CLINICAL STUDY PROTOCOL

A Feasibility Study for the use of Multispectral Optoacoustic Tomography in the Detection of Solid Tumors and Lymph Nodes (MSOT)

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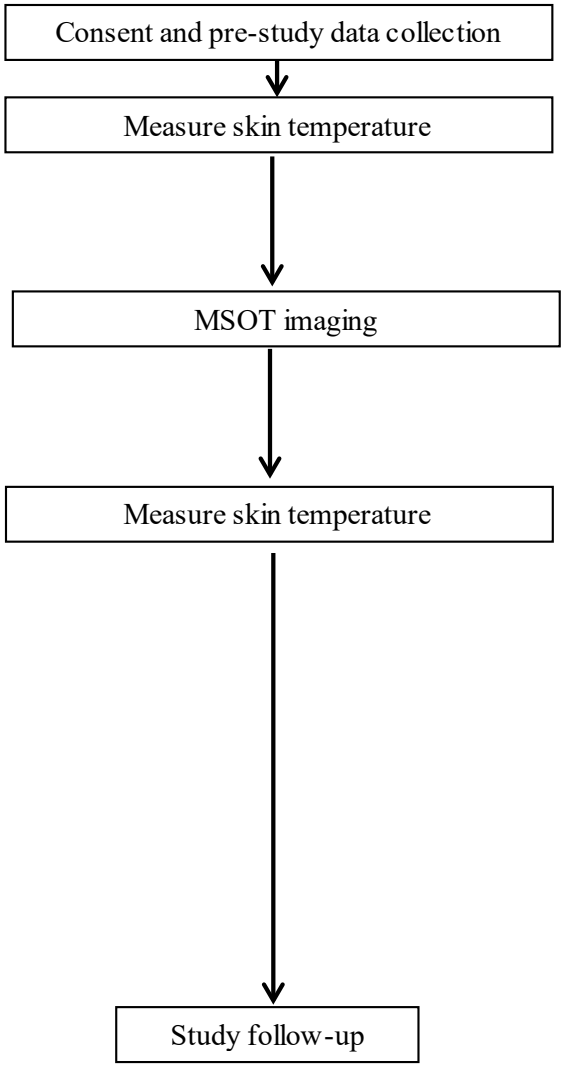
1.0 **SCHEMA**


Consent and pre-study data collection
Measure skin temperature
MSOT imaging
Measure skin temperature
Study follow-up

2.0 Introduction and Background

2.1 Introduction and Background

The accurate detection and localization of cancers *in vivo* are critical to medical decisions and improved treatments. Unfortunately, limitations of contrast (reporter) agents, resolution, and restrictions of depth reduce the ability of most imaging methods to detect and localize multiple contrast agents simultaneously. Alternatively, optical-fluorescent imaging can simultaneously detect multiple biomarkers using fluorescently labeled peptides or antibodies as contrast agents; however, these approaches are restricted to superficial detection, as light scattering degrades the spatial resolution at increased penetration depths greater than 8 mm. Multispectral optoacoustic tomography (MSOT) is emerging as an alternative modality that is not restricted by many of the limitations of the imaging used in diagnosis and treatment of diseases[1]. MSOT was initially developed for research, but has been adapted for clinical uses[2-4] (see Table 1).

Optoacoustic imaging is based upon a "light-in, sound-out," i.e. shining a light into the tissue and when the light hits the tissue, it can send a sound wave which we will capture using the MSOT device, approach through which absorption of near-infrared light (NIR) within biological tissues generates ultrasonic waves with much less scattering, longer range of detection, and higher accuracy compared with traditional optical imaging. The optoacoustic approach is unique, with increased optical contrast and signal-to-noise ratios[1]. Optoacoustic imaging retains the advantages of optical imaging, including high specificity to identify functional and molecular processes in living organisms with high sensitivity. Most tissues are relatively transparent to NIR light in the range of 600 to 900 nm; therefore, use of NIR light excitation and ultrasound signals renders photon scattering irrelevant to image formation, enabling high-resolution images of the biological function of tumors and organs. Once the sound waves are generated, they obey the physical laws of sound transmission; the intensity of the sound increases with the number of molecules excited, but is reduced by distance and the extent of ultrasound diffraction due to different densities of tissue. In MSOT, multiple spectral components of NIR light are varied automatically to excite specific molecules, permitting accurate tomographic images to be constructed from the resulting ultrasonic signals. MSOT is also unique in its ability to detect multiple contrast agents simultaneously based upon differential spectral shape.

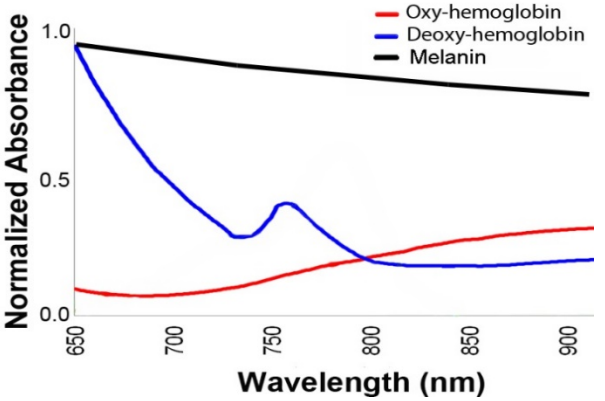


Figure 1 Spectra of endogenous absorbers that serve as endogenous contrast for MSOT

Changes in vasculature are often associated with both tumor development and progression, but microvascular changes occur below the resolution of common clinical imaging modalities. MSOT identifies microvascularity and tissue oxygenation by hemoglobin absorption of multiple wavelengths of light to generate high optoacoustic contrast[1,4-6].

Because oxy- and deoxy-hemoglobin each generate a unique optoacoustic signal, both oxy- and deoxy-hemoglobin can be observed simultaneously without the addition of exogenous contrast agents using MSOT (Figure 1). MSOT can distinguish between oxygenation states of hemoglobin, i.e. oxy-hemoglobin vs deoxy-hemoglobin, allowing visualization of differential blood saturation by oxygen within tissues, including the capability to differentiate between tumor and the surrounding tissue[2,7] as well as inflammation associated with Crohn's disease[3].

