Abbreviated Title: ICG for Hepatic Biopsies

Protocol #: 20CC0039

Version Date: 09/09/2025

NCT Number: Pending

Title: A Feasibility Study to Evaluate the Combination of Electromagnetic Tracking and Optical Imaging with Indocyanine Green (ICG) for Hepatic Biopsies

NIH Principal Investigator: Peter Pinto M.D.
Urologic Oncology Branch
National Cancer Institute
Building 10, Room 2-5952
10 Center Drive
Bethesda, MD 20892
Phone: 240-858-3700
E-mail: pp173u@nih.gov

Investigational Agents: N/A

Commercial Products:

	Autoclavable Camera Head (device)	UreteroRenoscope/ Ureteroscope (device)	Stryker® IRF Light Source and SafeLight Cable (device)	PercuNav and Uronav (device)	Indocyanine Green (ICG) (contrast)
Sponsor:	N/A				
Manufacturer:	Karl Storz Endoscopy Imaging, Inc	Karl Storz Endoscopy Imaging, Inc.	Stryker	Philips	Generic

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

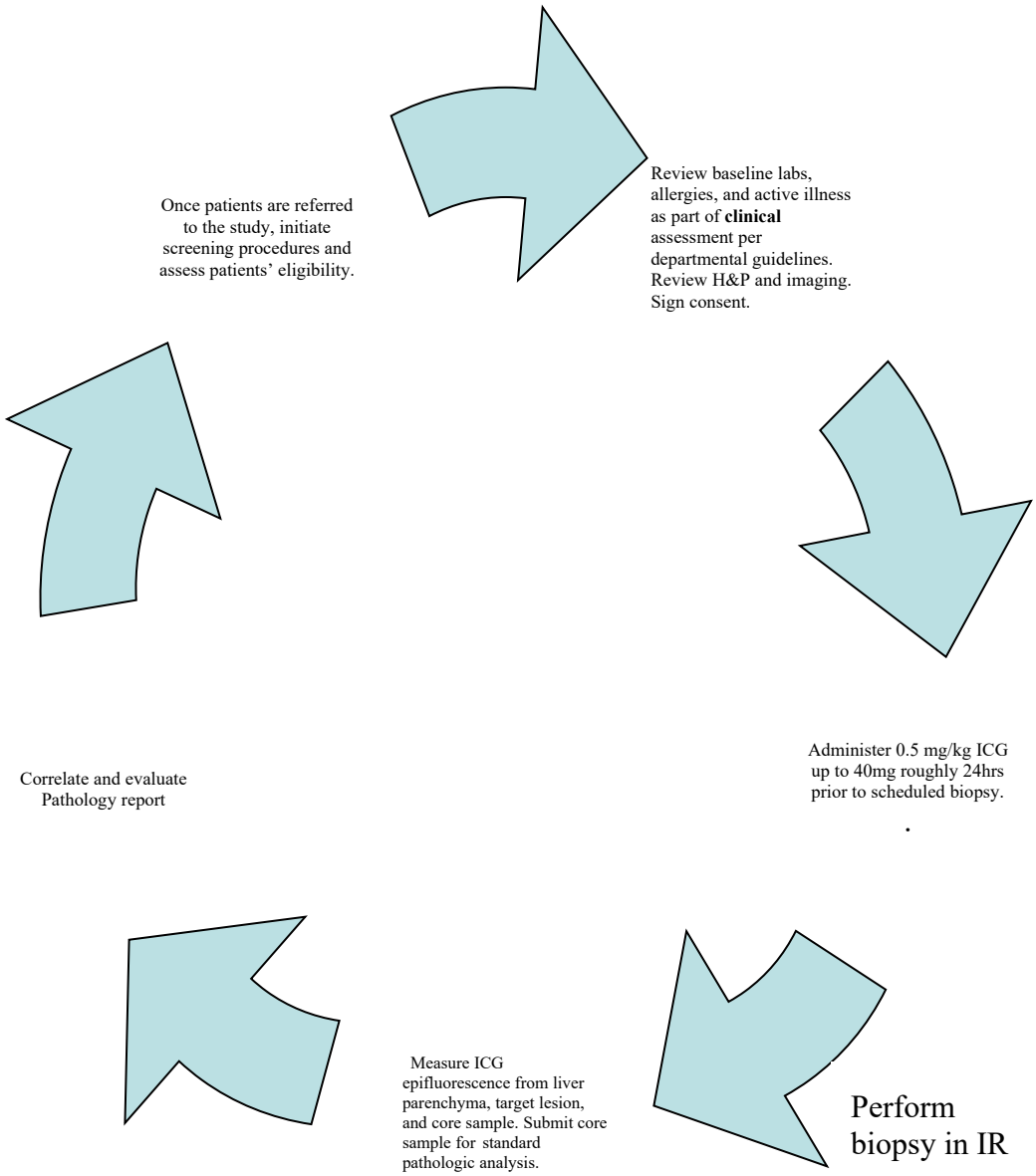
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A Feasibility Study to Evaluate the Combination of Electromagnetic Tracking and Optical Imaging with Indocyanine Green (ICG) for Hepatic Biopsies
Study Description:	This is a pilot study to evaluate the feasibility of the combination of optical molecular imaging (OMI) and electromagnetic (EM) tracking for use during biopsy of intrahepatic lesions to assess the concordance between fluorescence ICG and histopathology for the diagnosis of liver cancer.
Objectives:	Primary Objective: • To evaluate the feasibility of using both optical molecular imaging technique and electromagnetic tracking during standard liver biopsy. Secondary Objectives: 1. To assess concordance of ICG fluorescence signal at the in vivo site of biopsy with the presence or absence of malignancy, as defined by pathology 2. To assess, in biopsy tissue, the concordance of ICG florescence signal ex vivo (outside of the body with camera) with the presence or absence of malignancy as defined by pathology
Endpoints:	Primary Endpoint: • The determination of ICG fluorescent signal at the in-vivo site of biopsy using combination OMI and EM tracking will be based on Target-to-Background (TBR) such that TBR of ≥2 will produce a malignant diagnosis and TBR will produce a benign diagnosis. Secondary Endpoints: 1. To evaluate the agreement between histopathology and ICG fluorescent signal in-vivo biopsy site using combined OMI and EM tracking using histopathology results as the gold standard. 2. To evaluate the agreement between histopathology and ICG fluorescent signal of the specimen ex-vivo using a combination of OMI and EM tracking.
Study Population:	Patients ≥ 18 years of age whose imaging findings suggest liver cancer (hepatocellular carcinoma) or other metastatic liver malignancy for whom image-guided percutaneous biopsy is separately indicated or planned to be performed.
Phase:	Non-randomized, Open Label, prospective pilot study.
Description of Sites/Facilities Enrolling Participants:	Recruitment and investigational procedures will take place at the NIH Clinical Research Center in the Department of Radiology, Special Procedures.
Description of Study Intervention:	This is a non-randomized, prospective pilot study enrolling 77 patients with liver foci suspicious for cancer by invitation only. Patients will be referred by their NIH primary care team and will need to be registered to an existing NIH clinical study prior to enrolling in this study. Liver biopsy will be performed 24 hours after administration of clinically approved ICG 0.5 mg/kg IV. Biopsy will be performed using EM

	technology and evaluating OMI as a bystander in liver cancer biopsies. The fluorescence signal of the tissue cores will be evaluated ex vivo and the signal will be correlated to histopathological characteristics of the tissues
Study Duration:	We anticipate that 100% of the projected enrollment will be completed by 4 years. Data collection (including correlative pathology) will be completed by 4 years. We anticipate global primary and secondary analysis along with the final report will be completed in 5 years.
Participant Duration:	Participants will be considered "Off Treatment" upon completion of the hepatic biopsy and four (4) to six (6) hour post procedure monitoring period. Participants will be considered "Off Study" within one (1) ± two (2) months post correlative pathological assessment to result.

1.2 SCHEMA


Once patients are referred to the study, initiate screening procedures and assess patients' eligibility.
Review baseline labs, allergies, and active illness as part of **clinical** assessment per departmental guidelines. Review H&P and imaging. Sign consent.
Administer 0.5 mg/kg ICG up to 40mg roughly 24hrs prior to scheduled biopsy.
Perform biopsy in IR
Measure ICG epifluorescence from liver parenchyma, target lesion, and core sample. Submit core sample for standard pathologic analysis.
Correlate and evaluate Pathology report

1.3 SCHEDULE OF ACTIVITIES (SOA)

Table 1. Study Elements

Procedures	Screening	Pre-Procedure	Study Day 1 Intervention	Off Treatment Biopsy and post procedure monitoring completed	Off Study 1±2 months for all correlative pathological assessments
Informed consent [a]	X				
Review of imaging	X	X			
Review of medical history	X				
Physical examination	X				
Review of laboratory values	X				
ICG Administration		X			
Percutaneous Liver Biopsy			X		
Adverse Event monitoring		X	X		
Remove from protocol therapy				X	
Remove from study follow up					X
Completion of histopathology assessment					X

a. Informed consent obtained prior to screening procedures.

2 INTRODUCTION

2.1 STUDY RATIONALE

We propose a pilot study to determine the feasibility of using optical molecular imaging (a low cost, real-time, high resolution imaging discipline) for minimally invasive liver biopsy guidance in patients with diagnosed or suspected HCC or metastatic CRC. Patients who require a clinically indicated or separately IRB approved research liver biopsy would after consent, have the OMI performed during biopsy but not as the primary directorate to the biopsy site. The standard of care imaging and cognitive registration would be employed for navigation or guidance of needle and OMI would act as a bystander. Nonetheless, data from OMI imaging would be obtained and evaluated with ex vivo POC OMI and histopathology. Correlations from these studies might support a future larger clinical trial, with different goals that might not exclude efficacy and safety, or even allow for guidance of the needle if separately IRB-approved.

2.2 BACKGROUND

The advent of advanced imaging techniques including multi-phase contrast enhanced CT and MRI has improved the sensitivity for detection of focal hepatic lesions. However, focal lesions smaller than 3cm often lack specific features to allow for a reliable non-invasive characterization. Even molecular imaging modalities such as FDG-PET may be of limited utility given the intrinsic spatial resolution of PET as well as the heterogeneous background uptake of FDG by liver parenchyma. For this reason, the number of biopsies for small hepatic lesions has increased over the past decade (1). Compared to non-invasive imaging and serologic tests, biopsy is more sensitive and specific in diagnosing malignancy (2).

Optical molecular imaging is a low cost, real-time, high resolution imaging discipline that holds promise for minimally invasive procedure guidance. Optical molecular imaging as a field encompasses a vast array of imaging modalities; one such modality is the imaging of exogenously administered organic fluorochromes. For example, ICG is a common clinically used and FDA-cleared optical molecular imaging agent that fluoresces in the near infrared (NIR) and localizes with high target-to-background ratios to HCC and metastases from colorectal carcinoma CRC (5-8), whereas non-tumorous hepatic tissue clears the ICG. Recently the intra-operative imaging of ICG for the detection of intrahepatic malignancy has been demonstrated (6). Indeed, OMI maybe superior to standard visual inspection and intraoperative ultrasound for the detection of focal hepatic lesions during surgical resection.

Though still in its nascent stage, optical molecular imaging has the potential to make a tremendous impact in IR. With numerous fluorescently labeled molecular markers in preclinical and clinical development (not subject of this study), the opportunities to perform procedures guided by highly sensitive and specific molecular beacons of disease will continue to grow. In the context of percutaneous biopsy, optical imaging probes could improve operator confidence and decrease the risk of sampling error, which in turn would reduce the clinical concern of false negative following a benign biopsy, although this is speculative. Moreover, the improved operator confidence could enable sampling of smaller lesions, thereby potentially providing a diagnosis and appropriate management plan to patients earlier in the course of disease. Although speculative, given the molecular specificity of future fluorescent imaging agents, a quantitative assessment of the degree of molecular probe uptake could provide an “in vivo histology”

diagnosis, potentially without requiring tissue sampling, a feature that may be an important consideration for lesions in locations where obtaining a core specimen may be dangerous.

However, due to the short distance passage of light, the optical technique alone is not efficient at localizing a lesion if more than 0.5cm away from the target. Electromagnetic tracking (EM) and fusion co-display, on the other hand, can ensure the close proximity of the needle tip to lesion, by referencing and displaying the needle in relation to the pre-procedural imaging -CT or MRI. EM tracking for registration of preoperative images has been utilized during neurosurgery, orthopedic surgery, as well as in image-guided percutaneous biopsy at NIH for over 15 years. The PhD and MD researchers at NIH-CC-CIO have extensive experience in clinical use of a minimally-invasive image guidance navigation system that integrated electromagnetic tracking of needles and US probes to allow registration, fusion and co-display with 3D imaging data, to improve target visualization during needle-based procedures in interventional radiology. Multiple vendors and FDA cleared systems are currently marketed under the same basic principles (UroNav, PercuNav, Koelis, MIMS, Hitachi, Siemens, General Electric, Esaote BioSound, Imactis, Veran, Superdimension, Stealth Medtronic, Covidien Johnson and Johnson, Cascination to name a few). Multiple studies show that this technology can improve basic needle biopsy accuracy and minimize risks, complications and error during image guided percutaneous or transrectal biopsy.

In this “bystander” study for data acquisition and feasibility, we propose combining these two intrinsically synergistic, recent advances in the field of interventional radiology, in a feasibility study for data acquisition alone. “Bystander” is defined as a term to describe the study performed in the background during a standard procedure, performed in a very similar fashion to the standard methodology and workflow, while data will be gathered as described in this protocol. The final determination of malignancy will come from histopathology testing, accepted as the gold standard; that pathologic evaluation will proceed in the standard fashion Neither safety nor efficacy is a goal of this feasibility pilot study. However, the long range futuristic goals of the general approach may facilitate a better understanding of the perspective and scientific rationale underlying this feasibility pilot study. The following is not part of the study, and thus should not be factored in to the risk determination of this study, as it does not describe current method of use of this study outlined above and in the study protocol. Although not the subject of this study, the long range speculative goal might be to provide added information to potentially guide (in the future) intra-tumoral sampling towards the most viable and aggressive lesion components, or towards cancerous tissue instead of normal tissue, in order to attempt to improve the biopsy yield (but not in this study). In such a speculative future use, the EM navigation might navigate the biopsy needle generally close to suspicious (previously imaged) lesions; while the optical imaging devices provide real-time small scale, near field, feedback to assess whether needle cores are appropriately aimed and located precisely within small hepatic tumors, as opposed to normal liver or regenerative nodules. Again, this is not within the scope or methods for use of this study, and is provided for informational

2.3 PRELIMINARY STUDIES

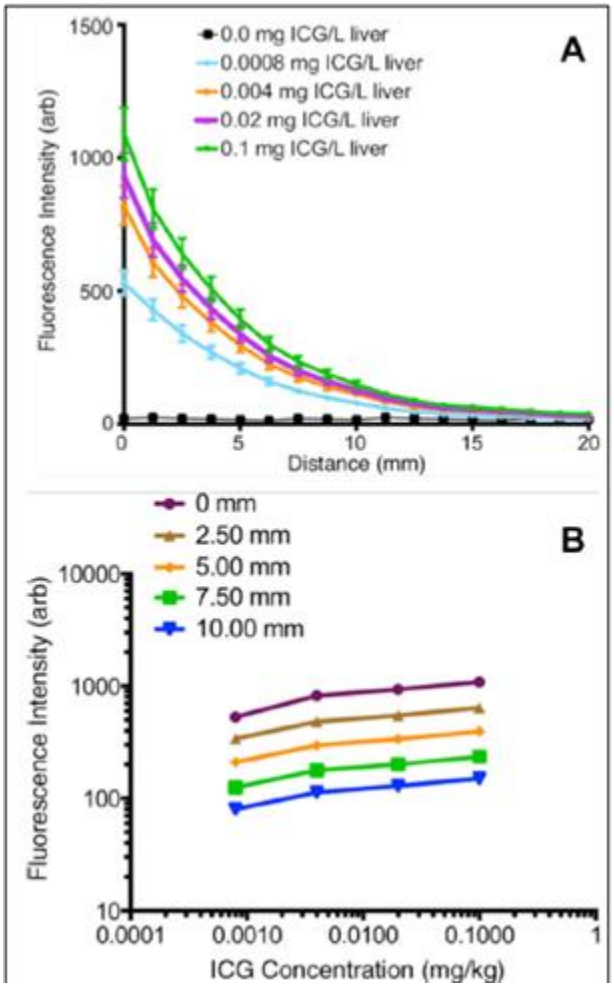


Figure 1. Graphs from a phantom experiment of ICG fluorescence as a function of concentration and distance through liver. **(A)** There is marked ICG fluorescence above background levels through 5-10 mm of homogenized liver tissue. **(B)** ICG fluorescence is measurable over two orders of magnitude of ICG concentration through liver tissue. arb = arbitrary units.

