Improving diagnostic US for reduction of benign breast biopsies using US-guided Optical Tomography

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1. STUDY OVERVIEW

1.1. Significance of Breast Cancer Diagnosis

Breast cancer is a heterogeneous disease with different histologic subtypes composed of different grades, growth rates, and metabolic activity levels resulting in a wide range of functional differences (1). Additionally, benign breast disease encompasses a heterogeneous group of lesions that vary in vascular content, proliferative index, and metabolic activity, all of which may or may not be associated with future risk of breast cancer (2). X-ray mammography is routinely used for breast cancer screening and diagnosis. Ultrasound (US) of the breast is frequently used as a complement to mammography and breast MRI (3), and may aid in screening dense breasts (4-5). Contrast enhanced mammography (CEM) is an FDA approved emerging technology indicated for lesion evaluation, determination of malignancy extent, neoadjuvant treatment response and as a substitute to MRI for high risk screening (6-9). While the characteristics of malignant and benign breast lesions are well established by conventional imaging techniques (10-12), the overlapping appearances of malignant and benign abnormalities result in hundreds of thousands of unnecessary biopsies, i.e. biopsies yielding benign results. (13) The positive predictive value of a screen detected abnormality recommended for biopsy is just over 25%, resulting in nearly 75% of unnecessary biopsy recommendations (14). An optical tomography system that adds functional information like tumor related hemoglobin concentration may improve the accuracy of the breast US exam. In particular, total Hemoglobin (tHb) values falling below a minimum value in the setting of a low or moderate suspicion abnormality (Breast Imaging and Database System, BI-RADS 4A or 4B assessment) may allow the diagnostic radiologist to downgrade the assessment to probably benign or benign (BI-RADS 3 or 2) and thereby reduce the rate of benign biopsies. In a previous study when a minimum threshold value of tHb<50μm was used 45% of BI-RADs 4A and 4B abnormalities that subsequently were proven to be benign could have safely avoided biopsy [34]. At this point in time we have accrued and preliminarily analyzed results from 35 patients (In addition to the initial 20 patients, 15 patients from phase 2 were also analyzed). When using tHb<50μm as a minimum threshold 12 patients with BI-RADS 4A or 4B abnormalities could be downgraded to a probably benign assessment and could have avoided biopsy, suggesting a 35% (12/35) reduction in benign biopsies.

Furthermore, there is an additional clinical scenario that US-guided DOT may have a beneficial impact, in cases with discordant imaging pathology correlation in the setting of a high suspicion US abnormality. In current practice, if a high suspicion (BI-RADS 4C) lesion has a benign pathology result, it may be considered to have discordant imaging pathology correlation and is frequently recommended for repeat biopsy or surgical excision (15). However, if the optical exam shows subthreshold tHb concentration, this may allow for less aggressive management, such as short term follow-up rather than additional tissue sampling. This is particularly applicable to a high suspicion irregular hypo-echoic mass, (which mimics malignancy on standard of care (SOC) US), with biopsy pathology of focal fibrosis. Focal fibrosis typically has low tHb (35).

1.2 Optical Tomography Using Near Infrared Light Guided or Assisted With Ultrasound (NIR/US)

Diffuse optical tomography (DOT) and spectroscopy (DOS) have been explored for diagnosis of breast cancer and for predicting and monitoring neoadjuvant therapy responses of advanced or aggressive breast cancer (17-25, 34). Utilizing DOT or DOS alone to perform breast cancer

diagnosis has been reported in many studies using different systems and optical wavelengths in the near infrared spectrum (17-25,34). However, due to intensive light scattering in tissue, lesion localization and light quantification accuracy may not be fully realized (21-22). New approaches taken by researchers in the field include ultrasound guided DOT (24-26, 28, 34), MRI-guided DOT or DOS (29-31), and x-ray guided DOT (32-33). These approaches utilize a conventional imaging modality to guide the DOT or DOS for lesion localization and image reconstruction in order to improve the light quantification accuracy for more accurate diagnosis.

Ultrasound-guided DOT, pioneered by our group, has demonstrated its potential role in differentiating malignant and benign breast abnormalities (24-25, 34), and in predicting and monitoring the neoadjuvant chemotherapy (NAC) response of breast cancer (26). Our unique approach employs a commercial US transducer and NIR optical imaging sensors mounted on a hand-held US probe. The co-registered US is used for lesion localization, and optical sensors are used for imaging tumor related vascularity.

The technical aspects of the NIR optical imaging system have been described in detail [24]. Briefly, NIR light is delivered from multiple source positions embedded within the probe with the central slot fitted with an ultrasound transducer and the reflected light (from normal and pathologic breast tissue) collected by multiple detection fibers within the probe that are coupled to photomultiplier tubes (PMTs). The transmitted NIR light is delivered to each transducer source position sequentially and reflected NIR light is detected in parallel from all PMT detectors. The entire acquisition from all source detector pairs takes approximately 3 to 4 seconds (figure 1). Specific light wavelengths in the NIR spectrum are selectively absorbed by oxygenated (HbO2) and deoxygenated Hemoglobin (Hb) and allow for the quantification of the concentrations and distributions of tHb, Hb and HbO2.

