

Unexpected Detection of a Sub-millimeter Early Hepatocellular Carcinoma Focus by Intraoperative Near-infrared Fluorescence Imaging

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Case report

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Abstract

Background: Intraoperative near-infrared fluorescence (NIRF) imaging became a great assistance to surgeons for precision cancer surgeries. Here, we demonstrate that NIRF was capable of detecting ultra-small hepatocellular carcinoma focus of about 430 μ m during real time monitored liver cancer surgery. Prognosis of hepatocellular carcinoma is closely related to residual tumor cells and tissues after tumor resection. Thus, close monitoring to ensure a complete removal of residual tumor is primordial. However, up to now, the identification of tiny lesions has not been reported. Herein, we report our findings about a case where tiny lesions were successfully identified by real-time ICG-NIRF imaging.

Case presentation: A 55-year-old man, with chronic hepatitis B infection, was preoperatively diagnosed with liver space-occupying lesion. A fluorescence signal was detected on the surface of the liver through the NIRF imaging system. We then tested the residual liver surface and observed a high signal point, less than 1 mm in the right anterior lobe of the liver. Histopathological examination revealed that the tiny fluorescent spot belongs to an early hepatocellular carcinoma focus.

Conclusion: Results strongly suggest that ICG-NIRF imaging system should be used as a routine intraoperative detection method for liver cancer surgery, in order to remove any residual tumor cells and tissue, hence minimizing further risk of remnant tumor regrowth.

1. Background And Introduction

Subsequently to the establishment of sentinel lymph nodes (SLNs) by Kitai using indocyanine green (ICG) (1), intraoperative near-infrared fluorescence (NIRF) imaging technology, which has boosted our comprehension in several domains of surgical oncology, has emerged as the research focus for precision cancer surgeries in the last decade. Numerous reported clinical cases, including detection of non-small cell lung cancers (2), thymic malignancies, neuroendocrine lung malignancies, pleural mesothelioma, thoracic metastases (3) and oral squamous cell carcinoma (4), have demonstrated that NIRF is capable of providing assistance to surgeons in three major ways: 1. Primary tumor Localization; 2. Identification of the positive surgical margin; and 3. Detection of the remote satellite cancerous lesions.

The residual ultra-small tumors have a major impact in determining patients' post-operative prognosis and survival. Once the dysplastic lesion is larger than 2 cm, the chance of cure decreases significantly (5). Sub-centimeter tumors have been previously identified on an ovarian cancer animal xenograft model (6) and in Phase I clinical trials using second window indocyanine green in thoracic cancer (7).

However, to the best of our knowledge, herein we are reporting for the first time, regardless of using electromagnetic, ultrasonic or fluorescence techniques, a case where an early hepatocellular carcinoma focus of about 430 μ m was visualized during real-time image-guided surgery. This sub-millimeter lesion was detected on the liver of a 55-year-old man diagnosed with liver space-occupying neoplasms. The case is a part of a clinical study weighing the role of the NIR imaging in guiding small lesions detection

during surgery. Up to now, the study involved 13 patients with an age range of 45 to 70yrs, who were diagnosed with an onset of hepatoblastoma.

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death in the world (8). For patients with early-stage liver cancer, surgery is still the main treatment method. However, the recurrence of liver cancer remains a major concern after resection. The 5-year recurrence rate of liver cancer reached more than 70% (9) and caused poor prognosis. Even patients with small HCC (< 3 cm) undergoing surgery have a 5-year survival rate of only 47–53% (10–12). Lack of effective intraoperative diagnosis is one of the key factors leading to residual tumor cells and postoperative recurrence. The near-infrared fluorescence (NIRF) imaging system has been widely used for the removal of sentinel lymph nodes during breast cancer surgery (13) and is currently used in the localization of tumors during liver cancer surgery (14, 15). However, to the best of our knowledge, the role of the NIRF imaging system in detecting tiny lesions in normal liver tissue has not been reported elsewhere.