A series of preclinical feasibility studies were performed of the combination optical and electromagnetic system, including phantom, tissue and in vivo animal were conducted, which included phantom ex vivo work, and in vivo animal trials. Separate distinct human in vivo clinical trials have been conducted of each technology in distinct trials: 1. EM tracking for biopsy (NIH CC) and 2. Optical imaging with ICG for liver biopsy (Massachusetts General Hospital). Prior work has included validation studies, semi-quantitative studies, software verification studies, accuracy studies, and ICG calibration testing.

2.3.1 Phantom study to measure ICG signal intensity relative to distance and ICG concentration in tissue.

A phantom was designed to measure ICG fluorescence signal intensity through varying thicknesses of liver tissue and at different ICG concentrations. The phantom was composed of two separate compartments; the upper compartment consisted of a cuvette filled with 25 mm of a homogenate of normal mouse liver tissue. The transparent bottom of the cuvette was in direct contact with a 1-mm layer of ICG mixed with liver tissue homogenate. Different concentrations of ICG mixture in liver tissue homogenate were created by using serial dilution (0.0008, 0.004, 0.02, and 0.1 mg/L of ICG) and were placed in the compartment below the cuvette. A 1.6-mm–diameter fiber-optic pediatric cystoscope-urethroscope was inserted into the cuvette. The pediatric cystoscope-urethroscope (Thorlabs, Newton, NJ) and withdrawn by increments of 1.25 mm from the cuvette dye interface. The light guide of the pediatric cystoscope-urethroscope was connected to a 785-nm fiber-coupled laser (BWTEK, Lubeck, Germany), and the eyepiece was connected through an 800-nm bandpass filter (Semrock, Rochester, NY) to an NIR camera (Allied Vision Technologies, Stadtroda, Germany). With each withdrawal of the pediatric

cystoscope-urethroscope, still images were recorded in triplicate with a 50-msec exposure for each ICG concentration.

To reduce the variability of measurement, a circular region of interest with an area of 20,000 square pixels was placed in the center of each image by using a standard image analysis software package (Image J; U.S. National Institutes of Health, Bethesda, MD). Signal intensities were measured independently and the mean of the triplicate measurements was used as the mean signal intensity. ICG fluorescence was detectable through 10 mm of homogenized tissue, suggesting that lesions within approximately 1 cm of the imaging system are potentially detectable. There was a monotonically increasing relationship between ICG concentration and fluorescence intensity over a range of ICG concentrations the spanned two orders of magnitude.

The BW & TEK 785 nm class IIIb laser system that was used for pre-clinical testing is not approved for use in human subjects, therefore an alternative system that complies with the FDA requirements was tested for substitute use in the in vivo component of the clinical trial. The camera of The ICG imaging system was tested in water and ICG solutions using (1) the original B&W Tek BWF1 laser generator (laser class IIIb) and (2) Stryker® IRF Light Source and Safelight Cable (ENV mode). The camera of the imaging system did not show presence of ICG in water regardless of which light source (or laser generator) to use. In ICG solutions, the camera showed stronger signals with the Stryker® IRF Light Source and Safelight Cable than the original BW&TEK laser generator. The experiment showed that the ENV mode of Stryker L10 light source is at least as good as the original BW&TEK laser generator which was already validated in ex vivo and in vivo animal experiments.

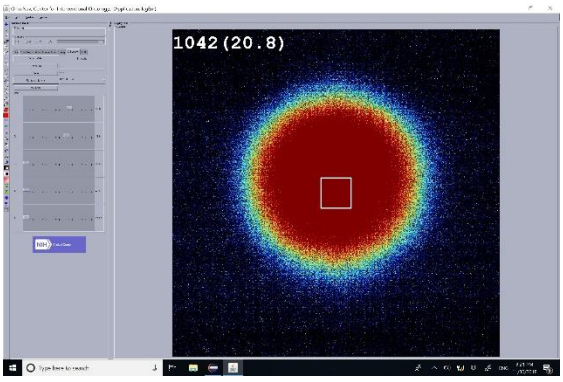


Figure 2. Stryker® IRF L10 Light Source and Safelight Cable generated a stronger fluorescence signal when tested with ICG contrast compared to the original device B&W Tek BWF1 laser system.

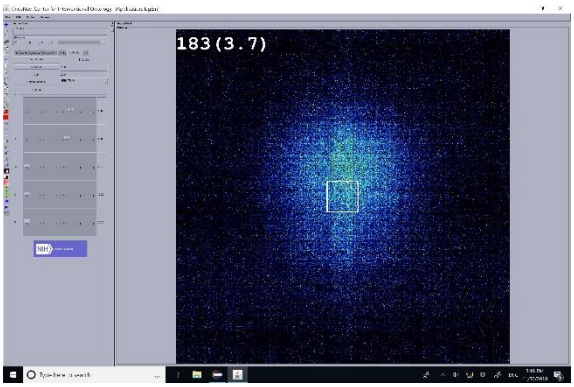


Figure 3. ICG contrast testing with B&W Tek BWF1 laser system. This device is not approved for use in human subjects.

Stryker® IRF L10 Light Source and Safelight Cable is an FDA 510 (k) cleared device that complies with IEC 60825-1:2007 and 21 CFR, Subchapter J, Parts 1040.10 and 1040.11. The 808 nm wavelength is the peak excitation wavelength for ICG and is the optimal excitation wavelength for the use proposed in the study. The wavelength, power output and the laser mode (continuous or pulsating) can be adjusted by the user in the Endoscopic Near-Infrared Visualization (ENV) mode. Additionally, this device is designed to ensure electromagnetic

compatibility with other electrical medical devices and complies with IEC 60601-1-2 requirements for EMC with other devices. In summary, L10 Light Source by Stryker is the ideal device for the in vivo component of the proposed study.

2.3.2 ICG uptake in a murine model of focal hepatic malignancy

A murine model of focal hepatic malignancy was generated by injecting human colorectal cancer cells (HT-29;ATCC, Manassas, VA) into the livers of athymic nude mice (nu/nu; Taconic, Germantown, NY). Three weeks later, 0.5mg/kg ICG was injected intravenously via tail vein injection. The mice were then sacrificed at 3, 6, and 24 hours post-injection, and surface reflectance NIR imaging of the excised livers was performed (Carestream, New Haven, CT).

ICG localizes to the intrahepatic lesions with high TBRs (**Figure 2** and **Figure 3**), with a TBR of approximately 4.0 at 24 hours (**Figure 4**). Of note, the injected ICG dose of 0.5mg/kg is equivalent to the manufacturer recommended dose.

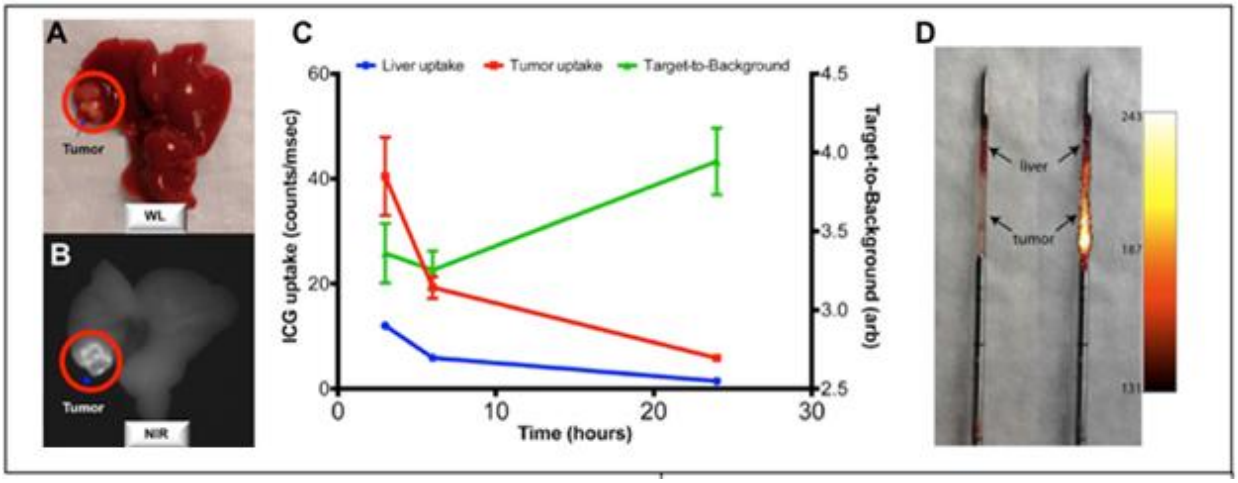


Figure 4. A, B) Intrahepatic xenografts demonstrate avid ICG update in a murine model. Gross appearance of the focal tumor within the liver (A) with elevated ICG update relative to liver on surface reflectance fluorescence imaging (B). C) ICG update in focal hepatic tumors over time. TBR for ICG update within intrahepatic tumors relative to adjacent liver parenchyma is maximal at 24 hours. D) Core biopsy sample from a preclinical hepatic tumor. Fluorescence signal from the tumor reflects accumulation of ICG.

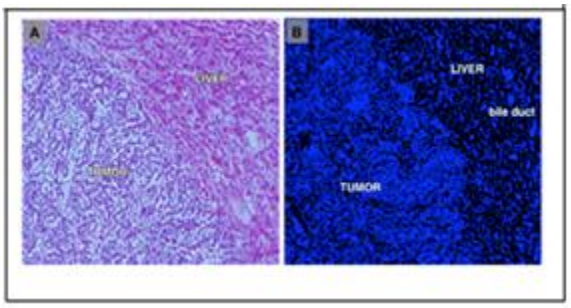


Figure 5. Hematoxylin-eosin-stained and fluorescence microscopy images of orthotopic hepatocellular tumor xenograft developed from HuH-7 cells. (A) H&E staining at 100x shows hepatocellular tumor with densely packed cells, and high nuclear-cytoplasmic ratios. The tumor-liver margin demonstrates the transition from normal to malignant cells. (B) The sharp tumor-liver margin is evident at ICG fluorescence imaging (100x), with increased ICG update within the tumoral cells. Of note, pools of ICG within the hapatic parenchyma reflect normal excretion of ICG into biliary canaliculi.

2.3.3 Xenograft Fluorescence Microscopy of ICG

A model of focal hepatic malignancy was generated by injecting human hepatocellular carcinoma cells (HuH-7, Japanese Collection of Research Bioresources) into the livers of athymic nude mice. Four weeks later, 0.5mg/kg ICG was injected intravenously via tail vein injection. The mice were then sacrificed at 24 hours post-njection. The excised livers were stored at −80°C. Two serial cryostat sections were cut from the tissue samples for fluorescence microscopy (10-µm section) and hematoxylin-eosin staining (50-µm section). The fluorescence microscopy was performed using laser scanning confocal microscopy (LSM 5 PASCAL; Zeiss, Oberkochen, Germany) with an excitation wavelength of 633 nm and 100× magnification.

The histologic specimens were evaluated qualitatively to determine tumor-liver boundaries.

Histologic findings in the tumor are remarkable for densely packed tumor cells with high nuclear-cytoplasmic ratios, an appearance in contrast to the adjacent normal hepatic sinusoidal architecture. Fluorescence microscopy similarly shows a sharp delineation between malignant and normal tissue, with increased ICG uptake in the xenograft. Notably, focal areas of ICG pooling are present in the biliary canaliculi in the adjacent liver—an expected finding given the hepatobiliary excretion of ICG (**Figure 5**).

2.3.4 Handheld miniaturized optical imaging device and the point-of care optical imaging system

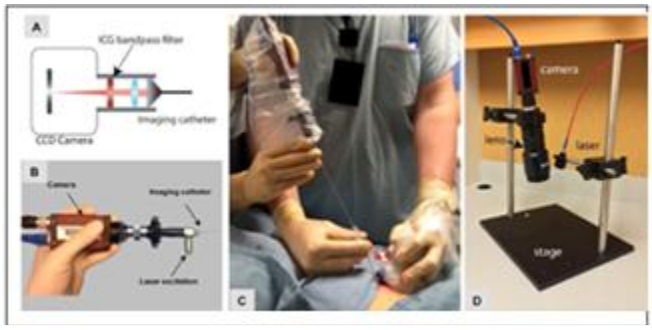


Figure 6. A) Schematic demonstrates design of handheld OMI device. B) Photograph of device, demonstrating handheld OMI system consists of a miniature endoscope coupled to a high resolution 12-bit camera. C) Handheld device advanced through a standard 17-gauge introducer needle US-guided percutaneous focal liver biopsy to perform real-time measurements of ICG localization. D) Point-of-care OMI system for acquisition of high resolution ICG fluorescence images of biopsy cores for real time assessment.

A model of focal hepatic malignancy was generated by injecting human hepatocellular carcinoma cells (HuH-7, Japanese Collection of Research Bioresources) into the livers of athymic nude mice. Four weeks later, 0.5mg/kg ICG was injected intravenously via tail vein injection. The mice were then sacrificed at 24 hours post-injection. The excised livers were stored at −80°C. Two serial cryostat sections were cut from the tissue samples for fluorescence microscopy (10-µm section) and hematoxylin-eosin staining (50-µm section). The fluorescence microscopy was performed using laser scanning confocal microscopy (LSM 5 PASCAL; Zeiss, Oberkochen, Germany) with an excitation wavelength of 633 nm and 100× magnification.

The histologic specimens were evaluated qualitatively to determine tumor-liver boundaries.

Histologic findings in the tumor are remarkable for densely packed tumor cells with high nuclear-cytoplasmic ratios, an appearance in contrast to the adjacent normal hepatic sinusoidal architecture. Fluorescence microscopy similarly shows a sharp delineation between malignant and normal tissue, with increased ICG uptake in the xenograft. Notably, focal areas of ICG pooling are present in the biliary canaliculi in the adjacent liver—an expected finding given the hepatobiliary excretion of ICG (**Figure 6**).

2.3.5 Results of pilot clinical trial for hepatic lesion sampling with optical guidance

Partners at MGH recently conducted the first-in-man trial of clinical liver biopsies using percutaneous OMI following injection of ICG (Figure 7). They recruited 6 patients for this pilot study [1]. The median size of the targeted lesions was 16 mm (range, 10–21 mm). Four of five biopsies (80%) yielded a positive pathologic diagnosis, and one biopsy specimen showed benign liver parenchyma.

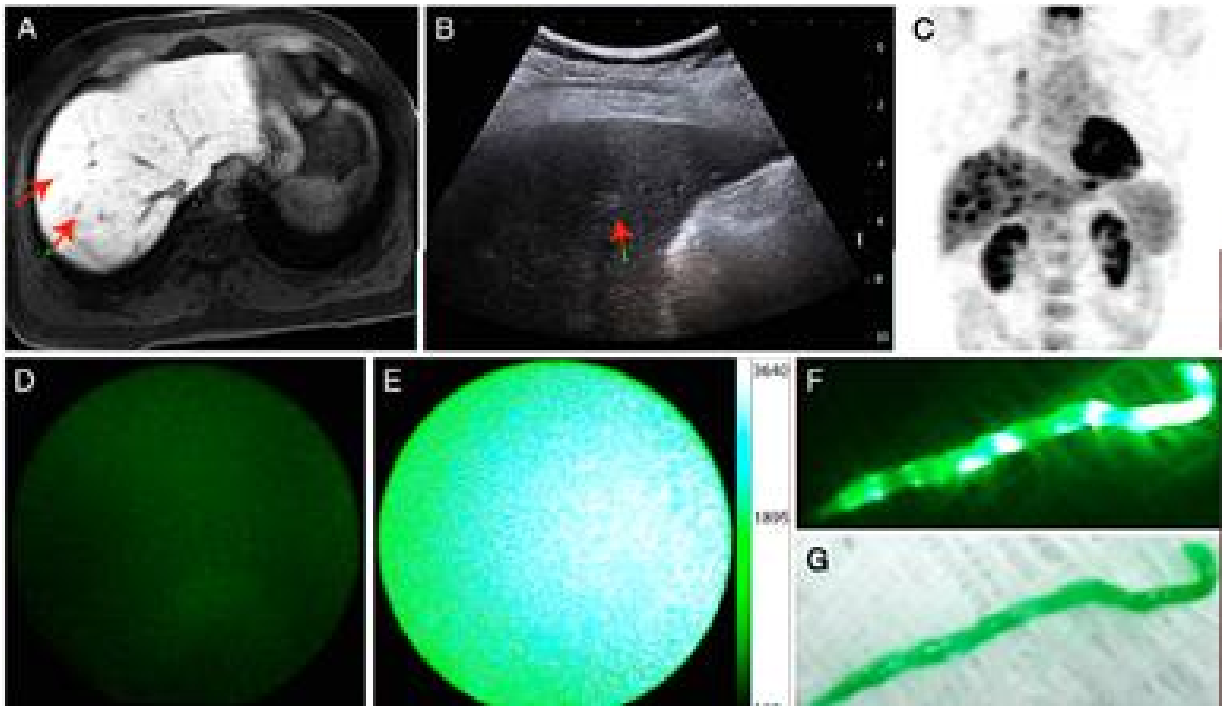


Figure 7. Focal hepatic lesions on, A, MR image and, B, US image. C, 18F FDG PET scan shows avid lesions suspected of being malignancy. Intraprocedural optical molecular images confirmed the accuracy of the biopsy needle placement, by measuring increased fluorescence, E, in the target lesion relative to, D, normal liver. Optical images of the core biopsy specimen show visualized area of, F, focally increaed ICG localization, G, in the core specimen. Final pathologic analysis of the core specimes revealed a granulomatous process.

