	1 (9)	.685
Histological grade			.638
I	1 (5)	0 (0)	
II	8 (42)	5 (45)	
II-III	1 (5)	0 (0)	
III	4 (21)	1 (9)	
No grading possible	5 (26)	5 (45)	
Preoperative chemotherapy			.015
Yes	3 (16)	7 (64)	
No	16 (84)	4 (36)	

Abbreviations: BCS, breast-conserving surgery; BMI, body mass index; DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; Her2/NEU, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; MB, methylene blue; MRM, modified radical mastectomy; MUC, mucinous adenocarcinoma; PR, progesterone receptor.

<sup>a</sup>N = 30.

human breast tissues during breast surgery. A MB-FI system specially developed for MB excitation and detection was used for imaging. Instead of conventional subcutaneous injection of MB for locating SLN in breast cancer surgeries,<sup>26</sup> MB was intravenously injected 3 hours before surgeries, with the dose of 1 mg/kg of the patient body weight. Thirty patients scheduled for surgeries (28 MRM and 2 BCS) were enrolled to evaluate whether MB could accumulate in breast tumor and be visualized by NIR-FI, as described by Tummers *et al* study.<sup>25</sup> Tummers *et al* showed that 20 of 24 breast tumors were detected by NIR-FI

method. The histological type of 4 nondetectable tumors were IDC, MUC, DCIS, and primary mucoepidermoid carcinoma, respectively.

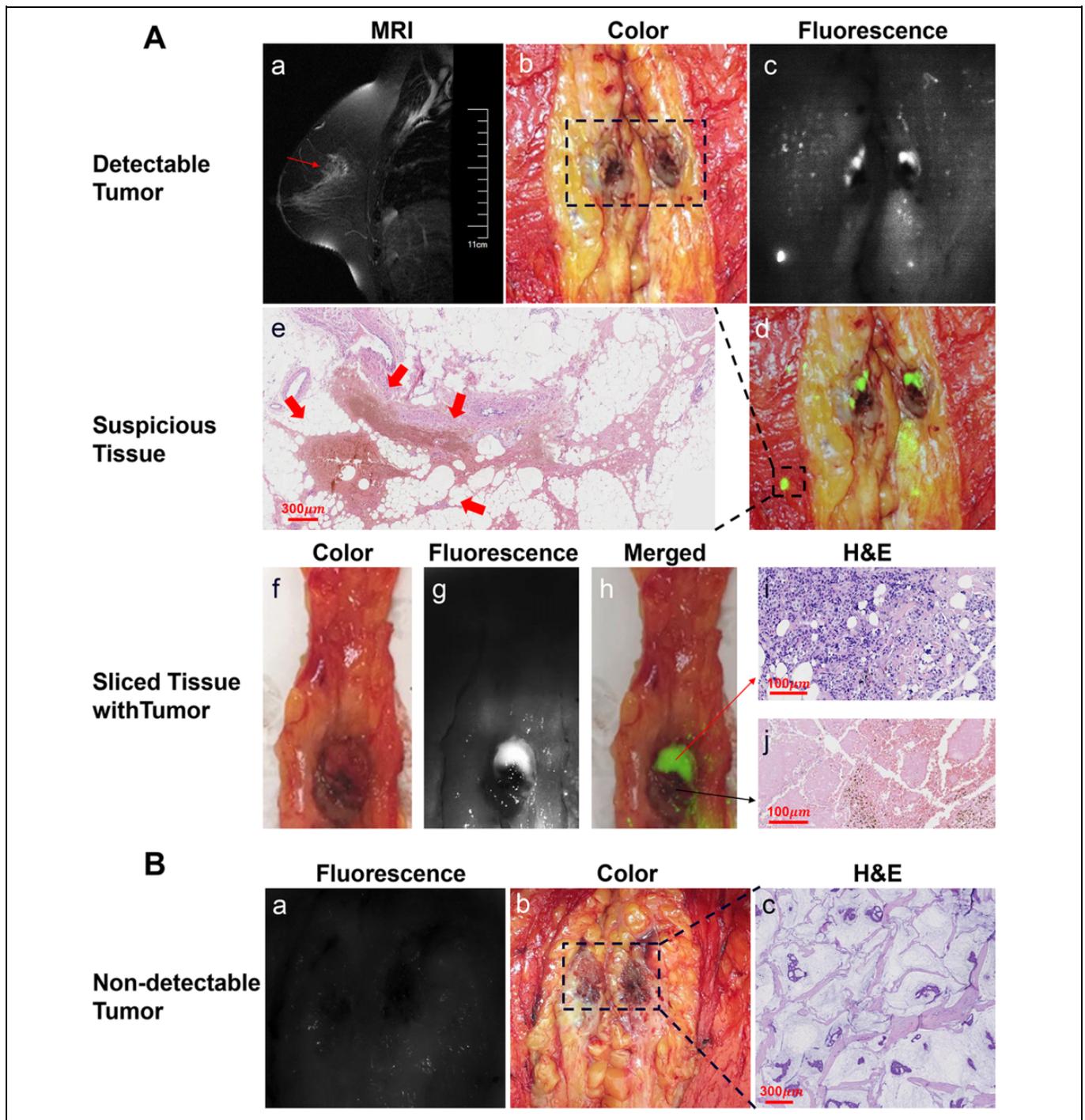
In our study, 19 of 30 patients exhibited fluorescent contrast on their resected tissues, which were lower than Tummers *et al* study. However, we found that preoperative chemotherapy affected the performance of MB-FI. The fluorescence detection rate was 80.0% and 30.0% for patients without and with chemotherapy ( $P < .05$ ), respectively (Table 1).