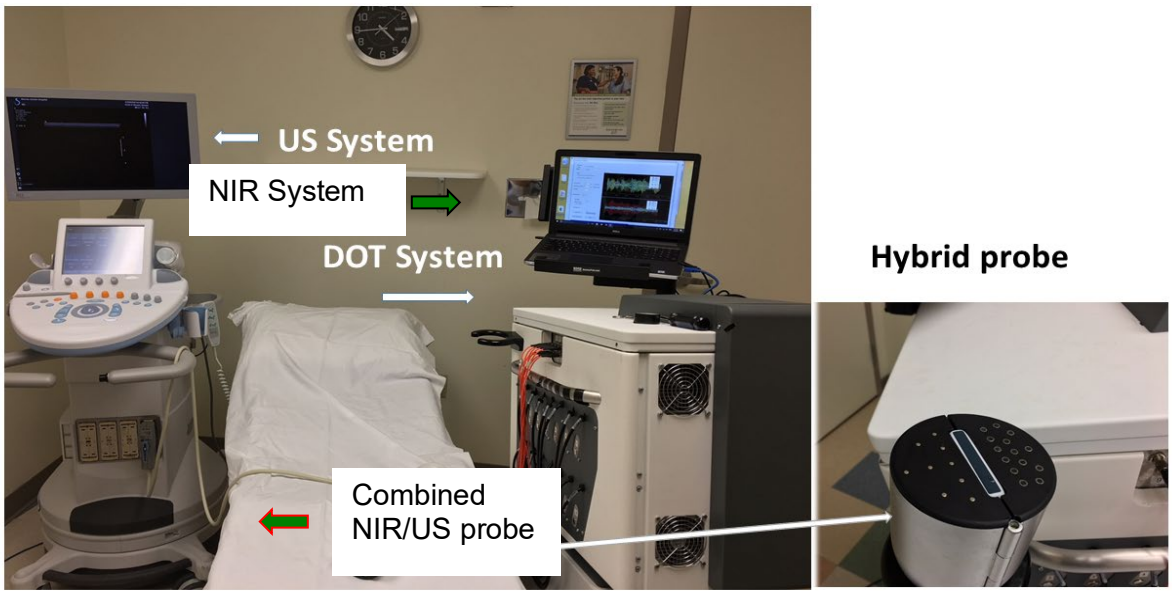


Figure 1: Hand-held US-guided Near Infrared system

Examples of using NIR/US for breast cancer diagnosis are given below [34].

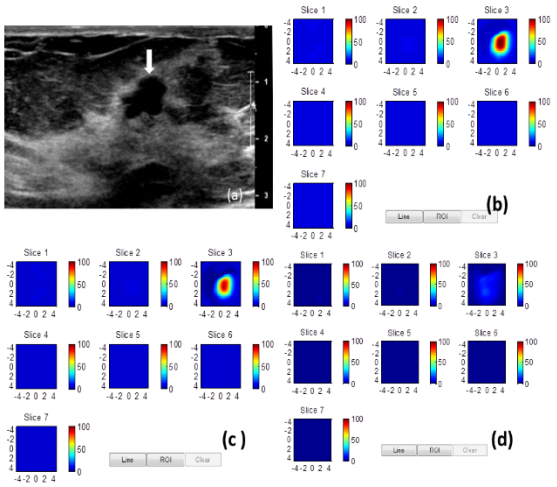


Figure 2. *(a) US image of a hypoechoic lobulated mass with internal echoes and septations located at 2 o'clock in the right breast of a 40-year-old woman. US readings from the two readers were 4B and 4C. (b) The tHb map shows a diffused mass with a maximum value of 52.5 μ mol/L. (c) oxyHb map, and (d) deoxyHb map. Core biopsy revealed DCIS and ADH.*

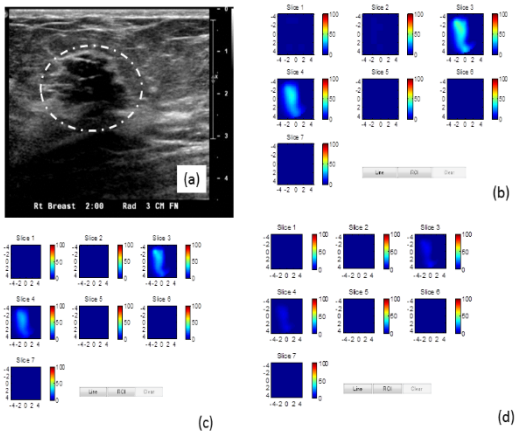


Figure 3*. (a) US image of a hypoechoic lobulated mass (arrow) in the right breast of a 72-year-old woman. US readings from the two readers were 4B and 4C. (b) tHb map showed an isolated mass, maximum 106.2 μ mol/L. (c) oxyHb map, and (d) deoxyHb map. OxyHb map follows tHb map closely, and the deoxyHb distribution is quite diffused. Core biopsy revealed DCIS, nuclear grade 2. Pathology at surgery revealed 0.6 cm DCIS with intraductal papilloma with atypia and microcalcification.*

2 OBJECTIVES

We have received 5-year NCI funding to support this study.