Imaging of tumors and cancer-related morphologic changes in tissues by MSOT is facilitated through exogenous contrast agents, including clinically approved optical dyes (e.g., isosulfan blue or indocyanine green)[8], markers targeted to cell surface molecules, for example, EGFR [11], the tumor microenvironment (e.g., pH)[9], and endogenous absorbers (i.e., oxyhemoglobin)[2,10]. Use of MSOT in multiple tissue types and at varying depths, i.e., at least 5 cm[3,8], can provide functional real-time three-dimensional (3D) information at high spatial resolution *in vivo*[1]. This ability will have a significant impact on clinical care in systemic diseases, including cancer involving multiple organs. The ability of MSOT to identify tumors indicates great potential for clinical applicability for solid tumors, such as melanoma, head and neck, breast, pancreatic, prostate, colon, and potentially, liver cancer.

2.2 Clinical Studies

MSOT has been used in research[1,6,11,12] and clinical studies[2,3,7,8] (including 6 clinical trials described in Table 1) and there has been no evidence of serious adverse events associated with the use of the device. Using a similar instrument in breast cancer, an optoacoustic imaging system from Seno Medical Instruments has received a CE mark in April 2014. Confirmatory studies for product approval of this particular device and specific indication have been started during the last years (with a focus on safety and performance). Presently, there has not been any association of phototoxicity associated with the use of MSOT in any clinical trial (Table 1).

Table 1 Clinical Trials using MSOT

Indication	Purpose of trial	Patients	References	Status
Peripheral athero-sclerosis – healthy volunteers	The current project comprises a proof of principal study to test the technical feasibility of the use of Multispectral Optoacoustic Tomography (MSOT) in healthy volunteers for non-invasive measurements and imaging of perfusion and oxygenation in the lower legs. By measuring peripheral blood oxygenation and perfusion with a prototype handheld MSOT probe and comparing that to standard ultrasound imaging, pulse-oximetry and standard ankle-brachial index as a gold standard, the diagnostic accuracy and measurement ranges in physiologic conditions as determined by MSOT will be tested.	10 (The Netherlands)	NTR4125 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4125	Published in Radiology
Limb ischemia, athero-sclerosis	This study aims to investigate the feasibility and clinical performance of MSOT imaging of the feet in Peripheral Vascular (PVD) patients. Our main objective is to investigate and obtain new information about the state of arteries in PVD patients using MSOT. Our second objective is to investigate the use of MSOT compared to duplex ultrasound and standard diagnostic work-up (including Fontaine classification) in providing more information on microvascular perfusion and oxygenation in the extremities in relation to severity of the PVD. The patients will be imaged on both feet at particular landmarks, which include large arteries (arteria tibialis posterior and dorsalis pedis) and microvasculature (on each of the toes). The images will be compared to duplex ultrasound images of the large arteries, which are acquired as part of the standard of care, as well as other available diagnostic information, including the Fontaine classification.	24 (The Netherlands)	NTR4461 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4461	Closed; Data analysis ongoing
Melanoma	This prospective study tests if photoacoustic tomography (PAT) and high-resolution ultrasound in combination with an indocyanine green injection is the first valid method to exactly assess the sentinel node status in melanoma patients in a non- invasive way and without exposing the patient to radioactivity. Therefore, preoperative, *in vivo* PAT results will be compared to the *in vivo* results based on standard diagnostics. Additionally, postoperative *ex vivo* PAT results followed by histological assessment will be compared.	40/120 (Germany)	DRKS00006329 http://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00006329 DRKS00005447 http://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00005447	First 20 patients: Published in Sci. Transl. Med. http://stm.sciencemag.org/content/7/317/317ra199 Next 60 patients: Data analysis
Crohn's disease/ Ulcerative colitis	The proposed study is designed as a pilot study to evaluate the usefulness of MSOT for the evaluation of disease activity in IBD patients. As current methods for the evaluation of intestinal inflammation in IBD have relevant restrictions, new, non- invasive, quantitative, and accurate modalities are urgently needed. In comparison to other techniques, MSOT provides relevant advantages such as non-invasive imaging, high spatial resolution (this is of special importance for the evaluation of the intestine, which can be only a	344 (Germany)	NCT02622139 https://clinicaltrials.gov/ct2/show/NCT02622139?term=MSOT&rank=1	Recruiting; First case study published in Gastro-enterology in motion

	few millimeters thick), and highly sensitive detection of specific molecules such as total hemoglobin or oxy- /deoxygenated hemoglobin. These advantages suggest MSOT as an outstanding technique for evaluating disease activity in IBD.			
Breast Cancer	Evaluation of breast cancer based upon oxy- and deoxy-hemoglobin. MSOT provided high special resolution of breast tissue at centimeter depths.	Germany		Clinical Cancer Research
Alopecia	Dermal Papilla cells (DPCs) of hair follicle units (HFU) derived from balding and non-balding regions of the scalp have differences in terms of their genetic, morphologic and functional characteristics. One of the study goals is to evaluate the ability of a new technology called multi-spectral optoacoustic tomography (MSOT) to non-invasively image the structural information of hairs and surroundings.	20 (Singapore)	Not available	First case study submitted for publication
Basal cell carcinoma	The purpose of this research study is to evaluate if MSOT can accurately map non-melanoma carcinomas with the objective to determine lesion dimensions to aide in surgical removal. Two- and three-dimensional handheld MSOT probes are compared in terms of their performance in mapping the non-melanoma lesions.	Not available (Singapore)	Not available	First case study submitted for publication

3.0 **Objectives**

This IDE FDA approved study will involve the interaction with patients that are scheduled for routine standard of care surgery. It will be a single-arm study that is designed to provide safety information regarding the use of the Acuity MSOT device in the clinical setting and the ability of MSOT imaging data to correlate with clinical findings identified via pathology. We will initially study the safety data in 10 patients, and will expand the study with FDA approval to include 40 more patients after an interim analysis (refer to section 10.3 for more details).

