Clinical Trial Protocol: PIONEER-01

Study Title:	A **P**ivotal Study of **I**maging with **O**pto-acoustics to diagnose breast masses detected by mammography and / or clinical findings: A **NE**w **E**valuation Tool for **R**adiologists
Study Number:	PIONEER-01
Investigational Device:	Imagio® breast imaging system
IDE Number:	***Not Applicable – FDA Concurrence with Non-Significant Risk Status effective October 28, 2011***
Investigators:	16 U.S. sites
Sponsor:	Seno Medical Instruments, Inc.
Sponsor Contact:	Shaan Schaeffer Seno Medical Instruments, Inc. 5253 Prue Road, Suite 315 San Antonio, TX 78240
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Date:	October 15, 2015
Protocol Version:	08

Confidentiality Statement

PIONEER-01 STUDY SYNOPSIS

Sponsor:	Seno Medical Instruments, Inc.
Study Title:	A **P**ivotal Study of **I**maging with **O**pto-acoustics to diagnose breast masses detected by mammography and/or clinical findings: A **NE**w **E**valuation Tool for **R**adiologists
Study Phase	Pivotal, Non-Significant Risk Study
Name of Finished Medical Device (Investigational Device):	Imagio, is an opto-acoustic (OA) imaging system designed to concurrently collect images in conjunction with diagnostic ultrasound (co-registered OA and B-mode imaging).
Indications for Use:	Imagio is indicated for use for opto-acoustic (OA) evaluation of breasts in women who are referred for a diagnostic breast ultrasound work-up due to a suspicious mass (including both palpable and non-palpable) or an imaging finding such as architectural distortion, asymmetry, or suspicious calcifications. This device is not intended to be used as a replacement for mammographic screening or for definitive pathologic diagnosis.
Study Objectives and Endpoints:	The objective of the study is to evaluate the safety and effectiveness of the Imagio for the visualization of suspicious masses when compared to the Imagio ultrasound component. The following outcomes will be evaluated to determine whether Imagio: 1. Has superior specificity (primary) relative to Imagio ultrasound (IUS) with non-inferior sensitivity (secondary) relative to IUS. 2. Has clinically acceptable predictive value of a positive test (PPV) and of a negative test (NPV) for a range of possible breast cancer prevalence. 3. Downgrades benign masses classified as BI-RADS 3, 4a, or 4b relative to IUS. 4. Downgrades masses classified as BI-RADS 3, 4a, or 4b according to the site investigator. 5. Upgrades malignant masses classified as BI-RADS 3, 4a, or 4b relative to IUS. 6. Achieves a high degree of concordance with the biopsy outcome. 7. Is safe.

Study Design: This is a prospective, controlled, multi-center, observational study that will compare Imagio OA versus the ultrasound component (IUS) for the visualization of suspicious masses.

Pilot/Pivotal Study: The Pilot Study (Pilot Phase of the Pivotal Study) data will be used to validate the existing algorithm to a pre-determined diagnostic rule set based on OA image characteristics established in a previously conducted independent Feasibility Study. The Pilot Study will collect imaging data from 100 subjects passing Quality Assurance review conducted by three central radiologists blinded to clinical history and histopathology outcome. Then a pool of at least 20 other independent radiologists (readers) not affiliated with any sites will be prospectively trained and then tested for proficiency using the image sets constructed by the central radiologists using the last 70 of the 100 Pilot cases. The first 30 cases will be used to familiarize readers with the image sets, the viewing logistics, and the eCRF to be completed, prior to reader proficiency testing. Upon establishing and meeting prospective rules for proficiency (based on Imagio sensitivity [missing at most one cancer] and Imagio specificity $\geq$30% after excluding the first 30 cases to allow for learning), a subset of 7 proficient readers will be selected for purposes of reading IUS and Imagio OA and 3 other readers will be selected to share the reimbursement reads of multiple combinations of imaging modalities with or without Imagio, The registration readers will be selected at random based on availability and once all readers are tested for proficiency; reimbursement readers will be selected by the Seno Medical Director. All registration readers will be proficiency tested and selected prior to making any Pivotal Study i.e., reads for subjects 101 and beyond, which will constitute the Pivotal Study. All effectiveness analyses will be conducted at the mass-level; it is projected that no more than 5% of all subjects will have multiple masses. A minimum of 100 Pilot Study masses and at least 1,700 Pivotal Study masses passing QA review and excluding enrollment failures are to be included. Enrollment will continue until there are 100 Pilot Study masses and at least 1,700 Pivotal Study masses that pass both criteria. The 100 Pilot Study cases will be used for education, training, and publication purposes; only the Pivotal Study cases will be used to support Imagio registration for this indication for use. All selected registration readers will evaluate all masses according to a MRMC design. To mimic the real world setting as closely as possible, all readers will receive feedback on the histopathology results in read only mode following their case reads after completing their locked evaluations; readers may not change results after receiving feedback. For the Pivotal Study, subsets of 5% of registration image sets and 2.5% of all reimbursement image sets will be reread to assess learning and consistency; the first reads will be used in the data analyses for registration and reimbursement. The PMA filing will be based

on the registration results with the reimbursement results to be submitted at a later date.

Study Population: A total of at least 2,000 female subjects, who are referred for a diagnostic breast ultrasound work-up due to a suspicious mass (including both palpable and non-palpable) or an imaging finding such as architectural distortion, asymmetry, or suspicious calcifications, will be enrolled at 16 sites in the U.S.

Patient Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Has a signed and dated informed consent, prior to initiation of any study activities.
2. Has had an undiagnosed suspicious finding within the previous 45 business days, by palpation or by a screening or diagnostic methodology other than ultrasound; this may include more than one suspicious mass.
3. Has at least one or up to three pre-selected and undiagnosed breast masses including suspicious solid masses and/or complex mixed cystic and solid masses that the investigator has characterized as either BI-RADS 3, BI-RADS 4, or BI-RADS 5 that have been scheduled for either biopsy or follow-up.
4. Has at least one undiagnosed breast mass that has been detected by one of the following five methodologies within 45 business days prior to enrollment with imaging results available for study utilization:
   a) Undiagnosed palpable masses found during a clinical exam including suspicious solid masses and complex cystic and solid masses that the investigator has characterized as either BI RADS 4 or BI RADS 5 that have been scheduled for biopsy or all BI RADS 3 subjects.
   b) Callbacks for additional evaluation of suspicious area(s) identified by imaging other than ultrasound.
   c) Diagnostic referral to assess focal physical symptoms and / or signs that were either a chief complaint of the subject or were elicited by the healthcare practitioner (excluding focal breast pain in the absence of other positive clinical findings).
   d) Interval clinical problems (symptoms or physical findings, excluding isolated focal breast pain, that have developed between yearly mammograms).
   e) Other referrals to conventional diagnostic ultrasound including subjects younger than 30 years old for a clinically suspicious area, or subjects referred from a screening MRI because of an abnormality.
5. Is at least 18 years of age.

6. Has received a recommendation to either biopsy or to not biopsy.
7. Is willing and able to comply with protocol-required procedures.

Exclusion Criteria:

1. Is male.
2. Has a condition or impediment which could interfere with the intended field of view (i.e., breast implants within the previous 12 months, or tattoos).
3. Has or has had cancer in the ipsilateral breast or prior breast surgeries in the same quadrant of the ipsilateral breast that would interfere with the ability to capture or interpret images.
4. Has had prior benign excisional breast biopsy within the immediate vicinity of the currently evaluated suspicious mass within the past 18 months (benign excisional biopsy not within immediate Imagio field-of - view will not exclude the subject from the study).
5. Has greater than three suspicious lesions.
6. Lesion(s) of interest is greater than 4 cm.
7. Has all mass (es) characterized as BI-RADS 1 and/or 2 as determined using a conventional diagnostic ultrasound.
8. Currently has mastitis.
9. Has focal pain without thickening or mass.
10. Is pregnant or lactating.
11. Has open sores including insect bites, rash, poison ivy, and chafing on the skin of the ipsilateral breast.
12. Has an acute or a chronic hematoma and/or acute ecchymosis of the ipsilateral breast.
13. Is experiencing photo-toxicity associated with currently taking, or having taken, photosensitizing agents within the previous 72 hours such as sulfa, ampicillin, tetracycline.
14. Is currently undergoing phototherapy.
15. Has a history of any photosensitive disease (e.g., porphyria, lupus erythematosus) or undergoing treatment for a photosensitive disease and is experiencing photosensitivity.
16. Has concurrent neoadjuvant therapy prior to the Imagio evaluation or the biopsy.
17. Has previously had image guided core biopsy, image guided DVAB, or surgical biopsy of the mass of interest.
18. Has nipple rings that cannot be removed or are not removed during Imagio evaluation.

Investigational Device: Imagio is an OA imaging system that has been designed to concurrently collect opto-acoustic images in conjunction with diagnostic gray-scale

(co-registered B-mode imaging) ultrasound. Use of color or power Doppler using a single linear conventional ultrasound probe will be separately assessed for research purposes and the Doppler information will not be used in the analysis of this study for determining the safety and effectiveness of Imagio.

Control:

The subject will serve as her own control for the study. Imagio OA evaluation results will be compared with the Imagio ultrasound component (IUS) for the registration read and Imagio will also be assessed in combination with Mammography and conventional diagnostic ultrasound (CDU) image sets for the reimbursement reads. The ultrasound component of Imagio, designated IUS, will serve as the "internal control." If a biopsy is performed, then the histology will serve as the gold standard; if a biopsy is not performed at study entry or for 12 months following study entry with no change in the target mass according to CDU (if performed), then the subject is considered to be a True-Negative for analysis purposes as confirmed by the Truth Panel.

Study Duration:

The duration of study participation for each subject will be up to 12 months depending on the screening BI RADS score and decision to biopsy. The duration of the entire study, from first subject enrolled until the last subject last visit is anticipated to be 24 months.

Table 1: SCHEDULE OF EVENTS

Evaluation	Screening Visit	Enrollment Visit Day 1	Imaging Visit Day 1-10	First Biopsy (within 12 Months)*	Follow-up for Subjects with No Biopsy (12 Months +/- 30 days)**
Informed Consent		X			
I/E Criteria		X##			
Demographics/ Medical History		X			
BI-RADS Score (including density)	X				
Conventional Diagnostic Ultrasound	X				
Biopsy Decision	X				
Mammogram	X#				
Imagio Evaluation			X*	X**+	X**+
Subject Completed Survey			X		
Directed Breast History			X		
Adverse Event Evaluation		X	X	X	X
Histopathology Assessment				X	X
Histopathology Report				X	X
Past Imaging Studies		X			

Subjects may have a mammogram at investigator discretion.
Subjects must have a conventional diagnostic ultrasound and all other diagnostic tests completed and decision to biopsy made before the Enrollment Visit.
***** Subjects who are scheduled to have a biopsy at any time during the 12 month Follow-Up (including baseline) will have an Imagio evaluation of the breast mass within 45 business days prior to the biopsy; for subjects requiring a second biopsy, additional time (up to 45 business days) will be permitted to perform the second biopsy if a waiver is granted by the study Medical Monitor, but a second Imagio Evaluation is not performed.
** Optional 6 month (+/- 30 Days) Follow-Up Visit at investigator discretion and planned 12 month Follow-Up Visit if no biopsy is performed within 12 months of Enrollment Visit.
+ The Follow-Up Visit will also include appropriate diagnostic imaging at the investigator's discretion.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACR	American College of Radiology
AE	Adverse Event
ANSI	American National Standards Institute
BCDR	Breast Cancer Detection Rate
BI-RADS	Breast Imaging-Reporting and Data System
BMI	Body Mass Index
CDU	Conventional Diagnostic Ultrasound
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report Form
CRO	Contract Research Organization
DCIS	Ductal Carcinoma In Situ
DVAB	Directional Vacuum – Assisted Biopsy
eCRF	Electronic CRF
EDC	Electronic Data Capture
EOS	End of Study
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FN	False Negative
FP	False Positive
FPR	False Positive Rate
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICC	Imaging Coordination Committee
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITD	Intention to Diagnose
IUS	Imagio Ultrasound
MedDRA	Medical Dictionary for Regulatory Activities

MPE	Maximum Permissible Exposure
MRI	Magnetic Resonance Imaging
MRMC	Multi-reader, Multi-case
NDU	Negative Diagnostic Ultrasound
NLR	Negative Likelihood Ratio
NPV	Predictive Values of a Negative Test
NSR	Non-significant Risk
OA	Opto-Acoustic
OEM	Original Equipment Manufacturer
OR	Odds Ratio
PDU	Positive Diagnostic Ultrasound
PET	Photon Emission Tomography
PLR	Positive Likelihood Ratio
POM	Probability of Malignancy
PPV	Positive Predictive Value
SAE	Serious Adverse Event
SDM	Standard Diagnostic Methods
Se	Sensitivity
Sp	Specificity
TN	True Negative
TP	True Positive
TPR	True Positive Rate
UADE	Unanticipated Adverse Device Effect
UL	Underwriters Laboratory
US	United States
UTMB	University of Texas Medical Branch at Galveston
WHO	World Health Organization

INTRODUCTION

1.1 BACKGROUND OF BREAST CANCER

Breast cancer has the highest percentage of new cases (28%) among all female cancer cases in the USA [1] and is the most common cause of cancer-related death for women between ages of 20-59 years. Yet, it remains difficult to definitively diagnose without performing a biopsy despite the existence of multiple screening and diagnostic imaging methodologies. According to the American College of Radiology (ACR), the ACR Appropriateness Criteria® are "evidence-based guidelines to assist referring physicians and other providers in making the most appropriate imaging or treatment decision for a specific clinical condition. By employing these guidelines, providers enhance quality of care and contribute to the most efficacious use of radiology. The guidelines are developed by expert panels in diagnostic imaging, interventional radiology, and radiation oncology. Each panel includes leaders in radiology and other specialties. There are more than 175 topics with over 850 variants in the March 2011 version" [2]. The focus of these guidelines is to address the on-going dilemma of the majority of biopsies performed resulting in a benign diagnosis, thus representing an excessive number of procedures and a significant burden on current delivery of healthcare and on the subject.

There are multiple reasons for the bias toward performing a biopsy beyond the psychological burden of a suspicious mass in the breast. Breast cancer is a heterogeneous disease with variability in genetic and hormonal influences, which contribute to its varied pathobiology [3]. Consequently, the clinical and radiographic manifestations and histopathology of breast cancer are variable. Furthermore, breast tissue is also heterogeneous containing fat, glandular and fibrous tissue. The structure of the breast and the nature of breast cancer present a great challenge for imaging technologies to detect cancerous masses reliably and to differentiate them from benign masses. Seno's imaging technology addresses this challenge by utilizing two very different real time temporally interleaved modalities, opto-acoustic (OA) and diagnostic ultrasound (US), thus providing co-registered and temporally fused functional and anatomical information. This combination has the potential to add diagnostic information to help the radiologist evaluate suspicious breast masses in an efficient manner.

Judah Folkman, MD, discovered that rapidly growing cancer cells need additional blood supply and gradually develop a dense microvascular network inside or around tumors required for tumor growth and progression. Vascularization of the tumor occurs through a series of sequential steps before or during the multistep progression to neoplasia [4]. This vascularization or angiogenesis is a marker of breast cancer development and has clinical implications in diagnosis and treatment. Experimental and clinical evidence suggests that the process of metastasis is also angiogenesis-dependent [5]. In this regard, pharmaceutical companies are pursuing tumor angiogenesis as one potential target for breast cancer chemotherapy agents [6].

In the early stages of cancer development, the tumor depends on blood vessels in the surrounding healthy tissue to support its continued growth. A tumor without its own independent supply of new blood vessels may be restricted in its growth to about 2 mm in size with just a few million cells and be non-life threatening [7].

Many of these breast tumors will remain small for years before transitioning to a rapid growth stage when the rate of the cell growth is much greater than the rate of apoptosis and requires new capillary blood vessels. These angiogenesis related micro-vessels have fragmented basement membranes and are leaky, making them more penetrable by malignant cells than are mature vessels. This new vascular phase of growth is usually followed by rapid tumor growth, vessel leakage and metastasis [7].

The ACR Practice Guideline for the Performance of Screening and Diagnostic Mammography, June 2008, states that "periodic mammography screening of age-appropriate asymptomatic women is currently the only imaging modality that has been shown by the preponderance of data to reduce breast cancer mortality" [8].

Mammographic sensitivity for breast cancer detection does decline significantly with increasing breast density, yet mammography is still of value in older women with dense breasts [9]. Mammography uses low-dose amplitude-X-rays (ionizing radiation at around 0.7 mSv) to examine the breast. A screening mammography is intended to detect unsuspected breast cancer in asymptomatic women based on mediolateral-oblique (MLO) and craniocaudal (CC) views of the breast. Based on the radiologists' reading of the screening mammogram, he/she determines the need for further diagnostic evaluation [8].

Accepted technologies for diagnostic evaluation include conventional diagnostic mammography, conventional diagnostic ultrasound, and MRI. Diagnostic mammography, which like screening mammography uses ionizing radiation, is used to evaluate subjects who have signs and/or symptoms of breast disease, imaging findings of concern, or prior imaging findings requiring specific follow-up. In addition to the MLO and CC views, a diagnostic mammogram includes views to evaluate a specific area of clinical concern, e.g., spot compression, spot compression with magnification, and others [8].

Indications for conventional diagnostic ultrasound include but are not limited to evaluation and characterization of palpable masses and other breast related signs and/or symptoms; evaluation of suspected or apparent abnormalities detected in other imaging studies; initial imaging evaluation of palpable masses in women $\leq$ 30 years of age; and, in lactating and pregnant women. Unlike mammography, conventional diagnostic ultrasound does not use ionizing radiation. ACR recommends the use of "a high-resolution, real-time linear array scanner operating at a center frequency of at least in the range of 10 MHz and preferably higher". The breast sonogram is correlated to the clinical signs or symptoms and with mammographic and other appropriate imaging studies [9].

Color Doppler has been used as an adjunct to conventional diagnostic ultrasound because once malignant masses achieve a certain size they need to generate neovascularity to sustain further growth. Color Doppler uses phase information to assess direction and velocity of blood flow. Color Doppler is very user dependent and has not been completely successful in demonstrating tumor neovascularity. Power Doppler assesses amplitude changes, does not assess direction of flow, but is more sensitive to low flow states and less angle dependent than color Doppler. It demonstrates tumor neovascularity slightly better than does color Doppler in most cases. However, power Doppler is still very operator dependent and is also not completely successful in demonstrating tumor neovascularity.