The primary objective of this study is to assess the ability of US-guided DOT to reduce the rate of benign biopsy. To achieve this objective we will simultaneously acquire co-registered US imaging data and US-guided DOT imaging data from a group of patients (~300) scheduled for US-guided breast biopsy. We hypothesize that the additional optical information of tHb concentration and distribution will lead to a downgrading of the assessment of a subset of low to moderate suspicion

abnormalities based on conventional imaging (i.e. breast ultrasound with or without mammography) and thereby could lead to a reduction in benign breast biopsies (i.e. false positives).

The secondary and complementary objective is to assess the impact on sensitivity of adjunctive optical data. In particular we hypothesize that by defining a conservative tHb threshold below which malignancy is so rare that the addition of US-guided DOT will not significantly alter the sensitivity of the combined US-guided DOT & CI assessment, i.e. adjunctive US-guided DOT will not decrease true positives.

A third objective is to assess the impact of adjunctive US-guided DOT data in the management of discordant pathology results. In current SOC, the radiologist performing the needle biopsy routinely compares the imaging with the pathology results for concordance. Highly suspicious abnormalities (BI-RAD 4C, 5) that yield benign results are typically considered discordant and are recommended for repeat biopsy or surgical excision. If the discordant abnormality has a tHb below the minimum threshold value, this might allow radiologists to forego a repeat biopsy or surgery. For example, focal fibrosis may appear highly suspicious on US. Our initial data show that fibrotic lesions have low vascularity (28).

Additionally as an exploratory objective, we will compare the results of US-guided DOT and in particular tHb concentration with contrast enhanced mammography (CEM) in the subset of patients who undergo CEM.

3 ELIGIBILITY CRITERIA

Inclusion Criteria:

- Female subjects ≥ 18 years old with ultrasound visible breast abnormalities (BI-RADS 3*, 4A, 4B, 4C, and 5) referred for ultrasound-guided core needle biopsy or fine needle aspiration
- Willing and able to provide informed consent

(*note that while a BI-RADS 3 assessment is probably benign, a subset of patients with this assessment choose to undergo biopsy rather than follow up imaging).

Exclusion Criteria:

- Lesions located in the darkly pigmented nipple-areolar complex area
- Subjects with breast implants
- Abnormality in the mirror image location of the contralateral breast.
- Additional abnormalities in the same region of the breast that would be included in US-guided DOT imaging of the abnormality undergoing biopsy
- Previous breast irradiation of the mirror image location of the contralateral breast
- Lesions located at previous biopsy sites when biopsy occurred within the last six months.
- Pregnancy
- Superficial abnormalities located entirely within (i.e. less than) 5mm of the overlying skin

3.1 Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for the study.

4. REGISTRATION PROCEDURES

The following steps must be taken in order to register patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5 STUDY PROCEDURES

The study will ensue in two phases: phase 1 – training set, and phase 2 prospective trial.

The first 20 patients undergoing US-DOT will comprise the training set to accomplish the aims of:
1) assessing US-DOT data,
2) assessing intra-observer variability in the assessment of US-DOT data
3) assessing inter-observer variability in the assessment of US-DOT data.

For phase 2, the prospective trial, up to 315 patients will be recruited with the expectation that approximately 5% will not successfully complete the study so that 300 patients will have valid US-DOT data for the ensuing reader study.

The study flow chart that integrates US-guided DOT collection into the standard of care (SOC) clinical workflow is shown below (figure 3). SOC clinical workflow includes review of patient information and diagnostic information, such as conventional imaging results with BIRADs

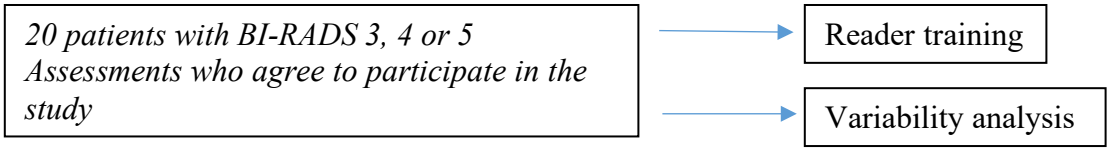
assessments. The study coordinator will approach eligible patients prior to biopsy to assess their willingness to participate in the study. Patients that are willing to participate will sign informed consent and undergo US-guided DOT imaging before biopsy. A subset of patients will also undergo CEM.