3.1 **Primary Objective(s)**

3.1.1 To collect safety data on patients in whom MSOT was used to image tumor or lymph nodes

3.1.2 To evaluate skin temperature pre and post imaging (pre- and post-surgery) as part of the safety evaluation of MSOT

3.2 **Secondary Objective(s)**

3.2.1 To detect tumor or lymph node by MSOT before and after surgical removal

3.2.2 To determine tumor positivity based on tissue pathology and detection of oxy- and deoxy-hemoglobin using MSOT localization

3.2.3 To determine tumor volume by MSOT before and after surgical resection

4.0 Patient Selection

4.1 Inclusion Criteria

4.1.1 Patients with an identified solid tumor, i.e. breast (Stage I-IV), melanoma (Stage I-IV), HNSCC (Stage I-III), pancreatic (Stage I-III), ovarian (Stage I-IV) that is scheduled for surgical removal of the tumor and completed standard imaging prior to surgery
4.1.2 Have acceptable hematologic status [total hemoglobin (tHb) ≥ 10 mg/dL]
4.1.3 Patients ≥ 18 yrs of age
4.1.4 Patient provided a signed and dated informed consent
4.1.5 Willing to comply with study procedures and be available for the duration of the study
4.1.6 Ability to understand and the willingness to sign an IRB-approved informed consent document.

4.2 Exclusion Criteria

4.2.1 Patients with central nervous system tumors
4.2.2 Patients with a tattoo over the surgical site
4.2.3 Pregnant women
4.2.4 Women who are breastfeeding
4.2.5 Systemic or local infection
4.2.6 Any systemic anomaly during the pre-op assessment preventing patient participation in the study
4.2.7 Any febrile illness that precludes or delays participation preoperatively
4.2.8 Anything that would put the participant at increased risk or preclude compliance with the study
4.2.9 Patients with Stage IV pancreatic cancer, Stage IV HNSCC are not surgical candidates and therefore excluded from this study

4.3 Inclusion of Women and Minorities

- Women and individuals of all races and ethnicities who meet the above-described eligibility criteria are eligible for this trial

- The study consent form will also be provided in Spanish for Spanish-speaking participants. Based on population estimates, we expect approximately 52% of participants to be women. Translating this to our expanded sample size estimate of 50, we plan to enroll at least 26 women. We plan to enroll at least 6% Native American, Hispanic/Latino, or African American (N=3). This would result in at least 6 patients of Fitzpatrick Skin Type I, 40 patients of Fitzpatrick Skin Type II-IV, and 4 patients of Fitzpatrick Skin Type V-VI. Because of the population distribution of our area we do not expect to accrue many individuals of Asian/Pacific Islander ancestry.

5.0 Subject Registration and Enrollment

5.1 Required protocol specific regulatory documents

This protocol, the Informed Consent document, any information to be given to the patient, and relevant supporting information must be submitted to the IRB by the Principal Investigator and reviewed and approved by the IRB before the study is activated.

5.2 Patient Registration and Enrollment

Patients must have signed and dated all applicable consents and authorization forms to be registered in the Clinical Trials Management System (CTMS) system, which is sponsored by The University of Oklahoma Stephenson Cancer Center (OU-SCC).

First, the study site should send the required documentation to SCC-IIT-Office@ouhsc.edu for confirmation of eligibility. A Screening identification (Screening ID) number will be provided by OU-SCC research staff. After all screening procedures and assessments have been completed and eligibility has been established, the subject Study ID number will be generated by OU-SCC. Once the patient has been provided a Study ID number, only the Study ID number should be used.

Patients must not start protocol procedures prior to registration and enrollment. A patient will start protocol procedures only after the pre-treatment evaluation is complete and eligibility criteria have been met.

NOTE: Per the Institutional Review Board (IRB) reporting, a patient is considered accrued once he or she signs a consent form for the study. A patient is considered enrolled once the patient begins treatment.

6.0 Study Outcomes and Study Measures

6.1 Primary Outcome

6.1.1 Adverse events as characterized by CTCAE v5.0 in patients that may result from MSOT imaging (≥44^{o}C)[13]

6.1.2 Measurement of skin temperature pre- and post-MSOT imaging (pre- and post-surgery 4 measurements total) with a touch thermometer as part of the safety evaluation of the MSOT device. The thermometer will be placed onto the skin until a temperature appears, about 1 minute, and the temperature will be recorded.

6.2 Secondary Outcomes

6.2.1 Tumor positivity as based on standard clinical laboratory techniques and signal detection of oxy- and deoxy-hemoglobin using MSOT localization
6.2.2 Tumor volumes as estimated by MSOT and by standard clinical laboratory techniques
6.2.3 Identification of tumor and/or lymph nodes based upon either Isosulfan Blue or IC Green dye

7.0 Study Plan

7.1 Study-Related Activities

Study Procedures

- Upon receipt of a signed consent form, the research study team will generate a unique identification number so that subjects are de-identified for analysis of data. *Any standard imaging measurements will be added in* Appendix A and B. This unique identification number will be used on the case report form (Appendix A and B).

- Patients will go through standard care procedures to prepare for surgery. Medically established diagnostic procedures will be used to detect tumor and lymph node margins.

Figure 2 The MSOT Acuity

- To evaluate the safety of MSOT (Figure 2), the device will be used to obtain images of the tumor or lymph node margins for investigational use only to compare to clinical pathology and patient's medical record.

- As part of the standard of care surgery preparation for cancer resection for all tumor types, patients will be injected with standard doses of Isosulfan Blue/ Indocyanine (IC) Green for either lymph node or tumor identification as part of the standard surgical procedure. The MSOT device will detect the presence of dye at the standard dose of dye used for surgical evaluation of tissue.

- Images will be obtained pre- and post-surgery (example simulation of operating room shown Figure 3). All imaging will be done in a closed surgical patient; no imaging will be performed on an open surgical patient. Dr. Lacey McNally has been trained in the use of the MSOT Acuity by the manufacturer, and she will be the MSOT operator for all MSOT imaging for this study. Dr. McNally will train the surgeon and surgical staff in the proper procedure for using the handheld detector and will maintain training records for this purpose. Only Dr. McNally and her research staff will perform post-imaging analysis on the de-identified digital images stored on the MSOT Acuity. The pathologist and clinician will perform all clinical evaluations of tumor samples as well as post-imaging assessment of treatment area and report where indicated on the imaging case report form. MSOT imaging will be for research only and no treatment decisions will be based on the MSOT images obtained.

