LCCC1915: High Frame Rate 3-D Super Resolution Ultrasound Microvascular Imaging

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name:_____

PI Signature: _____

Date:_____

Date of Protocol: August 19, 2022

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

Increasing the sensitivity and specificity of diagnostic imaging in patients at high risk for breast or thyroid cancer could provide substantial clinical benefit by improving diagnosis, preventing over-treatment, and reducing healthcare costs. Contrast enhanced super-resolution (CESR) imaging is a new type of contrast enhanced ultrasound imaging which is specifically sensitive to microvascular structure and density. It evaluates tumor micro-vasculature on the order of 10 – 100 microns in diameter and may provide a powerful prognostic tool for the diagnosis of breast cancer, and eventually for treatment evaluation.

This is a 3-arm single center study of 45 patients. These cohorts will include 15 breast patients scheduled to undergo a biopsy, and 15 thyroid patients scheduled to undergo fine needle aspiration, biopsy, or thyroidectomy that consent to undergo CESR imaging in conjunction with b-mode ultrasound prior to their scheduled biopsy. Prior to imaging clinical patients, the third arm will include 15 healthy volunteers that will be imaged to optimize imaging parameters. Despite the similarity to our previous study, LCCC 1748, optimization is required because we will employ a new imaging technique using a different US system. The optimization includes adjusting the frame rate, power, depth of imaging, and the linear translation rate. The primary objectives of this study are to evaluate the sensitivity and specificity of CESR imaging in the analysis of known breast and thyroid lesions by comparing image analyses to the pathological results for these lesions. The secondary objectives of this study are to compare the sensitivity and specificity of CESR imaging with traditional b-mode ultrasound in the distinction of malignant versus benign breast and thyroid lesions.

1.2 Disease Background

1.2.1 Breast Cancer

Breast cancer is the most common cancer type among women. Approximately 10% of women in the United States develop breast cancer during their lifetime, and 30% to 40% of these patients will die from it.[1, 2] Mammography is an effective tool for the early detection of breast cancer in the majority of women.[3] However, for women with dense breast tissue (considered an independent risk factor for breast cancer) and younger women, mammography performs poorly due to lower sensitivity and specificity in these groups.[4] For young women with heritable mutations who wish to begin screening at a younger age, these limitations are especially problematic.[5] Additionally, mammography is less sensitive in women who have undergone breast augmentation. Given these limitations, most of these women may undergo additional imaging with breast ultrasound or Magnetic Resonance Imaging (MRI).

MRI is time consuming, extraordinarily costly, and has limited availability, especially among rural and underserved populations. Another screening option, breast ultrasound (without contrast), is widely used when additional imaging beyond mammogram is required due to its real-time imaging capability (>30 images per second), portability, safety (does not involve radiation), and relatively low cost compared to breast MRI. Unfortunately, while breast ultrasound is highly sensitive (96%), it is less specific (70%), resulting in a high false positive rate. This results in unnecessary biopsies with associated complications, additional follow-up and negative psychosocial impacts on patients, e.g., significant anxiety. A significant clinical need exists to improve breast ultrasound sensitivity and specificity.

1.2.2 Thyroid Cancer

The most common endocrine cancer is thyroid cancer (approximately 1.0%–1.5% of all new cancers diagnosed each year in the USA). In the United States, 53,000 people are diagnosed with thyroid cancer each year.[6] In many countries in the developed world, thyroid cancer incidence has increased dramatically over the past three decades.[7] Increased frequency in use of sensitive diagnostic procedures, including ultrasound, Doppler examination and imaging techniques like CT, MRI, or PET scanning, has amplified the detection of thyroid cancer among other cancer types.[8] However, thyroid cancer incidence and mortality tendencies have been recognized as being consistent with overdiagnosis.[9] The financial impact of overdiagnosis in a thyroid cancer patient can range from hundreds to thousands dollars. It may also cause prospective harm in standings of avoidable distress and conceivable adverse consequences of avoidable treatment. [10]

1.3 Ultra-fast and Super-Resolution Imaging

Recent developments in ultrasound hardware and software have enabled a substantial leap forward in ultrasound imaging technology. New programmable ultrasound systems can utilize software beamformers, parallel and distributed computing architectures, and large onboard memory to perform ultra-fast imaging, on the order of thousands of frames per second, compared to ultrasound systems still utilized in the clinic which are limited to only slightly past 30 frames per second. This ‘quantum leap’ in system performance has enabled several revolutionary advancements that will ensure that ultrasound in the clinic will soon offer substantial capabilities beyond what is currently available. One such novel technology is ‘ultrasound localization microscopy’, also referred to in this proposal as contrast enhanced super-resolution (CESR) imaging.[11] This imaging approach has been developed by researchers in the medical ultrasound domain who have made an effort to mimic the revolutionary new technology of optical localization microscopy. Optical localization