Breast radiologist coinvestigators will perform (or supervise certified breast US technologists) in all NIR/US studies. As noted above, they will not be blinded to any clinical data. The function of the imaging specialist in US guided DOT imaging is to ensure that the correct anatomic location of the index lesion is imaged.

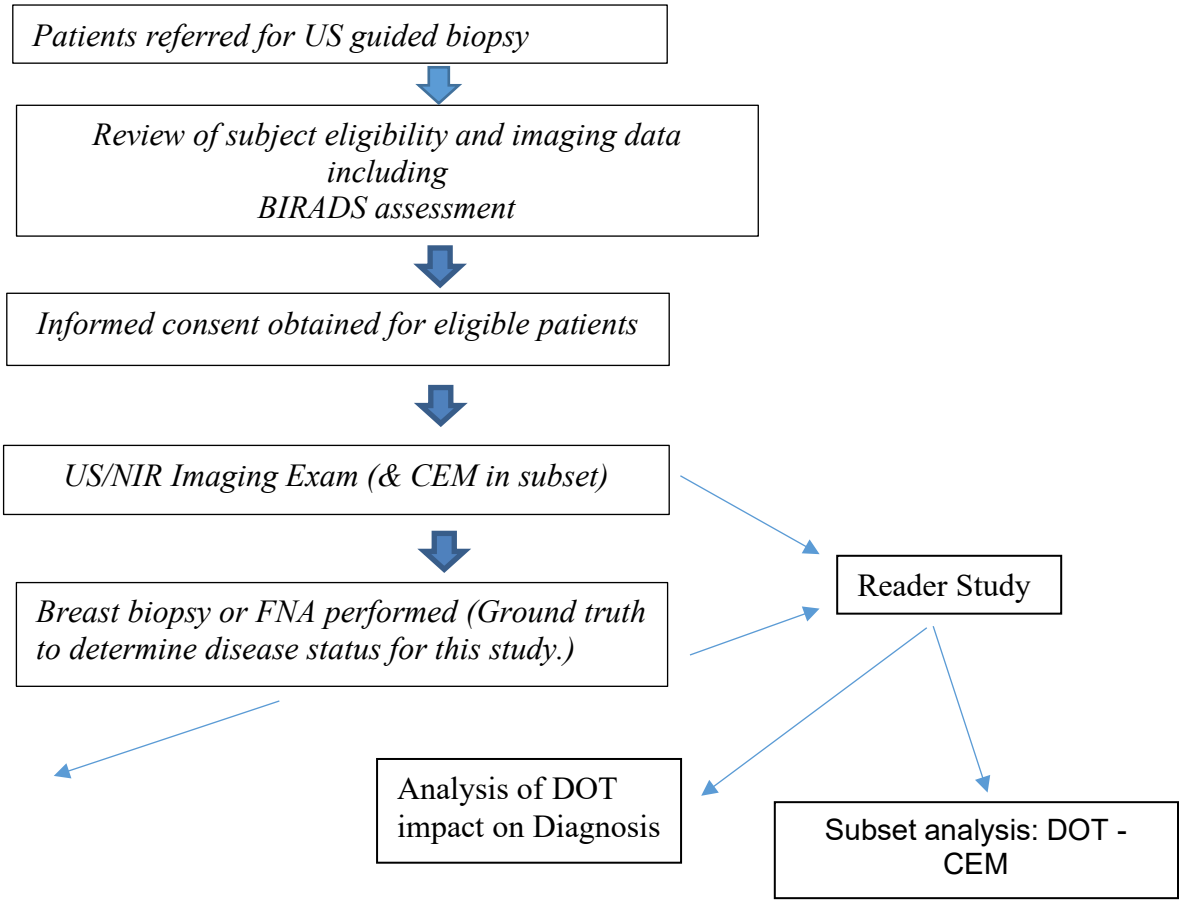
The study radiologist that performs/supervises the US-guided DOT imaging will complete CI and US-guided DOT case report forms (CRFs) blinded to the pathology result of the ensuing biopsy. In the event that the patient also undergoes CEM the study radiologist will complete CI and CEM CRFs blinded to the pathology result of the ensuing biopsy. Details of CRF completion and related reader study are noted in section 7 – Data Collection and Analysis below.

***Figure 3**. Study work flow*

Training Set:



Prospective Trial



6. US and OPTICAL IMAGING

A hand-held hybrid probe, consisting of a commercially available US transducer located in the middle and near-infrared source and detector optical fibers distributed at the periphery (see Figure 1), will be used for scans. For each patient, co-registered digital US images from the commercial ultrasound unit and optical measurements from our NIR optical imaging device are acquired simultaneously at multiple locations including the lesion of interest region and the same region in the mirror-image contralateral breast. The contralateral location is chosen as the reference site. The difference between measurements obtained from the lesion and the reference site is used for optical imaging reconstruction. Optical absorption distributions at four different wavelengths are reconstructed and lesion total hemoglobin concentration (tHb), and oxygenated and deoxygenated hemoglobin concentrations (HbO2 and Hb) are computed from absorption maps. The total data acquisition time is about 10 minutes once the lesion is identified by US. Following each patient study, the near infrared images will be reconstructed by Dr. Zhu' group.