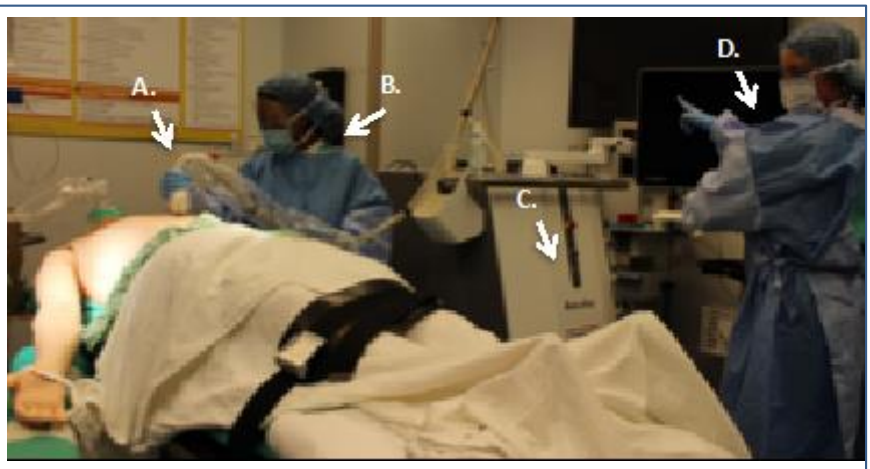
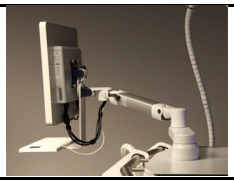
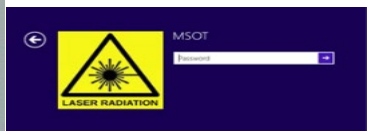
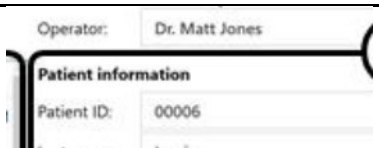
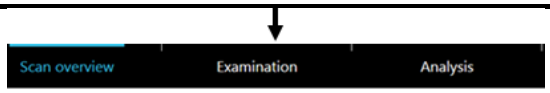
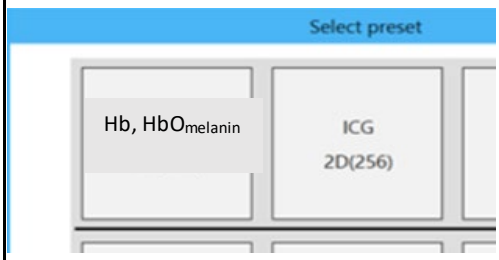
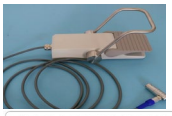


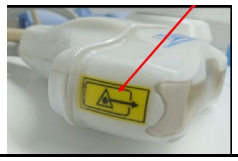
Figure 3: Demonstration of MSOT scanning procedure.A. Handheld detector covered with drape; B. surgeon; C. MSOT Acuity; D. trained MSOT operator.

- Logistics: The MSOT device will be used in The University of Oklahoma Health Sciences Center (OUHSC), and will reside in a temperature controlled and locked room.

Table 2 Clinical Protocol for MSOT Imaging

Step	Description
1	Prior to the time of surgery, the MSOT operator will ensure that the MSOT Acuity has been cleaned according to the manufacturer's recommendations found in the Instruction Manual -MSOT Acuity Cleaning & Disinfection.
2	A case report form with a unique identification number will be generated for each study participant (Appendix A).
3	The MSOT operator will maneuver the MSOT Acuity to the operating room and plug unit into proper electrical outlet. The MSOT Acuity will be positioned to allow easy access to the region of interest for imaging. The wheels will be locked into place to prevent acquisition unit from rolling during imaging.

4	The adjustable arm holding the monitor and keyboard will be locked into position for use by the MSOT operator for image acquisition.	
5	The MSOT operator will turn the key on the front panel to switch on the Acuity system, wait until the logon screen appears and log in.	 
6	Following the login, ViewMSOT™ automatically starts, initializes the hardware and opens the *Select patient* dialog.	
7	The MSOT operator will create a new patient record using the unique identification number as the Patient ID.	
8	The MSOT operator will Click on the *Examination* tab in the task bar to open the *Examination* screen.	
9	The Select preset* window will open, and the MSOT operator will choose the Hb, $HbO_{melanin}$ application to access the *Examination* screen. Check box when complete on Imaging Case Report form. ***NOTE**: Wavelengths and power settings are factory preset and cannot be changed manually by the user.	
10	The system performs an automatic system self-test each time the *Examination* screen is accessed for the first time after the system was switched on.	
11	The transducer must remain in the magnetic probe holder fixed to the acquisition unit until the system self-test has been completed. If the ultrasound probe is not in the probe holder, the MSOT operator will make sure that it is placed there.	
12	The staff will don safety goggles. The MSOT operator can control laser activation and deactivation via touch screen or keyboard in combination with the connected foot pedal.	 