microscopy exploits the stochastic blinking of specific fluorescent sources and super-localizes the center of each source by virtue of its separability. [12, 13] By accumulating these center positions over thousands of acquisitions, the resulting image achieves a ten-fold resolution improvement and enables imaging cell membrane and small organelles with a resolution beyond the diffraction limit. In the medical ultrasound domain, different techniques have been investigated to achieve an ultrasound super resolution image, using the blinking of contrast agent microbubbles as an acoustic equivalent of the fluorescent sources. Some groups used highly diluted contrast agents to meet the key requisite of separable microbubble detection.[14, 15] Although these groups obtained super-resolved images using conventional ultrasound scanners, the long acquisition time necessary to perform diluted microbubble super resolution imaging is likely to impinge upon its practicality. Tanter's group first demonstrated the application of ultrafast acquisition and spatiotemporal filtering to separate microbubbles even at a clinically-relevant concentration, by exploiting the decorrelation of microbubbles from a stack of images.[14, 16] This technique is a direct analog to fluorescence photoactivation localization microscopy (FPALM) in optics and the acquisition time is more reasonable for clinical translation. By localizing the centers of separable scattering microbubbles, this ultrasound localization microscopy technique allows imaging of microvessels at resolutions as small as ten micrometers, over an order of magnitude smaller than the ultrasound diffraction limit. Their team has recently published the use of this novel approach in Nature, demonstrating super-resolved vasculature maps of rat brain slices using this super resolution contrast ultrasound technique (Fig. 2.).[11] The Dayton Lab at UNC, following work from Tanter's group, has taken this approach a step further, applying it with a mechanically scanned system to obtain 3-D images of tumor-associated angiogenesis.[17] Our results illustrate that we can observe the same microvascular abnormalities we observe with Acoustic Angiography in tumors – supporting our hypothesis that we will be able to use super-resolution imaging to image cancer biomarkers in humans. It is of particular note that CESR can be performed effectively at clinical frequencies (our data to date has been acquired at 4.5 MHz with a clinical ATL probe), and depths up to 10 cm are theoretically achievable while still retaining resolution better than 100 microns, as long as microbubbles are detectable in original B mode imaging!

Another notable advantage of CESR is that it can be performed at low mechanical indices, less than 0.2, which means that it is a non-destructive imaging technique, and can readily be performed within guidelines for contrast in humans.

1.4 Software-based Ultrasound Systems

Within the last few years, computational power has finally achieved the performance required to design almost all components of an ultrasound system with dedicated programmable integrated circuits. Consequently, instead of large analog componentry with fixed capabilities, modern ultrasound systems can fit into the case of a simple PC, and still be highly programmable. Commercial vendors such as Verasonics, Cephasonics, and others, are now making programmable ultrasound systems widely available. The high-performance hardware means that these systems can transmit and receive data at rates up to the pulse repetition frequency limit based on speed of sound (thousands of frames per second, depending on tissue depth). It is this advancement which has enabled super-resolution imaging as described above.

1.5 Impact of ultrasound as a diagnostic tool

While there are clearly clinical applications for MRI, PET, SPECT, and CT that will never be supplanted by acoustics, there are clear clinical applications where ultrasound is well positioned to improve clinical outcome. For example, breast ultrasound has poor specificity and a high false positive rate, and hence it is not used as a screening tool for breast cancer. Furthermore, ultrasound is challenged to detect breast lesions smaller than a few millimeters. The same can be said about ultrasound's sensitivity to malignant thyroid and prostate cancer. Improvements in specificity in these fields would have a significant clinical impact. Furthermore, due to the low-cost and portability of the next generation of laptop or handheld-sized ultrasound systems, this modality is uniquely poised to expand diagnostic capability to rural and underserved locations and populations worldwide. Other clinical applications, such as assessing vasa vasorum in atherosclerotic plaques, or assessing angiogenesis in wound healing, may also benefit from high-resolution microvascular ultrasound imaging. Furthermore, the applications of high resolution microvascular imaging in pre-clinical cancer research are readily apparent.

1.6 Ultrasound-localization microscopy/Contrast Enhanced Super Resolution (CESR) Imaging

The methods described in this protocol are based on the novel imaging technique of Ultrasound Localization Microscopy, also called Contrast Enhanced Super-Resolution Imaging (CESR). This technology is only about five years old – with the seminal paper being published in Nature in 2015.[11] Its growth has been explosive because of the enormous potential– with at least 8 talks in this area at the 2016 International Ultrasonics Symposium. Further innovation from this technique arises from our 3-D CESR implementation, which has been published in November 2016.[17] In this project, we will advance this technology to the next step – fast 3-D CESR with a matrix transducer, combined with unique approaches such as multi-focus adaptive beamforming to increase

sensitivity, pushing its innovation to the next level with the goal of clinical translation.

1.7 Investigational super-resolution imaging methods

We will perform contrast enhanced super-resolution (CESR) imaging of tumors using a Verasonics Vantage system (Verasonics Inc., Redmond, WA, USA) with one of three different ultrasound probes, using plane-wave imaging at a pulse repetition frequency of 500 Hz. Two of the possible probes are 32x32 element matrix arrays with center frequencies of 1.5 and 3 MHz. The third probe is a hybrid dual-frequency array that transmits at 1.7 MHz with two single elements and receives at 20 MHz on a linear array. The transmitted pulses will be 1 cycle sinusoids at 1.5, 1.7, or 3 MHz with mechanical index less than 0.3. This low mechanical index was chosen to minimize bubble destruction under high frame rate insonification.

For data acquired with the matrix probes, a high-pass spatiotemporal singular value decomposition (SVD) filter is applied to detect the decorrelation of bubbles, yielding individual sources on the filtered images. This spatiotemporal filter can discriminate bubble signals whose spatial coherence is low from tissue signals whose spatial coherence is high because their temporal variations affect many neighboring pixels the same way.[18] For data acquired with the dual-frequency probe, bubbles are detected based on their nonlinear response relative to tissue. Hysteresis thresholding is used to localize the bubbles on the filtered images. Bubble centers are detected and center positions from all the frames are accumulated to get a super-resolution image, with a pixel size of 10 μm × 10 μm, for each scan slice.

1.8 Perflutren Lipid Background and Known Toxicities

See https://definityimaging.com/pdf/definity-pi.pdf for full prescribing information on perflutren when used according to its FDA-approved indication.

1.8.1 Background and Current Indications

Definity® (perflutren lipid) is an FDA-approved lipid-shell microbubble ultrasound (US) contrast agent that may be administered by an intravenous (IV) bolus or infusion. Currently, this contrast agent is approved for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve delineation of the left ventricular endocardial border. It is not approved as a contrast agent for microvascular imaging in the breast or for thyroid imaging.