In this study, after using the NIRF imaging system to locate the primary tumor, we further examined the surrounding normal liver tissue and surprisingly identified a tiny bright spot with a diameter of about 1 mm, which was excised with the guidance of the NIRF imaging system. Postoperative pathology confirmed that the tiny spot corresponds to an early hepatocellular carcinoma focus with a diameter of about 430 μm . This finding confirmed the complementary role of NIRF imaging during liver cancer resection. Furthermore, it can be used to perform intraoperative observation of the entire liver, identify tiny lesions and finally reduce the postoperative recurrence rate.

2. Case Presentation

The 55-year-old man, with chronic hepatitis B infection, was preoperatively diagnosed with liver space-occupying lesion. Table 1 lists the laboratory data before the operation. It can be seen that the concentration of the tumor marker alpha-fetoprotein concentration (AFP) 77.9 ng/ml was higher than normal limits. Liver function indexes were in normal range, whereas ICG R15 was 18.9% which was beyond 15%, indicating that liver metabolism was impaired. Therefore, our assessment was that this patient could only undergo hepatic lobectomy with the preoperative tumor location of CT (Fig. 1). We used an ICG-NIRF imaging system endowed with a qualitative detection limit of 0.488 nM, and a quantitative detection limit of 3.91 nM (Fig. 2) to observe the tumor and attempt to identify any other tiny tumors in the residual liver. Patient was first injected with ICG (0.5 mg/kg ICG) intravenously, however due to the patient impaired metabolism, the operation was scheduled four days after the ICG injection to obtain an optimal tumor to background ratio. The fluorescence signal that emitted from the liver surface could be detected through the NIRF system and could also be clearly recognized when the specimen was analyzed in-vitro (Fig. 3). We subsequently tested the residual liver surface and observed a high signal point of about 1 mm in the right anterior lobe of the liver which showed no differences from normal liver through naked-eye observation and palpation. After excision, the specimen sustainably expressed high fluorescence (Fig. 4). The operation time was 235 minutes and intraoperative bleeding was 200 ml. Histopathological examination revealed that this tiny fluorescent spot belongs to a single focus of an

early hepatocellular carcinoma.(Fig. 5). On the histopathological images, steatosis and mallory-denk bodies were identified. Meanwhile, multinucleation and nuclear atypia were also detected displaying nuclear overlapping, size enlargement and nuclear membrane distortion .

Table 1
list of the patient's laboratory tests results before surgery.

Laboratory tests	Results
ALT	42.1 U/L
AST	27.9 U/L
GGT	27.2 U/L
AKP	81.7 U/L
TBL	16.6 umol/L
DBL	6.2 umol/L
ALB	42.2 g/L
AFP	77.9 ng/ml
CEA	2.42 ng/ml
CA125	10.20 ng/ml
CA19-9	24.14 ng/ml
CA72-4	0.9 ng/ml
CA242	2.93 ng/ml
ICG _{R15}	18.9
ALT alanine aminotransferase, AST aspartate aminotransferase, GGT gamma-glutamyl transpeptidase, AKP alkaline phosphatase, TBL total bilirubin, DBL direct bilirubin, ALB albumin, AFP alpha-fetoprotein, CEA carcinoembryonic antigen, CA125 carbohydrate antigen 125, CA19-9 carbohydrate antigen 19 – 9, CA72-4 carbohydrate antigen 72 – 9, CA242 carbohydrate antigen 242, ICG _{R15} indocyanine green retention rate at 15 min	