Safety and Setup for Imaging

The US-guided NIR device has been used safely by Prof. Zhu's group in the past 14 years for ~500 patients [24,25,34]. There is no known risk associated with the use of the device. The light sources are low-power laser diodes with wavelengths in the NIR range and are fiberoptic coupled to the probe. The laser light sources can only be activated by pushing a button on the touch screen laptop when the surface of the probe, i.e., the tips of the fibers, is in contact with the skin.

7. DATA COLLECTION AND ANALYSIS

Study specific data will be collected from subjects after informed consent is obtained. The following data may be collected on the patient questionnaire: demographic information (name, DOB, Age, Race and Hispanic or Non-Hispanic) MRN#, imaging reports (e.g. mammography, US, PET, MRI, etc.), core biopsy pathology reports, surgical pathology reports if available, breast cancer risk information, etc.

In some cases, the pathologist may review existing biopsy specimens with special staining to look for signs of increased blood supply and vessel density. This will not require additional slicing of standard of care biopsy specimens; thus, samples will not be labeled or stored for the research study. The results of these tests will not be placed in the patient medical record. The results of these tests may be given to the investigators to correlate to the results of the NIR/US procedures.

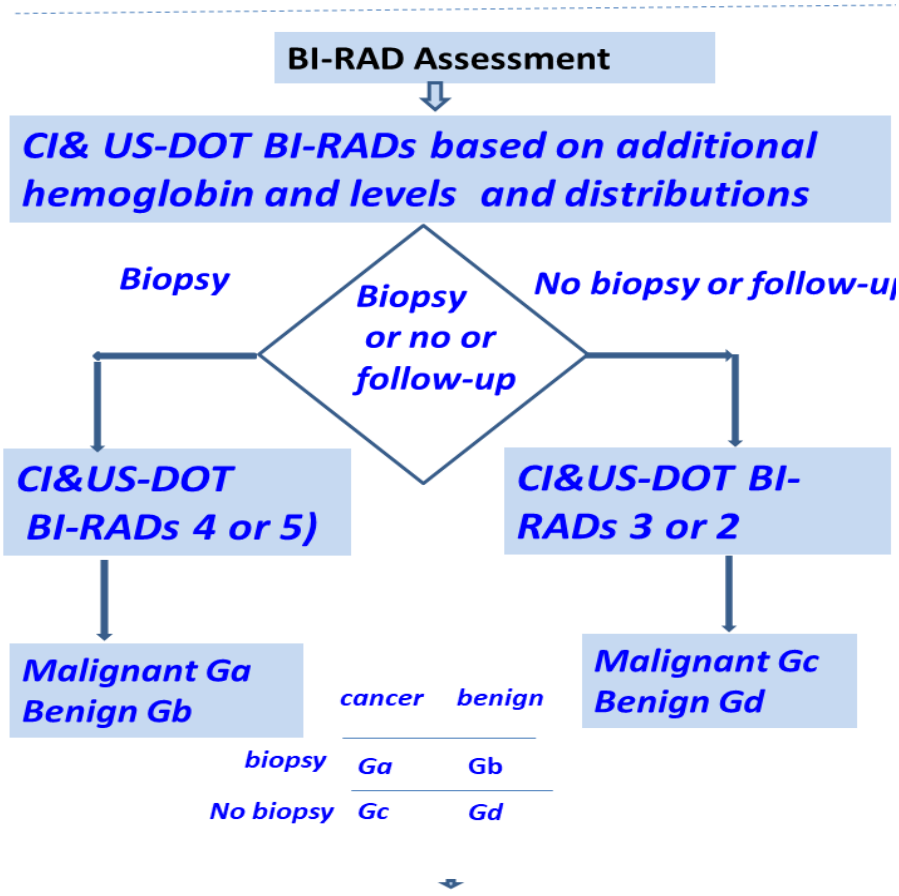
Dr. Ian Hagemann, specialist in breast pathology, will assess pathological findings.

The goal of this study is to assess the impact of adjunctive US-guided DOTon the accuracy of breast biopsy recommendations for abnormalities undergoing US-guided breast biopsy. In particular to assess the effect of combining US-guided DOT with CI to: 1. reduce the rate of benign breast biopsy, i.e. decrease false positive biopsy recommendations, and 2. to maintain sensitivity, i.e. maintain true positive recommendations.

Additional goalsof this study are to assess the role of US-guided DOT in managing discordant biopsy results and assess the correlation of optical imaging parameters with the presence

and intensity of contrast enhancement of the abnormality undergoing biopsy in patients who have undergone CEM.