13	The MSOT operator will hold the foot pedal until the self-test routine is finished. If the self-test is interrupted by releasing the foot pedal before the system self-test has been finalized, the test routine will need to be performed again.
14	A blue indicator in the signaling device on top of the system and in the front panel shows that the laser is ready for emission. 
15	The MSOT operator will record the self-test completion on the case report form and note the *Start scan time*. **NOTE: THE FOOT PEDAL WILL BE RELEASED ONCE SELF-TEST ROUTINE IS COMPLETE.**
16	The MSOT operator will tell the surgical team when the self-test routine has been successfully completed.
17	The MSOT operator will remove the probe from the magnetic holder to disinfect it using a germicidal disposable wipe (Sani-Cloth AF3, PDI, Orangeburg, NY), ensuring that the entire surface of the probe is moistened with the disinfectant. 
18	The MSOT operator will remove a second wipe from the package and repeat the disinfection process, ensuring that the entire surface of the probe is moistened with the disinfectant and allowed to dwell for at least 3 minutes. **NOTE: MSOT Operator will note dwell time on Imaging Case Report Form (Appendix A).**
19	After the 3-minute exposure time of the disinfectant, the MSOT operator will dry the probe surface with a commercially available, single-use, lint-free, low-germ towel.
20	A member of the surgical team will cover the handheld probe with a single-use ultrasonic probe drape (US.10.150.080, SonoGuard, Promecon GmbH, Hamburg, Germany), which will be the only part of the Acuity device to make contact with the patient, see (Figure 3).
21	Once time-out procedures have been completed, the surgical team will prepare the patient/region of interest on the skin for the initial MSOT scanning.
22	The skin surface will be cleaned following standard procedures. The skin will be dry without any alcohol pooling per manufacturer instructions, then ultrasound gel (#03-034, Aquasonic clear ultrasound gel, Parker Laboratories, Inc., Fairfield, NJ) will be applied to the area to be scanned.
23	The surgeon will place the imaging probe on the prepped skin area to be imaged BEFORE the laser is activated to avoid excess laser radiation.
24	Once the surgeon has the probe in place, the MSOT operator will press the foot pedal to activate the laser radiation for imaging. The MSOT operator will communicate with the surgeon as an image is acquired and coordinate the timing of the laser activation for additional scans. Release of the foot pedal will immediately shut down the laser system and stop the imaging procedure.
25	The MSOT operator will record *Scan comments* on the screen to be saved for each image acquired in the image sequence. **NOTE: Total number of images acquired in the image sequence will be recorded on the Imaging Case Report Form (Appendix A).**

26	A yellow indicator in the signaling device on top of the system and in the front panel shows that laser light is emitted through the aperture of the handheld probe. Keep the aperture of the probe on the region of interest or towards the floor. NEVER direct the aperture of the probe towards eyes while this indicator is on. Once the foot pedal is released laser emission is stopped and the laser emission indicator will turn off.  
27	The patient will undergo surgery for resection of the identified volume of tissue according to standard care.
28	After tumor or lymph node removal and incision has been closed, the area of interest will be reimaged by MSOT to obtain post-surgery images in the operating room by repeating steps 16 through 23.
29	An estimate of the percent amount of tumor remaining will be estimated by algorithms within in the MSOT computer programming for investigational use only.
30	The resected tumor will be taken to Pathology for volume assessment and pathological identification per medically established diagnostic standard care.
31	Data from the MSOT imaging is for research purposes only and not for treatment, and digital images will be kept on the MSOT computer, which will be stored in a secured room accessible only to study personnel.
32	Key parameters will be recorded on (Appendix A and B).

7.2 Duration of Follow Up

Subjects will be actively enrolled only for the duration of pre-op through surgical recovery, and until post-op discharge unless any study-related adverse event occurs. Medical record review will occur up to 6 months to allow for follow-up pathology assessments to be gathered and provide information to allow for analyses to be performed as previously described.

7.3 Criteria for Removal from Study

Patients can request to withdraw from the study at any time. Should patients consent to the study, but have tumor progression that prevents them from being a surgical candidate within 30 days, these patients may be removed at the Investigator's discretion. There is no reason to remove a patient otherwise from the study.

8.0 Adverse Events List and Reporting Requirements

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product, and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medical product, whether or not considered related to the medical product. All AEs are assessed by the center for severity, causality, outcome, seriousness, and if unanticipated.

8.1 Adverse Event List for MSOT

A slight, reversible reddening and temperature increase of sensitive skin. All adverse events and expected treatment effects, including mild events, will be monitored throughout the study until discharge.

8.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 8.1 above) for expedited reporting purposes only.

- **Attribution** of the AE:

- Definite – The AE **is clearly related** to the study treatment.
- Probable – The AE **is likely related** to the study treatment.
- Possible – The AE **may be related** to the study treatment.
- Unlikely – The AE **is doubtfully related** to the study treatment.
- Unrelated – The AE **is clearly NOT related** to the study treatment.

8.3 Serious Adverse Events (SAE)

Serious is defined as an adverse event that results in the following outcome:

- Death

- Life-threatening event (i.e. an event that places the patient, in the view of the investigator, at immediate risk of death from the event as it occurred). This does not include an event that, had it occurred in a more severe form, might have caused death

- Requires or prolongs hospitalization
- Results in persistent or significant disability/incapacity
- Congenital anomaly/birth defect.
- Important medical event that, based upon appropriate medical judgement, may jeopardize the patient, and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.4 Unanticipated Adverse Device Effects Reporting Requirements

Unanticipated Adverse Device Effect (UADE) means 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 application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

A medical review shall immediately occur with all reported Unanticipated Adverse Device Effects (UADE). The UADE shall be assessed for an unreasonable risks' determination. If an unreasonable risk has been determined, then the trial or the parts that cause the risk shall be stopped between 5-15 working days after making the determination.

8.5 Reporting of Unanticipated Problems, Adverse Events or Deviations

Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator to the IRB and sponsor or appropriate government agency, if appropriate and per institutional guidelines.

A description of all AEs and UADEs will be recorded on REDCap. All UADEs should be reported using the Sponsor Form (UADE Reporting Form) and MedWatch3500A to the following email or fax within 24 hours of the site notification of the event:

Email: SCCIITreporting@ouhsc.edu or
Fax: 1-405-271-1416

In compliance with FDA regulations 21 CFR 812.150, any UADEs occurring during the investigation should be reported to the sponsor and reviewing IRB within 10 days of first notice of the event. The sponsor will conduct an

evaluation of the UADE and report the results of such evaluation as an UADE Report to FDA, all reviewing IRBs, and participating investigators within 10 working days after the sponsor first receives notice of the effect.

An UADE Report will consist of a completed MedWatch 3500A form and a cover letter that provides an analysis of the event. Thereafter the sponsor shall submit such additional reports concerning the effect as per requests from FDA.