While the role of ultrasound has been primarily diagnostic, as described above, its role as an ancillary screening test in women who have dense tissue on mammography (where mammographic sensitivity is reduced and breast cancer risk is increased) is expanding. It has been shown repeatedly that ancillary screening ultrasound can find 3 to 4 per thousand cancers that are missed on mammography in women who have dense breasts, causing more and more centers to offer this service. One of the costs of ancillary ultrasound screening is false positive studies that ultimately result in biopsy of benign masses. As the role of ancillary screening breast ultrasound expands, the number of potential biopsies of benign masses will surely increase unless more accurate and appropriate characterization of benign masses is found thus obviating the need for biopsy.

MRI does not use ionizing radiation to produce computerized images of internal body tissues and is based on nuclear magnetic resonance of atoms within the body induced by the application of a strong magnetic field and radiofrequency waves. MRI is typically reserved for screening of high risk women, i.e., greater than 20% lifetime risk of breast cancer, assessing the extent of a new breast cancer prior to definitive intervention assessing the effect of chemotherapy or a known malignancy and additional evaluation of clinical or imaging findings as a problem solving technique. Ultrasound is often used to further characterize abnormalities detected by MRI.

According to ACR, MRI equipment specifications and performance should take into account resolution, contrast and field strength, simultaneous bilateral imaging, use of contrast agents, scanning time, and use of a dedicated MRI breast coil [10].

Positron emission tomography (PET) is a nuclear medicine imaging technique that produces a metabolic image and is used in some instances for diagnostic evaluation but not as widely as other modalities. Breast-specific gamma imaging is another problem-solving tool in breast imaging that uses injection of a gamma-emitting radioisotope to detect breast cancers but is not in wide use.

Depending on the outcome of the diagnostic evaluation, the subject may be referred for breast biopsy, to establish a histopathologic diagnosis. The breast imaging challenge, therefore, is to concurrently maximize the sensitivity and specificity to increase detection of breast cancer while decreasing the number of biopsies and while utilizing lower cost technologies and procedures.

1.2 Investigational Device – Mechanism of Action

OA imaging is based on the capability of optical pulses to generate diagnostic ultrasound (acoustic pressure) [11-13]. Pulses of near-infrared light at two wavelengths are applied sequentially to breast tissue. Internal optical absorption leads to the preferential deposition of energy in tissue volumes with an enhanced absorption coefficient (i.e., malignant masses and vasculature). Red light (757nm) is absorbed predominantly by hypoxic (de-oxygenated) blood, while near-infrared light (1064nm) is absorbed predominantly by normally oxygenated blood. The amount of light (optical fluence) is below the Maximum Permissible Exposure (MPE) for single pulse exposure and maximum power for a pulse train of continuous pulses according to the American National Standards Institute (ANSI) Standards for Safe Use of Lasers (ANSI Z136.1). The light excitation causes a mass to emit a pressure (acoustic) wave, which is detected by an array of acoustic sensors within the hand-held breast probe [14]. OA imaging technology has the merit of both the high contrast resolution of optical imaging and the high spatial resolution and penetration of ultrasound imaging which has been established in numerous studies and published in peer reviewed literature [15-21]. OA technology incorporates laser illumination and ultrasonic detection and is expected to achieve sensitivity and enhanced resolution in the breast tissue at depths more than sufficient to image any breast size. In prior studies Seno has demonstrated the capability of OA imaging to visualize a 1 mm diameter target at the depth of 8 cm in a custom-designed tissue phantom. The phantom used a 2% milk/distilled water mixture to obtain optical properties of $\mu_a = 0.1/cm$ (optical absorption), $\mu_s' = 9.8/cm$ (optical scattering), and $g = 0.8$ (anisotropy) with a 1 mm Teflon tube containing heparinized blood.

Proprietary algorithms perform co-registration of OA and diagnostic ultrasound images and provide much more detailed diagnostic information than the two technologies applied alone in a sequential manner; refer to Figure 1.

Figure 1

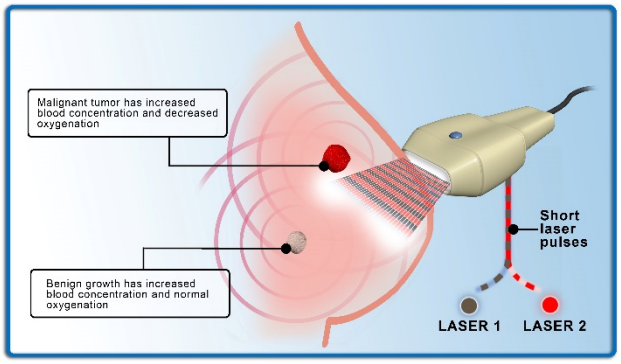
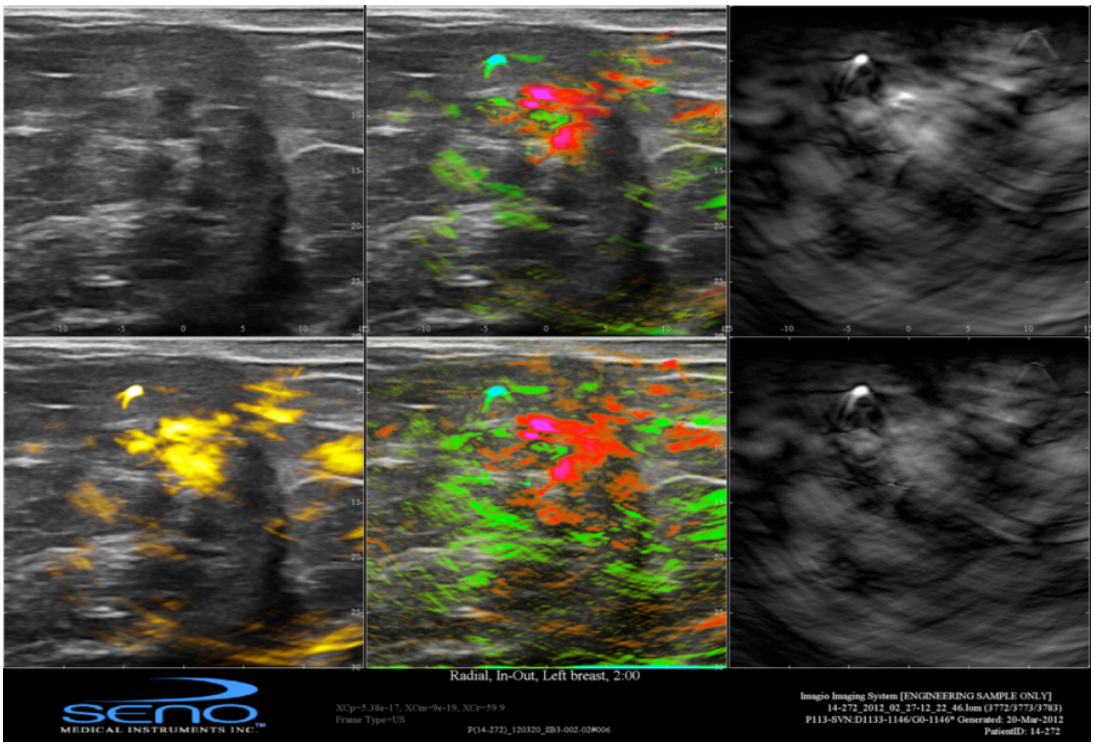


Figure 1: Schematic diagram showing the commercial implementation of a dual modality utilizing a hand-held opto-acoustic/ultrasonic probe.

The Imagio hand-held linear probe detects the time course of pressure signals resulting from the absorption of light which is preferential in malignant masses due to enhanced blood content. Each

optical pulse at each wavelength of light generates an OA image.

Figure 2 Example of Six-Up Display



Upper Left: B-mode	Lower Left: OA Total
Upper Middle: OA Combined	Lower Middle: OA Relative
Upper Right: OA Short	Lower Right: OA Long

Simultaneously, a diagnostic ultrasound image is acquired and co-registered with the OA image, providing a co-registered temporally interleaved and fused functional and anatomical map of the suspicious mass or abnormal tissue. This information, based on blood concentration in the neovasculature of the suspicious area and its oxygen saturation, is unique to OA technology.

The main chromophores in tissue that absorb illumination in the near-infrared range are oxygenated or deoxygenated molecules of hemoglobin in the blood. The contrast in OA imaging largely results from the paradigm of uneven vascular distribution inherent to cancer. Growing cancerous tumors develop an extensive network of blood vessels through the process of angiogenesis where there is increased blood flow to the cancerous tissue.

Seno's Imagio will be tested as an alternative to current commercially available ultrasound-only technology, which is used as the adjunctive technology for women found to have a suspicious mass after a screening mammography. Unlike other functional imaging modalities, such as MRI,

Seno's technology does not require any contrast agent. Seno's imaging modality is expected to uniquely increase diagnostic confidence by revealing the two functional hallmarks of a potential malignancy: the presence of abnormal blood vessels (angiogenesis) and the relative reduction in oxygen content of hemoglobin, in addition to the architecture and density of the suspicious area as defined by the diagnostic ultrasound component of the system.

1.3 Previous Studies

Early research proof of concept studies provided the company with supporting data to modify and enhance diagnostic performance of the Imagio by functional characteristics of real time opto-acoustic imaging performed at two wavelengths of Nd:YAG or Alexandrite lasers. Imagio was also tested on gelatin phantoms simulating dense breast with blood vessels having blood with various degrees of oxygen saturation. Experiments demonstrated that OA imaging is capable of not only visualizing shape and dimensions of blood vessels, but also differentiating deoxygenated blood from oxygenated blood.

Seno has completed enrollment into its Exploratory Study, "Clinical evaluation of Opto-Acoustic Tomography for detection and diagnostic differentiation of breast tumors", Cancer Therapy and Research Center (CTRC), at the University of Texas Science Center in San Antonio, TX, under the institution's IRB approval for a non-significant risk study.

The Exploratory Study collected data to refine the electro-mechanical design, including software and user interface so that a robust and consistent algorithm could be trained on a stable system configuration. A total of 55 subjects were enrolled at CTRC. Critical hardware and software components that affect signal acquisition (i.e., optical, probe, analog, and analog/digital components) were finalized at the close of this study and locked down.

Over 150 subjects were enrolled in the complete Feasibility Study to determine the ability of Imagio to help detect benign and malignant features to aid the radiologist in making more accurate diagnoses and the appropriate biopsy decision. This study was conducted at the Cancer Therapy and Research Center (CTRC) at The University of Texas Health Science Center in San Antonio, Texas and at South Texas Radiology Imaging Centers (STRIC) under the institutions IRB approvals for non-significant risk. The Feasibility Study provided data to train the algorithm and to identify separate sets of features that characterize benign and malignant disease. The Feasibility Study included subjects with negative diagnostic ultrasound (NDU) results and positive diagnostic ultrasound (PDU) results of varying histopathology diagnoses, sizes, locations, depths, ages, ethnicities, and races. These subject-specific characteristics are intended to identify any unusual subpopulations; to date, there are no such known exclusions other than subjects with obscured views and subjects on medications with the potential to activate dermatologic conditions. The Feasibility Study provided the estimates used to compute the Pivotal Study sample size.

1.4 Rationale for the PIONEER Study

Imagio is not yet approved for use and must be approved by the US Food and Drug Administration (FDA)

before it can be distributed for commercial use. If Imagio is approved for use, then potential benefits include sparing biopsies as well as improving the ability to diagnose cancers missed by conventional diagnostic ultrasound and mammography. There is also an unmet need in BI-RADs 4a subjects, where the risk of malignancy is low, but biopsy is mandatory, resulting in a large percentage of biopsies of benign masses. If Imagio can successfully downgrade benign masses classified as BI-RADS 3, 4a, or 4b to BI-RADS 2 or 3 by reducing the a priori risk of malignancy 5-fold (according to the POM), then subjects can be offered the option of short interval follow-up diagnostic ultrasound studies. Thus Imagio could improve efficiencies in diagnosis. In addition, the reduced discomfort of the procedure and minimal potential side effects are further considerations. For physicians, increased diagnostic certainty enhances subject care. Last, there may be cost savings for payers and for the healthcare system, resulting both from a reduction in the number of biopsies (i.e., those benign masses which have a reduced POM following Imagio), and from a greater diagnostic confidence when a biopsy is required that leads to earlier intervention. A more efficient diagnostic pathway may benefit all affected constituents in the screening and diagnosis of breast cancer.

The components used within the Imagio system have a proven safety record. Both lasers and conventional diagnostic ultrasound have been used in diagnostic procedure for decades with acceptable risk profiles. Imagio, which uniquely combines opto-acoustic and diagnostic ultrasound technology, will be tested to see if it will allow physicians to rapidly and effectively discern between benign and malignant masses with minimal potential side effects.

2 STUDY OBJECTIVES

The objective of the study is to evaluate the safety and effectiveness of the Imagio for the visualization and classification of suspicious masses when compared to the Imagio ultrasound component. The following outcomes will be evaluated to determine whether Imagio:

1. Has superior specificity relative to IUS (primary) with non-inferior sensitivity (secondary) relative to IUS.
2. Has clinically acceptable predictive value of a positive test (PPV) and of a negative test (NPV) for a range of possible breast cancer prevalence.
3. Downgrades benign masses classified as BI-RADS 3, 4a, or 4b according to IUS.
4. Downgrades benign masses classified as BI-RADS 3, 4a or 4b according to the site investigator.
5. Upgrades malignant masses classified as BI-RADS 3, 4a, or 4b relative to IUS.
6. Achieves a high degree of concordance with the biopsy outcome.
7. Is safe.

Color or power Doppler data may be optionally collected during the conventional diagnostic ultrasound examination at the discretion of the site investigator for research purposes. Data from Imagio evaluation of lymph nodes will also be optionally collected for research purposes.

3 INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This is a prospective, controlled, multi-center observational study that will compare the combined ultrasound and opto-acoustics results of Imagio with the ultrasound component of Imagio ("internal control"). Investigators will have the option to also collect color or power Doppler information during the conventional ultrasound examination on a case-by-case basis. The Doppler information will not be used in the analysis of this study for determining the safety and effectiveness of Imagio.

The study will initially consent subjects who have already had a suspicious finding within the previous 45 business days, by palpation or by a screening or diagnostic methodology other than ultrasound and are scheduled to undergo or already have had a conventional diagnostic ultrasound as per the site best practice guidelines. At least 2,000 subjects will be prospectively enrolled upon confirmation of being BI-RADS 3, BI-RADS 4, or BI-RADS 5 to obtain a sufficient number of benign masses (at least 1,000 masses) and, if there is not a sufficient number, then the total number of subjects may increase slightly to achieve the target number for benign masses. All selected subjects will then undergo an Imagio evaluation that must take place before any scheduled biopsy; if a biopsy is to be performed, then the biopsy specimen may be evaluated by an independent histopathologist. Following Seno's Medical Director review, in selected cases, histopathologic specimens from core and / or excisional biopsies will be sent to central pathology for review. Subjects that may be selected for central pathology review would include:

1. High risk lesions
2. Lesions with histologic-imaging discordance
3. Some with classically positive or classically negative OA studies
4. Some of whom are suspected of having false positive or false negatives OA studies.
5. Other lesions as designated by the Seno's Medical Director

All subjects not undergoing an immediate biopsy will be followed for an additional 12 months with an Imagio evaluation at the end of the 12 month follow-up. If possible, for subject management efficiency, once the subject agrees to participate in the study and her biopsy date is determined, then she can be scheduled for Imagio evaluation after the biopsy decision and scheduling, but prior to the biopsy. Follow-up will end when the subject undergoes a biopsy prior to 12 months or once the subject has been followed for 12 months. The decision to biopsy will be made solely by the site according to best practice without considering the Imagio evaluation outcome, but may include the CDU evaluation; if a biopsy is to be performed, then the biopsy specimen may be evaluated by an independent histopathologist.

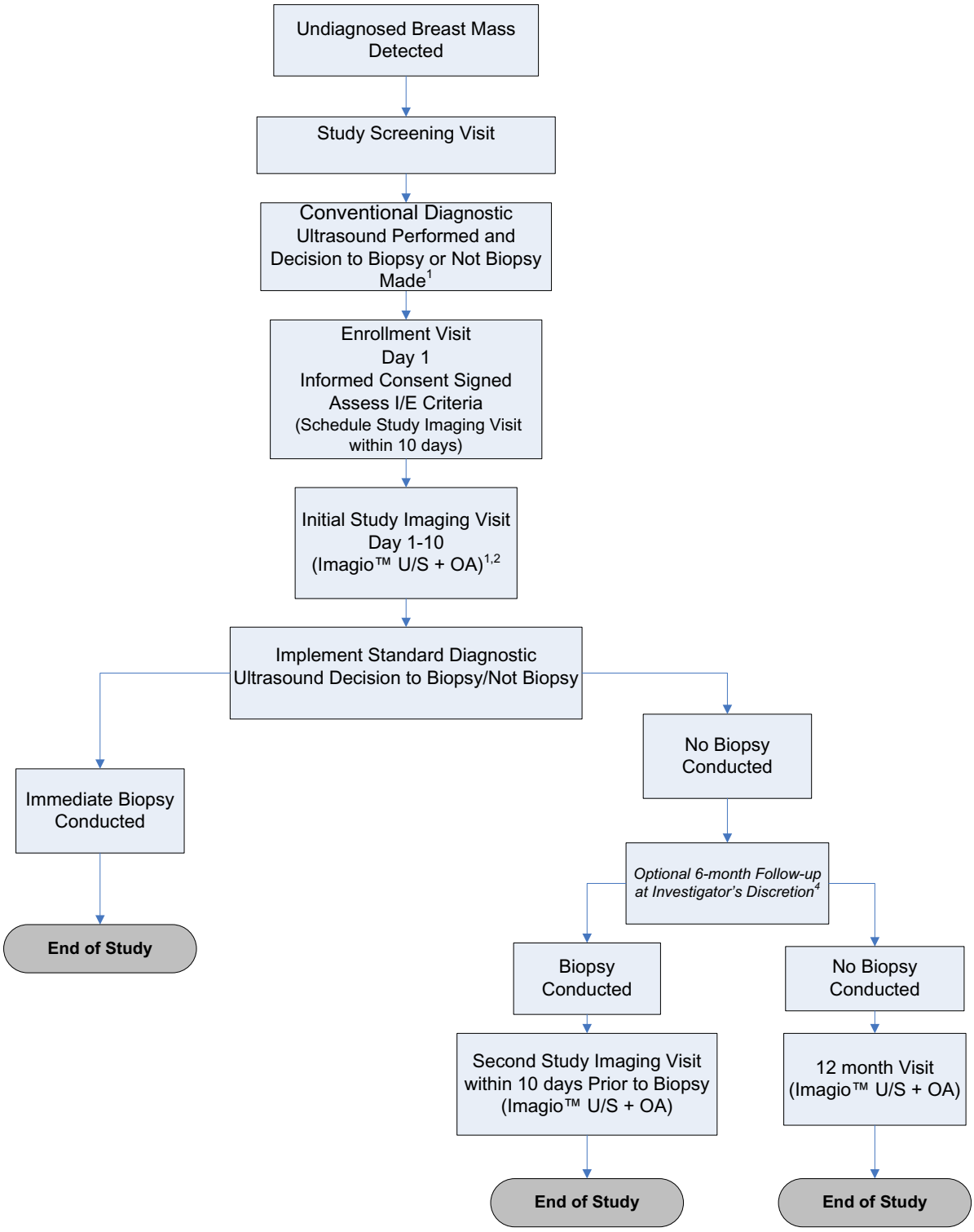


Figure 3: Subject Screening, Enrollment, Evaluation, and Possible Follow-up

[1] The decision to biopsy is made upon investigational site's standard of care and not based on the Imagio evaluation
[2] The Imagio evaluation is comprised of Imagio ultrasound and opto-acoustics
[3] The decision to delay biopsy due to observation of subject based upon investigational site's standard of care and not based on Imagio evaluation
[4] The optional 6 month follow-up visit is based upon Investigator's standard practice of medicine and not based on Imagio evaluation

A multi-reader, multi-case (MRMC) methodology will be used for the Pivotal Study where 7 independent radiologists passing prospective proficiency criteria will evaluate each of the Pivotal Study subjects for the registration reads (Imagio and IUS image sets) and 3 other qualified independent radiologists will share the evaluation of each of the Pivotal Study subjects for the reimbursement reads. The total number of subjects depends on accruing at least 1,000 benign masses and if there is not a sufficient number then the total number of masses may increase slightly to achieve the minimum number of 1,000 benign masses. The readers will also reread 5% of the registration cases and 2.5% of the reimbursement cases to assess temporal trends intra-observer variability and consistency.

