Evaluation to Differentiate between Tumor Lesions and the Parenchyma in Partial Nephrectomies for Renal Tumors Based on Quantitative Fluorescence Imaging Using Indocyanine Green Dye

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Abstract

Introduction: Near-infrared fluorescence imaging with indocyanine green is a useful tool during partial nephrectomy. Because an accurate method for judging hasn't been established yet, the success rate may be slightly different and inconsistent. ***Materials and Methods:*** A total of 21 cases with suspected renal cancers who had undergone a partial nephrectomy were enrolled. We examined differences in the success rate between malignant lesions and the parenchyma by quantifying fluorescence in the pre-resection and *ex vivo* phases. ***Results:*** Pre-resection imaging showed a significant degradation of fluorescence in the focused lesion in 76.2% (16/21) of cases. A significant degradation was observed in 73.7% (14/19) of the total malignant lesions, 70.5% (12/17) of cases with a clear cell lesion, 100% (2/2) of cases with non-clear cell lesions, and 100% (2/2) of benign angiomyolipomas. In contrast, imaging of the *ex vivo* resected specimens showed a significant degradation in fluorescence of the focused lesions in 85.7% (18/21) of cases. A significantly degradation was observed in 84.2% (16/19) of the total malignant lesions, 82.3% (14/17) of cases with a clear cell lesion, 100% (2/2) of cases with non-clear cell lesions, and 100% (2/2) of benign angiomyolipomas. ***Conclusion:*** We firstly evaluated the efficacy of quantitative indocyanine green-based fluorescence as an objective method.

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Introduction

During partial nephrectomy for renal cancer, it is important to maintain a resected margin between the tumor lesion and normal lesion in order to achieve a curable surgical procedure. In general, the shape of the resection is established by pre-operative radiological imaging, peri-operative ultrasound imaging, and endoscopic or optical imaging with incandescent light. However, only a few times can achieve a positive surgical margin and therefore a navigation system is indispensable for determining the appropriate shape of the resection design. To date such a system has not been established. One system of navigation, near-infrared fluorescence imaging with indocyanine green (ICG), has been evaluated in other fields such as hepatectomy [1] and for detecting sentinel lymph nodes in prostatectomy [2]. For renal surgery, ICG navigation was shown to be the optimal tool for identify-

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Fig. 1. Appearance of the HyperEye Medical System. **a** Overview of the HyperEye Medical System (Mizuho co.); **b** System for near-infrared fluorescent imaging; **c** Camera head used for fluorescent imaging.

ing the ischemic area [3], while transarterial superselective intrarenal mass delivery of ICG-lipiodol mixture was effective for identification and anatomical dissection [4]. In other aspect, the loss of fluorescence in renal cancers caused by functional impairment of bilitranslocase was reported in 2008 by Golijanin et al. [5]. This ICG-based fluorescence navigation system showed differences in color between tumors and normal lesions and provided helpful information for confirming a negative surgical margin determined by visual judgment in open surgery [6] and robotic-assisted surgery [7, 8]. However, the definitive criteria for evaluating the efficacy of ICG-based fluorescence navigation remains uncertain, because the efficacy achieved was different in each study [9].

To determine the definitive criteria of efficacy we performed minimum incision endoscopic partial nephrectomies guided by an ICG-based fluorescence navigation system and compared the objective quantitation of fluorescence between tumor lesions and the parenchyma of the kidney using ImageJ software.

Materials and Methods

Cases with suspicious renal cancers who underwent a partial nephrectomy between July 2013 and December 2017 were recruited for this cohort study at Aichi Cancer Center Hospital. We enrolled 21 cases with suspicious renal cancers who underwent a partial nephrectomy, using minimal invasive open surgery that involved an endoscopy-guided minimal incision, as reported previously [10]. Before the operation, informed consent to use ICG-based fluorescence navigation was obtained from all the patients, except for those with an allergic history against iodide component, because this component may cause an allergic response against ICG [11]. The study was approved by our institutional review board.