Preoperative chemotherapy, also termed as neoadjuvant chemotherapy, is defined as the administration of systemic chemotherapeutic agent prior to downstage tumors and local control of surgery.<sup>27</sup> During this treatment, circulation factors, which caused stimulation of similar tumor growth, were restricted and resulted in proliferation of breast cancer cells substantial reduction.<sup>28</sup> We hypothesize that the preoperative chemotherapy was likely to interfere with the accumulation of MB in tumors by restricting circulation factors, which remarkably reduced the efficacy of MB-FI.

For the 20 patients without chemotherapy, 4 resected tissues did not show sufficient fluorescence contrast. Three of them were confirmed to be IDC, and the other was MUC. We found that the majority (9 of 12) of patients with IDC showed fluorescent signals, but patients with MUC behaved very differently. Besides this nondetectable MUC, we found another patient carried mixed breast carcinoma (Supplementary Figure S1), including MUC, IDC, and ILC. Interestingly, IDC and ILC areas lighted up, whereas the MUC area was dark (Supplementary Figure S1B). Same phenomena of the hardly detectable tumor with MUC histological type were also reported by the previous studies.<sup>25,29</sup>

The MB-FI system captured 5 extra bright spots outside tumor areas from the 20 nonchemotherapy patients. However, pathological examinations confirmed that they were all benign tissues with hemorrhage. Therefore, we suspect that such fluorescent bright spots were caused by leakage of MB from blood vessels and retention in the tissue. After statistics of overall imaging results, a sensitivity of 0.63 and a positive predictive value of 0.79 were obtained from this study (Table 2). When we excluded the chemotherapy interferences, 16 tumors from 20 patients were detectable with a mean SBR of  $1.94 \pm 0.71$ ; thus, the tumor detectable rate of nonpreoperative chemotherapy group is 0.8. These results showed the feasibility of translating MB-FI for breast tumor visualization. With more clinical trials and injection dose studies of MB-FI performed, this technique would have a good potential of benefiting surgical outcomes. As to the mechanism of MB accumulation in breast tumors, it is still confused, which needs further studies to explore.