8.6 Post-imaging Procedural Events for MSOT

The post-imaging procedural events for MSOT are those events that are expected and standard to post-imaging as for ultrasound and include slight, reversible reddening and temperature increase of sensitive skin for a duration of ≤ 24 hours post-surgery. All unexpected events shall be captured. Post-imaging procedural events that occur in increased frequency and severity over what should normally occur in the medical opinion of the investigator will be considered adverse events (AE). AEs will be reported.

9.0 Data and Safety Monitoring

Safety oversight will be performed by Stephenson Cancer Center's (SCC) internal Data and Safety Monitoring Committee (DSMC). The DSMC is composed of individuals with the appropriate expertise in adult and pediatric hematology and medical oncology, radiation oncology, translational and correlative science, pharmacy, nursing and biostatistics. The DSMC operates under the rules of an approved data safety monitoring plan which complies with the National Cancer Institute (NCI) guidelines published as *Essential Elements of a Data and Safety Monitoring Plan for Clinical Trials Funded by NCI* as of January 2005 and the "*NIH Policy for Data and Safety Monitoring*," *NIH Guide for Grants and Contracts*, http://grants.nih.gov/grants/guide/notice-files/not98-084.html.

The Data Safety Monitoring Committee is charged with oversight of participant safety, study conduct and the validity and integrity of data for clinical trials at SCC. While the focus of the DSMC is to monitor interventional investigator-initiated trials (IITs) that are not subject to external monitoring, it has the authority to monitor any SCC protocol when potential concerns are identified. The DSMC also has the authority to suspend or close a study until the principal investigator addresses any issues that may cause harm or increase risks to subjects. The DSMC reports all findings to the Institutional Review Board (IRB).

Under the direction of the DSMC chair, a full board meeting is convened on a quarterly basis to review the accumulated safety data, accrual information, and additional information as stated in the DSMC plan.

9.1 DSMC Auditing

In addition to monitoring, the DSMC oversees an internal auditing process to ensure subject safety and data quality. All cancer-related clinical trials active at the SCC are eligible for audit; however, priority is placed on those clinical trials that are not monitored or audited by an outside entity. If an external entity conducts an audit of a clinical trial at the SCC, then the findings of that audit are reported to the DSMC, either through the formal audit report provided by the external auditing entity, if available, or from the PI, who will report any findings communicated during the audit process.

9.2 Data Handling and Record Keeping

9.2.1 Data Quality Assurance

Stephenson Cancer Center (SCC) will be responsible for clinical monitoring of all data for this study.

9.2.2 Electronic Database and Case Report Forms

The Principal Investigator and designated team will develop a study-specific electronic database in REDCap and study-specific case report forms for study data entry. All study data will be recorded into the REDCap database sponsored by OU-SCC and stored in a 21 CFR 11-compliant database. Appendix A and B will be paper based and later scanned into REDCAP. Only the Investigator and assigned research staff will have access to the study data. All case report forms will be available to Sponsor, IRB or regulatory authorities in event of an audit or inspection.

9.2.3 Data Disclosure and Subject Confidentiality

Subject medical information obtained as a result of this study is considered confidential. Disclosure to third parties other than those noted below is prohibited. All reports and communications relating to subjects in this study will identify each subject only by their initials and number. Medical information resulting from a subject's participation in this study may be given to the subject's personal physician or to the appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection on request by FDA or other government regulatory agency inspectors, the SCC clinical trial office auditors and monitors, Office of Compliance, and the Institutional Review Board (IRB).

All evaluation forms, reports, and other records that leave the site will be identified by a coded number, and/or initials to maintain subject confidentiality. All study records will be kept in a locked file cabinet or other secured area. All computer entry and networking programs will be identifiable only by coded numbers and/or initials. Subject personal medical information may be reviewed by representatives of the Sponsor, IRB, or regulatory authorities in the course of monitoring the progress of the clinical trial. Every reasonable effort will be made to maintain such information as confidential.

9.2.4 **Record Retention**

In accordance with 21 CFR 812.140, the Sponsor and Investigator will retain all research documents relating to the participation in the clinical trial. These documents must be retained for a period of two years after the date on which the investigation is terminated or completed, or the date that the records are no longer required for purposes of supporting a premarket approval application, a notice of completion of a product development protocol, a humanitarian device exemption application, a premarket notification submission, or a request for De Novo classification.

If the transfer of records custody during the retention period is accepted by any other person, notice of transfer must be given to FDA not later than 10 working days after transfer occurs.

9.3 **Study Monitoring**

All aspects of the study will be carefully monitored at periodic intervals throughout the study per FDA/ICH "Guidance for Industry – E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. All Case Report Forms (CRFs) will be up to 100% source verified against corresponding source documentation (e.g., office and clinical laboratory records) for each subject. The monitoring visits provide the PI with the opportunity to evaluate the progress of the study, to verify appropriate consent form procedures, and to verify the accuracy and completeness of CRFs, to resolve any inconsistencies in the study records and to assure that all protocol requirements, applicable FDA regulations, other requirements, and Investigator's obligations are being fulfilled.

Furthermore, this study will fall under the purview of the Stephenson Cancer Center Data Safety Monitoring Committee (DSMC).

10.0 **Statistical Considerations**

10.1 **Analysis of Primary Objective**

10.1.1 The primary objectives are to estimate the rate of adverse events and measure the skin temperature change following MSOT imaging both pre- and post-surgery. The adverse events will be estimated by grade, type and relatedness. The first analysis will pool all grade 3 unexpected events that are possibly, probably or definitely related to MSOT together and then construct a 95% exact Clopper-Pearson binomial confidence interval for this estimate. Additionally, measurement of skin temperature will be evaluated where skin temperature greater than 44^{o}C will be recorded[13]. A 95% exact Clopper-Pearson binomial confidence interval for the evaluation of skin temperature will be conducted

10.2 **Analysis of Secondary Objective**

10.2.1 There are three secondary outcomes of interest, two of these will be based upon a positivity (yes/no) (secondary outcomes 1 and 3) and one of these will assess on a continuous scale tumor size/ volume (secondary outcome 2). In each of these analyses, we will compare the information gathered from the MSOT approach (size, positivity tumor (yes/no), positivity of dye (yes/no)) for each patient and then compare these measures to those that are available from information gathered in the standard of care approach (i.e., from pathology reports after resection).