3. Discussion And Conclusions

Complete resection of tumor depends on the precise location of the tumor and the identification of the small lesions by preoperative imaging. Subjective judgment of the surgeon used to be the only method during the operation to ensure the negative margin and the removal of small lesions. Unfortunately, about 40% patients have residual tumor cells during surgery (16). It has been shown that the imaging tests before surgery such as CT and MRI were not suitable for the detection of micrometastases and nodules (< 5 mm) (17); due to the infiltration of cancer tissues, lack of differentiation, nerve involvement and complex veins. The characteristics of the tube system and other characteristics are based on the

surgeon's inspection and palpation, leading to a significant tumor residual rate (exceeding 50%) (18). Through the high permeability and high retention (EPR) effect of tumor tissue, fluorescent dyes can accumulate in tumor tissue, NIRF imaging system could provide high tumor background signal ratio using large amounts of ICG, thereby helping surgeons to effectively localize tumors. However, despite recent advances in imaging modalities, about 3–17% of HCCs can only be detected by microscopic examination(19, 20). Since it has been reported that tiny lesions lack of effective blood vessels and EPR effect, the principle of fluorescence imaging of small lesions needs further investigation.

In this study, we surprisingly discovered that the NIRF imaging system can detect tiny early hepatocellular carcinoma focus, which may be related to the ultra-high sensitivity of the detection instrument, the dose of fluorescent dye injection and the injection time. Even though, the mechanism requires further research, this technology can effectively improve the tumor clearance rate and reduce tumor recurrence, thus should be implemented as a routine intraoperative detection method. The use of ICG in several NIR-guided oncological surgeries has shown promising results. Nevertheless, further comprehensive research and clinical experiments are required in order to understand ICG's overall effects.

Declarations

Ethics and consent:

The experiment performed was conform with the ethical standards of Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School and the Informed consent was signed by the participant patient.

Consent for publication:

consent for publication was signed by the patient.

Availability of data and materials:

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest:

The authors declare no conflict of interest.

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Authors' contributions:

Xingyu Wu conducted clinical guidance and specific operations. Yiqing Wang provided the design and guidance to the preparation of the manuscript. NIDA El Islem Guissi performed the pathological analysis and contributed to the manuscript writing. Bo Dai wrote the whole manuscript. Ziyang Wang made significant revisions to the manuscript. Qian Lu Given fluorescence result analysis. Hucheng Ma and Huiming Cai took part in the experiment. All authors have read and approved the final manuscript.

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References

1. Kitai T, Inomoto T, Miwa M, Shikayama T. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. *Breast Cancer*. 2005;12(3):211-5.
2. Newton AD, Kennedy GT, Predina JD, Low PS, Singhal S. Intraoperative molecular imaging to identify lung adenocarcinomas. *J Thorac Dis*. 2016;8(Suppl 9):S697-S704.
3. Newton AD, Predina JD, Corbett CJ, Frenzel-Sulyok LG, Xia L, Petersson EJ, et al. Optimization of Second Window Indocyanine Green for Intraoperative Near-Infrared Imaging of Thoracic Malignancy. *J Am Coll Surg*. 2019;228(2):188-97.
4. Pan J, Deng H, Hu S, Xia C, Chen Y, Wang J, et al. Real-time surveillance of surgical margins via ICG-based near-infrared fluorescence imaging in patients with OSCC. *World J Surg Oncol*. 2020;18(1):96.
5. Sherman M. Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. *Semin Liver Dis*. 2010;30(1):3-16.
6. Tanyi JL, Cory, L., DeJesus, E., Keating, J., Predina, J., Nie, S., Deshpande, C., Low, P., & Singhal, S. . Intraoperative molecular imaging can identify sub-centimeter peritoneal implants during ovarian cancer cytoreductive surgery. . *European Journal of Gynaecological Oncology*. 2017;38(4):589-95.
7. Newton AD, Predina JD, Nie S, Low PS, Singhal S. Intraoperative fluorescence imaging in thoracic surgery. *Journal of Surgical Oncology*. 2018;118(2):344-55.
8. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69-90.
9. Llovet JM, Schwartz M, Mazzaferro V. Resection and Liver Transplantation for Hepatocellular Carcinoma. *Seminars in Liver Disease*. 2005;25(02):181-200.
10. Fong ZV, Tanabe KK. The clinical management of hepatocellular carcinoma in the United States, Europe, and Asia: A comprehensive and evidence-based comparison and review. *Cancer*. 2014;120(18):2824-38.