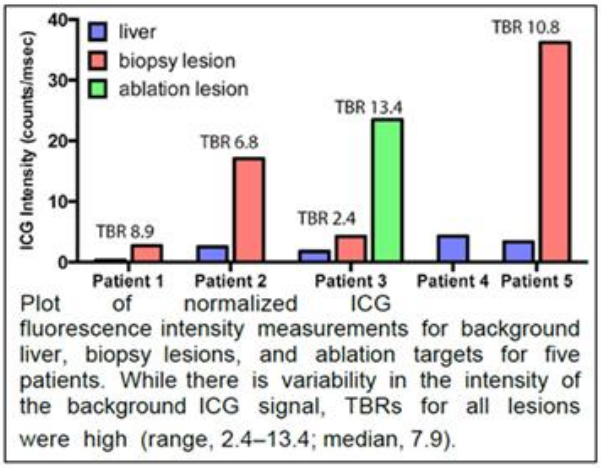


Figure 8. Plot of normalized ICG

Intraprocedural imaging of ICG fluorescence intensity at the tip of a standard introducer needle and within core biopsy specimens was feasible and added less than 5 minutes of procedure time in all patients. ICG was found to localize with TBRs greater than 2.0 (range, 2.4–13.4; median, 7.9) in all target lesions including primary HCC, metastatic CRC, and a non-neoplastic granulomatous process (**Figure 8**).

The difference between ICG fluorescence intensity between normal liver and focal hepatic lesions across the study cohort approached significance (P = .06). No trial-specific adverse events were observed up to 7 days following the procedures. We have shown that ICG imaging using these systems is a robust

adjunct to conventional image guidance for patients undergoing biopsy of primary HCC, hepatic CRC metastasis, and non-neoplastic inflammatory processes, as well as thermal ablations.

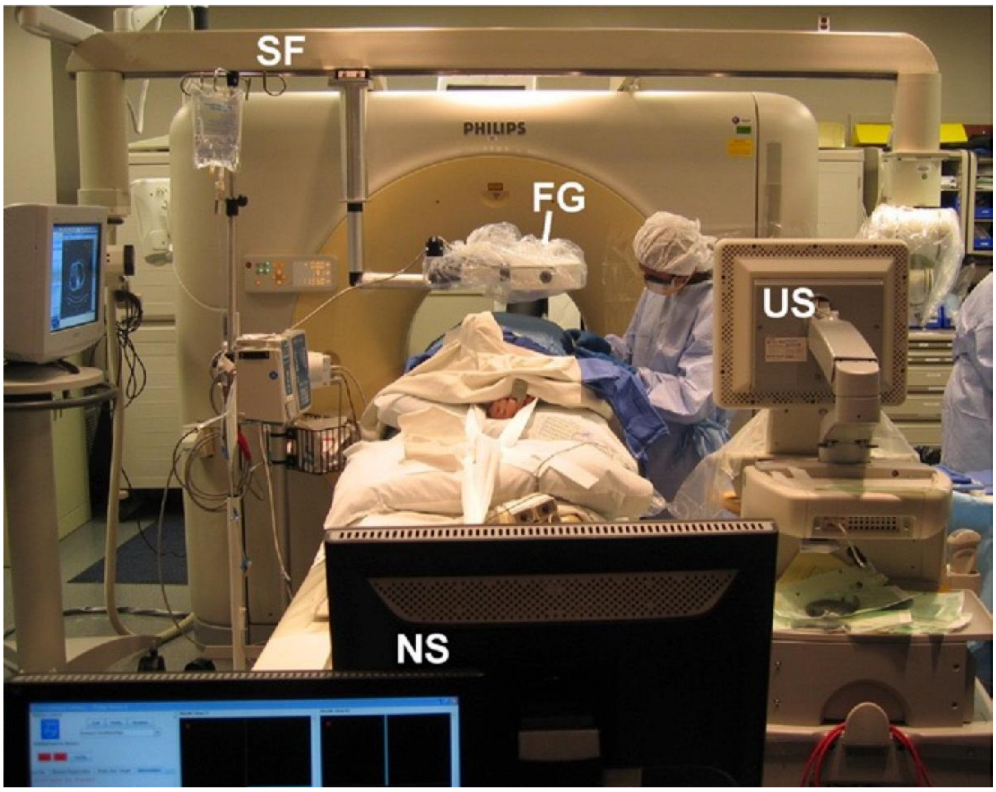


Figure 9. NIH Clinical Center setup for an ultrasound/computed tomography –guided biopsy procedure with electromagnetic tracking guidance. FG: electromagnetic filed generator, NS: navigation.

2.3.6 Electromagnetic navigation for liver biopsies

Electromagnetic (EM) tracking for registration of preoperative images during procedures maps conventional US and CT images for "fusion guidance" [7-19]. Over almost the past 2 decades, the NIH navigation system has integrated electromagnetic tracking of needles and US probes with custom software to allow registration and fusion with three-dimensional images to improve target visualization during needle-based procedures (**Figure 9** and **Figure 10**).

We have used anthropomorphic multimodality injectable interventional phantoms such as Model 57 (CIRS, Norfolk, VA) for a qualitative feasibility studies of EM tracking to demonstrate this application in different clinical scenarios using different imaging modalities to locate the lesion.

For instance, in a feasibility study over a decade ago, using EM tracking alone to navigate to a simulated hot PET lesion using 18F containing fiducials, PET/MR data set was acquired and the PET scan DICOM images were used to navigate a tracked needle to the internal target. DICOM images were transferred to navigation software and a tracked 22-gauge needle (with a sensor coil embedded in the tip) was placed toward the internal target with electromagnetic tracking guidance. MR scan verification was performed.

PET and MR datasets were also successfully loaded and registered to the phantom for navigation within metabolic and functional datasets. An internal radiopharmaceutical target was located with electromagnetic tracking with a tracked needle (**Figure 11**). This technique has been used successfully as a part of an NIH clinical trial for 15 years, and has also been commercialized or duplicated by at least 20 companies.

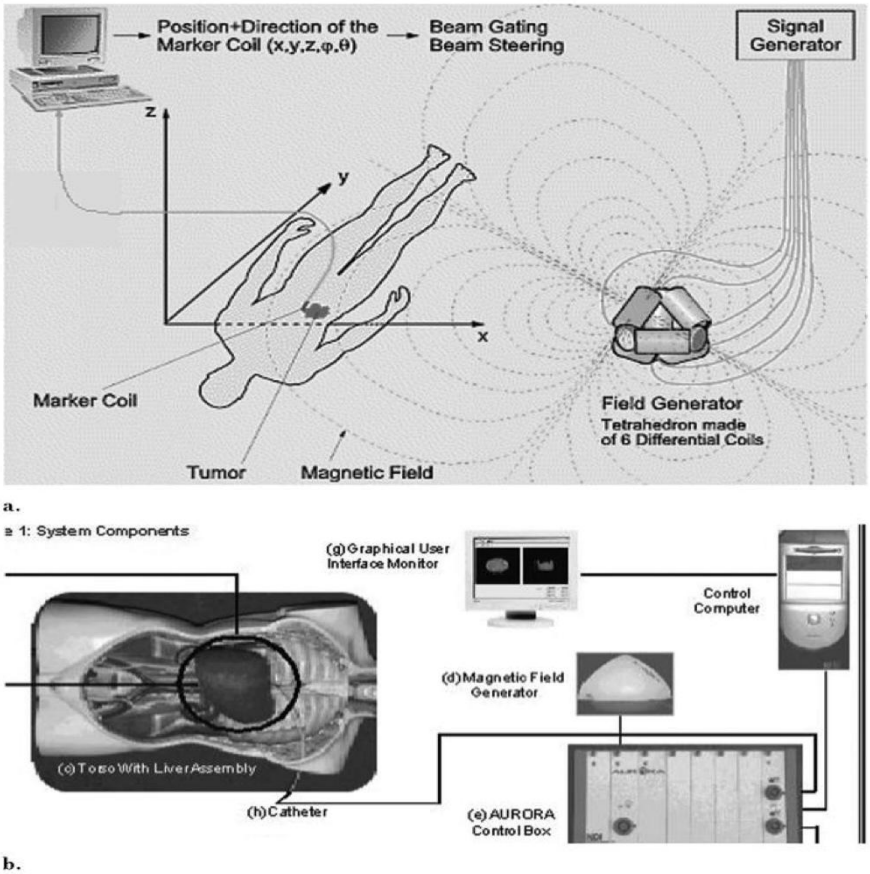


Figure 10. Electromagnetic tracking system components and setup: **(a)** electromagnetic tracking system set up and rationale. **(b)** Components of image-guided system incorporating electromagnetic tracking for abdominal interventions.

A swine model was used to evaluate the feasibility of EM tracking alone. Our studies showed that prospective use of the EM navigation system allows needle and electrode placement with higher confidence in all cases and provides valuable target information that is unavailable by conventional image guidance in most cases. For instance in one of our decade-old studies, the tracking technology enabled 22 needle and electrode placements in 19 procedures; these 22 placements would have had a high risk of technical failure with conventional image guidance only, owing to lack of target localization [11]. These were primarily cases in which the target lesions were visible only on an arterial phase CT scan or on prior PET or PET/CT scan and were then brought into the procedure by image registration with the navigation CT (Figure 11).

The technology was especially enabling for liver targets that were visible only during brief arterial phase enhancement on contrast-enhanced CT scan. The additional information allowed completion of biopsies in less time than they would have taken with conventional guidance, based on the number of failed needle positioning attempts with CT guidance only.

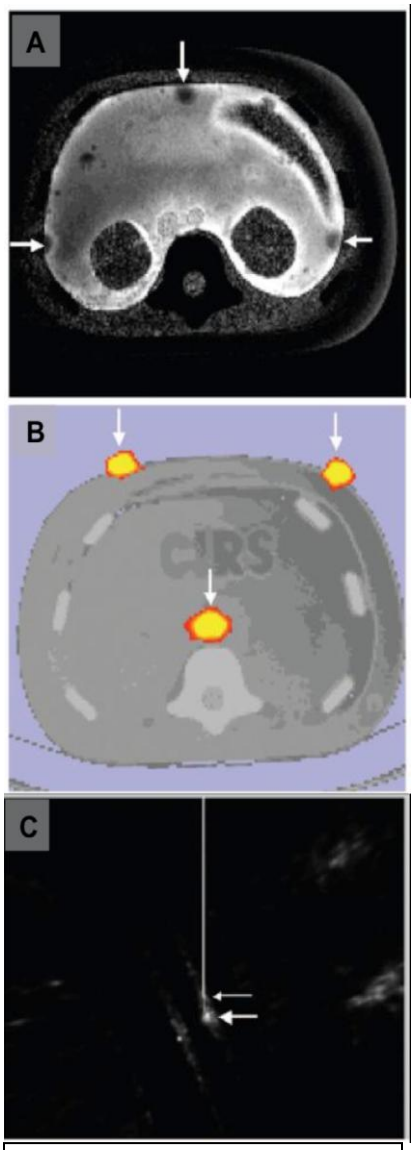


Figure 11. Use of pre-procedural PET and MR for needle navigation in a phantom. **(A)** T2-weighted MR image of phantom with gadolinium fiducials (arrows). **(B)** CT/PET image of interventional phantom with 6 mCi of 18F fiducials (arrows) could also be used for needle navigation. **(C)** Tracked needle position (small arrow) is displayed near internal target (large arrow) from pre-procedural PET during tracked needle insertion.

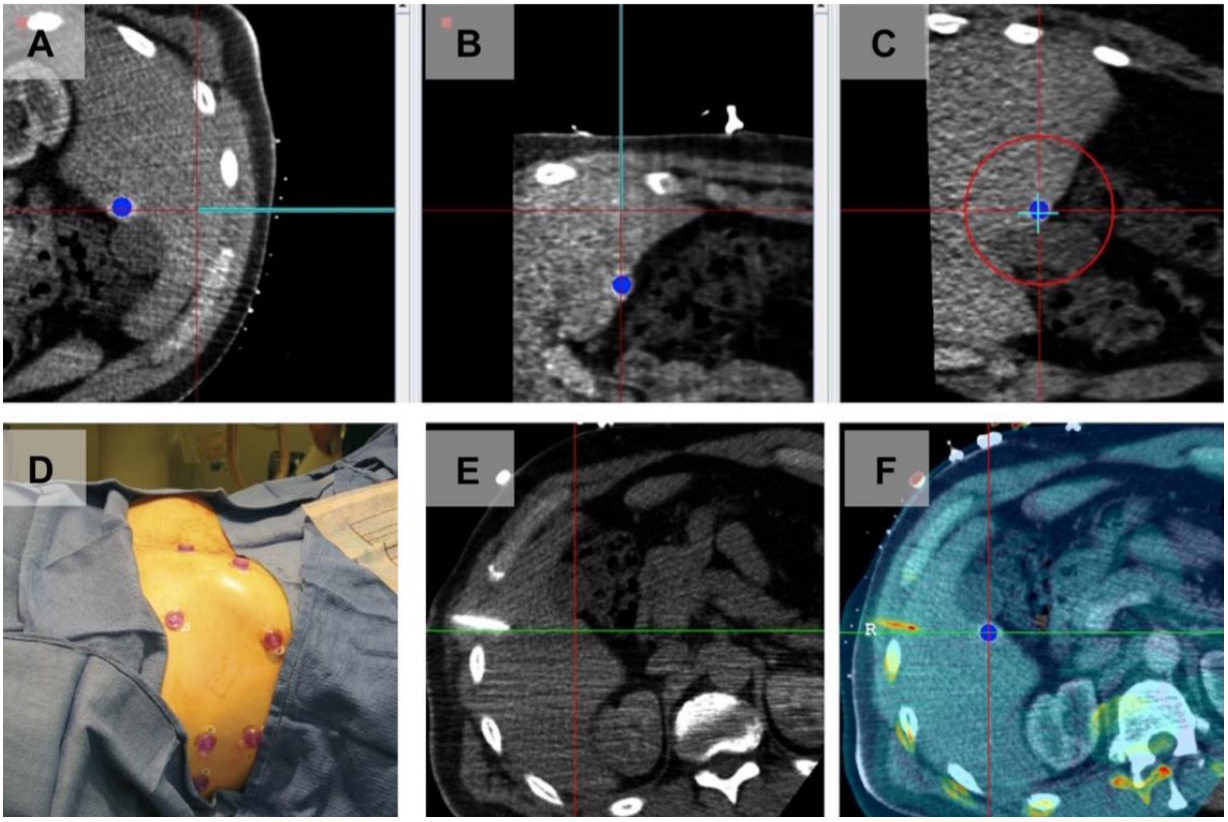


Figure 12. Navigation display at the NIH Clinical Center (A-C). Display showing the virtual needle aligned with the target identified on arterial phase-enhanced computed tomography scan. (D) Fiducial markers were placed the patient's skin to facilitate registration process. E) Non-contrast-enhanced confirmation scan of needle position does not show the target lesion. F) After rigid registration of the confirmation scan (pseudocolored) with the arterial contrast-enhanced scan (gray-scale background) in the vicinity of the target lesion, the correct alignment of the needle with the target is confirmed.

Additionally, fusion guidance significantly improves angle recent study, the addition of needle and US tracking improved needle path off-target error from 17.8 mm ± 17.1 to 3.3 mm ± 3.1 and changed the insertion angle by 13.3 degrees ± 6.5. We showed that registration and use of pre-procedural diagnostic or metabolic images enabled identification and localization of targets and had a direct impact on outcomes in interventions [11].

2.3.7 Combination EM and OMI System

The combined integrated platform provided simultaneous optical and EM feedback during simulated ex vivo and real in vivo animal biopsy. EM was used to place the needle roughly near the ICG target, and the optical signal was used to fine-tune and verify precise tip location at the optical source in phantoms and in live Woodchucks with liver tumors (data on file). The ICG signal was predictive of tumor presence on histology biopsy samples using a combined system.