This preliminary study also revealed some limitations of the MB-FI strategy. First, compared to ICG, MB showed an even less penetration depth due to its shorter wavelength of excitation and emission. For tumors deeper than 1 cm from the skin, it is impossible for the imaging system to detect any fluorescent signal. Therefore, it is more suitable to apply MB-FI during the



**Figure 3.** Imaging results of patient 15 with invasive ductal carcinoma (IDC) and patient 30 with mucinous adenocarcinoma (MUC). A, (a) magnetic resonance (MR) image, tumor pointed by red arrow, tumor size: 2 cm; (b-d) Tumor bisected in specimen, tumors pointed by black frame in (b), signal-to-background ratio (SBR):  $2.68 \pm 0.60$ , suspicious tissue circled by black frame in (d); (e) hematoxylin and eosin (H&E)-stained image of suspicious tissue indicated by black frame in (d) and hemorrhage area marked by large red arrows in (e). Scale bar: 300  $\mu\text{m}$ . (f-h) Sliced tissue with breast tumor and necrosis, grade III, estrogen receptor (ER)+, progesterone receptor (PR)+, and Her2/NEU+. (i) H&E-stained image of breast tumor in fluorescence signal detectable area (pointed by red arrow). Scale bar: 100  $\mu\text{m}$ ; (j) H&E-stained image of necrotic tissue (pointed by black arrow). Scale bar: 100  $\mu\text{m}$ . B, (a) Fluorescence image of MUC, tumor size: 2 cm; (b) Color image, tumors pointed by black frame; and (c) H&E-stained image. Scale bar: 300  $\mu\text{m}$ .

resection on incisal edges for identifying the residual tumor as well as applying it on resected tissues for selective specimen acquisition in rapid pathological examinations. Second, our

home-developed MB-FI system was not fully automated for intraoperative imaging and was not user-friendly enough for surgeons and pathologists, which still needs further improvements.

**Table 2.** Total Statistics of Sampled Tissues.<sup>a</sup>

Label	Nonpreoperative Chemotherapy, n = 25		Preoperative Chemotherapy, n = 10
	Malignant	Normal Tissue	Malignant
Breast tumors			
Signal detected	16	0	3
No signal detected	4	0	7
Other suspicious tissues			
Signal detected	0	5	0
Total (N = 35)			
Signal detected	19	5	
No signal detected	11	-	
Sensitivity	19/30	0.63	
Positive predictive value	19/24	0.79	

<sup>a</sup>N = 35.

## Conclusions

The MB-based NIR-FI strategy showed a good performance on the *ex vivo* tumor visualization from patients with breast cancer. It offered a sensitivity of 0.63 and a positive predictive value of 0.79. The effect of MB-FI was adversely influenced by preoperative chemotherapy in this study. More prospective researches are necessary to further explore its clinical values.

## Authors' Note

Chong Zhang, MSc, Daqing Jiang, MMed, and Bo Huang, MD, contributed equally to this work and should be considered as co-first authors.

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## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Supplemental Material

Supplemental material for this article is available online.

## References

- Harbeck N, Gnant M. Breast cancer. *Lancet*. 2017;389(1):1134-1150.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(1):7.
- Mclaughlin SA. Surgical management of the breast: breast conservation therapy and mastectomy. *Surg Clin North Am*. 2013;93(2):411-428.
- Tipirneni KE, Warram JM, Moore LS, et al. Oncologic procedures amenable to fluorescence-guided surgery. *Ann Surg*. 2016;266(1):36.
- Kryh CG, Pietersen CA, Rahr HB, Christensen RD, Wamberg P, Lautrup MD. Re-resection rates and risk characteristics following breast conserving surgery for breast cancer and carcinoma in situ: a single-centre study of 1575 consecutive cases. *Breast*. 2014;23(6):784-789.
- Adams BJ, Zoon CK, Stevenson C, Chitnavis P, Wolfe L, Bear HD. The role of margin status and reexcision in local recurrence following breast conservation surgery. *Ann Surg Oncol*. 2013;20(7):2250.
- Atkins J, Mushawah FA, Appleton CM, et al. Positive margins rates following breast-conserving surgery for stage I-III breast cancer: palpable versus non-palpable tumors. *J Surg Res*. 2012;177(1):109-115.
- Rizzo M, Iyengar R, Gabram SGA, et al. The effects of additional tumor cavity sampling at the time of breast-conserving surgery on final margin status, volume of resection, and pathologist workload. *Ann Surg Oncol*. 2010;17(1):228.
- Gray RJ, Pockaj BA, Garvey E, Blair S. Intraoperative margin management in breast-conserving surgery: a systematic review of the literature. *Ann Surg Oncol*. 2017;25(1):1-10.
- Cendán JC, Coco D. Accuracy of intraoperative frozen-section analysis of breast cancer lumpectomy-bed margins. *J Am Coll Surg*. 2005;201(2):194-198.
- Parrishnovak J, Holland EC, Olson JM. Image guided tumor resection. *Can J*. 2015;21(3):206.
- Vahrmeijer AL, Hutteman M, Jr, Frangioni JV. Image-guided cancer surgery using near-infrared fluorescence. *Nat Rev Clin Oncol*. 2013;10(9):507.
- Schaafsma BE, Mieog JS, Hutteman M, et al. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *J Surg Oncol*. 2011;104(3):323.
- He K, Chi C, Kou D, et al. Comparison between the indocyanine green fluorescence and blue dye methods for sentinel lymph node biopsy using novel fluorescence image-guided resection equipment in different types of hospitals. *Transl Res*. 2016;178:74-80.
- Chi C, Du Y, Ye J, et al. Intraoperative imaging-guided cancer surgery: from current fluorescence molecular imaging methods to