When used according to its approved indication, the maximum dose of perflutren is administered as either two bolus doses or one single intravenous infusion.

For our study, perflutren lipid will be administered intravenously by a nurse or trained medical personnel. We will request an investigational new drug (IND) exemption at the time of the IRB application because the investigators feel this study meets the criteria of an IND exempt study.

1.8.2 Associated Toxicities

In pre-market clinical trials, 1716 subjects were evaluated with activated perflutren lipid. Of the 1716 subjects, 144 subjects (8.4%) had at least one treatment-related adverse reaction. There were 26 serious adverse events and 15 (0.9%) subjects discontinued because of an adverse event. Nineteen subjects (1.1%) suffered serious cardiopulmonary adverse events including eight deaths. The deaths occurred several days after activated perflutren lipid administration and appear to be related to the course of underlying disease. Of the 11 other serious adverse events, which appeared within 2-15 days of the drug administration, all appeared to be a progression of underlying cardiac and non-cardiac disease. However, a role for perflutren lipid in the initiation or course of these adverse events cannot be ruled out.

There were 15 discontinuations reported. Nine of these patients were discontinued after the first injection. One patient experienced a hypersensitivity reaction with urticaria and pruritus and all the other patients experienced dizziness, chest pain, dyspnea or back pain. Adverse events (AEs) appeared within 1 – 15 minutes of the drug administration and were of moderate intensity resolving usually without treatment within minutes or hours after onset.

For all AEs, there were no differences in the overall incidence based on age, gender, or route of administration. The most common events were (% of patients experiencing): headache (2.3%), back and renal pain (1.2%), flushing (1.1%) and nausea (1.0%).

Cardiopulmonary Reactions
In 2007, in response to post-marketing reports of 4 deaths and 190 serious cardiopulmonary reactions, the FDA issued a black box warning for both Definity® and Optison® adding disease state contraindications and a mandatory 30 minute monitoring period following administration in all patients. Following this there have been several large scale safety studies looking into the records of a total of more than 200,000 patients who received one of these contrast agents. In all those studies a composite rate of serious adverse events was calculated to be 1 – 3 in

10,000,[19] compared to gadolinium-based MRI contrast which has an incidence of NSF of 2 – 5 in 100 patients with chronic kidney disease.[20]

Following a meeting of the FDA Cardio-renal Advisory Committee in 2008, the black box warning was revised. The revisions shortened the contraindications to include cardiac shunts and hypersensitivity to perflutren, and mandated the 30 minute monitoring period be limited to patients with pulmonary hypertension or unstable cardiopulmonary conditions. The black box warning was further revised in 2011, removing the mandatory 30 minute monitoring period, but stating that most serious cardiopulmonary reactions occur within 30 minutes of administration. For this reason, the label states that cardiopulmonary resuscitation personnel and equipment be readily available prior to perflutren administration, and that all patients be monitored for acute reactions.

Patients with a history of cardiac shunts, pulmonary hypertension or unstable cardiopulmonary conditions will be excluded from our study. In addition, all patients will be monitored for 15-minute post-perflutren administration by the research nurse or research physician.

Hypersensitivity Reactions
The real risk of perflutren in our study is to the small number of potential patients with undiagnosed allergy to perflutren. Post-marketing reports have included anaphylactoid events and other serious but non-fatal adverse reactions, typically within 30 minutes of drug administration (see the package insert for additional information. In order to avoid a potentially fatal event, epinephrine will be readily accessible in the code cart in the mammography clinic.

High Ultrasound Mechanical Index (MI)
High ultrasound MI values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. In addition, end-systolic triggering with high mechanical indices has been reported to cause ventricular arrhythmias.

Use in Patients with Known Breast Lesions
US contrast agents including perflutren should carry no additional risks in patients with a known breast lesions, as they are cleared by the lungs. The phospholipid component of perflutren lipid microspheres are thought to be metabolized to free fatty acids, while the octafluoropropane (OFP), as a stable gas, is not metabolized. In a small (n=8) pharmacokinetic study in healthy subjects, OFP was undetectable after 10 minutes in most subjects either in the blood or expired air, with a mean half-life of 1.3 minutes (Definity® Prescribing Information).

1.9 Rationale

One main limitation to the widespread use of contrast enhanced ultrasound clinically has been the lack of availability of state-of-the art contrast imaging approaches available to clinicians. However, contrast ultrasound will likely become far more widespread as new imaging techniques, such as CESR imaging, demonstrate their usefulness and become available on commercial ultrasound systems. We propose to evaluate a novel ultrasound method that could potentially improve the sensitivity and specificity of traditional breast and thyroid ultrasound. Increasing the accuracy of diagnostic imaging in high risk patients could provide substantial clinical benefit by improving diagnosis, preventing over-treatment, and reducing healthcare costs.

LCCC1915 is designed to evaluate the sensitivity and specificity of contrast enhanced super-resolution ultrasound imaging in the analysis of known breast and thyroid lesions in patients with pathologically confirmed diagnoses of breast or thyroid cancer comparted with pre-study imaging results. The gold standard for sensitivity and specificity, then, will be based on pathological results. The Breast Imaging Reporting and Data System (BIRADS) is used by radiologists who read mammograms, ultrasounds, and MRIs to indicate their level of suspicion of the possibility of breast cancer. Scores range from 0 to 6, with scores of 4-5 indicating suspicious results, while 6 indicates an existing diagnosis of breast cancer. For the breast cohort, this study is limited to women with a score of 4-5 based on pre-study imaging. We selected this group of patients because there is more likely to be a cancer diagnosis than BIRADS 1-3.