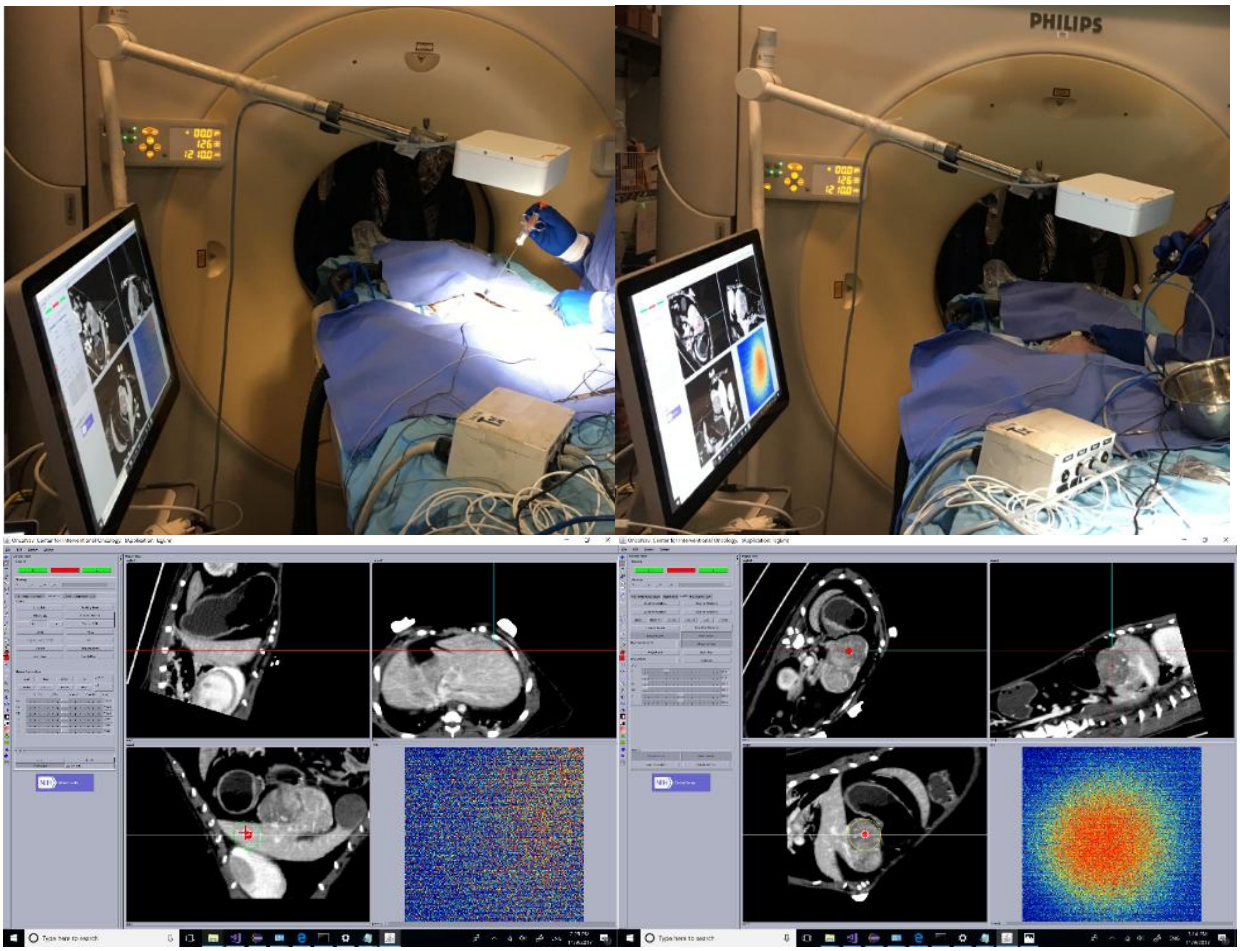
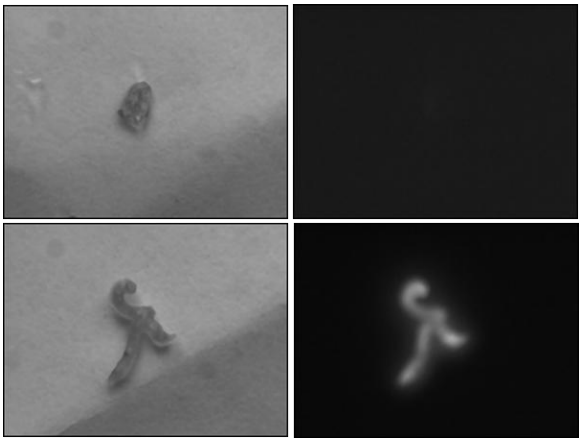


Figure 13. Color means ICG present = tumor



Top row = no ICG = normal liver
Bottom row = ICG present = tumor

Figure 14. White light Optical POC box ICG imaging

2.3.8 Handheld OMI

The OMI is a handheld endoscopic instrument consisting of an FDA-cleared and clinically used pediatric cystoscope-urethroscope (Model 27033 AA; Karl Storz, Tuttlingen, Germany) illuminated by a safelight cable attached to an 808 nm laser excitation source (Stryker IRF L10 Light Source and Safelight Cable) with an adjustable user feature in the Endoscopic Near-Infrared visualization (ENV) mode that can pass coaxially through the introducer of a standard 17-gauge core biopsy system (see **Figure 6**).

The eyepiece of the pediatric cystoscope-urethroscope is attached to high-definition endoscopic camera system sensitive to the visible and infrared spectrums. The optical image is transferred from the surgical site to the camera unit which is attached to the camera head (Mako; Allied Vision Technologies) and the light output is by a filtered band-pass filter that is compatible with the laser excitation source.

The imaging pediatric cystoscope-urethroscope tip is the only component that is in direct contact with the subject and will be sterilized according to manufacturer's guidelines, which the NIH Clinical Center sterilization have confirmed they can do as prescribed by the manufacturer in a standard fashion. The remainder of the handheld device is remote from the patient, but can be placed in sterile plastic surgical cover. The Stryker IRF L10 Light Source and Safelight Cable system are part of the Stryker® Infrared Fluorescence (IRF) Imaging System, which is an endoscopic illumination and imaging system for real-time high definition (HD) visible light and near-infrared dye fluorescence imaging of indocyanine green (ICG) during minimally invasive surgery/ procedures. The system complies with IEC 60825-1:2007 and 21 CFR, Subchapter J Parts 1040.10 and 1040.11. This device is designed to ensure electromagnetic compatibility with our electrical medical devices and complies with IEC 6060-1-1-2 requirements for EMC with other devices.

2.3.9 Point-of-care (POC) Optical Molecular Imager (OMI)

This is a camera in a black box taking images of the ex vivo tissue using a BWF1 laser to excite light to see the ICG fluorescence. Specifically, the core biopsy tissue specimen is placed in a sterile container and removed to a windowless room adjacent to the Interventional Radiology (IR) suite. The tissue is placed inside a 2 foot tall black box on a benchtop with the laser. The laser does not come in contact with the patient, nor in the same room as the patient, at any time. (**Figure 15**).

The BWF1 laser generator from the medical laser series will be used with the point of care OMI, which does not occur in vivo, and occurs remote from the patient and not in the procedure room. This system takes pictures of the ex vivo biopsy core itself with special lenses and filters, to display a signal of whether ICG is present in the tissue sample. Although speculative and not part of this study, this assessment might in the future, facilitate examination of future biopsy tissue quality or might point towards the absence or presence of tumor (although no such claims or hypotheses are made in this study).

In the POC epifluorescence OMI system, the fluorescence excitation light is provided by a 450-mW, 785-nm laser (Edmund Optics). Upon the absorption of laser light, the ICG in the vessels is excited and emits infrared light at a different wave length. The camera system captures the infrared emission, processes the image and displays it on a surgical display. Emitted fluorescent light is collected by a close focus video zoom lens (Edmund Optics) and filtered by a band-pass

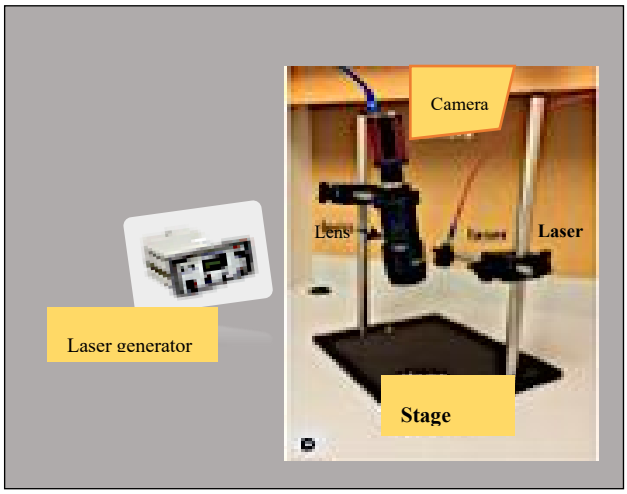


Figure 15. Black Box and contents for sample evaluation.

filter. The filtered fluorescent light is imaged with a near-infrared-optimized high temporal- and high-spatial resolution camera (Mako; Allied Vision Technologies) (**Figure 2**). A new filter slider, which allows for direct visualization of the sample by the POC system, is used for adjusting the sample in the field of view of the camera. A custom-built dark cabinet for t the POC eliminates or reduces signal from stray photons in the environment. (**Figure 15** and **Figure 16**).

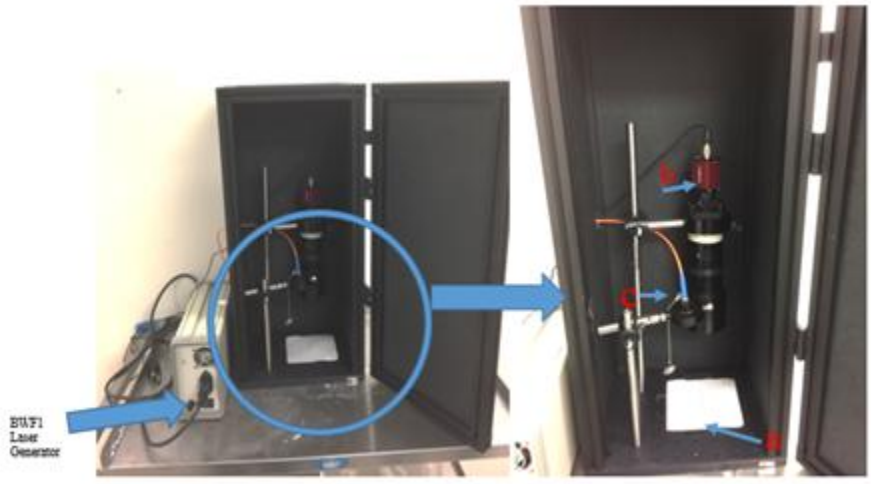


Figure 16. Outside imaging – the black box contents a. stage b. camera c. laser input to excite ICG for imaging.

2.3.10 EM tracking

Aurora (Northern Digital Inc, Waterloo, Ontario, Canada) EM tracking system is used for device tracking. A small electromagnetic field generator is mounted on a modified passive support and positioning arm, and produces an ultra-low electromagnetic field (<70μT) enabling simultaneous tracking of the several sensor coils in the inner needle stylet at the beginning of the biopsy, within a work volume of ~ 500mm3. Commercial and FDA-cleared "PercuNav" needles (Philips) have stylets that contain sensor coils in the needle tips. These needles allow for tracking and mapping location coordinates in relation to the pre-procedural CT or MRI when in the

presence of an EM field generator and a tracking system. During the biopsy (as described above), after both inner and outer biopsy needles are placed in the liver, the coaxial inner stylet is removed and replaced with the OMI scope. The outer needle remains in the liver after the inner stylet is removed, and provides a cannula lumen, through which is placed the scope (for data sampling of fluorescent signal). This occurs once along the way to the target liver lesion - likely in normal liver- and the process is repeated after advancement of the original needle and stylet to the target lesion, during the standard clinically-indicated biopsy (as described above). After optical ICG signal data acquisition, the scope is removed and a standard spring-loaded disposable FDA-cleared biopsy gun takes a tissue specimen from the same location as would have been biopsied in the absence of this study. Thus the FDA-cleared outer cannula introducer allows insertion of the biopsy needles and the pediatric cystoscope-urethroscope via the same outer needle / cannula, using a coaxial approach.

2.3.11 Software

The EM and optical display software components analyze the EM and optical data and signal outside of the body, and the information is not used to guide the needle placement or the biopsy sample. The commercial EM needle, described above, and the commercial EM field generator and its commercial advanced programming interface are analyzed by custom software in order to co-display the local optical signal as well as the EM tracking fusion biopsy location in relation to a pre-procedural CT or MRI image. This information will be analyzed post-procedure, but is not used for navigation of the device. This system and multiple commercial fusion biopsy platforms (UroNav & PercuNav, Philips, for example) have been in clinical use on IRB-approved protocols at NIH for 15 years. These NIH research studies are currently approved under an IDE exemption. The OMI/EM system in its entirety has been thoroughly tested in phantoms as well as live animals (woodchuck liver cancer tumors for biopsy). This in vivo testing, included the EM tracking / fusion display components, the OMI pediatric cystoscope-urethroscope, and the combined signal display. This custom software platform incorporates EM fusion co-display (that tracks, fuses, and displays the needle with respect to CT images). The system also combines this EM fusion co-display navigation with the software that adjusts the camera (optical signal) functions from the pediatric cystoscope-urethroscope, and co-displays the OMI camera feed (ICG dye) in real time. DICOM images are loaded onto workstation computer by network transfer directly from the DICOM server/client. The graphical user interface includes tri-planar reconstructed views (sagittal, coronal, and axial) next to the images. The coil sensor in the inner stylet guider needle tip gives position and orientation to reformat and display the corresponding CT display planes. The device location within the registered image is updated in real-time at 10 frames per second. Thus both EM and optical information provide position and ICG levels, respectively, in real time simultaneously. This information however, will NOT be used to guide the biopsy needle to a sample location for biopsy or data sampling. The optical and EM data will be analyzed after the procedure and do not impact the biopsy sampling location.

2.4 RISK/BENEFIT ASSESSMENT

2.4.1 Known Potential Risks

Liver biopsy is an invasive technique, with a well-described risk profile, and will be performed as clinically indicated and per the standards of the medical procedure. The biopsy procedure will be performed on the patients who have already been enrolled in other studies and required liver

image-guided biopsies, and we anticipate less than 5 minutes of added procedural time per participant using our designed biopsy device. We will exclude the patients who are not eligible for a routine image-guided biopsy, to minimize the adverse events. In case of any reactions, or detection of any potential risks to the participants or any predicted or un-predicted adverse events, the principal investigator will stop the procedure and all the adverse events will be reported in 24 hours. All the collected samples and data will be coded to maximize the participant's privacy. The records will be kept in locked drawers or password-protected electronic devices to ensure the safety of participants.

2.4.2 Known Potential Benefits

Patients with focal hepatic lesions need biopsies for accurate diagnosis, and the ones with small focal hepatic lesions approximately l< 3 cm required more accurate methods to guide the biopsy needle into the lesion. Accurate diagnosis of HCC or cancer is important in these patients since these patients are usually candidates for focal ablation and subsequent liver transplantation. Optical molecular imaging has been reported as a promising method for better detection of the tumors. Our null hypothesis is that there is discordance greater than 20% between the fluorescence signal during biopsy and histopathology. We anticipate that with the combination of both optical molecular imaging guidance and electromagnetic guidance, we can address issues of tumor heterogeneity, by guiding intra-tumoral sampling towards the most viable and aggressive lesion components to improve the biopsy yield.

2.4.3 Assessment of Potential Risks and Benefits

Since the selected participants are referred from other NIH research protocols that require an image-guided biopsy, they will be assessed for any risk of a percutaneous biopsy under anesthesia or conscious sedation per standard clinical care. Individuals eligible for biopsy based on standard clinical criteria and who have no contraindications to receiving ICG will be referred to this study and enrolled.

Participants will not be offered biopsy for the sole purpose of participation in this trial. Participation in this trial will not significantly alter clinical care. The procedure in this study requires intravenous administration of the FDA-approved agent (ICG) 24 hours before the intervention, and the biopsy procedure will be performed with FDA-cleared devices used as biopsy guides, which help the routine clinically used needle to enter the small lesion in the liver. Therefore, we anticipate that the participants in this study would be at minimal risk for using these devices compared to the ones who undergo a routine image-guided biopsy.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the feasibility of using both the optical molecular imaging technique and EM	Malignancy determination will be based on TBR: TBR≥2 will produce a malignant diagnosis,	The primary endpoint is the concordance between histopathology

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
tracking during standard liver biopsies.	and TBR<2 will produce a benign diagnosis.	outcomes and Target to Background Ratio (TBR) in vivo and ex vivo with a handheld OMI and PIC. Concordance is defined as a TBR ≥2. TBR will be calculated by dividing the mean fluorescence intensity within the lesion by the mean fluorescence intensity within the adjacent liver parencyhyma. This endpoint was *c*hosen because fluorescence microscopy similarly shows a sharp delineation between malignant and normal tissue, with increased ICG uptake in malignant cells in preclinical models. This additive marker aids in better diagnostic procedures to diagnose liver cancer.
Secondary		
To assess the concordance of ICG fluorescence signal at the in vivo site of biopsy with the presence or absence of malignancy, as defined by pathology.	ICG fluorescent signal at the in-vivo site of biopsy using a combination of OMI and EM tracking	We are assessing concordance with the histopathology and measured TBR from two different environments with

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To assess the concordance of ICG fluorescence signal ex vivo (outside of body with camera) of biopsy with the presence or absence of malignancy, as defined by pathology.	ICG fluorescent signal ex vivo of the specimen obtained using a combination of OMI and EM tracking.	approved devices. We expect that they will match but we will need to confirm.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a non-randomized, open-label prospective pilot study to evaluate the feasibility of the combination of optical molecular imaging (OMI) and EM tracking to guide liver biopsies and to detect and measure fluorescent ICG during intrahepatic biopsies. The primary endpoint is the concordance between the histopathology and measured ICG signal (typically TBR by handheld OMI for HCC). Concordance will be evaluated by confirming that all 4 of the following exist: 1.Diagnostic tissue specimen is obtained for conventional pathology analysis, defined by pathologist report of diagnostic liver lesion tissue 2. Imaging confirms that biopsy was taken from region desired 3. An ICG optical signal is obtained by ICG apparatus 4. EM tracking fusion image displays a location of biopsy.