- future multi-modality imaging technology. *Theranostics*. 2014;4(11):1072-1084.
16. Keereweer S, Van Driel PB, Snoeks TJ, et al. Optical image-guided cancer surgery: challenges and limitations. *Clin Cancer Res*. 2013;19(14):3745-3754.
  17. Hong G, Antaris AL, Dai H. Near-infrared fluorophores for biomedical imaging. 2017;1(1):0010.
  18. Murawa D, Hirche C, Dresel S, Hünerbein M. Sentinel lymph node biopsy in breast cancer guided by indocyanine green fluorescence. *Br J Surg*. 2009;96(11):1289-1294.
  19. Kitai T, Inomoto T, Miwa M, Shikayama TJBC. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. *Breast Cancer*. 2005;12(3):211-215.
  20. Troyan SL, Kianzad V, Gibbs-Strauss SL, et al. The FLARE™ intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping. *Ann Surg Oncol*. 2009;16(10):2943-2952.
  21. Vorst JRVD, Vahrmeijer AL, Hutteman M, et al. Near-infrared fluorescence imaging of a solitary fibrous tumor of the pancreas using methylene blue. *Worl J Gastroint Surg*. 2012;4(7):180-184.
  22. van der Vorst JR, Schaafsma BE, Verbeek FP, et al. Intraoperative near-infrared fluorescence imaging of parathyroid adenomas with use of low-dose methylene blue. *Head Neck*. 2014;36(6):853-858.
  23. Tummers QR, Schepers A, Hamming JF, et al. Intraoperative guidance in parathyroid surgery using near-infrared fluorescence imaging and low-dose methylene blue. *Surgery*. 2015;158(5):1323-1330.
  24. Tummers QRJG, Boonstra MC, Frangioni JV, Velde CJHVD, Vahrmeijer AL, Bonsing BA. Intraoperative near-infrared fluorescence imaging of a paraganglioma using methylene blue: a case report. *Int J Surg Case Rep*. 2015;6:150-153.
  25. Tummers QR, Verbeek FP, Schaafsma BE, et al. Real-time intraoperative detection of breast cancer using near-infrared fluorescence imaging and methylene blue. *Eur J Surg Oncol*. 2014;40(7):850-858.
  26. Simmons R, Thevarajah S, Brennan MB, Christos P, Osborne M. Methylene blue dye as an alternative to isosulfan blue dye for sentinel lymph node localization. *Breast J*. 2015;7(3):181-183.
  27. Ithimakin S, Chuthapisith S. Neoadjuvant chemotherapy for breast cancer. In: *Neoadjuvant Chemotherapy-Increasing Relevance in Cancer Management*. InTech; 2013.
  28. Cleator S, Parton M, Dowsett MJERC. The biology of neoadjuvant chemotherapy for breast cancer. *Endocr Relat Can*. 2002;9(3):183-195.
  29. Liberale G, Vankerckhove S, Caldon MG, et al. Fluorescence imaging after indocyanine green injection for detection of peritoneal metastases in patients undergoing cytoreductive surgery for peritoneal carcinomatosis from colorectal cancer: a pilot study. *Ann Surg*. 2016;264(6):1110-1115.