ICG-Based Fluorescence Navigation
We prepared a 2.5mg/ml ICG solution (Diagnogreen® 25 mg, Daiichi-Sankyo Pharmaceutical, Tokyo, Japan). The HyperEye Medical System (Mizuho Co., Tokyo, Japan) was used as the near-infrared device (wavelength 800–850 nm) (fig. 1a). This system provided illumination with near-infrared light of 760–780 nm (fig. 1b). The camera unit of this system (fig. 1c) enables visualization of invisible near-infrared rays on a monitor. ICG produced fluorescence reflected by near-infrared light at a wavelength of 800–850 nm.
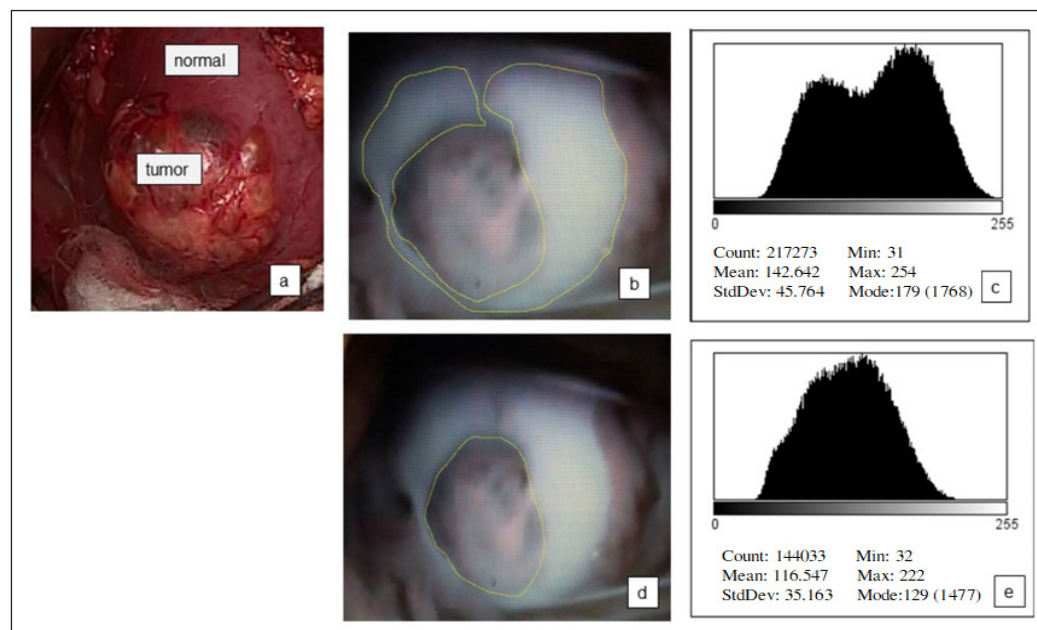
Fig. 2. Steps for quantifying fluorescence in the pre-operative image. **a** Appearance under the natural light before resection of the tumor; **b** Imaging under near-infrared fluorescence: trace of the normal area; **c** Histogram of the normal parenchyma; **d** Imaging under near-infrared fluorescence: trace of the tumor area; **e** Histogram of the tumor area.

Fig. 3. Steps for quantifying the fluorescence in the image of the resected specimens. **a** Appearance of the resected specimen under natural light; **b** Imaging under near-infrared fluorescence: trace of the normal area; **c** Histogram of the normal parenchyma; **d** Imaging under near-infrared fluorescence: trace of the tumor area; **e** Histogram of the tumor area.

Table 1. Baseline characteristics of the patients in the study

Patient demography	Values
Number of masses	21
Mean age, years (range)	63.7 ± 10.2 (39–74)
Gender, n (%)	
Male	16 (76.2%)
Female	5 (23.8%)
Tumor site, n (%)	
Right	9 (42.8%)
Left	12 (57.2%)
Mean maximum diameter, cm (range)	2.4 ± 0.9 (1.5–5.0)
Location, n (%)	
Endophytic	6 (28.6%)
Exophytic	13 (47.6%)
Mixed	2 (9.4%)
Surgical procedure	
Microwave	11 (52.4%)
Clamping	10 (47.6%)
Mean warm ischemic time, minutes (range)	
Microwave	24 (17–34)
Clamping	0
Benign total, n (%)	2 (9.5%)
Angiomyolipoma	2 (9.5%)
Malignancy total, n (%)	19 (90.5%)
Clear cell	17 (81.1%)
Papillary	1 (4.7%)
Chromophobe	1 (4.7%)

ICG-Based Fluorescence Navigation-Guided Partial Nephrectomy

When we suspected there was a high possibility of the resection margin extending into the renal pelvic area, the main renal artery was clamped. A 2 ml aliquot (5 mg) of ICG solution was injected intravenously before clamping in these cases. On the other hand, when the limitation of the extended margin of resection was estimated, a microwave system (Microtaze, Alfresa Co., Osaka, Japan) was prepared, without clamping of the main renal artery. To avoid bleeding in some cases of the microwave system, the ICG solution was injected before use of the microwave and resection.