Based on our preliminary data a TBR cut off value of 2.0 is typically considered positive for presence of malignant pathology. We expect that fluorescence intensity measurements will be able to accurately differentiate between benign and malignant lesions. This initial study will enroll 77 participants who have focal hepatic lesions suspicious for HCC or other metastatic liver disease, and for whom based on their primary research protocol, are directed to have a percutaneous diagnostic and or research biopsy.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Liver cancer is the sixth most common malignancy worldwide. This includes hepatocellular carcinoma (HCC) as the most common type of primary liver neoplasm and CRC as the major source of metastasis to the liver. The incidence of HCC and CRC is increasing rapidly, particularly in Western countries. Imaging plays a pivotal part in the diagnosis of HCC, intrahepatic colorectal cancer (CRC), and other liver malignancies; contrast-enhanced cross sectional imaging can often accurately diagnose the tumor non-invasively. Those lesions that are unable to be characterized non-invasively, however, require biopsy for tissue confirmation of malignancy. By providing a definitive diagnosis, focal image guided biopsy is an indispensable component of the care of patients with cirrhosis. Biopsy can also determine the suitability of minimally invasive therapies for HCC, intrahepatic CRC, and other liver malignancies such as thermal ablation or chemoembolization.

Biopsy is not considered a “perfect” test, and a negative biopsy does not exclude the diagnosis of liver malignancies such as HCC or metastatic CRC (3), due to the potential for sampling error. Small hepatic lesions less than 3cm can pose unique biopsy challenge. MRI visible liver lesions are not always visible by ultrasound or CT, the two available modalities in IR and thus may be

"invisible" to the Interventional Radiologist during the procedure. Patient habitus or underlying liver disease can hinder ultrasound detection of small lesions vis a vie overlying adipose tissue in obese patients and the echogenicity of hepatic parenchyma in cirrhotic patients. In CT-guided procedures, an important limitation is the beam-hardening artifact caused by the biopsy needle. At the critical moment when the biopsy needle is in the vicinity of the lesion, this artifact can obscure the lesion and decrease the Interventionalist's confidence in the accurate location of the biopsy target. Indeed, in a retrospective study, up to 45% of small hepatic lesions were insufficiently visualized with the biopsy needle in place (4). The negative predictive value (NPV) in one study was only 63%; that is, 37% of patients with benign biopsies were subsequently found to have malignant lesions at the attempted site of biopsy. In a large series from a partner hospital / institution, the NPV for biopsy of small hepatic lesions was 72% (1): over a quarter of the benign biopsies were in fact false negatives. Confidence in the accuracy of a focal hepatic biopsy is essential to diagnosis, staging, and triage of care, as a false negative biopsy can have a devastating impact on a patient's outcome. Multiple attempts have been made to improve biopsy accuracy. For example, some institutions perform cytological "wet reads" by examining the biopsy tissue under a microscope, while the patient is still on the scanner table. However, this additional step adds a considerable amount of procedural and sedation time and may not always predict the final biopsy result.

We propose a trial to determine the feasibility of using optical molecular imaging (a low cost, real-time, high resolution imaging discipline) for minimally invasive liver biopsy guidance in patients with diagnosed or suspected HCC or metastatic CRC. Patients who require a clinically indicated or separately IRB approved research liver biopsy would after consent, have the OMI performed during biopsy but not as the primary directorate to the biopsy site. The standard of care imaging and cognitive registration would be employed for navigation or guidance of needle and OMI would act as a bystander. Nonetheless, data from OMI imaging would be obtained and evaluated with ex vivo POC OMI and histopathology. Correlations from these studies might support a future larger clinical trial, with different goals that might not exclude efficacy and safety, or even allow for guidance of the needle if separately IRB-approved.

5 STUDY POPULATION

The target study population is patients with focal hepatic lesions with imaging features suspicious for hepatocellular carcinoma or other metastatic liver disease. A radiologist will review patients' imaging for disease appropriate for biopsy. The decision to refer the patient to enroll in this study may occur between during tumor boards or other clinical care meeting where the primary care team, consults with a CC Interventional Radiologist/ AI or PI, Chief of CC/Interventional Radiology.

5.1 INCLUSION CRITERIA

In order be eligible to participate in this study, an individual must meet all of the following criteria:

- Patients must have imaging findings consistent with hepatocellular carcinoma or other liver neoplasms or metastasis, for whom image-guided percutaneous biopsy is planned as clinically indicated or IRB-approved under a separate research protocol.
- Patients must have at least one lesion that can readily be biopsied per Principal Investigator.

- Age >18 years.
- Patients must have the ability to understand and the willingness to sign a written informed consent document.
- Evidence of post-menopausal status or negative urinary or serum pregnancy test for female pre-menopausal patients. Women will be considered post-menopausal if they verbally report amenorrhea for 12 months without an alternative medical cause, or have had surgery or received chemicals to induce menopause.

5.2 EXCLUSION CRITERIA

- History of hypersensitivity reactions to Indocyanine Green (ICG), iodinated contrast, or sulfur-containing compounds.
- Pregnant women and nursing mothers are excluded from this study because of exposure to radiation from CT scanning associated with the biopsy
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, or psychiatric illness/social situations that, in the opinion of the Principal Investigator, would limit compliance with study requirements

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Inclusion of children

HCC is rarely seen in children therefor we will enroll participants > 18 years of age. Children are excluded from this study.

5.3.2 Pregnant Women

Pregnant women and nursing mothers are excluded from this study because of exposure to radiation from CT scanning associated with the biopsy.

5.3.3 Decisionally Impaired

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, that subjects could become decisionally impaired. In these cases, the subject will be taken off study.

5.4 LIFESTYLE CONSIDERATIONS

5.4.1 Disease Community Engagement

The research framework of disease community engagement is not applicable to the proposed study. This feasibility study is at its earliest stages. The anticipated results may not be globally useful yet for the target population of the clinical trial.

5.5 SCREEN FAILURES

Recruitment for this protocol is by invitation only; as such referral to this protocol is dependent on the evaluation of the investigator of the primary protocol. Individuals who do not meet the criteria for participation in this study (screen failure) may be considered for rescreen at the discretion of the PI/ referral team. However, patients who have screened, found eligible, signed a consent, and assigned a unique study ID but become ineligible consequently for various reasons

prior to the biopsy, will be considered inevaluable. These patients will be considered a "screen failure" and his/ her unique study ID will be counted towards overall total accrual.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

This study will be "Enrolling by Invitation Only". Patients will be referred by their NIH primary care team and will need to be registered to an existing NIH clinical study prior to enrolling in this study. Any patient who is referred and found eligible for a standard liver biopsy will be able to screen for this study.

5.6.1 Costs

Potential participants will not incur any costs associated with this protocol.

5.6.2 Compensation

Eligible subjects who sign a consent form will be compensated when ICG is administered in accordance with NIH guidelines (**Table 2**).

All remuneration will be directed to be paid to the participant. These funds are intended to compensate for time away from work that maybe required to participate in this study and time spent receiving ICG. Payment will be sent after the biopsy procedure. Travel will not be provided or compensated for. We will also not provide remuneration for meals and lodging. If a participant withdraws from study, no future compensation will be made, but prior compensation will not be revoked.

Table 2. Subject Reimbursement Schedule

	Inconvenience units*	Pay for inconvenience	Time (h)	Pay for time	Total pay
Contrast IC/Oral	2	$20	3	$40	$60
Peripheral IV	1	$10	N/A	$0	$10
Screening	3	$30	N/A	$0	$30
TOTAL					$100
NOTE:	Patient will be paid $100 when he or she received ICG.				

* Inconvenience unit = $10

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS ADMINISTRATION

ICG is a clinical fluorescent imaging contrast agent and will be ordered from the CC Pharmacy.

Incidence of 1% to 10%: diaphoresis, headache, pruritus, skin discoloration at the intravenous site. In subjects allergic to iodine anaphylactic reactions have occurred. The total dose to be administered will not exceed 40 mg per day. Doses of up to 5 mg/kg have been administered routinely in Japan without adverse reactions. All adverse events experienced by participants will be collected from the time ICG is administered through the end of the procedure. Participants continuing to experience toxicity will be assessed until toxicity has resolved or deemed irreversible.

6.1.1 Study Intervention Description

The study has 2 steps:

a) In-procedure biopsy
b) Ex vivo tissue sample analysis outside of procedure (point of care)

6.1.2 In-procedure biopsy

Tumor biopsy is divided into several stages or parts, described below. The first step is the standard of care preparation of the site and imaging of the liver, infiltration of local anesthesia followed by placement of the PercuNav coaxial needle by ultrasound guidance and EM tracking and co-display into the liver parenchyma. The next step requires a very short pause to thread that pediatric scope inside the PercuNav sheath. This is done by removing the PercuNav inner stylet from the hollow sheath. Once the scope is in the sheath, it is advanced in order to allow for signal sampling from normal liver. After acquiring images from normal liver, the scope is removed and once again the PercuNav needle inner stylet is reinserted, and the needle is advanced and manipulated into the target lesion in the usual standard fashion with standard clinical image-guidance. When the target is reached, the same process ensues, with exchange of the inner stylet for the scope, and subsequent repeat ICG OMI imaging occurs again. Finally, the PercuNav stylet is removed and replaced with a standard spring-deployed biopsy needle, and tissue core biopsy sampling occurs. Except for the two pauses, the biopsy procedure occurs as per usual medical practice, with standard clinical image guidance determining the location of biopsy tissue sampling, as is standard practice for many decades at NIH and around the globe. Thus, in summary, in order to record the data (optical study has 2 steps).

6.1.3 Ex vivo tissue sample analysis outside of the procedure (point of care)

The second step of the study (Point of Care = POC) involves assessment of the tissue sample after it is removed from the body in the usual fashion, thus does not introduce risk to the patient, as it occurs outside of the procedure room itself. The sample is not perturbed, thus is totally analyzable after the study in the standard pathology fashion.

6.1.4 Dosing and Administration

6.1.4.1 Pre-Procedure and ICG Administration

ICG was first FDA cleared in 1959. ICG (IC-Green™ Akorn, Inc. Buffalo Grove, IL) (ICG C43H47N2NaO6S2 Molecular Weight 775) is a water-soluble, tricarbocyanine dye routinely used in dilution studies, hepatic and cardiac function studies and ophthalmic retinal fluoroscopy (20). ICG contains not more than 5% sodium iodide. ICG is indicated for determining cardiac output, hepatic function and liver blood flow, and for ophthalmic angiography. In this study, ICG may be used to roughly determine local tissue hepatic function, after the procedure, for data correlation.

ICG has been shown to localize to HCC and intrahepatic tumors 24-72 hours post-injection with high target to background ratios. Doses of ICG up to 5 mg/kg have been administered without adverse reactions. All patients will receive a dose of ICG 0.5mg/kg, with a maximum dose of 40mg, 18-30 hours (roughly 24 hours) prior to the scheduled procedure. Nurses from 3SE Day Hospital will administer ICG as per drug indications.

After receiving ICG, all patients will be monitored for 30 minutes following the injection to identify any acute adverse reaction, after which point they will be free to leave without any dietary or activity restrictions. Patients will receive contact information prior to injection and biopsy. See Section **6.1.4** for ICG administration procedure.

The usual fluoroscopy dose is 5 - 40 mg (adult dosage). ICG has been used to identify and monitor ocular melanoma (21), retinal metastases, and in the photodynamic therapy of cutaneous Kaposi's sarcoma (22). The recommended dose of up to 40 mg of dye in 1-2 mL of aqueous solvent is safe and in routine clinical use. The maximum dose for this trial will be 40mg ICG. The LD50 after IV administration in rodents is 50 – 80 mg/kg. A bolus of ICG can be administered through any venous access. Immediately following the bolus dose of dye, a 5 mL bolus of sodium chloride 0.9% is given; this regimen delivers a spatially limited dye bolus of optimal concentration to the arterial and arteriolar vasculature. Excitation of ICG occurs at 805 nm with emission maximum at 835 nm. In Japan, ICG is routinely administered to evaluate hepatic function prior to hepatic resection, as ICG is almost entirely and rapidly excreted via the hepatobiliary system. ICG is also used to evaluate vascular lesions of the gastrointestinal tract (e.g. esophageal varices) using NIR endoscopy systems. Recently, ICG, administered intravenously, has also been used in NIR endoscopy systems to successfully differentiate superficial gastric tumors from invasive cancers (23). ICG has also been shown to localize to hepatocellular carcinoma and intrahepatic colorectal cancer metastases 24 – 72 hours post-injection with high target to background ratios. Doses of ICG up to 5 mg/kg have been administered without adverse reactions. The abnormal liver tissue retains ICG, which does not washout and is thus detectable with OMI. See Section **6.2** for ICG preparation and administration.

Administration of ICG will be limited to the day prior to the period in which the biopsy is scheduled.

6.1.4.2 Intra-procedure and ICG fluorescence measurement

On the day of the biopsy, patients will be brought to the biopsy suite in the Interventional Radiology department, Clinical Center, NIH. After standard preparation for the biopsy, skin adhesive/removable fiducials (**Figure 18**) will be placed on the patient's abdomen for registration with electromagnetic imaging (EM). Using EM guidance static images (MRI, CT, PET) are fused to real time imaging (ultrasound, cone beam CT) and a PercuNav needle tracker that registers with EM is placed and directed towards the target lesion. The PercuNav needle is a coaxial needle with tracking capability. The PercuNav needle tracker (**Figure 17**) has a stylet and a hollow core sheath that allows passage of a biopsy needle and an optical guided pediatric cystoscope-urethroscope. There will be at least 3 or more measurements of the ICG fluorescence signal attempted.

Figure 18. Skin adhesives/ removable fiducials

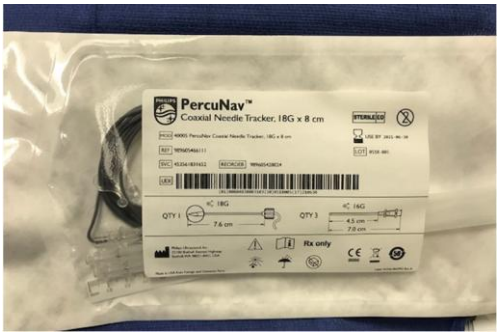


Figure 17. Philips PercuNav needle tracker

The procedure will follow a standard percutaneous liver biopsy using a PercuNav coaxial introducer and tracker along with a commercial needle biopsy device (e.g., Temno). The PercuNav commercial coaxial needle tracker devices have 2 parts – a blunt hollow cannula and a sharp inner needle stylet that punctures the skin and has a wire at its very end that connects to the EM tracking device. In this case, the PercuNav needle tracker provides the access to the skin and the biopsy needle.

The procedure for obtaining measurements of the ICG in vivo has 2 parts: a) In-procedure biopsy b) Ex vivo tissue sample analysis outside of procedure (point of care).

The biopsy is divided into several stages or parts. The first step (biopsy) allows for signal sampling by a commercial cystoscope-urethroscope just from the tip of a commercial coaxial needle cannula, during a percutaneous biopsy. This step is done by pausing during the standard coaxial percutaneous image-guided biopsy procedure, in order to allow for signal sampling from normal liver on the way to the target lesion. The standard needle inner stylet is then replaced, and the needle is manipulated into the target lesion in the usual standard fashion, followed by a repeat sample via the standard outer cannula introducer, after the endoscope is replaced a second time into the outer needle cannula (this time, with the tip residing in the target lesion). Except for the 2 pauses, the biopsy procedure occurs as per usual medical practice, with image guidance determining the location of biopsy tissue sampling, as is standard practice for many decades at NIH and around the globe. Thus, in summary, in order to record the data (optical signal) from 2 locations, there are 2 brief pauses, [no more than several minutes during a 20-60 minute procedure], where the pediatric cystoscope-urethroscope samples optical signal at 1) normal liver on the way to target lesion, and 2) from the target lesion itself (**Figure *19*** and **Figure *20***).