11. Altekruse SF, McGlynn KA, Dickie LA, Kleiner DE. Hepatocellular Carcinoma Confirmation, Treatment, and Survival in Surveillance, Epidemiology, and End Results Registries, 1992-2008. *Hepatology*. 2012;55(2):476-82.
12. Liu C-Y, Chen K-F, Chen P-J. Treatment of Liver Cancer. *Cold Spring Harbor Perspectives in Medicine*. 2015;5(9).
13. Wishart GC, Loh SW, Jones L, Benson JR. A feasibility study (ICG-10) of indocyanine green (ICG) fluorescence mapping for sentinel lymph node detection in early breast cancer. *European Journal of Surgical Oncology (EJSO)*. 2012;38(8):651-6.
14. Rossi G, Tarasconi A, Baiocchi G, De' Angelis GL, Gaiani F, Di Mario F, et al. Fluorescence guided surgery in liver tumors: applications and advantages. *Acta Biomed*. 2018;89(9-S):135-40.
15. Ishizawa T, Saiura A, Kokudo N. Clinical application of indocyanine green-fluorescence imaging during hepatectomy. *Hepatobiliary Surg Nutr*. 2016;5(4):322-8.
16. Aliperti LA, Predina JD, Vachani A, Singhal S. Local and systemic recurrence is the Achilles heel of cancer surgery. *Ann Surg Oncol*. 2011;18(3):603-7.
17. van der Vorst JR, Hutteman M, Mieog JSD, de Rooij KE, Kaijzel EL, Löwik CWGM, et al. Near-Infrared Fluorescence Imaging of Liver Metastases in Rats using Indocyanine Green. *Journal of Surgical Research*. 2012;174(2):266-71.
18. Burke K, Brown E. The Use of Second Harmonic Generation to Image the Extracellular Matrix During Tumor Progression. *IntraVital*. 2015;3(3).
19. Zhou J, Yang F, Jiang G, Wang J. Applications of indocyanine green based near-infrared fluorescence imaging in thoracic surgery. *Journal of Thoracic Disease*. 2016;8(S9):S738-S43.
20. Ishizawa T, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, et al. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. *Cancer*. 2009;115(11):2491-504.