White colored fluorescence imaging appeared in the parenchyma area a few seconds after the ICG injection. The entire fluorescence images were recorded to estimate the quantity of fluorescence for the next step. The fluorescence imaging of the resected specimen was recorded within 1 hour after resection.

Estimated Quantitation of Fluorescence

A freeze-frame picture of a video image after 1 minute was captured, followed by an ICG injection. A picture of the resected specimen was recorded after 1 hour followed by resection.

The images were used for quantitation in the next stage. For quantitation of fluorescence, we used an application tool, ImageJ [12]. A freeze-frame picture was captured by ImageJ and the tumor and normal areas were traced separately. The histogram of each traced area was produced, followed by quantification of the amount of fluorescence.

To explain the steps for quantifying fluorescence in detail, we have presented a significant case image of patient No. 2 (fig. 2, 3). Pre-operative imaging (fig. 2) and the *ex vivo* image (fig. 3)

were captured and the area of normal parenchyma (fig. 2b, 3b) and tumor lesion (fig. 2d, 3d) traced using the ImageJ program. A histogram of each area was prepared (fig. 2c, 2e, 3c, 3e), followed by statistical analysis to quantify the amount of fluorescence. Significant degradation of fluorescence in the tumor lesion was determined by comparison with a normal lesion ($p < 0.001$).

Statistical Analysis

The un-paired *t* test was used to compare the categorical variables. Differences with $p < 0.05$ were considered statistically significant. All statistical analyses were performed using SPSS version 15 software (SPSS, Chicago, IL, USA).

Results

Characteristic of the Patients in the Study

The characteristics of the 21 patients in the study are described in table 1. The distribution of the location of the lesions was 6 cases (28.6%) with an endophytic lesion, 13 cases (47.6%) with an exophytic lesion and 2 (9.4%) with a mixed lesion. In terms of surgical procedures, the microwave technique was used in 11 cases (52.4%) and the arterial clamping technique in 10 cases (47.6%).

The final pathological results revealed 2 cases (9.5%) of angiomyolipoma and 19 cases (90.5%) of malignant tumor. The absence of a positive surgical margin was accomplished in all cases with a partial nephrectomy. No case with an allergy against ICG was recorded.

Estimated Quantitation of Fluorescence

Table 2 provides a summary of quantitation of fluorescence, background of tumor shape, surgical technique, and pathology in all the patients. Pre-operative imaging demonstrated a significant degradation in fluorescence of the focused lesion in 16 (76.2%) cases, but not in the remaining 5 cases (23.8%).

A comparison based on the shape of the focused lesions showed significant degradation in 100% (6/6) of endophytic cases, but only 66.7% (10/15) of exophytic or mixed types. With regard to the surgical procedures, significant degradation was observed in 81.8% (8/11) of cases who received the microwave system, and 70% (7/10) who had the procedure with clamping.

Based on the pathological results, significant degradation was found in 73.7% (14/19) of cases with total malignant lesions, 70.5% (12/17) of cases with clear cell lesions, 100% (2/2) of cases with non-clear cell lesions, and 100% (2/2) with a benign angiomyolipoma.