Prior to sending the biopsy tissue to anatomic pathology for clinical diagnosis, the tissue will undergo the second part of the study via the Point of Care (POC) system. This optical imaging device a BWF1 laser generator from the medical laser series will be used with the point of care OMI, which occurs away from the patient, outside the procedure room. This system takes pictures of the ex vivo biopsy core itself, and may, at some point in the future, contribute as a "wet-read" by providing placement information as a cytology technician might.

A study physician will assist the operator and perform all manipulations of the imaging system. This ex vivo process occurs after the tissue is removed from the body in the usual fashion.

Neither the patient nor IR staff are exposed to laser energy, as it occurs outside of the procedure room itself. After completion of the biopsy, participants will recover as per standard clinical practice. Tissue will not be stored or specifically obtained for this study.

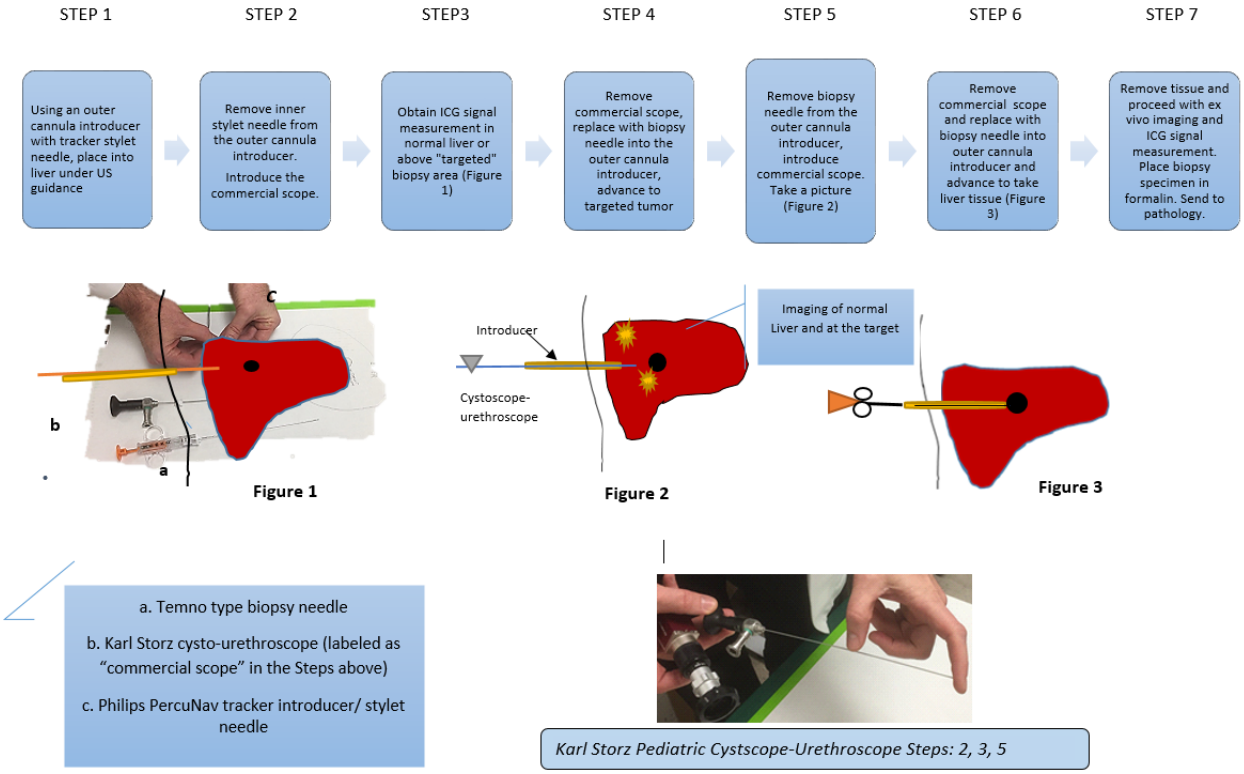
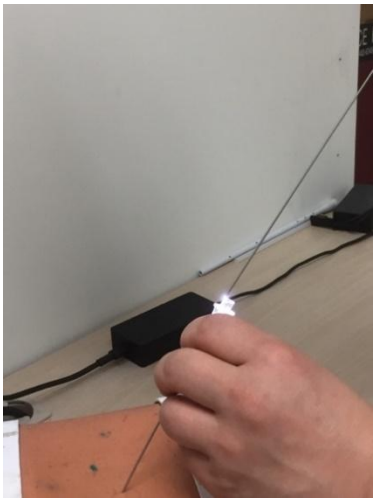


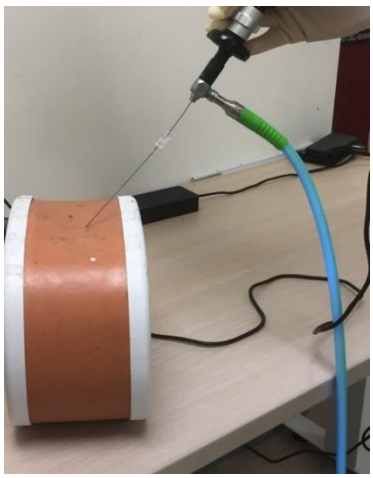
Figure 19. Percutaneous biopsy procedure flow with ICG signal measurement using a coaxial biopsy needle device, a tracked Percunav introducer/ stylet needle, and a pediatric cystoscope-urethroscope ("commercial scope").



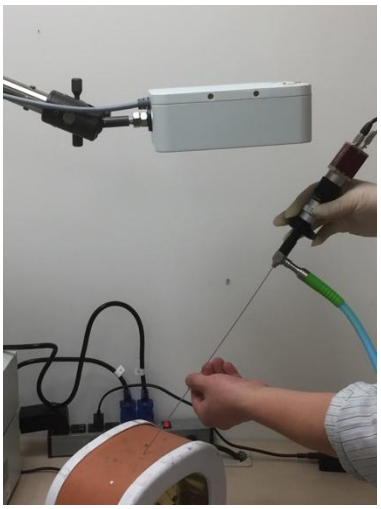
A.Inserting the pediatric scope through the co-axial introducer (after the stylet has been removed).

B.Advancing the scope to the first area of imaging (phantom liver).

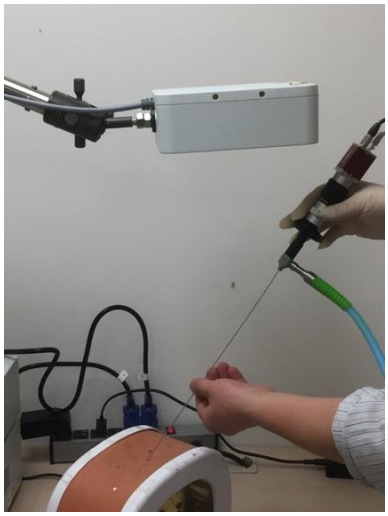
C. Take the first image.



D. Removing the scope.



E. 2nd image after further advancement of the needle



F. Full image of all parts in process with the electromagnetic generator in view

Figure 20. Demonstration on a phantom model of the insertion of the pediatric scope into the PercuNav Coaxial needle introducer**.**

6.1.4.3 Post-procedure care

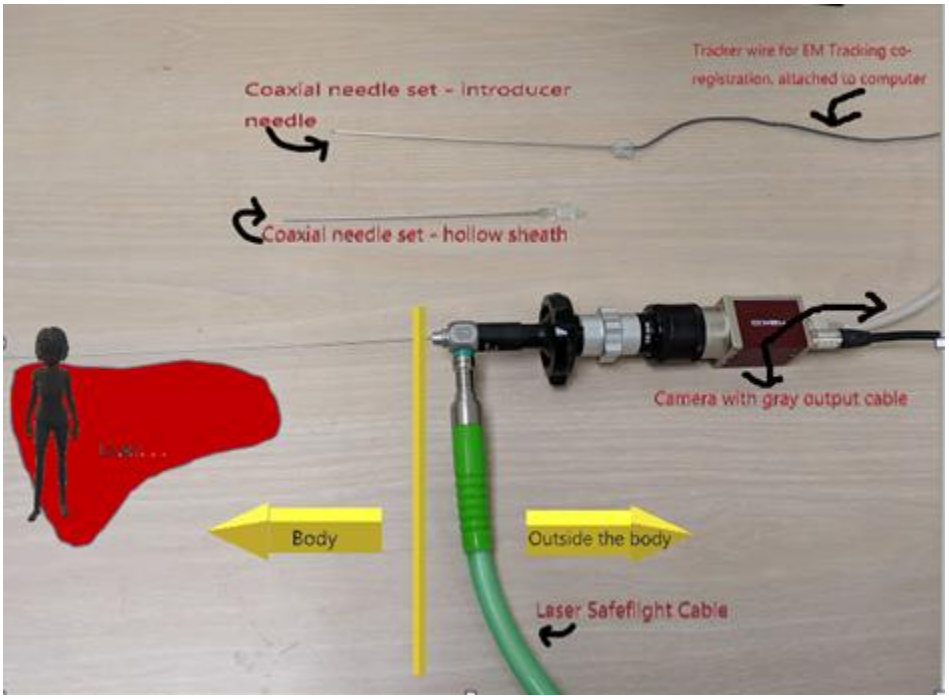
After completion of the procedure, the patients will recover as per standard clinical practice. Participants will be closely monitored for typically 4-6 hours after the procedure as part of their standard post liver biopsy clinical care. All pathological specimens will be evaluated by the NCI Laboratory of Pathology, who will classify the histology of the lesion according to standard criteria. After review, the results of the pathological analysis will be associated with the participant's code.

6.1.4.4 Duration of follow up

Participants will typically be closely observed for 4-6 hours after the biopsy procedure as part of their standard clinical care. Adverse event monitoring will be performed up to the completion of the biopsy monitoring period.

6.1.4.5 Study Device Description and Device Use

We will be using an FDA-cleared optical imaging device and EM tracking hardware devices for the proposed study. The software is not specifically FDA cleared, but the same platform has been extensively used in NIH Clinical Trials dating back to ~2003 in many thousands of patients. The hardware and software components are based upon the design of multiple pre-clinical studies as well as clinical imaging systems that we have previously constructed and tested in patients (EM at NIH and OMI at MGH). The modular design of the system allows for the seamless interchange of a variety of imaging catheters, expanding the depth and breadth of possible applications. The eye-piece of the Safelight cable imaging catheter connects to a laparoscopic or endoscopic camera and captures the infrared emission, processes the image in high resolution and displays it on a surgical display in real-time (Mako camera, Allied Vision Technologies, Stadtroda, Germany).



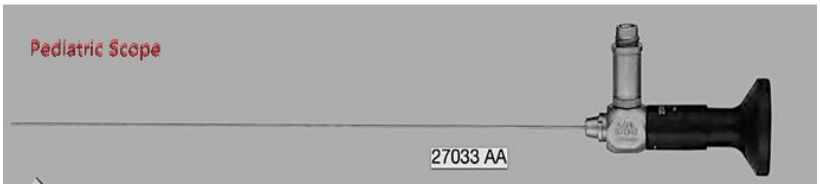


Figure 21. The OMI apparatus labeled, the coaxial needle (PercuNav) and the pediatric scope.

The laser system used for imaging ICG in vivo is a Stryker® IRF L10 Light Source and Safelight Cable which has an FDA 510K clearance and is approved for clinical use. This system has class 1M lasers at 808 and 830 nm. The 808 nm wavelength is the peak excitation wavelength for ICG and is the optimal excitation wavelength for the proposed study. The wavelength, power output and the laser mode (continuous or pulsating) can be by adjusted by the user in the Endoscopic Near-Infrared Visualization (ENV) mode. This system complies with IEC 60825-1:2007 and 21 CFR, Subchapter J, Parts 1040.10 and 1040.11. Additionally, this device is designed to ensure electromagnetic compatibility with other electrical medical devices and complies with IEC 60601-1-2 requirements for EMC with other devices.

Image display and camera functions are controlled on a laptop by a custom software program. We will use a clinically approved pediatric cystoscope-urethroscope (Model 27033 AA, Karl Storz, Tuttlingen, Germany) for the optical assessment. This component was selected due to its small outerdiameter, allowing it to pass through the introducer of a standard 17-gauge core biopsy system. The FDA cleared imaging catheter / scope and the FDA cleared biopsy needle will be the only components that are in contact with the patient. This scope piece will be autoclaved after each patient examination according to manufacturer's guidelines, and the remainder of the handheld device will be wrapped in a sterile drape. Sterilization of components will be performed based on manufacturer's recommendation (http://www.urologicservices.com/documentation/karl-storz-general-reprocessing-instructions.pdf) in consultation with the CC Central Sterile Service.

We will also be using a BWF1 laser manufactured by BW&Tek with the point of care system for *ex vivo* testing, which will occur outside of the procedure room. The excitation light is provided by a 450mW, 785nm laser. Fluorescent light collected by the imaging catheter is filtered to exclude reflected excitation light by a bandpass filter. The eyepiece of the fiber optic imaging catheter connects to a video endoscopic camera which can acquire high resolution, 12 bit images in real time. This laser system as part of the point of care device that will analyze tissue samples only, will not be near the patient, and will be located in a room separate from the clinical suite where the patient is having the biopsy. This laser system is not approved for in vivo use, therefore will only be used ex vivo, and will only be used to test for fluorescence signal after the tissue is obtained and transported to the ex vivo system for analysis.

6.1.4.6 Laser Safety

The NIH approved laser safety protocol and processes will be used only in reference to the B&W Tek BWF5 Coupled Fiber Laser System. A laser safety training SOP and process that is specific to this laser has been developed and approved by (CDR Dennis House), Laser Safety Officer who also conducts the NIH Laser Safety equipment inspections and audits. Persons using the laser will be trained on safety, as well as use. Laser safety training sessions will be conducted in

adherence with NIH policies and guidelines for safe use of laser systems. The laser waves are emitted only in the black box. The room in which the box is kept, will be windowless and the single external door will be placarded with signs "Laser in use", and laser safety goggles will be used by operating personnel and all personnel in the procedure room.

Point of Contact for Laser Safety:
Julie Peretti, RN
Research Nurse Specialist/CIO
10 Center Dr.
Building 10 Rm. 1C305
MSC 1182
Bethesda, Maryland, 20892
301-451-3287
jperetti@cc.nih.gov

The use of class 3b and 4 lasers is regulated by the Occupational Health and Safety Administration (OSHA). The laser will be registered with the NIH Laser Safety Program and reported to the Division of Occupational Health and Safety, NIH, as are all class 3b and 4 lasers.

(http://www.ors.od.nih.gov/sr/dohs/Documents/NIH%20Laser%20Safety%20Program.pdf).

Users will be trained in laser safety and laboratory-specific safe operating procedures will be followed. Additional information about the laser safety program is available at:

http://www.ors.od.nih.gov/sr/dohs/HealthAndSafety/IH/Pages/ih_laser.aspx.

6.1.4.7 Handheld Ocular Molecular Imaging (OMI) device

This is the FDA cleared endoscopic device that is placed through the standard outer cannula introducer (outer part of the FDA cleared coaxial needle system). At no time does an experimental device touch the patient, in any way. The standard flexible and blunt-tip pediatric cystoscope-urethroscope "stylet/camera" is placed inside the introducer cannula to the predetermined length, in order to sample the tissue just immediately at the tip of the outer needle (see **Figure 6**). This tip of the pediatric cystoscope-urethroscope is like a non-sharp blunt felt-tip pen tip that cannot pierce the skin or tissue with normal forces of gentle manual manipulation, as is intended. It is a hand-held device used by an experienced interventional radiology physician who is certified and credentialed to practice (image guided therapy) interventional radiology. The camera and safelight cable are never inserted into the patient's tissue beyond the ~ 1mm tip of the 17 Gauge outer cannula coaxial needle. The tissue (just beyond the pediatric cystoscope-urethroscope tip in the lesion) is sampled by the standard spring-loaded biopsy needle.

Liver biopsy is an invasive technique, with a well-described risk profile, and will be performed as clinically indicated and per the standards of the medical procedure. The 2 pauses to take signal sample with standard devices during the biopsy, is of non-significant risk (or minimal risk) in the opinion of the Principal Investigator the research team and others during the institutional scientific review process. The FDA-cleared pediatric cystoscope-urethroscope is not normally used via this needle cannula, tube, or lumen, but it is compatible.

The OMI is a handheld endoscopic instrument consisting of an FDA-cleared and clinically used pediatric cysto-urethroscope (Model 27033 AA; Karl Storz, Tuttlingen, Germany) illuminated by a safelight cable attached to an 808 nm laser excitation source (Stryker IRF L10 Light Source

and Safelight Cable) with an adjustable user feature in the Endoscopic Near-Infrared visualization (ENV) mode that can pass coaxially through the introducer of a standard 17-gauge core biopsy system (**Figure 6**).