Figures

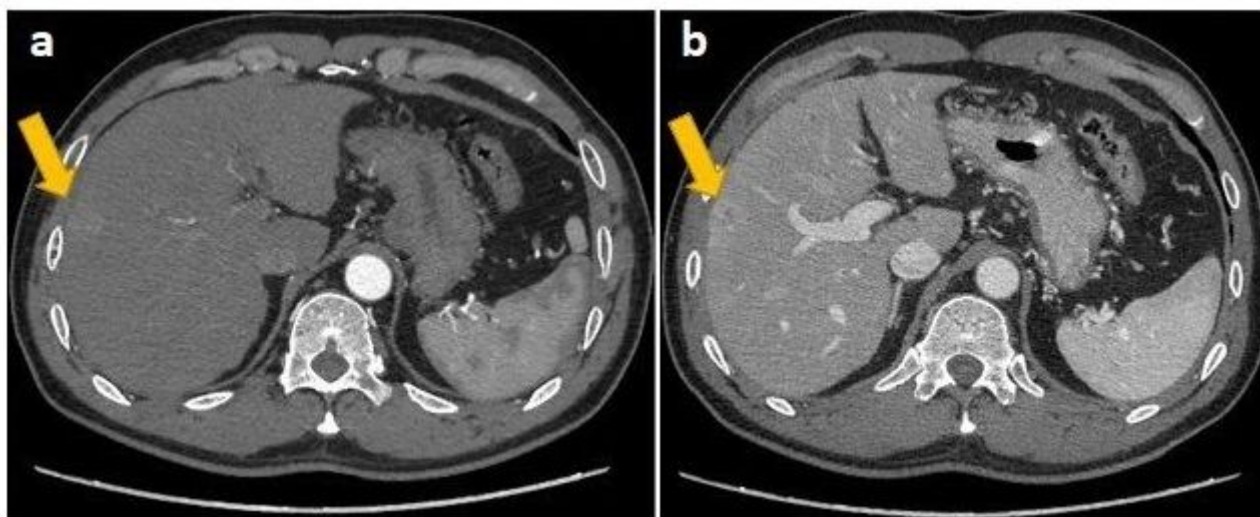


Figure 1

Enhanced computed tomography (CT) showed a subhepatic liver space-occupying lesion in the right lobe of the liver, and no obvious abnormalities were found. a. Early arterial phase. b Delayed phase.