For the thyroid cohort, we will include patients scheduled for a core needle or surgical thyroid biopsy, fine needle aspiration, or thyroidectomy of at least one sonographically visible thyroid lesion that is 3 cm in depth from the skin surface. We will recruit patients with high TIRADS risk scores (4c, moderately suspicious and 5, high risk), that have an anticipated risk range of 50% to in excess of 85%, which will provide an estimated relative risk of 75% to be congruent with the breast population.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To evaluate the sensitivity and specificity of contrast enhanced super-resolution imaging in the analysis of known breast lesions by comparing image analyses to the pathological results for these lesions.

2.1.2 To evaluate the sensitivity and specificity of contrast enhanced super-resolution imaging in the analysis of known thyroid lesions by comparing image analyses to the pathological results for these lesions.

2.2 Secondary Objectives

2.2.1 To compare (using a reader study) the sensitivity and specificity of contrast enhanced super-resolution imaging to the sensitivity and specificity of conventional b-mode ultrasound in evaluation of known breast lesions for predicting malignancy.

2.2.2 To compare (using a reader study) the sensitivity and specificity of contrast enhanced super-resolution imaging to the sensitivity and specificity of conventional b-mode ultrasound in evaluation of known thyroid lesions for predicting malignancy.

2.2.3 To compare the area under the curve (AUC) of contrast enhanced super-resolution imaging to the AUC of the b-mode ultrasound.

2.2.4 To compare radiologist preference of contrast enhanced super-resolution imaging to conventional b-mode ultrasound for each lesion characteristic (shape, margins, and vascularity).

2.3 Endpoints

We will utilize pathological status as derived from patient biopsy pathology reports.

3.0 PATIENT ELIGIBILITY

3.1 Arm 1: Healthy Volunteers

3.1.1 Inclusion Criteria

Subject must meet all of the inclusion criteria to participate in this study:

3.1.1.1 Adults ≥18 years old

3.1.1.2 Able to provide informed consent

3.1.1.3 Negative urine pregnancy test in women of child-bearing potential

3.1.2 Exclusion Criteria

All subjects meeting any of the exclusion criteria at baseline will be excluded from study participation

3.1.2.1 Institutionalized subject (prisoner or nursing home patient)

3.1.2.2 Critically ill or medically unstable and whose critical course during the observation period would be unpredictable (e.g., chronic obstructive pulmonary disease (COPD)

3.1.2.3 Known hypersensitivity to sulfur hexafluoride or to any component of perflutren lipid (Definity®)

3.1.2.4 Active cardiac disease including any of the following:

- Severe congestive heart failure (class IV in accordance with the classification of the New York Heart Association)
- Unstable angina.
- Severe arrhythmia (i.e. ventricular tachycardia, flutter fibrillation; ventricular premature complexes occurring close to the preceding T-wave, multifocal complexes).
- Myocardial infarction within 14 days prior to the date of proposed Definity® administration.
- Pulmonary hypertension
- Cardiac shunts

3.1.2.5 Any woman who is pregnant or has reason to believe she is pregnant or any woman who is lactating (the possibility of pregnancy has to be excluded by negative urine β-HCG results, obtained within 24 hours before the perflutren lipid administration, or on the basis of patient history, as defined by the UNC IRB SOP 4801.)

3.2 Arm 2: Breast Imaging Patients

3.2.1 Inclusion Criteria

Subject must meet all of the inclusion criteria to participate in this study:

3.2.1.1 Women ≥18 years old

3.2.1.2 Patient had a diagnostic breast ultrasound study performed at UNC

3.2.1.3 Scheduled for a core needle or surgical breast biopsy of at least one breast lesion that is 2 cm or less in size and 3 cm in depth from the skin surface

3.2.1.4 Lesion visualized on ultrasound

3.2.1.5 Able to provide informed consent

3.2.1.6 Negative urine pregnancy test in women of child-bearing potential

3.2.1.7 BIRADS score of 4 or 5.

3.2.2 Exclusion Criteria

All subjects meeting any of the exclusion criteria at baseline will be excluded from study participation

3.2.2.1 Male (it is uncommon for men to present for imaging and the overwhelming majority of findings are non-cancerous and do not lead to biopsy; male breast cancer represents <1% of newly diagnosed breast cancer)

3.2.2.2 Institutionalized subject (prisoner or nursing home patient)

3.2.2.3 Critically ill or medically unstable and whose critical course during the observation period would be unpredictable (e.g., chronic obstructive pulmonary disease (COPD)

3.2.2.4 Sonographically visible breast lesion larger than 2cm or greater than 3cm in depth from the skin surface

3.2.2.5 Known hypersensitivity to sulfur hexafluoride or to any component of perflutren lipid (Definity®)

3.2.2.6 Active cardiac disease including any of the following:

- Severe congestive heart failure (class IV in accordance with the classification of the New York Heart Association)
- Unstable angina.
- Severe arrhythmia (i.e. ventricular tachycardia, flutter fibrillation; ventricular premature complexes occurring close to the preceding T-wave, multifocal complexes).
- Myocardial infarction within 14 days prior to the date of proposed Definity® administration.
- Pulmonary hypertension
- Cardiac shunts

3.2.2.7 Any woman who is pregnant or has reason to believe she is pregnant or any woman who is lactating (the possibility of pregnancy has to be excluded by negative urine β-HCG results, obtained within 24 hours

before the perflutren lipid administration, or on the basis of patient history, as defined by the UNC IRB SOP 4801.)