Each registration reader will review the data from two image sets (IUS and Imagio) for each case; each independent reader will evaluate Imagio according to a pre-determined diagnostic rule set based on OA image characteristics and will similarly evaluate IUS according to another pre-determined diagnostic rule set where each reader will estimate the probability that the mass is cancer on a scale from 0% to 100% in 5% increments (1% if $\leq$10% or $\geq$90%). Readers will assess the image sets in the following pre-defined order during independent review sessions; this pre-defined order is consistent with medical practice:
Imagio diagnostic ultrasound alone (IUS)
Imagio (diagnostic ultrasound plus opto-acoustics).

The final sample size of the Pivotal Study will be determined by an interim analysis after the first 800 Pivotal Study subjects (cases 101-900 passing Quality Assurance review) have been evaluated with Imagio plus the results of any pre-scheduled biopsies; the final sample size will depend on the numbers of masses biopsied, the percent of biopsied masses with a cancer diagnosis, and the sensitivities and specificities for Imagio and IUS among biopsied masses. The interim analysis will be conducted once at least four of the seven registration readers complete the review of the 800 cases. Results will be shared with partners for business planning purposes but will not be shared with sites or published until the study is completed.

3.2. Independent Reader Selection

The study will utilize quality assurance readers (QARs) and independent readers (IRs) chosen based upon their education, experience, and training. Each receive training appropriate to their roles. QARs receive training how to check videos, image frames, and annotations. IRs receive training how to use reading stations, how to evaluate image sets based on standardized materials and case studies, how to complete and submit eCRFs, and how to receive real time feedback on their performance. All training is either led or supervised by the study Medical Director. The IR training process is conducted over a period of several months, and includes the following:

1. During off-site sessions, the IRs are trained in the protocol, GCP and 21 CFR Part 11, the Imagio device operation, the core imaging database, eCRF entry, and how to review Imagio images including the features.

2. During webinars, the IRs receive additional training how to evaluate Imagio videos and frames.

3. The IRs review image sets, enter their evaluations into a training database, and receive feedback in a group-setting.

4. The IRs receive additional training on the core imaging database (LifeRx and CLIB) and the process for eCRF completion.

A multi-stage process is implemented to establish reader proficiency and to select independent readers using Feasibility Study data, Validation and Verification Study data, and the Pilot Study data. The Pilot Study (Pilot Phase of the Pivotal Study) data are used to confirm the acceptability of the existing algorithm to be evaluated according to a pre-determined diagnostic rule set based on OA image characteristics (established in the previously conducted Feasibility Study). The Pilot Study collects imaging data from 100 subjects passing Quality Assurance review conducted by three central radiologists under the direction of the ICC. A pool of at least 20 other independent radiologists not affiliated with the ICC or any sites will be prospectively trained by the Seno Medical Director through face-to-face and webinar lectures as well as through reading practice sessions. All such trained readers are then screened by Seno for willingness to cooperate and ability to understand opto-acoustics. Equipment is then installed in their preferred reading environment and reading software is loaded for conducting further practice sessions. These readers are then tested for proficiency using the image sets constructed by the central radiologists using the last 70 of the 100 Pilot cases; the first 30 cases are not be used in order to let individual readers get familiar with the image sets, the viewing logistics, and the eCRF to be completed. Readers are not given a second chance to establish proficiency unless the pool of proficient readers is depleted due to reader dropouts.

Upon establishing and meeting prospective rules for proficiency (based on Imagio sensitivity [missing at most one cancer] and Imagio specificity $\geq$30% after excluding the first 30 cases to allow for learning), a subset of 7 proficient readers are selected for registration reads (IUS and Imagio) and 3 other readers are selected for reimbursement. The registration readers are selected among those meeting the proficiency standard; reimbursement readers are selected by the Seno Medical Director.

All registration readers will be tested and selected prior to making any further Pivotal Study reads for subjects 101 and beyond which will constitute the Pivotal Study. All effectiveness analyses will be conducted at the mass-level; it is projected that no more than 5% of all subjects will have multiple masses. A minimum of 100 Pilot Study cases and 1,700 Pivotal Study cases must pass QA review and must not be enrollment failures to be included in the respective Pilot and Pivotal Study analyses. Enrollment will continue until there are 100 Pilot Study cases and at least 1,700 Pivotal Study cases that pass both criteria. The 100 Pilot Study cases will be used for education, training, and publication purposes; only the Pivotal Study cases will be used to support Imagio registration for this indication for use.

All selected registration readers will evaluate all masses that pass QA review according to a MRMC design for the pivotal phase. To mimic the real world setting as closely as possible, all readers will receive respective feedback on the histopathology results after completing their locked evaluations; readers may not change results after receiving feedback.

For the Pivotal Study component, subsets of 5% of registration image sets and 2.5% of all reimbursement image sets will be reread to assess learning and consistency; the first reads will be used in the data analyses for registration and reimbursement. Readers will be required to turn in their evaluations before conducting the rereads which will be conducted once the respective reader completes their Pivotal Study reads.

3.3. Rationale for Study Design and Control Groups

Imagio will be assessed as a diagnostic test in the characterization of suspicious breast masses. The conventional diagnostic ultrasound will serve as the “external control” while the diagnostic ultrasound component of Imagio will serve as an “internal control.” The primary focus will be on the evaluation using the internal control.

The ultimate diagnosis depends on the histopathology examination of the biopsy from the suspected mass by an independent Histopathologist. The Histopathologist can elect to select slides for the final diagnosis review. This diagnosis will be included in the PMA submission. After designating the diagnosis, the independent Histopathologist may assess histopathologic specimens to better correlate specific internal and peritumoral findings (such as vessel density, neovessel count, cell density, type of host response to tumor, internal necrosis and hemorrhage, water content, etc.) with the opto-acoustic images and to help better understand the causes of opto-acoustic findings. Results of the detailed histopathology review will be published separately from the FDA submission but will be available to regulatory agencies upon request.

Following Seno’s Medical Director review, in selected cases, histopathologic specimens from core and / or excisional biopsies will be sent to central pathology for review. Subjects that may be selected for central pathology review would include:

1. High risk lesions
2. Lesions with histologic-imaging discordance
3. Some with classically positive or classically negative OA studies
4. Some of whom are suspected of having false positive or false negatives OA studies.

5. Other lesions as designated by Seno's Medical Director

In the absence of new findings, the decision to not recommend a biopsy during the first 12 months will be regarded as evidence of no disease, True Negative (TN) for those not biopsied; this is in accordance with FDA guidance regarding study analysis conventions. The study design will evaluate the specificity as the primary effectiveness endpoint and the sensitivity as secondary effectiveness endpoint for comparing Imagio relative to the pre-biopsy conventional diagnostic ultrasound images and histology results (if biopsy is performed). To ensure consistency, the assessment of all imaging studies including Imagio will be managed by an independent Imaging Coordination Committee (ICC) to coordinate the pool of potential readers for the Pilot/Pivotal Study and the subset of 7 qualifying readers for the Pivotal Study in accordance with FDA MRMC imaging review guidelines. Each registration reader will independently evaluate both image sets per subject once all imaging data are available per subject.

In addition, a Truth Panel will consist of at least two independent expert radiologists and a Histopathologist; the Truth Panel will work closely with the Sponsor and/or designee to adjudicate all cases and to review anomalies relating to pathology, imaging, subject history, and clinical course for the additional purpose of alerts regarding subjects at risk for malignancy, but not biopsied.

The major anticipated advantages of co-registered OA and B-mode imaging are as follows:

1. High optical contrast between potentially malignant and benign tissues that results from enhanced density of the microcirculation network within and around masses and enhanced thermo-elastic expansion coefficient in potentially malignant masses;
2. High sensitivity of detection due to efficient opto-thermal generation of pressure in masses, and sensitive detection of resulting ultrasonic waves with novel piezoelectric arrays; and
3. High (≤1 mm) spatial resolution that results from pulsed laser excitation and time-resolved detection of ultrasonic signal profiles with array detectors;
4. Substantial depth of monitoring (up to 8 cm in tissue phantoms) that results from deep penetration of the near-infra-red photons in tissues, efficient conversion of laser energy into acoustic pressure waves propagating through the breast, with negligible attenuation and minimal distortion, and from high sensitivity of piezoelectric arrays; and
5. Applicability to breasts of a broad range of mammographic density, including those with radiographically dense breasts.

3.4. Study Duration

Subjects are to be enrolled after decision to biopsy or not to biopsy. Each subject will undergo consent to have an Imagio evaluation, which must be completed within 10 business days of the Enrollment Visit; the Imagio evaluation must be performed prior to any biopsy. Thus all selected subjects will undergo an Imagio evaluation following conventional diagnostic ultrasound and in some cases mammography before any immediate biopsy. For subject management efficiency, once the subject agrees to participate in the study and her biopsy is scheduled, she can then be scheduled for Imagio prior to the biopsy. The subjects must have the Imagio evaluation within 45 business days before the biopsy. Core needle biopsy, image guided directional vacuum assisted needle biopsy (DVAB), or surgical biopsy will be performed. For

subjects requiring a second biopsy, additional time (up to 45 business days) will be permitted to perform the second biopsy. Any such decisions to biopsy will be made solely by the site without considering any Imagio evaluation outcomes.

All subjects not undergoing an immediate biopsy will be followed clinically for an additional 12 months. All subjects will have an Imagio evaluation at the first Imaging Visit and a subsequent Imagio evaluation before any subsequent biopsy that might be necessary because of an interval clinical finding during the 12 month follow-up or because of a 6 month follow-up conventional diagnostic ultrasound that has been recommended by the investigator. If the interval clinical problem does not result in a recommendation for biopsy, the investigator may schedule a 6 or 12 month follow-up visit at their discretion. Follow-up will end when either:

1. The subject undergoes a biopsy by 12 months, or
2. Once the subject has been followed for at least 12 months.

Any such decisions to biopsy will be made solely by the site investigator without considering the Imagio evaluation outcome.

Each subject will attend up to 4 visits:

- A Screening Visit.
- An Enrollment Visit (Day 1).
- 1 or 2 Imaging Visits (Day 1 to Day 10; a second Imaging Visit will occur for any biopsy within 12 months for those subjects with a biopsy scheduled before the first Imaging Visit).
- A Follow-Up Visit at 12 months (if no biopsy is scheduled within 12 months of the Enrollment Visit).

The Screening Visit, Enrollment Visit, and 1st Imaging Visit may all occur in proper sequential order in one day.

3.5. Ultrasound Visualization and Annotation

The conventional diagnostic ultrasound part of the study will include at least orthogonal viiew Radial and Anti-Radial views of the mass or longitudinal and transverse views). In some cases, additional views may be necessary (when the maximum diameter is not in Radial and Anti-radial planes, for example). The scan protocols will be those recommended in the “ACR 2011 Practice Guidelines for the Performance of a Breast Ultrasound Examination”, except that the subject name, hospital reference number, and birth date will be excluded from the image for purposes of maintaining anonymity. (The PDF file for the ACR guidelines is attached in Appendix C.)

The views will be properly annotated to be ACR BI-RADS breast ultrasound lexicon compliant and will include:

1. Breast side
2. Clock-face position
3. Distance from the nipple

4. Transducer orientation

A mass at 10:00 in the right breast 8 cm from the nipple scanned in the radial plane would be at R 10:00 N8 RAD.

Views will be obtained with and without measurement calipers. The presence of calipers may interfere with surface characteristics evaluation from standard diagnostic and Imagio gray scale images.

The views obtained during the Imagio scan and the labeling of the Imagio views must precisely replicate the views and annotation used for the diagnostic ultrasound part of the study. The exact views used for diagnostic ultrasound will be established during the Imagio scan. At a minimum, two orthogonal views (longitudinal/transverse or radial/anti-radial) will include both freeze frame images with and without calipers, and slow video "sweeps" through the lesion in both planes.

Color or power Doppler images may be collected during the conventional ultrasound examination for research purposes, but must be obtained in the same views used for diagnostic ultrasound and for the Imagio scan and must be annotated identically to the diagnostic gray scale and Imagio images.

The acoustic gel used will either be provided by or approved for use by Seno. The gels will be non-colored, as colored gel is anticipated to interfere with the optical part of the opto-acoustic examination.

All images used for this study will be anonymized to be HIPAA compliant prior to review by the independent QA evaluators and the independent readers. Subjects in this study will be assigned identifying subject numbers and their names and medical facility record numbers will be hidden on all accumulated images. The images will identify the subject only by subject numbers. The database that links the subject number with the subject names, medical facility record numbers, and birth dates will be maintained by the chief investigator and co-investigator and access to this database will be limited to the investigators and the Seno Medical Instruments, Inc. site monitor.

Informed consent will be obtained before Imagio imaging can be performed. The consent form template is included in Appendix B.

4. STUDY POPULATION SELECTION

4.1. Study Population

At least 2,000 subjects will be prospectively enrolled upon confirmation of being BI-RADS 3, BI-RADS 4, or BI-RADS 5 to obtain a sufficient number of benign masses (at least 1,000 masses) and, if there is not a sufficient number, then the total number of subjects may increase slightly to achieve the target number for benign masses. The following masses will be considered enrollment failures:

(a) masses for which the investigational device scans do not pass review (QAR failures) for Independent Review, or

(b) masses that have incomplete investigational device scans due to device malfunction, subject withdrawal or lesion being too deep for Imagio visualization.

Subjects with an undiagnosed suspicious finding within the previous 45 business days, by palpation or by a screening or diagnostic methodology other than ultrasound will be prospectively screened and consented. Once the investigator has made a biopsy decision (either no or yes), subjects will undergo an Imagio evaluation.

4.2. Inclusion Criteria

1. Has a signed and dated informed consent, prior to initiation of any study activities.
2. Has had an undiagnosed suspicious finding within the previous 45 business days, by palpation or by a screening or diagnostic methodology other than ultrasound; this may include more than one suspicious mass.
3. Has at least one or up to three pre-selected and undiagnosed breast masses including suspicious solid masses and/or complex mixed cystic and solid masses that the investigator has characterized as either BI-RADS 3, BI-RADS 4, or BI-RADS 5 that have been scheduled for either biopsy or follow-up.
4. Has at least one undiagnosed breast mass that has been detected by one of the following five methodologies within 45 business days prior to enrollment with imaging results available for study utilization:
    a. Undiagnosed palpable masses found during a clinical exam including suspicious solid masses and complex cystic and solid masses that the investigator has characterized as either BI-RADS 4 or BI-RADS 5 that have been scheduled for biopsy or any BI-RADS 3 subjects.
    b. Callbacks for additional evaluation of suspicious area(s) identified by imaging other than ultrasound.
    c. Diagnostic referral to assess focal physical symptoms and / or signs that were either a chief complaint of the subject or were elicited by the healthcare practitioner (excluding focal breast pain in the absence of other positive clinical findings).
    d. Interval clinical problems (symptoms or physical findings, excluding isolated focal breast pain, that have developed between yearly mammograms).
    e. Other referrals to conventional diagnostic ultrasound including subjects younger than 30 years old for a clinically suspicious area, or subjects referred from a screening MRI because of an abnormality.
5. Is at least 18 years of age.
6. Has received a recommendation to either biopsy or to not biopsy.
7. Is willing and able to comply with protocol-required procedures.

4.3 Exclusion Criteria

1. Is male.
2. Has a condition or impediment which could interfere with the intended field of view (i.e., breast implants within the previous 12 months, or tattoos).
3. Has or has had cancer in the ipsilateral breast or prior breast surgeries in the same quadrant of the ipsilateral breast that would interfere with the ability to capture or interpret images.
4. Has had prior benign excisional breast biopsy within the immediate vicinity of the currently evaluated suspicious mass within the past 18 months (benign excisional biopsy not within immediate Imagio field-of -view will not exclude the subject from the study).
5. Has greater than three suspicious lesions.
6. Lesion(s) of interest is greater than 4 cm.
7. Has all mass(es) characterized as BI-RADS 1 and/or 2 as determined using a conventional diagnostic ultrasound.
8. Currently has mastitis.
9. Has focal pain without thickening or mass.
10. Is pregnant or lactating.
11. Has open sores including insect bites, rash, poison ivy, and chafing on the skin of the ipsilateral breast.
12. Has an acute or a chronic hematoma and/or acute ecchymosis of the ipsilateral breast.
13. Is experiencing photo-toxicity associated with currently taking, or having taken, photosensitizing agents within the previous 72 hours such as sulfa, ampicillin, tetracycline.
14. Is currently undergoing phototherapy.
15. Has a history of any photosensitive disease (e.g., porphyria, lupus erythematosus) or undergoing treatment for a photosensitive disease and is experiencing photosensitivity.
16. Has concurrent neoadjuvant therapy prior to the Imagio evaluation or the biopsy.
17. Has previously had image guided core biopsy, image guided DVAB, or surgical biopsy of the mass of interest.
18. Has nipple rings that cannot be removed or are not removed during Imagio evaluation.