In contrast, imaging of the *ex vivo* resected specimens demonstrated significant degradation of fluorescence of the focused lesion in 18 (85.7%) cases, but not in 3 cases

Table 2. Results of quantitation of fluorescence

Case	Shape	Surgical procedure	Pathology	Pre-respective condition			Resected specimen in *ex vivo*		
				Normal lesions	Tumor lesions	Value	Normal lesions	Tumor lesions	Value
1	exophytic	microwave	clear	162.7 ± 25.4	129 ± 40.9	significant	154.1 ± 11.4	94.4 ± 25.4	significant
2	exophytic	microwave	clear	142.6 ± 45.6	116.5 ± 35.1	significant	214.1 ± 32.5	133.6 ± 20.8	significant
3	exophytic	microwave	clear	175.3 ± 31.6	193.1 ± 29.1	not significant	115.0 ± 16.4	118.3 ± 9.5	not significant
4	exophytic	clamping	clear	212.0 ± 21.5	192.0 ± 23.7	not significant	32.9 ± 6.9	7.5 ± 28.0	not significant
5	endophytic	microwave	clear	166.6 ± 36.2	121.1 ± 35.1	significant	132.8 ± 14.2	65.1 ± 19.3	significant
6	endophytic	clamping	clear	202 ± 25.1	148.5 ± 12.2	significant	150.0 ± 13.3	90.4 ± 11.8	significant
7	exophytic	microwave	clear	97.6 ± 8.9	90.5 ± 15.4	not significant	168.7 ± 21.1	97.7 ± 18.6	not significant
8	exophytic	clamping	clear	160.5 ± 13.6	79.2 ± 7.1	significant	171.0 ± 17.2	195.0 ± 5.2	significant
9	endophytic	clamping	AML	124.7 ± 20.5	95.2 ± 11.0	significant	91.3 ± 17.0	48.7 ± 8.1	significant
10	endophytic	clamping	AML	82.2 ± 9.0	50.7 ± 8.0	significant	202 ± 12.9	136.8 ± 9.6	significant
11	endophytic	clamping	clear	216.0 ± 24.1	143.0 ± 18.7	significant	186.8 ± 10.9	139.6 ± 20.7	significant
12	exophytic	microwave	clear	207.7 ± 15.9	172.0 ± 30.5	significant	111.5 ± 12.5	55.7 ± 18.3	significant
13	exophytic	microwave	clear	143.6 ± 29.3	103.0 ± 40.2	significant	171.9 ± 18.8	144.6 ± 13.0	significant
14	exophytic	microwave	papillary	133.1 ± 20.7	113.0 ± 8.2	significant	219.8 ± 11.1	169.4 ± 12.6	significant
15	exophytic	microwave	clear	210.5 ± 22.8	161.4 ± 9.0	significant	143.7 ± 19.0	94.6 ± 24.6	significant
16	exophytic	clamping	chromophobe	153.7 ± 31.5	55.3 ± 17.0	significant	139.0 ± 22.7	87.8 ± 17.6	significant
17	endophytic	clamping	clear	205.0 ± 34.8	124.2 ± 19.2	significant	120.4 ± 27.3	69.4 ± 13.8	significant
18	exophytic	microwave	clear	177.4 ± 30.7	114.2 ± 38.9	significant	236.4 ± 22.2	127.9 ± 34.1	significant
19	exophytic	clamping	clear	32.3 ± 11.0	39.7 ± 13.2	not significant	68.8 ± 21.9	40.5 ± 16.3	not significant
20	mixed	clamping	clear	191.3 ± 17.4	198.5 ± 23.2	not significant	109.6 ± 10.1	138.0 ± 13.3	not significant
21	mixed	microwave	clear	115.7 ± 20.3	33.9 ± 22.3	significant	118.6 ± 18.6	31.4 ± 33.0	significant

Table 3. Summary of the peri-operative success rate of the target lesions with hypofluorecence compared with the parenchyma, based on the pathological results

Author	Journal (published year)	% of malignant in total cases	Total	Clear cell carcinoma	Malignant pathological results				Benign pathological results			
					Chromophobe	Papillary	Cystic clear cell	Others	Oncocytoma	Angiomyolipoma	Cyst	Others
Tobis et al. [16]	Urology (2012)	82.3% (14/17)	94.1% (16/17)	100% (8/8)	100% (1/1)	100% (4/4)	100% (1/1)		100% (2/2)		0% (0/1)	
Tobis et al. [15]	J Endurol (2012)	68.5% (13/19)	68.4% (13/19)	100% (10/10)	100% (3/3)				0% (0/1)	0% (0/2)	0% (0/3)	
Angell et al. [8]	J Urol (2013)	78.9% (60/76)	85.5% (65/76)	83.3 (55/60)					54.4% (6/11)	66.6% (2/3)	100% (2/2)	
Manny et al. [13]	J Endurol (2013)	77.0% (77/100)	75% (75/100)	95.8% (46/48)	100% (6/6)	100% (13/13)	0% (0/7)	0% (0/3)	100% (8/8)	0% (0/8)	0% (0/5)	0% (0/2)
Our study	2018	90.5% (19/21)	76.1% (16/21)	70.5% (12/17)	100% (1/1))	100% (1/1)				100% (2/2)		