3.3 Arm 3: Thyroid Imaging Patients

3.3.1 Inclusion Criteria

Subject must meet all of the inclusion criteria to participate in this study:

3.3.1.1 Adults ≥18 years old

3.3.1.2 Patient had a diagnostic thyroid ultrasound study performed at UNC

3.3.1.3 TIRADS risk score of 4c or 5

3.3.1.4 Scheduled for a core needle or surgical thyroid biopsy, fine needle aspiration, or thyroidectomy of at least one sonographically visible thyroid lesion that is 3 cm in depth from the skin surface

3.3.1.5 Lesion visualized on ultrasound

3.3.1.6 Able to provide informed consent

3.3.1.7 Negative urine pregnancy test in women of child-bearing potential

3.3.2 Exclusion Criteria

All subjects meeting any of the exclusion criteria at baseline will be excluded from study participation

3.3.2.1 Institutionalized subject (prisoner or nursing home patient)

3.3.2.2 Critically ill or medically unstable and whose critical course during the observation period would be unpredictable (e.g., chronic obstructive pulmonary disease (COPD)

3.3.2.3 Known hypersensitivity to sulfur hexafluoride or to any component of perflutren lipid (Definity®)

3.3.2.4 Active cardiac disease including any of the following:

- Severe congestive heart failure (class IV in accordance with the classification of the New York Heart Association)
- Unstable angina.
- Severe arrhythmia (i.e. ventricular tachycardia, flutter fibrillation; ventricular premature complexes occurring close to the preceding T-wave, multifocal complexes).
- Myocardial infarction within 14 days prior to the date of proposed Definity® administration.
- Pulmonary hypertension
- Cardiac shunts

3.3.2.5 Any woman who is pregnant or has reason to believe she is pregnant or any woman who is lactating (the possibility of pregnancy has to be excluded by negative urine β-HCG results, obtained within 24 hours before the perflutren lipid administration, or on the basis of patient history, as defined by the UNC IRB SOP 4801.)

4.0 STUDY PLAN

4.1 STUDY SCHEMA

This is a 3-arm single center study of 45 patients scheduled to undergo a breast or thyroid biopsy that consent to undergo a CESR scan in conjunction with b-mode ultrasound prior to their scheduled biopsy.

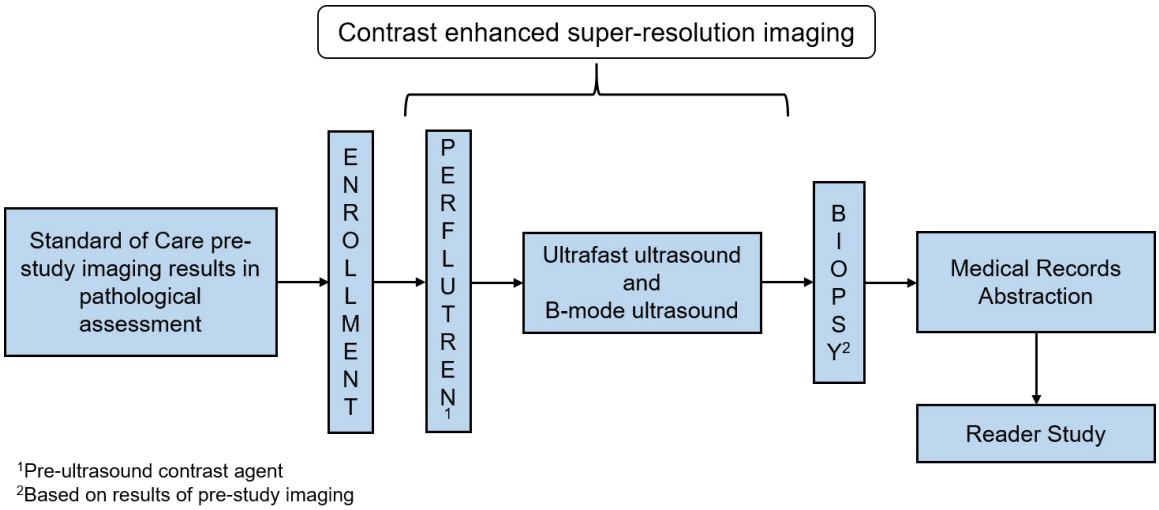

Figure 1. Study scheme.

4.2 Study Procedures

Study participants will be enrolled for 1 research imaging visit. In addition, their medical charts will be followed for 6 months.

4.2.1 Enrollment/Recruitment

A total of 45 adults will be enrolled to this study. The study subjects will be consecutively recruited from adults who are scheduled to undergo core needle or surgical biopsy to have pathological confirmation of malignancy status. Eligible patients will be identified by research staff review.
Once a patient has been referred, the patient will be approached by a coordinator from Radiology to assess interest in participation.

All eligible subjects who agree to participate in the study will be asked to come to their scheduled biopsy appointment thirty minutes early to complete the informed consent process.

Review of the consent will take place in the privacy of an exam room, or when possible, a sample consent form will be sent to the patient via email prior to the patient's visit to allow for ample review. Once the patient has consented, women of childbearing potential (WCBP) will be given a urine pregnancy test in order to ensure that they are not pregnant. If a urine pregnancy test shows a result positive for pregnancy, the patient will be excluded from the study per the exclusion criteria because the investigators cannot, in good conscience, expose a fetus to the contrast agent used. Women who consent for the study and are eligible will be escorted by the research coordinator to a dressing room, where the subject will change into a gown.

4.2.2 Research Imaging

4.2.2.1 Contrast Administration (if applicable)

At the time of imaging, the contrast agent perflutren lipid will be administered. See https://definityimaging.com/using-definity for instructions on perflutren lipid preparation and activation. Perflutren lipid is intended for intravenous (IV) administration only after activation in the Vialmix® apparatus. Cardiopulmonary resuscitation personnel and equipment will be readily available prior to perflutren administration, and all patients will be monitored for acute reactions.