For continuous outcomes we will examine three characteristics based on comparing the MSOT and standard of care ("gold standard") assessments. These include: 1) the mean difference in the measure (i.e., tumor volume or size), 2) the correlation between the measures taken using MSOT or standard of care; and 3) the comparison of variability of the measures from the two approaches (MSOT and standard of care). For the first approach we will calculate a paired t-test, for the second we will estimate a Pearson correlation and for the third we will perform an F-test to compare the equality of variances. With the expanded sample size of 50 patients we can detect a difference in values of volume (or size) equal to 0.4 standard deviations or less of the measure of difference in tumor volumes (or size) with 80% power using a paired t-test with alpha=0.05 (2-sided). In addition, there is 80% power to detect a correlation of 0.38 (or larger) with an alpha=0.05 (2-sided). Finally, there is 80% power to detect a ratio of 2.24 (or larger) when comparing variances between the two groups (note: this ratio corresponds to a ratio of 1.5 on the standard deviation scale).

If the study is terminated early with 45 patients scanned, we can still detect a difference in values of volume (or size) equal to 0.43 standard deviations or less of the measure of difference in tumor volumes (or size) with 80% power using a paired t-test with

alpha=0.05 (2-sided). In addition, there is 80% power to detect a correlation of 0.40 (or larger) with an alpha=0.05 (2-sided). Additionally, there is 80% power to detect a ratio of 2.35 (or larger) when comparing variances between the two groups (note: this ratio corresponds to a ratio of 1.53 on the standard deviation scale)

For binary outcomes (positivity and presence of dye), we will estimate the MSOT approach and the corresponding 95% Clopper-Pearson exact binomial confidence interval. Accuracy is defined as the number of patients where the MSOT and standard of care approach are in agreement (i.e., both are positive or both are negative). In addition, we can examine other characteristics of the two approaches including sensitivity, specificity, positive predictive value and negative predictive values.

10.3 Power and Sample Size

10.3.1 We will initially accrue 10 patients for safety analysis regarding the use of the MSOT device pre- and post-surgery. If there are no grade III-V AEs observed in the patients as defined in CTCAE v5.0 per Section 8.2, we will submit an IDE supplement to request an enrollment expansion for an additional 40 patients, for a total of 50 patients. Up to an additional 55 patients will be accrued (to account for patient dropouts) to enroll 40 patients. Thus, a total of 50 patients will be enrolled and scanned (10 patients in the initial analysis and 40 patients following the interim analysis) in order to gather sufficient data to evaluate the proportion of patients with adverse events that may be associated with the MSOT approach. The MSOT approach is not expected to be related to any adverse events except for a possible slight, reversible reddening and temperature increase of sensitive skin. Nevertheless, this study will allow us to estimate the proportion of unexpected adverse events of grade 3 or higher that are possibly, probably, or definitely related to the MSOT approach. With 50 patients, the upper bound of a 95% Clopper-Pearson exact confidence interval will be less than 20% if the observed rate of adverse events is 8% or less (4 out of 50 or less). We expect that the actual value will be 0 out of 50 – and if this is observed the upper bound of this confidence interval will be 7.1% which would provide a useful upper bound for the potential of adverse events.

The study will be terminated early, after 45 patients in total are enrolled and scanned, if 3 or fewer AEs are observed in the patients scanned by the MSOT device, based on the assumption that the upper limit of the 95% Clopper-Pearson exact confidence interval will be less than 20%. The upper limit of the 95% Clopper-Pearson exact confidence interval will be 7.9%, 11.7%, 15.1% or 18.3% respectively for 0, 1, 2, or 3 observed AEs.

10.3.2 **Interim analysis**: This study will initially accrue 10 patients to identify any potential grade III or IV AEs defined in CTCAE v5.0 per Section 8.2. As yet, no study using MSOT in over 600 patients has observed any grade III or IV AEs[2,3,14,15]. An interim analysis will be conducted after the first 10 patients. If there are no grade III or IV AEs observed in the patients, we will submit an IDE supplement with preliminary safety data, to request an enrollment expansion to 50 patients. If any grade V AEs occur during the investigation, the FDA will be notified immediately and the study halted for further accrual until discussed with FDA.

10.4 Estimated Accrual Rate

10.4.1 The estimated accrual rate is 10-20 patients per month after the initial accrual of 10 patients.

10.5 Estimated Study Length

10.5.1 Subjects will be actively enrolled only for the duration of pre-op through surgical recovery, and until post-op discharge unless any study-related adverse event occurs. Medical record review will occur up to 6 months to allow for follow-up pathology assessments to be gathered and provide information to allow for analyses to be performed as previously described.

11.0 ETHICAL AND REGULATORY CONSIDERATIONS

11.1 Ethical Conduct of the Study

The study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki in addition to the requirements of the ICH E2A guidelines. This study will also comply with U.S. FDA regulations under a U.S. Investigational Device Exemption (IDE) application in addition to local, state, and federal laws.

11.2 Informed Consent

The informed consent document will be in compliance with ICH GCP, local regulatory requirements, and legal requirements. The informed consent document used in this study, and any changes made during the course of the study, will be prospectively approved by the IRB of record.

The investigator must ensure that each study patient is fully informed about the nature and objectives of the study and possible risks associated with participation. The investigator, or a person designated by the investigator, will obtain informed consent from each patient before any study-specific activity is performed. The study site will retain the original of each patient's signed consent document. A copy of the informed consent document must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language.

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Appendix A – Imaging and Tissue Data Collection Form

Study Number: OU-SCC-MSOT	PID: 0 1 - __ __ __
Investigator: Lacey McNally, PhD	Surgery Date: __ __/__ __/__ __

1) Tumor or Lymph Node Assessments:

a) Target tissue

☐ Tumor

☐ Lymph Node

b) Standard of care method of imaging

☐ MRI _____tumor amount

☐ PET _____tumor amount

☐ CT _____tumor amount

☐ Other: _____ tumor amount

c) Did patient receive neoadjuvant therapy?