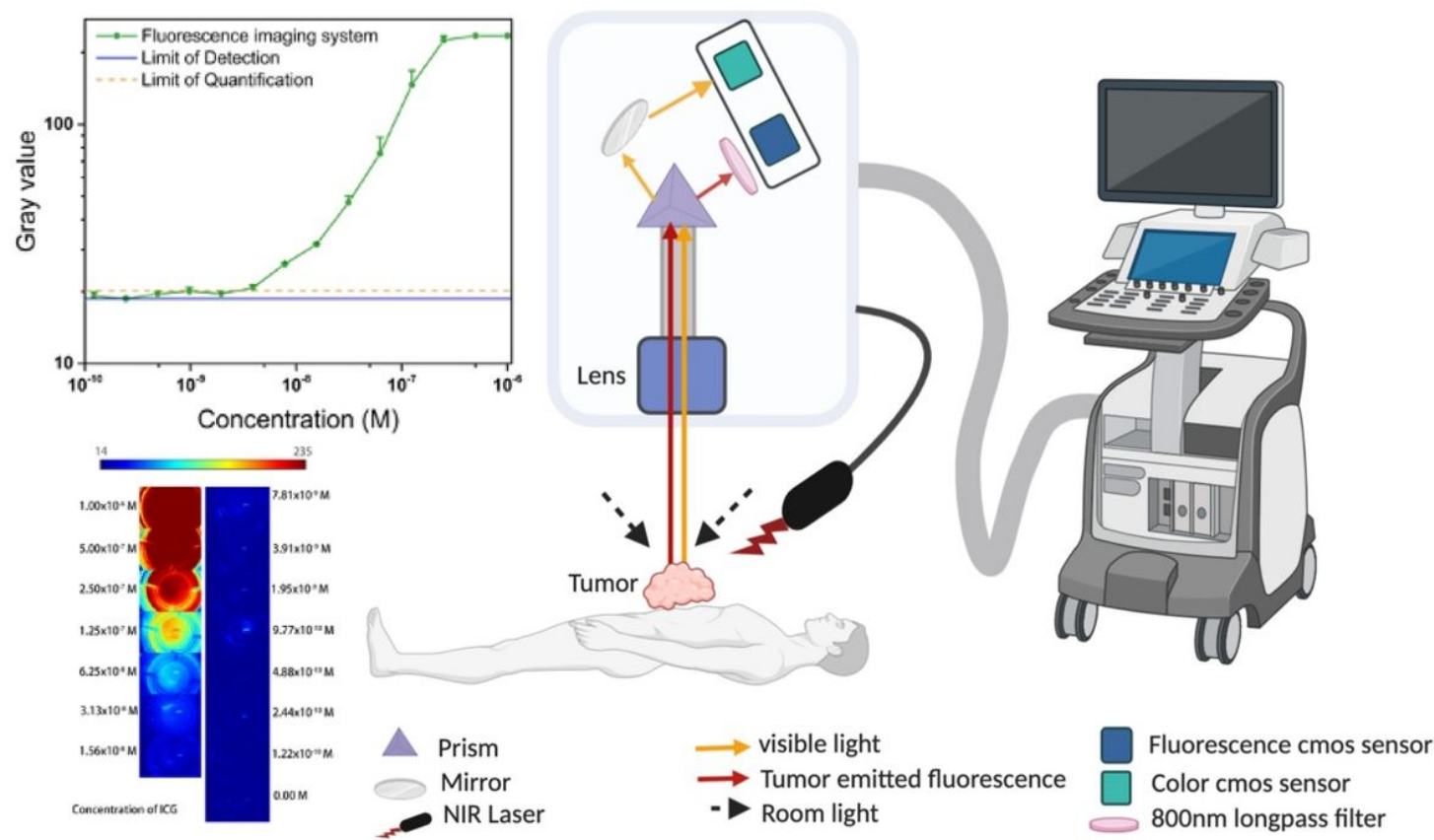


Figure 2

Diagrammatic sketch of NIR-real-time intraoperative guided liver resection using ICG. On the left are shown the results of the preoperative in vitro imaging of ICG solutions in 4% m/v BSA/PBS at different concentrations, the fluorescence signals of each well were quantified and the gray values were plotted against the concentrations, the qualitative detection limit was 0.488nM, and the quantitative detection limit was 3.91nM.

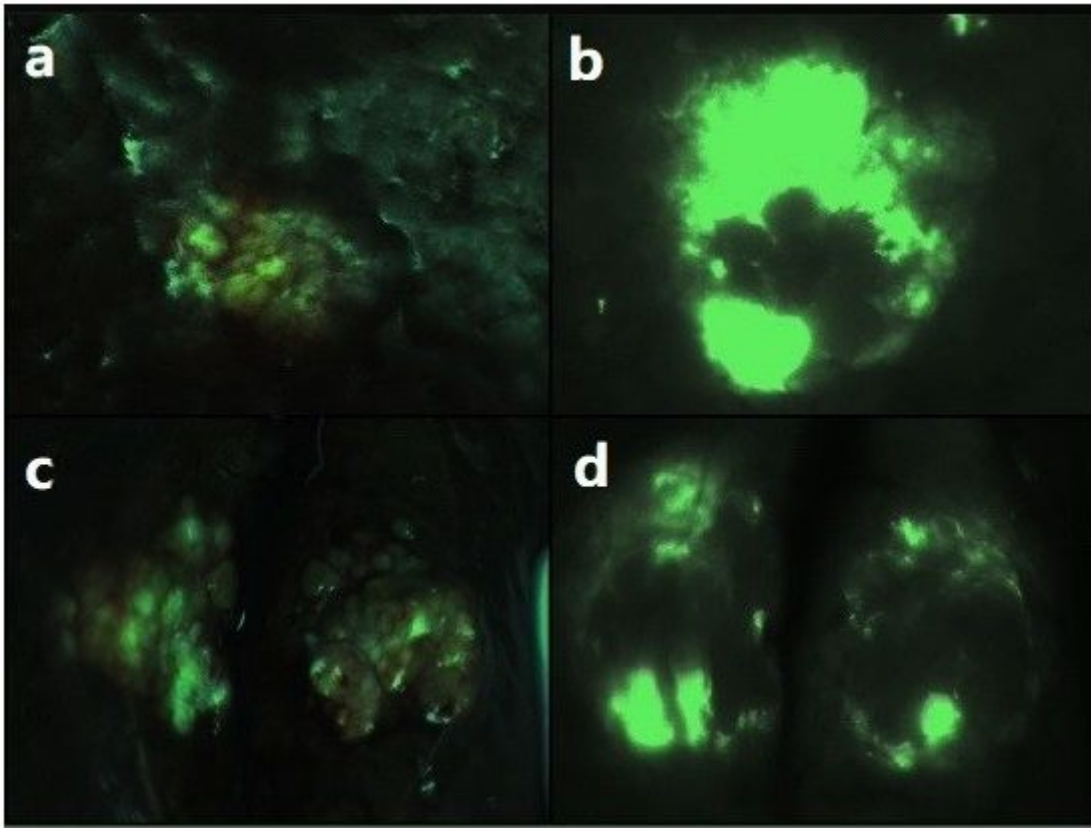


Figure 3

Findings using near-infrared fluorescence (NIRF) system. a and b. Recurrent lesions were located on the surface of the liver. c and d. In vitro imaging of dissected tumors.