Monitoring will include taking vitals (O_2 sat, HR, RR, BP). Vitals will first be collected at arrival to the clinic. All patients will be visually monitored for signs or symptoms of a contrast administration reaction once the drug is administered for a 15-minute period. Once 15 minutes have passed, vitals will be taken again. This study will be conducted in Mammography of the UNC Cancer Hospital, so trained medical personnel will be available as needed.

4.2.2.2 Imaging Procedures

CESR imaging involves a research ultrasound scanner as well as conventional b-mode ultrasound to guide the location of the imaging. The conventional ultrasound will be conducted just prior to CESR imaging for localization. Imaging will be performed within the package insert guidelines for ultrasound system mechanical index (a measurement of output power) when imaging perflutren contrast agent (less than 0.8).

CESR imaging will be performed by trained medical personnel using mild compression to eliminate motion. Total imaging time is estimated to be less than 15 minutes. All image data will be de-identified and transferred for off-line analysis based on a study ID. The research images will NOT be interpreted or analyzed for clinical decisions related to the patient. The field of view of the research images will be limited to clinically identified

lesions. Incidental findings are not anticipated due to the sensitivity limitations of this imaging modality. Information regarding additional lesions or other incidental findings discovered during research imaging procedures will not be recorded or communicated to the patient or their provider because this imaging modality is not standard of care.

4.2.3 Standard of Care Biopsy (if applicable)

The patient will then undergo his or her scheduled biopsy procedure based on the pre-study diagnostic imaging. The research CESR imaging will NOT be interpreted prior to the biopsy and therefore will not influence any clinical decision concerning the biopsy.

4.2.4 Medical Record Abstraction (if applicable)

The primary objective of this study is to determine the sensitivity and specificity of CESR imaging. In order to meet this objective, we will review each patient's clinical records, including their pathology report from biopsy. The malignancy will be determined as indicated by the pathology report.

4.2.5 Reader Study

A reader study will be performed after the completion of patient accrual to study lesion characteristics under CESR as compared to conventional b-mode ultrasound. In these pilot observational studies, the primary aim will be to evaluate the imaging approach for application in these two organs. We will also estimate the receiver operating characteristic (ROC) curve for the CESR system. For scientific rigor, a total of five readers who are not investigators on this study (radiologists trained in breast imaging or ultrasound imaging) will be recruited to participate for each reader study. The readers will be asked to assign a probability score (1 to 5) and confidence for each lesion for each modality (0 to 100%). ROC analysis will be performed as the primary analysis for the first aim. To compare the results from the two imaging modalities, we will adopt the mixed effect ANOVA based on the Dorfman-Berbaum-Metz method. The outcome variable is the Tukey's jackknife pseudovalues of the AUCs from each reader and each patient under either modality, and separately for each anatomic region. The fixed effect in the independent list will correspond to the difference between the two modalities, and the random effects will be used to account for within-patient and within-reader's correlations. Possible interactions between the modalities and the readers and the patients will also be included and tested for statistical significance. To test the main hypothesis, F-test statistic from the model parameter estimates will be used to compare the mean AUCs between the b-mode ultrasound images and the CESR ultrasound images only.

5.0 INVESTIGATIONAL DEVICE

5.1 Investigational Device Description

[redacted]

Research Ultrasound Scanner: Verasonics Vantage 1024

[redacted]

Figu [redacted]

Ultrasound 32 x 32 Transducer Arrays

[redacted]

similar [redacted]

Figure 3. Vermon 32 x 32 matrix array.

Hybrid Dual-frequency Ultrasound Array

[redacted] This dual-frequency imaging technique enables the detection of perflutren microbubbles without the need for spatiotemporal filtering.[22] Imaging with the dual-frequency probe will be performed with one Verasonics Vantage 256 system from the Vantage 1024 described above. For 3D acquisition, the transducer will be translated with a linear motion stage, as used in our previous study, LCCC 1748.

6.0 Perflutren Lipid Microspheres (Lantheus Medical Imaging)

[redacted]

[redacted]

6.1 Investigational Contrast Agent Description and Management

[redacted]

[redacted].com/pdf/vialmix-user-guide.pdf and the package insert for instructions on the use of Vialmix®.

6.1.1 Known and Associated Risks

For indication see https://www.definityimaging.com/pdf/definity-pi.pdf and see section 1.8.2 for a summary of toxicities reported in clinical trials. In addition, the following warnings and precautions are noted in the January 2017 labeling:

Serious Cardiopulmonary Reactions:
Serious cardiopulmonary reactions including fatalities have occurred uncommonly during or shortly following perflutren-containing microsphere administration, typically within 30 minutes of administration. The risk for these reactions may be increased among patients with unstable cardiopulmonary conditions (acute myocardial infarction, acute coronary artery syndromes, worsening or unstable congestive heart failure, or serious ventricular arrhythmias). Always have cardiopulmonary

resuscitation personnel and equipment readily available prior to Definity® administration and monitor all patients for acute reactions. The reported reactions include: fatal cardiac or respiratory arrest, shock, syncope, symptomatic arrhythmias (atrial fibrillation, tachycardia, bradycardia, supraventricular tachycardia, ventricular fibrillation, ventricular tachycardia), hypertension, hypotension, dyspnea, hypoxia, chest pain, respiratory distress, stridor, wheezing, loss of consciousness, and convulsions.

Hypersensitivity Reactions:
In postmarketing use, serious hypersensitivity reactions were observed during or shortly following perflutren-containing microsphere administration including: Shock, bronchospasm, throat tightness, angioedema, edema (pharyngeal, palatal, mouth, peripheral, localized), swelling (face, eye, lip, tongue, upper airway), facial hypoesthesia, rash, urticaria, pruritus, flushing, and erythema have occurred in patients with no prior exposure to perflutren-containing microsphere products. Always have cardiopulmonary resuscitation personnel and equipment readily available prior to Definity® administration and monitor all patients for hypersensitivity reactions.