5. DESCRIPTION OF EXPERIMENTAL DESIGN AND METHODS

5.1. Description of Investigational Device

Imagio utilizes acoustic pressure waves generated by stimulating the tissue with pulsed laser energy to obtain images of the tissue structures, including those containing concentrations of oxygenated and deoxygenated hemoglobin. It also uses diagnostic ultrasound transmitted and received signals to form diagnostic ultrasound images of the internal soft tissue. The investigational device consists of these sub-systems and components:

5.1.1 Opto-acoustic Probe: A handheld OA linear probe contains a wideband diagnostic ultrasound array that can be used as a traditional diagnostic ultrasound transducer over a range of frequencies from 4 MHz to 16 MHz at 20 dB power point and can also receive optically generated acoustic frequencies that

range from 0.1 MHz to 18 MHz at 20 db power point. With the integrated laser light delivery system, the hand-held probe can operate in a multiplexing mode so that the same probe can be used to create co-registered, temporally interleaved and fused functional OA and anatomic diagnostic ultrasound. The OA duplex probe footprint is no greater than 56 mm long by 36 mm wide, with the ultrasound transducer size being 38 mm long by 4 mm wide.

5.1.2 Laser: A Class IIIB laser subsystem generates the necessary output energy, pulse widths and wavelengths to provide the breast illumination to initiate the OA effect. The laser component is based on laser technology found in previously 510(k) cleared dermatological devices; the lasers are supplied by a FDA registered OEM manufacturer. The lasers are activated via a footswitch.

5.1.3 Electronics: The system electronics provide the necessary signal processing, control, digital processing and image reconstruction to convert OA and diagnostic ultrasound signals into the desired clinical displays and operations, and to combine those with similarly generated diagnostic ultrasound images.

5.1.4 Diagnostic Ultrasound: The diagnostic ultrasound subsystem is provided by an FDA registered OEM manufacturer. The system is capable of processing and displaying conventional diagnostic ultrasound images as well as co-registered OA and B-mode images.

5.1.5 Software: The software controls the overall data flow from the data acquisition process to the display of the final co-registered OA and diagnostic ultrasound images. The system continuously acquires both OA data from the dual lasers and diagnostic ultrasound images from the diagnostic ultrasound subsystem. Ancillary data such as the physician annotations are stored and saved in DICOM format.

5.2. Potential Risks

Risks associated with the intended use of Imagio include potential laser exposure, acoustic output exposure, and contamination due to insufficient cleaning/disinfection of subject/user contacting materials. Section 5.4 identifies potential risks to study subjects.

In addition to these risks, Seno has considered the following potential sources of risk to the subject or operator:

5.2.1 Applicable Energy Hazards: Electrical, heat, mechanical force/moving parts, non-ionizing radiation, and electromagnetic fields; comparable to other electro-mechanical software controlled devices, with the following exception:

When Imagio is prepared for imaging, the laser shutter can be opened and the laser will deliver near infrared pulses to the subjects' breast through a light delivery system. All subjects and study personnel in or near the experimental area will wear eyewear specially designed for protection against laser illumination in the near infrared spectral range. However, no leakage of laser light is anticipated from either laser system or light delivery system. Laser illumination at safe levels will be used for the proposed *in vivo* studies, according to FDA Performance Standards for Light-Emitting Products, 21 CFR Part 1040, 1998.

ANSI Z136.1-1993, “American National Standard for the Safe Use of Lasers,” published by the American National Standards Institute, Inc, defines the maximum permissible exposure (MPE) for laser irradiation of skin as equal to 20 to 100 mJ/cm^2 depending on the wavelength in the spectral range from 700 nm to 1100 nm (Nd:YAG laser or Alexandrite laser) and low repetition rate of nanosecond laser pulses [22]). The repetition rate of laser pulses must be limited to assure that the laser-induced pressure and heat dissipates from the irradiated volume during the time between two consecutive laser pulses. The OA functionality has been tested to be below the Maximum Permissible Exposure (MPE) for single pulse exposure and maximum power for a pulse train of continuous pulses.

In addition to testing the MPE for laser radiance, Seno has separately tested the acoustic output of the diagnostic ultrasound subsystem according to FDA’s “Guidance for Industry and FDA Staff: Information for Manufacturers Seeking Marketing Clearance of Diagnostic Ultrasound Systems and Transducers,” September 9, 2008.

Seno also assessed the cumulative non-ionizing radiation exposure from the laser and ultrasound subsystems as well as transient temperature effects (individual and combined thermal effect), on blood, blood vessels, and adjacent tissues in the laser/ultrasound propagation pathway.

Conclusion: On the basis of this information, FDA notified Seno on October 28, 2011 that the Imagio breast imaging system is non-significant risk for this study.

5.2.2. Applicable Biological Hazards: Bio-burden, bio-contamination, toxicity, cross-infection, inability to maintain hygienic safety, and degradation are comparable to other electro-mechanical software controlled devices. Subject/user contacting materials have been assessed and found to be commonly used in commercially available medical devices. Seno has contracted with NAMSA, Northwood, OH, to conduct the required biocompatibility testing to ISO 10933: Biological Evaluation of Medical Devices Part 1: Evaluation and Testing. This testing is currently on-going.

Cleaning and disinfection of the OA probe with Sani-Cloth® AF3 Germicidal Disposable Wipes is described in the Study Instructions for Use Manual. Cleaning and disinfection of the OA probe is required prior to the first subject of the day and after each subject.

5.2.3. Environmental Hazards: Electromagnetic, inadequate supply of power, restriction of cooling, likelihood of operation outside of prescribed environmental conditions, storage outside of prescribed environmental conditions, incompatibility with other devices, accidental mechanical damage, contamination due to waste products and/or device disposal are comparable to other electro-mechanical software controlled devices.

5.2.4. Hazards related to the Use of the Device: reasonably foreseeable misuse of the device, system noise, and use of the device by unskilled personnel, incorrect measurement and other metrological aspects, incorrect diagnosis, incompatibility with consumables/accessories/other devices, hazards from contraindicated pharmaceuticals, and hazards from contraindicated subject classes; comparable to other electro-mechanical software controlled devices.

5.2.5. Hazards arising from Functional Failure, Maintenance, and Aging: inadequacy of performance characteristics for intended use, lack of or inadequate specification for maintenance, including inadequate specification of post-maintenance functional checks, inadequate maintenance, lack of adequate determination of end of device life, loss of electrical /mechanical integrity, inadequate packaging (contamination/deterioration of the device), improper reuse, delayed cumulative effects from long-term use; comparable to other electro-mechanical software controlled devices.

5.2.6 General Hazards: hazards introduced by changes made to manage risk, hazards from design changes, and hazards from system assembly; comparable to other electro-mechanical software controlled devices.

5.2.7 Testing and Mitigation of Hazards: Seno Medical Instruments, Inc. has conducted appropriate design verification and design validation testing to ensure that device related risks are controlled and that the residual risk is at acceptable levels for this type of investigational device. Seno has performed testing according to the FDA recognized standards listed in the table below. In those cases where risk could not be mitigated via design, labeling and training serves to notify the investigator of potential hazards or hazardous situations in the instructions for use and/or in the exclusion criteria for this protocol, e.g., exclude women with or undergoing treatment for a photosensitive disease to mitigate risk of exacerbating a pre-existing condition such as lupus erythematosus.

Table 2 FDA Recognized Standards	
Medical Electrical Safety	IEC / ANSI/AAMI ES 60601-1:2005, Medical electrical equipment - Part 1: General requirements for basic safety and essential performance
Laser Safety (Class IIIB Laser)	IEC 60601-2-22:2007, Medical electrical equipment - Part 2-22: Particular requirements for basic safety and essential performance of surgical, cosmetic, therapeutic and diagnostic laser equipment
	FDA Performance Standards for Light- Emitting Products, Title 21 CFR Part 1040, 1998
Ultrasound Safety	IEC 60601-2-37:2007, Medical electrical equipment - Part 2-37: Particular requirements for the basic safety and essential performance of ultrasonic medical diagnostic and monitoring equipment
Electromagnetic Compatibility	IEC 60601-1-2:2007, Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral standard: Electromagnetic compatibility - Requirements and tests

Study Instructions for Use will include specific caution and warning statements to ensure that the investigational Imagio breast imaging system can be operated in a safe manner.

5.3 Operator Credentials and Training

Experienced clinicians who meet the following requirements will conduct all exams:

5.3.1 Physicians who have each of the following:

1. Completed residency and are board certified in diagnostic radiology
2. Formal training, e.g., per ACR, MQSA, or equivalent
3. A minimum of one year performing or demonstrated ability to perform breast imaging after radiology residency or breast imaging fellowship

5.3.2 Technologists who have the following:

1. Completed and hold current American Registry for Diagnostic Medical Sonography (ARDMS) Registered Diagnostic Medical Sonographer (RDMS) credential or
2. Registered mammography technologists who have also been certified to perform breast ultrasound (some breast centers use these people in addition to or instead of registered sonographers) and
3. At least 2 years of experience performing or demonstrated ability to perform breast imaging

Study personnel will receive training on safe operation of Imagio from Seno personnel or their agents. Each operator is trained on Imagio usage by incorporating both practical and didactic techniques. The Imagio Instructions for Use Manual is used as a training resource throughout the training session. Each training session is conducted by an ARDMS credentialed sonographer who are clinical applications specialists for Seno Medical Instruments, Inc.

Major Training Topics Include:

- An Introduction to Imagio
- Safety, EMC, and Regulatory Compliance
- Laser Safety
- As Low As Reasonably Achievable (ALARA) Principle and Output Displays
- Indications for Use
- Imagio Components and Controls
- Daily Operator Activities
- Laser Power Calibration
- Performing Quality Control Images
- Performing Patient Scanning

All Topics are reinforced with practical application where applicable.

A final return assessment is supervised by a Seno qualified ARDMS sonographer and clinical applications specialist and is performed by each operator to ensure they are competent to perform Imagio Quality Control and Patient Scanning Procedures.

5.4. Reader Training

The independent readers (IRs) were chosen based upon their education, experience, and ability to receive a passing score on a pre-read Proficiency Test. All IRs received the same training, using the same materials and IMAGIO images, and all trainings were either led or supervised by the study Medical Director. The process of training was conducted over a period of several months, and included the following:

1. During off-site sessions, the IRs are trained in the protocol, GCP and 21 CFR Part 11, the Imagio device operation, the core imaging database, eCRF entry, and how to review Imagio images including the features.

2. During webinars, the IRs receive additional training how to evaluate Imagio videos and frames.

3. The IRs review image sets, enter their evaluations into a training database, and receive feed back in a group-setting.

4. The IRs receive additional training on the core imaging database (LifeRx and CLIB) and the process for eCRF completion.

5.5 Truth Panel

The Truth Panel will consist of a histopathologist and at least two radiologists.

The histopathologist will review all pathology reports inclusive of core biopsy reports and surgical pathology reports for each subject with a biopsy for determining the definitive diagnosis and for identifying and resolving inconsistent information. Seno Medical representatives will provide the two reports to the histopathologist for review and adjudication. In addition, BMS and Seno Medical representatives will provide packets to the Truth Panel for selected cases as follows:

- Cases where the number or numbering of suspicious masses is under dispute (per IRs)
- Cases with questionable eligibility
- Cases with high risk histology's as identified by the Central Histopathologist
- Cases where individual IR's identified problems with specific image sets
- Cases not biopsied and being followed to determine if a mass was a True-Negative (TN)

BMS will provide selected images meeting the above criteria to the Truth Panel who will determine truth regarding benign or malignant for submitted imaging data.

The Truth Panel will collaborate to perform the following tasks:

1. Generate queries to sites to be managed by Seno Medical
2. Complete a CRF per patient deeming truth regarding:

- Number and location of specific lesions
- Histologic opinion and interpretation including benign or malignant classification
- Mass-specific True-Negative designation

5.6. Evaluation of Risk / Benefit

There is a minimal risk (0.4% or 1:269) that a subject may experience discomfort, including tingling or warmth, during the Imagio scan. The discomfort, if experienced, is not associated with any visible skin changes and resolves immediately at the completion of the scan without treatment. To date there have been no reports of unanticipated adverse device effects for this investigation. Previous studies using comparable prototype systems have been declared to be non-significant risk.

Conclusion: FDA notified Seno on October 28, 2011, that the agency believes that the Imagio breast imaging system is non-significant risk for this study.

Based on the recommendation of the QA evaluators, the independent readers, and the Truth Panel for missed masses not biopsied, the Medical Monitor will be immediately notified and will in turn notify the site investigator.

5.7. Central Histopathology Review

Following Seno's Medical Director review, in selected cases histopathologic specimens from core and / or excisional biopsies will be sent to central pathology for review. Subjects that may be selected for central pathology review would include:

1. High risk lesions
2. Lesions with histologic-imaging discordance
3. Some with classically true positive or classically true negative OA studies
4. Some of whom are suspected of having false positive or false negatives OA studies.
5. Other lesions as designated by Seno's Medical Director

This may include the initial biopsy, any second biopsy, and any subsequent biopsy or surgical excision during the first 12 months after study enrollment. The central histopathology review will be used for all analyses. All histopathology specimens will be labeled using the Study ID and the date that the specimen was collected.

All specimens should be formalin-fixed and paraffin-embedded. Sections should be cut at a thickness no greater than 4 microns, stained with Hematoxylin and Eosin, and cover-slipped in the routine manner. One original slide or recut from each paraffin block and one original slide of each immunohistochemical stain performed should be sent via Federal Express to:

F. Lee Tucker, MD, FCAP
500 River Creek Rd.
Wirtz, VA 24184
540-529-0719

Alternatively, slides may be sent USPS Express mail with tracking to:

F. Lee Tucker, MD, FCAP
PO Box 510
Wirtz, VA 24184
540-529-0719

5.8. Standard Diagnostic Measure (SDM) as Comparator

The diagnostic ultrasound of Imagio will serve as the "internal control". The conventional diagnostic control will service as the "external control"; the CDU must be completed within 45 business days prior to the Screening Visit and interpreted before the Enrollment Visit. If a biopsy is to be performed during the first 12 months, then any planned biopsy during the first 12 months must be scheduled to take place within 30 business days after the Imagio evaluation. The histologic findings of the biopsy procedure will serve as a gold standard for Imagio and the decision to not perform a biopsy during the first 12 months will be regarded as evidence of no malignant disease for those not biopsied, subject to review by the Truth Panel. Subjects requiring a second biopsy will be allowed additional time (up to 45 business days) to undergo a second biopsy.

The presented study design will evaluate all images that contribute to the decision to either perform a biopsy or to follow the subject for up to 12 months; this includes mammography will be read alone and in combination with conventional diagnostic ultrasound and Imagio which will be separately read by the reimbursement readers. The registration readers will focus on the IUS and Imagio image sets to be read in the following pre-defined order:

1. Imagio diagnostic ultrasound alone (IUS)
2. Imagio (diagnostic ultrasound plus opto-acoustics [OA]).

Specific image sets may include screening and diagnostic mammography as well as other diagnostic images but excluding the optional color or power Doppler. The images will be received, assigned, sent, recorded, and received back by the Imaging Coordination Committee. The ICC will manage the image set review process by the independent readers. To preserve their independence and objectivity as well as to minimize bias, the independent readers will NOT have access to any clinical data, any decisions to biopsy, or to any biopsy outcomes.

The Medical Monitor will be contacted regarding any biopsied cases where it is unclear whether the correct site was biopsied as well as have access to all unbiopsied cases where the QA evaluators or the

independent readers identified a new suspicious mass with a high probability of malignancy to be conveyed to the respective site investigator.

The Data Rights Document identifies the individuals who have rights and restrictions to the de-identified subject data. The investigator will not have access to the reader reviews (including the final interpretation). The independent readers will have access to relevant images used to make the decision to perform the diagnostic ultrasound in addition to the Imagio images in order to perform evaluations for the above-stated image series used to evaluate the subjects for the BI-RADs 4a or 4b classification and if a decision is made to biopsy. Seno will have access to reader results once the biopsy is completed and the image sets are read by all registration readers.

5.9. Comparator Device Components

Imagio is an investigational device that has not been approved for commercial use by the US FDA. However, FDA has notified Seno on October 28, 2011, that the agency believes that Imagio is non-significant risk for this study. Imagio includes subsystems and components that are comparable to commercially available medical devices:

5.**9**.1. Proprietary Device Name: PR3 Ultrasound System
Classification: Ultrasonic imaging system
Regulation Number: Title 21 CFR 892.1560
Medical Specialty: Radiology
Medical Device Regulatory Class: II (Special Controls)
510(K): K080935

Imagio is similar to the PR3 Ultrasound system in that both systems use ultrasound waves propagating through tissue to obtain images of soft tissue. The diagnostic ultrasound component of Imagio uses the standard parameters of the PR3 Ultrasound system. However, the opto-acoustic part of Imagio employs passive detection of low-amplitude ultrasound waves instead of ultrasound-emissions used by the PR3-USI.

5.**9**.2. Proprietary Device Name: Candela GentleLASE Laser
Classification: Dermatological Vascular Lesion Laser
Regulation Number: Title 21 CFR 878.4810
Medical Specialty: Dermatology
Medical Device Regulatory Class: II (Special Controls)
510(K): K083207

Imagio is similar to the Candela GentleLASE Laser in that both systems employ pulsed lasers operating in the near infrared spectral range, and both lasers are used for illumination of skin. Both lasers employ a Class IIIB laser, with the Candela GentleLASE Laser providing a higher energy per pulse.

5.10. Treatments Administered: *Not applicable.*

5.11. Selection and Timing of Dose for Each Subject: *Not applicable.*

5.12. Method of Assigning Subjects to Treatment Groups

This is a prospective, controlled, multi-center observational study where all subjects undergo a diagnostic ultrasound, to make a biopsy decision, and then are consented. All subjects will then undergo the Imagio evaluation following conventional diagnostic procedures. Thus, there is no randomization.

5.13. Blinding

Subjects will not be blinded to study procedures but will not have access to the Imagio evaluation result.