(14.3%). Comparison based on the shape of the focused lesions showed significant degradation in 100% (6/6) of endophytic cases, but only in 80% (12/15) of exophytic or mixed types. With regard to surgical procedures, significant degradation occurred in 90.9% (10/11) of cases with microwave surgery and 80% (8/10) of those who had clamping of the main renal artery. Comparison of pre-operative imaging and the resected specimens showed no difference in the degree of degradation in fluorescence.

The pathological results showed significant degradation in 84.2% (16/19) of cases with a total malignant lesion, 82.3% (14/17) of cases with a clear cell lesion, 100% (2/2) of cases with a non-clear cell lesion, and 100% (2/2) with a benign angiomyolipoma.

Discussion

The success rate for differentiating tumors from the parenchyma was reported to range between 73 and 88% [9]. Because an accurate method for this differentiation has not been established, the success rate for each case

is slightly different and inconsistent. On the basis of this background, we firstly evaluated objective quantitation of fluorescence using ImageJ. Pre-operative imaging showed significant degradation in fluorescence of tumor lesions in 16 of 21 cases (76.2%), while imaging of the resected specimens demonstrated significant degradation of fluorescence in tumor lesions in 18 of 21 cases (85.7%). This result was similar to the lower rate reported in previous studies, while the rate in resected specimen was approximately equal to the higher rate seen in earlier reports [9]. We therefore attempted to determine the reason for the instability observed with ICG-based fluorescence navigation.

The first reason for this discrepancy was uncertainties regarding the method for evaluating the efficacy of ICG-based fluorescence navigation. In all previous reports, efficacy was influenced by subjective judgment, and also the categories of the lesion were either Grade 2 [13] or Grade 3 [6, 14, 15]. Because objective evaluation may be the ideal method, in the future it is necessary to restrict the method of estimation to objective quantitation and evaluate using the same methods for determining efficacy.

The second reason for the discrepancy was related to the dose of ICG. It is possible that an overdose of ICG may cause all tissue to fluoresce. However, to date a standardized dosing regimen has not been defined [8]. According to our results, using the same dose of ICG (5 mg), the degree of fluorescence in both pre-operative imaging and the resected specimen was quite different in each case. In the future, because the dose of ICG is a key factor for quantifying fluorescence, an optimal dosing regimen of ICG is needed to determine the difference between normal and tumor lesions.

The third reason for the discrepancy depended on differences in the pathological results. In general, the mechanism contributing to differences in fluorescence of ICG remains uncertain. One hypothesis is that the difference in fluorescence is due to variations in the transport of ICG into proximal tubule cells of normal renal parenchyma and poor retention of ICG in cancerous cells that contribute to hypofluorecence of cancer components [5]. A second hypothesis is that differences in the activity of the carrier protein, bilitranslocase in normal lesions may be a key factor for altering fluorescence [5]. Therefore, we suspect that compared with normal parenchyma, the differences may be attributable to alterations in the cell structure of focused lesions that are dependent on the pathological structure. To investigate the validity of that hypothesis, we compared the peri-operative rate of hypo-fluorecence in target lesions with that in the parenchyma based on our pathological results, and those reported that in malignant non-clear cells with solid chromophobe and papillary components, all the cases had differences between focused lesions and the parenchyma, but inconsistent results in clear cells and benign lesions [13, 15, 16, 18] (table 3). Based on these findings, the pattern of ICG fluorescence was not useful for predicting malignant or benign lesions [13]. In addition, the rate of pathological distribution has been shown to be related to ethnic background [17]. As shown in table 3 (percentage of malignant 68.5–90.5%), our summary indicates the pathological distribution contributed to differences in the success rate of differentiating tumors from the parenchyma in all the cases. In the future, a molecular approach will be indispensable for determining the mechanism that results in different fluorescence under the same pathological conditions.