Figure 4 *– Reader Study Design*



To accomplish the aims of assessing the impact of adjunctive US-guided DOT on reducing benign biopsy recommendation (i.e. FPs), while maintaining sensitivity (i.e. TPs) we will conduct a reader study of the 300 subject trial. Three of four study radiologists will serve as study readers for each case. (To eliminate potential bias a study radiologist performing the biopsy will not serve as a reader for that case.) As noted above the study radiologist who performs or supervises the US-guided DOT exam will be one of the readers. Two additional study radiologist readers will subsequently and independently review the available pre-existing patient information and CI data, (excluding the US-guided biopsy exam) and complete CI and DOT CRFs blinded to the pathology result of the abnormality that underwent biopsy. The CRFs include a BI-RADS assessment. The reader will first complete the CI CRF blinded to the US-guided DOT result, then after reviewing the optical data (tHB concentration and distribution) the reader will complete the DOT CRF including the impact of US-guided DOT information on the BI-RADs assessment, i.e. no change, decreased or increased.

Similar to the CI plus DOT workflow, for cases that have CEM, a CI CRF will be completed first, followed by a CEM specific CRF including a BI-RADS assessment and the impact of CEM on the BIRADs assessment will be recorded. As noted above, the study radiologist that supervises the US-guided DOT and CEM exams will serve as one of the study readers. For the subsequent two readers we will alternate which adjunctive modality (i.e. CEM or US-DOT) is reviewed first and separate the subsequent review of the second adjunctive modality by a minimum of 4 weeks to prevent recollection bias).

Figure 4 diagrams the core analysis. Combined CI and US-guided DOT BI-RADS 2 and 3 assessments will be considered test negative, and BI-RADs 4A, 4B, 4C, and 5 assessments will be considered test positive. For negative tests, the biopsy proven malignant group (Gc, see Fig. 1(b)) represents false negative results, and the benign group Gd represents true negatives and reflects the potential reduction in benign biopsy recommendation if US-guided DOT were used as SOC. Ga represent true positives and Gb represent false positives utilizing adjunctive US-guided DOT. A comparison of the Ga groups i.e, CI alone vs. CI & DOT will constitute the assessment of the effect of adjunctive US-guided DOT on sensitivity.

A small minority of patients assessed as BIRADS 3-Probably Benign and recommended for short term follow up imaging on the basis of CI will elect to undergo biopsy. We will separately track the effect of supplemental US-DOT on this small subset.

7.1 Data Analysis and Validation

US-guided DOT database and Training:
Part 1 - Using typical tHb levels and features (e.g. peripheral enhancement, shadowing) from biopsy proven benign and malignant cases obtained from earlier studies we will train the four study radiologists. The goals of the training are to familiarize the study radiologists with US-guided DOT imaging and typical hemoglobin distributions (tHb, oxyHb, deoxyHb) of different benign and malignant disease processes and to assess intra- and inter-observer variability.
Data from the training study will not be used in the primary analysis.

Part 2 - Approximately 20 patients will be enrolled for the training phase. Inter-reader agreement will be assessed based on Fleiss's Kappa coefficient across the three radiologists and Cohen's Kappa between two radiologists, each associated with 95% confidence interval. The 20 patients will provide 83.32% power for testing agreement between two readers using the Kappa statistics to detect a true Kappa value of ≥0.75 against an unacceptable Kappa value of ≤0.3, based on 1-sided normal test at a 0.1 level.

Intra-observer variability will also be assessed in the training phase. Each radiologist will read each patient's images twice (or even three times). After the first round of reading, the patients' images will be stored and the radiologists will be asked to review the same patients' images some time later, after the image orders have been perturbed and patient ID and previous evaluation results have been blinded. Integrating data from repeated evaluations from multiple radiologists on the 20 patients, generalize linear mixed effects model will be used to fit the reading results to characterize inter- and intra-reader variability based on an intra-class correlation coefficient (ICC). If a persistent large intra-reader variability is found (e.g., with ICC >0.3), we will perform a focused interview with the radiologists to identify possible factors yielding inconsistency in reading results. Considering the influential factors, we will revise the training phase by providing more structured instructions illustrated with imaging examples to clarify potential issues. We expect improvement with structured instruction, but if intra-reader variability persists, we will consider requiring

repeated evaluations from the same radiologist on the same patient in order to robustly evaluate the diagnosis of US-guided DOT.

Up to 315 participants will be enrolled to yield 300 participants with valid US-DOT data to evaluate the primary and secondary objectives as below.