The investigator will have access to all conventional diagnostic ultrasound, mammography, and biopsy reports, but the interpretation of the Imagio evaluation will not be provided to the investigator. The investigator will also have the option to perform the color or power Doppler during the conventional ultrasound examination for research purposes. The Doppler information will not be used in the analysis of this study for determining the safety and effectiveness of the Imagio.

The 7 independent registration readers and the 3 other independent reimbursement readers per case will have separate access to the relevant images and videos used to make the decision to perform or defer a biopsy in addition to the Imagio diagnostic ultrasound image and opto-acoustic image. Imagio and IUS are regarded as the two most important image sets for comparisons; these will be the image sets to be reviewed by the registration readers. Mammography is regarded as the most likely referral methodology and will be read alone and in combination with CDU and Imagio by the reimbursement readers. Readers will not have access to the background clinical information or to the histopathology report. The Truth Panel will have access to all clinical data, QA evaluator, and independent reader results to be able to alert the study medical monitor regarding any high risk subjects; the Seno study coordinator will in turn alert the study investigator in the event that the subject was not biopsied or the biopsy report was missing.

5.14. Concomitant Therapy: *Not applicable.*

5.15. Restrictions: *None*

5.16. Treatment Compliance

Imagio will be operated only by study personnel trained by Seno personnel or their representatives. Subject compliance is ensured.

5.17. Packaging, Labeling, and Instructions for Use

Seno will ship investigational devices to participating sites utilizing packaging materials that protect the investigational devices from potential damage during transit. Seno personnel will unpack and install the study device as well as conduct and document operator training for study personnel.

Imagio will bear appropriate investigational device labeling, to include the statement: "Caution – Investigational Device. Limited by United States Law to Investigational Use."
Seno will also provide adequate instructions for use written at a comprehension level appropriate for the operators participating in this study; please refer to Section 5.3.

5.18. Storage and Accountability

Investigators will be responsible for ensuring that only authorized study personnel have access to the investigational device.

6. STUDY PROCEDURES

6.1. Study Procedure Overview

The study procedures and the sequence in which they will occur are defined below in Table 3 below and described in greater detail in Section 6.2:

Table 3: SCHEDULE OF EVENTS

Evaluation	Screening Visit	Enrollment Visit Day 1	Imaging Visit Day 1-10	First Biopsy (within 12 Months)*	Subjects with No Biopsy (12 Months +/- 30 days)**
Informed Consent		X			
I/E Criteria		X##			
Demographics/ Medical History		X			
BI-RADS Score (including density)	X				
Conventional Diagnostic Ultrasound	X				
Biopsy Decision	X				
Mammogram	X#				
Imagio Evaluation			X*	X**+	X**+
Subject Questionnaire			X		
Directed Breast History			X		
Adverse Event Evaluation			X	X	X
Histopathology Assessment				X	X
Histopathology Report				X	X
Past Imaging Studies		X			

Subjects may have a mammogram at investigator discretion.
Subjects must have a conventional diagnostic ultrasound and all other diagnostic tests completed and decision to biopsy made before the Enrollment Visit.
***** Subjects who are scheduled to have a biopsy at any time during the 12 month Follow-Up (including baseline) will have an Imagio evaluation of the breast mass within 30 business days prior to the biopsy; for subjects requiring a second biopsy, additional time (up to 45 business days) will be permitted to perform the second biopsy if a waiver is granted by the study Medical Monitor, but a second Imagio Evaluation is not performed.
** Optional 6 month (+/- 30 Days) Follow-Up Visit at investigator discretion and planned 12 month Follow-Up Visit if no biopsy is performed within 12 months of Enrollment Visit.
+ The Follow-Up Visit will also include appropriate diagnostic imaging at the investigator's discretion.

The investigator will check subject eligibility, including prescribed medications. The estimated total time for the Imagio evaluation is approximately 15 minutes depending on breast dimensions, mass location(s), mass size(s), mass type(s) and number of test positions required to fully visualize the mass(es).

6.2. Study Visits

6.2.1 Screening Visit

The following procedures will be conducted at the Screening Visit:

1. Qualify subject
2. Verify from images and/or reports that a suspicious mass was seen
3. Confirm that a conventional diagnostic ultrasound is to be performed or has been performed. The conventional diagnostic ultrasound should be performed prior to consenting the subject.
4. Confirm that the subject has an undiagnosed suspicious finding within the previous 45 business days, by palpation or by a screening or diagnostic methodology other than ultrasound

6.2.2. Enrollment Visit, Day 1

The following procedures will be conducted at the Enrollment Visit:

1. Verify from mammograms and reports that a suspicious mass was seen
2. Confirm that conventional diagnostic ultrasound and all other diagnostic tests were performed
3. Confirm that the biopsy decision has been made
4. Review HIPAA form and informed consent form with subject and obtain signature; consent to both forms must be obtained prior to performing any study procedures.
5. Full assessment of subject eligibility with inclusion/exclusion criteria
6. Record demographics and medical history
7. Document date of informed consent signature and HIPAA authorization in the eCRF.

Upon completion of the conventional diagnostic ultrasound:

8. Determine whether the subject had a conventional diagnostic ultrasound that resulted in a recommendation for biopsy or a recommendation not to biopsy.
9. Schedule Imagio evaluation to take place within 10 business days of the Enrollment Visit.

After completion of the Imagio evaluation and prior to the biopsy, the subject will complete the Subject Completed Survey.

Subjects may be asked to return to the clinic to repeat the Imagio scan if (a) the image quality is not acceptable for review by the independent QA evaluators and (b) they have not undergone a biopsy procedure.

Group Determination: For the purpose of subject follow-up activities, subjects will be categorized into the Biopsied Group or Unbiopsied Group. Classification will be determined by the respective site investigator based on the following criteria:

Biopsied Group Criteria:

Has at least one or up to three suspicious solid masses or suspicious complex mixed cystic and solid masses (as either the cause of the clinical or mammographic finding) that the investigator has characterized as either BI-RADS 4 or 5, a biopsy has been recommended for all masses and that subject has agreed to have a biopsy for each suspicious mass that has been identified.

Unbiopsied Group Criteria:

Has at least one solid mass, complex cystic and solid mass, or indeterminate cystic versus solid lesion (as either the cause of clinical or mammographic finding), that the investigator has characterized as BI-RADS 3 or has ≤2% POM, and for which the subject has chosen to have either biopsy or short interval follow-up. If greater than one mass is found and each mass is not recommended for biopsy, then only one mass of the site's choice will be designated as the primary mass and will be followed for up to 12 months; Ideally, the mass with the highest POM as per the investigator will be selected for follow-up.

6.2.3.Imaging Visit(s), Day 1 - 10

Within 10 business days of the completion of the Enrollment Visit and before any scheduled image-guided biopsy, the subject will undergo the Imagio study evaluation. The Imagio evaluation will be performed according to the Imagio Instructions for Use provided for this clinical study. Subjects may be asked to return to the clinic to repeat the Imagio scan if (a) the image quality is not acceptable for review by the independent evaluators and (b) they have not undergone a biopsy procedure. Seno Medical Instruments, Inc. or their designees may be present during the procedures to provide technical support.

The study data will be recorded using electronic data capture (EDC). Any adverse events that occur during this Visit will be entered into the eCRF.

The investigator is to ensure that the site histopathology report is obtained and the relevant results are entered into the eCRF. The central histopathology report will contain cell type, histologic grade for invasive malignant masses and nuclear grade for in situ carcinomas. Additionally, the Sponsor will assign a blinded sonographer to independently determine the maximum diameter of the index mass and extent of disease (which includes not only the maximum diameter of the index mass, but outside-to-outside dimensions between multifocal and/or multicentric invasive disease and/or maximum outside-to-outside largest dimension of combined invasive and in situ disease); the sonographer may be a Sponsor employee without access to diagnosis, histopathology results, reader scores, and nomogram results. Also included will be estrogen, progesterone, and Her-2 receptor status, lymph node status and number of positive lymph nodes. All of these histopathologic prognostic features will be entered into the eCRF. These histopathologic features are known to correlate with biologic behavior of the mass, as will be its OA characteristics. Thus, certain OA features are expected to correlate better with some prognostic histopathologic parameters than with some others.

If the subject does not undergo biopsy of the breast mass within 12 months of enrollment, a subsequent Imagio evaluation will be performed together with any other indicated imaging studies at the 12 month

follow-up Visit. In addition, the Imagio evaluation visit will be conducted prior to any scheduled biopsy that is indicated by interval clinical and/or imaging findings within the 12 month follow-up period.

6.2.4. Follow-up for Subjects with Subsequent Biopsy Within 12 Months Post-Enrollment

Subjects who are scheduled for biopsy because of new interval clinical or imaging findings that present within 12 months of the Enrollment Visit will have an Imagio evaluation in addition to (prior to) the biopsy being performed. The Imagio protocol will be performed according to the Imagio Instructions for Use provided for this clinical study. The study data will be recorded using electronic data capture (EDC). Any adverse events that occur during this visit will be reported. The investigator is to ensure that the histopathology report is obtained and the relevant results are entered into the eCRF.

6.2.5. 12 month Follow-up Visit for Subjects with No Biopsy

For non-biopsied subjects, a second and final Imagio evaluation and any other indicated imaging evaluations will be performed at the 12 month Follow-Up Visit. If an interval clinical and/or imaging problem arises before 12 months that would require a biopsy at that time, an Imagio evaluation and any other indicated imaging studies will be performed (45 days) before the biopsy. Truth Panel confirmation of mass change will be used to establish truth regarding mass changes; if no biopsy is recommended at the Month 12 Evaluation, and if there are no new symptoms, no new physical findings, and no new imaging abnormalities, the subject will be considered to be a True-Negative (TN), subject to truth panel review.

6.3. Study Evaluations and Procedures

6.3.1. Informed Consent

Prior to the initiation of any study activities, written informed consent and HIPAA authorization shall be obtained from all subjects; this will cover any additional data in the event that the subject is to be followed for 12 months and if a biopsy takes place during the subsequent 12 month follow-up. Subjects will authorize the investigator to submit those images used to make the decision to perform a biopsy or to continue follow-up.

6.3.2. Demographics

Demographics will be documented in the electronic Case Report Form (eCRF). This will include date of birth, race, and ethnicity.

6.3.3. Subject Survey

After completion of the Imagio evaluation and prior to the biopsy, the subject will complete the Subject

Data Form.

6.3.4. Adverse Events

Adverse events (AEs) will be collected as outlined in Section 6.5.

6.4. Effectiveness Assessments

Blinded Imagio results will be transferred to the independent ICC that will review all screening and diagnostic imaging conducted including all Imagio results at baseline, prior to any biopsy (i.e. Month 6), and at Month 12 if no biopsy is scheduled. Any imaging studies performed after the Enrollment Visit but before the biopsy will also be forwarded to the ICC for independent reader review.

The histopathology report will be used to establish the diagnosis; if no biopsy is recommended at the Month 12 evaluation, then the Truth Panel will consider all imaging and pathology findings to decide if a True Negative determination can be made. .

6.4.1 Statistical Endpoints

The following statistical metrics are to be determined for each image set relative to the histopathology diagnosis:

1. True-positive rate or sensitivity (Se)
2. Specificity (Sp)
3. Concordance (percent true positive [TP] or true negative [TN])
4. Positive likelihood ratio (PLR = Se/(1-Sp))
5. Negative likelihood ratio (NLR = Sp/(1-Se))
6. Predictive values of a negative test (NPV = TN/(FN+TN))
7. Predictive values of a positive test (PPV = TP/(FP+TP))
8. Odds ratio (OR = (TP/FP)/ (FN/TN)).

6.5. Safety Assessments

Criteria specified in this section will be used to define, evaluate, and report subject safety during the course of their participation in the study. This NSR study will be conducted under safety reporting requirements found in 21 CFR 812.150(a)(1) and 21 CFR 812.150(b)(1).

6.5.1 Defining, Recording and Reporting Events

6.5.1.1 Adverse Event (AE)

An AE is defined as any untoward medical occurrence in a subject. This means any clinically adverse sign, symptom, syndrome or illness that occurs or worsens during the course of the study, regardless of causality, that is not otherwise being measured in the study.

Each AE will be evaluated for its seriousness, severity and association with the Imagio study. Adverse events will be followed until resolution or for the duration of the study.

6.5.1.2 Serious Adverse Event (SAE)

The severity of AEs will be assessed according to the International Organization for Standardization (ISO) 14155-1 SAE definition. A device-related SAE will be defined as an SAE in which the Imagio device relationship cannot be ruled out.

An AE that meets any of the following criteria will be considered an SAE for protocol purposes:

1. Led to death
2. Led to a serious deterioration in the health of the subject that:

    - Resulted in a life-threatening illness or injury;
    - Resulted in permanent impairment of a body structure or a body function;
    - Required in-subject hospitalization or prolongation of an existing hospitalization; or,
    - Resulted in medical or surgical intervention to prevent permanent impairment of a body structure or a body function.

    - Resulted in persistent or significant disability or incapacity; or
    - Important AEs that are not immediately life-threatening or do not result in death or hospitalization but, based on the training physician's medical judgment, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Neither a biopsy nor a diagnosis of breast cancer is considered to be an adverse event.

6.5.1.3 Unanticipated Adverse Device Effect (UADE)

As defined by FDA regulation at 21 CFR 812.3(s):
Unanticipated adverse device effect means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

An observer in this study must report any suspected UADE to the Seno Medical Director within 24 hours of learning of the occurrence. Seno will then report any concerned UADEs, as required by FDA regulations.

6.5.2 Evaluation of Severity

The severity of AEs will be assessed according to the following definitions:

Mild: The AE is noticeable to the subject but does not interfere with routine activity. The AE does not require any action in connection with the study treatment.

Moderate: The AE interferes with routine activity, but responds to symptomatic therapy or rest. The AE may require action concerning the study treatment**.**

Severe: The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy. The AE may require action concerning the study treatment**.**

Life-Threatening: The AE is of a nature that the subject is at immediate risk of death, even if not related to the study treatment. The AE may require action concerning the study treatment.

6.5.3 Evaluation of AE Relationship to Investigational Device

The relationship between an AE and the study device (investigational device) will be determined by the investigator on the basis of his or her clinical judgment and the following definitions:

Table 4: Evaluation of AE Relationship to Investigational Device

Likely related	AEs with clear temporal and/or spatial relationship to study device, with no clear alternative underlying cause.
Probably related	AEs with reasonable temporal and/or spatial relationship to study device that cannot be readily explained by the subject's clinical state or other agents/therapies.
Possibly related	AEs with fair temporal and/or spatial relationship to study device, with no clear alternative underlying cause.

Not related	AEs that do NOT follow a temporal and/or spatial relationship to study device and can be readily explained by an alternative cause, such as an accidental injury or expected progression of an underlying or concomitant disease.

6.5.4 Reporting of Adverse Events

After the informed consent has been obtained, the investigator, or designee, will determine whether any AEs have occurred. Any AEs will be reported in the subject's medical record and on the AE CRF page, and each will be classified according to the aforementioned criteria.

The following categories of adverse events will be entered onto the eCRF:

1. Any adverse events related to the breast in which the lesion(s) is identified
2. Any procedure-related adverse events including adverse events associated with mammography, conventional diagnostic ultrasound, Imagio, or biopsy.

Any pre-existing conditions that are detected as part of the Enrollment procedures must be reported in the medical history and not as an AE. Regulatory reporting standards will be followed for qualifying serious or unexpected events as per 21 CFR 812.

As part of the safety evaluation, all AEs need to be captured in study records for analysis and inclusion of the analysis/findings in the study report. This includes following all AEs through resolution or study completion to assure subject well-being, safety and rights.

6.5.5 Serious Adverse Event (SAE) and Unanticipated Adverse Device Effect (UADE)

All SAEs and UADEs must be reported from the time the subject signs the informed consent form (ICF) through their study duration.

The investigator, or designee, must report all SAEs and all UADEs to the sponsor or sponsor's authorized representative within 24 hours of notification of the event. The completed SAE form must be sent to the study contact noted below:

Thomas Stavros, MD
Medical Director
Seno Medical Instruments, Inc.
Telephone: 210-615-6501
FAX: 210-615-6508

Necessary SAE forms will be provided to the investigator. If the event has been classified as a SAE or UADE, details of medical history, concomitant medications and an evaluation of compliance with study

evaluation should accompany the form. The investigator, or designee, must also report the resolution of reported SAEs/UADEs promptly.

The investigator will report all SAEs and UADEs to his/her IRB as per the IRB guidelines.

All SAE and UADE collection forms will be provided as part of the site files and placed within the site's regulatory binder as will be the appropriate contact information for safety reporting.

6.5.6 Reporting and Recording Adverse Events (AEs)

As part of the safety evaluation, all AEs need to be captured in study records for analysis and inclusion of the analysis/findings in the study report. This includes following all AEs through resolution or study completion to assure subject well-being, safety and rights.

The investigator, or designee must complete the UADE form and notify the sponsor or sponsor's authorized representative within 24 hours. The investigator will report all AEs and UADEs to his/her IRB, as per the IRB guidelines.

6.6 Removal of Subjects from the Study

The investigator may withdraw a subject from this study to promote subject safety and/or due to protocol non-compliance.

6.7 Appropriateness of Measurements

Imagio was developed to complement and to add additional knowledge to existing imaging modalities. The selected endpoints (sensitivity, specificity, concordance (percent TP and TN), positive likelihood ratio (Se/ (1-Sp)), negative likelihood ratio (Sp/ (1-Se)), NPV and PPV), and odds ratio give an adequate characterization of the Imagio System in the indications for use by:

- Decreasing the number (percent) of women potentially undergoing biopsy by identifying those unlikely to have a malignant mass while not increasing the number (percent) of false-negative to a clinically unacceptable level (see Non-Inferiority margin discussion).
- Showing that Imagio has a sufficiently high specificity relative to the Imagio ultrasound component.
- Showing that Imagio features differentiate between benign and cancer cases.
- Down classifying BI-RADS 3, 4a, and 4b subjects according to the site investigator.
- Down classifying BI-RADS 3, 4a, and 4b subjects according to IUS.
- Up classifying BI-RADS 3, 4a, and 4b subjects according to IUS

7. QUALITY CONTROL AND ASSURANCE

Seno or its designated representatives performs at least monthly quality assurance checks on all investigational devices at participating sites. Phantoms and testing protocols will be supplied. The

investigational devices will be delivered and installed by Seno. Seno will provide guidance on the daily operation, back-up, and maintenance of all investigational devices including data transfer.