The eyepiece of the pediatric cystoscope-urethroscope is attached to high-definition endoscopic camera system sensitive in the visible and infrared spectrum. (Stryker 1588 Camera Control Unit and Camera Head). The optical image is transferred from the surgical site to the camera unit by the aforementioned pediatric cysto-urethroscope which are attached to the camera head (Mako; Allied Vision Technologies) and the light output is by a filtered band-pass filter that is compatible with the laser excitation source. Expanded details of the device is described in Section **2.3.8**.

6.1.4.8 Point-of-care (POC) Optical Molecular Imager (OMI)

This is a “camera in a black box” taking images of the ex vivo tissue using a BWF1 laser to excite light to see the ICG fluorescence. Specifically, the core biopsy tissue specimen is placed in a sterile container and removed to a windowless room adjacent to the IR suite. The tissue is placed inside a 2 foot tall black box on a benchtop with the laser. The laser does not come in contact with the patient, nor is it in the same room as the patient at any time. Details about the POC device is found in Section **2.3.9**.

6.1.4.9 Non-significant Risk Determination Criteria

The proposed study meets the definition of a non-significant risk device and is subject to abbreviated IDE regulations as stated in 21 CFR Part 812.2(b):

Requirements for labeling the device (§812.5)

Maintaining IRB approval of the study as a Non-significant risk study (§56)

Ensuring all subjects provide informed consent (§50)

Monitoring the conduct and compliance of all sub-investigators and evaluating all adverse device effects to determine whether the study is safe to continue (§812.46 [a & b])

Maintaining records of the following (§812.140[b][4] and [5]):

a) basic device information
b) brief explanation of why the device is non-significant risk (See Below)
c) all adverse events, whether anticipated or unanticipated

Making reports of the following (§812.150[b][1-3] & [5-10]):

a) continuing review as required by the IRB
b) deviations from the investigational plan to the IRB
c) final report upon study termination/completion to IRBs
d) device (or parts) recall/returns/repairs

Restrictions on promoting (812.7[a]), charging subjects for the device (812.7[b]), prolonging an investigation (812.7[c]), and stating a device's safety/efficacy (812.7[d])

6.1.4.10 Brief explanation of why the device is non-significant risk and applicability of abbreviated IDE regulations

Regulatory issues for this study can be separated into invasive biopsy procedure risk, specific device risk and overall study risk. The biopsy risk will be undertaken regardless of this study, as it is clinically indicated or separately approved under a separate IRB approved protocol. However, because the research is related to the invasive biopsy procedure, the risk involving this invasive procedure pre-exists.

Devices used are FDA cleared, but the pediatric cystoscope-urethroscope is being used off-label, since its FDA indication-for-use clearance does not directly specify use through a cannula needle prior to liver biopsy sampling, during the overall liver biopsy procedure. The outer needle cannula is a sort of "lumen", but the use described is away from the original intended use.

The FDA's abbreviated IDE regulations are applicable to the study because although the devices to be used have received 510(k) FDA clearances, the research study itself in which they are to be used is related to an invasive procedure (regardless of it being clinically indicated). Thus, the research study involves an "invasive sampling" and has inherent risks associated with it. Despite the invasive sampling, we are confident that this research will not add significant risk because the added time required for the signal sampling (ICG signal) is short, and this added time will be a small fraction of the overall time required for a standard biopsy using the standard tools used for NIH biopsy. This is minimal added risk, if definable at all. The optical and molecular signal acquired from the tissue will be obtained via the coaxial biopsy cannula (through the outer biopsy needle conduit / cavity). Thus the study meets the IDE abbreviated requirements of the FDA, and the risk determination is non-significant risk (sub-category minimal risk).

The study determines the overall research determination and we will adhere to the FDA abbreviated IDE regulations per 21 CFR Part 812.2(b).

- This study will be using FDA 510(k) cleared devices. See APPENDICES for FDA 510(k) clearance letters to Karl Storz Imaging, Inc, Akorn, Inc., and Philips Invivo Corporation for permission to market and outlines for Indications for Use
- FDA 510 (k) Clearances for Karl Storz Imaging, Inc. The following Karl Storz Imaging, Inc. devices are FDA 510(k) cleared:
  1) Karl Storz Uretero-Renoscope/ Ureteroscope (Appendix A)
  a. June 24, 1994: FDA Clearance K940464/A
  2) Karl Storz-Endoscope Sterilization Trays (Appendix B)
  b. August 18, 2009: FDA Clearance K090818
- This study will be using FDA 510(k) cleared devices. See attached FDA 510(k) clearance letters to Karl Storz Imaging, Inc, Akorn, Inc., and Philips Invivo Corporation for permission to market and outlines for Indications for Use. The following Karl Storz Imaging, Inc. devices are FDA 510(k) cleared:
  1) Karl Storz Uretero-Renoscope/ Ureteroscope (Appendix A)
  a. June 24, 1994: FDA Clearance K940464/A
  2) Karl Storz-Endoscope Sterilization Trays (Appendix B)
  b. August 18, 2009: FDA Clearance K090818

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

ICG is a clinical fluorescent imaging contrast agent and will be ordered from the CC Pharmacy).

6.2.2 Formulation, Appearance, Packaging, and Labeling

(https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/011525s017lbl.pdf)

Injection: 25 mg freeze-dried powder in vials supplied with 10 ml aqueous solvent NDC 7478-701-02 (pH 5.5 – 6.5). Manufactured as IC-Green™ Akorn, Inc. Buffalo Grove, IL.

PREPARATION: PHARMACY PREPARATION INSTRUCTIONS (AS PER ICG PACKAGE INSERT): One or two vials of ICG 25 mg will be dissolved with 2 ml diluent, provided by the manufacturer (yielding ICG in solution at 12.5 mg/mL concentration). The CC Pharmacy will prepare the ICG for infusion.

Incidence of 1% to 10%: diaphoresis, headache, pruritus, skin discoloration at the intravenous

site. In subjects allergic to iodine anaphylactic reactions have occurred. The total dose to be administered will not exceed 40 mg per day. Doses of up to 5 mg/kg have been administered routinely in Japan without adverse reactions. All adverse events experienced by participants will be collected from the time ICG is administered through the end of the procedure. Participants continuing to experience toxicity will be assessed until toxicity has resolved or deemed irreversible.

6.2.3 Product Storage and Stability

Storage: Store at 15° to 25° C (59° to 77°).

Stability: Indocyanine Green for Injection USP is unstable in aqueous solution and must be used within 6 hours. However, the dye is stable in plasma and whole blood so that samples obtained in discontinuous sampling techniques may be read hours later. Sterile techniques should be used in handling the dye solution as well as in the performance of the dilution curves. If a precipitate is present, discard the solution.
https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/011525s017lbl.pdf.

6.2.4 Preparation

The CC Pharmacy will prepare ICG for infusion". The drug is supplied in a kit (NDC 17478-701-02) containing six 25 mg IC-GREEN vials and six 10mL Aqueous Solvent ampules. NDC 17478-701-25 IC-Green vial. 25 mg fill in 50 mL vial. NDC 17478-701.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Off-Treatment Criteria:

- Completion of biopsy and biopsy observation period (typically no more than a 6-hour stay in the recovery area after the biopsy procedure)
- Participant requests to be withdrawn from the biopsy procedure.

- Allergic reaction (as described in section **8.5**) and serious adverse event that is definitely related to the research.
- Unacceptable toxicity per PI
- Investigator discretion
- Loss of capacity to provide informed consent

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Off-Study Criteria:

- 1 ± 2 months of the completed biopsy (for all correlative pathological assessment of biopsy specimen availability)
- Participant requests to be withdrawn from study
- Death

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

The target study population is patients with focal hepatic lesions with imaging features suspicious for hepatocellular carcinoma or other metastatic liver disease. A radiologist will review patients' imaging for disease appropriate for biopsy. The decision to refer the patient to enroll in this study may occur between during tumor boards or other clinical care meeting where the primary care team, consults with a CC Interventional Radiologist/ AI or PI, Chief of CC/Interventional Radiology.

The research team will review and verify the following available information prior to patient registration on the protocol:

1. Clinical evaluation by referring team/ primary care team
2. Laboratory tests (blood work)
3. Radiology tests with report of focal lesions within the liver and confirmed or suspicious HCC other liver neoplasms or metastasis

8.2 REGISTRATION PROCEDURES

The Principal Investigator, along with key research personnel, will screen and enroll all eligible patients on the study. The screening and enrollment process will be documented in CRIS. All patients enrolled in the study will receive a unique patient identifier and will be entered in the study's screening and enrollment logs which are kept in our research database. The study research database will be maintained in an electronic folder in a larger central shared drive with a secure password and Clinical Center network back-up, in accordance with NIH CC policy.

8.3 TREATMENT ASSIGNMENT AND RANDOMIZATION/STRATIFICATION PROCEDURES COHORTS

Cohorts

Number	Name	Description
1	Patients	Patients scheduled for liver biopsy

Arms

Number	Name	Description
1	Interventional therapy	Liver biopsy with optical imaging and EM tracking guidance

8.4 EFFICACY ASSESSMENTS

8.4.1 Biospecimen Evaluations

We will be using concordance with pathology as gold standard by measuring TBR (See Statistical section). ICG measurements to obtain the TBR will be done in vivo and ex vivo inside the IR suite. Specimens for pathology will be evaluated for quality and for diagnosis by NCI Laboratory of Pathology. No biospecimens will be collected and stored specifically for our research.

8.5 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

To comply with the abbreviated IDE regulations 21 CFR 812.2(b), records concerning adverse device effects should include anticipated and unanticipated events and complaints.

Exceptions to adverse event data collection:

- Expected events related to the clinical biopsy procedure (for example, but incomplete list):
    - Pain or bruising at the biopsy site.
    - Increased redness, swelling or bloody or serosanguinous drainage at the biopsy site.
    - Events directly related to local anesthetic administration such as nausea, dizziness, numbness or tingling.
    - Self-resolved transient hypotension or bradycardia.
    - Diaphoresis
    - Headache
    - Pruritus
    - Skin discoloration at the intravenous site.

8.5.1 Definition of Adverse Event

8.5.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

8.5.1.2 Adverse Device Effect

An adverse device effect (ADE) is an AE related to the use of our devices under study: the Karl Storz devices, Stryker® IRF Light Source and Safelight Cable, UroNav, or PercuNav.

8.5.1.3 Unanticipated Adverse Device Effect

Defined as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or other unanticipated serious problem associated with a device that relates to the rights, safety and welfare of subjects.

8.5.2 Definition of Serious Adverse Events (SAE)

8.5.2.1 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in the following:

- death
- serious deterioration of the subject's health, leading to:
    - life-threatening illness or injury, or
    - permanent impairment of a body structure or function, or
    - in-patient or prolonged hospitalization, or
    - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment of a body structure or function

8.5.2.2 Serious Adverse Device Effect (SADE)

A serious adverse device effect (SADE) is an ADE that results in any of the consequences characteristic of an SAE. An unanticipated SADE (USADE) is a SADE which is not anticipated by the risk analysis, while an anticipated SADE (ASADE) is a SADE which is anticipated by the risk analysis.

8.5.3 Classification of an Adverse Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

8.5.3.1 Severity of the Event

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious"

8.5.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.5.3.3 Expectedness

The Principal Investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.5.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

As this is a feasibility study, only grade 3 and above adverse events that are possibly, probably and definitely related to the research procedure (i.e., use of biopsy assisting devices and ICG administration) will be recorded, and reported if applicable. Given the decades' long use of the ICG as an approved agent, and the dose range used, which is often less than what is used clinically, we do not anticipate any toxicity.

All applicable AEs as described above, including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All recordable AEs occurring while on study must be documented appropriately. All reportable AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

8.5.5 Adverse Event Reporting

Adverse event monitoring will be performed from the time the consent is obtained until the patient is off treatment (4-6 hours post biopsy procedure). Adverse event reporting will be performed during this timeframe. Any reported Grade 3 and above adverse events that are possibly, probably, and definitely related to the research procedure, will be followed until resolution or stabilization.

8.5.6 Serious Adverse Event Reporting

The study investigator shall complete an Unanticipated Adverse Device Effect Form and submit to the study sponsor and to the reviewing Institutional Review Board (IRB) as soon as possible, but in no event later than 7 calendar days after the investigator first learns of the effect. The study sponsor is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRBs and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effect as FDA requests.

8.6 UNANTICIPATED PROBLEMS

8.6.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a

greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.6.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

8.6.3 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NIH Intramural IRB.

9 STATISTICAL CONSIDERATIONS

Three medical procedures will be conducted to diagnose liver lesions as benign/malignant (negative/positive):

(1) Histopathology, using well-established standard procedures.
(2) ICG fluorescent signal at the in-vivo site of biopsy using a combination of OMI and EM tracking.
(3) ICG fluorescent signal ex vivo of the specimen obtained using a combination of OMI and EM tracking.

For (2) and (3), malignancy determination will be based on TBR: TBR≥2 will produce a malignant diagnosis, and TBR<2 will produce a benign diagnosis.

One main objective is to evaluate the agreement between (1) and (2), and between (1) and (3), with (1) considered as the gold standard. Since determinations are dichotomous, Cohen's kappa will be used to assess agreement.

9.1 Statistical Hypothesis

In terms of concordance, i.e. the proportion of EM+optical imaging and histopathology test results that match, the null hypothesis is that concordance ≤ 80%, and that the expected concordance (or concordance worth detecting) is 90%. Based on a positivity prevalence of 80%, i.e. 80% true malignant and 20% true benign, an 80% concordance can produce a kappa value ranging from 0 to 0.55; and a 90% concordance can produce a kappa value ranging from 0.62 to 0.74. Based on these numbers, the null and alternative hypotheses for this study are set in terms of kappa as follows:

- H_0: kappa ≤ 0.4
- H_1: kappa > 0.4

9.2 Sample Size Determination

Based on the null kappa value k_0=0.4, a kappa value k_1=0.7 worth detecting, alpha (Type I error) equal to 0.05, 80% power, and a prevalence (true positive rate) of 80%, the resulting sample size from a one-sided test is 77.

Generally, kappa=0.4 is considered as fair/moderate agreement, and kappa=0.7 as substantial agreement.[1] With an 80% true positive rate, kappa=0.4 corresponds to an 86% sensitivity and 55% specificity, and kappa=0.7 corresponds to a 93% sensitivity and 80% specificity

With data on 77 cases, it is worthwhile determining the optimal cut-off point for TBR to confirm whether TBR=2 is optimal. This can be done based on Youden's index in receiver operating characteristic (ROC) analysis.

9.3 POPULATIONS FOR ANALYSES

The target study population is patients with focal hepatic lesions with imaging. Features are suspicious for hepatocellular carcinoma or other liver neoplasm. Patients whose imaging findings suggest liver cancer (hepatocellular carcinoma) or other metastatic liver cancer for whom image-guided percutaneous biopsy is planned.

Accurate diagnosis of HCC and other liver malignancies is important in these patients since these patients may be candidates for liver transplantation or bridging transarterial chemoembolization or palliative focal ablation with systemic chemotherapy. Eligible patients demonstrate a focal hepatic lesion for which percutaneous image-guided biopsy is planned. The subject selection based on the race, ethnicity, sex and age has been considered based on the reported statistics of the HCC population.

Recruitment of the participants will be through the referring clinicians at NIH. The accrual will extend to different race and ethnicities and both sex. Since the safety of EM methods and ICG is not reported in children, we will enroll participants > 18 years to avoid any possible adverse events in children

9.3.1 Evaluable for toxicity

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Incidence of 1% to 10%: diaphoresis, headache, pruritus, skin discoloration at the intravenous site. In subjects allergic to iodine, anaphylaxis reactions have occurred. The total dose administered will not exceed 40 mg per day. Of note, doses of up to 5 mg/kg have been administered as routine in Japan without adverse reactions. All adverse events experienced by participants will be collected from the time ICG is administered, through the study, and until the patient is off treatment.

9.3.2 Evaluable for objective response

The metric to evaluate where ICG localizes is TBRs and will be calculated by dividing the mean fluorescence intensity within the lesion by the mean fluorescence intensity within the adjacent

[1] Landis, J.R. & Koch, G.G. (1977). "The measurement of observer agreement for categorical data". Biometrics, 33 (1): 159–174.

liver parenchyma with in vivo and ex vivo samples. All specimens will be sent to NCI Laboratory of Pathology for diagnosis and research as defined by the patient's primary protocol.

9.3.3 Evaluable Non-Target Disease Response

The intervention (guided biopsy) is performed at a single point, and will not alter disease burden. Thus, no patients will be classified as evaluable for non-target disease response.