Aim 1.1 Assess the impact of US-guided DOT on potential reduction of benign biopsies
The primary objective of this prospective study is to assess the impact of US-guided DOT **on the** potential reduction of benign biopsies, a.k.a, improve specificity. We hypothesize that the adjunctive use of US-guided DOT will reduce the rate of benign breast biopsy. Using these criteria, the first endpoint, specificity = Gd/(Gb+Gd), will be calculated as the proportion of subjects with a negative assessment (BI-RADS 2 or 3) and with no cancer at biopsy. With the exception of a small minority of patients with BI-RADS 3 assessments who elect to undergo biopsy and who will be tracked separately, all subjects in this study are considered positive using the current SOC, because they have been recommended for biopsy. Hence, 1) the specificity in this group for the SOC is 0% by definition; 2) the estimate of specificity for the DOT system represents the improvement in specificity; and 3) the estimate of specificity is also an indication of the percentage of benign biopsies that might be avoided if the NIR data were used by the radiologist in the biopsy decision making process.

Aim1.2 Assess the impact of US-guided DOT as an adjunct to CI on maintaining high sensitivity
To also ensure that the potential for false negatives is not a significant risk with the use of the US-guided DOT, we will also assess the probability of potentially misclassifying a malignant abnormality. By definition, FN=Gc/(Gc+Ga), which is 1 minus sensitivity. Our earlier study [Zhu reference] indicated that both radiologists reached 96.6% to 100% sensitivity with the tHb data included, and therefore had a 0-3.4% probability of missing a malignant lesion.

All CRFs including CI, DOT, CEM and biopsy results will be entered by our study radiologists, or study coordinator or study pathologist into the OnCore data base to be exported to Dr. Luo for statistical analysis. An ad hoc analysis of the CI, CEM and optical imaging characteristics and histopathologic features of false negative cases will be performed to identify suspicious features that always indicate a level of suspicion warranting biopsy.

Aim1.3 Explore the potential benefit of managing post biopsy BI-RADS 4C and 5 lesions
From a total of 300 patients who undergo the US-DOT exam, we expect to recruit a small subset of patients with BI-RADS 4C or 5 lesions whose biopsy results are benign. We will correlate initial biopsy results, optical data and gold standard final pathology for this subset of patients. This pilot data will allow us to assess the potential benefit of US-guided DOT in managing discordant abnormalities, i.e. reducing additional invasive procedures of a subset of benign abnormalities.

Exploratory Aim 1.4 Explore and compare US-guided DOT and CEM
In this exploratory aim, we will evaluate the agreement (Kappa coefficient) of US-guided DOT and CEM, as both modalities measure alterations in lesion vascularity. We will compare BI-RADS assessments of CI&CEM vs. CI&US-guided DOT as well as correlate imaging data such as intensity of enhancement in CEM and the total hemoglobin level of US-DOT.

Alternative plan: *If US-guided DOT is not comparable with CEM we will evaluate the capability of US-guided DOT in improving the accuracy* of combined CI&CEM because CEM may become SOC in a significant subset of patients.

8. ADVERSE EVENT MONITORING AND REPORTING

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 8.2.

There are minimal side effects associated with the NIR/US, therefore we will not be tracking AEs. All SAEs that occur within 24 hours of an NIR/US exam will be brought to the attention of the PI and a determination will be made if they are related or unrelated to study participation. Any SAE that is related to the NIR/US intervention, will be reported to the IRB.

8.1 Definitions

8.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Attribution (Relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website at: http://www.hhs.gov/ohrp/policy/advevntguid.html.

8.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

8.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

8.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

8.1.5 Unanticipated Problems

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, 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 subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.6. Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subject research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

8.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, and results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

8.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation. Pre-approval of all protocol exceptions must be obtained prior to the event.

8.2 Reporting to the Human Research Protection Office (HRPO)

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

8.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

8.4 Time Frame for Reporting Required Events

As noted above minimal adverse events are associated with NIR/US. If we are made aware of an AE/SAE then we will report this to the HRPO in the timeline noted in section 8.2.

9. DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual

- Protocol activation date
- Average rate of accrual observed in month 1, month 2, and subsequent months
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor any potential issues on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

10. Statistical Considerations

10. 1 Study design.

The study consists of two phases. The training phase consists of 20 eligible participants as detailed in Section "**US-guided DOT database and Training**" to assess sources of variabilities. The formal phase is a prospective study to enroll up to 315 eligible participants who will undergo SOC biopsy and fall in the BIRAD categories of 3, 4A, 4B, 4C, or 5 to yield 300 with valid US-DOT data.

10. 2 Study hypotheses and endpoints.

Study Hypothesis 1 for aim 1.1: The first hypothesis is that the measured tHb distribution and values can be potentially used as an adjunct for radiologists to reduce unnecessary biopsies in low risk patients with benign pathological results. The benign biopsy spare rate can be calculated as Gd/(Gb+Gd) in Fig.11, which is equivalent to the specificity, the primary endpoint of Aim1.1. We observed in previous studies that specificity could be improved by 45% on average if NIR data were used in the decision-making process. Note that the specificity in this group of biopsied patients under the SOC is 0% by definition. In this study, we conservatively set the minimum acceptable specificity at 30%. The null and alternative hypotheses, which demonstrate superiority when the NIR imaging data are used as an adjunct to the diagnostic assessment versus when the SOC diagnostic data are used alone, are given by

$$H_0: \text{Specificity} \leq 30\% \text{ versus } H_1: \text{Specificity} > 30\%$$

This aim will be considered a success if the lower limit of the 95% confidence interval on specificity exceeds 30%. This hypothesis will be tested at the one-sided 0.025 level, rather than 0.05, to account for the fact that two hypothesis tests (on specificity and sensitivity) are planned. This aim is considered a success if the lower limit of the 97.5% confidence interval on specificity exceeds 30%.