A Truth Panel will consist of two independent expert radiologists and a histopathologist; the Truth Panel will work closely with the Sponsor and/or designee to adjudicate all cases and to review anomalies relating to pathology, imaging, subject history, and clinical course for the additional purpose of alerts regarding subjects at risk for malignancy, but not biopsied.

- Quality Control and Assurance are conducted by the core imaging lab. A QA evaluator who is a QA radiologist (QAR) (separate from the independent reviewers) will perform an image/lesion pairing assessment once the ICC performs the QA of all demographic data, checks image completeness and quality, and resolves all discrepancies. The ICC must perform this QA before the independent review can take place. The QARs will also check if lesions scanned with Imagio at the site match the same lesion(s) seen on all imaging modalities at the site. The QA Radiologists review and select the relevant images and videos to allow unbiased and least burdensome IR review and ensure the imaging data can be read consistently, without ambiguity. In addition, criteria are in place to identify unbiopsied cases with a risk of malignancy according to the independent registration readers.

For the Pivotal Study component, subsets of 5% of registration image sets and 2.5% of all reimbursement image sets will be reread to assess learning and consistency.

In addition, Seno or its designated representatives will monitor all clinical studies that it sponsors. Before the enrollment of subjects in this study, the sponsor or sponsor's authorized representative and the investigator will review the protocol, the eCRF and instructions for completing them, the procedure for obtaining informed consent and the procedure for reporting AEs/UADEs/SAEs. Site monitoring visits will be performed by the sponsor or sponsor's authorized representatives on a regular basis. During these visits, information recorded in the eCRFs will be verified against source documents. The eCRF will be reviewed for device performance and compliance issues, safety information, legibility, completeness, accuracy and logical consistency. The data will reside in an electronic database. Additional computer programs that identify selected protocol violations, out-of-range data and other data errors may be used to help monitor the study. As necessary, requests for clarification or correction will be sent to the investigator.

Independent auditors from the sponsor or sponsor's authorized representative will be allowed by the investigator to audit previously monitored data. In addition, audits may be conducted by appropriate regulatory authorities.

Data entered in the eCRF will be source verified for accuracy and completeness. In addition, protocol compliance and compliance with FDA and EU regulations, including GCP, ICH, and ISO requirements, will be verified.

8. STATISTICAL METHODS

8.1 General Considerations

8.1.1 Statistical Strategy

This is a MRMC design involving two image sets (Imagio and its' ultrasound component) for registration (according to 7 independent readers) and multiple combinations of image sets for reimbursement (according to 3 other independent readers). The registration readers will review all cases while the reimbursement readers will read multiple combinations of image sets. The PMA filing will be based on the registration results with the reimbursement results to be submitted at a later date.

A total of at least 1700 women at 16 US sites are planned to undergo an Imagio evaluation following prospective enrollment, provided there are a sufficient number of benign masses (at least 1,000 masses) and, if there is not a sufficient number, then the total number of subjects will increase to achieve at least 1,000 benign masses. Subjects with an undiagnosed suspicious finding within the previous 45 business days, by palpation or by a screening or diagnostic methodology other than ultrasound, and scheduled to undergo a conventional diagnostic ultrasound will be prospectively screened. Subjects with only a BI-RADS 1 or BI-RADS 2 classification will be regarded as screen failures. This strategy reduces referral bias and accounts for a consecutive series of BI-RADS 3 and BI RADS 4 cases not discovered according to CDU. Once a decision has been made to biopsy (yes or no), the subject will then undergo an Imagio evaluation of all masses to be biopsied (up to a total of three per subject from both breasts). The Imagio results will not be used to decide whether or where to biopsy or to reverse the decision to biopsy; also no study-related follow-up is planned after the biopsy.

The analyses will be performed at the mass level since some subjects will have more than one mass biopsied. It is expected that no more than 5% of all subjects will have multiple masses so no repeated measures analyses are planned, that is, all lesions will be treated as independent observations.

Subjects will be drawn from the following four subpopulations selected by the site investigator to undergo diagnostic ultrasound and then Imagio:

- Callbacks for additional evaluation of suspicious area(s) identified by imaging other than ultrasound.
- Diagnostic referral to assess focal physical symptoms and / or signs that were either a chief complaint of the subject or were elicited by the healthcare practitioner (excluding focal breast pain in the absence of other positive clinical findings).
- Interval Clinical Problems (symptoms or physical findings excluding isolated focal breast pain, that have developed between yearly mammograms).
- Other referrals to diagnostic conventional ultrasound including subjects younger than 30 years old for a clinically suspicious area, or subjects referred from a screening MRI because of an

abnormality.

These subpopulations will be analyzed in aggregate as well as individually.

The Imagio diagnostic ultrasound component will serve as the “internal control”.

The specificity determined by a 2% POM is the primary effectiveness endpoint while the sensitivity is the secondary effectiveness endpoint.

Multiple effectiveness endpoints will be assessed (one primary and four secondary) so the overall 5% Type I error will be controlled by requiring two-sided p=0.01 to establish statistical significance. Specificity will be tested first according to a superiority test while sensitivity will then be tested according to non-inferiority tests. Testing will be based on the specificity (primary endpoint) and sensitivity (secondary endpoint), both based on a 2% Probability of Malignancy (POM). The overall specificity exceeding 40% will also be tested. In addition, downgrading benign masses and upgrading malignant masses using Imagio relative to the site and relative to IUS will be evaluated.

The superiority test and the non-inferiority tests will each have at least 80% power to test their respective hypotheses according to a two-sided test with overall 1% Type I error. The histopathology findings of the biopsy procedure will serve as a gold standard for Imagio. The decision to not recommend a biopsy during the first 12 months together with a negative 12 month clinical and imaging follow-up and no evidence of a >20% increase in the mass will be regarded as evidence of no disease (True-Negative for a negative OA/FN for a positive screening OA) for those initially not recommended for biopsy.

A total of 7 independent radiologists will be assigned per each case to independently evaluate both image sets (IUS and Imagio) per subject. In addition, 3 other independent readers will perform the reimbursement reads. All independent readers will evaluate all Pivotal Study cases; the readers will also review 5% of all cases to assess temporal trends and consistency; the initial evaluation will be used in all analyses.

Each registration reader will review the data from the two image sets (IUS, Imagio) for each case; each reader will evaluate Imagio according to a pre-determined diagnostic rule set based on OA image characteristics and will similarly evaluate IUS according to another pre-determined diagnostic rule set and each reader will estimate the probability that the mass is cancer on a scale from 0% to 100% in 5% increments **(**1% if ≤10% or ≥90%**)**. Readers will assess IUS and Imagio in this pre-defined order during independent review sessions; this pre-defined order is consistent with medical practice.

Registration readers will also have access to the results of two locked nomograms to separately predict the POM and the probability of cancer. These nomograms were constructed from the Pilot Study cases using a linear model for the POM and a logistic regression model for the probability of cancer. The nomograms are constructed from the five feature scores for Imagio from a blinded review performed by Dr. Stavros. The registration readers do not need to use the results of the nomograms in making their POM judgments, but they will immediately see the two predictions as soon as they score the five

features. The sensitivity and specificity of the nomograms will also be evaluated using the average predicted score; if the average is ≤0.1, then the case is predicted to be benign.

The final sample size of the Pivotal Study will be determined by an interim analysis after the first 800 Pivotal Study subjects (cases 101-900 passing Quality Assurance review) have been evaluated with Imagio plus the results of any pre-scheduled biopsies; the final sample size will depend on the numbers of masses biopsied, the percent of biopsied masses with a cancer diagnosis, and the sensitivities and specificities for Imagio and IUS among biopsied masses. The interim analysis will be conducted once at least four of the seven registration readers complete the review of the 800 cases. Results will be shared with partners for business planning purposes based on all 7 registration readers but will not be shared with sites or published until the study is completed. The final sample size will not exceed 2,400 masses.

The study design will require all images to be managed by an independent ICC according to FDA guidelines (FDA Clinical Performance Evaluation: Considerations for Computer-Assisted Detection Devices Applied to Radiology Images and Radiology Device Data – Pre-market Approval [PMA] and Pre-market Notification [510k] Submissions, October 21, 2009). The ICC will assign multiple readers per team to review all images leading to the decision to either perform a biopsy or to follow the subject for up to 12 months. To best replicate clinical relevance where mammography and conventional diagnostic ultrasound will be read first in practice, the image sets will be read in the following order:

1. Imagio diagnostic ultrasound alone
2. Imagio (diagnostic ultrasound plus opto-acoustics).

To preserve their independence and objectivity as well as to minimize bias, the independent readers must lock their IUS evaluations before being allowed to begin their Imagio evaluations; in addition, readers will NOT have access to any clinical data but will have access to biopsy outcomes once they record and lock their evaluations. Then readers will have controlled access to biopsy outcomes in units of 25 cases to simulate the real world setting where practicing radiologists receive outcomes.

Variable referral patterns across sites are expected. The referral pattern also varies depending on study site (academic vs. private practice), age, risk factors (family history of breast cancer, obesity), and breast BI-RADS density. Note that pre-screening and referral patterns influence diagnostic performance, helping to explain a breast cancer detection rate (BCDR) ranging between 18% and 40%. Endpoints of family history of breast cancer and obesity were not collected in this study.

The histologic findings of the biopsy procedure will serve as a gold standard for Imagio and the decision to not recommend a biopsy during the first 12 months will be regarded as evidence no of disease (True-Negative) for those not recommended for biopsy. Images will be assessed according to if the biopsy was performed immediately, delayed, or irrespective of timing through the 12 month follow-up interval.

High risk histologies will be analyzed separately from benign and malignant histologies in all data analyses. This reflects current practices where high risk masses are considered to be benign histologies but are always removed.

In general, superiority will be assessed using a two-sided 99% lower bound ≥ 0 while non-inferiority will be assessed using a one-sided 99.5% lower bound greater than or equal to the non-inferiority margin.

8.2 Study Hypotheses

The above considerations motivate multiple hypotheses. As pre-specified below, Hypothesis 1 tests Imagio specificity superiority versus IUS (H1) while Hypothesis 2 tests Imagio sensitivity non-inferiority versus IUS (H2). Hypotheses 3 and 4 test if the stand alone Imagio specificity is at least 40% (H3) and the stand alone sensitivity is at least 90% (H4). Hypothesis 5 tests the hypotheses for using Imagio to downgrade benign masses to BI-RADS 2 and 3 from BI-RADS 3, 4a, and 4b using IUS (H5) and upgrade malignant masses relative to IUS (H6).

The study hypotheses are as follows:

8.2.1. Hypothesis 1: The Imagio specificity is superior to IUS specificity

Null hypothesis) Ho: Imagio specificity – IUS specificity < 0%

versus

Alternative hypothesis) Ha: Imagio specificity – IUS specificity > 0%

A 6% absolute advantage in specificity is regarded as being the minimum meaningful advantage to help spare biopsies. Specificity superiority will be tested against the null hypothesis of no difference.

8.2.2. Hypothesis 2: The Imagio sensitivity is non-inferior to IUS

Null hypothesis) H_o: Imagio sensitivity – IUS sensitivity ≤ -5%

versus

Alternative hypothesis) H_a: Imagio sensitivity – IUS sensitivity > -5%

Sensitivities are expected to be at least 95% for both Imagio and IUS. A 5% sensitivity non-inferiority margin is regarded as being clinically meaningful.

8.2.3. Hypothesis 3: Imagio has acceptable stand-alone specificity

Null hypothesis) Ho: Specificity = 40%

versus

Alternative hypothesis) Ha: Specificity > 40%

A stand-alone specificity exceeding 40% is regarded as being clinically meaningful.

8.2.4. Hypothesis 4: Imagio has acceptable stand-alone sensitivity

Null hypothesis) H_o: Sensitivity = 90%

versus

Alternative hypothesis) H_a: Sensitivity > 90%

A stand-alone sensitivity exceeding 95% is regarded as being clinically meaningful.

8.2.5. Hypothesis 5: Imagio evaluation improves the overall diagnostic efficiency for benign masses as measured by the relative risk in the BI-RADS 3 and BI RADS 4a and BI RADS 4b subgroups according to IUS. A reduction of BI-RADS 4a and BI-RADS 4b according to IUS to BI-RADS 2 or 3 according to Imagio would be a major advance as would a reduction of BI-RADS 3 according to IUS to BI-RADS 1 or 2 according to Imagio. Specifically, the cancer prevalence ranges from 2% to 10% for the BI-RADS 4a category and 11% to 50% for the BI-RADS 4b category so a reduction to 1% or 2% would be equivalent to BI-RADS 2 or 3 which would be sufficient to prevent negative biopsies and to delay interval follow-up. A similar benefit would be to reduce cancer prevalence from 2% to <2% for the BI-RADS 3 subgroup.

Null hypothesis) H_o: No difference in BI-RADS according to Imagio vs. IUS

versus

Alternative hypothesis) H_a: Decreased BI-RADS according to Imagio vs. IUS

A 25% reduction to BI-RADS 2/3 from BI-RADS 3/4a/4b is regarded as being clinically meaningful for benign masses.

8.2.6. Hypothesis 6: Imagio evaluation improves the overall diagnostic efficiency for malignant masses as measured by the relative risk in the BI-RADS subgroups according to IUS. An increase in BI-RADS according to IUS to higher BI-RADS according to Imagio would be a major advance.

Null hypothesis) H_o: No difference BI-RADS according to Imagio vs. IUS

versus

Alternative hypothesis) H_a: Increased BI-RADS according to Imagio vs. IUS

A 10% increase in BI-RADS 3/4a/4b/4c to higher BI-RADS 4c/5 is regarded as being clinically meaningful for malignant masses.

8.3. Analysis Populations

All analyses will be performed with the intention to diagnose (ITD) study population which consists of all subjects coming in for their scheduled Imagio evaluation, having Imagio data declared evaluable, and having a biopsyat 12 months, or declared to be TN (or FP) at 12months.

There will be Truth Panel review to identify mass-specific subgroups and TN outcomes as well as reader-specific exclusions for the following:

1. Cases with QAR concerns (including cases with previously unidentified suspicious masses and cases where the Imagio evaluation was incomplete, unreliable, or unusable) for exclusions
2. Cases where the number or numbering of suspicious masses is under dispute (per QARs or IRs) for exclusions
3. Cases with questionable eligibility for exclusions
4. Cases with high risk histologies for separate analyses
5. Cases where individual IRs identified problems with specific image sets for exclusions
6. Cases with NDUs to determine if a mass was a True-Negative (TN) for specificity calculations.

8.4. Demographics and Baseline Characteristics

The age, race, ethnicity, menopausal status, breast density, and BI-RADS score will be presented using descriptive statistics for the overall ITD population, by study population type, by sites (individual, private practice, academic), by biopsy status (immediate, delayed, never), and by cancer status (benign, high risk, malignant).

8.5. Analysis Definitions

The final data for diagnostic test sensitivity and specificity will be presented in terms of a 2 × 2 table as follows; the threshold for defining a positive POM will be 2% corresponding to clinical practice standards:

Table 5: 2 x 2 Table

	Cancer	**Benign**
Test +	True Positive (*TP*)	False Positive (*FP*)
Test -	False Negative (*FN*)	True Negative (*TN*)

The above 2x2 table will also be constructed separately for Imagio and the Imagio diagnostic ultrasound component relative to a cancer or benign diagnosis. In addition, these 2x2 tables will also be generated for immediate biopsies, for delayed biopsies, for all biopsies, and for all cases including those not biopsied; discordant pairs for sensitivity as well as specificity will be analyzed. Those subjects without a biopsy will be excluded if they were not followed without a subsequent clinical evaluation.

A subject will be assigned to a row in the table depending on the respective diagnostic test result. Subjects with a diagnosis (biopsy or 11 month follow-up with no biopsy) will be assigned a column in the table according to the following rules:

1. If the result of the biopsy results in a cancer diagnosis, then the subject will be assigned to the Cancer column.
2. If the result of the biopsy results in a benign diagnosis, then the subject will be assigned to the Benign column.
3. If the biopsy was not performed and the subject was evaluated after 11 months post-baseline (nominal 12 month evaluation), then the subject will be counted as TN as long as there were no contrary clinical or radiologic findings (not to be based on Imagio); any mass declared to have any contrary clinical or radiologic findings will be declared to be a False-Positive if the Imagio POM was $\leq$2%; otherwise the subject will not be included in the table
4. If the baseline Imagio evaluation was not performed or declared to not be useable, then the subject will not be included in the table.

In addition, the cancer group will be further divided into invasive carcinoma and DCIS categories and each of these two categories will be assessed separately within a 2x2 table.

The use of the terms *sensitivity* and *specificity* is justified in this study because the biopsy and 11 month evaluation (12 month nominal evaluation) is the gold standard. The following statistical endpoints will be assessed for each image set:

- The *true positive rate* (TPR) or *sensitivity* of the test is defined as *TP/ (TP + FN)*
- The *specificity* of the test is defined as *TN/ (TN + FP)*
- The *concordance* of the test is defined as *(TP + TN)/N*
- The *positive likelihood ratio* is defined as TPR/FPR
- The *negative likelihood ratio* is defined as (1 – TPR)/(1 – FPR)
- The *positive predictive value* (PPV) is defined as *TP/ (TP + FP)*
- The *negative predictive value* (NPV) is defined as *TN/ (TN + FN)*
- The *odds ratio* (OR) is defined as *(TP/FP)/(FN/TN)*

Given that biopsy decisions are made using the 2% POM, POM levels >2% have limited relevance to the 2% threshold now widely used for making biopsy decisions.

8.6. Sample Size Calculations

The sample size calculations for the Pivotal Study may be increased based on the n=800 interim analysis results. The Pilot Study results will solely be used to validate the algorithm and will not count towards the Pivotal Study sample size.

The interim analysis will provide key information to check the preliminary assumptions regarding the degree of correlations between Imagio and IUS. Imagio can theoretically succeed with improvements relative to the above two diagnostic gray scale citations (25, 26) with success

optimized if Imagio can rule out suspicious benign cases (minimizing the false-positive percents). The following table summarizes the specificity superiority tests to detect a conservative absolute 6% advantage which can be performed with 90% power for 860 benign masses assuming 20% discordant.

Table 6: 99.5% Superiority: McNemar's test (Specificity Difference: Paired Proportions

	1
Test significance level, α	0.5%
Expected difference, $\delta = \lvert\pi_1 - \pi_2\rvert$	6%
Proportion discordant, $\eta = \pi_{10} + \pi_{01}$	20%
Power (%)	90
n (number of pairs)	860

The following table summarizes the sensitivity non-inferiority margin (5%) which can be ruled out with 90% power for 420 cancer masses.