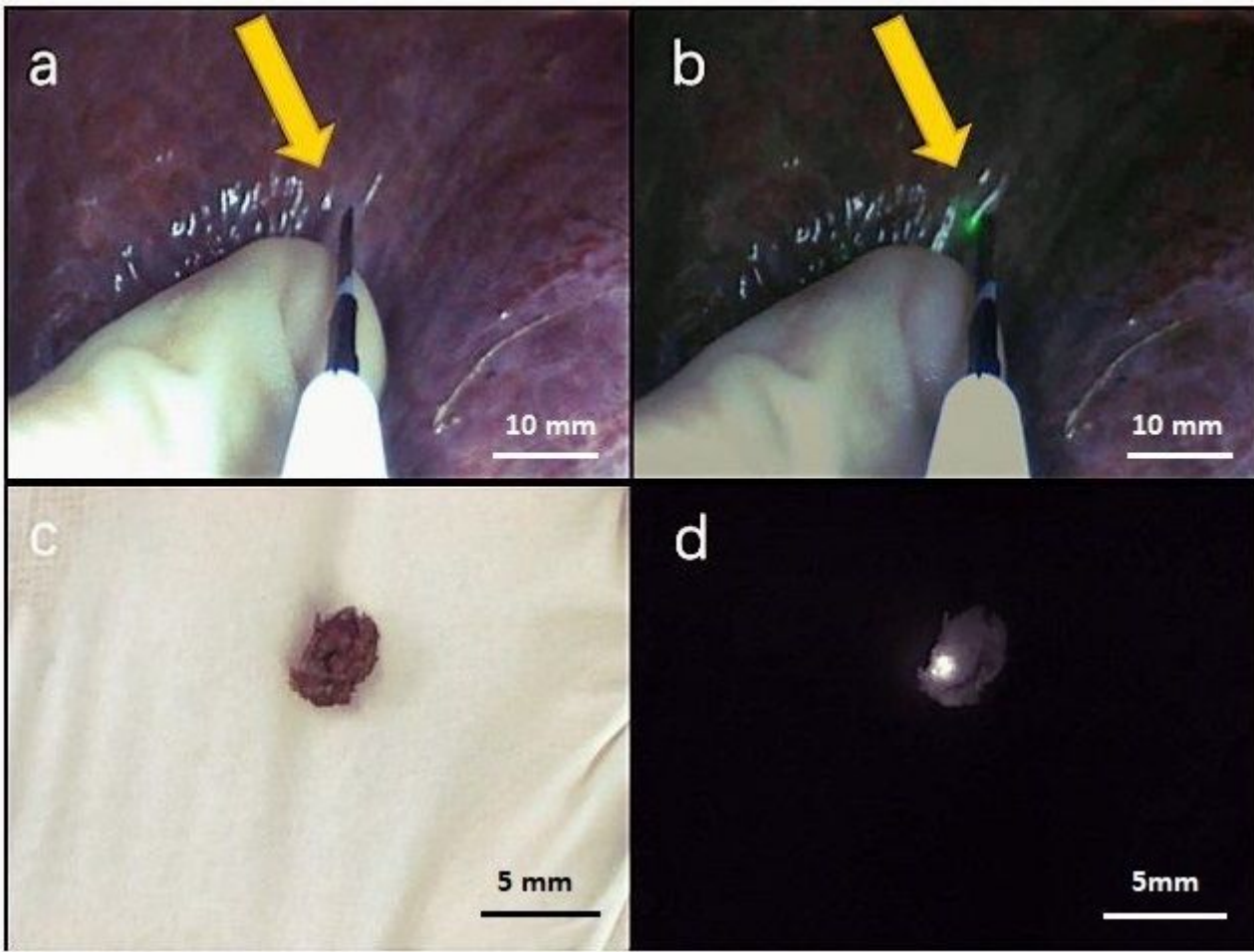


Figure 4

Detection of tiny lesions using near-infrared fluorescence (NIRF) system. a. Visual inspection of the lesion during the surgery b. System fluorescence detection of the lesion. c. Lesion resection d. In vitro fluorescence of the tiny lesion.