Systemic Embolization of Perflutren in Patients with Cardiac Shunts:
When administering Definity® to patients with a cardiac shunt, the microspheres can bypass filtering by the lung and enter the arterial circulation. Assess patients with shunts for embolic phenomena following Definity® administration. Definity® is only for intravenous administration; do not administer Definity® by intra-arterial injection.

Ventricular Arrhythmia Related to High Mechanical Index:
High ultrasound mechanical index values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. Additionally, end-systolic triggering with high mechanical indices has been reported to cause ventricular arrhythmias. Definity® is not recommended for use at mechanical indices greater than 0.8.

6.2 Return and Retention of Study Contrast Agent

Incomplete vials of perflutren lipid remaining at the completion of the study, or expired perflutren lipid will be destroyed by UNC IDS.

7.0 UNANTICIPATED CONCERNS (DEVICES)

7.1.1 Unanticipated Adverse Device Effect (UADE)

The investigational device exemption (IDE) regulations define an unanticipated adverse device effect (UADE) as “any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or 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" (21 CFR 812.3(s)).

7.1.2 Unanticipated Problems (UP)

As defined by UNC's IRB, unanticipated problems involving risks to study subjects refers to any incident, experience, or outcome that:

- Is 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;
- Is related or possibly related to a subject's participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

7.1.3 Reporting

7.1.4 UADEs

UADEs must be reported by the clinical investigator to the sponsor and the reviewing IRB, as described below:

For this device study, investigators are required to submit a report of a UADE to the FDA, the manufacturer of the device and the UNC IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the event (§ 812.150(a)(1)), using the MedWatch Form 3500A. Sponsors must immediately conduct an evaluation of a UADE and must report the results of the evaluation to FDA, the UNC IRB, and participating investigators within 10 working days after the sponsor first receives notice of the effect (§§ 812.46(b), 812.150(b)(1)).

For this device study, we will submit a report of a UADE to the manufacturer and the IRB as soon as possible, but no later than 10 working days after the investigators first learn of the event.

7.1.5 UP

Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the Study Coordinator using the IRB's web-based reporting system.

Any unanticipated problem that occurs during the conduct of this study and that meets **at least** the first two criteria listed in section 7.1.2 must be reported to the UNC IRB using the IRB's web-based reporting system.

8.0 ADVERSE EVENTS (DRUGS- CONTRAST AGENTS)

8.1 Definitions

8.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

8.1.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the sponsor could

determine that there is *reasonable possibility* that the drug caused the event.

- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

8.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.4 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization; *
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

8.2 Documentation of non-serious AEs or SARs

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through the 30-day follow-up period after treatment is discontinued.

Collected information should be recorded in the Case Report Forms (CRF) for that patient. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

8.3 SAEs or Serious SARs

8.3.1 Timing

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout.

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin from day 1 of study treatment and continue through the 30-day follow-up period after treatment is discontinued.

8.3.2 Documentation and Notification

These events (SAEs or Serious SARs) must be recorded in the SAE console within Oncore™ for that patient within 24 hours of learning of its occurrence.

8.3.3 Reporting

IRB Reporting Requirements:

- The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system within 7 days of the Investigator becoming aware of the problem.

Pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study should be recorded as SAEs. The patient is to be discontinued immediately from the study. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy and must document the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy was

abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

8.3.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight (Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following information for review: 1) safety and accrual data including the number of study participants treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

9.0 STATISTICAL CONSIDERATIONS

This is a nonrandomized, single-center study. The primary purpose and endpoint of this study is to evaluate the sensitivity and specificity of CESR imaging in the analysis of known breast and thyroid lesions by comparing image analyses to the pathological results for these lesions.

9.1 Study Design/Study Endpoints

This is a prospective study to assess the diagnostic performance of the newly developed device. The **primary outcome of interest is the sensitivity and specificity of the super-resolution imaging device in the analysis of known breast and thyroid lesions as compared to pathological results of these lesions.** . The sensitivity and specificity can be estimated non-parametrically as the proportion of the lesions which are distinguished as malignant versus non-malignant compared to the reference. This estimate will be calculated for each reader then averaged over all the readers. The standard error of the average sensitivity and specificity estimate will be calculated using the bootstrap method, where each patient is treated as independent unit with 5 ratings. The confidence interval of the final estimate will be provided using the normality assumption.

Furthermore, we will estimate the sensitivity and specificity of the b-mode ultrasound in this study and compare it with the sensitivity and specificity estimate of the CESR imaging using the bootstrap approach and the confidence interval of their differences will be provided using the normality assumption.

Malignancy scores of +1 and +2 will be considered malignant. Scores of -2, -1, and 0 will be considered not malignant. The confidence of malignancy will be used independently

9.2 Data Analysis Plans

ROC analysis will be performed as the primary analysis for the first aim. To compare the results from the two imaging modalities, we will adopt the mixed effect ANOVA based on the Dorfman-Berbaum-Metz method. The outcome variable is the Tukey's jackknife pseudovalues of the AUCs from each reader and each patient under either modality, and separately for each anatomic region. The fixed effect in the independent list will correspond to the difference between the two modalities and the random effects will be used to account for within-patient and within- reader's correlations.

Possible interactions between the modalities and the readers and the patients will also be included and tested for statistical significance. To test the main hypothesis, F-test statistic from the model parameter estimates will be used to compare the mean AUCs between the b- mode ultrasound images and the CESR ultrasound images only.