Table 7: 99.5% Non-inferiority Confidence Bound: Sensitivity Difference: Paired Proportions (simulation)

	1
Confidence level, 1-α (one-sided)	99.5%
Expected difference, $\pi_T - \pi_S$, Δ_1	0%
Proportion discordant, $\eta = \pi_{10} + \pi_{01}$	5%
Proportion both yes, π_{11}	94%
Lower limit for $\pi_T - \pi_S$, LL	-5%
Power (%)	90
n (number of pairs)	420

The following table displays the threshold needed to reject the null hypothesis of 40% specificity with 80% power according to a 99.5% lower bound for 1,160 benign masses. An observed 45% specificity would reject the 40% null hypothesis.

Table 8: 99.5% Exact Lower Bound: Specificity

	1
Test significance level, 1-α	99.5%
Null hypothesis proportion, π_0	40%
Alternative proportion, π_A	45%
Power (%)	80
N	1160

The following table displays the threshold needed to reject the null hypothesis of 90% sensitivity with 80% power according to a 99.5% lower bound for 360 cancer masses. An observed 95% sensitivity would reject the 90% null hypothesis.

Table 9: 99.5% Exact Lower Bound: Sensitivity

	1
Test significance level, 1-α	99.5%
Null hypothesis proportion, π_0	90%
Alternative proportion, π_A	95%
Power (%)	80
N	360

8.6.1 Sample Size Re-estimation

The sensitivity and specificity need to be estimated in an unbiased manner using the most accurate assumptions. This will be accomplished using an interim analysis after the first 800 subjects are evaluated by the independent readers; specific attention will be paid to the "internal control." The specificity will be the primary effectiveness endpoint and the sensitivity will be the secondary effectiveness endpoint for this sample size recalculation; the sample size will not be decreased under any circumstances. The final sample size of the Pivotal Study will be determined by an interim analysis after the first 800 subjects have been evaluated with Imagio relative to the results of any pre-scheduled biopsies; the final sample size will depend on the numbers of masses biopsied, the percent of biopsied masses with a cancer diagnosis, and the sensitivities and specificities for Imagio and IUS. The interim analysis will be conducted once at least four of the seven registration readers complete the review of the 800 cases. The final sample size will not exceed 2,400 masses.

To preserve the Type I error, the observed specificity differences will not be used in the sample size re-estimation and there will not be an interim stopping rule for the study.

8.7. Analyses

8.7.1 Sensitivity and Specificity

The specificity for Imagio relative to the Imagio ultrasound component (IUS) will be assessed using a one-sided 99.5% lower confidence bound. The goal is to establish at least a 6% absolute advantage in specificity for Imagio vs IUS.

The sensitivities and specificities will be displayed for Imagio relative to IUS; in these displays, the consequences of Imagio (positive vs. negative) will be considered and compared using a generalized linear model (GENMOD) to account for correlated outcomes among the readers..
Additional analyses will be performed using the nomograms for the POM and the diagnosis based on the five Imagio features. The first nomogram models the probability of malignancy (NPP) while the second nomogram models the probability of cancer (NPC). The sensitivity and specificity of the averaged prediction (NPP and NPC) being $\geq$0.1 or $\leq$0.1 will be evaluated. The ability of the nomogram to downgrade benign masses with IUS POM $\leq$30% and CDU POM $\leq$30% will also be assessed. The linear regression (NPP) and the logistic regression (NPC) models behind the predictions are based solely upon the Pilot Study data.

8.7.2 Histologic Considerations

The nature of the malignancies will be analyzed. While DCIS is considered malignant, many of the vascular and metabolic changes that might be detectable with OA may be limited to invasive carcinoma. Thus, the degree of invasive malignant and non-invasive (DCIS) malignant masses will be analyzed separately. It may be that OA is very effective in detecting invasive malignancy, but not as effective at detecting DCIS. The malignant potential becomes a consideration with OA risk benefit if it performed better for more invasive and more extensive malignancies, while a missed DCIS carries reduced risk. Other lower risk masses would include atypical ductal hyperplasia, atypical lobular hyperplasia, lobular carcinoma in situ, and lobular neoplasia which will be considered benign for the purposes of this study.

High risk masses will also be considered from the perspective of where to distinguish between benign and malignant disease. Specifically, histopathologic diagnoses of atypical ductal hyperplasia, atypical lobular neoplasia, and lobular carcinoma in situ, etc. are considered “in between” benign and malignant. These cases will be considered high risk as confirmed by the central pathologist. Since the features of high risk masses are nonspecific, usually reflecting the features of the underlying benign mass in which they arose, including such masses in the malignant category would reduce sensitivity. As a practical matter, such diagnoses are almost always require re-biopsy, e.g. a surgical excision, which often results in an upgrade to DCIS or invasive cancer. This information will be collected and included in the malignant category if so classified by the independent pathologist who will make the final determination. However, some cases, even after excision, may remain high risk so high risk masses will always be analyzed separately.

8.7.3 Feature Analysis

Additional analyses will be performed using the specific features from the Pivotal Study to discriminate benign from cancer using classification methodology. Six specific pre-defined OA features will be scored by the independent readers using a 0-5 or 0-6 ordinal scale to evaluate: 1) internal vascularity and de-oxygenation, 2) internal deoxygenated blush, 3) internal total blood 4) peri-tumoral boundary zone vascularity and deoxygenation,), 5) external peri-tumoral radiating vessels, and 6) interfering artifact. The first three features are considered to be internal features and will also be summed to derive a total internal feature score. Similarly, the fourth and fifth feature will be summed to derive a total external feature score. Also the five feature scores will also be summed to derive a total score. The distributions

of the benign vs. malignant masses will be performed using a Wilcoxon Rank Sum test for individual features and ANOVA for the three total scores.

8.7.4 Other Secondary Analyses

The sensitivity, specificity, concordance, PPV, NPV, PLR, NLR, and odds ratio for Imagio and the Imagio ultrasound component will be displayed for 2% POM and assessed using 99% confidence bounds.

Additionally, sensitivity and specificity results will be separately displayed for Imagio and the Imagio ultrasound component for the pre-defined subpopulations as descriptive statistics.

8.7.5 BI-RADS Analyses

Results will be displayed according to the BI-RADS classification to provide insight into currently used guidelines. The matched pairs cross tabulation of Imagio versus its' ultrasound component will be assessed.
The ability of Imagio to downgrade BI-RADS 4a and 4b (or BI-RADS 3) relative to IUS and separately according to the site investigator to BI-RADS 3 (or BI-RADS 1-2) for the benign cases and to then symmetrically upgrade BI-RADS 3, 4a, 4b, and 4c according to Imagio relative to IUS and separately relative to the site investigator for the cancer cases; the discordant pairs will be compared using a McNemar's test for individual readers and a generalized linear model for grouping readers. In addition, the ability of Imagio to improve the site investigator classification for the BI-RADS 4ab (or BI-RADS 3) subgroup will be assessed separately for the cancer and benign cases; the percents (Imagio positive vs. Imagio negative) will be separately evaluated using a two-sided Fisher Exact Test.

8.7.6 Histopathology Analyses

Results for cancers, benign cases, and high risk cases will be separately displayed to see if Imagio features are correlated with histopathology outcomes.

8.7.7 Other Analyses

The sensitivity and specificity for Imagio and the Imagio ultrasound component will be generated for individual readers. The learning curve of individual readers will also be assessed in units of at least 100 cancers and at least 200 benigns to check if sensitivities and specificities improve as more cases are read.

Inter-reader variation will be evaluated separately for cancer cases and for benign cases for the readers. Inter-reader variability for these two subsets (cancers, benigns) will be analyzed using an ANOVA model where the readers are considered to be fixed effects; a random effects model will also be run. Intra-reader variation will be evaluated using paired t-tests applied to the paired POMs, and BI-RADS separately for cancer cases and for benign cases.

8.8. Other Assessments or Analyses

Specific treatment-emergent adverse events will also be displayed for each subject.

9. ADMINISTRATIVE CONSIDERATIONS

9.1. Investigators and Study Administrative Structure

Each site will have a designated investigator and one or more study coordinators collectively responsible for the conduct of the study inclusive of referral, screening, enrollment, evaluation, documentation, and subsequent biopsy in accordance with Good Clinical Practice (GCP) compliance.

9.2. Institutional Review Board (IRB)

The protocol and the informed consent form, and any other institution-specific documents, must have the approval of a properly constituted IRB committee responsible for approving clinical studies. The signed IRB approval letter must specify the date of protocol and informed consent form approval and identify the documents approved including the investigator's name, the protocol version, date and title. Any subject materials or advertisements used to recruit subjects should also be reviewed and approved by the IRB. Clinical supplies will not be shipped until a signed approval letter from the IRB has been received and a contractual agreement has been signed by the sponsor or sponsor's authorized representative and the clinical site.

9.3. Ethical Conduct of the Study

This study will be conducted in accordance with Title 21 Code of Federal Regulations (CFR) Parts 50, 54, 56 812, and 820.30 as well as the ethical principles outlined in the ICH GCP guidelines, the standard ISO 14155; ICH E6, E9, E14, E15 and in accordance with all local requirements.

9.4. Informed Consent

A written Informed Consent Form (ICF) must be obtained from the subject prior to performing any study-related procedures. The investigator or investigator's designee will provide background information on the study, including the benefits and risks of all study-related procedures. The investigator or investigator's designee will also encourage the prospective subject to ask questions about the study and will provide the subject with sufficient opportunity to consider whether or not to participate. After written consent/assent has been documented, previously obtained clinical radiographic data can be used for the study.

Original signed informed consent forms must be filed in the subject records at the site. A copy of the signed consent/assent form should also be provided to the subject.

The ICF template for this study may be revised by an investigator or an IRB based on the institution's requirements for a non-significant risk device. However, all changes requested by an investigator or an IRB, even those that may not be considered substantial and/or do not affect the rights, safety or welfare of a subject, must be approved by the sponsor. If the sponsor determines that the revisions are substantial and/or affect the rights, safety or welfare of a subject, the ICF must be reviewed and approved by both the sponsor and IRB before the ICF can be utilized. Copies of the current ICF's are attached in Appendix B.

9.5. Subject Confidentiality

Confidentiality will be maintained in accordance with HIPAA as per Title 45 CFR Part 164.508. Subject names must not be revealed to the sponsor or sponsor's authorized representatives. Only the subject identifier (number and initials) will be recorded in the eCRF and if the subject's name appears on any other document, it must be redacted and replaced with the subject identifier before a copy of the document is supplied to the sponsor or sponsor's authorized representatives. Study findings stored on a computer will be stored in accordance with local data protection laws. In the event of inadvertent communication of such information, immediate steps to redact the information from all study files will be implemented, with appropriate documentation in the subject study file.

9.6. Study Monitoring

Seno or their designate will schedule and conduct regular on-site visits for monitoring of study activities and data recording in order to verify data accuracy as well as protocol and regulatory compliance. Review of the eCRFs at the investigational site will be conducted to confirm completeness and clarity, to verify eCRF data against source documents and to clarify administrative matters. Formal reports of these visits will be generated with copies provided to the Seno Director of Clinical Operations or designee for review and approval and/or designated sponsor representatives.

9.7. Case Report Forms and Study Records

Data will be recorded using an electronic data capture (EDC) system. An EDC Database Programmer will develop and test the database in a validated environment according to the requirements of 21 CFR Part 11, Electronic Records; Electronic Signatures. Highlights of the software development lifecycle process include database development according to a validation protocol that documents the design requirements and specifications, database testing by EDC programmers and the CRO Data Management end users using test eCRFs, and documented review by the CRO and acceptance by Seno. The database is considered validated when the expected results are the same as the actual results, and the end users verify that the database performs according to the requirements.

The validation report, design procedures, testing results, source code, and test eCRF cases are filed in the central files. Database maintenance will be provided throughout the study, as well as user support and administration (access and site user rights set-up, removal, etc.)

The MedDRA dictionary will be used to code adverse events.

The investigator is responsible for maintaining adequate and accurate medical records from which information will be transferred into the study database. Study-specific eCRFs should be completed by the investigator or designated personnel. Detailed instructions will be provided with the implementation of access to the eCRF.

9.8. Imaging Coordination Committee (ICC)

An independent ICC will coordinate the reviews of all possible image sets consisting of data used to make the diagnosis leading to biopsy as well as Imagio. The ICC will oversee three QA reviewers to ensure that the image sets were collected by sites as instructed and to make sure that cases are properly blinded. A separate ICC protocol will describe and govern the image review process. There will be 7 independent registration readers and 3 independent reimbursement readers for the Pivotal Study to perform these reviews. Readers will be directed to read the image sets in a predefined order to correspond to real world setting and to also receive real time feedback every 25 completed cases to further correspond to real world setting.

9.9. Protocol Deviations

A protocol deviation occurs when the clinical investigator or site personnel do not conduct the study according to the protocol, including the investigational device instructions for use, or the investigator agreement. No deviations from the protocol should occur except where necessary to protect the life or physical well-being of a subject during an emergency. These emergency deviations should be reported to the IRB and sponsor in writing or verbally as soon as possible, but no later than five (5) business days after the emergency occurred.

There will be unforeseen circumstances that are beyond the investigator's control (e.g., subject refused subsequent biopsy). Prior approval will not be granted in these situations, but the investigator should report these events upon determining that a deviation has occurred.

The investigator is responsible for complying with and adhering to IRB procedures for reporting deviations. All protocol deviations should be documented and forwarded to the sponsor or sponsor's authorized representative.

Significant protocol deviations will be reported by the sponsor or sponsor's authorized representative to the IRB yearly in the annual report. Participating investigators will receive regular updates regarding their site's deviations.

9.10 Device or Software Recalls, Malfunctions, and Modifications

The sponsor will notify the site in the event of any malfunctions with respect to their device as well as any recalls or modifications of their device or software. Sites will not modify the study device or use it beyond this study unless otherwise authorized under an approved protocol.

9.11 Source Documentation

Information on the eCRF should be verifiable in source documents. Surveys completed by the subject and/or information that may be directly entered by the investigator or his/her representative on the eCRFs will be considered source documents. Other records that will be considered source documents are hospital records, clinic charts, x-rays, CT scans, laboratory reports and pathology reports. Copies of source documents that should be sent to the sponsor or sponsor's authorized representative include operative summaries and discharge reports. Other source documents may include hospital discharge summaries, if available, or information in lieu of a discharge summary, such as discharge orders or progress notes; any relevant notes pertaining to AEs, including additional biopsies involving the breast.

9.12 Data Generation and Analysis

Data will be collected in accordance with Title 21 CFR Part 11 and will be analyzed in accordance to a prospective statistical analysis plan developed in accordance with the statistical section of this protocol and the latest FDA guidance documents for imaging modalities.

9.13. Retention of Data

All records, including compact disks of images, must be retained at each clinical site and by the sponsor or sponsor's authorized representative for a period of two years after the latter of the following two dates: the date the study is completed or terminated or the date that the records are no longer needed to support a regulatory submission. All study records may be relevant to regulatory inspection.

The sponsor or sponsor's authorized representative should be contacted in advance if there is a desire to discard or relocate any records or if there are any questions regarding record retention.

The sponsor or sponsor's authorized representative will maintain all records related to this investigation, according to Title 21 CFR 812.140 (b) and ICH GCP guidelines.

9.14 Financial Disclosure

Investigators will be asked to provide financial disclosure prior to authorization to begin the study as well as after the study is completed. Investigators will also be expected to share any situations which could introduce site-specific bias.

9.15 Publication and Disclosure Policy

The results of the study are the property of Seno Medical Instruments, Inc. All publications (manuscripts, abstracts or other modes of presentation) must be submitted at a time determined by Seno Medical Instruments, Inc. and must be reviewed and approved in writing by Seno Medical Instruments, Inc., in advance of submission. Co-authorship with any Seno Medical Instruments, Inc. personnel will be discussed and mutually agreed upon before submission of a manuscript to a publisher.

10. REFERENCE LIST

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Investigator's Signature

Study Title: A **P**ivotal Study of **I**maging with **O**pto-acoustics to diagnose breast masses detected by mammography and/or clinical findings: A **NE**w **E**valuation Tool for **R**adiologists

Study Number: PIONEER-01

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Investigational Site	Title	Investigator Printed Name	Investigator Signature	Date
Address			**Phone:**	
			Fax:	
			E-mail:	

Appendix A: Photo-sensitive Dermatoses and Photo-sensitizing Agents

Photo-sensitive Dermatoses

PHOTO-AGGRAVATED DERMATOSES
• Acne vulgaris • Atopic dermatitis • Bullous pemphigoid • Carcinoid syndrome • Cutaneous T-cell lymphoma • Dermatomyositis • Disseminated superficial actinic porokeratosis • Erythema multiforme • Familial benign chronic pemphigus (Hailey–Hailey disease) • Hartnup syndrome • Keratosis follicularis (Darier disease) • Lichen planus • Lupus erythematosus • Pellagra • Pemphigus, including pemphigus foliaceus (erythematosus) • Pityriasis rubra pilaris • Psoriasis • Reticular erythematous mucinosis (REM) • Rosacea • Seborrheic dermatitis • Transient acantholytic dermatosis (Grover's disease) • Viral infections, including herpes simplex, exanthems • Vitiligo

Clinical Features

The basic dermatosis may be mildly or severely exacerbated by UVR exposure, usually within minutes to hours. With sunlight avoidance, masses gradually improve over a period of days to weeks. The UVR-exacerbated eruption may be limited to sites characteristic of the underlying condition, or, less commonly, involve all sun-exposed sites. Photo-testing results are often normal, except in subjects with LE. Occasionally, broad-spectrum irradiation may evoke the eruption.

Pathology

The histology is that of the underlying dermatosis.

Differential Diagnosis

In general, photo-aggravated dermatoses are recognized by the presence of the underlying skin disease. Differentiation from PMLE (polymorphous light eruption) by history alone may be difficult. Clinically, the latter is typically papular and pruritic, has negative lupus serology, and is often characterized by abnormal photo-provocation tests. It is important to note that PMLE may coexist with other dermatoses, in particular with photosensitive psoriasis. Photo-aggravated atopic dermatitis, if widespread, may be confused with CAD; however, the latter subjects have markedly positive photo-test results. It is also important to distinguish UVR exacerbation of dermatoses from aggravation due to heat.