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

All patients who enroll in the study and complete the biopsy procedure will be evaluable for an objective response. Response is defined as localization of the target lesion and lends information regarding the feasibility of using the optical imaging device and EM tracking tool to guide liver biopsies. Fluorescence microscopy showing a clear delineation between malignant and normal tissue is also an objective response.

The main intervention of the study is the ability to improve biopsy guidance. Please note that standard pathology results of the hepatic biopsies will be part of routine clinical care and will be the responsibility of the NIH primary team to review and to provide appropriate follow up and clinical management. The tracking and fluorescence results will not be shared with participants, as these results are unlikely to have direct effect clinically on patient care. In addition to routine histological analysis, additional analysis for specific molecular markers will be performed in the NCI Laboratory of Pathology, as clinically indicated. However, all study-specific results are bench studies and will not be used in patient care decision making, thus we do not anticipate obtaining any incidental findings since the intervention is technical in nature regarding the biopsy procedure itself.

The main intervention of the study is the ability to improve biopsy guidance. Please note that standard pathology results of the hepatic biopsies will be part of routine clinical care and will be the responsibility of the NIH primary team to review and to provide appropriate follow up and clinical management. The tracking and fluorescence results will not be shared with participants, as these results are unlikely to have direct effect clinically on patient care. In addition to routine histological analysis, additional analysis for specific molecular markers will be performed in the NCI Laboratory Pathology, as clinically indicated. However, all study-specific results are bench studies and will not be used in patient care decision making, thus we do not anticipate obtaining any incidental findings since the intervention is technical in nature regarding the biopsy procedure itself.

9.4.2 Analysis of the Primary Endpoints

Completion of primary end and secondary endpoint analysis – The majority of data analysis can be performed on a per patient basis, thus analysis will be ongoing along with enrollment and does not need to wait until all subjects are enrolled to begin analysis.

Final Report - All primary and secondary data analysis along with completion of the final study report will be completed within one month of data collection completion. We anticipate that 100% of the projected enrollment will be completed by 4 years. Data collection (including correlative pathology) will be completed by 4 years. We anticipate global primary and secondary analysis along with the final report will be completed in 5 years.

9.4.3 Analysis of the Secondary Endpoints

Concordance will be evaluated by confirming that all 4 of the following exist:

1. Diagnostic tissue specimen is obtained for conventional pathology analysis, defined by pathologist report of diagnostic liver lesion tissue
2. Imaging confirms that biopsy was taken from region desired
3. An ICG optical signal is obtained by ICG apparatus
4. EM tracking fusion image displays a location of biopsy.

9.4.4 Safety Analyses

As this is a feasibility study, only grade 3 and above adverse events that are possibly, probably and definitely related to the research procedure (i.e., use of biopsy assisting devices and ICG administration) will be recorded, and reported if applicable. Given the decades' long use of the ICG as an approved agent, and the dose range used, which is often less than what is used clinically, we do not anticipate any toxicity.

To comply with the abbreviated IDE regulations 21 CFR 812.2(b), records concerning adverse device effects should include anticipated and unanticipated events and complaints.

Exceptions to adverse event data collection:

- Expected events related to the clinical biopsy procedure (for example, but incomplete list)
    - Pain or bruising at the biopsy site.
    - Increased redness, swelling or bloody or serosanguinous drainage at the biopsy site.
    - Events directly related to local anesthetic administration such as nausea, dizziness, numbness or tingling.
    - Self-resolved transient hypotension or bradycardia.
    - Diaphoresis
    - Headache
    - Pruritus
    - Skin discoloration at the intravenous site.

9.4.5 Baseline Descriptive Statistics

The following will be collected while the patient remains on the study:

- Demographics – age, ethnicity, sex, diagnosis
- Imaging pertaining to liver disease diagnosis
- Lesion information– included but not limited to size, number, location
- Intra-procedural details (included but not limited to)
    - start and end time of the procedure
    - intraprocedural scans used (ex: Ultrasound, Cone beam CT or Fluoroscopy)
    - ICG fluorescence signal (Target to Background/ TBR measurement)
- Post-procedural information
    - Specimen information – cores or fine needle aspirations, location specimen was collected, histopathology and diagnosis, H&E and immunohistochemistry (IHC) staining results

The following device information will be collected and kept in the study's regulatory binder while the study is ongoing:

- Basic device information – labeling of the device should include the name and place of business of the manufacturer (in accordance with 21 CFR 801.1), the quantity of the contents if applicable and the following statement, "CAUTION – Investigational device. Limited by Federal law to investigational use." The label should also describe all relevant contraindications, hazards, adverse effects, interfering substances or devices, warnings, and precautions.
- Any information regarding a return, repair or disposition of the device or any units of the device.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent Procedures and Documentation

All patients who are being considered for this trial will undergo informed consent prior to being enrolled in the trial, as well as evaluation by multi-disciplinary review. A forthright and detailed discussion of the biopsy options available to the patient and family members, shall occur at the time of consent. The experimental nature of the treatment, its theoretical advantages and disadvantages, and an overview of the procedure and anticipated convalescence are presented. The Informed Consent document is given to the patient and they are asked to review it, make notes and follow-up with a phone call to the physician or nurse investigator to have any additional questions answered prior to considering treatment on protocol.

When the patient is admitted to the Clinical Center for treatment, the PI, or AI will perform the consenting process.

The Investigator will obtain written informed consent from each patient. The informed consent form will contain all the Essential Elements of Informed Consent set forth in 21CFR, Part 50, the ICH Guideline for Good Clinical Practice, and the terms of the Declaration of Helsinki. Copies of the signed document should be given to the patient and filed in the Investigator's study file, as well as the patient's medical record if in conformance with Standard Operating Procedures.

10.1.2 Participation of Subjects Who Are/Become Decisionally Impaired

Adults unable to give consent are excluded from enrolling in the protocol as outlined in section **5.3.3**.

10.1.3 Telephone Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was returned.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented on a progress note by the consenting investigator.

The investigator will confirm that, when required, written legally effective consent has been obtained prior to initiating any study interventions.

10.2 Study Discontinuation and Closure

Below are the criteria to be met before the study could be discontinued:

- Completion of data collection time – The intervention, a hepatic biopsy guided with novel combination of devices, is concluded the same day as the scheduled biopsy. All correlative pathologic assessment of biopsy specimens will occur within 1 ± 2 months of the completed biopsy. We expect to complete all data collection for a single subject within 1 ± 2 months of the study procedure.
- Completion of primary end and secondary endpoint analysis – The majority of data analysis can be performed on a per patient basis, thus analysis will be ongoing along with enrollment and does not need to wait until all subjects are enrolled to begin analysis.
- Final Report - All primary and secondary data analysis along with completion of the final study report will be completed within one month of data collection completion. We anticipate that 100% of the projected enrollment will be completed by 4 years. Data collection (including correlative pathology) will be completed by 4 years. We anticipate global primary and secondary analysis along with the final report will be completed in 5 years.
- Plan of action if study fails to meet accrual milestones - If the study fails to meet accrual milestones, we will review our recruitment strategy and consider changing the status to "Open – Recruiting" to improve accrual.

10.3 Confidentiality and Privacy

All the collected samples and data will be coded to maximize the participant's privacy. The records will be kept in locked drawers or password-protected electronic devices to ensure the safety of subjects.

10.4 Future use of Stored Specimens and Data

Stored specimens are not anticipated with this protocol.

10.5 Safety Oversight

Safety oversight will be performed through the NIH Clinical Center's Quality Assurance Program.

10.6 Clinical Monitoring

The clinical research team will meet on a regular basis when patients are actively receiving an intervention on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

Accrual and safety data will be monitored by the principal investigator, who will provide oversight to the conduct of this study. The PI will continuously evaluate implementation of the protocol for any unusual or unpredicted complications that occur and will review the data for accuracy and completeness.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10.7 Quality Assurance and Quality Control

The NIH Clinical Center's Quality Assurance Program will conduct study monitoring at least annually or more frequently as required per 21 CFR 812.46. Patient consent documents, primary outcome and safety laboratory results and diagnostic test results will be monitored for accuracy, correct dating, and agreement between case report forms and source documents. All regulatory reports, reviews and amendments, adverse events and problem reports related to study, along with investigator credentials, training records, and the delegation of responsibility log will also be reviewed during monitoring visits. Monitoring visits will also include evaluation of conduct and compliance of all sub-investigators. Any major findings will be summarized in writing and reported to the study PI who will be responsible for submitting the monitoring report to the IRB.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Completion of data collection time – The intervention, a hepatic biopsy guided with novel combination of devices, is concluded the same day as the scheduled biopsy. All correlative pathologic assessment of biopsy specimens will occur within 1 ± 2 months of the completed biopsy. We expect to complete all data collection for a single subject within 1 ± 2 months of the study procedure.

Completion of primary end and secondary endpoint analysis – The majority of data analysis can be performed on a per patient basis, thus analysis will be ongoing along with enrollment and does not need to wait until all subjects are enrolled to begin analysis.

Accrual data will be monitored by the Principal Investigator, who will provide oversight to the conduct of this study. As this is a feasibility study, safety data collection is minimal and will be concentrate on ICD dye administration side effects, if any. The PI will continuously evaluate implementation of the protocol for any unusual or unpredicted complications that occur and will review the data for accuracy and completeness.

10.8.2 Study Records Retention

Study record retention will conform to regulatory requirements as per 21CFR 312.57. The investigator must maintain the required records for a period of two years after the date the investigation is completed or terminated or the records are no longer required to support a PMA or PDP, whichever date is later.

10.9 PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to Clinical Center Program Official. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.9.2 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; other study documents, and
  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1 Human Data Sharing Plan

De-identified human data generated in this research for future research may be shared with the following:

- In an NIH-funded or approved public repository clinicaltrials.gov
- In BTRIS
- In publication and/ or public presentations at the time of publication or shortly thereafter

De-identified data may be shared with approved outside collaborators under appropriate agreements and upon IRB approval.

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Cooperative Research and Development Agreement (*number pending*)

The study devices, PercuNav and Uronav, are provided by the company, Philips under a Collaborative Agreement [Cooperative Research and Development Agreement (CRADA)].

10.11.2Material Transfer Agreement (*number pending*)

Coded (linked) data will be shared with Umar Mahmood, MD at Massachusetts General Hospital under MTA. Decryption code to re-link data will not be shared with Dr. Mahmood.

10.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH Clinical Center has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

3D	Three Dimentional
AE	Adverse Event
ADE	Adverse Device Effect
ASADE	Anticipated Serious Adverse Device Effect
CC	Clinical Center
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CRC	Colorectal Cancer
CRF	Case Report Form
DHHS	Department of Health and Human Services
DICOM	Digital Imaging and Communications in Medicine
EM Tracking	Electromagnetic Tracking
FDA	Food and Drug Administration
FDG	Fludeoxyglucose
GMP	Good Manufacturing Practices
HCC	Hepatocellular Cancer
HD	High Definition
HIPAA	Health Insurance Portability and Accountability Act
ICG	Indocyanine Green
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IR	Interventional Radiology
IRB	Institutional Review Board
IRF	Infrared Fluorescence
IV	Intravenous
MR	Magnetic Resonance
NCI	National Cancer Institute
NIH	National Institutes of Health
IC	Institute or Center
NIR	Near Infrared
OHRP	Office for Human Research Protections
OMI	Optical Molecular Imaging
OSHA	Occupational Health and Safety Administration
PET	Positron Emission Tomography
PI	Principal Investigator
POC	Point of Care
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SADE	Serious Adverse Device Effect

SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TBR	Tumor to Background Ratio
UP	Unanticipated Problem

12 REFERENCES

1. Ma X (1), Arellano RS, Gervais DA, Hahn PF, Mueller PR, Sahani DV. Success of image-guided biopsy for small (≤ 3 cm) focal liver lesions in cirrhotic and non-cirrhotic individuals. J Vasc Interv Radiol. 2010 Oct;21(10):1539-47; quiz 1547.
2. Bialecki ES, Ezenekwe AM, Brunt EM, Collins BT, Ponder TB, Bieneman BK, et al. Comparison of liver biopsy and noninvasive methods for diagnosis of hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2006 Mar;4(3):361–8.
3. de Lope CR, Tremosini S, Forner A, Reig M, Bruix J. Management of HCC. J. Hepatol. 2012;56 Suppl 1:S75–87.
4. Stattaus J, Kuehl H, Ladd S, Schroeder T, Antoch G, Baba HA, et al. CT-guided biopsy of small liver lesions: visibility, artifacts, and corresponding diagnostic accuracy. Cardiovasc Intervent Radiol. 2007 Sep;30(5):928–35.
5. Kokudo N, Ishizawa T. Clinical Application of Fluorescence Imaging of Liver Cancer Using Indocyanine Green. Liver Cancer. Karger Publishers; 2012;1(1):15–21.
6. 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 Jun 1;115(11):2491–504.
7. Morita Y, Sakaguchi T, Unno N, Shibasaki Y, Suzuki A, Fukumoto K, et al. Detection of hepatocellular carcinomas with near-infrared fluorescence imaging using indocyanine green: its usefulness and limitation. Int J Clin Oncol. 2011 Dec 27.
8. Schaafsma BE, Mieog JSD, Hutteman M, van der Vorst JR, Kuppen PJK, Löwik CWGM, et al. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. J Surg Oncol. 2011 Sep 1;104(3):323–32.
9. Funovics MA, Alencar H, Montet X, Ralph Weissleder, Mahmood U. Simultaneous fluorescence imaging of protease expression and vascularity during murine colonoscopy for colonic lesion characterization. Gastrointest Endosc. 2006 Oct 1;64(4):589–97.
10. Kar S, Kumar A, Gao F, Qiu B, Zhan X, Yang X. Percutaneous optical imaging system to track reporter gene expression from vasculatures in vivo. Journal of biomedical optics. 2006 May;11(3):34008.
11. Sarantopoulos A, Beziere N, Ntziachristos V. Optical and opto-acoustic interventional imaging. Ann Biomed Eng. 2012 Feb;40(2):346–66.
12. Taruttis A, Ntziachristos V. Translational optical imaging. American Journal of Roentgenology. 2012 Aug;199(2):263–71.
13. Yang X. Interventional molecular imaging. Radiology. 2010 Mar;254(3):651–4.
14. Funovics MA, Ralph Weissleder, Mahmood U. Catheter-based in vivo imaging of enzyme activity and gene expression: feasibility study in mice. Radiology. 2004 Jun 1;231(3):659–66.
15. Sheth RA, Tam JM, Maricevich MA, Josephson L, Mahmood U. Quantitative endovascular fluorescence-based molecular imaging through blood of arterial wall Inflammation. Radiology. 2009 Jun;251(3):813–21.
16. Sheth RA, Maricevich M, Mahmood U. In vivo optical molecular imaging of matrix metalloproteinase activity in abdominal aortic aneurysms correlates with treatment effects on growth rate. Atherosclerosis. 2010 Sep;212(1):181–7.
17. Sheth RA, Upadhyay R, Stangenberg L, Sheth R, Weissleder R, Mahmood U. Improved

detection of ovarian cancer metastases by intraoperative quantitative fluorescence protease imaging in a pre-clinical model. Gynecologic Oncology. 2009 Mar;112(3):616–22.

18. Sheth RA, Upadhyay R, Weissleder R, Mahmood U. Real-time multichannel imaging framework for endoscopy, catheters, and fixed geometry intraoperative systems. Molecular imaging : official journal of the Society for Molecular Imaging. 2007 Apr;6(3):147–55.
19. Upadhyay R, Sheth RA, Weissleder R, Mahmood U. Quantitative real-time catheter-based fluorescence molecular imaging in mice. Radiology. 2007 Nov;245(2):523–31.
20. Regillo CD. The present role of indocyanine green angiography in ophthalmology. Curr Opin Ophthalmol. 1999 Jun;10(3):189–96.
21. Bischoff PM, Flower RW. Ten years experience with choroidal angiography using indocyanine green dye: a new routine examination or an epilogue? Doc Ophthalmol. 1985 Sep 30;60(3):235–91.
22. Abels C, Karrer S, Bäumler W, Goetz AE, Landthaler M, Szeimies RM. Indocyanine green and laser light for the treatment of AIDS-associated cutaneous Kaposi's sarcoma. Br J Cancer. 1998 Mar;77(6):1021–4.
23. Kimura T, Muguruma N, Ito S, Okamura S, Imoto Y, Miyamoto H, et al. Infrared fluorescence endoscopy for the diagnosis of superficial gastric tumors. Gastrointest Endosc. 2007 Jul 1;66(1):37–43.
24. Sheth RA, Arellano RS, Uppot RN, Samir AE, Goyal L, Zhu AX, et al. Prospective trial with optical molecular imaging for percutaneous interventions in focal hepatic lesions. 2015 Mar 274(3):917-26.
25. Sheth, R.A., Heidari A, Esfahani SA, Mahmood U. Interventional optical molecular imaging guidance during percutaneous biopsy.