9.3 Sample Size Considerations in ROC Analysis

For each lesion type, we assume 8 malignant and 7 benign and the AUC for conventional imaging to be 0.7 based on our previous breast studies. We will recruit patients with high TIRADS risk scores (4c, moderately

suspicious and 5, high risk), that have an anticipated risk range of a 50% to in excess of 85%, which will provide an estimated relative risk of 75% to be congruent with the breast population. The power to detect 0.2 AUC difference is about 30%. However, our primary goal is to evaluate the technical ability of the CESR approach to examine lesions in each of these superficial anatomic regions. We will also be evaluating the number of vessels visualized, the effects of respiratory motion and radiologist confidence in interpreting the CESR microvascular images. These studies will guide further development of the imaging approach for a prospective trial.

9.4 Secondary Analyses

Radiologist Preference

The secondary analysis will be to evaluate reader preference for specific lesion characteristics. To assess the reader preference for modality for each characteristic including shape, margins and vascularity, we will fit a random effect model with only intercept and random terms for patients and readers while the outcomes are the confidence scores (-3 to +3). By testing the intercept significantly larger than zero, we will conclude that the new modality provides more confidence for readers than the conventional one.

10.0 STUDY MANAGEMENT

10.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s) and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

10.2 Registration Procedures

Study participants will be registered into OnCore®, a web based clinical research platform by one of the Study Coordinators.

10.3 Data Management and Monitoring/Auditing

The images of all eligible enrolled subjects that are obtained and contribute to the ultimate diagnosis leading to biopsy will be de-identified for inclusion in the reader study. Copies of the clinical report forms as well as the de-identified images described in the preceding will be submitted for each case to the Study Coordinators for maintaining the study record and entering the data into a spreadsheet in preparation for the reader study.

As an investigator-initiated study, this trial may also be audited by the Lineberger Cancer Center audit committee as appropriate.

10.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

10.4.1 Emergency Modifications

UNC investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC's IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, an IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

10.4.2 Single Subject Exceptions

10.4.3 Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy. Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants

- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs, please follow the guidelines below:

Protocol Deviations: UNC personnel will record the deviation in OnCore®, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

Unanticipated Problems:
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the study team using the IRB's web-based reporting system.

10.5 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

10.6 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring

logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

10.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study participants. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

11.0 REFERENCES

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12.0 APPENDICES

12.1 Appendix A: Reader Study Data Collection Form

Specimen: _____ **Reader:_____**

Date:_____

Overall Assessment:

Primary

Overall specimen malignancy score (-2 to +2): _____
Malignancy confidence (0-100) _____%

Secondary

Overall specimen malignancy score (-2 to +2): _____
Malignancy confidence (0-100)_____%

1. Primary versus Secondary Shape/Morphology

+3	I am significantly more confident in the **Primary** representation of the lesion shape/morphology I described as compared to **Secondary** representation of the same lesion
+2	I am more confident in the **Primary** representation of the lesion shape/morphology I described as compared to **Secondary** representation of the same lesion
+1	I am slightly more confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.
0	I have the same confidence in the **Primary** representation of the lesion shape/morphology I described as I do in the **Secondary** representation of the same lesion
-1	I am slightly less confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.
-2	I am less confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.
-3	I am significantly less confident in the **Primary** representation of the lesion shape/morphology I described as compared to the **Secondary** representation of the same lesion.

2. Primary versus Secondary: Vascularity

+3	I am significantly more confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
+2	I am more confident in the **Primary** representation of the lesion vascularity I described as compared to **Secondary** representation of the same lesion
+1	I am slightly more confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
0	I have the same confidence in the **Primary** representation of the lesion vascularity I described as I do in the **Secondary** representation of the same lesion.
-1	I am slightly less confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
-2	I am less confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.
-3	I am significantly less confident in the **Primary** representation of the lesion vascularity I described as compared to the **Secondary** representation of the same lesion.

3. Primary versus Secondary Margins /Distribution

+3	I am significantly more confident in the **Primary** representation of the lesion margins/distribution I described as compared to **Secondary** representation of the same lesion
+2	I am more confident in the **Primary** representation of the lesion margins/distribution I described as compared to **Secondary** representation of the same lesion
+1	I am slightly more confident in the **Primary** representation of the lesion margins/distribution I described as compared to the **Secondary** representation of the same lesion.
0	I have the same confidence in the **Primary** representation of the lesion margins/distribution I described as I do in the **Secondary** representation of the same lesion
-1	I am slightly less confident in the **Primary** representation of the lesion **Secondary** representation of the same lesion.
-2	I am less confident in the **Primary** representation of the lesion margins/distribution I described as compared to the **Secondary** representation of the same lesion.
-3	I am significantly less confident in the **Primary** representation of the lesion margins/distribution I described as compared to the **Secondary** representation of the same lesion.

12.2 Appendix B: Previous Clinical Investigation Use of Verasonics System

The Verasonics Vantage investigational device and its predecessors have been utilized in a number of clinical research studies deemed non-significant risk by the respective IRB or other regulatory agency responsible for human subject safety.

UNITED STATES

- Duke University, Durham, NC: Improved Visualization of Endocardial Borders with Short-Lag Spatial Coherence Imaging of Fundamental and Harmonic Ultrasound Data. DOI: 10.1109/ULTSYM.2012.0531. This study used a Verasonics scanner to implement a new imaging scheme to improve endocardial delineation.
- University of Washington, Seattle, WA: Ultrasonic propulsion of kidney stones: preliminary results of human feasibility study. DOI: 10.1109/ULTSYM.2014.0126. This study utilized a Verasonics system to perform ultrasonic propulsion of kidney stones as an alternative to surgery.
- Mayo Clinic College of Medicine, Rochester, MN: Pediatric Cardiac Shear Wave Elastography for Quantitative Assessment of Myocardial Stiffness: A Pilot Study in Healthy Controls. DOI: 10.1016/j.ultrasmedbio.2016.03.009. This study implemented shear wave elastography on a Verasonics Vantage system to evaluate myocardial stiffness in children.
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