Treatment

Treatment consists primarily of topical or systemic therapy aimed at the underlying disorder. If significant photosensitivity persists, low-dose broad- or narrowband UVB or PUVA therapy (as for PMLE) may on occasion be effective. However, the latter is contraindicated in subjects with LE or dermatomyositis, because systemic exacerbation may conceivably occur. Photo-protection is also important.

Chemical- and Drug-Induced Photo-sensitivity

Key Features
• Phototoxicity is characterized by an exaggerated sunburn reaction and is usually caused by systemic agents • Photoallergy presents with eczematous masses and is most commonly associated with topical photoallergens • The cutaneous porphyrias are an example of phototoxicity induced by endogenous agents • Management consists of identification and avoidance of the offending agent

Common Photo-toxic and Photo-allergic Agents

Common Phototoxic Agents	Common Photo-allergic Agents
Anti-arrhythmics • Amiodarone • Quinidine Triazole antifungals • Voriconazole Diuretics • Furosemide • Thiazides Non-steroidal Anti-inflammatory Drugs • Nabumetone • Naproxen • Piroxicam	Topical Agents: • Sunscreens (e.g. oxybenzone, [benzophenone-3]) Fragrances • 6-Methylcoumarin • Musk ambrette • Sandalwood oil Antimicrobial Agents • Bithionol • Chlorhexidine • Fenticlor • Hexachlorophene
Phenothiazines • Chlorpromazine • Prochlorperazine Psoralens • 5-Methoxypsoralen • 8-Methoxypsoralen • 4,5„,8-Trimethylpsoralen Quinolones • Ciprofloxacin • Lomefloxacin • Nalidixic acid • Sparfloxacin St. John's Wort • Hypericin • Tar (topical) Tetracyclines • Doxycycline • Demeclocycline	Non-steroidal Anti-inflammatory Drugs • Diclofenac • Ketoprofen Phenothiazines • Chlorpromazine • Promethazine **Systemic Agents:** Anti-arrhythmics • Quinidine Antifungal • Griseofulvin Antimalarial • Quinine Antimicrobials • Quinolones (e.g. enoxacin, lomefloxacin) • Sulfonamides Non-steroidal Anti-Inflammatory Drugs • Ketoprofen • Piroxicam

History

Phototoxic agents have been used as medications from as early as 4000 BC, with the inhabitants of Mesopotamia (and later the Indians and Egyptians) employing plant-derived psoralens for the treatment of leukoderma. In 1913, hematoporphyrin-induced photo-toxicity was demonstrated by Meyer-Betz, while the current method of PUVA therapy was introduced in the 1970s. Photo-allergy was first recognized in 1939 based on a classic study by Epstein on sulfanilamide.

Epidemiology

While the exact prevalence of photo-toxicity and photo-allergy in the general population is unknown, their frequency in photo-dermatology referral centers has ranged from 7% to 15% for photo-toxicity and from 4% to 8% for photo-allergy.

Pathogenesis

The pathogenesis of phototoxicity involves the generation of oxygen free radicals, superoxide anions, hydroxyl radicals and singlet oxygen, which leads to a host of cytotoxic effects. Other mechanisms of tissue damage include the generation of stable photoproducts (reported with chlorpromazine and tetracyclines), the formation of photoadducts (reported with psoralens), and the generation of inflammatory mediators (reported with porphyrins and demethylchlortetracycline).

The pathogenesis of photoallergy is identical to that of allergic contact dermatitis, with the exception of the requirement for the presence of UVR to induce photoallergen formation.

Clinical Features

Photo-toxicity

Following cutaneous or systemic exposure to a phototoxic agent *and* appropriate UVR, phototoxicity develops within hours; for the vast majority of such agents, the action spectrum is in the UVA range. Erythema and edema as well as burning and stinging sensations characterize the initial presentation, with vesicles and bullae seen in severely affected subjects. Phototoxic reactions due to oral medications resemble exaggerated sunburn. These reactions resolve spontaneously with desquamation and hyperpigmentation.

Other less common manifestations of phototoxicity include pseudoporphyria (frequently caused by non-steroidal anti-inflammatory drugs [NSAIDs], especially naproxen), photo-onycholysis (reported with tetracyclines and psoralens), slate-gray hyperpigmentation (reported with amiodarone, tricyclic antidepressants and diltiazem), and lichenoid eruptions (seen with quinine and quinidine). Evolution of phototoxicity reactions into chronic actinic dermatitis (see above) has rarely been reported following exposure to thiazides, quinidine, quinine or simvastatin. The medications most commonly associated with phototoxicity are listed in Table 86.5.

5-Fluorouracil (5-FU), methotrexate and retinoids, three commonly used medications in dermatology, have been associated with 'photosensitivity'; however, none is associated with UVR-induced activation of the drug. UV-exacerbated erythema in subjects receiving 5-FU usually occurs in sites with actinic keratoses. While no systematic photo-testing study has been performed in subjects taking 5-FU, this reaction most likely represents an exacerbation of 5-FU-induced cutaneous inflammation by UVR. Administration of methotrexate is known to occasionally cause a recurrence of UV-induced erythema. Photo-testing in subjects taking methotrexate has been normal, and the mechanism for the 'recall erythema' reaction remains unclear. Photo-testing in subjects taking isotretinoin or etretinate is also usually a routine practice. The propensity for these subjects to develop UV-induced erythema is most likely due to retinoid-induced thinning of the stratum corneum.

Phytophotodermatitis is characterized by linear streaks of erythema that occur a day or so after contact with plants (frequently furocoumarin-containing) plus exposure to sunlight. Linear post-inflammatory hyperpigmentation is a classic finding. Plants capable of inducing phytophotodermatitis include yarrow, parsley, celery, parsnips, milfoil, lime, lemon and fig. Therefore, this condition most commonly occurs in individuals whose outdoor activities expose them to such plants (e.g. vegetable harvesters, bartenders).

Roofers and road workers may occasionally develop phototoxicity secondary to exposure to tar plus UVA from sunlight. St. John's wort (*Hypericum perforatum*), a product widely available in health food stores that is used to treat minor cuts, diarrhea, fever and depression, contains hypericin, a known phototoxic agent.

Photo-allergy

In previously sensitized individuals, exposure to photo-allergens plus sunlight results in the development of a pruritic eczematous eruption. In more severely affected subjects, vesicles and bullae may develop (but less commonly than in phototoxic reaction). While there are clear differences between the clinical manifestations of photo-toxicity versus photo-allergy, such differences may not always be obvious, and careful history taking and examination are essential.

Currently, in the US, UK and France, sunscreen agents (especially benzophenone-3) are the most common cause of photo-allergy, while NSAIDs are the leading photo-allergen in Germany, Austria, and Switzerland. Other common photo-allergens include fragrances and anti-bacterial products.

Subjects undergoing Photodynamic Therapy

Agents containing:

- aminolevulinic acid (levulin and metvix)
- methoxsalen (oxsoralen lotion or capsules)

Appendix B: ACR Guidelines

Revised 2011 (Resolution 11)*

ACR PRACTICE GUIDELINE FOR THE PERFORMANCE OF A BREAST ULTRASOUND EXAMINATION

PREAMBLE

These guidelines are an educational tool designed to assist practitioners in providing appropriate radiation oncology care for patients. They are not inflexible rules or requirements of practice and are not intended, nor should they be used, to establish a legal standard of care. For these reasons and those set forth below, the American College of Radiology cautions against the use of these guidelines in litigation in which the clinical decisions of a practitioner are called into question.

The ultimate judgment regarding the propriety of any specific procedure or course of action must be made by the physician or medical physicist in light of all the circumstances presented. Thus, an approach that differs from the guidelines, standing alone, does not necessarily imply that the approach was below the standard of care. To the contrary, a conscientious practitioner may responsibly adopt a course of action different from that set forth in the guidelines when, in the reasonable judgment of the practitioner, such course of action is indicated by the condition of the patient, limitations of available resources, or advances in knowledge or technology subsequent to publication of the guidelines. However, a practitioner who employs an approach substantially different from these guidelines is advised to document in the patient record information sufficient to explain the approach taken.

The practice of medicine involves not only the science, but also the art of dealing with the prevention, diagnosis, alleviation, and treatment of disease. The variety and complexity of human conditions make it impossible to always reach the most appropriate diagnosis or to predict with certainty a particular response to treatment.

Therefore, it should be recognized that adherence to these guidelines will not assure an accurate diagnosis or a successful outcome. All that should be expected is that the practitioner will follow a reasonable course of action based on current knowledge, available resources, and the needs of the patient to deliver effective and safe medical care. The sole purpose of these guidelines is to assist practitioners in achieving this objective.

I. INTRODUCTION

This guideline has been developed to assist practitioners performing ultrasound examination of the breast. When ultrasound is used as guidance for interventional procedures or biopsy, relevant ACR guidelines should be consulted.

II. INDICATIONS

Appropriate indications for breast sonography include, but are not limited to:

1. Evaluation and characterization of palpable masses and other breast related signs and/or symptoms [1-4].
2. Evaluation of suspected or apparent abnormalities detected on other imaging studies, such as mammography or magnetic resonance imaging (MRI) [5].
3. Initial imaging evaluation of palpable masses in women under 30 years of age who are not at high risk for development of breast cancer, and in lactating and pregnant women.
4. Evaluation of problems associated with breast implants [6].
5. Evaluation of breasts with microcalcifications and/or architectural distortion suspicious for malignancy or highly suggestive of malignancy in a setting of dense fibroglandular tissue, for detecting an underlying mass that may be obscured on the mammogram [6].
6. Guidance of breast biopsy and other interventional procedures [7].
7. Treatment planning for radiation therapy [6].
8. As a supplement to mammography, screening for occult cancers in certain populations of women (such as those with dense fibroglandular breasts who are also at elevated risk of breast cancer or with newly suspected breast cancer) who are not candidates for MRI [8-9] or have no easy access to MRI.
9. Identification and biopsy guidance of abnormal axillary lymph node(s), for example in patients with newly diagnosed or recurrent breast cancer [10-11] or with findings highly suggestive of malignancy or other significant etiology.

III. QUALIFICATIONS AND RESPONSIBILITIES OF THE PHYSICIAN

A. Physician

Physicians who supervise, perform, and/or interpret breast ultrasound examinations should be licensed medical practitioners who have a thorough understanding of the indications for ultrasound examinations as well as a familiarity with the basic physical principles and limitations of the technology of ultrasound imaging. They should be familiar with alternative and complementary imaging and diagnostic procedures and should be capable of correlating the results of these other procedures with the sonographic findings. They should have a thorough understanding of ultrasound technology and instrumentation, ultrasound power output, equipment calibration, and safety. Physicians responsible for breast ultrasound examinations should demonstrate familiarity with breast anatomy, physiology, and pathology. These physicians should provide evidence of the training and competence needed to perform breast ultrasound examinations successfully.

Physicians performing and/or interpreting breast ultrasound examinations should meet at least one of the following criteria:

Certification in Radiology or Diagnostic Radiology by the American Board of Radiology, the American Osteopathic Board of Radiology, the Royal College of Physicians and Surgeons of Canada, or the Collège des Médecins du Québec, and involvement with the supervision and/or performance, interpretation, and reporting of 300 breast ultrasound examinations within the last 36 months.[1]

or

Completion of an Accreditation Council for Graduate Medical Education (ACGME) approved diagnostic radiology residency program and involvement with the supervision and/or performance, interpretation, and reporting of 300 breast ultrasound examinations in the past 36 months.[1]

or

Physicians not board certified in radiology or not trained in a diagnostic radiology residency program, and who assume these responsibilities for sonographic imaging of the breast, should meet the following criteria: completion of an ACGME approved residency program in specialty practice plus 200 hours of Category I continuing medical education (CME) in breast ultrasound; and supervision and/or performance, interpretation, and reporting of 500 breast ultrasound examinations during the past 36 months in a supervised situation.

Maintenance of Competence

All physicians performing ultrasound examinations should demonstrate evidence of continuing competence in the interpretation and reporting of those examinations. If competence is assured primarily based on continuing experience, a minimum of 100 examinations per year is recommended in order to maintain the physician's skills. Because a physician's practice or location may preclude this method, continued competency can also be assured through monitoring and evaluation that indicates acceptable technical success, accuracy of interpretation, and appropriateness of evaluation.

Continuing Medical Education

The physician's continuing education should be in accordance with the ACR Practice Guideline for Continuing Medical Education (CME) and should include CME in ultrasonography as is appropriate to his or her practice.

B. Diagnostic Medical Sonographer

When a sonographer performs the examination, he or she should be qualified by appropriate training to do so. This qualification can be demonstrated by certification or eligibility for certification by a nationally recognized certifying body.

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[1]Completion of an accredited radiology residency in the past 24 months will be presumed to be satisfactory experience for the performance, reporting, and interpreting requirement.

IV. WRITTEN REQUEST FOR THE EXAMINATION

The written or electronic request for a breast ultrasound examination should provide sufficient information to demonstrate the medical necessity of the examination and allow for its proper performance and interpretation. Documentation that satisfies medical necessity includes 1) signs and symptoms and/or 2) relevant history (including known diagnoses). Additional information regarding the specific reason for the examination or a provisional diagnosis would be helpful and may at times be needed to allow for the proper performance and interpretation of the examination.

The request for the examination must be originated by a physician or other appropriately licensed health care provider. The accompanying clinical information should be provided by a physician or other appropriately licensed health care provider familiar with the patient's clinical problem or question and consistent with the state's scope of practice requirements. (ACR Resolution (35), adopted in 2006)

V. SPECIFICATIONS FOR INDIVIDUAL EXAMINATIONS

A. Image labeling should include a permanent identification label that contains:

1. Facility name and location.
2. Examination date.
3. Patient's first and last name.
4. Identifying number and/or date of birth.
5. Designation of right or left breast.
6. Anatomic location using clock face notation or a labeled diagram of the breast. Transducer orientation and distance from the nipple to the abnormality, if present, are required.
7. Sonographer's and/or physician's identification number, initials, or other symbol.

B. Lesion Characterization and Technical Factors [3]

1. The breast sonogram should be correlated with clinical signs and/or symptoms and with mammographic and other appropriate breast imaging studies. If sonography has been performed previously, the current examination should be compared with prior sonograms, as appropriate. A lesion or any area of the breast being studied should be viewed in 2 perpendicular projections, and real-time scanning by the interpreter is encouraged.

2. The size of a lesion should be determined by recording its maximal dimensions in at least 2 planes; orthogonal planes are recommended. At least 1 set of images of a lesion should be obtained without calipers.

3. The images should be labeled as to right or left breast, location of lesions, and the orientation of the transducer with respect to the breast (e.g., transverse or longitudinal, radial or antiradial). The location of the lesion should be recorded using clock face notation and distance from the nipple, and/or shown on a diagram of the breast. The length of the transducer face (footprint), usually between 3.5 cm and 5 cm, can be used to estimate the distance from the nipple. Measurements should not be made from the edge of the areola, as areolar width is widely variable.

4. Sonographic features are helpful in characterizing breast masses. These feature categories and their descriptors are listed and exemplified in the ACR Breast Imaging Reporting and Data System® (BI-RADS®). The BI-RADS sonographic categories include size, shape, orientation, margin, echogenicity, lesion boundary, attenuation (e.g., shadowing or enhancement), special cases, vascularity, and surrounding tissue [3].

5. Elastography, or tissue stiffness assessment, is among the new feature categories applicable to sonographic analysis of masses, to be included in the Associated Findings section in BI-RADS – Ultrasound, edition 2. To minimize errors in communication or interpretation, if elastography is performed, the color scales should be annotated to denote hardness or softness.

6. Mass characterization with ultrasonography is highly dependent on technical factors.

Breast ultrasound should be performed with a high-resolution scanner (see section VII). Gain settings, focal zone selections, and fields of view should be optimized to obtain high-quality images. The patient should be positioned to minimize the thickness of the portion of the breast being evaluated. For evaluation of lesions in, on, or just beneath the skin, a stand-off device or thick layer of gel may be helpful.

C. Guidance of Interventional Procedures

(See the ACR Practice Guideline for the Performance of Ultrasound-Guided Percutaneous Breast Interventional Procedures.)

When ultrasound guidance is used to assist in needle placement for interventional procedures, care should be taken to ensure that scanning geometry and transducer placement permit adequate visualization of the needle and the needle tip.

VI. DOCUMENTATION

Images of all important findings, including, in the case of interventional procedures, the relationship of the needle to the lesion, should be recorded in a retrievable and reviewable image storage format. It is recommended that documentation of a negative targeted or whole breast ultrasound examination be performed.

Adequate documentation is essential for high-quality patient care. There should be a permanent record of the ultrasound examination and its interpretation. Comparison with prior relevant imaging studies may prove helpful. Images of all appropriate areas, both normal and abnormal, should be recorded. Variations from normal size should generally be accompanied by measurement. The initials of the operator should be accessible on the images or electronically on PACS. Images should be labeled with the patient identification, facility identification, examination date, and image orientation. An official interpretation (final report) of the ultrasound examination should be included in the patient's medical record. It is recommended that the report include a description of the area scanned. Retention of the ultrasound examination images should be based on clinical need and with relevant legal and local health care facility requirements.

If ultrasound is performed for evaluating clinical signs and/or symptoms or a finding on mammography, MRI, or other breast imaging modality, the finding(s) should be referred to in the report. Reporting of lesions should generally include measurements. Use of an accepted reporting system, such as BI-RADS® US, is recommended.

Reporting should be in accordance with ACR Practice Guideline for Communication of Diagnostic Imaging Findings.

VII. EQUIPMENT SPECIFICATIONS

Breast ultrasound should be performed with a high- resolution real-time linear array scanner operating at a center frequency of at least 10 MHz and preferably higher. Other transducers may be utilized in special circumstances. Focal zones should be electronically adjustable. In general, the highest frequency capable of adequate penetration to the depth of interest should be used. For evaluating superficial lesions, scanning through a thin stand-off device or thick layer of gel may be helpful in offsetting the transducer face from the uppermost layer of skin, to bring it into the focal zone of the transducer.

VIII. QUALITY CONTROL AND IMPROVEMENT, SAFETY, INFECTION CONTROL, AND PATIENT EDUCATION

Policies and procedures related to quality, patient education, infection control, and safety should be developed and implemented in accordance with the ACR Policy on Quality Control and Improvement, Safety, Infection Control, and Patient Education appearing under the heading *Position Statement on QC & Improvement, Safety, Infection Control*, *and Patient Education* on the ACR web site (http://www.acr.org/guidelines).

Equipment performance monitoring should be in accordance with the ACR Technical Standard for Diagnostic Medical Physics Performance Monitoring of Real Time Ultrasound Equipment.

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Page 1 Results

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