Study Hypothesis 2 for aim 1.2: The second hypothesis is that, while reducing unnecessary benign biopsies, the addition of adjunct information from US-DOT still maintains a high sensitivity

in capturing nearly all malignant tumors. This can be evaluated based on sensitivity, which is calculated as Ga/(Gc+Ga), as shown in Fig.11.

H_o: sensitivity ≤ 90% versus H_1: sensitivity>90%

Based on previous study, we anticipate the sensitivity will be in the range of 96-100%. This aim is considered a success if the lower limit of the 95% confidence interval for sensitivity is at least 90%. This hypothesis will be tested at the one-sided 0.025 level, rather than 0.05, to stringently account for the fact that two hypothesis tests (on specificity and sensitivity) are planned. This aim is considered a success if the lower limit of the 97.5% confidence interval for sensitivity is at least 90%.

10.3 Sample size justifications.

We expect ~300 eligible women who will undergo SOC biopsy with valid US-DOT data and fall in the BIRAD categories of 3, 4A, 4B, 4C, or 5, rendering ~210 pathologically benign biopsies and 90 malignant biopsies. **For Study Hypothesis 1,** prior study results indicate the specificity was estimated to be no less than 45%. A total of 83 benign biopsies are needed to achieve 80.18% power to test Study Hypothesis 1 at a 2.5% level. This calculation is based on a 1-sided exact binomial test by conservatively assuming the true specificity is 45%, while 210 benign biopsies achieve 99.4% power to test Hypothesis 1. We also calculated the sample size based on the more stringent requirement that the lower limit of the 1-sided 97.5% conservative Clopper-Pearson binomial exact confidence interval (CI) (60) to the estimated specificity (of 45%) should exceed 30%. The 210 benign biopsies will allow us to estimate the 1-sided 97.5% exact CI to be between 37.7%~100%, thus satisfying this more stringent requirement. **For Study Hypothesis 2,** 90 malignant biopsies will achieve 89.33% power to conduct Study Hypothesis 2 at a 1-sided 2.5% level, based on a 1-sided exact binomial test. With 90 malignant biopsies, the sensitivity can be estimated at 98% with a 1-sided 97.5% exact CI of 92.06%~100%, thus satisfying the stringent criterion requiring a lower limit of >90%.

In calendar year 2016, over 7,000 women came to the Siteman Cancer Center at WashU for diagnostic evaluation. Approximately 17% had suspicious or highly suggestive malignancy findings and were recommended for tissue sampling. Over 700 women underwent US guided biopsy, which is more than 10 times larger than the potential study subjects each year.

10.4 Data Analysis

All data will be analyzed as it is without imputations. Summary statistics will be used to summarize data including mean, median, standard deviation, inerquartile range for continuous data and count (percentage) for categorical data.

All evaluable subjects will be analyzed. In addition to the specificity and sensitivity of US-guided DOT results, PPV, NPV, and overall accuracy will be calculated. The two study hypotheses will be tested by a binomial exact test and by constructing the one-sided 97.5% Clopper-Pearson exact confidence intervals for specificity and sensitivity. The lower limit will determine the achievement of study goals. We will explore the data further to refine the classification algorithm and the cut-off threshold. ROC analysis will evaluate the quantitatively measured tHb for pathological results of malignant biopsies. Besides tHb and its distribution, other parameters (such as oxyHb, deoxyHb, and distributions) will be either linearly incorporated with tHb to maximize the ultimate area under ROC or non-linearly through a tree-based classification model (e.g., using the R package “rpart”) (62).

The area under the ROC will be calculated to gauge the model's overall discriminative ability for biopsy results.

For the exploratory study (discordant biopsy results), the imaging data (e.g., tHb, oxyHb etc) will be summarized as appropriate in the subset of participants whose BIRAD results yield highly suspicious abnormalities (BI-RAD 4C, 5) but pathology yield benign results. Two sample t-test or Wilcoxon rank sum test as appropriate will be applied to compare this subset versus those with a malignant biopsy.

For the exploratory Study (CEM DOT correlation), we will compare BI-RADS assessments of CI&CEM vs. CI&US-guided DOT as well as correlate imaging data such as intensity of enhancement in CEM and the total hemoglobin level of US-DOT using Pearson or Spearman correlation coefficients. We will evaluate concordance/disconcordance between US-guided DOT and CEM using agreement measure (Kappa coefficient) and will test the disconcordance using McNemar test.

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