☐ Yes

☐ No

d) MSOT Image acquired?

☐ Yes

☐ No

e) Was an indicator dye used during surgery?

☐ Yes

☐ No

f) If an indicator dye used during surgery, please indicate which dye.

☐ Isosulfan Blue ☐ IC Green

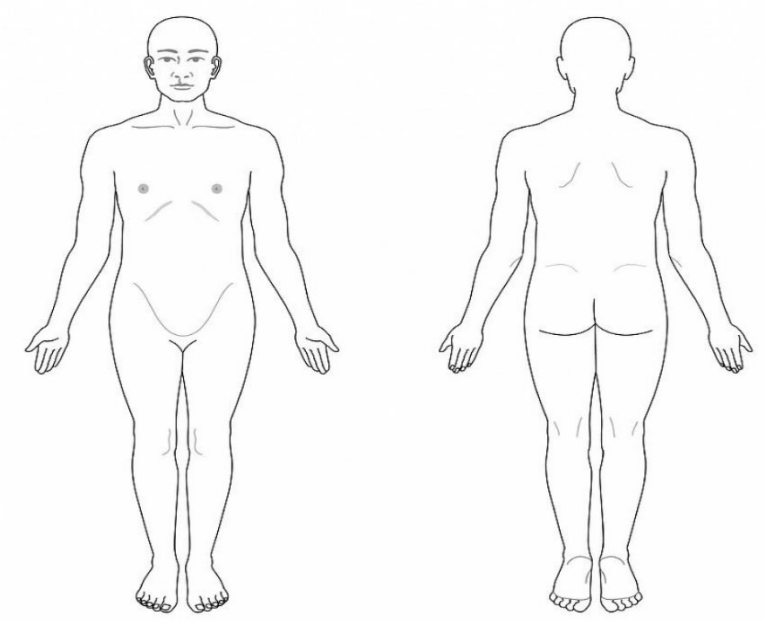
2) Location of Imaging

a) Please circle the location of the imaging (below) and provide further description as necessary

_____

_____

_____



https://anatomyclass123.com /

b) Erythema post-MSOT Imaging (RTOG)

i. Time after imaging: (hr: min) _____

ii. Grade (RTOG)

- ☐ 0 No erythema present
- ☐ 1 Follicular, faint or dull erythema, epilation, dry desquamation, decreased sweating
- ☐ 2 Tender or bright erythema, patchy moist desquamation, moderate edema
- ☐ 3 Confluent, moist desquamations other than skin folds, pitting, edema
- ☐ 4 Ulceration, hemorrhage, necrosis

3) Clinical Pathology Assessment

a) Date or diagnosis (month / year): ☐☐

b) <u>Positive tumor tissue</u>**:**

☐ Yes ☐ No

c) For ☐ Pancreatic ☐ melanoma ☐ breast ☐ HNSCC ☐ ovarian (check one) indicate stage at diagnosis (**TNM classification**):

☐ Tx ☐ T0 ☐ Tis ☐ T1 ☐ T2 ☐ T3 ☐ T4

☐ Nx ☐ N0 ☐ N1 ☐ N2 ☐ N3

☐ Mx ☐ M0 ☐ M1

d) Tumor subtype (if applicable) _____

4) Skin temperatures

Pre-Surgery MSOT imaging			
Pre-imaging Skin Temperature (°C)		Time	
Post-imaging Skin Temperature (°C)		Time	

Post-Surgery MSOT imaging			
Pre-imaging Skin Temperature (°C)		Time	
Post-imaging Skin Temperature (°C)		Time	

5) Patient Monitoring (*please note that patients must be monitored daily for adverse events until discharge). Please answer yes or no if Erythema at grade III or higher or change in skin pigmentation occurs.**

☐ Yes ☐ No

_____Post surgery
_____1 day
_____2 day
_____3 day
_____4 day
_____5 day
_____6 day
_____7 day
_____ 8 day
_____9 day
_____10 day
_____11 day
_____12 day
_____13 day
_____14 day
_____15 day

Discharge date: _____

6) **Adverse event. Please answer yes or no if Erythema at grade III or higher or change in skin pigmentation occurs.**

☐ Yes ☐ No

If yes, please fill out details below:

_____ Erythema _____ Time _____Date

_____ Changes in skin pigmentation _____ Date

7) **MSOT Scan Times:**

MSOT Scan Time (hr: mm)	
Select the preset Hb, HbO, IsoBlue, and/or ICG for wavelengths settings	☐ Check when complete
Pre-Surgery	Scan Start Time: Scan End Time: Total Scan time:
Post-Surgery	Scan Start Time: Scan End Time: Total Scan Time:
TOTAL scan time (Pre-Surgery+ Post-Surgery Scan times)	

Treating Physician Signature: _____ Date: ___ ___ / ___ ___ / ___ ___

PI Signature: _____ Date: ___ ___ / ___ ___ / ___ ___

Appendix B – Image Analysis Form

Study Number: OU-SCC-MSOT	PID: 0 1 - __ __ __
Investigator: Lacey McNally, PhD	Surgery Date: __ __/__ __/__ __

In vivo MSOT Size/Volume Assessment:

Tumor depth (mm):		
MSOT parameters		
Hb, a.u.	Pre-Surgery:	Post-Surgery:
Hb_{total}	Pre-Surgery:	Post-Surgery:
HbO	Pre-Surgery:	Post-Surgery:
Isosulfan Blue	☐ Yes ☐ No	Tissue: Value:
IC Green	☐ Yes ☐ No	Tissue: Value:

Duration of disease control: The duration of time from the date measurement criteria are first met for irCR, irPR, or irSD, until the first date that irPD is confirmed by independent radiology review or death, whichever comes first.

Treating Physician Signature: _____ Date: ___ ___ / ___ ___ / ___ ___

PI Signature: _____ Date: ___ ___ / ___ ___ / ___ ___