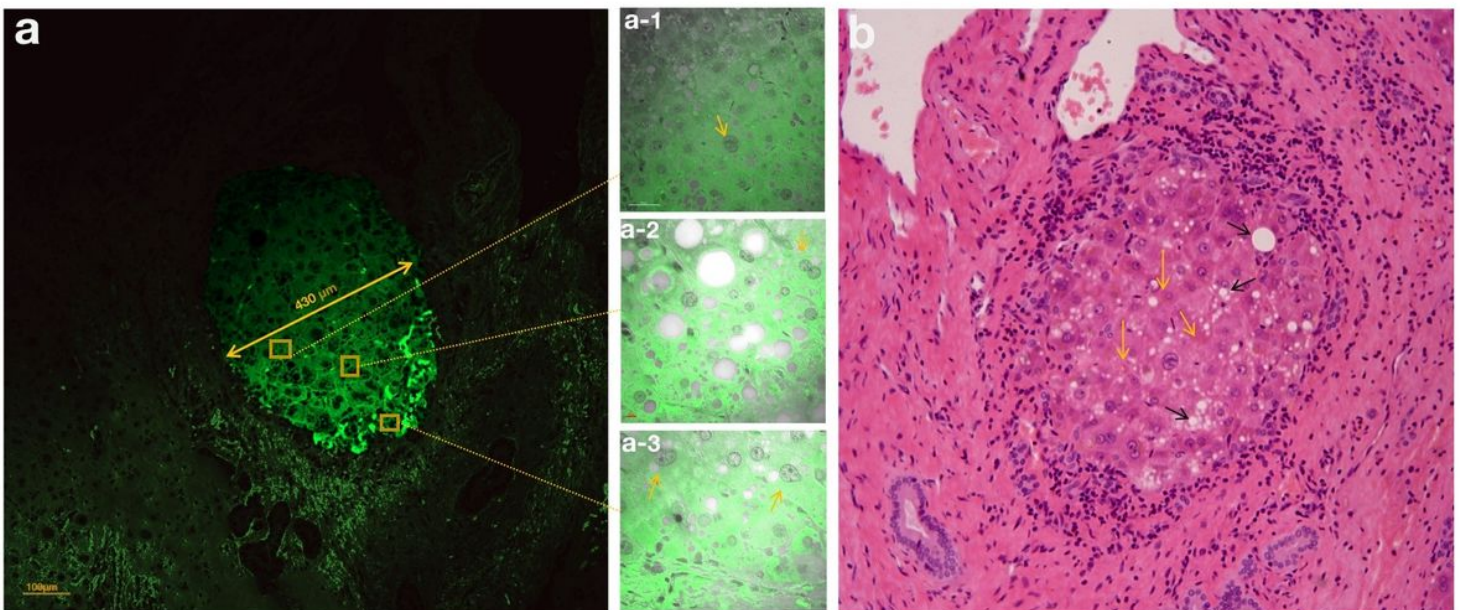


Figure 5

Pathological and fluorescence images of the resected specimen. a-confocal fluorescence image of the focus. a-1,a-2 and a-3 are confocal bright field and fluorescence overlay images, showing nuclear pleomorphism with overlapping, mitosis and nuclear size enlargement, and nuclear membrane distortion. b-H&E stain image of the focus showing steatosis (black arrows) and presence of mallory-denk bodies (yellow arrows), At high magnification, hyperchromasia and multinucleation were also detected.