The fourth reason for the discrepancy was dependent on the effect induced by the position of the focused lesions. For the position of the tumor, differences between the tumor and normal parenchyma were easier to distinguish for more exophytic tumors than for endophytic tumors. And once the resection had commenced, the hypofluorescent nature of tumor will be appreciated better [15]. We evaluated the efficacy of ICG-based fluorescence navigation depending on the shape of the focused lesion. Based on the shape of the focused lesion, significant degradation was observed in all endophytic cases, but only in 66.7% (10/15) of exophytic or mixed types. Therefore the endophytic location may not be a disadvantage for determining efficacy, whereas the fluorescence of focused lesions may affect the efficacy of ICG-based fluorescence navigation.

What is the beneficial outcome of ICG-based fluorescence navigation? Because outcome may be influenced by two crucial points, the phase of the resection and evaluation of the *ex vivo* surgical margin, it is necessary to separately evaluate the efficacy of ICG-based fluorescence navigation during each phase. According to a previous report, ICG-based fluorescence navigation contributes to a reduction in warm ischemia time in the resection phase, but does not improve negative surgical margin or decreased complication in the *ex vivo* surgical margin [14].

Regarding evaluation during resection, easier handing of the camera in ICG-based fluorescence navigation should be indispensable, especially when switching from white light to the fluorescence image. The main advantage of the advanced system is that surgeons can switch

from a standard white light to fluorescence imaging in the surgical console [9]. In contrast, the HyperEye Medical System is inappropriate for monitoring during resections due to less flexibility of the camera head and design of the structure itself. Therefore, improvements in the handling of the camera head will be needed in the future.

In general, evaluation of *ex vivo* lesions showed a better rate of diffraction (100%, 16/16) than that measured in pre-resected tumors (62.5%, 10/16) [6]. However, it is necessary to discuss the effects in normal parenchyma induced by microwaves, because no study has evaluated the effectiveness of ICG fluorescence in partial nephrectomy using microwaves, while the effects of microwaves on ICG fluorescence remain uncertain.

In general, microwaves cause coagulation in both sides of the parenchymal structure at the margins of the resection. Our results show that because of the alternation in color of both surfaces of the resected specimen due to coagulation, the stability of the homogeneous color of the resected specimen evaluated by optical judgment was insufficient to evaluate the remaining surgical margin. Accordingly, another method that optimizes evaluation of the resection margins needs to be developed.

In our study, we firstly evaluated whether the damage of normal parenchyma caused by microwaves was ignored or not. Our results showed the rate of significant degradation was 81.8% (8/11) and 90.9% (10/11) in cases before and after the use of microwaves, respectively. This finding indicates that the damage induced by microwaves in the normal parenchyma may be ignored, thus, ICG fluorescence will therefore be a useful tool for estimating the surgical margin in cases using the microwave system.

However, because the basic rate of a positive surgical margin during partial nephrectomy is extremely low [9], it is difficult to show an advantage by focusing on the degradation of a positive surgical margin.

In terms of management of safe use of ICG, the compound has the potential to induce an allergic reaction, because there is evidence that systemic reactions to ICG may be attributable to iodide components [11]. In fact, anaphylactic shock in response to intravenous ICG during a robotic partial nephrectomy has been reported [18]. In our study, a case with a history of an allergic reaction against iodide components was withdrawn from the study and therefore fortunately no severe adverse events have occurred. Therefore, it is important for future studies to pay attention to allergic events against ICG.

The main limitation of our study was its retrospective nature and the relatively small number of cases.

Unfortunately, the system for evaluating quantitative fluorescence imaging by ImageJ could not be provided as real time information, but only post-operative information. As a consequence, real time assessment of margin status could not be established using this version.

However, we were able to evaluate the efficacy of ICG-based fluorescence navigation using an objective quantitative method. We started the project using the same objective method to evaluate the robotic-assisted procedure, including the customized optimal dose of ICG for navigation. For the future, if the quantitation system using ImageJ is established as a real time assessment, this would provide surgeons with helpful information to estimate the status of the margin from 2 aspects. Firstly, in the pre-operative image, the design of the resection could be decided by quantitative changes in the area of fluorescence. Secondly, for post resection images, if stable quantitation of fluorescence in both surfaces of the resected specimens and parenchymal side is estimated, this will allow surgeons to confirm whether complete resection had been achieved.

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